Electronic Supporting Information
for

6-Alkyl-, 6-Aryl- and 6-Hetaryl-7-deazapurine Ribonucleosides as Inhibitors of Human or MTB Adenosine Kinase and Potential Antimycobacterial Agents

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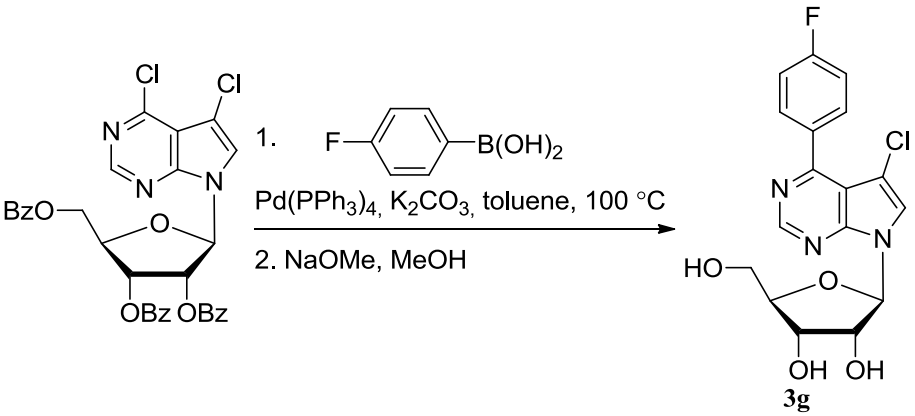
Table of contents

Experimental part - chemistry

NMR spectra were recorded on a 400 MHz (^{1}H at 400 MHz, ^{13}C at 100.6 MHz), or a 500 MHz (^{1}H at 500 MHz, ^{13}C at 125.7 MHz), or a 600 MHz (^{1}H at 600 MHz, ^{13}C at 150.9 MHz) spectrometers. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured at 25 °C, $[\alpha]_D^{20}$ values are given in 10^{-1} deg cm^2 g^{-1}. The high resolution mass spectra were measured using electrospray ionization. Reverse phase high performance flash chromatography (HPFC) purifications were performed on KP-C18-HS columns with Biotage SP1 system. The sample was loaded as a solution preferably in pure water or in water/DMSO (5:1) mixture. FT IR spectra were measured on Bruker Alpha machine using ATR technique. The purity of tested compounds was confirmed either by combustion analyses or by HPLC and was > 95%. Syntheses and characterization of compounds **1b-1t**, **2e,j-m,o-r** and **3e,g,j-m**[1] were reported earlier.

4-Methyl-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1a).
This compound was prepared as described.[2] Mp 179-181 °C (lit.[2] mp 175-176 °C). $[\alpha]_D$ -65.9 (*c* 0.390, DMSO). Spectroscopic data are as described.[2] Calcd for $C_{12}H_{15}O_4N_3$: C, 54.33; H, 5.70; N, 15.84. Found: C, 54.03; H, 5.49; N, 15.60.

Synthesis of compound 3g



3g

5-Chloro-4-(4-fluorophenyl)-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (3g). An argon purged mixture of 4,5-dichloro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine[3] (948 mg, 1.5 mmol), 4-

fluorophenylboronic acid (315 mg, 2.25 mmol), K_2CO_3 (414 mg, 3 mmol), $Pd(PPh_3)_4$ (87 mg, 75 μmol) in toluene (10 mL) was stirred at 100 °C for 4 h. After cooling the mixture was diluted with chloroform (60 mL), washed with saturated aqueous NH_4Cl (20 mL). Aqueous layer was re-extracted with chloroform (2 × 15 mL). Combined organics were dried over $MgSO_4$, co-evaporated with silica and column chromatography (SiO_2, hexane-EtOAc, 5:1) afforded benzoyl protected nucleoside as wellowish foam. This material was dissolved in THF (12 mL) and treated with sodium methoxide (1 M in MeOH, 4.5 mL, 4.5 mmol) at rt for 2 h. The mixture was co-evaporated with silica, chromatographed (SiO_2, 3% MeOH in chloroform) and crystallized from MeOH to afford product **3g** (362 mg, 64% in two steps) as off-white crystalline solid. Mp 175-176 °C. $[\alpha]_D$ -42.2 (*c* 0.249, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR (400 MHz, DMSO-d_6): 3.58 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.5, $J_{5'b,4'}$ = 3.8, H-5′b); 3.67 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 4.0, H-5′a); 3.96 (ddd, 1H, $J_{4',5'}$ = 3.8, 3.9, $J_{4',3'}$ = 3.2, H-4′); 4.14 (ddd, 1H, $J_{3',2'}$ = 5.0, $J_{3',OH}$ = 4.9, $J_{3',4'}$ = 3.2, H-3′); 4.43 (ddd, 1H, $J_{2',OH}$ = 6.3, $J_{2',1'}$ = 6.1, $J_{2',3'}$ = 5.0, H-2′); 5.12 (dd, 1H, $J_{OH,5'}$ = 5.5, 5.3, OH-5′); 5.23 (d, 1H, $J_{OH,3'}$ = 4.9, OH-3′); 5.46 (d, 1H, $J_{OH,2'}$ = 6.3, OH-2′); 6.32 (d, 1H, $J_{1',2'}$ = 6.1, H-1′); 7.39 (m, 2H, H-*m*-C_6H_4F); 7.82 (m, 2H, H-*o*-C_6H_4F); 8.17 (s, 1H, H-6); 8.94 (s, 1H, H-2). ^{13}C NMR (100.5 MHz, DMSO-d_6): 61.31 (CH_2-5′); 70.38 (CH-3′); 74.17 (CH-2′); 85.38 (CH-4′); 86.60 (CH-1′); 103.00 (C-5); 112.75 (C-4a); 114.81 (d, $J_{C,F}$ = 21.8, CH-*m*-C_6H_4F); 125.25 (CH-6); 132.37 (d, $J_{C,F}$ = 8.7, CH-*o*-C_6H_4F); 132.72 (d, $J_{C,F}$ = 3, C-*i*-C_6H_4F); 150.42 (C-7a); 151.40 (CH-2); 157.41 (C-4); 163.13 (d, $J_{C,F}$ = 247.2, C-*p*-C_6H_4F). ^{19}F NMR (470.3 MHz, DMSO-d_6): -111.96. MS (ESI) *m/z* 380 (M+H), 402 (M+Na). HRMS (ESI) for $C_{17}H_{16}N_3O_4ClF$ [M+H] calcd: 380.08079; found: 380.08080. Calcd for $C_{17}H_{15}O_4N_3ClF \cdot ½H_2O$: C, 52.52; H, 4.15; N, 10.81. Found: C, 52.57; H, 4.04; N, 10.77.

4-(Dibenzofuran-4-yl)-7-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (5u)

