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Discovery of AB680 – A Potent and Selective Inhibitor of CD73

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Abstract:

Extracellular adenosine (ADO), present in high concentrations in the tumor microenvironment (TME), suppresses immune function via inhibition of T cell and NK cell activation. Intra-tumoral generation of ADO depends on the sequential catabolism of ATP by two ecto-nucleotidases, CD39 (ATP→AMP) and CD73 (AMP→ADO). Inhibition of CD73 eliminates a major pathway of ADO production in the TME and can reverse ADO-mediated immune suppression. Extensive interrogation of structure activity relationships (SAR), structure-based drug design, and optimization of pharmacokinetic properties culminated in the discovery of AB680, a highly potent (K_i = 5 pM), reversible, and selective inhibitor of CD73. AB680 is further characterized by very low clearance and long half-lives across preclinical species, resulting in a PK profile suitable for long-acting parenteral administration. AB680 is currently being evaluated in Phase 1 clinical trials. Initial data show AB680 is well tolerated and exhibits a pharmacokinetic profile suitable for biweekly (Q2W) IV-administration in human.

Introduction.

The introduction of monoclonal antibody (mAb) therapies acting as immune checkpoint inhibitors, such as those targeting programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), has revolutionized cancer immunotherapy treatment.[1,2] However, these agents only elicit strong responses in a minority of patients.[3] For non-responders, tumors exploit additional immunosuppressive mechanisms to facilitate immune escape.[4] As such, combination therapies designed to block multiple immunosuppressive pathways may be required to improve clinical responses.[5] One mechanism by which

tumors evade the immune system is via depletion or increased production of small molecule signaling compounds within the tumor microenvironment (TME). Falling into the latter category, adenosine (ADO) has been shown to accumulate in tumors and exert profound immunosuppressive effects on a variety of tumor-infiltrating leukocytes (TILs) via the A_{2A}/A_{2B} adenosine receptors.[6] Intra-tumoral production of ADO is regulated by the ecto-nucleotidase CD73, which converts extracellular adenosine monophosphate (AMP) to ADO.[7,8,9,10] CD73 is expressed on a variety of TILs and has been found to be overexpressed on a variety of human cancers. Furthermore, high CD73 expression has been associated with poor clinical prognosis.[11] Inhibition of CD73 is expected to reverse ADO-mediated immune suppression by blocking the major mechanism for ADO production in the TME.[12,13,14,15]

CD73 is a glycosylphosphatidylinositol (GPI-) anchored homodimeric *ecto*-nucleotidase, that undergoes an extensive conformational switch between open and closed forms upon substrate binding and release.[16] The binding mode of nucleotide-derived inhibitor adenosine 5'-(α,β-methylene)diphosphate (AMPCP, **1**, K_i = 88.4 nM, Figure 1) has been elucidated and proven instrumental in the design of nucleoside derived CD73 inhibitors.[16,17] Müller and co-workers reported the initial structure-activity relationships (SAR) of this series, which resulted in the discovery of PSB-12379 (**2**, K_i = 2.2 nM, Figure 1),[18,19,26] and, following completion of our CD73 inhibitor discovery effort[20,21], have reported optimized CD73 inhibitors based on that scaffold.[22,23]

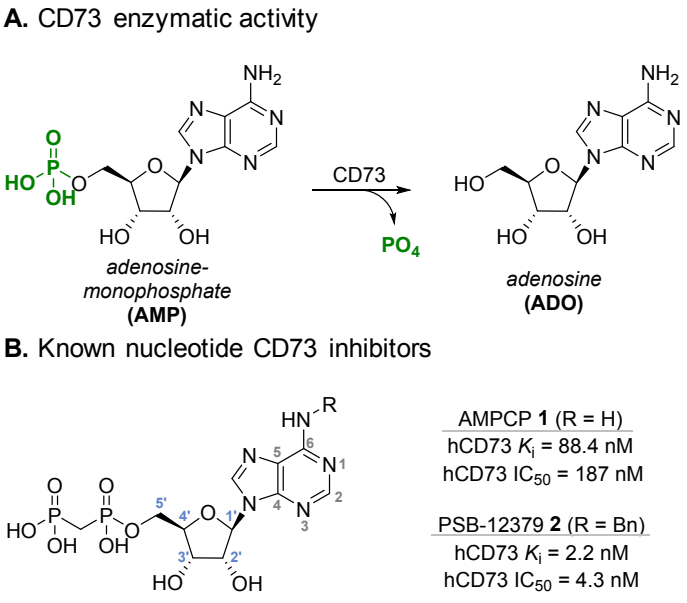

Figure 1. A. CD73 catalyzes the conversion of AMP to ADO with loss of inorganic phosphate (Pi). **B.** Structure of known CD73 inhibitors AMPCP and PSB-12379

AMPCP is a competitive inhibitor that binds the active site of CD73 via a concert of ionic, polar and hydrophobic interactions.[16] The ribose moiety forms bifurcated hydrogen bonds with D506 and N390, while the adenine heterocycle is sandwiched between F417 and F500 in a face-to-face π-stacking interaction (Figure 2A). Lastly, the bisphosphonate group forms an ionic interaction with the di-Zn catalytic domain with an additional ion-pair with R354 (Figure 2B). Our early investigations revealed modification or replacement of the bisphosphonate moiety was poorly tolerated within the nucleotide-mimetic scaffold. This observation confounded our goal of developing an orally bioavailable CD73 inhibitor as phosphate-containing small molecules are notorious for their poor oral bioavailability. Furthermore, high throughput and *in silico* screening techniques generated few or low-quality leads for further optimization. With these revelations we were faced with the option of guiding our discovery effort towards alternative routes of administration or rely upon pro-drug strategies.[24] The latter challenge appeared insurmountable due to the likely necessity to mask three ionizable positions and the sparsity of relevant precedent to do so.

We undertook medicinal chemistry efforts in pursuit of a molecule suitable for IV-administration at extended dosing intervals. For the convenience of patients, our goal was to identify a candidate which could be administered biweekly (Q2W), concomitant with typical mAb checkpoint inhibitor dosing schedules.[25] Regardless of the degree of potency we would ultimately be able to achieve, we realized that extraordinarily low clearance would be a necessity to continually inhibit CD73 activity over the desired dosing interval. As such, our discovery effort relied heavily on the optimization of pharmacokinetic parameters and the early development of tractable structure-pharmacokinetic relationships to ascertain the feasibility of this approach.

Results and Discussion. Our initial investigations into the SAR of the adenosine scaffold was focused on modification of the ribose moiety to avoid the known pharmacokinetic liabilities of the AMPCP scaffold. This work was performed with the 2-chloroadenine base, as in **3**, as this has been shown to improve potency.[26] In our assay, we found that incorporation of chlorine at the C2-position (**3**, Figure 2C) of adenine resulted in a 35-fold improvement in potency relative to AMPCP. With both the 2’ and 3’ hydroxyl groups of **3** putatively engaged in hydrogen bonds with CD73, we were pleased to find that replacement of the 2’ hydroxyl group with fluorine was well tolerated. The fluorine stereochemistry appeared to be important as incorporation of 2’-*(R)*-fluoro in earlier scaffolds (**5**) had resulted in substantial loss in potency.

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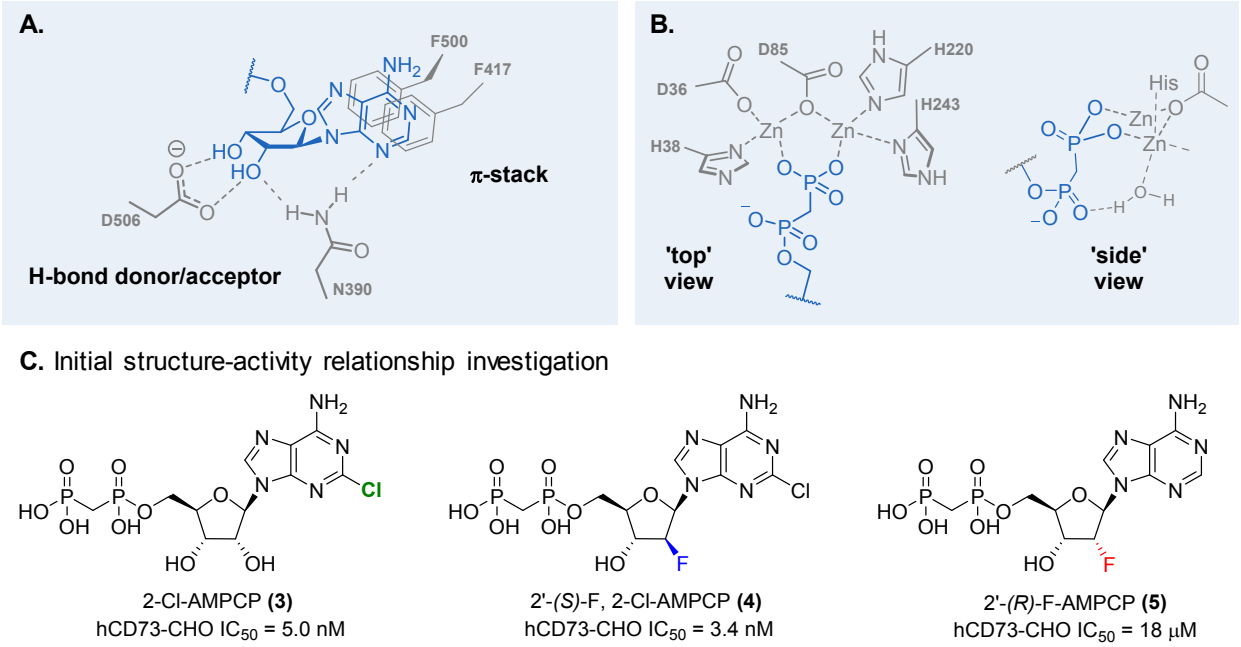
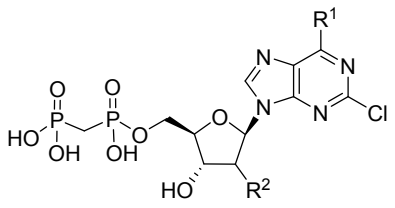


Figure 2. A. Schematic of key binding interactions between AMPCP and CD73 (closed form) based on PDB 4H2I which guided structure-based design and initial SAR. **B.** Schematic of ionic interaction of bisphosphonate moiety and di-Zn catalytic site. **C.** Initial SAR investigation.

Work by Müller and co-workers demonstrated that considerable potency gains could be achieved by incorporation of hydrophobic groups at the C6-position.[18,26] Our efforts identified a similar trend within the β-fluoroarabinose scaffold and we prepared numerous ribose examples for direct comparison. Although incorporation of small motifs, such as methyl-, ethyl-, or cyclopropylamine (Table 1, entries **6**-**9**) did not improve potency, the larger cyclopentylamine group (Table 1, entries **12**-**13**) resulted in a 10-fold potency improvement, providing subnanomolar CD73 inhibitors in both the ribose and β-fluoroarabinose scaffolds. Substitution with benzylamine (entries **16**-**17**) also resulted in similarly improved potency. Beyond these initial discoveries, the SAR at this position was relatively flat. Extensive elaboration of the benzylamine analogs was performed (e.g. **22-24**), the majority of which resulted in equivalent or slightly reduced potency. Increasing the polarity of the C6-substituent (entries **26-27**) resulted in moderate loss in potency, and disubstituted amines (e.g. pyrrolidine, **25**) were well tolerated, indicating that the N-H was not involved in a hydrogen bond donor interaction. A similar conclusion was made by the Muller lab working on a related scaffold.[18]



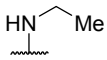
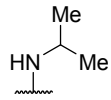
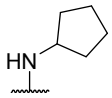
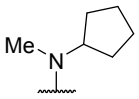
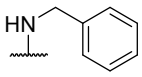
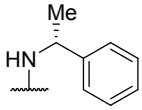
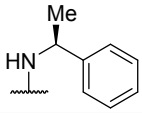
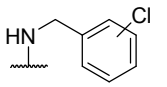
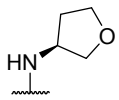
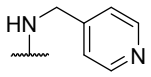
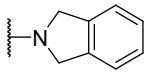
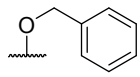
Entry	R^1	R^2	IC_{50} (nM)
3[a]		*(R)*-OH	5.0
4		*(S)*-F	3.4
6		*(S)*-F	12.0
7		*(S)*-F	5.0
8		*(S)*-F	2.6
9		*(R)*-OH	1.7
10		*(S)*-F	3.6
11		*(R)*-OH	0.70
12		*(S)*-F	0.40
13		*(R)*-OH	0.20
14		*(S)*-F	0.15
15		*(R)*-OH	0.12
16		*(S)*-F	0.70
17[a]		*(R)*-OH	0.30
18		*(S)*-F	1.6
19[a]		*(R)*-OH	0.70
20		*(S)*-F	4.1
21[a]		*(R)*-OH	0.30
22			*(o)* 4.2
23		*(S)*-F	*(m)* 1.3
24			*(p)* 2.4
25		*(S)*-F	0.90
26		*(S)*-F	0.80
27		*(S)*-F	3.6
28		*(S)*-F	1.1
29		*(S)*-F	1.3

Table 1. Modification of C6 position (R^1) in ribose [R^2 = *(R)*-OH] and β-fluoroarabinose [R^2 = *(S)*-F] scaffolds. [a]Reference 26.

Completion of this study revealed a consistent trend between the two furanose scaffolds, in which the β-fluoroarabinose scaffold generally demonstrated only slightly reduced potency relative to equivalently functionalized ribose analogs. This came as a surprise given that the 2'-hydroxyl group of AMPCP is reported to form two hydrogen bond interactions with D506 and N390 that would presumably be absent in β-fluoroarabinose analogs.[9] Furthermore, despite the modest loss in potency, the β-fluoroarabinose scaffold offered a compelling program direction, as compounds in this series generally exhibited improved physicochemical and pharmacokinetic properties. As such, we sought to further understand the molecular interactions of the β-fluoroarabinose core with the CD73 catalytic site. To accomplish this, we obtained a high-resolution co-crystal structure of **16** bound to the closed form of CD73.

The key interactions of **16** with CD73 are depicted in Figure 3A. Consistent with the binding interactions of AMPCP, the bisphosphonate group binds the di-zinc catalytic site and the purine heterocycle is sandwiched between F417 and F500 in a π-stacking interaction. In contrast, key interactions of the β-fluoroarabinose moiety were unique. The arabinose ring exhibited a unique puckered conformation moving the 3'-hydroxyl group into close proximity with N390. This

conformation allowed for a similar bifurcated hydrogen bond between the side-chain of N390 and both the 3'-OH and adenine-N3 (Figure 3A). The consequence of this altered ring conformation is best displayed by the overlay of AMPCP with **16** (Figure 3B). The 3'-OH of **16** is located roughly equidistant between the 2'-OH and 3'-OH of AMPCP. This provides a strong rationale for the retained potency of this series, which lacks a hydrogen bond acceptor at the 2'-position. If the ribose conformation of **16** was conserved with that of AMPCP, a hydrogen bond with N390 would be unattainable.

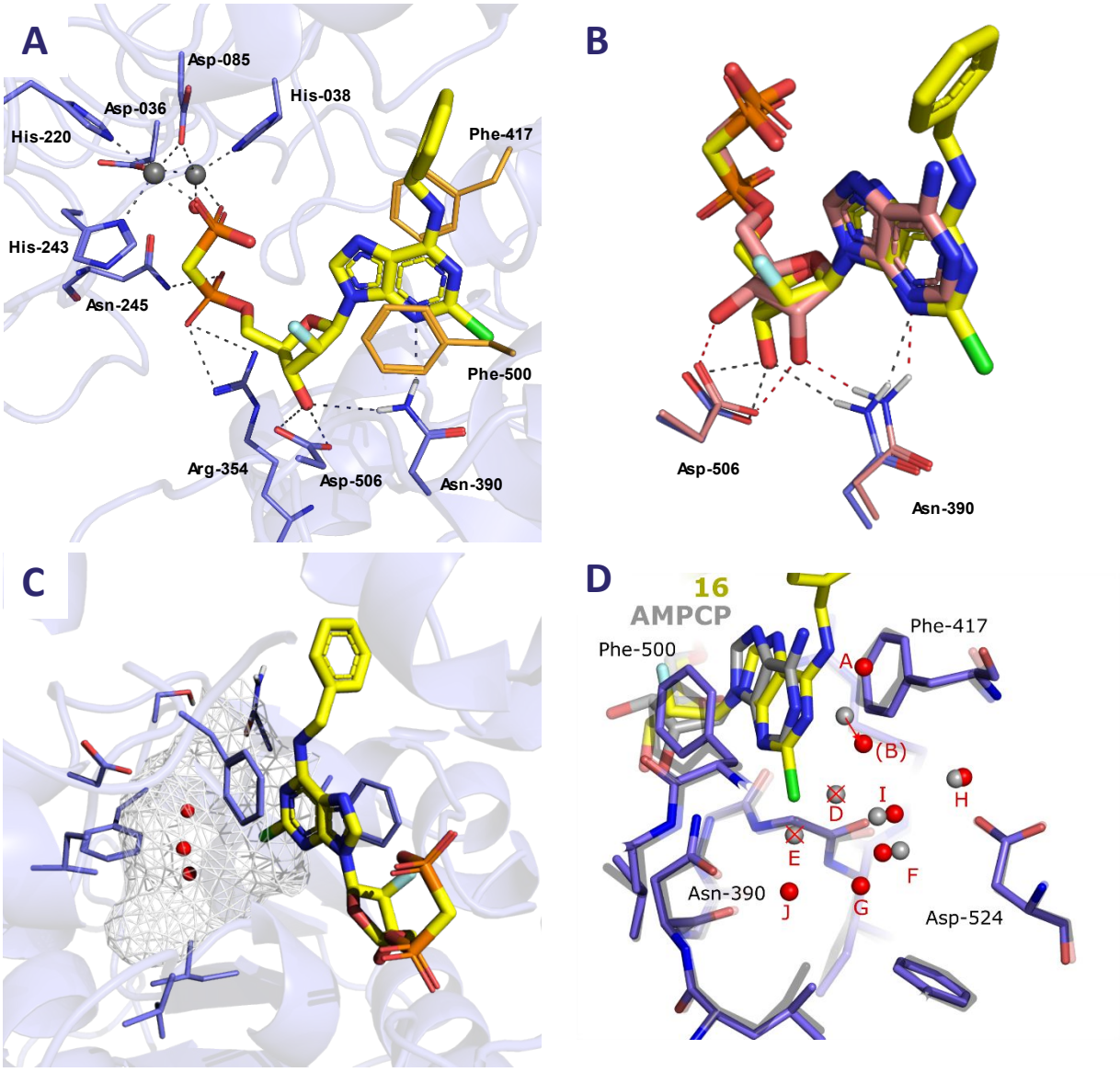


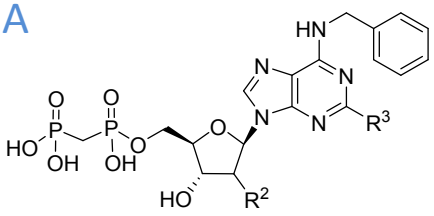
Figure 3. A. Key interactions between **16** and CD73 (closed form), 1.94 Å resolution, PDB ID: 6Z9B. **B.** Overlay of **16** (yellow) and AMPCP (pink, PDB ID: 4H2I). Key interaction with N390 and D506 shown. (Superposition based on matched atom pairs) **C.** C2-pocket cavity surface (grey mesh) is lined predominantly by hydrophobic residues. **D.** Changes in the water structure of the C2-pocket in a comparison of the binding modes of AMPCP (ligand, protein residues and water molecules in grey) and **16** (ligand yellow, protein residues blue-purple, waters red).

