Electronic Supplementary Information Part I

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

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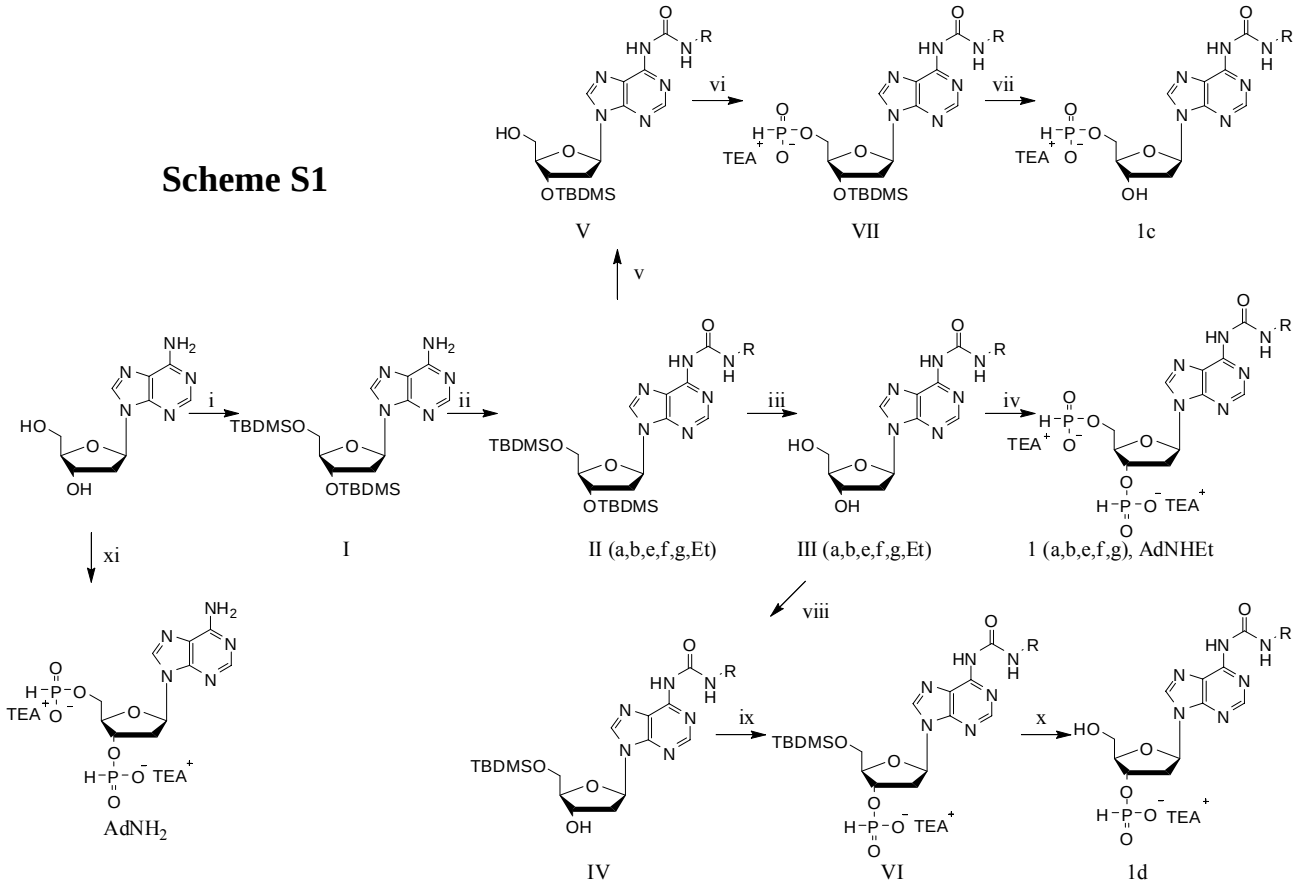
Materials and Methods

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.

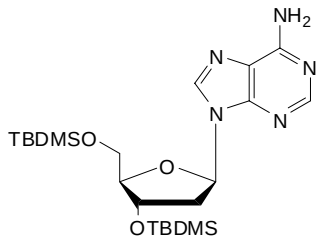
All the chromatographic runs were performed on Waters 600 HPLC System at room temperature using XTerra RP18 column, 5μm, 3.9 mm × 150 mm. A Waters 2996 diode array UV detector was used to monitor signals in the range from 190-400 nm. Samples were dissolved in 1:1 metanol/water in a concentration range 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.



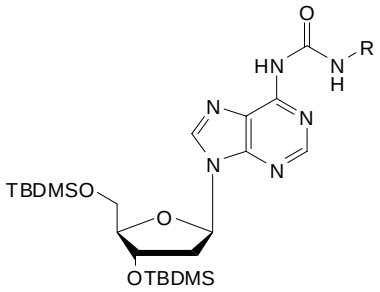
I

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^+)].

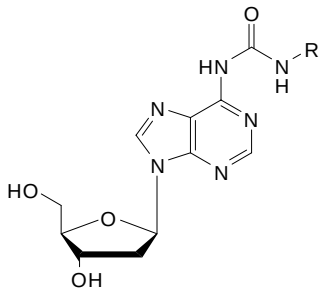
IIb. Yield, 93.1%; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ_{ppm} = 12.28 (1H, s, NH-carbamoyl), 9.14 (1H, s, NH-Carbamoyl), 8.70 (1H, s, H-purine), y 8.45 (1H, s, H-purine), 8.24 (2H, t, J_{HH} = 7.2 Hz, H-Naphthyl), 7.89 (1H, d, J_{HH} = 8.8 Hz, H-Naphthyl), 7.69-7.55 (4H, m, H-Naphthyl), 6.52 (1H, dd, J_{HH} = 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 [$(M+H^+)$].

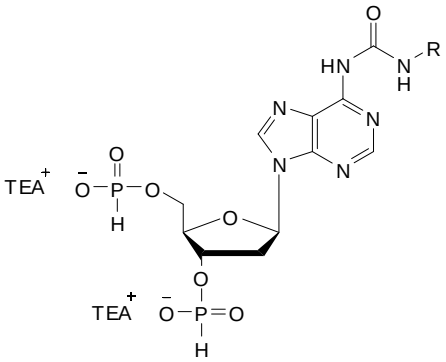
IIIb. Yield 90%. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ_{ppm} = 12.37 (1H, s, NH-carbamoyl), 10.39 (1H, s, NH-carbamoyl), 8.80 (1H, s, H-purine), y 8.67 (1H, s, H-purine), 8.18 (2H, t, J_{HH} = 6.8Hz, H-Naphthyl), 7.92 (1H, d, J_{HH} = 8Hz, H-Naphthyl), 7.69-7.53 (4H, m, H-Naphthyl), 6.43 (1H, dd, J_{HH} = 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$^+$)].



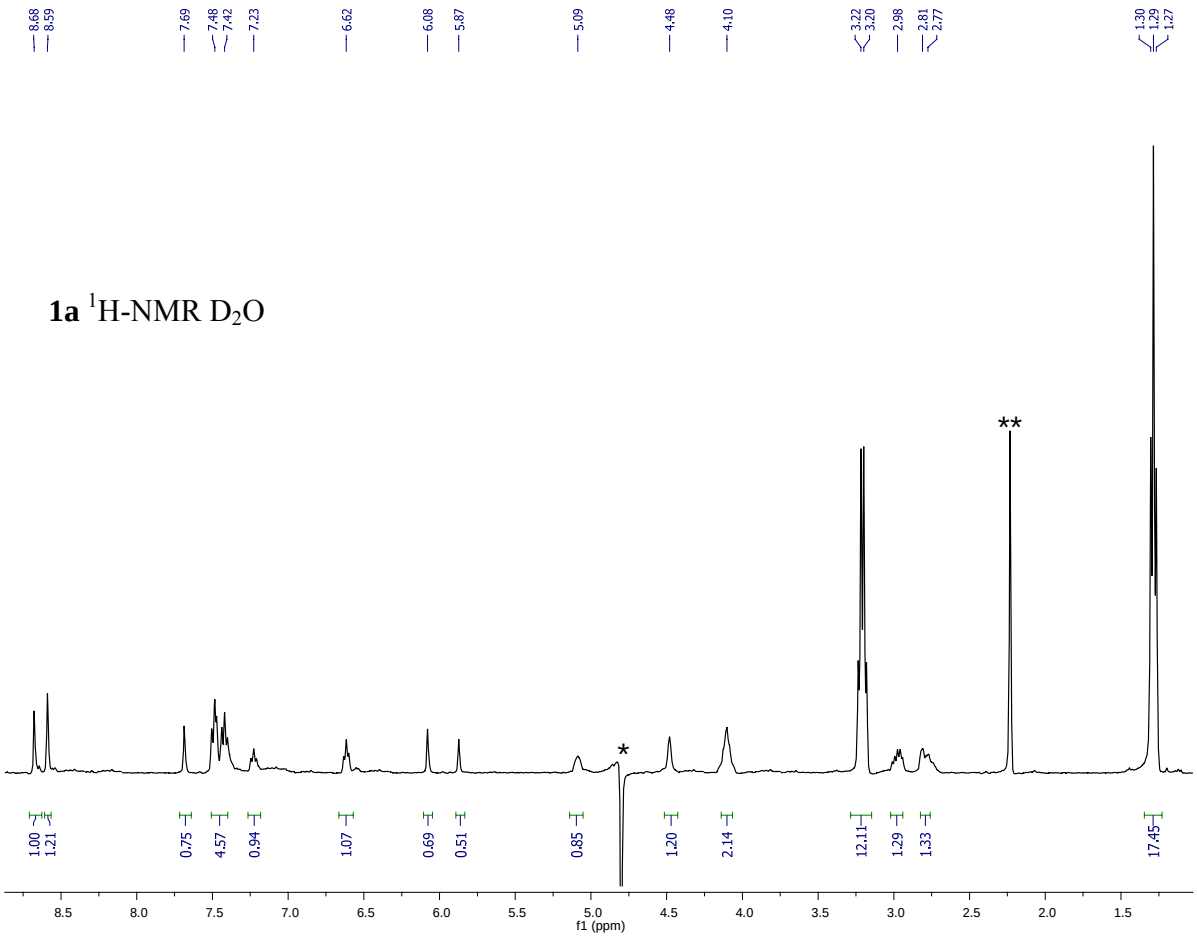
1a, 1b, 1e, 1f, 1g, AdNH$_2$ and AdNHEt

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.

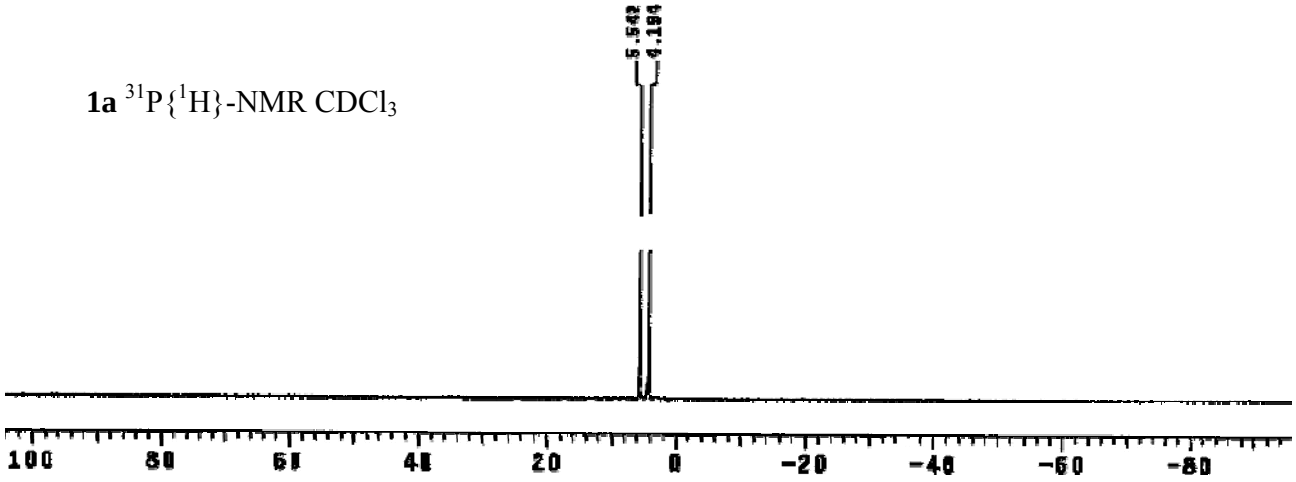
1a. Yield 87%. ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.68 (1H, s, H-purine), 8.59 (1H, s, H-purine), 7.69, 6.08 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.48 (2H, d, J_{HH} = 8 H-Phenyl), 7.42, 5.87 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.42 (2H, t, J_{HH} = 8. Hz, H-Phenyl), 7.23 (1H, t, J_{HH} = 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).



1a ^{1}H-NMR D_2O

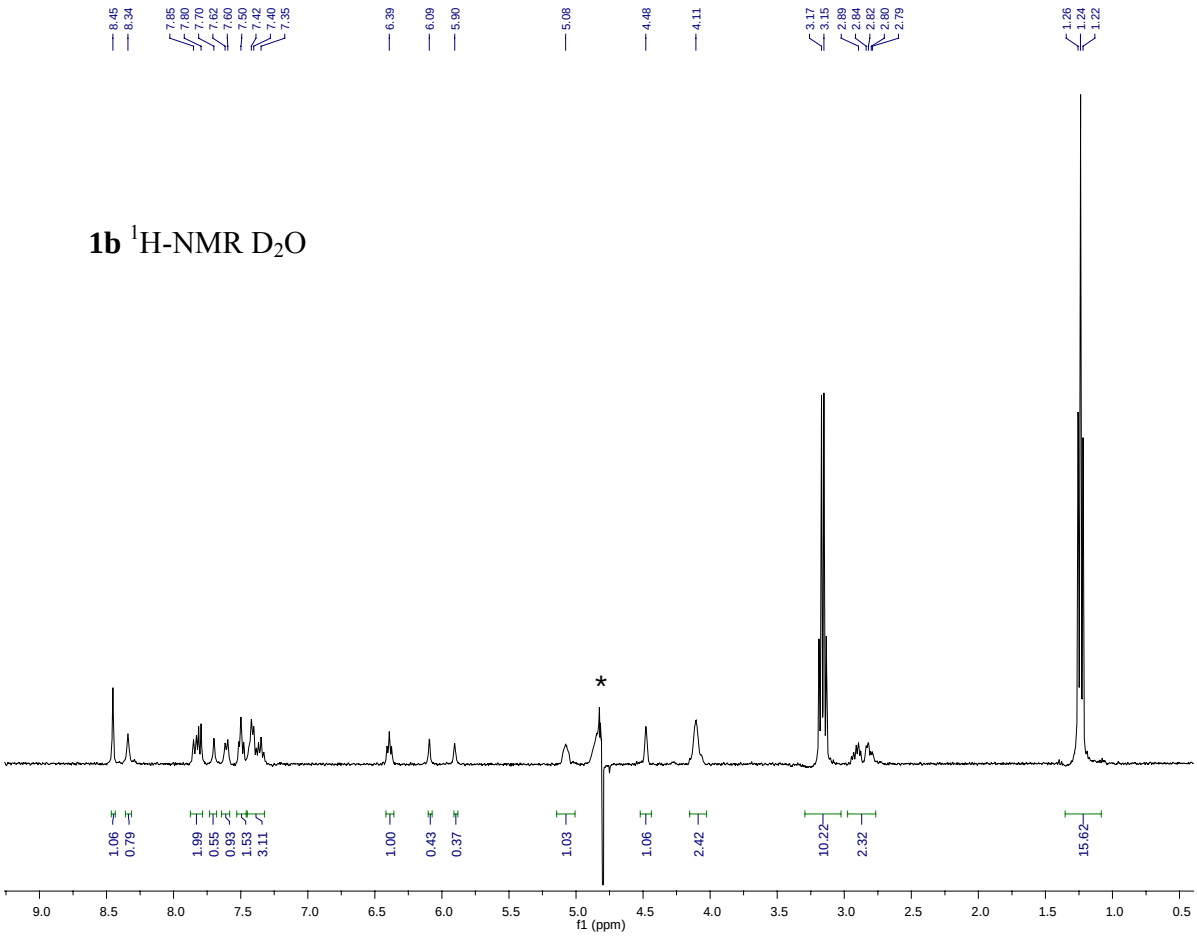
* HDO suppression. ** Acetone

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



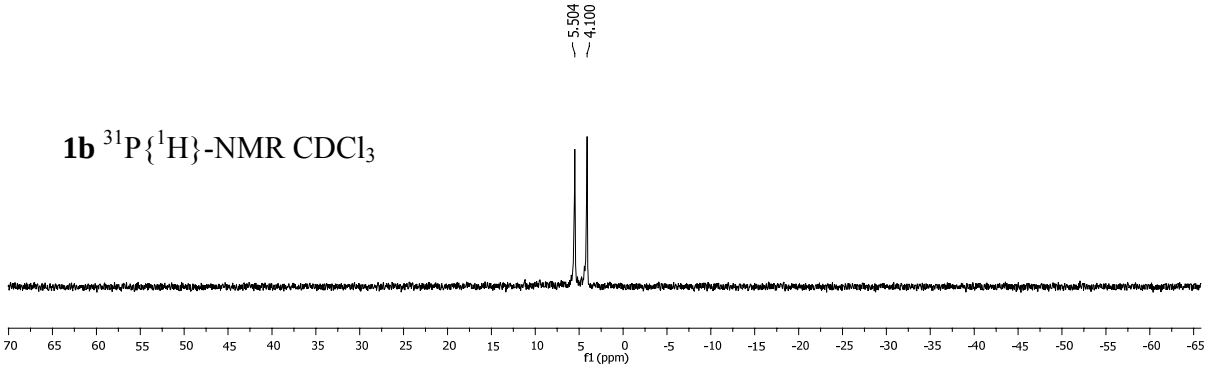
1b. Yield, 85 %; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.45 (1H, s, H-purine), y 8.34 (1H, s, H-purine), 7.85,7.83 (1H, d, J_{HH} = 8 Hz, H-Naphthyl), 7.81, 7.80 (1H, d, J_{HH} = 4 Hz, H-Naphthyl), 7.70, 6.09 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.62, 7.60 (1H, d, J_{HH} = 8 Hz, H-Naphthyl), 7.50 (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

