Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information

Synthesis of DNA-coupled isoquinolones and pyrrolidines by solid phase ytterbium- and silver-mediated imine chemistry

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Chemistry

General Methods and Materials

Unless otherwise noted, chemicals were purchased from *abcr, Acros Organics, Alfa Aesar, Fisher Scientific, Merck, Sigma Aldrich, TCI* and *VWR* and were used as provided without further purifications. Dry solvents (CH_2Cl_2, DMF, MeOH, ACN, EtOH, THF, toluene) were used as commercially available.

5'-Aminolinker-modified DNA oligonucleotides bound to controlled pore glass solid support (CPG, 1000 Å porosity) and the hairpin oligonucleotide were synthesized by *IBA* (Göttingen, Germany). The branched oligonucleotide-alkyne conjugate was prepared by DNA solid phase synthesis on a DNA Synthesizer H-8 from *K&A Laborgeraete GbR* on a 1 µmol scale using standard CPG-based phosphoramidite methods. The DNA phosphoramidites were used with the 4,4'-dimethoxytrityl (DMT) and β-cyanoethyl (CE) protecting groups (DMT-dA-CEP, DMT-dT-CEP, DMT-dG-CEP, DMT-dC-CEP and DMT-Ethynyl-dU-CEP). 5-(Benzylthio)-1*H*-tetrazole (BTT) was used as activator.

Oligonucleotide-small molecule conjugates bound to CPG were filtered and washed through synthesis columns using a vacuum manifold (*Vac-Man*®) from *Promega*.

Oligonucleotide concentrations. Concentrations were determined by UV spectroscopy using a *NanoDrop 2000* spectrophotometer from *Thermo Fisher Scientific*.

Semi-preparative ion pair RP-HPLC. Compound purification was performed on a *Shimadzu Prominence* HPLC System equipped with a C_{18} stationary phase (*Phenomenex*, Gemini, 5 µm, C_{18}, 110 Å, 100 x 4.6 mm). A gradient from 100 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) to MeOH (eluent B) was used at a flow rate of 5 mL/min. Fractions containing the desired product were pooled and concentrated.

> **Method:** Step gradient of 20 % to 70 % B within 13 min, then 70 % to 100 % B within 1 min followed by 100 % B for 3 min using 100 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) and MeOH (eluent B) at a flow rate of 5 mL/min.

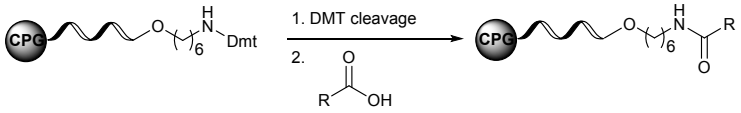
Analytical RP-HPLC. HPLC analysis was performed on an *Agilent 1100 series* chromatograph equipped with 1100 Quaternary Pump (*G1311A*), a 1100 Multi-Wavelength Detector (*G1365B*) and an *Agilent Eclipse Plus* C_{18} (4.6 x 100 mm, 3.5 µm) column. The conversion and purity of DNA conjugates were determined by integration of peaks recorded at 254 nm wavelength.

> **Method-I:** Step gradient of 10 % to 60 % B within 10 min, then 60 % to 100 % B within 2 min followed by 100 % B for 2 min using 10 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) and MeOH (eluent B) at a flow rate of 0.6 mL/min.
>
> **Method-II:** A linear gradient of 10 % to 100 % B within 10 min followed by 100 % B for 4 min using 10 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) and MeOH (eluent B) at a flow rate of 0.6 mL/min.

MALDI-TOF. Mass analysis was performed on a MALDI TOF/TOF MS from *Bruker Daltonics* using 2',4',6'-trihydroxyacetophenone (THAP) matrix (*Dichrom*).

Procedures

Amide coupling A (RP-01)



Step 1: The DMT-protective group of the CPG-bound oligonucleotide (250 nmol, 9-10 mg of solid phase material) was removed by addition of 200 µL 3 % trichloroacetic acid in dry CH_2Cl_2 for 1 min. An orange coloring of the solution indicated successful removal of the protecting group. The deprotection was repeated 3-5 times until no coloring of the solution was observed anymore. The CPG containing deprotected DNA was washed three times with each 200 µL of 1 % TEA in ACN, DMF, MeOH, ACN and CH_2Cl_2 and then dried *in vacuo*.

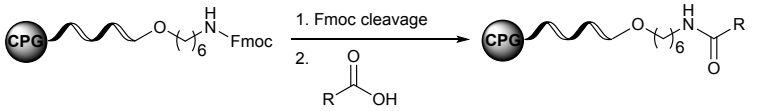
Step 2:

The CPG-bound oligonucleotide, the acid and HATU were dried *in vacuo* for 15 min. Stock solutions of all reactants in dry DMF were prepared before the reaction was started. To the solution of the acid (25 µmol, 100 equiv.) in 75 µL dry DMF, HATU (25 µmol, 100 equiv.) dissolved in 75 µL dry DMF and DIPEA (62.5 µmol, 250 equiv.) were added. The mixture was shaken for 5 min and added to the solid support-bound DNA suspended in 75 µL dry DMF (250 nmol, 1 equiv.). The amide coupling reaction was shaken at ambient temperature for 2 hours. Then, the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*. The amide coupling was repeated two times.

The completeness of the amide coupling was controlled by test cleavage of a small portion (0.7–0.9 mg, ~20 nmol) of the CPG-bound oligonucleotide conjugate with 500 µL AMA (AMA = aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol) for 30 min (hexT) or 4 h (ATC- and ATGC-sequences) at ambient temperature. To this solution 20 µL of 1 M Tris-buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and re-dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. In case of uncompleted coupling (<90%) the reaction was repeated a third time.

Unreacted amines were capped with acetic acid anhydride (three times 200 µL, 30 s, 1:1 mixture of THF/methylimidazole, 9:1, vol/vol, and THF/pyridine/acetic acid anhydride 8:1:1, vol/vol). The CPG-bound oligonucleotide conjugate was washed afterwards again three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*.

Amide coupling B (RP-02)



Step 1:

The Fmoc-protecting group of the CPG-bound oligonucleotide (250 nmol, 9-10 mg) was cleaved off by addition of 200 µL 20 % piperidine in dry DMF and shaking for 5 min. Afterwards, the CPG-bound deprotected oligonucleotide was washed three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and then dried *in vacuo*.

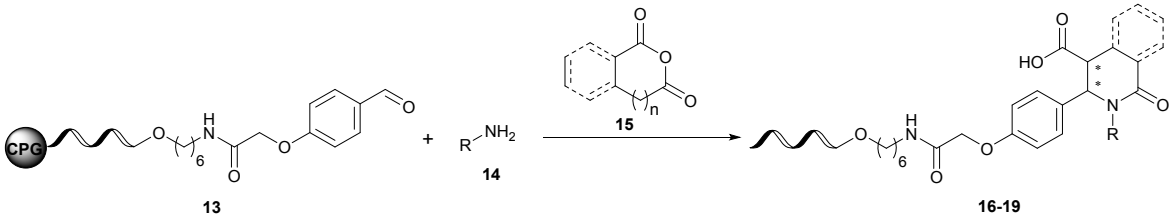
Step 2:

The CPG-bound oligonucleotide, the acid and HATU were dried *in vacuo* for 15 min. Stock solutions of all reactants in dry DMF were prepared before the reaction was started. To the solution of the acid (25 µmol, 100 equiv.) in 75 µL dry DMF, HATU (25 µmol, 100 equiv.) dissolved in 75 µL dry DMF and DIPEA (62.5 µmol, 250 equiv.) were added. The mixture was shaken for 5 min and added to the solid support-bound DNA suspended in 75 µL dry DMF (250 nmol, 1 equiv.). The amide coupling reaction was shaken at ambient temperature for 2 hours. Then, the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*. The amide coupling was repeated two times.

The completeness of the coupling reaction was controlled by test cleavage of a small portion (0.7–0.9 mg, ~20 nmol) of the CPG-bound oligonucleotide conjugate with 500 µL AMA (AMA = aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol) for 30 min (hexT) or 4 h (ATC- and ATGC-sequences) at ambient temperature. To this solution 20 µL of 1 M Tris-buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and re-dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. In case of uncompleted coupling the reaction was repeated a third time.

Unreacted amines were capped with acetic acid anhydride (three times 200 µL, 30 s, 1:1 mixture of THF/methylimidazole, 9:1, vol/vol, and THF/pyridine/acetic acid anhydride 8:1:1, vol/vol). The CPG-bound oligonucleotide conjugate was washed afterwards again three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*.

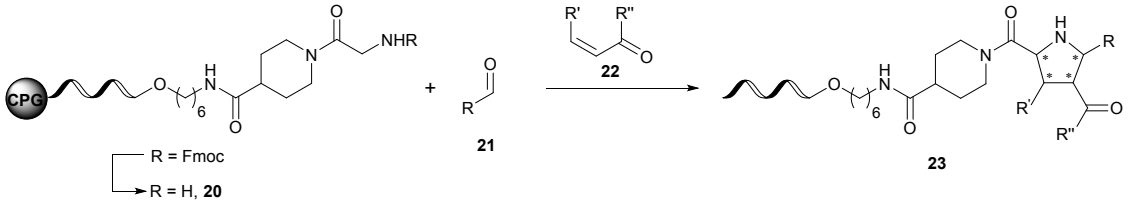
Castagnoli-Cushman reaction on CPG-bound oligonucleotides (RP-03)



The amine **14** (10 µmol, 500 equiv.) was dissolved in 24 µL CH_2Cl_2. The solution was added to the CPG-bound oligonucleotide-aldehyde conjugate **13** (20 nmol) suspended in 12 µL triethyl orthoformate. The

suspension was shaken at ambient temperature for 4 h. Afterwards 30 µL of a suspension of $Yb(OTf)_3$ (1 µmol, 50 equiv.) in CH_2Cl_2 was added, followed by 30 µL of a suspension of anhydride **15** (10 µmol, 500 equiv.) in CH_2Cl_2. Prior addition to the reaction vessel both suspension were vortexed and pipetted up and down to obtain homogeneous suspensions. The reaction mixture was shaken for 1 h at ambient temperature. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, 0.1 M $MgCl_2$ solution, water, DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*. CPG-bound oligonucleotide conjugates **16-19** (**16** = hexT, **17** = 10mer ATC, **18** = 10mer ATGC and **19** = 31mer ATGC) were then cleaved from the solid support and deprotected with 500 µL AMA solution (AMA = aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol) for 30 min (hexT) or 4 h (ATC- and ATGC-sequences) at ambient temperature. To this solution 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and afterwards re-dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC (Method I) and MALDI-MS. The product was purified by preparative RP-HPLC.

1,3-Dipolar cycloaddition on CPG-bound oligonucleotides (RP-04)



Step 1:

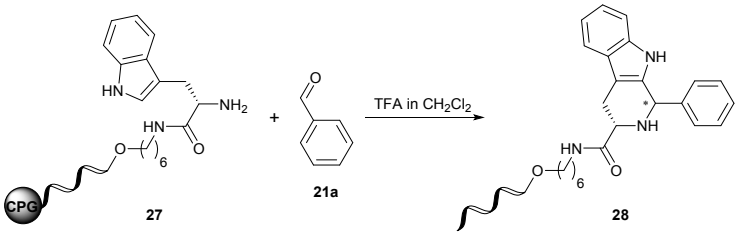
The Fmoc-protecting group of the CPG-bound oligonucleotide-glycine (20 nmol) was removed by addition of 200 µL 20 % piperidine in dry DMF and shaking for 5 min. Afterwards, the CPG-bound deprotected oligonucleotide-glycine conjugate **20** was washed three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*.

Step 2:

The aldehyde **21** (20 µmol, 1000 eq.) was either directly added to the CPG-bound DNA-glycine conjugate **20** and it was filled up to a final volume of 50 µL with ACN/triethyl orthoformate (2:1) or the aldehyde **21** was dissolved in 50 µL ACN/triethyl orthoformate (2:1) and then added to the DNA conjugate **20**. The reaction mixture was shaken at ambient temperature for 6 h. Afterwards, 30 µL of a suspension of AgOAc (2 µmol, 100 equiv.) in ACN/triethyl orthoformate (2:1) were added followed by dipolarophile **22** (80 µmol, 4000 equiv.) and TEA (80 µmol, 4000 equiv.). Prior addition to the reaction vessel the AgOAc suspension was vortexed and pipetted up and down to obtain a homogeneous suspension. The reaction mixture was shaken overnight at 50 °C. The CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, DMF, MeOH, ACN and CH_2Cl_2 and then dried *in vacuo*. The CPG-bound DNA conjugate **20** (**20a** = hexT, **20b** = 10mer ATC, **20c** = 10mer ATGC, **20d** = 31mer ATGC) was cleaved from the solid phase and deprotected by adding 500 µL AMA solution and shaking for 30 min (hexT) or 4 hours (ATC- and ATGC-sequences) at ambient temperature. Afterwards 20 µL 1 M

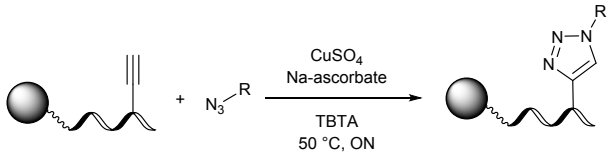
aqueous Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and the remaining DNA pellet was dissolved in 200 µL of distilled water. To remove residual silver ions, an additional washing step using Chelex 100 resin was introduced. For this, 5 mg Chelex resin were washed three times with 10 mM aqueous triethylammoinum acetate (pH = 8.0). The aqueous DNA solution was added to the resin and the suspension was shaken overnight at ambient temperature. The supernatant was taken off, dried in a SpeedVac and re-dissolved in 200 µL distilled water. The crude was analyzed by analytical RP-HPLC (Method I) and MALDI-MS. The product was isolated by preparative RP-HPLC.

TFA-mediated Pictet-Spengler reaction (RP-05)



The Fmoc-protecting group of CPG-bound DNA-Trp-NHFmoc (20 nmol) was removed by treatment with 500 µL of 20 % piperidine in DMF for 10 min at ambient temperature. The deprotected CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and then dried *in vacuo*. Afterwards the CPG-bound DNA-Trp-NH_2 (20 nmol) was suspended in dry CH_2Cl_2 (20 µL). Benzaldehyde (20 µmol, 1000 equiv.) was added followed by trifluoroacetic acid (0.5 %). The suspension was diluted with dry CH_2Cl_2 to a total volume of 50 µL and shaken at ambient temperature for 18 h. The CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of DMF, MeOH, ACN and CH_2Cl_2 and then dried *in vacuo*. The CPG-bound DNA conjugate **27** (**27a** = hexT, **27b** = 10mer ATC, **27c** = 10mer ATGC) was cleaved off from the solid phase by adding 500 µL AMA solution and shaking for 30 min (hexT) or 4 hours (ATC- and ATGC-sequences) at ambient temperature. Afterwards 20 µL 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and the residue was re-dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC (Method I) and MALDI-MS. The product was isolated by preparative RP-HPLC.

Copper(I)-catalyzed alkyne-azide cycloaddition (RP-06)



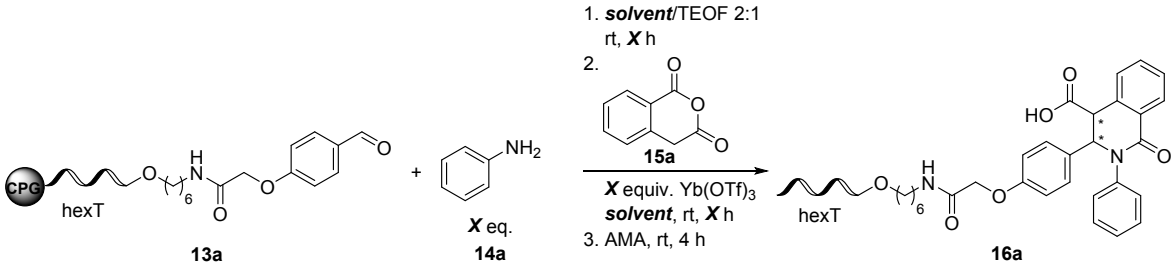
The CPG-bound oligonucleotide alkyne conjugate (50 nmol) was suspended in 100 µL DMF and diluted with 380 µL of H_2O/MeOH (1:1). Subsequently, the azide (125 µmol, 2500 equiv.) dissolved in 100 µL of DMF/H_2O (9:1), TBTA (6.25 µmol, 125 equiv.) dissolved in 20 µL of DMF, Na-ascorbate (6.25 µmol, 125 equiv.) dissolved in 10 µL of H_2O, and $CuSO_4$*$5H_2O$ (0.625 µmol, 12.5 equiv.) dissolved in 10 µL of

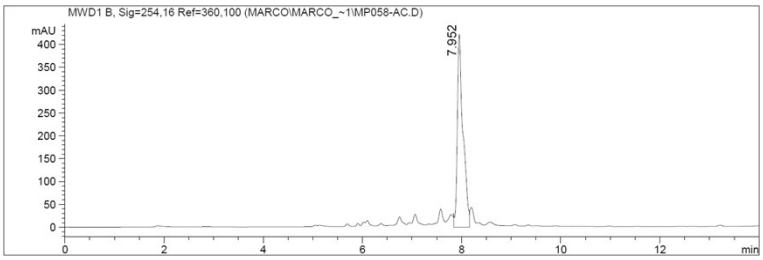
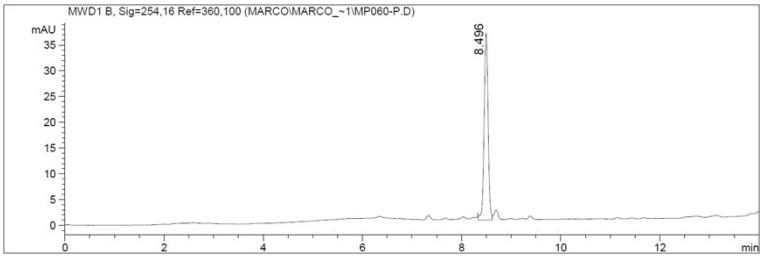
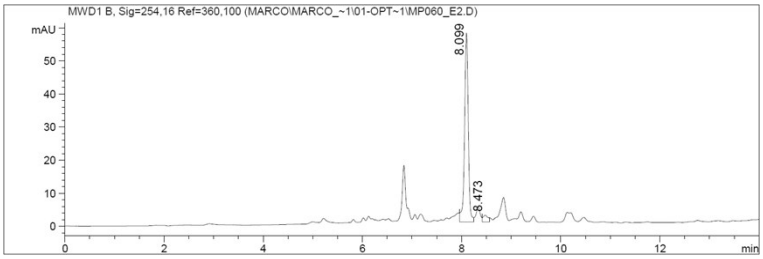
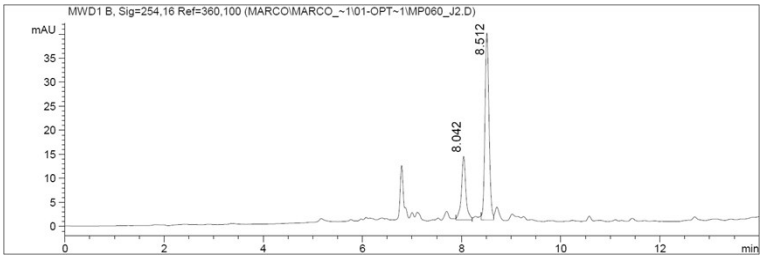
H_2O were added to the suspension in this order. Stock solutions of all reactants were prepared before the reaction was started. The reaction mixtures were shaken at 50 °C overnight. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, 0.1 M $MgCl_2$ solution, water, DMF, MeOH, ACN and CH_2Cl_2 and dried *in vacuo*.

The completeness of the reaction was controlled by cleavage of a small portion (~20 nmol) of CPG-bound oligonucleotide conjugate with 500 µL AMA (AMA = aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol) for 4 h at ambient temperature. To this solution 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac, re-dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC (Method II) and MALDI-MS.

$Yb(OTf)_3$-mediated Castagnoli-Cushman reaction on CPG-bound oligonucleotides

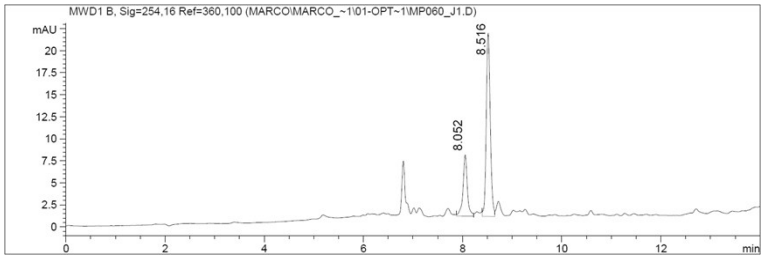
Table S1 Optimization of $Yb(OTf)_3$-mediated Castagnoli-Cushman reaction on CPG-bound hexT.[a]



Entry	Reaction conditions[b]	HPLC trace of crude reaction mixture[c]
1	hexT-aldehyde conjugate **13a**	
2	hexT-isoquinolone conjugate **16a**	
3	1. 500 equiv. **14a** CH_2Cl_2/TEOF (2:1), 4 h, rt ***2. washing step (3x 200 µL DMF, MeOH, MeCN, CH_2Cl_2)*** 3. 500 equiv. **15a** 100 equiv. $Yb(OTf)_3$ CH_2Cl_2, 2 h, rt => conversion <5 %	
4	1. 500 equiv. **14a** CH_2Cl_2/TEOF (2:1), 4 h, rt 2. 500 equiv. **15a** 100 equiv. $Yb(OTf)_3$ CH_2Cl_2, ***2 h***, rt => conversion 72 %	

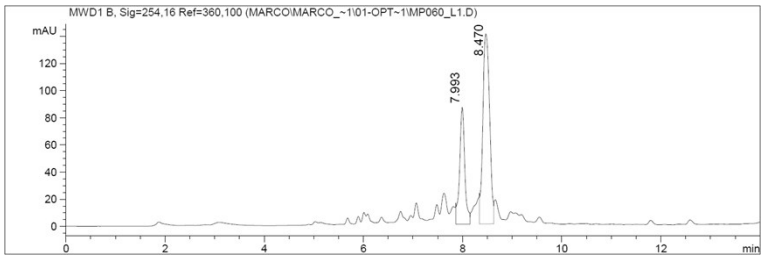
1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, ***1 h***, rt

   => conversion 73 %



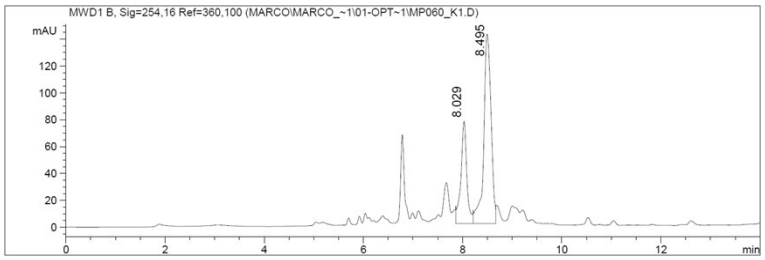
1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, ***0.5 h***, rt

   => conversion 68 %



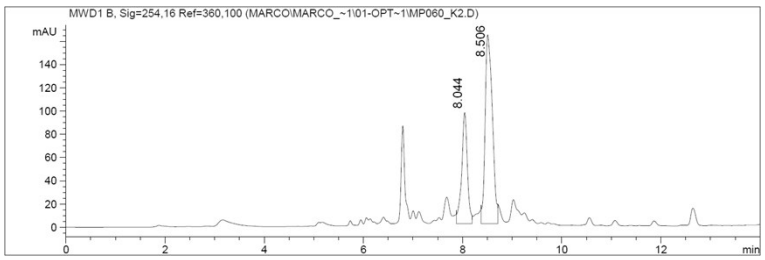
1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), ***3 h***, rt
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

   => conversion 69 %



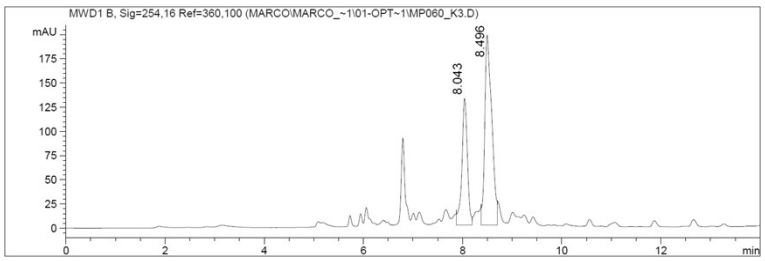
1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), ***2 h***, rt
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

   => conversion 68 %



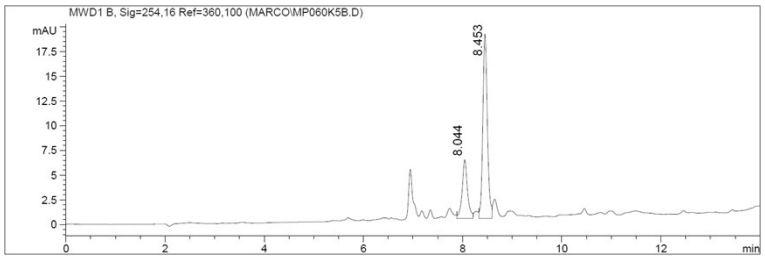
1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), ***1 h***, rt
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

   => conversion 65 %



1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), ***16 h***, rt
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

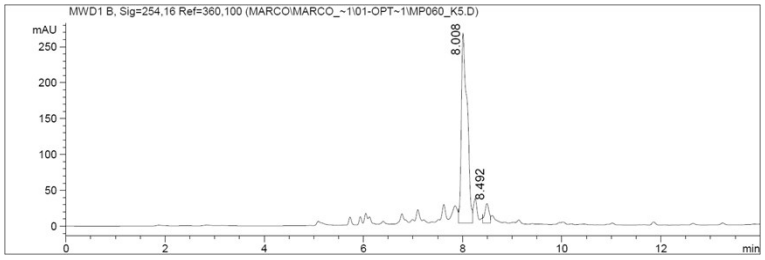
   => conversion 73 %



11

1. 500 equiv. **14a**
   CH_2Cl_2, 4 h, rt
   step without TEOF
2. 500 equiv. **15a**
   100 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

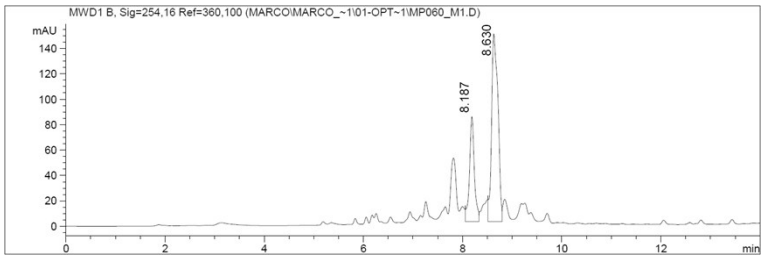
=> conversion 7 %



12

1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   50 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

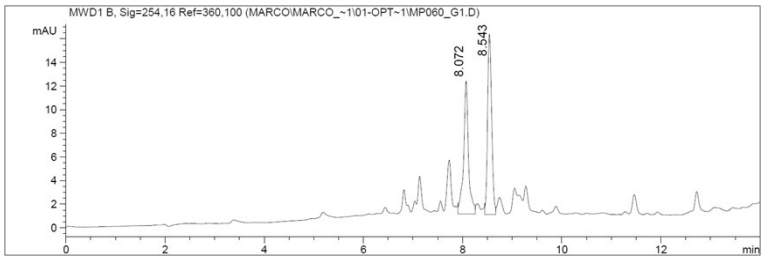
=> conversion 70 %



13

1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   25 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

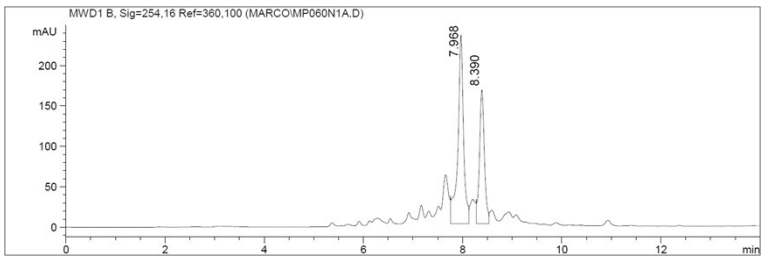
=> conversion 54 %



14

1. 500 equiv. **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   0 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

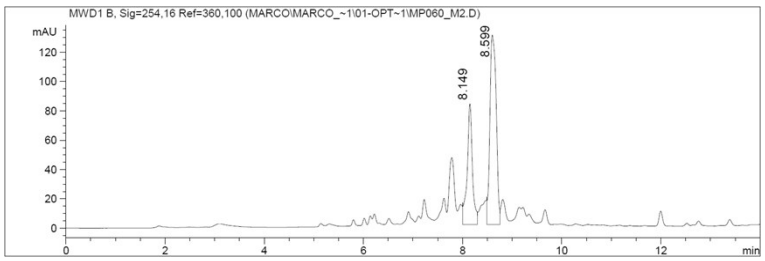
=> conversion 38 %



15

1. ***1000 equiv.*** **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. ***1000 equiv.*** **15a**
   50 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

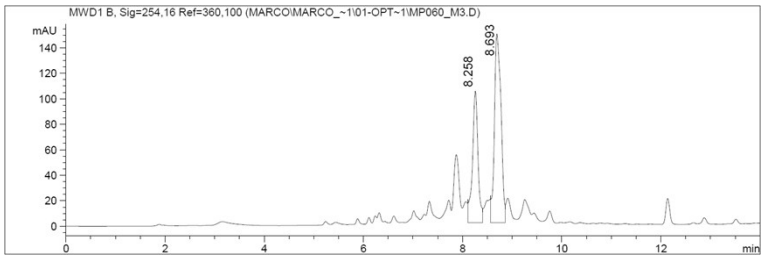
=> conversion 65 %



16

1. ***1500 equiv.*** **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. ***1500 equiv.*** **15a**
   50 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

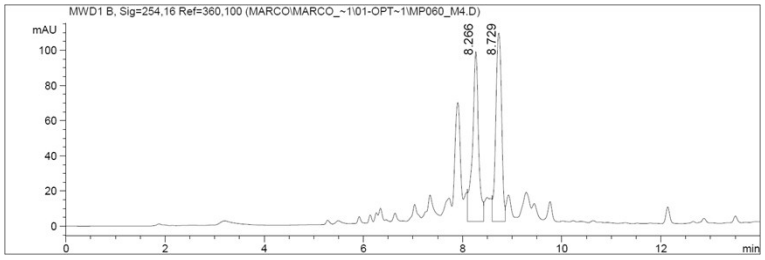
=> conversion 63 %



17

1. ***2000 equiv.*** **14a**
   CH_2Cl_2/TEOF (2:1), 4 h, rt
2. ***2000 equiv.*** **15a**
   50 equiv. $Yb(OTf)_3$
   CH_2Cl_2, 1 h, rt

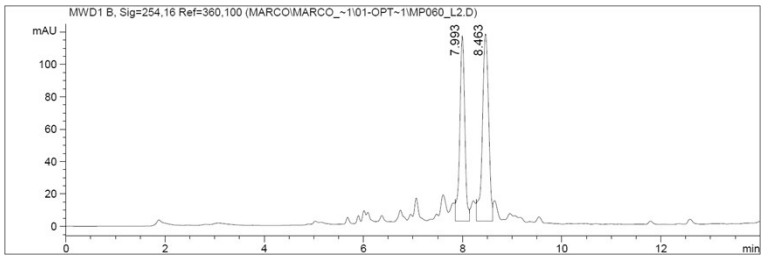
=> conversion 51 %



18

1. 500 equiv. **14a**
   THF/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   50 equiv. $Yb(OTf)_3$
   THF, 1 h, rt

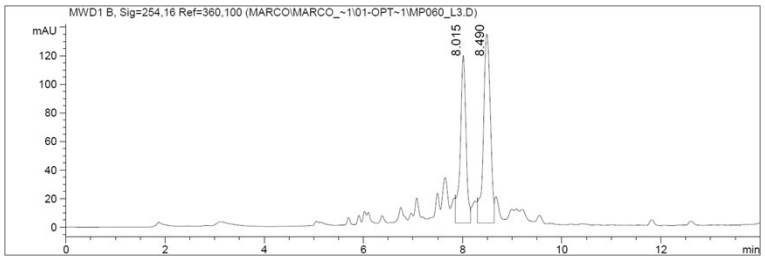
=> conversion 56 %



19

1. 500 equiv. **14a**
   DCE/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   50 equiv. $Yb(OTf)_3$
   DCE, 1 h, rt

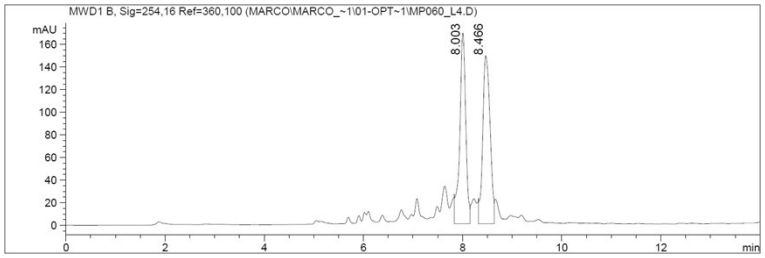
=> conversion 58 %



20

1. 500 equiv. **14a**
   MeCN/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   50 equiv. $Yb(OTf)_3$
   MeCN, 1 h, rt

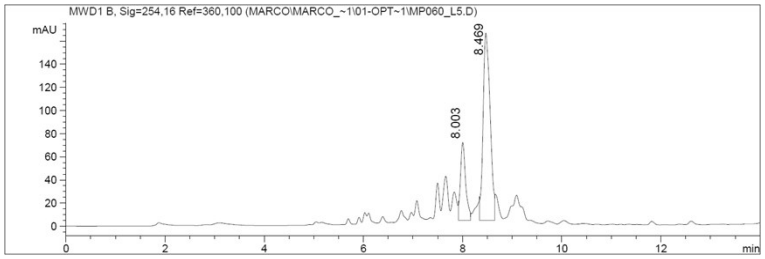
=> conversion 52 %



21

1. 500 equiv. **14a**
   MeOH/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   50 equiv. $Yb(OTf)_3$
   MeOH, 1 h, rt

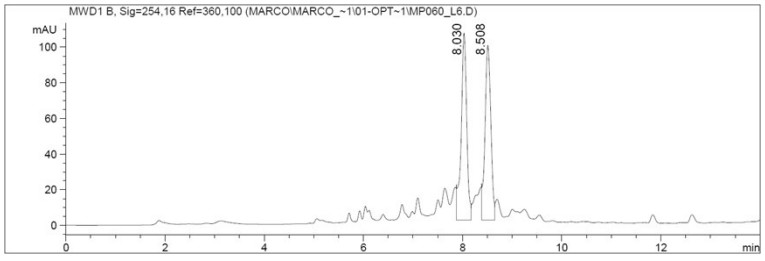
=> conversion 68 %

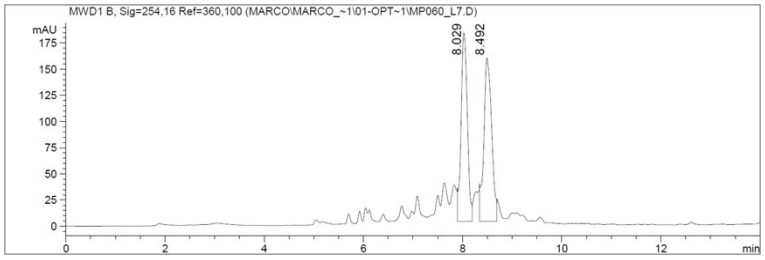
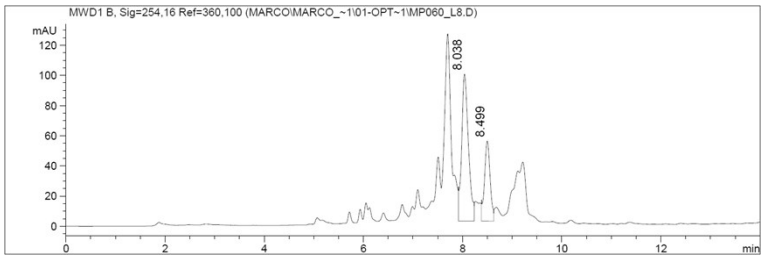
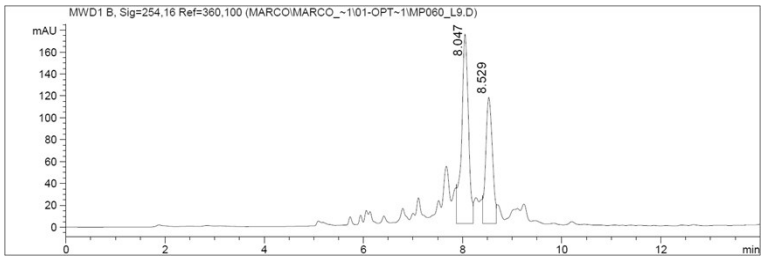


22

1. 500 equiv. **14a**
   toluene/TEOF (2:1), 4 h, rt
2. 500 equiv. **15a**
   50 equiv. $Yb(OTf)_3$
   toluene, 1 h, rt

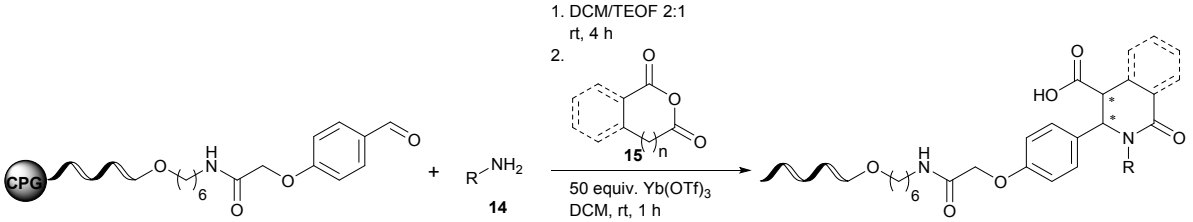
=> conversion 50 %



23	1. 500 equiv. **14a** ***1,4-dioxane***/TEOF (2:1), 4 h, rt 2. 500 equiv. **15a** 50 equiv. $Yb(OTf)_3$ ***1,4-dioxane***, 1 h, rt => conversion 52 %	
24	1. 500 equiv. **14a** ***DMF***/TEOF (2:1), 4 h, rt 2. 500 equiv. **15a** 50 equiv. $Yb(OTf)_3$ ***DMF***, 1 h, rt => conversion 31 %	
25	1. 500 equiv. **14a** ***EtOAc***/TEOF (2:1), 4 h, rt 2. 500 equiv. **15a** 50 equiv. $Yb(OTf)_3$ ***EtOAc***, 1 h, rt => conversion 40 %	

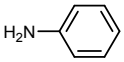
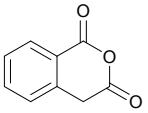
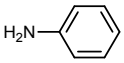
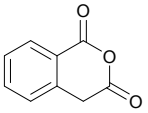
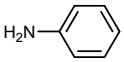
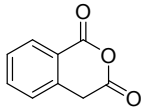
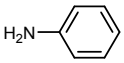
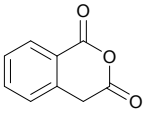
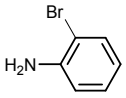
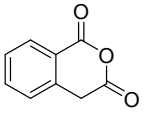
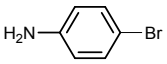
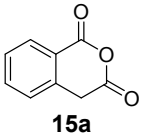
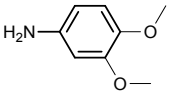
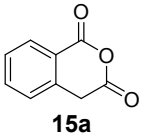
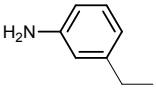
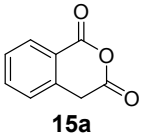
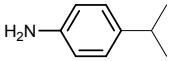
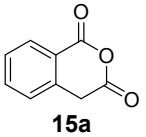
[a] CPG-bound hexT conjugate **13a** (20 nmol) and aniline **14a** (X equiv.) were condensed in 36 µL of indicated solvent/triethyl orthoformate (2:1) at ambient temperature, then $Yb(OTf)_3$ (X equiv.) and anhydride **15a** (X equiv.) each suspended in 30 µL of indicated solvent were added, the reaction mixture was shaken at ambient temperature. [b] parameters that were changed are in bold and italic. [c] Analytical RP-HPLC, Method-I. TEOF = triethyl orthoformate.

