Supporting Information to

The Synthesis of Methylated, Phosphorylated, and Phosphonated 3'-Aminoacyl-tRNA^{Sec} Mimics

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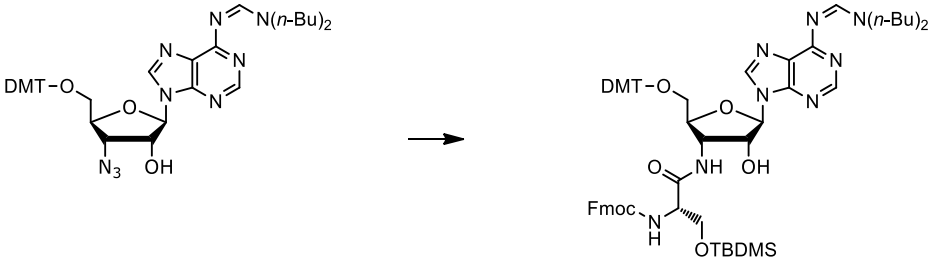
1 Supporting Methods

1.1 General remarks

^{1}H and ^{13}C NMR spectra were recorded on a *Bruker* DRX 300 MHz or *Bruker* UltraShield™ Plus 600 MHz instrument. The chemical shifts (*δ*) are reported relative to tetramethylsilane (TMS) and referenced to the residual proton signal of the deuterated solvent $CDCl_3$: 7.26 ppm for ^{1}H NMR spectra or 77.1 ppm for ^{13}C NMR spectra. ^{1}H and ^{13}C assignments are based on COSY and HSQC experiments. MS experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrumentation (*Thermo Fisher Scientific*) with an electrospray ion source. Samples were analyzed in the positive- or negative-ion mode. Reaction control was performed *via* analytical thin-layer chromatography (TLC, *Macherey-Nagel*) with fluorescent indicator. Column chromatography was carried out on silica gel 60 (70-230 mesh). Chemical reagents and solvents were purchased from commercial suppliers (*Sigma-Aldrich*, *IRIS Biotech GmbH*) and used without further purification. Custom Primer Support™ 200 Amino was purchased from *GE Healthcare*. Organic solvents for reactions were dried overnight over freshly activated molecular sieves (4Å). The reactions were carried out under argon atmosphere.

1.2 Synthesis of 3'-serinylamino-3'-deoxyadenosine functionalized solid support (**4**)

1.2.1 6-*N*-[(Di-*n*-butylamino)methylene]-3'-{[*N*-(9-fluorenyl)methoxycarbonyl-*O*-(*tert.*-butyldimethylsilyl)-L-serinyl]amino}-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-adenosine (**2**)



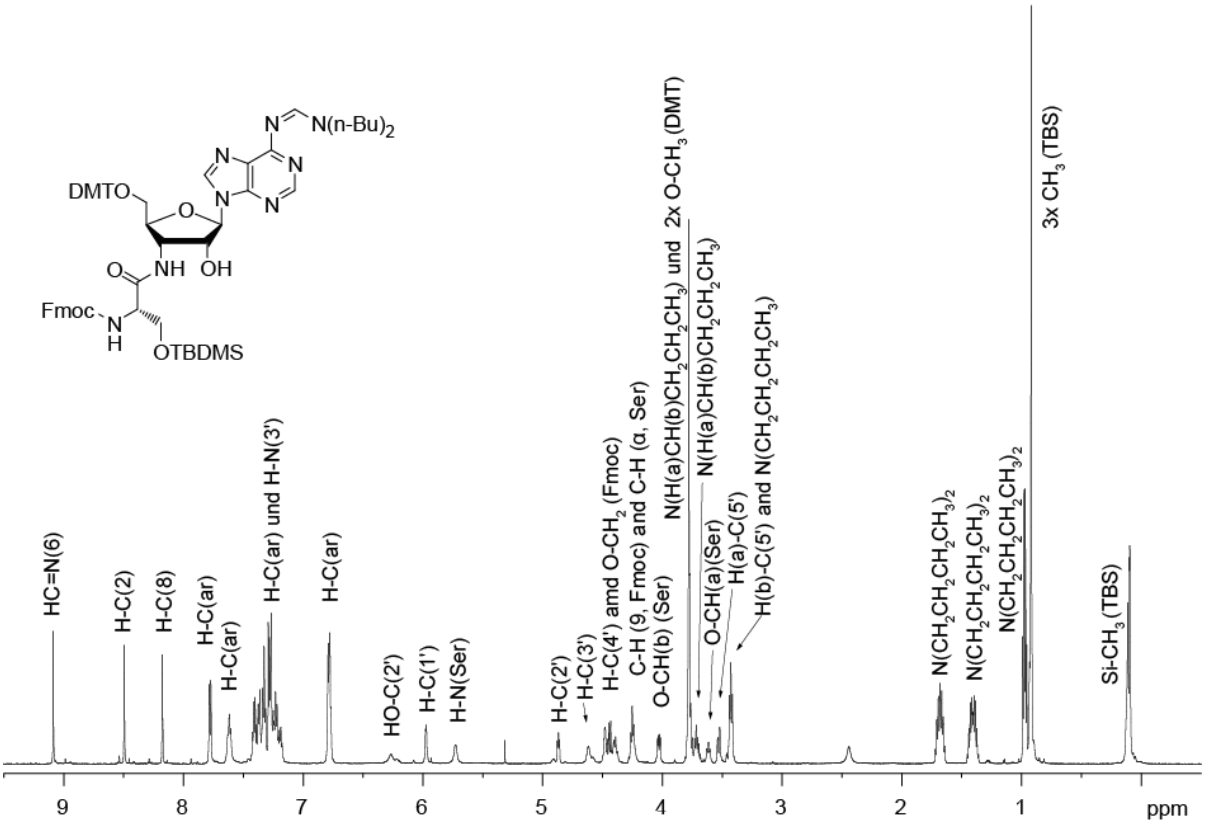
1 **2**

Fmoc-L-Ser(TBDMS)-OH[1] (172 mg, 0.39 mmol) was coevaporated three times with THF and dissolved in 6.3 mL THF. The solution was cooled to 0°C followed by the addition of 1-hydroxybenzotriazole hydrate (78 mg, 0.49 mmol). After 10 min *N*,*N*-diisopropylcarbodiimide (79 µL, 0.49 mmol) was added and stirred for another 10 min. Then, 3'-azido adenosine **1** (220 mg, 0.29 mmol), which was dissolved in 8.6 mL THF, was added dropwise. After another 15 min, trimethylphosphine (1M in THF, 644 µL, 0.64 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred overnight. Subsequently, the solvent were removed under reduced pressure, the residue was dissolved in CH_2Cl_2. The solution was extracted with water and half saturated $NaHCO_3$ solution. The organic layers were collected, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH = 99/1–96/4) to yield 316 mg of compound **2** as white foam (93 %).

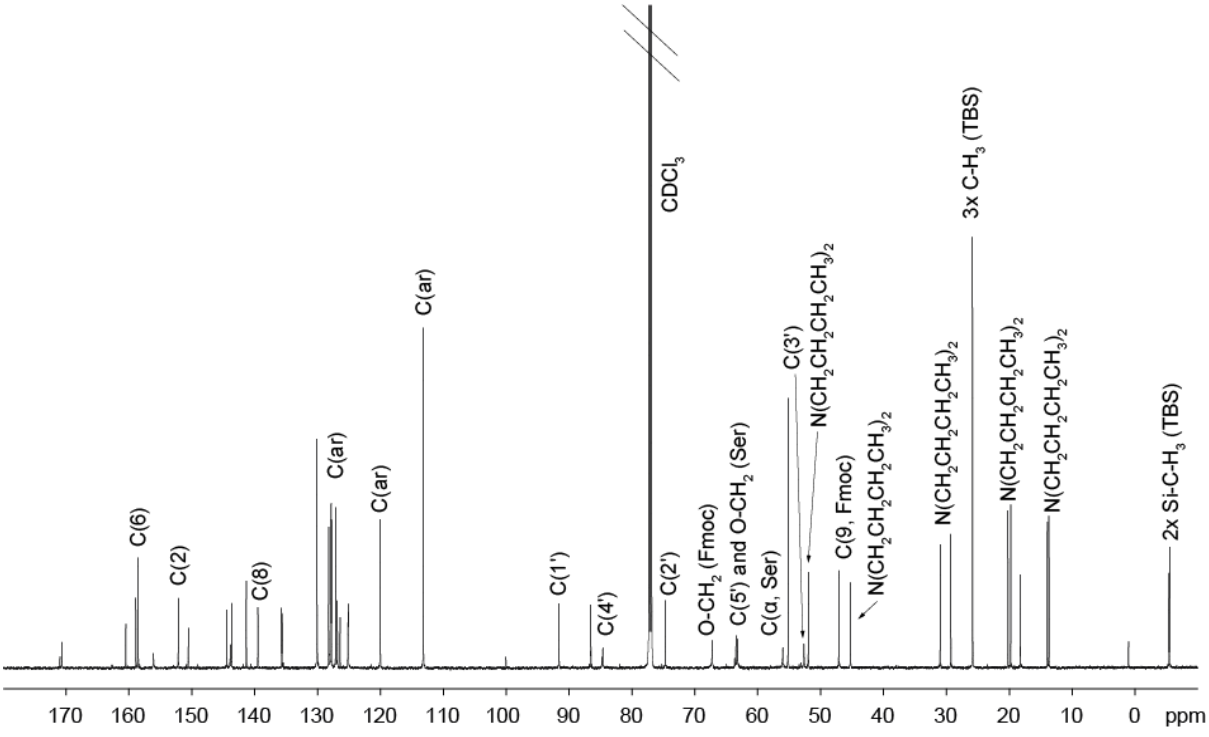
^{1}H-NMR (600 MHz, $CDCl_3$): δ 9.08 (s, 1H, HC=N(6)); 8.49 (s, 1H, HC(2)); 8.17 (s, 1H, HC(8)); 7.77 (d, 2H, J = 7.5, HC(ar)); 7.61 (t, 2H, J = 7.6, HC(ar)); 7.41-7.18 (m, 17 H, HC(ar) and HN(3')); 6.78 (d, 4H, J = 7.9, HC(ar)); 6.26 (s, 1H, HOC(2')); 5.97 (d, 1H, J = 3.8, HC(1')); 5.72 (s, 1H, HN(Ser)); 4.86 (t, 1H, J = 5.9 and J = 4.8, HC(2')); 4.61 (s, 1H, HC(3')); 4.49-4.39 (m, 3H, HC(4') and OCH_2(Fmoc)); 4.25 (t, 2H, J = 7.2 and J = 7.1, CH(9, Fmoc) and CH(α, Ser)); 4.02 (d-d, 1H, J = 4.0 and J = 9.8, OCH(b, Ser)); 3.78 (m, 7H, N(H(a)CH(b)$CH_2CH_2CH_3$) and 2x OCH_3(DMT)); 3.72 (m, 1H, N(C$H_2$$CH_2CH_2CH_3$)); 3.61 (t, 1H, J = 8.9, OCH(a, Ser)); 3.53 (d, 1H, J=9.3, H(a)-C(5')); 3.43 (t, 3H, J=6.8 and J=7.7, H(b)-C(5') and N(C$H_2$$CH_2CH_2CH_3$)); 1.68 (m, 4H, N($CH_2CH_2$$CH_2CH_3$)$_2$); 1.42 (m, 4H, N($CH_2CH_2CH_2$$CH_3$)$_2$); 0.98 (q, 6H, N($CH_2CH_2CH_2CH_3$)$_2$); 0.92 (s, 9H, 3x CH_3(TBDMS)); 0.10 (s, 6H, Si-CH_3(TBDMS)). ^{13}C-NMR (150 MHz, $CDCl_3$): δ 170.9, 170.6, 160.5, 158.9, 158.5 (C(6)), 156.1, 152.1 (C(2)), 150.5, 144.4, 143.8, 143.7, 141.3, 139.4 (C8), 135.7, 135.6, 130.1 (C(ar)), 128.2, 127.8 (C(ar)), 127.7 (C(ar)), 127.1

(C(ar)), 126.8 (C(ar)), 126.4, 125.1 (C(ar)), 120.0 (C(ar)), 113.1, (C(ar)), 91.6 (C(1')), 86.5, 84.6 (C(4')), 74.7 (C(2')), 67.2 (OCH_2 (Fmoc)), 63.5, (C(5')), 63.2 (OCH_2(Ser)), 56.0 (C(α, Ser)), 55.2, 52.7 (C(3')), 52.0 ($N(CH_2CH_2CH_2CH_3)_2$), 47.1 (C(9, Fmoc)), 45.3 ($N(CH_2CH_2CH_2CH_3)_2$), 31.0 and 29.3 ($N(CH_2CH_2CH_2CH_3)_2$), 25.8 (CH_3 (TBDMS)), 20.2 and 19.8 ($N(CH_2CH_2CH_2CH_3)_2$), 18,2, 13,9 and 13,7 ($(N(CH_2CH_2CH_2CH_3)_2$), 1,0, -5,4 and -5,5 (2x $SiCCH_3$ (TBDMS)). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{64}H_{79}N_8O_9Si$, 1131.57; found 1131.71.

