Supplementary Data.

The isoprenoid derivative N6-benzyladenosine (CM223) exerts antitumor effect in glioma patient-derived primary cells through the mevalonate pathway.

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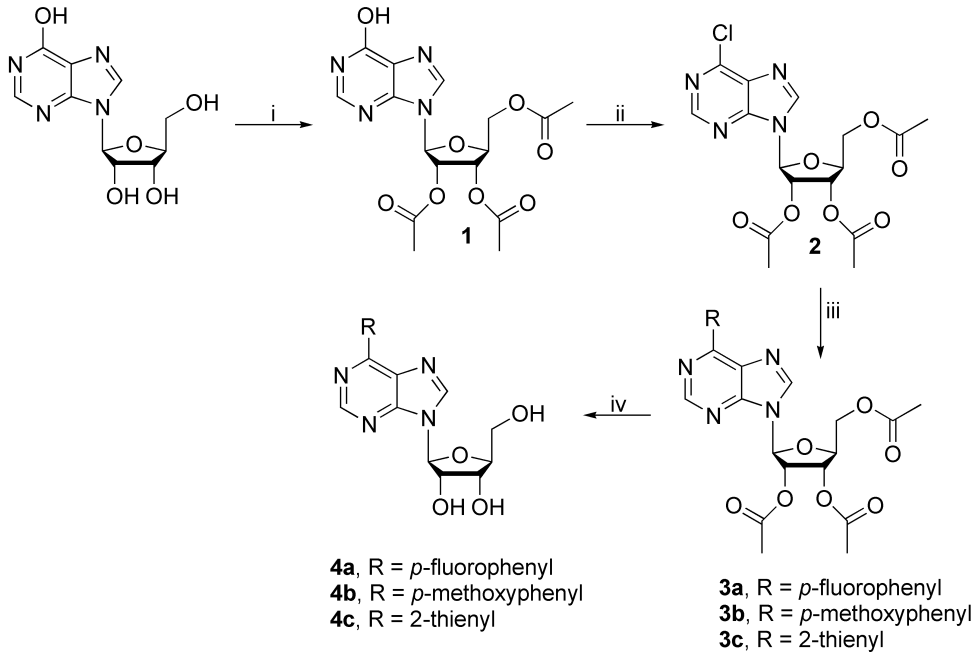
Chemistry

Compounds **FP11**, **FP13** and **FP16** were obtained by the synthetic route described in the **Scheme 1**. The reaction between inosine and acetic anhydride in pyridine at 0°C for 16 h led to the peracetylated derivative **1** which was converted into the corresponding 6-chloroderivative **2** by treatment with N,N,N,-triethylamine and $POCl_3$ at room temperature for 7 min and then at reflux for 12 min. The cross-coupling reaction Suzuki-Miyaura, between the 6-chloroderivative **2** and 1.5 eq of the suitable boronic acid in toluene with $Pd(PPh_3)_4$ as catalysist (5%) and 1.5 eq of K_2CO_3 as base was performed using microwave reactor at 140°C for 17 min to obtain the purine derivatives **FP11**, **FP13** and **FP16**. The acetyl functions of compounds **FP11**, **FP13** and **FP16** were cleaved by methanolysis with MeONa 1.0 M to afford **FP11**, **FP13** and **FP16**.

Scheme 1. Synthesis of 6-substituted purine derivatives **FP11**, **FP13** and **FP16.**



Reagents and conditions: (i) Ac_2O, Piridine, r.t., 16h; (ii) $POCl_3$, N,N,N- Triethylamine, r.t., 7 min, reflux,12 min; (iii) $Pd(PPh_3)_4$, toluene, K_2CO_3, suitable boronic acid, microwave 140°C, 17 min, (power 200 W, pressure 100 psi, stirring on), (iv) MeONa 1 M, MeOH.

Experimental section

General Procedures. Organic solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). and dried in the presence of appropriate drying agents and were stored over suitable molecular sieves. Melting points were determined on a Kofler® hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance-500® spectrometer in δ units with TMS as an internal standard. Mass spectra were performed with a Hewlett-Packard MS system 5988®. TLC was performed on silica gel sheets (Silica Gel 60 F254, Merck, Germany). Microwave-assisted reactions were run in a CEM microwave synthesizer. The system for isocratic flash chromatography includes a Buchi® Pump Module C-601 (continuous flow of solvents up to 250 ml/min at max 10 bar) and Buchi® prepacked cartridges (silica gel 60, particle size 40-63 μm). The purity was determined by high performance liquid chromatography (HPLC) Shimadzu system using analytic C18 Phenomenex column. Purity of all final compounds was 99%.

