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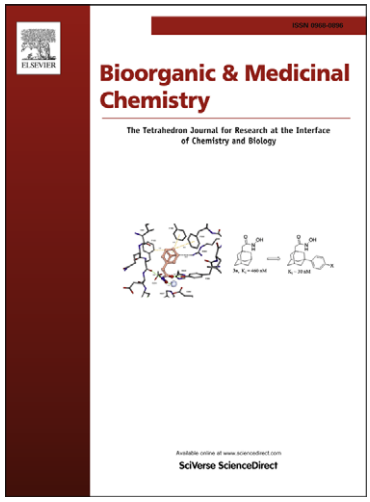

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Acyclic phosph(on)ate inhibitors of *Plasmodium falciparum* hypoxanthine-guanine-xanthine phosphoribosyltransferase

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Keywords: Phosphoribosyltransferase; malaria; HGXPRTase; purine salvage; protozoa

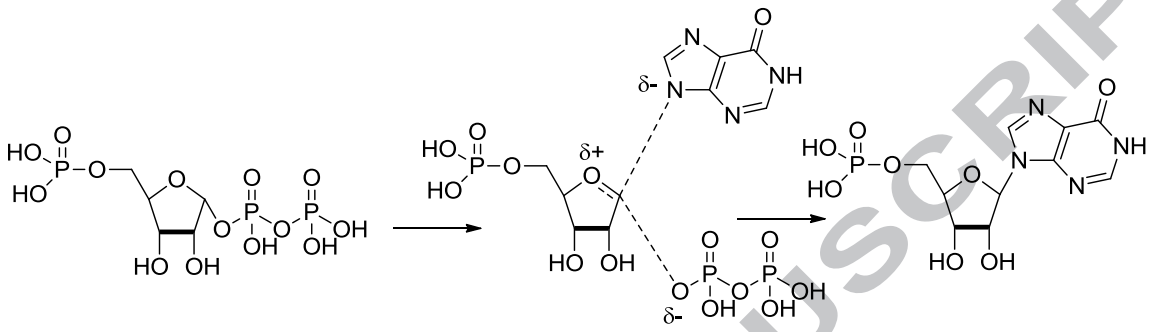
Abstract.

The pathogenic protozoa responsible for malaria lack enzymes for the *de novo* synthesis of purines and rely on purine salvage from the host. In *Plasmodium falciparum (Pf)*, hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT) converts hypoxanthine to inosine monophosphate and is essential for purine salvage making the enzyme an anti-malarial drug target. We have synthesized a number of simple acyclic aza-*C*- nucleosides and shown that some are potent inhibitors of *Pf* HGXPRT while showing excellent selectivity for the *Pf* versus the human enzyme.

Introduction.

Malaria is endemic in many parts of the world and there were over 200 million cases resulting in >600,000 deaths in 2010.[1] The disease is caused by *Plasmodium* protozoa of which *Pf* is the most deadly. Drug resistance[2] and the limitations of current vaccines[3] makes new drug development important for effective malaria control. Several pathogenic protozoa lack the enzymes for *de novo* synthesis of purines and are dependent on the purine salvage pathway.[4-6] In *Pf* purine salvage requires purine nucleoside phosphorylase (*Pf*PNP) to generate hypoxanthine and then hypoxanthine-guanine-xanthine phosphoribosyltransferase (*Pf* HGXPRT) to convert the hypoxanthine to inosine monophosphate.[7] Inhibition of *Pf*PNP has been shown to kill *Pf* in cell culture[8] and in an

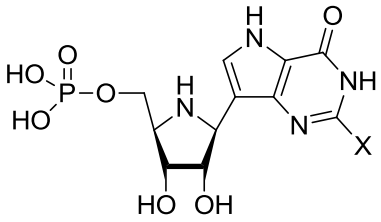
Aotus monkey model.[9] We have been interested in extending this work to the discovery of inhibitors of *Pf* HGXPRT as they are also expected to be lethal to the parasite. A number of purine analogues were reported some time ago as potential inhibitors of *Plasmodium berghei*, but none showed significant inhibition of the enzyme.[10-11]



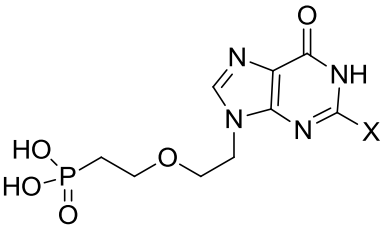
Scheme 1 Possible transition state of the HGXPRTase-catalysed reaction.

Pf HGXPRT is an *N*-ribosyltransferase and the transition states of such enzymes have been characterized with strong ribooxacarbenium ion properties and low bond order to both the purine and the nucleophile.[12-13] Kinetic commitment factors have prevented the transition state analysis of *Pf* HGXPRT to date but it would be reasonable to predict a dissociative transition state with ribooxacarbenium ion character similar to that shown (Scheme 1). Enzyme inhibitor design based on incorporating features of the transition state will often lead to exceptionally potent compounds.[14-18] Immucillin-H (and G) 5'-phosphate **1** and **2** are transition state analogue inhibitors of *Pf* HGXPRT and also of the human hypoxanthine-guanine phosphoribosyltransferase (HGPRT).[19-21] They mimic the ribooxacarbenium ion positive charge as well as other substrate properties. However, they are not selective for the parasite enzyme, would be expensive to synthesize and are unlikely to penetrate the erythrocyte and parasite cell membranes – so ruling them out as potential malaria therapeutics.

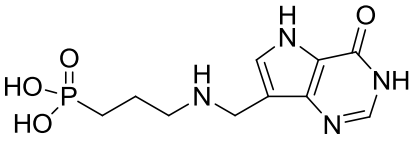
We have been interested in extending inhibitors **1** and **2** to compounds that are easier to synthesize and with selectivity for the parasite over the human enzyme and were intrigued by a report of some acyclic nucleoside phosphonates (such as **3**) which were moderate inhibitors of *Pf* HGXPRT with some selectivity over the human enzyme.[22] We



1 X = H
2 X = NH_2



3 X = H, NH_2



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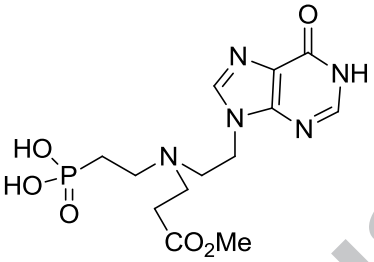

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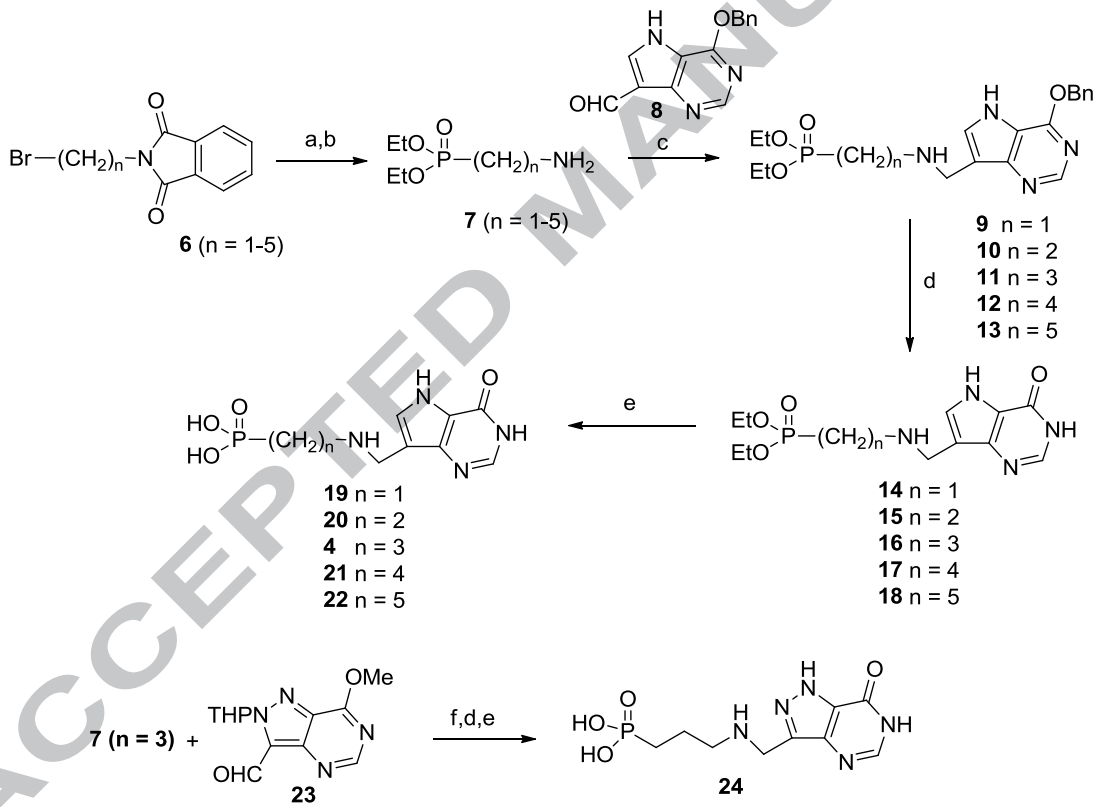
envisaged that acyclic nucleoside phosphonates such as **4** with a nitrogen to mimic the ribooxacarbenium ion with a methylene bridge to a 9-deazapurine might capture some transition state features. After this work was complete, some nitrogen-containing acyclic nucleoside phosphonates were described (e.g **5**)[23] which offered some improvements but failed to capture the full benefits of the nitrogen. Here we present the synthesis and enzyme inhibition properties of a number of acyclic aza-*C*-nucleoside phosphonates and phosphates; some of which are potent and selective inhibitors of *Pf* HGXPRT. A preliminary account of the biology of some of these compounds has been reported.[24]

Results and Discussion.

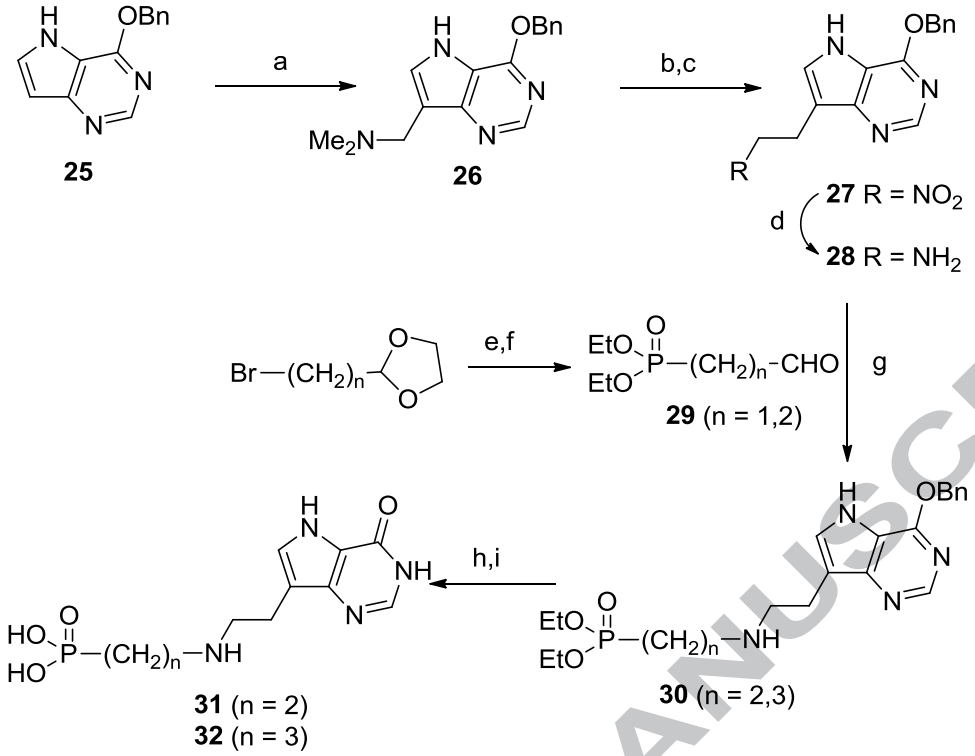
Target Selection and Synthesis.

We decided to prepare a series of linear aminophosphonates and to this end the commercially available bromoalkyl phthalamides **6** were treated with triethyl phosphite and then hydrazine hydrate[25] to give the known aminophosphonates **7**.[26] Coupling of aldehyde **8**[27] with these aminophosphonates and reduction of the resulting imines afforded **9** – **13** (Scheme 2). Acidic hydrolysis of the *O*-benzyl group in the deazapurines provided **14** – **18** and then dealkylation of the phosphonate ester groups gave the target aza-*C*-nucleoside mimics **4**, **19** – **22**. In a similar manner the 8-azapurine analogue **24** was also prepared from **23**. While it was likely that the positioning of the nitrogen in

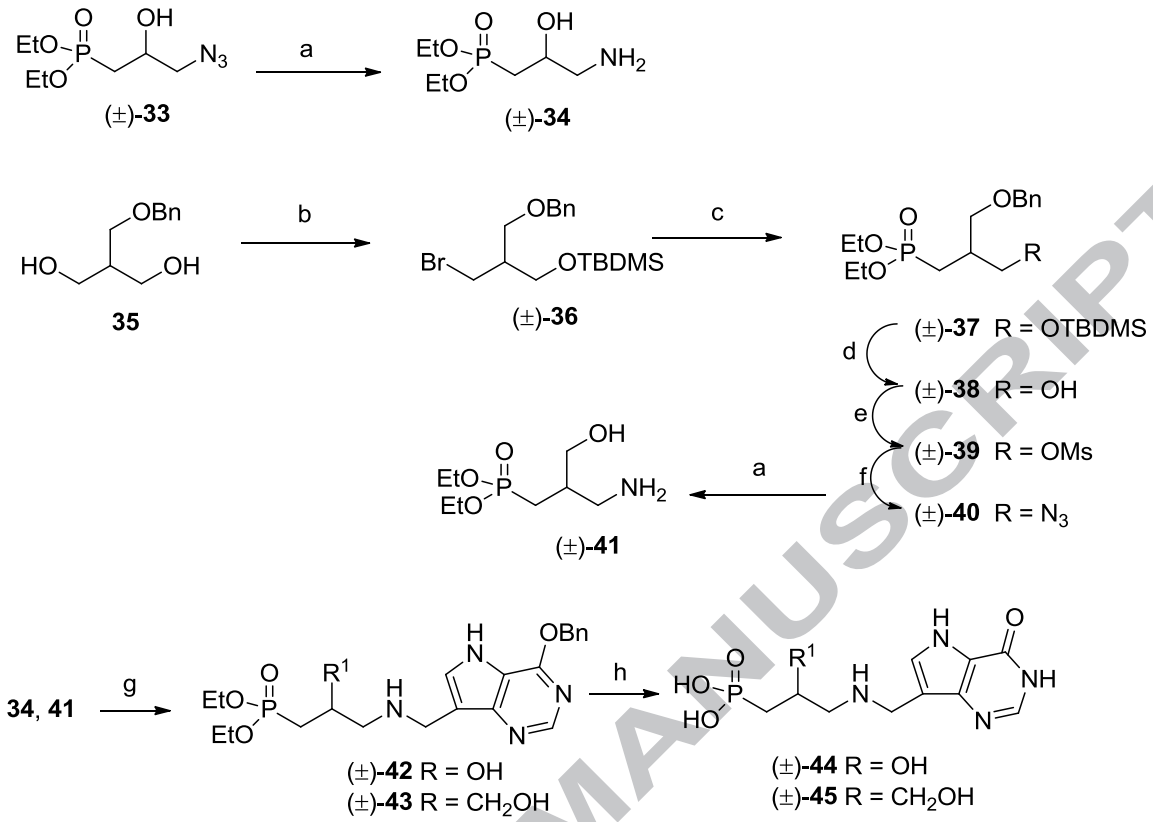
compounds **4**, **19** – **22** was optimal for mimicking the positive charge of a ribooxacarbenium ion transition state, we decided to explore the effect of moving the nitrogen along the chain. To this end, treatment of **25**[28] with dimethylamine and formaldehyde in a Mannich reaction afforded **26** (Scheme 3), which after reaction with methyl iodide and then basic nitromethane gave **27**. Reduction of the nitro group generated amine **28** which underwent reductive alkylation with phosphonate aldehydes **29** to give **30**. Deprotection as before gave the targets **31** and **32**. It was possible that substitution of the aminophosphonate alkyl chain with hydroxy or hydroxymethyl groups would offer improved properties as ribose mimics and so the known azide (±)-**33**[29] was reduced to the aminophosphonate (±)-**34** (Scheme 4). In addition the monobenzyl ether **35**[30] was converted into bromide



Scheme 2 (a) triethyl phosphite, 120°C; (b) H_2NNH_2, EtOH; (c) $NaBH_4$, EtOH; (d) 35% aq HCl, 60 °C; (e) 48% HBr 90 °C; (f) 2-picoline borane, MeOH.



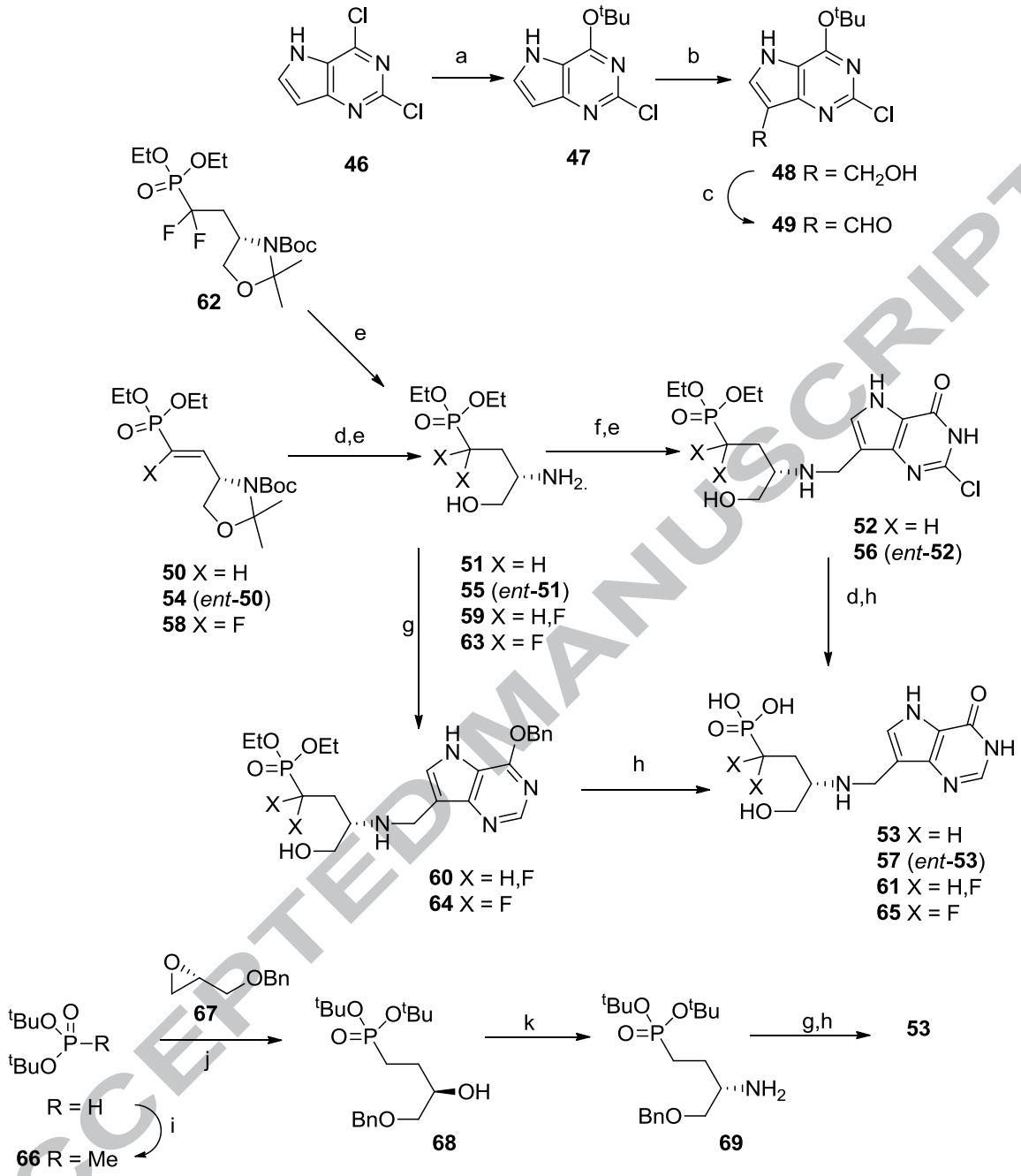
Scheme 3 (a) HCHO, $Me_2N.HCl$, NaOAc; (b) MeI; (c) CH_3NO_2, NaOMe; (d) $CoCl_2$, $NaBH_4$; (e) $(EtO)_3P$, 160 ^oC; (f) 1M HCl, reflux; (g) $NaBH_4$, EtOH; (h) conc HCl, 60 ^oC; (i) 48% HBr, 90 ^oC.



