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Synthesis and antiviral activity of N^9-[3-fluoro-2-(phosphonomethoxy)propyl] analogues derived from N^6-substituted adenines and 2,6-diaminopurines

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ABSTRACT

An efficient method for the synthesis of N^9-[3-fluoro-2-(phosphonomethoxy)propyl] (FPMP) derivatives of purine bases has been developed. Both (*R*)- and (*S*)-enantiomers of the N^6-substituted FPMP derivatives of adenine and 2,6-diaminopurine were prepared and their anti-human immunodeficiency virus (HIV) and anti-Moloney murine sarcoma virus (MSV) activity was evaluated. Whereas none of the 6-substituted FPMPA derivatives showed any antiviral activity, several FPMPDAP derivatives had a moderate antiretroviral activity. Moreover, the data obtained from the study of the substrate activity of the active derivatives towards N^6-methyl-AMP aminohydrolase support the notion that the studied N^6-substituted FPMPDAP derivatives act as prodrugs of the antiretroviral FPMPG analogues.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) are nucleotide analogs with phosphorous atom attached to the side aliphatic chain through a stable P–C bond.[1] ANPs exhibit various antiviral,[2] cytostatic,[3] antiparasitic,[4] and immunomodulatory properties.[5] Three ANPs (cidofovir, adefovir, tenofovir) are active components of potent antivirals used in human medicine for treatment of hepatitis B, AIDS, and other diseases caused by DNA viruses.[6]

An interesting subclass of ANPs is represented by the purine N^9-[3-fluoro-2-(phosphonomethoxy)propyl] (FPMP) derivatives **1** (Fig. 1).[2,7] In contrast to the purine N^9-[3-hydroxy-2-(phosphonylmethoxy)propyl] (HPMP) derivatives **2** (Fig. 1), which are active against a broad spectrum of DNA viruses,[1] the FPMP compounds **1** exhibit potent and selective activity against retroviruses (HIV-1 and HIV-2).[7,8] Thus, replacement of the hydroxyl group at the C′-3 position of an aliphatic chain by fluorine leads to a completely different pattern of antiviral activity where the loss of activity against DNA viruses is compensated by high and selective antiretroviral activity. In addition, (*S*)-FPMPA also showed interesting activity (EC_{50}: 1.2 μM) against hepatitis B virus (HBV).[9]

Moreover, fluorinated nucleoside analogs also drew attention from the pharmaceutical industry due to improved pharmacokinetic properties (absorption, distribution, metabolism, and excretion) and diminished side effects (toxicity).[10]

In analogy with the other ANPs, the virus-inhibitory activity of the FPMP analogs is based on their intracellular phosphorylation to give their diphosphates, which subsequently act as terminators of the growing DNA chain.[7,11] In regard of the biological properties of the fluorinated ANPs, main attention was aimed at the derivatives containing adenine, guanine, and 2,6-diaminopurine moieties (Table 1).[12] The anti-HIV effects of both the (*R*) and (*S*) enantiomers of the corresponding guanine (FPMPG) and diaminopurine (FPMPDAP) derivatives are comparable, whereas the activity of the adenine analogue (FPMPA) is strictly enantiospecific.[12] (*S*)-FPMPA is 30-fold more effective an inhibitor of HIV-1 and HIV-2 replication than its (*R*) counterpart and the difference in the antiviral activity of the enantiomers in the adenine series is probably caused by preferential phosphorylation of (*S*)-FPMPA by cellular AMP kinases.[12,13]

In order to improve the antiviral properties of the FPMP derivatives we have decided to perform structure–activity relationship (SAR) study focused on the enantiomeric N^6-substituted FPMP derivatives of adenine and 2,6-diaminopurine represented by the general structure **3** (Fig. 1). Our reasoning was based on the fact that N^6-substitution can considerably increase the antiviral and cytostatic activity[14] of ANPs or enhance their immunostimulatory and immunomodulatory activity.[5e] It has been found that the N^6-substituted 2,6-diaminopurine analogues are metabolized to

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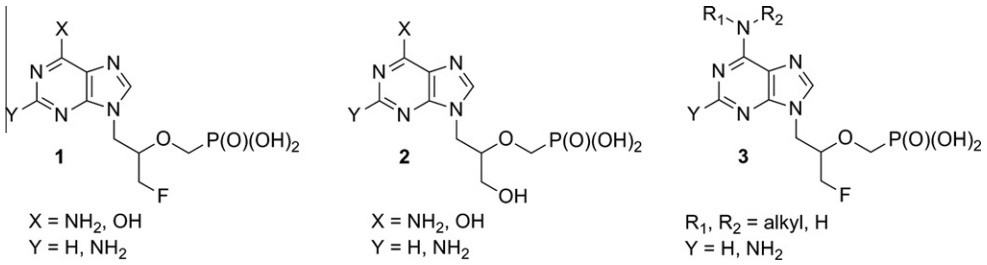


Figure 1. Acyclic nucleoside phosphonates.

Table 1
Anti-HIV-1 activity [EC_{50} (μM)] of purine FPMP analogues in MT-4 cells[12]

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)
(*R*)-FPMPA	272 ± 23.0	>300
(*S*)-FPMPA	8.9 ± 0.03	>300
(*R*)-FPMPG	5.9 ± 1.2	>300
(*S*)-FPMPG	3.9 ± 1.1	103 ± 90
(*R*)-FPMPDAP	4.3 ± 0.9	>300
(*S*)-FPMPDAP	15.0 ± 9.0	>300

[a] Compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cells by 50%.
[b] Compound concentration required to reduce MT-4 cell viability by 50%.

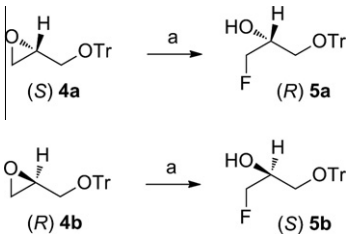
the corresponding guanine counterparts by N^6-methyl-AMP aminohydrolase and thus can be considered as prodrugs.[15]

From a chemical viewpoint, an improved synthetic strategy for the convenient preparation of the purine FPMP analogues has been developed since the original approach was quite laborious and rather expensive.[8b]

2. Results and discussion

2.1. Chemistry

Enantiomeric 3-fluoro-1,2-propanediols[16] were used in the original synthesis of the FPMP derivatives.[8b] In the present work, commercially available enantiomeric O-tritylated glycidols **4a** and **4b** were selected as a convenient starting material (Scheme 1). Glycidols **4** were converted regioselectively and in high yields to the corresponding fluorohydrines **5** by the treatment with potassium hydrogendifluoride and catalytic amounts of tetrabutylammonium dihydrogentrifluoride[17] under solid–liquid PTC (phase transfer catalysis) conditions.[18] Although the stereocenter at the C-2 position of glycidols **4** is not attacked during the reaction,[18] the formal configuration of fluorohydrines **5** is changed to the opposite one (Scheme 1). This fact was confirmed by the X-ray structure of fluorohydrine **5a** (Fig. 2). The data from X-ray crystallography analysis also showed that intramolecular O–H···O hydrogen bonds of the C-2 hydroxyl group play a dominant role in the crystal packing of the compound **5a** (Fig. 3).



Scheme 1. Synthesis of the fluorohydrines **5a** and **5b**. Reagents and conditions: (a) KHF_2, PhCl, cat. $Bu_4NH_2F_3$, 135 °C.

Alkylation of the fluorohydrines **5** with diisopropyl *O*-(*p*-toluensulphonyloxy)methylphosphonate[19] (**6**) with excess of NaH in DMF under standard conditions afforded phosphonates **8** in good yields (Scheme 2). Enantiomer **8a** was also obtained in the same yield (67%) by an alternative procedure using bromomethylphosphonate **7** instead of tosylate **6** under similar reaction conditions.[20]

Detritylation of compounds **8** was carried out in 80% aqueous acetic acid and gave acceptable yields of the derivatives **9**.

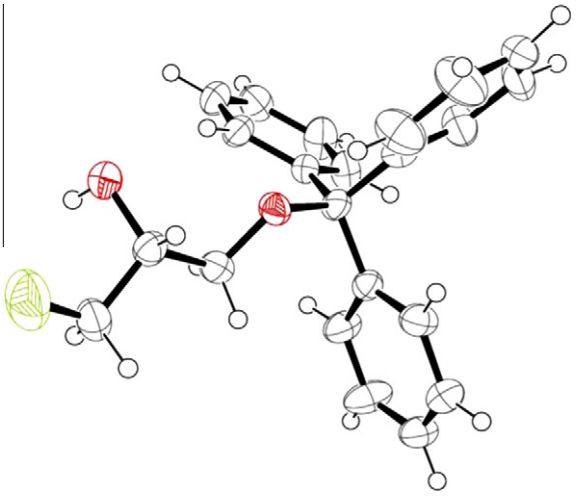


Figure 2. X-ray structure of fluorohydrine **5a**.

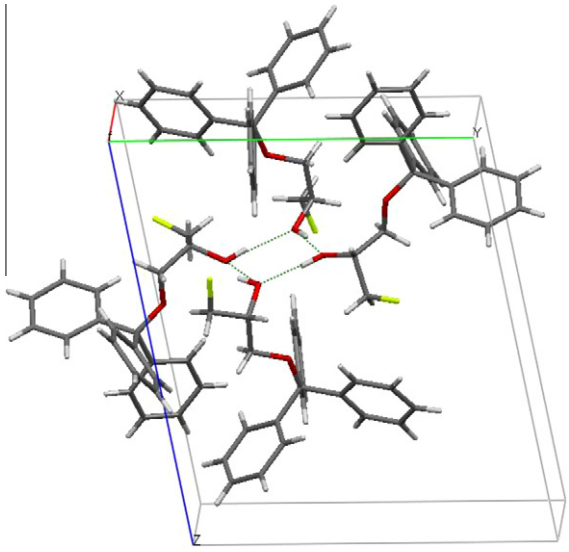
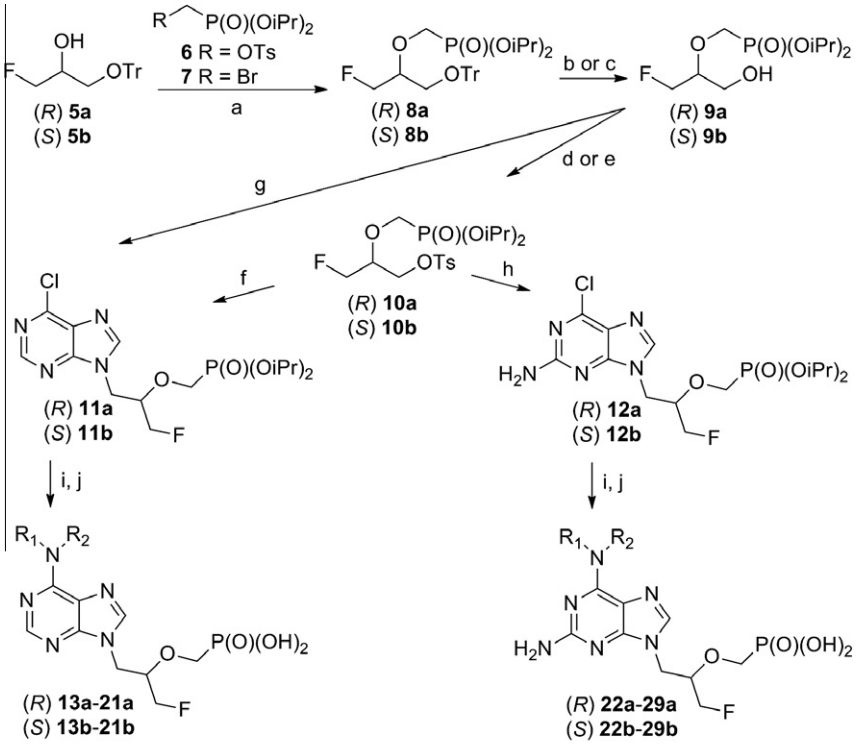


Figure 3. Crystal packing of fluorohydrine **5a**.



