Electronic Supplementary Information Part I

Molecular Recognition of Cationic Phenothiazinium and Phenoxazinium Dyes with $\pi$-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$_3$PO$_4$ in CDCl$_3$ (external standard). The $^{19}$F NMR spectra were referenced to KF in D$_2$O (internal standard). $^1$H NMR spectra acquired in D$_2$O 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, AdNH$_2$ and AdNHEt according to the method reported by Lombardo and Abraham.$^1$ These results will be published elsewhere in due course.

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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:H2O 15 min.; v) TFA:H2O, THF, rt, 2 h.; vi) (a) DPP, Py, rt, 15 min., (b) TEA:H2O, 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:H2O 15 min.; x) TBAF, THF, rt, 2 h.; xi) (a) DPP, Py, rt, 15 min., (b) TEA:H2O, rt, 15 min.

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): $\delta_{ppm} = 8.35$ (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): $\delta_{ppm} = 156.02$, 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$^+$)].

![Chemical structure of IIa, IIb, Ile, IIf, IIg and IIe](image)

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

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): $\delta_{ppm} = 11.85$ (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): $\delta_{ppm} = 151.67$, 150.91, 150.38,
IIb. Yield, 93.1%; $^1$H-NMR (CDCl$_3$, 200 MHz): $\delta$ppm = 12.28 (1H, s, NH-carbamoyl), 9.14 (1H, s, NH-Carbamoyl), 8.70 (1H, s, H-purine), 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), 0.90 (9H, s, t-butyl-TBDMS), 0.92 (9H, s, t-butyl-TBDMS), 0.117 (6H, s, di-Me-TBDMS), 0.117 (6H, s, di-Me-TBDMS). $^{13}$C-NMR (CDCl$_3$, 50 MHz): $\delta$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 599 [(M+H$^+$)].

Iic. Yield, 80%; $^1$H-NMR (CDCl$_3$, 200 MHz): $\delta$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, $J_{HH} = 8.4$Hz, 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): $\delta$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$_2$O, 188 MHz): $\delta$ppm = -116.35 (1F, t, F$^\text{para}$-Phenyl); FAB-MS: m/z 617 [(M+H$^+$)].

IIIf. Yield 85%; $^1$H-NMR (CDCl$_3$, 200 MHz): $\delta$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’ y 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): $\delta$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$_2$O, 376 MHz): $\delta$ppm = -111.58 (2F, t, F$^\text{meta}$-Phenyl); FAB-MS: m/z 635 [(M+H$^+$)].
**IIIg.** Yield 90.1%; $^1$H-NMR (CDCl$_3$, 200 MHz): $\delta_{ppm} = 11.73$ (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). 13C-NMR (CDCl$_3$, 50 MHz): $\delta_{ppm} = 152.00, 150.70, 149.74, 143.04, 121.111, 88.27, 84.68, 71.94, 62.85, 41.19, 26.15, 26.01, 18.65, 18.28, -4.43, -4.57, -5.35, -5.25. 19F-NMR (D$_2$O, 376 MHz): $\delta_{ppm} = -147.92$ (2F, d, F$_{ortho}$-Phenyl), -158.18 (1F, t, F$_{para}$-Phenyl), -164.85 (2F, t, F$_{meta}$-Phenyl), FAB-MS: m/z 689 [(M+H$^+$)].

**IIIEt.** Yield 94%; $^1$H-NMR (CDCl$_3$, 200 MHz): $\delta_{ppm} = 9.35$ (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), 0.02 (6H, s, di-Me-TBDMS) 0.11 (6H, s, di-Me-TBDMS); 13C-NMR (CDCl$_3$, 50 MHz): $\delta_{ppm} = 156.78, 156.02, 152.99, 149.53, 138.86, 120.02, 87.99, 84.49, 72.02, 62.94, 41.50, 34.15, 26.19, 26.00, 18.66, 18.24, 14.52, -4.37, -4.52, -5.09, -5.20; FAB-MS: m/z 551 [(M+H$^+$)].