2',3',5'-Tri-O-acetyl-inosine (1). Acetic anhydride (9.0 mL, 94.5 mmol) was added to a suspension of inosine (3,00 g, 11.2 mmol) in dry pyridine (20 mL) at 0 °C and stirred for 16 h. The solvent was evaporated *in vacuo*. The residue was dissolved in 200 mL of methylene chloride, and the organic layer was washed with water (2 x 100 mL), saturated $NaHCO_3$ solution (1 x 100 mL), and brine (1 x 100 mL), and dried over anhydrous magnesium sulfate. The organic layer was concentrated *in vacuo* to obtain derivative **2** as a white solid, which was used for the next reaction without purification. Yield 79%; mp: 161- 164°C. ^{1}H-NMR ($CDCl_3$) 12.49 (br, 1H, CONH); 8.23 (s, 1H, H2); 8.08 (s, 1H, H8); 6.19 (d, 1H, H1'α); 5.88 (tr, 1H, H2'β); 5.60 (tr, 1H, H3'β); 4.50-4.30 (m, 3H, H4', H5'a, H5'b); 2.16 (s, 3H, CH_3); 2.15 (s, 3H, CH_3); 2.11 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 170.50; 169.72; 169.46; 158.74; 148.82; 145.78; 138.80; 125.26; 86.85; 80.68; 73.60; 70.76; 63.26; 21.09; 20.83; 20.70.

9-(2,3,5-Tri-*O*-acetyl-*β*-D-ribofuranosyl)-6-chloropurine (2). To a suspension of inosine derivative **1** (2.45 g, 6.22 mmol) in triethylamine (0.72 mL, 6.53 mmol) at 0 °C was added phosphorus oxychloride (12.2 mL, 131 mmol). The mixture was stirred at room temperature for 7 min and then reflux for 12 min in a preheated oil bath. The solution was concentrated and the obtained oil was stirred in $CHCl_3$ (20 mL) and ice (20 mL). The aqueous layer was extracted with $CHCl_3$ (3 x 25 mL). The combined organic layers were washed with HCl 2M (4 x 20 mL)) and brine (1 x 20 mL), dried over dry $MgSO_4$ and evaporated to obtain 3.30 g of brown yellow oil. Purification by flash chromatography (AcOEt/ n-Hexane 2:1) afforded the chloro-derivative **2** as yellow oil. Yield 82%; ^{1}H-NMR ($CDCl_3$) δ 8,75 (s, 1H, H2); 8.30 (s, 1H, H8); 6.22 (d, 1H, H1'α); 5.91 (tr, 1H, H2'β); 5.62 (tr, 1H, H3'β); 4.46-4.39 (m, 3H, H4', H5'a, H5'b); 2.13 (s, 3H, CH_3); 2.10 (s, 3H, CH_3); 2.06 (s, 3H, CH_3). ^{13}C-NMR: ($CDCl_3$) δ 170.38; 169.68; 169.48; 152.46; 151.47; 151.40; 143.79; 132.52; 87.11; 80.75; 73.34; 70.70; 63.13; 21.01; 20.79; 20.63.

General procedure for the synthesis of 6-substituted purine derivatives 3a-c.

To a solution of chloro-derivative **2** (500 mg, 1.21 mmol) in toluene (10 mL), $Pd(PPh_3)_4$ (70 mg, 0.061 mmol), K_2CO_3 (242 mg 1.75 mmol) and suitable boronic acid (1.82 mmol) were added. The mixture was heated by the microwave irradiation at 140 °C, stirring for 17 min, (power 200W, pressure 100 PSI). After cooling to room temperature the brown mixture was filtrated and evaporated *in vacuo* to obtain an oil residue, which was purified by flash chromatography.

9-(2,3,5-Tri-*O*-acetyl-*β*-D-ribofuranosyl)-6-(4-fluorophenyl)purine (3a). The crude mixture was purified by flash chromatography (*n*-Hexane/AcOEt 1:1). Yield: 97%; ^{1}H-NMR ($CDCl_3$) δ 9.00 (s, 1H, H2); 8.83 (m, 2H, Ar); 8.28 (s, 1H, H8); 7.25 (m, 2H, Ar); 6.30 (d, 1H, H1'α); 6.00 (tr, 1H, H2'β); 5.71 (tr, 1H, H3'β); 4.52-4.41 (m, 3H, H4', H5'a, H5'b); 2.14 (s, 3H, CH_3); 2.10 (s, 3H, CH3); 2.05 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ 170.48; 169.75; 169.55; 167.44; 162.42; 154.40; 152.82; 152.20; 142.71; 132.35; 132.75; 116.22; 115,79; 86.67; 80.62; 73.34; 70.88; 63.31; 21.08; 20.87; 20.72.