(R)	(S)	R_1	R_2
13a	**13b**	Methyl	H
14a	**14b**	Cyclopropyl	H
15a	**15b**	Propyl	H
16a	**16b**	Butyl	H
17a	**17b**	sec-Butyl	H
18a	**18b**	Cyclopentyl	H
19a	**19b**	Methyl	Methyl
20a	**20b**	Ethyl	Ethyl
21a	**21b**	$-(CH_2)_4-$	

(R)	(S)	R_1	R_2
22a	**22b**	Cyclopropyl	H
23a	**23b**	Propyl	H
24a	**24b**	Allyl	H
25a	**25b**	2-(dimethylamino)ethyl	H
26a	**26b**	2-(methyloxy)ethyl	H
27a	**27b**	Methyl	Methyl
28a	**28b**	Ethyl	Ethyl
29a	**29b**	$-(CH_2)_4-$	

Scheme 2. Reagents and conditions: (a) NaH, DMF, −20 °C to rt; (b) 80% CH_3COOH, 90 °C; (c) Dowex D50W × 8 (H^+ form), aq MeOH, reflux; (d) TsCl, Et_3N, CH_2Cl_2, 0 °C to rt; (e) TsCl, Py, DMAP, 0 °C; (f) 6-chloropurine, NaH, DMF, 60 °C (conventional) or 120 °C (microwave); (g) 6-chloropurine, Ph_3P, DIAD, THF, rt to 60 °C; (h) 2-amino-6-chloropurine, NaH, DMF, 90 °C; (i) amine, CH_3CN, 70 °C or 80 °C; (j) TMSBr, CH_3CN, rt.

Compound **9b** was also obtained in similar yield (71%) by treatment of the trityl derivative **8b** with Dowex D 50 (H^+ form) and this method seems to be more practical with respect to the easier work up.

Both enantiomers **9a** and **9b** were tosylated with *p*-toluene sulphonyl chloride in either dichloromethane/TEA mixture or in pyridine and gave the desired alkylating agents **10** in good preparative yields.

Compounds **11a** and **11b** were prepared in moderate yields (44% and 53%, respectively) by condesation of the sodium salt of 6-chloropurine with the corresponding alkylating agents **10a** and **10b**. Slightly better yield of the alkylated product **11b** (59%) was obtained when the alkylation was conducted under microwave-assisted conditions.[21] The alkylation of 6-chloropurine was also carried out directly with the alcohol **9b** under the Mitsunobu reaction conditions,[22] but the compound **11b** was obtained in 26% yield only.[23]

Condensation of the sodium salt of 2-amino-6-chloropurine with the tosylates **10a** and **10b** afforded the desired products **12a** and **12b** in satisfactory yields (67% and 60%, respectively, Scheme 2). The structure and the configuration on the C-2′ chiral center of the (*R*)-enantiomer **12a** was confirmed by X-ray diffraction (Fig. 4).

Heating of 6-chloropurine intermediates **11** and **12** with the appropriate alkylamine or dialkylamine in acetonitrile, methylamine in ethanol, or dimethylammonium *N*,*N*-dimethylcarbamate

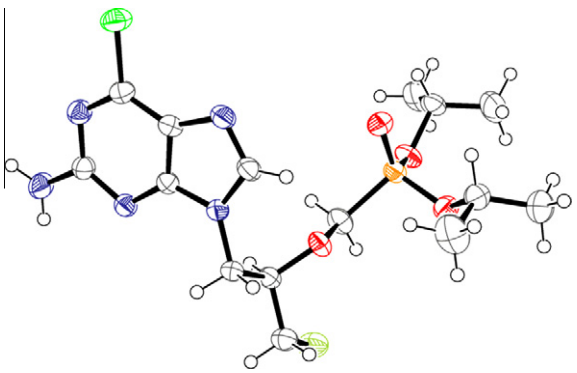


Figure 4. X-ray structure of compound **12a**.

in acetonitrile,[14b] followed by the standard removal of the isopropyl ester groups with TMSBr in acetonitrile at room temperature,[14b] afforded the crude products **13**–**29**. Final purification of the products **13–29** was performed by two approaches: (a) standard deionization by means of the ion-exchange resins and subsequent crystallization from aqueous ethanol to get the final free phosphonic acids;[14b] (b) reverse phase HPLC chromatography of the corresponding triethylammonium salts of the products followed by the conversion to their sodium salts on Dowex D50 × 8 (Na^+ form), which can be either crystallized from aqueous ethanol or lyophilized. The structure of the final products **13–29** was routinely confirmed by ^1H and ^{13}C NMR spectra and by mass spectrometry. Their purity was determined by elemental analysis and by assignment of the optical rotation.

2.2. Biological activity

None of the 6-substituted FPMPA derivatives (**13–21**) showed neither anti-HIV nor anti-MSV activity in cell culture (Scheme 2). In contrast, several N^6-substituted FPMPDAP derivatives were endowed with a moderate anti-HIV/MSV activity (Scheme 2, Table 2). The cyclopropyl derivatives, both (*R*) and (*S*) (**22a,b**), showed comparable activity against HIV, but they were less potent than (*R*)-PMPA and (*S*)-FPMPA. Among the propyl derivatives, only the (*R*)-enantiomer (**23a**) showed anti-HIV activity, and likewise, among the allyl derivatives, only the (*R*)-allyl derivative (**24a**) proved active. Some albeit weak anti-HIV activity was noted for the (*R*)-dimethyl derivative (**27a**). It was also striking to notice that the compounds showed a different SAR against MSV than HIV. This might be due to different activating enzymes and/or kinetic properties of their RTs.

With respect to the recently shown metabolic activation of N^6-substituted ANPs by N^6-methyl-AMP aminohydrolase[15] we tested the substrate activity of the N^6-substituted FPMPDAP derivatives that possess antiviral activity, together with their enantiomeric congeners, toward this enzyme. Data in Table 2, which represent the conversion to the corresponding enantiomer of FPMPG, show that the deamination of the anti-HIV active (*R*)-FPMPDAP derivatives is decreasing in the order of **22a** > **27a** > **24a** ⩾ **23a**, where the compounds **24a** and **23a** are poor substrates. This order is in full agreement with the previously published data in the series of N^6-substituted PMEDAP analogues (cyclopropyl > dimethyl > allyl ∼ propyl).[15b] It can be concluded that the studied N^6-substituted FPMPDAP derivatives are prodrugs of the antiretroviral FPMPG analogues (Table 1) although the substrate activity of the compounds does not exhibit a very good correlation with their anti-HIV potency. This discrepancy could be explained by a different intracellular uptake of the compounds and/or by diverse enzyme affinities in the subsequent metabolic pathways (e.g., enantiospecificity of phosphorylation). Various prodrugs of the active N^6-substituted FPMPDAP derivatives are being synthesized to help elucidate these correlations further.

3. Conclusions

Compared to the original laborious procedure,[8b] a simple and more efficient synthesis of (*R*)- and (*S*)-N^9-[3-fluoro-2-(phosphonomethoxy)propyl] (FPMP) derivatives of adenine and 2,6-diaminopurine has been developed, using the corresponding enantiomeric fluorohydrines **5** prepared from the O-tritylated glycidols **4**. This novel approach proved to be a method of choice for the synthesis of ANPs with the FPMP moiety. In total, 34 new FPMP derivatives were prepared, 17 in each of (*R*)- and (*S*)-series. Stereochemistry of the (*R*)-enantiomer **12a**, an intermediate in the synthesis of the N^6-substituted (*R*)-FPMPDAP analogues **22a**–**29a**, was confirmed by X-ray diffraction. The N^6-substituted FPMPA analogues displayed no activity against the viruses tested. Several derivatives in the N^6-substituted FPMPDAP series showed moderate anti-HIV/MSV activity at subtoxic concentrations, although the best analogues **22a** and **24a** proved 3–4 times less active than the parent (*R*)-FPMPDAP. The anti-HIV activity is apparently enantiospecific to the (*R*)-FPMPDAP series, with exception of the N^6-cyclopropyl derivatives, where both enantiomers **22a** and **22b** displayed

Table 2
Antiviral activity (HIV-1, HIV-2, MSV) and cytostatic properties in human T-lymphocyte (CEM) cells of the FPMPDAP derivatives and their substrate activity toward human N^6-methyl-AMP deaminase

Compound	EC_{50} (μM)[a]			CC_{50}[b] (μM)	MCC[c] (μM)	Human meAMP deaminase[d]
	HIV-1	HIV-2	MSV			Reaction rate, nmol min^{-1} mg^{-1}
22a	14.2 ± 4.4	22.8 ± 7.2	50 ± 3	>278	>100	64.54 ± 1.28
22b	25.5 ± 5.8	11.1 ± 0.0	6.9 ± 4.5	>278	>100	1.98 ± 0.14
23a	41.4 ± 19.9	22.4 ± 11.3	>100	>276	>100	4.17 ± 0.26
23b	>276	>276	>100	>276	⩾100	NP[e]
24a	18.0 ± 0.8	36.0 ± 2.0	69 ± 13	>278	>100	5.64 ± 0.38
24b	>247	>247	>100	>247	>100	NP
25a	>53	—	—	>53	—	ND[f]
25b	>100	>100	>100	>100	>100	ND
26a	>100	>100	>100	>100	>100	ND
26b	>100	>100	>100	>100	>100	ND
27a	51.7 ± 46.0	103.4 ± 2.0	>20	>287	⩾100	23.64 ± 0.36
27b	>255	114.7 ± 30.6	45 ± 25	>255	>100	8.07 ± 0.32
28a	>100	>100	>100	>100	>100	ND
28b	>100	>100	>100	>100	>100	ND
29a	>100	>100	>100	>100	>100	ND
29b	>100	>100	>20	>100	⩾100	ND
PMEA	7.4 ± 1.7	7.0 ± 1.1	2.1 ± 0.62	⩾250	⩾100	NA[g]
(*R*)-PMPA	4.6 ± 1.8	3.2 ± 1.4	—	>250	>100	NA
(*S*)-FPMPA	2.3 ± 0.3	2.9 ± 0.2	0.13 ± 0.04	>100	>100	NA

[a] Effective concentration required to protect CEM cells against the cytopathogenicity of HIV by 50% or to inhibit MSV-induced transformation of C3H/3T3 cells by 50%.
[b] Cytotoxic concentration required to inhibit CEM cell proliferation by 50%.
[c] Minimal cytotoxic concentration required to cause a microscopically visible morphological alteration of the cell cultures.
[d] The values are means ± SEM of four independent experiments (50 μM substrate, 12 μg mL^{-1} of enzyme, 60 min at 37 °C).
[e] NP, no product detected.
[f] ND, not determined.
[g] NA, not applicable.

pronounced anti-HIV activity. These results confirm the N^6-cyclopropyl group being a substituent of choice for the potential guanine prodrug approach. Although not sufficient as a requirement, the conversion of the active N^6-substituted FPMPDAP analogues to the corresponding guanine congeners by N^6-methyl-AMP aminohydrolase seems to be prerequisite condition to exert their anti-HIV activity.

4. Experimental

4.1. Methods and material

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. TLC was performed on TLC aluminium sheets—Silica Gel 60 F_{254} (Merck). Column chromatography was performed on silica gel 230–400 mesh, 60 Å (Merck). Reverse phase HPLC separation was performed on a Waters Delta 600 instrument with a Waters 486 Tunable Absorbance Detector using column Phenomenex Gemini C-18 (10 μm, 250 × 21.2 mm, flow 10 mL/min preparative column). ^{1}H and ^{13}C NMR and ^{19}F NMR spectra were measured on a Bruker Avance II 600 (^{1}H at 600 MHz and ^{13}C at 151 MHz) and/or Bruker Avance II 500 (^{1}H at 500 MHz and ^{13}C at 126 MHz and ^{19}F at 470 MHz) spectrometers in $CDCl_3$ or D_2O (NaOD additive) and referenced to TMS, ^{13}C chloroform signal (δ 77.0) or dioxane used as internal standard (δ 3.75 and δ 67.19). ^{19}F spectra were referenced to C_6F_6 external standard (δ −163). The numbering system for assignment of NMR signals is outlined in Figure 5. NMR spectra of the enantiomeric compounds are identical and are reported for the (*S*)-enantiomers only. Mass spectra were measured on a ZABEQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) or by ESI technique. The purity of compounds was ⩾95%, and it was determined from elemental analyses. Optical rotations were measured on an AUTOPOL IV polarimeter (Rudolph research analytical) at 20 °C; $[\alpha]_D$ values are given [10^{-1} deg cm^2 g^{-1}] and concentrations *c* are [g/100 mL]. Microwave experiment was carried out in 10 mL vial in CEM Discover (Explorer) microwave apparatus operated at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W.

The diffraction data of single crystals of **5a** (colorless, 0.20 × 0.50 × 0.53 mm) and **12a** (colorless, 0.16 × 0.31 × 0.42 mm) were collected on Xcalibur X-ray diffractometer with CuKα (λ = 1.54180 Å) at 150 K. The structures were solved by direct methods with SIR92[24] and refined by full-matrix least-squares on *F* with CRYSTALS.[25] All hydrogen atoms were located in a difference map, but those attached to carbon atoms were repositioned geometrically and then refined with riding constraints, while all other atoms were refined anisotropically in both cases.