6-Chloro derivative **4** (60 mg, 0.18 mmol), dibenzofuran-4-boronic acid (59 mg, 0.28 mmol), K_2CO_3 (51 mg, 0.37 mmol) and $Pd(PPh_3)_4$ (10.6 mg, 9.2 μmol) were dissolved in anhydrous toluene (1.5 mL) to 85 °C and stirred overnight. Then, solvents were removed in vacuo. The residue was dissolved in EtOAc (20 mL) and extracted

with aqueous HCl (1 M, 10 mL) and water. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexane-EtOAc 5:1). 4-Dibenzofuryl derivative **5u** (59 mg, 70 %) was obtained as a yellowish solid. Mp 175-177 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (s, 3H, $(CH_3)_2C$)); 1.67 (s, 3H, $(CH_3)_2C$)); 3.86 (dd, 1H, $J_{5'a,5'b}$ = 12.5 Hz, $J_{5'a,4'}$ = 1.7 Hz H-5´a); 4.04 (dd, 1H, $J_{5'a,5'b}$ = 12.6, $J_{5'b,4'}$ = 1.7 Hz, H-5´b); 4.54 (q, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 1.8 Hz, H-4'); 5.19 (dd, 1H, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 1.6 Hz, H-3'); 5.39 (dd, 1H, $J_{2',1'}$ = 4.9 Hz, $J_{2',3'}$ = 6.0 Hz, H-2'); 5.96 (d, 1H, $J_{1',2'}$ = 4.9 Hz, H-1'); 6.73 (d, 1H, $J_{5,6}$ = 3.6 Hz, H-5); 7.37 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-6); 7.40 (dt, $J_{8,9}$ = $J_{8,7}$ = 7.5 Hz, $J_{8,6}$ = 1.0 Hz, H-8-dibenzofuryl); 7.49 (ddd, 1H, $J_{7,8}$ = 7.2 Hz, $J_{7,6}$ = 6.9 Hz, $J_{7,9}$ = 1.3 Hz, H-7-dibenzofuryl); 7.55 (t, 1H, $J_{2,1}$ = $J_{2,3}$ = 6.9 Hz, H-2-dibenzofuryl); 7.57 (bd, 1H, $J_{3,2}$ = 6.9 Hz, H-3-dibenzofuryl); 7.99-8.04 (m, 2H, H-6-dibenzofuryl, H-9-dibenzofuryl); 8.13 (dd, 1H, $J_{1,2}$ = 7.7, $J_{1,3}$ = 1.2 Hz, H-1-dibenzofuryl); 9.05 (s, 1H, H-2). MS (ESI) m/z (%): 480 (17) [M + Na], 458 (100) [M + H]. HRMS (ESI) calcd for $C_{26}H_{24}O_5N_3$ [M + H]: 458.1710; found 458.1710.

4-(Benzofuran-2-yl)-7-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (5v)

Protected nucleoside **5v** was prepared as described for compound **5u**. 6-Chloro derivative **4** (200 mg, 0.61 mmol) and benzofuran-2-boronic acid (149 mg, 0.92 mmol) were used. Purification by column chromatography (SiO_2, hexane-EtOAc 2:1). 2-Benzofuryl derivative **5v** (216 mg, 86 %) was obtained as a yellowish amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 1.39 (s, 3H, $(CH_3)_2C$)); 1.67 (s, 3H, $(CH_3)_2C$)); 3.84 (bd, 1H, $J_{5'a,5'b}$ = 12.6 Hz, H-5´a); 4.02 (dd, 1H, $J_{5'a,5'b}$ = 12.6, $J_{5'b,4'}$ = 1.6 Hz, H-5´b); 4.53 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 1.6 Hz, H-4'); 5.16 (dd, 1H, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 1.6 Hz, H-3'); 5.33 (dd, 1H, $J_{2',1'}$ = 5.1 Hz, $J_{2',3'}$ = 5.9 Hz, H-2'); 5.93 (d, 1H, $J_{1',2'}$ = 4.9 Hz, H-1'); 7.21 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5); 7.32 (dt, 1H, $J_{5,4}$ = $J_{5,6}$ = 7.6 Hz, $J_{5,7}$ = 0.8 Hz, H-5-benzofuryl); 7.39-7.45 (m, 2H, H-6, H-6-benzofuryl); 7.63 (bd, 1H, $J_{4,5}$ = 8.2 Hz, H-4-benzofuryl); 7.73 (bd, 1H, $J_{7,6}$ = 7.6 Hz, H-7-benzofuryl); 7.85 (bs, 1H, H-3-benzofuryl); 8.88 (s, 1H, H-2). MS (ESI) m/z (%): 430 (100) [M + Na], 408 (10) [M + H]. HRMS (ESI) calcd for $C_{22}H_{22}O_5N_3$ [M + H]: 408.1554; found 408.1554.

4-(Dibenzofuran-4-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1u)

Protected nucleoside **5u** (55 mg, 0.12 mmol) was dissolved in 90% aqueous TFA (2 mL) and stirred at rt for 2 h. Then the reaction mixture was co-evaporated repeatedly with MeOH. The residue was purified by HPFC (C-18 column, 0→100% MeOH in water). Nucleoside **1u** (38 mg, 76 %) was obtained as a pale yellow crystalline solid after crystallization (water). Mp 231-235 °C. $[\alpha]_D^{20}$ -47.6 (*c* 0.212, DMSO). ^{1}H NMR (500 MHz, DMSO-d$_6$): 3.59 (ddd, 1H, J_{gem} = 11.9 Hz, $J_{5'a,OH}$ = 5.6 Hz, $J_{5'a,4'}$ = 4.0 Hz, H-5′a); 3.67 (ddd, 1H, J_{gem} = 11.9 Hz, $J_{5'b,OH}$ = 5.3 Hz, $J_{5'b,4'}$ = 4.1 Hz, H-5′b); 3.98 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.0 Hz, $J_{4',3'}$ = 3.1 Hz, H-4'); 4.17 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 4.9 Hz, $J_{3',4'}$ = 3.1 Hz, H-3'); 4.52 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 6.3 Hz, $J_{2',3'}$ = 5.0 Hz, H-2′); 5.10 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.4 Hz, OH-5′); 5.23 (d, 1H, $J_{OH,3'}$ = 4.9 Hz, OH-3′); 5.44 (d, 1H, $J_{OH,2'}$ = 6.4 Hz, OH-2′); 6.34 (d, 1H, $J_{1',2'}$ = 6.3 Hz, H-1'); 6.54 (bd, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.46 (td, 1H, $J_{8,7}$ = $J_{8,9}$ = 7.5 Hz, $J_{8,6}$ = 1.0 Hz, H-8-$C_{12}H_7O$); 7.56 (ddd, 1H, $J_{7,6}$ = 8.3 Hz, $J_{7,8}$ = 7.3 Hz, $J_{7,9}$ = 1.4 Hz, H-7-$C_{12}H_7O$); 7.62 (t, 1H, $J_{2,1}$ = $J_{2,3}$ = 7.6 Hz, H-2-$C_{12}H_7O$); 7.72 (dt, 1H, $J_{6,7}$ = 8.2 Hz, $J_{6,8}$ = $J_{6,9}$ = 0.8 Hz, H-6-$C_{12}H_7O$); 7.97 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 7.98 (dd, 1H, $J_{3,2}$ = 7.6 Hz, $J_{3,1}$ = 1.3 Hz, H-3-$C_{12}H_7O$); 8.26 (ddd, 1H, $J_{9,8}$ = 7.7 Hz, $J_{9,7}$ = 1.4 Hz, $J_{9,6}$ = 0.7 Hz, H-9-$C_{12}H_7O$); 8.36 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{1,3}$ = 1.3 Hz, H-1-$C_{12}H_7O$); 9.00 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): 61.81 (CH_2-5′); 70.87 (CH-3'); 74.27 (CH-2'); 85.48 (CH-4'); 86.88 (CH-1'); 101.74 (CH-5); 112.02 (CH-6-$C_{12}H_7O$); 117.54 (C-4a); 121.54 (CH-9-$C_{12}H_7O$); 122.63 (C-4-$C_{12}H_7O$); 122.98 (CH-1-$C_{12}H_7O$); 123.44 (C-9a-$C_{12}H_7O$); 123.60 and 123.63 (CH-2,8-$C_{12}H_7O$); 124.94 (C-9b-$C_{12}H_7O$); 127.92 (CH-6); 128.18 (CH-7-$C_{12}H_7O$); 128.69 (CH-3-$C_{12}H_7O$); 151.34 (CH-2); 151.84 (C-7a); 152.94 (C-4a-$C_{12}H_7O$); 153.76 (C-4); 155.71 (C-5a-$C_{12}H_7O$). IR (ATR): 3551, 1570, 1519, 1450, 1409, 1361, 1232, 1182, 1116, 1066, 1058, 1042. MS (ESI) m/z (%): 418 (100) [M + H]. HRMS (ESI) calcd for $C_{23}H_{20}O_5N_3$ [M + H]: 418.1397; found 418.1397. Calcd for $C_{23}H_{19}O_5N_3 \cdot 0.75H_2O$: C, 64.11; H, 4.79; N, 9.75. Found: C, 64.01; H, 4.66; N, 9.55.