**IIIa, IIIb, IIIe, IIIf and 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): $\delta_{ppm} = 11.66$ (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, $J_{HH} = 8$ Hz, H-Phenyl), 6.97 (1H, t, $J_{HH} = 7.2$ Hz, 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). 13C-NMR (CDCl$_3$-MeOH, 50 MHz): $\delta_{ppm} = 151.61, 150.35, 149.42, 142.40, 137.37, 128.86, 124.09, 121.14, 120.43, 88.80, 86.62, 71.82, 62.61, 40.75$. FAB-MS: $m/z$ 371 [(M+H$^+$)].

IIIb. Yield 90%. $^1$H-NMR (DMSO-d$_6$, 200 MHz): $\delta_{ppm} = 12.37$ (1H, s, NH-carbamoyl), 10.39 (1H, s, NH-carbamoyl), 8.80 (1H, s, H-purine), 8.18 (2H, t, $J_{HH} = 6.8$ Hz, H-Naphthyl), 7.92 (1H, d, $J_{HH} = 8$ Hz, H-Naphthyl), 7.69-7.53 (4H, m, H-Naphthyl), 6.43 (1H, dd, $J_{HH} = 6.8$ Hz, 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). 13C-NMR (CDCl$_3$-MeOH, 50 MHz): $\delta_{ppm} = 157.01, 155.99, 155.65, 155.55, 147.72, 139.11, 139.06, 133.96, 132.01, 131.43, 131.28, 131.01, 129.06, 126.44, 126.03, 122.74, 93.52, 89.33, 76.15, 67.09, 54.08$. FAB-MS: $m/z$ 421 [(M+H$^+$)].

IIIe. Yield, 80%; $^1$H-NMR (CDCl$_3$, 200 MHz): $\delta_{ppm} = 12.26$ (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, $J_{HH} = 8.4$ Hz, H-Phenyl), 6.58 (1H, dd, $J_{HH} = 6.6$ Hz, 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). 13C-NMR (CDCl$_3$, 50 MHz): $\delta_{ppm} = 151.60, 151.29, 150.77, 150.35, 150.63, 147.72, 139.11, 139.06, 133.96, 132.01, 131.43, 131.12, 129.06, 126.44, 126.03, 122.74, 93.52, 89.33, 76.15, 67.09, 54.08$. FAB-MS: $m/z$ 389 [(M+H$^+$)].

IIIIf. Yield, 83%; $^1$H-NMR (DMSO-d$_6$, 200 MHz): $\delta_{ppm} = 12.18$ (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). 13C-NMR (CDCl$_3$, 50 MHz): $\delta_{ppm} = 151.63, 151.29, 150.77, 150.22, 143.11, 142.12, 142.04, 141.95, 121.28, 103.23, 88.73, 84.49, 71.32, 62.25, 45.11$. 19F-NMR (D$_2$O, 376 MHz): $\delta_{ppm} = -111.58$ (2F, t, F$_{para}$-Phenyl), FAB-MS: $m/z$ 407 [(M+H$^+$)].
IIlg. Yield, 90.1%; $^1$H-NMR (DMSO-d$_6$, 200 MHz): $\delta_{ppm} = 11.44$ (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), 13C-NMR (CDCl$_3$-MeOH, 50 MHz): $\delta_{ppm} = 151.79, 151.21, 150.11, 143.28, 121.36, 88.75, 84.53, 71.32, 62.25, 60.47$. $^{19}$F-NMR (D$_2$O, 376 MHz): $\delta_{ppm} = -146.98$ (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): $\delta_{ppm} = 9.38$ (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); 13C-NMR (CDCl$_3$, 50 MHz): $\delta_{ppm} = 156.75, 156.06, 152.97, 149.55, 138.83, 120.05, 87.93, 84.52, 71.98, 62.85, 41.62, 34.23, 14.53$, FAB-MS: $m/z$ 323 [(M+H$^+$)].