9-(2,3,5-Tri-*O*-acetyl-*β*-D-ribofuranosyl)-6-(4-methoxyphenyl)purine (3b). The crude mixture was purified by flash chromatography (*n*-Hexane/AcOEt 1:1). Yield 75%; ^{1}H-NMR ($CDCl_3$) δ 8.97 (s, 1H, H2); 8.78 (d, 2H, Ar); 8.25 (s, 1H, H8); 7.07 (d, 2H, Ar); 6.29 (d, 1H, H1'α); 6.00 (tr, 1H, H2'β); 5.71 (tr, 1H, H3'β); 4.48-4.40 (m, 3H, H4', H5'a, H5'b); 3.89 (s, 3H, OCH3) 2.13 (s, 3H, CH3); 2.08 (s, 3H, CH3); 2.03 (s, 3H, CH3). ^{13}C-NMR: CDCl3 δ 170.50; 169.77; 169.56; 162.35; 155.28; 152.84; 151.99; 142.17; 132.39; 131.79; 128.22, 114.33; 86.53; 80.61; 73.33; 70.92; 63.35; 55.88; 21.11; 20.87; 20.72.

9-(2,3,5-Tri-*O*-acetyl-*β*-D-ribofuranosyl)-6-(2-thienyl)purine (3c). Purified by flash chromatography (*n*-Hexane/AcOEt 1:1). Yield 37%. ^{1}H-NMR ($CDCl_3$) δ 8.88 (s, 1H, H2); 8.65 (d, 1H, Th); 8.26 (s, 1H, H8); 7.62 (d, 1H, Th); 7.27 (tr, 1H, Th); 6.27 (d, 1H, H1'α); 5.97 (tr, 1H, H2'β); 5.70 (tr, 1H, H3'β); 4.48-4.40 (m, 3H, H4', H5'a, H5'b); 2.14 (s, 3H, CH_3); 2. 08 (s, 3H, CH_3); 2.04 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ 170.50; 169.75; 169.54; 152.95; 151.75; 150.82; 142.68; 139.88; 133.17; 131.35; 129.64; 129.06; 86.54; 80.66; 73.36; 70.90; 63.33; 21.08; 20.87; 20.70.

General procedure for the synthesis of 6-substituted purine derivatives FP11, FP13 and FP16.

A solution of NaOMe 1 M (600 μL, 0.6 mmol) was added to a solution of 1.48 mmol of compound **FP11, FP13 and FP16**in MeOH (20 mL), and the mixture was stirred at room temperature overnight. The suspension was filtrated under *vacuo* and then the precipitate was crushed with diisopropyl ether and filtrated again to give **FP11, FP13** and **FP16**. Tested compounds were characterized by mass spectroscopy and liquid chromatography using HPLC Shimadzu system (using analytic C18 Phenomenex column, at a flow rate of 1 mL/min using a solvent system from 90% of H_2O + 0.1% TFA (A) to 90% of acetonitrile + 0.1% TFA (B) in 30 min.