4-(Benzofuran-2-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1v)

Nucleoside **1v** was prepared as described for compound **1u**. Protected nucleoside **5v** (181 mg, 0.44 mmol) was used. Purification by column chromatography (SiO_2, 3%

MeOH in chloroform). Nucleoside **1v** (121 mg, 75 %) was obtained as a pale yellow cotton after crystallization (water-MeOH 1:1). Mp 223-224 °C. $[\alpha]_D$ -76.7 (*c* 0.249, DMSO). ^{1}H NMR (500 MHz, DMSO-d_6): 3.59 (ddd, 1H, J_{gem} = 11.9 Hz, $J_{5'a,OH}$ = 5.7 Hz, $J_{5'a,4'}$ = 4.0 Hz, H-5′a); 3.68 (ddd, 1H, J_{gem} = 11.9 Hz, $J_{5'b,OH}$ = 5.3 Hz, $J_{5'b,4'}$ = 4.0 Hz, H-5′b); 3.96 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 3.7 Hz, H-4'); 4.16 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 5.0 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'); 4.48 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 6.2 Hz, $J_{2',3'}$ = 5.1 Hz, H-2′); 5.10 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.5 Hz, OH-5′); 5.21 (d, 1H, $J_{OH,3'}$ = 4.9 Hz, OH-3′); 5.41 (d, 1H, $J_{OH,2'}$ = 6.3 Hz, OH-2′); 6.29 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1'); 7.29 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.36 (ddd, 1H, $J_{5,4}$ = 7.8 Hz, $J_{5,6}$ = 7.2 Hz, $J_{5,7}$ = 0.9 Hz, H-5-benzofuryl); 7.48 (ddd, 1H, $J_{6,7}$ = 8.3 Hz, $J_{6,5}$ = 7.2 Hz, $J_{6,4}$ = 1.3 Hz, H-6-benzofuryl); 7.80 – 7.84 (m, 2H, H-7,4-benzofuryl); 7.93 (d, 1H, $J_{3,7}$ = 1.0 Hz, H-3-benzofuryl); 8.04 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.89 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.73 (CH_2-5′); 70.75 (CH-3'); 74.26 (CH-2'); 85.43 (CH-4'); 86.92 (CH-1'); 101.63 (CH-5); 109.13 (CH-3-benzofuryl); 112.08 (CH-7-benzofuryl); 114.14 (C-4a); 122.59 (CH-4-benzofuryl); 123.97 (CH-5-benzofuryl); 126.66 (CH-6-benzofuryl); 127.94 (C-3a-benzofuryl); 128.91 (CH-6); 146.42 (C-4); 151.22 (CH-2); 152.54 (C-7a); 154.23 (C-2-benzofuryl); 155.47 (C-7a-benzofuryl). IR (ATR): 3168, 1596, 1566, 1461, 1358, 1345, 1104, 1057, 1027. MS (ESI) m/z (%): 390 (100) [M + Na], 368 (33) [M + H]. HRMS (ESI) calcd for $C_{19}H_{18}O_5N_3$ [M + H]: 368.1241; found 368.1240. Calcd for $C_{19}H_{17}O_5N_3 \cdot ½H_2O$: C, 60.63; H, 4.82; N, 11.16. Found: C, 60.69; H, 4.59; N, 10.95.

4-(Benzothiophen-2-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1w)

Nucleoside **6** (100 mg, 0.35 mmol), benzothiophene-2-boronic acid (78 mg, 0.44 mmol), Na_2CO_3 (111 mg, 1.1 mmol), $Pd(OAc)_2$ (4 mg, 18 µmol) and TPPTS (30 mg, 53 µmol) were dissolved in mixture of acetonitrile and water (2:1, 2 mL) and the mixture was heated to 100 °C and stirred for 7 h. Then solvents were removed in vacuo. The residue was purified by column chromatography (SiO_2, 1% MeOH in chloroform). Nucleoside **1w** (95 mg, 71 %) was obtained as a white crystalline solid after recrystallization (water-MeOH 1:1). Mp 208-210 °C. $[\alpha]_D$ -71.4 (*c* 0.266, DMSO). ^{1}H NMR (500 MHz, DMSO-d_6): 3.59 (ddd, 1H, J_{gem} = 11.9 Hz, $J_{5'a,OH}$ = 5.6 Hz, $J_{5'a,4'}$ = 4.0 Hz, H-5′a); 3.68 (ddd, 1H, J_{gem} = 11.9 Hz, $J_{5'b,OH}$ = 5.3 Hz, $J_{5'b,4'}$ = 4.0 Hz,

H-5´b); 3.96 (q, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 3.7 Hz, H-4'); 4.15 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 4.9 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'); 4.47 (btd, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 6.2 Hz, $J_{2',3'}$ = 5.1 Hz, H-2'); 5.11 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.5 Hz, OH-5´); 5.21 (d, 1H, $J_{OH,3'}$ = 4.9 Hz, OH-3´); 5.42 (d, 1H, $J_{OH,2'}$ =6.3 Hz, OH-2´); 6.29 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1'); 7.36 (d, 1H, $J_{5,6}$ = 3.9 Hz, H-5); 7.46 (m, 1H, H-5-benzothienyl); 7.47 (m, 1H, H-6-benzothienyl); 8.02 (m, 1H, H-4-benzothienyl); 8.05 (m, 1H, H-7-benzothienyl); 8.06 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.59 (bd, 1H, $J_{3,7}$ = 0.6 Hz, H-3-benzothienyl); 8.85 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.74 (CH_2-5´); 70.76 (CH-3'); 74.34 (CH-2'); 85.45 (CH-4'); 86.98 (CH-1'); 101.06 (CH-5); 114.02 (C-4a); 122.85 (CH-7-benzothienyl); 125.11 (CH-5-benzothienyl); 125.27 (CH-4-benzothienyl); 126.42 (CH-6-benzothienyl); 126.79 (CH-3-benzothienyl); 129.01 (CH-6); 140.33 and 140.63 (C-3a,7a-benzothienyl); 142.74 (C-2-benzothienyl); 150.19 (C-4); 150.96 (CH-2); 152.26 (C-7a). IR (ATR): 3215, 1560, 1446, 1422, 1338, 1247, 1232, 1189, 1100, 1081, 1021. MS (ESI) m/z (%): 406 (100) [M + Na], 384 (100) [M + H]. HRMS (ESI) calcd for $C_{19}H_{17}O_4N_3NaS$ [M + Na]: 406.08320; found 406.08317. Calcd for $C_{19}H_{17}O_4N_3S\cdot\frac{1}{2}H_2O$: C, 58.15; H, 4.62; N, 10.71. Found: C, 58.22; H, 4.68; N, 10.26.

