Electronic Supplementary Information Part I

Molecular Recognition of Cationic Phenothiazinium and Phenoxazinium Dyes with π-Extended 2-Deoxyadenosine Nucleotides

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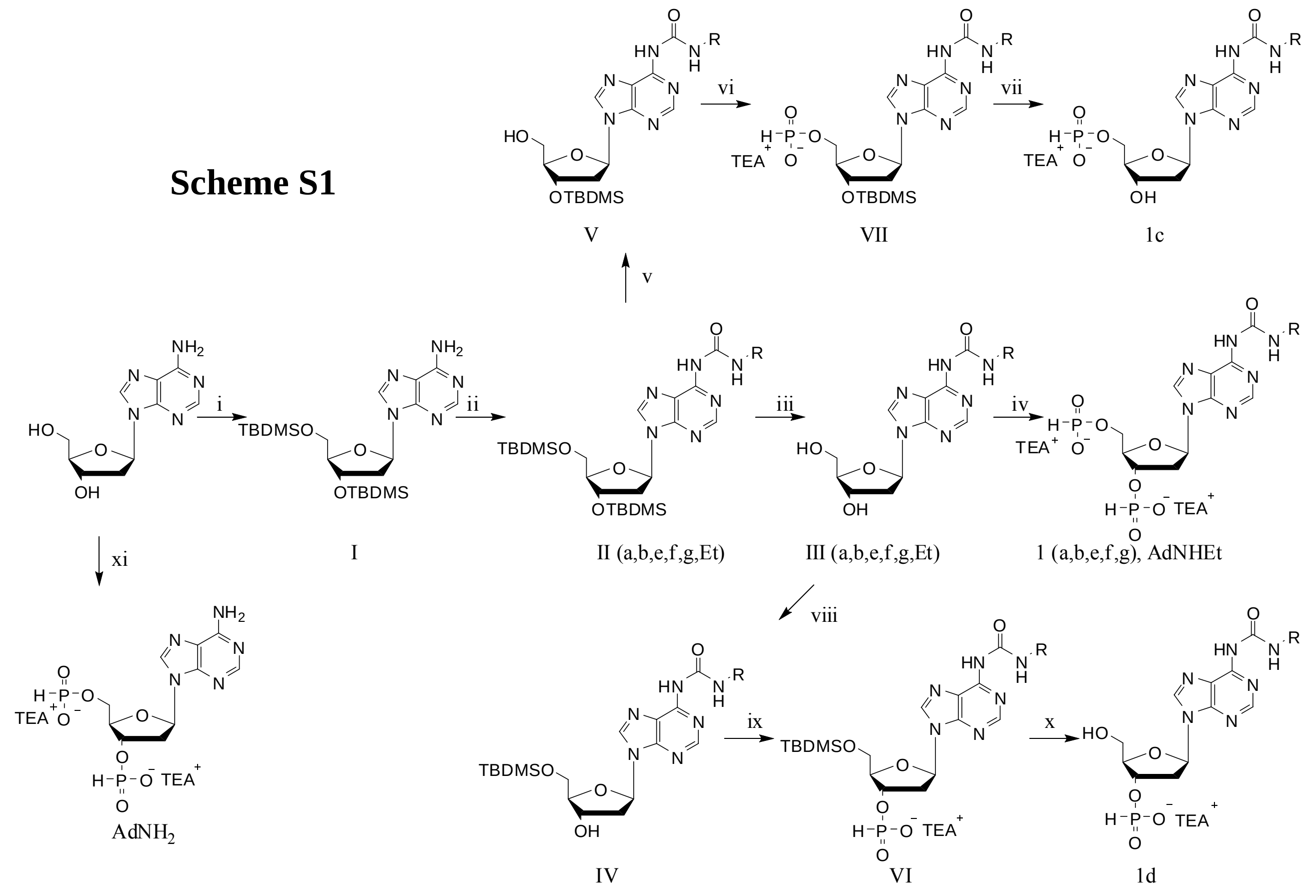
Materials and Methods

Chemicals and anhydrous solvents from commercial sources were used as received. Compounds **1a-1g**, **AdNH$_2$** and **AdNHEt** were synthesized according to Scheme S1 and procedures described below. Mass spectra (FAB and HR-FAB) were taken on a JEOL MStation Mass-Spectrometer. ^{1}H, ^{13}C, ^{31}P and ^{19}F NMR spectra were obtained on a Varian Mercury and Inova Spectrometers at 200 MHz and 400 MHz, respectively. Spectra were in ppm using TMS as reference for ^{1}H in DMSO-d_6 and $CDCl_3$, and solvent signal for ^{13}C nuclei. The ^{31}P NMR spectra were referenced to H_3PO_4 in $CDCl_3$ (external standard). The ^{19}F NMR spectra were referenced to KF in D_2O (internal standard). ^{1}H NMR spectra acquired in D_2O were presaturated to eliminate HDO solvent residual signal as indicated in the figures.

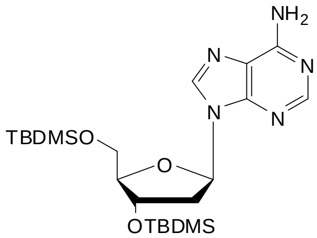
All the chromatographic runs were performed on Waters 600 HPLC System at room temperature using XTerra RP18 column, 5μm, 3.9 mm × 150 mm. A Waters 2996 diode array UV detector was used to monitor signals in the range from 190-400 nm. Samples were dissolved in 1:1 metanol/water in a concentration range of 1-2 mM. The mobile phase consisted in all cases of mixtures in varying proportions of 20 mM MOPS buffer at pH 7.4 previously saturated with 1-octanol and methanol containing 0.25 % (v/v) of 1-octanol. Experiments were conducted under isocratic conditions with MeOH proportions from 20 to 60 % v/v. These experimental conditions were used in order to obtain ELog P_{oct} values for nucleotides **1a-1g**, **AdNH2** and **AdNHEt** according to the method reported by Lombardo and Abraham.[1] These results will be published elsewhere in due course.

[1] F. Lombardo, M. Y. Shalaeva, K.A. Tupper, F. Gao and M.H. Abraham *J. Med. Chem.*, 2000, **43**, 2922.

Scheme S1



i) TBDMS-Cl, TEA, DMF, rt, 8 h., 95%; **ii)** Arylisocyanate, TEA, DMF, rt, 8 h.; **iii)** TBAF, THF, rt, 2 h.; **iv)** (a) DPP, Py, rt,, 15 min., (b) TEA:H_2O 15 min.; **v)** TFA:H_2O, THF, rt, 2 h.; **vi**) (a) DPP, Py, rt, 15 min., (b) TEA:H_2O, rt, 15 min.; **vii)** TBAF, THF, rt, 2 h.; **viii)** TBDMS-Cl, TEA, DMF, rt, 8 h., **ix)** (a) DPP, Py, rt, 15 min., (b) TEA:H_2O 15 min.; **x)** TBAF, THF, rt, 2 h.; **xi**) (a) DPP, Py, rt, 15 min., (b) TEA:H_2O, rt, 15 min.

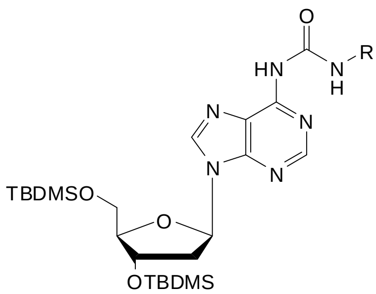


I

Synthesis of I

2-Deoxyadenosine monohydrated (5.4g, 20 mmol) was co-evaporated in dry pyridine (3×10 mL). The solid was dissolved in dry DMF (30 mL) followed by addition of triethylamine (70 mmol) via syringe. The solution was stirred at room temperature for 5 minutes, then excess TBDMS-Cl (9g, 60 mmol) was added as a solid in one portion and the mixture was stirred at room temperature under nitrogen atmosphere. The progress of the reaction was followed by TLC. After 8 h the reaction was quenched by addition of methanol (2 mL). The solvent was evaporated under vacuum and the residue was purified by precipitation with hexane.

I. Yield 95%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 8.35 (1H, s, H-Purine), 8.20 (1H, s, H-Purine), 6.46 (1H, dd, J_{HH} = 6.2 Hz, H1'-Deoxyribose), 6.19 (2H, s, NH_2-Deoxyribose), 4.63-4.60 (1H, m, H3'-Deoxyribose), 4.06-4.01 (1H, m, H4'-Deoxyribose), 3.94-3.75 (2H, m, H5'-Deoxyribose), 2.67-2.52 (1H, m, H2'-Deoxyribose), 2.25- 2.43 (1H, m, H2''-Deoxyribose), 0.93 (9H, s, *t*-butyl-TBDMS), 0.94 (9H, s, *t*-butyl-TBDMS), 0.12 (6H, s, *di*-Me-TBDMS) 0.11 (6H, s, *di*-Me-TBDMS); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 156.02, 152.99, 149.53, 138.86, 120.02, 87.99, 84.49, 72.02, 62.94, 41.50, 26.19, 26.00, 18.66, 18.24, -4.37, -4.52, -5.09, -5.20; FAB-MS: *m*/*z* 480 [(M+H$^+$)].



IIa, IIb, IIe, IIf, IIg and IIEt

General procedure for synthesis of IIa, IIb, IIe, IIf, IIg and IIEt

Nucleoside I (1 mmol) was co-evaporated in dry pyridine (3×5 mL). The solid was dissolved in dry DMF (5 mL) followed by addition of triethylamine (1.7 mmol) via syringe. The corresponding arylisocyanate (1.5 mmol) was added in one portion to the stirred solution under nitrogen atmosphere. The mixture was stirred at room temperature. The progress of the reaction was followed by TLC. After 8 h the reaction was quenched by addition of methanol (1 mL). The solvent was evaporated under vacuum and the residues were purified by precipitation with methanol.

IIa. Yield. 71%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 11.85 (1H, s, NH-carbamoyl), 8.66 (1H, s, NH-Carbamoyl), 8.62 (1H, s, H-purine), 8.46 (1H, s, H-purine), 7.66 (2H, d, J_{HH} = 8.8 Hz H-Phenyl), 7.36 (2H, t, J_{HH} = 8 Hz, H-Phenyl), 7.11 (1H, t, J_{HH} = 6.3 Hz, H-Phenyl), 6.51 (1H, dd, J_{HH} = 6.4 Hz, H1'-Deoxyribose), 4.67-4.64 (1H, m, H3'-Deoxyribose), 4.06-4.02 (1H, m, H4'-Deoxyribose), 3.92-3.80 (2H, m, H5'-Deoxyribose), 2.71-2.68 (1H, m, H2'-Deoxyribose), 2.52-2.49 (1H, m, H2''-Deoxyribose), 0.91 (9H, s, *t*-butyl-TBDMS), 0.90 (9H, s, *t*-butyl-TBDMS), 0.12 (6H, s, *di*-Me-TBDMS), 0.07 (6H, s, *di*-Me-TBDMS); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.67, 150.91, 150.38, 150.15, 142.10, 138.28, 129.17, 124.02, 120.51, 88.30, 84.79, 72.11, 62.99, 41.35, 26.23, 26.08, 18.72, 18.33, -4.30, -4.43, -5.07, -5.13. FAB-MS: *m*/*z* 599 [(M+H$^+$)].

