Supporting information

Design of an Automated Reagent-Dispensing System for Reaction Screening and Validation with DNA-tagged Substrates

Jens Bobers,[a‡] Mateja Klika Škopić,[b‡] Robin Dinter,[a] Piriyanth Sakthithasan,[a] Laura Neukirch,[b] Christian Gramse,[c] Ralf Weberskirch,[c] Andreas Brunschweiger,[b] Norbert Kockmann[a *]*

[a]TU Dortmund University, Department of Chemical and Biochemical Engineering, Laboratory of Equipment Design, Emil-Figge-Straße 68, 44227 Dortmund, Germany; [b]TU Dortmund University, Faculty of Chemistry and Chemical Biology, Otto-Hahn-Straße 6, 44227 Dortmund, Germany; [c]Faculty of Chemistry and Chemical Biology, Polymer Hybrid Systems, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

Corresponding authors:

norbert.kockmann@tu-dortmund.de

andreas.brunschweiger@tu-dortmund.de

Content

1. Mechanical design of the automated dosing system *ADoS*

The mechanical design builds up on an open source template of a 3D printer named Hypercube Evolution.[1] This 3D printer utilizes the CoreXY framework and is based on freely available parts such as GT belt profiles and stepper motors. The design offers a working platform, which is driven by two stepper motors at the same time to prevent tilting of the platform. The CAD model has a specification of a printable volume of 220 mm x 220 mm x 200 mm and has open-source 3D CAD files, which can be modified using the software Autodesk Inventor® (Autodesk, San Rafael, USA).

Main framework

Compared to the template design the area of the workspace was increased to 280 mm x 280 mm and the working platform size was set to 300 mm x 300 mm. These dimensions allow for accommodating up to six standard 96well microtiter plates. The main framework, which carries the whole weight and gives the *ADoS* its structural integrity, is based on lightweight, customizable aluminum extrusion profiles. They have a grid size of 30 mm x 30 mm and contain grooves on each side to provide flexibility for building structures (Aluprofiltechnik Kohl, Wadgassen, Germany). The joints and holder assemblies were 3D printed using an Ultimaker 3 3D printer (Ultimaker, Utrecht, Netherlands). The CAD files were converted to a machine-readable STL format for the Ultimaker 3 using the slicing software Ultimaker Cura (Ultimaker, Utrecht, Netherlands)

Modified CoreXY design

To redirect the belts around the frame 2 mm pitch GT profile pulleys with 20 teeth, if the toothed side of the belt is redirected, and without teeth, if the smooth side of the belt is redirected, have been ordered from the supplier Bohrers Computer Service (Bohrers Computer Service, Langerwehe, Germany). The linear guide assemblies for the y-axis have been built using linear rail guides made out of hardened steel, with a diameter of 10 mm and a length of 390 mm.

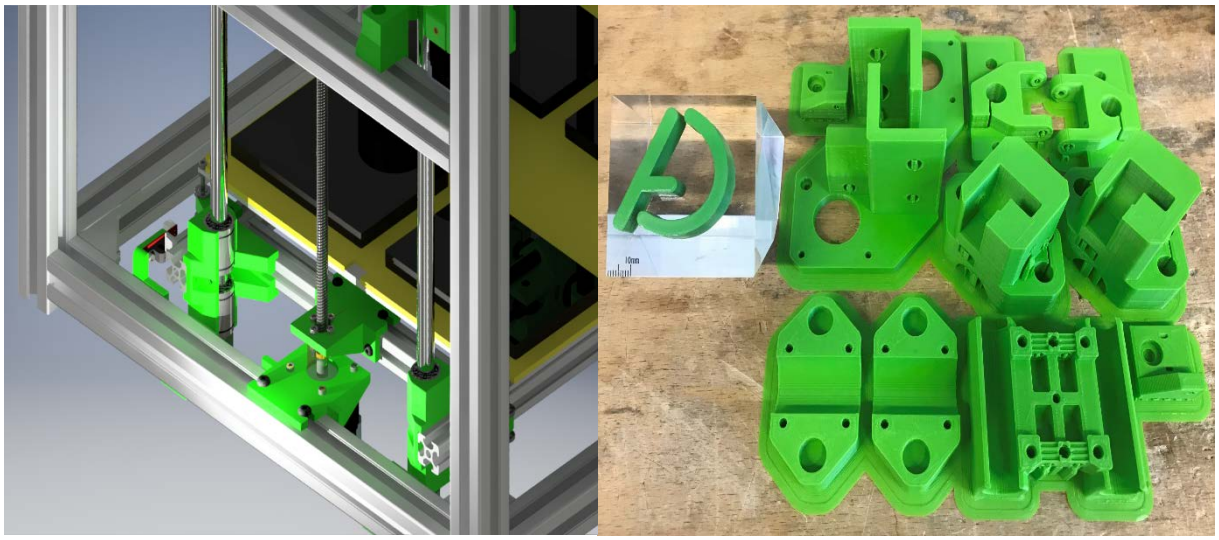


Figure S1. CAD file of 3D printed joints for the z axis (left). 3D printed holder assemblies and joints made from PLA (right)

The linear guide assemblies for the y-axis were built from rail guides made of hardened steel, with a diameter of 10 mm and a length of 390 mm (Dold Mechatronik, Steinach, Germany). The bearings for the y-axis are plain polypropylene polymer bearings (RJ4JP-01-10, igus GmbH, Cologne, Germany). The x-axis utilizes linear rail guides (8 mm diameter, 430 mm length, Dold Mechatronik). The x-axis carriage has a 3D printed main body. In this main body, igus® plain polymer bearings RJ4JP-01-08 were inserted at the back by a sliding mechanism. Four holes were placed at the front of the main body. These holes were used for guide pins to position the automated injection unit. NEMA 17 sized stepper motors were used to perform the motions in the XY-plane (SY42STH38-1684A, Pololu Corporation, Las Vegas, USA). They have a maximum holding torque of 0,363 Nm. GT2 profile belts were used to drive the carriage (EXP-GmbH, Saarbrücken, Germany). The corresponding drive wheels with 20 teeth have been mounted onto the shaft of the stepper motors (EXP-GmbH, Saarbrücken, Germany). Since the original design of the Hypercube Evolution uses an inductive switch as a limit switch to determine the zero point of the z-axis, modifications were carried out. The minimum distance between the x-carriage and the working platform can vary because the printer head of the automated injection unit may be substituted to different injection units, such as different

syringes or needles. Thus, the positioning of the limit switch can be adapted accordingly. A small flag marking the zero point was installed at the working platform serving as optical limit switch. The ball bearings were replaced by plain bearing. This modification tolerates longer maintenance intervals and the overall bearing system was less sensitive to dust.

Automated Injection Unit (AIU)

Since the template was a 3D printer design, it carried an extruder head on the carriage to perform 3D printing. In the *ADoS* design, the extruder assembly was replaced by a Hamilton GC syringe Microliter™ 800 series with a maximum volume of 50.00 µL for mixing and dispensing of reactants and reagents (Hamilton Company, Reno, USA, Figure S2).

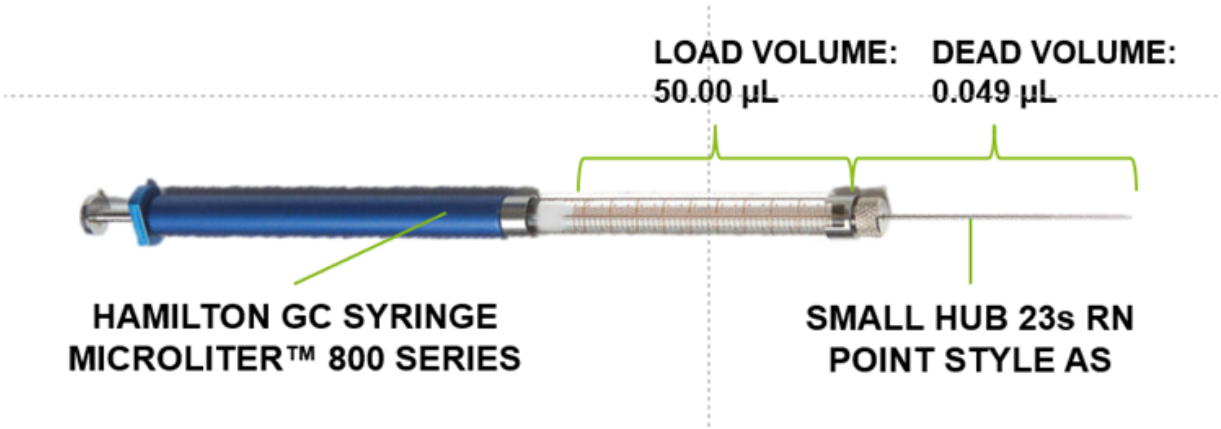


Figure S2. Setup of a Hamilton GC syringe Microliter™ 800 series with needle type 23s RN with conical point style.

The plunger of the syringe is moved linearly by a helically toothed rack and pinion translating the rotational movement of the NEMA 11 stepper motor (SY28STH32-0674A, Polulu, Las Vegas, USA). The assembly of the AIU is presented in Figure S3. Heat sinks made of aluminum (ABL Aluminium Components, Birmingham, UK) were mounted on the case of the stepper motor with heat-conducting adhesive to avoid overheating (Figure S3 A).

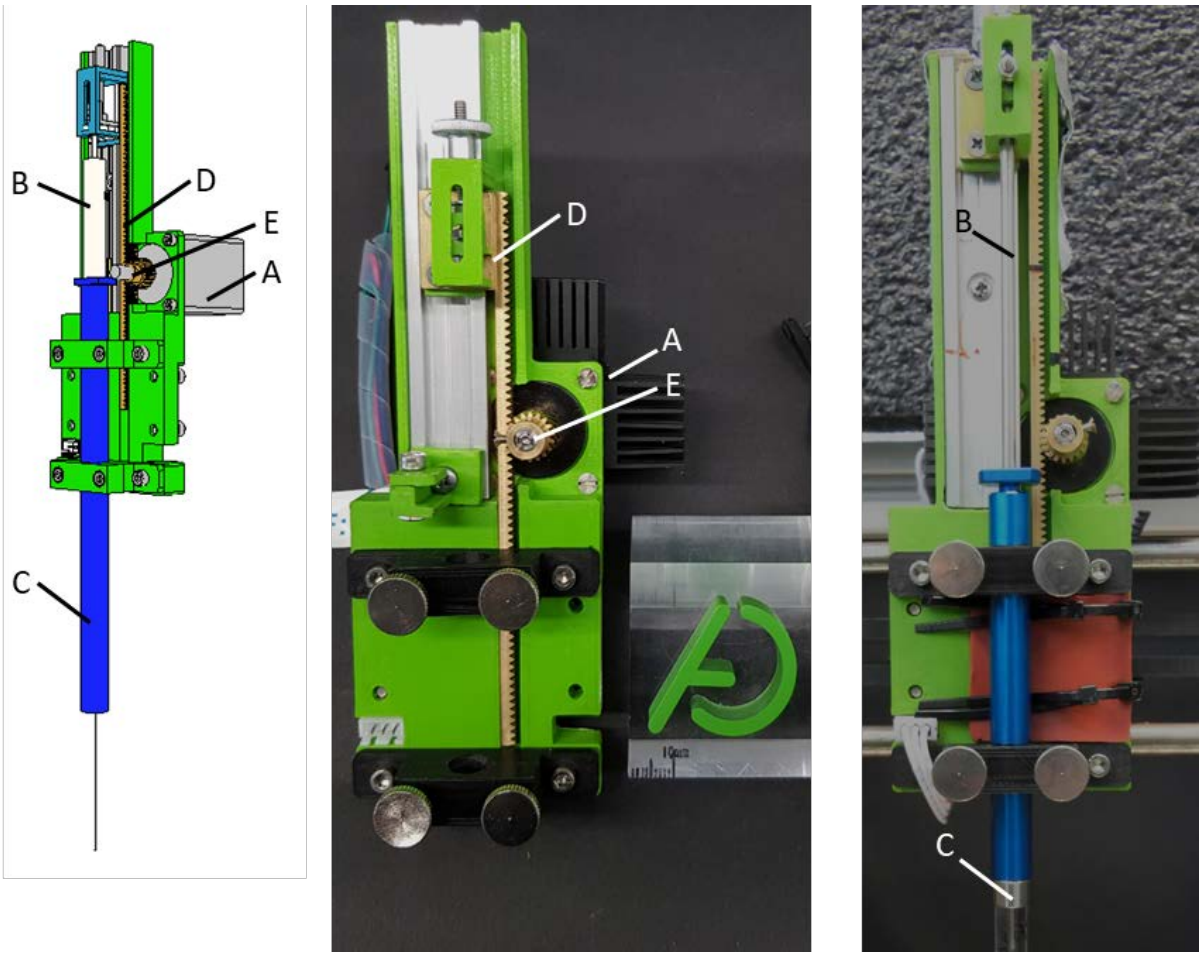


Figure S3. Assembly of the AIU consisting of stepper motor with cooling fins (A), helically toothed rack (D) and pinion (E), plunger of the syringe (B), and GC syringe (C). Green joints and holder assemblies are 3D printed and made from PLA.

2. Design of the *ADoS* control unit

As control unit of the *ADoS* we used an Arduino MEGA microcontroller (Arduino S.r.l., Monza, Italy), which manages all incoming G-code and P-code commands. G-code and P-code commands can be sent to the Arduino MEGA via an SD card slot and via a graphical user interface (GUI) designed with Matlab (Mathworks, Natick, USA). The G-code instructions were streamed to a G-code parser named GRBL via an Arduino UNO (Arduino S.r.l., Monza, Italy). This microcontroller was connected with a CNC Shield V3 (Joy-It, Neukirchen, Germany) containing four DRV8825 (Pololu, Las Vegas, USA) step driver boards, which was an expansion board for the Arduino providing the control circuit for 3D movement of printers or milling machines. The GRBL translates the G-code commands into binary signals to control the stepper motors in XYZ direction for the Cartesian motion. A feedback of the zero position was transmitted from the mechanical system to the CNC-shield via optical limit switches (TCST2103, Bohrers Computer Service, Langerweh, Germany). The P-code instructions were executed directly on the Arduino MEGA, which was connected to the step driver board SilentStepStick TMC2208 (Watterott, Leinefelde-Worbis, Germany TMC2208). The board translates the P-code commands into binary signals for the AIU. Since the Arduino MEGA cannot perform the P-code commands for the AIU and the peristaltic pumps at the same time due to low processor power, a second Arduino UNO (Arduino S.r.l., Monza, Italy) was introduced. This second Arduino UNO controlled the movement of the stepper motors for the peristaltic pumps and was connected to ULN2003 motor driver boards (Adafruit Industries, New York, USA). The Arduino MEGA switched the power supply for these step driver boards on and off by the P-code command P8. The electrical case including the case is shown in Figure S4.

