Supporting Information for

Fingerprinting Differential Active Site Constraints of ATPases

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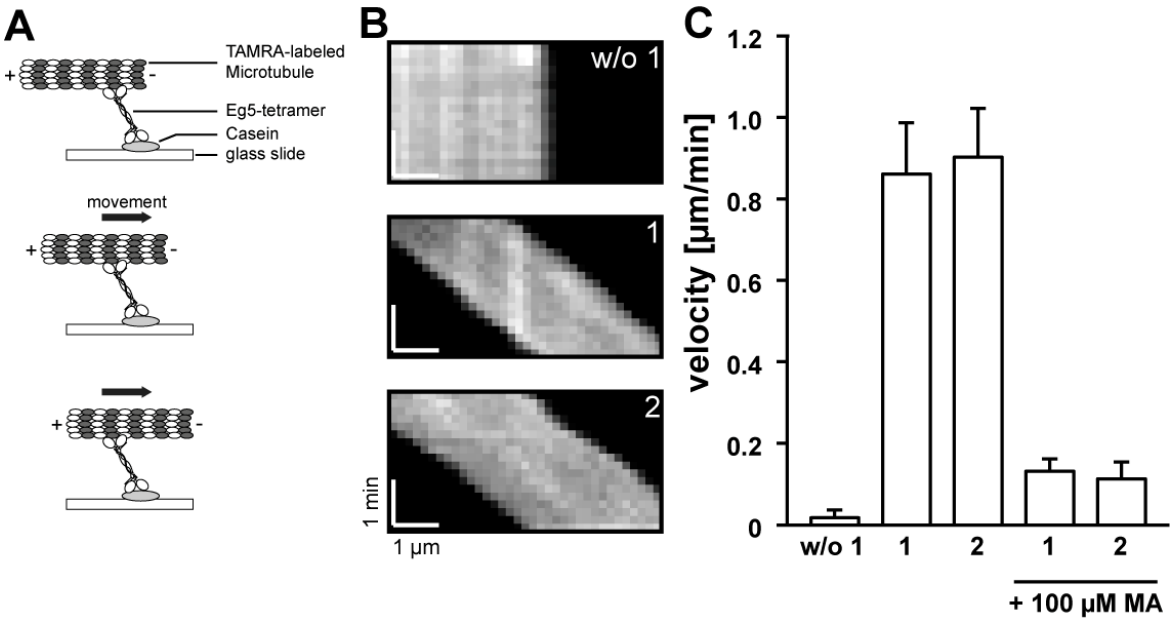
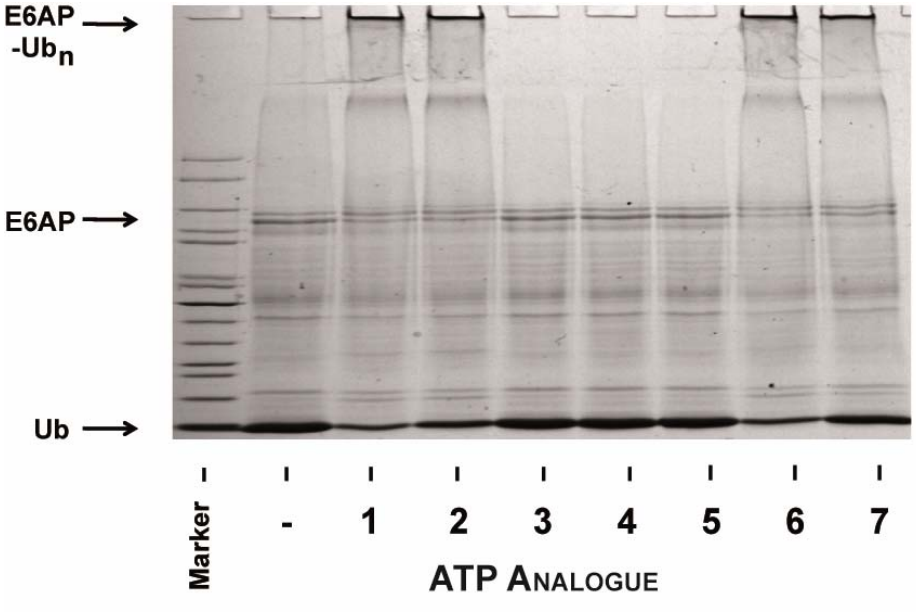
Supplementary Figure S1



Supplementary Fig. S1: Eg5 can utilize ATP or O2'-modified ATP analogue **2** to mediate microtubule gliding. (A) Schematic drawing of the experimental setup. Immobilized, tetrameric Eg5 moves towards the plus-ends of microtubules resulting in the gliding of microtubules with their minus-ends leading. (B) Representative kymographs showing Eg5-dependent micrutubule displacement in the presence of ATP (**1**) and O2'-modified ATP analogue (**2**), but not without ATP (**w/o 1**). Y-axes and x-axes showing time and distance, respectively. (C) Quantification of Eg5-driven microtubule movement in the presence of different nucleotides and/or Monastrol (MA). Monastrol significantly reduces Eg5-driven microtubule motility for both ATP and *O*2'-modified analogue **2**. Per condition, the movement of 100- (**1**; **2**), 50- (**w/o 1**), or 25 (**1** and **2** plus 100 µM Monastrol) microtubules from two independent experiments were analyzed. Bars represent the average velocity over three minutes plus standard deviation.

Supplementary Figure S2



Supplementary Fig. S2: Utilization of ATP (**1**) and the six novel ATP analogues (**2**-**7**) by UBA1. All ATP analogues were incubated in the presence of 150 nM UBA1, UbcH5b (E2), E6AP and ubiquitin for 1 h at 37°C. *O*2'-modified (**2**), *N*6-modified (**6**) and *C*8-modified (**8**) ATP analogues can clearly act as substrate for UBA1.

General experimental details

All temperatures quoted are uncorrected. All reagents are commercially available and used without further purification. All solvents are dried over molecular sieves and used directly without further purification. All reactions were conducted under exclusion of air and moisture. Purification of triphosphates was performed on a BioLogic DuoFlow System (Bio-Rad Laboratories) with DEAE Sephadex™ A-25 (GEHealthcare Bio-SciencesAB) column using a linear gradient of triethylammonium bicarbonate buffer (TEAB, pH 7.5) (0.1–1.0 M, flow 2 mL/min, pH= 7.5). For medium pressure liquid chromatography (MPLC), a Büchi unit with a Büchi controller C-620, two pumps C-605, a UV monitor C-630 (λ = 254 nm) and fraction collector C-660 was used. For the purification of nucleosides and nucleotides, a 310-25 LiChroprep® RP-18 ready-to-use column (Merck, 40–63 mm) with a linear gradient (5 to 100%) of acetonitrile in 50 mM aqueous triethylammonium acetate (TEAA buffer, pH 7.0) was used. Reversed phase high pressure liquid chromatography (RP-HPLC) was performed using a Shimadzu unit. For the purification of nucleotides EC 250/4 NUCLEODUR 100-5 C18 ec (Macherey-Nagel), VP 250/10 NUCLEODUR 100-5 C18 ec (Macherey-Nagel) or VP 250/21 NUCLEODUR C18 HTec, 5µm (Macherey-Nagel) column and a linear gradient (5 to 100%) of acetonitrile in 50 mM TEAA buffer (pH 7.0) were used. Analytical HPLC was performed using a EC 250/4 NUCLEODUR 100-5 C18 ec (Macherey-Nagel) column and a linear gradient (5 % to 35 % within 40 min) of acetonitrile in 50 mM TEAA buffer (pH 7.0). NMR spectra: Bruker Avance III 400 MHz spectrometer and Bruker AVIII 600 MHz spectrometer. ^{1}H and ^{13}C chemical shifts are reported relative to the residual solvent peak and are given in ppm (δ). A BBFOplus probe with actively shielded z-gradient was used with its inner (BB-) coil tuned to ^{19}F and ^{31}P, respectively. Flash chromatography: Merck silica gel G60. TLC: Merck precoated plates (silica gel 60 F_{254}). ESI-IT: Bruker Esquire 3000 plus. HRMS: Bruker Daltronics micrOTOF-Q II ESI-Qq-TOF. The reported yield refers to the analytically pure substance and is not optimized. Compound **3a[1], 6-Trifluoroacetamidohexane-1-iodide**[2], **4a**[3], **4a'[2], 2-Iodoadenosine**[4], **6a[5], 6b[6], 6c[7], 7a[8]** were synthesized according to corresponding literature.

Synthesis of Compounds

2'-O-(6-Azidohexyl)-adenosine 2a

Adenosine (0.86 g, 3.22 mmol, 1 eq.) was dissolved in 40 mL hot DMF and cooled to room temperature. NaH (103 mg, 4.30 mmol, 1.3 eq.) was added and the mixture was stirred for 2 hours. 6-Azido-1-bromohexane (0.96 g, 4.68 mmol, 1.5 eq.) was dissolved in 10 mL DMF and added to the reaction mixture. The solution was stirred at room temperature over night and quenched by the addition of 10 mL methanol. The mixture was stirred at room temperature for 30 minutes and solvents were evaporated under reduced pressure. Column chromatography (5% methanol in dichloromethane) gave 457 mg (1.17 mmol, 36%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.38 (s, 1H, H8), 8.14 (s, 1H, H2), 7.34 (bs, 2H, NH_2), 5.98 (d, J=6.5 Hz, 1H, H1'), 5.43 (dd, J=6.7 Hz, J=4.7 Hz, 1H, OH5'), 5.16 (d, J=3.5 Hz, 1H, OH3'), 4.47 (dd, J=6.2 Hz, J=4.9 Hz, 1H, H2'), 4.34 – 4.25 (m, 1H, H3'), 3.99 (pq, J=3.2 Hz, 1H, H4'), 3.73 – 3.63 (m, 1H, H5'a), 3.55 (m, 2H, H5'b, $O^{2'}$-CH_2a), 3.34 – 3.29 (m, 1H, $O^{2'}$-CH_2b), 3.21 (t, J=6.9 Hz, 2H, N_3-CH_2), 1.45 – 1.31 (m, 4H, 2x CH_2-linker), 1.25 – 1.06 (m, 4H, 2x CH_2-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 156.2, 152.5, 149.0, 139.8, 119.3, 86.5, 86.1, 80.7, 69.5, 69.0, 61.5, 50.5, 28.8, 28.1, 25.8, 24.8.

HR-ESI-MS: found: 393.1977; calculated: 393.1993 ($M+H^+$, $C_{16}H_{25}N_8O_4^+$); deviation: 4.1 ppm.

2'-*O*-(6-Trifluoroacetamidohexyl)-adenosine 2b[2]

2'-*O*-(6-Azidohexyl)-adenosine **2a** (120 mg, 306 µmol, 1 eq.) and triphenylphosphine (160 mg, 612 µmol, 2 eq.) were dissolved in 8 mL THF and 2 mL water. The solution was stirred at room temperature for 12 hours. The solvents were evaporated and the residue was coevaporated with methanol three times. The product was dissolved in 10 mL methanol and triethylamine (50 µl 36.5 mg, 361 µmol, 1.2 eq.) and ethyl trifluoroacetate (50 µl, 59.5 mg, 420 µmol, 1.4 eq.) were added. The solution was stirred at room temperature for 12 hours and the solvents were evaporated. Column chromatography gave 132 mg (286 µmol, 93%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.33 (s, 1H, NHTFA), 8.38 (s, 1H, H8), 8.14 (s, 1H, H2), 7.34 (bs, 2H, NH_2), 5.98 (d, J=6.4 Hz, 1H, H1'), 5.42 (dd, J=7.1 Hz, J=4.6 Hz, 1H, OH5'), 5.15 (d, J=5.1 Hz, 1H, OH3'), 4.49 (dd, J=5.9 Hz, J=4.9 Hz, 1H, H2'), 4.34 – 4.26 (m, 1H, H3'), 3.99 (pq, J=3.2 Hz, 1H, H4'), 3.73 – 3.65 (m, 1H, H5'a), 3.62 – 3.49 (m, 2H, H5'b, $O^{2'}$-CH_2a), 3.40 – 3.30 (m, 1H, $O^{2'}$-CH_2b), 3.10 (pq, J=6.7 Hz, 2H, NHTFA-C**H**$_2$), 1.44 – 1.31 (m, 4H, 2x CH_2-linker), 1.21 – 1.05 (m, 4H, 2x CH_2-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 156.2, 156.1 (q, J=36 Hz), 152.5, 149.0, 139.8, 119.3, 115.9 (q, J=286 Hz), 86.5, 86.1, 80.8, 69.6, 69.0, 61.5, 39.1, 28.9, 28.1, 25.9, 24.9.

^{19}F NMR (d_6-DMSO, 376 MHz): δ -74.4 (s, 3F).

HR-ESI-MS: found: 463.1894; calculated: 463.1911 ($M+H^+$, $C_{18}H_{26}F_3N_6O_5^+$); deviation: 3.7 ppm.

2'-*O*-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 2

Compound **2b** (90 mg, 194 µmol, 1 eq.) and proton sponge (65 mg, 303 µmol, 1.6 eq.) were coevaporated with acetonitrile twice and dried at high vacuum. The solids were dissolved in 5 mL trimethylphosphate and cooled to 0°C. Phosphorous oxychloride (25.2 µl, 43 mg, 279 µmol, 1.4 eq.) was added dropwise. The reaction was stirred at 0°C for 1 hour. Tributylamine (502 µl, 390 mg, 2.11 mmol, 11 eq.) and bis-(tributylammonium)-pyrophosphate (2 mL, 0.5 M in DMF, 1 mmol, 5 eq.) were added simultaneously, the solution was warmed to room temperature and stirred for 30 minutes. 5 mL 0.1 M TEAB buffer (pH 7.5) were added and the reaction stirred for 30 minutes. The mixture was extracted with ethyl acetate three times and the solvents were evaporated under reduced pressure. The compound was purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-HPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 110 µmol (57%) of colourless oil.

^{1}H NMR (D_2O, 400 MHz): δ 8.55 (s, 1H, H8), 8.23 (s, 1H, H2), 6.13 (d, J=7.0 Hz, 1H, H1'), 4.56 (dd, J=5.0 Hz, J=2.2 Hz, 1H, H3'), 4.44 (dd, J=6.8 Hz, J=5.2 Hz, 1H, H2'), 4.31 – 4.24 (m, 1H, H4'), 4.22 – 4.14 (m, 1H, H5'a), 4.14 – 4.07 (m, 1H, H5'b), 3.75 – 3.64 (m, 1H, $O^{2'}$-CH_2a), 3.54 – 3.43 (m, 1H, $O^{2'}$-CH_2b), 2.96 (t, J=7.1 Hz, 2H, NHTFA-C**H**$_2$), 1.35 – 1.06 (m, 4H, 2x CH_2-linker), 1.01 – 0.74 (m, 4H, 2x CH_2-linker).

^{19}F NMR (D_2O, 376 MHz): δ -75.9 (s, 3F).

^{31}P NMR (D_2O, 162 MHz): δ -10.8 (d, J=20.4 Hz, 1P), -11.5 (d, J=19.0 Hz, 1P), -23.3 (t, J=19.8 Hz, 1P).

HR-ESI-MS: found: 701.0729; calculated: 701.0745 ($M\text{-}H^+$, $C_{18}H_{27}F_3N_6O_{14}P_3^-$); deviation: 2.3 ppm.

2',5',*N*-Tritrityl-adenosine 3a[1]

Adenosine (4.53 g, 17.0 mmol, 1 eq.) and trityl chloride (18.9 g, 68.0 mmol, 4 eq.) were dissolved in 150 mL pyridine and stirred at 100°C for 4 hours. The solution was poured into ice-water and the precipitate was collected by filtration. The solid was redissolved in chloroform and extracted with 1 M HCl and saturated $NaHCO_3$ and dried over $MgSO_4$. The solvent was evaporated and the product was isolated by column chromatography (0-3% ethyl acetate in dichloromethane) to give 3.63 g (7.7 mmol, 45%) of a yellowish solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.10 (s, 1H, H8), 7.60 (s, 1H, H2), 7.44-6.98 (m, 46H, H-Trt, NH), 5.94 (d, J=6.1 Hz, 1H, H1'), 5.09 – 4.96 (m, 2H, H2', OH3'), 4.03 – 3.93 (m, H4'), 3.27 – 3.19 (m, 1H, H-3'), 3.13 – 3.14 (m, 1H, H5'a), 2.99 – 2.88 (m, 1H, H5'b).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 153.4, 150.8, 148.2, 145.0, 143.8, 143.2, 140.8, 128.6, 128.3, 128.2, 127.7, 127.5, 127.0, 126.6, 121.1, 86.8, 86.1, 84.3, 75.2, 70.3, 69.7, 63.4.