4-(1*H*-Indol-2-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1x)

Nucleoside **6** (100 mg, 0.35 mmol), *N*-Boc-indole-2-boronic acid (114 mg, 0.44 mmol), Na_2CO_3 (111 mg, 1.1 mmol), $Pd(OAc)_2$ (4 mg, 18 µmol) and TPPTS (30 mg, 53 µmol) were dissolved in mixture of acetonitrile and water (2:1, 2 mL) and the mixture was heated to 100 °C and stirred for 2.5 h. Then solvents were removed in vacuo. The residue was partially purified by HPFC (C-18 column, 0→100% MeOH in water). The mixture of products was dissolved in 90% TFA (4 mL) and stirred for 2 h (TLC analysis). The reaction mixture was repeatedly co-evaporated with MeOH. The product was purified using column chromatography (SiO_2, 2% MeOH in chloroform). Nucleoside **1x** (109 mg, 85 %) was obtained as a beige crystalline solid after recrystallization (water-MeOH 2:1). Mp 224-227 °C. $[\alpha]_D^{20}$ -78.3 (*c* 0.198, DMSO). ^{1}H NMR (500 MHz, DMSO-d_6): 3.59 (bdm, 1H, J_{gem} = 11.8 Hz, H-5´a); 3.68 (bdm, 1H, J_{gem} = 11.8 Hz, H-5´b); 3.96 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.9 Hz, $J_{4',3'}$ = 3.4 Hz, H-4'); 4.16 (dd, 1H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'); 4.48 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.6 Hz, H-2'); 5.13 (bs, 1H, OH-5´); 5.20 (bs, 1H, OH-3´); 5.40 (bs, 1H, OH-2´); 6.28 (d, 1H,

$J_{1',2'}$ = 6.2 Hz, H-1'); 7.07 (ddd, 1H, $J_{5,4}$ = 8.0 Hz, $J_{5,6}$ = 7.0 Hz, $J_{5,7}$ = 1.0 Hz, H-5-indolyl); 7.21 (ddd, 1H, $J_{6,7}$ = 8.2 Hz, $J_{6,5}$ = 7.0 Hz, $J_{6,4}$ = 1.2 Hz, H-6-indolyl); 7.25 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.55 (dq, 1H, $J_{7,6}$ = 8.2 Hz, $J_{7,5}$ = $J_{7,4}$ = $J_{7,NH}$ = 0.9 Hz, H-7-indolyl); 7.57 (dd, 1H, $J_{3,NH}$ = 2.2 Hz, $J_{3,4}$ = 0.9 Hz, H-3-indolyl); 7.68 (dq, 1H, $J_{4,5}$ = 8.0 Hz, $J_{4,3}$ = $J_{4,6}$ = $J_{4,7}$ = 0.9 Hz, H-4-indolyl); 7.98 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.86 (s, 1H, H-2); 11.92 (bd, 1H, $J_{NH,3}$ = 2.1 Hz, NH-indolyl). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.80 (CH_2-5′); 70.84 (CH-3'); 74.29 (CH-2'); 85.43 (CH-4'); 86.93 (CH-1'); 101.13 (CH-5); 105.77 (CH-3-indolyl); 112.61 (CH-7-indolyl); 113.98 (C-4a); 120.00 (CH-5-indolyl); 121.42 (CH-4-indolyl); 123.79 (CH-6-indolyl); 128.21 (CH-6); 128.68 (C-3a-indolyl); 134.78 (C-2-indolyl); 137.35 (C-7a-indolyl); 148.84 (C-4); 150.88 (CH-2); 152.02 (C-7a). IR (ATR): 3308, 1580, 1561, 1455, 1421, 1340, 1231, 1124, 1097, 1050, 1015. MS (ESI) m/z (%): 389 (100) [M + Na]. HRMS (ESI) calcd for $C_{19}H_{18}O_4N_4Na$ [M + Na]: 389.12203; found 389.12197. Calcd for $C_{19}H_{18}O_4N_4 \cdot 0.75H_2O$: C, 60.07; H, 5.17; N, 14.75. Found: C, 60.45; H, 4.95; N, 14.36.

4-(Dibenzothiophene-4-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1y)

Nucleoside **1y** was prepared as described for compound **1w**. Nucleoside **6** (100 mg, 0.35 mmol) and dibenzothiophene-4-boronic acid (100 mg, 0.44 mmol) were used. Purification by HPFC (C-18 column, 0→100% MeOH in water). Nucleoside **1y** (111 mg, 73 %) was obtained as a yellowish crystalline solid after recrystallization (water-MeOH 2:1). Mp 141-145 °C. $[\alpha]_D^{20}$ -36.2 (*c* 0.279, DMSO). ^{1}H NMR (500 MHz, DMSO-d_6): 3.60 (dm, 1H, J_{gem} = 11.9 Hz, H-5′a); 3.69 (dm, 1H, J_{gem} = 11.9 Hz, H-5′b); 3.98 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 3.6 Hz, H-4'); 4.17 (m, 1H, H-3'); 4.50 (bq, 1H, $J_{2',1'}$ = $J_{2',3'}$ = $J_{2',OH}$ = 5.6 Hz, H-2'); 5.11 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.4 Hz, OH-5′); 5.23 (d, 1H, $J_{OH,3'}$ = 4.6 Hz, OH-3′); 5.44 (d, 1H, $J_{OH,2'}$ = 6.1 Hz, OH-2′); 6.36 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1'); 7.20 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.54 (m, 1H, H-8-dibenzothienyl); 7.55 (m, 1H, H-7-dibenzothienyl); 7.79 (t, 1H, $J_{2,1}$ = $J_{2,3}$ = 7.7 Hz, H-2-dibenzothienyl); 8.068 (m, 1H, H-6-dibenzothienyl); 8.072 (bd, 1H, $J_{6,5}$ = 3.9 Hz, H-6); 8.45 (m, 1H, H-9-dibenzothienyl); 8.47 (dd, 1H, $J_{3,2}$ = 7.6 Hz, $J_{3,1}$ = 1.0 Hz, H-3-dibenzothienyl); 8.60 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{1,3}$ = 1.0 Hz, H-1-dibenzothienyl); 9.08 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.73 (CH_2-5′); 70.78 (CH-3'); 74.37 (CH-2'); 85.44 (CH-4'); 86.97 (CH-1'); 101.40 (CH-5); 115.52 (C-4a); 122.10

(CH-9-dibenzothienyl); 122.74 (CH-6-dibenzothienyl); 123.99 (CH-1-dibenzothienyl); 124.76 (CH-8-dibenzothienyl); 125.35 (CH-2-dibenzothienyl); 127.45 (CH-7-dibenzothienyl); 128.30 (CH-3-dibenzothienyl); 128.69 (CH-6); 131.81 (C-4-dibenzothienyl); 134.35 (C-9a-dibenzothienyl); 137.03 (C-9b-dibenzothienyl); 137.96 (C-4a-dibenzothienyl); 141.31 (C-5a-dibenzothienyl); 150.07 (CH-2); 152.26 (C-7a); 154.90 (C-4). IR (ATR): 3235, 1559, 1513, 1141, 1227, 1080, 1044. MS (ESI) m/z (%): 456 (100) [M + Na], 434 (15) [M + H]. HRMS (ESI) calcd for $C_{23}H_{20}O_4N_3S$ [M + H]: 434.11690; found 434.11682. Calcd for $C_{23}H_{19}O_4N_3S \cdot 0.75H_2O$: C, 61.80; H, 4.62; N, 9.40. Found: C, 63.65; H, 4.44; N, 9.32.

4-(Phenoxanthiin-4-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (1z)