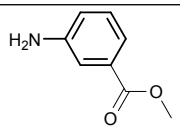
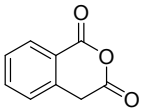
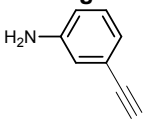
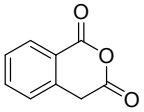
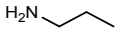
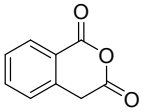
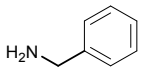
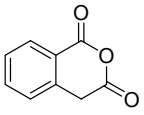
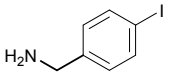
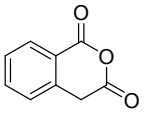
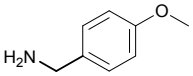
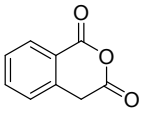
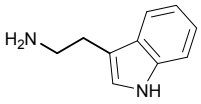
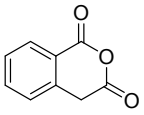
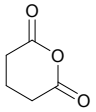
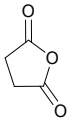
Table S2 – Scope of $Yb(OTf)_3$-mediated Castagnoli-Cushman reaction on CPG-bound oligonucleotides using different amines and anhydrides.[a]



hexT **13a**
10mer ATC **13b**
10mer ATGC **13c**
31mer ATGC **13d**

hexT **16a**
10mer ATC **17a**
10mer ATGC **18a-o**
31mer ATGC **19a**

Entry	Product	Amine	Anhydride	Conversion [%][b]	Yield [nmol][c]	$Mass_{calc.}$ $Mass_{found}$[d]
1	**16a**	 **14a**	 **15a**	70	4.9	2341.8 2342.3
2	**17a**	 **14a**	 **15a**	68	5.3	3540.6 3539.8
3	**18a**	 **14a**	 **15a**	69	4.7	3596.6 3596.1
4	**19a**	 **14a**	 **15a**	63[e]	---	9953.5 9942.6
5	**18b**	 **14b**	 **15a**	<5	---	3675.5 n.d.
6	**18c**	 **14c**	 **15a**	52	1.6	3675.5 3674.6
7	**18d**	 **14d**	 **15a**	46	2.5	3656.6 3654.1
8	**18e**	 **14e**	 **15a**	65	5.3	3624.6 3622.1
9	**18f**	 **14f**	 **15a**	72	4.6	3638.7 3640.0

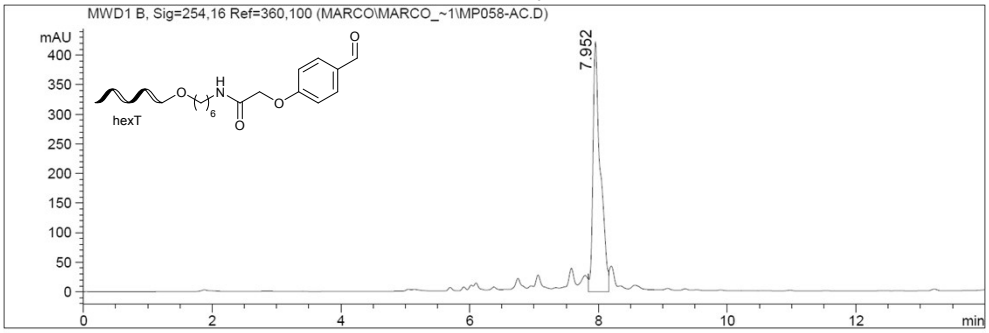
10	**18g**	 **14g**	 **15a**	54	5.1	3654.6 3655.3
11	**18h**	 **14h**	 **15a**	50	2.8	3620.6 3619.7
12	**18i**	 **14i**	 **15a**	89	5.2	3562.6 3564.3
13	**18j**	 **14j**	 **15a**	62	3.9	3610.6 3609.1
14	**18k**	 **14k**	 **15a**	74	5.0	3736.5 3734.8
15	**18l**	 **14l**	 **15a**	83	5.7	3640.6 3639.1
16	**18m**	 **14m**	 **15a**	78	3.8	3663.7 3661.8
17	**18n**	 **14i**	 **15b**	n.d.	---	3548.5 n.d.
18	**18o**	 **14i**	 **15c**	n.d.	---	3520.5 n.d.

[a] CPG-bound oligonucleotide conjugate **13** (20 nmol) and amine **14** (500 equiv., 10 µmol) were condensed in 36 µL CH_2Cl_2/triethyl orthoformate (2:1) at ambient temperature for 4 h, then addition of $Yb(OTf)_3$ (50 equiv., 1 µmol) and anhydride **15** (500 equiv., 10 µmol) each suspended in 30 µL CH_2Cl_2 at ambient temperature for 1 h. DNA cleavage with AMA (30 % aqueous ammonia/ 40 % aqueous methylamine, 1:1 (vol/vol)) at ambient temperature for 4 h. [b] Determined by analytical RP-HPLC analysis. [c] Determined by NanoDrop. [d] Measured by MALDI-MS. [e] Determined by analysis of preparative RP-HPLC chromatogram. 10mer ATC = 5'-TTA CTA CCT A-3', 10mer ATGC = 5'-GTC ATG ATC T-3', 31mer ATGC = 5'-CAA ATC CGT TCA CAC CGA CCT GTC ATG ATC T-3'. n.d. = not detected.

Starting materials for Castagnoli-Cushman reaction on CPG-bound oligonucleotides

DNA conjugate 13a: Following DMT removal, CPG-bound hexT-C_6-NH_2 was reacted with 2-(4-formylphenoxy)acetic acid according to RP-01.

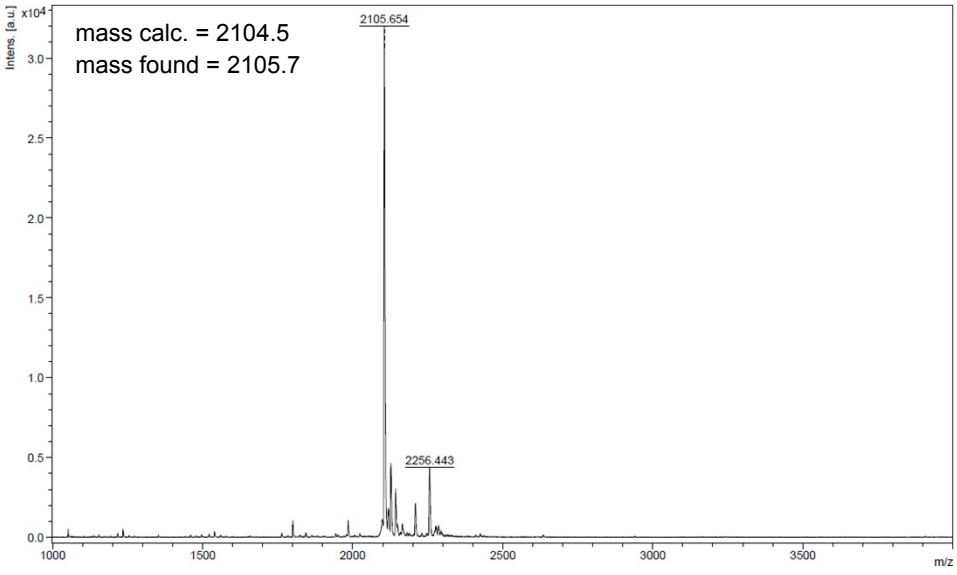
HPLC trace of crude reaction mixture 13a (Analytical RP-HPLC, Method-I)



Peak list:

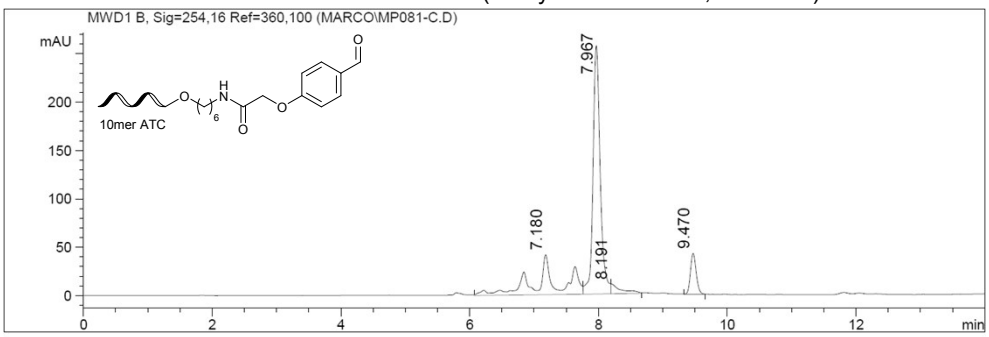
Ret. Time	Width min	Height	Area	Area %
7.952	0.113	422.406	3381.665	100.000

MALDI-MS spectrum of crude reaction mixture 13a



DNA conjugate 13b: Following DMT removal, CPG-bound ATC-C_6-NH_2 was reacted with 2-(4-formylphenoxy)acetic acid according to RP-01.

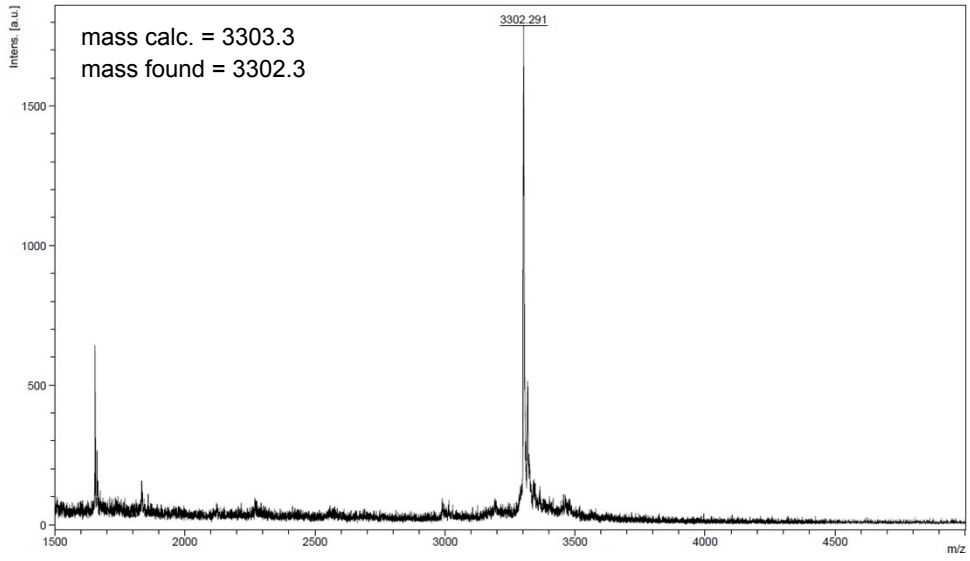
HPLC trace of crude reaction mixture 13b (Analytical RP-HPLC, Method-I)



Peak list:

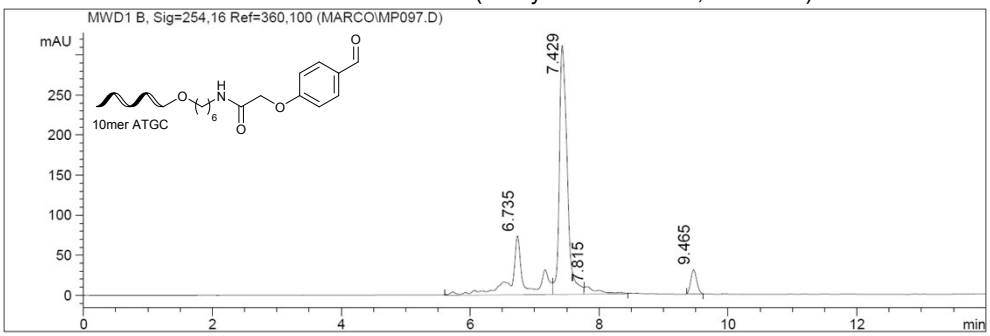
Ret. Time	Width min	Height	Area	Area %
7.180	0.379	41.251	938.027	28.436
7.967	0.129	257.082	1987.073	60.237
8.191	0.181	10.391	112.757	3.418
9.470	0.103	42.417	260.912	7.909

MALDI-MS spectrum of isolated product 13b



DNA conjugate 13c: Following DMT removal, CPG-bound ATGC-C_6-NH_2 was reacted with 2-(4-formylphenoxy)acetic acid according to RP-01.

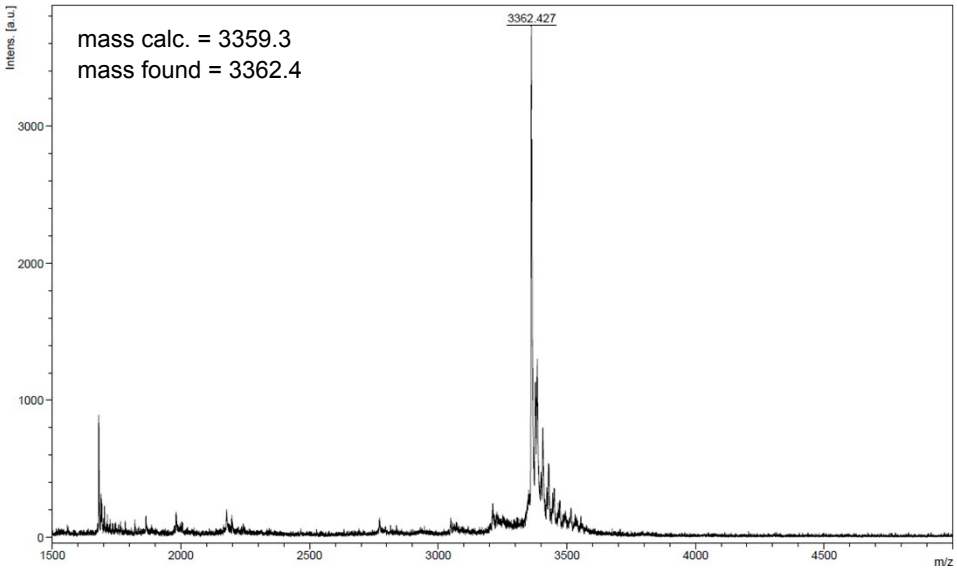
HPLC trace of crude reaction mixture 13c (Analytical RP-HPLC, Method-I)



Peak list:

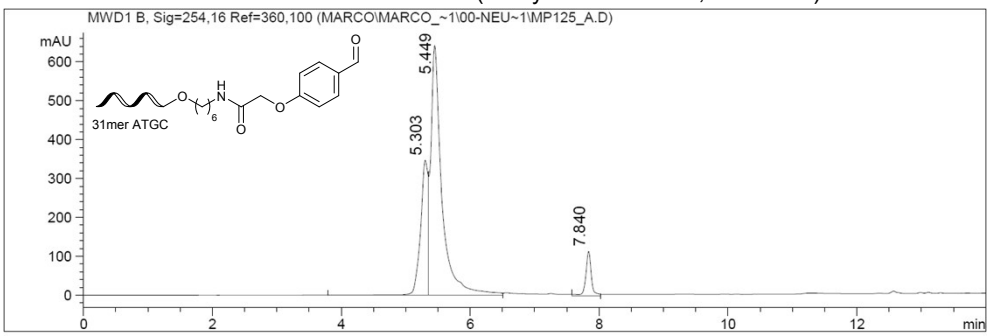
Ret. Time	Width min	Height	Area	Area %
6.735	0.266	73.641	1173.848	28.178
7.429	0.142	311.345	2652.366	63.670
7.815	0.249	9.363	139.647	3.352
9.465	0.108	30.728	199.962	4.800

MALDI-MS spectrum of isolated product 13c



DNA conjugate 13d: Following DMT removal, CPG-bound ATGC-C_6-NH_2 was reacted with 2-(4-formylphenoxy)acetic acid according to RP-01.

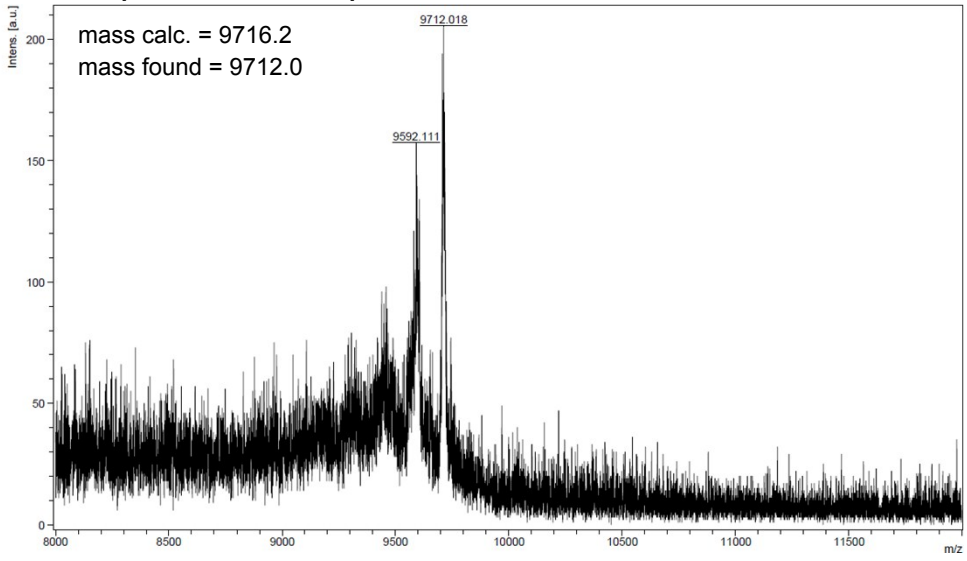
HPLC trace of crude reaction mixture 13d (Analytical RP-HPLC, Method-II)



Peak list:

Ret. Time	Width min	Height	Area	Area %
5.303	0.117	346.872	2818.422	25.313
5.449	0.167	642.035	7635.373	68.574
7.840	0.099	114.803	680.658	6.113

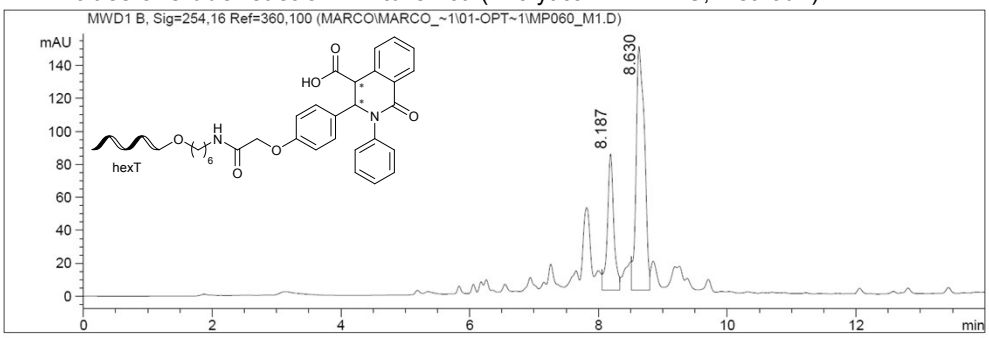
MALDI-MS spectrum of isolated product 13d



Products of Castagnoli-Cushman reaction on CPG-bound oligonucleotides

DNA conjugate 16a: CPG-bound hexT-aldehyde conjugate **13a** was reacted with aniline **14a** and homophthalic anhydride **15a** according to RP-03.

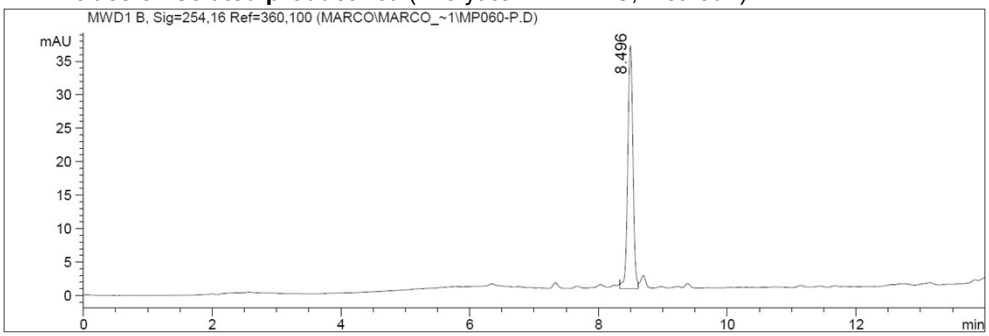
HPLC trace of crude reaction mixture 16a (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
8.187	0.113	82.678	560.013	29.804
8.630	0.149	147.883	1318.988	70.196

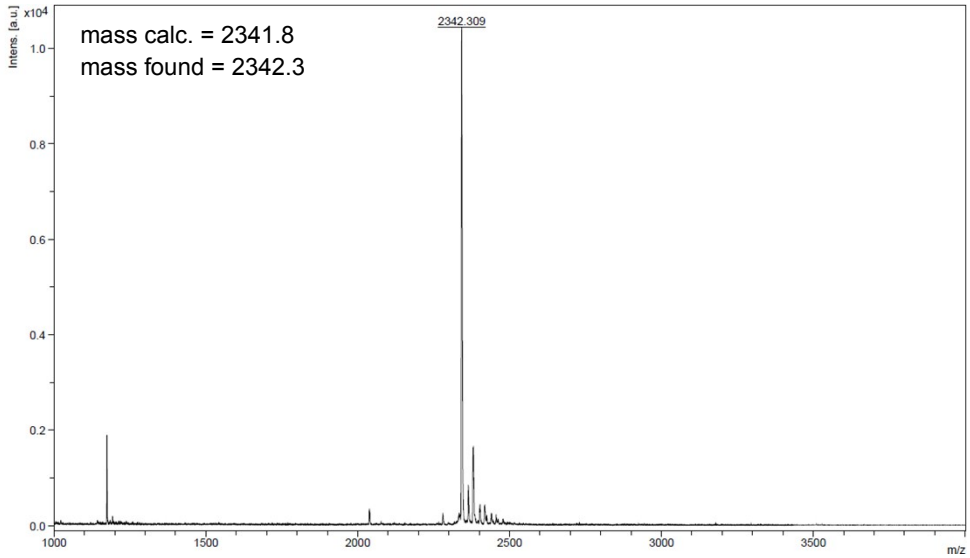
HPLC trace of isolated product 16a (Analytical RP-HPLC, Method-I)



Peak list:

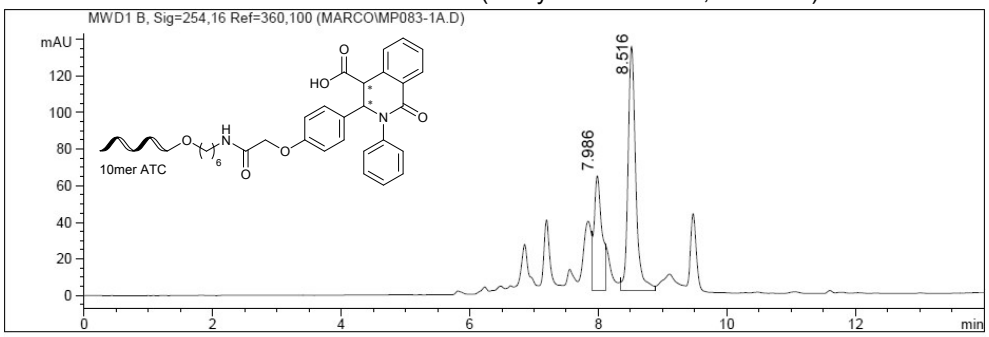
Ret. Time	Width min	Height	Area	Area %
8.496	0.092	36.476	201.526	100.000

MALDI-MS spectrum of isolated product 16a



DNA conjugate 17a: CPG-bound 10mer ATC-aldehyde conjugate **13b** was reacted with aniline **14a** and homophthalic anhydride **15a** according to RP-03.

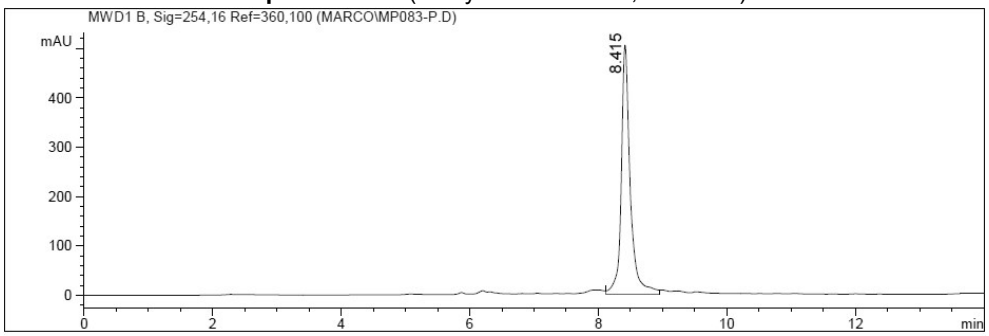
HPLC trace of crude reaction mixture 17a (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.986	0.140	62.787	526.326	32.230
8.516	0.138	133.548	1106.728	67.770

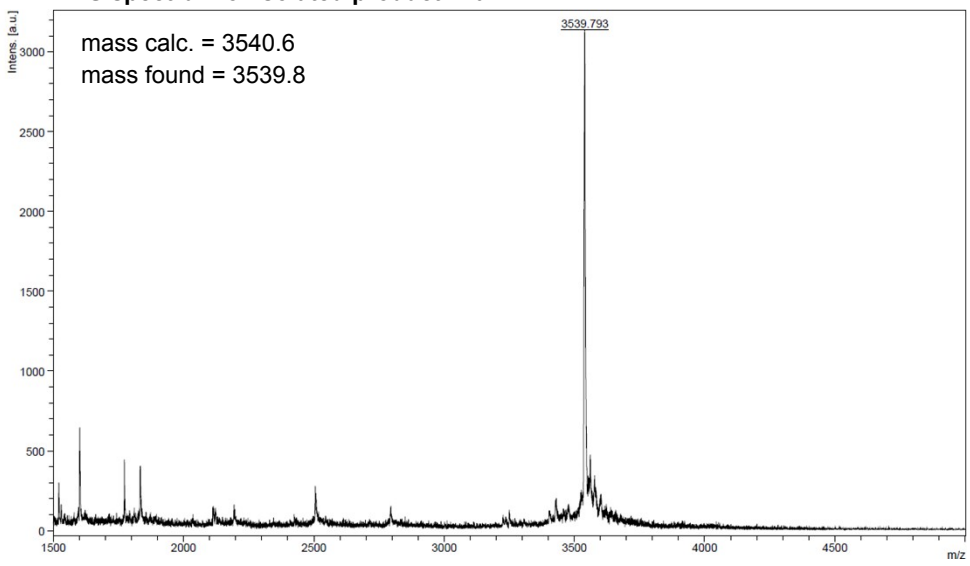
HPLC trace of isolated product 17a (Analytical RP-HPLC, Method-I)



Peak list:

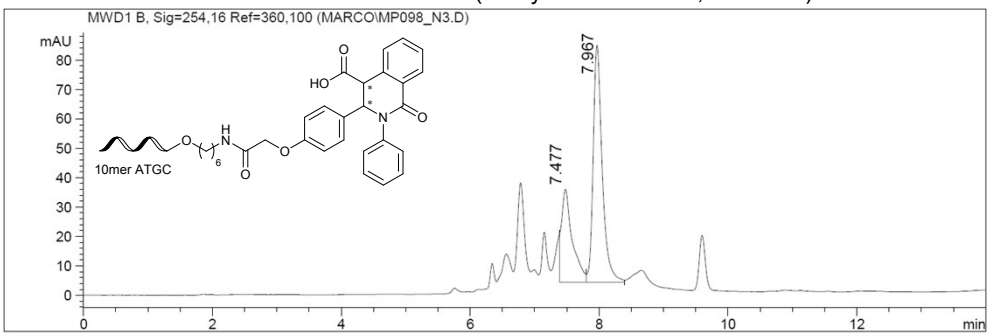
Ret. Time	Width min	Height	Area	Area %
8.415	0.140	506.541	4965.755	100.000

MALDI-MS spectrum of isolated product 17a



DNA conjugate 18a: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with aniline **14a** and homophthalic anhydride **15a** according to RP-03.

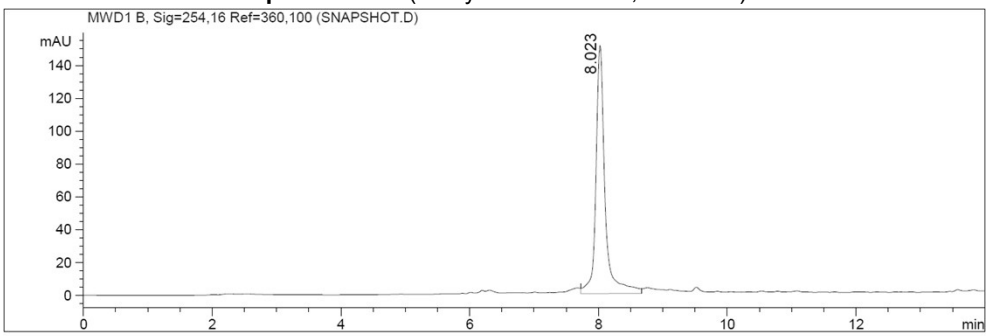
HPLC trace of crude reaction mixture 18a (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.477	0.196	31.684	372.399	32.195
7.967	0.162	80.722	784.286	67.805

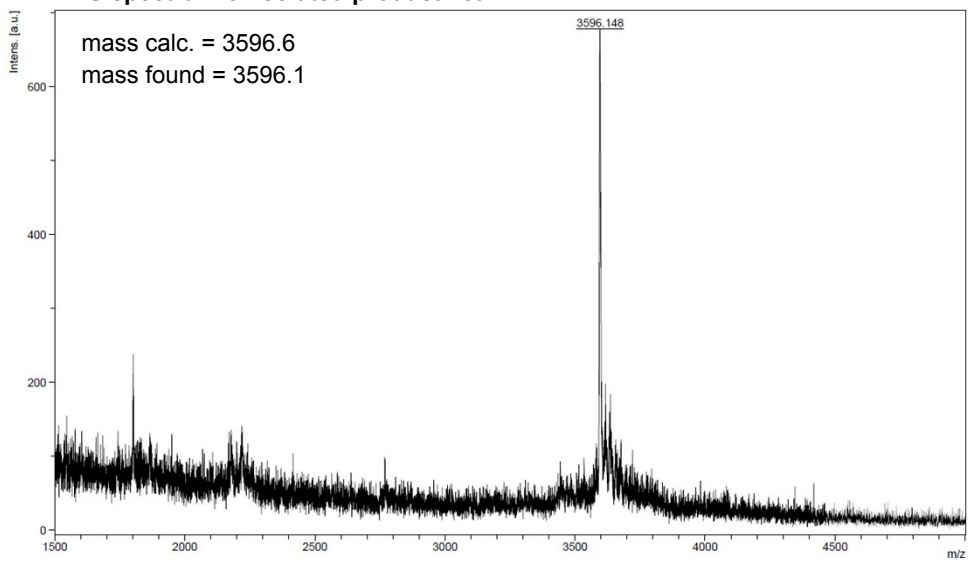
HPLC trace of isolated product 18a (Analytical RP-HPLC, Method-I)



Peak list:

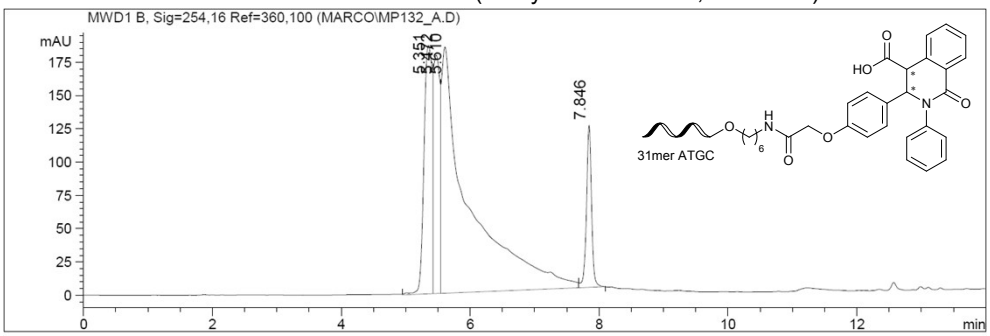
Ret. Time	Width min	Height	Area	Area %
8.023	0.144	151.055	1476.153	100.000

MALDI-MS spectrum of isolated product 18a



DNA conjugate 19a: CPG-bound 31mer ATC-aldehyde conjugate **13d** was reacted with aniline **14a** and homophthalic anhydride **15a** according to RP-03.

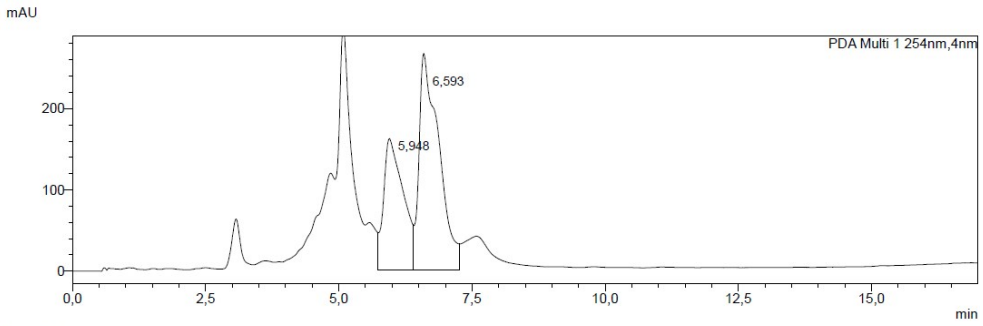
HPLC trace of crude reaction mixture 19a (Analytical RP-HPLC, Method-II)



Peak list:

Ret. Time	Width min	Height	Area	Area %
5.351	0.149	186.595	1665.541	18.264
5.472	0.116	179.091	1242.943	13.630
5.610	0.500	184.914	5543.173	60.787
7.846	0.091	122.275	667.405	7.319

HPLC trace of crude reaction mixture 19a (Preparative RP-HPLC)

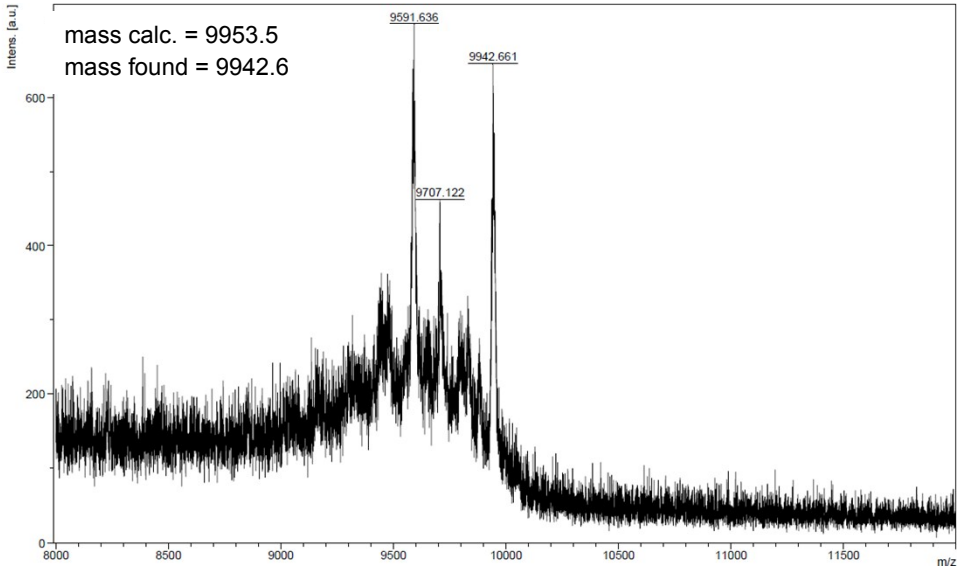


Peak Table

PDA Ch1 254nm

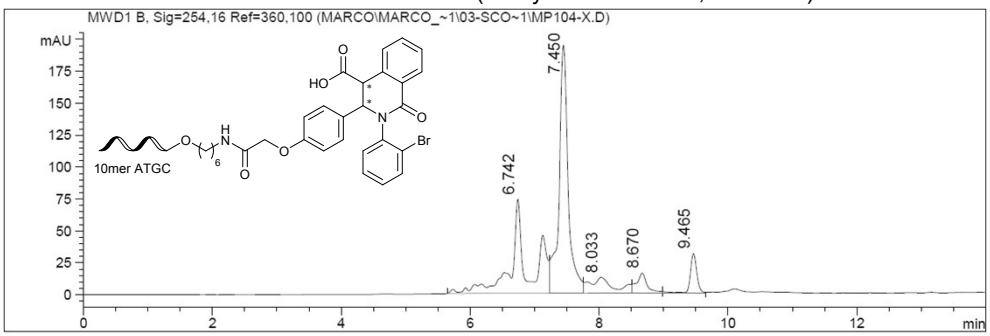
Peak#	Ret. Time	Area	Area%
1	5,948	4140713	37,471
2	6,593	6909770	62,529
Total		11050482	100,000

MALDI-MS of crude reaction mixture 19a



DNA conjugate 18b: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 2-bromoaniline **14b** and homophthalic anhydride **15a** according to RP-03.