HR-ESI-MS: found: 994.4316; calculated: 994.4327 ($M+H^+$, $C_{67}H_{56}N_5O_4^+$); deviation: 1.1 ppm.

3'-*O*-(6-Azidohexyl)-2',5',*N*-tritrityl-adenosine 3b

Compound **3a** (3.5 g, 3.5 mmol, 1 eq.) was dissolved in 150 mL acetonitrile and NaH (1.63 g, 60% in mineral oil, 41 mmol, 12 eq.) was added. The mixture was stirred at room temperature for 10 minutes and NaI (175 mg, 1.2 mmol, 0.3 eq.) and 6-azido-1-bromohexane (2.15 g, 10.5 mmol, 3 eq.) were added. The mixture was stirred for 48 hours and quenched with 30 mL ethanol for 30 minutes at room temperature. The solvents were evaporated. The residue was redissolved in ethyl acetate and extracted with 1 M phosphate buffer (pH 7.0, 1 M) three times. The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. Column chromatography (4:1 to 3:1 hexane in ethyl acetate) gave 1.96 g (1.75 mmol, 50%) of a yellowish solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.37 (s, 1H, H8), 7.49 (s, 1H, H2), 7.44 (s, 1H, NH), 7.40 – 6.99 (m, 45H, H-Trt), 6.14 (d, J=7.5 Hz, 1H, H1'), 5.18 (dd, J=7.4 Hz, J=5.0 Hz, 1H, H2'), 3.96 (t, J=5.3 Hz, H4'), 3.27 (t, J=6.9 Hz, 2H, N_3-CH_2), 3.25 – 3.12 (m, 2H, H-5'a, $O^{3'}$-CH_2a), 2.96 – 2.86 (m, 1H, H5'b), 2.79 – 2.70 (m, 1H, $O^{3'}$-CH_2b), 2.50 – 2.45 (m, 1H, H3'), 1.54 – 1.41 (m, 4H, 2x CH_2-linker), 1.41 – 1.26 (m, 4H, 2x CH_2-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 153.4, 150.6, 148.4, 144.9, 143.4, 141.1, 128.6, 128.2, 128.1, 127.7, 127.6, 127.4, 127.1, 127.0, 126.6, 121.4, 86.6, 86.4, 86.2, 81.2, 77.4, 73.5, 70.3, 68.4, 63.2, 50.6, 29.2, 28.2, 26.0, 25.3.

HR-ESI-MS: found: 1119.5274; calculated: 1119.5280 ($M+H^+$, $C_{73}H_{67}N_8O_4^+$); deviation: 0.5 ppm.

3'-*O*-(6-Azidohexyl)-adenosine 3c

Compound **3b** (1.96 g, 1.75 mmol, 1 eq.) was dissolved in 80 mL 80% acetic acid and stirred at 100°C for 90 minutes. The solvent was evaporated and the residue was purified by column chromatography (dichloromethane - 10% methanol in dichloromethane) to give 400 mg (1.02 mmol, 58%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.35 (s, 1H, H8), 8.14 (s, 1H, H2), 7.34 (bs, 2H, NH_2), 5.88 (d, J=6.2 Hz, 1H, H1'), 5.45 (pt, J=5.8 Hz, 1H, OH5'), 5.40 (d, J=6.1 Hz, 1H, OH2'), 4.74 (q, J=5.6 Hz, 1H, H2'), 4.04 (q, 3.5 Hz, 1H, H4'), 3.97 – 3.92 (m, 1H, H-3'), 3.72 – 3.60 (m, 1H, H5'a, $O^{3'}$-CH_2a), 3.60 – 3.45 (m, 2H, H5'b, $O^{3'}$-CH_2b), 3.32 (t, J=6.9 Hz, 2H, N_3-CH_2), 1.62 – 1.47 (m, 4H, 2x CH_2-linker), 1.42 – 1.28 (m, 4H, 2x CH_2-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 156.2, 152.4, 149.0, 139.8, 119.4, 88.0, 83.8, 78.4, 72.8, 69.7, 61.6, 50.6, 29.3, 28.2, 26.0, 25.1.

HR-ESI-MS: found: 393.1990; calculated: 393.1993 ($M+H^+$, $C_{16}H_{25}N_8O_4^+$); deviation: 0.8 ppm.

3'-*O*-(6-Trifluoroacetamidohexyl)-adenosine 3d

3'-*O*-(6-Azidohexyl)-adenosine **3c** (360 mg, 918 µmol, 1 eq.) and triphenylphosphine (480 mg, 1.8 mmol, 2 eq.) were dissolved in 16 mL THF and 4 mL water. The solution was stirred at room temperature for 12 hours. The solvents were evaporated and the residue was coevaporated with methanol three times. The product was dissolved in 10 mL methanol and triethylamine (150 µl, 110 mg, 1.08 mmol, 1.2 eq.) and ethyl trifluoroacetate (150 µl, 179 mg, 1.26 mmol, 1.4 eq.) were added. The solution was stirred at room temperature for 12 hours and the solvents were evaporated. Column chromatography gave 240 mg (520 µmol, 57%) of a white solid.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.30 (s, 1H, H8), 8.17 (s, 1H, H2), 5.97 (d, J=6.3 Hz, 1H, H1'), 4.84 – 4.79 (m, 1H, H2'), 4.23 (q, 2.7 Hz, 1H, H4'), 4.09 (dd,J=5.2, J=2.9, 1H, H-3'), 3.90 (dd, J=12.6 Hz, J=2.7 Hz, 1H, H5'a), 3.73 (dd, J=12.6 Hz, J=2.7 Hz, 2H, H5'b), 3.70 – 3.64 (m, 2H, $O^{3'}$-CH_2a), 3.64 – 3.56 (m, 2H, $O^{3'}$-CH_2b), 3.28 (t, J=6.9 Hz, 2H, NHTFA-C**H**$_2$), 1.70 – 1.51 (m, 4H, 2x CH_2-linker), 1.51 – 1.31 (m, 4H, 2x CH_2-linker).

^{13}C NMR (MeOD-d_4, 101 MHz): δ 158.9 (q, J=37 Hz), 157.5, 153.5, 150.0, 141.8, 120.9, 91.3, 85.9, 80.1, 75.0, 71.7, 63.4, 40.6, 30.7, 29.8, 27.6, 26.7.

^{19}F NMR (MeOD-d_4, 376 MHz): δ -77.3 (s, 3F).

HR-ESI-MS: found: 463.1903; calculated: 463.1911 ($M+H^+$, $C_{18}H_{26}F_3N_6O_5^+$); deviation: 1.7 ppm.

3'-*O*-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 3

Compound **2b** (180 mg, 388 µmol, 1 eq.) and proton sponge (130 mg, 606 µmol, 1.6 eq.) were coevaporated with acetonitrile twice and dried at high vacuum. The solids were dissolved in 10 mL

trimethylphosphate and cooled to 0°C. Phosphorous oxychloride (50.4 µl, 86 mg, 558 µmol, 1.4 eq.) was added dropwise. The reaction was stirred at 0°C for 1 hour. Tributylamine (1.04 mL, 780 mg, 4.22 mmol, 11 eq.) and bis-(tributylammonium)-pyrophosphate (4 mL, 0.5 M in DMF, 2 mmol, 5 eq.) were added simultaneously, the solution was warmed to room temperaure and stirred for 30 minutes. 10 mL 0.1 M TEAB buffer (pH 7.5) were added and the reaction stirred for 30 minutes. The mixture was extracted with ethyl acetate three times and the solvents were evaporated under reduced pressure. The compound was purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-HPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 191 µmol (49%) of colourless oil.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.60 (s, 1H, H8), 8.20 (s, 1H, H2), 6.09 (d, J=7.1 Hz, 1H, H1'), 4.94 – 4.86 (m, 1H, H2'), 4.35 –4.31 (m, 1H, H4'), 4.30 – 4.20 (m, 3H, H-3', H5'a, H5'b), 3.80 – 3.72 (m, 2H, $O^{3'}$-CH_2a), 3.72 – 3.64 (m, 2H, $O^{3'}$-CH_2b), 3.29 (t, J=7.2 Hz, 2H, NHTFA-C**H**$_2$), 1.72 – 1.54 (m, 4H, 2x CH_2-linker), 1.53 – 1.36 (m, 4H, 2x CH_2-linker).

^{19}F NMR (MeOD-d_4, 376 MHz): δ -77.3 (s, 3F).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.2 (m, 1P), -11.3 (d, J=21.8 Hz, 1P), -23.4 (m, 1P).

HR-ESI-MS: found: 701.0740; calculated: 701.0745 (M-H$^+$, $C_{18}H_{27}F_3N_6O_{14}P_3^-$); deviation: 0.7 ppm.

3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-4-*C*-formyl-1,2-*O*-isopropylidene-α-D-ribofuranose 4a[3]

Compound 4a was synthesized according to literature.

3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-4-*C*-(Z)-(7-(trifluoroacetamido)-hept-6-enyl)-1,2-*O*-isopropylidene-α-D-ribofuranose 4b

To a stirred solution of 6-Trifluoroacetamidohexane-1-iodide[2] (1.00 g, 3.10 mmol, 1 eq.) in anhydrous toluene, PPh_3 (2.81 g, 10.71 mmol, 3.5 eq.) was added and the mixture was refluxed for 16 h. After completion of the reaction, the mixture was allowed to cool to room temperature. The precipitate was collected by filtration, washed with toluene and dried in vacuo to give 1.83 g of compound triphenyl-(6-(trifluoroacetamido)hexyl)-phosphonium iodide **4a'** (3.06 mmol, 99%) as a pale yellow solid; R_f 0.80 (4:1 hexane in ethyl acetate).

Intermediate Iodide **4a'**

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (bs, 1H, NHTFA), 7.86 – 7.76 (m, 9H, ArH), 7.76 – 7.67 (m, 6H, ArH), 3.64 – 3.54 (m, 2H, NHTFA-C**H**$_2$), 3.43 – 3.36 (m, 2H, C**H**$_2$$PPh_3$), 1.65 – 1.57 (m, 6H, 3x CH_2-linker), 1.52 – 1.40 (m, 2H, CH_2-linker).

^{13}C NMR ($CDCl_3$, 101 MHz): δ 135.2, 135.2, 133.7, 133.6, 130.7, 130.6, 118.6, 117.7, 39.1, 27.9, 25.5, 22.5, 22.3.

^{31}P NMR ($CDCl_3$, 162 MHz): δ 24.39 (s, 1P)

Next, intermediate **4a'** (1.61 g, 2.74 mmol, 1.1 eq.) and *t*-BuOK (0.72 g, 6.41 mmol, 3.5 eq.) were suspended in 50 mL dry THF under nitrogen atmosphere and stirred at room temperature for 1.5 hours. The synthesized aldehyde **4a** (1.00 g, 1.83 mmol, 1 eq.), dissolved in dry THF (10 mL) was added and stirring was continued for 15 hours. Reaction mixture was quenched with 20 mL saturated $NaHCO_3$ solution and extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$, concentrated and purified by column chromatography (6:1 hexane in ethyl acetate) to give 1.14 g of compound **4b** (1.57 mmol, 86%) as a pale yellow foam; R_f 0.55 (4:1 hexane in ethyl acetate).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 – 7.56 (m, 4H, ArH), 7.50 – 7.29 (m, 11H, ArH), 6.52 (bs, 1H, NHTFA), 5.88 (d, J = 11.8 Hz, 1H, linker-CH_b=C**H**$_a$), 5.76 (d, J = 3.9 Hz, 1H, H1), 5.41 (dt, J = 10.6, 7.5 Hz, 1H, linker-C**H**$_b$=CH_a), 4.87 (d, J = 12.3 Hz, 1H, BnH_a), 4.72 (d, J = 12.3 Hz, 1H, BnH_b), 4.66 (t, J = 4.3 Hz, 1H, H2), 4.43 (d, J = 4.7 Hz, 1H, H3), 3.68 (d, J = 11.8 Hz, 1H, H5$_a$), 3.52 (d, J = 11.8 Hz, 1H, H5$_b$), 3.34 (td, J = 13.7, 6.5 Hz, 1H, NHTFA-C**H**$_2$a), 3.21 (td, J = 13.4, 6.9 Hz, 1H, NHTFAC**H**$_2$b), 2.31 – 2.16 (m, 1H, C**H**$_2$b-CH=CH), 2.10 – 1.95 (m, 1H, C**H**$_2$a-CH=CH), 1.55 (s, 3H, CH_3), 1.52 – 1.40 (m, 2H, CH_2-linker), 1.40 – 1.28 (m, 2H, CH_2-linker), 1.32 (s, 3H, CH_3), 1.25 – 1.11 (m, 2H, CH_2-linker), 1.00 (s, 9H, 3x CH_3).

^{13}C NMR ($CDCl_3$, 101 MHz): δ 138.1, 136.1, 135.7, 134.1, 133.8, 133.2, 129.8, 129.7, 128.6, 128.1, 128.0, 127.8, 127.7, 126.4, 113.3, 103.9, 86.1, 78.2, 77.4, 72.7, 64.6, 39.9, 28.7, 28.5, 28.4, 26.9, 26.3, 26.2, 25.7, 19.4.

^{19}F NMR ($CDCl_3$, 376 MHz): δ -75.83 (s, 3F).

HR-ESI-MS: found: 748.32206; calculated: 748.32517 ($M+Na^+$, $C_{40}H_{50}F_3NaNO_6Si^+$); deviation: 4.2 ppm.

5-*O*-*tert*-butyldiphenylsilyl-4-*C*-(7-(trifluoroacetamido)-heptyl)-1,2-*O*-isopropylidene-α-D-ribofuranose 4c

Compound **4b** (0.1g, 0.14 mmol) and one weight equivalent of 10% Pd/C were dissolved in 4 mL THF and stirred under H_2-atmosphere (balloon) for 1.5 hours at room temperature. After completion of the reaction, the mixture was filtered through celite on a sintered funnel and washed with acetone thoroughly. The solvent was removed under reduced pressure and the resulting pale yellow foam was purified by column chromatography (3:1 hexane in ethyl acetate) to give compound **4c** in quantitative yields as a white foam; R_f 0.4 (3:1 hexane in ethyl acetate).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74-7.58 (m, 4H, ArH), 7.51-7.32 (m, 6H, ArH), 6.23 (bs, 1H, NHTFA), 5.93 (d, J = 4.2 Hz, 1H, H1), 4.76 (dd, J = 6.3, 4.2 Hz, 1H, H2), 4.37 (dd, J = 7.5, 6.4 Hz, 1H, H3), 3.63 (d, J = 10.6 Hz, 1H, H5a), 3.53 (d, J = 10.5 Hz, 1H, H5b), 3.25 – 3.40 (m, 2H, NHTFA-C**H**$_2$), 2.64 (d, J = 7.7 Hz, 1H, 3OH), 1.82-0.78 (m, 12H, 6x CH_2-linker), 1.54 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.04 (s, 9H, 3x CH_3).

^{13}C NMR ($CDCl_3$, 101 MHz): δ 135.8, 135.7, 130.0, 129.9, 128.0, 127.9, 113.6, 105.1, 100.1, 90.0, 81.1, 72.5, 68.2, 40.1, 31.7, 30.2, 29.0, 27.1, 27.0, 26.9, 26.7, 23.4, 19.3.

^{19}F NMR ($CDCl_3$, 376 MHz): δ -75.95 (s, 3F).

HR-ESI-MS: found: 660.29255; calculated: 660.29387 ($M+Na^+$, $C_{33}H_{46}F_3NaNO_6Si^+$); deviation: 2.0 ppm.

1,2,3-Tri-*O*-acetyl-5-*O*-*tert*-butyldiphenylsilyl-4-*C*-(7-(trifluoroacetamido)-heptyl)-α-D-ribofuranose 4d

To a solution of compound **4c** (1.25 g, 1.96 mmol, 1 eq.) in a mixture of 25.6 mL acetic acid and acetic anhydride (2.2 mL, 19.6 mmol, 10 eq.) was added 100 µl of concentrated H_2SO_4 and the mixture was stirred for 24 hours at room temperature. After completion of the reaction, the mixture was concentrated and coevaporated with toluene. The residue was diluted with 100 mL dichloromethane and washed with 25mL saturated $NaHCO_3$ and 25 mL water, dried over $MgSO_4$, concentrated, and purified by column chromatography (4:1 hexane in ethyl acetate) to give 1.19 g of compound **4d** (1.65 mmol, 84%) as a pale yellow foam; R_f 0.4 (2:1 hexane in ethyl acetate).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.72 – 7.65 (m, 4H, ArH), 7.46 – 7.38 (m, 6H, ArH), 6.44 (bs, 1H, NHTFA), 6.41 (d, *J* = 4.7 Hz, 1H, H1), 5.65 (d, *J* = 6.3 Hz, 1H, H3), 5.58 (dd, *J* = 6.3, 4.7 Hz, 1H, H2), 3.60 – 3.54 (m, 2H, H5a,b), 3.37 – 3.28 (m, 2H, NHTFA-CH_2) 2.15 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.74 – 1.62 (m, 2H, CH_2-linker), 1.54 (m, 4H, 2x CH_2-linker), 1.33 – 1.16 (m, 6H, 3x CH_2-linker), 1.07 (s, 9H, 3x CH_3).