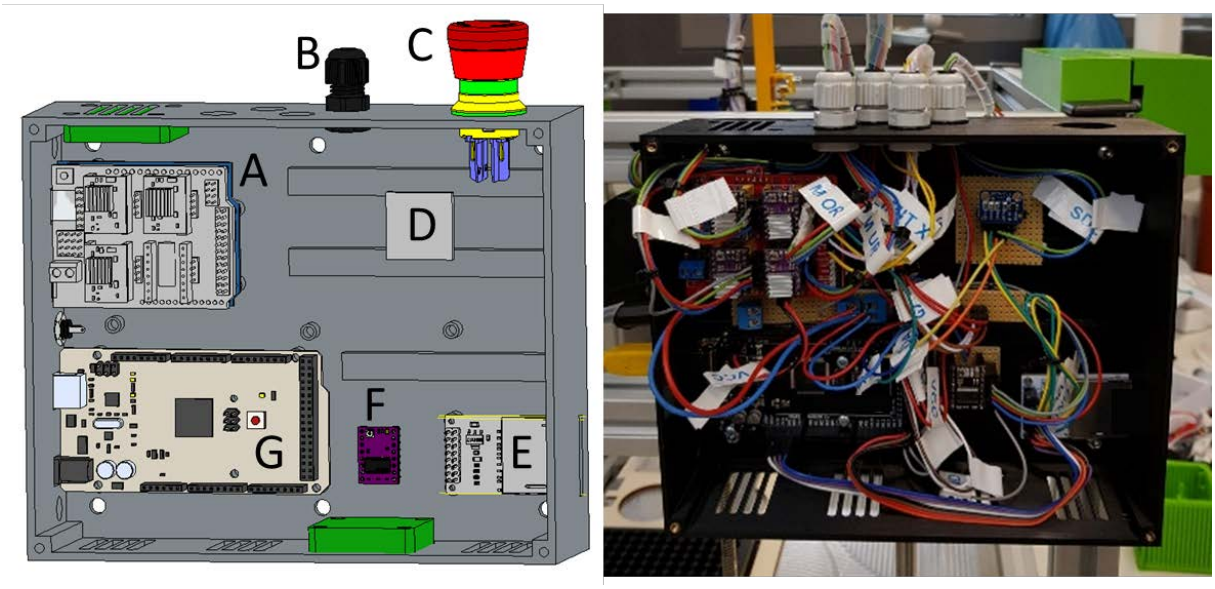


Figure S4. CAD file of the eletrical setup of the *ADoS* (left) containing Arduino UNO with CNC shield (A), cable guide (B), emergency swith (C), digital analogue converter (D), SD shield and slot (E), driver board (F), and Arduino MEGA (G). 3D printed case for eletronic setup without emergency switch and cooling fan (right)

If required, the user receives feedback about the XYZ positions as well as the content of the AIU via a LCD unit. The user can also use this unit to perform manual control by a user menu with five buttons. An emergency switch can stop the operation of the *ADoS* manually. The power supply for the stepper motors was provided by a VOLTCRAFT LPS1305 laboratory power supply (Conrad Electronic SE, Wernberg-Köblitz, Germany) and for the microcontrollers by a USB connection to the Arduino MEGA. For external pump control the electrical design contained a digital-analog converter.

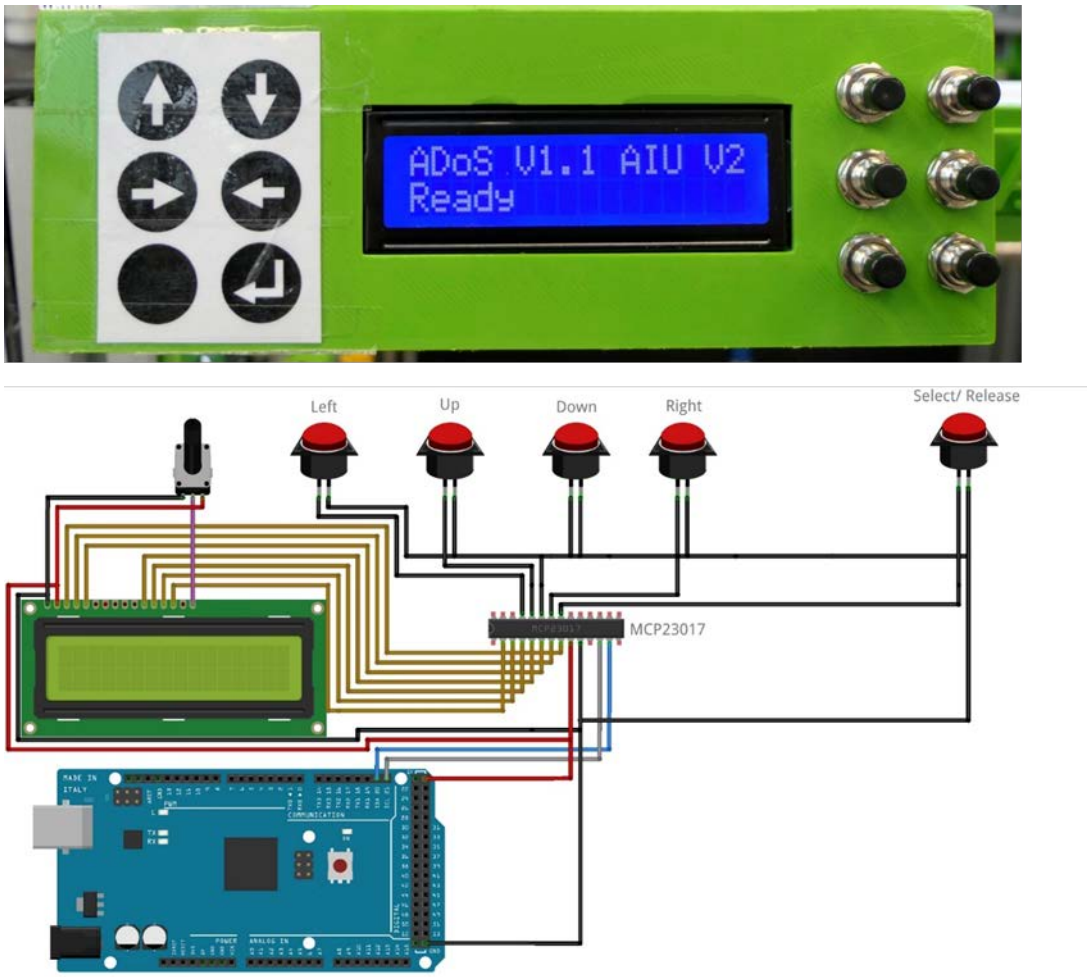


Figure S5. LC display with 3D printed case and buttons for manual control (top). Electrical circuit for the LC display and buttons (bottom)

Figure S5 displays the electrical circuit for the LC display and the 3D printed case with buttons for manual control of the *ADoS*. Additionally, a circuit to insert SD cards and to control an external pump was implemented. A simplified circuit without power supply and the third Arduino UNO is shown in Figure S6.

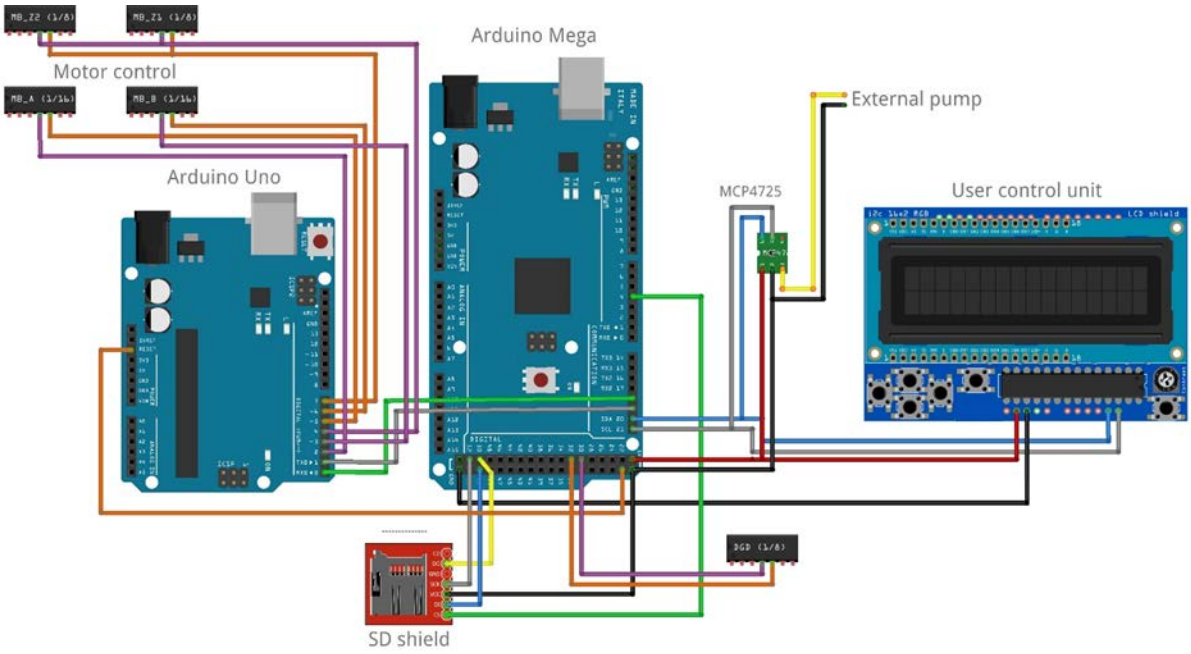


Figure S6. Simplified electric circuit consisting of Arduino UNO and MEGA, motor control, external pump control, SD shield, and LC display

3. Software design of the *ADoS*

The library Grbl, which is a free, open source, high performance, and low-cost alternative software for controlling the motion of machines, was used for performing the Cartesian motion of the *ADoS*. The Grbl library was downloaded from its open-source repository and flashed to the Arduino Uno. The config.h file of the Grbl library was modified to use the CoreXY framework. The search of the zero points, also called homing cycle, was adjusted to fit the CoreXY framework. The code snippets for the homing procedure of the z axis are shown in Figure S7. Using this code snippet, Grbl searches for the zero points of the z-, x- and y-axes in this order.

```
#define HOMING_CYCLE_0 (1<<Z_AXIS)
//#define HOMING_CYCLE_1 ((1<<X_AXIS)|(1<<Y_AXIS
// #define HOMING_CYCLE_

// NOTE: The following are two examples to setup homing for 2-axis machines.
// #define HOMING_CYCLE_0 ((1<<X_AXIS)|(1<<Y_AXIS))

#define HOMING_CYCLE_1 (1<<X_AXIS)
#define HOMING_CYCLE_2 (1<<Y_AXIS)
```

Figure S7. Example of a open-source Grbl code snippet for activating the homing cycle of the z axis.

Before Grbl can be used for controlling the movement of the *ADoS*, the translation from motor steps to mm needed to be implemented. This corresponds with the velocity and acceleration of each motor and, thus, for each axis. The values determined for each axis and the AIU are listed in Table S1.

Table S1. Characteristic parameters of stepper motors of each axis and the AIU for Grbl

axis	steps per mm [mm^{-1}]	velocity [mm s^{-1}]	acceleration [mm s^{-2}]
X	80	400	400
Y	80	400	400
Z	100	80	80
AIU	50.92	400	400

For positioning, different commands were defined, which are case-sensitive. Exemplary, the G0 command was used for moving to explicit coordinates. The command “G0 X100 Y150” would position the carriage to the x-position 100 mm and the y-position 150 mm. However, all axes will be moved at the same time. If the position sent is out of the range of the *ADoS*, Grbl will go to an alarm state.

4. Workspace design of the *ADoS*

Two types of standard 96-well microtiter plates made out of polypropylene (PP) were used for the presented experiments. The first one was the 96 Nunc™ MicroWell™ plate (Thermo Fisher Scientific Inc., Waltham, USA) with a working volume range from 50 µL to 300 µL. The second one was the 96 Eppendorf Deepwell Plate (Eppendorf AG, Hamburg, Germany) with a maximum working volume of 2000 µL. Four operation plates were defined that can be dedicated to different functions. The footprint of the operation plates was standardized to the geometry of 96-well microtiter well plates. The operation plates are presented in Figure S8.

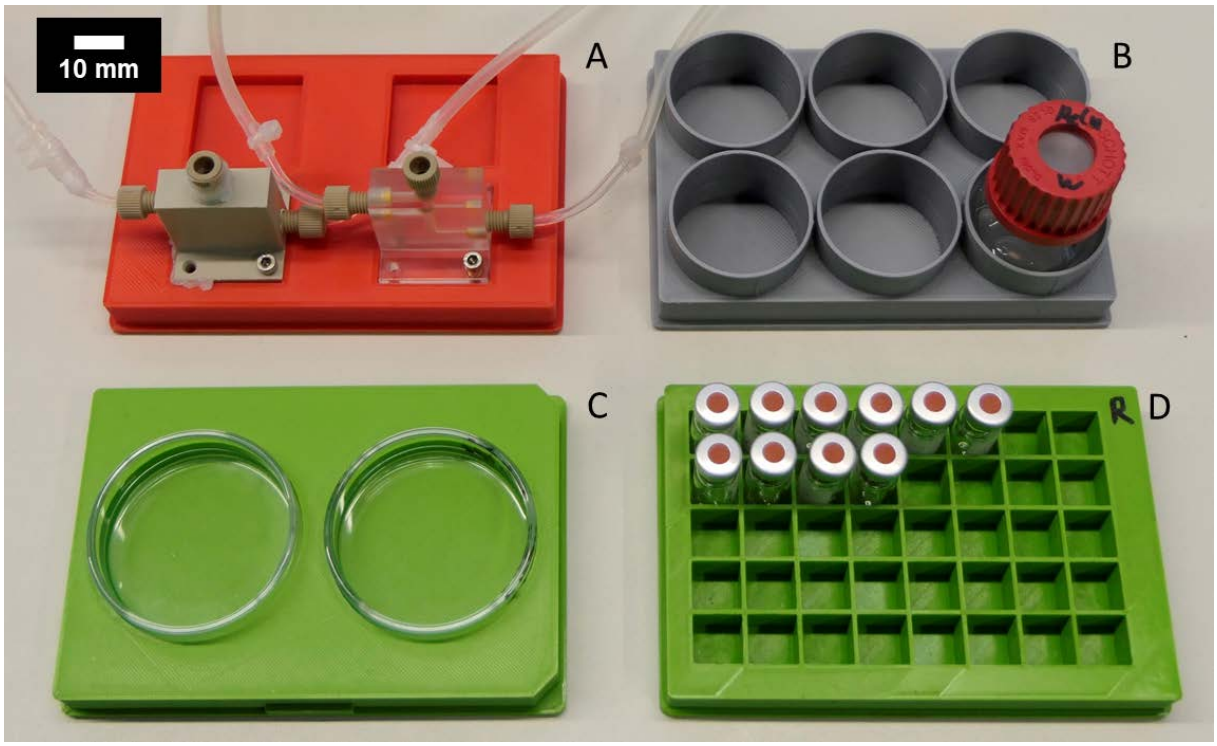


Figure S8. Operation plates for the *ADoS*. Continuous cleaning stations (A), Schott flasks (B), Petri dishes (C), and GC vials (D). The base plates were 3D printed, made from poly lactic acid (PLA), and adapted to the dimensions of a 96-well microtiter plate.

Z pieces for continuous cleaning were designed and manufactured from PMMA and PP. Schott glasses, GC vials, Petri dishes and microtiter plates can all be used and placed onto the base plate of the *ADoS*.

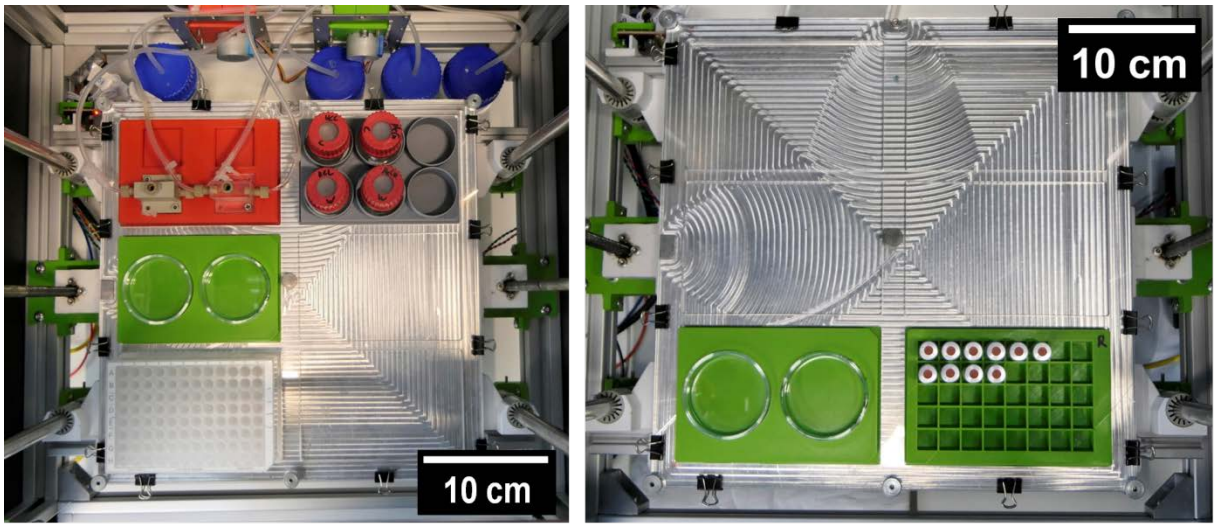


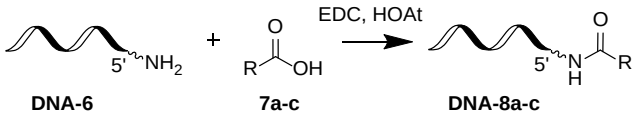
Figure S9. Experimental setup for performing amide bond formation as validation for the cleaning procedure and experimental setup for screening of the Povarov reaction including continuous and discontinuous syringe cleaning, water supply, and microtiter plate as reaction plate (left). Setup for the characterization of the GC syringe with deionized water containing water supply and GC vials (right)

As shown in Figure S9, any operation plates can be combined for different operations. The limits and positions of each operation plate were calibrated and implemented into the source code of the *ADoS*. Combining the codes for movement in x, y, and z direction with the pumps for cleaning agent and pipetting procedure translated from the manual lab work, services were defined to be fulfilled by each operation plate.