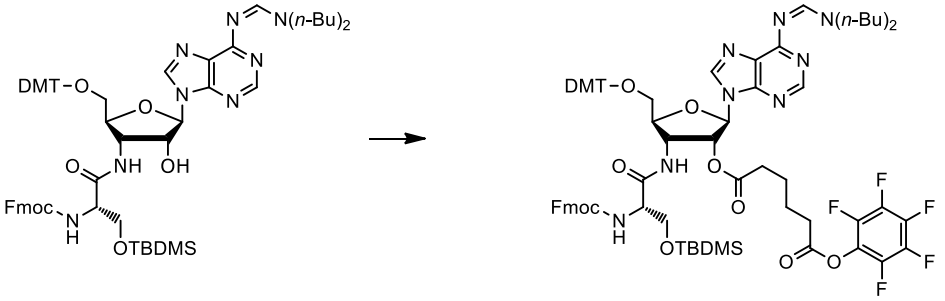
^{1}H-NMR (600 MHz, $CDCl_3$):



^{13}C-NMR (150 MHz, $CDCl_3$):



1.2.2 6-*N*-[(Di-*n*-butylamino)methylene]-3'-{[*N*-(9-fluorenyl)methoxycarbonyl-*O*-(*tert.*-butyldimethylsilyl)-L-serinyl]amino}-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[1,6-dioxo-6-(pentafluorophenyloxy)hexyl]-*β*-D-adenosine (**4.1**)

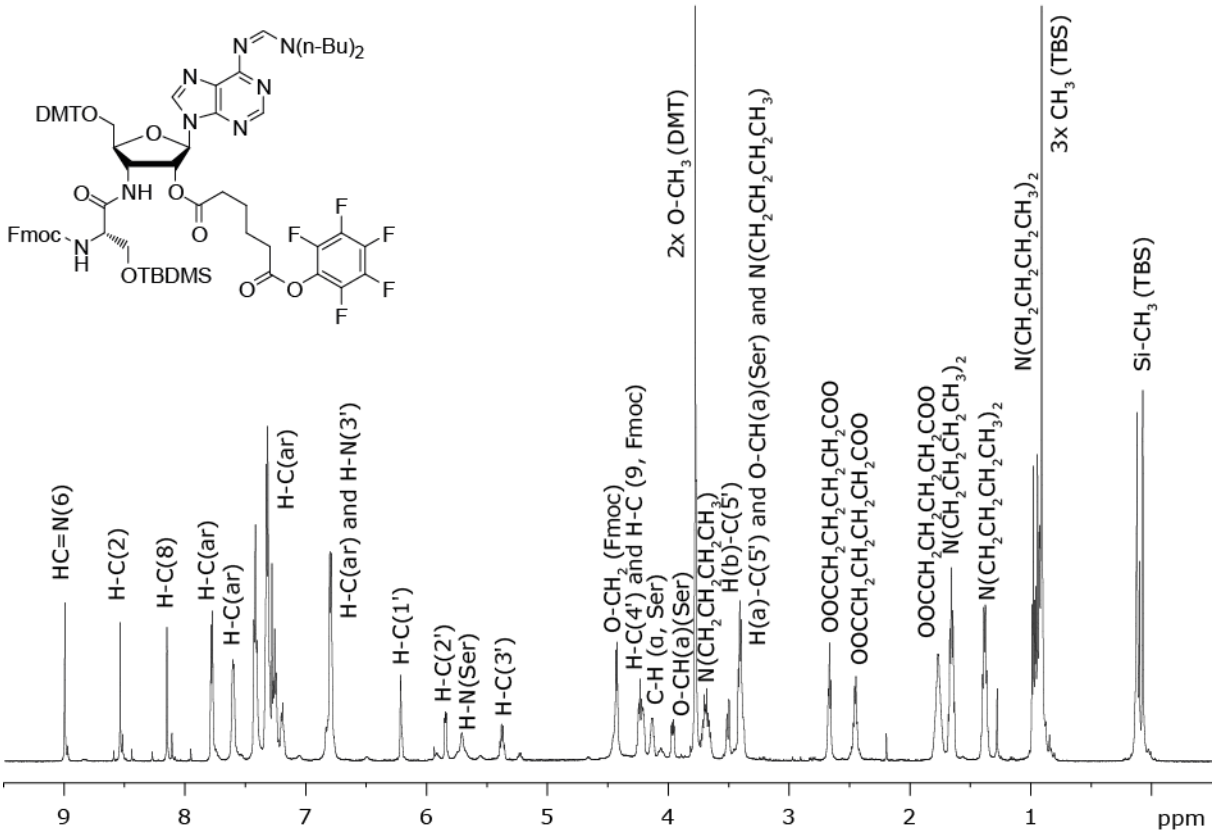


2 **4.1**

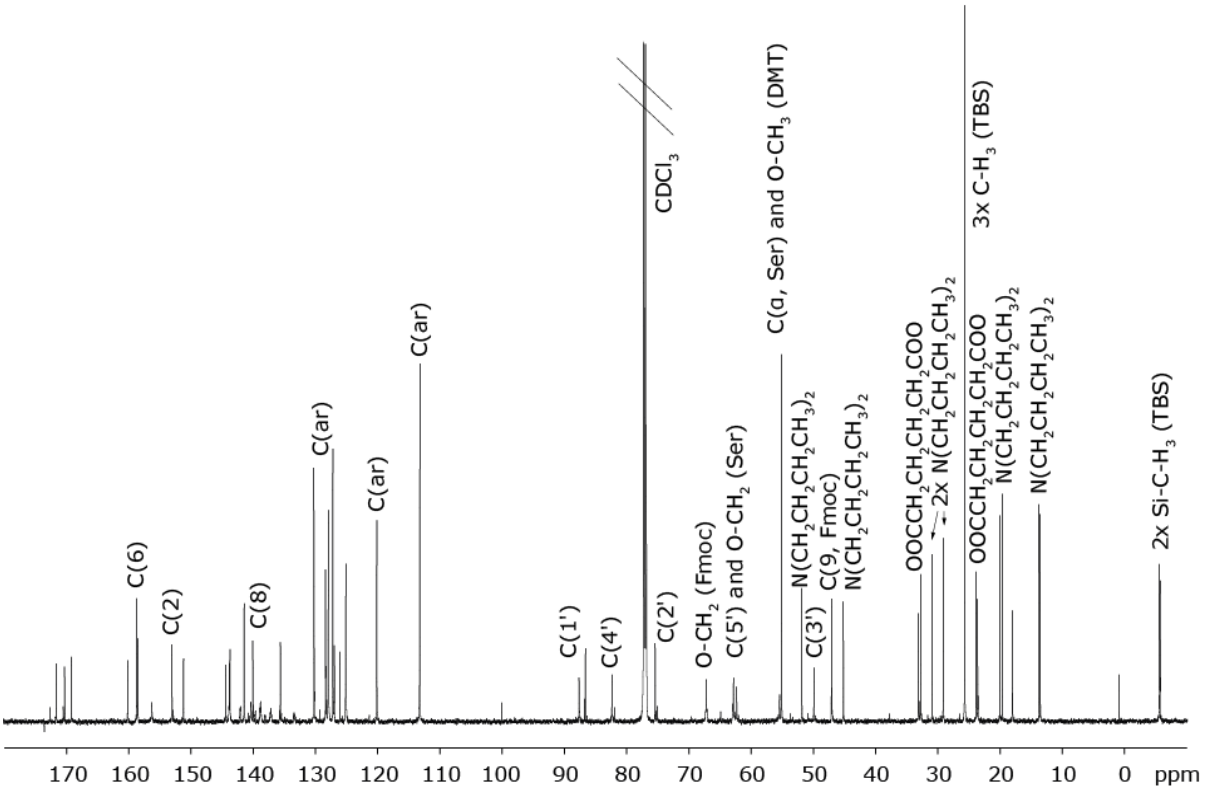
Compound **2** (50 mg, 0.04 mmol) was dissolved in 1.1 mL DMF/pyridine (1/1), 4-(*N*,*N*-dimethylamino)pyridine (6 mg, 0.05 mmol) and adipic acid pentafluorophenyl ester[2] (68 mg, 0.14 mmol) were added and the mixture was stirred one hour at room temperature. Then, the solvents were removed under reduced pressure and the crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/acetone = 95/5 – 85/15) to yield 40 mg of compound **4.1** as white foam (63 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ 9.00 (s, 1H, HC=N(6)), 8.54 (s, 1H, HC(2)), 8.16 (s, 1H, HC(8)), 7.78 (d, 3H, J = 7.4, HC(ar)), 7.76 (d, 3H, J = 4.8, HC(ar)), 7.42 (m, 5H, HC(ar)), 7.33-7.20 (m, 14H, HC(ar)), 6.80 (d, 6H, J = 7.1, HC(ar) and HN(3')), 6.22 (m, 1H, HC(1')), 5.85 (m, 1H, HC(2')), 5.71 (s, 1H, HN(Ser)), 5.38 (q, 1H, J = 14.7 and J = 7.9, HC(3')), 4.43 (d, 2H, J = 7.0, OCH_2(Fmoc)), 4.24 (m, 3H, HC(4') and HC(9, Fmoc)), 4.13 (s, 1H, CH(α, Ser)), 3.97 (m, 1H, OCH(a)(Ser)), 3.78 (s, 6H, 2x OCH_3(DMT)), 3.70 (m, 3H, N($CH_2CH_2CH_2CH_3$)), 3.51 (d, 1H, J = 8.8, H(b)C(5')), 3.40 (m, 4H, H(a)C(5') and OCH(a)(Ser) and N($CH_2CH_2CH_2CH_3$)), 2.66 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$), 2.45 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$), 1.77 (s, 4H, $OOCCH_2CH_2CH_2CH_2COO$), 1.65 (m, 5H, N($CH_2CH_2CH_2CH_3$)$_2$), 1.38 (m, 5H, N($CH_2CH_2CH_2CH_3$)$_2$), 0.98 (m, 6H, N($CH_2CH_2CH_2CH_3$)$_2$), 0.91 (s, 9H, 3x CH_3(TBDMS)), 0.10 (m, 6H, 3x CH_3(TBDMS)). ^{13}C-NMR (600 MHz, $CDCl_3$): δ 172.4, 171.5, 170.32, 170.1, 169.0, 160.0, 158.5 (C(6)), 156.1, 152.9 (C(2)), 151.0, 144.3, 143.7, 143.6, 141.9, 141.3, 140.3 (C(8)), 139.9, 139.8, 138.7, 137.0, 135.5, 130.1 (C(ar)), 128.3 (C(ar)), 127.8 (C(ar)), 127.1 (C(ar)), 126.9 (C(ar)), 125.9 (C(ar)), 125.0 (C(ar)), 120.0 (C(ar)), 113.1 (C(ar)), 100.0, 87.6 (C(1')), 86.6, 82.3 (C(4')), 75.4 (C(2')), 67.2 (OCH_2(Fmoc)), 62.9 and 62.4 (C(5') and OCH_2(Ser)), 55.5 and 55.2 (C(α, Ser) and OCH_3 (DMT)), 52.0 (N($CH_2CH_2CH_2CH_3$)$_2$), 50.0 (C(3')), 47.1 (C(9, Fmoc)), 45.2 (N($CH_2CH_2CH_2CH_3$)$_2$), 33.2 and 32.8 ($OOCCH_2CH_2CH_2CH_2COO$), 31.0 and 29.2 (N($CH_2CH_2CH_2CH_3$)$_2$), 25.8 (3x CH_3 (TBDMS)), 24.0 ($OOCCH_2CH_2CH_2CH_2COO$), 20.1 (N($CH_2CH_2CH_2CH_3$)$_2$), 20.0, 18.1, 13.9 (N($CH_2CH_2CH_2CH_3$)$_2$), 13.7, 1.0, -5.5 (2x $SiCH_3$(TBDMS)).
ESI-MS (m/z): $[M+H]^+$ calcd for $C_{76}H_{86}F_5N_8O_{12}Si$, 1425.61; found 1425.57.

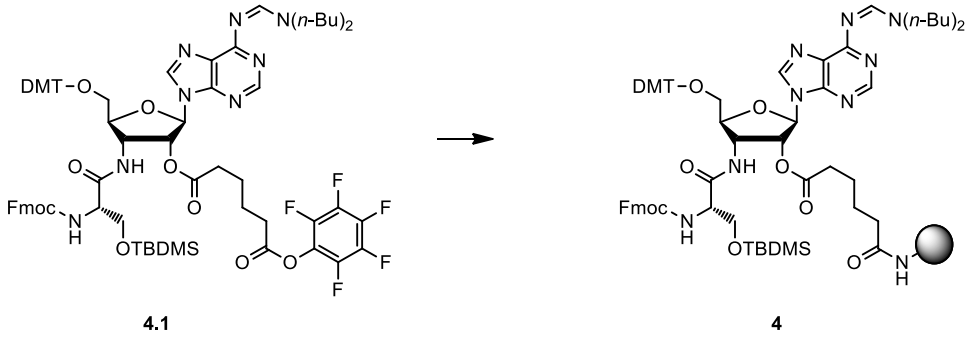
^{1}H-NMR (600 MHz, $CDCl_3$):



^{13}C-NMR (150 MHz, $CDCl_3$):



1.2.3 DMTO-rA-3'-NH-[Fmoc-Ser(TBDMS)] solid support (**4**)

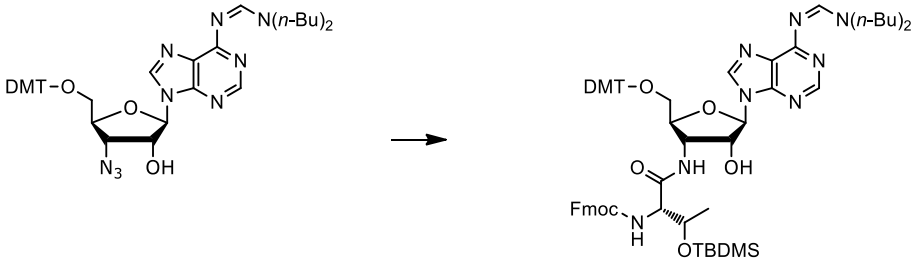


4.1 **4**

To a solution of active ester **4.2** (84 mg, 0.06 mmol) in 1.7 mL DMF was added amino-functionalized support (*GE Healthcare*, Custom Primer Support™ 200 Amino, 200 mg) and pyridine (6 µL, 0.07 mmol). The suspension was agitated for 20 hours at room temperature. The beads were collected on a Büchner funnel and washed with DMF, methanol and CH_2Cl_2. For capping of unreacted amino groups, the beads were treated with 10 ml of solution A (0.2 M phenoxy acetic anhydride in THF) and 10 ml of solution B (0.2 M *N*-methyl imidazole, 0.2 M *sym*-collidine in THF) and agitated for 10 min at room temperature. The suspension was filtrated again, the beads were washed with THF, methanol and CH_2Cl_2 and dried under vacuum. Loading of the solid support **4** was 52 µmol/g.

