Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 965–980
Bioorganic & Medicinal Chemistry

Synthesis of guanidino analogues of PMPDAP and their immunobiological activity

Michal Česnek,[a,*] Antonín Holý,[a] Milena Masojídková,[a] Eva Kmoníčková[b] and Zdeněk Zídek[b]

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2, 166 10 Praha 6, Czech Republic*

[b]*Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, v.v.i. Vídeňská 1083, 142 20 Praha 4, Czech Republic*

Received 20 August 2007; accepted 4 October 2007
Available online 9 October 2007

Abstract—A series of novel 9-, 7- and 3-substituted 2- or 6-guanidinopurines as analogues of potent antiviral and immunobiologically active compound enantiomers of PMPDAP was synthesized and evaluated for their biological activity. Compounds containing the combination of guanidino and amino group at the purine moiety enhanced the interferon-γ-triggered NO production in murine macrophages and stimulated the secretion of cytokines and chemokines in both murine macrophages and human peripheral blood mononuclear cells. The most active compounds are **27** and **54**. None of the compounds tested exhibited any significant cytostatic effect or antiviral effect.

1. Introduction

Acyclic nucleotide analogues are an important class of antivirals effective against replication of both DNA-viruses and retroviruses.[1] Therapeutically most important compounds of this class are prodrugs of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; adefovir) (**1**), approved by FDA for treatment of hepatitis B, and 9-(*R*)-[2-(phosphonomethoxy)propyl] adenine [(*R*)-PMPA (**2**); tenofovir], one of the most frequently prescribed anti-AIDS drugs. The antiviral activity of acyclic nucleotide analogues is acquired after transformation by intracellular kinases to the mono- and diphosphoryl derivatives, and it is assumed to be mainly due to suppression of viral DNA synthesis mediated by inhibition of viral DNA-polymerases. Some of these compounds, including tenofovir, possess also immunomodulatory properties that are relevant to antiviral activity.[2]

The immunostimulatory effect of compounds in murine macrophages has been found to be enhanced with certain substitutions of (*R*)-PMPDAP (9-(*R*)-[2-(phosphonomethoxy)propyl]-2,6-diaminopurine) (**3**) at the N^6-position.[2a]

In guanidinopurines the amino group is replaced and mimicked by the guanidino group. Interestingly, while the antiviral activity of guanidinopurines against DNA or RNA viruses, including 6- or 2-guanidino analogues of PMEDAP (9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine) and PMPDAP, is very low,[3] the immunostimulatory activity of 6-guanidino analogues of PMEDAP is preserved.[4] In an attempt to elucidate the influence of the position of guanidino group at the purine moiety on the intrinsic immunostimulatory potential of acyclic nucleotide analogues, we have prepared the series of 6- or 2-guanidinopurine 7- or 9-PMP analogues. We investigated also the influence of combination of additional amino group with guanidino group at the purine moiety. It seems that the presence of an amino group at the position 2 of the purine moiety is necessary for biological properties of guanidinopurines. This assumption is supported by our recent study of 2-amino-6-guanidino derivatives with the short aliphatic chain at the position 9 of the purine moiety.[5] While one of these compounds showed the immunostimulatory effect, a number of others were cytocidal.

Keywords: Guanidinopurines; Acyclic nucleotide analogues; Immunobiological activity; PMPDAP.
* Corresponding author. Tel.: +420 220183262; fax: +420 220183560; e-mail: cesnek@uochb.cas.cz

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.10.010

2. Synthesis

An experience from the synthesis of 6-guanidinopurines with various substituents in position 9 at the purine moiety was applied for the preparation of (*R*) and (*S*) 9, 7 and 3-{2-[(diisopropylphosphoryl)methoxy]propyl} derivatives. There are two possible approaches to the synthesis of guanidinopurines, the first is the alkylation of an appropriate halopurine **4** or **5** with (*R*) or (*S*)-2-[(diisopropoxyphosphoryl)methoxy]propyl 4-methylbenzenesulfonate (**6**) and (**7**) with subsequent guanidinolysis, the other one consists in an direct alkylation of guanidinopurine base.

The first approach was used for the synthesis of 2-amino-6-chloro-9-(*R*) and (*S*)-[2-(diisopropoxyphosphoryl)methoxy]propylpurines[6] (**13**), (**14**) and 2-amino-6-chloro-7-(*R*) or (*S*)-[2-(diisopropoxyphosphoryl) methoxy]propylpurines (**9**) and (**10**) (Scheme 1). These compounds were treated with guanidine at room temperature as described in the literature.[7]

The desired 6-guanidinopurine 9-(*R*) and (*S*)-[2-(diisopropoxyphosphoryl)methoxy]propyl derivatives **20** and **21** were obtained in good yield. On the other hand, 2-amino-7 (*R*) and (*S*) [(diisopropoxyphosphoryl)methoxy]propyl-6-guanidinopurines (**16**), (**17**) and compound **15** were obtained in low yields. This was probably caused by lower reactivity of the 6-chloro at 7-regioisomer.

The same synthetic procedure was applied also for the preparation of 6-chloro-7-(*R*)-[2-(diisopropoxyphosphoryl)methoxy]propylpurine (**8**) and 6-chloro-9-(*R*) and (*S*)-[2-(diisopropoxyphosphoryl)methoxy]propylpurine[8] (**11**) and (**12**).

The desired 6-guanidinopurine 9-(*R*) and (*S*)-[2-(diisopropoxyphosphoryl)methoxy]propyl derivatives **18** and **19** were obtained in good yield.

As it was difficult to isolate the intermediate compound 6-chloro-7-(*S*)-[2-(diisopropoxyphosphoryl)methoxy] propylpurine, we have chosen a different approach—direct alkylation of 6-guanidinopurine (**29**).[3b] Yield of this reaction is low, but the ratio of 7-regioisomer to the 9-regioisomer is better than the alkylation of 6-chloropurine derivative. The alkylation of 6-guanidinopurine gave the 9-regioisomer **31** which was identical with compound **19** and the desired compound **30** in 2:1 ratio (Scheme 2).

Synthesis of 2-guanidinopurine derivatives began from 2-chloropurine **33** which was transformed into the intermediate *R* and *S* 2-chloroderivatives **35**, **37** and **34**, **36**, respectively. 2-Chloropurine[9] **33** was treated with (*R* or *S*)-[2-(diisopropoxyphosphoryl)methoxy]propyl 4-methylbenzenesulfonate (**6**) or (**7**) in the presence of NaH (Scheme 3). 7-Regioisomers **34** and **35** were transformed by guanidinolysis at elevated temperature into the 2-guanidino derivatives **38** and **39**, also 9-regioisomers **40** and **41** were transformed from corresponding 6-chloro derivatives **36** and **37** by the same procedure. The guanidinolysis of 9-regioisomers and also 7 regioisomers affords satisfactory yield.

9-*R* and *S*-[2-(Diisopropoxyphosphoryl)methoxy]propyl regioisomers of 6-amino-2-guanidinopurine **47** and **48** were prepared by the alkylation of 6-amino-2-guanidinopurine (**46**) by (*R*) or (*S*)-[2-(diisopropoxyphosphoryl)methoxy]propyl 4-methylbenzenesulfonate (**6**) or (**7**) and Cs_2CO_3 (Scheme 4). These reactions afforded also the second regioisomer: 3-regioisomers **49** and **50** were isolated instead of expected 7-regioisomers.

The first approach is preferable for synthesis of the 9-regioisomer as a major product. If a 7-regioisomer is preferable, the direct alkylation of guanidinopurine base can be used. The ratio of the regioisomers is 1:1. Surprisingly, the alkylation of 6-amino-2-guanidinopurine affords 3-regioisomer instead of 7-regioisomer.

Free phosphonic acids **22**–**28**, **32**, **42**–**45** and **51**–**54** were obtained from compounds **15**–**21**, **30**, **31**, **38**–**41** and **47**–**50** by the transesterification reaction of phosphonomethoxypropyl esters with bromotrimethyl silane and subsequent hydrolysis.

3. Materials and methods

3.1. Compounds

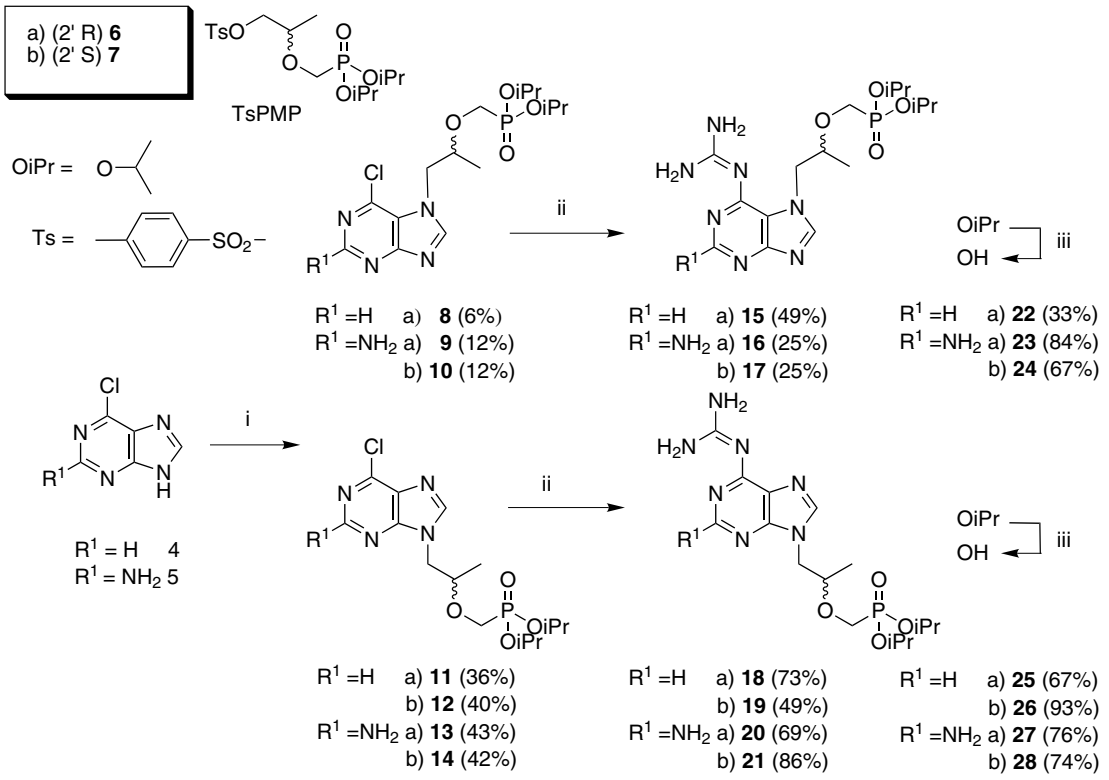
Stock solutions of acyclic guanidinopurines (5 mM) were prepared in incomplete $NaHCO_3$-containing, phenol red-free RPMI-1640 medium (Sigma–Aldrich, Praha, Czech Republic). They were sterile filtered using non-pyrogenic 0.22 μm filters (Costar, Cambridge, MA), used fresh or kept no longer than four weeks at −20 °C. Required working concentrations were prepared by diluting the stock solution in complete RPMI-1640 culture medium (described below).

The chromogenic Limulus Amoebocyte Lysate assay (Kinetic-QCL; Cambrex Bio Science, Walkersville, MD) was used to check for possible contamination with lipopolysaccharide (LPS). The final 50-μM concentrations of test acyclic nucleoside phosphonates contained <10 pg/ml of LPS, an amount that is virtually ineffective to activate secretion of cytokines.[2a]

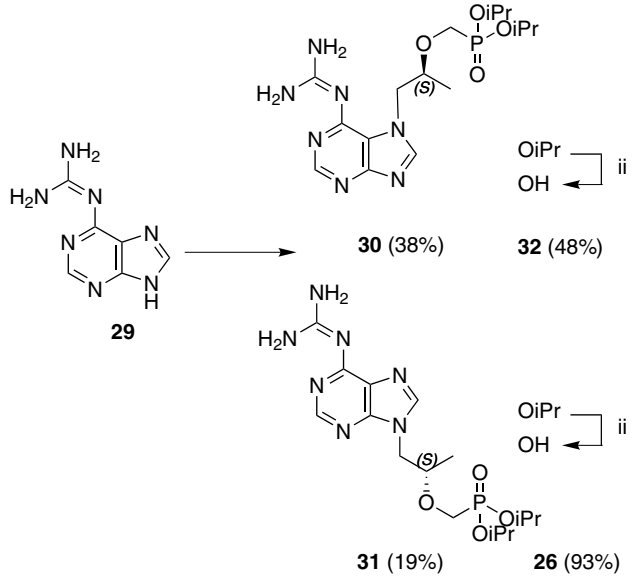
3.2. Origin of cells and their culture

Female mice of the inbred strain C57BL/6, 8–10 weeks old, were purchased from Charles River Deutschland (Sulzfeld, Germany). They were kept in transparent plastic cages and maintained in an Independent Environmental Air Flow Animal Cabinet (ESI Flufrance, Wissous, France). Lighting was set on 06–18 h, temperature at 22 °C.

Animals, killed by cervical dislocation, were ip injected with 8 ml of sterile saline. Pooled peritoneal cells collected from 4 to 7 animals were washed, resuspended in culture medium and seeded into 96-well round-bottomed microplates (Costar) in 100-μl volumes,



Scheme 1. Reagents and conditions: (i) TsPMP, NaH, DMF, 110 °C; (ii) guanidine solution, DABCO, rt; (iii) TMSBr, CH_3CN, rt.



Scheme 2. Reagents and conditions: (i) TsPMP, Cs_2CO_3, DMF, 110 °C; (ii) TMSBr, CH_3CN, rt.

2×10^6 cells/ml. Adherent cells (macrophages) were isolated by incubating the cells for 2 h at 37 °C, 5% CO_2, and then vigorously shaking the plate and washing the wells three times to remove non-adherent cells. Cultures were maintained at 37 °C, 5% CO_2 in humidified Heraeus incubator.

