Supporting Information

Synthesis of Ester Prodrugs of 9-(S)- [3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (HPMPDAP) as Anti-Poxvirus Agents

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CONTENT:

Characterization of some intermediates in preparation of (S)-HPMPDAP	S2
Spectral data of tested compounds	S3
Elemental analyses of compounds	S16
HPLC Analysis of Compounds 20 and 21	S 17

CHARACTERIZATION OF SOME INTERMEDIATES IN PREPARATION OF (S)-HPMPDAP

9-(S)-[(2-Hydroxy-3-trityloxy)propyl]-2,6-diaminopurine (3). An analytically pure sample was isolated as a white solid after chromatography on silica gel in system chloroform – methanol (85:15). $[\alpha]_D -11.3$ (c 0.248, CHCl_3). ESIMS: 467.1 (MH^+) (77), 489.1 ($\text{M}+\text{Na}^+$) (50), 954.5 ($2\text{M}+\text{Na}^+$) (100).

^1H NMR (500.0 MHz, $\text{DMSO}-d_6$): 2.89 (dd, 1H, $J_{\text{gem}} = 9.5$, $J_{3'b,2'} = 5.0$, H-3'b); 2.93 (dd, 1H, $J_{\text{gem}} = 9.5$, $J_{3'a,2'} = 5.0$, H-3'a); 3.97 (dd, 1H, $J_{\text{gem}} = 13.0$, $J_{1'b,2'} = 7.2$, H-1'b); 4.03 (m, 1H, H-2'); 4.08 (dd, 1H, $J_{\text{gem}} = 13.0$, $J_{1'a,2'} = 3.7$, H-1'a); 5.50 (br, 1H, OH); 5.71 (brs, 2H, NH_2 -2); 6.63 (brs, 2H, NH_2 -6); 7.25 (m, 3H, H-*p*-Tr); 7.31 (m, 6H, H-*m*-Tr); 7.40 (m, 6H, H-*o*-Tr); 7.57 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$): 46.45 (C-1'); 65.94 (C-3'); 68.17 (C-2'); 86.11 (C-Tr); 113.23 (C-5); 127.24 (C-*p*-Tr); 128.12 (C-*m*-Tr); 128.54 (C-*o*-Tr); 138.60 (C-8); 143.92 (C-*i*-Tr); 152.01 (C-4); 156.27 (C-6); 160.28 (C-2).

6-Amino-2-[(dimethylamino)methylene]amino]-9-[(S)-2-hydroxy-3-trityloxypropyl]purine (4).

Isolated as a yellow foam without chromatographic purification (partial degradation to the starting compound **3** on silica gel). TLC purity approx. 80 % in system ethyl acetate – acetone – ethanol – water (15:3:4:3), R_F 0.29 (starting compound **3**: R_F 0.70). ESIMS: 522.2 (MH^+) (100), 1064.9 ($2\text{M}+\text{Na}^+$) (44). HRMS (ESI): For $\text{C}_{30}\text{H}_{32}\text{N}_7\text{O}_2$ (MH^+) calculated: 522.2612; found: 522.2607. NMR spectra were not obtained in sufficient quality for reliable characterization of the compound.

Diisopropyl [[(1S)-2-(2,6-diamino-9H-purin-9-yl)-1-(hydroxymethyl)ethyl]oxy]methylphosphonate (6).

An analytical sample was isolated as a white foam after chromatography on silica gel in system ethyl acetate – acetone – ethanol – water (15:3:4:3).

^1H NMR (500.0 MHz, $\text{DMSO-}d_6$): 1.16, 1.19 and 1.22 ($3 \times \text{d}$, 12H, $J_{\text{vic}} = 6.2$, $\text{CH}_3\text{-ipr}$); 3.44 (m, 2H, PCH_2); 3.82 (m, 3H, H-2' and H-3'); 4.02 (dd, 1H, $J_{\text{gem}} = 14.5$, $J_{1'\text{b},2'} = 6.5$, H-1'b); 4.18 (dd, 1H, $J_{\text{gem}} = 14.5$, $J_{1'\text{a},2'} = 3.7$, H-1'a); 4.53 (m, 2H, CH-ipr); 5.10 (br s, 1H, OH); 5.79 (s, 2H, $\text{NH}_2\text{-2}$); 6.68 (s, 2H, $\text{NH}_2\text{-6}$); 7.63 (s, 1H, H-8).

SPECTRAL DATA OF TESTED COMPOUNDS

9-[[(5S)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine (7).

^{13}C NMR (125.7 MHz, D_2O): 42.39 (C-1'); 64.62 (d, $J_{\text{P,C}} = 143.0$, CH_2P); 69.30 (d, $J_{\text{P,C}} = 6.4$, C-3'); 73.76 (d, $J_{\text{P,C}} = 4.0$, C-2'); 112.22 (C-5); 141.10 (C-8); 150.54 (C-4); 155.52 (C-6); 159.52 (C-2).

3-(Hexadecyloxy)propyl Ester of 9-[[(5S)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine (8a).

trans-isomer

^1H NMR (500.0 MHz, CDCl_3): 0.87 (t, 3H, $J_{\text{vic}} = 7.0$, CH_3); 1.28 (m, 26H, CH_2); 1.54 (m, 2H, CH_2); 1.97 (p, 2H, $J_{\text{vic}} = 6.2$, CH_2); 3.38 (m, 2H, OCH_2); 3.48 (t, 2H, $J_{\text{vic}} = 6.1$, OCH_2); 3.91 (dd, $J_{\text{gem}} = 14.8$, $J_{\text{H,P}} = 0.5$, $\text{CH}_a\text{H}_b\text{P}$); 3.99 (dd, 1H, $J_{\text{gem}} = 14.8$, $J_{\text{H,P}} = 6.9$, $\text{CH}_a\text{H}_b\text{P}$); 4.08 (m, 1H, H-2'); 4.28-4.13 (m, 4H, H-3' and P- OCH_2); 4.38 (m, 2H, H-1'); 5.10 (bs, 2H, NH_2 -2); 6.10 (bs, 2H, NH_2 -6); 7.58 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, CDCl_3): 14.08 (CH_3); 22.64, 26.13, 29.31, 29.42 and 29.49 (CH_2); 29.60 (CH_2); 29.65, 29.68 (CH_2); 30.67 (d, $J_{\text{P,C}} = 5.9$, CH_2); 31.86 (CH_2); 42.69 (C-1'); 63.44 (d, $J_{\text{P,C}} = 145.0$, PCH_2); 71.17 and 66.22 (OCH_2); 71.85 (d, $J_{\text{P,C}} = 9.1$, C-3'); 74.07 (d, $J_{\text{P,C}} = 5.0$, C-2'); 113.48 (C-5); 138.96 (C-8); 151.75 (C-4); 155.15 (C-6); 158.99 (C-2).

