Electronic Supporting Information

Development of a Two-Phase Flow Reaction System for DNA-Encoded Amide Coupling

Robin Dinter,[*a] Suzanne Willems,[*b] Mahdi Hachem,[a] Yana Streltsova,[b] Andreas Brunschweiger[b] and Norbert Kockmann[a]

a) TU Dortmund University, Department of Chemical and Biochemical Engineering, Laboratory of Equipment Design, Emil-Figge-Str. 68, 44227 Dortmund, Germany

b) TU Dortmund University, Department of Chemistry and Chemical Biology, Medicinal Chemistry, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

Content

1. Materials and instruments 2
2. Coiled flow inverter (CFI) design 3
   Theory and equations for calculation 3
   Technical drawings for construction 5
3. Experimental setup 6
   Batch reactions 6
   Slug flow generation 8
4. Oligonucleotide RP-HPLC chromatograms and MALDI-MS spectra 12
   DNA-recovery 12
   Flow rate optimization 12
   Profiling 16
5. References 21

1. Materials and instruments

Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany), Bachem (Bubendorf, Switzerland), Enamine (Kyiv, Ukraine), Fluka (München, Germany), abcr (Karlsruhe, Germany), Thermo Fisher Scientific (Karlsruhe, Germany), TCI Chemicals (Eschborn, Germany) and VWR (Langenfeld, Germany). DNA in solution was purchased from Integrated DNA Technologies (Leuven, Belgium). The modified DNA used in this work is a 14mer-Aminolink-DNA (Integrated DNA Technologies, Coralville, Iowa, USA) with the sequence 5'-/5AmMC6/GTC TTG CCG AAT TC-3'. The modification on the 5'-end is an Amino Modifier C6 characterized by the NH_2 molecule at the very end. The letters A, C, G, and T correspond to the nucleobases Adenine, Cytosine, Guanine, and Thymine, respectively. This 14mer-DNA has a molar mass of 4409 g mol^{-1} and is stored at -20 °C. Since the DNA solution comes very concentrated from the manufacturer, it needs to be diluted before use. This is done by taking 400 µL of the DNA solution and diluting it in 1000 µL of dH_2O (distilled water). The resulting concentration amounts to about 180 µM, measured in NanoDrop, which measures the UV-Vis absorption of DNA at 260 nm. For a reaction containing 500 pmol of DNA, about 2.78 µL of the diluted solution is used per sample, compared to 8.33 µL for a reaction containing 1500 pmol of DNA.

As continuous phase (CP), which does not interfere with the reactants, and provides a reliable spatial separation between slugs, the inert liquid Fluorinert™ FC-40 (Sigma-Aldrich, F9755-100ML, Germany) was chosen. It is a thermally stable, colorless, fluorinated liquid which can be used as a CP in liquid-liquid two-phase flow reactions due to its immiscibility with other liquids and its inert nature. The density of FC-40 amounts ρ (25°C) = 1855 kg m^{-3}, much denser than water, the viscosity is v = 2.2 cSt and its molecular weight is equal to 553.1 g mol^{-1} according to the data sheet[1]. Since FC-40 is quite expensive and has a high global warming potential (GWP) of over 6000, recycling the CP is needed. Therefore, the FC-40 phase settles down at the bottom of the reagent glass, while the aqueous phase consisting of black food coloring (DECOCINO, Berlin, Germany) settles down at the top in a matter of seconds after the two phases encounter each other, as shown in Figure S1.

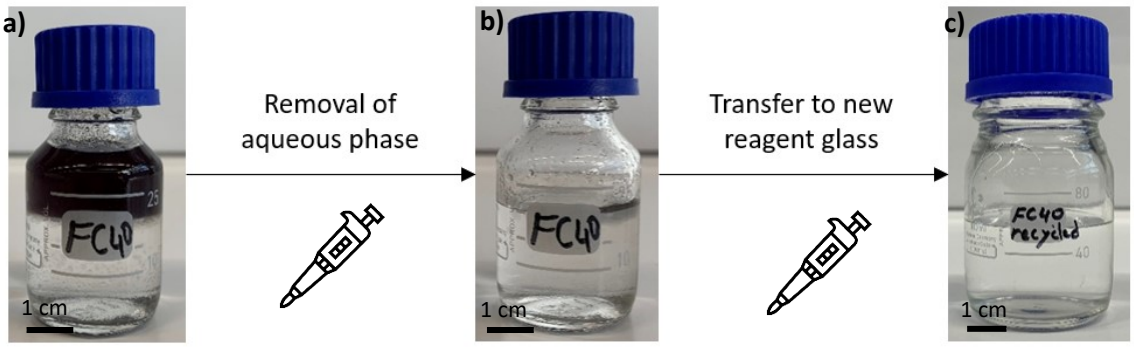


Figure S1 - Recycling of FC-40. a) shows the FC-40 phase settling at the bottom when it is mixed with a black aqueous phase (food coloring). b) shows the FC-40 phase after removal of the aqueous phase. c) shows the fully recycled FC-40 phase.

The aqueous phase can then be dismissed with a syringe or a pipette, and the now recycled FC-40 can be transferred to a clean reagent glass to be used again.

Oligonucleotide-small molecule conjugates were analyzed by ion pair reverse phase high-pressure liquid chromatography (HPLC, Infinity II 2460, Agilient) using a C18 stationary phase (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*4.6 mm) and a gradient of 10 mM aqueous triethylammonium acetate/MeOH. HPLC traces were recorded at 254 nm wavelength. Oligonucleotide concentrations were determined by UV-Vis spectroscopy using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). Oligonucleotides were analyzed by MALDI-MS (Bruker Daltonics) using HPA matrix.

2. Coiled flow inverter (CFI) design

For designing the CFI modules, the parameters and geometries are calculated by the equations in the first subsection[2–4]. The technical drawings of the CFI and modules used in this work is elaborated in the second subsection.

Theory and equations for calculation

All equations and calculations for designing CFI are given in the following literature and includes the number of bends n_b between the modules, the pitch distance p between two turns, the coil diameter d_c, the inner diameter d_i of the chosen tube, and the length of a single coil L_c[2–4].

First, the coil tube diameter d_{ct} with Equ. S1, where s is the wall thickness of the tube:[2–4]

$$d_c = d_i + 2s + d_{ct} \qquad \text{Equ. S1}$$

Due to their coiled structures, the flow inside the tube of a CFI creates a centrifugal force, which perturbs the laminar velocity profile. This perturbation leads to a secondary flow profile known as the Dean vortices. These vortices enhance the radial mixing inside the fluid and lead to a narrower RTD at laminar flow regimes. [2–4]

The flow regime is described by the Reynolds number, which can be defined as the ratio of inertial to viscous forces. The Re number is defined in Equ. S2, where *u* is the flow speed, *L* is the characteristic dimension of the reactor and *v* the kinematic viscosity of the fluid.[2–4]

$$Re = \frac{u\,L}{v} \qquad \text{Equ. S2}$$

Low Re numbers below 2000 indicate that the flow is laminar, whereas higher Re numbers above 2000 indicate that the flow is turbulent. In addition to the Reynolds number, the curvature λ and the torsion parameter T^* of a CFI play a defining role in inducing Dean vortices inside the coiled tube. The secondary flow in CFI is therefore characterized by the Dean number (Dn*)*, which is shown in Equ. S3. For Dean vortices to occur, the Dn number has to be above 3.[2–4]

$$Dn = Re\sqrt{\frac{1}{\lambda}} = Re\sqrt{\frac{d_c}{d_i}} \qquad \text{Equ. S3}$$

Along with the curvature, the formation of Dean vortices is affected by the torsion of a CFI, which is given by the modified torsion parameter T^* in Equ. S4. In order to ensure high radial mixing, the modified torsion parameter should be above 1000.[2–4]

$$T^* = Re\frac{\pi\, d_c}{p} \qquad \text{Equ. S4}$$

As can be seen, the Dean number Dn and the modified torsion parameter T^*, which are related to the geometrical aspects of a CFI, are directly proportional to the Reynolds number (Re), which is related to the properties of the fluid.[2–4]

The capillary microreactor was made of an FEP tube with an inner diameter d_i of 1 mm. Further dimensions and a wall thickness *s* of 0.3 mm, around the CFI modules with a pitch distance *p* of 1.6 mm which was determined by the tube geometry with the minimal pitch distance being the outer tube diameter d_o[5,6]. Another crucial change in the original design was an L-shaped connector between two modules, ensuring a fixed 90° angle between two CFI modules. In Figure S2, the design-space diagram shows an area by specifying the coil tube diameter d_{ct} and inner tube diameter d_i as a function of the volumetric flow rate[5]. For producing Dean vortices, two design criteria for Dean number Dn > 3 and modified torsion parameter T* > 1000 needed to be met, which depended on the volumetric flow rate[3,4]. In Figure S2, the gray area indicates the feasible CFI area, whereas the dark gray area shows the range at the minimum volumetric flow rate. The design space was increased with rising volumetric flow rates and was limited by a geometric constraint depending on material and fabrication[5,6]. Figure S2 confirms that the tailored CFI for on-DNA reactions still satisfied the two design criteria (Dn > 3 & T* > 1000) and produced Dean Vortices, which represent a secondary flow profile[7]. Based on this information, the volumetric flow rates $\dot{V}$, in which Dean vortices occurred was calculated as

depicted in Figure S2. For volumetric flow rates above 1.3 mL min^{-1} enhanced mixing and a narrow RTD can be ensured. The operation range for the tailored CFI for DNA-tagged substrates is represented by a slightly gray area in Figure S2.