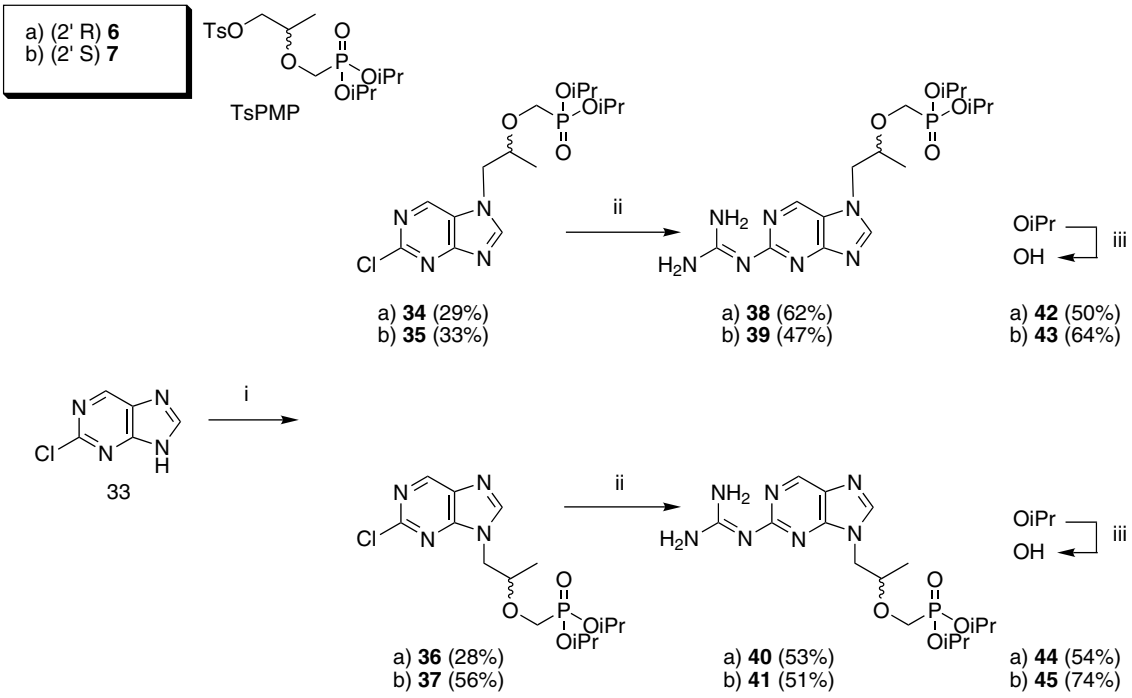
The sources of human peripheral blood mononuclear cells (PBMC) were buffy coats acquired from healthy donors (provided by the Institute of Hematology and Blood Transfusion, Prague). PBMC were separated by Ficoll-Paque gradient centrifugation (GE Health Care Bio-Sciences AB, Uppsala, Sweden) according to the manufacturer' instructions.

The cells were seeded into 96-well round-bottomed microplates (Costar) and maintained at 37 °C, 5% CO_2 in humidified Heraeus incubator. The mouse macrophages were cultured at final density of 2.0×10^6/ml, the human PBMC at density of 1.5×10^6/ml in complete RPMI-1640 culture medium. It contained 10% heat-inactivated foetal bovine serum, 2 mM L-glutamine, 50 μg/ml gentamicin and 5×10^{-5} M 2-mercaptoethanol (all from Sigma–Aldrich, Prague, CR).

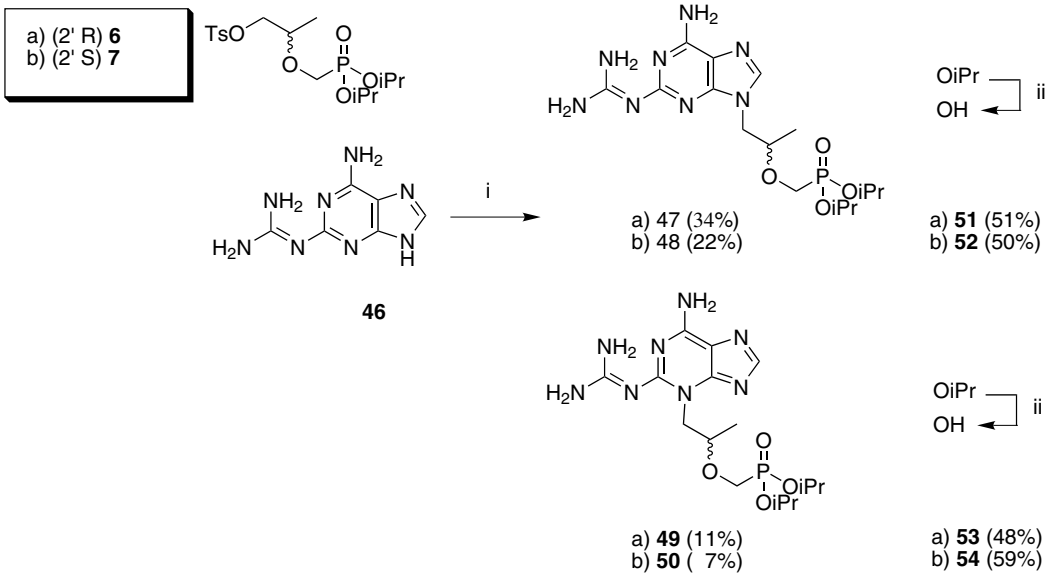
All protocols were approved by the Institutional Ethics Committee.

3.3. Nitric oxide assay

The mouse macrophages were cultured for 24 h in presence of test compounds, applied either alone or in the presence of mouse recombinant interferon-γ (IFN-γ; R&D Systems, Minneapolis, MN). The concentration of nitrites in supernatants of cells was taken as a measure of NO production.[10] It was detected in individual, cell-free samples (50 μl) incubated for 5 min at ambient temperature with an aliquot of a Griess reagent (1% sulfanilamide/0.1% naphthylenediamine/2.5% H_3PO_4). The absorbance at 540 nm was recorded using a microplate spectrophotometer (Tecan, Austria). A nitrite



Scheme 3. Reagents and conditions: (i) TsPMP, NaH, DMF, 110 °C; (ii) guanidine solution, DABCO, rt; (iii) TMSBr, CH_3CN, rt.



Scheme 4. Reagents and conditions: (i) TsPMP, Cs_2CO_3, DMF, 90 °C; (ii) TMSBr, CH_3CN, rt.

calibration curve was used to convert absorbance to μM nitrite.

3.4. Cytokine assays

Concentration of cytokines (pg/ml) in supernatants of cells was determined by ELISA kits, following the manufacturer's instructions (R&D Systems).

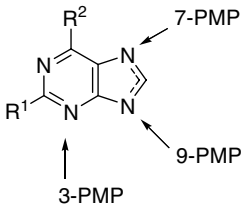
3.5. Statistical analysis

Analysis of variance (ANOVA) with subsequent Bonferroni multiple comparison test and graphical presentation of data were done using the Prism program (GraphPad Software, San Diego, CA).

4. Results and discussion

In the present study, selected guanidinopurines (listed in Table 1), that is, 2- or 6-guanidino-9-, 7- and 3-(*R*) or (*S*)-PMP purine derivatives, were tested for immunostimulatory activity, with the emphasis on structure–activity relationship. To this point, the combination of guanidino and amino group at the purine moiety was followed. Possible immunomodulatory effects were

Table 1. List of compounds subjected to immunobiological screening



Compound	Substituents		Isomer
	R1	**R2**	
25	H	Guanidino	9*R*
27	NH_2	Guanidino	9*R*
44	Guanidine	H	9*R*
51	Guanidine	NH_2	9*R*
26	H	Guanidino	9*S*
28	NH_2	Guanidino	9*S*
45	Guanidine	H	9*S*
52	Guanidine	NH_2	9*S*
22	H	Guanidino	7*R*
23	NH_2	Guanidino	7*R*
42	Guanidine	H	7*R*
32	H	Guanidino	7*S*
24	NH_2	Guanidino	7*S*
43	Guanidine	H	7*S*
53	Guanidine	NH_2	3*R*
54	Guanidine	NH_2	3*S*

detected following our recently described screening platform.[11]

Mouse macrophages produced no **NO** spontaneously, and it remained unchanged when they were cultured in the presence of the compounds alone (data not shown).

However, all guanidine analogues of the PMPDAP—compounds with guanidino group in the position 6 and amino group in the position 2 ($R^1 = NH_2$; R^2 = guanidine), that is, **27**, **28**, and also both 7-regioisomers **23** and **24** and double regioisomeric 3-PMP derivatives **53** and **54** significantly enhanced production of NO which was primarily activated with IFN-γ (Fig. 1a). The highest NO-up-regulatory potential was bound to the compounds **27** and **54**. Their effects became apparent with doses of 10–25 μM (Fig. 1b). Other acyclic nucleotides did not enhance the IFN-γ-triggered NO biosynthesis.

4.1. Cytokine secretion

Chemokines and cytokines were determined in supernatants of macrophages cultured for 5 h in the presence of acyclic nucleoside phosphonates alone, applied at concentration of 100 μM. The compounds that were found to increase production of NO also activated macrophages for secretion of TNF-α, MIP-1α and RANTES (Fig. 2a, b, c, respectively). The data are very similar to the NO results shown in Figure 1. Compounds, which were ineffective to stimulate the IFN-γ-induced production of NO, did not influence cytokine secretion. Similar to NO, the highest cytokine-stimulatory potential was possessed by compounds **27** and **54**.

Augmentation of IFN-γ-triggered NO biosynthesis by guanidinopurines can be explained by their ability to stimulate secretion of TNF-α in mouse macrophages (Fig. 2a). Similar to the NO production, the compounds **27** and **54** proved to be prominent stimulators of TNF-α, being able to enhance its levels in concentration of 10–25 μM (Fig. 2d). Although IFN-γ is known to have a direct moderate stimulatory effect on NO production in many cell types,[12] its effects can be synergistically enhanced by a number of cytokines[13] including TNF-α.[14] Among a plethora of their biological activities, NO and TNF-α also play significant roles in the host defence against various infections. NO inhibits replication of many viruses,[15], for example, cytomegalovirus,[16] Epstein-Barr virus,[17] herpes simplex virus,[18] polio virus type 1,[19] vaccinia viruses,[20] hepatitis B virus[21] and hepatitis C virus.[22] TNF-α, usually in combination with IFN-γ and

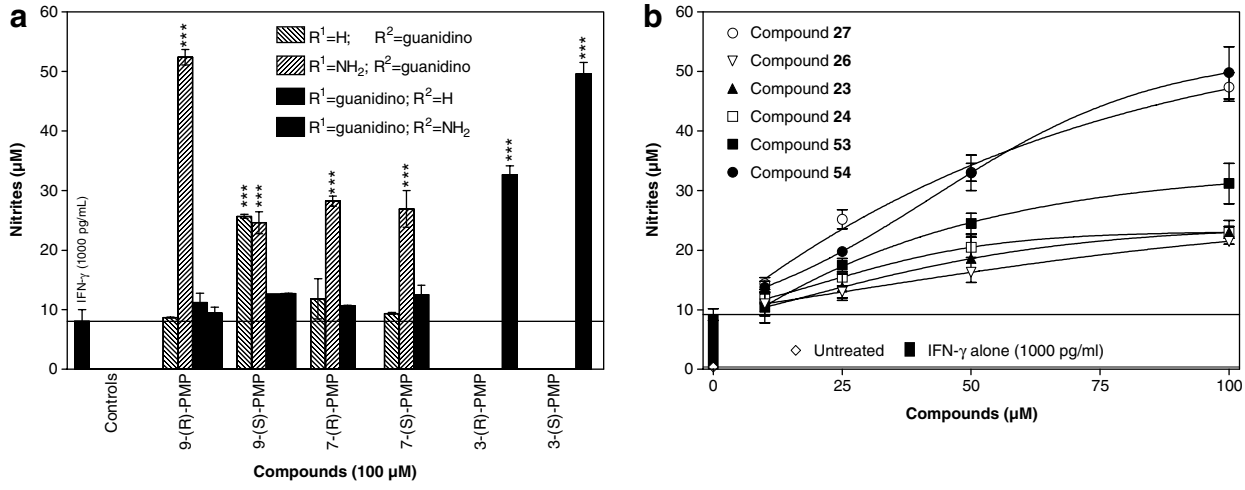


Figure 1. Production of NO by mouse peritoneal macrophages (2×10^6/ml) cultured 24 h in the presence of guanidinopurines (100 μM) applied together with mouse interferon-γ (IFN-γ; 1000 pg/ml). (a) The IFN-γ-primed NO biosynthesis is up-regulated by the test compounds in a dose-dependent manner. (b) Nitrite levels were determined spectrophotometrically using Griess reagent. The bars are means ± SEM representing one of three identical experiments. For identification of compounds see Table 1. Statistical significance: $^{***}P < 0.001$.

