Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information for

Vitamin B_{12} transports modified RNA into *E. coli* and *S.* Typhimurium cells

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1. General information

All solvents and chemicals were used of reagent grade and were used without further purification. Tested compounds were greater than 95% chemical purity as measured by elemental or HPLC analysis. High resolution mass spectra were recorded on SYNAPT or Waters Q-TOF Premier spectrometers. ^{1}H and ^{13}CNMR spectra were recorded at rt on Bruker 400 and 500 MHz instruments with TMS or residual solvent peak as an internal standard. ^{31}P NMR spectra were recorded without the standard. Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness and reverse phase-18 modified silica gel coated with flourescent indicator F254s. DCVC (dry column vacuum chromatography)[1] was performed using Merck Silica Gel 60 H and reverse phase Silica Gel 90 C18 (Fluka). The exact concentrations of solutions of oligonucleotides were determined by Spectrophotometer DeNovix DS-11.

Following compounds were synthesized according to the reported procedures:

N-(Cobalamin-5'-yl)-hept-6-ynamide (**2**)[2]

5'-Azido-thymidine (**3c**)[3]

5'-*O*-*tert*-Butyldimethylsilyl-2'-deoxycytidine (**8**)[4]

6-Bromohexyl 2-cyanoethyl diisopropylphosphoramidite[5]

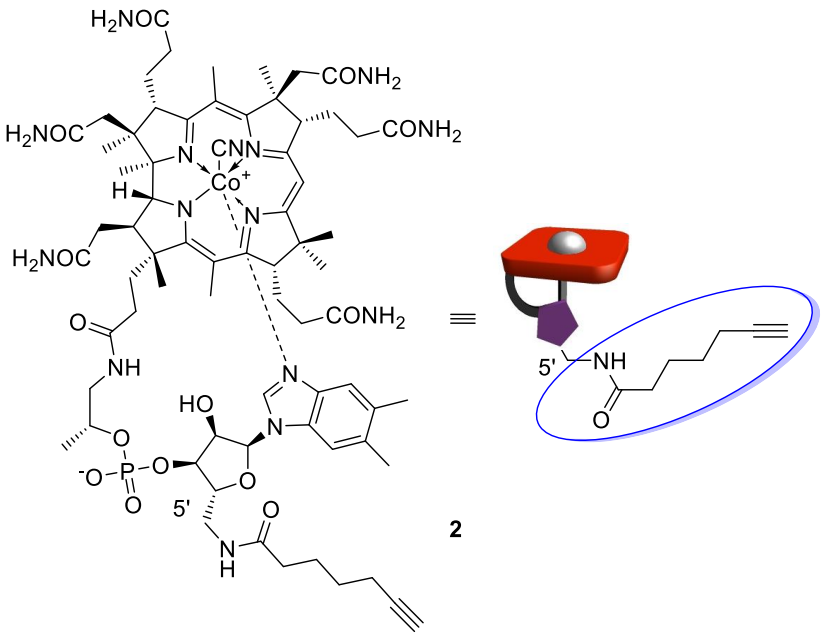


Figure 1. A simplified representation of *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**).

Following compounds were provided by FutureSynthesis Sp. z o. o.:

5’-N_3-deoxythymidine-CAU CUA GUA UUU CU-3’ (**5a**):

MW: 4855.2 g/mol; Molar Ext.coeffic.: 148400 L· mol^{-1}· cm^{-1}

5’-N_3-hexyl-CAU CUA GUA UUU CU-3’ (**5b**):

MW: 4730.2 g/mol; Molar Ext.coeffic.: 138900 L·mol^{-1}·cm^{-1}

5’-N_3-deoxythymidine-UUU CUA GUC UCA UA-3’ (**5c**):

MW: 4855.2 g/mol; Molar Ext.coeffic.: 148600 L· mol^{-1}· cm^{-1}

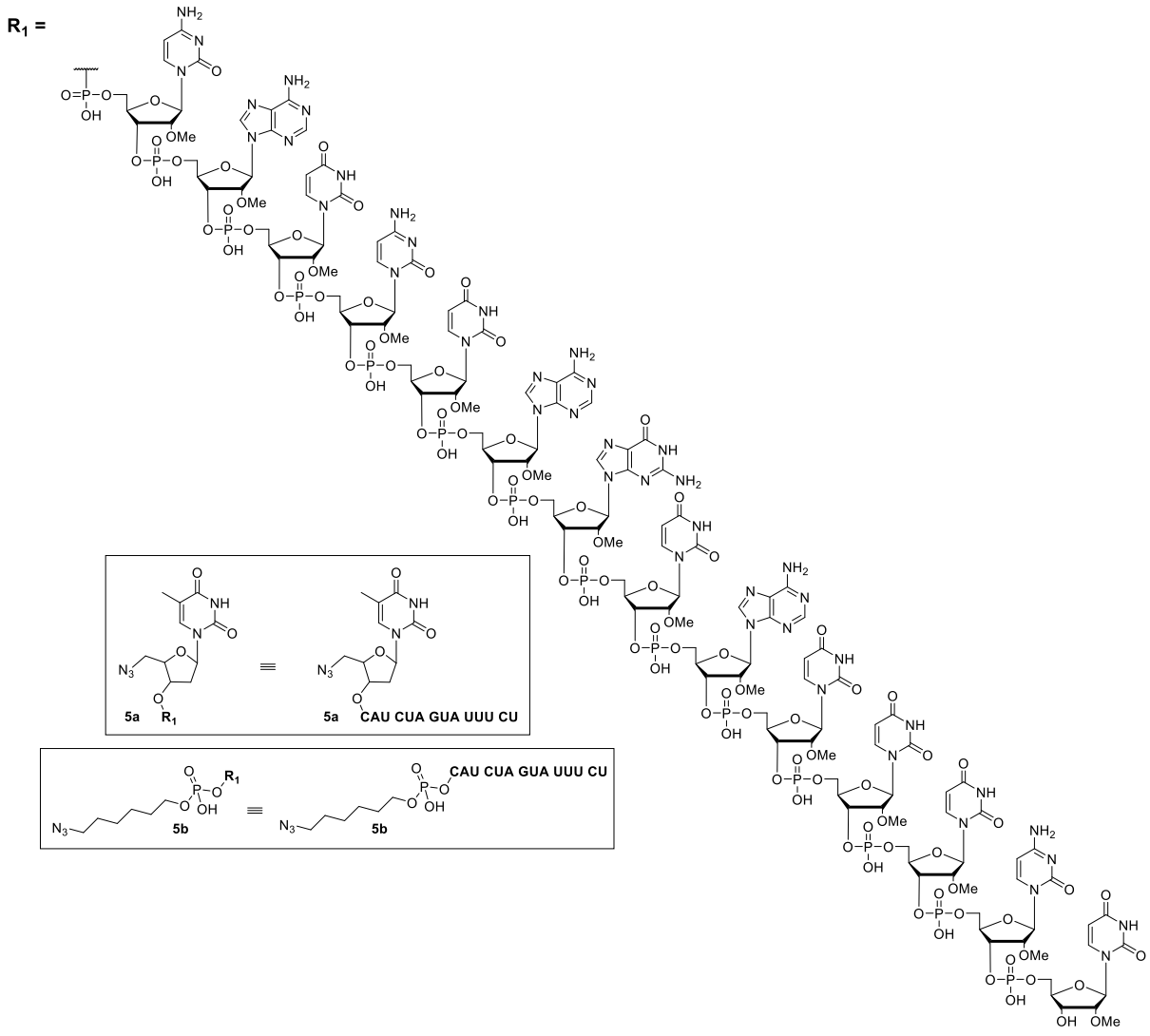


Figure 2. A simplified representation of 2’OMeRNA **5a** and **5b**.

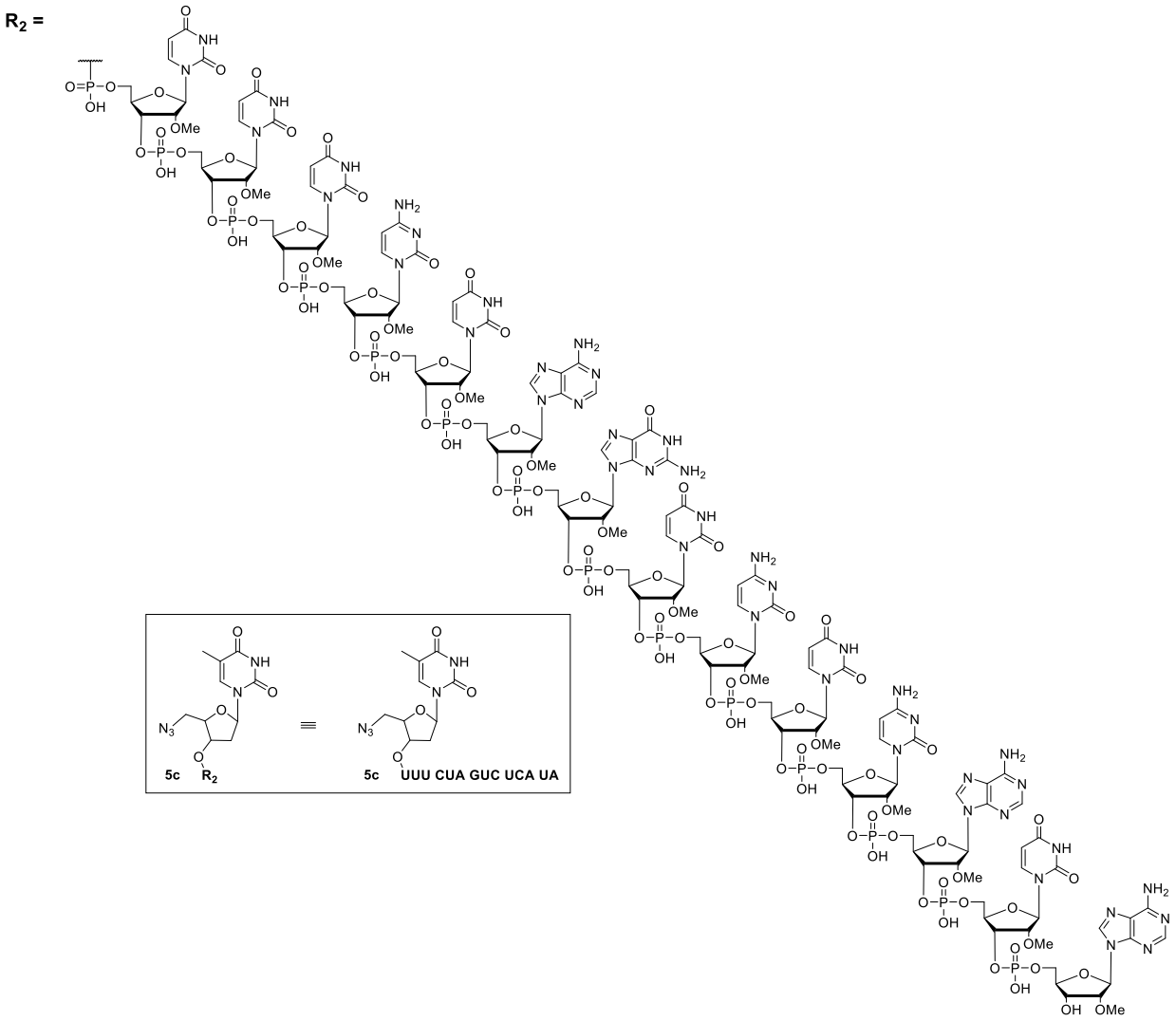


Figure 3. A simplified representation of scrambled 2'OMeRNA **5c**.

2. Biological information

Bacterial strains and determination of the level of fluorescence

To determine if vitamin B_{12} transports 2'OMeRNA oligomers to bacterial cells we used bacteria carrying a plasmid encoding red fluorescent protein (RFP) as previously described.[6] *Escherichia coli* K-12 MG1655[7] and *Salmonella enterica subsp. enterica* serovar Typhimurium LT2-R[6] cells encoding the *mrfp1* gene were grown 20 h in Davis Minimal Broth at 37 °C with shaking. Culture was supplemented with kanamycin to prevent plasmid loss. The effect of vitamin B_{12}-2'OMeRNA conjugates on the production of the red fluorescence protein (RFP) was determined using a standard microdilution method at concentration range 0–16 μM. To obtain relative fluorescence values (RFU) the previously described method was used.[6] Statistical significance was determined using the ANOVA with the probability value of $P \leq 0.05$ without further correction for multiple comparisons.

MTT based colorimetric assay for cytotoxic effect of 2'OMeRNA conjugated to vitamin B_{12}

The cytotoxic effect of 2'OMeRNA conjugated to vitamin B_{12} was tested on HEK-293 cells. HEK-293 were cultured in DMEM medium with high glucose (Lonza) suplemented with 10% FBS (Biowest) and Penicillin-Streptomycin (50U/ml, Gibco) in 37 °C and 5% CO_2 . Cells were seeded 10^4 per well on 96 well plates. In next 24 hours the 2'OMeRNA oligomers conjugates were added in 16μM concentration. The treatment time was 48 hours. Furthermore 10 μl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Merck) was added to each well, in order to measure the cytotoxicity effect of tested compunds to HEK-293. 4 hours later cells were incubated overnight in 100 μl of 10% SDS in 0,01 M HCl to solubilize formazan produced from MTT (Tada et al. 1986). The plates were read by Synergy H1MFDG (Biotek) at 590 nm. The obtained results of cells incubated in 2'OMeRNA conjugated to vitamin B_{12} were compared to results from untreated control cells.[8]

3. HPLC conditions

Following conditions were used for HPLC:

Column A: Kromasil C18, 5 µm, 250 mm × 4.0 mm; pressure – 20 MPa; flow rate – 1 mL/min; room temperature; detection – UV/vis at wavelengths (λ) of 361 and 267 nm.
Column B: Kromasil Eternity-5-C18, 250 mm × 4.6 mm; pressure – 10 MPa; flow rate – 1 mL/min; room temperature; detection – UV/vis at wavelengths (λ) of 361 and 267 nm.
Column C: Knauer C18, 5 µm, 250 mm × 8 mm; pressure – 190 MPa; flow rate – 4.5 mL/min; room temperature; detection – UV/vis at wavelengths (λ) of 267 nm.

HPLC method 1:

Time [min]	H_2O + 0.2‰ TFA [%]	MeCN [%]
Initial	90	10
15	30	70

HPLC method 2:

Time [min]	H_2O + 0.2‰ TFA [%]	MeCN [%]
Initial	90	10
15	30	70
25	30	70

HPLC method 3:

Time [min]	Buffer pH = 7.5 [%]	MeCN [%]
Initial	90	10
13	75	25
15	10	90
17	10	90

HPLC method 4:

Time [min]	Buffer pH = 7.5 [%]	MeCN [%]
Initial	90	10
10	80	20
18	80	20
19	10	90
20	10	90

4. Preparation of stock solutions

10 mM Cu-TBTA complex in DMSO/water (v/v 11:9): Copper(II) sulfate pentahydrate (50 mg, 0.20 mmol) was dissolved in water (10 mL). TBTA ligand (116 mg, 0.22 mmol) was dissolved in DMSO (11 mL). Two solution were then mixed.

2 M Triethylammonium acetate buffer, pH 7.0: Triethylamine (2.80 mL, 20 mmol) and acetic acid (1.10 mL, 20 mmol) were mixed. Water was added to 10 mL volume, and pH was adjusted to 7.0 with diluted solutions of triethylamine or acetic acid.

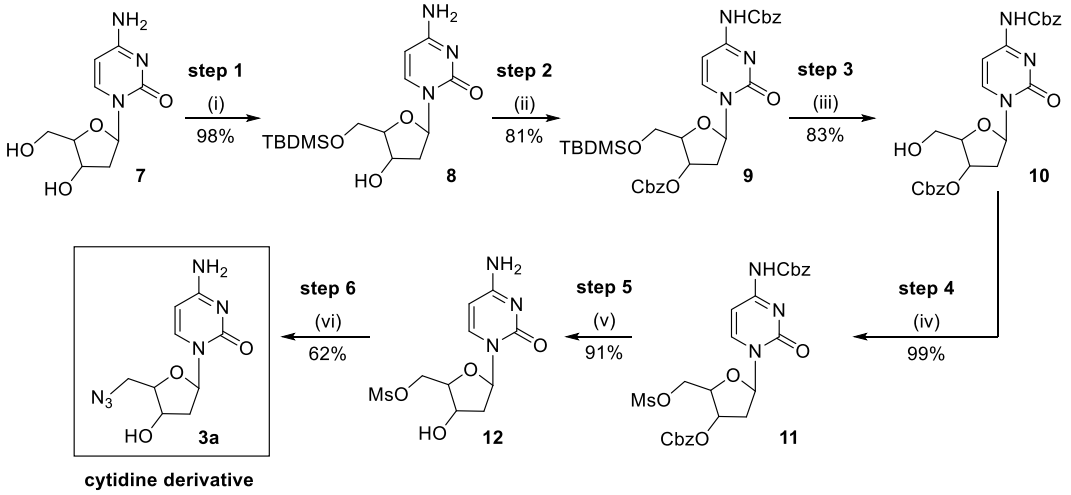
10 mM Ascorbic acid: Ascorbic acid (18 mg, 0.1 mmol) was dissolved in water (10 mL) and the mixture was degassed by bubbling argon and sonication for 15 minutes.

0.1 M Triethylammonium acetate buffer, pH 7.5: Glacial acetic acid (5.6 mL, 97 mmol) and trimethylamine (13.86 mL, 100 mmol) were dissolved in water (950 mL). pH was adjusted to 7.5 with diluted solutions of triethylamine or acetic acid.

5. Synthesis of 5'-azido-2'-deoxycytidine (3a)

2'-Deoxycytidine (**7**) was protected in 5' position with TBDMS according to the reported procedure (step 1),[23] followed by double protection of N^4 and 3' positions with benzyloxycarbonyl group (step 2) and subsequent acidic removal of the silyl group (step 3). Resulting compound **10** displays only moderate solubility in typically used organic solvents (DCM, AcOEt, MeOH). This feature allows to easily monitor the progress of mesylation (step 4) since the initial suspension changes into transparent solution along with the formation of mesylate **11**. Hydrogenation of compound **11** in the presence of Pd/C deprotects both benzyloxycarbonyl groups (step 5). In the last step, 5' mesylate **12** undergoes substitution with the azide anion, yielding desired, model nucleoside **3a**. This approach presents a reliable and relatively high yielding route towards 5'-azide **3a** from commercially available 2'-deoxycytidine (**7**).

Scheme 1. The multistep synthesis of 5'-azido-2'-deoxycytidine (**3a**).[a]

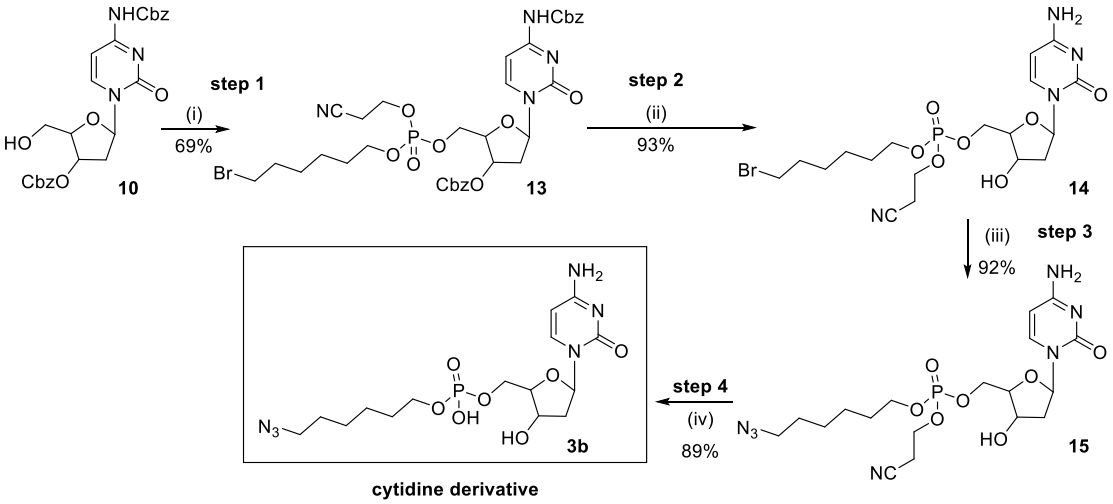


[a](i): 2'-deoxycytidine (**7**) (1 equiv.), TBDMSCl (1.1 equiv.), imidazole (1.3 equiv.), pyridine, rt, 9 h; (ii): nucleoside **8** (1 equiv.), CbzCl (4 equiv.), DMAP (6 equiv.), DCM, 0 °C to rt, 20 h; (iii): nucleoside **9**, TFA, H_2O, THF, 0 °C to rt, 20 h; (iv): nucleoside **10** (1 equiv.), MsCl (2 equiv.), Et_3N (2 equiv.), DCM, 0 °C to rt, 15 min.; (v): nucleoside **11**, H_2, Pd/C, EtOH, THF, rt, 30 min; (vi): nucleoside **12** (1 equiv.), NaN_3 (4 equiv.), DMF, 40 °C, 20 h.

6. Synthesis of cytidine derivative 3b

To verify the reactivity of azide located at the terminal C-atom of a labile linker, we synthesized a cytidine analog **3b**. Subjecting 2'-deoxycytidine derivative **10** protected at N^4 and 3' positions to the standard phosphoramidite method of nucleotide synthesis alowed for phosphate **13** decorated with bromine at the terminus of the hexyl chain (Scheme 2, step 1). At this point, no attempts were made to identify and separate potential diasteroisomers. After removal of Cbz protecting groups (step 2) bromide **14** was used as a substrate in nucleophilic substitution with $-N_3^-$ to afford azide **15** (step 3). The last step involved basic deprotection of the phosphate group (step 4) which led to desired product **3b** in overall yield (steps 1-4) of 53%.

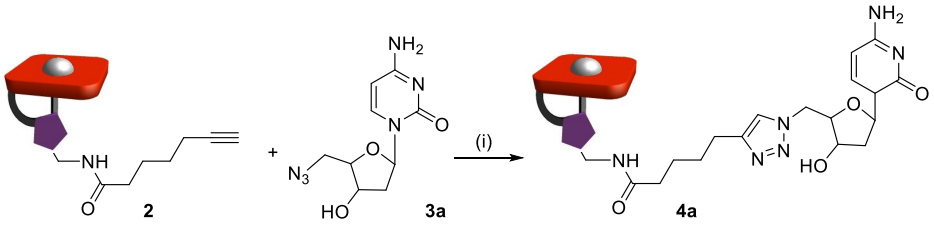
Scheme 2. The multistep synthesis of cytidine derivative **3b**.[a]



[a](i): *N*[4]-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (**10**) (1 equiv.), 6-Bromohexyl-2-cyanoethyl diisopropylphosphoramidite[5] (1.2 equiv.), 4,5-dicyanoimidazole (3 equiv.), dry DCM, dry MeCN, rt, 4 h; (ii): nucleotide **13**, H_2, Pd/C, EtOH, THF, rt, 15 min; (iii): nucleotide **14** (1 equiv.) NaN_3 (5 equiv.), dry DMF, rt, 2 h; (iv): nucleotide **15**, Et_3N, MeOH, rt, 30 min.

7. Optimization of the model CuAAC reaction

Table 1. Optimization of the model CuAAC reaction conditions directed at high yield and low concentration of 5'-azido-2'-deoxycytidine (**3a**).[a]

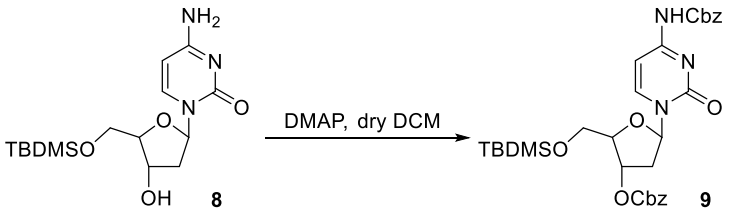


no	catalyst (concentration)	reducing agent	solvent	azide 3a [mM]	time [h]	yield [%]
1	$CuSO_4$-TBTA (0.5 mM)	AA	buffer/DMSO 1 : 1	20	48	44
2	$CuSO_4$-TBTA (1.5 mM)	AA	buffer /DMSO 1 : 1	20	4	92
3	$CuSO_4$-TBTA (3.0 mM)	AA	buffer /DMSO 1 : 1	20	4	89
4	$CuSO_4$-TBTA (1.5 mM)	AA	buffer /DMSO 4 : 1	20	4	74
5	$CuSO_4$-TBTA (1.5 mM)	AA	buffer /DMSO 11 : 1	20	4	62
6	$CuSO_4$ (1.5 mM)	AA	buffer /DMSO 1 : 1	20	4	64
7	$CuSO_4$ (1.5 mM)	AA	buffer	20	4	67
8	CuI-TBTA (1.5 mM)	-	buffer /DMSO 1 : 1	20	4	90
9	CuI (1.5 mM)	-	buffer /DMSO 1 : 1	20	4	62
10	CuOAc (1.5 mM)	-	buffer /DMSO 1 : 1	20	4	41
11	$CuSO_4$-TBTA (0.15 mM)	AA	buffer /DMSO 1 : 1	2	4	76
12	$CuSO_4$-TBTA (0.03 mM)	AA	buffer /DMSO 1 : 1	0.4	4	traces
13	$CuSO_4$-TBTA (0.15 mM)	AA	buffer /DMSO 1 : 1	2	20	84
14	$CuSO_4$-TBTA (0.08 mM)	AA	buffer /DMSO 1 : 1	1	20	61
15	$CuSO_4$-TBTA (0.15 mM)	AA	buffer /DMSO 1 : 1	1	20	96
16[b]	$CuSO_4$-TBTA (0.15 mM)	AA	buffer /DMSO 1 : 1	0.5	20	95

[a](i): azide **3a**, alkyne **2** (1 equiv.), solvent (0.5 ml), buffer pH = 7.0 (2 M), rt; L-ascorbic acid (AA) is used in an equimolar amount to the catalyst. [b]2 equiv. of alkyne **2** were used.

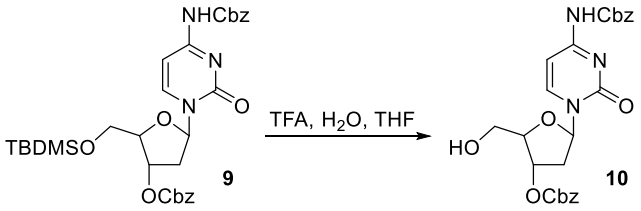
8. Experimental procedures

5'-*O*-*tert*-Butyldimethylsilyl-N^4-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (9)



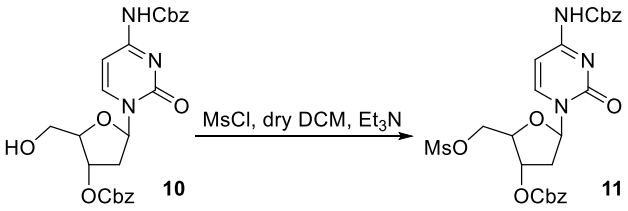
A Schlenk flask equipped with a stirring bar was charged with 5'-*O*-*tert*-butyldimethylsilyl-2'-deoxycytidine (**8**, 340 mg, 1.0 mmol) and DMAP (730 mg, 6.0 mmol). Dry DCM (12.5 mL) was added under an Ar atmosphere and the reaction mixture was cooled down to 0 °C. Benzyl chloroformate (570 µL, 4.0 mmol) was added dropwise and the resulting mixture was allowed to warm-up to room temperature and stirred for 20 h. Then it was diluted with DCM (50 mL) and washed consecutively with cold 1.0 M HCl_{aq} (10 mL), water (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified using DCVC, normal phase, gradually from 0% to 2% MeOH (v/v) in DCM to afford product **9** as a white solid (493 mg, 81%). R_f 0.62, 1:30 MeOH in DCM (normal phase). t_R (reverse phase-HPLC, column A, method 2): 16.73 min. m.p. 47-48 °C. Anal. calcd for $C_{31}H_{39}N_3O_8Si$: C 61.06, H 6.45, N 6.89, found: C 61.12, H 6.51, N 7.00. HRMS ESI calcd for $C_{31}H_{39}N_3O_8SiNa$ $[M+Na]^+$ 632.2404, found: 632.2399. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.24 (d, *J* = 7.5 Hz, 1H), 7.55 (s, 1H), 7.45 – 7.30 (m, *J* = 2.5 Hz, 10H), 7.18 (d, *J* = 7.5 Hz, 1H), 6.32 (dd, *J* = 7.8, 5.7 Hz, 1H), 5.26 – 5.11 (m, 5H), 4.33 – 4.21 (m, *J* = 1.9 Hz, 1H), 3.91 (ddd, *J* = 31.3, 11.4, 2.2 Hz, 2H), 2.82 (dd, *J* = 14.1, 5.4 Hz, 1H), 2.13 (ddd, *J* = 14.1, 7.8, 6.2 Hz, 1H), 0.89 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 162.3, 154.9, 154.6, 152.4, 144.2, 135.1, 134.9, 128.8, 128.8, 128.5, 128.4 (signals from phenyl rings overlap), 94.7, 87.3, 85.7, 78.7, 70.1, 68.0, 63.5, 39.7, 26.0, 18.3, -5.4, -5.5.