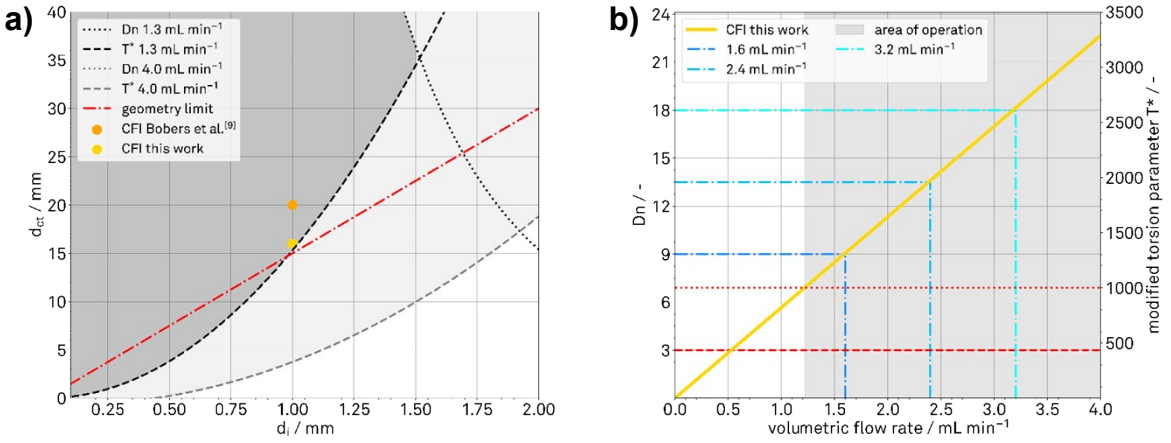


Figure S2 - *Calculation of the tailored CFI for DNA-tagged substrates: a) Design-space diagram for the two volumetric flow rates of 1.3 mL min^{-1} and 4.0 mL min^{-1}. b) Dn number and modified torsion parameter T* depending on the volumetric flow rate for the newly designed CFI module using an FEP-tube with an inner diameter of 1 mm.*

Technical drawings for construction

After the tailored CFI modules were theoretically calculated and designed according to the requirements of DNA-encoded chemistry, the computer-aided design (CAD) objects were created in Autodesk Inventor 360 (Autodesk, California, USA), as shown in Figure S3.

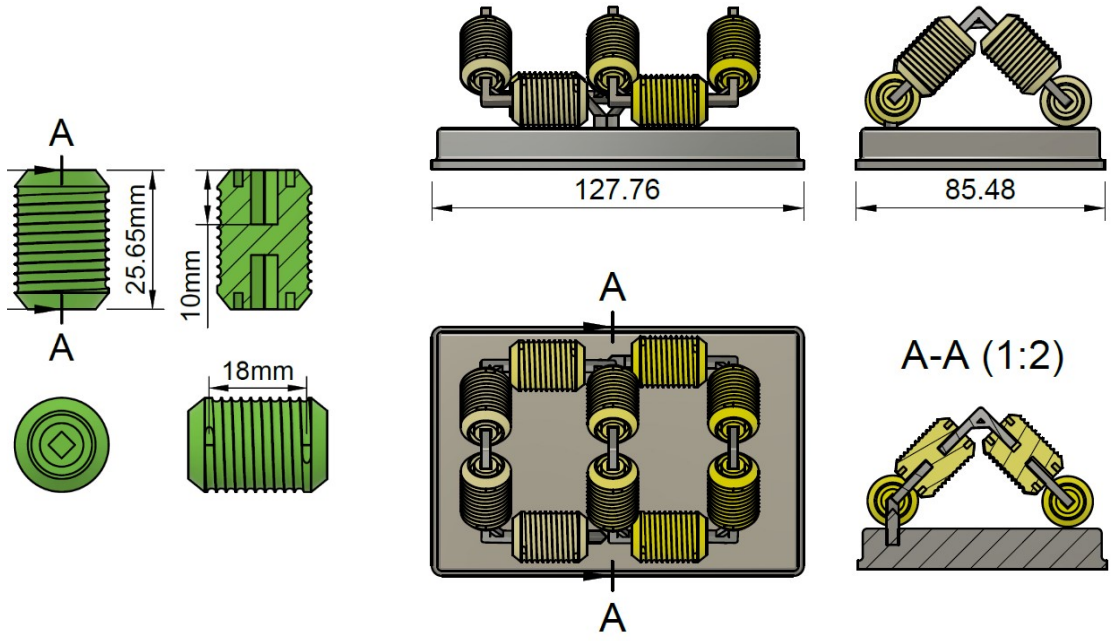


Figure S3 - CFI modules and plate holder with 10 modules (9 bends).

The CAD objects were manufactured by fused deposition modeling (FDM) 3D printing with polylactide (PLA) using an Ultimaker S5 (Ultimaker, Utrecht, Netherlands) 3D-printer.

3. Experimental setup

In this subsection, the experimental setup used during the scope of this work is described. First, the conduction of the amide coupling reactions in batch mode is presented. Second, the liquid-liquid two-phase flow setup is elaborated.

Batch reactions

For the conduction of batch reactions, the protocol written by Li *et al.*[8] is used. At first, a stock solution of 27 µmol of a carboxylic acid dissolved in 450 µL of DMSO is prepared in a 1.5 mL microliter tube. A stock solution of the organic coupling agents is then made. For that, 120 µmol (23 mg) of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 24 µmol (3.26 mg) of 1-Hydroxy-7-azabenzotriazole (HOAt), and 120 µmol (20.76 µL) of N,N-Diisopropylethylamine (DIPEA) are each dissolved in 400 µL of Dimethyl sulfoxide (DMSO). Afterward, the DNA solution is prepared by dissolving the desired amount of substance of **DNA-1** in 72 µL of 3-(N-morpholino)propanesulfonic acid (MOPS) buffer. This is done in order to neutralize and stabilize the pH value in the solution, since an acidic environment leads to loss of genetic information. In this work, a **DNA-1** amount of 500 pmol and 1500 pmol were used. When the stock solutions have been prepared, 40 µL of each of EDC, HOAt, and DIPEA are added to the carboxylic acid stock solution, as seen in Figure S4.

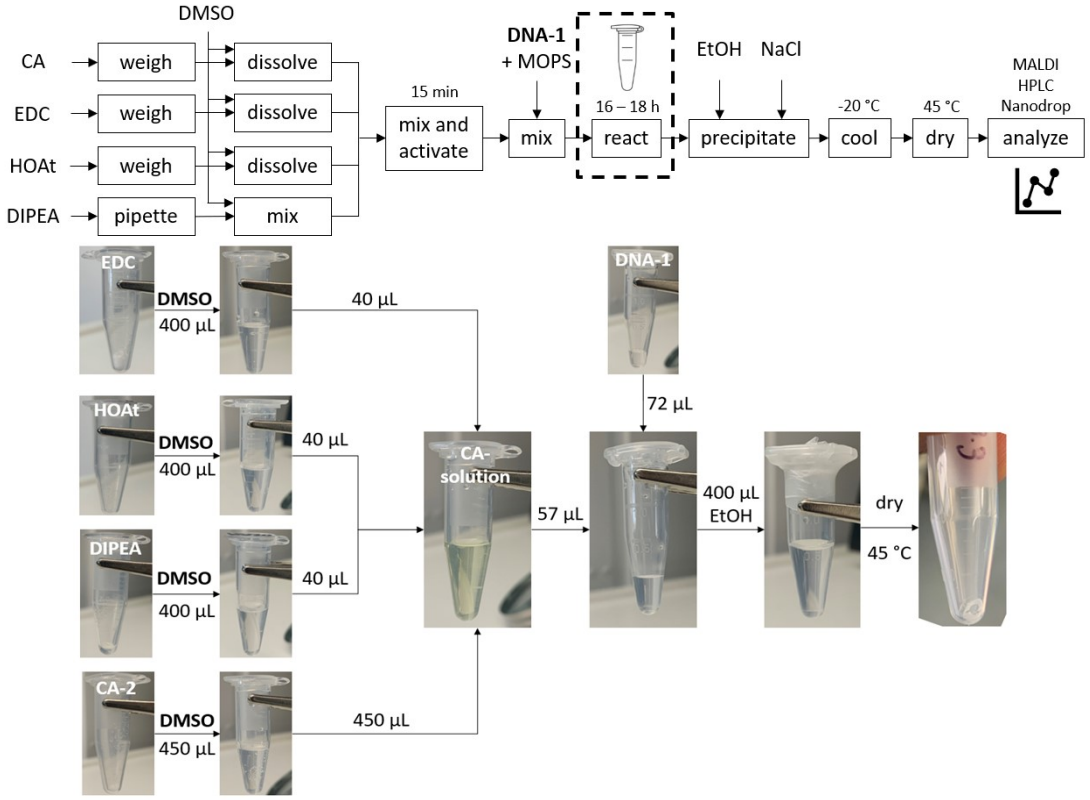


Figure S4 - Overview of the batch procedure used to conduct the amide coupling reaction of **DNA-1** with a carboxylic acid. A) This scheme is universal and can be used for the amide coupling reaction with all carboxylic acids. B) Amide coupling reaction with the **DNA-1** shown in the microliter tubes.

This addition is characterized by a yellowish solution color of the carboxylic acid solution. The mixture is then stirred on a shaker (VWR, Thermal Shake *lite*, Germany) for 15 min at room temperature of 23 °C to activate the solution. After the activation is completed, 72 µL of **DNA-1** solution and 57 µL of the carboxylic acid solution are added to each reaction sample. Thus, the total reaction volume amounts to 129 µL. The microliter tubes containing the reaction samples are transferred into a shaker, which rotates at room temperature (23 °C) at a speed of 1100 rpm. According to the literature[8], the amide coupling reaction lasts for about 16-18 h. After the reaction has finished, 400 µL of EtOH and 15 µL of NaCl are added to the reaction, in order to let the DNA-product precipitate, hindering any further reaction between the **DNA-1** and the carboxylic acid. The microliter tubes are secured with Parafilm to hinder any water from sneaking into the Ethanol phase. The samples are then stored at -20 °C overnight and then centrifuged for 30 min at 4 °C at a speed of 11,000 rpm. After centrifugation, the organic phase is removed with a Pipette, and the samples are placed in a SpeedVac (Thermo Fisher Scientific Inc., Massachusetts, USA) to dry at 45 °C. Afterward, only white pellets should be visible inside each microliter tube. These pellets are then dissolved in 40 µL dH2O and analyzed using NanoDrop, MALDI-MS, and RP-HPLC.

For the batch reactions conducted during this work, some changes to the protocol written by Li *et al.*[8] were made. For example, the necessity of the 15 min activation of the carboxylic acid was questioned. The effect of not using NaCl for precipitation was examined. Reactions were conducted for not only 16-18 h, but also 5 min, 23 min, and 45 min to check the progression of the amide coupling reaction of **DNA-1** for the different examined carboxylic acids.

Table S1 - Results of carboxylic acid and time optimization.

Entry	Carboxylic Acid	Time [min]	Yield [%]
1	**3a**	5	6
2	**3a**	45	46
3	**3a**	960	65
4	**3b**	5	70
5	**3b**	23	92
6	**3b**	45	93
7	**3c**	5	0
8	**3c**	23	0
9	**3c**	45	0
10[a]	**3a**	23	13
11[a]	**3a**	45	14
12[a]	**3a**	960	33
13	**3b**	10	49
14[b]	**3b**	10	57

a) coupling agents were left dissolved on the bench for one week. b) The carboxylic acid 3 and coupling agents HATU, HOAt and DiPEA were mixed for 15 min before addition to the DNA.