^{13}C NMR ($CDCl_3$, 101 MHz): δ 170.2, 170.1, 169.3, 135.8, 135.8, 132.8, 132.5, 130.0, 130.0, 128.0, 94.5, 89.5, 71.6, 71.0, 67.3, 40.0, 32.8, 30.2, 29.4, 28.9, 26.9, 26.6, 23.4, 21.4, 20.8, 20.5, 19.2.

^{19}F NMR ($CDCl_3$, 376 MHz): δ -75.90 (s, 3F).

HR-ESI-MS: found: 746.29209; calculated: 746.29426 ($M+Na^+$, $C_{36}H_{48}F_3NaNO_9Si^+$); deviation: 2.9 ppm.

2'-*O*-Acetyl-3'-*O*-acetyl-5'-*O*-tert-butyldiphenylsilyl-4'-*C*-(7-(trifluoroacetamido)-heptyl)-*N*6-benzoyl-adenosine 4e

Compound **4d** (60 mg, 0.083 mmol, 1 eq.) and *N*6-benzoyl protected adenine (22 mg, 0.091 mmol, 1.1 eq.) were solved in 6 mL anhydrous acetonitrile and *N,O*-bis(trimethylsilyl)acetamide (120 µl, 0.71 mmol, 8.5 eq.) were added. The mixture was refluxed for 2 hours and after cooling to 0°C, Me_3SiOTf (30 µl, 0.07 mmol, 0.85 eq.) was added. After refluxing over night the mixture was quenched with 10 mL saturated $NaHCO_3$ solution, evaporated and extracted with dichloromethane. The organic layer was dried over $MgSO_4$, concentrated and purified by column chromatography (1:1 hexane in ethyl acetate) to give 67 mg of compound **4e** (0.075 mmol, 90%) as a white foam; R_f 0.4 (1:2 hexane in ethyl acetate).

^{1}H NMR (d_6-DMSO, 400 MHz): δ 11.23 (bs, 1H, NHBz), 9.38 (t, *J* = 5.4 Hz, 1H, NHTFA), 8.63 (s, 1H, H8), 8.42 (s, 1H, H2), 8.07 – 7.99 (m, 2H, ArH), 7.69 – 7.32 (m, 13H, ArH), 6.42 – 6.36 (m, 1H, H2'), 6.33 (d, *J* = 6.8 Hz, 1H, H1'), 5.95 (d, *J* = 5.6 Hz, 1H, H3'), 4.09 (d, *J* = 10.7 Hz, 1H, H5'), 3.72 (d, *J* = 10.7 Hz, 1H, H5''), 3.19 – 3.11 (m, 2H, NHTFA-C**H**$_2$), 2.19 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.86 – 1.68 (m, 2H, CH_2-linker), 1.45 (d, *J* = 6.3 Hz, 2H, CH_2-linker), 1.22 (s, 8H, 4x CH_2-linker), 1.02 (s, 9H, 3x CH_3).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 208.4, 172.0, 169.3, 169.2, 156.3, 156.0, 151.7, 151.5, 150.7, 144.0, 135.2, 135.1, 132.5, 132.5, 132.4, 130.0, 128.5, 128.5, 128.0, 127.8, 126.1, 117.4, 114.6, 87.0, 85.1, 71.9, 71.5, 68.5, 65.1, 55.8, 32.1, 30.6, 29.6, 29.5, 28.5, 28.2, 26.5, 26.1, 22.3, 21.1, 20.3, 20.2, 18.9.

^{19}F NMR (d_6-DMSO, 376 MHz): δ -74.38 (s, 3F).

HR-ESI-MS: found: 903.36700; calculated: 903.36731 ($M+H^+$, $C_{46}H_{54}F_3N_6O_8Si^+$); deviation: 3.4 ppm.

2'-*O*-Acetyl-3'-*O*-acetyl-5'-*O*-hydroxy-4'-*C*-(7-(trifluoroacetamido)-heptyl)-*N*6-benzoyl-adenosine 4f

To a solution of compound **4e** (750 mg, 0.83 mmol, 1 eq.) in 50 mL THF, 100% acetic acid (60 µl, 1.0 mmol, 1.2 eq.) and a 1 M solution of tetrabutylammonium fluoride (TBAF) (1.0 mL, 1.0 mmol, 1.2 eq.) were slowly added. The mixture was stirred at room temperature for 12 hours, concentrated and purified by column chromatography (0-5% methanol in dichloromethane) to give 384 mg of compound **4f** (0.58 mmol, 70%) as a white foam; R_f 0.3 (5% methanol in dichloromethane).

^{1}H NMR (d_6-DMSO, 400 MHz): δ 11.28 (bs, 1H, NHBz), 9.39 (bs, 1H, NHTFA), 8.79 (s, 1H, H8), 8.75 (s, 1H, H2), 8.05 (d, *J* = 7.2 Hz, 2H, ArH), 7.66 (t, *J* = 7.4 Hz, 1H, ArH), 7.56 (t, *J* = 7.6 Hz, 2H, ArH), 6.32 (d, *J* = 7.7 Hz, 1H, H1'), 6.22 (dd, *J* = 7.7, 5.4 Hz, 1H, H2'), 5.65 (d, *J* = 5.3 Hz, 1H, H3'), 5.60 (t, *J* = 5.4 Hz, 1H, OH), 3.71 (dd, *J* = 11.7, 4.8 Hz, 1H, H5'), 3.60 (dd, *J* = 11.7, 6.0 Hz, 1H, H5''), 3.20 – 3.12 (m, 2H, NHTFA-C**H**$_2$), 2.20 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.71 – 1.56 (m, 2H, CH_2-linker), 1.54 – 1.42 (m, 2H, CH_2-linker), 1.33 – 1.20 (m, 8H, 4x CH_2-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 208.3, 169.1, 169.0, 156.7, 156.3, 155.9, 155.6, 134.5, 132.4, 129.1, 128.4, 127.5, 117.4, 114.6, 88.2, 85.9, 74.5, 72.5, 68.5, 64.3, 55.8, 32.0, 31.8, 29.6, 29.5, 28.3, 28.1, 26.5, 26.0, 22.7, 20.0.

^{19}F NMR (d_6-DMSO, 376 MHz): δ -74.41 (s, 3F).

HR-ESI-MS: found: 665.25206; calculated: 665.25412 ($M+H^+$, $C_{30}H_{36}F_3N_6O_8^+$); deviation: 3.1 ppm.

2'-*O*-Acetyl-3'-*O*-acetyl-4'-*C*-(7-(trifluoroacetamido)-heptyl)-*N*6-benzoyl-adenosine-triphosphate 4g

The protected nucleoside **4f** (100 mg, 0.15 mmol, 1 eq.) and proton sponge (48 mg, 22.5 mmol, 1.5 eq.) were coevaporated with acetonitrile twice and dried at high vacuum. The solids were dissolved in 3 mL trimethylphosphate and cooled to -25°C. Phosphorous oxychloride (45 µl, 76 mg, 50 mmol, 2 eq.) was added dropwise. The reaction was allowed to warm up to 0°C and stirred for 1 hour. Tributylamine (740 µl, 575 mg, 3.11 mmol, 16 eq.) and bis-(tributylammonium)-pyrophosphate (2 mL, 0.5 M in DMF, 1 mmol, 5 eq.) were added simultaneously, the solution was warmed to room temperature and stirred for 30 minutes. 5 mL 0.1 M TEAB buffer (pH 7.5) were added and the reaction stirred for 30 minutes. The mixture was extracted with ethyl acetate three times and the solvents were evaporated under reduced pressure. The compound was purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-

MPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 20.62 µmol of product **4g** (14%) as white fluffs.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 9.03 (s, 1H, H8), 8.73 (s, 1H, H2), 8.09 (d, *J* = 7.3 Hz, 2H, ArH), 7.66 (t, *J* = 7.4 Hz, 1H, ArH), 7.57 (t, *J* = 7.6 Hz, 2H, ArH), 6.41 (d, *J* = 7.5 Hz, 1H, H1'), 6.11 (dd, *J* = 7.3, 5.8 Hz, 1H, H2'), 5.82 (d, *J* = 5.5 Hz, 1H, H3'), 4.32 – 4.22 (m, 2H, H5',H5''), 3.27 (t, *J* = 7.1 Hz, 2H, NHTFA-C**H**$_2$), 2.22 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.85 – 1.72 (m, 2H, CH_2-linker), 1.60 – 1.49 (m, 2H, CH_2-linker), 1.42 – 1.34 (m, 8H, 4x CH_2-linker).

^{19}F NMR (MeOD-d_4, 376 MHz): δ -77.32 (s, 3F).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.24 (d, *J* = 20.8 Hz, 1P), -11.95 (d, *J* = 20.0 Hz, 1P), -23.19 (t, *J* = 18.0 Hz, 1P).

HR-ESI-MS: found: 903.14040; calculated: 903.13750 (M-H$^+$, $C_{30}H_{37}F_3N_6O_{17}P_3^-$); deviation: 3.2 ppm.

4'-*C*-(7-(trifluoroacetamido)-heptyl)-adenosine-triphosphate 4

Protected nucleotide **4g** (20.62 µmol) was dissolved in 3.0 mL water and 6.0 mL ammonium hydroxide (33%) was added to the solution and stirred for 3.5 hours at room temperature. After completion of the reaction ammonia was removed in vacuo. The crude product was converted without further purification. The deprotected nucleotide (6.72 µmol, 33%, 1 eq.) was dissolved in 7.0 mL methanol. To the solution ethyl trifluoroacetate (8.40 µL, 70.60 µmol, 11 eq.) and freshly distilled triethylamine (8.40 µL, 60.6 µmol, 9 eq.) were added and the reaction mixture was stirred 3 hours at room temperature. The reaction mixture was evaporated to dryness and purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-MPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 6.22 µmol of product **4** (30% over 2 steps) as white fluffs.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.68 (s, 1H, H8), 8.18 (s, 1H, H2), 6.06 (m, 1H, H1'), 5.06 – 5.01 (m, 1H, H2'), 4.48 (m, 1H, H3'), 4.15 (m, 1H, H5'), 4.03 (m, 1H, H5''), 3.25 (m, 2H, NHTFA-C**H**$_2$), 1.84 – 1.70 (m, 2H, CH_2-linker), 1.58 – 1.49 (m, 2H, CH_2-linker), 1.47 – 1.38 (m, CH_2-linker), 1.37 – 1.31 (m, 6H, 3x CH_2-linker).

^{19}F NMR (MeOD-d_4, 376 MHz): δ -77.35 (s, 3F).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -9.74 (m, 1P), -11.19 (m, 1P), -22.83 (m, 1P).

HR-ESI-MS: found: 715.09100; calculated: 715.09010 (M-H$^+$, $C_{19}H_{29}F_3N_6O_{14}P_3^-$); deviation: 1.3 ppm.

2-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine 5a

2-Iodo-adenosine[4] (1.97 g, 4.94 mmol, 1 eq.) was dissolved in 25 mL dry DMF and CuI (200 mg, 1.05 mmol, 0.2 eq.) was added. The reaction mixture was degassed under vacuo before $Pd(PPh_3)_4$ (600 mg, 0.52 mmol, 0.1 eq.), trifluoro-*N*-(pent-4-ynyl)-acetamide (840 mg, 4.69 mmol, 0.95 eq.) and triethylamine (1.5 mL, 10.82 mmol, 2.2 eq.) were added. The reaction mixture was stirred for 16

hours at room temperature, quenched with 25 mL saturated $NaHCO_3$ solution and extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$, concentrated and purified by column chromatography (10% methanol in dichloromethane) to give 2.07 g of compound **5a** (4.64 mmol, 94%) as a pale yellow foam; R_f 0.2 (5% methanol in dichloromethane).

^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.52 (t, *J* = 5.0 Hz, 1H, NHTFA), 8.40 (s, 1H, H8), 7.44 (bs, 2H, NH_2), 5.85 (d, *J* = 6.1 Hz, 1H, H1'), 5.46 (d, *J* = 6.2 Hz, 1H, OH2'), 5.25 (dd, *J* = 6.7, 4.8 Hz, 1H, OH5'), 5.19 (d, *J* = 4.8 Hz, 1H, OH3'), 4.55 – 4.50 (m, 1H, H2'), 4.14 – 4.10 (m, 1H, H3'), 3.95 (t, *J* = 3.3 Hz, 1H, H4'), 3.66 (dt, *J* = 12.0, 4.1 Hz, 1H, H5'), 3.60 – 3.51 (m, 1H, H5''), 3.33 – 3.27 (m, 2H, NHTFA-C**H**$_2$), 2.45 (t, *J* = 7.1 Hz, 2H, C**H**$_2$-CC), 1.77 (q, *J* = 7.1 Hz, 2H, CH_2-C**H**$_2$-CH_2).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 155.8, 149.3, 145.6, 140.3, 118.6, 117.4, 114.5, 87.4, 85.8, 84.5, 81.3, 73.7, 70.5, 61.5, 38.5, 27.0, 15.8.

^{19}F NMR (d_6-DMSO, 376 MHz): δ -74.31 (s, 3F).

HR-ESI-MS: found: 445.14206; calculated: 445.14418 ($M+H^+$, $C_{17}H_{20}F_3N_6O_5^+$); deviation: 4.8 ppm.

2-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine-triphosphate 5

Nucleoside **5a** (50 mg, 0.11 mmol, 1eq.) and proton sponge (35 mg, 0.16 mmol, 1.5 eq.) were coevaporated with acetonitrile twice and dried at high vacuum. The solids were dissolved in 2 mL trimethylphosphate and cooled to -30°C. Phosphorous oxychloride (40 µl, 67 mg, 0.55 mmol, 5 eq.) was added dropwise. The reaction was allowed to warm up to 0°C and stirred for 1 hour. Tributylamine (300 µl, 233 mg, 1.26 mmol, 11 eq.) and bis-(tributylammonium)-pyrophosphate (1.2 mL, 0.5 M in DMF, 0.6 mmol, 5 eq.) were added simultaneously, the solution was warmed to room temperature and stirred for 30 minutes. 5 mL 0.1 M TEAB buffer (pH 7.5) were added and the reaction stirred for 30 minutes. The mixture was extracted with ethyl acetate three times and the solvents were evaporated under reduced pressure. The compound was purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-MPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 44.4 µmol of product **5** (40%) as white fluffs.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.59 (s, 1H, H8), 7.19 (bs, 1H, NHTFA), 6.08 (d, *J* = 5.4 Hz, 1H, H1'), 4.69 (t, *J* = 5.1 Hz, 1H, H2'), 4.57 – 4.44 (m, 1H, H3'), 4.39 – 4.23 (m, 3H, H4', H5', H5''), 3.50 – 3.44 (m, 2H, NHTFA-C**H**$_2$), 2.52 (t, *J* = 7.1 Hz, 2H, C**H**$_2$-CC), 1.97 – 1.85 (m, 2H, CH_2-C**H**$_2$-CH_2).

^{19}F NMR (MeOD-d_4, 376 MHz): δ -75.79 (s, 3F).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.38 (d, *J* = 21.0 Hz, 1P), -11.39 (d, *J* = 21.2 Hz, 1P), -23.65 (t, *J* = 20.7 Hz, 1P).

HR-ESI-MS: found: 683.02920; calculated: 683.02750 ($M-H^+$, $C_{17}H_{21}F_3N_6O_{14}P_3^-$); deviation: 2.5 ppm.