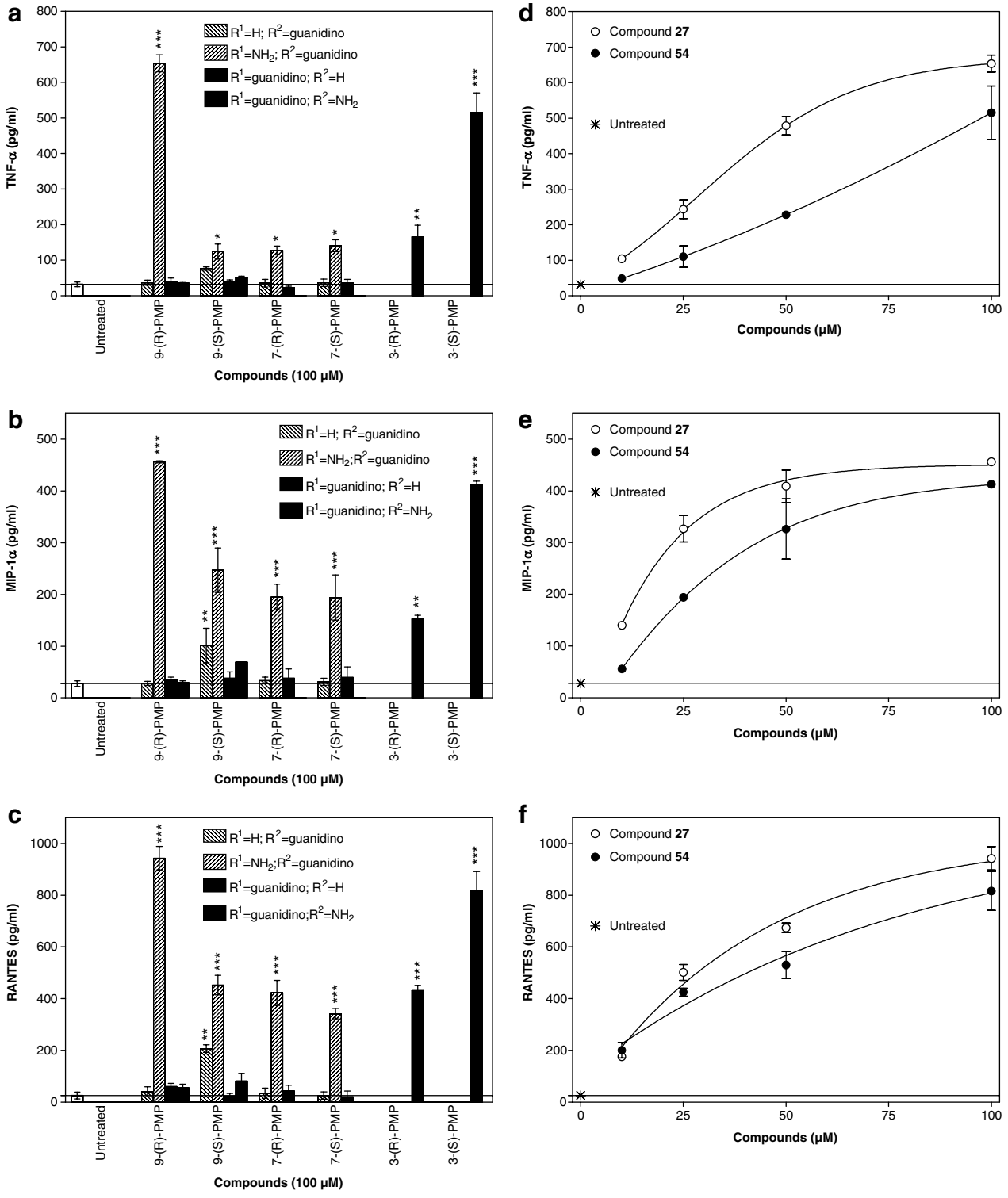


Figure 2. Several of the test guanidinopurines activate secretion of cytokine TNF-α (a), and chemokines MIP-1α (b) and RANTES (c) in mouse peritoneal macrophages. The in vitro immunostimulatory effects of highly effective compounds **A2 27** and **F4 54** are strictly dose-dependent (d, e, f, respectively). The concentration of cytokines was determined by ELISA in supernatants of cells (2×10^6/ml) cultured for 16 h in the presence of the compounds. The bars and points are means ± SEM and are representative of two-independent experiments. For identification of compounds see Table 1. Statistical significance: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

presumably via NO-related mechanisms, has been reported to suppress replication of herpes simplex virus,[23] cytomegalovirus,[24] leukaemia virus C,[25] varicela-zoster virus[26] and adenovirus.[27]

Except for NO and TNF-α we also investigated the effects of guanidinopurines on secretion of chemokines macrophage inflammatory protein-1α (MIP-1α/CCL3) and 'regulated on activation of normal T-cell expressed

and secreted' (RANTES/CCL5). It has been found that both MIP-1α (Fig. 2b) and RANTES (Fig. 2c), the constitutive levels thereof being negligible in control mouse macrophages, are stimulated by the same compounds that are effective to activate secretion of TNF-α (Fig. 2a) and up-regulate production of NO (Fig. 1a). The doses required for stimulation of chemokines were as low as <10 μM (Fig. 2e and f for MIP-1α and RANTES, respectively).

Importantly, the present findings show that the compounds also exhibit strong immunostimulatory properties in human PBMC. Secretion of both MIP-1α (Fig. 3a) and RANTES (Fig. 3b) is significantly enhanced by those compounds that have been found to possess immunobiological activity in the mouse screening design. The lack or lower effectiveness of guanidinopurines to induce RANTES in human PBMC may be due to the fact that the constitutive production of this chemokine in human cells is very high as compared to mouse macrophages. The effects of compounds are strictly dose-dependent; as low concentrations as approximately 5-μM are sufficient to activate secretion of the chemokines in human PBMC (Fig. 3c and d for MIP-1α and RANTES, respectively). MIP-1α and RANTES are ligands for the chemokine receptor CCR5 which is used as a co-receptor by macrophage-tropic HIV-1 strains to infect the cells.[28] Blocking the appropriate β-chemokine receptors on both macrophages and lymphocytes is thus presently considered to be a promising therapeutic approach against HIV.[28c,29] RANTES and MIP-1α have been shown to inhibit HIV infection.[30] Mechanisms of the inhibition include internalization of CCR5[31] and blockage of the cell–cell membrane fusion.[32]

5. Antiviral and cytostatic activity

None of these tested guanidinopurines exhibit any cytotoxicity in vitro in the L929, L1210, and HeLaS3 cell lines under standard conditions.[33] Compounds were also tested for their antiviral activity against selected DNA viruses, RNA viruses, and retroviruses: (MSV,

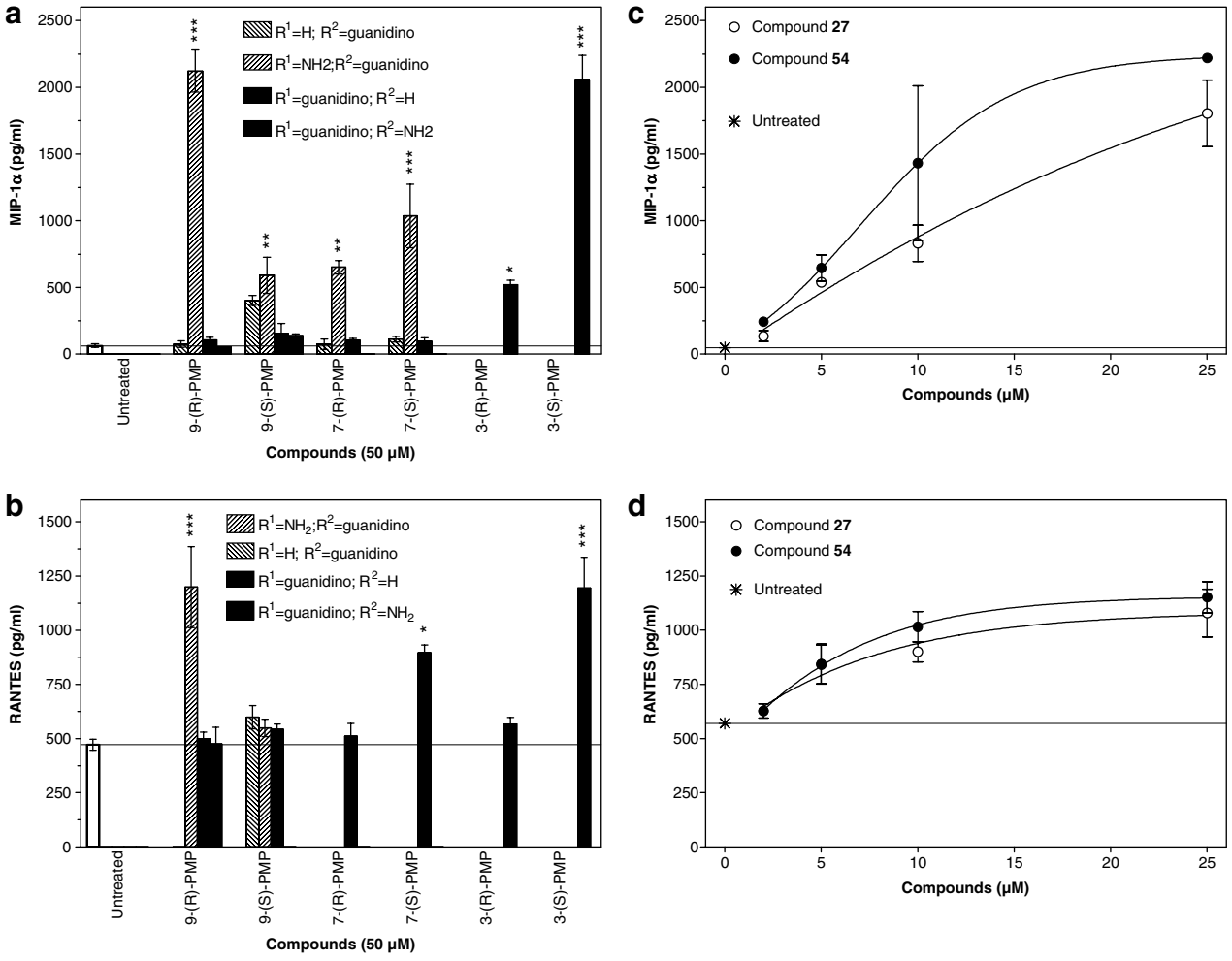


Figure 3. In vitro stimulation of chemokine secretion in human peripheral blood mononuclear cells (PBMC). Concentration of chemokines was assayed by ELISA after 16-h culture of PBMC (1.5×10^6/ml) in the presence of test guanidinopurines. Applied in 50-μM concentration, several of them are potent stimulators of MIP-1α (a) and RANTES (b). The magnitude of the effects depends on the dose applied (c, d for MIP-1α and RANTES, respectively). The bars and points are means ± SEM obtained of two healthy donors of PBMC. For identification of compounds see Table 1. Statistical significance: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

HIV-1, and HIV-2). None of the compounds showed any significant activity in these assays.[34]

6. Conclusion

The assumption that the amino-guanidinopurines possess immunobiological properties was confirmed. This effect is based also on the position and structure of side chain at the purine ring. The present study showed that the most effective is side chain at the position 9—compound **27** but the aliphatic phosphonate chain located at the position 3, compound **54** possess also a good immunological activity. In the contrary, the compounds bearing the chain at the position 7 did not show a significant immunobiological activity.

7. Experimental

7.1. General procedure

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried over P_2O_5 at 2 kPa. Chromatography systems S1: $CHCl_3$/MeOH (97:3); S2: EtOAc/EtOH/acetone/H_2O/NH_3 (4:1:1:1) containing 1% NH_3; EtOAc/EtOH/acetone/H_2O (6:1:1:0.5) containing 1% NH_3. Preparative TLC was carried out on $40 \times 17 \times 0.4$ cm loose layers of silica gel containing a UV indicator. Paper electrophoresis was performed on Whatman paper No. 3 MM at 40 V/cm for 1 h in 0.05 M triethylammonium hydrogencarbonate (TEAB) at pH 7.5 and the electrophoretic mobilities (E_{up}) are referenced to uridine 3′-phosphate. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB ionization by Xe (accelerating voltage 8 kV, glycerol matrix). Optical rotation was measured on Autopol IV (Rudolph Research Analytical). NMR spectra (J, Hz; δ, ppm) were measured on a Bruker DRX 500 (500 MHz for ^{1}H, 125.7 MHz for ^{13}C NMR spectra) in dimethyl sulfoxide-d_6. Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). Preparative HPLC purifications were performed on columns packed with 7 μm C18 reversed phase (Waters Delta 600 chromatograph column), 17×250 mm; in ca. 200 mg batches of mixtures using a linear gradient of 0.1 M tetraethylammonium hydrogencarbonate buffer in H_2O/CH_3OH (0–100% CH_3OH) or linear gradient MeOH/H_2O (1:4 to 9:1) as eluent.

Deionisation was performed on Dowex 50×8 (H^+-form) columns by the following procedure: after application of crude product the column was washed with water until the UV absorption dropped. Thereafter, the column was eluted with 2.5% aqueous NH_3 or with a MeOH/Et_3N/H_2O (1:1:3) mixture. Chromatography on Dowex 1×2 (acetate form) was made as follows: after application of the aqueous solution of the crude product onto the column, it was washed with water until the UV absorption dropped. The column was then eluted with a gradient of dilute acetic acid (0–1 M).

7.1.1. 6-Chloro-7-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-7H-purine (8), 6-chloro-9-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-9H-purine (11). NaH (60%) dispersion in paraffin oil, (0.47 g, 11.8 mmol) was added to a solution of 6-chloropurine (**4**) (1.5 g, 9.7 mmol) in DMF (45 ml) and the resulting mixture was stirred at room temperature for 2 h. Compound **6**[6] (4.75 g, 11.6 mmol) was added and the reaction mixture was stirred for 23 h at 80 °C. The solvent was removed, the residue was codistilled with toluene (3× 30 ml), extracted by boiling chloroform (150 ml) and purified on a silica gel column [90 g, S1] to give two compounds:

Compound **8** (0.22 g, 6%). Yellowish oil, FAB-MS: 391 [MH^+] (100). FAB-HRMS calcd for $C_{15}H_{25}N_4ClO_4P$ (MH^+) 391.1302, found 391.1293. ^{1}H NMR (DMSO-d_6): 1.02, 1.06, 1.12, 1.15 and 1.19 (d, 15H, J(CH_3, CH) = 6.1, CH_3); 3.58 (dd, 1H, J(P, CH) = 9.0, J_{gem} = 14.0, P–CH_2); 3.77 (dd, 1H, J(P, CH) = 8.8, J_{gem} = 14.0, P–CH_2); 3.98 (m, 1H, H-2′); 4.35 (dsept, 1H, J(CH, CH_3) = 6.1, J(P–CH) = 7.7, P–OCH); 4.44 (dsept, 1H, J(CH, CH_3) = 6.1, J(P–CH) = 7.7, P–OCH); 4.46 (dd, 1H, J(1′b, 2′) = 8.5, J_{gem} = 14.8, H-1′b); 4.60 (dd, 1H, J(1′a, 2′) = 3.2, J_{gem} = 14.8, H-1′a); 8.68 and 8.80 2× (s, 1H), (H-2 and H-8). ^{13}C NMR (DMSO-d_6): 16.23 (3′-CH_3); 23.62 (d, J(P, C) = 4.4, CH_3); 23.75 (d, J(P, C) = 4.4, CH_3); 23.78 (d, J(P, C) = 3.9, CH_3); 23.86 (d, J(P, C) = 3.9, CH_3); 50.94 (C-1′); 62.45 (d, J(P, C) = 165.0, P–C); 70.07, 70.16 (d, J(P, C) = 6.3, P–OCH); 76.10 (d, J(P, C) = 11.2, C-2′); 122.44 (C-5); 142.32 (C-6); 151.58 and 151.72 (C-2 and C-8); 161.67 (C-4).

