Supporting Information

Development of N^6-methyl-2-(1,2,3-triazol-1-yl)-2'-deoxyadenosine as a novel fluorophore and its application in nucleotide synthesis

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Materials and Methods

Yields refer to chromatographically and spectroscopically homogeneous materials. Dry MeCN, DMF, DCM and pyridine were obtained by distillation over CaH_2. Commercial reagents were used as received.

^{1}H-NMR, ^{31}P-NMR and ^{13}C-NMR spectra were recorded at 300 MHz, 122 MHz and 75.5 MHz, respectively. The proton signals for residual non-deuterated solvents (δ 7.26 for $CDCl_3$ and δ 2.50 for DMSO-d_6) and carbon signals (δ 77.1 for $CDCl_3$ and δ 39.5 for DMSO-d_6) were used as an internal references for ^{1}H-NMR and ^{13}C-NMR spectra, respectively. For ^{31}P spectra in aqueous solutions chemical shifts were measured using 85% H_3PO_4 for external reference calibration (0 ppm); the signal from the external references was then recorded in the computer of the spectrometer. In $CDCl_3$ solutions triethyl phosphate was used as ^{31}P internal reference (0 ppm). Coupling constants are reported in Hz. 600 MHz spectra were recorded on 600 MHz Varian Unity Inova spectrometer.

Infrared spectra were registered using Perkin Elmer Spectrum BX spectrometer.

The UV-Vis absorption spectra were acquired using *Perkin-Elmer 35* UV/Vis spectrometer with a 1 cm path length quartz cell. Emission spectra and absolute photoluminescence quantum yields were measured by the *Quanta Master 40* steady state spectrofluorometer using 1 cm and 0.5 cm path length quartz cuvettes, correspondingly. In the quantum yield measurements, 6 inch integrating sphere by *LabSphere* was employed.

Analytical thin layer chromatography (TLC) was performed on *Merck* silica gel *60 F_{254}* aluminium plates precoated with a 0.25 mm layer of silica gel. For preparative chromatography commercial silica gel (60 Å, 40-63 μm, ROCC) or (C18) was used.

For HPLC analyses *Agilent Technologies 1200 Series* system was used (*X Bridge* C18 column, 4.6×150 mm, particle size 3.5 μm). Eluent A – 0.01 M KH_2PO_4 water solution/MeCN (94/6, V/V), eluent B – 0.1% TFA water solution/MeCN (95/5, V/V), eluent C – MeCN. Flow rate: 1 mL/min. Wavelength of detection was set to 254 nm. Elution programs are described in Table 1.

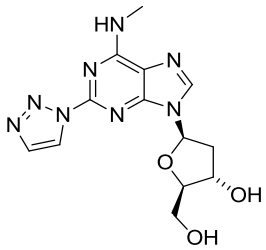
Table 1

Elution program	Content of C, %	Time, min
E_1 (A-C)	10-95-95-10	0-7-10-12
E_2 (A-C)	10-90-90-10	0-7-10-12
E_3 (B-C)	10-95-95-10	0-7-10-12
E_4 (B-C)	0-0-95-0	0-7-10-12
E_5 (A-C)	0-45-95-0	0-7-10-12
E_6 (A-C)	0-95-95-0	0-7-10-12

Waters Acquity UPLC system was used to perform LC/MS analyses; column: *Acquilty UPLC BEH C18* 1.7 μm, 2.1×50 mm; 0.1% TFA/H_2O (eluent D) and MeCN (eluent C) were selected as the mobile phase with eluation regime E_7 (D-C); content of eluent C was changed as follows: 0-0-100-0% (0-1-7.5-8.5 min).

HRMS analyses were performed on *Agilent1290 Infinity series* UPLC systemconnected to *Agilent 6230 TOF LC/MS* masspectrometer; column *Extend C18 RRHD* 2.1×50 mm, 1.8 μm. Formic acid in MeCN (0.1%) and formic acid in water (0.1%) were used as eluents.

9-(2'-Deoxy-β-D-ribofuranosyl)-2-(1*H*-1,2,3-triazol-1-yl)-6-methylamino-9*H*-purine (4)



25% Aqueous methylamine solution (40.0 mL, 0.32 mol, 50 equiv.) was added to a solution of bis-triazolyl derivative **12** (2.87 g, 6.33 mmol, 1.0 equiv.) in THF (15 mL). The resulting reaction mixture was stirred for 3 h (HPLC control) at 45 ^oC. The solution was evaporated and the obtained solid that was heated at 50 ^oC with MeOH (30 mL), kept

at -20 ^{o}C overnight, filtered and afterwards washed with cold (0 ^{o}C) MeOH (3×7 mL) to obtain the product **4** (1.63 g, 77%) as a white powder.

R_f = 0.34 (MeOH/DCM = 1/9).

HPLC t_R = 2.06 min, 97% purity, eluent E_3.

IR (KBr) ν (cm^{-1}): 3371, 2938, 1588, 1497, 1452, 1366, 1234, 1096, 1053.

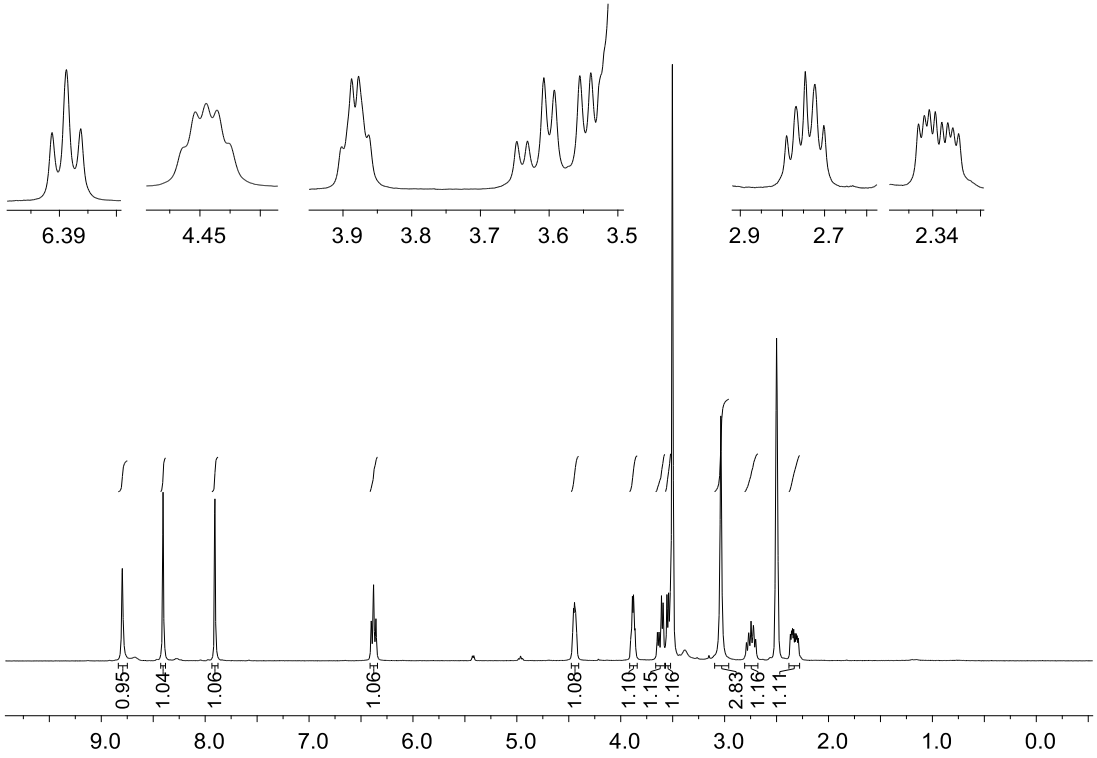
^{1}H-NMR (300 MHz, DMSO-d$_6$ + D$_2$O) δ (ppm): 8.80 (s, 1H, H-C(triazole)), 8.41 (s, 1H, H-C(8)), 7.91 (s, 1H, H-C(triazole)), 6.38 (dd, 1H, $^{3}J_{1'\text{-}2a'}$ = 7.4 Hz, $^{3}J_{1'\text{-}2b'}$ = 6.2 Hz, H-C(1')), 4.44 (ddd, 1H, $^{3}J_{2a'\text{-}3'}$ = 5.8 Hz, $^{3}J_{2b'\text{-}3'}$ = 3.2 Hz, $^{3}J_{3'\text{-}4'}$ = 2.8 Hz, H-C(3')), 3.88 (dt, 1H, $^{3}J_{4'\text{-}5a'}$ = $^{3}J_{4'\text{-}5b'}$ = 4.7 Hz, $^{3}J_{3'\text{-}4'}$ = 2.8 Hz, H-C(4')), 3.63, 3.54 (2dd, 2H, $^{2}J_{5a'\text{-}5b'}$ = 11.8 Hz, $^{3}J_{4\text{-}5a'}$ = $^{3}J_{4'\text{-}5b'}$ = 4.7 Hz, H_2-C(5')), 3.04 (t, 3H, (H_3C-)), 2.74 (ddd, 1H, $^{2}J_{2a'\text{-}2b'}$ = 13.3 Hz, $^{3}J_{1'\text{-}2a'}$ = 7.4 Hz, $^{3}J_{2a'\text{-}3'}$ = 5.8 Hz, H_a-C(2')), 2.33 (ddd, 1H, $^{2}J_{2a'\text{-}2b'}$ = 13.3 Hz, $^{3}J_{1'\text{-}2b'}$ = 6.2 Hz, $^{3}J_{2b'\text{-}3'}$ = 3.2 Hz, H_b-C(2')).

^{13}C-NMR (75.5 MHz, DMSO-d$_6$) δ (ppm): 155.5, 149.3, 148.7, 140.4, 133.8, 124.1, 119.2, 88.2, 83.5, 70.9, 61.8, 39.4*, 27.3.

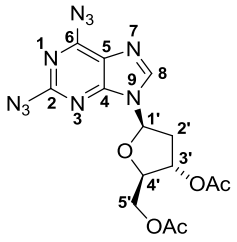
HRMS (ESI): calcd for $C_{13}H_{17}N_8O_3$ $[M+H]^+$, 333.1418; found 333.1406 (3.60 ppm).

* Determined from HSQC spectrum

^{1}H-NMR (300 MHz, DMSO-d6 + D_2O) spectrum of 4:



9-(3',5'-Di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)-2,6-diazido-9*H*-purine (7)



2,6-Dichloropurine nucleoside **6**[2] (2.00 g, 5.14 mmol, 1.0 equiv.) was dissolved in ethanol (100 mL) upon stirring at 45 °C oil. A solution of NaN_3 (1.32 g, 20.0 mmol, 4.0 equiv.) in water (5 mL) was added. Stirring and heating was continued for 2 h to yield a single product on TLC and HPLC. The reaction mixture was then evaporated to dryness. The residue was dissolved in DCM and washed with water (3×10 mL). After removal of DCM and drying under vacuum, product **7** (2.05 g, 98%) was obtained as a yellow foam in high purity (98% by HPLC).

R_f = 0.65 (Hex/EtOAc = 1/4).

HPLC t_R = 4.60 min, 98% purity, eluent E_1.

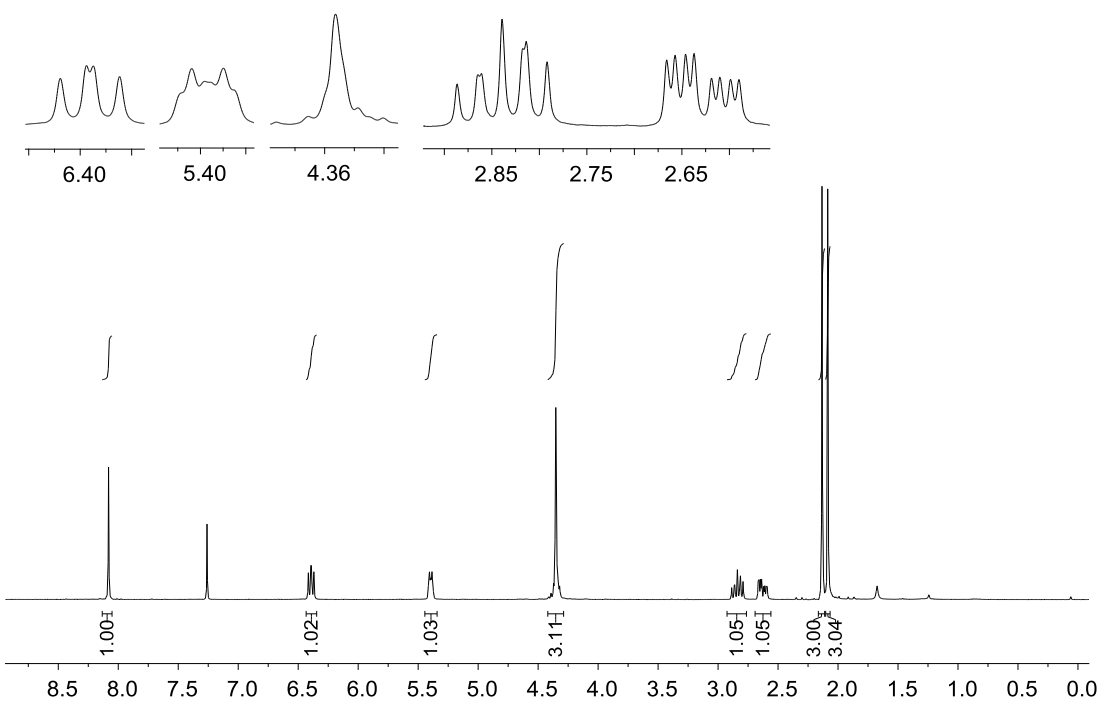
IR (KBr) ν (cm^{-1}): 3125, 2955, 2375, 2125, 1740, 1580, 1435, 1065.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.08 (s, 1H, H-C(8)), 6.39 (dd, 1H, $^{3}J_{1'\text{-}2a'}$ = 7.7 Hz, $^{3}J_{1'\text{-}2b'}$ = 6.1 Hz, H-C(1')), 5.40 (dt, 1H, $^{3}J_{2a'\text{-}3'}$ = 6.5 Hz, $^{3}J_{2b'\text{-}3'}$ = $^{3}J_{3'\text{-}4'}$ = 2.5 Hz, H-C(3')), 4.41−4.31 (m, 3H, H-C(4',5')), 2.85 (ddd, 1H, $^{2}J_{2a'\text{-}2b'}$ = 14.2 Hz, $^{3}J_{1'\text{-}2a'}$ = 7.7 Hz, $^{3}J_{2a'\text{-}3'}$ = 6.5 Hz, H_b-C(2')), 2.63 (ddd, 1H, $^{2}J_{2a'\text{-}2b'}$ = 14.2 Hz, $^{3}J_{1'\text{-}2b'}$ = 6.1 Hz, $^{3}J_{2b'\text{-}3'}$ = 2.5 Hz, H_a-C(2')), 2.13, 2.09 (2s, 6H, H_3CC(O)O-C(3',5')).