6-Chloro-9-(*β*-D-ribofuranosyl)-purine 6a[5]

Inosine (2 g, 7.5 mmol, 1 eq.) was suspended in 40 mL dichloromethane. To this trifluoroacetic anhydride (12 mL, 17.8 g, 85.0 mmol, 11 eq.) was added. The reaction mixture was stirred at room temperature for 12 hours. The solvents were evaporated under reduced pressure and the residue was redissolved in 200 mL dichloromethane. The solution was cooled to 0°C and a mixture of DMF (1.52 mL, 1.4 g, 20 mmol, 2.6 eq.) and thionyl chloride (3.03 mL, 5.0 g, 42 mmol, 5.6 eq.) in 80 mL dichloromethane was added dropwise. The solution was refluxed for 12 hours. The mixture was evaporated to a volume of 60 mL and extracted with saturated sodium hydrogencarbonate solution. The aqueous layer was reextracted with dichloromethane twice. The combined organic phases were dried over $MgSO_4$ and evaporated to dryness. The residue was dissolved in 6 mL methanol and refluxed for 12 hours. To the resulting suspension 12 mL diethyl ether were added to complete precipitation and the product was collected by filtration to afford 1.4 g (4.9 mmol, 65%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.95 (s, 1H, H8), 8.81 (s, 1H, H2), 6.05 (d, J=5.2 Hz, 1H, H1'), 5.56 (d, J=5.7 Hz, 1H, OH2'), 5.24 (d, J=5.1 Hz, 1H, OH3'), 5.08 (t, J=4.6 Hz, 1H, OH5'), 4.59 (pq, J=4.6 Hz, 1H, H2'), 4.20 (pq, J=4.4 Hz, 1H, H3'), 3.99 (pq, J=4.0 Hz, 1H, H4'), 3.75 – 3.67 (m, 1H, H5'a), 3.63 – 3.55 (m, 1H, H5'b).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 151.8, 151.6, 149.3, 145.8, 131.4, 88.2, 85.7, 74.0, 70.1, 61.0.

HR-ESI-MS: found: 321.0136; calculated: 321.0152 ($M+Cl^-$, $C_{10}H_{11}Cl_2N_4O_4^-$); deviation: 5.0 ppm.

N^6-(6-Aminohexyl)-adenosine 6b[6]

6-Chloro-9-(*β*-D-ribofuranosyl)-purine **6a** (1 g, 3.5 mmol, 1 eq.) was suspended in 30 mL ethanol. Triethylamine (0.48 mL, 0.35 g, 3.5 mmol, 1 eq.) and 1,6-diaminohexane (2.92 g, 22.6 mmol, 6 eq.) were added and heated to 100°C for 3 hours. The reaction mixture was cooled to room temperature and stirred for 12 hours to complete precipitation. The product was collected by filtration to give 1.06 g (2.9 mmol, 83%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 8.33 (s, 1H, H8), 8.19 (s, 1H, H2), 7.86 (s, 1H, C6-N**H**), 5.87 (d, J=6.1 Hz, 1H, H1'), 5.42 (bs, 2H, OH), 5.18 (bs, 1H, OH), 4.61 (t, J=5.4 Hz, 1H, H2'), 4.17 – 4.11 (m, 1H, H3'), 3.96 (q, J=3.4 Hz, 1H, H4'), 3.67 (dd, J=3.4 Hz, J=12.0 Hz, 1H, H5'a), 3.55 (bd, J=12.0 Hz, 1H, H5'b), 3.46 (bs, 2H, C^6-NH-C**H**$_2$), 2.49 – 2.45 (m, 2H, NH_2-C**H**$_2$), 1.64 – 1.53 (m, 2H, C^6-NH-CH_2-C**H**$_2$), 1.39 – 1.21 (m, 6H, 3x C**H**$_2$-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 154.5, 152.3, 148.0, 139.5, 119.6, 87.9, 85.8, 73.3, 70.5, 61.6, 41.5, 39.9, 33.2, 29.0, 26.2, 26.1.

HR-ESI-MS: found: 367.2081; calculated: 367.2088 ($M+H^+$, $C_{16}H_{27}N_6O_4^+$); deviation: 1.9 ppm.

N^6-(6-Trifluoroacetamidohexyl)-adenosine 6c[7]

Compound **6b** (1.06 g, 2.9 mmol, 1 eq.) was suspended in 30 mL methanol. Triethylamine (0.51 mL, 0.37 g, 3.7 mmol, 1.3 eq.) and ethyl trifluoroacetate (0.51 mL, 0.61 g, 4.3 mmol, 1.5 eq.) were added. The reaction mixture was stirred at room temperature for 12 hours. The product was collected by filtration to give 780 mg (1.7 mmol, 58%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.37 (bt, J=5.7 Hz, 1H, NHTFA), 8.33 (s, 1H, H8), 8.19 (bs, 1H, H2), 7.87 (s, 1H, C6-N**H**), 5.87 (d, J=6.1 Hz, 1H, H1'), 5.47 – 5.36 (m, 2H, OH2', OH5'), 5.20 – 5.13 (m, 1H, OH3'), 4.65 – 4.55 (m, 1H, H2'), 4.19 – 4.11 (m, 1H, H3'), 4.00 – 3.93 (m, 1H, H4'), 3.72 – 3.62 (m, 1H, H5'a), 3.60 – 3.51 (m, 1H, H5'b), 3.46 (bs, 2H, C^6-NH-C**H**$_2$), 3.16 (pq, J=6.5 Hz, 2H, NHTFA-C**H**$_2$), 1.64 – 1.53 (m, 2H, C^6-NH-CH$_2$-C**H**$_2$), 1.53 – 1.42 (m, 2H, NHTFA-CH$_2$-C**H**$_2$), 1.40 – 1.21 (m, 4H, 2x C**H**$_2$-linker).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 156.1 (q, J=35 Hz), 154.7, 152.4, 148.2, 139.6, 119.8, 115.9 (q, J=289 Hz), 87.9, 85.9, 73.4, 70.6, 61.7, 40.1, 39.2, 28.9, 28.2, 26.0, 25.9.

^{19}F NMR (d_6-DMSO, 376 MHz): δ -74.4 (s, 3F).

HR-ESI-MS: found: 463.1894; calculated: 463.1911 (M+H$^+$, $C_{18}H_{26}F_3N_6O_5^+$); deviation: 3.7 ppm.

N^6-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 6

Compound **6c** (250 mg, 541 µmol, 1 eq.) and proton sponge (163 mg, 761 µmol, 1.4 eq.) were coevaporated with acetonitrile twice and dried at high vacuum. The solids were dissolved in 12.5 mL trimethylphosphate and cooled to 0°C. Phosphorous oxychloride (55 µl, 93 mg, 609 µmol, 1.1 eq.) were added dropwise. The reaction was stirred at 0°C for 1 hour. Tributylamine (1.21 mL, 941 mg, 5.08 mmol, 10 eq.) and bis-(tributylammonium)-pyrophosphate (5.08 mL, 0.5 M in DMF, 2.54 mmol, 5 eq.) were added simultaneously, the solution was warmed to room temperature and stirred for 30 minutes. 15 mL 0.1 M TEAB buffer (pH=7.5) were added and the reaction stirred for 30 minutes. The mixture was extracted with ethyl acetate three times and the solvents were evaporated under reduced pressure. The compound was purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-HPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 184 µmol (34%) of colourless oil.

^{1}H NMR (D_2O, 400 MHz): δ 8.45 (s, 1H, H8), 8.16 (bs, 1H, H2), 6.06 (d, J=6.1 Hz, 1H, H1'), 4.69 – 4.63 (m, 1H, H2'), 4.50 – 4.43 (m, 1H, H3'), 4.30 – 4.23 (m, 1H, H4'), 4.21 – 4.13 (m, 1H, H5'a), 4.13 – 4.06 (m, 1H, H5'b), 3.46 (bs, 2H, C^6-NH-C**H**$_2$), 3.24 (t, J=6.9 Hz, 2H, NHTFA-C**H**$_2$), 1.58 – 1.47 (m, 2H, C^6-NH-CH$_2$-C**H**$_2$), 1.46 – 1.35 (m, 2H, NHTFA-CH$_2$-C**H**$_2$), 1.33 – 1.18 (m, 4H, 2x C**H**$_2$-linker).

^{19}F NMR (D_2O, 376 MHz): δ -75.8 (s, 3F).

^{31}P NMR (D_2O, 162 MHz): δ -10.9 (d, J=20.9 Hz, 1P), -11.6 (d, J=18.7, 1P), -23.4 (t, J=19.8, 1P).

HR-ESI-MS: found: 701.0723; calculated: 701.0745 (M-H$^+$, $C_{18}H_{27}F_3N_6O_{14}P_3^-$); deviation: 3.1 ppm.

N^6-(6-Cy5-amidohexyl)-adenosine-5'-triphosphate 6d:

118 µmol Compound **6** were dissolved in 5.5 ml 0.1 M NaOH and kept at room temperature for 4 hours. 1 ml of 1 M sodium hydrogencarbonate buffer (pH 8.7) was added and the solution was readjusted to pH 8.7. 121 mg (141 µmol, 1.2 eq.) Cy5 NHS ester (triethylammonium salt) in 1 ml DMF were added and the solution was stirred at room temperature for 12 hours. Purification by RP-HPLC gave 53 µmol (45%) of dark blue oil.

^{1}H NMR (MeOD, 400 MHz): δ 8.51 (s, 1H, H8), 8.31 (t, J=13.1 Hz, 1H, HB-Cy5), 8.30 (t, J=13.0 Hz, 1H, HB'-Cy5), 8.21 (s, 1H, H2), 7.89 (m, 4H, H_{Ar}-Cy5), 7.36 (m, 2H, H_{Ar}-Cy5), 6.71 (t, J=12.4 Hz, 1H, HC-Cy5), 6.37 (d, J=13.6 Hz, 1H, HA-Cy5), 6.35 (d, 13.6 Hz, 1H, HA'-Cy5), 6.09 (d, J=5.7 Hz, 1H, H1'), 4.71 (t, J=5.1 Hz, 1H, H2'), 4.51 (m, 1H, H3'), 4.32-4.07 (m, 7H, H4', H5'a, H5'b, 2x N-C**H**$_2$-Cy5), 3.56 (bs, 2H, C^6-NH-C**H**$_2$), 3.14 (m, 2H, CO-NH-C**H**$_2$), 2.19 (t, J=7.3 Hz, 2H, NH-CO-C**H**$_2$), 1.85-1.35 (m, 29H, 7x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD, 162 MHz): δ -9.7 (d, J=18.6 Hz, 1P), -11.0 (d, J=19.9 Hz, 1P), -22.5 (bs, 1P).

HR-ESI-MS: found: 621.1489; calculated: 621.1479 (M-2H$^+$, $C_{49}H_{65}N_8O_{20}P_3S_2^{2-}$); deviation: 1.6 ppm.

8-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine 7a

8-Bromo-adenosine (200 mg, 0.58 mmol, 1 eq.) and triethylamine (3.37 mL, 2.46 g, 24 mmol, 40 eq.) were dissolved in 25 mL DMF. $PdCl_2(PPh_3)_2$ (12.0 mg, 17.0 µmol, 0.03 eq.) and CuI (1 mg, 5.8 µmol, 0.01 eq.) were added. The solution was stirred at room temperature for 5 minutes. 5-Trifluoroacetamido-pentyne (268 mg, 1.5 mmol, 2.6 eq.) was added. The solution was stirred at room temperature for 96 hours and the solvents were evaporated under reduced pressure. The residue was triturated with dichloromethane and the resulting solid was collected by filtration to give 205 mg (0.46 mmol, 80%) of a white solid.

^{1}H NMR (d_6-DMSO, 400 MHz): δ 9.52 (bs, 1H, NHTFA), 8.15 (s, 1H, H2), 7.57 (bs, 2H, NH_2), 5.95 (d, J=6.9 Hz, 1H, H1'), 5.59 (dd, J=8.3 Hz, J=3.4 Hz, 1H, OH5'), 5.42 (bs, 1H, OH2'), 5.20 (bs, 1H, OH3'), 4.99 (bt, J=5.8 Hz, 1H, H2'), 4.24 – 4.17 (m, 1H, H3'), 3.99 (dd, J=6.0 Hz, J=3.6 Hz, 1H, H4'), 3.73 – 3.63 (m, 1H, H5'a), 3.59 – 3.48 (m, 1H, H5'b), 3.36 – 3.29 (m, 2H, NHTFA-C**H**$_2$), 2.63 (t, J=7.1 Hz, 2H,C**H**$_2$-CC), 1.84 (p, J=7.1 Hz, 2H, CH_2-C**H**$_2$-CH_2).

^{13}C NMR (d_6-DMSO, 101 MHz): δ 156.5 (q, 36 Hz), 156.1, 153.1, 148.4, 133.9, 119.2, 116.0 (q, 288 Hz), 96.6, 89.4, 86.7, 71.7, 71.1, 70.4, 62.3, 38.5, 26.9, 16.3.

^{19}F NMR (d_6-DMSO, 376 MHz): δ -74.3 (s, 3F).

HR-ESI-MS: found: 445.1427; calculated: 445.1442 (M+H$^+$, $C_{17}H_{20}F_3N_6O_5^+$); deviation: 3.4 ppm.

8-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine-5'-triphosphate 7

Compound **7a** (40 mg, 90 µmol, 1 eq.) and proton sponge (28.9 mg, 135 µmol, 1.5 eq.) were coevaporated with acetonitrile twice and dried at high vacuum. The solids were dissolved in 3 mL trimethylphosphate and cooled to 0°C. Phosphorous oxychloride (10.6 µl, 17.7 mg, 108 µmol, 1.2 eq.)

was added dropwise. The reaction was stirred at 0°C for 1 hour. Tributylamine (209 µl, 163 mg, 0.9 mmol, 10 eq.) and bis-(tributylammonium)-pyrophosphate (0.9 mL, 0.5 M in DMF, 0.45 mmol, 5 eq.) were added simultaneously, the solution was warmed to room temperature and stirred for 30 minutes. 10 mL 0.1 M TEAB buffer (pH 7.5) were added and the reaction stirred for 30 minutes. The mixture was extracted with ethyl acetate three times and the solvents were evaporated under reduced pressure. The compound was purified by anion-exchange chromatography. Fractions containing the product were evaporated and further purified by RP-HPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give 27.6 µmol (31%) of colourless oil.

^{1}H NMR (D_2O, 400 MHz): δ 8.20 (s, 1H, H2), 6.13 (d, J=5.9 Hz, 1H, H1'), 5.23 (t, J=6.1 Hz, 1H, H2'), 4.59 (m, 1H, H3'), 4.36 – 4.27 (m, 2H, H4', H5'a), 4.27 – 4.16 (m, 1H, H5'b), 3.50 (t, J=6.9 Hz, 2H, NHTFA-C**H**$_2$), 2.69 (t, J=6.9 Hz, 2H, C**H**$_2$-CC), 1.97 (p, J=6.9 Hz, 2H, CH_2-C**H**$_2$-CH_2).

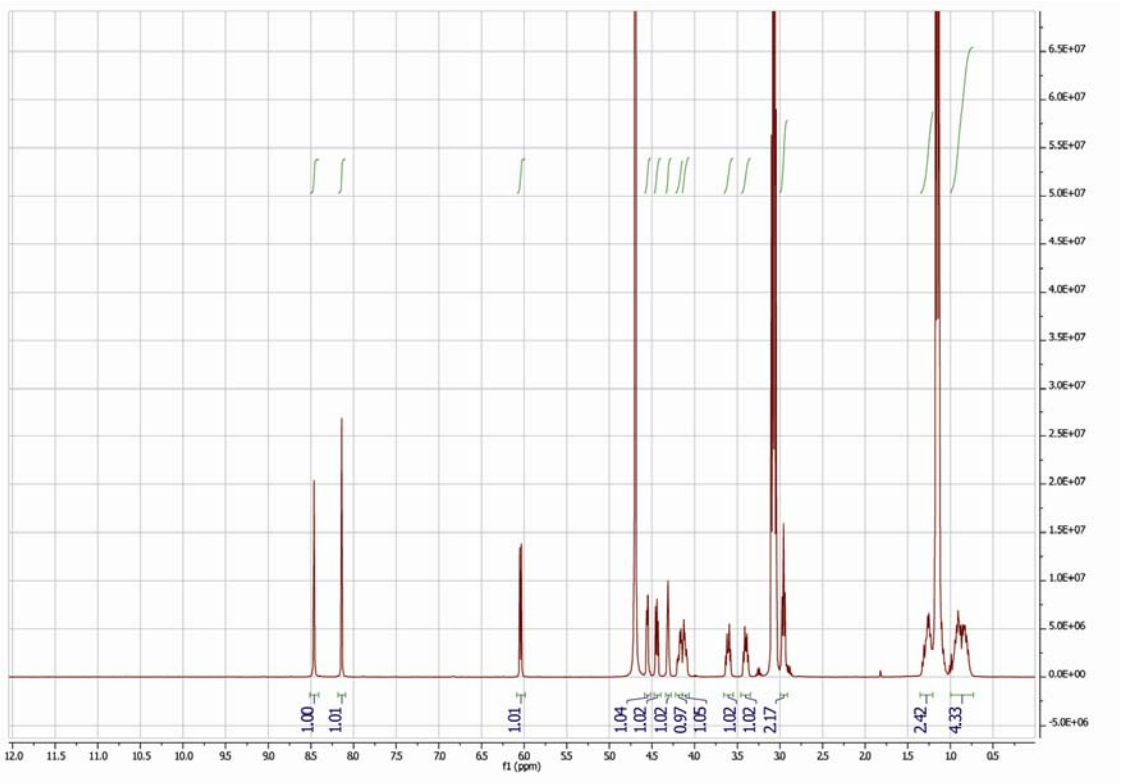
^{19}F NMR (D_2O, 376 MHz): δ -75.7 (s, 3F).