^{31}P (^1H dec.) NMR (202.3 MHz, CDCl_3): 11.08.

cis-isomer

^1H NMR (500.0 MHz, CDCl_3): 0.88 (t, 3H, $J_{\text{vic}} = 7.0$, CH_3); 1.26 (m, 26H, CH_2); 1.54 (m, 2H, CH_2); 1.94 (p, 2H, $J_{\text{vic}} = 6.2$, CH_2); 3.38 (t, 2H, $J_{\text{vic}} = 6.7$, OCH_2); 3.48 (t, 2H, $J_{\text{vic}} = 6.1$, OCH_2); 3.86 (dd, 1H, $J_{\text{gem}} = 14.3$, $J_{\text{P,H}} = 3.3$, $\text{CH}_a\text{CH}_b\text{P}$); 4.12 (dd, 1H, $J_{\text{gem}} = 14.3$, $J_{\text{P,H}} = 0.9$, $\text{CH}_a\text{CH}_b\text{P}$); 4.13 (m, 1H, H-2'); 4.28-4.18 (m, 4H, H-3' and P- OCH_2); 4.42 (m, 2H, H-1'); 4.76 (bs, 2H, NH_2); 5.53 (bs, 2H, NH_2); 7.56 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, CDCl_3): 159.83 (C-2); 155.79 (C-6); 151.91 (C-4); 138.60 (C-8); 114.06 (C-5); 73.42 (d, $J_{\text{P,C}} = 4.9$, C-2'); 71.20 (OCH_2); 70.54 (d, $J_{\text{P,C}} = 6.9$, C-3'); 66.16 (OCH_2); 64.31 (d, $J_{\text{P,C}} = 6.6$, OCH_2); 63.24 (d, $J_{\text{P,C}} = 148.1$, PCH_2); 42.35 (C-1'); 31.87 (CH_2); 30.82 (d, $J_{\text{P,C}} = 5.5$, CH_2); 29.65 (6C), 29.62, 29.60, 29.57, 29.45, 29.31, 26.09 and 22.64 (CH_2); 14.09 (CH_3).

^{31}P (^1H dec) NMR (202.3 MHz, CDCl_3): 12.79.

2-(Octadecyloxy)ethyl Ester of 9-[[*(5S)*-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine (8b).

trans-isomer

^1H NMR (500.0 MHz, CDCl_3): 7.55 (s, 1H, H-8); 5.53 (bs, 2H, NH_2); 4.77 (bs, 2H, NH_2); 4.37- 4.12 (m, 4H, H-1', H-3'); 4.06 (m, 1H, H-2'); 3.98 (dd, 1H, $J_{\text{P,CHax}} = 7.2$, $J_{\text{gem}} = 14.8$, PCH_{ax}); 3.88 (d, $J_{\text{P,CHeq}} \leq 0.5$, PCH_{eq}); 3.64 (m, 2H, OCH_2), 3.44 (t, 2H, $J_{\text{CH}_2,\text{CH}_2} = 6.8$, OCH_2); 3.37 (m, OCH_2); 1.55 (m, 2H, CH_2); 1.25 (m, 30H, CH_2); 0.99 (t, 3H, $J_{\text{CH}_3,\text{CH}_2} = 7.0$, CH_3).

^{13}C NMR (125.7 MHz, CDCl_3): 159.88 (C-2); 155.79 (C-6); 151.91 (C-4); 138.63 (C-8); 113.90 (C-5); 74.10 (d, $J_{\text{P,C}} = 5.3$, C-2'); 71.86 (d, $J_{\text{P,C}} = 8.7$, C-3'); 71.38 (OCH_2); 69.42 (d, $J_{\text{P,C}} = 4.9$, OCH_2); 65.00 (d, $J_{\text{P,C}} = 6.6$, OCH_2); 63.64 (d, $J_{\text{P,C}} = 145.6$, PCH_2); 42.61 (C-1'); 31.85, 29.63, 29.42, 29.37; 29.28; 25.99 and 22.61 (CH_2); 14.05 (CH_3).

cis-isomer

^1H NMR (500.0 MHz, CDCl_3): 7.56 (s, 1H, H-8); 5.54 (bs, 2H, NH_2); 4.76 (bs, 2H, NH_2); 4.45-4.08 (m, 6H, H-1', H-2', H-3', PCH_{eq}); 3.91 (dd, 1H, $J_{\text{P,CHax}} = 3.3$, $J_{\text{gem}} = 14.4$, PCH_{ax}); 3.58 (m, 2H, OCH_2); 3.43 (m, 4H, OCH_2); 1.55 (m, 2H, CH_2); 1.26 (m, 30H, CH_2); 0.87 (t, 3H, $J_{\text{CH}_3,\text{CH}_2} = 7.0$, CH_3).

^{13}C NMR (125.7 MHz, CDCl_3): 159.85 (C-2); 155.80 (C-6); 151.91 (C-4); 138.59 (C-8); 114.00 (C-5); 73.37 (d, $J_{\text{P,C}} = 5.0$, C-2'); 71.45 (OCH_2); 70.63 (d, $J_{\text{P,C}} = 6.9$, C-3'); 69.39 (d, $J_{\text{P,C}} = 4.4$, OCH_2); 65.84 (d, $J_{\text{P,C}} = 6.2$, OCH_2); 64.09 (d, $J_{\text{P,C}} = 147.5$, PCH_2); 42.39 (C-1'); 31.86, 29.60, 29.54, 29.45, 29.38, 29.29, 26.57 and 22.62 (CH_2); 14.06 (CH_3).

2-(Hexadecyloxy)ethyl Ester of 9-[[*(5S)*-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine (8c).

trans-isomer

^1H NMR (500.0 MHz, CDCl_3): 7.54 (s, 1H, H-8); 5.78 (bs, 2H, NH_2); 4.88 (bs, 2H, NH_2); 4.40-4.12 (m, 6H, H-1', H-3', POCH_2); 4.06 (m, 1H, H-2'); 3.97 (dd, 1H, $J_{\text{P,CHax}} = 7.3$, $J_{\text{gem}} = 14.8$, PCH_{ax}); 3.89 (d, 1H, $J_{\text{gem}} = 14.8$, $J_{\text{P,CHeq}} \leq 0.5$, PCH_{eq}); 3.65 (m, 2H, OCH_2); 3.46 (t, 2H, $J_{\text{CH}_2,\text{CH}_2} = 6.7$, OCH_2); 1.56 (br pent, 2H, CH_2); 1.25 (m, 26H, CH_2); 0.87 (t, 3H, $J_{\text{CH}_3,\text{CH}_2} = 7.0$, CH_3).