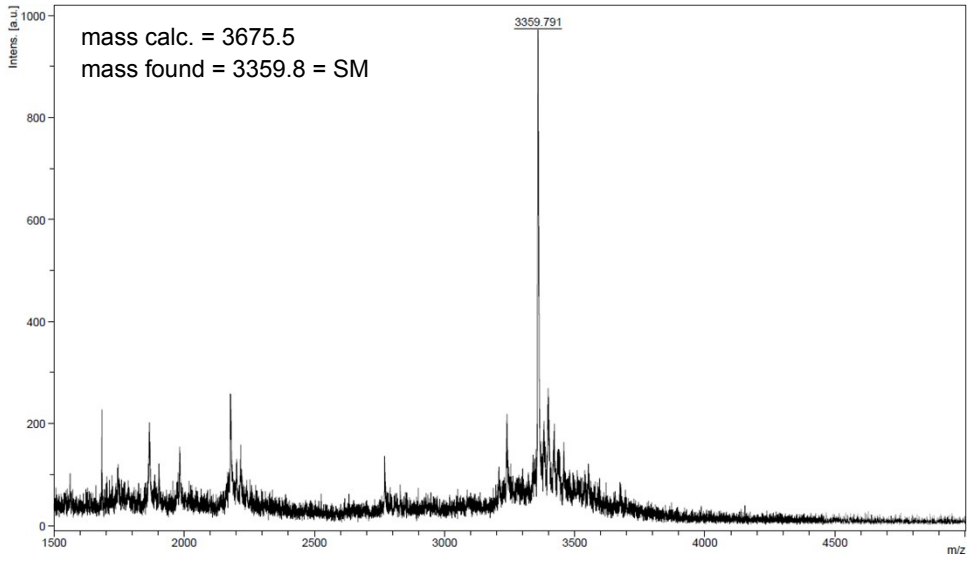
HPLC trace of crude reaction mixture 18b (Analytical RP-HPLC, Method-I)



Peak list:

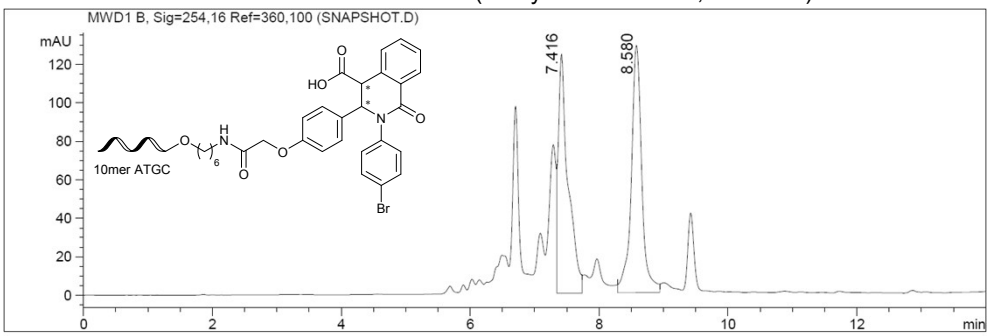
Ret. Time	Width min	Height	Area	Area %
6.742	0.298	73.922	1321.518	33.363
7.450	0.165	194.477	1931.022	48.751
8.033	0.425	12.362	315.422	7.963
8.670	0.189	15.643	177.239	4.475
9.465	0.117	30.828	215.774	5.448

MALDI-MS spectrum of crude reaction mixture 18b



DNA conjugate 18c: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 4-bromoaniline **14c** and homophthalic anhydride **15a** according to RP-03.

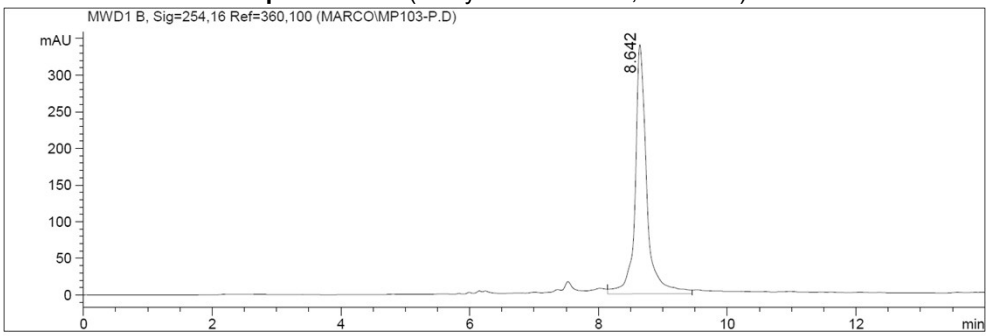
HPLC trace of crude reaction mixture 18c (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.416	0.176	124.158	1311.339	47.587
8.580	0.187	128.492	1444.323	52.413

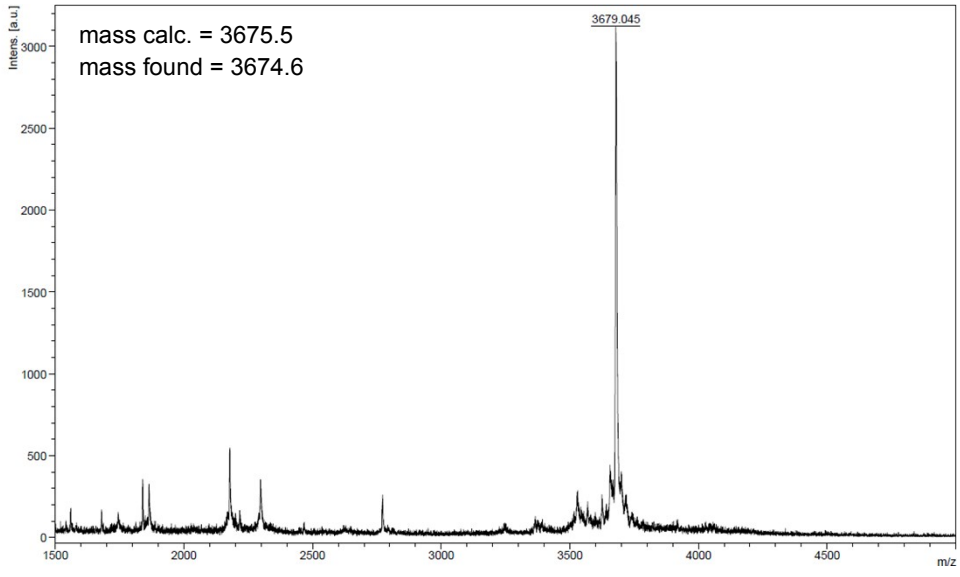
HPLC trace of isolated product 18c (Analytical RP-HPLC, Method-I)



Peak list:

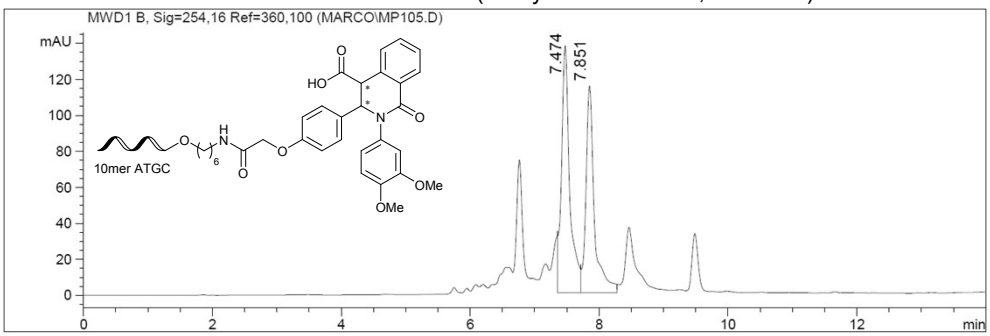
Ret. Time	Width min	Height	Area	Area %
8.642	0.190	340.304	4268.066	100.000

MALDI-MS spectrum of isolated product 18c



DNA conjugate 18d: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 3,4-dimethoxyaniline **14d** and homophthalic anhydride **15a** according to RP-03.

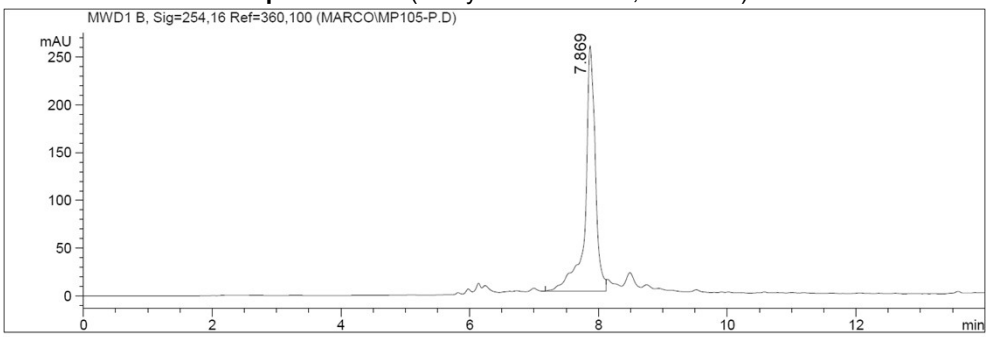
HPLC trace of crude reaction mixture 18d (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.474	0.147	137.451	1213.843	53.931
7.851	0.150	115.115	1036.903	46.069

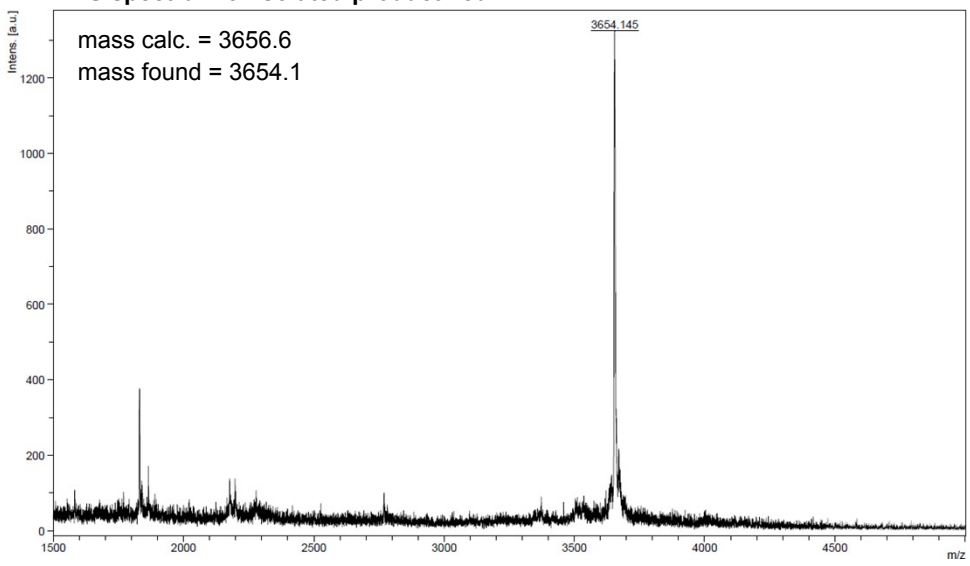
HPLC trace of isolated product 18d (Analytical RP-HPLC, Method-I)



Peak list:

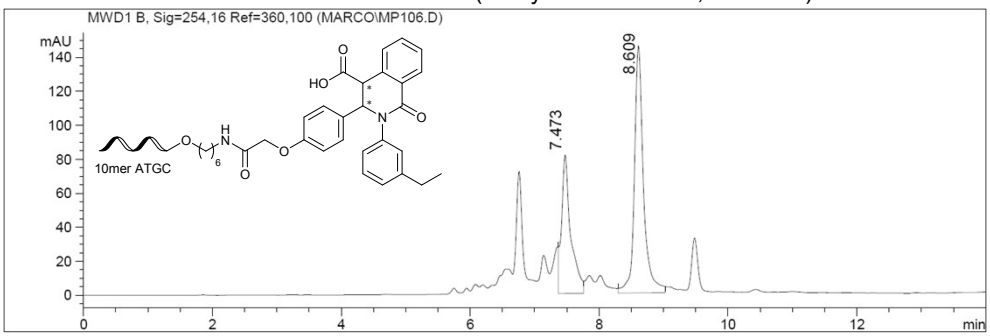
Ret. Time	Width min	Height	Area	Area %
7.869	0.183	257.443	2826.038	100.000

MALDI-MS spectrum of isolated product 18d



DNA conjugate 18e: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 3-ethylaniline **14e** and homophthalic anhydride **15a** according to RP-03.

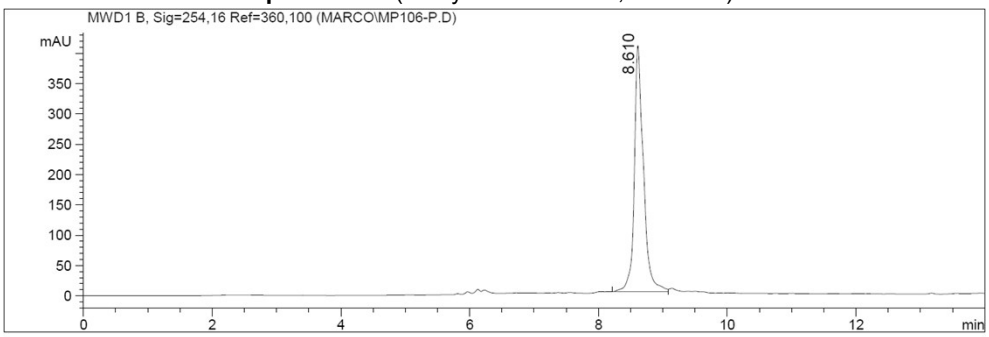
HPLC trace of crude reaction mixture 18e (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.473	0.164	81.625	803.562	35.752
8.609	0.165	145.597	1444.009	64.248

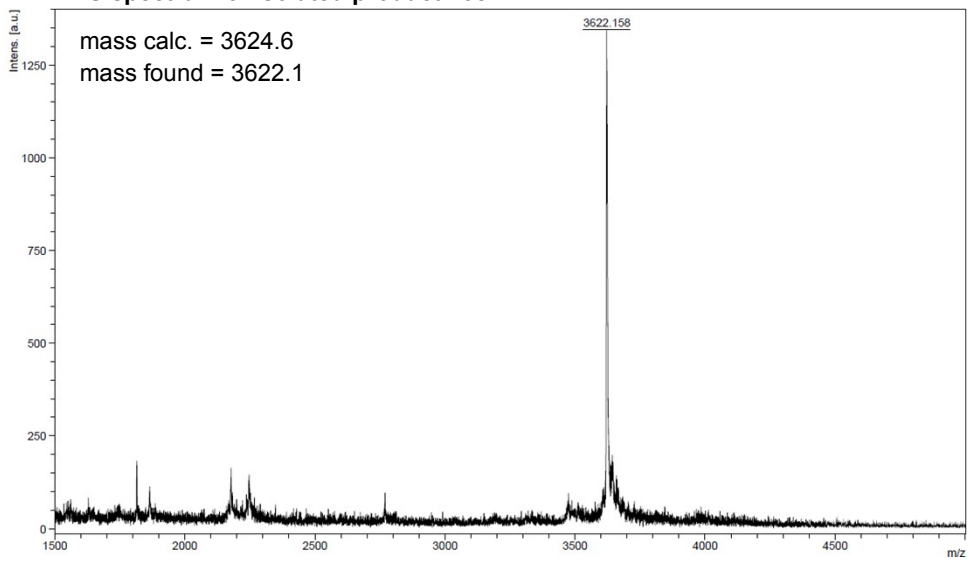
HPLC trace of isolated product 6e (Analytical RP-HPLC, Method-I)



Peak list:

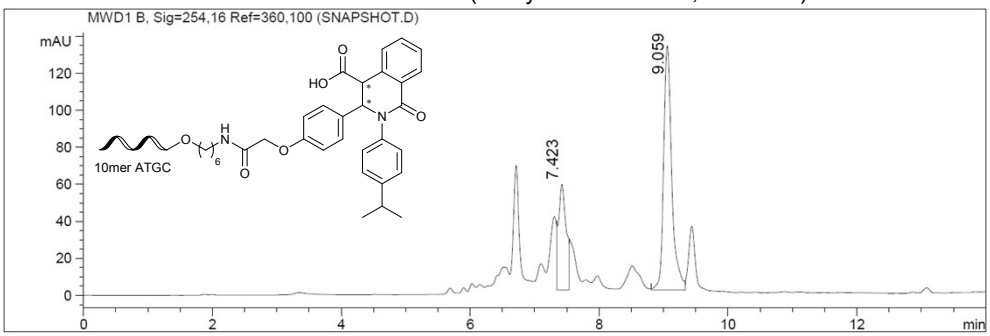
Ret. Time	Width min	Height	Area	Area %
8.610	0.164	407.221	4013.044	100.000

MALDI-MS spectrum of isolated product 18e



DNA conjugate 18f: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 4-isopropylaniline **14f** and homophthalic anhydride **15a** according to RP-03.

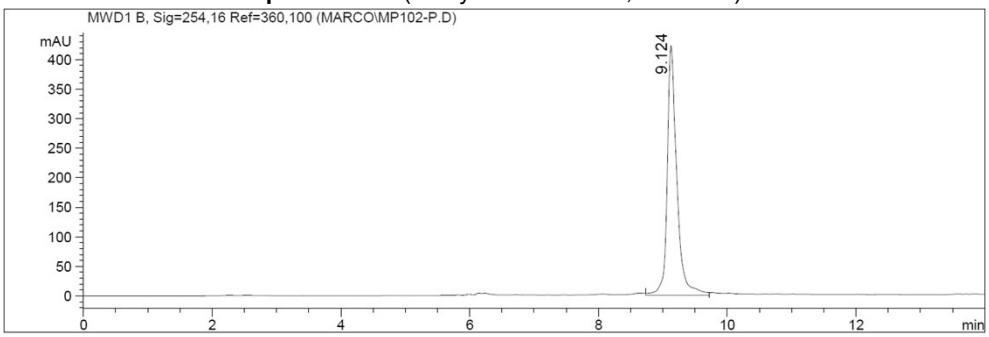
HPLC trace of crude reaction mixture 18f (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.423	0.133	56.845	454.404	28.199
9.059	0.146	131.883	1157.017	71.801

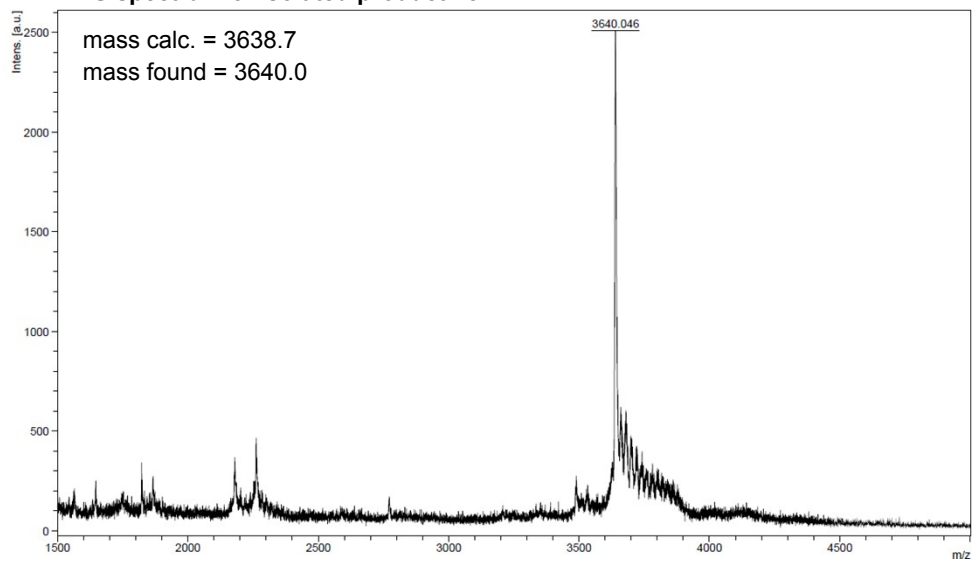
HPLC trace of isolated product 18f (Analytical RP-HPLC, Method-I)



Peak list:

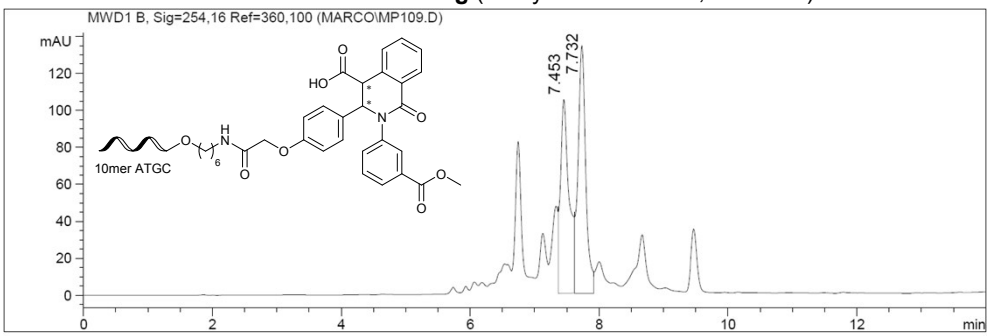
Ret. Time	Width min	Height	Area	Area %
9.124	0.148	422.379	4413.368	100.000

MALDI-MS spectrum of isolated product 18f



DNA conjugate 18g: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with methyl 3-aminobenzoate **14g** and homophthalic anhydride **15a** according to RP-03.

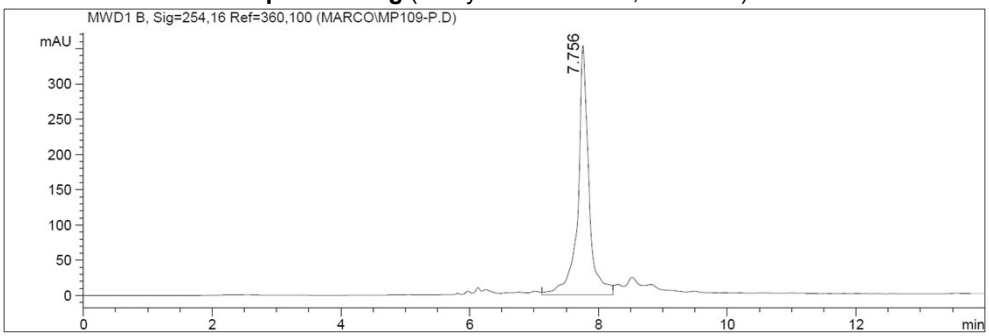
HPLC trace of crude reaction mixture 18g (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.453	0.153	104.916	962.440	46.147
7.732	0.140	133.932	1123.134	53.853

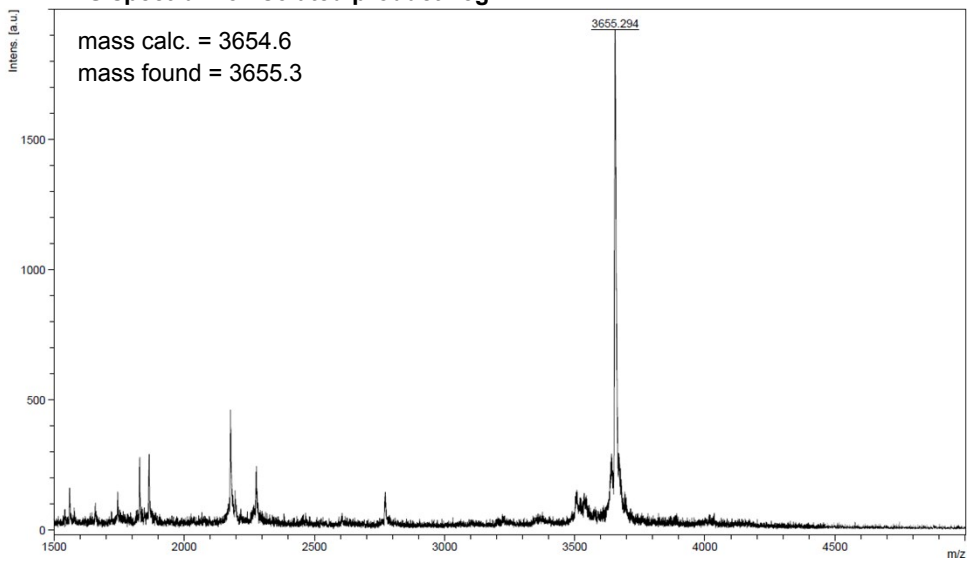
HPLC trace of isolated product 18g (Analytical RP-HPLC, Method-I)



Peak list:

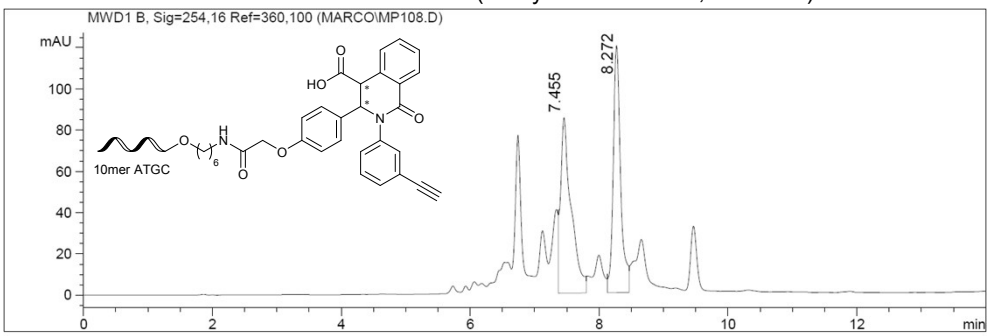
Ret. Time	Width min	Height	Area	Area %
7.756	0.160	353.485	4181.745	100.000

MALDI-MS spectrum of isolated product 18g



DNA conjugate 18h: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 3-ethynylaniline **14h** and homophthalic anhydride **15a** according to RP-03.

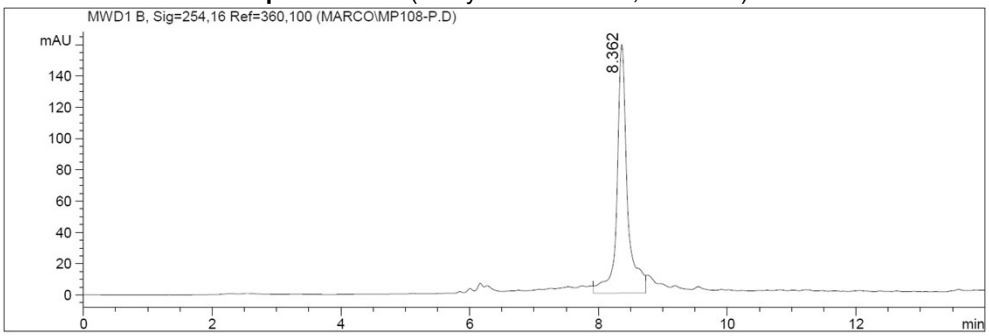
HPLC trace of crude reaction mixture 18h (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.455	0.192	85.166	978.952	50.342
8.272	0.134	119.972	965.663	49.658

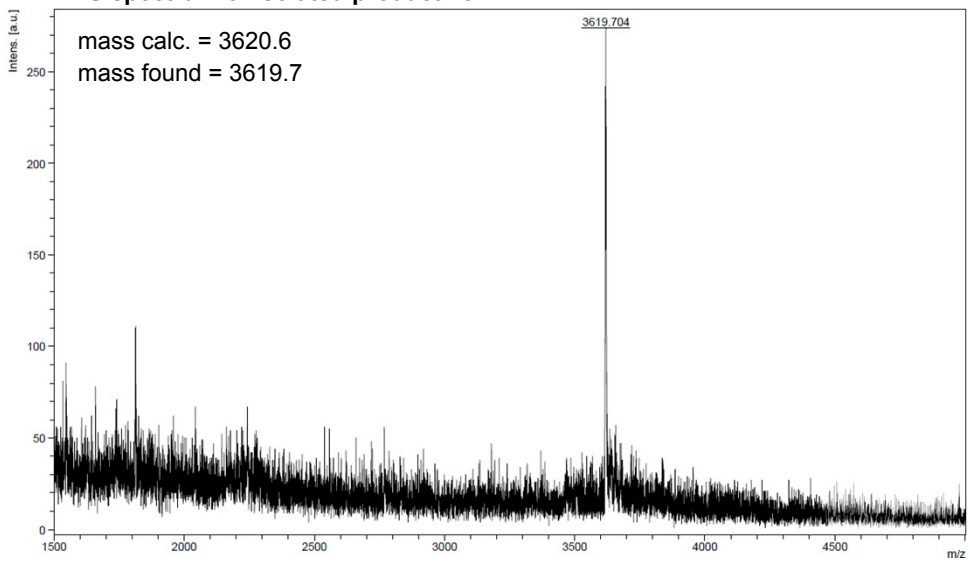
HPLC trace of isolated product 18h (Analytical RP-HPLC, Method-I)



Peak list:

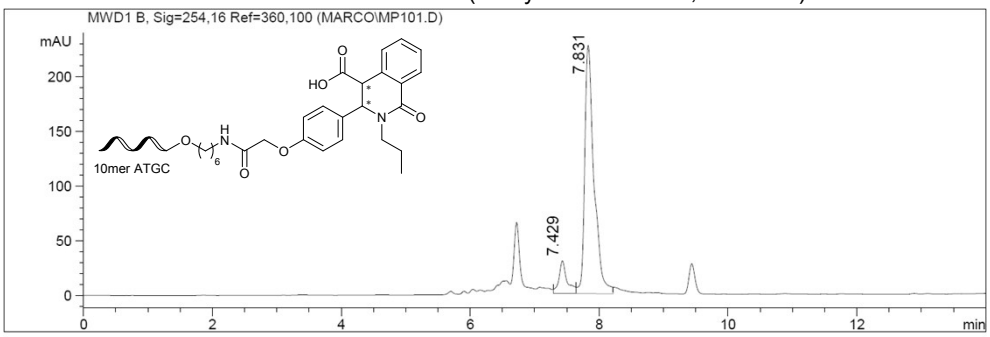
Ret. Time	Width min	Height	Area	Area %
8.362	0.163	158.998	1779.070	100.000

MALDI-MS spectrum of isolated product 18h



DNA conjugate 18i: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with propylamine **14i** and homophthalic anhydride **15a** according to RP-03.

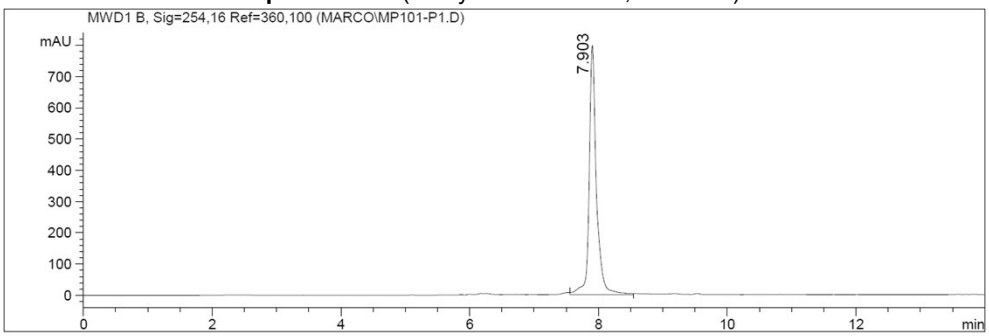
HPLC trace of crude reaction mixture 18i (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.429	0.147	29.976	263.737	10.437
7.831	0.166	227.281	2263.170	89.563

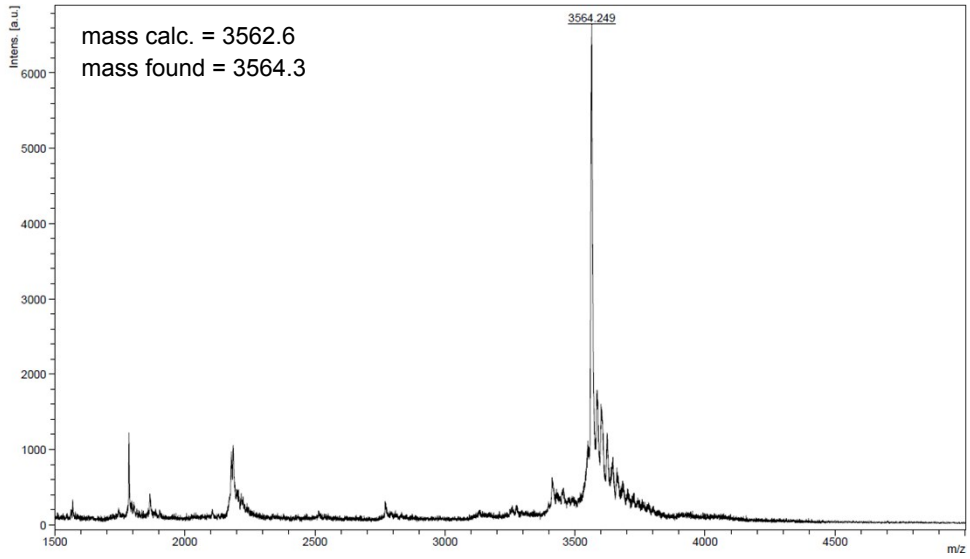
HPLC trace of isolated product 18i (Analytical RP-HPLC, Method-I)



Peak list:

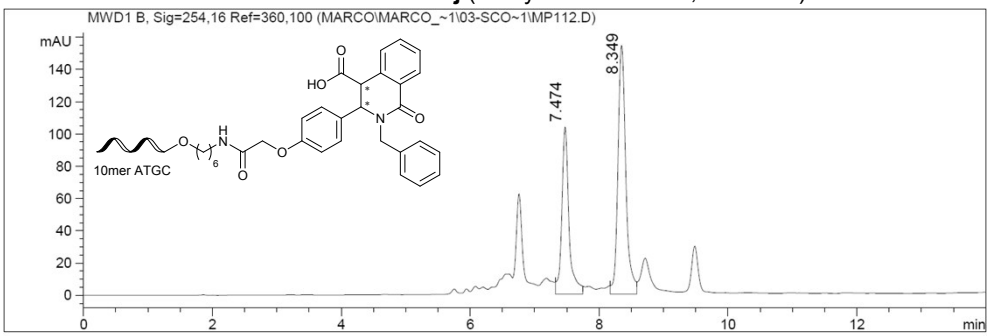
Ret. Time	Width min	Height	Area	Area %
7.903	0.114	800.325	6296.851	100.000

MALDI-MS spectrum of isolated product 18i



DNA conjugate 18j: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with benzylamine **14j** and homophthalic anhydride **15a** according to RP-03.

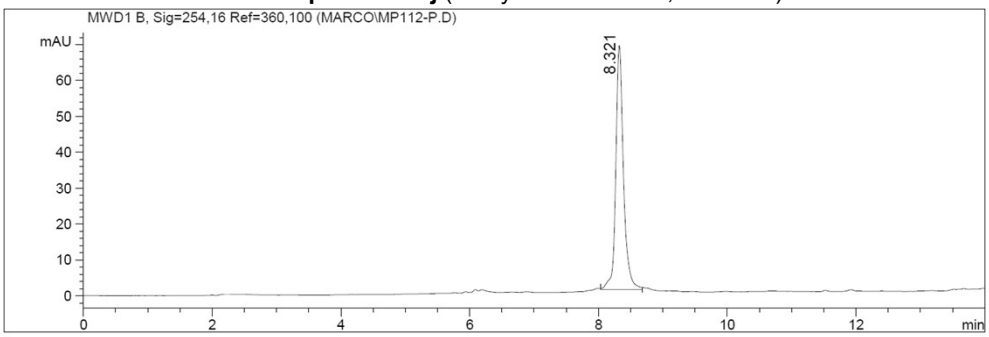
HPLC trace of crude reaction mixture 18j (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.474	0.129	103.798	802.549	38.078
8.349	0.141	154.408	1305.097	61.922

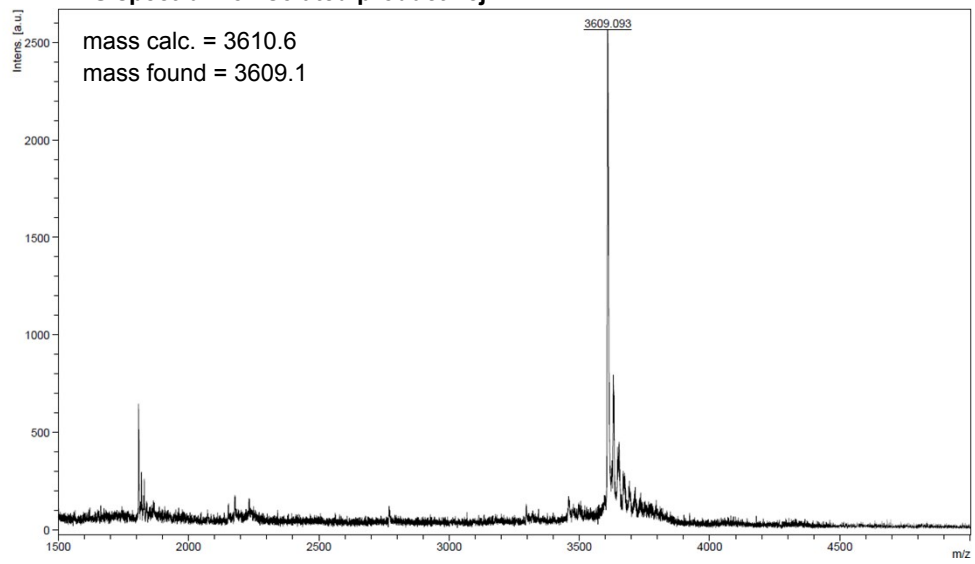
HPLC trace of the isolated product 18j (Analytical RP-HPLC, Method-I)



Peak list:

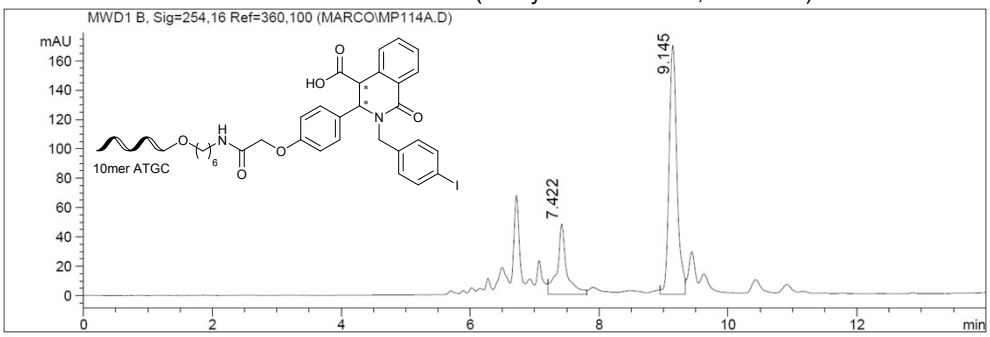
Ret. Time	Width min	Height	Area	Area %
8.321	0.139	68.131	566.380	100.000

MALDI-MS spectrum of isolated product 18j



DNA conjugate 18k: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 4-iodobenzylamine **14k** and homophthalic anhydride **15a** according to RP-03.

