

Supporting Information

A mild, DNA-Compatible nitro reduction using $B_2(OH)_4$

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1. General Information	
1a) Materials and equipment	2
1b) General procedure for the analysis of oligonucleotide compositions	3
1c) General procedure for ethanol precipitation and DNA reconstitution	3
1d) Representative general procedure for DNA ligation	3
1e) Elaboration of "DTSU" S1 to "HP" S2 for substrate preparation	4
1f) Representative chemical procedures for attaching substrates	4
2. Nitro reduction	
2a) Preparation of nitro substrates	5
2b) General procedure for nitro reduction	5
3. Deconvoluted mass spectra	6
4. Preparation of substrates	45
5. DNA stability and ligation test	51
6. Benchtop test of nitro reduction	55
7. Synthesis of a DNA-Encoded chemical library using nitro reduction	58
8. DECL sequencing and selection experiments	60
9. References	63

1. General Information

Some of the general materials, equipment and procedures used in this study are adapted from those our group has reported previously¹ or other DNA-encoded library publications.²

1a. Materials and equipment used for the synthesis and analysis of oligonucleotides and DNA-encoded chemical libraries. The chemically-modified DNA oligonucleotide DTSU (“DEC-Tec Starting Unit”, **S1**, Figure S1) and 5'-phosphorylated oligonucleotides were obtained from LGC Biosearch Technologies. All DNA oligonucleotides were assessed for purity through the general analytical procedure and sequences were designed on principles designed to maximize sequence-reads. High-concentration T4 DNA ligase was obtained from Enzymatics (Qiagen) and its activity was determined through test DNA oligomer ligations on DTSU. Chemical building blocks and reagents were sourced from a variety of vendors, and were generally used from aliquots dissolved in acetonitrile or mixed aqueous acetonitrile solutions. Building block aliquots for DECL synthesis were stored in Tracetraq barcoded tubes (Biozero) with either screw- or septa-caps. Barcoded tubes were read using a SampleScan 96 scanner (Biomicrolab) and decoded using Vortex software (Dotmatics). All buffers and ionic solutions, including HEPES 10X ligation buffer (300 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, 100 mM MgCl₂, 100 mM dithiothreitol, 10 mM adenosine triphosphate, pH 7.8), aq. NaOH (1.5 M), aq. NaCl (5M) and basic borate buffer (250 mM sodium borate/boric acid, pH 9.5), were prepared in-house. DNA working solutions were prepared using DNase free ultra-pure water (Invitrogen), HPLC-grade acetonitrile (Fisher) or high-purity absolute ethanol (Koptec). LC/MS running solvents were made from Optima LC/MS grade water (Fisher), Optima LC/MS grade methanol (Fisher), 99+% purity hexafluoroisopropanol (Sigma) and HPLC-grade triethylamine (Fisher). Solutions were generally transferred or pooled utilizing Biotix brand pipette tips and reservoirs (various sizes), reactions were generally performed in polypropylene, 96-well, deep-well plates (USA Scientific, various sizes) or polypropylene, 96-well PCR plates (Phenix) or polypropylene Eppendorf tubes (various brands), plates were sealed for incubation with AlumaSeal II foil seals (Excel Scientific) and large volume DNA precipitations were performed in polypropylene 250 mL screw-cap bottles, Eppendorf tubes or Falcon tubes (from various vendors). Heated reactions were either performed in ep384 Mastercyclers (Eppendorf), benchtop heating blocks or in laboratory ovens (Fisher). Solutions were centrifuged in either Avanti J-30I or Allegra X-15R centrifuges (Beckman-Coulter). Optical density measurements were made using a Biophotometer (Eppendorf). A Vanquish UHPLC system was integrated with LTQ XL ion trap mass spectrometer (ThermoFisher Scientific) for LC/MS analysis of oligonucleotides.

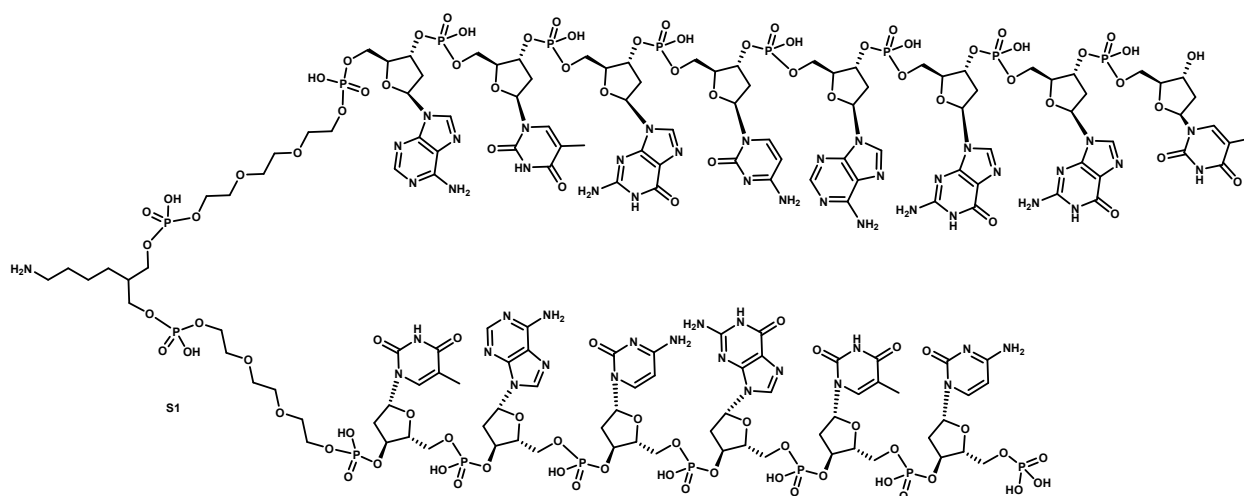


Figure S1. Structure of “DTSU” **S1** (5'-Phos-CTGCAT-Spacer 9-Amino C7-Spacer 9-ATGCAGGT 3').

1b. General procedure for the analysis of oligonucleotide compositions. Diluted samples of DNA stocks or reaction mixtures were injected on a Vanquish/LTQ system in amounts of 5–10 μL containing 50–200 pmol DNA analyte.

LC/MS Parameters for Thermo Vanquish UHPLC with LTQ Ion Trap MS Instrument

(i) LC settings

Column: Thermo DNAPac RP (2.1 x 50 mm, 4 μm)
Solvent A: 15mM triethylamine (TEA)/100mM hexafluoroisopropanol (HFIP) in water
Solvent B: 15mM TEA/100mM HFIP in 50% methanol
Solvent C: Methanol
Flow rate: 0.65 mL/min
Run time: 2 mins (gradient)
Column temperature: 100 °C (post column cooler at 40 °C)

(ii) MS settings

Source: ESI in negative mode
Spray voltage: 4100 V
Source heater temperature: 390 °C
Sheath Gas: 28 (instrument units)
Auxiliary Gas: 8 (instrument units)
Sweep Gas: 2 (instrument units)
Capillary temperature: 350 °C
Capillary voltage: -33.0 V
Tube lens: -92.0 V
MS Scan: 500 – 2000 m/z

Samples were analyzed on a Thermo Vanquish UHPLC system coupled to an electrospray LTQ ion trap mass spectrometer. An ion-pairing mobile phase comprising of 15mM TEA/100mM HFIP in a water/methanol solvent system was used in conjunction with an oligonucleotide column Thermo DNAPac RP (2.1 x 50 mm, 4 μm) for all the separations. All mass spectra were acquired in the full scan negative-ion mode over the mass range of 500–2000 m/z . The data analysis was performed by exporting the raw instrument data (.RAW) to an automated biomolecule deconvolution and reporting software (ProMass) which uses a novel algorithm known as ZNova to produce artifact-free mass spectra. **Deconvoluted mass spectra were standardized/compared against co-currently run samples of DTSU S1 and HP S2 to account for any drift from theoretical mass during deconvolution.**

1c. General procedure for ethanol precipitation and DNA reconstitution. To a DNA reaction mixture was added 5% (v/v) 5 M NaCl solution and 2.5–3 times the volume of absolute ethanol. The colloidal solution was then incubated at -20 °C overnight. After centrifugation, the supernatant was decanted, the pellet was centrifuged with 70% aq. ethanol, the supernatant again was decanted and the DNA pellet was dried in air or under gentle vacuum. Water was added to reconstitute the DNA to 0.5–1 mM. Ethanol precipitation was generally performed after each chemical reaction.