Slug flow generation

The liquid-liquid two-phase flow reactions consist of two liquid phases, a continuous phase (CP), and a dispersed phase (DP). The CP is continuously pumped into the flow reaction system (CFI) during the reaction, while the DP is pumped at the beginning along with the CP to produce the aqueous slugs with the DNA-reaction mixture. For the reaction system at hand, FC-40 is used as a CP and is placed in a 10 mL syringe on a syringe pump (LAMBDA Instruments GmbH, VIT-FIT, Switzerland). Both a glass syringe (FORTUNA, Metal Luer Lock, Germany), as well as a plastic syringe (HSW Henke-Ject, 5100.X00V0, Germany), were used for the CP. For the DP, a 250 µL GC-syringe (Hamilton, Model 825 RN, USA) is used. The correlation between the pump setting and the volumetric flow rates of the respective syringes can be found in Figure S5.

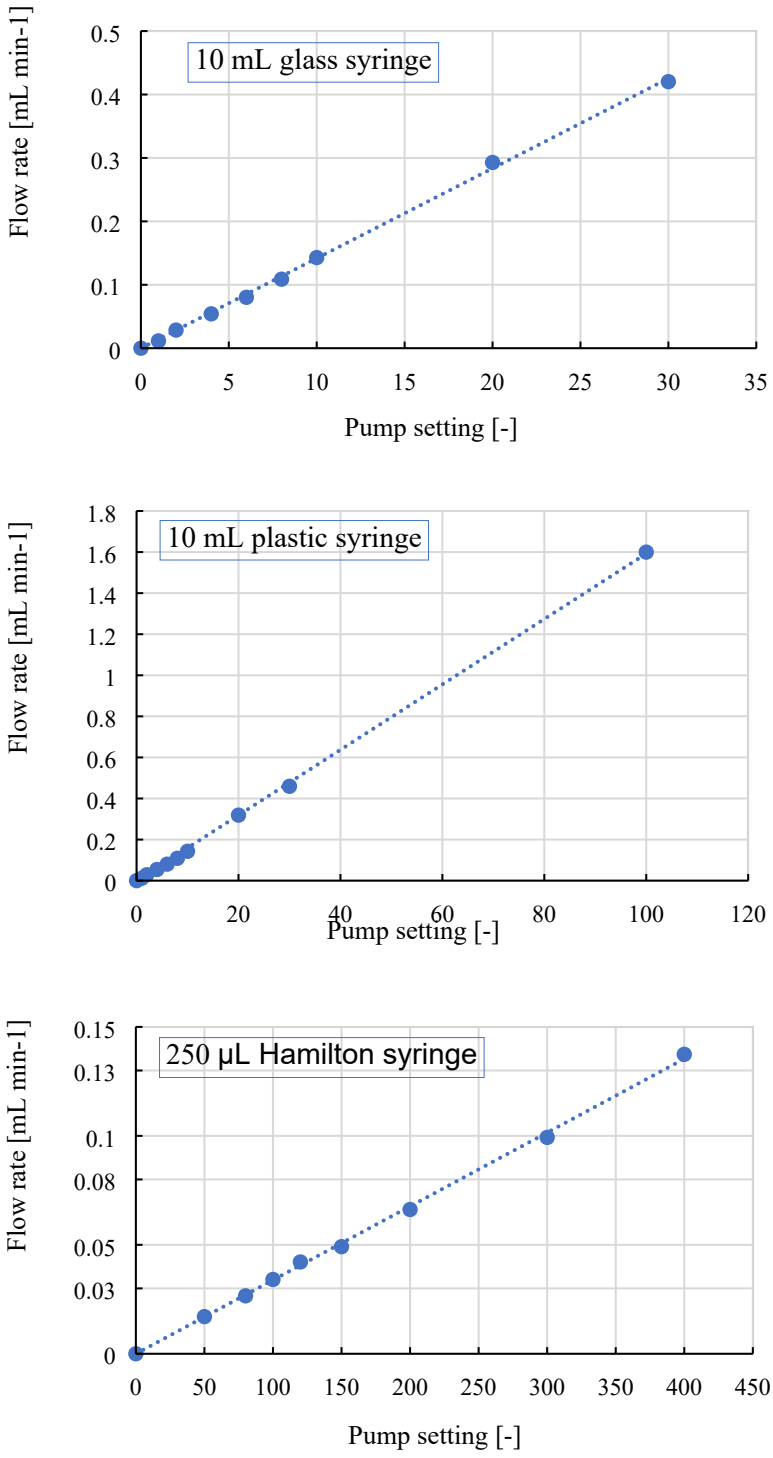


Figure S5 - Correlation between the pump setting and the volumetric flow rate. A) 10 mL glass syringe for the CP. B) 10 mL plastic syringe for the CP. C) 250 µL GC-syringe for the DP.

The weighing of the materials up to the activation of the carboxylic acid solution remains the same for the liquid-liquid two-phase flow setup as well. After the syringes have been filled, the Hamilton syringe is then connected to a T-junction and placed on the syringe pump. Also connected to the T-junction are the CP, placed on a second syringe pump, and the CFI reactor. Connected to the inlet and the outlet of the flow reactor are photoresistors developed by Höving *et al.*[2] to detect the slugs inside the system, as well as to determine the RTD in the reactor. The two most important components of the sensor

used are the LED (Tru Components, Germany) and photoresistor NSL-19M51 (Luna Innovations Inc., Roanoke, USA), as shown in Figure S6.

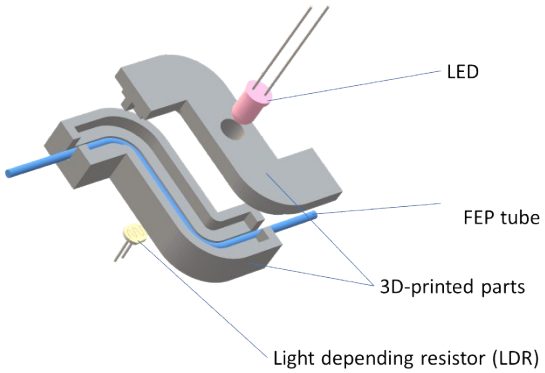


Figure S6 - 3D model of the photosensor developed by Höving *et al.*[2]. The most important components of the sensor are the LED and the photoresistor, which are built into the case. The FEP-tube is placed in an S-shape between the two 3D-printed cases.

The LED and the photoresistor are built on two opposite sides of a 3D-printed PLA housing case. The FEP tube goes inside the housing case. The S-shape of the sensor reduces stray light since the photoresistor changes its resistance depending on the incident light. The sensors are connected to an Arduino Uno microcontroller board to record the measured resistance of the sensor.[2]

For the generation of slugs the chosen flow rate for both phases is 37.6 µL min^{-1}. For that, the pump setting of the syringe pump holding the CP needs to be set at 2, and the one holding the DP needs to be set at 85, due to the different volumes of the syringes. With the chosen flow rate of 37.6 µL min^{-1}, 18 slugs of approximately 7 µL each are produced, as shown in Figure S7.

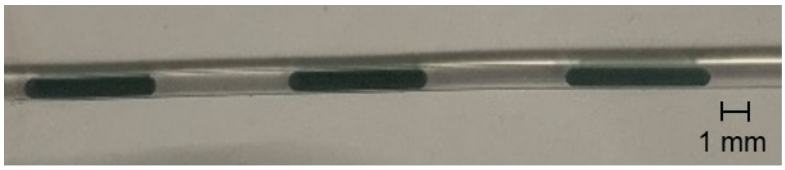


Figure S7 - Generation of slugs when both the continuous phase and the dispersed phase flow at low volumetric flows of 37.6 µL min^{-1}.

This is confirmed in Table S2, where the slug volumes were calculated using the photosensors and ImageJ depending on the volumetric flow rate of both the CP and the DP.

Table S2 - Slug volume and volume between two slugs determined with ImageJ and the photosensors depending on the volumetric flow rate of both the CP and the DP.

Flow rate [µL min^{-1}]		**ImageJ**	**Sensor**	**ImageJ**	**Sensor**
continuous phase	disperse phase	Slug volume / µL	Slug volume / µL	Volume between 2 slugs / µL	Volume between 2 slugs / µL
28.2	28.2	5.03	4.07	4.18	4.73
37.6	37.6	6.79	6.50	6.63	7.41
47.0	47.0	4.86	4.31	4.46	4.89
70.5	70.5	5.87	5.86	6.23	6.04
94.0	94.0	5.83	6.24	6.42	5.68
47.0	141.0	13.51	14.04	4.81	5.15
47.0	188.0	14.40	15.64	4.04	4.40
47.0	282.0	20.29	21.74	4.01	3.92

When all slugs have been generated, the syringe pump holding the DP is turned off, and the volumetric flow rate of the CP is adjusted according to the required reaction parameters marks the official starting time of the reaction. Since only the syringe pump of the CP remains active after the slug generation, the flow rate of the pump can be assumed to be equal to the volumetric flow of the liquids inside the reactor. Depending on the volumetric flow rate used during the reaction, the slugs can be pumped back and forth using the photosensors placed at the inlet and the outlet of the reactor. Since both the aqueous DNA reaction solution (DP) and the continuous FC-40 phase are colorless and have a very similar refractive index, it is difficult for the sensor to detect the slugs. A solution to this obstacle is injecting black food coloring, which can easily be detected by the sensors, before and after the slugs. When the food coloring is detected, the syringe pump of the CP changes the flow direction. This way, the slugs with the **DNA-1** reaction mixture situated between two food coloring slugs remain in the reactor while being pumped back and forth.

After the desired reaction time has been reached, the slugs are collected in microliter tubes containing 400 µL of Ethanol to be purified later. Naturally, the CP is also collected along with the reaction solution. Due to its immiscibility and high density, it can be carefully removed from the bottom of the microliter tube, leaving only the aqueous DNA-product mixture and Ethanol. Lastly, the samples are

secured with Parafilm and stored in the freezer overnight at -20 °C. The purification steps are the same as in the batch protocol.

