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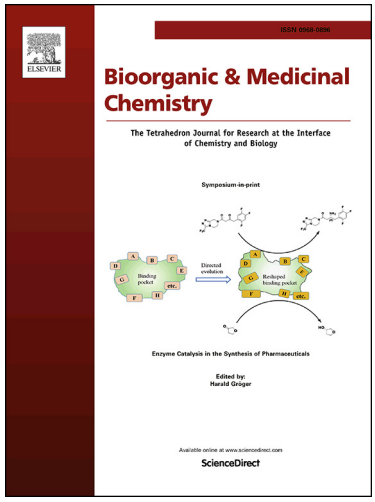

Synthesis and anti-HCV activity of β-D-2'-deoxy-2'-α-chloro-2'-β-fluoro and β-D-2'-deoxy-2'-α-bromo-2'-β-fluoro nucleosides and their phosphoramidate prodrugs

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ABSTRACT

We report herein the synthesis and evaluation of a series of β-D-2'-deoxy-2'-α-chloro-2'-β-fluoro and β-D-2'-deoxy-2'-α-bromo-2'-β-fluoro nucleosides along with their corresponding phosphoramidate prodrugs. Key intermediates, lactols **11** and **12,** were obtained by a diastereoselective fluorination of protected 2-deoxy-2-chloro/bromo-ribonolactones **7** and **8**. All synthesized nucleosides and prodrugs were evaluated with a hepatitis C virus (HCV) subgenomic replicon system.

1. Introduction

According to WHO, an estimated 71 million people are chronically infected worldwide with Hepatitis C Virus (HCV) and among them a significant number will develop cirrhosis or liver cancer.[1] However, since 2011 and the first approved direct acting antivirals (DAAs), boceprevir and telaprevir, a revolution occurred in the treatment of HCV infection. Today, several oral regimens combining different pangenotypic DAAs with different modes of action are available and a sustained virologic response (SVR) of almost 100% can be reached after 8 to 12 weeks of treatment.[2] To date, sofosbuvir (SOF) is the only nucleoside analog approved for the treatment of HCV infection and it is the pillar of the most prescribed treatments (Vozevi and Epcluza). Because nucleoside analogs targeting the HCV NS5B polymerase generally display broader activity against HCV genotypes (GT) along with a favorable safety profile and a high genetic barrier to resistance, they remain the gold standard for the treatment of HCV.[3] This idea was reinforced by the recent discovery of treatment naïve patients from Equatorial Guinea, with a new HCV GT-1 subtype naturally bearing resistance-associated mutations to NS5A inhibitors, another class of DAA.[4]

Based on the success of 2'-halogeno compounds such as SOF[5] but also the anticancer drug gemcitabine **2**[6], our group, and others, focused on the development of new 2'-dihalogeno nucleosides as potential anti-HCV agents. In 2017, Pinho et al. first reported the discovery of the β-D-2'-deoxy-2'-dichlorouridine nucleotide prodrug **3** as a potent inhibitor of HCV replication.[7] Our group also reported the discovery of β-D-2'-deoxy-2'-β-chloro,2'-α-fluoro-, β-D-2'-deoxy-2'-dibromo- and β-D-2'-deoxy-2'-β-bromo,2'-α-fluoro-uridine prodrugs **4a**[8], **5**[9] and **4b**,[10] as three potent anti-HCV agents (Figure 1). As part of our ongoing HCV drug discovery program, we wish to report herein, the synthesis and antiviral evaluation of new series of 2'-α-chloro,2'-β-fluoro and 2'-α-bromo,2'-β-fluoro nucleosides and their corresponding phosphoramidate prodrugs.

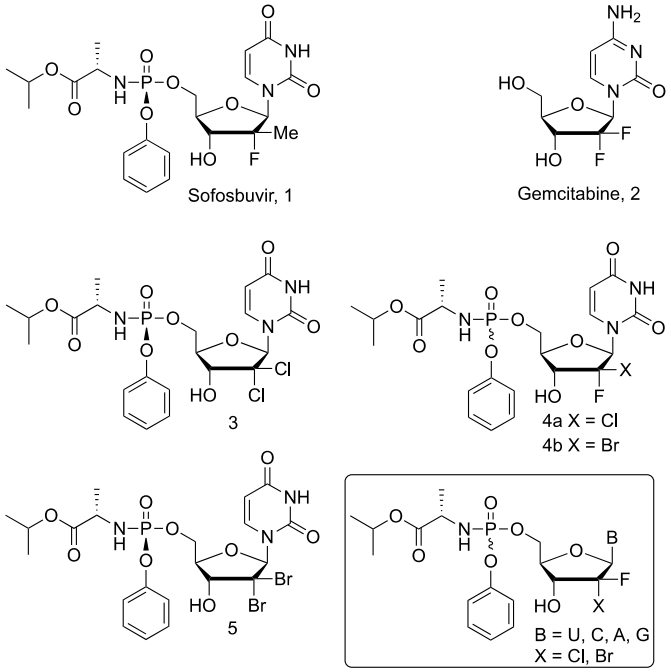
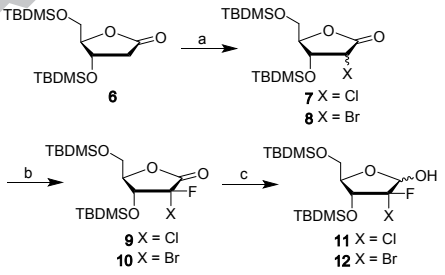


Fig. 1. Structures of sofosbuvir, gemcitabine, known 2'-deoxy-2'-dihalogeno nucleoside prodrugs, **3**-**5** and targeted 2'-β-chloro-2'-α-fluoro and 2'-β-bromo,2'-α-fluoro nucleotides analogs.

2. Results and Discussion

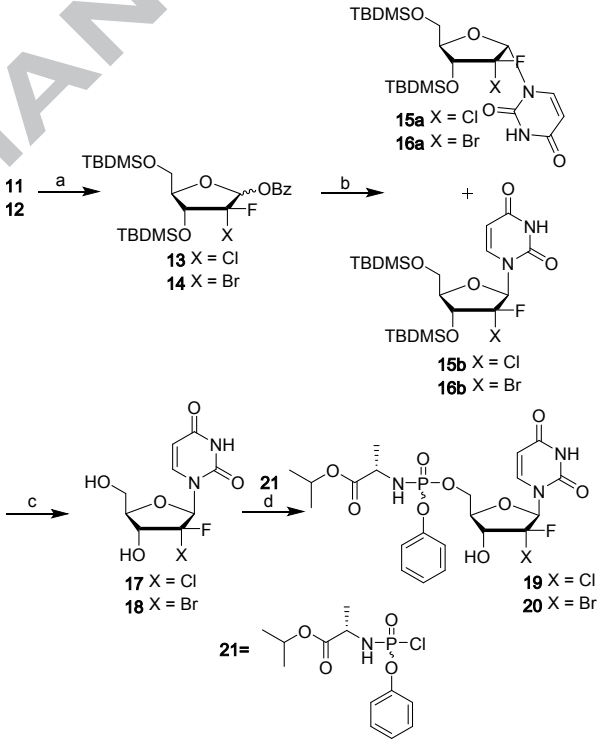
2.1. Chemistry

Diastereoselective synthesis of key 2-chloro-2-fluoro and 2-bromo-2-fluoro lactols **11** and **12** was performed by adapting the chemistry developed by Cen *et al.* for the stereoselective synthesis of the 2-Br, 2-F lactone **10**[11] (Scheme 1). Thus, protected lactone **6** was first converted to its corresponding trimethylsilyl enol ether followed by direct chlorination or bromination in the presence of either *N*-chlorosuccimimide (NCS) or *N*-bromosuccimimide (NBS) to give the chloro and bromo lactones **7** and **8**, as diastereomeric mixtures. Subsequent fluorination in presence of lithium hexamethyldisilazide (LiHMDS) and *N*-fluorobenzenesulfonimide (NFSI) afforded selectively the dihalogeno lactones **9** and **10**. Reduction with lithium tri-*tert*-butoxyaluminium hydride in tetrahydrofuran (THF) gave the corresponding lactols **11** and **12** as a mixture of α- and β- anomers (ratio ~2:1). With these two key lactols in hand, the corresponding pyrimidine and purine nucleoside analogs along with their phosphoramidate prodrugs were prepared following the chemistry depicted in Schemes 2-7.



Scheme 1. Synthesis of ribolactols **11** and **12**. (a) i) TEA, TMSOTf, DCM, 0 ºC, 30 min; ii) NCS or NBS, DCM, 0 ºC, 1 h, 77% (**7**), 82% (**8**) over two steps; (b) NFSI, LiHMDS, THF, -78 ºC, 1 h, 55% (**9**), 64% (**10**); (c) $LiAl(OtBu)_3H$, THF, 0 ºC to rt, 1 h, 97% (**11**), 98% (**12**).

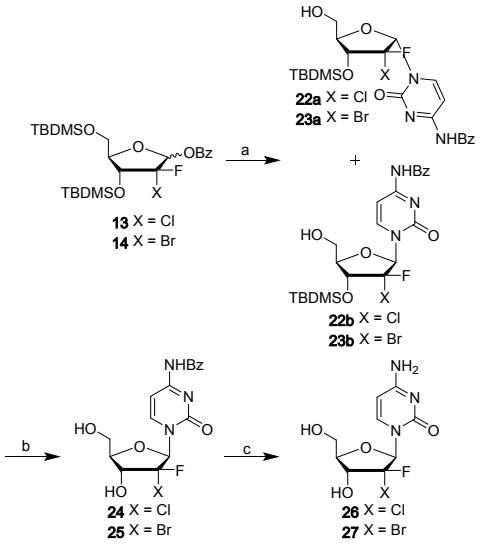
Thus, benzoylated derivatives **13** and **14**, formed by reaction of benzoyl chloride with lactols **11** and **12**, were reacted under classical Vorbrüggen type conditions in presence of persilylated uracil and trimethylsilyl trifluoromethanesulfonate (TMSOTf) under microwave irradiation to give α and β anomers **15a-b** and **16a-b**. At this stage, α and β isomers were separated by column chromatography and the isolated β isomers **16a-b** were deprotected by treatment with tetra-*N*-butylammonium fluoride (TBAF) to give nucleosides **17** and **18** in 70% and 90% yield respectively. In order to express their therapeutic effect, nucleoside analogs need to be sequentially phosphorylated onto their corresponding 5'-triphosphate forms. However, the first phosphorylation step has often been identified as the limiting step and that is why nucleoside monophosphate prodrugs, which are able to deliver intracellularly a nucleoside monophosphate, have been developed.[12] Among all the prodrugs that have emerged, McGuigan's Protides remains the most popular and have been clinically validated with the FDA-approval of drugs such as sofosbuvir **1** (HCV) or tenofovir disoproxyl fumarate (TDF) (HIV and HBV). Hence, phosphoramidate prodrugs **19** and **20** (R_P:S_p ~ 1:1) were prepared from nucleosides **17** and **18** by reaction with the phenyl L-isopropylalaninyl phosphorochloridate reagent **21** in presence of *N*-methylimidazole (Scheme 2).



Scheme 2. Synthesis of uridine analog phosphoramidates **19** and **20**. (a) BzCl, TEA, DCM, 0 ºC to rt, 16 h, 96% (**13**) and 83% (**14**); (b) uracil, BSA, CH_3CN, 60 ºC, 15 min, then TMSOTf, MW, 150 ºC, 6 min (**15**), 10 min (**16**), 30% (**15a**), 12% (**15b**), 22% (**16a**), 12% (**16b**); (c) TBAF, THF, rt, 1 h, 70% (**17**), 90% (**18**); (d) **21**, NMI, THF, rt, 1 h, 34% (**19**), 46% (**20**).

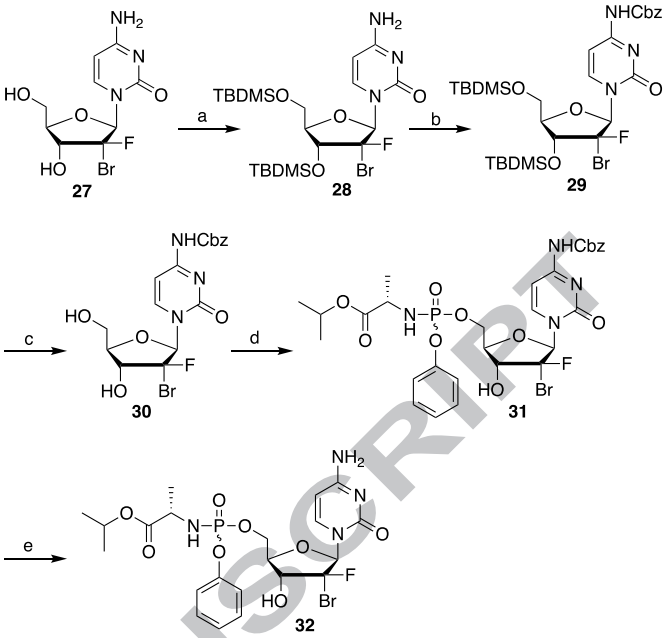
In a similar manner, glycosylation of benzoylated derivatives **13** and **14** with persilylated *N*-Bz-cytosine in the presence of TMSOTf at 150 °C for 12 min under microwave irradiation gave compounds **22** and **23** as a mixture of α/β anomers, easily separable by flash column chromatography on silica gel. In this case, *in-situ* 5'-TBDMS cleavage was observed probably due to the longer reaction time. Removal of the remaining TBDMS group with TBAF followed by debenzoylation in a saturated solution of ammonia in methanol

afforded nucleosides **26** and **27** in 71% and 96% yield, respectively (Scheme 3).



