Isopolar phosphonate analogue of adenosine diphosphate ribose.

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Supporting Information

Experimental section

HPLC runs were performed on a Gilson Anachem system with UV detector operating at 254 nm. The HPLC chromatography columns, purchased from Supelco. Anion exchange chromatography was run on a SAX (200 x 4.5 mm, 5µ) column with an isocratic elution using phosphate buffer (KH_2PO_4 50mM, pH=3.5, 5% MeOH) at a flow rate of 1 mL/min while reversed-phase chromatography was run on a RP-C_{18} column. For method 1, elutions were achieved by increasing the methanol content of a formic acid solution (50 mM, pH=6.0) from 5% to 30 % in a linear gradient over 30 min. For method 2, a solution of formic acid (50 mM, pH=6.0) containing 5% of methanol was used isocratically. The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded in $CDCl_3$ or in D_2O on a Brucker AC 300 MHz spectrometer. HMQC and ^{1}H-^{1}H COSY experiments were performed on a Brucker AMX 500 spectrometer. TMS (0 ppm, ^{1}H NMR), $CDCl_3$ (77 ppm, ^{13}C NMR) and $(EtO)_3PO$ (0 ppm, ^{31}P NMR) were used as internal references. The chemical shifts are reported in δ p.p.m. *J* values are recorded in Hz. Liquid secondary ion mass spectra (LSIMS) were recorded in a 3-nitrobenzylalcohol matrix with an effective accelerating voltage of 17 kV on a VG Autospec (Micromass, UK) instrument. Electrospray mass spectra (ES-MS) was performed on a VG Quattro Triple Quadrupole Mass Spectrometer with a flow rate of 20 µL/min and a capillary voltage of 3.5 kV. Reactions were performed under dry argon atmosphere.

Allyloxy diisopropylamino trimethylsilylethynylphosphine (**3**)

Trimethylsilylacetylene (0.34 g, 3.45 mmol) dissolved in THF (40 mL) was treated with *n*-butyllithium (2 M in hexane, 1.9 mL, 3.80 mmol) at -78°C. The formed trimethylsilylacetylide anion was added dropwise via cannula to a solution of allyloxychlorodiisopropyl-aminophosphine in THF (40 mL) cooled at -78° C. The resulting mixture was stirred at -78° C for 2h, diluted with CH_2Cl_2 (40 mL) and washed with $NaHCO_3$ (5%). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in a mixture of petroleum ether and diethylether (9:1) and applied on a short silica column (5g silicagel) to obtain the title compound **3** as a viscous oil in 80 % yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.72-5.78 (m, 1H, H_2C=C*H*-CH_2), 5.14-5.08 (m, 1H, *H*C=CH-CH_2), 4.92-4.98 (m, 1H, *H*C=CH-CH_2), 3.98-4.03 (m, 2H, CH-C*H*$_2$-O), 3.32-3.65 (m, 2H, *N*-[C*H*$(CH_3)_2]_2$), 1.01 (dd, *J*= 2.5, 10.0 Hz, 6H, CH(C*H*$_3)_2$), 0.99 (dd, *J*= 2.5, 10.5 Hz, 6H, CH(C*H*$_3)_2$), -0.02 (s, 9H, Si(C*H*$_3)_3$). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 135.9 (d, *J* = 5.1 Hz, H_2C=*C*H-CH_2), 116.2 (H_2C=CH-CH_2), 111.0 (d, $^3J_{PC}$=1.8 Hz, C-2 acetylene), 107.5 (d, $^2J_{PC}$= 20.3 Hz, C-1 acetylene), 67.6 (d, *J*= 17.1 Hz, P-O*C*H$_2$-CH_2), 43.1 (*C*H$(CH_3)_2$), 43.3 (*C*H$(CH_3)_2$), 24.4 (CH(*C*H$_3)_2$), 24.9 (CH(*C*H$_3)_2$), -0.0 (Si$(CH_3)_3$). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ 94.3. MS *m/z* (+ v/e, FAB) [23%, M+H]$^+$ 286, [81%, M+H- C_3H_5]$^+$ 244, [100%, M+H-C_3H_5O]$^+$ 228. HRMS (LSIMS, positive) calcd for $C_{11}H_{23}NPSi$ [M+H-C_3H_5O] 228.1337 found 228.1329

β – 5 - *O* – Allyl – 1 -*O* - methyl - 2,3 - *O*, *O* – isopropylidene –D - ribofuranoside (**4**)[21]