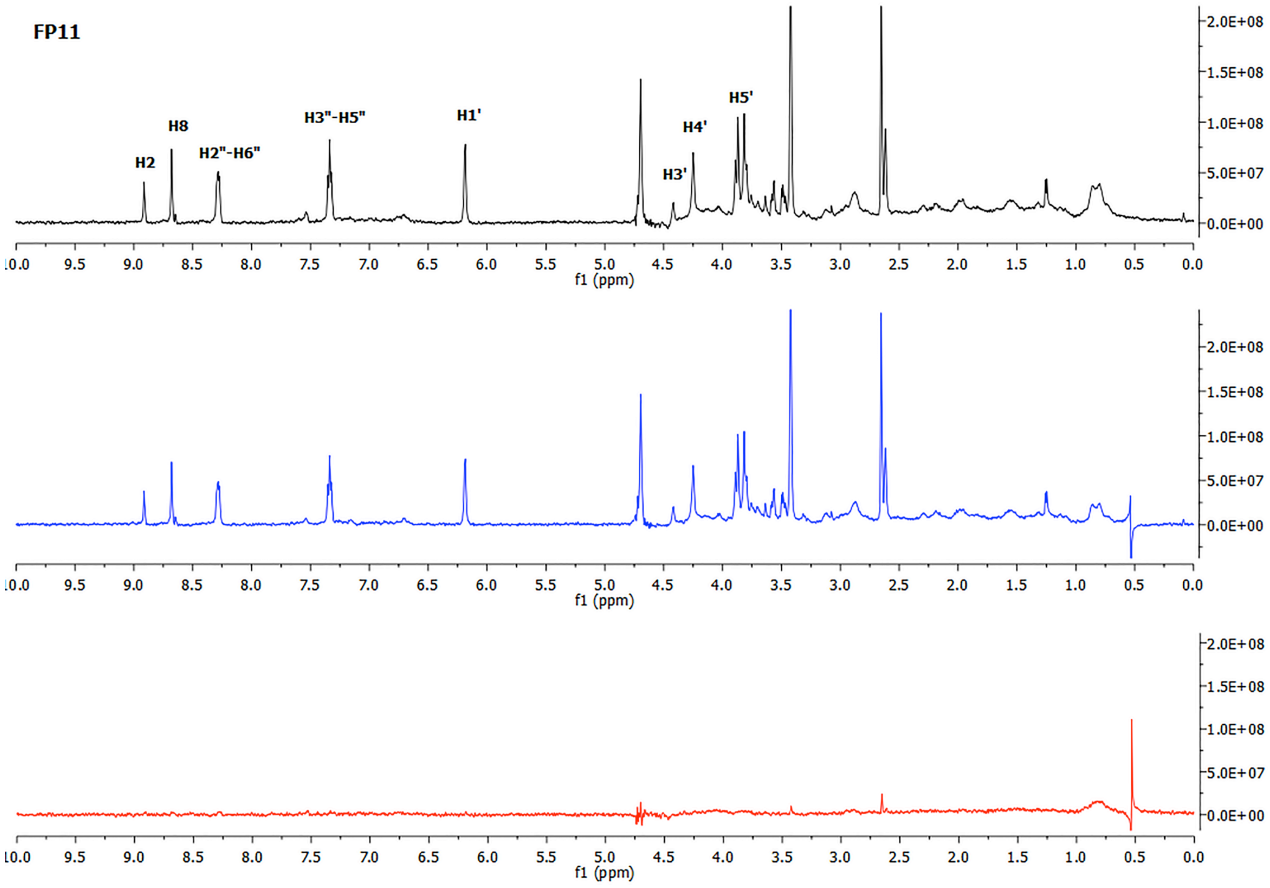
6-(4-Fluorophenyl)-9-(*β* -D-ribofuranosyl)purine (FP11). Yield: 75%; mp: 165-168 °C. ^{1}H-NMR (DMSO) δ 8,99 (s, 1H, H2); 8.92 (s, 1H, H8); 8.85 (m, 2H, Ar); 7.45 (m, 2H, Ar); 6.06 (d,