1d. Representative general procedure for DNA ligation.

To DNA conjugate **7a** (1.5 nmol, 5 μL , 1.0 equiv) was added DNA_1 (5'-CGGCTACAGTGT-3', 1.95 nmol, 1.95 μL , 1.3 equiv), DNA_2 (5'-ACTGTAGCCGGA-3', 1.95 nmol, 1.95 μL , 1.3 equiv), and nuclease-free water (3.6 μL), followed by the addition of 10 \times HEPES buffer (1.5 μL) and T4 DNA ligase (1.0 μL). The reaction mixture was incubated at room temperature overnight before performing gel electrophoresis. The crude material was purified by EtOH precipitation and then analyzed by gel electrophoresis. Gel electrophoresis was executed using precast 10% TBE acrylamide gel from Invitrogen (12 wells). The gel box was filled with 1 \times TBE buffer until the gel was covered. The purified DNA (by EtOH precipitation) was diluted to the concentration of 12 ng/ μL . To a tube was added 10 μL of one DNA sample and 2 μL of 6 \times DNA loading dye to make a DNA dye loading sample. The first lane of the gel was loaded with a DNA molecular weight ladder, and 5 μL of DNA-dye mixed samples was loaded into each lane. Gels

were ran at 160 V for 35 min and then stained in a container with 0.5 ng/mL ethidium bromide in 1× TBE buffer for 50 min. DNA fragments were visualized under a UV light device, and assessed for completed ligation.

1e. Elaboration of “DTSU” S1 to “HP” S2 or S3 for substrate preparation.

All substrates were prepared on DTSU **S1** that had been further elaborated by the ligation of additional oligonucleotides and a longer amino-terminating linker. This elaborated DNA, “HP” **S2**, was prepared through ligation of two duplexed 11-mer oligonucleotides through the general DNA ligation procedure (final sequence: 5' d TGA GTG AAT ACC TGC AT -Spacer 9-Amino C7-Spacer 9-ATG CAG GTA TTC ACT GAG G 3') followed by amidation of Fmoc-15-amino-4,7,10,13-tetraoxapentadecanoic acid through the general acylation procedure and Fmoc deprotection. A special, longer DNA oligonucleotide **S3** for ligation tests was prepared through ligation of two 39-mer duplexed oligonucleotides by the general procedure (final sequence 5' d TAT GAT ACT AAA GTA AGT CAC ACA CAA TTG GAG CAG TCC TGA GTG AAT ACC TGC AT -Spacer 9-Amino C7- Spacer 9-ATG CAG GTA TTC ACT GAG GAC TGC TCC AAT TGT GTG TGA CTT ACT TTA GTA TCA TAT C 3').

1f. Representative chemical procedures for attaching substrates.

Acylation: A carboxylic acid building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv), *N,N*-Diisopropylethylamine (DIPEA, 1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv), and 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT, 1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) were premixed for 10 min. The mixture was then added to a solution of amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (4000 nmol, 16 μ L, 250 mM, 400 equiv), which was allowed to sit at room temperature for 2–4 h before being quenched by EtOH precipitation. In some cases, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was substituted for DEPBT/DIPEA and used without premixing.

Sulfonylation: A sulfonyl chloride building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) was added to a solution of amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (4000 nmol, 16 μ L, 250 mM, 400 equiv), which was allowed to sit at room temperature for 2–8 h before being quenched by EtOH precipitation.

Reductive Alkylation: An aldehyde building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) was added to a solution of amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 5.8 MES buffer (4000 nmol, 16 μ L, 250 mM, 400 equiv) followed by a solution of NaCNBH₃ (400 nmol, 2 μ L, 200 mM in H₂O, 40 equiv) which was allowed to sit at 30 °C for 8–12 h before being quenched by EtOH precipitation.

Nucleophilic Aromatic Substitution: An electrophilic aryl fluoride building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) was added to a solution of amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (4000 nmol, 16 μ L, 250 mM, 400 equiv) and the reaction was heated to 60 °C for 6–12 h before being quenched by EtOH precipitation.

Urea formation: Two methods: a) An isocyanate building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) was added to a solution of amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (4000 nmol, 16 μ L, 250 mM, 400 equiv), which was allowed to sit at room temperature for 2–8 h before being quenched by EtOH precipitation. b) amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (5000 nmol, 20 μ L, 250 mM, 500 equiv), dipyrindin-2-yl-carbonate (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv), and triethylamine (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) were premixed for 2 h before amine building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) was added. The reaction was sit at room temperature for 16 h before being quenched by EtOH precipitation.

Carbamate formation: amine-DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (5000 nmol, 20 μ L, 250 mM, 500 equiv), *N,N'*-Disuccinimidyl carbonate (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv), and triethylamine (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) were premixed for 2 h before alcohol building block (1000 nmol, 5 μ L, 200 mM in MeCN, 100 equiv) was added. The reaction was sit at room temperature for 16 h before being quenched by EtOH precipitation.

N-Fmoc deprotection: 10% (v/v) aq. piperidine (5000 nmol, 5 μ L, ~1 M in H₂O, 500 equiv) was added to a solution of N-Fmoc DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O and the reaction was incubated at room temperature for 4–8 h. The solution was diluted with H₂O (40 μ L) and then quenched by EtOH precipitation.

N-Boc deprotection: To a solution of N-Boc DNA (10 nmol, 10 μ L, 1.0 mM, 1 equiv) in H₂O with pH 9.5 borate buffer (4000 nmol, 16 μ L, 250 mM, 400 equiv), additional H₂O (60 μ L) was added and the reaction was heated to 80 °C for 36–48 h before being quenched by EtOH precipitation.

2. Nitro reduction

2a) Preparation of nitro substrates. From HP **2**, compounds **1a–1g**, **1j–1o**, **1x**, **1z–1ee**, and **1gg–1ll** were prepared with the appropriate acid building block through the general acylation procedure, compounds **1h**, **1i**, **1v**, **1w**, and **1y** were prepared with the appropriate sulfonyl chloride building block through the general sulfonation procedure; compounds **1p**, **1q**, and **1u** were prepared with the appropriate aldehyde through the general reductive alkylation procedure; compounds **1r–1t** were prepared from the general nucleophilic aromatic substitution procedure; compound **1k** was prepared using urea formation method a, and **1l** was prepared using urea formation method b; compound **1ff** was prepared using the carbamate formation procedure.

2b) General procedure for nitro reduction. To a solution of DNA-conjugated nitro substrate (3 nmol, 3 μ L, 1.0 mM, 1 equiv) in H₂O was added NaOH (1500 nmol, 1 μ L, 1500 mM, 500 equiv), H₂O (2 μ L) and EtOH (4.5 μ L), followed by the addition of B₂(OH)₄ (450 nmol, 4.5 μ L, 100 mM in H₂O, 150 equiv). The reaction mixture was incubated at room temperature for 2 h prior to EtOH precipitation. The solution of B₂(OH)₄ in neutral water was prepared freshly from vortexing or brief sonication before use. B₂(OH)₄ was purchased from Ark Pharm, Inc. and used without further purification. When conducting a subsequent ligation, steps should be taken to ensure maximum removal of small molecules/salts before ligation, as large residual amounts of these may inhibit the function of DNA ligase. In DECL production, a double ethanol precipitation is recommended.