To a solution of D-ribose (6.30 g, 0.042 mol) in 126 mL methanol, *H*-Dowex resin (1.4 g) was added. The reaction was stirred 48 h at room temperature. The *H*-Dowex resin was filtrated off and rinsed portionly with ether. The filtrate was dried over Na_2SO_4 and concentrated under reduced pressure. The oil was dissolved in a mixture of acetone (47.2 mL) and 2,2 dimethoxypropane (0.084 mole, 10 mL). *H*-Dowex resin (1.4 g) was added and the mixture was stirred overnight. The resin was then removed by filtration and the filtrate evaporated under reduced pressure. β–1-*O*-Methyl-2,3-*O*,*O*– isopropylidene –D-ribofuranoside[21] was obtained in quantitative yields as a colourless oil, R_f = 0.4 ($CHCl_3$/Acetone, 9:1) and data match ref. 20. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.05 (s, 1H, H-1), 4.85 (d, *J* = 3.9 Hz, 1H, H-2), 4.59 (d, *J* = 3.4 Hz, 1H, H-3), 4.44 (t, *J* = 2.6 Hz, 1H, H-4), 3.6-3.3 (m, 2H, H-5), 3.44 (s, 3H, OCH_3), 1.49 (s, 3H, isopropylidene-CH_3), 1.32 (s, 3H, isopropylidene-CH_3). ^{13}C NMR ($CDCl_3$, 125.75 MHz) δ 112.1 (isopropylidene *C* $(CH_3)_2$), 110.0 (C-1), 88.8 (C-4), 86.2 (C-2), 81.9 (C-3), 64.4 (C-5), 55.9 (OCH_3), 26.7 (isopropylidene -CH_3), 25.0 (isopropylidene-CH_3).

To a solution of β–1-*O*-methyl-2,3-*O*,*O*– isopropylidene –D-ribofuranoside (5 g, 0.025 mole) in 100 mL DMF, BaO (14.5 g, 0.095 mol) and allylbromide (12.1 g, 0.1 mol) were added. The reaction mixture was stirred at rt, overnight. The solution was filtrated through a celite path and rinsed with chloroform. The filtrate was successively washed with water and aq. KCl (10%, w/v), dried over Na_2SO_4 and concentrated to obtain **4** as a colorless oil (5.5 g, 0.023 mol) in 92 % yield. This compound was used without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.85-5.92 (m, 1H, H_2C=C*H*-CH_2), 5.28 (dd, J_{gem}= 1.5 Hz, J_{trans}= 17.2 Hz, 1H, *H*$_2$C=CH-CH_2), 5.19 (dd, J_{gem}= 1.5 Hz, J_{cis} = 10.4 Hz, 1H, *H*$_2$C=CH-CH_2), 4.96 (s, 1H, H-1, β), 4.68 (d, *J* = 6.0 Hz, 1H, H-2), 4.58 (d, *J* = 6.8 Hz, 1H, H-4), 4.02 (d, *J* = 5.5 Hz, 2H, H_2C=CH-C*H*$_2$), 3.41-3.48 (m, 3H, H-3, H-5), 3.32 (s, 3H, O-Me), 1.48 (s, 3H, isopropylidene-CH_3), 1.34 (s, 3H, isopropylidene-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 136.1(H_2C=*C*H-CH_2), 117.5 (H_2C=CH-CH_2), 112.7 (isopro- pylidene-CH_3), 109.6 (C-1), 85.5 (C-4), 82.5 (C-2), 77.6 (C-3), 72.5 (H_2C=CH-*C*H$_2$O), 71.3 (C-5), 55.8 (O-Me), 26.3 (isopropylidene-CH_3), 25.3 (isopropylidene CH_3).

α/β 1,2,3-Tri-*O, O, O*-acetyl D-ribofuranose (**5**)[22]