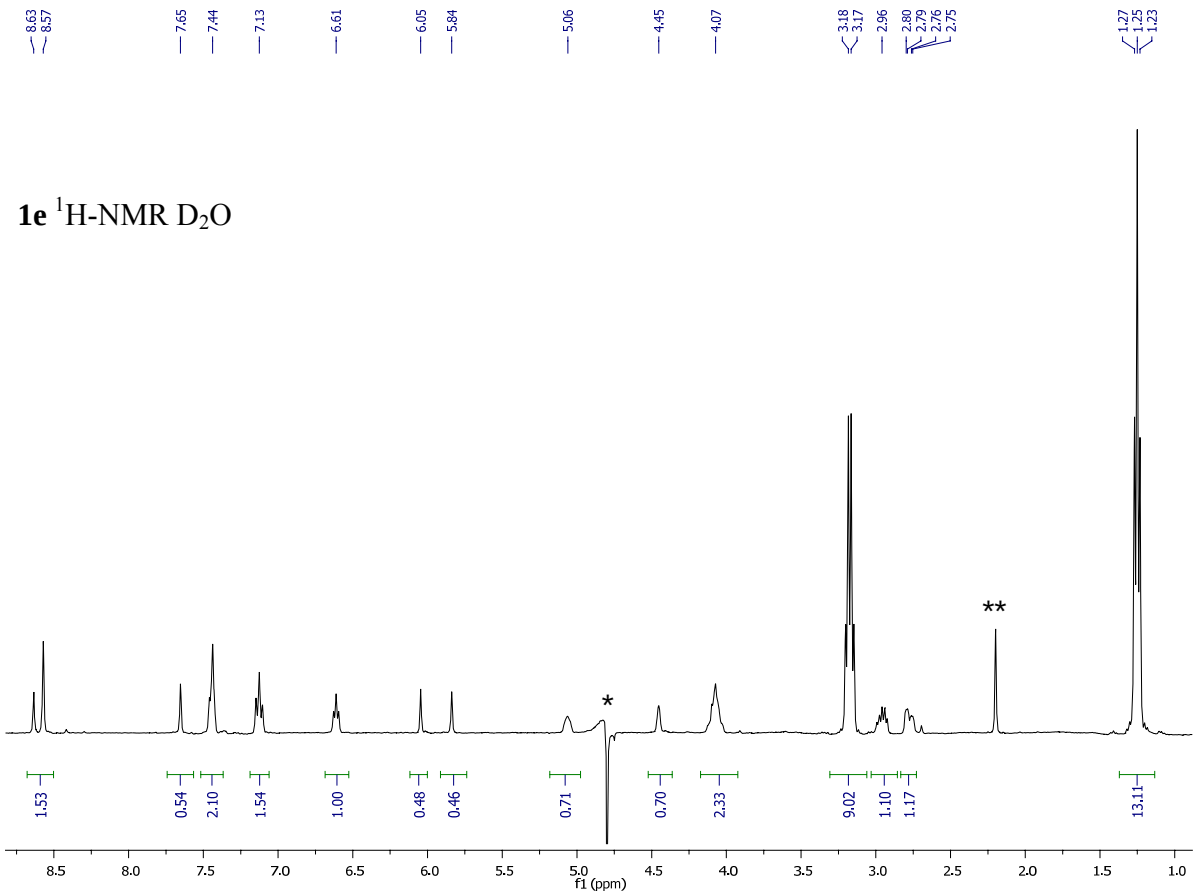


* HDO suppression

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

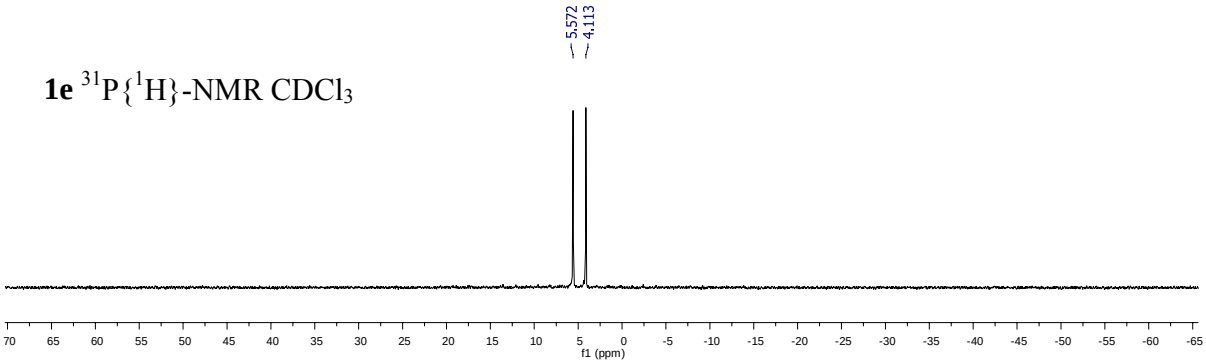


1e. Yield, 85%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.63 (1H, s, H-purine), 8.57 (1H, s, H-purine), 7.65,6.05 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.44 (2H, m, H-Phenyl), 7.44, 5.84 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.13 (2H, t, J_{HH} = 8.8 Hz, H-Phenyl), 6.61 (1H, dd, J_{HH} = 6.8 H1'-Deoxyribose), 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).

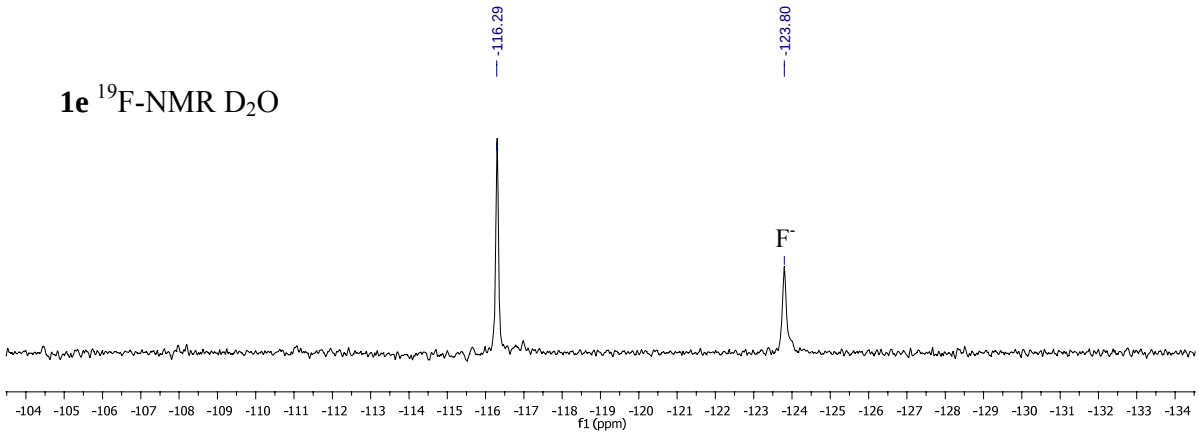


* HDO suppression. ** Acetone

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



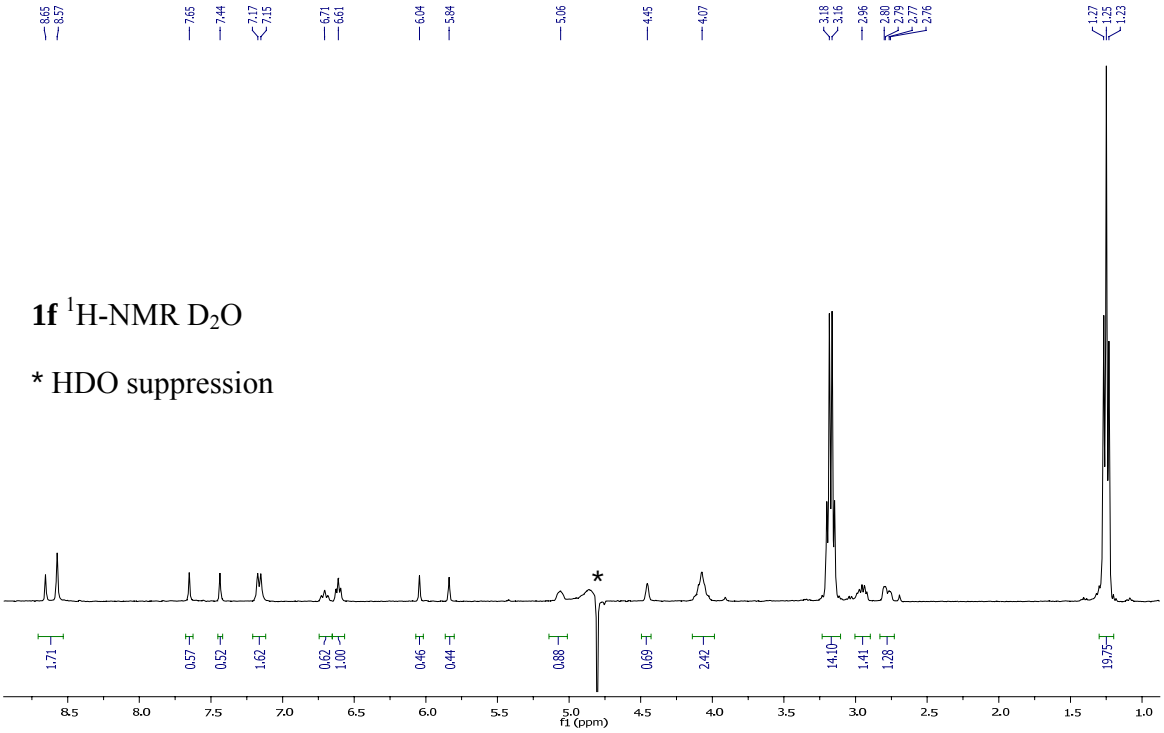
1e ^{19}F-NMR D_2O



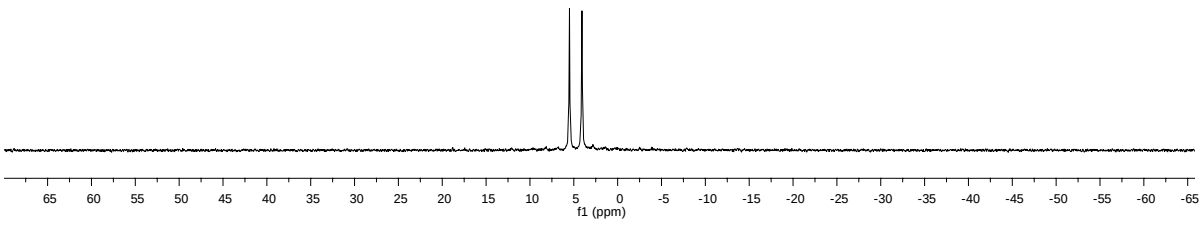
1f. Yield, 79%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.65 (1H, s, H-purine), 8.57 (1H, s, H-purine), 7.65, 6.04 (1H, d, J_{HP} = 644 Hz, H- Phosphonate), 7.44, 5.84 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 7.17, 7.15 (2H, d, J_{HH} = 8 Hz, H-Phenyl), 6.71 (1H, t, J_{HH} = 8.8 Hz, H-Phenyl), 6.61 (1H, 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

