An amphiphilic polymer-supported strategy enables chemical transformations under anhydrous conditions for DNA-encoded library synthesis.

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Supporting information:

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Abbreviations.

ACN: Acetonitrile

DIC: Diisopropylcarbodiimide

DMF: Dimethylformamide

DMT-MM: 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (CAS 3945-69-5)

DCM: Dichloromethane

HFIP: Hexafluoroisopropanol

HOAt: 1-Hydroxy-7-azabenzotriazole

LED: Light-Emitting Diode

MeOH: Methanol

TEA: Triethylamine

Rt: Retention time

SnAP: tin (Sn) amine protocol

TOF: time-of-flight mass spectrometry

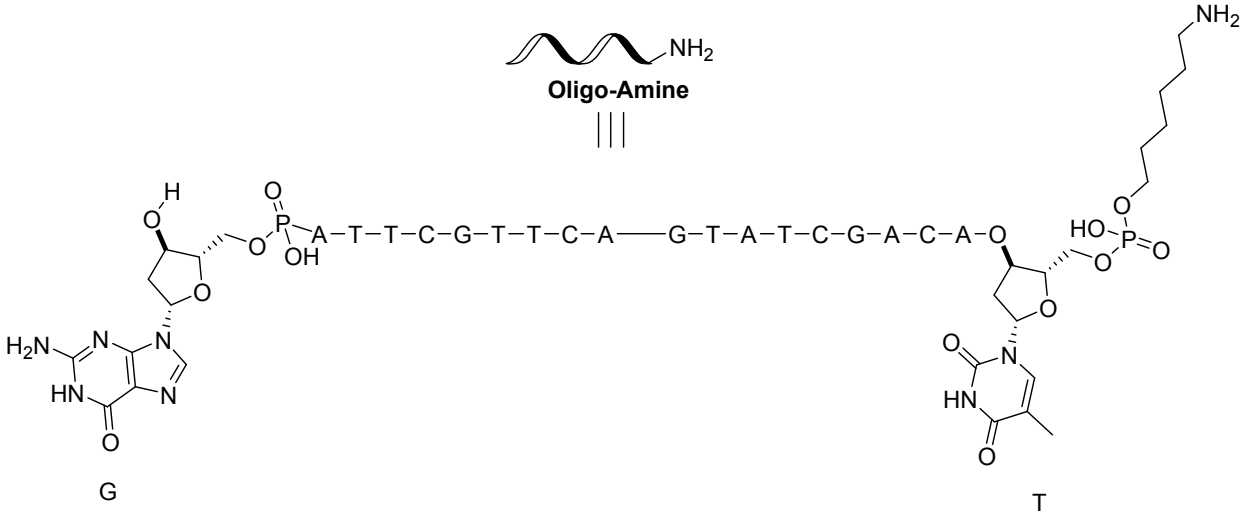
SI I Materials and methods

All reagents and solvents were purchased from ABCR, ChemBridge, Chemcia Scientific, Trans World Chemicals, Sigma-Aldrich, Fluka, Enamine, TCI, STREM, OXCHEM, Acela Pharmatech and Alfa Aesar at the highest commercial quality and used without further purification unless stated otherwise.

Oligonucleotides were purchased from Integrated DNA Technologies (Synthesis scale: 5 μmoles, standard desalting) or Sigma Aldrich (Synthesis scale: 5-10 μmoles, standard desalting) and used without additional purification. The derivatized oligonucleotides staring materials **1a**-**n** were purified by reverse-phase HPLC.

SI II Analysis and purification of oligonucleotides-conjugates

SI II1 Structure of the standard model oligonucleotide-amines

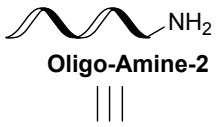


Chemical Formula: $C_{202}H_{262}N_{72}O_{123}P_{20}$
Exact Mass: 6283.12
Molecular Weight: 6286.17
GC content 40%

Figure SI II1: Molecular structure of the **Oligo-Amine** starting material.

The sequence of this model oligonucleotide (5'-TACAGCTATGACTTGCTTAG-3') was randomized.

A small number of optimization experiments were performed on oligonucleotide-amines **Oligo-Amine-2** with identical number of bases and linkers analogs to **Oligo-Amine** but with a proprietary oligonucleotide sequence.



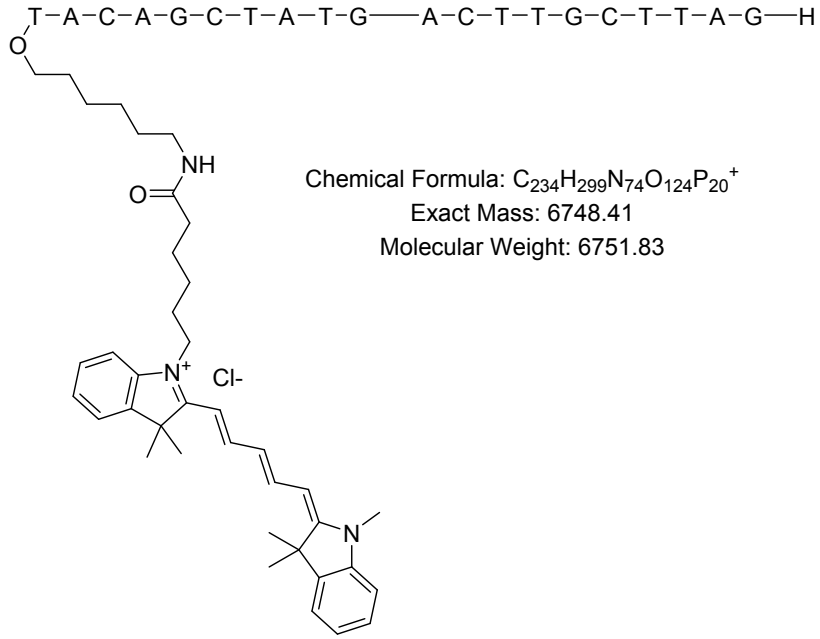
Oligo-Amine-2

Chemical Formula: $C_{199}H_{255}N_{79}O_{118}P_{20}$
Exact Mass: 6258.11
Molecular Weight: 6261.14
GC content 35%

Figure SI II2: Structure of the **Oligo-Amine-2** starting material.

SI II2 Synthesis and Characterization of DNA conjugates 1Cy5 and 1a-1n

Synthesis of 1Cy5



Chemical Formula: $C_{234}H_{299}N_{74}O_{124}P_{20}^{+}$
Exact Mass: 6748.41
Molecular Weight: 6751.83

Figure SI II3: Molecular structure of the starting material **1Cy5**.

For the synthesis of **1Cy5** the standard **Oligo-Amine** was reacted with commercially available Cyanine5 NHS ester (abcam CAS 1032678-42-4).
Oligo-Amine as a solution in water (50.0 µL, 0.050 µmol) was evaporated in a 250 µL PCR tube in a SpeedVac vacuum concentrator (Thermofisher). The residue was dissolved in 200 mM sodium phosphate buffer pH 8 (Volume: 25 µL). A solution of Cyanine5 NHS ester (Cy5) (1.9 mg, 3.08 µmol) in DMSO (Volume: 25 µL) was then added.
The resulting solution was homogenized and incubated at 40°C in a PCR thermocycler for 12 hours.

Workup

The sample was desalted by eluting the sample through two consecutive illustra NAP 25 columns (GE Healthcare cat number 17-0852-01), recovered and evaporated to dryness in a SpeedVac vacuum concentrator. The residue was dissolved in 1 mL of water, analyzed by TOF LC-MS and quantified by absorbance at 260 nm and 646 nm.

Analysis for 1Cy5

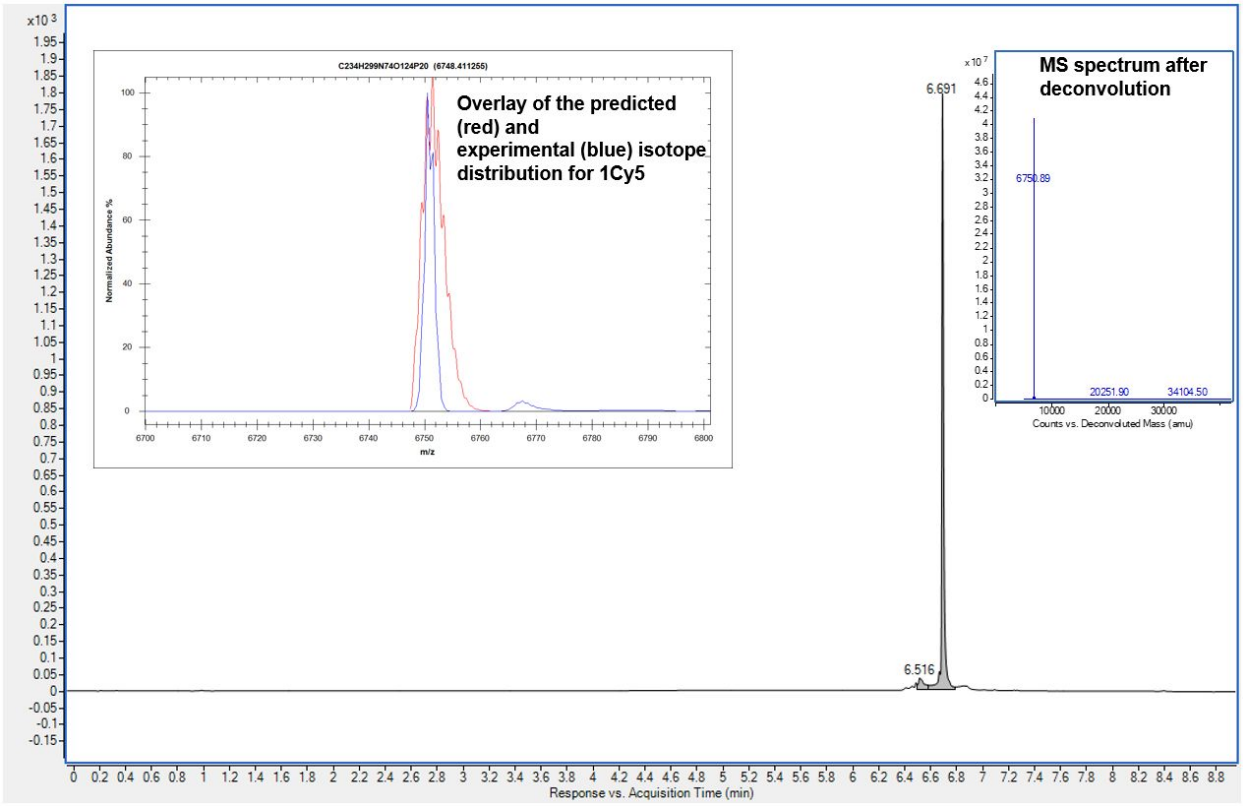


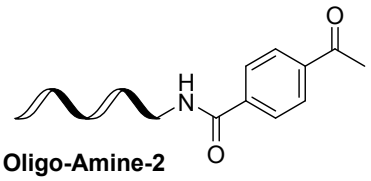
Figure SI II4: UPLC-TOF analysis and characterization of **1Cy5**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 um at 60°C:, Flow 0.6 mL/min, Gradient: 10% B for 0.2min, 10-23%B in 5.8min; 23-100%B in 1min; 100% for 0.8min with A= water + 10mM TEA + 200mM HFIP and B= MeOH, Rt= 6.691 min, m/z =6750.89 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6750.41), interpreted as compatible with the structure of the final compound **1Cy5**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1a

The acylation protocol on DNA immobilized on DEAE was adapted from Harbury *et al*.[1]



Oligo-Amine-2

Chemical Formula: $C_{208}H_{261}N_{79}O_{120}P_{20}$
Exact Mass: 6404.15
Molecular Weight: 6407.28

GC content 35%

Figure SI II5: Molecular structure of the starting material **1a**. **Oligo-Amine-2** is 20 bases long and has linker analogs to **Oligo-Amine** but with a proprietary oligonucleotide sequence.

For the synthesis of **1a,** the standard acylation protocol was performed using 4-Acetylbenzoic acid (Aldrich CAS 586-89-0)(100 mM in DMF).
5000 µL of a suspension of DEAE sepharose (DEAE Sephadex™ A-25, GE Healthcare 17-0170-02) was washed with 10 mL of DNA-bind solution (10 mM Acetic acid in water + 0.005 % triton-X100).
5000ul of a solution of **Oligo-Amine-2** as a solution (0.5 nmole/µL) (3.14 mg, 0.5 µmol) was then added to the resin together with 5 mL of DNA bind solution. This suspension was agitated for 2 min, followed by filtration and washing with water (10 mL) and MeOH (10 mL). The resin was then washed for 2 min with 10 mL of a 5% 4-methylpiperidine in MeOH to neutralize and remove any remaining acetic acid.
A solution of activated acid was prepared by mixing HOAt (CAS 39968-33-7)(100 mM in MeOH) (3000 µL, 300 µmol), 4-Acetylbenzoic acid (Aldrich CAS 586-89-0) (3000 µL, 300 µmol) and DIC (46.7 µL, 300 µmol). This solution was agitated 30 seconds before addition to the DEAE sepharose resin. The resin was agitated 15 min with the activated acid then drained. This process was repeated once.

A few beads were then incubated in 65 µL of DNA-elute solution (1.5 M NaCl, 50 mM pH8 phosphate buffer in water+0.005% triton-X100) and the released DNA purified by P6 chromatography (Micro Bio-Spin™ P-6 Gel Columns) before HPLC-TOF analysis.

Workup and Purification

At this stage the reaction was complete, and the DNA was released by treatment with 2 mL of DNA elute solution (1.5M NaCl, 50 mM phosphate buffer pH 8, 0.005 % triton-X) for 30 min, followed by 3 treatments with 2000 µL of the same solution for 15 min.
The mixture was concentrated using a 3K MWCO Ultrafiltration device (PALL, Nanosep 3K-30K Omega Centrifugal Device, 5 mL, 5000 G, 40°C, 30 min).
The concentrated sample was diluted to 4 mL with water and concentrated again (5000 G, 40°C, 30 min) (twice).

The final solution was purified by reverse phase HPLC (Column Xbridge® Prep C18 5 µm OBD™ 19x50 mm, Solvent A: 100 mM HFIP 86 mM TEA, Solvent B: MeOH/ACN 1/1). The fractions were combined and concentrated using a 3K MWCO centrifugal device (5000 G, 40°C, 30 min) and adjusted to 4 mL to give a solution containing 412 nmoles of pure product **1a** (82%) as determined by the UV absorbance at 260 nm.

Analysis for 1a

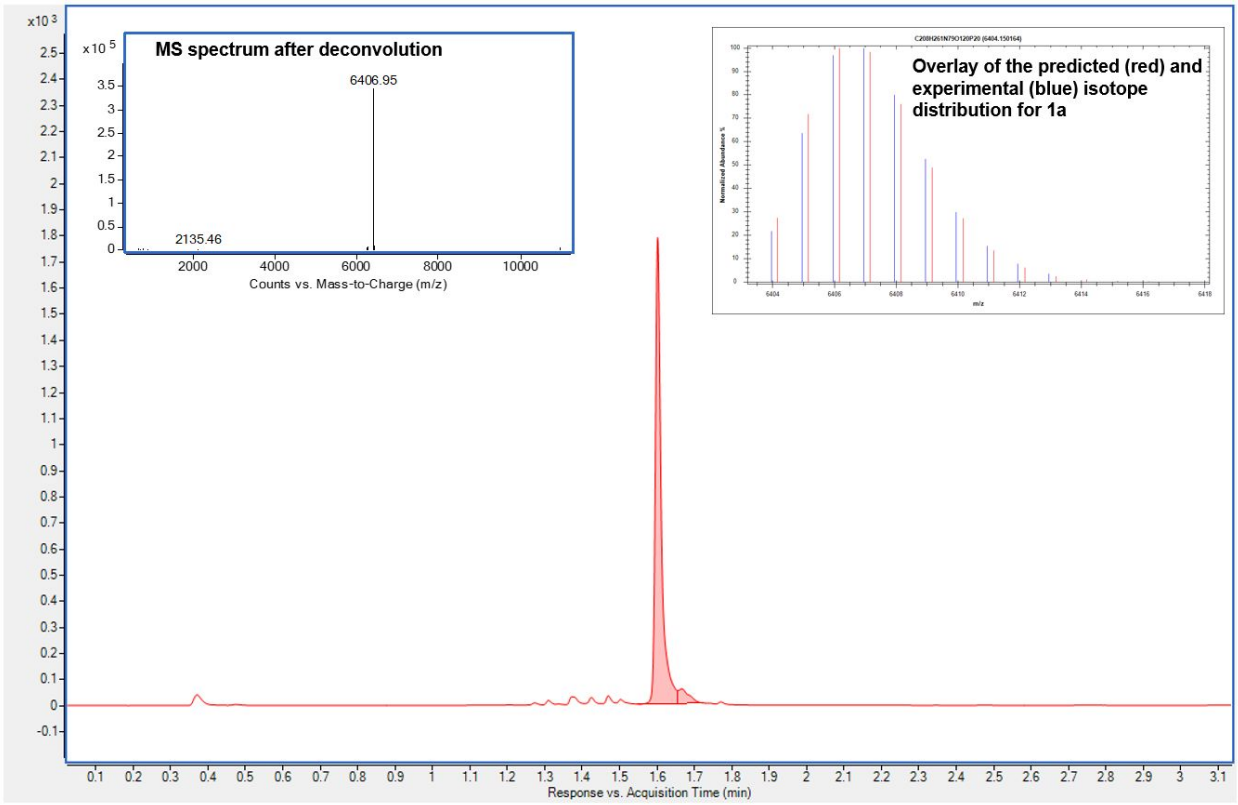
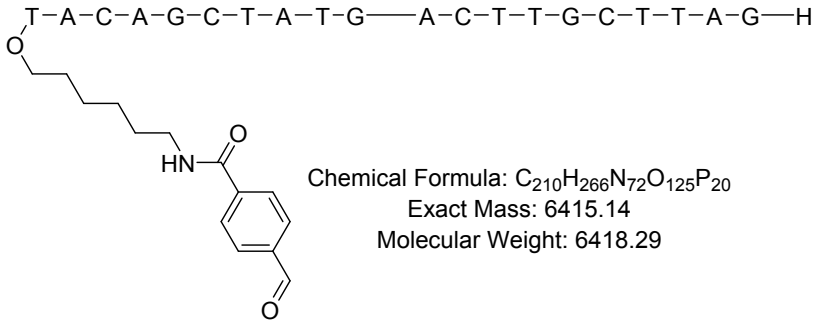


Figure SI II6: UPLC-TOF analysis of **1a**.

- **UPLC/MS** 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.602 min, m/z =6406.95 amu (M deconvoluted), interpreted as compatible with the structure of the final compound **1a** (M calculated 6407.15)

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1b



Chemical Formula: $C_{210}H_{266}N_{72}O_{125}P_{20}$
Exact Mass: 6415.14
Molecular Weight: 6418.29

Figure SI II7: Molecular structure of the starting material **1b**.

For the synthesis of **1b** the acylation was performed using DMT-MM and 4-formylbenzoic acid (Aldrich CAS 619-66-9). The acylation protocol using DMT-MM was adapted from Neri *et al*.[2]

Procedure

The **Oligo-Amine** (0.2 µmole) was dissolved in 200 mM pH 8 phosphate buffer (Volume: 255 µL) and treated with a solution of 4-formylbenzoic acid (Aldrich CAS 619-66-9) (200 µL, 20 µmol) and DMT-MM (in water) (50 µL, 20 µmol). The sample was homogenized by vortexing and then incubated at 30°C for 2 h.

Workup

The volume was adjusted to 2 mL with water and the mixture was desalted using 5 mL single filtration devices 3K MWCO Ultrafiltration device (PALL, Nanosep 3K-30K Omega Centrifugal Device, 5 mL, 5000 G, 40°C, 30 min).

The concentrated sample was diluted to 4 mL with water and concentrated again (5000 G, 40°C, 30 min) (twice).

Analysis for 1b

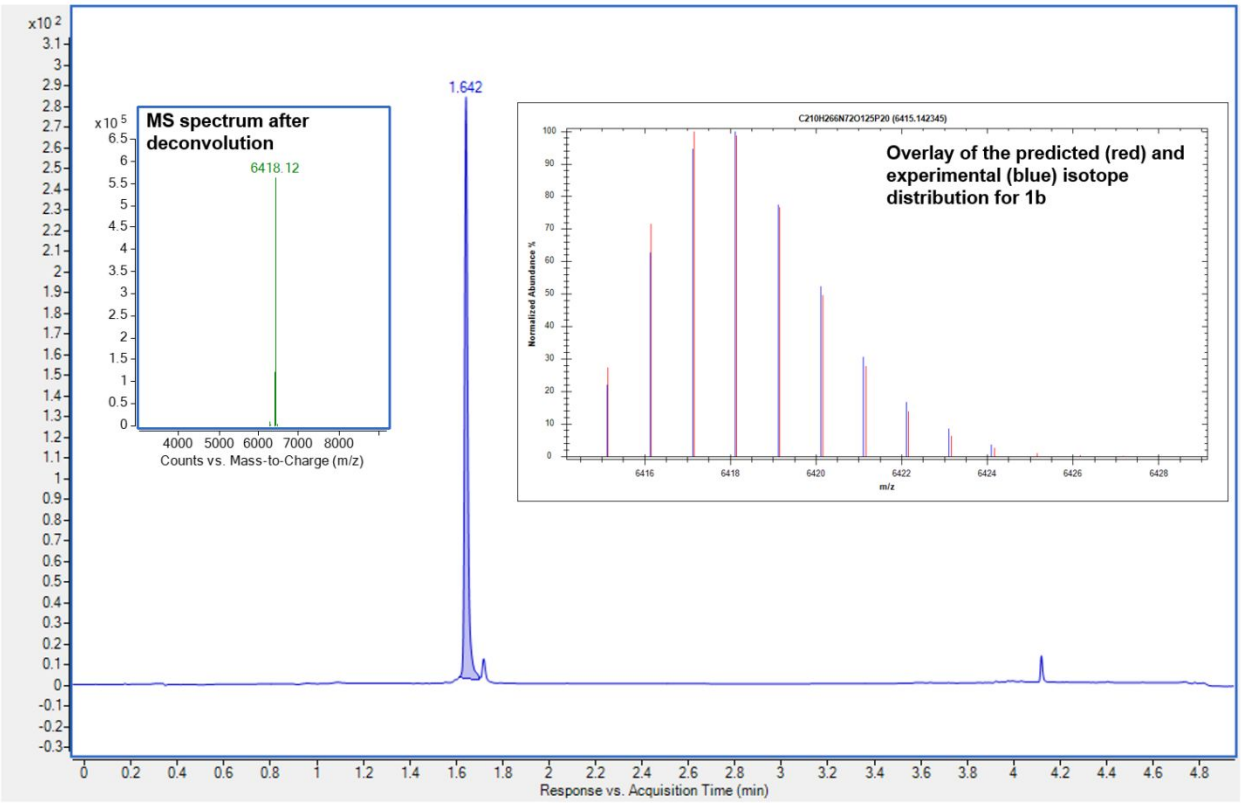


Figure SI II8: UPLC-TOF analysis of **1b**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05

min; 100% for 0.8min with A= water + 10mM TEA + 200mM HFIP and B= MeOH, Rt= 1.642 min, m/z =6418.12 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6418.15), interpreted as compatible with the structure of the final compound **1b**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1c:
The acylation protocol on DNA immobilized on DEAE was adapted from Harbury *et al*.[1]

Representative acylation protocol for the synthesis of 1c:

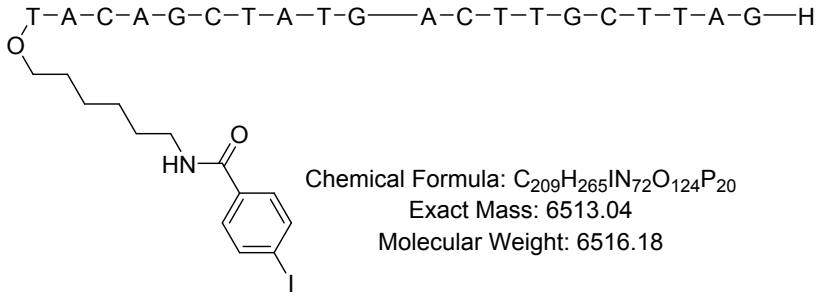


Figure SI II9: Molecular structure of the starting material **1c**.

5000 µL of a suspension of DEAE sepharose (DEAE Sephadex™ A-25, GE Healthcare 17-0170-02) was washed with 10 mL of DNA bind solution (10 mM Acetic acid in water+0.005% triton-X100).
5000 µL of a solution of **Oligo-Amine** as a solution (0.5 nmole/µL) (3.14 mg, 0.5 µmol) was then added to the resin together with 5 mL of DNA bind solution. This suspension was agitated for 2 min, followed by filtration and washing with water (10 mL) and MeOH (10 mL). The resin was then washed for 2 min with 10 mL of a 5% 4-methylpiperidine in MeOH to neutralize and remove any remaining acetic acid.
A solution of activated acid was prepared by mixing HOAt (CAS 39968-33-7)(100mM in MeOH) (3000 µL, 300 µmol), 4-iodobenzoic acid (100mM in DMSO) (3000 µL, 300 µmol) and DIC (46.7 µL, 300 µmol). This solution was agitated 30 seconds before addition to the DEAE sepharose resin. The resin was agitated 15min with the activated acid and this process repeated once.

A few beads were then incubated in 65 µL of DNA elute solution (1.5M NaCl, 50mM pH8 phosphate buffer in water+0.005% triton-X100) and the released DNA purified by P6 chromatography (Micro Bio-Spin™ P-6 Gel Columns) before HPLC-TOF analysis.

Workup and Purification

At this stage the reaction was complete, and the DNA was released by treatment with 2 mL of DNA elute solution (1.5M NaCl, 50 mM phosphate buffer pH8, 0.005% triton-X) for 30 min, followed by 3 treatments with 2000 µL of the same solution for 15 min.
The mixture was concentrated using a 3K MWCO Ultrafiltration device (PALL, Nanosep 3K-30K Omega Centrifugal Device, 5 mL, 5000 G, 40°C, 30 min).
The concentrated sample was diluted to 4 mL with water and concentrated again (5000 G, 40°C, 30 min) (twice).

The final solution was purified by reverse phase HPLC (Column Xbridge® Prep C18 5 µm OBD™ 19x50 mm, Solvent A: 100 mM HFIP 86 mM TEA, Solvent B: MeOH/ACN 1/1). The fractions were combined and concentrated using a 3K MWCO centrifugal device (5000 G, 40°C, 30 min) and adjusted to 4 mL to give a solution containing 445 nmoles of pure product **1c** (89%) as determined by the UV absorbance at 260 nm.

Analysis for 1c

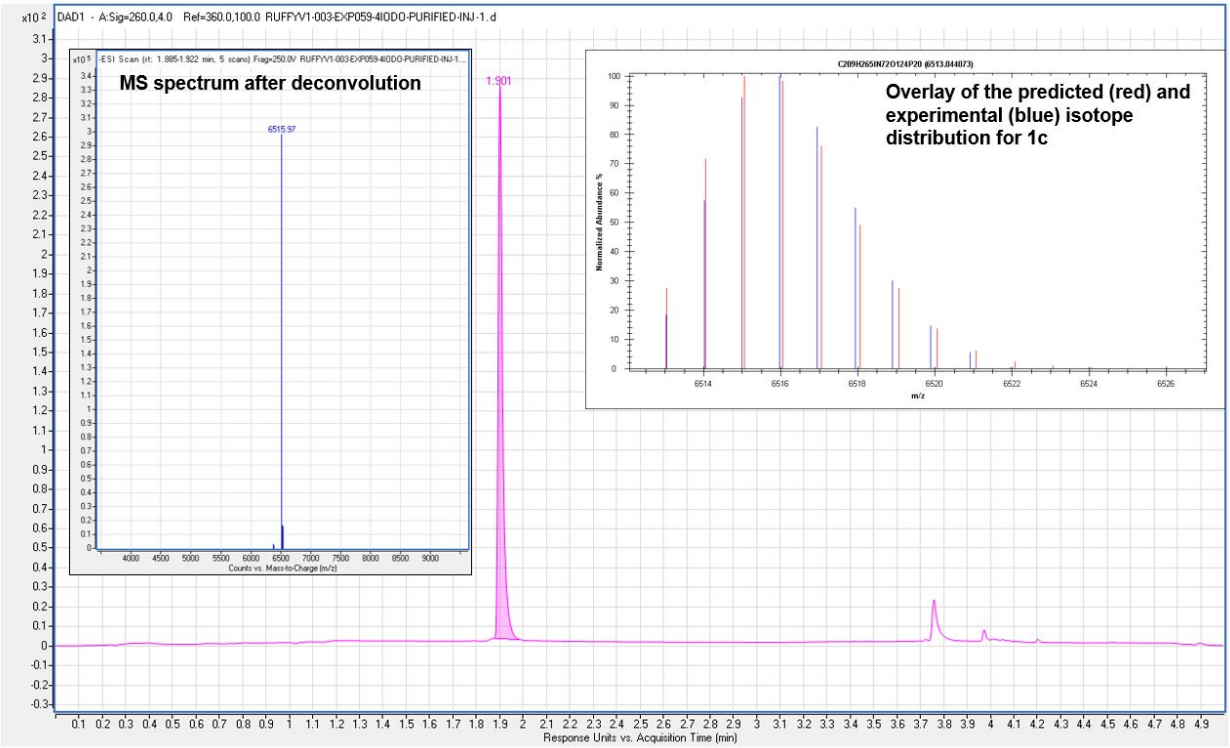


Figure SI II10: UPLC-TOF analysis of **1c**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.90 min, m/z =6515.97 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6516.05), interpreted as compatible with the structure of the final compound **1c**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1d

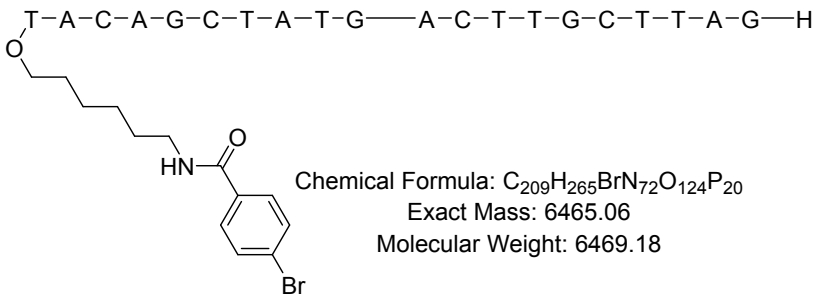


Figure SI II11: Molecular structure of the starting material **1d**.

For the synthesis of **1d** the acylation was performed using DMT-MM and 4-bromobenzoic acid (100 mM in DMSO) (200 µL, 20.00 µmol) using DMT-MM. The acylation protocol using DMT-MM was adapted from Neri *et al*.[2]

Analysis of 1d

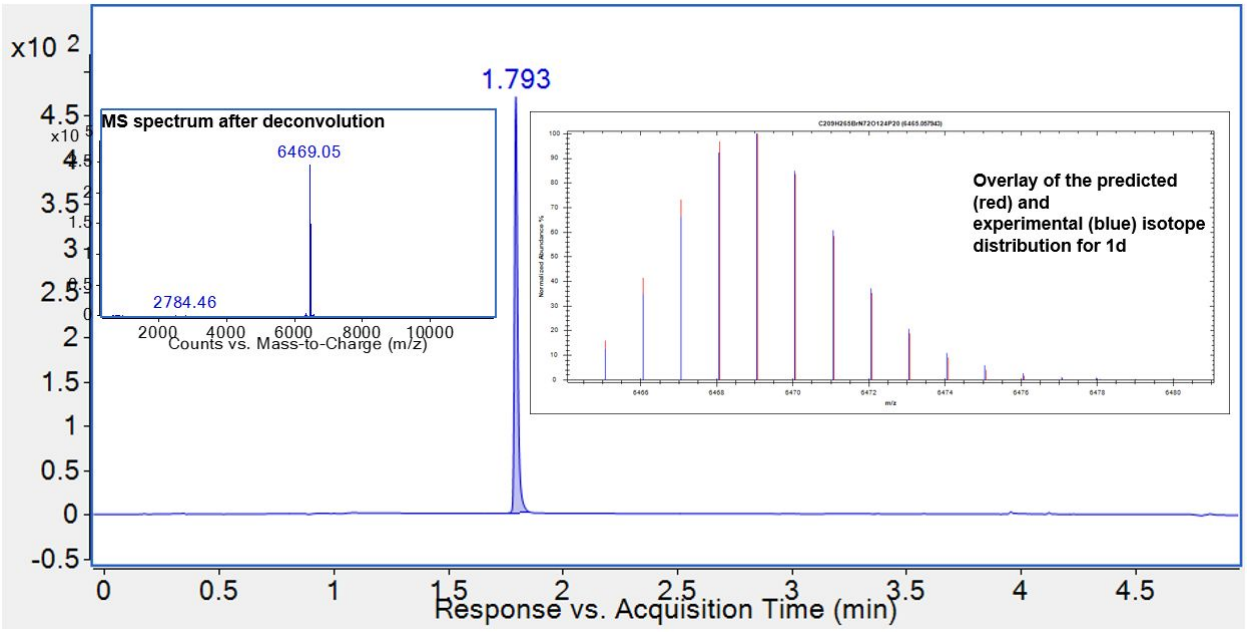


Figure SI II12: UPLC-TOF analysis of **1d**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.793 min, m/z =6469.05 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6469.06), interpreted as compatible with the structure of the final compound **1d**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1e

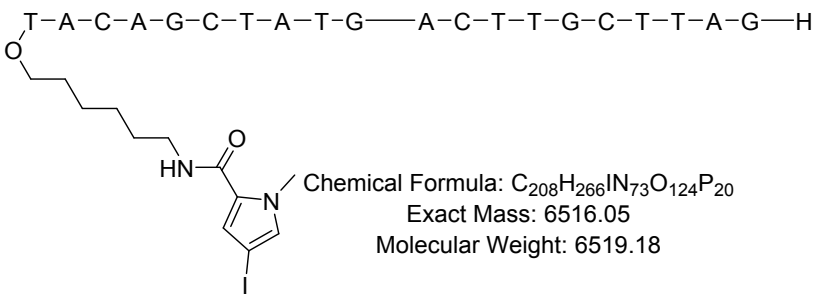


Figure SI II13: Molecular structure of the starting material **1e**.

For the synthesis of **1e** the standard acylation on DNA immobilized on DEAE was performed using 4-iodo-1-methyl-1H-pyrrole-2-carboxylic acid (BIONET FG-0709).

Analysis for 1e

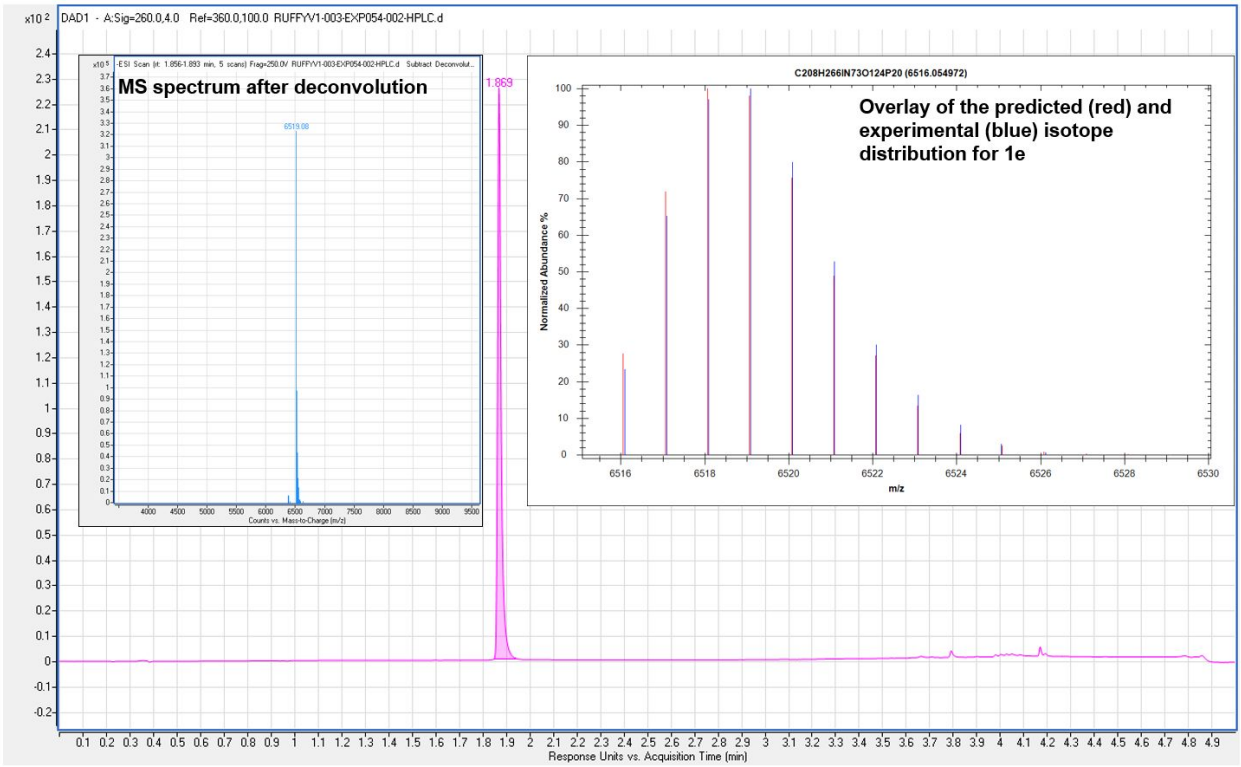
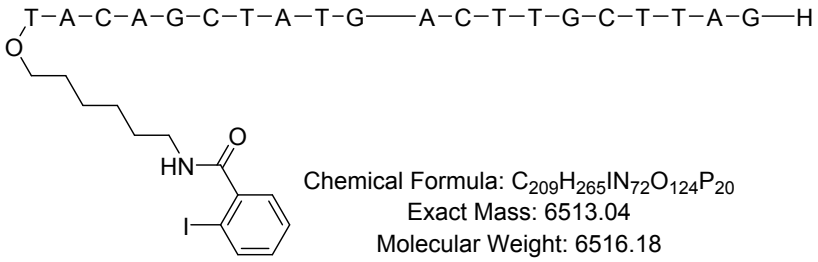


Figure SI II14: UPLC-TOF analysis of **1e**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.869 min, m/z =6519.03 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6519.06), interpreted as compatible with the structure of the final compound **1e**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1f



Chemical Formula: $C_{209}H_{265}IN_{72}O_{124}P_{20}$
Exact Mass: 6513.04
Molecular Weight: 6516.18

Figure SI II15: Molecular structure of the starting material **1f**.

For the synthesis of **1f** the standard acylation on DNA immobilized on DEAE was performed using 2-iodobenzoic acid (Fluka CAS 88-67-5).

Analysis for 1f

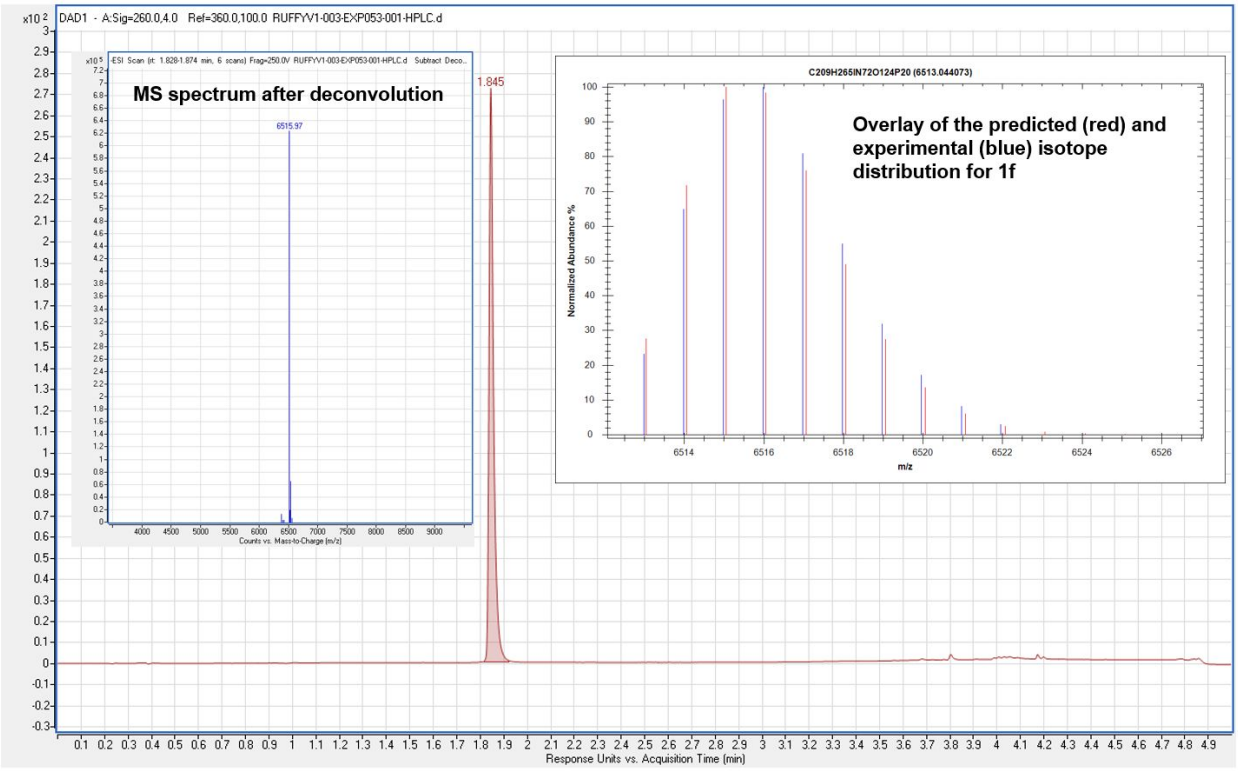


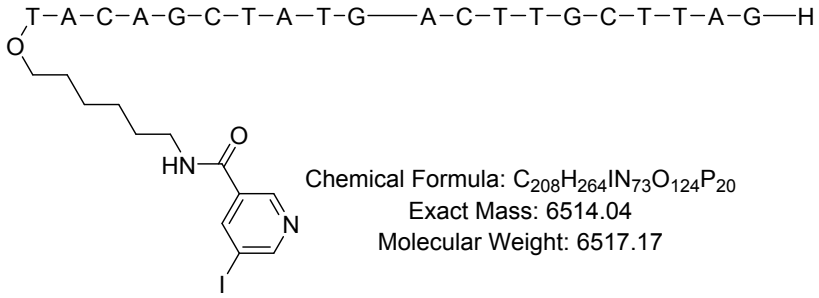
Figure SI II16: UPLC-TOF analysis of **1f**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05

min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.845 min, m/z =6515.97 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6516.05), interpreted as compatible with the structure of the final compound **1f**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1g



Chemical Formula: $C_{208}H_{264}IN_{73}O_{124}P_{20}$
Exact Mass: 6514.04
Molecular Weight: 6517.17

Figure SI II17: Molecular structure of the starting material **1g**.

For the synthesis of **1g** the standard acylation on DNA immobilized on DEAE was performed using 5-iodopyridine-3-carboxylic acid (Ark- Pharm. CAS 15366-65-1).