1.3 Synthesis of 3'-threonylamino-3'-deoxyadenosine functionalized solid support (5)

1.3.1 6-*N*-[(Di-n-butylamino)methylene]-3'-{[*N*-(9-fluorenyl)methoxycarbonyl-*O*-(*tert.*-butyldimethylsilyl)-L-threonyl]amino}-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-adenosine (**3**)

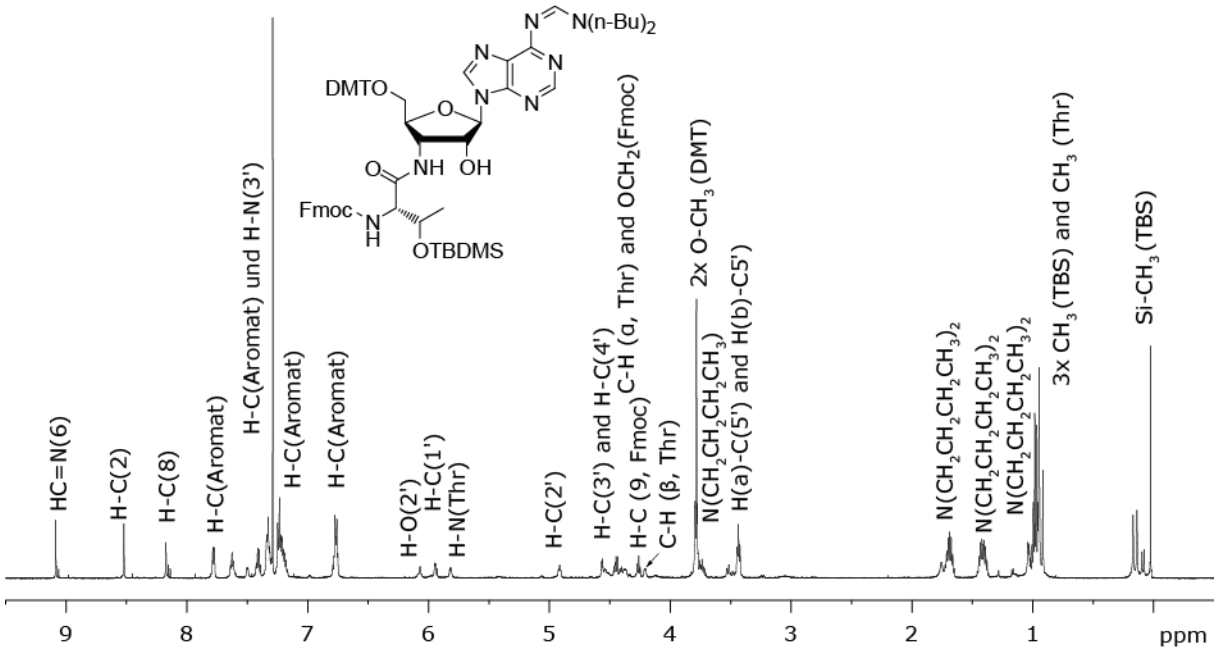


1 **3**

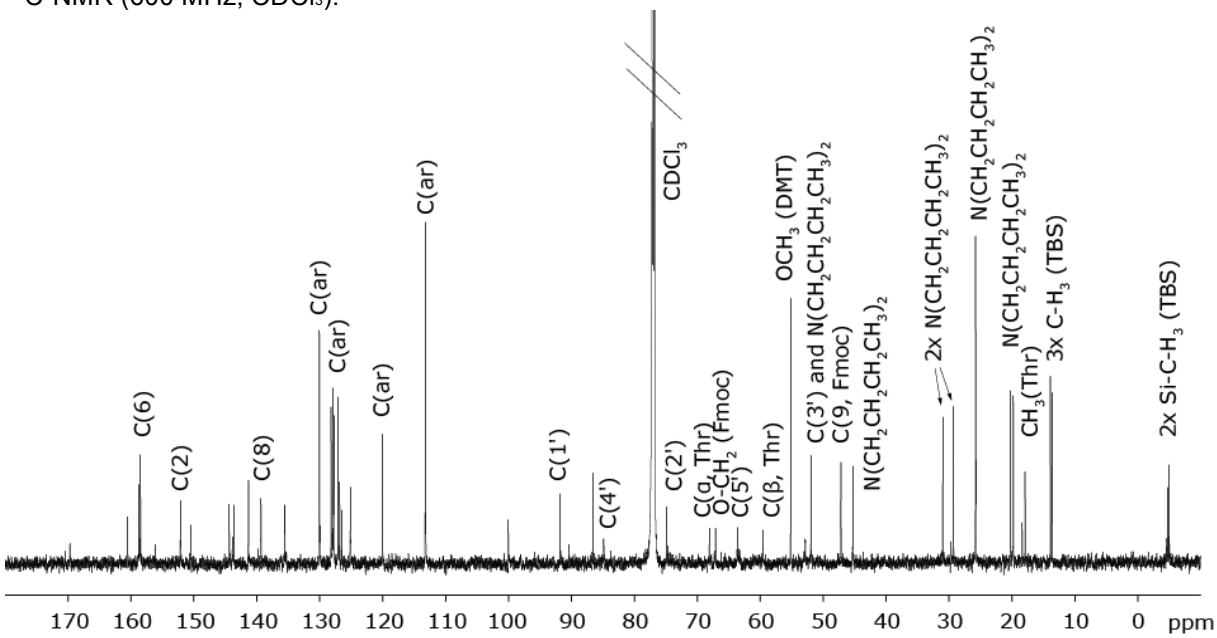
Fmoc-L-Thr(TBMDS)-OH[1] (81 mg, 0.18 mmol) was coevaporated three times with THF and then dissolved in 3 mL THF. The solution was cooled to 0°C, followed by addition of 1-hydroxybenzotriazole hydrate (36 mg, 0.23 mmol). After ten min, *N*,*N*-diisopropylcarbodiimide (36 µL, 0.23 was added. Ten min later, 9-(3'-azido-3'-deoxy-*β*-D-arabinofuranosyl)adenine **1** (100 mg, 0.14 mmol) dissolved in 4 mL THF was added dropwise. The reaction was stirred 15 min at 0°C, followed by addition trimethylphosphine solution (1M in THF, 300 µL). Stirring was continued for 16 hours at room temperature. Subsequently, the solvent was evaporated and the residue dissolved in CH_2Cl_2. The solution was washed with water and half saturated $NaHCO_3$ solution. The organic layers were collected, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by chromatography on SiO_2 (CH_2Cl_2/methanol = 98/2 – 95/5) to yield 150 mg of compound **3** as white foam (96 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ 9.05 (s, 1H, HC=N(6)), 8.49 (s, 1H, HC(2)), 8.14 (s, 1H, HC(8)), 7.75 (d, 2H, J = 7.4, H-C(ar)), 7.60 (t, 2H, J = 7.0, H-C(ar)), 7.41-7.10 (m, 17H, HC(ar) and HN(3')), 6.74 (m, 4H, H-C(ar)), 6.04 (s, 1H, HOC(2')), 5.91 (d, 1H, J = 4.8 Hz, H-C(1')), 5.79 (d, 1H, J = 5.8, HN(Thr)), 4.89 (m, 1H, HC(2')), 4.54–4 49 (m, 2H , HC(3') and CH(4')), 4.44–4.35 (m, 3H, OCH_2(Fmoc) and CH(α, Thr)), 4.23 (t, 1H, J = 7.2, CH(9, Fmoc)), 4.18 (m, 1H, CH(β, Thr)), 3.77–3.67 (m, 8H, $N(CH_2CH_2CH_2CH_3)_2$ and 2x O-CH_3 (DMT)), 3.50–5.46 (m, 2H, $N(CH_2CH_2CH_2CH_3)$), 3.41 (t, 2H, J=7.3, H(a)C(5') and H(b)C(5')), 1.69–1.62 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$), 1.41–1.35 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$), 1.01 (d, 3H, J = 6.2, CH_3(γ, Thr)), 0.97–0.89 (m, 15H, $N(CH_2CH_2CH_2CH_3)_2$ and 3x CH_3 (TBDMS)), 0.00-0.15 (m, 6H, Si-CH_3 (TBDMS)). ^{13}C-NMR (600 MHz, $CDCl_3$): 169.8, 160.7, 159.0, 158.7 (C(6)), 152.3 (C(2)), 150.7, 144.6, 144.1, 143.8, 141.5, 139.5, 135.8, 130.2 (C(ar)), 128.3 (C(ar)), 128.0 (C(ar)), 127.9, 127.2 (C(ar)), 127.0(C(ar)), 126.6, 125.3 (C(ar)), 120.2 (C(ar)), 113.3 (C(ar)), 100.2, 92.0 (C(1')), 86.7, 85.0 (C(4')), 75.0 (C(2')), 68.2 C(α, Thr), 67.3 (O-CH_2 (Fmoc)), 63.8 (C(5')), 59.7 (C(β,Thr), 55.3, 53.0, 52.1 (C(3') and $N(CH_2CH_2CH_2CH_3)_2$)), 47.3 (C(9, Fmoc)), 45.4 ($N(CH_2CH_2CH_2CH_3)_2$), 31.2 ($N(CH_2CH_2CH_2CH_3)_2$), 29.4 ($N(CH_2CH_2CH_2CH_3)_2$), 26.0 ($N(CH_2CH_2CH_2CH_3)_2$), 20.4, 20.0 ($N(CH_2CH_2CH_2CH_3)_2$), 18.5, 18.1 (C(γ,Thr), 14.1 (CH_3(TBDMS)), 13.8, -4 8 (2x Si-C (TBDMS)). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{65}H_{81}N_8O_9Si$, 1145.59; found 1145.63.

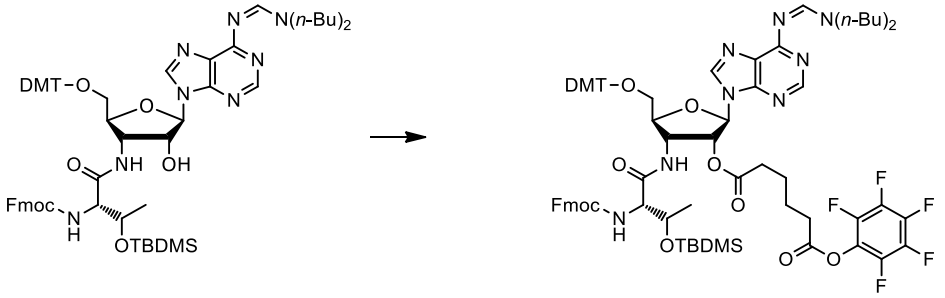
^{1}H-NMR (600 MHz, $CDCl_3$):



^{13}C-NMR (600 MHz, $CDCl_3$):



1.3.2 6-*N*-[(Di-*n*-butylamino)methylene]-3'-{[*N*-(9-fluorenyl)methoxycarbonyl-*O*-(*tert.*-butyldimethylsilyl)-L-threonyl]amino}-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[1,6-dioxo-6-(pentafluorophenyloxy)hexyl]-*β*-D-adenosine (**5.1**)

