Supporting Information

Synthetic Studies towards DNA Encoded Heterocycles Based on the On-DNA formation of α,β-Unsaturated Ketones

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Abbreviated Terms

ACN: acetonitrile

DMA: dimethylacetamide

HATU: 1-bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate

DIPEA: N, N-diisopropylethylamine

TosMIC: (1-((isocyanomethyl)sulfonyl)-4-methylbenzene)

DDQ: (2,3-dicyano-5,6-dichloro-p-benzoquinone)

TBHP: (tert-butylhydroperoxide)

DBU: (1,8-diazabicyclo[5.4.0] undec-7-ene)

NIS: (1-iodopyrrolidine-2,5-dione)

Scavenger：diethyldithiocarbamic acid sodium salt

pH=9.4 Buffer: 500 mM sodium borate in water

pH=5.5 Buffer: 500 mM sodium phosphate in water

General procedure for "on-DNA" Synthesis

Materials

Headpiece (5'-/5phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3', Figure S1) was obtained from Biosearch Technologies, Novato, CA.

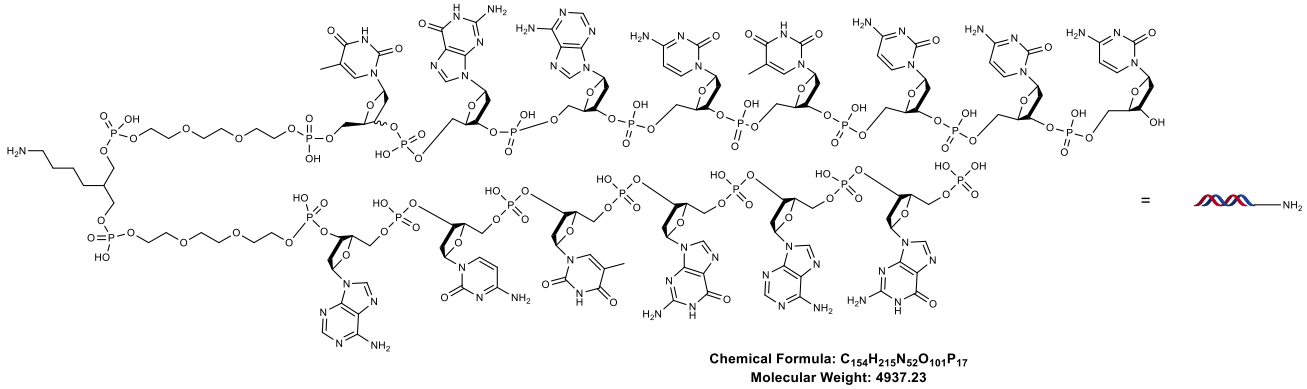


Figure S1. Headpiece: 5'-/5phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3', MW = 4937.2

Dimethylsulfoxide (DMSO), acetonitrile (ACN), 1-((isocyanomethyl)sulfonyl)-4-methylbenzene (TosMIC), N, N-dimethylacetamide (DMAc), 2,3-dicyano-5,6-dichloro-p-benzoquinone (DDQ), tert-butylhydroperoxide (TBHP), 1,8-diazabicyclo [5.4.0] undec-7-ene(DBU), 1-iodopyrrolidine-2,5-dione (NIS) and EtOH were purchased from Sigma-Aldrich. HATU (CAS: 148893-10-1), N, N-Diisopropylethylamine (DIPEA), NaCl, NaOAc were purchased from TCI. The MgCl2 was purchased from J&K. The ddH2O was obtained by passing the Milli-Q Direct. The buffer was purchased from Vazyme.

Analysis for DNA Compounds

The conversion rate was used to calculate the effect off one-step reaction according to the proportion of peak height of mass spectrum.

Analysis was performed by HPLC/ESI-MS. After reaction, an aliquot of the reaction mixture solution was diluted (typically a 1 μL aliquot diluted with 20 μL of water) for LC/MS. Reversephase chromatography column (XBridge C18, 3.5um 3.0*50mm) with monitoring at 260 nm.

Solvent A: 2.25% hexafluoroisopropanol(v/v) and 0.114% Triethylamine(v/v) in deionized water.

Solvent B: 2.25% hexafluoroisopropanol(v/v) and 0.114% Triethylamine(v/v) in 90/10 methanol/water. Flow rate: 0.450 mL/min; Time: 6.00 min.

Ethanol Precipitation for DNA

To a DNA reaction mixture was added 10% (v/v) 5 M NaCl solution and 2.5−3 times the volume of absolute ethanol. The colloidal solution was then allowed to sit at −80 °C for 0.5-1 hour. After centrifugation for 30 minutes at 4 ℃ in a microcentrifuge at 10 K rpm. The above supernatant was removed and the pellet (precipitate) was cooled in liquid nitrogen and then placed on a lyophilizer. After lyophilization, the dry pellet was recovered.

Quantification for DNA

The on-DNA Intermediate were purified by a 3 K Spinfilter tube after centrifugation for 30 min at 4 ℃ in a microcentrifuge at 10 K rpm. three times. Use the Thermo ScientificTM NanoDropTM One to quantify concentration of products under the ds-DNA mode.

Heating for the on-DNA reactions

All the on-DNA reactions were heating using the pcr thermocycler.

Experimental Procedure for on-DNA Reactions

Preparation of HP-Ar-CHO 1

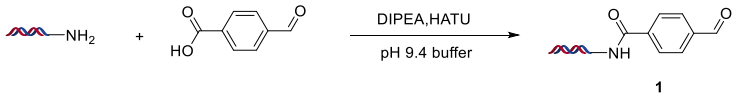


Figure S2. HP-Ar-CHO preparation

Materials

Headpiece: 2 mM in water

4-formyl benzoic acid: 200 mM in DMA

DIPEA: N, N-Diisopropylethylamine 200 mM in DMA

HATU: 2-(7-Azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate 200mM in DMA

pH 9.4 Buffer: 500 mM sodium borate in water (pH 9.4)

Procedure

1) Mixing 4-formyl benzoic acid with equal volume HATU and DIPEA to prepare the solution IV.
2) To the headpiece solution (200 nmol, 100 μL), was added 100 μL pH 9.4 buffer solution, 40 equiv. of solution IV (120 μL), and mix.
3) React at room temperature for 2 hours.
4) The product was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 1

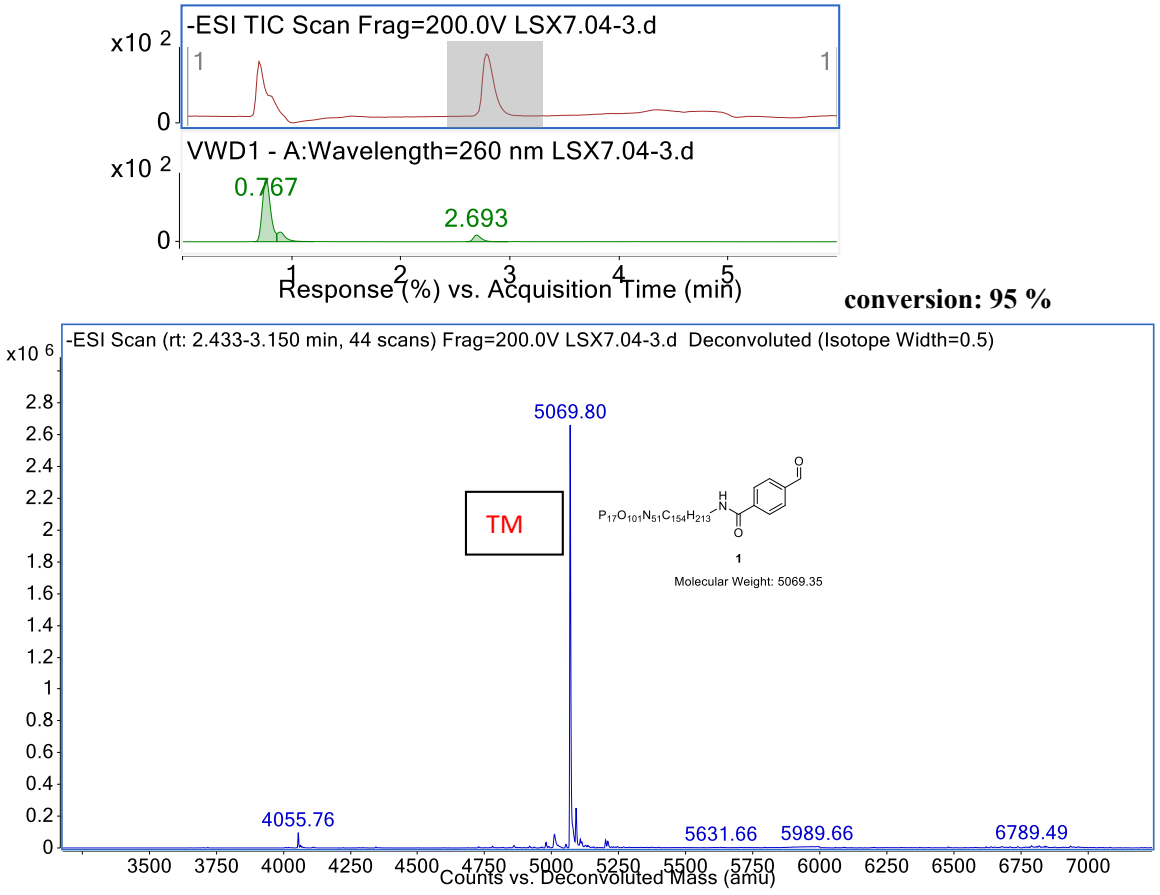


Figure S3. Deconvoluted mass of on-DNA product **1**

Preparation of on-DNA Product α,β-Unsaturated Ketones:

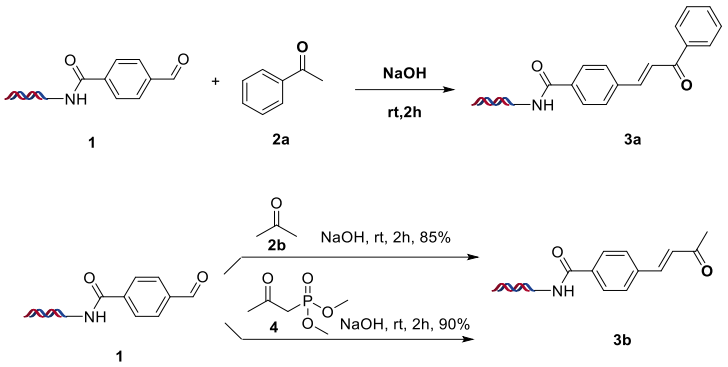


Figure S4. Compound 3a-3t preparation

Materials

1: 1 mM in water

Acetophenone **2**:200 mM in DMA

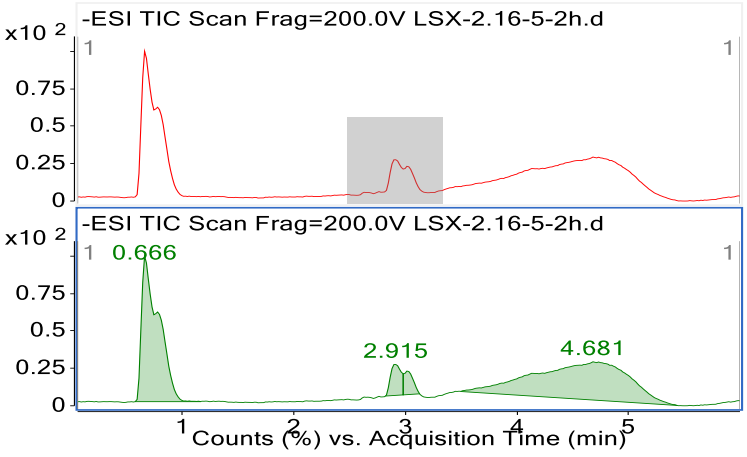
Dimethyl (2-oxopropyl) phosphonate **4**: 200 mM in DMA

NaOH: 200 mM in water

Procedure

1) To **1** solution (5 nmol, 5 μL), was added 5 μL acetophenone (200 equiv) or 5 μL Dimethyl (2-oxopropyl)phosphonate (200 equiv), 5ul NaOH (200 equiv). The mixture was vortexed.
2) The reaction mixture at 25 °C for 2 h.
3) After the reaction, the product was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 3a



conversion: 92 %

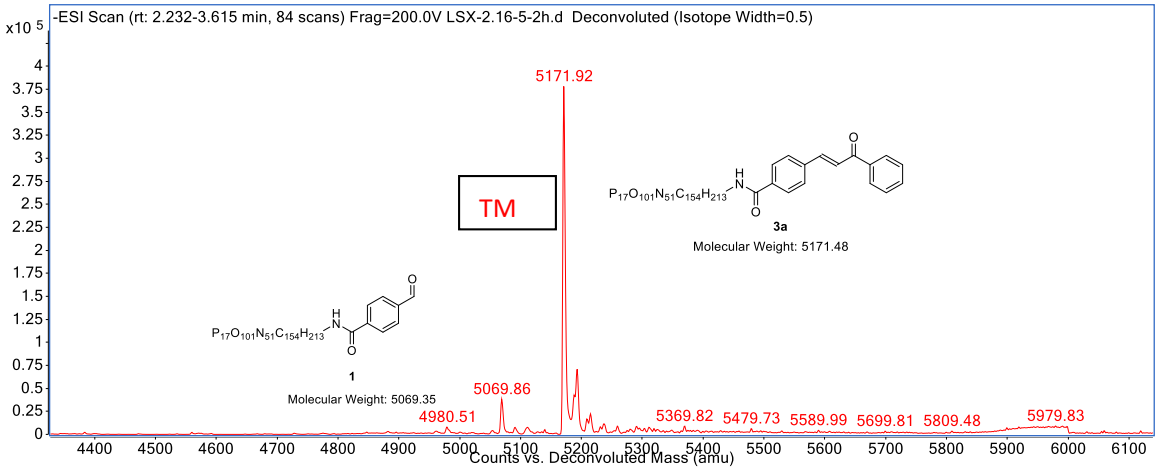
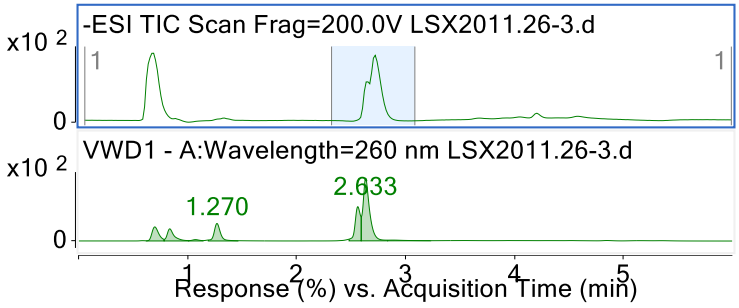