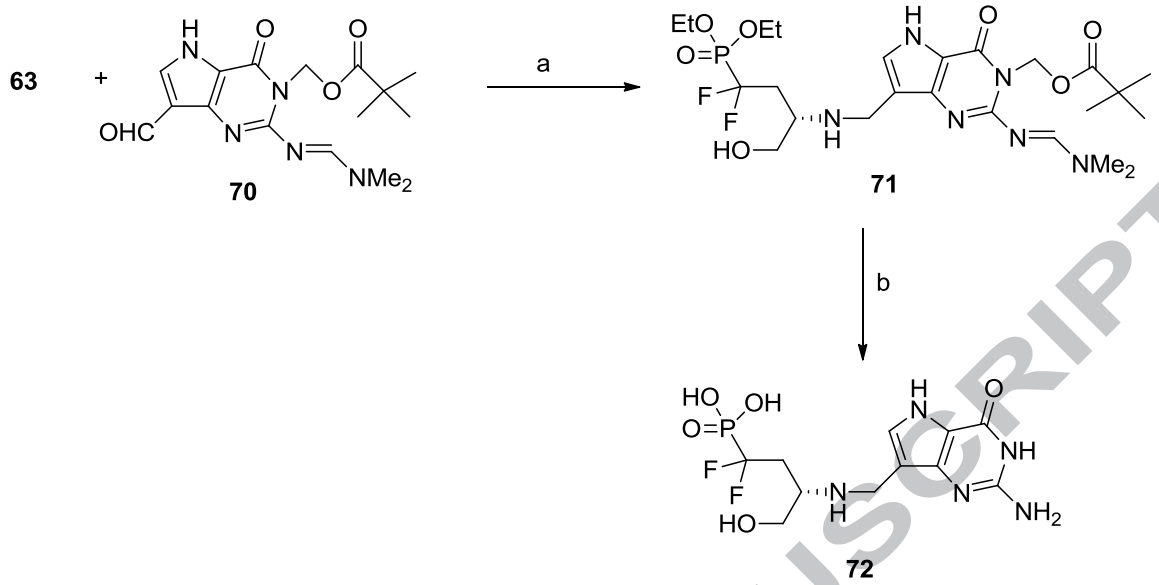
Scheme 4 (a) H_2, Pd/C; (b) i, NaH, TBDMSCl, ii, Ph_3P, CBr_4; (c) $(EtO)_3P$, 175 °C; (d) MeOH/35% aq HCl; (e) MsCl, Et_3N; (f) NaN_3, DMF, 80 °C; (g) 2-picoline borane, **8**, EtOH; (h) i, 35% aq HCl, 70 °C, ii, 48% HBr, 90 °C.

(±)-**36** and then the phosphonate (±)-**37**, from which the aminophosphonate (±)-**41** was derived. Reductive alkylation of aminophosphonates (±)-**34** and (±)-**41** with **8** gave, after deprotection, the targets (±)-**44** and (±)-**45**.

We have observed when designing inhibitors of nucleoside phosphorylases and hydrolases that serinol can be a good mimic of a ribooxacarbenium ion.[14, 31-32] Consequently we wanted to make serinol phosphonate analogues. For these compounds we chose to derivatise 9-deaza-2,6-dichloropurine (**46**) *via* **47** and **48** into aldehyde **49** (Scheme 5). This was done with the intention that both 9-deazahypoxanthine and 9-deazaguanine derivatives would be synthesized from **49** or its derivatives. Unfortunately attempted displacements of the Cl by NH_3 were unsuccessful for those compounds (data not given). The D-serine-derived vinyl phosphonate **50**[33] was reduced and deprotected to

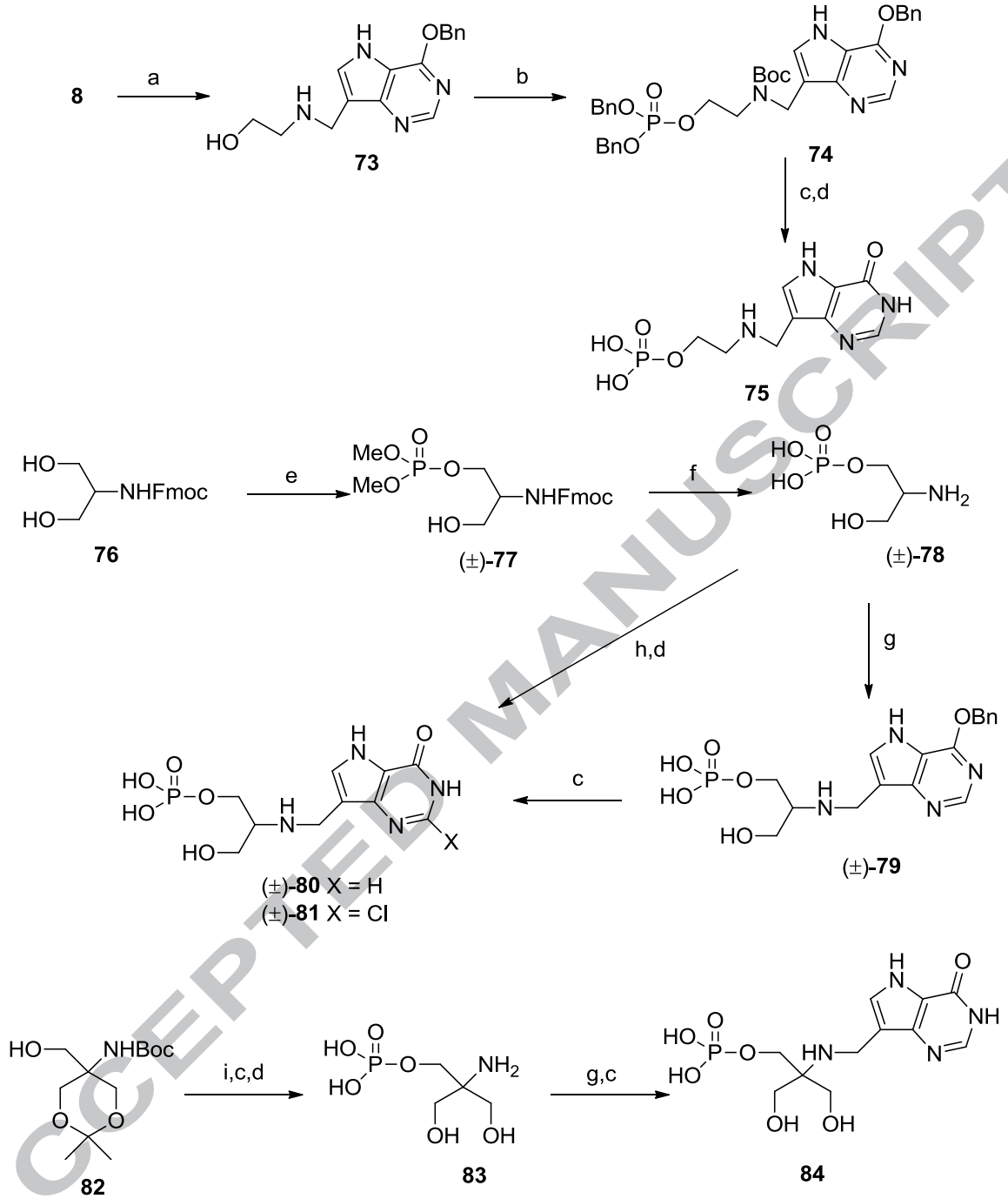


Scheme 5 (a) KO^{t}Bu, THF; (b) K_2CO_3, HCHO, aq dioxane; (c) Dess-Martin periodinane, Et_3N, THF; (d) H_2, Pd/C; (e) aq HCl; (f) **49**, 2-picoline borane, MeOH; (g) **8**, 2-picoline borane, MeOH; (h) 48% HBr, 80 ^oC; (i) NaH, MeI; (j) BuLi, $BF_3{\cdot}OEt_2$; (k) i, ^{i}PrOCON$_2$COO^{i}Pr, $(PhO)_2P(O)N_3$, Ph_3P; ii, $LiAlH_4$.

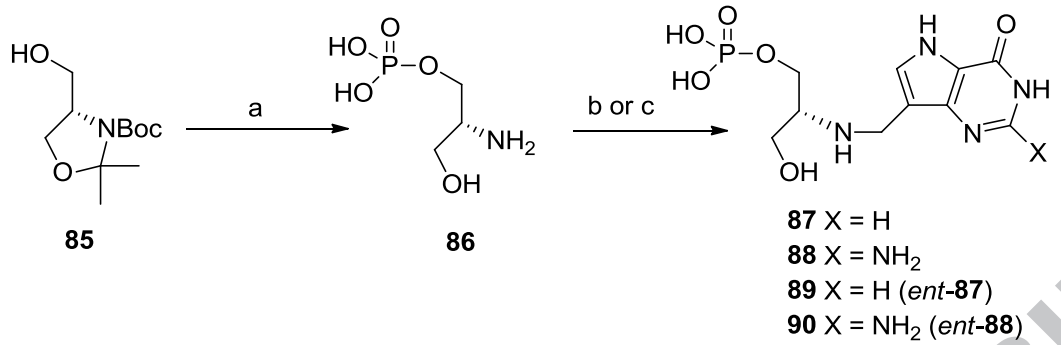


Scheme 6 (a) 2-picoline borane, Et_3N, MeOH; (b) 48% HBr 80 ^oC.

aminophosphonate **51** which was coupled with aldehyde **49** to give **52**. This was hydrogenolyzed and further deprotected to the target **53**. The L-serine-derived phosphonate **54** and the fluorophosphonate **58** were processed in the same way to the enantiomer **57** and the monofluoro compound **61**, while the difluorophosphonate **62**[34-35] similarly afforded **65**. We wanted to be sure that partial racemization had not occurred during reduction of the double bond in **50** and to this end we prepared **53** by an alternative method. Treatment of di-*tert*-butylphosphite with sodium hydride and methyl iodide gave **66**, and after deprotonation of **66** the anion was condensed with epoxide **67** to give **68**. This was converted to the amine **69** under conditions for inversion of configuration and the amine was converted into **53** as before. Both preparations of **53** had the same $[\alpha]_D$ value. Additionally the 9-deazaguanine derivative **70**[36] was coupled to difluorophosphonate **63** (Scheme 6) to afford, after deprotection, the alternative deazaguanine-containing inhibitor **72**.



Scheme 7 (a) ethanolamine, 2-picoline borane; (b) i, Boc_2O, ii, $(BnO)_2PN(iPr)_2$, tetrazole, iii, ^tBuOOH; (c) H_2, Pd/C; (d) 80% aq TFA; (e) $P(OMe)_3$, I_2, py; (f) i, TmsBr, ii, piperidine; (g) $NaBH_3CN$, **8**, Et_3N, MeOH 50 ^oC; (h) **49**, 2-picoline borane, Et_3N, MeOH; (i) i, $(BnO)_2PN^iPr_2$, tetrazole, ii, MCPBA.



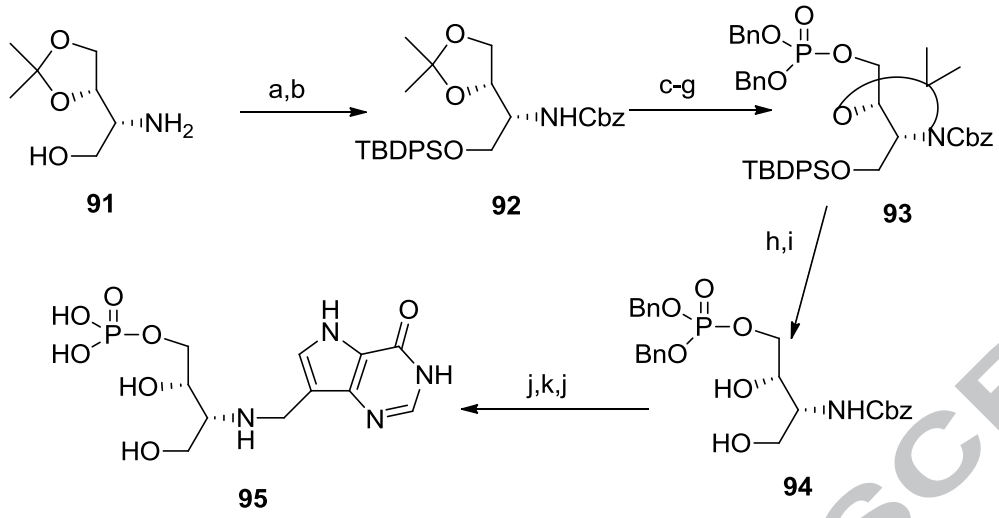
85 **86**

87 X = H
88 X = NH_2
89 X = H (*ent*-**87**)
90 X = NH_2 (*ent*-**88**)

Scheme 8 (a) i, $(BnO)_2PN(iPr)_2$, ii, MCPBA, iii, H_2, Pd/C, iv, aq TFA; (b) i, 2-picoline borane, **8**, ii, H_2, Pd/C; (c) i, 2-picoline borane, **70**, ii, 6M aq HCl.

We decided to make the corresponding phosphate esters of some of the phosphonates described above to compare the relative inhibitory properties. Reductive amination of aldehyde **8** with ethanolamine afforded **73** (Scheme 7), which was phosphorylated to **74** and deprotected to phosphate ester **75**. Treatment of the Fmoc-protected serinol **76** [37] with a mixture of iodine and trimethylphosphite[38] afforded the monophosphate (±)-**77** in moderate yield which was deprotected to the serinol phosphate (±)-**78**. Reductive amination of aldehyde **8** with amine (±)-**78** gave (±)-**79** which was hydrogenolyzed to the serinol target (±)-**80**. In addition, use of aldehyde **49** in the reductive coupling step followed by acid hydrolysis gave the 2-chloropurine derivative (±)-**81**. The Tris derivative **82**[39] was also phosphorylated and deprotected to the aminoalkyl phosphate **83** which was treated as described for **78** to give **84**. The chiral serinol phosphate esters were also prepared; phosphorylation of serinol derivative **85**[40-41] and deprotection gave aminoalcohol phosphate **86** which was coupled with the appropriate 9-carbaldehyde-9-deazapurine derivatives (**8** and **70**) under reductive conditions to afford, after deprotection, targets **87** and **88**. The enantiomers **89** and **90** were also prepared from the corresponding enantiomer of **86**[41] in the same way.

The aminoalcohol **91**[42] (Scheme 9) can be viewed as a chain extended serinol derivative. It was converted using standard procedures into carbamate **92** and then phosphate **93**, the diol **94** and finally the chain-extended target **95**.



Scheme 9 (a) TBDPSCl, imidazole; (b) BnOCOCl, $NaHCO_3$; (c) $HOAc/H_2O/THF$; (d) BzCl, py; (e) Me_2CO, $Me_2C(OMe)_2$, TsOH; (f) NaOMe, MeOH; (g) $(BnO)_2PNiPr_2$, tetrazole, then MCPBA; (h) Bu_4NF, THF; (i) $TFA/THF/H_2O$; (j) H_2, Pd black; (k) $NaBH_3CN$, **8**, Et_3N, MeOH.

Biological Results.

The compounds were assayed as inhibitors of *Pf*HGXPRT using spectrophotometric methods to observe the conversion of either xanthine or guanine to xanthosine-5'-monophosphate or guanosine-5'-monophosphate in the presence of 5-phospho-α-D-ribose-1-pyrophosphate. Analysis of the results from the simple phosphonates with different chain lengths (**4**, **19** – **22**) showed that **4** is a potent inhibitor and with excellent selectivity for the parasite over the human enzyme. That **4** is the most potent is consistent with the fact that there are the same number of bonds between the phosphonate and the deazapurine in **4** as in immucillin-H phosphate **1**. Moving the nitrogen along the chain (in **31**) was not beneficial and neither was incorporating the 8-azapurine (in **24**). Adding the hydroxyl group to the phosphonate alkyl chain (in **44**) may have conferred a small benefit in that **44** was made as the racemate and so one of the enantiomers may be better. However, it is a small benefit compared to the extra costs of introducing the chirality. Interestingly the selectivity margin of **44** was even better than for **4**. Adding a hydroxymethyl group (in **45**) was not beneficial for inhibitor potency, however the serinol phosphonate **53** proved to be an exceptionally potent inhibitor, showing again that serinol can act as an effective mimic for the ribooxacarbenium ion while the selectivity

Table 1[a]

Activity of synthesized acyclic aza-*C*-nucleoside phosph(on)ates as inhibitors of *Pf* and human (*Hs*) HGXPRT.

	***Pf* HGXPRT K_i (nM)**	***Hs* HGPRT K_i (nM)**		***Pf* HGXPRT K_i (nM)**	***Hs* HGPRT K_i (nM)**
19	> 20,000	> 10,000	**61**	1.2 ± 0.1	420 ± 10
20	1,900 ± 100	> 10,000	**65**	87 ± 8	> 10,000
4	10.6 ± 1.3[24]	4,900 ± 300[24]	**72**	100 ± 11	> 10,000
21	490 ± 35	> 10,000	**75**	14,400	> 10,000
22	8,600 ± 900	> 10,000	**80**	19 ± 2	> 10,000
24	27.6 ± 1.9	> 10,000	**81**	9,400	> 10,000
31	620 ± 61	> 10,000	**84**	15,300	> 10,000
32	> 20,000	11,320 ± 1,300	**87**	2.4 ± 0.2	> 10,000
44	9.1 ± 0.1	> 10,000	**88**	14.3 ± 1.7	> 10,000
45	490 ± 60	> 10,000	**90**	650 ± 150	> 10,000
53	0.65 ± 0.04[24]	380 ± 40[24]	**95**	980 ± 70	> 10,000
57	23.4 ± 2.3	16,000 ± 1,000			

[a] The K_i values were determined from analyses assuming competitive inhibition as in ref. 24.

for *Pf* over *Hs* enzymes was a factor of >500 for this compound. The enantiomer **57** was >30 times less active. Use of α-fluorophosphonates and α-α-difluorophosphonates has been proposed to change the pKa of the phosphonates to make them better mimics of phosphates, but in this situation the corresponding compounds **61** and **65** were less potent. Use of a deazaguanine moiety did not confer an advantage either, as in **72**. Making the phosphate analogue **75** of phosphonate **4** resulted in a surprising loss in activity with the phosphonate clearly being superior in activity. The phosphate analogues of the serinol phosphonates showed a similar but less dramatic trend with all being less potent than the corresponding phosphonates.

Conclusions.

We have synthesized and tested a range of acyclic aza-*C*-nucleoside phosph(on)ates and we discovered some relatively simple compounds that are potent and selective inhibitors of the *Pf* HGXPRT enzyme. These compounds offer promise as potential therapeutics for malaria by blocking purine salvage. The next step will be to devise a membrane permeable prodrug approach that will deliver drug through the erythrocyte and into the parasite. We have described[24] some preliminary experiments to this end and further work will be published in due course.

Experimental.

General Methods.

Chromatography solvents are distilled prior to use. Anhydrous solvents are those commercially available. Organic solutions are dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. Air sensitive reactions are performed under Ar. Analytical TLC is performed on Merck pre-coated silica gel 60 F254, detection by UV absorption and/or by heating after dipping in a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (5 wt%) and $Ce(SO_4)_2 \cdot 4\ H_2O$ (0.1 wt%) in 5% aq. H_2SO_4. Flash column chromatography is performed on silica gel (40-63 μm) or on an automated system with continuous gradient facility. ^{13}C-, ^{31}P- and ^{19}F-NMR spectra are ^{1}H decoupled, chemical shifts are in ppm and coupling constants in Hz if not already stated. ^{1}H NMR in $CDCl_3$ (internal TMS, δ 0), CD_3OD (internal TMS, δ 0), DMSOd_6 (internal TMS, δ 0) or D_2O (internal HOD) ^{13}C NMR in $CDCl_3$ (centre line of $CDCl_3$), DMSOd_6 (centre line of DMSOd_6) or D_2O, ^{31}P NMR in $CDCl_3$ or D_2O (external H_3PO_4, δ 0), ^{19}F NMR in $CDCl_3$ or D_2O (external CHF_3, δ 0). Assignments of ^{1}H and ^{13}C resonances are based on 2D (^{1}H-^{1}H DQF-COSY, ^{1}H-^{13}C HSQC, HMBC) and DEPT experiments. High resolution positive and negative electrospray mass spectra (ESI-HRMS) are recorded on a Q-TOF Premier tandem mass spectrometer. Melting points are uncorrected. Microanalyses are performed by the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

Diethyl {[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}(methyl)amino]methyl}phosphonate (14). Diethyl aminomethylphosphonate (**7** n=1,

98.5 mg, 1.5 eq.) and the aldehyde **8**[27] (100 mg, 1 eq.) are heated to 60 °C in EtOH (6 mL) for 30 min until dissolved. The solution is then cooled to room temperature, $NaBH_4$ (29.5 mg, 2eq) is added and the mixture is stirred for 30 min. The mixture is evaporated onto silica gel and flash chromatography (9/1/0.1 v/v/v DCM/MeOH/conc. NH_3), gives diethyl [({[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}amino)methyl]phosphonate **(9)**, (76 mg). This product is heated with conc. HCl (0.5 mL) at 60 °C for 1 h and the mixture is evaporated in vacuo twice from water and purified by flash chromatography (9/1/0.1 and 8/2/0.2 v/v/v DCM/MeOH/conc. NH_3) to give **14**, (45 mg, 36%). ^{1}H NMR (500 MHz, MeOD) δ 7.93 (s, 1H), 7.51 (s, 1H), 4.20-4.14 (m, 4H), 3.26-3.35 (m, 4H), 3.34 (m, 6H). ^{13}C NMR (125MHz, MeOD) δ155.8, 145.0,143.3, 129.8, 119.5, 111.8, 64.4, 44.5, 44.4, 43.5, 42.3, 16.7. ^{31}P NMR (202 MHz, MeOD) δ 22.5. ESI-HRMS for $C_{12}H_{19}N_4O_4NaP$ $[M+Na]^+$ calcd 337.1042; found 337.1039.