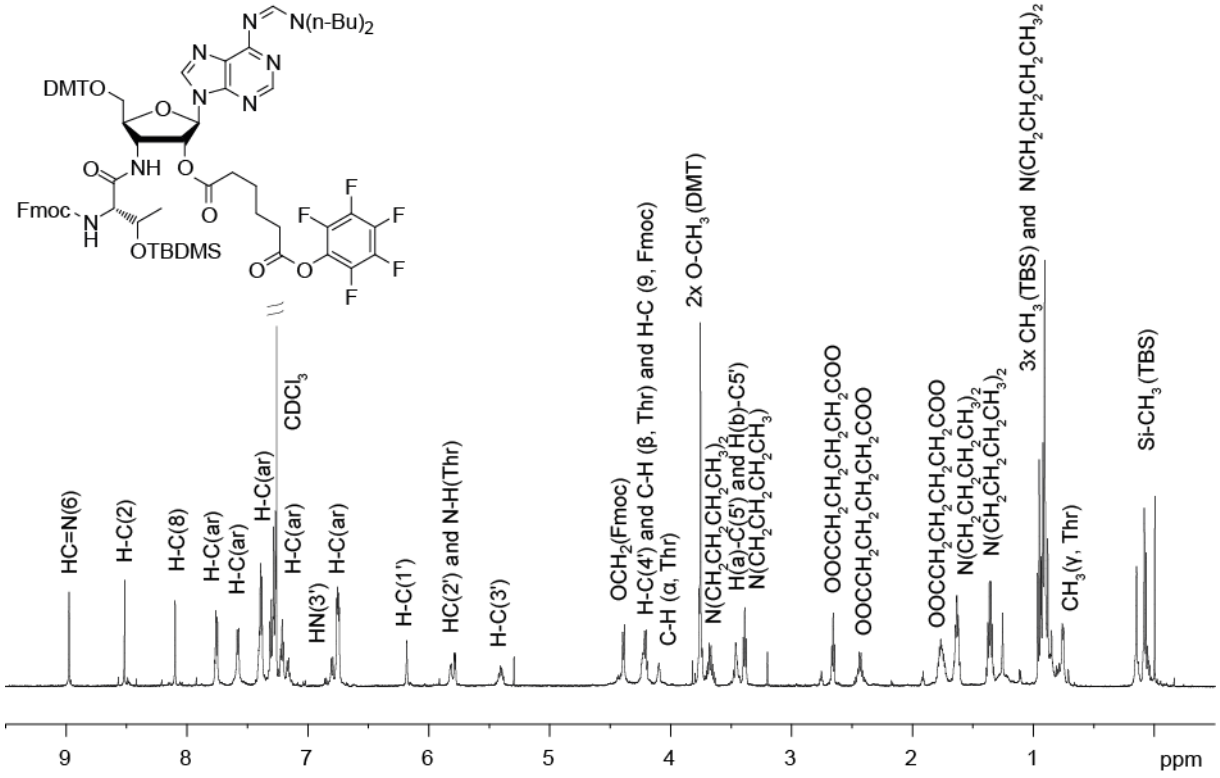


3 **5.1**

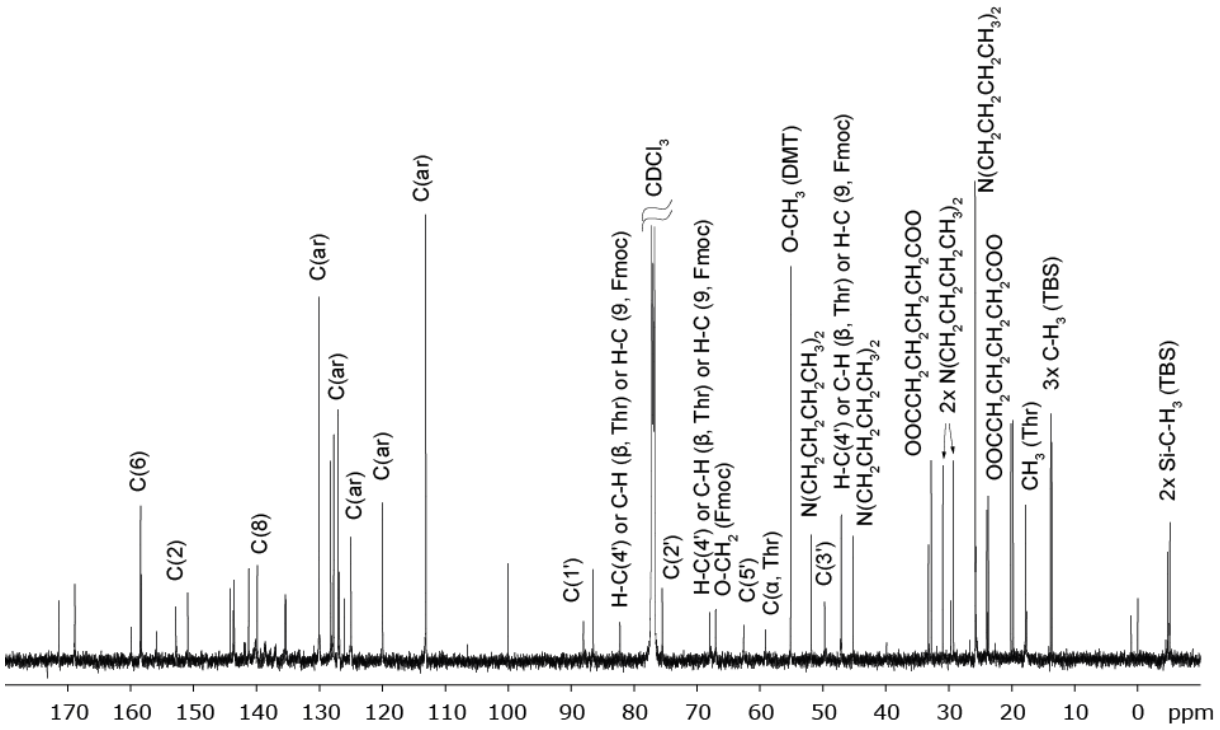
Compound **3** (60 mg, 0.05 mmol) was dissolved in 1.4 mL DMF/pyridine (1/1), 4-(*N*,*N*-dimethylamino)pyridine (7 mg, 0.06 mmol) and adipic acid pentafluorophenyl ester[2] (80 mg, 0.17 mmol) were added, and the mixture was stirred one hour at room temperature. Subsequently, the solvents were removed under reduced pressure. The crude product was purified by chromatography on SiO_2 (CH_2Cl_2/acetone = 95/5 – 85/5) to yield 37 mg of compound **5.1** as white foam (50 %).

^{1}H-NMR (600 MHz, $CDCl_3$): δ 8.97 (s, 1H, HC=N(6)), 8.52 (s, 1H, HC(2)), 8.10 (s, 1H, HC(8)), 7 75 (d, 2H, J = 6.8, HC(ar)), 7.57 (d, 2H, J= 7.1, HC(ar), 7.39 (m, 4H, HC(ar)), 7.32-7.16 (m, 8H, HC(ar)), 6.81-6.74 (m, 4H, HC(ar) and HN(3')), 6.18 (d, 1H, J = 1.9, HC(1')), 5.82 (d, 1H, J = 5.5, HN(Thr)), 5.78 (q, 1H, J = 2.3 and J = 6.1, HC(2')), 5.41 (m, 1H, HC(3'), 4.39 (d, 2H, J = 7.3, OCH_2(Fmoc)), 4.21 (m, 3H, HC(4') and HC(β, Thr) and HC(9, Fmoc)), 4.10 (m, 1H, HC(α, Thr)), 3.75 (s, 6H, 2 x OCH_3(DMT)), 3.68 (m, 2H, N($CH_2CH_2CH_2CH_3$)$_2$), 3.46 (m, 2H, H(a)C(5') and H(b)C(5')), 3.39 (t, 2H, J = 7.3, N($CH_2CH_2CH_2CH_3$)$_2$), 2.66 (t, 2H, J = 6.7, $OOCCH_2CH_2CH_2CH_2COO$), 2.43 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$), 1.76 (m, 4H, $OOCCH_2CH_2CH_2CH_2COO$), 1.64 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$), 1.35 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$), 0.97-0.85 (m, 15 H, N($CH_2CH_2CH_2CH_3$)$_2$ and 3 x CH_3(TBDMS)), 0.75 (m, 3H, CH_3(γ, Thr)), 0.08 (m, 6H, $SiCH_3$(TBDMS)). ^{13}C-NMR (600 MHz, $CDCl_3$): 171.6, 169.1, 160.2, 158.7 (C(6)), 156.1, 153.0 (C(2)), 151.2, 144.4, 143.9, 143.8, 141.5, 140.1 (C(6)), 135.7, 135.5, 130.3 (C(ar)), 128.4, 128.0, 127.9, 127.2 (C(ar)), 127.0, 126.2, 125.2 (C(ar)), 120.2 (C(ar)), 113.3 (C(ar)), 100.2, 88.2, 86.7, 82.40 (HC(4') or HC(β, Thr) or HC(9, Fmoc)), 75.7 (C2'), 68.1 (HC(4') or HC(β, Thr) or HC(9, Fmoc)), 67.2 (OCH_2, Fmoc), 62.8 (C5'), 59.3 (C(α, Thr)), 55.3 (OCH_3(DMT)), 52.0 (N($CH_2CH_2CH_2CH_3$)$_2$), 49.9 (C(3')), 47.3 (HC(4') or HC(β, Thr) or HC(9, Fmoc)), 45.4 (N($CH_2CH_2CH_2CH_3$)$_2$), 33.4, 33.0 ($OOCCH_2CH_2CH_2CH_2COO$), 31.1, 29.8 (N($CH_2CH_2CH_2CH_3$)$_2$), 29.4 (N($CH_2CH_2CH_2CH_3$)$_2$), 26.0 (N($CH_2CH_2CH_2CH_3$)$_2$), 24.1, 24.0 ($OOCCH_2CH_2CH_2CH_2COO$), 23.9, 20.3, 19.9, 18.0, 17.8 (CH_3(Thr)), 14.0 (CH_3(TBDMS)), 13.8 (CH_3(TBDMS)), 1.2, 0.1, -4.6 (Si-CH_3), -5.0 (Si-CH_3). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{77}H_{88}F_8N_8O_{12}Si$, 1439.62; found 1439.60.

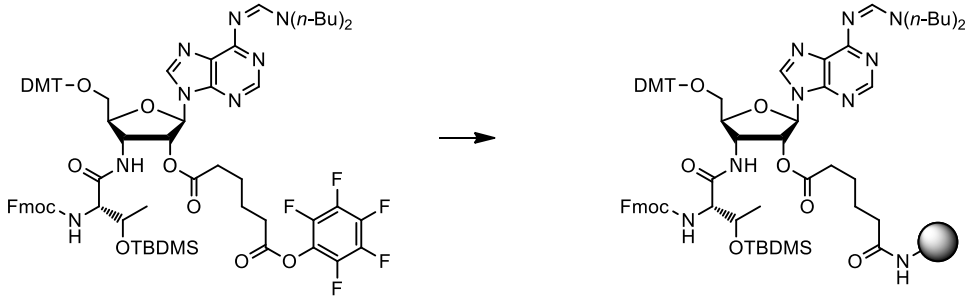
^{1}H-NMR (600 MHz, $CDCl_3$):



^{13}C-NMR (600 MHz, $CDCl_3$):



1.3.3 DMTO-rA-3'-NH-[Fmoc-Thr(TBDMS)] solid support (**5**)

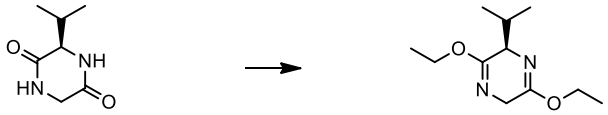


5.1 **5**

To a solution of active ester **4.2** (37 mg, 0.33 mmol) in 1.5 mL DMFwas added amino-functionalized support (*GE Healthcare*, Custom Primer Support^{TM} 200 Amino, 111 mg) and pyridine (5 µL, 0.06 mmol). The suspension was agitated for 20 hours at room temperature. The beads were collected on a Büchner funnel and washed with DMF, methanol and CH_2Cl_2. For capping of unreacted amino groups, the beads were treated with 10 ml of solution A (0.2 M phenoxy acetic anhydride in THF) and 10 ml of solution B (0.2 M *N*-methyl imidazole, 0.2 M *sym*-collidine in THF) and agitated for 10 min at room temperature. The suspension was filtrated again, the beads were washed with THF, methanol and CH_2Cl_2 and dried under vacuum. Loading of the solid support **5** was 63 µmol/g.

1.4 Synthesis of 3'-{[(2*S*)-2-amino-4-(diallyloxyphosphoryl)butyryl]amino}.3'-deoxyadenosine functionalized solid support (**10**)

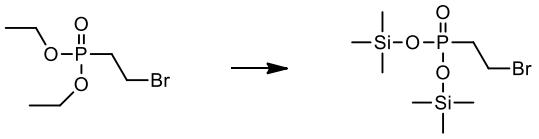
1.4.1 (3*R*)-3,6-Dihydro-2,5-diethoxy-3-isopropylpyrazine [3] (**6**)



6

To a suspension of (3*R*)-isopropylpiperazine-2,5-dione (1.0 g, 6.4 mmol) in 33 mL CH_2Cl_2 was added 4,136 g $BF_4 \cdot OEt_3$ (4.136 g, 21.8 mmol). The mixture was stirred for 5 days at room temperature, resulting in a yellowish solution that was added to cold (4°C) mixture of saturated $NaHCO_3$ solution (33 mL) and CH_2Cl_2 (33 mL). The pH value was kept basic (pH >8) by the addition of 10% NaOH solution. The aqueous phase was extracted with CH_2Cl_2 twice. The combined organic layers were washed with saturated NaCl solution, dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by column chromatography (ethyl acetate/hexane, 15/1 – 9/1) to yield 1.22 g of compound **6** as colorless oil (90 %).

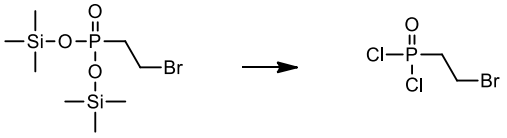
1.4.2 Bis(trimethylsilyl) (2-bromoethyl)phosphonate (**7.1**) [4]



7.1

Diethyl 2-bromoethylphosphonate (2.000 g, 8.2 mmol) was dissolved in 20 mL CH_2Cl_2 and cooled to 0°C. Then, bromotrimethylsilane (2.3 mL, 17.1 mmol) was added dropwise. After 2 hours at 0°C, the mixture was warmed to room temperature and stirred overnight. The volatile compounds were evaporated and the residue containing compound **7.1** was used for the next step without further purification.

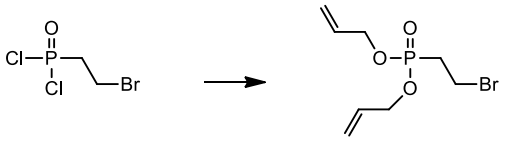
1.4.3 Dichloro (2-bromoethyl)phosphonate (**7.2**) [5]



7.1 **7.2**

The crude bis(trimethylsilyl) (2-bromoethyl)phosphonate **7.1** from the previous step was dissolved in 10 mL CH_2Cl_2 and a few drops of DMF. With vigorous stirring, oxalyl chloride (2.1 mL, 24.5 mmol) was added dropwise. After the addition was complete, the mixture was stirred for one more hour at room temperature. Subsequently, the solvents were evaporated, resulting in brownish oil of crude bis(chloro) (2-bromoethyl)phosphonate **7.2**.