Figure S5. Deconvoluted mass of on-DNA product **3a**

Mass Spectrum of on-DNA Product 3b from 2b



conversion: 85 %

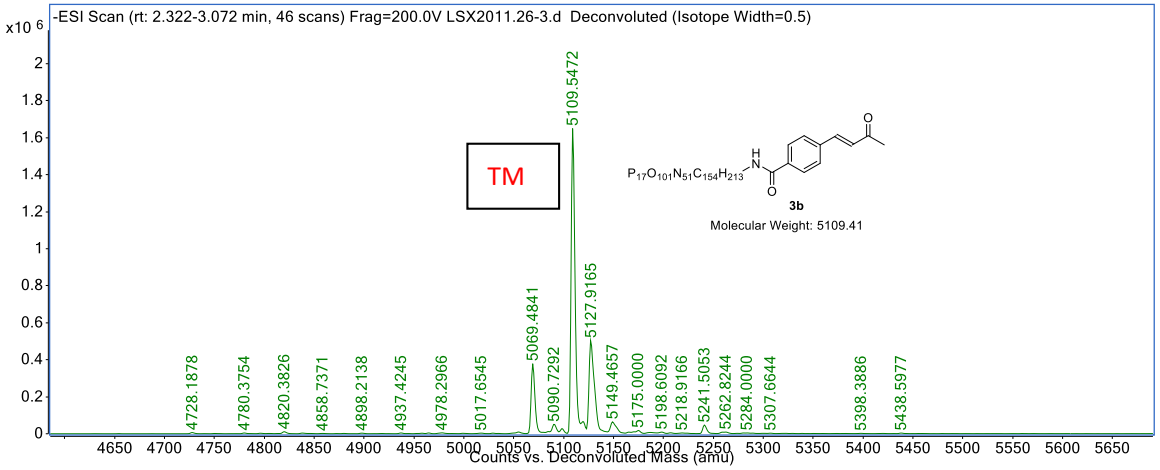


Figure S6. Deconvoluted mass of on-DNA product **3b** from 2b

Mass Spectrum of on-DNA Product 3b from 4

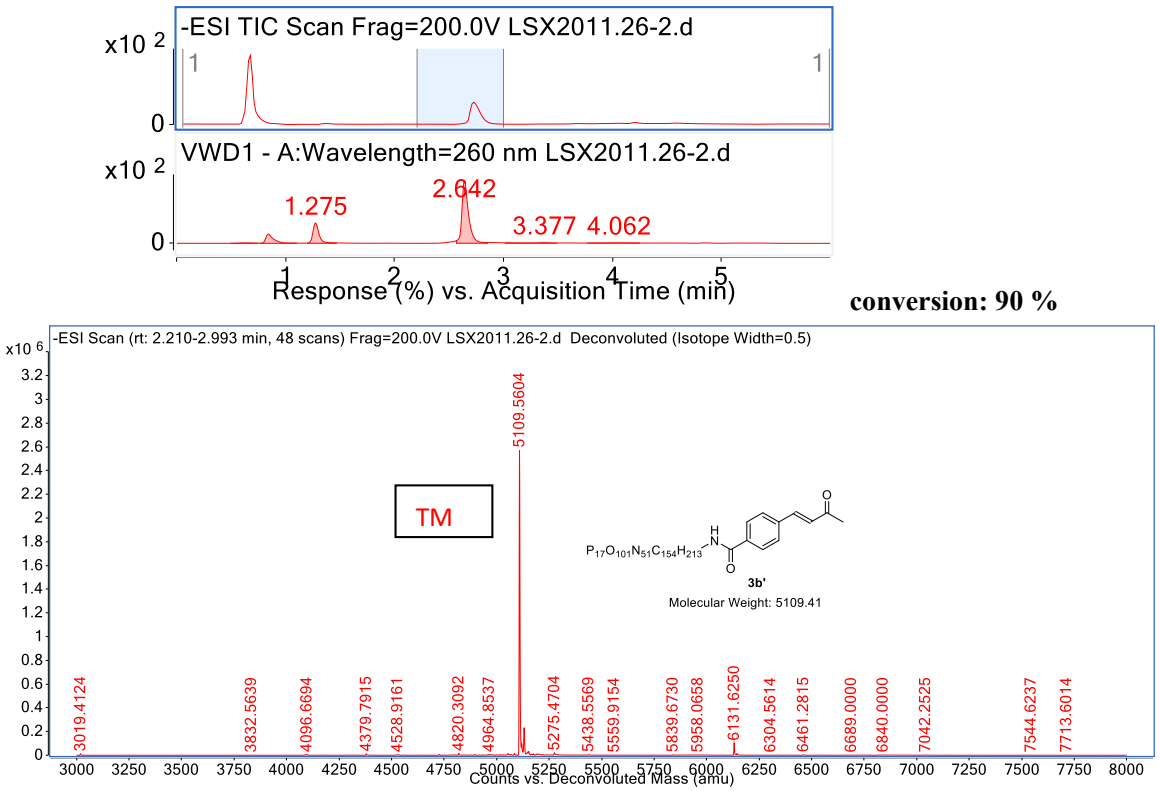


Figure S7. Deconvoluted mass of on-DNA product **3b** from 4

Mass Spectrum of on-DNA Product 3c

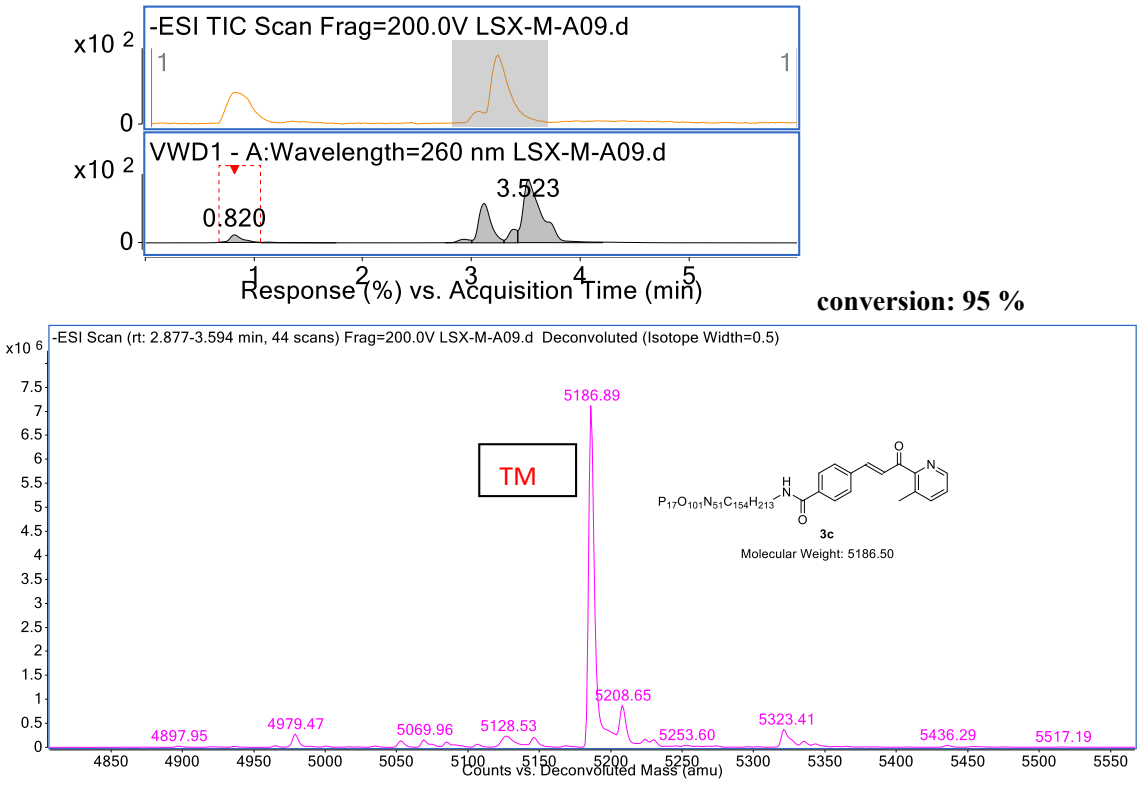


Figure S8. Deconvoluted mass of on-DNA product **3c**

Mass Spectrum of on-DNA Product 3d

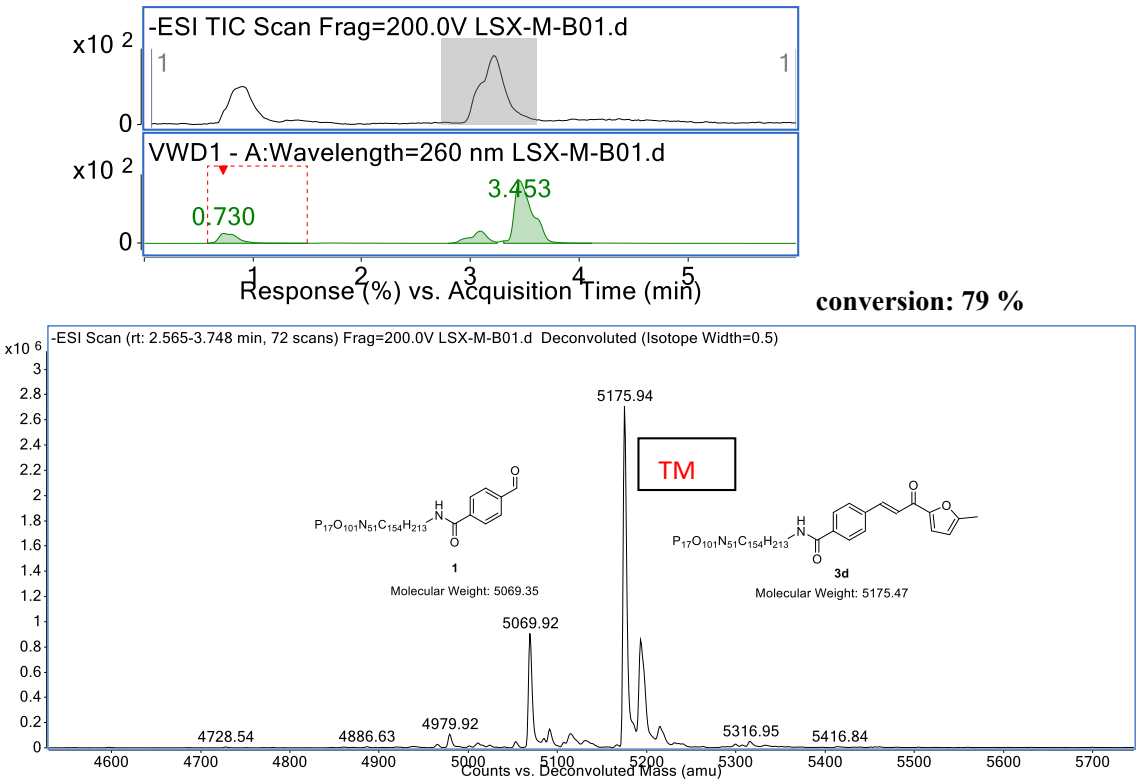


Figure S9. Deconvoluted mass of on-DNA product **3d**

Mass Spectrum of on-DNA Product 3e

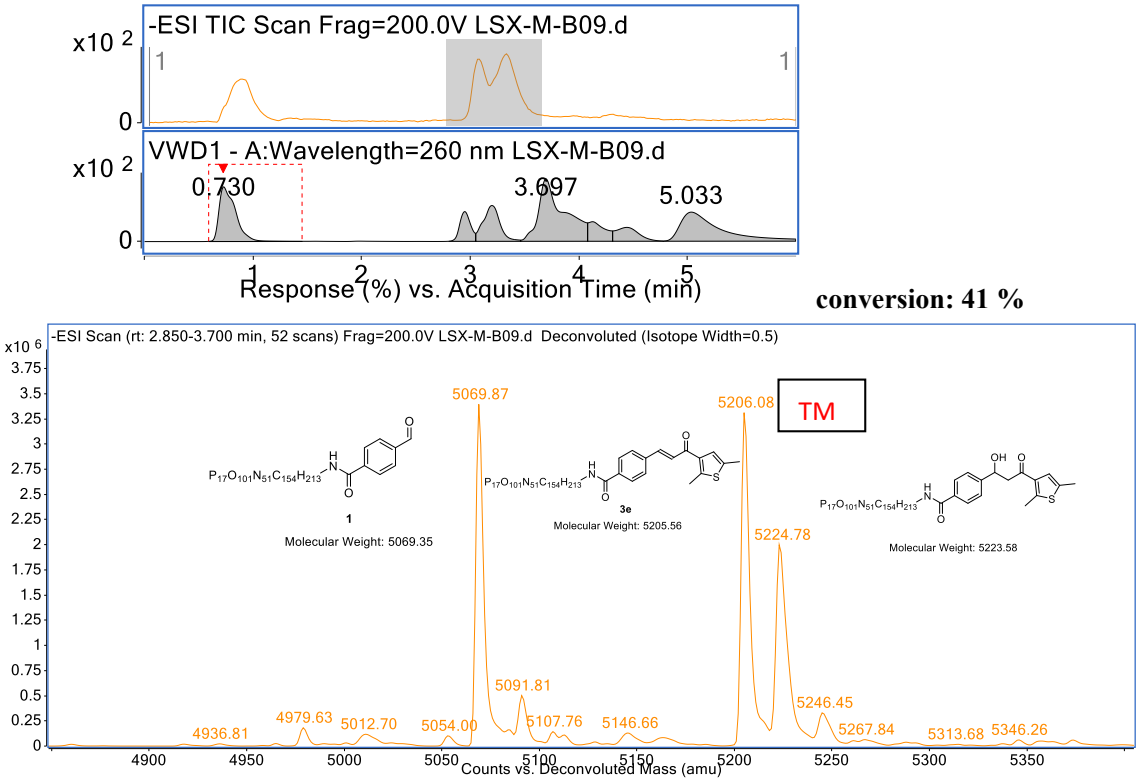


Figure S10. Deconvoluted mass of on-DNA product **3e**

Mass Spectrum of on-DNA Product 3f

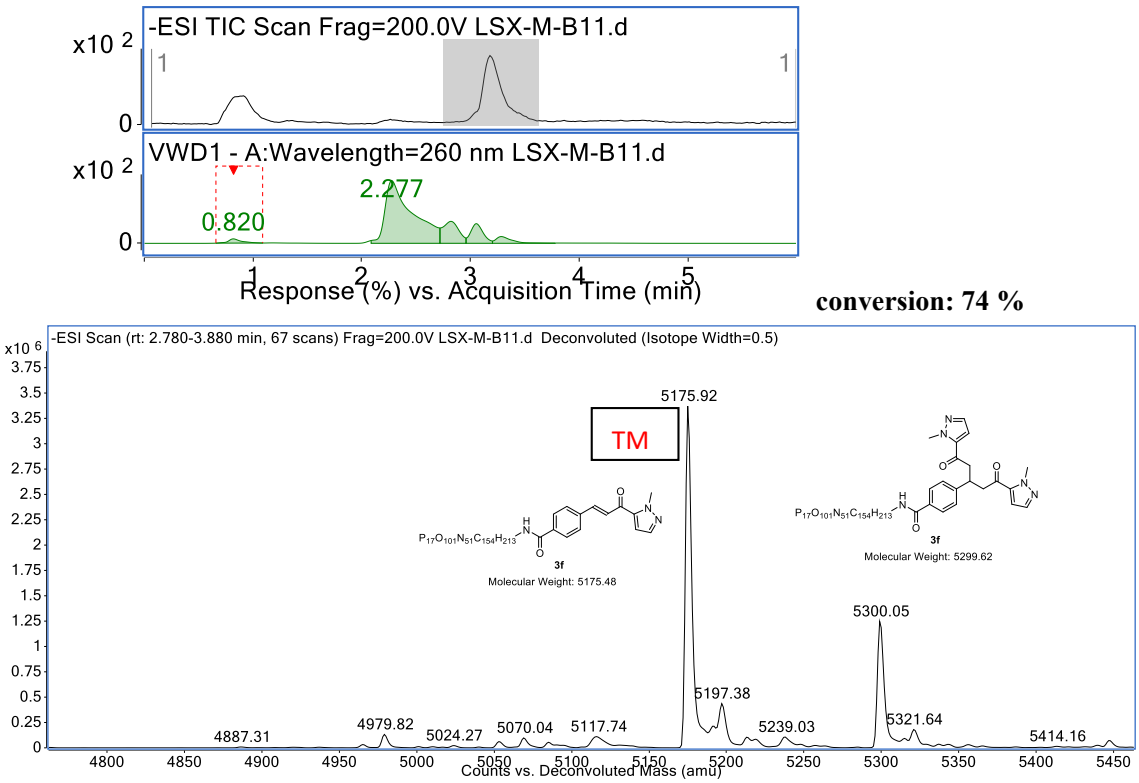


Figure S11 Deconvoluted mass of on-DNA product **3f**

Mass Spectrum of on-DNA Product 3g

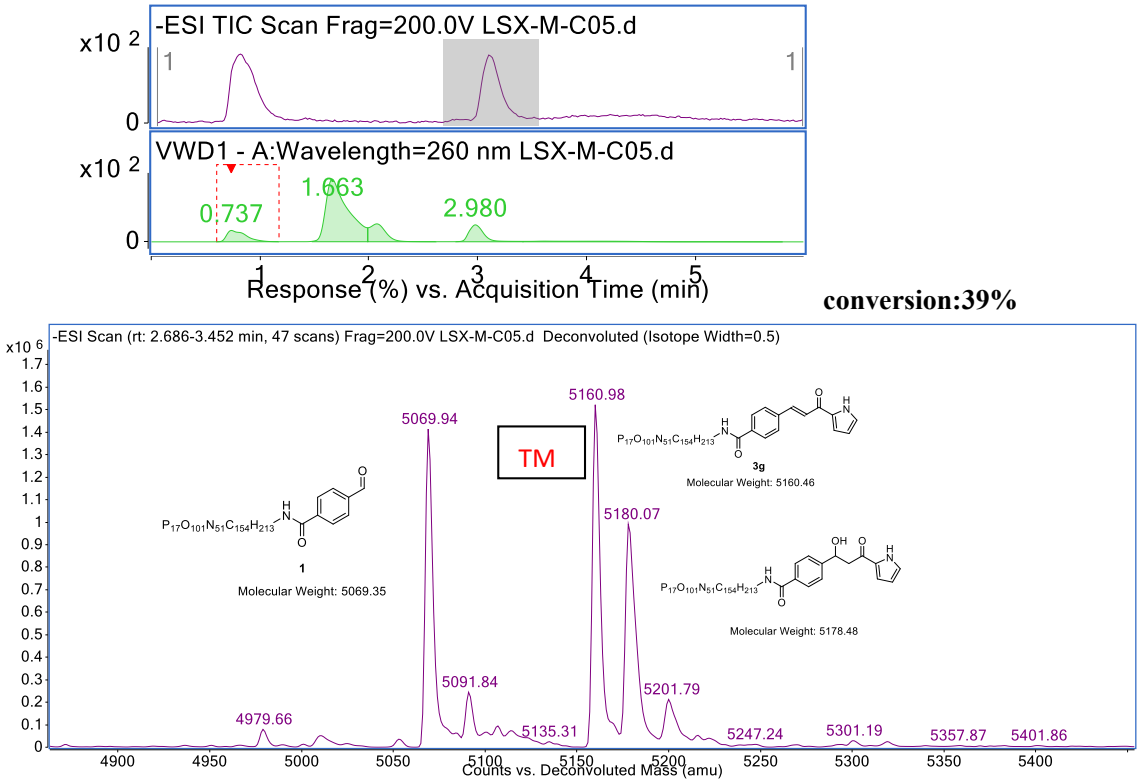


Figure S12 Deconvoluted mass of on-DNA product **3g**

Mass Spectrum of on-DNA Product 3h

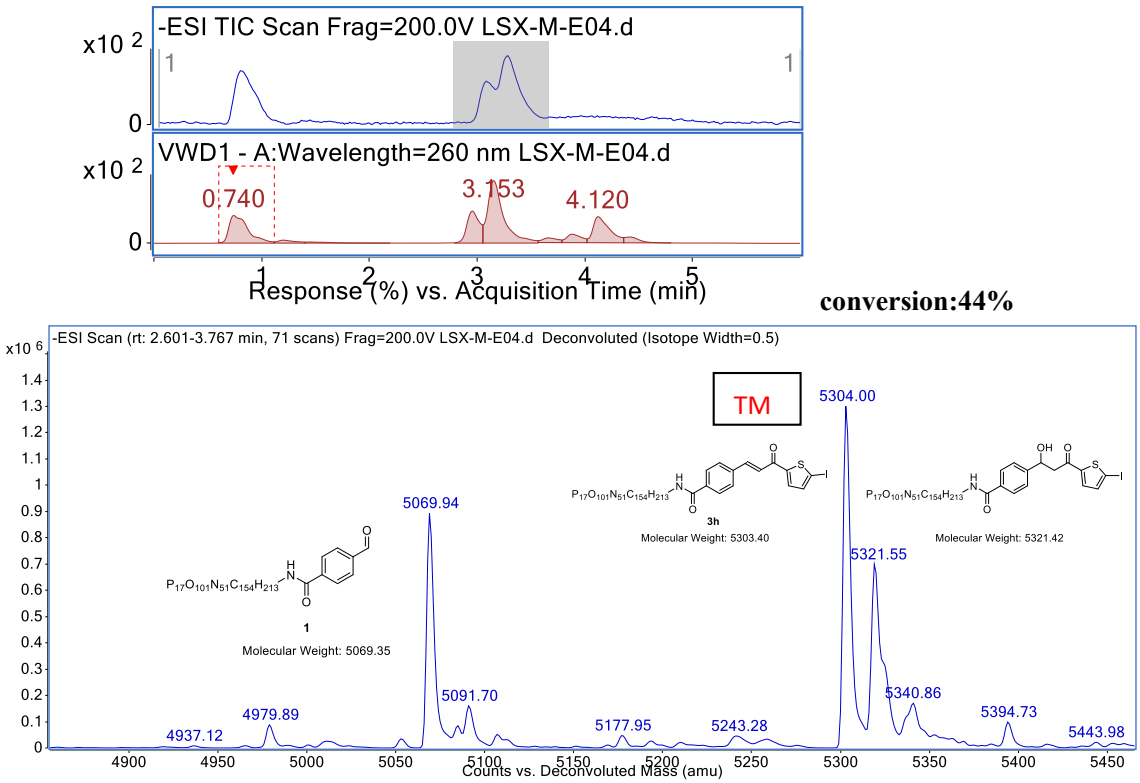


Figure S13 Deconvoluted mass of on-DNA product **3h**

Mass Spectrum of on-DNA Product 3i

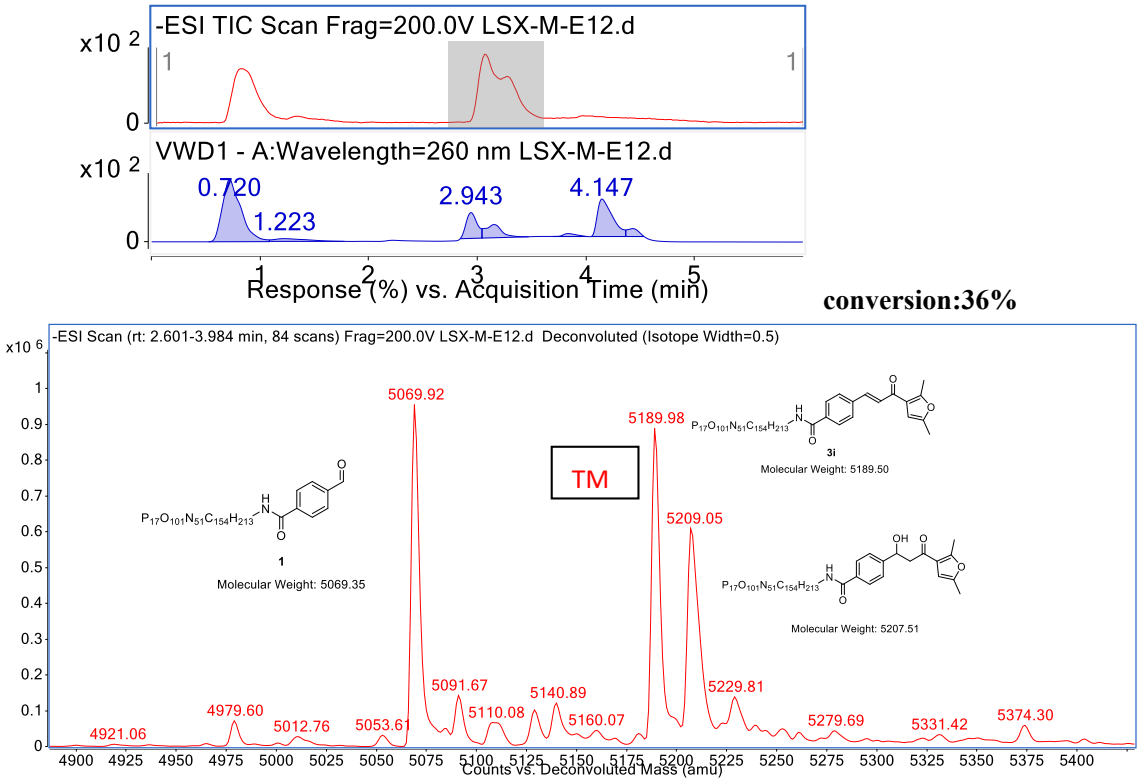


Figure S14 Deconvoluted mass of on-DNA product **3i**

Mass Spectrum of on-DNA Product 3j

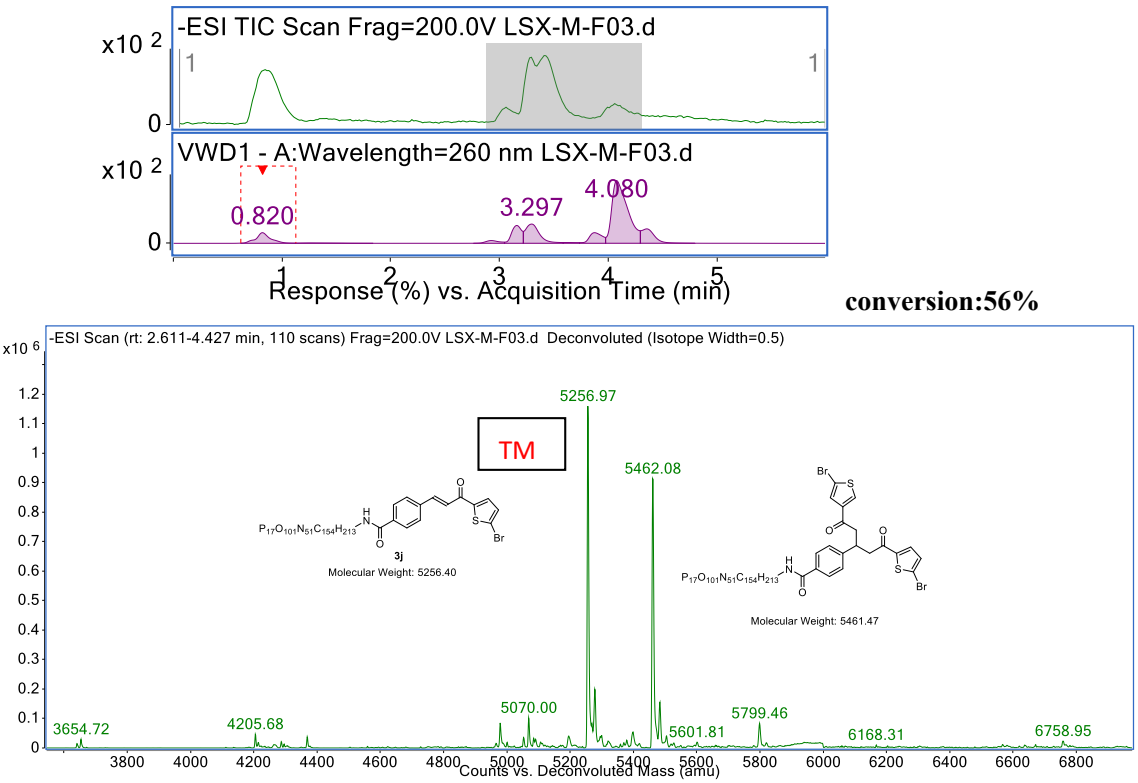


Figure S15 Deconvoluted mass of on-DNA product **3j**

Mass Spectrum of on-DNA Product 3k

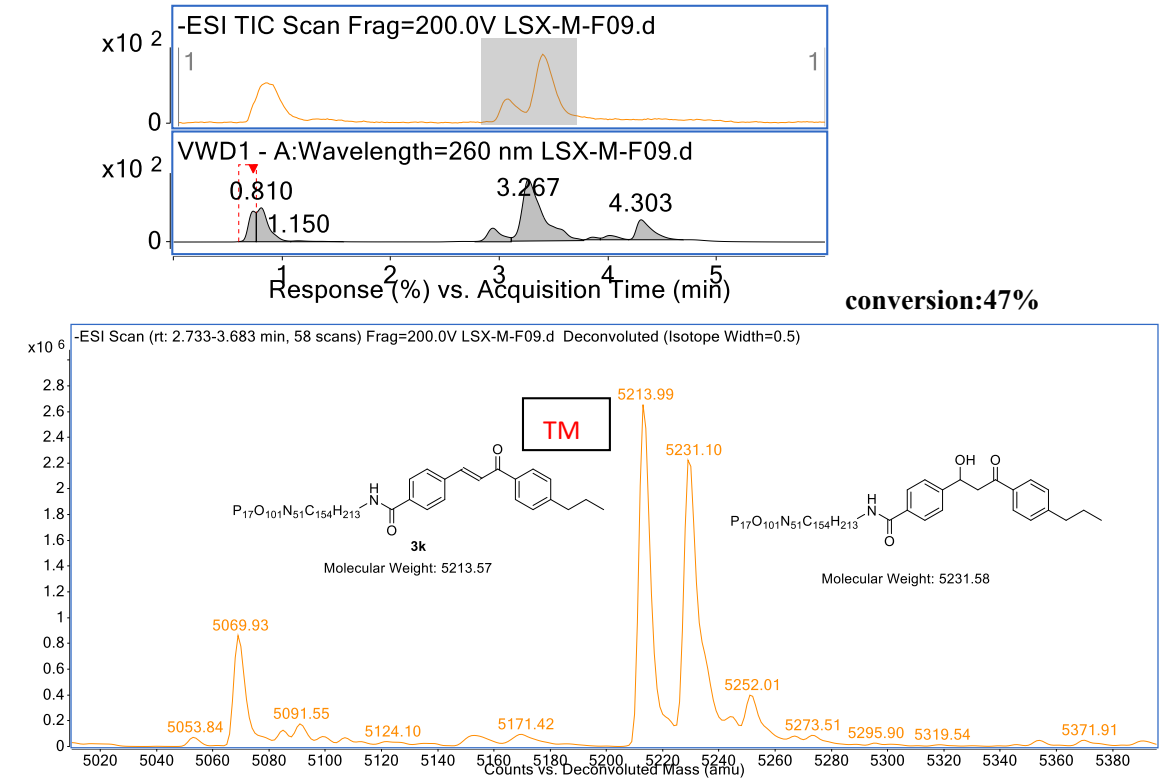


Figure S16 Deconvoluted mass of on-DNA product **3k**

Mass Spectrum of on-DNA Product 3l

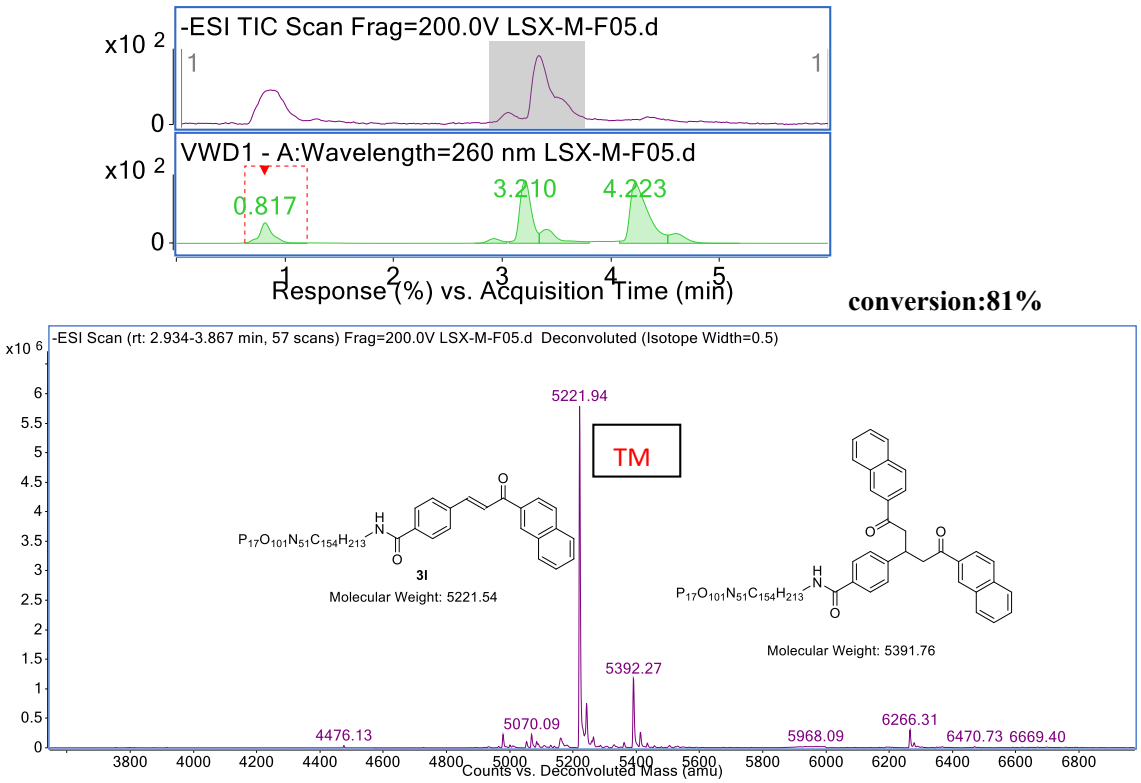


Figure S17 Deconvoluted mass of on-DNA product **3l**

Mass Spectrum of on-DNA Product 3m

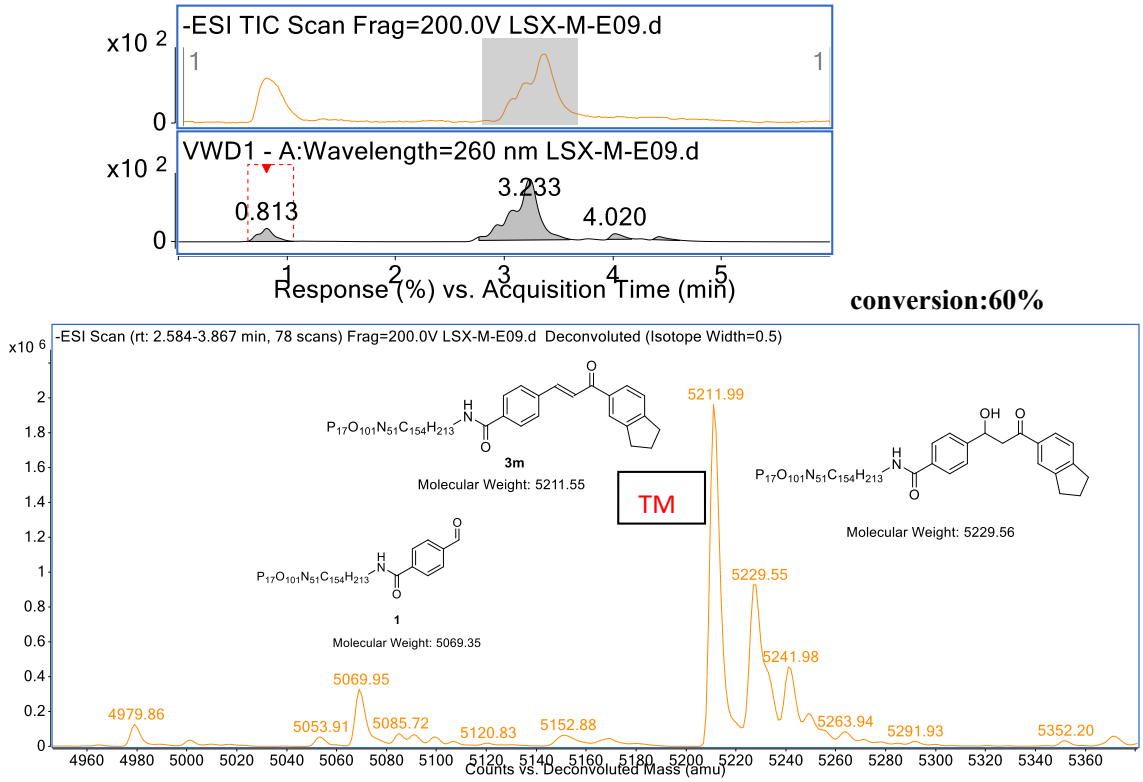


Figure S18 Deconvoluted mass of on-DNA product **3m**

Mass Spectrum of on-DNA Product 3n

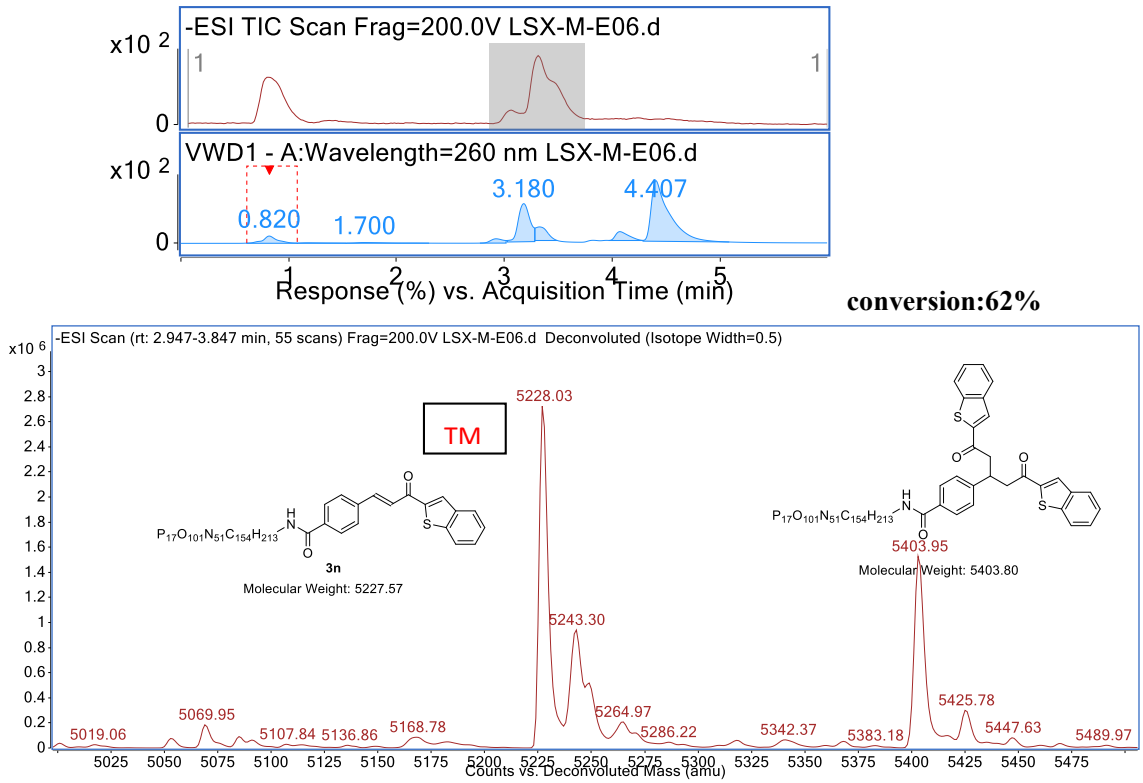


Figure S19 Deconvoluted mass of on-DNA product **3n**

Mass Spectrum of on-DNA Product 3o

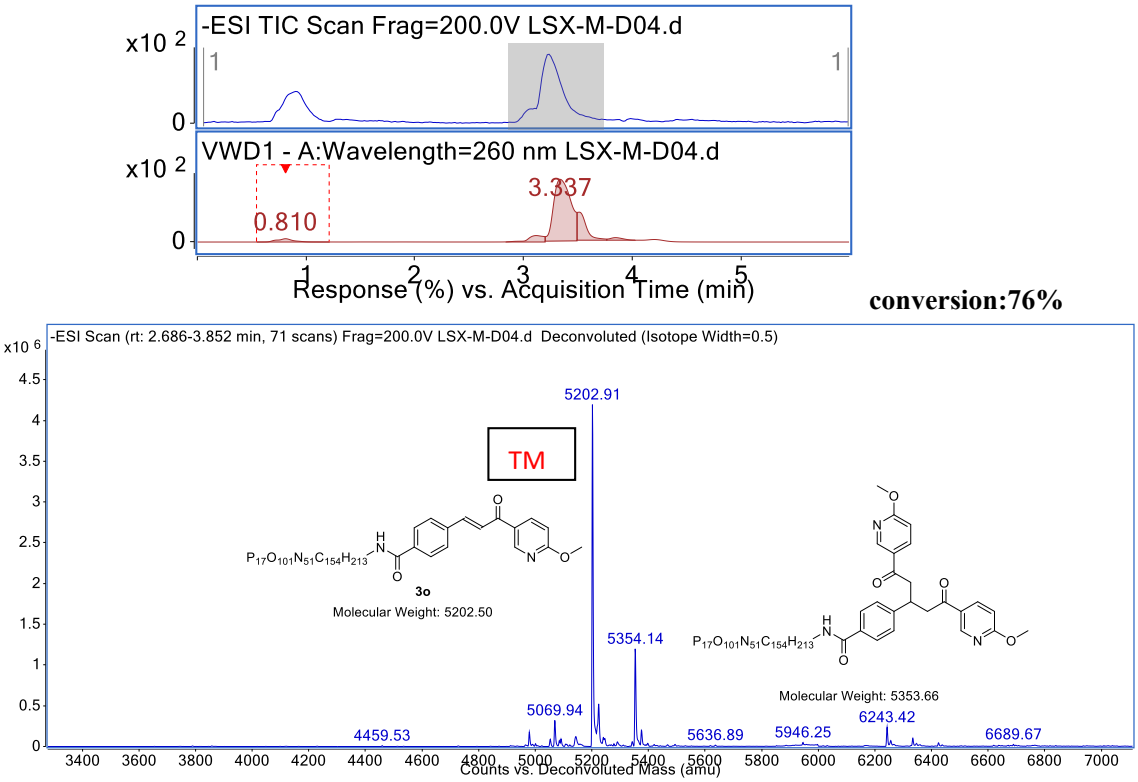


Figure S20 Deconvoluted mass of on-DNA product **3o**

Mass Spectrum of on-DNA Product 3p

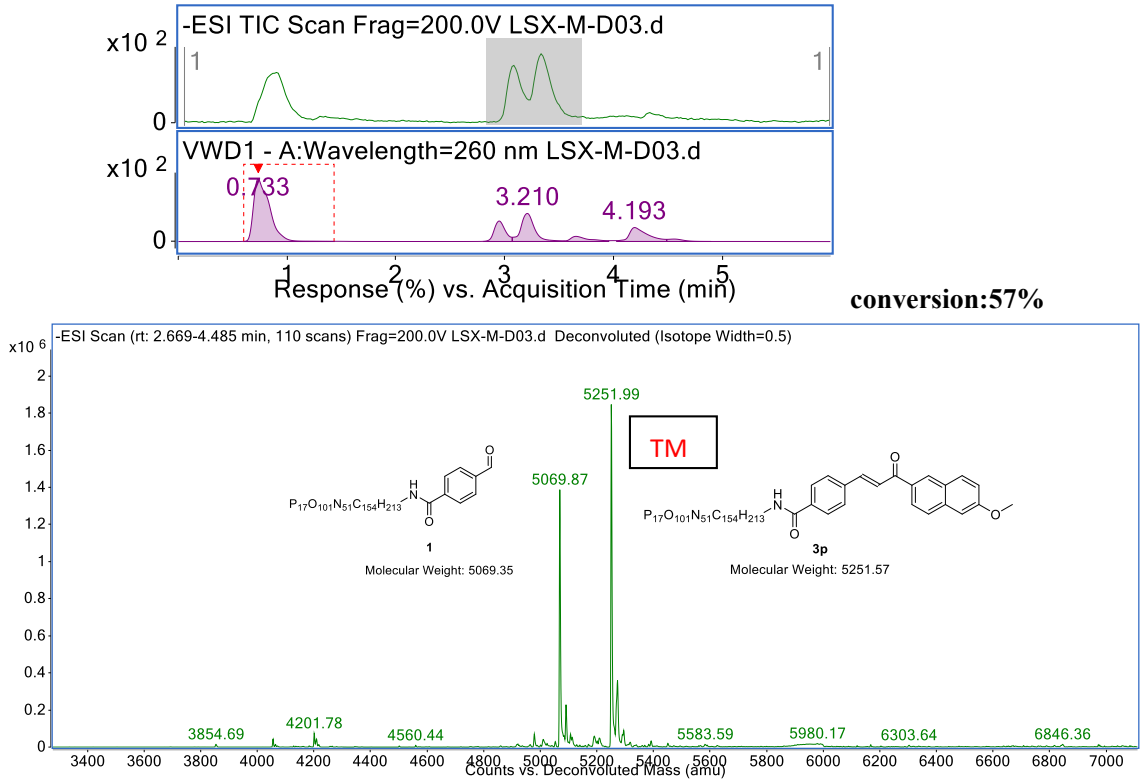


Figure S21 Deconvoluted mass of on-DNA product **3p**

Mass Spectrum of on-DNA Product 3q

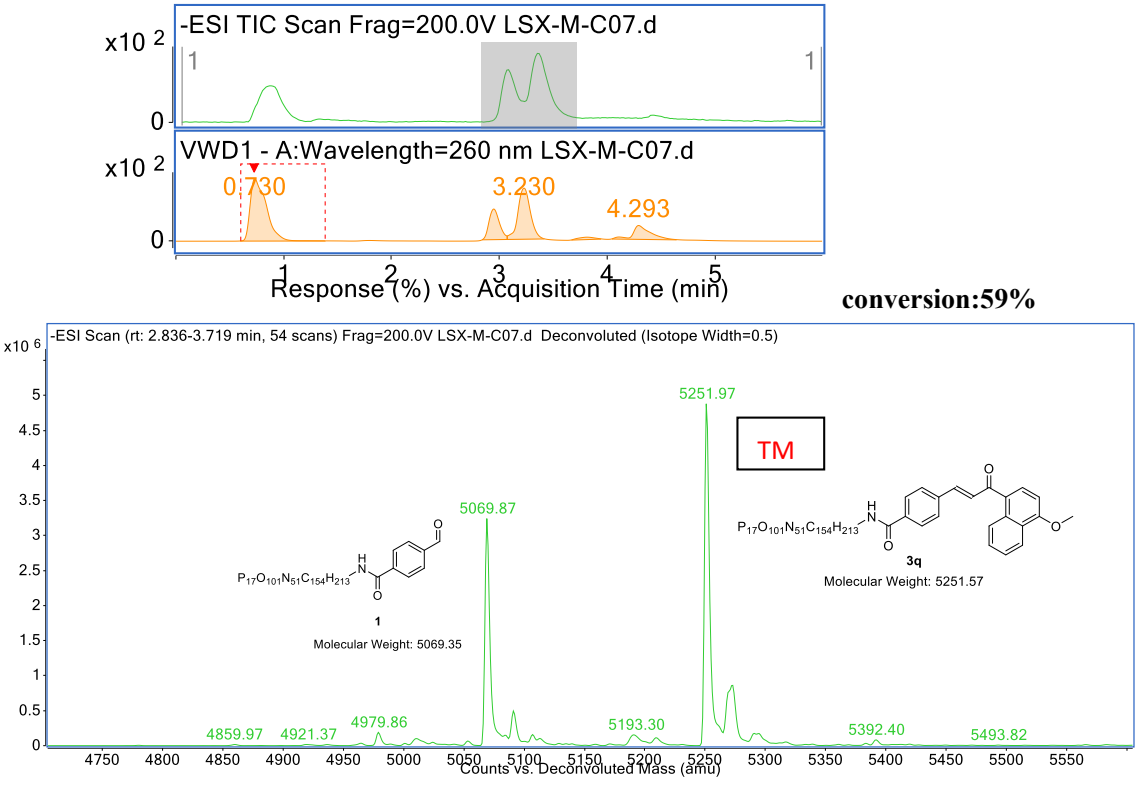


Figure S22 Deconvoluted mass of on-DNA product **3q**

Mass Spectrum of on-DNA Product 3r

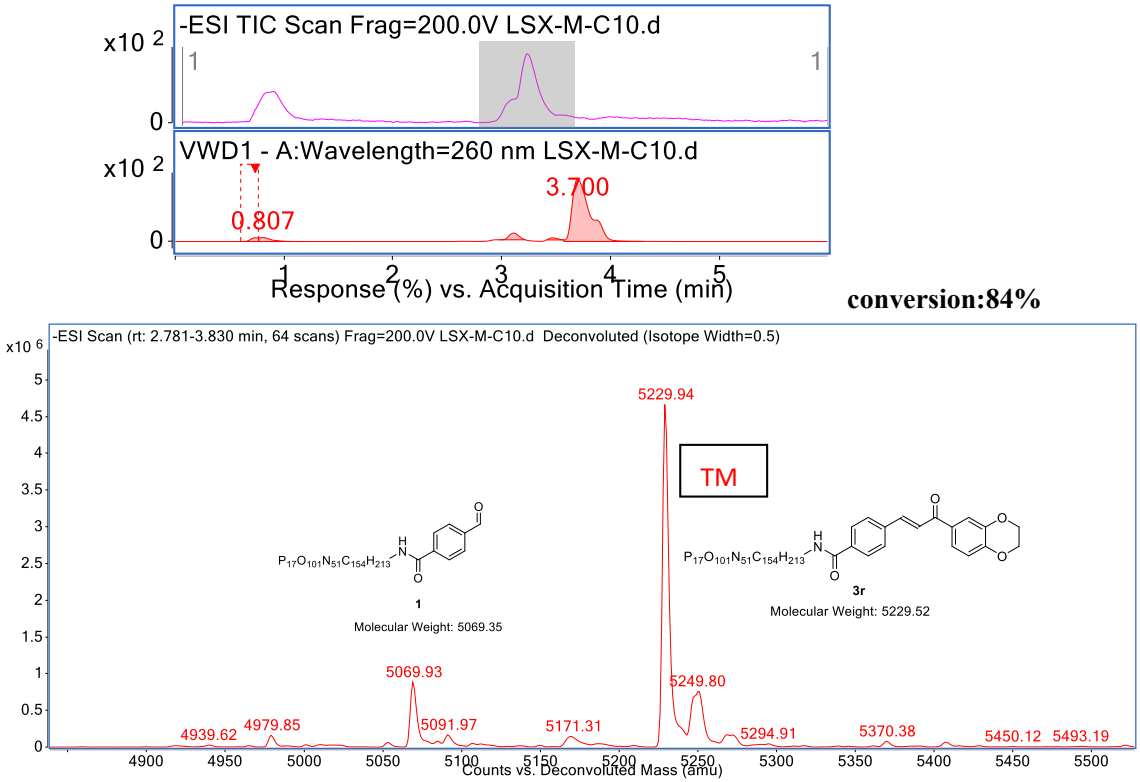


Figure S23 Deconvoluted mass of on-DNA product **3r**

Mass Spectrum of on-DNA Product 3s

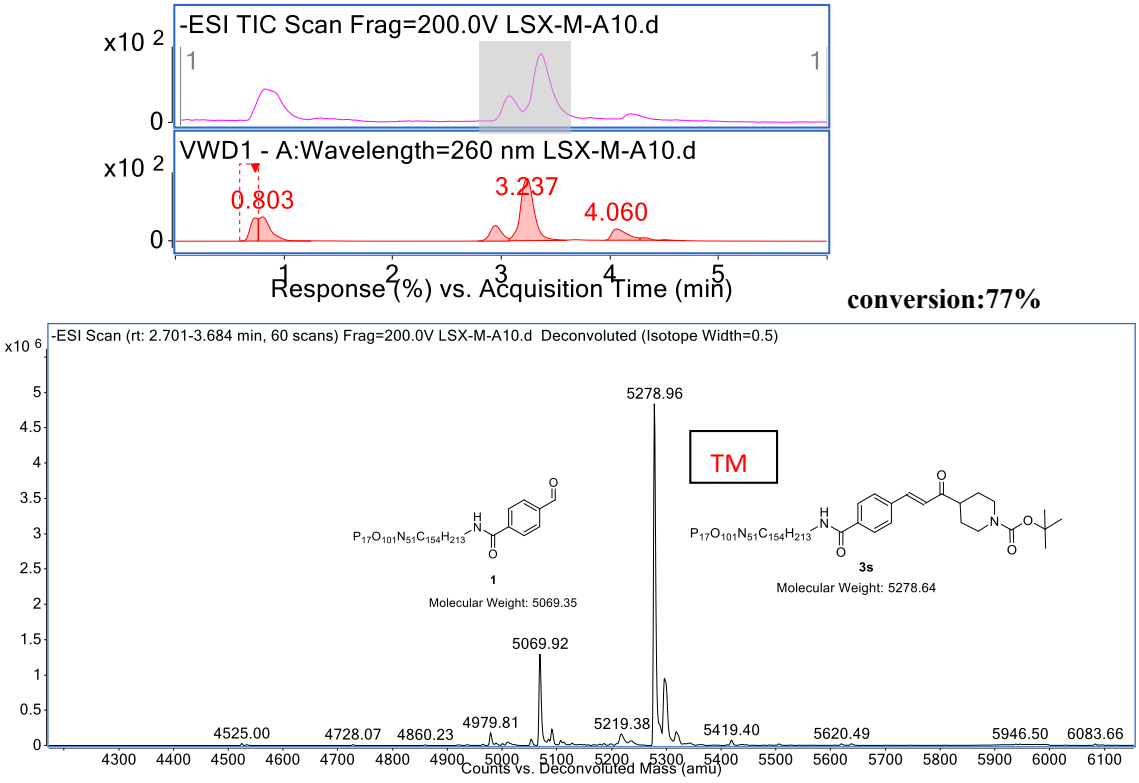


Figure S24 Deconvoluted mass of on-DNA product **3s**

Mass Spectrum of on-DNA Product 3t

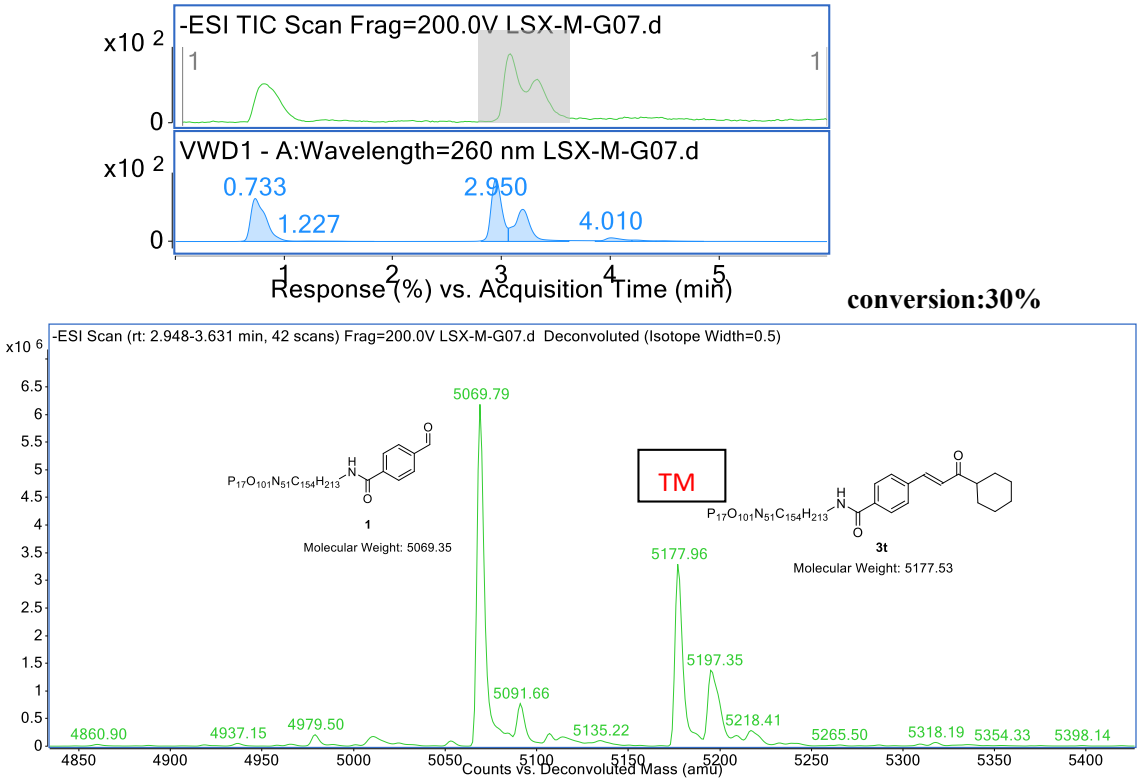


Figure S25 Deconvoluted mass of on-DNA product **3t**

Preparation of on-DNA Product 5a-5g

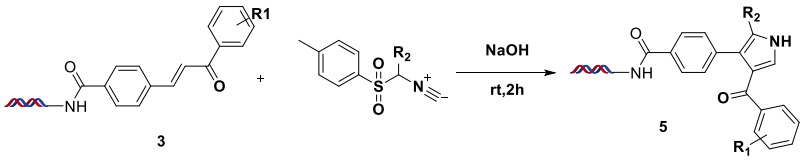


Figure S26 Synthetic route of on-DNA product **5a-g**

Materials

3: 1 mM in water

TosMIC (1-((isocyanomethyl)sulfonyl)-4-methylbenzene): 200 mM in DMA

NaOH: 200 mM in water

Procedure

1) To **3** solution (5 nmol, 5 μL), was added 5 μL TosMIC (200 equiv) , 5ul NaOH (200 equiv). The mixture was vortexed.
2) The reaction mixture at 25 °C for 2 h.
3) After the reaction, the product was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 5a