^{13}C NMR (125.7 MHz, CDCl_3): 159.86 (C-2); 155.84 (C-6); 151.85 (C-4); 138.61 (C-8); 113.81 (C-5); 74.11 (d, $J_{\text{P,C}} = 5.2$, C-2'); 71.87 (d, $J_{\text{P,C}} = 8.7$, C-3'); 71.41 (OCH_2); 69.44 (d, $J_{\text{P,C}} = 4.9$, OCH_2); 65.03 (d, $J_{\text{P,C}} = 6.6$, OCH_2); 63.65 (d, $J_{\text{P,C}} = 145.5$, PCH_2); 42.64 (C-1'); 31.87, 29.65 (6C), 29.60 (2C), 29.55, 29.45, 29.31, 26.02 and 22.64 (CH_2); 14.08 (CH_3).

cis-isomer

^1H NMR (500.0 MHz, CDCl_3): 7.55 (s, 1H, H-8); 5.82 (bs, 2H, NH_2); 4.90 (bs, 2H, NH_2); 4.48-4.02 (m, 8H, H-1', H-2', H-3', P-OCH_2 , PCH_{eq}); 3.90 (dd, 1H, $J_{\text{P,CHax}} = 3.2$, $J_{\text{gem}} = 14.4$, PCH_{ax}); 3.58 (m, 2H, OCH_2); 3.43 (m, 2H, OCH_2); 1.55 (br pent, 2H, CH_2); 1.25 (m, 26H, CH_2); 0.97 (t, 3H, $J_{\text{CH}_3,\text{CH}_2} = 7.0$, CH_3).

^{13}C NMR (125.7 MHz, CDCl_3): 159.86 (C-2); 155.88 (C-6); 151.85 (C-4); 138.56 (C-8); 113.91 (C-5); 73.40 (d, $J_{\text{P,C}} = 4.9$, C-2'); 71.47 (OCH_2); 70.65 (d, $J_{\text{P,C}} = 6.7$, C-3'); 69.40 (d, $J_{\text{P,C}} = 4.4$, OCH_2); 65.86 (d, $J_{\text{P,C}} = 6.2$, OCH_2); 63.78 (d, $J_{\text{P,C}} = 147.8$, PCH_2); 42.40 (C-1'); 31.87, 29.65 (5C), 29.61, 29.57, 29.56, 29.45, 29.39, 29.31, 25.94 and 22.64 (CH_2); 14.08 (CH_3).

3-(Hexadecyloxy)propyl Ester of 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine, sodium salt (9a).

^1H NMR (500.0 MHz, CD_3OD): 7.89 (s, 1H, H-8); 4.64 (m, 1H, H-2'); 4.31 (dd, 1H, $J_{1'a,2'} = 4.0$, $J_{\text{gem}} = 14.6$, H-1'a); 4.24 (dd, 1H, $J_{1'b,2'} = 6.0$, H-1'b); 3.93 (m, 2H, OCH_2); 3.69 (dd, 1H, $J_{\text{P,CH}} = 9.3$, $J_{\text{gem}} = 13.6$, PCH_a); 3.66 (dd, 1H, $J_{\text{P,CH}} = 9.3$, PCH_b); 3.64 (dd, 1H, $J_{3'a,2'} = 4.2$, $J_{\text{gem}} = 12.4$, H-3'a); 3.51 (dd, 1H, $J_{3'b,2'} = 4.6$, H-3'b); 3.49 (t, 2H, $J = 6.5$, OCH_2); 3.38 (t, 2H, $J = 6.7$, OCH_2); 1.81 (pent, 2H, CH_2); 1.53 (m, 2H, CH_2); 1.30 (m, 26H, CH_2); 0.91 (t, 3H, $J_{\text{CH}_3, \text{CH}_2} = 7.2$, CH_3).

^{13}C NMR (CD_3OD): Data not obtained due to insufficient solubility of the compound.

2-(Octadecyloxy)ethyl Ester of 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine, sodium salt (9b).

^1H NMR (500.0 MHz, $\text{DMSO}-d_6$): 7.67 (s, 1H, H-8); 6.65 (bs, 2H, NH_2); 5.80 (bs, 2H, NH_2); 4.03 (dd, 1H, $J_{1'a,2'} = 4.3$, $J_{\text{gem}} = 14.2$, H-1'a); 3.93 (dd, 1H, $J_{1'b,2'} = 7.0$, H-1'b); 3.73 (m, 4H, OCH_2); 3.65 (m, 1H, H-2'); 3.45 (dd, 1H, $J_{\text{P,CH}_a} = 7.3$, $J_{\text{gem}} = 13.6$, PCH_a); 3.41 (dd, 1H, $J_{\text{P,CH}_b} = 7.8$, PCH_b); 3.33 (t, 2H, $J_{\text{CH}_2, \text{CH}_2} = 6.4$, OCH_2); 3.24 (dd, 1H, $J_{3'a,2'} = 5.4$, $J_{\text{gem}} = 13.8$, H-3'a); 3.21 (dd, 1H, $J_{3'b,2'} = 7.5$, H-3'b); 1.45 (m, 2H, CH_2); 1.22 (m, 30H, CH_2); 0.85 (t, 3H, $J_{\text{CH}_2, \text{CH}_3} = 7.0$, CH_3).

^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$): 160.37 (C-2); 156.28 (C-6); 152.02 (C-4); 138.50 (C-8); 113.06 (C-5); 80.70 (d, $J_{\text{P,C}} = 4.9$, C-2'); 70.75 (d, $J_{\text{P,C}} = 6.0$, OCH_2); 70.48 (OCH_2); 66.66 (d, $J_{\text{P,C}} = 152.7$, PCH_2); 62.93 (d, $J_{\text{P,C}} = 5.6$, OCH_2); 60.69 (C-3'); 43.72 (C-1'); 31.53, 29.53, 29.27 (30 C), 29.19, 28.94, 25.93 and 22.33 (CH_2); 14.20 (CH_3).

2-(Hexadecyloxy)ethyl Ester of 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine, sodium salt (9c).