1.4.4 Diallyl (2-bromoethyl)phosphonate (**7**) [5]

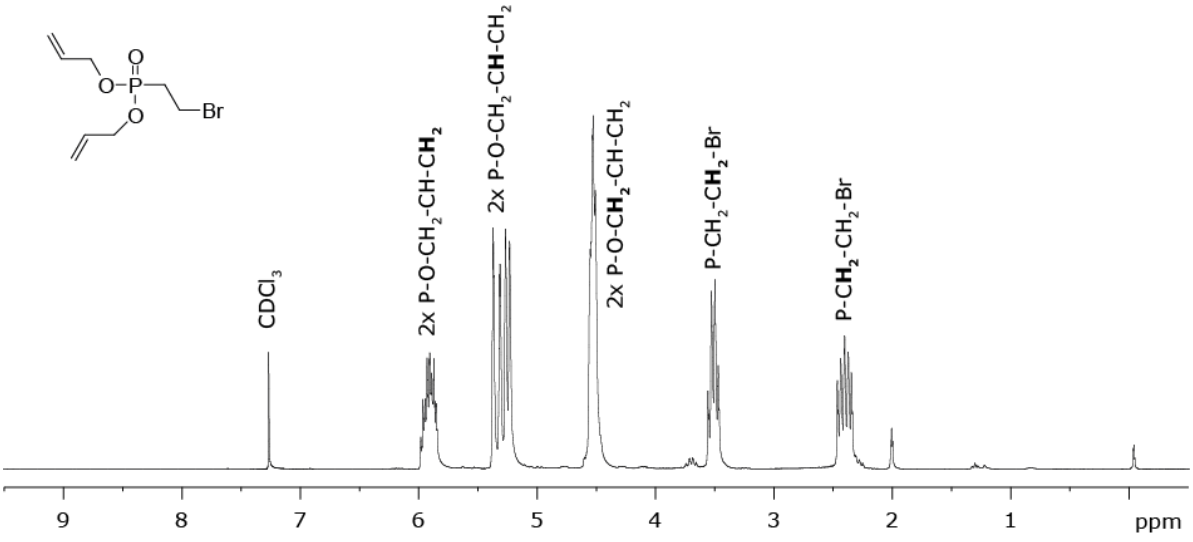


7.2 **7**

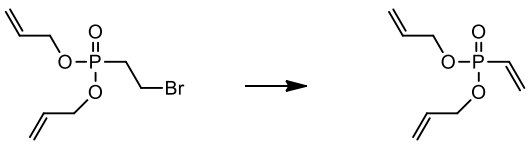
Allyl alcohol (1.2 mL, 17.1 mmol) and pyridine (1.4 mL, 17.1 mmol) were dissolved in 10 mL CH_2Cl_2 containing little 4-(dimethylamino)pyridine (DMAP) (2 mg; 0.1 mmol). The solution was stirred at -10 °C for 30 min and then cooled further to -20°C. The crude dichloro (2-bromoethyl)phosphonate **7.2** from the previous step, dissolved in 5 mL CH_2Cl_2, was added dropwise. The mixture was stirred at -20°C for one more hour, and continued overnight at room temperature. After evaporation, the crude product was purified by chromatography on SiO_2 (CH_2Cl_2/ethyl acetate = 8/2) to yield 2.1 g of compound **7** as yellowish oil (95 % over three steps).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (s, $CDCl_3$), 5.96-5.85 (m, 4 H, 2 x $POCH_2CH=CH_2$), 5.37-5.23 (m, 4 H, 2 x $POCH_2CH=CH_2$), 4.55-4.50 (m, 2 H, 2 x $POCH_2CH=CH_2$), 3.56-3.46 (m, 2H, PCH_2CH_2-Br), 2.47-2.35 (m, 2H, PCH_2CH_2-Br).

^{1}H NMR (300 MHz, $CDCl_3$):



1.4.5 Diallyl vinylphosphonate (**7.3**)

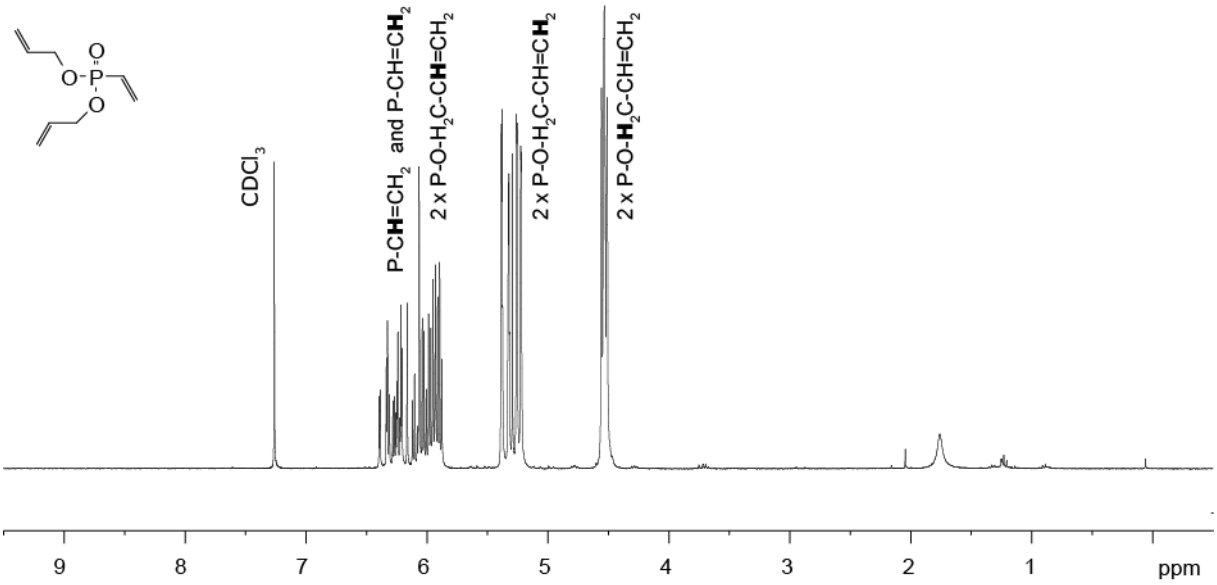


7 **7.3**

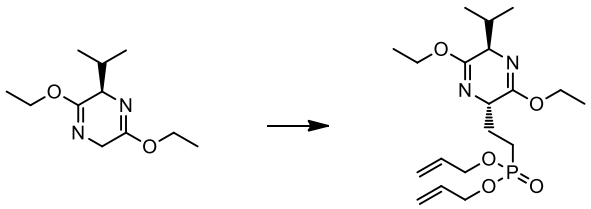
Diallyl-(2-bromoethyl)phosphonate (**7**) (1.0 g, 3.7 mmol) was dissolved in 10 mL CH_2Cl_2. To this solution, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was added dropwise and the reaction was stirred overnight. Subsequently, the solvent was removed. The crude product was purified by chromatography on SiO_2 (20% ethyl acetate in CH_2Cl_2) to yield 430 mg of compound **7.3** as clear oil (61 %).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (s, $CDCl_3$), 6.39-6.10 (m, 3 H, PC*H*=CH_2 and PCH=C*H*$_2$), 6.06-5.87 (m, 2 H, $POCH_2CH$=CH_2), 5.38-5.21 (q, 4 H, $POCH_2CH$=C*H*$_2$), 4.56-4.50 (t, 4 H, POC*H*$_2$CH=CH_2).

^{1}H NMR (300 MHz, $CDCl_3$):



1.4.6 Diallyl (2-((2*S*,5*R*)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)ethyl)phosphonate [6] (**8**)

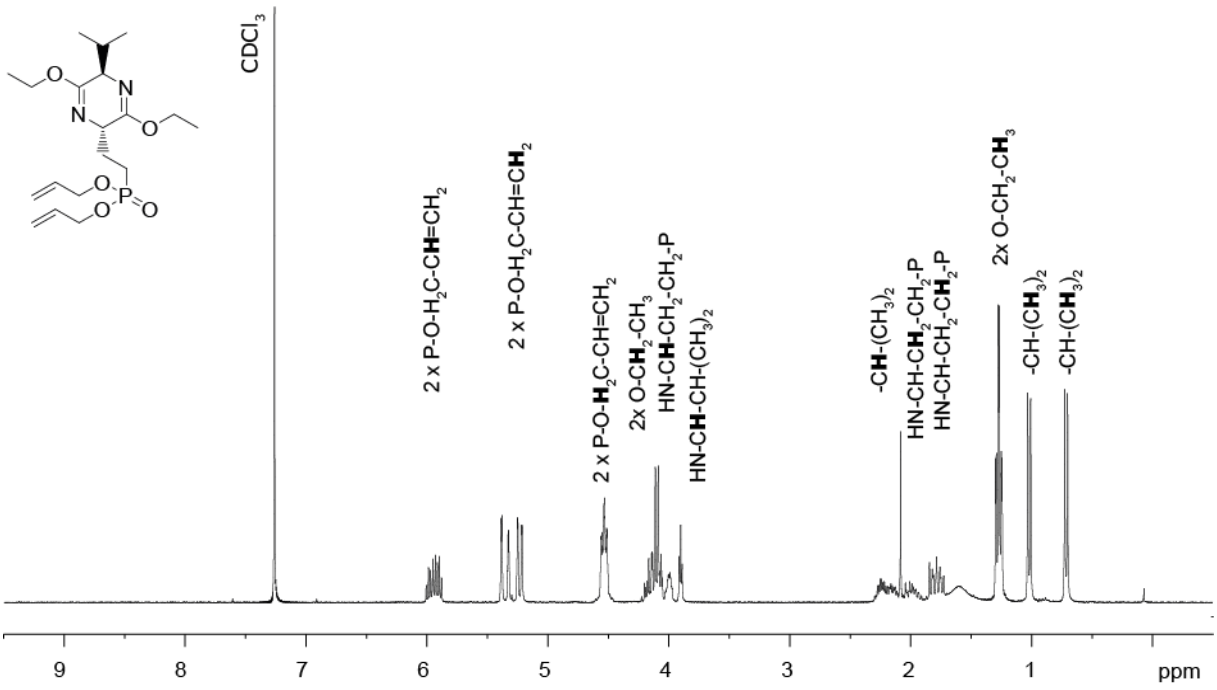


6 **8**

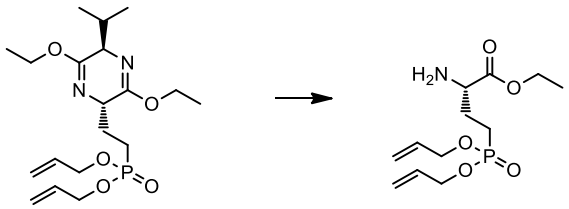
Schöllkopf bislactim ether **6** (295 mg, 1.4 mmol) was dissolved in 6 ml THF and cooled to -78°C. After dropwise addition of *n*-butyllithium (2.5 M in hexane, 556 µl), the solution was stirred for 30 min at -78°C. Then a mixture of diallyl vinylphosphonate **7.3** (26 mg, 0.1 mmol) and diallyl (2-bromoethyl)phosphonate **7** (337 mg, 1.3 mmol) in 4 mL THF was added dropwise. The mixture was stirred at -78°C for another 10 min and then allowed to warm to room temperature. Subsequently, the solvents were removed under reduced pressure. The resulting material was diluted with water and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The crude material was purified by chromatography on SiO_2 (ethyl acetate/hexane = 1/1) to yield 435 mg of compound **8** as colorless oil (85 %).

^{31}P-NMR (121 MHz, $CDCl_3$): δ 34.40. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 ($CDCl_3$), 6.01-5.88 (m, 2H, 2 x POH_2CCH=CH_2), 5.39-5.21 (q, 4H, PO*H*$_2$CCH=CH_2) , 4.56-4.51 (t, 4H, POH_2CCH=C*H*$_2$), 4.17-4.06 (m 4H, 2 x OC*H*$_2$$CH_3$), 4.00 (m, 1H, HNC*H*$CH_2CH_2P$), 3.90 (t, 1H, HNC*H*$CH(CH_3)_2$), 2.27-2.11 (m, 1H, C*H*$(CH_3)_2$), 2.04-1.95 (m, 2H, HNCHC*H*$_2$$CH_2P$), 1.84-1.73(m, 2H, $HNCHCH_2$C*H*$_2$P), 1.30 - 1.24 (t, 3H, 2 x OCH_2C*H*$_3$), 1.02 (d, 3H, CH(C*H*$_3$)$_2$), 0.72 (d, 3H, CH(C*H*$_3$)$_2$).

^{1}H NMR (300 MHz, $CDCl_3$):



1.4.7 (2*S*)-2-Amino-4-(diallyloxyphosphoryl)butanoic acid ethyl ester (**8.1**) [6]

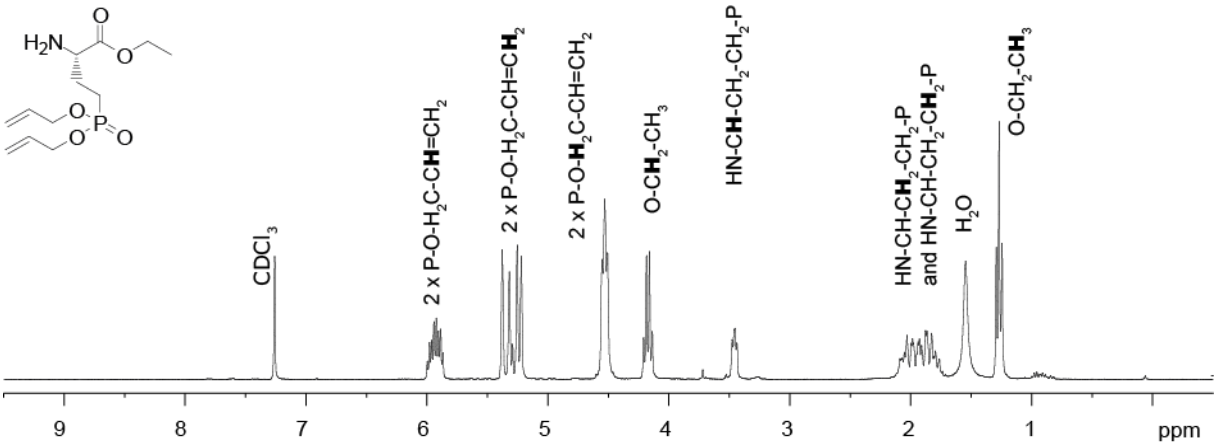


8 **8.1**

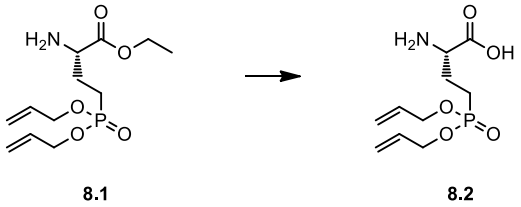
To a solution of diallyl (2-((2S,5R)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)ethyl)phosphonate (**8**) (435 mg, 1.08 mmol) in 10 mL THF/acetonitrile (1/1) was added 10 mL HCl solution (0.25 M). The mixture was stirred for 2 h at room temperature. The reaction was quenched with saturated Na_2CO_3 solution and the reaction mixture was extracted with ethyl acetate. The organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH, 99/1 – 95/5) yielding 229 mg of compound **8.1** as colorless oil (72%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 ($CDCl_3$), 6.00–5.87 (m, 2H, PO*H*$_2$CCH=CH_2), 5.38–5.22 (dd, 4H, POC*H*$_2$CH=CH_2), 5.53 (t, J = 6.9, 4H POCH$_2$CH=C*H*$_2$), 4.17 (q, J = 14.1, 3H, OC*H*$_2$CH$_3$), 3.45 (t, J = 6.7, 1H, CH(α, ABU)), 2.05–1.80 (m, 4H, CH_2(β, ABU) and CH_2(γ, ABU)), 1.27 (t, J = 7.0, 3H, OCH$_2$C*H*$_3$).