Nucleoside **1z** was prepared as described for compound **1w**. Nucleoside **6** (80 mg, 0.28 mmol) and phenoxanthiin-4-boronic acid (85 mg, 0.35 mmol) were used. Purification by HPFC (C-18 column, 0→100% MeOH in water). Nucleoside **1z** (89 mg, 71 %) was obtained as a yellow crystalline solid after recrystallization (water-MeOH 2:1). Mp 123-131 °C. $[\alpha]_D^{20}$ -44.8 (*c* 0.172, DMSO). ^{1}H NMR (500 MHz, DMSO-d_6): 3.58 (m, 1H, H-5′a); 3.67 (m, 1H, H-5′b); 3.96 (bq, 1H, $J_{4',5'a} = J_{4',5'b} = J_{4',3'}$ = 3.6 Hz, H-4'); 4.15 (bdd, 1H, $J_{3',2'}$ = 5.0 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'); 4.50 (bt, 1H, $J_{2',1'} = J_{2',3'}$ = 5.7 Hz, H-2'); 5.09 (bs, 1H, OH-5′); 5.24 (bs, 1H, OH-3′); 5.44 (bs, 1H, OH-2′); 6.30 (d, 1H, $J_{1',2'}$ = 6.3 Hz, H-1'); 6.49 (bd, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 6.69 (bdd, 1H, $J_{6,7}$ = 8.0 Hz, $J_{6,8}$ = 1.4 Hz, H-6-phenoxanthiinyl); 7.13 (btd, 1H, $J_{8,7} = J_{8,9}$ = 7.5 Hz, $J_{8,6}$ = 1.5 Hz, H-8-phenoxathiinyl); 7.18 (bddd, 1H, $J_{7,6}$ = 8.0 Hz, $J_{7,8}$ = 7.4 Hz, $J_{7,9}$ = 1.7 Hz, H-7-phenoxanthiinyl); 7.29 (t, 1H, $J_{2,1} = J_{2,3}$ = 7.7 Hz, H-2-phenoxanthiinyl); 7.32 (bdd, 1H, $J_{9,8}$ = 7.6 Hz, $J_{9,7}$ = 1.7 Hz, H-9-phenoxanthiinyl); 7.45 (dd, 1H, $J_{3,2}$ = 7.8 Hz, $J_{3,1}$ = 1.6 Hz, H-3-phenoxanthiinyl); 7.46 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{1,3}$ = 1.6 Hz, H-1-phenoxanthiinyl); 7.92 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.94 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.79 (CH_2-5′); 70.82 (CH-3'); 74.24 (CH-2'); 85.45 (CH-4'); 87.04 (CH-1'); 101.38 (CH-5); 118.13 (C-4a); 118.27 (CH-6-phenoxanthiinyl); 119.71 (C-9a-phenoxanthiinyl); 120.95 (C-4-phenoxanthiinyl); 125.17 (CH-2-phenoxanthiinyl); 125.54 (CH-8-phenoxanthiinyl); 127.16 (CH-9-phenoxanthiinyl); 127.73 (C-10a-phenoxanthiinyl); 127.93 (CH-6); 128.42 and 128.46 (CH-1,7-phenoxanthiinyl); 130.05 (CH-3-phenoxanthiinyl); 149.04 (C-4a-phenoxanthiinyl); 151.18 (CH-2); 151.33

and 151.37 (C-7a, C-5a-phenoxanthiinyl); 154.66 (C-4). IR (ATR): 3174, 1567, 1515, 1424, 1220, 1075, 1042. MS (ESI) m/z (%): 472 (100) [M + Na], 450 (20) [M + H]. HRMS (ESI) calcd for $C_{23}H_{20}O_5N_3S$ [M + H]: 450.11182; found 450.111680. Calcd for $C_{23}H_{19}O_5N_3S{\cdot}0.75H_2O$: C, 59.67; H, 4.46; N, 9.08. Found: C, 59.49; H, 4.40; N, 8.83.

4-Chloro-7-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (7)

Octadecyl dichlorophosphate (**10**) (1.07 g, 2.8 mmol) was dissolved in mixture of water and pyridine (1:5, 36 mL) and stirred at rt for 30 min. Then, the solvents were removed in vacuo. The crude octadecyl phosphate (**11**) was directly used in the next step without further purification. The crude octadecyl phosphate (**11**) was dissolved in pyridine (90 mL) and MtsCl (2.42 g, 11 mmol) was added, the reaction mixture was stirred for 30 min and then solution of ribonucleoside **4** (600 mg, 1.84 mmol) in pyridine (5 mL) was added. The reaction was stirred overnight at rt. Reaction was quenched with water (30 mL) and stirred for another 30 min, then solvents were removed in vacuo. The residue was dissolved in EtOAc and extracted with water. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The cude product was purified by column chromatography on silica ($CHCl_3$-MeOH-H_2O-NH_3 90:10:4:4) to obtain nucleoside octadecylphosphate **7** (698 mg, 58%) as a colourless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 0.87 (t, 3H, J_{vic} = 7.0 Hz, ($CH_3(CH_2)_{17}O$);); 1.17 - 1.32 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.34 (s, 3H, $(CH_3)_2C$); 1.48 (m, 2H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.59 (s, 3H, $(CH_3)_2C$); 3.72 (q, 2H, J_{vic} = $J_{H,P}$ = 6.7 Hz, ($CH_3(CH_2)_{16}CH_2O$); 3.96 – 4.02 (m, 2H, H-5´); 4.33 (bdd, 1H, $J_{4',5'}$ = 8.5, $J_{4',3'}$ = 4.4, H-4'); 5.03 (dd, 1H, $J_{3',2'}$ = 6.4 Hz, $J_{3',4'}$ = 3.7 Hz, H-3'); 5.16 (dd, 1H, $J_{2',1'}$ = 2.9, $J_{2',3'}$ = 6.4 Hz, H-2'); 6.31 (d, 1H, $J_{1',2'}$ = 2.8 Hz, H-1'); 6.62 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5); 7.51 (d, 1H, $J_{6,5}$ = 3.7 Hz, H-6); 8.64 (s, 1H, H-2). 642 (50) MS (ESI) m/z (%): 682 (10) [(^{37}Cl)M + Na], 680 (20) [(^{35}Cl)M + Na], 660 (50) [(^{37}Cl)M + H], 658 (100) [(^{35}Cl)M+H]. HRMS (ESI) calcd for $C_{32}H_{54}O_7N_3ClP$ [M + H]: 658.33824; found 658.33833.

4-Chloro-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (8)

Protected nucleoside octadecylphosphate **7** (650 mg, 0.99 mmol) was dissolved in 90% aqueous TFA (5 mL). The reaction was stirred for 45 min. The reaction mixture was repeatedly co-evaporated with MeOH. The residue was purified by column chromatography (SiO_2, $CHCl_3$-MeOH-H_2O-NH_3 80:20:4:4). 6-Chloroderivative **8** (573 mg, 94 %) was obtained as colourless amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): 0.86 (t, 3H, J_{vic} = 6.9 Hz, ($CH_3(CH_2)_{17}O$);); 1.14 - 1.26 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.48 (pent, 2H, J_{vic} = 7.1, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.79 (q, 2H, J_{vic} = $J_{H,P}$ = 6.6 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.01 – 4.04 (m, 2H, H-5´); 4.16 (bt, 1H, $J_{4',5'}$ = $J_{4',3'}$ = 3.2, H-4'); 4.34 (dd, 1H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 3.1 Hz, H-3'); 4.54 (bt, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.2 Hz, H-2'); 6.38 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1'); 6.68 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.99 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.54 (s, 1H, H-2). MS (ESI) m/z (%): 642 (50) [(^{37}Cl)M + Na], 640 (100) [(^{35}Cl)M + Na], 620 (15) [(^{37}Cl)M + H], 618 (32) [(^{35}Cl)M + H]. HRMS (ESI) calcd for $C_{29}H_{50}O_7N_3ClP$ [M+H]: 618.30694; found 618.30655.

4-Phenyl-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (9e)

6-Chloro derivative **8** (100 mg, 0.16 mmol), phenylboronic acid (30 mg, 0.24 mmol), K_2CO_3 (45 mg, 0.32 mmol) and $Pd(PPh_3)_4$ (9 mg, 8 μmol) were dissolved in mixture of DMF and water (8:1, 4 mL) and heated to 105 °C and stirred for 2 h. The reaction was quenched with 1 M HCl (1 mL) and solvents were removed in vacuo. The residue was purified by column chromatography (SiO_2, $CHCl_3$-MeOH-H_2O-NH_3 80:20:4:4). Octadecylphosphate **9e** (85 mg, 80 %) was obtained as a white lyophilizate (*t*BuOH-benzene 1:1). Mp 75°C (dec). ^{1}H NMR (500 MHz, CD_3OD): 0.90 (t, 3H, J_{vic} = 7.0 Hz, ($CH_3(CH_2)_{17}O$); 1.10 - 1.35 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.55 (bpent, 2H, J_{vic} = 7.2 Hz, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.83 (bq, 2H, J_{vic} = $J_{H,P}$ = 6.4 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.05 – 4.10 (m, 2H, H-5´); 4.22 (m, 1H, H-4'); 4.42 (dd, 1H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'); 4.62 (bt, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.6 Hz, H-2'); 6.50 (d, 1H, $J_{1',2'}$ = 6.0 Hz, H-1'); 6.94 (bd, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.53 – 7.62 (m, 3H, H-*m*,*p*-Ph); 8.16 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.06 (m, 2H, H-*o*-Ph); 8.83 (s, 1H, H-2). ^{13}C NMR