^1H NMR (500.0 MHz, $\text{CD}_3\text{OD} + \text{NaOD}$): 7.90 (s, 1H, H-8); 4.28 (bd, 2H, $J_{1',2'} = 5.6$, H-1'); 3.90-3.35 (m, 11H, H-2', H-3', PCH_2 , OCH_2); 1.50 (m, 2H, CH_2); 1.24 (m, 26H, CH_2); 0.87 (t, 3H, $J_{\text{CH}_3, \text{CH}_2} = 7.0$, CH_3).

^{13}C NMR (125.7 MHz, $\text{CD}_3\text{OD} + \text{NaOD}$): 160.32 (C-2); 156.19 (C-6); 151.52 (C-4); 138.70 (C-8); 112.61 (C-5); 81.62 (d, $J_{\text{P,C}} = 12.0$, C-2'); 70.95 (s, OCH_2); 70.13 (d, $J_{\text{P,C}} = 6.7$, OCH_2); 64.82 (d, $J_{\text{P,C}} = 158.21$, PCH_2); 63.67 (d, $J_{\text{P,C}} = 5.5$, OCH_2); 59.98 (C-3'); 43.81 (C-1'); 31.57, 29.27 (6C), 29.24 (2C), 29.17, 29.13, 28.95, 25.68 and 22.27 (CH_2).

Pivaloyloxymethyl Ester of 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (10).

^1H NMR (500.0 MHz, CD_3OD): 7.88 (s, 1H, H-8); 5.54 (dd, 1H, $J_{\text{P,OCH}} = 5.4$, $J_{\text{gem}} = 12.2$, OCH_2O); 5.50 (dd, 1H, $J_{\text{P,OCH}} = 4.9$, OCH_2O); 4.60 (m, 1H, H-2'); 4.28 (dd, 1H, $J_{1'a,2'} = 4.1$, $J_{\text{gem}} = 14.5$, H-1'a); 4.20 (dd, 1H, $J_{1'b,2'} = 6.4$, H-1'b); 3.71 (dd, 1H, $J_{\text{P,CHa}} = 9.3$, $J_{\text{gem}} = 13.0$, PCH_a); 3.65 (dd, 1H, $J_{\text{P,CHb}} = 9.1$, PCH_b); 3.60 (dd, 1H, $J_{3'a,2'} = 4.6$, $J_{\text{gem}} = 12.4$, H-3'a); 3.49 (dd, 1H, $J_{3'b,2'} = 4.9$, H-3'b); 1.15 (s, 9H, *t*-butyl).

^{13}C NMR (125.7 MHz, CD_3OD): 178.78 (C=O); 161.71 (C-2); 159.34 (C-6); 157.50 (C-4); 139.50 (C-8); 113.76 (C-5); 81.93 (d, $J_{\text{P,C}} = 10.9$, C-2'); 81.11 (d, $J_{\text{P,C}} = 5.0$, OCH_2O); 67.81 (d, $J_{\text{P,C}} = 159.9$, P-C); 61.58 (C-3'); 44.44 (C-1'); 39.66 and 27.56 (*t*-butyl).

Pivaloyloxymethyl Ester of 9-[[*(5S)*-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine (11).

trans-isomer

^1H NMR (500.0 MHz, CDCl_3): 7.48 (s, 1H, H-8); 5.72 and 5.69 (2x dd, 2H, $J_{\text{P,CH}} = 5.3$, $J_{\text{gem}} = 12.2$, OCH_2O); 5.60 (bs, 1H, NH_2); 4.81 (bs, 1H, NH_2); 4.41-4.13 (m, 4H NCH_2 , OCH_2); 4.07 (m, 1H, H-2'); 3.97 (dd, 1H, $J_{\text{gem}} = 14.4$, $J_{\text{P,CHax}} = 7.1$, PCH_{ax}); 3.92 (bd, 1H, $J_{\text{gem}} = 14.4$, PCH_{eq}); 1.21 (s, 9H, *tert*-Bu).

^{13}C NMR (125.7 MHz, CDCl_3): 177.0 (C=O); 159.91 (C-2); 155.81 (C-6); 151.84 (C-4); 138.53 (C-8); 114.0 (C-5); 81.43 (d, $J_{\text{P,C}} = 6.2$, OCH_2O); 74.12 (d, $J_{\text{P,C}} = 5.3$, C-2'); 72.50 (d, $J_{\text{P,C}} = 8.9$, C-3'); 64.09 (d, $J_{\text{P,C}} = 143.6$, PCH_2); 42.55 (C-1'); 38.65 (*tert*-Bu); 26.76 (3C, *tert*-Bu).

cis-isomer

^1H NMR (500.0 MHz, CDCl_3): 7.50 (s, 1H, H-8); 6.28 (bs, 1H, NH_2); 5.74 (dd, 1H, $J_{\text{P,CH}} = 5.6$, $J_{\text{gem}} = 12.3$, OCH_2O); 5.60 (dd, 1H, $J_{\text{P,CH}} = 5.1$, OCH_2O); 5.13 (bs, 1H, NH_2); 4.45-5.03 (m, 5H, H-1', OCH_2 , H-2'); 3.94 (bd, 1H, $J_{\text{P,CHax}} \leq 1.0$, $J_{\text{gem}} = 14.4$, PCH_{ax}); 3.85 (dd, 1H, $J_{\text{gem}} = 14.4$, $J_{\text{P,CHeq}} = 3.2$, PCH_{eq}); 1.19 (s, 9H, *tert*-Bu).

^{13}C NMR (125.7 MHz, CDCl_3): 176.96 (C=O); 160.02 (C-2); 156.04 (C-6); 151.70 (C-4); 138.40 (C-8); 113.69 (C-5); 81.70 (d, $J_{\text{P,C}} = 4.9$, OCH_2O); 73.24 (d, $J_{\text{P,C}} = 4.6$, C-2'); 70.95 (d, $J_{\text{P,C}} = 6.9$, C-3'); 63.57 (d, $J_{\text{P,C}} = 142.8$, PCH_2); 42.19 (C-1'); 38.60 (*tert*-Bu); 26.70 (3C, *tert*-Bu).

2,2,2-(Trifluoro)ethyl Ester of 9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine (12).

^1H NMR (500.0 MHz, CD_3OD): 7.89 (s, 1H, H-8); 4.33 – 4.20 (m, 5 H, H-1', H-3'b and OCH_2CF_3); 4.05 (dd, 1 H, $J_{\text{gem}} = 11.7$, $J_{3'a,2'} = 4.8$, H-3'a); 4.02 (m, 1H, H-2'); 3.82 (dd, 1H, $J_{\text{gem}} = 12.9$, $J_{\text{P,CHb}} = 9.6$, PCH_b); 3.69 (dd, 1H, $J_{\text{gem}} = 12.9$, $J_{\text{P,CHa}} = 9.8$, PCH_a).