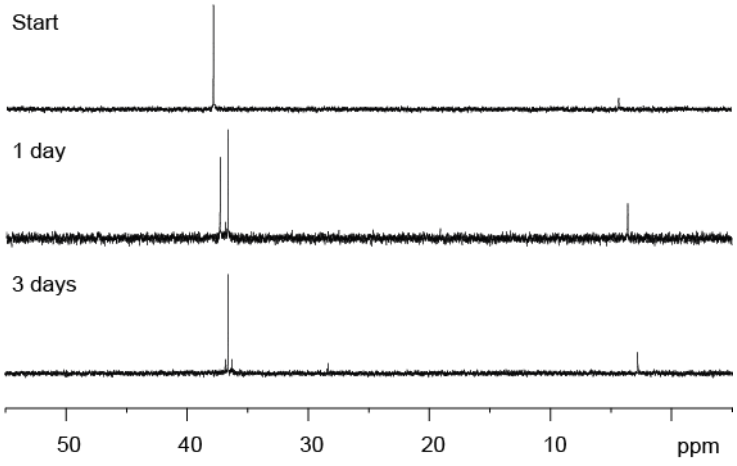
^{1}H NMR (300 MHz, $CDCl_3$):



1.4.8 (2*S*)-2-Amino-4-(diallyloxyphosphoryl)butanoic acid (**8.2**) [6]



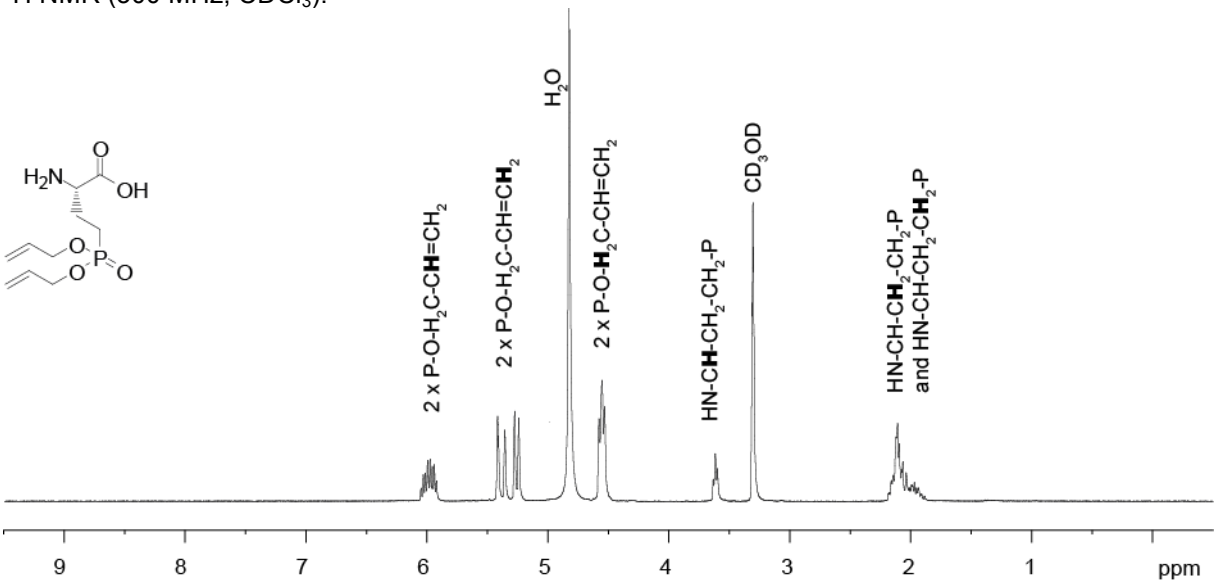
In an Eppendorf tube (2 mL volume), compound **8.1** (114 mg, 0.39 mmol) was dissolved in 1 mL of phosphate buffer (100 mM, pH 5.5) and chymotrypsin (5 mg as lyophilized powder) was added. The reaction mixture was incubated at 37°C for 4 days and the reaction progress was monitored via ^{31}P-NMR spectroscopy.



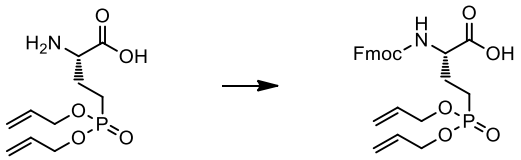
After the reaction was completed, the reaction mixture was applied to a RP18 Sep-Pak column (Waters) and eluated with water. The aqueous product fraction was further purified using reversed-phase chromatography on a *GE* Healthcare Äktaprime system using a commercial Merck Lobar 310-25 LiChroprep RP-18 (40–63 µm) column. The LC separation was monitored by conductivity. Solvent systems: A: water, B: CH_3CN. A linear gradient of 0–60% B was applied, flow rate 5 mL/min.). Compound **8.2** was isolated as white solid (48 mg, 45 %).

^{1}H NMR (300 MHz, CD_3OD): δ 6.05–5.92 (m, 2H, POCH_2CH=CH_2), 5.42–5.25 (dd, 4H, POCH_2CH=CH_2), 4.83 (s, H_2O), 4.56 (t, J = 7.8, 4H POCH_2CH=CH_2), 3.62 (t, J = 5.1, 1H, CH(α, ABU)), 3.31 (t, CD_3OD, 2.16 – 1.94 (m, 4H, CH_2(β ,ABU) and CH_2(γ, ABU)).

^{1}H NMR (300 MHz, $CDCl_3$):



1.4.9 *N*-Fmoc-(2*S*)-2-amino-4-(diallyloxyphosphoryl)butanoic acid (**9**)

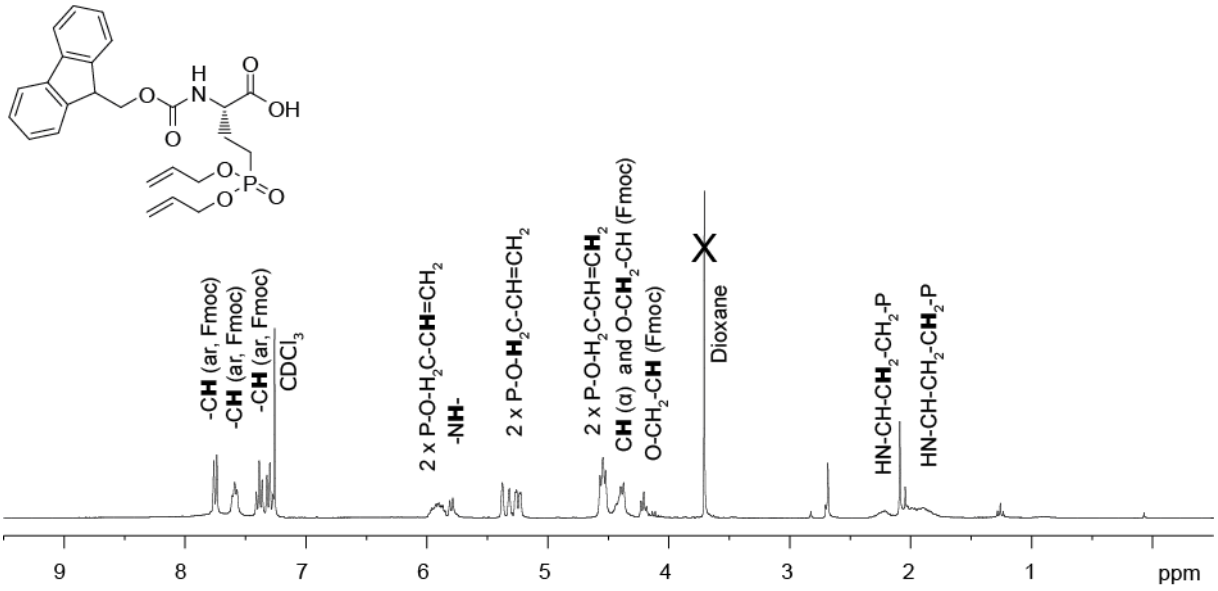


8.2 **9**

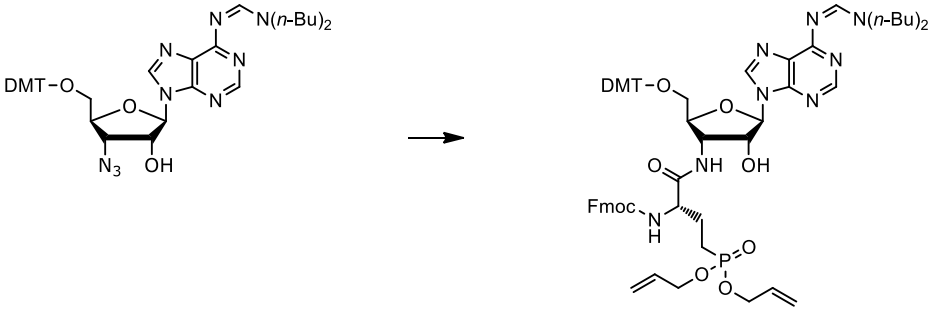
The amino acid **8.2** (96 mg, 0,4 mmol) and sodium carbonate (213 mg, 2.0 mmol) were suspended in 2.4 mL H_2O/dioxane (1/1). At 0°C, *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-OSu) (135 mg, 0.4 mmol) was added. The reaction was allowed to warm to room temperature and stirred overnight. Subsequently, the solution was acidified by addition of aqueous HCl solution (10% in water) and extracted with ethyl acetate. The organic layers were washed with saturated NaCl, dried over Na_2SO_4, filtered, and evaporated. The product was of sufficient quality and did not require further purification. Yield: 175 mg of **9** (98 %).

^{31}P-NMR (121 MHz, $CDCl_3$): δ 34.04. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (d, 2H CH(ar, Fmoc)), 7.59 (t, 2H CH(ar, Fmoc)), 7.41-7.27 (m, 4H CH(ar, Fmoc)), 7.26 ($CDCl_3$), 5.96-5.86 (m, 2H, 2 x POCH_2C*H*=CH_2), 5.81-5.78 (d, 1H, NH), 5.38-5.22 (q, 4H, POCH_2CH=CH_2), 4.57-4.52 (q, 4 H, POCH_2CH=CH_2), 4.40-4.37 (m, 3H, OCH_2CH(Fmoc) and C*H*(α)), 4.23-4.18 (t, 1H, OCH_2C*H*(Fmoc)), 3.71 (s, dioxane), 2.25-2.21 (m, 2H, CH_2(β)), 1.97-1.83 (m 2H, CH_2(γ)). ESI-MS (m/z): $[M-H]^-$ calcd for $C_{25}H_{27}NO_7P$, 484.15; found 483.97.

^{1}H NMR (300 MHz, $CDCl_3$):



1.4.10 6-*N*-[(Di-*n*-butylamino)methylene]-3'-[(2*S*)-2-(*N*-(9-fluorenyl)methoxycarbonylamino)-4-(dialloxyphosphoryl)butyrylamino]-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-adenosine

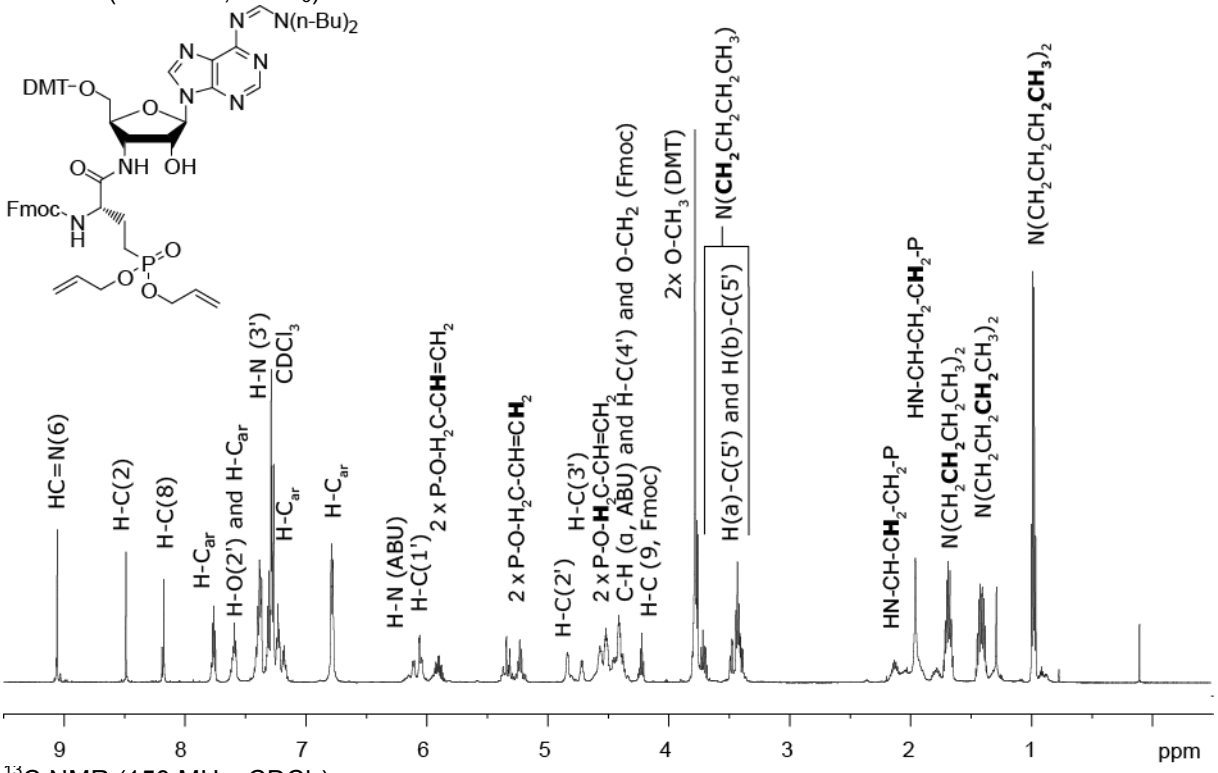


N-Fmoc-(2*S*)-2-amino-4-(diallyloxyphosphoryl)butanoic acid **9** (95 mg, 0.19 mmol) was coevaporated three times with THF and dissolved in 4 mL THF. The solution was cooled to 0°C, followed by the addition of 1-hydroxybenzotriazole hydrate (39 mg, 0.25 mmol). After ten min, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) in 1 mL CH_2Cl_2 was added and stirred for another ten min. Then, 3'-azido adenosine **1** (110 mg, 0.15 mmol) dissolved in 4 mL THF was added dropwise. After another 15 min, trimethylphosphine (1M in THF, 330 µL, 0.33 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred overnight. Subsequently, the solvents were removed under reduced pressure, the residue was dissolved in ethyl acetate and washed with water and half saturated $NaHCO_3$ solution. The organic layers were collected, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH = 99.5/0.5 – 95/5) to yield 62 mg of compound **10.1** as white foam (61 %).