***N*^{4}-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (10)**



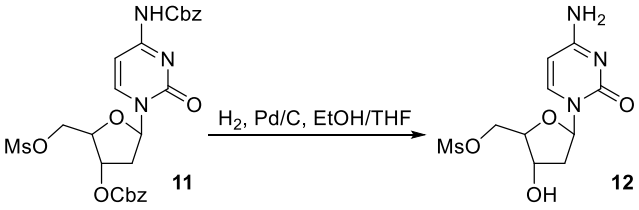
A round-bottomed flask equipped with a stirring bar was charged with 5'-*O*-*tert*-butyldimethylsilyl-*N*^{4}-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (**9**, 914 mg, 1.5 mmol). The substrate was dissolved in THF (8 mL). The solution was cooled down to 0 °C and aqueous TFA solution (2 mL, v/v 50%) was added. The resulting mixture was allowed to warm-up to room temperature and then stirred for 20 h. During this time precipitation of white solid was observed. The suspension was quenched with saturated $NaHCO_{3aq}$ (20 mL) and extracted with AcOEt (4 x 50 mL). Combined organic layers were washed with H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified using DCVC, normal phase, gradually from 3% to 5% MeOH in DCM (v/v) to afford product **10** as a white solid (616 mg, 83%). R_f 0.39, 1:30 MeOH in DCM (normal phase); R_f 0.31, 1:3 H_2O in MeOH (reverse phase). t_R (reverse phase-HPLC, column A, method 2): 15.02 min. m.p. 188-189 °C. Anal. calcd for $C_{25}H_{25}N_3O_8$: C 60.60, H 5.09, N 8.48, found: C 60.85, H 4.86, N 8.58. HRMS ESI calcd for $C_{25}H_{25}N_3O_8Na$ $[M+Na]^+$ 518.1539, found: 518.1531. ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.81 (s, 1H), 8.26 (d, *J* = 7.6 Hz, 1H), 7.50 – 7.21 (m, 10H), 7.05 (d, *J* = 7.5 Hz, 1H), 6.10 (dd, *J* = 7.9, 5.9 Hz, 1H), 5.32 – 5.00 (m, *J* = 5.1 Hz, 6H), 4.21 – 4.08 (m, *J* = 1.7 Hz, 1H), 3.63 (s, 2H), 2.61 – 2.49 (m, 1H), 2.34 – 2.12 (m, 1H). ^{13}C NMR ($(CD_3)_2SO$, 101 MHz) δ 163.4, 154.6, 154.2, 153.6, 145.0, 136.4, 135.7, 129.0, 128.91, 128.90, 128.7, 128.6, 128.4, 95.0, 86.7, 85.7, 79.1, 69.7, 67.0, 61.6, 38.6.

5'-Methanesulfonyl-*N*^{4}-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (11)



A Schlenk flask equipped with a stirring bar was charged with N^4-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (**10**, 250 mg, 0.5 mmol). Dry DCM (20 mL) and triethylamine (140 µL, 1 mmol) were added under an Ar atmosphere forming a suspension. The reaction mixture was cooled down to 0 °C and methanesulfonyl chloride (77 µL, 1 mmol) was added dropwise. The resulting mixture was allowed to warm-up to room temperature and stirred for 15 min. Cloudy mixture gradually transformed into transparent solution, indicating the reaction progress. After that it was diluted with DCM (30 mL) and washed consecutively with water (10 mL), saturated NH_4Cl_{aq} (10 mL), saturated $NaHCO_{3aq}$ (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified using DCVC, normal phase, gradually from 0% to 2% MeOH in DCM (v/v) to afford product **11** as a thick, transparent oil (284 mg, 99%). R_f 0.58, 1:30 MeOH in DCM (normal phase). t_R (reverse phase-HPLC, column A, method 2): 16.27 min. Anal. calcd for $C_{26}H_{27}N_3O_{10}S$: C 54.45, H 4.74, N 7.33, found: C 54.43, H 4.81, N 7.38. HRMS ESI calcd for $C_{26}H_{27}N_3O_{10}SNa$ $[M+Na]^+$ 596.1315, found: 596.1309. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, *J* = 7.4 Hz, 1H), 7.65 (s, 1H), 7.50 – 7.13 (m, *J* = 25.2, 12.7 Hz, 11H), 6.23 (dd, *J* = 7.9, 5.8 Hz, 1H), 5.31 – 5.06 (m, *J* = 14.5, 9.9 Hz, 5H), 4.53 (ddd, *J* = 14.9, 11.3, 3.2 Hz, 2H), 4.42 – 4.27 (m, *J* = 3.0 Hz, 1H), 3.05 (s, 3H), 2.90 – 2.72 (m, *J* = 10.0 Hz, 1H), 2.21 (dt, *J* = 14.7, 7.3 Hz, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 162.5, 154.6, 154.4, 152.2, 143.8, 135.0, 134.6, 128.8, 128.69, 128.65, 128.6, 128.4, 128.3, 95.2, 87.3, 82.4, 77.3, 70.3, 68.4, 68.0, 38.3, 37.7.

5'-Methanesulfonyl-2'-deoxycytidine (12)

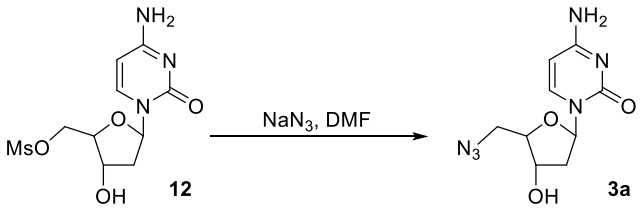


To a Schlenk flask equipped with a stirring bar palladium on carbon (10 wt. %, 50 mg) was added. It was then closed with a septum with two needles and purged with Ar for 10 min. followed by hydrogen for an additional 10 min. in order to activate the catalyst.

Attention: Hydrogen is highly flammable. No oxygen can be present in the flask as it may cause rapid ignition in the presence of Pd/C. Therefore initial purging with argon is absolutely necessary!

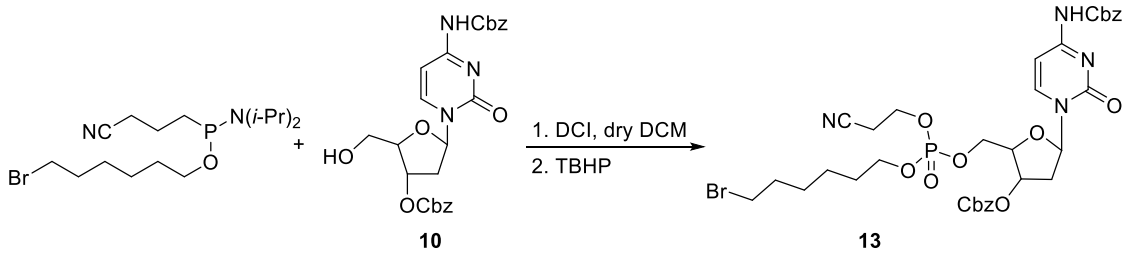
In a separate flask 5'-methanesulfonyl-*N*^{4}-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (**11**, 143 mg, 0.25 mmol) was dissolved in THF/EtOH (20 mL, v/v 1:3) and the resulting solution was purged with Ar for 15 min. It was added drop-wise to the activated Pd/C under a hydrogen atmosphere. The solution was purged with hydrogen for 30 min. It was then filtered through cotton wool and concentrated *in vacuo*. The residue was purified using DCVC, normal phase, gradually from 10% to 20% MeOH in DCM (v/v) to afford product **12** as a thick, transparent oil (69 mg, 91%). R_f 0.34, 1:6 MeOH in DCM (normal phase). t_R (reverse phase-HPLC, column A, method 1): 3.92 min. HRMS ESI calcd for $C_{10}H_{15}N_3O_6S$ Na $[M+Na]^+$ 328.0579, found: 328.0567. ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 7.57 (d, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 15.9 Hz, 2H), 6.19 (t, *J* = 6.8 Hz, 1H), 5.72 (d, *J* = 7.4 Hz, 1H), 5.45 (d, *J* = 4.3 Hz, 1H), 4.43 – 4.24 (m, *J* = 27.6, 11.0 Hz, 2H), 4.24 – 4.13 (m, *J* = 8.7 Hz, 1H), 4.01 – 3.86 (m, *J* = 6.5 Hz, 1H), 3.19 (s, 3H), 2.17 – 1.92 (m, 2H). ^{13}C NMR ($(CD_3)_2SO$, 101 MHz) δ 166.0, 155.4, 141.3, 94.8, 85.6, 83.8, 70.7, 70.1, 49.1, 37.2

5'-Azido-2'-deoxycytidine (3a)



A Schlenk flask equipped with a stirring bar was charged with 5'-methanesulfonyl-2'-deoxycytidine (**12**, 76 mg, 0.25 mmol) and sodium azide (65 mg, 1 mmol). Dry DMF (10 mL) was then added under an Ar atmosphere and the reaction mixture was stirred at 40 °C for 20 h. The solvent was removed under reduced pressure. The residue was purified using DCVC, normal phase, 10% MeOH (v/v) in DCM to afford product **3a** as a white solid (39 mg, 62%). R_f 0.58, 1:6 MeOH in DCM (normal phase). t_R (reverse phase-HPLC, column A, method 1): 4.48 min. m.p. 175-177 °C. Anal. calcd for $C_9H_{12}N_6O_3$: C 42.86, H 4.80, N 33.32, found: C 42.87, H 4.88, N 33.10. HRMS ESI calcd for $C_9H_{12}N_6O_3Na$ $[M+Na]^+$ 275.0869, found: 275.0863. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.75 (d, *J* = 7.5 Hz, 1H), 6.24 (t, *J* = 6.6 Hz, 1H), 5.91 (d, *J* = 7.5 Hz, 1H), 4.29 (dt, *J* = 6.8, 4.2 Hz, 1H), 3.97 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.69 – 3.48 (m, 2H), 2.41 – 2.28 (m, 1H), 2.23 – 2.09 (m, *J* = 13.7 Hz, 1H). ^{13}C NMR (CD_3OD, 101 MHz) δ 166.2, 156.7, 140.8, 94.8, 86.0, 84.9, 71.0, 52.0, 39.9.

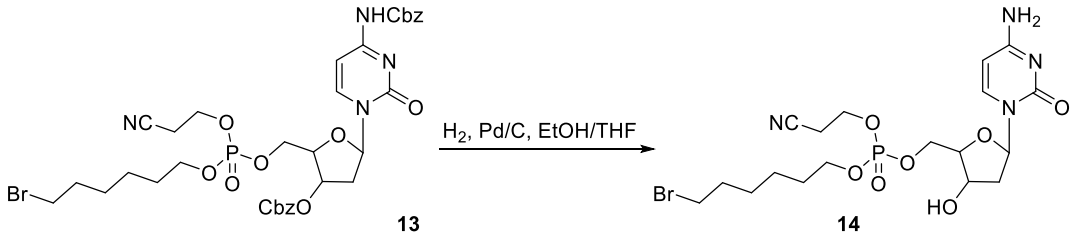
5'-(6-Bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-*N*^{4}-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (13)



10 **13**

A Schlenk flask equipped with a stirring bar was charged with 6-bromohexyl-2-cyanoethyl diisopropylphosphoramidite[4] (113 mg, 0.3 mmol) and dried under vacuum for 3 h. Dry DCM (10 mL) and dry MeCN (5 mL) were added under an Ar atmosphere, followed by *N*^{4}-3'-*O*-bis- benzyloxycarbonyl-2'-deoxycytidine (**10**, 124 mg, 0.25 mmol) and 4,5-dicyanoimidazole (89 mg, 0.75 mmol), which were dried under vacuum for 3 h prior to use. The reaction mixture was stirred at room temperature for 4 h. It was then cooled to 0 °C and *tert*-butyl hydroperoxide solution in decane (5.5 M, 270 µL, 1.5 mmol) was added. The resulting mixture was allowed to warm-up to room temperature and stirred for 2 h. It was diluted with DCM (30 mL) and washed consecutively with 10% Na_2SO_{3aq} (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified using DCVC, reverse phase, gradually from 66% to 75% MeOH in water (v/v) to afford product **13** as a thick, colorless oil (136 mg, 69%). R_f 0.44, 1:40 MeOH in DCM (normal phase); R_f 0.30, 1:3 H_2O in MeOH (reverse phase); t_R (reverse phase-HPLC, column A, method 2): 18.35 min. Anal. Calcd for $C_{34}H_{40}BrN_4O_{11}P$: C 51.59, H 5.09, N 7.08, found: C 51.45, H 5.31, N 6.82. HRMS ESI calcd for $C_{34}H_{40}BrN_4O_{11}PNa$ $[M+Na]^+$ 813.1512, found: 813.1508. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.17 (d, *J* = 7.6 Hz, 1H), 7.61 – 7.09 (m, 11H), 6.26 – 6.12 (m, 1H), 5.33 – 5.26 (m, *J* = 4.0, 2.7 Hz, 1H), 5.22 (s, 2H), 5.20 (s, 2H), 4.47 – 4.33 (m, *J* = 13.9, 7.3 Hz, 3H), 4.27 (dt, *J* = 7.3, 5.9 Hz, 2H), 4.13 (dd, *J* = 13.7, 6.5 Hz, 2H), 3.41 (t, *J* = 6.7 Hz, 2H), 2.87 (t, *J* = 5.9 Hz, 2H), 2.81 – 2.68 (m, *J* = 14.6, 8.3 Hz, 1H), 2.39 – 2.23 (m, *J* = 14.5 Hz, 1H), 1.89 – 1.76 (m, *J* = 14.0 Hz, 2H), 1.75 – 1.62 (m, 2H), 1.51 – 1.34 (m, 4H). ^{13}C NMR (CD_3OD, 101 MHz) δ 163.7, 156.0, 154.4, 144.0, 135.7, 135.3, 128.3, 128.26, 128.25, 128.1, 128.0, 127.9, 117.1, 95.7, 87.6, 83.4, 83.3, 77.4, 69.7, 68.74, 68.67, 67.2, 62.9, 62.8, 48.0, 47.8, 47.6, 47.4, 47.2, 37.9, 32.9, 32.3, 29.7, 29.6, 27.2, 24.2, 18.8, 18.7. ^{31}P NMR (CD_3OD, 162 MHz) δ 1.88, 1.93.

5'-(6-Bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-2'-deoxycytidine (14)



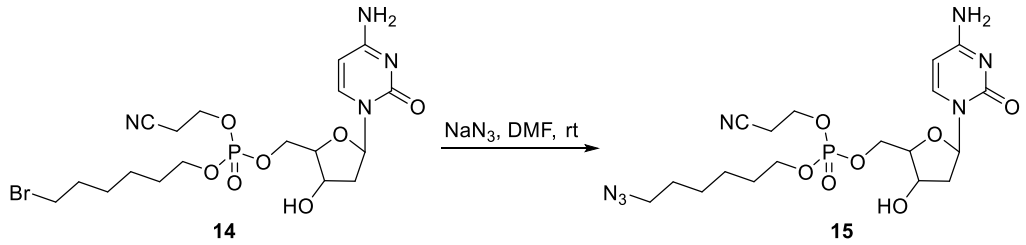
13 **14**

In a Schlenk flask palladium on carbon (10 wt. %, 25 mg) and a stirring bar were placed. It was then equipped with a septum, two needles and purged with Ar for 10 min. followed by hydrogen for an additional 10 min. in order to activate the catalyst.

Attention: Hydrogen is highly flammable. No oxygen can be present in the flask as it may cause rapid ignition in the presence of Pd/C. Therefore initial purging with argon is absolutely necessary!

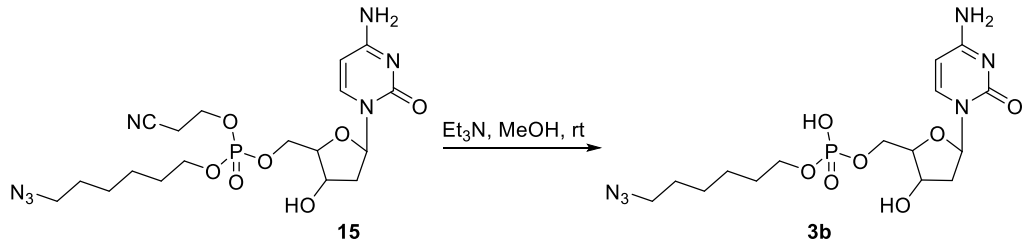
In a separate flask 5'-(6-bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-N^4-3'-*O*-bis-benzyloxycarbonyl-2'-deoxycytidine (**13**, 80 mg, 0.1 mmol) was dissolved in THF/EtOH (10 mL, v/v 1:3) and the resulting solution was purged with Ar for 15 min. It was added drop-wise to the activated Pd/C under a hydrogen atmosphere. The solution was purged with hydrogen for 30 min. It was then filtered through cotton wool and concentrated *in vacuo*. The residue was purified using DCVC, normal phase, gradually from 5% to 10% MeOH in DCM (v/v) to afford product **14** as a thick, colorless oil (49 mg, 93%). R_f 0.39, 1:6 MeOH in DCM (normal phase); t_R (reverse phase-HPLC, column A, method 1): 10.13 min. Anal. Calcd for $C_{18}H_{28}BrN_4O_7P$: C 41.31, H 5.39, N 10.71, found: C 41.28 H 5.55 N 10.64. HRMS ESI calcd for $C_{18}H_{29}BrN_4O_7P$ $[M+H]^+$ 523.0957, found: 523.0950. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.76 (dd, *J* = 7.5, 2.0 Hz, 1H), 6.26 (t, *J* = 6.5 Hz, 1H), 5.92 (dd, *J* = 7.5, 2.0 Hz, 1H), 4.41 – 4.22 (m, 5H), 4.18 – 4.05 (m, 3H), 3.49 – 3.38 (m, *J* = 11.2, 4.5 Hz, 2H), 2.93 – 2.83 (m, *J* = 10.2, 5.5 Hz, 2H), 2.45 – 2.33 (m, *J* = 16.8, 8.9 Hz, 1H), 2.19 – 2.08 (m, 1H), 1.91 – 1.78 (m, 2H), 1.77 – 1.66 (m, 2H), 1.54 – 1.35 (m, 4H). ^{13}C NMR (CD_3OD, 101 MHz) δ 166.2, 156.7, 140.8, 117.1, 94.8, 86.2, 84.8, 84.7, 70.3, 68.6, 68.5, 67.4, 62.74, 62.69, 40.2, 32.9, 32.3, 29.7, 29.6, 27.2, 24.2, 18.8, 18.7. ^{31}P NMR (CD_3OD, 162 MHz) δ 1.88, 1.90.

5'-(6-Azidohex-1-yloxy-2-cyanoethoxy)phosphoryl-2'-deoxycytidine (15)



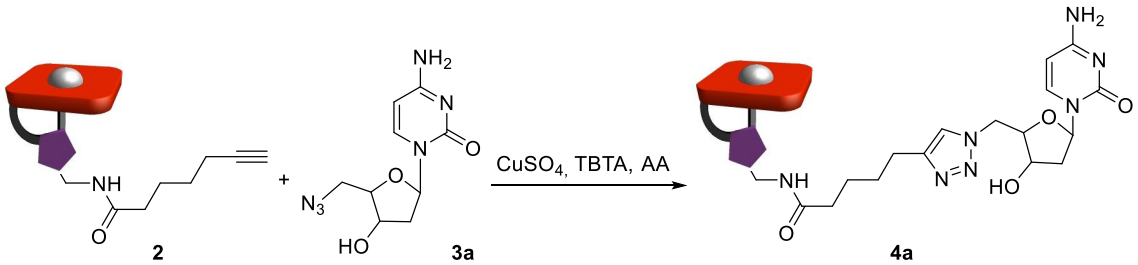
A Schlenk flask equipped with a stirring bar was charged with 5'-(6-bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-2'-deoxycytidine (**14**, 42 mg, 0.08 mmol) and sodium azide (26 mg, 0.4 mmol). Dry DMF (2 mL) was added under an Ar atmosphere and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure at 30 °C. The residue was purified using DCVC, normal phase, gradually from 5% to 20% MeOH in DCM (v/v) to afford product **15** as a thick, colorless oil (36 mg, 92%). R_f 0.25, 1:6 MeOH in DCM (normal phase); t_R (reverse phase-HPLC, column A, method 1): 9.73 min. HRMS ESI calcd for $C_{18}H_{28}N_7O_7PNa$ $[M+Na]^+$ 508.1686, found: 508.1672. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.77 (dd, *J* = 7.5, 1.8 Hz, 1H), 6.27 (t, *J* = 6.6 Hz, 1H), 5.95 (d, *J* = 7.5 Hz, 1H), 4.45 – 4.20 (m, 5H), 4.14 (dd, *J* = 13.8, 6.5 Hz, 2H), 4.10 – 4.02 (m, *J* = 8.5, 4.2 Hz, 1H), 3.30 – 3.20 (m, 2H), 2.95 – 2.84 (m, 2H), 2.44 – 2.30 (m, 1H), 2.15 (dt, *J* = 13.6, 6.7 Hz, 1H), 1.80 – 1.66 (m, 2H), 1.63 – 1.52 (m, *J* = 14.6, 7.4 Hz, 2H), 1.48 – 1.33 (m, 4H). ^{13}C NMR (CD_3OD, 101 MHz) δ 166.1, 156.8, 140.9, 117.1, 95.0, 86.2, 84.9, 84.8, 70.3, 68.62, 68.55, 67.42, 67.36, 62.8, 62.7, 50.9, 40.1, 29.74, 29.67, 28.3, 25.9, 24.7, 18.8, 18.7. ^{31}P NMR (CD_3OD, 162 MHz) δ 1.90, 1.94.

5'-(6-Azidohex-1-yloxy)phosphoryl-2'-deoxycytidine (3b)



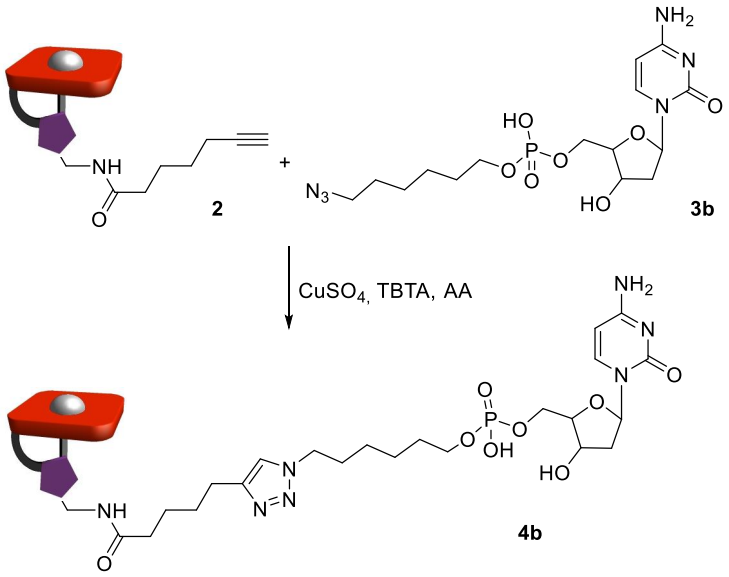
A round-bottomed flask equipped with a stirring bar was charged with 5'-(6-azidohex-1-yloxy-2-cyanoethoxy)phosphoryl-2'-deoxycytidine (**15**, 24 mg, 0.05 mmol). MeOH (2 mL) was added followed by Et_3N (0.5 mL). The reaction mixture was stirred at room temperature for 30 min. The solvent and amine were removed under reduced pressure and the residue was purified using DCVC, normal phase: 1) first 50 mL of 15% MeOH in DCM + 0.5% TFA (v/v) was used as an eluent, 2) then the chromatography was continued with MeOH in DCM, gradually from 25% to 50% to afford product **3b** as a white solid (19 mg, 89%). R_f 0.36, 2:3 MeOH in DCM (normal phase); t_R (reverse phase-HPLC, column A, method 1): 7.35 min. m.p. 134-136 °C. HRMS ESI calcd for $C_{15}H_{25}N_6O_7PNa$ $[M+Na]^+$ 455.1420, found: 455.1411. ^1H NMR (CD_3OD, 400 MHz) δ 8.31 (d, *J* = 7.8 Hz, 1H), 6.23 (t, *J* = 6.4 Hz, 1H), 6.11 (d, *J* = 7.8 Hz, 1H), 4.51 – 4.43 (m, 1H), 4.16 – 3.98 (m, *J* = 15.8 Hz, 3H), 3.88 (q, *J* = 6.4 Hz, 2H), 3.27 (t, *J* = 6.9 Hz, 2H), 2.44 – 2.34 (m, *J* = 13.6, 9.8 Hz, 1H), 2.32 – 2.21 (m, *J* = 13.2 Hz, 1H), 1.71 – 1.53 (m, 4H), 1.49 – 1.33 (m, 4H). ^{13}C NMR (CD_3OD, 101 MHz) δ 160.9, 148.9, 144.5, 93.7, 86.9, 86.8, 86.6, 70.7, 65.3, 65.2, 64.4, 51.0, 40.5, 30.33, 30.25, 28.4, 26.1, 25.1. ^{31}P NMR (CD_3OD, 162 MHz) δ 0.05.

