Discovery of IACS-8803 and IACS-8779, potent agonists of stimulator of interferon genes (STING) with robust systemic antitumor efficacy

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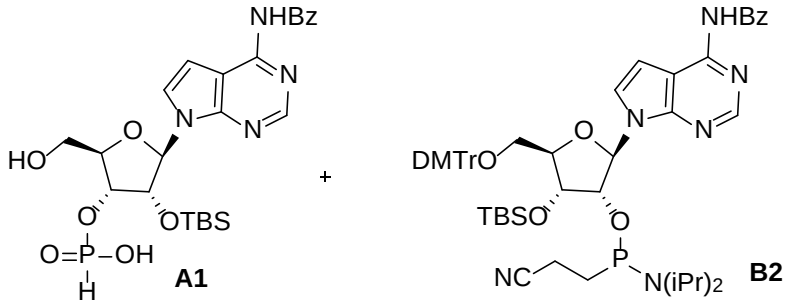
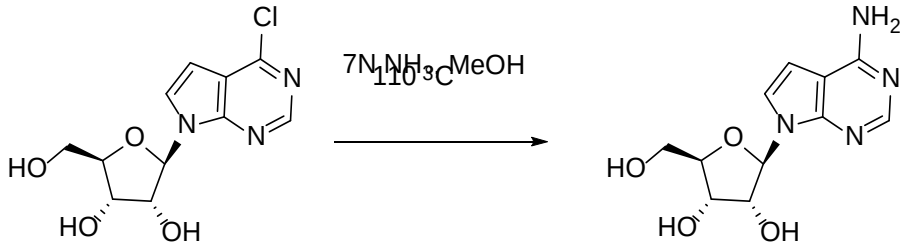
Supporting Information

Synthesis of Intermediates A_1 and B_2.



Step 1



A mixture of (2*R*,3*R*,4*S*,5*R*)-2-(4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (Wuxi catalog, 7.0 g, 24.5 mmol) and 7 N NH_3 in MeOH (70 mL) was stirred at 110 °C for 16 h in a pressure safe steel vessel –CAUTION!-. The mixture

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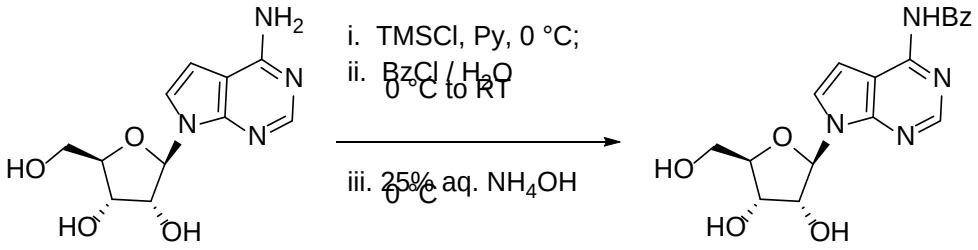
was cooled to RT and the volatiles were removed under reduced pressure. Ten batches of this reaction were run in parallel. The residues were combined and triturated with MeOH (500 ml) to give the title compound as an off-white solid.

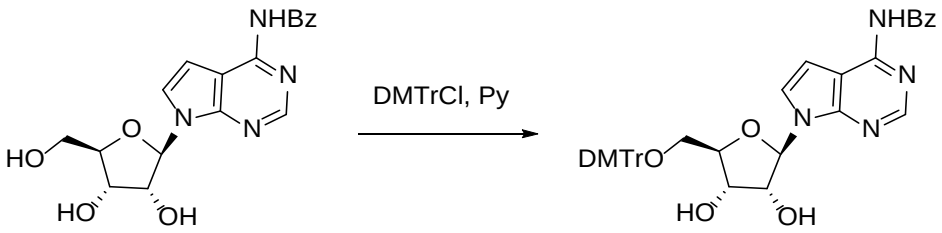
Step 2



To a solution of the product from the previous step (65.2 g, 245 mmol) in pyridine (1.14 L) at 0°C was added TMSCl (119.8 g, 1.10 mol, 4.5 eq) dropwise over 30 minutes. The mixture was stirred for further 30 minutes at 0 °C, and BzCl (6 g, 34.9 mmol, 1.5 eq) was then added dropwise. The resulting mixture was allowed to stir at RT for 16 h, cooled to 0 °C and then quenched with H_2O (200 mL), followed by 25% aq. NH_4OH (500 mL). The volatiles were removed under reduced pressure; the residue was diluted in H_2O (1.5 L) and extracted with EtOAc (3x 2.0 L). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (DCM: MeOH = 20:1) to afford the title compound (60.7 g, 0.164 mol, 67% over two steps).

[001] MS(ES^+) $C_{18}H_{19}N_4O_5$ requires: 371, found: 370.8 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.15 (s, 1H), 8.61 (s, 1H), 8.07 (d, *J*=7.4 Hz, 2H), 7.74 (d, *J*=3.8 Hz, 1H), 7.61 - 7.67 (m, 1H), 7.52 - 7.58 (m, 2H), 6.69 (d, *J*=3.6 Hz, 1H), 6.24 (d, *J*=6.4 Hz, 1H), 5.38 (d, *J*=6.4 Hz, 1H), 5.18 (d, *J*=4.8 Hz, 1H), 5.08 (t, *J*=5.5 Hz, 1H), 4.43 (q, *J*=6.1 Hz, 1H), 4.09 - 4.15 (m, 1H), 3.93 (q, *J*=3.6 Hz, 1H), 3.52 - 3.68 (m, 2H).

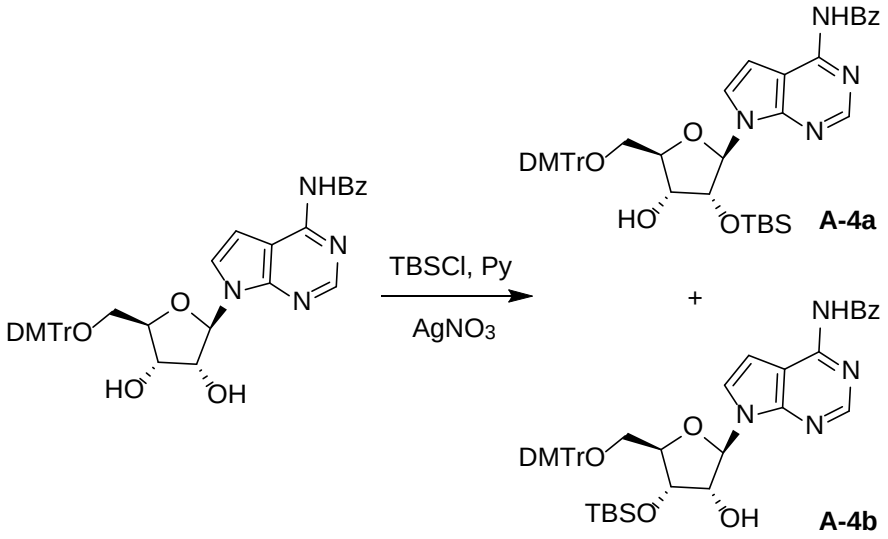
Step 3



To a solution of the product from the previous step (60.0 g, 162.0 mmol) in pyridine (420 mL) was added DMTrCl (65.87 g, 194.4 mmol, 1.2 *eq*) .The mixture was stirred at RT for 16 h, diluted with

CH_2Cl_2 (1.0 L), washed with sat $NaHCO_3$ (2x 500 mL), H_2O (500 mL) and brine (500 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (5/1 petroleum ether / EtOAc to 100% EtOAc) to afford the title compound (89.3 g, 132.8 mmol, 82%) as a white foam.

Step 4



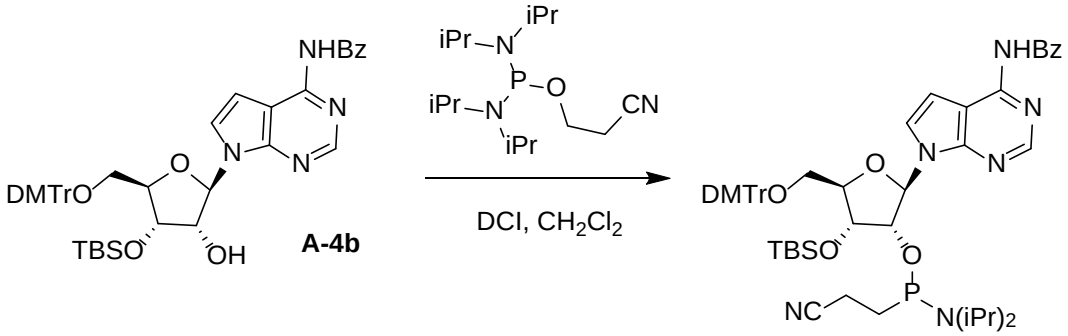
To a mixture of the product from the previous step (55 g, 81.76 mmol) and $AgNO_3$ (22.92 g, 134.9 mmol, 22.7 mL, 1.65 eq) in THF (400 mL) was added TBSCl (78.75 g, 522.48 mmol, 1.76 eq). The reaction mixture was stirred at RT for 5 h, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (Petroleum Ether / EtOAc=10/1 to 2/1) to afford **A-4a** (70 g, 88.9 mmol, 54.4%) and **A-4b** (7 g, 8.89 mmol, 5.4%).