Analysis for 1g

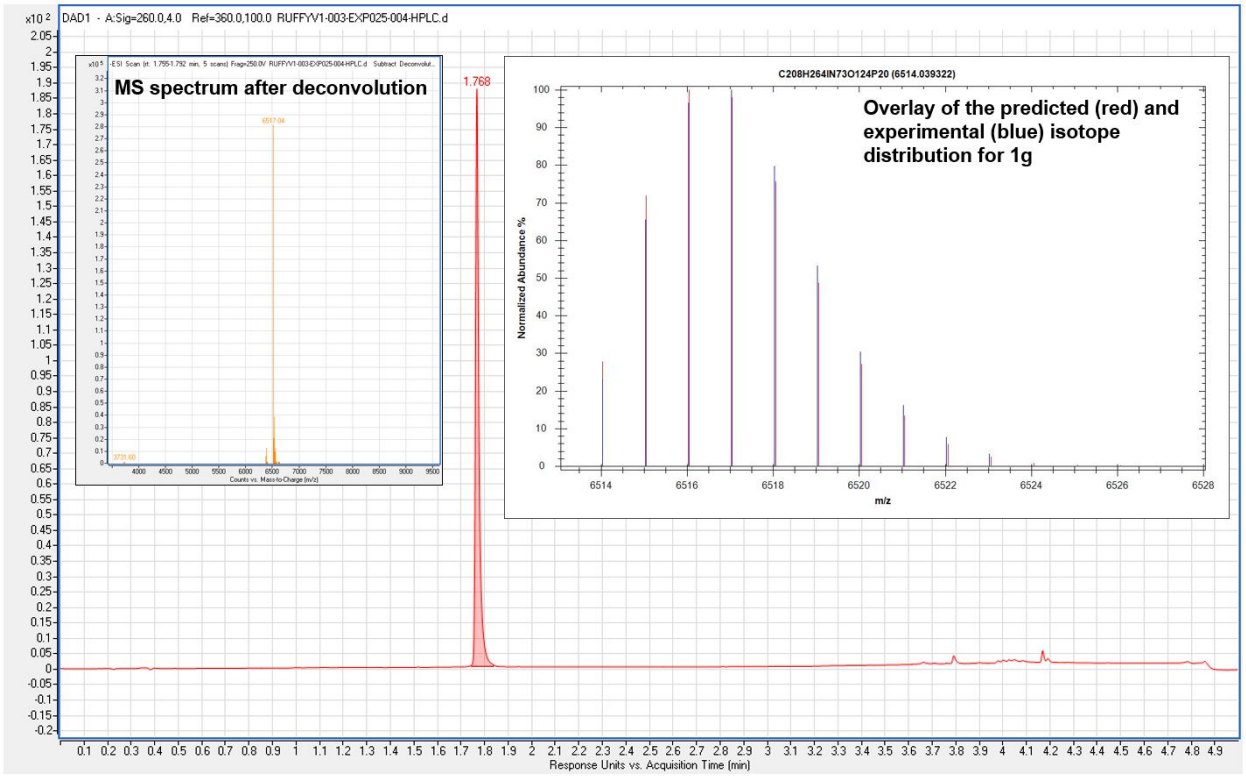
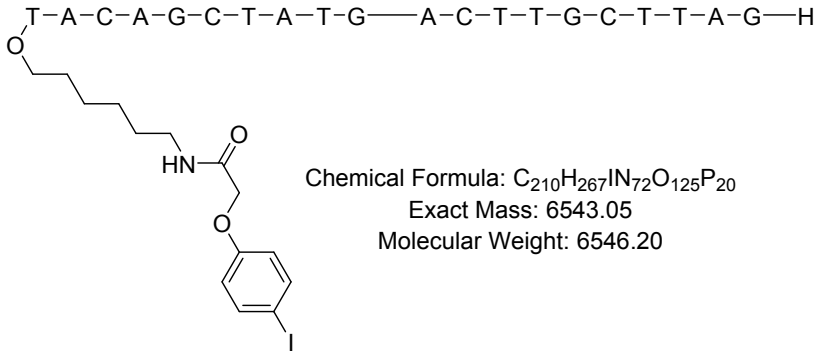


Figure SI II18: UPLC-TOF analysis of **1g**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.768 min, m/z =6517.00 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6517.04), interpreted as compatible with the structure of the final compound **1g**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1h



Chemical Formula: $C_{210}H_{267}IN_{72}O_{125}P_{20}$
Exact Mass: 6543.05
Molecular Weight: 6546.20

Figure SI II19: Molecular structure of the starting material **1h**.

For the synthesis of **1h** the standard acylation on DNA immobilized on DEAE was performed using 4-iodophenoxyacetic acid (Aldrich CAS 1878-94-0).

Analysis for 1h

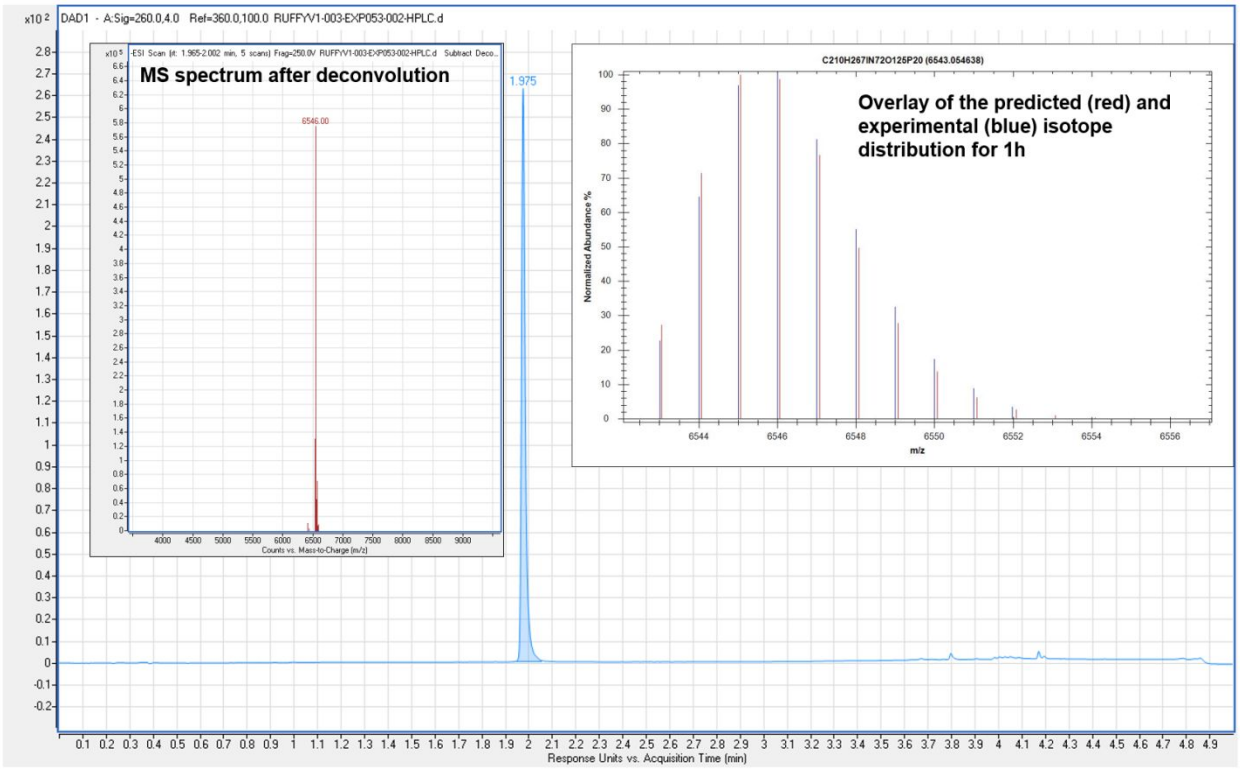
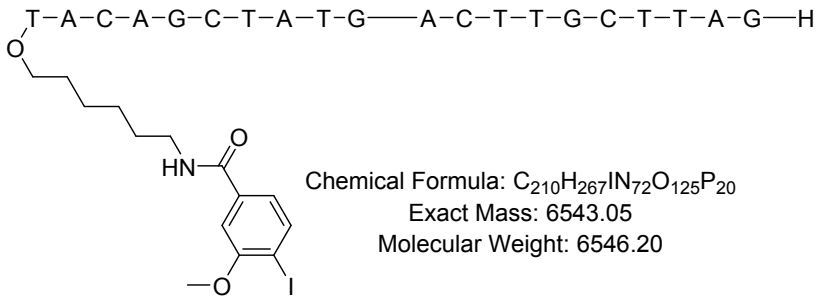


Figure SI II20: UPLC-TOF analysis of **1h**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.975 min, m/z =6546.00 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00) (Calc. 6546.06), interpreted as compatible with the structure of the final compound **1h**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1i



Chemical Formula: $C_{210}H_{267}IN_{72}O_{125}P_{20}$
Exact Mass: 6543.05
Molecular Weight: 6546.20

Figure SI II21: Molecular structure of the starting material **1i**.

For the synthesis of **1i** the standard acylation on DNA immobilized on DEAE was performed using 4-iodo-3-methoxybenzoic acid (Chemcia Scientific CAS 282087-44-9).

Analysis for 1i

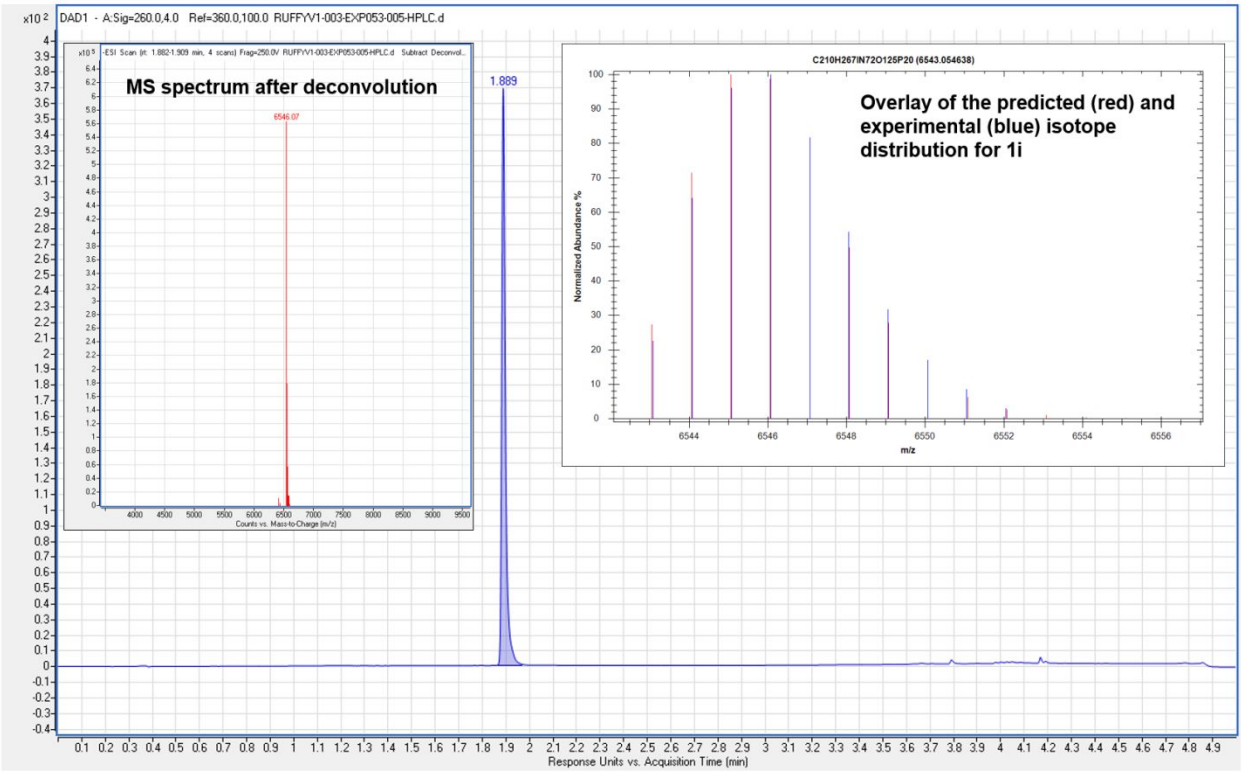
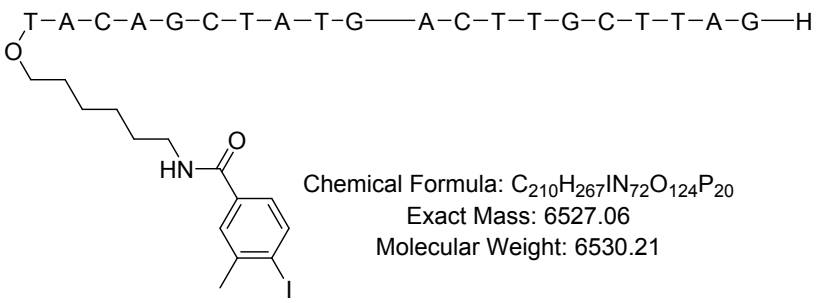


Figure SI II22: UPLC-TOF analysis of **1i**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7min; 30-85%B in 0.95min; 85-100%B in 0.05min; 100% for 0.8min with A= water + 10mM TEA + 200mM HFIP and B= MeOH, Rt= 1.889 min, m/z =6546.07 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00) (Calc. 6546.06), interpreted as compatible with the structure of the final compound **1i**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1j



Chemical Formula: $C_{210}H_{267}IN_{72}O_{124}P_{20}$
Exact Mass: 6527.06
Molecular Weight: 6530.21

Figure SI II23: Molecular structure of the starting material **1j**.

For the synthesis of **1j** the standard acylation on DNA immobilized on DEAE was performed 4-iodo-3-methylbenzoic acid (Trans World Chemicals I1188-J).

Analysis for 1j

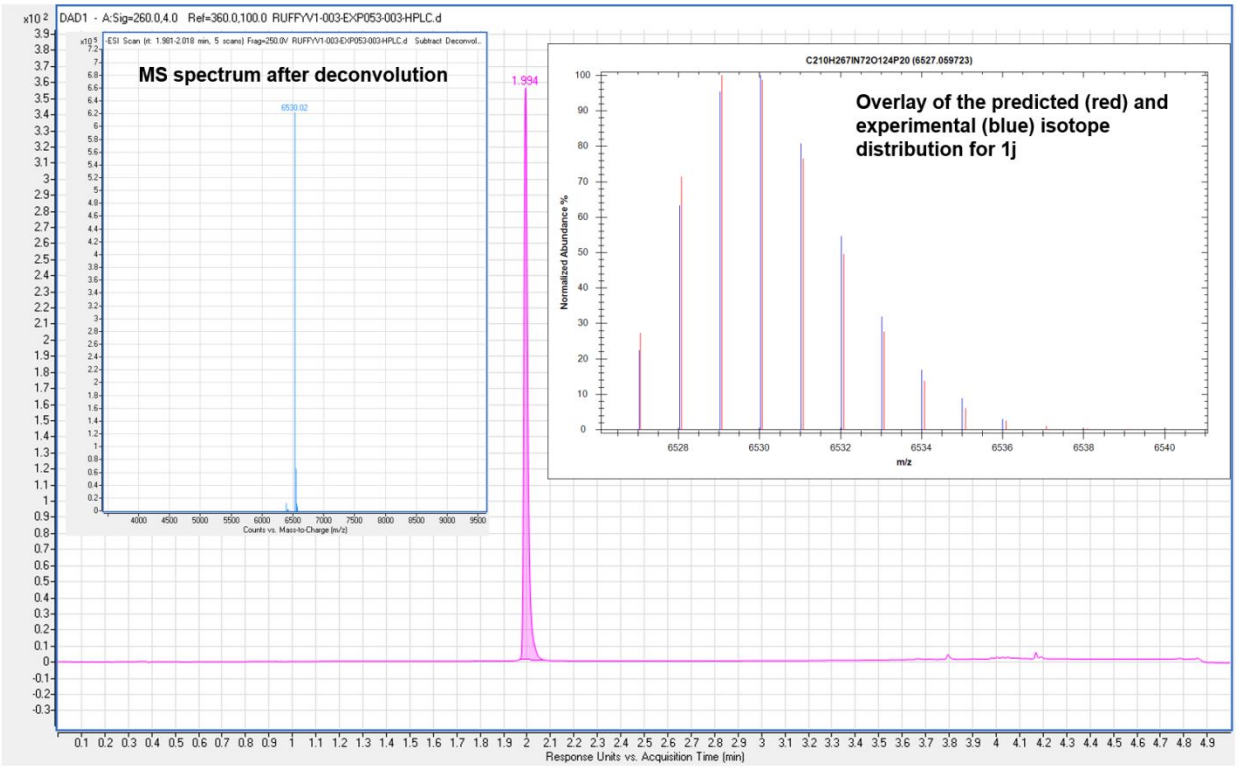
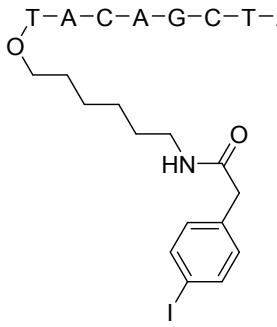


Figure SI II24: UPLC-TOF analysis of **1j**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.994 min, m/z =6530.02 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6530.06), interpreted as compatible with the structure of the final compound **1j**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1k



Chemical Formula: $C_{210}H_{267}IN_{72}O_{124}P_{20}$
Exact Mass: 6527.06
Molecular Weight: 6530.21

Figure SI II25: Molecular structure of the starting material **1k**.

For the synthesis of **1i** the standard acylation on DNA immobilized on DEAE was performed using 4-iodophenylacetic acid (Alfa Aesar CAS 1798-06-7).

Analysis for 1k

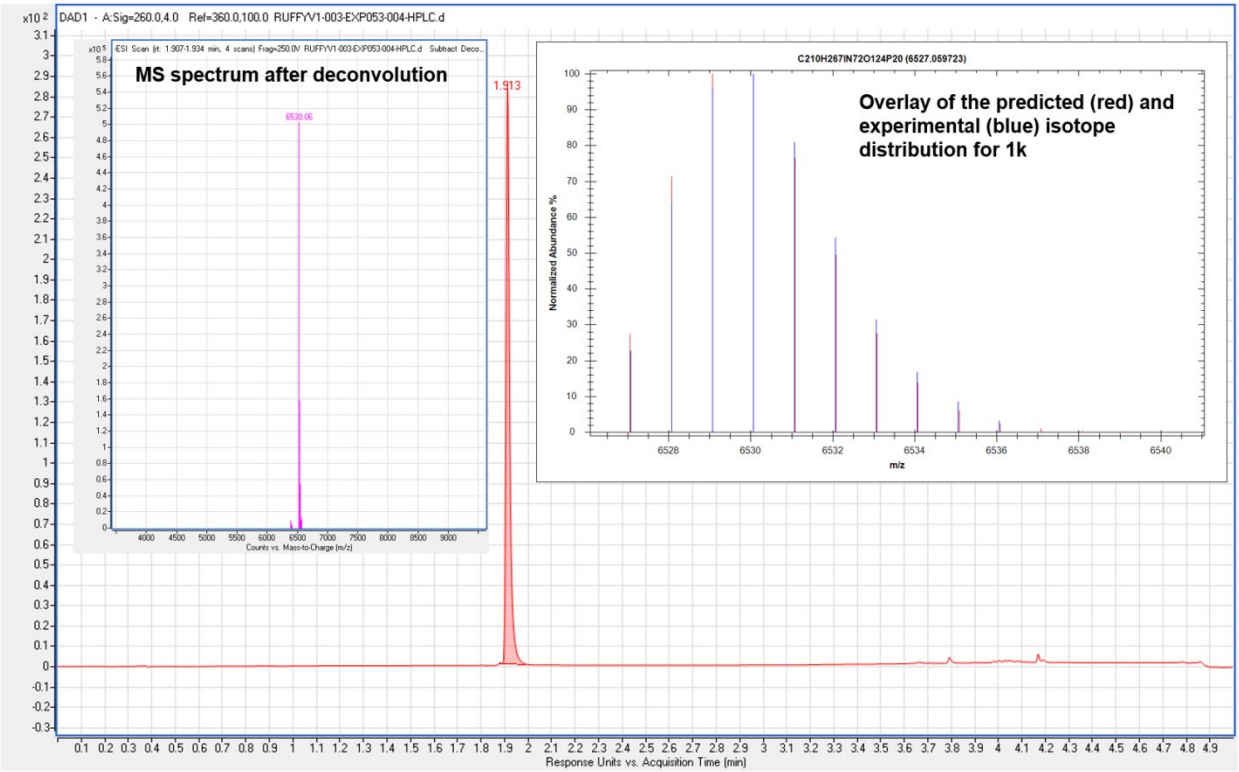


Figure SI II26: UPLC-TOF analysis of **1k**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.913 min, m/z =6530.06 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6530.06), interpreted as compatible with the structure of the final compound **1k**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1l

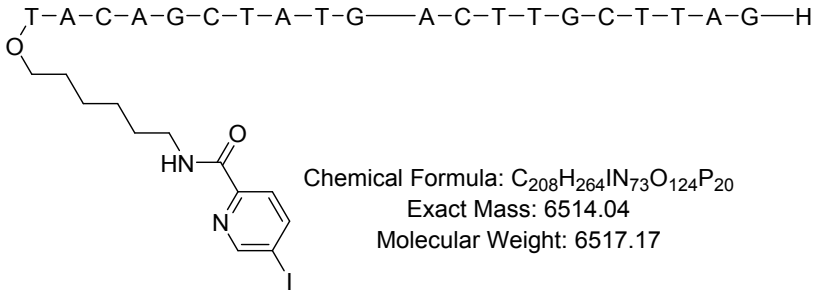


Figure SI II27: Molecular structure of the starting material **1l**.

For the synthesis of **1l** the standard acylation protocol using DMT-MM was performed using 5-iodopicolinic acid (OXCHEM CAS 32046-43-8).

Analysis for 1l

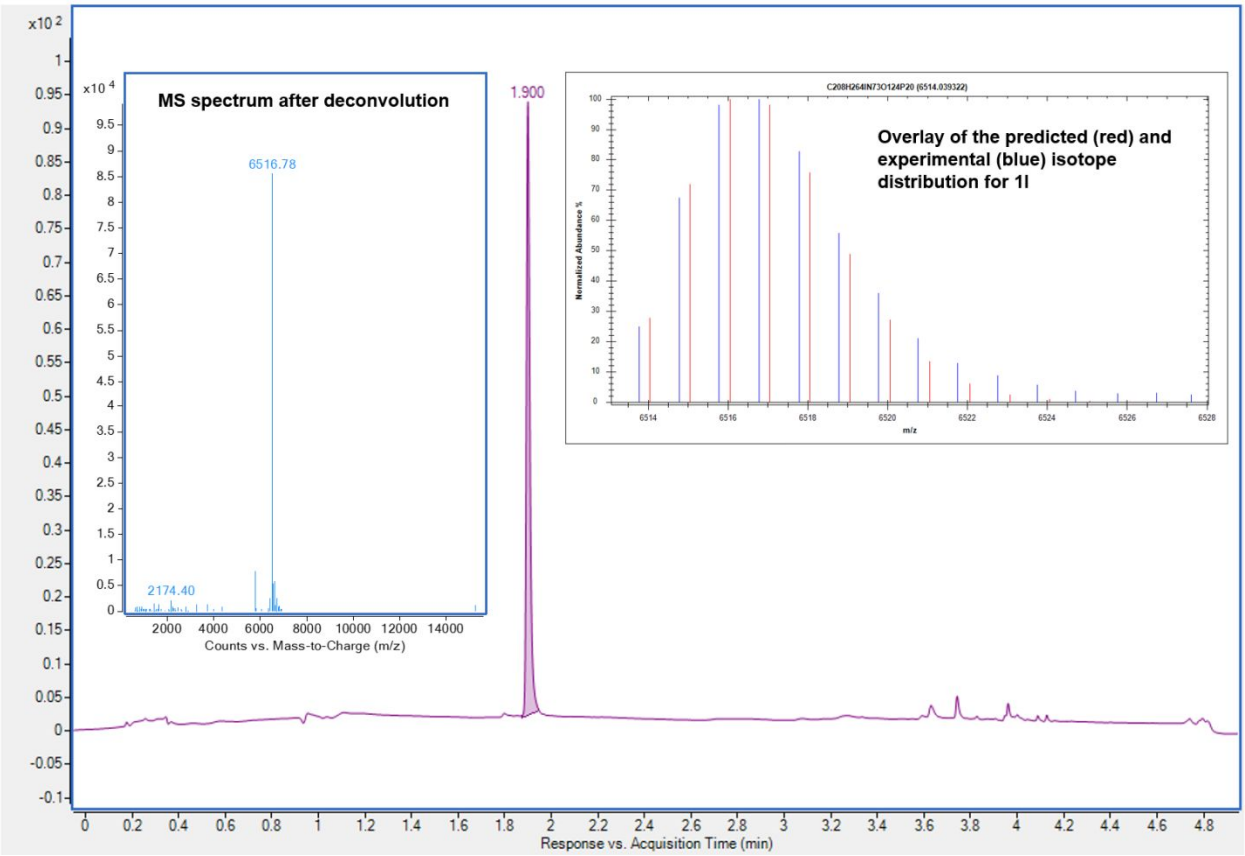
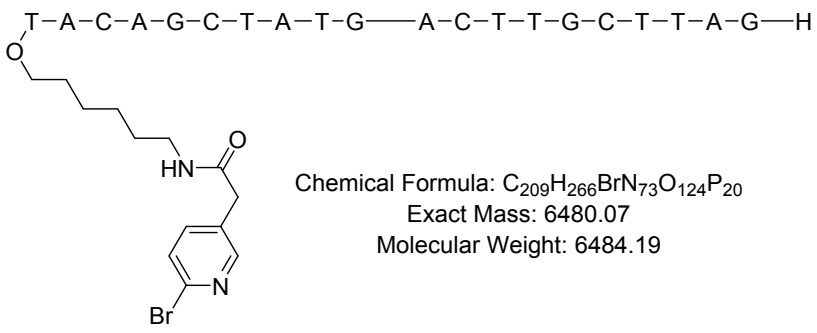


Figure SI II28: UPLC-TOF analysis of **1l**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.900 min, m/z =6516.78 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6517.04), interpreted as compatible with the structure of the final compound **1l**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1m



Chemical Formula: $C_{209}H_{266}BrN_{73}O_{124}P_{20}$
Exact Mass: 6480.07
Molecular Weight: 6484.19

Figure SI II29: Molecular structure of the starting material **1m**.

For the synthesis of **1m** the standard acylation protocol using DMT-MM was performed using 2-(6-bromopyridin-3-yl)acetic acid (Acela Pharmatech (Cat n° K19724)).

Analysis for 1m

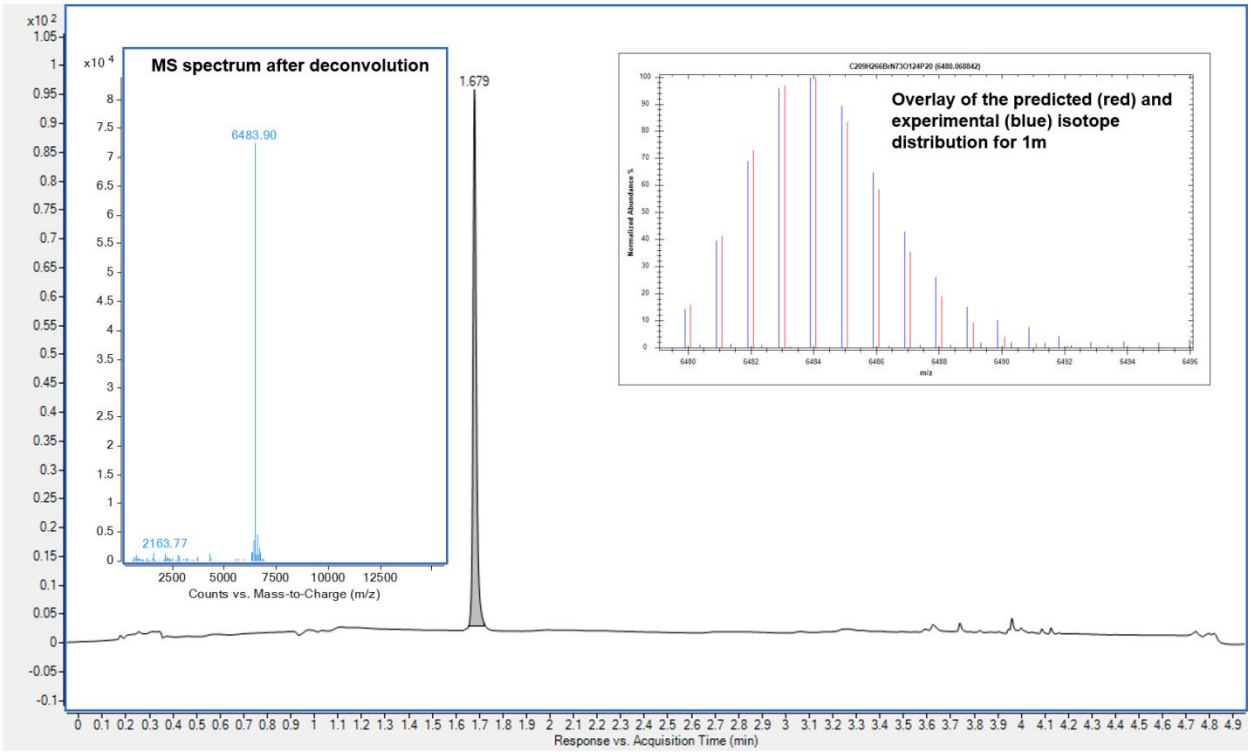
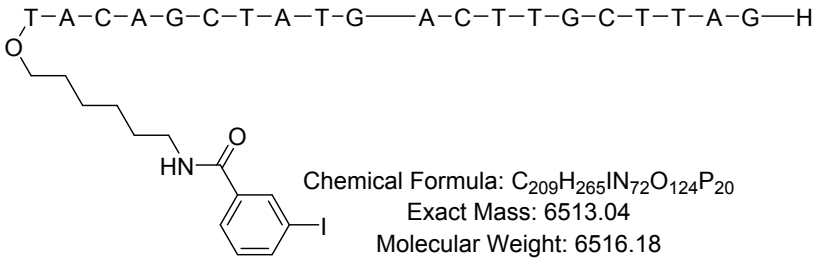


Figure SI II30: UPLC-TOF analysis of **1m**.

- **UPLC/MS** Agilent 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100% for 0.8 min with A= water + 10mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.679 min, m/z =6483.90 amu (M after deconvolution with Agilent MassHunter Qualitative Analysis 6.00)(Calc. 6484.07), interpreted as compatible with the structure of the final compound **1m**.

The predicted isotope distribution (ChemBioDraw Ultra 14.00) of the product (red) was overlapped with the experimental isotope distribution (Blue) using the Isotope Distribution Calculator add-on from the Agilent MassHunter 6.00 software suite.

Synthesis of 1n



Chemical Formula: $C_{209}H_{265}IN_{72}O_{124}P_{20}$
Exact Mass: 6513.04
Molecular Weight: 6516.18

Figure SI II31: Molecular structure of the starting material **1n**.

For the synthesis of **1n** the standard acylation on DNA immobilized on DEAE was performed using 3-iodobenzoic acid (Fluka CAS 618-51-9) (100 mM in DMF) (3000 µL, 300 µmol).

Analysis for 1n

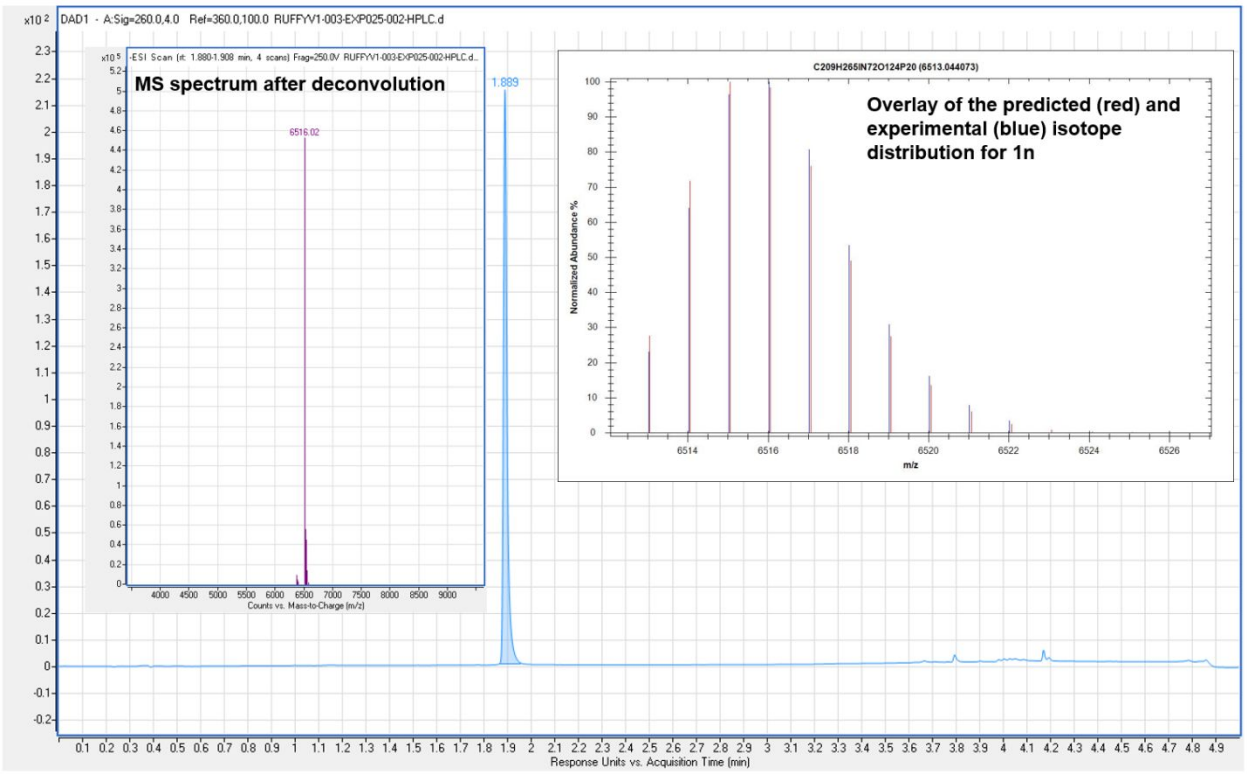


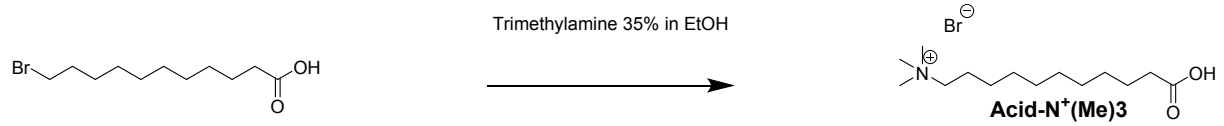
Figure SI II32: UPLC-TOF analysis of **1n**.

- **UPLC/MS** 6200 series TOF/6500 series Q-TOF, Column ACQUITY BEH OST C18 2.1x50 mm 1.7 µm at 60°C:, Flow 0.6 mL/min, Gradient: 15-30%B in 2.7 min; 30-85%B in 0.95 min; 85-100%B in 0.05 min; 100%

for 0.8 min with A= water + 10 mM TEA + 200 mM HFIP and B= MeOH, Rt= 1.889 min, m/z =6516.0 amu (M deconvoluted), interpreted as compatible with the structure of the final compound **1n** (M calculated 6516.05).

SI III Synthesis and characterization of the resin PEG+

Synthesis of the cationic acid 10-carboxy-N,N,N-trimethyldecan-1-aminium bromide Acid-N$^+$(Me)$_3$:



11-Bromoundecanoic acid (Fluka CAS 2834-05-1)

Chemical Formula: $C_{14}H_{30}NO_2^+$
Exact Mass: 244.23
Molecular Weight: 244.40

Yield: 55%, as a white powder

Figure SI III1: Synthesis of the quaternary ammonium-acid **Acid-N$^+$(Me)$_3$**

11-Bromoundecanoic acid (Fluka CAS 2834-05-1) (8 g, 30.2 mmol) was dissolved in trimethylamine (35% in EtOH) (60 mL, 224 mmol) and the reaction vessel sealed with a septum. The solution was stirred at room temperature for 48 hours. A white precipitated formed at this stage. **Workup**: The white precipitate was filtered and washed with cold EtOH. **Purification**: The collected white solid was recrystallized from EtOH, filtered and dried under reduced pressure to afford pure 10-carboxy-*N,N,N*-trimethyldecan-1-aminium bromide as a white powder (yield 54%). UPLC-MS: 0.51 min; 244.3 (M)+; Waters UPLC Acquity; column: Acquity UPLC BEH C18, 1.7 µm, 2.1x50 mm at 80°C, Eluent A: H2O + 0.05 % HCOOH + 3.75 mM ammonium acetate, B: iPrOH + 0.05 % HCOOH, Gradient: 5-98 % B in 1.7 min, flow: 0.6 mL/min. Rt 0.51min observed Mw 244.3 (M)+ interpreted as compatible with the structure of 10-carboxy-*N,N,N*-trimethyldecan-1-aminium bromide.

Analysis of the Acid-N$^+$(Me)$_3$

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.30 - 3.24 (m, 2H), 3.04 (s, 9H), 2.19 (t, J = 7.3 Hz, 2H), 1.71 - 1.60 (m, 2H), 1.48 (t, J = 7.1 Hz, 2H), 1.27 (d, J = 11.5 Hz, 12H). Interpreted as compatible with the structure of 10-carboxy-*N,N,N*-trimethyldecan-1-aminium bromide.
^{13}C NMR (101 MHz, DMSO-d_6) δ 174.43, 65.20, 52.07, 33.64, 28.73, 28.67, 28.65, 28.49, 28.42, 25.70, 24.44, 21.99.

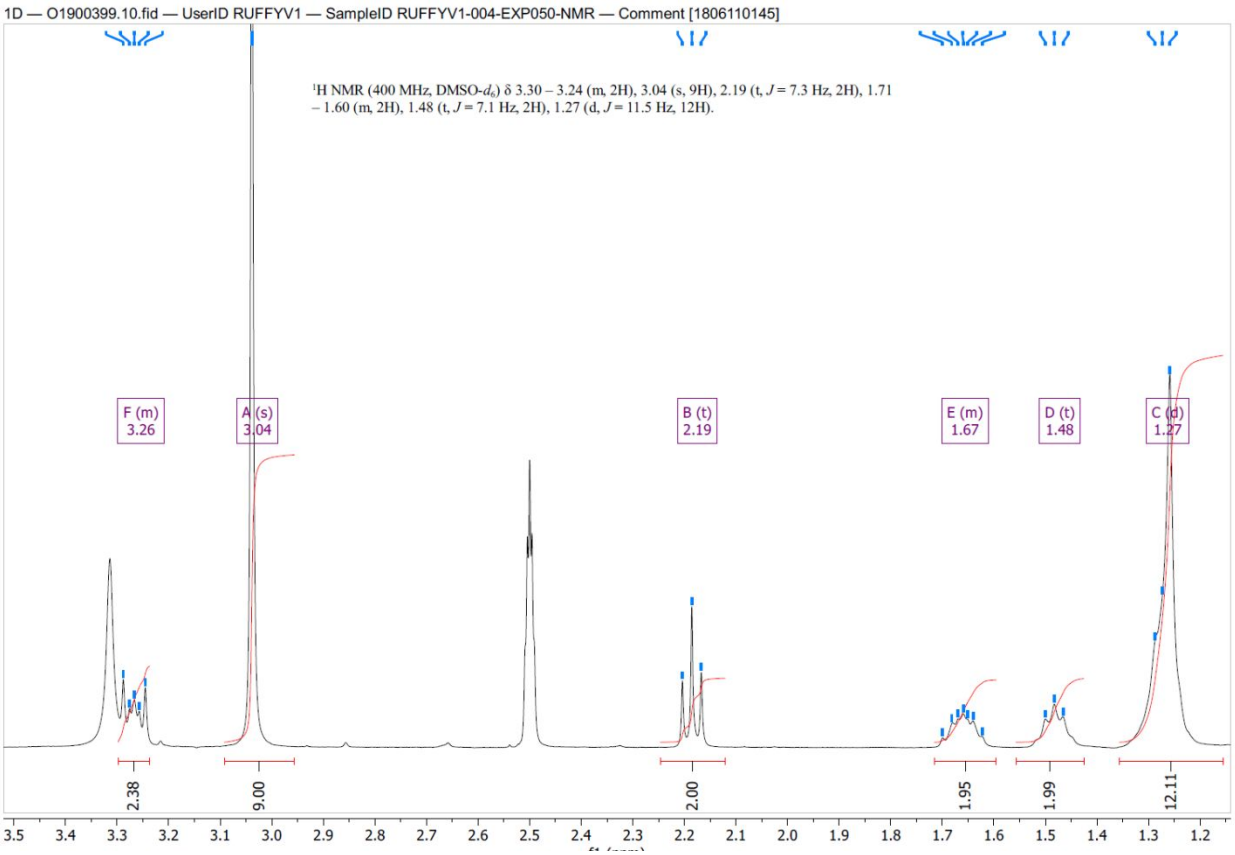


Figure SI III2: ^{1}H NMR Analysis of **Acid-N^{+}(Me)$_3$** (10-carboxy-*N,N,N*-trimethyldecan-1-aminium bromide)

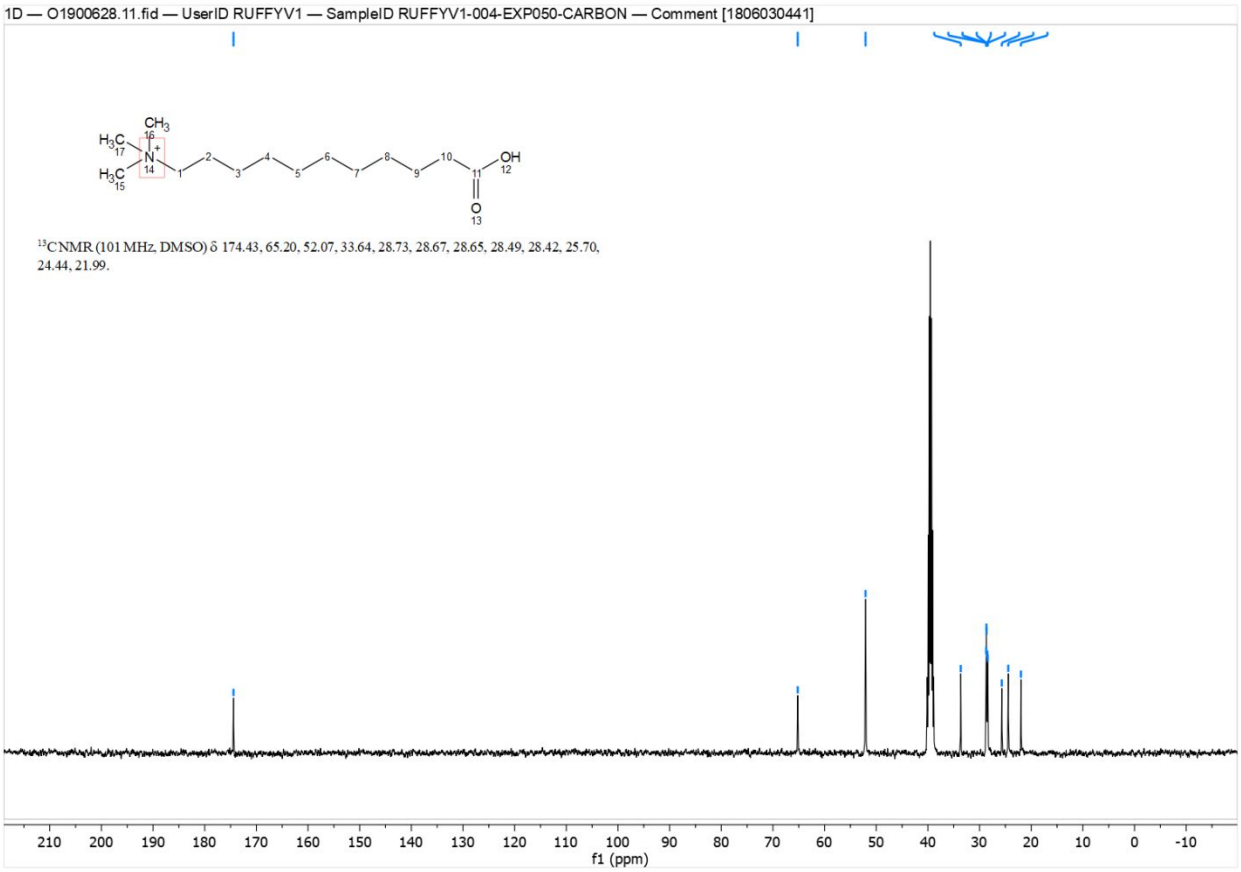


Figure SI III3: ^{1}H NMR Analysis of **Acid-N^{+}(Me)$_3$** (10-carboxy-*N,N,N*-trimethyldecan-1-aminium bromide)

General protocol for the Kaiser Test: detection of unreacted amines remaining after amide coupling

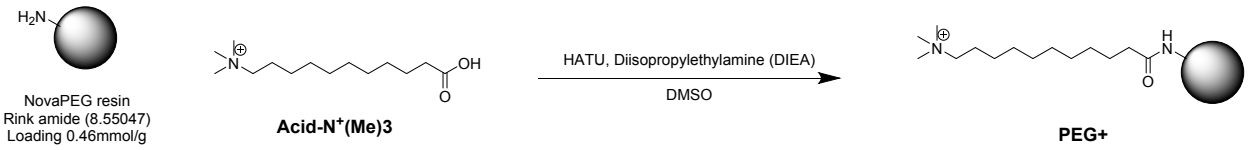
Solution 1: 5 g ninhydrin in 100mL ethanol

Solution 2: 80 g phenol in 20mL ethanol

Solution 3: 2 mL 0.001 M aqueous KCN in 98 mL pyridine

A few beads are placed in an eppendorf tube and 50 µL of each solution 1-2-3 are added. The tube is placed in a thermomixer and the reaction left to incubate for 5 min at 100°C. Resin and solution blue (variable intensity - from light to dark blue): positive (reaction isn't complete). Resin and solution colourless to light yellow: negative (reaction is complete, no more free primary amines are present).

Protocol for the synthesis of PEG+



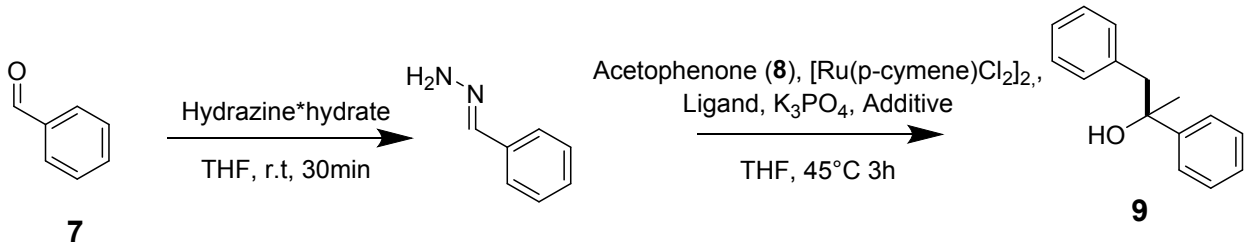
The resin NovaPEG resin Rink amide (catalog number 8.55047, loading 0.46 mmol/g) (4 g, 1.840 mmol) was swelled in DCM for 3 hours. The acid was activated by mixing **Acid-N$^+$(Me)$_3$** (2.387 g, 7.36 mmol, 4 eq.), HATU (2.80 g, 7.36 mmol, 4 eq.) and DIEA (1.285 mL, 7.36 mmol, 4 eq.) in DMSO (Volume: 40 mL). The resulting solution was added to the resin and allowed to react for 12 hours. A Kaiser test for the detection of free amines was negative.