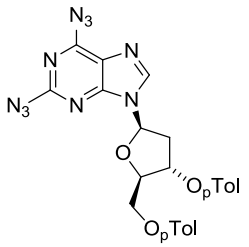
^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 170.4, 170.3, 156.3, 154.1, 153.4, 141.5, 121.9, 84.7, 82.8, 74.3, 63.7, 37.9, 21.0, 20.8.

HRMS (ESI): calcd for [$C_{14}H_{14}N_{10}O_5$+Na^+] 425.1041, found 425.1043 (0.47 ppm).

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 7:



9-(3',5'-Di-*O*-*p*-toluoyl-2'-deoxy-β-D-ribofuranosyl)-2,6-diazido-9*H*-purine (11)



Dry acetonitrile (20 mL) was added to an ice-NaCl cooled mixture of 2,6-diazidopurine[3] **8** (480 mg, 2.38 mmol, 1.0 equiv., dried by coevaporation with dry MeCN (3×10 mL)) and NaH (86 mg, 3.44 mmol, 1.5 equiv.) under argon. The resulting mixture was stirred for 30 min at 0 °C. Then a solution of furanosyl chloride **10**[4] (1.67 g, 4.30 mmol, 1.8 equiv., dried for 2 days at 5 Torr) in dry DCM (20 mL) was added to the previous mixture. In 5 hours a complete conversion of starting material was observed (TLC control). Volatiles were evaporated *in vacuo*. The resulting solid was purified on silica gel column (DCM/MeCN/Tol = 60/2/1) with a following crystalization from EtOAc to give product **11** (260 mg, 20%) as white crystals.

R_f = 0.5 (DCM/MeCN/Tol = 60/6/1).

HPLC t_R = 7.41 min, 93% purity, eluent E_1.

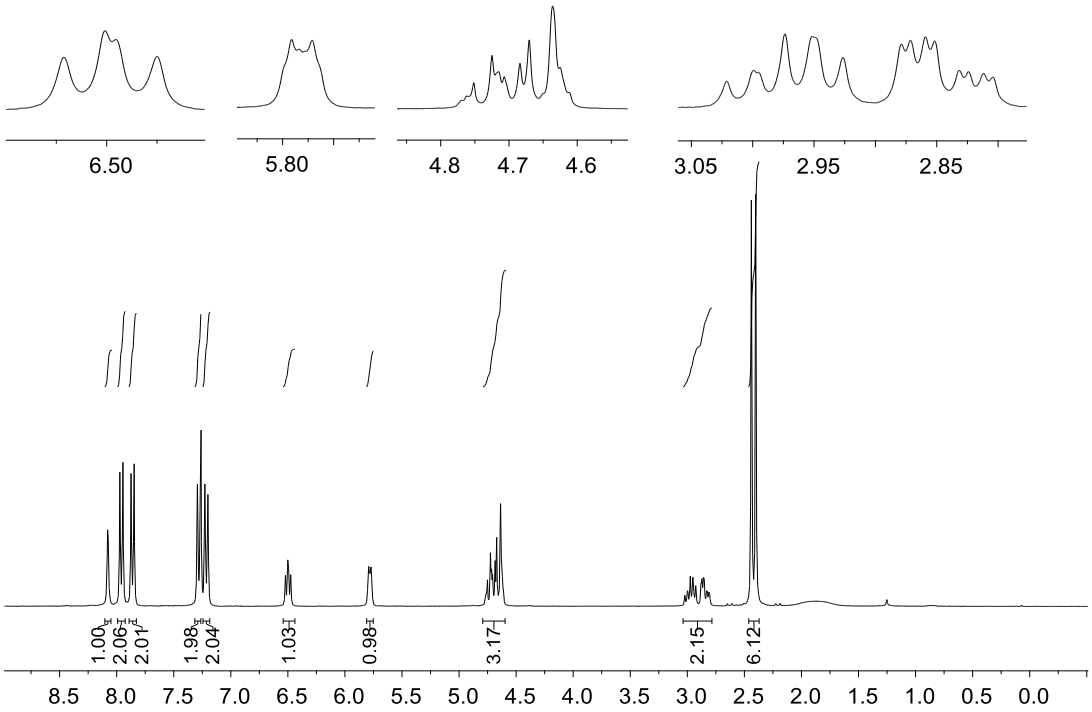
IR (KBr) ν (cm^{-1}): 3093, 2920, 2163, 2128, 1722, 1704, 1610, 1547, 1392, 1359.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.08 (s, 1H, H-C(8)), 7.96, 7.86 (2d, 4H, ^3J = 8.1 Hz, (Ar)), 7.28, 7.21 (2d, 4H, ^3J = 8.1 Hz, (Ar)), 6.50 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 7.8 Hz, $^3J_{1'\text{-}2b'}$ = 6.0 Hz, H-C(1')), 5.81−5.75 (m, 1H, H-C(3')), 4.78−4.60 (m, 3H, H-C(4'), H_2-C(5')), 2.97 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.2 Hz, $^3J_{1'\text{-}2a'}$ = 7.8 Hz, $^3J_{2a'\text{-}3'}$ = 6.6 Hz, H_a-C(2')), 2.84 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.2 Hz, $^3J_{1'\text{-}2b'}$ = 6.0 Hz, $^3J_{2b'\text{-}3'}$ = 2.2 Hz, H_b-C(2')), 2.44, 2.40 (2s, 6H, H_3C-(3',5'-$_p$Tol)).

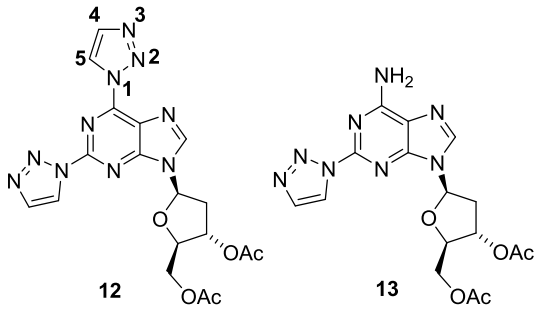
^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 166.1, 165.9, 156.3, 153.8, 153.1, 144.6, 144.2, 141.5, 129.8, 129.6, 129.3, 126.5 (2×C), 126.3, 121.0, 84.9, 84.0, 74.8, 63.8, 38.2, 21.7, 21.6.

HRMS (ESI): calcd for $C_{26}H_{22}N_{10}O_5Na$ $[M+Na]^+$, 577.1667; found 577.1666 (0.17 ppm).

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 11:



A mixture of 9-(3',5'-Di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)-2,6-bis-(1*H*-1,2,3-triazol-1-yl)-9*H*-purine (12) and 9-(3',5'-Di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)-2-(1*H*-1,2,3-triazol-1-yl)-6-amino-9*H*-purine (13)



TMS-acetylene (10.5 mL, 74 mmol, 10.0 eq.) and a solution of 10% acetic acid in water (34 mL) was added to a stirred solution of diazide **7** (2.96 g, 7.36 mmol, 1.0 equiv.) in *tert*-butanol (105 mL) at 45 ^oC. Then a solution of $CuSO_4 \cdot 5H_2O$ (102 mg, 0.408 mmol, 5.5 mol-%) in water (3 mL) and a solution of sodium ascorbate (136 mg, 0.687 mmol, 9.3 mol-%) in water (3 mL) were sequentially added to the previous mixture. The pre-

catalysts were added repeatedly every 2 hours to mantain their activity. After the starting material had fully transformed into products **12** and **13** (approximately 12 h, HPLC control) the reaction mixture was evaporated under reduced pressure and the residue was dissolved in acetone. The insolubles were removed by filtration through celite. The filtrate was evaporated and the residue was purified on silica gel column (MeCN/Tol; gradient 60→65%) to afford the product **12** (2.04 g, 61%) as a white foam.

Analytical data for compound **12:**

R_f = 0.43 (MeCN/Tol = 2/1).

HPLC t_R = 3.17 min, 95% purity, eluent E_1.

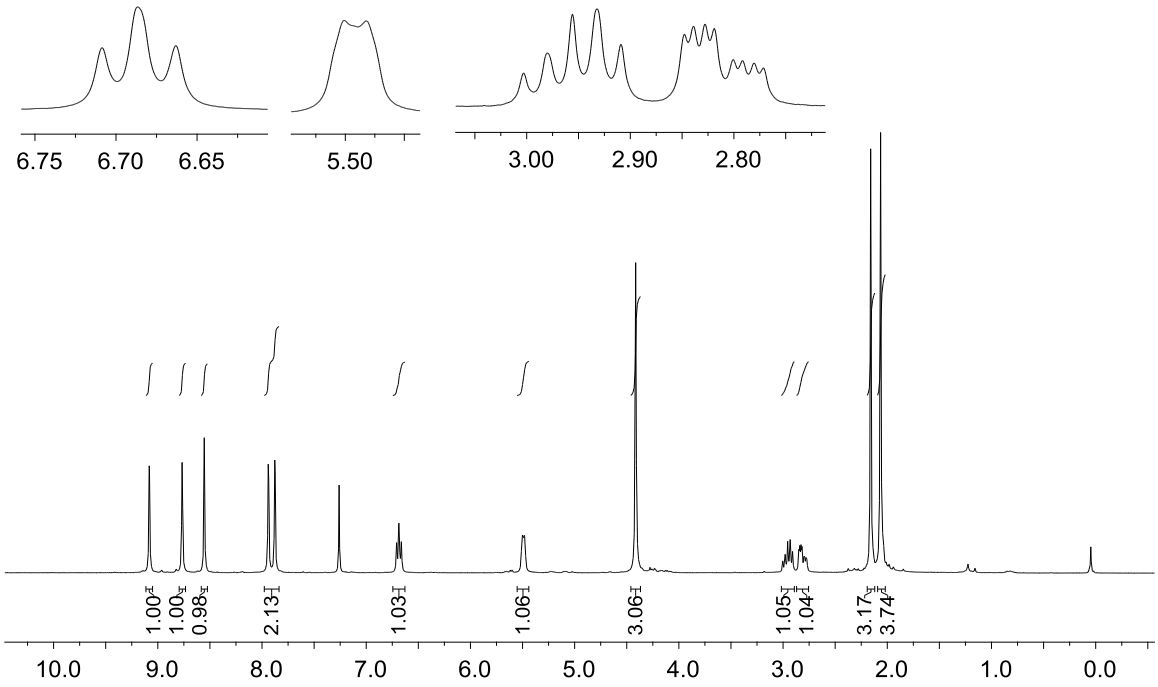
IR (KBr) ν (cm^{-1}): 3128, 2957, 1741, 1611, 1587, 1474, 1370, 1232, 994.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 9.08, 8.77 (2d, 2H, $^3J_{4\text{-}5}$ = 1.2 Hz, H-C(triazole)), 8.54 (s, 1H, H-C(8)), 7.94, 7.88 (2d, 2H, $^3J_{4\text{-}5}$ = 1.2 Hz, H-C(triazole)), 6.69 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 7.5 Hz, $^3J_{1'\text{-}2b'}$ = 6.0 Hz, H-C(1')), 5.52−5.46 (m, 1H, H-C(3')), 4.43−4.40 (m, 3H, H-C(4'), H_2-C(5')), 2.95 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.3 Hz, $^3J_{1'\text{-}2a'}$ = 7.5 Hz, $^3J_{2a'\text{-}3'}$ = 6.3 Hz, H_a-C(2')), 2.82 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.3 Hz, $^3J_{1'\text{-}2b'}$ = 6.0 Hz, $^3J_{2b'\text{-}3'}$ = 2.4 Hz, H_b-C(2')), 2.16, 2.07 (2s, 6H, $H_3CC(O)O$-C(3',5')).

^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 170.3, 170.3, 155.2, 148.8, 145.6, 145.5, 134.5, 134.5, 124.3, 123.7, 122.8, 85.1, 83.1, 74.2, 63.6, 38.3, 21.0, 20.8.

HRMS (ESI): calcd for $C_{18}H_{19}N_{10}O_5$ $[M+H]^+$, 455.1534; found 455.1516 (3.95 ppm).

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 12:



Analytical data for compound **13:**

White foam, R_f = 0.33 (MeCN/EtOH = 20/1).

HPLC t_R = 2.88 min, 98% purity, eluent E_1.