Compound **11** (1.35 g, 36%) the data are consistent with lit.[6]

7.1.2. 7-(*R*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-6-guanidino-7H-purine (15). A guanidine solution (7.5 ml, 2.5 mmol) prepared as mentioned in lit.[3b] was added to a flask containing compound **8** (0.19 g, 0.5 mmol) and DABCO (0.06 g, 0.5 mmol). The mixture was stirred at room temperature for 6 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3× 5 ml) and purified on a loose layer silicagel plate [S3] to give a compound **15**: (0.1 g, 49%). Yellowish foam, $[\alpha]_D$ −56.5° (c 0.36, methanol); FAB-HRMS calcd for $C_{16}H_{29}N_7O_4P$ (MH^+) 414.2019, found 414.2039. FAB-MS: 414 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.10, 1.12, 1.16, 1.17 and 1.20 (d, 15H, J(CH_3, CH) = 6.2, CH_3); 3.62 (dd, 1H, J(P, CH) = 9.3, J_{gem} = 13.9, P–CH_2); 3.73 (dd, 1H, J(P, CH) = 8.7, J_{gem} = 13.9, P–CH_2); 3.91 (m, 1H, H-2′); 4.49 (m, 2H, P–OCH); 4.60 (dd, 1H, J(1′b, 2′) = 6.8, J_{gem} = 13.9, H-1′b); 4.71 (dd, 1H, J(1′a, 2′) = 3.7, J_{gem} = 13.9, H-1′a); 7.30 (br, 4H, NH); 8.06 and 8.27 2× (s, 1H) (H-2 and H-8). ^{13}C NMR (DMSO-d_6): 16.42 (3′-CH_3); 23.74 (d, J(P, C) = 4.4, CH_3); 23.83 (d, J(P, C) = 4.9, CH_3); 23.90 and 23.95 (d, J(P, C) = 3.4, CH_3); 50.36 (C-1′); 62.62 (d, J(P, C) = 165.0, P–C); 70.27 (d, 2C, J(P, C) = 6.3, P–OCH); 76.82 (d, J(P, C) = 11.7, C-2′); 116.38 (C-5); 145.70 (C-8); 150.85 (C-2); 156.64 (C-6); 158.95 (C-4); 159.62 (N–C).

7.1.3. 2-Amino-7-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-7H-purine (16). A guanidine solution (9.9 ml, 3.5 mmol) prepared as mentioned in lit.[3b] was added to a flask containing compound **9**[6] (0.27 g, 0.7 mmol) and DABCO (0.078 g, 0.7 mmol). The mixture was stirred at room temperature for 6 h; The solvent was removed in vacuo, the residue was codistilled with toluene (3× 10 ml) and purified on a silica gel column [30 g, grad 5–15% EtOH vs EtOAc and S2] to give a compound **16** (0.080 g, 25%). Yellowish foam, FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 429.2118. FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ −37.9° (*c* 0.30, methanol); ^{1}H NMR (DMSO-d_6): 1.08, 1.16, 1.18, 1.19 and 1.21 (d, 15H, *J*(CH_3, CH) = 6.1, CH_3); 3.60 (dd, 1H, *J*(P, CH) = 9.0, J_{gem} = 13.8, P–CH_2); 3.70 (dd, 1H, *J*(P, CH) = 8.7, J_{gem} = 13.8, P–CH_2); 3.89 (m, 1H, H-2′); 4.43 (dd, 1H, *J*(1′b, 2′) = 6.6, J_{gem} = 13.8, H-1′b); 4.50 (dd, 1H, *J*(1′a, 2′) = 3.2, J_{gem} = 13.8, H-1′a); 4.51 (m, 2H, P–OCH); 5.63 (br s, 2H, NH); 7.10 90(br, 4H, NH); 7.73 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 16.57 (3′-CH_3); 23.81, 23.87 (d, *J*(P, C) = 4.4, CH_3) and 23.96, 23.99 (d, *J*(P, C) = 3.9, CH_3); 50.27 (C-1′); 62.82 (d, *J*(P, C) = 165.5, P–C); 70.31 (d, *J*(P, C) = 6.3, P–OCH); 70.33 (d, *J*(P, C) = 6.3, P–OCH); 76.91 (d, *J*(P, C) = 11.7, C-2′); 110.70 (C-5); 144.16 (C-8); 156.86 (C-6); 159.11 (C-2); 159.52 (N–C); 161.29 (C-4).

7.1.4. 2-Amino-7-(*S*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-7H-purine (17). A guanidine solution (12.5 ml, 4.12 mmol) prepared as mentioned in lit.[3b] was added to a flask containing compound **10**[6] (0.35 g, 0.82 mmol) and DABCO (0.09 g, 0.82 mmol). The mixture was stirred at room temperature for 4 h; The solvent was removed in vacuo, the residue was codistilled with toluene (3× 10 ml) and purified on a silica gel column [30 g, grad 5–15% EtOH vs EtOAc and S3] to give a compound **17** (0.092 g, 25%). Yellowish oil, FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 429.2117. FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ +94.0° (*c* 0.19, methanol); ^{1}H NMR (DMSO-d_6): 1.08, 1.16, 1.18, 1.19 and 1.21 (d, 15H, *J*(CH_3, CH) = 6.1, CH_3); 3.60 (dd, 1H, *J*(P, CH) = 9.0, J_{gem} = 13.8, P–CH_2); 3.70 (dd, 1H, *J*(P, CH) = 8.7, J_{gem} = 13.8, P–CH_2); 3.89 (m, 1H, H-2′); 4.43 (dd, 1H, *J*(1′b, 2′) = 6.6, J_{gem} = 13.8, H-1′b); 4.50 (dd, 1H, *J*(1′a, 2′) = 3.2, J_{gem} = 13.8, H-1′a); 4.51 (m, 2H, P–OCH); 5.63 (br s, 2H, NH); 7.10 90 (br, 4H, NH); 7.73 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 16.57 (3′-CH_3); 23.81, 23.87 (d, *J*(P, C) = 4.4, CH_3) and 23.96, 23.99 (d, *J*(P, C) = 3.9, CH_3); 50.27 (C-1′); 62.82 (d, *J*(P, C) = 165.5, P–C); 70.31 (d, *J*(P, C) = 6.3, P–OCH); 70.33 (d, *J*(P, C) = 6.3, P–OCH); 76.91 (d, *J*(P, C) = 11.7, C-2′); 110.70 (C-5); 144.16 (C-8); 156.86 (C-6); 159.11 (C-2); 159.52 (N–C); 161.29 (C-4).

7.1.5. 9-(*R*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-6-guanidino-9H-purine (18). A guanidine solution (56 ml, 18.5 mmol) prepared as mentioned in lit.[3b] was added to a flask containing compound **11**[8] (1.45 g, 3.7 mmol) and DABCO (0.41 g, 3.7 mmol). The mixture was stirred at room temperature for 6 h; The solvent was removed in vacuo, the residue was codistilled with toluene (3× 30 ml) and purified on a silica gel column [grad 5–15% EtOH vs EtOAc and S2] to give a compound **18** (1.12 g, 73%). Yellowish oil, FAB-HRMS calcd for $C_{16}H_{29}N_7O_4P$ (MH^+) 414.2019, found 414.2010. FAB-MS: 414 [MH^+] (100). $[\alpha]_D$ −20.4° (*c* 0.27, methanol); ^{1}H NMR (DMSO-d_6): 1.07, 1.13, 1.16, 1.17 and 1.20 (d, 15H, *J*(CH_3, CH) = 6.2, CH_3); 3.72 (dd, 1H, *J*(P, CH) = 9.6, J_{gem} = 13.8, P–CH_2); 3.79 (dd, 1H, *J*(P, CH) = 9.3, J_{gem} = 13.8, P–CH_2); 3.96 (m, 1H, H-2′); 4.17 (dd, 1H, *J*(1′b, 2′) = 6.5, J_{gem} = 14.4, H-1′b); 4.26 (dd, 1H, *J*(1′a, 2′) = 3.8, J_{gem} = 14.4, H-1′a); 4.50 (m, 2H, P–OCH); 7.30 (br, 4H, NH); 8.01 and 8.26 (s, 1H), (H-2 and H-8). ^{13}C NMR (DMSO-d_6): 16.94 (3′-CH_3); 23.79, 23.83 (d, *J*(P, C) = 4.9, CH_3) and 23.93, 23.97 (d, *J*(P, C) = 3.9, CH_3); 46.68 (C-1′); 62.61 (d, *J*(P, C) = 166.0, P–C); 70.33 (d, *J*(P, C) = 6.3, P–OCH); 70.36 (d, *J*(P, C) = 6.3, P–OCH); 75.58 (d, *J*(P, C) = 12.7, C-2′); 125.03 (C-5); 141.52 (C-8); 150.45 (C-4); 150.72 (C-2); 160.14 and 160.19 (N–C and C-6).

7.1.6. 9-(*S*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-6-guanidino-9H-purine (19). The data are identical with compound **31** starting compound **12**[6] yield (0.54 g, 49%). For $C_{16}H_{28}N_7O_4P$ (413.41) calcd: C, 46.49; H, 6.83; N, 23.72; P, 7.49. Found: C, 46.45; H, 6.81; N, 23.88; P, 7.23. $[\alpha]_D$ +22.4° (*c* 0.32, MeOH).

7.1.7. 2-Amino-9-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-9H-purine (20). A guanidine solution (37.5 ml, 12.5 mmol) prepared as mentioned in lit.[3b] was added to a flask containing compound **13**[6] (1.0 g, 2.5 mmol) and DABCO (0.28 g, 2.5 mmol). The mixture was stirred at room temperature for 6 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3× 20 ml) and purified on a silica gel column [40 g, grad 5–15% EtOH vs EtOAc, S3 and S2] to give a compound **20**.

(0.73 g, 69%). Yellowish foam, $[\alpha]_D$ −31.4° (*c* 0.25, methanol); for $C_{16}H_{29}N_8O_4P$ (428.43) calcd: C, 44.86; H, 6.82; N, 26.15; P, 7.23. Found: C, 45.07; H, 6.94; N, 25.85; P, 6.99. FAB-MS: 429 [MH^+] (100). ^{1}H NMR (DMSO-d_6): 1.06, 1.16, 1.18, 1.19 and 1.22 (d, 15H, *J*(CH_3, CH) = 6.1, CH_3); 3.68 (dd, 1H, *J*(P, CH) = 9.5, J_{gem} = 13.7, P–CH_2); 3.76 (dd, 1H, *J*(P, CH) = 9.3, J_{gem} = 13.7, P–CH_2); 3.90 (m, 1H, H-2′); 3.97 (dd, 1H, *J*(1′b, 2′) = 6.6, J_{gem} = 14.3, H-1′b); 4.04 (dd, 1H, *J*(1′a, 2′) = 3.9, J_{gem} = 14.3, H-1′a); 4.52 (m, 2H, P–OCH); 5.94 (br s, 2H, NH) and 7.45 (br, 4H, NH); 7.62 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 16.94 (3′-CH_3); 23.83 (d, *J*(P, C) = 3.9, CH_3); 23.86 (d, *J*(P, C) = 3.9, CH_3); 23.97 and 24.00 (d, *J*(P, C) = 3.9, CH_3); 46.41 (C-1′); 62.73 (d, *J*(P, C) = 165.5, P–C); 70.38, 70.37 (d, *J*(P, C) = 6.3, P–OCH); 75.67 (d, *J*(P, C) = 12.7, C-2′); 118.84 (C-5); 138.31 (C-8); 152.59 (C-4); 159.06 (C-6); 159.87 (C-2); 159.95 (N–C).

7.1.8. 2-Amino-9-(*S*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-9H-purine (21). A guanidine solution (23.5 ml, 23.5 mmol) prepared as mentioned in lit.[3a] was added to a flask containing compound **14**[6] (1.9 g, 4.7 mmol) and DABCO (0.53 g, 4.7 mmol). The mixture was stirred at room temperature for 3 h. The

solvent was removed in vacuo, the residue was codistilled with toluene (3× 20 ml) and purified on a silica gel column [90 g, grad 5–15% EtOH vs EtOAc and S3] to give a compound **21** (1.72 g, 86%). Yellowish foam, FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ +38.9° (*c* 0.01, methanol); FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 429.2135. ^{1}H NMR (DMSO-d_6): 1.06, 1.16, 1.18, 1.19 and 1.22 (d, 15H, $J(CH_3, CH)$ = 6.1, CH_3); 3.68 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 13.6, P–CH_2); 3.76 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 13.6, P–CH_2); 3.90 (m, 1H, H-2′); 3.97 (dd, 1H, J(1′b, 2′) = 6.5, J_{gem} = 14.2, H-1′b); 4.04 (dd, 1H, J(1′a, 2′) = 3.9, J_{gem} = 14.2, H-1′a); 4.52 (m, 2H, P–OCH); 5.92 (br s, 2H, NH) and 7.30 (br, 4H, NH); 7.61 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 16.94 (3′-CH_3); 23.82 (d, J(P, C) = 4.9, CH_3); 23.85 (d, J(P, C) = 4.9, CH_3); 23.96 and 23.99 (d, 2C, J(P, C) = 3.9, CH_3); 46.38 (C-1′); 62.73 (d, J(P, C) = 165.5, P–C); 70.34, 70.36 (d, J(P, C) = 6.3, P–OCH); 75.67 (d, J(P, C) = 12.7, C-2′); 118.96 (C-5); 138.20 (C-8); 152.54 (C-4); 159.06 (C-6); 159.95 and 160.16 (C-2) and (N–C).

7.1.9. 7-(*S*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-6-guanidino-7H-purine (30), 9-(*S*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-9H-purine (31). (*S*)-2-((Diisopropoxyphosphoryl)methoxy)propyl 4-methylbenzenesulfonate[6] (**7**) (1.96 g, 4.8 mmol) was added to 6-guanidinopurine[3b] (**29**) (0.71 g, 4 mmol) in DMF (26 ml) the mixture was warmed to 110 °C and Cs_2CO_3 (0.65 g, 2 mmol) was added. The resulting mixture was stirred at 110 °C for 26 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3× 20 ml) and purified on a silica gel column [grad 5–15% EtOH vs EtOAc then: S3] to give two compounds:

Compound **30** (0.63 g, 38%). Yellowish oil, FAB-MS: 414 [MH^+] (100). $[\alpha]_D$ +67.3° (*c* 0.25, methanol); FAB-HRMS calcd for $C_{16}H_{29}N_7O_4P$ (MH^+) 414.2019, found 414.2009. ^{1}H NMR (DMSO-d_6): 1.10, 1.12, 1.16, 1.17 and 1.20 (d, 15H, $J(CH_3, CH)$ = 6.2, CH_3); 3.62 (dd, 1H, J(P, CH) = 9.3, J_{gem} = 13.9, P–CH_2); 3.73 (dd, 1H, J(P, CH) = 8.7, J_{gem} = 13.9, P–CH_2); 3.91 (m, 1H, H-2′); 4.49 (m, 2H, P–OCH); 4.60 (dd, 1H, J(1′b, 2′) = 6.8, J_{gem} = 13.9, H-1′b); 4.71 (dd, 1H, J(1′a, 2′) = 3.7, J_{gem} = 13.9, H-1′a); 7.30 (br, 4H, NH); 8.06 and 8.27 2× (s, 1H), (H-2 and H-8). ^{13}C NMR (DMSO-d_6): 16.42 (3′-CH_3); 23.74 (d, J(P, C) = 4.4, CH_3); 23.83 (d, J(P, C) = 4.9, CH_3); 23.90 and 23.95 (d, J(P, C) = 3.4, CH_3); 50.36 (C-1′); 62.62 (d, J(P, C) = 165.0, P–C); 70.27 (d, 2C, J(P, C) = 6.3, P–OCH); 76.82 (d, J(P, C) = 11.7, C-2′); 116.38 (C-5); 145.70 (C-8); 150.85 (C-2); 156.64 (C-6); 158.95 (C-4); 159.62 (N–C).