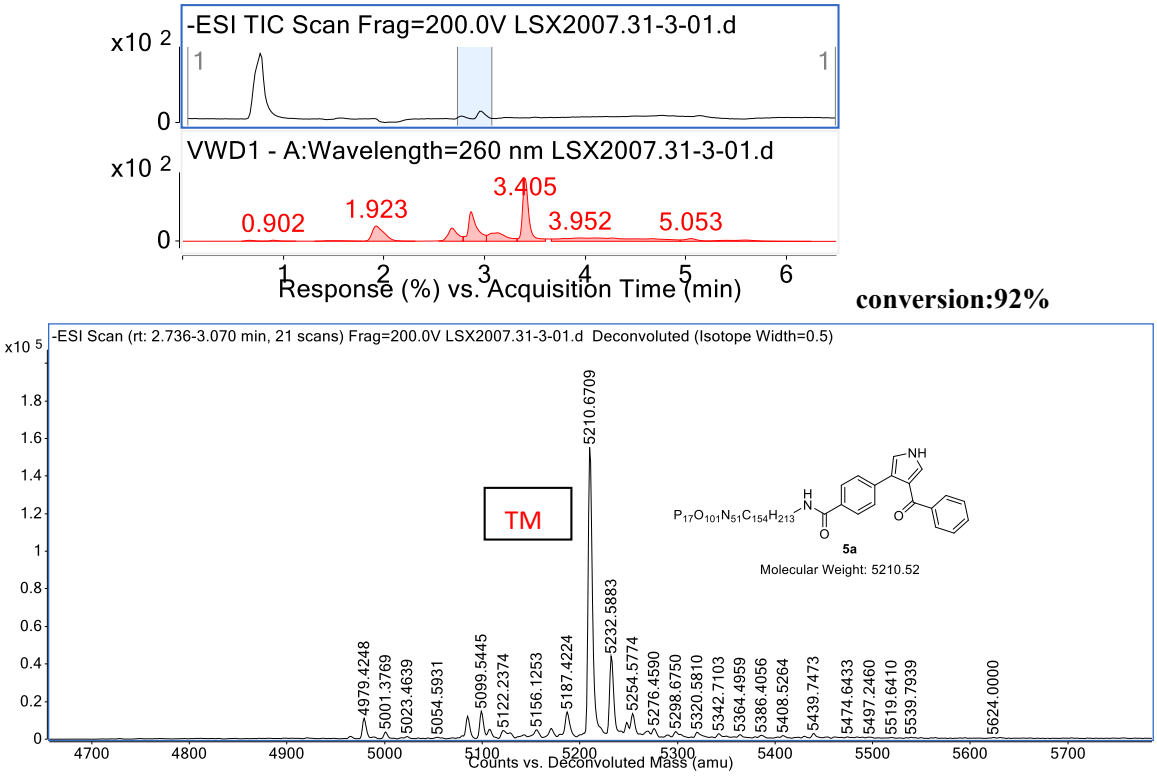


Figure S27 Deconvoluted mass of on-DNA product **5a**

Mass Spectrum of on-DNA Product 5b

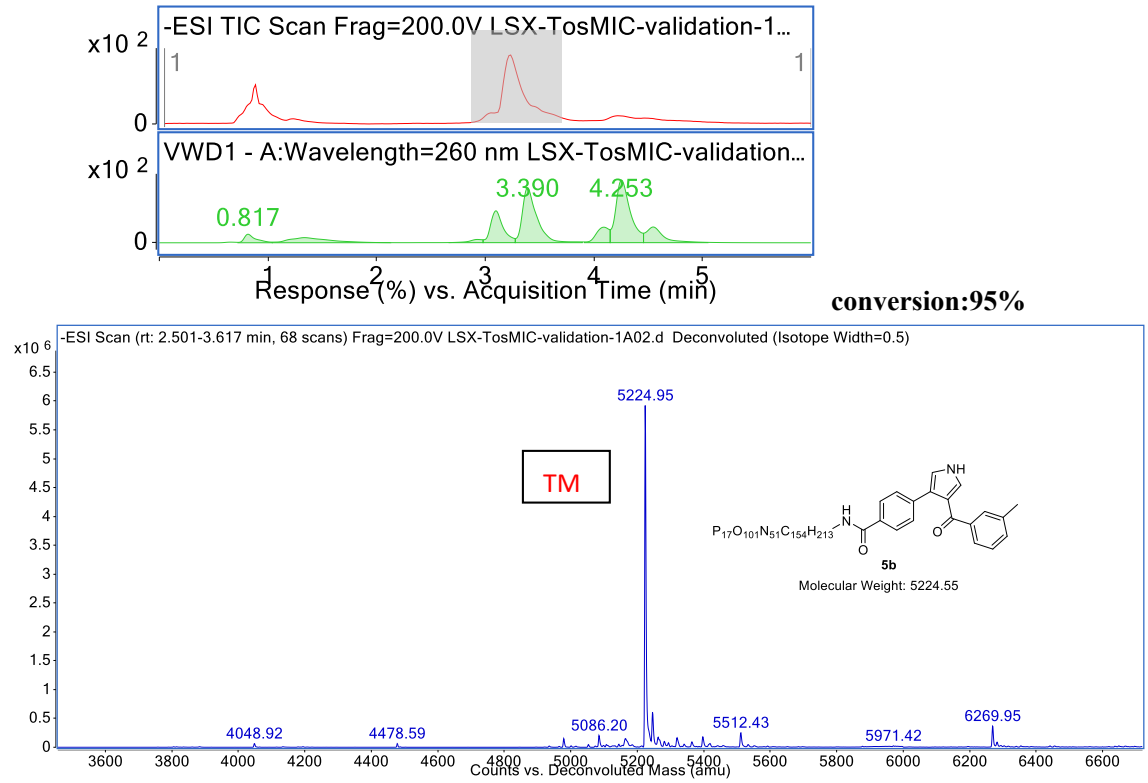


Figure S28 Deconvoluted mass of on-DNA product **5b**

Mass Spectrum of on-DNA Product 5c

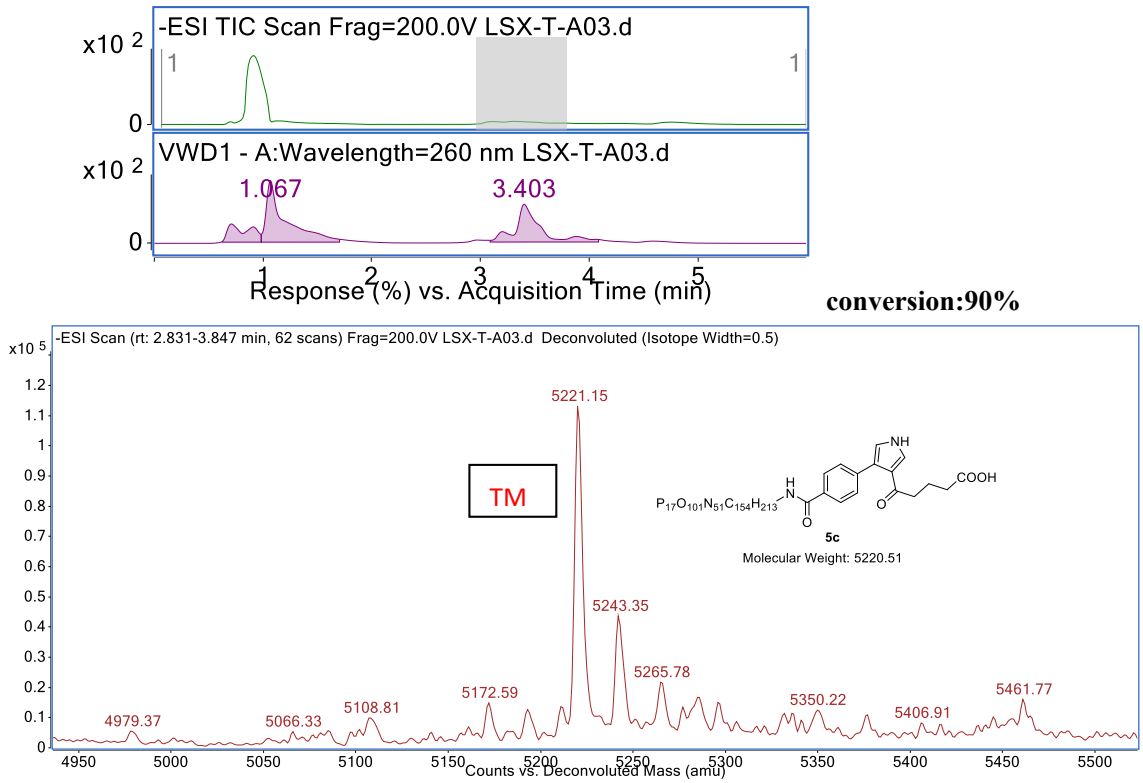


Figure S29 Deconvoluted mass of on-DNA product **5c**

Mass Spectrum of on-DNA Product 5d

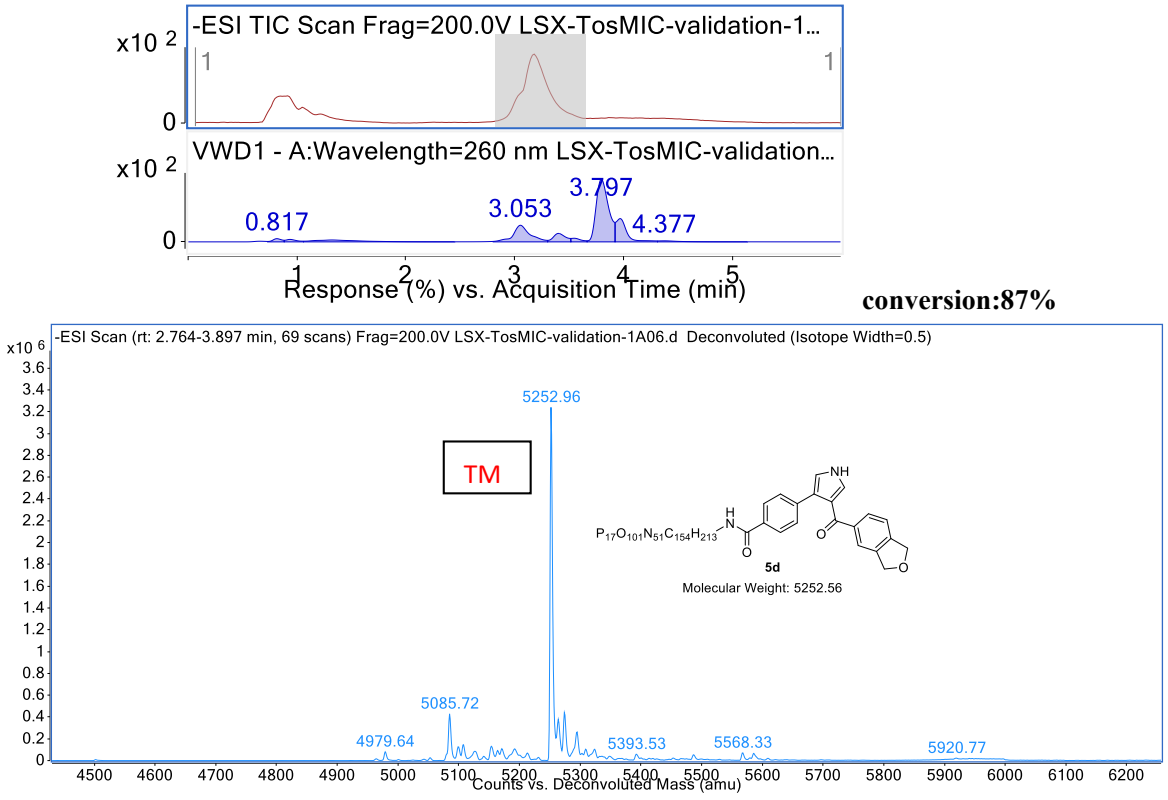


Figure S30 Deconvoluted mass of on-DNA product **5d**

Mass Spectrum of on-DNA Product 5e

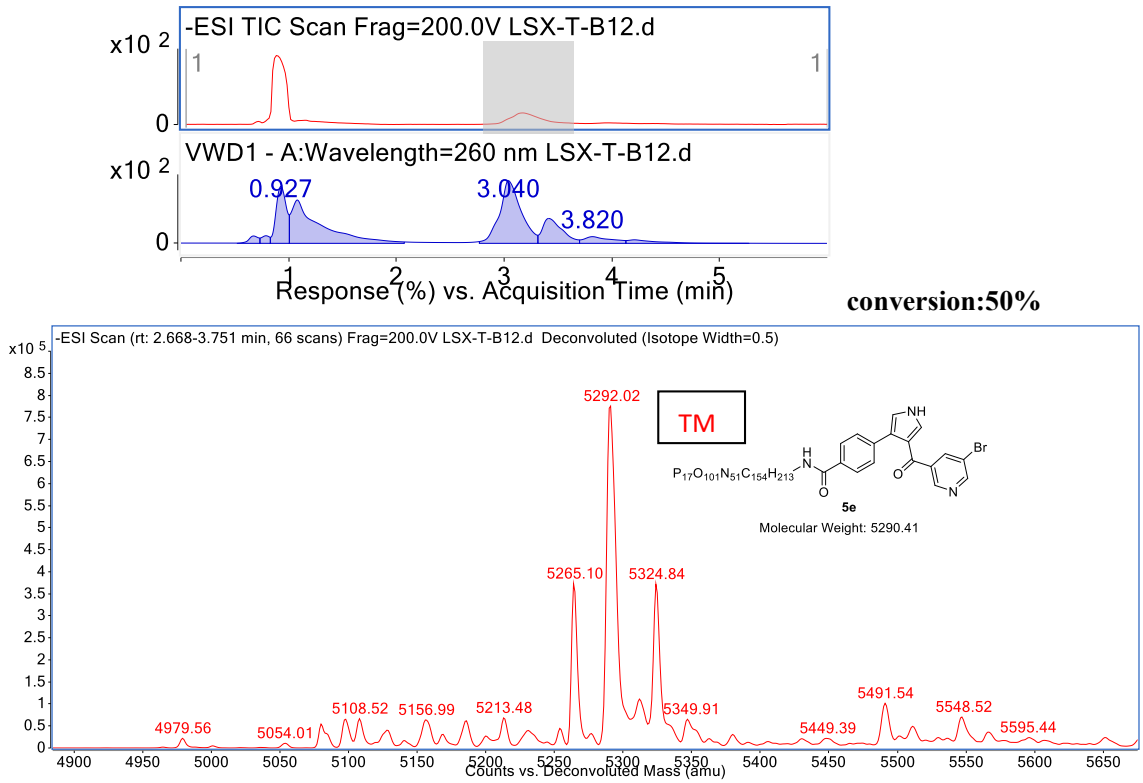


Figure S31 Deconvoluted mass of on-DNA product **5e**

Mass Spectrum of on-DNA Product 5f

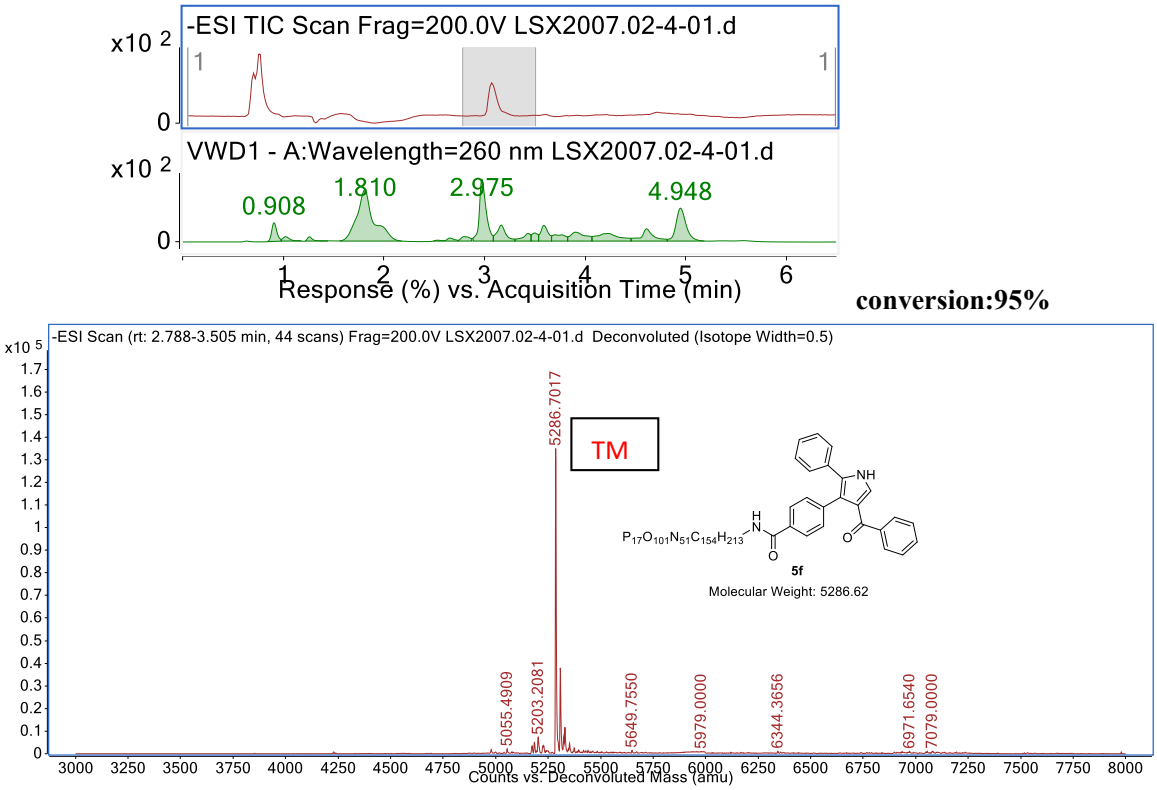


Figure S32 Deconvoluted mass of on-DNA product **5f**

Mass Spectrum of on-DNA Product 5g

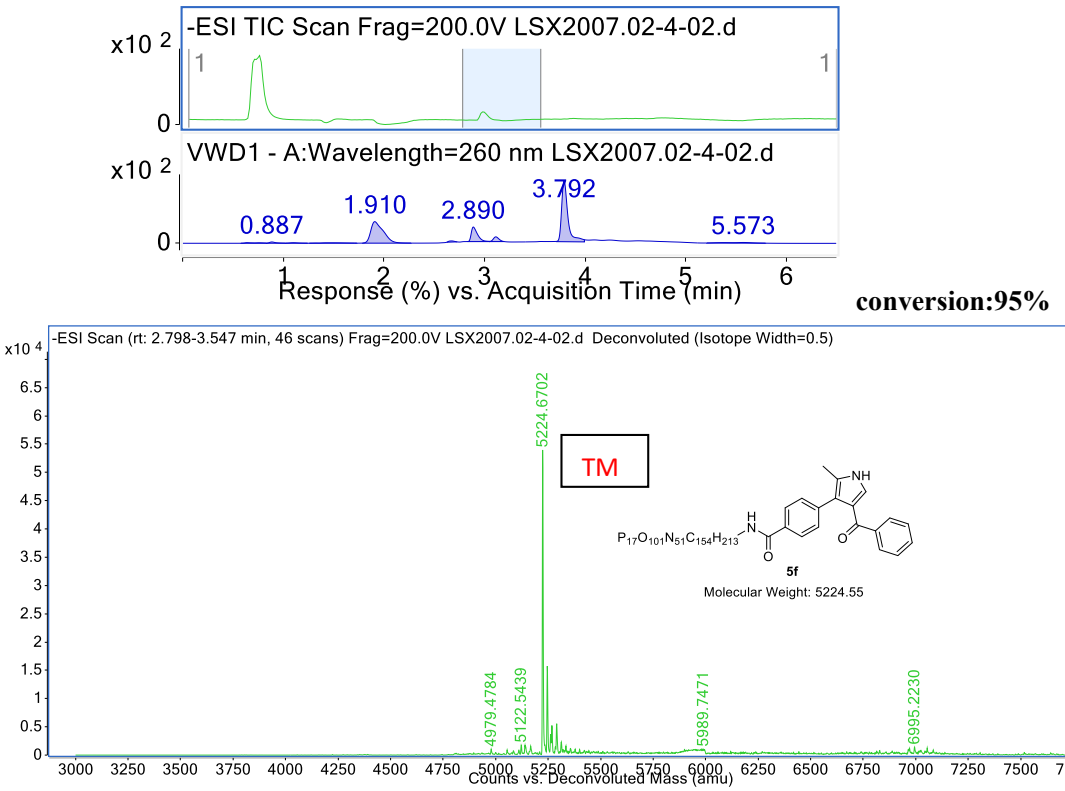


Figure S33 Deconvoluted mass of on-DNA product **5g**

Preparation of on-DNA Product Pyrrole

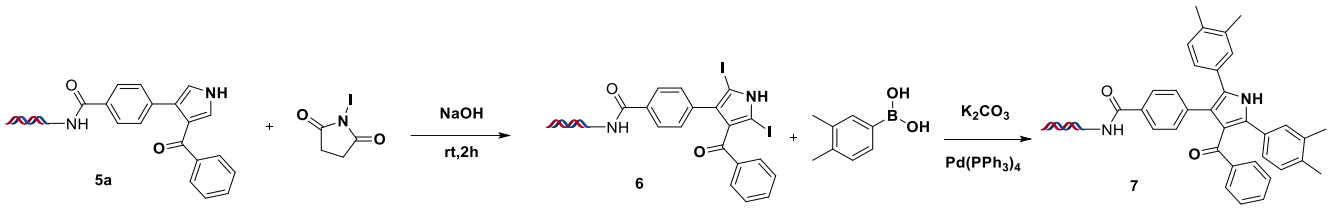


Figure S34 Synthetic route of on-DNA product **7**

Materials

5a: 1 mM in water

NIS (1-iodopyrrolidine-2,5-dione): 10 mM in DMA

NaOH: 200 mM in water

(3,4-dimethylphenyl) Boronic Acid: 200 mM in DMA

K_2CO_3: 500mM in water

$Pd(PPh_3)_4$: 5mM

Scavenger: 100mM in water

Procedure

1) To **5a** solution (5 nmol, 5 μL), was added 5 μL NIS (10 equiv) , 2.5ul NaOH (100 equiv). The mixture was vortexed.
2) The reaction mixture at 25 °C for 2 h.
3) After the reaction, the product **6** was obtained by ethanol precipitation as described above.
4) To **6** solution (5 nmol, 5 μL), was added 5 μL (3,4-dimethylphenyl) Boronic Acid (200 equiv) , 5uL K_2CO_3 (500 equiv), and 2uL $Pd(PPh_3)_4$ (2 equiv). The mixture was vortexed.

5) The reaction mixture at 80 °C for 4 h.

6) Add scavenger reagent (6 equiv, 0.6 μL) at 70 °C for 20min to remove the metal catalyst.

7) The product **7** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 6

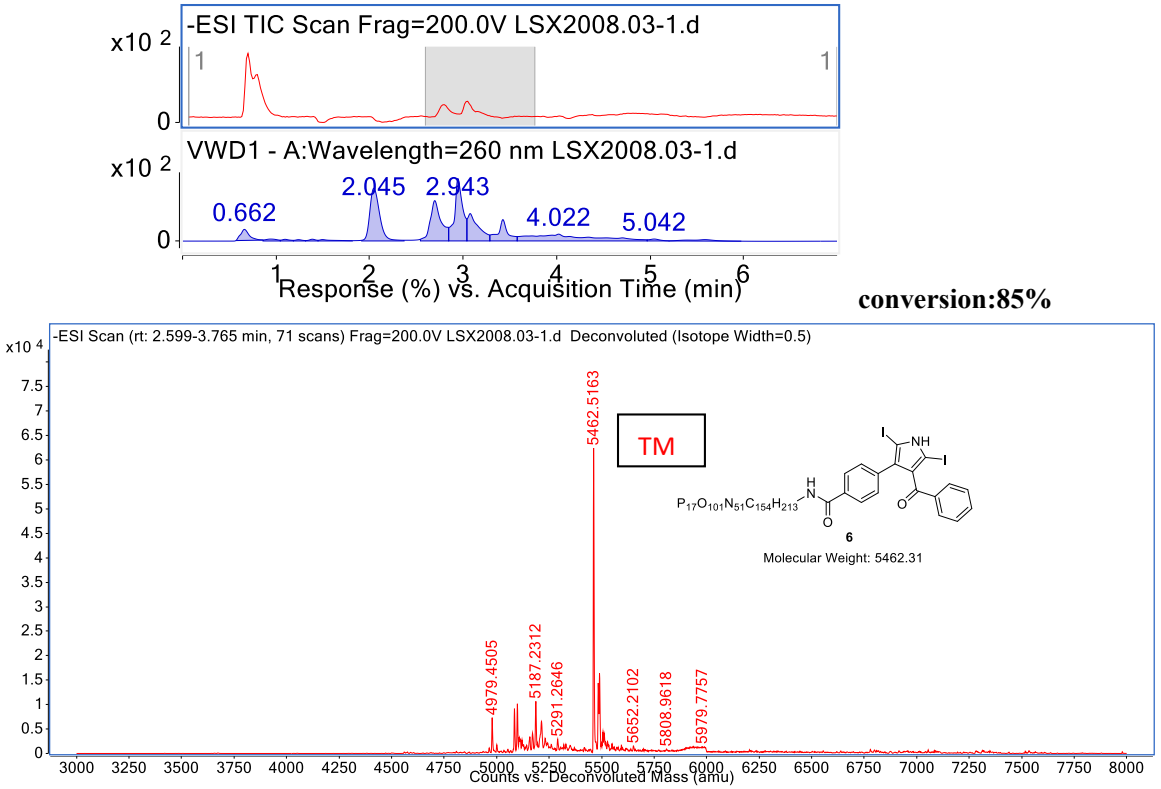
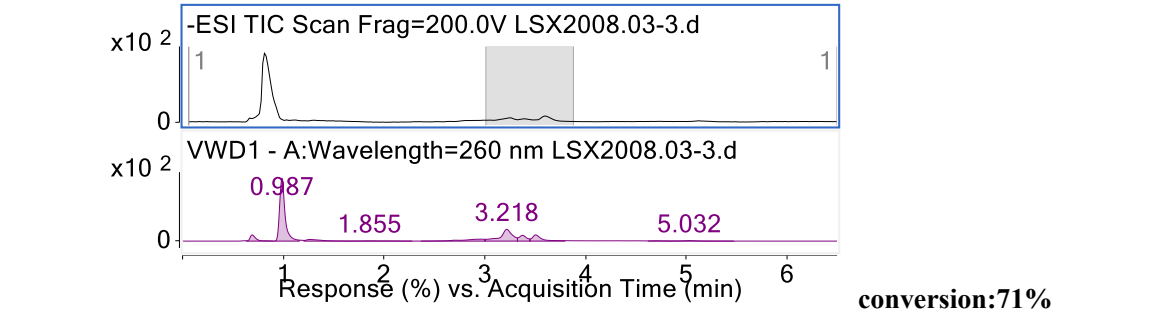


Figure S35 Deconvoluted mass of on-DNA product **6**

Mass Spectrum of on-DNA Product 7a



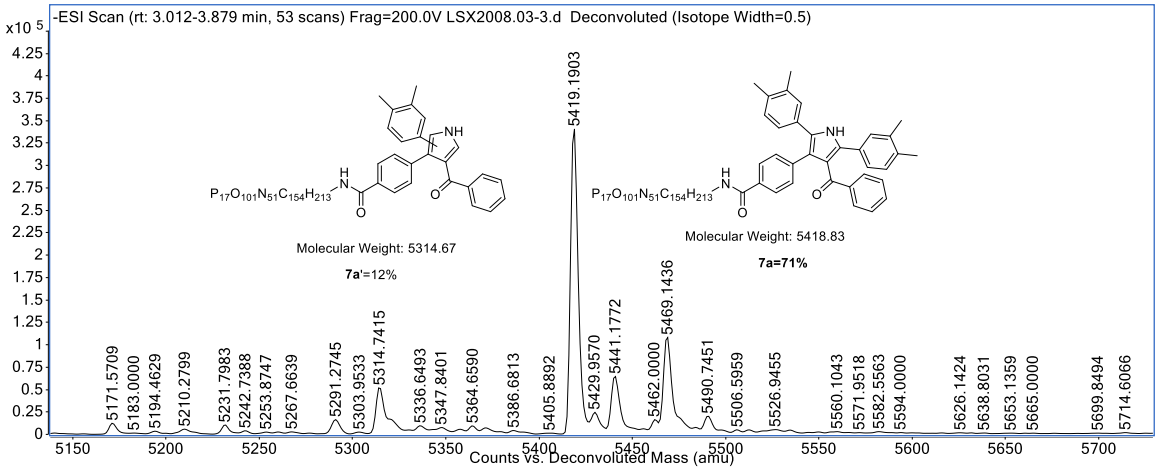
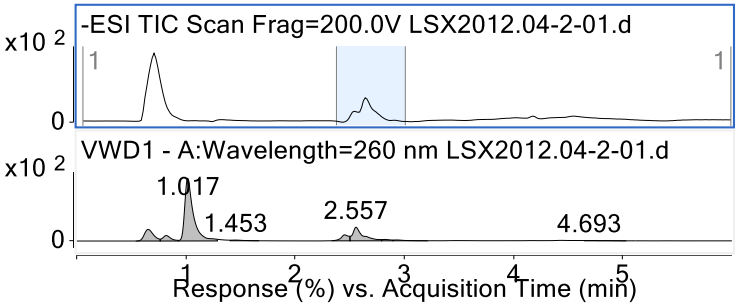


Figure S36 Deconvoluted mass of on-DNA product **7a** and **7a'**

Mass Spectrum of on-DNA Product 7b



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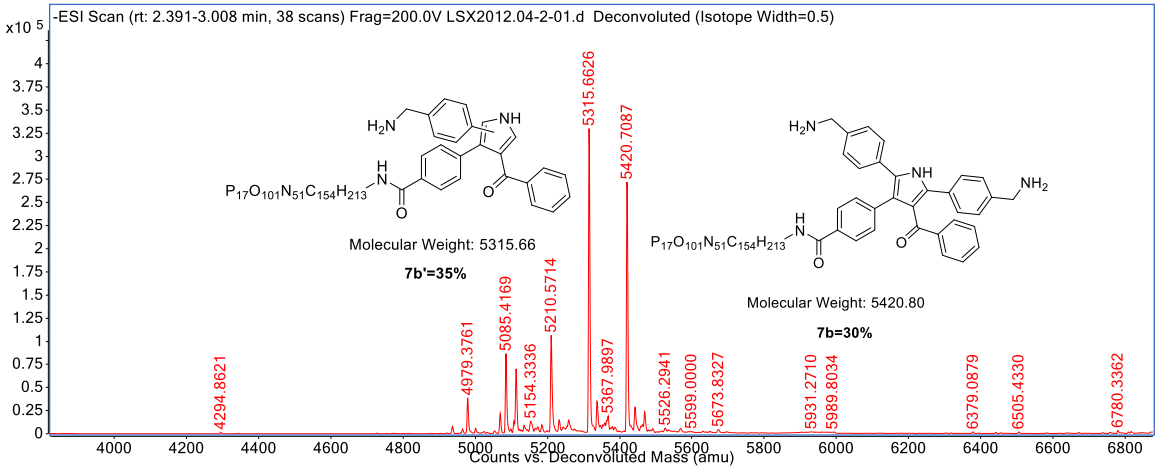
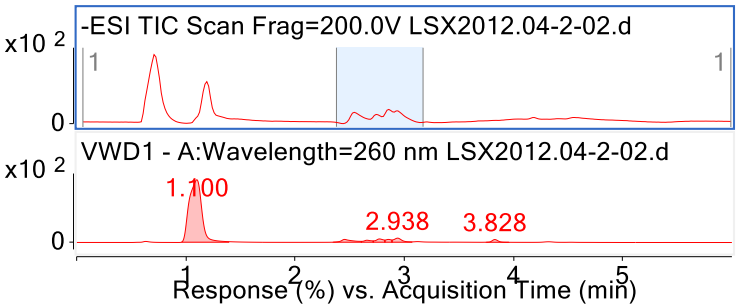


Figure S37 Deconvoluted mass of on-DNA product **7b** and **7b'**

Mass Spectrum of on-DNA Product 7c



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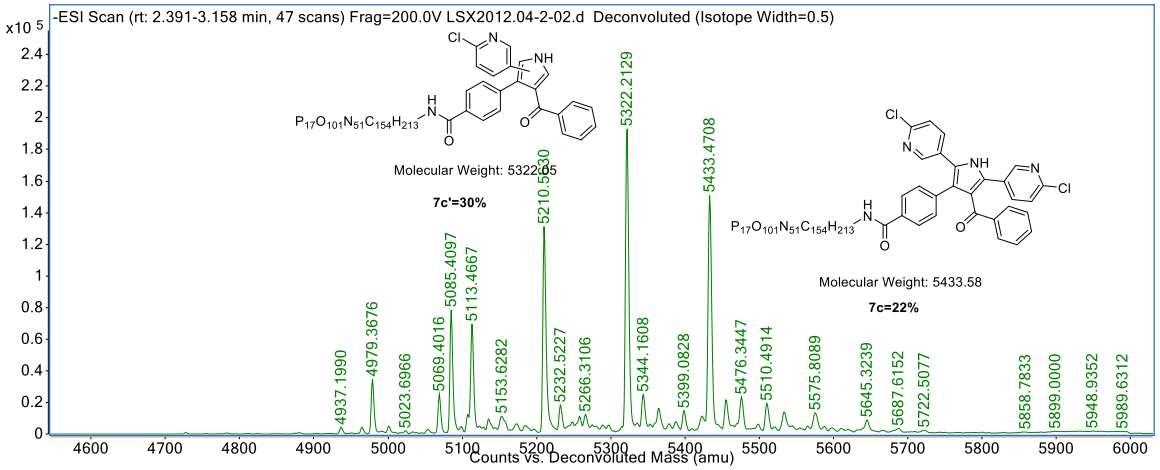
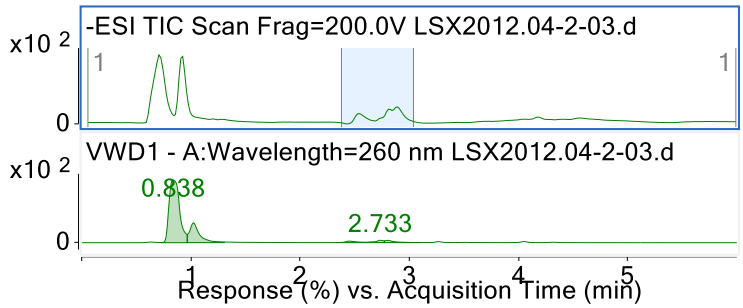


Figure S38 Deconvoluted mass of on-DNA product **7c** and **7c'**

Mass Spectrum of on-DNA Product 7d



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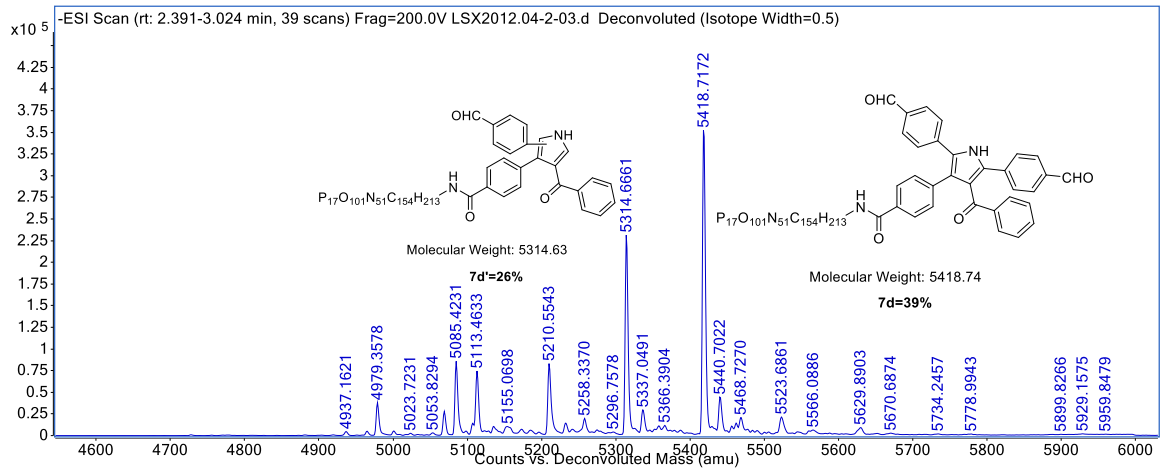
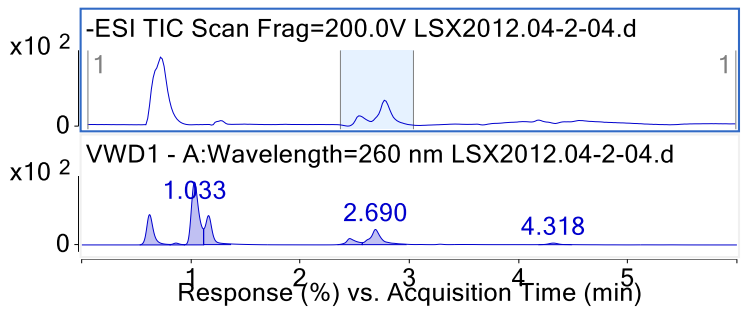


Figure S39 Deconvoluted mass of on-DNA product **7d** and **7d'**

Mass Spectrum of on-DNA Product 7e



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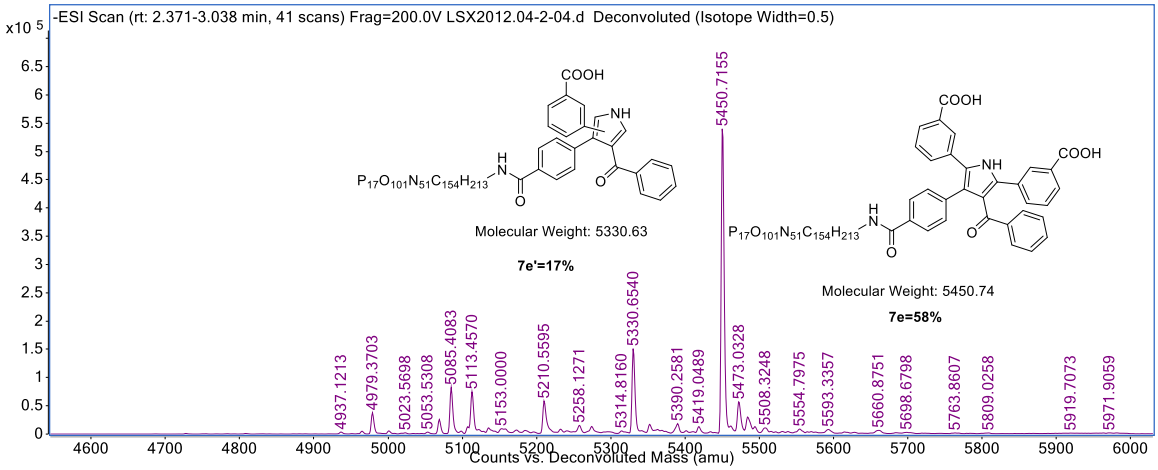
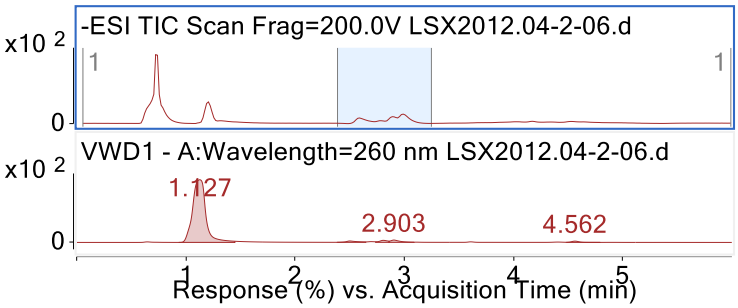


Figure S40 Deconvoluted mass of on-DNA product **7e** and **7e'**

Mass Spectrum of on-DNA Product 7f



conversion:46%

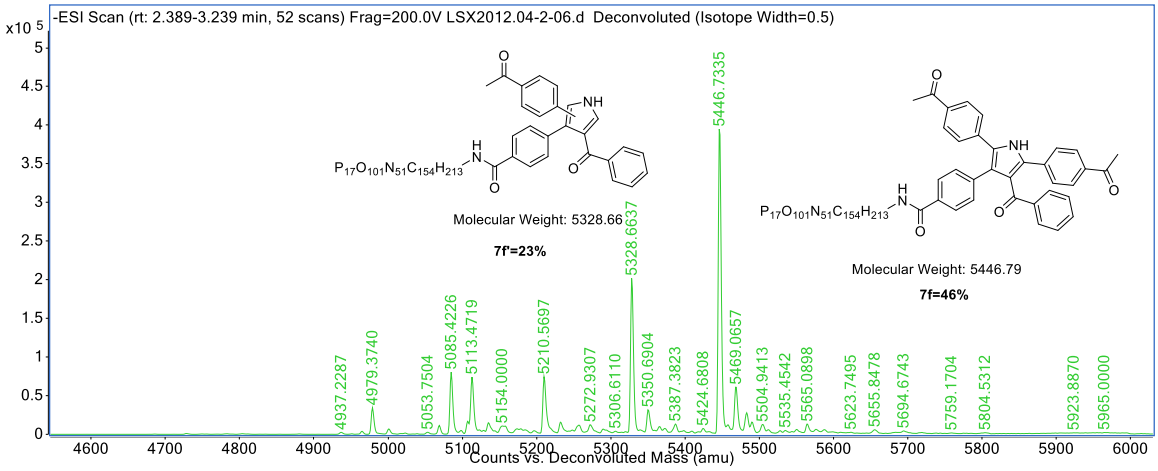
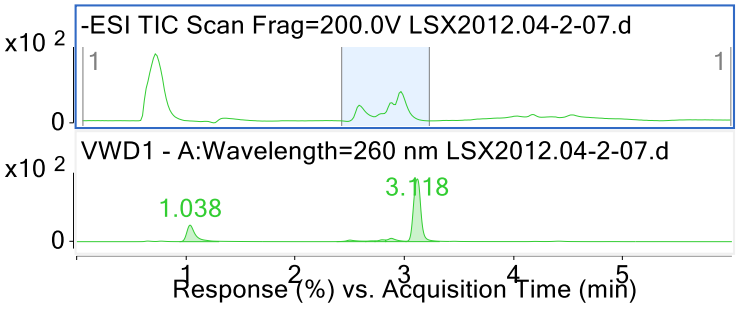


Figure S41 Deconvoluted mass of on-DNA product **7f** and **7f'**

Mass Spectrum of on-DNA Product 7g



conversion:47%

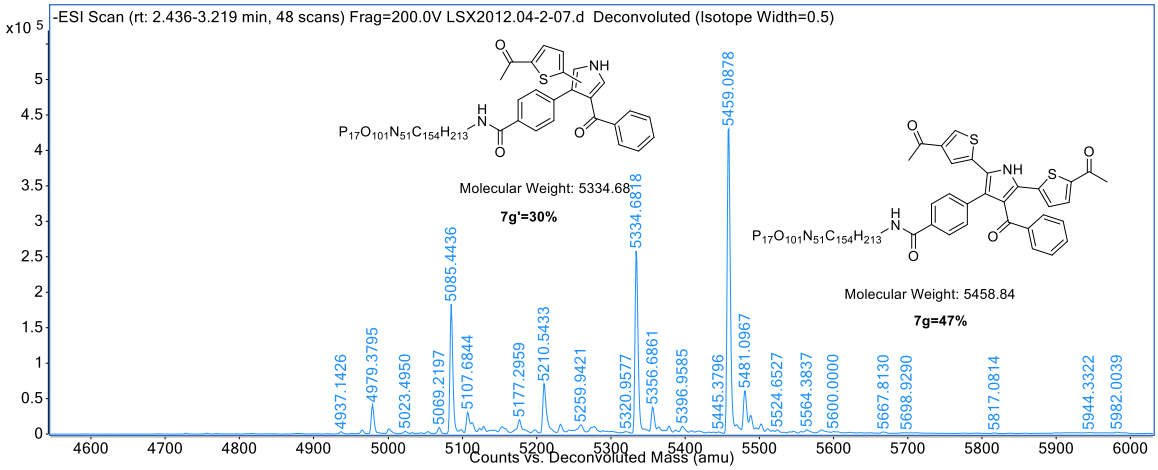
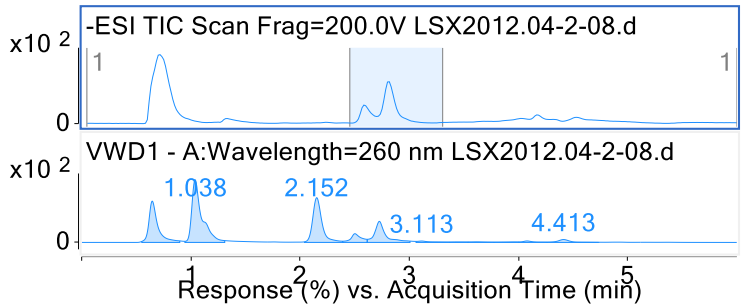