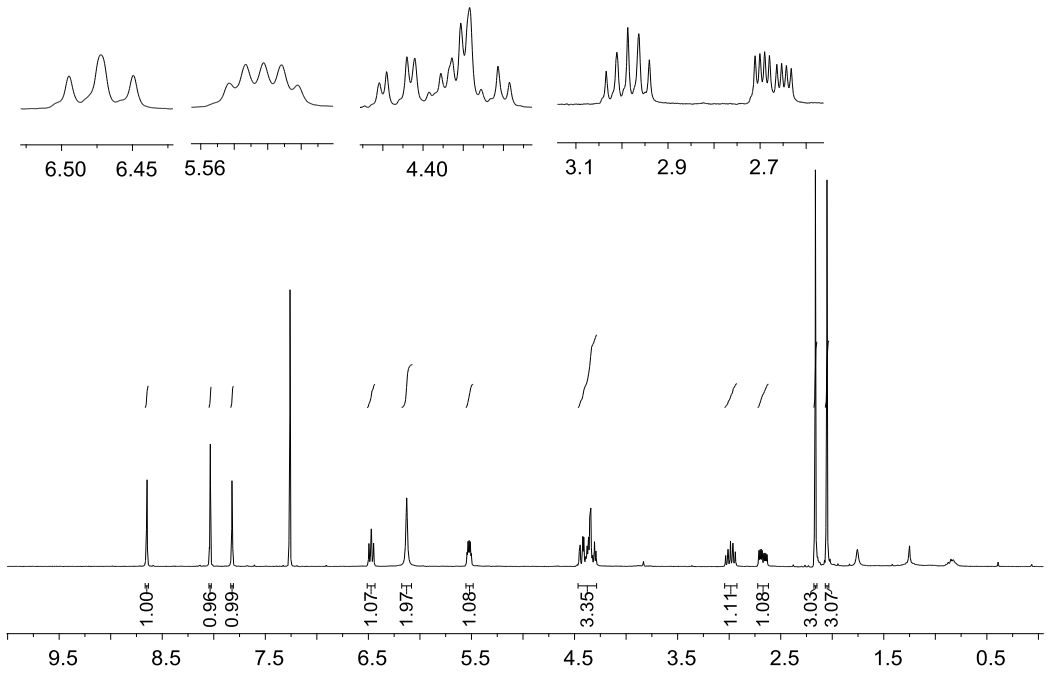
IR (KBr) ν (cm^{-1}): 3453, 3320, 3147, 1740, 1651, 1588, 1445, 1374, 1243.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.65 (d, 1H, $^3J_{4\text{-}5}$ = 1.2 Hz, H-C(triazole)), 8.03 (s, 1H, H-C(8)), 7.82 (d, 1H, $^3J_{4\text{-}5}$ = 1.2 Hz, H-C(triazole)), 6.47 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 7.2 Hz, $^3J_{1'\text{-}2b'}$ = 6.3 Hz, H-C(1')), 6.13 (s, 2H, (H_2N-)), 5.52 (ddd, 1H, $^3J_{2a'\text{-}3'}$ = 6.6 Hz, $^3J_{2b'\text{-}3'}$ = 3.2 Hz, $^3J_{2b'\text{-}4'}$ = 2.8 Hz, H-C(3')), 4.34 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 9.9 Hz, $^3J_{4'\text{-}5a'}$ = 3.1 Hz, 1H, H_a-C(5')), 4.42−4.35 (m, 1H, H-C(4')), 4.22 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 9.9 Hz, $^3J_{4'\text{-}5b'}$ = 3.6 Hz, 1H, H_b-C(5'), 3.01 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.1 Hz, $^3J_{1'\text{-}2a'}$ = 7.2 Hz, $^3J_{2a'\text{-}3'}$ = 6.6 Hz, H_a-C(2')), 2.67 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.1 Hz, $^3J_{1'\text{-}2b'}$ = 6.3 Hz, $^3J_{2b'\text{-}3'}$ = 3.2 Hz, H_b-C(2')), 2.16, 2.05 (2s, 6H, H_3CC(O)O-C(3',5')).

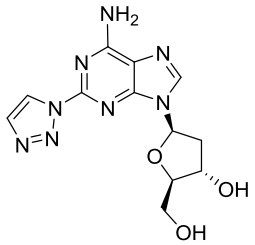
^{13}C-NMR (75.5 MHz, DMSO-d_6) δ (ppm): 170.2, 170.1, 156.7, 149.4, 149.2, 140.8, 133.6, 123.6, 118.8, 83.7, 81.7, 74.2, 63.5, 35.2, 20.8, 20.4.

HRMS (ESI): calcd for $C_{16}H_{19}N_8O_5$ $[M+H]^+$, 403.1473; found 403.1464 (2.23 ppm).

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 13:



9-(2'-deoxy-β-D-ribofuranosyl)-2-(1*H*-1,2,3-triazol-1-yl)-6-amino-9*H*-purine (14)



25% Aqueous methylamine solution (1 mL) was added to a solution of compound **13** (40 mg, 0.10 mmol, 1.0 equiv.) in acetone (1 mL). The reaction mixture was stirred at 45 ^{o}C for 1.5 h (TLC control). Solvents were removed *in vacuo* and the resulting solid was washed two times with cold methanol (-20 ^{o}C, 2 and 1 mL, respectively). Deprotected nucleoside **14** (23 mg, 73%) was obtained as a white powder.

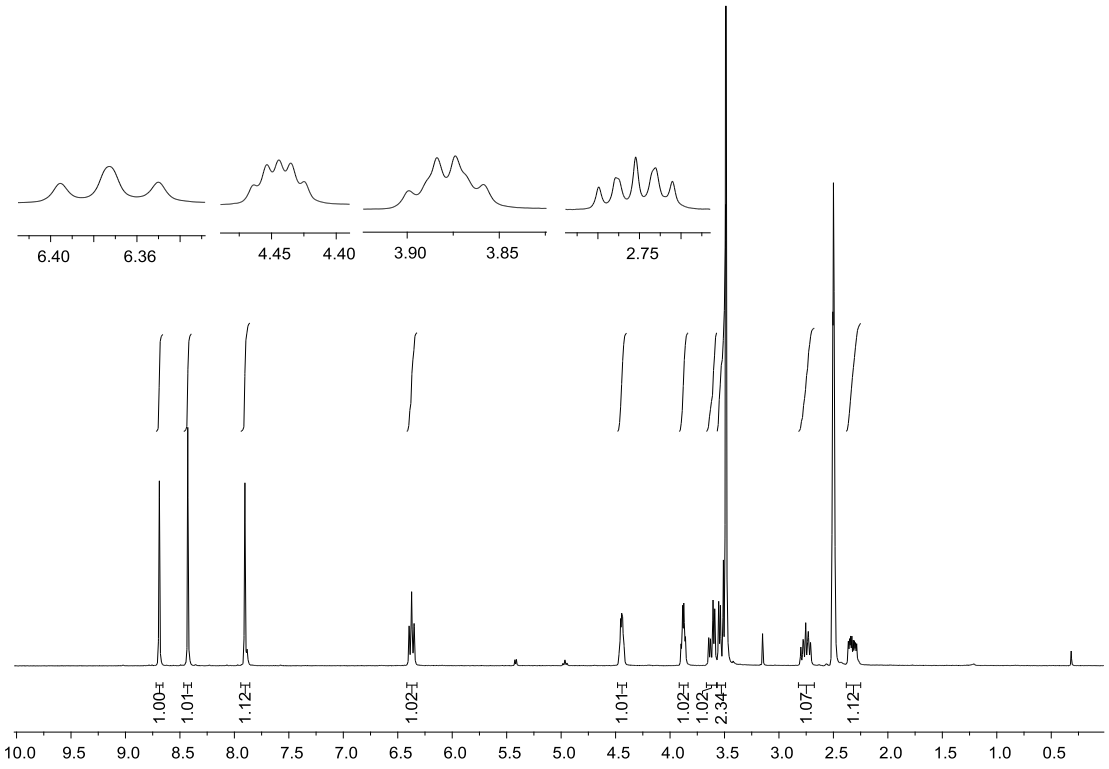
HPLC t_R = 2.97 min, 99% purity, eluent E_5.

IR (KBr) ν (cm^{-1}): 3440, 3325, 3210, 2930, 1650, 1375, 1235, 1025.

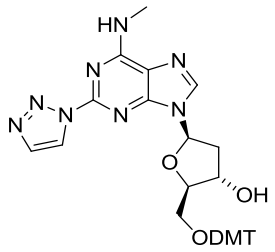
^{1}H-NMR (300 MHz, DMSO-d6 + D_2O) δ (ppm): 8.69 (d, 1H, ^3J = 1.1 Hz, H-C(triazole)), 8.43 (s, 1H, H-C(8)), 7.91 (d, 1H, ^3J = 1.1 Hz, H-C(triazole)), 6.37 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 7.4 Hz, $^3J_{1'\text{-}2b'}$ = 6.2 Hz, H-C(1')), 4.44 (ddd, 1H, $^3J_{2a'\text{-}3'}$ = 6.1 Hz, $^3J_{2b'\text{-}3'}$ = 3.3 Hz, $^3J_{3'\text{-}4'}$ = 3.0 Hz, H-C(3')), 3.88 (dt, 1H, $^3J_{4'\text{-}5a'}$ = $^3J_{4'\text{-}5b'}$ = 4.7 Hz, $^3J_{3'\text{-}4'}$ = 3.0 Hz, H-C(4')), 3.62 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 11.7 Hz, $^3J_{4'\text{-}5a'}$ = 4.7 Hz, H_a-C(5')), 3.53 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 11.7 Hz, $^3J_{4'\text{-}5b'}$ = 4.7 Hz, H_b-C(5')), 2.76 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 13.4 Hz, $^3J_{1'\text{-}2a'}$ = 7.4 Hz, $^3J_{2a'\text{-}3'}$ = 6.1 Hz, H_a-C(2')), 2.32 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 13.4 Hz, $^3J_{1'\text{-}2b'}$ = 6.2 Hz, $^3J_{2b'\text{-}3'}$ = 3.3 Hz, H_b-C(2')).

^{13}C-NMR (75.5 MHz, DMSO-d6) δ (ppm): 156.6, 149.6, 149.3, 140.7, 133.7, 123.8, 118.6, 87.9, 83.7, 70.8, 61.7, 39.4.

^{1}H-NMR (300 MHz, DMSO-d6 + D_2O) spectrum of 14:



9-(5'-*O*-Dimethoxytrityl-2'-deoxy-β-D-ribofuranosyl)-2-(1*H*-1,2,3-triazol-1-yl)-6-methylamino-9*H*-purine (15)



Compound **4** (1.50 g, 4.51 mmol, 1.0 equiv.) was partially dissolved in dry pyridine (15 mL) and evaporated, then dissolved in dry pyridine (20 mL) under nitrogen and placed in an ice bath. To the cold solution, dimethoxytrityl chloride (1.51 g, 4.51 mmol, 1.0 equiv.) was added in two portions with time interval of 2.5 h. After 22 h more dimethoxytrityl chloride (0.30 g, 0.90 mmol, 0.2 equiv.) was added. Next day, the TLC showed almost no presence of the starting material. The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (MeOH/DCM 0%→3.5%). Product **15** (2.79 g, 87%) was isolated as a white foam.

R_f = 0.7 (MeOH/DCM = 1/9).

IR (KBr) ν (cm^{-1}): 3300, 3100, 2933, 1634, 1585, 1509, 1496, 1447, 1366, 1250, 1176, 1046.

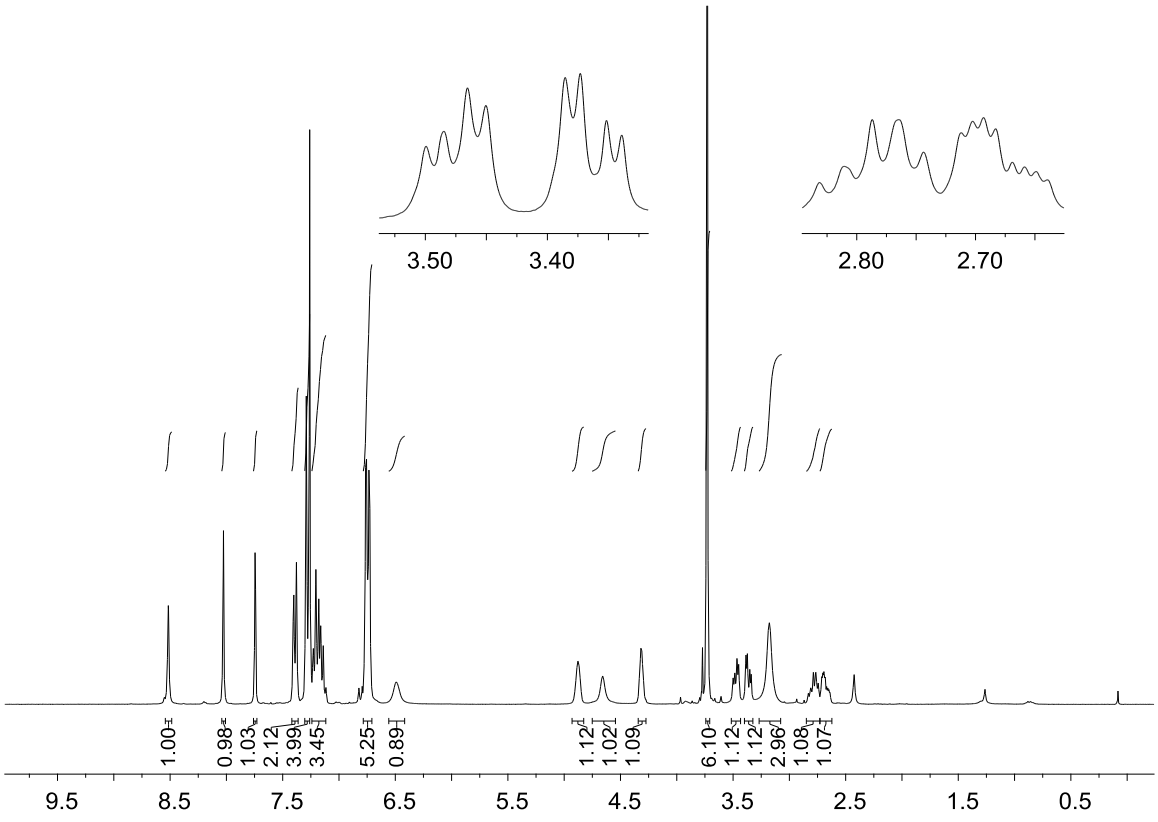
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.53 (s, 1H, H-C(triazole)), 8.03 (s, 1H, H-C(8)), 7.74 (s, 1H, H-C(triazole)), 7.42−7.11 (m, 9H, (Ar)), 6.79−6.71* (m, 1H, H-C(1')), 6.75 (2d, 4H, ^3J = 8.9 Hz, (Ar)), 6.49 (br. s, 1H, (H_3C-HN-)), 4.93−4.83 (m, 1H, H-C(3')), 4.66 (br. s, 1H, HO-C(3')), 4.31 (dt, 1H, $^3J_{4'\text{-}5a'}$ = 4.5 Hz, $^3J_{4'\text{-}5b'}$ = 3.7 Hz, $^3J_{3'\text{-}4'}$= 2.2 Hz, H-C(4')), 3.73 (s, 6H, 2×(H_3CO-)), 3.47 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 10.2 Hz, $^3J_{4'\text{-}5a'}$ = 4.5 Hz, H_a-C(5')), 3.36 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 10.2 Hz, $^3J_{4'\text{-}5b'}$ = 3.7 Hz, H_b-C(5')), 3.18 (s, 3H, (H_3C-HN-)), 2.79 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 13.0 Hz, $^3J_{1'\text{-}2a'}$ = 7.4 Hz, $^3J_{2a'\text{-}3'}$ = 5.8 Hz, H_a-C(2')), 2.67 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 13.0 Hz, $^3J_{2b'\text{-}3'}$ = 5.7 Hz, $^3J_{1'\text{-}2b'}$ = 3.1 Hz, H_b-C(2')).

* Determined from HSQC spectrum

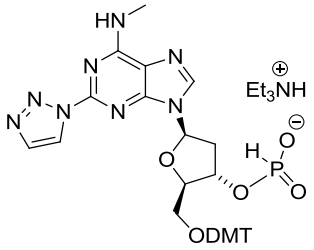
^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 158.5, 155.8, 149.5, 149.0, 144.7, 139.4, 135.8, 133.5, 130.1, 128.2, 127.8, 126.8, 123.1, 119.5, 113.1, 87.1, 86.5, 84.5, 72.6, 64.2, 55.2, 40.9, 27.6.