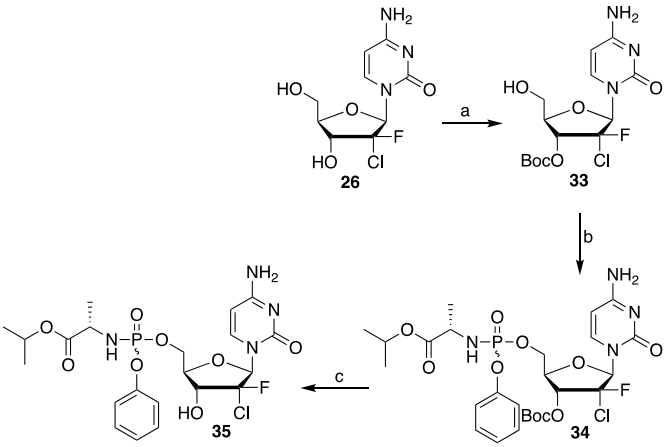
Scheme 3. Synthesis of cytidine nucleoside analogs **26** and **27**. (a) *N*-Bz-cytosine, BSA, CH_3CN, 60 ºC, 15 min, then TMSOTf, 150 ºC, 12 min, MW, 37% (**22a**), 12% (**22b**), 21% (**23a**), 14% (**23b**); (b) TBAF, THF, rt, 1 h, 73% (**24**), 90% (**25**); (c) NH_3/MeOH, rt,16 h, 71% (**26**), 96% (**27**).

Due to the low solubility of nucleosides **26** and **27** in solvents ,and combination of solvents, commonly used for the formation of phosphoramidates (THF, THF/ACN or THF/DMF), alternative approaches were investigated to synthesize the corresponding cytidine phosphoramidate prodrugs **32** and **35**. We first introduced the prodrug moiety onto the more soluble *N*-benzoylated intermediates **24** and **25** but were finally unable to find suitable conditions to debenzoylate the protected prodrug without substantial degradation. The preparation of prodrug **32** was eventually achieved by introduction of a more labile benzyloxycarbonyl group (Cbz).[13] Thus, nucleoside **27** was first reacted with TBDMSCl in presence of imidazole followed by treatment with CbzCl and DMAP to give the fully protected nucleoside **29**. TBDMS groups were then removed in the presence of $Et_3N.3HF$ to give the desired 4-*N*-Cbz protected nucleoside **30**. Finally, reaction with phenyl L-isopropylalaninyl phosphorochloridate reagent **21** in the presence of *N*-methyl imidazole and hydrogenolysis of the Cbz-protected cytidine ProTide intermediate **31** gave the corresponding phosphoramidate prodrug **32** (Scheme 4).



Scheme 4. Synthesis of cytidine analog phosphoramidate **32**. (a) TBDMSCl, imidazole, DCM, rt, 16 h, 83%; (b) CbzCl, DMAP, DCM, rt, 1.5 h, 74%; (c) $Et_3N.3HF$, THF, rt, 36 h 67%; (d) **21**, NMI, THF, rt, 4 h, 20%; (e) Pd/C, EtOH, 1,4-cyclohexadiene, rt, 2 h, 55%.

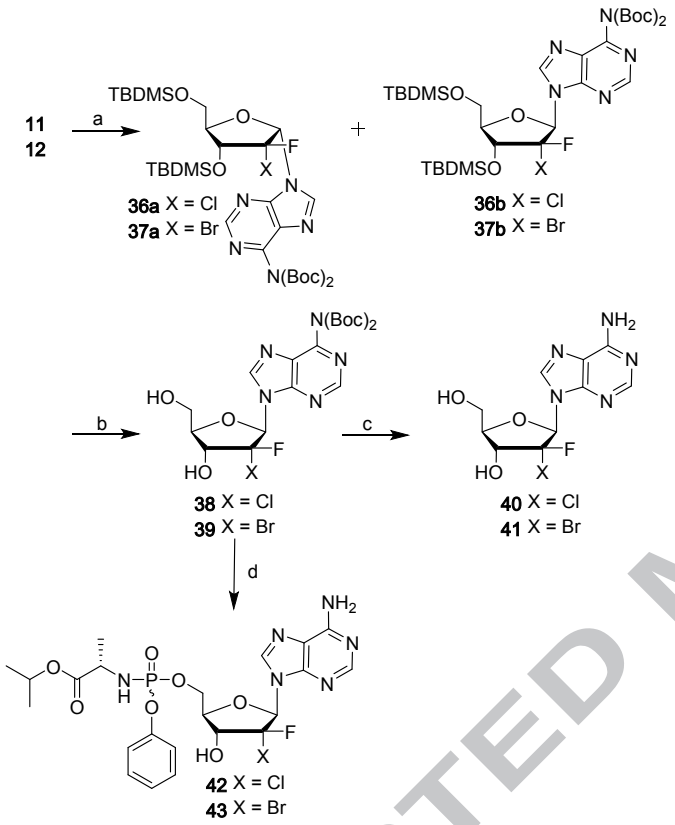
For the synthesis of 2'-β-chloro-2'-α-fluoro cytidine phosphoramidate prodrug **35**, we investigated the protection of the 3'-hydroxyl with a Boc group in order to favor the 5'-regioselectivity of the phosphorylation and increase the solubility of the nucleoside.[14] Thus, nucleoside **26** was selectively protected in the presence of di-*tert*-butyl dicarbonate in dioxane/water (4:1) to give compound **33** in 30% yield. Compound **33** was then reacted with phenyl L-isopropylalaninyl phosphorochloridate reagent **21** in the presence of *tert*-butyl magnesium chloride to give the phosphoramidate prodrug **34**, in 89% yield. Final treatment of compound **34** with a 4 M solution of HCl in dioxane to remove the Boc group gave the desired phosphoramidate prodrug **35** (Scheme 5).



Scheme 5. Synthesis of cytidine analog phosphoramidate **35**. (a) Boc_2O, Na_2CO_3, dioxane/water 4:1, rt, 48 h, 30%; (b) **21**, *t*BuMgCl, THF, 0 ºC to rt, 1 h, 89%; (c) HCl/dioxane 4M, 2 h, rt, 50%.

Purine nucleosides **36**, **37**, **44** and **45** were prepared according to the chemistry described in Scheme 6 and 7. Thus, coupling of lactols **11** and **12** with bis-*N*-Boc protected adenine in the presence of DIAD and PPh_3 gave **36** and **37** as a mixture of α/β anomers (ratio 4:1) separable by flash chromatography on silica

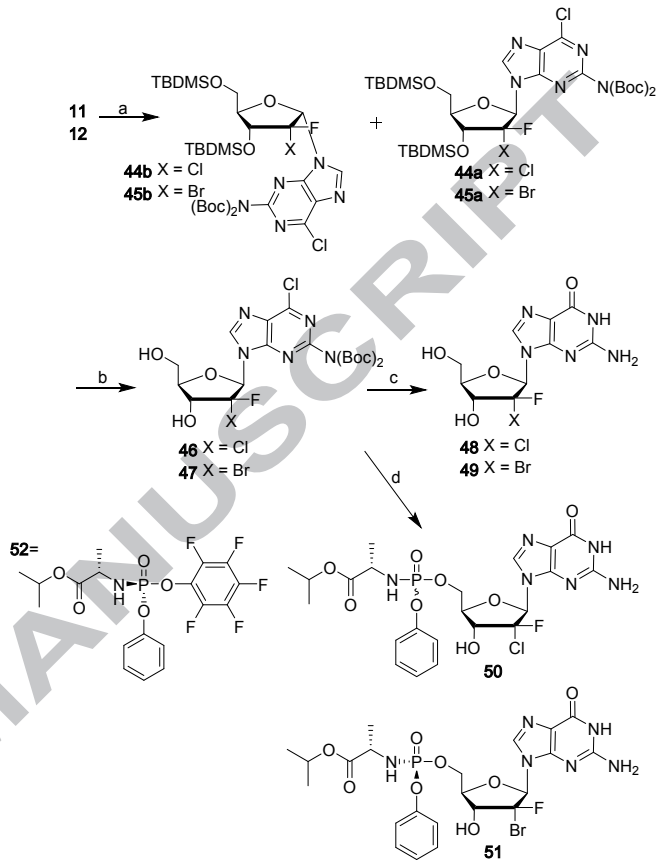
gel. Deprotection of the silyl groups with TBAF in THF lead to the bis-*N*-Boc protected adenine derivatives **38** and **39**, which were treated with a 4 M solution of HCl in dioxane to give the desired adenosine nucleosides **40** and **41**. The corresponding monophosphate prodrugs **42** and **43** were obtained by reaction of protected intermediates **38** and **40** with phenyl L-isopropylalaninyl phosphorochloridate **21** in presence of *tert*-butyl magnesium chloride followed by treatment with a 4 M solution of HCl in dioxane (Scheme 6).



Scheme 6. Synthesis of adenosine analog phosphoramidates **42** and **43**. (a) bis-*N*-Boc A, PPh_3, DIAD, 70 ºC, 1 h, 27% (**36a**), 7% (**36b**), 44% (**37a**), 12% (**37b**); (b) TBAF, THF, rt, 1 h, 99% (**38**), 70% (**39**); (c) 4 M HCl/dioxane, rt, 1 h, 61% (**40**), 60% (**41**); (d) i) **21**, *t*BuMgCl, THF, rt, 1 h; ii) 4 M HCl/dioxane, rt, 1 h, 29% (**42**), 20% (**43**) over two steps.

Similarly, reaction of the 2-bis-*N*-Boc-amino-6-chloro-9H-purine with lactols **11** and **12** under Mitsunobu conditions gave compounds **44** and **45** as a mixture of α/β anomers (ratio 4:1) separable by flash chromatography. Treatment of **44a** and **44b** with TBAF afforded bis-*N*-Boc protected nucleosides **46** and **47** which were treated with 75% aqueous formic acid to give targeted nucleosides **48** and **49**.[15] Compound **46** was reacted with L-isopropylalaninyl phosphorochloridate **21** in presence of *tert*-butyl magnesium chloride and then treated with 75% aqueous formic acid to give the targeted phophoramidate prodrug **50** as a S_p/R_p mixture, in 24% yield over two steps**.**

Interestingly, our first attempts to prepare monophosphate prodrugs **51** from protected intermediates **47** using phenyl L-isopropylalaninyl phosphorochloridate **21** were unsuccessful and led to the formation of 3',5'-di-phosphorylated derivatives. In order to avoid this problem, we turned our attention to the pentafluoro phenyl reagent **52**. Indeed, this diastereomerically pure reagent (S_p isomer), used to synthesize sofosbuvir on a large scale, is known to be selective to the 5'-position when used at low temperature.[16] Thus, reaction of compound **47** with pentafluoro phenyl phosphoramidate **52** in the presence of *tert*-butyl magnesium chloride followed by treatment with an aqueous solution of formic acid gave the desired phosphoramidate prodrug **51 (**S_p isomer) in 22% yield over two steps (Scheme 7).



Scheme 7. Synthesis of guanosine analog phosphoramidates **50** and **51**. (a) 2-bis-*N*-Boc-6-Cl purine, PPh_3, DIAD, 70 ºC, 1 h, 54% (**44a**), 12% (**44b**), 20% (**45a**), 6% (**45b**); (b) TBAF, THF, rt, 1 h, 89% (**46**), 60% (**48**); (c) HCOOH/H_2O (4/1), 60 ºC, 16 h, 54% (**48**), 50% (**49**); (d) i) **21** or **52**, *t*BuMgCl, THF, 0 ºC to rt, 1 h; ii) HCOOH/H_2O (4/1), 60 ºC, 4 h, 24% (**50**), 22% (**51**) over two steps.

All α and β anomers were identified using 1H 2D-NOESY experiments. Fig. 2 shows an example of the NOE effect observed for each isomers. In the case of the β-anomer clear NOE interaction were observed between H2 of the adenine nucleobase and H3' and H5' of the sugar as well as between H1' and H4'. On the other hand, NOE interactions between the H2 and H4', H1' and H5' were observed for the α-anomer (Fig. 2).

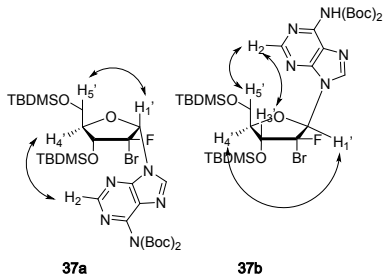


Fig. 2. Anomer assignment for compound **37a** and **37b** *via* NOE experiments.

Despite what was reported by Cen *et al.*,[11] the stereochemistry at the 2'-position could not be clearly established through common NMR experiments. Therefore, suitable crystals were grown from compounds **15a** and **22a** which allowed us to

confirm the *R*-configuration of C-2' of our nucleosides through X-ray diffraction analysis (Fig. 3).

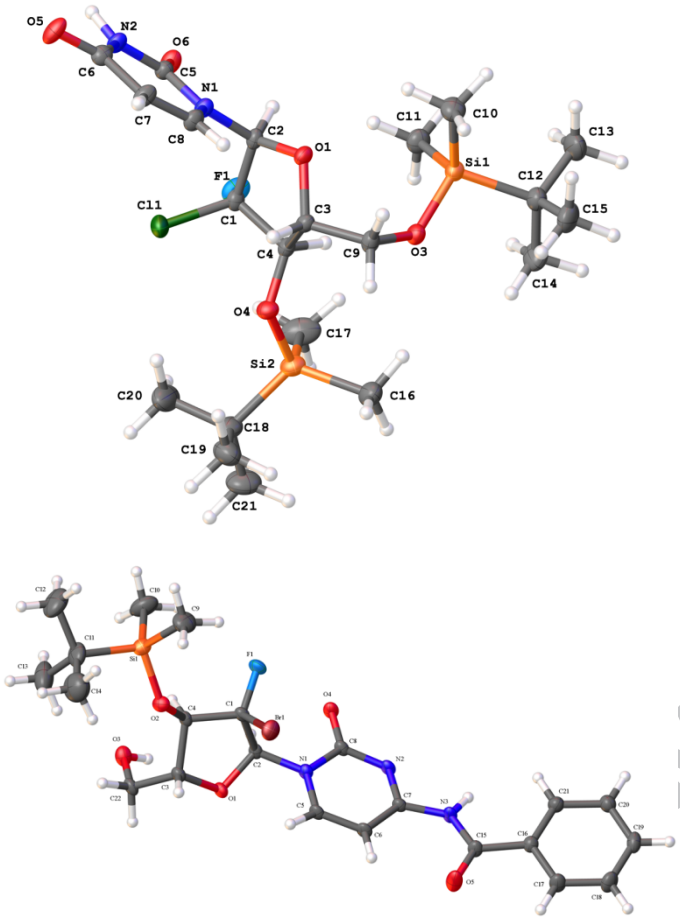


Fig. 3. Single crystal X-ray structure of compound **15a** (top) and **22a** (bottom).