Diethyl {2-[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]ethyl}phosphonate (15). Using the procedure above for the preparation of compound **14**, compound **7**, (n=2) is converted to title compound **15**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.77 (s,1H), 7.27 (s, 1H), 3.95 (m, 4H), 3.76 (s, 2H), 2.72 (m, 2H), 2.50 (s, 2H),1.92 (m, 2H), 1.19 (m, 6H). ^{13}C NMR (125MHz, MeOD) δ153.6, 143.0, 141.3, 125.8, 117.6, 115.3, 60.8, 42.1, 26.0, 24.9, 16.2. ^{31}P NMR (202 MHz, DMSO-d_6) δ30.3. ESI-HRMS for $C_{13}H_{21}N_4O_4NaP$ $[M+Na]^+$ calcd 351.1198; found 351.1194.

Diethyl {2-[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]propyl}phosphonate (16). Using the procedure above for the preparation of compound **14**, compound **7**, (n=3) is converted to title compound **16**. ^{1}H NMR (500 MHz, D_2O) δ 8.79 (s,1H), 7.70 (s,1H), 4.29 (s, 2H), 3.98-3.92 (m, 4H), 3.07-3.04 (m,2H), 1.86-183 (m, 2H), 1.16-1.10 (m,6H). ^{13}C NMR (125MHz, D_2O) δ 152.7, 144.8, 133.0, 132.6, 118.2, 103.3, 63.6, 46.7, 40.4, 21.8, 20.7, 18.9, 15.6. ^{31}P NMR (202 MHz, D_2O) δ 33.4. ESI-HRMS for $C_{14}H_{24}N_4O_4P$ $[M+H]^+$ calcd 343.1535; found 343.1528.

Diethyl {2-[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]butyl}phosphonate (17). Using the procedure above for the preparation of compound **14**, compound **7**, (n=4) is converted into first diethyl [4-({[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}amino) butyl]phosphonate **(12)**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.72 (s, 1H), 7.44-7.27 (bm, 5H), 5.53 (s, 2H), 4.19 (s, 2H), 4.03-3.98 (m, 4H), 2.80-2.77 (m, 2H), 1.72-1.51 (bm, 6H), 1.30-1.23 (m, 6H). ^{13}C NMR (125MHz, $CDCl_3$) δ 149.7, 139.4, 130.5, 129.3, 128.5, 128.4, 128.2, 128.1, 127.7, 115.0, 108.5, 67.7, 61.6, 47.1, 41.5, 27.8, 27.7, 25.6, 24.5, 20.9, 16.4. ^{31}P NMR (202 MHz, $CDCl_3$) δ 31.6. ESI-HRMS for $C_{22}H_{32}N_4O_4P$ $[M+H]^+$ calcd 447.2161; found 447.2153. This is then converted to title compound **17**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (s, 1H), 7.78 (s, 1H), 4.36 (s, 2H), 4.01-4.07 (m, 4H), 3.10-3.08 (m, 2H), 1.91-1.85 (m, 2H), 1.81-1.75 (m, 2H), 1.65-1.54 (m, 2H), 1.26-1.20 (m, 6H).

Diethyl {2-[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]pentyl}phosphonate (18). Using the procedure above for the preparation of compound **14**, compound **7**, (n=5) is converted first into diethyl [5-({[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}amino) pentyl]phosphonate **(13)**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.33 (s, 1H), 7.47-7.28 (bm, 5H), 5.57(s, 2H), 4.09-4.00 (m, 4H), 4.01(s, 1H), 1/70-1.51 (bm, 6H), 1.39-1.35(m, 2H), 1.32-1.27 (m, 6H). ^{13}C NMR (125MHz, $CDCl_3$) δ 155.4, 149.5, 149.1, 136.3, 128.5, 128.4, 128.3, 128.0, 127.7, 115.3, 113.8, 67.8, 61.4, 48.8, 43.2, 29.0, 28.2, 26.0, 25.0, 22.2, 16.4. ^{31}P NMR (202 MHz, $CDCl_3$) δ 32.2. ESI-HRMS for $C_{23}H_{34}N_4O_4P$ $[M+H]^+$ calcd 461.2318; found 461.2311. This is then converted to title compound **18**. ^{1}H NMR (300 MHz, D_2O) δ 8.53 (s, 1H), 7.68 (s,1H), 4.36 (s, 2H), 4.11-3.97(m, 4H), 3.09-3,01 (m, 2H), 1.90-1.40 (bm, 8H), 1.28-1.22 (m, 6H).

{[({4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]methyl}phosphonic acid (19). The diethyl phosphonate (**14**, 43mg) is heated with 48% HBr for 5 h at 90 °C and the solution is evaporated in vacuo twice from water. Reverse phase (C_{18}) chromatography eluting with water gives title compound **(19**, 40 mg) as a white water insoluble solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (s, 1H), 7.73 (s, 1H), 3.39 (s, 2H), 3.36 (s, 2H). ^{13}C (125 MHz, DMSO-d_6) δ 152.3, 144.0, 137.2, 130.7, 117.7, 104.3, 42.2,

41.0. ^{31}P (202 MHz, DMSO-d_6) δ 11.7. ESI-HRMS for $C_8H_{12}N_4O_4P$ $[M+H]^+$ calcd 259.0596; found 259.0599.

{[({4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]ethyl}phosphonic acid (20). Using the procedure for the preparation of compound **19**, compound **15** is converted to title compound **20**. ^{1}H NMR (500 MHz, D_2O) δ 8.69 (s, 1H), 7.69 (s,1H), 4.34 (s,2H), 3.23-3.19 (m, 2H), 2.02-1.95 (m, 2H). ^{13}C NMR (125MHz, D_2O) δ 153.2, 144.5, 134.8, 132.3, 118.2, 103.6, 42.0, 40.2, 25.0, 23.9. ^{31}P NMR (202 MHz, D_2O) δ 20.6. ESI-HRMS for $C_9H_{12}N_4O_4P$ $[M-H]^-$ calcd 271.0596; found 271.0594.

{[({4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]propyl}phosphonic acid (4). Using the procedure for the preparation of compound **19**, compound **16** is converted to title compound **4**. ^{1}H NMR (500 MHz, D_2O) δ 8.68 (s, 1H), 7.69 (s, 1H), 4.30 (s, 2H), 3.07-3.01 (m, 2H), 1.88-1.80 (m, 2H), 1.74-1.68 (m 2H). ^{13}C NMR (125MHz, D_2O) δ 152.5, 144.9, 132.8, 131.8, 118.3, 103.0, 47.1, 40.5, 24.1, 23.0, 19.4. ^{31}P NMR (202 MHz, D_2O) δ 29.2. ESI-HRMS for $C_{10}H_{16}N_4O_4P$ $[M+H]^+$ calcd 287.0909; found 287.0904.

{[({4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]butyl}phosphonic acid (21). Using the procedure for the preparation of compound **19**, compound **17** is converted to title compound **21**. ^{1}H NMR (500 MHz, D_2O) δ 8.93 (s, 1H), 7.75 (s, 1H), 4.31 (s, 2H), 3.02-3.06 (m, 2H), 1.75-1.67 (m, 4H), 1.55-1.51 (m, 2H). ^{13}C NMR (125 MHz, D_2O) δ 152.5, 144.9, 132.8, 131.7, 118.3, 103.0, 46.6, 40.4, 26. 3, 26.2, 25.0, 19.3. ^{31}P NMR (202 MHz, D_2O)δ 31.2. ESI-HRMS for $C_{11}H_{18}N_4O_4P$ $[M+H]^+$ calcd 301.1066; found 301.1064.

{[({4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]pentyl}phosphonic acid (22). Using the procedure for the preparation of compound **19**, compound **18** is converted to title compound **22**. ^{1}H NMR (500 MHz, D_2O) δ 8.93 (s, 1H), 7.76 (s, 1H), 4.31 (s, 2H), 3.01-3.05 (m, 2H), 1.71-1.58 (bm, 4H), 1.49-1.42 (m, 2H), 1.38-1.32 (m, 2H). ^{13}C NMR (125MHz, D_2O) δ 152.4, 144.9, 132.8, 131.7, 118.3, 103.1, 46.9, 40.3,

26.6, 26.5, 26.3, 25.1, 21.4. ^{31}P NMR (202 MHz, D_2O) δ 32.3. ESI-HRMS for $C_{12}H_{20}N_4O_4P$ $[M+H]^+$ calcd 315.1222; found 315.1221.

3-((7-Hydroxy-1H-pyrazolo[4,3-d]pyrimidin-3-yl)methylamino)propylphosphonate ammonium salt (24). Anhydrous HCl in MeOH (1.0 M, 0.11 mL, 0.11 mmol) is added dropwise to a solution of the diethyl phosphonate (**7**, n=3) (100 mg, 0.51 mmol) in anhydrous MeOH (2 mL) followed by the addition of the aldehyde **23**[43] (90 mg, 0.34 mmol) and 2-picoline borane complex (50 mg, 0.44 mmol). The resulting suspension is left to stir for 2 h and the then homogeneous solution absorbed onto silica gel and the resulting residue subjected to flash chromatography (EtOAc then 5 to 10% v/v MeOH in $CHCl_3$) to afford diethyl 3-((7-methoxy-2-(tetrahydro-2H-pyran-2-yl)-2H-pyrazolo[4,3-d]pyrimidin-3-yl)methylamino)propylphosphonate (123 mg, 82 % yield) as a white solid, which is committed to the next step without further characterisation or purification. Concentrated HCl (1.5 mL, 18 mmol) is added to a solution of this material (90 mg, 0.204 mmol) in MeOH (3 mL, 0.204 mmol) and the resulting mixture heated to reflux for 6 h. The reaction mixture is concentrated in vacuo and partitioned between chloroform and water, the water layer is extracted with additional chloroform, the organic layers are combined, dried and concentrated in vacuo. The residue is dissolved in aqueous hydrobromic acid (2 mL, 17.68 mmol) and heated for a further 6 h at 80 ºC. The reaction mixture is concentrated in vacuo and the resulting residue co-distilled with water (2 x 20 mL). Flash chromatography (1:1 v/v dioxane/conc. NH_4OH) afforded the title compound **24** (35 mg, 60%) as a foam. ^{1}H NMR (500 MHz, D2O): δ = 7.98 (s, 1H), 4.47 (s, 2H), 3.16 (t, *J* = 6.9 Hz, 2H), 1.91 (septet, *J* = 7.0 Hz, 2H), 1.56 (m, 2H). ^{13}C NMR (125 MHz, D_2O): δ = 155.2, 148.4, 137.5, 135.6, 128.6, 66.6, 48.7, 48.6 (d, *J* = 13.4 Hz), 40.8, 26.1 (d, *J* = 132 Hz), 25.6, 20.7(d, *J* = 3.8 Hz). ^{31}P (202 MHz, D_2O): δ = 21.9.

{[4-(Benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}dimethylamine (26). The deazapurine **25**[28] (178 mg,1 eq.) in dioxane (1 mL) and water (1 mL) with NaOAc (64.6 mg, 1 eq.), dimethylamine hydrochloride (64.6 mg,1 eq.) and formaldehyde (65.5 µL, 1.1 eq., 37% aq) is heated at 95 °C for 4 h and then evaporated under reduced pressure. The residue is evaporated twice from ammonia in MeOH onto silica gel. Flash chromatography (DCM/MeOH-NH_3 9:1 v/v) gives title compound **26** (163 mg). ^{1}H NMR

(500 MHz, MeOD) δ 8.42 (s, 1H), 7.54 (s, 1H), 7.53-7.31 (m, 5H), 5.68 (s, 2H), 3.75 (s, 2H), 2.28 (s, 6H). ^{13}C NMR (125 MHz, MeOD) δ 157.2, 150.3, 137.8, 131.8, 129.4, 116.4, 112.1, 69.1, 52.4, 44.8. ESI-HRMS for $C_{15}H_{19}N_4O$ $[M+H]^+$ calcd 283.1559; found 283.1560.

4-(Benzyloxy)-7-(2-nitroethyl)-5H-pyrrolo[3,2-d]pyrimidine (27). The dimethylamine **26** (548 mg,1 eq.) in tetrahydrofuran (25 mL) is treated with methyl iodide (1.2 mL, 10 eq.) at room temperature for 18 h and then the solution is evaporated under high vacuum to give an orange foam of the quaternary ammonium salt of **26**. Nitromethane (3.34 mL, 30 eq.) is added to methanol (32 mL) and methanolic sodium methoxide (7 mL, 30%, 20 eq.) and the mixture is stirred under argon for 10 min and then the crude quaternary ammonium salt is added. The mixture is stirred for 2 h and then the methanol is evaporated. The residue is diluted with water, the pH adjusted to 3 with 1 N HCl and extracted 3x with ethyl acetate. The organic extract is washed with brine, dried and evaporated. Flash chromatography (20-50% v/v EtOAc in hexanes) gives title compound **27** (310 mg, 53%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (s, 1H), 7.54 (d, 1H), 7.42-7.33 (m, 5H), 5.61 (s, 2H), 4.93 (m, 2H), 3.37 (m, 2H). ^{13}C NMR (125MHz, DMSO-d_6) δ 154.9, 148.7, 136.6, 128.8, 128.4, 114.4, 109.7, 75.0, 67.0, 22.1. ESI-HRMS for $C_{15}H_{14}N_4O_3Na$ $[M+Na]^+$ calcd 321.0964; found 321.0970.

2-[4-(Benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl] ethan-1-amine (28). Sodium borohydride (393 mg, 10 eq.) is added in small portions to **27** (310 mg 1 eq.) in methanol (20 mL) with $CoCl_2.6H_2O$ (495 mg, 2 eq.) at 0 °C and then the mixture is stirred at 0 °C for 30 min. The product is evaporated onto silica gel and flash chromatography (9/1/0.1 and 8/2/0.2 v/v/v DCM/MeOH/conc. NH_3) gives title compound **28** (195 mg, 70%). ^{1}H NMR (500 MHz, MeOD) δ 8.4 (s, 1H), 7.36 (s, 1H), 7.53-7.30 (m, 5H), 5.62 (s, 2H), 2.98-2.92 (m, 4H).^{13}C NMR (125MHz, MeOD) δ 157.1, 149.8, 137.9, 129,3,129.6,128.3, 116.8, 114.2, 69.1, 43.0, 28.1. ESI-HRMS for $C_{15}H_{17}N_4O$ $[M+H]^+$ calcd 269.1402; found 269.1404.

Diethyl (2-oxoethyl)phosphonate (29, n=1) and diethyl (3-oxopropyl)phosphonate (29, n=2). Triethylphosphite (8.4 mL) and 2-(2-bromoethyl)-1,3-dioxolane (10 g) are

heated at 160 °C for 18 h and then evaporated under high vacuum at 100 °C . The residue is refluxed with 1 N HCl under argon for 15 min and cooled to room temperature. The product is salted out with solid NaCl and extracted with DCM, washed with saturated $NaHCO_3$, brine, dried and concentrated. Flash chromatography (EtOAc-MeOH 95:5 v/v) gives title compound **29, n=2** (2.5 g). The NMR spectral data was identical with literature values.[44] The same method applied to 2-(bromomethyl)-1,3-dioxolane afforded title compound **29, n=1** with the same NMR data as reported.[45]

Diethyl [2-({2-[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]ethyl}amino)ethyl]phosphonate (30, n=2). The amine **28** (67 mg, 1.5 eq) and phosphonoaldehyde **29, n=1** (40 mg, 1 eq.) in ethanol (2 mL) are heated at 70 °C for 30 min and cooled to room temperature. $NaBH_4$ (17 mg, 2 eq.) is added and the mixture stirred for 30 min. The mixture is evaporated onto silica gel and subjected to flash chromatography (9/1/0.1 and 8/2/0.2 v/v/v DCM/MeOH/conc.NH_3) to give title compound **30, n=2** (30 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.48-7.27 (m, 5H), 7.19 (s, 1H), 5.55 (s, 2H), 4.07-4.01 (m, 4H), 3.04-2.95 (m, 6H), 2.08-2.02 (m, 2H), 1.28-1.25 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) 155.3, 149.1, 148.8, 136.3, 128.4, 127.4, 115.4, 113.7, 67.9, 61.8, 49.1, 42.8, 26.1, 25.0, 24.0, 16.4. ^{31}P NMR (202 MHz, $CDCl_3$) 29.2. ESI-HRMS for $C_{21}H_{30}N_4O_4P$ $[M+H]^+$ calcd 433.2005; found 433.1999.

Diethyl [3-({2-[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]ethyl}amino)propyl]phosphonate (30, n=3). The amine **28** (60 mg, 1.5 eq.) and phosphonoaldehyde **29, n=2** (36 mg, 1 eq.) in ethanol (2 mL) are heated at 70 °C for 30 min and cooled to room temperature. $NaBH_4$ (15 mg, 2 eq.) is added and the mixture stirred for 30 min. Evaporation onto silica gel and flash chromatography (9/1/0.1 and 8/2/0.2 v/v/v DCM/MeOH/conc. NH_3) gives title compound **30, n=3** (50 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.26 (s, 1H), 7.49-7.19 (m, 5H), 5.57 (s, 2H), 4.09-4.01 (m, 4H), 2.98 (s, 4H), 2.70 (m, 2H), 1.78-1.71 (m, 4H), 1.31-1.26 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.3, 149.3, 136.3, 128.5, 126.8, 115.4, 114.8, 67.9, 61.5, 49.7, 49.6, 24.5, 24.0, 22.8, 22.7, 16.4. ^{31}P NMR (202 MHz, $CDCl_3$) δ 32.1. ESI-HRMS for $C_{22}H_{32}N_4O_4P$ $[M+H]^+$ calcd 447.2161; found 447.2160.

{2-[(2-{4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}ethyl)amino]ethyl}phosphonic acid (31). The diethyl phosphonate **30, n=2** (52 mg) is heated at 60 °C with conc. HCl (0.5 mL) for 1 h, evaporated under reduced pressure twice from water and the heated with 48% HBr (1 mL) at 90 °C for 5 h and evaporated again twice from water. The product is chromatographed on reverse phase C_{18} silica gel eluting with water to give title compound **31** (39 mg). ^{1}H NMR (500M Hz, D_2O) δ 8.78 (s, 1H), 7.50 (s, 1H), 3.29-3.24 (m, 2H), 3.22-3.18 (m, 2H), 3.05-3.02 (m, 2H), 2.04-1.97 (m, 2H). ^{13}C NMR (125MHz , D_2O) δ 152.8, 144.0, 132.2, 130.3, 117.9, 108.0, 47.0, 42.9, 24.9, 23.8, 20.3. ^{31}P NMR (202 MHz, D_2O) δ 21.3. ESI-HRMS for $C_{10}H_{16}N_4O_4P$ $[M+H]^+$ calcd 287.0909; found 287.0912.

{3-[(2-{4-Hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}ethyl)amino]propyl}phosphonic acid (32). The diethyl phosphonate **30, n=3** (50 mg) is heated at 60 °C with conc. HCl (0.5 mL) for 1 h and then evaporated under reduced pressure twice from water. The residue is then heated with 48% aq. HBr (1 mL) at 90 °C for 5 h and evaporated again twice from water. The product is chromatographed on reverse phase C_{18} silica gel eluting with water to give title compound **32** (30mg). ^{1}H NMR (500 MHz, D_2O) δ 8.76 (s, 1H), 7.49 (s, 1H), 3.26-3.17 (m, 2H), 3.07-2.98 (m, 4H), 1.88-1.79 (m, 2H), 1.74-1.67 (m, 2H). ^{13}C NMR (125MHz , D_2O) δ 152.9, 144.0, 132.4, 130.2, 117.9, 108.2, 47.9, 47.2, 24.3, 23.2, 20.3, 19.5. ^{31}P NMR (202 MHz, D_2O) δ 28.3. ESI-HRMS for $C_{11}H_{18}N_4O_4P$ $[M+H]^+$ calcd 301.1066; found 301.1062.