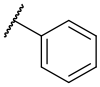
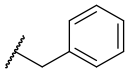
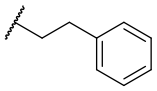
The adenine-C2-chlorine is positioned at the entry to a large pocket, occupied by water molecules and lined primarily by hydrophobic residues (grey mesh, Figure 3C). The substantial potency enhancement resulting from chlorine incorporation may be due in part to Van der Waals forces with hydrophobic residues lining the entry to this pocket, polar interactions with N499, and rearrangements of the C2-pocket water structure. In a comparison of the CD73 cocrystal structures with AMPCP and **16**, introduction of the C2 chlorine substituent in **16** displaces two water molecules (D and E in Figure 4D) and two new water positions (J and G) are occupied instead, close to the hydrophobic bottom of the pocket.

We believed further potency gain could be achieved by occupying a larger fraction of the pocket. We prepared a number of analogs to interrogate this hypothesis. Unfortunately, it became evident that increasing the size of substituents at the C2 position was poorly tolerated (Table 2). This may be explained by the narrow entrance to the hydrophobic cavity imparted by the phenylalanine residues. Increasing the size of the C2 substituent may, in turn, partially disrupt the favorable π-stacking interaction. Indeed, increasing size from -methyl (**30**, IC_{50} = 1.3 nM) to -ethyl (**31**, IC_{50} = 12.0 nM) resulted in a nearly 10-fold potency loss while a C2-ethyne substituent, featuring a smaller steric footprint, was comparatively well-tolerated (**39**, IC_{50} = 2.0 nM). Electron-donating groups -NHMe, -pyrrolidine, and -OMe (**34-36**, respectively) were also poorly tolerated (7 to 160-fold loss of potency). These comparatively electron-rich heterocycles may weaken the π-stacking interaction with F417 and F500. A similar steric argument could also account for their diminished potency.

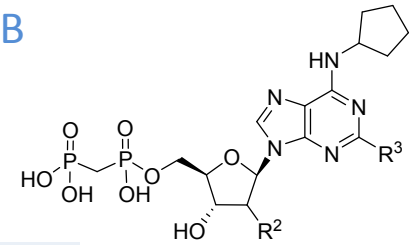
Interestingly, a benzyl group at the C2-position (**38**, IC_{50} = 1.5 nM) was comparatively well tolerated considering its increased size. The arene likely forms favorable interactions with residues deeper in the C2-pocket and would be expected to displace additional water from the pocket. We believe this may offset the aforementioned unfavorable interactions of a hydrophobic group with the narrow entrance to the C2-pocket. Increasing the size to a phenethyl substituent (**40**) led to a considerable loss in potency, consistent with the limited size of the C2-pocket.

A



Entry	R^3	R^2	IC_{50} (nM)
30		*(S)*-F	4.0
31		*(R)*-OH	1.3
32		*(R)*-OH	12.0
33		*(S)*-F	18.0
34		*(R)*-OH	5.0
35		*(R)*-OH	48
36		*(R)*-OH	2.2
37		*(S)*-F	10.0
38		*(S)*-F	1.5
39		*(S)*-F	2.0
40		*(S)*-F	18.0

B



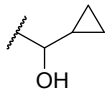
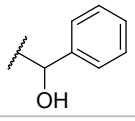
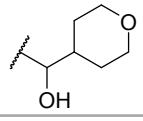
Entry	R^3	R^2	IC_{50} (nM)
41		*(S)*-F	0.6
42		*(S)*-F	1.4
43		*(S)*-F	3.3
44		*(S)*-F	202

Table 2. A. Modification of C2 position (R^3) with constant C6-benzyl group. **B.** Modification of C2 position (R^3) with constant C6-cyclopentyl group.

With a basic understanding of the nucleoside SAR and potent compounds in hand, we began optimization of PK parameters in pursuit of a drug which was suitable for infrequent IV-administration. Compounds in the β-fluoroarabinose series were generally characterized by favorable pharmacokinetic profiles, exhibiting very low clearance and long half-lives in rat (Table 3). To further optimize for low clearance compounds, four potent analogs with varied C6-substituents (R1, Table 3) were chosen. Compounds with benzylamine at R^1 (**16**, Table 3) exhibited reduced clearance rates relative to cyclopentylamine analogs (**12**). Clearance in rats could be further reduced by substituting the C6 position with (S)-(−)-α-methylbenzylamine (**20**, CL = 0.021 L/h/kg). The stereochemistry at the benzylic position was critical for improving metabolic stability. Interestingly, the opposite diastereomer, featuring a (R)-(+)-α-methylbenzylamine (**18**) at C6, displayed higher clearance than the unsubstituted benzylamine analog (**16**).

To continue to explore opportunities to improve potency and PK properties, we began investigating alternative heterocycles to replace the adenine base. In the β-fluoroarabinose series, replacement of adenine with a pyrazolopyrimidine heterocycle provided compounds **45** - **48**, all of which displayed equivalent or reduced potency against CD73. Gratifyingly, this relationship of C6-substitutent and clearance was conserved relative to compounds featuring the

adenine base. This clear and predictable relationship was integral in our optimization towards very low clearance CD73 inhibitors.

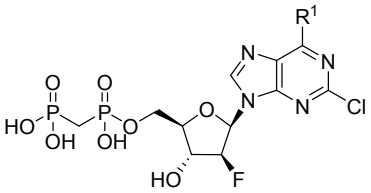
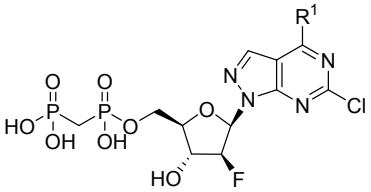
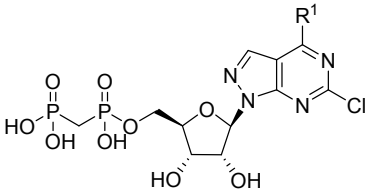
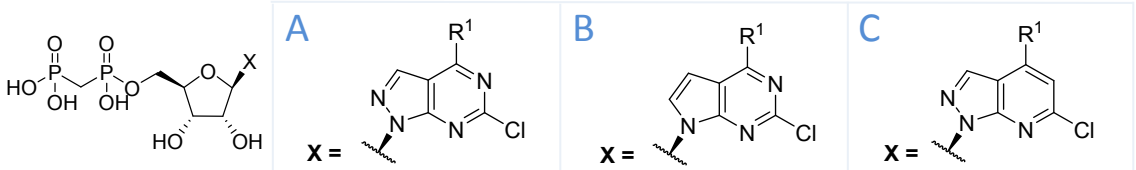
Nucleoside Core	Assay	R^1 = HN	R^1 = HN	R^1 = HN, Me	R^1 = HN, Me
	**ID**	**12**	**16**	**20**	**18**
	hCD73 IC_{50} (nM)	0.4	0.7	4.1	1.6
	CL (rat) (L/h/kg)	0.15	0.060	0.021	0.28
	**ID**	**45**	**46**	**47**	**48**
	hCD73 IC_{50} (nM)	0.15	5.9	5.4	2.1
	CL (rat) (L/h/kg)	0.27	nd	0.029	0.56
	**ID**	**49**	**50**	**51**	**52**
	hCD73 IC_{50} (nM)	0.027	0.052	0.045	0.058
	CL (rat) (L/h/kg)	0.63	0.20	0.064	0.17

Table 3. Concise optimization of potency and pharmacokinetic properties by variation of C6 substituent (R^1) and purine heterocycle. Rat clearance determined in male SD rats following an IV dose of 0.5 mg/kg.

The analogous pyrazolopyrimidines (**49** - **52**) featuring a ribose core exhibited remarkably improved potency. Relative to analogs possessing the β-fluoroarabinose core, ribose analogs were 10 to 100-fold more potent. For example, compound **51** displayed extraordinary potency (IC_{50} = 45 pM) and very low clearance in rats (0.064 L/h/kg). With this discovery, the development of a long-active IV-administered drug appeared within reach.

The introduction of the pyrazolopyrimidine core was, however, associated with worsened chemical stability. Anomeric cleavage of the heterocycle was prevalent under acidic conditions.[27] Furthermore, the electrophilicity at the C2 position was evidenced by displacement of the C2-Cl by water under basic conditions. Lastly, metabolic profiling identified a potential for oxidation of the unsubstituted C6-benzylamine group. We believed we could mitigate each of these issues by further modification of the purine heterocycle and modification of the C6-benzylamine moiety. We investigated three heterocycles, pyrazolopyrimidine (control, Table 4A), pyrrolopyrimidine (Table 4B), and pyrazolopyridine (Table 4C) in combination with *ortho*, *meta* or *para*-fluorine substituted (S)-(−)-α-methylbenzylamines at the C6 position. Incorporation

of fluorine at these positions had little effect on potency. The pyrazolopyridine analogs (**55** and **60**) demonstrated the lowest *in vivo* rat clearance of the analogs examined. For example, **60** exhibited remarkably low clearance (0.009 L/h/kg) in rats. Furthermore, **55** and **60** exhibited improved chemical stability under both acidic and basic conditions. Given their favorable properties, **55** and **60**, as well as **51**, were selected for further characterization.



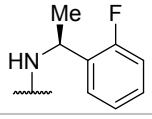
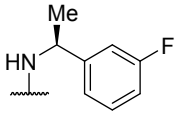
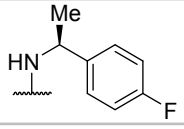
R^1	ID	IC_{50} (nM)	CL_{rat} (L/h/kg)	ID	IC_{50} (nM)	CL_{rat} (L/h/kg)	ID	IC_{50} (nM)	CL_{rat} (L/h/kg)
	**53**	0.070	0.04	**54**	0.028	0.05	**55**	0.041	0.02
	**56**	0.031	0.05	**57**	0.032	0.04	-	-	-
	**58**	0.095	-	**59**	0.031	0.03	**60**	0.115	0.009

Table 4. Further optimization of purine heterocycle and C6-substituent in parallel. Rat clearance determined in male SD rats following an IV dose of 0.5 mg/kg.

CD73 is expressed on the surface of cancer cells as well as a variety of cells which comprise the immune infiltrate. Additionally, soluble CD73 is abundant in human serum. As such, we examined the potency of **51**, **55** and **60** against human and mouse $CD8^+$ T cells, human peripheral blood monocytic cells (PBMCs), and soluble recombinant CD73. All compounds retained potency against soluble CD73 and were extraordinarily potent against human $CD8^+$ T cells, with **51** and **55** exhibiting IC_{50} values of less than 10 pM. Similar potencies were observed for human PBMCs. In contrast, a near 100-fold loss in potency was observed against mouse $CD8^+$ T cells for the three compounds. The relative ranking of the three compounds was conserved across all cell-based assays. In spite of the extraordinary potency, **55** was confirmed to be a reversible, competitive inhibitor of CD73 with a K_i of 5 pM.[28] None of the compounds showed any off-target activity against the ecto-nucleotidases NTPDase-1 (CD39), -2, -3, and -8 up to 10 µM.[29] Compounds **51**, **55,** and **60** were also screened against 60 targets in a wide ligand profile screen (CEREP) with no off-target activity observed up to 10 µM.

Compound	hCD73-Soluble (IC_{50}, nM)	$hCD8^+$ T Cells (IC_{50}, nM)	$mCD8^+$ T Cells (IC_{50}, nM)	hPBMC (IC_{50}, nM)	CD39 (IC_{50}, μM)	NTPDase 2 (IC_{50}, μM)	NTPDase 3 (IC_{50}, μM)	NTPDase 8 (IC_{50}, μM)
51	0.053	0.0027	0.16	0.0085	> 10	> 10	> 10	> 10
55	0.043	0.0080	0.66	0.011	> 10	> 10	> 10	> 10
60	n.d.	0.010	0.83	0.021	> 10	> 10	> 10	> 10

Table 5. Potency of select compounds against soluble CD73 and primary immune cell types. Selectivity against related ecto-nucleotidases.

Mouse, rat, dog and monkey IV-administered PK data were collected for **51**, **55,** and **60** (Table 6). Low clearance was observed across all species for each compound. Allometric scaling was employed to project human PK parameters. The pyrazolopyridine analogs, **55** and **60**, were projected to possess 98- and 167-hour human half-lives, respectively, suitable for intravenous administration every 2 weeks (Q2W). Due to its superior potency *in vitro* and favorable pharmacokinetic properties, **55** was selected for further characterization and ultimately became our clinical development candidate AB680.

	51				AB680 (55)				60			
Species	**CL** (L/h/kg)	**Vss** (L/kg)	**$T_{1/2}$** (h)	**fu** (%)	**CL** (L/h/kg)	**Vss** (L/kg)	**$T_{1/2}$** (h)	**fu** (%)	**CL** (L/h/kg)	**Vss** (L/kg)	**$T_{1/2}$** (h)	**fu** (%)
Mouse	0.076	0.18	2.0	2.9	0.025	0.12	3.5	0.89	0.015	0.11	6.5	0.65
Rat	0.064	0.12	1.7	0.95	0.020	0.12	5.3	0.17	0.0093	0.12	9.6	0.16
Dog	0.071	0.84	13	18.7	0.050	1.3	22	8.3	0.034	0.63	15	10.2
Monkey	0.0024	0.096	28	0.20	0.0025	0.099	27	0.59	0.0017	0.078	34	0.34
Human (projected)	0.0036	0.14	27	0.89	0.0012	0.17	98	0.42	0.00054	0.13	167	0.29

Table 6. Pharmacokinetic data for select compounds in preclinical species following an IV dose of 0.5 mg/kg. Projected human PK parameters determined by allometric scaling. V_{ss} prediction determined by Øie-Tozer method.

AB680 was assessed for its potential to cause drug-drug interactions (Table 7). No inhibition was observed against a panel of CYP isoforms up to 100 μM and no potential for time-dependent CYP inhibition was observed. Potential for CYP induction, assessed via enzymatic activity, mRNA expression levels, or PXR reporter gene assay, was not observed. Furthermore, no evidence for cardiovascular liabilities (hERG, IC_{50} > 10 μM AB680) or genotoxicity (AMES negative) was found.

Assay	Result
CYP inhibition (1A2, 2C9, 2C19, 2D6, 3A4)	All > 100 μM
P450 TDI (1A2, 2C9, 2C19, 2D6, 3A4)	No issues
AMES	No issues
hERG (automatic patch clamp)	IC_{50} > 10 μM
CYP Induction, mRNA (1A2, 2B6, 3A4)	No issues

Table 7. Additional in vitro profiling of AB680

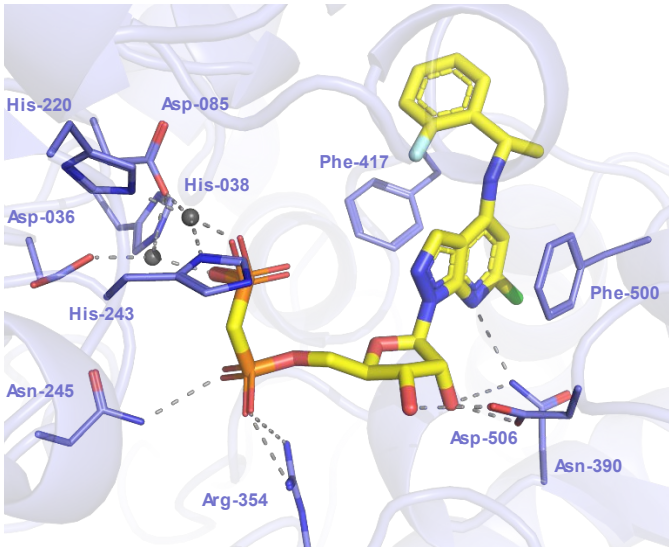
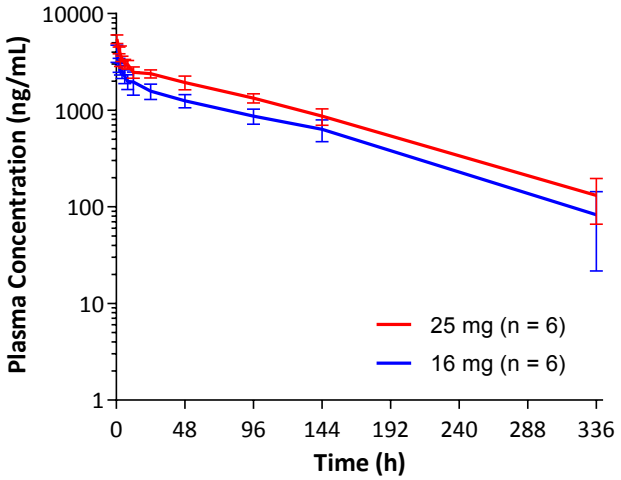


Figure 4. X-ray structure of AB680 bound to CD73 (closed form), PDB ID: 6Z9D.