Chemicals and ion-exchange resins (Dowex D50 × 8, Dowex 1 × 2 400) were purchased from Sigma–Aldrich (Prague, Czech Republic). Acetonitrile and DMF were distilled from P_2O_5 and stored over molecular sieves (4 Å). Diisopropyl [(*p*-toluene sulphonyloxy)methyl]phosphonate (**6**)[19] and diisopropyl bromomethylphosphonate (**7**)[20] were prepared according to the literature. (2*R*) and (2*S*)-[(*O*-trityloxy)methyl]oxirane were purchased from DAISO Co. Ltd. (Japan).

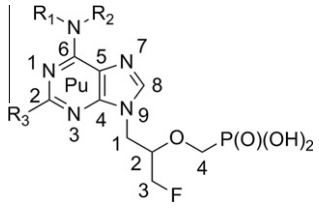


Figure 5. The general numbering scheme for assignment of NMR signals.

4.2. General procedure for purification of the free phosphonic acids

Method A:[14b] Water (20 mL) was added to the crude phosphonic acid and the solution was made alkaline with aqueous ammonia and evaporated to dryness. The residue in water (10 mL) was applied onto a column (50 mL) of Dowex 50 × 8 (H^+ form) equilibrated in water. Column was rinsed by water (300 mL) to remove redundant salts and eluted by 2% aqueous ammonia. UV absorbing fractions were collected and evaporated. The residue was dissolved in water (20 mL) and the solution was applied onto a column (50 mL) of Dowex 1 × 2 (acetate form) equilibrated in water. The column was washed with water (300 mL) and eluted with a linear gradient of aqueous acetic acid (1–30%). UV absorbing fractions containing product were evaporated, codistilled with water (3 × 20 mL), and dried under vacuum (30 °C, 2 mmHg) overnight. The products were either crystallized (aqueous EtOH) or lyophilized. *Method B*: Water (20 mL) was added to the crude phosphonic acid and the solution was made alkaline with aqueous TEAB solution (2 M, 2 mL) and evaporated. The triethylammonium salt thus obtained was purified by preparative HPLC. Product was eluted by linear gradient from H_2O to 50% MeOH in H_2O with addition of TEAB (0.1%). UV absorbing fractions containing product were collected and evaporated. The residue was applied onto a column (30 mL) of Dowex 50 × 8 (Na^+ form) equilibrated in water. Elution with water and evaporation in vacuo gave the corresponding sodium salt of the phosphonic acid. Compounds were either crystallized or in some cases lyophilized.

4.2.1. (*R*)-1-Fluoro-3-(trityloxy)propan-2-ol (5a)[18]

Procedure A: Mixture of compound **4a** (38.9 g, 122.9 mmol), KHF_2 (28.8 g, 368.8 mmol), tetrabutylammonium dihydrogentrifluoride (11.0 g, 36.4 mmol) in chlorobenzene (25 mL) was heated under argon with stirring at 135 °C for 15 h. The reaction was cooled down, diluted with EtOAc (100 mL) and filtered through a Celite. The filtrate was stabilized with Et_3N (0.5 mL) and evaporated in vacuo. Silica gel chromatography (hexane–toluene (1:1) with 0.1% Et_3N) gave compound **5a** (33.8 g, 82%) as oil which crystallized on standing, mp = 80–82 °C; $[\alpha]_D$ −8.6 (*c* 0.3, $CHCl_3$); MS (ESI) *m/z*: 359 [M+Na]$^+$. Anal. Calcd ($C_{22}H_{21}FO_2$): C, 78.55; H, 6.29; F, 5.65. Found: C, 78.29; H, 6.23; F, 5.83. *Crystal data for* **5a**: $C_{22}H_{21}F_1O_2$, triclinic, space group *P*1, *a* = 8.9301(17) Å, *b* = 13.241(2) Å, *c* = 15.211(3) Å, α = 79.151(15)°, β = 88.847(16)°, γ = 89.288(14)°, *V* = 1766.1(6) Å^3, *Z* = 4, *M* = 1345.62, 27873 reflections measured, 12670 independent reflections. Final *R* = 0.069, *wR* = 0.088, *GoF* = 1.134 for 10361 reflections with $I > 2\sigma(I)$ and 903 parameters. The asymmetric unit contains four independent molecules of **(5a)**, which are connected via hydrogen bonds. CCDC 789968.

4.2.2. (*S*)-1-Fluoro-3-(trityloxy)propan-2-ol (5b)

Treatment of compound **4b** (38.9 g, 122.9 mmol) by procedure A gave 36.6 g, (88%) of **5b** as oil which crystallized on standing, mp = 79–81 °C; $[\alpha]_D$ +9.0 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.25 (m, 2H, C3), 3.98 (dm, 1H, $J_{(2\text{-F})}$ = 18.5, C2), 4.45 (ddd, 1H, $J_{(gem)}$ = 9.5, $J_{(1a\text{-F})}$ = 47.5, $J_{(1a\text{-2})}$ = 5.6, $C1_a$), 4.48 (ddd, 1H, $J_{(gem)}$ = 9.6, $J_{(1b\text{-F})}$ = 47.2, $J_{(1b\text{-2})}$ = 4.1, $C1_b$), 7.24 (m, 3H, Ph4), 7.3 (m, 6H, Ph3), 7.42 (m, 6H, Ph2); ^{13}C NMR ($CDCl_3$): δ 63.53 (d, $J_{(3\text{-F})}$ = 7.1, C3), 69.71 (d, $J_{(2\text{-F})}$ = 19.9, C2), 84.29 (d, $J_{(1\text{-F})}$ = 169.2, C1), 86.91 (C_{Tr}), 127.19 (Ph4), 127.90 (Ph3), 128.57 (Ph2), 143.56 (Ph1); ^{19}F NMR ($CDCl_3$): -231.85 (dt, $J_{(F\text{-}1)}$ = 47.2 Hz, $J_{(F\text{-}2)}$ = 18.9 Hz); MS (ESI) m/z: 359 [M+Na]$^+$. Anal. Calcd ($C_{22}H_{21}FO_2$): C, 78.55; H, 6.29; F, 5.65. Found: C, 78.35; H, 6.25; F, 5.72.

4.2.3. Diisopropyl (*R*)-({[1-fluoro-3-(trityloxy)propan-2-yl]oxy}methyl)phosphonate (8a)

Method C: To a solution of compound **5a** (33.0 g, 98.1 mmol) in anhydrous DMF (400 mL) under argon atmosphere at -20 °C was added NaH (4.42 g, 110.4 mmol, 60% dispersion in mineral oil) and the mixture was stirred for 0.5 h at -20 °C. Then, tosylate **6** (27.3 ml, 110.4 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and brown residue was dissolved in chloroform and filtered through Celite. The filtrate was concentrated in vacuo and purified on a silica gel column (hexane–toluene, 1:1) to afford a colorless oil which after standing for several days crystallized to white solid **8a** (33.1 g, 66%), mp = 82–83 °C; $[\alpha]_D$ −13.5 (*c* 0.3, $CHCl_3$); MS (ESI) *m/z* 515 $[M+H]^+$. Anal. Calcd ($C_{29}H_{36}FO_5P$): C, 67.69; H, 7.05; F, 3.69; P, 6.02. Found: C, 67.75; H, 7.20; F, 3.73; P, 6.10.

Method D: Compound **5a** (2.6 g, 7.6 mmol) and bromo derivative **7** (2.4 mL, 9.8 mmol) were dissolved in anhydrous DMF (25 mL) and the solution was cooled to −20 °C. Then NaH (452 mg, 11.3 mmol) was added in several portions. The mixture was stirred at −20 °C for 2 h, 0 °C for another 2 h, and then at room temperature overnight. The product **8a** was isolated by the same way as in method C to give 2.61 g (67%) of **6a** as white solid.

4.2.4. Diisopropyl (*S*)-({[1-fluoro-3-(trityloxy)propan-2-yl]oxy}methyl)phosphonate (8b)

Treatment of compound **5b** (36.0 g, 107.0 mmol) by method C gave 35.4 g (64%) of **8b**, viscous oil which crystallized on standing, mp = 82–83 °C; $[\alpha]_D$ +12.1 (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.28–1.33 (m, 12H, CH_3-*i*Pr), 3.24–3.29 (m, 2H, C1), 3.79 (dm, 1H, $J_{(2\text{-}F)}$ = 19.0, C2), 3.83–3.90 (m, 2H, C4), 4.51 (m, 1H, C3a), 4.61 (m, 1H, C3b), 4.68–4.80 (m, 2H, CH-*i*Pr), 7.23 (m, 3H, Ph4), 7.30 (m, 6H, Ph3), 7.43 (m, 6H, Ph2); ^{13}C NMR ($CDCl_3$): δ 23.87–24.07 (m, CH_3-*i*Pr), 61.99 (d, $J_{(1\text{-}F)}$ = 7.9, C1), 65.24 (d, $J_{(C\text{-}P)}$ = 167.9, C4), 71.07 (d, $J_{(C\text{-}O\text{-}P)}$ = 6.5, CH-*i*Pr), 79.79 (dd, $J_{(2\text{-}F)}$ = 18.6, $J_{(2\text{-}P)}$ = 11.4, C2), 83.54 (d, $J_{(3\text{-}F)}$ = 171.2, C3), 86.89 (C_{Tr}), 127.09 (Ph4), 127.83 (Ph3), 128.57 (Ph2), 143.56 (Ph1); MS (ESI) *m/z*: 515 $[M+H]^+$. Anal. Calcd ($C_{29}H_{36}FO_5P$): C, 67.69; H, 7.05; F, 3.69; P, 6.02. Found: C, 67.68; H, 7.15; F, 3.61; P 5.84.

4.2.5. Diisopropyl (*R*)-{[(1-fluoro-3-hydroxypropan-2-yl)oxy]methyl}phosphonate (9a)

Method E: A solution of compound **8a** (33.0 g, 64.1 mmol) in 80% aqueous acetic acid (400 mL) was heated at 90 °C for 30 min. After cooling to room temperature the mixture was evaporated in vacuo and codistilled with water (2 × 50 mL). The residue was extracted with boiling mixture of water–EtOH (9:1, 150 mL) and filtered through Celite. The water phase was extracted with boiling hexane (3 × 100 mL) and concentrated to dryness. The residue was chromatographed on a silica gel column (98:2 chloroform–methanol) to give 12.03 g (69%) of **9a** as colorless oil: $[\alpha]_D$ −9.6 (*c* 0.4, $CHCl_3$); MS (ESI) *m/z*: 295 $[M+Na]^+$. Anal. Calcd ($C_{10}H_{22}FO_5P$): C, 44.12; H, 8.15; F, 6.98; P 11.38. Found: C, 43.88; H, 8.33; F, 6.85; P, 11.41.

4.2.6. Diisopropyl (*S*)-{[(1-fluoro-3-hydroxypropan-2-yl)oxy]-methyl}phosphonate (9b)

Treatment of compound **8b** (35.0 g, 68.0 mmol) by method E afforded 14.32 g (77%) of **9b** as colorless oil; $[\alpha]_D$ +9.8 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.27 (m, 12H, CH_3-*i*Pr), 3.68 (m, 2H, C1), 3.79 (dd, 1H, $J_{(gem)}$ = 13.9, $J_{(H\text{–}C\text{–}P)}$ = 8.7, $C4_b$), 3.94 (dd, 1H, $J_{(gem)}$ = 13.9, $J_{(H\text{–}C\text{–}P)}$ = 8.1, $C4_a$), 4.09 (m, 1H, C2), 4.45 (m, 2H, C3), 4.68 (m, 2H, CH-*i*Pr); ^{13}C NMR ($CDCl_3$): δ 23.82 (m, CH_3-*i*Pr), 60.51 (d, $J_{(1\text{-}F)}$ = 8.1, C1), 65.06 (d, $J_{(C\text{-}P)}$ = 168.9, C4), 71.22 (d, $J_{(C\text{-}O\text{-}P)}$ = 6.8, CH-*i*Pr), 71.59 (d, $J_{(C\text{–}O\text{–}P)}$ = 6.6, CH-*i*Pr), 81.74 (dd, $J_{(2\text{-}F)}$ = 18.5, $J_{(2\text{-}P)}$ = 9.3, C2), 82.89 (d, $J_{(3\text{-}F)}$ = 170.6, C3); MS (ESI) *m/z*: 295 $[M+Na]^+$. Anal. Calcd ($C_{10}H_{22}FO_5P$): C, 44.12; H, 8.15; F, 6.98; P 11.38. Found: C, 44.01; H, 8.35; F, 7.01; P 11.35.

Method F: A mixture of compound **8b** (5.7 g, 11.08 mmol) and Dowex D50 × 8 (H^+ form, 40 ml) in 90% aqueous methanol (200 mL) was refluxed until hydrolysis of the trityl group was completed (TLC, 2 h). After cooling to room temperature the mixture was filtered through Celite and solvent was taken down in vacuo. The residue was chromatographed on a silica gel column (98:2 chloroform–methanol) to give 2.17 g (71%) of **9b** with spectra identical to product prepared by the method E.