5. Materials and instruments for chemical reactions

Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany), Bachem (Bubendorf, Switzerland), Carbolution (St. Ingbert, Germany), Fluka (München, Germany), abcr (Karlsruhe, Germany), Thermo Fisher Scientific (Karlsruhe, Germany), TCI Chemicals (Eschborn, Germany) and VWR (Langenfeld, Germany). Controlled pore glass solid phase was filtered on a synthesis column plugged onto a vacuum manifold (Vac-Man®, Promega). Oligonucleotide-small molecule conjugates were purified by ion pair reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C_{18} stationary phase (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*10.0 mm) and a gradient of 100 mM aqueous triethylammonium acetate/MeOH. The triethylammonium acetate buffer was set to pH= 8. Oligonucleotide-small molecule conjugates were analyzed by ion pair reverse phase high-pressure liquid chromatography (HPLC, Shimadzu Prominence) using a C_{18} 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 spectroscopy using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific). Oligonucleotides were analyzed by MALDI-MS (Bruker Daltonics) using THAP matrix (Dichrom) or HPA matrix, with the laser operating at 355 nm, 532 nm, 1064 nm, and 808 ± 15 nm. Laser power varied between 95 % and 100 %. MALDI spectra were acquired from 200 - 250 laser shots. The polymer micelle **4** was synthesized and characterized according to a previously published procedure.[2]

6. Coupling of carboxylic acids to DNA-6



Scheme S1. Reaction of 5'-aminolinker-modified **DNA-6** with carboxylic acid building blocks **7a-c**.

Protocol for manual pipetting**:**[3] To a solution of **DNA-6** (500 pmol) dissolved in 50 µL of MOPS buffer (50 mM, pH= 8, 0.5 M NaCl) were added a carboxylic acid **7a-c** (2.7 µmol, 5400 eq.) dissolved in 45 µL of DMSO (taken from a stock solution: 27 µmol dissolved in 450 µL of DMSO), EDC x HCl (1.2 µmol, 2400 eq.) dissolved in 4 µL of DMSO (taken from a stock solution: 12 µmol dissolved in 40 µL of DMSO), HOAt (240 nmol, 480 eq.) dissolved in 4 µL of DMSO (taken from a stock solution: 24 µmol dissolved in 400 µL of DMSO), and DIPEA (1.2 µmol, 2400 eq.) dissolved in 4 µL of DMSO (taken from a stock solution: 12 µmol dissolved in 40 µL of DMSO). The reaction mixture was filled with 22 µL of MOPS buffer to a final volume of 129 µL. The reaction mixture was shaken at room temperature for 18 hours. The amide coupling products **DNA-8a-c** were isolated by ethanol precipitation (twice). The DNA pellet was redissolved in 40 µL of distilled water, and analyzed by RP-HPLC (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (10% - 60% of methanol over 9 min), and by MALDI-MS to analyze product conversion and product identity.

ADoS protocol: For each pipetting step, the GC syringe was filled up to 50 µL. To obtain the required accuracy, 5 µL were discharged. From the total volume of 45 µL, the following volumes were injected into the corresponding wells.

50 µL of **DNA-6** (500 pmol) dissolved in MOPS buffer (50 mM, pH= 8, 0.5 M NaCl) were filled into the wells. Then, 22 µL of MOPS buffer were added. A carboxylic acid **7a-c** (2.7

µmol, 5400 eq.) dissolved in 45 µL of DMSO (stock solution: 27 µmol in 450 µL of DMSO), EDC x HCl (1.2 µmol, 2400 eq.) dissolved in 4 µL of DMSO (stock solution: 12 µmol in 40 µL of DMSO), HOAt (240 nmol, 480 eq.) dissolved in 4 µL of DMSO (stock solution: 24 µmol in 400 µL of DMSO), and DIPEA (1.2 µmol, 2400 eq.) dissolved in 4 µL of DMSO (stock solution: 12 µmol in 40 µL of DMSO) were added to a total volume of 129 µL. Shaking, reaction time, and analytics were done as described above.

In between each pipetting step, the outer surface of the needle was cleaned with DMSO and DI water for 5 s with the continuous cleaning station. Changing between two components, the outer and inner surfaces of the needle were cleaned with DMSO and DI water using the continuous cleaning station. The total volume of the syringe was filled three times for each cleaning agent.

6.1. Investigation of reproducibility of amide coupling reaction on DNA in solution

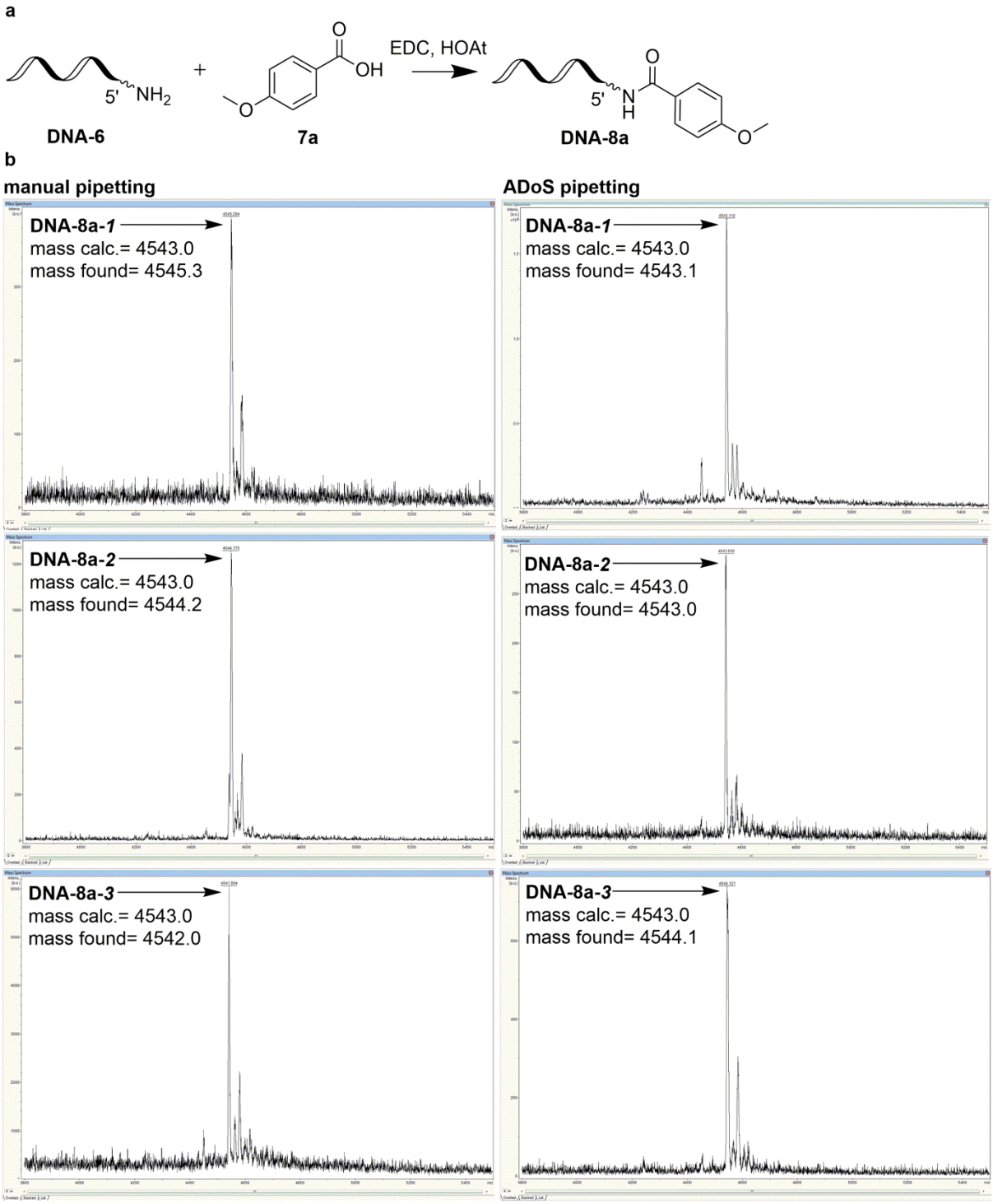


Figure S10. Coupling of carboxylic acid **7a** to **DNA-6** by amide bond formation. The reaction was set up in triplicate manually and with the *ADoS*. (a) Scheme for the synthesis of DNA conjugate **DNA-8a** by amide coupling. (b) MALDI-MS spectra of the crude product **DNA-8a-*1-3*** obtained by setting up experiments manually (left hand spectra) and MALDI-MS spectra of the crude product **DNA-8a-*1-3*** obtained by setting up experiments with *ADoS* (right hand spectra). Replicate numbers are indicated in italics.

6.2. Investigation of reproducibility and potential cross-contamination in amide coupling reaction on DNA in solution

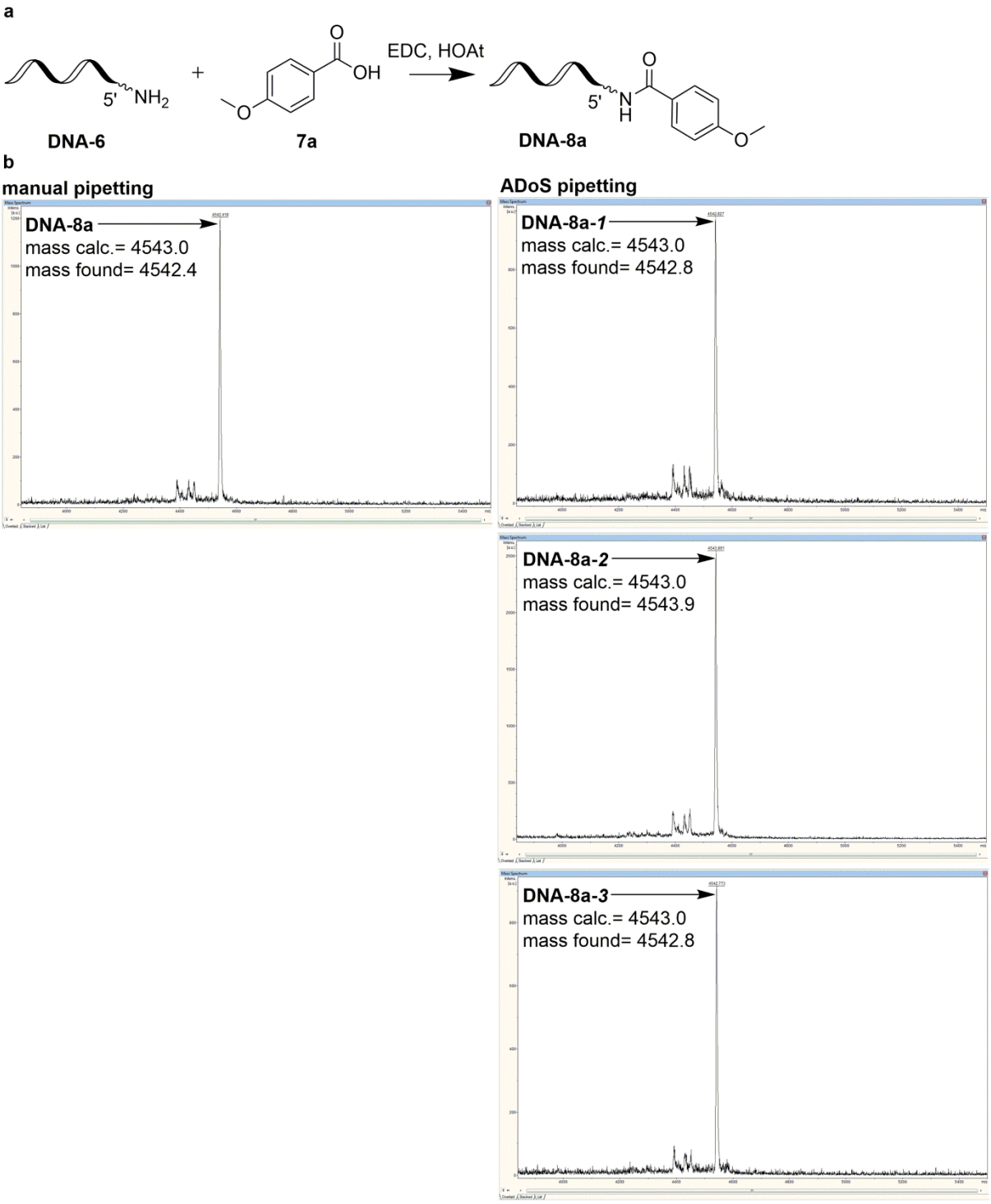


Figure S11. Coupling of carboxylic acid **7a** to **DNA-6** by amide bond formation. The reaction was set up manually and in triplicate with the *ADoS*. (a) Scheme for the synthesis of DNA conjugate **DNA-8a** by amide coupling. (b) MALDI-MS spectrua of the crude product **DNA-8a-*1-3*** obtained by setting up experiment manually (left hand spectrum) and MALDI-MS spectra of the crude product **DNA-8a-*1-3*** obtained by setting up experiments with *ADoS* (right hand spectra). Replicate numbers are indicated in italics.