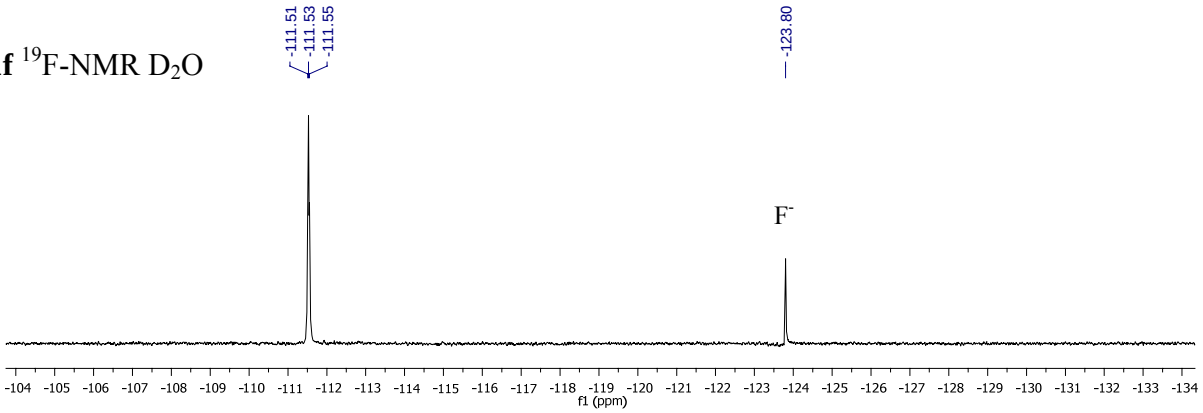
* HDO suppression



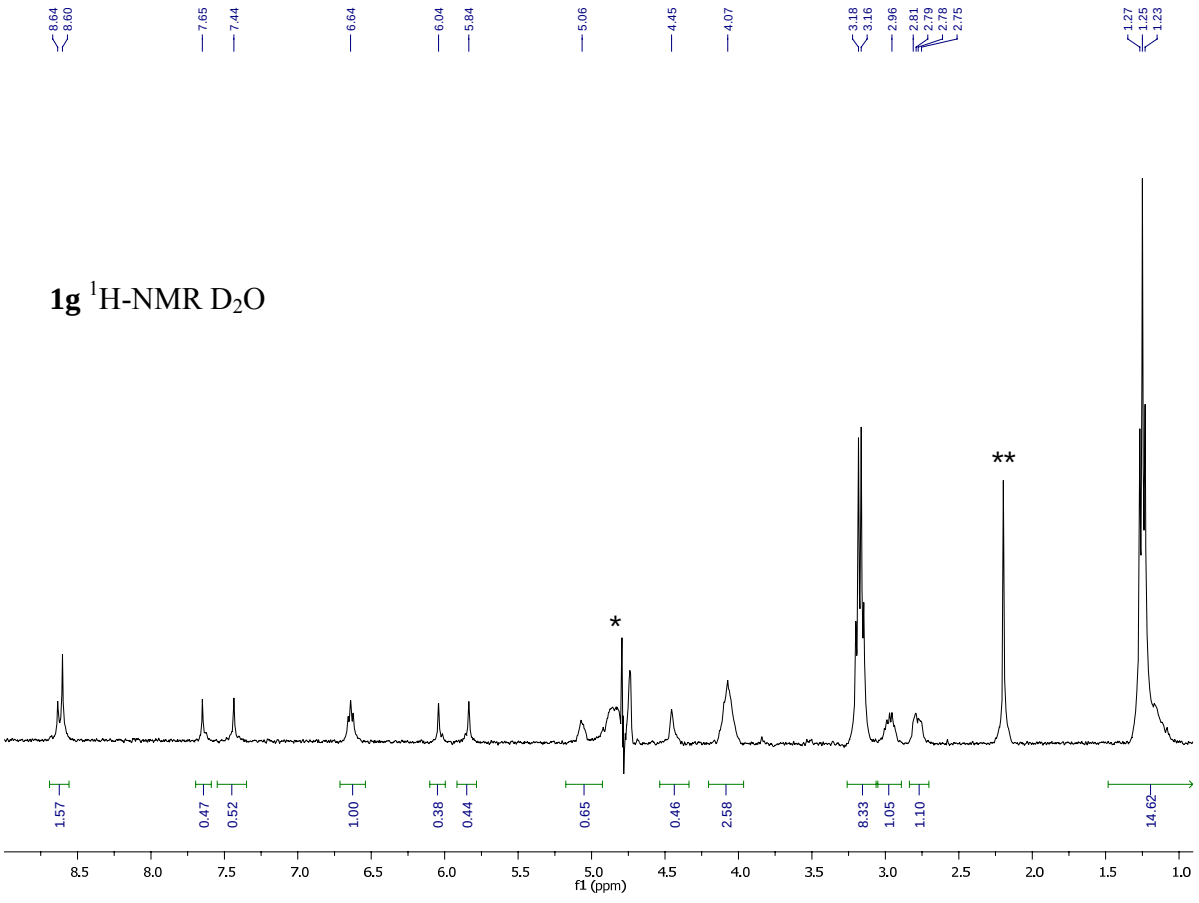
1f $^{31}P\{^{1}H\}$-NMR $CDCl_3$



1f ^{19}F-NMR D_2O

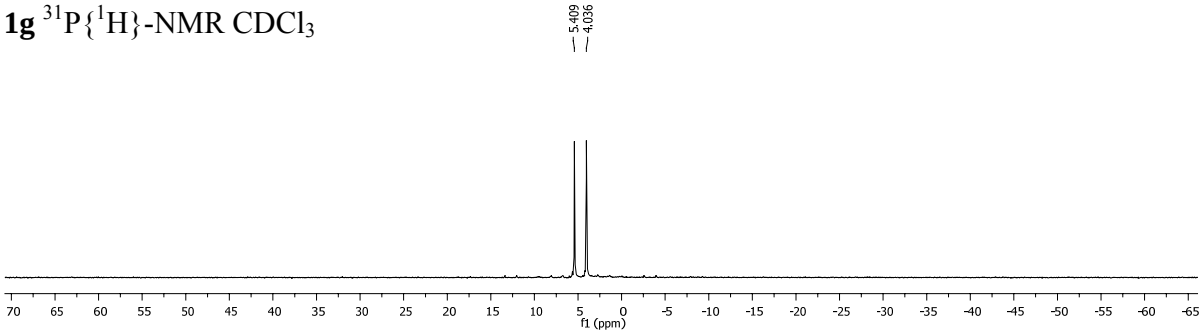


1g. Yield. 90.1%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.64 (1H, s, H-purine), 8.60 (1H, s, H-purine), 7.65, 6.04 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.44, 5.84 (1H, d, J_{HP} = 640 Hz, H-Phosphonate), 6.64 (1H, dd, J_{HH} = 6.8 Hz, H1'-Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.45 (1H, m, H4'-Deoxyribose), 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$^-$)].

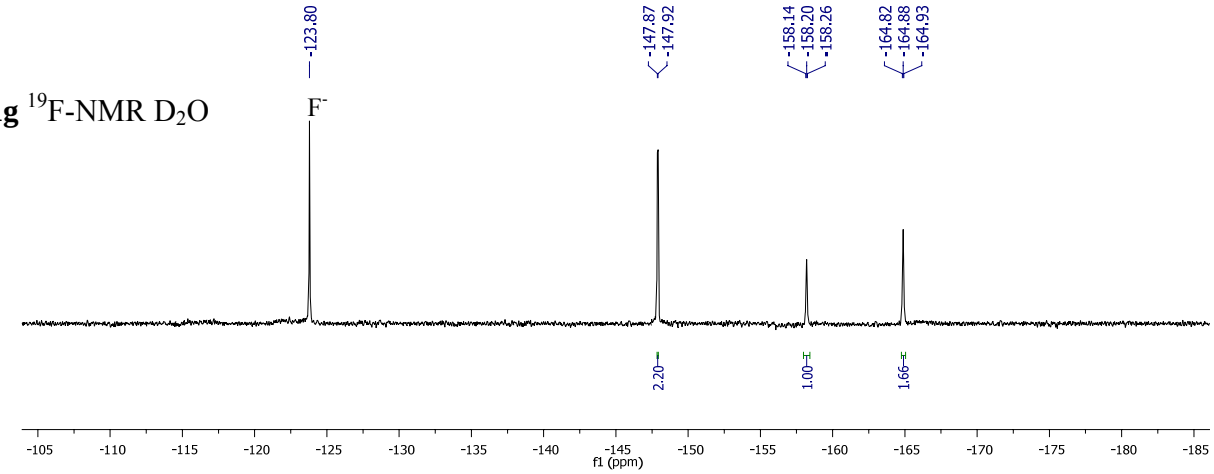


* HDO suppression. ** Acetone

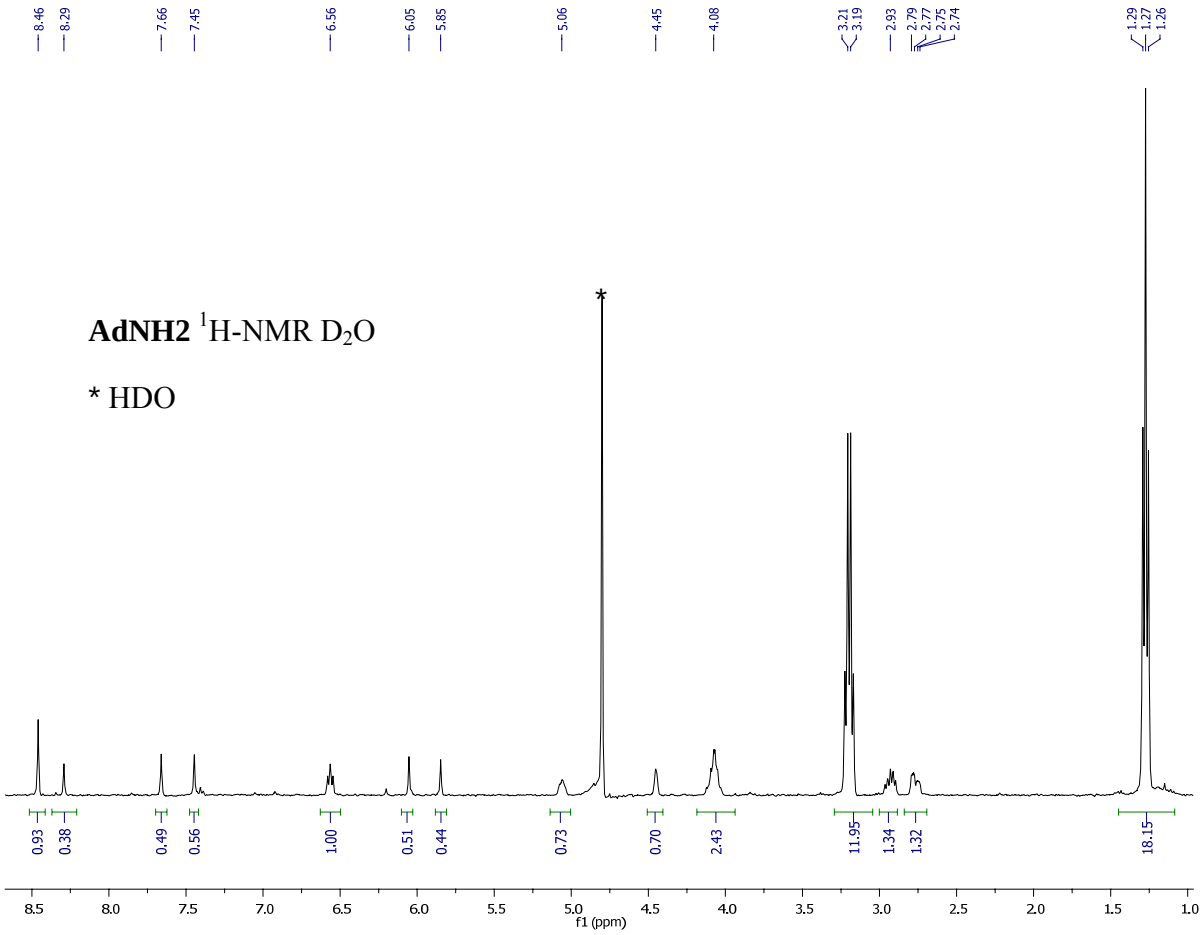
1g $^{31}P\{^{1}H\}$-NMR $CDCl_3$



1g ^{19}F-NMR D_2O

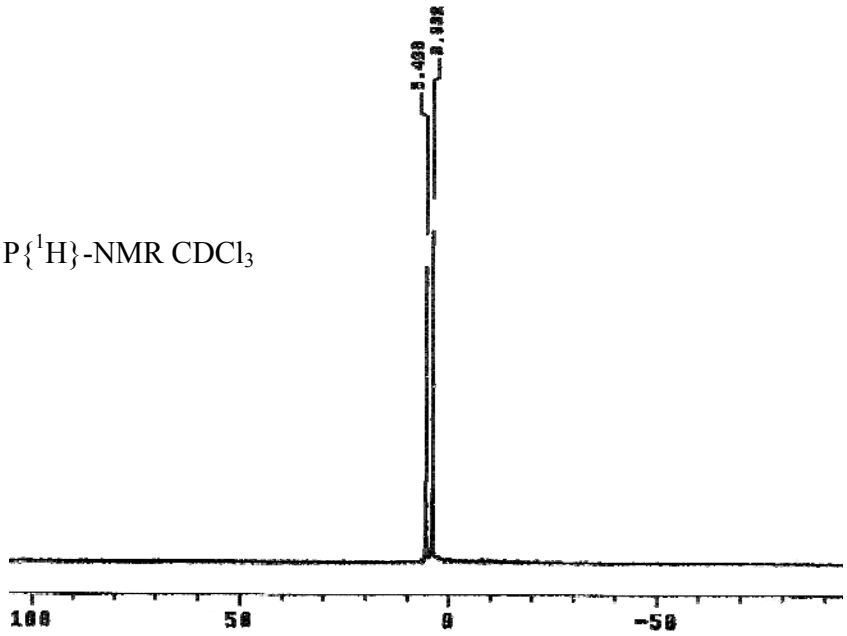


$AdNH_2$. Yield, 91%; ^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.46 (1H, s, NH-Purine), 8.29 (1H, s, NH-Purine), 7.66, 6.05 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.45, 5.85 (1H, d, J_{HP} = 6340 Hz, H-Phosphonate), 6.56 (1H, dd, J_{HH} = 7.2 Hz, H1'-Deoxyribose), 5.06 (1H, m, H3'-Deoxyribose), 4.45 (1H, 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^-)].