4.2.7. Diisopropyl (*R*)-({[1-fluoro-3-(tosyloxy)propan-2-yl]oxy}methyl)phosphonate (10a)

Method G: A mixture of compound **9a** (6.0 g, 22.04 mmol) and Et_3N (3.36 mL, 24.2 mmol) in dry CH_2Cl_2 (60 mL) was cooled at 0 °C and a solution of tosylchloride (5.04 g, 26.4 mmol) in CH_2Cl_2 (150 mL) was added dropwise. After stirring for 2 h at room temperature DMAP (27 mg, 0.22 mmol) was added and the mixture was stirred at room temperature overnight. Solvent was evaporated in vacuo and the residue was purified on a silica gel column ($CHCl_3$, followed by 1% MeOH in $CHCl_3$) to give 6.75 g (72%) of **10a** as yellowish oil; $[\alpha]_D$ −13.2 (*c* 0.4, $CHCl_3$); MS (ESI) *m/z*: 449 $[M+Na]^+$. Anal. Calcd ($C_{17}H_{28}FO_7PS$): C, 47.88; H, 6.62; F, 4.46; P, 7.26. Found: C, 48.01; H, 6.89; F, 4.38; P, 7.35.

4.2.8. Diisopropyl (*S*)-({[1-fluoro-3-(tosyloxy)propan-2-yl]oxy}methyl)phosphonate (10b)

Treatment of compound **9b** (6.6 g, 24.2 mmol) by method G gave 8.45 g (81%) of **10b** as yellowish oil; $[\alpha]_D$ + 14.0 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.31 (d, 6H, $J_{(CH3\text{–}CH)}$ = 6.2 Hz, CH_3-*i*Pr), 28 1.32 (d, 3H, $J_{(CH3\text{–}CH)}$ = 6.2 Hz, CH_3-*i*Pr), 3.83 (d, 2H, $J_{(H\text{–}C\text{–}P)}$ = 8.6, C4), 3.94 (m, 1H, C2), 4.09–4.16 (m, 2H, C1), 4.47 (ddd, 1H, $J_{(3\text{-}F)}$ = 46.8, $J_{(gem)}$ = 10.2, $J_{(3\text{-}2)}$ = 4.9, C3), 4.5 (ddd, 1H, $J_{(3\text{-}F)}$ = 47.0, $J_{(gem)}$ = 10.2, $J_{(3\text{-}2)}$ = 4.2), C3), 4.69–4.76 (m, 2H, CH-*i*Pr), 7.37 (m, 2H, Ph3), 7.79 (m, 2H, Ph2); ^{13}C NMR ($CDCl_3$): δ 21.61 (CH_3-Tos), 23.88 (d, $J_{(C\text{–}C\text{–}O\text{–}P)}$ = 4.4, CH_3-*i*Pr), 24.01 (d, $J_{(C\text{–}C\text{–}O\text{–}P)}$ = 3.6, CH_3-*i*Pr), 65.32 (d, $J_{(C\text{-}P)}$ = 168, C4), 67.22 (d, $J_{(1\text{-}F)}$ = 7.3, C1), 71.31 (d, $J_{(C\text{–}O\text{–}P)}$ = 6.5, CH-*i*Pr), 77.45 (dd, $J_{(2\text{-}F)}$ = 20.0, $J_{(2\text{-}P)}$ = 9.8, C2), 81.35 (d, $J_{(3\text{-}F)}$ = 173.1, C3), 127.93 (Ph2), 129.95 (Ph3), 132.33 (Ph1), 145.17 (Ph4); MS (ESI) *m/z*: 449 $[M+Na]^+$. Anal. Calcd ($C_{17}H_{28}FO_7PS$): C, 47.88; H, 6.62; F, 4.46; P, 7.26. Found: C, 47.96; H, 6.93; F, 4.41; P, 7.36.

Method H: A solution of tosylchloride (3.54 g, 18.51 mmol) in pyridine (30 mL) was added dropwise to a solution of **9b** (4.2 g, 15.42 mmol) in pyridine (50 mL) at 0 °C. The reaction mixture was stirring at 0 °C for 1 h and then allowed to stand in a refrigerator overnight. Then, the reaction mixture was poured into water-ice mixture (250 mL) and extracted with diethylether (4 × 150 mL). Combined organics were washed successively with 1 M HCl (200 mL), water (200 mL), saturated aqueous solution of $NaHCO_3$ (100 mL), and water (200 mL). Organic layer was dried over $MgSO_4$ and filtered. Solvents were evaporated and the residue was chromatographed as in method G to give 4.74 g (72%) of **10b**.

4.2.9. (*R*)-6-Chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}purine (11a)

Method I: Sodium hydride (720 mg, 18 mmol, 60% dispersion in mineral oil) was added to a solution of 6-chloropurine (2.55 g, 16.5 mmol) in DMF (50 mL) and the mixture was stirred at room temperature for 1 h. Then, a solution of **10a** (6.4 g, 15.00 mmol) in DMF (20 mL) was added dropwise and the mixture was stirred at 60 °C for 8 h. Solvent was removed in vacuo and the remaining residue was extracted in chloroform (300 mL). The chloroform solution was filtered through Celite and evaporated to dryness. Silica gel column chromatography (5% MeOH in $CHCl_3$) afforded 2.78 g (45%) of **11a** as white foam; $[\alpha]_D$ +26.0 (*c* 0.4, $CHCl_3$); MS (ESI) *m/z*: 409 $[M+H]^+$. Anal. Calcd ($C_{15}H_{23}ClFN_4O_4P$): C, 44.07; H,

5.67; Cl, 8.67; F, 4.65; N, 13.71; P, 7.58. Found: C, 44.29; H, 5.66; N, 14.00; F, 4.73; P, 7.42.

4.2.10. (*S*)-6-Chloro-9-{2-[(diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}purine (11b)

Treatment of compound **10b** (6.4 g, 15 mmol) by method I gave 3.26 g (53%) of **11b** as white foam; $[\alpha]_D$ −23.5 (*c* 0.4, $CHCl_3$); ^1H NMR: δ 1.25 and 1.29 and 1.31 and 1.33 (4 × d, 4 × 3H, $J_{(CH3\text{-}CH)}$ = 6.2, CH_3-*i*Pr), 3.76 (dd, 1H, $J_{(gem)}$ = 13.9, $J_{(CH2-P)}$ = 8.7, $C4_b$), 4.16 (m, 1H, 2), 3.92 (dd, 1H, $J_{(gem)}$ = 13.9, $J_{(CH2-P)}$ = 8.5, $C4_a$), 4.47 (dd, 1H, $J_{(gem)}$ = 14.7, $J_{(1b\text{-}2)}$ = 7.1, $C1_b$), 4.48 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b\text{-}2)}$ = 4.7, $J_{(3b\text{-}F)}$ = 46.7, $C3_b$), 4.62 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a\text{-}2)}$ = 4.0, $J_{(3a\text{-}F)}$ = 47.1, $C3_a$), 4.70 (m, 2H, CH-*i*Pr), 4.63 (ddd, 1H, $J_{(gem)}$ = 14.7, $J_{(1a\text{-}2)}$ = 3.8, $J_{(1a\text{-}F)}$ = 0.9, $C1_a$), 8.35 (s, 1H, Pu8), 8.75 (s, 1H, Pu2); ^{13}C NMR: 23.86 (m, CH_3-*i*Pr), 43.97 (d, $J_{(1\text{-}F)}$ = 7.6 Hz, C1), 65.12 (d, $J_{(C\text{-}P)}$ = 168.2, C4), 71.29 (m, CH-*i*Pr), 77.76 (dd, $J_{(2\text{-}F)}$ = 19.7, $J_{(2\text{-}P)}$ = 9.1, C2), 81.37 (d, $J_{(3\text{-}F)}$ = 174.3, C3), 131.26 (Pu5), 146.35 (Pu8), 150.94 (Pu6), 151.83 (Pu4), 151.86 (Pu2); ^{19}F NMR: δ -227.70 (td, $J_{(F\text{-}3a)} = J_{(F\text{-}3b)}$ = 46.7, $J_{(F\text{-}2)}$ = 18.6); MS (ESI) *m/z*: 409 $[M+H]^+$. Anal. Calcd ($C_{15}H_{23}ClFN_4O_4P$): C, 44.07; H, 5.67; Cl, 8.67; F, 4.65; N, 13.71; P, 7.58. Found: C, 44.22; H, 5.86; N, 13.56; F, 4.59; P, 7.73.

Method J. Microwave-assisted: Sodium hydride (56 mg, 1.4 mmol) was added to a solution of 6-chloropurine (199 mg, 1.29 mmol) in DMF (5 mL) and the mixture was stirred for 15 min at room temperature. A solution of tosylate **10b** (0.5 g, 1.17 mmol) in DMF (2 mL) was added, the reaction vial was sealed with teflon septum and microwave irradiated at 120 °C for 30 min. After cooling to room temperature the solvent was removed in vacuo and the residue was chromatographed as in the method I to give 282 mg (59%) of **11b**.

Method K. Mitsunobu reaction: A solution of DIAD (0.97 mL, 4.78 mmol) in THF (20 mL) was added dropwise to a mixture of alcohol **9b** (1 g, 3.67 mmol), 6-chloropurine (739 mg, 4.78 mmol), and Ph_3P (1.25 g, 4.78 mmol) in THF (50 mL) and the reaction mixture was stirred at 25 °C for 2 h and then at 60 °C for 6 h. The reaction was monitored by TLC (5% MeOH in $CHCl_3$). After the reaction was completed, the solvent was removed in vacuo and the residue was chromatographed as in the method I to give 396 mg (26%) of **11b**.

4.2.11. (*R*)-2-Amino-6-chloro-9-{2-[(diisopropoxyphosphoryl)-methoxy]-3-fluoropropyl}purine (12a)

Procedure B: A mixture of 2-amino-6-chloropurine (3.5 g, 20.64 mmol) and sodium hydride (750 mg, 18.76 mmol, 60% suspension in mineral oil) in DMF (250 mL) was stirred at 90 °C for 5 min. Tosylate **10a** (8.0 g, 18.76 mmol) was added and the reaction mixture was heated at 90 °C for 2 h. Then solvent was taken down in vacuo and the residue in chloroform (150 mL) was sonicated and filtered through Celite. The filtrate was concentrated in vacuo and chromatographed on a silica gel column (10% MeOH in $CHCl_3$) to give 5.29 g (67%) of **12a** as white solid, mp = 138 °C; $[\alpha]_D$ +23.7 (*c* 0.4, $CHCl_3$); MS (ESI) *m/z*: 447 $[M+Na]^+$. Anal. Calcd ($C_{15}H_{24}ClFN_5O_4P$): C, 42.51; H, 5.71; F, 4.48; N, 16.52; P, 7.31. Found: C, 42.64; H, 5.87; F, 4.40; N, 16.45; P, 7.45. *Crystal data for* **12a**: $C_{15}H_{24}C_{l1}F_1N_5O_4P_1$, orthorhombic, space group $P2_12_12_1$, a = 8.21290(10) Å, b = 10.50670(10) Å, c = 22.9181(3) Å, V = 1977.61(4) Å^3, Z = 4, M = 423.81, 34008 reflections measured, 4149 independent reflections. Final R = 0.030, wR = 0.033, GoF = 1.101 for 3565 reflections with $I > 2\sigma(I)$ and 246 parameters. CCDC 789969.

4.2.12. (*S*)-2-Amino-6-chloro-9-{2-[(diisopropoxyphosphoryl)-methoxy]-3-fluoropropyl}purine (12b)

Treatment of **10b** (6.34 g, 14.87 mmol) by procedure B gave 3.77 g (60%) of **12b** as white powder, mp = 138 °C; $[\alpha]_D$ −22.7 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.26 and 1.30 and 1.31 and 1.34 (4 × d, 4 × 3H, $J_{(CH3\text{-}CH)}$ = 6.2, CH_3-*i*Pr), 3.79 (dd, 1H, $J_{(gem)}$ = 13.8, $J_{(H-C-P)}$ = 8.8, $C4_b$), 3.87 (dd, 1H, $J_{(gem)}$ = 13.8, $J_{(H-C-P)}$ = 8.6, $C4_a$), 4.08 (m, C2), 4.23 (dd, 1H, $J_{(gem)}$ = 14.4, $J_{(1b\text{-}2)}$ = 6.9, $C1_b$), 4.37 (ddd, 1H, $J_{(gem)}$ = 14.6, $J_{(1a\text{-}2)}$ = 3.9, $J_{(1a\text{-}F)}$ = 1.1, $C1_a$), 4.45 (ddd, 1H, $J_{(gem)}$ = 10.4, $J_{(3b\text{-}2)}$ = 4.7, $J_{(3b\text{-}F)}$ = 46.8, $C3_b$), 4.58 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a\text{-}2)}$ = 4.0, $J_{(3a\text{-}F)}$ = 47.2, $C3_a$), 4.71 (m, 2H, CH-*i*Pr), 5.20 (br s, 2H, NH_2), 7.91 (s, Pu8); ^{13}C NMR ($CDCl_3$): δ 23.99 (m, CH_3-*i*Pr), 43.45 (d, $J_{(1\text{-}F)}$ = 7.7, C1), 65.25 (d, $J_{(C\text{-}P)}$ = 168.4, C4), 71.41 (d, $J_{(C-O-P)}$ = 6.6, CH-*i*Pr), 78.00 (dd, $J_{(2\text{-}F)}$ = 19.6, $J_{(2\text{-}P)}$ = 9.8, C2), 81.65 (d, $J_{(3\text{-}F)}$ = 173.9, C3), 124.95 (Pu5), 143.33 (Pu8), 151.34 (Pu6), 153.83 (Pu4), 159.05 (Pu2); MS (ESI) *m/z*: 447 $[M+Na]^+$. Anal. Calcd ($C_{15}H_{24}ClFN_5O_4P$): C, 42.51; H, 5.71; F, 4.48; N, 16.52; P, 7.31. Found: C, 42.35; H, 5.83; F, 4.49; N, 16.33.