Nucleoside-vitamin B_{12} conjugate 4a



A round-bottomed flask equipped with a stirring bar was charged with 5'-azido-2'-deoxycytidine (**3a**, 2.5 mg, 0.01 mmol) and *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**, 29 mg, 0.02 mmol). DMSO (9.84 mL) was added, followed by water (8.6 mL), triethylammonium acetate buffer stock solution (2 M, 1 mL), Cu-TBTA (10 mM in DMSO/water: v/v 11:9) stock solution (300 µL) and ascorbic acid stock solution (10 mM, 300 µL). The reaction mixture was stirred for 20 h at room temperature. It was then concentrated under reduced pressure, transferred to Falcon tube, and acetone/hexane (50 mL, v/v 1:1) was added. The mixture was centrifuged and a colorless supernatant was decantated. Acetone (50 mL) was added and the suspension was sonicated and centrifuged once more. Solid precipitate was purified using DCVC, reverse phase, gradually from 10% to 20% MeCN in water (v/v) to afford product **4a** as a purple solid (17 mg, 95%). R_f 0.44, 1:3 MeCN in H_2O (reverse phase); t_R (reverse phase-HPLC, column B, method 1): 6.73 min. Anal. calcd for $C_{79}H_{109}CoN_{21}O_{17}P \cdot 8H_2O$: C 51.04, H 6.78, N 15.82, found: C 50.94, H 6.76, N 15.89. HRMS ESI calcd for $C_{79}H_{109}CoN_{21}O_{17}PNa$ $[M+Na]^+$ 1736.7278, found: 1736.7258. UV-Vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 551 (7.3×10^3), 517 (6.6×10^3), 362 (2.3×10^4), 274 (1.9×10^4). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.84 (s, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.25 (s, 1H), 7.05 (s, 1H), 6.57 (s, 1H), 6.29 (d, *J* = 2.8 Hz, 1H), 6.20 (t, *J* = 6.4 Hz, 1H), 6.04 (s, 1H), 5.87 (d, *J* = 7.4 Hz, 1H), 4.71 (qd, *J* = 14.5, 5.1 Hz, 2H), 4.59 – 4.44 (m, 2H), 4.35 (dd, *J* = 10.6, 4.9 Hz, 1H), 4.30 (dd, *J* = 14.7, 7.6 Hz, 1H), 4.20 (dd, *J* = 9.6, 4.7 Hz, 1H), 4.18 – 4.15 (m, 1H), 4.12 (d, *J* = 11.4 Hz, 1H), 3.94 (t, *J* = 7.3 Hz, 1H), 3.85 (d, *J* = 13.3 Hz, 1H), 3.66 (d, *J* = 13.9 Hz, 1H), 3.61 (dd, *J* = 10.6, 5.0 Hz, 1H), 3.39 (d, *J* = 11.0 Hz, 1H), 2.89 – 1.84 (m, 26H), 2.58 (s, 6H), 2.28 (s, 3H), 2.27 (s, 3H), 1.89 (s, 3H), 1.82 – 1.64 (m, 6H), 1.50 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H), 1.27 (d, *J* = 5.8 Hz, 3H), 1.20 (s, 3H), 1.33 – 1.04 (m, 3H), 0.43 (s, 3H). ^{13}C NMR (CD_3OD, 126 MHz) δ 181.6, 180.1, 177.7, 177.3, 176.6, 175.9, 175.5, 175.3, 174.6, 174.0, 167.6, 167.3, 166.9, 158.1, 149.0, 143.4, 142.5, 138.2, 135.7, 133.9, 131.4, 124.6, 117.8, 112.5, 108.7, 105.1, 96.3, 95.7, 87.7, 87.6, 86.4, 85.6, 80.5, 78.3, 76.3, 73.8, 72.4, 70.8, 60.3, 57.7, 57.0, 54.5, 52.6, 52.5, 49.4, 48.4, 46.9, 44.0, 43.0, 41.6, 40.6, 40.1, 36.9, 36.2, 35.4, 33.7, 33.1, 32.9, 32.4, 32.3, 30.0, 29.5, 27.41, 27.35, 26.2, 26.0, 20.9, 20.5, 20.4, 20.3, 19.9, 17.5, 17.0, 16.4, 16.1.

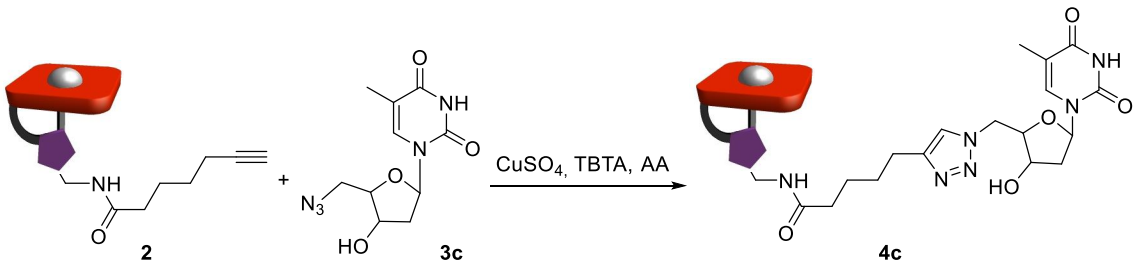
Nucleotide-vitamin B_{12} conjugate 4b



A round-bottomed flask equipped with a stirring bar was charged with 5'-(6-azidohex-1-yloxy)phosphoryl-2'-deoxycytidine (**3b**, 2.2 mg, 0.005 mmol) and *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**, 14.6 mg, 0.01 mmol). DMSO (5 mL) was added, followed by water (4.28 mL), triethylammonium acetate buffer stock solution (2 M, 500 µL), Cu-TBTA (10 mM in DMSO/water: v/v 11:9) stock solution (150 µL) and ascorbic acid stock solution (10 mM, 150 µL). The reaction mixture was stirred for 20 h at room temperature. It was then concentrated under reduced pressure, transferred to Falcon tube, and acetone/hexane (50 mL, v/v 1:1) was added. The mixture was centrifuged and a colorless supernatant was decantated. Acetone (50 mL) was added and the suspension was sonicated and centrifuged once more. Solid precipitate was purified using DCVC, reverse phase: 1) first 30 mL of 10% MeCN in water + 0.5% TFA (v/v) was used as an eluent, 2) then the chromatography was continued with MeCN in water, gradually from 15% to 20% to afford product **4b** as a purple solid (7.4 mg, 78%). R_f 0.43, 1:3 MeCN in H_2O (reverse phase); t_R (reverse phase-HPLC, column B, method 1): 7.03 min. Anal. Calcd for $C_{85}H_{122}CoN_{21}O_{21}P_2 \cdot 9H_2O$: C 49.63, H 6.86, N 14.30, found: C 49.27, H 6.72, N 13.91. HRMS ESI calcd for $C_{85}H_{122}CoN_{21}O_{21}P_2Na_2$ $[M+2Na]^{2+}$ 1939.7716, found: 969.8898. UV-Vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 550 (6.7×10^3), 516 (6.1×10^3), 361 (2.1×10^4), 277 (1.9×10^4). ^{1}H NMR (CD_3OD, 500 MHz) δ 8.28 (d, *J* = 7.8 Hz, 1H), 7.79 (s, 1H), 7.24 (s, 1H), 7.06 (s, 1H), 6.58 (s, 1H), 6.30 (d, *J* = 3.2 Hz, 1H), 6.24 (t, *J* = 6.4 Hz, 1H), 6.11 (d, *J* = 7.7 Hz, 1H), 6.05 (s, 1H), 4.60 – 4.54 (m, 1H), 4.54 – 4.49 (m, 1H), 4.49 – 4.44 (m, 1H), 4.36 (t, *J* = 7.1 Hz, 2H), 4.34 – 4.26 (m, 1H), 4.18 (t, *J* = 3.4 Hz, 1H), 4.12 (d, *J* = 11.6 Hz, 1H), 4.11 – 4.00 (m, 3H), 3.96 (t, *J* = 8.3 Hz, 1H), 3.91 – 3.81 (m, 3H), 3.72 (t, *J* = 6.3 Hz, 1H), 3.65 (d, *J* = 13.9 Hz, 1H), 3.61 (dd, *J* = 10.7, 5.1 Hz, 1H), 3.38 (d, *J* = 11.0 Hz, 1H), 2.90 – 1.85 (m, 27H), 2.57 (s, 6H), 2.28 (s, 3H), 2.26 (s, 3H), 1.89 (s, 3H), 1.82 – 1.68 (m, 5H), 1.66 – 1.58 (m, 2H), 1.50 (s, 3H), 1.44 (m, 2H), 1.40 – 1.23 (m, 5H), 1.37 (s, 3H), 1.36 (s, 3H), 1.26 (d, *J* = 6.2 Hz, 3H) 1.20 (s, 3H), 1.12 (m, 1H), 0.44 (s, 3H). ^{13}C NMR (CD_3OD, 126 MHz) δ 181.7, 180.1, 177.8, 177.7, 177.4, 176.6, 176.0, 175.6, 175.4, 174.7, 174.1, 167.3, 166.9, 162.8, 148.9, 145.7, 143.4, 138.3, 135.7, 133.9, 131.5, 123.3, 117.9, 112.5, 108.8, 105.1, 95.7, 95.3, 88.3, 88.2, 88.0, 87.7,

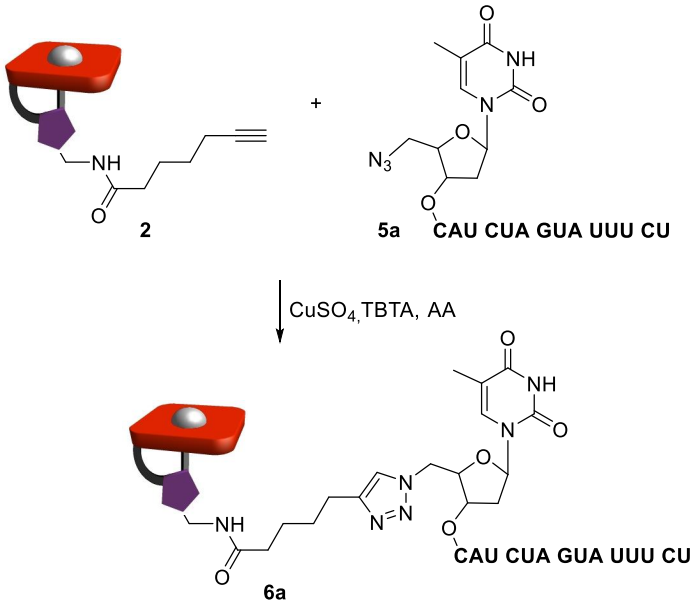
86.5, 80.63, 80.59, 78.2, 76.4, 73.92, 73.86, 72.2, 70.9, 68.9, 66.62, 66.57, 65.92, 65.87, 60.3, 57.7, 57.1, 54.7, 52.6, 51.2, 49.5, 49.3, 49.2, 48.5, 46.93, 46.89, 44.1, 43.1, 41.9, 41.6, 40.1, 36.9, 36.3, 35.5, 33.8, 33.1, 33.0, 32.5, 32.3, 31.7, 31.6, 31.3, 30.2, 29.6, 27.5, 27.4, 27.2, 26.5, 26.4, 26.3, 26.1, 20.9, 20.51, 20.46, 20.33, 20.29, 20.27, 20.0, 17.5, 17.2, 16.4, 16.2. ^{31}P NMR (CD_3OD, 202 MHz) δ 0.29, 0.06.

Nucleoside-vitamin B_{12} conjugate 4c



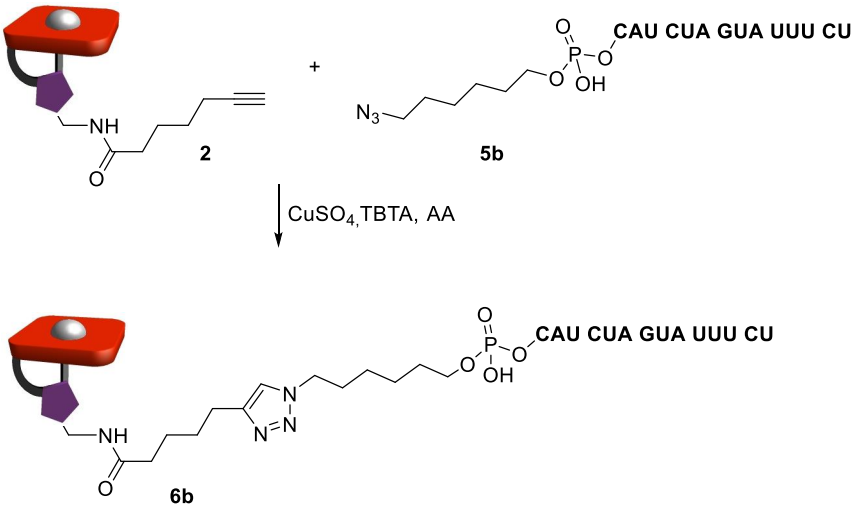
A round-bottomed flask equipped with a stirring bar was charged with 5'-azido-thymidine (**3c**, 5.4 mg, 0.02 mmol) and *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**, 29 mg, 0.02 mmol). DMSO (470 µL) was added, followed by water (380 µL), Triethylammonium acetate buffer stock solution (2 M, 50 µL), Cu- TBTA (10 mM in DMSO/water: v/v 11:9) stock solution (50 µL) and freshly prepared ascorbic acid solution (10 mM, 50 µL). The reaction mixture was stirred for 48 h at room temperature. It was then concentrated under reduced pressure, transferred to Falcon tube, and acetone/hexane (50 mL, v/v 1:1) was added. The mixture was centrifuged and a colorless supernatant was decantated. Acetone (50 mL) was added and the suspension was sonicated and centrifuged once more. Solid precipitate was purified using DCVC, reverse phase, gradually from 10% to 20% MeCN in water (v/v) to afford product **4c** as a purple solid (12 mg, 69%). R_f 0.18, 1:4 MeCN in H_2O (reverse phase); t_R (reverse phase-HPLC, column B, method 1): 7.23 min. Anal. calcd for $C_{80}H_{110}CoN_{20}O_{18}P \cdot 9H_2O$: C 50.79, H 6.82, N 14.81, found: C 50.80, H 6.81, N 14.90. HRMS ESI calcd for $C_{80}H_{109}CoN_{20}O_{18}P$ $[M-H]^-$ 1727.7298, found: 1727.7280. UV-Vis (H_2O) λ_{max} (nm) (ε, L mol^{-1} cm^{-1}) 548 (7.8×10^3), 518 (6.9×10^3), 362 (2.4×10^4), 274 (2.0×10^4). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.83 (s, 1H), 7.23 (d, *J* = 16.4 Hz, 2H), 7.05 (s, 1H), 6.57 (s, 1H), 6.27 (d, *J* = 3.3 Hz, 1H), 6.20 (t, *J* = 6.7 Hz, 1H), 6.04 (s, 1H), 4.76 – 4.61 (m, 2H), 4.55 – 4.45 (m, 2H), 4.43 – 4.36 (m, 1H), 4.35 – 4.24 (m, 1H), 4.22 – 4.07 (m, 3H), 3.98 – 3.89 (m, 1H), 3.82 (dd, *J* = 13.5, 2.0 Hz, 1H), 3.66 (d, *J* = 14.1 Hz, 1H), 3.61 (dd, *J* = 10.6, 5.2 Hz, 1H), 3.39 (d, *J* = 10.9 Hz, 1H), 2.91 – 1.61 (m, 35H), 2.58 (s, 3H), 2.57 (s, 3H), 2.28 (s, 3H), 2.26 (s, 3H), 1.88 (s, 3H), 1.50 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H), 1.26 (d, *J* = 6.3 Hz, 3H), 1.20 (s, 3H), 1.42 – 1.07 (m, 3H), 0.43 (s, 3H). ^{13}C NMR (CD_3OD, 126 MHz) δ 181.6, 180.1, 177.73, 177.71, 177.3, 176.6, 175.9, 175.52, 175.51, 175.3, 174.6, 174.0, 167.3, 166.9, 166.2, 152.2, 149.0, 143.4, 138.2, 138.0, 135.7, 133.8, 131.4, 124.5, 117.8, 112.5, 112.0, 108.7, 105.1, 95.7, 87.5, 86.6, 86.4, 85.5, 80.6, 80.5, 78.2, 78.1, 76.3, 73.82, 73.77, 72.4, 70.77, 70.75, 60.3, 57.7, 57.0, 54.5, 52.6, 52.4, 49.4, 48.4, 46.89, 46.85, 44.0, 43.1, 41.5, 40.1, 39.6, 36.9, 36.2, 35.4, 33.7, 33.1, 32.9, 32.4, 32.3, 30.0, 29.5, 27.39, 27.35, 26.2, 26.0, 20.9, 20.5, 20.4, 20.3, 20.23, 20.21, 19.9, 17.5, 17.1, 16.4, 16.2, 12.5.

2'OMeRNA-vitamin B_{12} conjugate 6a



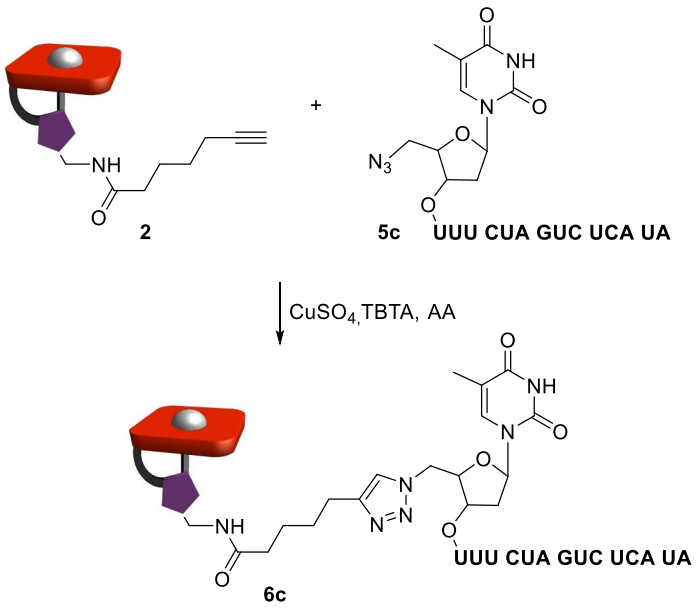
Solution A: *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**, 2.3 mg, 1.6 µmol) was dissolved in distilled water (320 µL) to afford 5 mM solution. **Solution B**: 2'OMeRNA oligonucleotide **5a** (5'-N_3-deoxythymidine-CAU CUA GUA UUU CU-3', 8 mg, 1.6 µmmol) was dissolved in distilled water (500 µL). The exact concentration of the solution was determined by NanoDrop UV-Vis Spectroscopy to be 14580 ng / µL. To a 1.5 mL Eppendorf tube equipped with a stirring bar DMSO (197 µL), distilled water (28 µL) and triethylammonium acetate buffer stock solution (2 M, 20 µL) were added. They were followed by solution **A** (80 µL) and solution **B** (63 µL). Cu-TBTA (10 mM in DMSO/water: v/v 11:9) stock solution (6 µL) and ascorbic acid stock solution (10 mM, 6 µL) were added. The reaction mixture was stirred at room temperature. After 1.5 h, 3 h and 4.5 h portions of ascorbic acid stock solution (10 mM, 3 x 6 µL) were added. After 20 h the HPLC analysis indicated 97% conversion. The reaction mixture was diluted with distilled water (4.5 mL) and purified using preparative reverse phase-HPLC, column C, method 3. The eluate was freeze-dried to afford product **6a** as a pink solid. t_R (reverse phase-HPLC, column C, method 3): 11.22 min. MS ESI calcd for $C_{224}H_{298}CoN_{63}O_{120}P_{15}$ $[M-H]^-$ 6316.65, found: 6318.50.

2'OMeRNA-vitamin B_{12} conjugate 6b



Solution A: *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**, 2.3 mg, 1.6 µmol) was dissolved in distilled water (320 µL) to afford 5 mM solution. **Solution B**: 2'OMeRNA oligonucleotide **5b** (5'-N_3-hexyl-CAU CUA GUA UUU CU-3', 8 mg, 1.6 µmmol) was dissolved in distilled water (500 µL). The exact concentration of the solution was determined by NanoDrop UV-Vis Spectroscopy to be 19943 ng / µL. To a 1.5 mL Eppendorf tube equipped with a stirring bar DMSO (197 µL), distilled water (37 µL) and triethylammonium acetate buffer stock solution (2 M, 20 µL) were added. They were followed by solution **A** (80 µL) and solution **B** (54 µL). Cu-TBTA (10 mM in DMSO/water: v/v 11:9) stock solution (6 µL) and ascorbic acid stock solution (10 mM, 6 µL) were added. The reaction mixture was stirred at 45 °C. After 1.5 h and 3 h portions of ascorbic acid stock solution (10 mM, 3 x 6 µL) were added. After 24 h the HPLC measurement indicated 71% conversion. The reaction mixture was diluted with distilled water (4.5 mL) and purified using preparative reverse phase-HPLC, column C, method 4. The eluate was freeze-dried to afford product **6b** as a pink solid. t_R (reverse phase-HPLC, column C, method 4): 10.45 min.

Scrambled 2'OMeRNA-vitamin B_{12} conjugate 6c

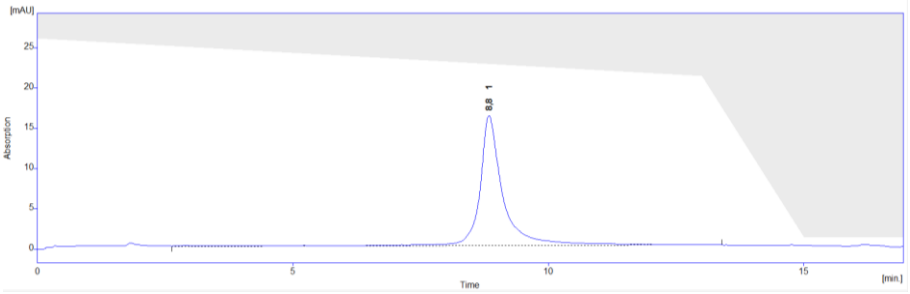


Solution A: *N*-(cobalamin-5'-yl)-hept-6-ynamide (**2**, 2.3 mg, 1.6 μmol) was dissolved in distilled water (320 μL) to afford 5 mM solution. **Solution B**: 2'OMeRNA oligonucleotide **5c** (5'-N_3-deoxythymidine-UUU CUA GUC UCA UA-3', 8 mg, 1.6 μmmol) was dissolved in distilled water (500 μL). The exact concentration of the solution was determined by NanoDrop UV-Vis Spectroscopy to be 17801 ng / μL. To a 1.5 mL Eppendorf tube equipped with a stirring bar DMSO (197 μL), distilled water (29 μL) and triethylammonium acetate buffer stock solution (2 M, 20 μL) were added. They were followed by solution **A** (80 μL) and solution **B** (62 μL). Cu-TBTA (10 mM in DMSO/water: v/v 11:9) stock solution (6 μL) and ascorbic acid stock solution (10 mM, 6 μL) were added. The reaction mixture was stirred at 45 °C. After 1.5 h and 3 h portions of ascorbic acid stock solution (10 mM, 2 x 6 μL) were added. After 48 h the HPLC analysis indicated 80% conversion. The reaction mixture was diluted with distilled water (4.5 mL) and purified using preparative reverse phase-HPLC, column C, method 3. The eluate was freeze-dried to afford product **6c** as a pink solid. t_R (reverse phase-HPLC, column C, method 3): 11.72 min. MS ESI calcd for $C_{224}H_{298}CoN_{63}O_{120}P_{15}$ $[M-H]^-$ 6316.65, found: 6318.00.

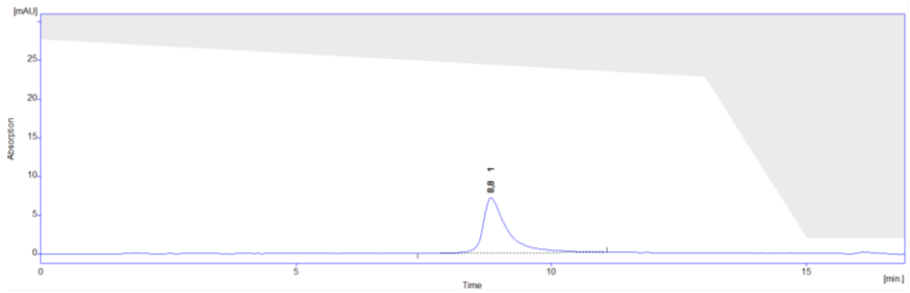
9. Stability of conjugates in bacteria medium

The stability tests were performed in a qualitative manner. Prior to the addition to the bacterial medium, the purity of a conjugate was ensured by HPLC analysis. Then the conjugate was added to the bacterial medium (50 μM concentration of a conjugate in the media). After 24 h a sample was taken and analysed by HPLC. As no decomposition was observed, quantitative experiments were not required.

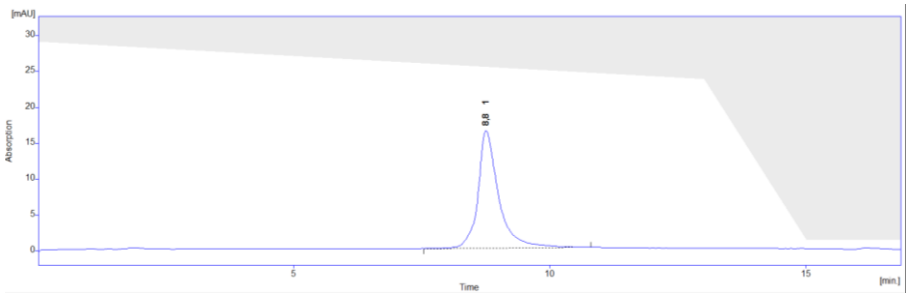
2'OMeRNA-vitamin B_{12} conjugate **6a** (pure compound before the addition to bacteria medium)



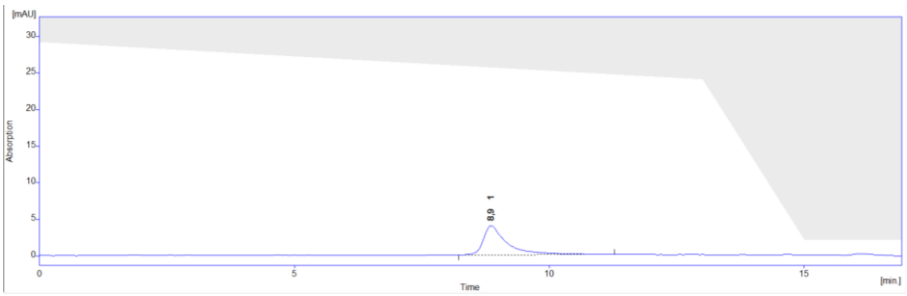
2'OMeRNA-vitamin B_{12} conjugate **6a** after 24 h in bacteria medium



2'OMeRNA-vitamin B_{12} conjugate **6c** (pure compound before the addition to bacteria medium)

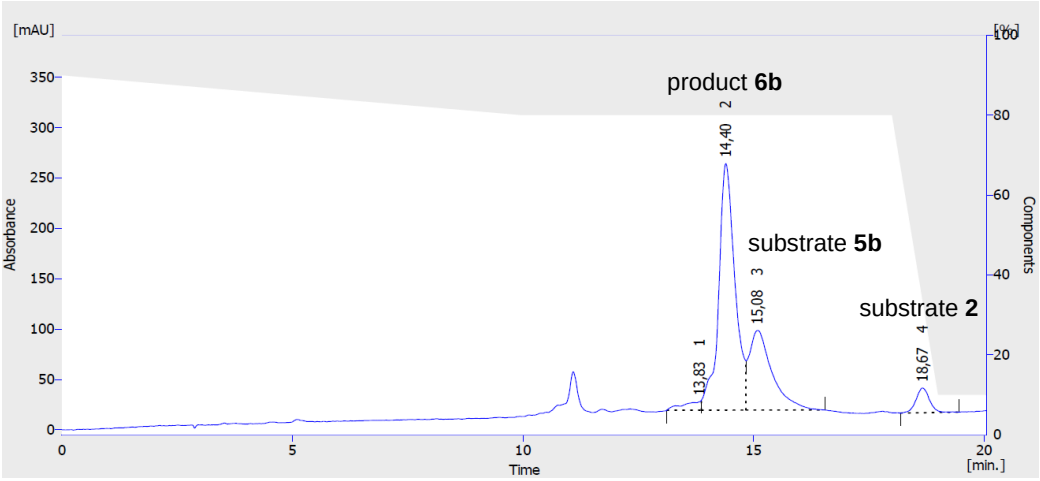


2'OMeRNA-vitamin B_{12} conjugate **6c** after 24 h in bacteria medium



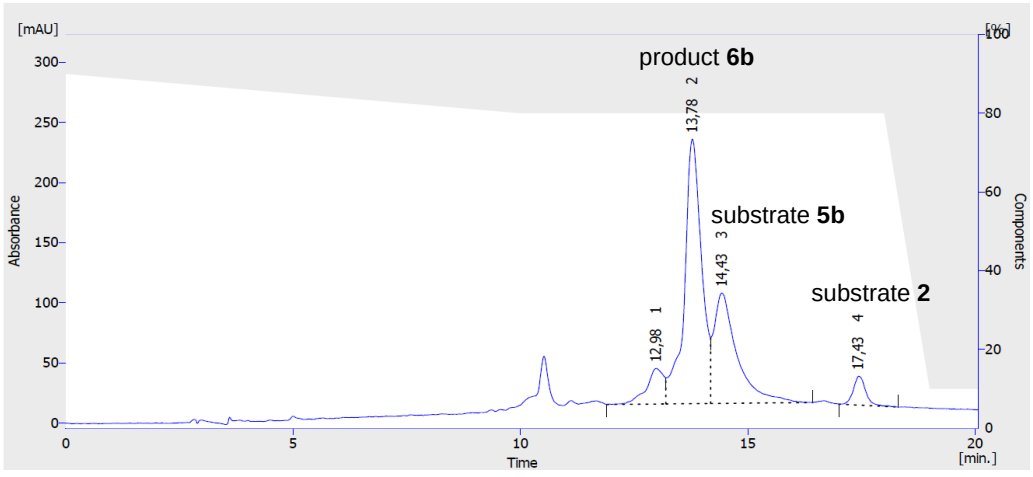
Vitamin B_{12}-hexyl-2'OMeRNA conjugate 6b

After purification, conjugate **6b** was analysed by ESI MS and the signal at m/z = 6193.50 corresponds to a pseudomolecular ion $[M-H]^-$, but RP-HPLC (column A, method 4) analysis showed that during the freeze-drying procedure its purity decreased to 75%. Nevertheless, the stability test in bacteria medium was performed. Conjugates **6b** was added at 50 µM concentrations to Davis Minimal Broth.[9] Following overnight incubation at 37 °C with shaking, RP-HPLC analysis was performed. The resulting chromatogram did not show any important differences before and after incubation. Therefore, this conjugate was considered stable in the presence of the tested medium.