Diethyl (3-amino-2-hydroxypropyl)phosphonate (34). Diethyl (3-azido-2-hydroxypropyl)phosphonate **(33)**[29] (620 mg) is reduced with 10% palladium on charcoal (250 mg) in ethanol (10 mL) under hydrogen at 1 atm. for 1 h, filtered and concentrated. Flash chromatography (9/1/0.1 and 8/2/0.2 v/v/v DCM/MeOH/conc. NH_3) gives title compound **34** (388 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.18-4.09 (m, 4H), 3,98-3.92 (m, 1H), 2.88-2.68 (m, 2H), 2.15-1.95 (bm, 3H),1.98-1.89 (m, 2H), 1.35-1.33 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 67.4, 61.9, 48.1, 31.6, 31.0, 16.4. ^{31}P NMR (202 MHZ,$CDCl_3$)δ 30.0. ESI-HRMS for $C_7H_{19}NO_4P$ $[M+H]^+$ calcd 212.1052; found 212.1049.

[3-(Benzyloxy)-2-(bromomethyl)propoxy](tert-butyl)dimethylsilane (36). A solution of **35**[30] (1.04 g,1 eq.) in dry THF (3 mL) is added to NaH (60%, 127 mg, 5.3 eq.) in dry THF (12 mL) with ice cooling. The mixture is stirred at room temperature for 30 min and then $^{t}BuMe_2SiCl$ (800 mg, 5.3 eq.) in dry THF (2 mL) is added. After 2 h, water (6 mL) is added and the product extracted with EtOAc, washed with brine, dried and evaporated. Flash chromatography (hexanes-EtOAc 9:1 v/v) gives 2-[(benzyloxy)methyl]-3-[(tert-butyldimethylsilyl)oxy]propan-1-ol (1.25 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.30 (m, 5H), 4.51 (s, 2H), 3.80-3.71 (m, 4H), 3.64-3.53 (m, 2H), 2.07-2.01 (m, 1H), 0.86 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (125MHz,$CDCl_3$) δ 138.0, 128.4, 127.7, 73.2, 70.0, 64.5, 63.4, 43.0, 25.9, 18.2, 0.0. ESI-HRMS for $C_{17}H_{30}O_3NaSi$ $[M+Na]^+$ calcd 333.1826; found 333.1858. To this material (1.25 g, 1 eq.) in dry DCM (2 mL) and dry pyridine (0.35 mL) under argon is added CBr_4 (2 g, 1.5 eq.) followed by the dropwise addition of triphenylphosphine (1.07 g, 1.02 eq.) in dry DCM (2 mL). After 2.5 h, ice-cold hexanes (25 mL) are added and the precipitate filtered off. Flash chromatography (hexanes and then hexane with 5% v/v EtOAc) of the concentrated filtrate gives title compound **36** (788 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.21 (m, 5H), 4.45 (s, 2H), 3.67-3.59 (m, 2H), 3.55-3.41 (m, 2H), 2.16-2.09 (m, 1H), 0.83 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (125 MHz,$CDCl_3$) δ 138.3, 128.4, 127.6, 73.4, 69.1, 61.7, 43.8, 33.0, 25.9, 18.2, 0. ESI-HRMS for $C_{17}H_{29}O_2NaSi79Br$ $[M+Na]^+$ calcd 395.1018; found 395.1020.

Diethyl {2-[(benzyloxy)methyl]-3-[(tert-butyldimethylsilyl)oxy] propyl}phosphonate (37). Compound **36** (2.46 g, 1 eq.) and triethylphosphite (3.5 mL, 3 eq.) are heated at 175 °C for 18 h. Concentration of the product under high vacuum at 100 °C and flash chromatography (hexanes-EtOAc 20%-100% v/v) of the residue gives title compound **37** (2.07g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.22 (m, 5H), 4.45 (s, 2H), 4.04-4.03 (4H), 3.66-3.62 (m,2H), 3.52-3.50 (m,2H), 2.20-2.12 (m,1H), 1.87-1.72 (m, 2H), 1.28-1.24 (m, 6H), 0.84 (s, 9H), 0.0 (s, 6H). ^{13}C NMR (125 MHz,$CDCl_3$) δ 138.6, 128.3, 127.5, 73.0, 70.2, 63.0, 61.4, 36.6, 25.9, 24.3, 23.1, 18.3, 16.4, 0.0. ^{31}P NMR (202 MHZ, $CDCl_3$) δ.32.0. ESI-HRMS for $C_{21}H_{39}O_5NaSiP$ $[M+Na]^+$ calcd 453.2202; found 453.2205.

Diethyl [2-(azidomethyl)-3-(benzyloxy)propyl]phosphonate (40). Compound **37** (1.87 g) in MeOH (10 mL) and conc. HCl (10 mL) is stirred at room temperature for 1 h.

Evaporation at reduced pressure gives crude diethyl {2-[(benzyloxy)methyl]-3-hydroxypropyl}phosphonate **(38)** (1.45 g) which is dissolved in DCM (30 mL) and triethylamine (1.9 mL, 3 eq.) and cooled to 0 °C. Methanesulfonyl chloride (0.72 mL, 2 eq.) is added and the mixture stirred at room temperature for 1 h, diluted with DCM and the organic extract washed with satd. $NaHCO_3$, brine, dried and evaporated to give crude mesylate **39**. Displacement of the mesylate is effected by heating a solution of crude **39** (1.93 g) in DMF (20 mL) with NaN_3 (0.9 g) at 80 °C for 7 h. Addition of water and extraction with diethyl ether followed by flash chromatography (50%-100% v/v EtOAc in hexanes) gives title compound **40** (1.06 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.27 (m, 5H), 4.53 (s, 2H), 4.14-4.03 (m, 4H), 3.58-3.47 (m, 4H), 2.34-2.25 (m, 1H), 1.88-1.76 (m, 2H), 1.35-1.27 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.0, 129.6, 128.3, 73.2, 70.2, 61.6, 52.6, 34.4, 25.4, 24.3, 16.4. ESI-HRMS for $C_{15}H_{24}N_3O_4NaP$ $[M+Na]^+$ calcd 364.1402; found 364 1393.

Diethyl [2-(aminomethyl)-3-hydroxypropyl]phosphonate (41). Compound **40** (1.06 g) in MeOH (10 mL) with 20% $Pd(OH)_2$ /C (0.5 g) is hydrogenated at 1 atm for 18 h. Filtration and flash chromatography (9/1/0.1 and 8/2/0.2 v/v DCM/MeOH/conc. NH_3) gives title compound **41** (300 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.22-4.05 (m, 4H), 3.83-3.71 (m, 2H), 3.02-2.81 (m, 2H), 2.25-2.15 (m, 1H), 2.10-1.98 (m, 1H), 1.86-1.71 (m, 2H), 1.38-1.31 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 67.4, 61.7, 46.2, 37.1, 26.2, 25.1, 16.5. ^{31}P NMR (202 MHz, $CDCl_3$) δ.31.8. ESI-HRMS for $C_8H_{21}NO_4P$ $[M+H]^+$ calcd 226.1208; found 226.1206.

Diethyl [3-({[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}amino)-2-hydroxypropyl]phosphonate (42). The aminophosphonate **34** (95 mg,1.5 eq.) and aldehyde **8** (80 mg, 1 eq.) in ethanol (3 mL) are heated at 70 °C for 10 min and then picoline borane (64 mg, 2 eq.) is added and the mixture stirred for 3.5 h. The product is evaporated onto silica gel and subjected to flash chromatography (9/1/0.1 and 8/2/0.2 v/v/v DCM/MeOH/conc. NH_3) to give title compound **42** (20 mg). ^{1}H NMR (500 MHz,$CDCl_3$) δ 8.53 (s, 1H), 7.49-7.35 (m, 5H), 7.26 (s, 1H), 5.58 (s, 2H), 4.16-3.96 (m, 6H), 2.83-2.64 (m, 2H), 2.02-1.87 (m, 2H), 1.34-1.30(m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$)δ 155.4, 149.6, 149.0, 136.2, 128.5, 127.3, 115.4, 115.1, 67.9, 65.0, 61.8, 55.1,

43.0, 32.1, 31.0, 16.4. ^{31}P NMR (202 MHz, $CDCl_3$) δ.29.9. ESI-HRMS for $C_{21}H_{30}N_4O_5P$ $[M+H]^+$ calcd 449.1954; found 449.1951.

Diethyl {2-[({[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}amino)methyl]-3-hydroxypropyl}phosphonate (43). The aminophosphonate **41** (100 mg, 1.5 eq.) and aldehyde **8** (80 mg, 1 eq.) are heated together in ethanol (3 mL) at 70 °C for 10 min and then picoline borane (64 mg, 2 eq.) is added and heated at 70 °C for 2 h. Evaporation and flash chromatography (9/1/0.1v/v/v DCM/MeOH/conc. NH_3) gives title compound **43** (99 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.49-7.33 (m, 5H), 7.26 (s, 1H), 5.58 (s, 2H), 4.13-3.99 (m, 6H), 3.81-3.63 (m, 2H), 3.01-2.81 (m, 2H), 2.26-2.19 (m, 1H), 1.75-1.63 (m, 2H), 1.35-1.24 (m, 6H). ^{13}C NMR (125MHz, $CDCl_3$) δ 155.3, 149.8, 149.1, 136.2, 128.5, 127.3, 115.4, 114.3, 67.8, 61.7, 53.7, 43.3, 34.6, 26.6, 25.5, 16.4. ^{31}P NMR (202 MHz, $CDCl_3$) δ 31.3. ESI-HRMS for $C_{22}H_{32}N_4O_5P$ $[M+H]^+$ calcd 463.2110; found 463.2103.

{2-Hydroxy-3-[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]propyl} phosphonic acid (44). Compound **42** (20 mg) is heated with conc. HCl (0.5 mL) at 70 °C for 30 min and then evaporated under reduced pressure twice from water. The product is heated with 48% HBr (1 mL) at 90 °C for 5 h and then evaporated under reduced pressure twice from water. Reverse phase chromatography on C_{18} silica gel eluting with water gives title compound **44** (7 mg). ^{1}H NMR (500 MHz, D_2O) δ 8.89 (s, 1H), 7.44 (s, 1H), 4.38 (s, 2H), 4.24-4.10 (m, 2H), 3.27-2.99 (m, 2H), 2.04-1.97 (m, 2H). ^{13}C NMR (125 MHz, D_2O) δ 152.7, 144.9, 132.9, 132.4, 118.2, 103.3, 62.3, 52.0, 40.5, 33.2, 32.1. ^{31}P NMR (202 MHz, D_2O) δ 25.0. ESI-HRMS for $C_{10}H_{16}N_4O_5P$ $[M+H]^+$ calcd 303.0858; found 303.0862.

(3-Hydroxy-2-{[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]methyl}propyl)phosphonic acid (45). Compound **43** (45 mg) is heated at 70 °C in conc. HCl (0.5 mL) for 1 h and evaporated twice from water. The crude product is the heated with HBr (48%, 1 mL) at 90 °C for 3 h and then evaporated twice from water. Chromatography on reverse phase C_{18} silica gel eluting with water gives title compound **45** (23 mg).^{1}H NMR (500 MHz, D_2O) δ 8.19 (s, 1H), 7.58 (s, 1H),

4.30-4.21 (m, 2H), 3.56-3.37 (m, 2H), 3.15-3.04 (m, 2H), 2.19-2.11 (m, 1H), 1.68-1.46 (m, 2H). ^{13}C NMR (125 MHz, D_2O) δ 152.6, 144.9, 132.8, 118.4, 103.1, 63.8, 49.8, 40.9, 33.0, 27.3, 26.2. ^{31}P NMR (202 MHz, D_2O) δ.27.5. ESI-HRMS for $C_{11}H_{18}N_4O_5P$ $[M+H]^+$ calcd 317.1015; found 317.1007.

4-(*tert*-Butoxy)-2-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidine (47). 2,4-Dichloro-5*H*-pyrrolo[3,2-*d*]pyrimidine **(46)**[46] (0.5 g, 2.66 mmol) and sublimed potassium *tert*-butoxide (1.492 g, 13.30 mmol) are stirred in THF (25 mL) at 40 ^{o}C for 16 h. The solvent is evaporated, H_2O (10 mL) added and the mixture extracted with EtOAc (50 mL), washed with brine, then dried and the solvent evaporated to yield a cream coloured solid. Flash chromatography (EtOAc-Hexanes, 3:7 v/v) gives title compound **47** (0.33 g, 55%) as a colourless solid. ^{1}H NMR (500 MHz, $CDCl_3$) 8.58 (bs, exchanged to D_2O, 1H), 7.37 (t, J = 3.1, after D_2O became d, J = 3.1, 1H), 6.56 (dd, J = 3.1, 2.2, after D_2O became d, J = 3.1, 1H), 1.70 (s, 9H). ^{13}C NMR (125.7 MHz, CD_3OD) 157.3 (C), 152.4 (C), 150.4 (C), 132.0 (CH), 116.3 (C), 102.0 (CH), 85.0 (C), 28.7 (CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ESI-HRMS for $C_{10}H_{13}{}^{(35)}ClN_3O$ $[M+H]^+$ calcd 226.0747; found 226.0752.

[4-(*tert*-Butoxy)-2-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methanol (48). Compound **47** (0.330 g, 1.462 mmol) is dissolved in a mixture of 1,4-dioxane (6 mL) and H_2O (3 mL) then potassium carbonate (0.525 g, 3.80 mmol) and formaldehyde solution (37% aq., 1.53 mL, 19.10 mmol) are added and the mixture heated at 80 ^{o}C for 19 h. The mixture is cooled, extracted with EtOAc (50 mL), washed with brine, dried then the solvent evaporated to a gum which is dissolved in 7M NH_3-MeOH (10 mL) and left at room temperature for 30 min After evaporation of the solvent the residue is flash chromatographed (EtOAc-hexanes, 7:3 then EtOAc) to give title compound **48** (0.214 g, 57%) as a colourless solid. ^{1}H NMR (500 MHz, CD_3OD) 7.51 (s, 1H), 4.75 (s, 2H), 1.72 (s, 9H). ^{13}C NMR (125.7 MHz, CD_3OD) 157.3 (C), 150.5(C x 2), 131.0 (CH), 116.8 (C), 116.6 (C), 85.0 (C), 55.2 (CH_2), 28.8 (CH_3). Referenced to the centre line of CD_3OD at 49.0 ppm. ESI-HRMS for $C_{11}H_{14}{}^{(35)}ClN_3NaO_2$ $[M+Na]^+$ calcd 278.0672; found 278.0671.

4-(*tert*-Butoxy)-2-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidine-7-carbaldehyde (49). Dess-Martin periodinane (0.390 g, 0.921 mmol) is added to a mixture of triethylamine (0.47 mL, 3.35 mmol) and alcohol **48** (0.214 g, 0.837 mmol) in THF (6 mL) and the mixture is stirred at room temperature for 40 min. EtOAc (50 mL), sat.aq. $NaHCO_3$ (3 mL) and water (3 mL) are added successively and the mixture filtered through Celite. The organic layer is separated and washed with brine then dried and the solvent evaporated to leave a solid which is dissolved in a 1:1 v/v mixture of MeOH/$CHCl_3$, silica gel added and the solvent evaporated. The residue is flash chromatographed (EtOAc-hexanes,4:6 then 1:1 v/v) to give title compound **49** as a colourless solid which is triturated with a little EtOAc and dried (0.179 g, 84%). A sample is recrystallized from EtOAc. ^{1}H NMR (500 MHz, DMSO d_6) 13.08 (bs, 1H), 10.06 (s, 1H), 8.41 (s, 1H), 1.70 (s, 9H). ^{13}C NMR (125.7 MHz, DMSO d_6) 183.8 (CH), 156.2 (C), 151.0 (C), 149.6 (C), 137.7 (CH), 116.8 (C), 115.9 (C), 84.5 (C), 28.2 (CH_3). Referenced to the centre line of DMSOd_6 at δ 39.7. ESI-HRMS for $C_{11}H_{12}{}^{(35)}ClN_3NaO_2$ $[M+Na]^+$ calcd 276.0516; found 276.0511.

Diethyl [(3*S*)-3-amino-4-hydroxybutyl]phosphonate hydrochloride (51). Compound **50**[33] (0.460 g, 1.27 mmol) and 10% Pd-C (50 mg) are stirred in EtOH (20 mL) under a hydrogen atmosphere at ambient pressure and temperature for 6 h. The mixture is filtered through Celite and the solvent evaporated. The residue is dissolved in a mixture of EtOH (3 mL) and 37% aq. HCl (2 mL) and left at room temperature for 1.5 h then the solvent is evaporated to give title compound **51** as a colourless gum (330 mg, 100%). $[\alpha]_D^{22}$ +5.3 (c 0.57 MeOH). ^{1}H NMR (500 MHz, D_2O) 4.28-4.17 (m, 4H), 3.91 (dd, J = 12.5, 3.7, 1H), 3.74 (dd, J = 12.5, 6.4, 1H), 3.48 (m, 1H), 2.14-1.93 (m, 4H), 1.40 (t, J = 7.1, 6H). Referenced to HOD at 4.79 ppm. ^{13}C NMR (125.7 MHz, D_2O), 64.3 (d, J = 6.6, CH_2), 60.7 (CH_2), 53.5 (d, J = 18.2, CH), 22.3 (d, J = 4.1, CH_2), 21.0 (d, J = 141.2, CH_2), 16.2 (d, J = 5.7, CH_3). Referenced to internal CH_3CN at δ 1.47. ^{31}P NMR (202.4 MHz, D_2O), 33.3 (s). ESI-HRMS for $C_8H_{21}NO_4P$ $[M-HCl+H]^+$ calcd 226.1208; found 226.1213.

Diethyl [(3*R*)-3-amino-4-hydroxybutyl]phosphonate hydrochloride (55). Compound **54**[33] (0.35 g, 0.96 mmol) is treated as described above for the preparation of **51** to give title compound **55** (0.25 g, 99%). $[\alpha]_D^{22}$ -5.1 (c 0.63, MeOH). ESI-HRMS for $C_8H_{21}NO_4P$

$[M\text{-}HCl+H]^+$ calcd 226.1208; found 226.1205. The ^1H, ^{13}C and ^{31}P NMR spectra are identical to those of the enantiomer **51**.

Diethyl [(3*S*)-3-[({2-chloro-4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-4-hydroxybutyl]phosphonate (52). A mixture of **51** (0.055 g, 0.591 mmol), triethylamine (0.030 mL, 0.211 mmol), aldehyde **49** (0.1 g, 0.394 mmol) and 2-picoline borane complex (0.055 g, 0.512 mmol) are stirred together in MeOH (4 mL) at room temperature for 16 h. The solvent is evaporated and the residue chromatographed on silica gel ($CHCl_3$-MeOH-7M NH_3 in MeOH, 9.6:0.2:0.2 v/v/v) to give intermediate diethyl [(3*S*)-3-({[4-(*tert*-butoxy)-2-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-4-hydroxybutyl]phosphonate as a colourless gum (108 mg) which is dissolved in 1:1 mixture of 37% aq. HCl-THF (3 mL) and left at room temperature for 10 min. The solvent is evaporated and the residue dissolved in EtOH and neutralized with Amberlyst A21 resin. After filtering off the resin, the filtrate is evaporated and the residue is chromatographed on silica gel ($CHCl_3$-MeOH-7M NH_3 in MeOH, 7.0:2.75:0.25 v/v/v) to give title compound **52** (0.070 g, 44%) as a colourless amorphous solid. $[\alpha]_D^{22}$ +10.3 (c 0.555, MeOH) ^1H NMR (500 MHz, CD_3OD) 7.37 (s, 1H), 4.28 (d. *J* = 13.7, 1H), 4.23 (d, *J* = 13.7, 1H), 4.13-4.05 (m, 4H), 3.93 (dd, *J* = 12.3, 3.6, 1H), 3.72 (dd, *J* = 12.3, 4.6, 1H), 3.22 (m, 1H), 2.04-1.90 (m, 4H), 1.31 (dt, *J* = 7.0, 0.5, 6H). ^{13}C NMR (125.7 MHz, CD_3OD) 162.5 (C), 149.7 (C), 146.4 (C), 129.3 (CH), 119.4 (C), 107.7 (C), 63.5 (d, *J* = 6.4, CH_2), 60.1 (CH_2), 59.7 (d, *J* = 17.5, CH), 40.4 (CH_2), 22.8 (d, *J* = 3.7, CH_2), 22.2 (d, *J* = 142.3, CH_2), 16.7 (d, *J* = 5.8, CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (202.3 MHz, CD_3OD), 31.7 (s). ESI-HRMS for $C_{15}H_{25}{}^{(35)}ClN_4O_5P$ $[M+H]^+$ calcd 407.1251; found 407.1250.