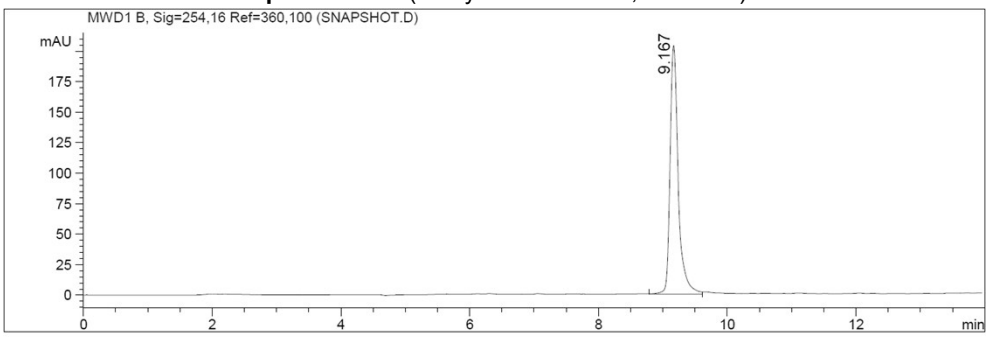
HPLC trace of crude reaction mixture 18k (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.422	0.169	48.195	489.194	25.385
9.145	0.141	169.850	1437.921	74.615

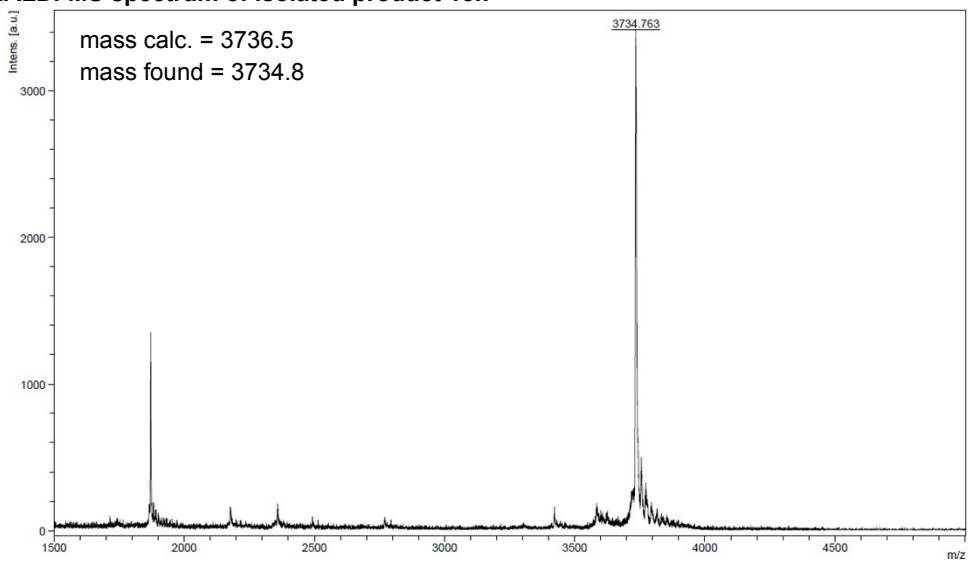
HPLC trace of isolated product 18k (Analytical RP-HPLC, Method-I)



Peak list:

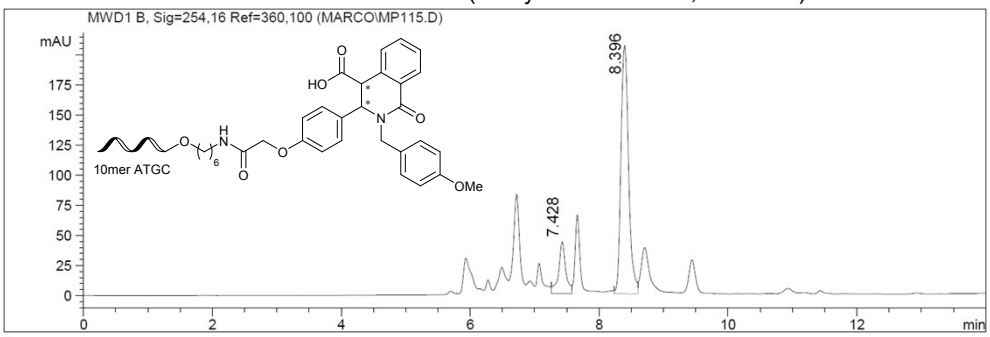
Ret. Time	Width min	Height	Area	Area %
9.167	0.136	204.183	1819.655	100.000

MALDI-MS spectrum of isolated product 18k



DNA conjugate 18l: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with 4-methoxybenzylamine **14l** and homophthalic anhydride **15a** according to RP-03.

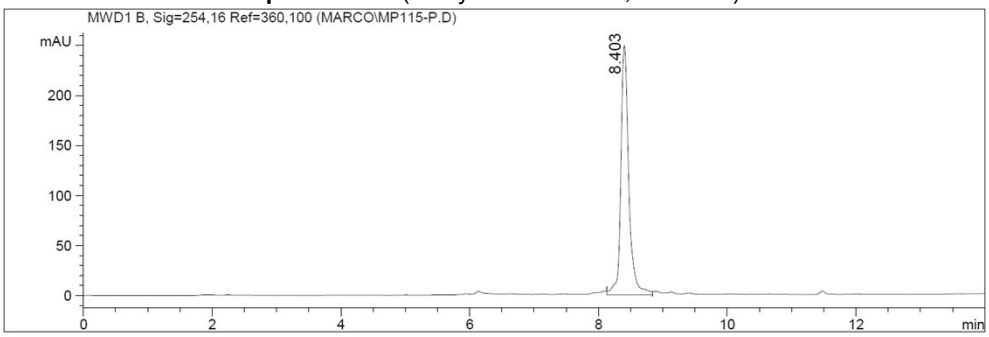
HPLC trace of crude reaction mixture 18l (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.428	0.129	43.345	334.211	16.405
8.396	0.137	206.483	1703.029	83.595

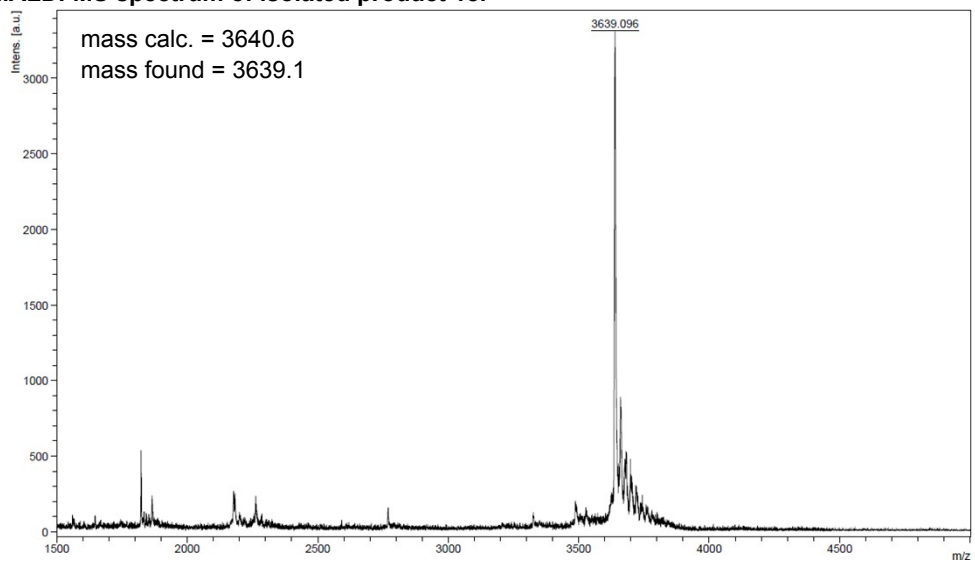
HPLC trace of isolated product 18l (Analytical RP-HPLC, Method-I)



Peak list:

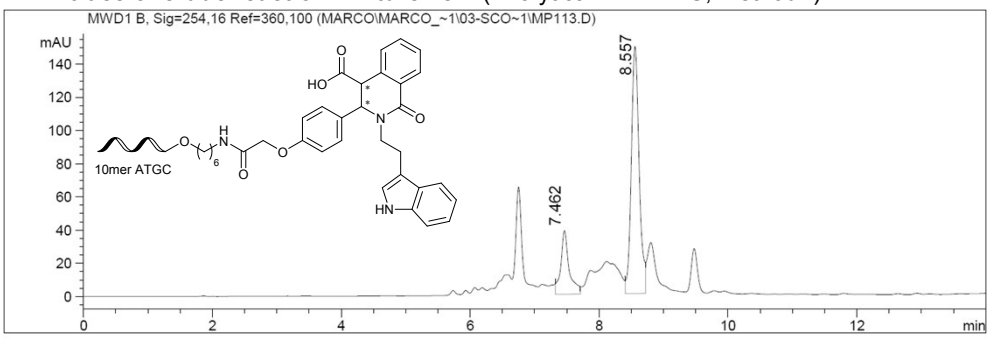
Ret. Time	Width min	Height	Area	Area %
8.403	0.128	249.320	2128.528	100.000

MALDI-MS spectrum of isolated product 18l



DNA conjugate 18m: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with tryptamine **14m** and homophthalic anhydride **15a** according to RP-03.

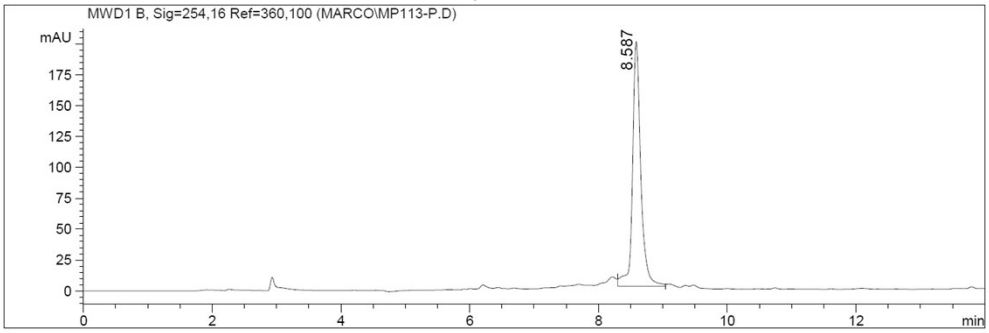
HPLC trace of crude reaction mixture 18m (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.462	0.150	38.387	345.767	21.599
8.557	0.140	149.195	1255.070	78.401

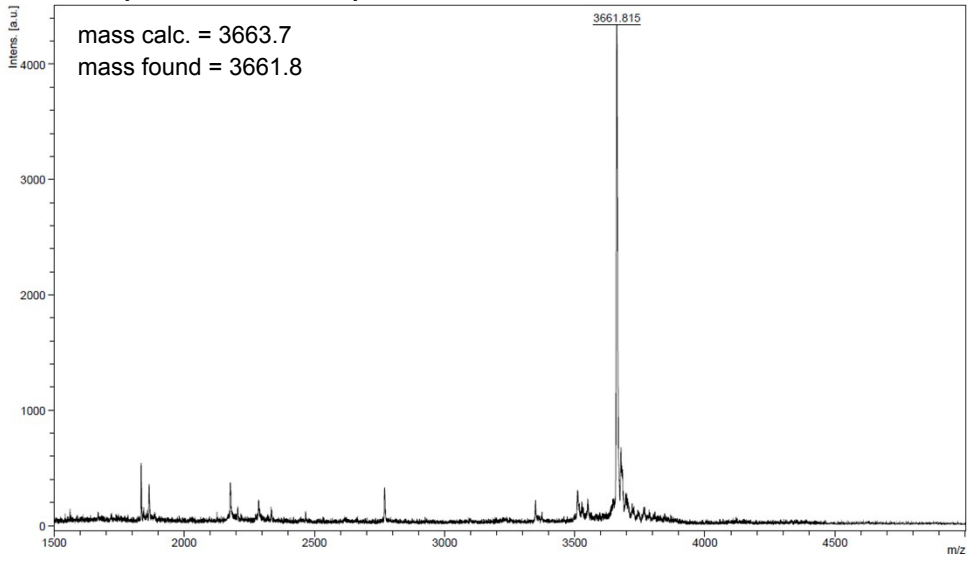
HPLC trace of isolated product 18m (Analytical RP-HPLC, Method-I)



Peak list:

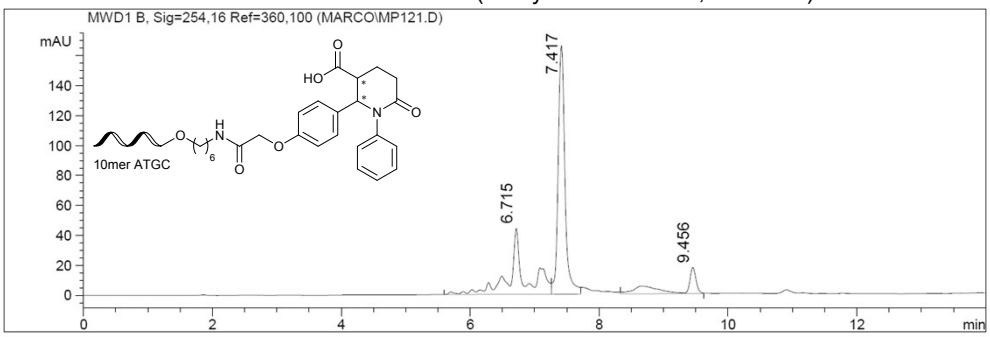
Ret. Time	Width min	Height	Area	Area %
8.587	0.148	198.422	1759.228	100.000

MALDI-MS spectrum of isolated product 18m



DNA conjugate 18n: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with propylamine **14i** and glutaric anhydride **15b** according to RP-03.

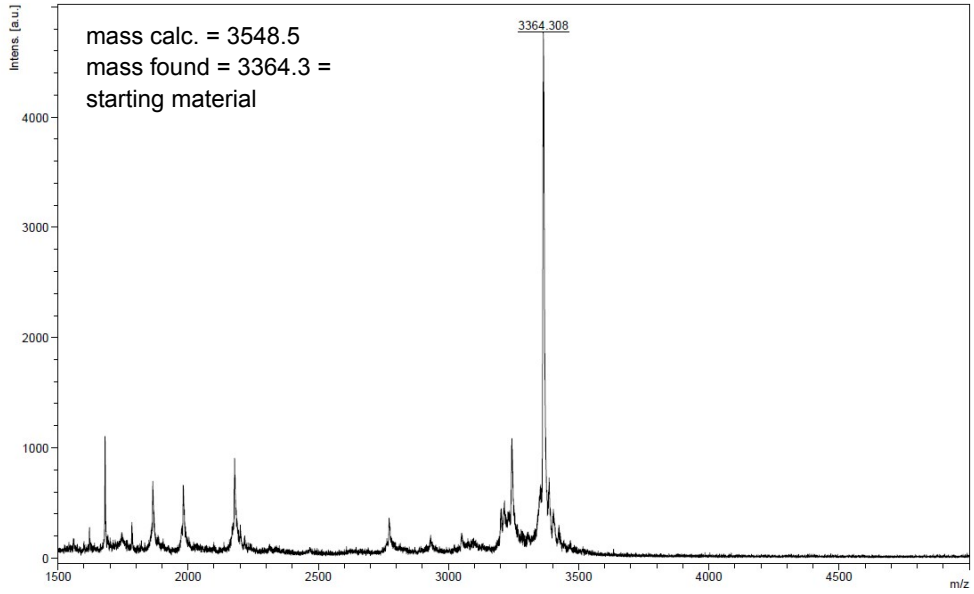
HPLC trace of crude reaction mixture 18n (Analytical RP-HPLC, Method-I)



Peak list:

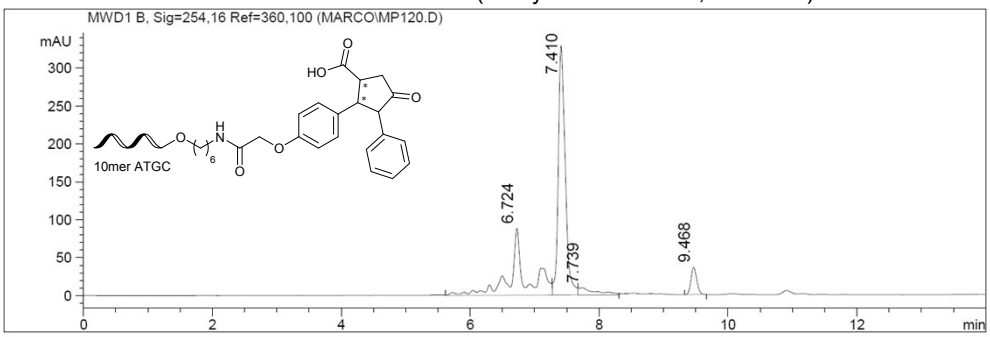
Ret. Time	Width min	Height	Area	Area %
6.715	0.276	44.096	729.753	33.003
7.417	0.122	166.128	1213.558	54.882
9.456	0.253	17.632	267.883	12.115

MALDI-MS spectrum of crude reaction mixture 18n



DNA conjugate 18n: CPG-bound 10mer ATGC-aldehyde conjugate **13c** was reacted with propylamine **14i** and succini anhydride **15c** according to RP-03.

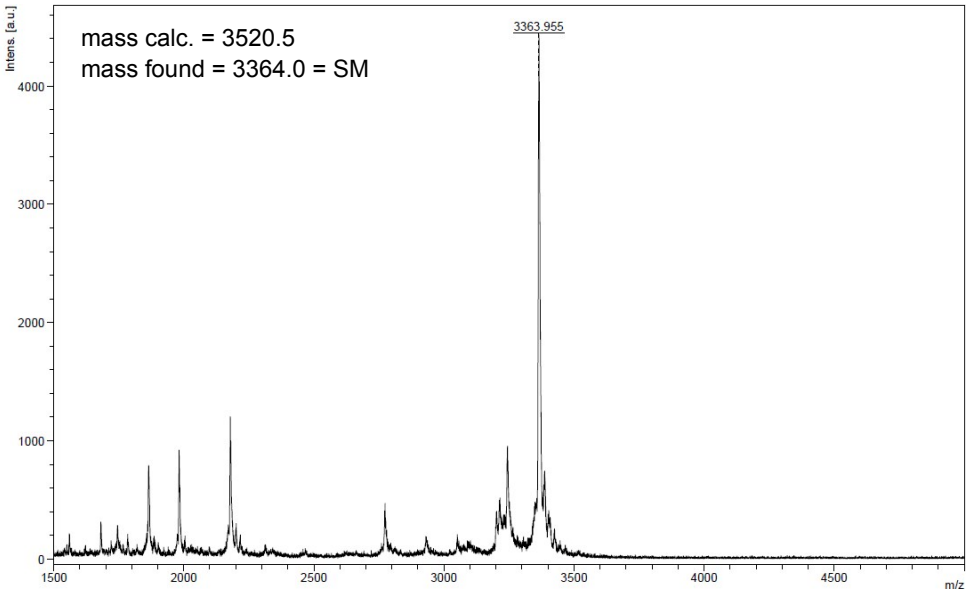
HPLC trace of crude reaction mixture 18o (Analytical RP-HPLC, Method-I)



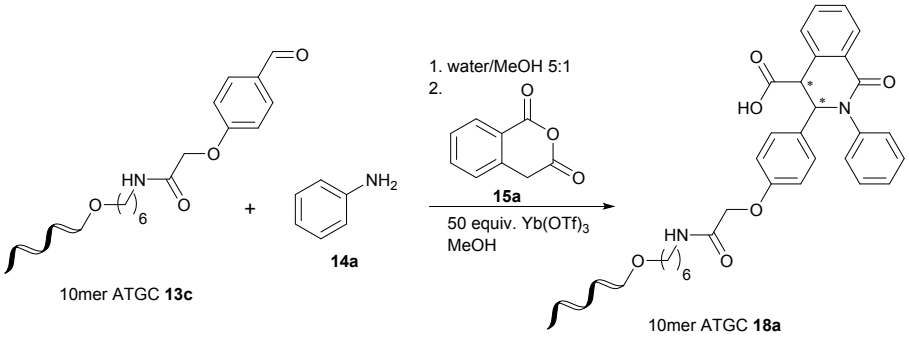
Peak list:

Ret. Time	Width min	Height	Area	Area %
6.724	0.277	88.331	1465.854	33.998
7.410	0.124	329.489	2444.827	56.704
7.739	0.312	9.436	176.636	4.097
9.468	0.104	35.974	224.242	5.201

MALDI-MS spectrum of crude reaction mixture 18o



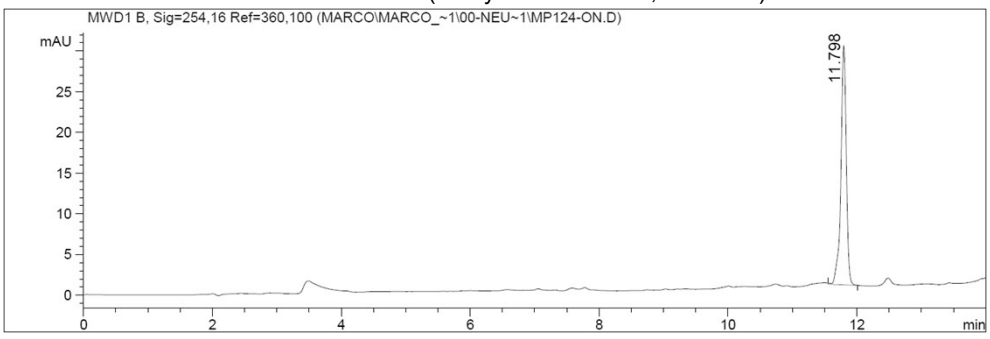
Castagnoli-Cushman reaction on a DNA-aldehyde conjugate in solution



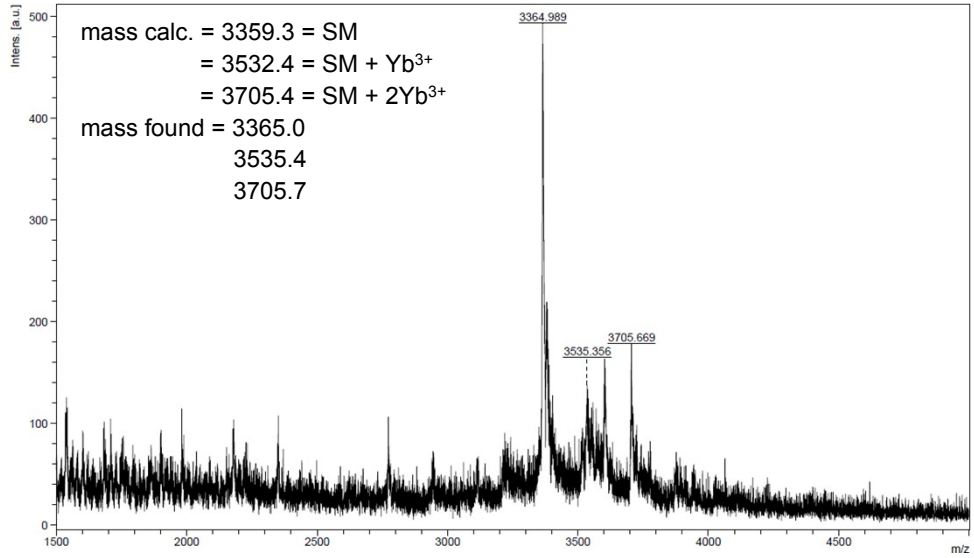
Scheme S1 $Yb(OTf)_3$-mediated Castagnoli-Cushman reaction on oligonucleotide in solution.

Oligonucleotide-aldehyde conjugate **13** (500 pmol) was dissolved in a final volume of 25 µL of water. To this solution amine **14** (250 nmol, 500 equiv.) pre-dissolved in 5 µL of methanol was added. The solution was shaken at ambient temperature for 4 h. Afterwards 5 µL of $Yb(OTf)_3$ (25 nmol, 50 equiv.) dissolved in methanol and 15 µL of anhydride **15a** (250 nmol, 500 equiv.) dissolved in methanol were added and the solution was shaken for 1 h at ambient temperature. Then the reaction mixture was diluted with 75 µL of water and extracted 6x with 200 µL ethyl acetate. The aqueous phase was dried in SpeedVac and the residue was dissolved in 40 µL of distilled water. Ethanol precipitation was performed by adding four volumes of ethanol and incubating this solution at -80 °C overnight. The DNA conjugate was centrifuged to obtain a pellet, the supernatant was taken off, the DNA was dried in a SpeedVac, re-dissolved in 100 µL distilled water and analyzed by analytical RP-HPLC (Method I) and MALDI-MS.

HPLC trace of crude reaction mixture (Analytical RP-HPLC, Method-I)

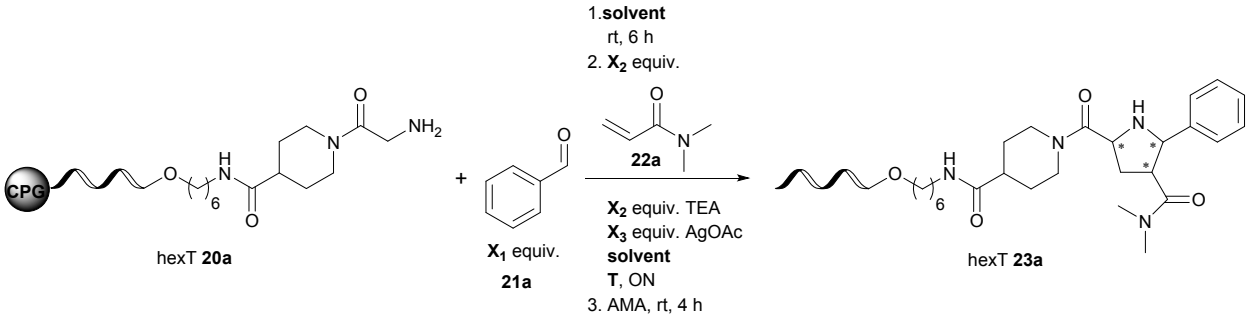


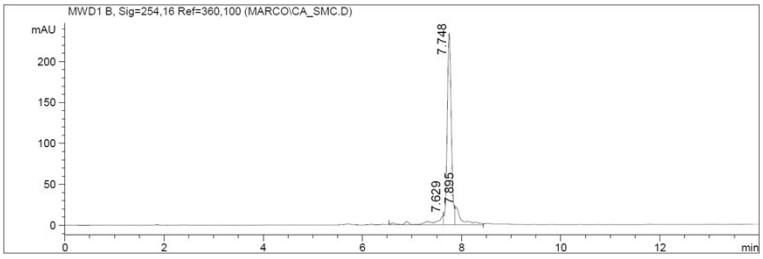
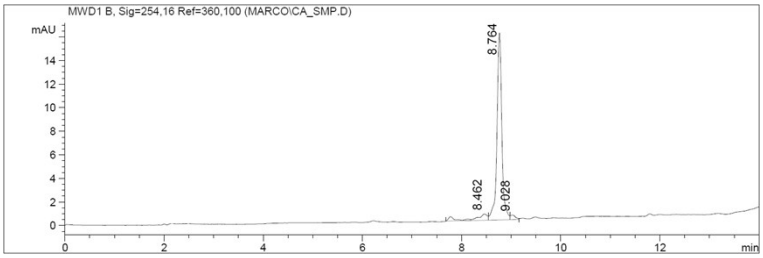
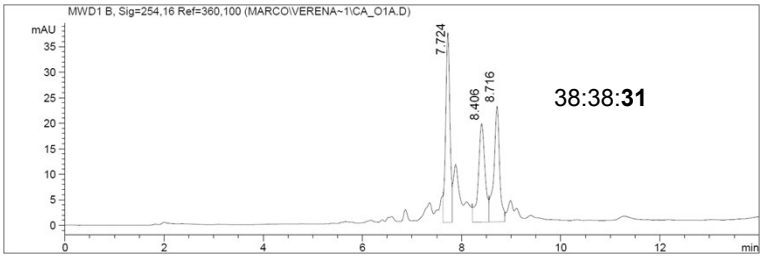
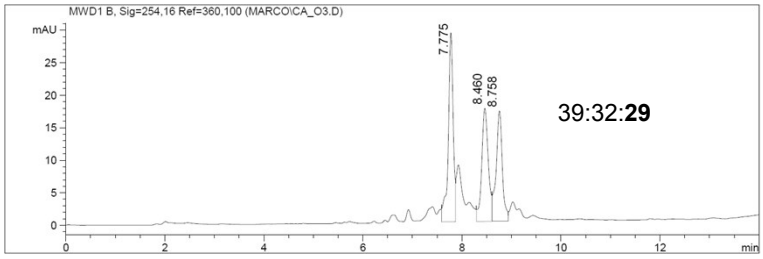
MALDI-MS spectrum of crude reaction mixture



AgOAc-mediated 1,3-dipolar cycloaddition on CPG-bound DNA oligonucleotides

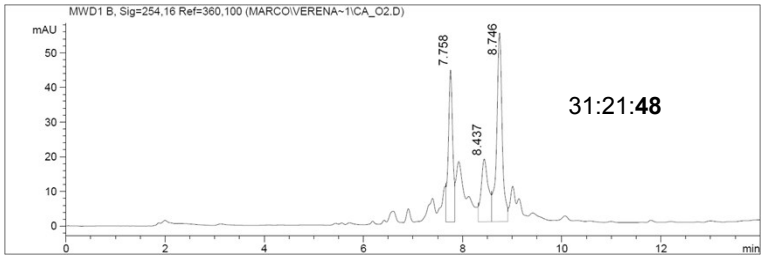
Table S3 Optimization of AgOAc-mediated 1,3-dipolar cycloaddition on CPG-bound hexT.[a]



Entry	Reaction conditions[b]	HPLC trace of crude reaction mixture[c]
1	hexT-glycine conjugate **20a**	
2	hexT-pyrrolidine conjugate **23a**	
3	1. 1000 equiv. **21a**, ACN, 6 h, rt 2. 1000 equiv. **22a** 1000 equiv. TEA, 100 equiv. AgOAc ACN, rt, ON => conversion 31 %	
4	1. 1000 equiv. **21a**, ACN, 6 h, rt 2. 1000 equiv. **22a**, 1000 equiv. TEA, ***200 equiv.*** AgOAc ACN, rt, ON => conversion 29 %	

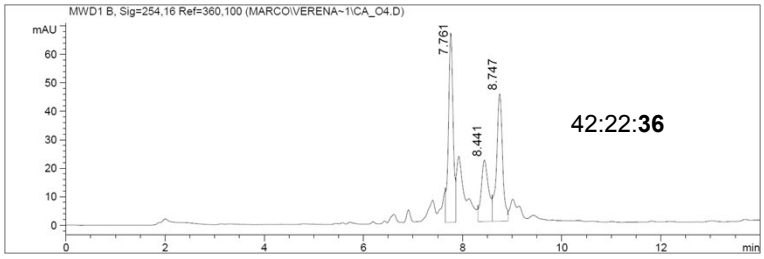
5 1. 1000 equiv. **21a**, ACN, 6 h, rt
2. 1000 equiv. **22a**
1000 equiv. TEA,
100 equiv. AgOAc
ACN, **40 °C**, ON

=> conversion 48 %



31:21:**48**

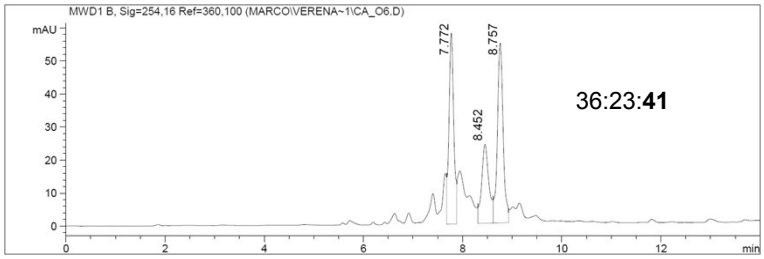
6 1. 1000 equiv. **21a**, ACN, 6 h, rt
2. 1000 equiv. **22a**
1000 equiv. TEA,
200 equiv. AgOAc
ACN, ***40 °C***, ON
=> conversion 36 %



42:22:**36**

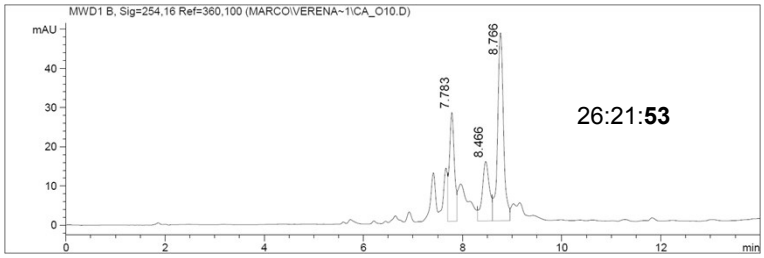
7 1. ***2000 equiv.*** **21a**, ACN, 6 h, rt
2. ***2000 equiv. 22a***
2000 equiv. TEA,
100 equiv. AgOAc
ACN, 40 °C, ON

=> conversion 41 %



36:23:**41**

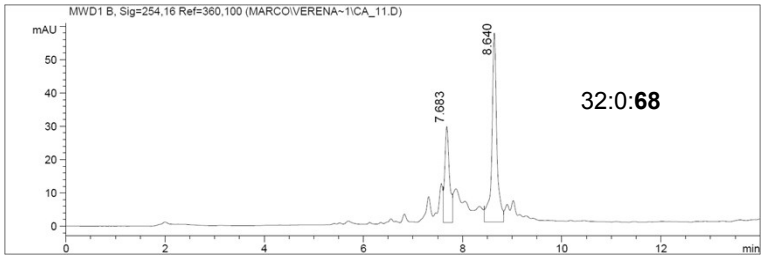
8 1. ***4000 equiv.*** **21a**, ACN, 6 h, rt
2. ***4000 equiv. 22a***
4000 equiv. TEA,
100 equiv. AgOAc
ACN, 40 °C, ON
=> conversion 53 %



26:21:**53**

9 1. 1000 equiv. **21a**, ACN, 6 h, rt
2. ***3000 equiv. 22a***
3000 equiv. TEA,
100 equiv. AgOAc
ACN, ***50 °C***, ON

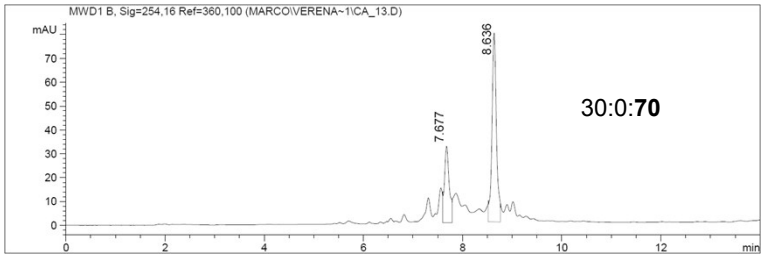
=> conversion 68 %



32:0:**68**

10 1. 1000 equiv. **21a**, ACN, 6 h, rt
2. ***4000 equiv. 22a***
4000 equiv. TEA,
100 equiv. AgOAc
ACN, ***50 °C***, ON

=> conversion 70 %

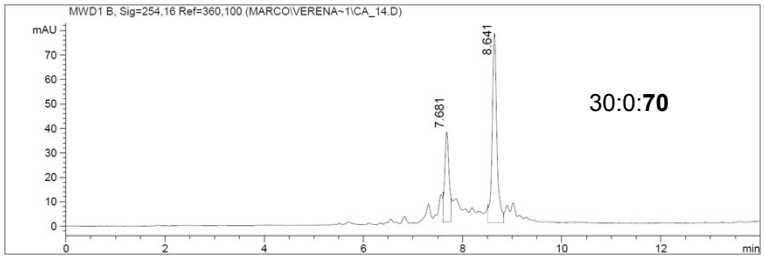


30:0:**70**

11

1. 1000 equiv. **21a**, ACN/TEOF (2:1), 6 h, rt
2. 4000 equiv. **22a**
   4000 equiv. TEA,
   100 equiv. AgOAc
   ACN/TEOF (2:1), 50 °C, ON

   => conversion 70 %

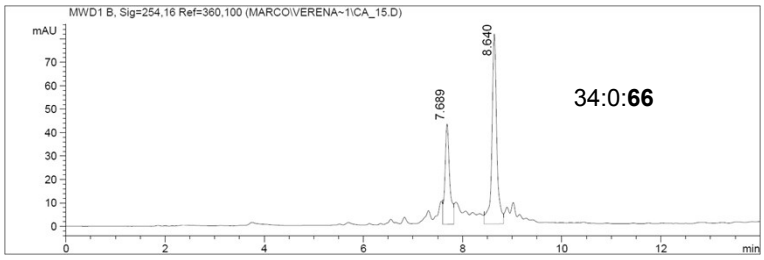


30:0:**70**

12

1. 1000 equiv. **21a**, ACN/TEOF (2:1), 6 h, rt
2. 4000 equiv. **22a**
   4000 equiv. TEA,
   100 equiv. ***AgTFA***
   ACN/TEOF (2:1), 50 °C, ON