Finally, X-ray co-crystallization of AB680 with CD73 revealed a binding mode analogous to AMPCP and **16**, described above. The density map indicated flexibility within the α-methylbenzylamine moiety, suggesting this group may bind as a mixture of rotational isomers. The major conformation (63% abundance) is shown in Figure 4. The π-stacking interaction of AB680 is slightly altered relative to that of AMPCP with the nucleobase of AB680 being shifted away from F417 towards F500. As a result, F417 no longer forms a parallel face-to-face π-stacking interaction with the nucleobase of AB680 but is tilted toward an edge-to-face interaction. As a result of this conformational change, F417 forms an additional face-to-face π-interaction with the fluorphenyl substituent of AB680. These interactions may be energetically more favorable and may partially explain the potency gain observed by incorporation of this nucleobase and the phenyl substituent.[30]

Based on the extraordinary potency of AB680 *in vitro* and excellent pharmacokinetic profile in preclinical species, AB680 was advanced to a phase I clinical study in healthy volunteers. This placebo-controlled study assessed the safety, tolerability, and PK/PD profile of AB680. Here we present the initial pharmacokinetic data of AB680 in humans. AB680 was well tolerated when administered as a single IV dose across the range of 0.1 to 25 mg (6 cohorts). All treatment-emergent adverse events were mild or moderate in severity, with no clear pattern of toxicity at these dose levels. AB680 displayed low clearance and a long half-life (67 – 74 hours) following an IV infusion of 16 or 25 mg over 30-60 minutes (Figure 6). The human PK profile of AB680 is consistent with the intended Q2W dosing schedule and validates the design strategy employed to discover a long-acting drug for IV-administration.

A.

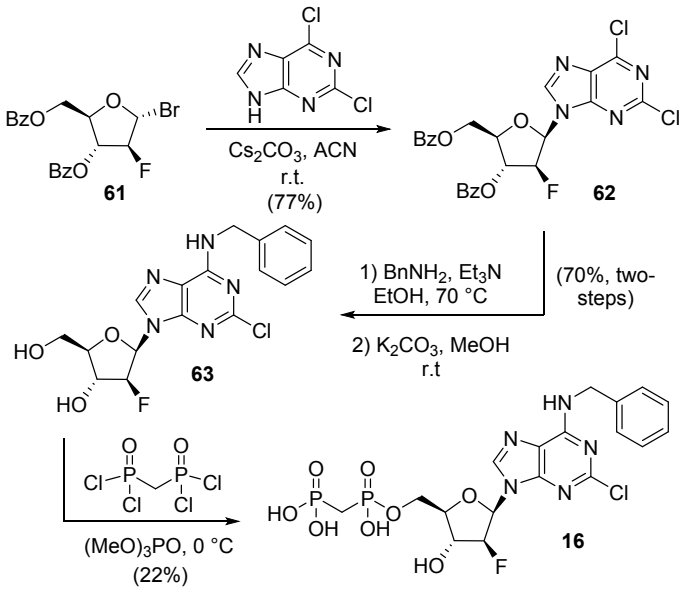


B.

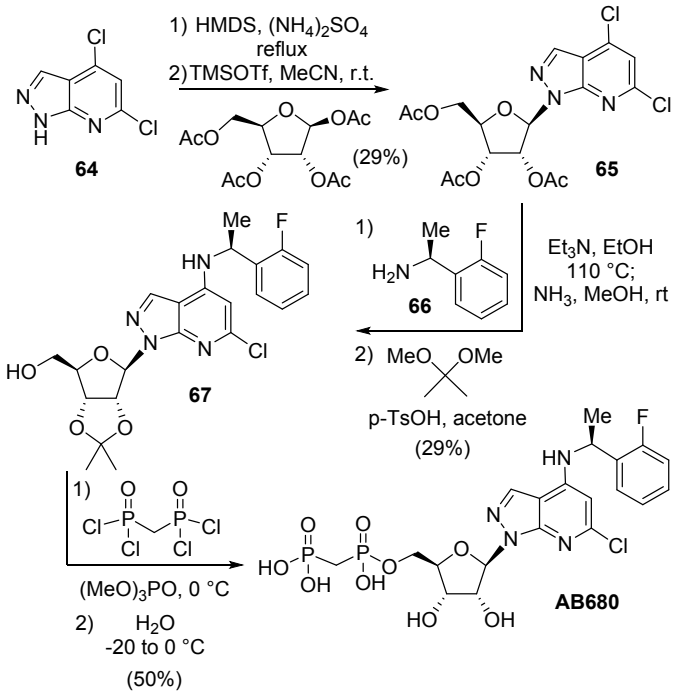
Cohort	16 mg	25 mg
n	6	6
C_{max} (ng/mL)	3,910	5,170
CL (L/h)	0.0672	0.0685
V_{ss} (L)	6.38	6.98
$T_{1/2}$ (h)	66.6	74.2

Figure 6. A. Mean (SD) plasma concentrations of AB680 versus time in healthy volunteers (HV) following an IV-infusion over 30 minutes at dose levels of 16 and 25 mg/kg. **B.** Non-compartmental PK parameters following a single IV dose of AB680.

Chemistry. *Synthesis of representative examples **16** and **AB680**.* The synthesis of analogs possessing a β-fluoroarabinose or ribose core followed a similar general strategy; building the nucleotide core followed by phosphonylation as the final step.[31] In the case of β-fluoroarabinose analogs, such as **16** (Scheme 1), glycosylation of the 2,6-dichloro-imidazolopyrimidine was achieved by treatment with Cs_2CO_3 followed by addition of benzoate protected 2-fluoro-α-D-arabinofuranosyl bromide (**61**) to the reaction mixture.[32] This cleanly provided **62** with complete regio- and diastereomeric control. S_NAr displacement of the C6-chloride was achieved by treatment with benzylamine and Et_3N in EtOH at elevated temperature. No displacement of the C2-chloride was observed. Global benzoate deprotection was realized by treatment with K_2CO_3 in MeOH at room temperature to provide the penultimate nucleoside **63** in 70 % yield.



Scheme 1. Preparation of **16** - Representative synthesis of β-fluoroarabinose analogs.

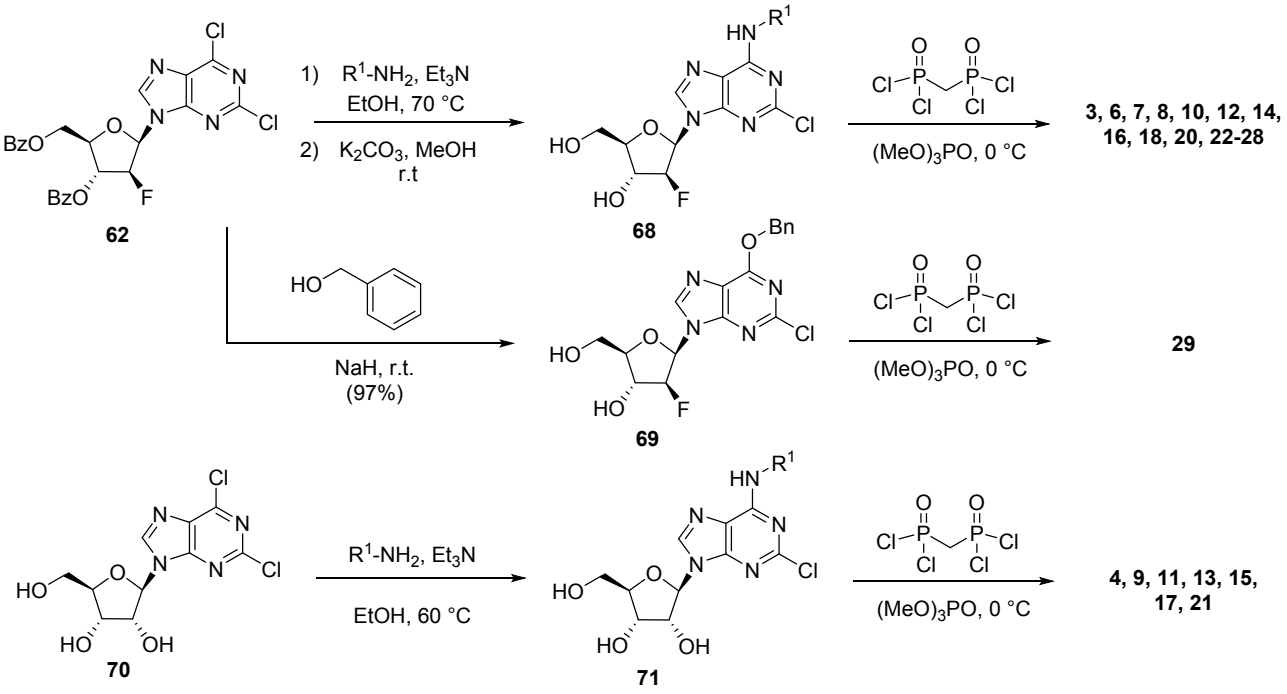


Scheme 2. Synthesis of AB680.

5'-phosphorylation and phosphonylation of nucleosides has been reported to be rapid and regioselective in the presence of $PO(OMe)_3$ without the necessity of protecting secondary alcohols.[33] Indeed, treatment of **63** with excess methylenebis(phosphonic dichloride) in $PO(OMe)_3$ resulted in the selective phosphonylation of the 5'-hydroxyl group. Following quenching the phosphonyl chloride with aqueous buffer, **16** was isolated by reverse phase preparative HPLC.

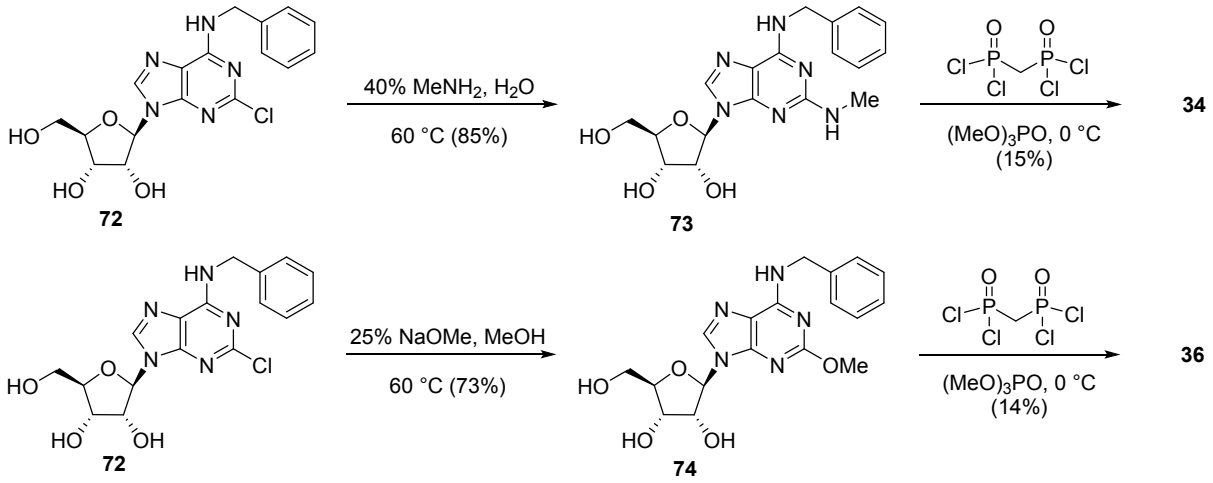
The synthesis of AB680 and related ribose analogs employed a similar strategy. The pyrazolopyridine nucleobase presented unique challenges in the glycosylation step. Selectivity for pyrazole-N1 glycosylation was achieved by exposure of **64** to catalytic ammonium sulfate in refluxing HMDS following established methods. After removal of excess HMDS, the crude silylated heterocycle was reconstituted in MeCN and alkylated with β-D-ribofuranose-1,2,3,5-tetraacetate promoted by TMS-triflate. Careful separation of the minor undesired anomer was required by column chromatography. Displacement of the C6-chloride with benzylamine **66** was achieved by heating in EtOH with excess Et_3N at 110 °C. Subsequent removal of the 2',3',5'-acetate groups was rapid in the presence of methanolic ammonia. Following acetonide protection of the 2',3'-hydroxyl groups, phosphonylation of the 5'-position was achieved under conditions analogous to those described above to prepare **16**. Acetonide deprotection, promoted by acid generated by quenching the intermediate phosphonyl chloride with water, afforded AB680. The remaining pyrazolopyridine-containing analog **60** was prepared via an analogous route.

Synthesis of C6 modified analogs (Table 1). C6-modified analogs were readily prepared in a high throughput manner via nucleophilic aromatic substitution with amines or alcohols (Scheme 3). Within the β-fluoroarabinose scaffold, benzoyl protected 2,6-dichloroadenosine intermediate **62** was treated with a variety of primary and secondary amines and triethylamine in EtOH at 70 °C. In every instance S_nAr occurred exclusively at the C6 position in moderate to good yield. Removal of the 3',5'-benzoyl protecting groups was achieved by treatment with K_2CO_3 in MeOH providing variably C6-amine substituted nucleosides **68**. The corresponding nucleotides were prepared under conditions identical to those employed to prepare **16** (Scheme 1). Ribose nucleosides **71** were prepared similarly from commercially available dichloride **70** (Scheme 3).



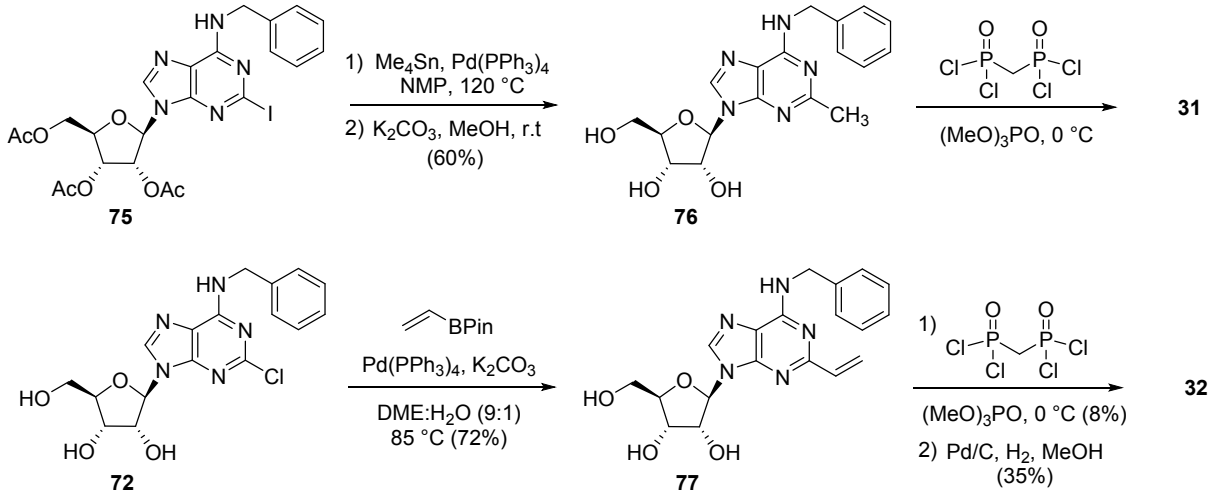
Scheme 3. General synthesis of C6-modified nucleotide analogs. See experimental section for details.

Synthesis of C2 modified analogs (Table 2). Synthetic access to C2 modified analogs was generally accomplished by nucleophilic substitution of a suitable aryl halide or transition metal-mediated catalysis. Compounds **34**-**36** were prepared by the former mechanism. Treatment of nucleoside **72** with excess methylamine in water at 60 °C provided the desired C2-substituted nucleoside in 85% yield (Scheme 4). The analogous C2-pyrrolidine analog (precursor to **35**) was prepared under similar conditions. Sodium methoxide was also a competent nucleophile. Treatment with excess NaOMe in MeOH displaced the C2-chloride in good yield providing nucleoside **74**. Phosphonylation under standard conditions provided the final nucleotide analogs **34** and **36**.



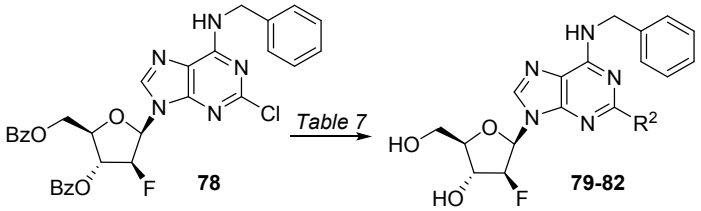
Scheme 4. Synthesis of C2-modified analogs **34** and **36** via SnAr with amine and alcohol nucleophiles

The remaining analogs presented in Table 2 were accessed via palladium-catalyzed cross coupling of suitably functionalized 2-halo-adenine intermediates and a nucleophilic coupling partner. The C2-methyl analog **31** was prepared from the corresponding C2-iodide **75** by Stille reaction with Me_4Sn and $Pd(PPh_3)_4$ in NMP at 120°C followed by deprotection and phosphonylation. C2-Ethyl analog **32** was prepared analogously via Suzuki coupling of vinylboronic acid pinacol ester with **72** followed by phosphonylation and reduction.