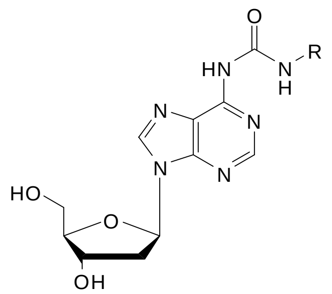
IIb. Yield, 93.1%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 12.28 (1H, s, NH-carbamoyl), 9.14 (1H, s, NH-Carbamoyl), 8.70 (1H, s, H-purine), y 8.45 (1H, s, H-purine), 8.24 (2H, t, J_{HH} = 7.2 Hz, H-Naphthyl), 7.89 (1H, d, J_{HH} = 8.8 Hz, H-Naphthyl), 7.69-7.55 (4H, m, H-Naphthyl), 6.52 (1H, dd, J_{HH} = 6.2 Hz H1'-Deoxyribose), 4.68-4.62 (1H, m, H3'-Deoxyribose), 4.07-4.02 (1H, m, H4'-Deoxyribose), 3.93-3.74 (2H, m, H5'-Deoxyribose), 2.76-2.63 (1H, m, H2'-Deoxyribose), 2.55-43 (1H, m, H2''-Deoxyribose), 0.90 (9H, s, *t*-butyl-TBDMS), 0.92 (9H, s, *t*-butyl-TBDMS), 0.117 (6H, s, *di*-Me-TBDMS), 0.082 (6H, s, *di*-Me-TBDMS). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.98, 150.88, 150.37, 150.19, 141.93, 134.30, 133.47, 128.87, 126.84, 126.39, 126.17, 126.012, 124.73, 121.405, 121.087, 119.06, 88.27, 84.80, 71.99, 62.91, 41.51, 26.21, 26.038, 18.70, 18.29, -4.33, -4.47, -5.09. FAB-MS: *m*/*z* 649 [(M+H$^+$)].

IIe. Yield, 80%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 12.13 (1H, s, NH-carbamoyl), 11.02 (1H, s, NH-Carbamoyl), 8.75 (1H, s, H-purine), 8.57 (1H, s, H-purine), 7.63 (2H, dd, J_{HH} = 4.8, 8.8 Hz, H-Phenyl), 6.74 (2H, t, J_{HH} = 8.4Hz, H-Phenyl), 6.56 (1H, dd, J_{HH} = 6.6 H1'-Deoxyribose), 5.04 (1H, m, H3'-Deoxyribose), 4.47 (1H, m, H4'-Deoxyribose), 4.09 (2H, m, H5' y H5"-Deoxyribose), 2.79-2.71 (2H, m, H2' y H2''-Deoxyribose), 0.95 (9H, s, *t*-butyl-TBDMS), 0.83 (9H, s, *t*-butyl-TBDMS), 0.12 (6H, s, *di*-Me-TBDMS), 0.51 (12H, s, *di*-Me-TBDMS). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.59, 150.75, 150.62, 149.67, 142.27, 134.16, 122.15, 121.89, 120.68, 115.85, 115.42, 85.85, 84.40, 74.52, 63.55, 40.39, 26.17, 26.12, 18.68, 18.23, -4.44, -4.59,-5.34, -5.29. ^{19}F-NMR (D_2O, 188 MHz): δ_{ppm} = -116.35 (1F, t, F_{para}-Phenyl); FAB-MS: *m*/*z* 617 [(M+H$^+$)].

IIf. Yield 85%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 12.23 (1H, s, NH-carbamoyl), 9.46 (1H, s, NH-Carbamoyl), 8.61 (1H, s, H-purine), 8.50 (1H, s, H-purine), 7.27 (2H, d, J_{HH} = 8 Hz, H-Phenyl), 6.50 (2H, m, J_{HH} = 6.2 Hz, H1'-Deoxyribose, H-Phenyl), 4.65-4.64 (1H, m, H3'-Deoxyribose), 4.04-4.02 (1H, m, H4'-Deoxyribose), 4.02-3.78 (2H, m, H5'-Deoxyribose), 2.74-2.64 (1H, m, H2'-Deoxyribose), 2.53-2.42 (1H, m, H2''-Deoxyribose), 0.92 (9H, s, *t*-butyl-TBDMS), 0.89 (9H, s, *t*-butyl-TBDMS), 0.11 (6H, s, *di*-Me-TBDMS); 0.04 (6H, s, *di*-Me-TBDMS); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.73, 150.73, 150.57, 149.88,142.42,140.89, 140.63, 140.35, 121.05, 103.54, 88.33, 84.81, 72.08, 62.97, 41.33, 26.19, 26.05, 18.68, 18.68, -4.33, -4.47, -5.12, -5.18. ^{19}F-NMR (D_2O, 376 MHz): δ_{ppm} = -111.58 (2F, t, F_{meta}-Phenyl); FAB-MS: *m*/*z* 635 [(M+H$^+$)].

IIg. Yield. 90.1%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 11.73 (1H, s, NH-carbamoyl), 9.68 (1H, s, NH-Carbamoyl), 8.61 (2H, s, H-purine), 6.51 (1H, dd, J_{HH} = 6.4 Hz, H1' -Deoxyribose), 4.66-4.64 (1H, m, H3'-Deoxyribose), 4.04-4.02 (1H, m, H4'-Deoxyribose), 3.83-3.73 (2H, m, H5'-Deoxyribose), 2.71-2.76 (1H, m, H2'-Deoxyribose), 2.50-2.48 (1H, m, H2''-Deoxyribose), 0.91 (9H, s, *t*-butyl-TBDMS), 0.87 (9H, s, *t*-butyl-TBDMS), 0.10 (6H, s, *di*-Me-TBDMS), 0.5 (12H, s, *di*-Me-TBDMS). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 152.00, 150.70, 149.74, 143.04, 121.111, 88.27, 84.68, 71.94, 62.85, 41.19, 26.15, 26.01, 18.65, 18.28, -4.43, -4.57,-5.35, -5.25. ^{19}F-NMR (D_2O, 376 MHz): δ_{ppm} = -147.92 (2F, d, F_{orto}-Phenyl), -158.18 (1F, t, F_{para} -Phenyl), -164.85 (2F, t, F_{meta} -Phenyl), FAB-MS: *m*/*z* 689 [(M+H$^+$)].

IIEt. Yield 94%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 9.35 (1H, t, NH-carbamoyl), 8.62 (1H, s, H-Purine), 8.42 (1H, s, H-Purine), 8.02 (1H, s, NH-Carbamoyl) 6.56 (1H, dd, J_{HH} = 6.2 Hz, H1'-Deoxyribose), 4.99 (1H, m, H3'-Deoxyribose), 4.36 (1H, m, H4'-Deoxyribose), 4.04 (2H, m, H5'-Deoxyribose), 3.42-3.35 (2H, m, CH_2-Et), 2.812-2.65 (2H, m, H2' y H2''-Deoxyribose), 1.02 (3H, m, CH_3-Et), 0.93 (9H, s, *t*-butyl-TBDMS),0.92 (9H, s, *t*-butyl-TBDMS), 0.12 (6H, s, *di*-Me-TBDMS) 0.11 (6H, s, *di*-Me-TBDMS); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 156.78, 156.02, 152.99, 149.53, 138.86, 120.02, 87.99, 84.49, 72.02, 62.94, 41.50, 34.15, 26.19, 26.00, 18.66, 18.24, 14.52, -4.37, -4.52, -5.09, -5.20; FAB-MS: *m*/*z* 551 [(M+H$^+$)].



IIIa, IIIb, IIIe, IIIf, IIIg and IIIEt

General procedure for synthesis of IIIa, IIIb, IIIe, IIIf and IIIg

The corresponding nucleoside (**IIa, IIb, IIe, IIf, or IIg**, 1 mmol) was co-evaporated in dry THF (3×5 mL). The solids were dissolved in dry THF (6 mL) followed by addition of 1M TBAF solution in THF (3 mmol) via syringe under nitrogen atmosphere. The mixture was stirred at room temperature. The progress of the reaction was followed by TLC. After 2 h the reaction was quenched by addition of methanol (1 mL). The solvent was evaporated under vacuum and the residues were purified by precipitation with methanol.

IIIa. Yield 85%; ^{1}H-NMR ($CDCl_3$-MeOD, 200 MHz): δ_{ppm} = 11.66 (1H, s, NH-carbamoyl), 9.41 (1H, s, NH-carbamoyl), 8.44 (1H, s, H-purine), 8.22 (1H, s, H-purine), 7.44 (2H, d, J_{HH} = 8 Hz, H-Phenyl), 7.21 (2H, t, J_{HH} = 8Hz, H-Phenyl), 6.97 (1H, t, J_{HH} = 7.2Hz, H-Phenyl), 6.32 (1H, dd, J_{HH} = 6.2 Hz, H1'-Deoxyribose), 4.05-4.48 (1H, m, H3'-Deoxyribose), 3.82-3.60 (3H, m, H4', H5'-Deoxyribose), 2.77-2.64 (1H, m, H2'-Deoxyribose), 2.34-2.25 (1H, m, H2''-Deoxyribose). ^{13}C-NMR ($CDCl_3$-MeOH, 50 MHz): δ_{ppm} = 151.61, 150.35, 149.42, 142.40, 137.37, 128.86, 124.09, 121.14, 120.43, 88.80, 86.62, 71.82, 62.61, 40.75. FAB-MS: *m*/*z* 371 [(M+H^{+})].

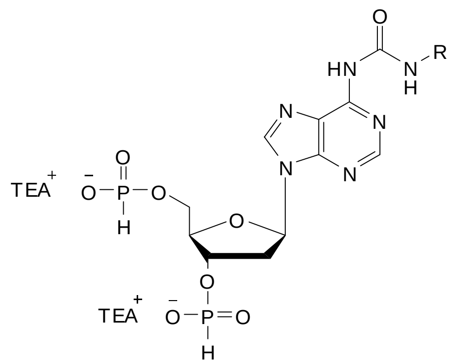
IIIb. Yield 90%. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ_{ppm} = 12.37 (1H, s, NH-carbamoyl), 10.39 (1H, s, NH-carbamoyl), 8.80 (1H, s, H-purine), y 8.67 (1H, s, H-purine), 8.18 (2H, t, J_{HH} = 6.8Hz, H-Naphthyl), 7.92 (1H, d, J_{HH} = 8Hz, H-Naphthyl), 7.69-7.53 (4H, m, H-Naphthyl), 6.43 (1H, dd, J_{HH} = 6.8Hz, H1'-Deoxyribose), 5.34 (1H, d, J_{HH} =4.4 Hz, OH3'), 5.014 (1H, t, J_{HH} =5.6 Hz, OH5'), 4.412 (1H, m, H3'-Deoxyribose), 3.87-3.86 (1H, m, H4'-Deoxyribose), 3.63-3.42 (2H, m, H5'-Deoxyribose), 2.77-2.67 (1H, m, H2'-Deoxyribose), 2.38-2.26 (1H, m, H2''-Deoxyribose), ^{13}C-NMR ($CDCl_3$-MeOH, 50 MHz): δ_{ppm} = 157.01, 155.99, 155.65, 155.55, 147.72, 139.11, 139.06, 133.96, 132.01, 131.43, 131.28, 131.01, 129.06, 126.44, 126.03, 122.74, 93.52, 89.33, 76.15, 67.09, 54.08. FAB-MS: *m*/*z* 421 [(M+H^{+})].

IIIe. Yield, 80%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 12.26 (1H, s, NH-carbamoyl), 11.38 (1H, s, NH-carbamoyl), 8.72 (1H, s, H-purine), 8.56 (1H, s, H-purine), 7.59 (2H, dd, J_{HH} = 4.8, 8.8 Hz, H-Phenyl), 6.72 (2H, d, J_{HH} =8.4Hz, H-Phenyl), 6.58 (1H, dd, J_{HH} =6.6 H1'-Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.42 (1H, m, H4'-Deoxyribose), 4.11 (2H, m, H5'-Deoxyribose), 2.78-2.72 (2H, m, H2' y H2'-Deoxyribose), ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.60, 150.80, 150.58, 149.69, 142.23, 134.13, 122.11, 121.90, 120.63, 115.87, 115.39, 85.87, 84.41, 74.49, 63.57, 40.38, ^{19}F-NMR (D_2O, 188 MHz): δ_{ppm} = -116.35 (1F, t, F_{para}-Phenyl; FAB-MS: *m*/*z* 389 [(M+H^{+})].