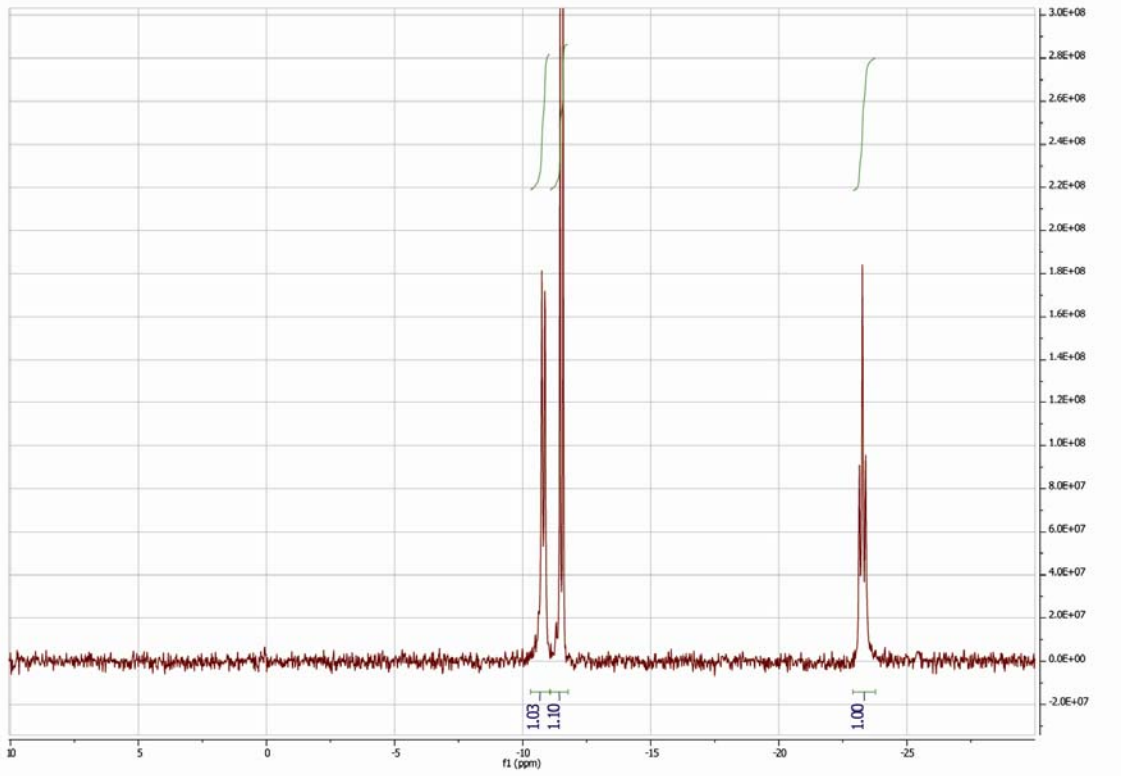
^{31}P NMR (D_2O, 162 MHz): δ -19.9 (m, 1P), -11.4 (d, J=18.2 Hz, 1P), -23.0 (m, 1P).

HR-ESI-MS: found: 683.0266; calculated: 683.0275 (M-H$^+$, $C_{17}H_{21}F_3N_6O_{14}P_3^-$); deviation: 1.3 ppm.

NMR spectra

2'-*O*-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 2:





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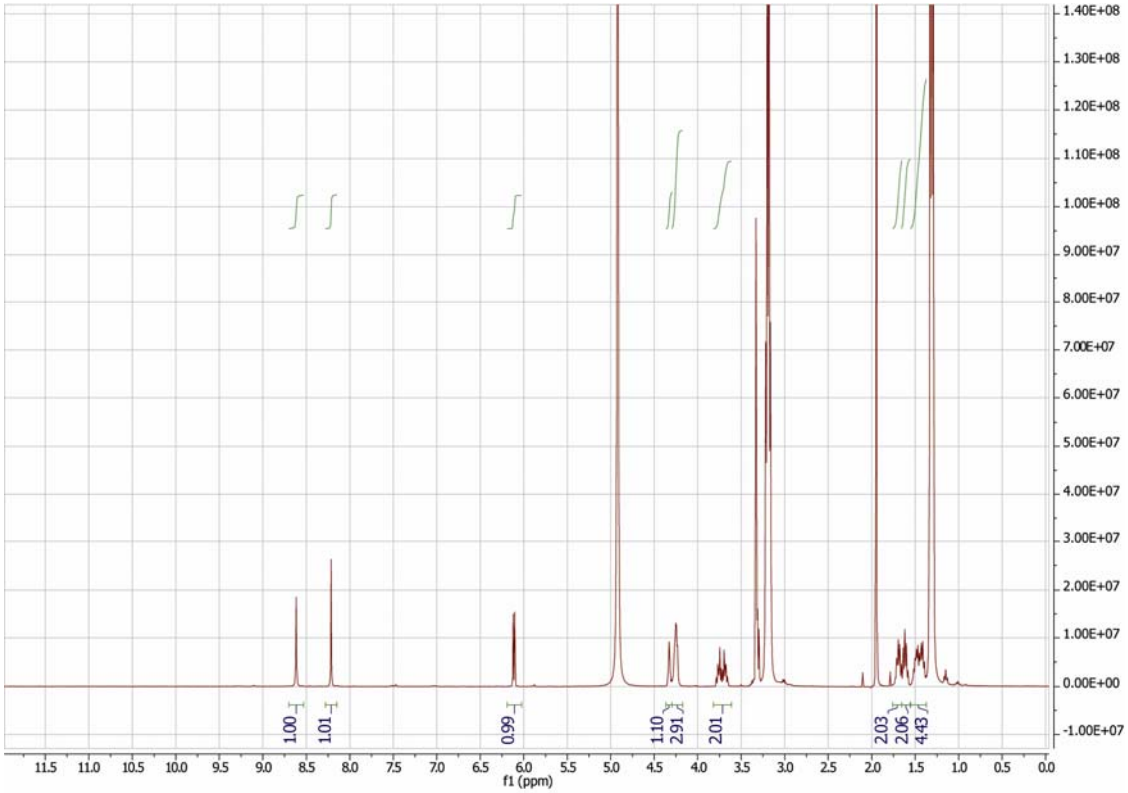
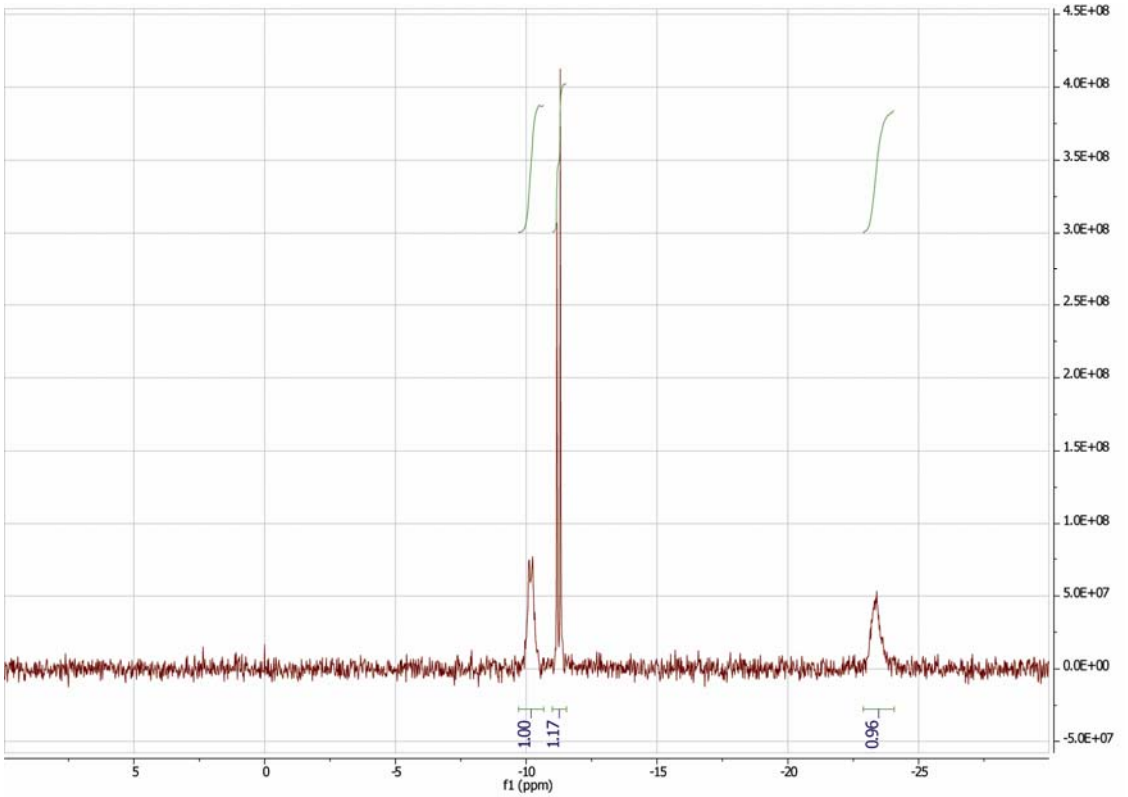
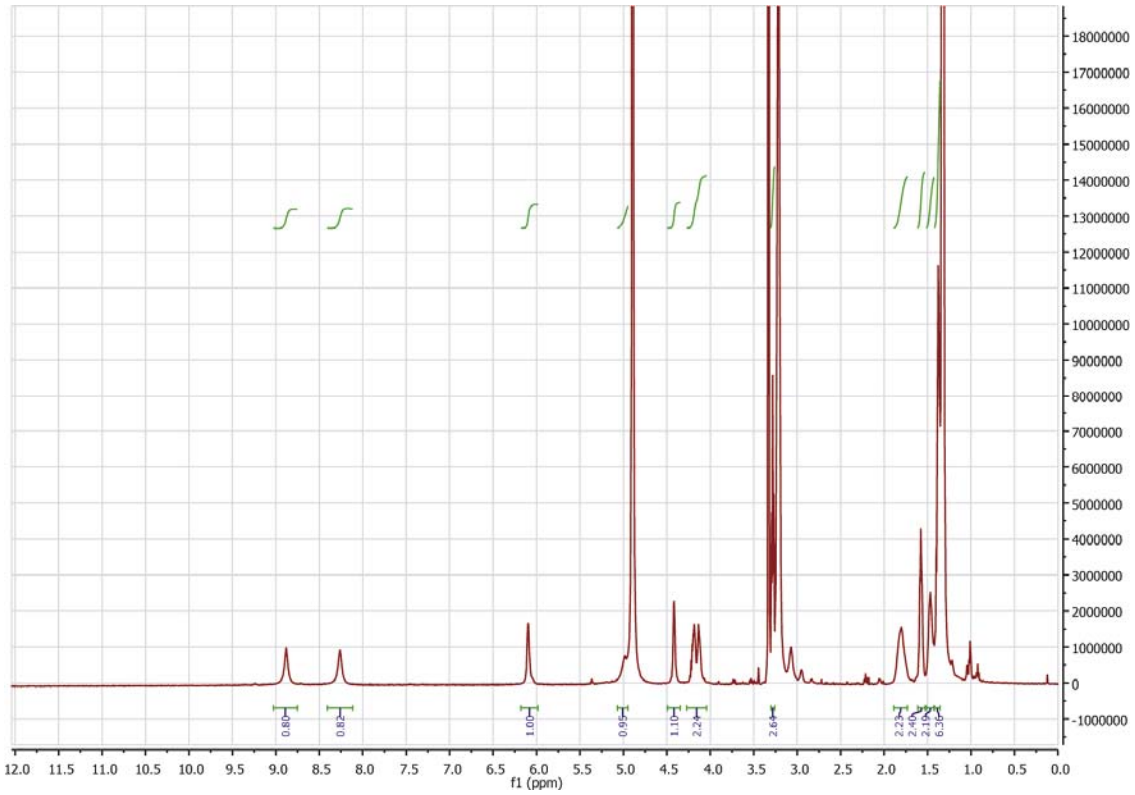
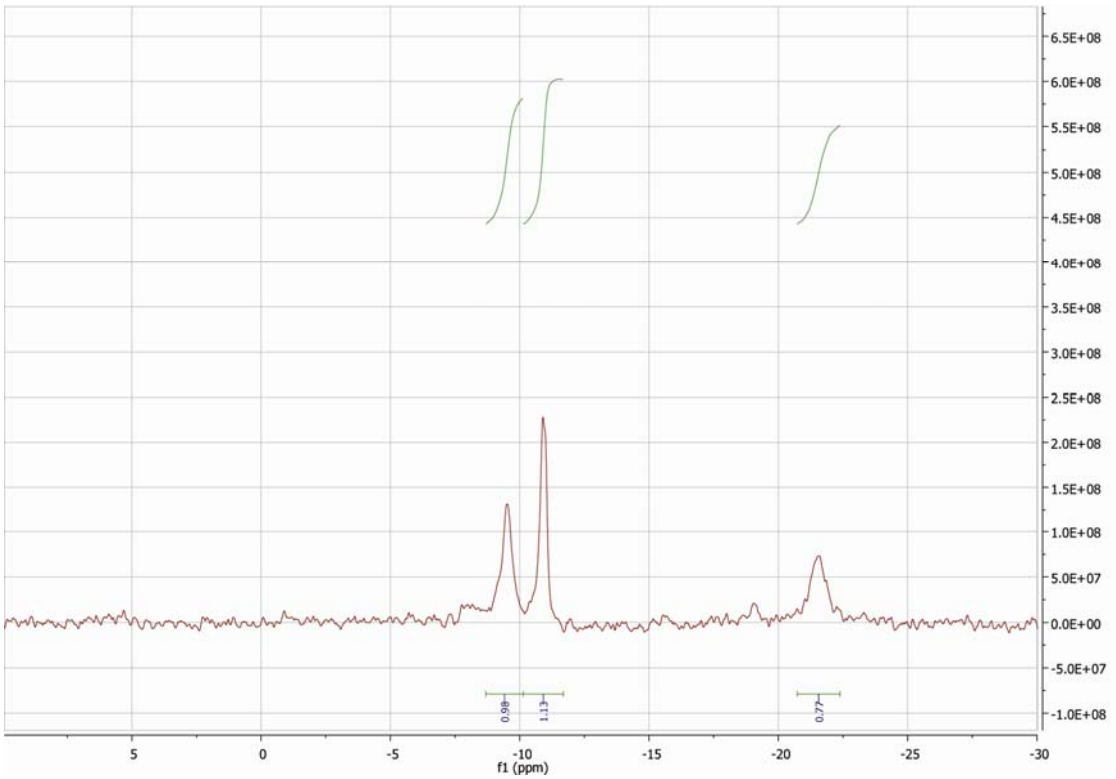