   => conversion 66 %

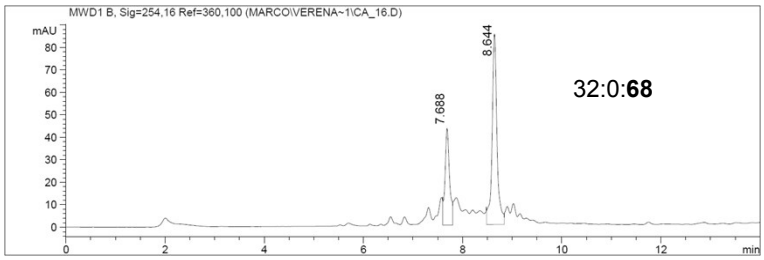


34:0:**66**

13

1. 1000 equiv. **21a**, ACN/TEOF (2:1), 6 h, rt
2. 4000 equiv. **22a**
   4000 equiv. TEA,
   100 equiv. ***$AgSbF_6$***
   ACN/TEOF (2:1), 50 °C, ON

   => conversion 68 %

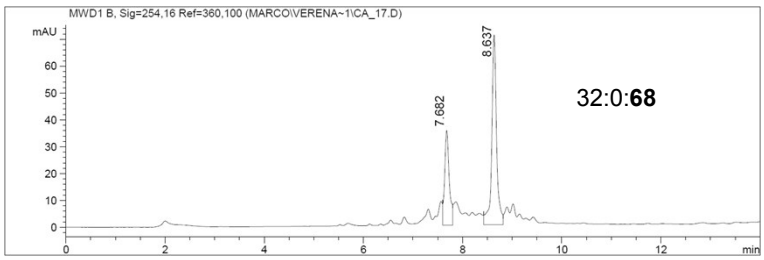


32:0:**68**

14

1. 1000 equiv. **21a**, ACN/TEOF (2:1), 6 h, rt
2. 4000 equiv. **22a**
   4000 equiv. TEA,
   100 equiv. ***AgOTf***
   ACN/TEOF (2:1), 50 °C, ON

   => conversion 68 %

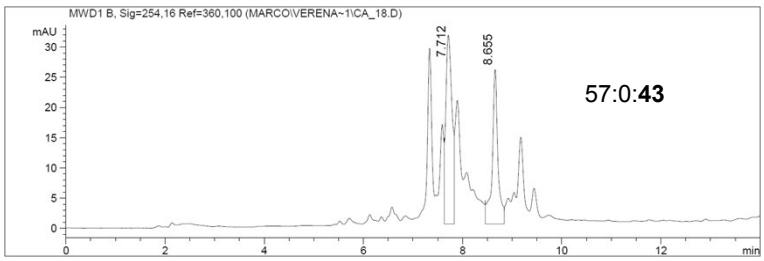


32:0:**68**

15

1. 1000 equiv. **21a**, ***MeOH***/TEOF (2:1), 6 h, rt
2. 4000 equiv. **22a**
   4000 equiv. TEA,
   100 equiv. AgOAc
   MeOH/TEOF (2:1), 50 °C, ON

=> conversion 43 %

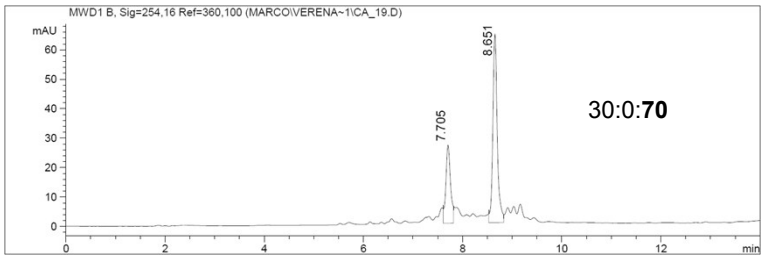


57:0:**43**

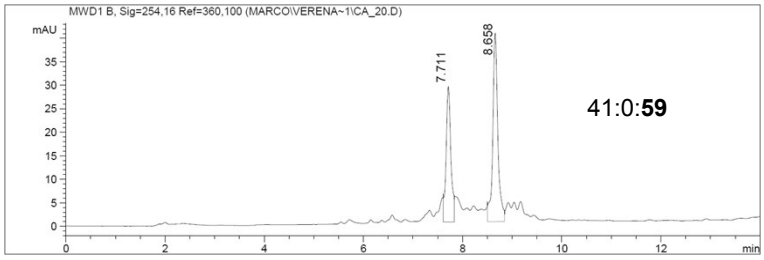
16

1. 1000 equiv. **21a**, ***THF***/TEOF (2:1), 6 h, rt
2. 4000 equiv. **22a**
   4000 equiv. TEA,
   100 equiv. AgOAc
   THF/TEOF (2:1), 50 °C, ON

   => conversion 70 %

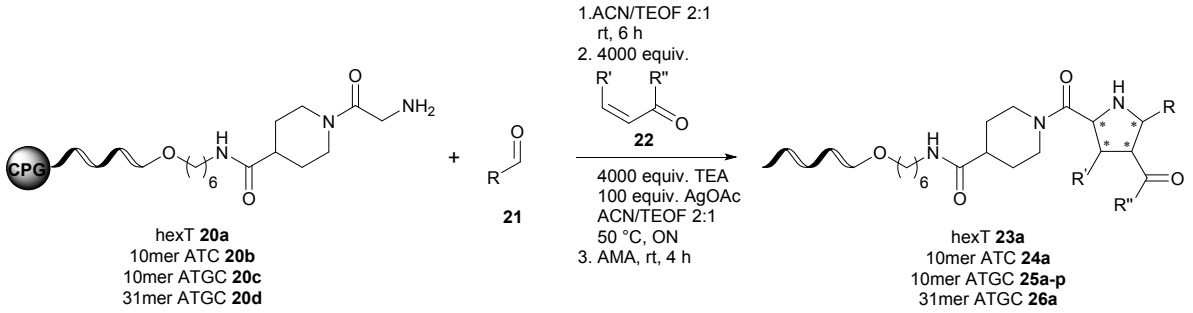


30:0:**70**

17	1. 1000 equiv. **21a**, ***toluene***/TEOF (2:1), 6 h, rt 2. 4000 equiv. **22a** 4000 equiv. TEA, 100 equiv. AgOAc ***toluene***/TEOF (2:1), 50 °C, ON => conversion 59 %		41:0:**59**

[a] Reaction of CPG-bound hexT-conjugate **20a** (20 nmol) and aldehyde **21a** (X equiv.) in 50 µL solvent at ambient temperature for 6 h, then heterocycle formation with AgOAc (X equiv.), dissolved in 30 µL solvent, triethylamine (X equiv.) and *N*,*N*-dimethylacrylamide **22a** (X equiv.), at T for overnight. [b] parameters that were changed are in bold and italic. [c] Analytical RP-HPLC, Method-I. TEOF = triethyl orthoformate.

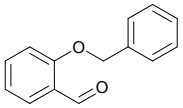
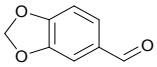
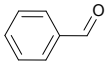
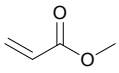
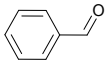
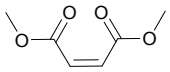
Table S4 – Scope of AgOAc-mediated 1,3-dipolar cycloaddition on CPG-bound oligonucleotides.[a]



hexT **20a**
10mer ATC **20b**
10mer ATGC **20c**
31mer ATGC **20d**

hexT **23a**
10mer ATC **24a**
10mer ATGC **25a-p**
31mer ATGC **26a**

Entry	Product	Aldehyde	Dipolarophile	Conversion [%][b]	Yield [nmol][c]	$Mass_{calc.}$ $Mass_{found}$[d]
1	**23a**	**21a**	**22a**	70	6.1	2297.7 2301.1
2	**24a**	**21a**	**22a**	50	3.4	3496.6 3499.3
3	**25a**	**21a**	**22a**	50	3.0	3552.6 3553.3
4	**26a**	**21a**	**22a**	45	---	9909.6 9908.6
5	**25b**	**21b**	**22a**	48	1.3	3631.5 3633.3
6	**25c**	**21c**	**22a**	41	1.9	3587.1 3589.8
7	**25d**	**21d**	**22a**	47	3.1	3570.6 3572.6
8	**25e**	**21e**	**22a**	24	1.1	3582.6 3583.7
9	**25f**	**21f**	**22a**	38	0.7	3608.7 3613.4
10	**25g**	**21g**	**22a**	26	0.6	3576.6 3578.6
11	**25h**	**21h**	**22a**	67	3.1	3577.6 3579.0
12	**25i**	**21i**	**22a**	54%	1.5	3628.7 3630.9
13	**25j**	**21j**	**22a**	58	4.0	3629.7 3630.0

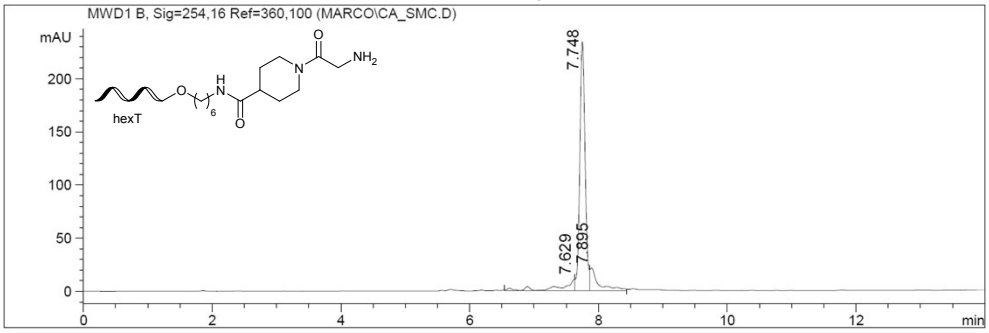
15	**25k**	 **21k**	 **22a**	48	0.6	3658.7 3659.1
16	**25l**	 **21l**	 **22a**	30	1.9	3596.6 3599.4
17	**25m**	 **21m**	 **22a**	n.d.	---	3504.6 n.d.
18	**25n**	 **21a**	 **22b**	20	1.6	3538.6 3541.2
19	**25o**	 **21a**	 **22c**	10	0.5	3595.6 3598.5

[a] CPG-bound oligonucleotide conjugate **20** (20 nmol) and aldehyde **21** (1000 equiv., 20 µmol) in 50 µL ACN/triethyl orthoformate (2:1) were condensed at ambient temperature for 6 h, then AgOAc (100 equiv., 2 µmol), dissolved in 30 µL ACN/triethyl orthoformate (2:1), triethylamine (4000 equiv., 80 µmol) and dipolarophile **22** (4000 equiv., 80 µmol) were added and reacted with the DNA-imine conjugate at 50 °C overnight. Cleavage with AMA (30 % aqueous ammonia/ 40 % aqueous methylamine, 1:1 (vol/vol)) at ambient temperature for 4 h. [b] Determined by analytical RP-HPLC analysis. [c] Determined by NanoDrop. [d] Measured by MALDI-MS. 10mer ATC = 5'-TTA CTA CCT A-3', 10mer ATGC = 5'-GTC ATG ATC T-3', 31mer ATGC = 5'-CAA ATC CGT TCA CAC CGA CCT GTC ATG ATC T-3'. n.d. = not detected, TEOF = triethyl orthoformate.

Starting materials for 1,3-dipolar cycloaddition on CPG-bound oligonucleotides

DNA conjugate 20a: Following DMT removal, CPG-bound hexT-C_6-NH_2 was reacted with 1-Fmoc-piperidine-4-carboxylic acid according to RP-01 and subsequently with Fmoc-glycine according to RP-02.

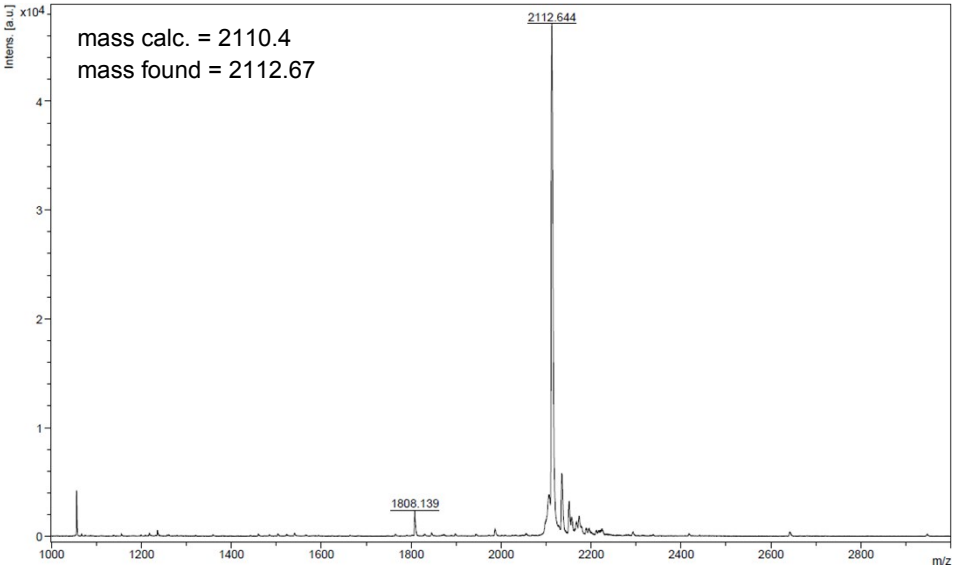
HPLC trace of crude reaction mixture 20a (Analytical RP-HPLC, Method-I)



Peak list:

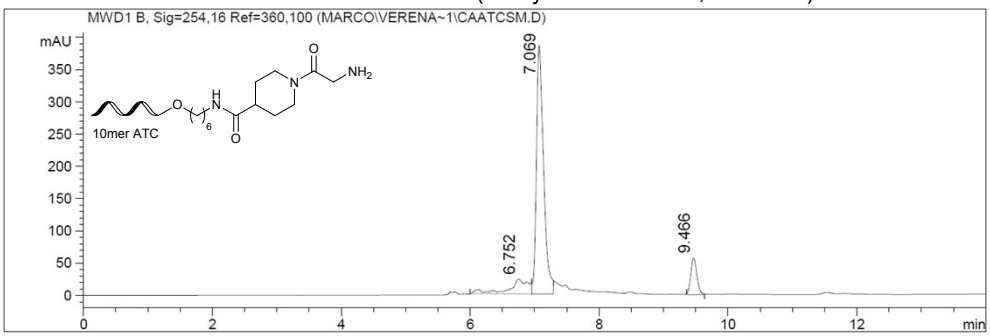
Ret. Time	Width min	Height	Area	Area %
7.629	0.229	10.626	146.193	8.730
7.748	0.095	234.639	1337.275	79.855
7.895	0.152	21.029	191.165	11.415

MALDI-MS spectrum of crude reaction mixture 20a



DNA conjugate 20b: Following DMT removal, CPG-bound ATC-C_6-NH_2 was reacted with 1-Fmoc-piperidine-4-carboxylic acid according to RP-01 and subsequently with Fmoc-glycine according to RP-02.

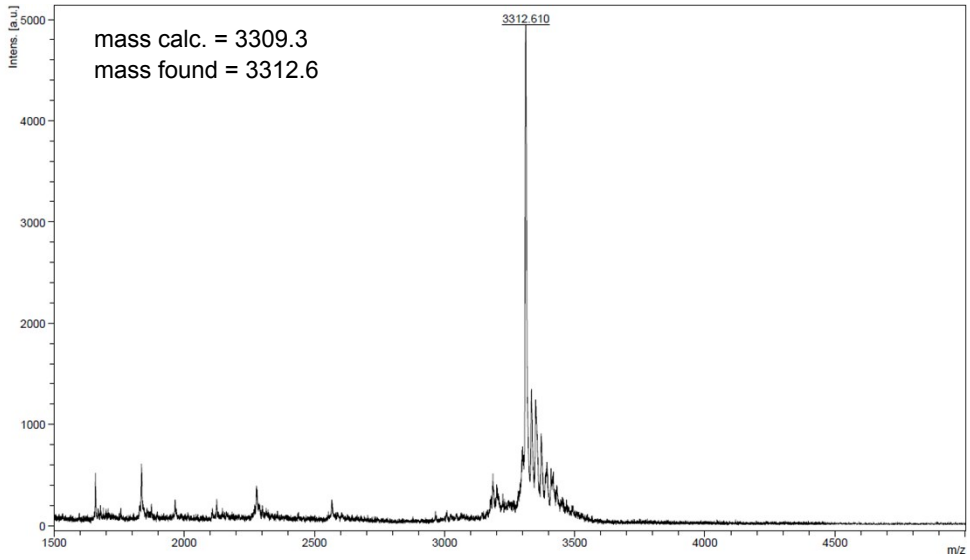
HPLC trace of crude reaction mixture 20b (Analytical RP-HPLC, Method-I)



Peak list:

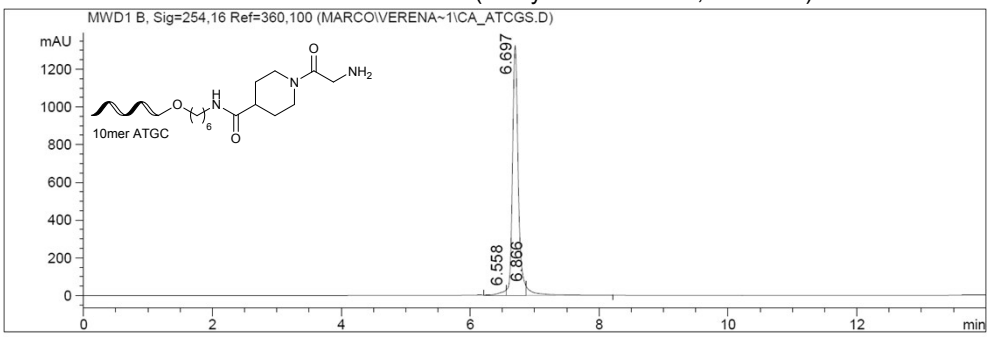
Ret. Time	Width min	Height	Area	Area %
6.752	0.347	22.790	474.446	12.437
7.069	0.128	386.304	2969.422	77.837
9.466	0.109	56.642	371.053	9.726

MALDI-MS spectrum of crude reaction mixture 20b



DNA conjugate 20c: Following DMT removal, CPG-bound ATGC-C_6-NH_2 was reacted with 1-Fmoc-piperidine-4-carboxylic acid according to RP-01 and subsequently with Fmoc-glycine according to RP-02.

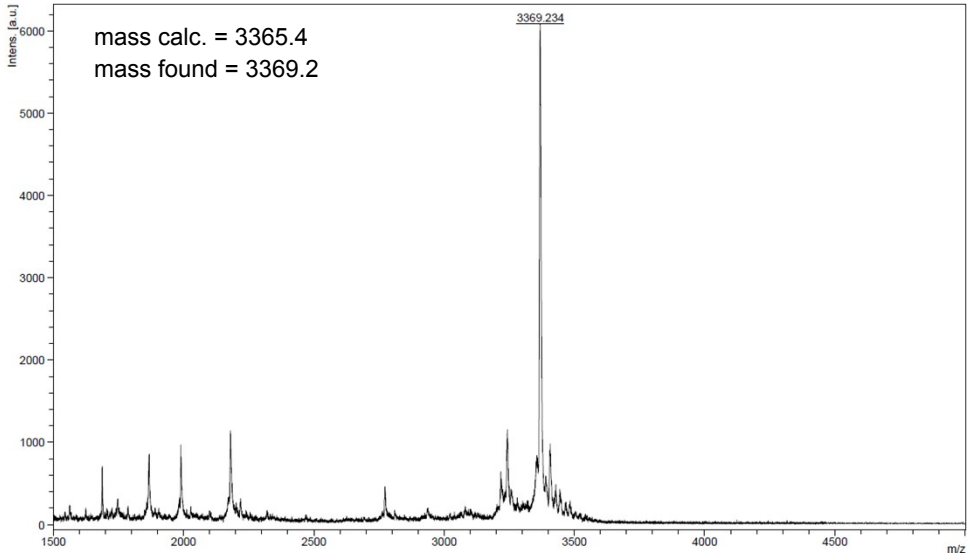
HPLC trace of crude reaction mixture 20c (Analytical RP-HPLC, Method-I)



Peak list:

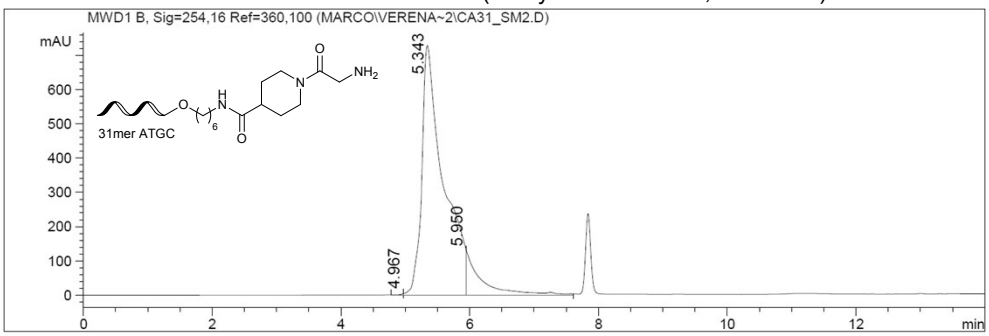
Ret. Time	Width min	Height	Area	Area %
6.558	0.113	28.109	189.879	2.189
6.697	0.102	1326.277	8087.587	93.226
6.866	0.136	48.641	397.785	4.585

MALDI-MS spectrum of crude reaction mixture 20c



DNA conjugate 20d: Following DMT removal, CPG-bound ATGC-C_6-NH_2 was reacted with 1-Fmoc-piperidine-4-carboxylic acid according to RP-01 and subsequently with Fmoc-glycine according to RP-02.

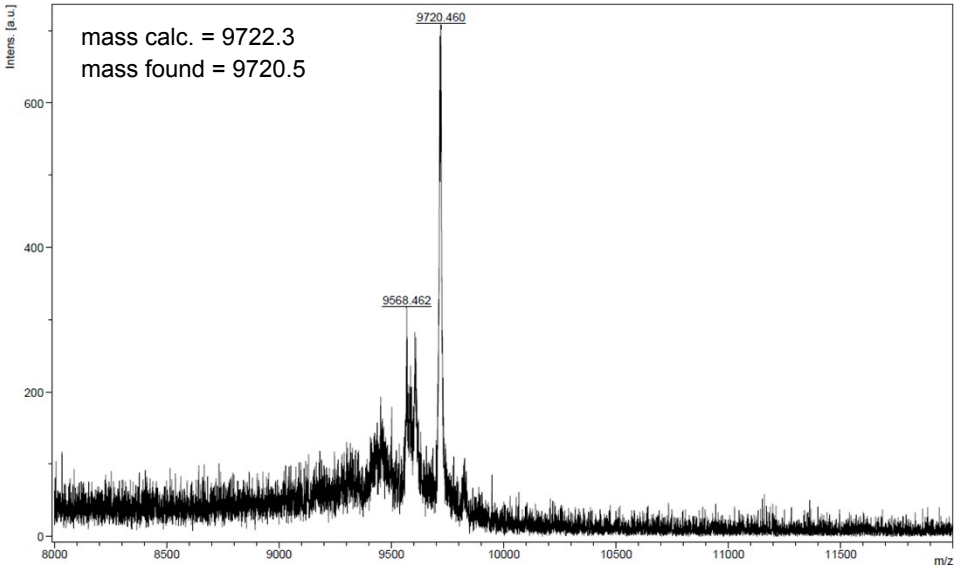
HPLC trace of crude reaction mixture 20d (Analytical RP-HPLC, Method-II)



Peak list:

Ret. Time	Width min	Height	Area	Area %
4.967	0.057	3.956	13.489	0.071
5.343	0.388	728.580	16961.625	89.245
5.950	0.264	128.350	2030.550	10.684

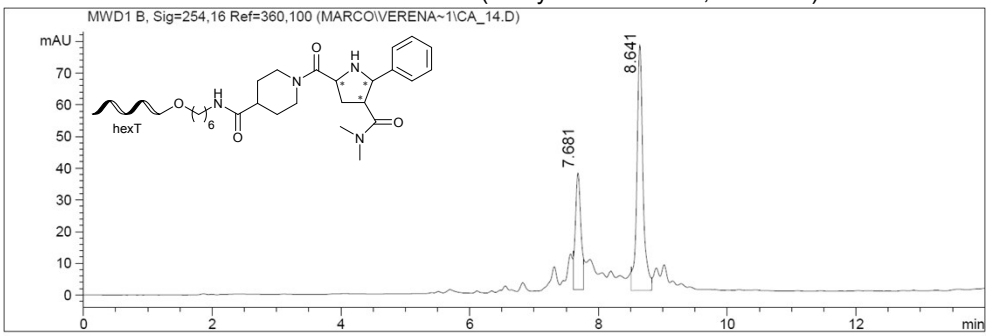
MALDI-MS spectrum of crude reaction mixture 20d



Products of 1,3-dipolar cycloaddition on CPG-bound oligonucleotides

DNA conjugate 23a: CPG-bound hexT-aldehyde conjugate **20a** was reacted with benzaldehyde **21a** and *N,N*-dimethylacrylamide **22a** according to RP-04.

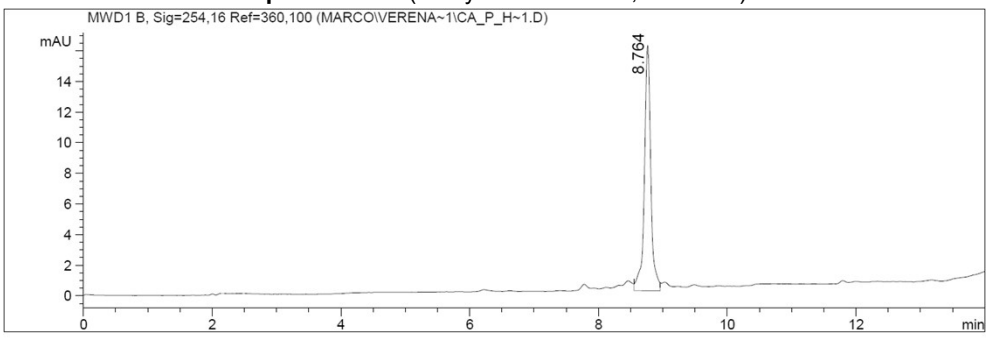
HPLC trace of crude reaction mixture 23a (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.681	0.098	36.972	216.657	30.022
8.641	0.108	77.644	505.000	69.978

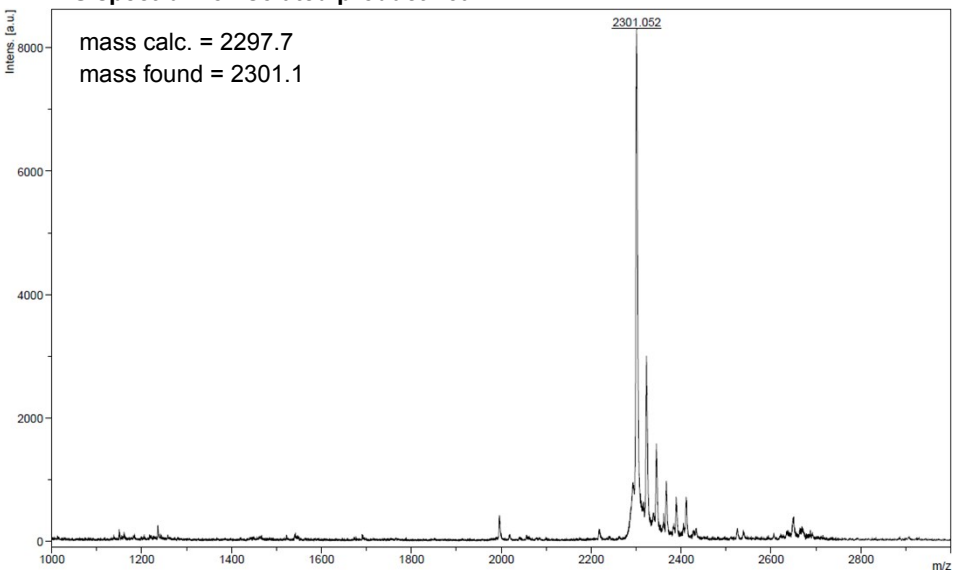
HPLC trace of isolated product 23a (Analytical RP-HPLC, Method-I)



Peak list:

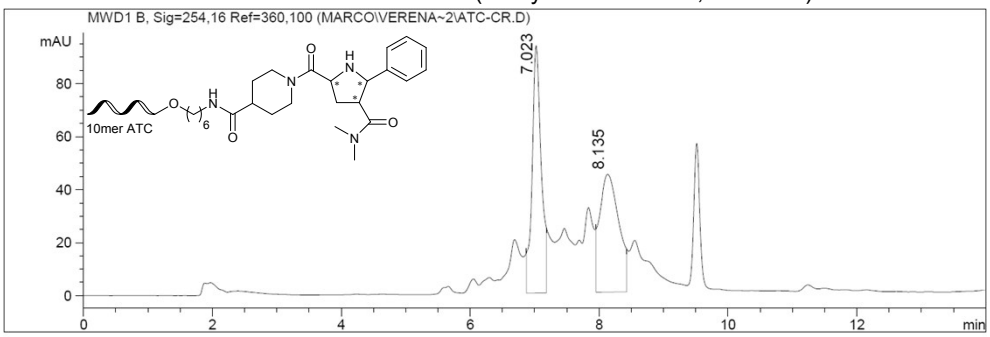
Ret. Time	Width min	Height	Area	Area %
8.764	0.116	16.033	111.293	100.000

MALDI-MS spectrum of isolated product 23a



DNA conjugate 24a: CPG-bound 10mer ATC-aldehyde conjugate **20b** was reacted with benzaldehyde **21a** and *N,N*-dimethylacrylamide **22a** according to RP-04.

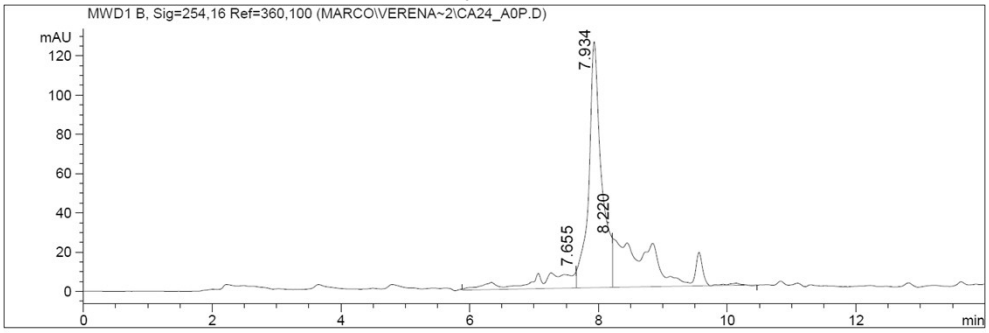
HPLC trace of crude reaction mixture 24a (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
7.023	0.163	93.485	912.917	49.929
8.135	0.343	44.504	915.518	50.071

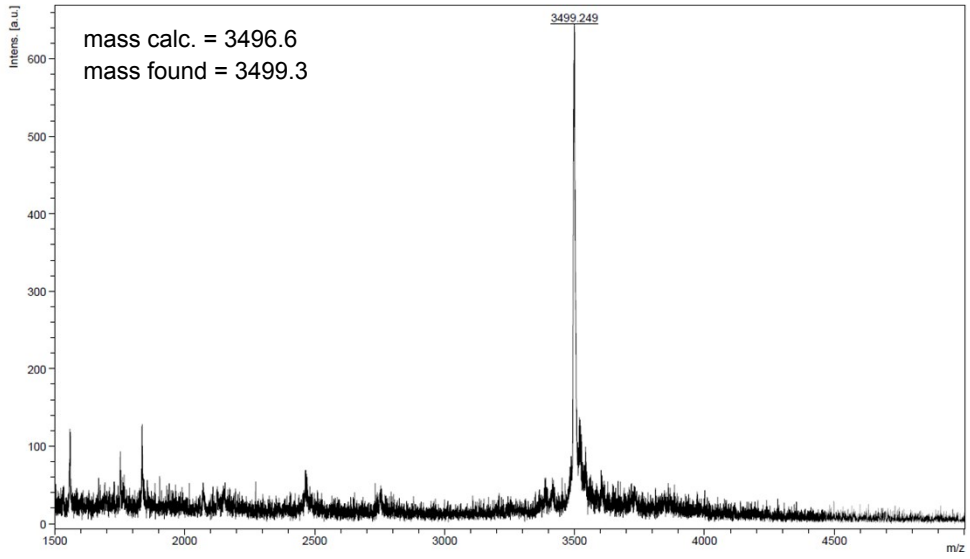
HPLC trace of isolated product 24a (Analytical RP-HPLC, Method-I)



Peak list:

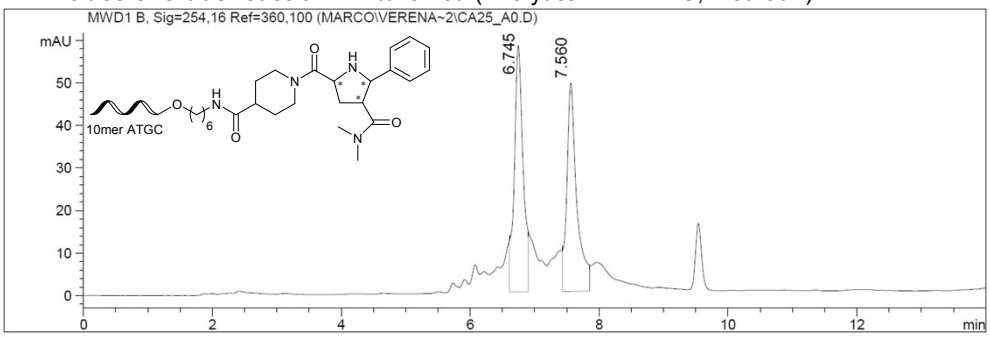
Ret. Time	Width min	Height	Area	Area %
7.655	0.701	8.792	369.922	11.700
7.934	0.229	125.606	1725.164	54.563
8.220	0.709	25.069	1066.726	33.738

MALDI-MS spectrum of isolated product 24a



DNA conjugate 25a: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with benzaldehyde **21a** and *N,N*-dimethylacrylamide **22a** according to RP-04.

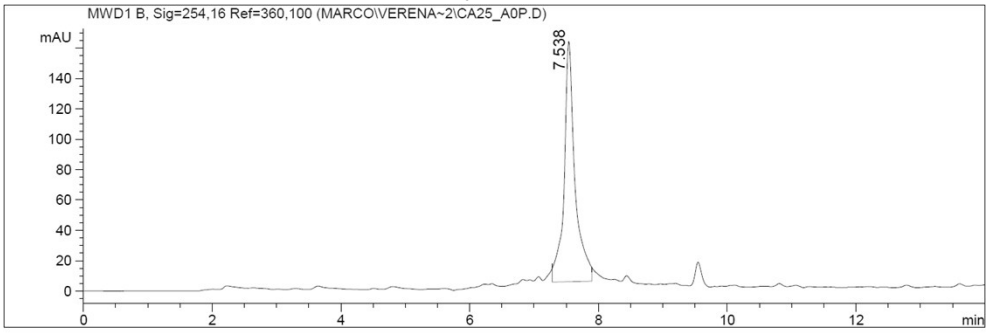
HPLC trace of crude reaction mixture 25a (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.745	0.157	57.984	547.737	50.801
7.560	0.180	49.041	530.466	49.199

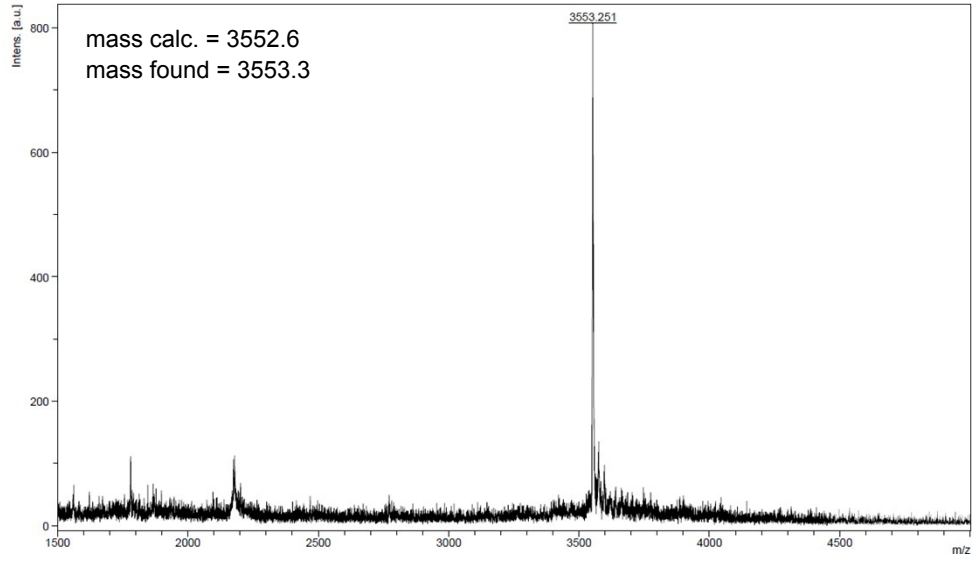
HPLC trace of isolated product 25a (Analytical RP-HPLC, Method-I)



Peak list:

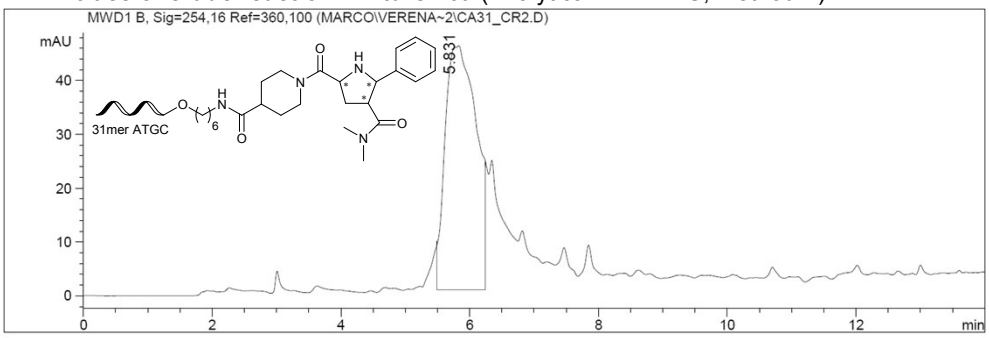
Ret. Time	Width min	Height	Area	Area %
7.538	0.196	158.621	1862.320	100.000

MALDI-MS spectrum of isolated product 25a



DNA conjugate 26a: CPG-bound 31mer ATGC-aldehyde conjugate **20d** was reacted with benzaldehyde **21a** and *N,N*-dimethylacrylamide **22a** according to RP-04.