Compound **31** (0.31 g, 19%). Yellowish oil, FAB-MS: 414 [MH^+] (100). FAB-HRMS calcd for $C_{16}H_{29}N_7O_4P$ (MH^+) 414.2019, found 414.2028. ^{1}H NMR (DMSO-d_6): 1.07, 1.13, 1.16, 1.17 and 1.20 (d, 15 H, $J(CH_3, CH)$ = 6.2, CH_3); 3.72 (dd, 1H, J(P, CH) = 9.5, J_{gem} = 13.7, P–CH_2); 3.79 (dd, 1H, J(P, CH) = 9.3, J_{gem} = 13.7, P–CH_2); 3.96 (m, 1H, H-2′); 4.17 (dd, 1H, J(1′b, 2′) = 6.5, J_{gem} = 14.4, H-1′b); 4.26 (dd, 1H, J(1′a, 2′) = 3.8, J_{gem} = 14.4, H-1′a); 4.50 (m, 2H, P–OCH); 7.30 (br, 4H, NH); 8.01 and 8.27 2× (s, 1H), (H-2 and H-8). ^{13}C NMR (DMSO-d_6): 16.93 (3′-CH_3); 23.77, 23.81 (d, J(P, C) = 4.9, CH_3) and 23.95, 23.91 (d, J(P, C) = 4.4, CH_3); 46.67 (C-1′); 62.61 (d, J(P, C) = 165.5, P–C); 70.30 and 70.33 (d, J(P, C) = 6.3, P–OCH); 75.56 (d, J(P, C) = 12.7, C-2′); 125.01 (C-5); 141.50 (C-8); 150.44 (C-4); 150.70 (C-2); 160.09, 160.17 (C-6, N–C).

7.1.10. 2-Chloro-7-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-7H-purine (34), 2-Chloro-9-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-9H-purine (36). The synthetic procedure same as compounds **35** and **37**.

Compound **34** (0.74, 29%). The spectral data are consistent with compound **35** yellowish foam, $[\alpha]_D$ −18.5° (*c* 0.67, methanol); FAB-HRMS calcd for $C_{15}H_{25}N_4O_4PCl$ (MH^+) 391.1302, found 391.1291.

Compound **36** yield (0.71 g, 28%). The spectral data are consistent with compound **37** yellowish foam, FAB-HRMS calcd for $C_{15}H_{25}N_4O_4PCl$ (MH^+) 391.1302, found 391.1315. $[\alpha]_D$ −39.9° (*c* 0.51, methanol).

7.1.11. 2-Chloro-7-(*S*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-7H-purine (35) and 2-chloro-9-(*S*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-9H-purine (37). NaH (60%) dispersion in paraffin oil, (0.31 g, 7.8 mmol) was added to a solution of 2-chloropurine[9] (**33**) (1 g, 6.5 mmol) in DMF (33 ml) and the resulting mixture was stirred at room temperature for 1 h. Then compound **7** (3.17 g, 7.7 mmol) was added and reaction mixture was stirred for 23 h at 110 °C. The solvent was removed, the residue was codistilled with toluene (3× 20 ml) and purified on a silica gel column [60 g, S1] to give two compounds:

Compound **35**, (0.84 g, 33%); white solid, FAB-MS: 391 [MH^+] (100). FAB-HRMS calcd for $C_{15}H_{25}N_4ClO_4P$ (MH^+) 391.1302, found 391.1308. ^{1}H NMR (DMSO-d_6): 1.03, 1.11, 1.13, 1.17 (d, 15H, $J(CH_3, CH)$ = 6.8, CH_3); 3.63 (dd, 1H, J(P, CH) = 9.6, J_{gem} = 13.8, P–CH_2); 3.80 (dd, 1H, J(P, CH) = 9.0, J_{gem} = 13.8, P–CH_2); 3.95 (m, 1H, H-2′); 4.36 (dd, 1H, J(1′b, 2′) = 7.3, J_{gem} = 14.6, H-1′b); 4.46 (m, 2H, P–OCH); 4.54 (dd, 1H, J(1′a, 2′) = 3.0, J_{gem} = 14.6, H-1′a); 8.70 (s, 1H, H-8); 9.14 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6): 16.17 (3′-CH_3); 23.60 (d, J(P, C) = 4.9, CH_3); 23.73 (d, J(P, C) = 4.4, CH_3); 23.76 (d, J(P, C) = 3.4, CH_3) and 23.83 (d, J(P, C) = 3.9, CH_3); 50.22 (C-1′); 62.49 (d, J(P, C) = 165.5, P–C); 70.09 and 70.15 (d, J(P, C) = 6.4, P–OCH); 75.93 (d, J(P, C) = 12.7, C-2′); 125.60 (C-5); 144.32 (C-6); 151.69 (C-8); 152.82 (C-2); 162.05 (C-4).

Compound **37**, (1.43 g, 56%); white solid, FAB-MS: 391 [MH^+] (100). FAB-HRMS calcd for $C_{15}H_{25}N_4ClO_4P$ (MH^+) 391.1302, found 414.1292. ^{1}H NMR (DMSO-d_6): 1.06, 1.15, 1.17, 1.20 and 1.21 (d, 15H, $J(CH_3, CH)$ = 6.8, CH_3); 3.69 (dd, 1H, J(P, CH) = 9.6, J_{gem} = 13.8, P–CH_2); 3.81 (dd, 1H, J(P, CH) = 9.0, J_{gem} = 13.8, P–CH_2); 4.00 (m, 1H, H-2′); 4.24 (dd, 1H, J(1′b, 2′) = 7.7, J_{gem} = 14.4, H-1′b); 4.39 (dd, 1H,

J(1′a, 2′) = 3.2, J_{gem} = 14.4, H-1′a); 4.44 (m, 2H, P–OCH); 8.57 (s, 1H, H-8); 9.10 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6): 16.68 (3′-CH_3); 23.66, 23.75 (d, J(P, C) = 4.4, CH_3) and 23.82, 23.87 (d, J(P, C) = 3.9, CH_3); 47.33 (C-1′); 62.38 (d, J(P, C) = 165.5, P–C); 70.21 and 70.14 (d, J(P, C) = 6.3, P–OCH); 75.14 (d, J(P, C) = 12.2, C-2′); 132.99 (C-5); 148.79 (C-8); 149.93 (C-6); 152.91 (C-2); 153.62 (C-4).

7.1.12. 7-(*R*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-2-guanidino-7H-purine (38). A guanidine solution (10 ml, 10 mmol) prepared as mentioned in lit.[3a] was added to a flask containing compound **34** (0.7 g, 1.8 mmol) and DABCO (0.20 g, 1.8 mmol). The mixture was stirred at 120 °C for 24 h; after that the reaction mixture was cooled to room temperature and poured into diethylether. The solvent was decanted and the residue was adsorbed on the silica-gel and applied onto the column of silica-gel (30 g, S3 and S2) to give a compound **38** (0.46 g, 62%). White powder, FAB-HRMS calcd for $C_{16}H_{29}N_7O_4P$ (MH^+) 414.2019, found 414.2029. FAB-MS: 414 [MH^+] (100). $[\alpha]_D$ −12.0° (*c* 0.16, methanol); ^{1}H NMR (DMSO-d_6): 1.09, 1.10, 1.15 and 1.19 (d, 15H, J(CH_3, CH) = 6.1, CH_3); 3.68 (dd, 1H, J(P, CH) = 9.5, J_{gem} = 13.8, P–CH_2); 3.80 (dd, 1H, J(P, CH) = 9.2, J_{gem} = 13.8, P–CH_2); 3.94 (m, 1H, H-2′); 4.24 (dd, 1H, J(1′b, 2′) = 6.5, J_{gem} = 14.6, H-1′b); 4.39 (dd, 1H, J(1′a, 2′) = 3.3, J_{gem} = 14.6, H-1′a); 4.48 (m, 2H, P–OCH); 7.10 (br s, 4H, NH); 8.29 (s, 1H, H-8); 8.87 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6): 16.45 (3′-CH_3); 23.74, 23.83 (d, J(P, C) = 4.4, CH_3) and 23.90, 23.94 (d, J(P, C) = 4.4, CH_3); 49.56 (C-1′); 62.70 (d, J(P, C) = 165.0, P–C); 70.26 and 70.28 (d, J(P, C) = 6.3, P–OCH); 75.93 (d, J(P, C) = 12.7, C-2′); 120.13 (C-5); 142.26 (C-6); 148.57 (C-8); 160.86, 2C, (C-2, N–C); 162.50 (C-4).

7.1.13. 7-(*S*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-2-guanidino-7H-purine (39). A guanidine solution (10.2 ml, 10.2 mmol) prepared as mentioned in lit.[3a] was added to a flask containing compound **35** (0.8 g, 2.04 mmol) and DABCO (0.25 g, 2.04 mmol). The mixture was stirred at 110 °C for 16 h; after that the reaction mixture was cooled to room temperature and poured into diethylether. The solvent was decanted and the residue was adsorbed on the silica-gel and applied onto the column of silica-gel (30 g, S2) to give a compound **39** (0.4 g, 47%). Yellowish oil, for $C_{16}H_{28}N_7O_4P{\cdot}1/3CO_3$ (413.41) calcd: C, 45.26; H, 6.51; N, 22.62; P, 7.15. Found: C, 45.35; H, 6.88; N, 22.66; P, 6.96; FAB-MS: 414 [MH^+] (100). $[\alpha]_D$ +46.5° (*c* 0.28, methanol); ^{1}H NMR (DMSO-d_6): 1.09, 1.10, 1.15 and 1.19 (d, 15H, J(CH_3, CH) = 6.2, CH_3); 3.67 (dd, 1H, J(P, CH) = 9.5, J_{gem} = 13.8, P–CH_2); 3.80 (dd, 1H, J(P, CH) = 9.2, J_{gem} = 13.8, P–CH_2); 3.95 (m, 1H, H-2′); 4.26 (dd, 1H, J(1′b, 2′) = 6.8, J_{gem} = 14.6, H-1′b); 4.41 (dd, 1H, J(1′a, 2′) = 3.2, J_{gem} = 14.6, H-1′a); 4.48 (m, 2H, P–OCH); 7.60 (br, 4H, NH); 8.34 (s, 1H, H-8); 8.92 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6): 16.39 (3′-CH_3); 23.70, 23.78 (d, J(P, C) = 4.4, CH_3) and 23.85, 23.89 (d, J(P, C) = 4.9, CH_3); 49.65 (C-1′); 62.69 (d, J(P, C) = 165.5, P–C); 70.22 and 70.24 (d, J(P, C) = 6.3, P–OCH); 75.87 (d, J(P, C) = 12.7, C-2′); 120.61 (C-5); 142.36 (C-6); 148.98 (C-8); 157.94 (N–C); 160.75 (C-4); 160.93 (C-2).

7.1.14. 9-(*R*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-2-guanidino-9H-purine (40). A guanidine solution (9 ml, 9 mmol) prepared as above[3a] was added to a flask containing compound **36** (0.7 g, 1.8 mmol) and DABCO (0.20 g, 1.8 mmol). The mixture was stirred at 120 °C for 24 h; after that the reaction mixture was cooled to room temperature and poured into diethylether. The solvent was decanted and the residue was adsorbed on the silica-gel and applied onto the column of silica-gel (30 g, S3 and S2) to give a compound **40** (0.39 g, 53%). Yellowish oil, FAB-HRMS calcd for $C_{16}H_{29}N_7O_4P$ (MH^+) 414.2019, found 414.2028. FAB-MS: 414 [MH^+] (100). $[\alpha]_D$ −25.0° (*c* 0.44, methanol); ^{1}H NMR (DMSO-d_6): 1.08, 1.12, 1.13 and 1.17 (d, 15H, J(CH_3, CH) = 6.1, CH_3); 3.70 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 13.9, P–CH_2); 3.81 (dd, 1H, J(P, CH) = 8.9, J_{gem} = 13.9, P–CH_2); 3.98 (m, 1H, H-2′); 4.30 (dd, 1H, J(1′b, 2′) = 7.0, J_{gem} = 14.6, H-1′b); 4.43 (dd, 1H, J(1′a, 2′) = 3.4, J_{gem} = 14.6, H-1′a); 4.44 (m, 1H, P–OCH); 4.48 (m, 1H, P–OCH); 8.49 (br s, 4H, NH); 8.45 (s, 1H, H-8); 9.03 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6): 16.77 (3′-CH_3); 23.71, 23.78 (d, J(P, C) = 4.4, CH_3) and 23.85, 23.90 (d, J(P, C) = 3.9, CH_3); 47.03 (C-1′); 62.50 (d, J(P, C) = 166.0, P–C); 70.21 and 70.26 (d, J(P, C) = 6.3, P–OCH); 75.37 (d, J(P, C) = 12.6, C-2′); 130.55 (C-5); 147.70 (C-8); 148.66 (C-6); 152.24 (C-4); 152.27 (C-2); 155.38 (N–C).