Workup
The resin was washed with DMSO 4 times, and water 4 times.
To facilitate the pipetting of a homogenous suspension, the resin was suspended in water/glycerol 1/1 and the volume adjusted to 80mL.

SI IV Umpolung addition of carbanion equivalents on immobilized DNA-ketone conjugates

SI IV1 Adaptation of the literature protocol to DNA conjugates immobilized on PEG+

The protocol for this reaction was adapted from the original report by Li *et al.*[3]



7 **9**

Ligand	Additive	Conversion (%)
dmpe	CsF	95
dmpe	-	85
dppp	CsF	92

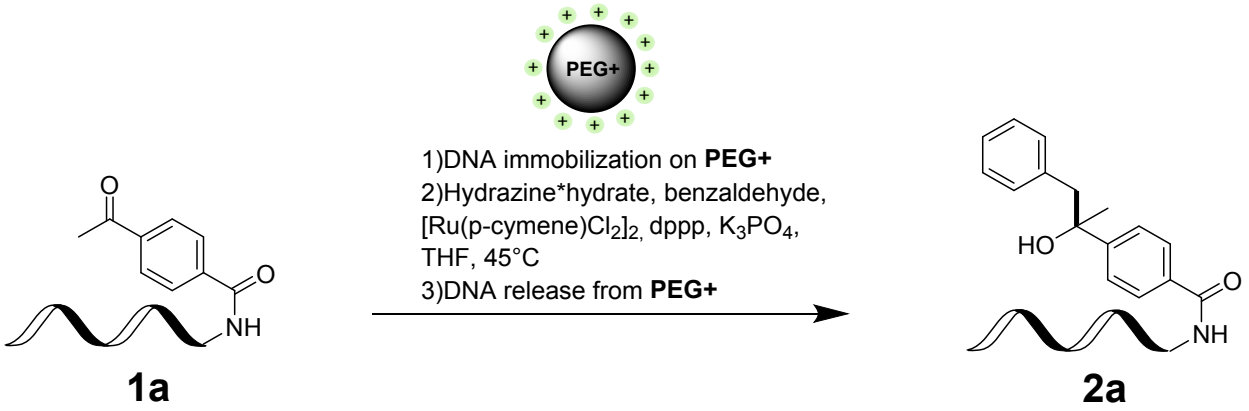
Scheme SI IV1: Literature reported yield and reaction conditions for the use of aldehydes as alkyl carbanion equivalents for additions to carbonyl compounds.[3] Reaction conditions: **7** (25 µl, 0.24 mmol, 1.2 eq.), $N_2H_4 \cdot H_2O$ (13 µl, 0.26 mmol, 1.3 eq.), THF (0.1 mL), room temperature (r.t.), 30 min; **8** (23.5 µl, 0.20 mmol, 1.0 eq.), $[Ru(p\text{-cymene})Cl_2]_2$ (0.9 mg, 0.0015 mmol, 0.75 mol%), ligand (0.003 mmol, 1.5 mol%), K_3PO_4 (0.05 mmol, 25 mol %), CsF (15 mg, 0.10 mmol, 50 mol%), 45 °C, 3 h, under N_2.

While the literature reports optimal conditions for this reaction using 1,2-bis(dimethylphosphino)ethane (dmpe) as ligand and CsF as an additive (see **Scheme SI IV 1**), the use of 1,3-bis(diphenylphosphino)propane (dppp) and running the reaction in the absence of CsF led to only a slight drop in conversion (92% and 85% respectively) compared to the optimal reported protocol (95%) (**Scheme SI IV1**).

Therefore, we attempted to replicate the reported conditions in the absence of CsF to simplify the reaction system. As our DNA susbtrate is immobilized on solid support, we prepared a solution in dry THF of all the other reagents and added the base K_3PO_4 as a solid to the reaction. According to the published protocol the concentration of the different reaction components should be 2.4 mM for benzaldehyde, 15 µM for $[Ru(p\text{-cymene})Cl_2]_2$ and 30 µM for the ligand 1,3-bis(diphenylphosphino)propane (dppp) (Aldrich CAS 6737-42-4).

The concentration of these reagents was therefore kept identical in our attempt to use these reactions conditions on **1a** immobilized on **PEG+**.

Protocol for the initial attempt using conditions directly taken from the literature: Main text Figure 2 B (a)



Scheme SI IV2: Reaction conditions for the use of aldehydes as alkyl carbanion equivalents for additions to **1a** after immobilization on the amphiphilic solid support **PEG+**. Reaction conditions: **1a** (10 nmol) immobilized on **PEG+** (12.5 mg), hydrazine hydrate (2.6 mM), benzaldehyde (2.4 mM), $[Ru(p\text{-cymene})Cl_2]_2$ (15 µM), dppp (30 µM), K_3PO_4 (25 mol% with respect to benzaldehyde), dry THF, 45°C 1 h.

Immobilization of the susbtrate 1a on PEG+: In a 2 mL syringe equipped with a filter 250 µL of a suspension of **PEG+** (resin swollen in water) (250 µL, 0.5 g in 10mL) was incubated with 97 µL of a solution of **1a** (as a solution in water) (0.064 mg, 0.01 µmol) for 15 min. The resin was washed with water and dry THF (4 times).

***In-situ* preparation of the hydrazone and catalyst solution:** Benzaldehyde (Aldrich CAS 100-52-7) (488 µL, 4.8 mmol) was dissolved in THF (Volume: 2 mL) followed by the addition of $N_2H_4*H_2O$ (253 µL, 5.2 mmol). This solution was stirred at room temperature for 30 min. A small amount of Na_2SO_4 (580 mg) was added to this solution. After drying with Na_2SO_4 the hydrazone solution was used to dissolve $[Ru(p\text{-cymene})Cl_2]_2$ (Aldrich CAS 52462-29-0) (18 mg, 29.4 µmol) and 1,3-bis(diphenylphosphino)propane (Aldrich CAS 6737-42-4) (25 mg, 60.6 µmol).

500 µL of this solution of hydrazone (2.4 mM), $[Ru(p\text{-cymene})Cl_2]_2$ (15 µM) and dppp (30 µM), was then added to the syringe containing the substrate **1a** immobilized on resin. K_3PO_4 (79 mg) was then added as a solid and the syringe sealed with a stopper and incubated with mechanical agitation at 45°C for 1 h.

Release of the crude 2a from the resin PEG+: The release procedure was adapted from Harbury *et al.*[1]

The resin was washed twice with THF and the DNA was released by treatment with 500 µL of DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X) for 10 min at room temperature. This solution was recovered and desalted using a NAP-5 (GE health Care) before UPLC-TOF analysis.

Analysis of the crude reaction:

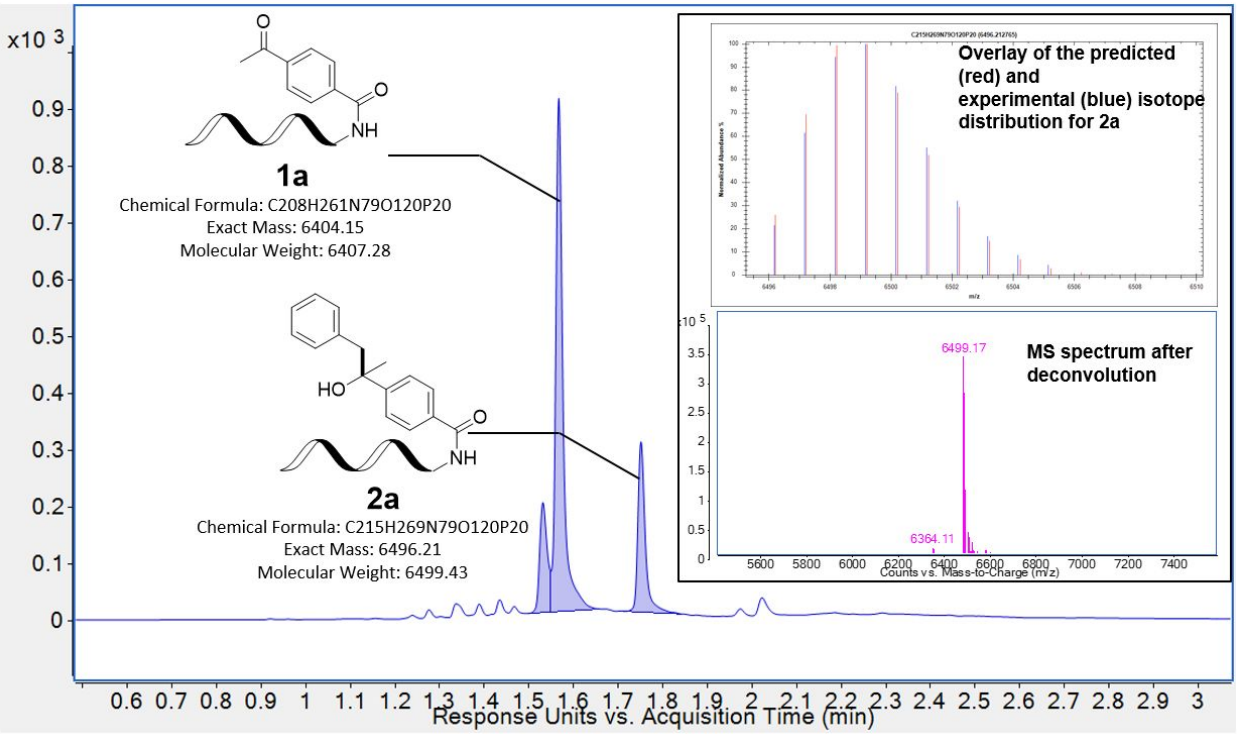


Figure SI IV1: UPLC-TOF analysis of the crude product of the first attempt to perform the Umpolung addition of on DNA-ketone conjugate **1a** immobilized on **PEG+**. Reaction conditions: **1a** (10 nmol) immobilized on **PEG+** (12.5 mg), hydrazine hydrate (2.6 mM), benzaldehyde (2.4 mM), $[Ru(p\text{-cymene})Cl_2]_2$ (15 µM), dppp (30 µM), K_3PO_4 (25 mol% with respect to benzaldehyde) in dry THF (0.5 mL), 45°C, 1 h.

Low intensity peaks with retention times between 1.2 and 1.5 min do not correspond to ruthenium adducts or degradation products according to UPLC-TOF analysis, but rather to impurities already present in the starting material **1a** as shown in Figure SI II6.

Protocol using conditions adapted to improve the conversion into 2a: Main text Figure 2 B (b)

These conditions are identical to the previous example except that more Na_2SO_4 and K_3PO_4 (39 mol% with respect to benzaldehyde) were used.

Immobilization of the substrate 1a on PEG+: In a 2 mL syringe equipped with a filter 250 µL of a suspension of **PEG+** (resin swollen in water) (250 µL, 0.5 g in 10 mL) was incubated with 97 µl of a solution of **1a** (as a solution in water) (0.064 mg, 0.01 µmol) for 15 min. The resin was washed with water and dry THF (4 times).

***In situ* preparation of the hydrazone and catalyst solution:** Benzaldehyde (Aldrich CAS 100-52-7) (488 µL, 4.8 mmol) was dissolved in THF (Volume: 2 mL) followed by the addition of $N_2H_4{*}H_2O$ (253 µL, 5.2 mmol). This solution was stirred at room temperature for 30 min. A larger amount of Na_2SO_4 (1000 mg)

was added to this solution. After drying with Na_2SO_4 the hydrazone solution was used to dissolve [Ru(p-cymene)Cl_2]$_2$ (Aldrich CAS 52462-29-0) (18 mg, 29.4 µmol) and 1,3-bis(diphenylphosphino)propane (Aldrich CAS 6737-42-4) (25 mg, 60.6 µmol).

500 µL of this solution of hydrazone, [Ru(p-cymene)Cl_2]$_2$ and dppp were then added to the syringe containing the substrate **1a** immobilized on resin. K_3PO_4 (100 mg) was then added as a solid. The syringe was sealed with a stopper and incubated with mechanical agitation at 45°C for 1 h.

Analysis of the crude reaction:

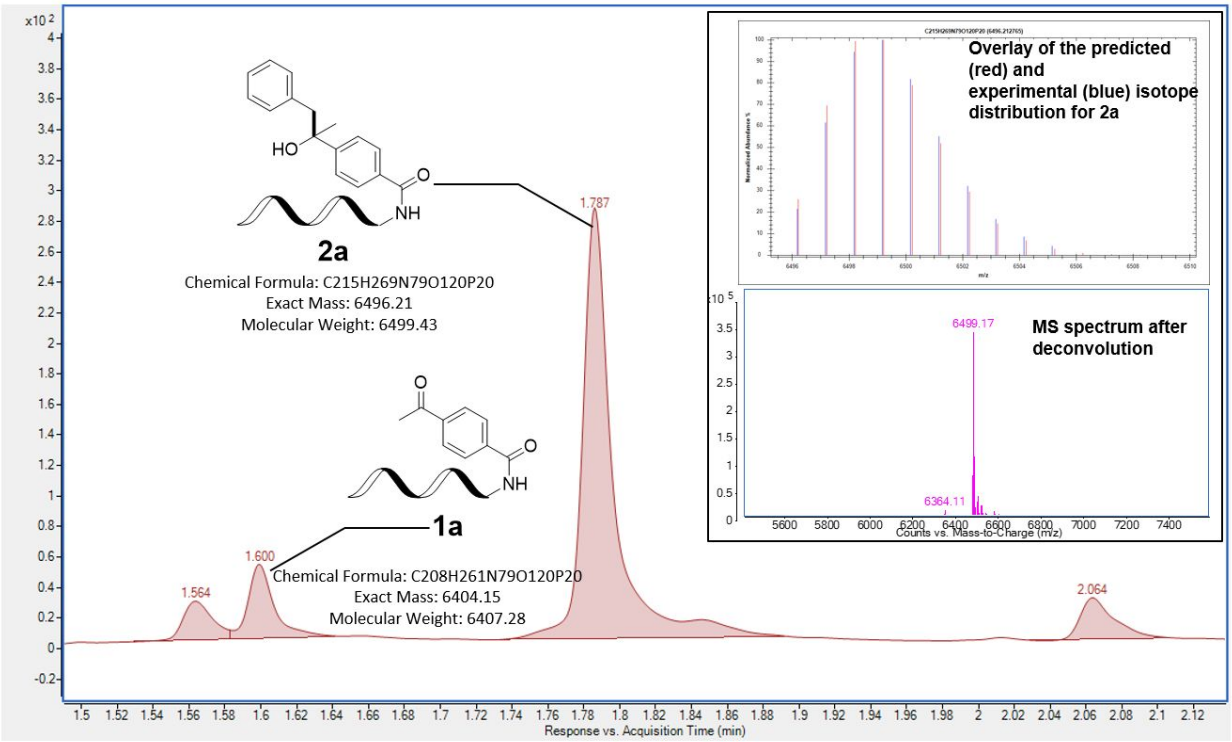


Figure SI IV2: UPLC-TOF analysis of the crude product of the first attempt to perform the Umpolung addition on DNA-ketone conjugate **1a** immobilized on **PEG+**. Reaction conditions: **1a** (10 nmol) immobilized on **PEG+** (12.5 mg), hydrazine hydrate (2.6 mM), benzaldehyde (2.4 mM), [Ru(p-cymene)Cl_2]$_2$ (15 µM), dppp (30 µM), K_3PO_4 (39 mol% with respect to benzaldehyde) in dry THF (0.5 mL), 45°C, 1 h.

Protocol used to perform this reaction on DEAE sepharose:

The protocol is identical as that for **Figure 2 B (b)** (see above) except that the DNA immobilization was performed on DEAE sepharose (DEAE Sephadex™ A-25, GE healthcare) in a 1 mL syringe equipped with a filter. 250 µL of a suspension of the DEAE Sepharose (2 g in 30 mL of water) was washed with water followed by wash with a 10 mM acetic acid solution and 0.005% Triton X-100 (the DNA immobilization was adapted from Harbury *et al.*[1]). The resin was incubated with a solution of **1a** (0.010 µmol) in 0.5 mL of a 10 mM acetic acid solution and 0.005% Triton X-100 for 15 min.

Analysis of the crude reaction:

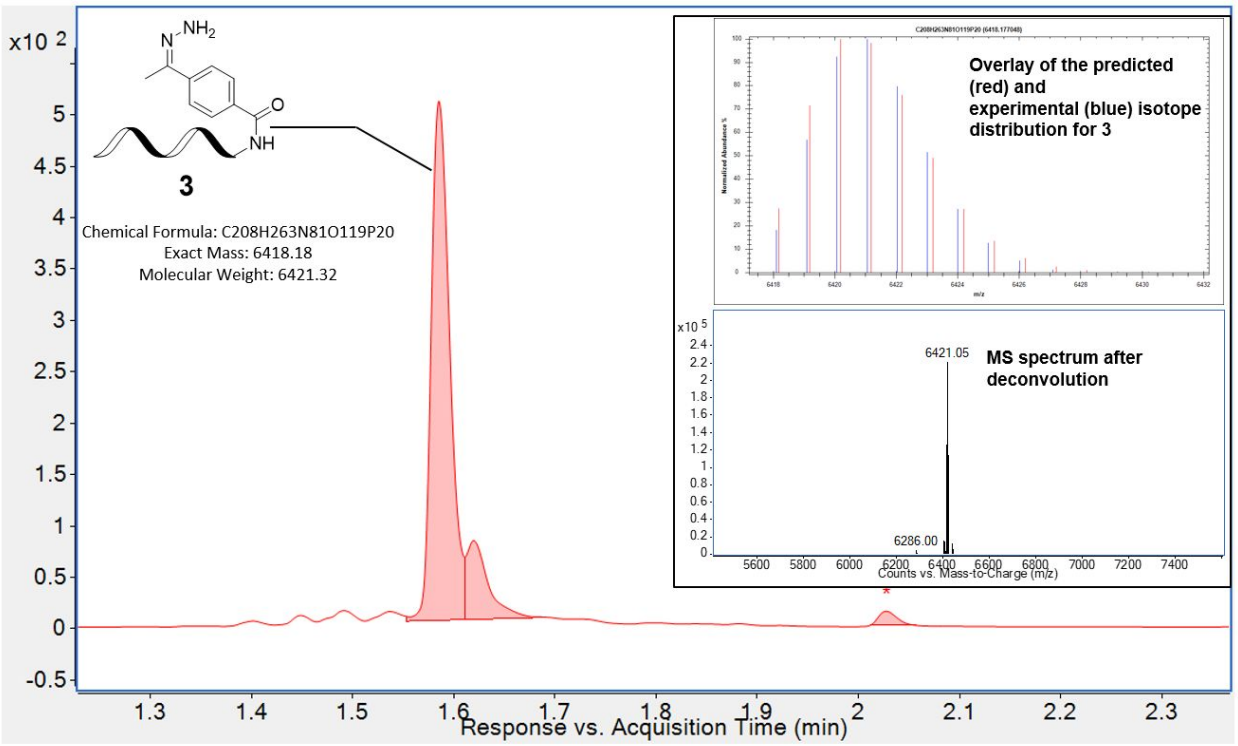
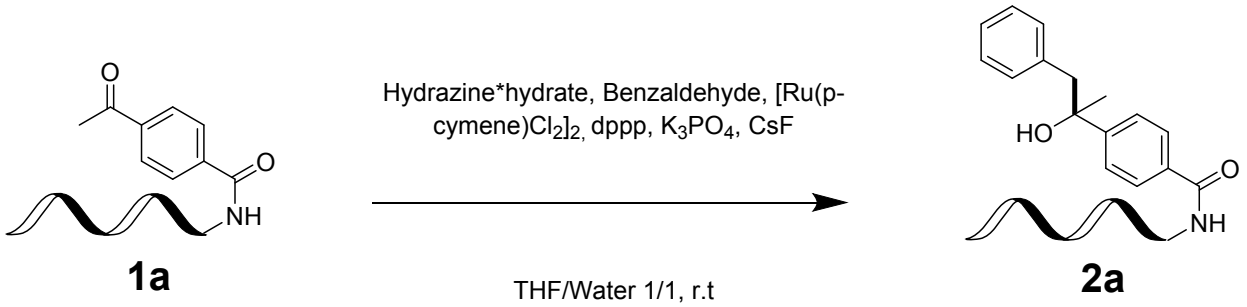


Figure SI IV3: UPLC-TOF analysis of the crude product of the first attempt to perform the Umpolung addition of on DNA-ketone conjugate **1a** immobilized on **DEAE sepharose**. Reaction conditions: **1a** (10 nmol) immobilized on **DEAE sepharose** (16 mg), hydrazine hydrate (2.6 mM), benzaldehyde (2.4 mM), $[Ru(p\text{-cymene})Cl_2]_2$ (15 µM), dppp (30 µM) and K_3PO_4 (39 mol% with respect to benzaldehyde) in dry THF (0.5 mL), 45°C 1h.

SI IV2 Attempt to perform the Umpolung addition of carbanion equivalents on DNA-ketone conjugates under aqueous conditions



Scheme SI IV3: Reaction conditions for the use of aldehydes as alkyl carbanion equivalents for additions to **1a** under aqueous conditions.

The protocol for this reaction was adapted from the original report by Li *et al.*[3]

***In situ* preparation of the hydrazone and catalyst solution:**:

Benzaldehyde (Aldrich CAS 100-52-7) (98 µL, 0.96 mmol) was dissolved in dry THF (Volume: 0.4 mL) followed by the addition of $NH_2NH_2*H_2O$ (51.0 µL, 1.04 mmol). This solution was stirred at room temperature for 30 min. A small amount of Na_2SO_4 was added to this solution before using it.

In an Eppendorf PCR tube® **1a** (as a dry pellet) (0.064 mg, 0.01 µmol) was dissolved in 50 µL of a solution of CsF (1.6 M) and K_3PO_4 (800 mM) (50 µL, 40.0 µmol) in water.

50 µL of the hydrazone solution prepared above was then added to this solution of **1a** and stirred at room temperature for 2 hours.

The reaction was not homogenous and two phases were present.

Workup
The reaction was diluted with water and desalted before UPLC-TOF analysis.

Analysis of the crude reaction:

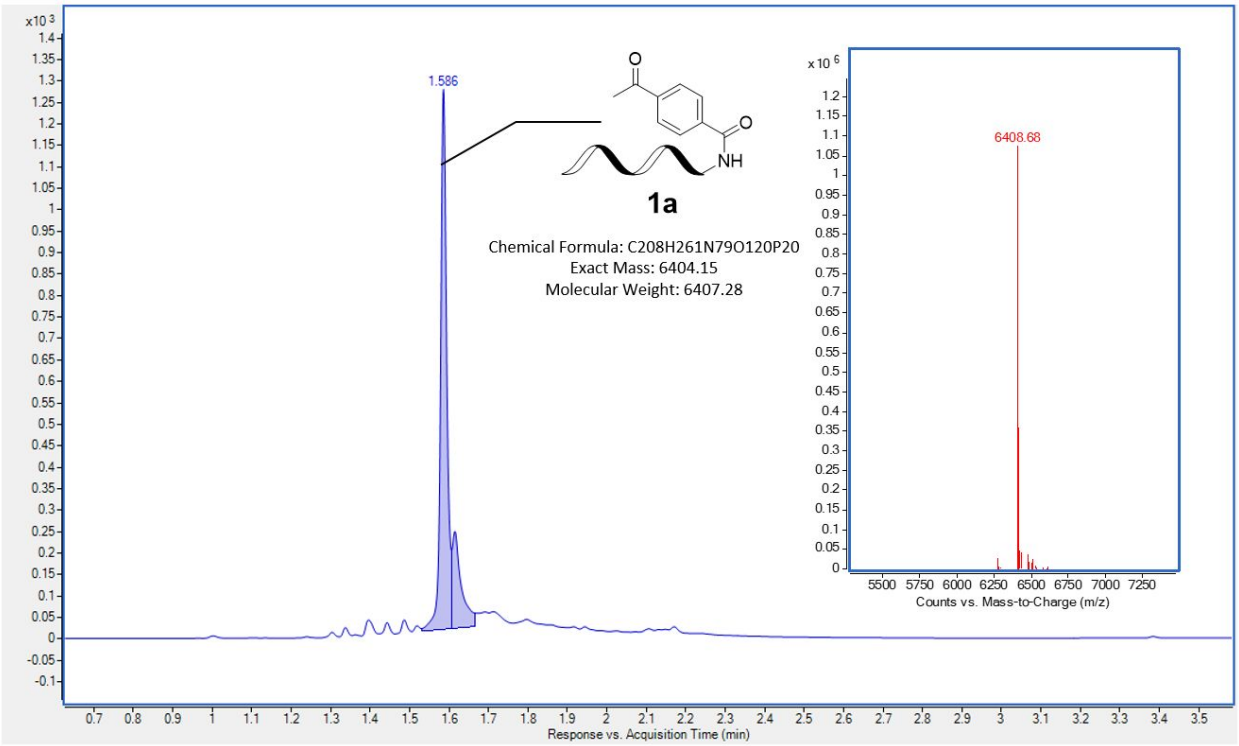


Figure SI IV4: UPLC-TOF analysis of the crude product of the attempt to perform the umpolung addition of on DNA-ketone conjugate **1a** under aqueous conditions reaction.

Only the starting material **1a** could be detected in the crude product. No trace of the expected product **2a** could be observed under these reaction conditions.

SI V Synthesis of saturated heterocycles on immobilized DNA-aldehyde conjugates using the SnAP reaction

The protocol for this reaction was adapted from the original report from Bode *et al.*[4]

Useful information on this reaction can be found on the Bode research group website:

http://www.bode.ethz.ch/research/snap-chemistry.htmL

SnAP reagents are available commercially. A set of 12 SnAP reagents were purchased from Aldrich, and used without any further purification. (Figure SI V1)

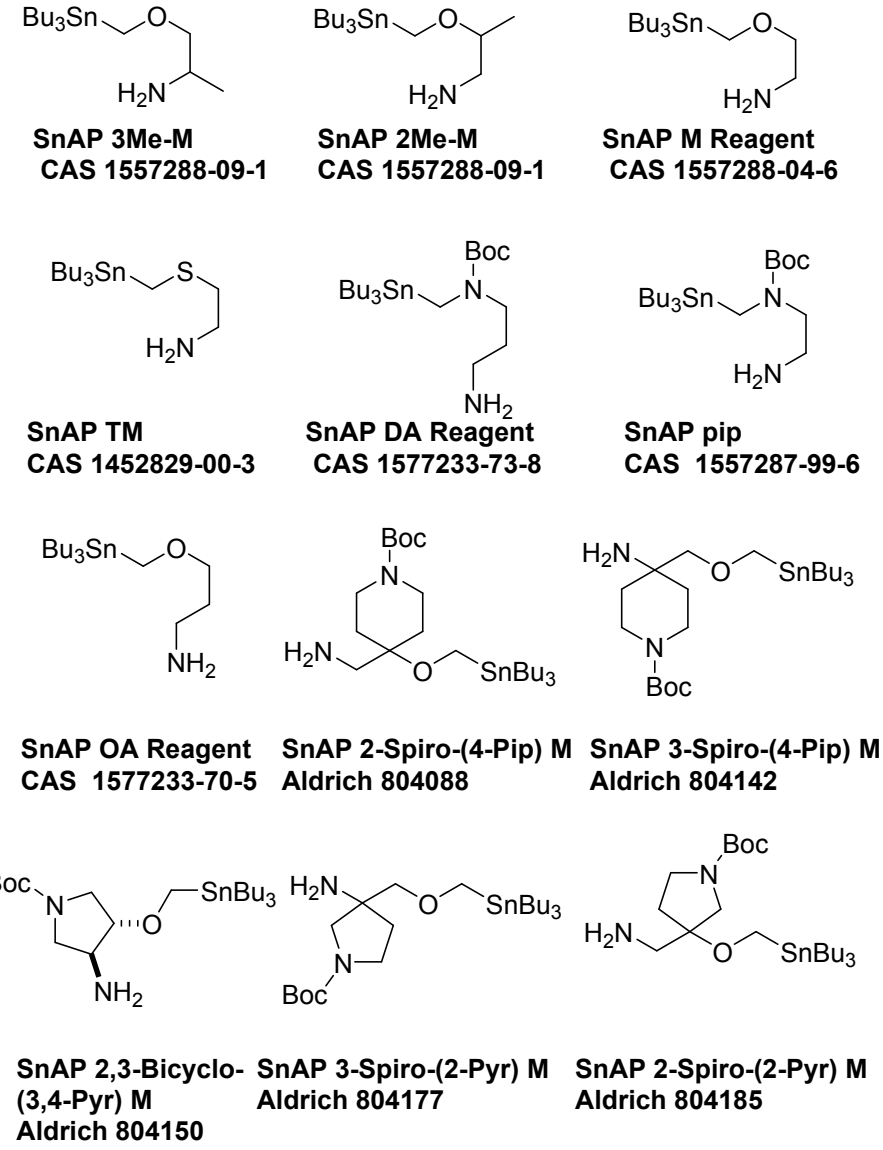


Figure SI V1: Structure and nomenclature of the commercial **SnAP** reagents used in this study.

SI V1 Adaptation of the SnAP reaction on immobilized DNA conjugate 1b

1st attempt to perform the SnAP reaction on 1b (Main text Figure 3B(a))

For this reaction a solution of copper(II) trifluoromethanesulfonate (STREM CAS 34946-82-2) (2.5 mM) and 2,6-lutidine (2.5 mM) in CH_2Cl_2/HFIP 4/1 was prepared in advance (1 hour) as the formation of the complex is not instantaneous due to the low solubility of copper(II) trifluoromethanesulfonate in the solvent mixture.

DNA immobilization:

In a 1 mL syringe equipped with a filter, 250 µL of a suspension of the resin **PEG+** (resin swollen in water) (250 µL, 12.5 mg) was incubated with an aqueous solution of **1b** (0.010 µmol) for 15 min. The resin was washed with water, THF and CH_2Cl_2 (4 times). 0.2mL of CH_2Cl_2 was added to the resin followed by the **SnAP pip** reagent (Aldrich CAS 1557287-99-6 , (tert-Butyl(2aminoethyl)((tributylstannyl)methyl)carbamate) (8.42 µL, 20.00 µmol). The suspension was mechanically agitated using an Eppendorf ThermoMixer® for 30 min at room temperature. At this stage the resin was washed with CH_2Cl_2 and treated with a solution of copper(II) trifluoromethanesulfonate (0.181 mg, 0.500 µmol) and 2,6-lutidine (0.058 µL, 0.500 µmol) in CH_2Cl_2 (Volume: 0.16 mL) and HFIP (Volume: 0.04 mL) (premixed at room temperature for 1h). The suspension was mechanically agitated at room temperature for 1h.

Workup

The resin was washed 3 times with THF.

The DNA was released from the resin by treatment with 150 µL of DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X). This solution was recovered and diluted with 100 µL of water before desalting and UPLC-TOF analysis. Analysis of the crude reaction mixture can be found in figure SI V2.

Analysis of the crude reaction:

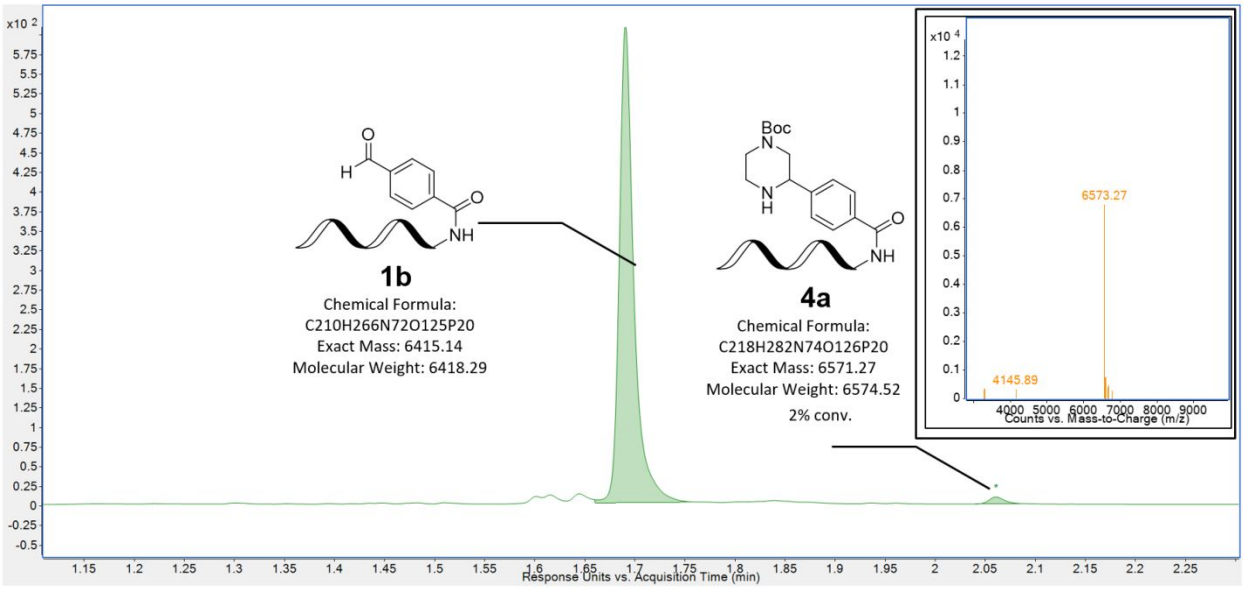


Figure SI V2: Analysis of the crude reaction (initial conditions) between **1b** and the SnAP reagent **SnAP pip** (Figure SIV1). Reaction conditions: 1) **1b** (10 nmol) immobilized on **PEG+**, SnAP reagent (100 mM) in CH_2Cl_2 (0.2 mL), 22°C, 1h. 2) $Cu(OTf)_2$(2.5 mM)/2,6-lutidine(2.5 mM) in CH_2Cl_2/HFIP 4/1 (0.2 mL), 22°C, 1 h.

Protocol using conditions adapted to improve the conversion into 4a (Main text Figure 2 B (b))

A solution of copper(II) trifluoromethanesulfonate (12.5 mM) and 2,6-lutidine (12.5 mM) in HFIP was prepared in advance (1 hour) as the formation of the complex is not instantaneous due to the low solubility of copper(II) trifluoromethanesulfonate in this solvent.

DNA immobilization:

In a 0.5 mL cartridge equipped with a filter, 250 µL of a suspension of the **PEG+** (resin swollen in water) (250 µL, 12.5 mg) was incubated with an aqueous solution of **1b** (0.010 µmol) for 15 min. The resin was washed with water, THF and CH_2Cl_2 (4 times). The resin was then dried under high vacuum.

0.2 mL of CH_2Cl_2 was added to the resin followed by a few beads of molecular sieve 4Å (Aldrich cat number 20,860-4, 8-12 mesh), and the **SnAP pip** reagent (see figure SI V1) (20.00 µmol). The suspension was mechanically agitated for 1 h at 40°C. The resin was washed with CH_2Cl_2. CH_2Cl_2 (Volume: 0.16 mL) was added and 40 µL of a suspension of copper(II) trifluoromethanesulfonate (0.181 mg, 0.500 µmol) and 2,6-lutidine (0.058 µL, 0.500 µmol) in HFIP (premixed for 1 h at room temperature) was added. The suspension was mechanically agitated at 40°C for 2 h.

Workup

The resin was washed 3 times with THF.

The DNA was released from the resin by treatment with 500 µL of DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH8, 0.005% triton-X). This solution was recovered and diluted with 500 µL of water and 50 µL of EDTA 200 mM before desalting and UPLC-TOF analysis. Analysis of the crude reaction mixture can be found in figure SI V3.

Analysis of the crude reaction:

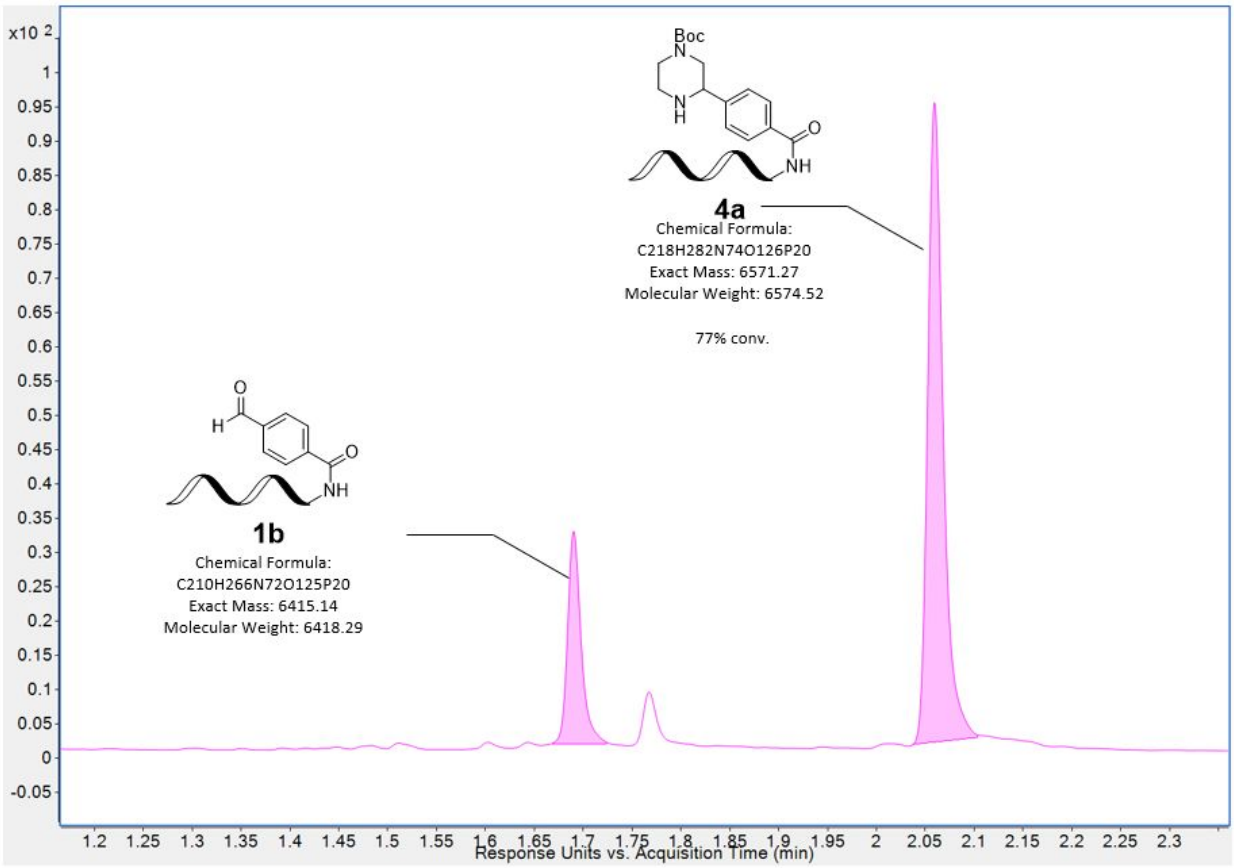


Figure SI V3: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP pip** (Figure SIV1). Reaction conditions: 1) **1b** (10 nmol) immobilized on **PEG+**, SnAP reagent (100 mM), 4Å molecular sieve in CH_2Cl_2 (0.2 mL), 40°C, 1 h. 2) $Cu(OTf)_2$ (2.5 mM)/2,6-lutidine (2.5 mM), in CH_2Cl_2/HFIP 4/1 (0.2 mL), 40°C, 2 h.

Protocol used to perform the reaction on DEAE sepharose:

Protocol identical as for **Figure 3 B (b)** (see above) except that the DNA immobilization was performed on DEAE sepharose (DEAE Sephadex™ A-25, GE healthcare). In a 1 mL syringe equipped with a filter, 250 µL of a suspension of the DEAE Sepharose (2 g in 30 mL of water) was washed with water then with a 10 mM acetic acid solution containing 0.005% Triton X-100 (protocol adapted from Harbury *et al.*[1]). The resin was then incubated with a solution of **1b** (0.010 µmol) in 0.5 mL of a 10 mM acetic acid solution and 0.005% Triton X-100 for 15 min.

Analysis of the crude reaction:

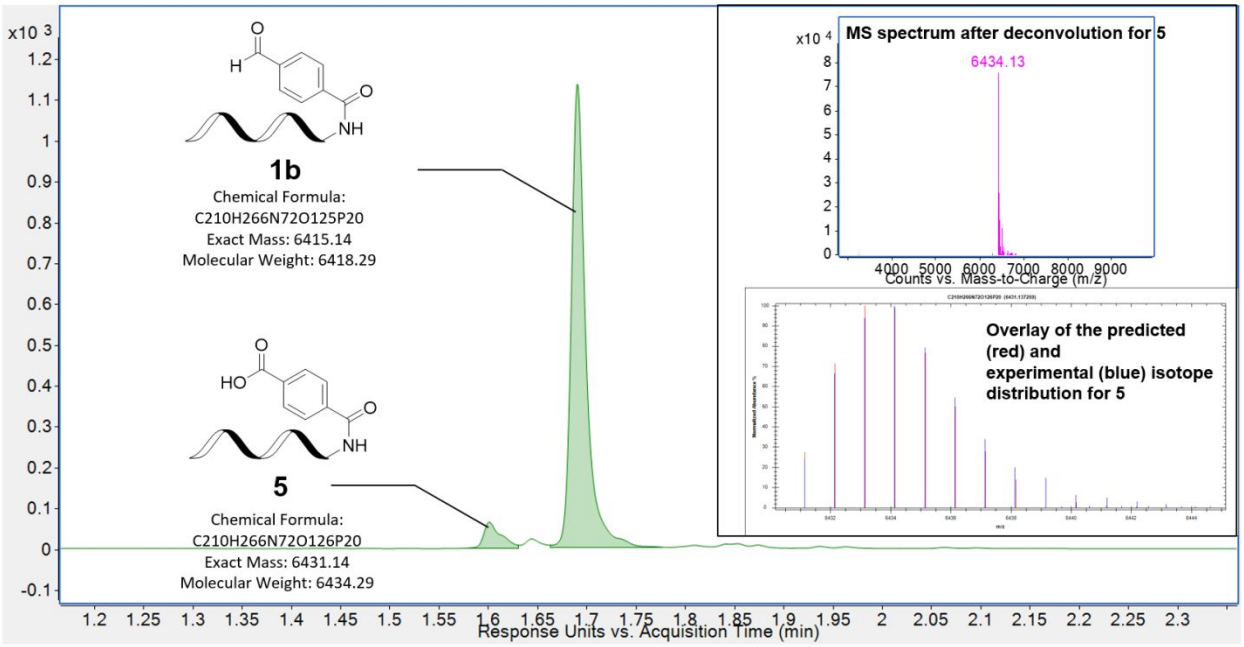


Figure SI V4: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP pip** (Figure SIV1) and characterization of compound **5**. Reaction conditions: 1) **1b** (10 nmoles) immobilized on **DEAE sepharose** (16 mg), SnAP reagent (100 mM), 4Å molecular sieve in CH_2Cl_2 (0.2 mL), 40°C, 1 h. 2) $Cu(OTf)_2$(2.5 mM)/2,6-lutidine(2.5 mM), CH_2Cl_2/HFIP 4/1 (0.2 mL) 40°C, 2 h.