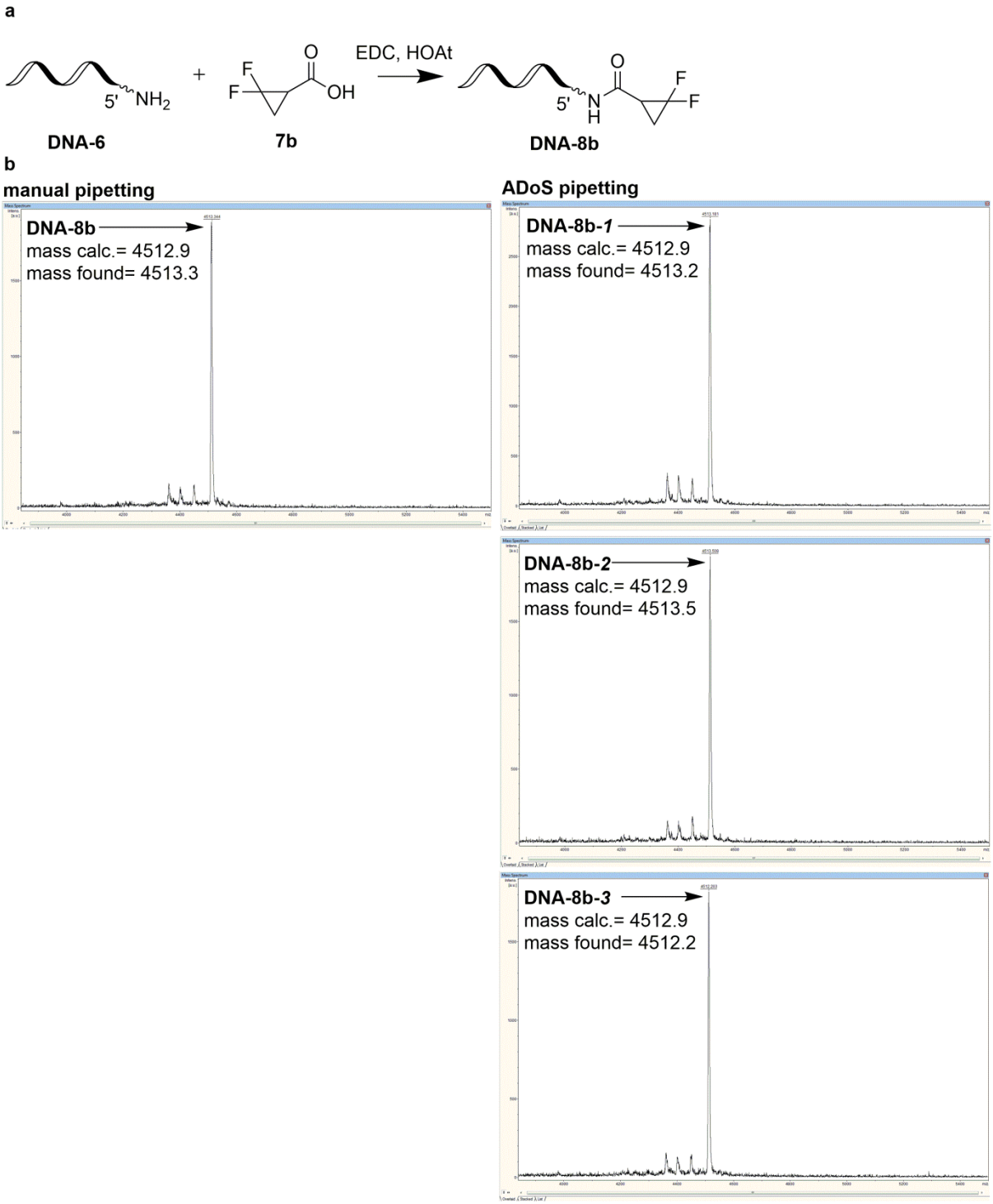


Figure S12. Coupling of carboxylic acid **7b** to **DNA-6** by amide bond formation. The reaction was set up manually and in triplicate with the *ADoS*. (a) Scheme for the synthesis of DNA conjugate **DNA-8b** by amide coupling. (b) MALDI-MS spectra of the crude product **DNA-8b-*1-3*** obtained by setting up experiment manually (left hand spectrum) and MALDI-MS spectra of the crude product **DNA-8b-*1-3*** obtained by setting up experiments with *ADoS* (right hand spectra). Replicate numbers are indicated in italics.

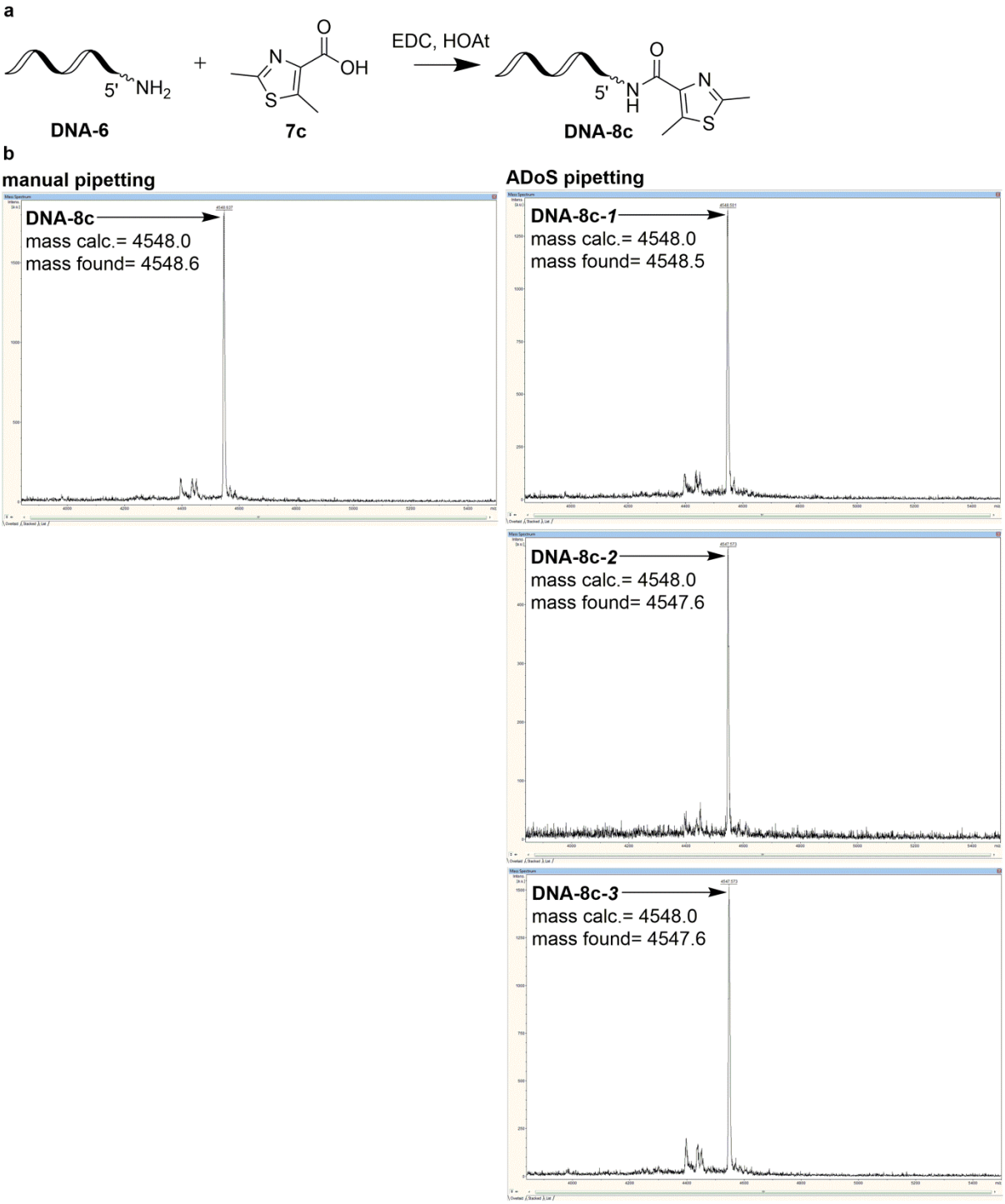


Figure S13. Coupling of carboxylic acid **7c** to **DNA-6** by amide bond formation. The reaction was set up manually and in triplicate with the *ADoS*. (a) Scheme for the synthesis of DNA conjugate **DNA-8c** by amide coupling. (b) MALDI-MS spectra of the crude product **DNA-8c-*1*-*3*** obtained by setting up experiment manually (left hand spectrum) and MALDI-MS spectra of the crude product **DNA-8c-*1*-*3*** obtained by setting up experiments with *ADoS* (right hand spectra). Replicate numbers are indicated in italics.

7. Polymer micelle 4-mediated Povarov reaction to DNA-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline conjugates

7.1. Synthesis of DNA–conjugates by amide coupling on solid support

7.1.1. Procedure for coupling of carboxylic acids to 5'-aminolinker-DNA–conjugates

The DMt-protective group of 5'-aminolinker-modified DNA (5`-GTC TTG CCG AAT TC) bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ca. 40 mg) was removed by addition of 3% trichloroacetic acid in dry CH_2Cl_2 (3 x 200 µL) for 3 x 1 min. A yellow to orange color indicated a successful removal of the protective group. The CPG containing the amine-deprotected DNA was washed three times with each 200 µL of 1% TEA in MeCN, DMF, MeOH, MeCN, and CH_2Cl_2. The CPG, a carboxylic acid, and HATU were then dried *in vacuo* for 15 min. Stock solutions of all reactants in dry DMF were prepared immediately before the reaction was started. To 300 µL of a solution of a carboxylic acid (100 µmol, 100 eq.) in dry DMF were added HATU (38 mg, 100 µmol, 100 eq.) dissolved in 300 µL of dry DMF and DIPEA (42 µL, 250 µmol, 250 eq.). This reaction mixture was shaken for 5 min and added to the solid support-bound DNA suspended in dry DMF (300 µL). The amide coupling reaction was shaken at room temperature for 4 h. Then, the CPG containing the DNA-conjugate was filtered over a filter column and washed subsequently with each 3 x 200 µL of DMF, MeOH, MeCN, and CH_2Cl_2. Unreacted amines were capped with acetic acid anhydride (a 1:1 mixture of THF/methylimidazole, 9:1, vol/vol, and THF/pyridine/acetic acid anhydride, 8:1:1, vol/vol was used), and the CPG was washed again with each 3 x 200 µL of DMF, MeOH, MeCN, and CH_2Cl_2, and dried *in vacuo* for 15 min. For purification, the DNA-conjugate was deprotected and cleaved from the CPG by treatment with 500 µL of AMA (AMA= aqueous ammonia (30%)/ aqueous methylamine (40%), 1:1, vol/vol) for 4 h at room temperature. To this solution 20 µL of 1 M Tris buffer (pH= 7.5) were added, the mixture was dried in a SpeedVac, redissolved in 200 µL of distilled water, and the product was purified by RP-HPLC (Gemini,

5u, C18, 110 Å column; 100*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20% - 70% of methanol over 13 min).

7.1.2. Synthesis of DNA-aldehyde conjugate DNA-1

DNA-6 (100 nmol, 5`-GTC TTG CCG AAT TC) was coupled with 2-(4-formylphenoxy)acetic acid **7d** (1.8 mg, 10 µmol, 100 eq.) to furnish **DNA-1**, according to the procedure for coupling of carboxylic acids to amino-modified DNA. For analysis, an aliquot of ca. 10 nmol of each conjugate was deprotected and cleaved from the CPG with 500 µL of AMA (AMA= aqueous ammonia (30%)/ aqueous methylamine (40%), 1:1, vol/vol) for 4 h at room temperature. Then, 20 µL of 1 M Tris buffer (pH= 7.5) were added, the products were dried in a SpeedVac, redissolved in 100 µL of distilled water, and purified by RP-HPLC (Gemini, 5u, C18, 110 Å column; 100*10.0 mm) with a gradient of aqueous triethylammonium acetate buffer (100 mM, pH= 8) and methanol (20% - 70% of methanol over 13 min). After purification, fractions containing the desired product were collected, evaporated, and co-evaporated with 3 x 200 µL of ethanol in a SpeedVac, and then precipitated twice from ethanol.

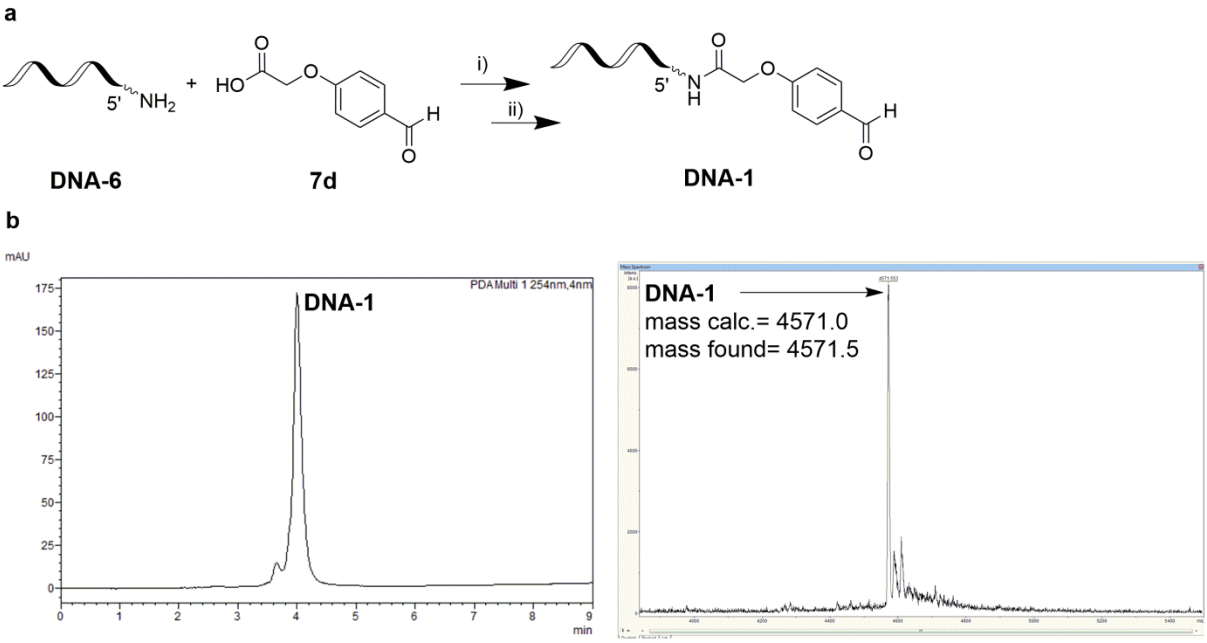


Figure S14. Synthesis of the DNA-aldehyde conjugate **DNA-1**. (a) Scheme of the synthesis of **DNA-1**. (b) HPLC trace of the aldehyde **DNA-1** (left hand trace) and MALDI-MS spectrum of the aldehyde **DNA-1** (right hand spectrum). Reagents and conditions: i) HATU, DIPEA, dry DMF, room temperature, 4 h; ii) AMA (aqueous ammonia (30%)/ aqueous methylamine (40%), 1:1, vol/vol), 4 h, room temperature.

7.2 Initial attempt of reagent dispensing for polymer micelle 4-mediated Povarov reaction to DNA-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline conjugate DNA-5a with *ADoS* robotic system

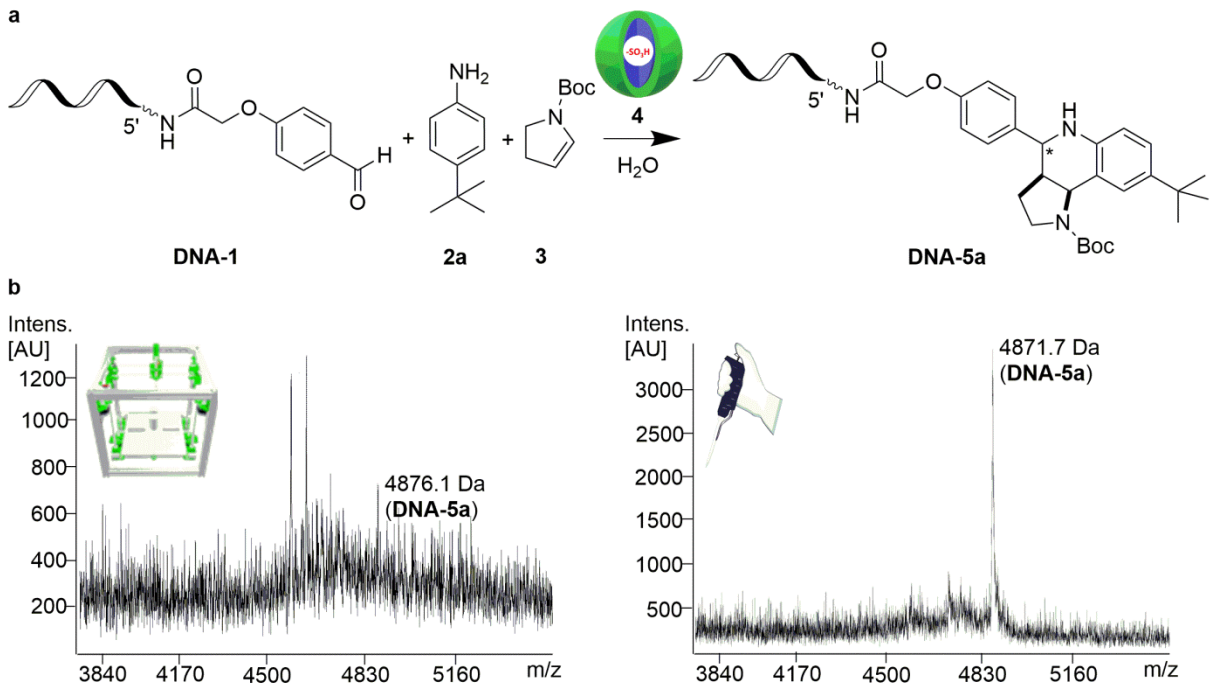


Figure S15. Initial attempt of reagent dispensing for micelle-mediated Povarov reaction of DNA-aldehyde **DNA-1** to DNA-hexahydropyrroloquinoline **DNA-5a** (5'-C6-aminolinker-GTC TTG CCG AAT TC, M (calc.): 4871.4 Da) with automated *ADoS* robotic system. (a) Scheme of the micelle-mediated Povarov reaction yielding **DNA-5a**. (b) Head-to-head comparison of reactions set up with automated *ADoS* robotic system and by hand pipetting. MALDI MS spectra of an attempted micelle-mediated reaction to **DNA-5a** set up with *ADoS* robotic system (left-hand trace) and by manual pipetting (right-hand trace).