Figure S42 Deconvoluted mass of on-DNA product **7g** and **7g'**

Mass Spectrum of on-DNA Product 7h



conversion:53%

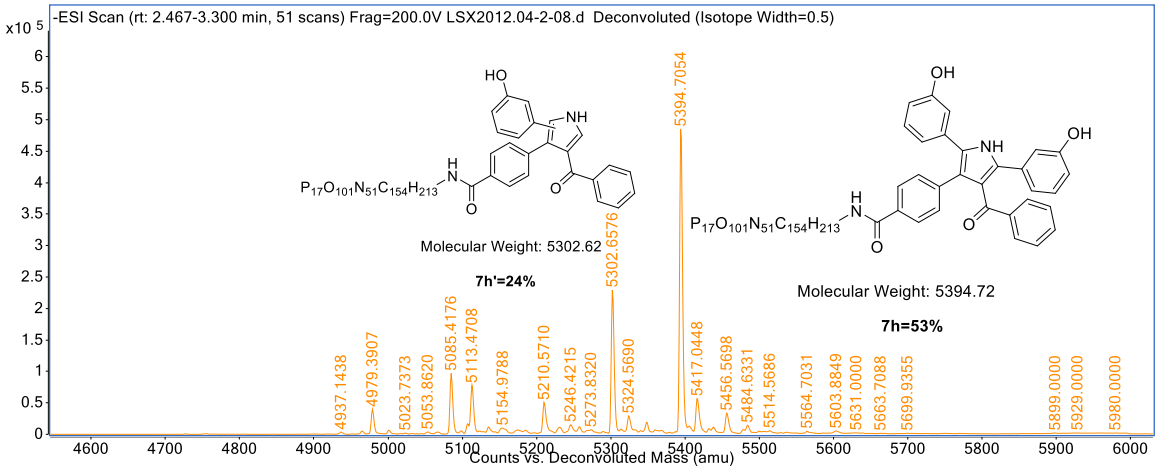
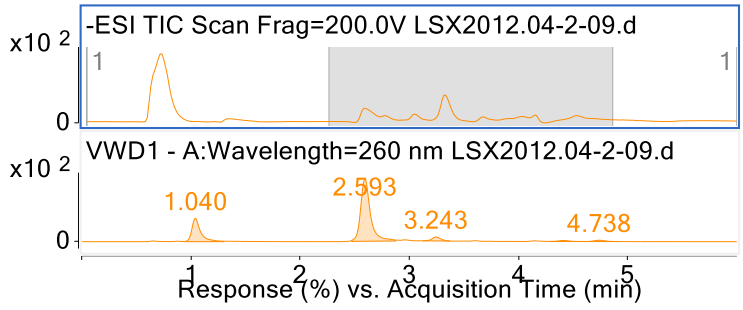


Figure S43 Deconvoluted mass of on-DNA product **7h** and **7h'**

Mass Spectrum of on-DNA Product 7i



conversion:60%

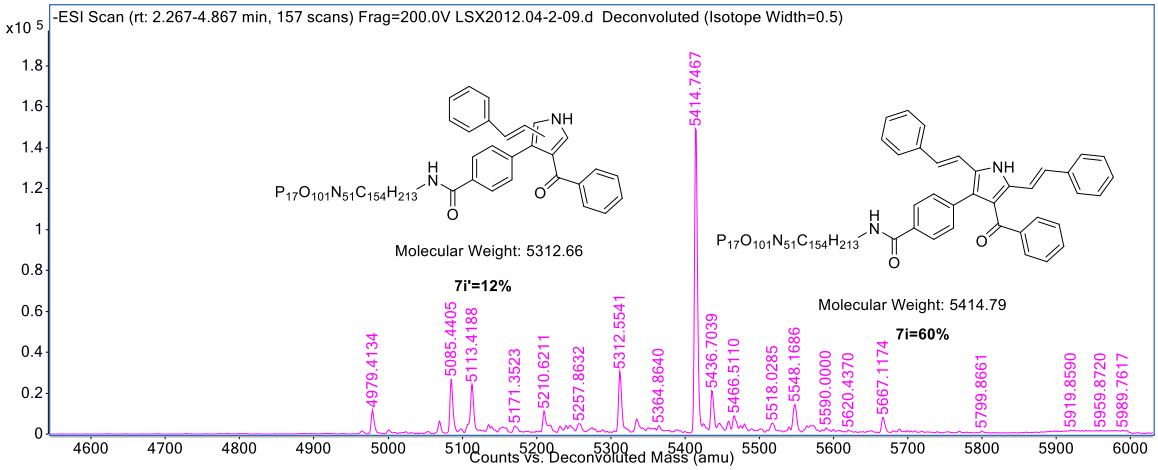


Figure S44 Deconvoluted mass of on-DNA product **7i** and **7i'**

Preparation of on-DNA Product Pyrrolidine

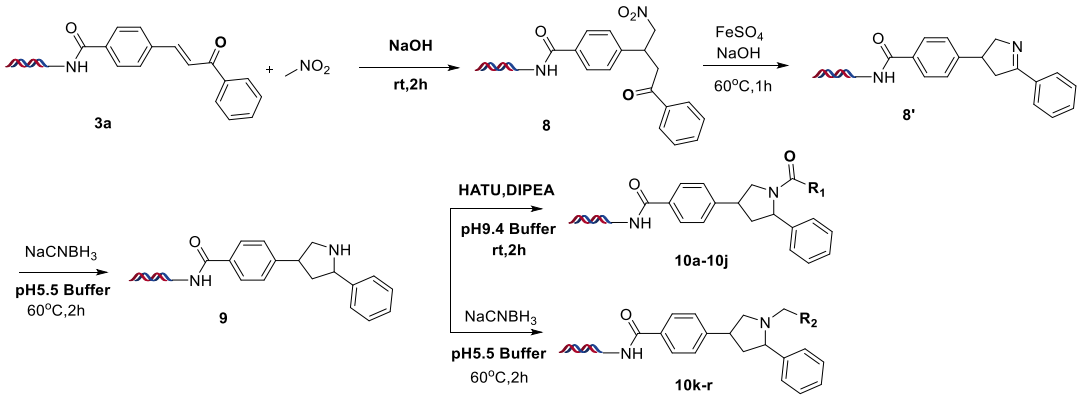


Figure S45 Synthetic route of on-DNA product **10a-10r**

Materials

3a: 1 mM in water

CH_3NO_2: 200 mM in DMA

NaOH: 200 mM in water

$FeSO_4$: 200 mM in water

$NaCNBH_3$: 400mM in water

HATU: 200mM in DMA

DIPEA: 200mM in DMA

Acid: 200mM in DMA

Aldehyde: 200mM in DMA

Procedure

1) To **3a** solution (5 nmol, 5 μL), was added 5 μL NaOH (200 equiv) , 5ul CH_3NO_2 (200 equiv). The mixture was vortexed.
2) The reaction mixture at 25 °C for 2 h.
3) After the reaction, the product **8** was obtained by ethanol precipitation as described above.
4) To **8** solution (5 nmol, 5 μL), was added 5 μL NaOH (200 equiv) , 5uL $FeSO_4$ (200 equiv). The mixture was vortexed.
5) The reaction mixture at 60 °C for 1 h.
6) After centrifugation for 15 minutes at 4 ℃ in a microcentrifuge at 10 K rpm, the product **8'** was obtained when the

supernatant was ethanol precipitation as described above.

7) The on-DNA Intermediate were purified by a 3 K Spinfilter tube after centrifugation for 30 min at 4 ℃ in a microcentrifuge at 10 K rpm. three times.
8) To **8′** solution (5 nmol, 5 μL,1mM in pH 5.5 Buffer), was added 2.5 μL $NaCNBH_3$ (200 equiv). The mixture was vortexed.
9) The reaction mixture at 60 °C for 2 h.
10) After the reaction, the product **9** was obtained by ethanol precipitation as described above.
11) Mixing acid with equal volume HATU and DIPEA to prepare the solution V.
12) To the **9** solution (5 nmol, 5 μL), was added 5 μL pH 9.4 buffer solution, 200 equiv. of solution V (15 μL), and mix.
13) The reaction mixture at 25 °C for 2 h.
14) After the reaction, the product **10a-10j** was obtained by ethanol precipitation as described above.
15) To the **9** solution (5 nmol, 5 μL), was added 5 μL pH5.5 buffer solution, 5uL aldehyde (200 equiv) and 2.5ul $NaCNBH_3$ (200 equiv). The mixture was vortexed.
16) The reaction mixture at 60 °C for 2 h.
17) After the reaction, the product **10k-10r** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 8