HRMS (ESI): calcd for $C_{34}H_{35}N_8O_5$ $[M+H]^+$, 635.2725; found 635.2713 (1.89 ppm).

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 15:



9-(5'-*O*-Dimethoxytrityl-2'-deoxy-β-D-ribofuranosyl)-2-(1*H*-1,2,3-triazol-1-yl)-6-methylamino-9*H*-purine 3'-*H*-hosphonate triethylammonium salt (16)



The 5'-protected nucleoside **15** (1.19 g, 1.87 mmol, 1.0 equiv.) and imidazole (1.46 g, 21.4 mmol, 11.5 eq.) were dried seperately by co-evaporation with anhydrous pyridine. Imidazole was stirred with dry acetonitrile (16.5 mL) in an ice-NaCl bath for 15 minutes under nitrogen. Phosphorous trichloride (0.58 mL, 6.7 mmol, 3.6 equiv.) and Et_3N (dried on molecular sieves, 2.91 mL, 20.9 mmol, 11.2 equiv.) were added to the chilled solution to give a white suspension. Solution of nucleoside **15** in dry acetonitrile (18.5 mL) was added dropwise to the pre-obtained suspension in 30 minutes. The resulting reaction mixture was stirred for 20 minutes at room temperature (20 ^oC). Then water (5 mL) was added, the resulting clear solution was evaporated to thick consistency and partitioned between DCM (50 mL) and TEAB solution (30 mL, 1M, pH = 8.5). The aqueous layer was extracted with dichloromethane (3×10 mL). Organic layers were once more washed with TEAB (20 mL) that was back-extracted with DCM as previously. The combined DCM layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/DCM 4%→12% with additive of 1% Et_3N) to yield **16** (1.54 g, 91% with 2 eq. Et_3N) as a white foam.

R_f = 0.47 (MeOH/DCM = 1/9 + 2 drops of Et_3N).

IR (KBr) ν (cm^{-1}): 3409, 2939, 2637, 2487, 1632, 1586, 1509, 1447, 1372, 1221, 1178, 1064.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 12.51 (br. s, 1H, H-N($TEAH^+$)), 8.56 (d, 1H, $^3J_{4\text{-}5}$ = 0.9 Hz, H-C(triazole)), 7.96 (s, 1H, H-C(8)), 7.75 (d, 1H, $^3J_{4\text{-}5}$ = 0.9 Hz, H-C(triazole)), 7.41−7.12 (m, 9H, (Ar)), 6.91 (d, 1H, $^1J_{P\text{-}H}$ = 617 Hz, H-P), 6.75 (2d, 4H, ^3J = 8.8 Hz, (Ar)), 6.55 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 7.6 Hz, $^3J_{1'\text{-}2b'}$ = 6.0 Hz, H-C(1')), 6.26 (quartet,

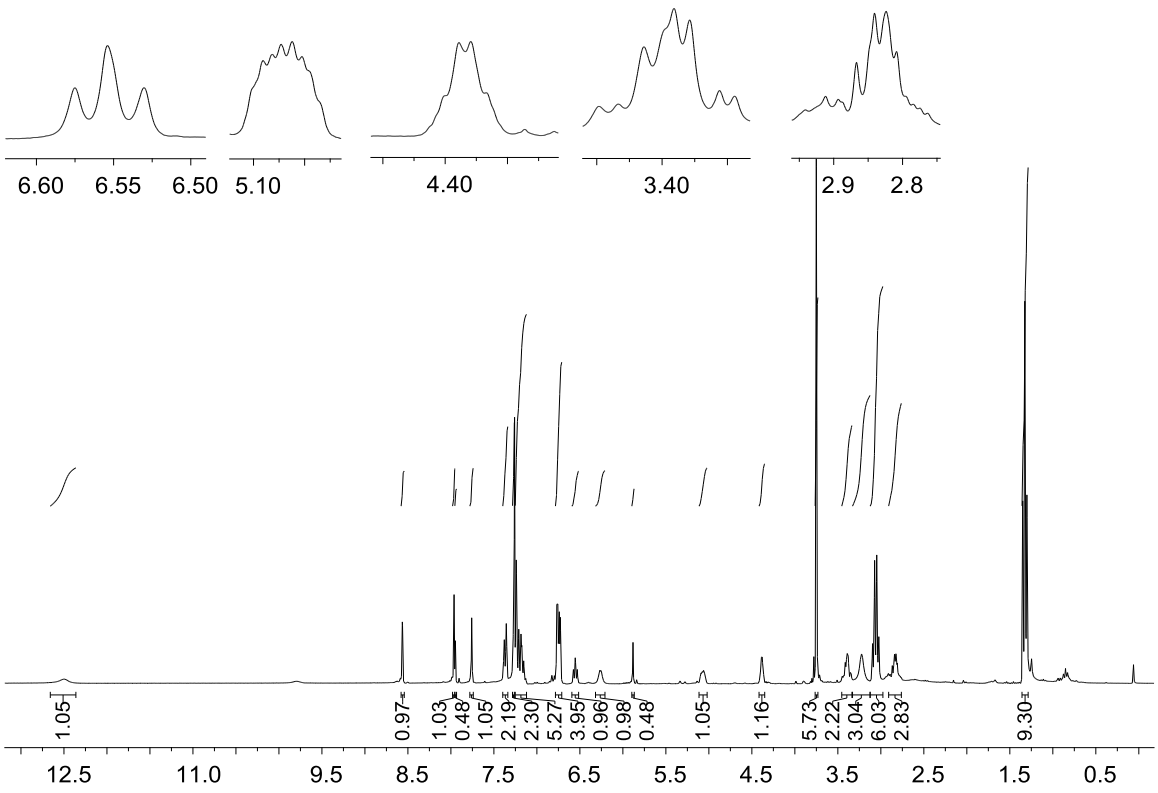
1H, ^{3}J = 4.5 Hz, (H_3C-<u>H</u>N-)), 5.07 (dddd, 1H, $^{3}J_{2a'-3'}$ = 5.6 Hz, $^{3}J_{2b'-3'}$ = 3.0 Hz, $^{3}J_{3'-4'}$ = 2.3 Hz, $^{3}J_{3'-P}$ = 9.0 Hz, H-C(3')), 4.39 (ddd, 1H, $^{3}J_{4'-5a'}$ = 4.5 Hz, $^{3}J_{4'-5b'}$ = 3.5 Hz, $^{3}J_{3'-4'}$ = 2.3 Hz, H-C(4')), 3.75 (s, 6H, 2×(H_3CO-)), 3.42 (dd, 1H, $^{2}J_{5a'-5b'}$ = 10.4 Hz, $^{3}J_{4'-5a'}$ = 4.5 Hz, H_a-C(5')), 3.37 (dd, 1H, $^{2}J_{5a'-5b'}$ = 10.4 Hz, $^{3}J_{4'-5b'}$ = 3.5 Hz, H_b-C(5')), 3.28−3.16 (m, 3H, (<u>H</u>$_3$C-HN-)), 3.06 (quartet, 6H, ^{3}J = 7.3 Hz 3×(-CH_2-($TEAH^+$))), 2.89 (ddd, 1H, $^{2}J_{2a'-2b'}$ = 13.9 Hz, $^{3}J_{1'-2a'}$ = 7.6 Hz, $^{3}J_{2a'-3'}$ = 5.6 Hz, H_a-C(2')), 2.82 (ddd, 1H, $^{2}J_{2a'-2b'}$ = 13.9 Hz, $^{3}J_{1'-2b'}$ = 6.0 Hz, $^{3}J_{2b'-3'}$ = 3.0 Hz, H_b-C(2')), 1.33 (t, 9H, ^{3}J = 7.3 Hz, 3×(H_3C-($TEAH^+$))).

^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 158.5, 155.8, 149.7, 149.0, 144.5, 139.1, 135.7, 133.5, 130.1, 128.1, 127.8, 126.9, 123.2, 119.5, 113.1, 86.5, 85.8 ($^{3}J_{P-4'}$ = 6.3 Hz), 84.3, 74.1 ($^{2}J_{P-3'}$ = 4.0 Hz), 63.8, 55.2, 46.0, 40.1, 27.6, 10.1.

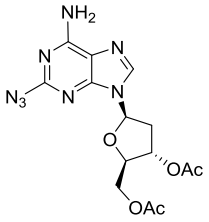
^{31}P-NMR (122 MHz, $CDCl_3$) δ (ppm): 4.0 (dd, $^{1}J_{P-H}$ = 617 Hz, $^{3}J_{P-H}$ = 9 Hz).

HRMS (ESI): calcd for $C_{34}H_{34}N_8O_7P$ $[M\text{-}Et_3NH]^-$, 697.2288; found 697.2286 (0.29 ppm).

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 16:



9-(3',5'-Di-*O*-acetyl-2'-deoxy-β-D-ribofuranosyl)-2-azido-6-amino-9*H*-purine (18)



Solid sodium ascorbate (147 mg, 0.750 mmol, 1.0 equiv.) was added to a solution of diazido nucleoside **7** (300 mg, 0.750 mmol, 1.0 equiv.) in a mixture of *tert*-butanol, acetone (6.5 mL and 1 mL, respectively) and 10% aqueous acetic acid (2 mL). Then a solution of $CuSO_4 \cdot 5H_2O$ (11.3 mg, 0.0450 mmol, 6.0 mol-%) in water (1 mL) was added and the resulting mixture was stirred at ambient temperature. Formation of gaseous product was observed. According to HPLC analysis, after 15 min the mixture consists of

2-azido-6-amino, 2,6-diamino- and 2,6-diazido purine derivatives in the ratio 66:11:16. The reaction mixture was evaporated, partitioned between water (2 mL) and DCM (5 mL). Aqueous layer was extracted with DCM (4×1.5 mL) and the combined organic phase was evaporated after drying on anhydrous Na_2SO_4. Silica gel column chromatography (DCM/MeOH 1.5→2.1%) afforded the product **18** (181 mg, 65%) as a white solid.

R_f = 0.4 (DCM/MeOH 19:1).

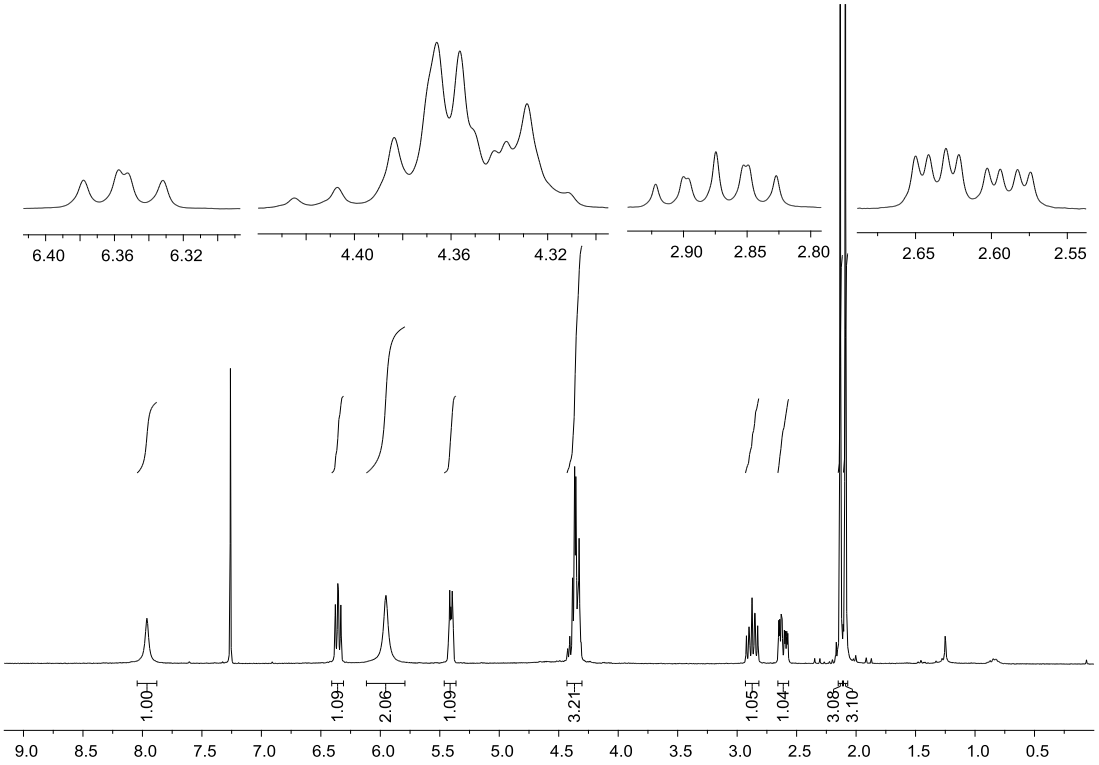
HPLC t_R = 5.97 min, 98% purity, eluent E_5.

IR (KBr) ν (cm^{-1}): 3350, 3185, 2135, 1745, 1650, 1585, 1350, 1230, 1035.

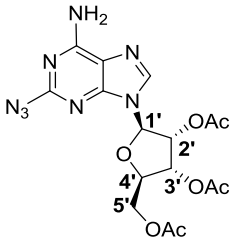
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.96 (s, 1H, H-C(8)), 6.36 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 7.7 Hz, $^3J_{1'\text{-}2b'}$ = 6.1 Hz, H-C(1')), 5.95 (br.s, 2H, H_2N-), 5.41 (ddd, 1H, $^3J_{2a'\text{-}3'}$ = 6.5 Hz, $^3J_{2b'\text{-}3'}$ = 2.6 Hz, $^3J_{3'\text{-}4'}$ = 2.4 Hz, H-C(3')), 4.43-4.30 (m, 3H, H-C(4'), H_2C(5')), 2.87 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.1 Hz, $^3J_{1'\text{-}2a'}$ = 7.7 Hz, $^3J_{2a'\text{-}3'}$ = 6.5 Hz, H_a-C(2')), 2.61 (ddd, 1H, $^2J_{2a'\text{-}2b'}$ = 14.1 Hz, $^3J_{1'\text{-}2b'}$ = 6.1 Hz, $^3J_{2b'\text{-}3'}$ = 2.6 Hz, H_b-C(2')), 2.13, 2.09 (2s, 6H, H_3CC(O)O-C(3', 5')).