A-4a: ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.16 (br s, 1H), 8.58 (s, 1H), 8.07 (d, *J*=7.7 Hz, 2H), 7.52 - 7.67 (m, 4H), 7.42 (d, *J*=7.7 Hz, 2H), 7.22 - 7.32 (m, 7H), 6.88 (d, *J*=8.8 Hz, 4H), 6.69 (d, *J*=3.8 Hz, 1H), 6.30 (d, *J*=5.4 Hz, 1H), 5.12 (d, *J*=5.8 Hz, 1H), 4.59 (t, *J*=5.3 Hz, 1H), 4.07 - 4.21 (m, 2H), 3.73 (s, 6H), 3.28 (br s, 2H), 0.75 (s, 9H), -0.04 (s, 3H), -0.16 (s, 3H).

A-4b: ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.76 (brs, 1H), 8.59 (s, 1H), 8.07 (d, *J*=7.8 Hz, 2H), 7.61 - 7.66 (m, 2H), 7.52 - 7.57 (m, 2H), 7.38 (br d, *J*=7.8 Hz, 2H), 7.19 - 7.32 (m, 7H), 6.86 (d, *J*=8.7 Hz, 4H), 6.68 (d, *J*=3.6 Hz, 1H), 6.21 (d, *J*=5.6 Hz, 1H), 5.38 (br d, *J*=5.9 Hz, 1H), 4.57 (br d, *J*=5.1 Hz, 1H), 4.34 (t, *J*=4.4 Hz, 1H), 4.00 (br d, *J*=4.1 Hz, 1H), 3.72 (s, 6H), 3.30 - 3.39 (m, 1H), 3.12 - 3.16 (m, 1H), 3.14 (br dd, *J*=4.7, 10.4 Hz, 1H), 0.84 (s, 9H), 0.08 (s, 3H), 0.03 (s, 3H).

Intermediate B_2

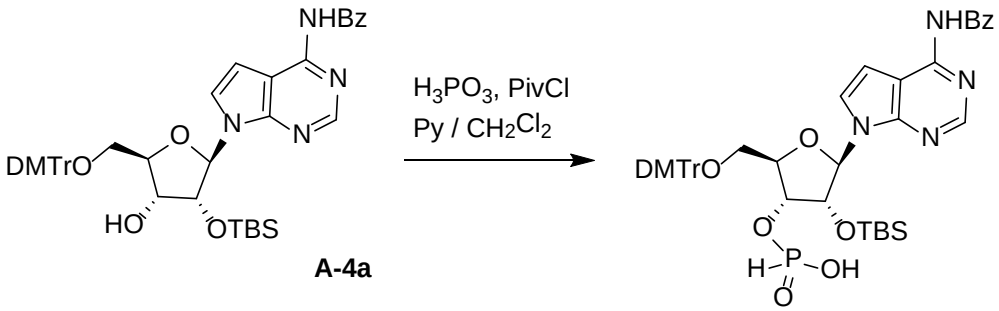
Step 5



To a solution of **A-4b** (10.0 g, 12.71 mmol) in CH_2Cl_2 (100 mL) were added were added 3-((bis(diisopropylamino)phosphanyl)oxy)propanenitrile (4.21 g, 14 mmol, 1.1 eq) and DCI (2.25 g, 19.07 mmol, 1.5 eq). The mixture was stirred at RT for 5 h, diluted with CH_2Cl_2 (100 mL) and washed with sat $NaHCO_3$ (3x 100mL).The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (petroleum ether / EtOAc=10/1 to 3/1; 0.5% TEA) to afford **Intermediate B_2** (10.0 g, 9.72 mmol, 76%) as a white foam.; ^{1}H-NMR (400 MHz, CD_3CN) δ ppm 9.23 (br s, 1H), 8.52 (br s, 1H), 8.01 (br d, *J*=5.8 Hz, 2H), 7.60 - 7.68 (m, 1H), 7.51 - 7.58 (m, 2H), 7.40 - 7.50 (m, 3H), 7.18 - 7.34 (m, 7H), 6.79 - 6.92 (m, 5H), 6.34 - 6.48 (m, 1H), 4.97 - 4.70 (m, 1H), 4.63 - 4.48 (m, 1H), 4.13 (br d, *J*=3.9 Hz, 1H), 3.84 - 3.69 (m, 7H), 3.62 - 3.41 (m, 4H), 3.27 - 3.15 (m, 1H), 2.58 (t, *J*=6.2 Hz, 1H), 2.41 (t, *J*=6.2 Hz, 1H), 1.12 - 1.03 (m, 9H), 0.91 - 0.84 (m, 12H), 0.13 (d, *J*=16.2 Hz, 3H), 0.05 (s, 3H); ^{31}P NMR (162MHz, CD_3CN) δ ppm 149.92, 149.53.

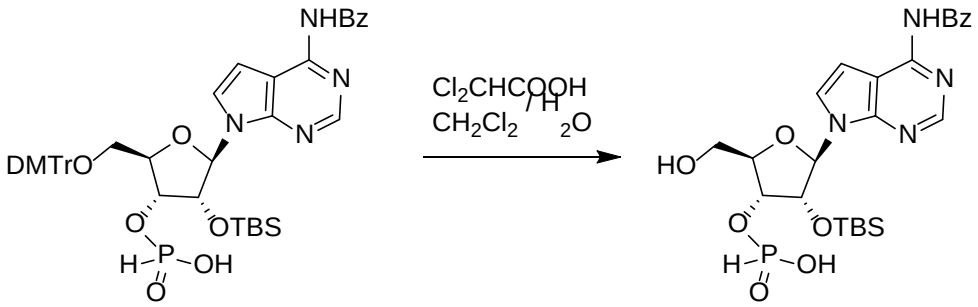
Intermediate A_1

Step 6



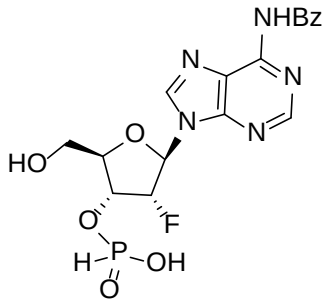
Phosphorous acid (15.63 g, 190.6 mmol, 15 eq) was co-evaporated three times with anhydrous pyridine (5 mL) and then dissolved in anhydrous pyridine (75 mL) upon heating to *ca.* 45 °C. The mixture was allowed to cool to RT. **A-4a** (10.0 g, 12.7 mmol) was added and the mixture was cooled to 0°C. Pivaloyl chloride (15.32 g, 127.07 mmol, 10.0 eq) was slowly added at 0 °C and the resulting mixture was allowed to warm to RT and stirred for 16 h. The reaction mixture was then quenched by 1 M aq. TEAB (100 mL) and extracted with EtOAc (3x 1000 mL). The combined organic layers were washed with 0.5 M aq. TEAB (100 m L), and brine (1000 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (CH_2Cl_2 / MeOH = 50/1) to afford the title compound as a white foam (8.0 g, 8.38 mmol, 66%).

Step 7

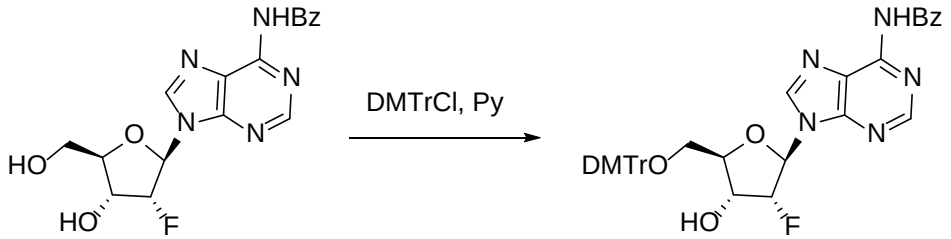


The product from the previous step (40.0 g, 42.01 mmol) and H_2O (4.0 g, 222 mmol, 4.0 mL, 5.3 eq) were added to a solution of $Cl_2CHCOOH$ in CH_2Cl_2 (6% v/v, 400 mL) and the reaction mixture was stirred at RT for 0.5 h, then washed with H_2O (4x 200 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. SiO_2 gel (80 g, previously treated with TEA) was combined with the filtrate, and the mixture was concentrated under reduced pressure to obtain free flowing SiO_2 gel powder. The residue was purified by SiO_2 gel column chromatography (CH_2Cl_2 / MeOH = 50 / 1 to 30 / 1) to give **Intermediate A_1** as a white solid (15.0 g, 23.08 mmol, 55%); uMS(ES^+) $C_{24}H_{34}N_4O_7PSi$ requires: 549, found: 549.1 $[M+H]^+$; ^{1}H-NMR (400 MHz, 400 MHz, DMSO-d_6) δ ppm 11.15 (br s, 1H), 8.49 - 8.74 (m, 1H), 8.08 (d, J=7.6 Hz, 2H), 7.78 (d, J=3.6 Hz, 1H), 7.60 - 7.67 (m, 1H), 7.51 - 7.57 (m, 2H), 6.74 (d, J=3.6 Hz, 1H), 6.28 (d, J=6.2 Hz, 1H), 5.75 (s, 1H), 4.56 - 4.74 (m, 2H), 4.16 (br s, 1H), 3.61 - 3.76 (m, 2H), 3.03 (q, J=7.2 Hz, 5H), 1.19 (t, J=7.4 Hz, 7H), 0.69 (s, 9H), -0.09 (s, 3H), -0.27 (s, 3H); ^{31}P NMR (162 MHz, DMSO-d_6) δ ppm 0.72.