2.2. Antiviral activity and cytotoxicity

Nucleoside analogs **17**, **18**, **26**, **27**, **40**, **41**, **48**, **49** and their corresponding phosphoramidate prodrugs **19**, **20**, **32**, **35**, **42**, **43**, **50**, **51** were evaluated for inhibition of HCV genotype 1b RNA replication in Huh-7 cells using a subgenomic HCV replicon system.[17] Cytotoxicity in Huh-7 cells was determined simultaneously by extraction and amplification of both HCV RNA and cellular ribosomal RNA (rRNA).[18] In addition, cytotoxicity was determined in primary human peripheral blood mononuclear (PBM) cells, human lymphoblastoid cells (CEM), and African Green monkey Vero cells (Table 1).[19,20] Except for 2'-Br,2'-F cytidine derivative **27**, which displayed an EC_{50} of 5.1 μM, none of the nucleosides showed anti-HCV activity at concentration up to 10 μM. On the other hand, monophosphate prodrugs **19**, **20**, **32**, **35** and **42** had $EC_{50's}$ in the low to sub-micromolar range (EC_{50} between 0.4 and 2.7 μM). This difference of potency between the nucleosides and their prodrug counterparts indicates that, once more, the monophosphorylation of modified nucleosides can be problematic and that making prodrug is an unavoidable step in the development of nucleoside analogs. Unfortunately, the increase in potency also came with an increase of cytotoxicity, especially towards Huh-7 cells. Indeed, compounds **19**, **20**, **27**, **32**, **35** and **42** displayed CC_{50} in the low micromolar range in this specific cell line. Interestingly, neither the guanosine analogs **48** and **49** nor their corresponding monophosphate prodrugs (**50** and **51**) showed anti-HCV activity at concentration up to 10 μM. The unusual anti-HCV activity profile observed in these two series of 2'-dihalo nucleoside analogs versus what we have reported previously[8,**Error! Bookmark not defined.**] for related 2'-halo series of nucleoside analogs is a strong indicator that the observed anti-HCV activity presented here is largely, if not completely, due to the cytotoxicity toward the Huh-7 replicon cell line.

Table 1
HCV genotype 1b replicon activity and cytotoxicity of nucleosides **17**, **18**, **26**, **27**, **40**, **41**, **48**, **49** and their phosphoramidate prodrugs **19**, **20**, **32**, **35**, **42**, **43**, **50**, **51**.

Compound	Anti-HCV activity (μM)		Cytotoxicity, CC_{50} (μM)			
	EC_{50}	EC_{90}	Huh-7	PBM	CEM	Vero
17	> 10	> 10	> 100	> 100	> 100	> 100
18	> 10	> 10	> 100	> 100	> 100	> 100
19	0.9 ± 0.3	3.7 ± 1.1	1.3	> 100	90	> 100
20	0.4 ± 0.3	2.0 ± 0.9	8.0	> 100	> 100	> 100
26	> 10	> 10	12	9.3	11	63
27	5.1 ± 0.9	> 10	2.9	> 100	3.2	2.9
32	2.4 ± 0.1	≥ 10	6.8	> 100	> 100	> 100
35	0.7 ± 0.2	3.4 ± 1.7	23	> 100	> 100	> 100
40	> 10	> 10	> 100	> 100	> 100	> 100
41	> 10	> 10	> 100	> 100	> 100	> 100
42	2.7 ± 1.3	≥ 10	1.6	26	15	> 100

43	> 10	> 10	6.9	19	29	71
48	> 10	> 10	> 100	> 100	> 100	> 100
49	> 10	> 10	> 100	> 100	> 100	> 100
50	> 10	> 10	> 100	> 100	84	> 100
51	> 10	> 10	> 100	> 100	> 100	> 100
SOF	0.05 ± 0.02	0.37 ± 0.25	> 100	> 100	> 100	> 100

3. Conclusion

Sixteen 2'-α-chloro,2'-β-fluoro and 2'-α-bromo,2'-β-fluoro nucleosides analogs along with their corresponding phosphoramidate prodrugs were prepared from key dihalogeno lactols **11** and **12**. Among them, compounds **19**, **20**, **27**, **32**, **35** and **42** displayed anti-HCV activity in the low to sub-micromolar range. Unfortunately cytotoxicity was observed for most of these compounds in Huh-7 cells, precluding them for further development for HCV. Based on this study as well as previously reported studies,[21] having a 2'-F group in the beta position seems to significantly increase the probability of cytotoxicity.

4. Experimental section

4.1. General

Anhydrous solvents were purchased from Aldrich Chemical Company, Inc. (Milwaukee). Reagents were purchased from commercial sources. Unless noted otherwise, the materials used in the examples were obtained from readily available commercial suppliers or synthesized by standard methods known to one skilled in the art of chemical synthesis. Microwave reactions were performed with a CEM discover explorer 12 hybrid. ^{1}H, ^{13}C and ^{31}P NMR spectra were taken on a Bruker Ascend™ 400 spectrometer at rt, and reported in ppm downfield from internal tetramethylsilane (for ^{1}H-NMR). NMR processing was performed with Mnova (Mestrelab Research). Deuterium exchange and decoupling experiments were utilized to confirm proton assignments. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), m (multiplet). All J-values are in Hz and calculated by Mnova programs. Mass spectra were determined on a Micromass Platform LC spectrometer using electrospray ionization. Analytic TLC was performed on Analtech GHLF silica gel plates, and preparative TLC on Analtech GF silica gel plates. Column chromatography was performed on Combiflash R_f200.

4.2. Synthetic procedures

*4.2.1 (3R/S,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chlorodihydrofuran-2(3H)-one (**7**)*

To a solution of compound **6** (5 g, 13.9 mmol) in DCM (180 mL) at 0 °C was added triethylamine (11.6 mL, 83.1 mmol) followed by TMSOTf (7.54 mL, 41.6 mmol). The reaction mixture was stirred at 0 °C for 30 minute and a solution of *N*-chlorosuccinimide (2.8 g, 21.0 mmol) in DCM (36 mL) was added. After stirring at 0 °C for another 1 h, the reaction mixture was poured into a saturated solution of $NaHCO_3$ (200 mL) and extracted with DCM (2 x 150 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate 40:1) to afford compound **7** as a 1:1.3 mixture of diastereoisomers (4.22 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): 4.59-4.62 (m, 1.5H), 4.48 (m, 0.5H), 4.38-4.42 (m, 1.5H), 4.16-4.19 (m, 1H), 3.90-3.95 (m, 1.5H), 3.76-3.81 (m, 1.5H), 0.8 and 0.9 (each s, 27H), 0.14-0.08 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 169.7, 86.0, 83.8, 75.1, 70.6, 61.5, 59.9, 59.2, 55.9, 25.9, 25.7, 18.4, 18.0, -4.9, -5.3, -5.4, -5.5. MS (HR-ESI) for $C_{17}H_{36}ClO_4Si_2$ [$(M+H)^+$], Calcd: m/z 395.1841, Found: m/z 395.1834.

*4.2.2 (3R/S,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-bromodihydrofuran-2(3H)-one (**8**)*

To a solution of compound **6** (7.0 g, 19.4 mmol) in DCM (250 mL) at 0 °C was added triethylamine (16.3 mL, 117 mmol) followed by TMSOTf (10.6 mL, 58.3 mmol). The solution was stirred at 0 °C for 30 minutes then, at 0 °C, a solution of *N*-bromosuccinimide (5.2 g, 29.1 mmol) in DCM (75 mL) was added. After stirring at 0 °C for another 1 h, the reaction mixture was poured into a saturated solution of $NaHCO_3$ (300 mL) and extracted with DCM (2 x 200 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate 40:1) to afford compound **8** as a 1:1.5 mixture of diastereoisomers (7.0 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.71 – 4.67 (m, 1H), 4.49 (d, J = 5.6 Hz, 1H), 4.42 – 4.37 (m, 3H), 4.33 (dt, J = 4.7, 2.2 Hz, 1H), 4.25 – 4.20 (m, 1H), 3.98 – 3.92 (m, 1H), 3.83 – 3.74 (m, 3H), 0.93 – 0.86 (m, 48H), 0.20 – 0.04 (m, 33H).^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (d, J = 80.9 Hz), 85.2 (d, J = 28.2 Hz), 75.6, 69.6, 68.8, 60.3, 46.13 (d, J = 3.6 Hz), 39.0, 25.8, 25.6, 21.1, 18.2 18.0, 14.21, -4.9, -5.3, -5.4, -5.5. MS (HR-ESI) for $C_{17}H_{36}BrO_4Si_2$ [$(M+H)^+$]. Calcd: m/z 439.1335. Found: m/z 439.1330.

*4.2.3 (3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chloro-3-fluorodihydrofuran-2(3H)-one (**9**)*

Compound **7** (4 g, 10.1 mmol) and NFSI (4.78 g, 15.2 mmol) were dissolved in anhydrous THF (80 mL). The solution was cooled to -78 °C, and a 1 M solution of LiHMDS in THF (13.2 mL, 13.2 mmol) was added dropwise. The reaction mixture was stirred at -78 °C for 1 h, and quenched with a saturated solution of NH_4Cl (100 mL). The mixture was allowed to warm to rt, and the aqueous layer was extracted with ethyl acetate (3 x 100 mL). The organic layers were combined, washed with a saturated solution of $NaHCO_3$, water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate 40:1) to afford compound **9** (2.25 g, 55%). ^{1}H NMR (400 MHz $CDCl_3$): 4.79 (dd, J = 14.5 Hz, J = 8.4 Hz, 1H), 4.09 (dt, J = 1.9 Hz, 1H), 4.01 (dt, J = 2.3 Hz, 1H), 3.80 (two d, J = 1.9 Hz, 1H), 0.9 (ds, 18H), 0.09 (four s, 12H). ^{19}F

NMR (376 MHz, $CDCl_3$): -127.5 (d, J = 14.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5, 104.4 (d, J = 264 Hz), 80.9, 71.9, 58.6, 25.9, 25.6, 18.4, 18.2, -4.6, -5.1, -5.3, -5.4. MS (HR-ESI) for $C_{17}H_{35}ClFO_4Si_2$ [$(M+H)^+$], Calcd: m/z 413.1740. Found: m/z 413.1746.

*4.2.4 (3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-bromo-3-fluorodihydrofuran-2(3H)-one (**10**)*

Compound **8** (7.0 g, 15.9 mmol) and NFSI (7.5 g, 23.8 mmol) were dissolved in anhydrous THF (140 mL). The solution was cooled to -78 °C and a 1 M solution of LiHMDS in THF (20.7 mL, 20.7 mmol) was added dropwise. The reaction mixture was stirred at -78 °C for another 1 h, and was quenched with a saturated solution of NH_4Cl (200 mL). The mixture was allowed to warm to rt, and the aqueous layer was extracted with ethyl acetate (3 x 150 mL). The organic layers were combined, washed with a saturated solution of $NaHCO_3$, water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate 40:1) to afford compound **10** (4.6 g, 64%). ^1H NMR (400 MHz $CDCl_3$): δ 4.54 (dd, J = 15.3, 8.0 Hz, 1H), 4.04 – 4.00 (m, 1H), 3.98 (dd, J = 2.6, 1.8 Hz, 1H), 3.81 – 3.75 (m, 1H), 0.94 (s, 9H), 0.87 (s, 9H), 0.18 (s, 3H), 0.14 (s, 3H), 0.07 (d, J = 2.6 Hz, 6H).^{19}F NMR (376 MHz, $CDCl_3$): δ -126.98 (dd, J = 15.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 100.7, 97.9, 80.6, 72.1, 58.1, 25.7, 25.6, 18.2, 18.0, -4.6, -5.1, -5.4, -5.5. MS (HR-ESI) for $C_{17}H_{35}BrO_4Si_2$ [$(M+H)^+$]. Calcd: m/z 457.1241. Found: m/z 457.1236.

*4.2.5 (2R/S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-ol (**11**)*

To a solution of compound **9** (5.37 g, 13 mmol) in anhydrous THF (90 mL) was added dropwise $LiAlH[OC(CH_3)_3]_3$ (32.5 mL, 32.48 mmol, 1 M in THF) at 0 °C. The reaction mixture was stirred at rt for 1 h. The reaction was quenched with a saturated solution of NH_4Cl (50 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate 20:1) to afford compound **11** (5.23 g, 97%). ^1H NMR (400 MHz, $CDCl_3$): 5.25 (ddd, J = 12.5 Hz, J = 6.3 Hz, J = 0.9 Hz, 0.5H), 5.16 (d, J = 9.6 Hz, 1H), 4.66 (dd, J = 12.5 Hz, J = 6.7 Hz, 1H), 4.39 (ddd, J = 11.7 Hz, J = 4.0 Hz, J = 0.9 Hz, 0.5H), 4.07-4.11 (m, 0.5H), 3.92 (dt, J = 1.9 Hz, 1H), 3.82 (d, J = 9.3 Hz, 1H), 3.78 (dt, J = 2.5 Hz, 1H), 3.72 and 3.69 (each dd, J = 4.0 Hz, J = 1.8 Hz, 0.5H), 3.61-3.66 (m, 1.5H), 3.50 (d, J = 12.2 Hz, 0.5H), 0.93, 0.92, 0.90 (each s, 27 H), 0.19, 0.16, 0.15, 0.1, 0.07, 0.00 (each s, 18 H). ^{19}F NMR (376 MHz, $CDCl_3$): -122.59 (dd, J = 12.2 Hz, J = 6.4 Hz), -130.55 (d, J = 12.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 116.1 (d, J = 249 Hz), 115.1 (d, J = 263 Hz), 99.5, 99.2, 98.9, 84.5, 82.7, 82.6, 74.7, 74.5, 72.2, 72.0, 62.0, 61.2, 26.0, 25.7, 18.49, 18.47, 18.2, 18.1, -4.4, -4.7, -4.8, -5.3, -5.3, -5.4. MS (HR-ESI) for $C_{17}H_{36}ClFNaO_4Si_2$ [$(M+Na)^+$], Calcd: m/z 437.1722, Found: m/z 437.1717.