SI V2 Scope of the SnAP reaction using 12 commercially available SnAP reagents

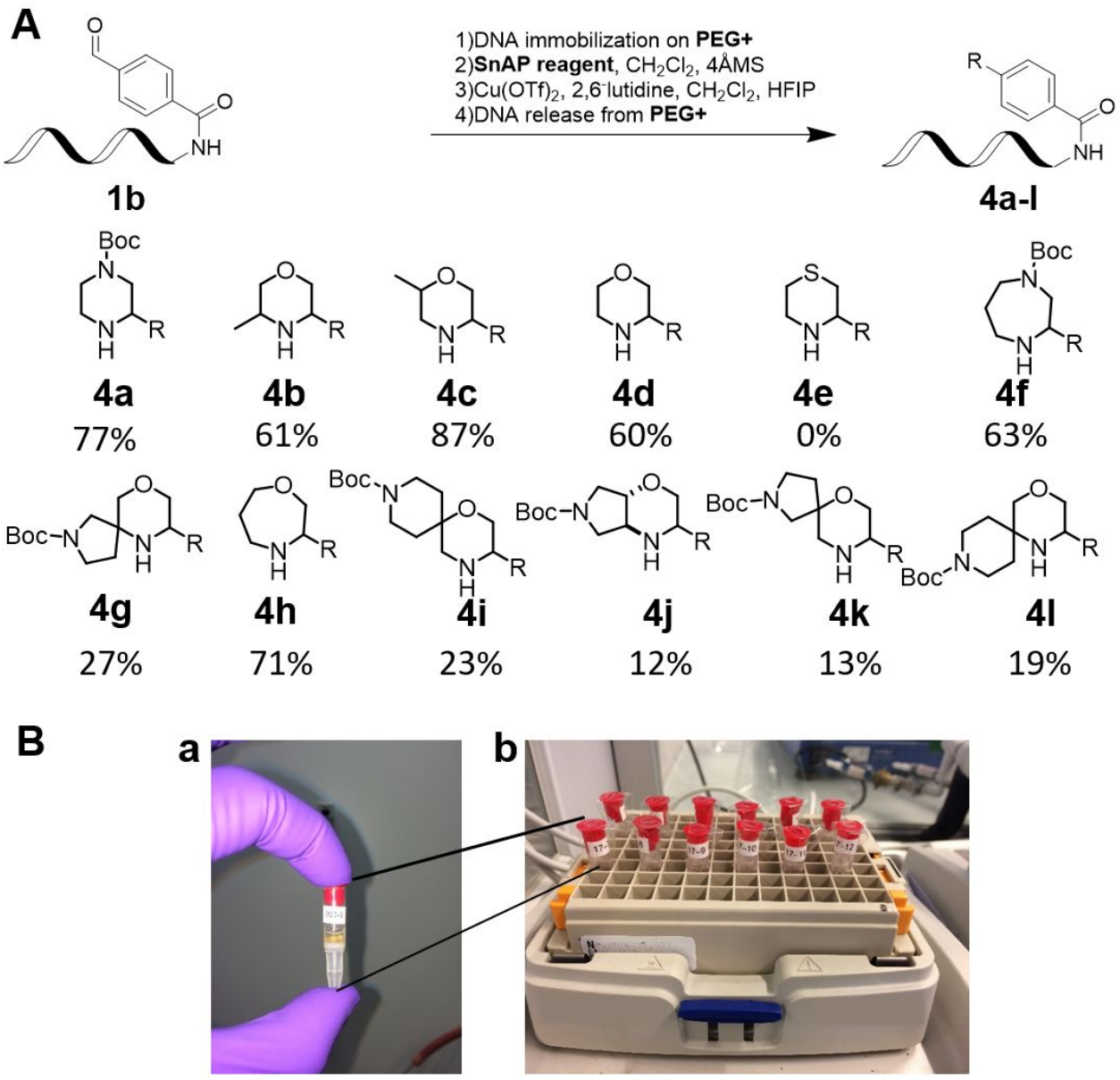


Figure SI V5: A SnAP reaction on **1b** using a set of 12 commercially available SnAP reagents. Reaction conditions: 1) SnAP reagent (100 mM), 4Å molecular sieve in CH_2Cl_2 (0.2 mL), 40°C, 1 h. 2) $Cu(OTf)_2$(2.5 mM)/2,6-lutidine(2.5 mM) in CH_2Cl_2/HFIP 4/1 (0.2 mL) 40°C, 2 h. **B** Experimental setup used to perform these reactions in parallel (**b**). On the left picture (**a**), beads of molecular sieve can be seen in the reaction cartridge.

Protocol:

For these reaction a solution of copper(II) trifluoromethanesulfonate (12.5 mM) and 2,6-lutidine (12.5 mM) in HFIP was prepared in advance (1 hour) as the formation of the complex is not instantaneous due to the low solubility of copper(II) trifluoromethanesulfonate in this solvent.

DNA immobilization:

In a 0.5 mL cartridge equipped with a filter, 250 µL of a suspension of the **PEG+** (resin swollen in water) (250 µL, 12.5 mg) was incubated with an aqueous solution of **1b** (0.010 µmol) for 15 min. The resin was washed with water, THF and CH_2Cl_2 (4 times). The resin was then dried under high vacuum.
0.2 mL of CH_2Cl_2 was added to the resin followed by a few beads of molecular sieve 4Å (Aldrich cat number 20,860-4, 8-12 mesh), and the SnAP reagent (see figure SI V1) (20.00 µmol). The suspension was mechanically agitated for 1 h at 40°C. The resin was then washed with CH_2Cl_2. CH_2Cl_2 (Volume: 0.16 mL) was added and 40 µL of a suspension of copper(II) trifluoromethanesulfonate (0.181 mg, 0.500 µmol) and 2,6-lutidine (0.058 µL, 0.500 µmol) in HFIP (premixed for 1 h at room temperature) was added. The suspension was mechanically agitated at 40°C for 2 h.

Workup
The resin was washed 3 times with THF.
The DNA was released from the resin by treatment with 500 µL of DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH8, 0.005% triton-X). This solution was recovered and diluted with 500 µL of water before desalting and UPLC-TOF analysis. Analysis of the crude reaction mixtures can be found in figures SI V6-17.

Results:

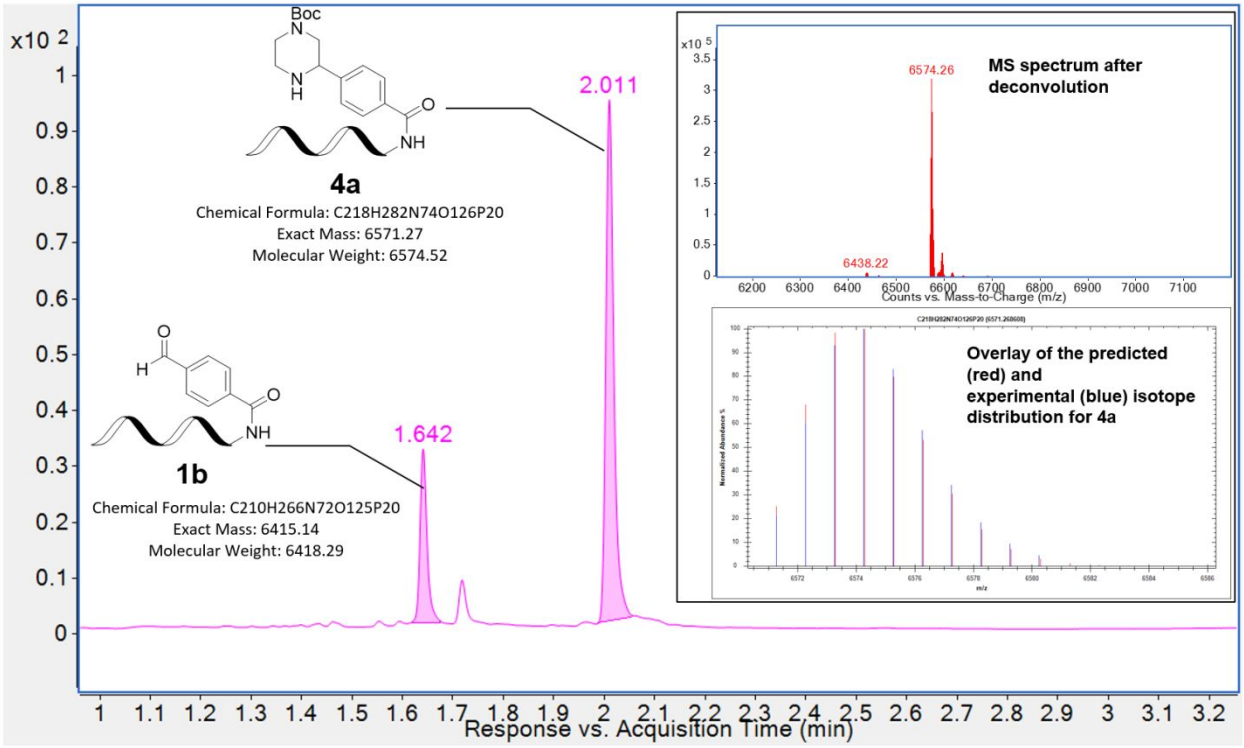


Figure SI V6: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP pip** (figure SI V1)

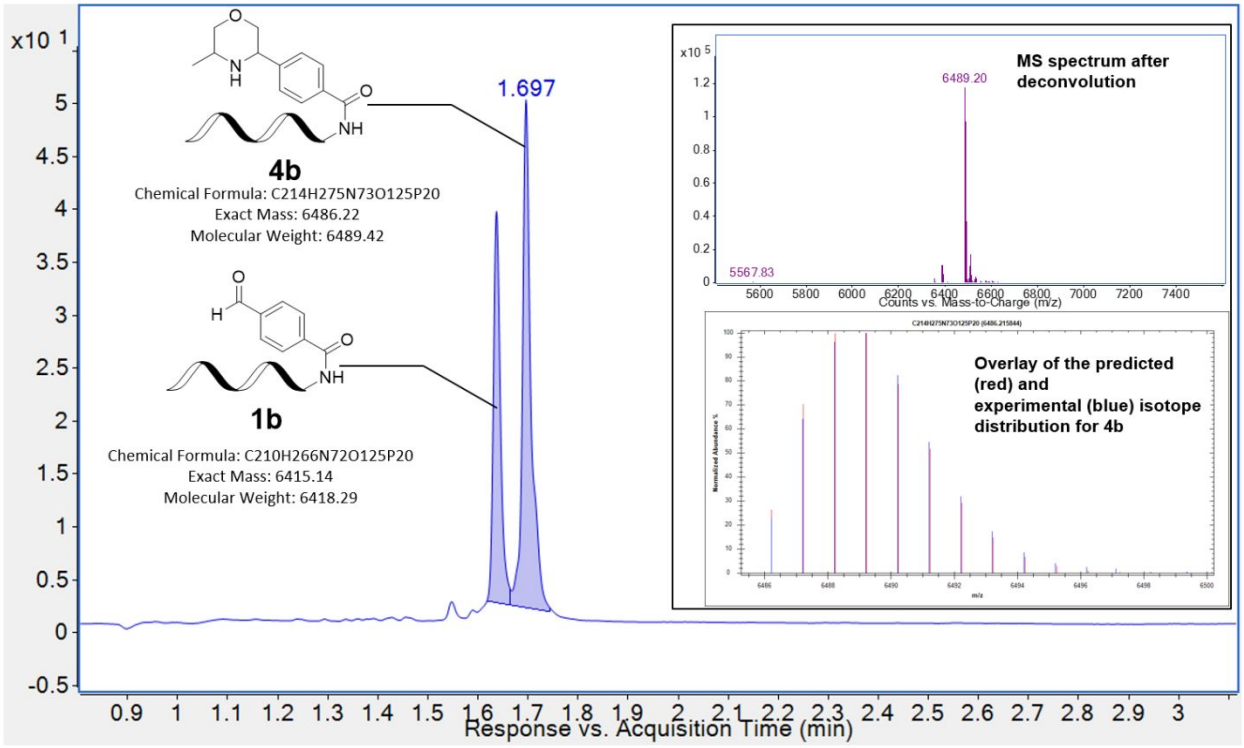


Figure SI V7: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 3Me-M** (figure SI V1)

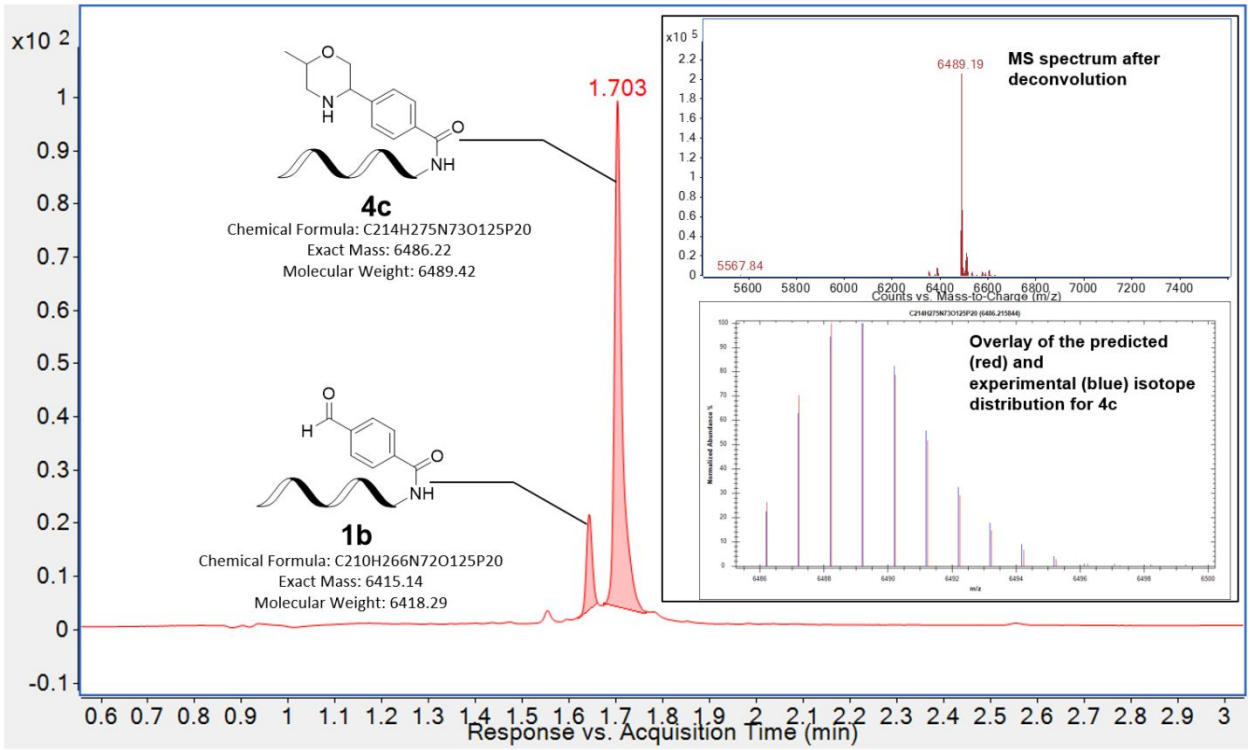


Figure SI V8: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 2Me-M** (figure SI V1)

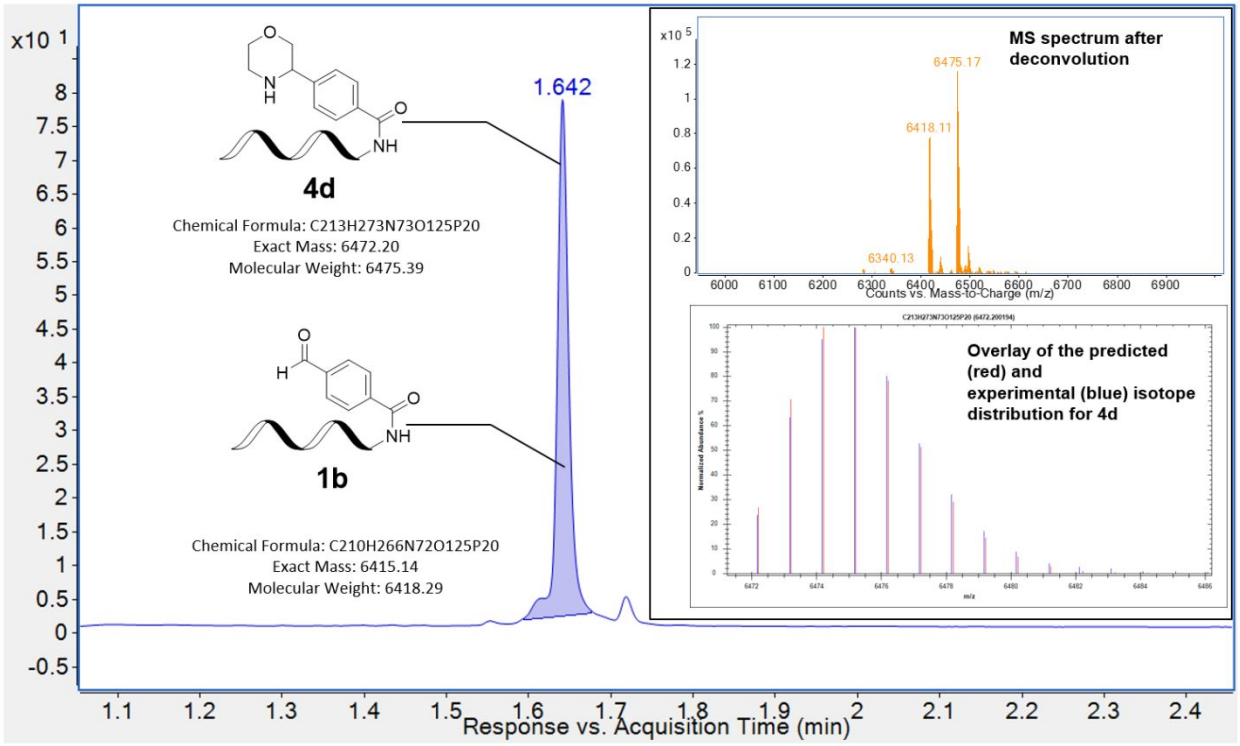


Figure SI V9: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP M** (figure SI V1). Due to the coelution of **1b** and **4d**, the ratio of MS signal was used to determine the conversion for this substrate.

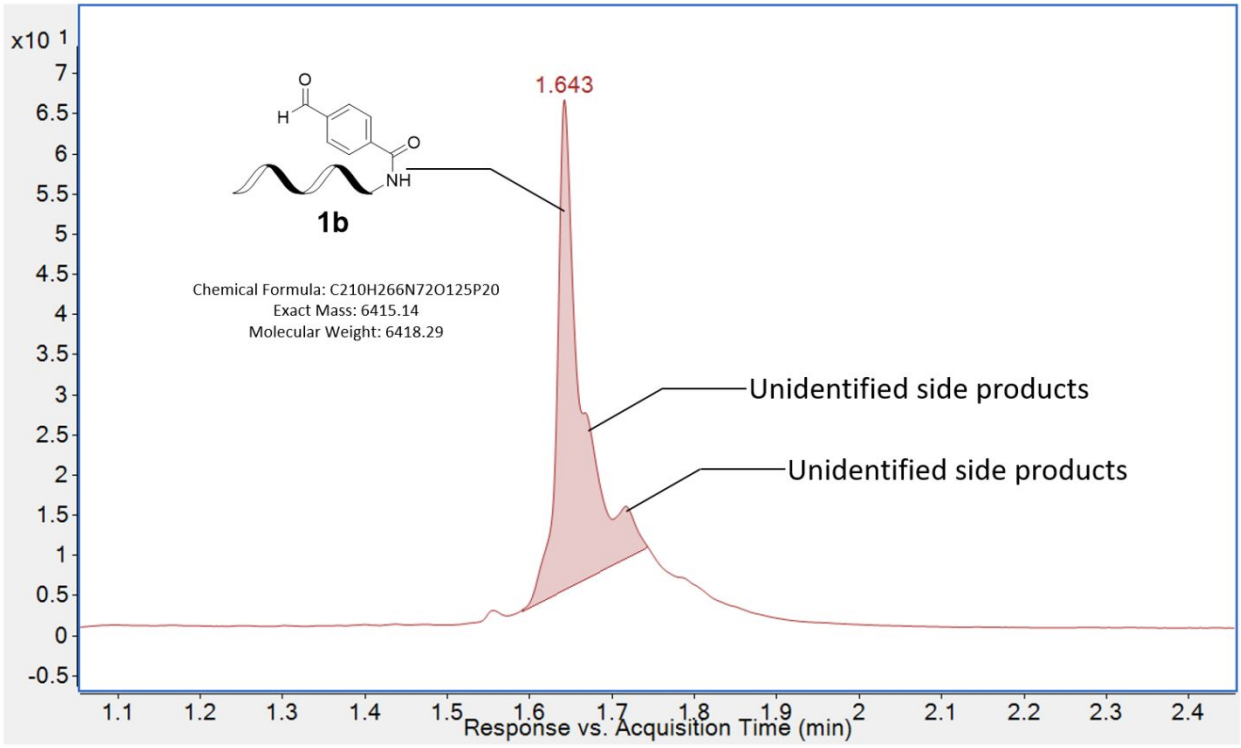


Figure SI V10: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP TM** (figure SI V1)

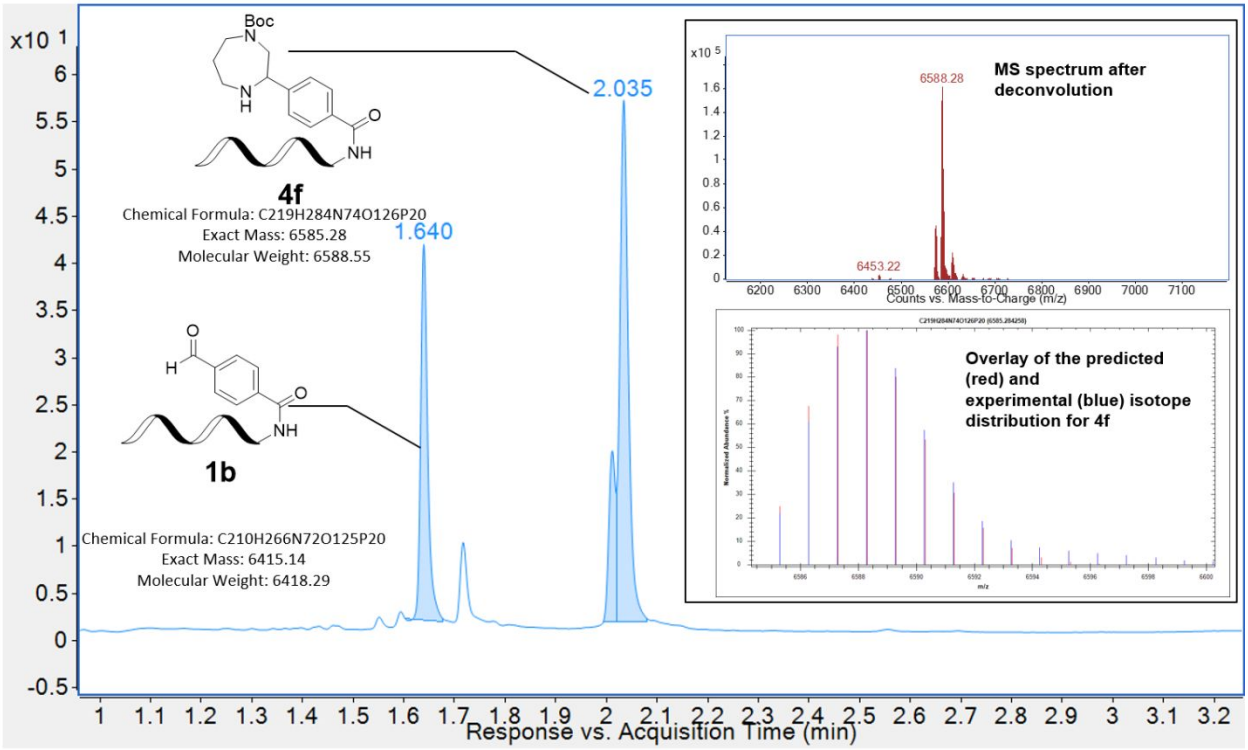


Figure SI V11: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP DA** (figure SI V1)

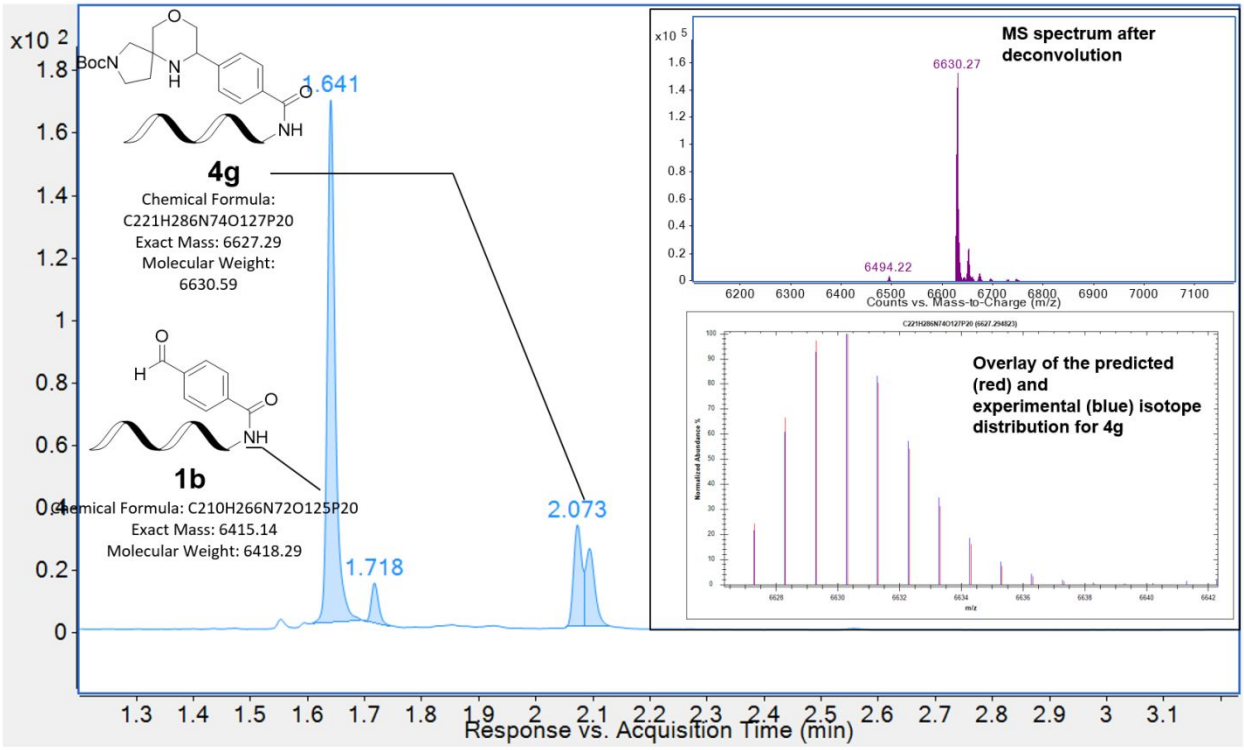


Figure SI V12: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 3-Spiro-(2-Pyr) M** (figure SI V1)

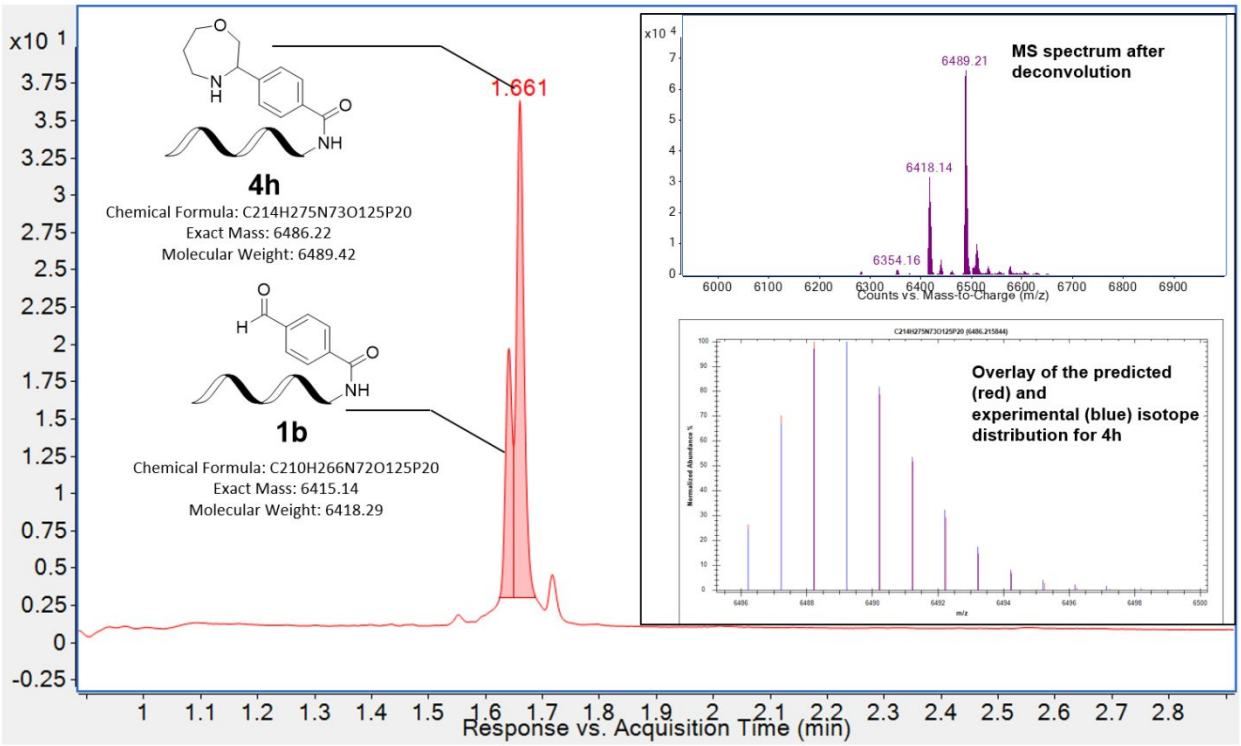


Figure SI V13: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP OA** (figure SI V1)

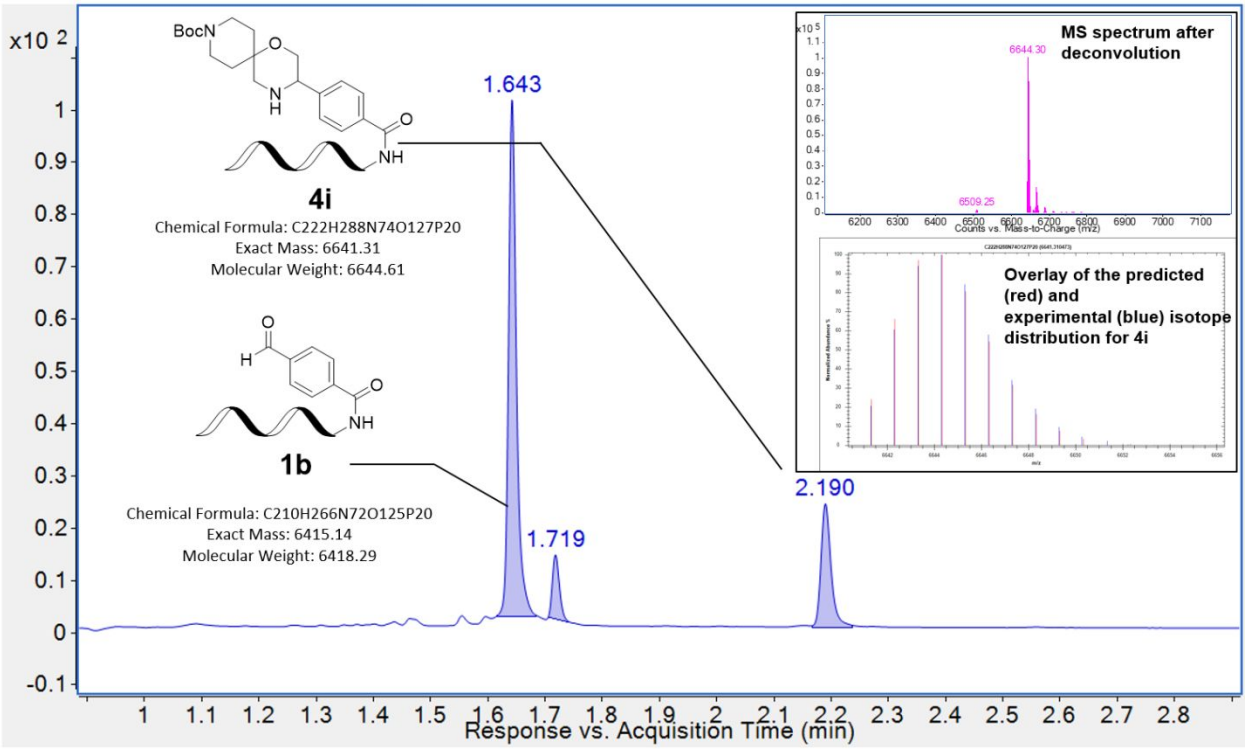


Figure SI V14: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 2-Spiro-(4-Pip) M** (figure SI V1)

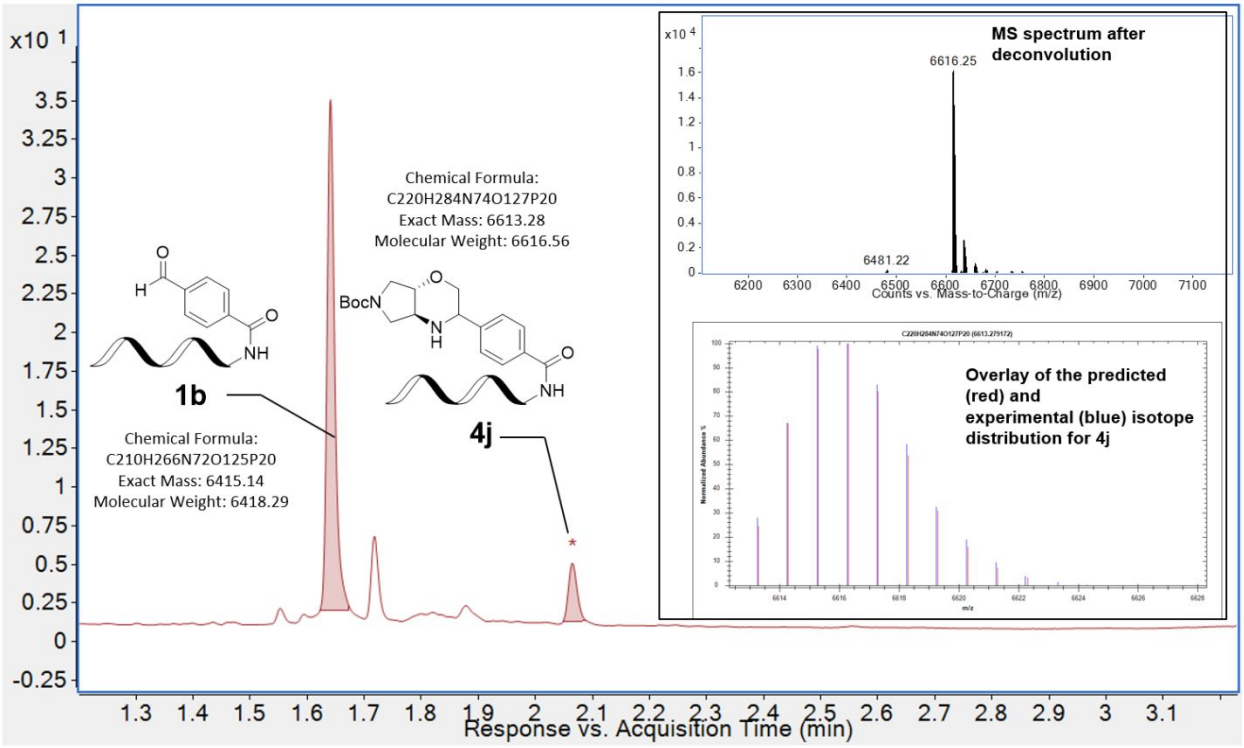


Figure SI V15: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 2,3-Bicyclo-(3,4-Pyr) M** (figure SI V1)

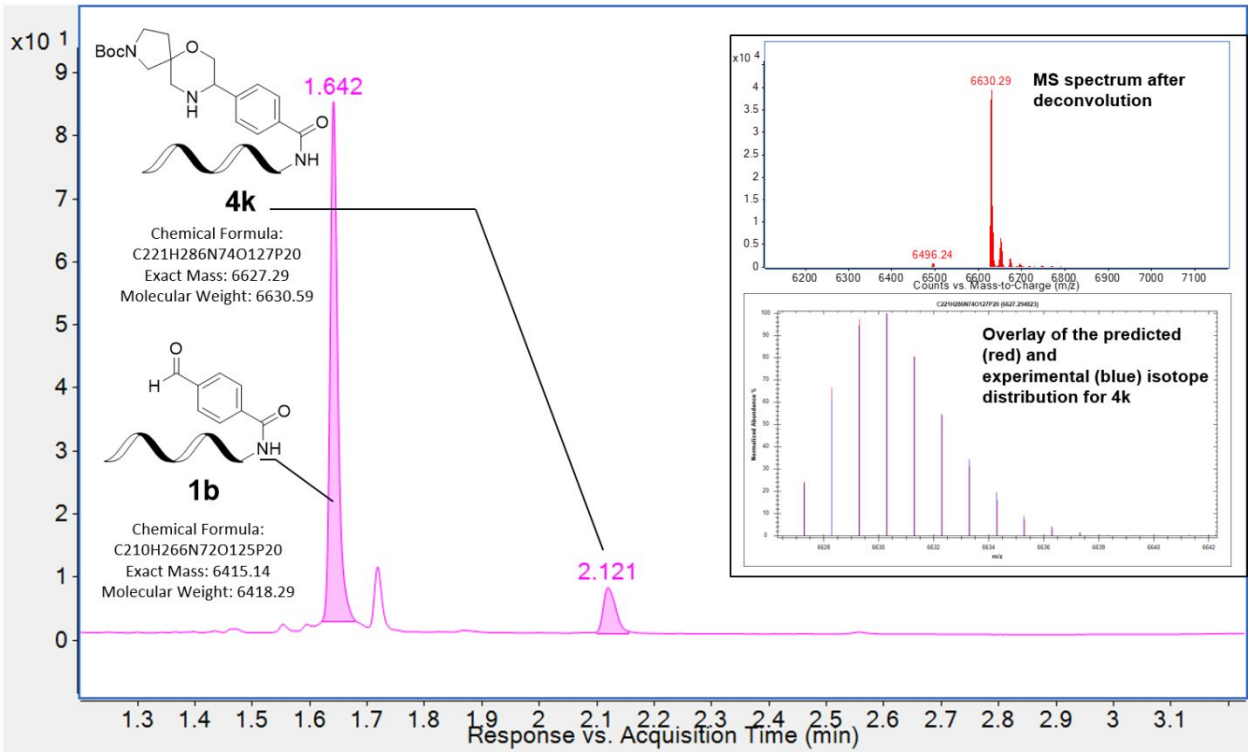


Figure SI V16: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 2-Spiro-(2-Pyr) M** (figure SI V1)

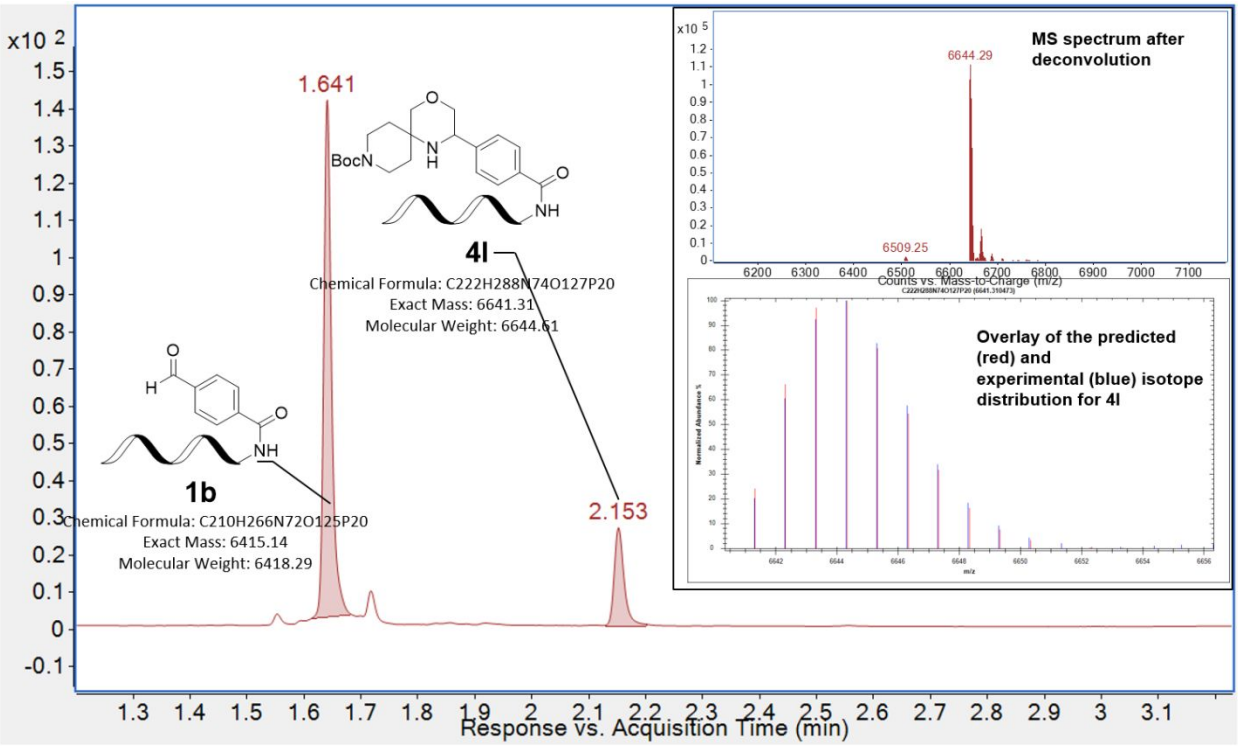


Figure SI V17: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 3-Spiro-(4-Pyp) M** (figure SI V1)

SI V3 Implementation of multiple reaction cycles to improve conversions and shorten reaction times

Protocol:

For these reaction a solution of copper(II) trifluoromethanesulfonate (12.5 mM) and 2,6-lutidine (12.5 mM) in HFIP was prepared in advance (1 hour) as the formation of the complex is not instantaneous due to the low solubility of copper(II) trifluoromethanesulfonate in this solvent.

DNA immobilization:

In a 2 mL syringe equipped with a filter, 125 µL of a suspension of the **PEG+** (resin swollen in water) (125 µL, 6.25 mg) was incubated with an aqueous solution of **1b** (5 nmol) for 15 min. The resin was washed with water, THF (4 times) and CH_2Cl_2 (4 times).

The immobilized **1b** was then submitted to 1, 2 or 3 reaction cycles (see below).

A SnAP reaction cycle: 0.4 mL of CH_2Cl_2 was added to the resin followed by a few beads of molecular sieve 4Å (Aldrich cat number 20,860-4, 8-12 mesh), and the SnAP reagent (see figure

SI V1) (40.00 µmol). The suspension was mechanically agitated for 15 min at 40°C. The resin was washed with CH_2Cl_2 three times. CH_2Cl_2 (Volume: 0.32 mL) was added and 80 µL of a suspension of copper(II) trifluoromethanesulfonate (0.362 mg, 1 µmol) and 2,6-lutidine (0.116 µL, 1 µmol) in HFIP (premixed for 1 h at room temperature) was added. The suspension was mechanically agitated at 40°C for 15 min. The resin was then washed 3 times with THF.

The above procedure is repeated for additional reaction cycles.

Workup

The DNA was released from the resin by treatment with 500 µL of DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH8, 0.005% triton-X, 5% 4-Me-piperidine). The resin was washed again with 250 µL of the same solution. The washing solutions were combined before desalting using a NAP 10 column and analysis by UPLC-TOF. Analysis of the crude reaction mixtures can be found in figure SI V18 and main text figure 3 D.

Results:

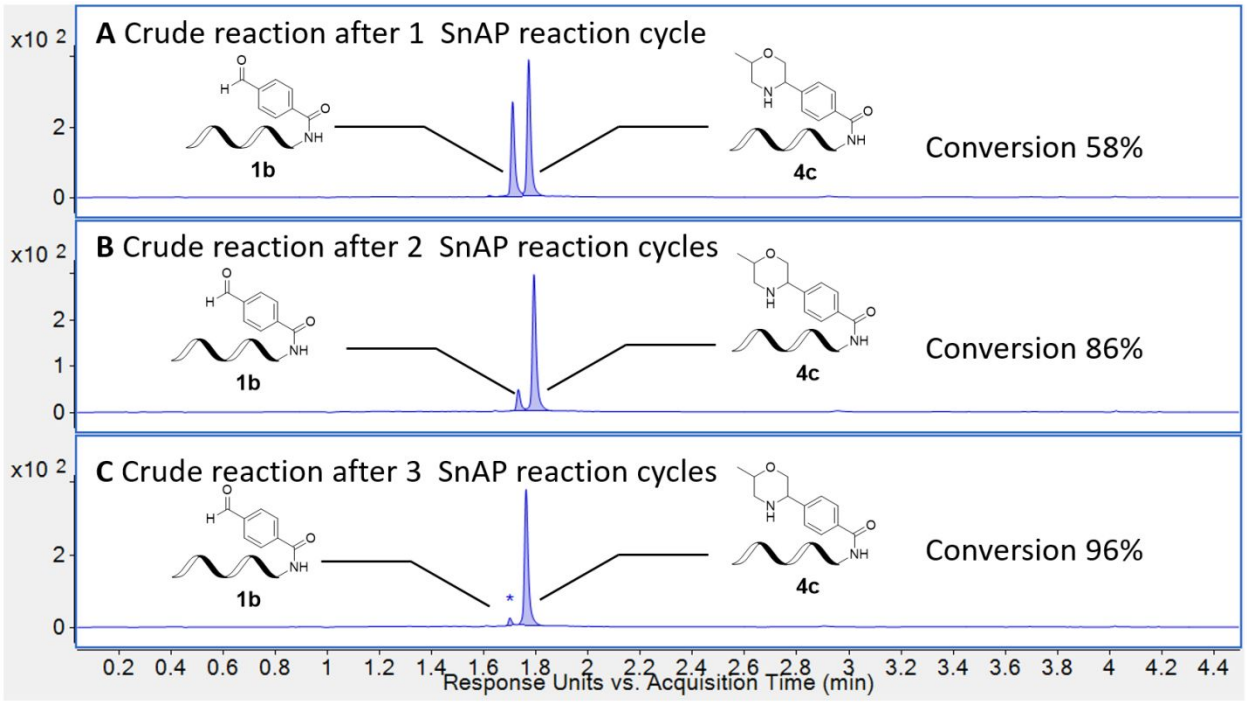


Figure SI V18: Analysis of the crude reaction between **1b** and the SnAP reagent **SnAP 2Me-M** (figure SI V1) after 1, 2 or 3 reaction cycles.