Synthesis of Intermediate A_3.

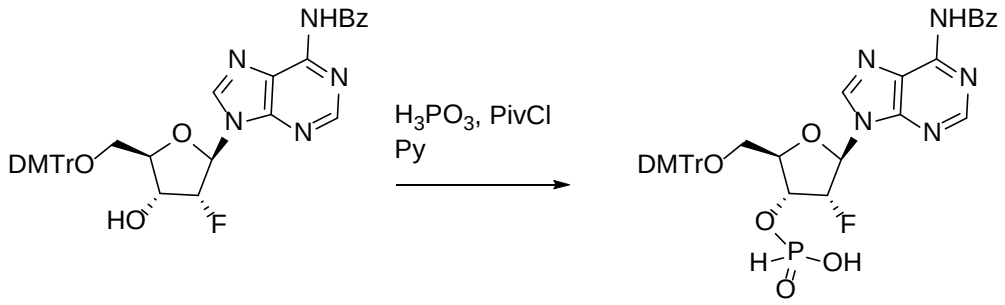


Step 1



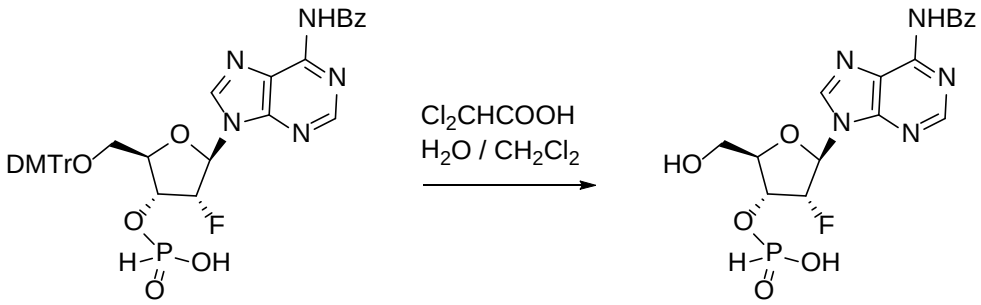
To a solution of *N*-(9-((2*R*,3*R*,4*R*,5*R*)-3-fluoro-4-hydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (Carbosyth, 35 g, 93.7 mmol) in pyridine (180 mL) was added DMTrCl (38.12 g, 112.5 mmol, 1.2 eq) and the resulting mixture was stirred at RT for 16 h. The mixture was then diluted with CH_2Cl_2 (800 mL), washed with sat $NaHCO_3$ (2x 400 mL) and brine (400 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (petroleum ether / EtOAc=10/1 to 1/4) to give the title compound as a white solid (53.0 g, 78.4 mmol, 84%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.26 (br s, 1H), 8.74 (s, 1H), 8.62 (s, 1H), 8.05 (d, J=7.4 Hz, 2H), 7.60 - 7.72 (m, 1H), 7.48 - 7.58 (m, 2H), 7.32 (d, J=7.2 Hz, 2H), 7.14 - 7.24 (m, 7H), 6.80 (dd, J=6.2, 8.7 Hz, 4H), 6.43 (d, J=20.0 Hz, 1H), 5.73 - 5.85 (m, 1H), 5.61 (d, J=4.4 Hz, 1H), 4.76 - 4.99 (m, 1H), 4.14 (br d, J=5.4 Hz, 1H), 3.64 - 3.79 (m, 7H), 3.19 - 3.33 (m, 2H).

Step 2



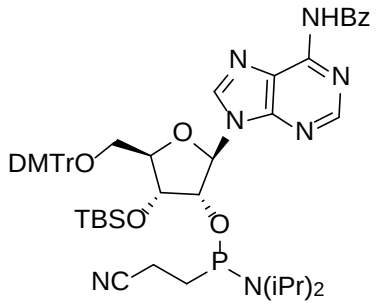
Phosphorous acid (18.2 g, 222 mmol, 15 eq) was co-evaporated three times with anhydrous pyridine (15 mL) and then dissolved with heating in anhydrous pyridine (150 mL). The mixture was allowed to cool to RT. The product from the previous step (10 g, 14.8 mmol) was added, and the resulting mixture was cooled to 0 °C. Pivaloyl chloride (17.85 g, 148 mmol, 10 eq) was slowly added at 0 °C and the resulting mixture was allowed to warm to RT and stirred for 16 h. The reaction mixture was then quenched with 1 M aq. TEAB (150 mL) and extracted with EtOAc (3x 900 mL).The combined organic layers were washed with 0.5 M aq. TEAB (900 mL), brine (900 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (CH_2Cl_2 / MeOH = 50/1 to 20/1; 1% TEA) to give the title compound as a white foam (38 g).

Step 3



The product from the previous step (38 g, 45.19 mmol) and H_2O (4.0 g, 222 mmol, 4.0 mL, 5 eq) were added to a solution of $Cl_2CHCOOH$ in CH_2Cl_2 (6% v/v, 380 mL) and the reaction mixture was stirred at RT for 0.5 h. The reaction mixture was filtered to give a red solid, which was washed with CH_2Cl_2 (2x 20 mL) to give the title compound as a white solid (15 g, 30.87 mmol, 68%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 11.24 (br s, 1H), 8.78 (s, 1H), 8.73 (s, 1H), 8.02 - 8.08 (m, 2H), 7.76 (d, J=1.2 Hz, 0.5H), 7.62 - 7.68 (m, 1H), 7.53 - 7.59 (m, 2H), 6.69 (s, 1H), 6.46 (dd, J=3.2, 16.6 Hz, 1H), 6.07 (d, J=1.4 Hz, 0.5H), 5.87 - 5.91 (m, 1H), 5.73 - 5.78 (m, 1H), 5.17 - 5.28 (m, 1H), 4.22 - 4.28 (m, 1H), 3.64 - 3.84 (m, 2H).

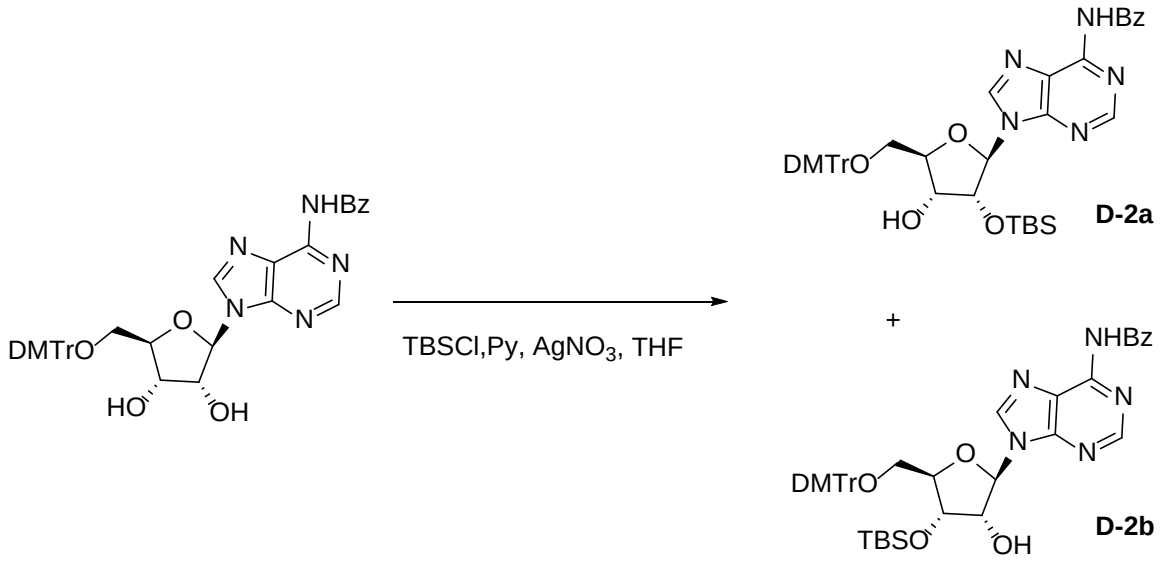
Synthesis of Intermediate B_1.



Step 1

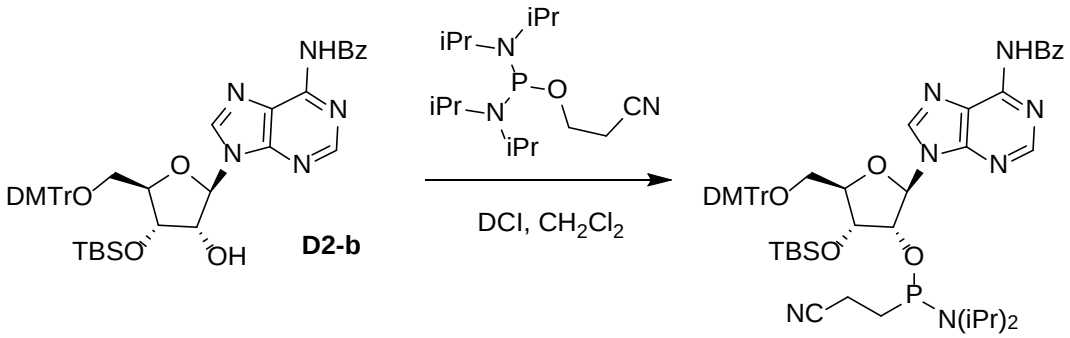
To a solution of N-(9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (100 g, 269.3 mmol) in pyridine (500 mL) at 0°C were added DMAP (1.64 g, 13.46 mmol, 0.05 eq) and DMTrCl (100.4 g, 296.2 mmol, 1.1 eq). The reaction mixture was stirred at RT for 16 h, then quenched by addition of MeOH (500 mL). The volatiles were removed under reduced pressure and the residue was purified by SiO_2 gel chromatography (1/1 petroleum ether / EtOAc to 100% EtOAc) to give the title compound as a white foam (150 g, 223 mmol, 83%).