4.3. N^6-Mono- and N^6,N^6-disubstituted 9-[3-fluoro-2-(phosphonomethoxy)propyl]adenines. General procedure

Procedure C: A mixture of compound **11a** or **11b** (0.5 g, 1.22 mmol) and the corresponding primary or secondary amine (2 mL) or dimethylammonium *N*,*N*-dimethylcarbamate (2 mL) in acetonitrile (20 mL) was heated with stirring at 70 °C for 4–6 h. Reactions with ethanolic solution of methylamine (5.6 M) were carried out in a steel autoclave. The reactions were monitored by TLC (10% or 20% MeOH in $CHCl_3$). After completion, the solvents were evaporated in vacuo and the residue was codistilled with EtOH (2 × 10 mL). The residue was purified by a flash chromatography on silica gel (10% MeOH in $CHCl_3$). The crude intermediate was dissolved in acetonitrile (20 mL) and Me_3SiBr (2 mL) was added. The mixture was allowed to stand at ambient temperature overnight. The reaction was monitored by TLC (mixture 2-propanol/NH_3/H_2O, 7:2:1). The solvents were evaporated in vacuo and codistilled with toluene (2 × 5 mL) and with EtOH (1 × 5 mL). The crude products **13**–**21** were purified method A or method B.

4.3.1. (*R*)-9-[3-Fluoro-2-(phosphonomethoxy)propyl]-N^6-methyladenine (13a)

Procedure C and method A afforded 125 mg (33%) of **13a** as white solid; $[\alpha]_D$ +6.7 (*c* 0.2, H_2O); MS (ESI) *m/z* 320 $[M-H]^-$. Anal. Calcd ($C_{10}H_{15}FN_5O_4P$): C, 37.62; H, 4.47; F, 5.95; N, 21.94; P, 9.70. Found: C, 37.33; H, 4.75; F, 5.69; N, 21.84.

4.3.2. (*S*)-9-[3-Fluoro-2-(phosphonomethoxy)propyl]-N^6-methyladenine (13b)

Procedure C and method A afforded 186 mg (45%, monohydrate) of **13b** as white solid; $[\alpha]_D$ −8.0 (*c* 0.2, H_2O); ^1H NMR (D_2O+NaOD): δ 3.00 (br s, 3H, CH_3-R_1), 3.54 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.6, $C4_b$), 3.72 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.2, $C4_a$), 4.04 (dm, 1H, $J_{(2\text{-}F)}$ = 22.7, C2), 4.34 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1b\text{-}2)}$ = 7.2, $C1_b$), 4.42 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1a\text{-}2)}$ = 4.3, $C1_a$), 4.50 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b\text{-}2)}$ = 4.0, $J_{(3b\text{-}F)}$ = 46.4, $C3_b$), 4.66 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a\text{-}2)}$ = 3.6, $J_{(3a\text{-}F)}$ = 47.1, $C3_a$), 8.04 (s, 1H, Pu2); 8.07 (s, 1H, Pu8); ^{13}C NMR (D_2O+NaOD): δ 27.69 (CH_3-R_1), 44.04 (d, $J_{(1\text{-}F)}$ = 7.6, C1), 67.02 (d, $J_{(C\text{-}P)}$ = 156.9, C4), 78.49 (dd, $J_{(2\text{-}F)}$ = 18.8, $J_{(2\text{-}P)}$ = 11.6, C2), 82.59 (d, $J_{(3\text{-}F)}$ = 168.3, C3), 118.71 (Pu5), 142.78 (Pu8), 147.84 (Pu4), 152.54 (Pu2), 151.11 (Pu6); MS (ESI+) *m/z* 320 $[M+H]^+$. Anal. Calcd ($C_{10}H_{15}FN_5O_4P.H_2O$): C, 35.61; H, 5.08; F, 5.63; N, 20.77; P, 9.18. Found: C, 35.77; H, 5.20; F, 5.48; N, 20.51.

4.3.3. (*R*)-N^6-Cyclopropyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (14a)

Procedure C and method A afforded 231 mg (55%) of **14a** as white solid; $[\alpha]_D$ +8.1 (*c* 0.3, H_2O); MS (ESI) *m/z* 346 $[M+H]^+$. Anal. Calcd ($C_{12}H_{17}FN_5O_4P$): C, 41.74; H, 4.96; F, 5.50; N, 20.28; P, 8.97. Found: C, 41.47; H, 4.92; F, 5.23; N, 20.05.

4.3.4. (*S*)-N^6-Cyclopropyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (14b)

Procedure C and method A afforded 152 mg (36%) of **14b** as white solid; $[\alpha]_D$ −7.2 (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 0.88 and 1.09 (2 × m, 2 × 2H, CH_2-(2, 2')-R_1), 2.89 (br s, 1H, CH-(1)-R_1), 3.56 (dd, 1H, $J_{(gem)}$ = 13.2, $J_{(H-C-P)}$ = 9.6, $C4_b$), 3.77 (dd, 1H, $J_{(gem)}$ = 13.2, $J_{(H-C-P)}$ = 9.0, $C4_a$), 4.13 (dm, 1H, $J_{(2-F)}$ = 22.7, C2), 4.50–4.75 (m, 4H, $C1_a$, $C1_b$, $C3_a$, $C3_b$), 8.39 (s, 1H, Pu8), 8.47 (s, 1H, Pu2); ^{13}C NMR (D_2O): δ 7.23 (CH_2-R_1), 23.34 (CH-R_1), 44.73 (d, $J_{(1-F)}$ = 7.2, C1), 66.67 (d, $J_{(C-P)}$ = 157.5, C4), 78.52 (dd, $J_{(2-F)}$ = 18.7, $J_{(2-P)}$ = 11.4, C2), 82,51 (d, $J_{(3-F)}$ = 168.4, C3), 118.85 (Pu5), 144.74 (Pu2), 146.04 (Pu8), 148.36 (Pu4), 150.45 (Pu6); MS (ESI) *m/z* 346 $[M+H]^+$. Anal. Calcd ($C_{12}H_{17}FN_5O_4P$): C, 41.74; H, 4.96; F, 5.50; N, 20.28; P, 8.97. Found: C, 41.38; H, 5.08; F, 5.35; N, 20.18.

4.3.5. (*R*)-9-[3-Fluoro-2-(phosphonomethoxy)propyl]-N^6-propyl-adenine (15a)

Procedure C and method A afforded 231 mg (52%, monohydrate) of **15a** as white solid; $[\alpha]_D$ +7.3 (*c* 0.3, H_2O); MS (ESI) *m/z* 348 $[M+H]^+$. Anal. Calcd ($C_{12}H_{19}FN_5O_4P.H_2O$): C, 39.46; H, 5.79; F, 5.20; N, 19.17; P, 8.48. Found: C, 39.43; H, 5.80; F, 5.38; N, 19.37.

4.3.6. (*S*)-9-[3-Fluoro-2-(phosphonomethoxy)propyl]-N^6-propyl-adenine (15b)

Procedure C and method A afforded, 207 mg (49%) of **15b** as white solid; $[\alpha]_D$ −8.0 (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 0.97 (t, 3H, $J_{(CH3-CH2)}$ = 7.4, CH_3-(3)-R_1), 1.67 (m, 2H, CH_2-(2)-R_1), 3.46 (br s, 2H, CH_2-(1)-R_1), 3.49 (dd, 1H, $J_{(gem)}$ = 12.3, $J_{(H-C-P)}$ = 9.1, $C4_b$), 3.66 (dd, 1H, $J_{(gem)}$ = 12.3, $J_{(H-C-P)}$ = 9.3, $C4_a$), 4.04 (dm, 1H, $J_{(2-F)}$ = 23.2, C2), 4.45 (m, 3H, $C1_a$, $C1_b$, $C3_b$), 4.61 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a-2)}$ = 3.8, $J_{(3a-F)}$ = 47.3, $C3_a$), 8.13 (s, 1H, Pu2), 8.21 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 11.21 (CH_3-(3)-R_1), 22.70 (CH_2-(2)-R_1), 45.24 CH_2-(1)-R_1), 43.78 (d, $J_{(1-F)}$ = 6.5, C1), 68.96 (d, $J_{(C-P)}$ = 150.1, C4), 78.03 (dd, $J_{(2-F)}$ = 18.8, $J_{(2-P)}$ = 10.8, C2), 82.76 (d, $J_{(3-F)}$ = 167.4, C3), 118.61 (Pu5), 143.18 (Pu8), 148.26 (Pu4), 152.97 (Pu2), 155,09 (Pu6); MS (FAB) *m/z* 348.1 $[M+H]^+$. Anal. Calcd ($C_{12}H_{19}FN_5O_4P$): C, 41.50; H, 5.51; F, 5.47; N, 20.17; P, 8.92. Found: C, 41.21; H, 5.71; F, 5.26; N, 19.99.

4.3.7. (*R*)-N^6-Butyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]-adenine (16a)

Procedure C and method A afforded 133 mg (30%) of **16a** as white solid; $[\alpha]_D$ +7.5 (*c* 0.3, H_2O); MS (ESI) *m/z* 362 $[M+H]^+$. Anal. Calcd ($C_{13}H_{21}FN_5O_4P$): C, 43.21; H, 5.86; F, 5.26; N, 19.38; P, 8.57. Found: C, 42.95; H, 6.08; F, 5.03; N, 19.09.

4.3.8. (*S*)-N^6-Butyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]-adenine (16b)

Procedure C and method B afforded 264 mg (53%, sodium salt) of **16b** as white solid; $[\alpha]_D$ −5.8 (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 0.92 (t, 3H, $J_{(CH3-CH2)}$ = 7.4, CH_3-R_1), 1.40 (m, 2H, CH_2-(3)-R_1), 1.63 (m, 2H, CH_2-(2)-R_1), 3.46 (br s, 2H, CH_2-(1)-R_1), 3.54 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.5, $C4_b$), 3.72 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.2, $C4_a$), 4.06 (dm, 1H, $J_{(2-F)}$ = 22.7, C2), 4.39 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1b-2)}$ = 7.1, $C1_b$), 4.48 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1a-2)}$ = 4.2, $C1_a$), 4.51 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b-2)}$ = 4.0, $J_{(3b-F)}$ = 46.4, $C3_b$), 4.66 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a-2)}$ = 3.6, $J_{(3a-F)}$ = 47.1, $C3_a$), 8.13 (s, 1H, Pu2), 8.15 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 13.68 (CH_3-R_1), 20.08 (CH_2-(3)-R_1), 31.12 CH_2-(2)-R_1), 41.39 (CH_2-(1)-R_1), 44.18 (d, $J_{(1-F)}$ = 7.3, C1), 67.01 (d, $J_{(C-P)}$ = 157.1, C4), 78.56 (dd, $J_{(2-F)}$ = 18.7, $J_{(2-P)}$ = 11.6, C2), 82.60 (d, $J_{(3-F)}$ = 168.4, C3), 118.78 (Pu5), 143.31 (Pu8), 147.96 (Pu4), 151.54 (Pu2), 153.86 (Pu6); MS (ESI) *m/z* 429 $[M+Na]^+$. Anal. Calcd ($C_{13}H_{19}FN_5Na_2O_4P$): C, 38.53; H, 4.73; F, 4.69; N, 17.28 P, 7.64. Found: C, 38.25; H, 5.03; F, 4.92; N, 17.57.