Scheme 5. Synthesis of C2-modified analogs **31** and **32**.

The syntheses of β-fluoroarabinose C2-modified nucleosides **79**-**82** are described in Table 7. Each was prepared from compound **78** via Suzuki or Sonogashira coupling under standard conditions followed by benzoate deprotection with K_2CO_3 in MeOH. Subsequent alkyne hydrogenation was required to prepare C2-phenethyl nucleoside analog **82**. Nucleosides **79**-**82** were converted to their corresponding nucleotide analogs **37**-**40** under standard conditions.



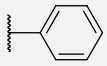
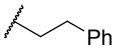
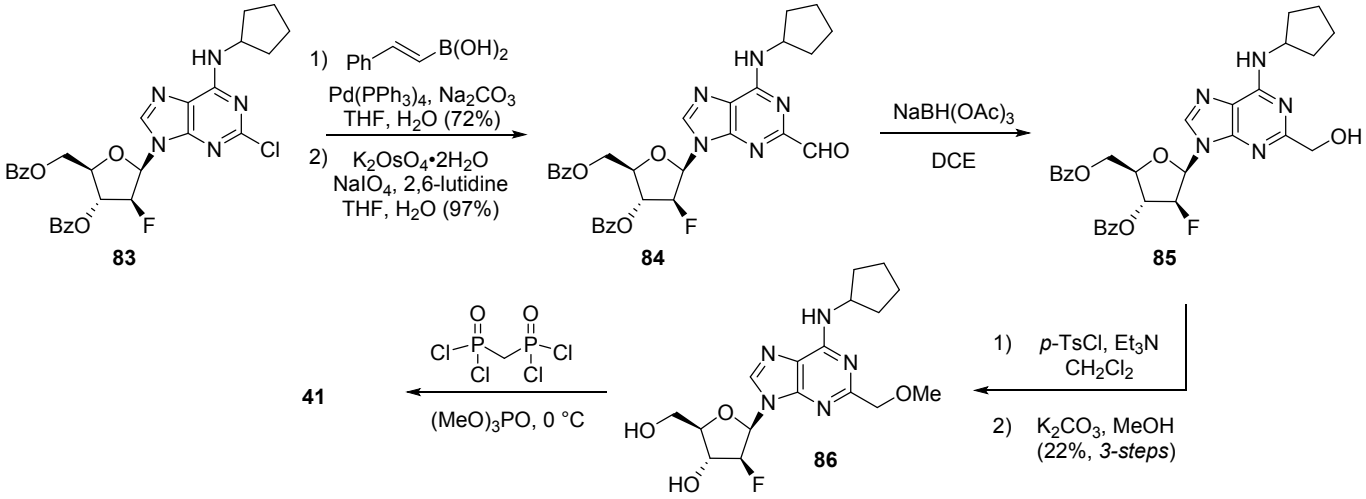
Pdt #	R^2	Conditions	Yield (%)
79		1) Phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, THF, H_2O 2) K_2CO_3, MeOH	8 *(two steps)*
80		1) K-benzyltrifluoroborate, $PdCl_2(dppf)$, Cs_2CO_3, THF, H_2O 2) K_2CO_3, MeOH	40 86
81		1) TMS-acetylene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF 2) K_2CO_3, MeOH	43 42
82		1) Phenylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF 2) K_2CO_3, MeOH 3) Pd/C, H_2	92 79 *(steps 2-3)*

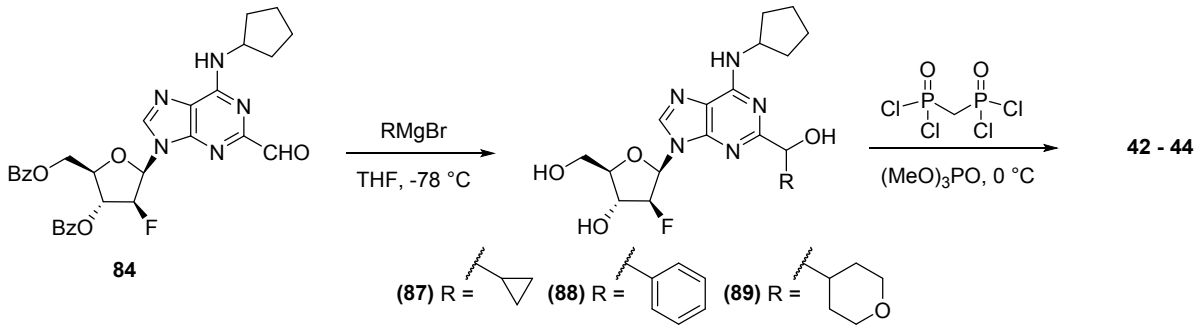
Table 7. Summary of conditions employed for C2-modification of β-fluoroarabinose nucleosides. Products **79**-**82** were converted to nucleotides **37**-**40**, respectively, under standard conditions.

The analogs described in Table 2B were prepared in a divergent manner from C2-aldehyde **84** which was prepared via Suzuki coupling of **83** with *trans*-2-phenylvinylboronic acid and subsequent Lemieux-Johnson oxidative olefin cleavage. Intermediate **84** was reduced with $NaBH(OAc)_3$ to give benzyl alcohol **85**. Activation with p-TsCl followed by methanolysis provided **86**, the nucleoside precursor to **41**.



Scheme 6. Synthesis of **41**.

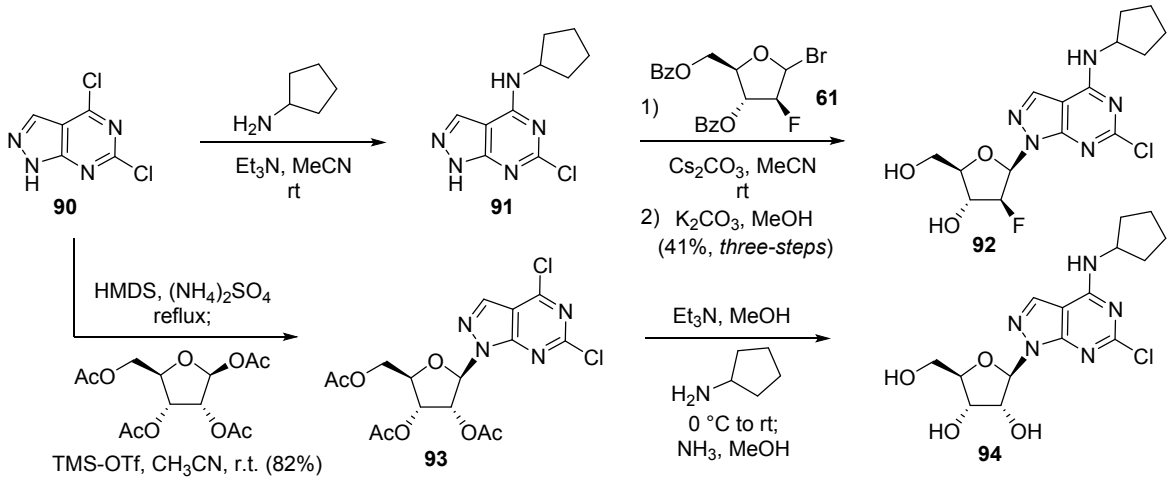
Aldehyde **84** was also treated independently with cyclopropyl, phenyl, and tetrahydropyranyl Grignard reagents to provide secondary alcohols **87**-**89**, respectively, as a mixture of diastereomers. Excess organomagnesium reagent was utilized to remove the 3' and 4'-benzoate groups in situ. Phosphonylation under standard conditions provided analogs **42**-**44**.



Scheme 7. Synthesis of **42**-**44**.

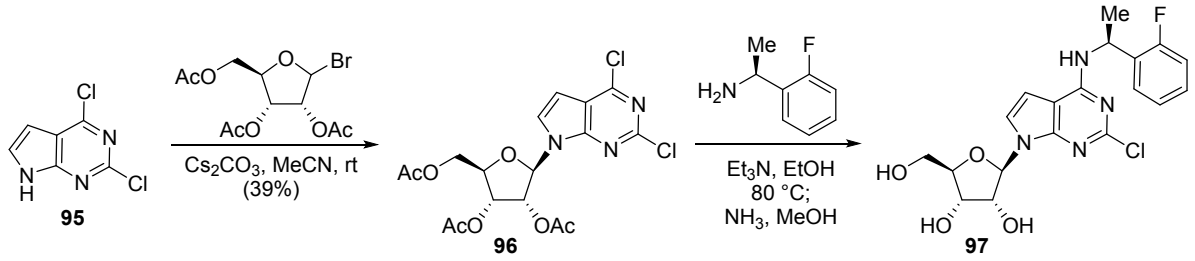
Representative syntheses of analogs with pyrazolopyrimidine and pyrrolopyrimidine nucleobases (Tables 3 and 4). The syntheses of ribose and β-fluoroarabinose containing a pyrazolopyrimidine nucleobase began from commercially available 2,6-dichloropyrazolopyrimidine **90** (Scheme 8). In contrast to β-fluoroarabinose adenine analogs, such as **16**, nucleophilic substitution of the 6-position with alkyl groups was performed prior to glycosidic bond formation. This was necessary due to the high relative electrophilicity of **90**. In fact, exposure of **90** to Cs_2CO_3 caused rapid polymerization of **90** at room temperature.

In one example, treatment of **90** with cyclopentylamine rapidly formed **91**. Glycosylation of **91** was achieved by treatment with 2-fluoro-α-*D*-arabinofuranosyl bromide **61** in the presence of Cs_2CO_3. Under these conditions, both pyrazolopyrimidine *N1*- and *N2*-alkylated products were generated, which were readily separated after deprotection with K_2CO_3 in MeOH. Nucleoside **92** was converted to **45** under phosphonylation conditions identical to those described for the synthesis of **16**. Analogs **46**-**48** were prepared by an analogous route where cyclopropylamine was replaced with the suitable benzylamine in the first step of the sequence.



Scheme 8. Synthesis of pyrazolopyrimidine nucleosides.

Pyrazolopyrimidine nucleosides featuring a ribose moiety as exemplified by **94** were accessed via a synthetic sequence analogous to that employed for the synthesis of AB680 (*vide supra*). In short, **90** was refluxed with HMDS in the presence of catalytic ammonium sulfate followed by treatment with β-*D*-ribofuranose-1,2,3,5-tetraacetate and TMS-triflate to prepare protected nucleoside **93**. Treatment with cyclopentylamine and Et_3N in MeOH resulted in rapid and selective displacement of the C6-chloride. Global deprotection of the intermediate 2',3',5'-triacetate was achieved by exposure to ammonia in MeOH, affording nucleoside **94**. Under standard phosphonylation conditions described above, **94** was converted to **49**. Analogs **50-52**, **53**, **56**, and **58** were prepared by an analogous route where cyclopropylamine was replaced with the suitable benzylamine.



Scheme 9. Synthesis of pyrrolopyrimidine nucleosides

Analogs containing a pyrrolopyrimidine nucleobase (**54**, **57**, and **59**) were prepared as described in Scheme 9. 2,6-Dichloropyrrolopyrimidine was glycosylated by treatment with *D*-arabinofuranosyl bromide-2,3,5-triacetate and Cs_2CO_3 in MeCN at room temperature affording **96** in moderate yield. Nucleophilic substitution of the C6-chloride with (*S*)-1-(2-fluorophenyl)ethanamine was achieved by heating in EtOH in the presence of Et_3N. Subsequent treatment with ammonia

in MeOH deprotected the intermediate 2',3',5'-triacetate to afford nucleoside **97**. Under standard phosphonylation conditions, described above, **97** was converted to **54**. Analogs **57** and **59** were prepared by an analogous route.

Conclusion. Inhibition of CD73 eliminates a major pathway of ADO production in the TME and can reverse ADO-mediated immune suppression. A series of potent and selective CD73 inhibitors were optimized using structure-based design and interrogation of structure activity relationships. Trend analysis of rat IV pharmacokinetic data established clear and consistent structure-pharmacokinetic relationships. Taken together, structure-activity and pharmacokinetic relationships led to the discovery of AB680, which demonstrates extraordinary potency and very low clearance across preclinical species. AB680 is currently being evaluated in Phase 1 clinical trials. Initial data show AB680 is well tolerated and exhibits a pharmacokinetic profile suitable for Q2W IV-administration in human. Even though AB680 was optimized for parenteral delivery, an extensive formulation effort identified proprietary formulations that allow for the oral delivery of AB680[34]. Details of this oral formulation will be published in due course.

Experimental.

General Chemistry. All reactions were performed using a Teflon-coated magnetic stir bar at the indicated temperature and were conducted under an inert atmosphere when stated. All chemicals were used as received. Reactions were monitored by TLC (silica gel 60 with fluorescence F254, visualized with a short wave/long wave UV lamp) and/or LCMS (Agilent 1100 series LCMS with UV detection at 254 nm using a binary solvent system [0.1% TFA in MeCN/0.1% TFA in H_2O] using either of the following column: Agilent Eclipse Plus C18 [3.5 µm, 4.6 mm i.d. x 100 mm]). Flash chromatography was conducted on silica gel using an automated system (CombiFlash RF+ manufactured by Teledyne ISCO), with detection wavelengths of 254 and 280 nm. Reverse phase preparative HPLC was conducted on an Agilent 1260 Infinity series HPLC. Samples were eluted using a binary solvent system (0.1% TFA in MeCN/0.1% TFA in H_2O) with gradient elution on a Gemini C18 110 Å column (21.2 mm i.d. x 250 mm) with detection at 254 nm. Final compounds obtained through preparative HPLC were concentrated through lyophilization. All reported yields are isolated yields. All assayed compounds were purified to ≥95% purity as determined by HPLC or LCMS (Agilent 1100 series LCMS with UV detection at 254 nm using a binary solvent system [0.1% TFA in MeCN/0.1% TFA in H_2O or MeCN/50 mM sodium phosphate in water, pH 8.5] using the following column: Agilent Eclipse Plus C18 column [3.5 µm, 4.6 mm i.d. x 100 mm]). ^{1}H NMR spectra were recorded on a Varian 400 MHz NMR spectrometer equipped with an Oxford AS400 magnet. Chemical shifts (δ) are reported as parts

per million (ppm) relative to residual undeuterated solvent as an internal reference. The abbreviations s, br. s, d, t, q, dd, dt, ddd, and m stand for singlet, broad singlet, doublet, triplet, quartet, doublet of doublets, doublet of triplets, doublet of doublet of doublets, and multiplet, respectively.

Clinical Study.

The phase I clinical study was a first-in-human, double-blind, randomized, placebo-controlled combined single-ascending-dose (SAD) and multiple-ascending-dose (MAD) study to evaluate the safety, tolerability, PK and potential PD effects of AB680 in healthy volunteers. Participants were randomly to receive AB680 (n=6) or matching placebo (n = 2) in each of 7 dosing cohorts in the SAD part and a single dose cohort in the MAD part. In the SAD part of the study, participants received a single IV infusion of 0.1, 0.6, 2, 4, 8, 16, 25 mg AB680 or placebo. In the MAD part of the study, participants received IV infusion of 8 mg AB680 or placebo once daily on 3 days (Days 1, 8, and 15).

Compound Synthesis.

[(2*R*,3*R*,4*S*,5*R*)-3-Benzoyloxy-5-(2,6-dichloro-9*H*-purin-9-yl)-4-fluorotetrahydrofur-2-yl]methyl benzoate (62). 2,6-dichloropurine (3.6 g, 18.8 mmol) was dissolved in 90 mL of MeCN and treated with Cs_2CO_3 (7.5 g, 23 mmol, 1.2 equiv.). The mixture was stirred at room temperature for 30 min. (2*R*,3*R*,4*S*,5*R*)-2-(Benzoyloxymethyl)-5-bromo-4-fluorotetrahydrofur-3-yl benzoate (8.75 g, 21 mmol, 1.1 equiv.) was dissolved in 100 mL of MeCN and added to the mixture dropwise via an addition funnel. The mixture was allowed to stir overnight at room temperature. The mixture was filtered on a pad of silica gel and concentrated. The residue was adsorbed on silica and purified using column chromatography (hexanes / EtOAc) to provide the product (**62**) as a white solid in 77% yield (7.72g). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 (d, *J* = 3.0 Hz, 1H), 8.10 (ddt, *J* = 8.5, 3.1, 0.9 Hz, 4H), 7.74 – 7.36 (m, 6H), 6.64 (dd, *J* = 21.8, 2.8 Hz, 1H), 5.83 – 5.69 (m, 1H), 5.40 (ddd, *J* = 49.9, 2.8, 0.8 Hz, 1H), 4.89 – 4.77 (m, 2H), 4.62 (q, *J* = 4.0 Hz, 1H). ESI MS $[M+H]^+$ for $C_{24}H_{17}Cl_2FN_4O_5$, calcd 531.1, found 531.1.

(2*R*,3*R*,4*S*,5*R*)-5-(9a-benzyl-2-chloro-9-adenineyl)-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (63). Compound **62** (9.0 g, 17 mmol), benzylamine (3 mL, 26 mmol, 1.5 equiv.), and Et_3N (5 mL, 34 mmol, 2.0 equiv.) in anhydrous EtOH (60 mL) was stirred at 70 °C for 4 hours. The reaction mixture was then cooled to room temperature and the product was collected by filtration and used without further purification (white solid, 8.9 g, 87%). ESI MS $[M+H]^+$ for $C_{31}H_{25}ClFN_5O_5$, calcd 602.2, found 602.0.