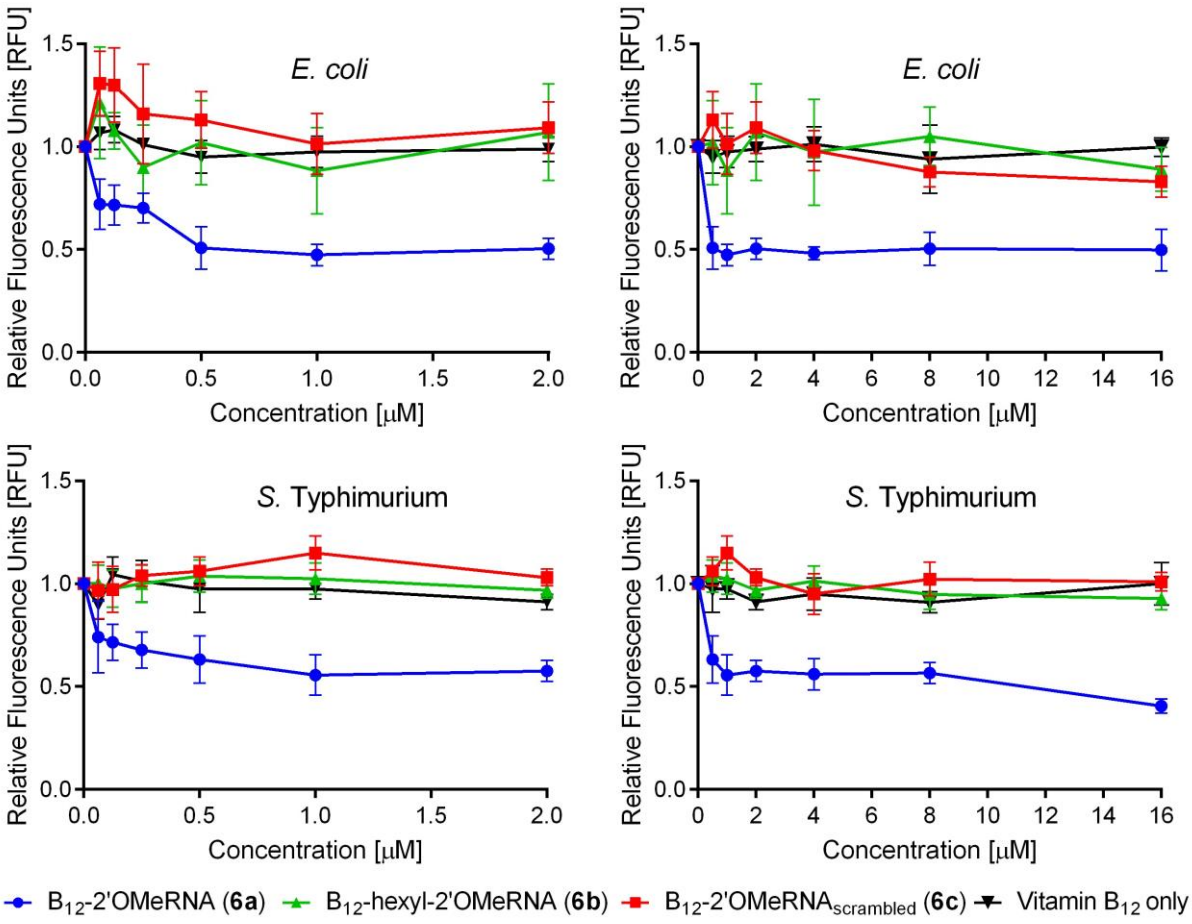
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	13,833	239,666	8,509	2,5	2,4	0,43	856
2	14,400	6058,605	244,938	63,3	68,6	0,37	856
3	15,083	2778,944	79,085	29,0	22,1	0,55	903
4	18,667	499,526	24,548	5,2	6,9	0,32	931
	Total	9576,741	357,079	100,0	100,0		

Vitamin B_{12}-hexyl-2'OMeRNA conjugate 6b after 24 h in bacteria medium



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	12,983	781,729	29,666	7,6	8,1	0,40	987
2	13,783	5780,549	220,073	56,0	60,2	0,38	995
3	14,433	3307,602	91,722	32,0	25,1	0,55	973
4	17,433	461,408	24,121	4,5	6,6	0,28	722
	Total	10331,289	365,582	100,0	100,0		

10. Relative fluorescence measured after treatment of *E. coli* and *S.* Typhimurium cells with vitamin B_{12}-2'OMeRNA conjugates 6a, 6b, 6c and vitamin B_{12} only



Left graphs show data for finer concentrations in the 0 – 2 μM range and right graphs up to 16 μM concentrations. Error bars represent standard errors from three independent biological experiments. The differences between the conjugate **6a** and **6b**, **6c** or vitamin B_{12} only are significant with $P \leq 0.05$ (determined with ANOVA method without correction for multiple comparisons). The level of fluorescence of cells treated with vitamin B_{12}-hexyl-2'OMeRNA (**6b**) was not reduced, which might stem from the fact that: the compound was obtained as a mixture, was not stable in cells, its three dimensional structure prevented efficient interactions with the vitamin B_{12} membrane receptors or the mRNA target.

11. References

(1) D. S. Pedersen, C. Rosenbohm, *Synthesis (Stuttg).* **2001**, *2001* (16), 2431.

(2) A. Jackowska, M. Chromiński, M. Giedyk, D. Gryko, *Org. Biomol. Chem.* **2018**, *16*, 936.

(3) I. Van Daele, H. Munier-Lehmann, M. Froeyen, J. Balzarini, S. Van Calenbergh, *J. Med. Chem.* **2007**, *50* (22), 5281.

(4) S. Debarge, J. Balzarini, A. R. Maguire, *J. Org. Chem.* **2011**, *76* (1), 105.

(5) J. Lietard, A. Meyer, J.-J. Vasseur, F. Morvan, *Tetrahedron Lett.* **2007**, *48* (50), 8795.

(6) M. Równicki, M. Wojciechowska, A. J. Wierzba, J. Czarnecki, D. Bartosik, D. Gryko, J. Trylska, *Sci. Rep.* **2017**, *7* (1), 7644.

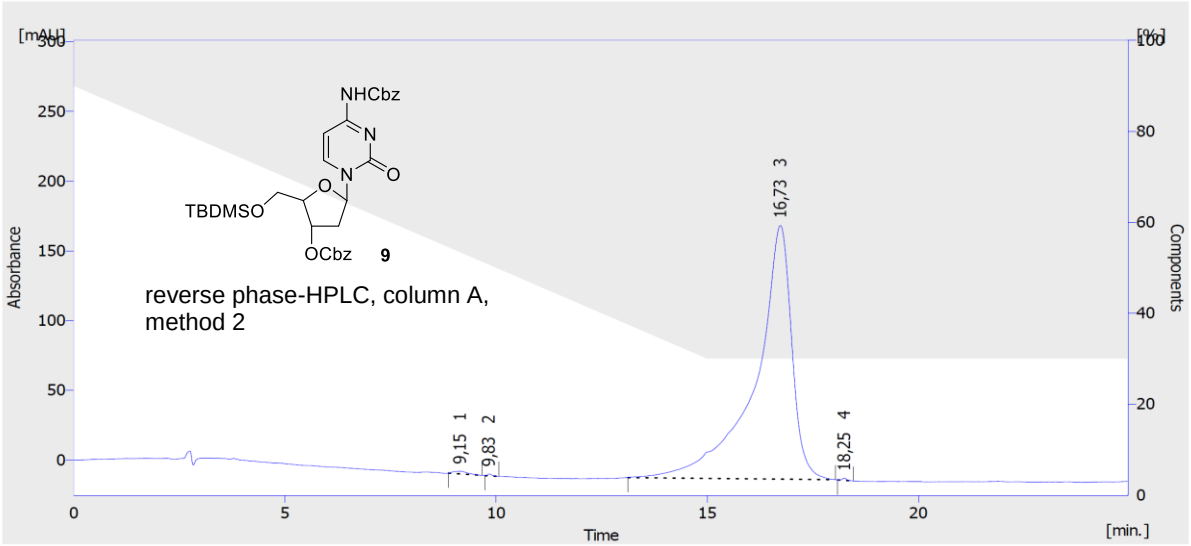
(7) M. S. Guyer, R. R. Reed, J. A. Steitz, K. B. Low, *Cold Spring Harb. Symp. Quant. Biol.* **1981**, *45*, 135.

(8) H. Tada, O. Shiho, K. Kuroshima, M. Koyama, K. Tsukamoto, *J Immunol Methods.* **1986**, *6;93(2)*, 157.

(9) B. D. Davis, *Proc. Natl. Acad. Sci.*, **1949**, *35 (1)*, 1.

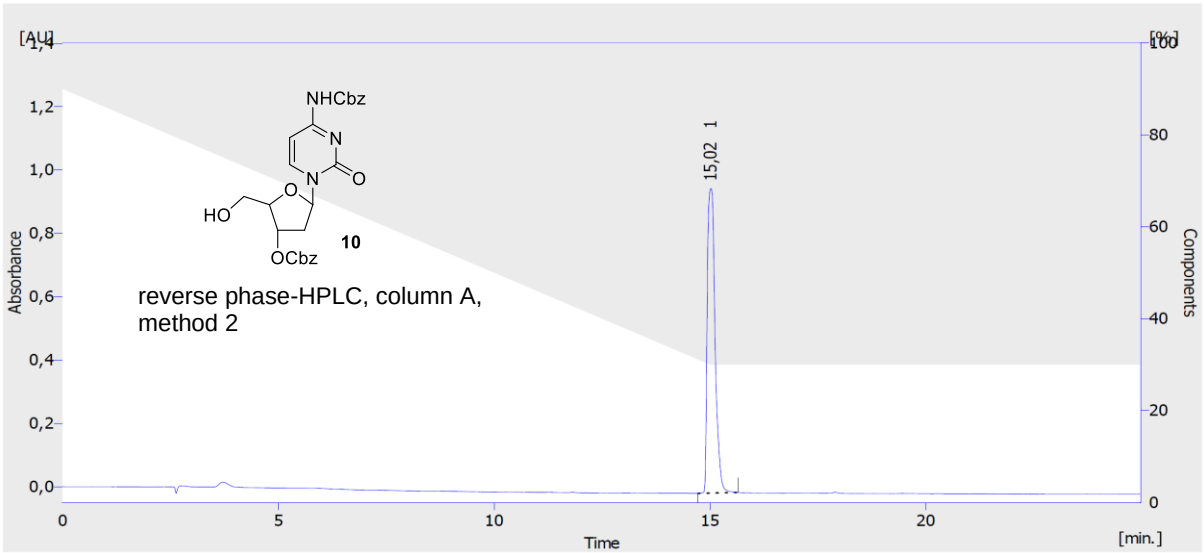
12. HPLC chromatograms

5'-*O-tert*-Butyldimethylsilyl-N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (9)



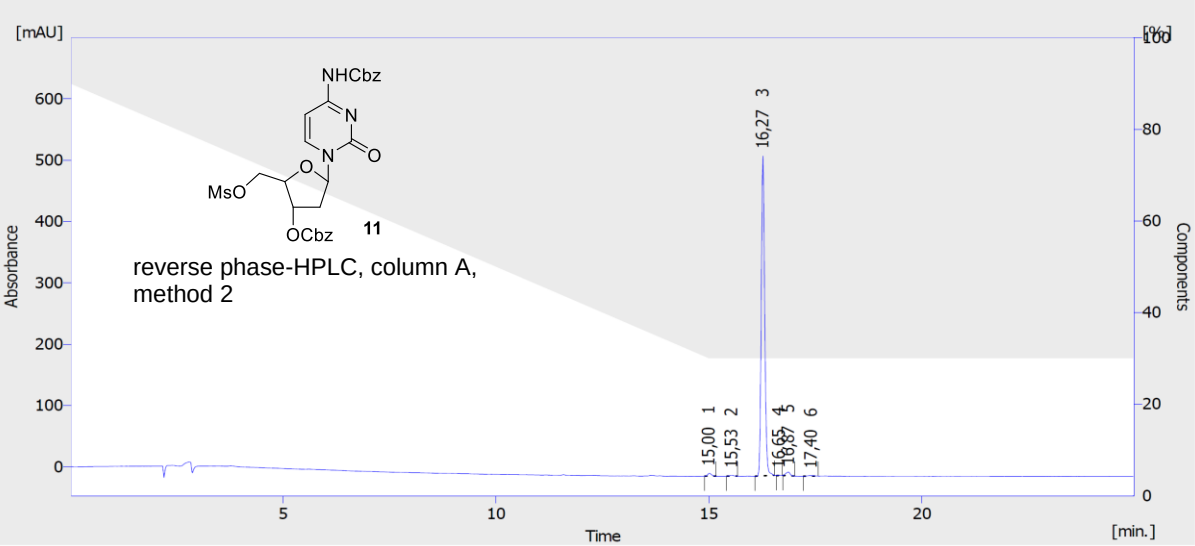
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,150	46,473	1,988	0,4	1,1	0,38	995
2	9,833	10,035	1,443	0,1	0,8	0,12	997
3	16,733	10937,983	182,003	99,4	97,3	0,70	491
4	18,250	11,544	1,656	0,1	0,9	0,12	1000
	Total	11006,035	187,089	100,0	100,0		

N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (10)



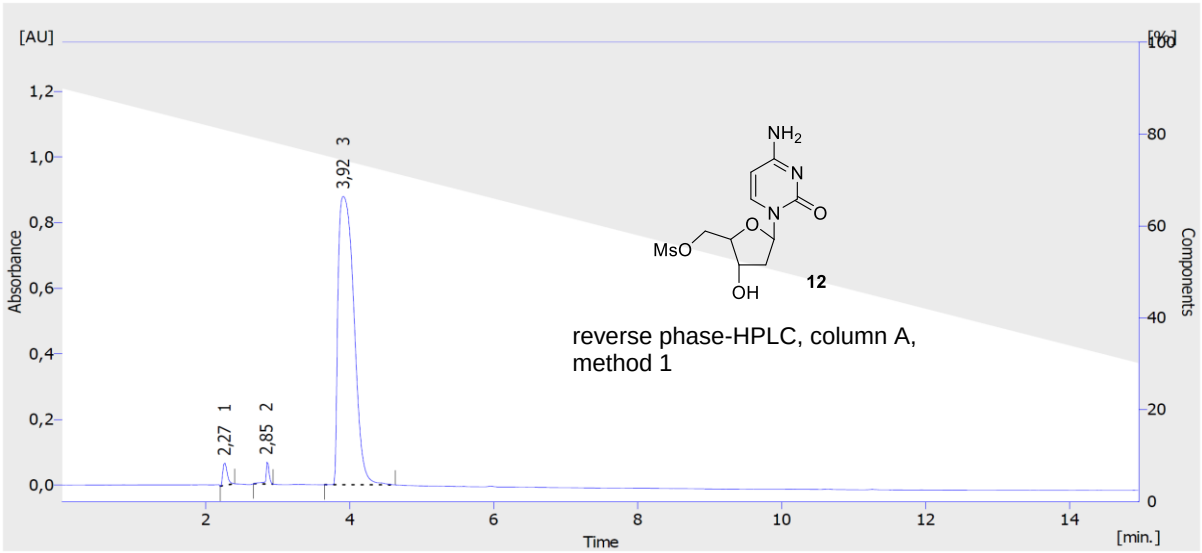
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	15,017	11998,984	962,307	100,0	100,0	0,20	459
	Total	11998,984	962,307	100,0	100,0		

5'-Methanesulfonyl-N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (11)



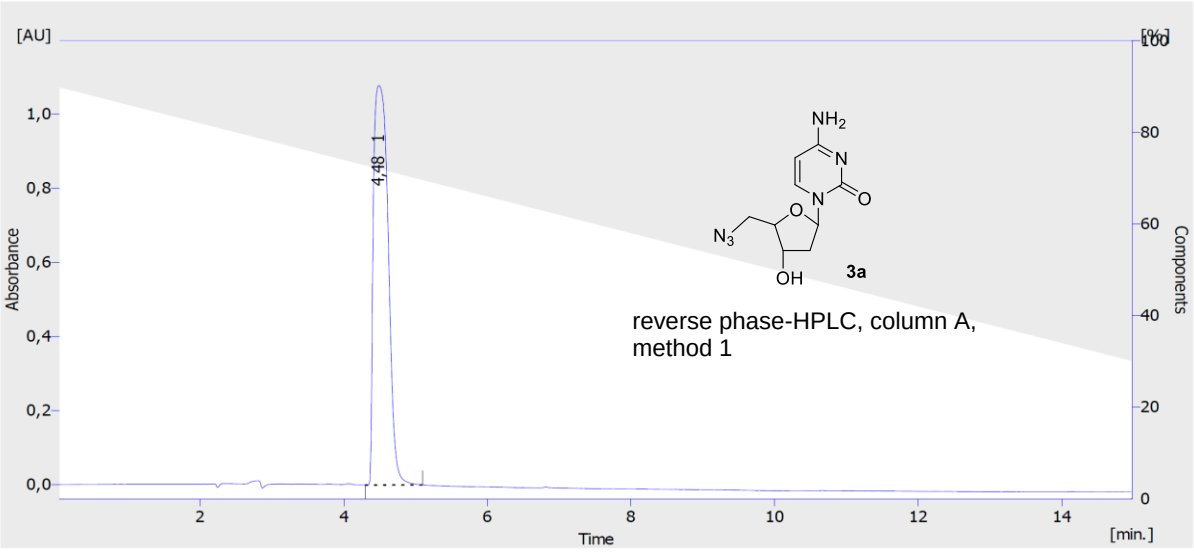
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	15,000	27,399	4,495	0,9	0,8	0,10	998
2	15,533	7,751	1,223	0,3	0,2	0,12	1000
3	16,267	2927,720	521,286	97,0	97,5	0,10	498
4	16,650	3,194	0,781	0,1	0,1	0,08	1000
5	16,867	43,015	6,173	1,4	1,2	0,13	799
6	17,400	7,855	0,711	0,3	0,1	0,22	1000
	Total	3016,933	534,670	100,0	100,0		

5'-Methanesulfonyl-2'-deoxycytidine (12)



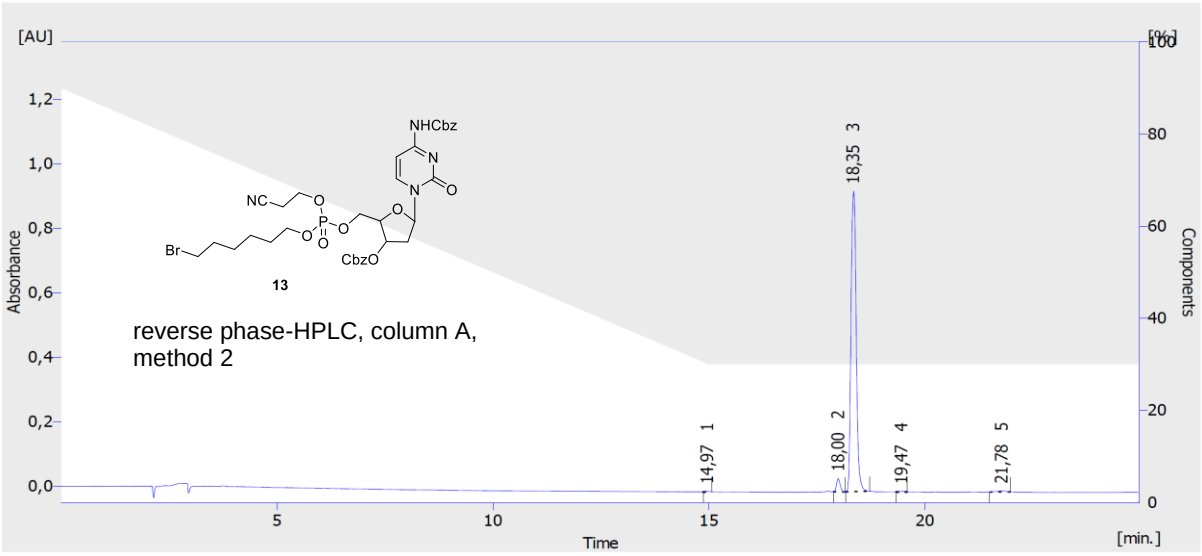
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	2,267	291,103	68,003	2,1	6,7	0,10	302
2	2,850	209,602	67,062	1,5	6,6	0,05	828
3	3,917	13430,950	879,191	96,4	86,7	0,27	744
	Total	13931,655	1014,255	100,0	100,0		

5'-Azido-2'-deoxycytidine (3a)



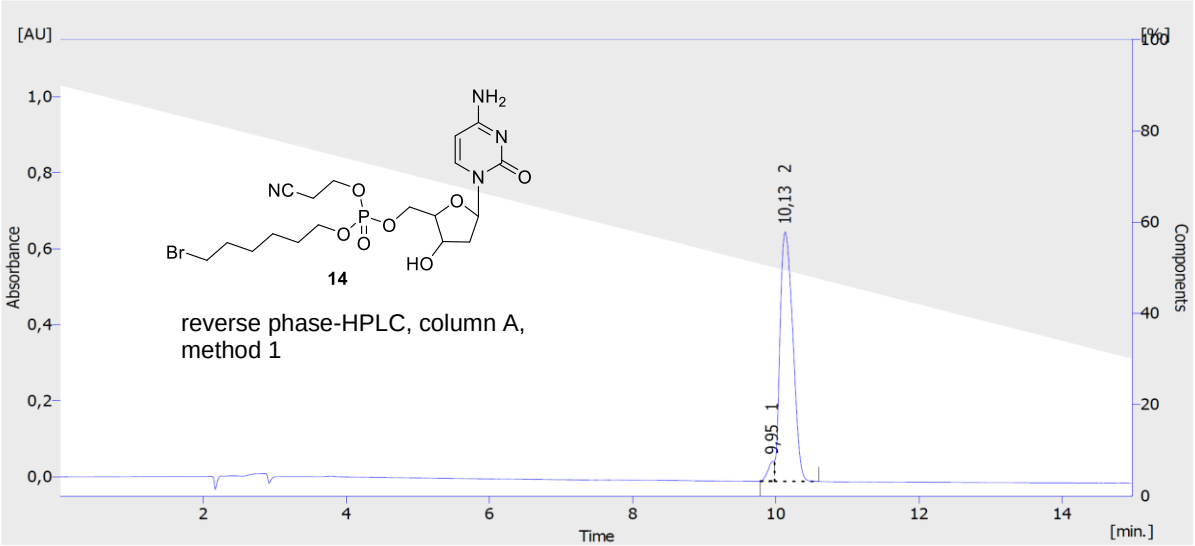
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	4,483	12808,952	898,168	100,0	100,0	0,27	762
	Total	12808,952	898,168	100,0	100,0		

5'-(6-bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (13)



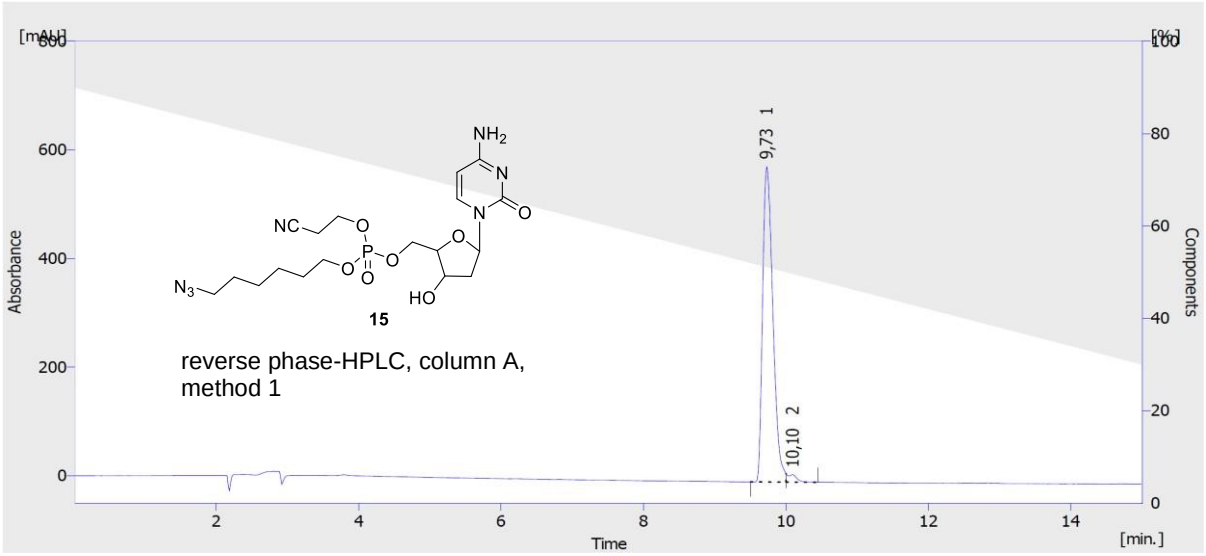
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	14,967	10,393	1,761	0,1	0,2	0,12	1000
2	18,000	237,242	40,898	3,0	4,2	0,10	881
3	18,350	7711,331	932,325	96,1	95,1	0,13	500
4	19,467	14,866	1,796	0,2	0,2	0,15	989
5	21,783	51,240	3,727	0,6	0,4	0,22	699
	Total	8025,071	980,507	100,0	100,0		

5’-(6-bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-2’-deoxycytidine (14)



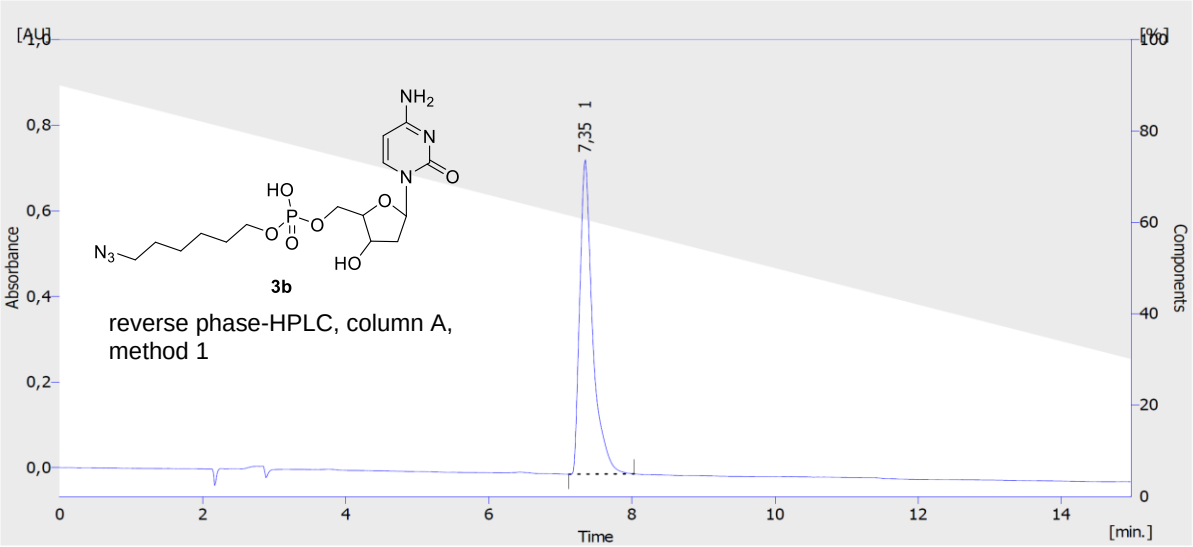
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,950	299,004	51,853	3,5	7,3	0,10	744
2	10,133	8172,340	656,444	96,5	92,7	0,23	796
	Total	8471,344	708,297	100,0	100,0		