3. Deconvoluted Mass Spectra of DNA-Conjugates

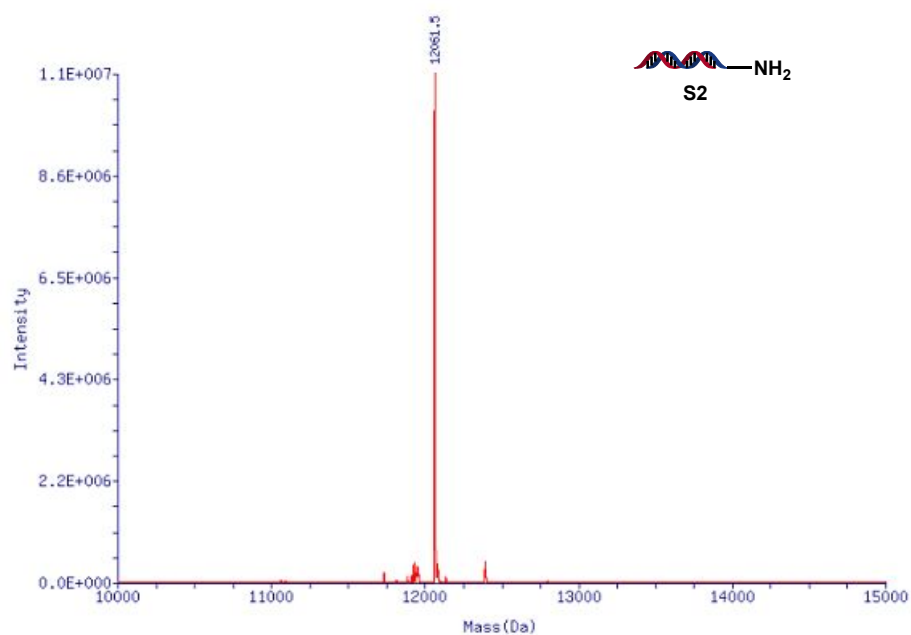


Figure S2. Deconvoluted mass spectrum of DNA **S2** , expected: 12060.0; observed 12061.5.

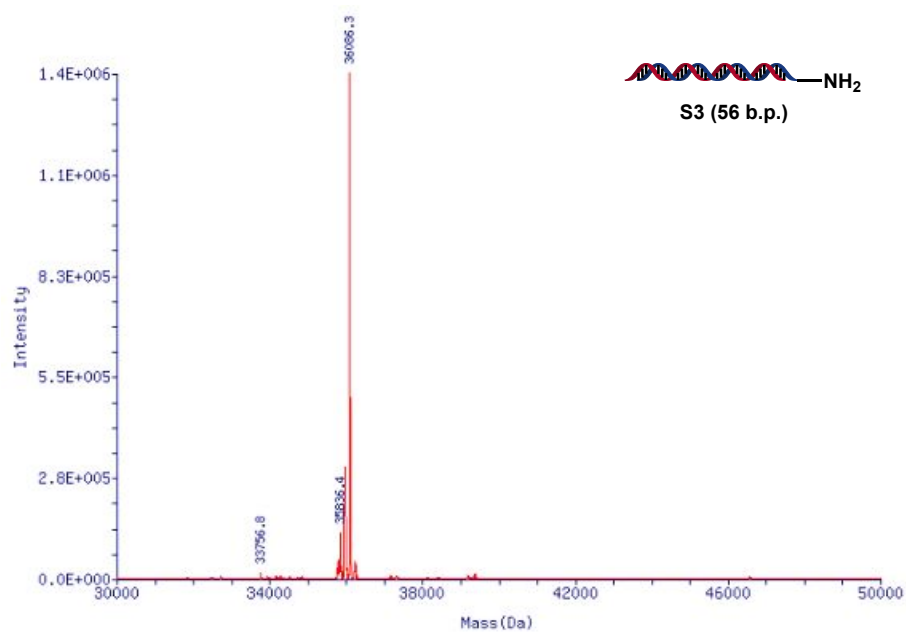


Figure S3. Deconvoluted mass spectrum of the 56 b.p DNA **S3**, expected: 36088.7; observed 36086.3.

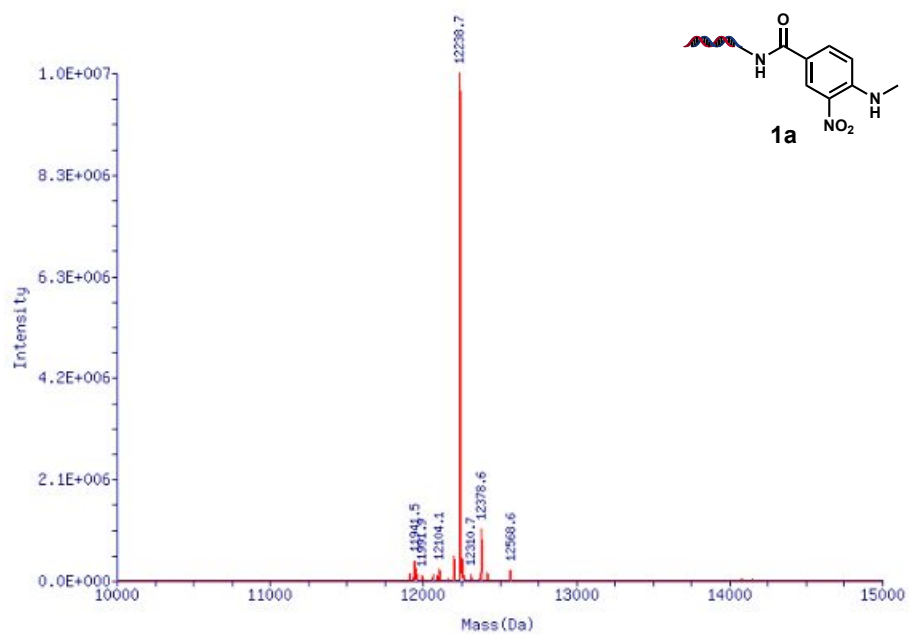


Figure S4. Deconvoluted mass spectrum of compound **1a**, expected: 12238.2; observed 12238.7.

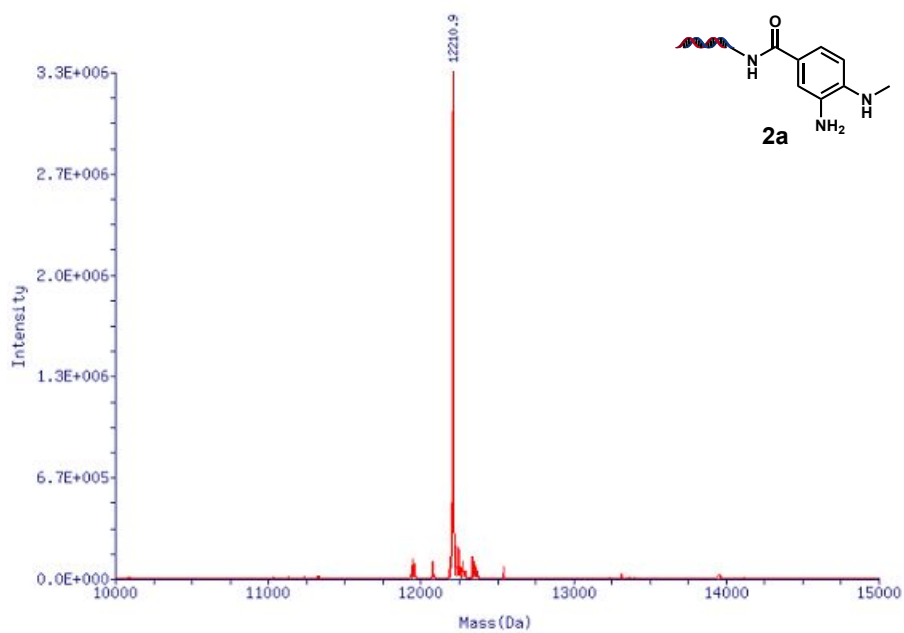


Figure S5. Deconvoluted mass spectrum of compound **2a**, expected: 12208.2; observed 12210.9.