The above product (10.2 g, 17 mmol) and K_2CO_3 (7 g, 51 mmol, 3 equiv) were dissolved in 170 mL of MeOH and stirred at room temperature for 4 hours. The reaction mixture was then filtered and concentrated on a pad of silica gel. The reaction mixture was purified using column chromatography (CH_2Cl_2 / MeOH) to provide the product **63** as a white solid in 80% yield (5.3 g): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (t, *J* = 6.3 Hz, 1H), 8.31 (d, *J* = 2.0 Hz, 1H), 7.36 – 7.18 (m, 5H), 6.34 (dd, *J* = 13.6, 4.7 Hz, 1H), 5.23 (dt, *J* = 52.6, 4.3 Hz, 1H), 4.66 (q, *J* = 7.3, 5.7 Hz, 2H), 4.43 (dt, *J* = 19.0, 4.8 Hz, 1H), 3.84 (q, *J* = 4.9 Hz, 1H), 3.65 (tq, *J* = 12.0, 6.2, 5.2 Hz, 2H).). ESI MS $[M+H]^+$ for $C_{17}H_{18}ClFN_5O_3$, calcd 394.1, found 394.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(benzylamino)-2-chloropurin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (16). Compound **63** (800 mg, 2 mmol) was dissolved in trimethyl phosphate (15 mL) and cooled to 0 °C (ice bath), then a cold solution of methylenebis(phosphonic dichloride) (2.5 g, 10 mmol, 5 equiv.) in trimethyl phosphate (5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h, and was then carefully quenched with 0.5 M triethylammonium bicarbonate solution (15 mL) and stirred at 0 °C for 15 min, and then 2 h at room temperature. The reaction mixture was purified by reverse phase HPLC (C18 column, 0 to 40% gradient of MeCN and water with 0.1% TFA) to give the product **16** as a white solid in 22% yield (290 mg): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (t, *J* = 6.3 Hz, 1H), 8.30 (d, *J* = 2.2 Hz, 1H), 7.40 – 7.18 (m, 5H), 6.38 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.45 – 5.04 (m, 1H), 4.65 (t, *J* = 5.5 Hz, 2H), 4.54 – 4.42 (m, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.04 (t, *J* = 5.1 Hz, 1H), 2.26 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{18}H_{21}ClFN_5O_8P_2$, calcd 550.8, found 550.2.

[(2*R*,3*R*,4*R*,5*R*)-3,4-diacetoxy-5-(4,6-dichloro-1,2,7-triaza-1*H*-inden-1-yl)tetrahydrofur-2-yl]methyl acetate (65). 4,6-Dichloro-*1H*-pyrazolo[3,4-*d*]pyrimidine **64** (7.0 g, 37 mmol) and ammonium sulfate (100 mg, 5.3 mmol, 1 mol %) were dissolved in hexamethyldisilizane (40 mL). The mixture was heated to reflux and stirred for 3 h. Following this time, the mixture was concentrated in vacuo and then dried under high vacuum (~1 h). The residue was then taken up in MeCN (70 mL), β-*D*-ribofuranose 1,2,3,5-tetraacetate (12.9 g, 40.7 mmol, 1.1 equiv) was added. This mixture was cooled to 0 °C and TMSOTf (10 mL, 55.5 mmol, 1.5 equiv) was added dropwise. The reaction was stirred for an additional hour at 0 °C then quenched by slow addition of saturated aqueous $NaHCO_3$ (1 L) at 0 °C. The mixture was diluted with EtOAc. The aqueous phase was extracted with EtOAc and then the combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, 0→40% EtOAc/Hex) to afford the product **4** as a foam (4.8 g, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.72 (s, 1H), 6.49 (s, 1H), 5.99 – 5.75 (m, 1H),

5.73 – 5.54 (m, 1H), 4.48 – 4.25 (m, 3H), 4.12 – 3.96 (m, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 1.97 (s, 3H). ESI MS $[M+H]^+$ for $C_{17}H_{17}Cl_2N_3O_7$, calcd 446.0, found 446.2.

[(1*R*,5*R*,6*R*,8*R*)-8-{4-[(*S*)-1-(*o*-Fluorophenyl)ethylamino]-6-chloro-1,2,7-triaza-1*H*-inden-1-yl}-3,3-dimethyl-2.4.7-trioxabicyclo[3.3.0]oct-6-yl]methanol (67). A solution of **65** (3.1 g, 6.9 mmol), (1*S*)-1-(2-fluorophenyl)ethylamine (1 g, 7.2 mmol) and triethylamine (1 mL, 7.2 mmol) in anhydrous EtOH (7 mL) was sealed in a screw-top flask and heated to 110 °C overnight. After cooling to room temperature, 7M NH_3/MeOH (5 mL) was added and the mixture was stirred overnight. The reaction mixture was concentrated to dryness under reduced pressure, then reconstituted in acetone (10 mL). 2,2-Dimethoxypropane (10 mL) and p-TsOH (1.6 g, 8.6 mmol) was added and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated to dryness under reduced pressure and purified by column chromatography (SiO_2, 0→50% EtOAc/Hex) to provide **67** (950 mg, 29% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (br. s, 1H), 8.24 (d, *J* = 7.2 Hz, 1H), 7.37 (td, *J* = 7.8, 1.8 Hz, 1H), 7.32 – 7.25 (m, 1H), 7.23 – 7.11 (m, 2H), 6.21 (d, *J* = 1.8 Hz, 1H), 5.98 (br. s, 1H), 5.26 (dd, *J* = 6.1, 1.9 Hz, 1H), 5.03 (br .s, 1H), 4.89 (dd, *J* = 6.2, 2.2 Hz, 1H), 4.85 (t, *J* = 5.8 Hz, 1H), 4.07 (ddd, *J* = 7.8, 5.9, 2.2 Hz, 1H), 3.52 – 3.37 (m, 1H), 3.33 – 3.22 (m, 1H), 2.47 (p, *J* = 1.8 Hz, 2H), 1.54 (d, *J* = 6.7 Hz, 3H), 1.51 – 1.44 (m, 3H), 1.33 – 1.27 (m, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{20}ClFN_4O_4$, calcd 423.1, found 423.3.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1S)-1-(2-fluorophenyl)ethyl]amino]pyrazolo[3,4-b]pyridin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid, AB680 (55). Compound **67** (200 mg, 0.43 mmol) was dissolved in trimethyl phosphate (2 mL) and cooled to 0 °C (ice bath), then a cold solution of methylenebis(phosphonic dichloride) (325 mg, 1.3 mmol, 3 equiv.) in trimethyl phosphate (1 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h, and was then treated with water (1 mL) and stirred overnight at room temperature. The reaction mixture was purified by reverse phase HPLC (C18 column, 0 to 40% gradient of MeCN and water with 0.1% TFA) to give the product **AB680** as a white powder in 50% yield (150 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 8.23 (d, *J* = 6.9 Hz, 1H), 7.42 – 7.34 (m, 1H), 7.33 – 7.09 (m, 3H), 6.06 (d, *J* = 4.3 Hz, 1H), 5.97 (s, 1H), 5.04 (s, 1H), 4.53 – 4.47 (m, 1H), 4.25 (t, *J* = 4.7 Hz, 1H), 4.13 – 3.97 (m, 2H), 3.92 – 3.82 (m, 1H), 2.16 (d, *J* = 20.5 Hz, 2H), 1.56 (d, *J* = 6.4 Hz, 3H). ESI MS $[M+H]^+$ for $C_{20}H_{25}ClFN_4O_9P_2$, calcd 581.1, found 581.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-(6-amino-2-chloropurin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (4). The title compound was synthesized in similar fashion to Example **16**

using commercially available alcohol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, *J* = 2.2 Hz, 1H), 7.92 (s, 2H), 6.36 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.26 (dt, *J* = 52.5, 4.3 Hz, 1H), 4.51 (dt, *J* = 18.6, 4.7 Hz, 1H), 4.19 (t, *J* = 6.0 Hz, 2H), 4.04 (t, *J* = 5.0 Hz, 1H), 2.26 (t, *J* = 20.5 Hz, 2H); MS: (ES) m/z calculated for $C_{11}H_{15}ClFN_5O_8P_2$ $[M-H]^-$ 460.1, found 460.1.

[[(2*R*,3*R*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (5): The title compound was synthesized in similar fashion to **step c** of Example **16** using corresponding alcohol: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.26 (s, 1H), 7.92 (s, 2H), 6.27 (dd, *J* = 17.2, 2.8 Hz, 1H), 5.50 (ddd, *J* = 52.5, 4.5, 2.8 Hz, 1H), 4.64 – 4.52 (m, 1H), 4.29 – 4.08 (m, 3H), 2.25 (t, *J* = 20.4 Hz, 2H). ESI MS $[M+H]^+$ for $C_{11}H_{16}FN_5O_8P_2$, calcd 428.1, found 428.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(methylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (6): The title compound was synthesized in similar fashion to Example **16:** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (q, *J* = 4.6 Hz, 1H), 8.27 (s, 1H), 6.45 (brs, 2H), 6.37 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.25 (dt, *J* = 52.4, 4.3 Hz, 1H), 4.50 (dt, *J* = 18.6, 4.6 Hz, 1H), 4.19 (t, *J* = 5.9 Hz, 2H), 4.04 (q, *J* = 5.2 Hz, 1H), 3.33 (brs, 1H), 2.93 (d, *J* = 4.5 Hz, 3H), 2.26 (t, *J* = 20.4 Hz, 2H). ESI MS $[M-H]^-$ for $C_{12}H_{17}ClFN_5O_8P_2$, calcd 474.7, found 474.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(ethylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (7): The title compound was synthesized in similar fashion to Example **16**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (t, *J* = 5.7 Hz, 1H), 8.26 (s, 1H), 7.28 (brs, 2H), 6.37 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.25 (dt, *J* = 52.4, 4.3 Hz, 1H), 4.50 (dt, *J* = 18.5, 4.6 Hz, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.03 (q, *J* = 5.1 Hz, 1H), 3.87 (brs, 1H), 3.45 (m, 1H), 2.27 (t, *J* = 20.5 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 3H). ESI MS $[M+H]^+$ for $C_{13}H_{19}ClFN_5O_8P_2$, calcd 490.7, found 490.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(cyclopropylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (8): The title compound was synthesized in similar fashion to Example **16**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.28 (d, *J* = 2.1 Hz, 1H), 6.38 (dd, *J* = 14.2, 4.6 Hz, 1H), 5.26 (ddd, *J* = 52.5, 4.3, 4.3 Hz, 1H), 4.51 (dt, *J* = 18.5, 4.5 Hz, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.03 (q, *J* = 5.0 Hz, 1H), 2.98 (s, 1H), 2.36 – 2.15 (m, 2H), 0.82 – 0.48 (m, 4H). ESI MS $[M-H]^-$ for $C_{14}H_{18}ClFN_5O_8P_2$, calcd 500.03, found 500.0.

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-6-(cyclopropylamino)purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (9): The title compound was synthesized in similar fashion to Example 13

using cyclopropylamine in place of cyclopentylamine: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.42 (s, 1H), 5.86 (d, *J* = 5.8 Hz, 1H), 4.52 (t, *J* = 5.4 Hz, 1H), 4.28 – 4.03 (m, 4H), 2.97 (s, 1H), 2.25 (t, *J* = 20.5 Hz, 2H), 0.75 (s, 2H), 0.64 (s, 3H). ESI MS $[M+H]^+$ for $C_{14}H_{20}ClN_5O_9P_2$, calcd 500.1, found 500.1

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(propan-2-ylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (10): The title compound was synthesized in similar fashion to Example **16**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (m, 2H), 6.37 (d, *J* = 13.9 Hz, 1H), 5.28 (brs, 2H), 5.25 (d, *J* = 52.1 Hz, 1H), 4.98 (brs, 1H), 4.51 (d, *J* = 18.3 Hz, 1H), 4.35 (sept, *J* = 7.9 Hz, 1H), 4.19 (m, 2H), 4.04 (m, 1H), 2.26 (t, *J* = 20 Hz, 2H), 1.21 (dd, *J* = 6.6, 2.1 Hz, 6H). ESI MS $[M-H]^-$ for $C_{14}H_{21}ClFN_5O_8P_2$, calcd 502.7, found 502.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-6-(propan-2-ylamino)purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (11): The title compound was synthesized in similar fashion to Example **13** using isopropylamine in place of cyclopentylamine: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 5.85 (d, *J* = 5.9 Hz, 1H), 4.51 (t, *J* = 5.5 Hz, 1H), 4.36 (s, 1H), 4.24 – 4.03 (m, 4H), 2.25 (t, *J* = 20.5 Hz, 2H), 1.21 (dd, *J* = 6.6, 2.0 Hz, 5H). ESI MS $[M+H]^+$ for $C_{14}H_{22}ClN_5O_9P_2$, calcd 502.1, found 502.1

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(cyclopentylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (12): The title compound was synthesized in similar fashion to Example **16**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, *J* = 7.8 Hz, 1H), 8.27 (s, 1H), 6.37 (dd, *J* = 14.4, 4.6 Hz, 1H), 5.25 (dt, *J* = 52.4, 4.3 Hz, 1H), 4.55 – 4.37 (m, 2H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.03 (q, *J* = 5.1 Hz, 1H), 2.26 (t, *J* = 20.5 Hz, 2H), 1.93 (s, 2H), 1.64 (d, *J* = 62.5 Hz, 6H). ESI MS $[M+H]^+$ for $C_{16}H_{23}ClFN_5O_8P_2$, calcd 530.1, found 530.2

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-6-(cyclopentylamino)purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (13): A mixture of 2,6-dichloropurine riboside **70** (321 mg, 1 mmol), cyclopentylamine (103 µL, 1.05 mmol, 1.05 equiv.), and triethylamine (146 µL, 1.05 mmol, 1.05 equiv.) in anhydrous EtOH (3 mL) was stirred at 60 °C for overnight. Reaction mixture was evaporated and the crude product was used in the next step without purification. ESI MS $[M+H]^+$ for $C_{15}H_{21}ClN_5O_4$, calcd 370.8, found 370.2.

The above product (370 mg, 1 mmol) was dissolved in trimethyl phosphate (5 mL) and cooled to 0 °C (ice bath), then a cold solution of methylenebis(phosphonic dichloride) (1.25 g, 5 mmol, 5 equiv.) in trimethyl phosphate (2 mL) was added

dropwise. The reaction mixture was stirred at 0 °C for 3 h, and was then carefully quenched with 0.5 M triethylammonium bicarbonate solution (7 mL) and stirred at 0 °C for 15 min, and then 2 h at room temperature. The reaction mixture was purified by reverse phase HPLC (C18 column, 0 to 30% gradient of MeCN and water with 0.1% TFA) to give the product as a white solid in 28% yield (181 mg): ^{1}H NMR (400 MHz, DMSO) δ 8.45 – 8.32 (m, 2H), 5.85 (d, *J* = 5.5 Hz, 1H), 4.55 – 4.36 (m, 2H), 4.23 – 4.07 (m, 4H), 2.26 (t, *J* = 20.5 Hz, 2H), 2.04 – 1.85 (m, 2H), 1.77 – 1.46 (m, 6H). ESI MS $[M+H]^+$ for $C_{16}H_{25}ClN_5O_9P_2$, calcd 528.8, found 528.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[cyclopentyl(methyl)amino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (14): This compound was obtained similar fashion to Example **16** using N-methylcyclopentylamine in place of benzylamine. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.7 (brs, 2H), 8.30 (d, *J* = 2.1 Hz, 1H), 6.40 (dd, *J* = 14.3, 4.6 Hz, 1H), 6.09 (brs, 1H), 5.25 (dt, *J* = 52.5, 4.3 Hz, 1H), 4.53-4.43 (m, 1H), 4.23-4.14 (m, 2H), 4.09 – 3.98 (m, 1H), 2.28 (dd, *J* = 20.5 Hz, *J* = 20.5 Hz, 2H), 2.5 (s, 3H), 1.96 – 1.44 (m, 9H). ESI MS $[M+H]^+$ for $C_{17}H_{25}ClFN_5O_8P_2$, calcd 544.8, found: 544.2

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-6-[cyclopentyl(methyl)amino]purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (15): The title compound was synthesized in similar fashion to Example **13** with *N*-methylcyclopentylamine in place of cyclopentylamine. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 5.88 (d, *J* = 5.9 Hz, 1H), 4.53 – 4.46 (m, 1H), 4.19 (dd, *J* = 5.0, 3.1 Hz, 1H), 4.15 – 4.06 (m, 3H), 3.17 (brs, 3H), 2.26 (t, *J* = 20.5 Hz, 2H), 1.94 – 1.53 (m, 9H). ESI MS $[M+H]^+$ for $C_{17}H_{27}ClN_5O_9P_2$, calcd 542.1, found 542.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-(benzylamino)-2-chloropurin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (17): The title compound was synthesized in similar fashion to Example **13**. ^{1}H NMR (400 MHz, DMSO) δ 8.95 (t, *J* = 6.2 Hz, 1H), 8.44 (s, 1H), 7.46 – 7.14 (m, 5H), 5.86 (d, *J* = 5.8 Hz, 1H), 4.75 – 4.58 (m, 2H), 4.52 (t, *J* = 5.4 Hz, 1H), 4.26 – 3.97 (m, 4H), 2.26 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{18}H_{21}ClN_5O_9P_2$, calcd 548.8, found 548.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[[(1*R*)-1-phenylethyl]amino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (18): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (d, *J* = 8.3 Hz, 1H), 8.28 (s, 1H), 7.42 (d, *J* = 7.6 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 7.19 (bs,

1H), 6.34 (dd, *J* = 14.8, 4.4 Hz, 1H), 5.39 (bs, 1H), 5.23 (d, *J* = 52.6 Hz, 1H), 4.49 (d, *J* = 19.2 Hz, 2H), 4.17 (bs, 2H), 4.01 (d, *J* = 5.1 Hz, 1H), 2.24 (t, *J* = 20.6 Hz, 2H), 1.52 (d, *J* = 7.0 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_8P_2$, calcd 566.1, found 566.1