Step 2



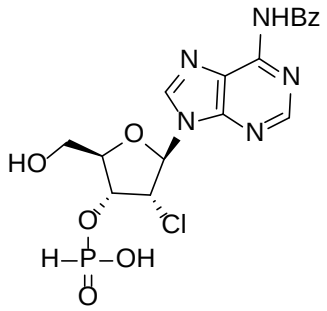
To a solution of the product from the previous step (200 g, 296.9 mmol) in THF (800 mL) and pyridine (15 mL) were added $AgNO_3$ (83.2 g, 489.8 mmol, 82 mL, 1.65 eq) and TBSCl (78.7 g, 522.5 mmol, 1.76 eq), and the mixture was stirred at RT for 16 h. The reaction mixture was filtered, diluted with H_2O (1.0 L) and extracted with EtOAc (3x 1.0 L). The combined organic layers were washed with brine (2x 1.0 L), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (petroleum ether / EtOAc=3/1 to 1/1) to give **D-2a** (114 g, 145 mmol, 49%) and **D-2b** (53 g, 68 mmol, 23%).

Step 3

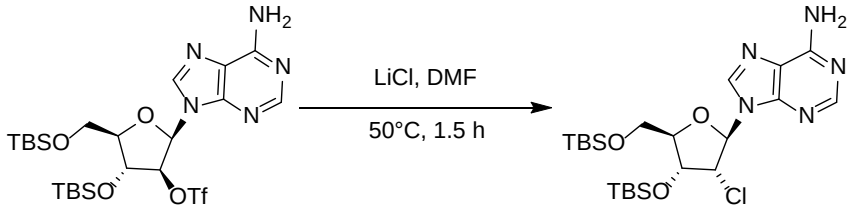


To a solution of **D-2b** (19.0 g, 24.1 mmol) in MeCN (200mL) at 0 °C were added 3-((bis(diisopropylamino)phosphanyl)oxy)propanenitrile (7.99 g, 26.5 mmol, 1.1 eq) and DCI (3.42 g, 28.9 mmol, 1.2 eq), and the resulting mixture was stirred for 3 h at RT under N_2 atmosphere. The volatiles were removed under reduced pressure and the residue was purified by SiO_2 gel chromatography (petroleum ether / EtOAc=4/1 to 1.5/1; 1% TEA) to give the title compound as a white foam (20.5 g, 20.74 mmol, 86%). MS(ES^+) $C_{53}H_{67}N_7O_8PSi$ requires: 988, found: 987.8 $[M+H]^+$; ^{1}H-NMR (400 MHz, CD_3CN) δ ppm 8.57 (s, 1H), 8.26 (s, 1H), 7.91 (br d, J=7.6 Hz, 2H), 7.57 (m, 1H), 7.47 - 7.49 (m, 2H), 7.17 - 7.21 (m, 9H), 6.73 – 6.76 (m, 4H), 6.10 - 6.19 (m, 1H), 5.10-5.14 (m, 1H), 4.64 - 4.68 (m, 1H), 4.10-4.14 (m, 1H), 3.25-3.75 (m, 7H), 3.40-3.51 (m, 4H), 3.15-3.18 (m, 1H), 2.58 (t, J=6.0 Hz, 1H), 2.41 (t, J=6.0 Hz, 1H), 1.03-1.06 (m, 9H), 0.76-0.86 (m, 12H), 0.00-0.93(m, 6H); ^{31}P NMR (162 MHz, CD_3CN) δ ppm 150.32, 149.58.

Synthesis of Intermediate A4.

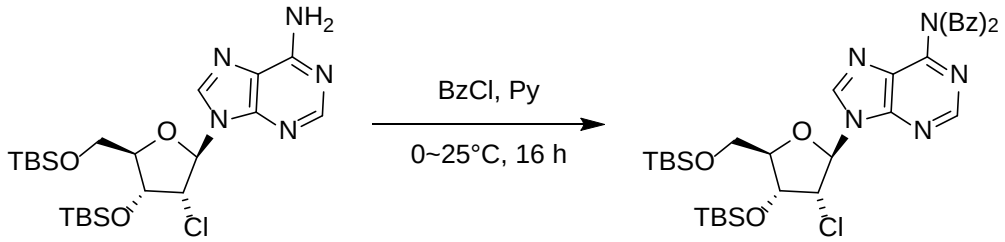


Step 1



A mixture of (2*R*,3*S*,4*R*,5*R*)-2-(6-amino-9*H*-purin-9-yl)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl trifluoromethanesulfonate (Ref. 27; 69.0 g, 0.11 mol, 1.0 eq) and LiCl (51.2 g, 1.21 mol, 11.0 eq) in DMF (600 mL) was heated at 50 °C for 1.5 h. The reaction mixture was diluted with DCM (500 mL) and washed with H_2O (300 mL × 3). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (PE: EtOAc = 1:1) to give the title compound (46.0 g, 89.5 mmol, 81.4% yield) as a yellow solid. **^{1}H NMR** (400 MHz $CDCl_3$) δ ppm 8.30-8.38 (m, 1H), 8.06 (s, 1H), 6.20 (d, *J* = 5.9 Hz, 1H), 5.82-5.95 (m, 2H), 4.96-5.04 (m, 1H), 4.56-4.63 (m, 1H), 4.17 (q, *J* = 3.5 Hz, 1H), 3.96 (dd, *J* = 11.4, 4.3 Hz, 1H), 3.77 (dd, *J* = 11.4, 3.0 Hz, 1H), 0.92 (d, *J* = 13.9 Hz, 18H), 0.14 (d, *J* = 8.8 Hz, 6H), 0.08 (s, 6H).

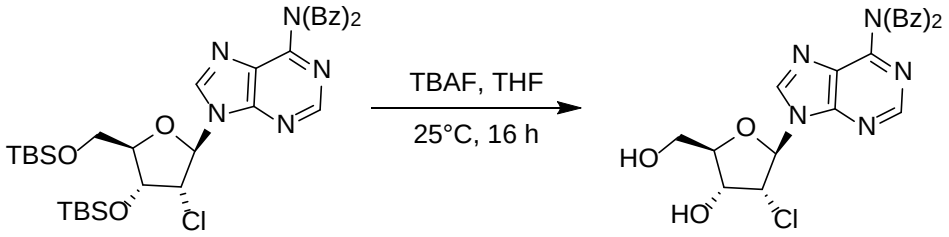
Step 2



To a solution of the product from previous step (46.0 g, 89.4 mmol, 1.0 eq) in Py (460 mL) at 0°C was added BzCl (25.2 g, 179 mmol, 20.5 mL, 2.0 eq) dropwise under N_2, and the resulting mixture was stirred at 25°C for 16 hrs. The reaction was diluted with H_2O (200 mL) and extracted with

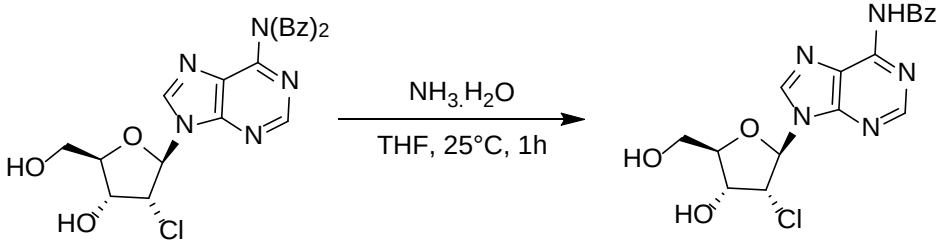
EtOAc (200 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give N-benzoyl-N-(9-((2*R*,3*R*,4*R*,5*R*)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chlorotetrahydrofuran-2-yl)-9H-purin-6-yl)benzamide (60.0 g) as yellow oil which was used for the next step without further purification. MS(ES$^+$) $C_{36}H_{48}ClN_5O_5Si_2$ requires: 721, found: 722 $[M+H]^+$;