^{13}C-NMR (75.5 MHz, $CDCl_3$) δ (ppm): 170.5, 170.4, 157.1, 155.9, 150.9, 138.2, 117.1, 84.7, 82.7, 74.4, 63.8, 37.7, 21.0, 20.9.

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 18:



9-(2',3',5'-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-azido-6-amino-9*H*-purine (19)



Solid sodium ascorbate (129 mg, 0.650 mmol, 1.0 equiv.) was added to a solution of diazide **17** (300 mg, 0.650 mmol, 1.0 equiv.) in a mixture of *tert*-butanol (6.3 mL) and 10% aqueous acetic acid (2.1 mL). Then a solution of $CuSO_4 \cdot 5H_2O$ (9 mg, 0.04 mmol, 6.0 mol-%) in water (0.5 mL) was added and the reaction mixture was stirred at room temperature (20^{o}C). Intensive evolution of gas was observed. According to HPLC analysis, after 10 min the mixture consists of 2-azido-6-amino-, 2,6-diamino- and 2,6-diazido purine derivatives in the ratio 75:7:18. The reaction mixture was kept at 0 ^{o}C for an hour. The insoluble material was then removed by filtration, washed with cold

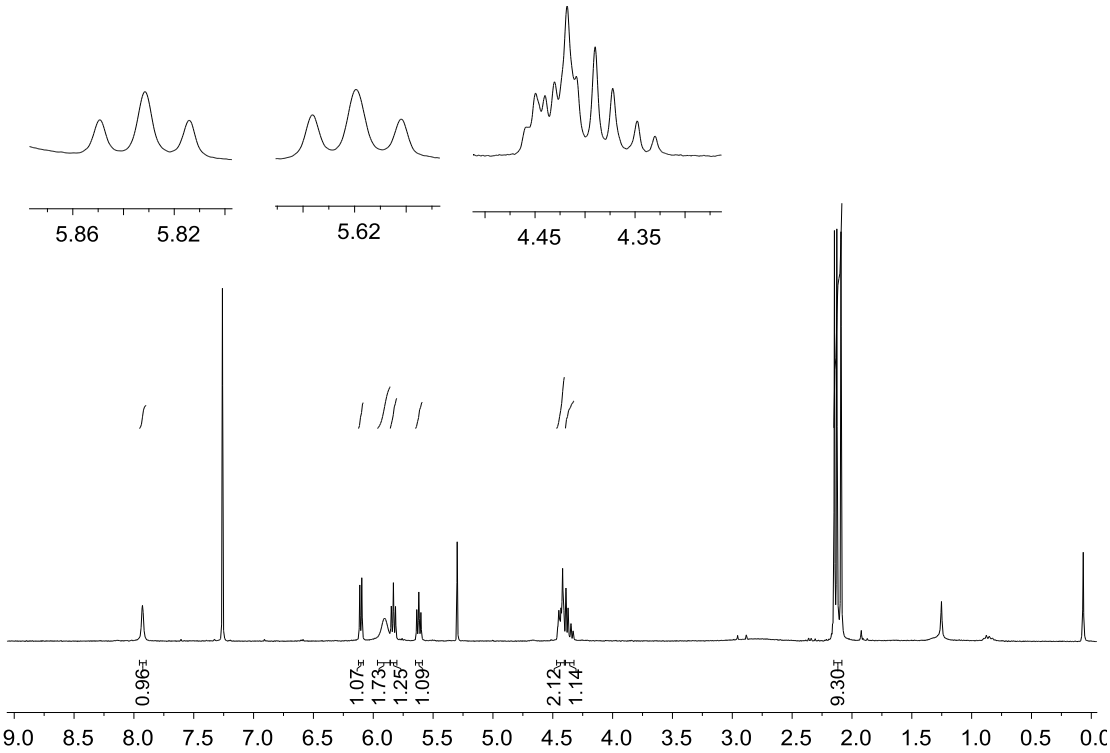
mathanol (-20 ^{o}C, 3×1.5 mL) and water (3×0.5 mL). Product **19** (186 mg, 66 %) was obtained as a white powder.

HPLC t_R = 6.42 min, 93% purity, eluent E_5.

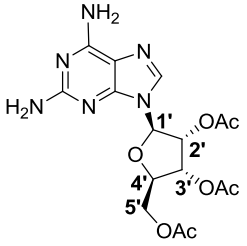
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.93 (s, 1H, H-C(8)), 6.10 (d, 1H, $^3J_{1'\text{-}2'}$ = 5.0 Hz, H-C(1')), 5.91 (br.s, 2H, H_2N-), 5.83 (dd, 1H, $^3J_{2'\text{-}3'}$ = 5.5 Hz, $^3J_{1'\text{-}2'}$ = 5.0 Hz, H-C(2')), 5.62 (dd, 1H, $^3J_{2'\text{-}3'}$ = 5.5 Hz, $^3J_{3'\text{-}4'}$ = 4.8 Hz, H-C(3')), 4.47-4.40 (m, 2H, H-C(4'), H_a-C(5')), 4.36 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 12.6 Hz, $^3J_{4'\text{-}5b'}$ = 5.3 Hz, H_b-C(5')), 2.15, 2.13, 2.09 (3s, 9H, H_3CC(O)O-C(2', 3', 5')).

The analytical data for compound **19** corespond to those previously reported.[5]

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 19:



9-(2',3',5'-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-6-amino-9*H*-purine (20)



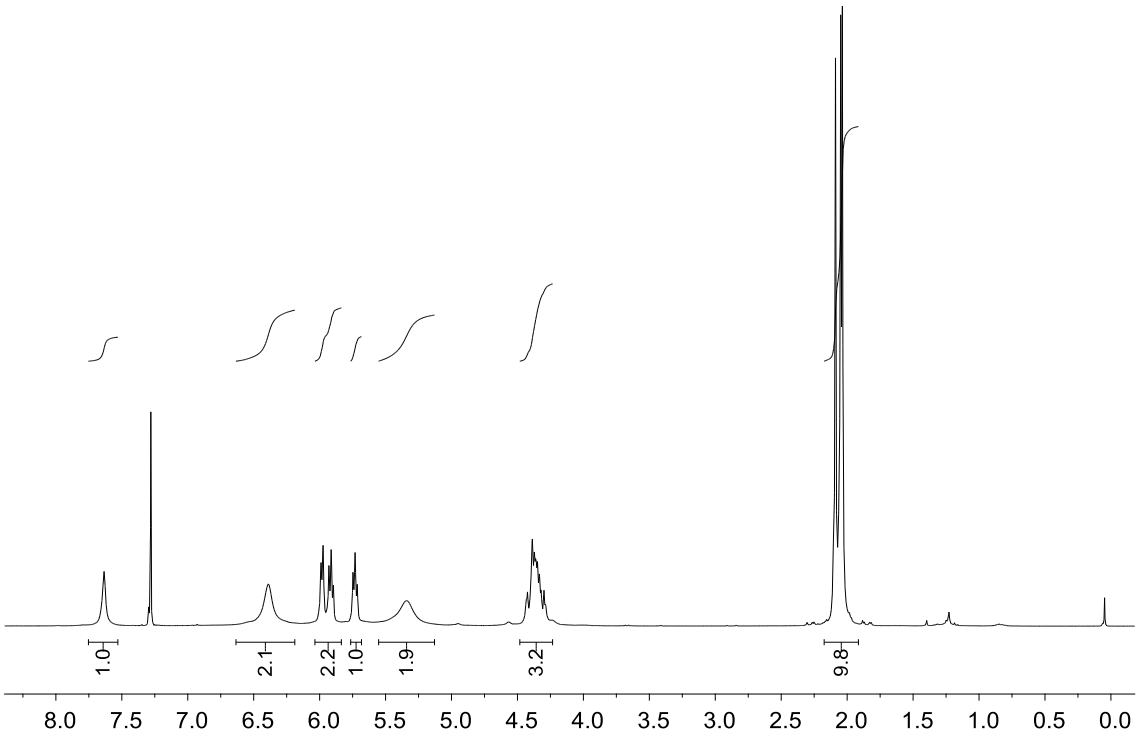
Solid sodium ascorbate (170 mg, 0.860 mmol, 2.0 equiv.) was added to a solution of diazide **17** (200 mg, 0.430 mmol, 1.0 equiv.) in a mixture of *tert*-butanol (4.2 mL) and 10% aqueous acetic acid (1.4 mL). Then a solution of $CuSO_4 \cdot 5H_2O$ (5 mg, 0.02 mmol, 6.0 mol-%) in water (0.5 mL) was added and the reaction mixture was stirred for 2 h with heating (45°C). Intensive evolution of gas was observed. The mixture was then partitioned between DCM (10 mL) and water (10 mL). The water layer was extracted with DCM (6×5 mL). Combined organic phase was evaporated and purified by silica gel column (MeOH/DCM 3→10%). Product **20** (165 mg, 93 %) was obtained as a white foam.

HPLC t_R = 4.67 min, 96% purity, eluent E_5.

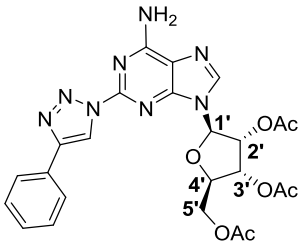
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 7.64 (s, 1H, H-C(8)), 6.39 (br.s, 2H, H_2N-), 5.98 (d, 1H, $^3J_{1'-2'}$ = 4.7 Hz, H-C(1')), 5.91 (dd, 1H, $^3J_{2'-3'}$ = 5.3 Hz, $^3J_{1'-2'}$ = 4.7 Hz, H-C(2')), 5.73 (dd, 1H, $^3J_{2'-3'}$ = 5.3 Hz, $^3J_{3'-4'}$ = 4.4 Hz, H-C(3')), 5.34 (br.s, 2H, H_2N-), 4.45-4.27 (m, 3H, H-C(4'), $H_{a,b}$-C(5')), 2.09, 2.05, 2.04 (3s, 9H, $H_3CC(O)O$-C(2', 3', 5')).

The analytical data for compound **20** corespond to those previously reported.[5]

^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of 20:



9-(2',3',5'-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-6-amino-9*H*-purine (21)



A solution of $CuSO_4 \cdot 5H_2O$ (6 mg, 0.02 mmol, 5.5 mol-%) in water (0.1 mL) and a solution of sodium ascorbate (8 mg, 0.04 mmol, 9.3 mol-%) in water (0.1 mL) were sequentially added to a suspension of 2-azido nucleoside **19** (183 mg, 0.420 mmol, 1.0 equiv.) and phenyl acetylene (0.90 mL, 0.840 mmol, 2.0 equiv.) in methanol (5 mL) and 10% aqueous acetic acid. The reaction vessel was then placed in an oil bath (45 ^{o}C) and stirred overnight. HPLC revealed a complete conversion of the starting material. The inhomogeneous reaction mixture was cooled in ice for 3 h and filtered, washed with cold methanol (0 ^{o}C, 3×2 mL), water (1.5 mL) and again with the cold methanol (1.5 mL) to give product **21** as a white powder (130 mg). Filtrate was evaporated and purified by silica gel column chromatography (MeOH/DCM 1→40%) and 38 mg of product was obtained. The combined yield is 75%.

R_f = 0.67 (DCM/MeCN 1:1)

HPLC t_R = 8.04 min, 97% purity, eluent E_5.

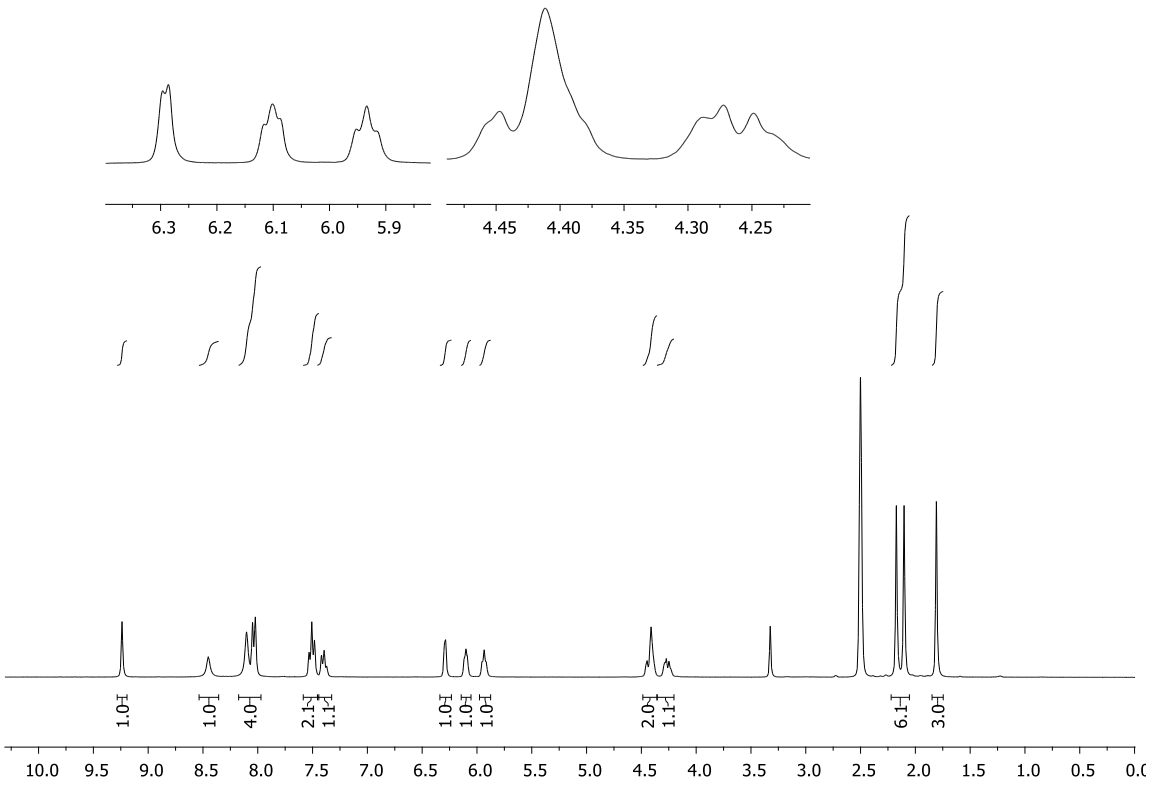
IR (KBr) ν (cm^{-1}): 3335, 3130, 1750, 1670, 1610, 1420, 1240, 1225, 1010.