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-6-[[(1*R*)-1-phenylethyl]amino]purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (19): The title compound was synthesized in similar fashion to Example **13**. ^{1}H NMR (400 MHz, DMSO) δ 8.94 – 8.80 (m, 1H), 8.48 – 8.37 (m, 1H), 7.49 – 7.15 (m, 5H), 5.84 (d, *J* = 5.8 Hz, 1H), 5.41 (q, *J* = 7.0 Hz, 1H), 4.57 – 4.44 (m, 1H), 4.23 – 4.01 (m, 4H), 2.26 (t, *J* = 20.5 Hz, 2H), 1.54 (d, *J* = 7.0 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{25}ClN_5O_9P_2$, calcd 564.1, found 564.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[[(1*S*)-1-phenylethyl]amino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (20): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, *J* = 8.3 Hz, 1H), 8.28 (s, 1H), 7.41 (d, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.20 (d, *J* = 7.6 Hz, 1H), 6.34 (d, *J* = 14.1 Hz, 1H), 5.39 (bs, 1H), 5.21 (d, *J* = 52.5 Hz, 1H), 4.47 (d, *J* = 18.3 Hz, 2H), 4.17 (s, 2H), 4.01 (s, 1H), 2.24 (t, *J* = 20.6 Hz, 2H), 1.52 (d, *J* = 7.1 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_8P_2$, calcd 566.1, found 566.1

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-6-[[(1*S*)-1-phenylethyl]amino]purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (21): The title compound was synthesized in similar fashion to Example **13**. ^{1}H NMR (400 MHz, DMSO) δ 8.94 – 8.78 (m, 1H), 8.48 – 8.38 (m, 1H), 7.47 – 7.13 (m, 5H), 5.84 (d, *J* = 5.8 Hz, 1H), 5.41 (q, *J* = 7.1 Hz, 1H), 4.54 – 4.46 (m, 1H), 4.22 – 4.05 (m, 4H), 2.26 (t, *J* = 20.5 Hz, 2H), 1.54 (d, *J* = 7.1 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{25}ClN_5O_9P_2$, calcd 564.1, found 564.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[(2-chlorophenyl)methylamino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (22): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (t, *J* = 6.1 Hz, 1H), 8.35 (s, 1H), 7.47 (dd, *J* = 6.0, 3.3 Hz, 1H), 7.35 – 7.22 (m, 3H), 6.40 (dd, *J* = 14.2, 4.6 Hz, 1H), 5.27 (dt, *J* = 52.4, 4.3 Hz, 1H), 4.73 (d, *J* = 5.2 Hz, 2H), 4.52 (d, *J* = 18.5 Hz, 1H), 4.20 (t, *J* = 6.2 Hz, 2H), 4.05 (q, *J* = 5.1 Hz, 1H), 2.27 (t, *J* = 20.5 Hz, 2H). ESI MS $[M+H]^+$ for $C_{18}H_{20}Cl_2FN_5O_8P_2$, calcd 586.0, found 586.1

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[(3-chlorophenyl)methylamino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (23): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (t, *J* = 6.2 Hz, 1H), 8.33 (d, *J* = 2.2 Hz, 1H), 7.45 – 7.27 (m, 4H), 6.39 (dd, *J* = 14.4, 4.6

Hz, 1H), 5.26 (dt, *J* = 52.4, 4.2 Hz, 1H), 4.74 – 4.58 (m, 2H), 4.51 (dt, *J* = 18.5, 4.6 Hz, 1H), 4.20 (t, *J* = 6.1 Hz, 2H), 4.04 (q, *J* = 5.1 Hz, 1H), 2.27 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{18}H_{19}Cl_2FN_5O_8P_2$, calcd 584.0, found 584.0

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[(4-chlorophenyl)methylamino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (24): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, *J* = 6.2 Hz, 1H), 8.32 (d, *J* = 2.1 Hz, 1H), 7.64 – 7.08 (m, 4H), 6.38 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.26 (dt, *J* = 52.5, 4.3 Hz, 1H), 4.64 (q, *J* = 7.3, 5.4 Hz, 2H), 4.51 (dt, *J* = 18.7, 4.6 Hz, 1H), 4.28 – 4.11 (m, 2H), 4.04 (q, *J* = 5.1 Hz, 1H), 2.27 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{18}H_{20}Cl_2FN_5O_8P_2$, calcd 584.0, found 584.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-(2-chloro-6-pyrrolidin-1-ylpurin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (25): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, *J* = 2.1 Hz, 1H), 6.39 (dd, *J* = 14.1, 4.7 Hz, 1H), 5.26 (dt, *J* = 52.5, 4.3 Hz, 1H), 4.50 (dt, *J* = 18.5, 4.6 Hz, 1H), 4.19 (t, *J* = 5.9 Hz, 2H), 4.05 (q, *J* = 5.3, 4.1 Hz, 3H), 3.60 (t, *J* = 6.8 Hz, 2H), 2.27 (t, *J* = 20.5 Hz, 2H), 2.01 (p, *J* = 6.7 Hz, 2H), 1.92 (q, *J* = 6.7 Hz, 2H).ESI MS $[M+H]^+$ for $C_{15}H_{21}ClFN_5O_8P_2$, calcd 516.1, found 516.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-[[(3*S*)-oxolan-3-yl]amino]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (26): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, *J* = 6.3 Hz, 1H), 8.31 (s, 1H), 6.38 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.26 (dt, *J* = 52.4, 4.2 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.58 – 4.35 (m, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.04 (q, *J* = 5.0 Hz, 1H), 3.89 (dt, *J* = 15.3, 7.9 Hz, 2H), 3.78 – 3.68 (m, 1H), 3.61 (dd, *J* = 8.9, 4.4 Hz, 1H), 2.43 – 1.87 (m, 4H). ESI MS $[M-H]^-$ for $C_{15}H_{21}ClFN_5O_9P_2$, calcd. 530.1, found 530.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(pyridin-4-ylmethylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (27): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.66 (d, *J* = 5.7 Hz, 2H), 8.37 (s, 1H), 7.65 (d, *J* = 5.6 Hz, 2H), 6.40 (dd, *J* = 14.0, 4.6 Hz, 1H), 5.40 – 5.08 (m, 1H), 4.80 (d, *J* = 6.1 Hz, 2H), 4.53 (d, *J* = 18.3 Hz, 1H), 4.19 (s, 2H), 4.04 (d, *J* = 5.2 Hz, 1H), 2.25 (t, *J* = 20.4 Hz, 2H). ESI MS $[M+H]^+$ for $C_{17}H_{20}ClFN_6O_8P_2$, calcd 553.1, found 553.2

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-chloro-6-(1,3-dihydroisoindol-2-yl)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (28): The title compound was synthesized in similar fashion to Example **16**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, *J* = 2.1 Hz, 1H), 7.48 (dt, *J* = 9.9, 4.7 Hz, 2H), 7.43 – 7.28 (m, 2H), 6.44 (dd, *J* = 13.8, 4.7 Hz, 1H), 5.41 (s, 2H), 5.29 (dt, *J* = 52.6, 4.4 Hz, 1H), 4.98 (s, 2H), 4.54 (dt, *J* = 18.7, 4.7 Hz, 1H), 4.21 (t, *J* = 5.9 Hz, 2H), 4.05 (q, *J* = 4.9 Hz, 1H), 2.27 (t, *J* = 20.5 Hz, 2H). ESI MS $[M+H]^+$ for $C_{19}H_{21}ClFN_5O_8P_2$, calcd 564.1, found 564.1

[[(2*R*,3*R*,4*S*,5*R*)-5-(2-chloro-6-phenylmethoxypurin-9-yl)-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (29): Under a nitrogen atmosphere, sodium hydride (90 mg, 2.26 mmol, 1.2 equiv., 60% in oil) and benzyl alcohol (10 mL) were stirred at r.t. for 15 min. Compound **62** (1.00 g, 1.88 mmol) was added and the mixture stirred at r.t. for 2 h. The reaction mixture was purified directly by column chromatography (0-10% MeOH in CH_2Cl_2) to afford **69** as a white solid (721 mg, 97%). ESI MS $[M+H]^+$ for $C_{17}H_{17}ClFN_4O_4$, calcd 395.1, found 395.1.

Intermediate **69** (197 mg, 0.5 mmol) was dissolved in trimethyl phosphate (2.5 mL) and cooled to 0 °C. A solution of methylenebis(phosphonic dichloride) (624 mg, 2.5 mmol, 5 equiv.) in trimethyl phosphate (1.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h and then carefully quenched at -20 °C with 0.5 M triethylammonium bicarbonate solution (3.6 mL). The mixture was stirred at -20 °C for 15 min, then stirred at 0 °C for 15 min, then stirred at r.t. for 15 min. The mixture was washed with EtOAc (10 mL) three times. The aqueous layer was purified directly by reverse phase HPLC (C18 column, 0 to 50% gradient of MeCN and water with 0.1% TFA) to afford the desired product as a white solid (40.2 mg, 15%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, *J* = 2.1 Hz, 1H), 7.57 – 7.51 (m, 2H), 7.46 – 7.35 (m, 3H), 6.49 (dd, *J* = 13.6, 4.7 Hz, 1H), 5.61 (s, 2H), 5.30 (dt, *J* = 52.4, 4.4 Hz, 1H), 4.53 (dt, *J* = 18.6, 4.7 Hz, 1H), 4.21 (t, *J* = 6.0 Hz, 2H), 4.06 (q, *J* = 5.0 Hz, 1H), 2.27 (t, *J* = 20.6 Hz, 2H). ESI MS $[M-H]^-$ for $C_{18}H_{19}ClFN_4O_9P_2$, calcd 551.0, found 551.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(benzylamino)-2-methylpurin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (30): The title compound was synthesized in similar fashion to Example **16** using 6-chloro-2-methylpurine in place of 2,6-dichloropurine: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.23 (s, 1H), 7.44 – 7.19 (m, 5H), 6.44 (dd, *J* = 15.0, 4.6 Hz, 1H), 5.41 – 5.13 (m, 1H), 4.72 (s, 2H), 4.53 (dd, *J* = 18.4, 4.7 Hz, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.04 (t, *J* = 5.1 Hz, 1H), 2.46 (s, 3H), 2.26 (t, *J* = 20.5 Hz, 2H). ESI MS $[M+H]^+$ for $C_{19}H_{24}FN_5O_8P_2$, calcd 532.1, found 532.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-(benzylamino)-2-methylpurin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (31): To a nitrogen purged reaction mixture of the N^6-benzyl-2-iodopurine riboside **72** (1.03 g, 1.7 mmol) and tetramethyltin (470 µL, 3.34 mmol) in NMP (10 mL) was added $Pd(PPh_3)_4$ (196 mg, 0.17

mmol, 10 mol%) and the reaction mixture was heated at 120 °C for overnight. LCMS indicated product formation. It was cooled to room temperature, diluted with water, extracted with EtOAc, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column to get the product (1 g). ESI MS $[M+H]^+$ for $C_{24}H_{27}N_5O_7$, calcd 498.2, found 498.3

To a solution of the acetate derivative above (1 g, 2.01 mmol) in MeOH (5 mL) was added K_2CO_3 (276 mg, 2 mmol) and the reaction mixture was stirred at r.t for 1 h. Then, it was diluted with CH_2Cl_2, filtered through a pad of silica. The filtrate was concentrated and purified by flash column (ISCO, 40 g column, 0 to 20 % MeOH in dichloromethane, 20 min) to afford **75** as off white solid (450 mg, 60%) ESI MS $[M+H]^+$ for $C_{18}H_{21}N_5O_4$, calcd 372.2, found 372.2

Intermediate **75** (150 mg, 0.4 mmol) was dissolved in trimethyl phosphate (3 mL) and cooled to 0 °C (ice bath), then an ice cold solution of methylenebis(phosphonic dichloride) (504 mg, 2 mmol, 5 equiv.) in trimethyl phosphate (1 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 3 h, and was then carefully quenched with 0.5 M triethylammonium bicarbonate solution (8 mL) and stirred at 0 °C for 15 min, and then 2 h at room temperature. The reaction mixture was purified by reverse phase HPLC (C18 column, 0 to 30% gradient of MeCN and water with 0.1% TFA) to give the product as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 – 8.32 (m, 2H), 7.38 – 7.18 (m, 5H), 5.92 (d, *J* = 6.0 Hz, 1H), 4.71 (s, 2H), 4.55 (t, *J* = 5.5 Hz, 1H), 4.19 – 3.98 (m, 4H), 2,44 (s, 3H), 2.23 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{19}H_{25}N_5O_9P_2$, calcd 528.1, found 528.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-(benzylamino)-2-ethylpurin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (32): A mixture of *N*^{6}-benzyl-2-chloropurine riboside **74** (783 mg, 2 mmol), vinylboronic acid pinacol ester (462 mg, 3 mmol, 1.5 equiv.), K_2CO_3 (828 mg, 6 mmol, 3 equiv.) and $Pd(PPh_3)_4$ in 1,2-dimethoxyethane:H_2O (9:1, 10 mL) was stirred under N_2 at 85 °C for 1 day. Reaction mixture was cooled down to room temperature, diluted with EtOAc (100 mL) and washed with H_2O (50 mL). Organic layer was separated, dried over $MgSO_4$, filtered and evaporated to give yellow solid. Crude product **75** was washed with MTBE (50 mL) and used directed in the next step (550 mg, 72%).

Phosphonylation of **75** proceeded in similar fashion to example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.38 (d, *J* = 7.0 Hz, 2H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.25 – 7.15 (m, 1H), 6.64 (dd, *J* = 17.2, 10.4 Hz, 1H), 6.39 (dd, *J* = 17.2, 2.4 Hz, 1H), 5.94 (d, *J* = 6.0 Hz, 1H), 5.55 (d, *J* = 10.5 Hz, 1H), 4.73 (s, 2H), 4.63 (t, *J* = 5.5 Hz, 1H), 4.28 – 4.00 (m, 4H), 2.25 (t, *J* = 20.4 Hz, 2H). ESI MS $[M+H]^+$ for $C_{20}H_{26}N_5O_9P_2$, calcd 542.1, found 542.2.

The phosphonylated product above (40 mg, 0.06 mmol) was dissolved in MeOH (10 mL), purged with N_2 and 10% Pd/C (50% wet, 30 mg) was added. Reaction mixture was vigorously stirred under H_2 (balloon) for 2h and after filtration the product was purified by RP18 HPLC (H_2O+0.1% TFA/MeCN+0.1% TFA) to give **32** as white solid (14 mg, 35%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 – 8.18 (m, 2H), 7.33 – 7.27 (m, 2H), 7.27 – 7.18 (m, 2H), 7.15 (t, *J* = 7.2 Hz, 1H), 5.86 (d, *J* = 6.0 Hz, 1H), 4.64 (s, 2H), 4.55 (t, *J* = 5.5 Hz, 1H), 4.19 – 3.98 (m, 4H), 2.70 – 2.61 (m, 2H), 2.16 (t, *J* = 20.5 Hz, 2H), 1.16 (t, *J* = 7.6 Hz, 3H). ESI MS $[M+H]^+$ for $C_{20}H_{27}N_5O_9P_2$, calcd 544.1, found 544.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(benzylamino)-2-(trifluoromethyl)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (33): The title compound was synthesized in similar fashion to Example **16**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (d, *J* = 6.3 Hz, 1 H), 8.49 (d, *J* = 2.1 Hz, 1 H), 7.39 – 7.35 (m, 2 H), 7.34 – 7.27 (m, 2 H), 7.25 – 7.20 (m, 1 H), 6.48 (dd, *J* = 14.0, 4.7 Hz, 1 H), 5.30 (dt, *J* = 52.4, 4.3 Hz, 1 H), 5.20 (bs, 1 H), 4.70 (t, *J* = 5.7 Hz, 1 H), 4.56 (dt, *J* = 18.6, 4.7 Hz, 1 H), 4.21 (t, *J* = 6.2 Hz, 2 H), 4.06 (q, *J* = 5.1 Hz, 1 H), 2.26 (t, *J* = 20.5 Hz, 2 H). ESI MS $[M+H]^+$ for $C_{19}H_{21}F_4N_5O_8P_2$, calcd 586.1, found 586.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-(benzylamino)-2-(methylamino)purin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (34): The known riboside **72** (250 mg, 0.64 mmol) was dissolved in 40% $MeNH_2$ in H_2O solution (2 mL) and stirred at 60 °C for overnight. The reaction mixture was then concentrated under reduced pressure and the residue was diluted with H_2O (15 mL). The product **73** was collected by filtration (white solid, 210 mg, 85%). ESI MS $[M+H]^+$ for $C_{18}H_{23}N_6O_4$, calcd 387.4, found 387.3.

The nucleotide **34** was obtained using a similar procedure as for Example **13** to give white solid (38 mg, 15%): ^{1}H NMR (400 MHz, DMSO) δ 8.08 (s, 1H), 7.42 – 7.19 (m, 5H), 5.79 (d, *J* = 6.1 Hz, 1H), 4.75 – 4.45 (m, 3H), 4.24 – 4.02 (m, 4H), 2.81 (s, 3H), 2.22 (t, *J* = 20.4 Hz, 2H). ESI MS $[M-H]^-$ for $C_{19}H_{26}N_6O_9P_2$, calcd 543.4, found 543.2.