Diethyl [(3*R*)-3-[({2-chloro-4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-4-hydroxybutyl]phosphonate (56). In the same way as that described for the synthesis of enantiomer (**52** (Scheme 18), compound **55** (0.083 g, 0.317 mmol) is treated with triethylamine (0.030 mL, 0.211 mmol), aldehyde **49** (0.054 g, 0.21 mmol) and 2-picoline borane complex (0.029 g, 0.275 mmol) to give after treatment with a 1:1 mixture of 37% aq. HCl-MeOH, title compound **56** (0.039 g, 45%). $[\alpha]_D^{22}$ -10.6 (c

0.455, MeOH). ESI-HRMS for $C_{15}H_{25}{}^{(35)}ClN_4O_5P$ $[M+H]^+$ calcd 407.1251; found 407.1250. The ^1H, ^{13}C and ^{31}P NMR spectra are identical to those of the enantiomer **52**.

Diethyl [(1*R*/*S*, 3*S*)-3-amino-1-fluoro-4-hydroxybutyl]phosphonate hydrochloride (59). The α-fluoro-vinylphosphonate **58**[33] (0.222 g, 0.582 mmol) and 10% Pd/C (50 mg) are stirred in EtOH (10 mL) under a hydrogen atmosphere at ambient temperature and pressure for 5.5 h. The mixture is filtered through Celite and the solvent evaporated to a colourless gum (225 mg) which is dissolved in a mixture of EtOH (3 mL) and 37% aq. HCl (1 mL) and left at room temperature for 1.5 h. The solvent is evaporated to give title compound **59** as an ~1:1 mixture of diastereomers as a dark yellow gum. ^1H NMR (500 MHz, D_2O), 5.38 (m, 0.5H), 5.28 (m, 0.5H), 4.38-4.30 (m, 4H), 3.96 (t, J = 3.4, 0.5H), 3.94 (t, J = 3.4, 0.5H), 3.81-3.70 (m, 2H), 2.46-2.24 (m, 2H), 1.43 (t, J = 7.1, 6H). Referenced to HOD at δ 4.79. ^{13}C NMR (125.7 MHz, D_2O) 86.9 (dd, J = 175.6, 173.7, CHF), 85.9 (dd, J = 177.2, 173.3, CHF), 66.0 (d, J = 7.0, CH_2), 65.8 (d, J = 6.7, CH_2), 61.6 (CH_2), 61.0 (CH_2), 51.3 (d, J = 16.0, CH), 50.6 (d, J = 14.7 CH), 29.9 (d, J = 19.8, CH_2), 29.8 (d, J = 19.2, CH_2), 16.3 (d, J = 4.9, CH_3). Referenced to internal CH_3CN at δ 1.47. ^{31}P NMR (202. MHz, D_2O), 18.0 (d, J = 76.0), 17.8 (d, J = 76.0). ^{19}F NMR (470.5 MHz, D_2O) -209.7 (d, J = 76.0). ESI-HRMS for $C_8H_{20}FNO_4P$ $[M-HCl+H]^+$ calcd 244.1114; found 244.1111. The NMR spectra indicated **59** is a ~1:1 mixture of diastereomers.

Diethyl [(1*R*/*S*, 3*S*)-3-({[4-(benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-1-fluoro-4-hydroxybutyl]phosphonate (60). Compound **59** (0.157 g, 0.561 mmol), aldehyde **8** (0.113 g, 0.401 mmol), Et_3N (0.034 mL, 0.241 mmol) and 2-picoline borane complex (0.056 g, 0.521 mmol) are stirred together in MeOH (15 mL) at 50 °C for 24 h. The solvent is evaporated and the residue flash chromatographed ($CHCl_3$-7M NH_3 in MeOH, 99:1 then 99:2 v/v) to give title compound **60** as an ~1:1 mixture of diastereomers as a colourless gum. ^1H NMR (500 MHz, CD_3OD) 8.42 (s, 1H), 7.52-7.49 (m, 3H), 7.39-7.29 (m, 3H), 5.61 (s, 2H), 5.13 (ddt, J = 46.4, 4.8, 2.4, 0.5H), 5.07 (ddt, J = 46.7, 6.9, 3.4, 0.5H), 4.23-4.12 (m, 4H), 4.08 (d, J = 13.5, 0.5H), 4.01 (s, 1H), 3.98 (d, J = 13.5, 0.5H), 3.72 (dt, J = 11.5, 4.7, 1H), 3.56 (dt, J = 10.7, 5.5, 1H), 2.97 (pent, J = 5.6, 0.5H), 2.91 (sext, J = 4.6, 0.5H), 2.14-1.81 (m, 2H), 1.36-1.30 (m, 6H). ^{13}C NMR

(125.7 MHz, CD_3OD) 157.0 (C), 150.1 (CH), 150.0 (CH), 149.6 (C), 137.9 (C), 130.3 (CH), 129.5 (CH), 129.4 (CH), 129.2 (CH), 116.7 (C), 115.2 (C), 114.8 (C), 87.8 (dd, *J* = 177.5, 171.9 CHF), 87.5 (dd, *J* = 177.5, 171.9 CHF), 69.0 (CH_2), 64.8 (t, *J* = 8.4, CH_2), 64.5 (t, *J* = 7.5, CH_2), 64.1 (CH_2), 63.7 (CH_2), 56.4 (d, *J* = 13.6, CH), 55.7 (d, *J* = 13.9, CH), 41.3 (CH_2), 41.2 (CH_2), 33.8 (d, *J* = 20.1, CH_2), 32.7 (d, *J* = 19.7, CH_2), 16.7 (CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (202.3 MHz, CD_3OD) 19.0 (d, *J* = 77.6), 18.5 (d, *J* = 77.6). ^{19}F NMR (470.5 MHz, CD_3OD) -209.9 (d, *J* = 77.7), -210.7 (d, *J* = 77.4). ESI-HRMS for $C_{22}H_{31}FN_4O_5P$ $[M+H]^+$ calcd 481.2016; found 481.2014. The NMR spectra indicated **60** is a ~1:1 mixture of diastereomers.

Diethyl [(3*S*)-3-amino-1,1-difluoro-4-hydroxybutyl]phosphonate hydrochloride (63). Compound **62**[34-35] (0.345 g, 0.860 mmol) is dissolved in a mixture of EtOH (3 mL) and 37% aq. HCl (1 mL) and left to stand at room temperature for 1.5 h. The solvent is evaporated to give title compound **63** as a yellow gum (0.256 g, 100%). ^1H NMR (500 MHz, $CDCl_3$) 8.24 (bs, 3H), 4.60 (bs, 1H), 4.35-4.28 (m, 4H), 4.02 (d, *J* = 9.1, 1H), 3.92-3.81 (m, 2H), 2.91-2.74 (m, 1H), 2.63-2.47 (m, 1H), 1.39 (dt, *J* = 7.0, 1.0, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$) 119.3 (dt, *J* = 261.3, 216.5, CF_2), 65.5 (d, *J* = 4.5, CH_2), 61.5 (CH_2), 48.2 (CH), 33.5 (m, CH_2), 16.3 (d, *J* = 5.0, CH_3). Referenced to the centre line of $CDCl_3$ at δ 77.0. ^{31}P NMR (202.3 MHz, $CDCl_3$), 5.4 (t, *J* = 104.9). ^{19}F NMR (470.5 MHz, $CDCl_3$) -107.7 (dd, *J* = 301.1, 102.5), -111.2 (dd, *J* = 301.3, 105.9). ESI-HRMS for $C_8H_{19}F_2NO_4P$ $[M\text{-}HCl+H]^+$ calcd 262.1020; found 262.1017.

Diethyl [(3*S*)-3-({[4-(benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-1,1-difluoro-4-hydroxybutyl]phosphonate (64). Compound **63** (0.140 g, 0.470 mmol), aldehyde **8** (0.095 g, 0.336 mmol), Et_3N (0.042 mL, 0.302 mmol) and 2-picoline borane complex (0.047 g, 0.437 mmol) are stirred in MeOH (15 mL) at 50 °C for 24 h. The solvent is evaporated and the residue flash chromatographed ($CHCl_3$-7M NH_3 in MeOH, 99:1 then 98:2 v/v) to give title compound **64** as a colourless gum (0.088 g, 53%). $[\alpha]_D^{22}$ +7.5 (c 0.475, MeOH). ^1H NMR (500 MHz, CD_3OD) 8.42 (s, 1H), 7.53 (s, 1H), 7.51 (d, *J* = 7.3, 2H), 7.38-7.30 (m, 3H), 5.60 (s, 2H), 4.29-4.22 (m, 4H), 4.04 (d, *J* = 13.6, 1H), 3.99 (d, *J* = 13.6, 1H), 3.73 (dd, *J* = 11.3, 4.3 1H), 3.53 (dd, *J* = 11.3, 6.2, 1H), 3.18 (m, 1H), 2.34-2.24 (m, 2H), 1.34 (t, *J* = 7.0, 6H). ^{13}C NMR (125.7 MHz, CD_3OD) 157.0 (C),

150.1 (CH), 149.5 (C), 137.8 (C), 130.4 (CH), 129.5 (CH), 129.4 (CH), 129.2 (CH), 122.0 (dt, J = 259.1, 218.5, CF_2), 116.7 (C), 114.5 (C), 69.0 (CH_2), 66.2 (d, J = 6.7, CH_2), 64.2 (CH_2), 53.6 (CH), 41.1 (CH_2), 36.3 (dt, J = 19.8, 14.3, CH_2), 16.7 (CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (202.4 MHz, CD_3OD) 6.7 (t, J = 109.9). ^{19}F NMR (470.5 MHz, CD_3OD) -110.3 (dd, J = 299.3, 109.9, -111.6 (dd, J = 299.3, 109.9). ESI-HRMS $C_{22}H_{30}F_2N_4O_5P$ $[M+H]^+$ calcd 499.1922; found 499.1927.

[(3*S*)-4-Hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonic acid hydrobromide (53).

Compound **52** (0.060 g, 0.147 mmol) is dissolved in EtOH (5 mL), 10% Pd-C (40 mg) added and the mixture stirred under a hydrogen atmosphere at ambient pressure and temperature for 3 h. The mixture is filtered through Celite and the solvent evaporated to give diethyl [(3*S*)-4-hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonate hydrochloride (0.055 g, 91%) as a colourless solid. $[\alpha]_D^{22}$ +12.2 (c 0.54, MeOH). ^{1}H NMR (500 MHz, CD_3OD) 7.97 (s, 1H), 7.67 (bs, 1H), 4.47 (d, J = 13.9, 1H), 4.43 (d, J = 13.9, 1H), 4.15-4.06 (m, 4H), 4.00 (dd, J = 12.4, 2.9, 1H), 3.76 (dd, J = 12.4, 4.1, 1H), 3.35 (m, partially hidden by residual CD_3OD, 1H), 2.11-1.88 (m, 4H), 1.32 (t, J = 7.1, 6H). ^{13}C NMR (125.7 MHz, CD_3OD) 155.7 (C), 145.2 (C), 143.8 (CH), 130.8 (CH), 119.6 (C), 108.0 (C), 63.6 (d, J = 4.3, CH_2), 59.6 (d, J = 17.7, CH), 59.0 (CH_2), 39.7 (CH_2), 22.3 (d, J = 3.7, CH_2), 22.2 (d, J = 142.7, CH_2), 16.7 (d, J = 5.8, CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (202.3 MHz, CD_3OD) 31.2 (s). ESI-HRMS for $C_{15}H_{26}N_4O_5P$ $[M-HCl+H]^+$ calcd 373.1641; found 373.1642. This material (0.052 g, 0.127 mmol) is heated at 80 ^{o}C in hydrobromic acid (48% aq., 1.002 mL, 12.72 mmol) for 4 h. The solvent is evaporated and the residue eluted from a column of RP 18 silica gel (H_2O) to afford title compound **53** as a colourless glass which slowly crystallized. $[\alpha]_D^{23}$ +9.3 (c 0.355, H_2O). ^{1}H NMR (500 MHz, D_2O + DCl): δ 8.93 (s, 1H), 7.84 (s, 1H), 4.51 (d, J = 14.7 Hz, 1H), 4.48 (d, J = 14.7 Hz, 1H), 3.97 (dd, J = 13.1, 2.7 Hz, 1H), 3.81 (dd, J = 13.1, 4.7 Hz, 1H), 3.46 (m, 1H), 2.08-1.77 (m, 4H). Referenced to HOD at 4.79 ppm. ^{13}C NMR (125.7 MHz, D_2O +DCl) 153.4 (C), 145.2 (CH), 133.8 (C) 133.7 (CH), 118.8 (C), 104.0 (C), 59.8 (d, J = 17.1 Hz, CH), 58.5 (CH_2), 38.9 (CH_2), 23.3 (d, J = 137.2 Hz, CH_2), 21.5 (d, J = 3.2 Hz,

CH_2). Referenced to internal CH_3CN at 1.47 ppm. ^{31}P NMR (202.3 MHz, D_2O + DCl) 28.5 (s). ESI-HRMS for $C_{11}H_{16}N_4O_5P$ $[M\text{-}HBr\text{-}H]^-$ calcd 315.0858; found 315.0855.

[(3*R*)-4-Hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonic acid hydrobromide (57). In the same way as that described for the synthesis of enantiomer **53** (Scheme 18), compound **56** (0.065 g, 0.160 mmol) is stirred under a hydrogen atmosphere in the presence of 10% Pd/C (40 mg) to give [(3*R*)-4-hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonate hydrochloride (0.063 g, 96%). $[\alpha]_D^{25}$ -12.4 (c 0.37, MeOH). ESI-HRMS for $C_{15}H_{26}N_4O_5P$ $[M\text{-}HCl+H]^+$ calcd 373.1641; found 373.1647. This material (0.058 g, 0.142 mmol) is treated with 48% aq. hydrobromic acid (1.12 mL. 14.2 mmol) to give title compound **57** (0.04 g, 71%). $[\alpha]_D^{25}$ -9.9 (c 0.365, H_2O). ESI-HRMS for $C_{11}H_{16}N_4O_5P$ $[M\text{-}HBr\text{-}H]^-$ calcd 315.0858; found 315.0857. The ^1H, ^{13}C and ^{31}P NMR spectra are identical to those of the enantiomer **53**.

[(1*R*/*S*, 3*S*)-1-Fluoro-4-hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonic acid; triethylamine (61). Compound **60** (0.1 g, 0.208 mmol) and 10% Pd/C (30 mg) are stirred in EtOH (6 mL) under a hydrogen atmosphere at ambient temperature and pressure for 3 h. The mixture is filtered and the solvent evaporated. The residue is dissolved hydrogen bromide (48% aq. 1.639 mL, 20.81 mmol) and heated to 80 ^oC for 6 h. The solvent is evaporated several times from H_2O then the residue dissolved in hot water and chromatographed on RP 18 silica gel (H_2O) to give the sparingly soluble product hydrobromide salt. It is dissolved in 5% aq. Et_3N and the solvent evaporated then the residue flash chromatographed (1,4-dioxane-H_2O-Et_3N, 80:20:3 then 70:30:3 v/v/v) to give title compound **61** as an ~1:1 mixture of diastereomers as a colourless amorphous solid. ^1H NMR (500 MHz, D_2O) 7.97 (s, 1H), 7.69 (s, 1H), 4.79 (HOD + 0.5H), 4.69 (bs, 0.5H), 4.46 (dd, J = 13.8, 1.9, 1H), 4.36 (dd, J = 13.8, 2.7, 1H), 4.08 (dd, J = 12.8, 3.6, 1H), 3.91 (dt, J = 12.8, 5.0, 1H), 3.66-3.58 (m, 1H), 3.22 (q, J = 7.4, 6H), 2.44-2.24 (m, 2H), 1.30 (t, J = 7.4, 9H). Referenced to HOD at 4.79 ppm. Approximately 1 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, D_2O) 155.5 (C), 143.8 (C), 143.3 (CH), 131.3 (CH), 131.2 (CH), 118.0 (C), 107.0 (C), 106.9 (C), 89.7

(bm, CHF), 60.4 (CH_2), 59.5 (CH_2), 57.6 (CH), 56.1 (CH), 47.3 (CH_2), 38.7 (CH_2), 38.5 (CH_2), 31.1 (d, J = 20.9, CH_2), 30.6 (d, J = 19.7, CH_2), 8.9 (CH_3). Referenced to internal CH_3CN at δ 1.47. ^{31}P NMR (202.3 MHz, CD_3OD + drop Et_3N) 11.0 (d, J = 61.6), 10.9 (d, J = 63.1). ^{19}F NMR (470.5 MHz, D_2O) -197.9 (d, J = 63.4), -203.9 (d, J = 62.1). ESI-HRMS for $C_{11}H_{15}FN_4O_5P$ $[M\text{-}Et_3N\text{-}H]^-$calcd. 333.0764; found 333.0754. The NMR spectra indicated compound **61** is an ~1:1 mixture of diastereomers.

[(3*S*)-1,1-Difluoro-4-hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonic acid; triethylamine (65). Compound **64** (0.088 g, 0.177 mmol) and 10% Pd/C (30 mg) are stirred in EtOH (5 mL) under a hydrogen atmosphere at ambient temperature and pressure for 3 h. The mixture is filtered through Celite and the solvent evaporated. The residue is heated at 80 ^oC in hydrobromic acid (48% aq., 1.39 mL, 17.65 mmol) for 10 h. The solvent is evaporated and the residue dissolved in 5% aq. Et_3N, evaporated, dissolved in H_2O and loaded on to a column of Dowex 50WX8 (H^+) resin (4 cm x 1 cm) and eluted with water (50 mL, discarded) then 5% aq. Et_3N (250 mL). After leaving the column standing in 5% aq. Et_3N overnight it is eluted with more 5% aq. Et_3N (100 mL). The combined basic fractions are evaporated and the residue flash chromatographed (1,4-dioxane-H_2O-Et_3N, 85:15:3 then 80:20:3 v/v/v)to give title compound **65** as a cream coloured solid (0.058 g, 73%). $[\alpha]_D^{22}$ +6.7 (c 0.55, 3% aq. Et_3N). ^{1}H NMR (500 MHz, D_2O) 7.94 (s, 1H), 7.68 (s, 1H), 4.46 (d, J = 13.8, 1H), 4.32 (d, J = 13.8, 1H), 4.10 (dd, J = 12.8, 3.8, 1H), 3.91 (dd, J = 12.8, 5.0, 1H), 3.72 (m, 1H), 3.22 (q, J = 7.3, 6.5H), 2.62 (m, 1H), 2.43 (m, 1H), 1.3 (t, J = 7.3, 9.75H). Referenced to HOD at δ 4.79. Approximately 1.1 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, D_2O) 155.4 (C), 143.6 (C), 143.2 (CH), 131.3 (CH), 123.0 (dt, J = 260.5, 179.1, CF_2), 117.9 (C), 106.9 (C), 60.4 (CH_2), 54.6 (CH), 47.3 (CH_2), 38.4 (CH_2), 34.9 (dt, J = 23.1, 13.8, CH_2), 8.9 (CH_3). Referenced to internal CH_3CN at δ 1.47. ^{31}P NMR (202.4 MHz, D_2O) 4.6 (t, J = 83.1). ^{19}F NMR (470.5 MHz, D_2O) -104.1 (dd, J = 286.6, 83.5, -110.6 (dd, J = 286.4, 82.8). ESI-HRMS for $C_{11}H_{14}F_2N_4O_5P$ $[M\text{-}Et_3N\text{-}H]^-$ calcd 351.0670; found 351.0671.

di-*tert*-Butyl [(3*R*)-4-(benzyloxy)-3-hydroxybutyl]phosphonate (68). In a modification of a known method,[47] sodium hydride (60% in oil, 2.039 g, 51.0 mmol) is added in one

portion to a solution of di-*tert*-butyl phosphonate (6.6 g, 34.0 mmol) in CH_3CN (30 mL) at room temperature and the mixture stirred 30 min. Iodomethane (4.25 mL, 68.0 mmol) is added in 0.5 mL portions controlling the resulting exotherm with an ice-bath so that the temperature did not go above 50 ^oC. After the temperature has dropped back to room temperature, H_2O (1 mL) is added and the solvent evaporated. The residue is suspended in DCM and filtered through Celite then the solvent evaporated. The residue is flash chromatographed (EtOAc-hexanes, 4:6 then 6:4 v/v) to give di-*tert*-butyl methylphosphonate **(66)** (5.4 g, 76%) as a colourless oil. The ^1H and ^{31}P NMR are in agreement with that reported in the literature[47] except for the sign in the ^{31}P NMR which is +ve and opposite to that reported. ^{13}C NMR (500 MHz, $CDCl_3$) δ 81.4 (d, J = 89.6 Hz, C), 30.4 (d, J = 3.7 Hz, CH_3), 17.0 (d, J = 148.5 Hz, CH_3). Referenced to the centre line of $CDCl_3$ at 77.0 ppm. ESI-HRMS for $C_9H_{21}NaO_3P$ $[M+Na]^+$ calcd 231.1126; found 231.1132. n-Butyllithium (1.5M, 17.05 mL, 25.6 mmol) is added dropwise to a solution of di- *tert* butyl methylphosphonate **(66)** (5.33 g, 25.6 mmol) in THF (25 mL) keeping the temperature below -70 ^oC throughout the addition. After 15 min, boron trifluoride diethyl etherate (3.35 mL, 26.4 mmol) is added dropwise to the dark coloured mixture and after 10 min (2*R*)-2-[(benzyloxy)methyl]oxirane (**67**)[48] (1.4 g, 8.53 mmol) in THF (0.5 mL) added dropwise. After 30 min, sat. aq. $NaHCO_3$ (5 mL) is added then triethylamine (23.97 mL, 171 mmol) and the mixture warmed to room temperature. The solvent is evaporated and the residue dissolved in Et_2O (150 mL) and washed with sat. aq. $NaHCO_3$, dried and the solvent evaporated. The residue is flash chromatographed ($CHCl_3$-MeOH, 99:1 v/v) to give **68** as a yellow oil (2.53 g, 80%). $[\alpha]_D^{20}$ + 6.3 (c, 0.82, $CHCl_3$). ^1H NMR (500 MHz, CDCl3) δ 7.36-7.27 (m, 5H), 4.55 (s, 2H), 3.85 (m, 1H), 3.48 (dd, J = 9.5 Hz, 4.1, 1H), 3.40 (dd, J = 9.5, 7.0 Hz, 1H), 3.07 (d, J = 3.0, exchanged to D_2O, 1H), 1.86-1.67 (m, 4H), 1.49 (s, 18H). ^{13}C NMR (125.7 MHz, $CDCl_3$) 138.0 (C), 128.4 (CH), 127.7 (CH), 81.6 (d, J = 8.5 Hz, C), 74.1 (CH_2), 73.3 (CH_2), 70.4 (d, J = 13.7 Hz, CH), 30.4 (CH3), 27.3 (d, J = 5.1 Hz, CH_2), 26.5 (d, J = 146.5 Hz, CH_2). Referenced to the centre line of $CDCl_3$ at δ 77.0. ^{31}P NMR (202.4 MHz, $CDCl_3$) 24.4 (s). ESI-HRMS for $C_{19}H_{33}NaO_5P$ $[M+Na]^+$ calcd 395.1963, found 395.1969.