4. Oligonucleotide RP-HPLC chromatograms and MALDI-MS spectra

DNA-recovery

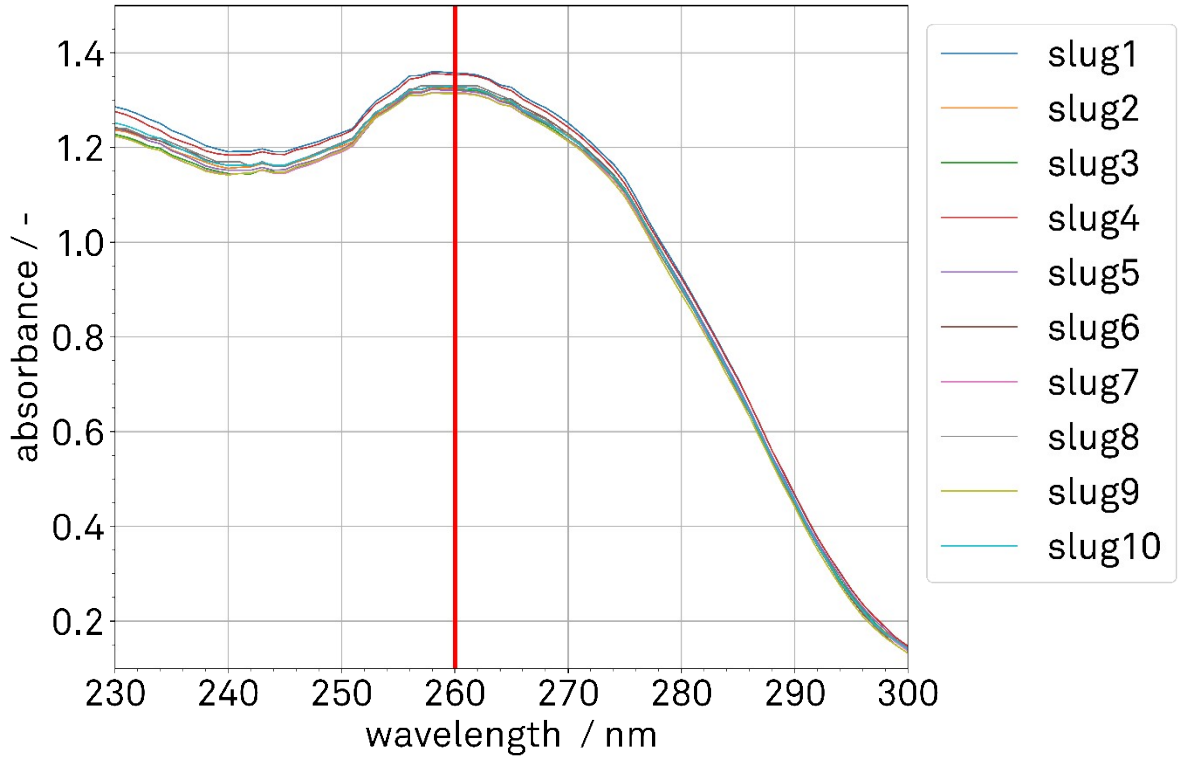


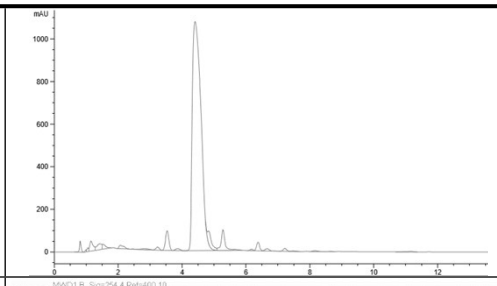
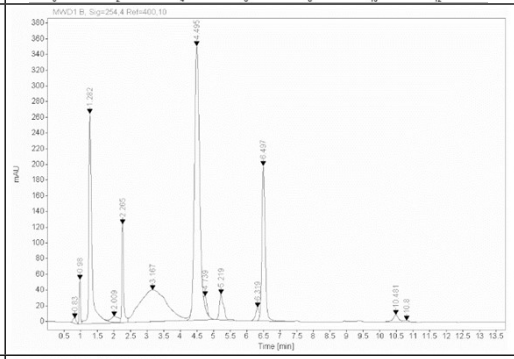
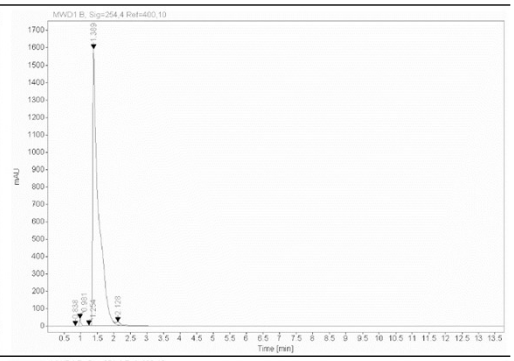
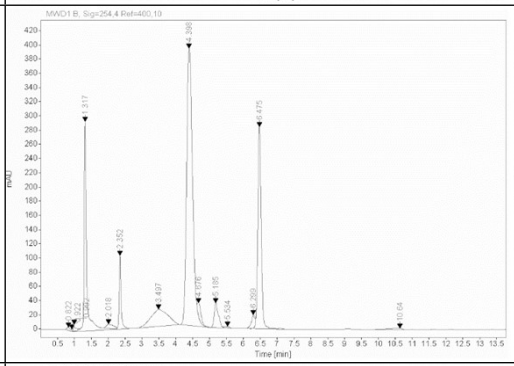
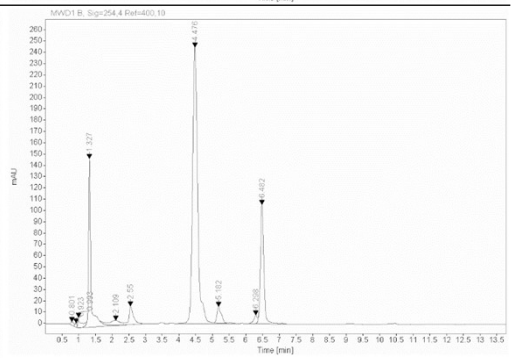
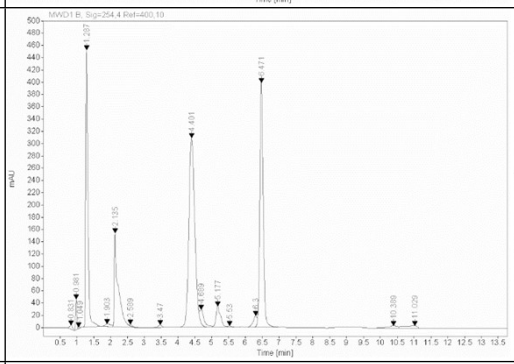
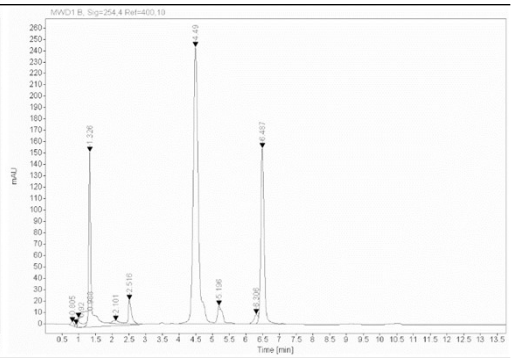
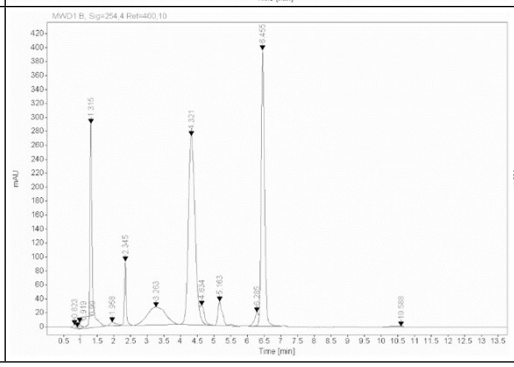
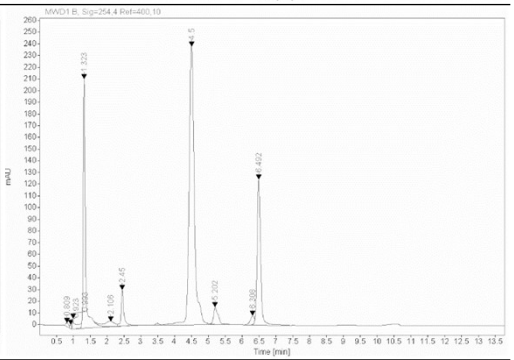
Figure S8 - UV-Vis spectrum of ten generated slugs to investigate DNA-recovery. The absorption at 260 nm is used to determine the DNA concentration in each slug.

Flow rate optimization

Table S3 - Conversions to the product **DNA-4a** depending on different flow rates.

	$\dot{V}$ = 1.6 mL min^{-1} (Dn = 9)		$\dot{V}$ = 2.4 mL min^{-1} (Dn = 13.5)		$\dot{V}$ = 3.2 mL min^{-1} (Dn = 18)	
Time [min]	**Batch [%]**	**Flow [%]**	**Batch [%]**	**Flow [%]**	**Batch [%]**	**Flow [%]**
10	26.3	-	26.8	25.4	30.7	25.4
23	29.3	22.6	32.2	26.3	31.1	25.4
45	42	22.6	39.8	40.4	34.8	25.4
60	42.6	25.6	42.4	32	31.8	23.2
110	56.3	22.6	46.2	32.2	46.3	32.2

Table S4 - RP-HPLC-traces of batch and flow reactions at the volumetric flow rate of 1.6 mL min^{-1} and Dn numbers 9.