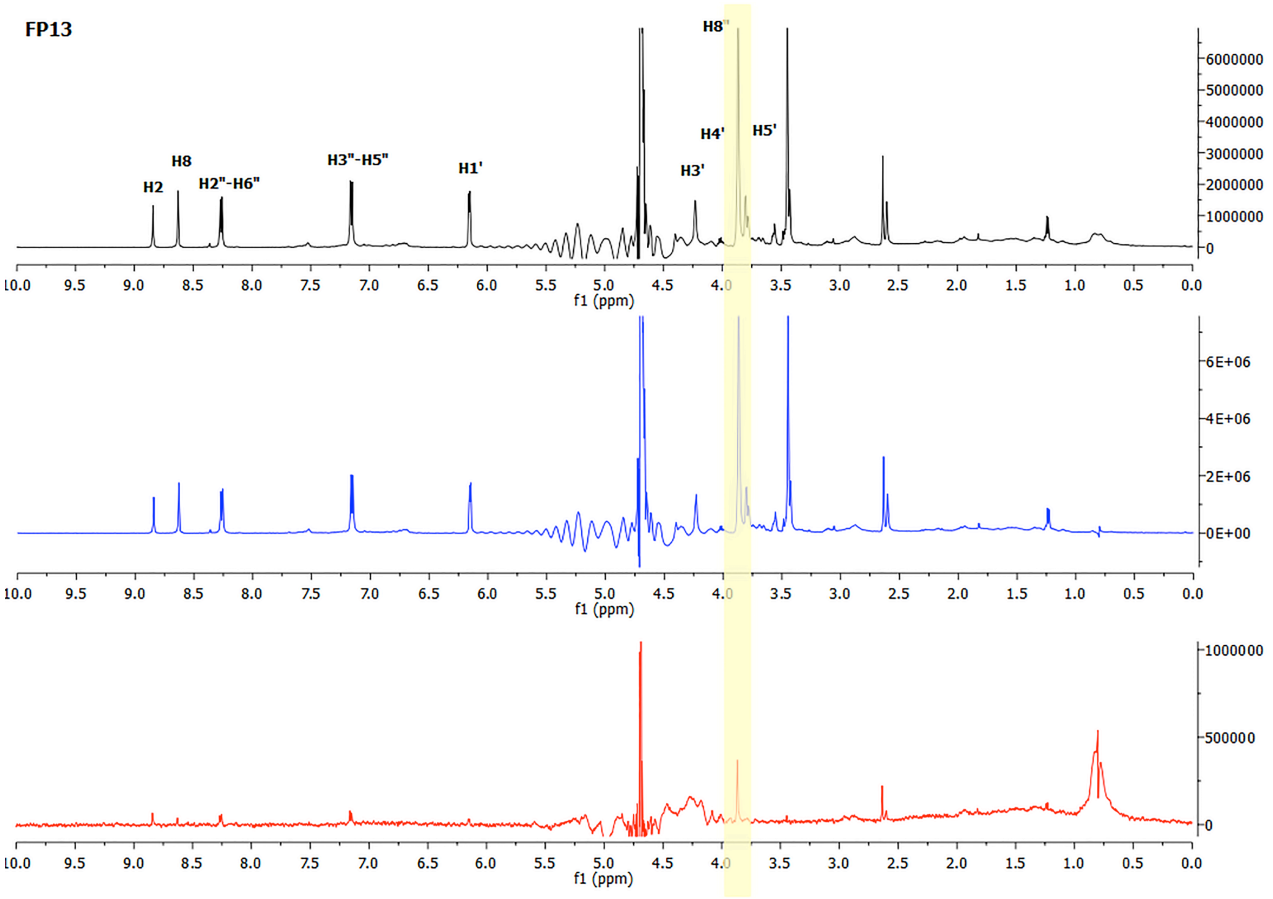
1H, H1'α); 5.00-5.60 (br, 2H, OH2'β; OH3'β); 4.62 (tr, 1H, OH5'β); 4.17 (br, 1H, H2'β); 3.97 (br, 1H, H3'β); 3.66-3.53 (m, 3H, H4', H5'a, H5'b). ^{13}C-NMR (DMSO) 151.51; 151.22; 144.32; 131.23; 131.06; 129.90; 115.35; 114.93; 87.04; 85.03; 73.13; 69.59; 60.56. MS *m/z* 347 (M^+). HPLC t_R= 10.10 min, purity 98.7%.

6-(4-Methoxyphenyl)-9-(*β*-D-ribofuranosyl)purine (FP13). Yield: 75%; mp 170-173 °C. ^{1}H-NMR (DMSO) δ 8,96 (s, 1H, H2); 8.85 (s, 1H, H8); 8.80 (d, 2H, Ar); 7.13 (d, 2H, Ar); 6.05 (d, 1H, H1'α); 5.00-5.60 (br, 3H, OH2'β; OH3'β; OH5'β); 4.62 (tr, 1H, H2'β); 4.19 (d, 1H, H3'β); 3.97 (d, 1H, H4'β); 3.85 (s, 3H, OCH3); 3.66-3.53 (m, 2H, H5'a, H5'b). ^{13}C-NMR: (DMSO) 161.05; 152.94; 151.23; 151.66 143.74; 130.50; 127.02; 113.53; 87.00; 85.03; 73.11; 69.66; 63.35; 54.79. MS *m/z* 359 (M^+). HPLC t_R= 9.19 min, purity 99.4%.

6-(2-Thienyl)-9-(*β*-D-ribofuranosyl)purine (FP16). Yield: 54%**;** mp 169-172 °C. ^{1}H-NMR (DMSO) δ 8,89 (s, 1H, H2); 8.85 (s, 1H, H8) 8,61 (m, 1H, Th); 7.91 (m, 1H, Th); 7.33 (tr, 1H, Th); 6.05 (d, 1H, H1'α); 4.62 (tr, 1H, H2'β); 4.19 (br, 1H, H3'β); 3.98 (br, 1H, H4'β); 3.70-3.20 (br, 5H, OH2', OH3', OH5', H5'a, H5'b). ^{13}C-NMR **(**DMSO) 151.33; 150.94; 148.3; 144.37; 138.87; 131.86; 131.19; 128.46; 127.91; 87.09; 85.05; 73.16; 69.63; 60.59. MS *m/z* 334 (M^+). HPLC t_R= 8.84 min, purity 95%.