7.1.15. 9-(*S*)-{2-[(Diisopropylphosphoryl)methoxy]propyl}-2-guanidino-9H-purine (41). A guanidine solution (16.6 ml, 16.6 mmol) prepared as mentioned in lit.[3a] was added to a flask containing compound **37** (1.3 g, 3.3 mmol) and DABCO (0.40 g, 3.3 mmol). The mixture was stirred at 110 °C for 16 h; after that the reaction mixture was cooled to room temperature and poured into diethylether. The solvent was decanted and the residue was adsorbed on the silica-gel and applied onto the column of silica-gel (30 g, S3) to give a compound **41**, (0.7 g, 51%). Yellowish oil, for $C_{16}H_{28}N_7O_4P{\cdot}0.5H_2O$ (413.41) calcd: C, 45.49; H, 6.92; N, 23.21; P, 7.33. Found: C, 45.52; H, 6.79; N, 23.18; P, 6.90; FAB-MS: 414 [MH^+] (100). $[\alpha]_D$ +79.2° (*c* 0.28, methanol); ^{1}H NMR (DMSO-d_6): 1.09, 1.11, 1.16 and 1.20 (d, 15H, J(CH_3, CH) = 6.8, CH_3); 3.72 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 13.7, P–CH_2); 3.80 (dd, 1H, J(P, CH) = 9.2, J_{gem} = 13.7, P–CH_2); 3.95 (m, 1H, H-2′); 4.15 (dd, 1H, J(1′b, 2′) = 6.6, J_{gem} = 14.4, H-1′b); 4.24 (dd, 1H, J(1′a, 2′) = 3.8, J_{gem} = 14.4, H-1′a); 4.50 (m, 2H, P–OCH); 7.00 (br, 4H, NH); 8.10 (s, 1H, H-8); 8.74 (s, 1H, H-6). ^{13}C NMR (DMSO-d_6): 16.94 (3′-CH_3); 23.73, 23.80 (d, J(P, C) = 4.4, CH_3) and 23.89, 23.92 (d, J(P, C) = 4.4, CH_3); 46.31 (C-1′); 62.57 (d, J(P, C) = 165.5, P–C); 70.28 and 70.32 (d, J(P, C) = 6.3, P–OCH); 75.44 (d, J(P, C) = 12.7, C-2′); 127.28 (C-5); 144.71 (C-8); 147.60 (C-6); 152.86 (C-4); 158.50 (N–C); 162.31 (C-2).

7.1.16. 6-Amino-9-(*R*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-9H-purine (47), 6-amino-3R-{2-[(diisopropylphosphoryl)methoxy]propyl}-6-guanidino-3H-purine (49). Compound **6**[6] (2.94 g, 7.2 mmol) was added to the mixture of 6-amino-2-guanidinopurine[3a] (**46**) (1.15 g, 6 mmol) in DMF (39 ml) and Cs_2CO_3 (0.98 g, 3 mmol) at 90 °C. The resulting mixture was stirred at

90 °C for 21 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3× 30 ml) and purified on a silica gel column [grad 5–15% EtOH vs EtOAc, S3 and S2] to give two compounds:

Compound **49** (0.28 g, 11%). Yellowish oil, FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 429.2135. FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ −25.8° (*c* 0.65, methanol); ^{1}H NMR (DMSO-d_6): 1.07, 1.15, 1.16, 1.17 and 1.20 (d, 15H, $J(CH_3, CH)$ = 6.1, CH_3); 3.74 (dd, 1H, J(P, CH) = 9.1, J_{gem} = 13.8, P–CH_2); 3.84 (dd, 1H, J(P, CH) = 8.9, J_{gem} = 13.8, P–CH_2); 4.22 and 4.44 2× (m, 1H), (H-1′and H-2′); 4.50 (m, 2H, P–OCH); 6.69 (br s, 2H, NH); 7.40 (br, 4H, NH); 7.58 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 17.46 (3′-CH_3); 23.78 (d, J(P, C) = 4.4, CH_3); 23.81 (d, J(P, C) = 4.4, CH_3); 23.94 and 23.98 (d, J(P, C) = 3.9, CH_3); 48.57 (C-1′); 62.66 (d, J(P, C) = 164.6, P–C); 70.28 (d, J(P, C) = 6.3, P–OCH); 70.33 (d, J(P, C) = 6.3, P–OCH); 74.87 (d, J(P, C) = 12.2, C-2′); 113.11 (C-5); 148.56 (C-8); 151.96 (C-4); 153.04 (C-2); 153.51 (C-6); 159.60 (N–C).

Compound **47** (0.88 g, 34%). Yellowish oil, FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 414.2119. FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ −14.6° (*c* 0.46, methanol); ^{1}H NMR (DMSO-d_6): 1.08, 1.12, 1.16, 1.17 and 1.95 (d, 15H, $J(CH_3, CH)$ = 6.1, CH_3); 3.70 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 13.8, P–CH_2); 3.79 (dd, 1H, J(P, CH) = 9.2, J_{gem} = 13.8, P–CH_2); 3.93 (m, 1H, H-2′); 4.10 (dd, 1H, J(1′b, 2′) = 6.8, J_{gem} = 14.3, H-1′b); 4.20 (dd, 1H, J(1′a, 2′) = 3.5, J_{gem} = 14.3, H-1′a); 4.49 (m, 2H, P–OCH); 7.96 (s, 1H, H-8); 6.70 (br s, 1H, NH); 7.30 (br s, 1H, NH); 7.60 (br s, 3H, NH); 8.80 (br, 1H, NH). ^{13}C NMR (DMSO-d_6): 16.88 (3′-CH_3); 23.75, 23.82 (d, J(P, C) = 4.4, CH_3) and 23.93, 23.96 (d, J(P, C) = 3.9, CH_3); 46.97 (C-1′); 62.63 (d, J(P, C) = 165.0, P–C); 70.28 and 70.33 (d, J(P, C) = 6.3, P–OCH); 75.49 (d, J(P, C) = 12.7, C-2′); 115.27 (C-5); 141.17 (C-8); 150.38 (C-4); 153.06 (C-6); 155.71 (C-2); 156.28 (N–C).

7.1.17. 6-Amino-9-(*S*)-{2-[(diisopropylphosphoryl)methoxy]propyl}-2-guanidino-9H-purine (48), 6-amino-3S-{2-[(diisopropylphosphoryl)methoxy]propyl}-2-guanidino-3H-purine (50). Compound **7**[6] (1.96 g, 4.8 mmol) was added to 6-amino-2-guanidinopurine[3a] (**46**) (0.77 g, 4 mmol) in DMF (26 ml) the mixture was warmed to 110 °C and Cs_2CO_3 (0.65 g, 2 mmol) was added. The resulting mixture was stirred at 110 °C for 24 h. The solvent was removed in vacuo, the residue was codistilled with toluene (3× 20 ml) and purified on a silica gel column [30 g, grad 5–15% EtOH vs EtOAc then: S3 and S2] to give two compounds:

Compound **50** (0.11 g, 7%). Yellowish oil, FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ +50.0° (*c* 0.05, methanol); FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 429.2112. ^{1}H NMR (DMSO-d_6): 1.06, 1.16, 1.17, 1.18 and 1.21 (d, 15H, $J(CH_3, CH)$ = 6.1, CH_3); 3.76 (dd, 1H, J(P, CH) = 8.9, J_{gem} = 13.8, P–CH_2); 3.85 (dd, 1H, J(P, CH) = 8.9, J_{gem} = 13.8, P–CH_2); 4.23 (m, 2H, H-1′); 4.44 (m, 1H, H-2′); 4.51 (m, 2H, P–OCH); 7.30 (br, 4H, NH); 7.43 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 17.59 (3′-CH_3); 23.80 and 23.81 (d, J(P, C) = 4.4, CH_3); 23.95 (d, J(P, C) = 3.4, CH_3); 24.00 (d, J(P, C) = 3.9, CH_3); 48.51 (C-1′); 62.66 (d, J(P, C) = 164.6, P–CH_2); 70.28, 70.32 (d, J(P, C) = 6.3, P–OCH); 74.95 (d, J(P, C) = 12.2, C-2′); 115.18 (C-5); 150.19 (C-8); 152.46 (C-4); 152.70 (C-2); 153.80 (C-6); 159.44 (N–C).

Compound **48** (0.38 g, 22%). Yellowish oil, FAB-MS: 429 [MH^+] (100). $[\alpha]_D$ +35.7° (*c* 0.27, methanol); FAB-HRMS calcd for $C_{16}H_{30}N_8O_4P$ (MH^+) 429.2128, found 429.2135. ^{1}H NMR (DMSO-d_6): 1.08, 1.14, 1.15, 1.16 and 1.19 (d, 15H, $J(CH_3, CH)$ = 6.2, CH_3); 3.69 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 13.8, P–CH_2); 3.79 (dd, 1H, J(P, CH) = 9.2, J_{gem} = 13.8, P–CH_2); 3.93 (m, 1H, H-2′); 4.11 (dd, 1H, J(1′b, 2′) = 6.8, J_{gem} = 14.4, H-1′b); 4.22 (dd, 1H, J(1′a, 2′) = 3.3, J_{gem} = 14.4, H-1′a); 4.48 (m, 2H, P–OCH); 6.69 (br s, 2H, NH); 7.30 (br s, 2H, NH); 7.70 (br, 2H, NH); 8.00 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 17.87 (3′-CH_3); 23.73 (d, J(P, C) = 4.9, CH_3); 23.80 (d, J(P, C) = 4.4, CH_3); 23.89 (d, 2C, J(P, C) = 3.9, CH_3); 47.05 (C-1′); 62.60 (d, J(P, C) = 165.5, P–C); 70.25, 70.29 (d, J(P, C) = 6.3, P–OCH); 75.46 (d, J(P, C) = 12.7, C-2′); 115.48 (C-5); 141.42 (C-8); 150.28 (C-4); 152.32 (C-6); 155.38 and 155.70 (C-6) and (N–C).

7.2. Cleavage of the phosphonate esters: General procedure

The ester (1 mmol) was suspended in acetonitrile (30 ml) and TMSBr (3 ml) was added. The reaction mixture was stirred at room temperature overnight. After evaporation of the volatiles and co-distillation with acetonitrile (20 ml), the residue was treated with water (20 ml) and 35% aqueous NH_3 (3 ml) for 5 min and evaporated. The residue was dissolved in water and applied onto a column of Dowex 50X8 (H^+ form). The column was washed with water and eluted with 2.5% aqueous NH_3. The product-containing UV-absorbing fractions were evaporated, the residue was dissolved in 35% aqueous NH_3 and applied onto a column of Dowex 1X2 (acetate form), washed with water and eluted with a linear gradient of aqueous acetic acid (0–1 mol/l). The product-containing fractions were evaporated, codistilled with water (5× 30 ml) and the residue was crystallized to give a pure free phosphonate. This procedure affords compounds **22**–**28**, **32**, **42**–**45**, **51**–**54.**

7.2.1. 6-Guanidino-7-(*R*)-[2-(phosphonomethoxy)propyl]-7H-purine (22). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **15** (0.075 g, 0.18 mmol); Dowex 50X8, HPLC TEAB isocratic; yield (0.020 g, 33%); white powder, E_{up} = 0.68; FAB-MS: 330 [MH^+] (40). ^{1}H NMR (D_2O+NaOD): 1.11 (d, 3H, J(3′, 2′) = 6.3, CH_3); 3.41 (dd, 1H, J(P, CH) = 9.3, J_{gem} = 12.3, P–CH_2); 3.52 (dd, 1H, J(P, CH) = 9.4, J_{gem} = 12.3, P–CH_2); 3.91 (m, 1H, H-2′); 4.59 (dd, 1H, J(1′b, 2′) = 4.3, J_{gem} = 14.2, H-1′b); 4.66 (dd, 1H, J(1′a, 2′) = 5.9, J_{gem} = 14.2, H-1′a); 8.32 and 8.35 2× (s, 2H), (H-8 and H-2). ^{13}C NMR (D_2O+NaOD): 14.36 (3′-CH_3); 53.00 (C-1′); 69.57 (d, J(P, C) = 150.5, P–C); 78.70 (d, J(P, C) = 10.2, C-2′);

119.27 (C-5); 144.86 (C-8); 154.29 (C-2); 156.52 (C-6); 159.03 (C-4); 160.28 (N–C).

7.2.2. 2-Amino-6-guanidino-7-(*R*)-[2-(phosphonomethoxy) propyl]-7H-purine (23). Workup viz. cleavage of the phosphonate esters–general procedure; starting compound **16** (0.059 g, 0.13 mmol); Dowex 1X2 eluted with 0.1 M AcOH; HPLC yield (0.040 g, 84%); white powder, $E_{up} = 0.60$; FAB-MS: 345 [MH^+] (55). ^1H NMR (D_2O+NaOD): 1.09 (d, 3H, $J(3', 2') = 6.4$, CH_3); 3.39 (dd, 1H, J(P, CH) = 9.3, $J_{gem} = 12.4$, P–CH_2); 3.49 (dd, 1H, J(P, CH) = 9.3, $J_{gem} = 12.4$, P–CH_2); 3.93 (m, 1H, H-2′); 4.51 (dd, 1H, $J(1'b, 2') = 4.6$, $J_{gem} = 13.9$, H-1′b); 4.60 (dd, 1H, $J(1'a, 2') = 5.6$, $J_{gem} = 13.9$, H-1′a); 8.11 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 16.14 (3′-CH_3); 50.50 (C-1′); 67.06 (d, J(P, C) = 151.4, P–C); 76.37 (d, J(P, C) = 11.2, C-2′); 111.65 (C-5); 146.00 (C-8); 157.43 (C-6); 156.20 and 159.46 (C-2, C-4); 159.91 (N–C).

7.2.3. 2-Amino-6-guanidino-7-(*S*)-[2-(phosphonomethoxy) propyl]-7H-purine (24). Workup viz. cleavage of the phosphonate esters—general procedure. Starting compound **17** (0.065 g, 0.15 mmol); Dowex 1X2 eluted with 0.05 M AcOH; yield (0.035 g, 67%); white crystals (aqueous EtOH); $E_{up} = 0.62$; FAB-MS: 345 [MH^+] (65). $[\alpha]_D$ +6.5° (*c* 0.1, H_2O+TEAB); ^1H NMR (D_2O+NaOD): 0.83 (d, 3H, $J(3', 2') = 6.3$, CH_3); 3.12 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.2$, P–CH_2); 3.22 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.2$, P–CH_2); 3.63 (m, 1H, H-2′); 4.20 (dd, 1H, $J(1'b, 2') = 4.5$, $J_{gem} = 14.3$, H-1′b); 4.27 (dd, 1H, $J(1'a, 2') = 6.3$, $J_{gem} = 14.3$, H-1′a); 7.81 (s, 1H, H-8). ^{13}C NMR (D_2O+ NaOD): 16.14 (3′-CH_3); 50.32 (C-1′); 66.95 (d, J(P, C) = 150.4, P–C); 76.34 (d, J(P, C) = 11.2, C-2′); 111.33 (C-5); 145.70 (C-8); 157.24 (C-6); 159.19, 159.26 and 159.61 (C-6, C-2 and N–C).