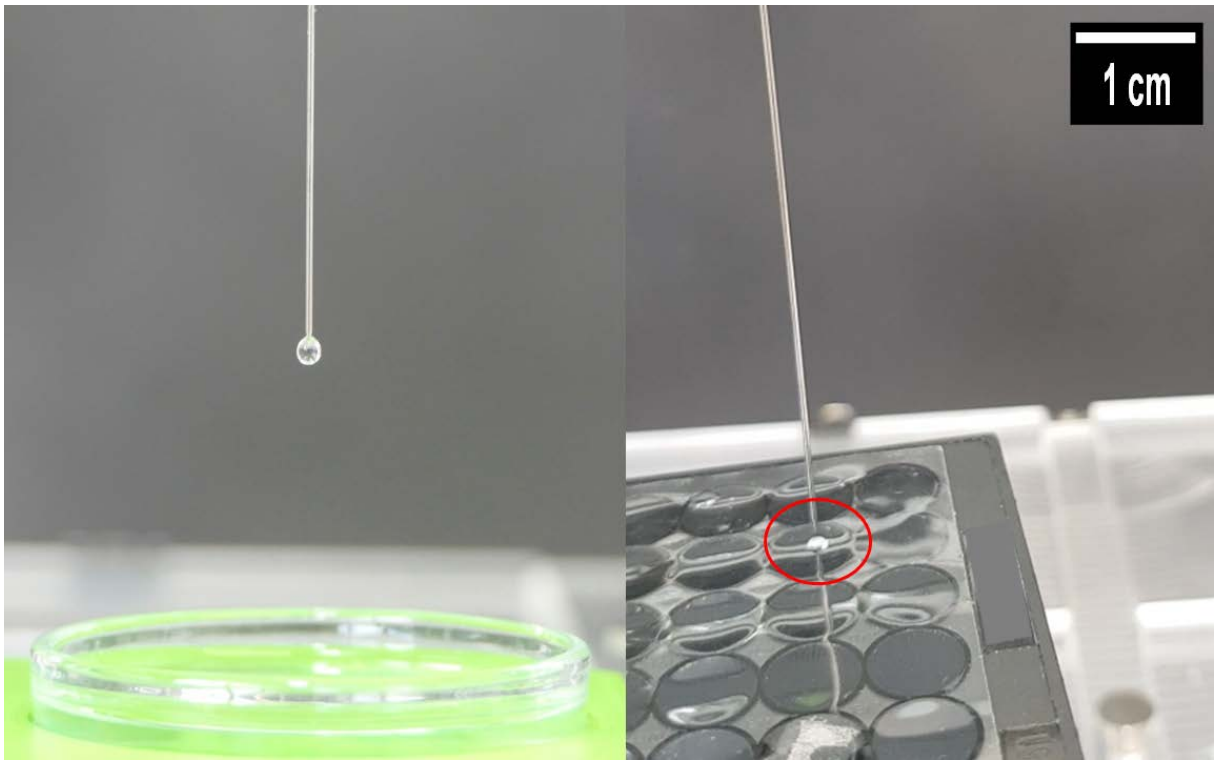


Figure S16. Image of 1 µL droplet at the tip of the needle of the GC syringe (left); 1 µL droplet attached to the surface of cover foil (right)

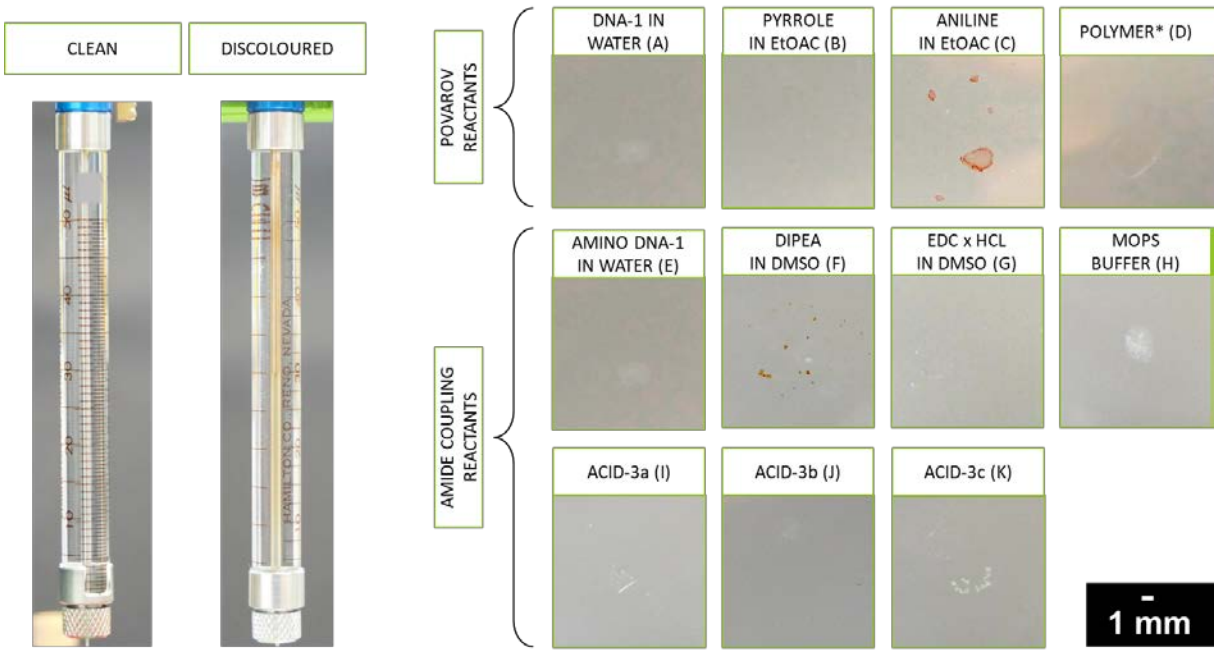
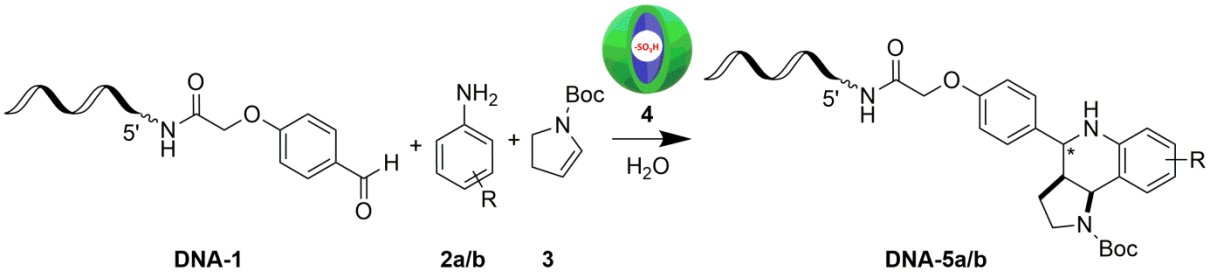


Figure S17. Clogged glass cylinder of the syringe after 2 days of down time in comparison to the clean syringe (left); investigation of dried reagents for amide coupling and Povarov reaction revealed that the anilines caused syringe clogging.

7.3. Polymer micelle 4-mediated Povarov reaction – screening of different co-solvents



Scheme S2. Polymer micelle **4**-mediated Povarov reaction of DNA-aldehyde **DNA-1**, anilines **2a**/**b**, and olefin **3** to DNA-hexahydropyrroloquinoline conjugates **DNA-5a**/**b**.

Protocol for manual pipetting[2]**:** To a solution of DNA-aldehyde conjugate **DNA-1** (500 pmol) in distilled water (5 µL) were added an aniline **2a** or **2b** (1 µmol, 2000 eq.) dissolved in 1.5 µL of a co-solvent (stock solution: 60 µmol in 90 µL of co-solvent), *N*-Boc-2,3-dihydro-1*H*-pyrrole **3** (1 µmol, 2000 eq.) dissolved in 1 µL of a co-solvent (stock solution: 150 µmol in 150 µL of co-solvent), and block copolymer **4** (25 nmol, 50 eq.) dissolved in 12 µL of distilled water. Polymer **4** was taken from an aqueous stock solution (0.5 µmol in 240 µL). The reaction mixture was filled with 30.5 µL of distilled water to a volume of 50 µL giving a final concentration of 500 µM of polymer **4**. The reaction mixtures were shaken at room temperature for 6 hours. Then, 70 µL of distilled water were added and the reaction mixture was thoroughly extracted with ethyl acetate (6 x 400 µL). The aqueous solution was evaporated in a SpeedVac, the residue was redissolved in 45 µL of distilled water, and coupling products **DNA-5a**/**b** were analyzed by RP-HPLC (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (10% - 60% of methanol over 9 min), and by MALDI-MS analysis. The product conversion was estimated based on the area under the curve of the product peak versus the starting material peak in the HPLC-trace (analytical HPLC) and by MALDI-MS.

ADoS protocol: For each pipetting step, the GC syringe was filled up to 50 µL. To obtain the highest accuracy, 5 µL were discharged. From the total volume of 45 µL, the following volumes were injected into the corresponding wells.

The wells were filled with DNA-aldehyde conjugate **DNA-1** (500 pmol) in 5 µL distilled water. Aniline **2a** or **2b** (1 µmol, 2000 eq.) dissolved in 1.5 µL of a co-solvent (stock solution: 60 µmol in 90 µL of a co-solvent), *N*-Boc-2,3-dihydro-1*H*-pyrrole **3** (1 µmol, 2000 eq.) dissolved in 1 µL of a co-solvent (stock solution: 150 µmol in 150 µL of co-solvent), and block copolymer **4** (25 nmol, 50 eq.) dissolved in 12 µL of distilled water taken from a aqueous stock solution (0.5 µmol in 240 µL) were added. 30.5 µL DI water was added to a total volume of 50 µL with a polymer concentration of 500 µM. Shaking, reaction time, and analytics were done as described above.

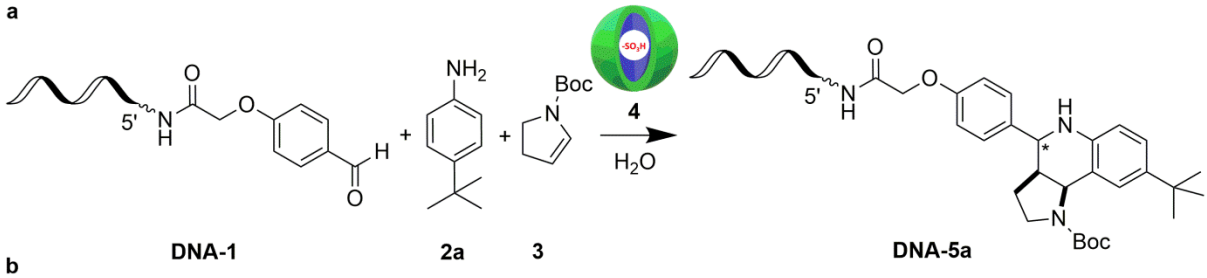
In between each pipetting step, the outer surface of the needle was cleaned with DMSO and deionized water for 5 s on the continuous cleaning station. Changing between two components, the outer and inner surface of the needle were cleaned with DMSO and deionized water on the continuous cleaning station. The total volume of the syringe was pulled up three times for each cleaning agent.

Table S2. Impact of co-solvents on the block copolymer **4**-mediated Povarov reaction to DNA-hexahydro-1*H*-pyrrolo[3,2-*c*]quinoline conjugate **DNA-5a/b**.

No.	aniline	polymer **4** [eq.][a]	polymer **4** concentration [μM]	co-solvent[b]	**DNA-5a/b** (manual pipetting) [%][c]	**DNA-5a/b** (*ADoS* pieptting) [%][c]
1	**2a**	50	500	DMF	95	92
2	**2a**	50	500	MeOH	96	96
3	**2a**	50	500	MeCN	96	96
4	**2a**	50	500	EtOAc	92	92
5	**2a**	50	500	1,2-DCE	93	95
6	**2b**	50	500	DMF	62	50
7	**2b**	50	500	MeOH	61	58
8	**2b**	50	500	MeCN	64	60
9	**2b**	50	500	EtOAc	64	62
10	**2b**	50	500	1,2-DCE	52	48

[a] versus the DNA-aldehyde conjugate **DNA-1**; [b] each co-solvent was used in volume corresponding to a 5% of total reaction volume; [c] HPLC analysis of the crude, missing percentage to 100%: mainly **DNA-1**.