^{13}C NMR (125.7 MHz, CD_3OD): 161.52 (C-2); 157.40 (C-6); 153.09 (C-4); 141.14 (C-8); 125.45 (qd, $J_{\text{C,F}} = 276.9$, $J_{\text{C-C-O-P}} = 9.4$, CF_3); 113.86 (C-5); 78.89 (d, $J_{\text{P,C}} = 12.5$, C-2'); 67.41(d, $J_{\text{P,C}} = 161.3$, PCH_2); 64.16 (C-3'); 63.42 (qd, $J_{\text{C-C-F}} = 36.1$, $J_{\text{C-O-P}} = 4.5$, OCH_2CF_3); 45.09 (C-1').

2,2,2-(Trifluoro)ethyl Ester of 9-[(5S)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}-2,6-diaminopurine (13).

trans-isomer

^1H NMR (600 MHz, CD_3OD): 4.06 (dd, 1H, $J_{\text{gem}} = 15.2$, $J_{\text{H,P}} = 1.1$, $\text{CH}_a\text{H}_b\text{P}$); 4.13 (dd, 1H, $J_{\text{gem}} = 15.5$, $J_{1'b,2'} = 8.1$, H-1'b); 4.21-4.29 (m, 2H, H-1'a and H-2'); 4.33 (ddd, 1H, $J_{\text{gem}} = 11.8$, $J_{3'b,2'} = 8.9$, $J_{\text{H,P}} = 1.2$, H-3'b); 4.36 (dd, 1H, $J_{\text{gem}} = 15.2$, $J_{\text{H,P}} = 11.2$, $\text{CH}_a\text{H}_b\text{P}$); 4.54 (ddd, 1H, $J_{\text{H,P}} = 17.4$, $J_{\text{gem}} = 11.8$, $J_{3'a,2'} = 2.2$, H-3'a); 4.55-4.64 (m, 2H, OCH_2CF_3); 7.77 (s, 1H, H-6).

^{13}C NMR (150.9 MHz, CD_3OD): 43.58 (C-1'); 62.66 (qd, $J_{\text{C,F}} = 37.7$, $J_{\text{C,P}} = 5.4$, CH_2CF_3); 63.91 (d, $J_{\text{C,P}} = 144.3$, CH_2P); 74.45 (d, $J_{\text{C,P}} = 9.0$, C-3'); 75.38 (d, $J_{\text{C,P}} = 5.4$, C-2'); 113.45 (C-5); 124.37 (qd, $J_{\text{C,F}} = 276.8$, $J_{\text{C,P}} = 8.0$, CF_3); 141.08 (CH-8); 152.97 (C-4); 156.24 (C-6); 160.14 (C-2).

^{31}P (^1H dec.) NMR (202.3 MHz, CD_3OD): 13.50.

^{19}F NMR (470.3 MHz, CD_3OD): -73.48 (t, $J_{\text{F,H}} = 8.3$).

cis-isomer

^1H NMR (600 MHz, CD_3OD): 4.11 (dd, 1H, $J_{\text{gem}} = 14.8$, $J_{\text{H,P}} = 3.2$, $\text{CH}_a\text{H}_b\text{P}$); 4.21-4.29 (m, 2H, H-1' b and H-2'); 4.32 (dd, 1H, $J_{\text{gem}} = 15.4$, $J_{1'a,2'} = 8.8$, H-1' a); 4.40 (dd, 1H, $J_{\text{gem}} = 14.8$, $J_{\text{H,P}} = 7.7$, $\text{CH}_a\text{H}_b\text{P}$); 4.46 (ddd, 1H, $J_{\text{gem}} = 12.0$, $J_{3'b,2'} = 7.7$, $J_{\text{H,P}} = 6.0$, H-3' b); 4.55-4.64 (m, 3H, H-3' a and OCH_2CF_3); 7.81 (s, 1H, H-6).

^{13}C NMR (150.9 MHz, CD_3OD): 43.15 (C-1'); 63.43 (qd, $J_{\text{C,F}} = 37.7$, $J_{\text{C,P}} = 5.5$, CH_2CF_3); 63.48 (d, $J_{\text{C,P}} = 146.0$, CH_2P); 73.09 (d, $J_{\text{C,P}} = 7.2$, C-3'); 74.60 (d, $J_{\text{C,P}} = 5.1$, C-2'); 113.45 (C-5); 124.20 (qd, $J_{\text{C,F}} = 276.9$, $J_{\text{C,P}} = 7.7$, CF_3); 140.97 (CH-8); 153.08 (C-4); 156.14 (C-6); 160.01 (C-2).

^{31}P (^1H dec.) NMR (202.3 MHz, CD_3OD): 15.09.

^{19}F NMR (470.3 MHz, CD_3OD): -73.74 (t, $J_{\text{F,H}} = 8.3$).

Bis[(2,2,2-(Trifluoro)ethyl] tosyloxymethanephosphonate (15).

^1H NMR (500.0 MHz, CDCl_3): 7.81 (dm, 2H, H-arom.); 7.40 (dm, 2H, H-arom.); 4.36 (d, 2H, $J_{\text{P,CH}} = 9.8$, PCH_2); 4.43 (m, 4H, P-OCH₂); 2.48 (s, 3H, CH_3).

^{13}C NMR (125.7 MHz, CDCl_3): 146.06, 131.13, 130.17 (2C) and 128.20 (2C) (C-arom.); 122.06 (qd, 2C, $J_{\text{C,F}} = 277.6$, $J_{\text{C,P}} = 7.5$, CF_3); 62.90 (qd, 2C, $J_{\text{C,F}} = 38.5$, $J_{\text{C,P}} = 5.9$, P-OCH₂); 60.70 (d, $J_{\text{P,C}} = 175.7$, PCH_2); 21.70 (CH_3).

Butylsalicyl Ester of 9-[(5S)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine (16).