*4.2.6 (2R/S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-bromo-3-fluorotetrahydrofuran-2-ol (**12**)*

To a solution of compound **10** (3.5 g, 7.65 mmol) in anhydrous THF (70 mL) was added dropwise $LiAlH[OC(CH_3)_3]_3$ (11.5 mL, 11.5 mmol, 1 M in THF) at 0 °C. The reaction mixture was stirred at rt for 1 h. The reaction was quenched with a saturated solution of NH_4Cl (50 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (hexane/ethyl acetate 20:1) to afford compound **12** (3.43 g, 98%). ^1H NMR (400 MHz, $CDCl_3$): δ 5.32 (dd, J = 9.0, 1.3 Hz, 1H), 5.18 (ddd, J = 12.4, 6.3, 0.9 Hz, 0.5H), 4.49 (dd, J = 13.5, 6.7 Hz, 1H), 4.39 (ddd, J = 12.1, 4.6, 1.0 Hz, 0.5H), 4.06 – 3.99 (m, 0.5H), 3.92 (dt, J = 6.7, 2.0 Hz, 1H), 3.87 (d, J = 9.2 Hz, 1H), 3.79 (t, J = 2.4 Hz, 0.5H), 3.76 (d, J = 2.5 Hz, 1H), 3.67 (m, 1H), 3.63 (d, J = 1.7 Hz, 1H), 3.61 (d, J = 1.7 Hz, 0.5H), 3.49 (d, J = 12.4 Hz, 0.5H), 0.96 – 0.87 (m, 27H), 0.22 – 0.04 (m, 18H).^{19}F NMR (376 MHz, $CDCl_3$): -120.06 (dd, J = 12.2 Hz, J = 6.4 Hz), -127.23 (d, J = 13.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 113.5, 110.8, 99.9 (d, J = 21.4 Hz), 99.0 (d, J = 31.9 Hz), 83.6, 82.3 (d, J = 8.7 Hz), 74.5 (d, J = 24.9 Hz), 72.1 (d, J = 22.3 Hz), 61.7, 60.9, 28.4 – 24.1 (m), 18.2 (d, J = 29.8 Hz).

*4.2.7 (2R/S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl benzoate (**13**)*

To a solution of compound **11** (4.72 g, 11.4 mmol) in DCM (100 mL) was added triethylamine (3.96 mL, 28.4 mmol). The reaction was cooled to 0 °C and benzoyl chloride (1.97 mL, 17.0 mmol) was introduced dropwise. After being stirred for 30 min at 0 °C, the mixture was warmed to rt and stirred for 16 h. The reaction was quenched with methanol (1 mL) and then was washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (hexane/ethyl acetate 20:1) to afford compound **13** (5.65 g, 96%). ^1H NMR (400 MHz, $CDCl_3$): 8.10 and 8.05 (m, 2.2H), 7.57-7.62 (m, 1.2H), 7.42-7.47 (m, 2.4H), 6.54 and 6.46 (m, 1.25H), 4.80 (dd, J = 12.5 Hz, J = 7.9 Hz, 1H), 4.37 (dd, J = 18.1 Hz, J = 4.7 Hz, 0.25 H), 4.21 (m, 0.25 H), 3.85-3.91 (m, 2H), 3.76-3.84 (m, 0.5H), 3.68-3.72 (m, 1H), 0.95 and 0.78 (each s, 22H), 0.07, 0.08, 0.16, 0.20 and 0.22 (each s, 15 H). ^{19}F NMR (376 MHz, $CDCl_3$): -115.15 (dd, J = 17.8 Hz, J = 7.7 Hz) and 128.53 (d, J = 12.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 133.7, 130.3, 130.2, 129.4, 128.6, 114.0 (d, J = 273 Hz), 97.7, 87.4, 82.5, 72.0, 62.0, 60.8, 25.7, 25.8, 25.9, 26.0, 18.5, 18.2, -4.5, -4.7, -4.8, -5.2, -5.3, -5.4. MS (HR-ESI) for $C_{24}H_{40}ClFNaO_5Si_2$ [$(M+Na)^+$], Calcd: m/z 541.1985, Found: m/z 541.1978.

*4.2.8 (2R/S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-bromo-3-fluorotetrahydrofuran-2-yl benzoate (**14**)*

To a solution of compound **12** (744 mg, 1.62 mmol) in DCM (40 mL) was added triethylamine (0.34 mL, 2.43 mmol). The reaction was cooled to 0 °C and benzoyl chloride (0.23 mL, 1.94 mmol) was introduced dropwise. After being stirred for 30 min at 0 °C, the mixture was warmed to rt and stirred for 16 h. The reaction was quenched with methanol (0.20 mL) and then washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (hexane/ethyl acetate 20:1) to afford compound **14** (760 mg, 83%). ^1H NMR (400 MHz, $CDCl_3$): 8.13-8.11 (m, 1H), 8.06-8.03 (m, 2H), 7.62-7.58 (m, 2H), 7.47-7.43 (m, 3H), 6.64 (m, 1H), 6.56 (m, 0.5H), 4.59 (dd, J = 12.5 Hz, J = 7.9 Hz, 1H), 4.37 (dd, J = 18.1 Hz, J = 4.7 Hz, 1H), 4.20 (m, 1H), 3.90-3.86 (m, 2H), 3.81-3.77 (m, 1H), 3.72-3.68 (m, 1H), 0.95 and 0.78 (each s, 22H), 0.25, 0.20, 0.18, 0.09, 0.08, 0.07 (each s, 15 H). ^{19}F NMR (376 MHz, $CDCl_3$): -115.18 (dd, J = 17.8 Hz, J = 7.7 Hz) and

127.00 (d, *J* = 12.1 Hz). MS (HR-ESI) for $C_{24}H_{40}BrFO_5Si_2$ [(M+H)$^+$], Calcd: m/z 563.1582, Found: m/z 563.1588.

*4.2.9 1-((2S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**15a**) and 1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**15b**)*

A solution of compound **13** (1.14 g, 2.19 mmol), uracil (0.5 g, 4.46 mmol) and BSA (2.65 mL, 10.8 mmol) in acetonitrile (10 mL) was stirred at 60 °C for 15 minutes before addition of TMSOTf (2.22 mL, 12.3 mmol). The reaction vessel was then placed into the cavity of microwave reactor and irradiated for 6.5 min at 150 °C. The reaction was quenched by addition of a 5% aqueous solution of $NaHCO_3$ at 0 °C. The aqueous layer was extracted with ethyl acetate (20 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 2:1) to afford **15a** (350 mg, 32%) and **15b** (140 mg, 13%). **15a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (s, 1H), 7.49 (d, *J* = 8.04 Hz, 1H), 6.45 (d, *J* = 10.5 Hz, 1H), 5.72 (dd, *J* = 8.3 Hz, *J* = 2.3 Hz, 1H), 4.61 (dd, *J* = 12.3 Hz, *J* = 4.8 Hz, 1H) 4.20 (m, 1H), 3.75 (m, 2H), 0.93 (two s, 18H), 0.08 (four s, 12H). ^{19}F NMR (376 MHz, $CDCl_3$): -114.05 (t, *J* = 11.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 150.2, 140.4, 117.9 (d, *J* = 252 Hz), 102.0, 88.2, 86.1, 74.8, 61.3, 26.0, 25.7, 18.4, 18.1, -4.7, -4.9, -5.3, -5.32. MS (HR-ESI) for $C_{21}H_{38}ClFN_2NaO_5Si_2$ [(M+Na)$^+$], Calcd: m/z 531.1890, Found: m/z 531.1882. **15b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.73 (dd, *J* = 8.3 Hz, *J* = 1.5 Hz, 1H), 6.37 (d, *J* = 5.6 Hz, 1H), 5.72 (dd, *J* = 8.3 Hz, *J* = 2.4 Hz, 1H), 4.42 (dd, *J* = 16.3 Hz, *J* = 7.3 Hz, 1H, 3.96 (dt, *J* = 2.7 Hz 1H), 3.86 (m, 1H), 3.79 (m, 1H), 0.93 (two s, 18H), 0.08 (four s, 12H). ^{19}F NMR (376 MHz, $CDCl_3$): -122.39 (dd, *J* = 16.04 Hz, *J* = 4.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 150.1, 139.6, 114.6 (d, *J* = 259 Hz), 102.7, 87.8, 82.0, 73.2, 60.3, 26.0, 5.7, 18.5, 18.2, -4.39, -4.91, -5.29, -5.39. MS (HR-ESI) for $C_{21}H_{38}ClFN_2NaO_5Si_2$ [(M+Na)$^+$], Calcd: m/z 531.1890. Found: m/z 531.1884.

*4.2.10 1-((2S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-bromo-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**16a**) and 1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-bromo-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**16b**)*

A solution of compound **14** (1.00 g, 1.77 mmol), uracil (0.22 g, 1.95 mmol) and BSA ((1.29 mL, 5.31 mmol) in acetonitrile (10 mL) was stirred at 60 °C for 15 min before addition of TMSOTf (0.96 mL, 5.31 mmol). The reaction vessel was then placed into the cavity of microwave reactor and irradiated for 6.5 min at 150 °C. The reaction was quenched by addition of a 5% aqueous solution of $NaHCO_3$ at 0 °C. The aqueous layer was extracted with ethyl acetate (20 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 2:1) to afford **16a** (220 mg, 22%) and **16b** (120 mg, 12%). **16a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, *J* = 8.24 Hz, 1H), 6.42 (d, *J* = 10.6 Hz, 1H), 5.73 (d, *J* = 8.3 Hz, 1H), 4.53 (dd, *J* = 12.3 Hz, *J* = 4.8 Hz, 1H) 4.18 (m, 1H), 3.73 (m, 2H), 0.95 (two s, 18H), 0.15 (three s, 12H). ^{19}F NMR (376 MHz, $CDCl_3$): -111.15 (t, *J* = 11.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.3, 150.3, 140.3, 116.7 (d, *J* = 252 Hz), 114.1 101.8, 88.4 (d, *J* = 150 Hz), 85.3, 74.8 (d, *J* = 103 Hz), 61.1, 25.9, 25.7, 18.5, 18.0, -4.5, -4.7, -4.9, -5.4, -5.5. **16b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.05 (s, 1H), 7.76 (dd, *J* = 8.3 Hz, *J* = 1.5 Hz, 1H), 6.56 (d, *J* = 5.6 Hz, 1H), 5.71 (d, *J* = 8.0 Hz, 1H), 4.25 (dd, *J* = 16.3 Hz, *J* = 7.3 Hz, 1H, 3.97 (dt, *J* = 2.7 Hz 1H), 3.85 (m, 1H), 3.77 (m, 1H), 0.93 (two s, 18H), 0.12 (three s, 12H). ^{19}F NMR (376 MHz, $CDCl_3$): -120.56 (dd, *J* = 16.04 Hz, *J* = 4.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 150.1, 139.5, 111.8, 109.1, 102.5, 88.7, 81.6, 73.2, 60.0, 25.7, 18.4, 18.0, -4.4, -4.9, -5.4, -5.5 MS (HR-ESI) for $C_{21}H_{38}BrFN_2O_5Si_2$ [(M+H)$^+$], Calcd: m/z 553.1565, Found: m/z 553.1567.

*4.2.11 1-((2R,3R,4R,5R)-3-Chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**17**)*

To a solution of compound **15b** (21 mg, 0.041 mmol) in anhydrous THF (1 mL) was added TBAF (91 µL, 0.091 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and then quenched with a saturated solution of NH_4Cl. The aqueous layer was extracted with ethyl acetate (3 x 5 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **17** (8 mg, 70%). ^{1}H NMR (400 MHz, MeOD): δ 7.95 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 6.33 (d, *J* = 8.4 Hz, 1H), 5.72 (d, *J* = 8.8 Hz, 1H), 4.36 (dd, *J* = 17.0 Hz, *J* = 6.9 Hz, 1H), 3.9 (m, 2H), 3.78 (m, 1H). ^{19}F NMR (376 MHz, MeOD): -124.82 (dd, *J* = 17.4 Hz, *J* = 8.5 Hz). ^{13}C NMR (100 MHz, MeOD): δ 165.7, 152.1, 142.2, 116.3 (d, *J* = 258 Hz), 102.8, 88.8, 83.6, 74.1, 60.6. MS (HR-ESI) for $C_9H_{11}ClFN_2O_5$ [(M+H)$^+$], Calcd: m/z 281.0341, Found: m/z 281.0333.

*4.2.12 1-((2R,3R,4R,5R)-3-Bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (**18**)*

To a solution of compound **16b** (60 mg, 0.14 mmol) in anhydrous THF (4 mL) was added TBAF (310 µL, 0.31 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and then quenched with a saturated solution of NH_4Cl. The aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **18** (41 mg, 90%). ^{1}H NMR (400 MHz, MeOD): δ 7.97 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 5.72 (d, *J* = 8.8 Hz, 1H), 4.22 (dd, *J* = 17.0 Hz, *J* = 6.9 Hz, 1H), 3.90 (m, 2H), 3.78 (m, 1H). ^{19}F NMR (376 MHz, MeOD): -124.12 (dd, *J* = 17.4 Hz, *J* = 8.5 Hz). ^{13}C NMR (100 MHz, MeOD): δ 164.3, 153.5, 140.8, 128.6, 122.2, 120.1, 112.5, 101.3, 88.3, 81.9, 72.7, 68.1, 59.1, 50.5, 20.4. MS (HR-ESI) for $C_9H_{310}BrFN_2O_5$ [(M+H)$^+$], Calcd: m/z 324.9835, Found: m/z 324.9833.