IIIf. Yield, 83%; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ_{ppm} = 12.18 (1H, s, NH-carbamoyl), 10.38 (1H, s, NH-carbamoyl), 8.7 (2H, s, H-purine), 7.4 (2H, d, J_{HH} =8 Hz, H-Phenyl), 6.80 (1H, t, Hz, H-Phenyl), 6.41 (1H, dd, H1'-Deoxyribose), 5.36 (1H, d, -OH3'), 5.03 (1H, s, -OH5'), 4.45 (1H, m, H3'-Deoxyribose), 3.92 (1H, m, H4'-Deoxyribose), 3.46 (2H, m, H5'-Deoxyribose), 2.78 (1H, m, H2'-Deoxyribose), 2.38 (1H, m, H2''-Deoxyribose), ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.63, 151.29, 150.77, 150.22, 143.11, 142.12, 142.04, 141.95, 121.28, 103.23, 88.73, 84.49, 71.32, 62.25, 45.11. ^{19}F-NMR (D_2O, 376 MHz): δ_{ppm} = -111.58 (2F, t, F_{meta}-Phenyl), FAB-MS: *m*/*z* 407 [(M+H^{+})].

IIIg. Yield, 90.1%; ^{1}H-NMR (DMSO-d$_6$, 200 MHz): δ_{ppm} = 11.44 (1H, s, NH-carbamoyl), 10.78 (1H, s, NH-carbamoyl), 8.71 (1H, s, H-purine), 8.62 (1H, s, H-purine), 6.45 (1H, dd, J_{HH} = 6.6 Hz, H1' - Deoxyribose), 5.37 (1H, d, -OH3'), 5.03 (1H, t, -OH5'), 4.40 (1H, m, H3'-Deoxyribose), 3.90-3.89 (1H, m, H4'-Deoxyribose), 3.66-3.50 (2H, m, H5'-Deoxyribose), 2.82-2.69 (1H, m, H2'-Deoxyribose), 2.49-2.29 (1H, m, H2''-Deoxyribose), ^{13}C-NMR ($CDCl_3$-MeOH, 50 MHz): δ_{ppm} = 151.79, 151.21, 150.11, 143.28, 121.36, 88.75, 84.53, 71.32, 62.25, 60.47. ^{19}F-NMR (D_2O, 376 MHz): δ_{ppm} -146.98 (2F, d, F_{ortho}-Phenyl), -157.36 (1F, t, F_{para} -Phenyl), -162.46 (2F, t, F_{meta}-Phenyl), FAB-MS: *m*/*z* 461 [(M+H$^+$)].

IIIEt. Yield 86%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 9.38 (1H, t, NH-carbamoyl), 8.59 (1H, s, H-Purine), 8.58 (1H, s, H-Purine), 8.04 (1H, s, NH-Carbamoyl) 6.58 (1H, dd, J_{HH} = 6.2 Hz, H1'-Deoxyribose), 4.89 (1H, m, H3'-Deoxyribose), 4.28 (1H, m, H4'-Deoxyribose), 4.06 (2H, m, H5'-Deoxyribose), 3.41-3.36 (2H, m, CH_2-Et), 2.80-2.63 (2H, m, H2' y H2''-Deoxyribose), 1.03 (3H, m, CH_3-Et); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 156.75, 156.06, 152.97, 149.55, 138.83, 120.05, 87.93, 84.52, 71.98, 62.85, 41.62, 34.23, 14.53, FAB-MS: *m*/*z* 323 [(M+H$^+$)].

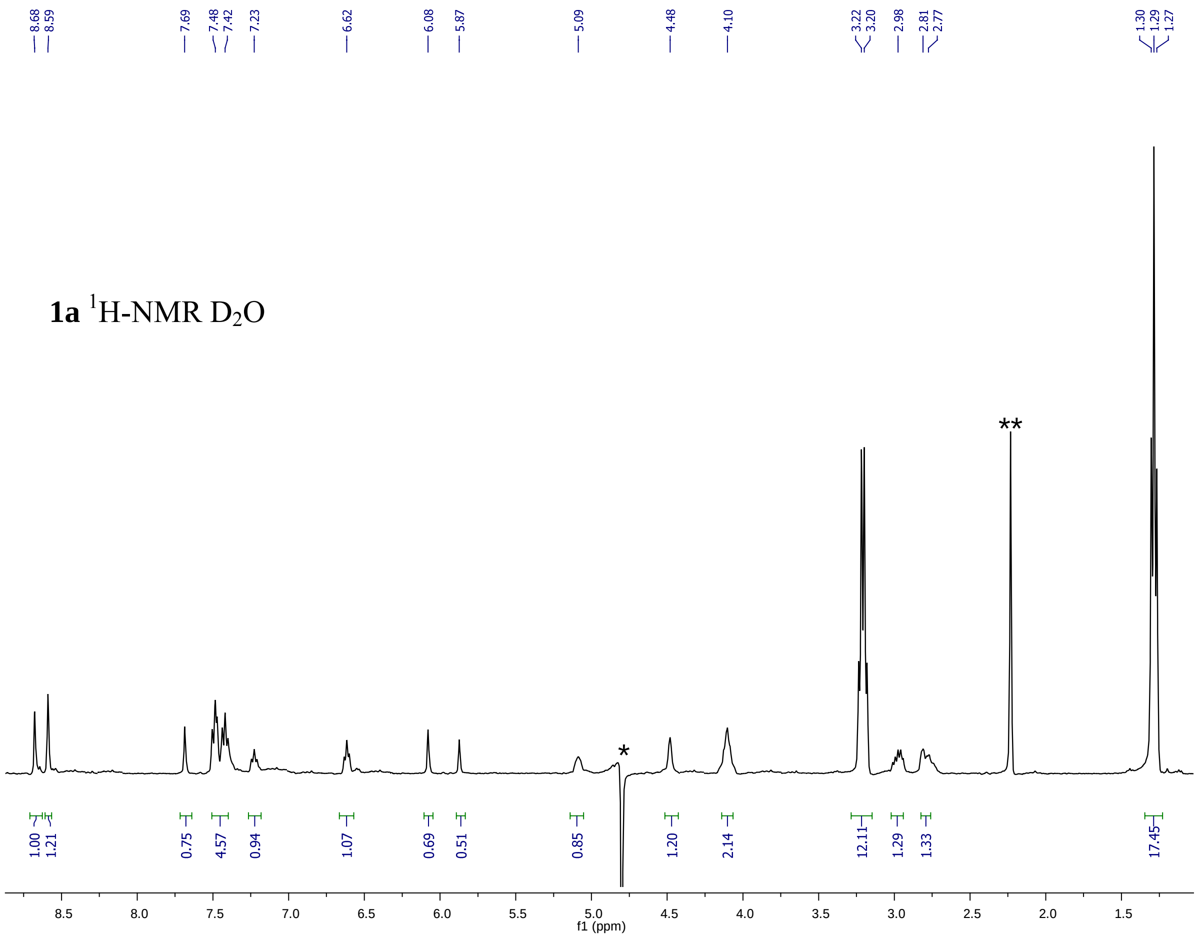


1a, 1b, 1e, 1f, 1g, AdNH$_2$ and AdNHEt

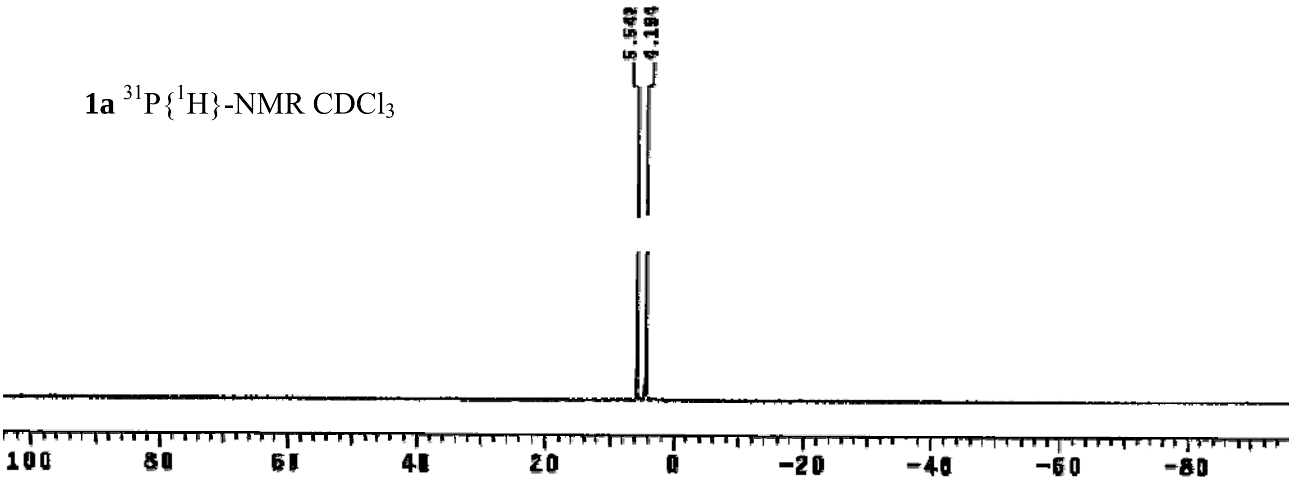
General procedure for synthesis of 1a, 1b, 1e, 1f, 1g, AdNH2 and AdNHEt

The corresponding nucleoside (IIIa, IIIb, IIIe, IIIf, IIIg, IIIEt or 2-Deoxyadenosine monohydrated (1 mmol) was co-evaporated in dry pyridine (3 x 5 mL). The solids were dissolved in dry pyridine (5 mL) followed by addition of diphenylphosphate (7 mmol) via syringe. The reaction mixture was stirred under nitrogen atmosphere at room temperature. The progress of the reaction was followed by TLC. After 15 minutes the reaction was quenched by addition of the mixture of water-triethylamine (1:1 v/v, 2 ml) and was stirred for 15 minutes. The solvent was evaporated to give a yellow oil. The products were purified by column chromatography on silica gel using a stepwise gradient of methanol (0:60%) in methylenechloride.

1a. Yield 87%. ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.68 (1H, s, H-purine), 8.59 (1H, s, H-purine), 7.69, 6.08 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.48 (2H, d, J_{HH} = 8 H-Phenyl), 7.42, 5.87 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.42 (2H, t, J_{HH} = 8. Hz, H-Phenyl), 7.23 (1H, t, J_{HH} = 7.2 Hz, H-Phenyl), 6.62 (1H, dd, J_{HH} = 6.4 Hz, H1'-Deoxyribose), 5.09 (1H, m, H3'-Deoxyribose), 4.48 (1H, s, H4'-Deoxyribose), 4.10 (2H, m, H5'-Deoxyribose), 3.21 (12H, q, J_{HH} = 7.2 Hz, CH_2-TEA), 2.98, 2.81, 2.77 (2H, 2m, H2' y H2''-Deoxyribose), 1.29 (18H, q, J_{HH} =7.2 Hz, CH_3-TEA); ^{13}C-NMR ($CDCl_3$-MeOH, 50 MHz): δ_{ppm} = 151.63, 150.33, 149.45, 142.47, 137.33, 128.89, 124.06, 121.12, 120.45, 88.88, 86.69, 71.82, 62.64, 45.67, 40.70, 8.73, ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} = 5.54 (1P, H-Phosphonate) 4.19 (1P, H-Phosphonate). HR-MS: *m/z* (FAB$^-$) 497.1088 (M^- $C_{17}H_{19}O_8N_6P_2^-$ requires 497.0745).