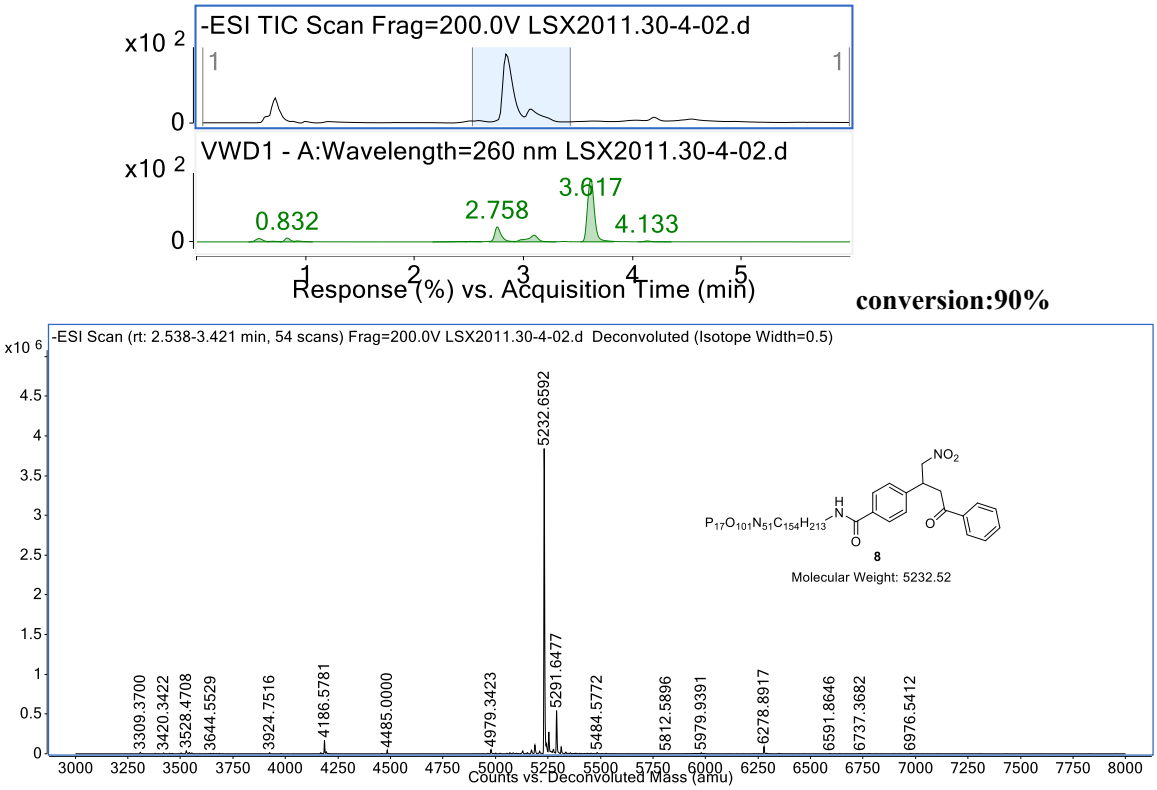


Figure S46 Deconvoluted mass of on-DNA product **8**

Mass Spectrum of on-DNA Product 8′

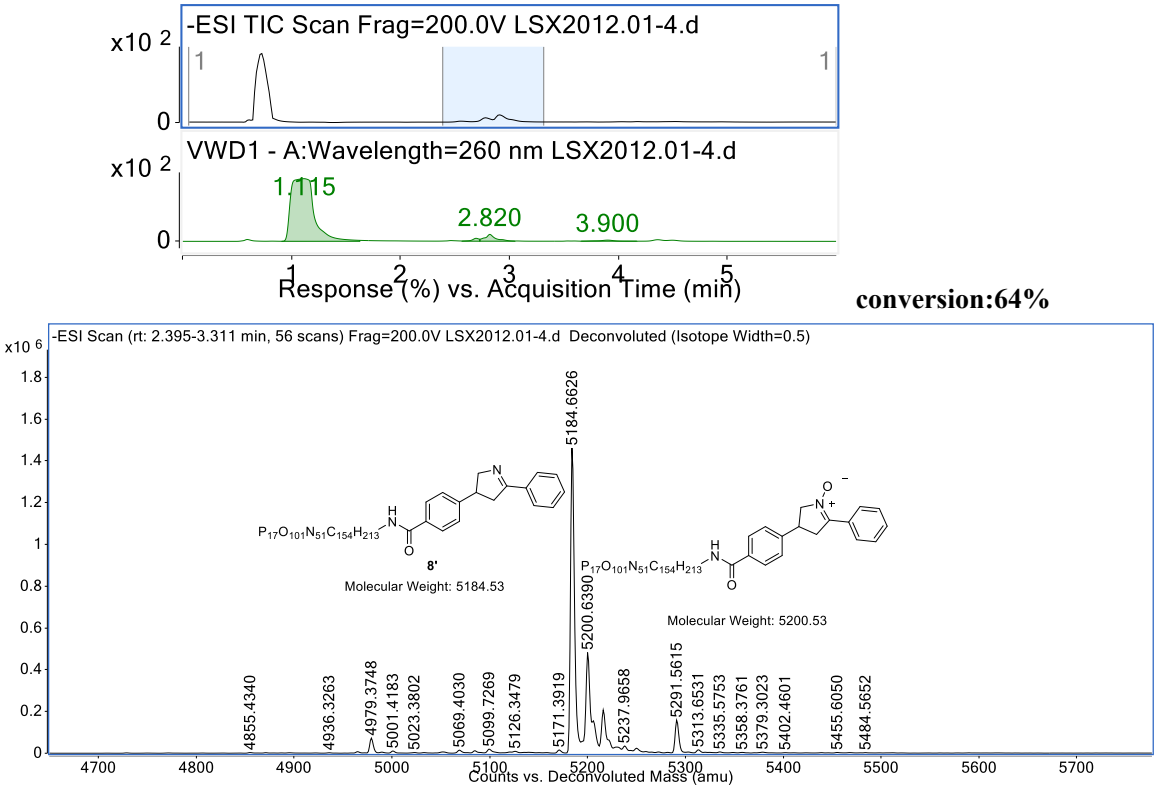


Figure S47 Deconvoluted mass of on-DNA product **8′**

Mass Spectrum of on-DNA Product 9

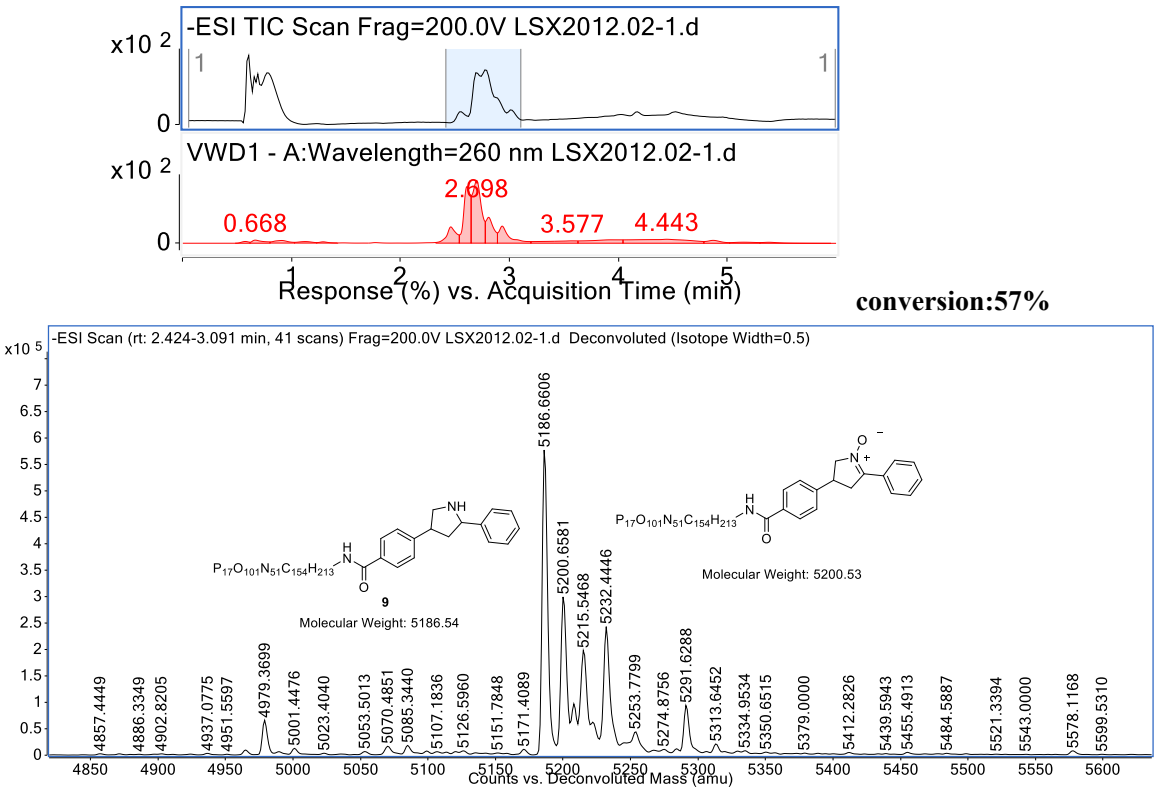


Figure S48 Deconvoluted mass of on-DNA product **9**

Mass Spectrum of on-DNA Product 10a

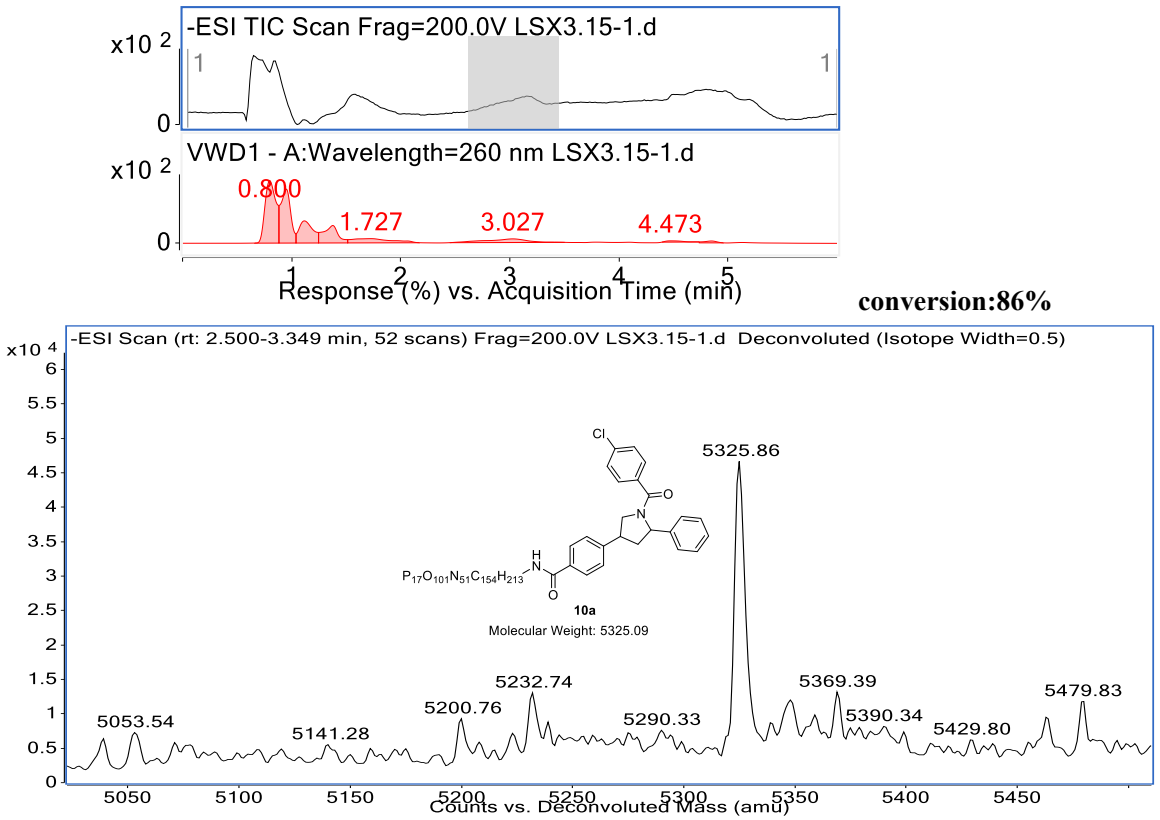


Figure S49 Deconvoluted mass of on-DNA product **10a**

Mass Spectrum of on-DNA Product 10b

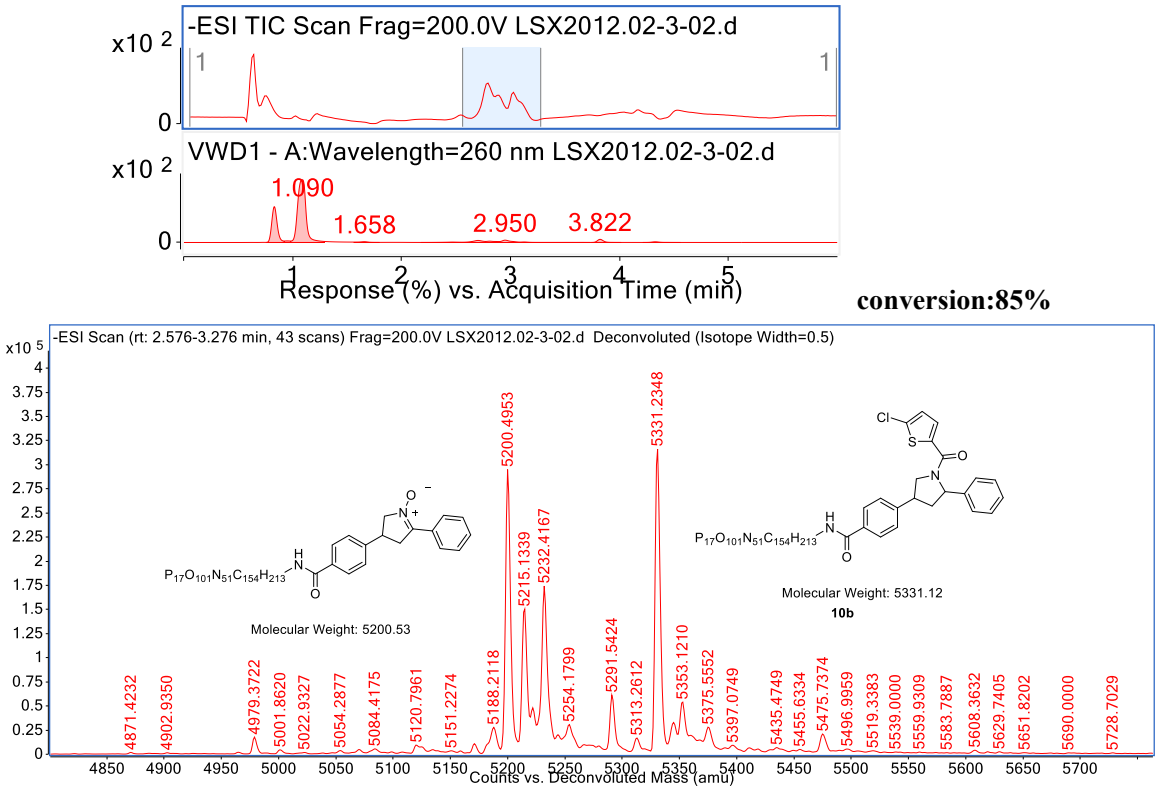


Figure S50 Deconvoluted mass of on-DNA product **10b**

Mass Spectrum of on-DNA Product 10c

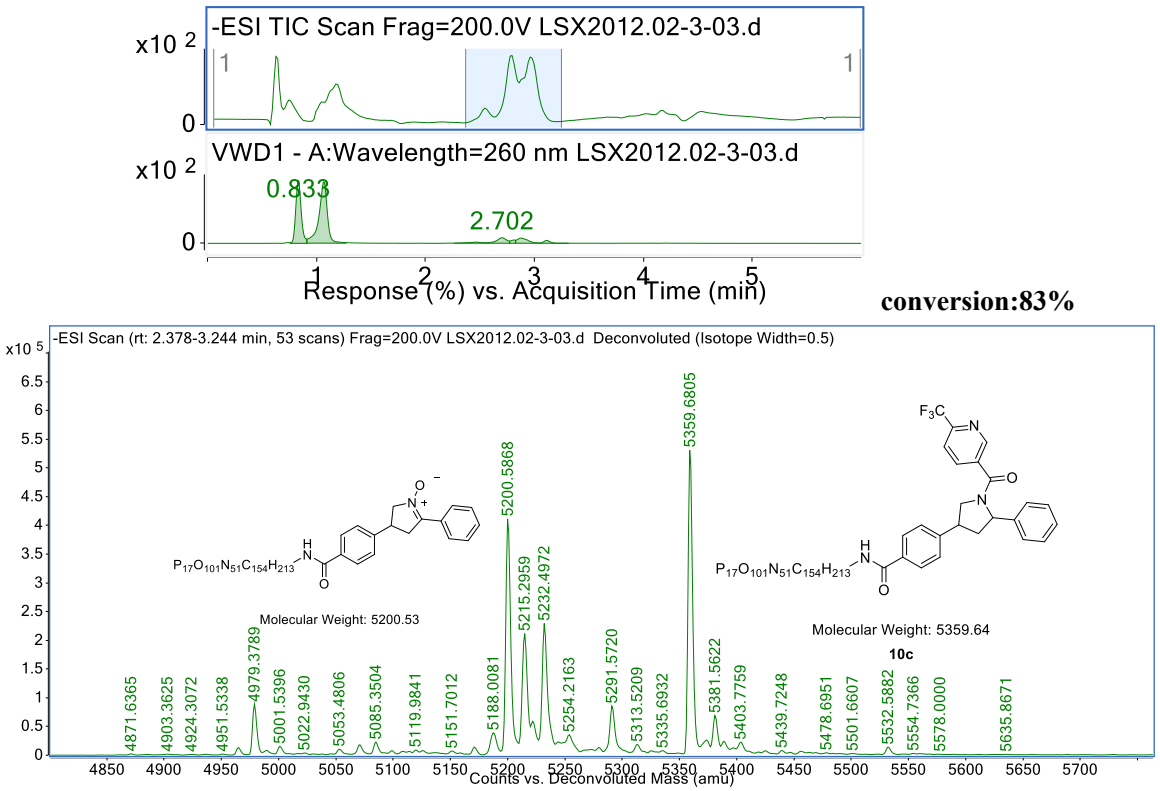


Figure S51 Deconvoluted mass of on-DNA product **10c**

Mass Spectrum of on-DNA Product 10d

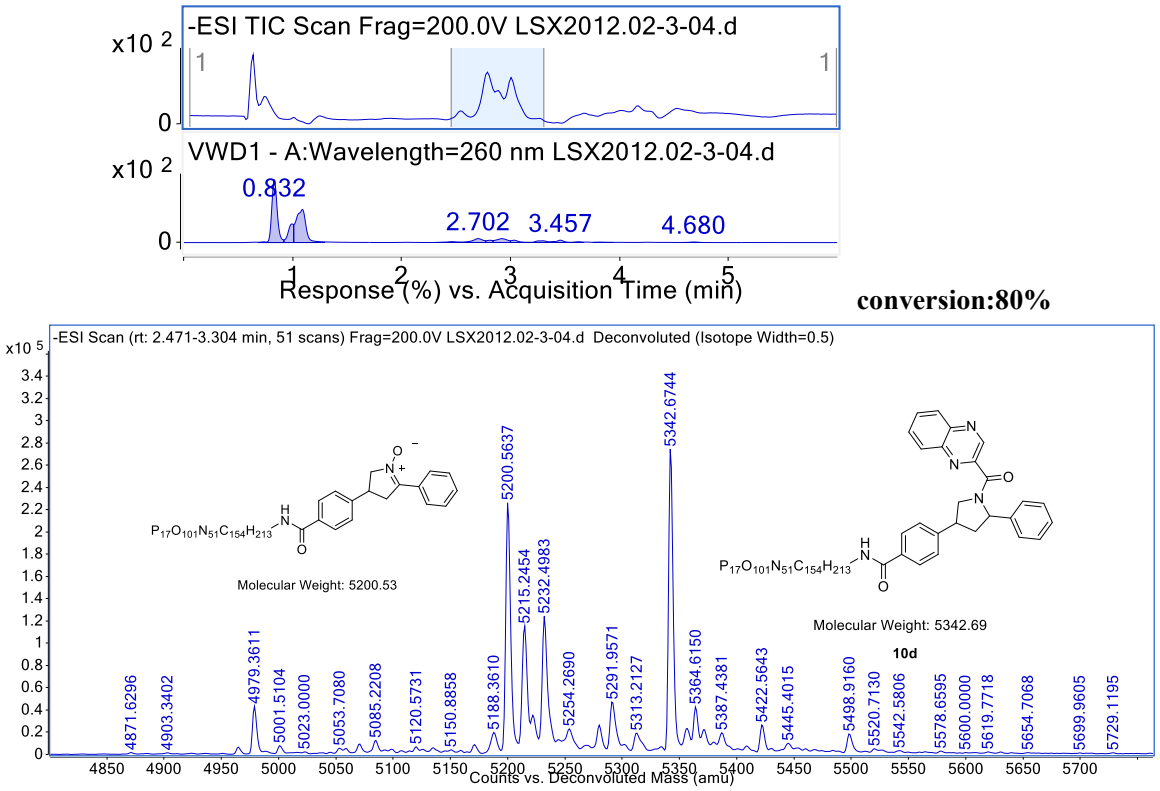


Figure S51 Deconvoluted mass of on-DNA product **10d**

Mass Spectrum of on-DNA Product 10e

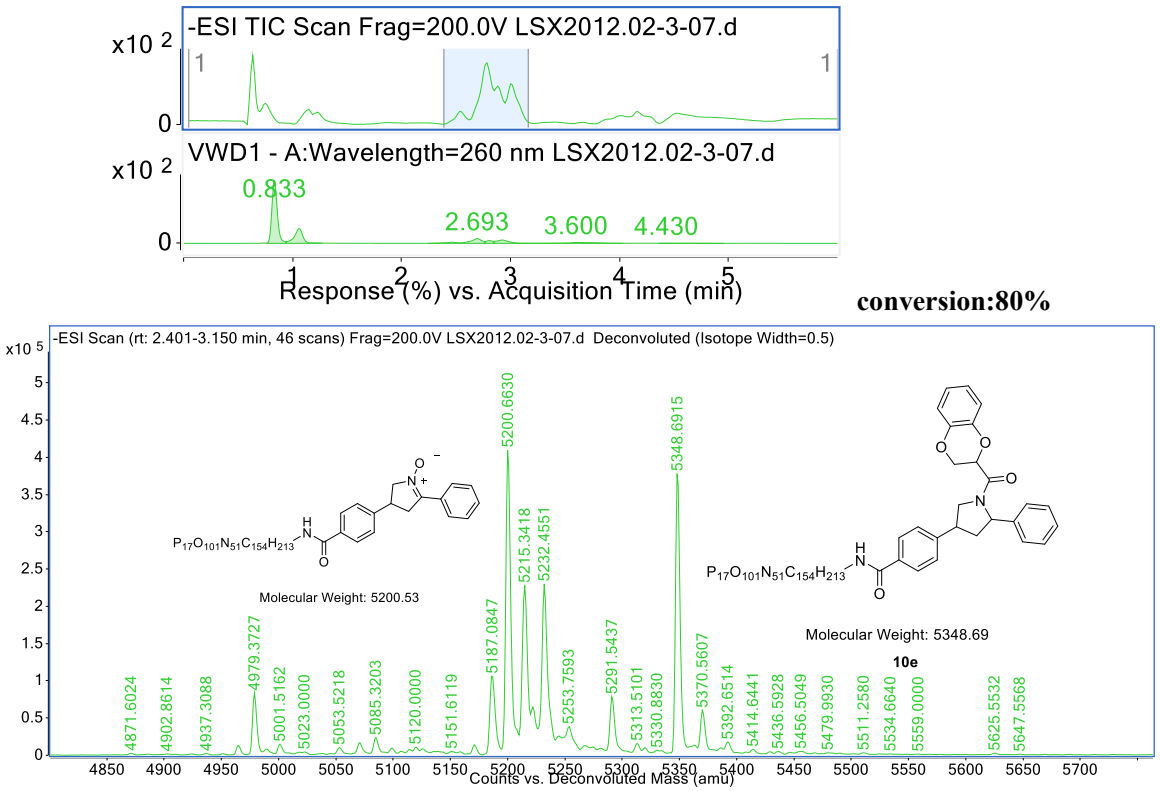


Figure S52 Deconvoluted mass of on-DNA product **10e**

Mass Spectrum of on-DNA Product 10f

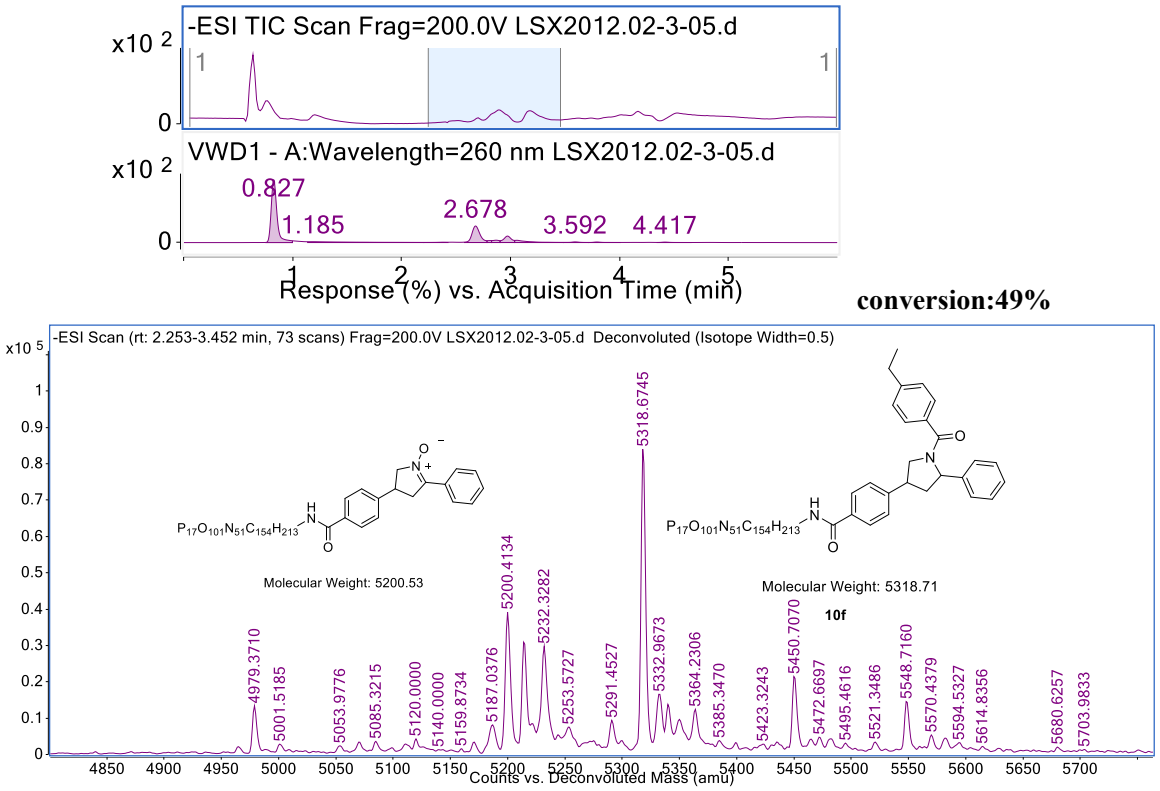


Figure S53 Deconvoluted mass of on-DNA product **10f**

Mass Spectrum of on-DNA Product 10g

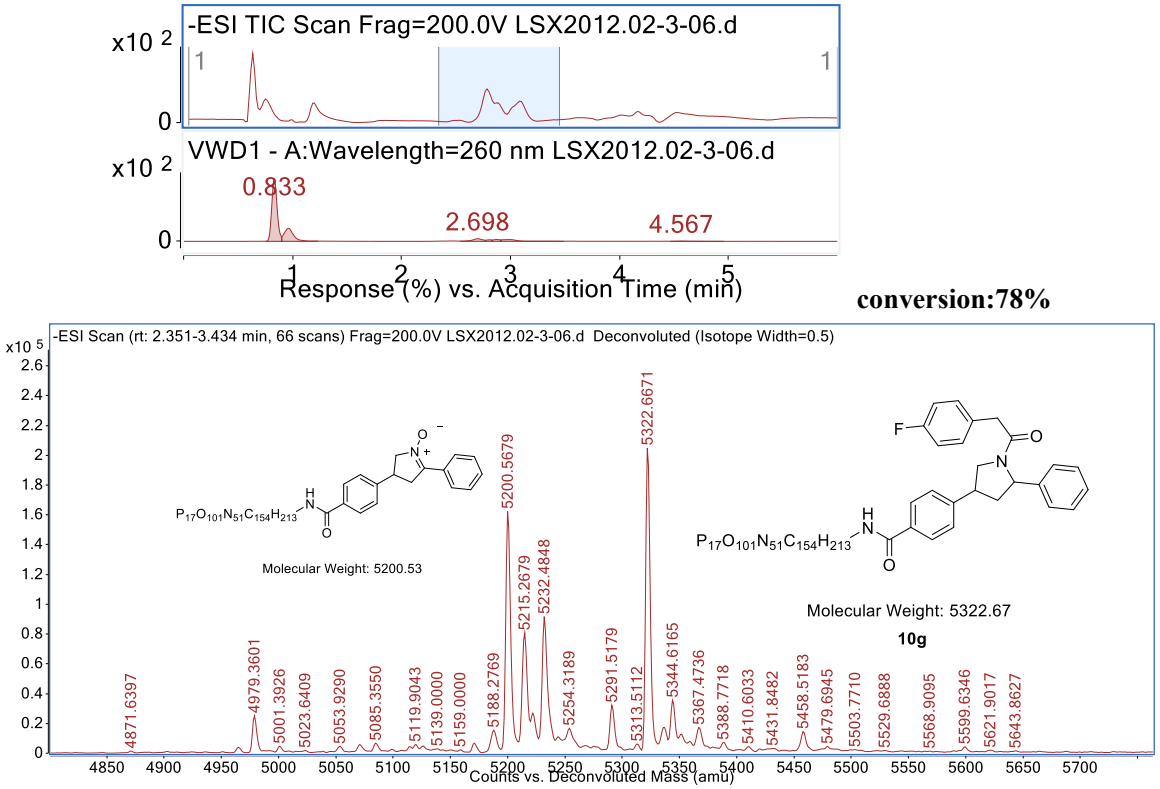


Figure S54 Deconvoluted mass of on-DNA product **10g**

Mass Spectrum of on-DNA Product 10h

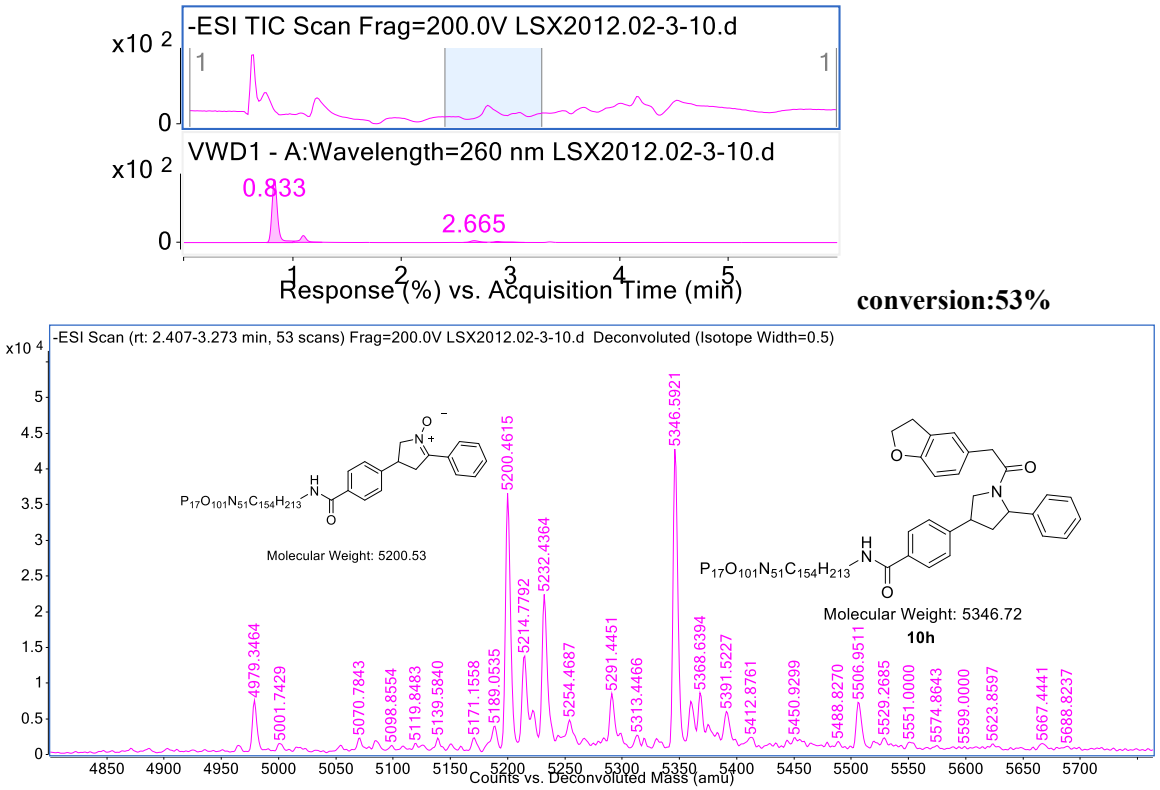


Figure S55 Deconvoluted mass of on-DNA product **10h**

Mass Spectrum of on-DNA Product 10i

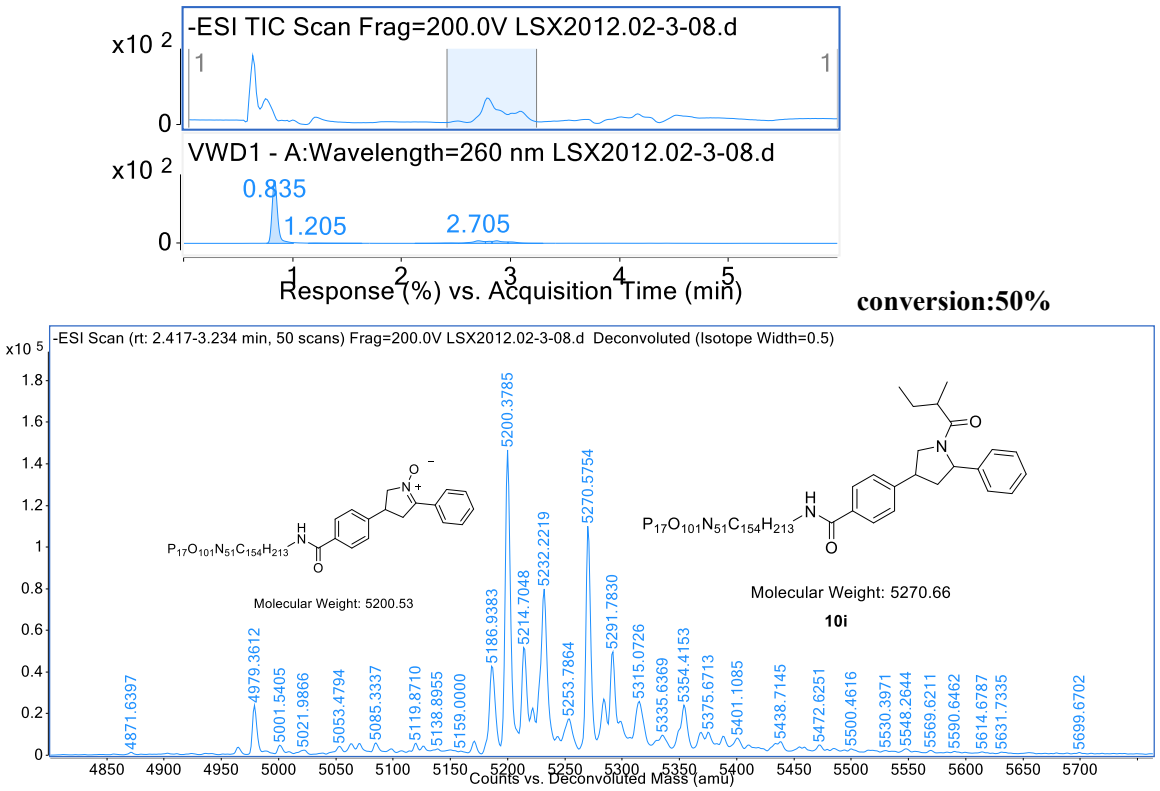


Figure S56 Deconvoluted mass of on-DNA product **10i**

Mass Spectrum of on-DNA Product 10j

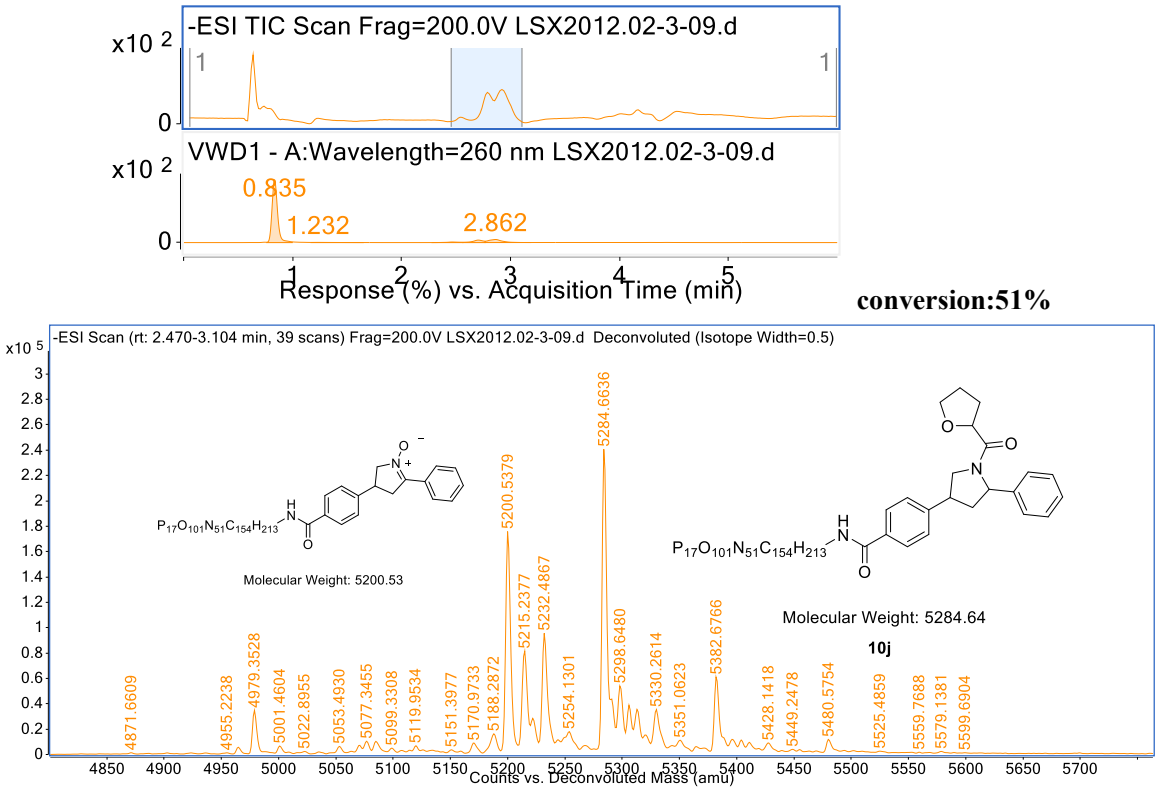


Figure S57 Deconvoluted mass of on-DNA product **10j**

Mass Spectrum of on-DNA Product 10k

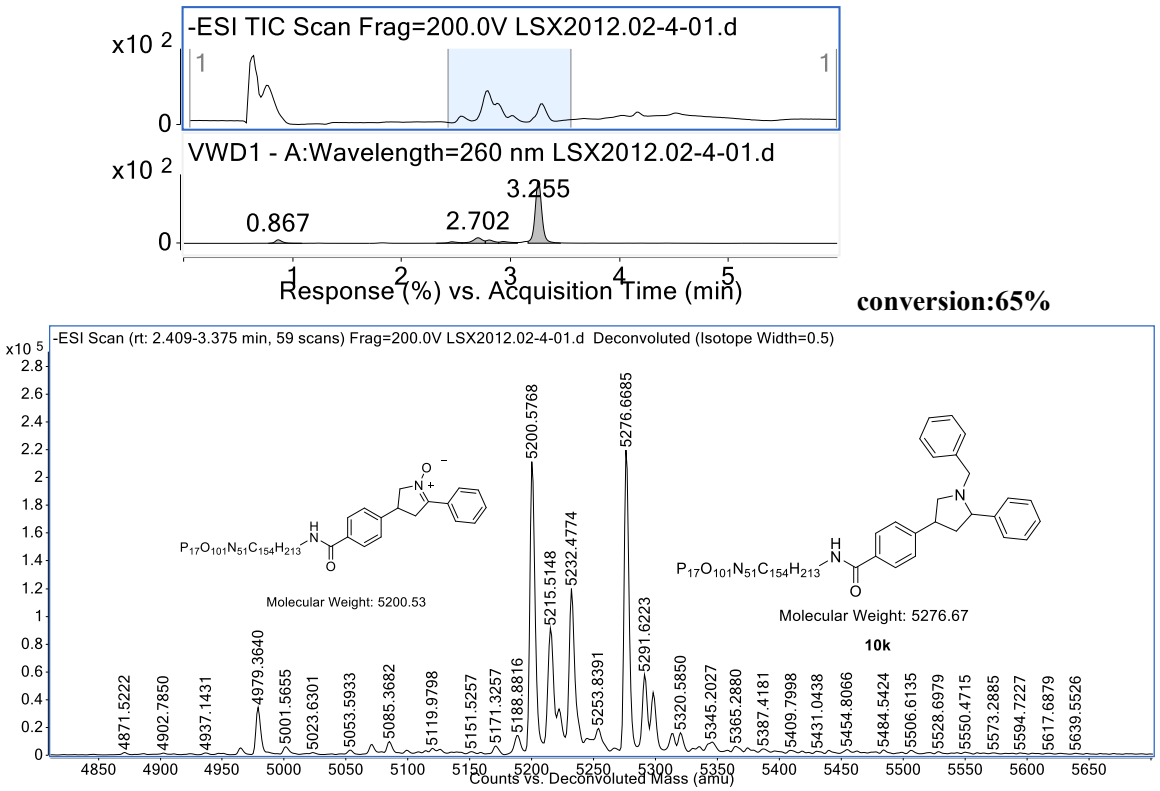


Figure S58 Deconvoluted mass of on-DNA product **10k**

Mass Spectrum of on-DNA Product 10l

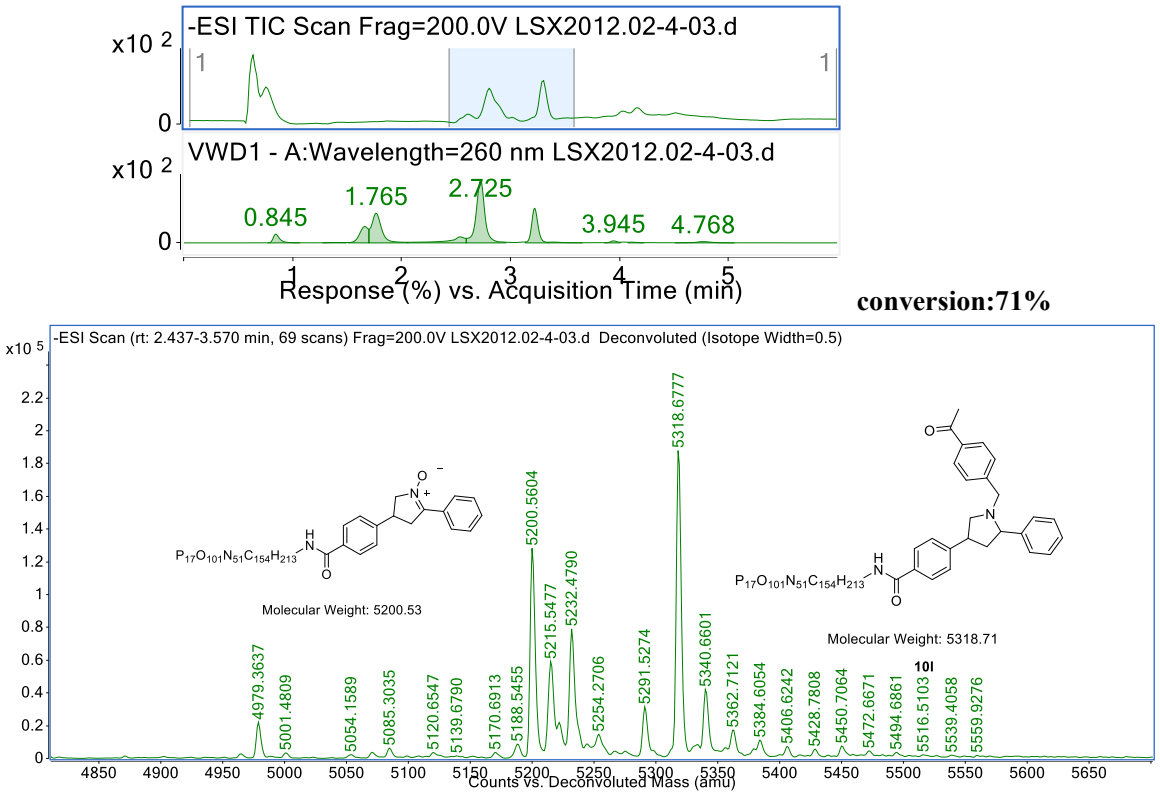


Figure S59 Deconvoluted mass of on-DNA product **10l**

Mass Spectrum of on-DNA Product 10m

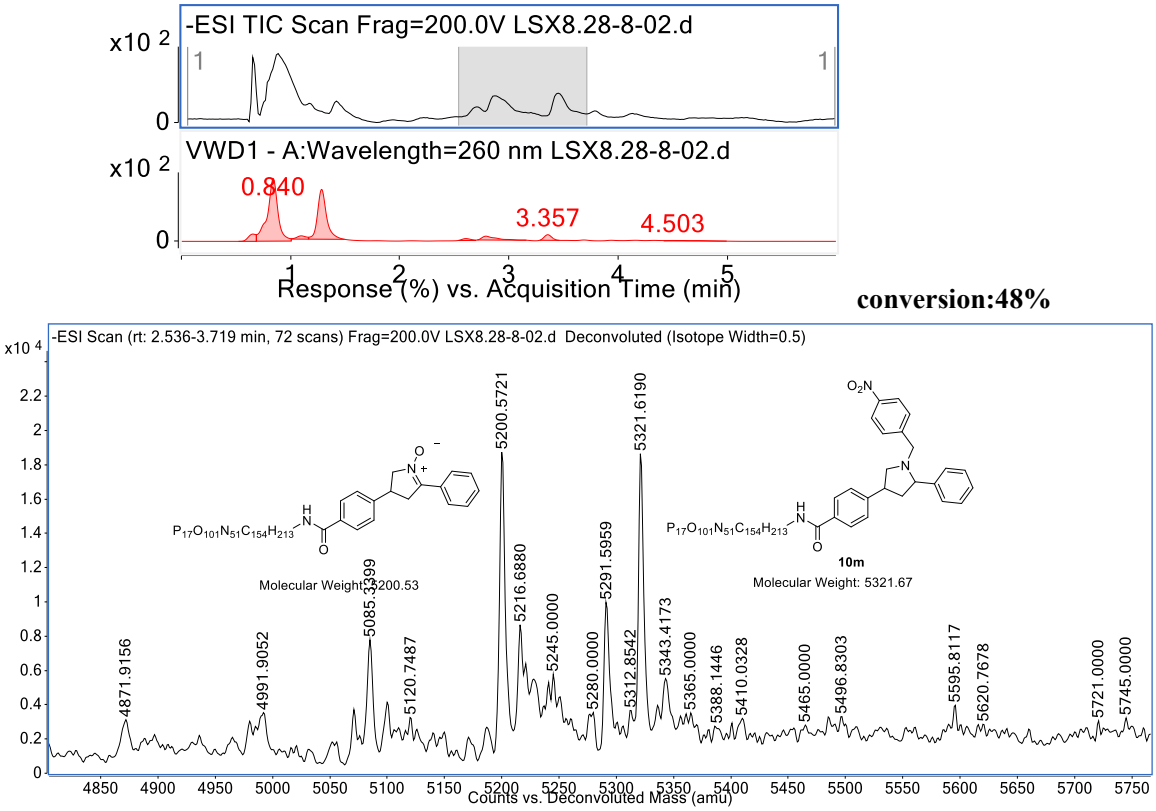


Figure S60 Deconvoluted mass of on-DNA product **10m**

Mass Spectrum of on-DNA Product 10n

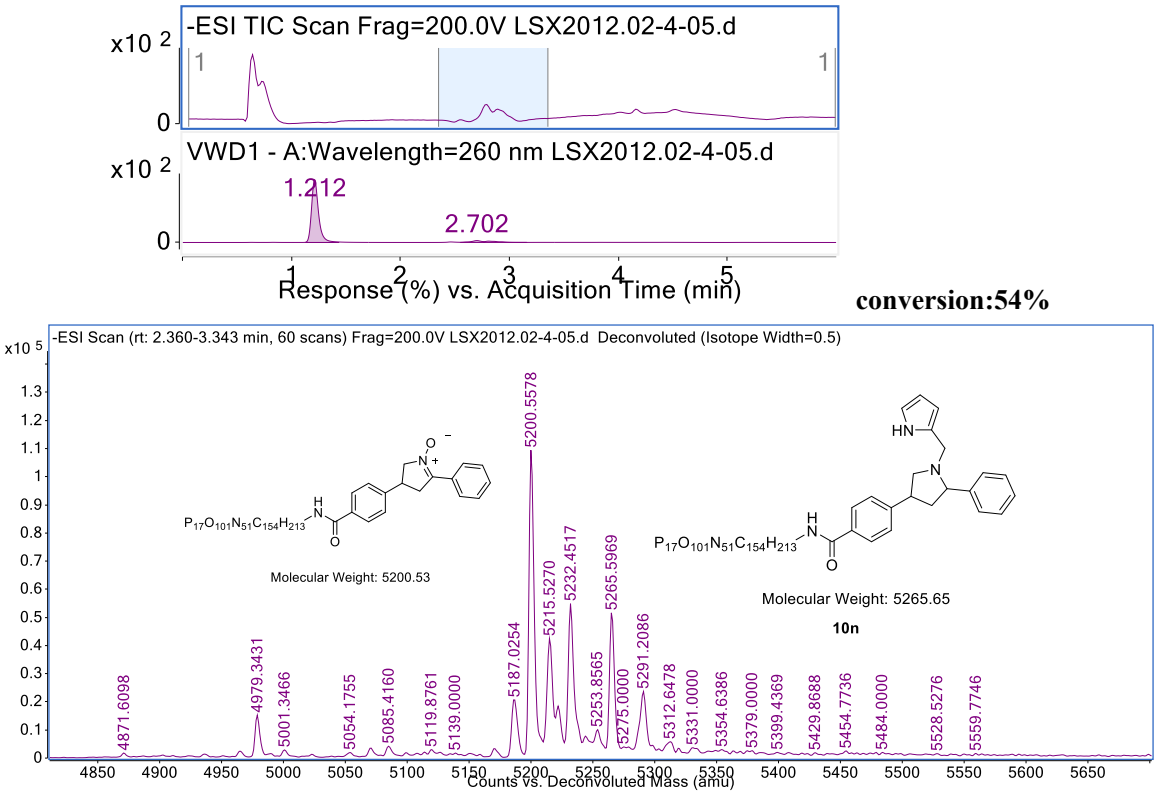


Figure S61 Deconvoluted mass of on-DNA product **10n**

Mass Spectrum of on-DNA Product 10o

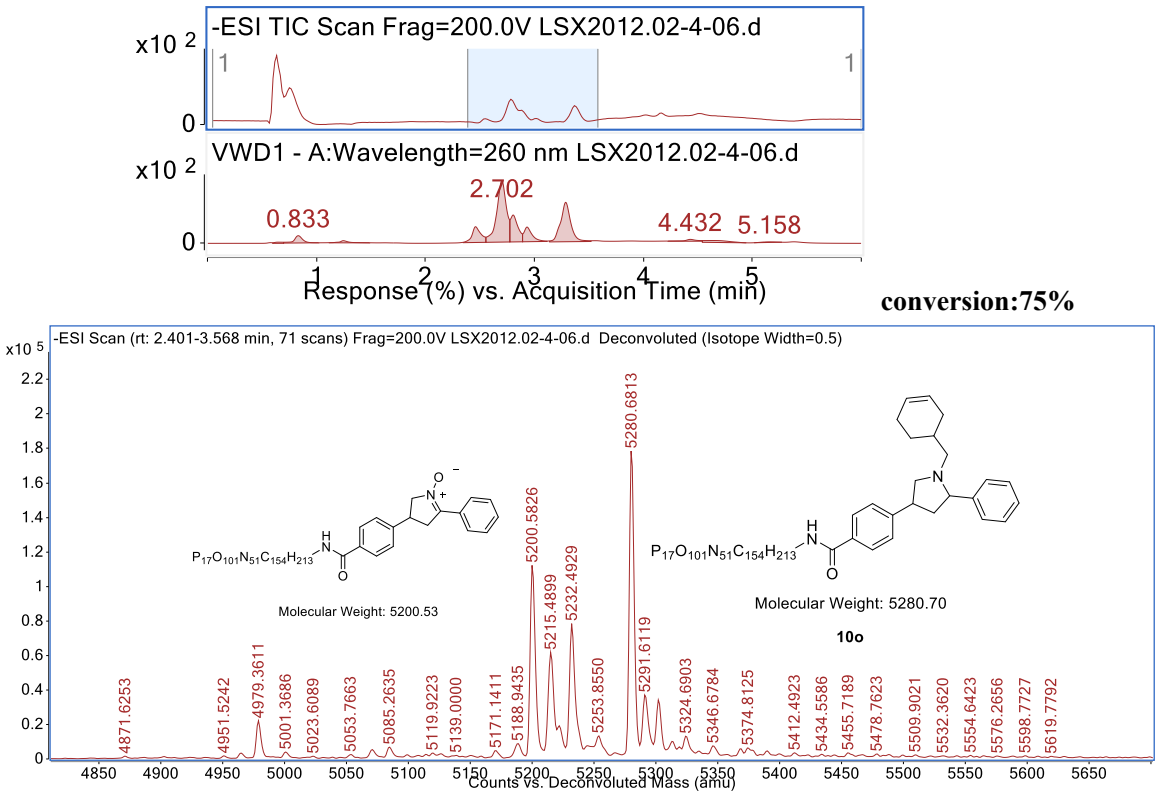


Figure S62 Deconvoluted mass of on-DNA product **10o**

Mass Spectrum of on-DNA Product 10p

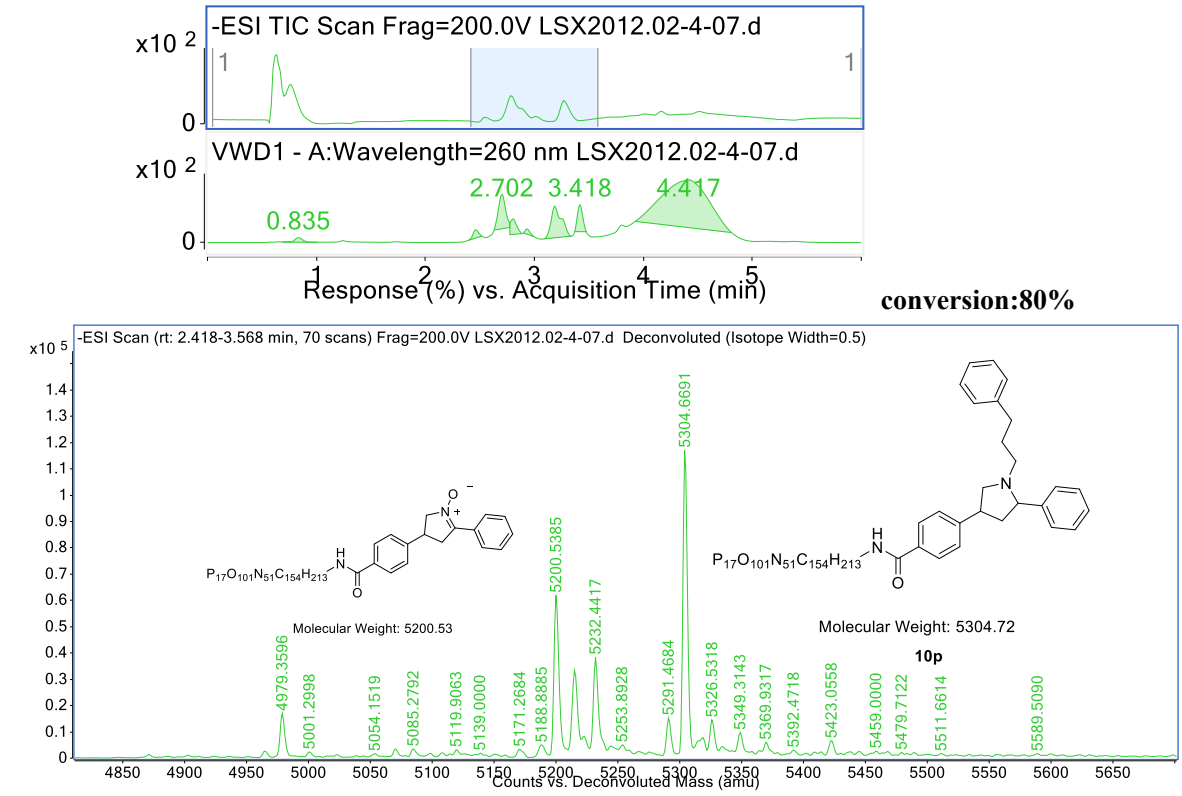


Figure S63 Deconvoluted mass of on-DNA product **10p**

Mass Spectrum of on-DNA Product 10q

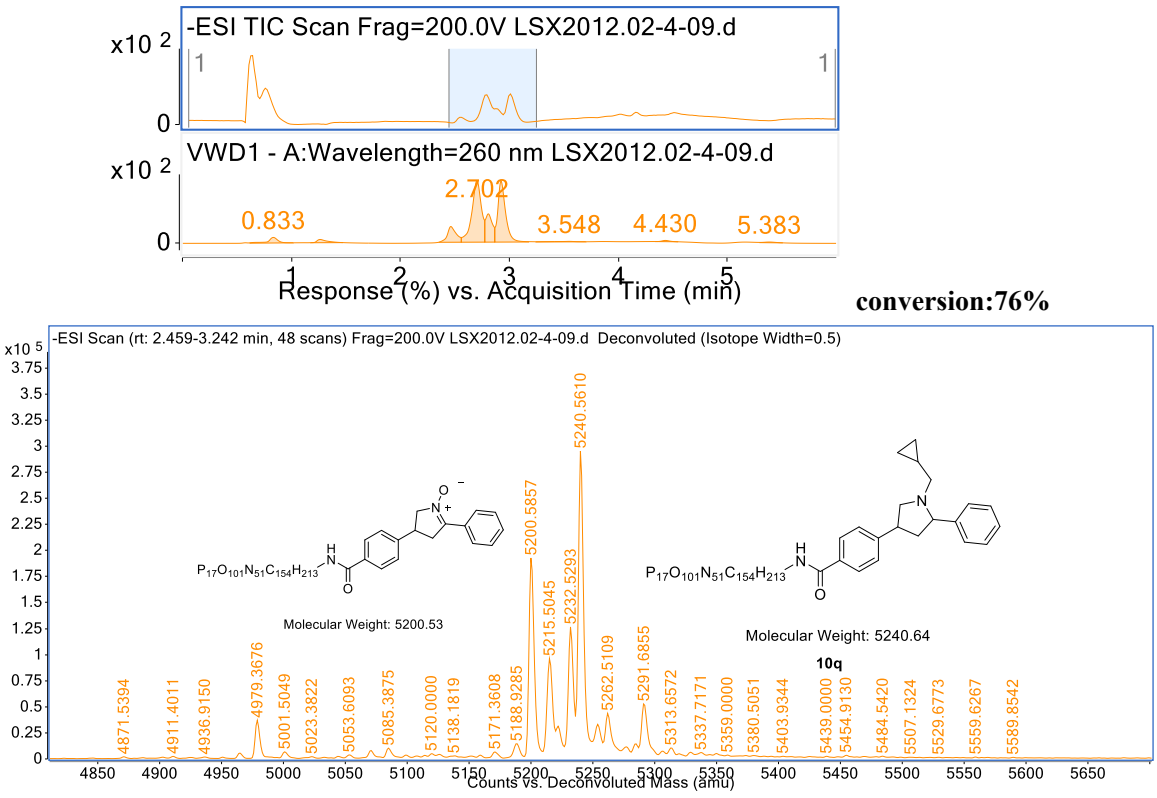


Figure S64 Deconvoluted mass of on-DNA product **10q**

Mass Spectrum of on-DNA Product 10r

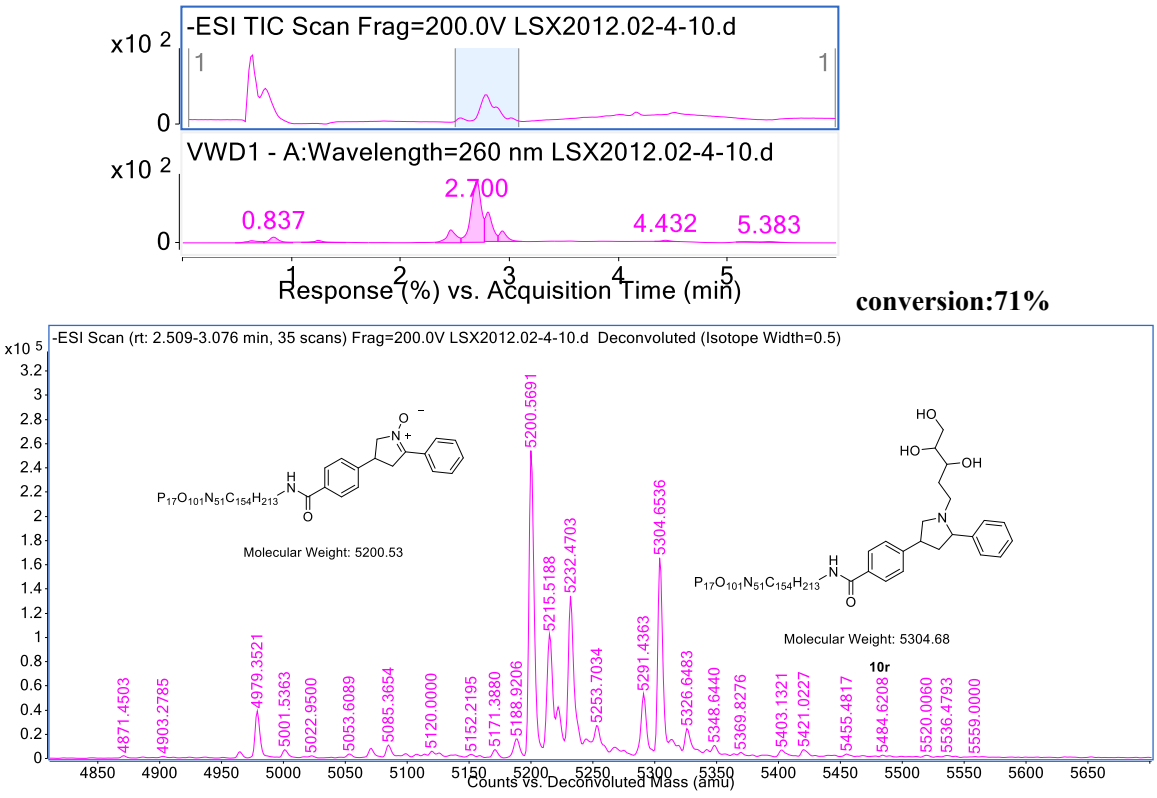


Figure S65 Deconvoluted mass of on-DNA product **10r**

Preparation of on-DNA Product Isoxazole

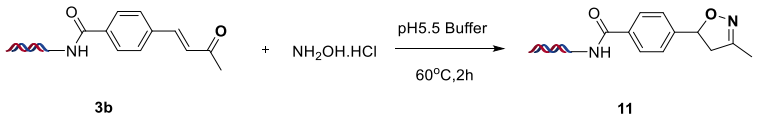


Figure S66 Synthetic route of on-DNA product **11**

Materials

3b: 1 mM in water

NH_2OH. HCl: 200 mM in water

Procedure

1) To **3b** solution (5 nmol, 5 μL), was added 5 μL pH5.5 Buffer , 5ul NH_2OH. HCl (200 equiv). The mixture was vortexed.
2) The reaction mixture at 60°C for 2 h.
3) After the reaction, the product **11** was obtained by ethanol precipitation as described above.

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Mass Spectrum of on-DNA Product 11

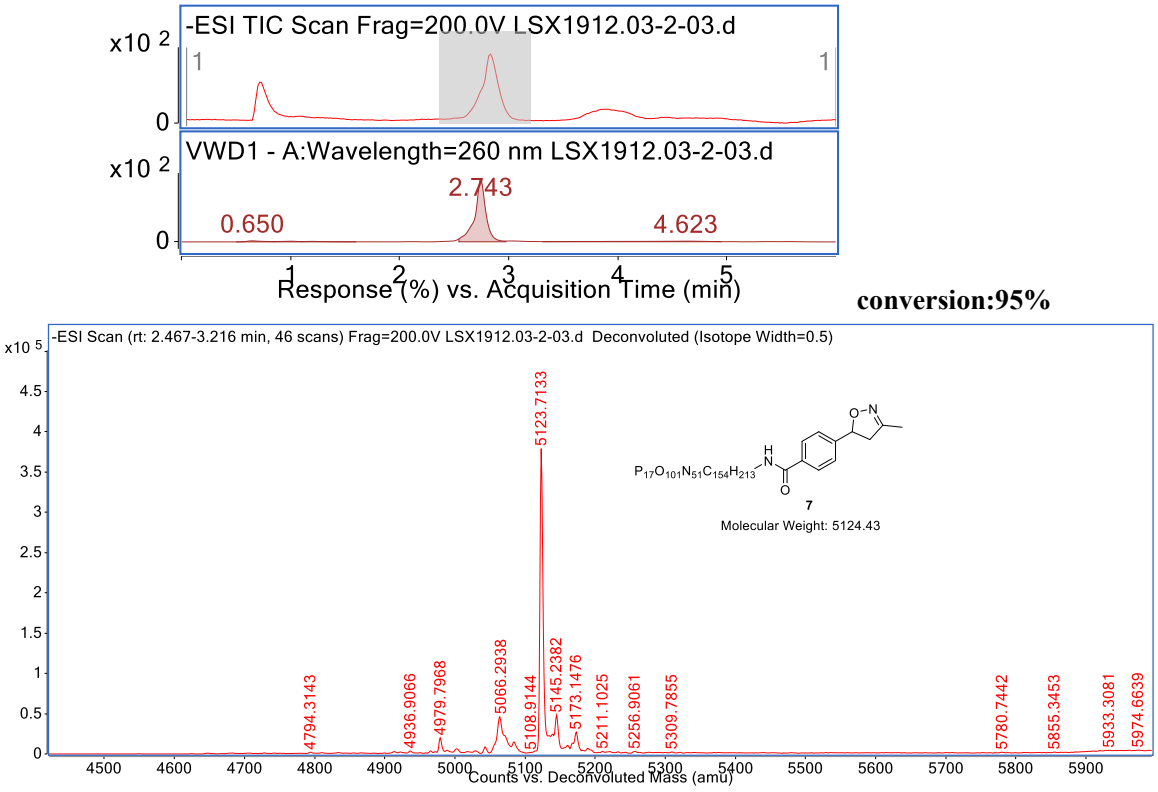


Figure S67 Deconvoluted mass of on-DNA product **11**

Preparation of on-DNA Product Pyrazole

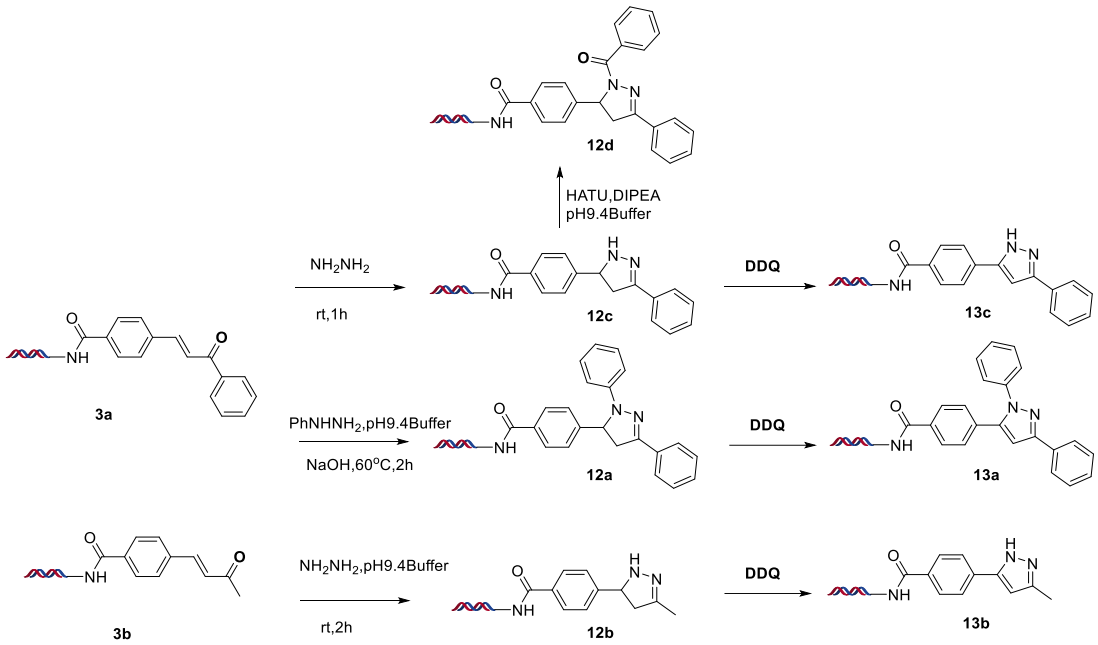


Figure S68 Synthetic route of on-DNA product **12a-d, 13a-c**

Materials

3a: 1 mM in water

3b: 1 mM in water

NH_2NH_2: 200mM in water

$PhNHNH_2$: 200mM in DMA

NaOH: 200mM in water

DDQ: (2,3-dicyano-5,6-dichloro-p-benzoquinone) 200mM in DMA

HATU: 200mM in DMA

DIPEA: 200mM in DMA

Benzoic acid: 200 mM in DMA

Procedure 1

1) To **3a** solution (5 nmol, 5 μL), was added 1.25 μL NaOH(50 equiv), 5 μL $PhNHNH_2$ (200 equiv). The mixture was vortexed.
2) The reaction mixture at 25 °C for 2 h.
3) After the reaction, the product **12a** was obtained by ethanol precipitation as described above.
4) To **12a** solution (5 nmol, 5 μL), was added 5 μL pH 5.5 Buffer , 5uL DDQ (200 equiv). The mixture was vortexed.
5) The reaction mixture at 60 °C for 2 h.
6) After the reaction, the product **13a** was obtained by ethanol precipitation as described above.