The allylated ribose **4** (4.8 g, 0.02 mol) was dissolved at 0°C in solution containing glacial acetic acid (7mL), acetic anhydride (5 mL) and H_2SO_4 (1.67 mL). The reaction mixture was allowed to warm up progressively and stirred overnight. The solution was poured in a mixture of ice and $NaHCO_3$. The neutralized solution was extracted three times with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated. α/β–1,2,3-Tri-*O,O,O*-acetyl 5-*O*-allyl D-ribofuranose was obtained as a colourless oil in 75 % yield (0.015 mol, 4.75 g). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.21 (s, 0.5 H, α/β), 6.15 (s, 0.5 H, α/β), 5.41-5.46 (m, H-2, α/β), 5.32-5.36 (m, H-2, α/β), 5.25-5.31 (m, 1H, *H*C=CH-CH_2), 5.19-5.21 (m, 1H, *H*C=CH-CH_2), 5.18 (s, H-3, α/β), 5.13 (dd, *J* = 1.2, 5.1 Hz, H-3, α/β), 4.29-4.32 (m, 1H, H-4), 4.02-4.07 (m, 2H, H_2C=CH-C*H_2*), 3.60-3.62 (m, 2H, H-5), 2.07, 2.08, 2.10, 2.11, 2.12, 2.13, 2.14 (m, 9H, COOC*H_3*). ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 169.3, 169.4, 169.6, 169.8, 170.0 (C=O), 134.3 (H_2C=*C*H-CH_2), 117.3 (H_2*C*=CH-CH_2), 99.4, 98.3 (C_1, α/β), 83.7, 81.0 (C_4, α/β), 80.9, 76.9 (C_3, α/β), 74.3, 69.6 (C_2, α/β), 72.6 (H_2C=CH-*C*H_2), 70.4, 69.0 (C_5, α/β), 20.5, 20.7, 20.8, 21.0, 22.5, 25.3 (COOC*H_3*). MS *m/z* (+ v/e, EI) [5%, M-H] 315, [100%, M-$C_2H_3O_2$], [64%, M-2x$C_2H_3O_2$-C_2H_3O] 155, [13%, M-3x$C_2H_3O_2$] 139. HRMS (LSIMS, positive) calcd for $C_{14}H_{20}O_8$ [M-H] 315.1079 found 315.1077.

α/β –5-*O*-Allyl-1, 2, 3- *O*, *O, O* - triacetyl D-ribofuranoside (1.17 g, 3.70 mmol) was dissolved in a mixture of methanol (25 mL) and CH_2Cl_2 (25 mL) and the solution was cooled at 0°C. Palladium chloride (0.23 g, 1.30 mmol) was added and the reaction mixture was warmed up to room temperature and vigorously stirred for 4h. The reaction was quenched with triethylamine (1.7 mL, 12 mmol) and evaporated under pressure. The residue was dissolved in EtOAc and filtrated through a celite path. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/acetone). Title compound **5** was obtained as a yellowish oil in 58 % (0.60 g, 2.16 mmol, mixture of α and β anomers) and data match ref. 21. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.06 (H-1, 0.5 H, α/β), 6.08 (H-1, 0.5 H, α/β), 5.31-5.33 (m, 0.5 H, H-2, α/β), 5.27 (d, J = 4.9 Hz, 0.5 H, H-2, α/β), 5.14 (d, *J* = 1.8 Hz, 0.5 H, H-3, α/β), 5.04 (dd, *J* = 1.8, 5.2 Hz, 0.5H, H-3, α/β), 4.16-4.18 (m, 1H, H-4), 3.71-3.78 (m, 2H, H-5), 2.00, 2.01, 2.03, 2.04, 2.05, 2.06 (m, 9H, COOC*H_3*). ^{13}C NMR ($CDCl_3$, 125.75 MHz) δ 169.3, 169.0, 168.9, 168.7, 168.6 (C=O), 98.2, 97.1 (C_1 α/β), 83.8, 81.4 (C_4 α/β), 80.0, 76.0 (C_3 α/β), 73.5, 68.9 (C_2 α/β), 60.8, 60.6 (C_5 α/β), 20.0, 19.8, 19.7, 19.6, 19.5, 19.4, 19.3 (COOCH_3). HRMS (LSIMS, positive) calcd for $C_{11}H_{16}O_8$ [M-OH]$^+$ 259.0817 found 259.0830.

2, 3- *O*, *O* -Triacetyl-5-*O*-[(allyl ethynephosphonyl]-D-ribofuranoside (**6**)