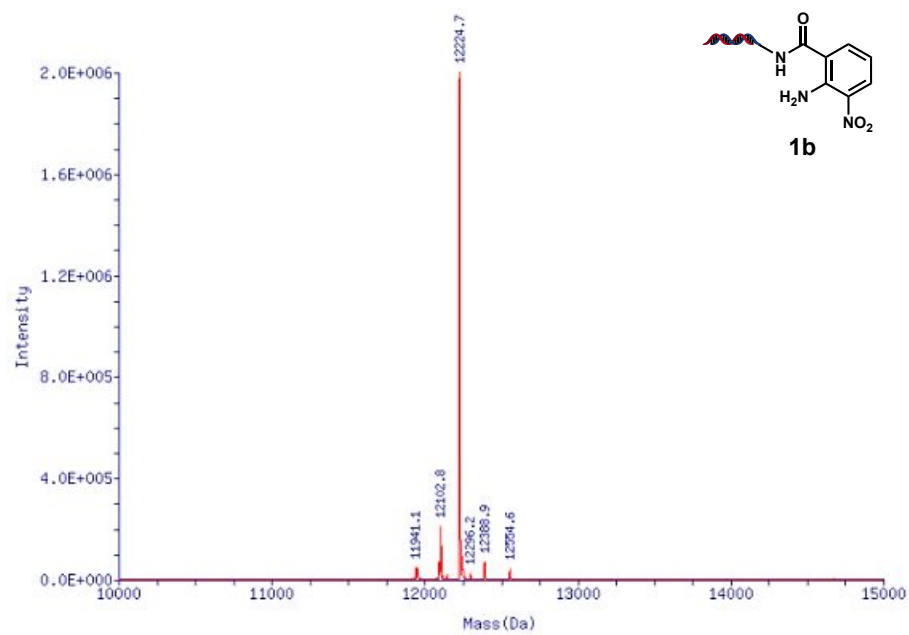


Figure S6. Deconvoluted mass spectrum of compound **1b**, expected: 12224.1; observed 12224.7.

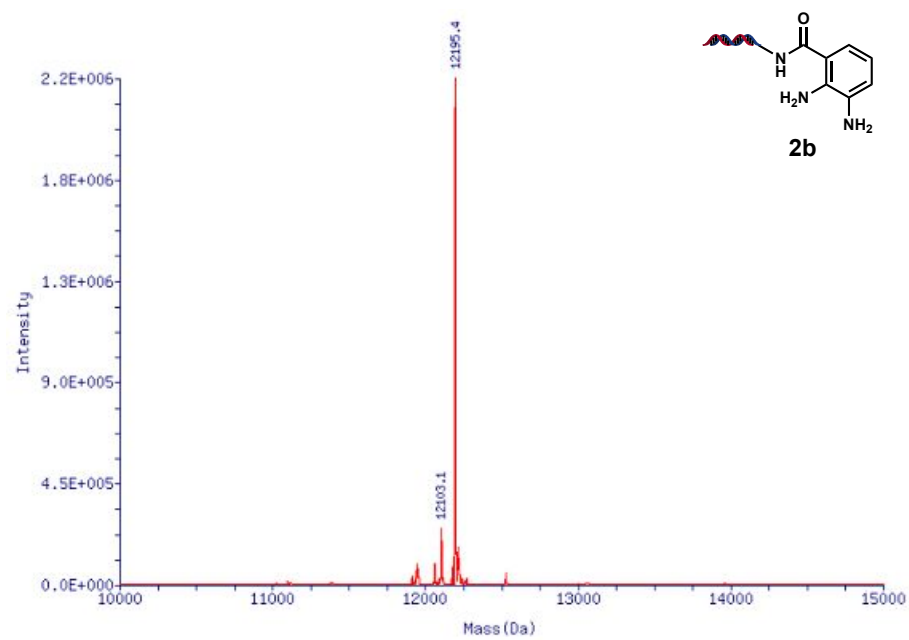


Figure S7. Deconvoluted mass spectrum of compound **2b**, expected: 12194.1; observed 12195.4.

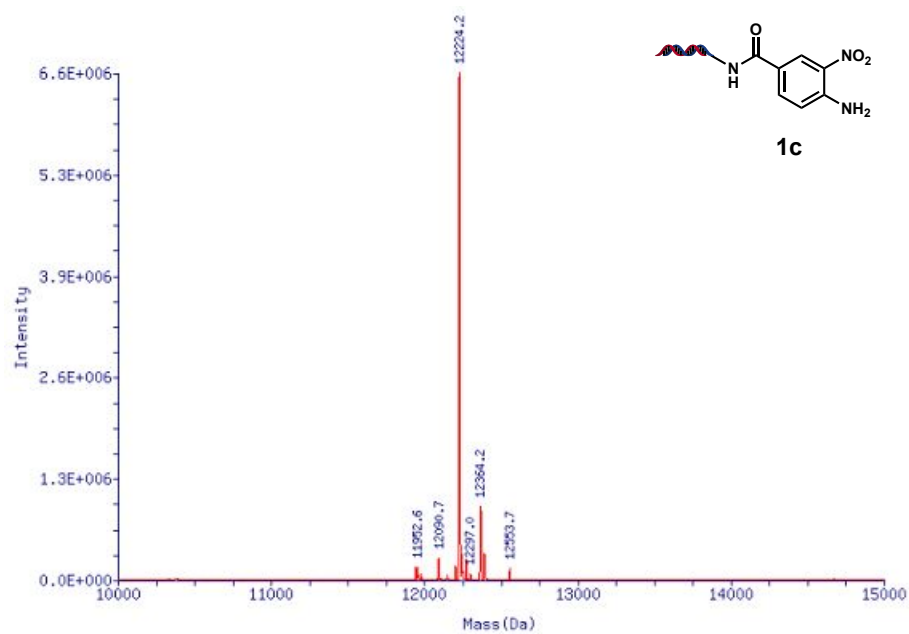


Figure S8. Deconvoluted mass spectrum of compound **1c**, expected: 12224.1; observed 12224.2.

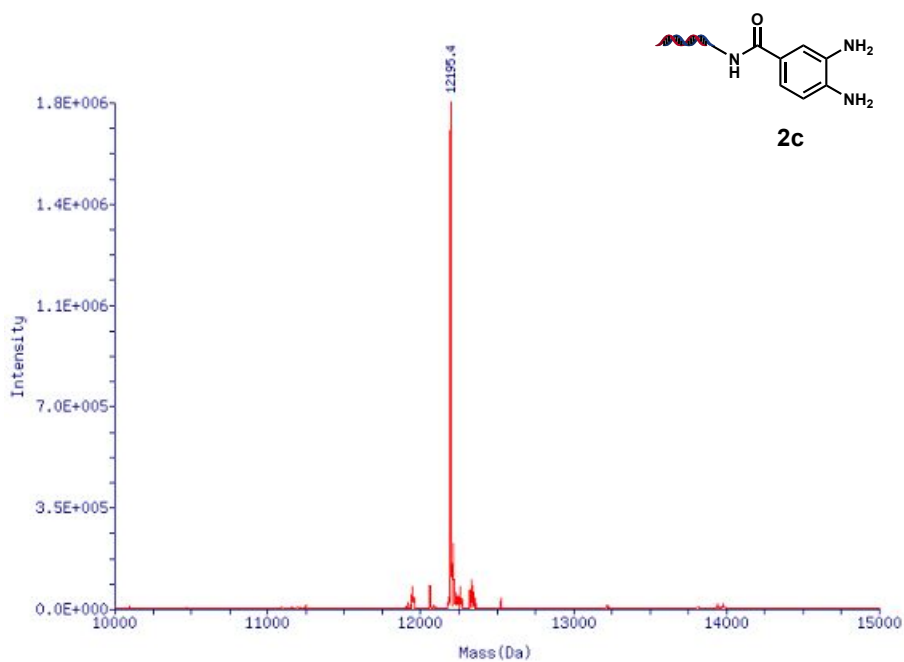


Figure S9. Deconvoluted mass spectrum of compound **2c**, expected: 12194.1; observed 12195.4.