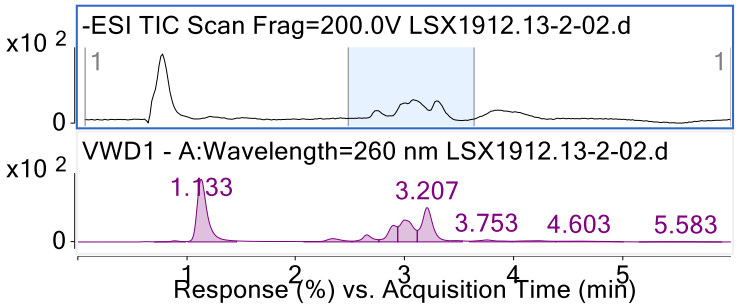
Procedure 2

1) To **3b** solution (5 nmol, 5 μL), was added 5 μL pH 9.4 Buffer, 5 μL $NHNH_2$ (200 equiv). The mixture was vortexed.
2) The reaction mixture at 60 °C for 2 h.
3) After the reaction, the product **12b** was obtained by ethanol precipitation as described above.
4) To **12b** solution (5 nmol, 5 μL), was added 5 μL pH 5.5 Buffer , 5uL DDQ (200 equiv). The mixture was vortexed.
5) The reaction mixture at 60 °C for 2 h.
6) After the reaction, the product **13b** was obtained by ethanol precipitation as described above.

Procedure 3

1) To **3a** solution (5 nmol, 5 μL NH_2NH_2 (200 equiv). The mixture was vortexed.
2) The reaction mixture at 25 °C for 1 h.
3) After the reaction, the product **12c** was obtained by ethanol precipitation as described above.
4) To **12c** solution (5 nmol, 5 μL), was added 5 μL pH 5.5 Buffer , 5uL DDQ (200 equiv). The mixture was vortexed.
5) The reaction mixture at 60 °C for 2 h.
6) After the reaction, the product **13c** was obtained by ethanol precipitation as described above.
7) Mixing benzoic acid with equal volume HATU and DIPEA to prepare the solution VI.
8) To **12c** solution (5 nmol, 5 μL), was added 5 μL pH 9.4 Buffer, 200 equiv. of solution VI (15 μL), and mix.
9) The reaction mixture at 25 °C for 2 h.
10) After the reaction, the product **12d** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 12a



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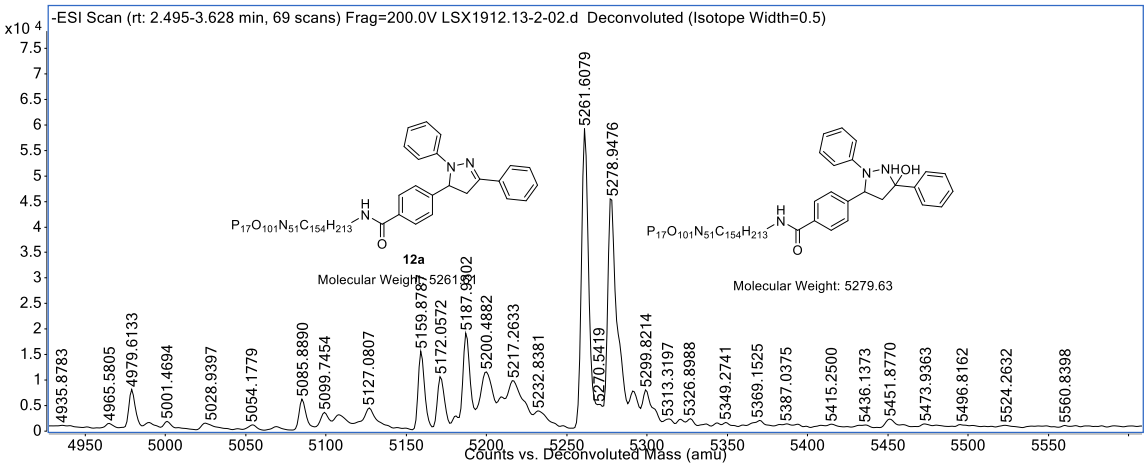
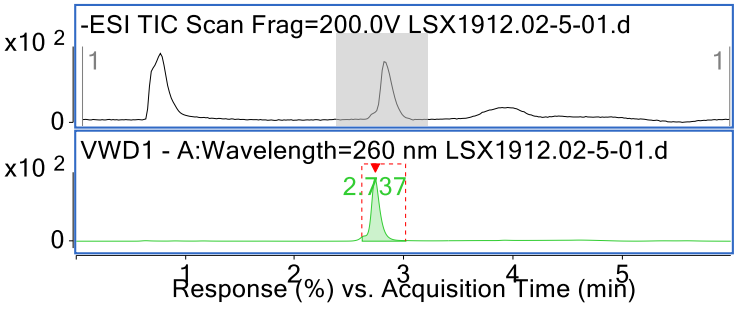


Figure S69 Deconvoluted mass of on-DNA product **12a**

Mass Spectrum of on-DNA Product 12b



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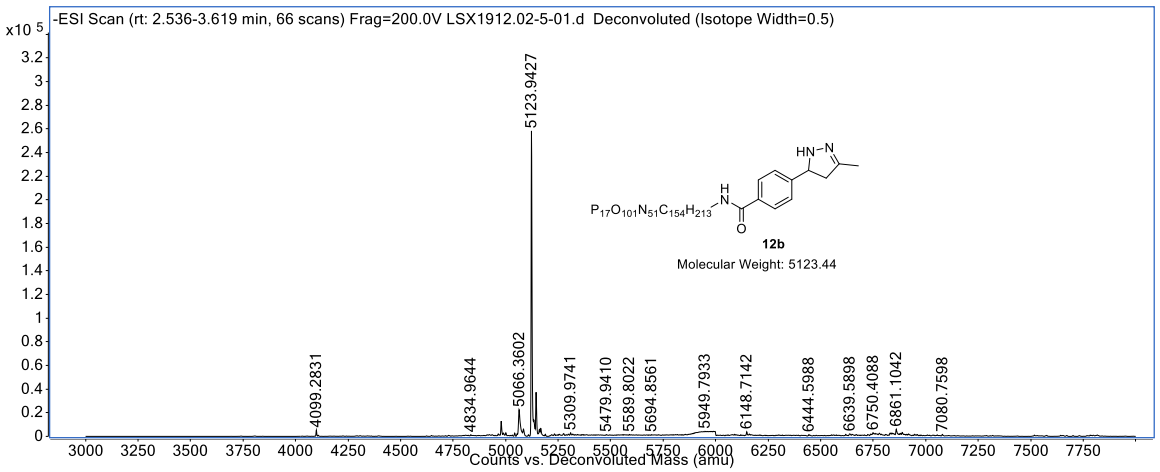
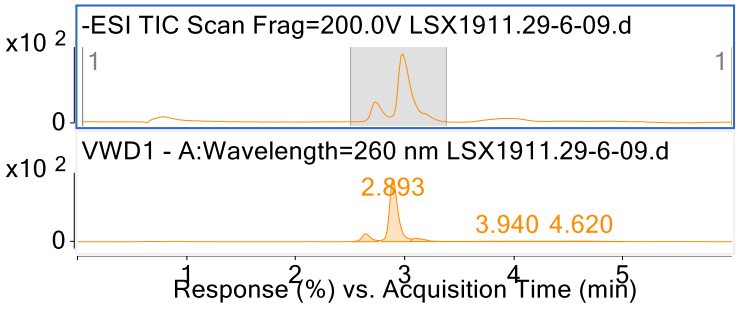


Figure S70 Deconvoluted mass of on-DNA product **12b**

Mass Spectrum of on-DNA Product 12c



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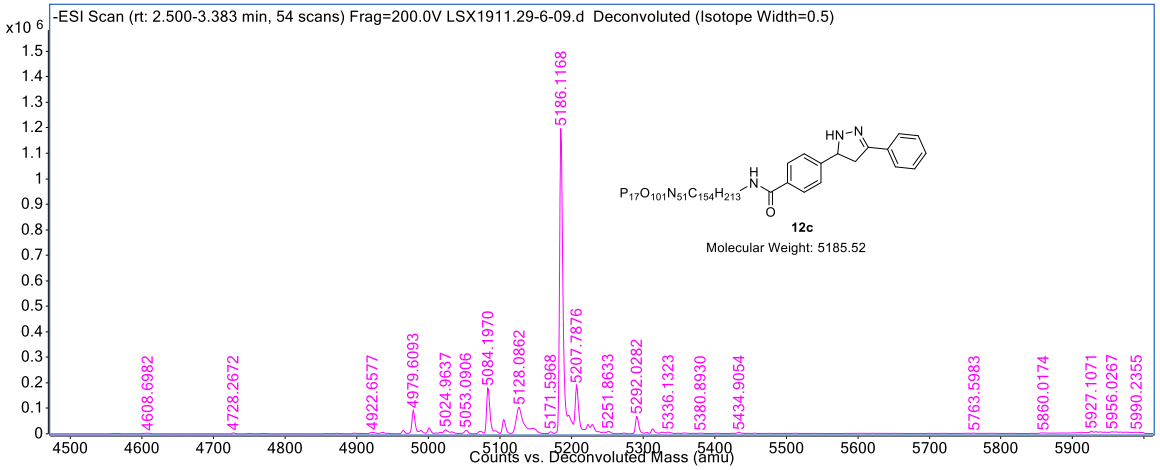
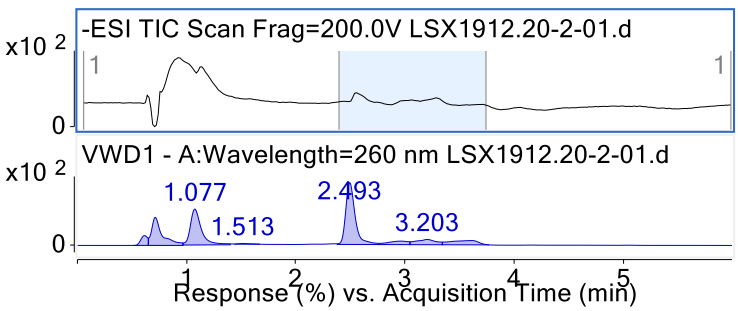


Figure S71 Deconvoluted mass of on-DNA product **12c**

Mass Spectrum of on-DNA Product 13a



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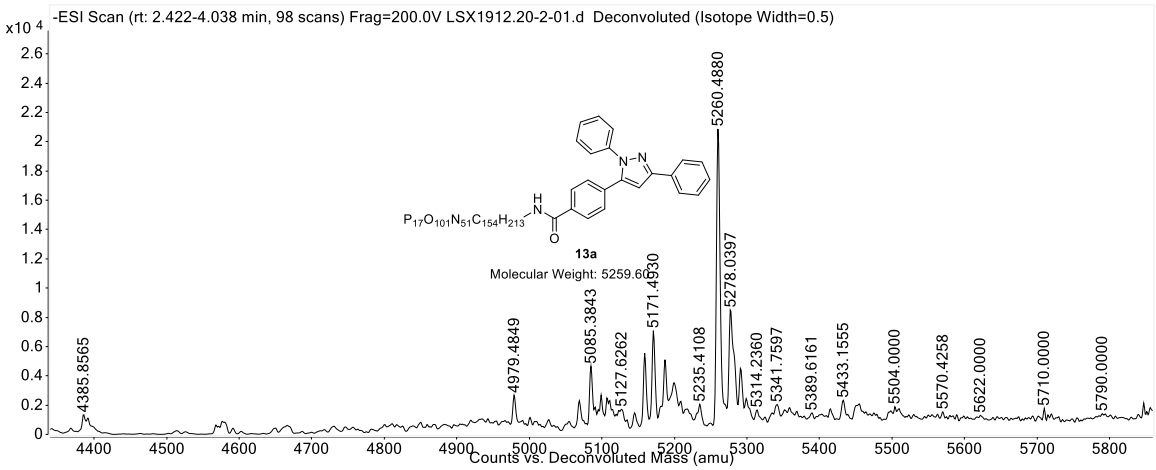
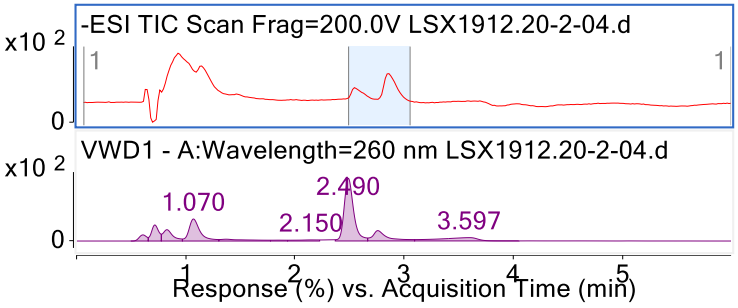


Figure S72 Deconvoluted mass of on-DNA product **13a**

Mass Spectrum of on-DNA Product 13b



conversion:40%

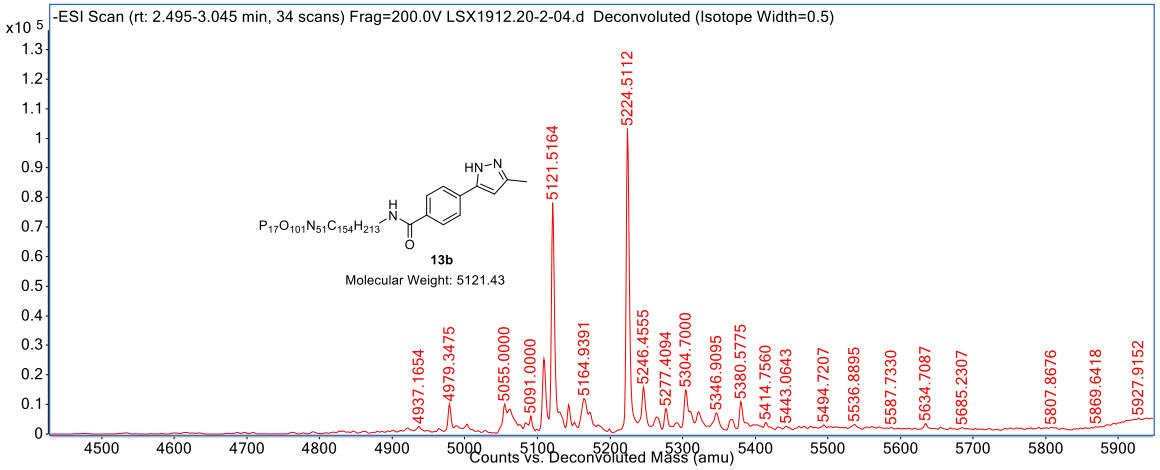
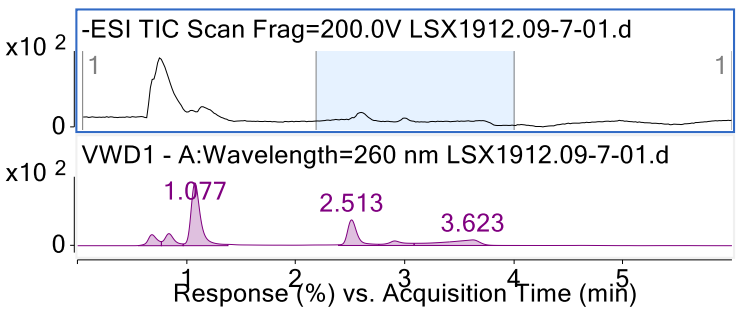


Figure S73 Deconvoluted mass of on-DNA product **13b**

Mass Spectrum of on-DNA Product 13c



conversion:76%

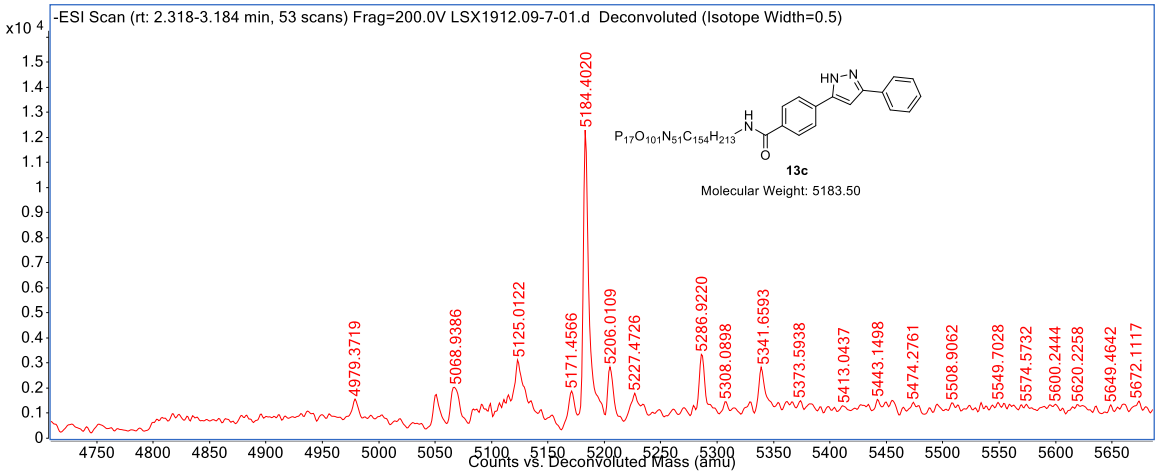
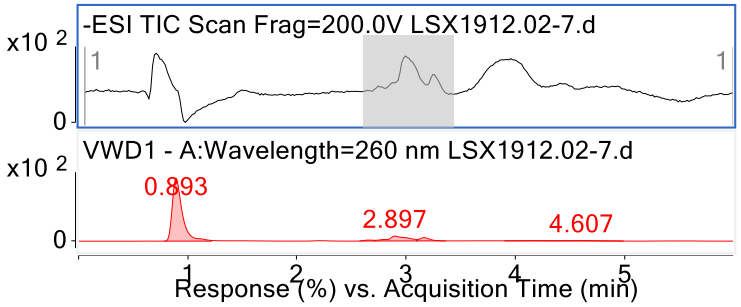


Figure S74 Deconvoluted mass of on-DNA product **13c**

Mass Spectrum of on-DNA Product 12d



conversion:30%

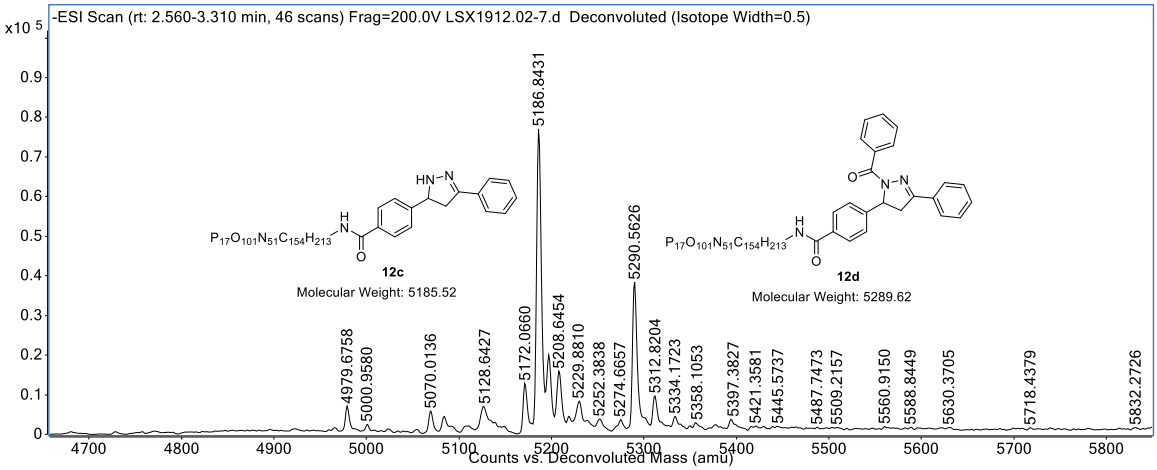


Figure S75 Deconvoluted mass of on-DNA product **12d**

Preparation of on-DNA Product Piperidine

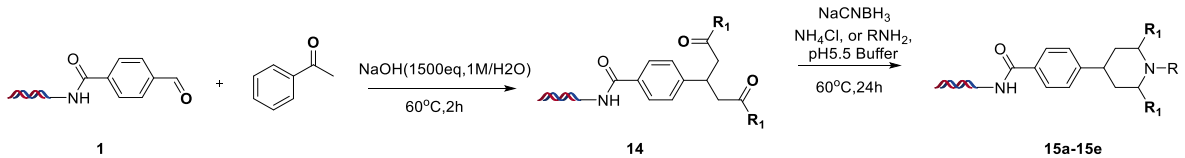


Figure S76 Synthetic route of on-DNA product **15a-e**

Materials

1: 1 mM in water

Acetophenone: 200mM in DMSO

NaOH: 1M in water

NH_4Cl: 3M in water

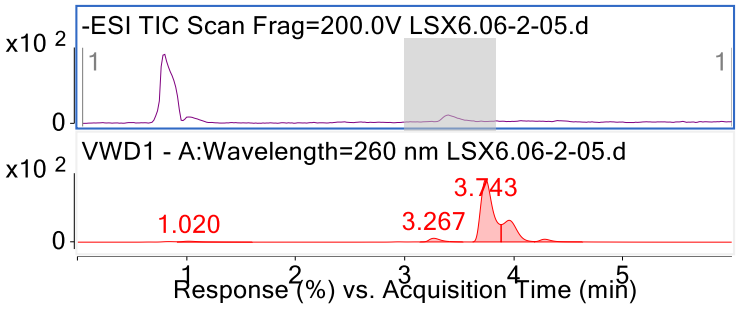
RNH_2: 3M in DMA

$NaCNBH_3$: 3M in water

Procedure

1) To **1** solution (5 nmol, 5 μL), was added 20 μL acetophenone (800 equiv) , 7.5ul NaOH (1500 equiv). The mixture was vortexed.
2) The reaction mixture at 60 °C for 2 h.
3) After the reaction, the product **14** was obtained by ethanol precipitation as described above.
4) To **14** solution (5 nmol, 5 μL), was added 5 μL pH5.5 Buffer , 10ul NH_4Cl (6000 equiv) or 10uL RNH_2 (6000 equiv) and 10uL $NaCNBH_3$(6000 equiv) . The mixture was vortexed.
5) The reaction mixture at 60 °C for 16 h
6) After the reaction, the product **15** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 14



conversion:95%

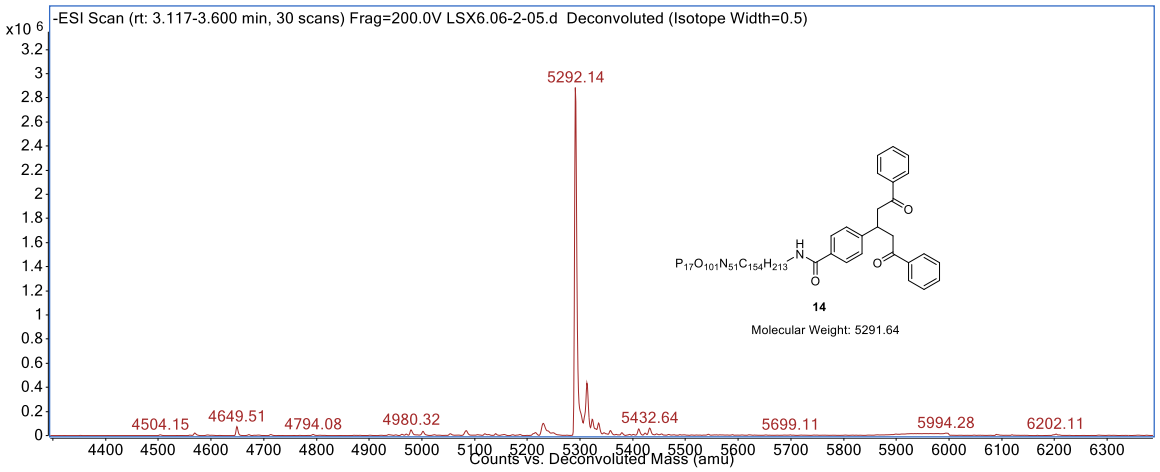


Figure S77 Deconvoluted mass of on-DNA product **14**

Mass Spectrum of on-DNA Product 15a

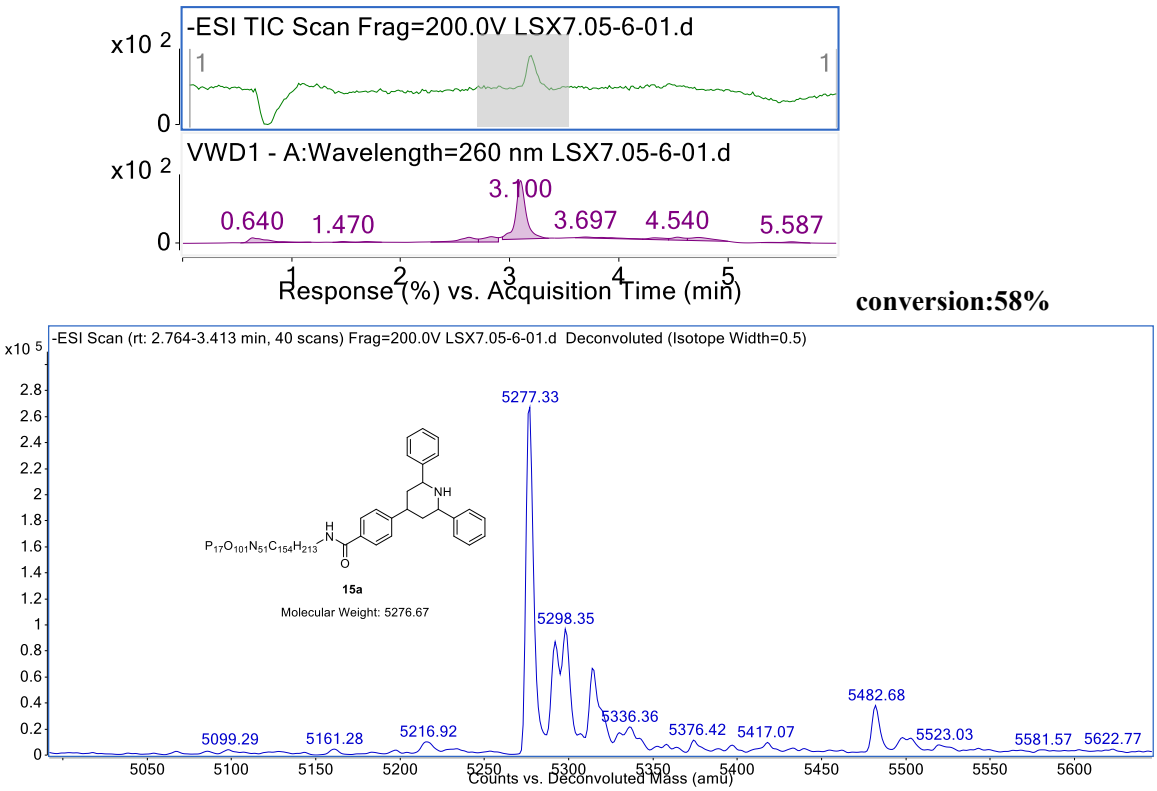


Figure S78 Deconvoluted mass of on-DNA product **15a**

Mass Spectrum of on-DNA Product 15b

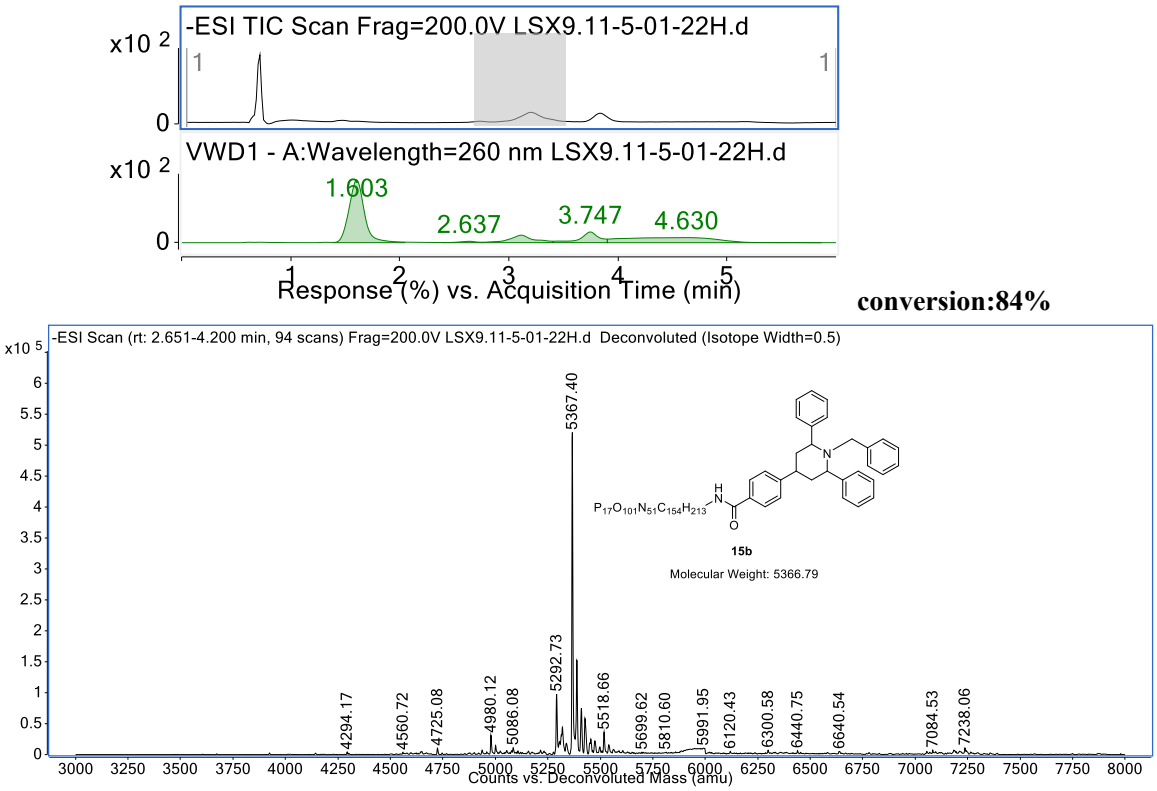


Figure S76 Deconvoluted mass of on-DNA product **15b**

Mass Spectrum of on-DNA Product 15c

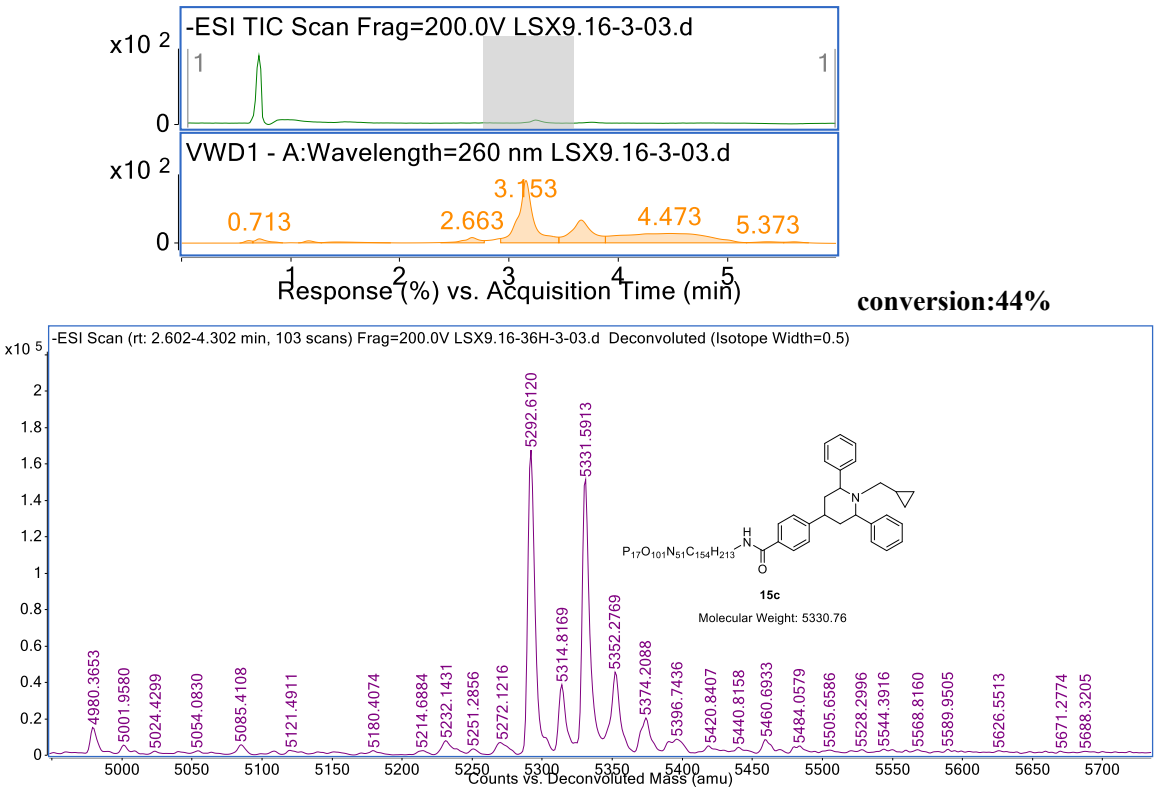


Figure S79 Deconvoluted mass of on-DNA product **15c**

Mass Spectrum of on-DNA Product 15d

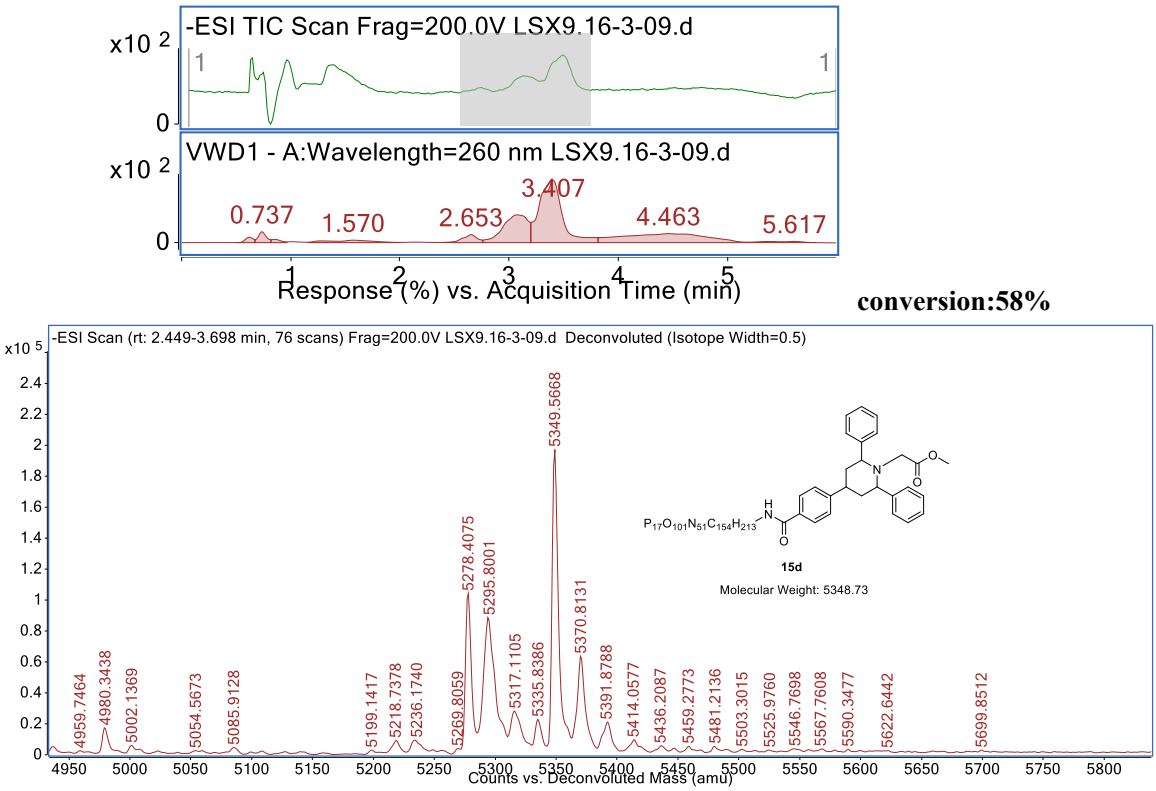


Figure S80 Deconvoluted mass of on-DNA product **15d**

Mass Spectrum of on-DNA Product 15e

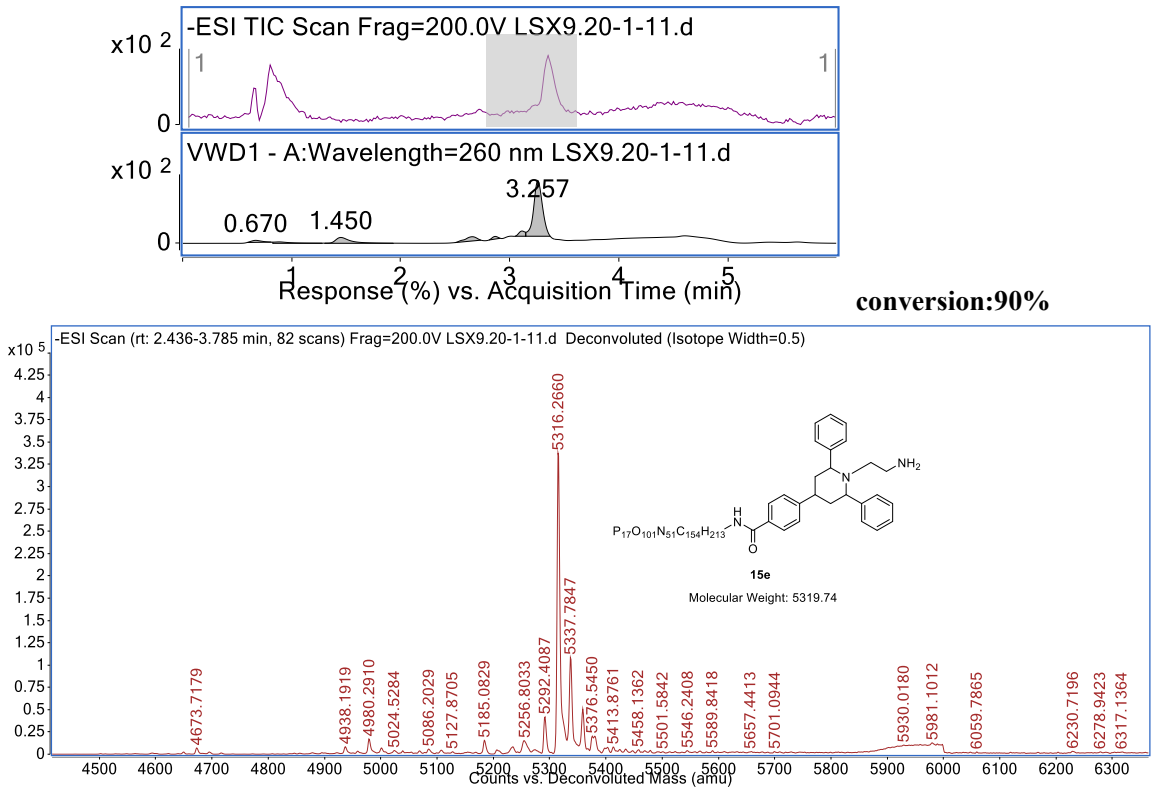


Figure S81 Deconvoluted mass of on-DNA product **15e**

Preparation of on-DNA Product Pyridine

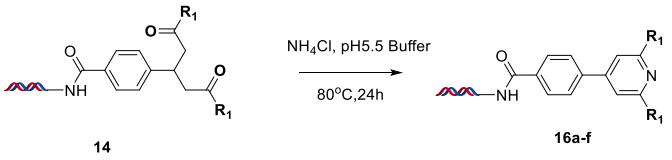


Figure S82 Synthetic route of on-DNA product **16a-16f**

Materials

14: 1 mM in water

NH_4Cl: 3M in water

Procedure

1) To **14** solution (5 nmol, 5 μL), was added 5 μL pH5.5 Buffer , 10ul NH_4Cl (6000 equiv). The mixture was vortexed.
2) The reaction mixture at 80 °C for 24 h
3) After the reaction, the product **16** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 16a