The compound was isolated as a mixture of diastereoisomers in ratio *cis* : *trans* 88:12. The ratio was determined from ^{31}P NMR spectra and HPLC. Full characterization by ^1H and ^{13}C

NMR spectra was possible for *cis* isomer only; intensities of signals of a minor *trans*- isomer were not sufficient for full NMR characterization.

cis-isomer

^1H NMR (600 MHz, CDCl_3): 0.96 (t, 3H, $J_{\text{vic}} = 7.4$, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$); 1.45, 1.72 ($2 \times \text{m}$, $2 \times 2\text{H}$, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$); 4.14 (dd, 1H, $J_{\text{gem}} = 13.5$, $J_{1'b,2'} = 7.2$, H-1' b); 4.20 (dd, 1H, $J_{\text{gem}} = 14.6$, $J_{\text{H,P}} = 3.0$, $\text{CH}_a\text{H}_b\text{P}$); 4.21 (m, 1H, H-2'); 4.23 (dd, 1H, $J_{\text{gem}} = 13.5$, $J_{1'a,2'} = 3.6$, H-1' a); 4.27 (m, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$); 4.49 (m, 1H, H-3' b); 4.55 (dd, 1H, $J_{\text{gem}} = 14.6$, $J_{\text{H,P}} = 9.0$, $\text{CH}_a\text{H}_b\text{P}$); 4.59 (ddd, 1H, $J_{\text{H,P}} = 14.1$, $J_{\text{gem}} = 11.9$, $J_{3'a,2'} = 2.7$, H-3' a); 4.76 (bs, 2H, NH_2 -2); 5.55 (bs, 2H, NH_2 -6); 7.26 (dddd, 1H, $J_{4,3} = 7.8$, $J_{4,5} = 7.5$, $J_{4,6} = 1.3$, $J_{\text{H,P}} = 0.7$, H-4-Ph); 7.30 (dt, 1H, $J_{6,5} = 8.4$, $J_{6,4} = J_{\text{H,P}} = 1.3$, H-6-Ph); 7.50 (ddd, 1H, $J_{5,6} = 8.4$, $J_{5,4} = 7.5$, $J_{5,3} = 1.8$, H-5-Ph); 7.58 (s, 1H, H-6); 7.92 (dd, 1H, $J_{3,4} = 7.8$, $J_{3,5} = 1.8$, H-3-Ph).

^{13}C NMR (150.9 MHz, CDCl_3): 13.72 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$); 19.18, 30.59 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$); 42.47 (C-1'); 64.52 (d, $J_{\text{C,P}} = 148$, CH_2P); 65.11 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$); 71.20 (d, $J_{\text{C,P}} = 7$, C-3'); 73.47 (d, $J_{\text{C,P}} = 5$, C-2'); 114.05 (C-5); 122.69 (d, $J_{\text{C,P}} = 5$, C-2-Ph); 122.99 (d, $J_{\text{C,P}} = 3$, CH-6-Ph); 125.49 (CH-4-Ph); 131.70 (CH-3-Ph); 133.84 (CH-5-Ph); 138.64 (CH-8); 149.09 (d, $J_{\text{C,P}} = 8$, C-1-Ph); 151.94 (C-4); 155.83 (C-6); 159.87 (C-2); 164.38 (CO).

^{31}P (^1H dec.) NMR (202.3 MHz, CDCl_3): 10.07 (*cis*-isomer), 7.87 (*trans*-isomer).

***N*-[*N*-(*t*-Butyloxycarbonyl)-*L*-Valyl]-*O*-Prop-2-yl-*L*-Seryl Ester of 9-[[*(5S)*-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine, *trans*-isomer (20).**

^1H NMR (500.0 MHz, $\text{DMSO}-d_6$): 0.83 (d, 3H, $J_{\text{vic}} = 6.8$, CH_3 (Val)); 0.88 (d, 3H, $J_{\text{vic}} = 6.9$, CH'_3 (Val)); 1.17 (d, 3H, $J_{\text{vic}} = 6.2$, $\text{CH}_3\text{CH}(\text{O})\text{CH}'_3$); 1.18 (d, 3H, $J_{\text{vic}} = 6.2$, $\text{CH}_3\text{CH}(\text{O})\text{CH}'_3$); 1.37 (s, 9H, *t*-Bu); 1.97 (octet, 1H, $J_{\text{vic}} = 6.8$, CHMe_2 (Val)); 3.89-3.95 (m, 3H, NCH (Val), OCHP , H-1'a); 4.04 (dd, 1H, $J_{\text{gem}} = 14.6$, $J_{\text{vic}} = 2.9$, H-1'b); 4.13-4.19 (m, 2H, H-2', H-3'a); 4.22-4.30 (m, 3H, CH_2 (Ser), OCHP); 4.38 (m, 1H, H-3'b); 4.62 (m, 1H, NCH (Ser)), 4.90 (septet, 1H, $J_{\text{vic}} = 6.2$, OCHMe_2); 5.78 (bs, 2H, NH_2 (2)); 6.69 (bs, 2H, NH_2 (6)); 6.73 (bd, 1H, $J_{\text{vic}} = 9.0$, NH (Val)); 7.61 (s, 1H, H-8); 8.41 (bd, 1H, $J_{\text{vic}} = 7.7$, NH (Ser)).
 ^{13}C NMR (125.7 MHz, $\text{DMSO}-d_6$): 18.16, 19.38 ($(\text{CH}_3)_2$ (Val)); 21.63, 21.66 ($(\text{CH}_3)_2\text{CHO}$); 28.39 ($(\text{CH}_3)_3\text{C}$); 30.73 (Me_2CH (Val)); 42.14 (C-1'); 52.56 (d, $J_{\text{C,P}} = 6.6$, NCH (Ser)); 59.52 (NCH (Val)); 62.63 (d, $J_{\text{C,P}} = 141.2$, OCH_2P); 64.26 (d, $J_{\text{C,P}} = 4.4$, CH_2 (Ser)); 69.06 (OCHMe_2); 72.48 (d, $J_{\text{C,P}} = 9.0$, C-3'); 73.90 (d, $J_{\text{C,P}} = 5.3$, C-2'); 78.29 (CMe_3); 113.05 (C-5); 137.86 (C-8); 152.01 (C-4); 155.66 (CO (Boc)); 156.28 (C-6); 160.48 (C-2); 168.63 (CO (Ser)); 171.91 (CO (Val)).

^{31}P (^1H dec.) NMR (202.3 MHz, $\text{DMSO}-d_6$): 12.83.