Step 3



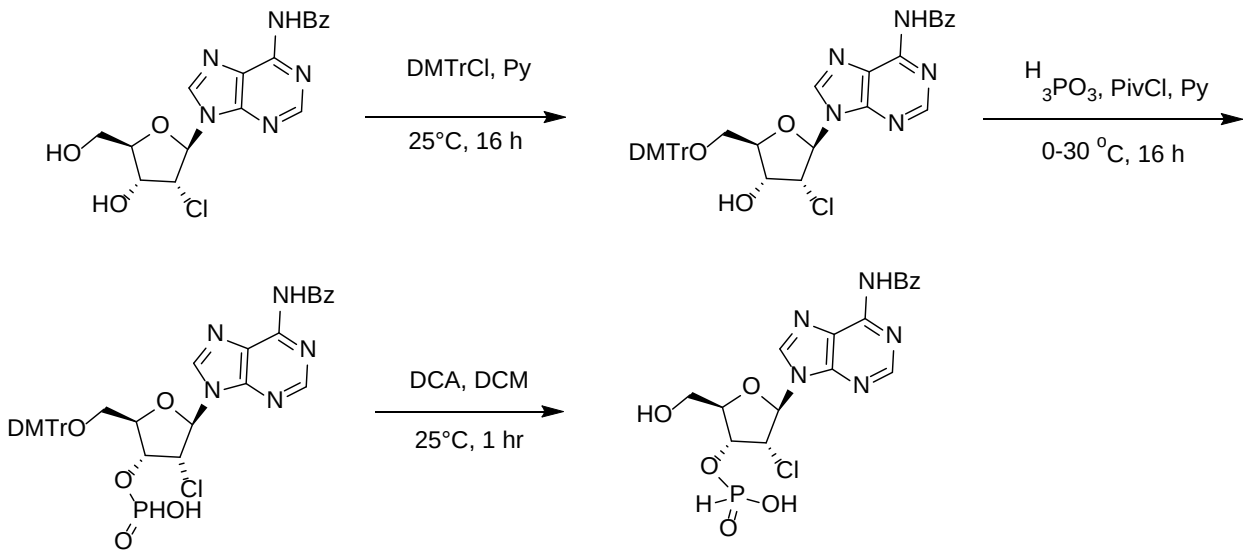
To a solution of the product from previous step (60.0 g, 97 mmol, 1.0 eq) in THF (600 mL) was added TBAF (1 M in THF, 290 mL, 3.0 eq). Then the mixture was stirred at 25°C for 16 hrs. The volatiles were removed under reduced pressure to give N-benzoyl-N-(9-((2*R*,3*R*,4*R*,5*R*)-3-chloro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9*H*-purin-6-yl)benzamide (48.0 g) as a yellow oil which was used for the next step without further purification. MS(ES$^+$) $C_{24}H_{20}ClN_5O_5$ requires: 493, found: 494 $[M+H]^+$;

Step 4



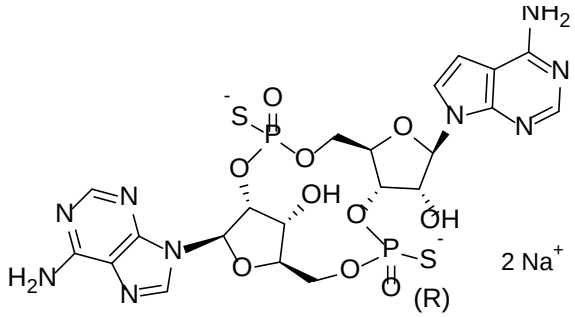
To a solution of the product from previous step (48.0 g, 97.2 mmol, 1.0 eq) in THF (500 mL) was added $NH_3.H_2O$ (28% in water, 8.0 mL, 7.28 g, 54.0 mmol, 0.5 eq). The resulting mixture was stirred at 25°C for 1 hr, during which time a solid product was formed. The suspension was filtered and the filter cake was washed with THF (300 mL × 3). The filtrate was concentrated under reduced pressure to give N-(9-((2*R*,3*R*,4*R*,5*R*)-3-chloro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9H-purin-6-yl)benzamide (36 g, 92.3 mmol, 95% yield) as a white solid product; MS(ES$^+$) $C_{17}H_{16}ClN_5O_4$ requires: 389, found: 390 $[M+H]^+$;

Steps 5 to 7

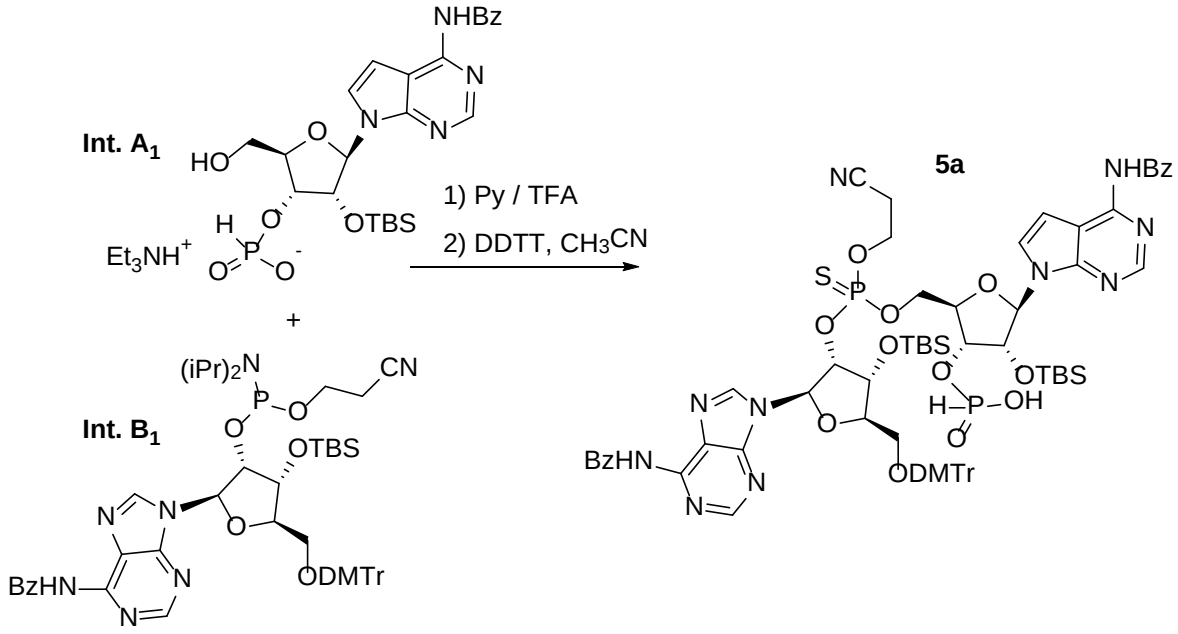


The transformations depicted in the above scheme were performed according to the procedures previously described for the synthesis of Intermediate A_3, steps 1 to 3. **Intermediate A_4**: white solid (13.0 g, 27.8 mmol, 30% over three steps); ^{1}H NMR: (400 MHz DMSO-d_6) δ ppm 11.28 (br s, 1H), 8.78 (br d, *J* = 4.0 Hz, 2H), 8.05 (br d, *J* = 7.4 Hz, 2H), 7.60-7.68 (m, 1H), 7.55 (br t, *J* = 7.5 Hz, 2H), 6.34 (br d, *J* = 5.9 Hz, 1H), 5.27 (br d, *J* = 5.1 Hz, 1H), 5.02 (br s, 1H), 4.30 (br s, 1H), 3.74 (br s, 2H), 3.01-3.11 (m, 2H); ^{31}P NMR: (162 MHz DMSO-d_6) δ 0.63 ppm.

Synthesis of IACS-8779 (1a) and 1b

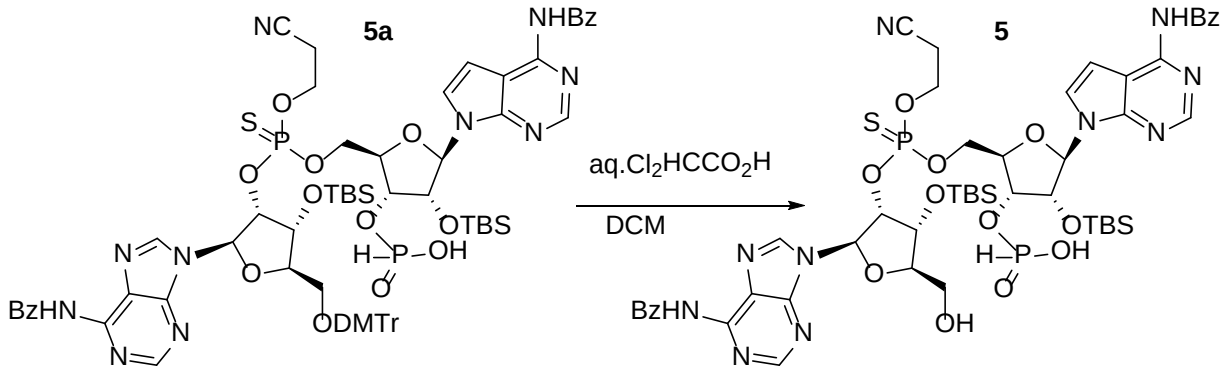


Step 1



To a solution of Intermediate A_1 (4.00 g, 6.16 mmol) in CH_3CN (50 mL) was added pyridine-TFA (2.38 g, 12.3 mmol, 2.0 eq) followed by a mixture of Intermediate B_1 (6.70 g, 6.78 mmol, 1.10 eq) and 3Å molecular sieves (1.0 g, 24.6 mmol, 4.0 eq) in CH_3CN (50 mL), and the resulting mixture was stirred for 30 minutes at RT. DDTT (1.52 g, 7.39 mmol, 1.20 eq) was then added and the mixture was stirred at RT for further 30 minutes. The volatiles were removed under reduced pressure to afford the crude compound **5a** (9.04 g), which was used without further purification in the next step.