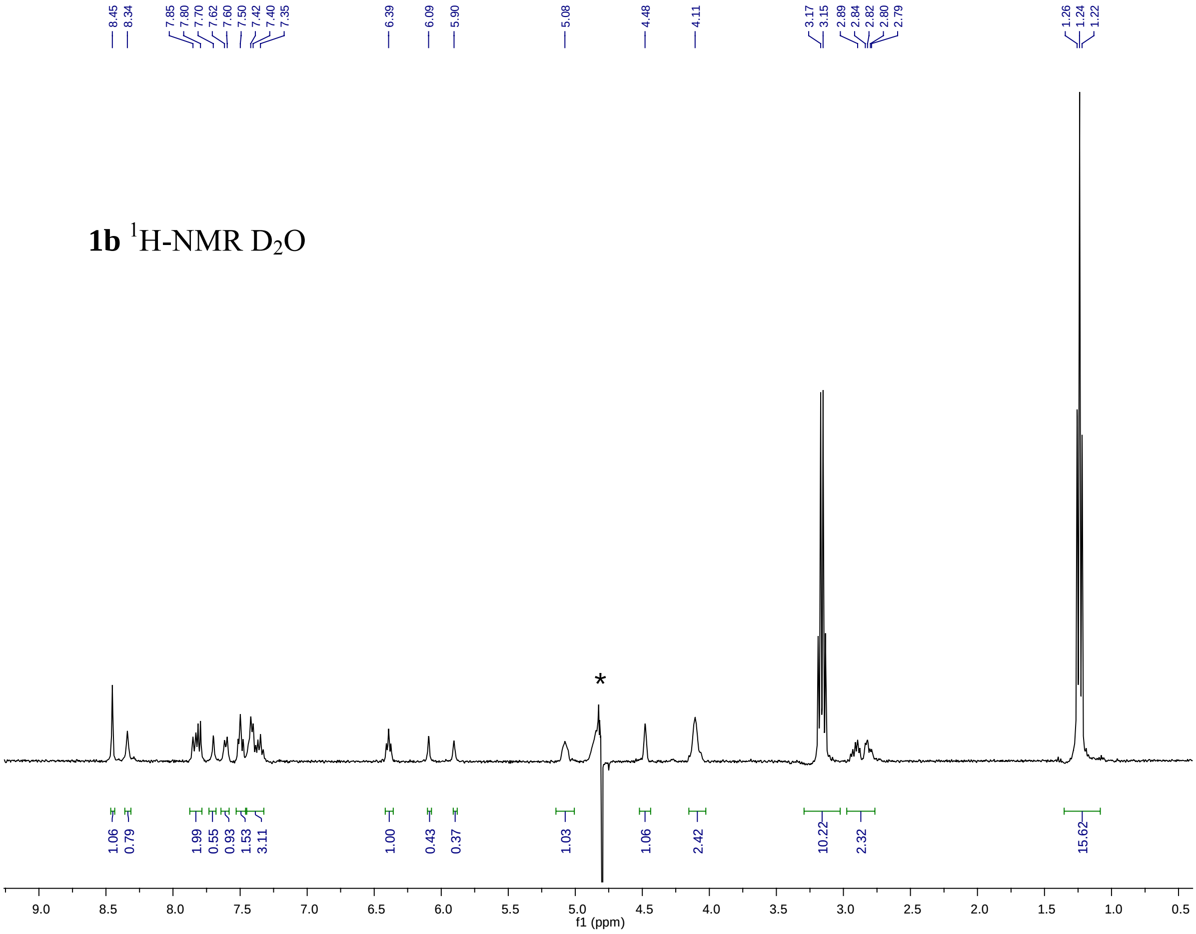
* HDO suppression. ** Acetone



1a $^{31}P\{^{1}H\}$-NMR $CDCl_3$

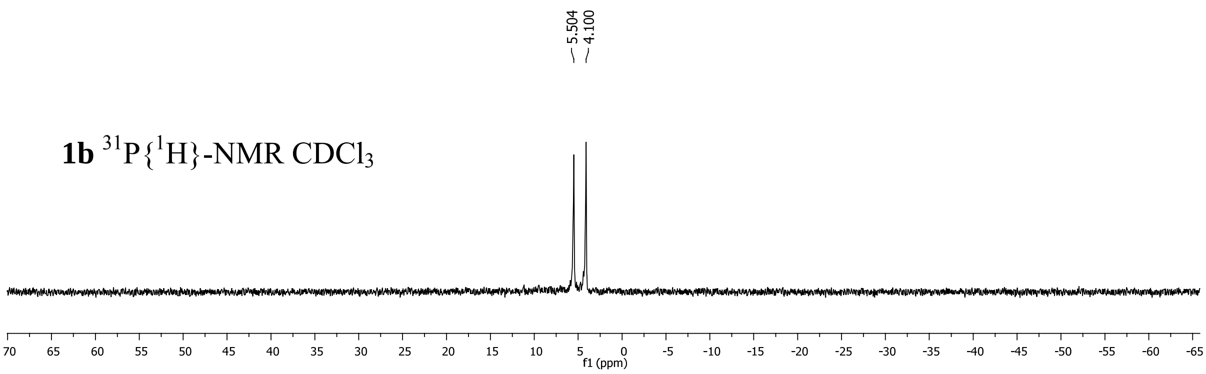
1b. Yield, 85 %; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.45 (1H, s, H-purine), y 8.34 (1H, s, H-purine), 7.85,7.83 (1H, d, J_{HH} = 8 Hz, H-Naphthyl), 7.81, 7.80 (1H, d, J_{HH} = 4 Hz, H-Naphthyl), 7.70, 6.09 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.62, 7.60 (1H, d, J_{HH} = 8 Hz, H-Naphthyl), 7.50 (1H, t, J_{HH} = 7.4 Hz, H-Naphthyl), 7.50, 5.90 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.42-7.35 (3H, m, H-Naphthyl), 6.39 (1H, dd, J_{HH} = 6.8Hz, H1'-Deoxyribose), 5.08 (1H, m, H3'-Deoxyribose), 4.48 (1H, m, H4'-Deoxyribose), 4.11 (2H, m, H5'-Deoxyribose), 3.16 (12H, s, J_{HH} = 7.2 Hz, CH_2-TEA), 2.89, 2.84-2.79 (2H, m, H2'-Deoxyribose), 1.24 (18H, s, J_{HH} = 7.2 Hz, CH_3-TEA) ^{13}C-NMR ($CDCl_3$-MeOH, 50 MHz): δ_{ppm} = 156.98, 155.87, 155.63, 155.60, 147.71, 139.09, 139.07, 133.95, 131.99, 131.45, 131.31, 130.99, 129.04, 126.48, 126.01, 122.77, 93.55, 89.313, 76.13, 67.11, 54.09, 45.56, 8.72. ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} 5.50 (1P, H-Phosphonate), 4.10 (1P, H-Phosphonate). HR-MS: *m/z* (FAB^-) 547.1467 (M^- $C_{21}H_{21}O_8N_6P_2^-$ requires 547.0902).