5’-(6-azidohex-1-yloxy-2-cyanoethoxy)phosphoryl-2’-deoxycytidine (15)



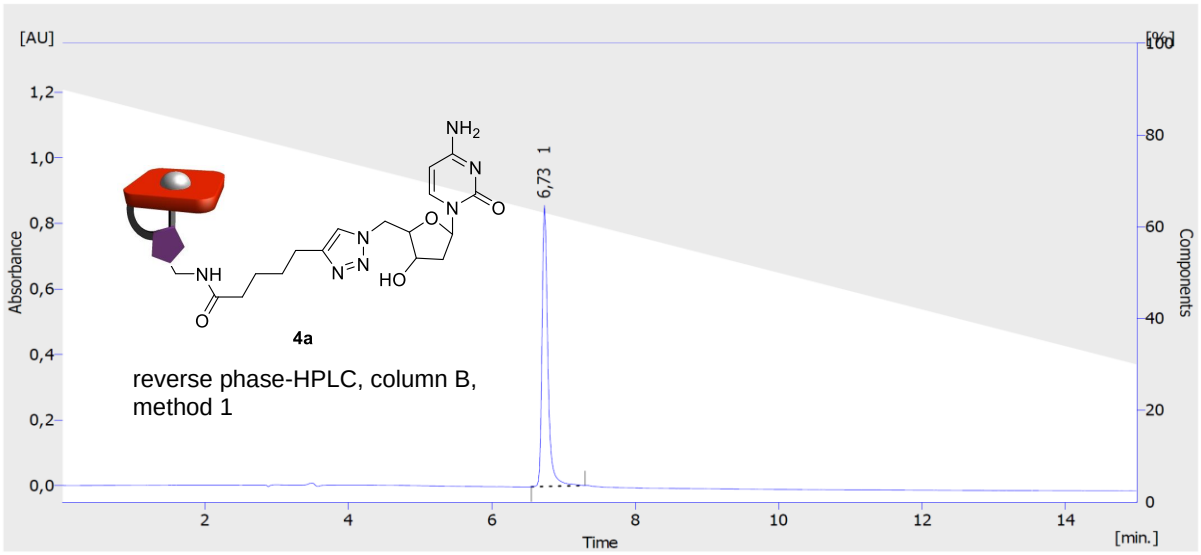
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,733	5812,349	580,791	97,7	97,7	0,18	832
2	10,100	138,450	13,784	2,3	2,3	0,17	770
	Total	5950,800	594,576	100,0	100,0		

5’-(6-azidohex-1-yloxy)phosphoryl-2’-deoxycytidine (3b)



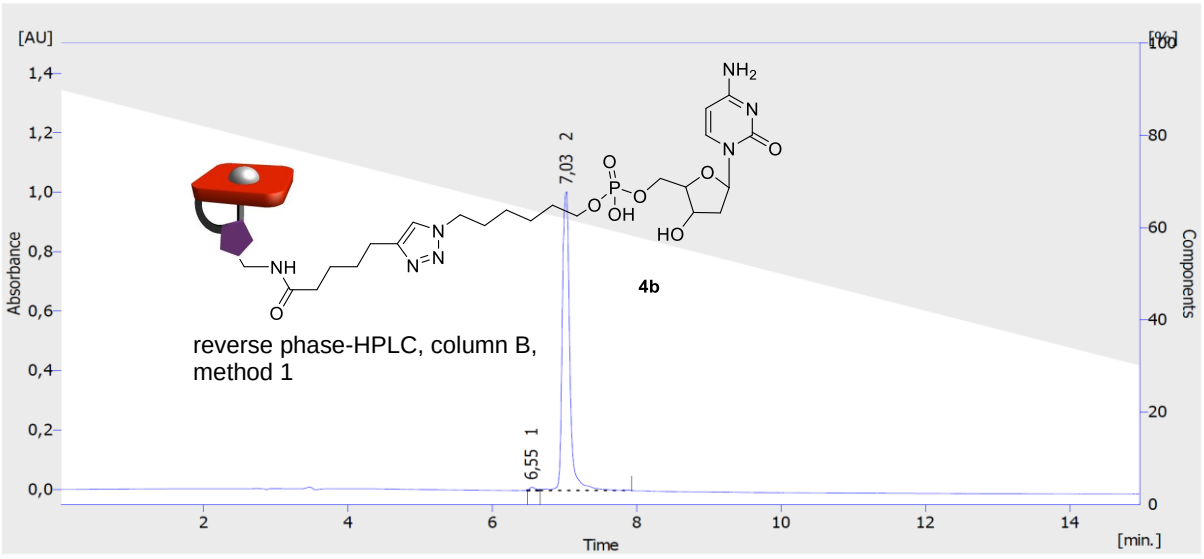
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	7,350	7653,302	612,135	100,0	100,0	0,20	692
	Total	7653,302	612,135	100,0	100,0		

Nucleoside-vitamin B_{12} conjugate 4a



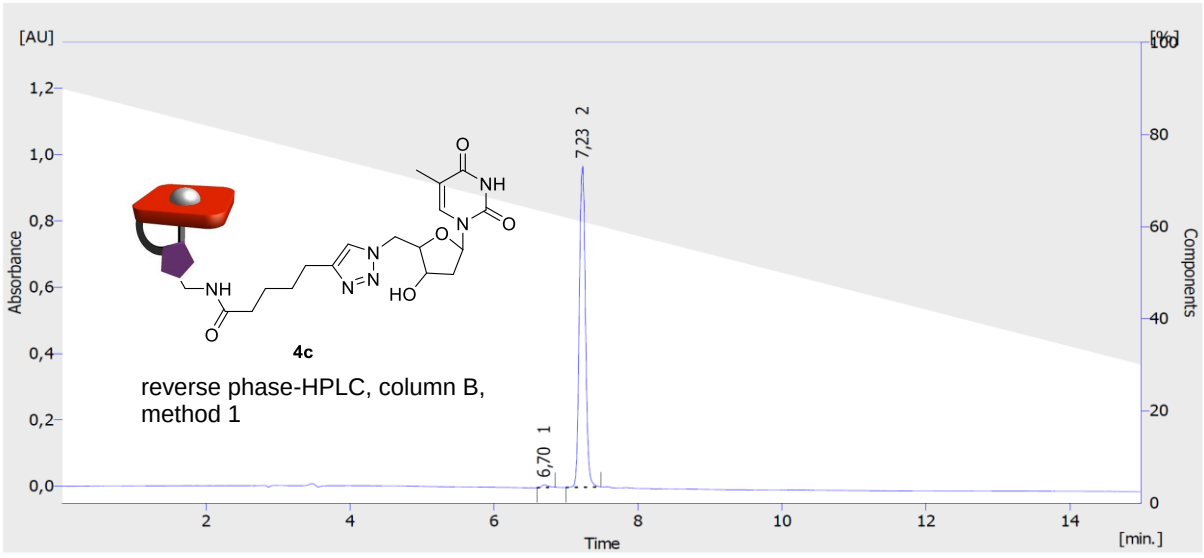
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	6,733	4857,770	857,980	100,0	100,0	0,10	604
	Total	4857,770	857,980	100,0	100,0		

Nucleoside-vitamin B_{12} conjugate 4b



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	6,550	60,816	11,422	0,8	1,1	0,08	952
2	7,033	7253,234	1004,825	99,2	98,9	0,12	379
	Total	7314,050	1016,247	100,0	100,0		

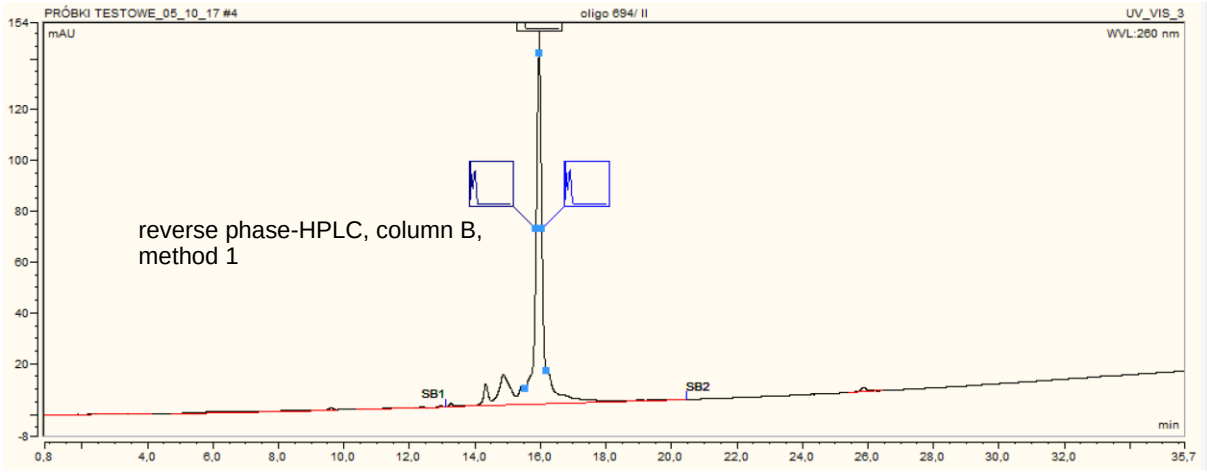
Nucleoside-vitamin B_{12} conjugate 4c



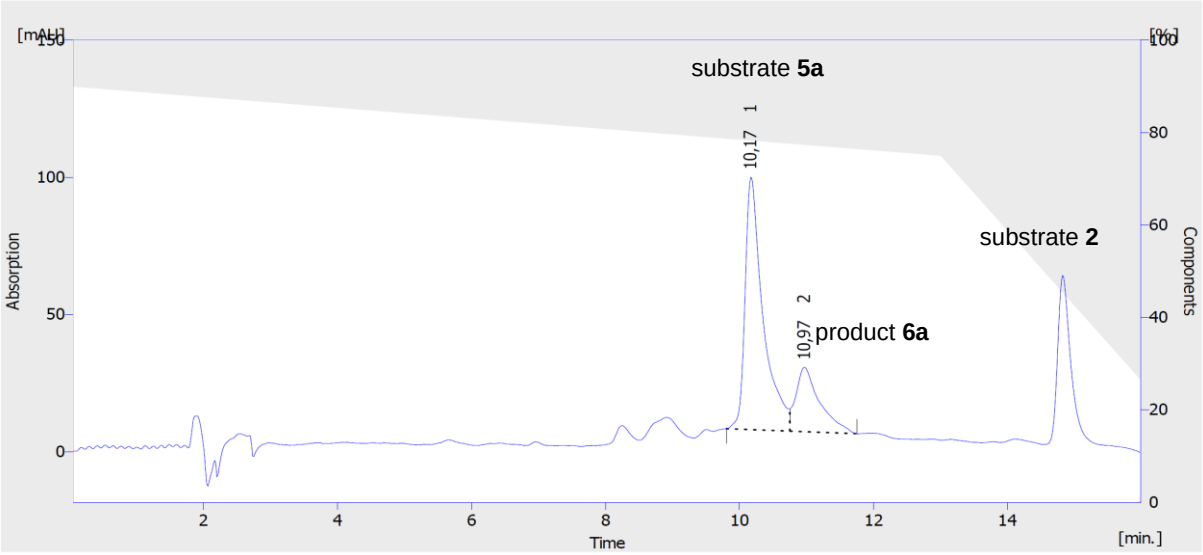
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	6,700	50,251	8,521	0,9	0,9	0,12	949
2	7,233	5739,435	968,627	99,1	99,1	0,12	582
	Total	5789,686	977,148	100,0	100,0		

2'OMeRNA-vitamin B_{12} conjugate 6a

Substrate 5a:

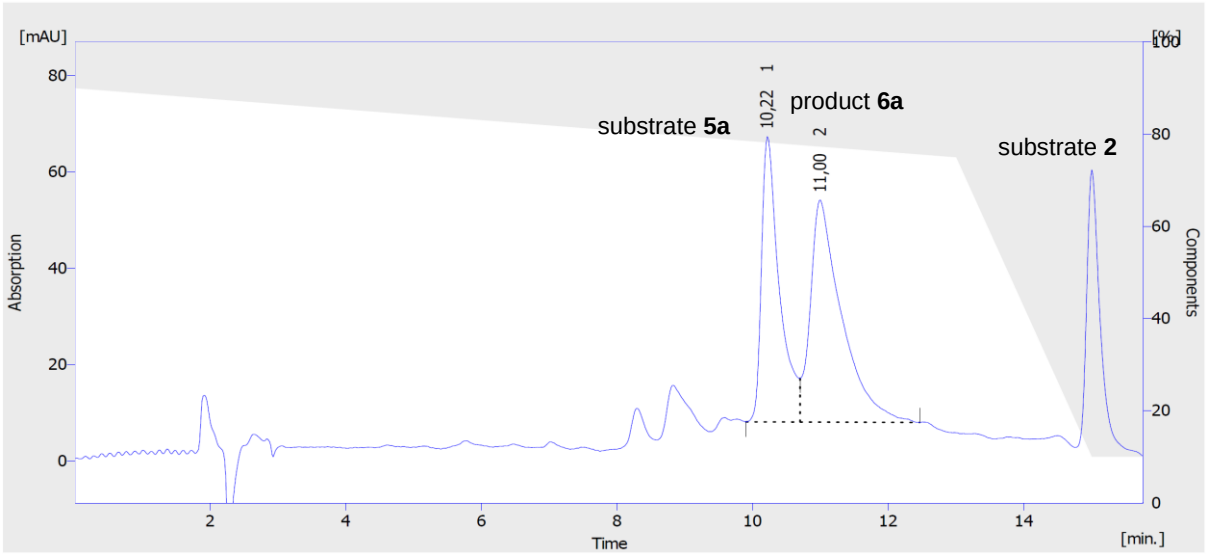


After 1.5 h:



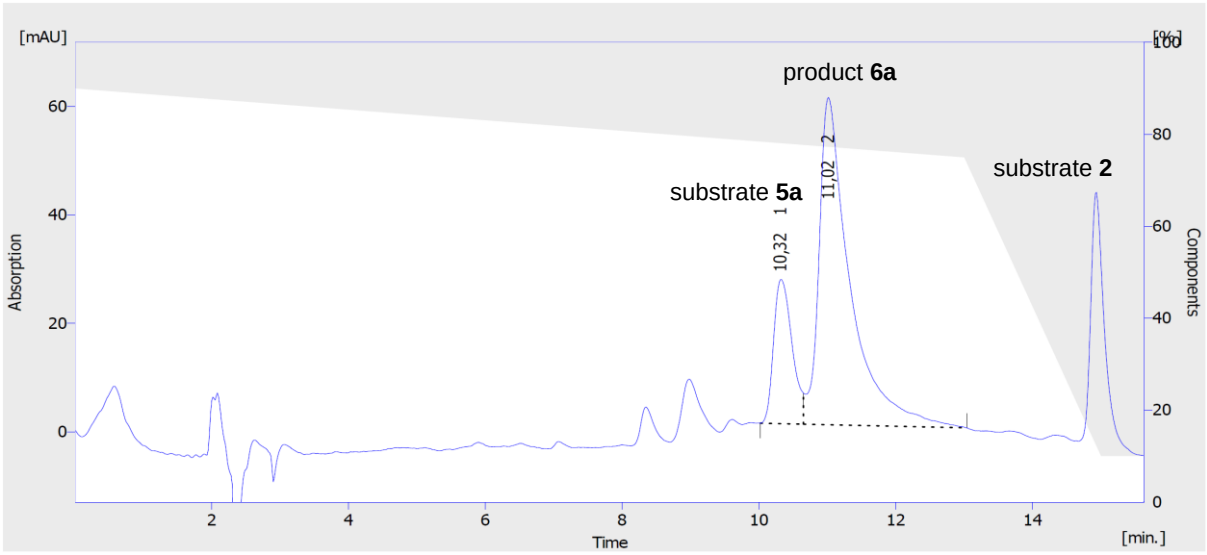
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	10,167	1713,674	92,052	74,6	79,7	0,27	916
2	10,967	584,849	23,476	25,4	20,3	0,37	918
	Total	2298,523	115,529	100,0	100,0		

After 3 h:



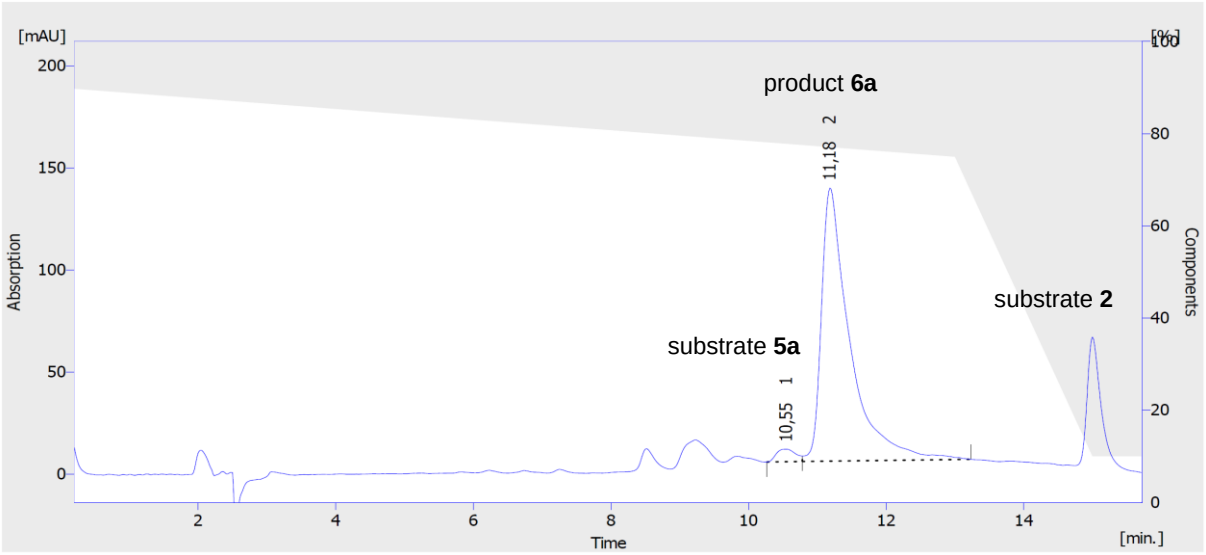
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	10,217	1087,028	59,241	42,2	56,2	0,27	954
2	11,000	1489,490	46,115	57,8	43,8	0,47	856
	Total	2576,518	105,356	100,0	100,0		

After 4.5 h:



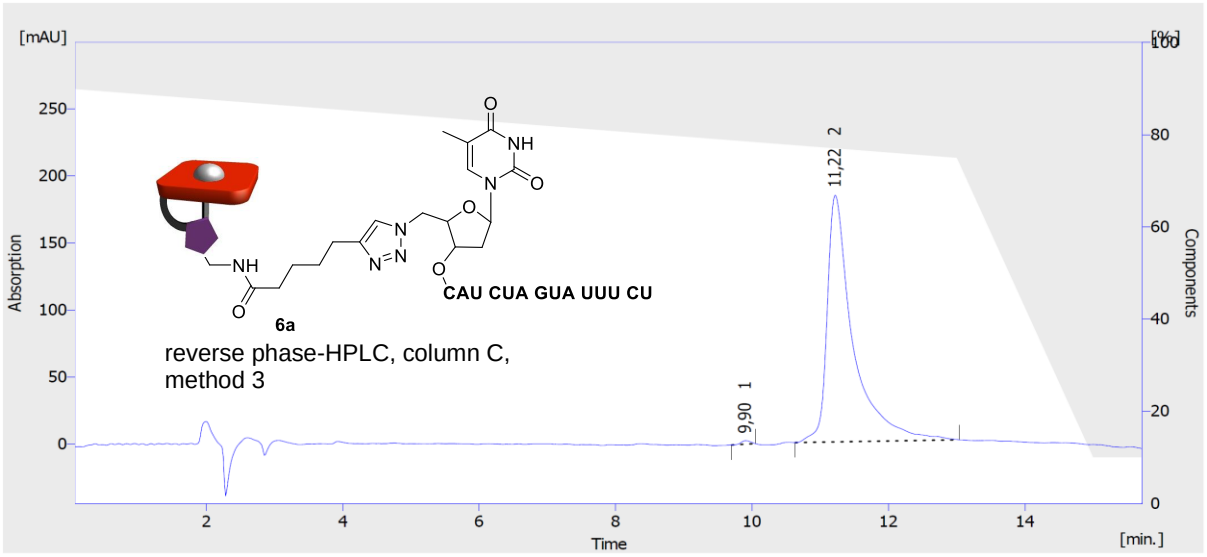
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	10,317	498,018	26,618	20,6	30,6	0,30	988
2	11,017	1919,819	60,373	79,4	69,4	0,43	950
	Total	2417,837	86,991	100,0	100,0		

After 20 h:



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	10,550	120,430	6,177	3,0	4,4	0,37	996
2	11,183	3869,033	133,833	97,0	95,6	0,40	242
	Total	3989,463	140,010	100,0	100,0		

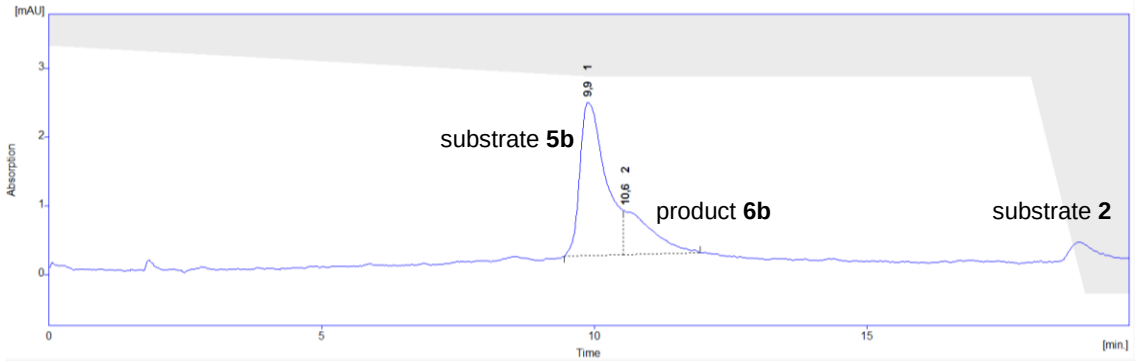
Purified product 6a:



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,900	20,019	2,221	0,5	1,4	0,18	734
2	11,217	4067,330	153,404	99,5	98,6	0,35	529
	Total	4087,349	155,625	100,0	100,0		

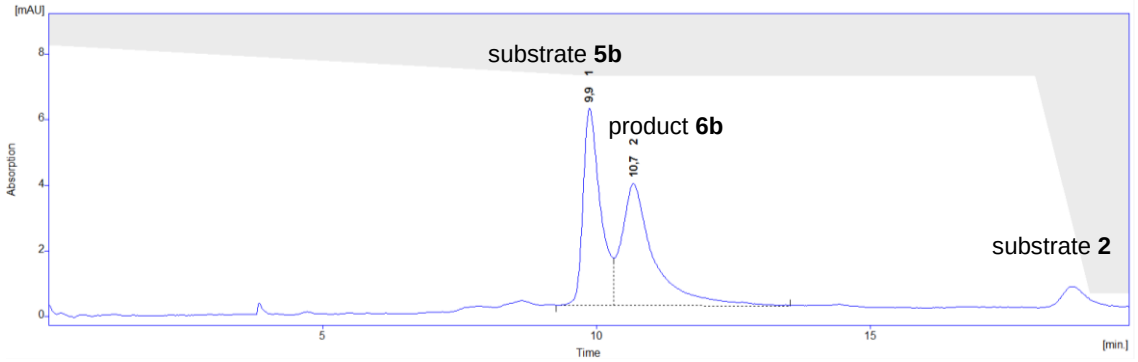
2'OMeRNA-vitamin B_{12} conjugate 6b

After 1.5 h:



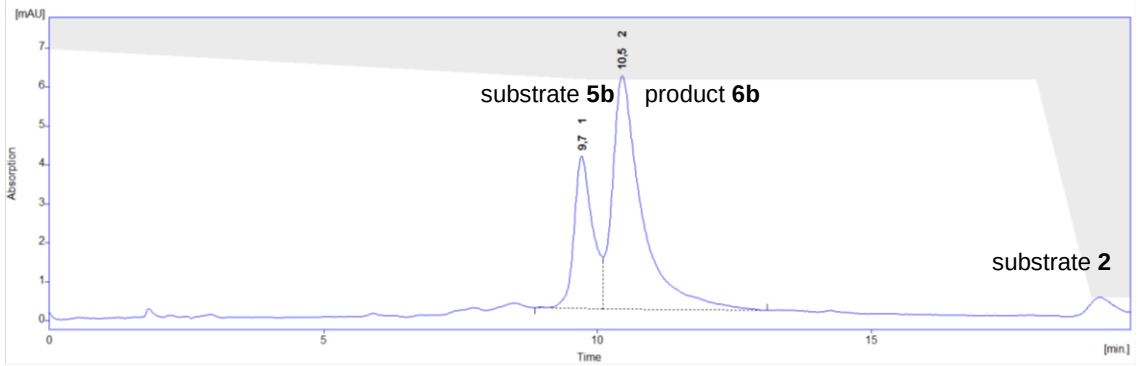
	Reten. Time [min]	Start Time [min]	End Time [min]	Start Value [mAU]	End Value [mAU]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,883	9,450	10,533	2,687	2,903	730,421	22,241	75,3	77,9	0,50	957
2	10,567	10,533	11,950	2,903	3,185	239,790	6,295	24,7	22,1	0,60	999
	Total					970,211	28,536	100,0	100,0		

After 3 h:



	Reten. Time [min]	Start Time [min]	End Time [min]	Start Value [mAU]	End Value [mAU]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,867	9,267	10,317	3,462	3,430	1319,437	59,943	46,3	61,8	0,35	932
2	10,683	10,317	13,533	3,430	3,330	1533,188	37,099	53,7	38,2	0,55	795
	Total					2852,625	97,042	100,0	100,0		

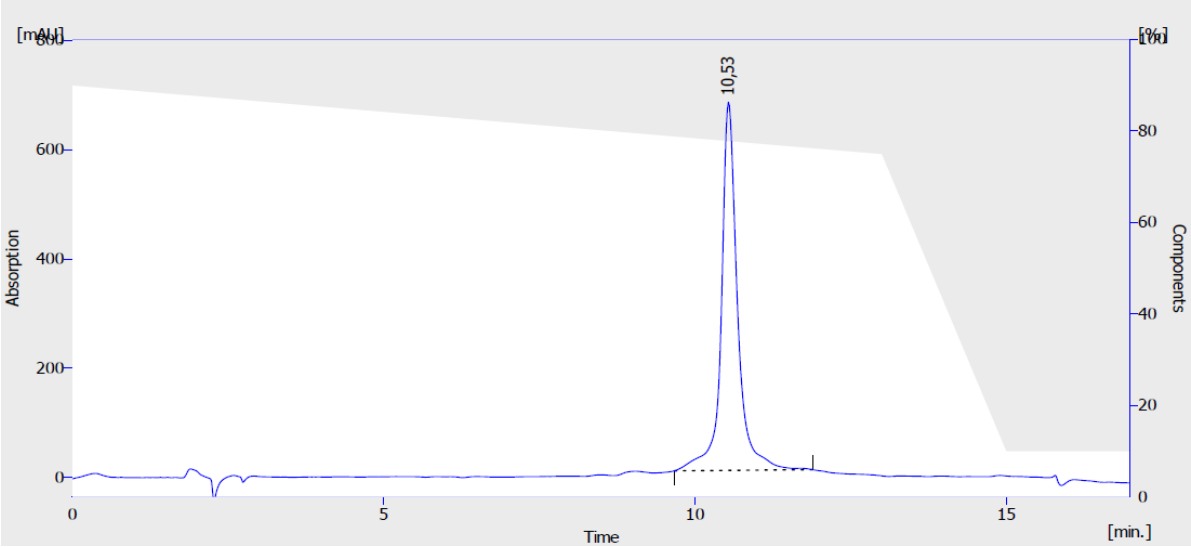
After 24 h:



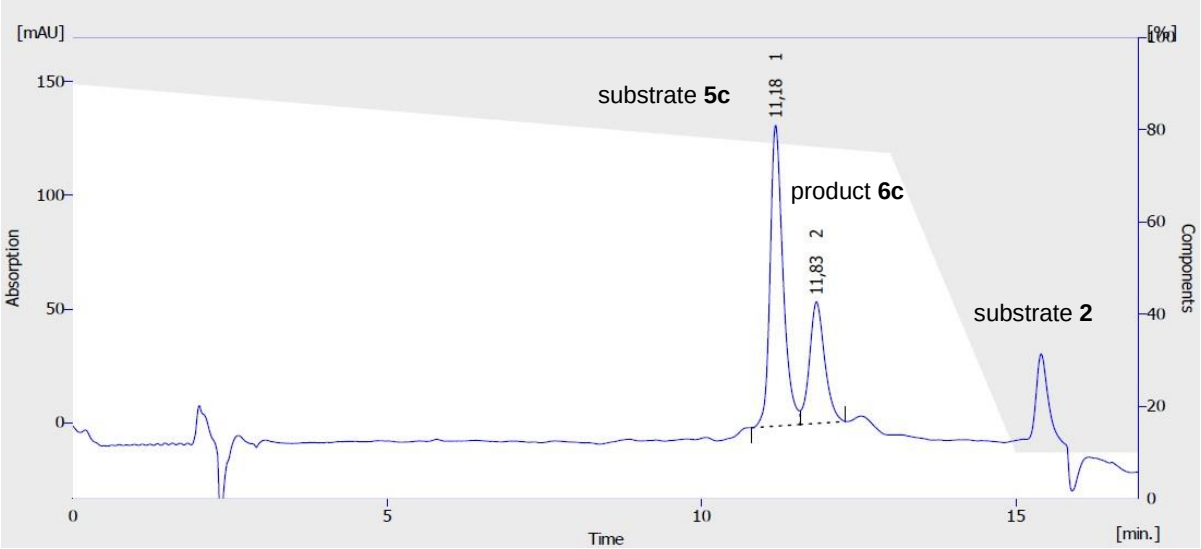
	Reten. Time [min]	Start Time [min]	End Time [min]	Start Value [mAU]	End Value [mAU]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	9,717	8,850	10,100	3,363	3,152	921,307	39,047	28,9	39,5	0,38	833
2	10,450	10,100	13,100	3,152	2,645	2268,160	59,845	71,1	60,5	0,50	818
	Total					3189,467	98,892	100,0	100,0		

2'OMeRNA-vitamin B_{12} conjugate 6c

Substrate 5c:

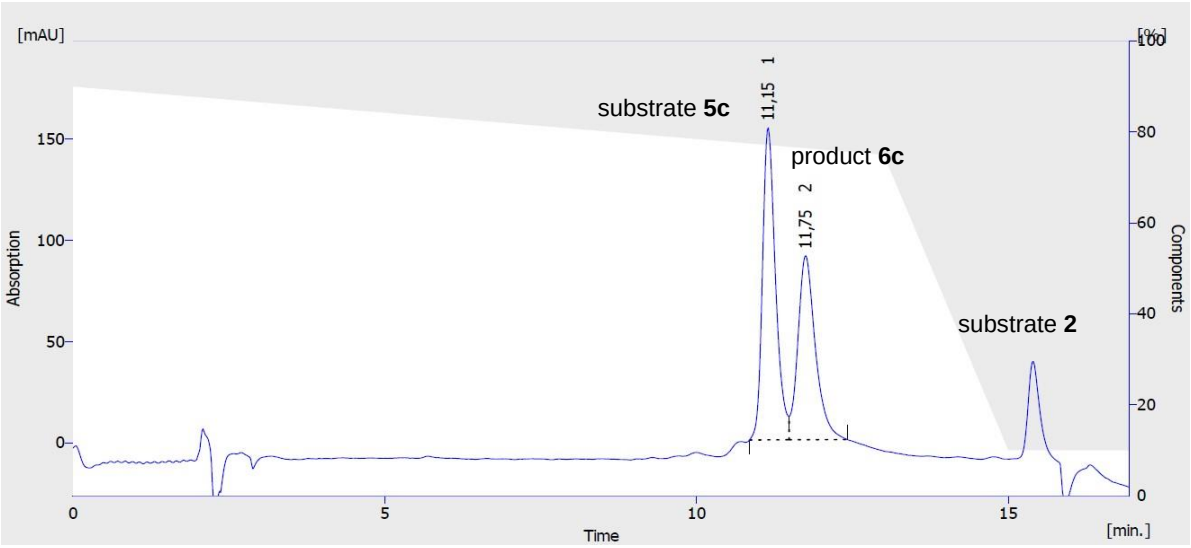


After 1.5 h:



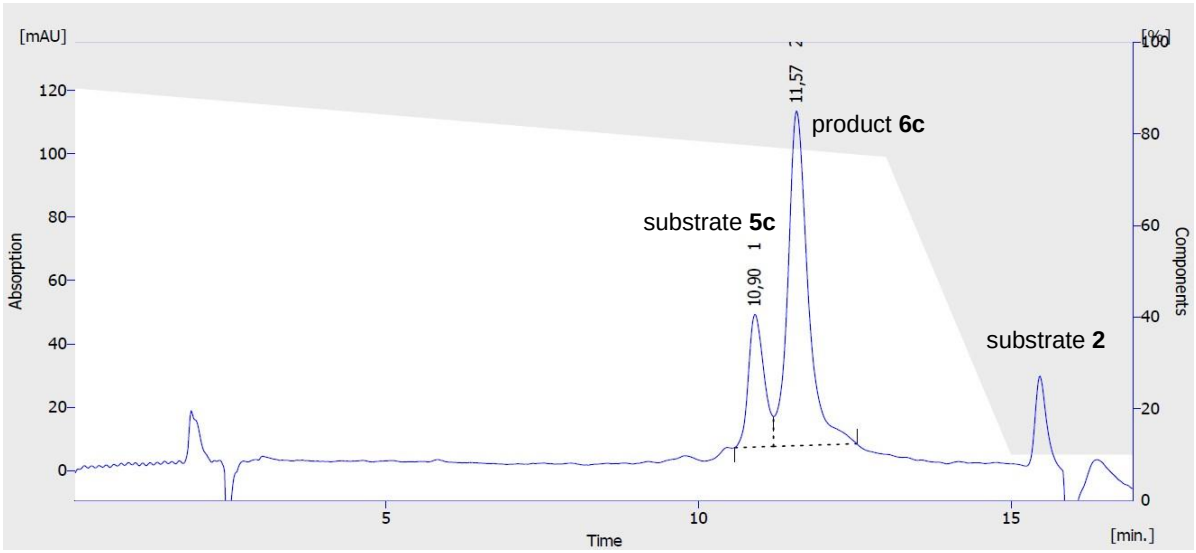
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,183	1890,771	132,193	67,6	71,2	0,22	895
2	11,833	906,791	53,422	32,4	28,8	0,27	979
	Total	2797,563	185,616	100,0	100,0		

After 3 h:



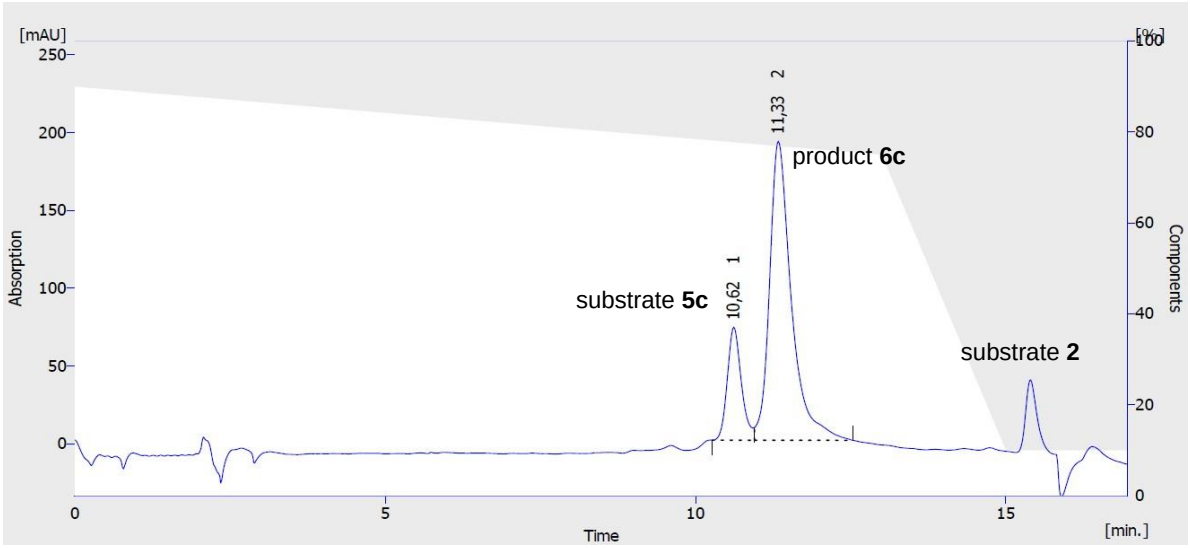
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,150	2237,585	154,013	55,6	62,9	0,25	854
2	11,750	1783,882	90,891	44,4	37,1	0,32	745
	Total	4021,466	244,904	100,0	100,0		

After 24 h:



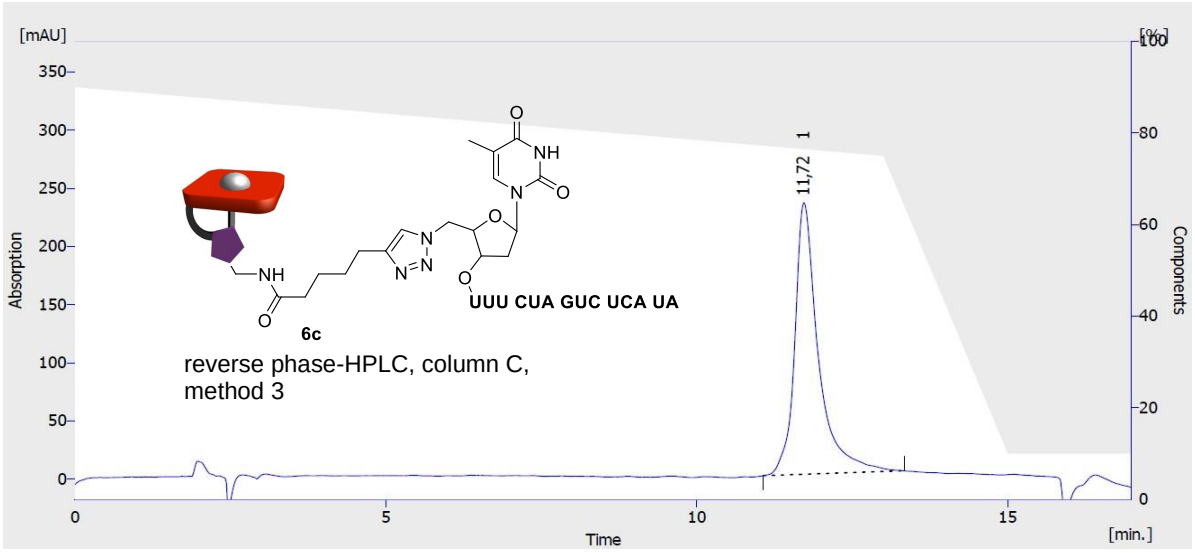
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	10,900	724,971	41,887	23,3	28,4	0,28	948
2	11,567	2388,673	105,682	76,7	71,6	0,33	881
	Total	3113,644	147,569	100,0	100,0		

After 48 h:



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	10,617	1141,768	72,639	19,5	27,4	0,25	926
2	11,333	4716,857	192,267	80,5	72,6	0,35	943
	Total	5858,625	264,905	100,0	100,0		

Purified product 6c:

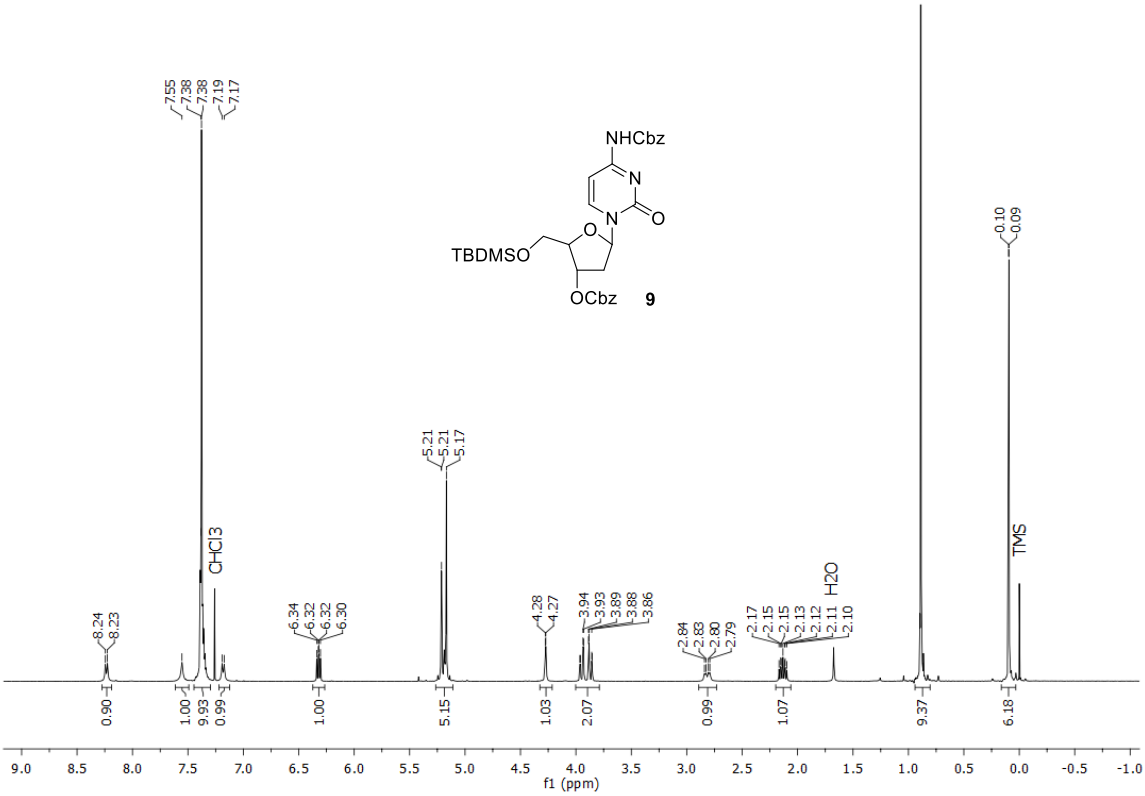


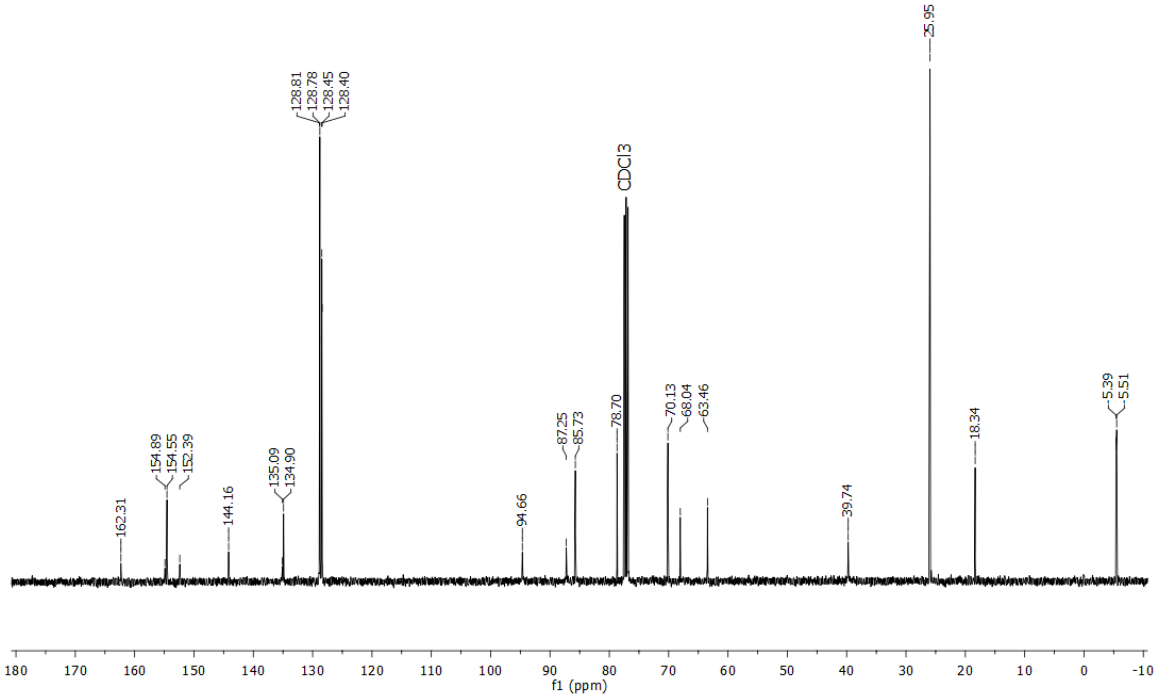
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	PDA Peak Purity
1	11,717	6190,010	233,377	100,0	100,0	0,37	645
	Total	6190,010	233,377	100,0	100,0		

13. ^{1}H, ^{13}C and ^{31}P NMR spectra

5'-*O-tert*-Butyldimethylsilyl-N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (9)

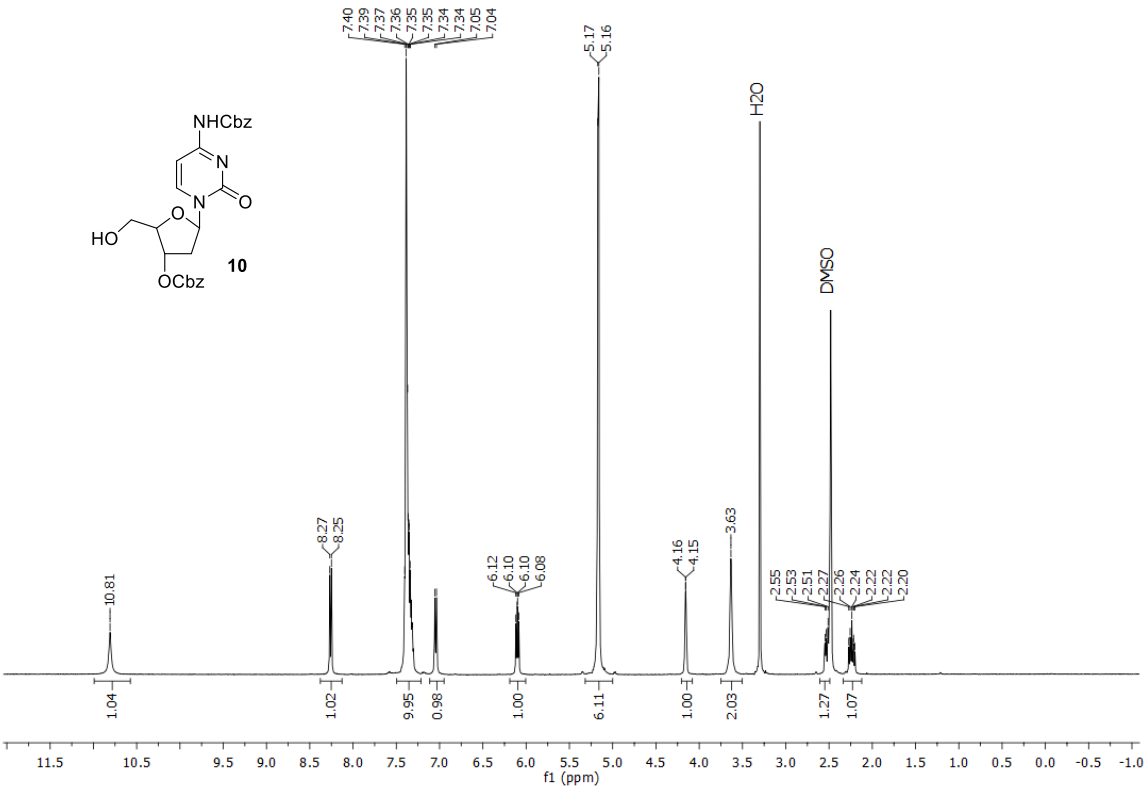
^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) in $CDCl_3$

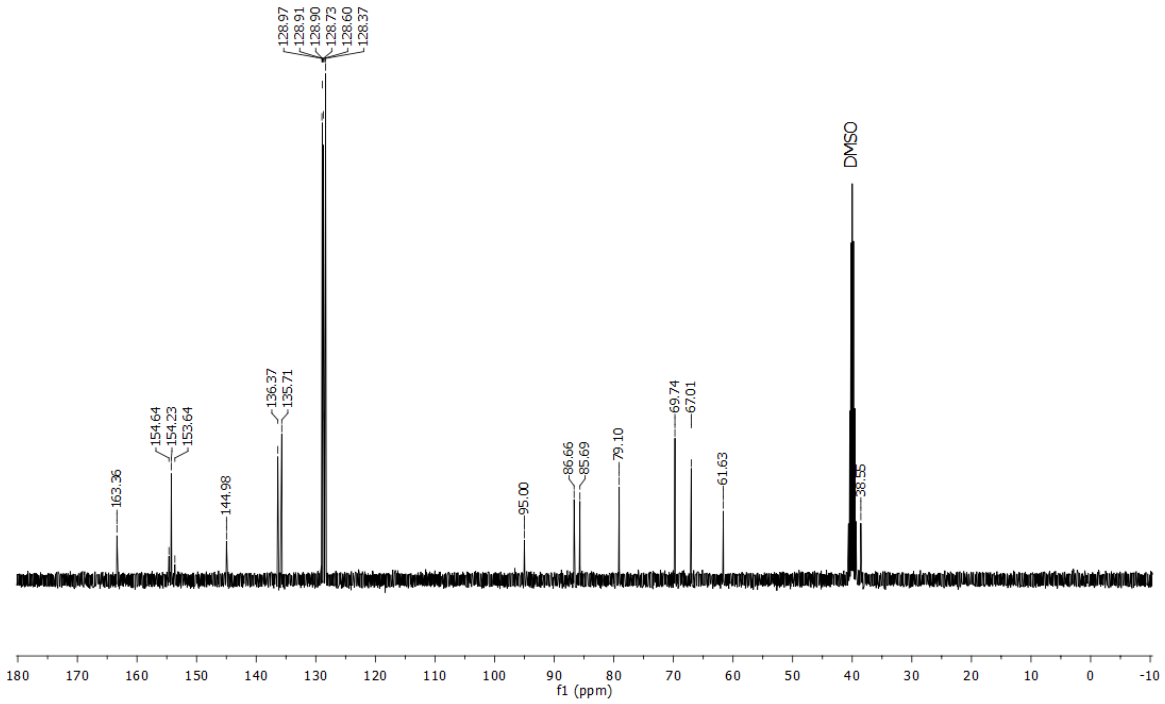




N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (10)

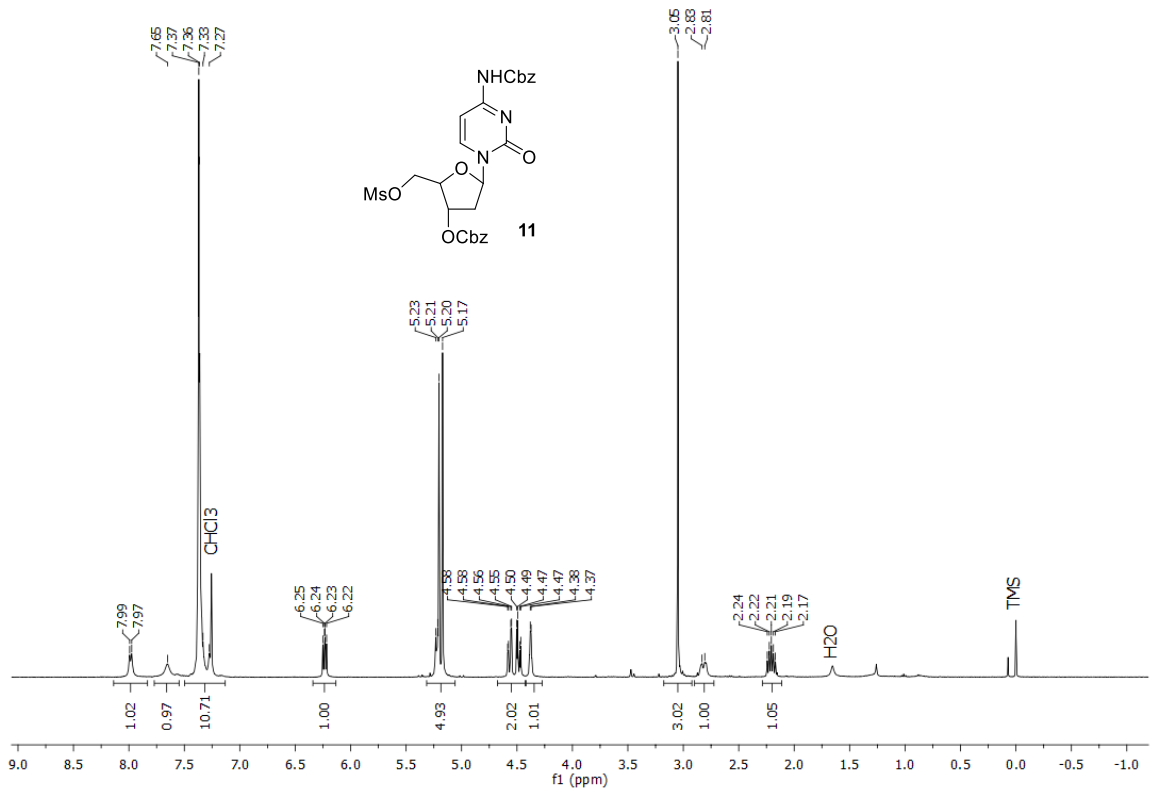
^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) in $(CD_3)_2SO$

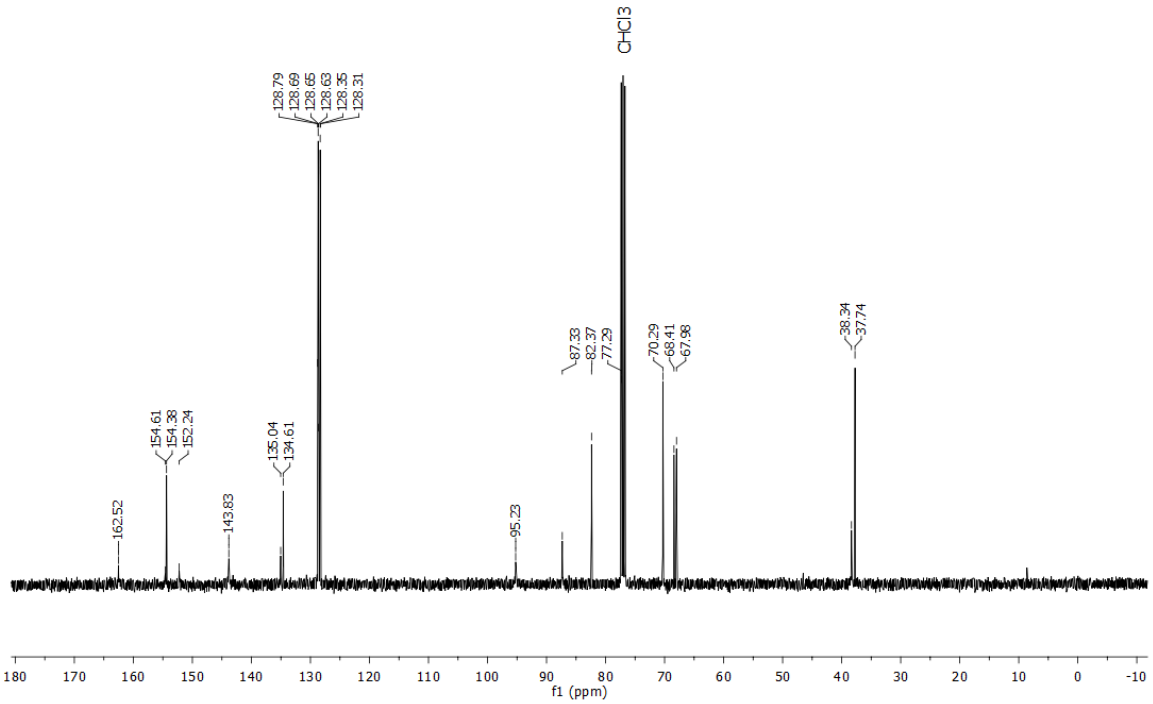




5'-Methanesulfonyl-N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (11)