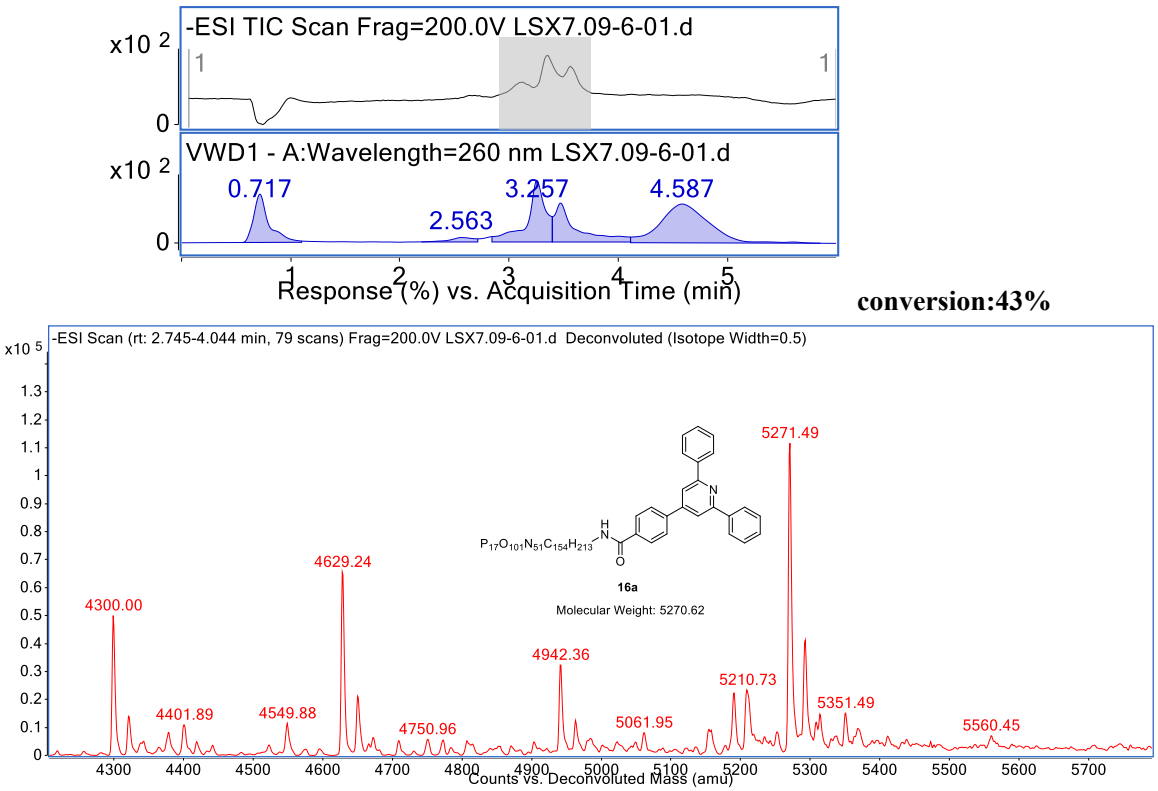


Figure S83 Deconvoluted mass of on-DNA product **16a**

Mass Spectrum of on-DNA Product 16b

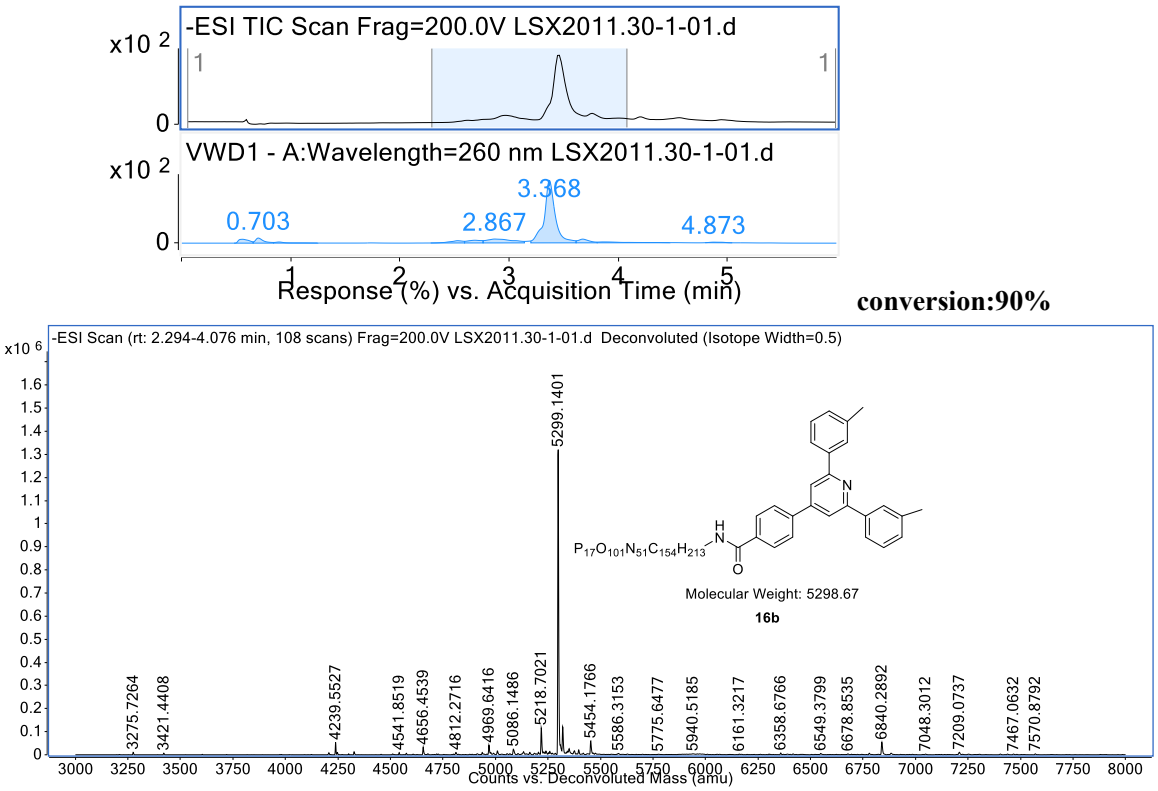


Figure S84 Deconvoluted mass of on-DNA product **16b**

Mass Spectrum of on-DNA Product 16c

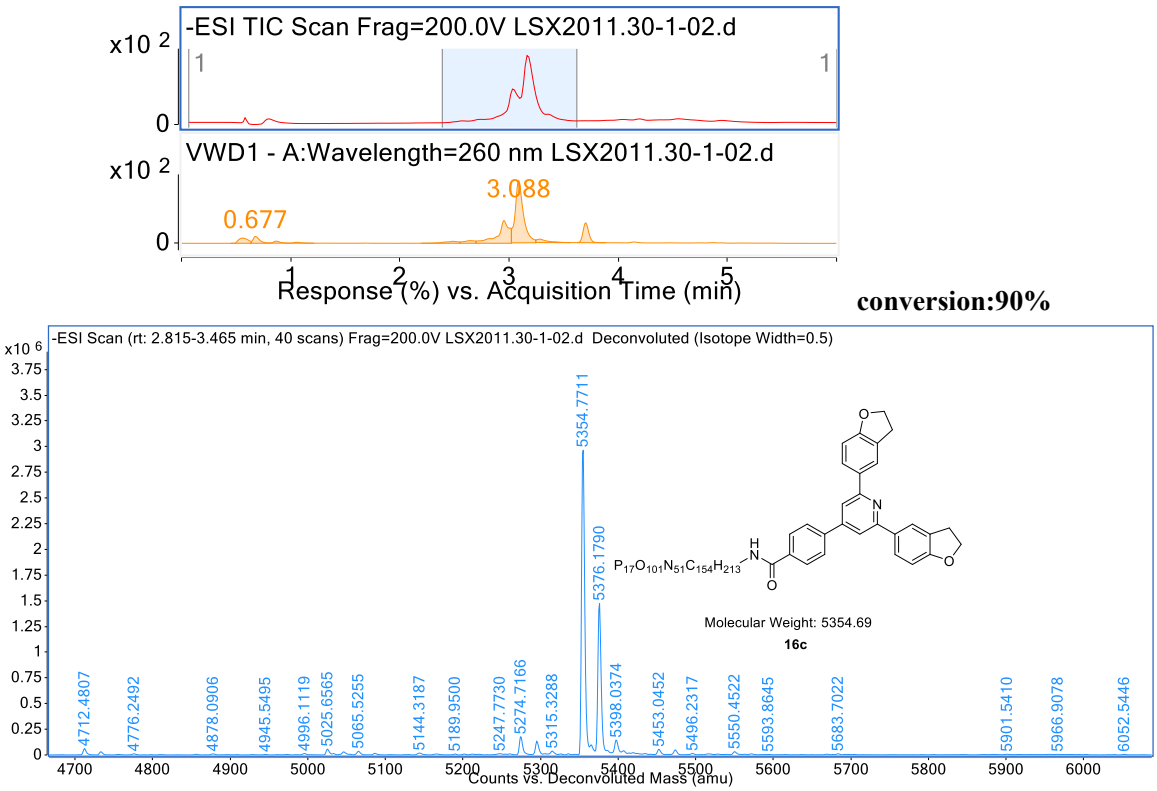


Figure S85 Deconvoluted mass of on-DNA product **16c**

Mass Spectrum of on-DNA Product 16d

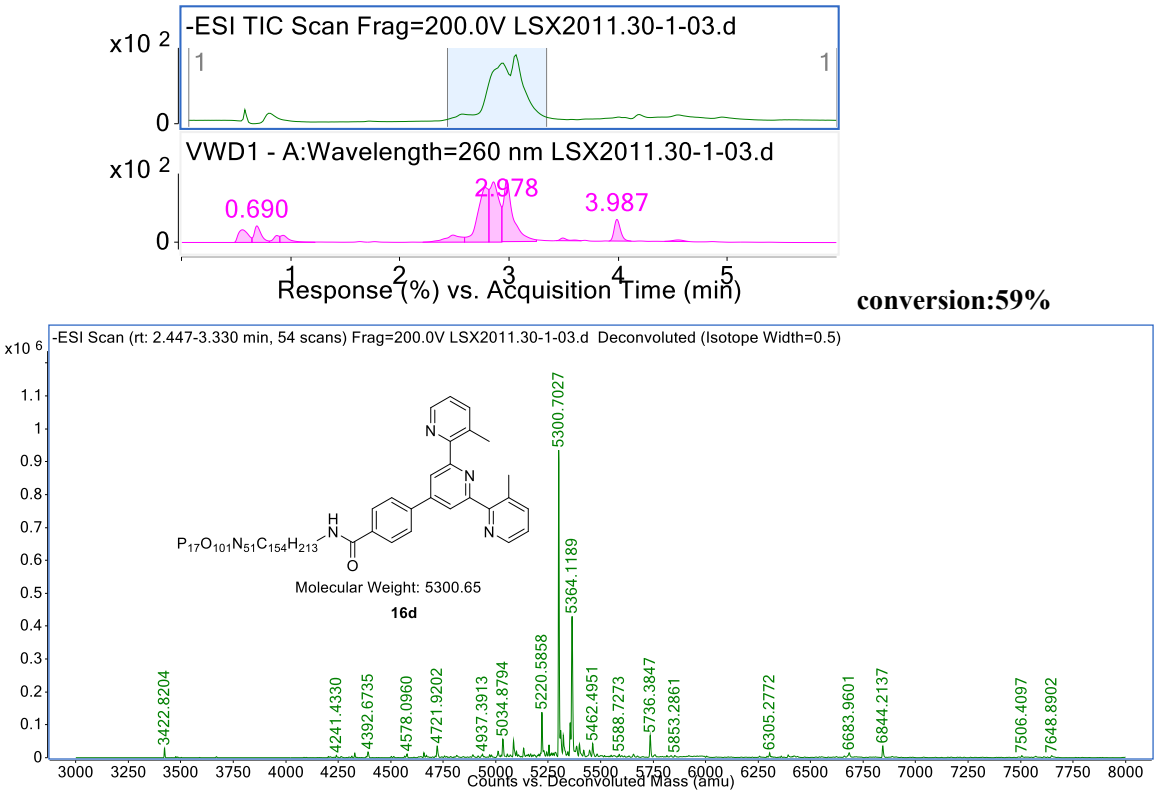


Figure S86 Deconvoluted mass of on-DNA product **16d**

Mass Spectrum of on-DNA Product 16e

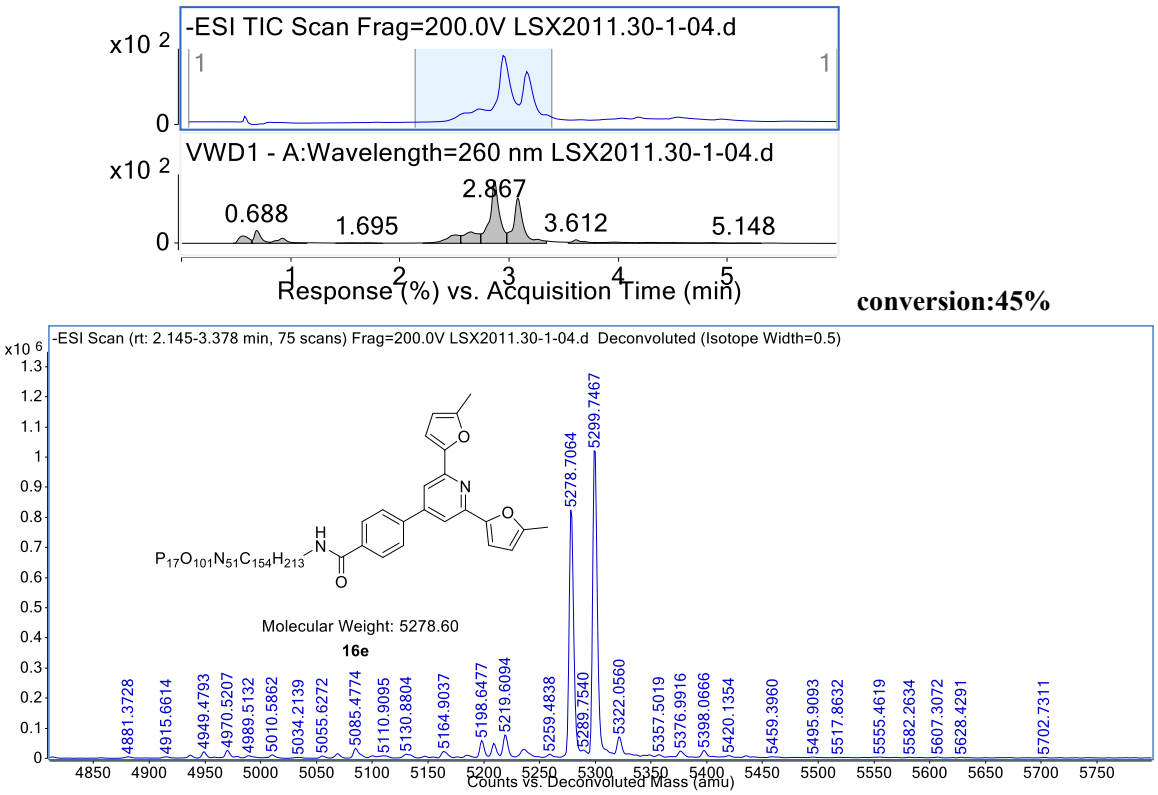


Figure S87 Deconvoluted mass of on-DNA product **16e**

Mass Spectrum of on-DNA Product 16f

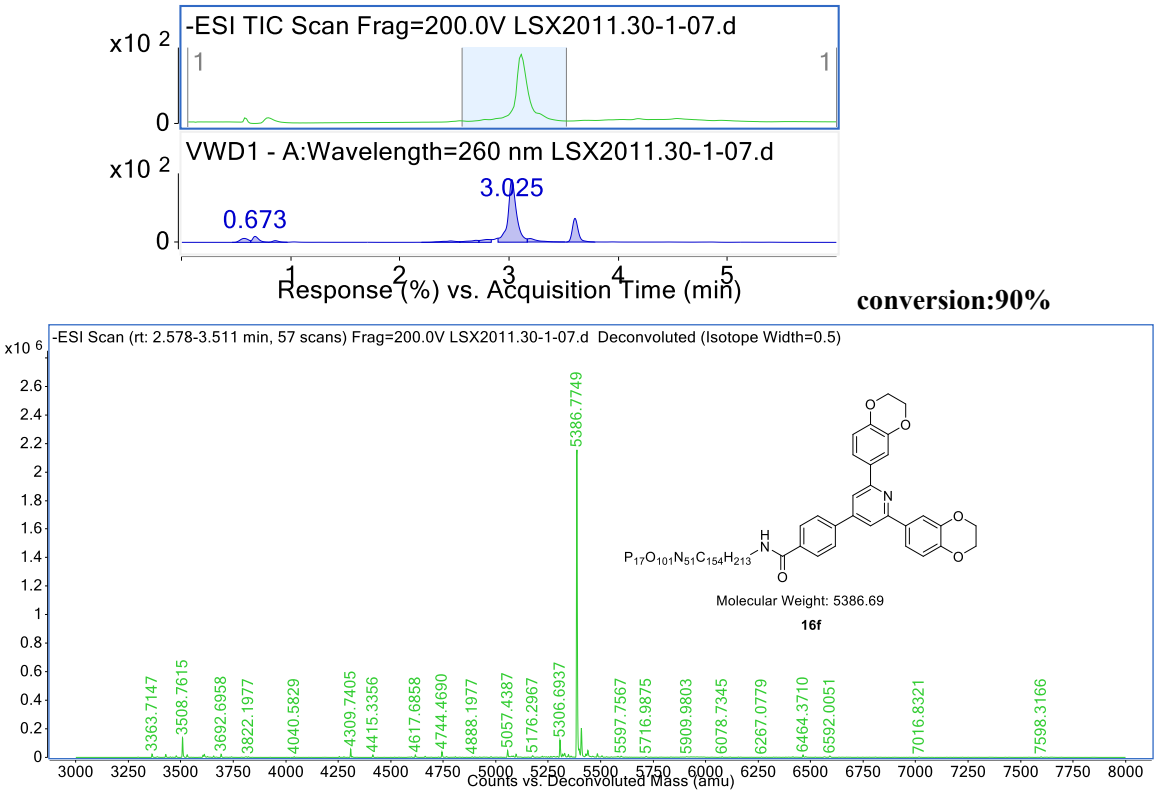


Figure S88 Deconvoluted mass of on-DNA product **16f**

Preparation of on-DNA Product 17a-d

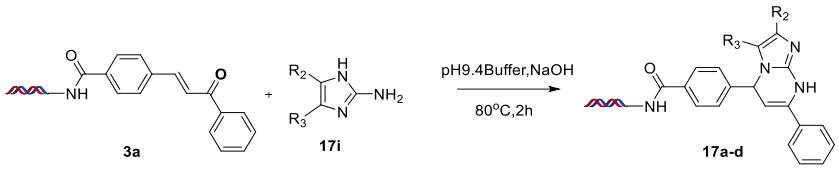


Figure S89 Synthetic route of on-DNA product **17a-d**

Materials

3a: 1 mM in water

17i: 200mM in EtOH

NaOH: 200mM in water

Procedure

1) To **3a** solution (5 nmol, 5 μL), was added 5 μL pH 9.4 Buffer , 5ul **17i** (200 equiv) and 1.25uL NaOH(50 equiv) . The mixture was vortexed.
2) The reaction mixture at 80 °C for 2 h
3) After the reaction, the product **17** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 17a

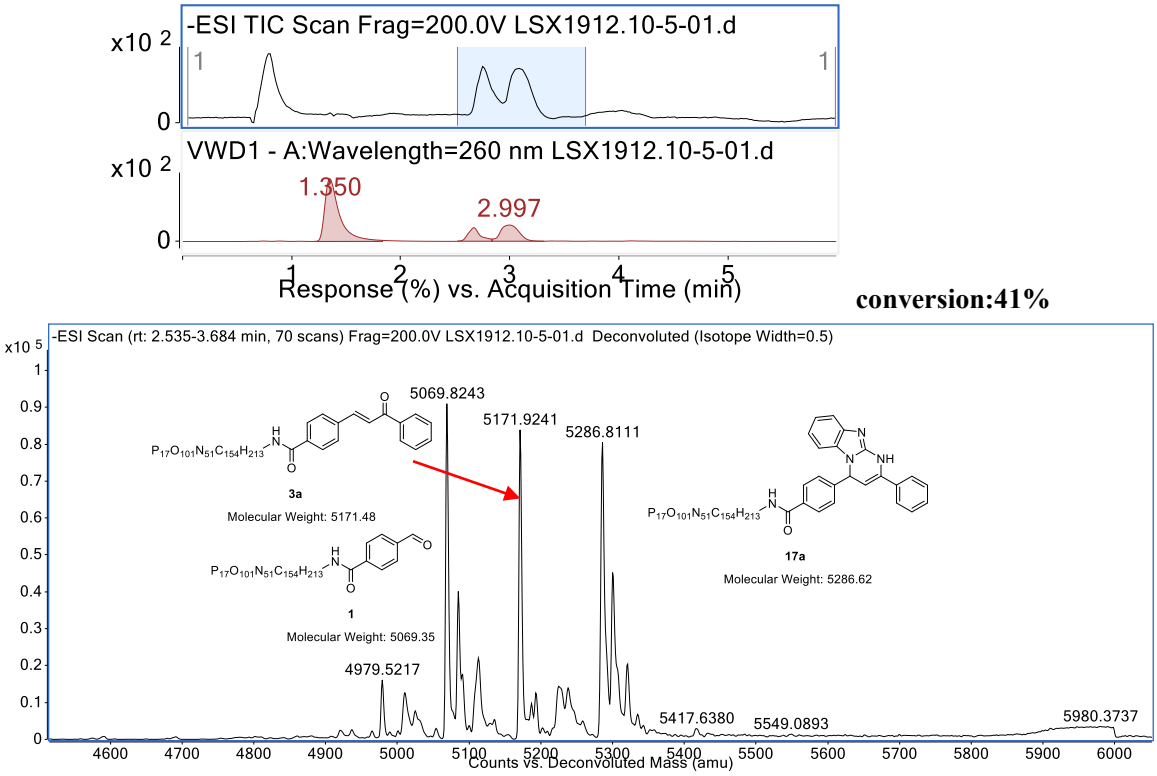
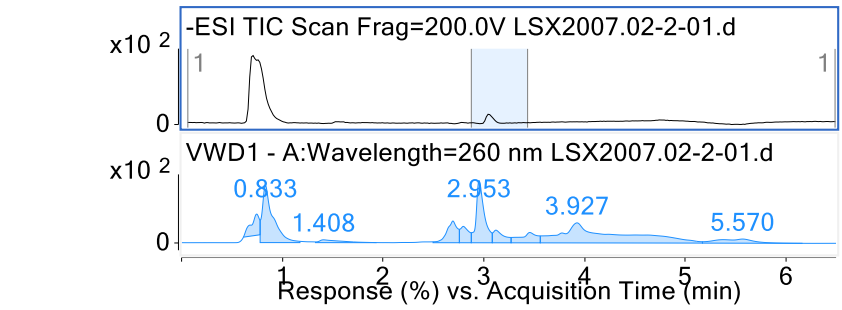


Figure S90 Deconvoluted mass of on-DNA product **17a**

Mass Spectrum of on-DNA Product 17b



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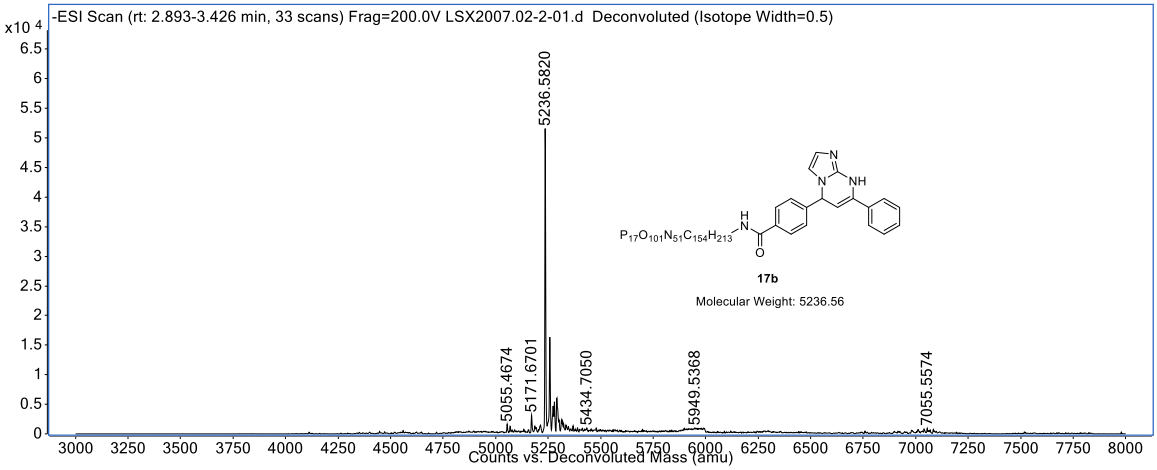
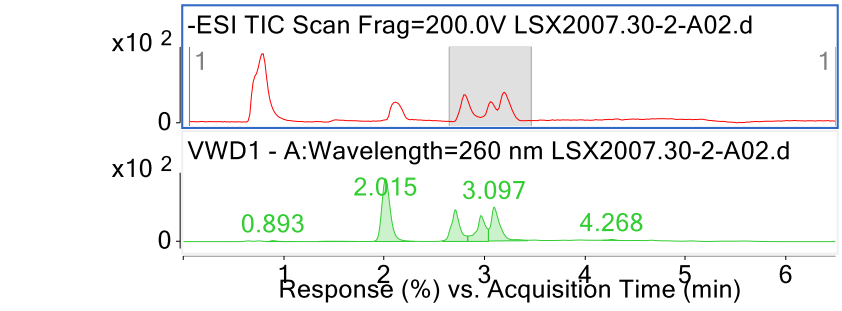


Figure S91 Deconvoluted mass of on-DNA product **17b**

Mass Spectrum of on-DNA Product 17c



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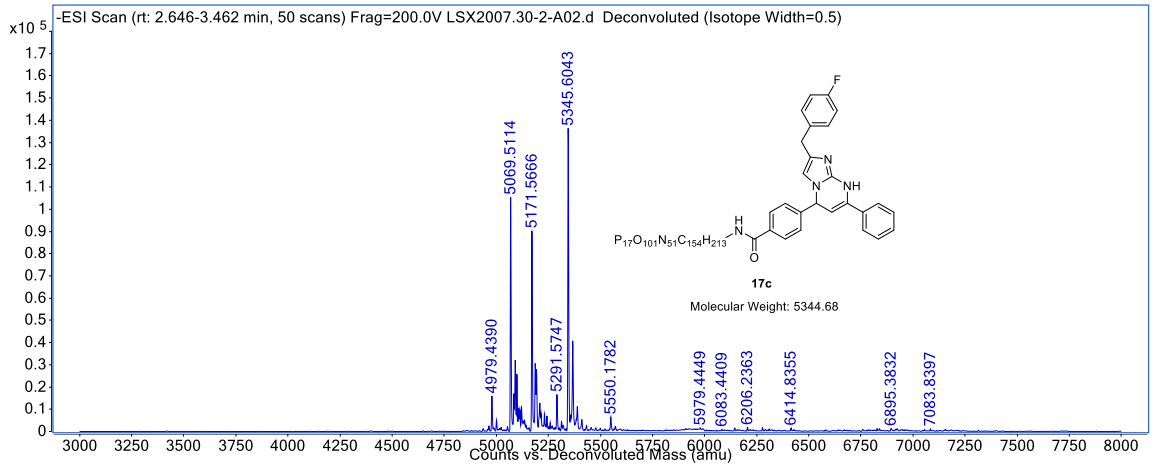


Figure S92 Deconvoluted mass of on-DNA product **17c**

Mass Spectrum of on-DNA Product 17d

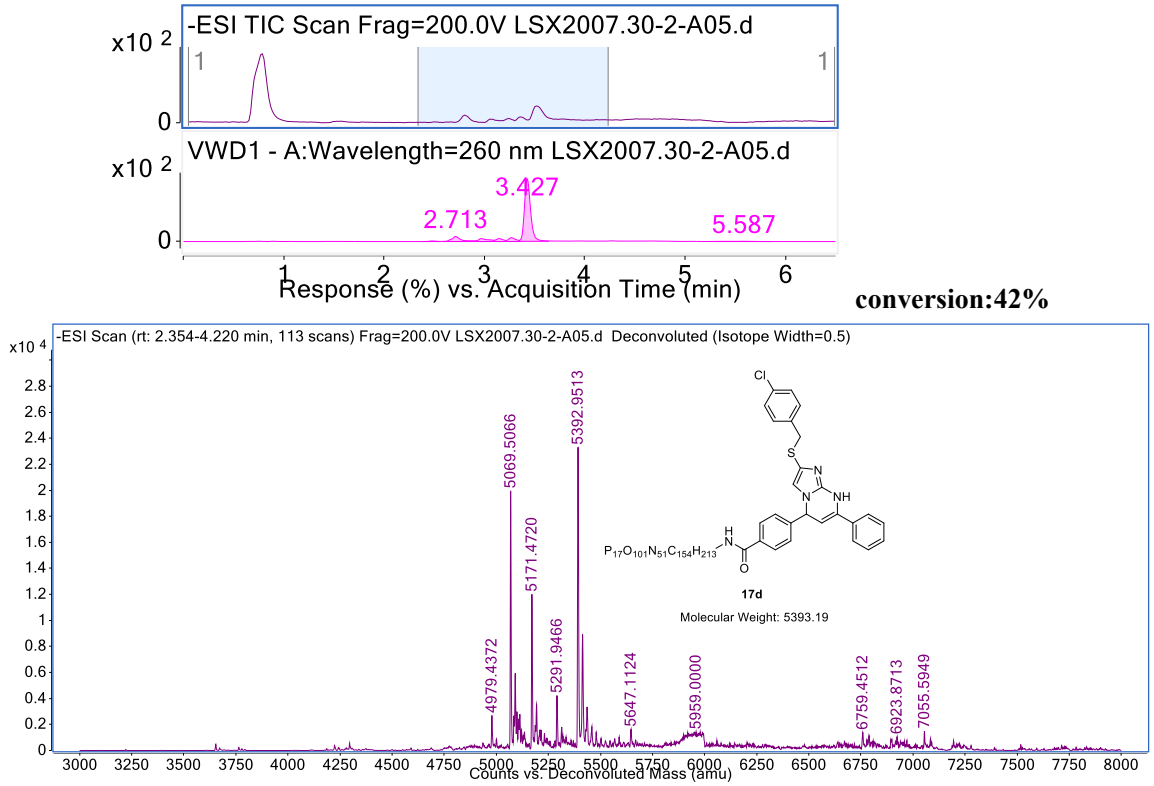


Figure S93 Deconvoluted mass of on-DNA product **17d**

Preparation of on-DNA Product 18a-b

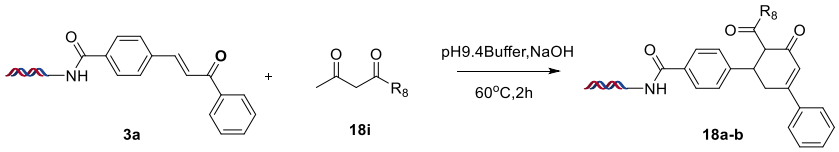


Figure S94 Synthetic route of on-DNA product **13a-b**

Materials

3a: 1 mM in water

18i: 200mM in DMA

NaOH: 200mM in water

Procedure

1) To **3a** solution (5 nmol, 5 μL), was added 5 μL pH9.4 Buffer , 5ul **18i** (200 equiv) and 1.25uL NaOH(50 equiv) . The mixture was vortexed.
2) The reaction mixture at 60 °C for 2 h
3) After the reaction, the product **18** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 18a

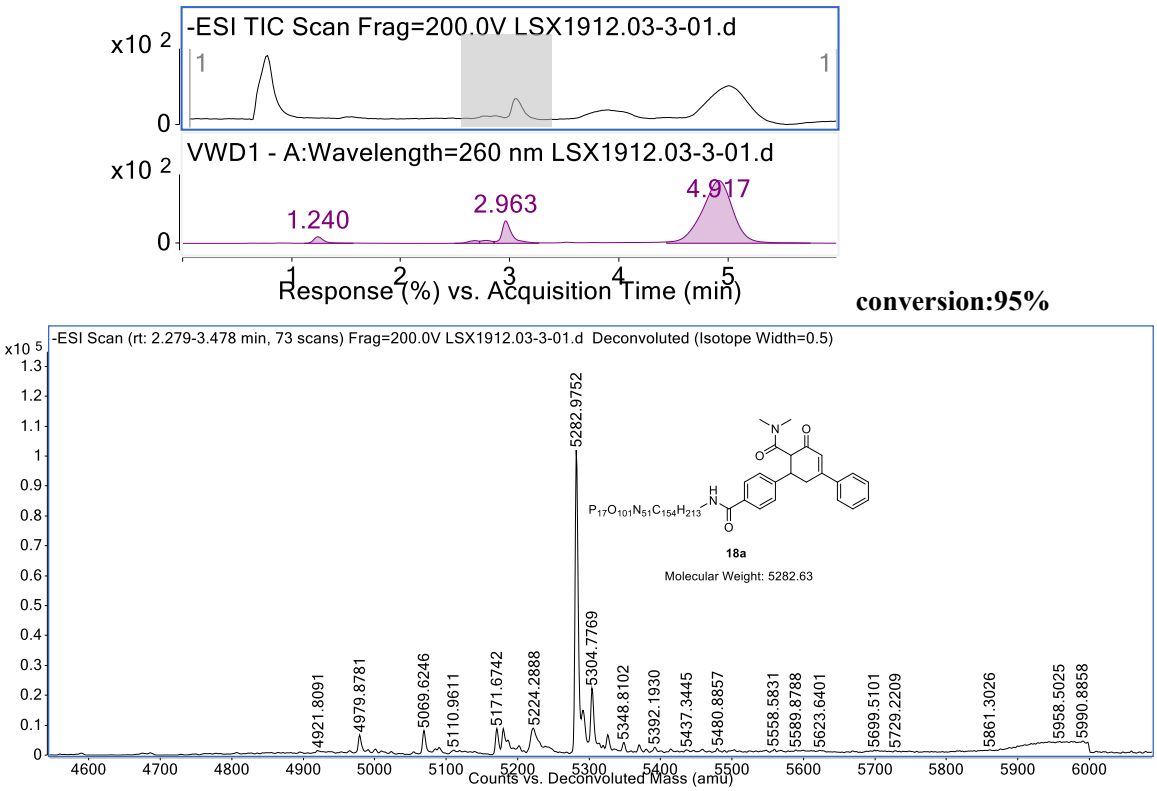


Figure S95 Deconvoluted mass of on-DNA product **18a**

Mass Spectrum of on-DNA Product 18b

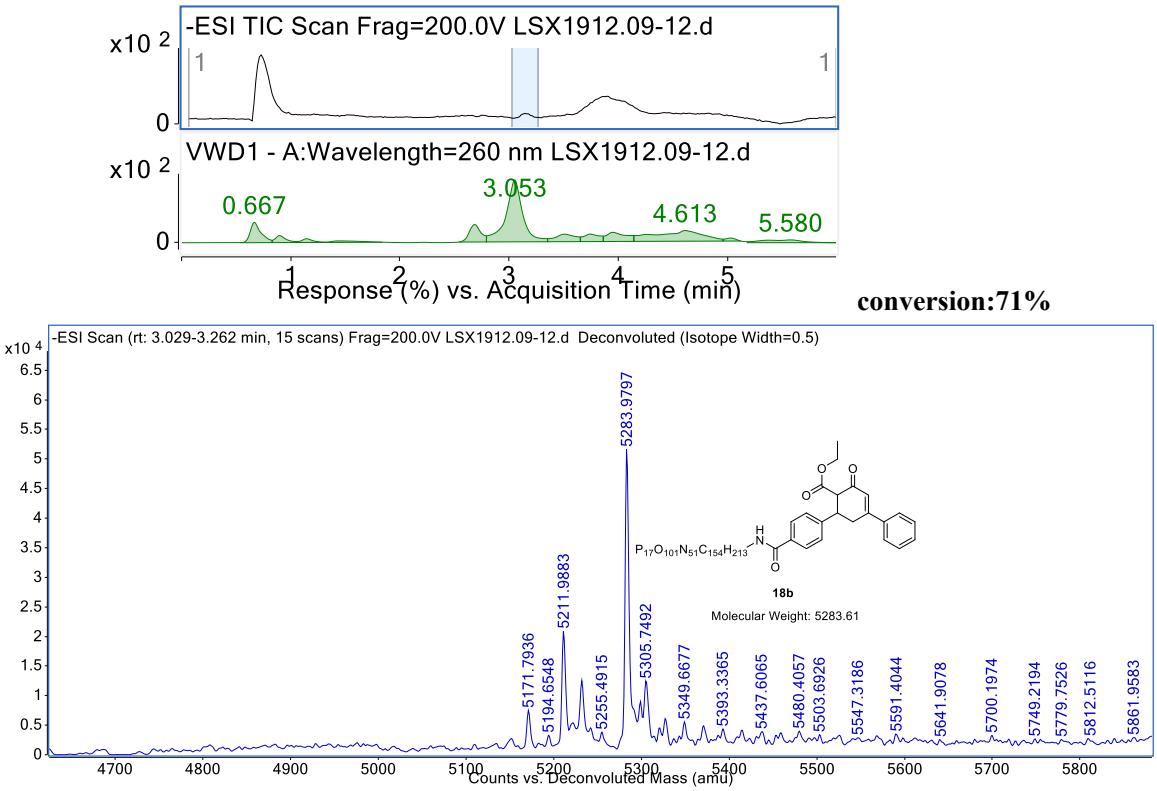


Figure S96 Deconvoluted mass of on-DNA product **18b**

Preparation of on-DNA Product 19a-b

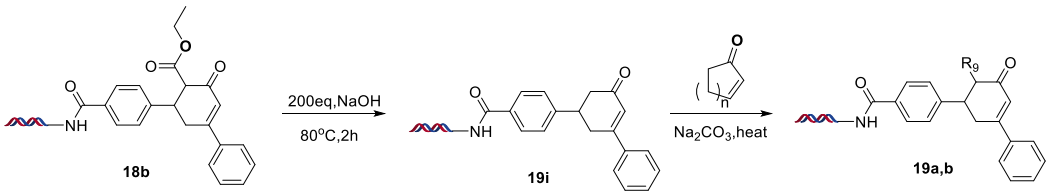


Figure S97 Synthetic route of on-DNA product **19a-b**

Materials

18b: 1 mM in water

NaOH: 200mM in water

Na_2CO_3: 200mM in water

Cyclohex-2-en-1-one or cyclopent-2-en-1-one: 500Mm in DMA

Procedure

1) To **18b** solution (5 nmol, 5 μL), was added 5 μL NaOH (200 equiv). The mixture was vortexed.
2) The reaction mixture at 80 °C for 2 h
3) After the reaction, the product **19i** was obtained by ethanol precipitation as described above.
4) To **19i** solution (5 nmol, 5 μL), was added 8 μL cyclohex-2-en-1-one or cyclopent-2-en-1-one (800 equiv) and 12.5 μL Na_2CO_3 (500 equiv). The mixture was vortexed.
5) The reaction mixture at 80 °C for 2 h
6) After the reaction, the product **19a-b** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 19i