(125.7 MHz, CD_3OD): 14.45 ($\mathbf{C}H_3(CH_2)_{17}O$); 23.74, 26.84, 30.39, 30.47, 30.66, 30.68, 30.71, 30.72, 30.745, 30.753, 30.76, 30.77 and 30.79 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 31.78 (d, $J_{C,P}$ = 7.2 Hz, $CH_3(CH_2)_{15}\mathbf{C}H_2CH_2O$); 33.07 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 66.21 (d, $J_{C,P}$ = 4.7 Hz, CH_2-5′); 66.76 (d, $J_{C,P}$ = 5.5 Hz, ($CH_3(CH_2)_{16}\mathbf{C}H_2O$); 72.41 (CH-3'); 76.14 (CH-2'); 85.25 (d, $J_{C,P}$ = 8.8 Hz, CH-4'); 88.46 (CH-1'); 102.71 (CH-5); 117.80 (C-4a); 129.22 (CH-6); 129.96 and 129.97 (CH-*o*,*m*-Ph); 131.42 (CH-*p*-Ph); 138.78 (C-*i*-Ph); 152.00 (CH-2); 153.68 (C-7a); 158.74 (C-4). ^{31}P NMR (202.3 MHz, CD_3OD): 1.78. IR (ATR): 3061, 2921, 2851, 1560, 1515, 1459, 1201, 1045. MS (ESI) m/z (%): 658 (100) [M - H]. HRMS (ESI) calcd for $C_{35}H_{53}O_7N_3P$ [M - H]: 658.36266; found 658.36092.

4-(Furan-2-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (9j)

6-Chloro derivative **8** (40 mg, 65 µmol), 2-(tributylstannyl)furan (35 mg, 98 µmol) and $PdCl_2(PPh_3)_2$ (2.3 mg, 3.3 µmol) were dissolved in anhydrous DMF (2 mL) and heated to 105 °C and stirred for 1 h. Then, solvents were removed in vacuo and the residue was purified by column chromatography (SiO_2, $CHCl_3$-MeOH-H_2O-NH_3 85:15:4:4). Octadecylphosphate **9j** (29 mg, 69 %) was obtained as a white lyophilizate (*t*BuOH-benzene 2:1). Mp 78-85°C. ^{1}H NMR (600 MHz, CD_3OD): 0.90 (t, 3H, J_{vic} = 7.1 Hz, ($CH_3(CH_2)_{17}O$);); 1.12 - 1.34 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.56 (m, 2H, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.85 (bq, 2H, J_{vic} = $J_{H,P}$ = 6.5 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.08 - 4.11 (m, 2H, H-5′); 4.21 (m, 1H, H-4'); 4.41 (bdd, 1H, $J_{3',2'}$ = 5.2 Hz, $J_{3,'4'}$ = 3.3 Hz, H-3'); 4.60 (dd, 1H, $J_{2',1'}$ = 6.0 Hz, $J_{2',3'}$ = 5.1 Hz, H-2'); 6.46 (d, 1H, $J_{1',2'}$ = 6.0 Hz, H-1'); 6.75 (dd, 1H, $J_{4,3}$ = 3.6 Hz, $J_{4,5}$ = 1.8 Hz, H-4-furyl); 7.19 (dd, 1H, $J_{5,6}$ = 3.8 Hz, $J_{5,LR}$ = 0.5 Hz, H-5); 7.53 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5}$ = 0.8 Hz, H-3-furyl); 7.92 (dd, 1H, $J_{5,4}$ = 1.8 Hz, $J_{5,3}$ = 0.8 Hz, H-5-furyl); 8.02 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.73 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, CD_3OD): 14.44 ($\mathbf{C}H_3(CH_2)_{17}O$); 23.74, 26.83, 30.39, 30.48, 30.66, 30.69, 30.73, 30.74, 30.76, 30.77, 30.78 and 30.79 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 31.76 (d, $J_{C,P}$ = 7.5 Hz, $CH_3(CH_2)_{15}\mathbf{C}H_2CH_2O$); 33.08 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 66.35 (d, $J_{C,P}$ = 5.5 Hz, CH_2-5′); 66.95 (d, $J_{C,P}$ = 5.9 Hz, ($CH_3(CH_2)_{16}\mathbf{C}H_2O$); 72.42 (CH-3'); 76.14 (CH-2'); 85.29 (d, $J_{C,P}$ = 8.8 Hz, CH-4'); 88.44 (CH-1'); 103.62 (CH-5); 113.76 (CH-4-furyl); 114.67 (C-4a); 115.37

(CH-3-furyl); 129.62 (CH-6); 147.19 (C-4); 147.56 (CH-5-furyl); 151.03 (CH-2); 152.91 (C-2-furyl); 153.90 (C-7a). ^{31}P NMR (202.3 MHz, CD_3OD): 1.64. IR (ATR): 3147, 2921, 2851, 1564, 1455, 1203, 1049. MS (ESI) m/z (%): 672 (18) [M + Na], 650 (100) [M + H]. HRMS (ESI) calcd for $C_{33}H_{53}O_8N_3P$ [M+H]: 650.35648; found 650.35622.

4-(Furan-3-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (9k)

Octadecylphosphate **9k** was prepared as described for compound **9e**. 6-Chloro derivative **8** (50 mg, 81 μmol) and furan-3-boronic acid (14 mg, 0.12 mmol) were used. Octadecylphosphate **9k** (33 mg, 67 %) was obtained as a white lyophilizate (*t*BuOH-benzene 2:1). Mp 87-90 °C. ^{1}H NMR (500 MHz, CD_3OD): 0.90 (t, 3H, J_{vic} = 7.0 Hz, ($CH_3(CH_2)_{17}O$); 1.13 - 1.36 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.57 (pent, 2H, J_{vic} = 7.1 Hz, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.85 (bq, 2H, $J_{vic} = J_{H,P}$ = 6.2 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.04 – 4.15 (m, 2H, H-5´); 4.22 (m, 1H, H-4'); 4.41 (m, 1H, H-3'); 4.60 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.5 Hz, H-2'); 6.46 (d, 1H, $J_{1',2'}$ = 5.9 Hz, H-1'); 7.01 (d, 1H, $J_{5,6}$ = 3.8 Hz, H-5); 7.22 (dd, 1H, $J_{4,5}$ = 2.0 Hz, $J_{4,2}$ = 0.9 Hz, H-4-furyl); 7.74 (t, 1H, $J_{5,4} = J_{5,2}$ = 1.7 Hz, H-5-furyl); 8.03 (d, 1H, $J_{6,5}$ = 3.9 Hz, H-6); 8.51 (bdd, 1H, $J_{2,5}$ = 1.4 Hz, $J_{2,4}$ = 1.0 Hz, H-2-furyl); 8.76 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.45 (**C**$H_3(CH_2)_{17}O$); 23.74, 26.85, 30.41, 30.48, 30.68, 30.71, 30.74, 30.76, 30.77, 30.78 and 30.79 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 31.78 (d, $J_{C,P}$ = 6.9 Hz, $CH_3(CH_2)_{15}\mathbf{C}H_2CH_2O$); 33.08 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 66.28 ($CH_2$-5´); 66.90 (($CH_3(CH_2)_{16}\mathbf{C}H_2O$); 72.38 (CH-3'); 76.19 (CH-2'); 85.31 (d, $J_{C,P}$ = 8.0 Hz, CH-4'); 88.54 (CH-1'); 102.76 (CH-5); 110.25 (CH-4-furyl); 116.54 (C-4a); 125.08 (C-3-furyl); 129.52 (CH-6); 145.82 (CH-5-furyl); 146.44 (CH-2-furyl); 150.89 (CH-2); 151.03 (C-4); 153.31 (C-7a). ^{31}P NMR (202.3 MHz, CD_3OD): 1.65. IR (ATR): 3141, 2921, 2851, 1595, 1567, 1454, 1183, 1047. MS (ESI) m/z (%): 672 (70) [M + Na], 650 (100) [M + H]. HRMS (ESI) calcd for $C_{33}H_{53}O_8N_3P$ [M + H]: 650.35618; found 650.35643.