^{1}H-NMR (300 MHz, DMSO-d6) δ (ppm): 9.24 (s, 1H, H-C(triazole)), 8.45 (s, 1H, H-C(8)), 8.10 (br.s, 2H, H_2N-), 8.04 (d, 2H, ^3J = 7.7 Hz, H-C(Ar)), 7.51 (dd, 2H, ^3J = 7.7 Hz, ^3J = 7.2 Hz, H-C(Ar)), 7.39 (t, 1H, ^3J = 7.2 Hz H-C(Ar)), 6.29 (d, 1H, $^3J_{1'\text{-}2'}$ = 4.0 Hz, H-C(1')), 6.10 (dd, 1H, $^3J_{2'\text{-}3'}$ = 5.6 Hz, $^3J_{1'\text{-}2'}$ = 4.0 Hz, H-C(2')), 5.93 (dd, 1H, $^3J_{2'\text{-}3'}$ = 5.6 Hz, $^3J_{3'\text{-}4'}$ = 6.3 Hz, H-C(3')), 4.44 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 11.7 Hz, $^3J_{4'\text{-}5a'}$ = 3.5 Hz, H_a-C(5')), 4.43-4.37* (m, 1H, H-C(4')), 4.23 (dd, 1H, $^2J_{5a'\text{-}5b'}$ = 11.7 Hz, $^3J_{4'\text{-}5b'}$ = 4.9 Hz, H_b-C(5')), 2.17, 2.10, 1.81 (3s, 9H, $H_3CC(O)O$-C(2', 3', 5')).

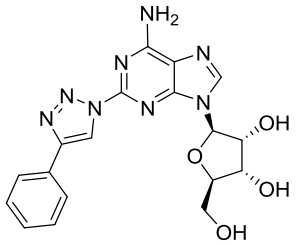
* Determined from HSQC spectrum

^{13}C-NMR (75.5 MHz, DMSO-d6) δ (ppm): 169.9, 169.8, 169.3, 156.8, 149.1 (2C), 146.4, 141.4, 130.0, 128.9, 128.3, 125.5, 119.5, 118.9, 86.4, 78.9, 72.3, 69.7, 62.4, 20.4, 20.3, 20.1.

^{1}H-NMR (300 MHz, DMSO-d6) spectrum of 21:



9-(β-D-ribofuranosyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-6-amino-9*H*-purine (22)



Methylamine water solution (25%, 1 mL) was added to the suspension of nucleoside **21** (110 mg, 0.210 mmol, 1.0 equiv.) in THF (2 mL). The clear solution was then placed in 45 ^{o}C oil bath. After 5 h HPLC showed a complete disappearance of the starting material. After evaporation the mixture was washed 3 times by addition of

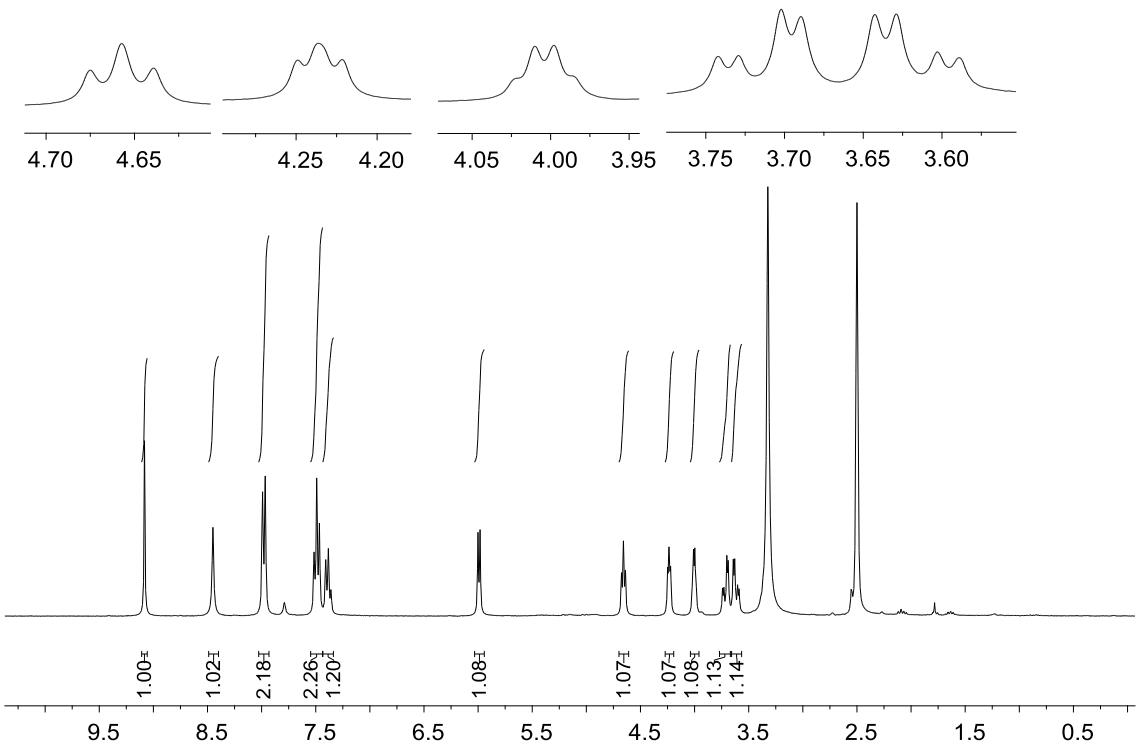
acetonitrile (1 mL), heating (60 ^{o}C), cooling (-20 ^{o}C). Product **22** (80 mg, 95%) was obtained as a white powder.

HPLC t_R = 5.37 min, 99% purity, eluent E_5.

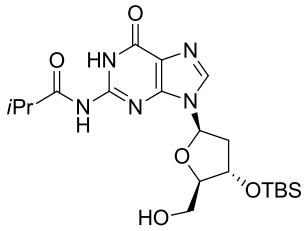
^{1}H-NMR (300 MHz, 50 ^{o}C, DMSO-d6 + D_2O) δ (ppm): 9.08 (s, 1H, H-C(triazole)), 8.45 (s, 1H, H-C(8)), 7.98 (d, 2H, ^{3}J = 7.4 Hz, H-C(Ar)), 7.49 (dd, 2H, ^{3}J = 7.4 Hz, ^{3}J = 7.1 Hz, H-C(Ar)), 7.38 (t, 1H, ^{3}J = 7.1 Hz H-C(Ar)), 5.99 (d, 1H, $^{3}J_{1'\text{-}2'}$ = 5.7 Hz, H-C(1')), 4.66 (dd, 1H, $^{3}J_{1'\text{-}2'}$ = 5.7 Hz, $^{3}J_{2'\text{-}3'}$ = 5.0 Hz, H-C(2')), 4.24 (dd, 1H, $^{3}J_{2'\text{-}3'}$ = 5.0 Hz, $^{3}J_{3'\text{-}4'}$ = 3.3 Hz, H-C(3')), 4.00 (dt, 1H, $^{3}J_{4'\text{-}5'}$ = 4.0 Hz, $^{3}J_{3'\text{-}4'}$ = 3.3 Hz, H-C(4')), 3.73, 3.62 (2dd, 2H, $^{2}J_{5a'\text{-}5b'}$ = 11.9 Hz, $^{3}J_{4'\text{-}5a,b'}$ = 4.0 Hz, H_a-C(5')).

^{13}C-NMR (75.5 MHz, DMSO-d6 + D_2O) δ (ppm): 156.6, 150.0, 149.2, 146.5, 140.9, 130.1, 129.1, 128.5, 125.6, 119.8, 118.7, 87.4, 85.8, 73.7, 70.4, 61.4.

^{1}H-NMR (300 MHz, 50 ^{o}C, DMSO-d6 + D_2O) spectrum of 22:



N^2-Isobutyryl-3'-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine (24)[1]



A mixture of 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-deoxyguanosine (10.23 g, 16.00 mmol, 1.0 equiv.) and imidazole (3.07 g, 45.0 mmol, 2.8 equiv.) was evaporated twice with dry acetonitrile and kept under reduced pressure (5 Torr) for 2 h. Dimethylformamide (7.1 mL, distilled over calcium hydride) was added under nitrogen and the reaction vessel was placed in 35 ^{o}C. Then TBS-Cl (2.86 g, 19.0 mmol, 1.2 equiv.) was added with stirring. The progress of the reaction was monitored by TLC (R_f = 0.7 (EtOAc/Tol = 20/1)). After 4.5 h DCM (100 mL) and saturated aqueous $NaHCO_3$ (30 mL) were added. Intensive stirring was applied for 1.5 h. The aqueous layer was separated and extracted with DCM (6×10 mL). The combined organic phase was dried on Na_2SO_4, filtered and evaporated. After drying overnight (5 Torr), the resulting material was dissolved in dry DCM and 5% solution of dichloroacetic acid in DCM was added under stirring. The resulting reaction mixture was stirred for 10 min, then neutralized and washed with saturated aqueous $NaHCO_3$ solution (3×40 mL). Each $NaHCO_3$ layer was extracted with DCM (3×7 mL). The combined DCM layers were evaporated and the residue was purified by silica gel column chromatography (MeOH/DCM = 4%) to yield product **24** (4.00 g, 55%, 2 steps) as an off-white foam.

R_f = 0.88 (MeOH/DCM = 1/9).

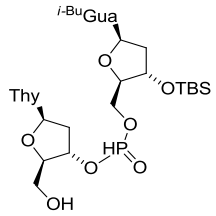
HPLC t_R = 5.32 min, 97% purity, eluent E_1.

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 12.06 (s, 1H, H-N(3)), 9.10 (s, 1H, H-N-C(2)) 7.80 (s, 1H, H-C(8)), 5.12 (dd, 1H, $^3J_{1'\text{-}2a'}$ = 8.3 Hz, $^3J_{1'\text{-}2b'}$ = 6.0 Hz, H-C(1')), 5.02 (d, 1H, $^3J_{5b'}$ = 7.9 Hz, HO-C(5')), 4.58 (ddd, 1H, $^3J_{2a'\text{-}3'}$ = 5.5 Hz, $^3J_{2b'\text{-}3'}$ = 1.8 Hz, $^3J_{3'\text{-}4}$ =

[1] Compound **24** has previously been reported: 1) Utagawa, E.; Sekine, M.; Seito, K. *J. Org. Chem.* **2006**, *71*, 7668-7677. 2) Manetto, A.; Georganakis, D.; Leondiadis, L; Gimisis, T.; Mayer, P.; Carell, T.; Chatgilialoglu, C. *J. Org. Chem.* **2007**, *72*, 3659-3666. 3) Patil, S. V.; Mane, R. B.; Salunkhe, M. M. *Synth. Commun.* **1994**, *24*, 2423-2428.

1.5 Hz, 1H, H-C(3')), 3.99 (dt, 1H, $^3J_{4'\text{-}5a'} = {}^3J_{4'\text{-}5b'} = 2.3$ Hz, $^3J_{3'\text{-}4'} = 1.5$ Hz, H-C(4')) 3.88 (dd, 1H, $^2J_{5a'\text{-}5b'} = 12.4$ Hz, $^3J_{4'\text{-}5a'} = 2.3$ Hz, H_a-C(5')), 3.37 (ddd, 1H, $^2J_{5a'\text{-}5b'} = 12.4$ Hz, $^3J_{HO\text{-}} = 7.9$ Hz, $^3J_{4'\text{-}5b'} = 1.5$ Hz, H_b-C(5')), 2.75 (ddd, 1H, $^2J_{2a'\text{-}2b'} = 13.2$ Hz, $^3J_{1'\text{-}2a'} = 8.3$ Hz, $^3J_{2a'\text{-}3'} = 5.5$ Hz, H_a-C(2')), 2.72 (septet, 1H, $^3J = 7.2$ Hz (Me_2-CH-CO(O)-), 2.20 (ddd, 1H, $^2J_{2a'\text{-}2b'} = 13.2$ Hz, $^3J_{1'\text{-}2b'} = 6.0$ Hz, $^3J_{2b'\text{-}3'} = 1.8$ Hz, H_b-C(2')), 1.23 un 1.21 (2d, 6H, $^3J = 7.2$ Hz, (Me_2-CH-CO(O)-)), 0.84 (s, 9H, *t*-Bu-$C(Me)_2$-Si), 0.04 (s, 6H, *t*-Bu-$C(Me)_2$-Si).

***H*-Phosphonate diester (S1)**



Procedure 1: Nucleoside **24** (507 mg, 1.12 mmol, 1.0 equiv.) and 5'-O-dimethoxytrityl protected thymidine *H*-phosphonate[6] (858 mg, 1.23 mmol, 1.1 equiv.) were placed in a round-bottom flask and dried by evaporation with dry acetonitrile (2×10 mL) and pyridine (2×7 mL). To the dried solids dry pyridine (23 mL) and pivaloyl chloride (0.41 mL, 4.77 mmol, 4.2 equiv.) were added under nitrogen. The resulting reaction mixture was stirred at ambient temperature for 10 min and then quenched by addition of 5% aqueous citric acid solution (50 mL) and DCM (10 mL). The obtained mixture was stirred intensively for another 10 minutes. The resulting aqueous fraction was extracted with DCM (3×10 mL). Organic layers were dried on anhydrous Na_2SO_4, filtered and evaporated to give white foam with product content of 63% according to HPLC ($t_R = 7.128$ min, eluent E_1). MS of the main product corresponds to the desired product that still contains 5'-*O*-trityl group (calcd for $C_{51}H_{63}N_7O_{13}PSi$ $[M\text{-}H]^-$, 1040.40; found 1040.64). It was stored at -20 ^oC overnight under nitrogen. $R_f = 0.5$ (MeOH/DCM = 1.3/20).