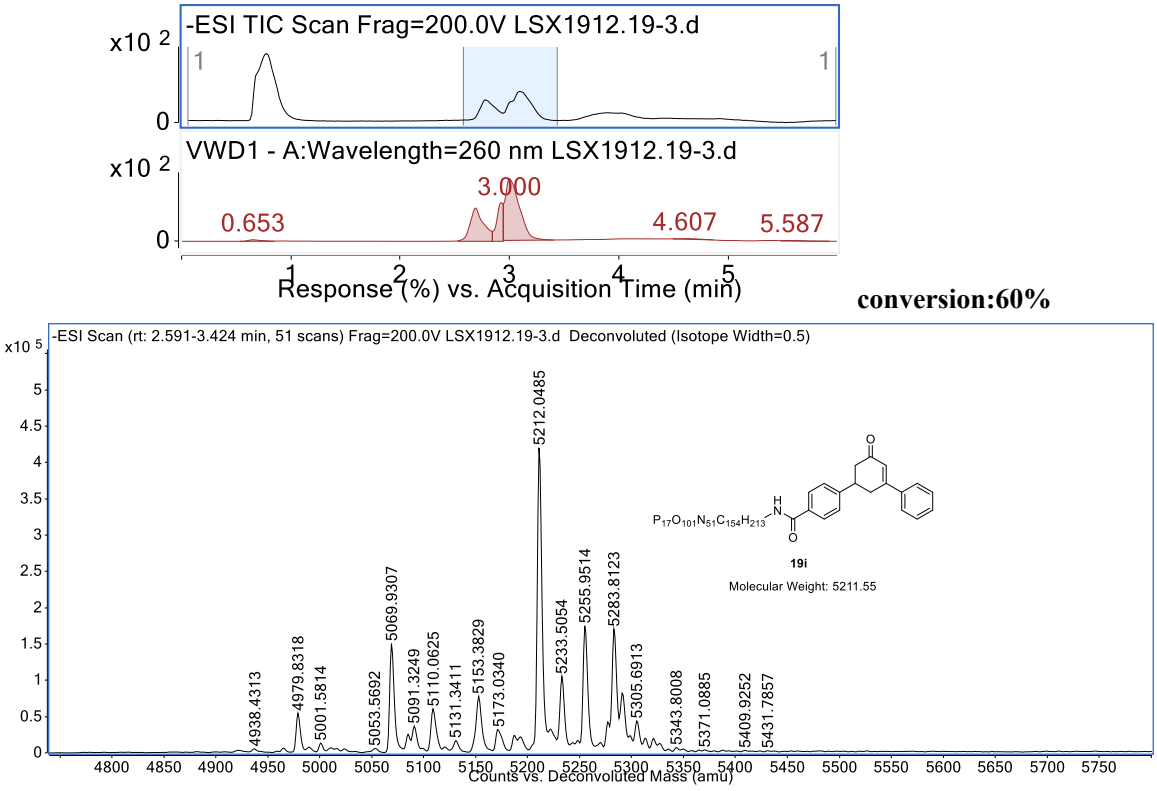


Figure S98 Synthetic route of on-DNA product **19i**

Mass Spectrum of on-DNA Product 19a

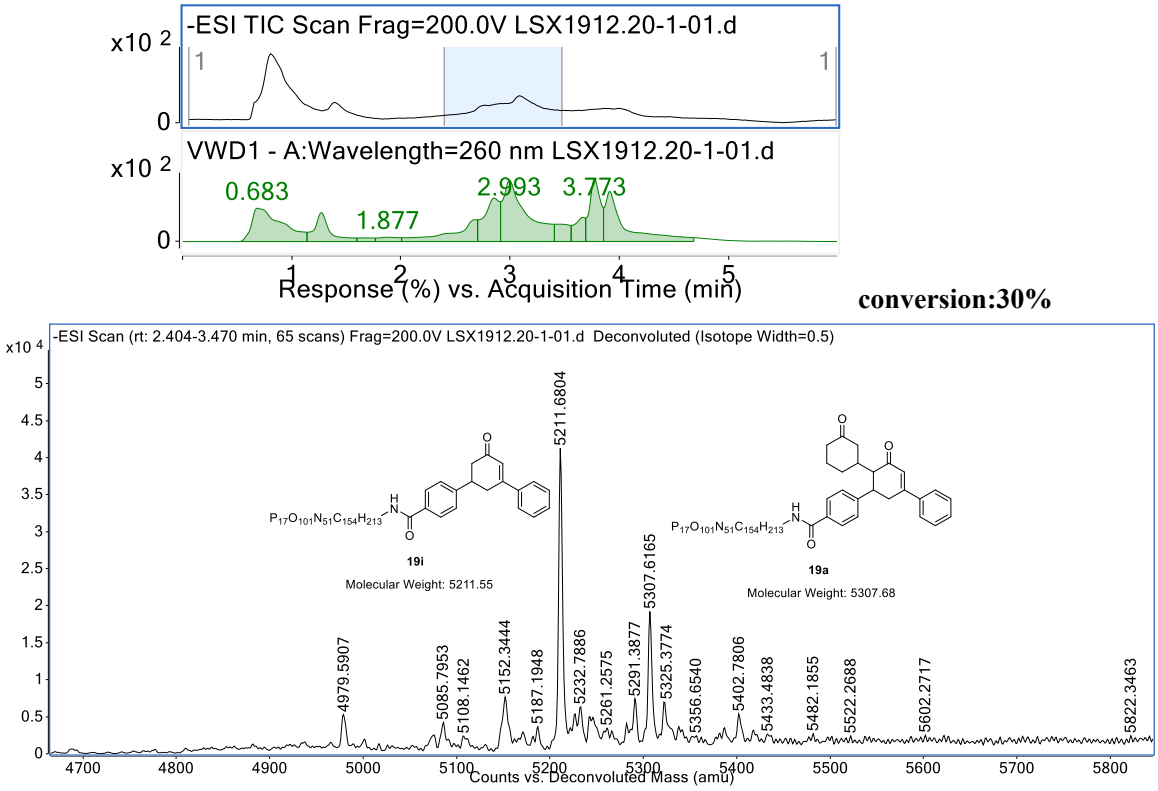


Figure S99 Synthetic route of on-DNA product **19a**

Mass Spectrum of on-DNA Product 19b

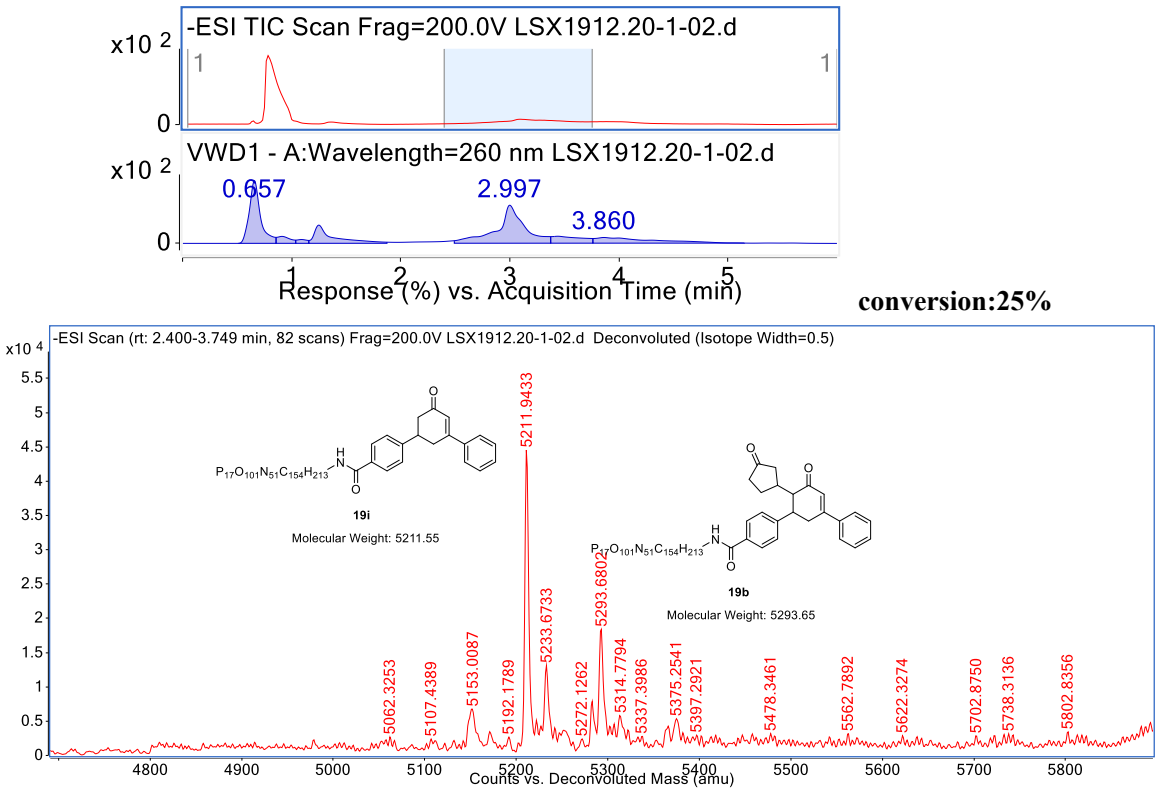
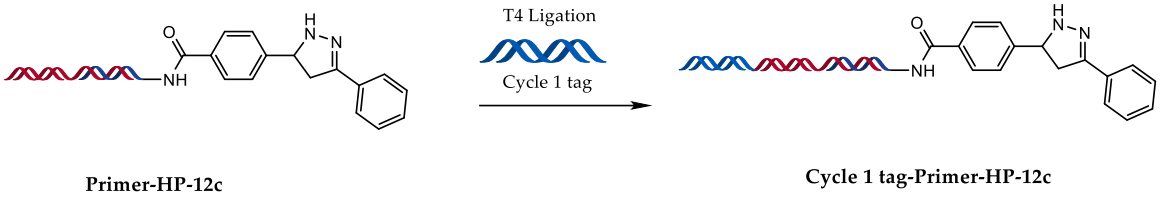


Figure S100 Synthetic route of on-DNA product **19b**

Oligonucleotide Ligation Experiment

Cycle 1 tag Ligation



Primer-HP-12c **Cycle 1 tag-Primer-HP-12c**

Figure S101 Cycle 1 tag ligation procedure

Procedure

For the ligation producer, the reaction material **12c** (contains the byproducts) was ligated with cycle 1 tag (2.0mM,1.3 equiv.) in the presence of T4 ligase (300U) for 12 h at r.t.

Mass Spectrum of T4 Ligation Product Cycle 1 tag-Primer-HP-12c:

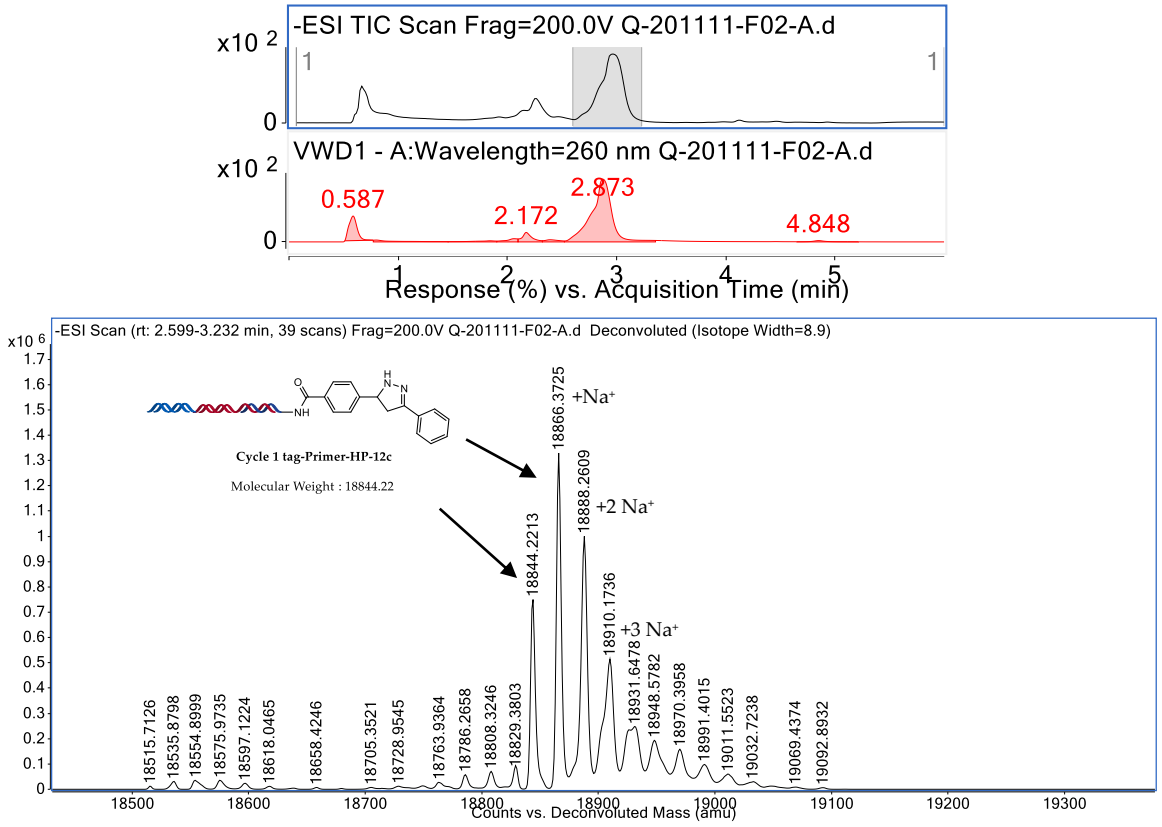
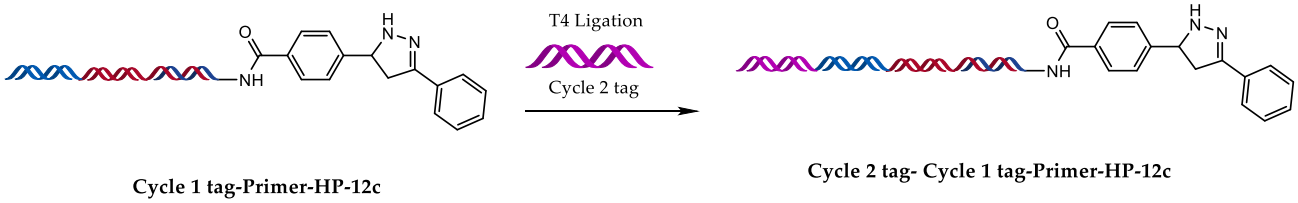


Figure S102 Deconvoluted mass of on-DNA product **Cycle 1 tag-Primer-HP-12c**

Cycle 2 tag Ligation



Cycle 1 tag-Primer-HP-12c **Cycle 2 tag- Cycle 1 tag-Primer-HP-12c**

Figure S103 Cycle 2 tag ligation procedure

Procedure

For the ligation producer,the reaction material 12c (contains the byproducts) was ligated with cycle 2 tag (2.0Mm,1.3 equiv.) in the presence of T4 ligase (300U) for 12 h at r.t.

Mass Spectrum of T4 Ligation Product Cycle 2 tag-Cycle 1 tag-Primer-HP-12c:

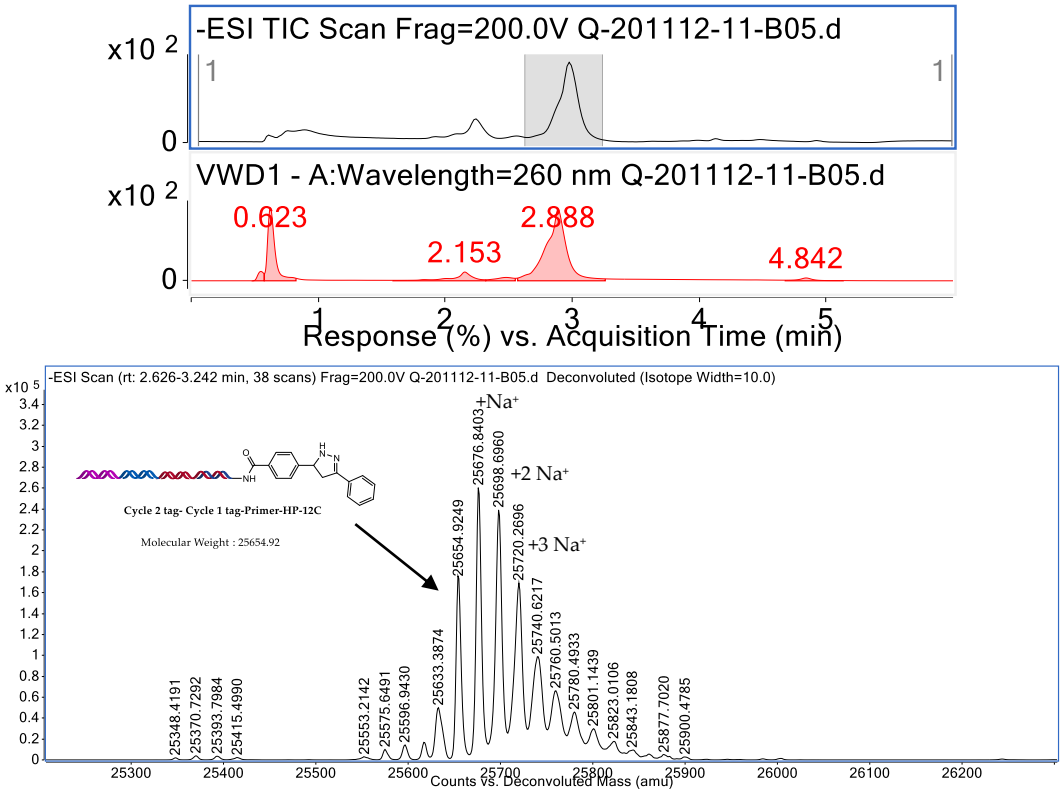


Figure S104 Deconvoluted mass of on-DNA product **Cycle 2 tag-Cycle 1 tag-Primer-HP-12c**

Closing tag Ligation

Cycle 2 tag- Cycle 1 tag-Primer-HP-13c

Closing tag-Cycle 2 tag- Cycle 1 tag-Primer-HP-13c

Figure S105 Closing tag ligation procedure

Procedure

For the ligation producer,the reaction product 13c (contains the byproducts) was ligated with closing tag (0.9Mm,1.0 equiv.) in the presence of T4 ligase (300U) for 12 h at r.t.

Mass Spectrum of T4 Ligation Product Closing tag-Cycle 2 tag-Cycle 1 tag-Primer-HP-13c:

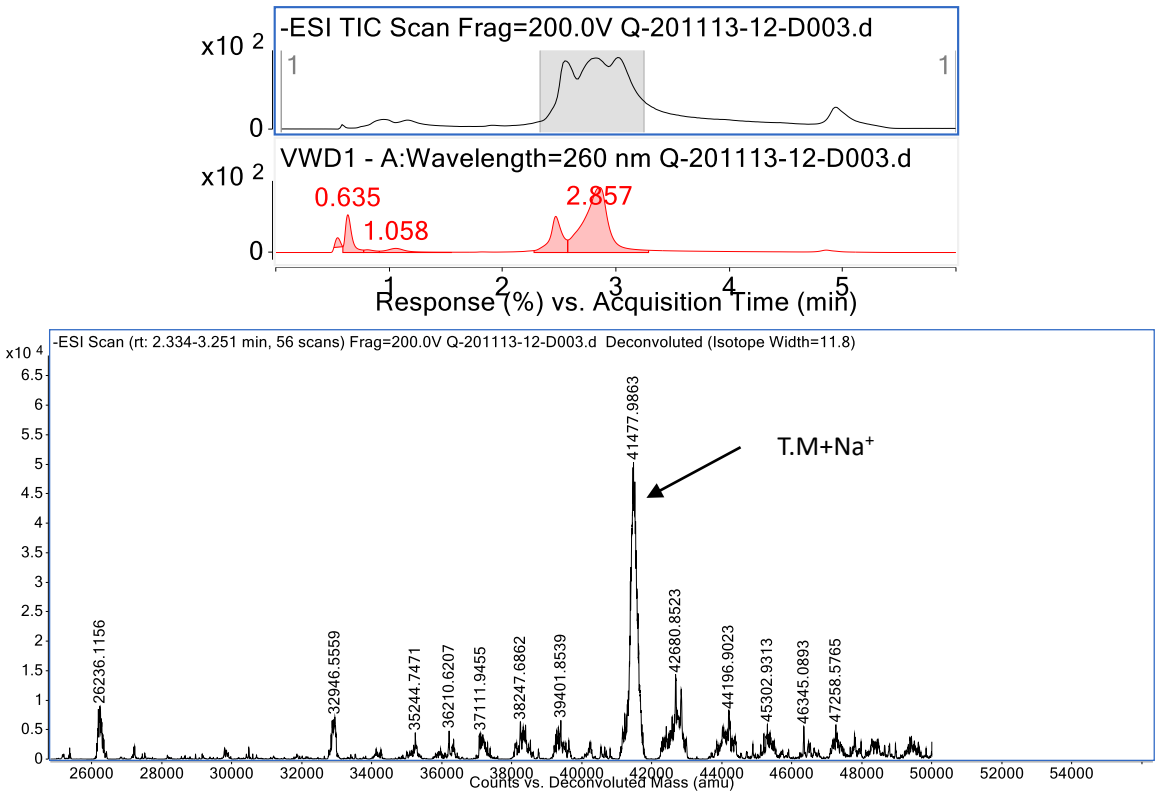


Figure S106 Deconvoluted mass of on-DNA product **Closing tag-Cycle 2 tag-Cycle 1 tag-Primer-HP-13c**

Sanger Sequencing

Materials

1 cycle prime

Forward primer:

5′- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGACTCCCAAATCGATGTG-3′

Reverse primers:

5′- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTCCCTCGCGCCATCAG -3′

2 cycle prime

Forward primer:

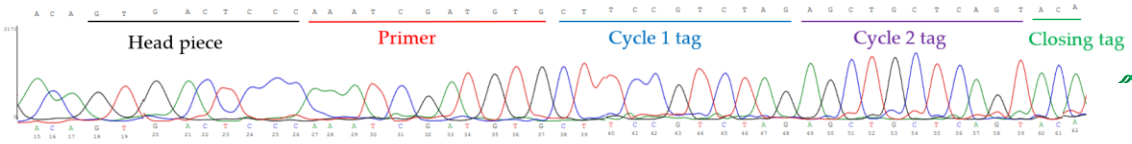
5′- CAAGCAGAAGACGGCATACGAGATTAAGGCGAGTCTCGTGGGCTCGGAGATG-3′

Reverse primers:

5′- AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGT -3′

Procedure

After 2 cycle PCR amplification, the oligonucleotide ligation products of 1a or 4a were purified with the AMPure XP beads purification kit according to the manufacturer's protocol. The purified PCR products were subjected to Sanger sequencing.



Closing tag-Cycle 2 tag- Cycle 1 tag-Primer-HP-12c

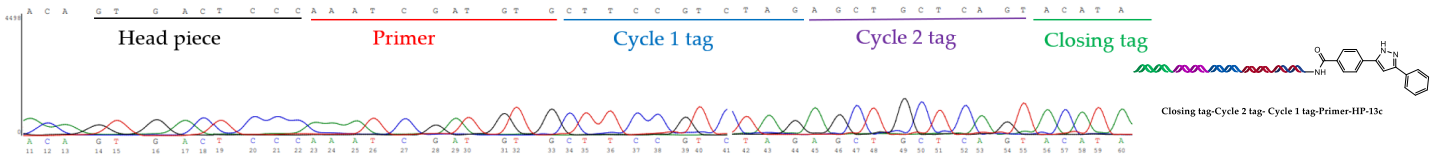


Figure S107 Results of Sanger sequencing

In conclusion, our data revealed that the chemical reactions used in this paper caused no damage to oligo sequence and potentially could be used in the encoded library construction.

Off-DNA Validation

Synthetic Scheme

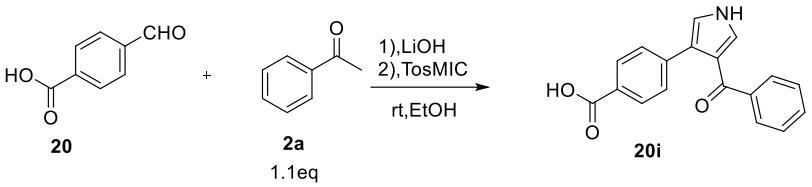


Figure S108 Synthetic Scheme of **20i**

Procedure

A mixture of 4-formylbenzoic acid (1.0 mmol), acetophenone (1.1 mmol) and lithium hydroxide monohydrate (1.1 mmol) in ethanol (3 mL) was stirred at RT. The progress of the reaction was monitored by TLC. Upon completion, lithium hydroxide monohydrate (1.1 mmol) and tosylmethyl isocyanide (1.2 mmol) were added and stirred. As the reaction progressed, the product precipitated. The precipitate was filtered, and washed with water and cold ethanol, successively. The product **20i** was characterized by spectroscopic methods.

δ 1H NMR (500 MHz, DMSO-d6) δ 12.86 (s, 1H), 11.85 (s, 1H), 7.87 (d, J = 7.9 Hz, 2H), 7.80 (d, J = 7.5 Hz, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.54 – 7.51 (m, 4H), 7.31 (d, J = 2.7 Hz, 1H), 7.27 (d, J = 2.3 Hz, 1H). **13C NMR** (126 MHz, DMSO) δ 190.05, 167.21, 139.73, 139.53, 131.52, 128.83, 128.75, 128.53, 128.03, 127.97, 127.53, 124.29, 120.51, 120.40. **LRMS** (ESI): 292.1 $(M+H)^+$; **HRMS** (ESI): calcd. For $C_{18}H_{14}NO_3$ $(M+H)^+$: 292.0974, found: 292.0966.

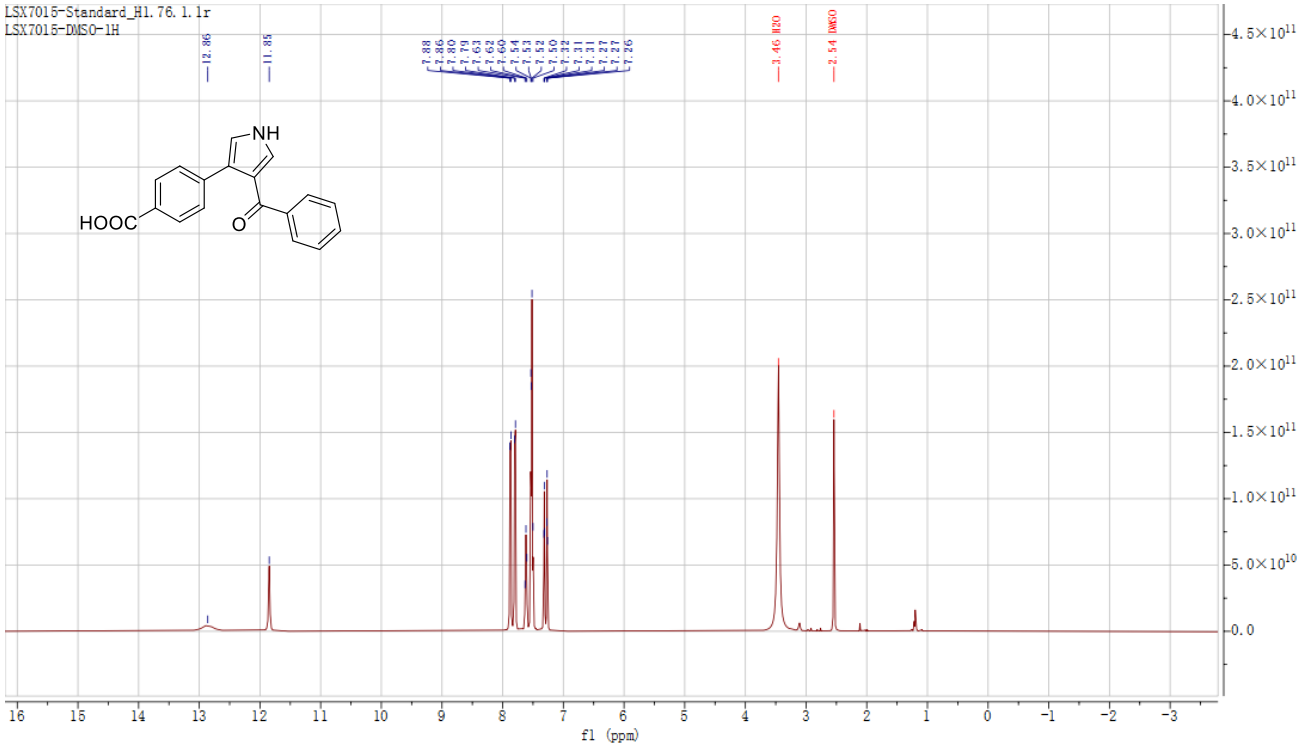


Figure S109 **1H NMR** of **20i**

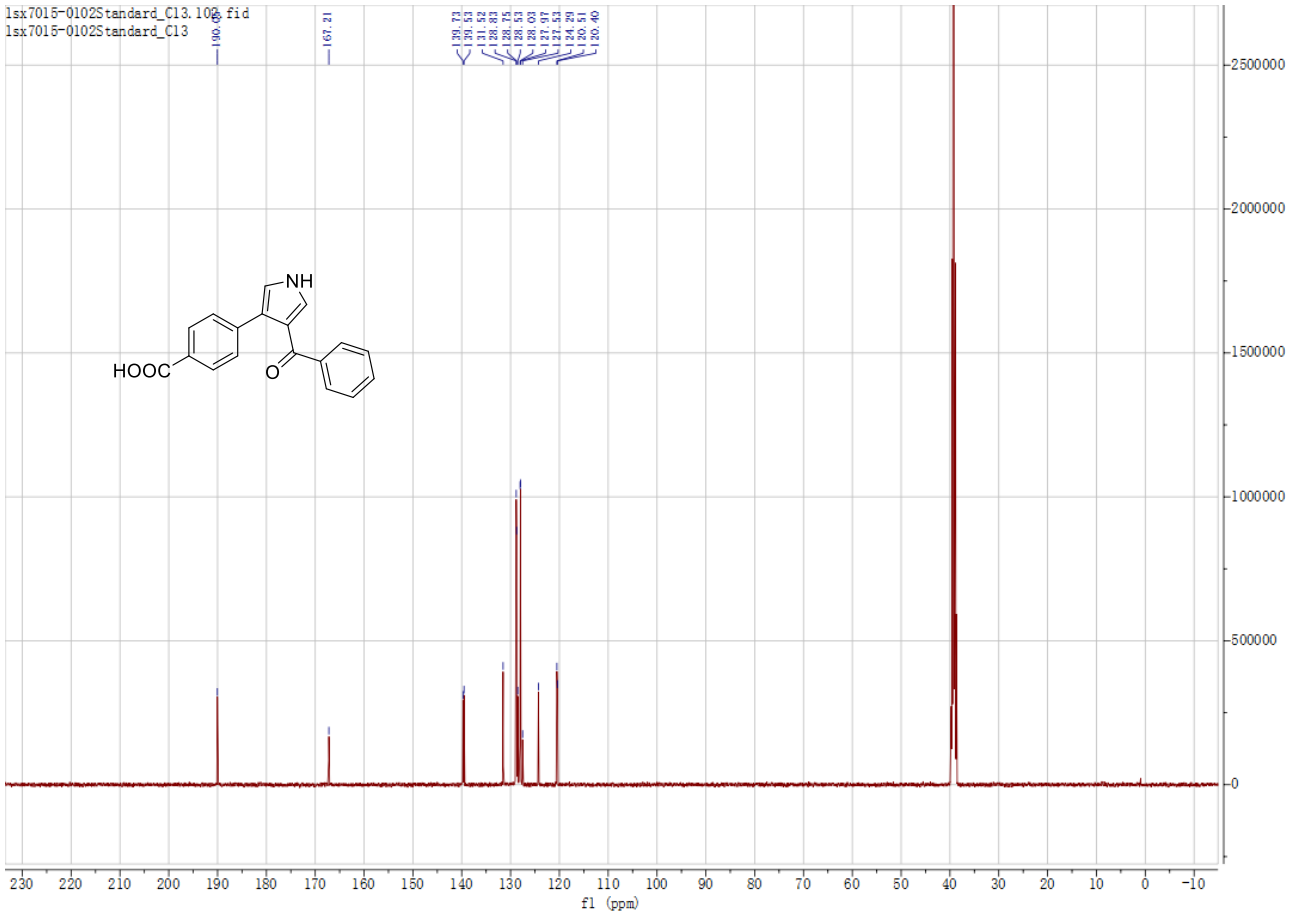


Figure S110 **13C NMR** of **20i**

Preparation of on-DNA Product 5a'

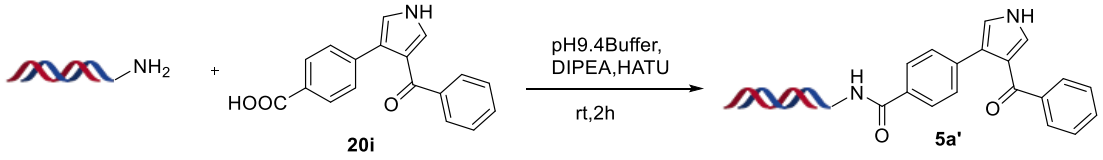


Figure S111 Synthetic Scheme of **5a'**

Materials

Headpiece: 2 mM in water

20i: 200 mM in DMA

DIPEA: N, N-Diisopropylethylamine 200 mM in DMA

HATU: 2-(7-Azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate 200mM in DMA

pH 9.4 Buffer: 500 mM sodium borate in water (pH 9.4)

Procedure

1) Mixing **20i** with equal volume HATU and DIPEA to prepare the solution V.
2) To the headpiece solution (20 nmol, 10 μL), was added 10 μL pH 9.4 buffer solution, 100 equiv. of solution V (30 μL), and mix.
3) React at room temperature for 2 hours.
4) The product **5a'** was obtained by ethanol precipitation as described above.

Mass Spectrum of on-DNA Product 5a'

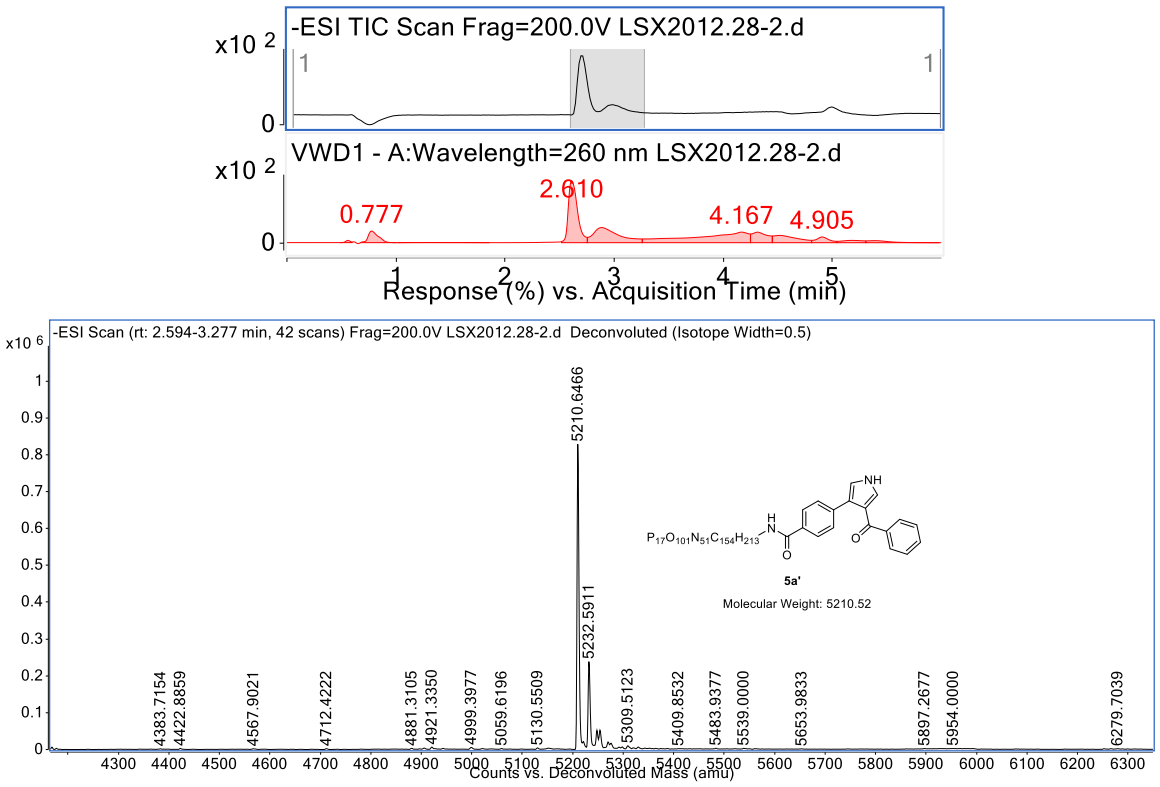


Figure S112 Deconvoluted mass of on-DNA product **5a'**

Mass Spectrum of Mixed on-DNA Product 5a and 5a′

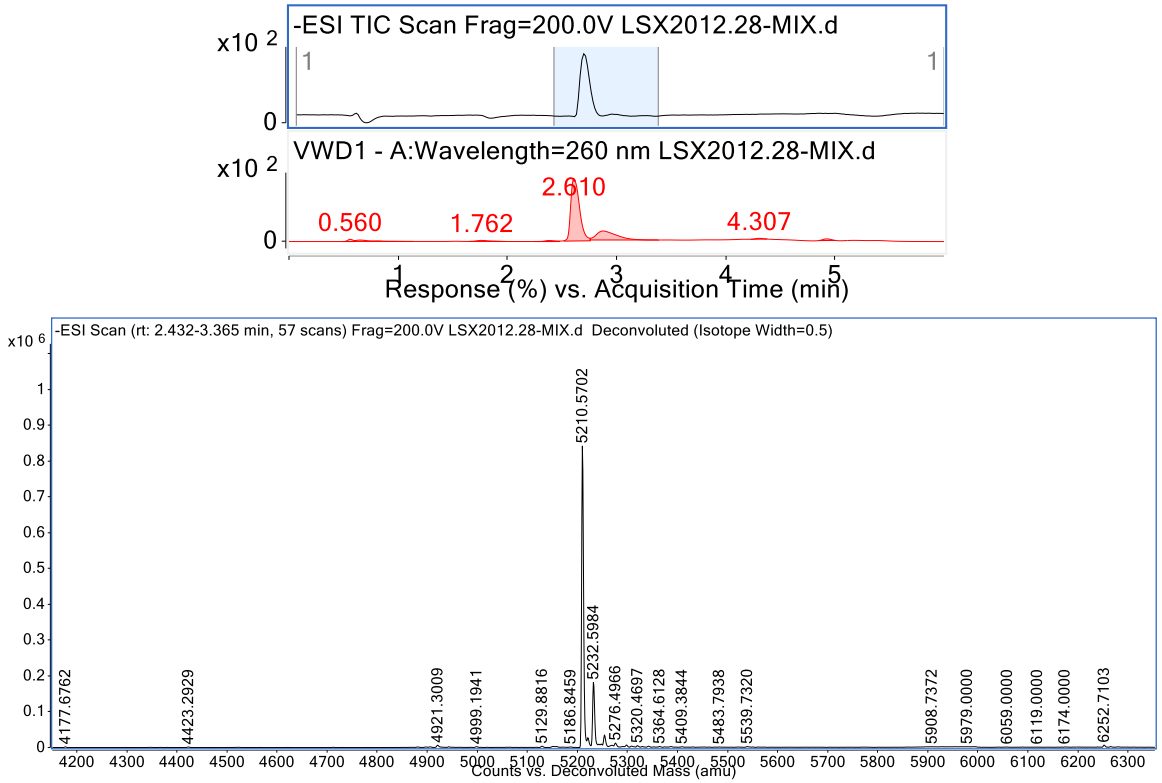


Figure S113 Deconvoluted mass of Mixed on-DNA product **5a and 5a′**

In conclusion, our data revealed that the structure of On DNA product **5a** is the same as **5a′.**