[[(2R,3S,4R,5R)-5-[6-(benzylamino)-2-pyrrolidin-1-ylpurin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (35): The title compound was synthesized in similar fashion to Example **34** but substituting methylamine for pyrrolidine: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 2H), 7.42 – 7.14 (m, 5H), 5.82 (d, *J* = 5.5 Hz, 1H), 4.71 – 4.51 (m, 3H), 4.26 (t, *J* = 4.3 Hz, 1H), 4.21 – 4.00 (m, 3H), 3.46 (s, 4H), 2.23 (t, *J* = 20.4 Hz, 2H), 1.89 (s, 4H). ESI MS $[M+H]^+$ for $C_{22}H_{31}N_6O_9P_2$, calcd 585.1, found 585.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-(benzylamino)-2-methoxypurin-9-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (36): The known riboside **72** (250 mg, 0.64 mmol) was dissolved in 25% NaOMe in MeOH solution (2 mL) and stirred at 60 °C for overnight. The reaction mixture was concentrated under reduced pressure and the residue was then diluted with H_2O (15 mL) and acetic acid until neutral pH. The product **74** was collected by filtration (white solid, 180 mg, 73%). ESI MS $[M+H]^+$ for $C_{18}H_{22}N_5O_5$, calcd 388.4, found 388.1.

The nucleotide **36** was obtained using a similar procedure as for example **13** to give a white solid (37 mg, 14%): ^{1}H NMR (400 MHz, DMSO) δ 8.48 (s, 1H), 8.20 (s, 1H), 7.37 – 7.17 (m, 5H), 5.82 (d, *J* = 5.9 Hz, 1H), 4.64 (d, *J* = 5.0 Hz, 3H), 4.28 – 4.00 (m, 4H), 3.80 (s, 3H), 2.23 (t, *J* = 20.5 Hz, 2H). ESI MS $[M+H]^+$ for $C_{19}H_{26}N_5O_{10}P_2$, calcd 546.4, found 546.1.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(benzylamino)-2-phenylpurin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (37): Compound **78** (750 mg, 1.25 mmol), phenylboronic acid (229 mg, 1.88 mmol), and K_2CO_3 (518 mg, 3.75 mmol) were suspended in 3:1 THF:H_2O (10.3 mL). This mixture was degassed by N_2 sparge for 10 minutes. Subsequently $Pd(PPh_3)_4$ (144 mg, 0.13 mmol) was added and the resulting mixture was degassed for an additional 5 minutes then sealed and heated to 80 °C overnight. After cooling to room temperature the reaction was diluted with EtOAc and washed with water and brine. The organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was comprised of a mixture mono- and di-debenzoylated products which was used directly in the next step.

The product above was dissolved in MeOH (12.5 mL) and K_2CO_3 (518 mg, 3.75 mmol) was added. The resulting suspension was stirred overnight at room temperature then portioned between EtOAc and water. The organics were washed with brine then dried ($MgSO_4$) and concentrated under reduced pressure. The desired product **79** was obtained following column chromatography (SiO_2, 0 to 10% gradient of MeOH and CH_2Cl_2) as a white solid (41 mg, 8% two-steps). ESI MS $[M+H]^+$ for $C_{23}H_{22}FN_5O_3$, calcd 436.2, found 436.3.

Nucleotide **37** was obtained using identical procedure as for Example **16** to give a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.44 – 8.32 (m, 2H), 8.29 (d, *J* = 2.4 Hz, 1H), 7.40 – 7.50 (m, 5H), 7.31 (dd, *J* = 8.3, 6.9 Hz, 2H), 7.24 – 7.15 (m, 1H), 6.59 (dd, *J* = 15.4, 4.6 Hz, 1H), 5.30 (dt, *J* = 52.4, 4.1 Hz, 1H), 4.82 (s, 2H), 4.69 – 4.48 (m, 1H), 4.22 (d, *J* = 6.6 Hz, 2H), 4.08 (q, *J* = 5.1 Hz, 1H), 2.27 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{24}H_{26}FN_5O_8P_2$, calcd 592.1, found 592.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[2-benzyl-6-(benzylamino)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (38): Compound **78** (391 mg, 0.659 mmol), potassium benzyltrifluoroborate (391 mg, 1.98 mmol), and cesium carbonate (1.07 g, 3.30 mmol) were suspended in 20:1 THF:H_2O (6.5 mL). This mixture was degassed by N_2 sparge for 10 minutes. Subsequently $Pd(PPh_3)_2Cl_2$ (96 mg, 0.132 mmol) was added and the resulting mixture was degassed for an additional 5 minutes then sealed and heated to 80 °C for 48 hours. After cooling to room temperature, the reaction was diluted with EtOAc and washed with water and brine. The organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was obtained following column chromatography (SiO_2, EtOAc/Hexane) as a beige solid (174 mg, 40%).

The product above (174 mg, 0.265 mmol) was dissolved in MeOH (2.65 mL) and K_2CO_3 (110 mg, 3.75 mmol) was added. The resulting suspension was stirred at room temperature for 1.5 hours then partioned between EtOAc and water. The organics were washed with brine then dried ($MgSO_4$) and concentrated under reduced pressure. The desired product **80** was obtained following column chromatography (SiO_2, 0 to 10% gradient of MeOH and CH_2Cl_2) as a white solid (102 mg, 86%). ESI MS $[M+H]^+$ for $C_{24}H_{24}FN_5O_3$, calcd 450.2, found 450.3.

Nucleotide **38** was obtained from **80** using identical procedure as for Example **16** to give white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.22 (s, 1H), 7.61 – 6.94 (m, 10H), 6.44 (dd, *J* = 15.1, 4.6 Hz, 1H), 5.23 (dt, *J* = 52.4, 4.1 Hz, 1H), 4.82 – 4.40 (m, 3H), 4.18 (t, *J* = 6.5 Hz, 2H), 4.03 (dd, *J* = 10.9, 5.9 Hz, 3H), 2.26 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{25}H_{28}FN_5O_8P_2$, calcd 606.1, found 606.3.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(benzylamino)-2-ethynylpurin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (39): Compound **78** (2.0 g, 3.32 mmol) was suspended in in DMF (7.4 mL) and diisopropylamine (2.3 mL) was added followed by trimethylsilyl-acetylene (703 uL, 4.98). This mixture was degassed by N_2 sparge for 10 minutes. Subsequently CuI (125 mg, 0. 66 mmol) and $Pd(PPh_3)_2Cl_2$ (233 mg, 0.0.33 mmol) was added and the resulting mixture was degassed for an additional 5 minutes then sealed and heated to 80 °C for 36 hours. After cooling to room temperature the reaction was diluted with EtOAc and washed with sat. NH_4Cl (aqueous), water and brine. The organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was obtained following column chromatography (SiO_2, 5% to 70% EtOAc/Hexane) as a beige solid (950 mg, 43%).

The product above (950 mg, 1.43 mmol) was dissolved in MeOH (14 mL) and K_2CO_3 (592 mg, 4.29 mmol) was added. The resulting suspension was stirred overnight at room temperature then partioned between EtOAc and water. The organics were washed with brine then dried (Na_2SO_4) and concentrated under reduced pressure. The desired product **81** was obtained following column chromatography (SiO_2, 0 to 10% gradient of MeOH and CH_2Cl_2) to afford the title compounds as a white solid (230 mg, 42%). ESI MS $[M+H]^+$ for $C_{19}H_{18}FN_5O_3$, calcd 384.1, found 384.2.

Nucleotide **39** was obtained from **81** using identical procedure as for Example **16** to give a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 8.36 (d, *J* = 2.2 Hz, 1H), 7.39 – 7.26 (m, 5H), 7.28 – 7.17 (m, 1H), 6.44 (dd, *J* = 14.8, 4.5 Hz, 1H), 5.25 (dt, *J* = 52.5, 4.1 Hz, 1H), 4.69 (s, 2H), 4.51 (d, *J* = 18.1 Hz, 1H), 4.19 (d, *J* = 7.1 Hz, 2H), 2.27 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{20}H_{22}FN_5O_8P_2$, calcd 540.1, found 540.2.

[(2*R*,3*R*,4*S*,5*R*)-5-[6-(benzylamino)-2-(2-phenylethyl)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (40): Compound **78** (750 mg, 1.24 mmol) was suspended in in DMF (8.3 mL) and Et_3N (260 µL) was added followed by phenyl acetylene (205 µL). This mixture was degassed by N_2 sparge for 10 minutes. Subsequently CuI (24 mg) and $Pd(PPh_3)_2Cl_2$ (44 mg) were added and the resulting mixture heated to 80 °C overnight. After cooling to room temperature, the reaction was diluted with EtOAc and washed with 10% citric acid (aqueous), water and brine. The organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was obtained following column chromatography (SiO_2, EtOAc/Hexane) as a tan oil (762 mg, 92%).

The product above (762 mg, 1.14 mmol) was dissolved in MeOH (11.4 mL) and K_2CO_3 (473 mg, 3.42 mmol) was added. The resulting suspension was stirred overnight at room temperature then portioned between EtOAc and water. The organics were washed with brine the brine then dried (Na_2SO_4) and concentrated under reduced pressure. The desired product was obtained following column chromatography (SiO_2, 0 to 10% gradient of MeOH and CH_2Cl_2) as a colorless oil. ESI MS $[M+H]^+$ for $C_{25}H_{22}FN_5O_3$, calcd 460.2, found 460.2.

To a solution of the product above (203 mg, 0.44 mmol) in EtOH (4.4 mL) under a nitrogen atmosphere was added palladium on carbon (10 wt% wet, 20 mg). The nitrogen atmosphere was displaced with hydrogen and the stirred at room temperature. After stirring overnight the reaction was diluted with EtOAc and filtered through celite. The filtrate was concentrated under reduced pressure to afford **82** (161 mg, 79%) which was used without further purification. ESI MS $[M+H]^+$ for $C_{25}H_{26}FN_5O_3$, calcd 464.2, found 464.4.

Nucleotide **40** was obtained from **82** using identical procedure as for Example **16** to give a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 – 8.14 (m, 2H), 7.58 – 6.91 (m, 11H), 6.44 (d, *J* = 15.0 Hz, 1H), 5.22 (d, *J* = 52.4 Hz, 1H), 4.71 (s, 2H), 4.54 (dt, *J* = 18.4, 4.4 Hz, 1H), 4.19 (t, *J* = 6.2 Hz, 2H), 4.11 – 3.96 (m, 1H), 3.23 – 2.83 (m, 5H), 2.26 (t, *J* = 20.5 Hz, 2H). ESI MS $[M-H]^-$ for $C_{26}H_{30}FN_5O_8P_2$, calcd 620.2, found 620.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(cyclopentylamino)-2-(methoxymethyl)purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (41): Compound **83** (10.0g, 17.24 mmol), phenylvinylboronic acid (3.83 g, 25.86 mmol), and sodium carbonate (5.44 mg, 51.72 mmol) were suspended in 3:1 THF:H_2O (100 mL). This mixture was degassed by N_2 sparge for 10 minutes. Subsequently $Pd(PPh_3)_4$ (1.99 g, 1.72 mmol) was added and the resulting mixture was degassed for an additional 5 minutes then heated to reflux overnight. After cooling to room temperature, the reaction was diluted with EtOAc and washed with water and brine. The organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The desired product was obtained following column chromatography (SiO_2, 5% to 50% EtOAc/Hexane) as a colorless solid (8.06 g, 72%).

To a suspension of the styrene product above (8.06 g, 12.04 mmol), sodium periodate (15.5 g, 72.4 mmol), and 2,6-lutidine (2.80 mL, 24.1 mmol) in 2:1 THF:H_2O (127.5 mL) was added potassium osmate dihydrate (100 mg, 0.30 mmol). The resulting thick suspension was stirred overnight at room temperature then partitioned between EtOAc and water. The organics were washed sequentially with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The desired product **84** was obtained following column chromatography (SiO_2, EtOAc/Hexane) as an off-white oil (6.74 g, 97%). ESI MS $[M+H]^+$ for $C_{30}H_{28}FN_5O_6$, calcd 574.2, found 574.4.

To a solution of **84** (1.0 g, 1.74 mmol) in dichloroethane (20 mL) was added sodium triacetoxyborohydride (443 mg, 2.09 mmol) in a single portion. The reaction was stirred at room temperature overnight then partitioned between EtOAc and water. The organics were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford **85** which was used without further purification. ESI MS $[M+H]^+$ for $C_{30}H_{30}FN_5O_6$, calcd 576.2, found 576.3.

To a solution of **85** in dichloromethane (10 mL) at 0 °C were added TsCl (436 mg, 2.29 mmol) and triethylamine (400 µL, 2.87 mmol). The reaction was allowed to warm to room temperature and stir overnight. The reaction was diluted with EtOAc and washed with sat. $NaHCO_3$, 10% citric acid, water and brine. The organics were dried over $MgSO_4$ and

concentrated under reduced pressure to afford the crude title compound (1.20 g, 94% two-steps) which was used directly in the next step.

To a flask charged with crude tosylate (700 mg, 0.959 mmol) and K_2CO_3 (662 mg, 4.8 mmol) was added MeOH (10 ml). The resulting suspension was stirred overnight then diluted with EtOAc and washed with water and brine. The organics were dried over MgSO4 and concentrated under reduced pressure. The desired product **86** (85 mg, 23%) was obtained following column chromatography (SiO_2, 0 to 15% gradient of MeOH and CH_2Cl_2). ESI MS $[M+H]^+$ for $C_{17}H_{24}FN_5O_4$, calcd 382.2, found 382.3.

Nucleotide **41** was obtained from **86** using identical procedure as for Example **16** to give a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H), 6.46 (dd, *J* = 14.9, 4.6 Hz, 2H), 5.24 (dt, *J* = 52.5, 4.2 Hz, 1H), 4.54 (dt, *J* = 18.3, 4.4 Hz, 2H), 4.40 (s, 2H), 4.20 (t, *J* = 6.1 Hz, 3H), 4.04 (t, *J* = 5.0 Hz, 1H), 3.37 (s, 5H), 2.26 (t, *J* = 20.5 Hz, 2H), 1.96 (s, 3H), 1.81 – 1.41 (m, 10H). ESI MS $[M-H]^-$ for $C_{18}H_{28}FN_5O_9P_2$, calcd 538.1, found 538.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[4-(cyclopentylamino)-6-[cyclopropyl(hydroxy)methyl]pyrazolo[3,4-d]pyrimidin-1-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (42): The title compound was synthesized in similar fashion to Example **44**. (1:1 mixture of diastereomers): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 – 7.85 (m, 1H), 6.60 – 6.41 (m, 1H), 5.27 (d, *J* = 53.7 Hz, 1H), 5.09 (s, 1H), 4.57 (dt, *J* = 18.4, 4.3 Hz, 1H), 4.29 – 4.14 (m, 2H), 4.05 (s, 2H), 2.26 (t, *J* = 20.5 Hz, 2H), 2.13 – 1.88 (m, 2H), 1.85 – 1.47 (m, 6H), 1.33 – 1.18 (m, 1H), 0.40 (s, 4H). ESI MS $[M-H]^-$ for $C_{20}H_{29}FN_5O_9P_2$, calcd 564.1, found 564.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(cyclopentylamino)-2-[hydroxy(phenyl)methyl]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (43): The title compound was synthesized in similar fashion to Example **44**. (1:1 mixture of diastereomers): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 – 7.85 (m, 4H), 7.49 (s, 4H), 7.41 – 7.08 (m, 8H), 6.47 (dd, *J* = 14.8, 4.6 Hz, 2H), 5.98 – 5.39 (m, 2H), 5.24 (dt, *J* = 52.4, 4.2 Hz, 1H), 5.07 (s, 1H), 4.54 (d, *J* = 14.1 Hz, 0H), 4.39 – 3.86 (m, 6H), 2.26 (t, *J* = 20.5 Hz, 3H), 1.99 (d, *J* = 34.0 Hz, 5H), 1.65 (d, *J* = 52.4 Hz, 13H). ESI MS $[M-H]^-$ for $C_{23}H_{30}FN_5O_9P_2$, calcd 600.2, found 600.3.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-(cyclopentylamino)-2-[hydroxy(oxan-4-yl)methyl]purin-9-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (44): Compound **84** (1.00 g, 1.75 mmol) was dissolved in THF (9

mL) and cooled to -78 °C. 4-tetrahydropyranylmagnesium bromide (9 mL, 8.75 mmol, 0.2M in THF) was added dropwise. The reaction mixture was allowed to warm to r.t. and stirred at r.t. for 3h. The reaction mixture was cooled to 0 °C, MeOH (50 mL) was added, and the mixture stirred at r.t. for 14h. The reaction mixture was dry loaded onto silica gel and purified by silica gel chromatography (0-10% MeOH in DCM) to afford the desired product **89** as a white solid (273 mg, 35%).

Nucleotide **44** was obtained from **89** using identical procedure as for Example **16** to give a white solid (1:1 mixture of diastereomers): ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.60 – 6.40 (m, 1H), 5.26 (d, *J* = 53.3 Hz, 1H), 4.63 – 4.39 (m, 2H), 4.30 – 4.13 (m, 2H), 4.13 – 3.97 (m, 1H), 3.94 – 3.75 (m, 2H), 3.38 – 3.13 (m, 2H), 2.26 (t, *J* = 20.4 Hz, 2H), 2.17 – 1.85 (m, 3H), 1.85 – 1.22 (m, 12H). ESI MS $[M-H]^-$ for $C_{23}H_{35}FN_5O_9P_2$, calcd 606.2, found 606.3.