SI VI Dual catalysis photoredox decarboxylative cross-coupling on immobilized DNA-halogenoaromatic conjugates

The protocol for this reaction was adapted from the original report from MacMillan *et al.*[5]

Useful information on this reaction can be found on the MacMillan group website: http://chemLabs.princeton.edu/macmillan/reactions/metallaphotoredox-decarboxylative-arylation-2/

SI VI1 Protocol for the photoredox decarboxylative cross-coupling on immobilized DNA-halogenoaromatic conjugates in single glass vials

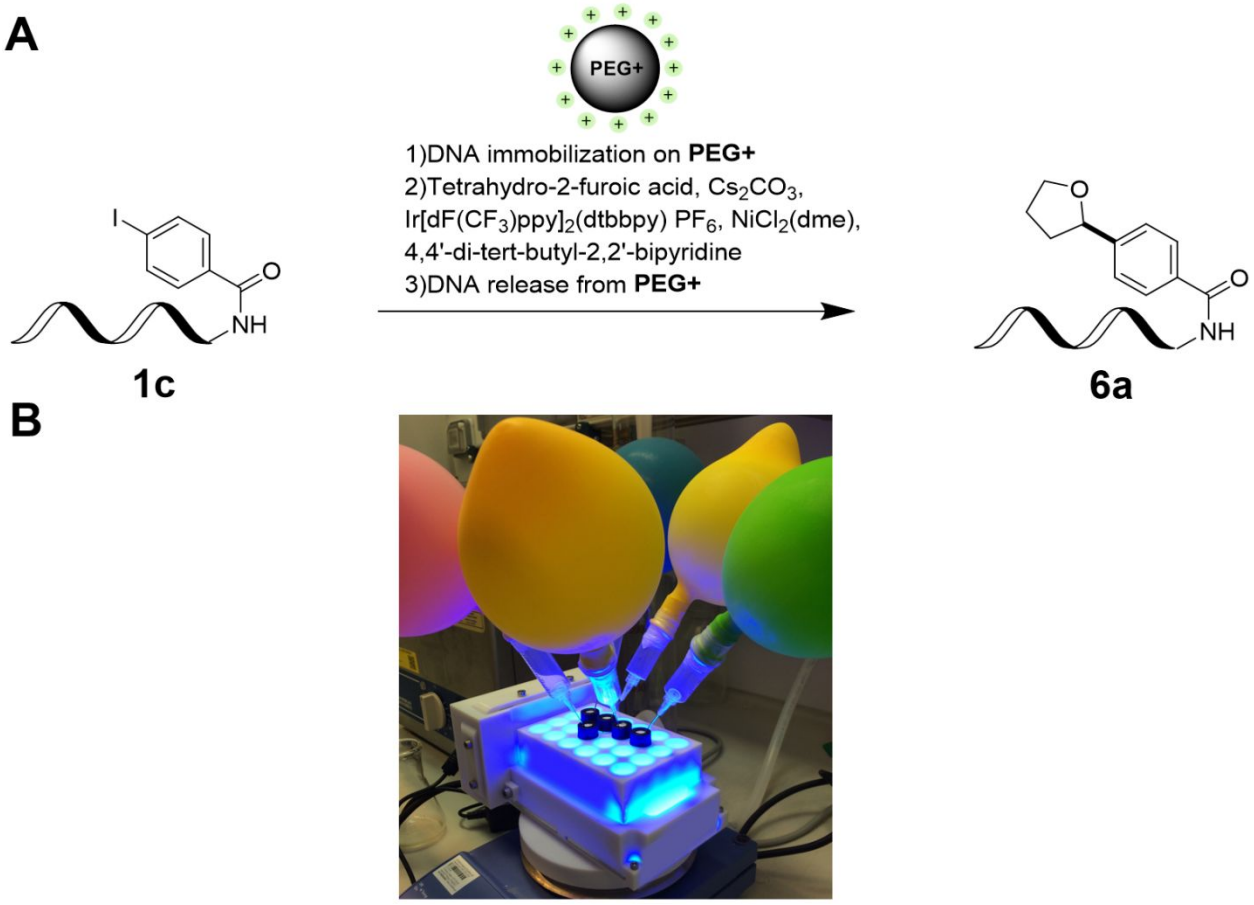


Figure SI VI1: **A** Dual catalysis photoredox decarboxylative cross coupling on **1c** in single glass vials. **B** Experimental setup used in this transformation. The vials are kept under positive pressure of argon and are equipped with a magnetic stir bar for efficient agitation during the reaction.

Solution A of catalysts and carboxylic acid substrate:
Tetrahydrofuran-2-carboxylic acid (Fluka CAS 16874-33-2) (9.57 µL, 0.100 mmol) was dissolved in MeOH (150 uL) and added to a solution of Cs_2CO_3 (16.29 mg, 0.05 mmol) in water (150 µL). The solution was homogenized by vortexing for 30 seconds and evaporated to dryness on a SpeedVac™.
The resulting dry Cesium salt of tetrahydrofuran-2-carboxylic acid was dissolved in DMSO (Volume: 2000 µL) and added to a solution of $NiCl_2$(dme) (Aldrich CAS 29046-78-4) (2.197 mg, 10 µmol), 4,4'-di-tert-butyl-2,2'-bipyridine (Aldrich CAS 72914-19-3) (2.68 mg, 10 µmol) and $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (Aldrich cat number 747793) (2.244 mg, 2 µmol) in DMSO (Volume: 3000 µL) in a 20-mL microwave vial. This solution containing Tetrahydrofuran-2-carboxylic acid

(20 mM), Cs_2CO_3 (10 mM), $NiCl_2$(dme) (2 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (2 mM), $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (0.4 mM) was degassed by argon bubbling for 15min.

DNA immobilization: The resin **PEG+** (1 g suspended in 20 mL with glycerol:water 1:1) (200 µL, 10 mg) was transferred in a 1 mL syringe equipped with a filter. The resin was dried by filtration and washed with 3x1 mL H_2O and then incubated for 15 min with **1c** (6.52 µg, 0.001 µmol) in 200µL of H_2O. The solvent was drained and the resin washed with DMSO 4 times.

Reaction setup: The immobilized **1c** was then slurried in DMSO (Volume: 500 µL) and transferred into a 2mL glass vial containing a magnetic stirrer. 500 µL of the solution **A** containing the catalysts and cesium salt of the carboxylic acid substrate was added to this suspension under argon. The vial was placed in a 3D-printed LED photoreactor (470 nm, 2 W) and irradiated for 60 min (see Figure SI VI1).

Workup

The resin was transferred back into a 1 mL filter syringe, the solvent filtered, and the resin washed 5 times with DMSO. The resin was incubated 15 min at 50°C with 100 µL of H_2O and filtered. Then 100 µL DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X, 5% 4-Me-piperidine) was added to the resin, incubated for 30 min at 50°C and filtered into the same tube. The combined aqueous elution samples were desalted by passage through an Illustra NAP-2 column (GE healthcare) and analyzed by UPLC-TOF LC-MS.

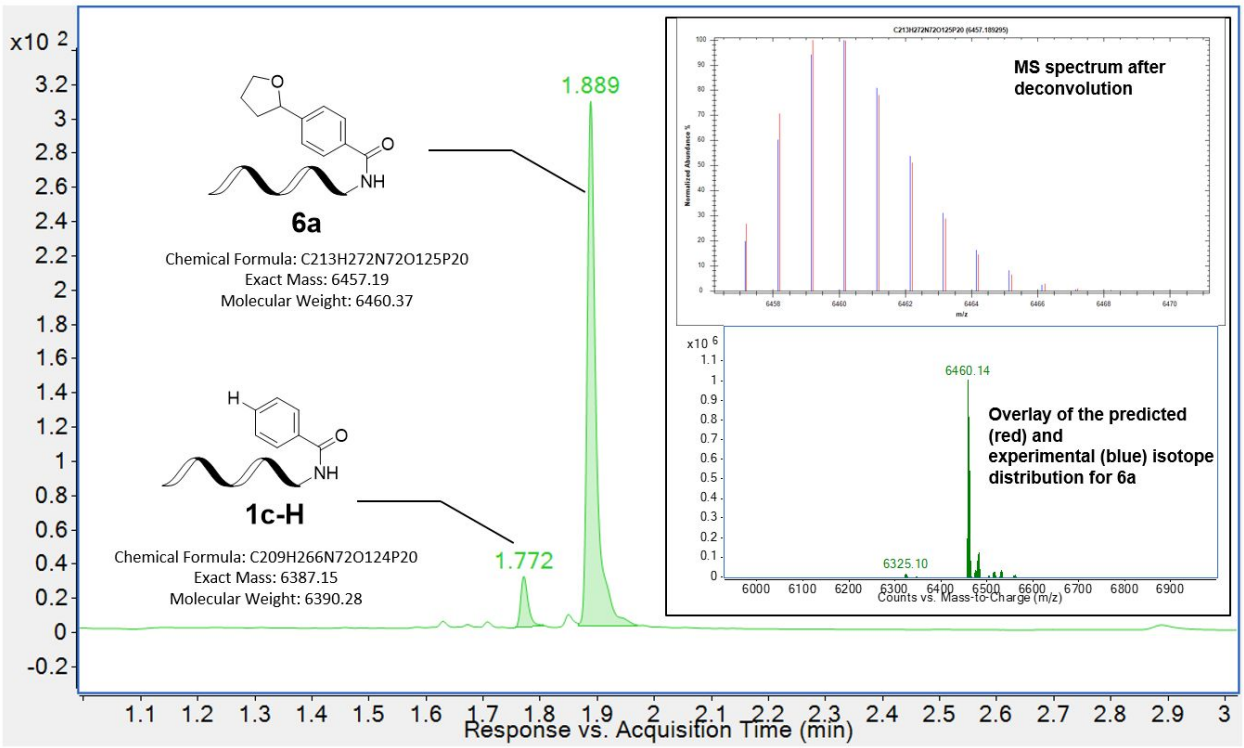


Figure SI VI2: UPLC-TOF analysis of the crude reaction mixture. Reaction conditions: **1c** (1 nmole) immobilized on **PEG+** (10 mg), tetrahydrofuran-2-carboxylic acid (10 mM), Cs_2CO_3 (5 mM),

$NiCl_2$(dme) (1 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (1 mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.2 mM) in DMSO (1 mL), 3D-printed LED photoreactor (470 nm, 2W), 60 min, room temperature.

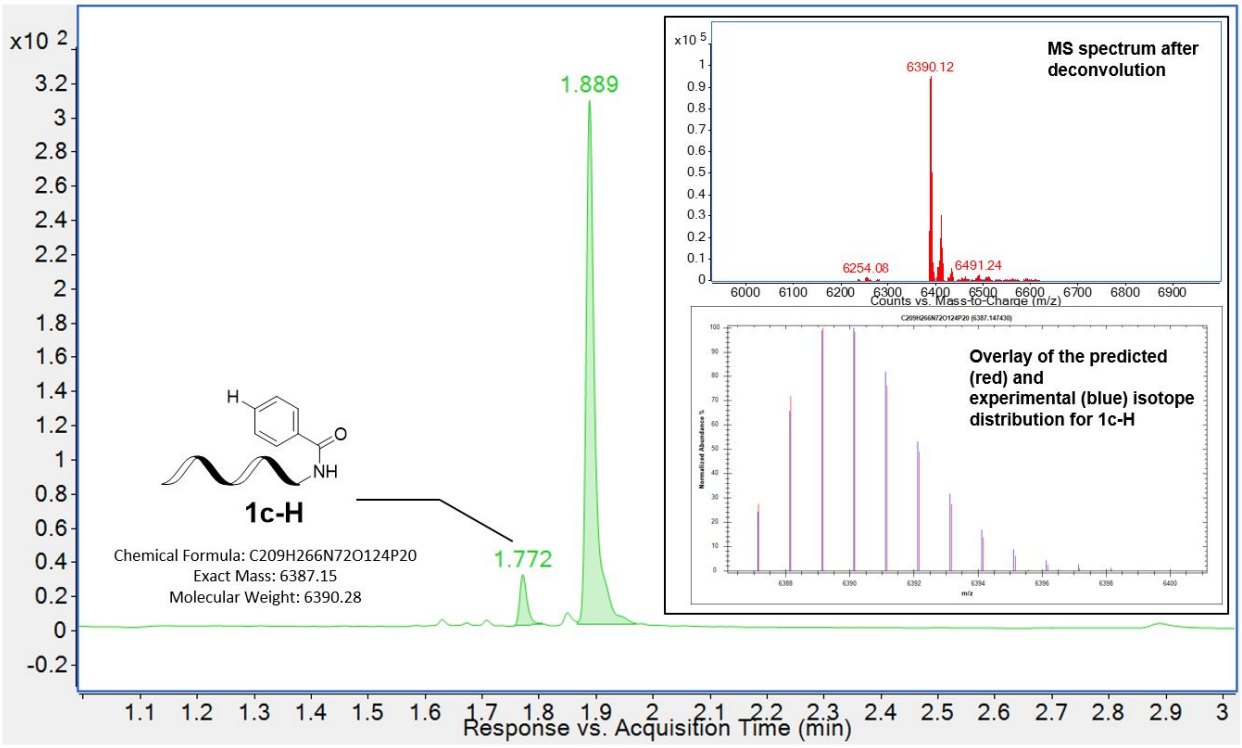
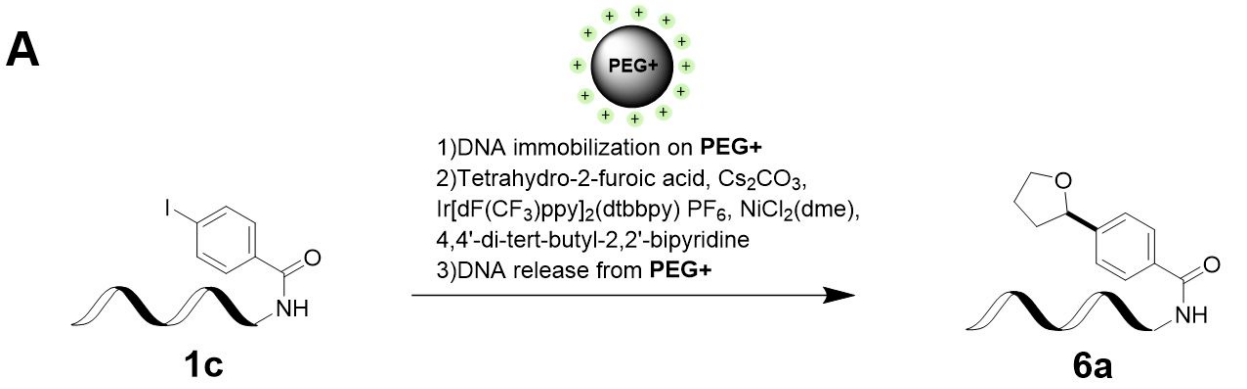


Figure SI VI3: UPLC-TOF characterization of 1c-H.

SI VI 2 Protocol for the photoredox decarboxylative cross coupling on immobilized DNA-halogenoaromatic conjugates in 96-well filter plates

Specific equipment

AcroPrep™ 1 mL 96-Well Filter Plates, Pall Laboratory, magnetic stirrer, LED photoreactor array of 96 LEDs (Abon, 96x833mW, 470nm).



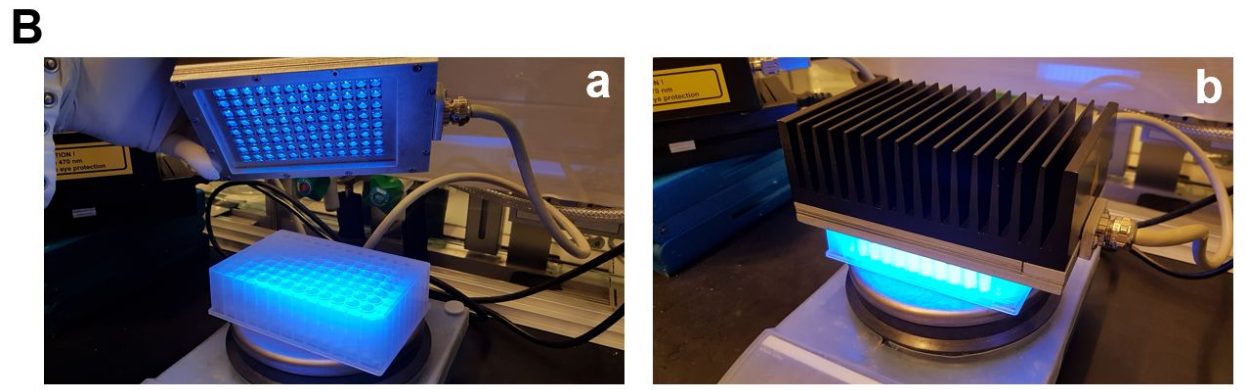


Figure SI VI4: **A** Dual catalysis photoredox decarboxylative cross coupling on **1c** in parallelizable plate format. **B** Experimental setup used in this transformation. **a** A custom array of 96 LEDs was used to control the irradiation with a single LED per well. **b** In this setup irradiation occurs from the top of the plate, and the samples can be magnetically stirred using a regular magnetic stir plate. The entire setup can be kept under inert atmosphere in a glove box in order to ensure maximum reproducibility between experiments.

Protocol

Solution A: Cesium salt of the acid substrate (50 mM) in DMSO

The acid tetrahydrofuran-2-carboxylic acid (9.57 µL, 0.100 mmol) was dissolved in MeOH (150 µL) and added to a solution of Cs_2CO_3 (16.29 mg, 0.05 mmol) in water (150 µL). The solution was homogenized by vortexing for 30 seconds and evaporated to dryness on a SpeedVac™. The resulting cesium salt of tetrahydrofuran-2-carboxylic acid was dissolved in dry DMSO (Volume: 2000 µL).

Solution B: Catalysts $NiCl_2$(dme) (3.33 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (3.33 mM), $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (0.66 mM) in DMSO.

The catalyst solution was prepared by dissolving $NiCl_2$(dme) (2.197 mg, 10 µmol), 4,4'-di-tert-butyl-2,2'-bipyridine (2.68 mg, 10 µmol) and $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (2.244 mg, 2 µmol) in DMSO (Volume: 3000 µL) in a sealed 5 mL microwave tube containing 4Å molecular sieve. The solution was degassed by argon bubbling.

DNA immobilization and resin preparation:

The resin **PEG+** (1 g suspended in 20 mL with glycerol:water 1:1) (100 µL, 5 mg) was transferred in a 1mL 96-well filter plate. The resin was dried by filtration and washed with 3x1 mL H_2O and then incubated for 15 min with **1c** (6.52 µg, 0.001 µmol) in 200 µL of H_2O. The solvent was drained and the resin washed with MeOH 3 times. The solvent was then evaporated to dryness in a speedvac. Two magnetic stir bars were added to each well and the bottom of the plate was heat-sealed with an aluminium foil.

Setup of the reaction:

Under inert atmosphere, 50 µL of the solution **A** containing the cesium salt of tetrahydrofuran-2-carboxylic acid (50 mM) were added to the immobilized DNA substrate **1c** on **PEG+** in each well, followed by 75 µL solution **B** in a glove box under argon. The plate was irradiated under argon for 5 min at room temperature by placing the photoreactor inversed on top of the plate.(Figure SI VI4 B(b))

Workup

The suspension was filtered by centrifugation and the resin was washed 5 times with DMSO. The resin was incubated 15 min at 50°C with 100 µL H2O and filtered into a 500 µL plate. Then 150 µL DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X, 5% 4-Me-piperidine) were added to the resin, incubated for 30 min at 50°C and filtered into the same tube.

The samples where purified by NAP2 and analyzed by TOF LC-MS.

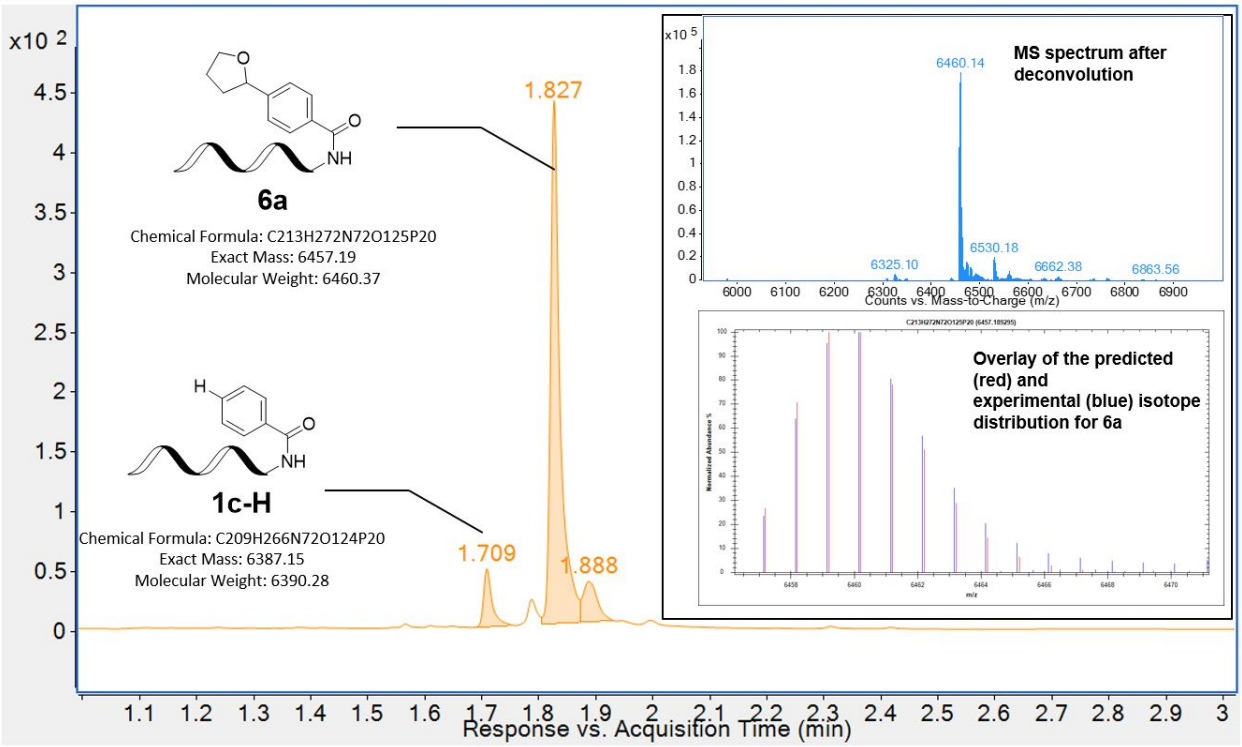


Figure SI VI5: UPLC-TOF analysis of the crude reaction mixture between 1c and tetrahydrofuran-2-carboxylic acid in a plate format. Reaction conditions: **1c** (1 nmol) immobilized on **PEG+** (5 mg), tetrahydrofuran-2-carboxylic acid (20 mM), Cs_2CO_3 (10 mM), $NiCl_2$(dme) (2 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (2 mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.4 mM) in DMSO (125 µL), custom 96-LED array photoreactor (470 nm, 96x833 mW), 5 min, room temperature.

SI VI3 Scope of the photoredox decarboxylative coupling using commercially available carboxylic acids and two different photoredox catalysts

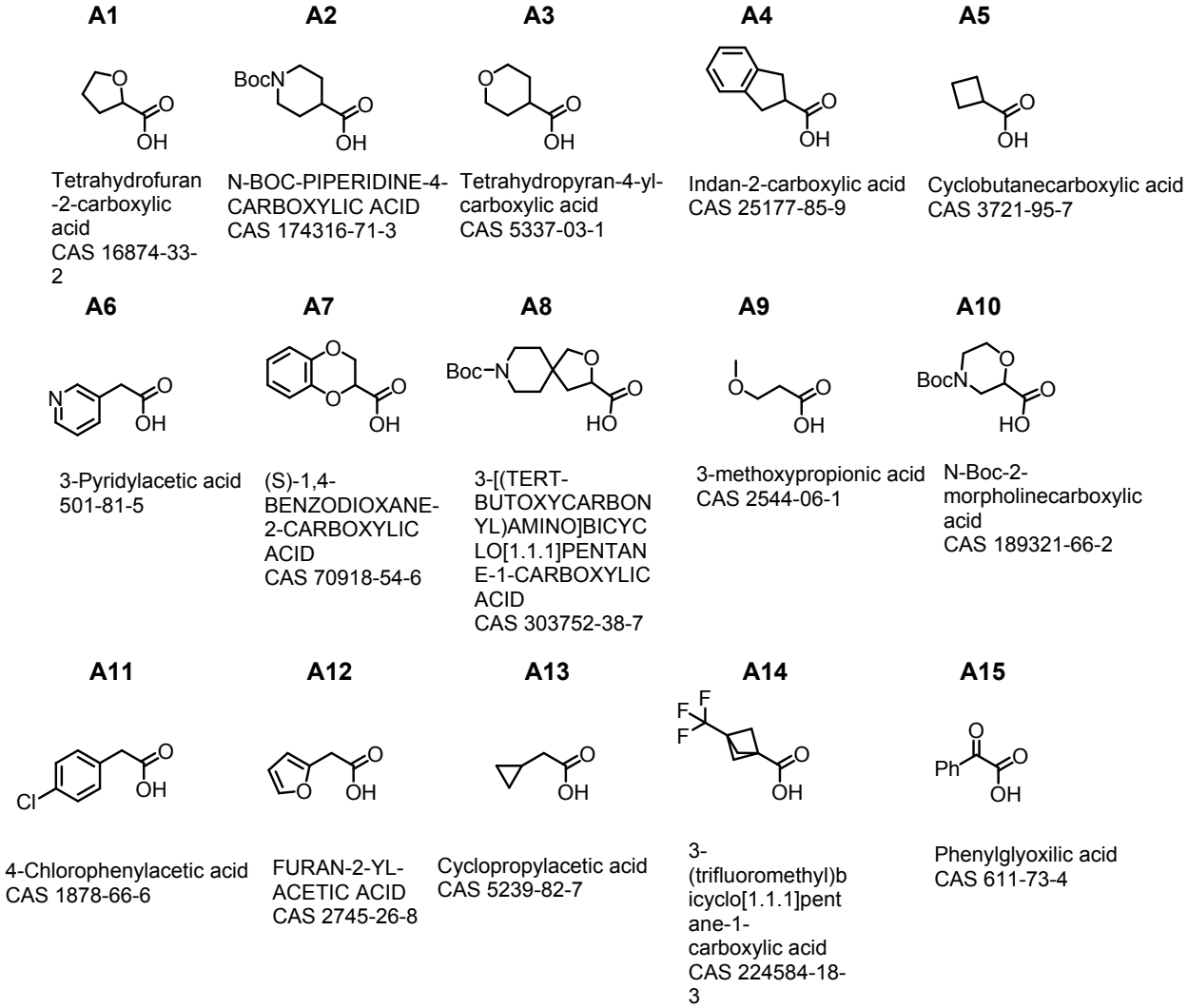


Figure SI VI6: Set of carboxylic acid that were used in this experiment.

Each of these acids was used as substrate for a reaction with **1c** using either the photoredox catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (Aldrich 901409).

Protocol

Solution A: cesium salt of the acid substrates (50 mM) in DMSO.

Each acid **A1-15** (0.1 mmol) was dissolved in MeOH (150 µL) and added to a solution of Cs_2CO_3 (16.29 mg, 0.05 mmol) in water (150 µL). The solution was homogenized by vortexing for 30 seconds and evaporated to dryness on a SpeedVac™. The resulting Cs salt of **A1-15** was dissolved in dry DMSO (Volume: 2000 µL).

Solution B: Catalysts $NiCl_2$(dme) (3.33 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (3.33 mM), $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (0.66 mM) in DMSO.

The catalyst solution was prepared by dissolving $NiCl_2$(dme) (2.197 mg, 10 µmol), 4,4'-di-tert-butyl-2,2'-bipyridine (2.68 mg, 10 µmol) and $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (2.244 mg, 2 µmol) in DMSO (Volume: 3000 µL) in a 5 mL microwave tube containing 4Å molecular sieve. The solution was degassed by argon bubbling.

Solution C: Catalysts $NiCl_2$(dme) (3.33 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (3.33 mM), $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (0.66 mM) in DMSO.

The catalyst solution was prepared by dissolving $NiCl_2$(dme) (2.197 mg, 10 µmol), 4,4'-di-tert-butyl-2,2'-bipyridine (2.68 mg, 10 µmol) and $Ir[dF(Me)ppy]_2(dtbbpy)\ PF_6$ (2.028 mg, 2 µmol) in DMSO (Volume: 3000 µL) in a 5 mL microwave tube containing 4Å molecular sieve. The solution was degassed by argon bubbling.

DNA immobilization and resin preparation:

The resin **PEG+** (1g suspended in 20mL with glycerol:water 1:1) (100 µL, 5 mg) was transferred in a 1mL 96-well filter plate. The resin was dried by filtration and washed with 3x1 mL H_2O and then incubated for 15min with **1c** (6.52 µg, 0.001 µmol) in 200 µL of H_2O. The solvent was drained and the resin washed with MeOH 3 times. The solvent was then evaporated to dryness in a speedvac™. Two magnetic stirrer were added to each well and the bottom of the plate was heat-sealed with an aluminium foil.

Setup of the reaction:

Under inert atmosphere, 50 µL of the solution **A** containing the cesium salt of the acid building blocks **A1-15** (Figure SI VI6) (50 mM) were added to the immobilized DNA substrate **1c** on **PEG+** in each well, followed by 75 µL solution **B** or solution **C** in a glove box under argon. The plate was irradiated under argon for 5 min at room temperature by placing the photoreactor inversed on top of the plate (Figure SI VI4).

Workup

The suspension was filtered by centrifugation and the resin was washed 5 times with DMSO. The resin was incubated 15 min at 50°C with 100 µL H_2O and filtered into a 500 µL collection plate. Then 150 µL DNA elute solution (1.5M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X, 5%

4-Me-piperidine) were added to the resin, incubated for 30 min at 50°C and filtered into the same plate. The crude mixture was purified by ethanol precipitation.

Results

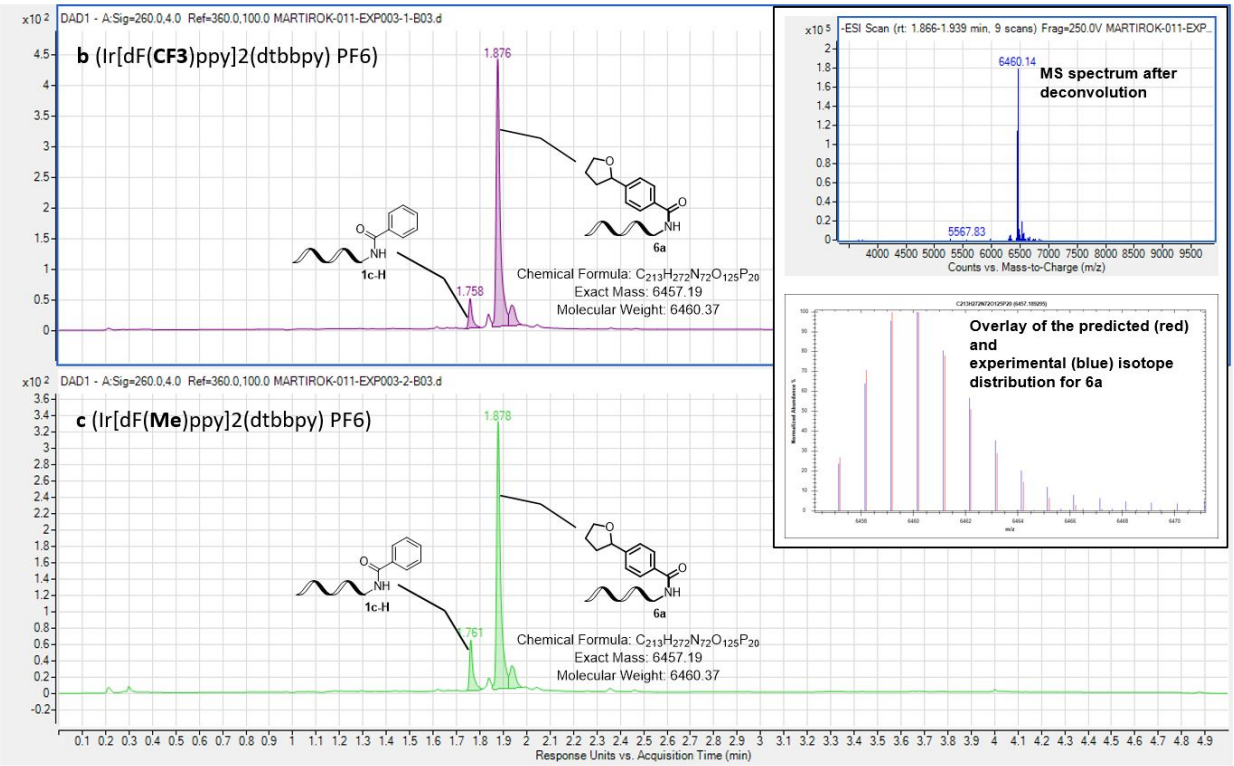


Figure SI VI7: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6a**.

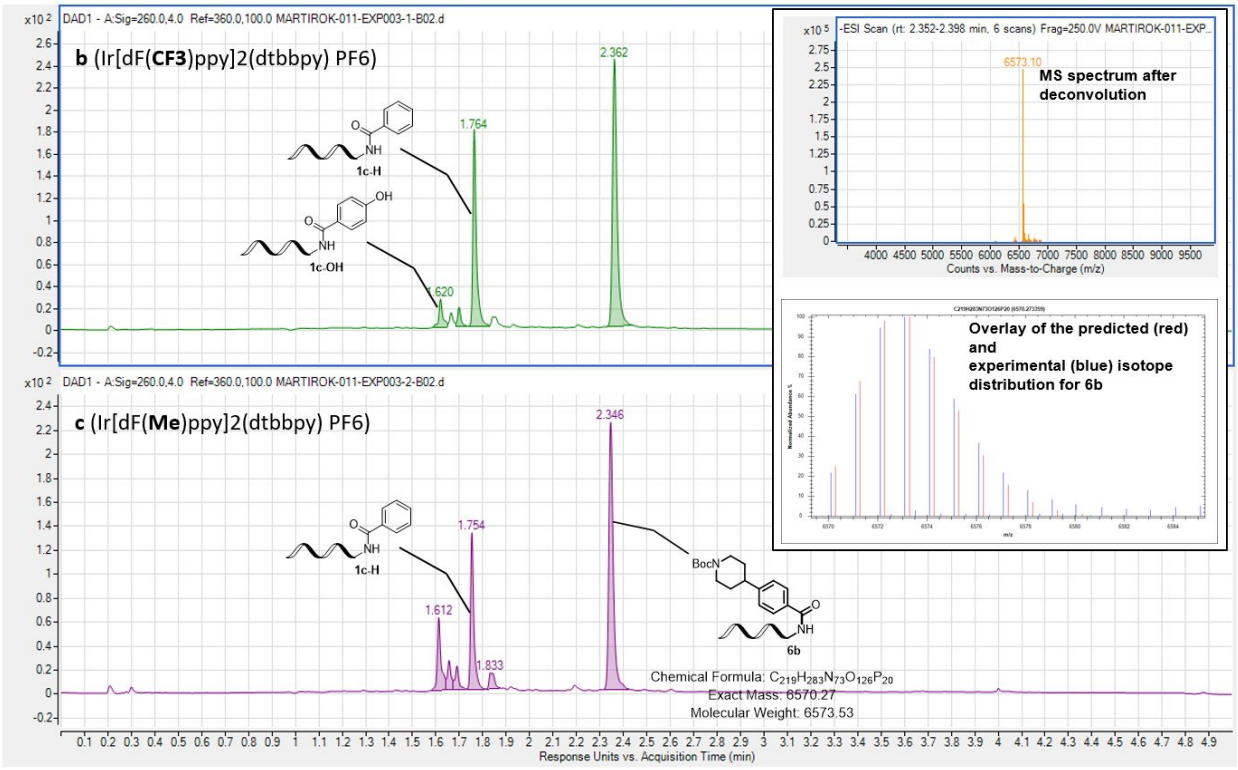


Figure SI VI8: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A2** N-Boc-piperidine-4-carboxylic acid (CAS 174316-71-3) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6b**.

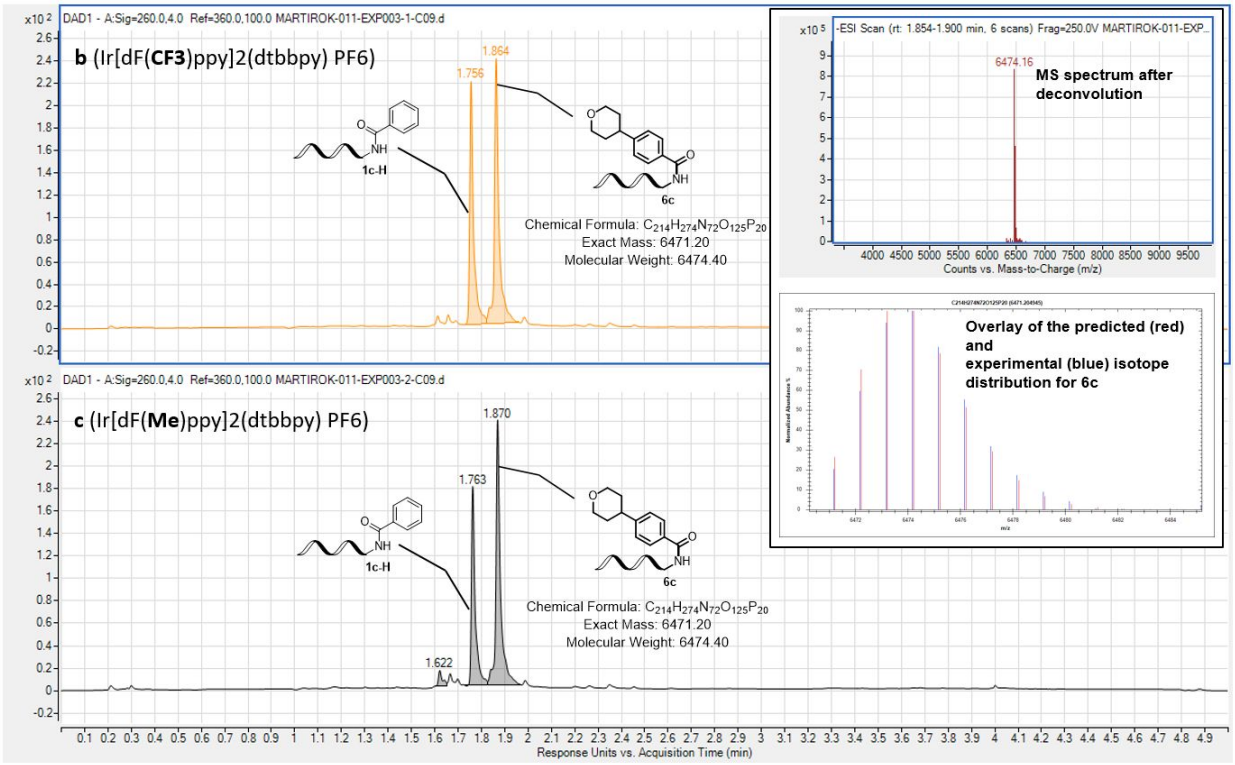


Figure SI VI9: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A3** tetrahydropyran-4-yl-carboxylic acid (CAS 5337-03-1) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6c**.

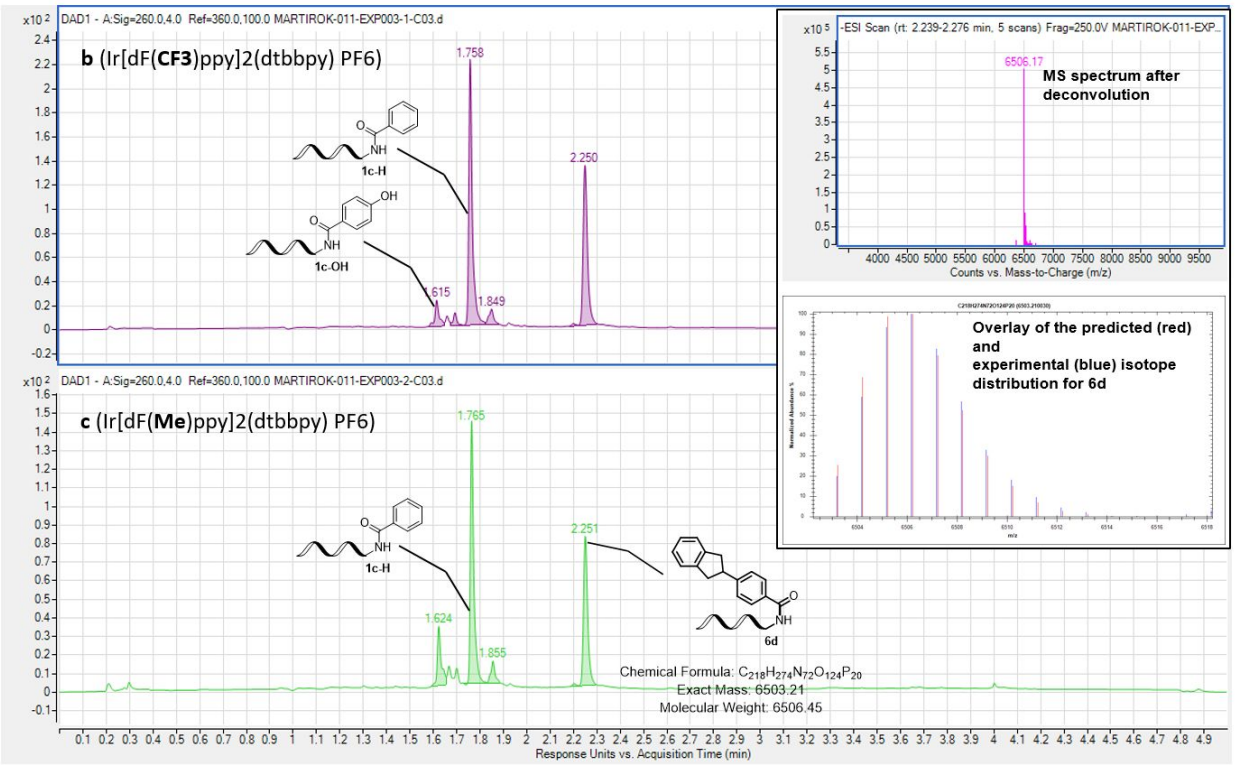


Figure SI VI10: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A4** indan-2-carboxylic acid (CAS 25177-85-9) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6d**.

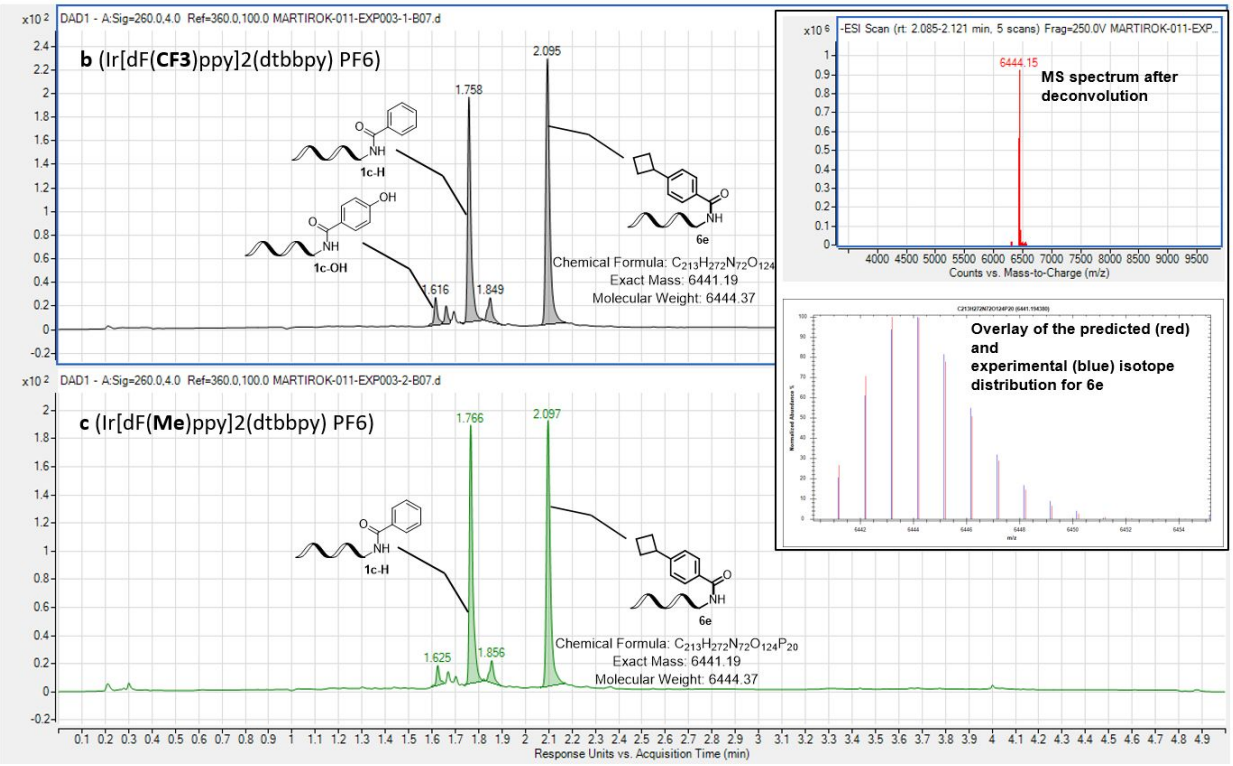


Figure SI VI11: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A5** cyclobutanecarboxylic acid (CAS 3721-95-7) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6e**.