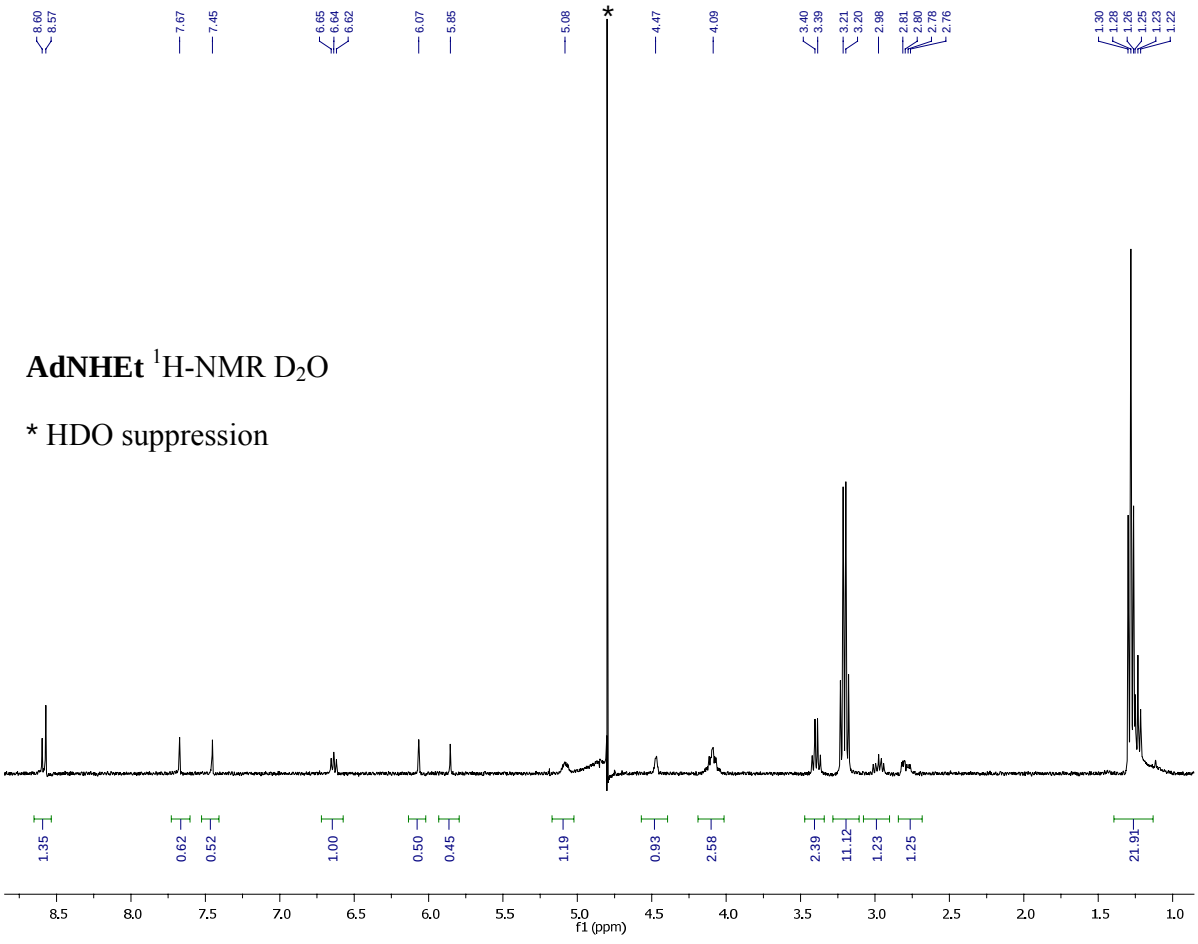
AdNH2 ^{1}H-NMR D_2O

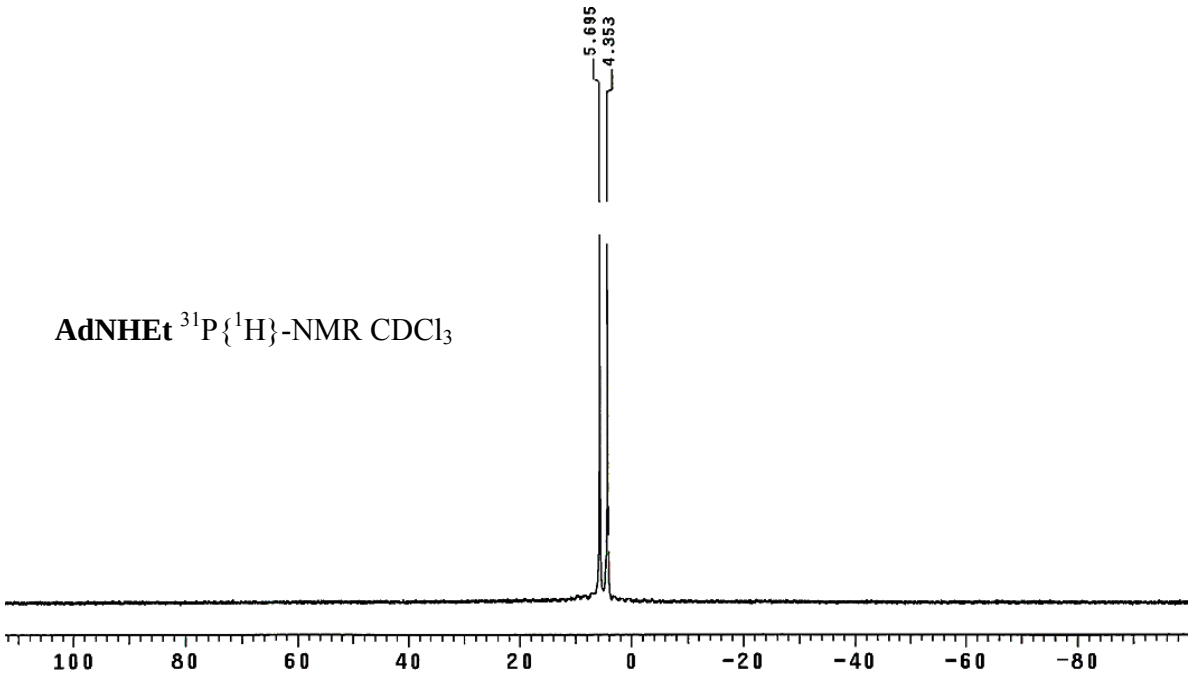
* HDO

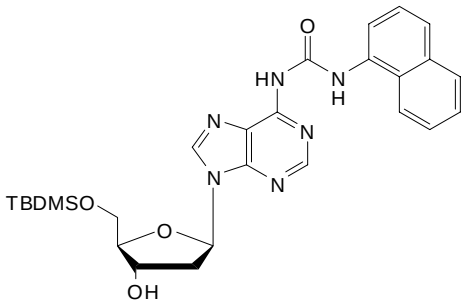


AdNH2 $^{31}P\{^{1}H\}$-NMR $CDCl_3$

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$^-$)].





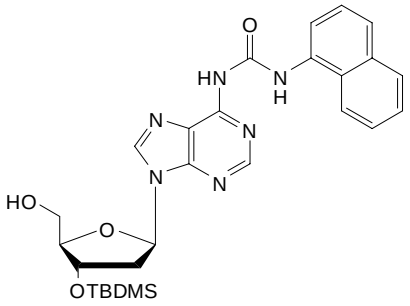


IV

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 [$(M+H^+)$].



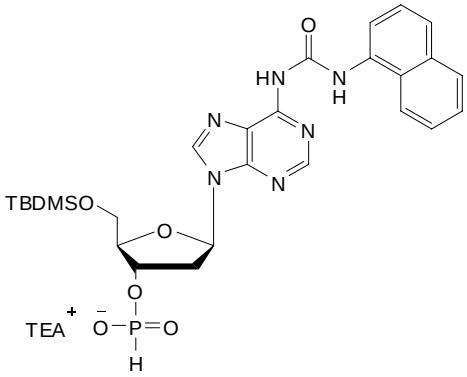
V

Synthesis of V

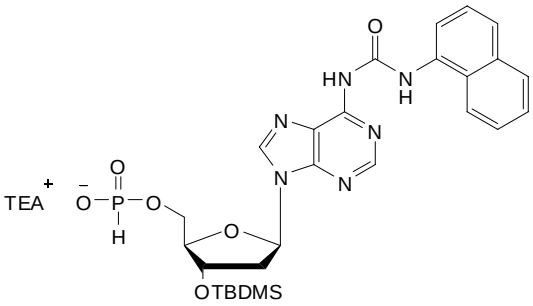
The nucleoside **IIb** (1 mmol) se was co-evaporated in THF (2×5 mL). The solid was dissolved in THF (12 mL). To a stirred solution was added aqueous TFA (6 ml, TFA:H_2O v/v 1:1) at 0ºC. After stirring for 2 h at 0ºC, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and diluted with 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

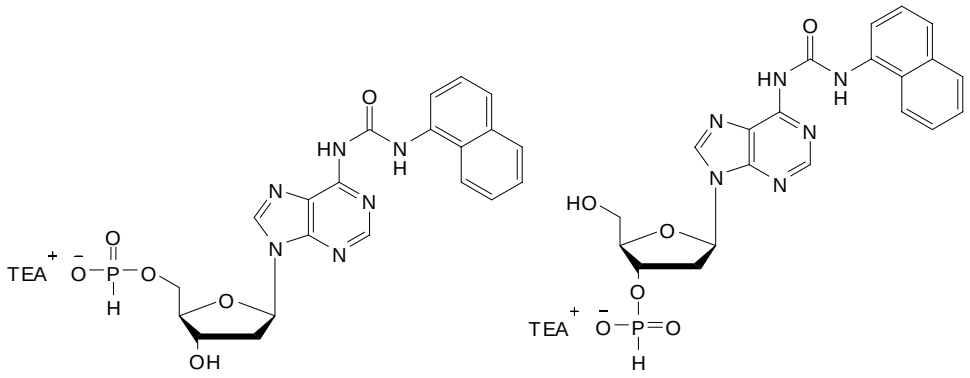
Synthesis of VI and VII

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$^-$)].



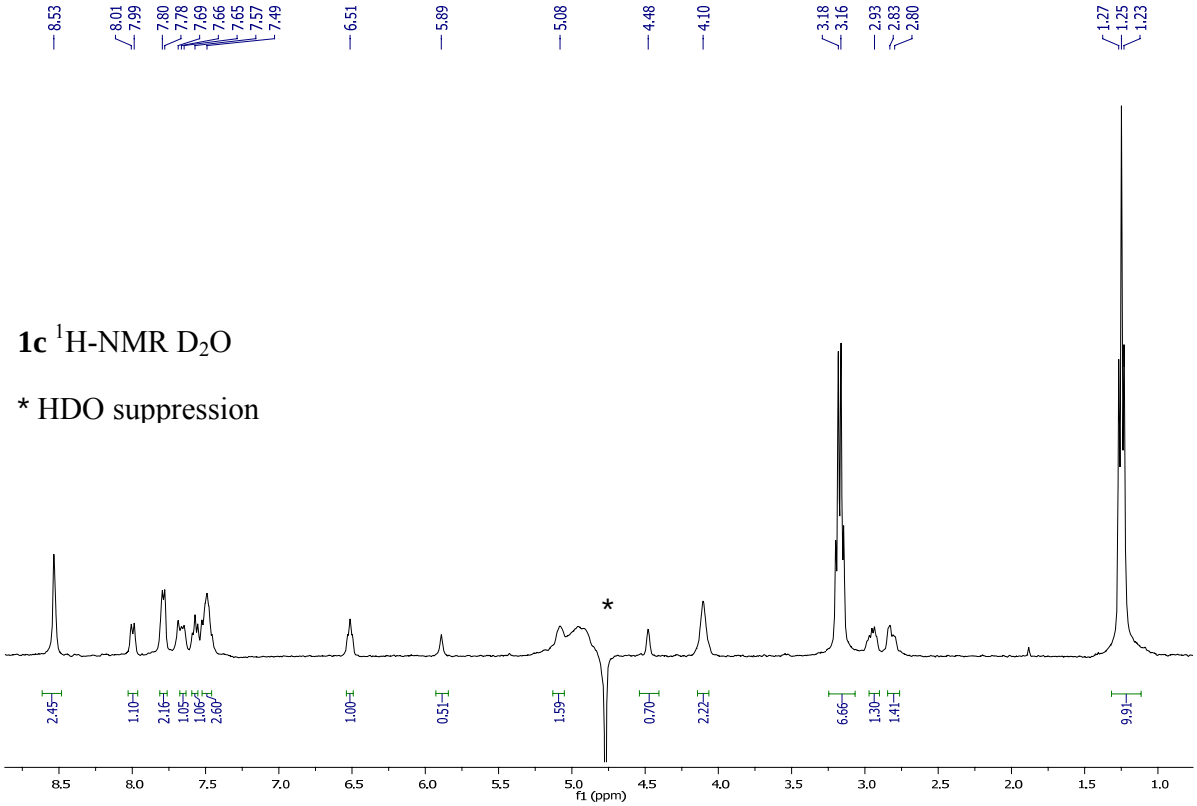
1c **1d**

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.

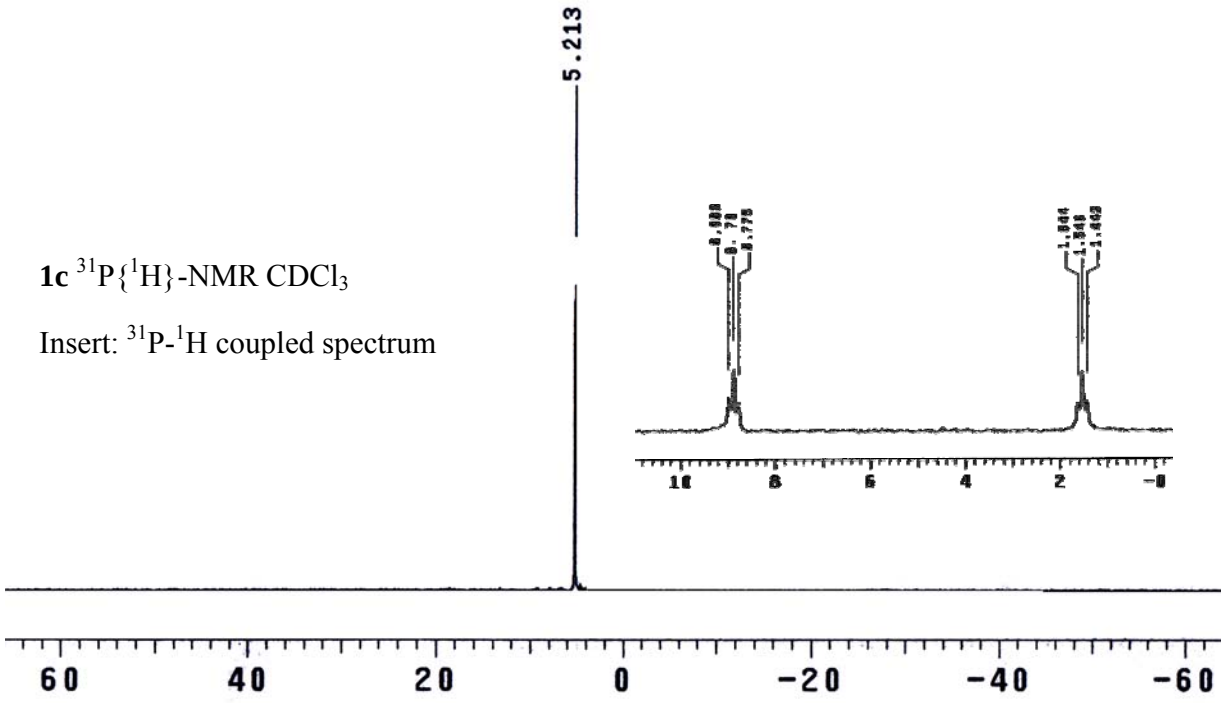
1c. 87 %; ^{1}H-NMR (D_2O, 200 MHz): δ_{ppm} = 8.53 (2H, s, H-purine), 8.0 1, 7.99 (1H, d, J_{HH} = 8 Hz H-Naphthyl), 7.80, 7.78 (2H, d, J_{HH} = 8 H-Naphthyl), 7.66 (1H, m, H-Naphthyl), 7.57 (1H, m, H-Naphthyl), 7.49 (2H, m, H-Naphthyl), 7.49, 5.89 (1H, d, J_{HP} = 639.6 Hz, H-Phosphonate), 6.51 (1H, 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,

J_{PH} =593.97, J_{HH} = 8 Hz, H-Phosphonate); HR-MS: *m/z* (FAB$^-$) 483.1624 (M^- $C_{21}H_{20}O_6N_6P^-$ requires 483.1187).