*4.2.13 Isopropyl ((((2R,3R,4R,5R)-4-chloro-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**19**)*

To a solution of compound **17** (30 mg, 0.11 mmol) in THF (2 mL) was added *N*-methylimidazole (68 µL, 0.85 mmol) then (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate (0.21 g, 0.69 mmol) in THF (0.69 mL). The mixture was stirred at rt for 1 h, quenched with water and extracted with ethyl acetate (5 mL). The organic layer was washed with water twice, dried

over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 20:1) to afford compound **19** as a R_P:S_P diastereomeric mixture (~1:1) (20 mg, 34%).^{1}H NMR (400 MHz, MeOD): δ 7.66 and 7.58 (each dd, *J* = 8.3 Hz, *J* = 2.4 Hz, 1H), 7.35-7.38 (m, 2H), 7.20-7.27 (m, 3H), 6.33-6.37 (m, 1H), 5.69 and 5.64 (each d, *J* = 8.3 Hz, 1H), 4.93-4.99 (m, 1H), 4.29-4.41 (m, 3H), 4.10 (m, 1H), 3.9 (m, 1H), 1.29-1.36 (m, 3H), 1.22-1.24 (m 6H). ^{19}F NMR (376 MHz, MeOD): -124.88 (m). ^{31}P NMR (162 MHz, MeOD): 3.65, 3.58. ^{13}C NMR (100 MHz, MeOD): δ 173.2, 162.8,150.5, 150.3, 140.1, 130.1, 125.6, 120.1, 113.7 (d, *J* = 257 Hz), 103.0, 87.3, 80.4, 73.4, 69.8, 64.2, 50.6, 21.8, 20.9. MS (HR-ESI) for $C_{21}H_{27}ClFN_3O_9P$ [(M+H)$^+$], Calcd: m/z 550.1157, Found: m/z 550.1149.

*4.2.14 Isopropyl ((((2R,3R,4R,5R)-4-bromo-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**20**)*

To a solution of compound **18** (22 mg, 0.077 mmol) in THF (2 mL) was added *N*-methylimidazole (31 µL, 0.39 mmol) then (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate **21** (0.071 g, 0.23 mmol) in THF (0.23 mL). The mixture was stirred at rt for 1 h, quenched with water and extracted with ethyl acetate (5 mL). The organic layer was washed with water twice, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 20:1) to afford compound **20** as a diastereomeric mixture of R_P:S_P (~1:1) (18 mg, 46%). ^{1}H NMR (400 MHz, MeOD): δ 7.66 and 7.60 (each dd, *J* = 8.3 Hz, *J* = 2.4 Hz, 1H), 7.38-7.34 (m, 2H), 7.27-7.20 (m, 3H), 6.52-6.47 (m, 1H), 5.69 and 5.64 (each d, *J* = 8.3 Hz, 1H), 4.99 (m, 1H), 4.40-4.30 (m, 1H), 4.29-4.20 (m, 1H), 4.15-4.05 (m, 1H), 3.95-3.85 (m, 1H) 1.35-1.31 (m, 3H), 1.25-1.21 (m 6H). ^{19}F NMR (376 MHz, MEOD): -124.53 (m). ^{31}P NMR (162 MHz, MeOD): 3.64, 3.57. ^{13}C NMR (100 MHz, MeOD): δ 173.2, 164.1, 150.5, 140.1, 129.4, 124.9, 120.0, 101.7, 88.0, 80.4, 68.8, 64.8, 50.3, 20.5, 18.9.: MS (HR-ESI) for $C_{21}H_{27}BrFN_3O_9P$ [(M+H)$^+$], Calcd: m/z 594.0652, Found: m/z 594.0652

*4.2.15 N-(1-((2S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-3-chloro-3-fluoro-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**22a**) and N-(1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-3-chloro-3-fluoro-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**22b**)*

A solution of compound **13** (1.0 g, 1.92 mmol), 4-*N*-benzoylcytosine (660 mg, 3.06 mmol) and BSA (1.9 mL, 7.66 mmol) in acetonitrile (10 mL) was stirred at rt for 15 min before addition of TMSOTf (3.11 mL, 17.23 mmol). The reaction vessel was then placed into the cavity of a microwave reactor and irradiated for 12 min at 150 °C. The reaction was quenched by addition of 5% aqueous solution of $NaHCO_3$ (20 mL) at 0 °C. The aqueous layer was extracted with ethyl acetate (3 x 20 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 2:1) to afford **22a** (352 mg, 37%) and **22b** (120 mg, 13%). **22a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (br s, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.61 (m, 2H), 7.51 (m, 2H), 6.77 (d, *J* = 8.1 Hz, 1H), 4.73 (dd, *J* = 12.3 Hz, *J* = 7.1 Hz, 1H), 4.20 (m, 1H), 3.92 (m, 1H), 3.75 (m, 1H), 2.76 (br s, 1H), 0.92 (s, 9H), 0.17 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$): -116.55 (s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 162.9, 155.2, 145.1, 133.5, 133.0, 129.2, 127.8, 117.7 (d, *J* = 258 Hz), 96.6, 88.2, 84.8, 74.5, 60.6, 25.7, 18.3, -4.6, -4.9. MS (HR-ESI) for $C_{22}H_{30}ClFN_3O_5Si$ [(M+H)$^+$], Calcd: m/z 498.1627, Found: m/z 498.1624. **22b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (br s, 1H), 8.17 (d, *J* = 7.3 Hz, 1H), 7.86 (d, *J* = 7.3 Hz, 1H), 7.60 (m, 2H), 7.49 (m, 2H), 6.55 (d, *J* = 6.8 Hz, 1H), 4.51 (dd, *J* = 16.6 Hz, *J* = 7.4 Hz, 1H), 4.09 (m, 1H), 3.95 (m, 1H), 3.84 (m, 1H), 2.7 (br s, 1H), 0.93 (s, 9H), 0.15 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$): -122.31 (s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 162.9, 155.1, 145.3, 133.4, 133.0, 129.1, 127.8, 114.5 (d, *J* = 260 Hz), 97.4, 89.2, 82.2, 73.4, 59.7, 25.8, 18.1, -4.6, -4.9. MS (HR-ESI) for $C_{22}H_{30}ClFN_3O_5Si$ [(M+H)$^+$], Calcd: m/z 498.1627, Found: m/z 498.1620.

*4.2.16 N-(1-((2S,3R,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-3-bromo-3-fluoro-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**23a**) and N-(1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-3-bromo-3-fluoro-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**23b**)*

A solution of compound **14** (1.5 g, 2,67 mmol), 4-*N*-benzoylcytosine (634 mg, 2.94 mmol) and BSA (1.94 mL, 8.01 mmol) in acetonitrile (10 mL) was stirred at rt for 15 min before addition of TMSOTf (1.45 mL, 8.01 mmol). The reaction vessel was then placed into the cavity of microwave reactor and irradiated for 12 min at 150 °C. The reaction was quenched by addition of a 5% aqueous solution of $NaHCO_3$ (20 mL) at 0 °C. The aqueous layer was extracted with ethyl acetate (3 x 20 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 2:1) to afford **23a** (300 mg, 21%) and **23b** (200 mg, 14%). **23a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89-7.84 (m, 3H), 7.59 (t, *J*= 14.8 Hz, 2H), 7.50 (t, *J* = 15.2 Hz, 2H), 6.68 (d, *J* = 8.0 Hz, 1H), 4.60 (dd, *J* = 13.5, 7.2 Hz, 1H), 4.18 – 4.15 (m, 1H), 3.92 – 3.70 (m, 2H), 2.52 (BR S, 1H), 0.91 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -113.25 (s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 162.9, 155.1, 144.9, 133.3, 129.1, 127.6, 117.1, 114.4, 96.2, 88.2 (d, *J*= 137.6 Hz), 83.9, 74.5 (d, J= 94.9 Hz), 60.3, 25.6, 18.0, -4.5, -4.9. . MS (HR-ESI) for $C_{22}H_{29}BrFN_3O_5Si$ [(M+H)$^+$]. Calcd: m/z 542.1122. Found: m/z 542.1117. **23b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 7.5 Hz, 1H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.63-7.58 (m, 2H), 7.52 – 7.48 (m, 2H), 6.71 (d, *J* = 5.6 Hz, 1H), 4.36 (dd, *J* = 17.1, 7.6 Hz, 1H), 4.14 – 4.08 (m, 1H), 3.95 (dt, *J* = 4.9, 2.2 Hz, 1H), 3.84 (dd, *J* = 12.4, 2.8 Hz, 1H), 0.94 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H). ^{19}F NMR ($CDCl_3$, 376 MHz) δ -121.28 (dd, *J* = 12.0, 7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 162.6, 155.2, 145.4, 133.5, 132.7, 129.0, 127.9, 111.5, 97.2, 81.6, 73.2, 59.4, 29.7, 25.6, 18.0, -4.5, -4.9. MS (HR-ESI) for $C_{22}H_{29}BrFN_3O_5Si$ [(M+H)$^+$]. Calcd: m/z 542.1122. Found: m/z 542.1117.

*4.2.17 N-(1-((2R,3R,4R,5R)-3-Chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**24**)*

To a solution of compound **22b** (70 mg, 0.14 mmol) in anhydrous THF (2 mL) was added TBAF (180 µL, 0.18 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and then quenched with a saturated solution of NH_4Cl (4 mL). The aqueous layer was extracted with ethyl acetate (3 x 4 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **24** (39 mg, 73%). ^{1}H NMR (400 MHz,

MeOD): δ 8.51 (d, *J* = 7.6 Hz, 1H), 7.97 (m, 2H), 7.64 (m, 2H), 7.52 (m, 2H), 6.51 (d, *J* = 6.1 Hz, 1H), 4.44 (dd, *J* = 16.1 Hz, *J* = 7.3 Hz, 1H), 3.93-4.01 (m, 2H), 3.83 (m, 1H). ^{19}F NMR (376 MHz, MeOD): -124.97 (dd, *J* = 16.6 Hz, *J* = 5.3 Hz). ^{13}C NMR (100 MHz, MeOD): δ 169.1, 165.4, 157.7, 146.4, 134.59, 134.17, 129.8, 129.2, 116.2 (d, *J* = 257 Hz), 98.5, 90.0, 83.4, 73.6, 60.3. MS (HR-ESI) for $C_{16}H_{16}ClFN_3O_5$ [(M+H)$^+$], Calcd: m/z 384.0763, Found: m/z 384.0753.

*4.2. 18 N-(1-((2R,3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**25**)*

To a solution of compound **23b** (40 mg, 0.074 mmol) in anhydrous THF (2 mL) was added TBAF (96 µL, 0.096 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and then quenched with a saturated solution of NH_4Cl (4 mL). The aqueous layer was extracted with ethyl acetate (3 x 4 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **25** (28 mg, 90%). ^{1}H NMR (400 MHz, MeOD) δ (ppm) 8.56 (d, *J* = 7.6 Hz, 1H), 7.98 (d, *J* = 6.8 Hz, 2H), 7.67 – 7.53 (m, 4H), 6.68 (d, *J* = 5.6 Hz, 1H), 4.28 (dd, *J* = 16.8, 7.6 Hz, 1H), 4.01 – 3.93 (m, 2H), 3.83 (dd, *J* = 12,0, 2.4 Hz, 1H). ^{19}F NMR (376 MHz, MeOD) δ -123.50 (d, *J* = 18.4 Hz). ^{13}C NMR (100 MHz, MeOD): δ 169.1, 164., 157.7, 144.9, 133.2, 132.7, 128.4, 127.8, 112.3, 97.1, 89.5, 81.7, 72.5, 58.7, 58.1. MS (HR-ESI) for $C_{16}H_{15}BrFN_3O_5$ [(M+H)$^+$]. Calcd: m/z 428.0257. Found: m/z 428.0249.

*4.2.19 4-Amino-1-((2R,3R,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1H)-one (**26**)*

A solution of compound **24** (39 mg, 0.1 mmol) in a saturated solution of ammonia in MeOH (2 mL) was stirred at rt for 16 h The solution was evaporated to dryness under reduced pressure and co-evaporated several times with methanol. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **26** (20 mg, 71%). ^{1}H NMR (400 MHz, MeOD): δ 7.95 (dd, *J* = 7.6 Hz, *J* = 1.9 Hz, 1H), 6.45 (d, *J* = 8.2 Hz, 1H), 5.93 (d, *J* = 7.3 Hz, 1H), 4.36 (dd, *J* = 17.2 Hz, *J* = 7.2 Hz, 1H), 3.94 (m, 1H), 3.89 (m, 1H), 3.8 (m, 1H). ^{19}F NMR (376 MHz, MeOD): -124.72 (dd, *J* = 16.7 Hz, *J* = 7.6 Hz). ^{13}C NMR (100 MHz, MeOD): δ 167.6, 158.0, 143.7, 116.4 (d, *J* = 258 Hz), 96.2, 89.3, 83.1, 74.1, 60.7. MS (HR-ESI) for $C_9H_{12}ClFN_3O_4$ [(M+H)$^+$], Calcd: m/z 280.0500, Found: m/z 280.0492.

*4.2.20 4-Amino-1-((2R,3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1H)-one (**27**)*

A solution of compound **25** (32 mg, 0.073 mmol) in a saturated solution of ammonia in MeOH (2 mL) was stirred at rt for 16 h. The solution was evaporated to dryness under reduced pressure and co-evaporated several times with methanol. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **27** (23 mg, 96%). ^{1}H NMR (400 MHz, MeOD) δ 7.96 (dd, *J* = 7.6, 1.8 Hz, 1H), 6.60 (d, *J* = 8.2 Hz, 1H), 5.90 (d, *J* = 7.6 Hz, 1H), 4.20 (dd, *J* = 17.6, 7.2 Hz, 1H), 3.96 – 3.89 (m, 1H), 3.89 – 3.84 (m, 1H), 3.78 (dd, *J* = 12.4, 3.3 Hz, 1H). ^{19}F NMR (376 MHz, MeOD) δ -123.93 (m). ^{13}C NMR (100 MHz, MeOD): δ 165.6, 155.8, 144.7, 141.6, 132.2, 130.9, 128.5, 112.5, 109.8, 94.7, 93.3, 88.9, 88.8, 81.6, 729, 72.6, 59.1, 29.3 MS (HR-ESI) for $C_9H_{12}BrFN_3O_4$ [(M+H)$^+$], Calcd: m/z 323.9995, Found: m/z 323.9993.