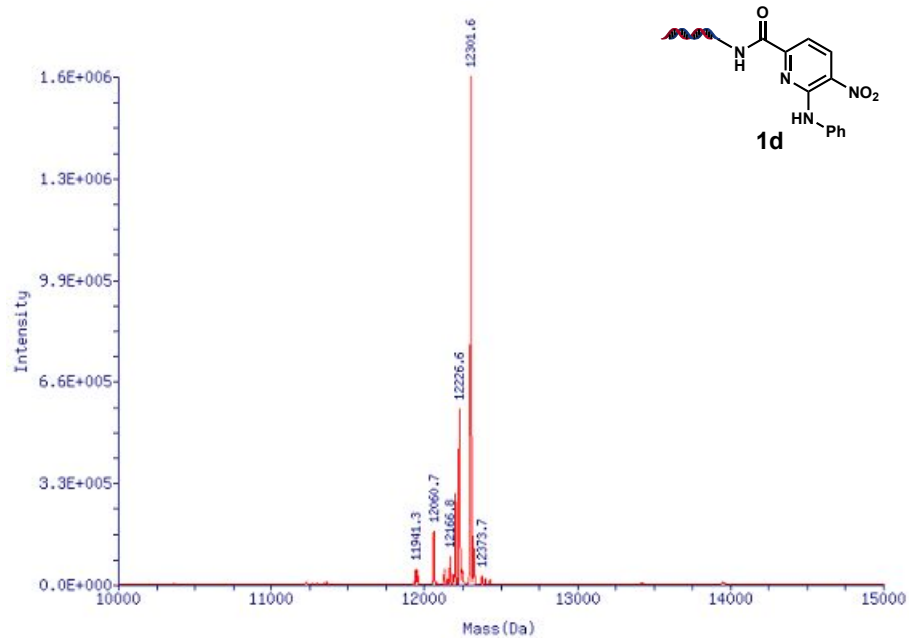


Figure S10. Deconvoluted mass spectrum of compound **1d**, expected: 12301.2; observed 12301.6.

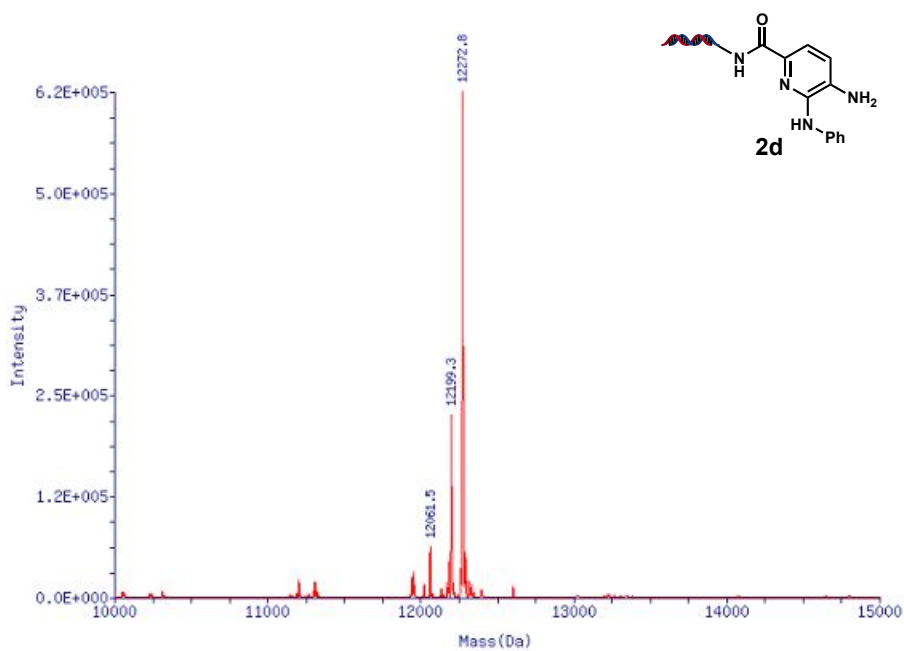


Figure S11. Deconvoluted mass spectrum of compound **2d**, expected: 12271.2; observed 12272.8.

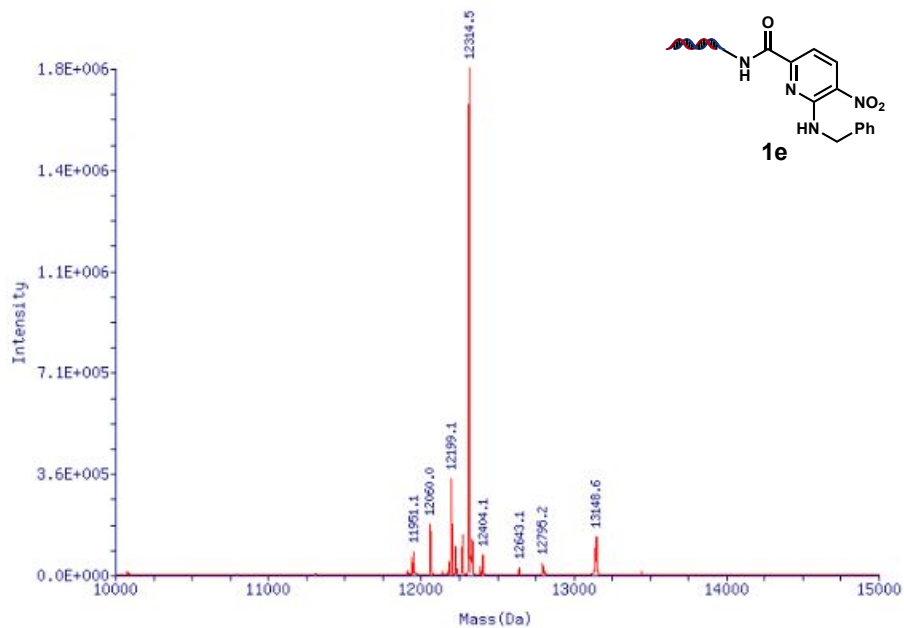


Figure S12. Deconvoluted mass spectrum of compound **1e**, expected: 12315.2; observed 12314.5.