1c ^{1}H-NMR D_2O

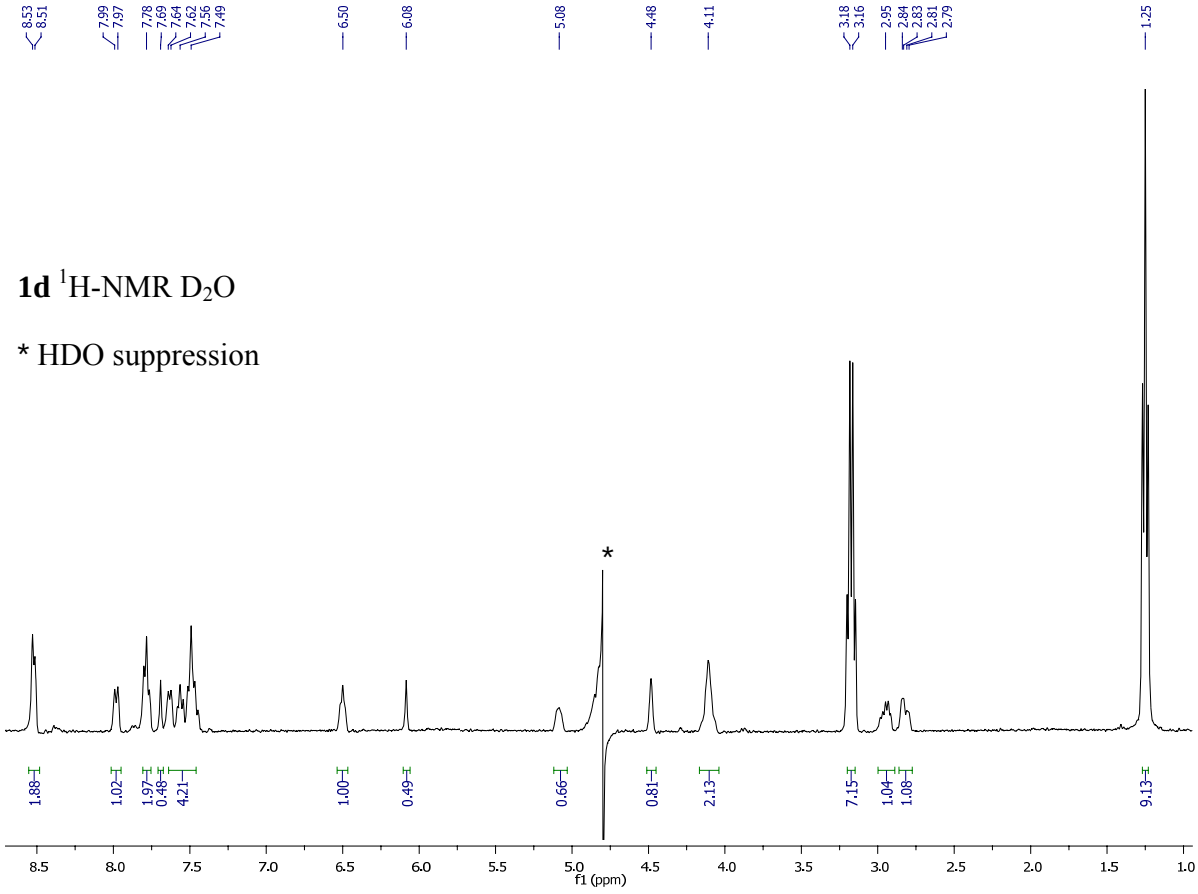
* HDO suppression



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

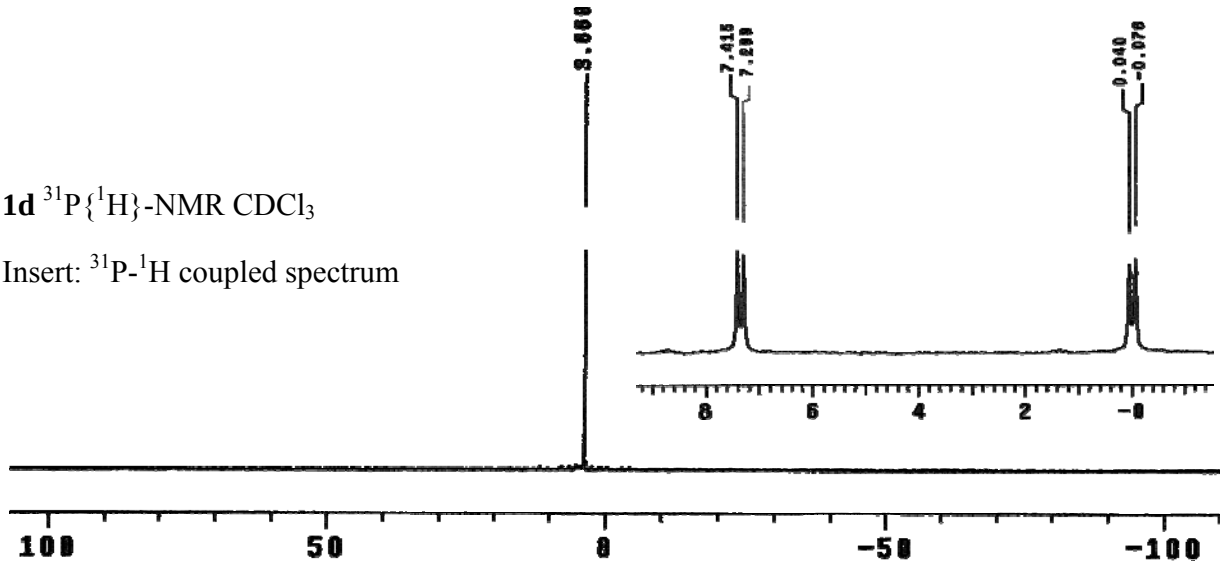
Insert: ^{31}P-^1H coupled spectrum

1d. Yield 89%;^{1}H-NMR (D_2O, 400 MHz): δ_{ppm} = 8.53 (1H, s, H-purine), 8.51 (1H, s, H-purine), 7.99, 7.97 (1H, t, J_{HH} = 8 Hz, H-Naphthyl), 7.78 (1H, t, J_{HH} = 7.2 Hz, H-Naphthyl), 7.69, 6.08 (1H, d, J_{HP} = 644 Hz, H-Phosphonate), 7.64, 7.62 (1H, d, J_{HH} = 8 Hz, H-Naphthyl), 7.56 (1H, t, J_{HH} = 8 Hz, H-Naphthyl), 7.49 (3H, m, H-Naphthyl), 6.50 (1H, dd, J_{HH} = 6.4 Hz, H1'-Deoxyribose), 5.08 (1H, m, H3'-Deoxyribose), 4.48 (1H, m, H4'-Deoxyribose), 4.11 (2H, m, H5'-Deoxyribose), 3.17 (6H, q, J_{HH} = 7.2 Hz, CH_3-TEA), 2.95, 2.84-2.79 (2H, m, H2', H2''-Deoxyribose), 1.25 (9H, t, J_{HH} = 7.2 Hz, CH_2-TEA), 0.82 ^{13}C-NMR ($CDCl_3$, 50 MHz): δ_{ppm} = 152.12, 151.35, 151.01, 150.62, 142.79, 134.35, 129.26, 127.29, 126.76, 126.54, 126.35, 124.45, 121.65, 121.20, 118.15, 88.09, 84.35, 70.92, 63.84, 45.30, 8.74 ^{31}P-NMR ($CDCl_3$, 81 MHz): δ_{ppm} 3.66 (1P, dd, J_{PH} = 597.37, J_{HH} = 9.4 Hz, H-Phosphonate); HR-MS: *m*/*z* (FAB$^-$) 483.1581 (M^- $C_{21}H_{20}O_6N_6P^-$ requires 483.1187).



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

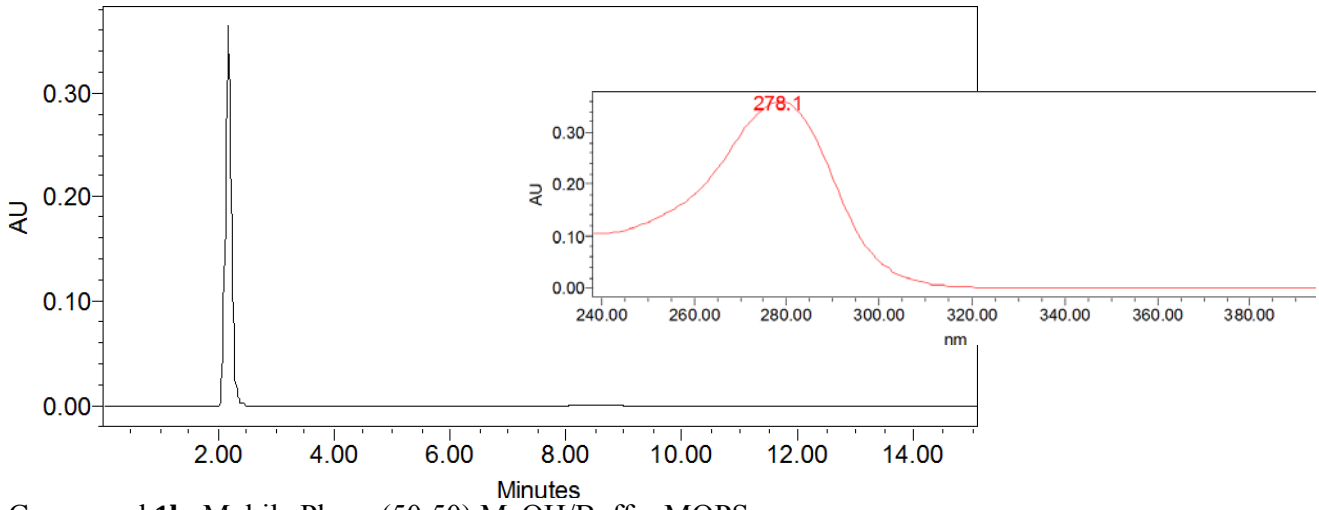
Insert: ^{31}P-^{1}H coupled spectrum



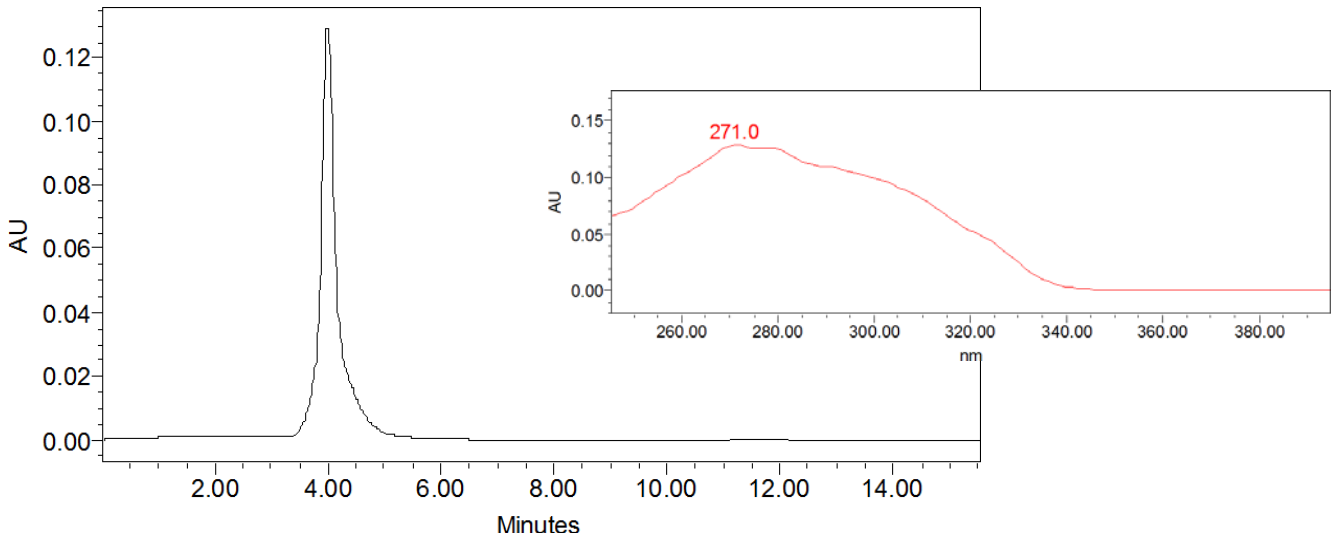
HPLC Chromatograms

Selected HPLC traces for compounds **1a-1g, AdNH2** and **AdNHEt** are shown with the corresponding UV spectra. Chromatographic conditions are indicated for each experiment.

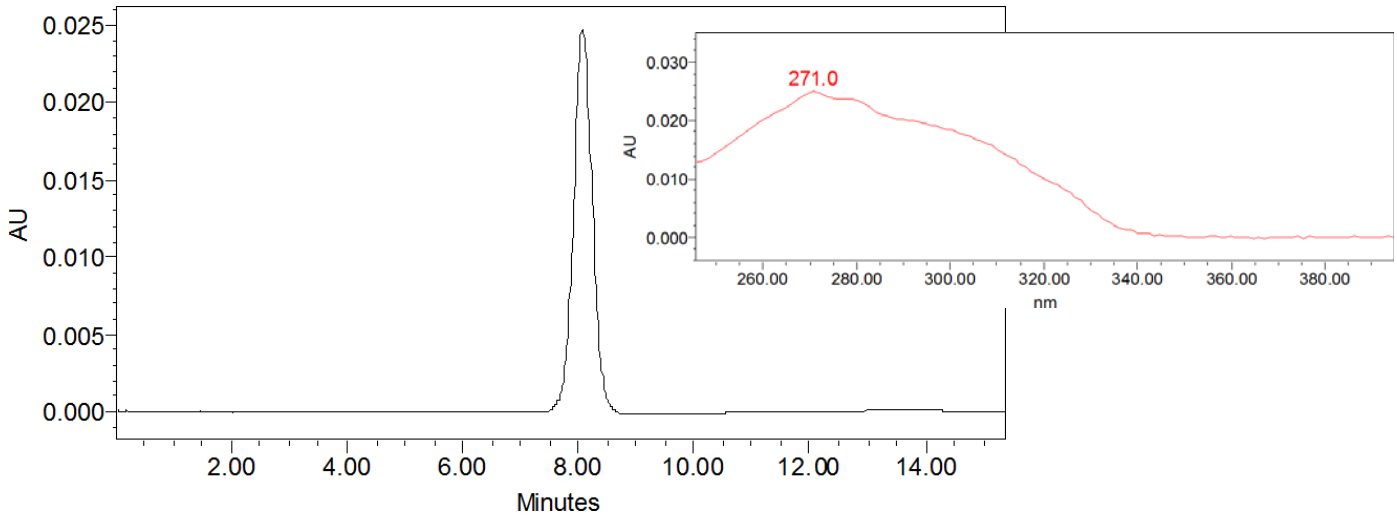
Compound **1a**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 2.170.



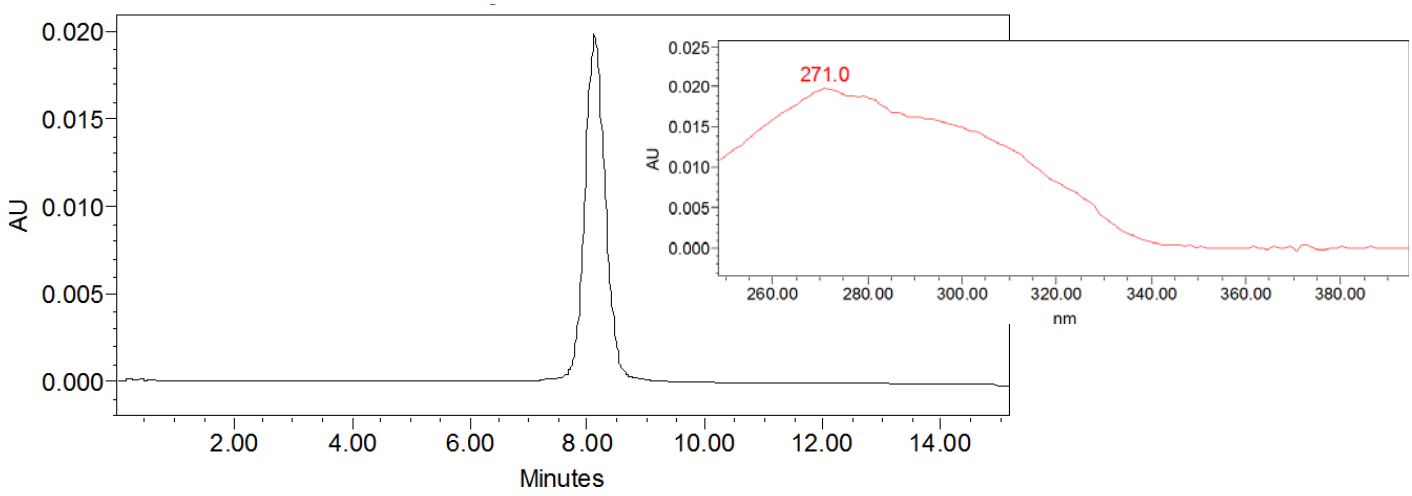
Compound **1b**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 3.994.



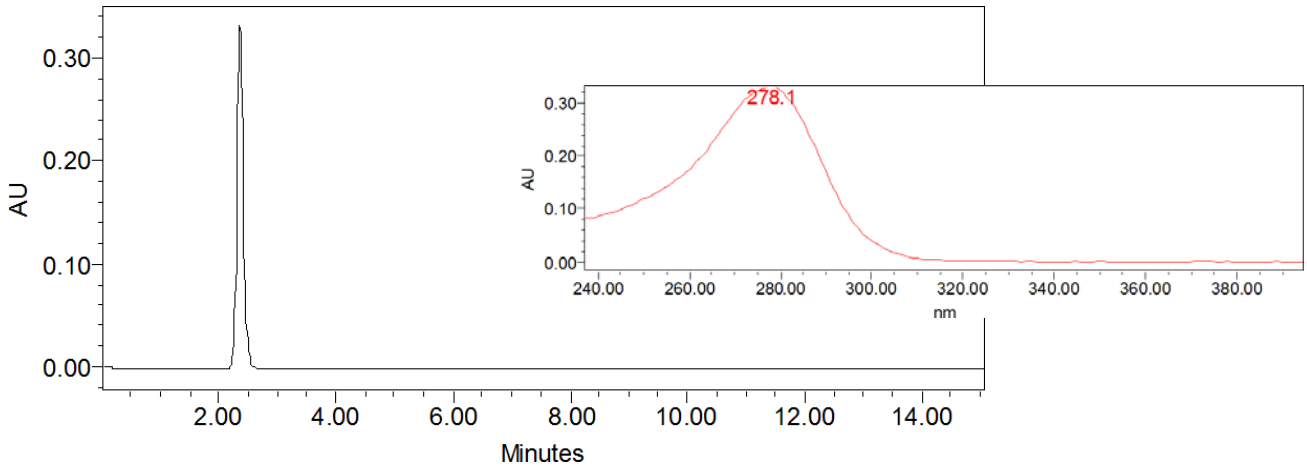
Compound **1c**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 8.073.