4.3.9. (*R*)-N^6-(Butan-2-yl)-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (17a)

Procedure C and method A afforded 95 mg (21%) of **17a** as white solid; $[\alpha]_D$ +11.8 (*c* 0.3, H_2O); MS (ESI) *m/z* 362 $[M+H]^+$. Anal. Calcd ($C_{13}H_{21}FN_5O_4P$): C, 43.21; H, 5.86; F, 5.26; N, 19.38; P, 8.57. Found: C, 43.01; H, 6.04; F, 5.14; N, 19.68.

4.3.10. (*S*)-N^6-(Butan-2-yl)-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (17b)

Procedure C and method B afforded 136 mg (27%, sodium salt) of **17b** as white solid; $[\alpha]_D$ −9.9 (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 0.92 (m, 3H, CH_3-(4)-R_1), 1.25 (m, 3H, CH_3-(1)-R_1), 1.62 (m, 2H, CH_2-(3)-R_1), 3.54 (m, 1H, $C4_b$), 3.71 (2 × dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.3, $C4_a$), 4.05 (m, 2H, C2 and CH-(2)-R_1), 4.37 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1b-2)}$ = 7.1, $C1_b$), 4.45 (m, 1H, $C1_a$), 4.49 (2 × ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b-2)}$ = 4.0, $J_{(3b-F)}$ = 46.4, $C3_b$), 4.65 (2 × ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a-2)}$ = 3.6, $J_{(3a-F)}$ = 47.1, $C3_a$), 8.12 (s, 2H, Pu2 and Pu8); ^{13}C NMR (D_2O): δ 10.31 (CH_3-(4)-R_1), 20.07 (CH_3-(1)-R_1), 29.54 CH_2-(3)-R_1), 44.06 (d, $J_{(1-F)}$ = 7.4, C1), 48.68 (CH-(2)-R_1), 67.16 (d, $J_{(C-P)}$ = 156.7, C4), 78.61 (dd, $J_{(2-F)}$ = 18.6, $J_{(2-P)}$ = 11.7, C2), 82.66 (d, $J_{(3-F)}$ = 168.3, C3), 118.70 (Pu5), 142.87 (Pu8), 148.23 (Pu4), 152.71 (Pu2), 154.43 (Pu6); MS (ESI) *m/z* 429 $[M+H]^+$. Anal. Calcd ($C_{13}H_{19}FN_5Na_2O_4P$): C, 38.53; H, 4.73; F, 4.69; N, 17.28 P, 7.64. Found: C, 38.75; H, 5.01; F, 4.51; N, 17.15.

4.3.11. (*R*)-N^6-Cyclopentyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (18a)

Procedure C and method A afforded 266 mg (58%) of **18a** as white solid; $[\alpha]_D$ +7.3 (*c* 0.2, H_2O); MS (ESI) *m/z* 374 $[M+H]^+$. Anal. Calcd ($C_{14}H_{21}FN_5O_4P$):, 45.04; H, 5.67; F, 5.09; N, 18.76; P, 8.30. Found: C, 44.83; H, 5.80; F, 5.28; N, 19.02.

4.3.12. (*S*)-N^6-Cyclopentyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (18b)

Procedure C and method A afforded 157 mg (34%) of **18b** as white solid; $[\alpha]_D$ −5.7 (*c* 0.2, H_2O); ^1H NMR (D_2O): δ 1.61–1.81 and 2.06 (m, 6H + 2H, CH_2-(2-5)-R_1), 3.55 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.5, $C4_b$), 3.73 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H-C-P)}$ = 9.2, $C4_a$), 4.08 (dm, 1H, $J_{(2-F)}$ = 22.7, C2), 4.30 (br s, 1H, CH-(1)-R1), 4.42 (dd, $J_{(gem)}$ = 14.8, $J_{(1b-2)}$ = 7.2, $C1_b$), 4.52 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1a-2)}$ = 4.1, $C1_a$), 4.53 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b-2)}$ = 3.9, $J_{(3b-F)}$ = 46.4, $C3_b$), 4.67 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a-2)}$ = 3.6, $J_{(3a-F)}$ = 47.1, $C3_a$), 8.21 (s, 2H, Pu2 and Pu8); ^{13}C NMR (D_2O): δ 23.91 (CH_2-(3, 4)-R_1), 32.96 (CH_2-(2, 5)-R_1), 44.31 (d, $J_{(1-F)}$ = 7.3, C1), 67.00 (d, $J_{(C-P)}$ = 156.9, C4), 78.56 (dd, $J_{(2-F)}$ = 18.8, $J_{(2-P)}$ = 11.6, C2), 82.63 (d, $J_{(3-F)}$ = 168.3, C3), 118.81 (Pu5), 143.82 (Pu8), 148.03 (Pu4), 149.81 (Pu2), 152.09 (Pu6); MS (ESI) *m/z* 374 $[M+H]^+$. Anal. Calcd ($C_{14}H_{21}FN_5O_4P$): C, 45.04; H, 5.67; F, 5.09; N, 18.76; P, 8.30. Found: C, 44.78; H, 5.79; F, 4.90; N, 18.53; P, 8.03.

4.3.13. (*R*)-N^6,N^6-Dimethyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (19a)

Procedure C and method A afforded 159 mg (39%) of **19a** as white solid; $[\alpha]_D$ +6.3 (*c* 0.3, H_2O); MS (ESI) *m/z* 334 $[M+H]^+$. Anal. Calcd ($C_{11}H_{17}FN_5O_4P$): C, 39.64; H, 5.14; F, 5.70; N, 21.01; P, 9.29. Found: C, 39.43; H, 5.33; F, 5.48; N, 20.90.

4.3.14. (*S*)-N^6,N^6-Dimethyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (19b)

Procedure C and method A afforded 208 mg (48%, monohydrate) of **19b** as white solid; $[\alpha]_D$ −7.2 (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 3.10 (br s, 6H, CH_3-R_1-R_2), 3.30 (dd, 1H, $J_{(gem)}$ = 13.0, $J_{(H-C-P)}$ = 9.7, $C4_b$), 3.48 (dd, 1H, $J_{(gem)}$ = 12.9, $J_{(H-C-P)}$ = 9.3, $C4_a$), 3.82 (dm, 1H, $J_{(2-F)}$ = 22.9, C2), 4.13 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1b-2)}$ = 7.2, $C1_b$), 4.20 (m, 1H, $C1_a$), 4.28 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b-2)}$ = 3.8, $J_{(3b-F)}$ = 46.6, $C3_b$), 4.44 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a-2)}$ = 3.3,

$J_{(3a\text{-}F)}$ = 47.1, $C3_a$), 7.80 (s, 1H, Pu2), 7.85 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 39.58 (CH_3-R_1-R_2), 44.07 (d, $J_{(1\text{-}F)}$ = 7.6, C1), 67.08 (d, $J_{(C\text{-}P)}$ = 157.0, C4), 78.56 (dd, $J_{(2\text{-}F)}$ = 18.7, $J_{(2\text{-}P)}$ = 11.8, C2), 82.68 (d, $J_{(3\text{-}F)}$ = 168.3, C3), 118.93 (Pu5), 141.77 (Pu8), 149.44 (Pu4), 151.71 (Pu2), 154.34 (Pu6); MS (ESI) *m/z* 334 $[M+H]^+$. Anal. Calcd ($C_{11}H_{17}FN_5O_4P.H_2O$): C, 37.61; H, 5.45; F, 5.41; N, 19.94; P, 8.82. Found: C, 37.90; H, 5.40; F, 5.33; N, 19.75.

4.3.15. (*R*)-*N*^{6},*N*^{6}-Diethyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (20a)

Procedure C and method A afforded 121 mg (27%) of **20a** as white solid; $[\alpha]_D$ +15.3 (*c* 0.3, H_2O); MS (ESI) *m/z* 362 $[M+H]^+$. Anal. Calcd ($C_{13}H_{21}FN_5O_4P$): C, 43.21; H, 5.86; F, 5.26; N, 19.38; P, 8.57. Found: C, 42.95; H, 6.05; F, 4.98; N, 19.10.

4.3.16. (*S*)-*N*^{6},*N*^{6}-Diethyl-9-[3-fluoro-2-(phosphonomethoxy)-propyl]adenine (20b)

Procedure C and method B afforded 152 mg (31%, sodium salt) of **20b** as white solid; $[\alpha]_D$ −12.8 (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 1.05 (t, 6H, $J_{(CH3\text{-}CH2)}$ = 7.1, CH_3-R_1-R_2), 3.73 (dd, 1H, $J_{(gem)}$ = 13.1, $J_{(H\text{–}C\text{–}P)}$ = 9.5, $C4_b$), 3.69 (dd, 1H, $J_{(gem)}$ = 13.0, $J_{(H\text{–}C\text{–}P)}$ = 9.4, $C4_a$), 3.77 (br s, 4H, CH_2-R_1), 4.02 (dm, 1H, $J_{(2\text{-}F)}$ = 22.8, C2), 4.35 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1b\text{-}2)}$ = 7.1, $C1_a$), 4.44 (dd, 1H, $J_{(gem)}$ = 15.0, $J_{(1a\text{-}2)}$ = 4.3, $C1_a$), 4.48 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b\text{-}2)}$ = 4.0, $J_{(3b\text{-}F)}$ = 46.4, $C3_b$), 4.65 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a\text{-}2)}$ = 3.6, $J_{(3a\text{-}F)}$ = 47.2, $C3_a$), 8.03 (s, 1H, Pu2), 8.06 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 13.38 (CH_3-R_1-R_2), 44.02 (d, $J_{(1\text{-}F)}$ = 7.4, C1), 44.47 (CH_2-R_1), 67.15 (d, $J_{(C\text{–}P)}$ = 156.8, C4), 78.58 (dd, $J_{(2\text{-}F)}$ = 18.7, $J_{(2\text{-}P)}$ = 11.8, C2), 82.65 (d, $J_{(3\text{-}F)}$ = 168.4, C3), 118.43 (Pu5), 141.75 (Pu8), 149.61 (Pu4), 152.13 (Pu2), 153.44 (Pu6); MS (ESI) *m/z* 429 $[M+Na]^+$. Anal. Calcd ($C_{13}H_{19}FN_5Na_2O_4P$): C, 38.53; H, 4.73; F, 4.69; N, 17.28 P, 7.64. Found: C, 38.82; H, 5.01; F, 4.48; N, 16.99.

4.3.17. (*R*)-9-[3-Fluoro-2-(phosphonomethoxy)propyl]-6-(pyrrolidin-1-yl)purine (21a)

Procedure C and method A afforded 289 mg (66%) of **21a** as white solid; $[\alpha]_D$ +11.5 (*c* 0.3, H_2O); MS (ESI) *m/z* 362 $[M+H]^+$. Anal. Calcd ($C_{13}H_{19}FN_5O_4P$): C, 43.46; H, 5.33; F, 5.29; N, 19.49; P, 8.62. Found: C, 43.21; H, 5.54; F, 5.25; N, 19.55.

4.3.18. (*S*)-9-[3-Fluoro-2-(phosphonomethoxy)propyl]-6-(pyrrolidin-1-yl)purine (21b)

Procedure C and method B afforded 214 mg (43%, sodium salt) of **21b** as white solid; $[\alpha]_D$ −13.4 (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 1.99 and 2.04 (br s, 2 × 2H, CH_2-(3, 4)-R_1-R_2), 3.49 (br s, 2H, CH_2-(2_b, 5_b)-R_1-R_2), 3.49 (dd, 1H, $J_{(gem)}$ = 12.8, $J_{(H\text{–}C\text{–}P)}$ = 9.4, $C4_b$), 3.62 (m, 1H, $C4_a$), 3.82 (br s, 2H, CH_2-(2_a,5_a)-R_1-R_2), 4.01 (dm, 1H, $J_{(2\text{-}F)}$ = 23.0, C2), 4.35 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1b\text{-}2)}$ = 6.8, $C1_b$), 4.42 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1a\text{-}2)}$ = 4.1, $C1_a$), 4.47 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b\text{-}2)}$ = 3.9, $J_{(3b\text{-}F)}$ = 46.4, $C3_b$), 4.64 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a\text{-}2)}$ = 3.7, $J_{(3a\text{-}F)}$ = 47.2, $C3_a$), 7.98 (s, 1H, Pu2), 8.07 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 24.37 and 26.26 (CH_2-(3, 4)-R_1-R_2), 43.96 (d, $J_{(1\text{-}F)}$ = 6.7, C1), 48.50 and 49.67 (CH_2-(2, 5)-R_1-R_2), 67.79 (d, $J_{(C\text{-}P)}$ = 149.6, C4), 78.36 (dd, $J_{(2\text{-}F)}$ = 18.7, $J_{(2\text{-}P)}$ = 11.1, C2), 82.67 (d, $J_{(3\text{-}F)}$ = 168.2, C3), 118.82 (Pu5), 141.98 (Pu8), 149.11 (Pu4), 152.30 (Pu2), 152.52 (Pu6); MS (ESI) *m/z* 427 $[M+Na]^+$. Anal. Calcd ($C_{13}H_{17}FN_5Na_2O_4P$): C, 38.72; H, 4.25; F, 4.71; N, 17.37 P, 7.68. Found: C, 38.86; H, 4.38; F, 4.79; N, 17.12.