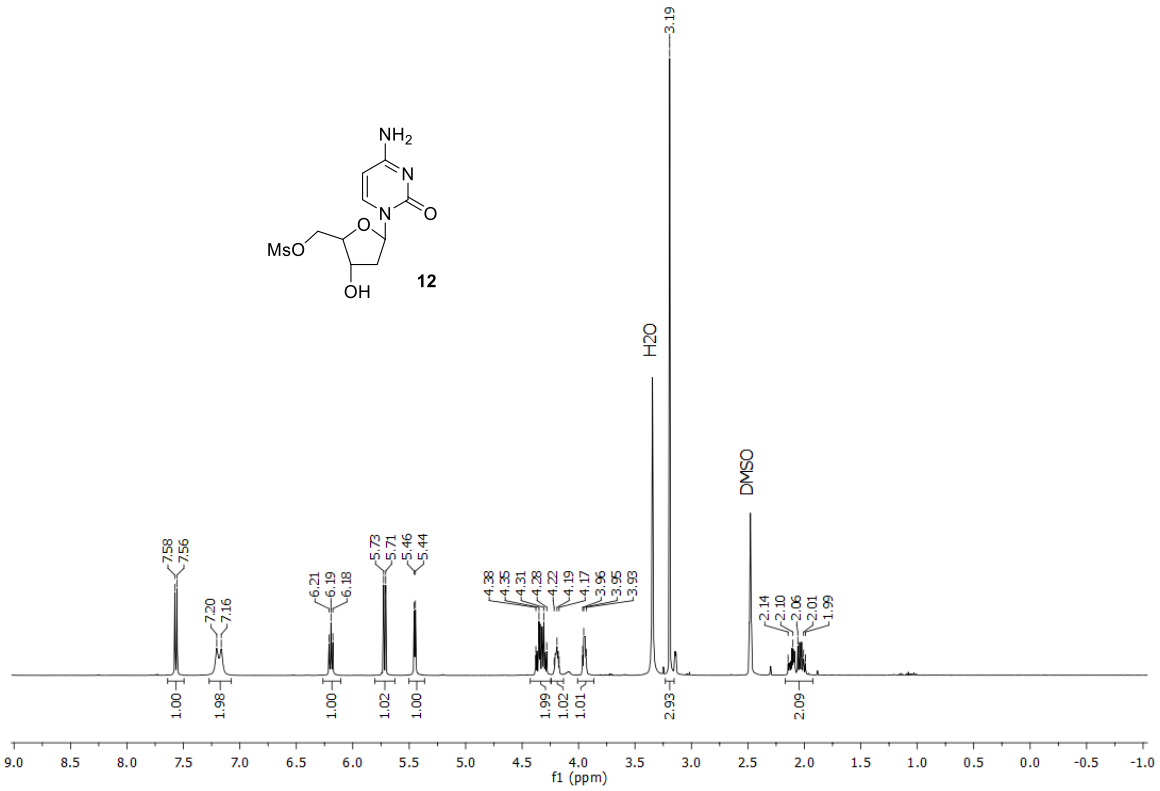
^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) in $CDCl_3$

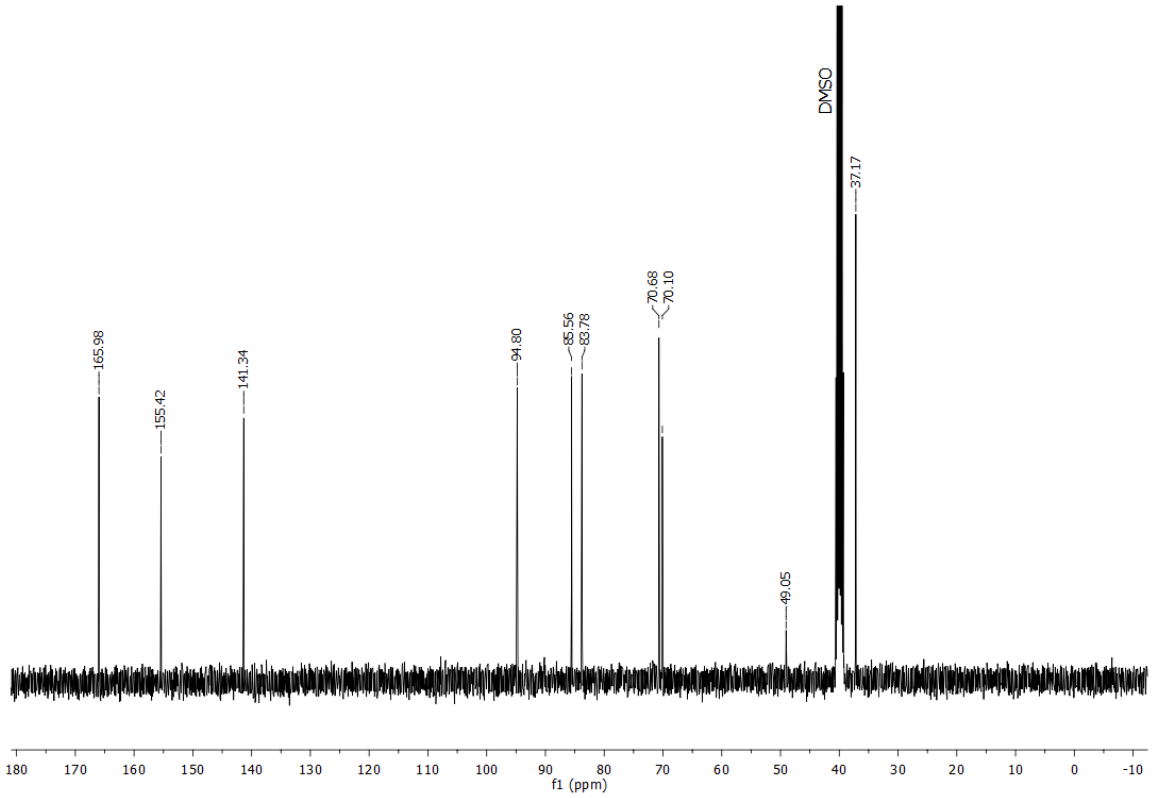




5'-Methanesulfonyl-2'-deoxycytidine (12)

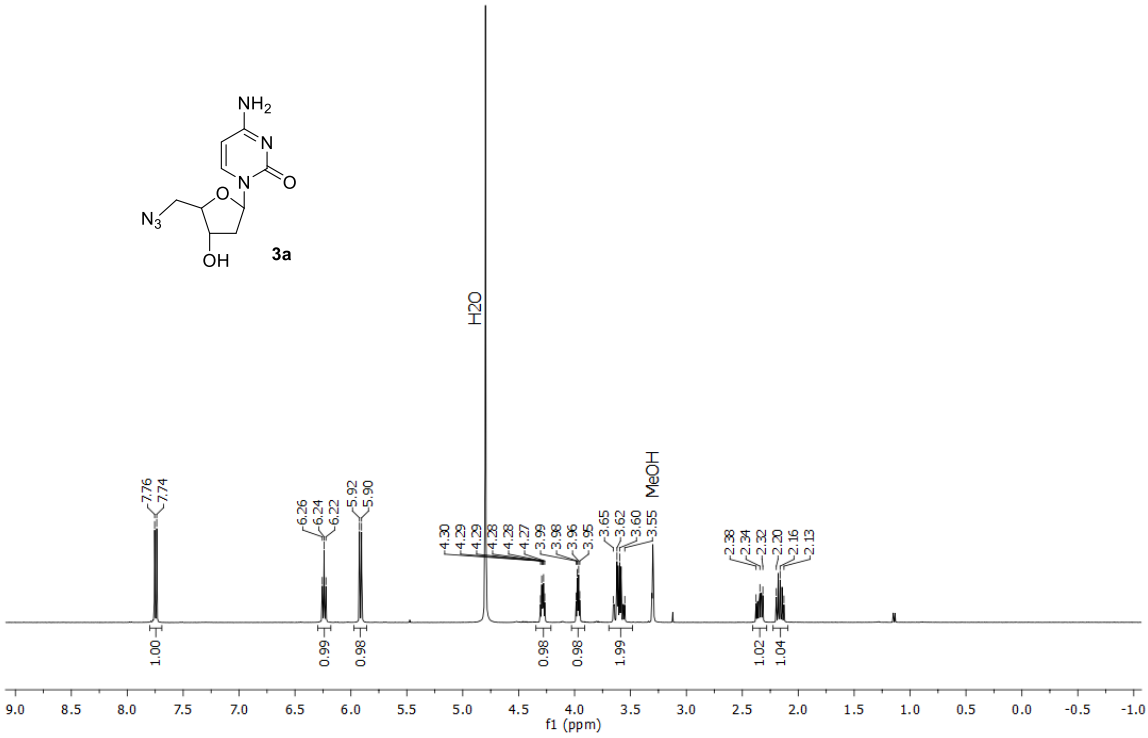
^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) in $(CD_3)_2SO$

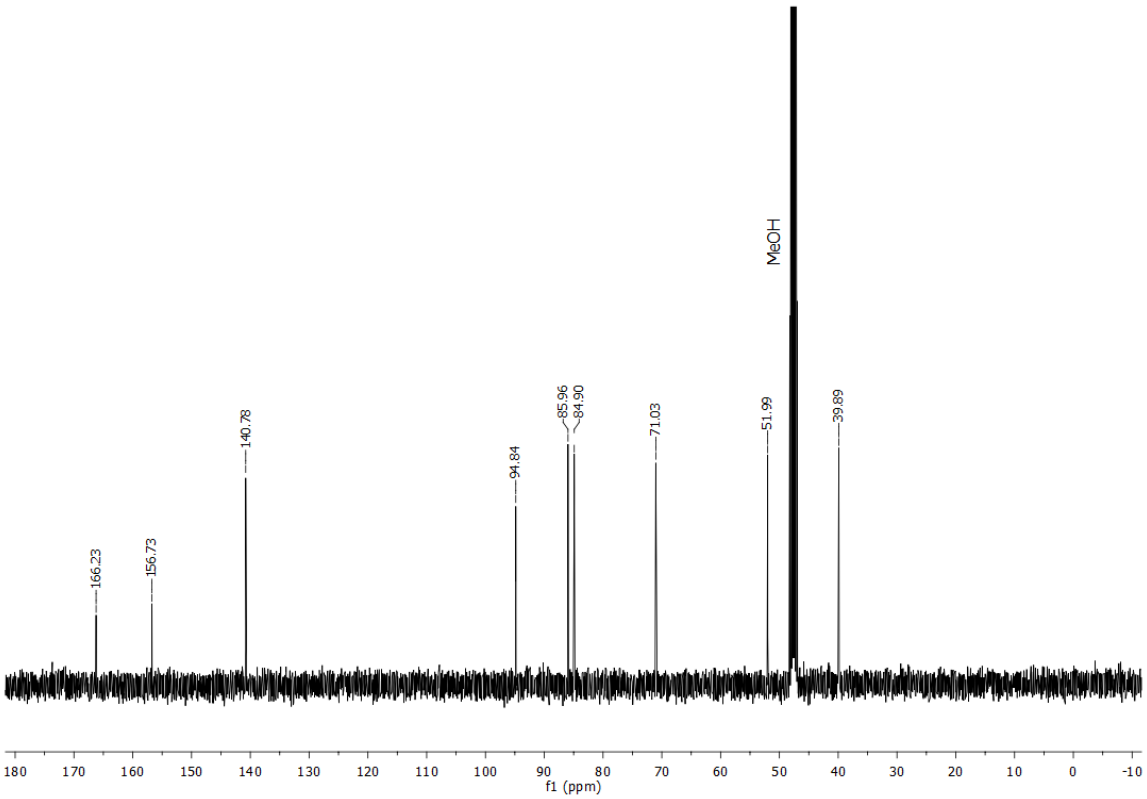




5'-Azido-2'-deoxycytidine (3a)

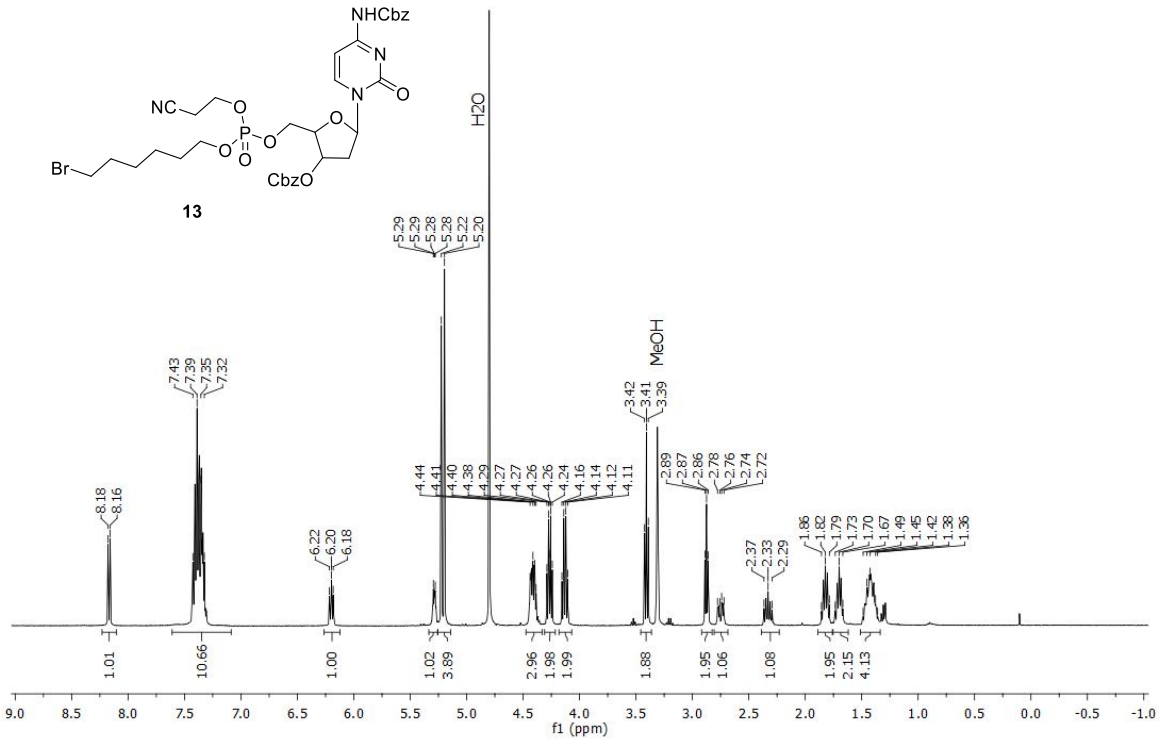
^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) in CD_3OD

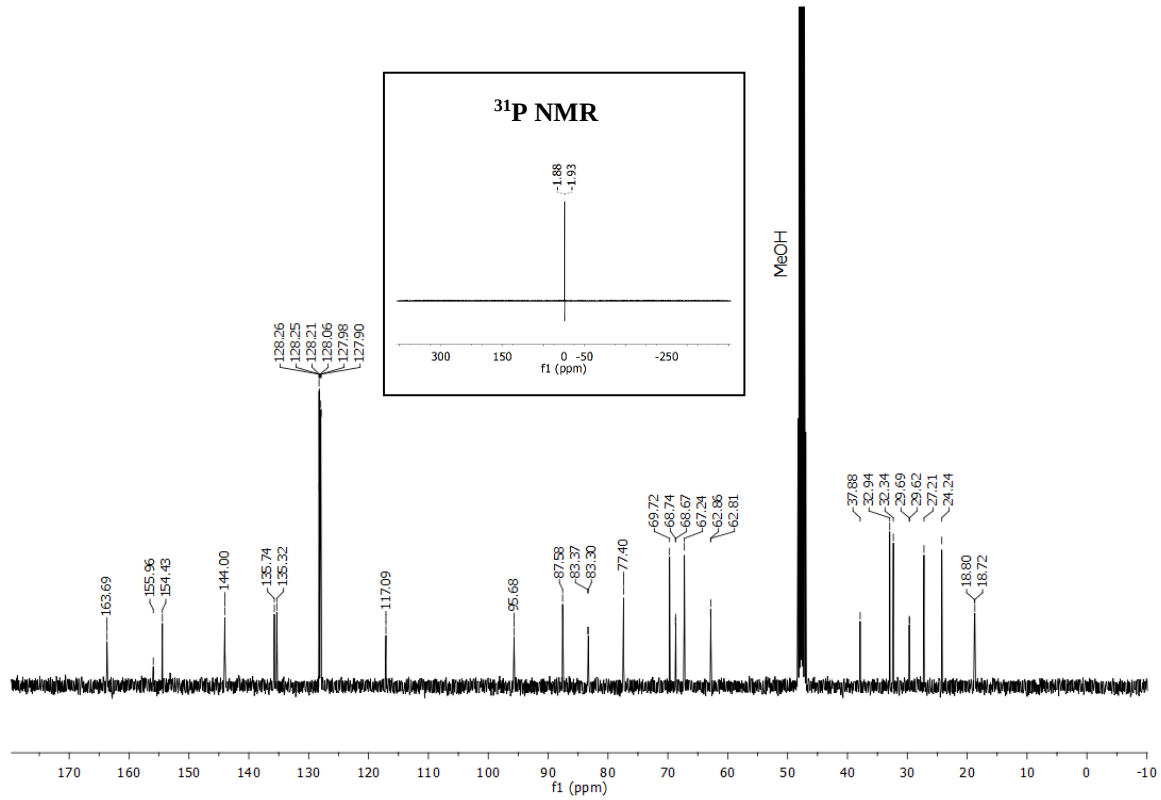




5'-(6-bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-N^4-3'-O-bis-benzyloxycarbonyl-2'-deoxycytidine (13)

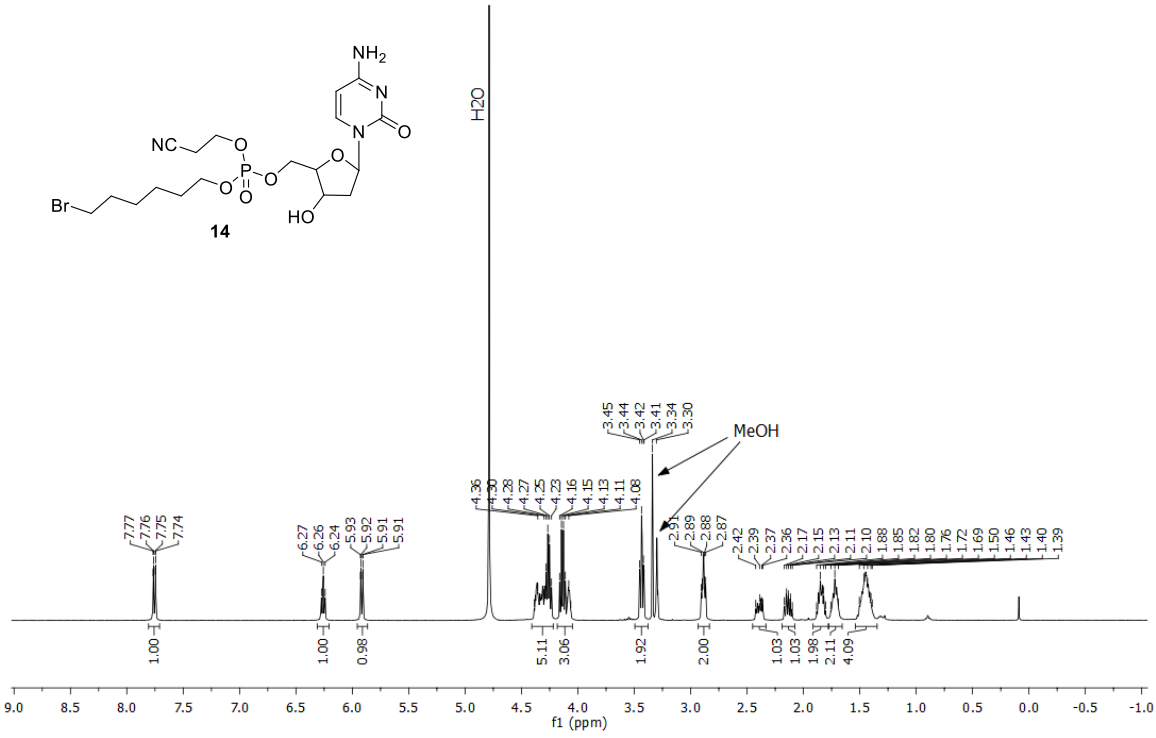
^{1}H NMR (400 MHz), ^{13}C NMR (101 MHz) and ^{31}P NMR (162 MHz) in CD_3OD

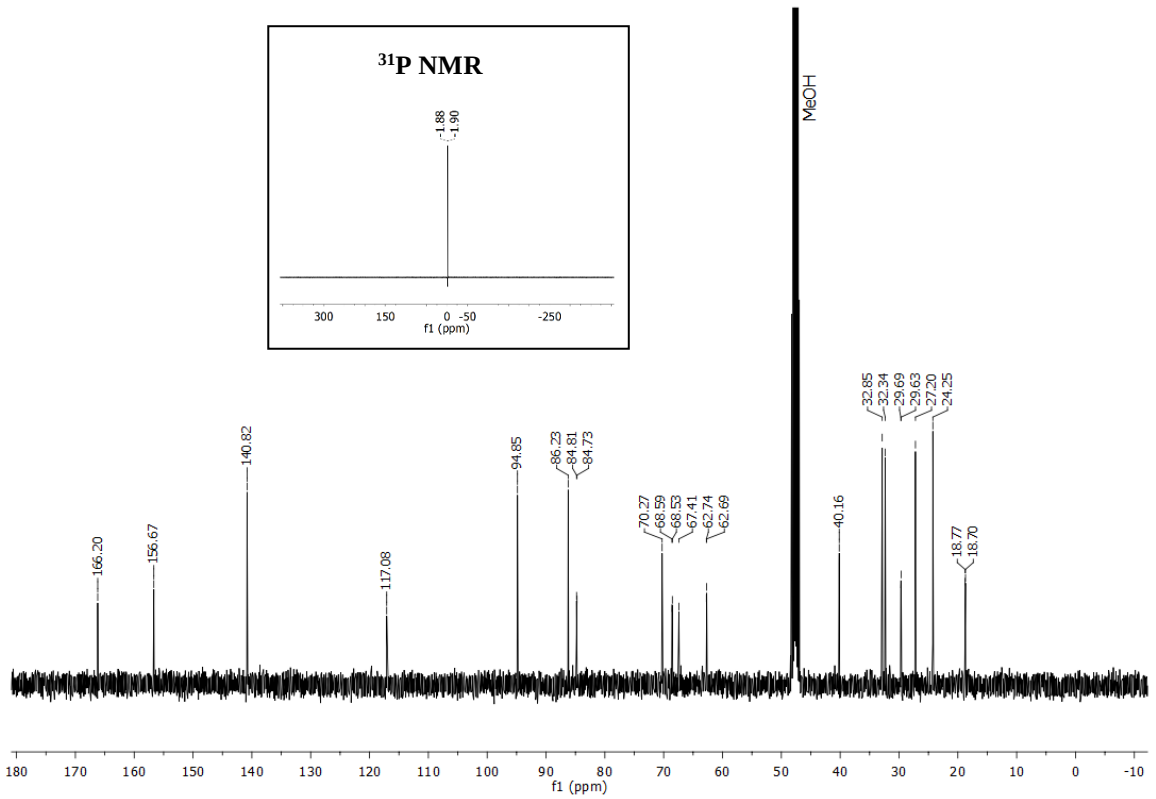




5’-(6-bromohex-1-yloxy-2-cyanoethoxy)phosphoryl-2’-deoxycytidine (14)

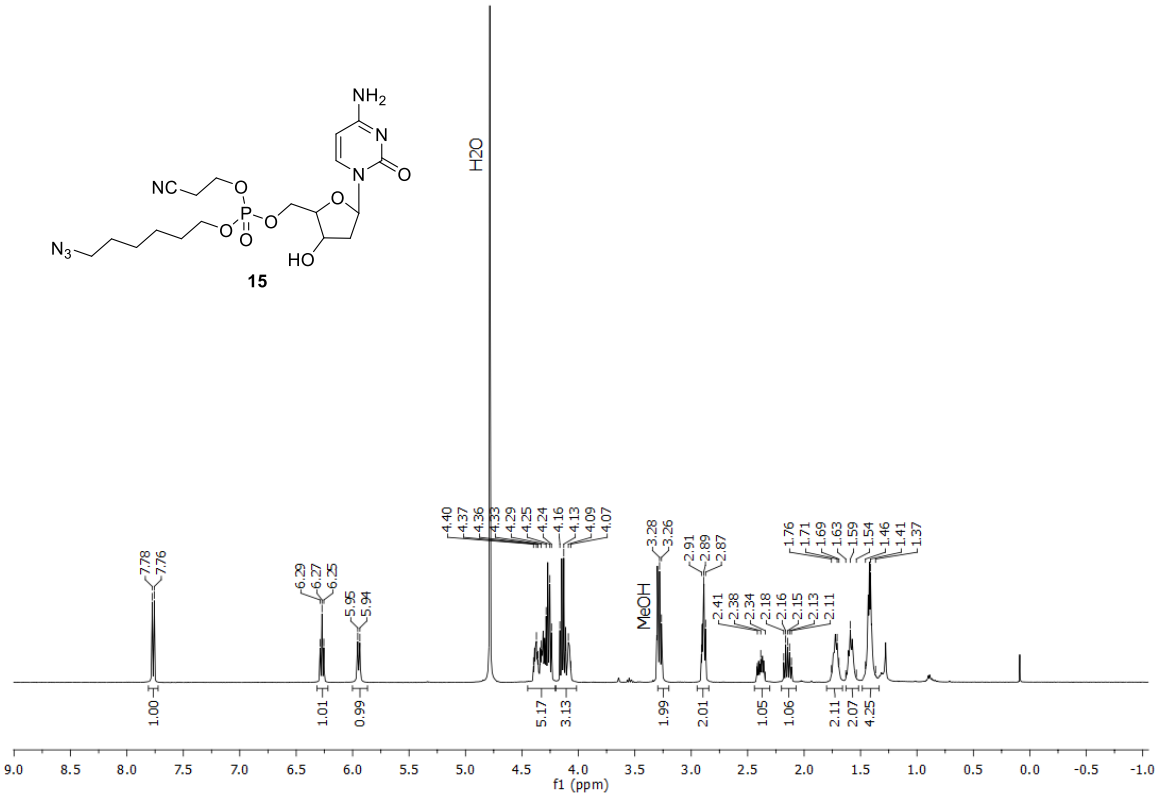
^{1}H NMR (400 MHz), ^{13}C NMR (101 MHz) and ^{31}P NMR (162 MHz) in CD_3OD