*4.2.21 4-Amino-1-((2R,3R,4R,5R)-3-bromo-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)pyrimidin-2(1H)-one (**28**)*

Imidazole (18 mg, 0.26 mmol) was added to a mixture of compound **27** (26 mg, 0.086 mmol) and TBDMSCl (26 mg, 0.17 mmol) in DCM (4 mL). The mixture was stirred for 16h at rt, quenched with water and the crude mixture was extracted with ethyl acetate (5 mL). The organic layer was washed with a saturated aqueous solution of NH_4Cl, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (10:1) to afford compound **28** (27 mg, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.75 (dd, *J* = 7.5, 1.0 Hz, 1H), 6.69 (d, *J* = 5.7 Hz, 1H), 5.72 (d, *J* = 7.5 Hz, 1H), 4.23 (dd, *J* = 16.8, 7.5 Hz, 1H), 3.97 (dt, *J* = 11.6, 2.6 Hz, 1H), 3.83 (dd, *J* = 7.5, 2.3 Hz, 1H), 3.78 (dd, *J* = 11.7, 2.2 Hz, 1H), 0.93 (s, 9H), 0.92 (s, 9H), 0.16 (s, 3H), 0.11 (s, 9H). ^{19}F NMR ($CDCl_3$, 376 MHz) δ -121.70 (dd, *J* = 16.9, 4.6 Hz). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.98 (s), 155.80 (s), 141.02 (s), 112.36 (s), 109.65 (s), 95.12 (s), 89.34 (d, *J* = 19.0 Hz), 81.23 (s), 73.50 (d, *J* = 25.3 Hz), 60.34 (s), 26.14 (s), 25.84 (s), 18.60 (s), 18.25 (s), -4.12 (s), -4.71 (s), -5.19 (s), -5.26 (s). . MS (HR-ESI) for $C_{21}H_{39}BrFN_3O_4Si_2$ [(M+H)$^+$]. Calcd: m/z 552.1647. Found: m/z 552.1642.

*4.2.22 Benzyl (1-((2R,3R,4R,5R)-3-bromo-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)carbamate (**29**)*

To a solution of compound **28** (27 mg, 0.049 mmol) and benzyl chloroformate (21 µL, 0.15 mmol) in DCM (5 mL) was added DMAP (36 mg, 0.29 mmol). The reaction was stirred at rt for 1.5 h, quenched with water (5 mL) and extracted with ethyl acetate (5 mL). The organic layer was washed with HCl 1N, $NaHCO_3$, brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane/ethyl acetate 1:1) to afford compound **29** (25 mg, 74%).^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.18 (d, *J* = 7.6 Hz, 1H), 7.75 (s, 1H), 7.37 (brs, 5H), 7.22 (d, *J* = 7.6 Hz, 1H), 6.72 (d, *J* = 4.5 Hz, 1H), 5.22 (brs, 2H), 4.26 (dd, *J* = 16.6, 7.8 Hz, 1H), 4.02 (d, *J* = 11.8 Hz, 1H), 3.90 (d, *J* = 7.7 Hz, 1H), 3.81 (dd, *J* = 11.8, 1.9 Hz, 1H), 0.96 (s, 9H), 0.93 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H), 0.11 (s, 3H). ^{19}F NMR ($CDCl_3$, 376 MHz) δ -122.22 (d, *J* = 16.6 Hz). MS (HR-ESI) for $C_{29}H_{46}BrFN_3O_6Si_2$ [(M+H)$^+$]. Calcd: m/z 686.2014. Found: m/z 686.2009.

*4.2.23 Benzyl (1-((2R,3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)carbamate (**30**)*

To a solution of compound **29** (25 mg, 0.036 mmol) in THF (4 mL) was added Et_3N.3HF (0.06 mL, 0.36 mmol). The reaction mixture was stirred for 36 h at rt then Et_3N (0.06 mL) was added to quench the reaction. After removal of the volatiles under reduced pressure, the crude product was purified using silica gel chromatography (CH_2Cl_2/MeOH 10:1) to afford compound **30** (11 mg, 67%).^{1}H NMR (MeOD, 400 MHz) δ (ppm) 8.44 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.50 – 7.26 (m, 6H), 6.64 (d, *J* = 6.2 Hz, 1H), 5.23 (s, 2H), 4.25 (dd, *J* = 17.2, 7.6 Hz, 1H), 3.98 – 3.91 (m, 2H), 3.81 (dd, *J* = 12.5, 2.9 Hz, 1H). ^{19}F NMR (MeOD, 376 MHz) δ -124.35 (dd, *J* = 17.0, 4.8 Hz). ^{13}C NMR (100 MHz, MeOD): δ 163.9, 156.1, 153.1, 144.5, 135.7, 128.2, 127.9, 112.2, 109.5, 95.5, 89.6, 89.4, 81.7 (d, *J*= 23.56 Hz), 72.4, 72.2, 67.3, 58.8. MS (HR-ESI) for $C_{17}H_{17}BrFN_3O_6$ [(M+H)$^+$]. Calcd: m/z 458.0363. Found: m/z 458.0355.

*4.2.24 Isopropyl ((((2R,3R,4R,5R)-5-(4-(((benzyloxy)carbonyl)amino)-2-oxopyrimidin-1(2H)-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**31**)*

To a solution of compound **30** (35 mg, 0.076 mmol) in THF (3 mL) was added NMI (30 µL, 0.38 mmol) then (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate **21** (70 mg, 0.23 mmol) in THF (0.23 mL). The mixture was stirred at rt for 4 h, quenched with water (1 mL) and extracted with ethyl acetate (2 mL). The organic layer was washed with water twice, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 20:1) to afford compound **31** (11 mg, 20%) as a diastereomeric mixture of R_P/S_P (~1:1).^{1}H NMR (MeOD, 400 MHz) δ (ppm) 8.00 (ddd, *J* = 43.8, 7.7, 1.8 Hz, 1H), 7.47 – 7.15 (m, 11H), 6.65 (dd, *J* = 9.0 Hz, 1H), 5.24 (s, 2H), 5.04 – 4.94 (m, 1H), 4.59 – 4.35 (m, 2H), 4.26 – 4.19 (m, 1H), 4.16 – 4.12 (m, 1H), 3.97 – 3.86 (m, 1H), 1.38 – 1.31 (m, 3H), 1.22 (dd, *J* = 6.2, 2.1 Hz, 6H). ^{19}F NMR (MeOD, 376 MHz) δ -124.22 – -124.67 (m). ^{31}P NMR (MeOD, 162 MHz) δ 3.66 (d, *J* = 13.5 Hz). LR-MS: calculated for $C_{29}H_{34}BrFN_4O_{10}P$ 727.12, found 726.11, 728.20,.

*4.2.25 Isopropyl ((((2R,3R,4R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**32**)*

To a round bottom flask charged with compound **31** (23 mg, 0.032 mmol) in ethanol (2 mL) was added 1,4-cyclohexadiene (0.1 mL, 0.70 mmol) and Pd/C (10 mg, 0.01 mmol). After 2 h at 25 °C, the mixture was filtrated through a pad of celite and the filtrate was then concentrated under reduced pressure. Purification by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) gave nucleotide **32** in 55% yield (13 mg) as a diastereomeric mixture of R_p/S_p (~1:1).^{1}H NMR (MeOD, 400 MHz) δ (ppm) 7.61 (ddd, *J* = 28.8, 7.6, 2.3 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.27 – 7.20 (m, 3H), 6.61 (dd, *J* = 11.2, 8.4 Hz, 1H), 5.86 (dd, *J* = 12.0, 7.6 Hz, 1H), 5.04 – 4.95 (m, 1H), 4.54 – 4.31 (m, 2H), 4.19 (ddd, *J* = 17.5, 6.5, 2.0 Hz, 1H), 4.10 (brs, 1H), 3.93 – 3.88 (m, 1H), 1.37 – 1.31 (m, 3H), 1.29 (brs, 2H), 1.25 – 1.20 (m, 4H). ^{19}F NMR (MeOD, 376 MHz) δ -124.46 (m). ^{31}P NMR (MeOD, 162 MHz) δ 3.60, 3.53. ^{13}C NMR (100 MHz, MeOD): δ 171.5, 165.5, 155.1, 150.2, 143.8, 130.1, 121.3, 120.3, 106.1, 98.1, 94.1, 78.4, 76.7, 69.5, 63.0, 46.9, 21.6, 19.1. MS (HR-ESI) for $C_{21}H_{28}BrFN_4O_8P$ [$(M+H)^+$], Calcd: m/z 593.0812, Found: m/z 593.0813.

*4.2.26 (2R,3R,4R,5R)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-4-chloro-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-yl tert-butyl carbonate (**33**)*

To a solution of compound **26** (109 mg, 0.39 mmol) and di-*tert*-butyl dicarbonate (85 mg, 0.39 mmol) in dioxane (8 mL) was added a solution of Na_2CO_3 (207 mg, 1.9 mmol) in water (2 mL). The reaction mixture was stirred at rt for 48 h and diluted with water (4 mL). The product was extracted with ethyl acetate (5 mL), washed with water, brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **33** (44 mg, 30%). ^{1}H NMR (400 MHz, MeOD): δ 7.80 (dd, *J* = 7.6 Hz, *J* = 2.8 Hz, 1H), 6.45 (d, *J* = 6.4 Hz, 1H), 5.93 (d, *J* = 7.9 Hz, 1H), 5.31 (dd, *J* = 16.9 Hz, *J* = 6.2 Hz, 1H), 4.10 (m, 1H), 3.92 (m, 1H), 3.78 (m, 1H), 1.50 (s, 9H). ^{19}F NMR (376 MHz, MeOD): -124.07 (t, *J* = 14.7 Hz). ^{13}C NMR (100 MHz, MeOD): δ 167.6, 157.8, 153.2, 143.0, 113.8 (d, *J* = 257 Hz), 96.5, 89.1, 85.0, 81.3, 76.9, 60.8, 27.8. MS (HR-ESI) for $C_{14}H_{20}ClFN_3O_6$ [$(M+H)^+$], Calcd: m/z 380.1025, Found: m/z 380.1017.

*4.2.27 Isopropyl ((((2R,3R,4R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3-((tert-butoxycarbonyl)oxy)-4-chloro-4-fluorotetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate diastereomeric mixture (**34**)*

To a solution of compound **33** (44 mg, 0.11 mmol) in THF (3 mL) was added *tert*-butylmagnesium chloride (180 µL, 0.18 mmol, 1 M in THF) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and a solution of L-isopropylalaninyl phosphorochloridate **21** (105 mg, 0.34 mmol) in THF (0.34 mL) was added. The reaction mixture was stirred for 1 h at rt. The solvent was evaporated and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **34** as a diastereomeric mixture of R_P/S_P (~1:1) (67 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.28-7.35 (m, 3H), 7.13-7.23 (m, 3H), 6.51-6.57 (m, 1H), 5.76 and 5.68 (each d, *J* = 7.6 Hz, 1H), 5.17-5.22 (m, 1H), 4.97-5.00 (m, 1H), 4.29-4.45 (m, 2H), 4.21 (m, 1H), 3.91-3.99 (m, 2H), 1.51 (s, 9H), 1.36-1.41 (m, 3H), 1.16-1.23 (m, 6H). ^{19}F NMR (376 MHz, $CDCl_3$): -125.8 (s), 126.1 (s). ^{32}P NMR (162 MHz, $CDCl_3$): 2.51, 2.38. ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 165.7, 155.5, 151.7, 150.7, 142.2, 129.93,129.88, 125.3, 120.3, 111.5 (d, *J* = 254 Hz), 95.5, 87.1, 84.7, 84.7, 69.5, 64.5, 50.5, 27.7, 21.8, 21.0. MS (HR-ESI) for $C_{26}H_{36}ClFN_4O_{10}P$ [$(M+H)^+$], Calcd: m/z 649.1842, Found: m/z 649.1837.

*4.2.28 Isopropyl ((((2R,3R,4R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**35**)*

Compound **34** (67 mg, 0.1 mmol) was treated with a 4 M solution of HCl in dioxane for 2 h at rt. Solvents were removed under vacuum and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **35** as a diastereomeric mixture of R_p/S_p (~1:1) (28 mg, 50%). ^{1}H NMR (400 MHz, MeOD): δ 7.64 & 7.56 (each dd, *J* = 7.6 Hz, *J* = 2.3 Hz, 1H), 7.34-7.40 (m, 2H), 7.20-7.27 (m, 3H), 6.44-6.49 (m, 1H), 5.85 & 5.89 (each d, *J* = 7.6 Hz, 1H), 4.96-5.00 (m, 1H), 4.35-4.43 (m, 2H), 4.30 (m, 1H), 4.10 (m, 1H), 3.91 (m, 1H), 1.34 (m, 3H), 1.22 (m, 6H). ^{19}F NMR (376 MHz, MeOD): -124.83 (s). ^{31}P NMR (162 MHz, MeOD): 3.60, 3.54. ^{13}C NMR (100 MHz, MeOD): δ 174.5, 167.6, 157.9, 152.1, 142.8, 130.8, 126.3, 121.4, 115.8 (d, *J* = 257 Hz), 96.5, 89.1, 81.4, 74.9, 70.2, 65.9, 51.8, 21.9, 20.5. MS (HR-ESI) for $C_{21}H_{28}ClFN_4O_8P$ [$(M+H)^+$], Calcd: m/z 549.1317, Found: m/z 549.1313.