1b ^{1}H-NMR D_2O

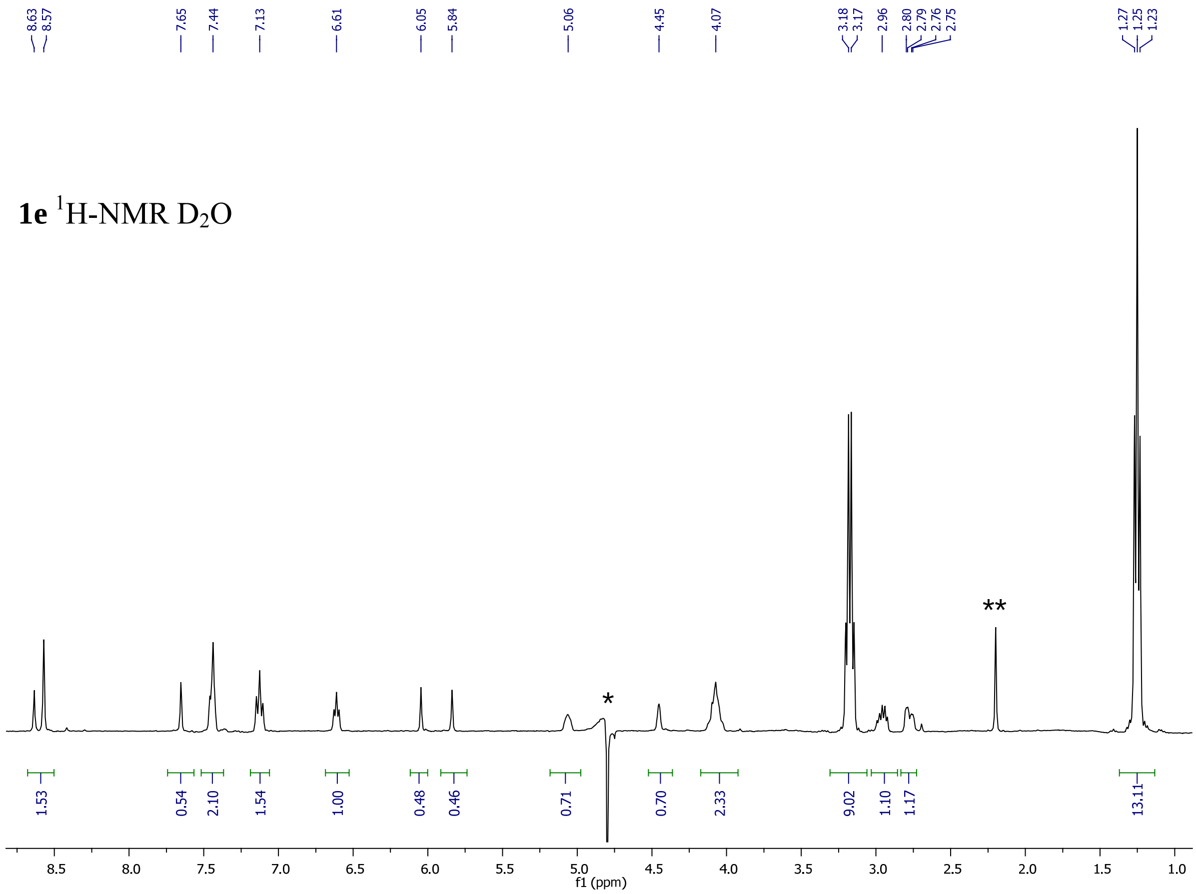


* HDO suppression

1b $^{31}P\{^{1}H\}$-NMR $CDCl_3$

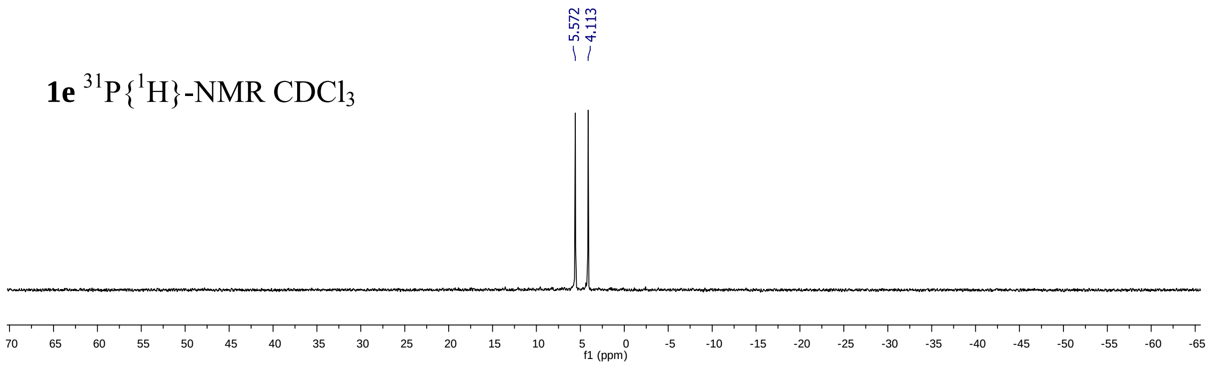


1e. Yield, 85%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.63 (1H, s, H-purine), 8.57 (1H, s, H-purine), 7.65,6.05 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.44 (2H, m, H-Phenyl), 7.44, 5.84 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.13 (2H, t, J_{HH} = 8.8 Hz, H-Phenyl), 6.61 (1H, dd, J_{HH} = 6.8 H1'-Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.45 (1H, m, H4'-Deoxyribose), 4.07 (2H, m, H5'-Deoxyribose), 3.17 (12H, q, J_{HH} =7.2 CH_2-TEA), 2.96, 2.80-2.75 (2H, m, H2' y H2''-Deoxyribose), 1.25 (18H, t, J_{HH} = 7.2 Hz CH_3-TEA). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.59, 150.79, 150.60, 149.67, 142.21, 134.15, 122.08, 121.92, 120.59, 115.85, 115.41, 85.90, 84.39, 74.51, 63.54, 45.69, 40.34, 8.76. ^{19}F-NMR (D_2O, 188 MHz): δ_{ppm} = -116.29 (1F, t, F_{para}-Phenyl); ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} = 5.77 (1P, H-Phosphonate), 4.11 (1P, H-Phosphonate); HR-MS: m/z (FAB$^-$) 515.0141 (M^- $C_{17}H_{18}O_8N_6FP_2^-$ requires 515.0651).

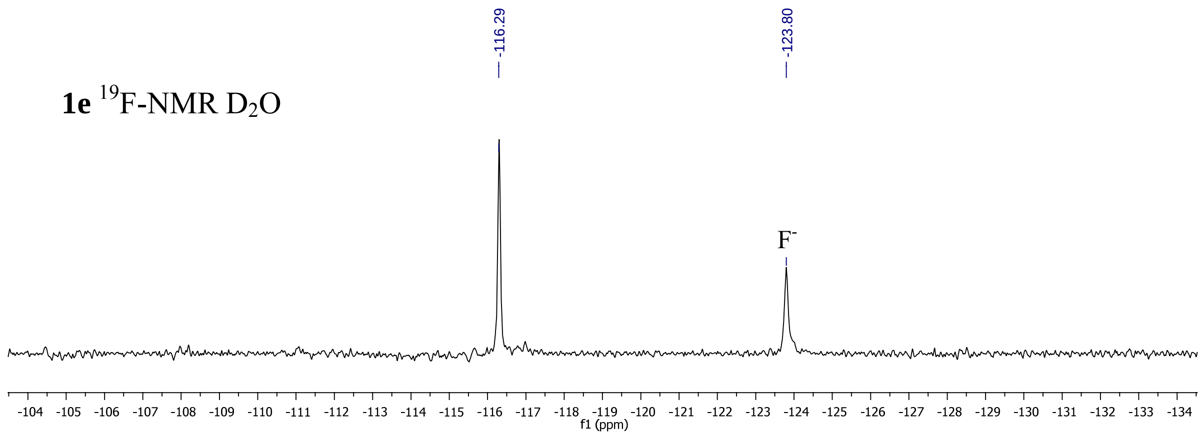


* HDO suppression. ** Acetone

1e $^{31}P\{^{1}H\}$-NMR $CDCl_3$



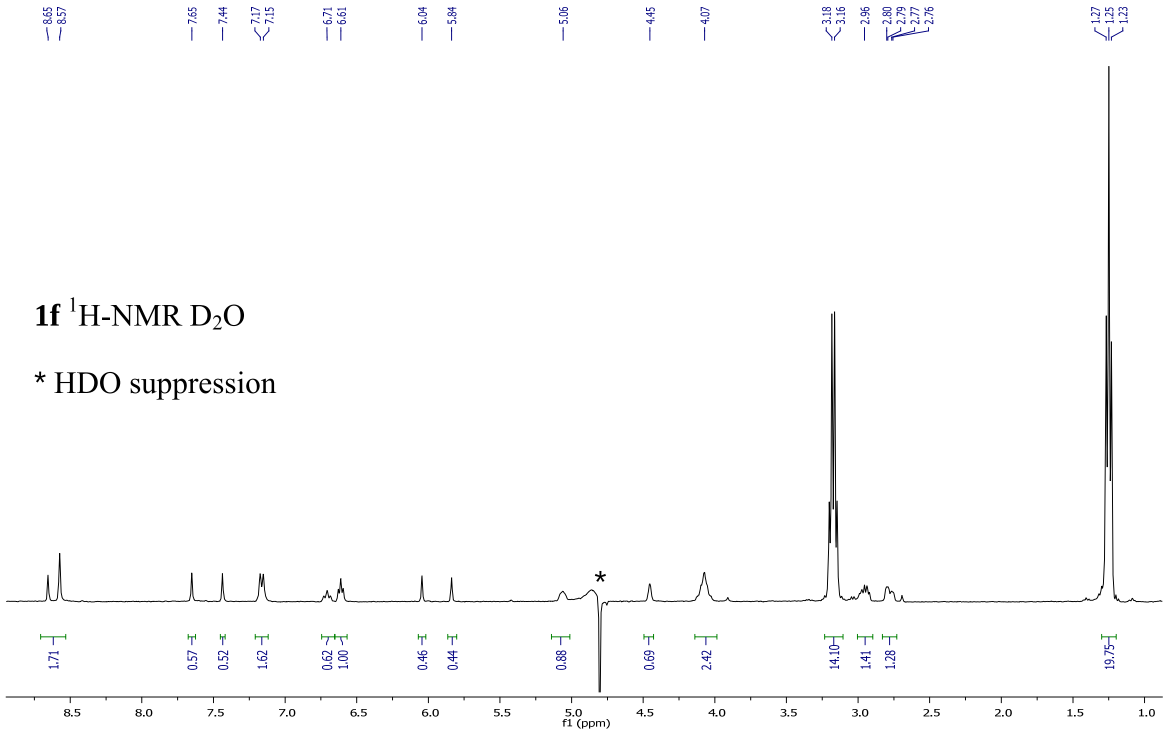
1e ^{19}F-NMR D_2O



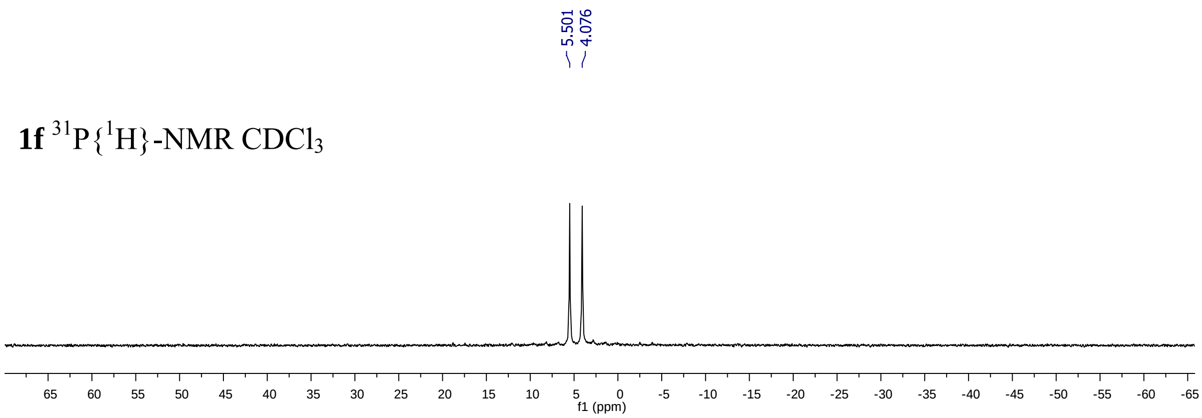
1f. Yield, 79%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.65 (1H, s, H-purine), 8.57 (1H, s, H-purine), 7.65, 6.04 (1H, d, J_{HP} = 644 Hz, H- Phosphonate), 7.44, 5.84 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.17, 7.15 (2H, d, J_{HH} = 8 Hz, H-Phenyl), 6.71 (1H, t, J_{HH} = 8.8 Hz, H-Phenyl), 6.61 (1H, dd, J_{HH} = 6.8 Hz, H1' Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.45 (1H, m, H4' Deoxyribose), 4.07 (2H, m, H5' Deoxyribose), 3.17 (12H, q, J_{HH} = 7.2 Hz, CH_2-TEA), 2.96, 2.80-2.76 (2H, m, H2'y H2'' Deoxyribose), 1.25 (18H, t, J_{HH} = 7.2 Hz, CH_3-TEA); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.65, 151.32, 150.75, 150.28, 143.08,142.16, 142.05, 141.96, 121.31, 103.21, 88.72, 84.52, 71.35, 62.27, 45.08, 44.51, 8.72. ^{19}F-NMR (D_2O, 376 MHz): δ_{ppm} = -111.53 (2F, t, F_{meta}-Phenyl), ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} =5.50 (1P, H-Phosphonate) 4.07 (1P, H-Phosphonate); HR-MS: m/z (FAB^-) 533.0247 (M^- $C_{17}H_{17}O_8N_6F_2P_2^-$ requires 533.0557).