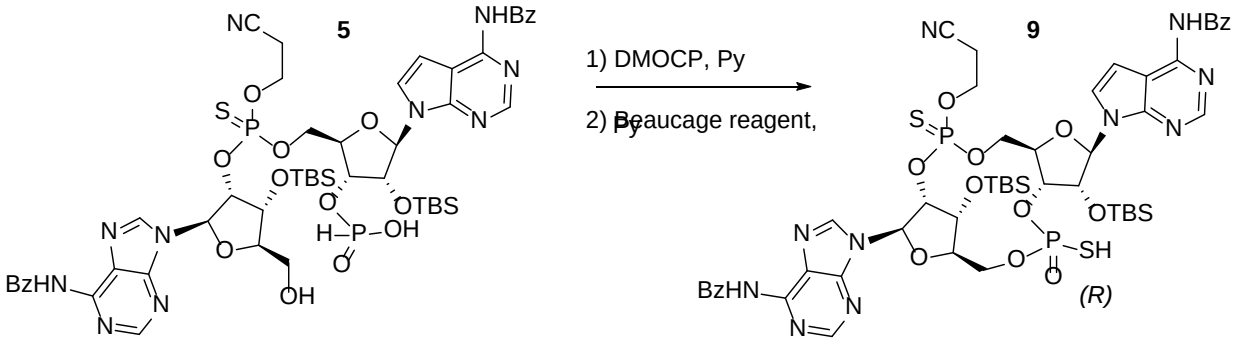
Step 2



To a solution of $Cl_2CHCOOH$ in CH_2Cl_2 (6% v/v, 200 mL) was added H_2O (2.0 g, 111 mmol, 2.0 mL, 18.0 eq) and compound **5a** from the previous step (9.04 g, assume 6.16 mmol). The reaction mixture was stirred at RT for 0.5 h, then quenched with pyridine (120 mL) and concentrated under

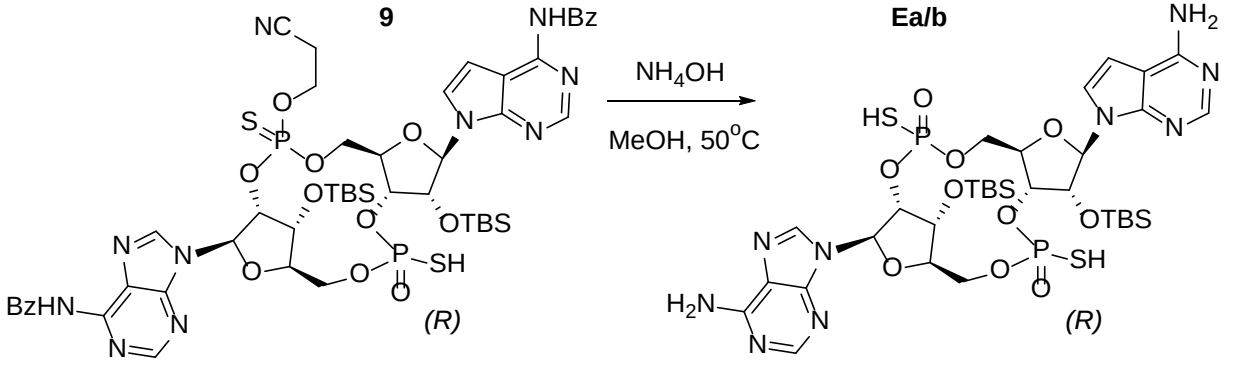
reduced pressure, to afford crude compound **5** (7.18 g), which was used without further purification in the next step.; MS(ES$^+$) $C_{50}H_{67}N_{10}O_{13}P_2SSi_2$ requires: 1165, found: 1165.3 [M+H]+.

Step 3



To a solution of compound **5** from the previous step (7.18 g, assume 6.16 mmol) in pyridine (50 mL) was added DMOCP (3.98 g, 21.6 mmol, 3.5 eq). The mixture was stirred for 0.5 h at RT. To the mixture was then added Beaucage reagent (3*H*-1,2-benzodithiol-3-one 1,1-dioxide, 1.85 g, 9.24 mmol, 1.5 eq), and the resulting mixture and stirred at RT for further 30 minutes. The reaction mixture was quenched by addition of 3.4% aq. $NaHCO_3$ (1.0 L), and then extracted with EtOAc (2x 500 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (CH_2Cl_2 / MeOH = 30/1 to 15/1) to give compound **9** (3.0 g, 2.04 mmol) as a mixture of diastereoisomers which was used as such in the following step.

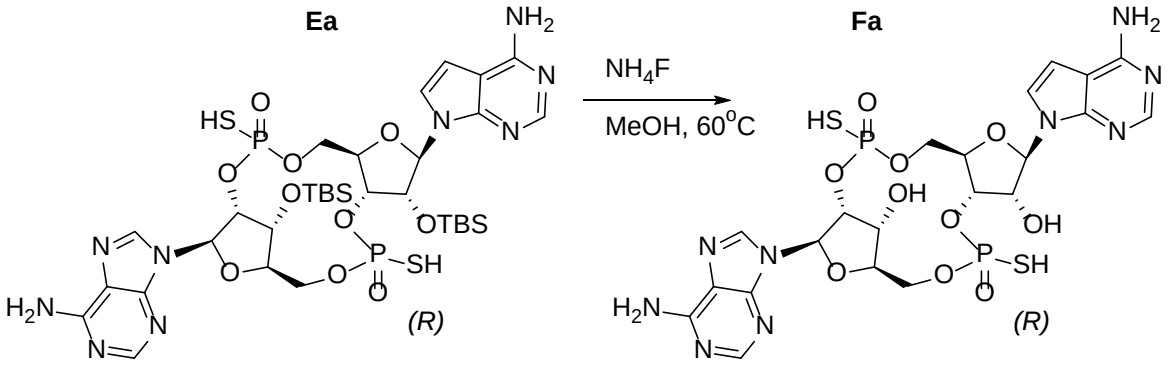
Step 4



To a solution of compound **9** from the previous step (3.0 g, 2.04 mmol) in MeOH (30 mL) was added NH_4OH (32.8 g, 935 mmol, 458 eq). The mixture was stirred at 50 °C for 12 h in a pressure safe steel vessel, then concentrated under reduced pressure. The residue was purified by prep-

HPLC (PHENOMENEX® LUNA® C18 250*50 10 um; mobile phase: A: H_2O (10mM NH_4HCO_3); B: MeCN; A%-B%= 20%-50%, 20 minutes) to give two products: compound **Ea** (R_pR_p or S_pR_p diastereoisomer; 380 mg, 0.391 mmol;) and compound **Eb** (S_pR_p or R_pR_p diastereoisomer; 350 mg, 0.349 mmol) as a white solids..

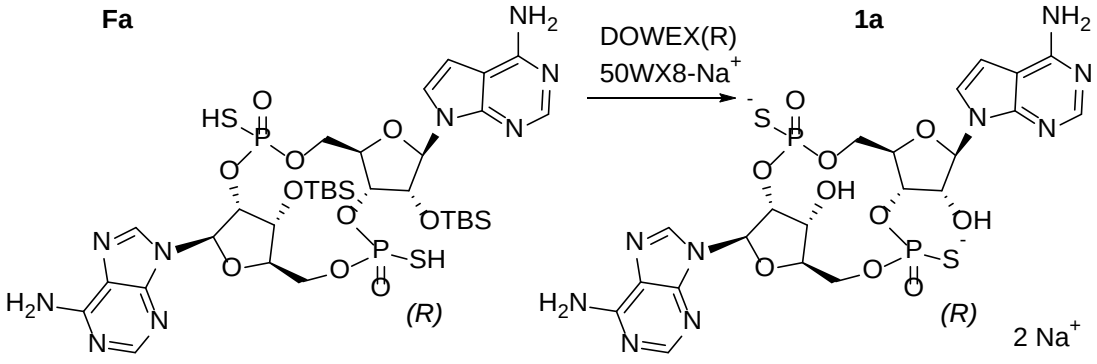
Step 5



To a solution of compound **Ea** (200 mg, 218 umol) in MeOH (5.0 mL) was added NH_4F (80.7 mg, 2.18 mmol, 10.0 eq) and the resulting mixture was stirred at 60 °C for 16 h. The volatiles were removed under reduced pressure and the residue was purified by prep-HPLC [Waters Xbridge 150*25 5um; mobile phase: A: H_2O (10 mM NH_4HCO_3); B: MeCN; A%-B%= 1%-20%, 10.5 minutes] to give compound **Fa** (30 mg, 40 umol) as a white solid.

Reaction of compound **Eb** in a similar manner afforded compound **Fb** (30 mg, 40 umol) as a white solid.