4.4. *N*^{6}-Mono- and *N*^{6},*N*^{6}-disubstituted *N*^{9}-[2-(diisopropyl)-phosphonomethoxy-3-fluoropropyl]-2,6-diaminopurines. General procedure

Procedure D: A mixture of compound **12a** or **12b** (0.5 g, 1.18 mmol) and the corresponding primary or secondary amine (2 mL) or dimethylammonium *N*,*N*-dimethylcarbamate (2 mL) in acetonitrile (25 mL) was heated with stirring at 80 °C for 6–8 h. The reactions were monitored by TLC (10% or 20% MeOH in $CHCl_3$). After completion, the work up, deprotection with TMSBr, and purification are analogous to the procedure C.

4.4.1. (*R*)-*N*^{6}-Cyclopropyl-2,6-diamino-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (22a)

Procedure D and method A afforded 195 mg (45%) of **22a** as white solid; $[\alpha]_D$ +14.4 (*c* 0.4, H_2O); MS (ESI) *m/z* 361 $[M+H]^+$. Anal. Calcd ($C_{12}H_{18}FN_6O_4P$): C, 40.01; H, 5.04; F, 5.27; N, 23.33; P, 8.60. Found: C, 40.02; H, 5.24; F, 5.25; N, 23.55.

4.4.2. (*S*)-*N*^{6}-Cyclopropyl-2,6-diamino-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (22b)

Procedure D and method A afforded 221 mg (52%)of **22b** as white solid; $[\alpha]_D$ −11.4 (*c* 0.4, H_2O); ^{1}H NMR (D_2O): δ 0.64 and 0.87 (2 × m, 2 × 2H, CH_2-(2, 2′)-R_1), 2.79 (br s, 1H, CH-(1)-R_1), 3.56 (dd, 1H, $J_{(gem)}$ = 12.9, $J_{(H\text{–}C\text{–}P)}$ = 9.5, $C4_b$), 3.70 (dd, 1H, $J_{(gem)}$ = 12.9, $J_{(H\text{–}C\text{–}P)}$ = 9,3, $C4_a$), 4.02 (dm, 1H, $J_{(2\text{-}F)}$ = 23.3, C2), 4.21 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1b\text{-}2)}$ = 6.9, $C1_b$), 4.29 (dd, 1H, $J_{(gem)}$ = 14.9, $J_{(1a\text{-}2)}$ = 4.5, $C1_a$), 4.48 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b\text{-}2)}$ = 3.9, $J_{(3b\text{-}F)}$ = 46.5, $C3_b$), 4.67 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a\text{-}2)}$ = 3.4, $J_{(3a\text{-}F)}$ = 47.2, $C3_a$), 7.81 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 7.34 (CH_2-(2, 2′)-R_1), 23.93 (CH-(1)-R_1), 43.68 (d, $J_{(1\text{-}F)}$ = 7.4, C1), 66.69 (d, $J_{(C\text{–}P)}$ = 155.5, C4), 78.54 (dd, $J_{(2\text{-}F)}$ = 18.6, $J_{(2\text{-}P)}$ = 11.6, C2), 82.79 (d, $J_{(3\text{-}F)}$ = 168.0, C3), 113.12 (Pu5), 140.83 (Pu8), 150.43 (Pu4), 155.96 (Pu6), 159.54 (Pu2); MS (ESI) *m/z* 361 $[M+H]^+$. Anal. Calcd ($C_{12}H_{18}FN_6O_4P$): C, 40.01; H, 5.04; F, 5.27; N, 23.33 P, 8.60. Found: C, 39.82; H, 5.18; F, 5.31; N, 23.38.

4.4.3. (*R*)-2,6-Diamino-*N*^{6}-propyl-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (23a)

Procedure D and method A afforded 267 mg (62%) of **23a** as white solid; $[\alpha]_D$ +20.3 (*c* 0.4, H_2O); MS (ESI) *m/z* 363 $[M+H]^+$. Anal. Calcd ($C_{12}H_{20}FN_6O_4P$): C, 39.78; H, 5.56; F, 5.24; N, 23.20; P, 8.55. Found: C, 39.55; H, 5.65; F, 4.96; N, 22.95.

4.4.4. (*S*)-2,6-Diamino-*N*^{6}-propyl-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (23b)

Procedure D and method A afforded 245 mg (57%) of **23b** as white solid; $[\alpha]_D$ −19.2 (*c* 0.4, H_2O); ^{1}H NMR (D_2O+NaOD): δ 0.95 (t, 3H, $J_{(CH3\text{-}CH2)}$ = 7.4, CH_3-R_1), 1.64 (sxt, 2H, $J_{(CH2\text{-}CH3)}$ = $J_{(CH2\text{-}CH2)}$ = 7.3, CH_2-(2)-R_1), 3.41 (br s, 2H, CH_2-(1)-R_1), 3.50 (dd, 1H, $J_{(gem)}$ = 12.4, $J_{(H\text{–}C\text{–}P)}$ = 9.2, $C4_b$), 3.58 (dd, 1H, $J_{(gem)}$ = 12.4, $J_{(H\text{–}C\text{–}P)}$ = 9.3, $C4_a$), 4.00 (dm, 1H, $J_{(2\text{-}F)}$ = 23.9, C2), 4.25 (dd, 1H, $J_{(gem)}$ = 14.7, $J_{(1b\text{-}2)}$ = 6.4, $C1_b$), 4.30 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1a\text{-}2)}$ = 5.2, $C1_a$), 4.45 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b\text{-}2)}$ = 3.7, $J_{(3b\text{-}F)}$ = 46.5, $C3_b$), 4.66 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a\text{-}2)}$ = 3.6, $J_{(3a\text{-}F)}$ = 47.3, $C3_a$), 7.86 (s, 1H, Pu8); ^{13}C NMR (D_2O+NaOD): δ 11.24 (CH_3-R_1), 22.81 (CH_2-(2)-R_1), 43.42 (d, $J_{(1\text{-}F)}$ = 6.9, C1), 68.67 (d, $J_{(C\text{-}P)}$ = 151.2, C4), 78.23 (dd, $J_{(2\text{-}F)}$ = 18.5, $J_{(2\text{-}P)}$ = 10.9, C2), 82.83 (d, $J_{(3\text{-}F)}$ = 167.4, C3), 113.36 (Pu5), 140.6 (Pu8), 150.26 (Pu4), 155.73 (Pu6); MS (ESI) *m/z* 363 $[M+H]^+$. Anal. Calcd ($C_{12}H_{20}FN_6O_4P$): C, 39.78; H, 5.56; F, 5.24; N, 23.20; P, 8.55. Found: C, 39.61; H, 5.60; F, 5.14; N, 23.03.

4.4.5. (*R*)-*N*^{6}-Allyl-2,6-diamino-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (24a)

Procedure D and method A afforded 271 mg (64%) of **24a** as white solid; $[\alpha]_D$ +9.1 (*c* 0.3, H_2O); MS (ESI) *m/z* 359 $[M-H]^-$. Anal. Calcd ($C_{12}H_{18}FN_6O_4P$): C, 40.72; H, 5.04; F, 5.27; N, 23.33 P, 8.60. Found: C, 40.52; H, 5.23; F, 5.07; N, 23.23.

4.4.6. (*S*)-*N*^{6}-Allyl-2,6-diamino-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (24b)

Procedure D and method B afforded 175 mg (37%, sodium salt) of **24b** as white solid; $[\alpha]_D$ −8.7 (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 3.56

(dd, 1H, $J_{(gem)}$ = 12.9, $J_{(H–C–P)}$ = 9.3, $C4_b$), 3.69 (dd, 1H, $J_{(gem)}$ = 12.9, $J_{(H–C–P)}$ = 9.2, $C4_a$), 4.03 (dm, 1H, $J_{(2-F)}$ = 23.0, C2), 4.12 (br s, 2H, CH_2-(1)-R_1), 4.23 (dd, 1H, $J_{(gem)}$ = 14.7, $J_{(1b-2)}$ = 6.8, $C1_b$), 4.31 (dd, 1H, $J_{(gem)}$ = 14.6, $J_{(1a-2)}$ = 4.6, $C1_a$), 4.48 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b-2)}$ = 4.0, $J_{(3b-F)}$ = 46.5, $C3_b$), 4.66 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a-2)}$ = 3.5, $J_{(3a-F)}$ = 47.2, $C3_a$), 5.17 (dq, 1H, $^3J_{(cis)}$ = 10.4, $J_{(gem)}$ = $^3J_{(trans)}$ = 1.5, $CH_{(trans)}$-(3)-R_1), 5.24 (dq, 1H, $^3J_{(cis)}$ = $J_{(gem)}$ = 1.5, $3J_{(trans)}$ = 17.3, $CH_{(cis)}$-(3)-R_1), 6.00 (ddt, 1H, $^3J_{(cis)}$ = 10.4, $J_{(CH–CH2)}$ = 4.9, $^3J_{(trans)}$ = 17.3, CH-(2)-R_1), 7.83 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 43.16 (CH_2-(1)-R_1), 43.68 (d, $J_{(1-F)}$ = 7.6, C1), 67.54 (d, $J_{(C-P)}$ = 155.3, C4), 78.56 (dd, $J_{(2-F)}$ = 18.7, $J_{(2-P)}$ = 11.3, C2), 82.81 (d, $J_{(3-F)}$ = 167.9, C3), 113.27 (Pu5), 116.07 (CH_2-(3)-R_1), 135.00 (CH-(2)-R_1), 140.69 (Pu8), 150.53 (Pu4), 155.44 (Pu6); MS (ESI) *m/z* 384 $[M+Na]^+$. Anal. Calcd ($C_{12}H_{16}FN_6Na_2O_4P$): C, 35.65; H, 3.99; F, 4.70; N, 20.79 P, 7.66. Found: C, 35.38; H, 4.28; F, 4.71; N, 20.66.

4.4.7. (*R*)-2,6-Diamino-N^6-(*N*,*N*-dimethylaminoethyl)-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (25a)

Procedure D and method A afforded 109 mg (24%, monohydrate) of **25a** as white solid; $[\alpha]_D$ +8.4 (*c* 0.2, H_2O); MS (ESI) *m/z* 390 $[M-H]^-$. Anal. Calcd ($C_{13}H_{23}FN_7O_4P{\cdot}H_2O$): C, 38.14; H, 6.16; F, 4.64; N, 23.95 P, 7.57. Found: C, 38.02; H, 5.89; F, 4.87; N, 23.76.

4.4.8. (*S*)-2,6-Diamino-N^6-(*N*,*N*-dimethylaminoethyl)-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (25b)

Procedure D and method B afforded 94 mg (18%, sodium salt) of **25b** as white solid; $[\alpha]_D$ −6.8 (*c* 0.2, H_2O); ^{1}H NMR (D_2O): δ 2.96 (s, 6H, CH_3-R_1), 3.43 (t, 2H, $J_{(CH2–CH2)}$ = 5.9, CH_2-(2)-R_1), 3.51 (dd, 1H, $J_{(gem)}$ = 12.7, $J_{(H–C–P)}$ = 9.4, $C4_b$), 3.64 (dd, 1H, $J_{(gem)}$ = 12.7, $J_{(H–C–P)}$ = 9.2, $C4_a$), 3.90 (m, 2H, CH_2-(1)-R_1), 4.02 (dm, 1H, $J_{(2-F)}$ = 23.6, C2), 4.21 (dd, 1H, $J_{(gem)}$ = 14.6, $J_{(1b-2)}$ = 6.9, $C1_b$), 4.28 (dd, 1H, $J_{(gem)}$ = 14.6, $J_{(1a-2)}$ = 4.5, $C1_a$), 4.49 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b-2)}$ = 3.8, $J_{(3b-F)}$ = 46.5, $C3_b$), 4.67 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a-2)}$ = 3.6, $J_{(3a-F)}$ = 47.3, $C3_a$), 7.86 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 36.23 (CH_3-R_1), 43.68 (C1), 43.71 (CH_3-R_1), 57.95 (CH_2-(2)-R_1), 67.96 (d, $J_{(C-P)}$ = 153.5, C4), 78.28 (dd, $J_{(2-F)}$ = 18.6, $J_{(2-P)}$ = 11.2, C2), 82.76 (d, $J_{(3-F)}$ = 167.9, C3), 113.37 (Pu5), 140.87 (Pu8), 151.04 (Pu4), 155.42 (Pu6), 160.38 (Pu2); MS (FAB) *m/z* 392 $[M+H]^+$. Anal. Calcd ($C_{13}H_{21}FN_7Na_2O_4P$): C, 35.87; H, 4.86; F, 4.36; N, 22.52 P, 7.12. Found: C, 35.60; H, 5.04; F, 4.34; N, 22.37.