*4.2.29 6-bis(tert-Butoxycarbonyl)amino-9-(3,5-di-O-tert-butyldimethylsilyl-2'-deoxy-2'-chloro-2'-fluoro-D-ribofuranosyl)purine (**36**)*

To a solution of compound **11** (0.5 g, 1.2 mmol), 6-bis(*tert*-butoxycarbonyl)amino-9H-purine (0.2 g, 1.29 mmol), triphenylphosphine (0.47 g, 1.79 mmol) in THF (10 mL) was added DIAD (0.36 mL, 1.79 mmol) dropwise at 0 °C. The reaction mixture was heated at 70 °C for 1 h, and then diluted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 9:1) to afford **36a** (236 mg, 27%) and **36b** (59 mg, 7%). **36a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.45 (s, 1H), 6.67 (d, *J* = 9.0 Hz, 1H), 4.77 (dd, *J* = 13.0 Hz, 4.8 Hz, 1H), 4.36 (m, 1H), 3.81 (m, 2H), 1.44 (s, 18H), 0.92 (two s, 18H), 0.10 (four s,

12H). ^{19}F NMR (MHz, $CDCl_3$): -116.34 (t, J = 11.2 Hz, 1F). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.6, 151.8, 151.34, 144.1, 131.5, 116.9 (d, J = 251 Hz), 87.6, 86.5, 74.9, 61.5, 26.0, 25.7, 18.5, 18.2, -4.6, -4.8, -5.3, -5.4. MS (HR-ESI) for $C_{32}H_{56}ClFN_5O_7Si_2$ [$(M+H)^+$], Calcd: m/z 732.3391, Found: m/z 732.3394. **36b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89(s, 1H), 8.44 (s, 1H), 6.56 (d, J = 10.6 Hz, 1H), 4.63 (dd, J = 15.8 Hz, 5.5 Hz, 1H), 4.03 (m, 1H), 3.97 (m, 1H), 3.87 (m, 1H), 1.44 (s, 18H), 0.95 (two s, 18H), 0.14 (four s, 12H). ^{19}F NMR (MHz, $CDCl_3$): -123.24 (t, J = 12.5 Hz, 1F). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.4,152.7, 150.7, 150.4, 143.0, 128.7, 114.1 (d, J = 262 Hz), 87.3, 83.9, 83,7, 74.1, 73.8, 61.1, 27.9, 26.1, 25.4, 18.6, 18.2, -3.4, -4.5, -4.8, -5.2. MS (HR-ESI) for $C_{32}H_{56}ClFN_5O_7Si_2$ [$(M+H)^+$], Calcd: m/z 732.3391, Found: m/z 732.3390.

*4.2.30 6-bis(tert-Butoxycarbonyl)amino-9-(3,5-di-O-tert-butyldimethylsilyl-2'-deoxy-2'-bromo-2'-fluoro-D-ribofuranosyl)purine (**37a** and **37b**)*

To a solution of compound **12** (1.0 g, 2.17 mmol), 6-bis(*tert*-butoxycarbonyl)amino-9H-purine (0.80 g, 2.39 mmol) and triphenylphosphine (0.85 g, 0.33 mmol) in THF (25 mL) was added DIAD (0.64 mL, 0.33 mmol) dropwise at 0 °C. The reaction mixture was heated at 70 °C for 1 h, and then diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 9:1) to afford **37a** (756 mg, 44%) and **37b** (190 mg, 12%). **37a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.87 (s, 1H), 8.41 (s, 1H), 6.65 (d, J = 9.6 Hz, 1H), 4.70 (dd, J = 13.5, 5.2 Hz, 1H), 4.46 – 4.26 (m, 1H), 3.91 – 3.73 (m, 2H), 1.41 (s, 18H), 0.93 (two s, 18H), 0.31 – 0.02 (m, 12H). ^{19}F NMR (MHz, $CDCl_3$): -113.87 (t, J = 11.2 Hz, 1F). ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.1, 152.5, 150.6, 150.2, 143.2, 128.8, 88.1, 87.7, 85.7, 83.8, 75.2, 75.0, 61.4, 31.6, 27.8, 25.9, 25.7, 22.7, 18.4, 18.1. -4.5, -4.8, -5.3, -5.4. MS (HR-ESI) for $C_{32}H_{55}BrFN_5O_7Si_2$ [$(M+H)^+$], Calcd: m/z 776.2885, Found: m/z 776.2883. **37b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 1H), 8.43 (s, 1H), 6.74 (d, J = 9.2 Hz, 1H), 4.55 (dd, J = 13.5, 5.2 Hz, 1H), 4.07 – 4.04 (m, 1H), 4.02 – 3.88 (m, 2H), 1.45 (s, 18H), 0.97 (s, 18H), 0.20 – 0.15 (m, 12H). ^{19}F NMR (MHz, $CDCl_3$): -122.96 (m, 1F). ^{13}C NMR (101 MHz, $CDCl_3$) : δ 153.2, 152.5, 150.5, 150.2, 143.9, 142.8, 128.5, 111.5, 108.6, 88.3, 88.1, 83.8, 83,2, 74.1, 73.8, 60.9, 27.7, 26.0, 25.6, 18.5, 18.0, -4.4, -4.8, -5.3, -5.4. MS (HR-ESI) for $C_{32}H_{55}BrFN_5O_7Si_2$ [$(M+H)^+$], Calcd: m/z 776.2885, Found: m/z 776.2883.

*4.2.31 6-bis(tert-Butoxycarbonyl)amino-9-(2'-deoxy-2'-chloro-2'-fluoro-β-D-ribofuranosyl)purine (**38**)*

To a solution of compound **36** (103 mg, 0.14 mmol) in anhydrous THF (5 mL) was added TBAF (350 µL, 0.35 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and quenched with a saturated solution of NH_4Cl (5 mL). The aqueous layer was extracted with ethyl acetate and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **38** (70 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.87 (s, 1H), 8.62 (s, 1H), 6.54 (d, J = 8.7 Hz, 1H), 5.19 (br s, 1H), 4.73 (dd, J = 15.4 Hz, 6.0 Hz, 1H), 4.37 (br s, 1H), 4.13 (m, 1H), 4.03 (m, 1H), 3.93 (m, 1H), 1.38 (s, 18H). ^{19}F NMR (MHz, $CDCl_3$): -123.52 (dd, J = 14.7 Hz, J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.2, 152.7, 150.4, 144.0, 128.8, 114.3 (d, J = 253.6 Hz), 88.3, 84.7, 83.4, 73.0, 72.7, 60.4, 27.8. MS (HR-ESI) for $C_{20}H_{28}ClFN_5O_7$ [$(M+H)^+$], Calcd: m/z 504.1661, Found: m/z 504.1658.

*4.2.32 6-bis(tert-Butoxycarbonyl)amino-9-(2'-deoxy-2'-bromo-2'-fluoro-β-D-ribofuranosyl)purine (**39**)*

To a solution of compound **37** (190 mg, 0.14 mmol) in anhydrous THF (10 mL) was added TBAF (610 µL, 0.61 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and quenched with a saturated solution of NH_4Cl (10 mL). The aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **39** (93 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 1H), 8.49 (d, J = 1.6 Hz, 1H), 6.69 (d, J = 9.5 Hz, 1H), 4.71 (dd, J = 15.7, 5.9 Hz, 1H), 4.19 (dt, J = 6.0, 3.0 Hz, 1H), 4.09 (d, J = 12.7 Hz, 1H), 3.98 (d, J = 12.4 Hz, 2H), 1.43 (s, 18H). ^{19}F NMR (MHz, $CDCl_3$): -121.02 (dd, J = 14.7 Hz, J = 8.8 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.1, 152.5, 150.7, 150.3, 143.4, 128.9, 111.8, 89.7, 89.5, 84.2, 83.1, 83.1, 73.5, 73.3, 60.6, 27.7. MS (HR-ESI) for $C_{20}H_{28}BrFN_5O_7$ [$(M+H)^+$], Calcd: m/z 548.1156, Found: m/z 548.1132.

*4.2.33 (2R,3R,4R,5R)-5-(6-Amino-9H-purin-9-yl)-4-chloro-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (**40**)*

Compound **38** (0.06 g, 0.12 mmol) was dissolved in a 4 M solution of HCl in dioxane (2 mL). The reaction mixture was stirred for 1 h at rt. Volatiles were removed under vacuum and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **40** (21 mg, 61%). ^{1}H NMR (400 MHz, MeOD): δ 8.41 (s, 1H), 8.23 (s, 1H), 6.43 (d, J = 11.2 Hz, 1H), 4.57 (dd, J = 16.7 Hz, J = 6.1 Hz, 1H), 4.1 (m, 1H), 3.98 (m, 1H), 3.88 (m, 1H). ^{19}F NMR (MHz, MeOD): -123.99 (t, J = 14.2 Hz). ^{13}C NMR (100 MHz, MeOD): δ 157.0, 154.2, 150.3, 141.2, 119.32 115.2 (d, J = 258 Hz), 88.5, 84.3, 74.3, 61.2. MS (HR-ESI) for $C_{10}H_{12}ClFN_5O_3$ [$(M+H)^+$], Calcd: m/z 304.0613, Found: m/z 304.0605.

*4.2.34 (2R,3R,4R,5R)-5-(6-Amino-9H-purin-9-yl)-4-bromo-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (**41**)*

Compound **39** (0.014 g, 0.026 mmol) was dissolved in a 4 M solution of HCl in dioxane (1 mL). The reaction mixture was stirred for 1 h at rt. Volatiles were removed under vacuum and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **41** (5.2 mg, 60%). ^{1}H NMR (400 MHz, MeOD) δ 8.34 (s, 1H), 8.13 (s, 1H), 6.51 (d, J = 10.5 Hz, 1H), 4.40 (dd, J = 16.7, 6.1 Hz, 1H), 3.94 (m, 1H), 3.86-3.73 (m, 1H), 3.63-3.54 (d, J = 4.8 Hz, 1H). ^{19}F NMR (MHz, MeOD): -122.50 (dd, J = 12.12 Hz, J = 12.0 Hz). ^{13}C NMR (101 MHz, MeOD) δ 156.0, 152.8, 149.4, 139.7, 139.7, 118.3, 112.0, 88.3, 88.1, 83.1, 83.1, 73.4, 73.1, 59.9. MS (HR-ESI) for $C_{10}H_{12}BrFN_5O_3$ [$(M+H)^+$], Calcd: m/z 348.0107, Found: m/z 348.0107.

*4.2.35 Isopropyl ((((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**42**)*

To a solution of compound **38** (50 mg, 0.099 mmol) in THF (2 mL) was added *tert*-butylmagnesium chloride (248 µL, 0.248 mmol, 1 M in THF) The reaction mixture was stirred for 20 minutes at 0 °C before addition of L-isopropylalaninyl phosphorochloridate **21** (90 mg, 0.29 mmol) in THF (0.29 mL). The reaction mixture was stirred 1 h at rt. The volatiles were evaporated and the residue was directly treated, without further purification, with a 4 M solution of HCl in dioxane (2 mL). The reaction mixture was stirred 1 h at rt. Solvents were removed under vacuum and the residue was purified by flash column

chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **42** as a diastereomeric mixture of R_p/S_p (~1:1) (16 mg, 29% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H), 8.07 & 7.98 (each s, 1H), 7.28-7.34 (m, 2H), 7.1-7.23 (m, 3H), 6.47 (dd, *J* = 12.4 Hz, *J* = 2.8 Hz, 1H), 6.04 (d, *J* = 11.6 Hz, 2H), 5.59 (br s, 1H), 4.94-5.01 (m, 1H), 4.55-4.68 (m, 1H), 4.38-4.48 (m, 2H), 4.09-4.28 (m, 2H), 3.92-4.04 (m, 1H), 1.33-1.38 (two s, 3H), 1.16-1.22 (m, 6H). ^{19}F NMR (MHz, $CDCl_3$): -123.44 (t, *J* = 14.7 Hz), -124.17 (t, *J* = 14.7 Hz). ^{31}P NMR (MHz, $CDCl_3$): 3.01, 2.88. ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2, 155.7, 153,7, 150.6, 150.3, 139.4, 129.9, 125.4, 120.3, 119.1, 113.5 (d, *J* = 256 Hz), 86.8, 81.1, 74.1, 69.7, 64.9, 60.6, 50.6, 21.7, 20.9. MS (HR-ESI) for $C_{22}H_{28}ClFN_6O_7P$ [(M+H)$^+$], Calcd: m/z 573.1430, Found: m/z 573.1424.

*4.2.36 Isopropyl ((((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**43**)*

To a solution of compound **39** (70 mg, 0.128 mmol) in THF (2 mL) *tert*-butylmagnesium chloride (319 µL, 0.319 mmol, 1 M in THF) was added and the reaction mixture was stirred for 20 minutes at 0 °C. A solution of the phosphorochloridate **21** (46 mg, 0.153 mmol) in THF (0.153 mL) was then added and the reaction mixture was stirred for 1 h at rt. The volatiles were removed under vacuum and the residue was directly treated, without further purification, with a 4 M solution of HCl in dioxane (2 mL). The reaction mixture was stirred 1 h at rt. Solvents were removed under vacuum and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **43** as a diastereomeric mixture of R_p/S_p (~1:1) (16 mg, 20% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 5.1 Hz, 1H), 8.05 (d, *J* = 2.8 Hz, 1H), 7.99 – 7.91 (m, 1H), 7.38 – 7.28 (m, 2H), 7.25 – 7.14 (m, 3H), 6.67 – 6.59 (m, 1H), 5.85 (s, 2H), 5.00 (q, *J* = 7.2, 6.7 Hz, 2H), 4.57 (d, *J* = 4.7 Hz, 1H), 4.48 – 4.39 (m, 3H), 4.25 (d, *J* = 5.0 Hz, 1H), 4.09 – 3.93 (m, 3H), 3.79 – 3.71 (m, 1H), 1.37 (dd, *J* = 6.9, 2.6 Hz, 4H), 1.30 – 1.15 (m, 14H). ^{19}F NMR (MHz, $CDCl_3$): -121.51 (t, *J* = 14.7 Hz), -122.53 (t, *J* = 14.7 Hz). ^{31}P NMR (MHz, $CDCl_3$): 3.42, 3.07. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 155.4, 153.5, 150.5, 150.2, 139.4, 129.8, 129.7, 125.4, 125.3, 120.1, 120.1, 87.6, 69.6, 50.5, 29.7, 21.7, 21.6, 20.9, 20.8. MS (HR-ESI) for $C_{22}H_{28}BrFN_6O_7P$ [(M+H)$^+$], Calcd: m/z 617.0924, Found: m/z 617.0925.