To a solution of **5** (0.60 g, 2.10 mmol) and **3** (0.58 g, 2.10 mmol) in acetonitrile (4.5 mL) was added drop wise 2,4-dinitrophenol (0.58 g, 3.15 mmol) dissolved in acetonitrile (3 mL). The reaction was stirred for 2 h, diluted with 160 mL of CH_2Cl_2 and cooled down to 0°C. The oxidation was carried out by addition of aq-H_2O_2 (30%, w/v, 2.5 mL, 12.40 mmol). The reaction was stirred for an additional 7 min at 0°C then quenched with 355 mL of a saturated $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the oily residue (0.75 g, 1.58 mmol) was dissolved in dry ethanol (7.5 mL) containing CsF (0.30 g, 1.58 mmol). The reaction mixture was stirred for 1 h. After addition of a saturated solution of $NaHCO_3$, the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The oily residue was purified by flash chromatography ($CHCl_3$/CH_3OH: 97.5/2.5, v/v) to obtain **6** as colourless oil in 50% yield (3 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.14 (s, 0.6 H, β-anomer, H-1'), 6.08 (s, 0.4 H, α -anomer, H-1'), 5.85-5.90 (m, 1H, H_2C=C*H*-CH_2), 5.34 (dd, *J*=17.2 Hz, 1.2 Hz, 1H, *H*C=CH-CH_2), 5.24-5.26 (m, 1H, H-2'), 5.22 (dd, *J* = 10.3 Hz, 0.8 Hz, 1H, *H*C=CH-CH_2), 5.13 (d, *J* = 1.3 Hz, H, H-3', α/β), 5.02 (d, *J* = 3.0 Hz, H, α/β), 4.56-4.59 (m, 2H, H_2C=CH-C*H_2*), 4.13-4.29 (m, 3H, H-4' & H-5'), 2.92 (d, J_{PH} = 13.6 Hz, 1H, *H*-acetylene), 1.99, 2.00, 2.01, 2.04, 2.05, 2.06 (9H, COOC*H_3*). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 169.6, 169.8, 169.9, 170.1, 170.4, 170.6 (C=O), 132.1 (d, $^4J_{PC}$ = 7.2 Hz, H_2C=*C*H-CH_2), 119.37 (d, $^5J_{PC}$= 4.4 Hz, H_2*C*=CH-CH_2), 99.74 (C-1'β anomer), 98.3 (C-1' α anomer), 89.3 (d, $^2J_{PC}$= 52.5 Hz, C-6 acetylene), 89.1 (d, $^2J_{PC}$ = 52.3 Hz, C-6 acetylene), 83.5 (dd , $^4J_{PC}$ = 8.1, 2.5 Hz , C-4', α/β, R or S), 80.8 (C-3', α/β), 80.0 (dd, $^3J_{PC}$ = 8.2, 5.0 Hz, C-4', α/β, R or S), 77.7 (C-3', α/β), , 74.6 (C-2', α/β), 73.9 (d, $^3J_{PC}$ = 297.5 Hz, C-7 acetylene), 70.5 (C-2', α/β), 68.2 (d, $^3J_{PC}$ = 5.1 Hz, H_2C=CH-*C*H_2), 65.8 (d, $^3J_{PC}$ = 3.3 Hz, C-5', α/β), 65.78 (d, $^3J_{PC}$ = 4.8 Hz, C-5', α/β), 20.8, 20.9, 21.0, 21.3, 21.4, 21.6 (COO*C*H_3). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ –7.28 (d, *J* = 4.5 Hz, α/β), -7.39 (d, *J* = 3.4 Hz, α/β). MS *m/z* (+ v/e, Electrospray) [M+Na]$^+$ 427.1. MS *m/z* (+ v/e, FAB), [100%, M-$C_2H_3O_2$] 345. HRMS (LSIMS, positive) calcd for $C_{13}H_{14}O_9P$ [M-$C_2H_3O_2$] 345.0375 found 345.0359.

P^1-[Allyloxy-diisopropylamino]-P^2-[(α/ β–1,2,3-*O*,*O*,*O*-triacetyl)-D-ribofuranosid-5-yl-allyl] -acetylene- (1-phosphine, 2-phosphonate) (**7**)

To a THF solution (5.7 mL) of **6** (0.22 g, 0.54 mmol) and diisopropylaminochlorophosphine (0.12 g, 0.54 mmol) cooled at –78°C was added *n*-butyllithium (2 M in hexane, 0.33 mL, 0.60 mmol). The solution was stirred for 50 min at –78°C and quenched with a mixture of CH_2Cl_2 and saturated $NaHCO_3$ solution (17 mL/12 mL). The aqueous layer was extracted three times with CH_2Cl_2 . The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in a mixture of diethylether-pentane (3/1) and applied on a short silica column (diethylether/pentane (3/1)