7.2.4. 6-Guanidino-9-(*R*)-[2-(phosphonomethoxy)propyl]-9H-purine (25). Workup viz. cleavage of the phosphonate esters—general procedure. Starting compound **18** (0.94 g, 2.3 mmol); Dowex 1X2 eluted with 0.05 M AcOH; yield (0.5 g, 67%); white crystals (aqueous EtOH); $E_{up} = 0.63$; for $C_{10}H_{16}N_7O_4P{\cdot}2/3H_2O$ (329.25) calcd: C, 35.20; H, 5.12; N, 28.73; P, 9.08. Found: C, 35.18; H, 4.92; N, 28.42; P, 9.11. $[\alpha]_D$ +2.9° (*c* 0.59, H_2O+TEAB); FAB-MS: 330 [MH^+] (100). ^1H NMR (D_2O+NaOD): 1.12 (d, 3H, $J(3', 2') = 6.3$, CH_3); 3.47 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.3$, P–CH_2); 3.57 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.3$, P–CH_2); 3.99 (m, 1H, H-2′); 4.25 (dd, 1H, $J(1'b, 2') = 5.5$, $J_{gem} = 14.6$, H-1′b); 4.35 (dd, 1H, $J(1'a, 2') = 4.0$, $J_{gem} = 14.6$, H-1′a); 8.25 (s, 1H) and 8.26 (s, 1H), (H-2 and H-8). ^{13}C NMR (D_2O+NaOD): 16.30 (3′-CH_3); 47.17 (C-1′); 66.76 (d, J(P, C) = 151.4, P–C); 75.39 (d, J(P, C) = 10.7, C-2′); 123.91 (C-5); 143.51 (C-8); 149.66 (C-4); 151.12 (C-2); 159.05 and 160.04 (C-6 and N–C).

7.2.5. 6-Guanidino-9-(*S*)-[2-(phosphonomethoxy)propyl]-9H-purine (26). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **19** (0.31 g, 0.75 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.4 g, 93%); white crystals, (H_2O); $E_{up} = 0.64$; for $C_{10}H_{16}N_7O_4P{\cdot}H_2O$ (329.25) calcd: C, 34.59; H, 5.22; N, 28.23; P, 8.92. Found: C, 34.59; H, 5.13; N, 27.90; P, 9.25. FAB-MS: 330 [MH^+] (50). $[\alpha]_D$ +16.8° (*c* 0.16, H_2O); ^1H NMR (D_2O+NaOD): 1.12 (d, 3H, $J(3', 2') = 6.1$, CH_3); 3.46 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.2$, P–CH_2); 3.56 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.2$, P–CH_2); 3.98 (m, 1H, H-2′); 4.25 (dd, 1H, $J(1'b, 2') = 5.6$, $J_{gem} = 14.7$, H-1′b); 4.35 (dd, 1H, $J(1'a, 2') = 4.2$, $J_{gem} = 14.7$, H-1′a); 8.25 and 8.27 2× (s, 2H), (H-8 and H-2). ^{13}C NMR (D_2O+NaOD): 16.34 (3′-CH_3); 47.18 (C-1′); 66.81 (d, J(P, C) = 150.4, P–C); 75.42 (d, J(P, C) = 11.2, C-2′); 123.97 (C-5); 143.57 (C-8); 149.74 (C-4); 151.24 (C-2); 159.12 (C-6); 160.11 (N–C).

7.2.6. 2-Amino-6-guanidino-9-(*R*)-[2-(phosphonomethoxy) propyl]-9H-purine (27). Workup viz. cleavage of the phosphonate esters—general procedure—without workup on D1 column; starting compound **20** (0.64 g, 2.5 mmol); Deionized on Dowex 50 and applied on HPLC in 0.1 M TEAB; yield (0.39 g, 76%); white powder; $E_{up} = 0.57$; for $C_{10}H_{17}N_8O_4P{\cdot}0.5\ H_2O$ (344.27) calcd: C, 34.00; H, 5.14; N, 31.72; P, 8.77. Found: C, 34.00; H, 4.80; N, 31.46; P, 9.19. $[\alpha]_D$ −3.2° (*c* 0.19, H_2O+TEAB); FAB-MS: 345 [MH^+] (60). ^1H NMR (D_2O+NaOD): 3.46 (dd, 1H, J(P, CH) = 9.3, $J_{gem} = 12.4$, P–CH_2); 3.55 (dd, 1H, J(P, CH) = 9.5, $J_{gem} = 12.5$, P–CH_2); 3.93 (m, 1H, H-2′); 4.08 (dd, 1H, $J(1'b, 2') = 7.9$, $J_{gem} = 14.6$, H-1′b); 4.16 (dd, 1H, $J(1'a, 2') = 4.2$, $J_{gem} = 14.6$, H-1′a); 7.92 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 16.33 (3′-CH_3); 46.76 (C-1′); 66.73 (d, J(P, C) = 151.4, P–C); 75.45 (d, J(P, C) = 10.7, C-2′); 118.40 (C-5); 140.95 (C-8); 151.50 (C-4); 158.98, 159.63 and 160.13 (C-6, C-2 and N–C).

7.2.7. 2-Amino-6-guanidino-9-(*S*)-[2-(phosphonomethoxy) propyl]-9H-purine (28). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **21** (1.7 g, 4 mmol); Dowex 1X2 eluted with 0.1 M AcOH; (Dowex 1 extracted by hot water—low solubility of compound **28** in 0.1 M AcOH); yield (1.01 g, 74%); white powder; $E_{up} = 0.58$; for $C_{10}H_{17}N_8O_4P{\cdot}2/3H_2O$ (344.27) calcd: C, 33.71; H, 5.19; N, 31.45; P, 8.69. Found: C, 33.65; H, 5.03; N, 31.02; P, 8.60. $[\alpha]_D$ +25.5° (*c* 0.23, H_2O+TEAB); FAB-MS: 345 [MH^+] (10). ^1H NMR (D_2O+ NaOD): 1.13 (d, 3H, $J(3', 2') = 6.2$, CH_3); 3.46 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.4$, P–CH_2); 3.55 (dd, 1H, J(P, CH) = 9.4, $J_{gem} = 12.4$, P–CH_2); 3.95 (m, 1H, H-2′); 4.11 (dd, 1H, $J(1'b, 2') = 5.9$, $J_{gem} = 14.4$, H-1′b); 4.19 (dd, 1H, $J(1'a, 2') = 4.3$, $J_{gem} = 14.4$, H-1′a); 7.94 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 16.34 (3′-CH_3); 46.91 (C-1′); 66.82 (d, J(P, C) = 151.4, P–C); 75.48 (d, J(P, C) = 11.2, C-2′); 118.52 (C-5); 141.13 (C-8); 151.71 (C-4); 159.14 (C-6); 159.78 (C-2); 160.14 (N–C).

7.2.8. 6-Guanidino-7-(*S*)-[2-(phosphonomethoxy)propyl]-7H-purine (32). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **30** (0.60 g, 1.5 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.23 g, 48%); white crystals, (H_2O); $E_{up} = 0.72$; for $C_{10}H_{16}N_7O_4P{\cdot}H_2O$ (329.25) calcd: C, 34.59; H, 5.22; N, 28.23; P, 8.92. Found: C, 34.77; H, 5.39; N, 28.01; P, 8.82. FAB-MS: 330 [MH^+] (15).

$[\alpha]_D$ +14.3° (*c* 0.23, H_2O+NaOH); ^1H NMR (D_2O+NaOD): 1.10 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.41 (dd, 1H, *J*(P, CH) = 9.4, J_{gem} = 12.3, P–CH_2); 3.53 (dd, 1H, *J*(P, CH) = 9.4, J_{gem} = 12.3, P–CH_2); 3.92 (m, 1H, H-2′); 4.59 (dd, 1H, *J*(1′b, 2′) = 4.4, J_{gem} = 14.2, H-1′b); 4.66 (dd, 1H, *J*(1′a, 2′) = 5.9, J_{gem} = 14.2, H-1′a); 8.32 and 8.33 2× (s, 2H), (H-8 and H-2). ^{13}C NMR (D_2O+NaOD): 14.35 (3′-CH_3); 52.99 (C-1′); 69.57 (d, *J*(P, C) = 150.4, P–C); 78.70 (d, *J*(P, C) = 10.2, C-2′); 119.27 (C-5); 144.86 (C-8); 154.29 (C-2); 156.50 (C-6); 159.03 (C-4); 160.28 (N–C).

7.2.9. 2-Guanidino-7-(*R*)-[2-(phosphonomethoxy)propyl]-7H-purine (42). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **38** (0.25 g, 0.6 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.1 g, 50%); white powder; E_{up} = 0.40; FAB-MS: 330 [MH^+] (60). $[\alpha]_D$ −5.3° (*c* 0.3, H_2O+TEAB); ^1H NMR (D_2O+NaOD): 1.09 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.50 (d, 2H, *J*(P, CH) = 8.9, P–CH_2); 4.07 (m, 1H, H-2′); 4.17 (dd, 1H, *J*(1′b, 2′) = 7.2, J_{gem} = 13.1, H-1′b); 4.47 (dd, 1H, *J*(1′a, 2′) = 5.9, J_{gem} = 13.1, H-1′a); 7.70 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 17.01 (3′-CH_3); 49.57 (C-1′); 67.33 (d, *J*(P, C) = 151.4, P–C); 75.14 (d, *J*(P, C) = 10.8, C-2′); 114.60 (C-5); 150.78 (C-8); 151.91 (C-4); 153.62 (C-2); 154.06 (C-6); 159.49 (N–C).

7.2.10. 2-Guanidino-7-(*S*)-[2-(phosphonomethoxy)propyl]-7H-purine (43). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **39** (0.31 g, 0.75 mmol); Dowex 1X2 eluted with 0.25 M AcOH; yield (0.16 g, 64%); white crystals, (H_2O) E_{up} = 0.43; for $C_{10}H_{16}N_7O_4P{\cdot}2/3H_2O$ (329.25) calcd: C, 35.20; H, 5.12; N, 28.73; P, 9.08. Found: C, 35.38; H, 5.26; N, 28.40; P, 8.81. FAB-MS: 330 [MH^+] (100). $[\alpha]_D$ +9.8° (*c* 0.43, H_2O+NaOH); ^1H NMR (D_2O+NaOD): 1.18 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.51 (dd, 1H, *J*(P, CH) = 9.1, J_{gem} = 12.4, P–CH_2); 3.61 (dd, 1H, *J*(P, CH) = 9.1, J_{gem} = 12.4, P–CH_2); 4.09 (m, 1H, H-2′); 4.36 (dd, 1H, *J*(1′b, 2′) = 5.4, J_{gem} = 14.9, H-1′b); 4.50 (dd, 1H, *J*(1′a, 2′) = 4.1, J_{gem} = 14.9, H-1′a); 8.48 (s, 1H, H-8); 8.87 (s, 1H, H-6). ^{13}C NMR (D_2O+NaOD): 16.40 (3′-CH_3); 49.82 (C-1′); 66.93 (d, *J*(P, C) = 150.8, P–C); 75.49 (d, *J*(P, C) = 10.7, C-2′); 120.78 (C-5); 142.71 (C-6); 149.82 (C-8); 159.03 (N–C); 160.28 (C-4); 161.58 (C-2).

7.2.11. 2-Guanidino-9-(*R*)-[2-(phosphonomethoxy)propyl]-9H-purine (44). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **40** (0.35 g, 0.8 mmol); Dowex 1X2 eluted with 0.1 M AcOH; HPLC yield (0.15 g, 54%); white powder, E_{up} = 0.46; for $C_{10}H_{16}N_7O_4P{\cdot}0.5\ H_2O$ (329.25) calcd: C, 35.51; H, 5.07; N, 28.99; P, 9.16. Found: C, 35.68; H, 5.05; N, 28.42; P, 8.90. FAB-MS: 330 [MH^+] (40). $[\alpha]_D$− 2.2° (*c* 0.46, H_2O+TEAB); ^1H NMR (D_2O+NaOD): 1.11 (d, 3H, *J*(3′, 2′) = 6.4, CH_3); 3.50 (dd, 1H, *J*(P, CH) = 9.3, J_{gem} = 12.4, P–CH_2); 3.57 (dd, 1H, *J*(P, CH) = 9.5, J_{gem} = 12.4, P–CH_2); 4.00 (m, 1H, H-2′); 4.27 (dd, 1H, *J*(1′b, 2′) = 5.1, J_{gem} = 14.6, H-1′b); 4.35 (dd, 1H, *J*(1′a, 2′) = 4.1, J_{gem} = 14.6, H-1′a); 8.35 (s, 1H, H-8); 8.72 (s, 1H, H-6). ^{13}C NMR (D_2O+NaOD): 16.45 (3′-CH_3); 46.52 (C-1′); 66.77 (d, *J*(P, C) = 150.9, P–C); 75.28 (d, *J*(P, C) = 10.3, C-2′); 127.03 (C-5); 146.46 (C-8); 148.33 (C-6); 152.26 (C-4); 159.16 (N–C); 161.43 (C-2).

7.2.12. 2-Guanidino-9-(*S*)-[2-(phosphonomethoxy)propyl]-9H-purine (45). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **41** (0.61 g, 1.5 mmol); Dowex 1X2 eluted with 0.25 M AcOH; yield (0.36 g, 74%); white crystals, (H_2O) E_{up} = 0.47; for $C_{10}H_{16}N_7O_4P{\cdot}2/3H_2O$ (329.25) calcd: C, 35.20; H, 5.12; N, 28.73; P, 9.08. Found: C, 35.29; H, 5.26; N, 28.69; P, 8.89. FAB-MS: 330 [MH^+] (100). $[\alpha]_D$ +18.9° (*c* 0.46, H_2O+NaOH); ^1H NMR (D_2O+NaOD): 1.11 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.51 (dd, 1H, *J*(P, CH) = 9.4, J_{gem} = 12.4, P–CH_2); 3.58 (dd, 1H, *J*(P, CH) = 9.4, J_{gem} = 12.4, P–CH_2); 3.99 (m, 1H, H-2′); 4.24 (dd, 1H, *J*(1′b, 2′) = 5.1, J_{gem} = 14.6, H-1′b); 4.31 (dd, 1H, *J*(1′a, 2′) = 4.1, J_{gem} = 14.6, H-1′a); 8.33 (s, 1H, H-8); 8.67 (s, 1H, H-6). ^{13}C NMR (D_2O+NaOD): 16.54 (3′-CH_3); 46.55 (C-1′); 66.84 (d, *J*(P, C) = 150.4, P–C); 75.33 (d, *J*(P, C) = 10.7, C-2′); 126.97 (C-5); 146.38 (C-8); 148.35 (C-6); 152.17 (C-4); 159.19 (N–C); 161.45 (C-6).