Figure S1: STD-NMR spectra of FPPS/**FP11**, **FP13** and **FP16**, respectively, in black the off-resonance spectra, in blue the on-resonance spectra and in red the STD spectra.




FP13
H2
H8
H2"-H6"
H3"-H5"
H1'
H3'
H4'
H5'
H8"
f1 (ppm)

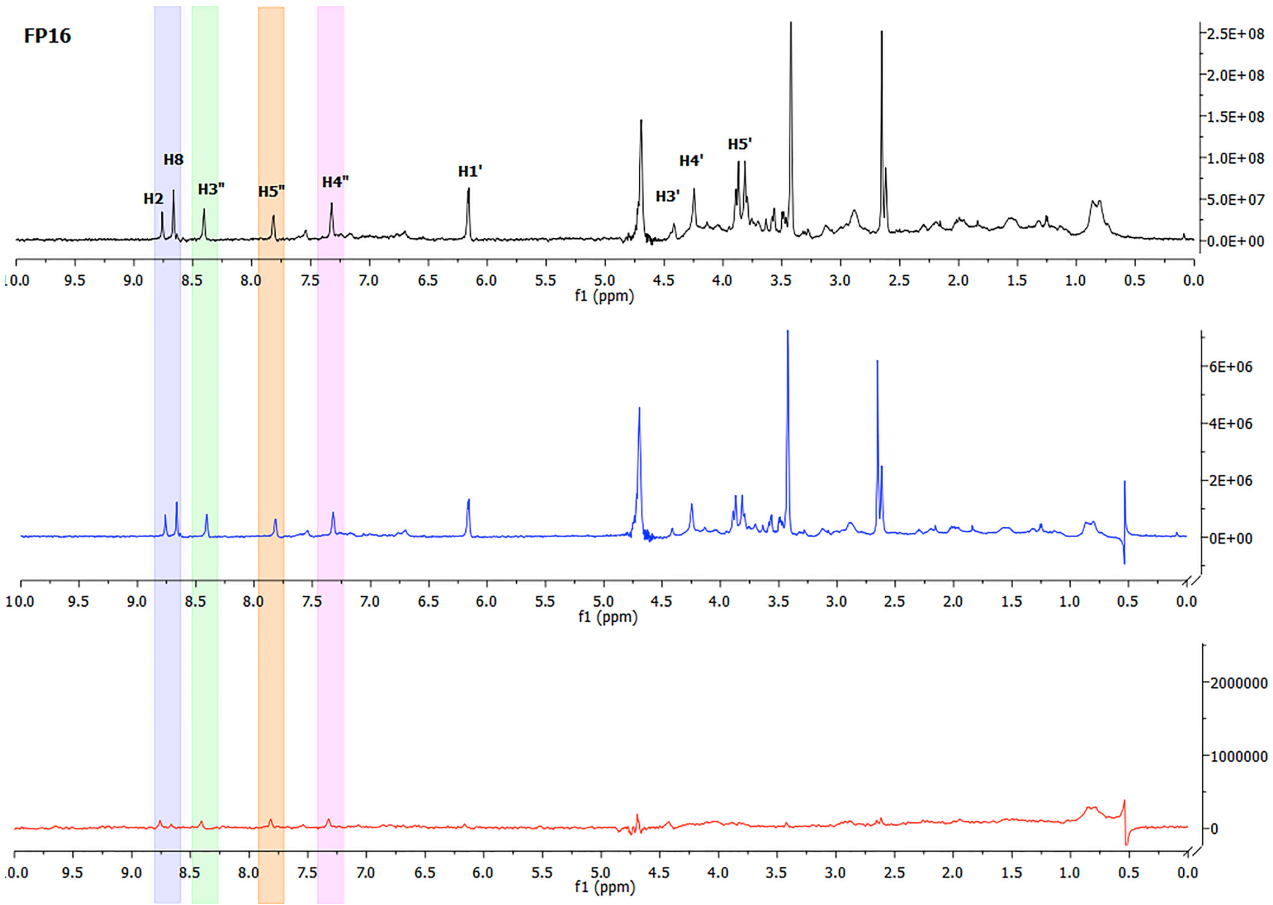

FP16
H2
H8
H3"
H5"
H4"
H1'
H3'
H4'
H5'
f1 (ppm)