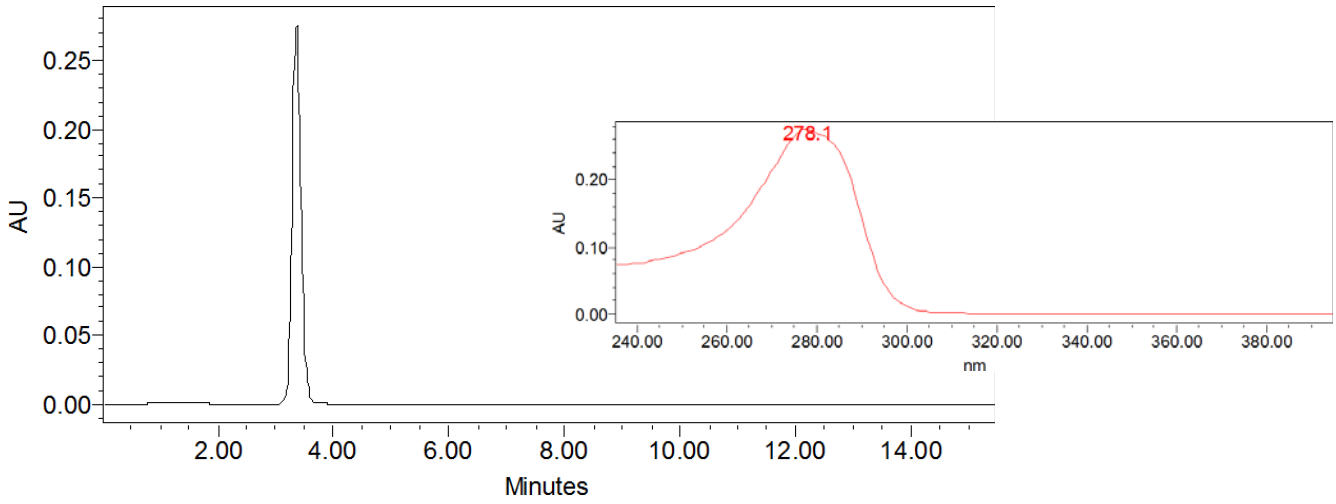
Compound **1d**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 8.121.



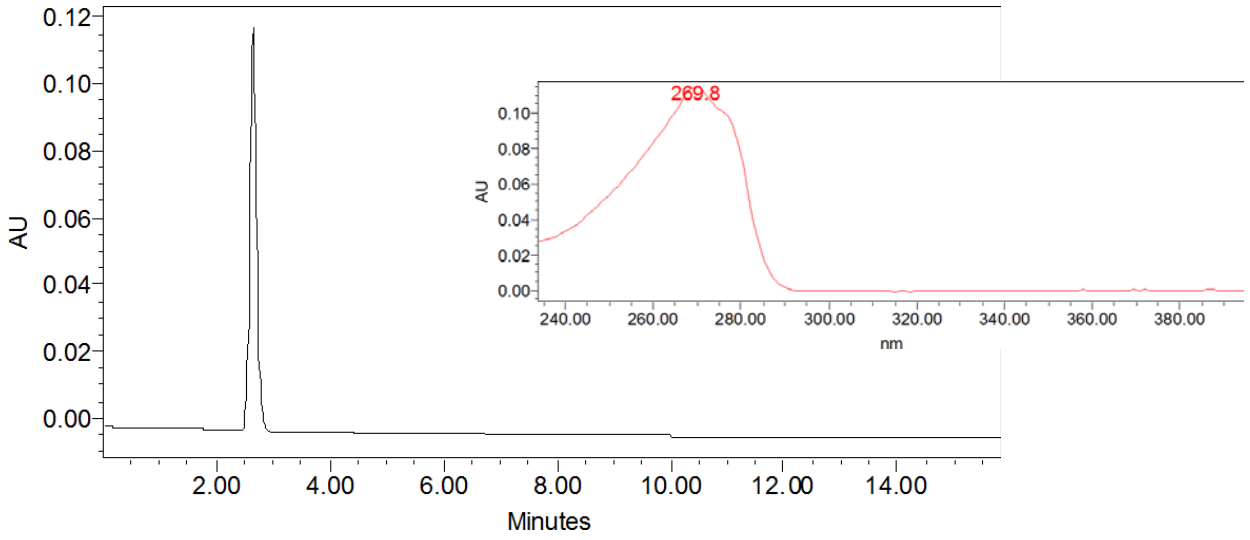
Compound **1e**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 2.355.



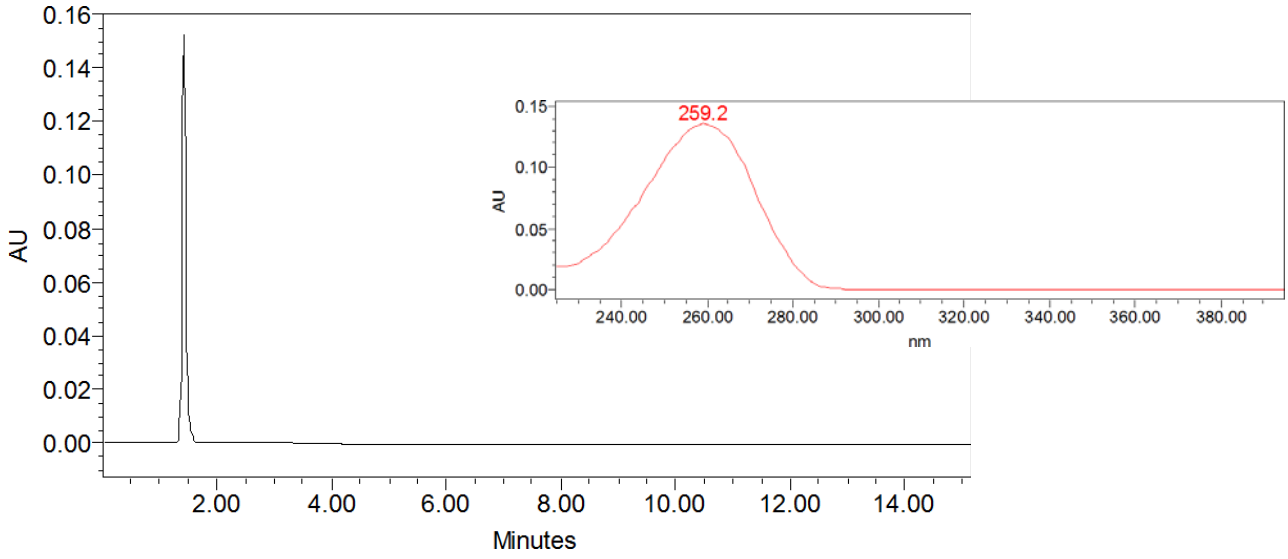
Compound **1f**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 3.360.



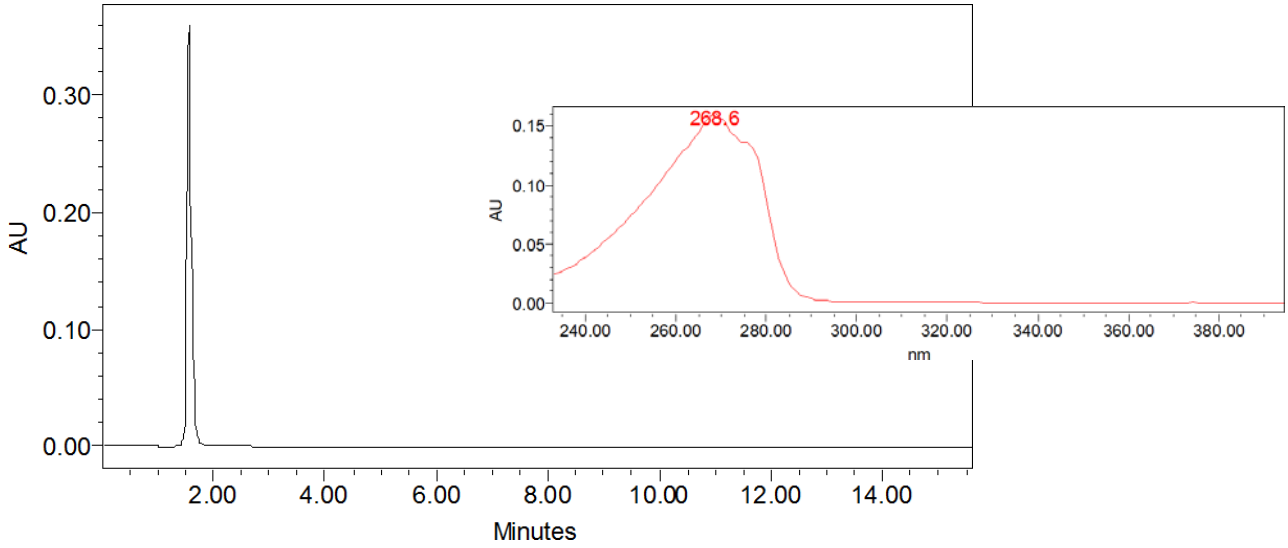
Compound **1g**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 2.654.



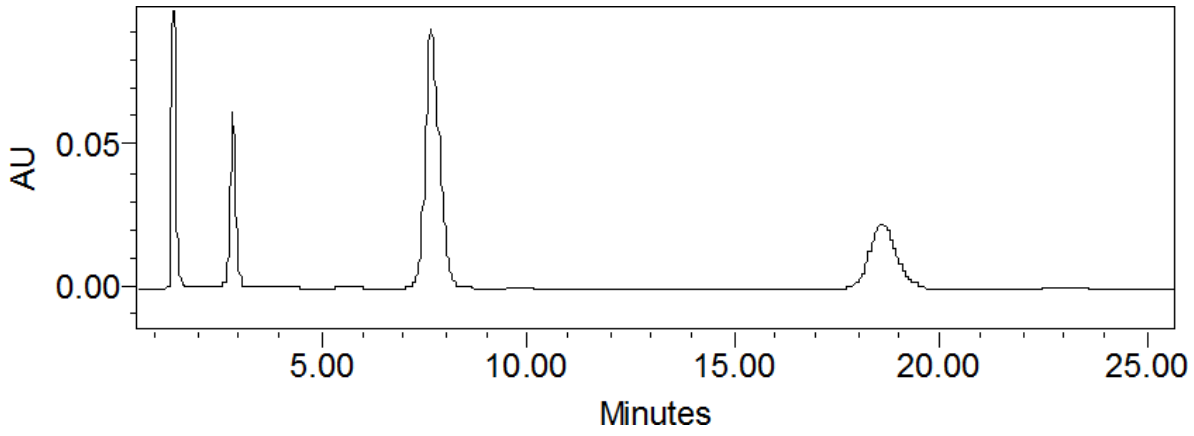
Compound **AdNH2**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 1.422.



Compound **AdNHEt**, Mobile Phase (50:50) MeOH/Buffer MOPS
Retention time (min): 1.564.



Mixture of compounds **AdNH2**, **1a**, **1b** and **1c**. Mobile Phase (40:60) MeOH/Buffer MOPS
Retention time (min): 1.418 (AdNH2), 2.866 (1a), 7.659 (1b) and 18.594 (1c).



Part II

Molecular Modeling. Molecular modeling was carried out with MacroModel 9.6 and Jaguar 7.5 using Maestro 8.5.207 interface, Schrödinger, LLC. On Macromodel, global conformational searches for nucleotides, dyes and complexes were carried out using the OPLS-2005 force field using water default as solvent. The starting geometries for these searches were obtained from minimization using the same force field in water. All structures within 25 kJ/mol were retained from a sampling of 20,000 structures obtained by a mixed Monte Carlo Multiple Minimum/Low-Mode Conformational Search method (MCMM/LMCS) that randomly changes torsion angles in the starting geometry, and minimizes the resulting structures to convergence.

Full geometry optimization for N^6-phenylcarbamoyl-2'-deoxyadenosine derivatives were also computed at LMP2/6-31G**//DFT/6-311+G** level both in gas phase and in water solvent using the standard Poisson-Boltzmann model (PBF) as implemented in Jaguar 7.5. Structures were characterized by 3N-6 real vibrational frequencies (see Figure S11 below).

UV-Visible Titrations

Salts of cationic dyes **TB**, **MB**, **AC**, **OX**, and **NB** were obtained from Sigma-Aldrich and used as received. All the measurements were conducted at 25ºC (except when the effect of temperature was investigated) on solutions buffered with 0.010 M MOPS adjusted to pH 7.0 containing 0.010 M NaCl. Buffer solutions were prepared using chemical grade salts and deionized water. Absorption spectra were measured using a Hewlett-Packard Model 8453 Diode Array UV-Vis spectrometer in quartz cuvettes of 1 cm pathlength using the buffer without dye as reference. A 1.5×10^{-5} M solution of the dyes and 5×10^{-3} M solutions of nucleotides were freshly prepared for titration experiments. Small aliquots (10 μl) of stock nucleotide solutions were added to the dye solution (3000 μL) until the absorbance no longer decreased. Nucleotide concentrations were thus varied from 2.5×10^{-6} to 1×10^{-4}

M. The mixture of dye and nucleotide solutions were stirred uniformly for 1 minute and allowed to equilibrate for 15 s before recording the absorption spectra. Experimental samples were prepared using calibrated micropipettes. For each concentration studied the data were acquired between 250-800 nm. Ten wavelengths between 560-680 nm were subsequently analyzed.

Equation 1 was used to calculate the equilibrium constants for each titration experiment; this equation assumes a 1:1 binding model in which concentrations of host and guest are of similar magnitude (see H.-J. Schneider and A.K. Yatsimirsky, *Principles and Methods in Supramolecular Chemistry*, Wiley, 2000. p.142). Non-linear curve fitting of the data was performed using Origin 5.0 Microcal TM® using least-squares minimization.

$$\mathbf{A_{obs} = A_S\,[S]_t + A_L\,[L]_t + 0.5\,\Delta A\,([S]_t + [L]_t + K_D - \{([S]_t + [L]_t + K_D)^2 - 4\,[S]_t\,[L]_t\}^{1/2})} \qquad \mathbf{(1)}$$

where $[L]_t$ = ligand concentration, $[S]_t$ = substrate concentration, A_S = absorbance of substrate, K_D = dissociation constant of complex SL, $\Delta A = A_{SL} - A_S$.

Autoassociation of both cationic dyes and nucleotides was tested by the concentration dependence of the UV-vis absorption spectra within the concentration range used for titration experiments. Linear relationships were found for all cases indicating negligible dimerization or higher complex formation.

Table S1 Measured binding constants K_{as} ($\times 10^5$, M^{-1}) for nucleotides **1a-1g** and cationic dyes[a]

Dye	1a	1b	1c	1d	1e	1f	1g
MB	0.89 ±0.11	2.4 ±0.5	0.21 ±0.07	0.16 ±0.01	0.36 ±0.05	0.35 ±0.05	0.23 ±0.02
AC	0.50 ±0.03	1.5 ±0.3	1.36 ±0.08	0.63 ±0.11	0.95 ±0.06	2.01 ±0.12	0.30 ±0.03
TB	3.00 ±0.37	7.8 ±2.1	1.79 ±0.16	1.43 ±0.08	0.59 ±0.03	9.39 ±0.69	0.89 ±0.02
NB	1.35 ±0.08	55.7 ±5.7	0.65 ±0.19	0.49 ±0.15	1.35 ±0.04	1.89 ±0.27	0.39 ±0.05
OX	0.83 ±0.09	5.0 ±0.9	0.62 ±0.08	0.40 ±0.07	0.38 ±0.04	1.42 ±0.15	0.27 ±0.01

[a] K_{as} measured by UV-vis titrations in MOPS aqueous buffer at pH 7, 25ºC and 0.010 M NaCl.