7.2.13. 6-Amino-2-guanidino-9-(*R*)-[2-(phosphonomethoxy)propyl]-9H-purine (51). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **47** (0.87 g, 2.0 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.36 g, 51%); white crystals (H_2O); E_{up} = 0.43; for $C_{10}H_{17}N_8O_4P{\cdot}1/3CO_3$ (344.27) calcd: C, 34.07; H, 4.70; N, 30.76; P, 8.50. Found: C, 34.07; H, 5.05; N, 30.80; P, 8.27. FAB-MS: 345 [MH^+] (51). $[\alpha]_D$ −14.8° (*c* 0.30, H_2O+TEAB); ^1H NMR (D_2O+NaOD): 1.05 (d, 3H, *J*(3′, 2′) = 6.4, CH_3); 3.44 (dd, 1H, *J*(P, CH) = 9.3, J_{gem} = 12.4, P–CH_2); 3.50 (dd, 1H, *J*(P, CH) = 9.3, J_{gem} = 12.4, P–CH_2); 3.89 (m, 1H, H-2′); 4.08 (dd, 1H, *J*(1′b, 2′) = 5.5, J_{gem} = 14.6, H-1′b); 4.14 (dd, 1H, *J*(1′a, 2′) = 4.4, J_{gem} = 14.6, H-1′a); 7.96 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 16.49 (3′-CH_3); 46.67 (C-1′); 66.81 (d, *J*(P, C) = 150.9, P–C); 75.47 (d, *J*(P, C) = 10.8, C-2′); 113.28 (C-5); 141.77 (C-8); 150.79 (C-4); 155.41 (C-6); 158.96 (C-2); 161.61 (N–C).

7.2.14. 6-Amino-2-guanidino-9-(*S*)-[2-(phosphonomethoxy)propyl]-9H-purine (52). Workup viz. cleavage of the phosphonate esters—general procedure +0.8 ml DMF in reaction mixture; starting compound **48** (0.38 g, 0.8 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.15 g, 50%); white powder; E_{up} = 0.4; for $C_{10}H_{17}N_8O_4P{\cdot}2H_2O$ (344.27) calcd: C, 31.58; H, 5.57; N, 29.46; P, 8.14. Found: C, 31.65; H, 5.68; N, 29.56; P, 8.11. $[\alpha]_D$ +21.6° (*c* 0.11, H_2O+NaOH); FAB-MS: 345 [MH^+] (15). ^1H NMR (D_2O+NaOD): 1.10 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.48 (dd, 1H, *J*(P, CH) = 9.1, J_{gem} = 12.4, P–CH_2); 3.55 (dd, 1H, *J*(P, CH) = 9.3, J_{gem} = 12.4, P–CH_2); 3.96 (m, 1H, H-2′); 4.17 (dd, 1H, *J*(1′b, 2′) = 5.4, J_{gem} = 14.2, H-1′b); 4.23 (dd, 1H, *J*(1′a, 2′) = 4.3, J_{gem} = 14.6, H-1′a); 8.05 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 16.46 (3′-CH_3); 46.76 (C-1′); 66.83 (d, *J*(P, C) = 150.4, P–C); 75.47 (d, *J*(P, C) = 10.7, C-2′); 113.41 (C-5); 141.97 (C-8); 150.98 (C-4); 155.57 (C-6); 158.94 (C-2); 161.70 (N–C).

7.2.15. 6-Amino-2-guanidino-3*R*-[2-(phosphonomethoxy) propyl]-3H-purine (53). Workup viz. cleavage of the phosphonate esters—general procedure; starting compound **49**, (0.26 g, 0.6 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.1 g, 48%); white crystals (H_2O); $E_{up} = 0.5$; for $C_{10}H_{17}N_8O_4P$ (344.27) calcd: C, 34.89; H, 4.98; N, 32.55; P, 9.00. Found: C, 34.73; H, 5.08; N, 32.32; P, 8.81. FAB-MS: 345 [MH^+] (30). $[\alpha]_D$ −15.4° (*c* 0.23, H_2O+NH_3); ^{1}H NMR (D_2O+NaOD): 1.09 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.50 (d, 2H, *J*(P, CH) = 8.9, P–CH_2); 4.07 (m, 1H, H-2′); 4.17 (dd, 1H, *J*(1′b, 2′) = 7.2, J_{gem} = 13.1, H-1′b); 4.47 (dd, 1H, *J*(1′a, 2′) = 5.9, J_{gem} = 13.1, H-1′a); 7.70 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 17.01 (3′-CH_3); 49.57 (C-1′); 67.33 (d, *J*(P, C) = 151.4, P–C); 75.14 (d, *J*(P, C) = 10.8, C-2′); 114.60 (C-5); 150.78 (C-8); 151.91 (C-4); 153.62 (C-2); 154.06 (C-6); 159.49 (N–C).

7.2.16. 6-Amino-2-guanidino-3*S*-[2-(phosphonomethoxy) propyl]-3H-purine (54). Workup viz. cleavage of the phosphonate esters—general procedure +0.2 ml DMF in reaction mixture; starting compound **50** (0.11 g, 0.25 mmol); Dowex 1X2 eluted with 0.1 M AcOH; yield (0.052 g, 59%); white powder; $E_{up} = 0.52$; FAB-MS: 345 [MH^+] (10). FAB-HRMS calcd for $C_{10}H_{18}N_8O_4P$ (MH^+) 345.1189, found 345.1185. ^{1}H NMR (D_2O+NaOD): 1.09 (d, 3H, *J*(3′, 2′) = 6.3, CH_3); 3.50 (d, 2H, *J*(P, CH) = 8.9, P–CH_2); 4.07 (m, 1H, H-2′); 4.17 (dd, 1H, *J*(1′b, 2′) = 7.2, J_{gem} = 13.1, H-1′b); 4.47 (dd, 1H, *J*(1′a, 2′) = 5.9, J_{gem} = 13.1, H-1′a); 7.70 (s, 1H, H-8). ^{13}C NMR (D_2O+NaOD): 17.01 (3′-CH_3); 49.57 (C-1′); 67.33 (d, *J*(P, C) = 151.4, P–C); 75.14 (d, *J*(P, C) = 10.8, C-2′); 114.60 (C-5); 150.78 (C-8); 151.91 (C-4); 153.62 (C-2); 154.06 (C-6); 159.49 (N–C).

Acknowledgments

This work is a part of the research projects of the Institute of Organic Chemistry and Biochemistry No. Z40550506 and the Institute of Experimental Medicine No. AV0Z50390512. It was supported by the Centre for New Antivirals and Antineoplastics (1M0508), by the Programme of Targeted Projects of Academy of Sciences of the Czech Republic (1QS400550501), by the Gilead Sciences, Inc. (Foster City (CA), USA), by the Grant Agency of the Czech Republic (Grant No. 203/05/2539), and by the financial support of the European Commission (René Descartes Prize-2001, Grant No. HPAW-2002-100096). The authors' thanks are also due to the staff of the mass spectrometry and analytical departments of the Institute of Organic Chemistry and Biochemistry.

References and notes

1. (a) De Clercq, E.; Holy, A. *Nat. Rev. Drug Disc.* **2005**, *11*, 928; (b) Holy, A. *Antiviral Res.* **2006**, *71*, 248.
2. (a) Zidek, Z.; Potmesil, P.; Kmonickova, E.; Holy, A. *Eur. J. Pharmacol.* **2003**, *475*, 149; (b) Zidek, Z.; Fankova, D.; Holy, A. *Antimicrob. Agents Chemother.* **2001**, *45*, 3381; (c) Potmesil, P.; Krecmerova, M.; Kmonickova, E.; Holy, A.; Zidek, Z. *Eur. J. Pharmacol.* **2006**, *540*, 191.
3. (a) Cesnek, M.; Masojidkova, M.; Holy, A.; Solinova, V.; Koval, D.; Kasicka, V. *Collect. Czech. Chem. Commun.* **2006**, *71*, 1303; (b) Cesnek, M.; Masojidkova, M.; Holy, A. *Tetrahedron* **2002**, *58*, 2985.
4. Zidek, Z. Unpublished results.
5. Cesnek, M.; Holy, A.; Masojidkova, M.; Zidek, Z. *Bioorg. Med. Chem.* **2005**, *13*, 2917.
6. Holy, A.; Dvorakova, H.; Masojidkova, M. *Collect. Czech. Chem. Commun.* **1995**, *60*, 1390.
7. Cesnek, M.; Holy, A.; Masojidkova, M. *Collect. Czech. Chem. Commun.* **2003**, *68*, 2201.
8. Holy, A.; Votruba, I.; Tloustova, E.; Masojidkova, M. *Collect. Czech. Chem. Commun.* **2001**, *66*, 1545.
9. Breshears, S. R.; Wang, S. S.; Bechtolt, S. G.; Christensen, B. E. *J. Am. Chem. Soc.* **1959**, *81*, 3789.
10. Marletta, M. A.; Yoon, P. S.; Iyengar, R.; Leaf, C. D.; Wishnok, J. S. *Biochemistry* **1988**, *27*, 8706.
11. Kmoníčková, E.; Melkusová, P.; Farghali, H.; Holý, A.; Zídek, Z. *Nitric Oxide* **2007**, *17*, 160.
12. (a) Stuehr, D. J.; Marletta, M. A. *J. Immunol.* **1987**, *139*, 518; (b) Liew, F. Y.; Li, Y.; Severn, A.; Millott, S.; Schmidt, J.; Salter, M.; Moncada, S. *Eur. J. Immunol.* **1991**, *21*, 2489; (c) Sakurai, S.; Kamachi, K.; Konda, T.; Miyajima, N.; Kohase, M.; Yamamoto, S. *Infect. Immun.* **1996**, *64*, 1309.
13. Bogdan, C.; Röllinghof, M.; Vodovotz, Y.; Xie, Q. w.; Nathan, C. In *Immunotherapy of Infections*; Masihi, K. N., Ed.; Marcel Dekker Inc.: New York, 1994; pp 37–54.
14. (a) Ding, A. H.; Nathan, C. F.; Stuehr, D. J. *J. Immunol.* **1988**, *141*, 2407; (b) Nussler, A. K.; Di Silvio, M.; Liu, Z.-Z.; Geller, D. A.; Freeswick, P.; Dorko, K.; Bartoli, F.; Billiar, T. R. *Hepatology* **1995**, *21*, 1552; (c) Silva, J. S.; Vespa, G. N.; Cardoso, M. A.; Aliberti, J. C.; Cunha, F. Q. *Infect. Immun.* **1995**, *63*, 4862.
15. Karupiah, G.; Harris, N. Gamma Interferon-Induced Nitric Oxide in Antiviral Defense and Immunopathology. In *Gamma Interferon in Antiviral Defense*; Karupiah, G., Ed.; R.G. Landes Comp.: Georgetown, TX, 1997; pp 119–142.
16. Bodaghi, B.; Goureau, O.; Zipeto, D.; Laurent, L.; Virelizier, J.-L.; Michelson, S. *J. Immunol.* **1999**, *162*, 957.
17. Gao, X. R.; Tajima, M.; Sairenji, T. *Virology* **1999**, *258*, 375.
18. Melkova, Z.; Esteban, M. *J. Immunol.* **1995**, *155*, 5711.
19. Komatsu, T.; Bi, Z.; Reiss, C. S. *J. Neuroimmunol.* **1996**, *68*, 101.
20. Esteban, M.; Patino, C. *J. Interferon Cytokine Res.* **2000**, *20*, 867.
21. Guidotti, L. G.; McClary, H.; Loudis, J. M.; Chisari, F. V. *J. Exp. Med.* **2000**, *191*, 1247.
22. Sharara, A. I.; Perkins, D. J.; Misukonis, M. A.; Chan, S. U.; Dominitz, J. A.; Weinberg, J. B. *J. Exp. Med.* **1997**, *186*, 1495.
23. Feduchi, E.; Alonso, M. A.; Carrasco, L. *J. Virol.* **1989**, *63*, 1354.
24. Orange, J. S.; Biron, C. A. *J. Immunol.* **1996**, *156*, 4746.
25. Imanishi, J.; Ike, H.; Yamada, A. *R. Soc. Biol.* **1987**, *181*, 722.
26. Ito, M.; Nakano, T.; Kamiya, T.; Kitamura, K.; Ihara, T.; Kamiya, H.; Sakurai, M. *Antiviral Res.* **1991**, *15*, 183.
27. Mayer, A.; Gelderblom, H.; Kumel, G.; Jungwirth, C. *Virology* **1992**, *187*, 372.
28. (a) Doranz, B. J.; Rucker, J.; Yi, Y.; Smyth, R. J.; Samson, M.; Peiper, S. C.; Parmentier, M.; Collman, R. G.; Doms, R. W. *Cell* **1996**, *85*, 1149; (b) Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; Mackay, C. R.; LaRosa, G.; Newman, W.;

Gerard, N.; Gerard, C.; Sodorski, J. *Cell* **1996**, *85*, 1135; (c) Samson, M.; Labbé, O.; Mollereau, C.; Vassart, G.; Parmentier, M. *Biochemistry* **1996**, *35*, 3362.
29. Arenzana-Seisdedos, A.; Virelizier, J. L.; Rousset, D.; Clark-Lewis, I.; Loescher, P.; Moser, B.; Baggiolini, M. *Nature* **1996**, *383*, 400; Simmons, G.; Clapham, P. R.; Picard, L.; Offord, R. E.; Rosenkilde, M. M.; Schwartz, T. W.; Buser, R.; Wells, T. N. C.; Proudfoot, A. E. I. *Science* **1997**, *276*, 276.
30. (a) Deng, H.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Di Marzio, P.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature* **1996**, *381*, 661; (b) Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. *Science* **1996**, *272*, 1955; (c) Capobianchi, M. R.; Abbate, I.; Antonelli, G.; Turriziani, O.; Dolei, A.; Dianzani, F. *AIDS Res. Hum. Retroviruses* **1998**, *14*, 233; (d) Yang, A. G.; Zhang, X.; Torti, F.; Chen, S. Y. *Hum. Gene Ther.* **1998**, *9*, 2005.
31. Signoret, N.; Pelchen-Matthews, A.; Mack, M.; Proudfoot, A. E. I.; Marsh, M. *J. Cell Biol.* **2000**, *151*, 1281.
32. Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. *Nature* **1996**, *381*, 667.
33. Votruba, I. Unpublished results.
34. De Clercq, E. Unpublished results.