a

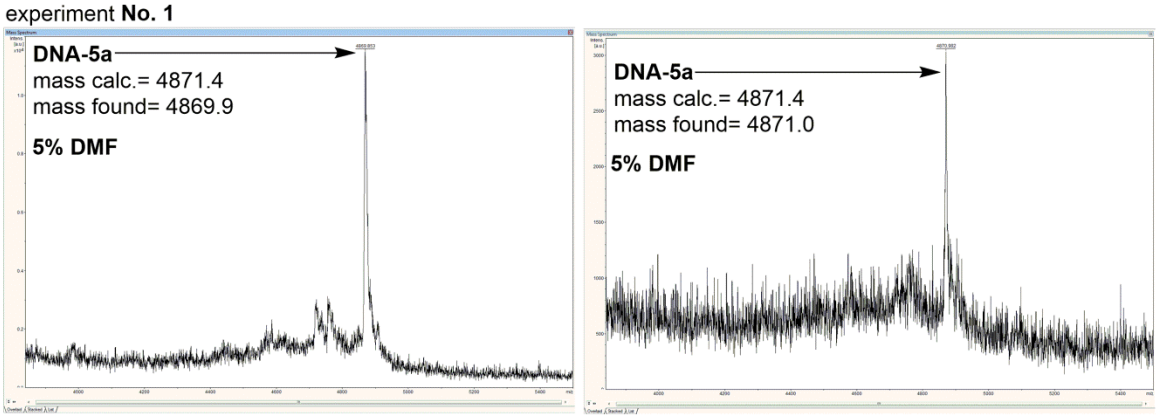


DNA-1 **2a** **3** **DNA-5a**

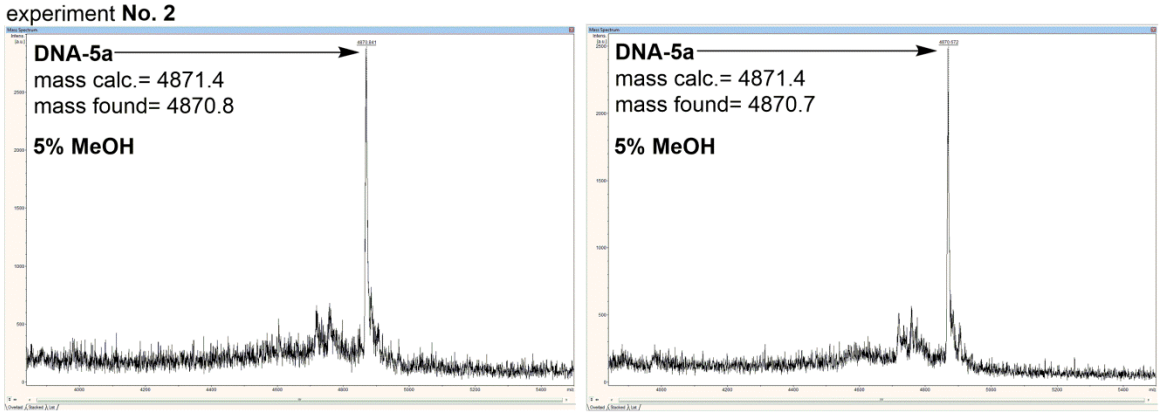
b

manual pipetting **ADoS pipetting**

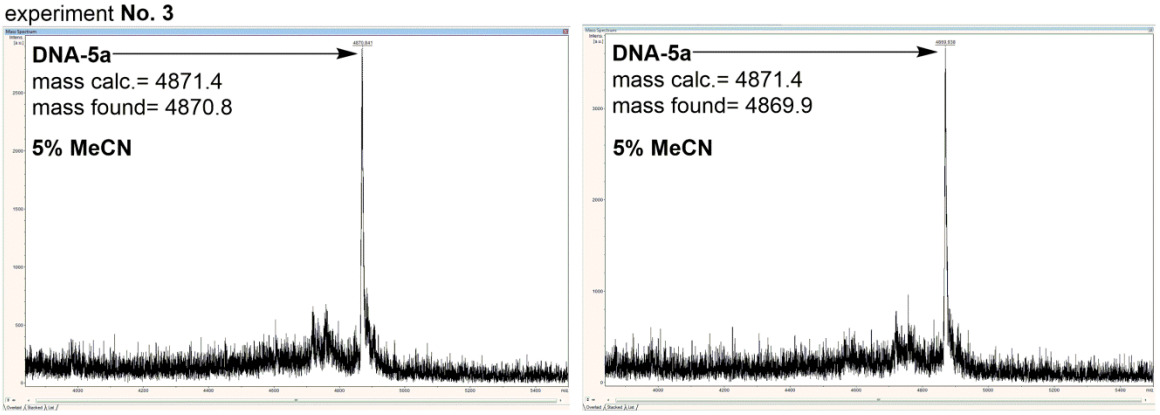
experiment **No. 1**



experiment **No. 2**



experiment **No. 3**



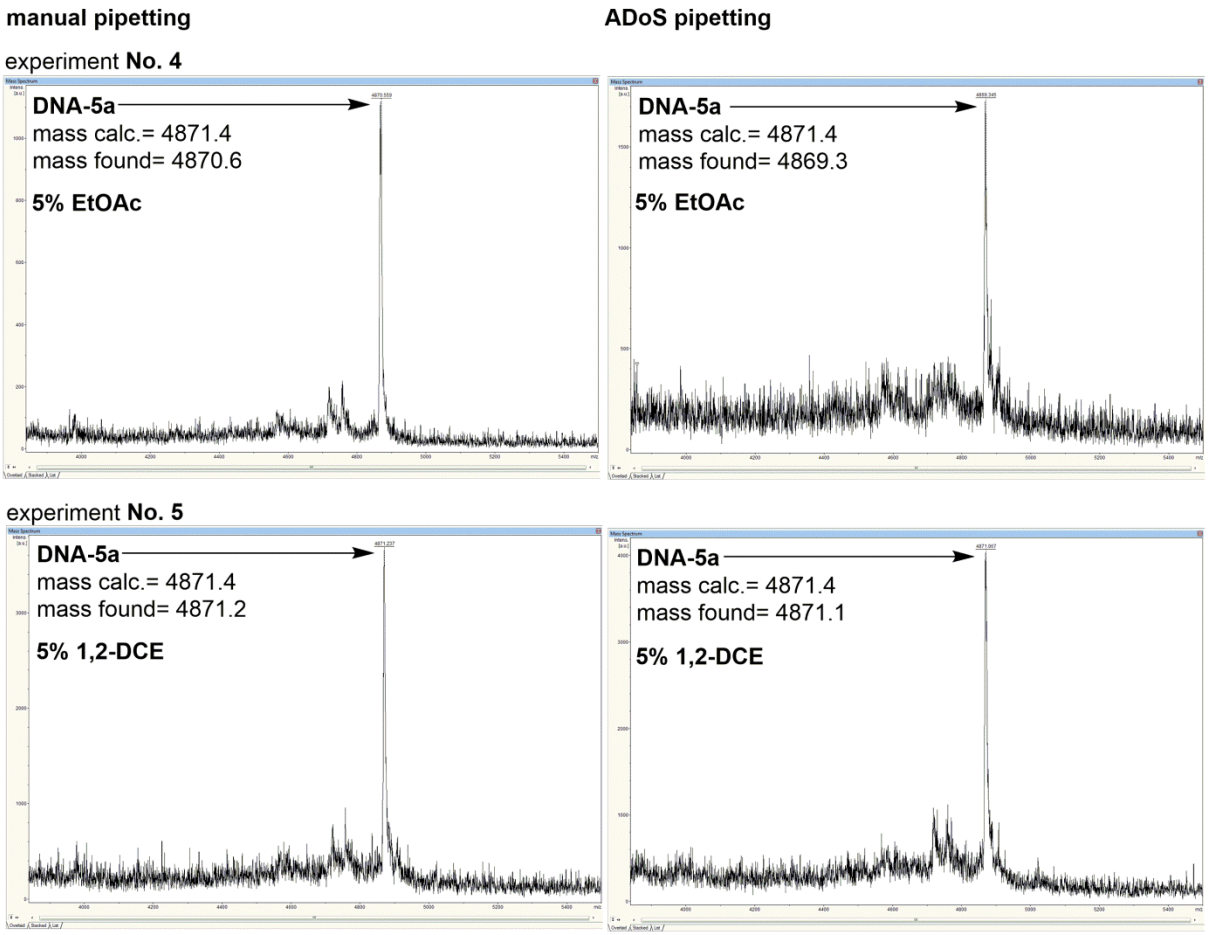
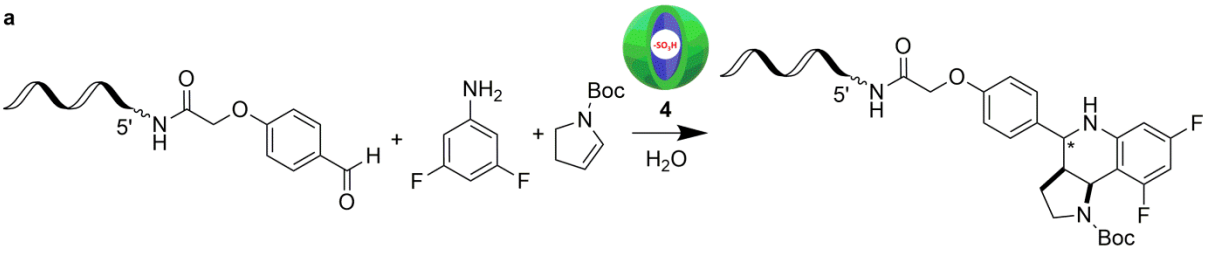


Figure S18. Effect of co-solvents on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2a**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table S2. The reactions were set up manually (left hand MALDI spectra) and by *ADoS* (right hand MALDI spectra). MALDI-MS spectra show experiments **No. 1** - **No. 5**.

a

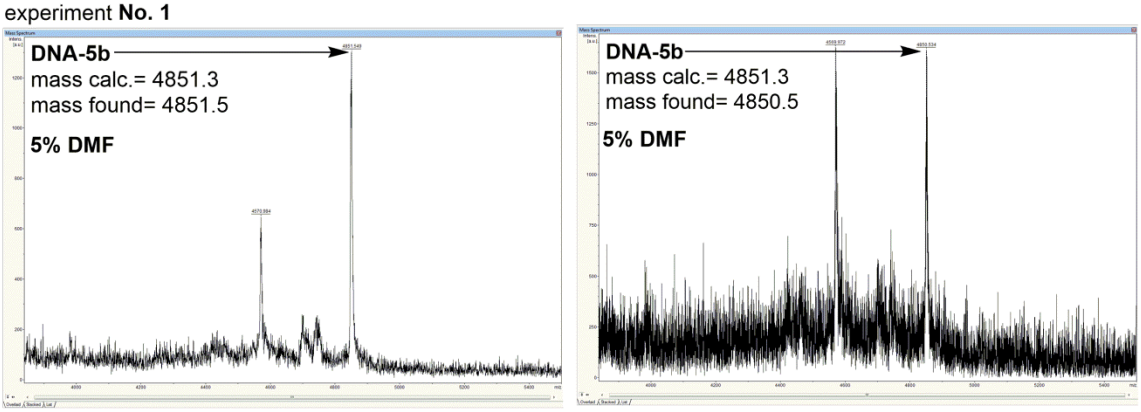


DNA-1 **2b** **3** **DNA-5b**

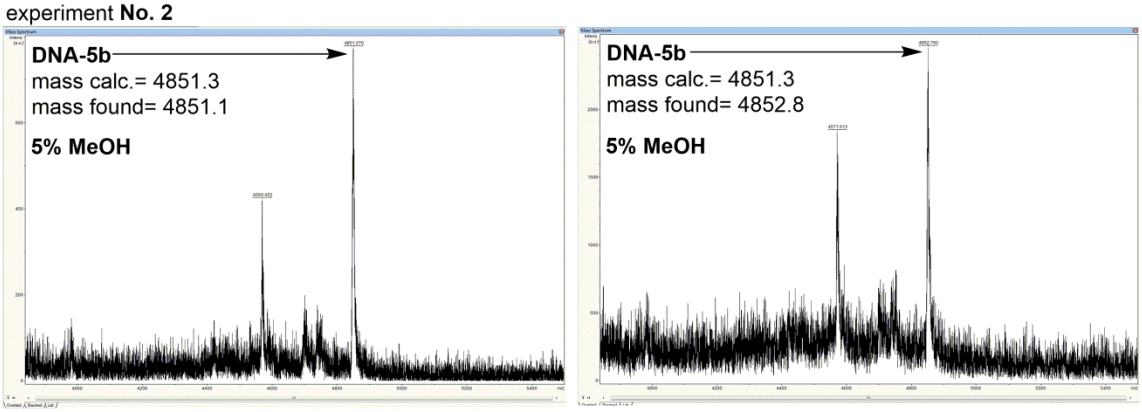
b

manual pipetting **ADoS pipetting**

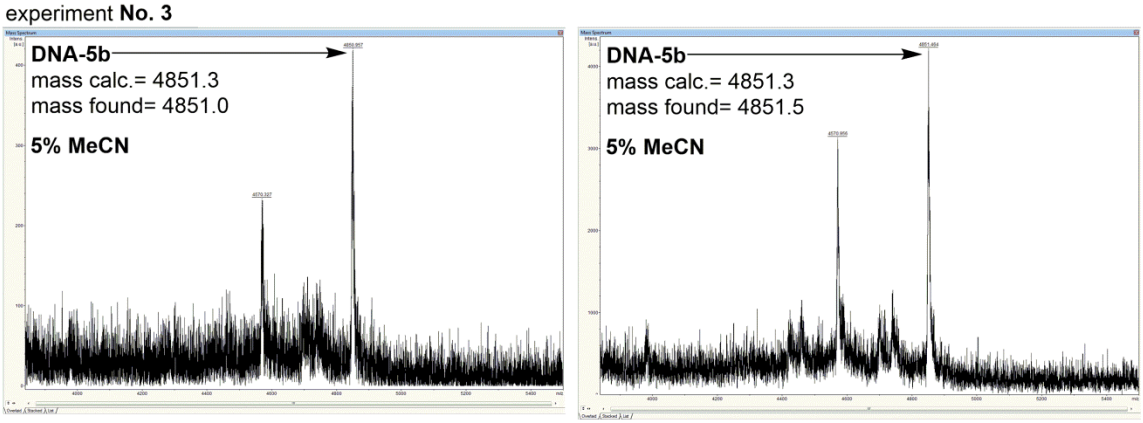
experiment **No. 1**



experiment **No. 2**



experiment **No. 3**



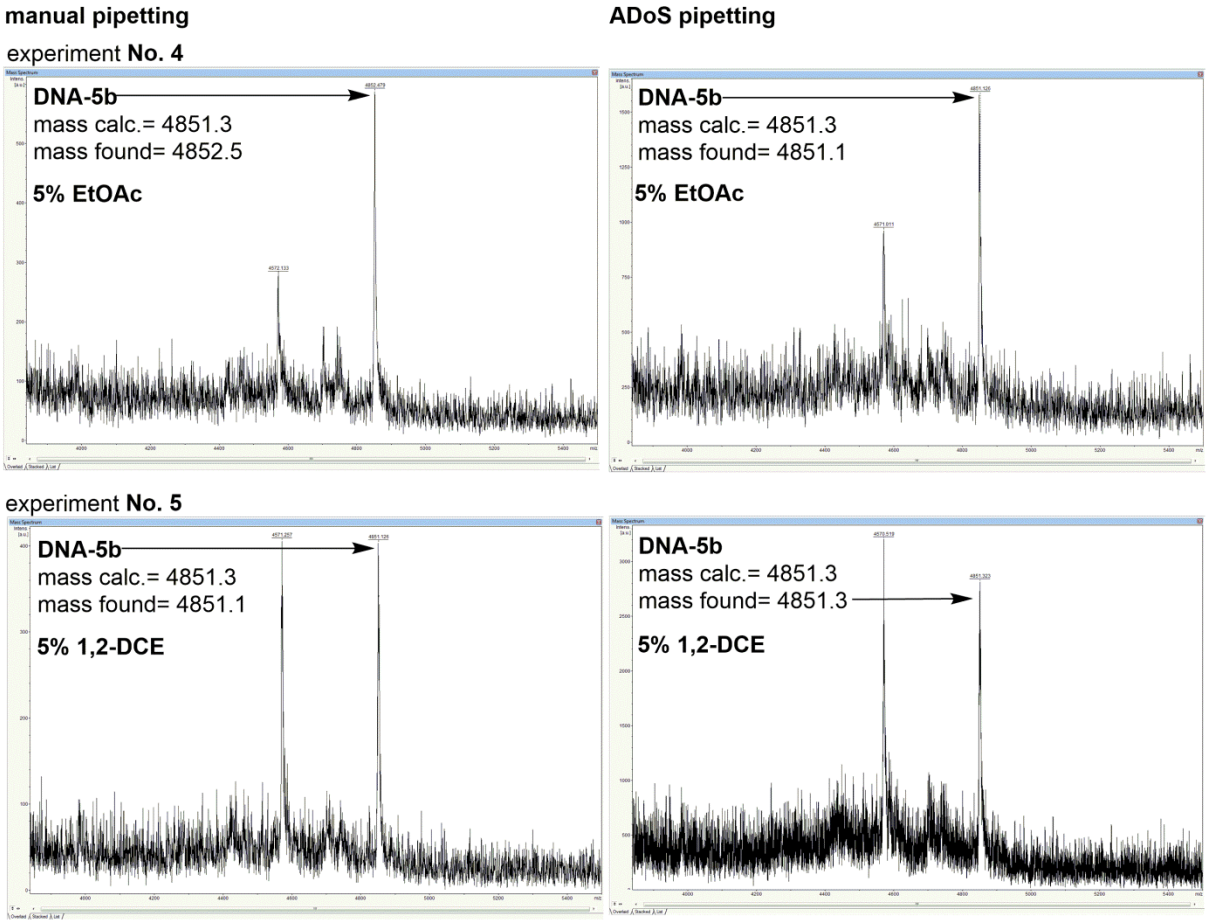


Figure S19. Effect of co-solvents on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2b**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table S2. The reactions were set up manually (left hand MALDI spectra) and by *ADoS* (right hand MALDI spectra). MALDI-MS spectra show experiments **No. 6** - **No. 10**.