7-(β-D-Ribofuranosyl)-4-(thiophen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (9l)

Octadecylphosphate **9l** was prepared as described for compound **9j**. 6-Chloro derivative **8** (50 mg, 81 μmol) and 2-(tributylstannyl)thiophene (45 mg, 0.12 mmol) were used. Octadecylphosphate **9l** (33 mg, 62 %) was obtained as a white lyophilizate (*t*BuOH-benzene 2:1). Mp 119-123 °C. ^{1}H NMR (500 MHz, CD_3OD): 0.90 (t, 3H, J_{vic} = 7.0 Hz, ($CH_3(CH_2)_{17}O$); 1.10 - 1.39 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.55 (pent, 2H, J_{vic} = 7.1 Hz, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.83 (bq, 2H, J_{vic} = $J_{H,P}$ = 6.5 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.06 – 4.10 (m, 2H, H-5´); 4.21 (m, 1H, H-4'); 4.41 (bdd, 1H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'); 4.60 (bt, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.5 Hz, H-2'); 6.46 (d, 1H, $J_{1',2'}$ = 5.9 Hz, H-1'); 7.10 (bd, 1H, $J_{5,6}$ = 3.9 Hz, H-5); 7.28 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.8 Hz, H-4-thienyl); 7.72 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.1 Hz, H-5-thienyl); 8.01 (d, 1H, $J_{6,5}$ = 3.9 Hz, H-6); 8.09 (dd, 1H, $J_{3,4}$ = 3.8 Hz, $J_{3,5}$ = 1.1 Hz, H-3-thienyl); 8.70 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.45 ($\mathbf{C}H_3(CH_2)_{17}O$); 23.74, 26.84, 30.41, 30.48, 30.68, 30.71, 30.74, 30.76, 30.77, 30.78 and 30.79 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 31.77 (d, $J_{C,P}$ = 7.5 Hz, $CH_3(CH_2)_{15}\mathbf{C}H_2CH_2O$); 33.08 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 66.24 (bd, $J_{C,P}$ = 4.7 Hz, CH_2-5´); 66.85 (bd, $J_{C,P}$ = 5.5 Hz, ($CH_3(CH_2)_{16}\mathbf{C}H_2O$); 72.39 (CH-3'); 76.13 (CH-2'); 85.23 (d, $J_{C,P}$ = 8.6 Hz, CH-4'); 88.44 (CH-1'); 102.55 (CH-5); 115.34 (C-4a); 129.21 (CH-6); 129.61 (CH-4-thienyl); 130.64 (CH-3-thienyl); 131.13 (CH-5-thienyl); 143.13 (C-2-thienyl); 151.68 (CH-2); 152.11 (C-4); 153.83 (C-7a). ^{31}P NMR (202.3 MHz, CD_3OD): 1.71. IR (ATR): 3182, 2920, 2851, 1562, 1447, 1199, 1044. MS (ESI) m/z (%): 664 (100) [M - H]. HRMS (ESI) calcd for $C_{33}H_{51}O_7N_3PS$ [M - H]: 664.31908; found 664.31920.

7-(β-D-Ribofuranosyl)-4-(thiophen-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (9m)

Octadecylphosphate **9m** was prepared as described for compound **9e**. 6-Chloro derivative **8** (100 mg, 0.16 mmol) and thiophene-3-boronic acid (31 mg, 0.24 mmol) were used. Octadecylphosphate **9m** (91 mg, 85 %) was obtained as a white lyophilizate (*t*BuOH-benzene 1:1). Mp 101-104 °C. ^{1}H NMR (500 MHz, CD_3OD): 0.89 (t, 3H, J_{vic} = 7.0 Hz, ($CH_3(CH_2)_{17}O$); 1.10 - 1.35 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.55 (pent, 2H, J_{vic} = 7.1 Hz, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.82 (q, 2H, J_{vic} = $J_{H,P}$ = 6.5 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.06 – 4.09 (m, 2H, H-5´); 4.21 (bpent, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = $J_{H,P}$ = 2.7 Hz, H-4'); 4.42 (dd, 1H, $J_{3',2'}$ = 5.2 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'); 4.61 (bt, 1H,

$J_{2',1'}$ = $J_{2',3'}$ = 5.6 Hz, H-2'); 6.47 (d, 1H, $J_{1',2'}$ = 6.0 Hz, H-1'); 7.05 (bd, 1H, $J_{5,6}$ = 3.9 Hz, H-5); 7.62 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,2}$ = 3.0 Hz, H-5-thienyl); 7.89 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,2}$ = 1.3 Hz, H-4-thienyl); 8.01 (d, 1H, $J_{6,5}$ = 3.9 Hz, H-6); 8.32 (dd, 1H, $J_{2,5}$ = 3.0 Hz, $J_{2,4}$ = 1.3 Hz, H-2-thienyl); 8.76 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.44 (**C**$H_3(CH_2)_{17}O$); 23.72, 26.83, 30.38, 30.46, 30.65, 30.68, 30.71, 30.73, 30.74, 30.745, 30.753 and 30.77 (CH_3(**C**$H_2)_{15}CH_2CH_2O$); 31.76 (d, $J_{C,P}$ = 7.5 Hz, $CH_3(CH_2)_{15}$**C**H_2CH_2O); 33.06 (CH_3(**C**$H_2)_{15}CH_2CH_2O$); 66.18 (d, $J_{C,P}$ = 5.2 Hz, CH_2-5´); 66.76 (d, $J_{C,P}$ = 6.0 Hz, ($CH_3(CH_2)_{16}$**C**H_2O); 72.40 (CH-3'); 76.11 (CH-2'); 85.23 (d, $J_{C,P}$ = 8.6 Hz, CH-4'); 88.37 (CH-1'); 102.71 (CH-5); 116.92 (C-4a); 127.69 (CH-5-thienyl); 128.45 (CH-4-thienyl); 129.05 (CH-6); 129.18 (CH-2-thienyl); 140.89 (C-3-thienyl); 151.83 (CH-2); 153.66 and 153.77 (C-4, 7a). ^{31}P NMR (202.3 MHz, CD_3OD): 1.76. IR (ATR): 3146, 2920, 2851, 1564, 1454, 1197, 1052. MS (ESI) m/z (%): 664 (100) [M - H]. HRMS (ESI) calcd for $C_{33}H_{51}O_7N_3PS$ [M - H]: 664.31908; found 664.31768.