Dichloroacetic acid (0.8 mL) was added to the solution of dimethoxytrityl-protected *H*-phosphonate diester in DCM (7 mL). After 20 minutes of stirring, more dichloroacetic acid (0.4 mL) was added. As shown by TLC, complete detritylation was achieved. The crude product was purified by silica gel column chromatography

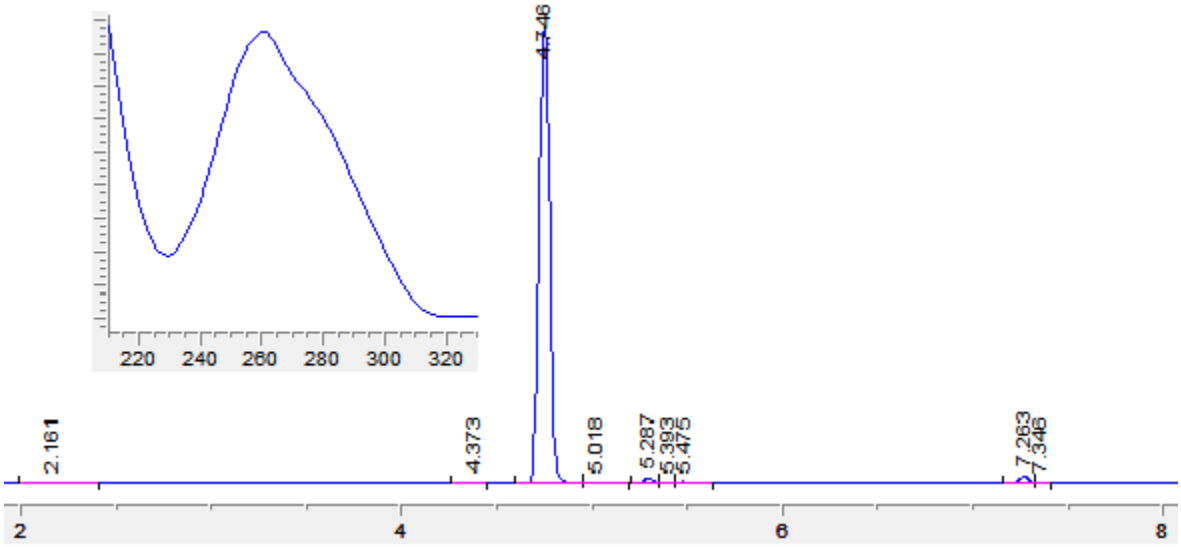
(MeOH/DCM 2→4.5%) without any other work-up to yield product **S1** (517 mg, 62%) as a white foam.

R_f = 0.25 (MeOH/DCM = 1.3/20).

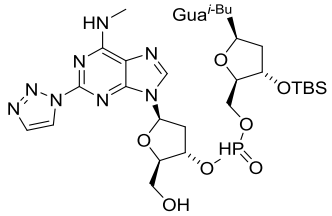
HPLC t_R = 4.75 min, 95% purity, eluent E_1.

MS: calcd for $C_{30}H_{45}N_7O_{11}PSi$ $[M-H]^-$, 738.27; found 738.07.

HPLC chromatogram and UV spectrum of S1:



***H*-Phosphonate diester (25)**



Compound **25** was obtained according to the **Procedure 1** using nucleoside **24** (508 mg, 1.12 mmol, 1.0 equiv.), *H*-phosphonate **16** (1114 mg, 1.24 mmol, 1.1 equiv.) pivaloyl chloride (0.41 mL, 4.78 mmol, 4.3 equiv.). Silica gel column chromatography (MeOH/DCM 0→3%) afforded partially purified product **S2** with product content of 50% according to HPLC (t_R = 7.128 min, eluent E_1). MS of the main product corresponds to the desired DMT-protected intermediate (calcd for $C_{54}H_{67}N_{13}O_{11}PSi$ $[M+H]^+$, 1132.56; found 1132.91). R_f = 0.6 (MeOH/DCM = 1.3/20).

Dichloroacetic acid (1.0 mL) was added to the solution of dimethoxytritylated condensation intermediate in DCM (5 mL). After 15 minutes of stirring no starting material was detectable by TLC. The crude product was purified by silica gel column chromatography (MeOH/DCM 1→5%) to yield product **25** (210 mg, 25%) as a white solid.

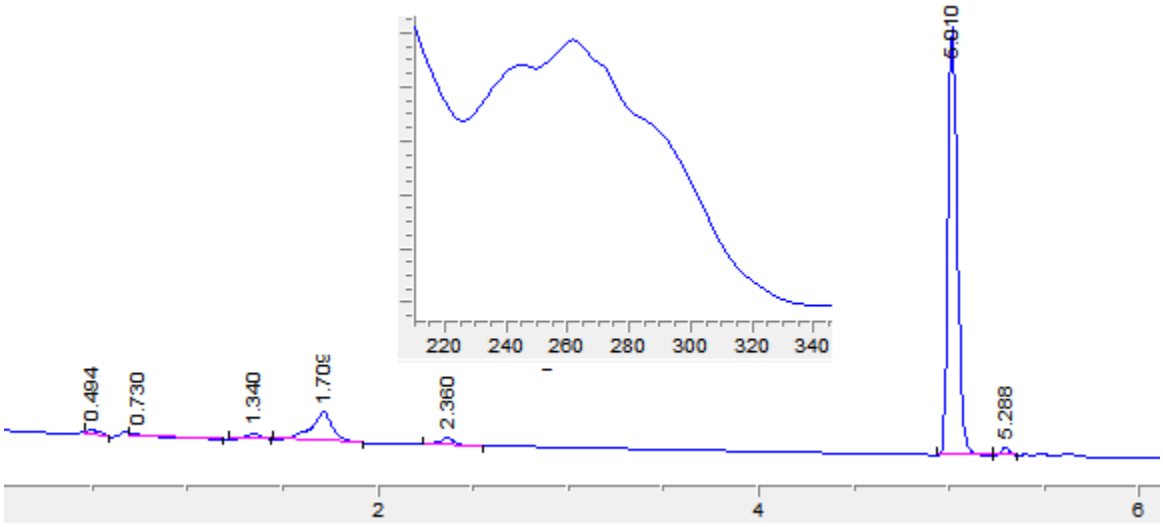
R_f = 0.13 (MeOH/DCM = 1.3/20).

HPLC t_R = 5.01 min, 82% purity, eluent E_1.

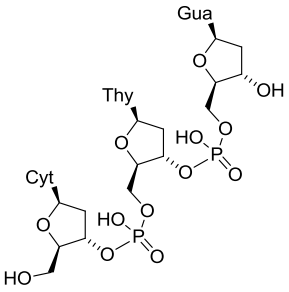
HRMS (ESI): calcd for $C_{33}H_{48}N_{13}O_9PSiNa$ $[M+Na]^+$, 852.3097; found 852.3110 (1.53 ppm).

^{31}P-NMR (122MHz, $CDCl_3$) δ (ppm): A mixture of diastereoisomers: 14.5 (d, $^1J_{P\text{-}H}$ = 727 Hz) and 7.7 (d, $^1J_{P\text{-}H}$ = 732 Hz).

HPLC chromatogram and UV spectrum of 25:



Trinucleotide S3



Procedure 2: *H*-Phosphonate diester **S1** (100 mg, 0.135 mmol, 1.0 equiv.) and *H*-phosphonate **26**[6] (118 mg, 0.148 mmol, 1.1 equiv.) were dried under reduced pressure (10 Torr) for 3 h and coevaporated with dry pyridine (3 mL). The resulting oil was dissolved in dry pyridine (3 mL) under nitrogen. Next, pivaloyl chloride (49 μL, 0.573 mmol, 4.2 equiv.) was added and the resulting solution was agitated for 15 min. Then the mixture was quenced by an addition of 5% aqueous citric acid solution (10 mL) and DCM (5 mL) with intensive stirring. The aqueous layer was extracted with DCM (4×5 mL). DCM fractions were dried on anhydrous Na_2SO_4, filtered and evaporated to viscous consistency. Iodine solution in pyridine-water[7] was added (approx. 3 mL of 2% I_2 solution in Py/H_2O (98/2)), to the point when no disappearance of the brown colour was observed in 5 min period of time. To remove the iodine excess $P(OEt_3)$ was then added. The mixture was chromatographically filtered trough a pad of silica gel (MeOH/DCM 3→16% with 0.5% Et_3N additive) to yield an intermediate product with 3'-*O*-TBS and 5'-*O*-DMT groups.

For the intermediate R_f = 0.63 (MeOH/DCM = 1/5 + Et_3N).

1M TBAF solution (1 mL) in H_2O/THF (5%) was added to the obtained product upon heating in 25 ^oC oil. Stirring was continued for 1 h when HPLC showed a complete conversion of the starting material (t_R = 3.96 min, 78% purity, eluent E_1). Volatiles were evaporated and the residue was dissolved in DCM (5 mL) and dichloroacetic acid (1 mL) was added. TLC showed a complete disappearance of the starting material in 15 min (R_f = 0.25 (MeOH/DCM = 5/1 + Et_3N)). The mixture was immediately purified by silica gel column chromatography (MeOH/DCM 6→25% with 2% Et_3N additive). 73 mg of oil was obtained after keeping in vacuum (10 Torr, 2 days).

HPLC t_R = 1.23 min, 95% purity, eluent E_7.

MS (ESI): clctd for $C_{40}H_{47}N_{10}O_{19}P_2$ $[M-H]^-$, 1033.25; found 1033.56.

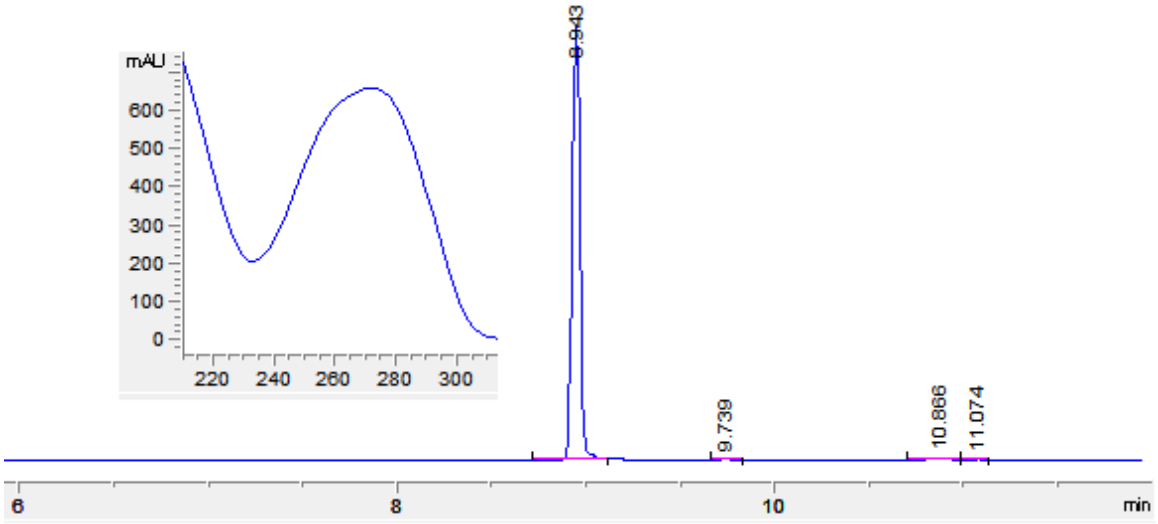
The obtained substance was dissolved in NH_3/H_2O (5 mL, 25%) and placed in 55 ^oC oil and stirred overnight (HPLC control). When the reaction had completed, the insolubles were filtered and the resulting clear solution was concentrated (to approx. 3 mL) for purification on C18 silica gel ($MeCN/H_2O$; 1→7% with 0.5% AcOH additive) to give product **S3** as transparent solid (22 mg, 19%, 5 steps).

HPLC t_R = 8.93 min, 97% purity, eluent E_4.

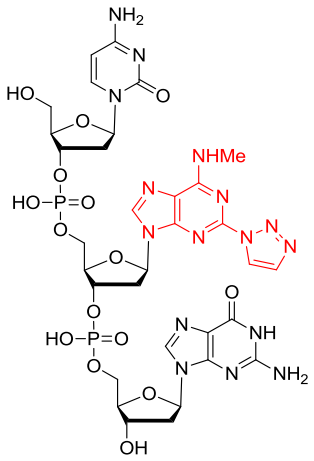
MS: calcd for $C_{29}H_{39}N_{10}O_{17}P_2$ $[M+H]^+$, 861.20; found 860.96.

^{31}P-NMR (122 MHz, D_2O) δ (ppm): 1.7 (2×P).

HPLC chromatogram and UV spectrum of S3:



Trinucleotide 27



Compound **27** was obtained according to the **Procedure 2** using *H*-phosphonate diester **25** (100 mg, 0.121 mmol, 1.0 equiv.), *H*-phosphonate **26** (119 mg, 0.132 mmol, 1.1 equiv.), pivaloyl chloride (44 µL, 0.511 mmol, 4.2 equiv.). After oxidation and chromatographic purification (MeOH/DCM 2.5→10%, 1% Et_3N additive) 80 mg of white substance **S4** was obtained (R_f = 0.25 (MeOH/DCM = 1/5 + Et_3N), HPLC t_R = 5.01 min, 89% purity, eluent E_6).

After the detritylation and desilylation by dichloroacetic acid and TBAF 44 mg of product **S5** was obtained by silica gel column chromatography (MeOH/DCM; 7→14%)

(R_f = 0.1 (MeOH/DCM = 1/5 + Et_3N)). According to LC/MS data, also the cleavage of benzoyl groups had occured (calcd for $C_{36}H_{47}N_{16}O_{16}P_2$ $[M+H]^+$, 1021.28; found 1021.18; HPLC t_R = 3.41 min, 50% purity, eluent E_7).

Deacylation by NH_3 followed by silica gel column chromatography (MeCN/H_2O 0.5→15% with AcOH additive of 0.5%) afforded the product **27** as white flakes (13 mg, 11%, 5 steps).

HPLC t_R = 9.12 min, 91% purity, eluent E_4.

Purification by HPLC was performed to achieve pure material for NMR analysis. Eluation programme D-F in this case (D = H_2O/MeCN/formic acid = 95.0/5.0/0.1; F = MeOH) was applied:

Time (min)	0	7.5	10	12
Content of F (%)	13	14.5	95	13

In this system t_R = 6.57 min.