Oligonucleotide	RP-HPLC-Chromatogram (Batch)	RP-HPLC-Chromatogram (Flow)
DNA-1 Retention Time 4.4 min		
DNA-4a Dn = 9 r.t. 10 min Retention time 6.4 min		
DNA-4a Dn = 9 r.t. 23 min Retention time 6.4 min		
DNA-4a Dn = 9 r.t. 45 min Retention time 6.4 min		
DNA-4a Dn = 9 r.t. 65 min Retention time 6.4 min		

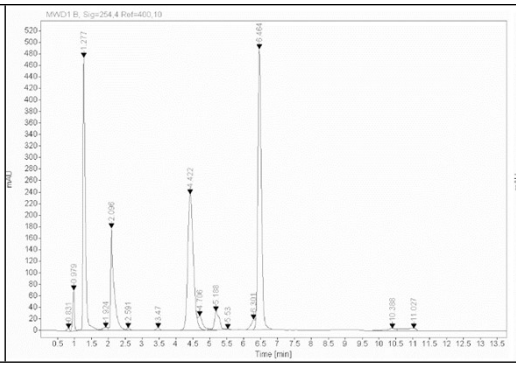
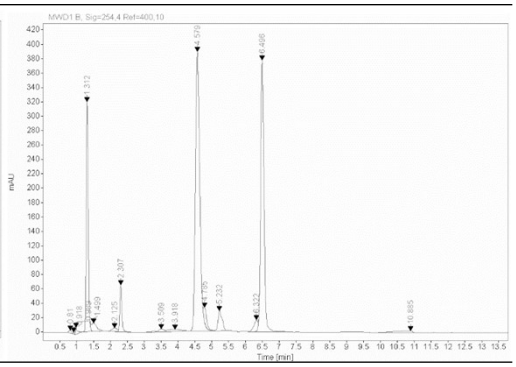
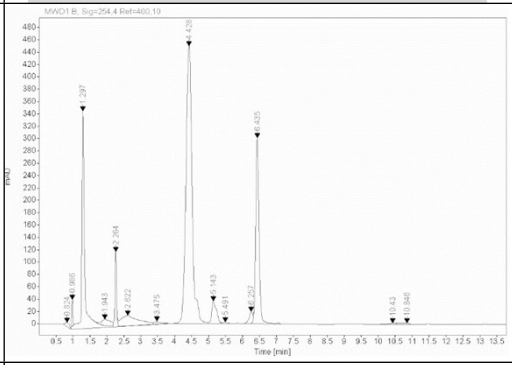
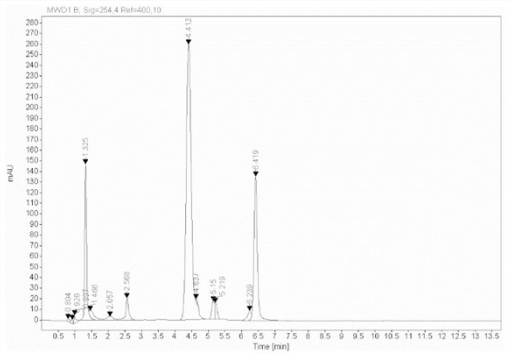
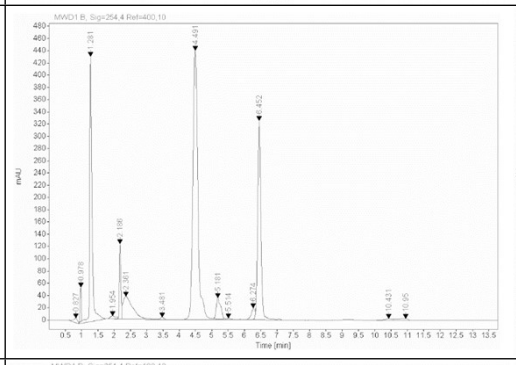
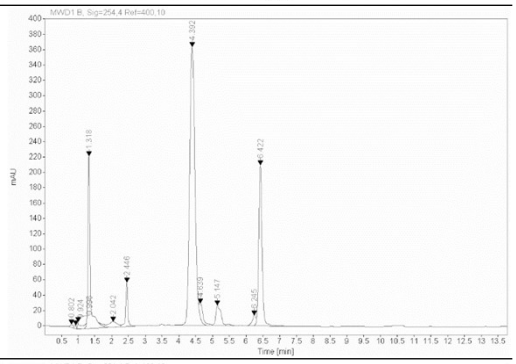
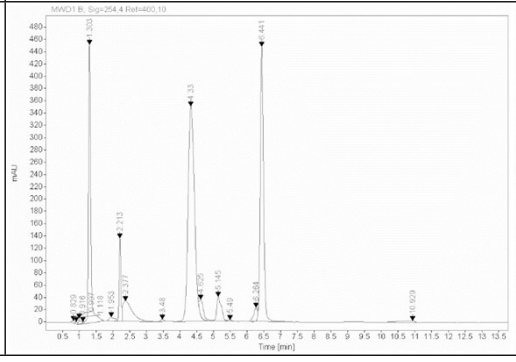
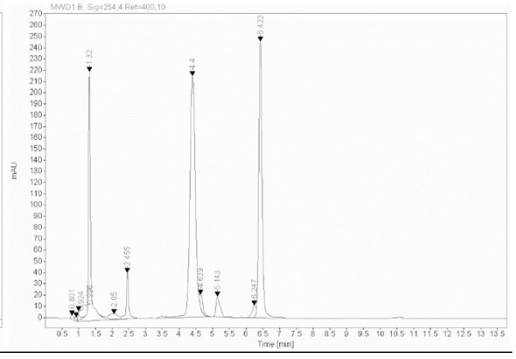
DNA-4a Dn = 9 r.t. 110 min Retention time 6.4 min		

Table S5 - RP-HPLC-traces of batch and flow reactions at the volumetric flow rate of 2.4 mL min^{-1} and Dn numbers 13.5.

Oligonucleotide	RP-HPLC-Chromatogram (Batch)	RP-HPLC-Chromatogram (Flow)
DNA-1 Retention Time 4.4 min		
DNA-4a Dn = 13.5 r.t. 10 min Retention time 6.4 min		
DNA-4a Dn = 13.5 r.t. 23 min Retention time 6.4 min		
DNA-4a Dn = 13.5 r.t. 45 min Retention time 6.4 min		

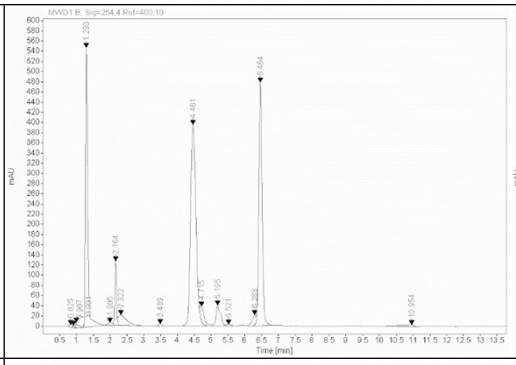
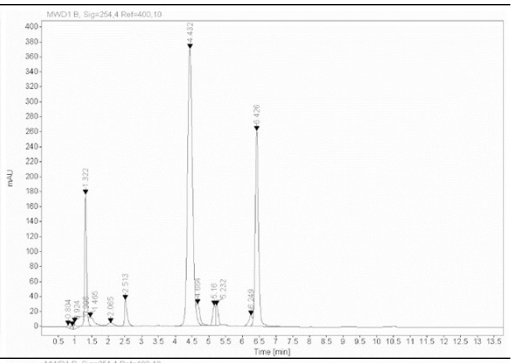
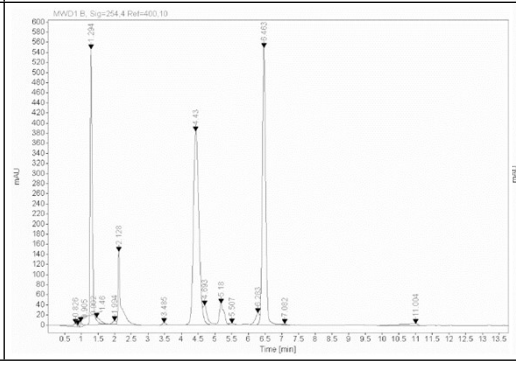
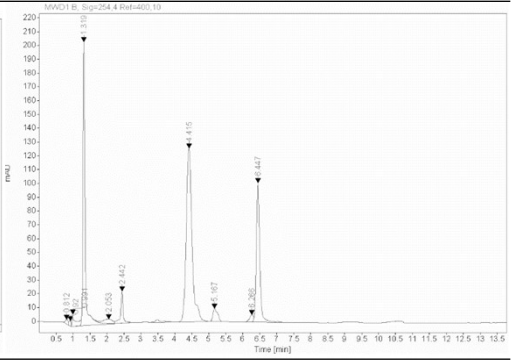
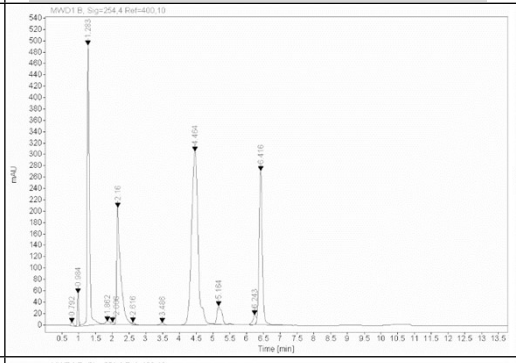
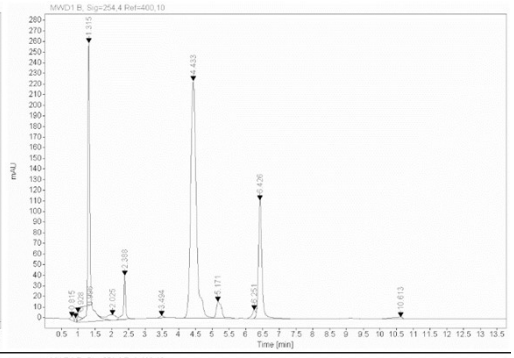
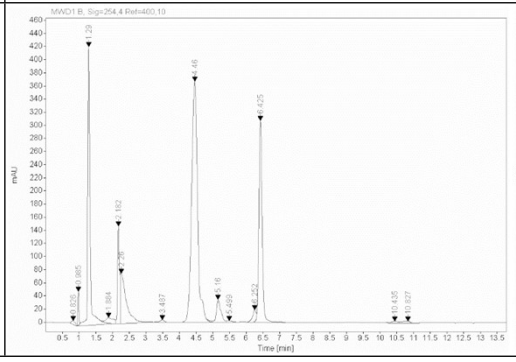
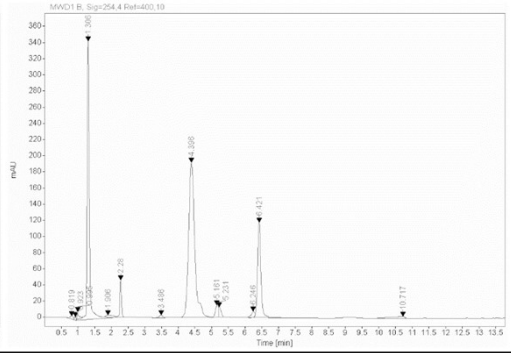
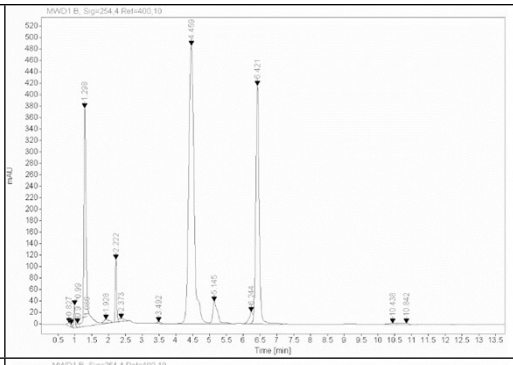
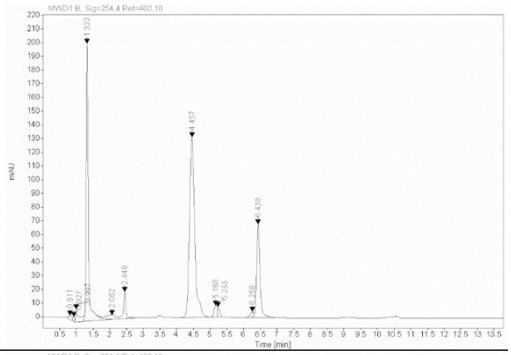
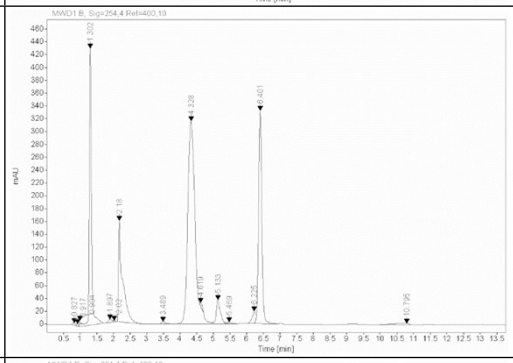
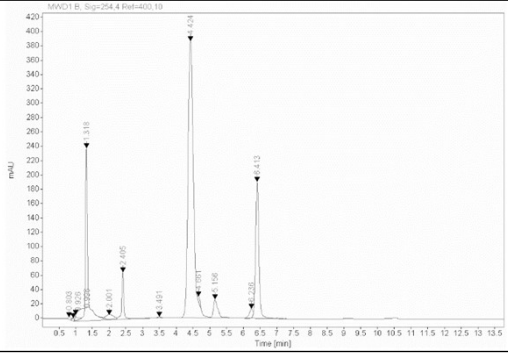
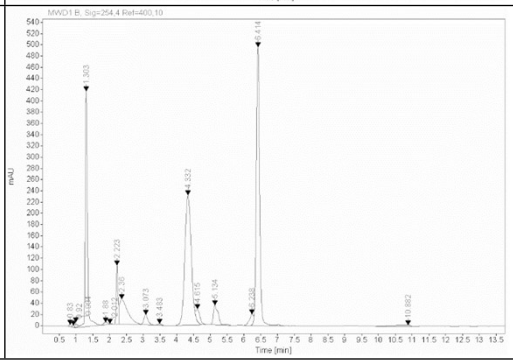
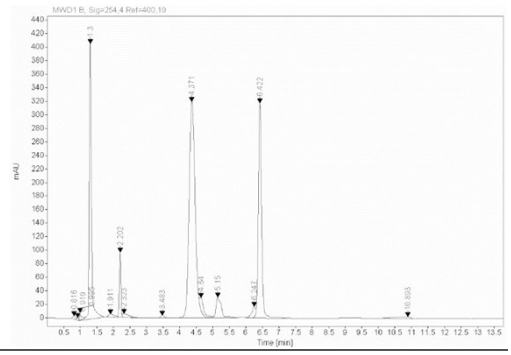
DNA-4a Dn = 13.5 r.t. 65 min Retention time 6.4 min		
DNA-4a Dn = 13.5 r.t. 110 min Retention time 6.4 min		