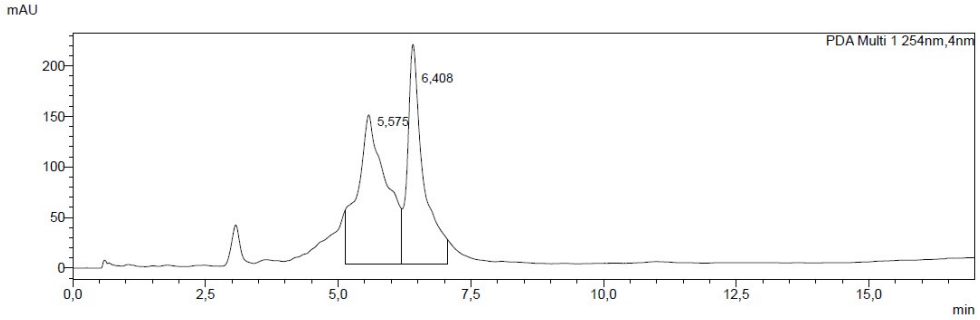
HPLC trace of crude reaction mixture 26a (Analytical RP-HPLC, Method-II)



Peak list:

Ret. Time	Width min	Height	Area	Area %
5.831	0.560	45.354	1525.251	100.000

HPLC trace of crude reaction mixture 26a (Preparative RP-HPLC)

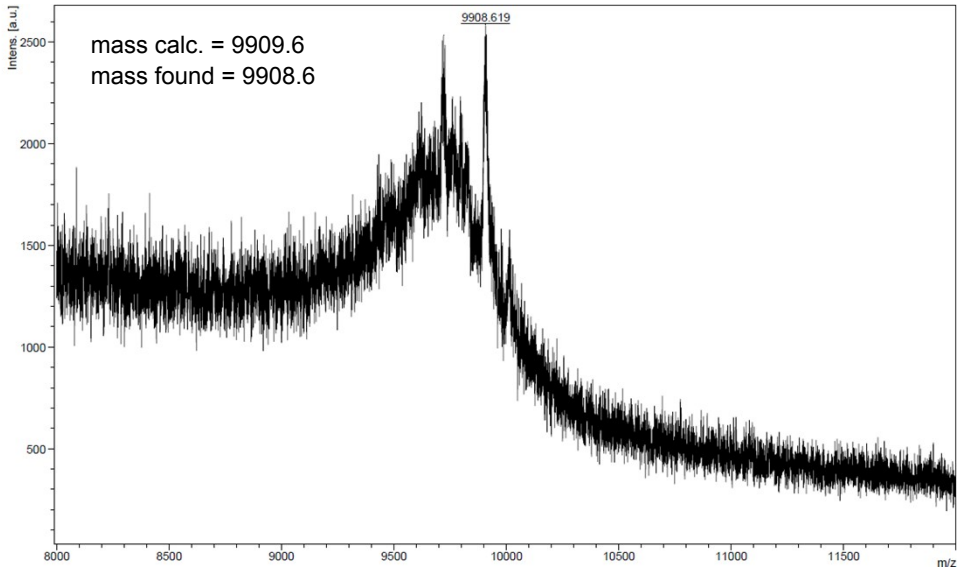


Peak Table

PDA Ch1 254nm

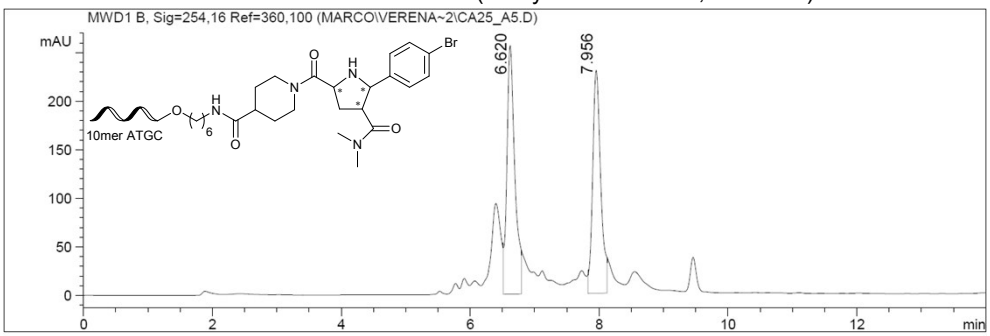
Peak#	Ret. Time	Area	Area%
1	5,575	5565738	55,205
2	6,408	4516294	44,795
Total		10082032	100,000

MALDI-MS spectrum of crude reaction mixture 26a



DNA conjugate 25b: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-bromobenzaldehyde **21b** and *N,N*-dimethylacrylamide **22a** according to RP-04.

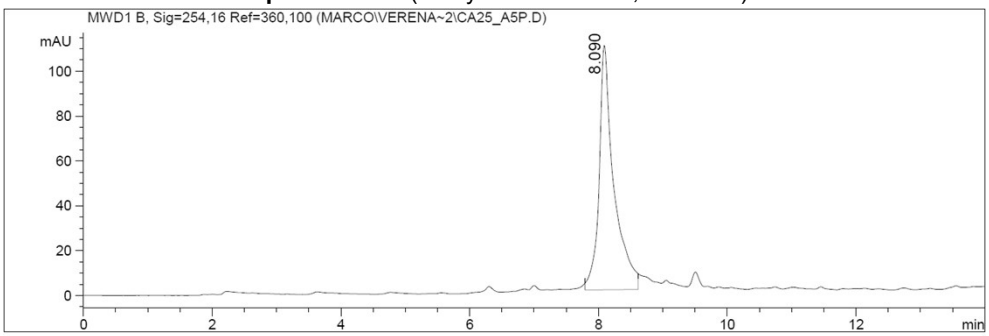
HPLC trace of crude reaction mixture 25b (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.620	0.142	256.333	2189.740	52.390
7.956	0.144	229.900	1989.920	47.610

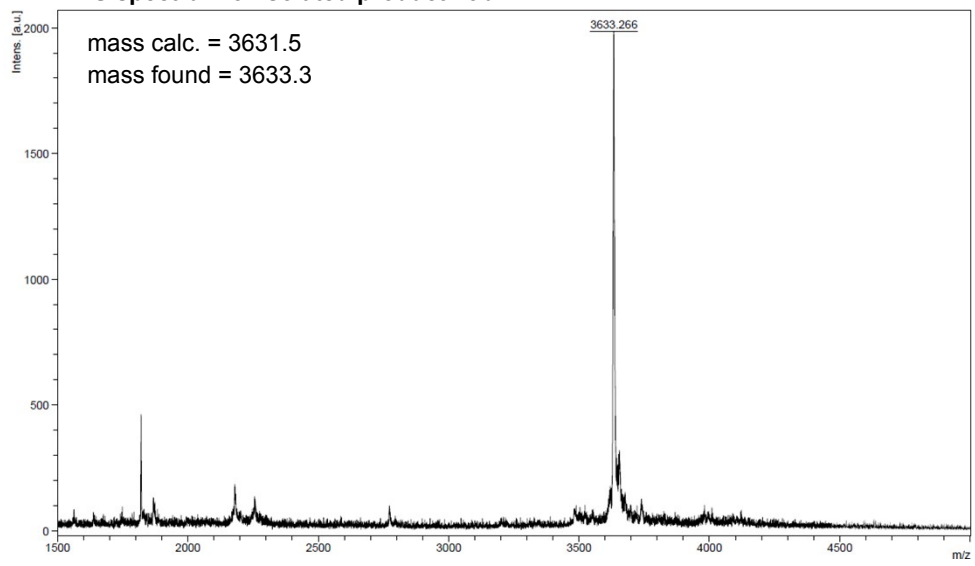
HPLC trace of isolated product 25b (Analytical RP-HPLC, Method-I)



Peak list:

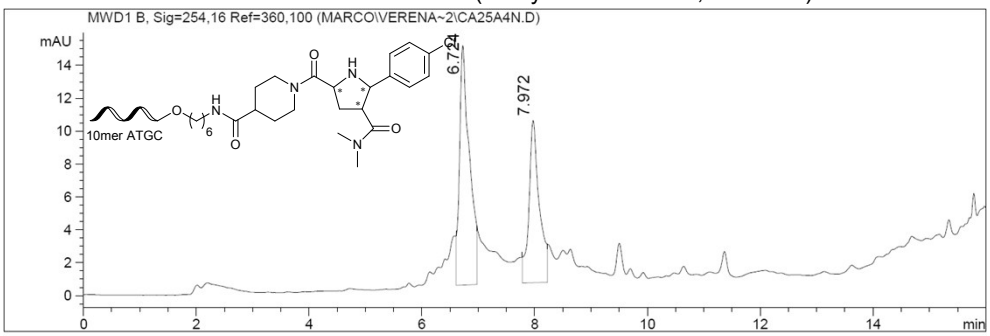
Ret. Time	Width min	Height	Area	Area %
8.090	0.267	109.030	1745.680	100.000

MALDI-MS spectrum of isolated product 25b



DNA conjugate 25c: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-chlorobenzaldehyde **21c** and *N,N*-dimethylacrylamide **22a** according to RP-04.

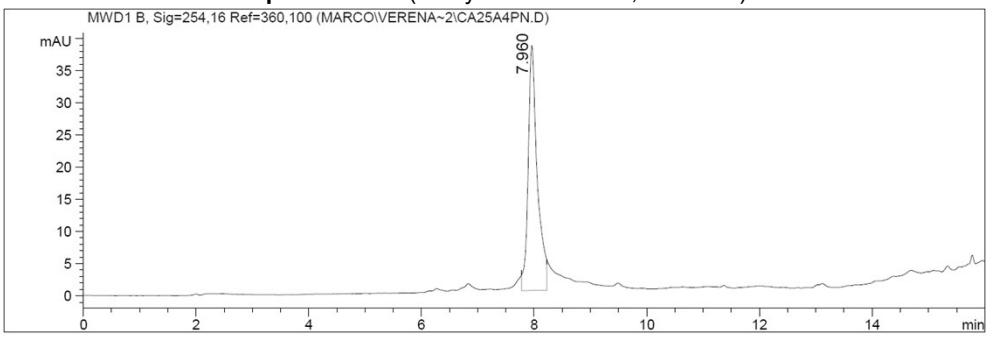
HPLC trace of crude reaction mixture 25c (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.724	0.205	14.574	179.067	58.672
7.972	0.213	9.881	126.132	41.328

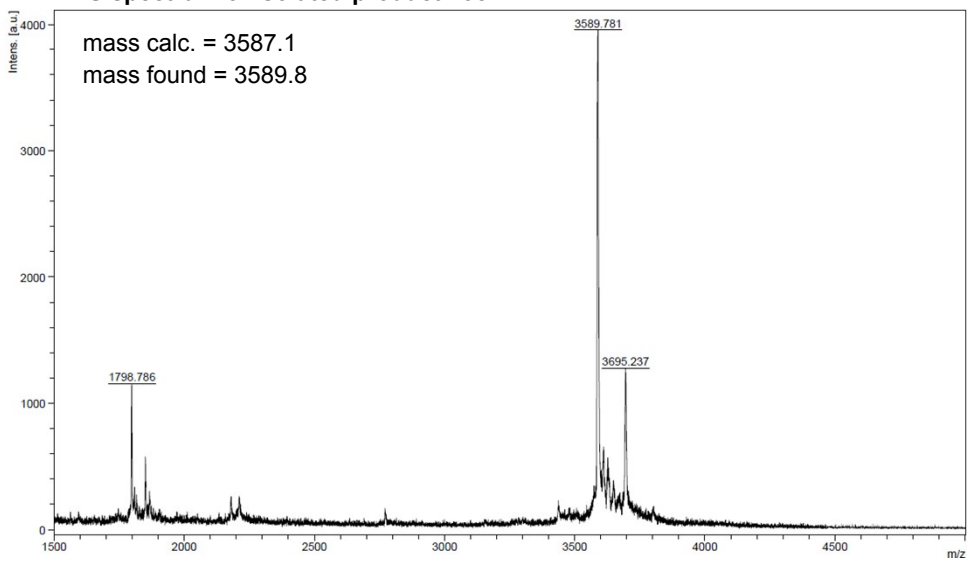
HPLC trace of isolated product 25c (Analytical RP-HPLC, Method-I)



Peak list:

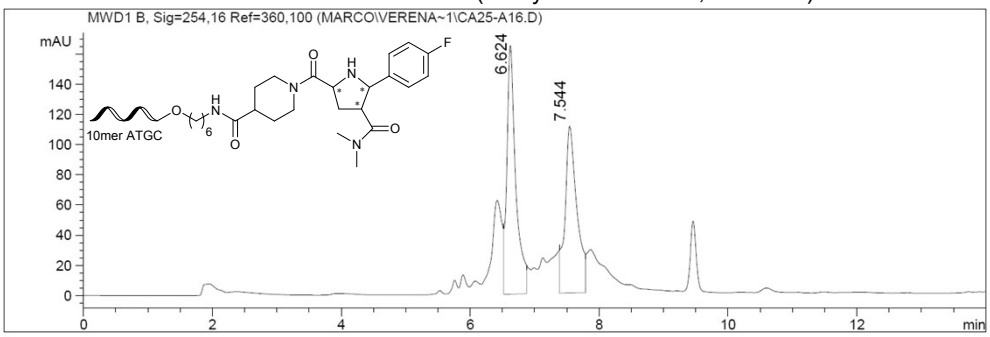
Ret. Time	Width min	Height	Area	Area %
7.960	0.188	38.136	429.028	100.000

MALDI-MS spectrum of isolated product 25c



DNA conjugate 25d: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-fluorobenzaldehyde **21d** and *N,N*-dimethylacrylamide **22a** according to RP-04.

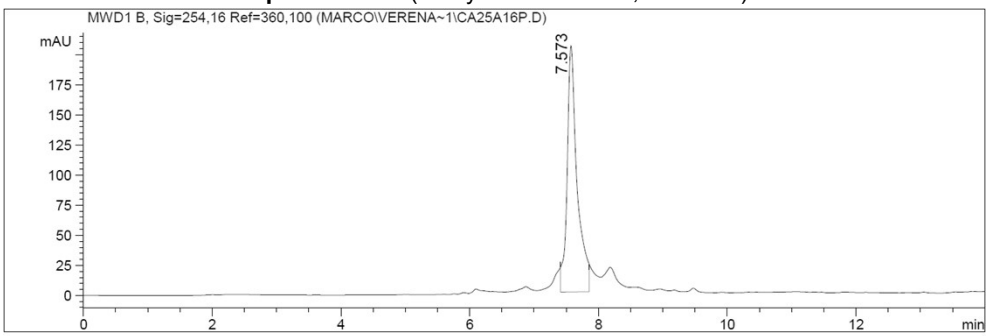
HPLC trace of crude reaction mixture 25d (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.624	0.159	164.699	1567.480	52.693
7.544	0.212	110.556	1407.250	47.307

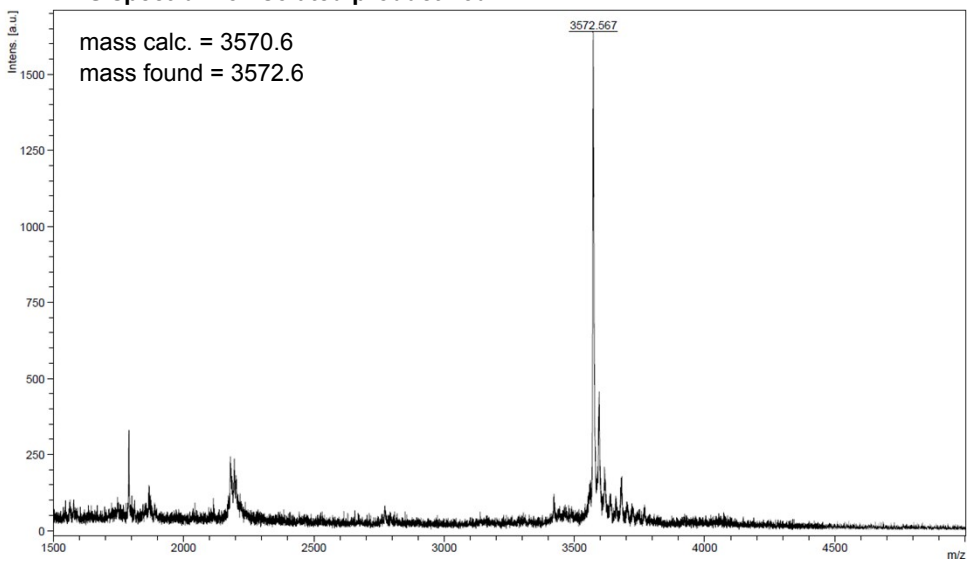
HPLC trace of isolated product 25d (Analytical RP-HPLC, Method-I)



Peak list:

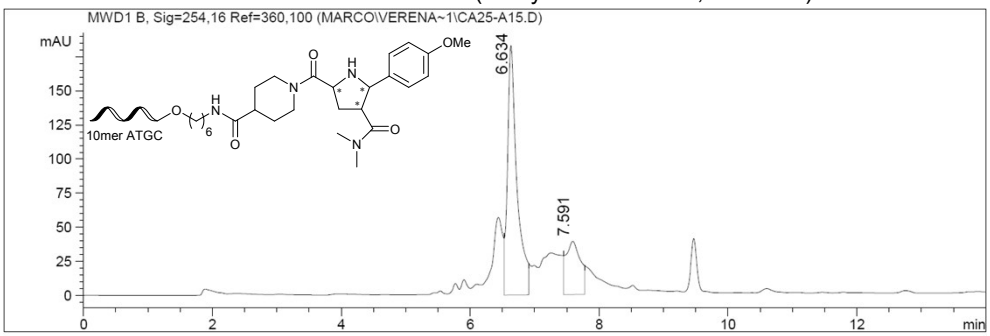
Ret. Time	Width min	Height	Area	Area %
7.573	0.178	204.819	2191.556	100.000

MALDI-MS spectrum of isolated product 25d



DNA conjugate 25e: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-methoxybenzaldehyde **21e** and *N,N*-dimethylacrylamide **22a** according to RP-04.

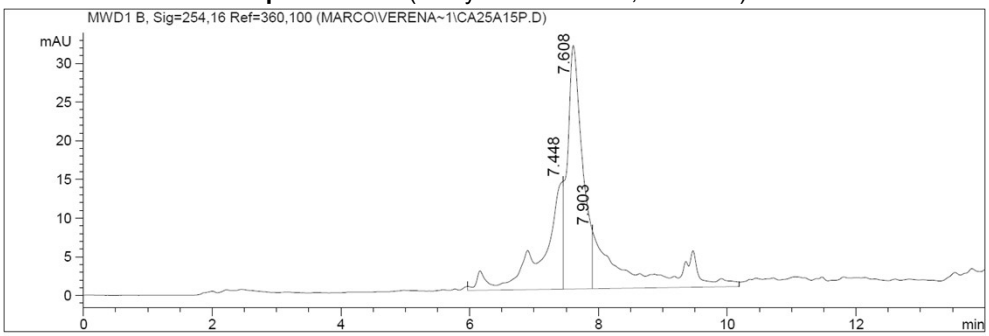
HPLC trace of crude reaction mixture 25e (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.634	0.170	183.070	1864.126	75.702
7.591	0.255	39.070	598.326	24.298

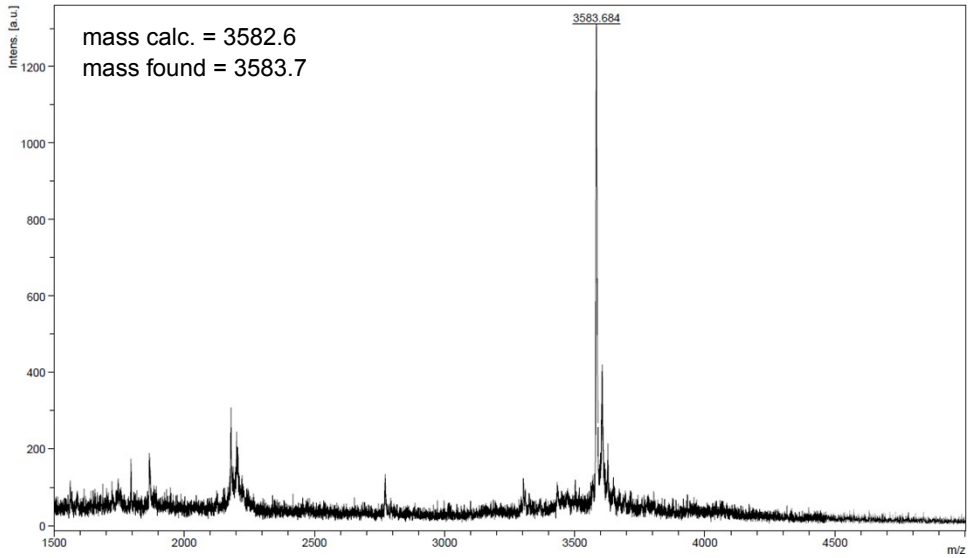
HPLC trace of isolated product 25e (Analytical RP-HPLC, Method-I)



Peak list:

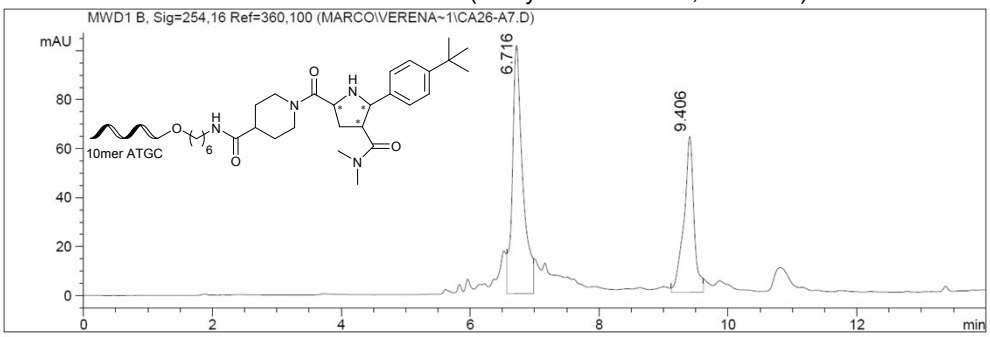
Ret. Time	Width min	Height	Area	Area %
7.448	0.366	13.906	305.698	27.798
7.608	0.268	31.476	506.568	46.064
7.903	0.630	7.609	287.447	26.138

MALDI-MS spectrum of isolated product 25e



DNA conjugate 25f: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-*tert*-butylbenzaldehyde **21f** and *N,N*-dimethylacrylamide **22a** according to RP-04.

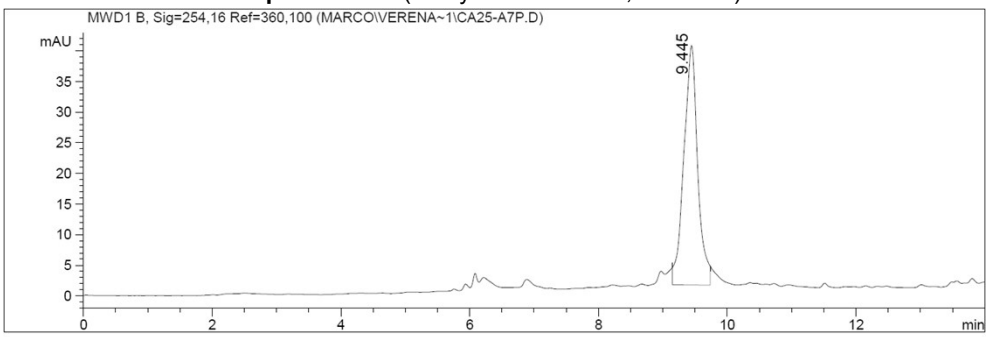
HPLC trace of crude reaction mixture 25f (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.716	0.186	101.417	1130.862	62.256
9.406	0.179	63.725	685.621	37.744

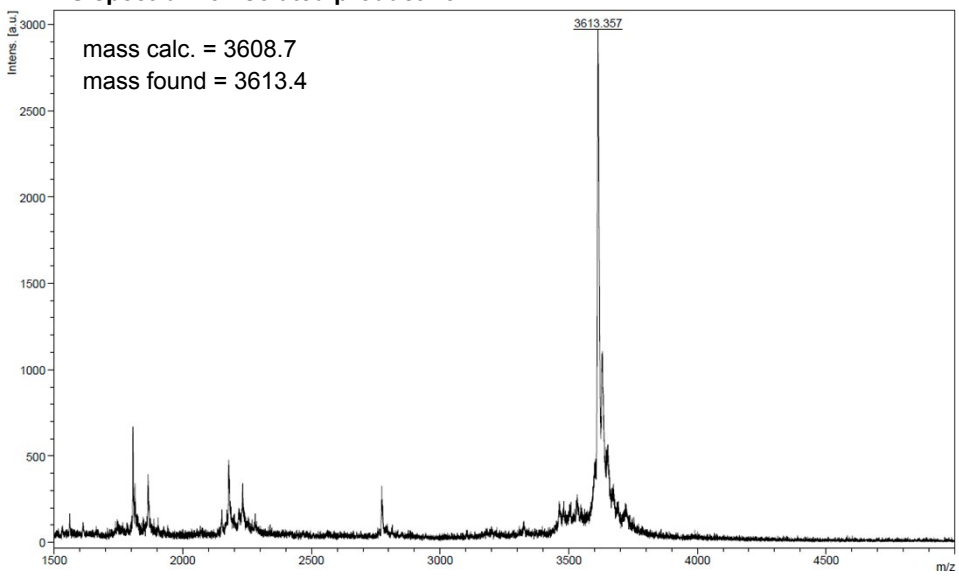
HPLC trace of isolated product 25f (Analytical RP-HPLC, Method-I)



Peak list:

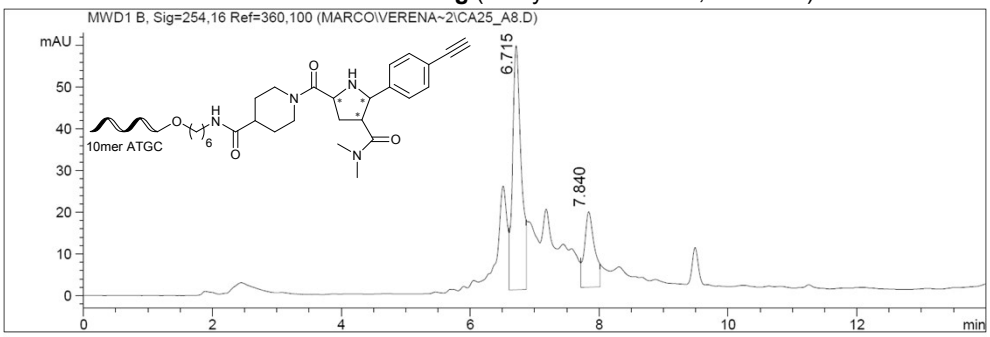
Ret. Time	Width min	Height	Area	Area %
9.445	0.248	39.097	581.896	100.000

MALDI-MS spectrum of isolated product 25f



DNA conjugate 25g: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-ethynylbenzaldehyde **21g** and *N,N*-dimethylacrylamide **22a** according to RP-04.

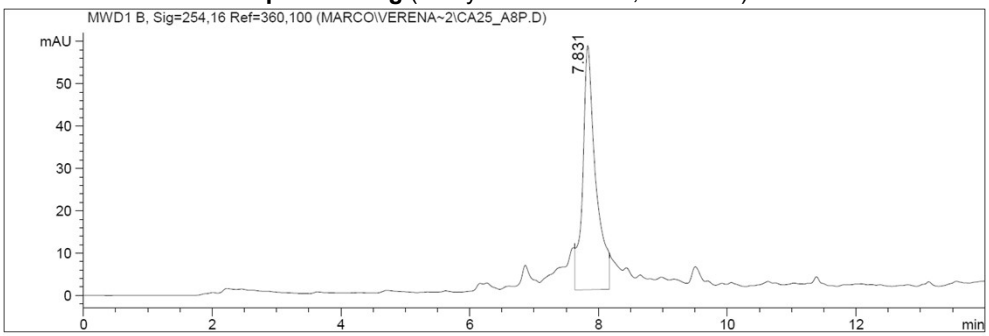
HPLC trace of crude reaction mixture 25g (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.715	0.155	58.577	545.278	73.632
7.840	0.180	18.108	195.268	26.368

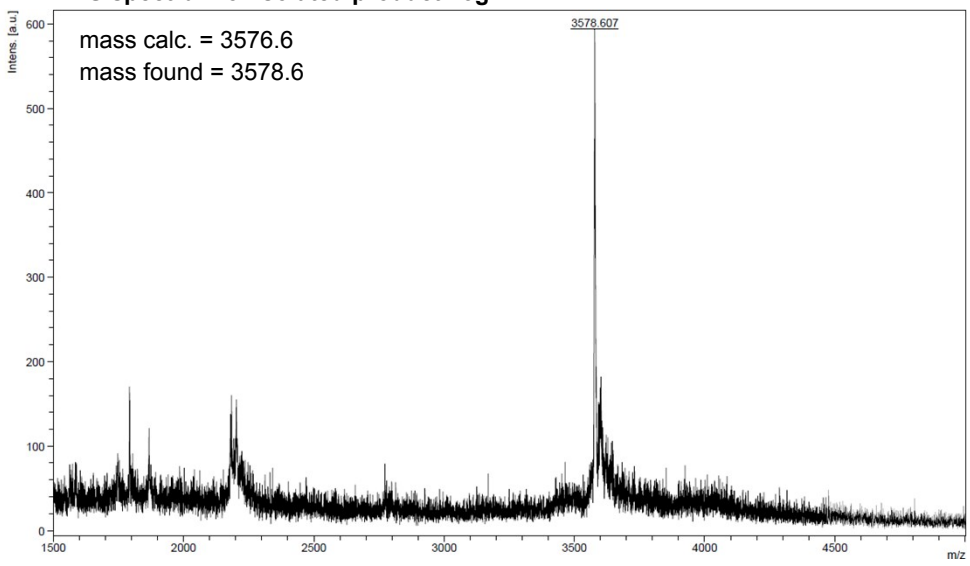
HPLC trace of isolated product 25g (Analytical RP-HPLC, Method-I)



Peak list:

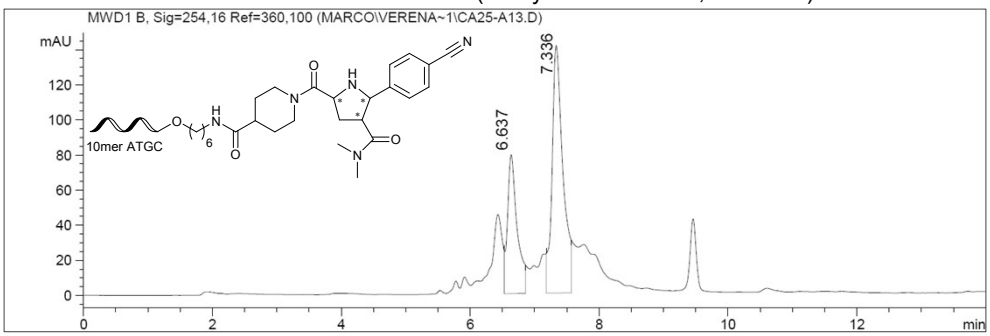
Ret. Time	Width min	Height	Area	Area %
7.831	0.234	57.706	808.712	100.000

MALDI-MS spectrum of isolated product 25g



DNA conjugate 25h: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-formylbenzonitrile **21h** and *N,N*-dimethylacrylamide **22a** according to RP-04.

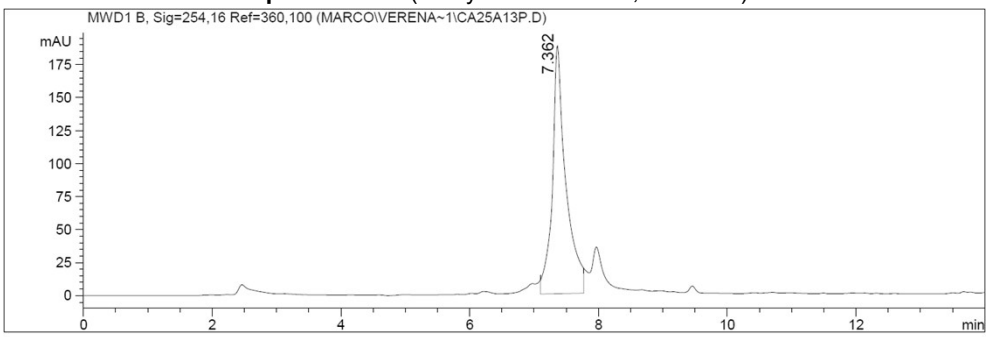
HPLC trace of crude reaction mixture 25h (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.637	0.174	79.215	828.028	33.236
7.336	0.197	141.059	1663.362	66.764

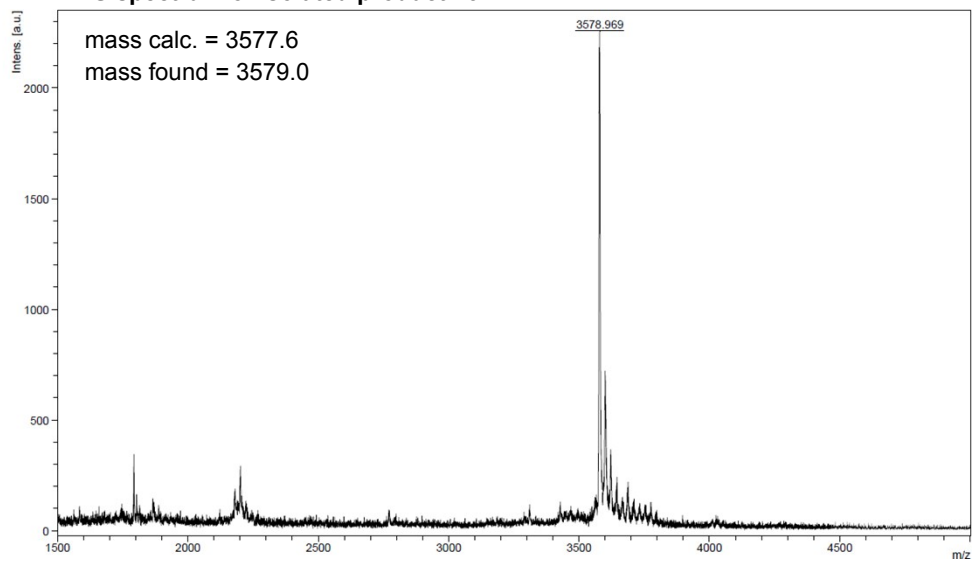
HPLC trace of isolated product 25h (Analytical RP-HPLC, Method-I)



Peak list:

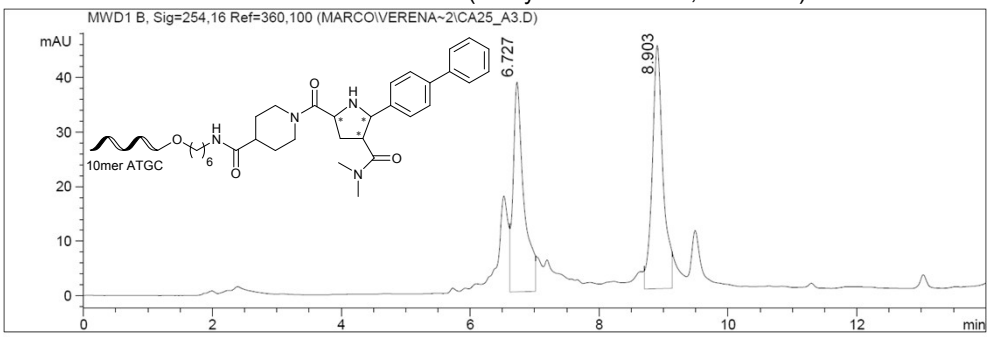
Ret. Time	Width min	Height	Area	Area %
7.362	0.242	188.094	2734.618	100.000

MALDI-MS spectrum of isolated product 25h



DNA conjugate 25i: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with biphenyl-4-carboxaldehyde **21i** and *N,N*-dimethylacrylamide **22a** according to RP-04.