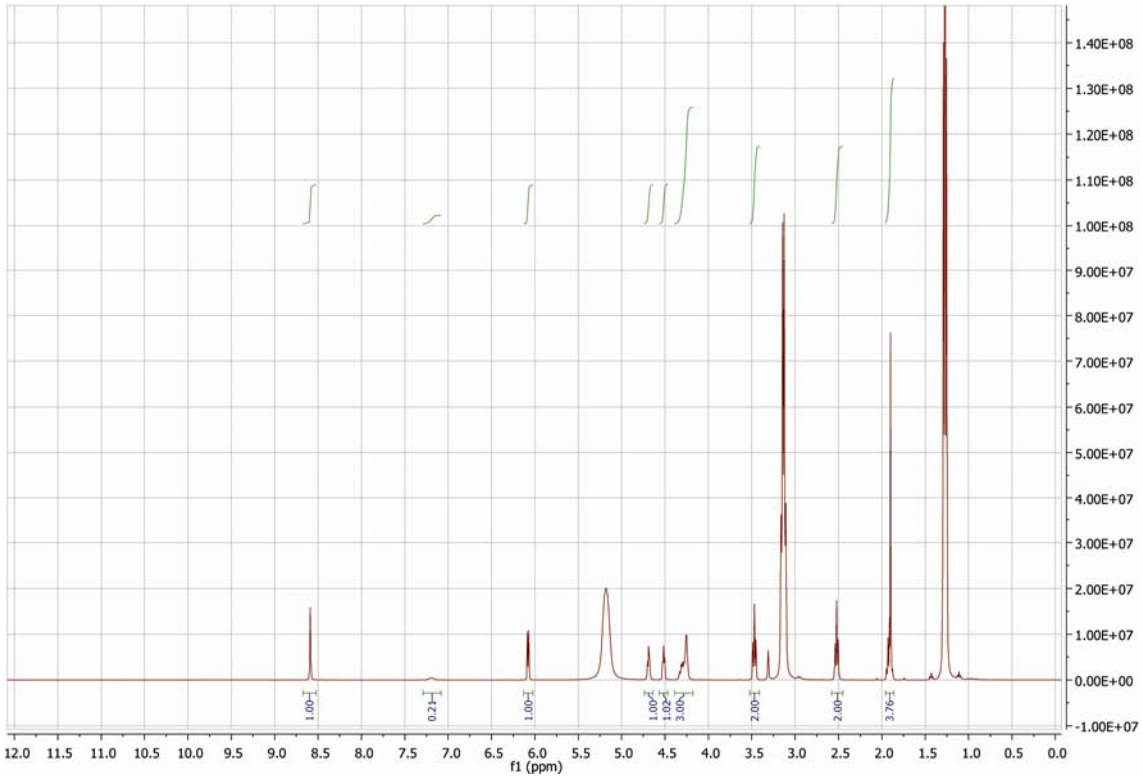


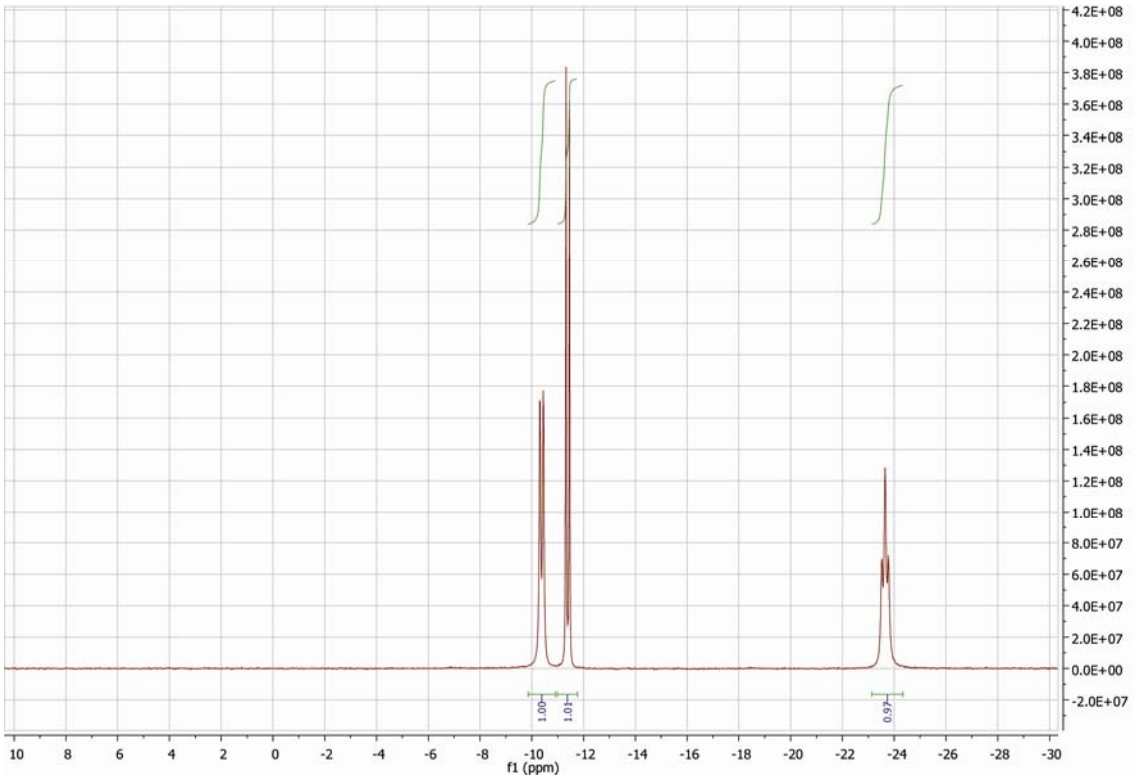
4'-*C*-(7-(trifluoroacetamido)-heptyl)-adenosine-triphosphate 4:



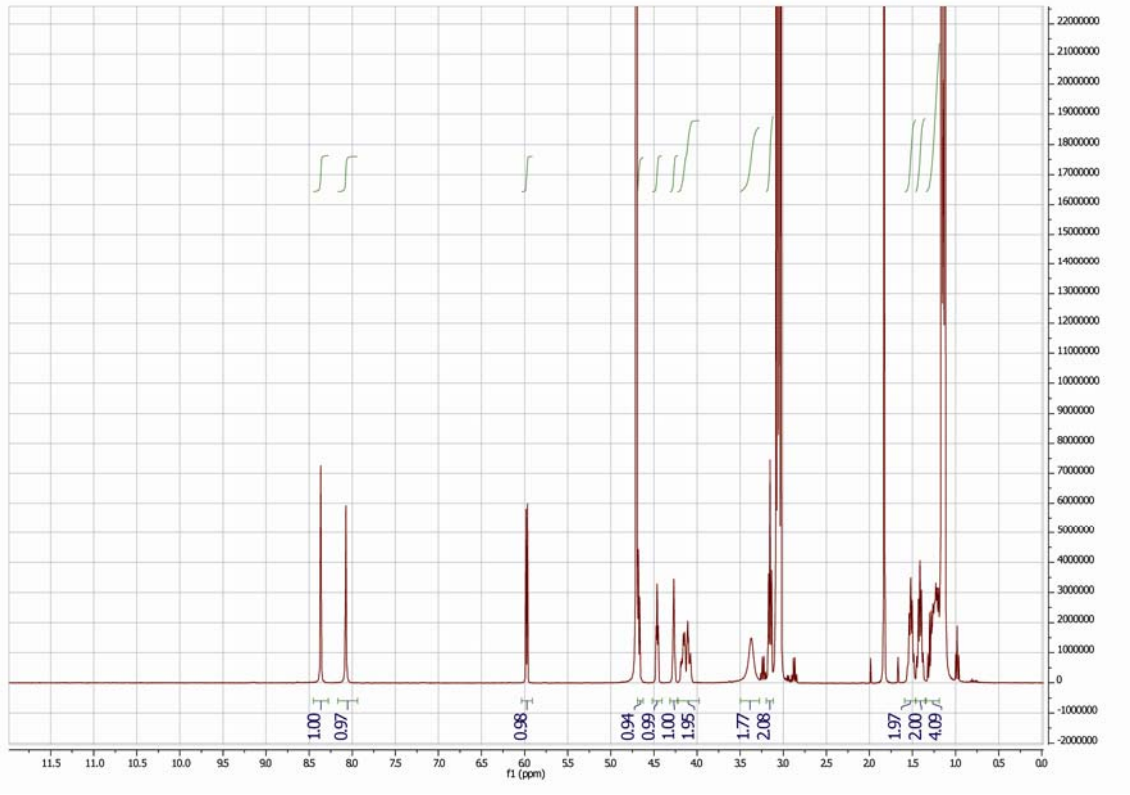


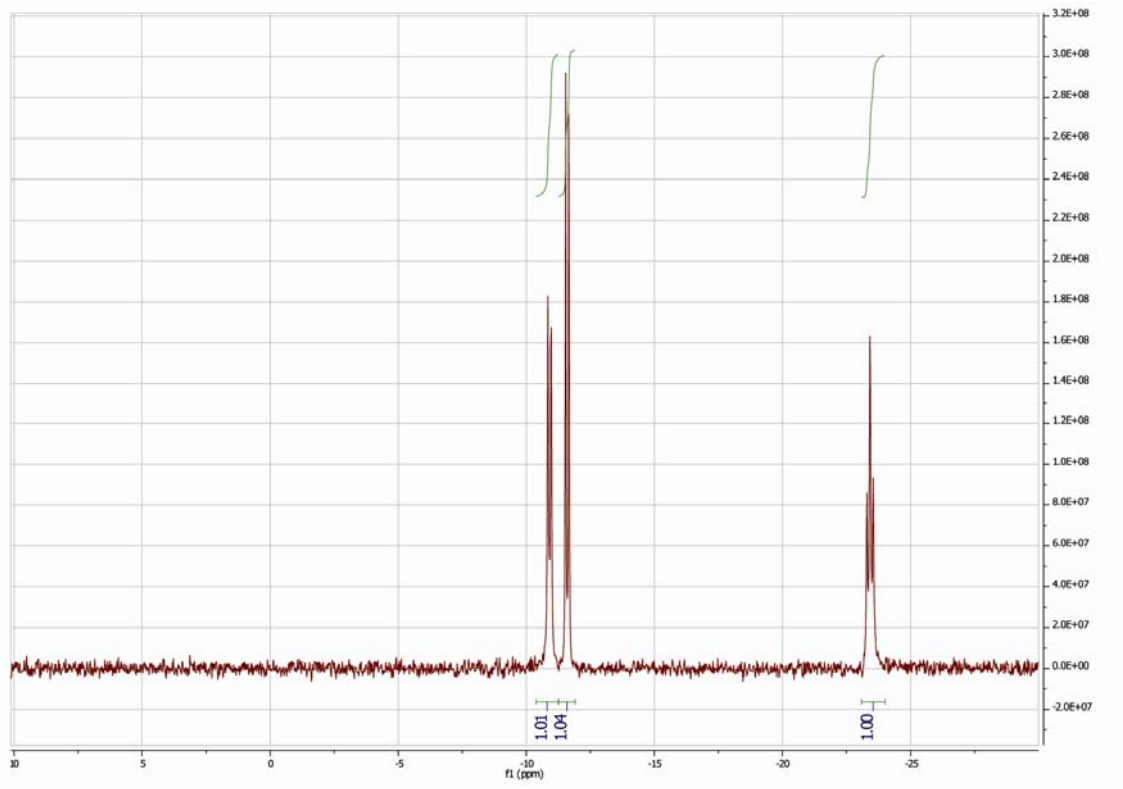
2-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine-triphosphate 5:



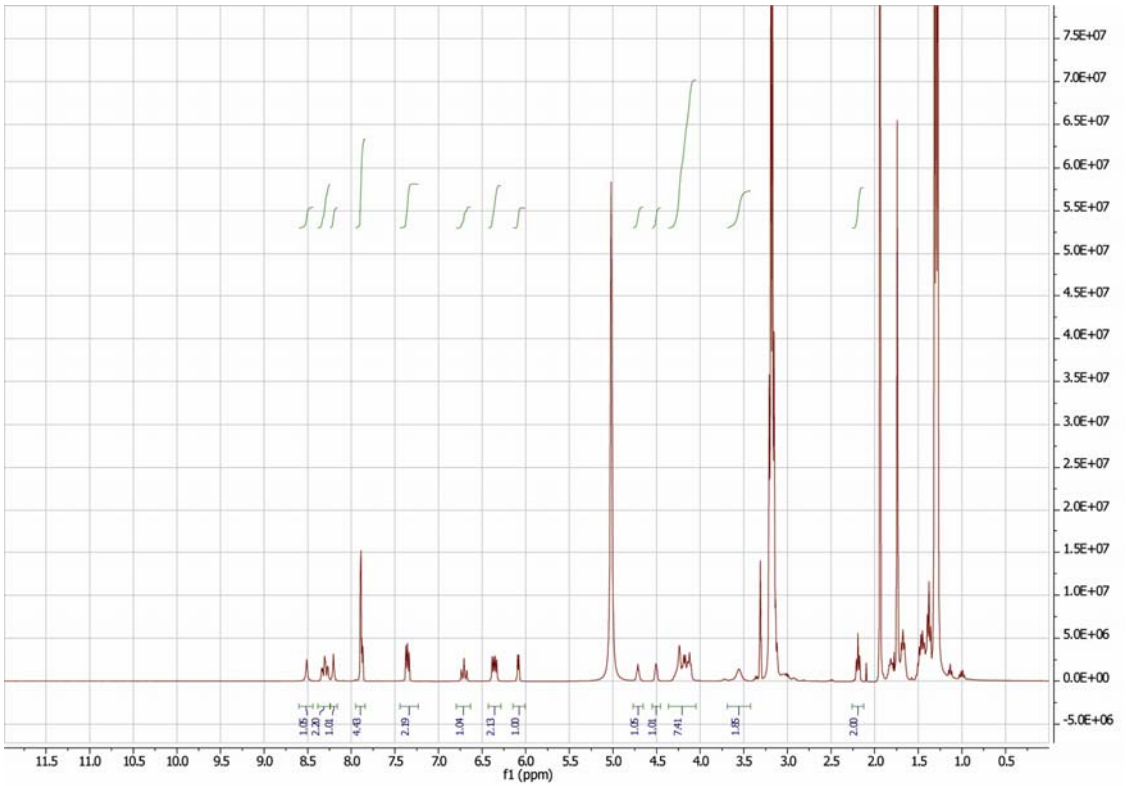


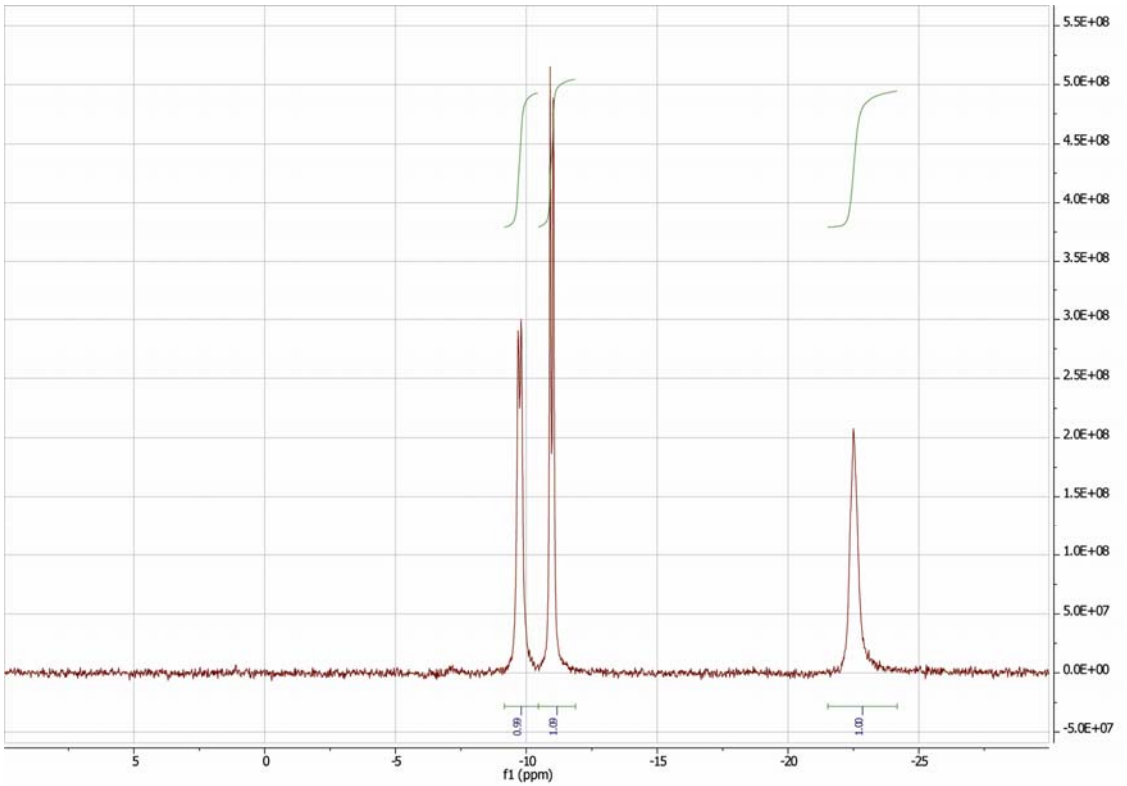
N^6-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 6:



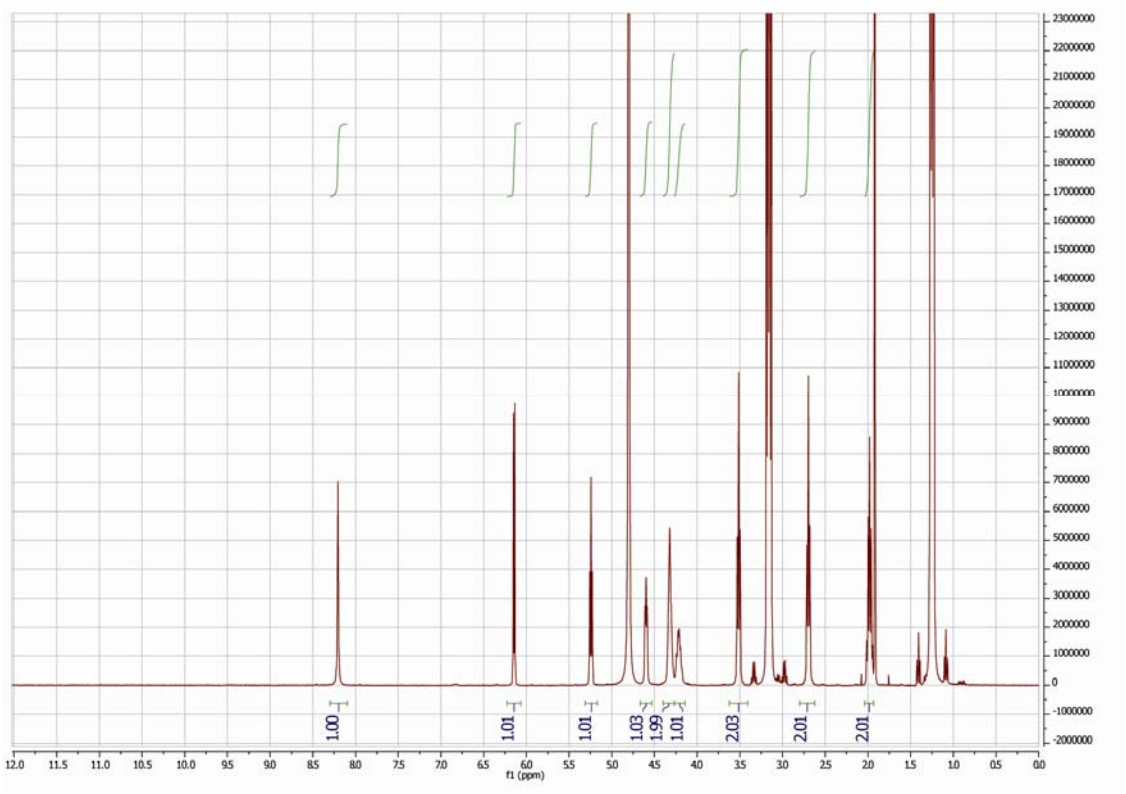


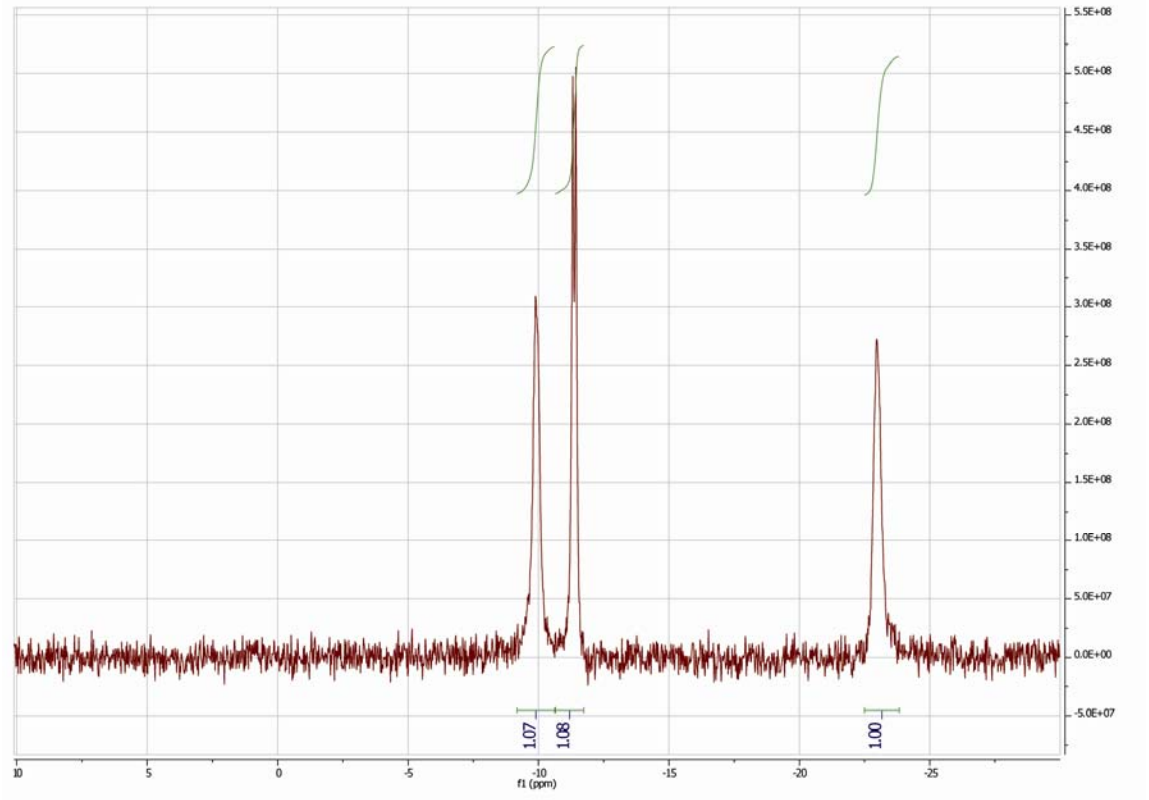
N^6-(6-Cy5-amidohexyl)-adenosine-5'-triphosphate 6d:





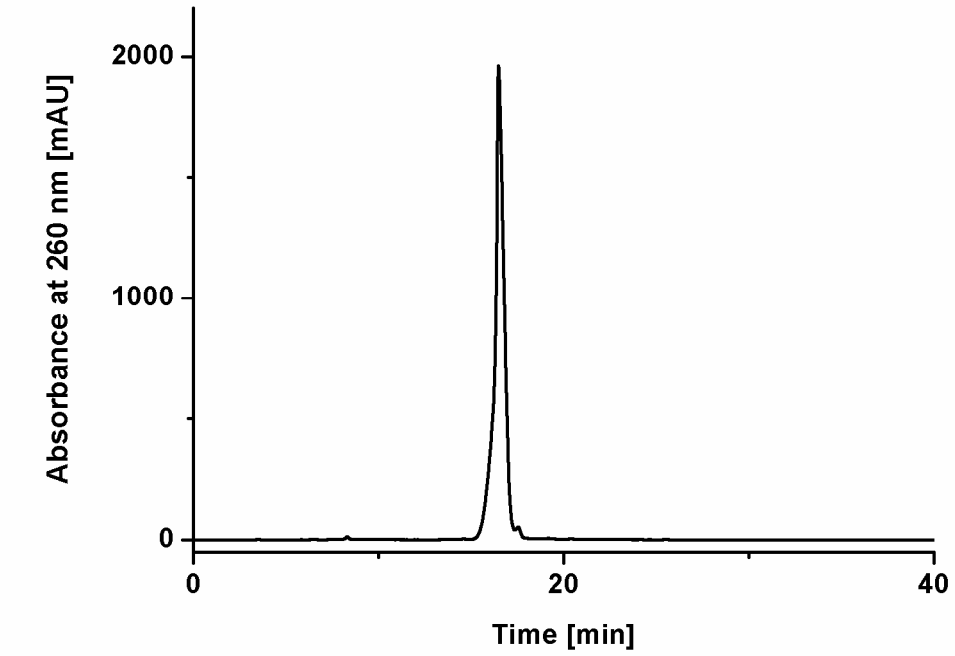
8-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine-5’-triphosphate 7:



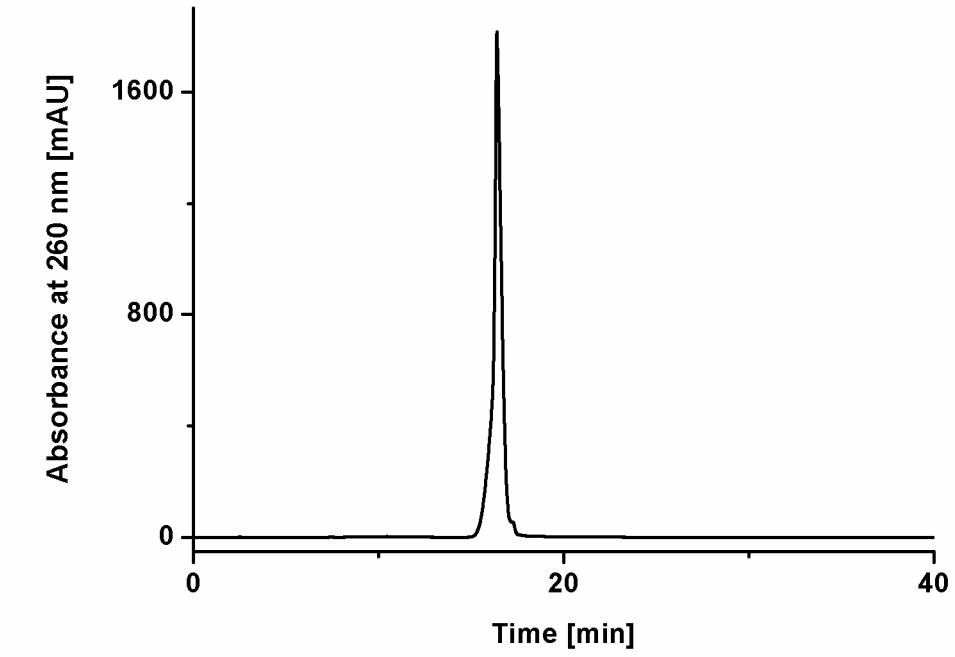


HPLC chromatograms

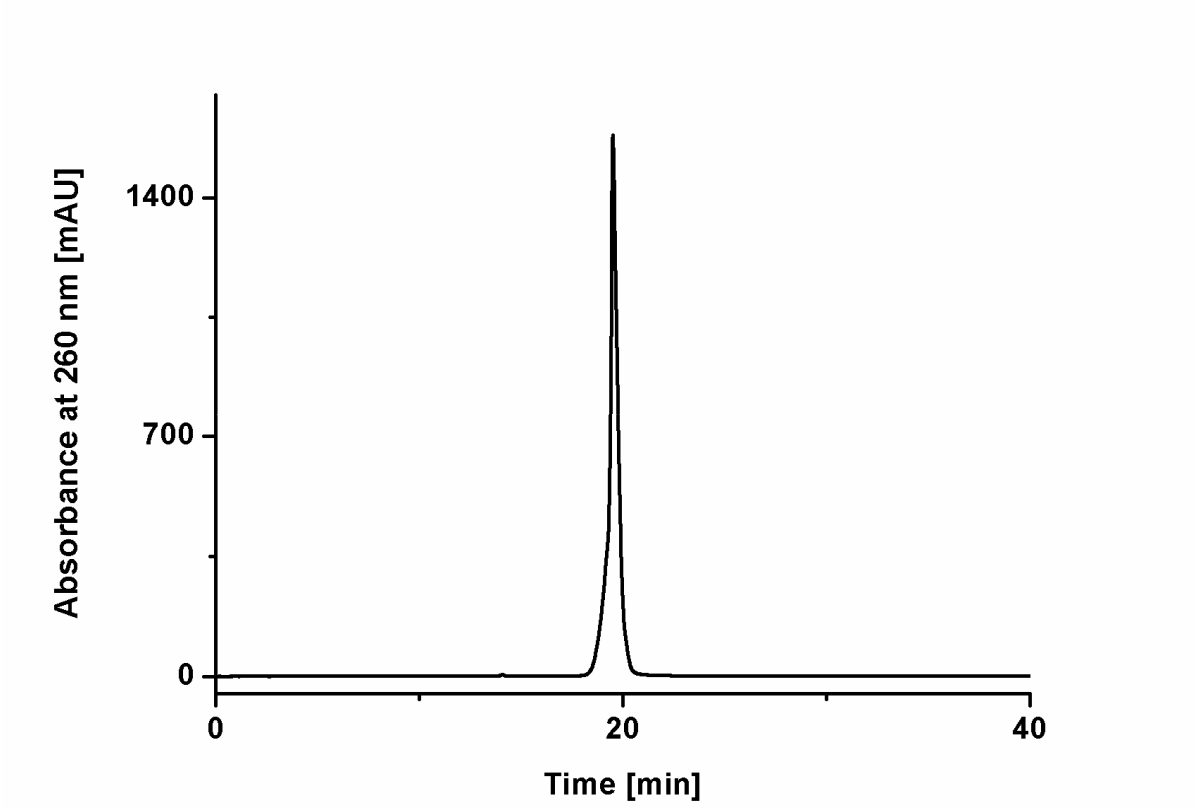
2'-*O*-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 2:



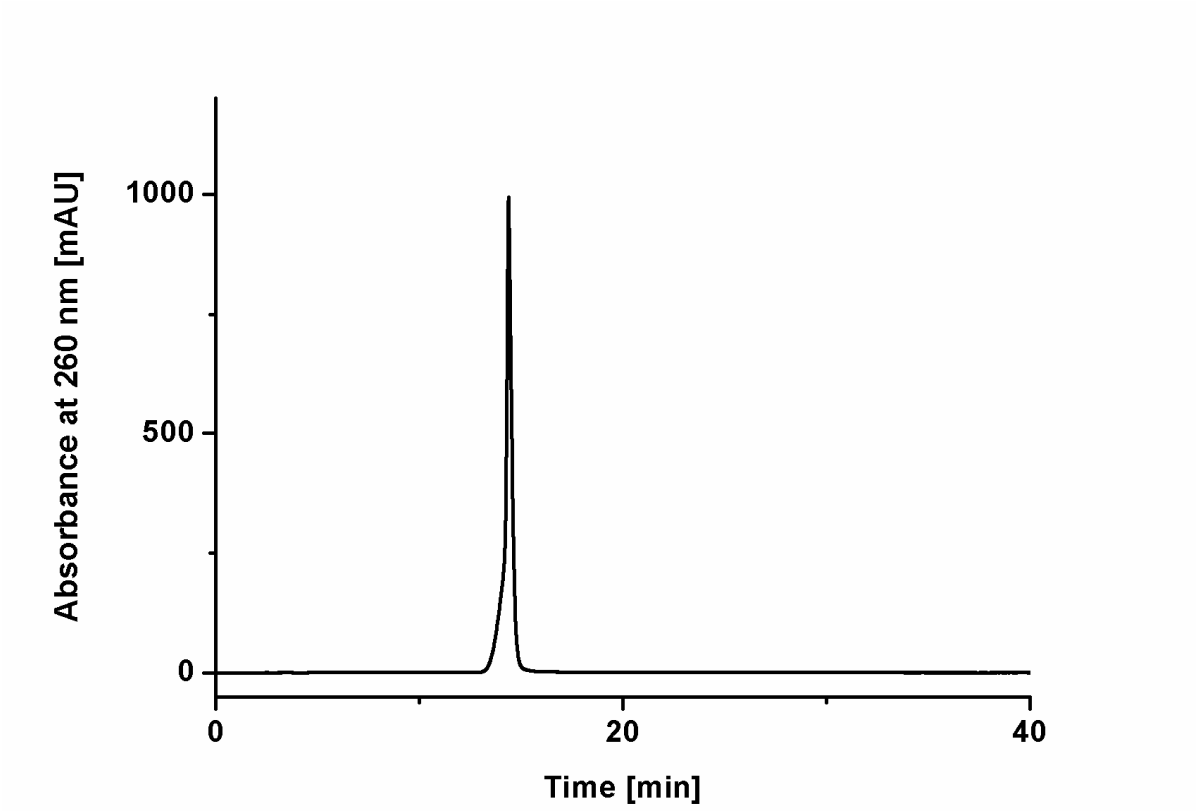
3'-*O*-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 3:



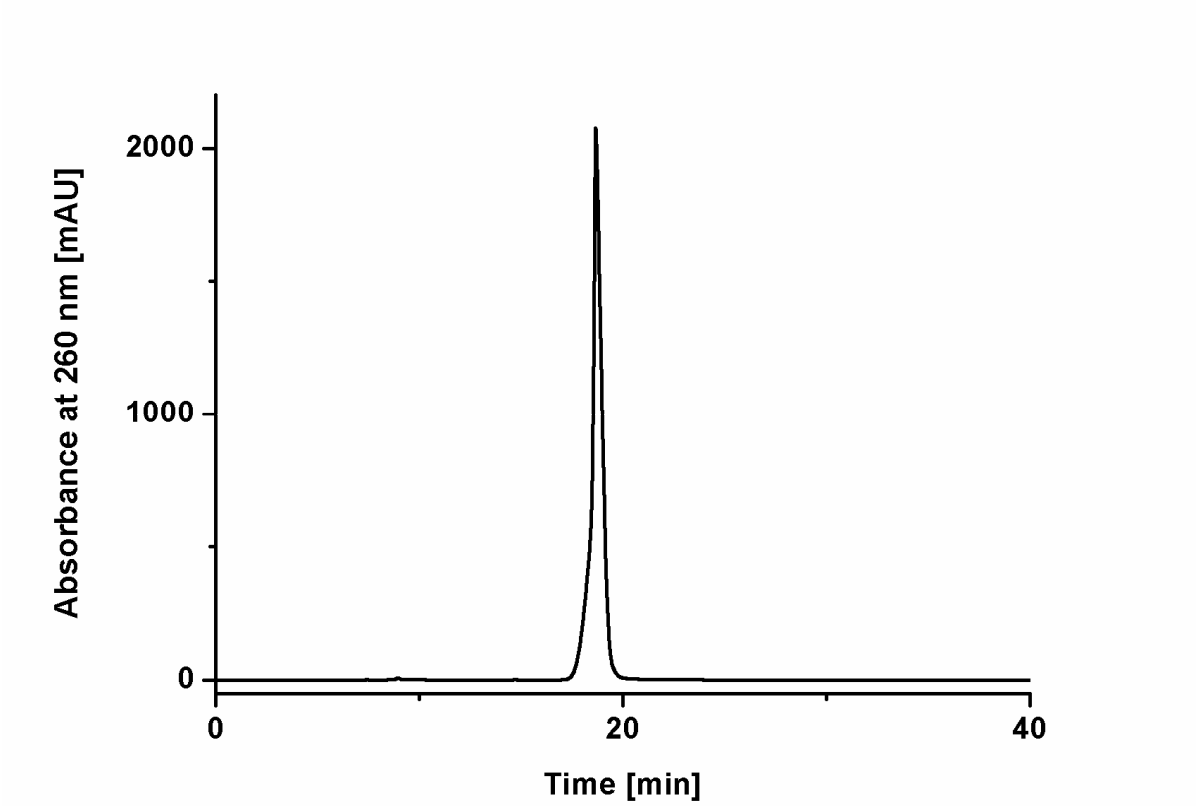
4'-*C*-(7-(trifluoroacetamido)-heptyl)-adenosine-triphosphate 4:



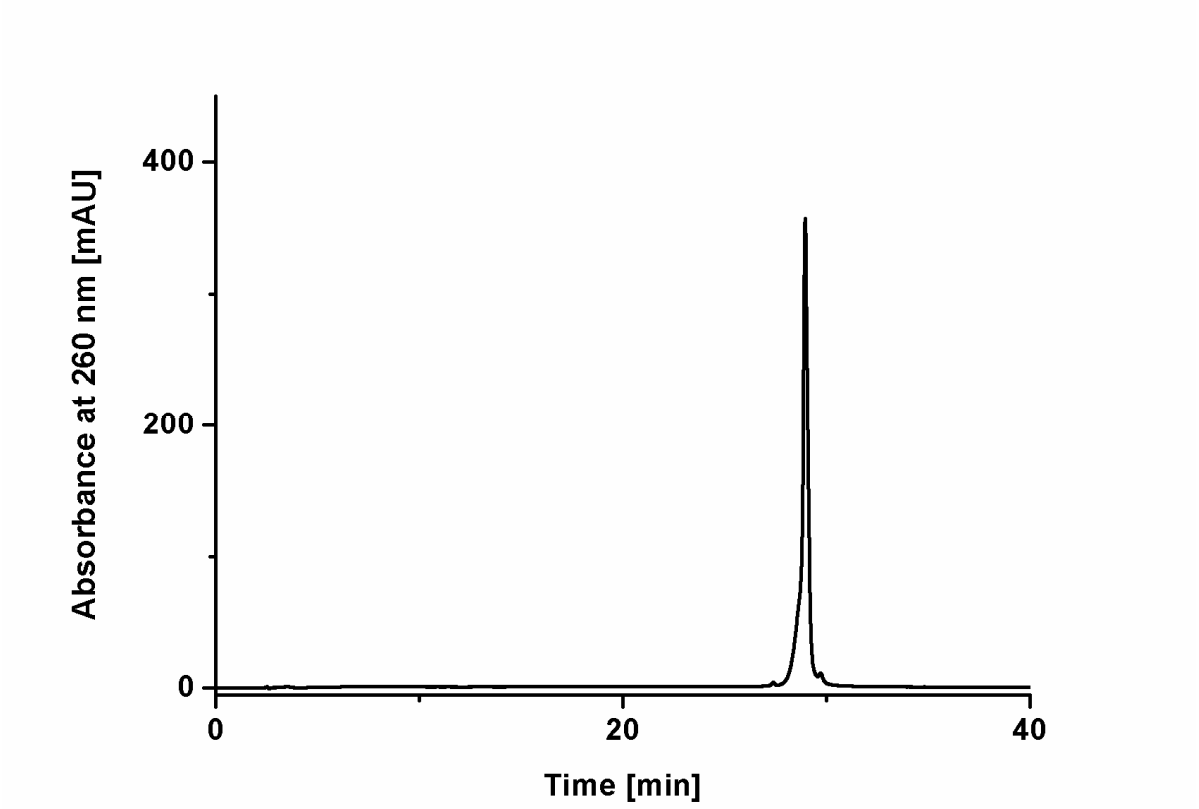
2-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine-triphosphate 5:



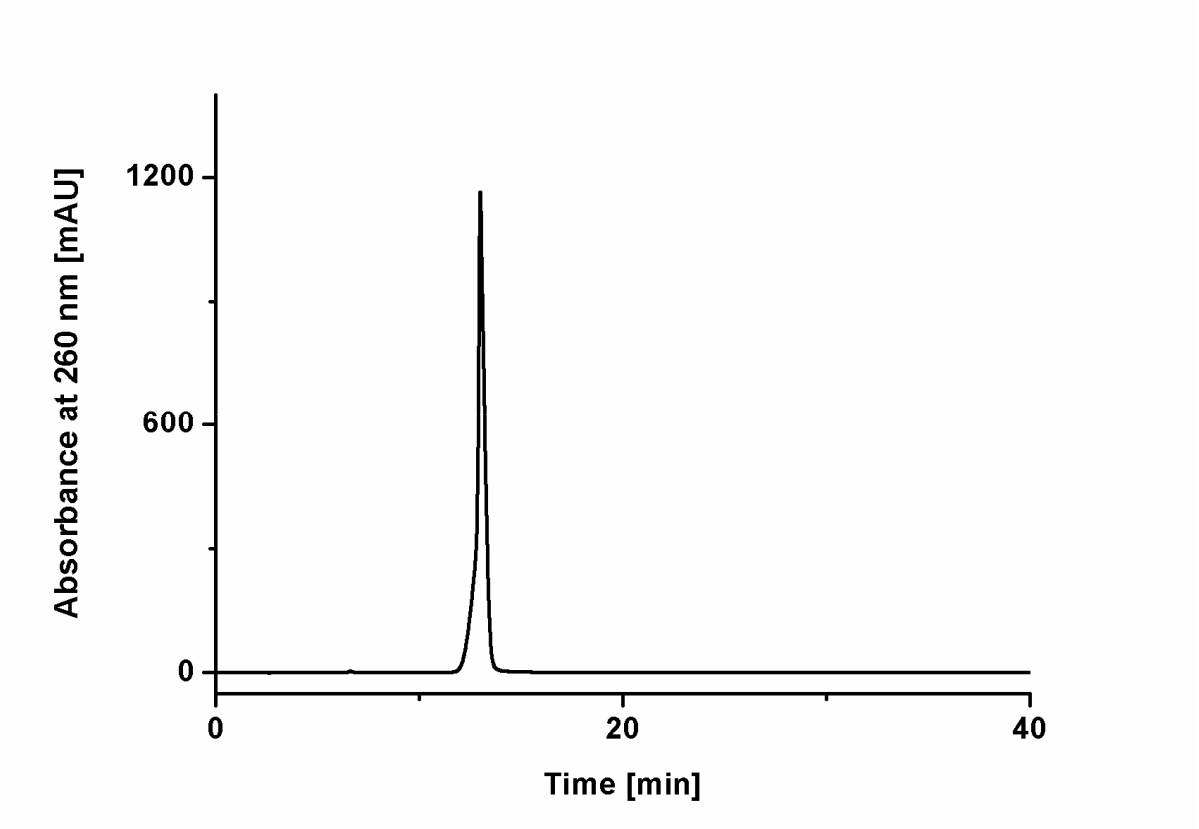
N^6-(6-Trifluoroacetamidohexyl)-adenosine-5'-triphosphate 6:



N^6-(6-Cy5-amidohexyl)-adenosine-5'-triphosphate 6d:



8-(5-Trifluoroacetamido-pent-1-yn-1-yl)-adenosine-5'-triphosphate 7:



Expression and purification of human FAK kinase domain

The kinase domain of human FAK (aa 411-689, hFAK-KD) was expressed with an amino-terminal 6xHis-tag and a carboxy-terminal PEYFK sequence (FYVE epitope-tag; tag-tools). Expression of the fusion protein was performed in Sf9 insect cells using the pVL1392 transfer vector and the BaculoGold Transfection Kit (BD Biosciences). Cells were lysed in Sf9 lysis buffer (0.33% Tween20, 1 mM EDTA, 500 mM NaCl, 100 µM PMSF, 1 mM β-mercaptoethanol in 25 mM Tris-HCl, pH 7.5) for 20 min at 4°C. The lysate was cleared for 30 min at 20000xg at 4°C. The recombinant hFAK-KD was purified by fast protein liquid chromatography using a HisTrapFFcrude column (GE Healthcare).

in vitro protein tyrosine kinase assay

A 96-well plate was coated with 1.0 µg per well of Poly-Glu-Tyr peptide (molar ratio of 4:1; Sigma) in MilliQ water for 1 h at 37°C and blocked with 1% BSA, 0.02% NaN_3 in PBS with 0.01% Tween (30 min, 37°C). 100 µM ATP (or ATP analogue) were added with or without purified hFAK-KD (0.4 µg per well) in kinase buffer (125 mM NaCl, 48 mM $MgCl_2$, 50 mM HEPES, pH 7.5) in a total volume of 50 µL. The plate was incubated at 37°C for indicated time intervals, then the kinase reaction was stopped by the addition of 100 µL 1 mM EDTA in PBS. The wells were washed three times with 100 µl 0.05% Triton X-100 in PBS. Phosphorylated peptide was detected by monoclonal anti-phosphotyrosine antibody (Upstate Biotechnology) followed by peroxidase-coupled goat anti-mouse antibody. TMB solution (0.5 mM 3,3´,5,5´-tetramethylbenzidine in 0.5% acetone, 4.5% ethanol and 1 mM H_2O_2 in 30 mM potassium citrate, pH 4.1) was added and the reaction was stopped with 2 M H_2SO_4 after 2-10 minutes. The absorbance was measured at 450 nm. The values were normalized to the absorbance after incubation with natural ATP for 1 h.

Expression and purification of Eg5 assay components

His-Eg5 (motor domain, aa 1 – 371) was expressed in *E. coli* and purified by Ni-NTA chromatography. N-terminally His_{10}-tagged, full length human Eg5 was expressed in SF9 cells using the Baculo™ virus system. Cells were lysed in lysis buffer (25 mM Tris-HCl pH 8.0, 250 mM NaCl, 1 mM ATP, 5 mM $MgCl_2$, 0.1% TX-100 and Complete™ protease inhibitors) by douncing on ice. Cleared lysate was incubated with Ni-NTA agarose beads for 2 h at 4°C, washed with lysis buffer incl. 20 mM Imidazole and eluted repetitively with 0.5x bed volumes of elution buffer (25 mM Tris-HCl pH 8.0, 250 mM NaCl, 1 mM ATP, 5 mM $MgCl_2$, 200 mM Imidazole). Elution fractions were analyzed by SDS-PAGE and subsequent coomassie staining / western blot analysis using anti-Eg5 antibody. Selected elution fractions were dialyzed (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 10% glycerol, 10 mM 2-mercaptoethanol, 1 mM ATP, 5 mM $MgCl_2$) over night at 4°C. Dialyzed His_{10}-Eg5 was aliquoted in small fractions, snap frozen and stored at -80°C. Microtubules were prepared from tubulin purified from pig brain.[9] 1 ml of tubulin (10 mg/ml) was cleared by centrifugation (186,000 g, 4°C, 10 min). 25% glycerol, 1.5 mM GTP and buffer containing 20 mM PIPES, 1 mM $MgCl_2$ and 1 mM EGTA were added to a final volume of 2 ml. The mixture was incubated at 37°C for 30 minutes. 40 µM taxol were added and incubation continued for additional 30 minutes. The microtubules were collected by centrifugation (186,000 g, 35°C, 30 min) and resuspended in buffer containing 20 mM PIPES, 1 mM $MgCl_2$, 1 mM EGTA and 25 µM taxol.

Malachite Green Assay for Eg5 activity

ATP or the indicated ATP analogue (100 µM), His-Eg5 (motor domain, 25 nM) and microtubules (100 nM) were incubated in buffer containing 20 mM PIPES (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mg/ml BSA and 0.1 µM taxol at room temperature. After 0 min, 3 min and 6 min 10 µl of reaction mixture were quenched by addition of 40 µl 1 M $HClO_4$. 50 µl malachite green solution (1.3 M HCl, 8.5 mM ammonium molybdate, 363 nM malachite green) were added. After 20 minutes at room temperature absorbance at 650 nm was measured. Relative absorbance was calculated by subtracting the absorbance after 0 min.. The relative absorbance for natural ATP after 6 min of incubation was normalized to 1.

Microtubule-gliding assay

Microtubule-gliding assays were performed as described[10], with the following exceptions: Cy3-labeled tubulin instead of rhodamine-labeled tubulin was used. The final concentration of precleared (25000 x g, 5 min, 4°C) His_{10}-Eg5 floated into the motility chamber was 5 µM. Gliding assays were performed in the presence of 10 mM ATP or 10 mM ATP-analogue. Measurements were performed on a Zeiss Axio Imager M1 with a Photometrics Cascade II:512 EMCCD camera (20ms acquisition, gain: 1000, 10 sec steps over 3 min) and Visiview Software from Visitron. Velocity measurements were performed using ImageJ and the plugin MTrackJ.

Expression and purification of enzyme components for the E6AP assay

Human recombinant UBA1 and His-E6AP were expressed SF9 cells and purified by anion-exchange chromatography or Ni-NTA chromatography, respectively. Human recombinant UbcH5b was expressed as His-tagged protein in *E. coli* and purified by Ni-NTA chromatography. Human recombinant ubiquitin was expressed in *E. coli* and isolated by heat denaturation (75°C, 30 min) and purification of the supernatant by anion-exchange chromatography followed by dialysis with 50 mM NaCl, 25 mM Tris (pH 7.6).

E6AP polyubiquitylation assay for UBA1 activity

For the initial screen of the ATP analogues, ATP or the indicated ATP analogue (500 µM), UBA1 (150 nM), His-UbcH5b (48 µg/ml), His-E6AP (300 nM) and ubiquitin (500 µg/ml) were incubated in buffer containing 25 mM Tris-HCl (pH 7.6), 50 mM NaCl, 5 mM $MgCl_2$ and 1.25 mM DTT at 37 °C for 1 hour. The solution was quenched by addition of SDS-loading buffer. The outcome of the reaction was monitored by SDS-PAGE (upper separation gel: 10% acrylamide, lower separation gel: 15% acrylamide) and staining with coomassie brilliant blue. The intensity of the ubiquitin band was quantified using AIDA software. For all reactions consumption of ubiquitin was calculated relative to incubation without ATP and this value was normalized to natural ATP.

For the time-dependent analysis using a rate-limiting amount of UBA1, UBA1 (10 nM), His-UbcH5b (20 µg/ml) and ubiquitin (250 µg/ml) were used. All other conditions were the same. The reaction

was quenched after 0 hours, 1 hour and 2 hours and the outcome of the reaction was monitored as described above. The intensity of the ubiquitin band was quantified using AIDA software. For all reactions consumption of ubiquitin was calculated relative to incubation for 0 hours and this value was normalized to consumption of ubiquitin after incubation with natural ATP for 2 hours.

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