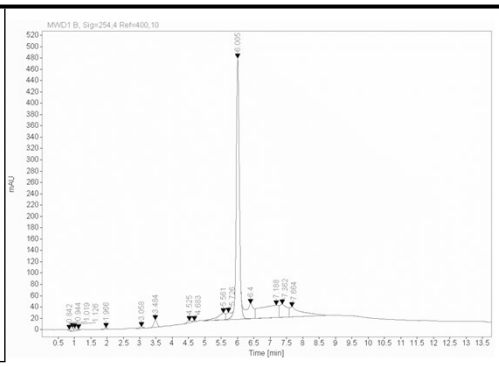
Table S6 - RP-HPLC-traces of batch and flow reactions at the volumetric flow rate of 3.2 mL min^{-1} and Dn numbers 18.

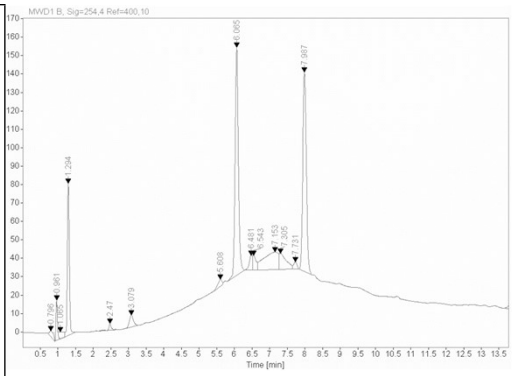
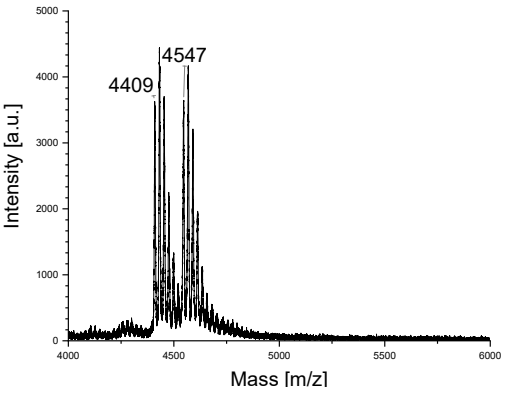
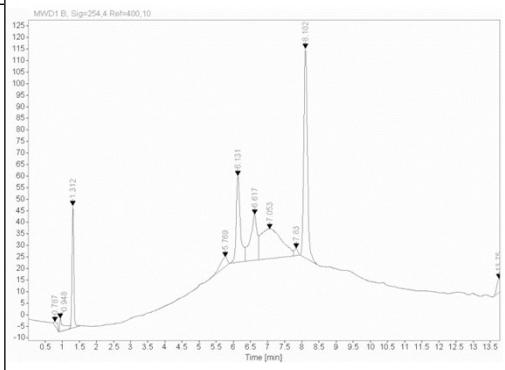
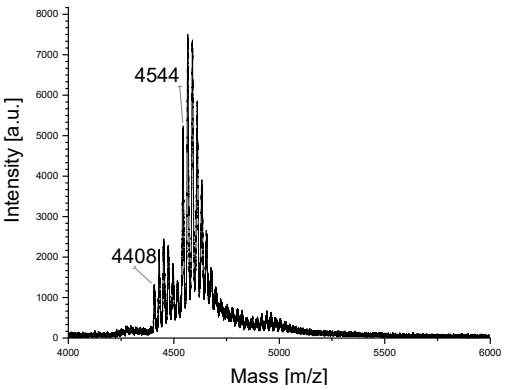
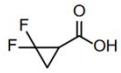
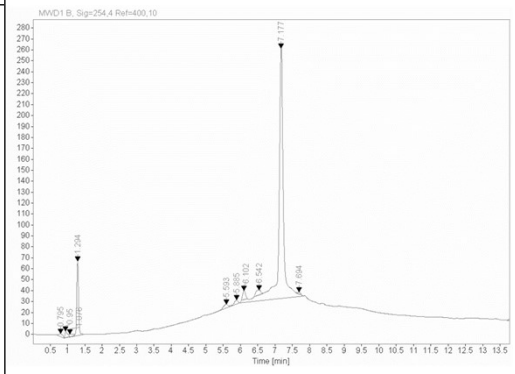
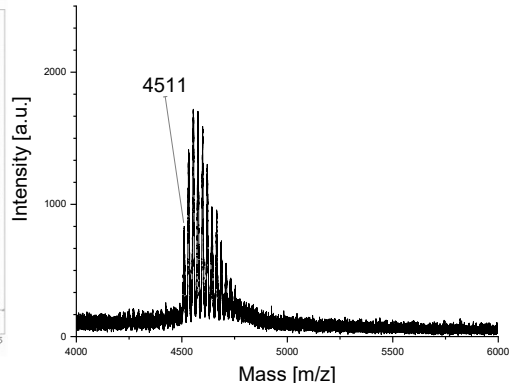
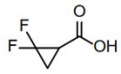
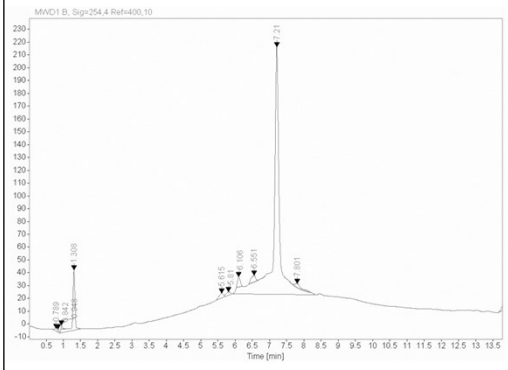
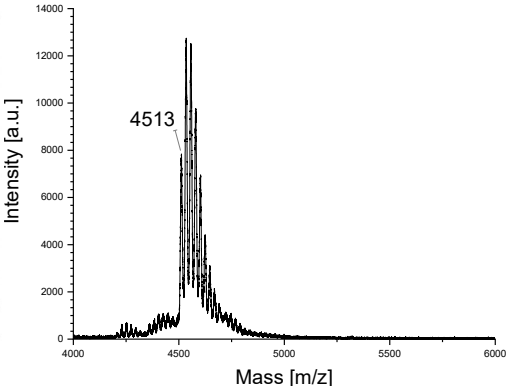
Oligonucleotide	RP-HPLC-Chromatogram (Batch)	RP-HPLC-Chromatogram (Flow)
DNA-1 Retention Time 4.4 min		
DNA-4a Dn = 18 r.t. 10 min Retention time 6.4 min		
DNA-4a Dn = 18 r.t. 23 min Retention time 6.4 min		