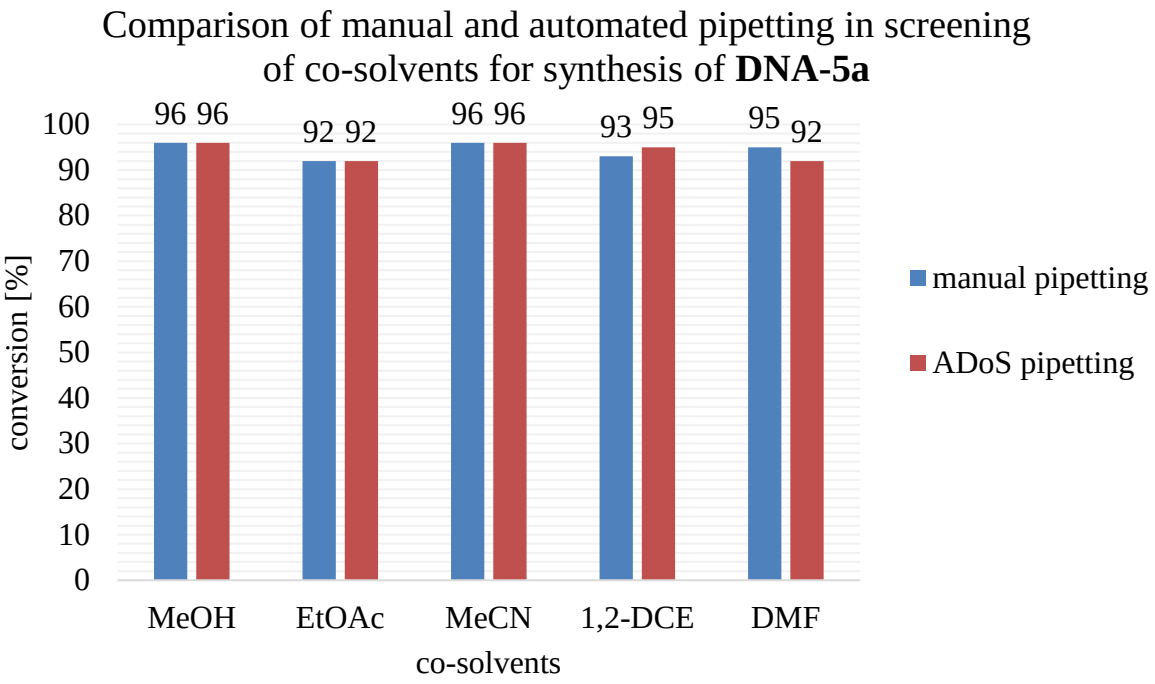


Figure S20. Effect of co-solvents on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2b**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table S2 (**No. 1** - **No. 5**).

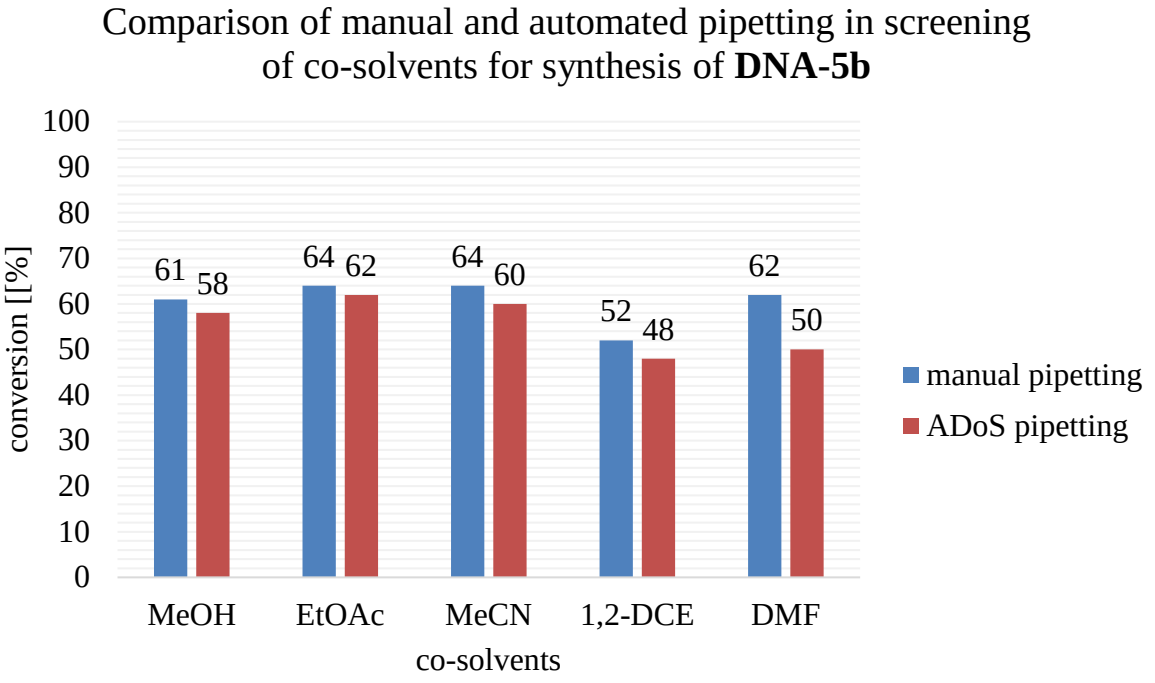


Figure S21. Effect of co-solvents on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2b**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table S2 (**No. 6** - **No. 10**).

7.4. Polymer micelle 4-mediated Povarov reaction – polymer dilution experiment

Protocol for manual pipetting:[2] To a solution of DNA-aldehyde conjugate **DNA-1** (500 pmol) in distilled water (5 µL) were added an aniline **2a** (1 µmol, 2000 eq.) dissolved in 1.5 µL of ethyl acetate (stock solution: 60 µmol in 90 µL of ethyl acetate), *N*-Boc-2,3-dihydro-1*H*-pyrrole **3** (1 µmol, 2000 eq.) dissolved in 1 µL of ethyl acetate (stock solution: 150 µmol in 150 µL of ethyl acetate), and block copolymer **4** at the amount given in Table 1. Polymer **4** was taken from an aqueous stock solution. The reaction mixture was filled with distilled water to reach the final concentration of polymer **4** given in Table 1. The reaction mixtures were shaken at room temperature for 18 hours or 3 hours. Then, 70 µL of distilled water were added and the reaction mixture was thoroughly extracted with ethyl acetate (6 x 400 µL). The aqueous solution was evaporated in a SpeedVac, the residue was redissolved in 45 µL of distilled water, and coupling products **DNA-5a** were analyzed by RP-HPLC (Phenomenex, Gemini; 5 µm, C18, 110 Å, 100*4.6 mm) with a gradient of aqueous triethylammonium acetate buffer (10 mM, pH= 8) and methanol (10% - 60% of methanol over 9 min), and by MALDI-MS analysis. The product conversion was estimated based on the area under the curve of the product peak versus the starting material peak in the HPLC-trace (analytical HPLC) and by MALDI-MS.

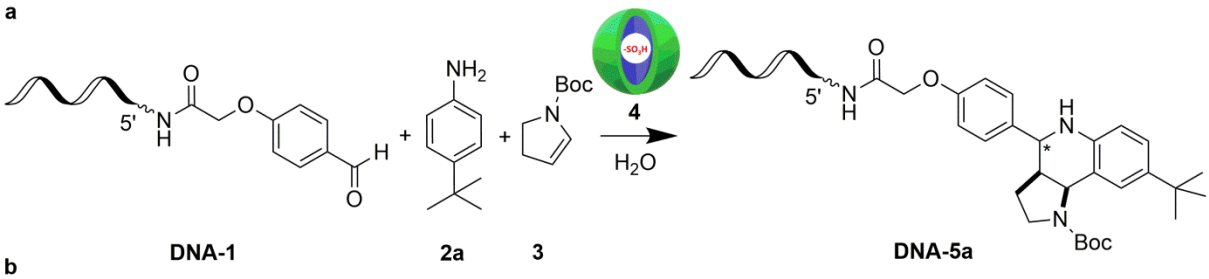
ADoS protocol: For each pipetting step, the GC syringe was filled up to 50 µL. To obtain the highest accuracy, 5 µL were discharged. From the total volume of 45 µL, the following volumes were injected into the corresponding wells.

The wells were filled with DNA-aldehyde conjugate **DNA-1** (500 pmol) in 5µL distilled water. Aniline **2a** or **2b** (1 µmol, 2000 eq.) dissolved in 1.5 µL of ethyl acetate (stock solution: 60 µmol in 90 µL of ethyl acetate), *N*-Boc-2,3-dihydro-1*H*-pyrrole **3** (1 µmol, 2000 eq.)

dissolved in 1 µL of ethyl acetate (taken from a stock solution: 150 µmol dissolved in 150 µL of ethyl acetate), and block copolymer **4** (Table 1) taken from a aqueous stock solution were added. 30.5 µL DI water was added to a total volume of 50 µL with a polymer concentration as given in Table 1. Shaking, reaction time, and analytics were done as described above.

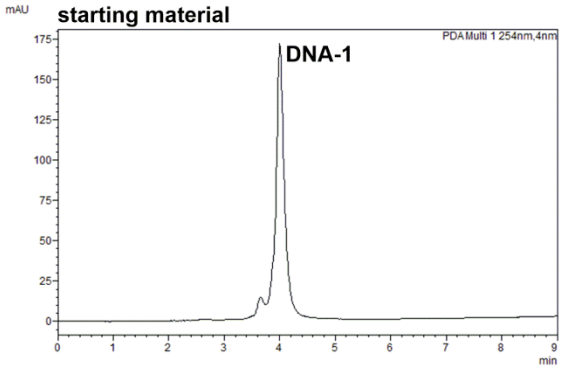
In between each pipetting step, the outer surface of the needle was cleaned with DMSO and DI water for 5 s on the continuous cleaning station. Changing between two components, the outer and inner surfaces of the needle were cleaned with DMSO and DI water on the continuous cleaning station. The total volume of the syringe was pulled up three times for each cleaning agent.

a

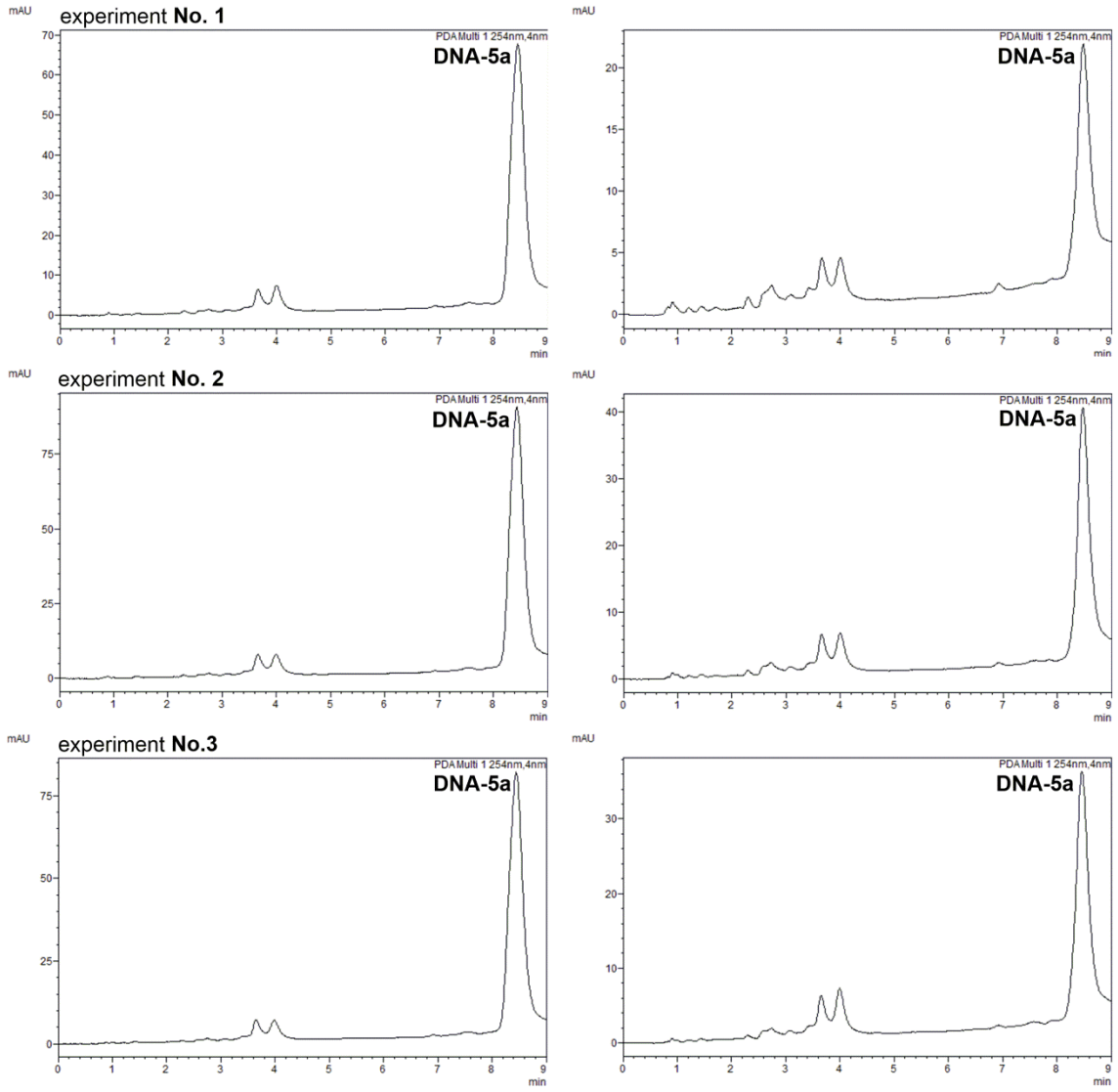


DNA-1 2a 3 DNA-5a

b



manual pipetting **ADoS pipetting**



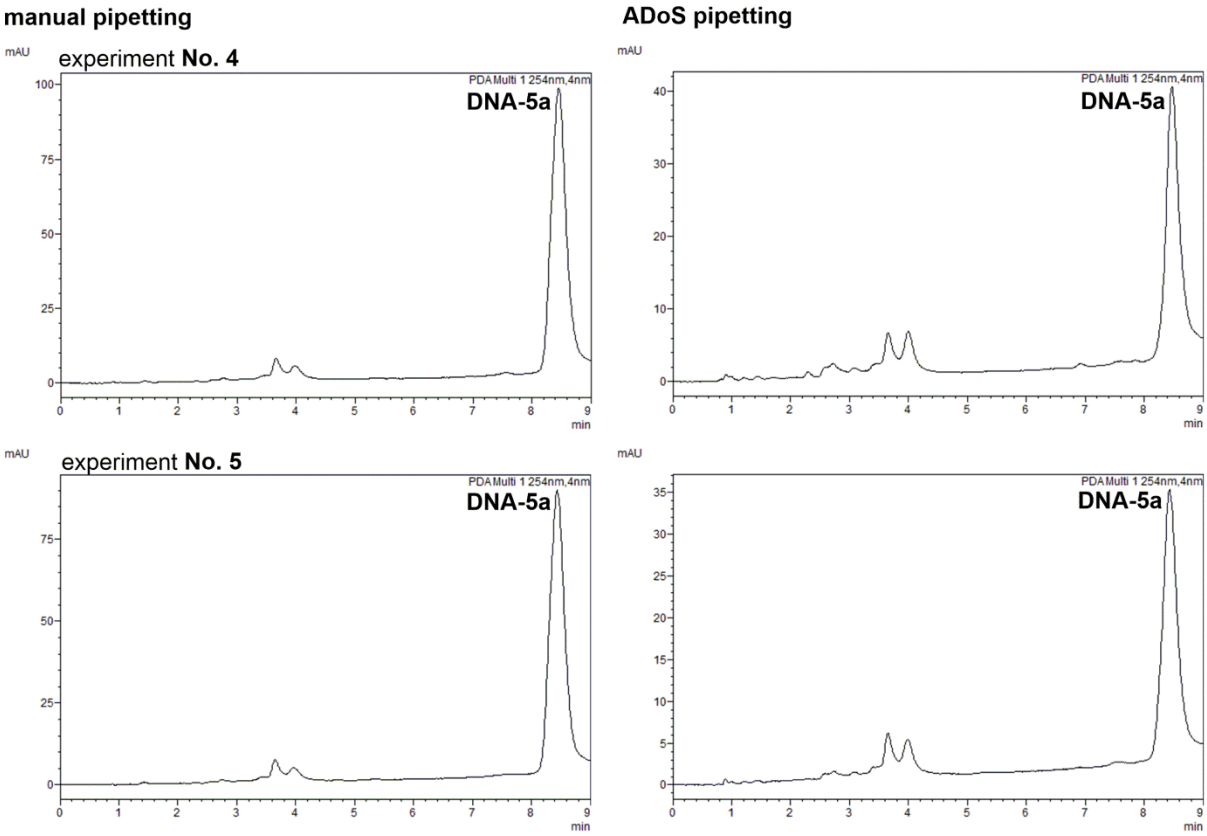
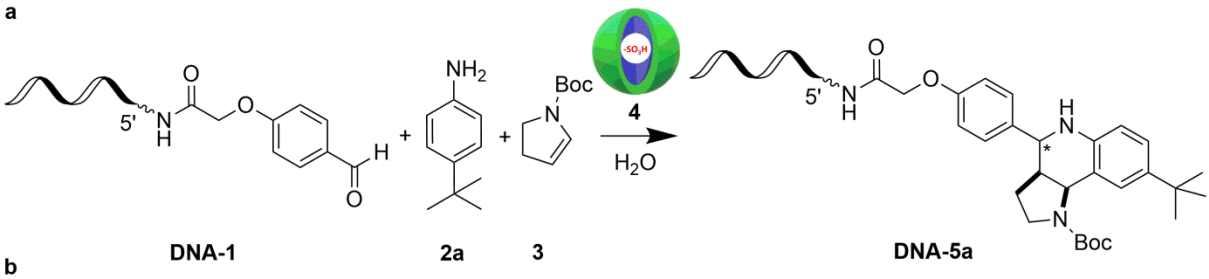