Step 6

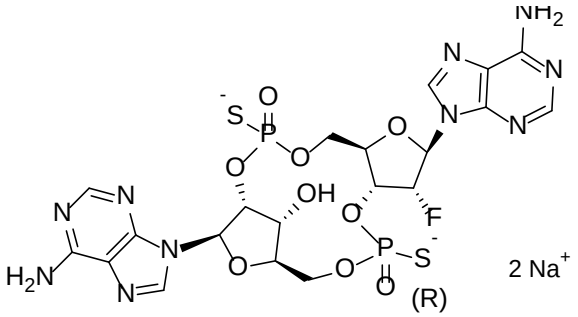


To a solution of compound **Fa** (30.0 mg, 41.5 umol) in H_2O (5.0 mL) was added DOWEX®-50WX8 (Na^+ form; 300 mg) and the mixture was stirred at RT for 0.5 h. The reaction was then filtered, and the filtrate was lyophilized to give **1a** (28.0 mg, 38.1 umol) as a white solid.; MS(ES^+) $C_{21}H_{26}N_9O_{10}P_2S_2$ requires: 690, found: 690.0 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm

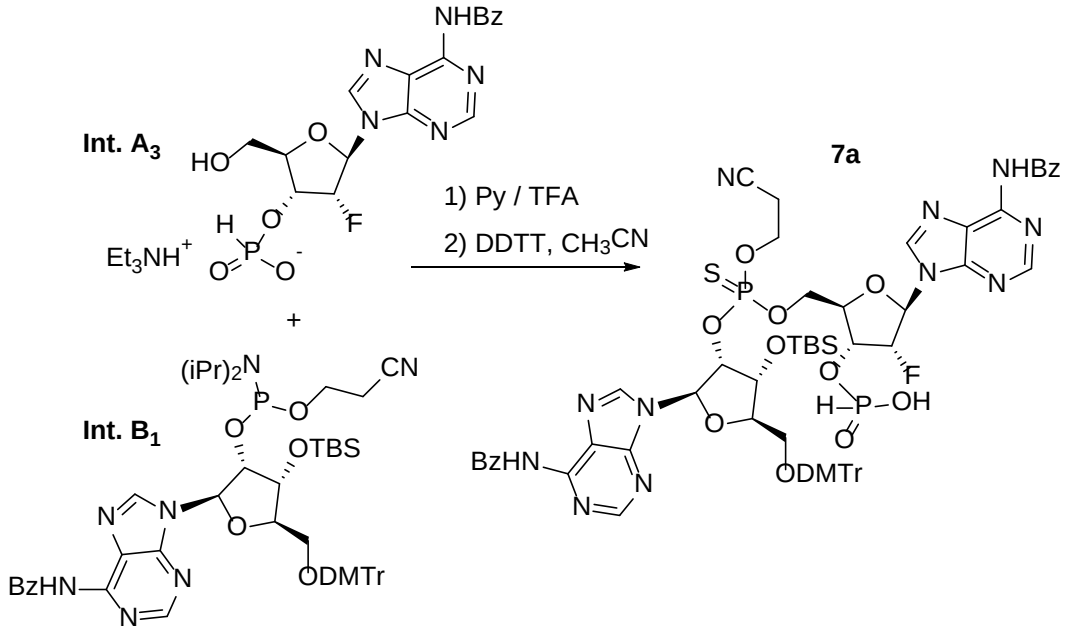
8.52 (s, 1H), 8.26 (s, 1H), 8.19 (s, 1H), 7.50 (d, J = 3.6 Hz 1H), 6.85 (d, J = 3.6 Hz, 1H), 6.09-6.13 (m, 2H), 5.46 (d, J = 8.8 Hz, 1H), 5.28-5.30 (m, 1H) 4.63 (d, J = 4 Hz, 1H), 4.03-4.27 (m, 5H), 3.64-3.69 (m, 2H); ^{31}P NMR (162 MHz, DMSO-d_6) δ ppm 60.19, 56.77; R_t = 1.797 minutes [Waters XBridge Shield RP18 2.1*50mm, 5um; mobile phase: A: H_2O + 10mM NH_4HCO_3; B: MeCN; A%-B%= 0%-30%, 5.2 minutes].

Reaction of compound **Fb** in a similar manner gave **1b** (30.0 mg, 39.6 umol) as a white solid; MS(ES$^+$) $C_{21}H_{26}N_9O_{10}P_2S_2$ requires: 690, found: 690.0 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 8.50 (s, 1H), 8.29 (s, 1H), 8.19(s, 1H), 7.62 (d, J = 3.2 Hz 1H), 6.85 (d, J = 4.0 Hz, 1H), 6.09-6.14 (m, 2H), 5.19-5.29 (m, 2H), 4.68 (dd, J = 7.6 Hz, 1H), 4.37 (d, J = 4.0 Hz, 1H), 4.11-4.20 (m, 3H), 3.95-3.99 (m, 1H), 3.69-3.81 (m, 2H); ^{31}P NMR (162 MHz, CD_3OD) δ ppm 59.29, 51.96; R_t = 2.101 minutes [Waters XBridge Shield RP18 2.1*50mm, 5um; mobile phase: A: H_2O + 10mM NH_4HCO_3; B: MeCN; A%-B%= 0%-30%, 5.2 minutes].

<u>Synthesis of IACS-8803 (3a) and 3b</u>

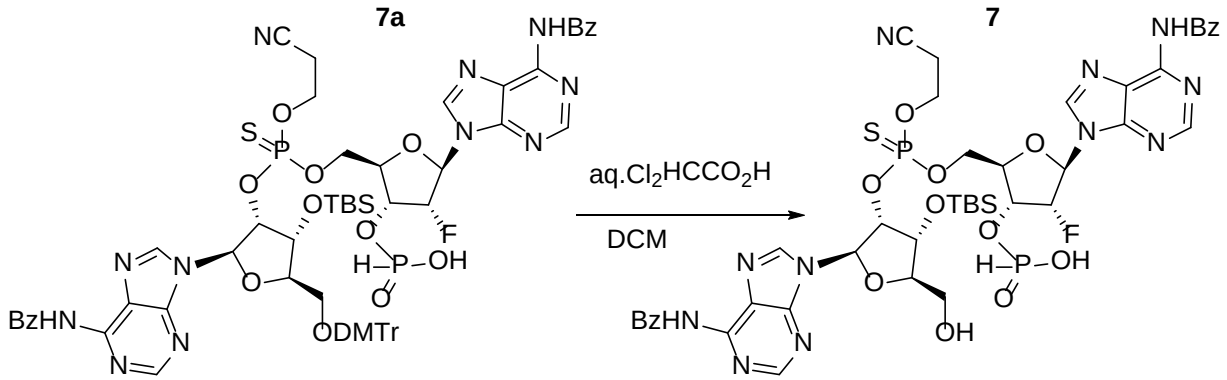


Step 1



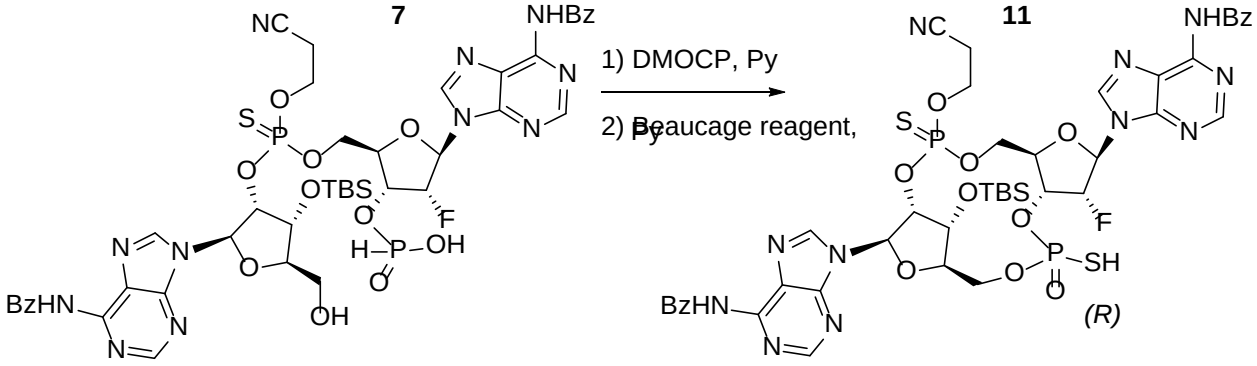
To a solution of **Intermediate A_3** (4.0 g, 9.15 mmol) in CH_2Cl_2 (40 ml) was added TEA (463 mg, 4.58 mmol, 0.50 eq). The mixture was stirrred for 5 minutes at RT and the volatiles were removed under reduced pressure. The residue was dissolved in CH_3CN (40.00 mL) and pyridine-TFA (3.53 g, 18.3 mmol, 2.0 eq) was added, followed by a mixture of **Intermediate B_1** (9.04 g, 9.15 mmol, 1.0 eq) and 3Å molecular sieves (1.48 g, 36.6 mmol, 4.0 eq) in CH_3CN (40 mL), and the resulting mixture was stirred for 30 minutes at RT. DDTT (2.25 g, 10.98 mmol, 1.2 eq) was added and the mixture was stirred at RT for further 30 minutes. The volatiles were removed under reduced pressure to afford the crude compound **7a** (12.4 g), which was used without further purification in the next step.