1f ^{1}H-NMR D_2O

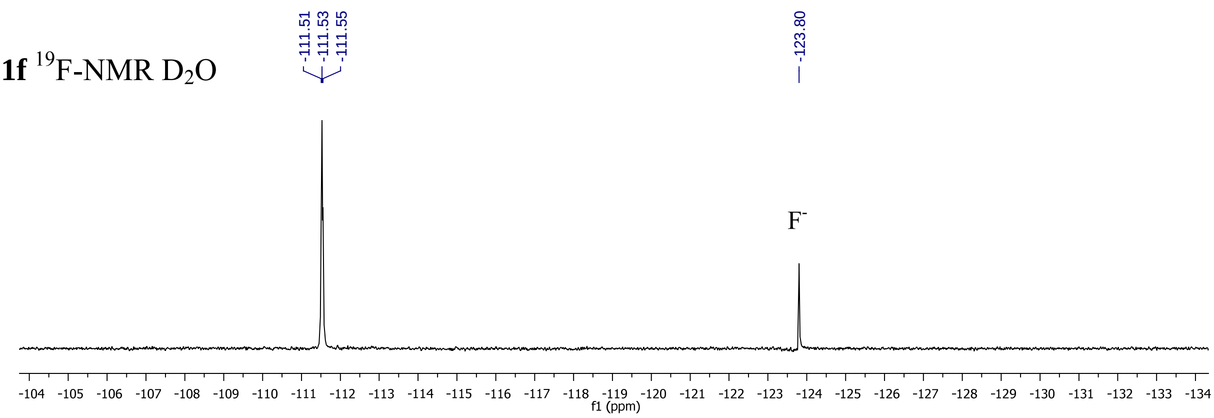
* HDO suppression



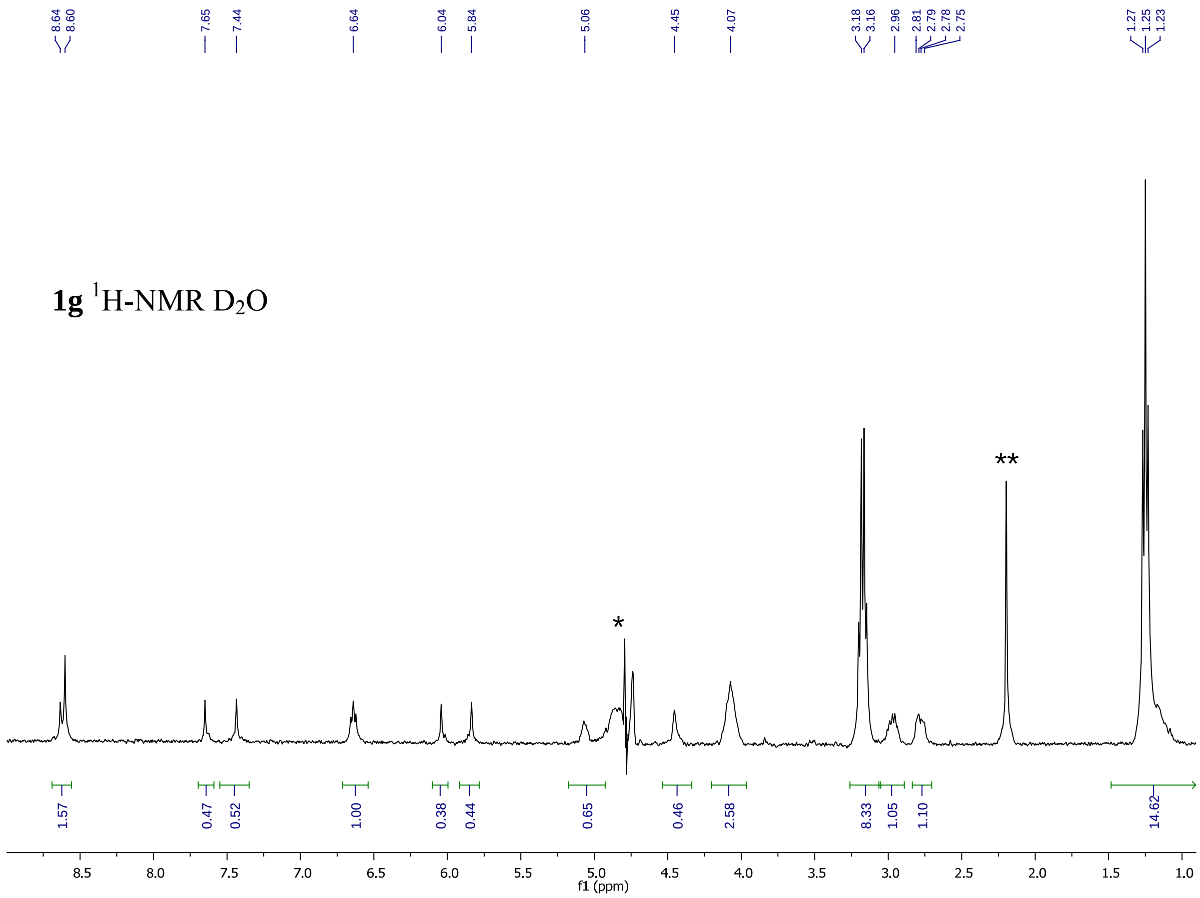
1f ^{31}P{^{1}H}-NMR $CDCl_3$



1f ^{19}F-NMR D_2O

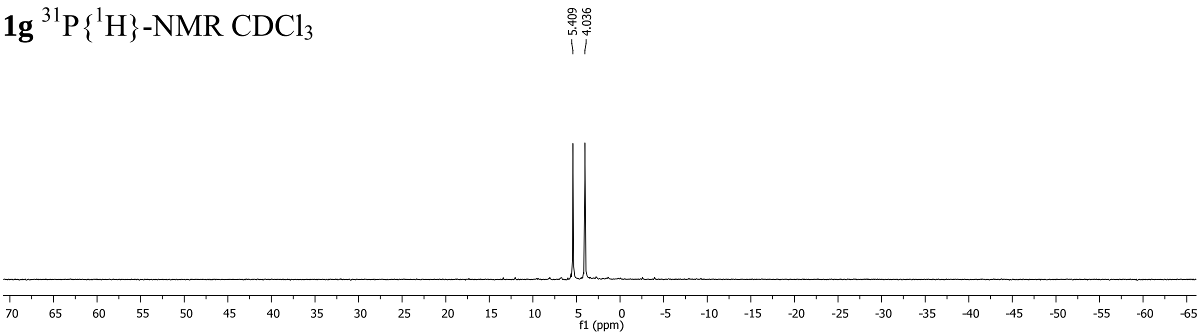


1g. Yield. 90.1%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.64 (1H, s, H-purine), 8.60 (1H, s, H-purine), 7.65, 6.04 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.44, 5.84 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 6.64 (1H, dd, J_{HH} = 6.8 Hz, H1'-Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.45 (1H, m, H4'-Deoxyribose), 4.07 (2H, m, H5'-Deoxyribose), 3.17 (12H, q, J_{HH} = 7.2 Hz, CH_2-TEA), 2.96, 2.81-2.75 (2H, m, H2', H2''-Deoxyribose), 1.25 (18H, t, J_{HH} = 7.2 Hz, CH_3-TEA) ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 151.78, 151.23, 150.13, 143.37, 121.40, 88.82, 84.47, 73.07, 61.87, 45.7, 8.71. ^{19}F-NMR (D_2O, 376 MHz): δ_{ppm} -147.87, 147.92 (2F, d, F_{ortho} -Phenyl), -158.20 (1F, t, F_{para} -Phenyl), -164.88 (2F, t, F_{meta} -Phenyl), ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} 5.40 (1P, H-Phosnonate), 4.03 (^{31}P, H-Phosnonate); FAB-MS: 587 [(M-TEA$^-$)].