4.4.9. (*R*)-2,6-Diamino-N^6-(2-methoxyethyl)-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (26a)

Procedure D and method A afforded 249 mg (56%, monohydrate) of **26a** as white solid; $[\alpha]_D$ +6.2 (*c* 0.2, H_2O); MS (ESI) *m/z* 377 $[M-H]^-$. Anal. Calcd ($C_{13}H_{23}FN_7O_4P.H_2O$): C, 38.10; H, 5.33; F, 5.02; N, 22.22 P, 8.19. Found: C, 37.89; H, 5.45; F, 5.00; N, 22.09.

4.4.10. (*S*)-2,6-Diamino-N^6-(2-methoxyethyl)-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (26b)

Procedure D and method B afforded 182 mg (37%, sodium salt) of **26b** as white solid; $[\alpha]_D$ −5.4 (*c* 0.2, H_2O); ^{1}H NMR (D_2O): δ 3.4 (s, 3H, CH_3-R_1), 3.56 (dd, 1H, $J_{(gem)}$ = 12.8, $J_{(H–C–P)}$ = 9.4, $C4_b$), 3.71 m (dd, 5H, CH_2-(1, 2)-R_1, $C4_a$), 4.03 (dm, $J_{(2-F)}$ = 23.2, C2), 4.24 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1b-2)}$ = 6.8, $C1_b$), 4.32 (dd, 1H, $J_{(gem)}$ = 14.7, $J_{(1a-2)}$ = 4.6, $C1_a$), 4.48 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b-2)}$ = 3.9, $J_{(3b-F)}$ = 46.4, $C3_b$), 4.65 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a-2)}$ = 3.5, $J_{(3a-F)}$ = 47.2, $C3_a$), 7.84 (s, Pu8); ^{13}C NMR (D_2O): δ 40.32 (CH_2-(1)-R_1), 43.68 (d, $J_{(1-F)}$ = 7.6, C1), 58.68 (CH_3-R_1), 67.51 (d, $J_{(C-P)}$ = 155.5, C4), 71.34 (CH_2-(2)-R_1), 78.60 (dd, $J_{(2-F)}$ = 18.7, $J_{(2-P)}$ = 11.2, C2), 82.80 (d, $J_{(3-F)}$ = 167.9, C3), 113.48 (Pu5), 140.73 (Pu8), 150.50 (Pu4), 155.78 (Pu6), 160.38 (Pu2); MS (ESI) *m/z* 446 $[M+Na]^+$. Anal. Calcd ($C_{12}H_{18}FN_6Na_2O_5P$): C, 34.13; H, 4.30; F, 4.50; N, 19.90 P, 7.43. Found: C, 33.89; H, 4.56; F, 4.53; N, 19.86.

4.4.11. (*R*)-2,6-Diamino-N^6,N^6-dimethyl-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (27a)

Procedure D and method A afforded 213 mg (52%) of **27a** as white solid; $[\alpha]_D$ +12.6 (*c* 0.3, H_2O); MS (ESI) *m/z* 349 $[M+H]^+$. Anal. Calcd ($C_{11}H_{18}FN_6O_4P$): C, 37.94; H, 5.21; F, 5.45; N, 24.13; P, 8.89. Found: C, 37.72; H, 5.44; F, 5.38; N, 23.94.

4.4.12. (*S*)-2,6-Diamino-N^6,N^6-dimethyl-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (27b)

Procedure D and method B afforded 122 mg (26%, sodium salt) of **27b** as white solid; $[\alpha]_D$ −9.7 (*c* 0.3, H_2O); ^{1}H NMR (D_2O+NaOD): δ 3.29 (br s, 6H, CH_3-R_1), 3.47 (dd, 1H, $J_{(gem)}$ = 12.3, $J_{(H–C–P)}$ = 9.1, $C4_b$), 3.52 (dd, 1H, $J_{(gem)}$ = 12.3, $J_{(H–C–P)}$ = 9.3, $C4_a$), 3.99 (dm, 1H, $J_{(2-F)}$ = 24.3, C2), 4.28 (m, 2H, C1), 4.44 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3b-2)}$ = 3.6, $J_{(3b-F)}$ = 46.5, $C3_b$), 4.66 (ddd, 1H, $J_{(gem)}$ = 10.5, $J_{(3a-2)}$ = 3.5, $J_{(3a-F)}$ = 47.4, $C3_a$), 7.85 (s, 1H, Pu8); ^{13}C NMR (D_2O+NaOD): δ 38.98 (CH_3-R_1), 43.40 (d, $J_{(1-F)}$ = 7.1, C1), 68.98 (d, $J_{(C-P)}$ = 150.2, C4), 78.11 (dd, $J_{(2-F)}$ = 18.4, $J_{(2-P)}$ = 10.8, C2), 82.87 (d, $J_{(3-F)}$ = 167.3, C3), 113.77 (Pu5), 139.68 (Pu8), 152.13 (Pu4), 155.58 (Pu6), 160.08 (Pu2); MS (ESI) *m/z* 416 $[M+Na]^+$. Anal. Calcd ($C_{11}H_{16}FN_6Na_2O_4P$): C, 33.68; H, 4.11; F, 4.84; N, 21.43 P, 7.90. Found: C, 33.56; H, 4.38; F, 4.72; N, 21.28.

4.4.13. (*R*)-2,6-Diamino-N^6,N^6-diethyl-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (28a)

Procedure D and method B afforded 220 mg (44%, sodium salt) of **28a** as white solid; $[\alpha]_D$ +21.9 (*c* 0.4, H_2O); MS (ESI) *m/z* 377 $[M+H]^+$. Anal. Calcd ($C_{13}H_{20}FN_6Na_2O_4P$): C, 37.15; H, 4.80; F, 4.52; N, 20.00; P, 7.37. Found: C, 37.42; H, 5.55; F, 4.25; N, 19.72.

4.4.14. (*S*)-2,6-Diamino-N^6,N^6-diethyl-9-[3-fluoro-2-(phosphonomethoxy)propyl]purine (28b)

Procedure D and method A afforded 372 mg (84%) of **28b** as white solid; $[\alpha]_D$ −18.4 (*c* 0.4, H_2O); ^{1}H NMR (D_2O+NaOD): δ 1.19 (m, 6H, CH_3-R_1), 3.57 (dd, 1H, $J_{(gem)}$ = 12.8, $J_{(H–C–P)}$ = 9.2, $C4_b$), 3.70 (m, 1H, $C4_a$), 3.77 (br s, 4H, CH_2-R_1), 4.02 (dm, 1H, $J_{(2-F)}$ = 22.9, C2), 4.24 (dd, 1 H, $J_{(gem)}$ = 14.9, $J_{(1b-2)}$ = 6.6, $C1_b$), 4.32 (dd, 1H, $J_{(gem)}$ = 15.0, $J_{(1a-2)}$ = 4.0, $C1_a$), 4.47 (ddd, 1H, $J_{(gem)}$ = 10.7, $J_{(3b-2)}$ = 3.8, $J_{(3b-F)}$ = 46.5, $C3_b$), 4.64 (ddd, 1H, $J_{(gem)}$ = 10.7, $J_{(3a-2)}$ = 3.5, $J_{(3b-F)}$ = 47.2, $C3_a$), 7.78 (s, 1H, Pu8); ^{13}C NMR (D_2O+NaOD): δ 13.34 (CH_3-R_1), 43.66 (CH_2-R_1), 43.97 (br s, C1), 67.52 (d, $J_{(C-P)}$ = 157.7, C4), 78.64 (dd, $J_{(2-F)}$ = 18.9, $J_{(2-P)}$ = 11.2, C2), 82.76 (d, $J_{(3-F)}$ = 168.3, C3), 113.20 (Pu5), 139.72 (Pu8), 150.68 (Pu4), 153.71 (Pu6), 158.81 (Pu2); MS (ESI) *m/z* 377 $[M+H]^+$. Anal. Calcd ($C_{13}H_{22}FN_6O_4P$): C, 41.49; H, 5.89; F, 5.05; N, 22.33 P, 8.23. Found: C, 41.14; H, 5.70; F; 4.87; N, 22.16.

4.4.15. (*R*)-2-Amino-9-[3-fluoro-2-(phosphonomethoxy)-propyl]-6-(pyrrolidin-1-yl)purine (29a)

Procedure D and method A afforded 238 mg (54%) of **29a** as white solid; $[\alpha]_D$ +10.9 (*c* 0.3, H_2O); MS (ESI) *m/z* 373 $[M-H]^-$. Anal. Calcd ($C_{13}H_{20}FN_6O_4P$): C, 41.71; H, 5.39; F, 5.08; N, 22.45; P, 8.27. Found: C, 41.64; H, 5.46; F, 4.87; N, 22.29.

4.4.16. (*S*)-2-Amino-9-[3-fluoro-2-(phosphonomethoxy)-propyl]-6-(pyrrolidin-1-yl)purine (29b)

Procedure D and method B afforded 249 mg (50%, sodium salt) of **29b** as white solid; $[\alpha]_D$ −9.4 (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 1.93 and 2.00 (br s, 2 × 2H, CH_2-(3, 4)-R_1-R_2), 3.44 (br s, 2H, CH_2-(2_b,5_b)-R_1-R_2), 3.52 (dd, 1H, $J_{(gem)}$ = 12.8, $J_{(H–C–P)}$ = 9.3, $C4_b$), 3.65 (dd, 1H, $J_{(gem)}$ = 12.8, $J_{(H–C–P)}$ = 9.3, $C4_a$), 3.77 (br s, 2H, CH_2-(2_a, 5_a)-R_1-R_2), 3.99 (dm, 1H, $J_{(2-F)}$ = 23.3, C2), 4.21 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1b-2)}$ = 6.9, $C1_b$), 4.28 (dd, 1H, $J_{(gem)}$ = 14.8, $J_{(1a-2)}$ = 4.5, $C1_a$), 4.47 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3b-2)}$ = 3.9, $J_{(3b-F)}$ = 46.5, $C3_b$), 4.66 (ddd, 1H, $J_{(gem)}$ = 10.6, $J_{(3a-2)}$ = 3.5, $J_{(3a-F)}$ = 47.2, $C3_a$), 7.75 (s, 1H, Pu8); ^{13}C NMR (D_2O): δ 24.38 and 26.17 (br s, CH_2-(3, 4)-R_1-R_2), 43.72

(d, $J_{(1\text{-}F)}$ = 7.5, C1), 48.27 and 49.72 (br s, CH_2-(2, 5)-R_1-R_2), 67.78 (d, $J_{(C\text{-}P)}$ = 154.6, C4), 78.49 (dd, $J_{(2\text{-}F)}$ = 18.6, $J_{(2\text{-}P)}$ = 11.3, C2), 82.78 (d, $J_{(3\text{-}F)}$ = 168.0, C3), 113.54 (Pu5), 139.72 (Pu8), 150.89 (Pu4), 152.82 (Pu6), 159.34 (Pu2); MS (ESI) *m/z* 375 $[M+H]^+$. Anal. Calcd ($C_{13}H_{18}FN_6Na_2O_4P$): C, 37.33; H, 4.34; F, 4.54; N, 20.09 P, 7.41. Found: C, 37.33; H, 4.64; F, 4.49; N, 19.86.

4.5. Biological assays

4.5.1. Anti-HIV activity assays

Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing $\sim 3 \times 10^5$ CEM cells/mL infected with 100 $CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4–5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

4.5.2. Cytostatic activity assay

All assays were performed in 96-well microtiter plates. To each well were added $(5-7.5) \times 10^4$ cells and a given amount of the test compound. The CEM cells were allowed to proliferate for 72 h at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

4.5.3. Anti-Moloney murine sarcoma sirus (MSV) assays

The inhibitory effect of the test compounds on MSV-induced transformation of murine embryo fibroblast C3H/3T3 cell cultures was examined microscopically at day 6 postinfection. MSV was added at 75 focus-forming units to monolayer cell cultures in 48-well microtiter plates.

4.5.4. Enzyme assay

Recombinant human N^6-methyl-AMP deaminase (human abacavir monophosphate deaminase) was prepared in Gilead Science (Foster City, CA, USA).[15b] The tested compounds were assayed in the reaction mixture containing 50 mM PIPES (pH 6.8), 2 mM DTT, 100 μg mL^{-1} BSA, 12 μg mL^{-1} of enzyme and 50 μM substrate. Mixture was incubated 60 min at 37 °C and then processed and analyzed by HPLC (see Ref. 24 for details). The substrate activity of studied compounds was expressed as the rate of product formation (nmol min^{-1} mg).

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