di-*tert*-Butyl [(3*S*)-3-amino-4-(benzyloxy)butyl]phosphonate (69). A mixture of diisopropyl azodicarboxylate (DIAD, 1.78 mL, 9.13 mmol) and diphenylphosphoryl azide (DPPA ,1.97 mL, 9.13 mmol) in dry toluene (10 mL) is added dropwise to a solution of **68** (2 g, 5.37 mmol) and triphenyl phosphine (2.39 g, 9.13 mmol) in toluene (20 mL) at 0 ^{o}C. After 10 mins, the mixture is allowed to warm to room temperature and then stirred overnight, filtered through Celite and the solvent evaporated. The residue is flash chromatographed (EtOAc-hexanes, 4:6 v/v) to give the crude azide as a yellow oil (1.6 g) which is dissolved in dry Et_2O (30 mL), cooled in an ice-bath and $LiAlH_4$ (1M in THF, 8.06 mL, 8.06 mmol) added dropwise. The mixture is warmed to room temperature and stirred for 30 min then cooled in an ice-bath and H_2O (0.3 mL), 15% aq. NaOH (0.3 mL) then H_2O (0.9 mL) added successively. After diluting with EtOAc, the mixture is filtered through Celite and the solvent evaporated. The residue is flash chromatographed ($CHCl_3$-7M NH_3 in MeOH, 98:2 then 96:4 v/v) to give **69** as a colourless oil (0.688 g, 35%). $[\alpha]_D^{20}$ + 1.2 (c, 1.09, $CHCl_3$). ^{1}H NMR δ (500 MHz, $CDCl_3$) 7.36-7.26 (m, 5H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J* = 12.1 Hz, 1H), 3.45 (dd, *J* = 9.1, 4.0 Hz, 1H), 3.27 (dd, *J* = 9.1, 7.3 Hz, 1H), 3.01 (m, 1H), 1.8-1.49 (m, 6H, after D_2O exchange, became 4H), 1.49 (d, *J* = 1.5 Hz, 18H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 138.2 (C), 128.3 (CH), 127.6 (CH), 81.4 (d, *J* = 8.6, C), 75.3 (CH_2), 73.2 (CH_2), 51.4 (d, *J* = 16.2 Hz, CH), 30.4 (d, *J* = 3.5 Hz, CH_3), 28.1 (d, *J* = 5.5 Hz, CH_2), 26.9 (d, *J* = 146.5 Hz, CH_2). Referenced to the centre line of $CDCl_3$ at δ 77.0. ^{31}P NMR (202.4 MHz, $CDCl_3$) 23.7 (s). ESI-HRMS for $C_{19}H_{35}NO_4P$ $[M+H]^+$ calcd 372.2304; found 372.2311. The % d.e. of compound **69** is determined in two ways, as follows. The (*S*, *R*) Mosher amide[49] is prepared by dissolution of di-*tert*-butyl [(3*S*)-3-amino-4-(benzyloxy)butyl]phosphonate (**69**) (6.3 mg, 0.017 mmol) in a mixture of $CDCl_3$ and Et_3N (3 eq.) and a solution of (*S*)-MTPACl (1.2 eq.) in $CDCl_3$ (prepared from (*R*)-MTPA, 99% e.e.) is added to give a total volume of 0.6 mL. The mixture is spiked with $CFCl_3$ and left for 30 min. ^{19}F NMR (470.5 MHz, $CDCl_3$, ref. $CFCl_3$ δ 0): δ -69.3 (s, 2%), -69.4 (s, 98%). The % d.e. is 96%. The (*S*, *S*) Mosher amide is prepared by dissolution of di-*tert*-butyl [(3*S*)-3-amino-4-(benzyloxy)butyl]phosphonate (**69**) (5.0 mg, 0.019 mmol) in a mixture of $CDCl_3$ and Et_3N (3.6 eq.) and a solution of (*R*)-MTPACl (1.2 eq.) in $CDCl_3$ (prepared from (*S*)-MTPA, 99% e.e.) added to give a total volume of 0.6 mL. The mixture is spiked with $CFCl_3$ and left for 30 min. ^{19}F NMR

(470.5 MHz, $CDCl_3$, ref. $CFCl_3$ δ 0): δ -69.3 (s, 97%), -69.4 (s, 3%). The % d.e. is 94%. The average % d.e. from these two assessments is 95%.

[(3*S*)-4-Hydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butyl]phosphonic acid hydrobromide (53). Compound **69** (0.15 g, 0.404 mmol), 2-picoline borane complex (0.043 g, 0.404 mmol), 4-benzyloxy-5*H*-pyrrolo[3,2-*d*]pyrimidine-7-carbaldehyde (**8**) (0.087 g, 0.311 mmol) and Et_3N (0.043 mL, 0.311 mmol) are stirred in MeOH (15 mL) at 40 °C overnight to give a clear colourless solution. The solvent is evaporated and the residue is chromatographed (0 → 10% continuous gradient of 0.5% v/v Et_3N/MeOH in EtOAc) to give di-*tert*-butyl [(3*S*)-4-(benzyloxy)-3-({[4-(benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)butyl]phosphonate as a colourless gum (0.135 g, 71%). $[\alpha]_D^{20}$ -1.0 (c, 0.6, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.13 (bs, exchanged D_2O, 1H), 8.53 (s, 1H), 7.48-7.46 (m, 2H), 7.40-7.23 (m, 9H), 5.57 (s, 2H), 4.51 (d, *J* = 11.9, 1H), 4.48 (d, *J* =11.9, 1H), 4.05 (s, 2H), 3.53 (dd, *J* = 9.5, 4.6, 1H), 3.46 (dd, *J* = 9.5, 6.0, 1H), 2.94-2.89 (m, 1H), 2.21 (bs, exchanged D_2O, 1H), 1.84-1.76 (m, 2H), 1.72-1.63 (m, 2H), 1.443, 1.442 (2s, 18H). ^{13}C NMR (125.7 MHz, $CDCl_3$) 155.3 (C), 149.6 (CH), 149.2 (C), 138.3 (C), 136.4 (C), 128.6 (CH), 128.4 (CH), 128.3 (CH), 127.6 (CH), 127.5 (CH), 127.1 (CH), 115.4 (C) 115.3 (C), 81.5 (d, *J* = 3.0, C), 81.4 (d, *J* = 2.6, C), 73.2 (CH_2), 71.7 (CH_2), 67.7 (CH_2), 56.8 (d, *J* = 16.5, CH), 40.9 (CH_2), 30.40 (CH_3), 30.37 (CH_3), 26.4 (d, *J* = 146.2, 2H), 25.2 (CH_2). Referenced to the centre line of $CDCl_3$ at δ 77.0. ^{31}P NMR (202.4 MHz, $CDCl_3$) δ 24.1 (s). ESI-HRMS for $C_{33}H_{46}N_4O_5P$ $[M+H]^+$ calcd 609.3206; found 609.3203. This material (0.120 g, 0.197 mmol) is heated at 80 °C in 48% aq. HBr (3 mL) for 16 h. After cooling, the solution is washed with $CHCl_3$ (2x) then the aqueous phase evaporated and the residue chromatographed on RP 18 silica gel (H_2O) to give **53** as a colourless glass. $[\alpha]_D^{20}$ +9.8 (c2.03, H_2O). ESI-HRMS for $C_{11}H_{18}N_4O_5P$ $[M+H]^+$ calcd 317.1015; found 317.1013. The ^{1}H, ^{13}C and ^{31}P NMR of compound **5**3 made by this route are the same as those reported above in an alternative synthesis.

[7-({[(2*S*)-4-(Diethoxyphosphoryl)-4,4-difluoro-1-hydroxybutan-2-yl]amino}methyl)-2-[(*E*)-[(dimethylamino)methylidene]amino]-4-oxo-3*H*,4*H*,5*H*-pyrrolo[3,2-

***d*]pyrimidin-3-yl]methyl 2,2-dimethylpropanoate (71).** The phosphonate **63** (0.135 g, 0.454 mmol), deazaguanine derivative **70**[36] (0.113 g, 0.324 mmol), Et_3N (0.041 mL, 0.292 mmol) and 2-picoline borane complex (0.045 g, 0.421 mmol) are stirred in MeOH (5 mL) at 50 ^oC for 1 h then at room temperature for 16 h. The solvent is evaporated and the residue flash chromatographed ($CHCl_3$-7M NH_3 in MeOH, 99:1 then 98:2 v/v) to give **71** as a yellow amorphous solid together with a mixture of **70** + **71**. The latter is chromatographed twice more to give a total amount of **71**, 0.109 g, 57%. $[\alpha]_D^{22}$ +5.4 (c 1.44, MeOH). ^1H NMR (500 MHz, CD_3OD) 8.58 (s, 1H), 7.25 (s, 1H), 6.31 (s, 2H), 4.31-4.24 (m, 4H), 3.90 (d, *J* = 13.2, 1H), 3.83 (d, *J* = 13.2, 1H), 3.73 (dd, *J* = 11.2, 4.6, 1H), 3.52 (dd, *J* = 11.2, 6.7, 1H), 3.22 (m, 1H), 3.18 (s, 3H), 3.06 (s, 3H), 2.37-2.19 (m, 2H), 1.37 (t, *J* = 7.0, 6H), 1.15 (s, 9H). ^{13}C NMR (125.7 MHz, CD_3OD) 179.0 (C), 158.7 (CH), 156.5 (C), 155.3 (C), 144.9 (C), 128.6 (CH), 121.1 (dt, *J* = 259.0, 218.3, CF_2), 115.3 (C), 114.7 (C), 67.0 (CH_2), 66.3 (d, *J* = 6.9, CH_2), 64.6 (CH_2), 53.8 (CH), 41.7 (CH_2), 41.1 (CH_3), 39.8 (C), 36.2 (dt, *J* = 20.0, 14.1, CH_2), 35.2 (CH_3), 27.4 (CH_3), 16.7 (d, *J* = 5.0, CH_3). Referenced to the centre line of CD_3OD at 49.0 ppm. ^{31}P NMR (202.4 MHz, CD_3OD) 6.6 (t, *J* = 109.6). ^{19}F NMR (470.5 MHz, CD_3OD) -110.3 (dd, *J* = 298.9, 109.4), -111.4 (dd, *J* = 298.9. 109.8). ESI-HRMS for $C_{24}H_{40}F_2N_6O_7P$ $[M+H]^+$ calcd 593.2664; found 593.2667.

[(3*S*)-3-[({2-Amino-4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-1,1-difluoro-4-hydroxybutyl]phosphonic acid; triethylamine (72). Compound **71** (0.1 g, 0.169 mmol) is heated at 80 ^oC in 48% aq. hydrobromic acid for 16 h then evaporated to the sparingly soluble hydrobromide salt form of **72**. It is dissolved in 5% aq. Et_3N, evaporated and the residue dissolved in H_2O and loaded on to a column of Dowex 50WX8 (H^+) resin (4 cm x 1 cm) and eluted with water (50 mL, discarded) then 5% aq. Et_3N (250 mL). After leaving the column standing in 5% aq. Et_3N overnight it is eluted with more 5% aq. Et_3N (100 mL). The combined basic fractions are evaporated and the residue flash chromatographed (1,4-dioxane-H_2O-Et_3N, 80:20:3 then 70:30:3 v/v/v) to give a yellow glassy solid which is dissolved in 3% aq. Et_3N and chromatographed on RP 18 silica gel (3% aq. Et_3N) to give **72** as a cream coloured amorphous solid (0.051 g, 65%). $[\alpha]_D^{21}$ +7.7 (c 0.62, 3% aq. Et_3N). ^1H NMR (500 MHz, D_2O) 7.58 (s, 1H), 4.43 (d,

J = 13.7, 1H), 4.32 (d, *J* = 13.7, 1H), 4.08 (dd, *J* = 12.8, 4.0, 1H), 3.92 (dd, *J* = 12.8, 6.0, 1H), 3.75 (m, 1H), 3.27 (q, *J* = 7.3, 7.3H), 2.65 (m, 1H), 2.44 (m, 1H), 1.35 (t, *J* = 7.3, 10.9H). Referenced to HOD at δ 4.79. Approximately 1.2 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, D_2O) 158.0 (C), 152.6 (C), 141.8 (C), 130.6 (CH), 122.8 (dt, *J* = 260.6, 180.2, CF_2), 112.9 (C), 103.1 (C), 60.4 (CH_2), 54.6 (CH), 47.2 (CH_2), 38.3 (CH_2), 34.4 (dt, *J* = 23.9, 14.5, CH_2), 8.8 (CH_3). ^{31}P NMR (202.4 MHz, D_2O) 4.8 (t, *J* = 83.7). ^{19}F NMR (470.5 MHz, D_2O) -103.8 (dd, *J* = 287.8, 84.2, -110.6 (dd, *J* = 287.6, 83.4). ESI-HRMS for $C_{11}H_{15}F_2N_5O_5P$ $[M\text{-}Et_3N\text{-}H]^-$ calcd 366.0779; found 366.0785.

2-({[4-(Benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}amino)ethan-1-ol (73). Ethanolamine (120 μL, 5 eq.) is added to acetyl chloride (142 μL, 5 eq.) in methanol (8 mL) and a few microdrops of acetyl chloride added to adjust the pH to 5. To this solution is added aldehyde **8** (100 mg, 1 eq.) and the mixture is stirred at 40 °C for 30 min. Picoline borane (64 mg, 1.5 eq.) is added and the mixture stirred at 40 °C for 4 h. The product is evaporated onto silica gel and subjected to flash chromatography (9/1/0.1 v/v/v DCM/MeOH/conc. NH_3) to give title compound **73** (47 mg). ^1H NMR (500 MHz, MeOD) δ 8.35 (s, 1H), 7.51 (s, 1H), 7.43-7.23 (m, 5H), 5.53 (s, 2H), 4.04 (s, 2H), 3.63 (m, 2H), 2.80 (m, 2H). ^{13}C NMR (125 MHz, MeOD) δ 157.2, 150.5, 149.6, 137.0, 131.4, 129.5, 116.8, 111.8, 69.2, 60.2, 51.0, 42.9. ESI-HRMS for $C_{16}H_{19}N_4O_2$ $[M+H]^+$ calcd 299.1508; found 299.1514.

tert-Butyl N-{[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}-N-(2-[bis(benzyloxy)phosphoryl]oxy} ethyl)carbamate (74). To a solution of **73** (47 mg, 1 eq.) in MeOH (5 mL) and Et_3N (22.5 μL) is added di-tert-butyl dicarbonate (35 mg, 1 eq.) and the mixture is stirred for 30 min. Evaporation and flash chromatography (EtOAc) gives tert-butyl N-{[4-(benzyloxy)-5H-pyrrolo[3,2-d]pyrimidin-7-yl]methyl}-N-(2-hydroxyethyl)carbamate (32 mg). To this carbamate and tetrazole (28 mg, 5 eq.) in DCM (2 mL) is added dibenzyl diisopropylphosphoramidite (52.5 μL, 1.5 eq.) and the mixture is stirred under argon for 30 min. tert-Butyl hydroperoxide (0.08 mL) is then added followed by Na_2SO_3 (10% aq., 0.08 mL) and the product is extracted with DCM, washed with brine, dried and evaporated. Flash chromatography (50%, 70%, then 100% v/v EtOAc in hexanes) gives **74** (32 mg). ^1H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1H),

7.46-7.26 (m, 6H), 5.56 (s, 2H), 5.03-4.97 (m, 4H), 4.60 (s, 2H), 4.14-4.08 (bm, 2H), 3.60 (bs, 2H), 1.42 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.5, 155.3, 149.9, 136.3, 135.9, 128.4, 127.9, 114.9, 114.4, 113.9, 80.0, 69.3, 67.8, 65.7, 47.3, 41.9, 41.1, 28.4. ^{31}P NMR (202 MHz, $CDCl_3$) δ -1.0. ESI-HRMS for $C_{35}H_{40}N_4O_7P$ $[M+H]^+$ calcd 659.2635; found 659.2640.

({2-[({4-Hydroxy-5H-pyrrolo[3,2-]pyrimidin-7-yl}methyl)amino]ethoxy}phosphonic acid (75). The carbamate **74** (23 mg) is hydrogenated at atmospheric pressure with 10% Pd/C (20 mg) in ethanol (5 mL) for 2 h, filtered and evaporated. The crude reduced product is hydrolysed with 80% aqueous TFA (5 mL) for 2 h and then evaporated twice from dioxane followed by evaporation from 1 N HCl to give **75** (14 mg). ^{1}H NMR (500 MHz, D_2O) δ 8.05 (s, 1H), 7.71 (s, 1H), 4.42 (s, 2H), 4.05-4.02 (m, 2H), 3.32-3.30 (m, 2H). ^{13}C NMR (125 MHz, D_2O) δ 153.2,144.4,134.7, 132.3,118.2,103.6,60.5, 46.9, 40.1. ^{31}P NMR (202 MHz, D_2O) δ 2.3. ESI-HRMS for $C_9H_{14}N_4O_5P$ $[M+H]^+$ calcd 289.0702; found 289.0699.

9*H*-Fluoren-9-ylmethyl *N*-{(±)-2(*R*/*S*)-1-[(dimethoxyphosphoryl)oxy]-3-hydroxypropan-2-yl}carbamate (±)-(77). Iodine (1.94 g, 7.66 mmol) is added in one portion to a solution of trimethyl phosphite (0.943 mL, 7.98 mmol) in CH_2Cl_2 (25 mL) at 0 ^{o}C, which is then warmed to room temperature and stirred for 5 min to give a colourless solution. A portion (6.7 mL, 1.3 eq) of this is added dropwise to a solution of compound **76**[37] (0.5 g, 1.596 mmol) in a mixture of CH_2Cl_2 (6 mL) and pyridine (3 mL) at 0 ^{o}C and stirred for 1 h. The mixture is warmed to room temperature and stirred for 30 min then the solvent is evaporated and the residue subjected to flash chromatography (EtOAc-hexanes, 9:1 v/v then EtOAc) to give first unreacted **76** (190 mg) then **(±)-77** (0.24 g, 36%) as a colourless gum. ^{1}H NMR (500 MHz, CD_3OD) 7.76 (d, J = 7.5, 2H), 7.63 (d, J = 7.3, 2H), 7.37 (t, J = 7.4, 2H), 7.29 (t, J = 7.4, 2H), 4.40-4.33 (m, 2H), 4.19-4.13 (m, 2H), 4.08 (m, 1H), 3.87 (m, 1H), 3.74 (d, J = 11.1, 3H), 3.72 (d, J = 11.2, 3H), 3.65-3.56 (m, 2H). ^{13}C NMR (125.7 MHz, CD_3OD) 158.4 (C), 145.3 (d, J = 4.8, C), 142.6 (C), 128.7 (CH), 128.1 (CH), 126.1 (CH), 120.9 (CH), 67.74 (CH_2), 67.70 (CH_2), 61.5 (CH_2), 55.3 (d, J = 6.1, CH_3), 54.2 (d, J = 7.0, CH), 48.4 (CH). Referenced to the centre line of

CD_3OD at 49.0 ppm. ^{31}P (121.5 MHz, CD_3OD) 2.2 (s). ESI-HRMS for $C_{20}H_{24}NNaO_7P$ $[M+Na]^+$ calcd 444.1188; found 444.1179.