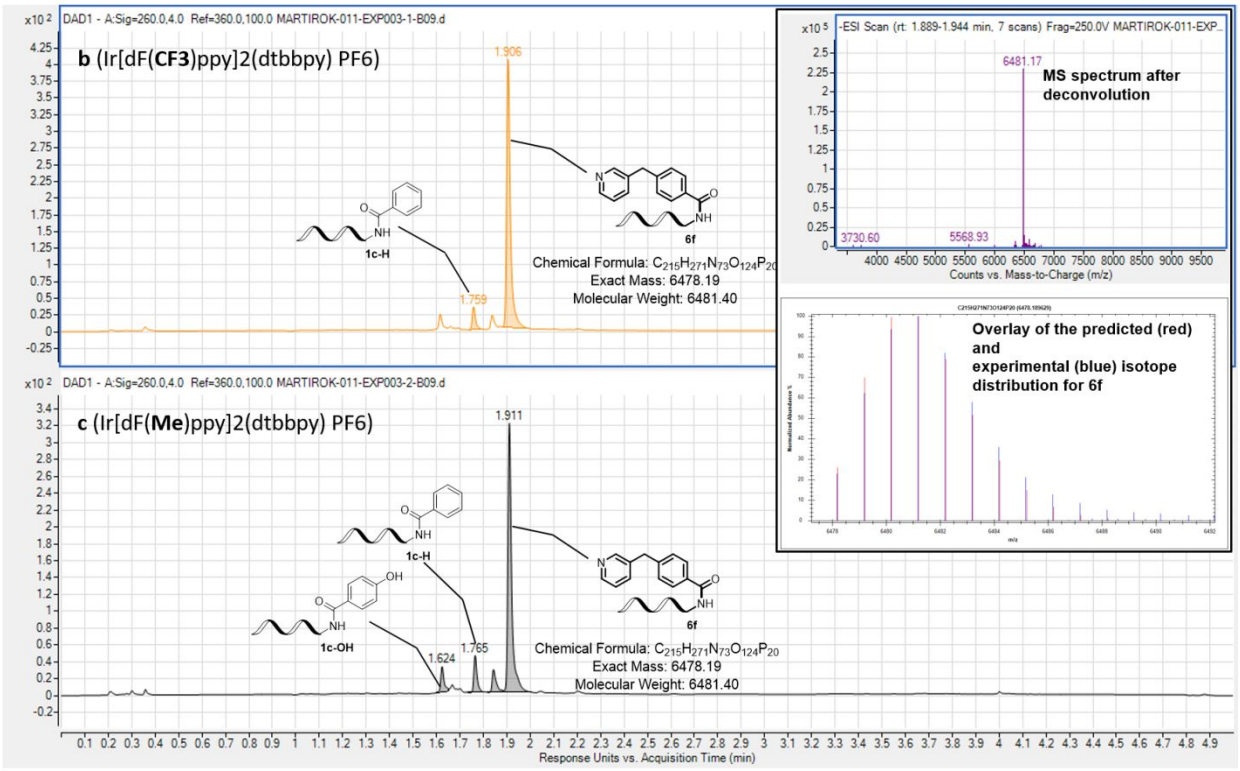


Figure SI VI12: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A6** 3-pyridylacetic acid (CAS 501-81-5) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6f**.

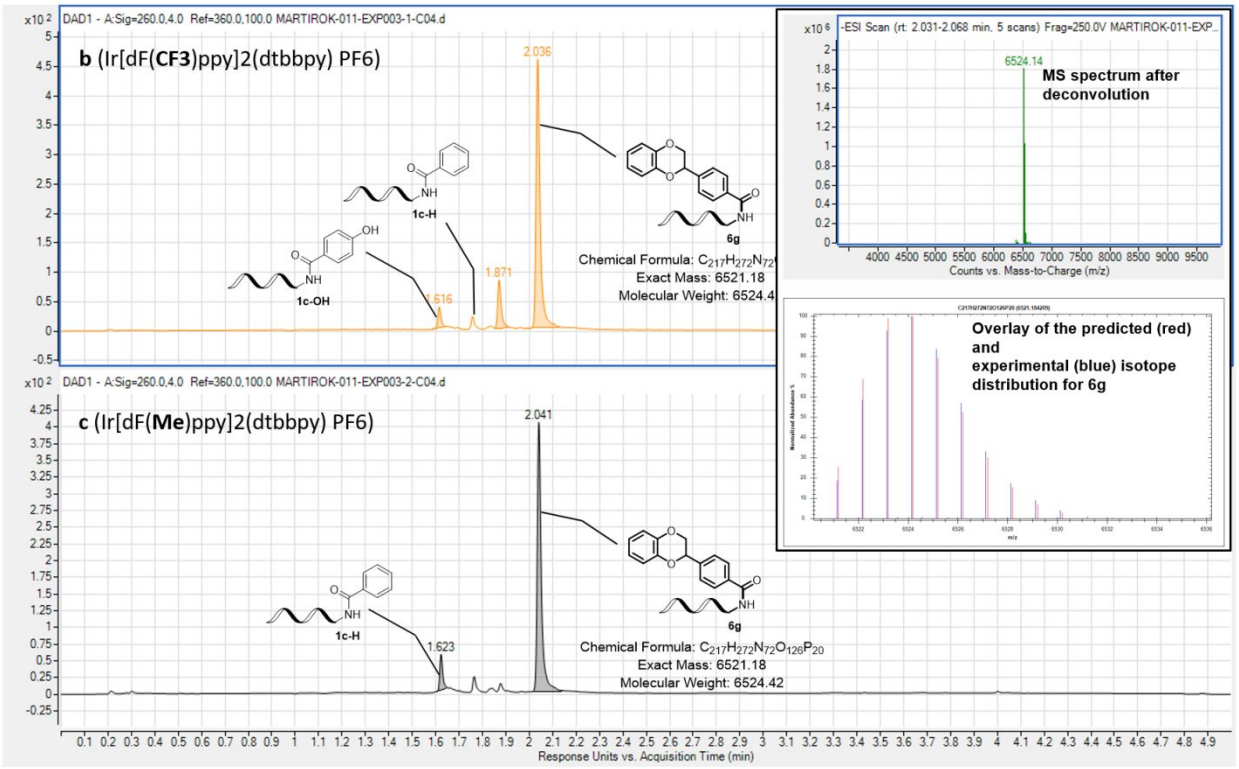


Figure SI VI13: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A7** (S)-1,4-benzodioxane-2-carboxylic acid (CAS 70918-54-6) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6g**.

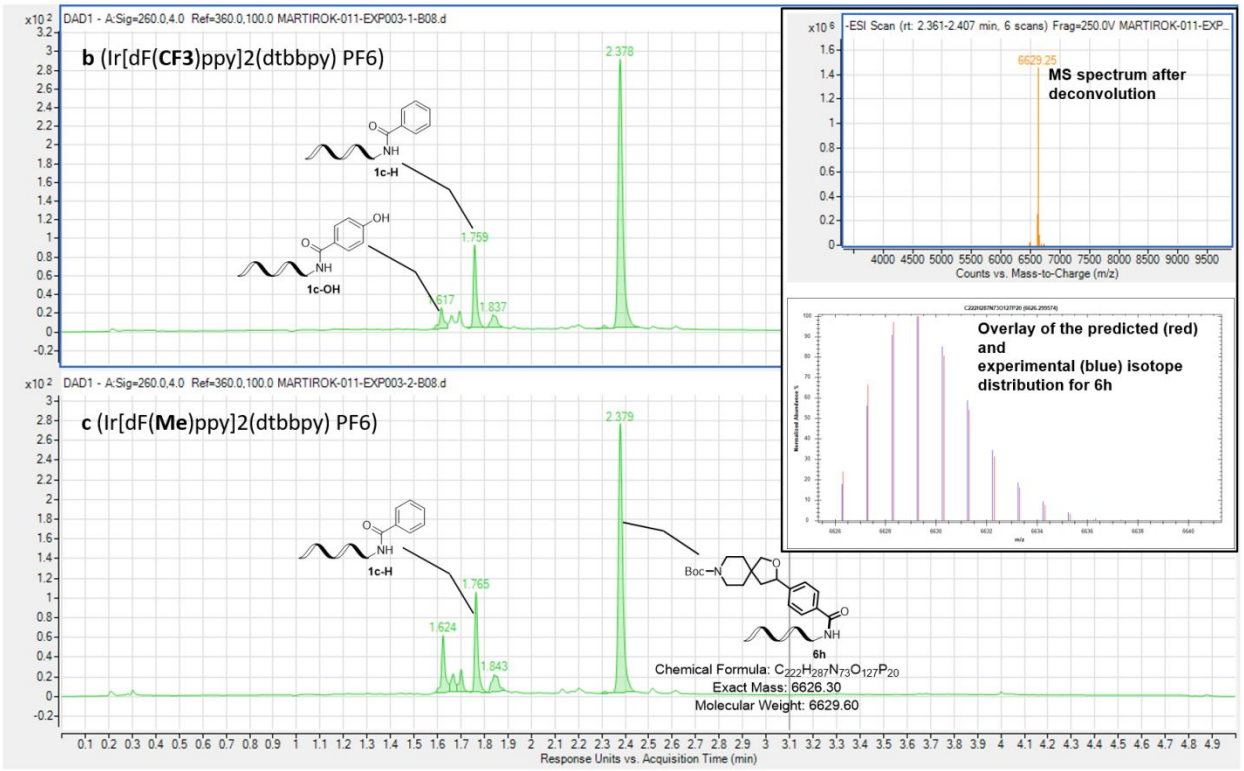


Figure SI VI14: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A8** 3-[(tert-butoxycarbonyl)amino]bicyclo[1.1.1]pentane-1-carboxylic acid (CAS 303752-38-7) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6h**.

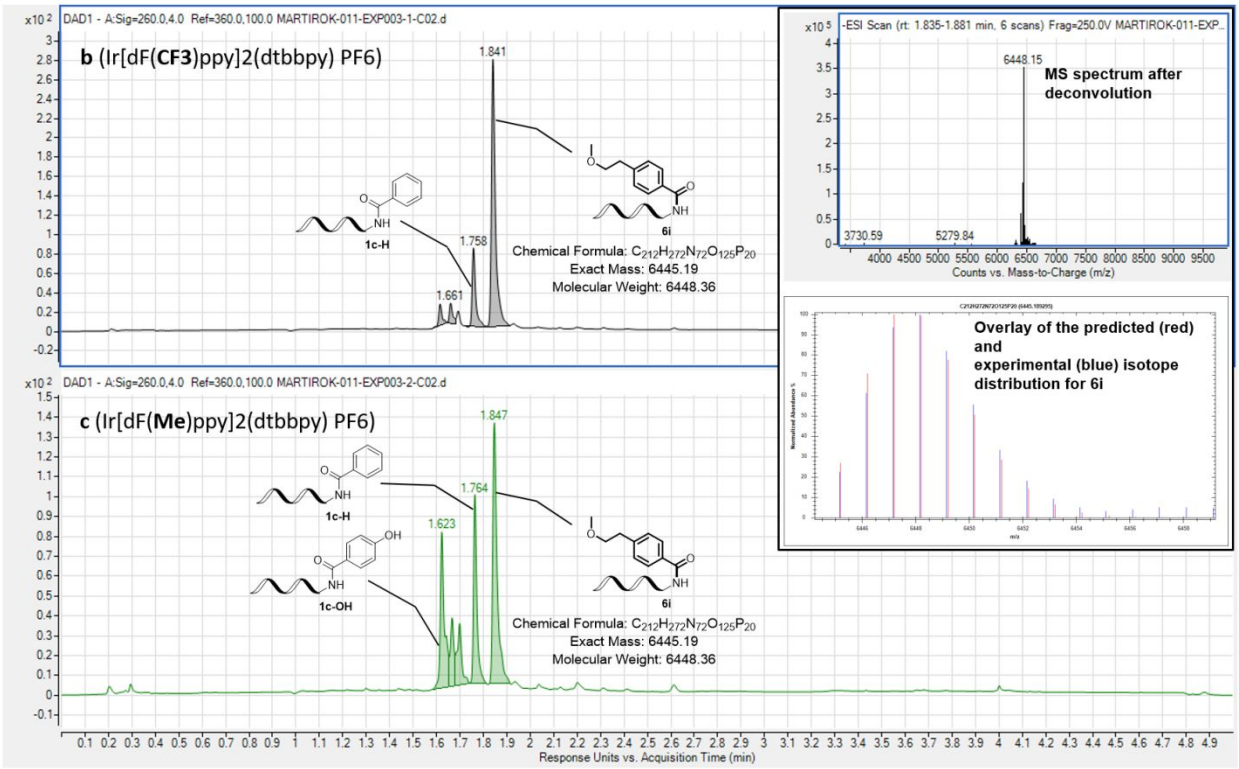


Figure SI VI15: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A9** 3-methoxypropionic acid (CAS 2544-06-1) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6i**.

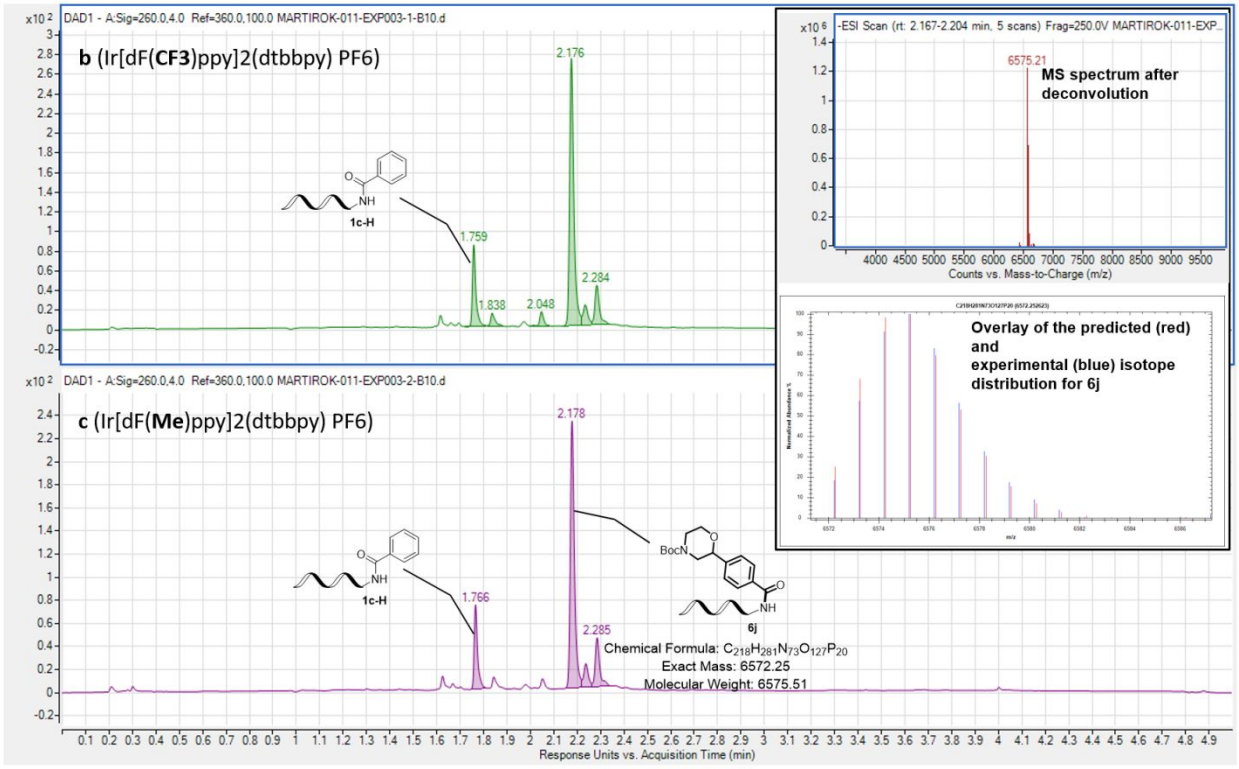


Figure SI VI16: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A10** *N*-Boc-2-morpholinecarboxylic acid (CAS 189321-66-2) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6j**.

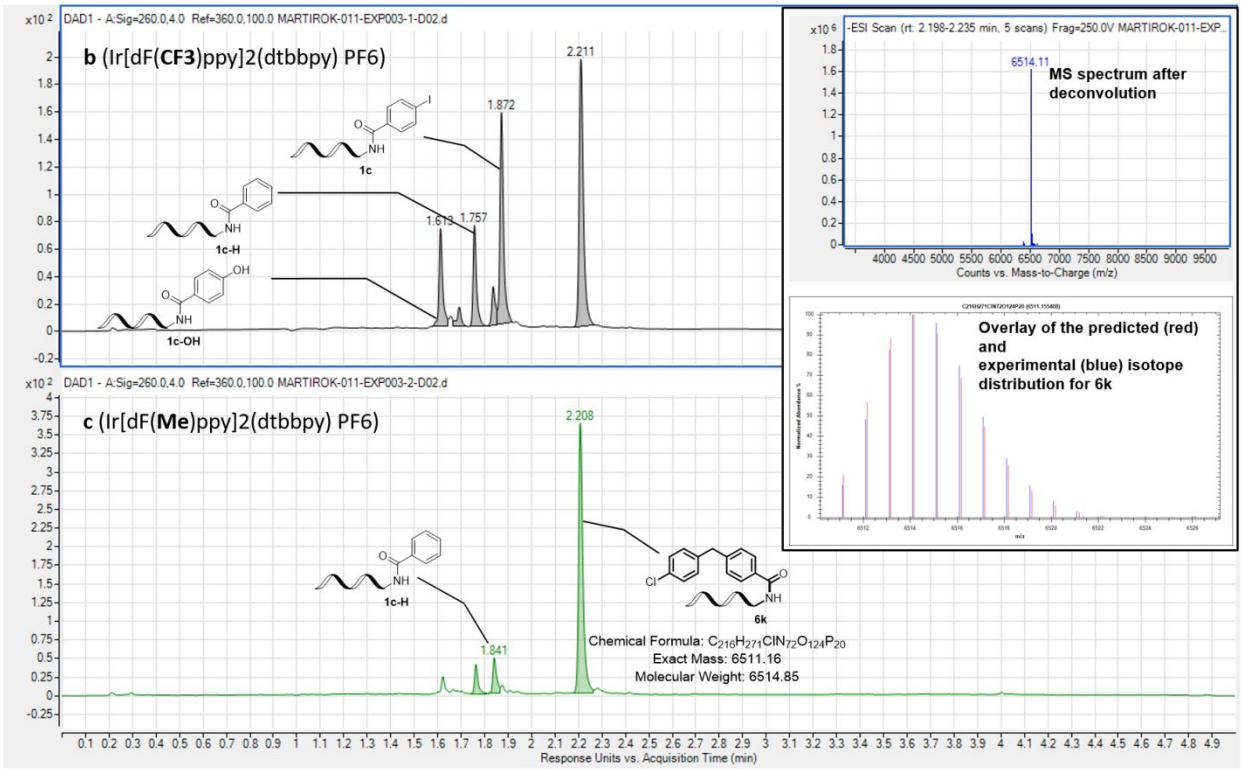


Figure SI VI17: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A11** 4-chlorophenylacetic acid (CAS 1878-66-6) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6k**.

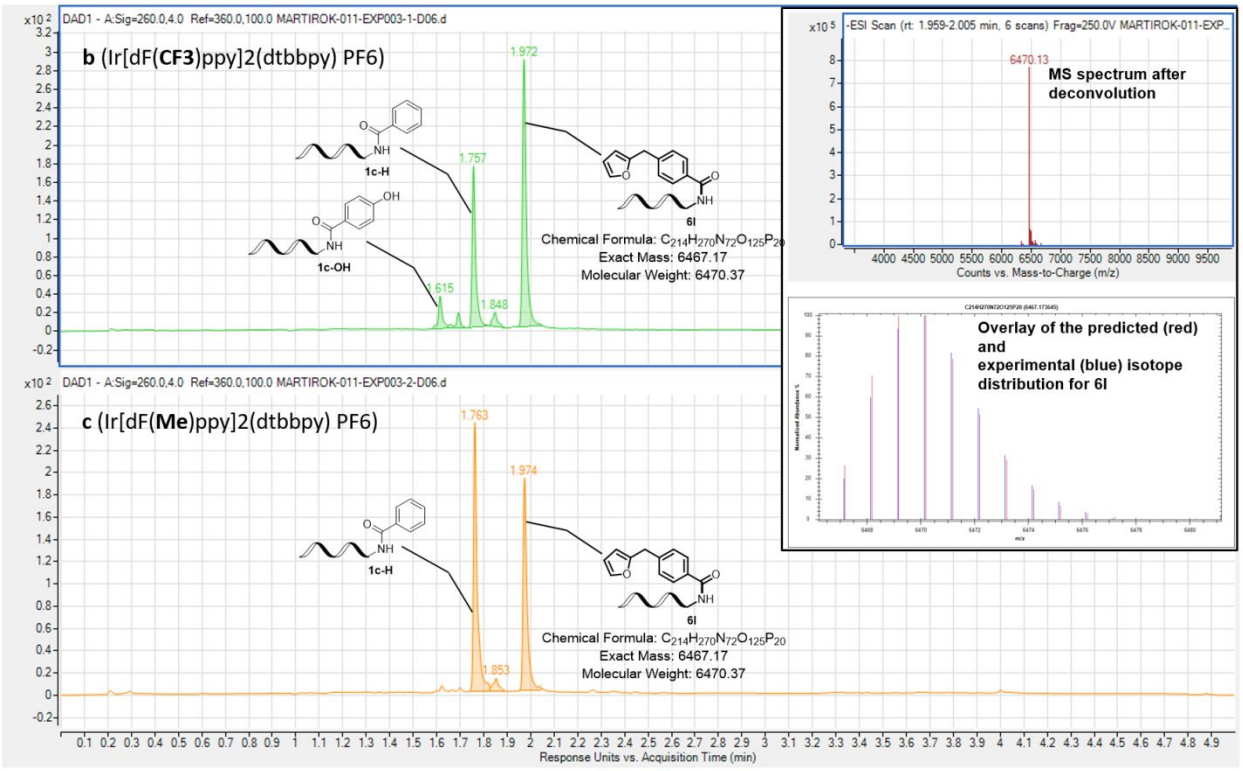


Figure SI VI18: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A12** furan-2-yl-acetic acid (CAS 2745-26-8) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6l**.

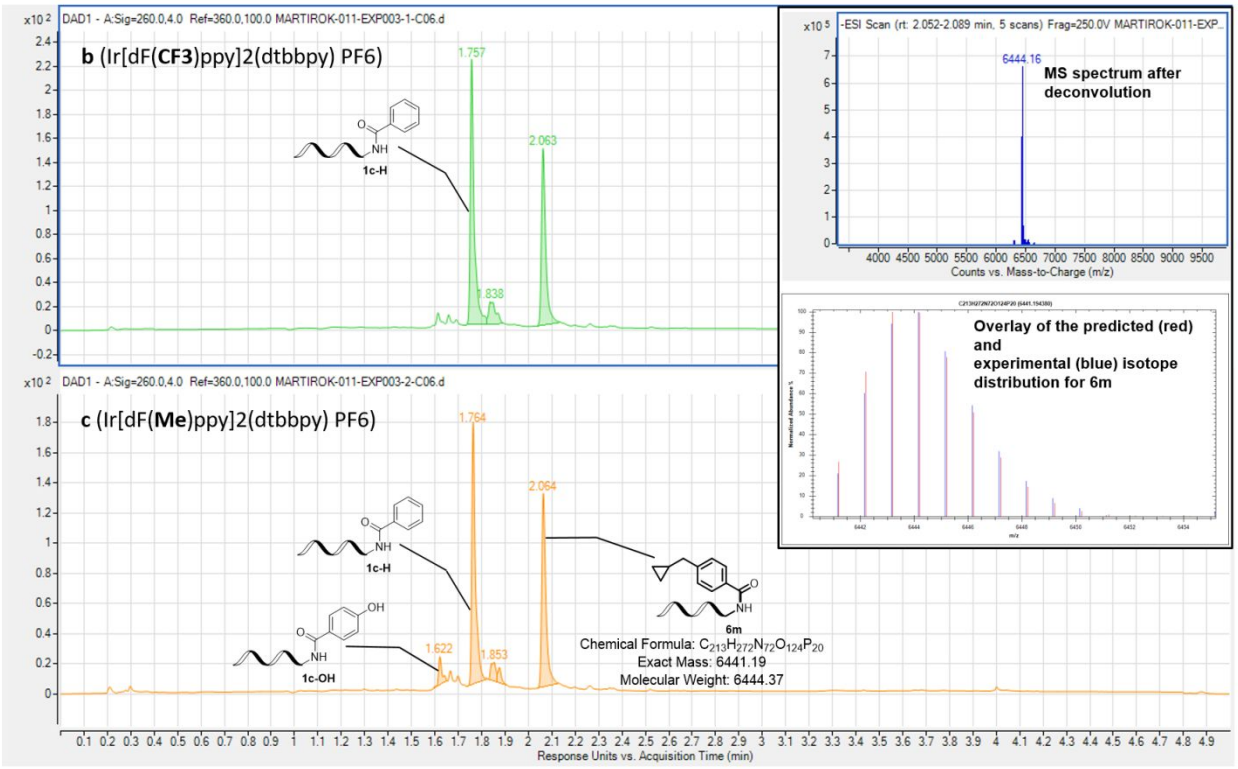


Figure SI VI19: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A13** cyclopropylacetic acid (CAS 5239-82-7) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6m**.

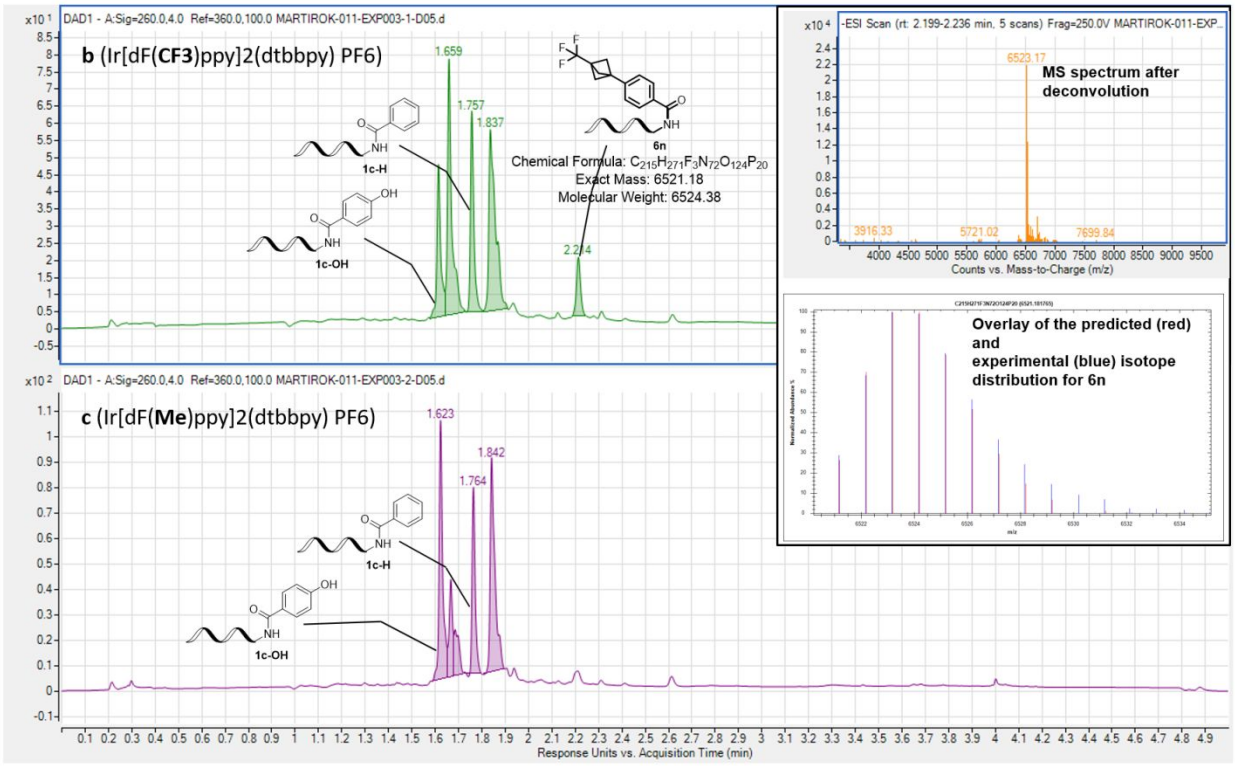


Figure SI VI20: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A14** 3-(trifluoromethyl)bicyclo[1.1.1]pentane-1-carboxylic acid (CAS 224584-18-3) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6n**.

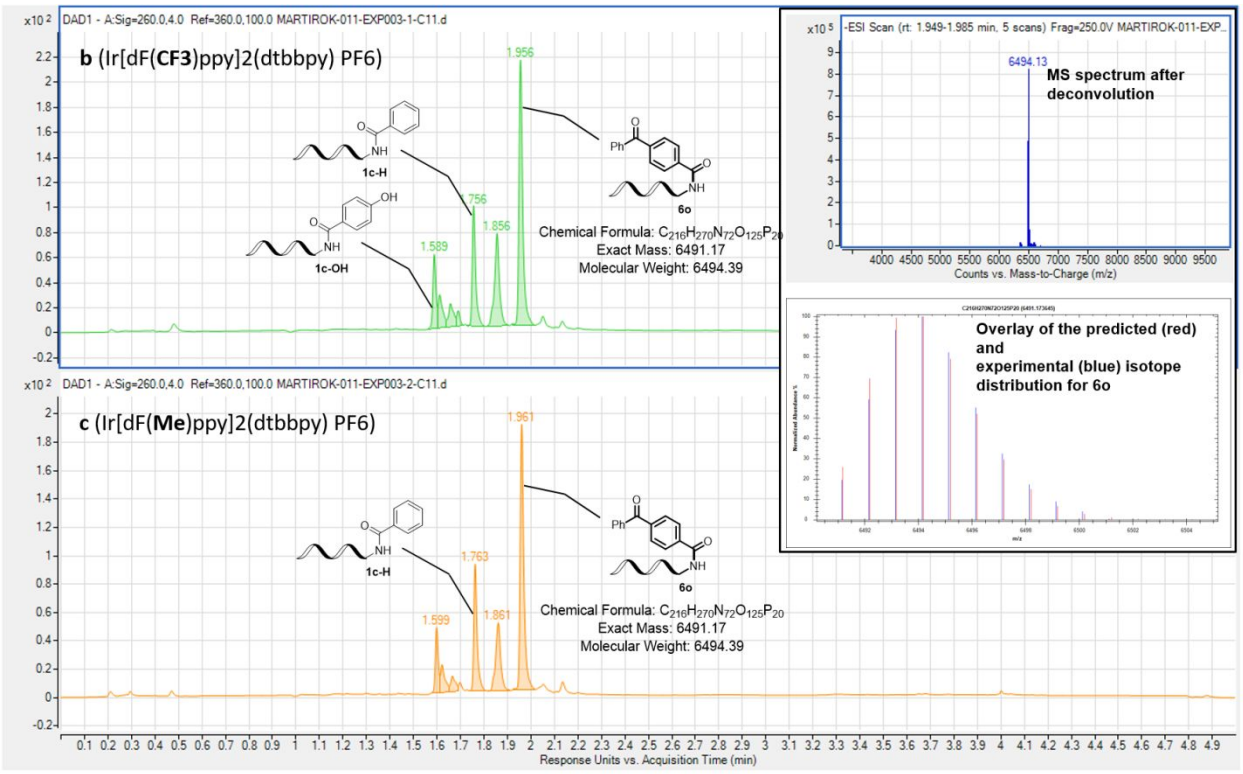


Figure SI VI21: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A15** phenylglyoxilic acid (CAS 611-73-4) using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6o**.

SI VI 4 Scope of the photoredox decarboxylative coupling using a set of DNA-conjugated halogenoaromatic substrates (1c-n) and two different photoredox catalysts

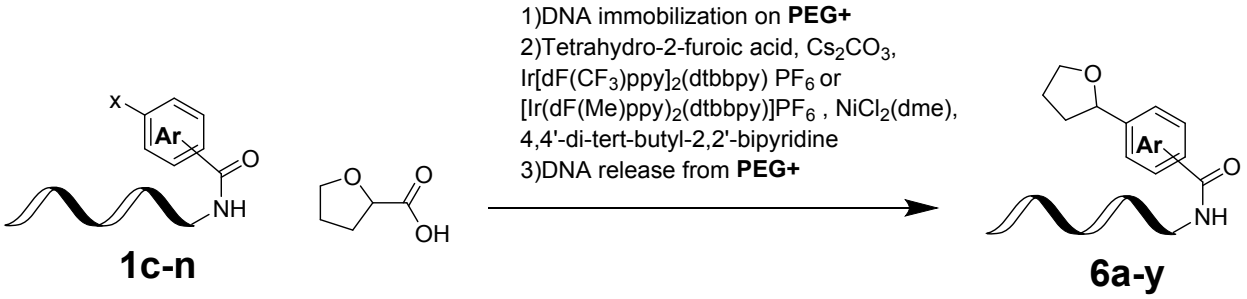


Figure SI VI22: Scope of the photoredox decarboxylative coupling using a set of DNA-conjugated halogenoaromatic substrates (1c-n) and two different photoredox catalysts

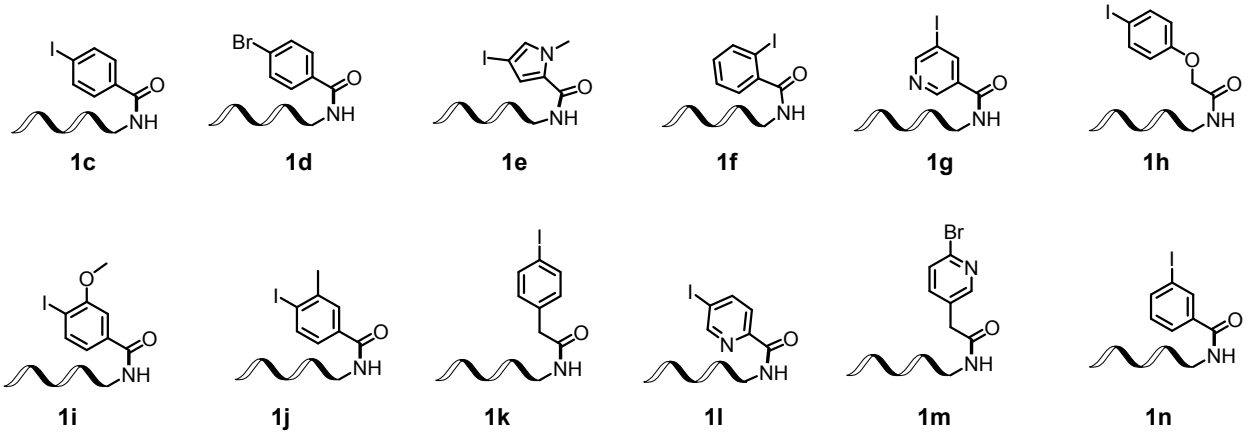


Figure SI VI23: Set of carboxylic acid DNA-conjugated halogenoaromatic substrates (1c-n) that were used in this experiment.

Each of these DNA-conjugated halogenoaromatic substrates (**1c-n**) was used as substrate for a reaction with tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ and under the same protocol as used for the scope determination for carboxylic acids.

Results

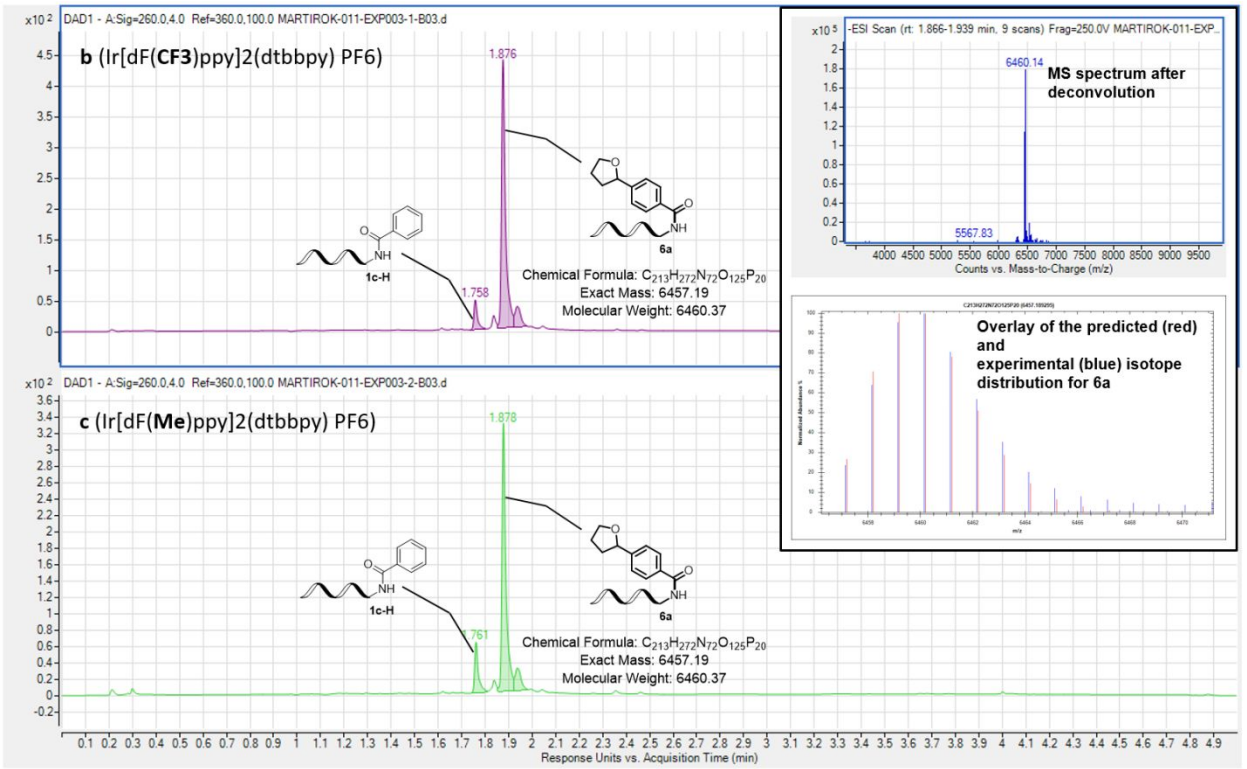


Figure SI VI24: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1c** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox

catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (b) or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (c) and characterization of the product **6a**.

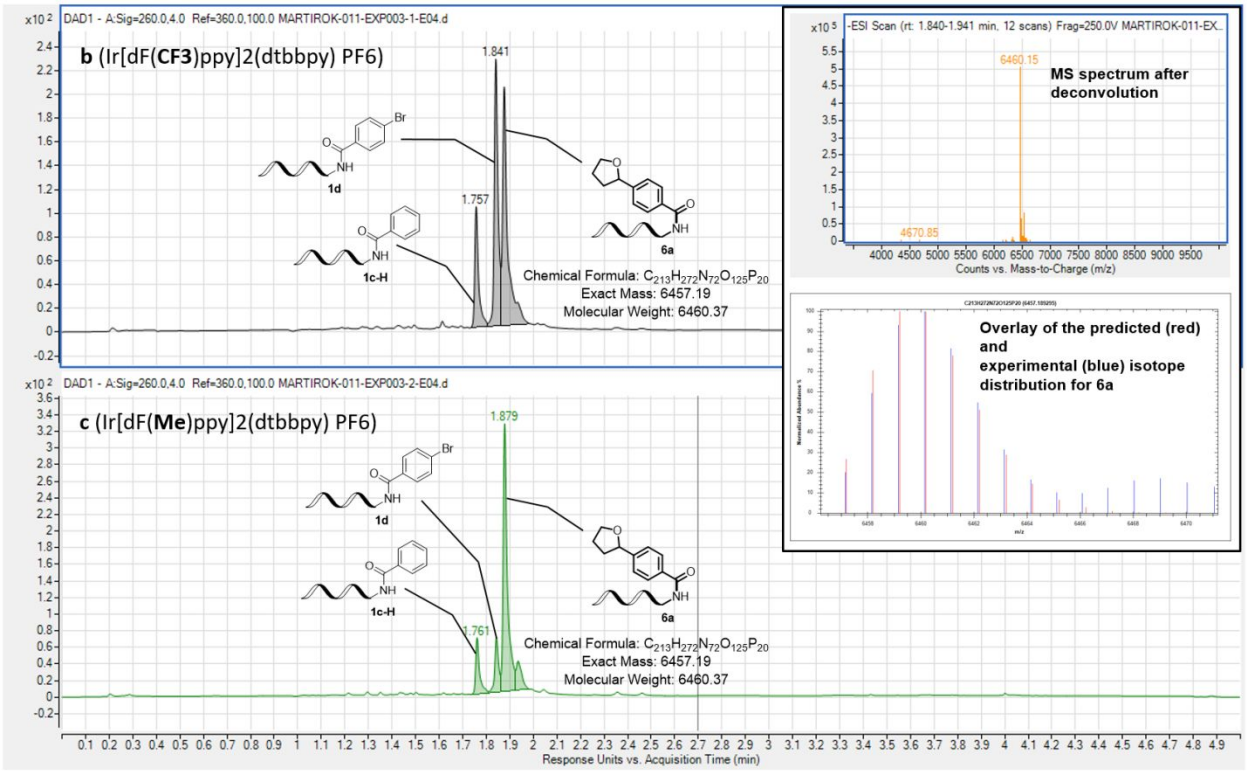


Figure SI VI25: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1d** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (b) or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (c) and characterization of the product **6a**.

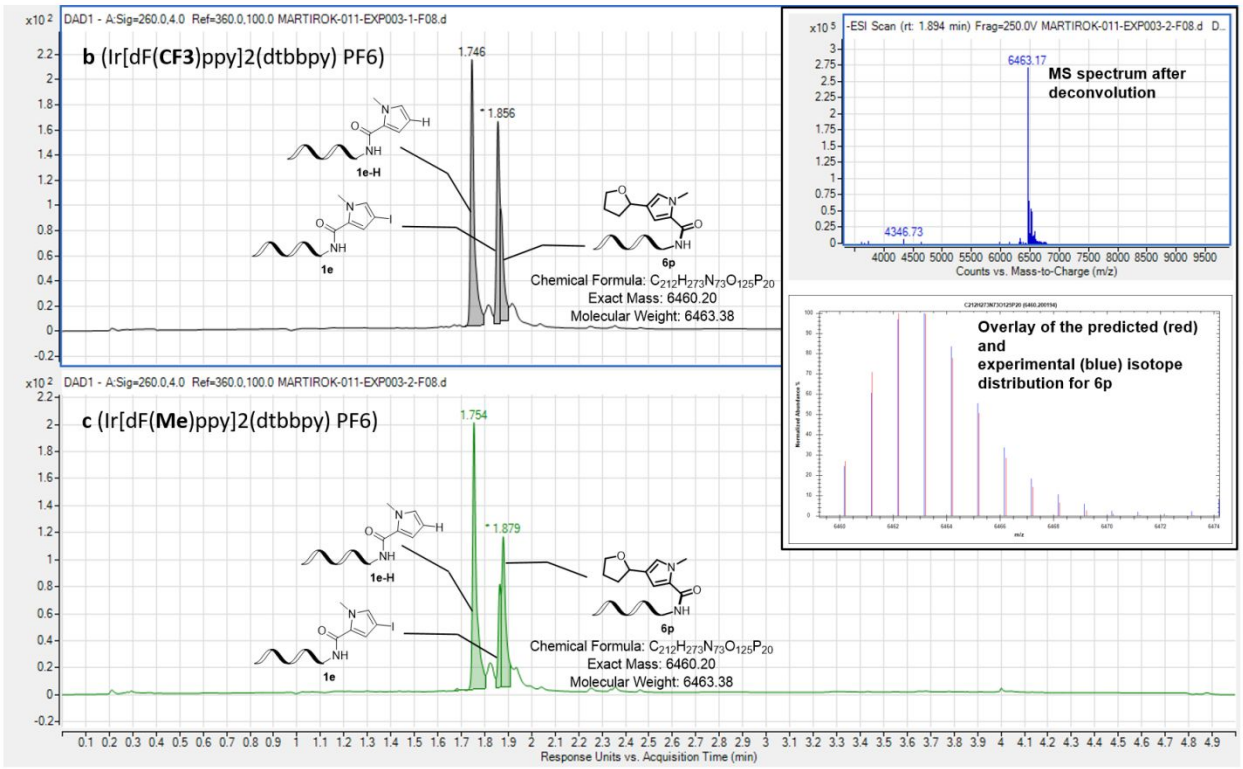


Figure SI VI26: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1e** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6p**.