Figure S2. ^{1}H NMR spectrum (600 MHz T= 298K) of **FP11** (5 mM) in 25 mM d-Tris (pH 7.4), 0.5 mM $MgCl_2$ and 25 mM NaCl**.**

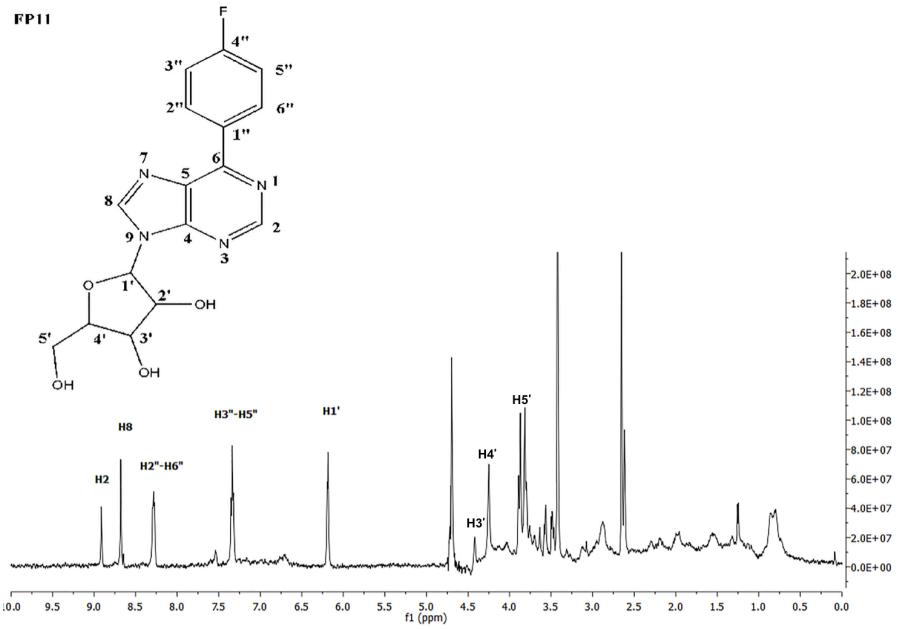


Figure S3. ^{1}H NMR spectrum (600 MHz T= 298K) of **FP13** (5 mM) in 25 mM d-Tris (pH 7.4), 0.5 mM $MgCl_2$ and 25 mM NaCl**.**

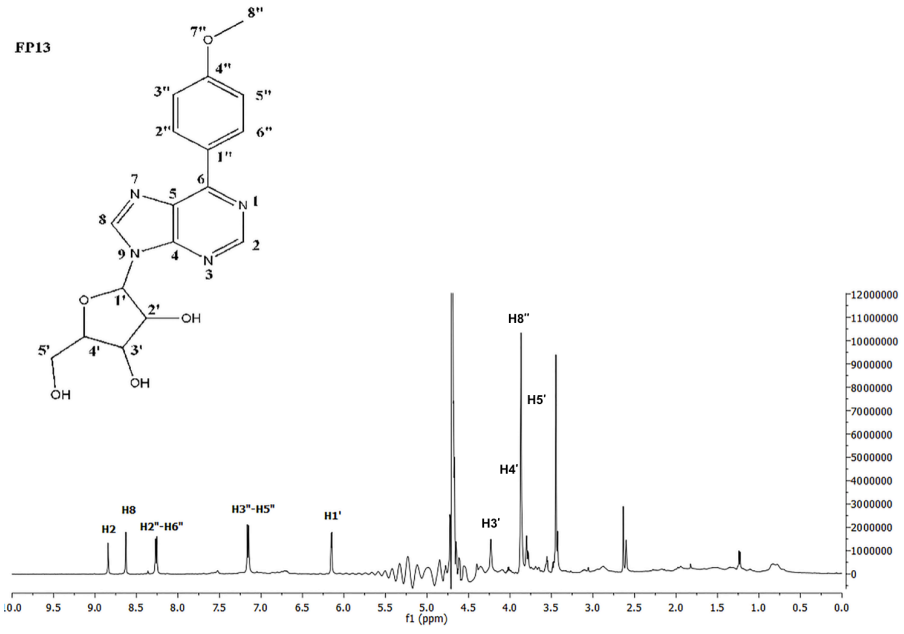


Figure S4. ^{1}H NMR spectrum (600 MHz T= 298K) of **FP16** (5 mM) in 25 mM d-Tris (pH 7.4), 0.5 mM $MgCl_2$ and 25 mM NaCl**.**