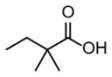
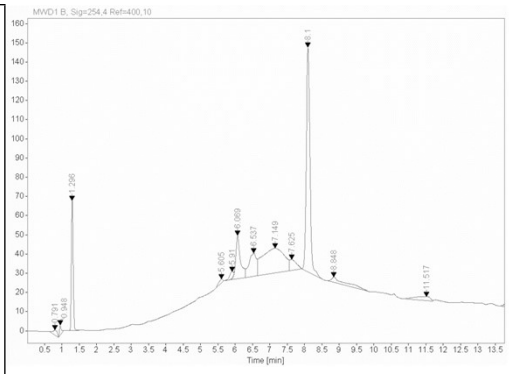
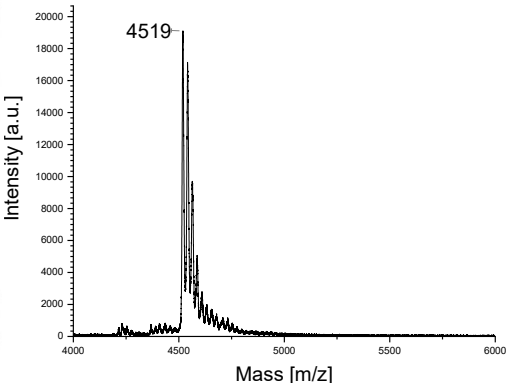
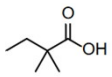
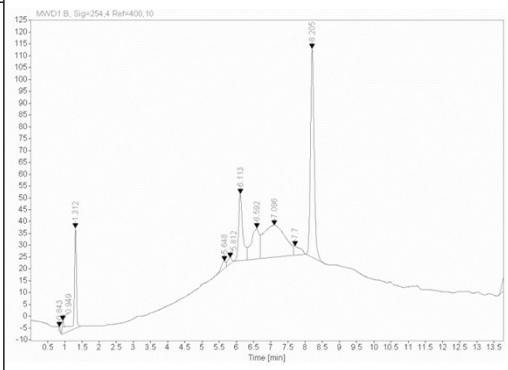
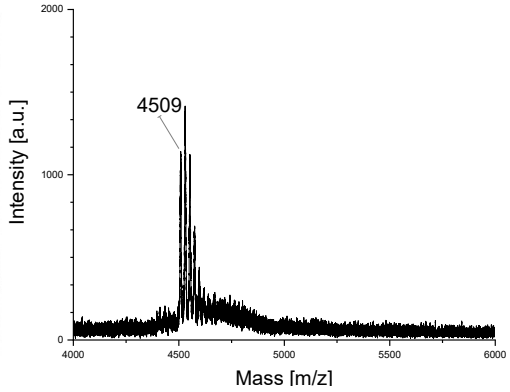
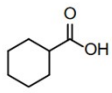
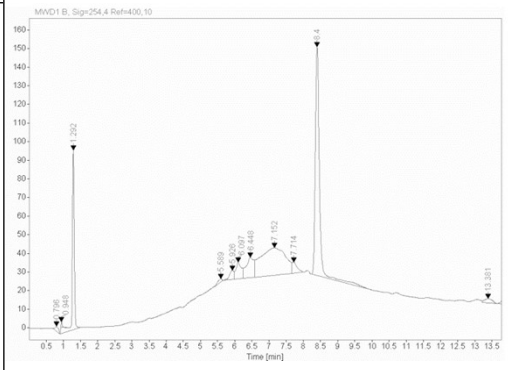
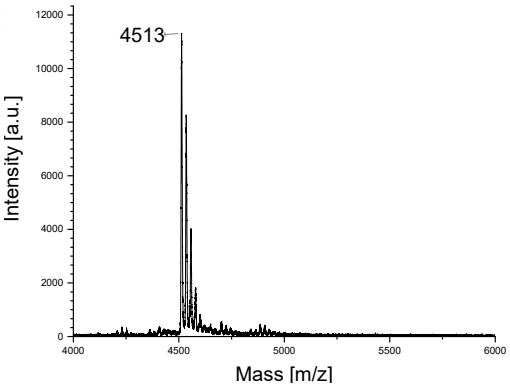
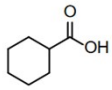
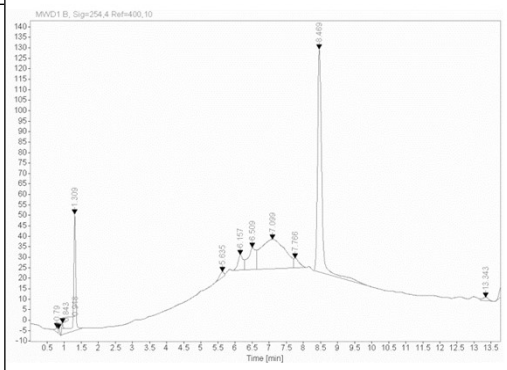
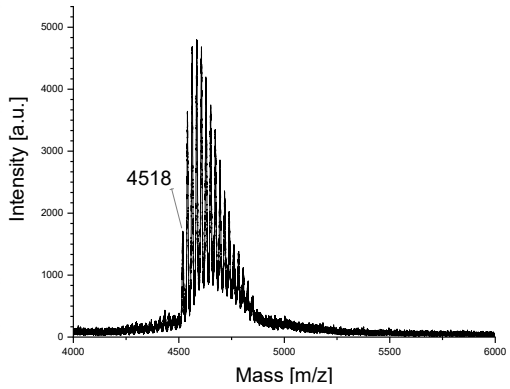
DNA-4a Dn = 18 r.t. 45 min Retention time 6.4 min		
DNA-4a Dn = 18 r.t. 65 min Retention time 6.4 min		
DNA-4a Dn = 18 r.t. 110 min Retention time 6.4 min		

Profiling

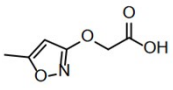
Table S7 - RP-HPLC and MALDI-traces of profiling experiments with carboxylic acids **3**.

Oligonucleotide	RP-HPLC-Chromatogram	MALDI Spectrum
DNA-1 Retention time 6 min Mass 4408 Da		

DNA-4a  **3a** Flow Retention time 8 min Mass 4545 Da		
DNA-4a  **3a** Batch Retention time 8 min Mass 4545 Da		
DNA-4d  **3d** Flow Retention time 7.1 min Mass 4513 Da		
DNA-4d  **3d** Batch Retention time 7.1 min Mass 4513 Da		

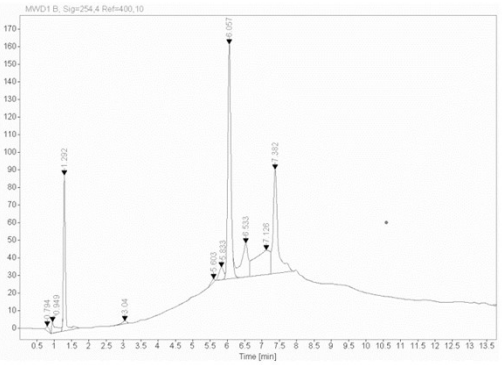
DNA-4e  **3e** Flow Retention time 8.1 min Mass 4507 Da		
DNA-4e  **3e** Batch Retention time 8.1 min Mass 4507 Da		
DNA-4f  **3f** Flow Retention time 8.4 min Mass 4519 Da		
DNA-4f  **3f** Batch Retention time 8.4 min Mass 4519 Da		