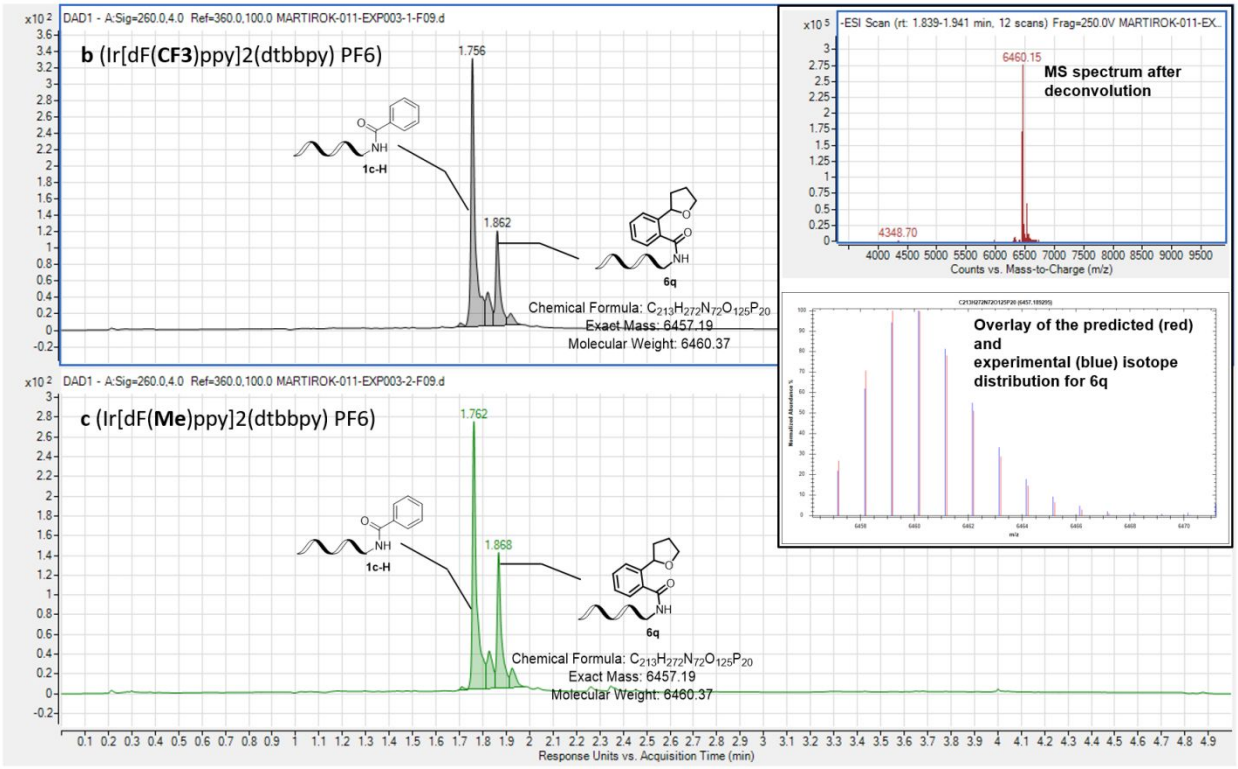


Figure SI VI27: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1f** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6q**.

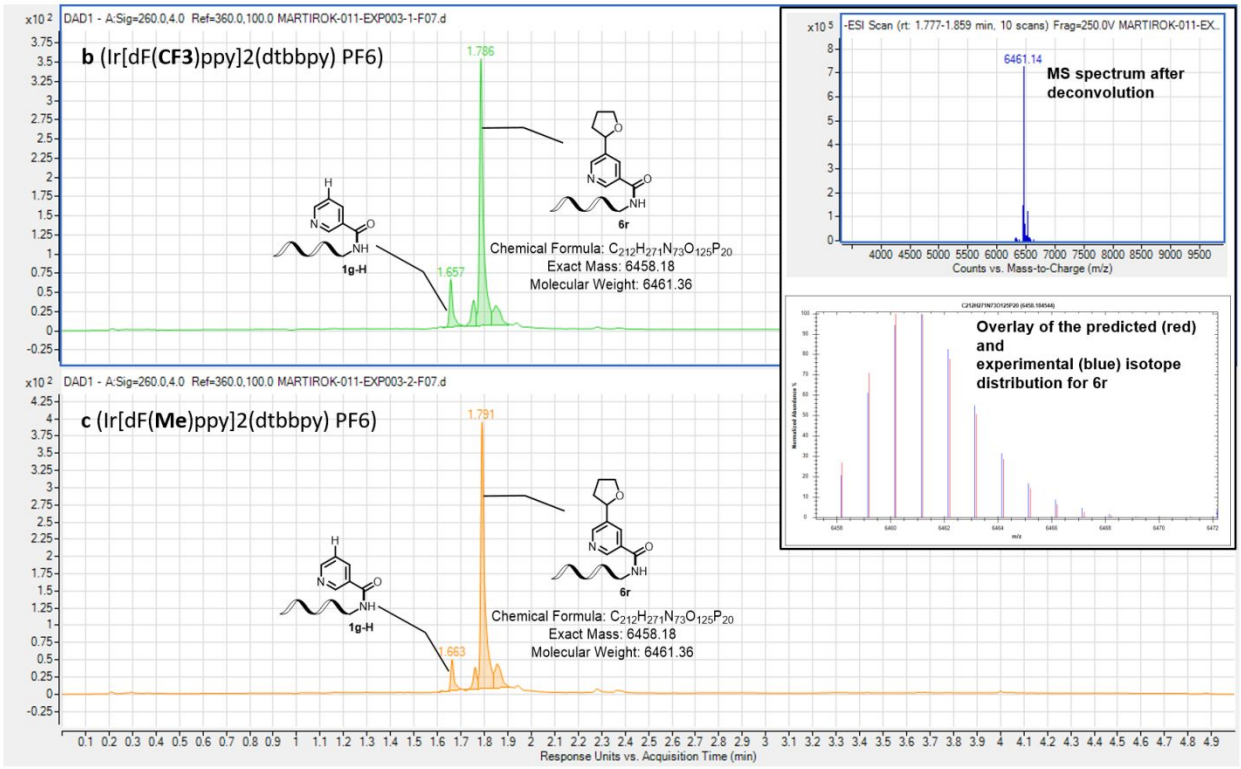


Figure SI VI28: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1g** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (b) or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (c) and characterization of the product **6r**.

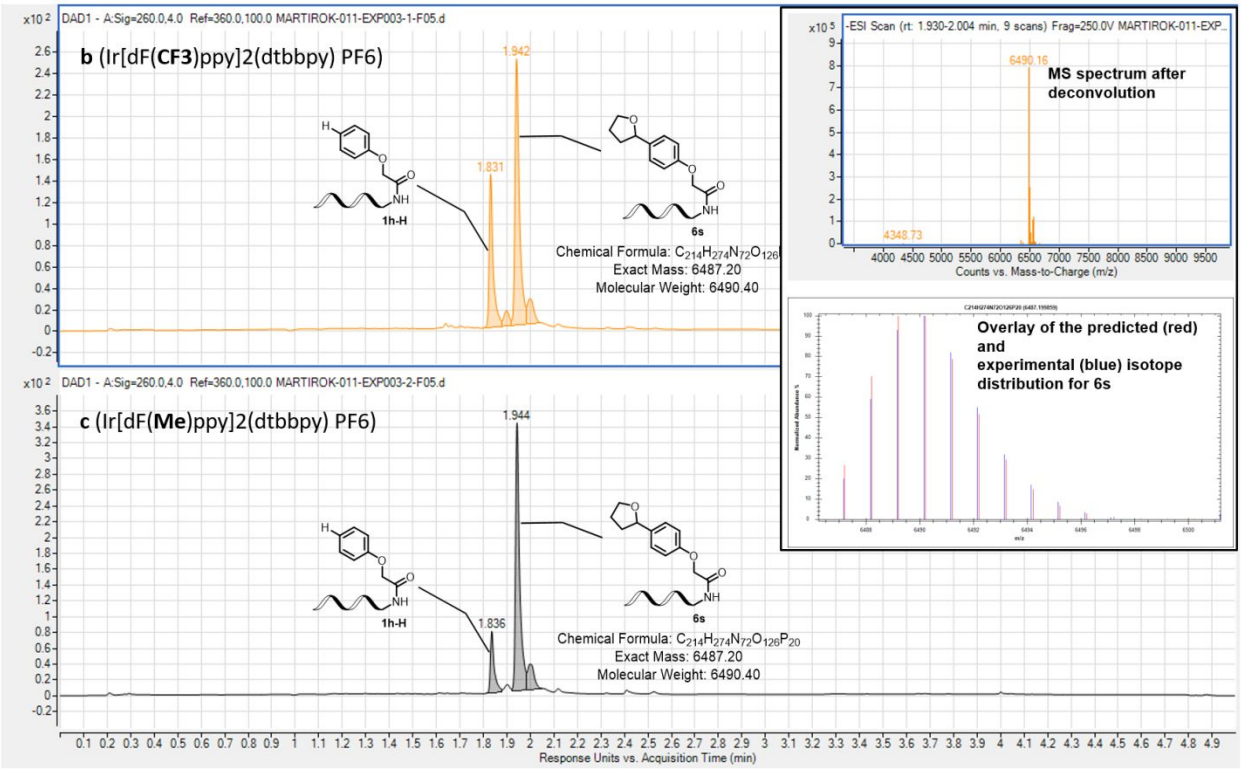


Figure SI VI29: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1h** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6s**.

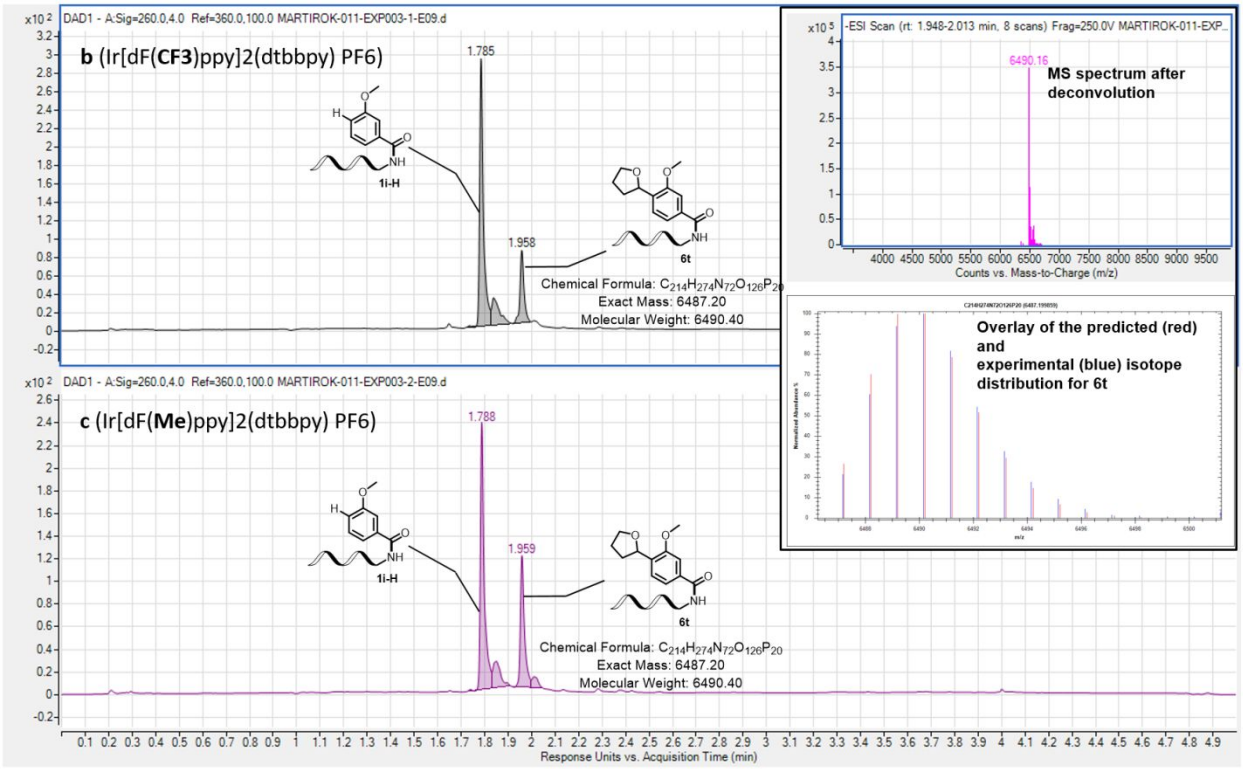


Figure SI VI30: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1i** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (b) or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (c) and characterization of the product **6t**.

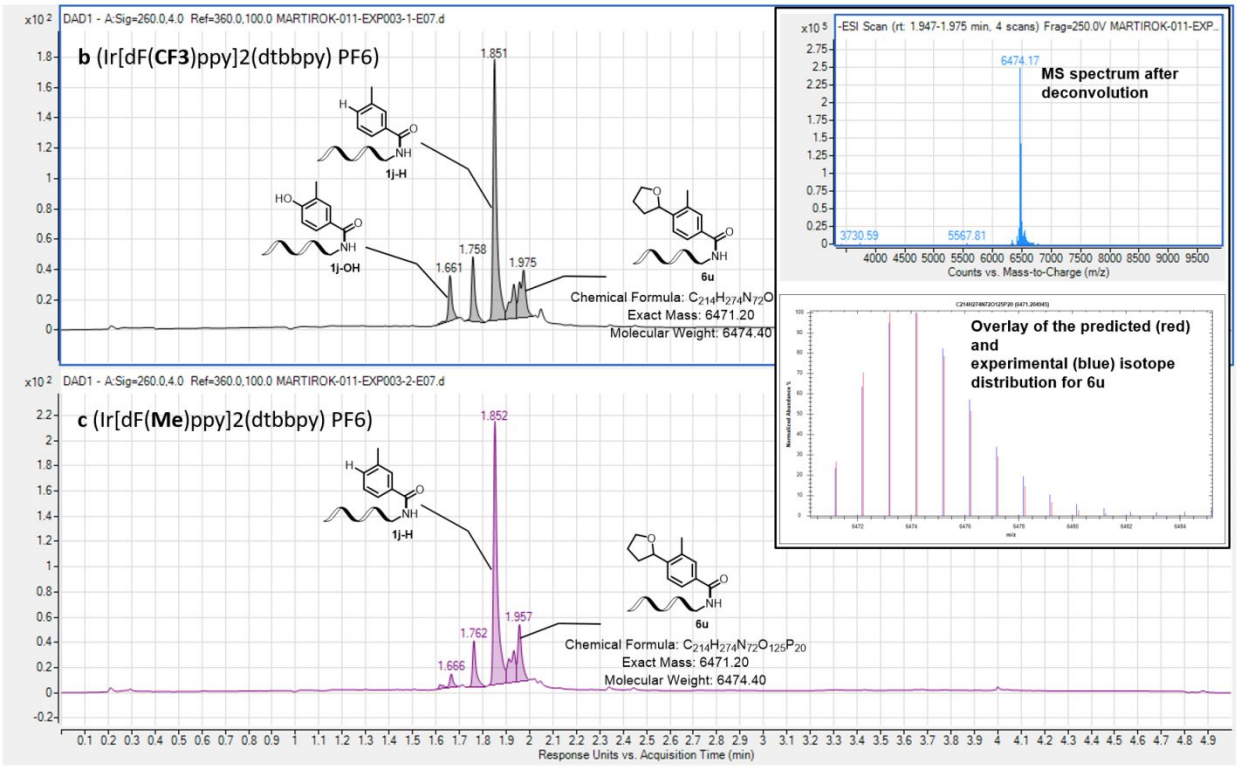


Figure SI VI31: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1j** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6u**.

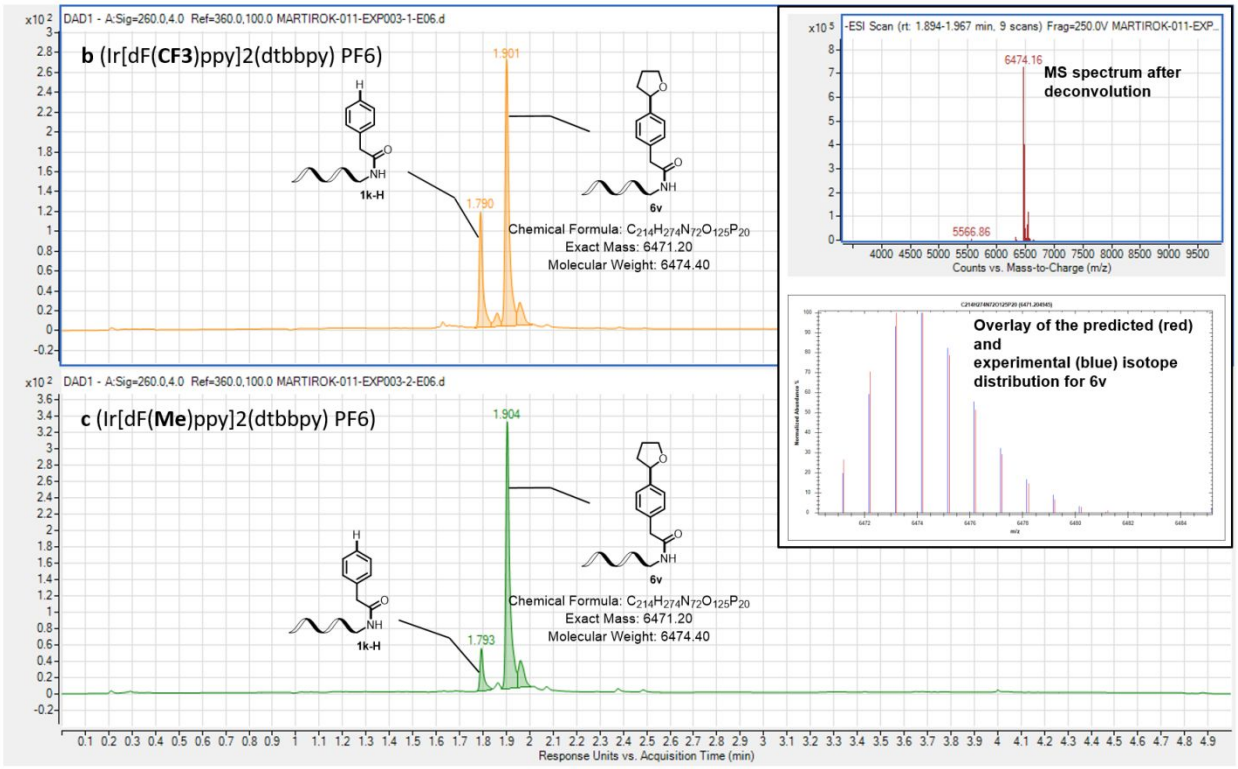


Figure SI VI32: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1k** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6v**.

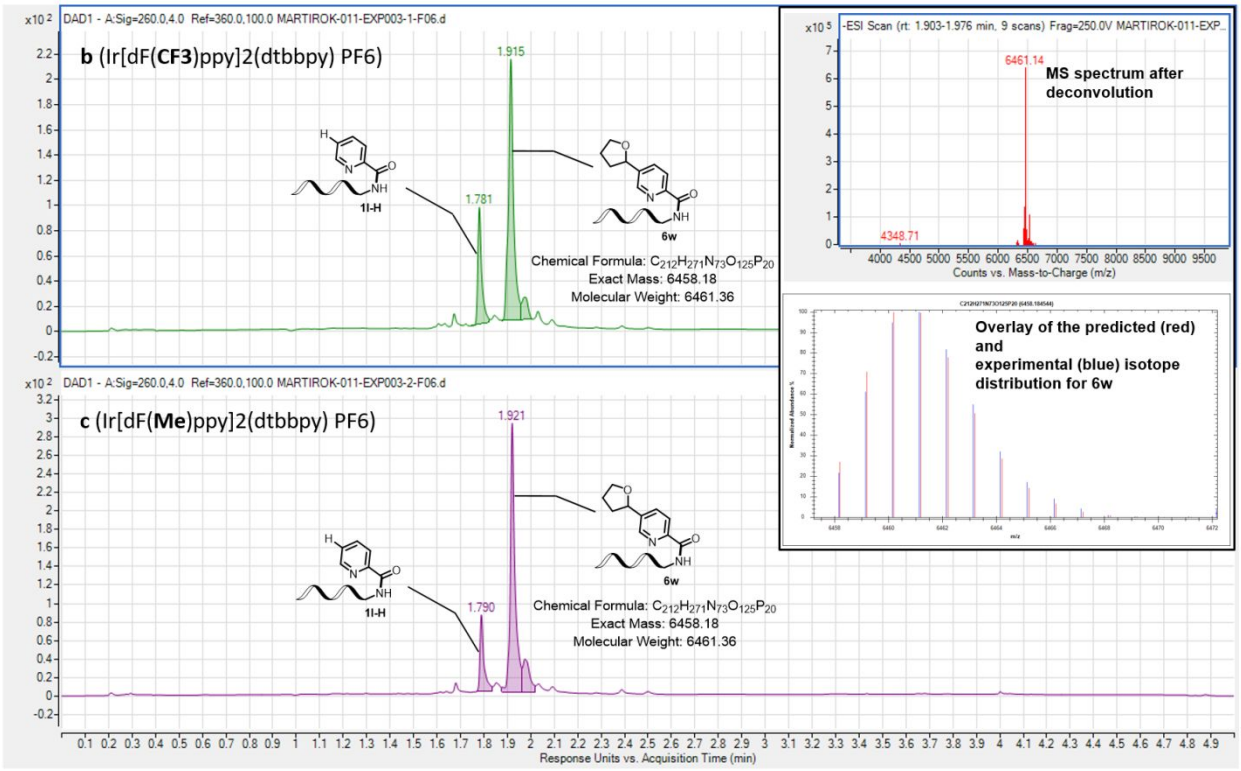


Figure SI VI33: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1l** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (b) or $[Ir(dF(Me)ppy)_2(dtbbpy)]PF_6$ (c) and characterization of the product **6w**.

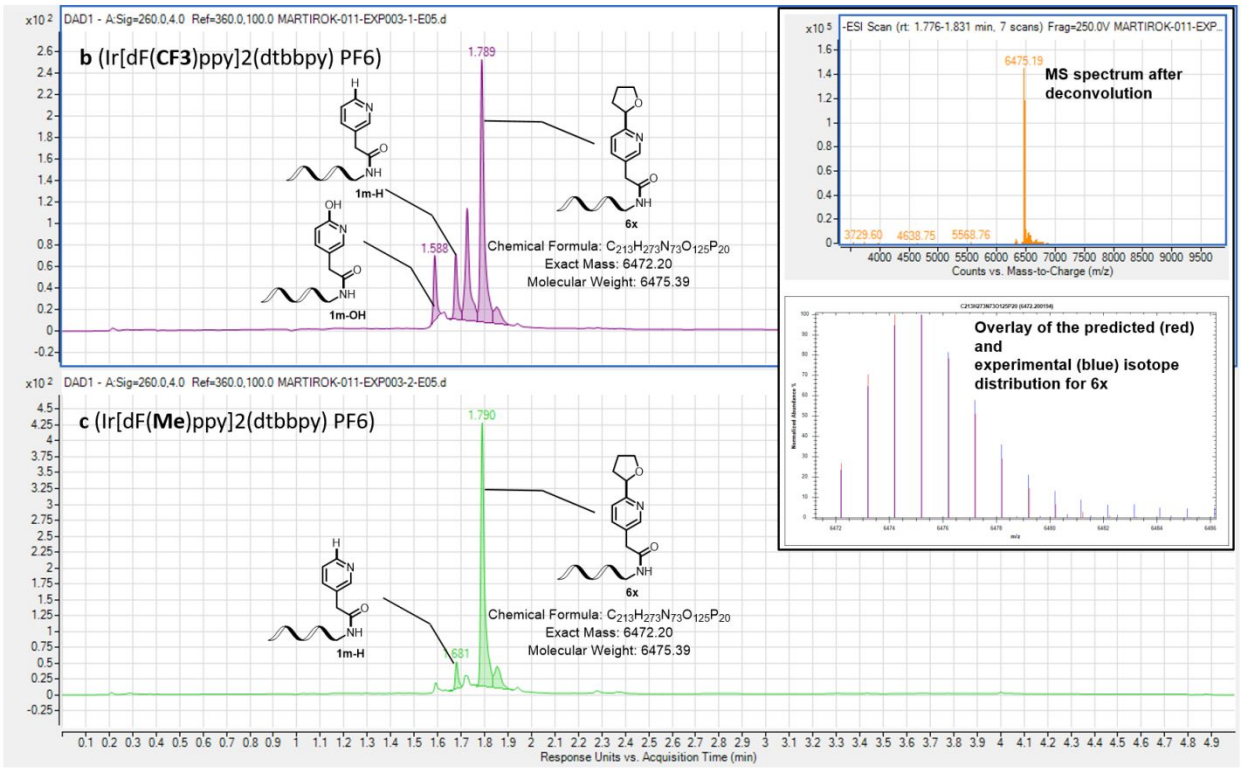


Figure SI VI34: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1m** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (b) or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (c) and characterization of the product **6x**.

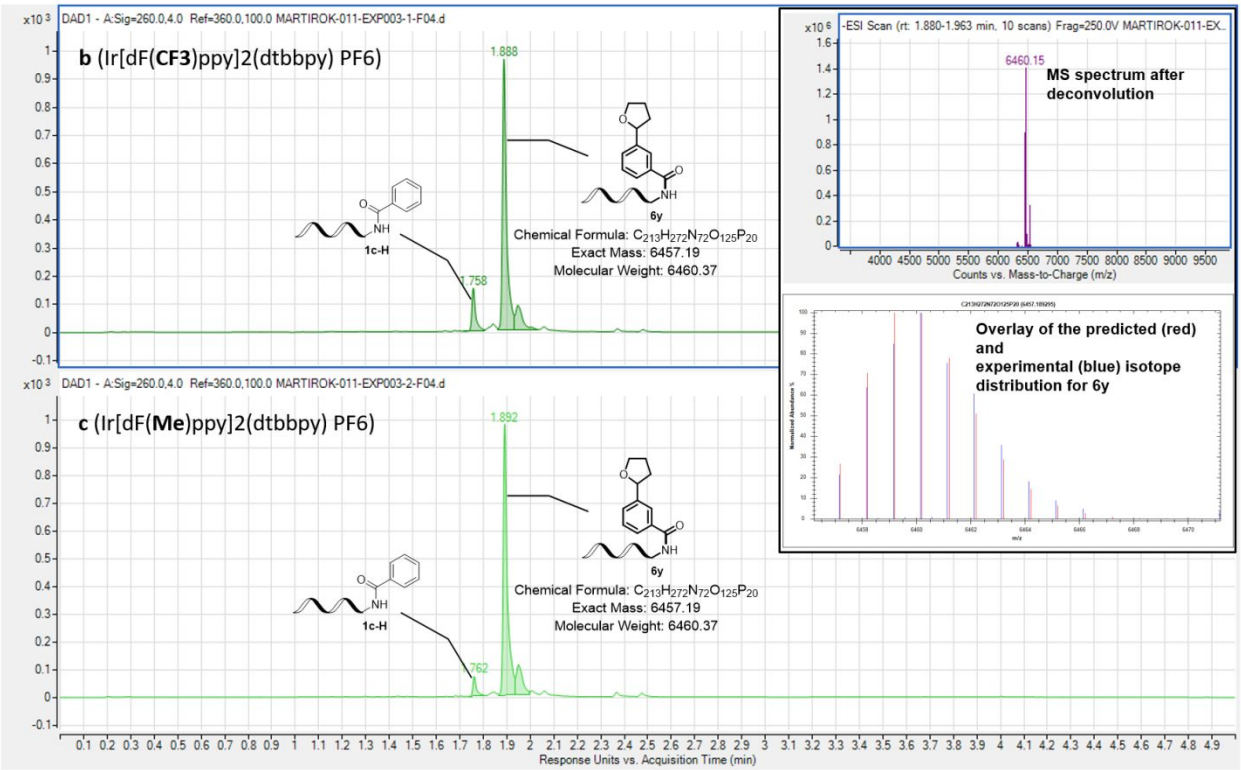


Figure SI VI35: UPLC-TOF analysis of the decarboxylative coupling reaction between the DNA conjugate **1n** and the acid **A1** tetrahydrofuran-2-carboxylic acid using either the photoredox catalyst Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (b) or [Ir(dF(Me)ppy)$_2$(dtbbpy)]PF_6 (c) and characterization of the product **6y**.

SI VI 5 Compatibility of the decarboxylative coupling with water and DEAE sepharose

An experiment in glass vials was performed in order to study if the decarboxylative coupling between **1m** and **A2** tolerates water and if this reaction can also be performed on DEAE sepharose. (Figure SI VI36)

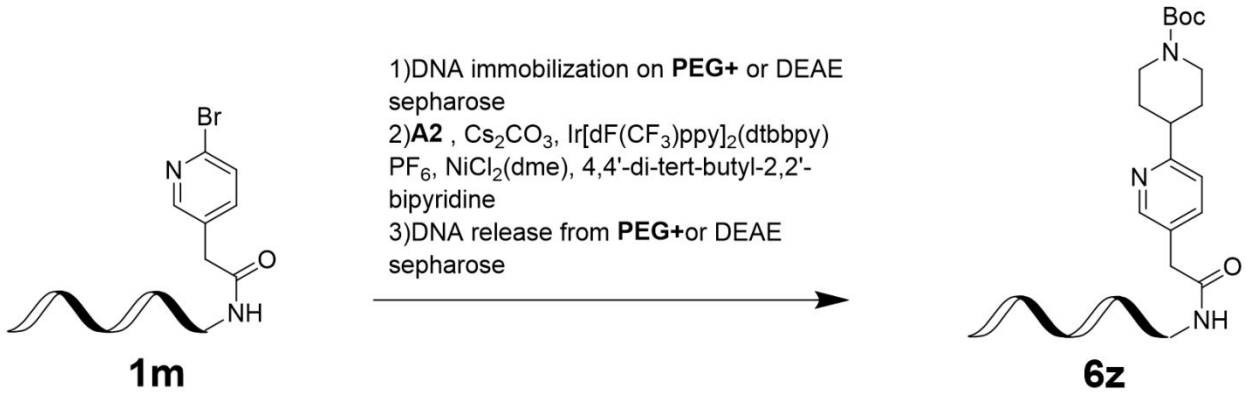


Figure SI VI36: Dual catalysis photoredox decarboxylative cross-coupling on **1m** and **A2** in single glass vials to test the tolerability of this reaction to the presence of water and alternative solid support DEAE sepharose.

Reaction protocol to test the effect of added water on 1m immobilized on PEG+:
The reaction protocol in glass vial was used to run a reaction in pure DMSO and a reaction in DMSO/Water 9/1 in parallel on **1m** immobilized on **PEG+**.
Solution A of catalysts and carboxylic acid substrate:
A2 N-Boc-Piperidine-4-carboxylic acid (CAS 174316-71-3) (22.93 mg, 0.100 mmol) was dissolved in MeOH (150 µL) and added to a solution of Cs_2CO_3 (16.29 mg, 0.05 mmol) in water (150 µL). The solution was homogenized by vortexing for 30 seconds and evaporated to dryness on a SpeedVac™.
This dry cesium salt of **A2** *N*-Boc-piperidine-4-carboxylic acid (CAS 174316-71-3) was dissolved in DMSO (Volume: 2000 µL) and added to a solution of $NiCl_2$(dme) (2.197 mg, 10 µmol), 4,4'-di-tert-butyl-2,2'-bipyridine (2.68 mg, 10 µmol) and Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (2.244 mg, 2 µmol) in DMSO (Volume: 3000 µL) in a 20 mL microwave vial. This solution containing **A2** *N*-Boc-piperidine-4-carboxylic acid (20 mM), Cs_2CO_3 (10 mM), $NiCl_2$(dme) (2 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (2 mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.4 mM) was degassed by argon bubbling for 15 min.

DNA immobilization: The resin **PEG+** (1 g suspended in 20 mL with glycerol/water 1/1) (200 µL, 10mg) was transferred to a 1mL syringe equipped with a filter. The resin was dried by filtration and washed 3 times with 1 mL H_2O and then incubated for 15min with **1m** (6.52 µg, 0.001 µmol) in 200 µL of H_2O. The solvent was drained and the resin was washed with DMSO 4 times.

Reaction setup in pure DMSO: The immobilized **1m** was then suspended in DMSO (Volume: 500 µL) and transferred into a 2mL glass vial containing a magnetic stir bar. 500 µL of the solution **A** containing the catalysts and cesium salt of the carboxylic acid substrate were added to the suspension under argon. The vial was placed in a 3D-printed LED photoreactor (470 nm, 2W) and irradiated for 60 min (see Figure SI VI1).

Reaction setup in DMSO/Water 9/1: The immobilized **1m** was suspended in DMSO/water 8/2 (Volume: 500 µL) and transferred to a 2 mL glass vial containing a magnetic stir bar. 500 µL of

the solution **A** containing the catalysts and cesium salt of the carboxylic acid substrate were added to the suspension under argon. The vial was placed in a 3D-printed LED photoreactor (470 nm, 2W) and irradiated for 60 min.(see Figure SI VI1)

Workup

The resin was transferred back into a 1 mL filter syringe, the solvent drained and the resin washed 5 times with DMSO. The resin was incubated 15 min at 50°C with 100µL of H2O and drained. Then, 100µL DNA elute solution (1.5M NaCl, 50mM phosphate buffer pH8, 0.005% triton-X, 5% 4-Me-piperidine) were added to the resin, incubated for 30min at 50°C and drained into the same tube. The combined aqueous elution samples were desalted by means of an Illustra NAP-2 column (GE healthcare) and analyzed by UPLC-TOF LC-MS.

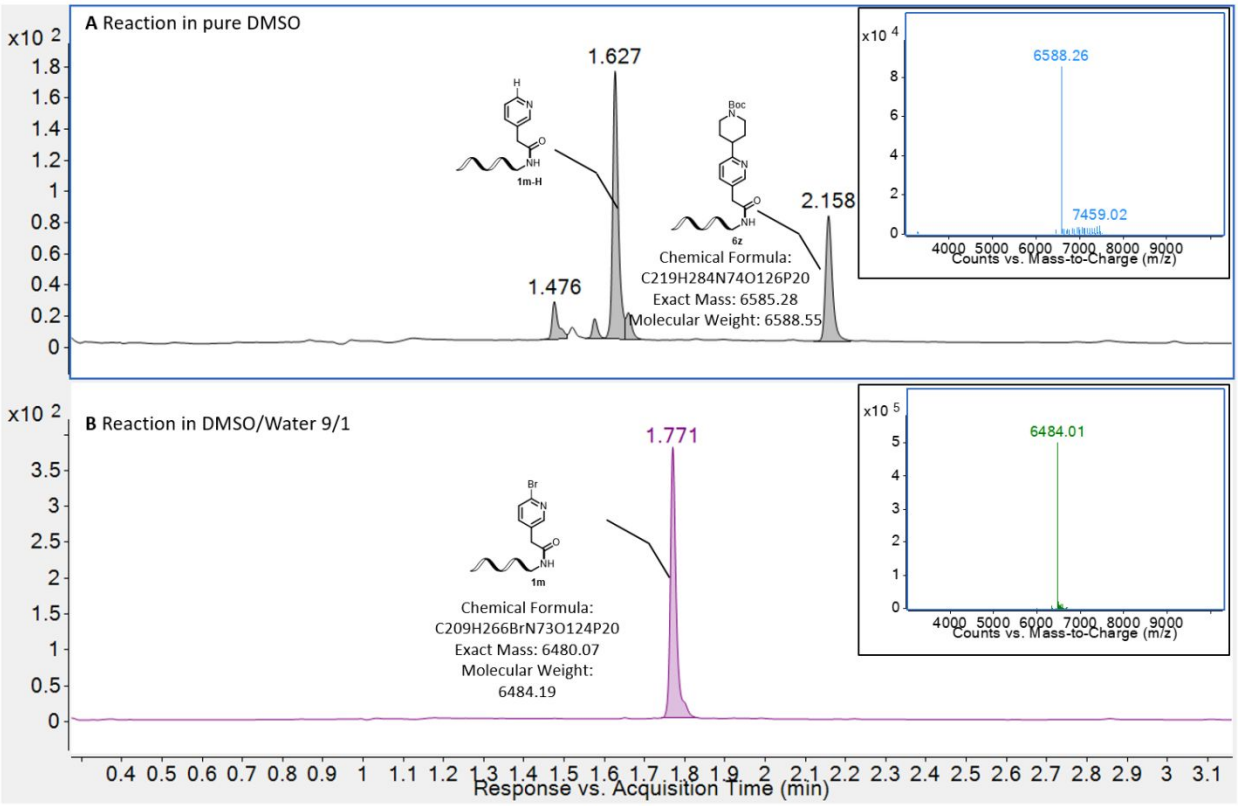


Figure SI VI37: UPLC-TOF analysis of the crude reaction mixture of A Reaction conditions: **1m** (1 nmole) immobilized on **PEG+** (10 mg) or DEAE sepharose, **A2** *N*-Boc-piperidine-4-carboxylic acid (CAS 174316-71-3) (10 mM), Cs_2CO_3 (5 mM), $NiCl_2$(dme) (1 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (1 mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.2 mM) in DMSO (1 mL), 3D-printed LED photoreactor (470nm, 2W), 60min, room temperature. **B** Reaction conditions: **1m** (1 nmole) immobilized on **PEG+** (10 mg) or DEAE sepharose, **A2** N-Boc-Piperidine-4-carboxylic acid (10 mM), Cs_2CO_3 (5 mM), $NiCl_2$(dme) (1 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (1mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.2 mM) in DMSO/Water 9/1 (1 mL), 3D-printed LED photoreactor (470nm, 2W), 60 min, room temperature.

Reaction protocol to test the effect of the immobilization of 1m immobilized on DEAE sepharose:

The reaction protocol in glass vial used on **1m** immobilized on **PEG+** was also deployed in parallel to run a reaction on **1m** immobilized on DEAE sepharose.

Solution A of catalysts and carboxylic acid substrate:

A2 *N*-Boc-piperidine-4-carboxylic acid (CAS 174316-71-3) (22.93 mg, 0.100 mmol) was dissolved in MeOH (150 µL) and added to a solution of Cs_2CO_3 (16.29 mg, 0.05 mmol) in water (150 µL). The solution was homogenized by vortexing for 30 seconds and evaporated to dryness on a SpeedVac™.

This dry cesium salt of **A2** *N*-Boc-piperidine-4-carboxylic acid (CAS 174316-71-3) was dissolved in DMSO (volume: 2000 µL) and added to a solution of $NiCl_2$(dme) (2.197 mg, 10 µmol), 4,4'-di-tert-butyl-2,2'-bipyridine (2.68 mg, 10 µmol) and $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (2.244 mg, 2 µmol) in DMSO (volume: 3000 µL) in a 20-mL microwave vial. This solution containing **A2** *N*-Boc-piperidine-4-carboxylic acid (20 mM), Cs_2CO_3 (10 mM), $NiCl_2$(dme) (2mM), 4,4'-di-tert-butyl-2,2'-bipyridine (2 mM), $Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ (0.4 mM) was degassed by argon bubbling for 15 min.

DNA immobilization: The resin **DEAE sepharose** (2 g suspended in 30 mL of water) (200 µL, 10 mg) was transferred to a 1 mL syringe equipped with a filter. The resin was drained by filtration and washed 3 times with 1 mL H_2O and then washed with 10 mM acetic acid solution and 0.005% Triton X-100 (protocol adapted from Harbury *et al.*[1]). The dried resin was then incubated for 15min with **1m** (6.52 µg, 0.001 µmol) in 200 µL of 10 mM acetic acid solution and 0.005% Triton X-100. The solvent was drained and the resin washed with DMSO 4 times.

Reaction setup in pure DMSO: 1m immobilized on DEAE sepharose was then suspended in DMSO (Volume: 500 µL) and transferred to a 2 mL glass vial containing a magnetic stir bar. 500 µL of the solution **A** containing the catalysts and cesium salt of the carboxylic acid substrate were added to this suspension under argon. The vial was placed in a 3D-printed LED photoreactor (470 nm, 2 W) and irradiated for 60 min (see Figure SI VI1).

Workup

The resin was transferred back into a 1 mL filter syringe, the solvent drained and the resin washed 5 times with DMSO. The resin was incubated 15 min at 50°C with 100 µL of H_2O and filtered. Then 100 µL DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X, 5% 4-Me-piperidine) was added to the resin, incubated for 30 min at 50°C and filtered into the same tube. The combined aqueous elution samples were desalted by means of an Illustra NAP-2 column (GE healthcare) and analyzed by UPLC-TOF LC-MS.

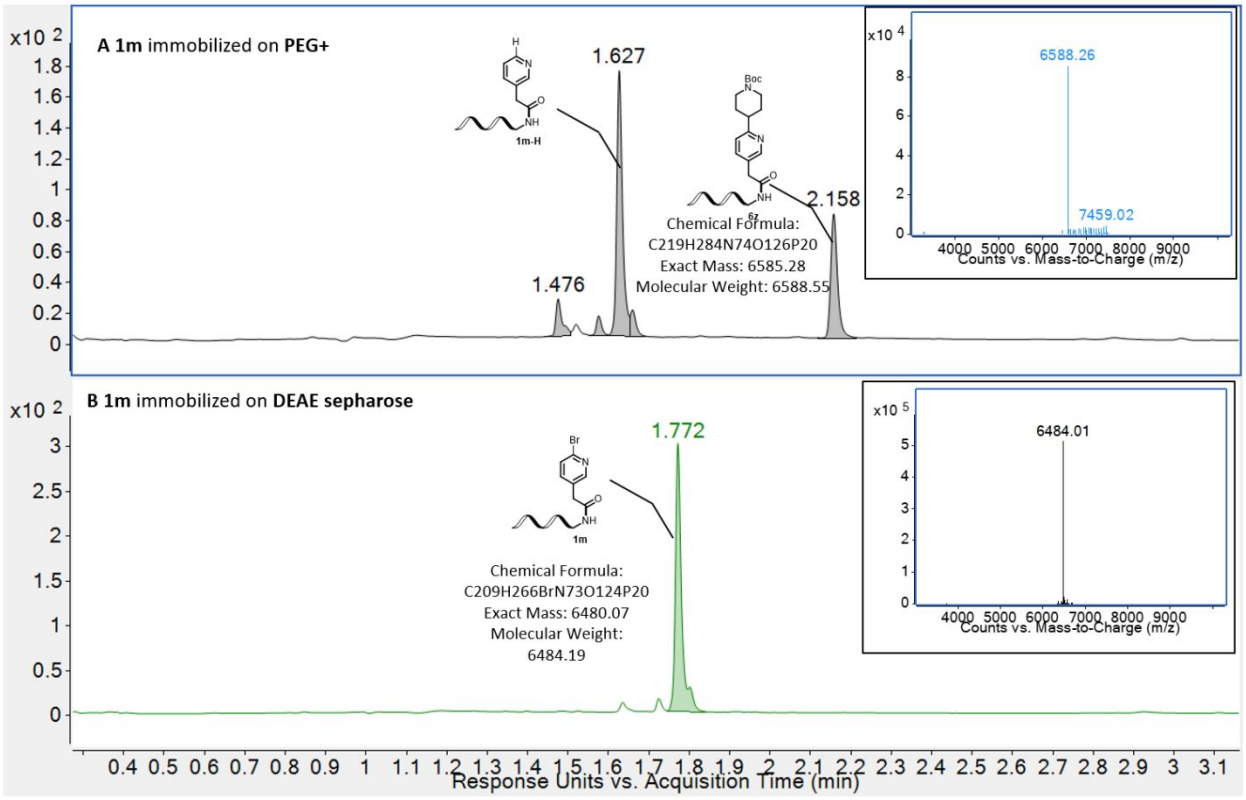


Figure SI VI38: UPLC-TOF analysis of the crude reaction mixture of A Reaction conditions: **1m** (1nmol) immobilized on **PEG+** (10 mg), **A2** *N*-Boc-piperidine-4-carboxylic acid (10 mM), Cs_2CO_3 (5 mM), $NiCl_2$(dme) (1 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (1 mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.2 mM) in DMSO (1 mL), 3D-printed LED photoreactor (470 nm, 2 W), 60 min, room temperature. **B** Reaction conditions: **1m** (1nmole) immobilized on **DEAE sepahrose** (13 mg), **A2** *N*-Boc-piperidine-4-carboxylic acid (10 mM), Cs_2CO_3 (5 mM), $NiCl_2$(dme) (1 mM), 4,4'-di-tert-butyl-2,2'-bipyridine (1 mM), Ir[dF(CF_3)ppy]$_2$(dtbbpy)PF_6 (0.2 mM) in DMSO (1 mL), 3D-printed LED photoreactor (470 nm, 2 W), 60 min, room temperature.

SI VII Protocol and analysis for the evaluation of the stability of the DNA tags during the SnAP reaction and photoredox decarboxylative cross-coupling on immobilized DNA substrates

In a first approach, evaluation of the DNA strands integrity is determined by UPLC-TOF analysis as shorter oligonucleotides resulting from the cleavage of the phosphodiester bond are easily detected. Other types of DNA damage, including depurination which typically occurs under acidic conditions or at elevated temperature, can also be detected by the analysis of the MS spectrum. Under our conditions for photoredox decarboxylative coupling and for the SnAP reaction, no such DNA degradation was detected as illustrated by the UPLC-TOF chromatogram and MS spectrum analysis depicted in the figures SI V2-18 and SI VI2-35.

However, we wanted to ensure that our conditions would still be compatible with the longer DNA tags which are used during DEL synthesis (in particular after the second and third cycles of library synthesis) and that sufficient amounts of DNA tags would remain after reaction and release from the support.