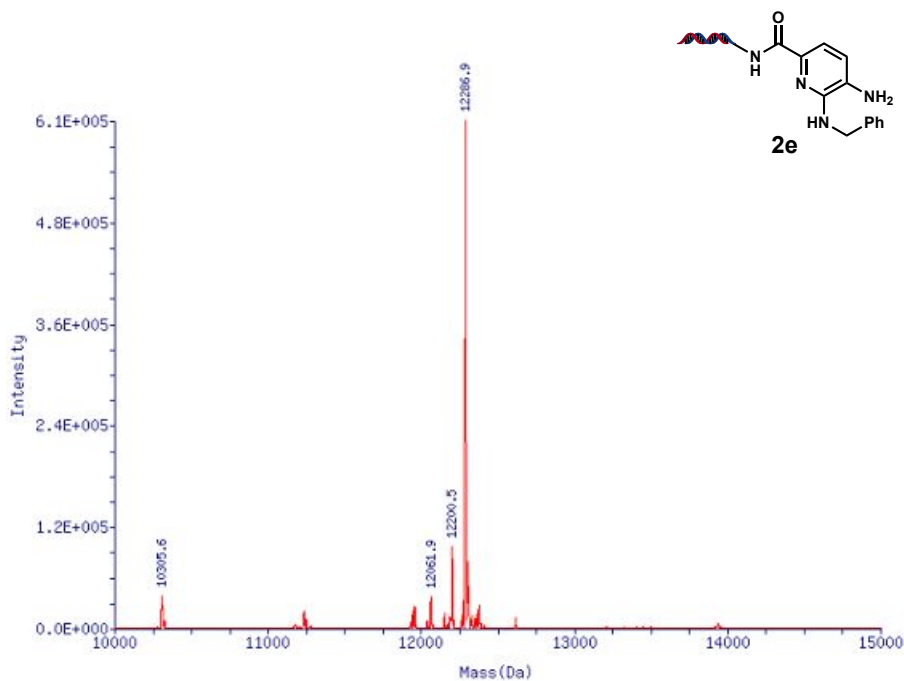


Figure S13. Deconvoluted mass spectrum of compound **2e**, expected: 12285.2; observed 12286.9.

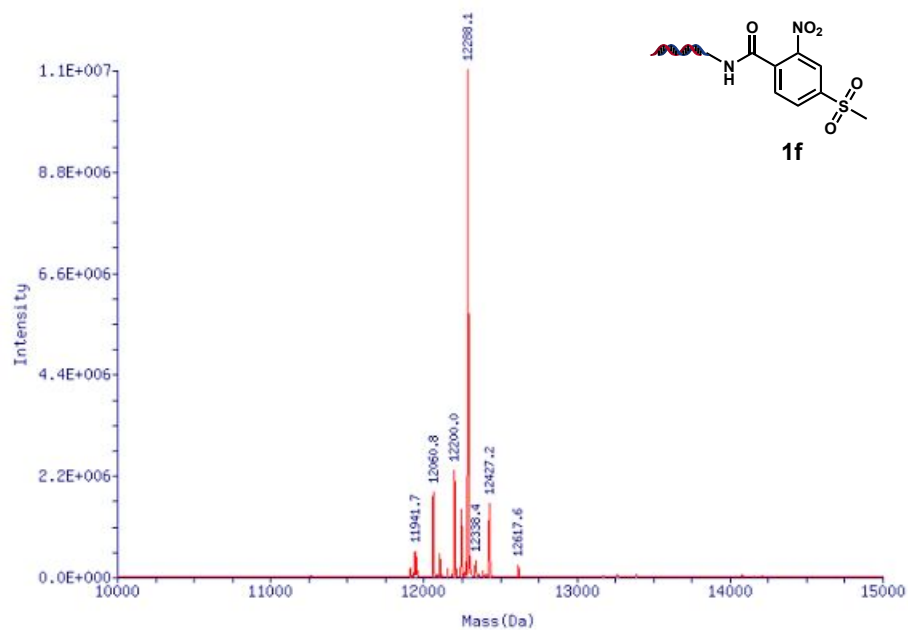


Figure S14. Deconvoluted mass spectrum of compound **1f**, expected: 12287.2; observed 12288.1.

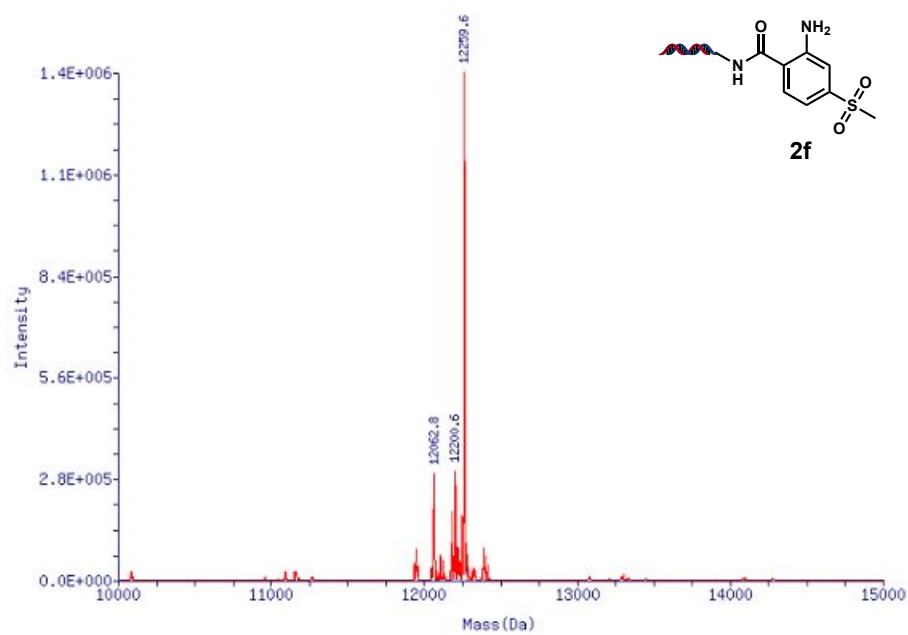


Figure S15. Deconvoluted mass spectrum of compound **2f**, expected: 12257.2; observed 12259.6.

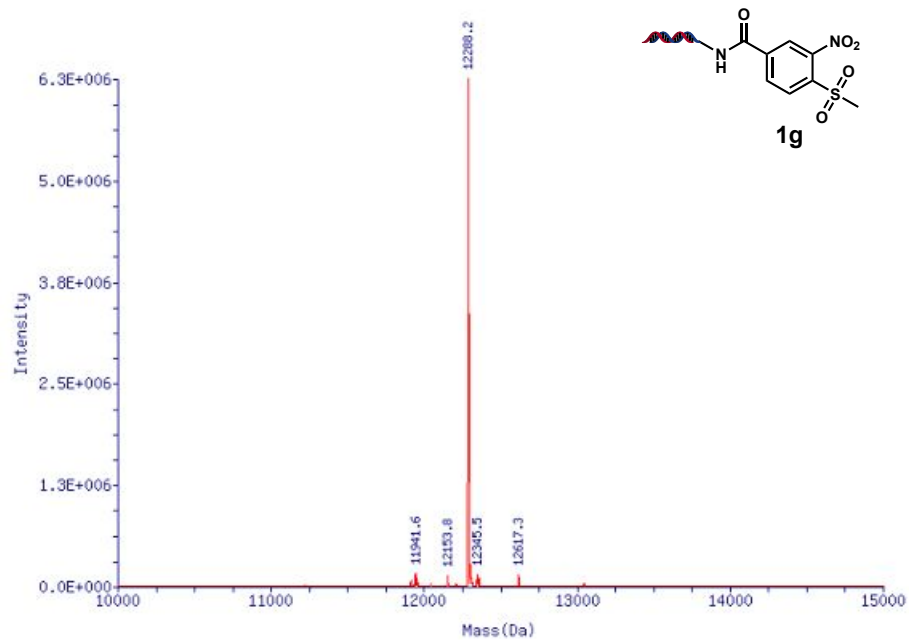


Figure S16. Deconvoluted mass spectrum of compound **1g**, expected: 12287.2; observed 12288.2.