[(±)-2-(*R*/*S*)-2-({[4-(Benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-3-hydroxypropoxy]phosphonic acid; triethylamine (±)-(79). Compound (±)-**77** (0.4 g, 0.949 mmol) is dissolved in CH_2Cl_2 (10 mL) and bromotrimethylsilane (1.232 mL, 9.49 mmol) added. The clear solution is left at room temperature for 16 h then the solvent is evaporated. 10% aq. Et_3N (2 mL) is added and the solution evaporated again. The residue is dissolved in DMF (4 mL) and piperidine (0.8 mL) added. After 30 min at room temperature the solvent is evaporated. The residue is dissolved in H_2O (10 mL) and washed with EtOAc (10 mL). After evaporating the aqueous solution, the residue is repeatedly evaporated from 1:1 v/v MeOH-Et_3N (6 mL) to exchange most of the piperidine with Et_3N. The residue is chromatographed on silica gel (1,4-dioxane-H_2O-Et_3N, 60:40:1 v/v/v) then on RP-18 silica gel (H_2O) to give **(±)-78** (160 mg, 0.428 mmol, ~ 80-85% pure) which is suspended in a mixture of MeOH (15 mL) and Et_3N (0.040 mL, 0.286 mmol). Aldehyde **8** (0.072 g, 0.286 mmol) and sodium cyanoborohydride (0.023 g, 0.371 mmol) are added and the mixture heated at 50 ^oC for 16 h. The solvent is evaporated and the residue flash chromatographed (THF-H_2O-Et_3N, 85:15:1 then 80:20:1 v/v/v) then on RP-18 silica gel (H_2O then MeOH-THF-Et_3N, 70:30:1 v/v/v) to give **(±)-79** as a colourless amorphous solid (71 mg, 49%). ^1H NMR (500 MHz, CD_3OD) 8.48 (s, 1H), 7.85 (s, 1H), 7.52 (d, J = 7.2, 2H), 7.39-7.30 (m, 3H), 5.63 (s, 2H), 4.44 (s, 2H), 4.18 (ddd, J = 12.4, 8.8, 3.4, 1H), 4.04 (ddd, J = 12.4, 9.7, 5.6 1H), 3.85 (dd, J = 12.0, 5.1, 1H), 3.79 (dd, J = 12.0, 6.1, 1H), 3.31 (m, residual CD_3OD + 1H), 3.07 (q, J = 7.3, 5.2H), 1.26 (t, J = 7.3, 8H). Approximately 0.9 eq of Et_3N present. ^{13}C NMR (125.7 MHz, CD_3OD) 157.1 (C), 150.8 (CH), 149.6 (C), 137.7 (C), 133.3 (CH), 129.5 (CH), 129.4 (CH), 129.3 (CH), 116.5 (C), 107.9 (C), 69.1 (CH_2), 62.6 (d, J = 3.8, CH_2), 60.7 (d, J = 3.6, CH), 59.9 (CH_2), 47.1 (CH_2), 40.2 (CH_2), 9.1 (CH_3). Referenced to the centre line of CD_3OD at δ 40.0. ^{31}P (202.3 MHz CD_3OD), 3.2 (s). ESI-HRMS for $C_{17}H_{20}N_4O_6P$ $[M-Et_3N-H]^-$ calcd 407.1120; found 407.1128.

{(±)-2(*R*/*S*)-3-Hydroxy-2-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]propoxy}phosphonic acid; triethylamine (±)-(80). Compound **(±)-79**

(0.07 g, 0.137 mmol) is dissolved in a 1:1 mixture of MeOH-H_2O (10 mL), 20% $Pd(OH)_2/C$ (40 mg) added and the mixture stirred under a hydrogen atmosphere at ambient pressure and temperature for 4 h. After filtering through Celite the solvent is evaporated to give **(±)-80** as a colourless solid (54 mg, 94%). ^{1}H NMR (500 MHz, D_2O) 8.14 (s, 1H), 7.82 (s, 1H), 4.59 (s, 2H), 4.23 (ddd, *J* = 12.4, 6.7, 3.5, 1H), 4.11 (ddd, *J* = 12.4, 7.5, 5.5, 1H), 4.02 (dd, *J* = 12.6, 5.0, 1H), 3.96 (dd, *J* = 12.6, 5.9, 1H), 3.60 (m, 1H), 3.27 (q, *J* = 7.4, 2.6H), 1.35 (t, *J* = 7.4, 3.9H). Referenced to HOD at 4.79 ppm. Approximately 0.4 eq. of Et_3N present. ^{13}C NMR (75.5 MHz, D_2O-CD_3OD, 3:1) 156.0 (C), 144.5 (C), 143.8 (CH), 131.7 (CH), 118.6 (C), 107.8 (C), 61.6 (CH_2), 60.2 (CH), 59.1 (CH_2), 47.6 (CH_2), 39.9 (CH_2), 9.2 (CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (121.5 MHz, D_2O) 3.0 (s). ESI-HRMS for $C_{10}H_{14}N_4O_6P$ $[M\text{-}Et_3N\text{-}H]^-$ calcd 317.0651; found 317.0644.

{(±)-2(*R*/*S*)-[({2-Chloro-4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-3-hydroxypropoxy}phosphonic acid; triethylamine (±)-(81). A mixture of **(±)-78** (0.19 g, 0.509 mmol), Et_3N (0.048 mL, 0.339 mmol), the aldehyde **49** (0.086 g, 0.339 mmol) and 2-picoline borane complex (0.047 g, 0.441 mmol) are stirred together in MeOH (15 mL) at 50 ^oC for 16 h then the solvent evaporated and the residue chromatographed on silica gel (1,4-dioxane-H_2O-1% aq. Et_3N, 75:25:1 v/v/v). The crude product is further chromatographed on silica gel (THF-H_2O-Et_3N, 85:15:1 then 80:20:1 v/v/v) to give [(±)-2(*R*/*S*)-2-({[4-(*tert*-butoxy)-2-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-3-hydroxypropoxy]phosphonic acid; triethylamine as a colourless amorphous solid (105 mg, 51%). ^{1}H NMR (500 MHz, CD_3OD) 7.80 (s, 1H), 4.32 (d, *J* = 13.9, 1H), 4.29 (d, *J* = 13.9, 1H), 4.13 (ddd, *J* = 12.4, 9.2, 3.6, 1H), 4.00 (ddd, *J* = 12.4, 10.3, 5.6, 1H), 3.82 (dd, *J* = 11.9, 5.2, 1H), 3.77 (dd, *J* = 11.9, 5.9, 1H), 3.16 (m, 1H), 2.89 (q, *J* = 7.3, 12H), 1.72 (s, 9H), 1.19 (t, *J* = 7.3, 18H). Approximately 2 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, CD_3OD) 157.4 (C), 151.2 (C), 150.9 (C), 133.2 (CH), 116.5 (C), 109.4 (C), 85.2 (C), 62.8 (d, *J* = 4.5, CH_2), 60.9 (d, *J* = 3.5, CH), 60.5 (CH_2), 47.0 (CH_2), 40.3 (CH_2), 28.7 (CH_3), 9.9 (CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (202.3 MHz, CD_3OD) 3.0 (s). ESI-HRMS for $C_{14}H_{21}{}^{(35)}ClN_4O_6P$ $[M\text{-}Et_3N\text{-}H]^-$ calcd 407.0887; found 407.0895. This material (0.095 g, 0.155 mmol) is dissolved in 80% aq. TFA (2

mL) and left at room temperature. After 15 min the solvent is evaporated to leave a colourless solid which is chromatographed on silica gel (1,4-dioxane-H_2O-Et_3N 75:25:1 v/v/v) to give **(±)-81** as a colourless gum (84 mg, 97%). ^{1}H NMR (500 MHz, CD_3OD) 7.53 (s, 1H), 4.36 (s, 2H), 4.18 (ddd, *J* = 12.4, 8.7, 3.4, 1H), 4.05 (ddd, *J* = 12.4, 9.7, 5.4, 1H), 3.86 (dd, *J* = 12.0, 5.2, 1H), 3.81 (dd, *J* = 12.0, 6.0, 1H), 3.31 (residual CD_3OD + 1H), 3.08 (q, *J* = 7.3, 10.8H), 1.26 (t, *J* = 7.3, 16.2H). Approximately 1.8 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, CD_3OD) 164.7 (C), 152.5 (C), 147.2 (C), 129.5 (CH), 119.8 (C), 106.7 (C), 62.2 (CH_2), 60.8 (CH), 59.9 (CH_2), 47.1 (CH_2), 40.7 (CH_2), 9.5 (CH_3). Referenced to the centre line of CD_3OD at 49.0 ppm. ^{31}P NMR (202.3 MHz, CD_3OD), 4.1 (s). ESI-HRMS for $C_{10}H_{13}{}^{(35)}ClN_4O_6P$ $[M\text{-}Et_3N\text{-}H]^-$ calcd 351.0261; found 351.0264.

[2-Amino-3-hydroxy-2-(hydroxymethyl)propoxy]phosphonic acid (83). Dibenzyl diisopropyl phoshoramidite (3.64 mL, 10.83 mmol) is added to an ice cold solution of compound **82**[39] (1.35g, 5.42 mmol) and 1*H*-tetrazole (1.52 g, 21.7 mmol) in CH_2Cl_2 (50 mL). The solution is stirred at room temperature for 1 h and then cooled to -30 ºC. A solution of MCPBA (4.67g, 16.3 mmol) in CH_2Cl_2 (25 mL) is added and the solution bought slowly to room temperature. The solution is partitioned between CH_2Cl_2 and 10% aqueous sodium bicarbonate. The organic phase is dried and concentrated under reduced pressure. The residue is flash chromatographed (CH_2Cl_2-MeOH, 9:1) to give crude *tert*-butyl *N*-[5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxan-5-yl]carbamate (2.81 g, 99%). A portion of this material (0.2 g) is further flash chromatographed (EtOAc-hexanes) and the purified product is then dissolved in methanol (40 mL) and stirred with palladium on charcoal (5%, 22 mg) under a balloon of hydrogen. After 2 h the catalyst is removed by filtration. The solution is concentrated to dryness and the residue dissolved in 90% aqueous TFA. After 30 min the solution is concentrated under reduced pressure and the residue dissolved in H_2O. Lyophilization gives **83** (90 mg, 0.29 mmol, 76%). ^{1}H NMR (500 MHz, D_2O, HOD 4.70 ppm) δ 3.99 (s, 2H), 3.75 (s, 4H). ^{13}C NMR (125.7 MHz, D_2O, referenced to internal acetone at δ 30.3) δ 62.8 (d, $J_{C,P}$ = 4.6 Hz), 60.8 (d, $J_{C,P}$ = 8.1 Hz), 59.3. ^{31}P NMR (202.4, MHz, D_2O) δ 0.0. ESI-HRMS $C_4H_{13}NO_6P$ $[M+H]^+$calcd. 202.0481, found 202.0484.

{3-Hydroxy-2-[({4-hydroxy-5H-pyrrolo[3,2-d]pyrimidin-7-yl}methyl)amino]-2-(hydroxymethyl)propoxy}phosphonic acid (84). Compound **83** (55 mg, 0.18 mmol), aldehyde **8** (93 mg, 0.26 mmol) and sodium cyanoborohydride (7.8 mg, 0.48 mmol) are stirred in MeOH (5 mL) at 40 ºC for 16 h and then at room temperature for 48 h. The solution is concentrated onto silica gel and then flash chromatographed on silica (MeCN-H_2O, 4:1 v/v), Further purification by flash chromatography (CH_2Cl_2–MeOH-28% aq. NH_4OH, 25:25:10 v/v/v then 50:80:20 v/v/v) gives [2-({[4-(benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-3-hydroxy-2-(hydroxymethyl)propoxy]phosphonic acid (7.0 mg, 0.015 mmol, 9%). ^{1}H NMR (500 MHz, D_2O, HOD 4.70 ppm) δ 8.35 (s, 1H), 7.79 (s, 1H), 7.46 (m, 2H), 7.41 (m, 3H), 5.45 (s, 2H), 4.47 (s, 2H), 4.09 (m, 2H), 3.86 (m, 4H). ^{13}C NMR (125.7 MHz, D_2O) δ 155.9, 149.3, 146.6, 135.6, 133.0, 128.8, 128.6, 127.8, 115.2, 104.6, 68.6, 65.7(d, $J_{C,P}$ = 7 Hz), 60.9, 57.9, 35.4. ^{31}P NMR (202.4 MHz, D_2O) δ 1.2. ESI-HRMSMS $C_{18}H_{24}N_4O_7P$ $[M+H]^+$ calcd. 439.1383, found 439.1385. This material in methanol (5 mL) and water (5 mL) is stirred with Pearlmann's catalyst (2 mg) under a balloon of hydrogen for 16 h. The solution is filtered and the solvent evaporated to give **84** (6.0 mg, 0.014 mmol) and crystallised from MeCN-H_2O. (2.0 mg, 41%) ^{1}H NMR (500 MHz, D_2O, HOD 4.70 ppm) δ 7.98 (s, 1H), 7.61 (s, 1H), 4.22 (s, 2H), 3.90 (d, J = 6.4 Hz, 2H,), 3.77 (m, 4H). ^{13}C NMR (125.7 MHz, D_2O) δ 155.6, 143.1, 142.7, 130.0, 117.4, 109.8, 63.7, 61.5, 59.0, 35.0. ^{31}P NMR (202.4 MHz, D_2O) δ 4.5. ESI-HRMS $C_{11}H_{18}N_4O_7P$ $[M+H]^+$ calcd. 349.0913, found 349.0911.

[(2*R*)-2-Amino-3-hydroxypropoxy]phosphonic acid (86). Dibenzyl diisopropylphosphoramidite (1.067 mL, 3.24 mmol) is added to compound **85**[40,41] (0.5 g, 2.162 mmol) and 1*H*-tetrazole (0.454 g, 6.49 mmol) in CH_3CN (10 mL) and the mixture stirred for 1 h. The solvent is evaporated and the residue flash chromatographed (EtOAc-hexanes, 1:9 v/v) to give tert-butyl (4*R*)-4-({[bis(benzyloxy)phosphanyl]oxy}methyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate as a colourless oil. This is dissolved in CH_2Cl_2 (10 mL) cooled in ice-water and MCPBA (*m*-chloroperoxybenzoic acid) (0.995 g, 4.32 mmol) added and stirred for 30 min. The mixture is diluted with CH_2Cl_2 (50 mL) and washed with sat. aq. Na_2SO_3, sat. aq. $NaHCO_3$ (3x) then brine, dried and the solvent

evaporated. The residue is flash chromatographed (DCM-hexanes-EtOAc, 4:3:1 v/v/v) to give *tert*-butyl (4*R*)-4-({[bis(benzyloxy)phosphoryl]oxy}methyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate as a colourless oil (0.816 g, 77%). $[\alpha]_D^{22}$ +21.5 (c 0.57, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) 7.34 (s, 10H), 5.08-5.00 (m, 4H), 4.22-4.16 (m, 0.5H), 4.13-4.06 (m, 1H), 3.97-3.83 (m, 3H), 3.77 (q, J = 8.6, 0.5H), 1.52-1.41 (m, 15H). ^{13}C NMR (125.7 MHz) 152.1, 151.4 (C), 135.7 (C), 128.6 (CH), 127.9 (CH), 94.1, 93.6 (C), 80.6, 80.3 (C), 69.4 (CH_2), 65.7, 65.3 (CH_2), 64.8, 64.6 (CH_2), 56.6, 56.5, 56.32, 56.26 (CH), 28.3 (CH_3), 27.4, 24.3 (CH_3), 26.6, 23.0 (CH_3). Referenced to the centre line of $CDCl_3$ at δ 77.0. ^{31}P NMR (202.3 MHz, $CDCl_3$) -1.0 (s), -1.1 (s). ESI-HRMS for $C_{25}H_{34}NNaO_7P$ $[M+Na]^+$ calcd 514.1971; found 514.1968. This material (0.77 g, 1.567 mmol) and 10% Pd-C (100 mg) are stirred in EtOH (15 mL) under a hydrogen atmosphere at ambient temperature and pressure for 16 h. The mixture is filtered through cellulose paper and the solvent is evaporated to give {[(4*R*)-3-[(*tert*-butoxy)carbonyl]-2,2-dimethyl-1,3-oxazolidin-4-yl]methoxy}phosphonic acid as a colourless gum (480 mg). This is dissolved in 80% aq. TFA (10 mL) and left at room temperature for 2 h. The solvent is evaporated and the residue dissolved in H_2O (10 mL) and washed with CH_2Cl_2 (2 x10 mL) then evaporated. The residue is dissolved in H_2O and chromatographed on RP 18 silica gel (H_2O) to give **86** as a colourless gum which solidified (0.26 g, 97%). $[\alpha]_D^{22}$ 0 (c 0.565, H_2O). No measurable rotation observed. ^{1}H NMR (500 MHz, D_2O) 4.19-4.12 (m, 1H), 4.11-4.03 (m, 1H), 3.90 (dd, *J* = 12.3, 4.7, 1H), 3.81 (dd, *J* = 12.3, 6.7, 1H), 3.63 (m, 1H). Referenced to HOD at δ 4.79. ^{13}C NMR (125.7 MHz, D_2O) 62.9 (d, *J* = 3.2, CH_2), 59.1 (CH_2), 53.5 (d, *J* = 7.4, CH). Referenced to internal CH_3CN at δ 1.47. ^{31}P NMR (202.3 MHz, D_2O) 0.0 (s). ESI-HRMS for $C_3H_9NO_5P$ $[M-H]^-$ calcd 170.0218; found 170.0211.

[(2*R*)-3-Hydroxy-2-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]propoxy]phosphonic acid; triethylamine (87). Compound **86** (25 mg, 0.146 mmol) is suspended in methanol (50 mL) and brought to pH7 with Et_3N. Aldehyde **8** (22 mg, 0.088 mmol) and then picoline borane complex (20.3 mg, 0.19 mmol) are added and the suspension stirred at 50 ºC for 60 h. The solution is evaporated onto silica gel. Flash chromatography (1,4-dioxane-H_2O-Et_3N, 30:10:0.4 v/v/v then 20:10:0.3 v/v/v)

gives [(2*R*)-3-hydroxy-2-[({4-benzyloxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-ylmethyl)amino]propoxy]phosphonic acid; triethylamine (18 mg, 40%). ^{1}H NMR (500 MHz, CD_3OD) 8.39 (s, 1H), 7.75 (s, 1H), 7.43 (m, 2H), 7.28 (m, 3H), 5.55 (s, 2H), 4.37 (m, 2H), 4.09 (m, 1H), 3.95 (m, 1H), 3.76 (dd, J = 12.1, 5.0 Hz, 1H), 3.69 (dd, J = 12.1, 6.2 Hz, 1H), 3.23 (m, 1H), 3.01 (q, J = 7.1 Hz, 7H), 1.18 (t, J = 7.1 Hz, 11H). Approximately 1.1 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, CD_3OD, centre line 49.0 ppm) 157.3, 150.8, 149.6, 137.8, 133.1, 129.7, 116.8, 108.2, 69.4, 62.7 (d, $J_{C,P}$ = 5 Hz), 60.7 (d, $J_{C,P}$ = 5 Hz), 60.0, 47.6, 40.4, 9.4. ^{31}P NMR (202.4 MHz, CD_3OD) δ 2.7 ESI-HRMS for $C_{17}H_{20}N_4O_6P$ $[M-H]^-$ calcd. 407.1120, found 407.1129. A solution of this material (14 mg, 0.028 mmol) in methanol (5 mL) and water (5 mL) is stirred with Pearlmann's catalyst (2 mg) under a balloon of hydrogen for 16 h. The solution is filtered and the solvent evaporated to give **87** (4 mg, 46%). ^{1}H NMR (500 MHz, D_2O-CD_3OD, 2:1) δ 7.91 (s, 1H), 7.61 (s, 1H), 4.39 (m, 2H), 4.08 (m, 1H), 3.96 (m, 1H), 3.81 (dd, J = 12.1, 5.5 Hz, 1H), 3.74 (dd, J = 12.1, 6.1, Hz, 1H), 3.41 (m, 1H), 3.11 (q, J = 7.1 Hz, 4H), 1.14 (t, J = 7.1 Hz, 11H). Approximately 0.7 eq. of Et_3N present. ^{13}C NMR (125.7 MHz, D_2O-CD_3OD, 2:1 centre line of CD_3OD 49.0 ppm) δ 155.0, 143.6, 142.8, 130.8, 117.7, 106.0, 60.9 (d, $J_{C,P}$ = 5 Hz), 58.3 (d, $J_{C,P}$ = 7 Hz), 57.6, 46.6, 38.7, 8.1. ^{31}P NMR (202.4 MHz, D_2O-CD_3OD, 2:1) δ 1.0. ESI-HRMS for $C_{10}H_{14}N_4O_6P$ $[M-H]^-$ calcd. 317.0651, found 317.0644.