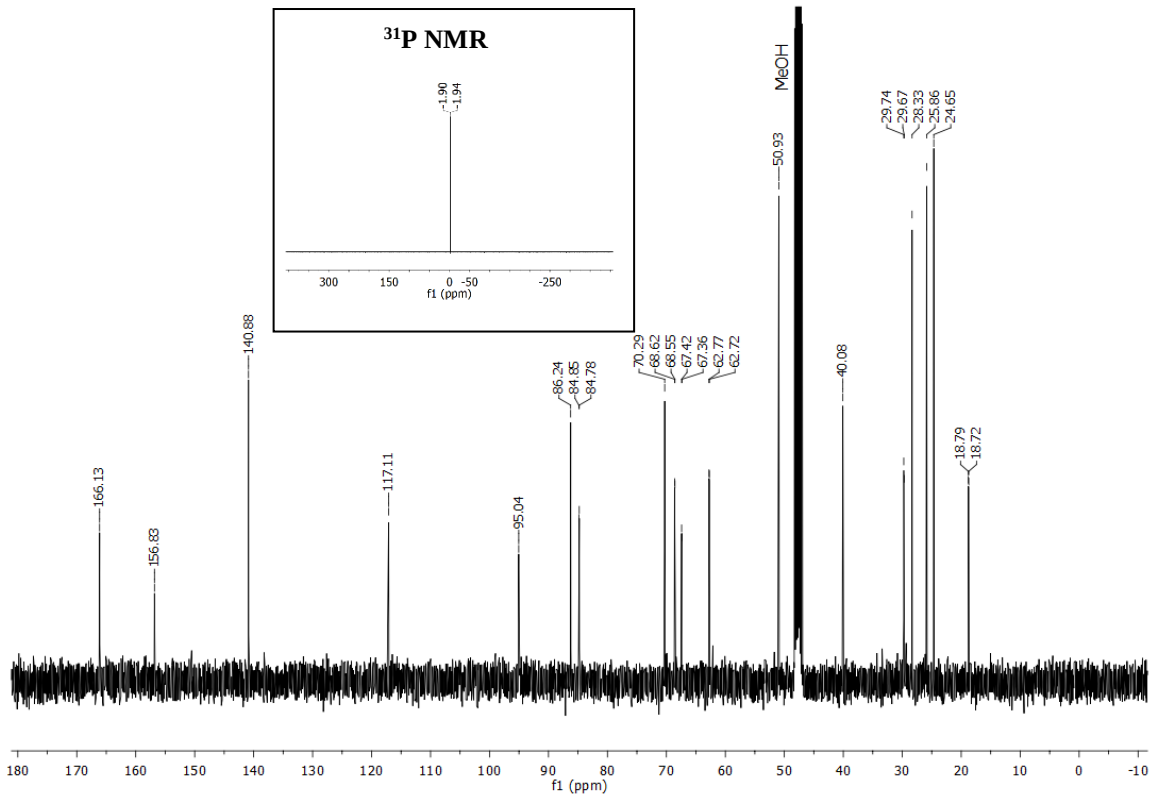




5’-(6-azidohex-1-yloxy-2-cyanoethoxy)phosphoryl-2’-deoxycytidine (15)

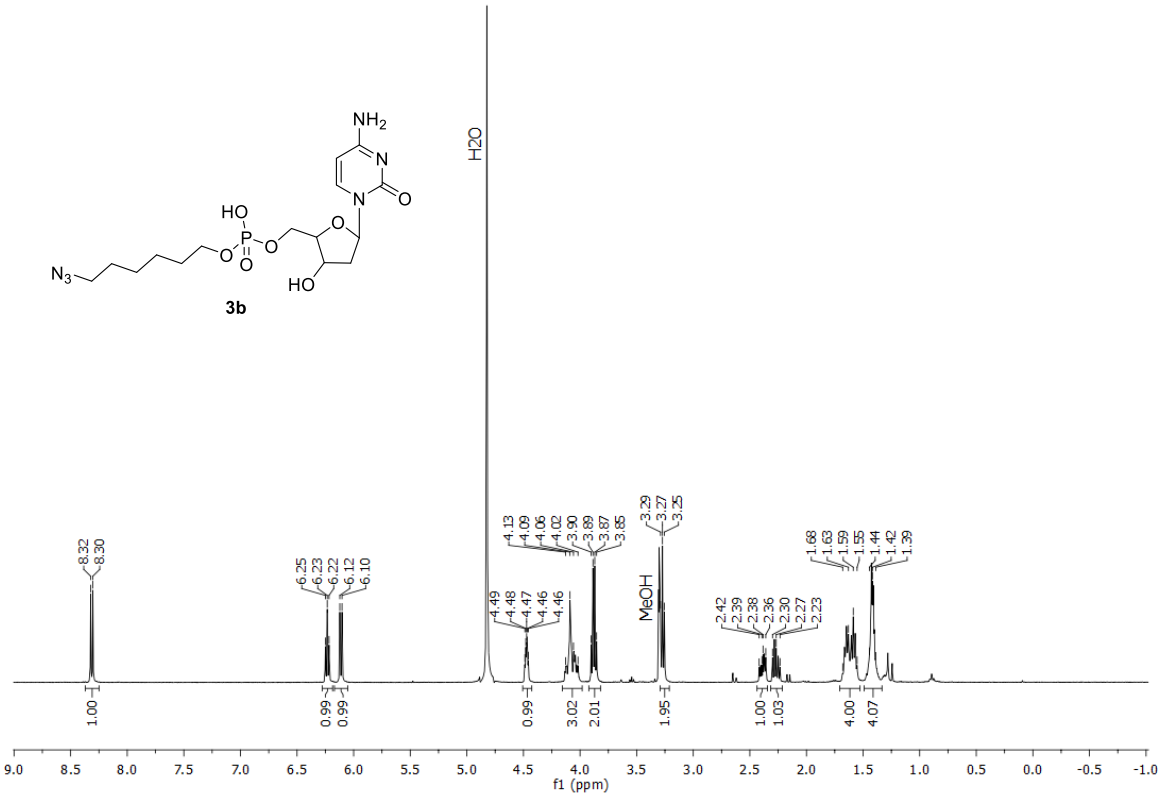
^{1}H NMR (400 MHz), ^{13}C NMR (101 MHz) and ^{31}P NMR (162 MHz) in CD_3OD

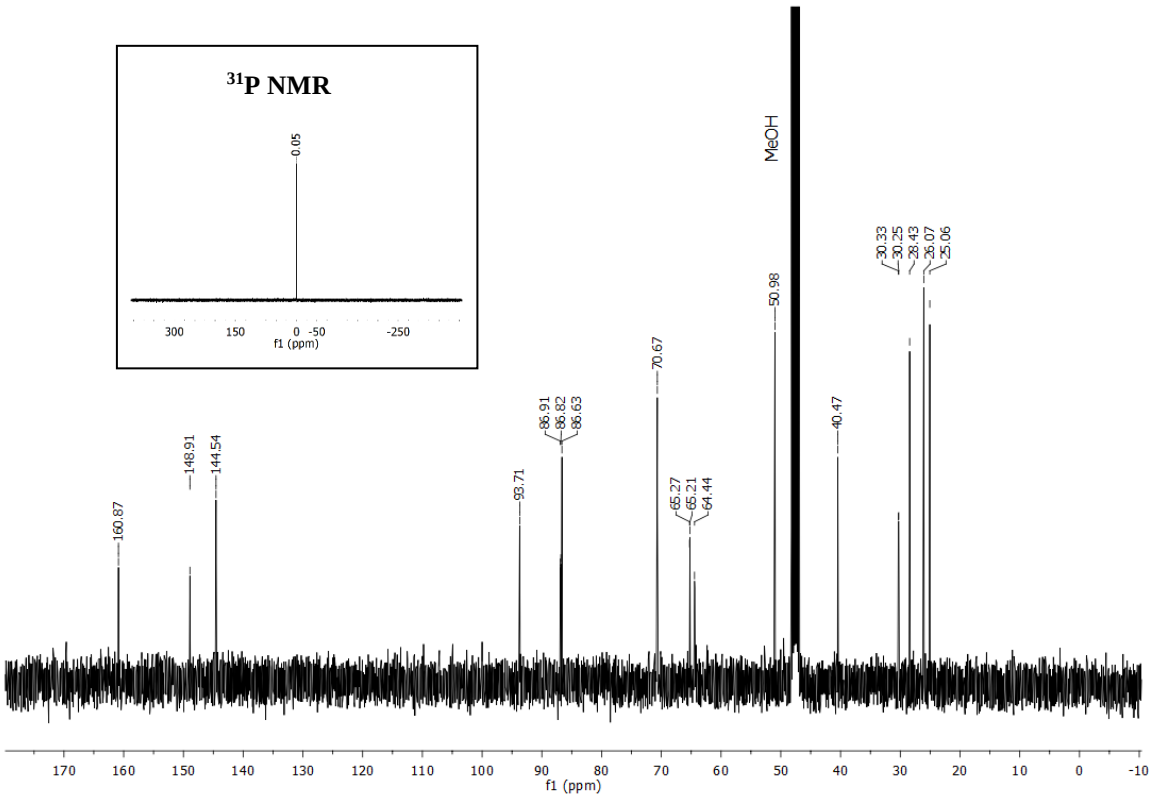




5'-(6-azidohex-1-yloxy)phosphoryl-2'-deoxycytidine (3b)

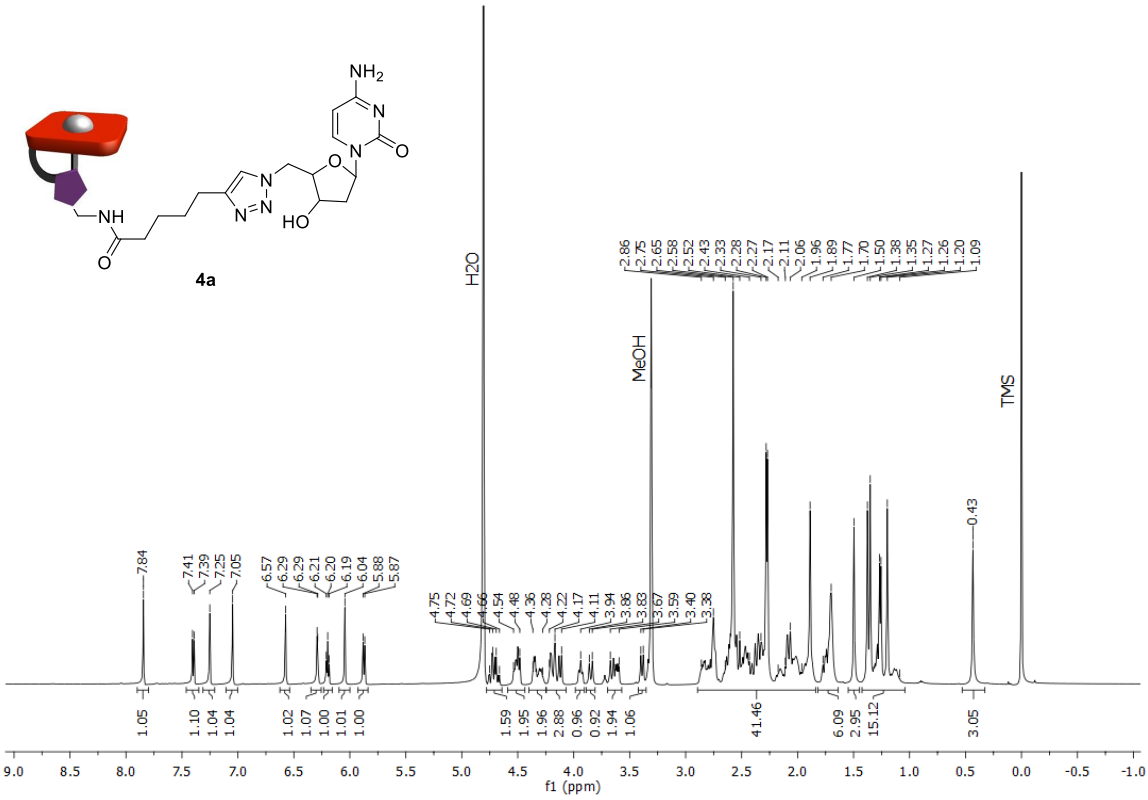
^{1}H NMR (400 MHz), ^{13}C NMR (101 MHz) and ^{31}P NMR (162 MHz) in CD_3OD

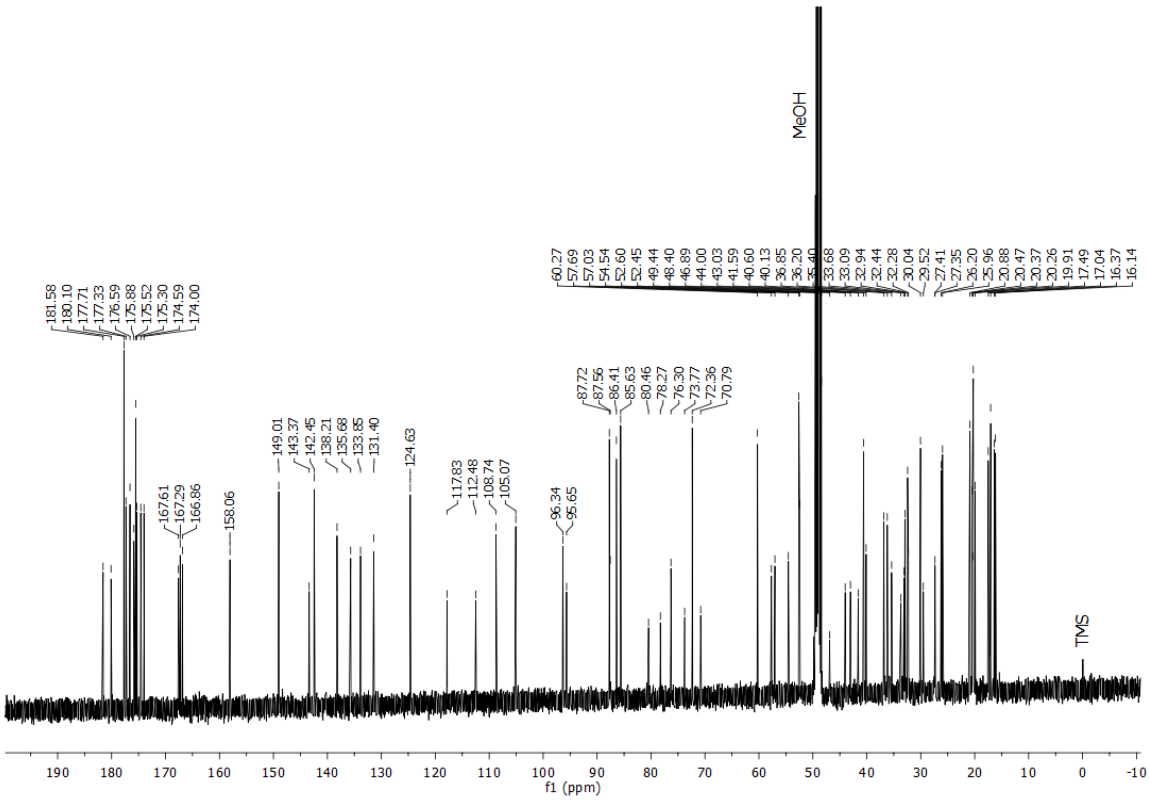




Nucleoside-vitamin B_{12} conjugate 4a

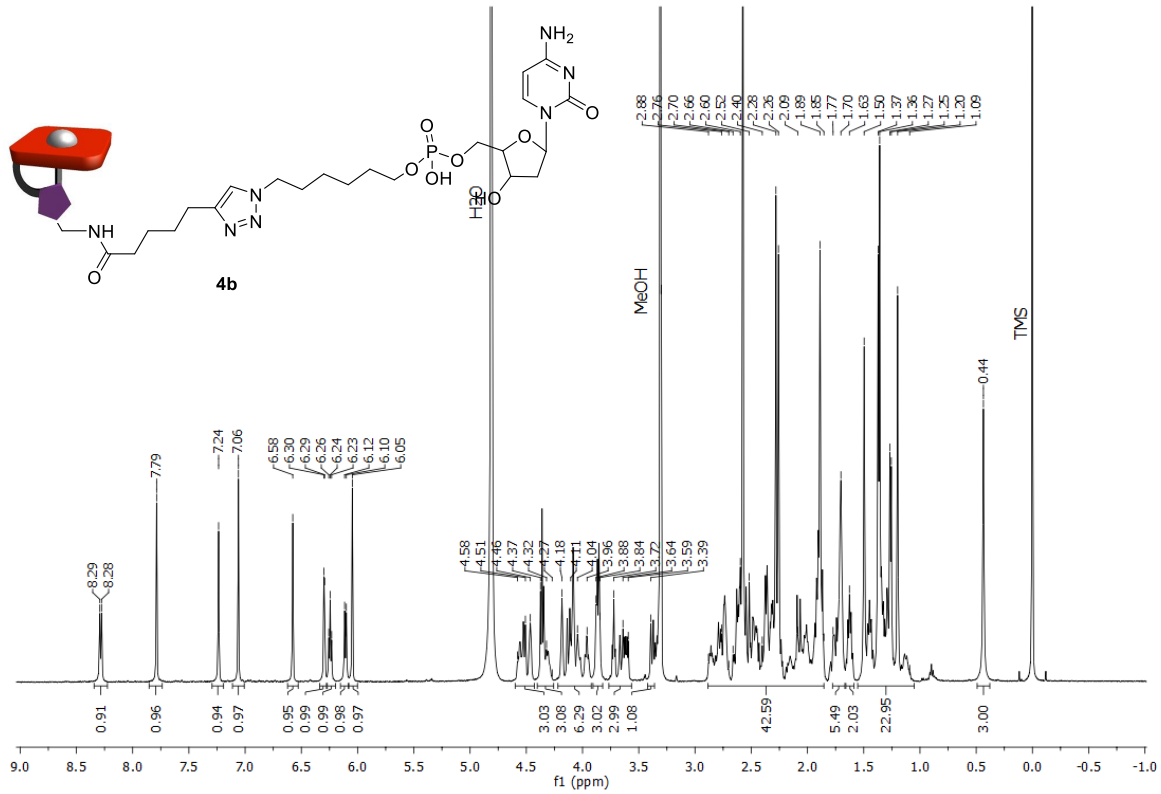
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) in CD_3OD

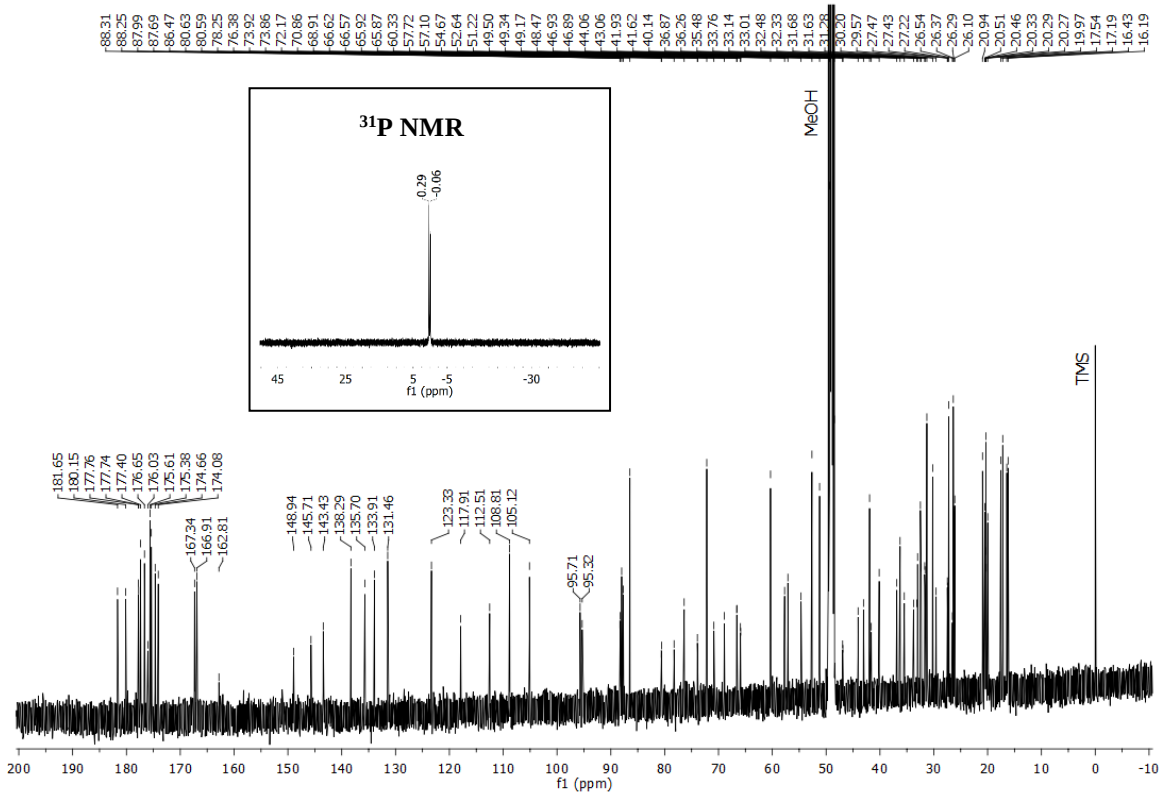




Nucleoside-vitamin B_{12} conjugate 4b

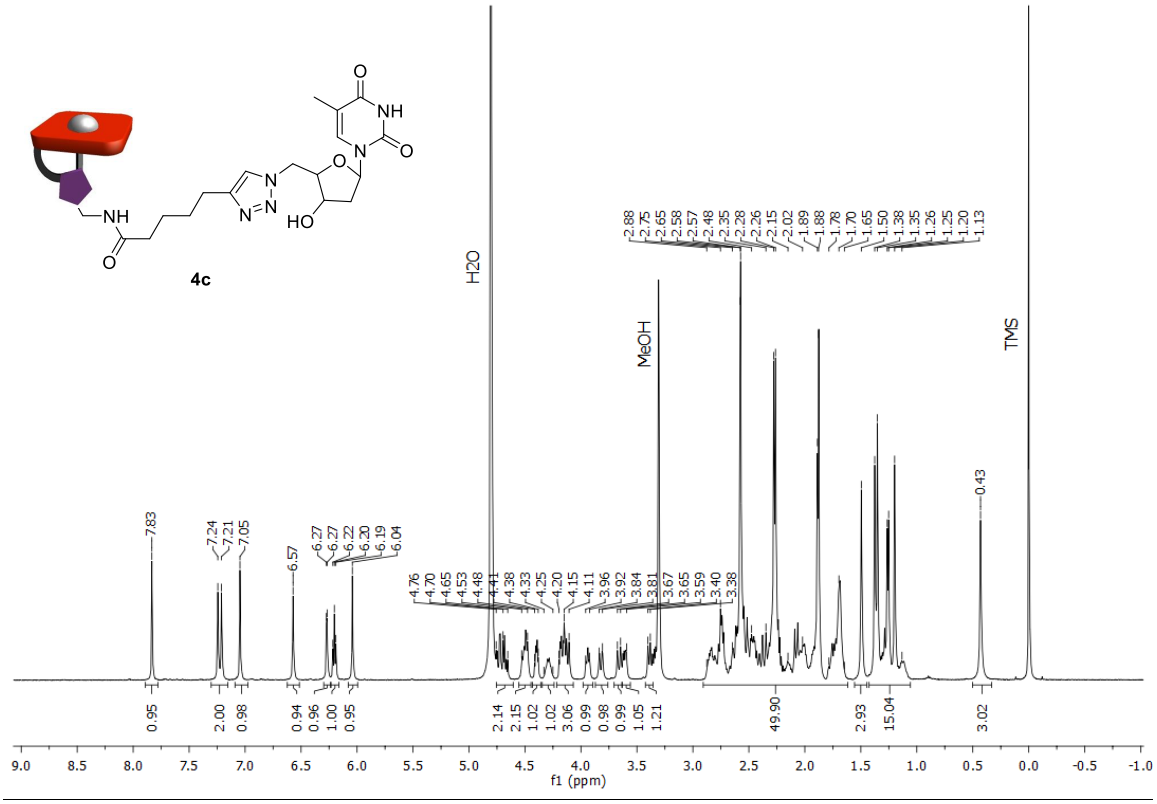
^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz) and ^{31}P NMR (202 MHz) in CD_3OD

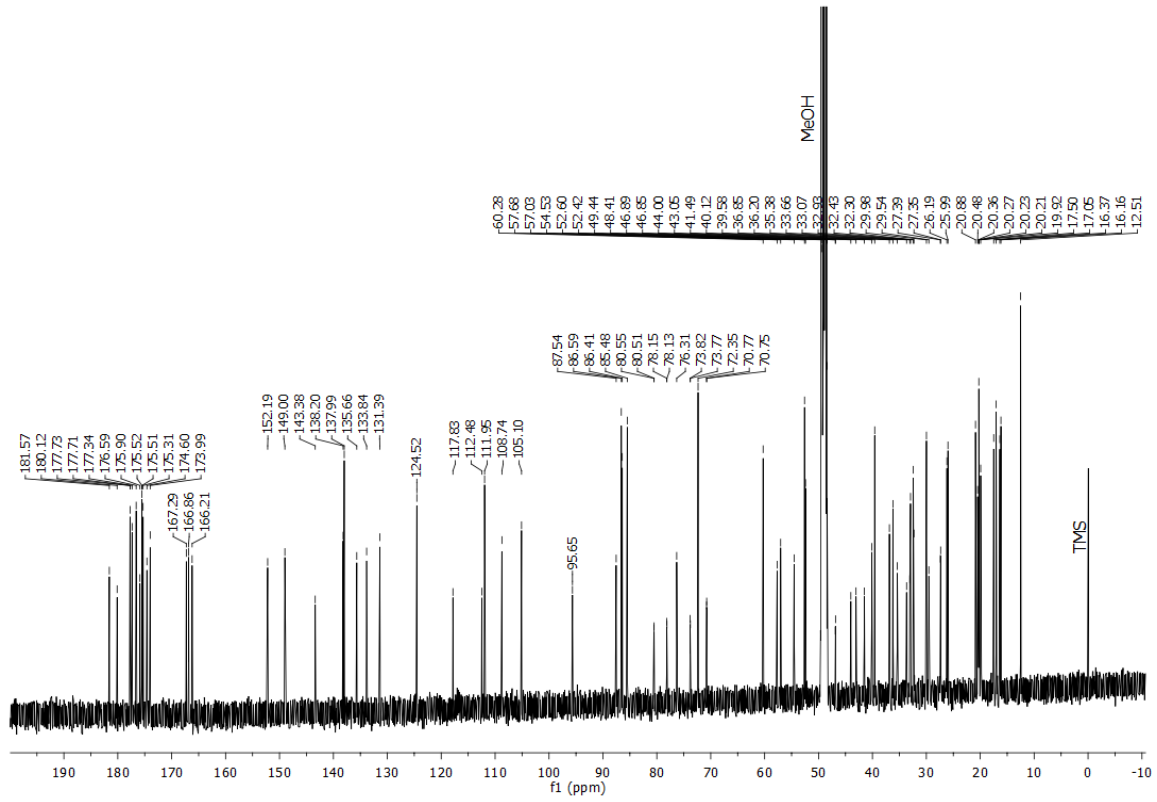




Nucleoside-vitamin B_{12} conjugate 4c

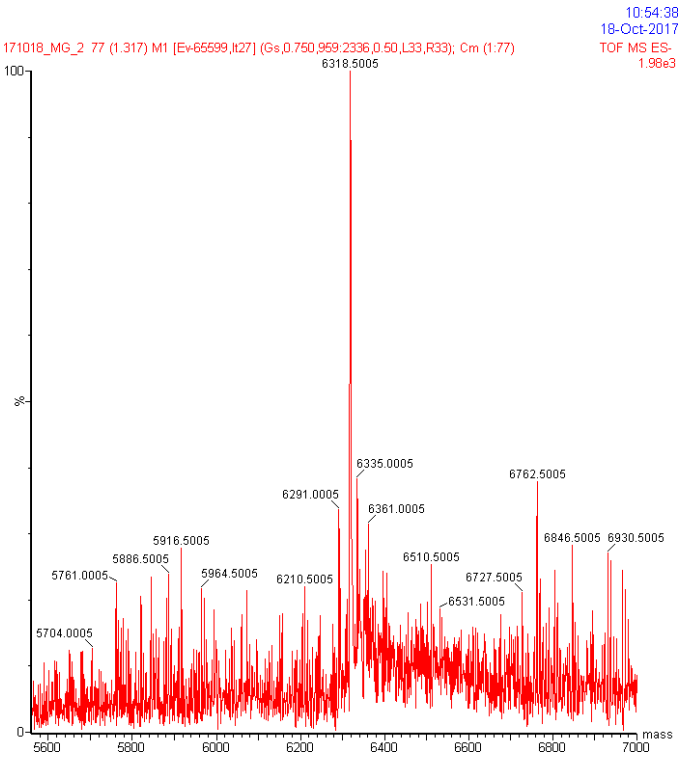
^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) in CD_3OD





14. MS spectra of conjugates B_{12}-2'OMeRNA

2'OMeRNA-vitamin B_{12} conjugate 6a



2'OMeRNA-vitamin B_{12} conjugate 6c