[[(2*R*,3*R*,4*S*,5*R*)-5-[4-(benzylamino)-6-chloropyrazolo[3,4-d]pyrimidin-1-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (45): 4,6-Dichloro-1H-pyrazolo[3,4-d]pyrimidine **90** (1.0 g, 5.3 mmol) was dissolved in anhydrous MeCN (10 mL) and cyclopentylamine (478 mg, 5.6 mmol, 1.05 equiv.) was added followed by TEA (779 µL, 5.6 mmol, 1.05 equiv.). The mixture was stirred at room temperature for overnight, then anhydrous Cs_2CO_3 (3.4 g, 10.6 mmol, 2 equiv.) and the bromide (2.2 g, 5.3 mmol) were added. Reaction mixture was stirred at room temperature for overnight then evaporated. Crude residue was dissolved in MeOH (20 mL) and anhydrous K_2CO_3 (2.2 g, 15.9 mmol, 3 equiv.) was added. The mixture was stirred at room temperature for overnight, evaporated with silica gel and purified by column chromatography (SiO_2, Hex→ 100% EtOAc) to give desired N1 product **92** first (800 mg, 41%) and then the N2 isomer (600 mg, 30%). **92**: ESI MS $[M+H]^+$ for $C_{15}H_{20}ClFN_5O_3$, calcd 372.1, found 372.2.

Nucleotide **45** was obtained from **92** using identical procedure as for Example **16** to give a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, *J* = 7.2 Hz, 1H), 8.29 (s, 1H), 6.52 (d, *J* = 6.5 Hz, 1H), 5.50 – 5.29 (m, 1H), 4.75 (dt, *J* = 18.7, 7.5 Hz, 1H), 4.43 (h, *J* = 6.9 Hz, 1H), 4.31 – 4.22 (m, 1H), 4.18 – 4.05 (m, 1H), 4.04 – 3.92 (m, 1H), 2.20 (t, *J* = 20.5 Hz, 2H), 2.05 – 1.93 (m, 2H), 1.80 – 1.46 (m, 6H). ESI MS $[M+H]^+$ for $C_{16}H_{24}ClFN_5O_8P_2$, calcd 530.1, found 530.2.

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-chloro-4-[[(1S)-1-phenylethyl]amino]pyrazolo[3,4-d]pyrimidin-1-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (47): The title compound was synthesized in similar fashion to Example **45**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (d, *J* = 8.1 Hz, 1H), 8.33 (s, 1H), 7.40 (d, *J* = 7.9 Hz, 2H), 7.33 (t, *J* = 7.5 Hz,

2H), 7.23 (t, *J* = 7.4 Hz, 1H),), 6.34 (dd, *J* = 14.3, 4.6 Hz, 1H), 5.39 (bs, 1H), 5.31 – 5.12 (m, 1H), 5.14 (bs, 1H), 4.48 (dt, *J* = 18.5, 4.5 Hz, 1H), 4.17 (s, 3H), 4.01 (d, *J* = 5.2 Hz, 2H), 2.24 (t, *J* = 20.4 Hz, 3H), 1.51 (d, *J* = 7.0 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_8P_2$, calcd 566.1, found 566.1

[[(2*R*,3*R*,4*S*,5*R*)-5-[6-chloro-4-[[(1*R*)-1-phenylethyl]amino]pyrazolo[3,4-d]pyrimidin-1-yl]-4-fluoro-3-hydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (48): The title compound was synthesized in similar fashion to Example **45**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (d, *J* = 8.0 Hz, 1H), 8.33 (d, *J* = 1.2 Hz, 1H), 7.40 (d, *J* = 7.9 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 2H), 7.24 (t, *J* = 7.6 Hz, 1H), 6.50 (d, *J* = 6.5 Hz, 1H), 5.51 – 5.23 (m, 2H), 4.82 – 4.66 (m, 1H), 4.22 (bs, 1H), 4.13 – 4.02 (m, 1H), 3.94 (bs, 1H), 2.17 (t, *J* = 20.5 Hz, 2H), 1.53 (d, *J* = 7.1 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_8P_2$, calcd 566.1, found 566.2

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-(cyclopentylamino)pyrazolo[3,4-d]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (49): 4,6-Dichloro-1H-pyrazolo[3,4-d]pyrimidine **90** (25g, 132 mmol) and ammonium sulfate (0.20 g, 1.5 mmol) were dissolved in 150 mL of hexamethyldisilziane. The mixture was then warmed to reflux and stirred for 3 h. The mixture was then concentrated to dryness. The solid residue was then taken up in 300 mL of MeCN, and the protected ribose (50.6 g, 159 mmol) was added. This mixture was cooled 0°C and TMSOTf (27 mL, 145 mmol) was added dropwise. The mixture was then warmed to room temperature and allowed to stir overnight. The mixture was then concentrated and taken up in EtOAc. The organics were washed with saturated $NaHCO_3$ and brine. The organics were dried with $MgSO_4$, filtered and concentrated. The crude residue was purified using column chromatography (Hexanes / EtOAc) to provide the desired compound **93** (48 g, 108 mmol) in 82% overall yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (s, 1H), 6.47 (d, *J* = 3.2 Hz, 1H), 5.82 (dd, *J* = 5.3, 3.2 Hz, 1H), 5.63 (t, *J* = 5.8 Hz, 1H), 4.47 – 4.40 (m, 1H), 4.37 – 4.30 (m, 1H), 4.12 – 4.02 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H). ESI MS $[M+Na]^+$ for $C_{16}H_{16}Cl_2N_4NaO_7$, calcd 469.0, found 469.0.

Intermediate **93** (22 g, 49.3 mmol) was dissolved in MeOH (100 mL) and cooled to 0 °C. Cyclopentylamine (5.1g, 51.8 mmol, 1.05 equiv.), and triethylamine (7.2 mL, 51.8 mmol, 1.05 equiv.) were added and reaction mixture was stirred at 0 °C for 15 min then at rt for 4 h. 7M NH_3 in MeOH (60 mL) was added and reaction was stirred at rt for 1 day. Reaction mixture was evaporated and the crude product **94** was used in the next step without purification. ESI MS $[M+H]^+$ for $C_{15}H_{21}ClN_5O_4$, calcd 370.1, found 370.2.

Nucleotide **49** was obtained from **94** using identical procedure as for Example **13** to give a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (d, *J* = 7.2 Hz, 1H), 8.24 (s, 1H), 6.00 (d, *J* = 4.2 Hz, 1H), 4.49 (t, *J* = 4.7 Hz, 1H), 4.41 (q, *J* = 6.7 Hz, 1H), 4.26 (t, *J* = 4.7 Hz, 1H), 4.15 – 4.00 (m, 2H), 3.94 – 3.84 (m, 1H), 2.16 (t, *J* = 20.5 Hz, 2H), 2.04 – 1.91 (m, 2H), 1.79 – 1.45 (m, 6H). ESI MS $[M+H]^+$ for $C_{16}H_{25}ClN_5O_9P_2$, calcd 528.1, found 528.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[4-(benzylamino)-6-chloropyrazolo[3,4-d]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (50): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 – 9.18 (m, 1H), 8.35 – 8.16 (m, 1H), 7.39 – 7.19 (m, 5H), 6.07 – 5.94 (m, 1H), 4.69 (d, *J* = 5.4 Hz, 2H), 4.58 – 4.44 (m, 1H), 4.30 – 4.20 (m, 1H), 4.15 – 4.01 (m, 2H), 3.96 – 3.80 (m, 1H), 2.17 (t, *J* = 20.9 Hz, 2H). ESI MS $[M-H]^-$ for $C_{18}H_{22}ClN_5O_9P_2$, calcd 548.1, found 548.1.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1*S*)-1-phenylethyl]amino]pyrazolo[3,4-d]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (51): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 – 8.95 (m, 1H), 8.35 – 8.17 (m, 1H), 7.48 – 7.28 (m, 4H), 7.28 – 7.09 (m, 1H), 6.09 – 5.87 (m, 1H), 5.42 (q, *J* = 6.9 Hz, 1H), 4.60 – 4.33 (m, 1H), 4.33 – 4.16 (m, 1H), 4.13 – 3.96 (m, 2H), 3.97 – 3.80 (m, 1H), 2.35 – 1.95 (m, 2H), 1.62 – 1.36 (m, 3H). ESI MS $[M-H]^-$ for $C_{19}H_{24}ClN_5O_9P_2$, calcd 562.1, found 562.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1*R*)-1-phenylethyl]amino]pyrazolo[3,4-d]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (52): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (d, *J* = 8.4 Hz, 1H), 8.32 (s, 1H), 7.48 – 7.30 (m, 4H), 7.28 – 7.15 (m, 1H), 6.09 – 5.79 (m, 1H), 5.47 – 5.36 (m, 1H), 4.58 – 4.42 (m, 1H), 4.32 – 4.19 (m, 1H), 4.17 – 3.95 (m, 2H), 3.95 – 3.79 (m, 1H), 2.18 (t, *J* = 20.8 Hz, 2H), 1.71 – 1.37 (m, 4H). ESI MS $[M-H]^-$ for $C_{19}H_{24}ClN_5O_9P_2$, calcd 562.1, found 562.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1*S*)-1-(2-fluorophenyl)ethyl]amino]pyrazolo[3,4-d]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (53): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 – 9.15 (m, 1H), 8.33 (dd, *J* = 1.5, 0.7 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.29 (dd, *J* = 7.8, 5.6 Hz, 1H), 7.23 – 7.08 (m, 2H), 6.00 (d, *J* = 4.2 Hz, 1H), 5.65 – 5.51 (m, 1H), 4.48 (t, *J* = 4.9 Hz, 1H), 4.26 (t, *J* = 4.5 Hz, 1H), 4.05 (dq, *J* = 10.1, 5.9, 5.2 Hz, 2H), 3.88 (dt, *J* = 11.3, 6.0 Hz, 1H), 2.29 – 2.08 (t, *J* = 20.4 Hz , 2H), 1.53 (d, *J* = 6.8 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_9P_2$, calcd 582.1, found 582.1

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-4-[[(1*S*)-1-(2-fluorophenyl)ethyl]amino]pyrrolo[2,3-*d*]pyrimidin-7-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (54): To the mixture of 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine **95** (14.8 g, 78.9 mmol) and *D*-arabinofuranosyl bromide-2,3,5-triacetate (40 g, 118.3 mmol, 1.5 equiv.) in anhydrous CH_3CN (600 mL), Cs_2CO_3 (38.6 g, 118.3 mmol, 1.5 equiv.) was added and the reaction mixture was stirred at room temperature for overnight. Evaporated with silica gel and purified by column chromatography (SiO_2, Hex→ Hex:EtOAc, 2:8) to give **96** as a white solid (13.8 g, 39%). ESI MS $[M+H]^+$ for $C_{17}H_{18}Cl_2N_3O_7$, calcd 446.0, found 446.1.

Deprotection to provide **97** and phosphonylation, providing **54**, were performed in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, *J* = 7.9 Hz, 1H), 7.47 – 7.36 (m, 2H), 7.32 – 7.23 (m, 1H), 7.20 – 7.09 (m, 2H), 6.81 (s, 1H), 5.96 (d, *J* = 6.0 Hz, 1H), 5.58 (t, *J* = 7.3 Hz, 1H), 4.29 (t, *J* = 5.7 Hz, 1H), 4.14 – 3.96 (m, 4H), 2.24 (t, *J* = 20.5 Hz, 2H), 1.51 (d, *J* = 6.9 Hz, 3H). ESI MS $[M+H]^+$ for $C_{20}H_{25}ClFN_4O_9P_2$, calcd 581.1, found 581.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1*S*)-1-(3-fluorophenyl)ethyl]amino]pyrazolo[3,4-*d*]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (56): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (d, *J* = 7.9 Hz, 1H), 8.31 (s, 1H), 7.50 – 7.30 (m, 1H), 7.22 (d, *J* = 8.2 Hz, 2H), 7.06 (td, *J* = 8.7, 2.5 Hz, 1H), 6.00 (d, *J* = 4.2 Hz, 1H), 5.41 (t, *J* = 7.3 Hz, 1H), 4.48 (t, *J* = 4.7 Hz, 1H), 4.26 (t, *J* = 4.8 Hz, 1H), 4.05 (dq, *J* = 11.7, 6.5 Hz, 2H), 3.88 (dt, *J* = 11.2, 6.2 Hz, 1H), 2.17 (t, *J* = 20.5 Hz, 2H), 1.53 (d, *J* = 7.0 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_9P_2$, calcd 582.1, found 582.1

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-4-[[(1*S*)-1-(3-fluorophenyl)ethyl]amino]pyrrolo[2,3-*d*]pyrimidin-7-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (57): The title compound was synthesized in similar fashion to Example **54**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.30 (m, 2H), 7.26 – 7.16 (m, 2H), 7.08 – 6.98 (m, 1H), 6.77 (s, 1H), 5.97 (d, *J* = 6.0 Hz, 1H), 5.45 – 5.33 (m, 1H), 4.29 (t, *J* = 5.5 Hz, 1H), 4.14 – 3.98 (m, 4H), 2.24 (d, *J* = 20.5 Hz, 2H), 1.51 (d, *J* = 6.8 Hz, 3H). ESI MS $[M+H]^+$ for $C_{20}H_{25}ClFN_4O_9P_2$, calcd 581.1, found 581.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1*S*)-1-(4-fluorophenyl)ethyl]amino]pyrazolo[3,4-*d*]pyrimidin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (58): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (d, *J* = 7.9 Hz, 1H), 8.30 (d, *J* = 1.2 Hz, 1H), 7.42 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.15 (td, *J* = 8.9, 1.2 Hz, 2H), 6.00 (d, *J* = 4.2 Hz, 1H), 5.40 (t, *J* = 7.3 Hz, 1H), 4.48 (t, *J* = 4.8 Hz, 1H), 4.25 (t,

J = 4.5 Hz, 1H), 4.18 – 3.95 (m, 2H), 3.95 – 3.82 (m, 1H), 2.16 (t, *J* = 20.4 Hz, 2H), 1.52 (d, *J* = 7.2 Hz, 3H). ESI MS $[M+H]^+$ for $C_{19}H_{24}ClFN_5O_9P_2$, calcd 582.1, found 582.1.

[[(2*R*,3*S*,4*R*,5*R*)-5-[2-chloro-4-[[(1*S*)-1-(4-fluorophenyl)ethyl]amino]pyrrolo[2,3-*d*]pyrimidin-7-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (59): The title compound was synthesized in similar fashion to Example **54**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, *J* = 8.0 Hz, 1H), 7.46 – 7.34 (m, 3H), 7.17 – 7.08 (m, 2H), 6.76 (s, 1H), 5.97 (d, *J* = 6.3 Hz, 1H), 5.44 – 5.33 (m, 1H), 4.29 (t, *J* = 5.8 Hz, 1H), 4.14 – 3.97 (m, 4H), 2.24 (d, *J* = 20.5 Hz, 2H), 1.50 (d, *J* = 6.9 Hz, 3H). ESI MS $[M+H]^+$ for $C_{20}H_{25}ClFN_4O_9P_2$, calcd 581.1, found 581.2.

[[(2*R*,3*S*,4*R*,5*R*)-5-[6-chloro-4-[[(1*S*)-1-(4-fluorophenyl)ethyl]amino]pyrazolo[3,4-*b*]pyridin-1-yl]-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]methylphosphonic acid (60): The title compound was synthesized in similar fashion to Example **49**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.18 (d, *J* = 7.2 Hz, 1H), 7.46 – 7.39 (m, 2H), 7.19 – 7.10 (m, 2H), 6.13 – 5.99 (m, 2H), 4.89 (s, 1H), 4.53 – 4.46 (m, 1H), 4.25 (t, *J* = 4.8 Hz, 1H), 4.12 – 3.97 (m, 2H), 3.92 – 3.81 (m, 1H), 2.18 (t, *J* = 20.5 Hz, 2H), 1.50 (d, *J* = 7.3 Hz, 3H). ESI MS $[M+H]^+$ for $C_{20}H_{25}ClFN_4O_9P_2$, calcd 581.1, found 581.2.

Associated Content

Supporting Information

The supporting information is available free of charge on the ACS publications website at DOI:

Molecular formula strings and some data (CSV)

Analogous synthetic procedures for the preparation of additional compounds. Further co-crystallographic details concerning compounds **16** and **AB680,** and various assay protocols. (PDF)

Accession Codes

Atomic coordinates have been deposited in the Protein Data Bank (https://www.rcsb.org) for CD73 complexed with **16** (PDB ID: 6Z9B) and **AB680** (PDB ID: 6Z9D). Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

The authors declare no competing financial interest.

Abbreviations Used

ADO, adenosine monophosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CD73, cluster of differentiation 73, ecto-5'-nucleotidase; Cl_{int}, intrinsic clearance; CYP, cytochrome P450; V_{ss}, steady-state volume of distribution; fu, unbound (free) drug fraction; MRT, mean resonance time; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; NMP, *N*-methylpyrrolidone; *p*TsOH, *para*-toluenesulfonic acid; SAR, structure-activity relationship; $T_{1/2}$, half-life; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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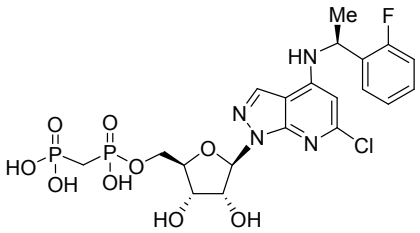
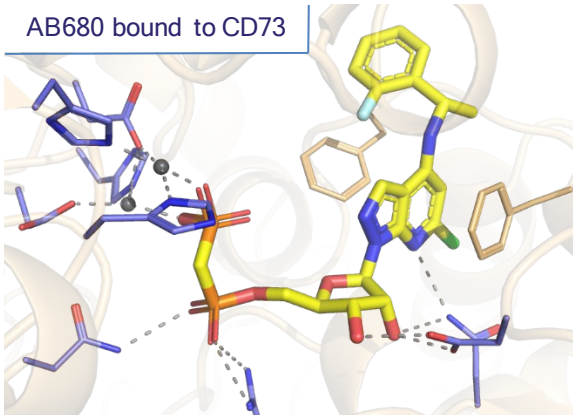


AB680

IC_{50} (Soluble CD73) = 43 pM

IC_{50} ($hCD8^+$) = 8 pM



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