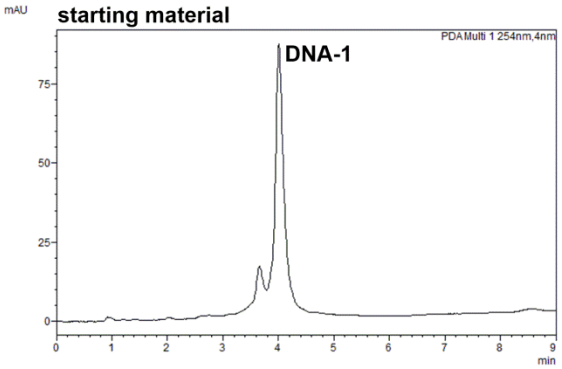
Figure S22. Effect of polymer **4** concentration on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2a**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table 1. The reactions were set up manually and with the *ADoS*. HPLC traces show starting material DNA-aldehyde conjugate **DNA-1** and experiments **No. 1** - **No. 5**.

a

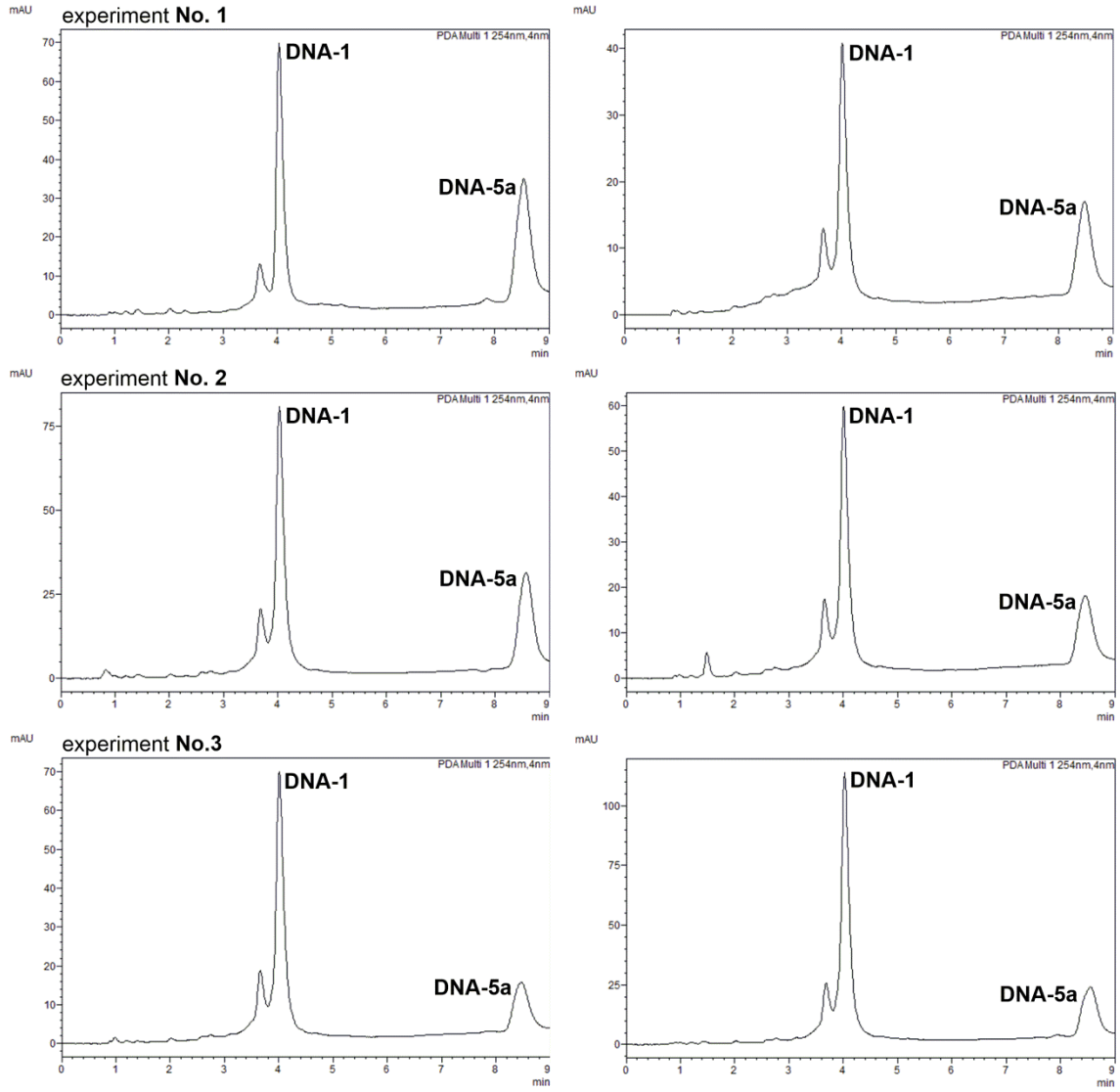


DNA-1 **2a** **3** **DNA-5a**

b



manual pipetting **ADoS pipetting**



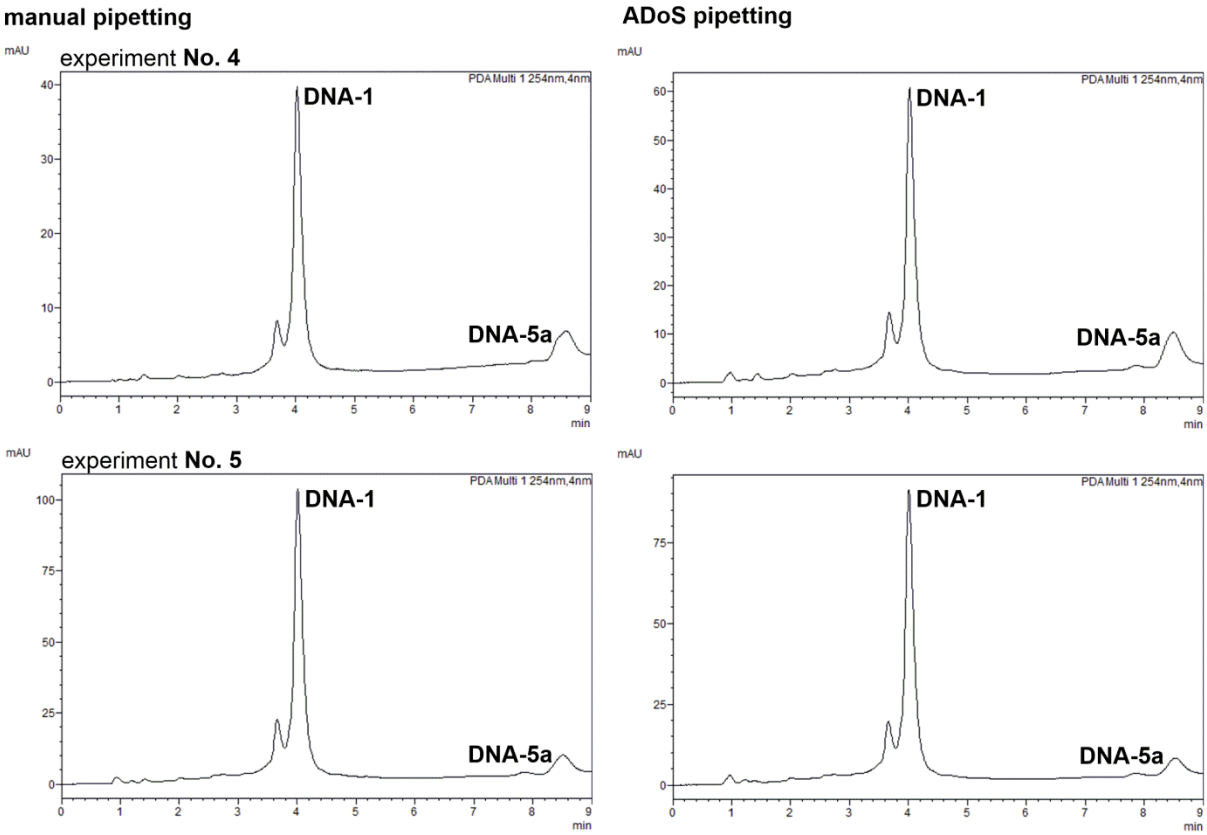


Figure S23. Effect of polymer **4** concentration on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2a**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table 1. Reactions were set up manually (left hand spectra) and with the *ADoS* (right hand spectra). HPLC traces show starting material DNA-aldehyde conjugate **DNA-1** and experiments **No. 6** - **No. 10**.

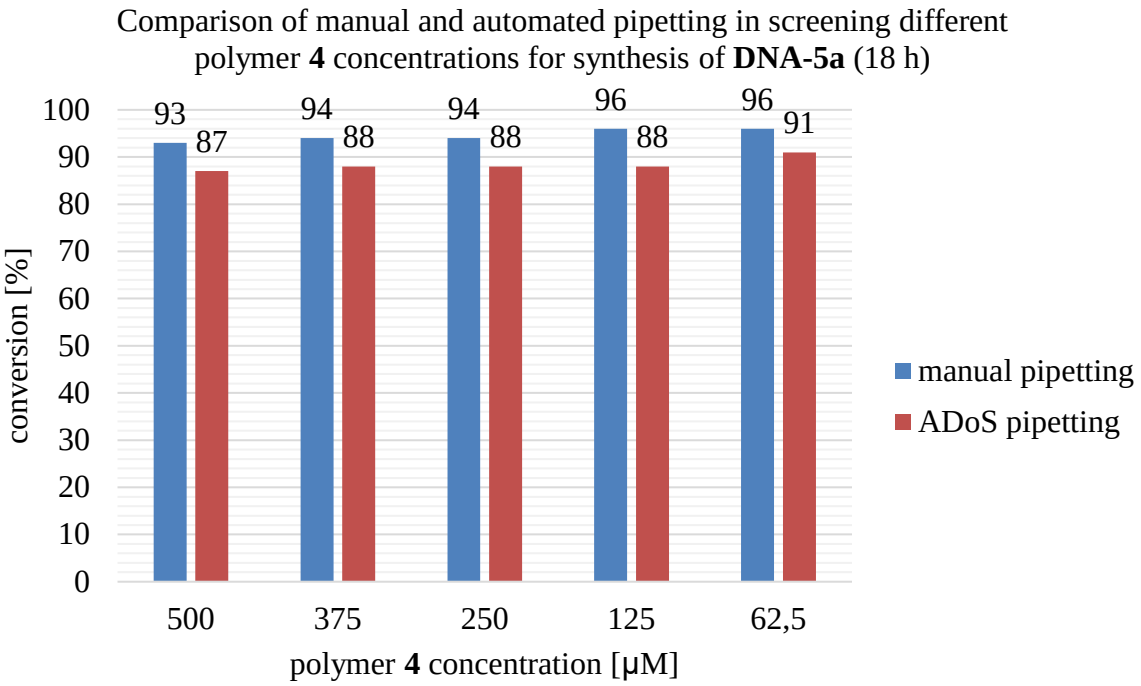


Figure S24. Effect of polymer **4** concentration on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2a**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table 1 (**No. 1** - **No. 5**).

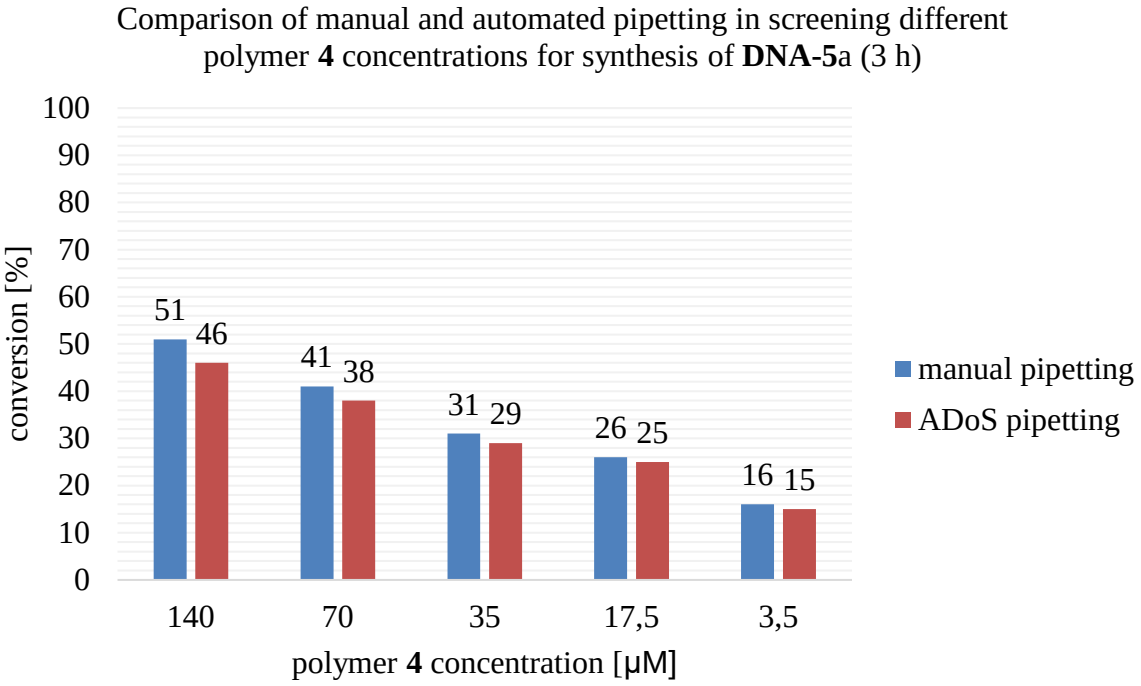


Figure S25. Effect of polymer **4** concentration on the polymer **4**-mediated Povarov reaction of the oligonucleotide-aldehyde conjugate **DNA-1**, 4-*tert*-butylaniline **2a**, and *tert*-butyl 2,3-dihydro-1*H*-pyrrole-1-carboxylate **3**, for reaction conditions see Table 1 (**No. 6** - **No. 10**).

8. Literature

1 SCOTT_3D, *HyperCube Evolution*. https://www.thingiverse.com/thing:2254103, last date accessed: 21. Nov. 2019.

2 Klika Škopić, M.; Götte, K.; Gramse, C.; Dieter, M.; Pospich, S.; Raunser, R.; Weberskirch, R.; Brunschweiger, A. Micellar brønsted acid mediated synthesis of DNA-tagged heterocycles. *J. Am. Chem. Soc.*, **2019**, *141*, 10546-10555.

3 Li, Y. Z.; Gabriele, E.; Samain, F.; Favalli, N.; Sladojevich, F.; Scheuermann, J.; Neri, D. Optimized reaction conditions for amide bond formation in DNA-encoded combinatorial libraries. *ACS Comb. Sci.*, **2016**, *18*, 438–443.