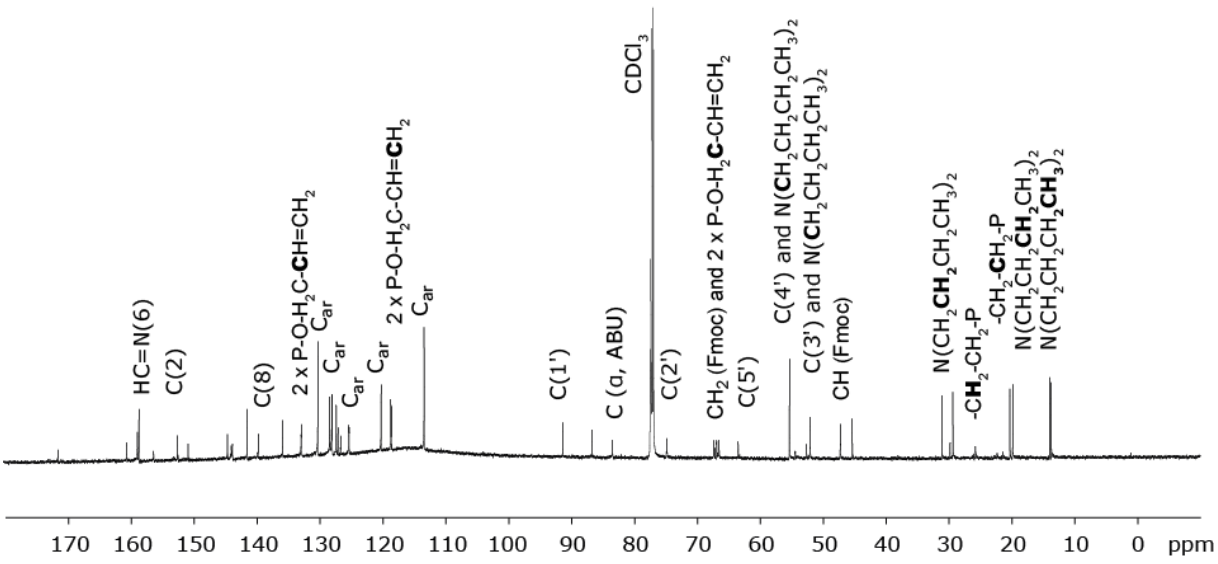
^{1}H NMR (600 MHz, $CDCl_3$): δ 9.03 (s, 1H, HC=N(6)), 8.46 (s, 1H, HC(2)), 8.15 (s, 1H, HC(8)),7.74–7.72 (m, 2H, HC(ar)) 7.56-7,55 (m, 2H, HO(2') and HC(ar)), 7.37–7.34 (m, 5H, HN(3') and HC_{ar}), 7.28–7.15 (m, 10H, HC(ar) and $CDCl_3$), 6.76–6.74 (m, 4H, HC(ar)), 6.08–6.07 (d, J = 7.7, 1H HN(ABU)), 6.03–6.01 (d, J = 3.2, 1H, HC(1')), 5.90–5.84 (m, 2H, 2 x $POCH_2CH$=CH_2), 5.34–5.18 (m, 4H, 2 x $POCH_2CH$=CH_2), 4.80 (s, 1H, HC(2')), 4.69–4.68 (d, J = 6.2, 1H, HC(3')), 4.53–4.47 (m, 4H, 2 x $POCH_2CH$=CH_2), 4.42–4.34 (m, 4H HC(α, ABU) and HC(4') and OCH_2(Fmoc)), 4.20–4.19 (m, 1H, HC(9, Fmoc)), 3.75 (s, 6H, 2x OCH_3), 3.70–3.66 (m, 2H, N($CH_2CH_2CH_2CH_3$)) 3.46–3.44 (dd, J = 2,5, J = 10.6, 2H H(a)C(5') and H(b)C(5')), 3.41–3.35 (m, 2H, N($CH_2CH_2CH_2CH_3$)), 2.12–2.00 (m, 2H,

NHCHCH_2CH$_2$P), 1.92–1.72 (m, 2H, HNCHCH$_2$CH_2P), 1.68–1.63 (m, 4H, 2 x N(CH$_2$CH_2CH$_2$CH$_3$)), 1.41–1.36 (m, 4H, 2 x N(CH$_2$CH$_2$CH_2CH$_3$), 0.95–0.94 (m, 4H, 2 x N(CH$_2$CH$_2$CH$_2$CH_3). ^{13}C NMR (150 MHz, CDCl$_3$): δ 171.43, 160.51, 158.84 (HC=N(6)), 158.61, 156.27, 152.47 (C(2)), 150.77, 144.54, 143.96, 143.77, 141.42, 139.67 (C(8)), 135.74, 132.79 (2 x POCH$_2$*C*H=CH$_2$), 130.17 (C(ar)), 128.30-126.97 (C(ar)), 126.54, 125.24 (C(ar)), 125.20, 120.14 (C(ar)), 118.59, 118.52 (2 x POCH$_2$CH=*C*H$_2$), 113.30 (C(ar)), 91.30 (C(1')), 86.66, 83.45 (C(α, ABU)), 77.37, 77.16 (CDCl$_3$), 76.95, 74.76 (C(2')), 67.32 (CH$_2$(Fmoc)), 66.87 and 66.51 (2 x PO*C*H$_2$CH=CH$_2$), 66.47, 63.45 (C(5')), 55.31 (N(*C*H$_2$CH$_2$CH_2CH$_3$)), 54.44 (C(4')), 52.62 (C(3')), 52.06 (N(*C*H$_2$CH$_2$CH_2CH$_3$)), 47.27 (CH(Fmoc)), 47.23, 45.36, 31.13 (N(CH$_2$CH$_2$CH_2CH$_3$)), 29.40, 25.83 (*C*H$_2$CH$_2$P), 20.35 (CH$_2$*C*H$_2$P), 19.91 (N(CH$_2$CH$_2$CH_2CH$_3$)), 14.06, 13.83(N(CH$_2$CH$_2$CH_2CH$_3$)). ESI-MS (m/z): [M+H]$^+$ calcd for $C_{65}H_{76}N_8O_{11}P$, 1175.54; found 1175.48.

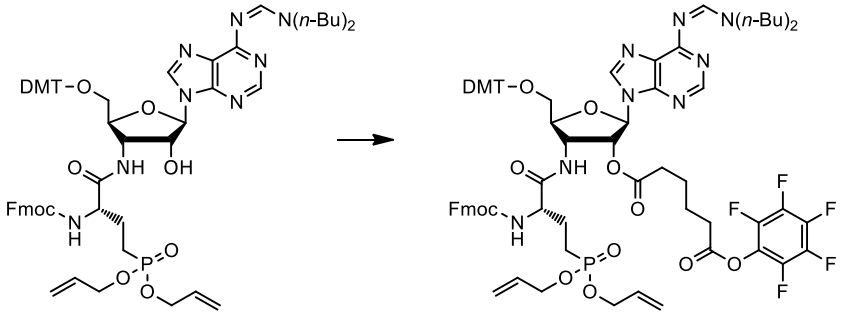
^{1}H NMR (600 MHz, CDCl$_3$):



^{13}C NMR (150 MHz, CDCl$_3$):



1.4.11 6-*N*-[(Di-*n*-butylamino)methylene]-3'-[(2*S*)-2-(*N*-(9-fluorenyl)methoxycarbonylamino)-4-(dialloxyphosphoryl)butyrylamino]-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[1,6-dioxo-6-(pentafluorophenyloxy)hexyl]-*β*-D-adenosine

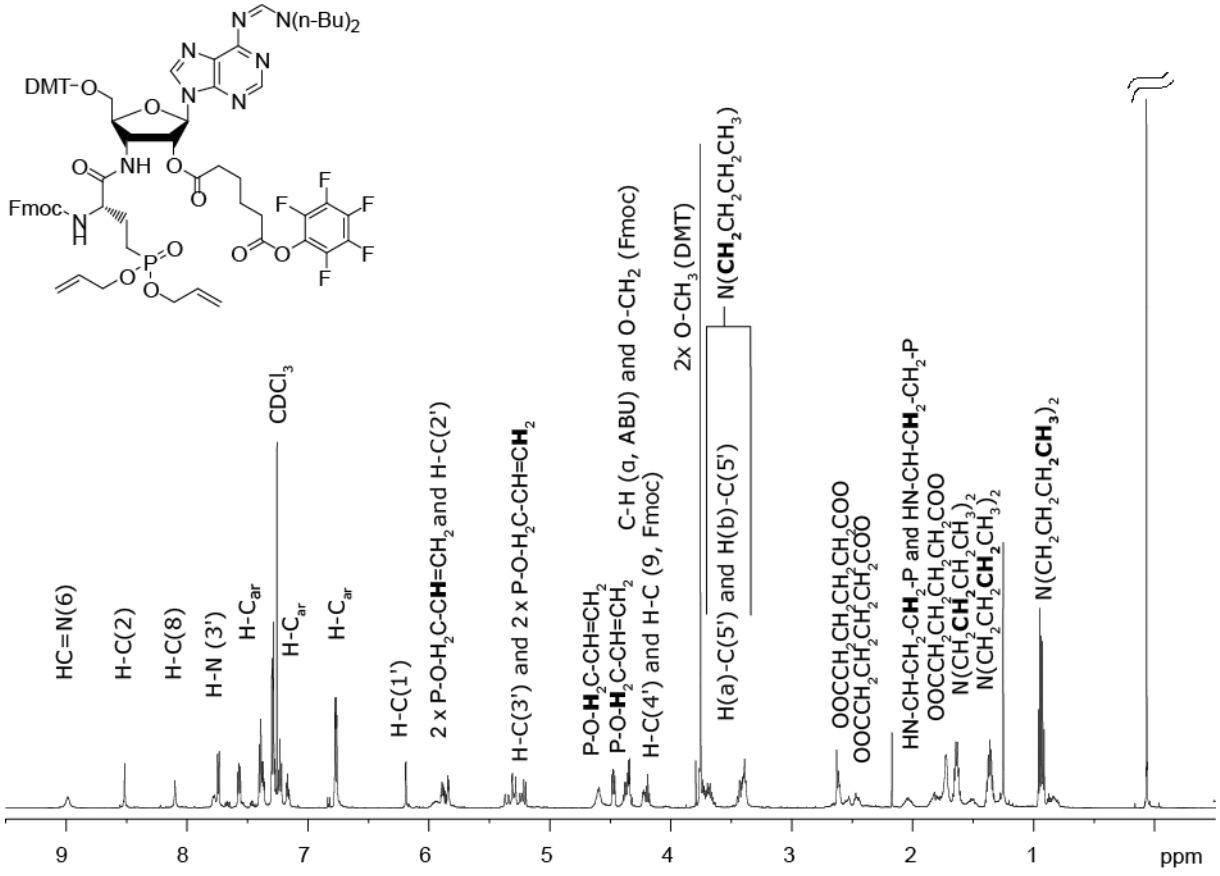


10.1 **10.2**

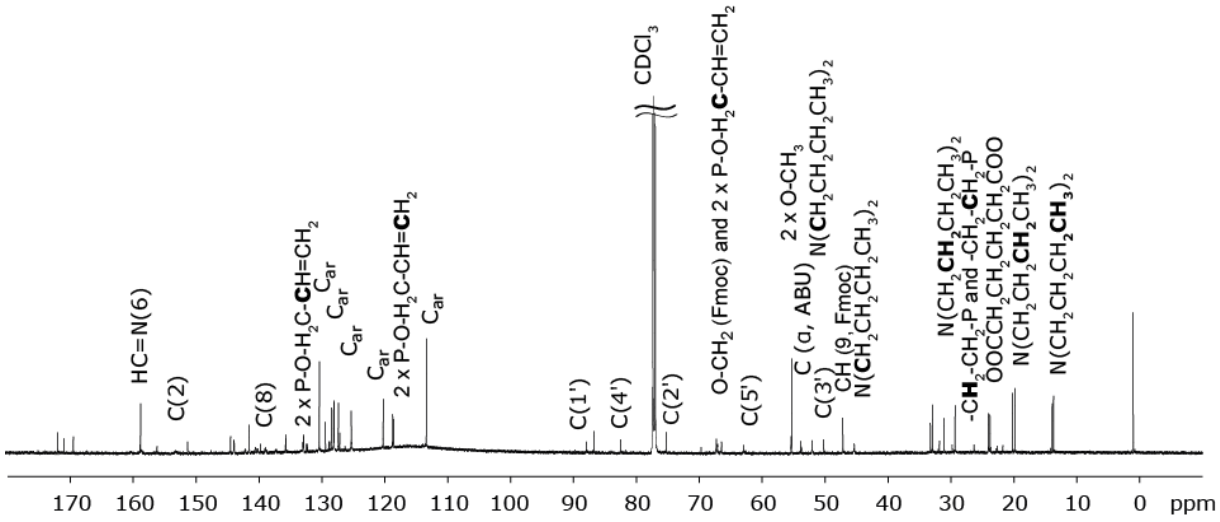
Compound **10.1** (25 mg, 0.02 mmol) was dissolved in 0.5 mL DMF and 0.4 mL pyridine. After addition of DMAP (3 mg, 0.02 mmol) and adipic acid bis pentafluorophenyl ester (20 mg, 0.04 mmol), the solution was stirred 60 min at room temperature. Subsequently, the solvents were evaporated and the crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/aceton = 95/5 – 85/15) to yield 13 mg of compound **10.2** as white foam (41 %).