***N*-[*N*-(*t*-Butyloxycarbonyl)-*L*-Valyl]-*O*-Prop-2-yl-*L*-Seryl Ester of 9-[(5*S*)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl}-2,6-diaminopurine, *cis*-isomer (20).**

^1H NMR (500.0 MHz, $\text{DMSO}-d_6$): 0.81 (d, 3H, $J_{\text{vic}} = 6.8$, CH_3 (Val)); 0.86 (d, 3H, $J_{\text{vic}} = 6.9$, CH'_3 (Val)); 1.16 (d, 3H, $J_{\text{vic}} = 6.2$, $\text{CH}_3\text{CH}(\text{O})\text{CH}'_3$); 1.16 (d, 3H, $J_{\text{vic}} = 6.2$,

CH₃CH(O)CH₃); 1.33 (s, 9H, *t*-Bu); 1.92 (octet, 1H, $J_{\text{vic}} = 6.9$, CHMe₂ (Val)); 3.86 (dd, 1H, $J_{\text{CH-CH}} = 7.0$, $J_{\text{CH-NH}} = 8.9$, NCH (Val)); 3.96 (dd, 1H, $J_{\text{gem}} = 15.0$, $J_{\text{H-P}} = 5.5$, OCHP); 4.09 (dd, 1H, $J_{\text{gem}} = 14.4$, $J_{\text{vic}} = 3.7$, H-1'a); 4.16-4.35 (m, 6H, H-3'a, CH₂ (Ser), OCH₂P, H-2', H-1'b); 4.46 (td, 1H, $J_{\text{gem}} = J_{\text{vic}} = 11.5$, $J_{\text{H-P}} = 2.6$, H-3'b); 4.58 (m, 1H, NCH (Ser)), 4.90 (septet, 1H, $J_{\text{vic}} = 6.2$, OCHMe₂); 5.79 (bs, 2H, NH₂ (2)); 6.67 (bd, 1H, $J_{\text{vic}} = 9.4$, NH (Val)); 6.69 (bs, 2H, NH₂ (6)); 7.71 (s, 1H, H-8); 8.42 (bd, 1H, $J_{\text{vic}} = 7.8$, NH (Ser)).

¹³C NMR (125.7 MHz, DMSO-*d*₆): 18.31, 19.39 ((CH₃)₂ (Val)); 21.64, 21.66 ((CH₃)₂CHO); 28.37 ((CH₃)₃C); 30.65 (Me₂CH (Val)); 41.29 (C-1'); 52.46 (d, $J_{\text{C,P}} = 6.9$, NCH (Ser)); 59.58 (NCH (Val)); 60.69 (d, $J_{\text{C,P}} = 140.8$, OCH₂P); 65.27 (d, $J_{\text{C,P}} = 5.5$, CH₂ (Ser)); 69.06 (OCHMe₂); 71.01 (d, $J_{\text{C,P}} = 6.8$, C-3'); 72.09 (d, $J_{\text{C,P}} = 5.1$, C-2'); 78.30 (CMe₃); 113.09 (C-5); 137.97 (C-8); 152.06 (C-4); 155.66 (CO (Boc)); 156.28 (C-6); 160.44 (C-2); 168.58 (CO (Ser)); 172.05 (CO (Val)).

³¹P (¹H dec.) NMR (202.3 MHz, DMSO-*d*₆): 13.94.

***N*-L-Valyl-*O*-Prop-2-yl-L-Seryl Ester of 9-[[*(5S)*-2-Hydroxy-2-oxido-1,4,2-dioxaphosphinan-5-yl]methyl]-2,6-diaminopurine, *trans*-isomer (21).**

Purity: 89 %.

¹H NMR (500.0 MHz, CD₃OD): 1.09 (d, 3H, $J_{\text{vic}} = 6.9$, CH₃ (Val)); 1.11 (d, 3H, $J_{\text{vic}} = 6.9$, CH₃' (Val)); 1.25 (d, 3H, $J_{\text{vic}} = 6.2$, CH₃CH(O)CH₃); 1.28 (d, 3H, $J_{\text{vic}} = 6.2$, CH₃CH(O)CH₃); 2.24 (octet, 1H, $J_{\text{vic}} = 6.5$, CHMe₂ (Val)); 3.78 (m, 1H, NCH (Val)); 4.03 (d, 1H, $J_{\text{gem}} = 14.8$, OCHP); 4.14 (dd, 1H, $J_{\text{gem}} = 14.1$, $J_{\text{vic}} = 5.3$, H-1'a); 4.18-4.35 (m, 4H, OCH₂P, H-3'a, H-2', H-1'b); 4.39 (ddd, 1H, $J_{\text{gem}} = 10.7$, $J_{\text{vic}} = 3.3$, $J_{\text{H-P}} = 7.2$, CHH' (Ser)); 4.47-4.53 (m, 2H, H-3'b, CHH' (Ser)); 4.85 (m, 1H, NCH (Ser)), 5.00 (septet, 1H, $J_{\text{vic}} = 6.2$, OCHMe₂); 7.73 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, CD_3OD): 17.92, 18.88 ($(\text{CH}_3)_2$ (Val)); 22.00 ($(\text{CH}_3)_2\text{CHO}$); 31.50 (Me_2CH (Val)); 43.64 (C-1'); 54.32 (d, $J_{\text{C,P}} = 6.1$, NCH (Ser)); 59.65 (NCH (Val)); 64.07 (d, $J_{\text{C,P}} = 144.9$, OCH_2P); 65.98 (d, $J_{\text{C,P}} = 6.5$, CH_2 (Ser)); 71.48 (OCHMe_2); 73.99 (d, $J_{\text{C,P}} = 8.7$, C-3'); 75.51 (d, $J_{\text{C,P}} = 5.5$, C-2'); 113.51 (C-5); 141.09 (C-8); 153.10 (C-4); 156.20 (C-6); 160.12 (C-2); 169.06 (CO (Ser)); 169.70 (CO (Val)).

^{31}P (^1H dec.) NMR (202.3 MHz, CD_3OD): 13.49.

***N*-L-Valyl-*O*-Prop-2-yl-L-Seryl Ester of 9-[(5*S*)-2-Hydroxy-2-oxido-1,4,2-dioxaphosphan-5-yl]methyl}-2,6-diaminopurine, *cis*-isomer (21).**