Table S2 Selected ΔG^o and $\Delta\Delta G^o$ (kJ mol^{-1} at 298 K) values for complexes of nucleotides **1a-1d** and cationic dyes

	ΔG^o				$\Delta\Delta G^o$		
Dye	**1a**	**1b**	**1c**	**1d**	**1b-1a**	**1c-1b**	**1d-1b**
MB	-28.23	-30.69	-24.65	-23.98	-2.46	+6.04	+6.71
AC	-26.80	-29.53	-29.29	-27.38	-2.73	+0.24	+2.15
TB	-31.24	-33.61	-29.97	-29.41	-2.37	+3.64	+4.20
NB	-29.27	-38.48	-27.46	-26.76	-9.21	+11.02	+11.72
OX	-28.06	-32.51	-27.34	-26.25	-4.45	+5.17	+6.26

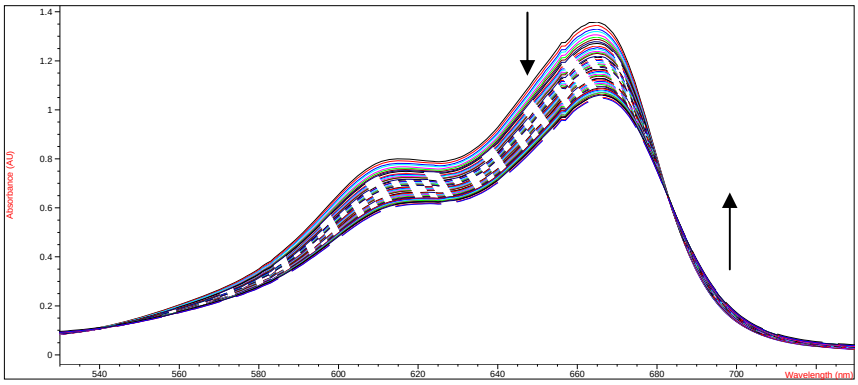


Fig. S1 Changes of the absorption spectra during titration of **MB** (2×10^{-5} M) with nucleotide **1a**. Arrows indicate the spectral changes occurring in response to an increasing concentration of **1a** (0-1.0×10^{-4} M).

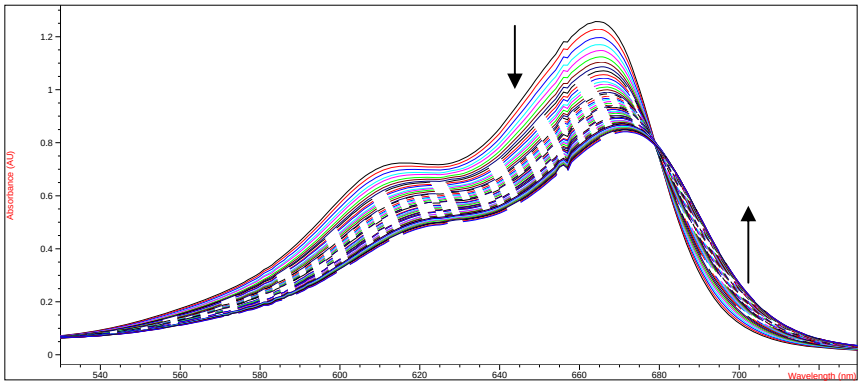


Fig. S2 Changes of the absorption spectra during titration of **MB** (2×10^{-5} M) with nucleotide **1b**. Arrows indicate the spectral changes occurring in response to an increasing concentration of **1b** (0-1.0×10^{-4} M).

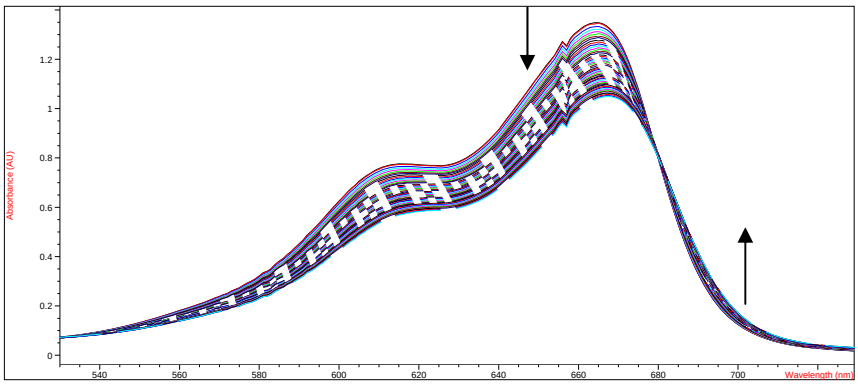


Fig. S3 Changes of the absorption spectra during titration of **MB** (2×10^{-5} M) with nucleotide **1f**. Arrows indicate the spectral changes occurring in response to an increasing concentration of **1f** (0-1.0×10^{-4} M).

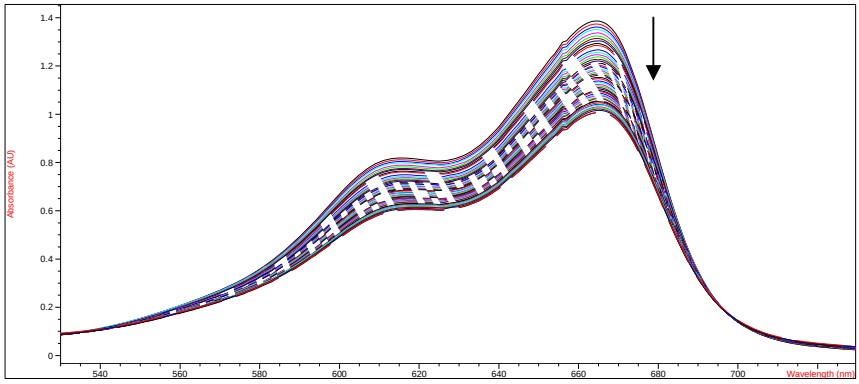


Fig. S4 Changes of the absorption spectra during titration of **MB** (2×10^{-5} M) with nucleotide **1g**. Arrows indicate the spectral changes occurring in response to an increasing concentration of **1g** ($0\text{-}1\times10^{-4}$ M).

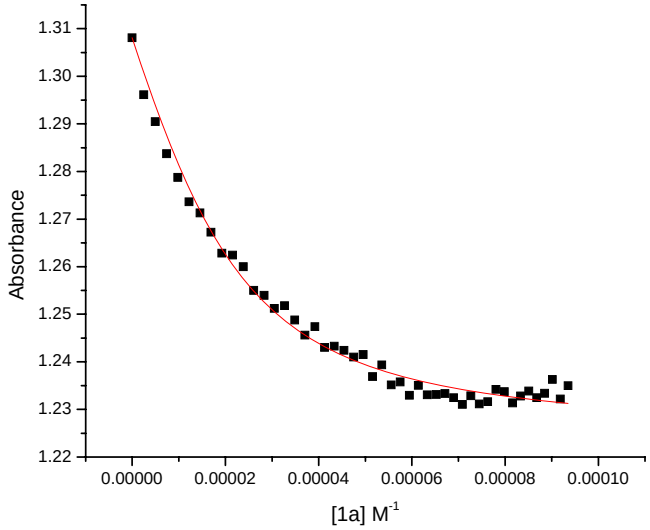


Fig. S5 Changes of the absorbance at 660 nm during titration of **MB** (2×10^{-5} M) with nucleotide **1a** ($0\text{-}9.3\times10^{-5}$ M). The solid curve is fitting to a 1:1 binding model according to equation 1 (see above).

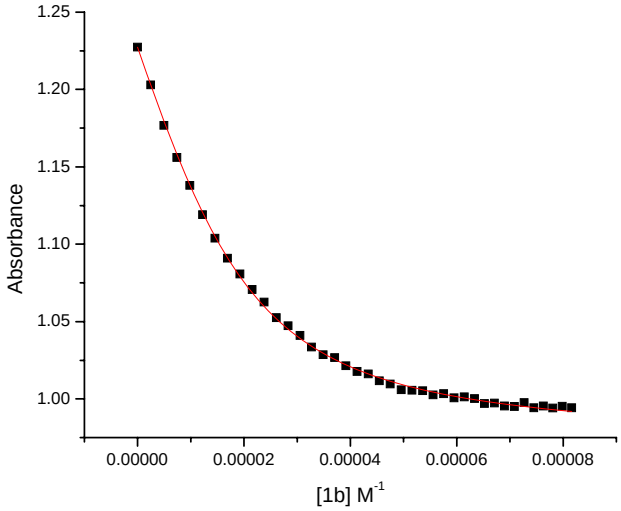


Fig. S6 Changes of the absorbance at 660 nm during titration of **MB** (2×10^{-5} M) with nucleotide **1b** ($0\text{-}8.1\times10^{-5}$ M). The solid curve is fitting to a 1:1 binding model according to equation 1 (see above).

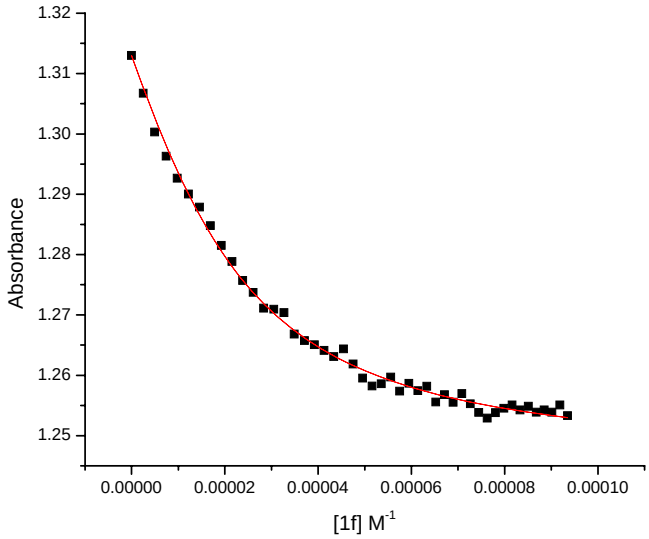


Fig. S7 Changes of the absorbance at 660 nm during titration of **MB** (2×10^{-5} M) with nucleotide **1f** (0-9.3×10^{-5} M). The solid curve is fitting to a 1:1 binding model according to equation 1 (see above).

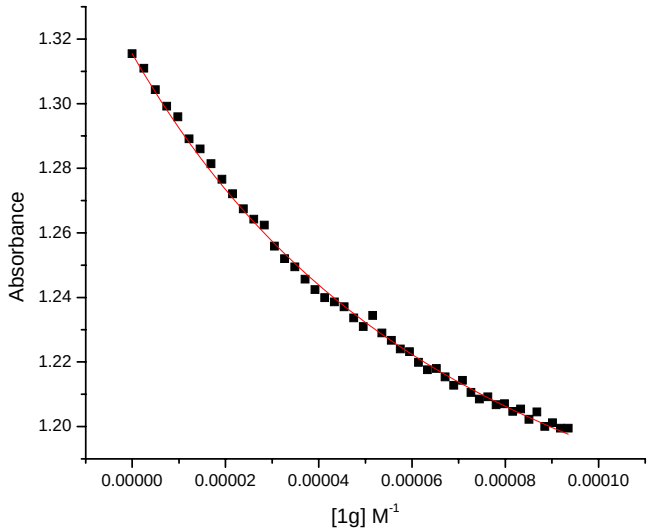


Fig. S8 Changes of the absorbance at 660 nm during titration of **MB** (2×10^{-5} M) with nucleotide **1g** (0-9.3×10^{-5} M). The solid curve is fitting to a 1:1 binding model according to equation 1 (see above).

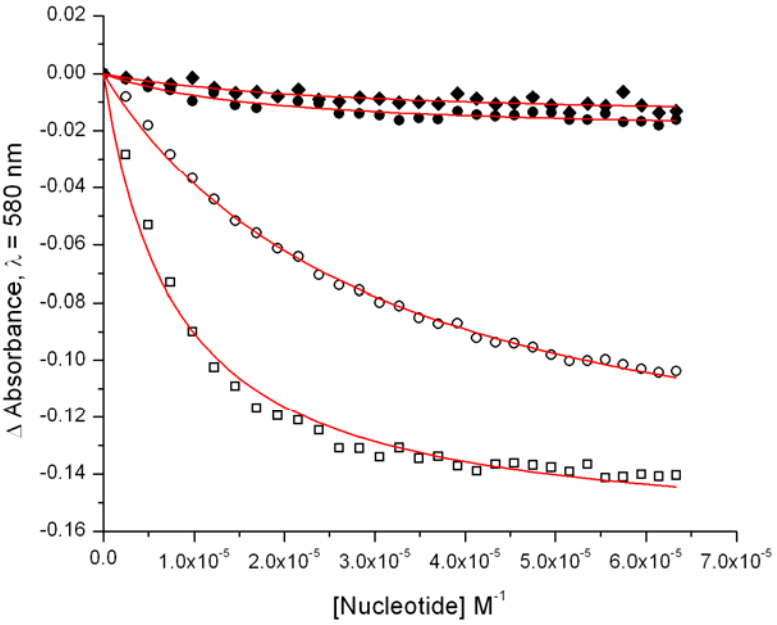


Fig. S9. Changes in the absorbance of **OX** upon addition of nucleotides **1a** (○) and **1b** (□). For comparison **AdNH$_2$** (●) and **AdNHEt** are included (♦). Solid line shows nonlinear fit to 1:1 binding model.

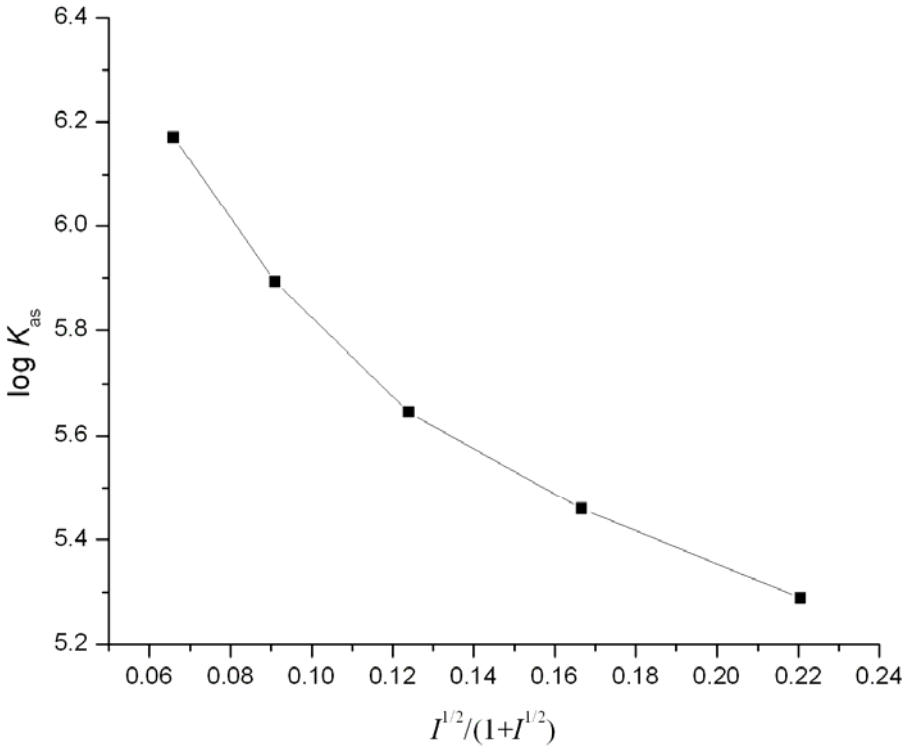


Figure S10. Ionic strength dependence for association of **1b**-**TB.**

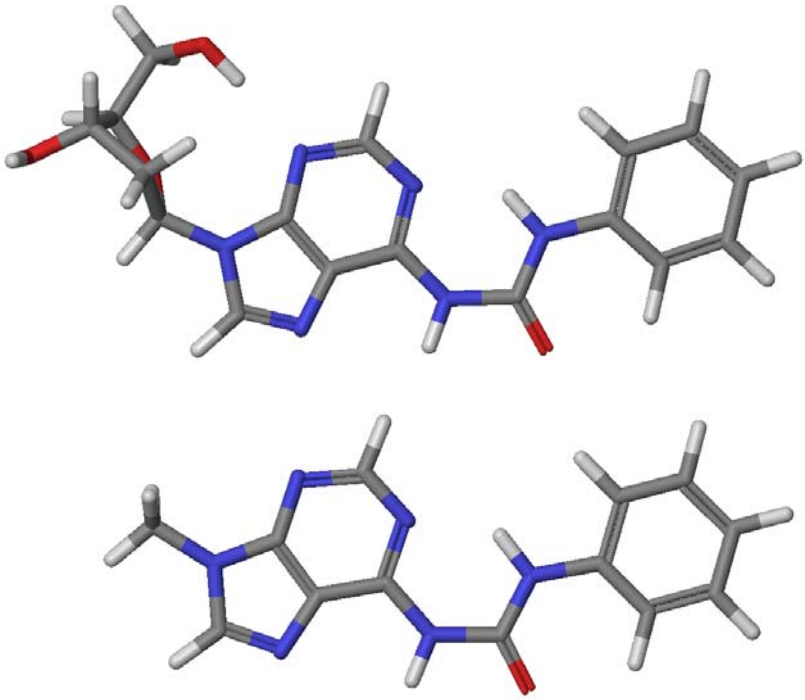


Figure S11. (Top) Optimized equilibrium geometry for N^6-(*N*'-phenylcarbamoyl)-2'-deoxyadenosine in gas phase and (bottom) for N^9-methyl-N^6-(*N*'-phenylcarbamoyl)adenine in water solvent using the standard Poisson-Boltzmann model (PBF) as implemented in Jaguar 7.5. Calculations were performed at LMP2/6-31G**//DFT/6-311+G** level and final structures were characterized by 3N-6 real vibrational frequencies.