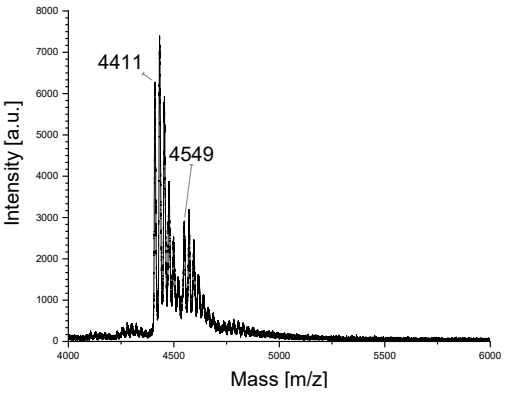
DNA-4g



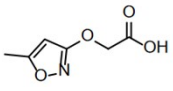
3g

Flow
Retention time
7.4 min
Mass
4548 Da



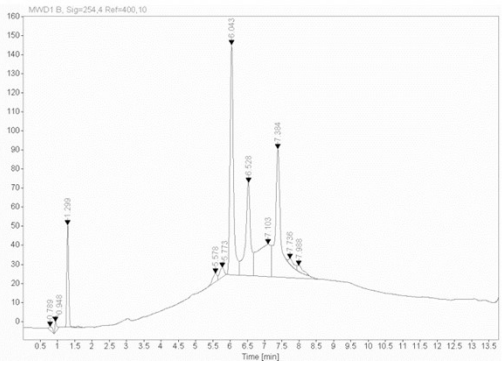


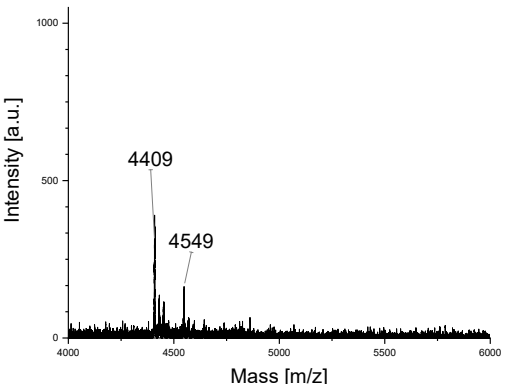
DNA-4g



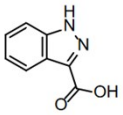
3g

Batch
Retention time
7.4 min
Mass
4548 Da



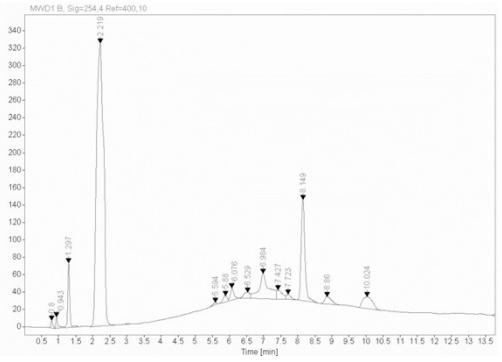


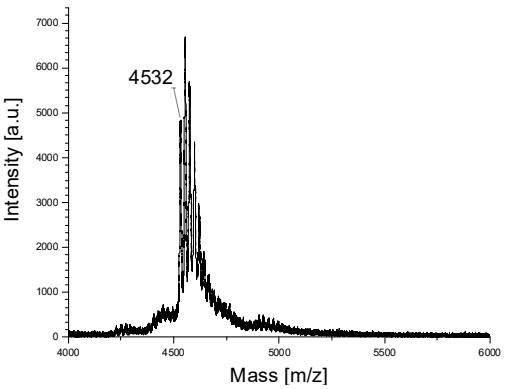
DNA-4h



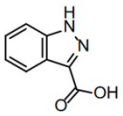
3h

Flow
Retention time
8.2 min
Mass
4553 Da



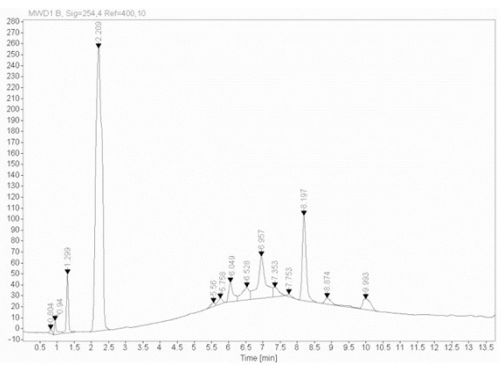


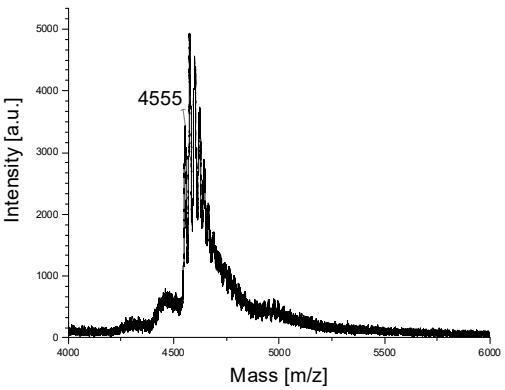
DNA-4h



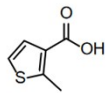
3h

Batch
Retention time
8.2 min
Mass
4553 Da



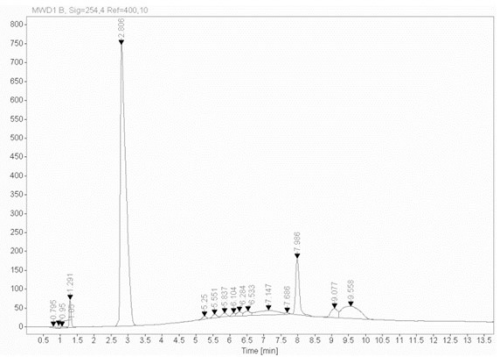


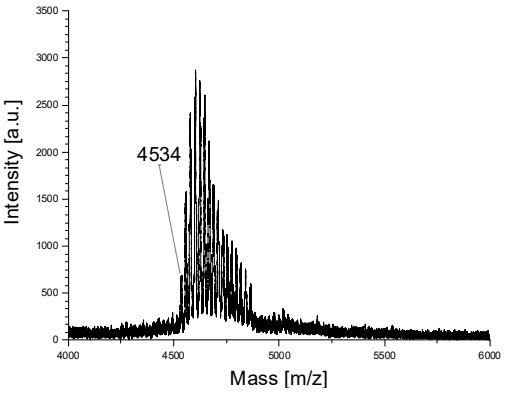
DNA-4i



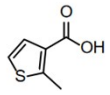
3i

Flow
Retention time
8 min
Mass
4533 Da



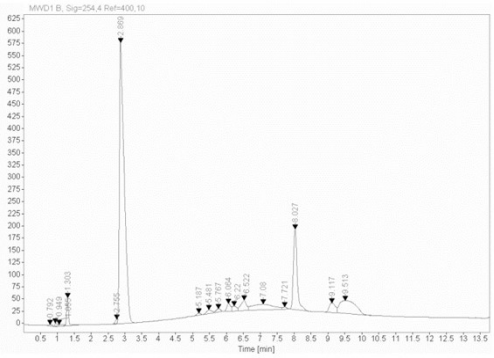


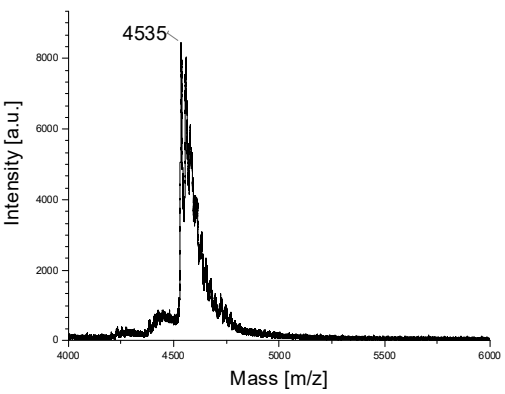
DNA-4i



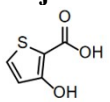
3i

Batch
Retention time
8 min
Mass
4533 Da



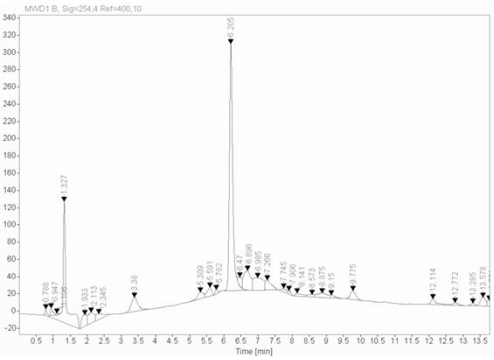


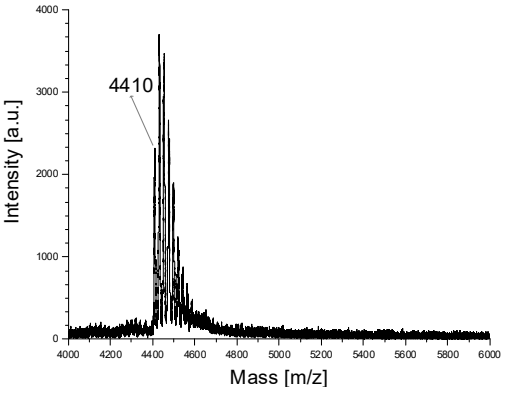
DNA-4j



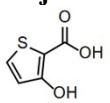
3j

Flow
Retention time
-
Mass
4535 Da



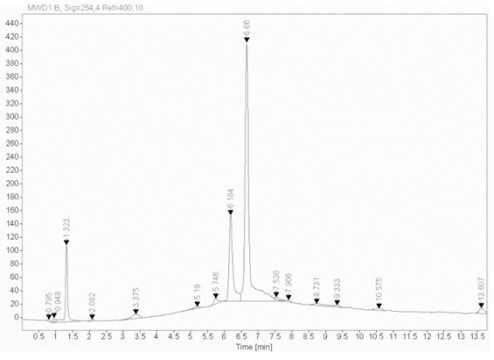


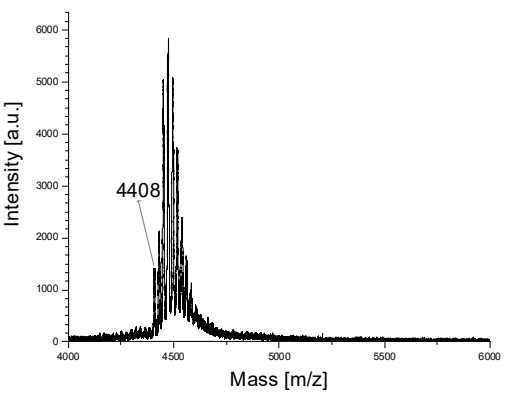
DNA-4j

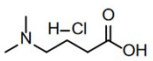
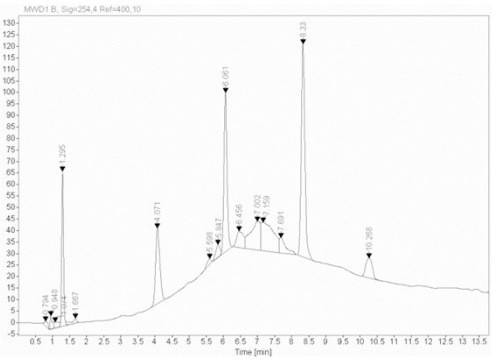
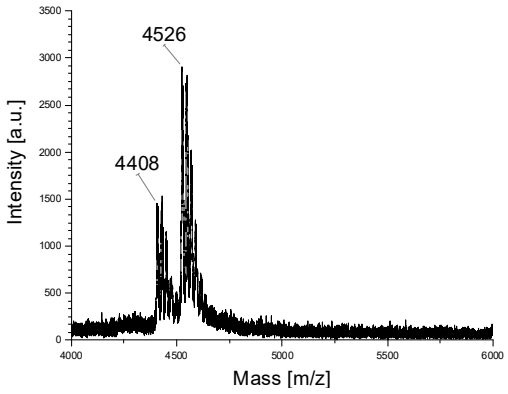
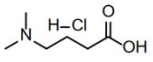
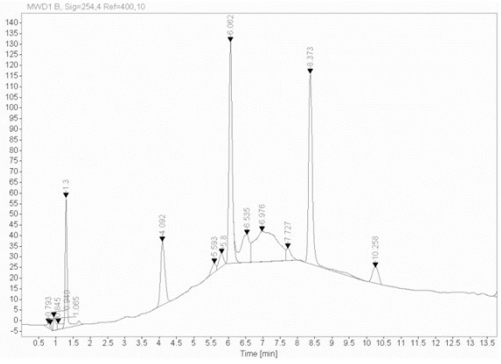
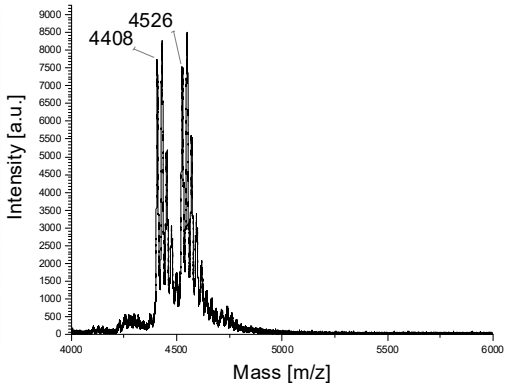


3j

Batch
Retention time
-
Mass
4535 Da





DNA-4k  **3k** Flow Retention time 8.3 min Mass 4524 Da		
DNA-4k  **3k** Batch Retention time 8.3 min Mass 4524 Da		

5. References

1 3M™ Fluorinert™ FC-40 Elektronik Flüssigkeit | 3M Deutschland, https://www.3mdeutschland.de/3M/de_DE/p/d/b40045180/, (accessed 16 November 2022).

2 S. Höving, J. Bobers and N. Kockmann, *J. Flow Chem.*, 2022, **12**, 185–196.

3 S. K. Kurt, M. Akhtar, K. D. P. Nigam and N. Kockmann, *Ind. Eng. Chem. Res.*, 2017, **56**, 11320–11335.

4 S. Klutz, S. K. Kurt, M. Lobedann and N. Kockmann, *Chem. Eng. Res. Des.*, 2015, **95**, 22–33.

5 N. Kockmann, *Chemie-Ingenieur-Technik*, 2020, **92**, 685–691.

6 M. Schmalenberg, W. Krieger and N. Kockmann, *Chemie-Ingenieur-Technik*, 2019, **91**, 567–575.

7 W. R. Dean, *Proc. R. Soc*, 1928, **5**, 402–420.

8 Y. Li, E. Gabriele, F. Samain, N. Favalli, F. Sladojevich, J. Scheuermann and D. Neri, *ACS Comb. Sci.*, 2016, **18**, 438–443.