^{31}P-NMR (121 MHz, $CDCl_3$): δ 33.76. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.98 (s, 1H, HC=N(6)), 8.51(s, 1H, H-C(2)), 8.10 (s, 1H, H-C(8)), 7.75 – 7.73 (m, 3H, H-N (3') and C-H_{ar}), 7.58 – 7.21 (m, 19H, C-H_{ar}), 7.26 (s, $CDCl_3$), 6.76 (d, 4H, H-C_{ar}), 6.19 (d, J = 2.7, 1H, H-C(1')), 5.94 – 5.83 (m, 3H, 2 x P-O-H_2C-CH=CH_2 and H-C(2')), 5.33 – 5.19 (m, 5H, H-C(3') and 2 x P-O-H_2C-CH=CH_2), 4.59 – 4.45 (m, 4H, 2 x P-O-H_2C-CH=CH_2), 4.38 – 4.34 (m, 3H, C-H (α, ABU) and O-CH_2 (Fmoc)), 4.24 – 4.19 (m, 2H, H-C(4') and H-C (9, Fmoc)), 3.76 (s, 6H, 2x O-CH_3 (DMT)), 3.69 – 3.67 (m, 2H, N($CH_2CH_2CH_2CH_3$)), 3.43 – 3.39 (m, 4H, H(a)-C(5') and H(b)-C(5') and N($CH_2CH_2CH_2CH_3$)), 2.63 (s, 2H, OOC$CH_2CH_2CH_2CH_2$COO), 2.51 – 2.48 (m, 2H, OOC$CH_2CH_2CH_2CH_2$COO), 2.02 – 1.96 (m, 4H, HN-CH-CH_2-CH_2-P and HN-CH-CH_2-CH_2-P), 1.72 (m, 4H, OOC$CH_2CH_2CH_2CH_2$COO), 1.66 – 1.62 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$), 1.41 – 1.32 (m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$), 0.95 – 0.93 (m, 6H, N($CH_2CH_2CH_2CH_3$)$_2$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.6, 170.6, 169.2, 158.5 (HC=N(6)), 155.9, 153.0 (C(2)), 151.1, 144.2, 143.8, 143.7, 141.9, 141.3, 140.3, 140.0, 139.5 (C(8)), 138.8, 138.7, 137.0, 135.5, 135.4, 132.7, 132.6 (2 x POCH$_2$*C*H=CH_2), 132.1, 130.1 (C(ar)), 129.1, 128.6 (C(ar)), 128.5 (C(ar)), 128.2 (C(ar)), 127.9 (C(ar)), 127.1 (C(ar)), 126.9 (C(ar)), 126.0(C(ar)), 125.1(C(ar)), 120.0 (C(ar)), 118.4 (2 x POCH$_2$CH=*C*H$_2$), 113.2 (C(ar)), 87.7 (C(1')), 86.6, 82.3 (C(4')), 77.16 ($CDCl_3$), 75.3 (C(2')), 69.7, 67.3 (OCH$_2$(Fmoc), 67.1 (2 x PO*C*H$_2$CH=CH_2), 66.4, 62.9 (C(5')), 55.4 (2 x OCH_3), 53.9, 53.8 (C(α, ABU), 52.0 (N(*C*H$_2$CH$_2$C*H*$_2$CH$_3$)), 50.2 (C(3')), 47.2 (CH(9, Fmoc)), 47.2 (N(*C*H$_2$CH$_2$C*H*$_2$CH$_3$)), 33.3, 32.9, 32.0, 31.1 (N(CH_2*C*H$_2$CH$_2$CH$_3$)), 29.8, 29.4, 26.4 (*C*H$_2$CH$_2$P and CH_2*C*H$_2$P), 24.1 (OOC$CH_2CH_2CH_2CH_2$COO), 23.8, 22.7, 21.8, 20.3 (N(CH_2CH_2*C*H$_2$CH$_3$)$_2$), 19.9, 14.3, 14.1 (N($CH_2CH_2CH_2$*C*H$_3$)$_2$). ESI-MS (m/z): $[M+H]^+$ calcd for $C_{77}H_{83}F_5N_8O_{14}P$, 1469.57; found 1469.53.

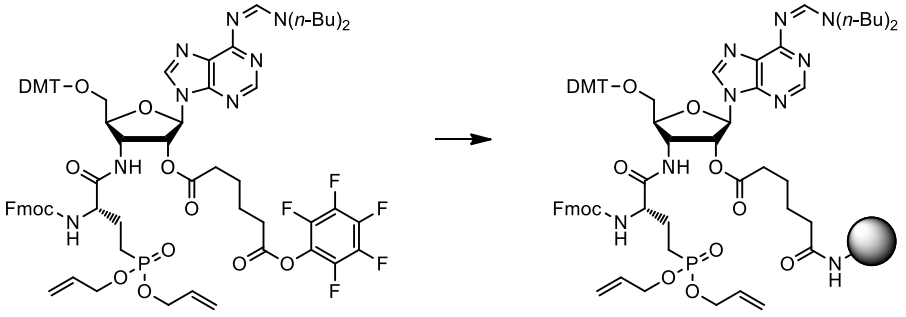
^{1}H NMR (600 MHz, $CDCl_3$):



^{13}C NMR (150 MHz, $CDCl_3$):



1.4.12 DMTO-rA-3'-NH-[Fmoc-Abu(pAll$_2$)] solid support (**10**)



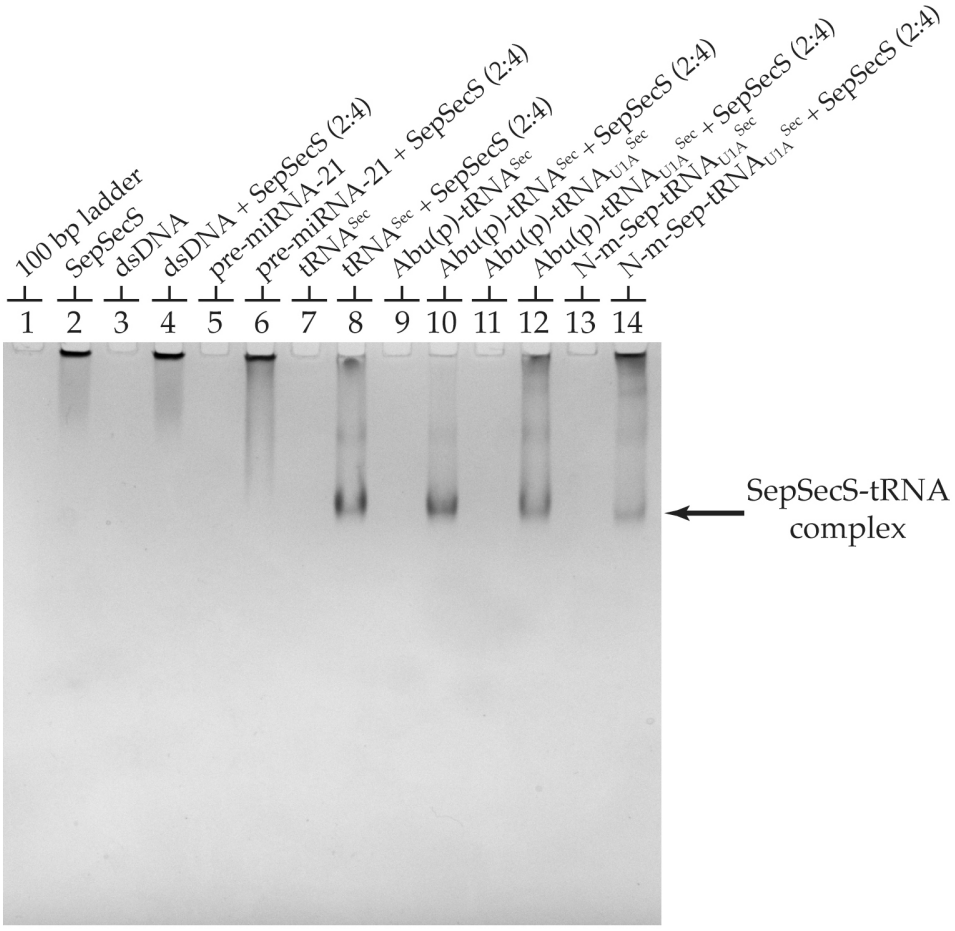
10.2 **10**

To a solution of active ester **10.2** (48 mg, 0.33 mmol) in 1.5 mL DMF was added amino-functionalized support (*GE Healthcare*, Custom Primer Support^{TM} 200 Amino, 200 mg) and pyridine (6 µL, 0.07 mmol). The suspension was agitated for 20 hours at room temperature. The beads were collected on a Büchner funnel and washed with DMF, methanol and CH_2Cl_2. For capping of unreacted amino groups, the beads were treated with 10 ml of solution A (0.2 M phenoxy acetic anhydride in THF) and 10 ml of solution B (0.2 M *N*-methyl imidazole, 0.2 M sym-collidine in THF) and agitated for 10 min at room temperature. The suspension was filtrated again, the beads were washed with THF, methanol and CH_2Cl_2 and dried under vacuum. Loading of the solid support **10** was 28 µmol/g.

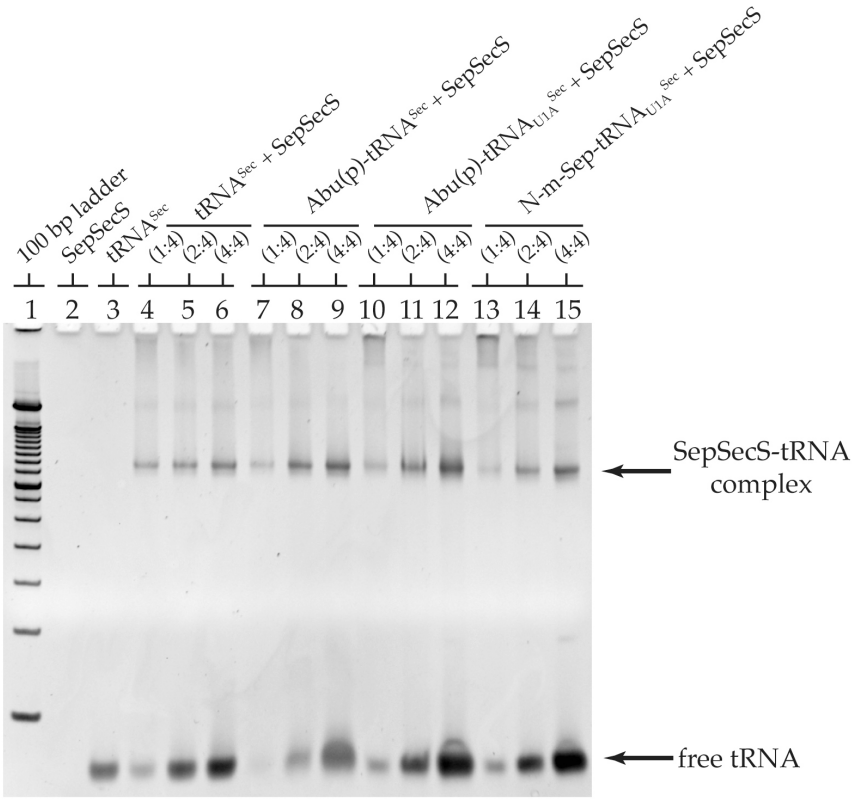
1.5 Mass spectrometry

All experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrumentation connected to an Amersham Ettan micro LC system. RNA sequences were analyzed in the negative-ion mode with a potential of -4 kV applied to the spray needle. LC: Sample (200 pmol RNA dissolved in 30 µL of 20 mM EDTA solution, average injection volume: 30 µL), column (Waters XTerra®MS, C18 2.5 µm, 1.0 x 50 mm) at 21°C, flow rate: 30 µL/min, eluant A: 8.6 mM TEA, 100 mM 1,1,1,3,3,3-hexafluoroisopropanol in H_2O (pH 8.0), eluant B: methanol, gradient: 0-100 % B in A within 30 min, UV-detection at 254 nm.

2 Supporting Figures



Supporting Figure S1. SepSecS forms a stable complex with the in vitro transcribed tRNA^{Sec} and tRNA^{Sec} mimics. Staining the native gel (Figure 5 in the main text) with Coomassie reveals that SepSecS enters the gel only in the presence of tRNA^{Sec} and tRNA^{Sec} derivatives (lanes 8, 10, 12, and 14). Moreover, SepSecS co-migrates with the slower tRNA^{Sec} band (see Figure 5, lanes 8, 10, 12, 14) suggesting formation of the stable binary complex. SepSecS does not enter the gel in the absence of tRNA^{Sec} and tRNA^{Sec} derivatives (lanes 2, 4, 6). No complex formation and change in the electrophoretic mobility of SepSecS is detected in the presence of DNA (lane 4) or pre-mi-RNA-21 (lane 6).



Supporting Figure S2. tRNA mimics and transcribed $tRNA^{Sec}$ bind to SepSecS with similar stoichiometry. Increasing amounts of $tRNA^{Sec}$ and derivatives were incubated with the constant amount of SepSecS. The samples were resolved on a 6% native polyacrylamide gel and the resulting gel was stained in ethidium bromide. The molar ratios are calculated based on the SepSecS monomer.

3 References

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