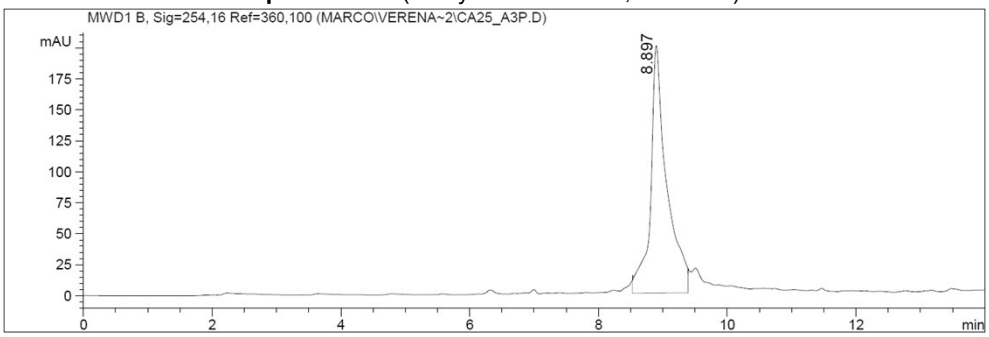
HPLC trace of crude reaction mixture 25i (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.727	0.191	38.479	441.614	46.180
8.903	0.192	44.631	514.677	53.820

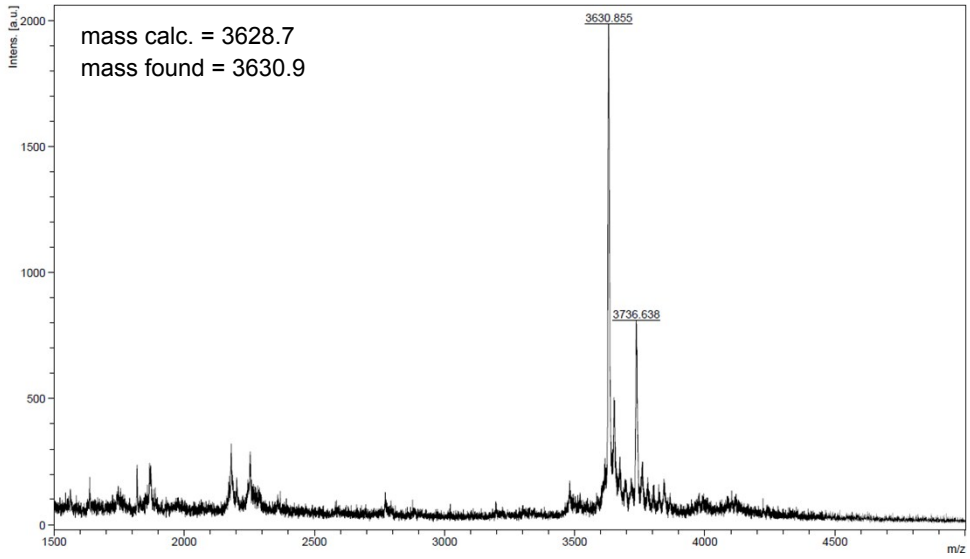
HPLC trace of isolated product 25i (Analytical RP-HPLC, Method-I)



Peak list:

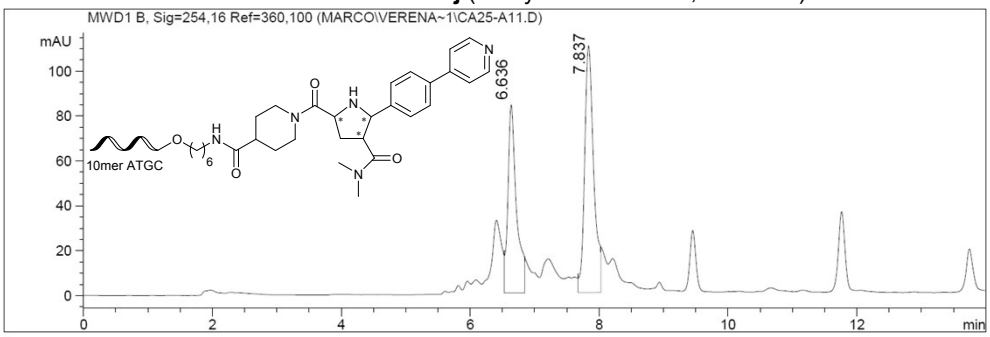
Ret. Time	Width min	Height	Area	Area %
8.897	0.290	199.680	3479.585	100.000

MALDI-MS spectrum of isolated product 25i



DNA conjugate 25j: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 4-pyridin-4-ylbenzaldehyde **21j** and *N,N*-dimethylacrylamide **22a** according to RP-04.

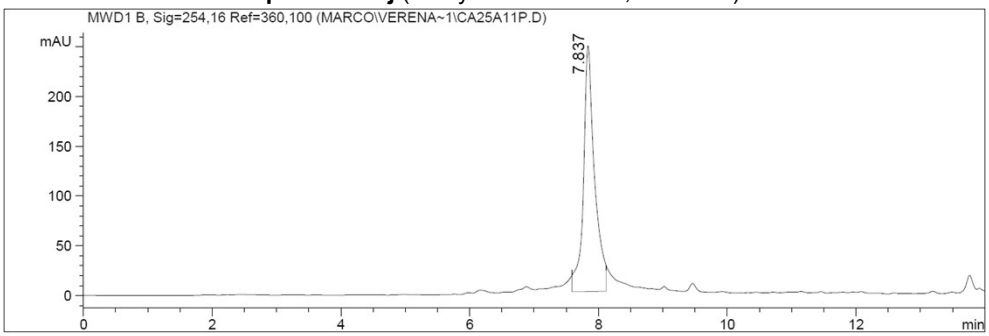
HPLC trace of crude reaction mixture 25j (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.636	0.153	84.040	771.274	41.742
7.837	0.163	110.072	1076.442	58.258

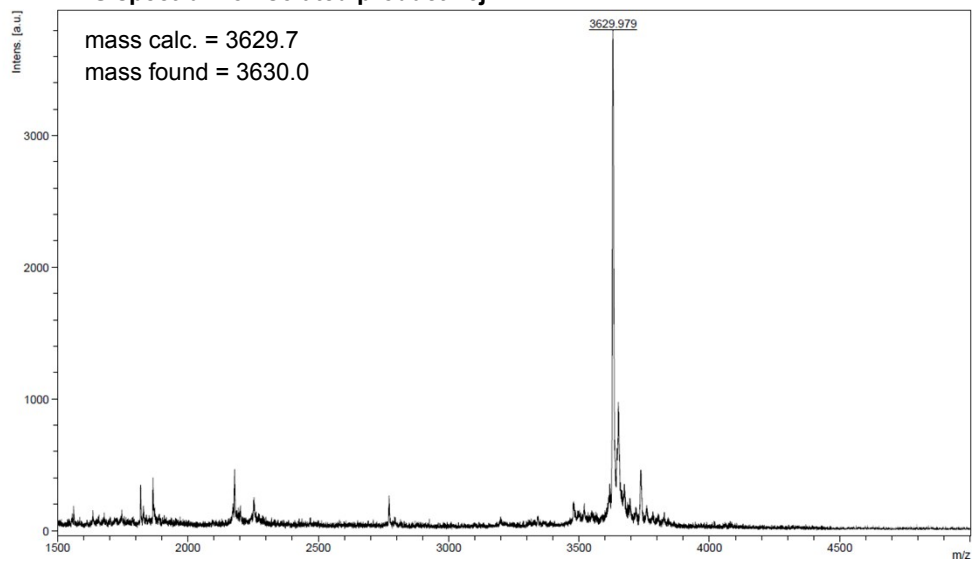
HPLC trace of isolated product 25j (Analytical RP-HPLC, Method-I)



Peak list:

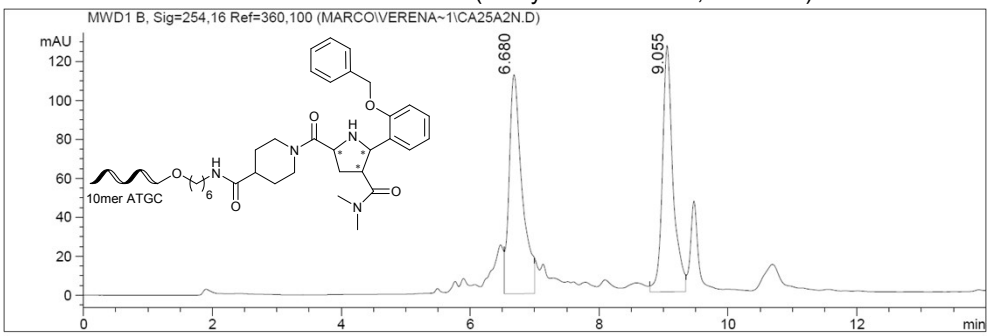
Ret. Time	Width min	Height	Area	Area %
7.837	0.202	247.394	2999.225	100.000

MALDI-MS spectrum of isolated product 25j



DNA conjugate 25k: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 2-phenylmethoxybenzaldehyde **21k** and *N,N*-dimethylacrylamide **22a** according to RP-04.

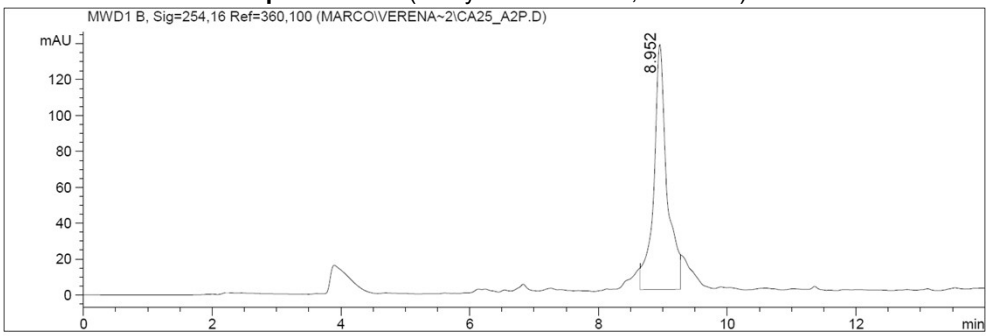
HPLC trace of crude reaction mixture 25k (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.680	0.233	112.505	1572.313	51.919
9.055	0.192	126.566	1456.082	48.081

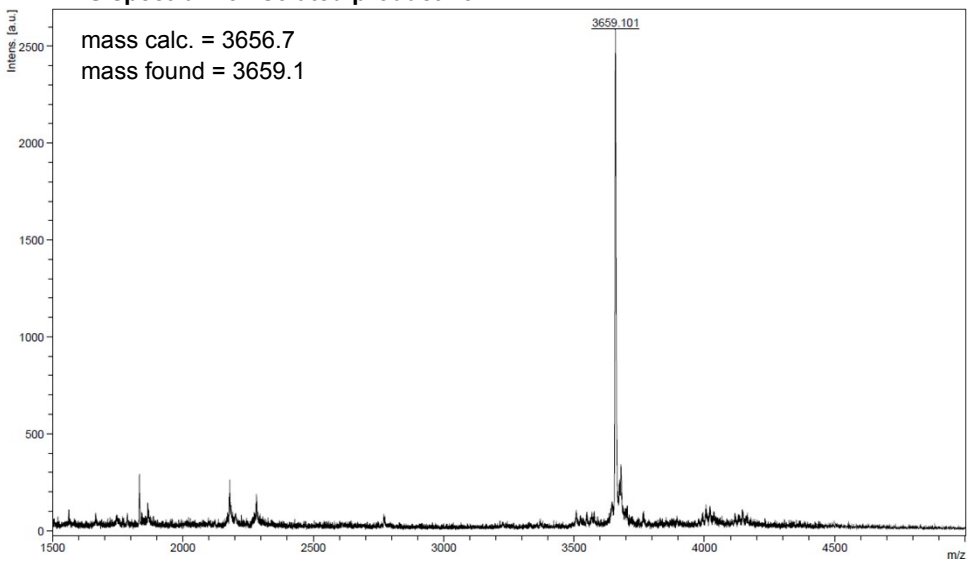
HPLC trace of isolated product 25k (Analytical RP-HPLC, Method-I)



Peak list:

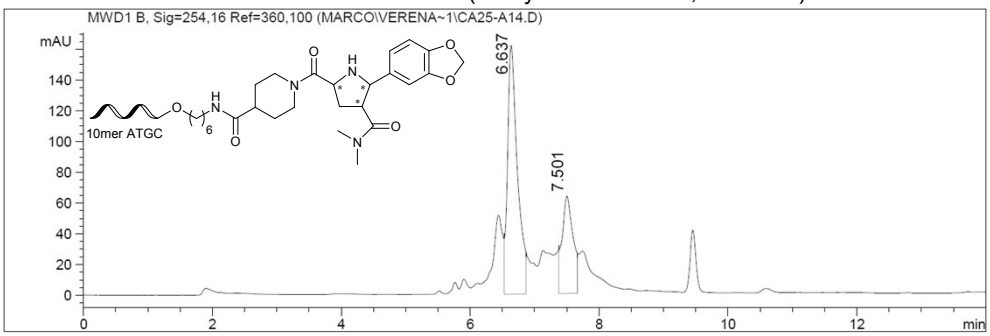
Ret. Time	Width min	Height	Area	Area %
8.952	0.238	136.650	1947.764	100.000

MALDI-MS spectrum of isolated product 25k



DNA conjugate 25l: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with 1,3-benzodioxole-5-carbaldehyde **21l** and *N,N*-dimethylacrylamide **22a** according to RP-04.

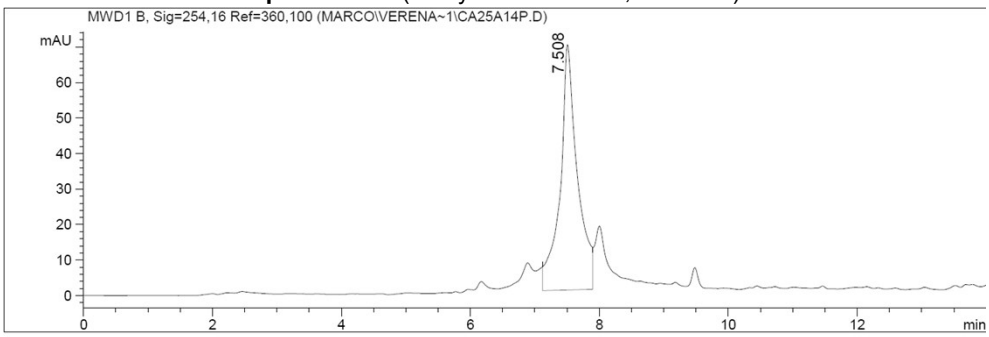
HPLC trace of crude reaction mixture 25l (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.637	0.178	161.991	1727.826	69.918
7.501	0.195	63.472	743.381	30.082

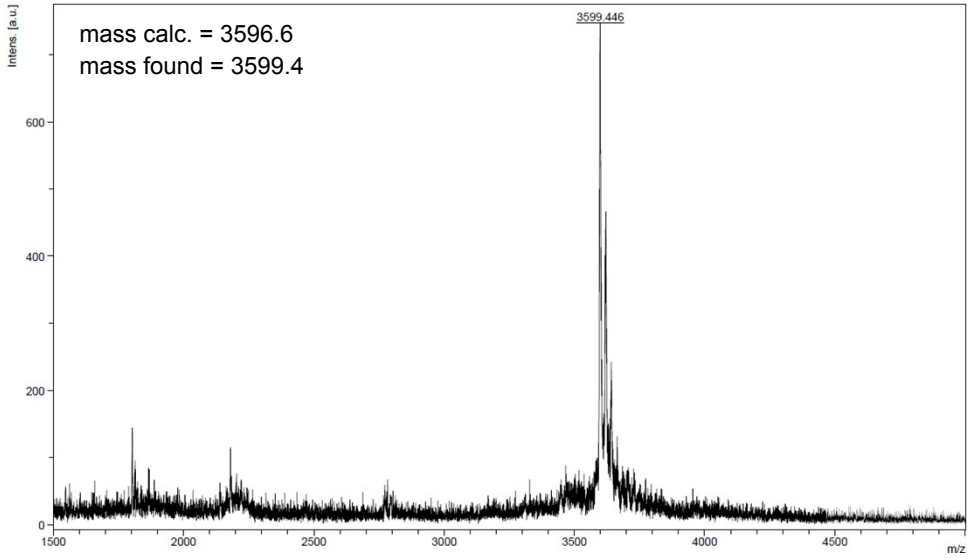
HPLC trace of isolated product 25l (Analytical RP-HPLC, Method-I)



Peak list:

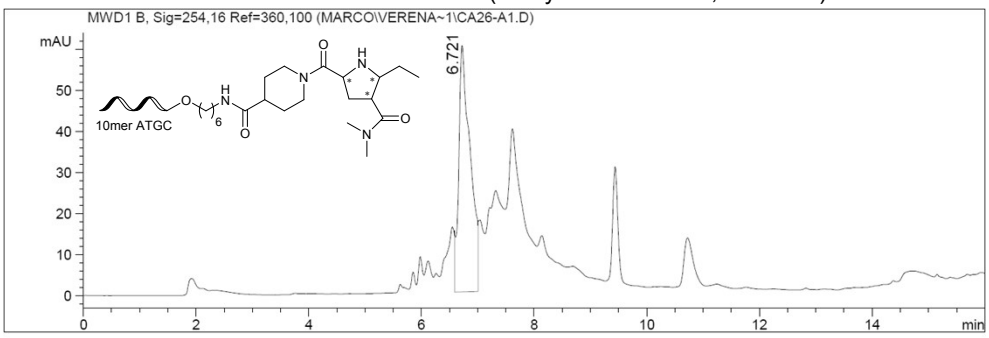
Ret. Time	Width min	Height	Area	Area %
7.508	0.310	69.119	1284.571	100.000

MALDI-MS spectrum of isolated product 25l



DNA conjugate 25m: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with propanal **21m** and *N,N*-Dimethylacrylamide **22a** according to RP-04.

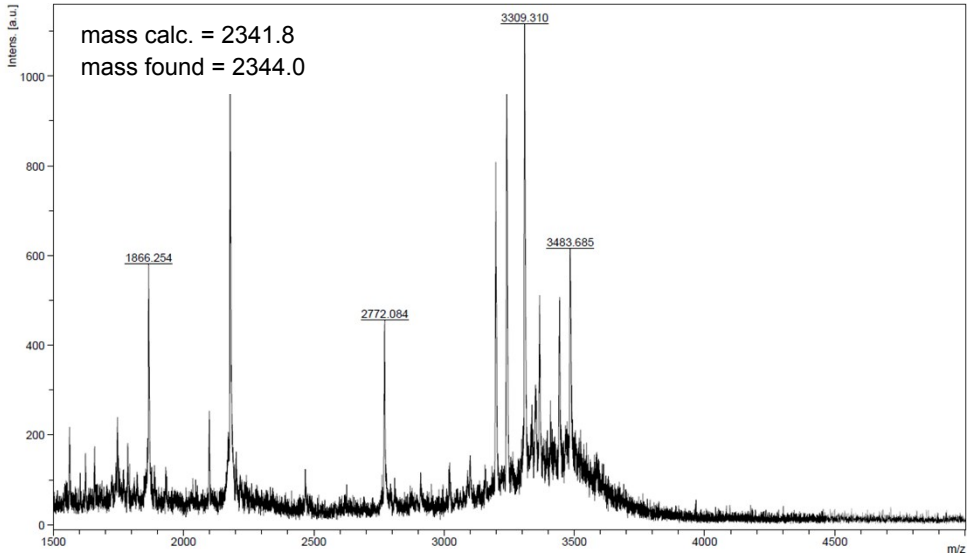
HPLC trace of crude reaction mixture 25m (Analytical RP-HPLC, Method-I)



Peak list:

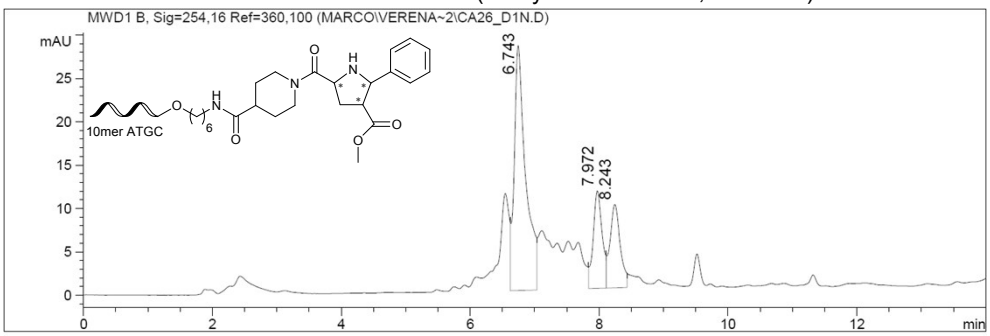
Ret. Time	Width min	Height	Area	Area %
6.721	0.234	60.108	845.161	100.000

MALDI-MS spectrum of crude reaction mixture 25m



DNA conjugate 25n: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with benzaldehyde **21a** and methyl prop-2-enoate **22b** according to RP-04.

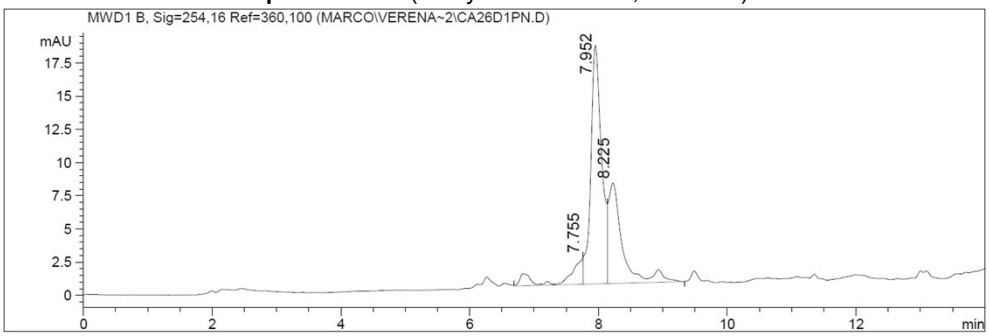
HPLC trace of crude reaction mixture 25n (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.743	0.208	28.257	352.390	62.056
7.972	0.162	11.238	109.190	19.229
8.243	0.184	9.626	106.275	18.715

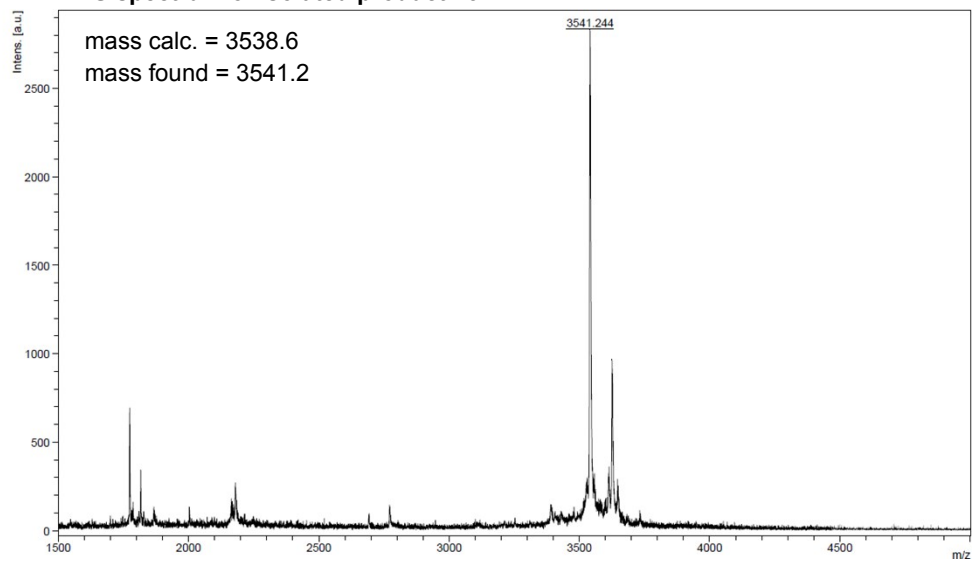
HPLC trace of isolated product 25n (Analytical RP-HPLC, Method-I)



Peak list:

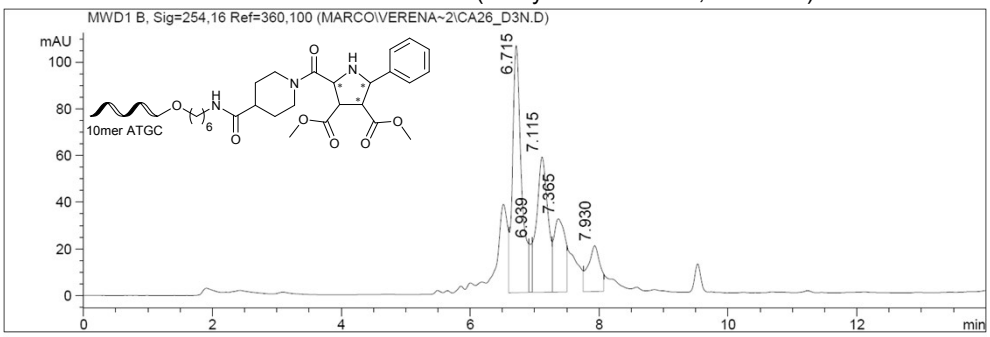
Ret. Time	Width min	Height	Area	Area %
7.755	0.278	1.988	33.168	9.313
7.952	0.193	17.973	208.665	58.589
8.225	0.252	7.564	114.320	32.099

MALDI-MS spectrum of isolated product 25n



DNA conjugate 25o: CPG-bound 10mer ATGC-aldehyde conjugate **20c** was reacted with benzaldehyde **21a** and dimethyl (*Z*)-but-2-enedioate **22c** according to RP-04.

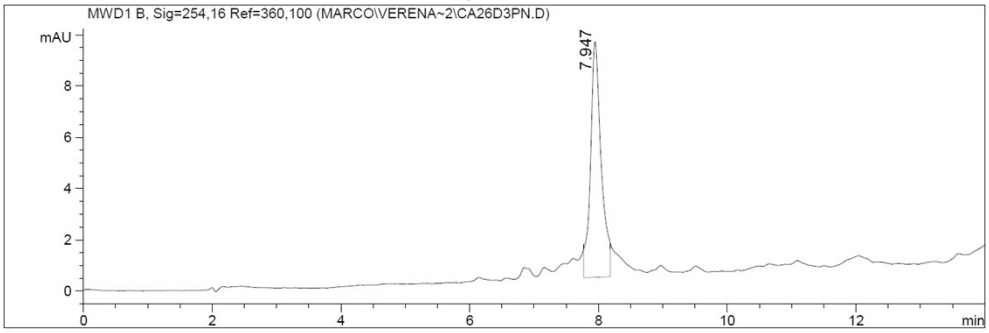
HPLC trace of crude reaction mixture 25o (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.715	0.159	105.996	1010.774	42.032
6.939	0.057	21.404	72.647	3.021
7.115	0.201	57.967	699.485	29.087
7.365	0.197	31.390	370.204	15.395
7.930	0.214	19.632	251.653	10.465

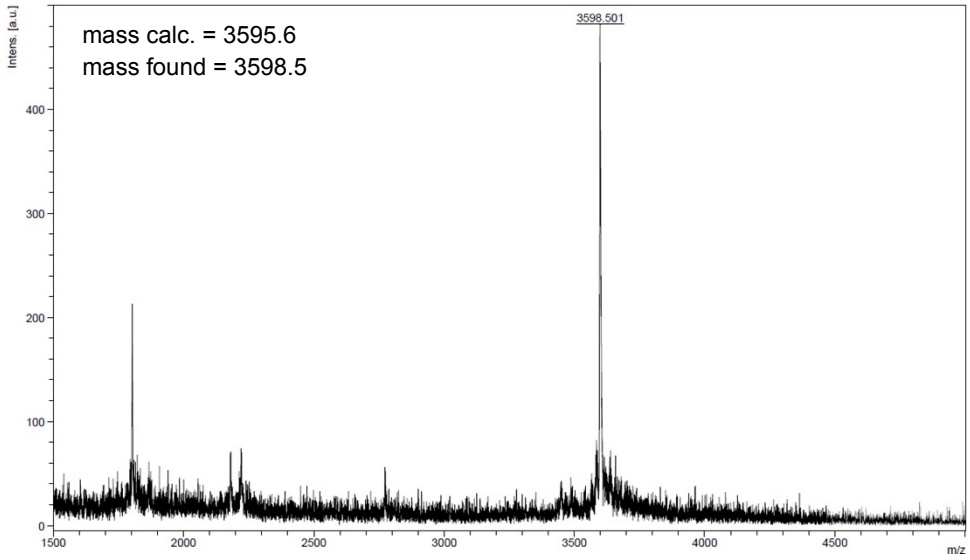
HPLC trace of isolated product 25o (Analytical RP-HPLC, Method-I)



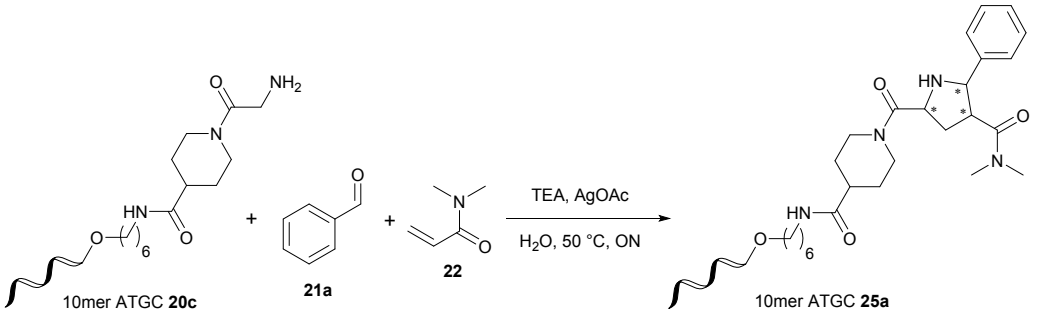
Peak list:

Ret. Time	Width min	Height	Area	Area %
7.947	0.191	9.203	105.396	100.000

MALDI-MS spectrum of isolated product 25o



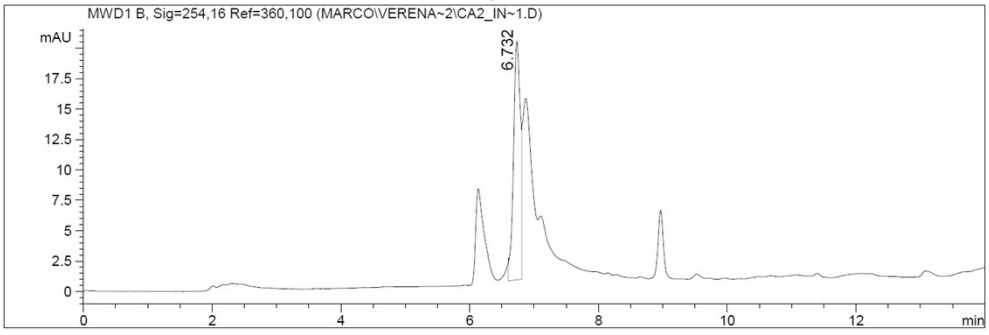
1,3-dipolar cycloaddition on oligonucleotides in solution



Scheme S2 AgOAc-mediated 1,3-dipolar cycloaddition on oligonucleotides in solution.

The DNA-glycine conjugate **20c** (500 pmol), aldehyde **21a** (500 nmol, 1000 equiv.), AgOAc (50 nmol, 1000 equiv.), *N,N*-dimethylacrylamide **22** (2 µmol, 4000 equiv.) and triethylamine (2 µmol, 4000 equiv.) were reacted in a total volume of 50 µL distilled water. The reaction mixture was shaken at 50 °C overnight. Afterwards, the mixture was diluted by adding 50 µL distilled water and extracted 6x with 200 µL ethyl acetate. The aqueous phase was dried *in vacuo* and the residue was re-dissolved in 30 µL distilled water. Ethanol precipitation was performed by adding three volumes of ethanol and storing the solution at -80 °C overnight. Centrifugation gave a DNA pellet, the supernatant was taken off, the precipitated DNA was dried in a SpeedVac and re-dissolved in 100 µL distilled water. A last purification step was performed by incubating the DNA conjugate with Chelex resin overnight. The supernatant was taken off, dried *in vacuo* and re-dissolved in 40 µL distilled water for analysis.

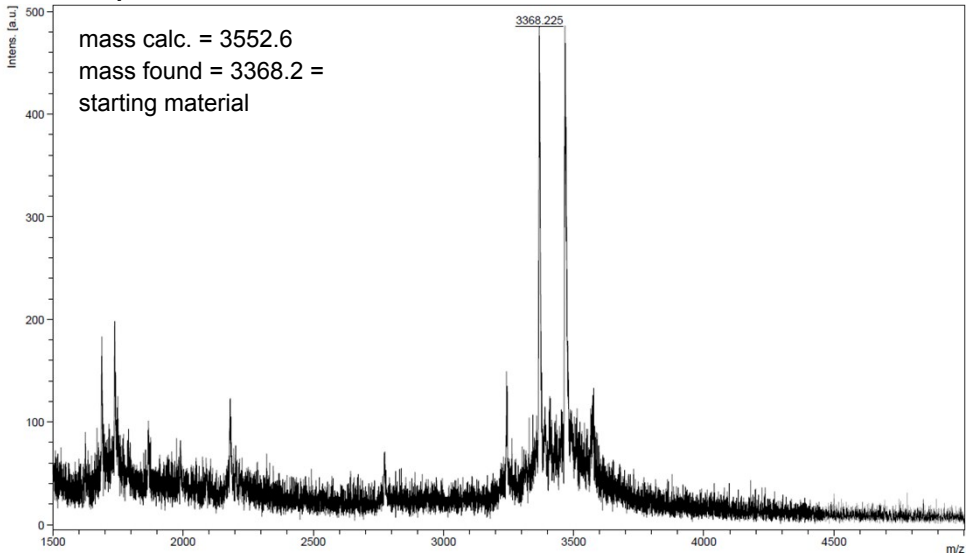
HPLC trace of crude reaction mixture (Analytical RP-HPLC, Method-I)



Peak list:

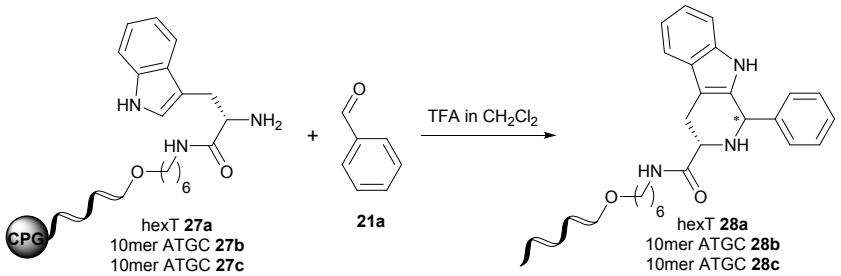
Ret. Time	Width min	Height	Area	Area %
6.732	0.124	19.656	146.322	100.000

MALDI-MS spectrum of crude reaction mixture



TFA-mediated Pictet-Spengler reaction on CPG-bound oligonucleotides

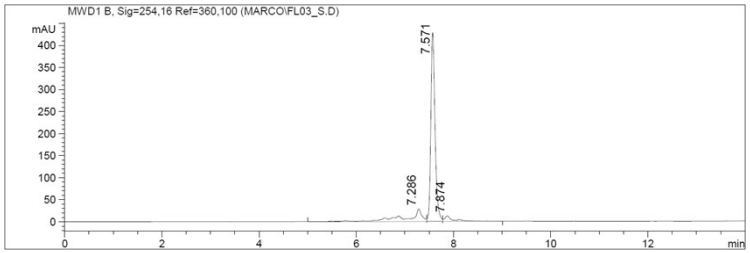
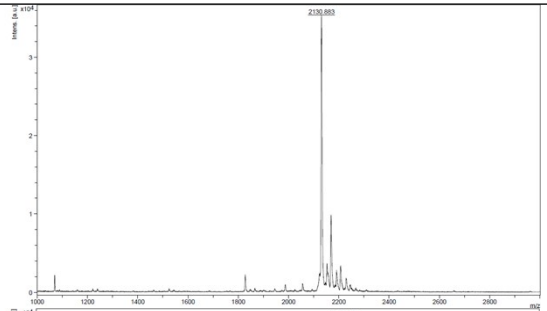
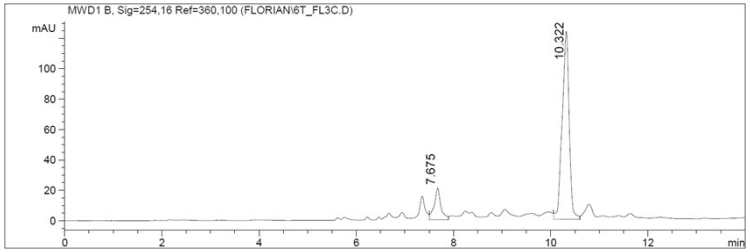
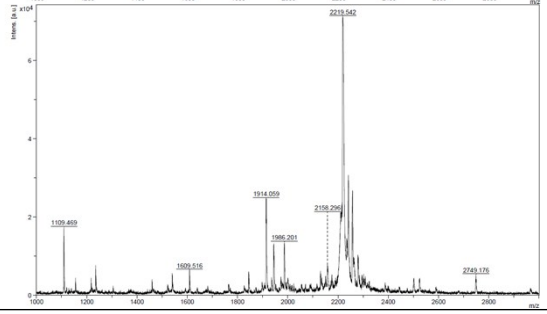
Table S5 Investigations towards TFA-mediated Pictet-Spengler reaction on CPG-bound oligonucleotides.[a]