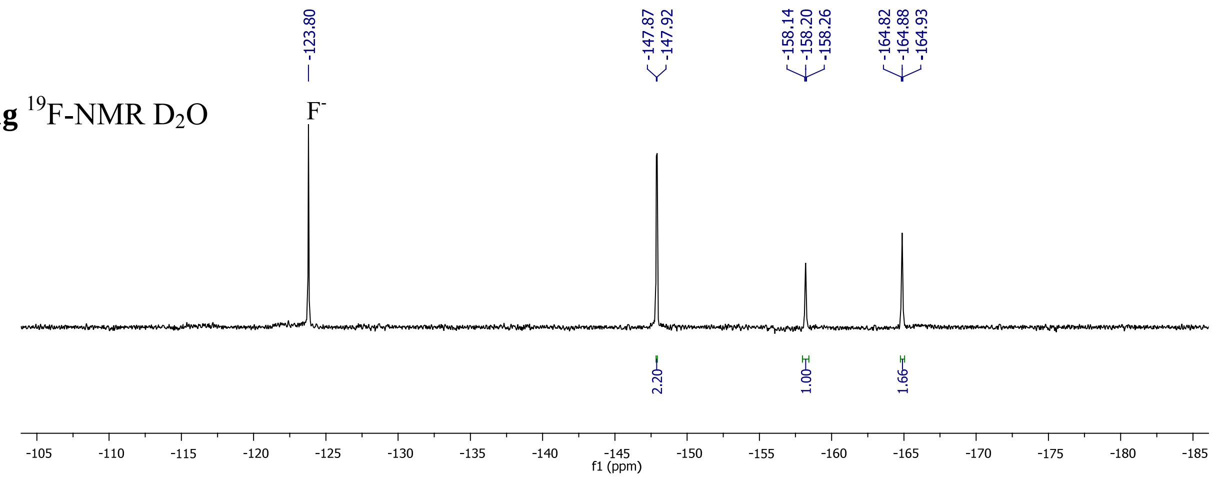


* HDO suppression. ** Acetone

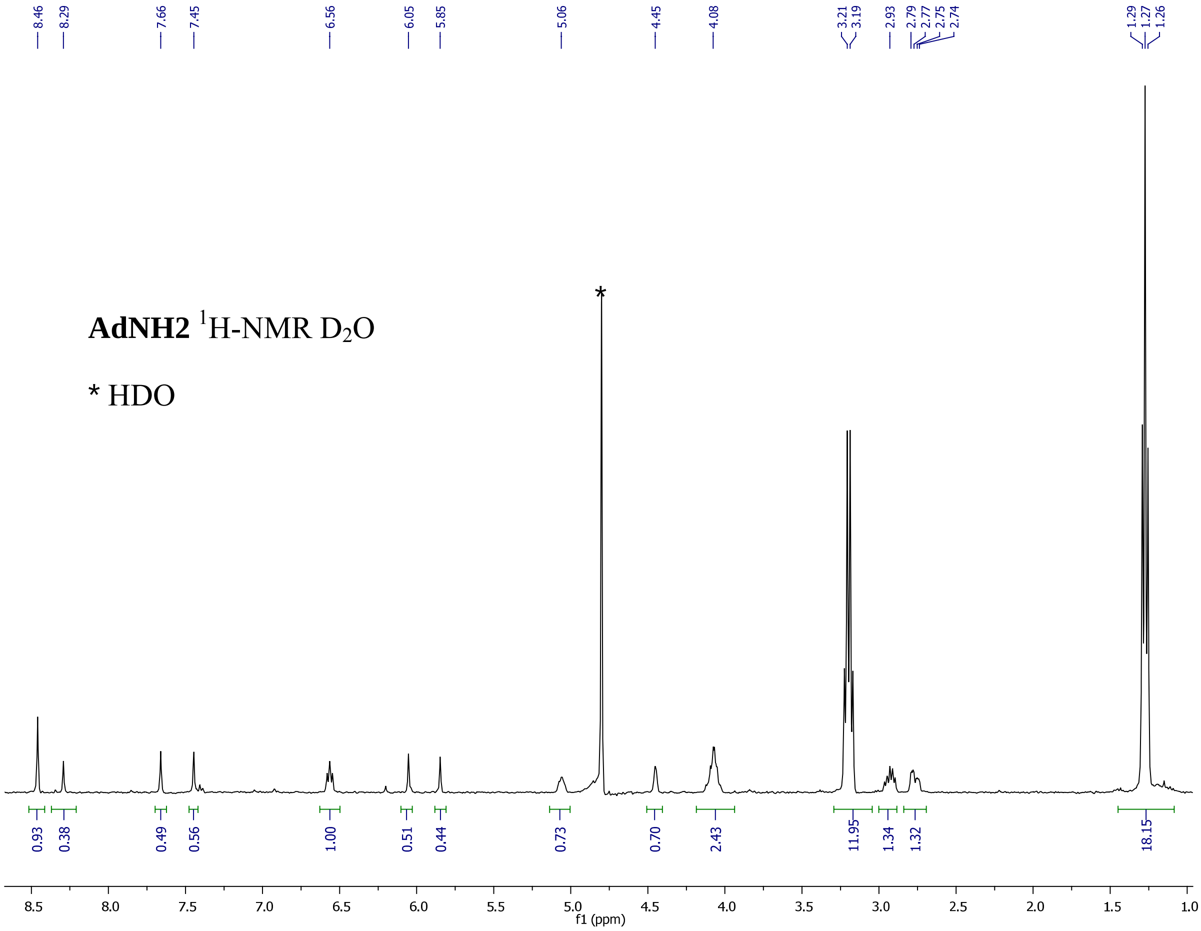
1g $^{31}P\{^1H\}$-NMR $CDCl_3$



1g ^{19}F-NMR D_2O

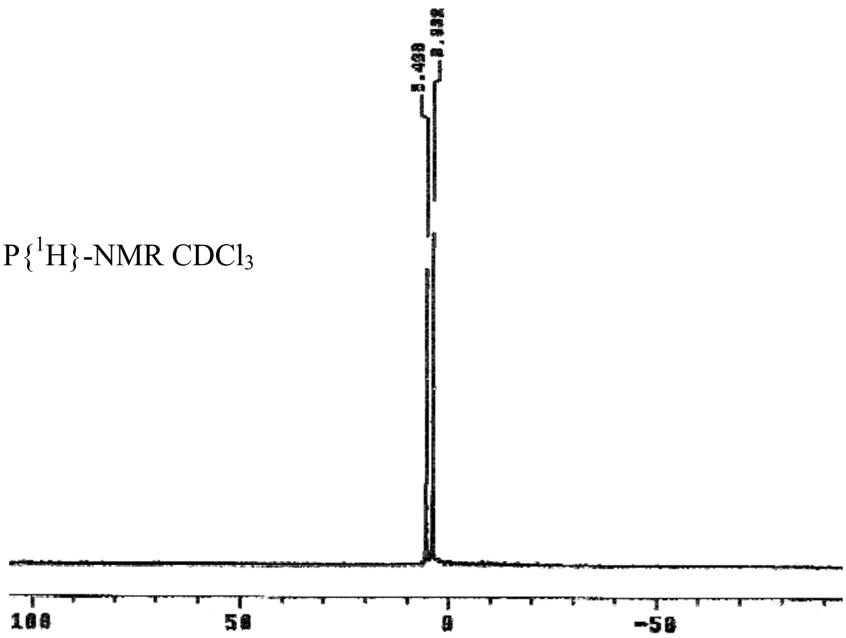


AdNH₂. Yield, 91%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.46 (1H, s, NH-Purine), 8.29 (1H, s, NH-Purine), 7.66, 6.05 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.45, 5.85 (1H, d, J_{HP} = 6340 Hz, H-Phosphonate), 6.56 (1H, dd, J_{HH} = 7.2 Hz, H1'-Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.45 (1H, m, H4'-Deoxyribose), 4.08 (2H, m, H5'-Deoxyribose), 3.20 (12H, q, J_{HH} = 7.4 Hz, CH_2-TEA), 2.93,2.79-2.74 (2H, m, H2' y H2''-Deoxyribose), 1.27 (18H, t, J_{HH} = 7.4 Hz, CH_3-TEA). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 156.01, 152.40, 149.79, 139.96, 119.04, 87.99, 84.09, 72.01, 61.94, 45.41, 40.50, 8.71. ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} = 5.43 (^{31}P, H-Phosphonate). 3.93 (1P, H-Phosphonate); FAB-MS: 378 [(M-TEA⁻)].



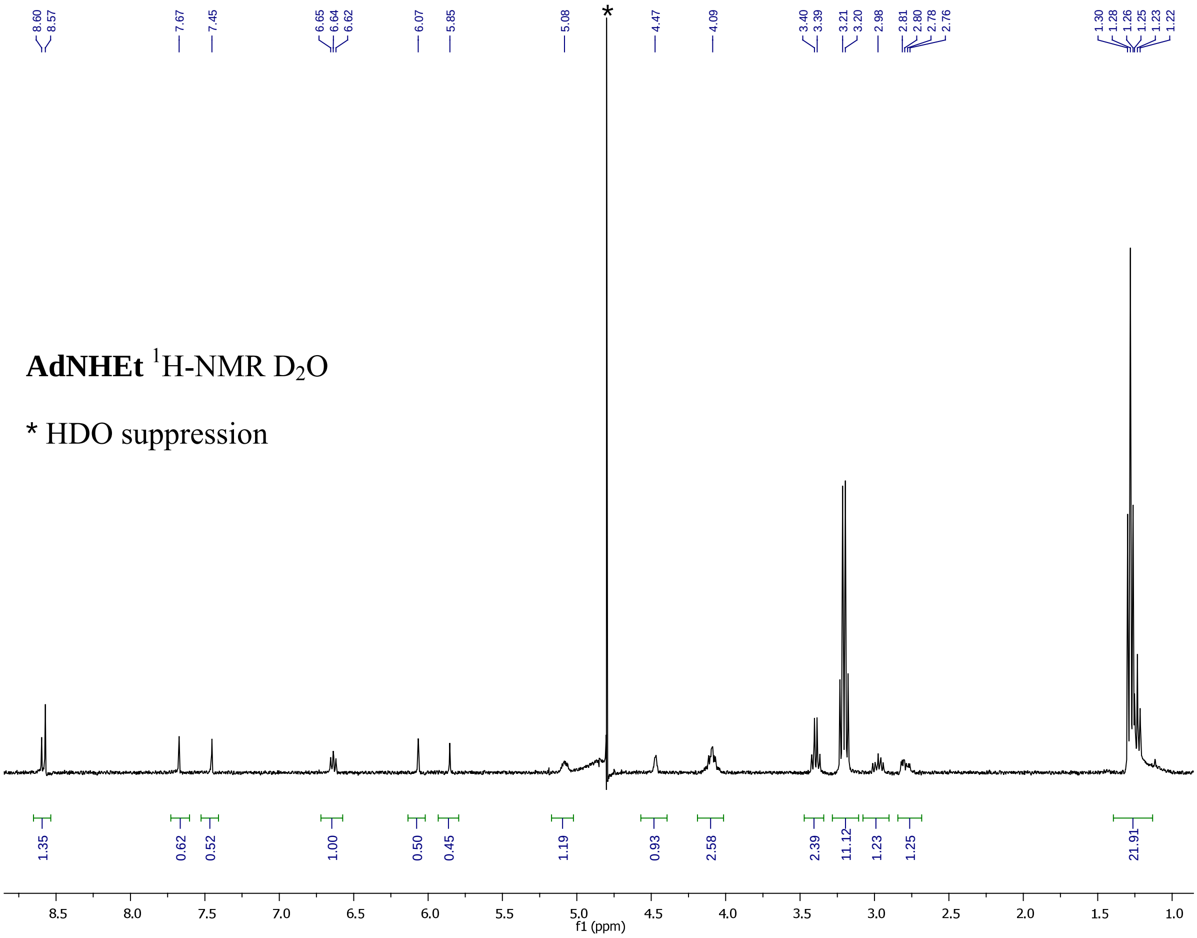
AdNH2 [1]H-NMR D_2O

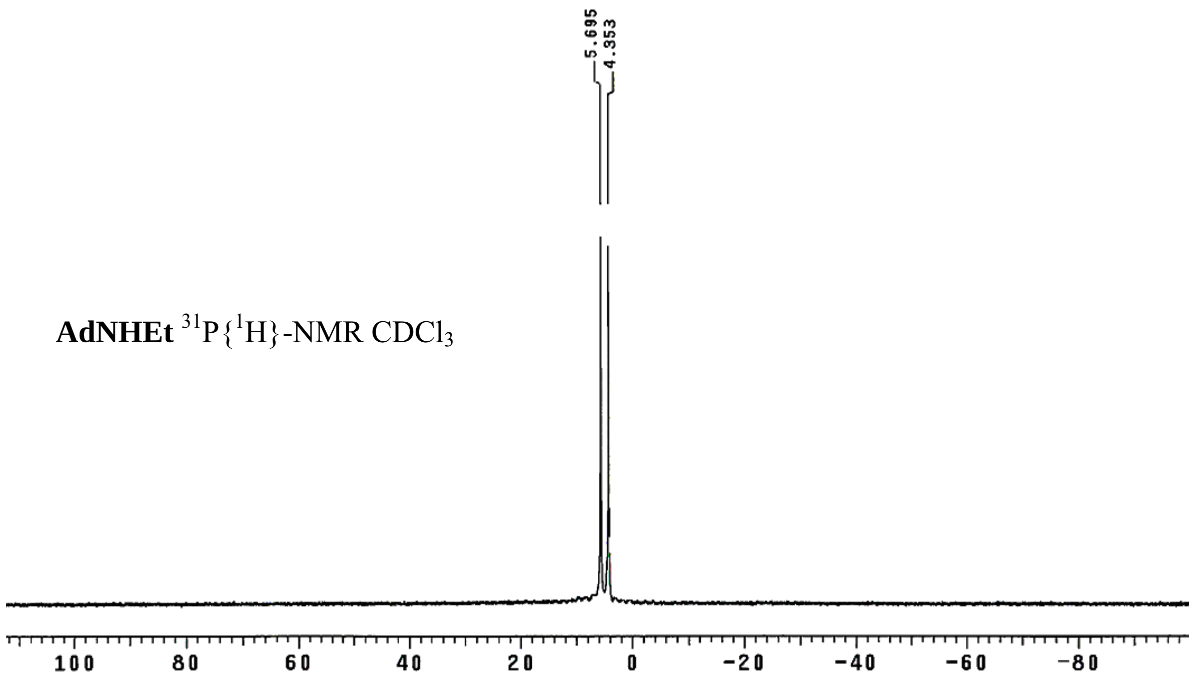
* HDO

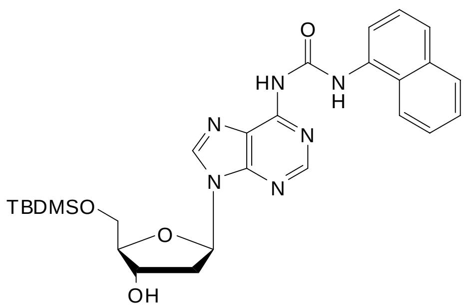


AdNH2 [31]P{[1]H}-NMR $CDCl_3$

AdNHEt. Yield, 79 %; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.60 (1H, s, H-purine), 8.57 (1H, s, H-purine), 7.67, 6.07 (1H, d, J_{PH} = 640 Hz, H-Phosphonate), 7.45, 5.85 (1H, d, J_{PH} = 640Hz, H-Phosphonate), 6.64 (1H, dd, J_{HH} = 7.6 Hz, H1'- Deoxyribose), 5.08 (1H, m, H3'-Deoxyribose), 4.47 (1H, m, H4'-Deoxyribose), 4.09 (2H, m, H5'-Deoxyribose), 3.40 (2H, q, J_{HH} = 7.2 Hz, CH_2-Et), 3.21 (12H, q, J_{HH} = 7.2 Hz, CH_2-TEA), 2.98, 2.81-2.76 (2H, m, H2', H2''-Deoxyribose), 1.28 (18H, t, J_{HH} = 7.2 Hz, CH_3-TEA), 1.23 (3H, t, J_{HH} = 7.2 Hz, CH_3-Et); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 155.37, 152.48, 150.86, 149.79, 140.42, 121.55, 88.09, 84.11, 71.96, 61.93, 45.51, 39.93, 34.56, 14.58, 8.77.^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} 5.69 (1P, H-Phosphonate), 4.35 (1P, H-Phosphonate); FAB-MS: 449 [(M-TEA$^-$)].





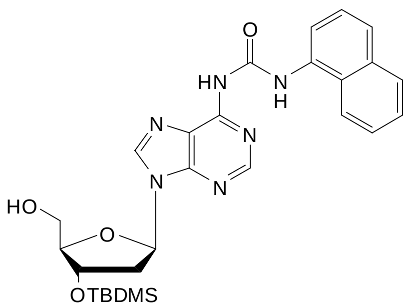


IV

Synthesis of IV

Nucleoside **IIIb** (1 mmol) was co-evaporated in dry pyridine (3×5 mL). The solid was dissolved in dry DMF (5 mL) followed by addition of triethylamine (1.5 mmols) via syringe. The solution was stirred at room temperature for 5 minutes. TBDMS-Cl (1.1 mmol) was added as a solid in one portion and the reaction mixture was stirred under nitrogen atmosphere at room temperature. The progress of the reaction was followed by TLC. After 8 h the reaction was quenched by addition of methanol (1 mL). The solvent was evaporated to a white solid. The compound was purified bye precipitation with hexane.