used as eluant) to obtain the title compound **7** as a colorless oil in 53 % yield. ^{1}H NMR ($CDCl_3$, 300 MHz) 6.13 (s, 1H, 2/3 H-1', β–anomer), 6.08 (s, 1H, 1/3 H-1', α–anomer), 5.84-5.87 (m, 2H, H_2C=C*H*-CH_2), 5.31-5.34 (m, 2H, *H*$_2$C=CH-CH_2), 5.23-5.28 (m, 1H, H-2'), 5.12-5.21 (m, 1H, *H*C=CH-CH_2), 5.08-5.1 (m, 1H, *H*C=CH-CH_2), 5.03 & 5.13 (s, 1H, H-3', α/β), 4.53-4.55 (m, 2H, H_2C=CH-C*H*$_2$), 4.31-4.32 (m, 1H, H-4'), 4.25-4.26 (m, 2H, H-5'), 4.13-4.15 (m, 2H, H_2C=CH-C*H*$_2$), 3.62 (m, 2H, C*H*(CH_3)$_2$), 2.00, 2.01, 2.02, 2.03, 2.04, 2.05, 2.06 (9H, COOC*H*$_3$), 1.15 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$), 1.13 (d, *J* = 6.8 Hz, 6H, CH(C*H*$_3$)$_2$). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 169.6, 169.8, 170.0, 170.2, 170.4 (C=O), 135.4 (d, $^4J_{PC}$= 7.2 Hz, H_2C=*C*H-CH_2), 132.7 (d, ^4J= 7.3 Hz, H_2C=*C*H-CH_2), 119.0 (d, ^5J= 8.6 Hz, H_2C=CH-CH_2), 117.6 (H_2C=CH-CH_2), 104.8 (d, $^2J_{P(III)-C}$= 39 Hz, P(V)-C*C*-P(III)), 99.9 (C-1', β-anomer), 98.5 (C-1', α-anomer), 91.3 (d, $^2J_{P(V)-C}$= 286.8 Hz P(V)-*C*C-P(III)), 83.5 (dd, $^4J_{PC}$ = 8.1, 4.0 Hz , C-4', α/β, R or S), 80.77 (C-3', α/β), 80.73 (C-3', α/β), 80.2 (d, $^4J_{PC}$ = 8.5 Hz, C-4', α/β), 74.5 (C-2', α/β)), 70.6 (C-2', α/β)), 68.4 (d, $^3J_{PC}$= 20.0 Hz, H_2C=CH-*C*H$_2$), 67.9 (d, $^3J_{PC}$= 9.1 Hz, H_2C=CH-*C*H$_2$), 65.7 (C-5', α/β), 65.57, 65.51, 65.45 (C-5', α/β) 44.1 (CH(C*H*$_3$)$_2$), 21.9 (*C*H(CH_3)$_2$), 19.4, 19.5, 19.7, 20.0, 20.1 (CH_3-acetate). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ +96.5 (d, *J* = 6.7 Hz, α/β), +96.3 (d, *J* = 8.4 Hz, α/β), -6.60, -6.66, -6.70, -6.73, -6.77, -6.83. MS *m/z* (+ v/e, FAB) [100%, M+H]$^+$ 592, [14%, M-C_2H_3O] 548, [52%, M-$C_2H_3O_2$] 532, [28%, M-$C_6H_{14}N$] 492, [41%, M-$C_{14}H_{24}P_2N$], 276. HRMS (LSIMS, positive) calcd for $C_{25}H_{39}NO_{11}P_2$ [M+H]$^+$ 592.2076 found 592.2102.

P^1-[Allyl (2',3'-*O*-isopropylidene)-adenosin-5'-yl]-P^2-[allyl (α/β –1",2",3"-*O,O,O*-triacetyl)-D-ribofuranosid-5"-yl]-acetylene-bis(phosphonate) (**8**).