4-(Benzofuran-2-yl)-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5'-*O*-octadecylphosphate (9v)

Octadecylphosphate **9v** was prepared as described for compound **9e**. 6-Chloro derivative **8** (100 mg, 0.16 mmol) and benzofuran-2-boronic acid (39 mg, 0.24 mmol) were used. Octadecylphosphate **9v** (95 mg, 84 %) was obtained as a white lyophilizate (*t*BuOH-benzene 1:1). ^{1}H NMR (500 MHz, CD_3OD): 0.89 (t, 3H, J_{vic} = 7.0 Hz, ($CH_3(CH_2)_{17}O$); 1.05 - 1.35 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2O$); 1.55 (m, 2H, $CH_3(CH_2)_{15}CH_2CH_2O$); 3.84 (bq, 2H, J_{vic} = $J_{H,P}$ = 6.5 Hz, ($CH_3(CH_2)_{16}CH_2O$); 4.07 - 4.10 (m, 2H, H-5´); 4.22 (bpent, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = $J_{H,P}$ = 2.8 Hz, H-4'); 4.43 (bdd, 1H, $J_{3',2'}$ = 5.2 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'); 4.64 (dd, 1H, $J_{2',1'}$ = 5.9 Hz, $J_{2',3'}$ = 5.3 Hz, H-2'); 6.49 (d, 1H, $J_{1',2'}$ = 6.0 Hz, H-1'); 7.329 (bdd, 1H, $J_{5,6}$ = 3.8 Hz, J_{LR} = 0.5 Hz, H-5); 7.333 (ddd, 1H, $J_{5,4}$ = 7.8 Hz, $J_{5,6}$ = 7.2 Hz, $J_{5,7}$ = 1.0 Hz, H-5-benzofuryl); 7.45 (ddd, 1H, $J_{6,7}$ = 8.4 Hz, $J_{6,5}$ = 7.2 Hz, $J_{6,4}$ = 1.3 Hz, H-6-benzofuryl); 7.69 (dq, 1H, $J_{7,6}$ = 8.3 Hz, $J_{7,5}$ = $J_{7,4}$ = $J_{7,3}$ = 0.9 Hz, H-7-benzofuryl); 7.66 (ddd, 1H, $J_{4,5}$ = 7.8 Hz, $J_{4,6}$ = 1.3 Hz, $J_{4,7}$ = 0.7 Hz, H-4-benzofuryl); 7.84 (d, 1H, $J_{3,7}$ = 1.0 Hz, H-3-benzofuryl); 8.07 (d, 1H, $J_{6,5}$ = 3.8 Hz, H-6); 8.80 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 14.45 (**C**$H_3(CH_2)_{17}O$); 23.74, 26.86, 30.41, 30.47,

30.65, 30.69, 30.70, 30.71, 30.72, 30.74, 30.749, 30.753 and 30.78 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 31.79 (d, $J_{C,P}$ = 7.7 Hz, $CH_3(CH_2)_{15}\mathbf{C}H_2CH_2O$); 33.07 ($CH_3(\mathbf{C}H_2)_{15}CH_2CH_2O$); 66.22 (d, $J_{C,P}$ = 5.2 Hz, CH_2-5´); 66.79 (d, $J_{C,P}$ = 6.0 Hz, $(CH_3(CH_2)_{16}\mathbf{C}H_2O$); 72.43 (CH-3'); 76.09 (CH-2'); 85.30 (d, $J_{C,P}$ = 8.9 Hz, CH-4'); 88.39 (CH-1'); 103.55 (CH-5); 110.31 (CH-3-benzofuryl); 112.67 (CH-7-benzofuryl); 116.06 (C-4a); 123.40 (CH-4-benzofuryl); 124.84 (CH-5-benzofuryl); 127.68 (CH-6-benzofuryl); 129.45 (C-3a-benzofuryl); 129.75 (CH-6); 148.04 (C-4); 151.89 (CH-2); 154.27(C-7a); 155.28 (C-2-benzofuryl); 157.34 (C-7a-benzofuryl). ^{31}P NMR (202.3 MHz, CD_3OD): 1.80. IR (ATR): 3293, 2919, 2850, 1603, 1569, 1463, 1235, 1182, 1128, 1069. MS (ESI) m/z (%): 722 (45) [M + Na], 700 (100) [M + H]. HRMS (ESI) calcd for $C_{37}H_{55}O_8N_3P$ [M + H]: 700.37213; found 700.37176.

Materials and methods for biological profiling

Cloning, expression and purification of human and MTB ADK was reported previously.[4]

Inhibition of adenosine kinases:

For determination of inhibition of MTB ADO the standard reaction mixture (50 μl) contained 50 mM Tris-HCl pH 8.0, 10 mM KCl, 10 mM $MgCl_2$, 5 mM ATP, 80 μg BSA, 1 μCi of [^{3}H]-adenosine (20 Ci/mmol), 1 μM unlabeled adenosine, various concentration of tested compounds and 1.15 ng of adenosine kinase. For inhibition of human adenosine kinase the standard reaction mixture (50 μl) contained 50 mM HEPES pH 6.2, 10 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 80 μg BSA, 1 μCi of [^{3}H]-adenosine (20 Ci/mmol), 1 μM unlabeled adenosine, various concentration of tested compounds and 0,94 ng of adenosine kinase.

The mixtures were incubated at 37ºC for 20 min and were separated on PEI cellulose plate (prespotted with 0.01 AMP). The plates were developed in the solvent system 2-propanol_NH4OH_water (7:1:2). The spots were visualized under UV light (254 nm) and cut out for radioactivity determination in the toluene-based scintillation cocktail.

Substrate activity enzyme assay:

The standard reaction mixture (100 μl) 50 mM HEPES pH 6.2, 10 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, 50 μM 7-deazaPu-nsd, BSA (200 μg/ml) and 150 ng of ADK. The mixtures were incubated at 37ºC for 20 min and following precipitation with cold 70% methanol, the superntants were evaporated and pellets were solubilized in 100 μl of water and components were separated using HPLC on Supelcosil LC-18-T column by a gradient of acetonitrile .

Cytotoxic MTT assay.[5] Human fibroblast cell lines BJ and MRC-5 were obtained from ATTC (American Tissue Culture Collection) and used as a prototype on non-malignant cells. Cell suspensions were prepared and diluted to expected target cell density (30 000 cells/well). Cells were added by pipette (80 μL) into 96-well microtiter plates.

Inoculates were allowed a pre-incubation period of 24 h at 37 °C and 5 % CO_2 for stabilisation. Four-fold dilutions, in 20-μL aliquots, of the intended test concentration were added to the microtiter plate wells at time zero. All test compound concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5 % CO_2 atmosphere at 100 % humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period the formazan produced was dissolved by the addition of 100 μL/well of 10 % aq SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density was measured at 540 nm on microarray reader. The 50% cytotoxic concentration (CC_{50}), was calculated from appropriate dose-response curves.

Anti-mycobacterial activity. *Mycobacterium bovis BCG* (strain isolated from commercially available live vaccine SSI was obtained from Statens Serum Institute) was used as a prototype mycobacterium to assess activity of the compounds on living and proliferating cells. Briefly, the mycobacterial cell suspensions were prepared and diluted in culture medium (Difco Middlebrook 7H9 Broth supplemented with Middlebrook ADC Growth Supplement FD019 and 0,04% of Tween 80) to approximate target cell density (100 000 microbes/well). Cells were added by pipette (80 μL) into 96-well microtiter plates. Four-fold dilutions, in 20-μL aliquots, of the intended test concentration were added to the microtiter plate wells at time zero. All test compound concentrations were examined in duplicate. Incubation of the cells with the test compounds lasted for 120 hrs. at 37 °C, 5% CO_2 and 100 % humidity. At the end of the incubation period, the living cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1–4 h. After this incubation period the formazan produced was dissolved by the addition of 100 μL/well of 10 % aq SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density was measured at 540 nm on microarray reader. The 50% growth inhibitory concentration (IC_{50}), was calculated from appropriate dose-response curves.

References

1 P. Nauš, R. Pohl, I. Votruba, P. Džubák, M. Hajdúch, R. Ameral, G. Birkus, T. Wang, A. S. Ray, R. Mackman, T. Cihlar, M. Hocek, *J. Med. Chem.*, 2010, **53**, 460–470.

2 R. Wu, E. D. Smidansky, H. S. Oh, R. Takhampunya, R. Padmanabhan, C. E. Cameron, B. R. Peterson, *J. Med. Chem.*, 2010, **53**, 7958-7966.

3 F. Seela, X. Ming, *Tetrahedron*, 2007, **63**, 9850-9861.

4 P. Spáčilová, P. Nauš, R. Pohl, I. Votruba, J. Snášel, H. Zábranská, I. Pichová, R. Ameral, G. Birkuš, T. Cihlář, M. Hocek, *ChemMedChem*, 2010, **5**, 1386–1396.

5 M. Hajdúch, V. Mihál, J. Minařík, E. Faber, M. Šafářová, E. Weigl, P. Antálek, *Cytotechnology*, 1996, **19**, 243-245.