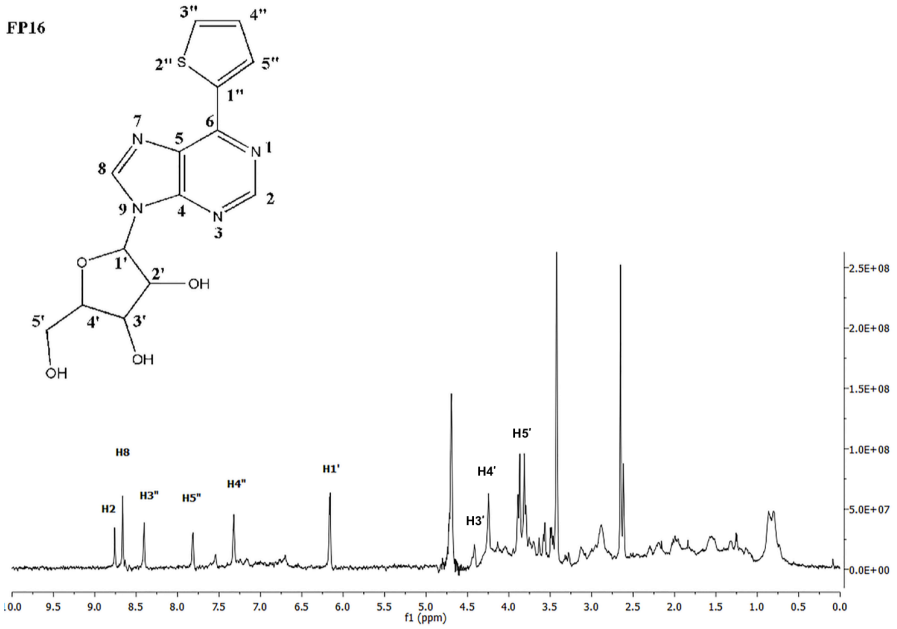


Figure S5. ^{1}H NMR spectrum (600 MHz T= 298K) of **CM223** (5 mM) in 25 mM d-Tris (pH 7.4), 0.5 mM $MgCl_2$ and 25 mM NaCl**.**

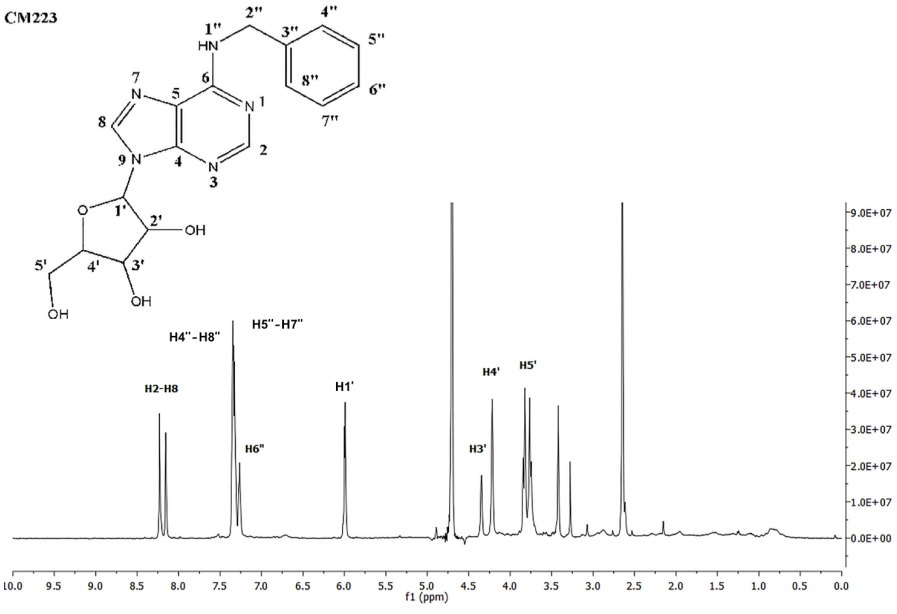


Figure S6. ^{1}H NMR spectrum (600 MHz T= 298K) of **II** (5 mM) in 25 mM d-Tris (pH 7.4), 0.5 mM $MgCl_2$ and 25 mM NaCl**.**