*4.2.37 2-bis(tert-Butoxycarbonyl)amino-6-chloro-9-(3,5-di-O-tert-butyldimethylsilyl-2'-deoxy-2'-chloro-2'-fluoro-D-ribofuranosyl)purine (**44a** and **44b**)*

To a solution of compound **11** (3.0 g, 7.22 mmol), 2-bis(*tert*-butoxycarbonyl)amino-6-chloro-9H-purine (2.94 g, 7.95 mmol) and triphenylphosphine (2.84 g, 10.83 mmol) in THF (50 mL) was added DIAD (2.13 mL, 10.83 mmol) dropwise at 0 °C. The reaction mixture was heated at 70 °C for 1 h, and then diluted with ethyl acetate (50 mL). The organic layer was washed with water, and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 9:1) to afford **44a** (3.0 g, 54%) and **44b** (0.67 g, 12%). **44a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 6.60 (d, *J* = 9.0 Hz, 1H), 4.74(dd, *J* = 12.9 Hz, *J* = 4.4 Hz, 1H), 4.37 (m, 2H), 3.82 (m, 1H), 1.41 (s, 18H), 0.93 (two s, 18H), 0.15 (four s, 12H). ^{19}F NMR (MHz, $CDCl_3$): -115.58 (t, *J* = 10.9 Hz, 1F). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.6, 151.4, 150.4, 144.8, 129.8, 117.1 (d, *J* = 251 Hz), 87.8, 85.6, 83.8, 75.0, 61.5, 28.0, 26.0, 25.7, 18.5, 18.2, -4.5, -4.9, -5.28, -5.31. MS (HR-ESI) for $C_{32}H_{55}Cl_2FN_5O_7Si_2$ [(M+H)$^+$], Calcd: m/z 766.3001, Found: m/z 766.2998. **44b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1H), 6.49 (d, *J* = 9.0 Hz, 1H), 4.60 (dd, *J* = 13.0 Hz, 4.8 Hz, 1H), 4.00 (m, 2H), 3.88 (m, 1H), 1.41 (s, 18H), 0.99 (two s, 18H), 0.15 (s, 12H). ^{19}F NMR (MHz, $CDCl_3$): -123.58 (t, *J* = 11.8 Hz, 1F). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.7, 151.5, 150.4, 144.3, 129.9, 113.9 (d, *J* = 258 Hz), 87.4, 83.8, 73.6, 61.0, 27.9, 26.1, 25.7, 18.8, 18.1, -4.5, -4.9, -5.28, -5.31. MS (HR-ESI) for $C_{32}H_{55}Cl_2FN_5O_7Si_2$ [(M+H)$^+$], Calcd: m/z 766.3001, Found: m/z 766.3001.

*4.2.38 2-bis(tert-Butoxycarbonyl)amino-6-chloro-9-(3,5-di-O-tert-butyldimethylsilyl-2'-deoxy-2'-bromo-2'-fluoro-D-ribofuranosyl)purine (**45a** and **45b**)*

To a solution of compound **12** (2.60 g, 5.66 mmol), 2-bis(*tert*-butoxycarbonyl)amino-6-chloro-9H-purine (2.29 g, 6.23 mmol), triphenylphosphine (2.19 g, 8.49 mmol) in THF (50 mL), DIAD (1.67 mL, 8.49 mmol) was added dropwise at 0 °C. The reaction mixture was heated at 70 °C for 1 h, and then diluted with ethyl acetate (50 mL). The organic layer was washed with water, and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/ethyl acetate 9:1) to afford **45a** (0.95 g, 20%) and **45b** (0.26 g, 6%). **45a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 6.58 (d, *J* = 9.7 Hz, 1H), 4.69 (dd, *J* = 13.5, 5.1 Hz, 1H), 4.41 – 4.31 (m, 1H), 3.83 (m, 2H), 1.43 (s, 18H), 0.94 (d, *J* = 11.1 Hz, 18H), 0.21-0.10 (m, 12H). ^{19}F NMR (MHz, $CDCl_3$): -113.37 (t, *J* = 10.9 Hz, 1F). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.25, 144.56, 129.80, 88.34, 87.97, 85.84, 83.66, 61.28, 60.40, 28.22, 27.83, 25.99, 25.86, 25.65, 25.60, 21.05, 18.34, 18.08, 14.20, -4.60, -4.87, -5.40, -5.44. [(M+H)$^+$] for $C_{32}H_{54}BrClFN_50_7Si_2$. Calcd: m/z 810.2496, Found: m/z 810.2495. **45b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 4.49 (dd, *J* = 15.3, 5.7 Hz, 1H), 4.06-3.89 (m, 1H), 3.86 (m, 1H), 1.41 (s, 18H), 0.95 (d, *J* = 8.6 Hz, 18H), 0.15 (m, 12H). ^{19}F NMR (MHz, $CDCl_3$): -121.88 (t, *J* = 11.8 Hz, 1F). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.6, 150.2, 144.9, 88.4, 83.7, 83.3, 73.8, 73.5, 60.8, 30.9, 27.8, 26.0, 25.6, 21.0, 18.5, 18.0, 14.2, -4.4, -4.7, -5.2, -5.3. [(M+H)$^+$] for $C_{32}H_{54}BrClFN_50_7Si_2$. Calcd: m/z 810.2496, Found: m/z 810.2495.

*4.2.39 2-bis(tert-Butoxycarbonyl)amino-6-chloro-9-(2'-deoxy-2'-chloro-2'-fluoro-β-D-ribofuranosyl)purine (**46**)*

To a solution of compound **44** (0.3 g, 0.39 mmol) in anhydrous THF (10 mL) was added TBAF (0.97 mL, 0.98 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at rt and quenched with a saturated solution of NH_4Cl (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **46** (188 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 6.49 (d, *J* = 7.2 Hz, 1H), 4.80 (dd, *J* = 14.8 Hz, *J* = 6.03 Hz, 1H), 4.68 (br s, 1H), 4.14 (m, 1H), 4.07 (m, 1H), 3.97 (m, 1H), 3.86 (br s, 1H), 1.39 (s, 18H). ^{19}F NMR (MHz, $CDCl_3$): -123.64 (dd, *J* = 13.88 Hz, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.5, 152.3, 151.7, 150.5, 145.1, 130.0, 114.2 (d, *J* = 260 Hz), 88.5, 84.4, 83.2, 72.5, 60.4, 27.9. MS (HR-ESI) for $C_{20}H_{26}Cl_2FN_5NaO_7$ [(M+Na)$^+$], Calcd: m/z 560.1091, Found: m/z 560.1084.

*4.2.40 2-bis(tert-Butoxycarbonyl)amino-6-chloro-9-(2'-deoxy-2'-bromo-2'-fluoro-β-D-ribofuranosyl)purine (**47**)*

To a solution of compound **45** (0.24 g, 0.296 mmol) in anhydrous THF (10 mL) was added TBAF (0.74 mL, 0.741 mmol, 1 M in THF). The reaction mixture was stirred for 1 h at

rt and quenched with a saturated solution of NH_4Cl (10 mL). The aqueous layer was extracted with ethyl acetate (3x10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford compound **47** (100 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 6.66 (d, *J* = 7.5 Hz, 1H), 4.70 (dd, *J* = 15.7, 6.6 Hz, 1H), 4.54 (br s, 1H), 4.14 (m, 1H), 4.09 (m, 1H), 3.99 (m, 1H), 1.40 (s, 18H). ^{19}F NMR (MHz, $CDCl_3$): -121.55 (dd, *J* = 13.88 Hz, *J* = 6.4 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.4, 152.1, 151.6, 150.3, 144.9, 129.9, 111.5, 108.8, 89.6, 89.4, 84.3, 82.8, 82.8, 72.8, 72.5, 60.2, 53.0, 27.8. MS (HR-ESI) for $C_{20}H_{26}BrClFN_5O_7$ [(M+H)$^+$], Calcd: m/z 582.0766. Found: m/z 582.0761.

*4.2.41 2-Amino-9-((2R,3R,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (**48**)*

Compound **46** (40 mg, 0.074 mmol) was dissolved in a mixture of formic acid/H_2O (4:1) and stirred at 60 °C for 16 h. Volatiles were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 8:2) to afford compound **48** (12.8 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 6.27 (d, *J* = 10.8 Hz, 1H), 4.53 (dd, *J* = 16.5 Hz, *J* = 6.04 Hz, 1H), 3.96 (m, 1H), 3.91 (m, 1H), 3.81 (m, 1H). ^{19}F NMR (MHz, $CDCl_3$): -124.85 (t, *J* = 12.9 Hz, 1F). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3, 155.8, 153.4, 137.8, 117.04, 115.82 (d, *J* = 258 Hz), 88.1, 84.3, 74.3, 61.3. MS (HR-ESI) for $C_{10}H_{12}ClFN_5O_4$ [(M+H)$^+$], Calcd: m/z 320.0562, Found: m/z 320.0554.

*4.2.42 2-amino-9-((2R,3R,4R,5R)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (**49**)*

Compound **47** (50 mg, 0.086 mmol) was dissolved in a mixture of formic acid/H_2O (4:1) (2 mL) and stirred at 60 °C for 16 h. Volatiles were removed under reduced pressure and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 8:2) to afford compound **49** (15.6 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (br s, 1H), 7.94 (s, 1H), 6.72 (d, *J* = 5.6 Hz, 1H), 6.62 (br s, 1H), 6.27 (d, *J* = 9.2 Hz, 1H), 5.26 (t, *J* = 5.5 Hz, 1H), 4.34 (dd, *J* = 16.2, 5.6 Hz, 1H), 3.83 (m, 1H), 3.73 – 3.58 (m, 2H). ^{19}F NMR (MHz, DMSO-d_6): -121.02 (t, *J* = 12.9 Hz, 1F). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.0, 154.6, 151.8, 135.6, 116.4, 87.2, 87.0, 59.9. MS (HR-ESI) for $C_{10}H_{12}BrFN_5O_4$ [(M+H)$^+$], Calcd: m/z 364.0056, Found: m/z 364.0054.

*4.2.43 Isopropyl ((((2R,3R,4R,5R)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**50**)*

To a solution of compound **46** (50 mg, 0.093 mmol) in THF (2 mL) *tert*-butylmagnesium chloride (232 µL, 0.232 mmol, 1 M in THF) was added and the reaction mixture was stirred for 20 minutes at 0 °C. Then L-isopropylalaninyl phosphorochloridate **21** (42 mg, 0.139 mmol) 1M in THF (0.139 mL) was added and the reaction mixture was stirred 1 h at rt. The solvent was evaporated and the residue was directly treated, without further purification, with a mixture of formic acid/H_2O (4:1) (2 mL). The reaction mixture was stirred 4 h at 60 °C. Solvents were removed under vacuum and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **50** as a diastereomeric mixture R_p/S_p (~1:1) (12.9 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 & 7.80 (each s, 1H), 7.35-7.31 (m, 2H), 7.25-7.17 (m, 3H), 6.29 & 6.26 (m, 1H), 4.98-4.93 (m, 2H), 4.60-4.50 (m, 1H), 4.48-4.39 (m, 2H), 4.20-4.13 (m, 1H), 3.93-3.86 (m, 1H), 1.32 (m, 3H), 1.22- 1.18 (m, 6H). ^{19}F NMR (MHz, $CDCl_3$): -124.55 (t, *J* = 12.9 Hz, 1F). ^{31}P NMR (MHz, $CDCl_3$): 3.66, 3.59. ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 159.31, 155.8, 153.4, 152.1, 137.8, 130.8, 126.2, 121.4, 117.1, 115.3 (d, *J* = 255 Hz), 88.0, 82.4, 74.9, 70.2, 66.3, 51.7, 21.9, 20.4. MS (HR-ESI) for $C_{22}H_{28}ClFN_6O_8P$ [(M+H)$^+$], Calcd: m/z 589.1379, Found: m/z 589.1372.

*4.2.44 Isopropyl ((((2R,3R,4R,5R)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate, diastereomeric mixture (**51**)*

To a solution of compound **47** (50 mg, 0.086 mmol) in THF (2 mL) *tert*-butylmagnesium chloride (215 µL, 0.215 mmol, 1 M in THF) was added and the reaction mixture was stirred for 20 minutes at 0 °C. Then (*S*)-2-[(*S*)-(2,3,4,5,6-pentafluorophenoxy)-phenoxyphosphorylamino] propionic acid isopropyl ester, **52** (58 mg, 0.120 mmol) 1M in THF (0.120 mL) was added and the reaction mixture was stirred 2 h at rt. The solvent was evaporated and the residue was directly treated, without further purification, with a mixture of formic acid/H_2O (4:1) (2 mL). The reaction mixture was stirred 4 h at 60 °C. Solvents were removed under vacuum and the residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 4:1) to afford compound **51** as a S_p isomer (11.9 mg, 22%). ^{1}H NMR (400 MHz, MeOD δ 7.72 (dd, *J* = 12.3, 2.9 Hz, 1H), 7.35-7.27 (m, 2H), 7.19 – 7.03 (m, 3H), 6.32 (m 1H), 4.73 – 4.25 (m, 4H), 4.08 – 4.03 (m, 1H), 3.82 – 3.77 (m, 1H), 3.70 – 3.50 (m, 1H), 1.35 – 1.03 (m, 10H). ^{19}F NMR (MHz, MeOD): -122.80 (d, *J* = 14.6 Hz, 1F). ^{31}P NMR (MHz, MeOD): 3.84. ^{13}C NMR (100 MHz, MeOD): δ 173.2, 157.8, 154.3, 152.0, 150.8, 136.4, 129.4, 124.8, 120.0, 115.7, 111.3, 87.6, 80.9, 74.0, 68.7, 65.4, 510.3, 20.5, 19.0. MS (HR-ESI) for $C_{22}H_{28}BrFN_6O_8P$ [(M+H)$^+$], Calcd: m/z 633.0873, Found: m/z 633.0874.

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