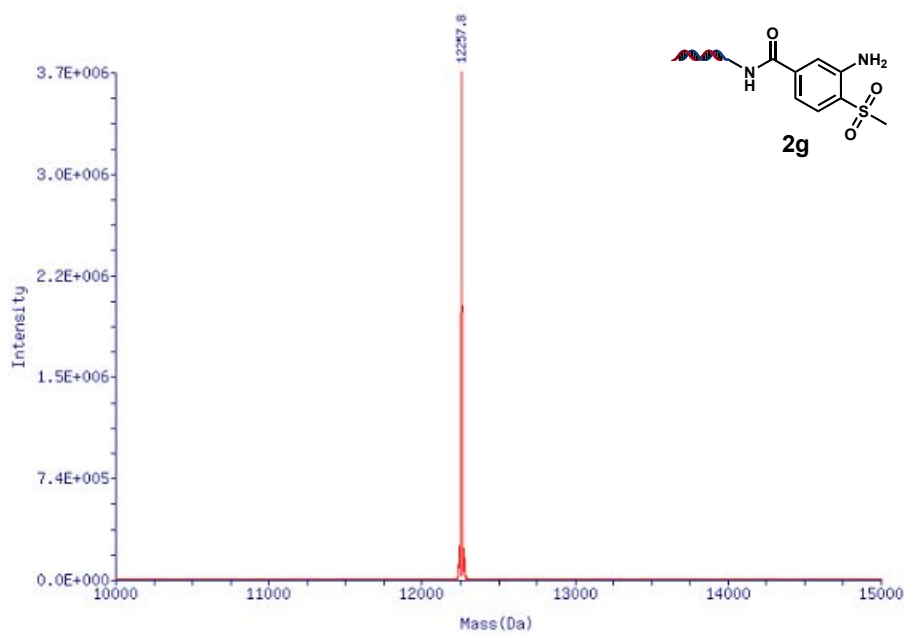


Figure S17. Deconvoluted mass spectrum of compound **2g**, expected: 12257.2; observed 12257.8.

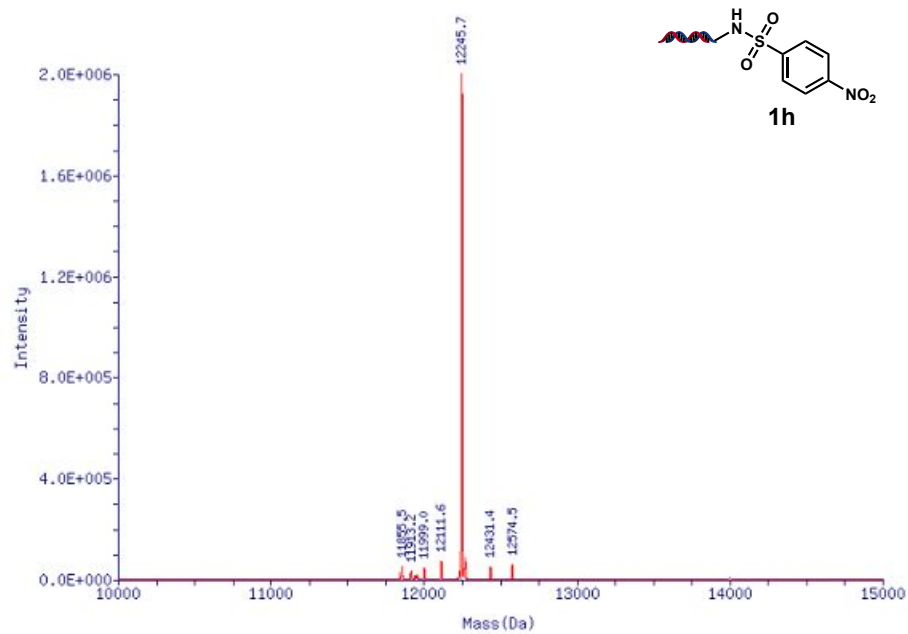


Figure S18. Deconvoluted mass spectrum of compound **1h**, expected: 12245.2; observed 12245.7.

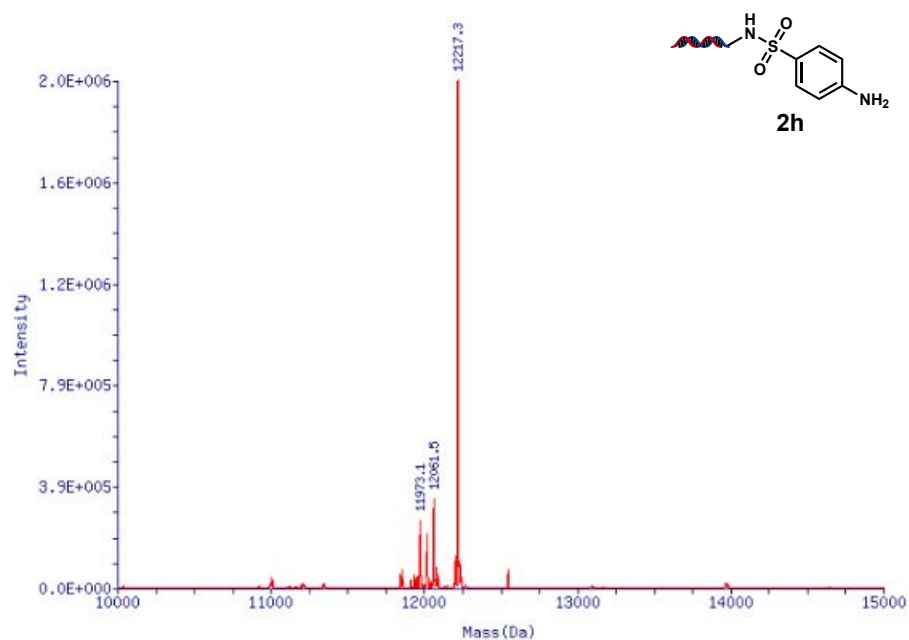


Figure S19. Deconvoluted mass spectrum of compound **2h**, expected: 12215.2; observed 12217.3.

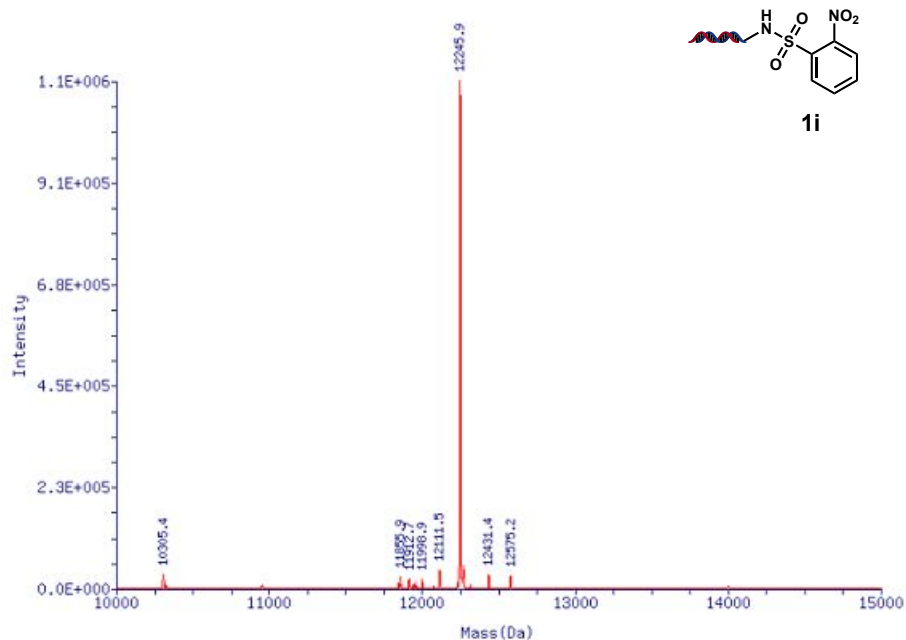


Figure S20. Deconvoluted mass spectrum of compound **1i**, expected: 12245.2; observed 12245.9.