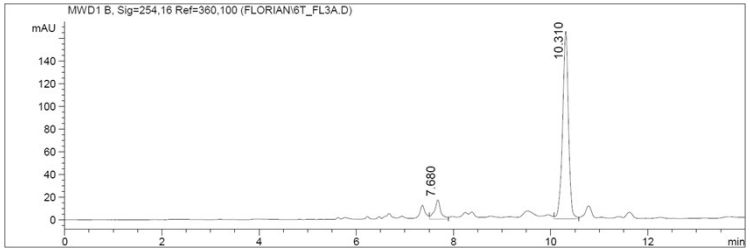
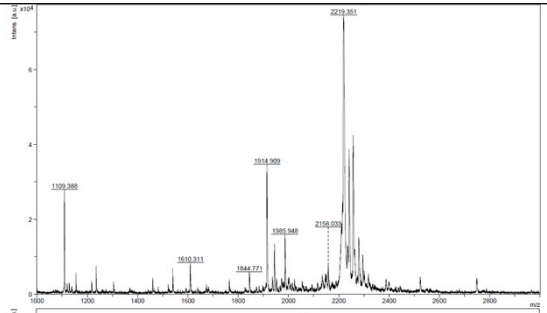
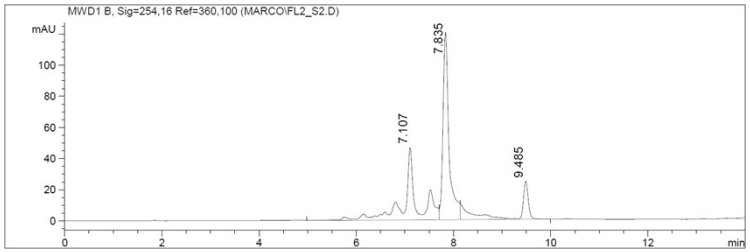
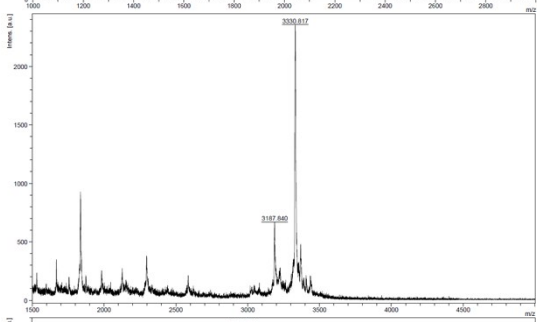
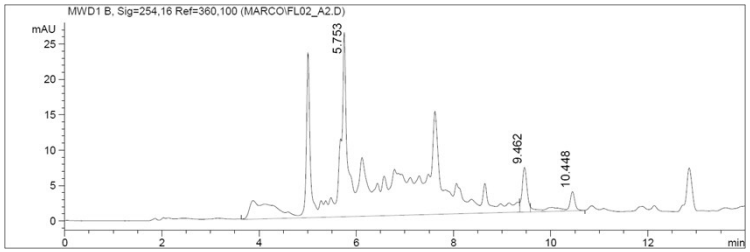
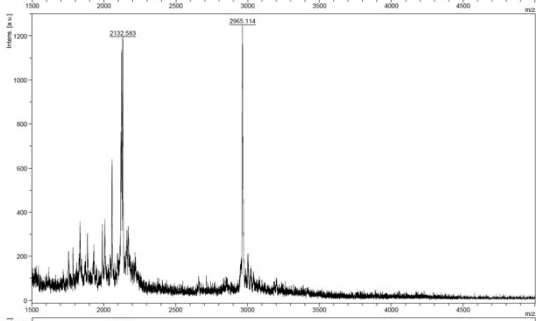
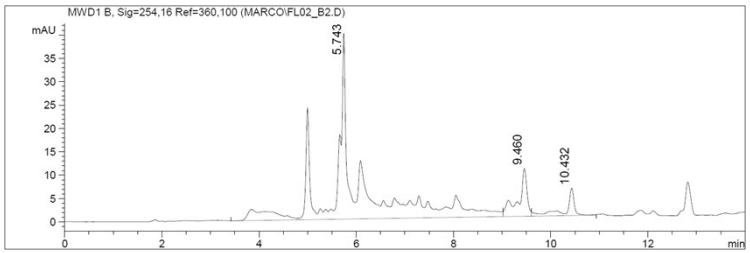
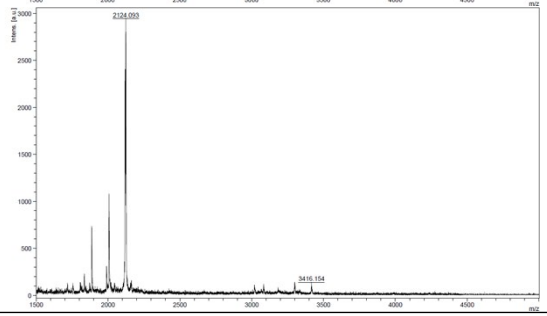


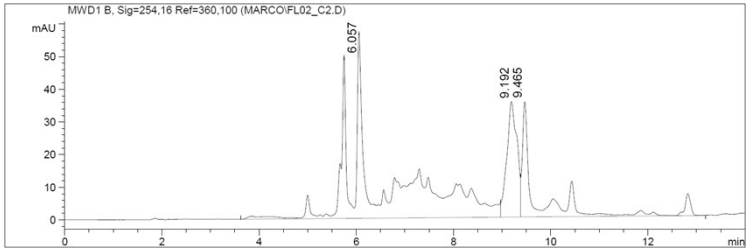
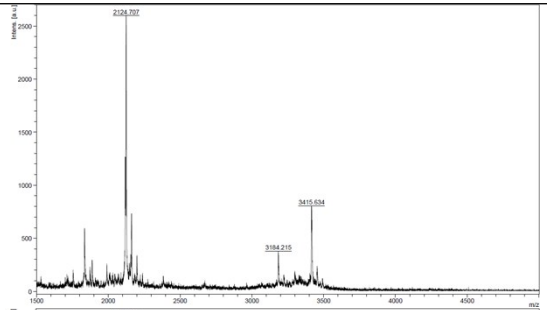
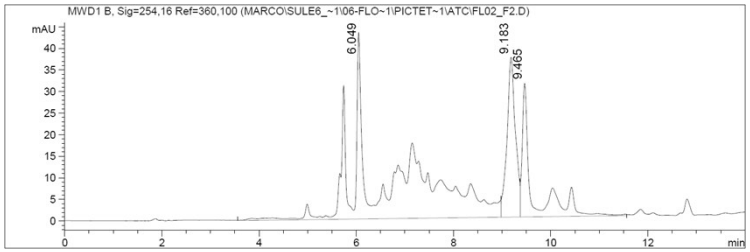
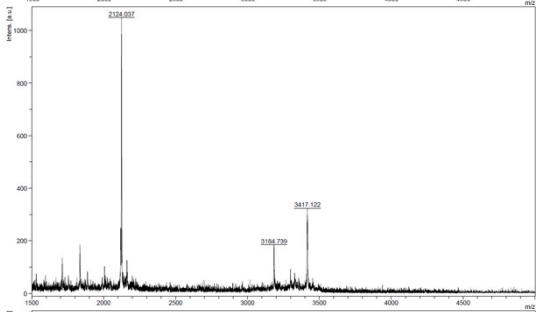
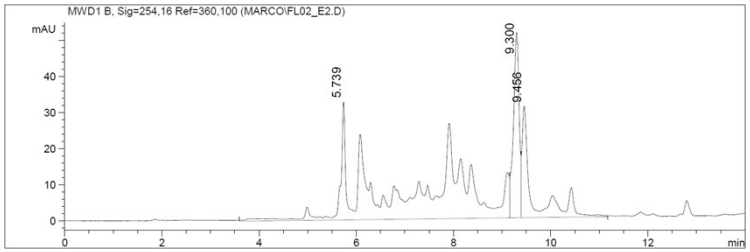
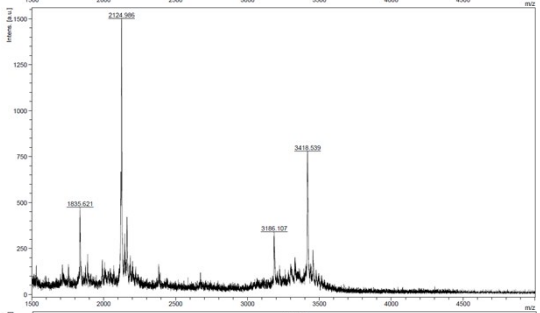
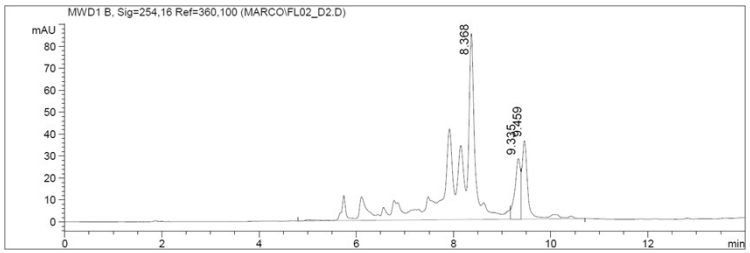
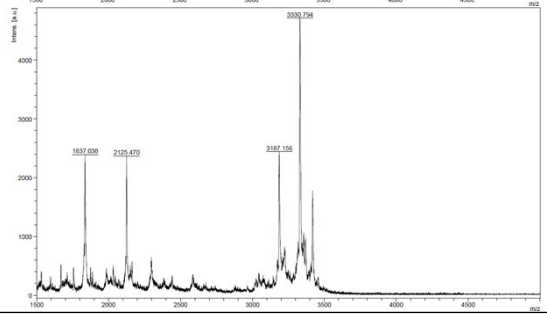
hexT **27a**
10mer ATGC **27b**
10mer ATGC **27c**

21a

hexT **28a**
10mer ATGC **28b**
10mer ATGC **28c**

Entry	Reaction conditions[b]	HPLC trace of crude reaction mixture[c]	MALDI-MS of crude reaction mixture
1	hexT-Trp **27a** mass calc. = 2128.6 mass found = 2130.9		
2	hexT-Trp **27a**, ***10 %*** TFA rt, 18 h => yield 52 % => degradation <5 % mass calc. = 2216.7 mass found = 2219.5		

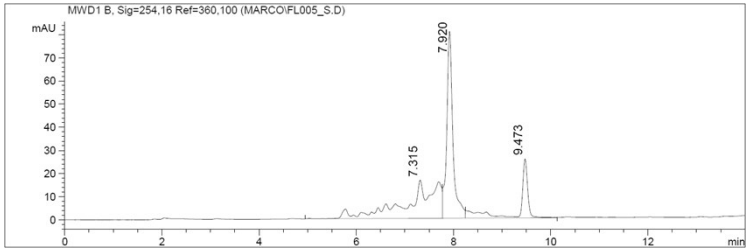
3	hexT-Trp **27a**, ***2 %*** TFA rt, 18 h => yield 60 % => degradation <5 % mass calc. = 2216.7 mass found = 2219.4		
4	ATC-Trp **27b** mass calc. = 3327.4 mass found = 3330.8		
5	ATC-Trp **27b**, ***2 %*** TFA rt, 18 h => yield = n.d. => degradation >95 % mass calc. = 3415.5 mass found = n.d.		
6	ATC-Trp **27b**, ***1 %*** TFA rt, 18 h => yield = n.d. => degradation >95 % mass calc. = 3415.5 mass found = n.d.		

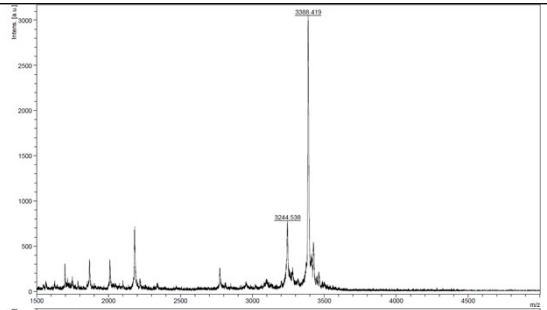
7	ATC-Trp **27b**, ***0.5 %*** TFA rt, 18 h => yield 17 % => degradation 73% mass calc. = 3415.5 mass found = 3415.6		
8	ATC-Trp **27b**, ***0.2 %*** TFA rt, 18 h => yield 17 % => degradation 73 % mass calc. = 3415.5 mass found = 3417.1		
9	ATC-Trp **27b**, 0.5 % TFA rt, ***4 h*** => yield 16 % => degradation 60 % mass calc. = 3415.5 mass found = 3418.5		
10	ATC-Trp **27b**, 0.5 % TFA rt, ***1 h*** => yield 8 % => degradation 40 % mass calc. = 3415.5 mass found = 3421.7		

ATGC-Trp **27c**

11

mass calc. = 3381.7
mass found = 3388.4

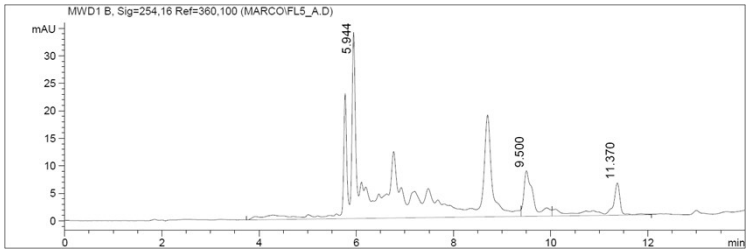


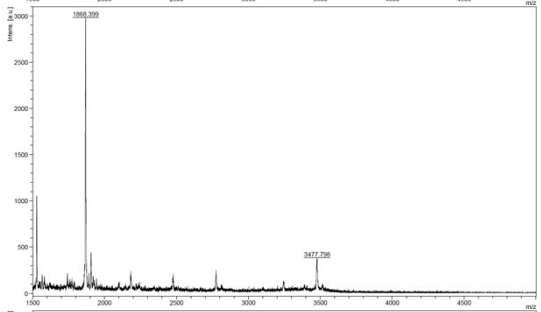


ATGC-Trp **27c**, 0.5% TFA
rt, ***18 h***

12 => yield = n.d.
=> degradation 91 %

mass calc. = 3469.7
mass found = n.d.

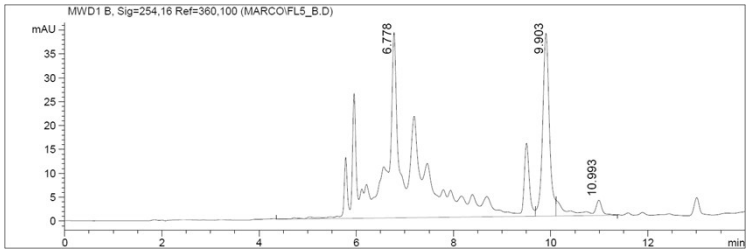


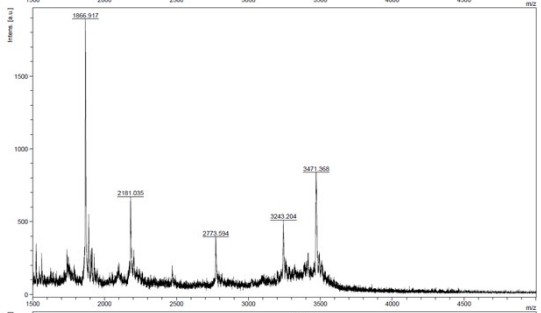


ATGC-Trp **27c**, 0.5 % TFA
rt, ***4 h***

13 => yield 18 %
=> degradation 73 %

mass calc. = 3469.7
mass found = 3471.4

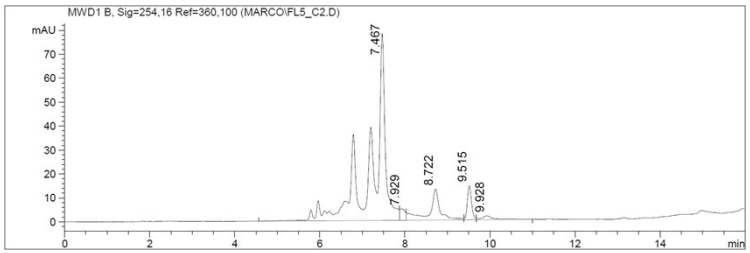


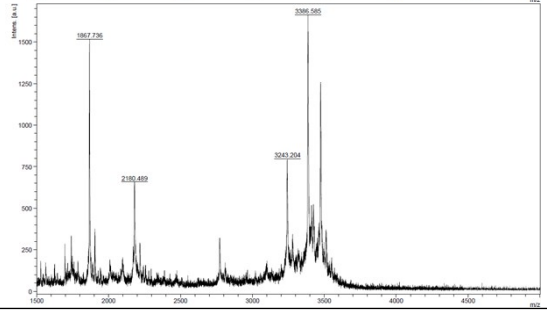


ATGC-Trp **27c**, 0.5 % TFA
rt, ***1 h***

14 => yield <5 %
=> degradation 73 %

mass calc. = 3469.7
mass found = n.d.

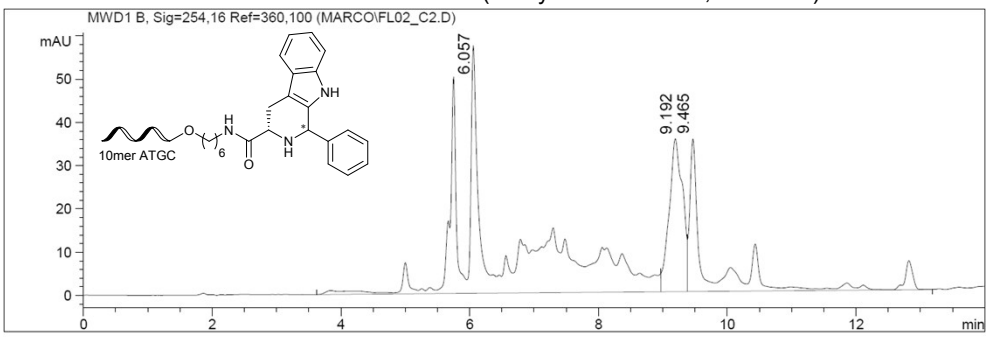




[a] CPG-bound oligonucleotide conjugate **27** (20 nmol), benzaldehyde **21a** (1000 equiv., 20 µmol) and trifluoroacetic acid (X %) in 50 µL CH_2Cl_2 at ambient temperature. Afterwards AMA (30 % aqueous ammonia/40 % aqueous methylamine, 1:1 (vol/vol)) at ambient temperature for 4 h. [b] parameters that were changed are in bold and italic.. [c] Analytical RP-HPLC, Method-I.

DNA conjugate 28b: CPG-bound 10mer ATGC-Trp-NHFmoc conjugate **27c** was reacted with benzaldehyde **21a** and trifluoroacetic acid according to RP-05.

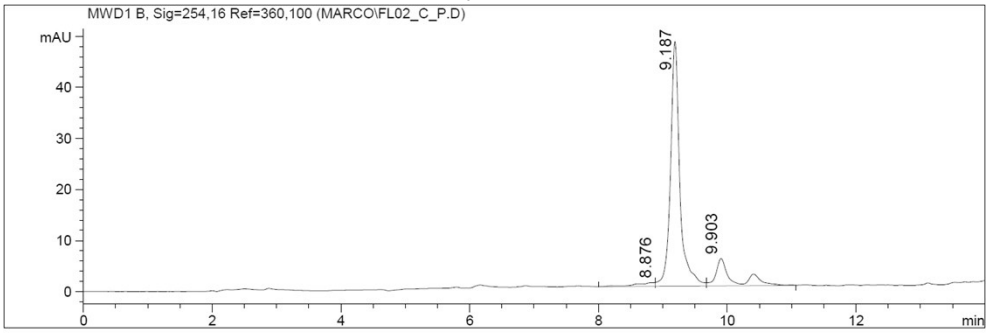
HPLC trace of crude reaction mixture 28b (Analytical RP-HPLC, Method-I)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.057	0.585	57.377	2013.654	64.616
9.192	0.243	35.354	515.638	16.546
9.465	0.277	35.347	587.048	18.838

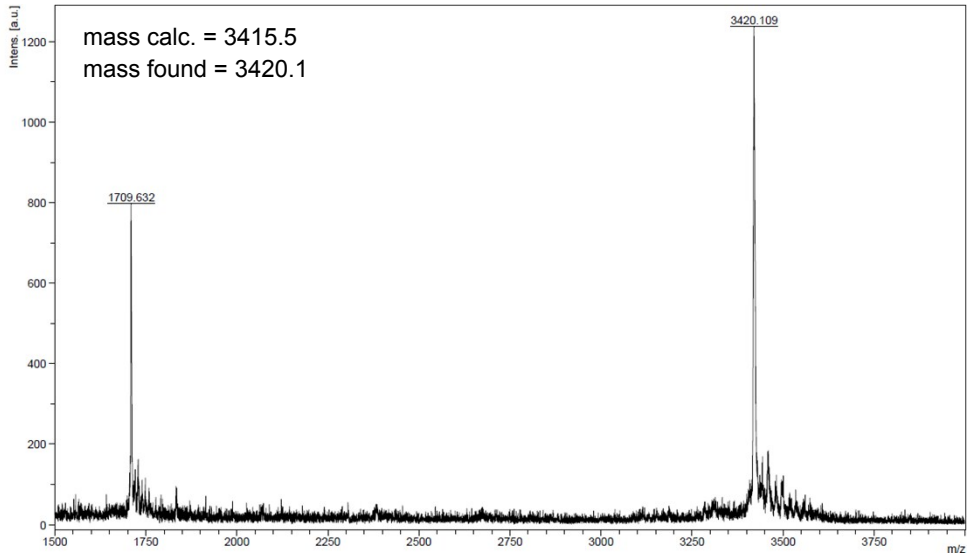
HPLC trace of isolated product 28b (Analytical RP-HPLC, Method-I)



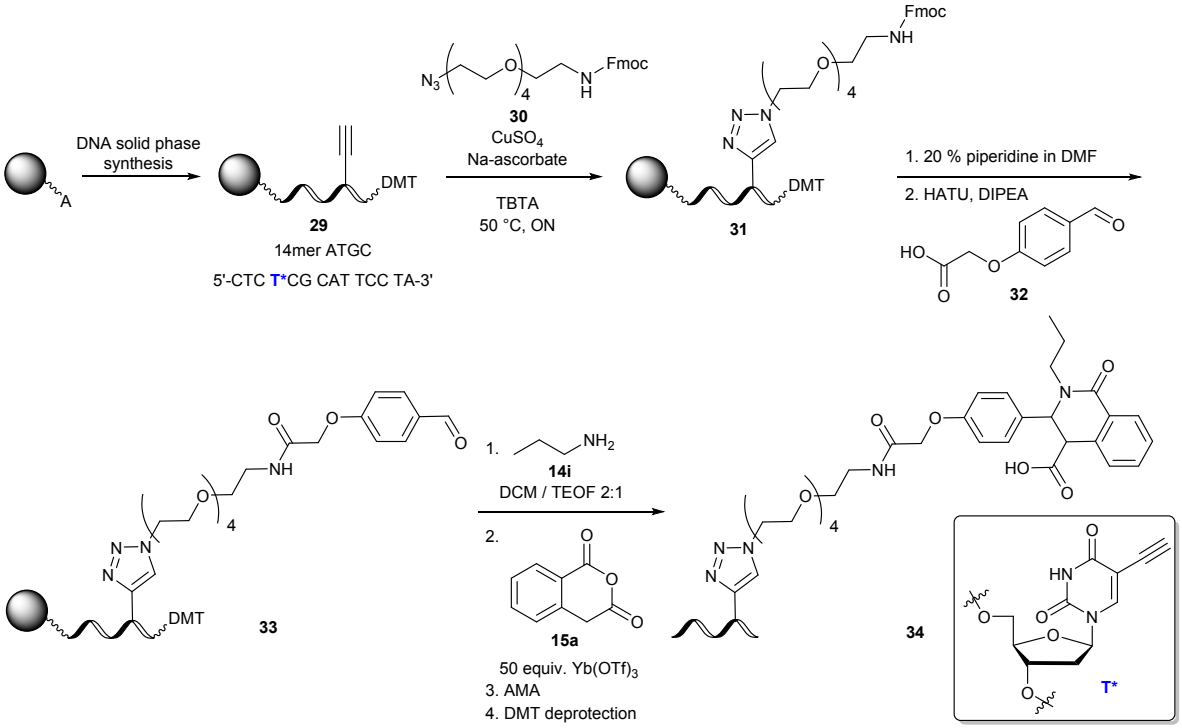
Peak list:

Ret. Time	Width min	Height	Area	Area %
8.876	0.348	0.717	14.958	2.521
9.187	0.168	47.965	483.235	81.437
9.903	0.296	5.359	95.195	16.043

MALDI-MS spectrum of isolated product 28b



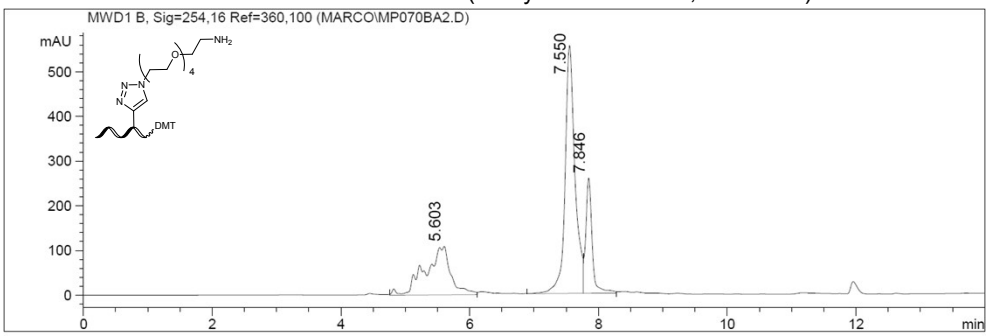
Castagnoli-Cushman reaction on a CPG-bound coding DNA oligonucleotide



Scheme S3 Castagnoli-Cushman reaction on a 14mer DNA oligonucleotide with linker moiety positioned in the sequence.

DNA conjugate 31: CPG-bound DNA-alkyne conjugate was reacted with 1-Fmoc-14-Azido-3,6,9,12-tetraoxatetradecan-1-amine according to RP-06.

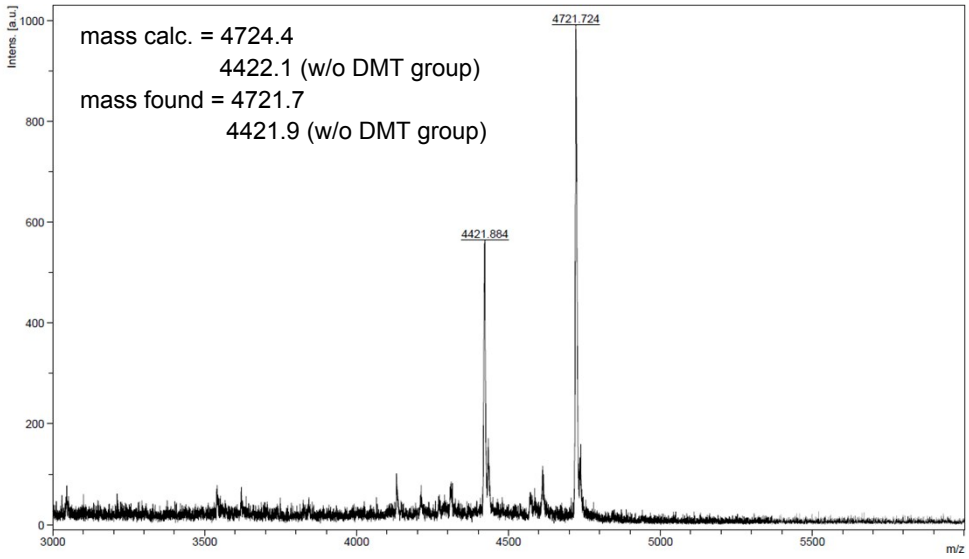
HPLC trace of crude reaction mixture 31 (Analytical RP-HPLC, Method-II)



Peak list:

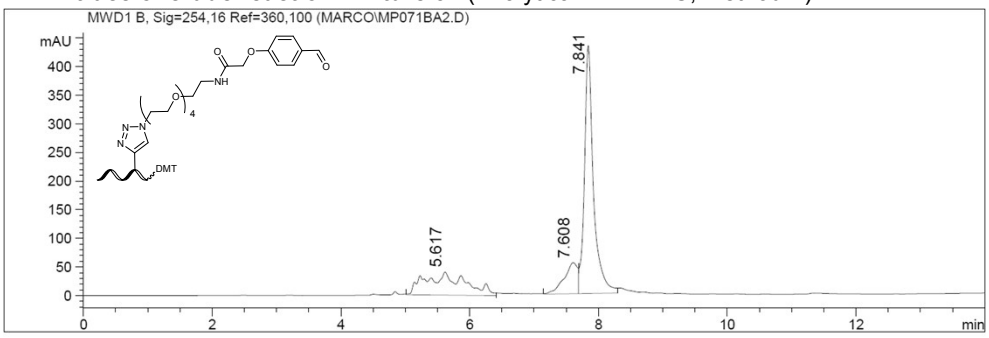
Ret. Time	Width min	Height	Area	Area %
5.603	0.452	108.794	2949.263	27.714
7.550	0.178	555.743	5921.981	55.649
7.846	0.114	258.728	1770.348	16.636

MALDI-MS spectrum of crude reaction mixture 31



DNA conjugate 32: CPG-bound DNA-PEG_4 conjugate was reacted with 2-(4-formylphenoxy)acetic acid according to RP-02.

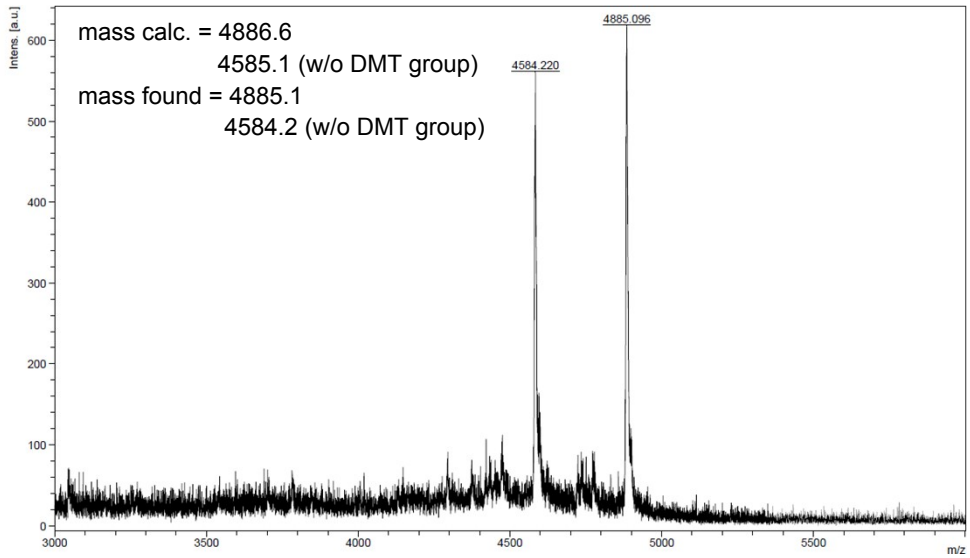
HPLC trace of crude reaction mixture 32 (Analytical RP-HPLC, Method-II)



Peak list:

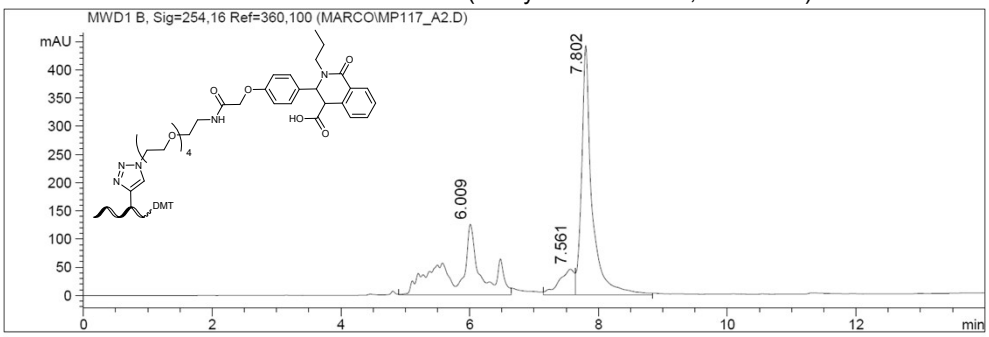
Ret. Time	Width min	Height	Area	Area %
5.617	0.710	40.448	1722.062	25.758
7.608	0.243	54.105	789.454	11.808
7.841	0.161	433.448	4174.140	62.434

MALDI-MS spectrum of crude reaction mixture 32



DNA conjugate 33: CPG-bound DNA-aldehyde conjugate **32** was reacted with aniline **14a** and homophthalic anhydride **15a** according to RP-03 followed by DMT removal using 40% AcOH for 30 min.

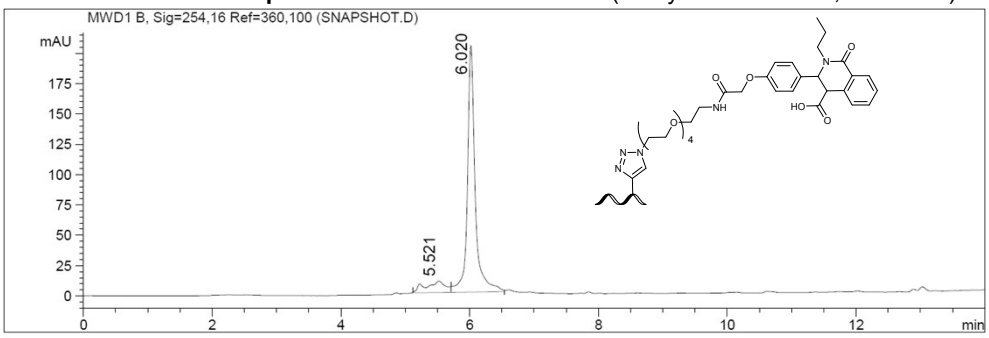
HPLC trace of crude reaction mixture 33 (Analytical RP-HPLC, Method-II)



Peak list:

Ret. Time	Width min	Height	Area	Area %
6.009	0.486	125.457	3657.017	39.281
7.561	0.277	45.529	757.085	8.132
7.802	0.184	442.589	4895.881	52.587

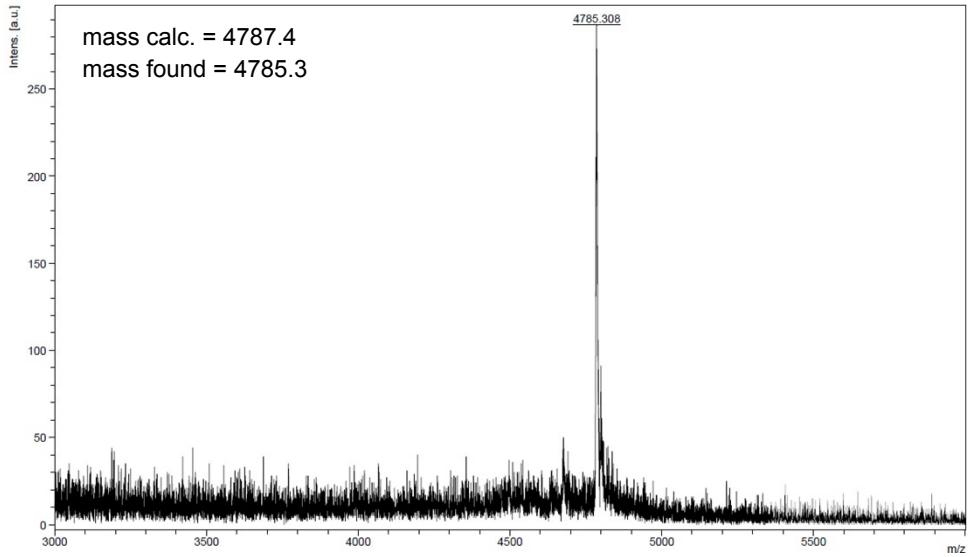
HPLC trace of isolated product 33 after DMT removal (Analytical RP-HPLC, Method-II)



Peak list:

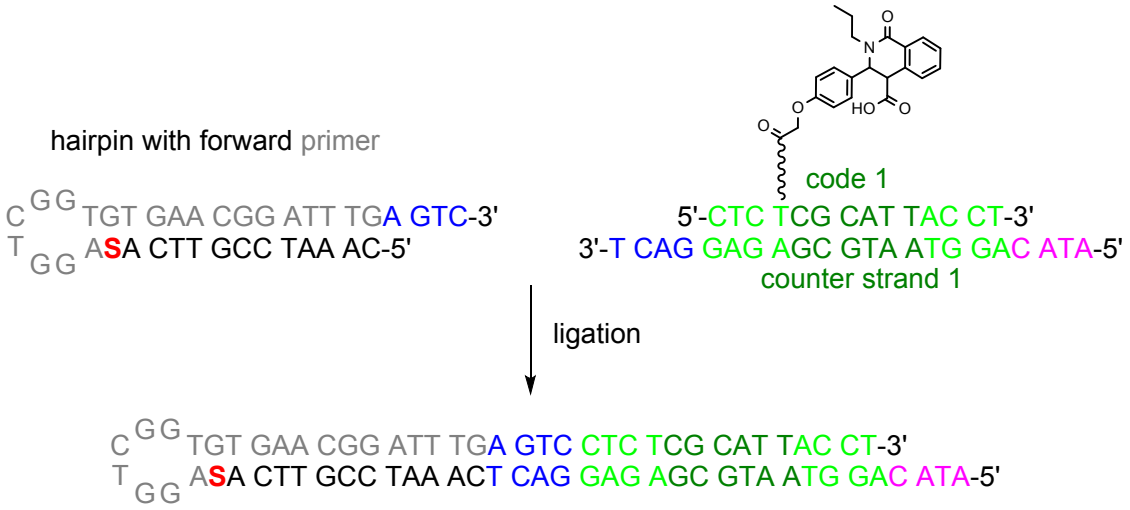
Ret. Time	Width min	Height	Area	Area %
5.521	0.354	9.477	201.100	10.568
6.020	0.139	203.478	1701.798	89.432

MALDI-MS spectrum of isolated product 33



DNA ligation

General Methods and Materials



	DNA sequence
Hairpin	5'-CAA ATC CGT TCA S AGG TCG GTG TGA ACG GAT TTG AGT C-3'
Code 1	5'-CTC TTT ACC TAC CT-3'
Counter strand 1	5'-ATA CAG GTA GGT AAA GAG GAC T-3'

S = C_6-Spacer

5'-phosphorylation of DNA

For 5'-phosphorylation of 280 pmol DNA in a total reaction volume of 20 µL, 10 units of T4 polynucleotide kinase (T4 PNK, *Thermo Fisher Scientific*), 1x PNK Buffer A (500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 1 mM spermidine, pH = 7.6, 25 °C, *Thermo Fisher Scientific*) and 1 mM ATP (*Thermo Fisher Scientific*) were used. Reaction mixtures were incubated at 37 °C for 20 min, then heat-inactivated at 75 °C for 15 min and slowly cooled down to 4°C.

Ligation of DNA

Prior to enzymatic ligation of DNA, the oligonucleotides were annealed by incubation at 85 °C for 10 min and cooling down to 4 °C. For ligation (20 µL scale), 100 pmol of each oligonucleotide, 600 units of T4 DNA Ligase (T4 DNA ligase rapid, *Biozym*) and 1x T4 DNA Ligase Buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, pH = 7.6 at 25°C, *Biozym*) were mixed. Ligation reactions were performed at 25 °C for 16 h, then stopped by heat inactivation at 75 °C for 15 min and cooled down to 4 °C.

Analysis of DNA ligation

For analysis of DNA ligation reactions, agarose gel electrophoresis was performed using a 5.5 % agarose gel. Electrophoresis was carried out in TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH = 8.3) at 100 V constant voltage for 15 min and then 150 V constant voltage for about 45 min. For staining of the DNA, Midori Green Direct (*NIPPON Genetics*) and as a reference, GeneRuler Ultra Low Range DNA Ladder (*Thermo Fisher Scientific*) was used. Imaging of the gels was performed using the *Bio-Rad Gel Doc™ XR system*.

Purification of DNA

DNA was precipitated by adding 1/10 volume of 3 M aq. sodium acetate (pH = 5.2) and 3 volumes of 100% ethanol and incubating this solution for about 4 h at -80 °C. Afterwards the samples were centrifuged at 4°C for 30 min (13200 rpm; *Centrifuge 5415 R, Eppendorf*), the supernatant was taken off and the DNA pellets were dried. The DNA samples were dissolved in water for analysis by agarose gel electrophoresis.