[(2*R*)-2-[({2-Amino-4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-3-hydroxypropoxy]phosphonic acid hydrochloride (88). A mixture of **86** (0.15 g, 0.877 mmol), triethylamine (0.123 mL, 0.877 mmol), **70**[36] (0.122 g, 0.351 mmol) and 2-picoline borane complex (0.049 g, 0.456 mmol) are stirred together in MeOH (15 mL) at 50 ^{o}C for 17 h then the solvent is evaporated and the residue flash chromatographed (3% aq. Et_3N in 1,4-dioxane-H_2O, 8:2 v/v) to give [(2*R*)-2-[({2-[(*E*)-[(dimethylamino)methylidene]amino]-3-{[(2,2-dimethylpropanoyl)oxy]methyl}-4-oxo-3*H*,4*H*,5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-3-hydroxypropoxy]phosphonic acid; triethylamine as a yellow solid (0.144 g, 68.0%). $[\alpha]_D^{22}$ -15.1 (c 0.51, MeOH). ^{1}H NMR (500 MHz, CD_3OD, 4 mg in 0.6 mL) 8.70 (s, 1H), 7.47 (s, 1H), 6.31 (s, 2H), 4.38 (d, J = 13.6, 1H), 4.32 (d. J = 13.6, 1H), 4.25 (ddd, J = 12.2, 8.6, 3.2, 1H), 4.03 (ddd, J =

12.2, 10.1, 5.1, 1H), 3.89 (dd, *J* = 12.0, 5.1, 1H), 3.84 (dd, *J* = 12.0, 6.7, 1H), 3.34 (m, 1H), 3.22 (s, 3H), 3.08 (s, 3H), 3.02 (q, *J* = 7.3, 5.4H), 1.24 (t, *J* = 7.3, 8H), 1.15 (s, 9H). Approximately 0.9 eq. of Et_3N present. ^{1}H NMR (500 MHz, CD_3OD, 25 mg in 0.6 mL) 8.69 (s, 1H), 7.47 (s, 1H), 6.27 (d, *J* =9.0, 1H), 6.22 (d, *J* = 9.1, 1H), 4.37 (d, *J* = 13.6, 1H), 4.32 (d. *J* = 13.6, partly overlapped with ddd at 4.28, 1H), 4.28 (ddd, J = 12.3, 9.0, 3.0, 1H, partly overlapped with d at 4.32), 4.08 (ddd, *J* = 12.6, 10.6, 5.3, 1H), 3.96-3.86 (m, 2H), 3.38 (m, 1H), 3.23 (s, 3H), 3.09 (s, 3H), 3.05 (q, *J* =7.3, 5.4H), 1.25 (t, *J* = 7.3, 8H), 1.13 (s, 9H). ^{13}C NMR (125.7 MHz, CD_3OD, 25 mg in 0.6 mL) 179.0 (C), 159.0 (CH), 156.2 (C), 155.6 (C), 144.9 (C), 130.5 (CH), 115.1 (C), 108.4 (C), 67.1 (CH_2), 62.1 (d, *J* = 4.7, CH_2), 61.4 (d, *J* = 2.9, CH), 60.3 (CH_2), 46.9 (CH_2), 41.3 (CH_3), 39.8 (C), 35.3 (CH_3), 27.4 (CH_3), 9.9 (CH_3). Referenced to the centre line of CD_3OD at δ 49.0. ^{31}P NMR (202.3 MHz, CD_3OD) 3.4 (s). ESI-HRMS for $C_{19}H_{30}N_6O_8P$ $[M\text{-}Et_3N\text{-}H]^-$ calcd 501.1863; found 501.1857. The ^{1}H and ^{13}C NMR spectra displayed significant concentration effects. This material (0.138 g, 0.229 mmol) is heated at 100 ^{o}C in 6M aq. HCl (6 mL) for 1.5 h. The solvent is evaporated and the residue flash chromatographed (1,4-dioxane-H_2O-Et_3N, 75:25:3 then 70:30:3 v/v/v) to give the triethylammonium form of the product as a light brown solid. This is dissolved in 5% HCl (10 mL), the solvent evaporated and the residue dissolved in hot H_2O and loaded on to an Amberlyst A21 resin column. The column is eluted first with H_2O then the product is eluted off with 1M HCl. The residue after evaporation of the solvent is chromatographed on RP 18 silica gel (H_2O) to give **88** as a colourless solid after freeze drying (56 mg, 66%). $[\alpha]_D^{22}$ +4.9 (c 0.45, 1.5M HCl). ^{1}H NMR (500 MHz, D_2O + drop of DCl) 7.62 (s, 1H), 4.49 (d, *J* = 14.2, 1H), 4.43 (d, *J* = 14.2. 1H), 4.31 (ddd, *J* = 12.1, 6.1, 3.6, 1H), 4.19 (ddd, *J* = 12.1, 6.0, 6.0, 1H), 3.94 (dd, *J* = 12.5, 4.9, 1H), 3.88 (dd, *J* = 12.5, 6.3, 1H), 3.65 (m, 1H). Referenced to HOD at 4.79 ppm. ^{13}C NMR (125.7 MHz, D_2O + drop of DCl) 154.2 (C), 151.2 (C), 133.1 (C), 132.3 (CH), 112.4 (C), 101.5 (C), 61.7 (d, *J* = 3.6, CH_2), 59.2 (d, *J* = 6.8, CH), 58.5 (CH_2), 39.3 (CH_2). Referenced to internal CH_3CN at 1.47 ppm. ^{31}P NMR (202.3 MHz, D_2O + DCl) -0.2 (s). ESI-HRMS for $C_{10}H_{15}N_5O_6P$ $[M\text{-}HCl\text{-}H]^-$ calcd 332.0760; found 332.0755. Found 10.1% Cl, $C_{10}H_{16}N_5O_6P$•HCl required 9.6% Cl indicating the product is a mono hydrochloride salt.

[(2*S*)-3-hydroxy-2-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-l}methyl)amino]propoxy]phosphonic acid; triethylamine (89) is prepared, in the same manner as that of the enantiomer **87** and has the same ^{1}H, ^{13}C and ^{31}P NMR as the enantiomer **87**. ESI-HRMS for $C_{10}H_{15}N_4NaO_6P$ $[M+Na]^+$ calcd. 341.0627, found 341.0626.

[(2*S*)-2-[({2-amino-4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]-3-hydroxypropoxy]phosphonic acid hydrochloride (90) is prepared, in the same manner as that of the enantiomer **88** as a colourless amorphous solid. $[\alpha]_D^{22}$ -4.6 (c 0.42, 1.5M HCl). ESI-HRMS for $C_{10}H_{15}N_5O_6P$ $[M\text{-}HCl\text{-}H]^-$ calcd 332.0760; found 332.0756. The ^{1}H, ^{13}C and ^{31}P NMR spectra are identical to those of the enantiomer **88**.

Benzyl *N*-[(1*R*)-2-[(*tert*-butyldiphenylsilyl)oxy]-1-[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl]carbamate (92). A solution of compound **91** (2.0g, 12.4 mmol) and imidazole (3.38 g, 49.6 mmol) in DMF (50 mL) is cooled in an ice-water bath whilst *tert*-butylchlorodiphenylsilane(11.5 mL, 43.4 mmol) is added from a syringe. The mixture is stirred at room temperature for 2 h and then diluted with water and extracted three times with EtOAc. The combined extracts are washed with saturated aqueous NaCl, dried and concentrated under reduced pressure. The residue is flash chromatographed EtOAc-CH_2Cl_2-Et_3N, 10:90:0.1 then stepwise to 40:60:0.1) to give *O*-silylated hydroxylamine (3.9 g, 79%). This material is dissolved in THF-water (2:1, 90 mL) and benzylchloroformate (1.53 mL, 10.7 mmol) and $NaHCO_3$ (2.46g, 29.3 mmol) are added. After stirring for 48 h the mixture is partitioned between EtOAc and water. The organic phase is washed with saturated aqueous NaCl, dried and concentrated under reduced pressure. The residue is flash chromatographed (EtOAc-hexanes, 1:9 v/v then stepwise to 2:8 v/v) to give **92** (4.0 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (m, 4H), 7.37 (m, 11H), 5.11 (d, J = 12.3 Hz, 1H), 5.05 (d, J = 12.3 Hz, 1H), 4.95 (bd, J = 9.4 Hz, 1H), 4.44 (m, 1H), 4.02 (m, 1H), 3.91 (m, 1H), 3.70 (m, 3H), 1.38 (s, 3H), 1.34 (s, 3H), 1.05 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$, centre line δ 77.0) δ 156.4, 136.5, 135.6, 133.2, 129.8, 128.5, 128.1, 127.7, 109.2, 73.9, 66.9, 66.2, 63.8, 52.7, 26.8, 26.3, 25.1, 19.2. ESI-HRMS for $C_{31}H_{39}NO_5SiNa$ $[M+Na]^+$ calcd. 556.2496, found 556.2486.

Benzyl (4*R*,5*S*)-5-({[bis(benzyloxy)phosphoryl]oxy}methyl)-4-{[(*tert*-butyldiphenylsilyl)oxy]methyl}-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (93). A solution of **92** (0.75g, 1.41 mmol) in AcOH- THF-water (3:1:1, 35 mL) is heated at 50 ºC for 5 h. The solvents are removed under reduced pressure. The residue is dissolved in, and evaporated from, ethanol and toluene to give benzyl *N*-[(2*R*,3*S*)-1-[(*tert*-butyldiphenylsilyl)oxy]-3,4-dihydroxybutan-2-yl]carbamate (0.71g, >100%) which is used without further purification. A portion of this material (0.33g, 0.67 mmol) and pyridine (0.16 mL, 2.0 mmol) are dissolved in CH_2Cl_2 (10 mL) and cooled in a dry ice-acetone bath then benzoyl chloride (0.135 mL, 1.15 mmol) is added. The solution is stirred for 30 min and then quenched with water, brought to room temperature and washed with water, 10% aqueous $NaHCO_3$ and saturated aqueous NaCl, dried and concentrated under reduced pressure. The resulting (2*S*,3*R*)-3-{[(benzyloxy)carbonyl]amino}-4-[(*tert*-butyldiphenylsilyl)oxy]-2-hydroxybutyl benzoate is taken up in acetone (16 mL) and 2,2-dimethoxypropane (4 mL), with a trace of toluenesulfonic acid, and stirred at 50 ºC for 3 h. The solution is cooled and concentrated under reduced pressure. The residue is taken up in EtOAc, washed with 10% aqueous $NaHCO_3$, dried and concentrated under reduced pressure. The resulting benzyl (4*R*,5*S*)-5-[(benzoyloxy)methyl]-4-{[(*tert*-butyldiphenylsilyl)oxy]methyl}-2,2-dimethyl-1,3-oxazolidine-3-carboxylate is dissolved in methanol (20 mL) and basified with sodium methoxide. After 1 h saturated aqueous ammonium chloride (0.5 mL) is added, the mixture is concentrated under reduced pressure, taken up in EtOAc, washed with water, dried ($MgSO_4$) and evaporated to dryness. Flash chromatography (EtOAc-hexanes 20:80 v/v, then stepwise to 25:75 v/v) gives benzyl (4*R*,5*S*)-4-{[(*tert*-butyldiphenylsilyl)oxy]methyl}-5-(hydroxymethyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (0.16 g, 43%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (m, 4H), 7.47-7.12 (m, 11H), 5.20-4.84 (m, 2H), 4.36 (m, 1H), 4.05-3.60 (m, 5H), 2.10 (m, 1H), 1.67 (s, 3H), 1.52 (s, 3H), 1.05 (s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$ centre line δ 77.0, signals split due to hindered rotation) δ 152.4, 136.3, 135.7, 133.1, 129.8, 128.4, 128.0, 127.8, 95.4, (78.5, 77.6), 66.9, (63.5, 61.7), (59.8, 58.9), 28.1, 26.9, 26.1, 19.2. ESI-HRMS $C_{31}H_{39}NO_5SiNa$ $[M+Na]^+$ calcd. 556.2495, found 556.2495. A solution of this material(160 mg, 0.30 mmol) and 1*H*-tetrazole (42 mg, 0.60 mmol) in MeCN (9 mL) is

cooled in an ice-H_2O bath and dibenzyl diisopropylphosphoramidite (0.168 mL, 0.51 mmol) is added. The mixture is briefly warmed to room temperature, then cooled again in the ice-water bath whilst MCPBA (138 mg, 0.60 mmol) is added. The solution is warmed to room temperature and partitioned between EtOAc and 10% aqueous $NaHCO_3$. The organic phase is concentrated under reduced pressure and the residue flash chromatographed (EtOAc-hexanes, 25:75) to give crude **93** (200 mg, 0.25 mmol, 83%). A portion of this is further purified by flash chromatography (EtOAc-CH_2Cl_2, 4:96) to give clean **93**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (bs, 4H), 7.30(m, 20H), 7.11 (bs, 1H), 5.20-4.82 (bm, 2H), 5.02(m, 4H), 4.46(m, 1H), 4.11 (m, 1H), 4.05(m, 1H), 3.85(bs, 1H), 3.71(bs, 2H), 1.61(bs, 3H), 1.50(bs, 3H), 1.09(s, 9H). ^{13}C NMR (125.7 MHz, $CDCl_3$, centre line δ 77.0, signals split due to hindered rotation) δ 152.2, 135.4, 129.9, 128.4, 128.1, 128.0, 127.8, (95.7, 95.1), (76.4, 75.6), 69.4, 67.7, (67.1, 66.8), (63.0, 61.5), (60.2, 59.2), 28.3, 26.9, 26.5, 14.1. ^{31}P NMR (202,4 MHz, $CDCl_3$) δ -1.0 ESI-HRMS for $C_{45}H_{52}NO_{8P}SiNa$ $[M+Na]^+$ calcd. 816.3098, found 816.3095.

Benzyl *N*-[(2R,3S)-4-{[bis(benzyloxy)phosphoryl]oxy}-1,3-dihydroxybutan-2-yl]carbamate (94). Compound **93** (200 mg, 0.25 mmol) is dissolved in THF (5 mL) and TBAF (1M, 0.44 mL, 0.44 mmol) is added. After a few minutes the solution is filtered through a pad of silica gel and concentrated under reduced pressure to give crude benzyl (4*R*,5*S*)-5-({[bis(benzyloxy)phosphoryl]oxy} methyl)-4-(hydroxymethyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate. The residue is stirred in THF-TFA-H_2O(1:1:1) for 4 h, concentrated under reduced pressure and flash chromatographed (EtOAc) to give **94** (46 mg, mmol, 36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (m, 15H), 5.48 (m, 1H), 5.06 (m, 6H), 4.08 (m, 1H), 3.99 (m, 2H), 3.79 (m, 1H), 3.71 (m, 2H), 2.10 (bs, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$, centre line δ 77.0) δ 156.7, 136.3, 135.4, 133.1, 128.8, 128.7, 128.2, 128.1, 128.1, 71.7, 70.2, 69.9, 67.1, 64.2, 52.6. ^{31}P NMR (202.4 MHz, $CDCl_3$) δ 0.5 ESI-HRMS for $C_{26}H_{30}NO_8PNa$ $[M+Na]^+$ calcd. 538.1067, found 538.1605.

[(2*S*,3*R*)-2,4-Dihydroxy-3-[({4-hydroxy-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl}methyl)amino]butoxy]phosphonic acid (95). A solution of compound **94** (100 mg,

0.19 mmol) in methanol (10 mL) is stirred with Pd black (5 mg) under a balloon of hydrogen. After 3 h the catalyst is removed by filtration, 7M NH_3 in MeOH (1 drop) added, and the solution concentrated to dryness to give [(2*S*,3*R*)-3-amino-2,4-dihydroxybutoxy]phosphonic acid; ammonia (33 mg, 86%). ^{1}H NMR (500 MHz, D_2O, HOD 4.70 ppm) δ 4.00 (m, 3H), 3.85 (dd, *J* = 12.4, 4.2 Hz, 1H), 3.74 (dd, *J* = 12.4, 7.1 Hz, 1H), 3.49 (m, 1H), 3.31 (s, 1H). ^{13}C NMR (125.7 MHz, D_2O) δ 66.9 (d, $J_{C,P}$ = 8 Hz), 65.1(d, $J_{C,P}$ = 6 Hz), 59.0, 54.6. ^{31}P NMR (202.4 MHz, D_2O) δ 4.8. ESI-HRMS for $C_4H_{12}NNaO_6P$ $[M+Na]^+$ calcd. 224.0300, found 224.0299. This material (15 mg, 0.075 mmol) is suspended in methanol and brought to pH 6 with triethylamine, aldehyde **8** (20 mg, 0.060 mmol) and sodium cyanoborohydride (7.0 mg, 0.11 mmol) are added and the mixture stirred at 50 ºC for 48 h. The solution is concentrated onto silica gel and the residue flash chromatographed (*i*-PrOH-H_2O, 8:2 v/v) to give crude give [(2*S*,3*R*)-3-({[4-(benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-2,4-dihydroxybutoxy]phosphonic acid which is dissolved in H_2O, and chromatographed on a Strata-X cartridge (Phenomenex) eluting with H_2O-methanol-28% aq. NH_4OH (5:4:1 v/v/v) to give [(2*S*,3*R*)-3-({[4-(benzyloxy)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl]methyl}amino)-2,4-dihydroxybutoxy]phosphonic acid (12 mg, 38%). ^{1}H NMR (500 MHz, D_2O-MeOD, 9:1, HOD 4.70 ppm) δ 8.38 (s, 2H) 8.34 (s, 1H), 7.81 (s, 1H), 7.46 (m, 2H), 7.37 (m, 3H), 5.50 (s, 2H), 4.54 (d, *J* = 14.1 Hz, 1H), 4.48 (d, *J* = 14.1 Hz, 1H), 4.02-3.92 (m, 3H), 3.86 (m, 2H), 3.40 (m, 1H). ^{13}C NMR (125.7 MHz, D_2O-MeOD, 9:1) δ 170.6 155.8, 149.6, 147.6, 135.9, 132.7, 128.7, 128.5, 127.9, 115.3, 104.6, 68.5, 67.1, 65.6, 59.9, 56.8, 39.0. ^{31}P NMR (202.4 MHz, D_2O) δ 1.3. ESI-HRMS for $C_{18}H_{24}N_4O_7P$ $[M+H]^+$ calcd. 439.1383, found 439.1378. A solution of this material (11 mg, 0.022 mmol) in 20% aqueous methanol (7mL) is stirred with Pd black (1 mg) under a balloon of hydrogen. After 14 h the catalyst is removed by filtration and the residue after evaporation purified by flash chromatography (*i*-PrOH-H_2O, 7:3 v/v) to give **95** (3.2 mg, 42%) ^{1}H NMR (500 MHz, D_2O, HOD 4.70 ppm) δ 8.05 (s, 1H), 7.73 (s, 1H), 4.56 (d, *J* = 14.1 Hz, 1H), 4.49 (d, *J* = 14.1 Hz, 1H), 4.00(m, 3H), 3.90 (m, 2H), 3.48 (m, 1H). ^{13}C NMR (125.7 MHz, D_2O) δ 155.2, 150.6, 143.1, 130.9, 117.7, 106.2, 67.3 (d, $J_{C,P}$ = 6 Hz), 65.4, 60.0, 56.8, 39.0. ^{31}P NMR (202.4 MHz, D_2O) δ 2.9. ESI-HRMS for $C_{11}H_{17}N_4NaO_7P$ $[M+Na]^+$ calcd. 371.0733, found 371.0733.

Enzyme Inhibition Assays

*Pf*HGXPRT activity was measured using spectrophotometric assays observing the conversion of xanthine and 5-phospho-α-D-ribose-1-pyrophosphate (PRPP) to xanthosine-5'-monophosphate and inorganic pyrophosphate (PPi) at 247 nm (ε_{257} – 6.8 mM^{-1} cm^{-1}) or the conversion of guanine and PRPP to guanosine-5'-monophosphate and PPi (ε_{257} – 5.8 mM^{-1} cm^{-1}) on a Varian Cary 100 spectrophotometer (Palo Alto, CA) at 37°C. All assays were conducted in 10 mM potassium phosphate (pH 7.6), 10 mM $MgCl_2$, 1 mM PRPP, 150 mM xanthine or 50 mM guanine, 0.5 mM DTT with varying amounts of inhibitor and concentrations of *Pf*HGXPRT between 2 and 10 nM or human HGPRT of 1 nM. As the dissociation constants of many of the inhibitors were near or below that of the enzyme concentration, the data were fit to the Morrison equation for tight-binding inhibitors.[50]

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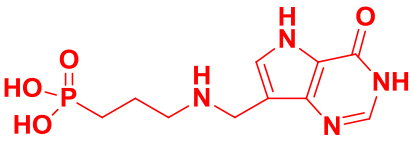
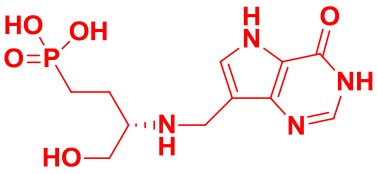
Graphical Abstract.



K_i = 10.6 nM



K_i = 0.65 nM