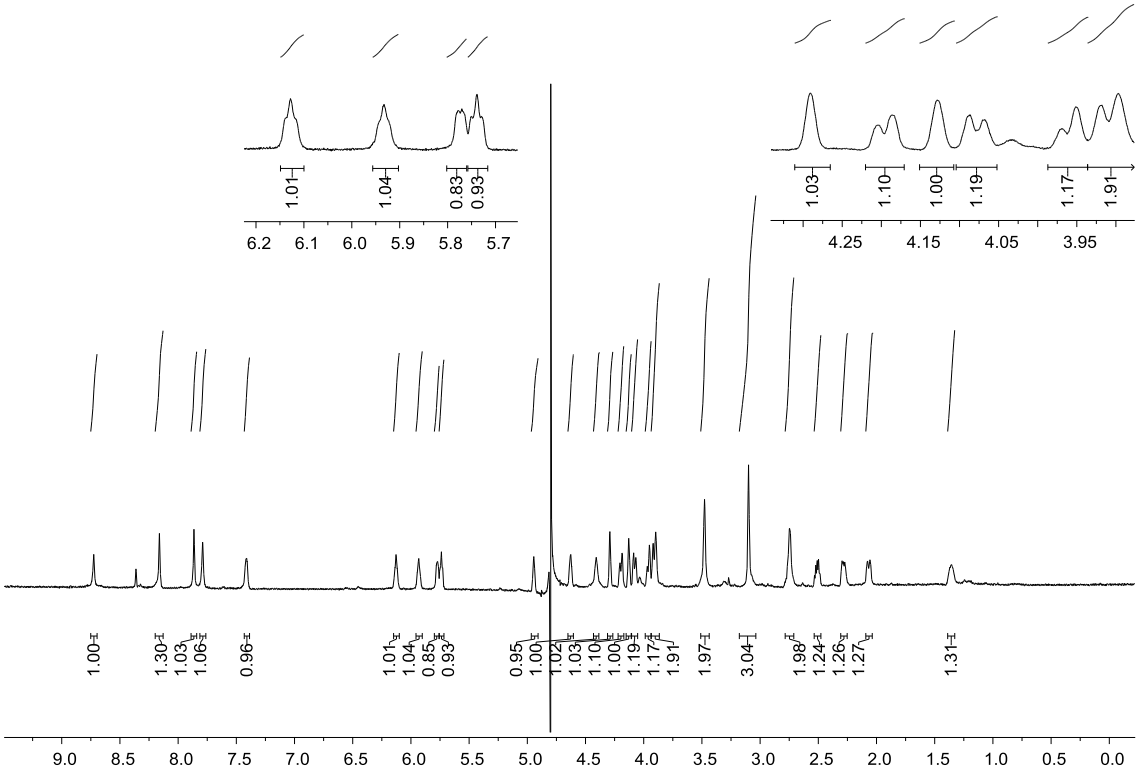
HRMS (ESI): calcd for $C_{32}H_{42}N_{16}O_{15}P_2$ $[M+2H]^{2+}$, 952.248/2 = 476.1240; found 476.1225 (3.15 ppm).

MS (ESI): calcd for $C_{32}H_{39}N_{16}O_{15}P_2$ $[M-H]^-$, 949.23; found 948.95; calcd for $C_{32}H_{41}N_{16}O_{15}P_2$ $[M+H]^+$, 951.24; found 951.55.

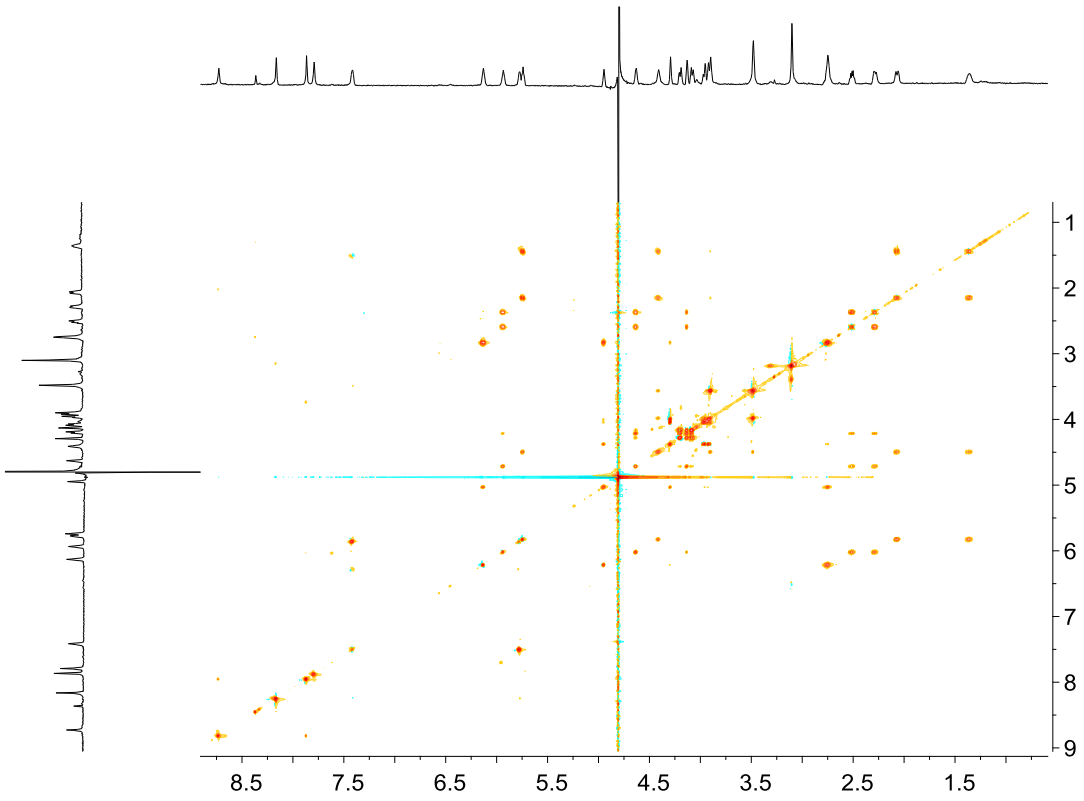
^{1}H-NMR (600 MHz, D_2O) δ (ppm): 8.71 (s, 1H, H-C(triazole)), 8.16 (s, 1H, H-C(8)), 7.87 (s, 1H, H-C(triazole)), 7.79 (s, 1H, H-C(8)), 7.41 (d, 1H, ^3J = 6.0 Hz, H-C(6, Cys)), 6.15-6.10 (m, 1H, H(1'a)), 5.96-5.91 (m, 1H, H(1'b)), 5.78 (d, 1H, ^3J = 6.0 Hz, H-C(5, Cys)), 5.76-5.72 (m, 1H, H(1'c)), 4.96-4.93 (m, 1H, H(3'c)), 4.65-4.61 (m, 1H, H(3'b)), 4.44-4.38 (m, 1H, H(3'c)), 4.31-4.27 (m, 1H, H(4'a)), 4.22-4.17 (dm, 1H, ^2J = 11.1 Hz, H(5'b)), 4.15-4.11 (m, 1H, H(4'b)), 4.10-4.05 (dm, 1H, ^2J = 11.1 Hz, H(5''b)), 3.98-3.94 (dm, 1H ^2J = 11.0 Hz, H(5'a)), 3.94-3.88 (m, 2H, H(4'c, 5''a)), 3.53-3.44 (m, 2H, H(5'c, 5''c)), 3.10 (s, 3H, $\underline{H_3}$C-N), 2.79-2.70 (m, 2H, H(2'a, 2''a)), 2.54-2.48 (m, 1H, H(2'b)), 2.31-2.25 (m, 1H, H(2''b)), 2.10-2.04 (m, 1H, H(2'c)), 1.40-1.32 (m, 1H, H(2''c)).

^{31}P-NMR (122 MHz, D_2O) δ (ppm): 2.9, 1.7.

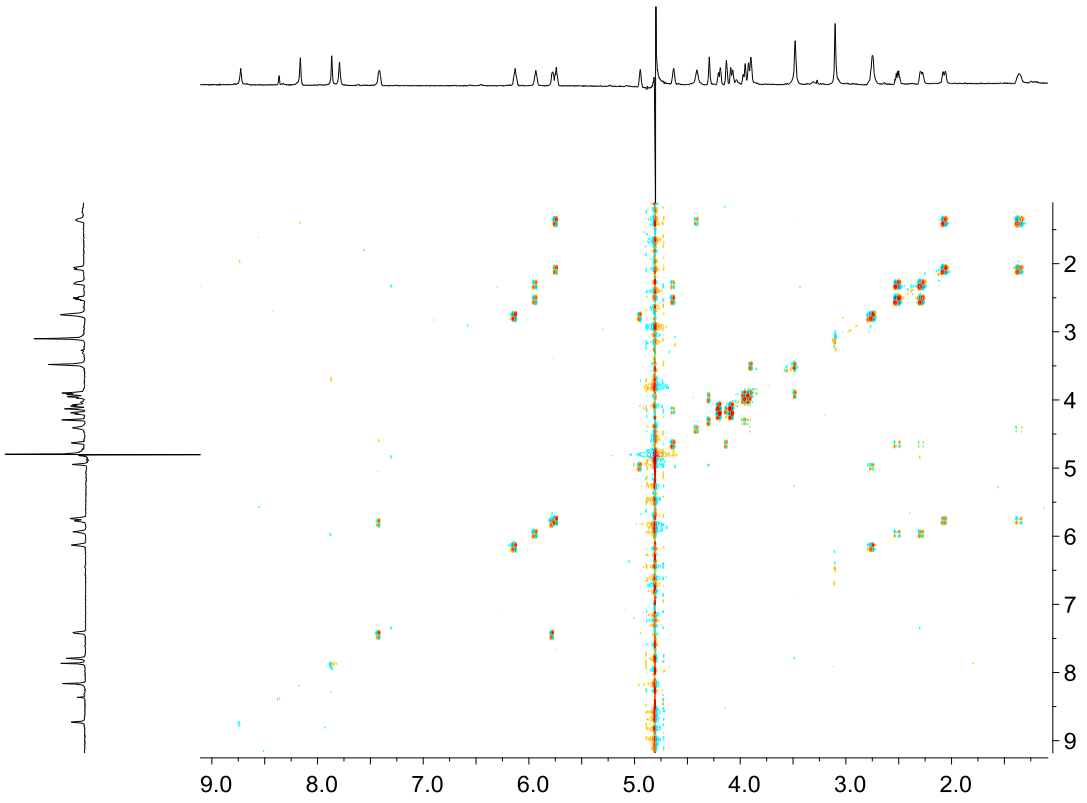
^{1}H-NMR (600 MHz, D_2O) spectrum of 27:



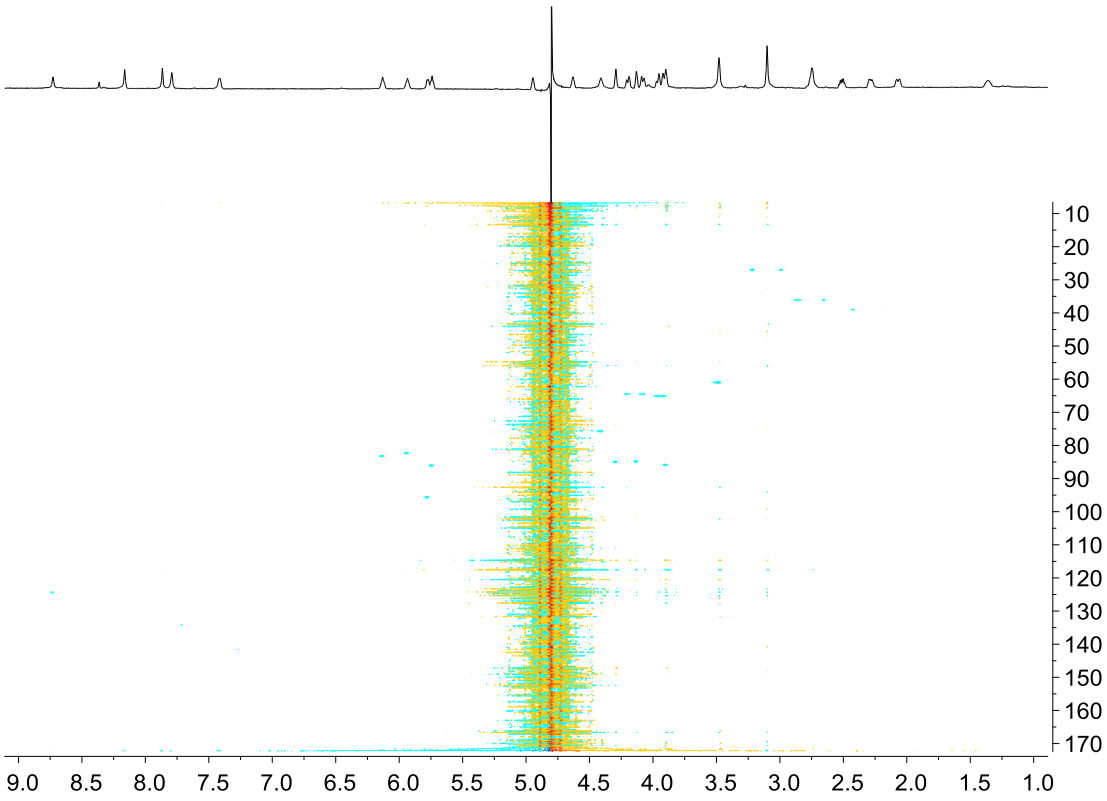
TOCSY spectrum of trinucleotide 27:



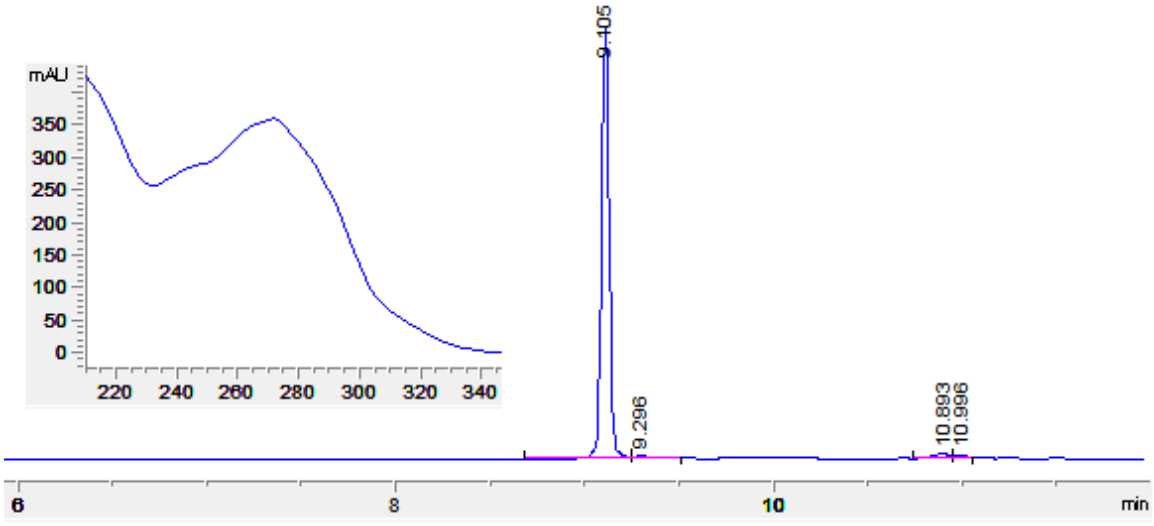
COSY spectrum of trinucleotide 27:



HSQC spectrum of trinucleotide 27:



HPLC chromatogram and UV spectrum of 27:



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