To this end, we designed a 85-base pair reporter double-stranded oligonucleotides **qPCR-oligo** to mimic the DNA tags used on substrates of a DEL synthesis at the second cycle. This oligonucleotide (obtained by T4 DNA ligase standard ligation techniques[6] starting from oligonucleotides obtained commercially from Sigma) was designed to contain two 23- and 24-base primers and has a similar covalent headpiece architecture as other double-stranded DNA tags described in the literature.[6]

We then used the DNA-encoded rehearsal methodology described by Malone *et al.* in which the yield of amplifiable DNA remaining after a chemical transformation is evaluated by quantitative PCR.[7-8] In this protocol, a known amount of a reporter oligonucleotide (0.1nmol of **qPCR-oligo** in our case) is initially added to the reactions to be evaluated. After a work up, the amount of intact/amplifiable DNA in the reaction samples is then quantified using qPCR using the Ct method and compared to standard samples. While in the original publication Malone *et al.* used an oligonucleotide covalently immobilized on magnetic beads,[7] we reversibly immobilized the free reporter oligonucleotide on our **PEG+** resin with or without substrate DNA conjugates prior to running the reactions. The DNA conjugates were used as reporters to facilitate UPLC-TOF analysis and ensure that the reaction of interest did took place. The amount of amplifiable DNA in photoredox decarboxylative couplings and SnAP reaction samples was then determined using standard qPCR protocols[7] after establishing a standard curve for reference samples containing known quantities of this reporter oligonucleotide (Figures SI VII1 and VII2).

SI VII1 Protocol for the evaluation of the recovery of cycle 2 DNA tag qPCR-oligo by qPCR after the SnAP protocol:

In a 1 mL syringe equipped with a filter, 250 µL of a suspension of the resin **PEG+** (resin swollen in water) (250 µL, 12.5 mg) was incubated with an aqueous solution of **qPCR-oligo** (0.1 nmol) (as a solution in 200 µL of water) at 60°C for 30 min. Importantly, the same stock solution of qPCR oligo was used to perform the immobilization and to prepare the calibration standards used in qPCR.

In parallel, a second reaction sample was prepared to be able to monitor the reaction by UPLC-TOF: In a 1 mL syringe equipped with a filter, 250 µL of a suspension of the resin **PEG+** (resin swollen in water) (250 µL, 12.5 mg) was incubated with an aqueous solution of **1b** (as a solution in water) (2 nmol) and **qPCR-oligo** (0.1 nmol) (as a solution in 200 µL of water) at 60°C for 30 min.

Three cycles of the SnAP protocol were then performed on these immobilized DNA substrates using the protocol described in SI V3.

After the reaction, the DNA species were released from the resin by treatment with 500 µL of DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH8, 0.005% triton-X5%, 4-Me-piperidine) at 60°C for 15min. The resin was then washed with 250 µL of DNA elute solution. The elution solutions were combined (750 µL) and desalted through a NAP-10 column, resulting in a final sample volume of 1200 µL.

Before qPCR analysis, the two reaction samples were diluted 1666-fold in order to match the concentration of the middle standard of the linear calibration curve, which was established using known amounts of **qPCR-oligo**.

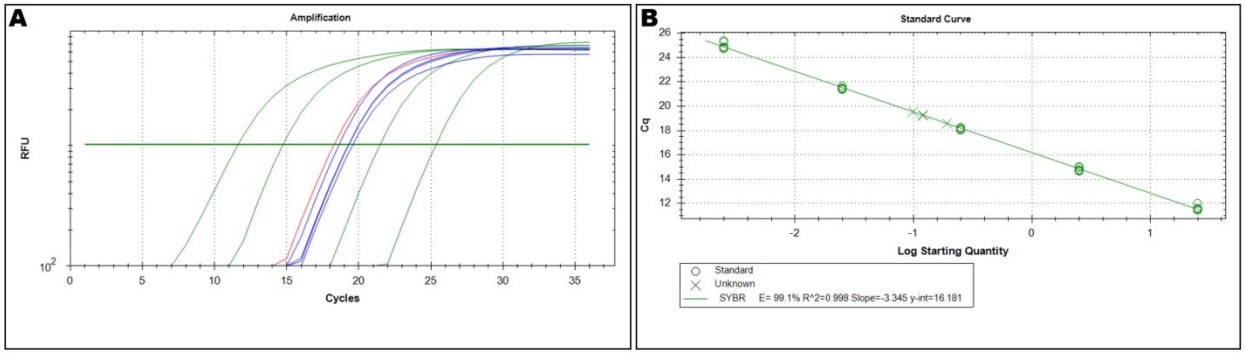


Figure SI VII1: Representative qPCR amplification curves (**A**) and Cq (Calibration cycles) calibration curve (**B**) used for the quantification of remaining amplifiable DNA for reactions samples after the SnAP protocol (blue) and reference sample (red). Samples (cross symbols) were run in duplicates and calibration standards (green curves (A) and circles (B)) sextuplicates but for clarity only one fluorescence trace is represented for each sample.

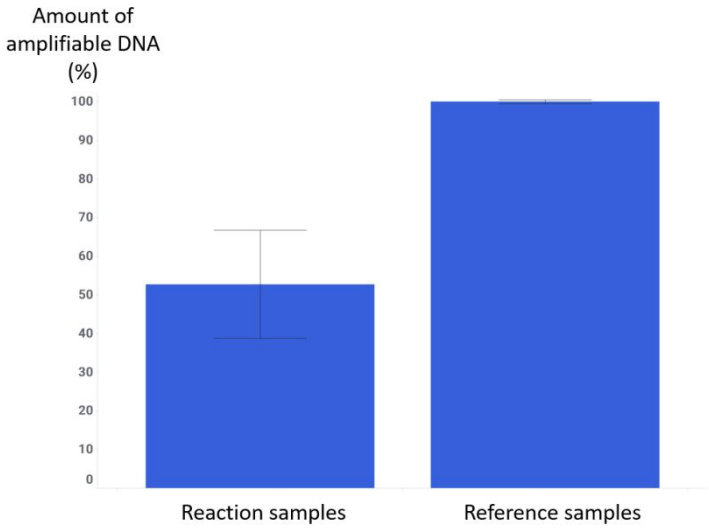


Figure SI VII2: Percentage of amplifiable DNA in reaction samples after the SnAP reaction under reaction conditions **SI V3**. The percentage of amplifiable DNA was calculated with respect to a set of reference samples of **qPCR-oligo** which were not submitted to reaction conditions **SI V3**

but contained the same amount of **qPCR-oligo** as the initial amount loaded in the reactions samples. Error bars correspond to the standard deviation of the samples.

After submitting the **qPCR-oligo** to our standard reaction conditions for the SnAP protocol, in average 53% of the reporter dsDNA **qPCR-oligo** remains amplifiable (Figure SI VII2).

SI VII2 Evaluation of the recovery of cycle 2 DNA tag qPCR-oligo by qPCR after the photoredox decarboxylative cross coupling protocol:

The resin **PEG+** (1 g suspended in 20 mL with glycerol/water 1/1) (100 µL, 5 mg) was transferred in a 1mL 96-well filter plate. The resin was dried by filtration and washed with 3x1 mL H2O and then incubated for 15 min with 1c (6.52 µg, 0.001 µmol) in 200 µL of H2O and **qPCR-oligo** (0.1 nmol) (as a solution in 200 µL of water) at 60°C for 30 min. Importantly, the same stock solution of **qPCR-oligo** was used to perform the immobilization and to prepare the calibration standards used for the qPCR quantification. The solvent was drained and the resin washed 3 times with MeOH. The solvent was then evaporated to dryness in a SpeedVac™. Two magnetic stir bars were added to each well and the bottom of the plate was heat-sealed with aluminum foil.

The photoredox decarboxylative coupling protocol was then performed on these immobilized DNA substrates using the protocol described in SI VI2 with tetrahydrofuran-2-carboxylic acid as coupling partner.

Workup

The suspension was filtered by centrifugation and the resin was washed 5 times with DMSO. The resin was incubated 15 min at 60°C with 100 µL H2O and filtered into a 500 µL plate. Then 200 µL DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X, 5% 4-Me-piperidine) were added to the resin, incubated for 30 min at 60°C and filtered into the same tube. The elution process was repeated once with 200 µL DNA elute solution. The combined elution samples (500 µL) were desalted with a NAP-5 column before UPLC-TOF and qPCR analysis resulting in a final sample volume of 1000 µL.

Before qPCR analysis the two reaction samples were diluted 2000-fold in order to match the concentration of the middle standard of the linear calibration curve, which was established using known amounts of **qPCR-oligo**.

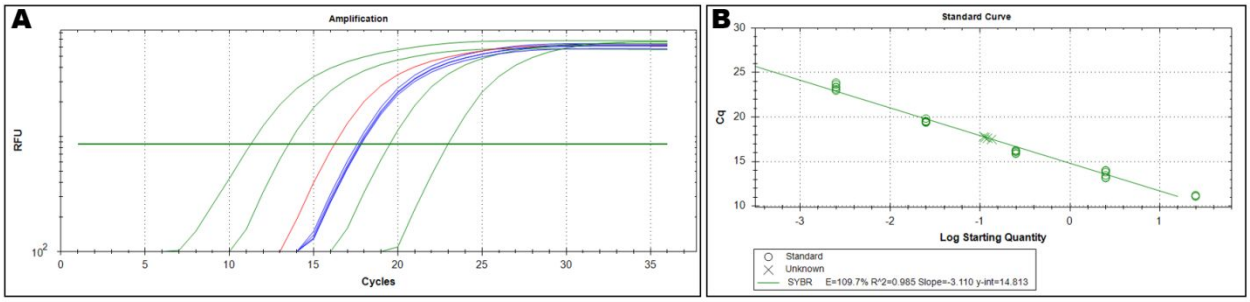


Figure SI VII3: Representative qPCR amplification curves (**A**) and Cq (Calibration cycles) calibration curve (**B**) used for the quantification of remaining amplifiable DNA for reactions samples after the photoredox decarboxylative cross coupling protocol (blue) and reference sample (red). Samples (cross symbols) were run in duplicates and calibration standards (green curves (A) and circles (B)) sextuplicates but for clarity only one fluorescence trace is represented for each sample.

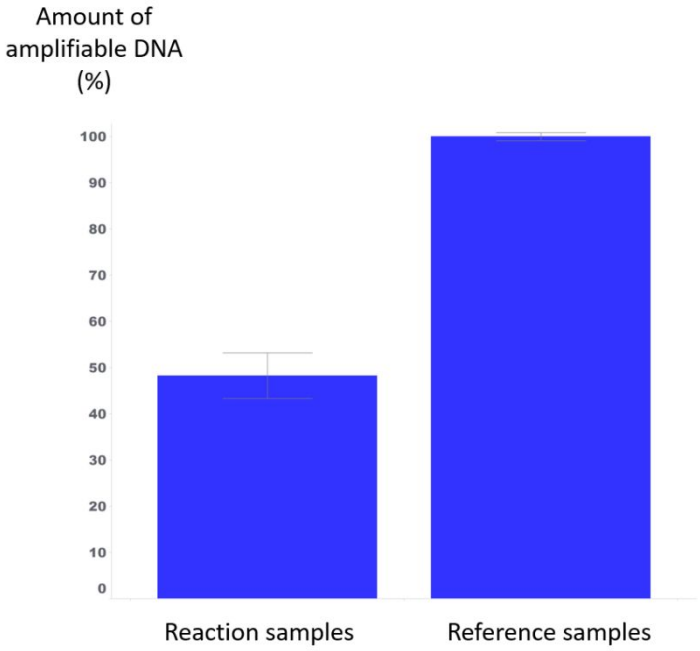


Figure SI VII4: Percentage of amplifiable DNA after photoredox decarboxylative cross coupling reaction samples under reaction conditions SI VI3. The percentage of amplifiable DNA was calculated with respect to a set of reference samples of **qPCR-oligo** which was not submitted to reaction conditions SI VI3 but contained the same amount of **qPCR-oligo** as the initial amount loaded in the reactions samples. Error bars correspond to the standard deviation of the samples.

After submitting the **qPCR-oligo** to the standard reaction conditions for decarboxylative cross coupling SI VI3, 48% of the reporter dsDNA remains amplifiable (Figure SI VII3-4).

Taken together, the DNA losses observed after catch-react-release cycles for both the SnAP and photoredox cross-coupling are comparable with those observed for other well-established DNA-compatible chemical transformations (such as acylation[7] or the Suzuki cross-coupling[7, 9]). These recoveries of amplifiable DNA fulfill the criteria dictated by our practice of the DNA-encoded library technology and should be generally compatible with library synthesis in the academic or industrial environment.

SI VII3 Evaluation of the impact of our reaction conditions on ligation efficiency

In an effort to further characterize the impact of our reaction conditions on the integrity of the DNA tags we also performed test ligations according to the method described by Ratnayake *et al.*[6] to evaluate the competency of the tags in subsequent encoding steps.

In these experiments, the samples of **qPCR-oligo** used for the previous qPCR quantification after either the SnAP reaction (SI VII1) or the photoredox decarboxylative cross-coupling (SI VII2) were used for a T4 mediated ligation[6] of additional oligonucleotides (17 and 11 bases) on the upper and lower strands of the **qPCR-oligo.** Thus, **qPCR-oligo** was elongated to a final ligation product with a length of 202 bases (Figure SIVII5-6).

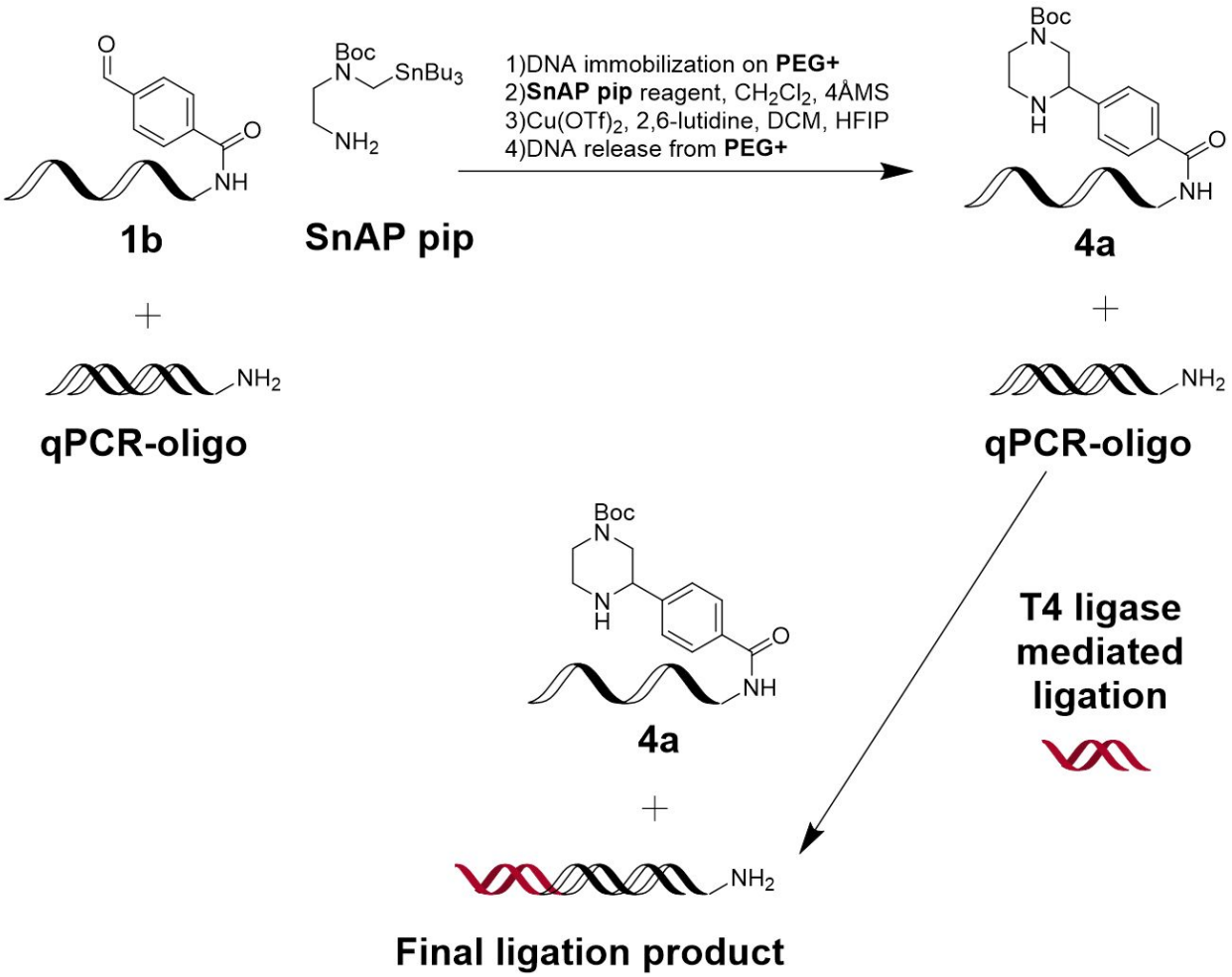


Figure SIVII5: Evaluation of the ligation efficiency of **qPCR-oligo** after being exposed to photoredox decarboxylative reaction conditions as described in SI VII1. Standard T4 ligation conditions[6] were used to ligate two oligonucleotides, one each to the upper and lower strands of **qPCR-oligo** (85 base-pairs) yielding a final ligation product with a length of 202 bases.

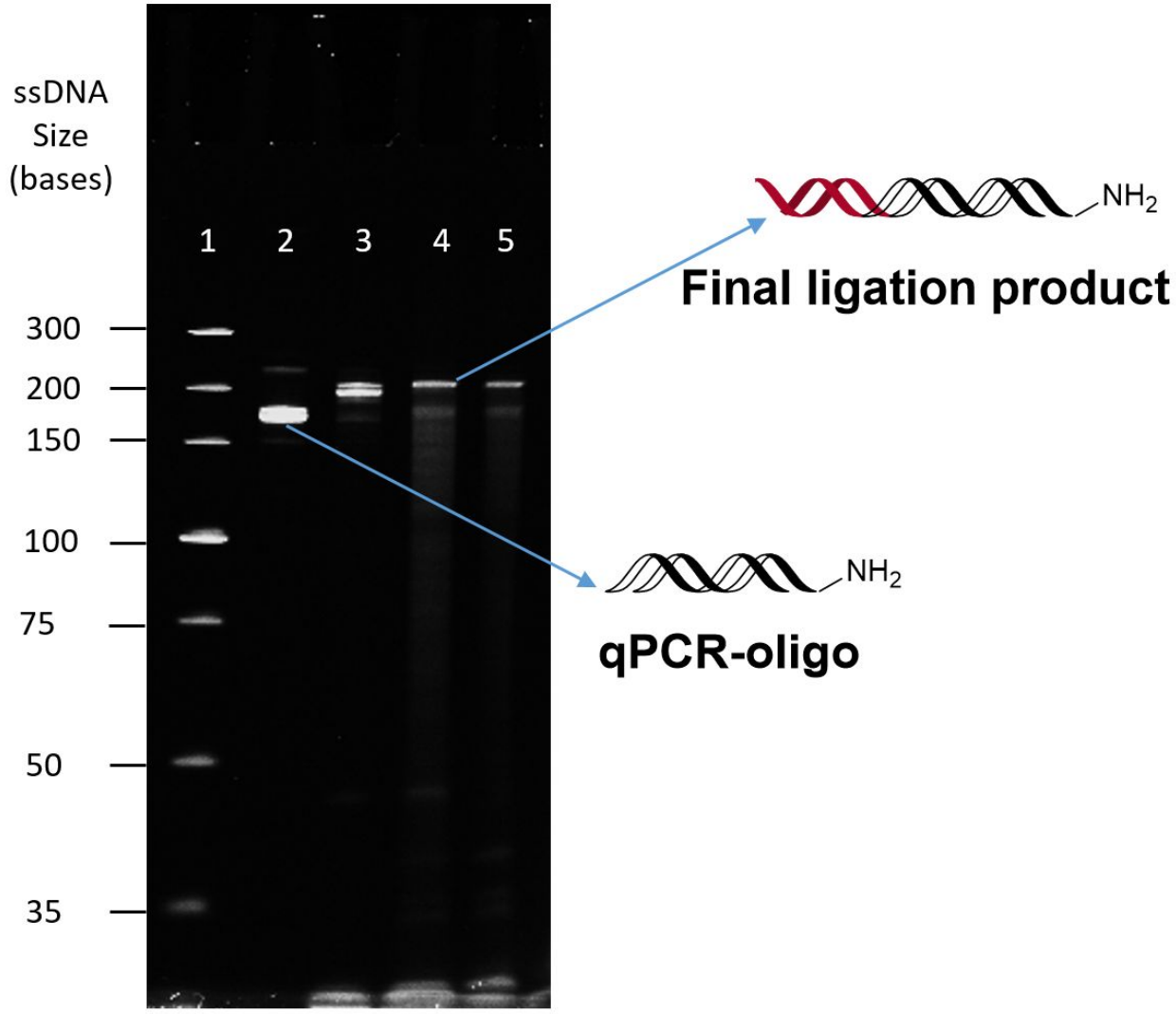


Figure SIVII6: Denaturing polyacrylamide gel electrophoresis analysis of the crude ligation mixture performed on **qPCR-oligo** recovered after being exposed to the SnAP protocol conditions as described in SI VII1. Standard T4 ligation conditions[6] were used to ligate two oligonucleotides to the upper and lower strands of qPCR-oligo (85bp) yielding a final product with a length of 202 bases. Lane 1: ssDNA molecular weight markers (bases); lane 2: reference **qPCR-oligo**; lane 3: ligation control on a reference **qPCR-oligo** which was not exposed to the SnAP reaction conditions; lane 4 and 5: crude ligation mixture on two different samples of **qPCR-oligo** recovered after being exposed to the SnAP reaction conditions as described SI VII1. As the gel was run under denaturing conditions (TBE-urea; 60°C) the 85bp **qPCR-oligo** and final ligation product migrate respectively as a 170 and 202 bases single-standed oligo as confirmed with molecular weight markers.

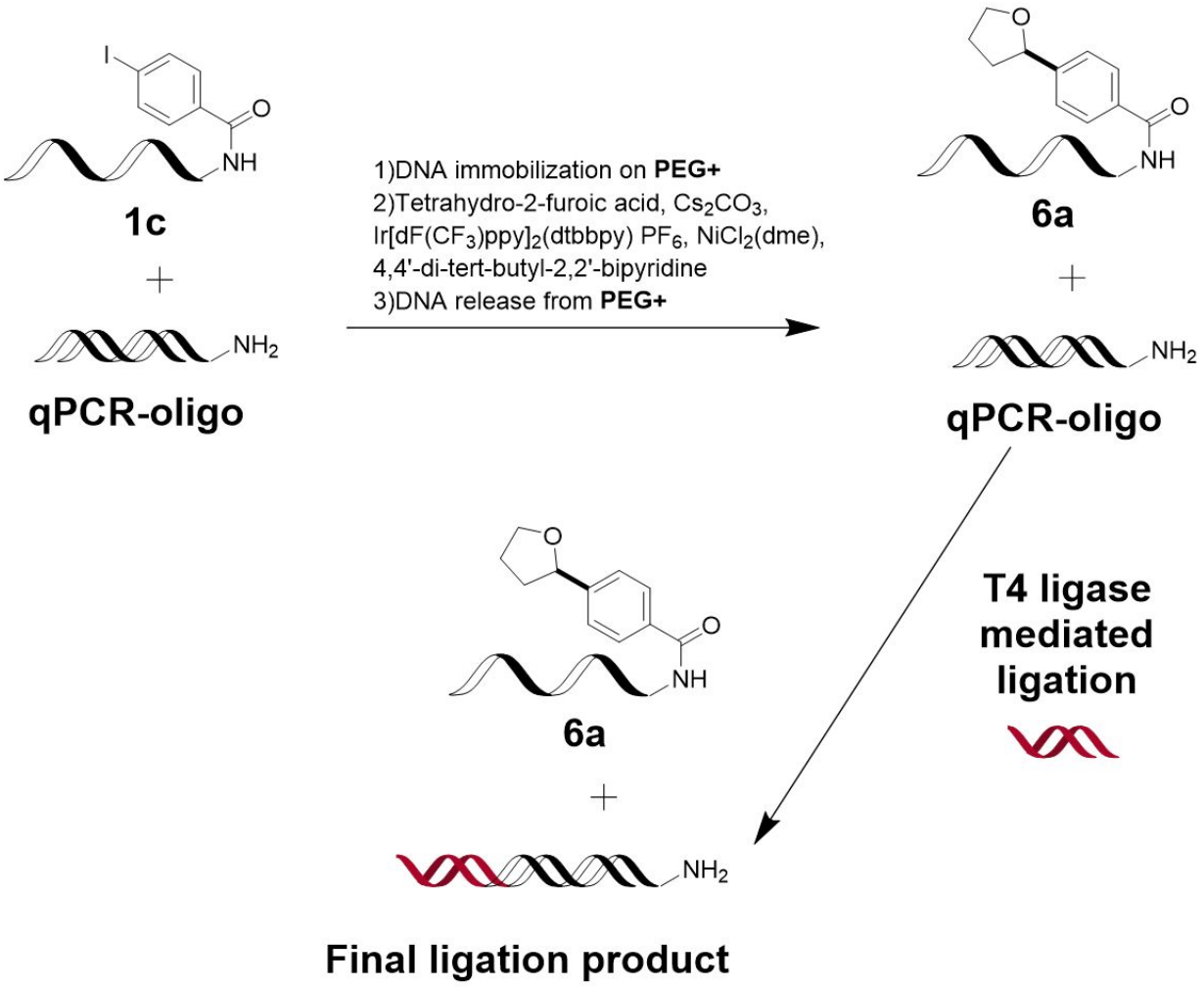


Figure SIVII7: Evaluation of the ligation efficiency of **qPCR-oligo** after being exposed to photoredox decarboxylative reaction conditions as described in SI VII2. Standard T4 ligation conditions[6] were used to ligate two oligonucleotides, one each to the upper and lower strands of qPCR-oligo (85bp) yielding a final product with a length of 202 bases.

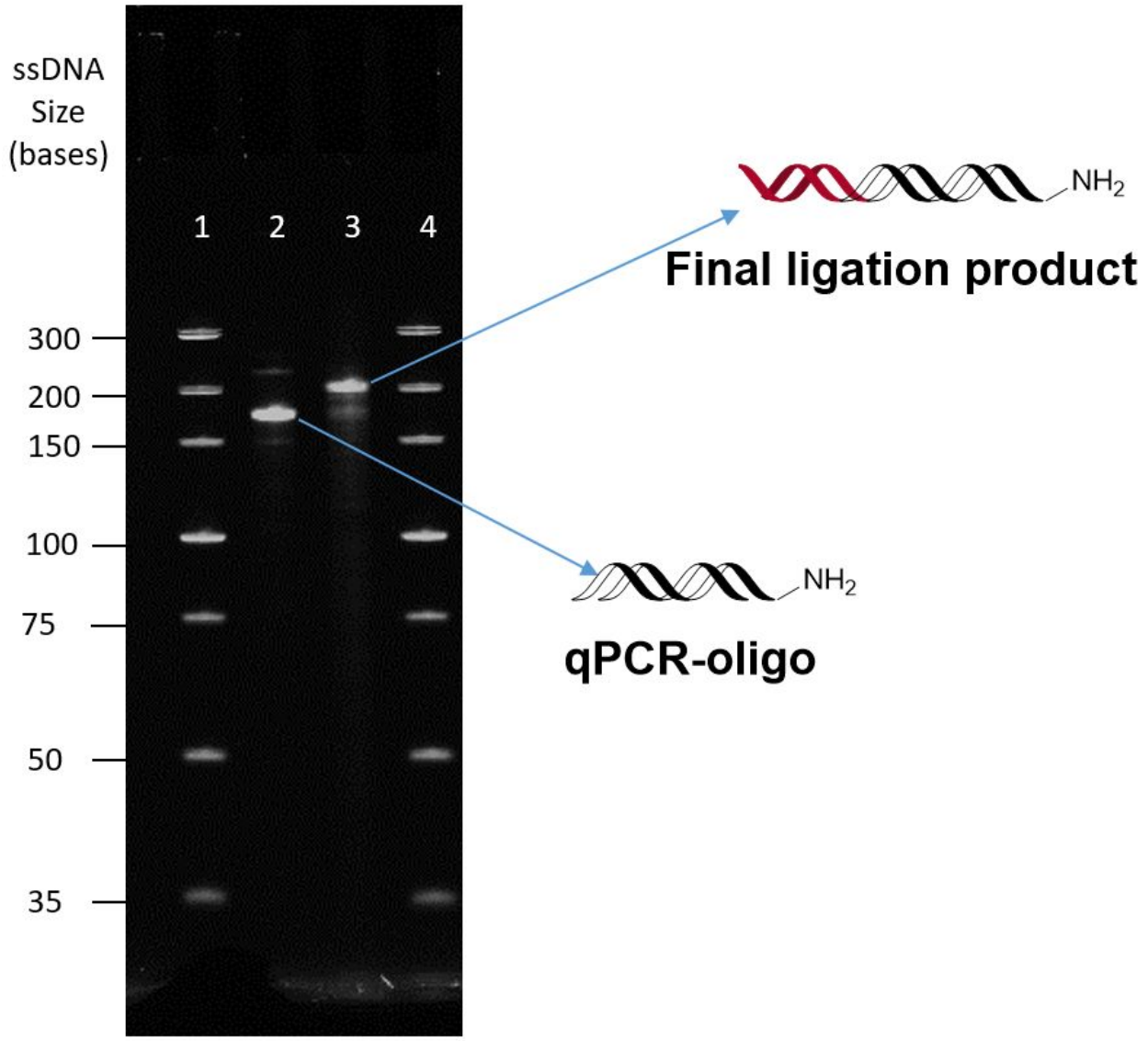


Figure SIVII8: Denaturing polyacrylamide gel electrophoresis analysis of the crude ligation mixture performed on **qPCR-oligo** recovered after being exposed to photoredox decarboxylative reaction conditions as described in SI VII2. Standard T4 ligation conditions[6] were used to ligate two oligonucletodies, one each to the upper and lower strands of qPCR-oligo (85bp) and yielding a final product with a length of 202 bases. Lane 1 and 4: ssDNA molecular weight markers (bases); lane 2: reference **qPCR-oligo**; lane 3: crude ligation mixture on a sample of **qPCR-oligo** recovered after being exposed to the photoredox decarboxylative reaction conditions as described in SI VII2. As the gel was run under denaturing conditions (TBE-urea; 60°C) the 85bp **qPCR-oligo** and final ligation product migrate respectively as a 170 and 202 bases single-standed oligo as confirmed with molecular weight markers.

In conclusion, the model second step library tag **qPCR –oligo** recovered after the catch-react-release cycle for both the SnAP and the photochemical cross-coupling is still competent in the ligation of additional encoding oligonucleotides.

SI VIII Determination of the loading capacity of the PEG+ resin and recovery for short single-stranded (20 bases) and model cycle 2 double-stranded DNA tags (85bp)

SI VIII1 Initial loading and recovery determination using a Cy5 labelled oligonucleotide

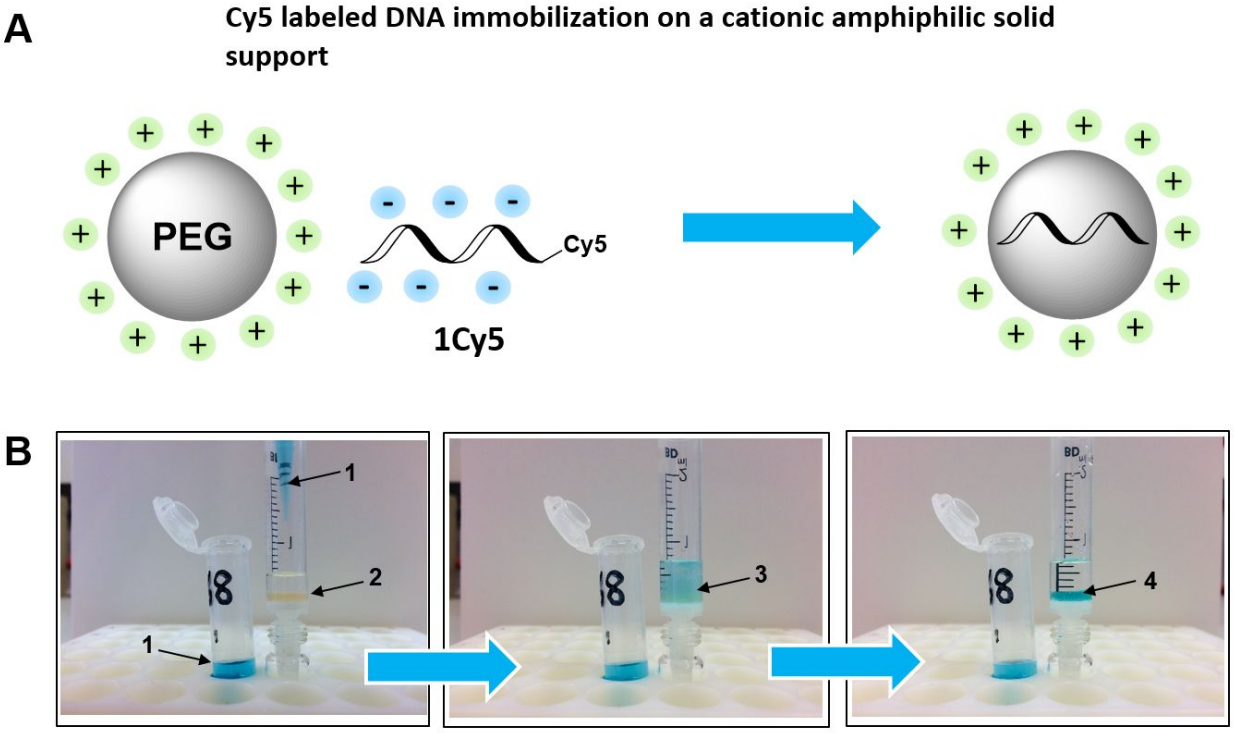


Figure SI VIII1: A Chemical structure and schematic representation of an amphiphilic polymeric support **PEG+** and its use for the transfer and immobilization of the DNA oligonucleotide **1Cy5**, here conjugated to a Cy5 dye. **B** Photographic representation of the different stages of immobilization. An aqueous solution of a Cy5-labelled oligonucleotide **1Cy5** (2 nmol) (1) is added to a colorless aqueous suspension of **PEG+** (2) resulting in a blue suspension (3) immediately after addition. After 5 minutes of agitation, the blue coloration indicative of the presence of Cy5 DNA conjugate **1Cy5** had transferred from the aqueous supernatant to the **PEG+** solid support (4).

Protocol for DNA loading and immobilization

The resin **PEG+** (1 g suspended in 20 mL with glycerol/water 1/1) (200 µL, 10 mg) was transferred in a 5 mL syringe with filter insert. The solvent was drained and the resin washed 5 times with water.

Then, **1Cy5** as a solution in water (200 µL, 0.002 µmol) was added to the resin. The suspension was then agitated in a Thermomixer for 30min at 60°C.

The suspension was filtered and the flow-through was quantified by absorption at 646nm (Eppendorf BioSpectrometer® basic), indicating complete absorption of **1cy5** onto the solid support.

Heating at 60°C during immobilization is not required for short single strand DNA oligonucleotides (20 bases) as for these substrates the immobilization is complete after 15min at room temperature. For longer DNA sequences such as **qPCR-oligo** the immobilization process is greatly accelerated at 60°C and requires between 15 and 30 min to be completed. Therefore we typically use a loading at 60°C for 30 min by default.

Protocol for DNA release and recovery determination

The dry resin was incubated 30min at 60°C with 200 µL of DNA elution solution (1.5 M NaCl, 50 mM phosphate buffer pH 8, 0.005% triton-X, 5% 4-Me-piperidine). The elution solution was collected and desalted using a NAP5 column (GE Healthcare illustra™ NAP™). The resulting desalted sample was collected in a 1.5 mL Eppendorf tube and evaporated to dryness.

The dry DNA pellet was then dissolved in 200 µL of water before quantification by absorption at 646 nm. The recovery yield was 1.5 nmol of **1Cy5**, equivalent to a recovery of 75%.

SI VIII2 Determination of the maximum loading capacity and recovery determination using oligonucleotides of different length

The maximum capacity of the resin was determined by sequential incubation of 100 µL (5 mg) of resin suspension with known amounts of **Oligo-Amine** (short 20 bases single strand DNA) or **qPCR-oligo** (85 bp double strand DNA, equivalent to a cycle 2 DNA tag) until saturation of the resin. Saturation was observed when some residual absorbance could be detected at 260 nm in the supernatant indicating that unbound DNA was left in solution.

Protocol for the determination of the loading capacity of the PEG+ resin for short single strand DNA oligonucleotide Oligo-Amine:

As set of 5 reference samples were prepared in order to determine the absorbance of solution containing 1, 2, 4, 6 and 10 nmoles of **Oligo-Amine** in 500 µL of water. As expected, the measured absorbance at 260 nm is linear with the concentration of the sample (Figure SI VIII2), and therefore the amount of residual amount of DNA in solution can then be quantified.

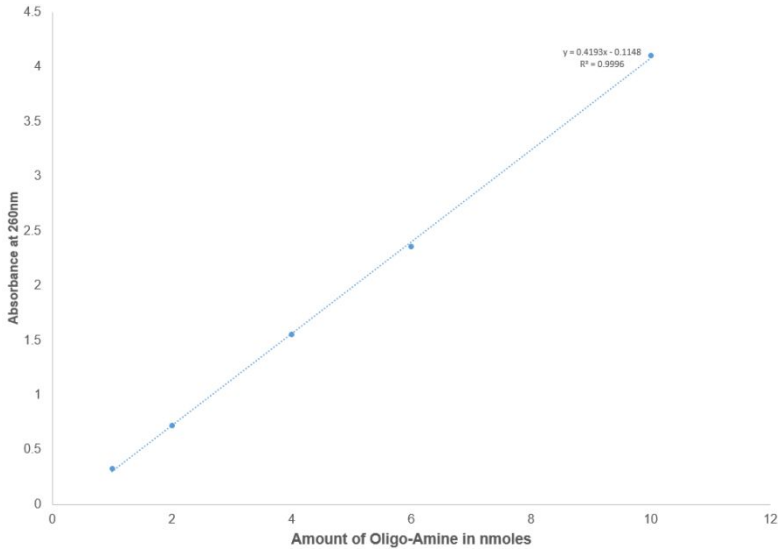


Figure SI VIII2: Absorbance at 260 nm of reference samples containing different amounts of **Oligo-Amines** in 500 µL of water. Absorbance above 1 were measured with an optical path length of 2 mm and extrapolated by the instrument to a path length of 10 mm.

In a 2 mL syringe equipped with a filter, the resin **PEG+** (1 g suspended in 20 mL with glycerol/water 1/1) (100 µL, 5 mg) was washed with water 5 times. The resin was then incubated sequentially with 500 µL of solution containing increasing amounts of **Oligo-Amine** for 15 min at 60°C. At the end of the loading cycle the absorbance at 260 nm of the supernatant was determined and is reported in the following table SI VIII1. Between each incubation, the water was drained and the resin was washed with 250 µL of water.

Loading cycle	Amount added (nmol)	Volume of loading solution (uL)	Absorbance of the loading solution at 260 nm	Absorbance of the supernatant at 260 nm at the end of the loading cycle	Calculated amount remaining in the supernatant (nmol)	Total amount loaded (nmol)
1	2	500	0.724	-0.09	0	2
2	2	500	0.724	-0.12	0	4
3	4	500	1.554	-0.004	0	8
4	4	500	1.554	-0.006	0	12
5	6	500	2.359	0.01	0	18
6	10	500	4.105	0	0	28
7	10	500	4.105	-0.002	0	38
8	10	500	4.105	-0.002	0	48
9	10	500	4.105	0.077	0.2	57.8

Table SI VIII1: Summary of the measured absorbance of the supernatant for the successive loading cycles using the resin **PEG+** (1 g suspended in 20 mL with glycerol:water 1:1) (100 µL, 5 mg). The absorbance of the supernatant can be compared to the absorbance of the reference samples (Figure SI VIII2).

The absorbance at 260 nm after the last loading cycle (cycle 9, table SI VIII1) indicated the presence of 0.2 nmoles left in solution, resulting in a total of 57.8 nmoles loaded onto 100 µL of PEG+ suspension (5 mg), corresponding to a capacity of 11.5 nmoles of **Oligo-Amine** for 1 mg of resin.

The loaded DNA was released by 3 successive treatment with 250 µL DNA elute solution (1.5 M NaCl, 50 mM phosphate buffer pH8, 0.005% triton-X, 5% 4-Me-piperidine) at 60°C for 30 min. The combined eluates were desalted using a NAP25 column (GE Healthcare illustra™ NAP™) resulting in a sample volume of 2500 µL with an absorbance at 260 nm of 3.372. This corresponded to an amount of 41 nmoles of **Oligo-Amine** recovered after release and a recovery yield of 71%.

Protocol for the determination of the loading capacity of the PEG+ resin for a cycle 2 model DNA tag qPCR-oligo (85 bp):

As set of 5 reference samples were prepared in order to determine the absorbance of solution containing 1, 2, 4, 6 and 10 nmoles of **qPCR-Oligo** in 500 µL of water. As expected the measured absorbance at 260 nm is linear with the concentration of the sample (Figure SI VIII3), and therefore the amount of residual amount of DNA in solution can then be quantified.

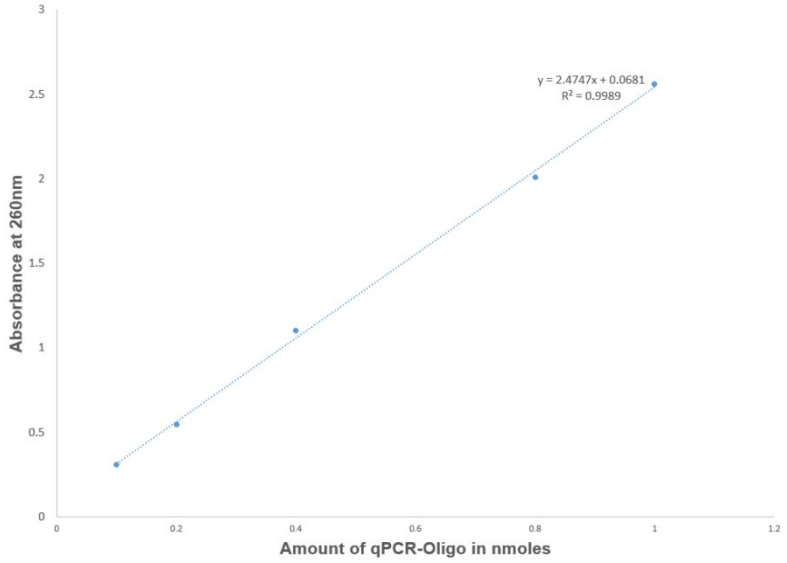


Figure SI VIII3: Absorbance at 260 nm of reference samples containing different amounts of **qPCR-Oligo** in 500 µL of water. Absorbance above 1 were measured with an optical path length of 2 mm and extrapolated by the instrument to a path length of 10 mm.

In a 2 mL syringe equipped with a filter, the resin **PEG+** (1 g suspended in 20 mL with glycerol:water 1:1) (100 µL, 5 mg) was washed with water 5 consecutive times. The resin was then incubated sequentially with 500 µL of solution containing increasing amounts of **qCR-oligo**

for 30 min at 60°C. At the end of the loading cycle the absorbance at 260 nm of the supernatant was determined and is reported in the following table SI VIII2. Between each incubation, the water was drained and the resin washed with 250 µL of water.

Loading cycle	Amount added (nmol)	Volume of loading solution (uL)	Absorbance of the loading solution at 260 nm	Absorbance of the supernatant at 260 nm at the end of the loading cycle	Calculated amount remaining in the supernatant (nmol)	Total amount loaded (nmol)
1	0.1	500	0.31	>0.05	0	0.1
2	0.1	500	0.31	>0.05	0	0.2
3	0.1	500	0.31	>0.05	0	0.3
4	0.1	500	0.31	>0.05	0	0.4
5	0.2	500	0.55	>0.05	0	0.6
6	0.2	500	0.55	>0.05	0	0.8
7	0.4	500	1.10	>0.05	0	1.2
8	0.8	500	2.01	>0.05	0	2
9	1	500	2.56	0.12	0.05	2.95

Table SI VIII2: Summary of the measured absorbance of the supernatant for the successive loading cycles using the resin **PEG+** (1 g suspended in 20 mL with glycerol:water 1:1) (100 µL, 5 mg) and the oligonucleotide **qPCR-oligo**. The absorbance of the supernatant can be compared to the absorbance of the references samples (Figure SI VIII3).

The absorbance at 260 nm after the last loading cycle (cycle 9, table SI VIII2) indicated the presence of 0.05 nmoles left in solution, resulting in a total of 2.95 nmoles loaded onto 100 µL of PEG+ suspension (5 mg), corresponding to a capacity of 0.59 nmoles of **qPCR-Oligo** for 1 mg of resin.

The recovery yield was determined on a different sample loaded in an identical manner with a total amount of **qPCR-oligo** of 2.2 nmoles. The loaded DNA was released by 3 successive treatment with 250 µL DNA elute solution (1.5 M NaCl, 50mM phosphate buffer pH8, 0.005% triton-X, 5% 4-Me-piperidine) at 60°C for 30 min. The combined eluates were desalted using a NAP10 column (GE Healthcare illustra™ NAP™) resulting in a sample volume of 1500 µL with an absorbance at 260 nm of 1.011. This corresponded to an amount of 1.18 nmoles of **qPCR-oligo** recovered after release and a recovery yield of 54%. The same release procedure was repeated resulting in the recovery of an additional 0.112 nmoles (5%) of **qPCR-oligo** and a total recovery yield of 59%.

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