Step 2



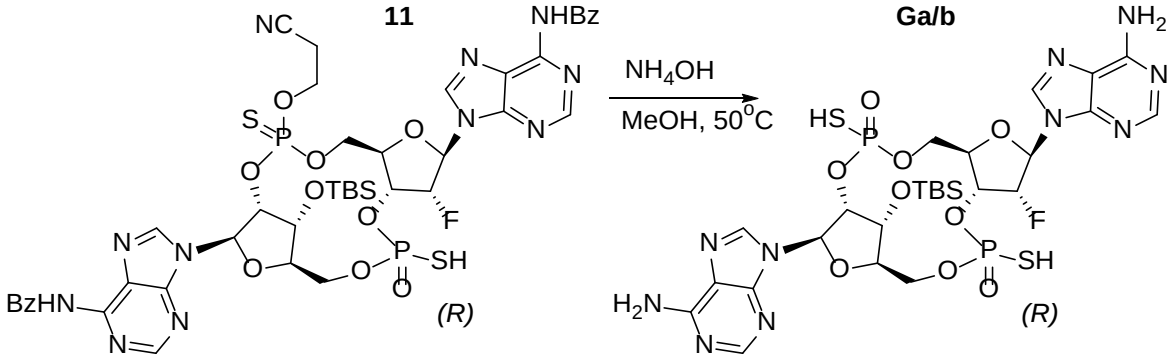
To a solution of $Cl_2CHCOOH$ in CH_2Cl_2 (6% v/v, 200 mL) was added compound **2-1** from the previous step (12.4 g, assume 9.15 mmol) and H_2O (2.00 g, 111 mmol, 2.0 mL, 12.1 eq). The reaction mixture was stirred at RT for 0.5 h, then quenched with pyridine (100 mL) and concentrated under reduced pressure to afford compound **2-2** (9.64 g), which was used without further purification in the next step.

Step 3



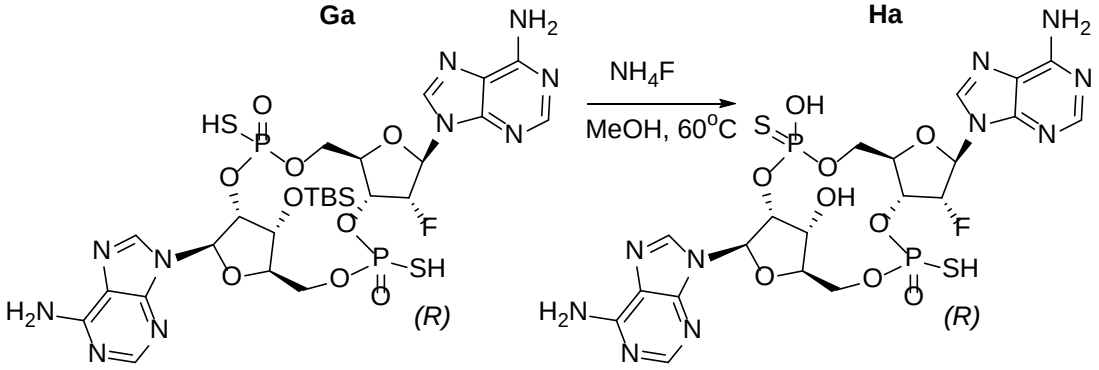
To a solution of compound **7** from the previous step (9.63 g, assume 9.15 mmol) in pyridine (200 mL) was added DMOCP (5.90 g, 32.0 mmol, 3.5 eq) and the mixture was stirred for 0.5 h at RT. 3H-1,2-Benzodithiol-3-one 1,1-dioxide (2.75 g, 13.7 mmol, 1.5 eq) was then added, and the resulting mixture and stirred at RT for further 30 minutes. The reaction mixture was quenched by addition of 3.4% aq. $NaHCO_3$ (1.0 L), and then extracted with EtOAc mL (2x 500 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (CH_2Cl_2/MeOH = 30/1 to 10/1) to give compound **11** (3.2 g, 2.1 mmol) as a mixture of diastereoisomers which was used as such in the following step.

Step 4



To a solution of compound **11** from the previous step (2.0 g, 1.87 mmol) in MeOH (10 mL) was added NH_4OH (18.2 g, 519 mmol, 277 eq). The mixture was stirred at 50 °C for 16 h in a pressure safe steel vessel, then concentrated under reduced pressure. The residue was purified by prep-HPLC [PHENOMENEX ® LUNA® C18 250*50 10 um; mobile phase: A: H_2O (10mM NH_4HCO_3); B: MeCN; A%-B%= 10%-40%, 20 minutes] to give two products: compound **Ga** (R_pR_p or S_pR_p diastereoisomer, 180 mg, 0.209 mmol) and compound **Gb** (S_pR_p or R_pR_p diastereoisomer, 200 mg, 0.228 mmol) as white solids.

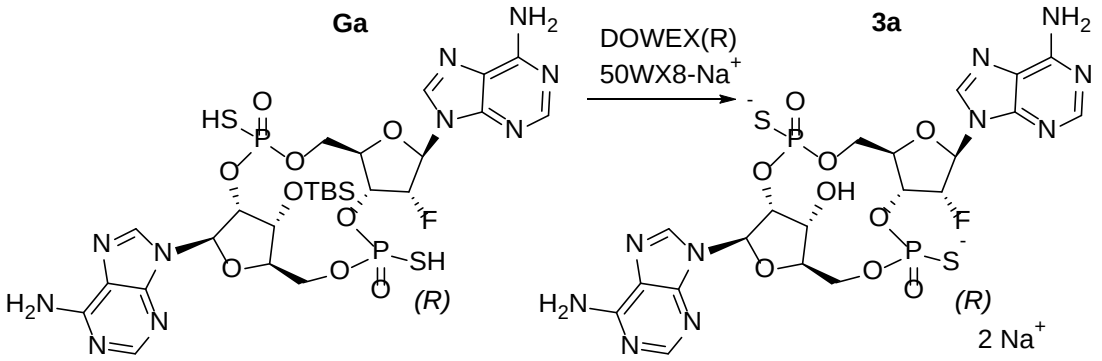
Step 5



To a solution of compound **Ga** (100 mg, 119 umol) in MeOH (3.0 mL) was added NH_4F (44.1 mg, 1.19 mmol, 10 eq) and the resulting mixture was stirred at 60 °C for 16 h. The reaction mixture was then allowed to cool to RT and concentrated under reduced pressure. The residue was taken up in H_2O (0.5 mL), cooled to 10°C and kept stirring for 30 minutes, then filtered and the filter cake was collected to give compound **Ha** (30.0 mg, 41.3 umol) as a white solid; MS(ES^+) $C_{20}H_{24}FN_{10}O_9P_2S_2$ requires: 693, found: 693.2 $[M+H]^+$; ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 8.98 (s, 1H), 8.22 (s, 2H), 7.82 (s, 1H), 6.45 (d, J = 14.4 Hz, 1H), 6.33 (d, J = 8.0 Hz, 1H),5.62 (d, J = 53.8 Hz, 1H), 5.32 (m, 1H), 5.07-5.13 (m, 1H), 4.36-4.46 (m, 5H), 4.06 (d, J = 11.2 Hz, 1H), 3.86 - 3.90 (m, 1H).

Reaction of compound **Gb** in a similar manner afforded compound **Hb** (30.0 mg, 41.3 umol) as a white solid; MS(ES^+) $C_{20}H_{24}FN_{10}O_9P_2S_2$ requires: 693, found: 693.2$[M+H]^+$; ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 8.76 (s, 1H), 8.49 (s, 1H), 8.24 (s, 1H), 8.18 (s, 1H), 6.34-6.41 (m, 2H), 5.70 (dd, J = 51.8 Hz, 1H), 5.22-5.239 (m, 2H), 4.50-4.59 (m, 4H), 4.32(s, 1H), 4.03-4.07 (m, 1H).

Step 6



To a solution of compound **Ga** (30.0 mg, 41.3 umol) in H_2O (10.0 mL) was added Dowex®-50WX8 (Na^+ form; 300 mg) and the mixture was stirred at RT for 4 h. The reaction was then filtered, and the filtrate was lyophilized to give **3a** (30 mg, 40.6 umol) as a white solid.; MS(ES^+) $C_{20}H_{24}FN_{10}O_9P_2S_2$ requires: 693, found: 693.0 $[M+H]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm 8.56 (s, 1H), 8.40 (s, 1H), 8.17 (s, 1H), 8.13 (s, 1H), 7.34 (s, 2H), 7.22 (s, 2H), 6.23(m, 1H), 6.09(d, J = 8.4 Hz 1H), 5.71(d, J = 52.8 Hz, 1H), 5.54(s, 1H), 5.15-5.30(m, 2H), 3.91-4.37(m, 5H), 3.67-3.70(m, 1H); ^{31}P NMR (162 MHz, CD_3OD) δ ppm 55.97, 53.66; R_t = 1.384 minutes [Waters XBridge Shield RP18 2.1*50mm, 5um; mobile phase: A: H_2O + 10mM NH_4HCO_3; B: MeCN; A%-B%= 0%-30%, 5.2 minutes].

Reaction of compound **Gb** in a similar manner gave **3b** (28.0 mg, 40.6 umol) as a white solid. MS(ES^+) $C_{20}H_{24}FN_{10}O_9P_2S_2$ requires: 693, found: 693.0 $[M+H]^+$; ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 8.86 (br s, 1H), 8.39 (s, 1H), 8.19 (s, 1H), 8.02 (br s, 1H), 6.35-6.41 (m, 2H), 5.70 (d, J = 51.8 Hz, 1H), 5.22-5.27 (m, 2H), 4.35-4.60 (m, 5H), 4.05-4.08 (m, 2H); ^{31}P NMR (162 MHz, CD_3OD) δ ppm 57.39, 52.28; R_t = 1.644 minutes [Waters XBridge Shield RP18 2.1*50mm, 5um; mobile phase: A: H_2O + 10mM NH_4HCO_3; B: MeCN; A%-B%= 0%-30%, 5.2 minutes].