**General procedure for synthesis of 1a, 1b, 1e, 1f, 1g, AdNH$_2$ and AdNHEt**

The corresponding nucleoside (IIla, IIlb, IIle, IIlf, IIlg, 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$_2$O, 400 MHz): $\delta_{ppm} = 8.68$ (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, $J_{HH} = 8$ Hz-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): $\delta_{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): $\delta_{ppm} = 5.54$ (1P, H-Phosphonate) 4.19 (1P, H-Phosphonate). HR-MS: $m/z$ (FAB’) 497.1088 (M’ C$_{17}$H$_{19}$O$_8$N$_6$P$_2$ requires 497.0745).

**1a $^1$H-NMR D$_2$O**

* HDO suppression. ** Acetone
1b. Yield, 85 %; $^1$H-NMR (D$_2$O, 400 MHz): $\delta_{ppm} = 8.45$ (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, $J_{HH} = 6.8$Hz, 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): $\delta_{ppm} = 156.98$, 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): $\delta_{ppm} = 5.50$ (1P, H-Phosphonate), 4.10 (1P, H-Phosphonate). HR-MS: $m/z$ (FAB') 547.1467 (M$^+$ C$_{21}$H$_{21}$O$_8$N$_6$P$_2$ 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): $\delta_{ppm} = 8.63$ (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, $J_{HH} = 6.8$ 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, 2.80-2.75 (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): $\delta_{ppm} = 151.59$, 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): $\delta_{ppm} = -116.29$ (1F, t, F$_{para}$-Phenyl); $^{31}$P-NMR ($CDCl_3$, 81 MHz): $\delta_{ppm} = 5.77$ (1P, H-Phosphonate), 4.11 (1P, H-Phosphonate); HR-MS: m/z (FAB’) 515.0141 (M’ C$_{17}$H$_{18}$O$_8$N$_6$FP$_2$ requires 515.0651).

* HDO suppression. ** Acetone
1f. Yield, 79%; $^1$H-NMR (D$_2$O, 400 MHz): $\delta_{ppm} = 8.65$ (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): $\delta_{ppm} = 151.65$, 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$_2$O, 376 MHz): $\delta_{ppm} = -111.53$ (2F, t, F$_{meta}$-Phenyl), $^{31}$P-NMR (CDCl$_3$, 81 MHz): $\delta_{ppm} = 5.50$ (1P, H-Phosphonate) 4.07 (1P, H-Phosphonate); HR-MS: m/z (FAB$^-$) 533.0247 (M’ C$_{17}$H$_{13}$OsN$_6$F$_2$P$_2$’ requires 533.0557).
1f $^1$H-NMR D$_2$O

* HDO suppression

1f $^{31}$P{$^1$H}-NMR CDCl$_3$

1f $^{19}$F-NMR D$_2$O
1g. Yield. 90.1%; $^1$H-NMR (D$_2$O, 400 MHz): $\delta_{ppm} =$ 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): $\delta_{ppm} =$ 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$_2$O, 376 MHz): $\delta_{ppm} =$ -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): $\delta_{ppm} =$ 5.40 (1P, H-Phosnonate), 4.03 ($^{31}$P, H-Phosnonate); FAB-MS: 587 [(M-TEA)].

* HDO suppression. ** Acetone
AdNH₂. Yield, 91%; ¹H-NMR (D₂O, 400 MHz): δppm = 8.46 (1H, s, NH-Purine), 8.29 (1H, s, NH-Purine), 7.66, 6.05 (1H, d, JHP = 644 Hz, H-Phosphonate), 7.45, 5.85 (1H, d, JHP = 6340 Hz, H-Phosphonate), 6.56 (1H, dd, JHH = 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, JHH = 7.4 Hz, CH₂-TEA), 2.93, 2.79-2.74 (2H, m, H2' y H2''-Deoxyribose), 1.27 (18H, t, JHH = 7.4 Hz, CH₃-TEA). ¹³C-NMR (CDCl₃, 50 MHz): δppm = 156.01, 152.40, 149.79, 139.96, 119.04, 87.99, 84.09, 72.01, 61.94, 45.41, 40.50, 8.71. ³¹P-NMR (CDCl₃, 81 MHz): δppm = 5.43 (³¹P, H-Phosphonate). 3.93 (1P, H-Phosphonate); FAB-MS: 378 [(M-TEA⁻)].
**AdNH2** $^1$H-NMR D$_2$O

* HDO

**AdNH2** $^{31}$P-$^1$H-NMR CDCl$_3$
AdNHEt. Yield, 79 %; $^1$H-NMR (D$_2$O, 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, $J_{PH} = 640$Hz, 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), 1.80 (18H, t, $J_{HH} = 7.2$ Hz, CH$_3$-TEA), 1.12 (3H, m, H3-TEA); $^{13}$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)].