The aminophosphine **7** (0.34 g, 0.57 mmol) was dissolved with 2',3'-*O,O*-isopropylidene adenosine (0.17 g, 0.57 mmol) in acetonitrile (23 mL) and stirred for 10 min at rt. To this mixture, 2,4-DNP (0.157 g, 0.855 mmol) dissolved in 0.95 mL of acetonitrile was added dropwise. After 5h, an additional equivalent of 2,4-DNP and of 2',3'-*O,O*-isopropylidene adenosine were added. The reaction was stirred overnight and then cooled to -40°C. *t*-BuOOH (solution 5-6 M in decane, 0.17 mL, 0.85 mmol) was added and the reaction was stirred for 7 min. A mixture of CH_2Cl_2 and a saturated $NaHCO_3$ solution (50:30 mL, v/v) was added and the aqueous layer was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The oily residue was chromatographed on silica gel (pentane/diethylether; 1/3; v/v) to obtain the bisphosphonate **8** as a colorless oil (0.145 g) in 31% yield (over two steps). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.34 (s, 1 H, H-2 ade), 7.99-8.03 (m, 1H, H-8 ade), 6.38 (bs, 2H, N*H*$_2$ ade), 6.23 (s, 0.6 H, H-1", β-anomer), 6.16 (s, 0.4 H, H-1", α-anomer) 6.15 (s, 1H, H-1'), 5.80-6.0 (m, 2H, H_2C=C*H*-CH_2O), 5.42-5.44 (m., 1H, H-2'), 5.33-5.42 (m, 1H, H), 5.21-5.33 (m, 5H, *H*$_2$C=CH-CH_2 ; H-2"), 5.20 (bs, H-3", α/β), 5.15-5.17 (m, 1H, H-3'), 5.13 (bs, H-3", α/β), 4.63-4.65 (m, 2H, H_2C=CH-C*H*$_2$), 4.55-4.57 (m, 2H, H_2C=CH-C*H*$_2$), 4.50-4.54 (m, 1H, H-4'), 4.39-4.45 (m, 2H, H-5'), 4.28-4.38 (m, 3H, H-4"& H-5"), 2.09, 2.10, 2.105, 2.11, 2.115, 2.121, 2.126, 2.127, 2.128, 2.132, 2.138 (COOC*H*$_3$), 1.62 (s, 3H, CH_3-isopropylidene), 1.41 (s, 3H, CH_3 isopropylidene).^{13}C NMR ($CDCl_3$, 75.5 MHz) 169.5, 169.8, 170.0, 170.5, 170.8 (C=O), 155.6 (C-6 ade), 153.0 (C-2 ade), 149.0 (C-4 ade), 139.7 (C-8 ade), 131.2 (d, *J*= 6.2 Hz, H_2C=*C*H-CH_2), 119.8 (C-5 ade), 119.5 (H_2C=CH-CH_2), 114.5 (*C*-isopropylidene), 99.2 (C-1", β anomer), 97.8 (C-1", α anomer) , 90.7 (d, *J* = 8.1 Hz, C-1'), 89.35, 89.6, 89.75, 89.9, 90.1, (P^1-CC-P^2), 85.9, 86.2, 86.5 (P^1-CC-P^2), 85.0 (d, *J*= 5.4 Hz, C-4', α/β), 84.0 (C-2'), 82.9 (d, *J* = 5.5 Hz, C-4"), 81.2 (d, *J* =3.5 Hz, C-3'), 80.2 (d, *J* =3.0 Hz, C-4", α/β), 78.6 (C-3", α/β), 77.2(C-3", α/β), 74.0 (d, *J* =7.1 Hz, C-2", α/β), 69.8 (d, *J* =7.7 Hz, C-2", α/β), 68.5 (H_2C=CH-*C*H$_2$), 66.9 (bs, C5'), 66.0, 66.1, 66.4, 66.8 (C-5"), 27.5 (*C*H$_3$-isopropylidene), 25.19 (*C*H$_3$-isopropylidene), 20.3, 20.4, 20.5, 20.9, (COO*C*H$_3$). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ from -9.41 to -9.86, multiplet (9 peaks: -9.41, -9.48, -9.54, -9.57, -9.63, -9.64, -9.67, -9.74, -9.77, -9.83, -9.86). MS *m/z* (+ v/e, FAB) [100%, M+H]$^+$ 814, [10%, M- $C_2H_3O_2$]$^+$ 754, [48%, M-$C_{19}H_{25}P_2O_{13}$]$^+$ 290. HRMS (LSIMS, positive) calcd for $C_{32}H_{42}O_{16}N_5P_2$ [M+H]$^+$ 814.2098 found 814.2085.

Allyl (2', 3'-*O*, *O* –isopropylidene)- D-adenosin-5'-yl-methyl phosphate (**10**)

Compound **8** (0.010 g, 0.0123 mmol) was dissolved in 2.5 mL methanol and added to a cooled (ice bath) methanol solution saturated with ammonia. Gaseous ammonia was bubbled through this solution during 5 min and the mixture was stirred for 1 h at RT. The solvent was removed under vacuum and the residue was dissolved in a minimum of chloroform and applied on a preparative TLC using as eluant a mixture of chloroform and methanol (10:1). Compound **10** was obtained in 55 % yield (0.003 g, 0.0068 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.36 (s, 1H, H-2 ade), 8.02 (s, 1H, H-8 ade), 6.14 (d, *J*= 2.3 Hz, H1-ade), 5.79-5.92 (m, 1H, CH_2=C*H*CH_2O), 5.70 (bs, 2H, NH_2-ade), 5.40-5.43 (m, 1H, H-2' ade), 5.23-5.34 (m, 2H, C*H*$_2$=$CHCH_2O$), 5.10-5.12 (m, 1H, H-3' ade), 4.44-4.51 (m, 3H, CH_2=CHC*H*$_2$O, H-4' ade), 4.20-4.25 (m, 2H, H-5' ade), 1.62 (s, 3H, CH_3-acetonide). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ 0.62, 0.65 ppm. MS *m/z* (+ ve Electrospray) [100% M+H]$^+$ 442 and [15% M+Na]$^+$ 464 ($C_{17}H_{24}O_7N_5P$ requires *M*, 441). MS *m/z* (+ v/e, FAB) [38%, M+H]$^+$ 442, [100%, M-$C_5H_4N_5$] 307, [23%, M+H-$C_5H_4N_5$]$^+$ 308. HRMS (LSIMS, positive) calcd for $C_{17}H_{25}O_7N_5P$ [M+H]$^+$ 442.1491 found 442.1507.