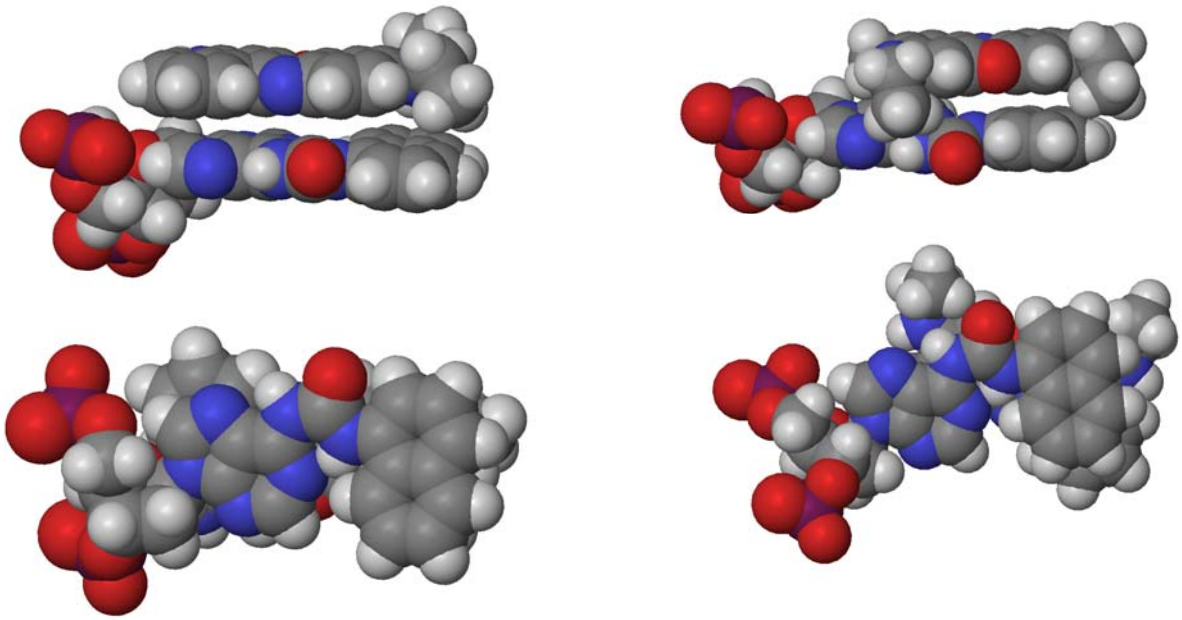
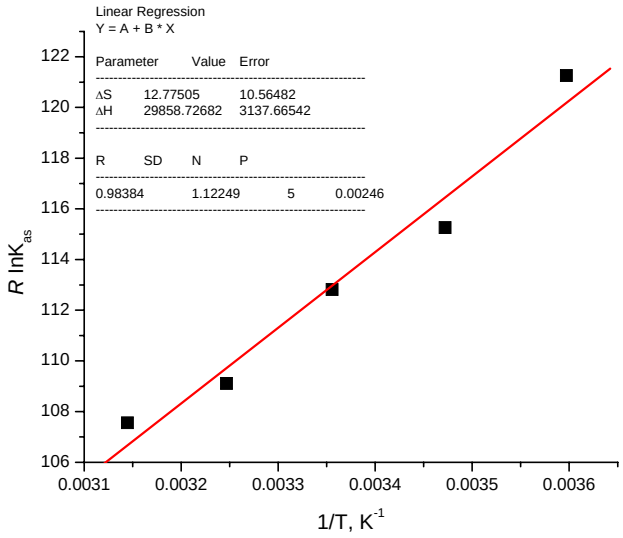


Figure S12. Minimum energy conformers for complexes **1b-NB** (left) and **1b-OX** (right). Calculations were carried out with MacroModel 9.6 (OPLS 2005 in water).



	ΔH° kJ mol^{-1}	TΔS° kJ mol^{-1}	ΔG° kJ mol^{-1}
1b-TB	**-29.8 ±3.1**	**- 3.7 ± 3.1**[a]	**-33.5**[b]

[a] at 298 K

[b] For comparison at 298 K a ΔG° = -33.6 kJ mol^{-1} was measured for **1b-TB** , see Table S2)

Figure S13. Temperature dependence of the equilibrium constant (R InK_{as}) for complex **1b-TB.** The solid line is the fitting to the van't Hoff equation. The thermodynamic parameters obtained are shown in the table.

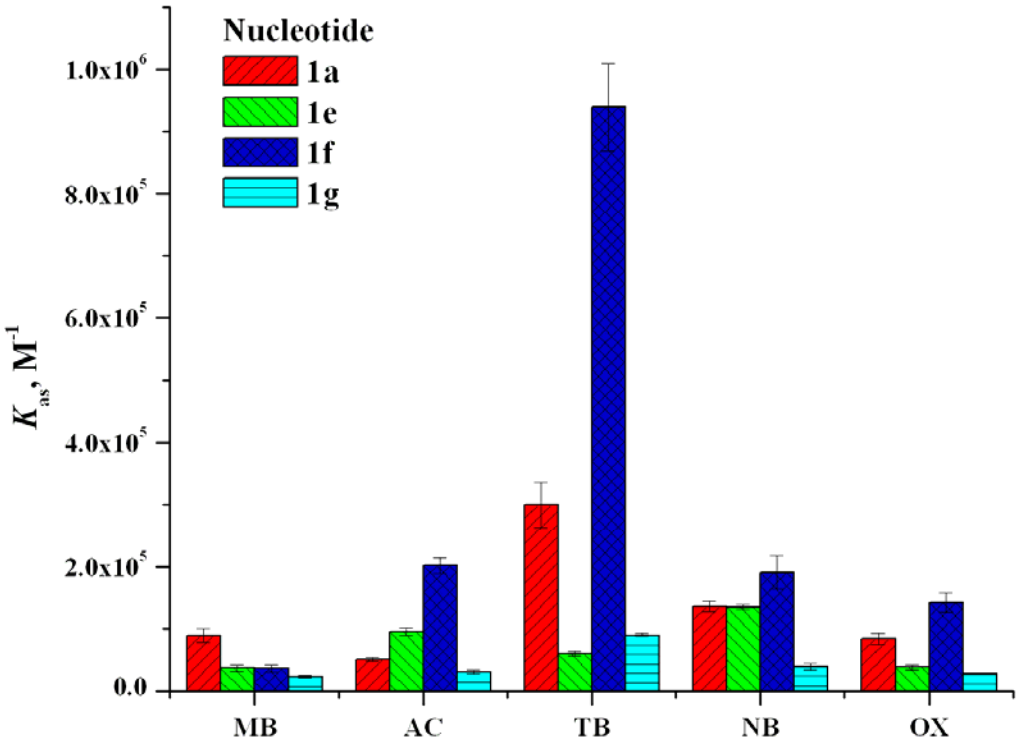


Figure S14. Comparison of association constants (K_{as}) for nucleotides **1a**, **1e**, **1f** and **1g** with phenothiazinium and phenoxazinium dyes.

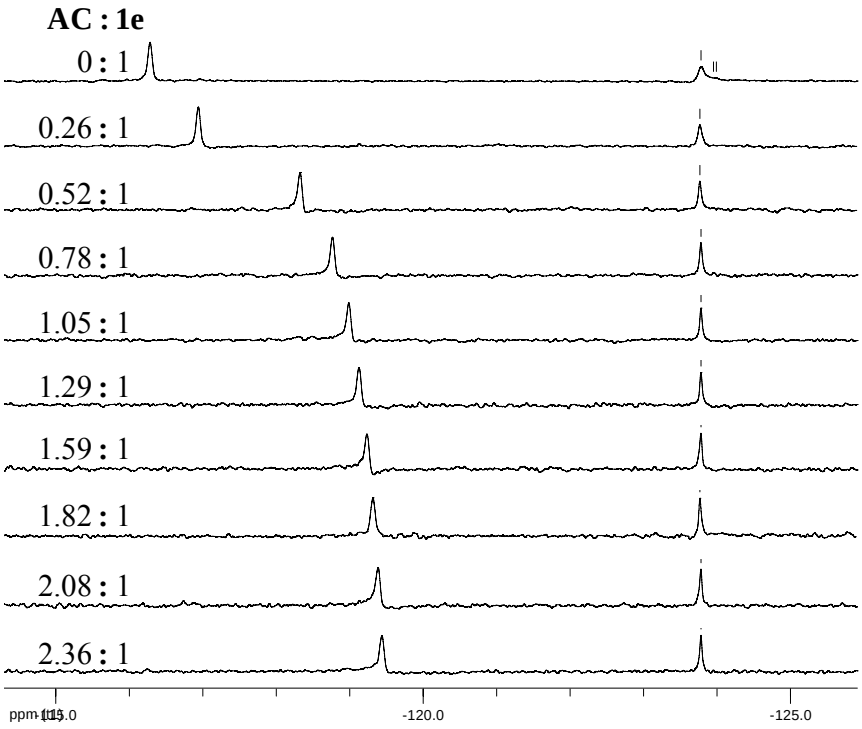


Figure S15. Changes in the ^{19}F NMR spectra (188 MHz) of **1e** (8.5×10^{-4} M in D_2O) upon addition of **AC**. Relative molar concentrations of **AC:1e** are indicated for each spectrum. Signal at -123.8 ppm belongs to KF.

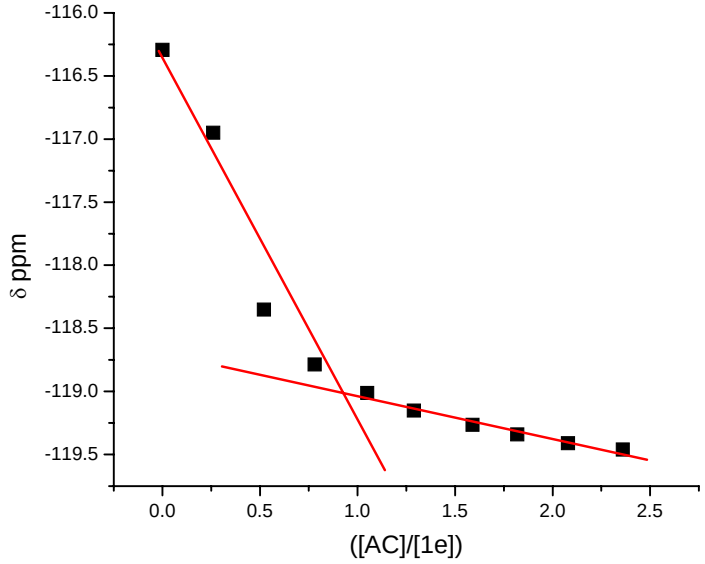


Figure S16. Plot of the ^{19}F chemical shift displacements of 4-fluorophenyl in **1e** (8.5×10^{-4} M in D_2O) upon addition of **AC**. Relative molar concentrations of **AC/1e** are indicated in x-axis.

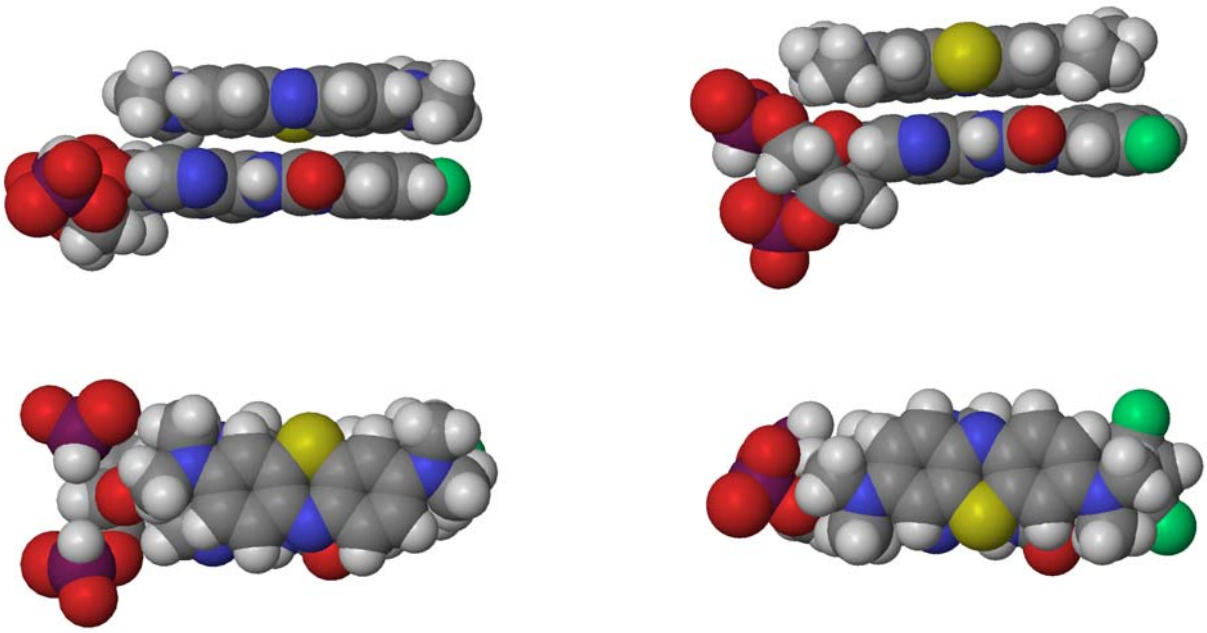


Figure S17. Minimum energy conformers for complexes **1e-MB** (left) and **1f-MB** (right). Calculations were carried out with MacroModel 9.6 (OPLS 2005 in water).

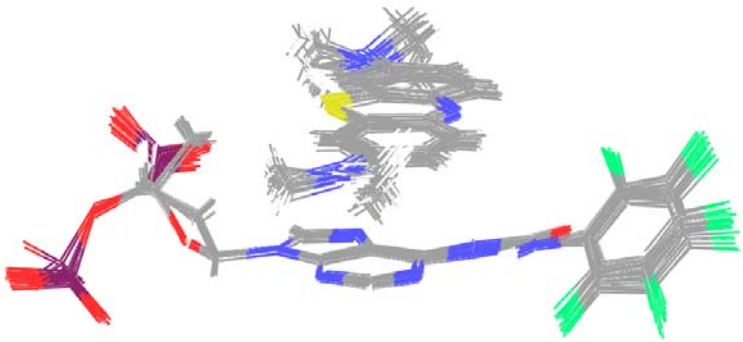


Figure S18. Twenty five lower energy conformers of complex **1g-MB** found within 3.5 kJ/mol from global minimum. Calculations were carried out with MacroModel 9.6 (OPLS 2005 in water).