AdNHEt $^1$H-NMR D$_2$O

* HDO suppression
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 by precipitation with hexane.
IV. Yield 75%; $^1$H-NMR (DMSO-d$_6$, 200 MHz): $\delta_{ppm}$ = 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, $J_{HH}$ = 6.4 Hz 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, s, t-butyl-TBDMS), -0.01 (6H, s, di-Me-TBDMS); $^{13}$C-NMR (CDCl$_3$, 50 MHz): $\delta_{ppm}$ = 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$^+$)].

![Structural diagram](image-url)

V. Yield, 70%; $^1$H-NMR (DMSO-d$_6$, 200 MHz): $\delta_{ppm}$ = 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): $\delta_{ppm}$ = 152.07, 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$^+$)].
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\(^+\)].

![structure VI](image1)

**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): \(\delta_{ppm} = 12.34\) (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, \(J_{HH} = 6.4\) Hz 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): \(\delta_{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\(_2\)O, 81 MHz): \(\delta_{ppm} = 5.19\) (1P, H-Phosphonate); FAB-MS: 597 [(M-TEA\(^-\)].

**VII.** Yield, 91%; \(^1\)H-NMR (DMSO-d\(_6\), 200 MHz): \(\delta_{ppm} = 12.39\) (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, \(J_{HH} = 7.4\) Hz 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, CH3-TEA), 1.55 (6H, t, CH2-TEA), 0.89 (9H, 1s, t-butyl -TBDMS), 0.13 (6H, 1s, di-Me-TBDMS); 13C-NMR (CDCl3, 50 MHz): $\delta_{ppm} =$ 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. 31P-NMR (CDCl3, 81 MHz): $\delta_{ppm}$ 5.21 (1P, H-Phosphonate); FAB-MS: 597 [(M-TEA)].

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 (D2O, 200 MHz): $\delta_{ppm} =$ 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, d, $J_{HH} = 7.0$ 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 CH3-TEA), 2.93, 2.83-2.80 (2H, m, H2’, H2” Deoxyribose), 1.25 (9 H, t, $J_{HH} = 7.2$ Hz CH2-TEA). 13C-NMR (CDCl3, 50 MHz): $\delta_{ppm} =$ 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, 31P-NMR (CDCl3, 81 MHz): $\delta_{ppm}$ 5.21 (1P, dt,
$J_{\text{PH}} = 593.97$, $J_{\text{HH}} = 8$ Hz, H-Phosphonate); HR-MS: $m/z$ (FAB$^-$) 483.1624 (M$^-$ C$_{21}$H$_{20}$O$_6$N$_6$P$^-$ requires 483.1187).

**$^{1c}$H-NMR D$_2$O**

* HDO suppression

**$^{1c}$P{$^{1H}$}-NMR CDCl$_3$**

Insert: $^{31}$P-$^1$H coupled spectrum
1d. Yield 89%; $^1$H-NMR (D$_2$O, 400 MHz): $\delta_{ppm} = 8.53$ (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): $\delta_{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): $\delta_{ppm} 3.66$ (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$_6$N$_6$P requires 483.1187).