P^1-[Allyl-adenosin-5'-yl]-P^2-[allyl (α/β –1",2",3"-*O,O,O*-triacetyl)-D-ribofuranosid-5"-yl]-acetylene-bis(phosphonate) (**9**).

Compound **8** (0.0195 g, 0.024 mmol) was dissolved in a mixture of water (0.40 mL) and TFA (0.76 mL). The solution was stirred for 40 min at RT and evaporated under

high vacuum. Residual TFA was removed by coevaporating three times with toluene. The residue was dissolved in minimum of chloroform and applied on a preparative TLC using as eluent a mixture of chloroform/methanol (10:1) to obtain the title compound as colourless oil in 65% yield. Unreacted starting material (4.5 mg, 23%) was recovered. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (s, 1 H, H-2 ade), 8.00 (m, 1H, H-8 ade), 6.07 (s, 0.6 H, H-1", β-anomer), 6.06 (s, 0.4 H, H-1", α-anomer), 5.97 (d, *J*= 4.7 Hz, 1 H, H-1'), 5.72-5.92 (m, 2H, H_2C=C*H*-CH_2O), 5.35-5.36 (m., 1H, H-2'), 5.21-5.33 (m, 6H, H_2C=CH-CH_2 ; m, 1H, H-2" ; m, 1 H, H-3'), 5.19 (bs, 1 H, H-3", 0.6 β), 5.18 (bs, 1 H, H-3", 0.4 α), 4.53-4.61 (m, 4H, H_2C=CH-C*H*$_2$), 4.50-4.52 (m, 2H, H-5'), 4.27-4.42 (m, 4H, H-4', H-4"& H-5"), 3.72 (dd, *J*= 4.35, 11.25 Hz, 3H, POC*H*$_3$), 1.99, 2.00, 2.01, 2.03, 2.04, 2.047,_2.05, 2.06, 2.07 (COOC*H*$_3$). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ - 8.50, - 8.52 ppm. MS *m/z* (+ ve Electrospray) $[M+H]^+$ 774 and $[M+Na]^+$ 796 ($C_{29}H_{37}N_5O_{16}P_2$ requires *M*, 773)

P^1-[(Adenosin-5'-yl]-P^2-[α/β -D-ribofuranosid-5"-yl]-acetylene-bis(phosphonate) (**2**).

Compound **9** (0.012 g, 0.0157 mmol), PPh_3 (0.0012 g, 0.0046 mmol), formic acid (0.0017 mL, 0.045 mmol) and triethylamine (0.007 mL, 0.043 mmol) were dissolved in 0.2 mL THF. To this solution, Pd[PPh_3] (0.003 g, 0.0026 mmol) was added and the resulting solution was stirred for 8 h at RT and the solution was evaporated under reduced pressure. The residue was dissolved in 0.5 mL water and three times extracted with 0.5 mL diethylether. The aqueous layer was evaporated under high vacuum and the residue was dried by coevaporating three times with toluene then dissolved in 0.5 mL of a 0.1 M Sodium methoxide in methanol solution (0.05 mmol) and vigorously stirred at RT for 25 min. The solution was quenched with a formate solution (50 mM formic acid, pH=3 with ammonia solution) and the resulting solution evaporated under high vacuum. The residue was purified by reversed phase column chromatography (method 2) and the fractions containing the title compound were evaporated under high vacuum. The excess of ammonium salts were removed by coevaporating several times with methanol. **2** (0.006 g, 0.0106 mole) was obtained in 67 % yield. ^{1}H NMR (D_2O, 300 Mhz) δ 8.31 (s, 1H, H-2 ade), 8.16 (s, 1H, H-8 ade), 5.83 (d, *J* =11.4 Hz, 1H, H-1'), 5.00 (d, *J* = 4.0 Hz, 0.3 H, H-1" β), 4.9 (d, *J* = 3.5 Hz, H-3'), 4.1 (bs, 1H, H-4'), 3.89 (bs, 2H, H-5'), 3.24-3.68 (m, 6H, H-2", H-3", H-4", H-5"). ^{31}P NMR (D_2O, 121.5 MHz ^{1}H-decoupled) δ -8.86. MS *m/z* (-ve Electrospray) [100% M-H]$^-$ 566, [49% M-2H+Na]$^-$ 588.2 ($C_{17}H_{23}N_5O_{16}P_2$ requires *M*, 567). MS *m/z* (+ve Electrospray) [95% M+H]$^+$ 568, [100% M+Na]$^+$, 590. HRMS (LSIMS, positive) calcd for $C_{17}H_{22}O_{13}N_5P_2$ [M+H]$^-$ 566.0689 found 566.0681.