^1H NMR (500.0 MHz, CD_3OD): 1.07 (d, 3H, $J_{\text{vic}} = 6.9$, CH_3 (Val)); 1.10 (d, 3H, $J_{\text{vic}} = 6.9$, CH_3 (Val)); 1.26 (d, 3H, $J_{\text{vic}} = 6.2$, $\text{CH}_3\text{CH}(\text{O})\text{CH}_3$); 1.27 (d, 3H, $J_{\text{vic}} = 6.2$, $\text{CH}_3\text{CH}(\text{O})\text{CH}_3$); 2.23 (octet, 1H, $J_{\text{vic}} = 6.6$, CHMe_2 (Val)); 3.78 (m, 1H, NCH (Val)); 4.05 (m, 1H, OCHP); 4.23 (m, 1H, H-2'); 4.26-4.35 (m, 3H, OCHP, H-1'); 4.40-4.44 (m, 2H, H-3'a, CHH' (Ser)); 4.53-4.61 (m, 2H, H-3'b, CHH' (Ser)); 4.82 (m, 1H, NCH (Ser)), 5.05 (septet, 1H, $J_{\text{vic}} = 6.3$, OCHMe_2); 7.83 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, CD_3OD): 17.85, 18.85 ($(\text{CH}_3)_2$ (Val)); 21.94 ($(\text{CH}_3)_2\text{CHO}$); 31.52 (Me_2CH (Val)); 43.25 (C-1'); 54.33 (d, $J_{\text{C,P}} = 6.4$, NCH (Ser)); 59.59 (NCH (Val)); 63.30 (d, $J_{\text{C,P}} = 145.2$, OCH_2P); 67.25 (d, $J_{\text{C,P}} = 6.2$, CH_2 (Ser)); 71.48 (OCHMe_2); 72.68 (d, $J_{\text{C,P}} = 7.1$, C-3'); 74.61 (d, $J_{\text{C,P}} = 5.1$, C-2'); 113.34 (C-5); 141.38 (C-8); 153.14 (C-4); 155.48 (C-6); 159.07 (C-2); 169.03 (CO (Ser)); 169.74 (CO (Val)).

^{31}P (^1H dec.) NMR (202.3 MHz, CD_3OD): 14.57.

Elemental analyses of compounds

No.	Formula (M _w)	Calculated				Found			
		%C	%H	%N	%P	%C	%H	%N	%P
3	C ₂₇ H ₂₆ N ₆ O ₂ ·½ H ₂ O (475.55)	68.19	5.72	17.67	-	68.08	5.75	17.86	-
8a	C ₂₈ H ₅₁ N ₆ O ₅ P (582.72)	57.71	8.82	14.42	5.32	57.40	9.08	14.60	5.59
8b	C ₂₉ H ₅₃ N ₆ O ₅ P (596.75)	58.37	8.95	14.05	5.19	58.12	9.11	13.76	5.01
8c	C ₂₇ H ₄₉ N ₆ O ₅ P (568.70)	57.02	8.68	14.78	5.45	56.75	8.72	14.47	5.29
9a	C ₂₈ H ₅₂ N ₆ O ₆ NaP (622.71)	54.00	8.42	13.50	4.97	54.36	8.69	13.19	4.88
9b	C ₂₉ H ₅₅ N ₆ O ₆ NaP (636.75)	54.70	8.84	13.20	4.86	54.97	8.99	12.98	4.56
9c	C ₂₇ H ₅₀ N ₆ O ₆ NaP (608.69)	53.28	8.28	13.81	5.09	52.99	8.42	13.42	4.80
10	C ₁₅ H ₂₅ N ₆ O ₇ P·½ H ₂ O (441.38)	40.82	5.94	19.04	7.02	40.84	5.93	18.83	7.14
11	C ₁₅ H ₂₃ N ₆ O ₆ P·H ₂ O (432.37)	41.67	5.83	19.44	7.16	41.61	6.03	19.18	7.32
12	C ₁₁ H ₁₆ N ₆ O ₅ F ₃ P (400.25)	33.01	4.03	21.00	7.74	33.39	4.31	21.20	7.88
13	C ₁₁ H ₁₄ N ₆ O ₄ F ₃ P·2H ₂ O (418.27)	31.59	4.34	20.09	7.41	31.88	4.73	20.39	7.06
15	C ₁₂ H ₁₃ F ₆ O ₆ PS (376.29)	33.50	3.05	-	7.20	33.54	3.05	-	7.54
16	C ₂₀ H ₂₅ N ₆ O ₆ P·0.6H ₂ O (486.97)	49.40	5.43	17.28	6.37	49.62	5.39	16.87	6.51
18	C ₆ H ₁₄ O ₃ NCI (183.64)	39.24	19.31	7.68	-	39.39	7.83	7.51	-
19	C ₁₆ H ₃₀ O ₆ N ₂ (346.49)	55.53	8.92	8.01	-	55.47	8.73	8.09	-

Additional analytical data

Compound **15**, calculated: 26.49 % F, 7.45 % S; found: 26.88 % F, 7.85 % S.

Compound **18**, calculated: 19.31 % Cl; found: 19.37 % Cl.

HPLC Analysis of Compounds 20 and 21

To determine purity of these compounds, HPLC was used instead of elemental analysis due to a hygroscopic character of the material.

1. Chromatogram of solvent injection

	ret. time (min)	area (V*sec)	area (%)
solvent peak 1	27.20	0.192	
solvent peak 2	29.43	0.123	
solvent peak 3	29.80	0.083	

2. Chromatogram of *cis* cHPMPDAP-Ser(OiPr)-Val.HCl (**21-cis**)

	ret. time (min)	area (V*sec)	area (%)
solvent peak 1	27.15	0.184	
solvent peak 2	29.41	0.128	
solvent peak 3	29.78	0.081	
impurity 1	1.66	1.031	3.98
impurity 2	17.09	0.141	0.55
cis cHPMPDAP-Ser(OiPr)-Val.HCl	11.80	24.714	95.47

3. Chromatogram of *trans* cHPMPDAP-Ser(OiPr)-Val.HCl (**21-trans**)

	ret. time (min)	Area (V*sec)	Area (%)
solvent peak 1	27.12	0.169	
solvent peak 2	29.39	0.123	
solvent peak 3	29.76	0.079	
Impurity 1	1.61	2.412	8.99

Impurity 2	16.85	0.481	1.79
Impurity 3	2.48	0.044	0.17
<i>trans</i> cHPMPDAP-Ser(OiPr)Val.HCl	11.64	23.892	89.05

4. Chromatogram of cHPMPDAP-Ser(OiPr)-Val(BOC) (20)

	ret. time (min)	area (V*sec)	area (%)
solvent peak 1	27.16	0.179	
solvent peak 2	29.43	0.138	
solvent peak 3	29.79	0.076	
impurity 1	1.70	0.100	2.35
impurity 2	2.59	0.062	1.46
impurity 3	26.14	0.041	0.97
cHPMPDAP-Ser(OiPr)-Val(BOC)	21.61	4.044	95.22

Experimental

Column: Nova Pack C18, 3.9 ×150 mm, C18 protection column

Flow: 1 ml/min

Mobile phase A: MeOH – 20 mM NaH₂PO₄ buffer pH 3.0 (1:9)

Mobile phase B: MeOH

Gradient: 100 % A (0 min) – 100 % B (30 min) – 100 % B (32 min) – 100 A (33 min) -100 A (40 min)

Detection wavelength: 254 nm

Samples were dissolved in mobile phase A and directly injected.

Peaks on the chromatogram of solvent injection were not considered for purity evaluation.