1d $^1$H-NMR D$_2$O

* HDO suppression
$^{31}$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 N6-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.

\[
A_{\text{obs}} = A_S [S]_t + A_L [L]_t + 0.5 \Delta A \left( ([S]_t + [L]_t + K_D - \sqrt{([S]_t + [L]_t + K_D)^2 - 4 [S]_t [L]_t}) \right) - 4 [S]_t [L]_t^{1/2} \quad (1)
\]

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 $K_a$ ($\times 10^5$, M$^{-1}$) for nucleotides 1a-1g and cationic dyes$^a$

<table>
<thead>
<tr>
<th>Dye</th>
<th>la</th>
<th>lb</th>
<th>lc</th>
<th>ld</th>
<th>le</th>
<th>lf</th>
<th>lg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>0.89 ±0.11</td>
<td>2.4 ±0.5</td>
<td>0.21 ±0.07</td>
<td>0.16 ±0.01</td>
<td>0.36 ±0.05</td>
<td>0.35 ±0.05</td>
<td>0.23 ±0.02</td>
</tr>
<tr>
<td>AC</td>
<td>0.50 ±0.03</td>
<td>1.5 ±0.3</td>
<td>1.36 ±0.08</td>
<td>0.63 ±0.11</td>
<td>0.95 ±0.06</td>
<td>2.01 ±0.12</td>
<td>0.30 ±0.03</td>
</tr>
<tr>
<td>TB</td>
<td>3.00 ±0.37</td>
<td>7.8 ±2.1</td>
<td>1.79 ±0.16</td>
<td>1.43 ±0.08</td>
<td>0.59 ±0.03</td>
<td>9.39 ±0.69</td>
<td>0.89 ±0.02</td>
</tr>
<tr>
<td>NB</td>
<td>1.35 ±0.08</td>
<td>55.7 ±5.7</td>
<td>0.65 ±0.19</td>
<td>0.49 ±0.15</td>
<td>1.35 ±0.04</td>
<td>1.89 ±0.27</td>
<td>0.39 ±0.05</td>
</tr>
<tr>
<td>OX</td>
<td>0.83 ±0.09</td>
<td>5.0 ±0.9</td>
<td>0.62 ±0.08</td>
<td>0.40 ±0.07</td>
<td>0.38 ±0.04</td>
<td>1.42 ±0.15</td>
<td>0.27 ±0.01</td>
</tr>
</tbody>
</table>

$^a K_a$ measured by UV-vis titrations in MOPS aqueous buffer at pH 7, 25°C and 0.010 M NaCl.

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

<table>
<thead>
<tr>
<th>Dye</th>
<th>la</th>
<th>lb</th>
<th>lc</th>
<th>ld</th>
<th>lb-1a</th>
<th>lc-1b</th>
<th>ld-1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>-26.80</td>
<td>-29.53</td>
<td>-29.29</td>
<td>-27.38</td>
<td>-2.73</td>
<td>+0.24</td>
<td>+2.15</td>
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<tr>
<td>TB</td>
<td>-31.24</td>
<td>-33.61</td>
<td>-29.97</td>
<td>-29.41</td>
<td>-2.37</td>
<td>+3.64</td>
<td>+4.20</td>
</tr>
<tr>
<td>NB</td>
<td>-29.27</td>
<td>-38.48</td>
<td>-27.46</td>
<td>-26.76</td>
<td>-9.21</td>
<td>+11.02</td>
<td>+11.72</td>
</tr>
</tbody>
</table>

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

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

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

Fig. S5 Changes of the absorbance at 660 nm during titration of MB (2×10⁻⁵ M) with nucleotide 1a (0-9.3×10⁻⁵ 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 (0-8.1×10⁻⁵ 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 AdNH₂ (●) 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 $N^\alpha$-($N^\prime$-phenylcarbamoyl)-2'-deoxyadenosine in gas phase and (bottom) for $N^\alpha$-methyl-$N^\alpha$-($N^\prime$-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).
Figure S13. Temperature dependence of the equilibrium constant (R ln$K_{as}$) 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta S$</td>
<td>12.77505</td>
<td>10.56482</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>29858.72682</td>
<td>3137.66542</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$\Delta H^\circ$</th>
<th>$\Delta S^\circ$</th>
<th>$\Delta G^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b-TB</td>
<td>-29.8 ±3.1</td>
<td>-3.7 ± 3.1</td>
<td>-33.5b</td>
</tr>
</tbody>
</table>

$^a$ at 298 K

$^b$ For comparison at 298 K a $\Delta G^\circ = -33.6$ kJ mol$^{-1}$ was measured for 1b-TB, see Table S2.

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$_2$O) 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$_2$O) 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).