Supplement: Acetylene β–(2', 3' *O*, *O* –isopropylidene)- 5'-*O*-(allyl ethynephosphonyl) D-adenosine

To a solution of **3** (0.12 g, 0.42 mmol) and 2', 3'-*O*, *O* –isopropylidene adenosine (0.13 g, 0.42 mmol) in acetonitrile (17 mL) was added 2,4-DNP as a solid (0.12 g, 0.63 mmol). The reaction was stirred for 2 h after which time further quantities of **3** (0.06g, 0.21 mmol) and 2,4-DNP (0.04 g, 0.21 mmol) were added. The reaction was stirred for a further 3h followed by further addition of the same amounts of **3** and 2,4-DNP. The reaction was almost completed after 10 h and the condensed intermediate was oxidized *in situ* by adding *t*BuOOH (solution 5-6 M in hexane, 0.084 mL). After 7 min, the reaction was quenched with a mixture of CH_2Cl_2 and a $NaHCO_3$ saturated solution. The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The oily residue was chromatographed (silica, $CHCl_3$/MeOH (92/8)) to obtain a colorless oil (0.14 g, 0.28 mmol) which was directly dissolved in ethanol (1.3 mL). To the solution, CsF was added (0.06 g, 0.39 mmol) and the reaction was stirred for 1 h at rt. A mixture DCM and saturated solution of $NaHCO_3$ was then added and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The oily residue was chromatographed on silica to obtain the title compound as colourless oil in 57 % yield (0.103 g, 0.24 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (d, *J* = 2.6 Hz, 1H, H-2 ade), 7.97 (d, *J* = 2.6 Hz, 1H, H-8 ade), 6.21 (bs, 2H, N*H*$_2$-ad), 6.16 (d, *J*= 2.0 Hz., 1H, H-1'), 5.82-5.96 (m, 1H, CH_2=C*H*CH_2), 5.42-5.46 (m, 1H, H-2'), 5.32-5.37 (m, 1H, C*H*=$CHCH_2$), 5.23-5.26 (m, 1H, C*H*=$CHCH_2$), 5.12-5.14 (m, 1H, H-3'), 4.53-4.57 (m, 2H, CH_2=CHC*H*$_2$), 4.50-4.52 (m, 1H, H-4'), 4.31-4.37 (m, 1H, H-5'), 4.28-4.29 (m, 1H, H-5') 2.93 (dd, *J*= 13.5 Hz, 18.6 Hz, 1H, H-acetylene), 1.63 (s, 3H, C*H*$_3$-isopropylidene), 1.39 (s, 3H, C*H*$_3$-isopropylidene). ^{13}C NMR ($CDCl_3$, 125.75 MHz) δ 155.6 (C-6 ade),153.1 (C-2 ade), 149.2 (C-4 ade), 139.6 (d, *J*= 3.4 Hz, C-8 ade), 131.6 (d, *J*= 6.9 Hz, H_2C=*C*H-CH_2O), 120.0 (C-5 ade), 119.0 (H_2*C*=CH-CH_2O), 114.5 (*C*-isopropylidene), 90.9 (d, *J*= 9.0 Hz, C-1 ade), 89.2 (d, *J*= 52.1 Hz, C-6 acetylene), 85.2 (d, *J* = 8.6 Hz, C-4'), 85.1 (d, *J*= 8.7 Hz, C-4'), 84.2 (C-2'), 81.4 (d, *J* = 4.9 Hz, C-3'), 74.4 (d, $^3J_{PC}$ = 9.7 Hz, C-7 acetylene), 67.92 (d, *J*= 4.8 Hz, H_2C=CH-*C*H_2), 67.88 (d, *J*= 4.7 Hz, H_2C=CH-*C*H_2), 66.2 (bs, C-5), 26.1 (*C*H_3-isopropylidene), 24.3 (*C*H_3-isopropylidene). ^{31}P NMR ($CDCl_3$, 121.5 MHz ^{1}H-decoupled) δ -7.16, -7.33, -7.36, HRMS (LSIMS, positive) calcd for $C_{18}H_{22}O_6N_5P$ $[M+H]^+$ 436.1385 found 436.1399.