IV. Yield 75%; ^{1}H-NMR (DMSO-d$_6$, 200 MHz): δ_{ppm} = 12.37 (1H, s, NH- Carbamoyl), 10.41 (1H, s, NH-Carbamoyl), 8.82 (1H, s, H-purine), 8.62 (1H, s, H-purine), 8.21 (2H, t, J_{HH} = 6.6 Hz, H-Naphthyl), 7.95 (1H, d, J_{HH} =8 Hz, H-Naphthyl), 7.71-7.47 (4H, m, H-Naphthyl), 6.46 (1H, dd, J_{HH} = 6.4Hz H1'-Deoxyribose), 5.42 (1H, d, J_{HH} = 4, OH3'), 4.44 (1H, m, H3'-Deoxyribose), 3.91-3.86 (1H, m, H4'-Deoxyribose), 3.80-3.68 (2H, m, H5'-Deoxyribose), 2.81-2.74 (1H, m, H2'-Deoxyribose), 2.48-2.39 (1H, m, H2'-Deoxyribose'), 0.81 (9H, 1s, *t*-butyl-TBDMS), -0.01 (6H, 1s, *di*-Me-TBDMS); ^{13}C-NMR (CDCl$_3$, 50 MHz): δ_{ppm} = 152.04, 151.35, 151.00, 150.65, 142.81, 134.34, 129.27, 127.30, 126.74, 126.56, 126.31, 124.43, 121.67, 121.17, 118.11, 88.04, 84.38, 70.93, 63.85, 26.55, 18.79, -4.62. FAB-MS: 535 [(M+H$^+$)].

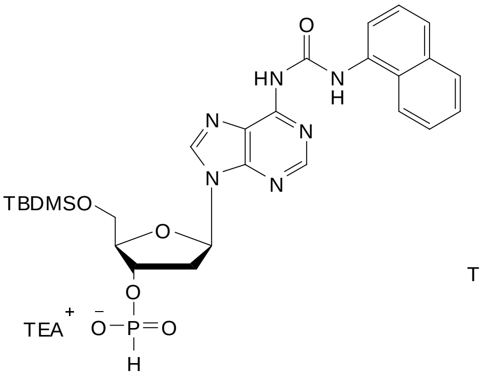


V

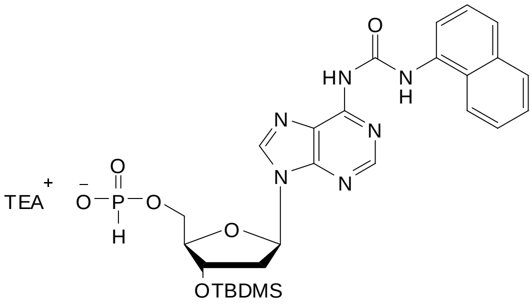
Synthesis of V

The nucleoside **IIb** (1 mmol) se was co-evaporated in THF (2×5 mL). The solid was dissolved in THF (12 mL). To a stirred solution was added aqueous TFA (6 ml, TFA:H_2O v/v 1:1) at 0ºC. After stirring for 2 h at 0ºC, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and diluted with ethyl acetate (50 ml). After separation, the organic phase was washed with H_2O (3 x 20 ml) and brine (3 x 20 ml), dried over anhydrous Na_2SO_4 and evaporated under vacuum and the residue was purified by precipitation with methanol.

V. Yield, 70%; ^{1}H-NMR (DMSO-d$_6$, 200 MHz): δ_{ppm} = 12.40 (1H, s, NH- Carbamoyl), 10.42 (1H, s, NH- Carbamoyl), 8.82 (1H, s, H-purine), y 8.72 (1H, s, H-purine), 8.26-8.20 (2H, m, H-Naphthyl), 7.95 (1H, d, J_{HH} =7.4 H-Naphthyl), 7.73-7.46 (4H, m, H-Naphthyl), 6.45 (1H, dd, J_{HH} =7.0 H1'-Deoxyribose), 5.11 (1H, t, OH5'), 4.65-4.63 (1H, m, H3'-Deoxyribose), 4.02-3.89 (1H, m, H4'-Deoxyribose), 3.62-3.56 (2H, m, H5'-Deoxyribose), 2.94-2.81 (1H, m, H2'-Deoxyribose), 2.39-2.28 (1H, m, H2''-Deoxyribose), 0.88(9H, s, *t*-butyl -TBDMS), 0.10 (6H, s, *di*-Me-TBDMS); ^{13}C-NMR (CDCl$_3$, 50 MHz): δ_{ppm} = 152.07, 151.25, 150.97, 150.66, 143.27, 134.33, 129.26, 127.31, 126.72, 126.55, 126.28, 124.42, 121.68, 121.26, 118.06, 88.86, 84.55, 73.14, 61.97, 26.47, 18.50, -3.98. FAB-MS: 535 [(M+H$^+$)].



VI



VII

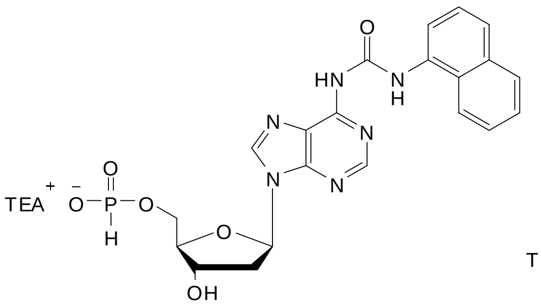
Synthesis of VI and VII

Nucleoside IV or V (1 mmol) was co-evaporated in dry pyridine (3 x 5 mL). The solids were dissolved in dry pyridine (5 mL) followed by addition of diphenylphosphate (7 mmol) via syringe. The reaction mixture was stirred under nitrogen atmosphere at room temperature. The progress of the reaction was followed by TLC. After 15 minutes the reaction was quenched by addition of the mixture of water-triethylamine (1:1 v/v, 2 ml) and was stirred for 15 minutes. The solvent was evaporated to yellow oil. The products were purified by precipitation with hexane.

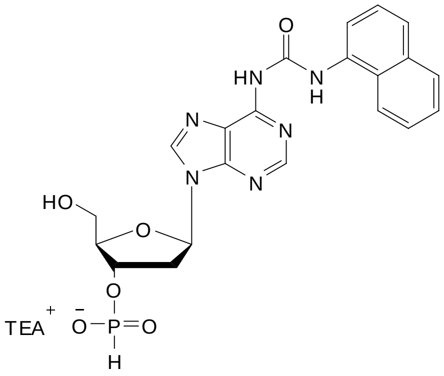
VI. Yield 95%; ^{1}H-NMR (DMSO-d$_6$, 200 MHz): δ_{ppm} = 12.34 (1H, s, NH-Carbamoyl), 10.44 (1H, s, NH-Carbamoyl), 8.84 (1H, s, H-purine), y 8.61 (1H, s, H-purine), 8.19 (2H, t, J_{HH} = 6.6 Hz, H-Naphthyl), 7.94 (1H, d, J_{HH} = 8 Hz, H-Naphthyl), 7.72-7.45 (4H, m, H-Naphthyl), 6.92 (1H, d, J_{HP} = 614 Hz, H-Phosphonate), 6.44 (1H, dd, J_{HH} = 6.4Hz H1'-Deoxyribose), 4.46 (1H, m, H3'-Deoxyribose), 3.92-3.88 (1H, m, H4'-Deoxyribose), 3.82-3.66 (2H, m, H5'-Deoxyribose), 3.31 (9H, q, CH_3-TEA), 2.85-2.76 (1H, m, H2'-Deoxyribose), 2.47-2.37 (1H, m, H2''-Deoxyribose), 1.52 (6H, t, CH_2-TEA), 0.79 (9H, s, *t*-butyl-TBDMS), -0.02 (6H, s, *di*-Me-TBDMS); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 152.10, 151.32, 151.05, 150.68, 142.86, 134.31, 129.25, 127.320, 126.77, 126.59, 126.35, 124.46, 121.61, 121.14, 118.17, 88.05, 84.39, 70.96, 63.89, 45.32 26.57, 18.74, 8.73 -4.61. ^{31}P-NMR (D_2O, 81 MHz): δ_{ppm} 5.19 (1P, H-Phosphonate); FAB-MS: 597 [(M-TEA$^-$)].

VII. Yield, 91%; ^{1}H-NMR (DMSO-d$_6$, 200 MHz): δ_{ppm} = 12.39 (1H, s, NH- Carbamoyl), 10.45 (1H, s, NH-Carbamoyl), 8.86 (1H, s, H-purine), y 8.79 (1H, s, H-purine), 8.25-8.21 (2H, m, H-Naphthyl), 7.98 (1H, d, J_{HH} = 7.4 H-Naphthyl), 7.75-7.49 (4H, m, H-Naphthyl), 6.94 (1H, d, J_{HP} = 618 Hz, H-

Phosphonate), 6.42 (1H, dd, J_{HH} =7.0 H1'-Deoxyribose), 4.64-4.62 (1H, m, H3'-Deoxyribose), 4.01-3.87 (1H, m, H4'-Deoxyribose), 3.61-3.59 (2H, m, H5'-Deoxyribose), 2.93-2.82 (1H, m, H2'-Deoxyribose), 2.38-2.27 (1H, m, H2''-Deoxyribose), 3.28 (9H, q, CH_3-TEA), 1.55 (6H, t, CH_2-TEA), 0.89 (9H, 1s, *t*-butyl -TBDMS), 0.13 (6H, 1s, *di*-Me-TBDMS); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 152.06, 151.24, 150.94, 150.64, 143.27, 134.34, 129.27, 127.39, 126.78, 126.52, 126.23, 124.43, 121.63, 121.29, 118.09, 88.83, 84.54, 73.11, 61.98, 45.73, 26.46, 18.52, 8.76, -3.95. ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} 5.21 (1P, H-Phosphonate); FAB-MS: 597 [(M-TEA$^-$)].



1c



1d

Synthesis of 1c and 1d

Nucleotide VI or VII (1 mmol) was co-evaporated in dry THF (3×5 mL). The solid was dissolved in dry THF (6 mL) followed by addition of TBAF of 1M solution in THF (1.5 mmol) via syringe under nitrogen atmosphere. The mixture was stirred at room temperature. The progress of the reaction was followed by TLC. After 2 h the reaction was quenched by addition of methanol (1 mL). The solvent was evaporated under vacuum and the residues were purified by precipitation with methanol.

1c. 87 %; ^{1}H-NMR (D_2O, 200 MHz): δ_{ppm} = 8.53 (2H, s, H-purine), 8.0 1, 7.99 (1H, d, J_{HH} = 8 Hz H-Naphthyl), 7.80, 7.78 (2H, d, J_{HH} = 8 H-Naphthyl), 7.66 (1H, m, H-Naphthyl), 7.57 (1H, m, H-Naphthyl), 7.49 (2H, m, H-Naphthyl), 7.49, 5.89 (1H, d, J_{HP} = 639.6 Hz, H-Phosphonate), 6.51 (1H, dd, J_{HH} =7.0 H1' Deoxyribose), 5.08 (1H, m, H3' Deoxyribose), 4.48 (1H, m, H4' Deoxyribose), 4.10 (2H, m, H5' Deoxyribose), 3.17 (6H, q, J_{HH} = 7.2 Hz CH_3-TEA), 2.93, 2.83-2.80 (2H, m, H2', H2'' Deoxyribose), 1.25 (9 H, t, J_{HH} = 7.2 Hz CH_2-TEA). ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 152.05, 151.23, 150.96, 150.68, 143.29, 134.36, 129.23, 127.37, 126.80, 126.55, 126.22, 124.44, 121.65, 121.27, 118.11, 88.84, 84.55, 73.12, 61.96, 45.72, 8.75, ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} 5.21 (1P,dt,