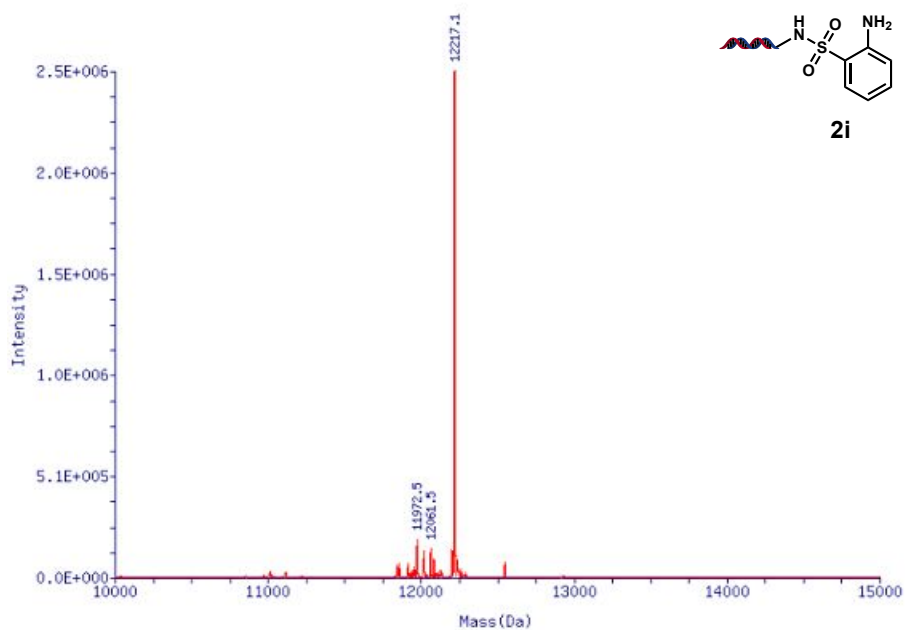


Figure S21. Deconvoluted mass spectrum of compound **2i**, expected: 12215.2; observed 12217.1.

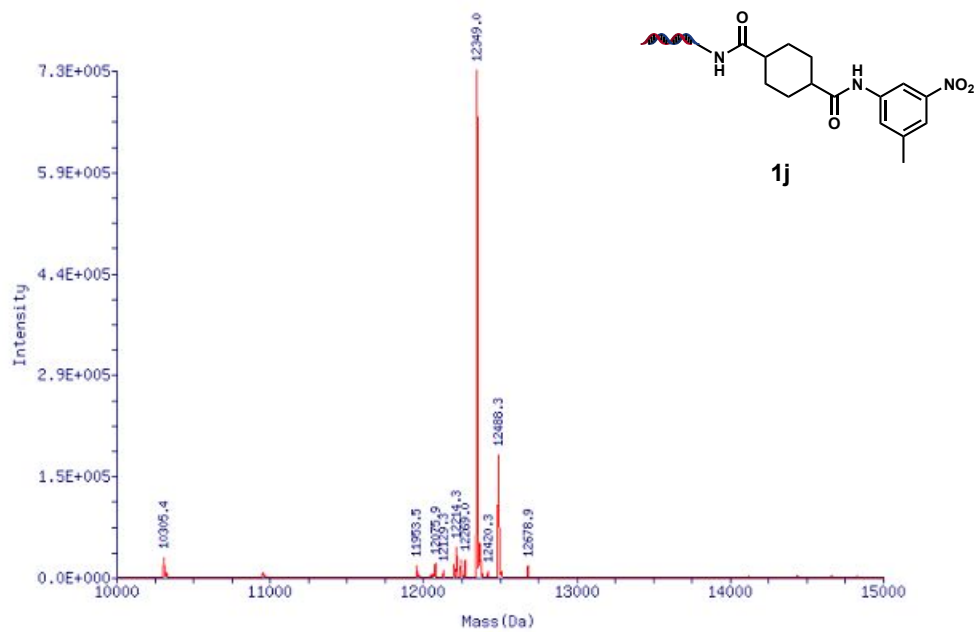


Figure S22. Deconvoluted mass spectrum of compound **1j**, expected: 12348.3; observed 12349.0.

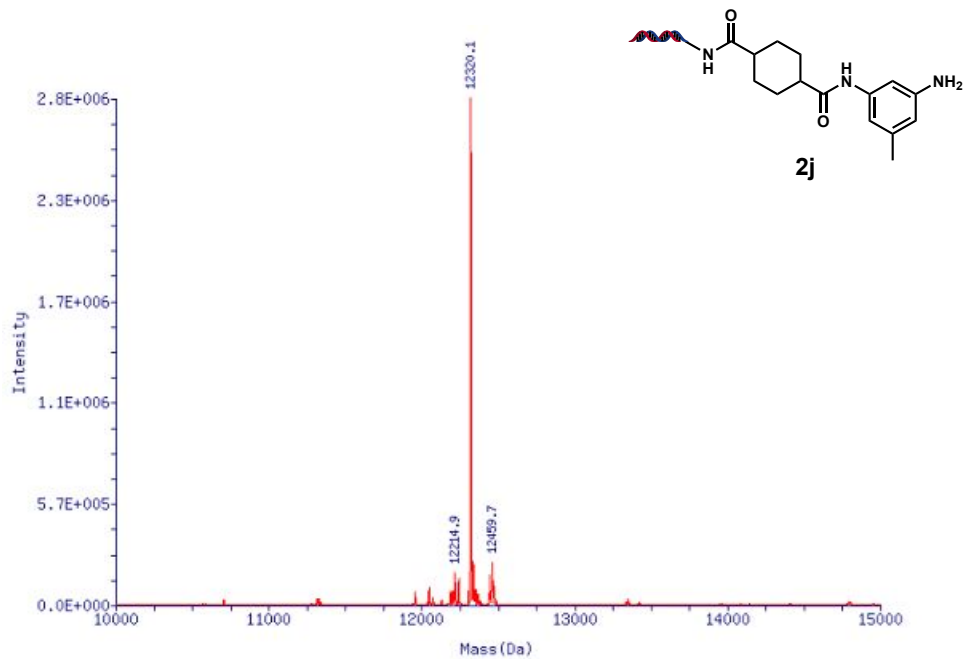


Figure S23. Deconvoluted mass spectrum of compound **2j**, expected: 12318.3; observed 12320.1.

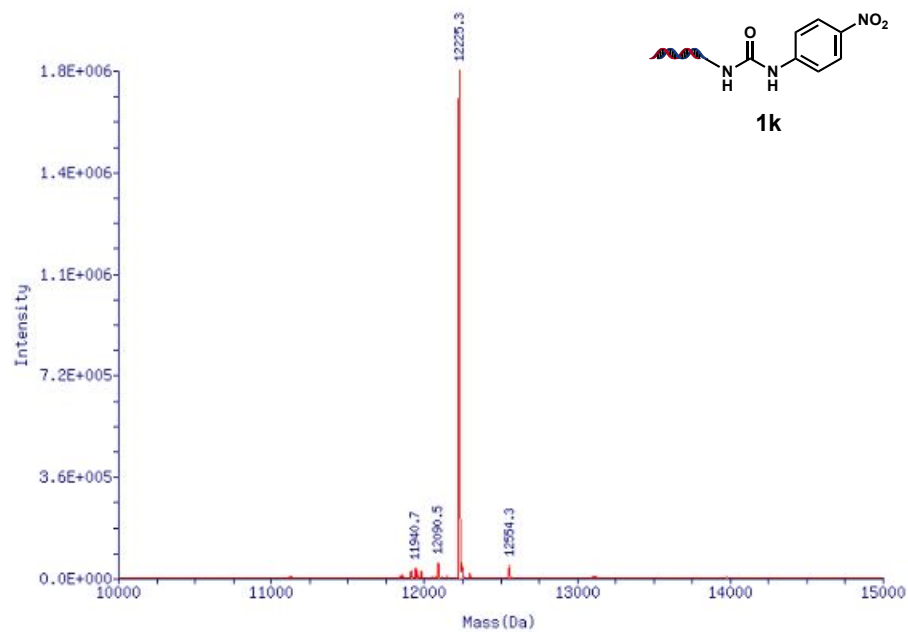


Figure S24. Deconvoluted mass spectrum of compound **1k**, expected: 12224.1; observed 12225.3.

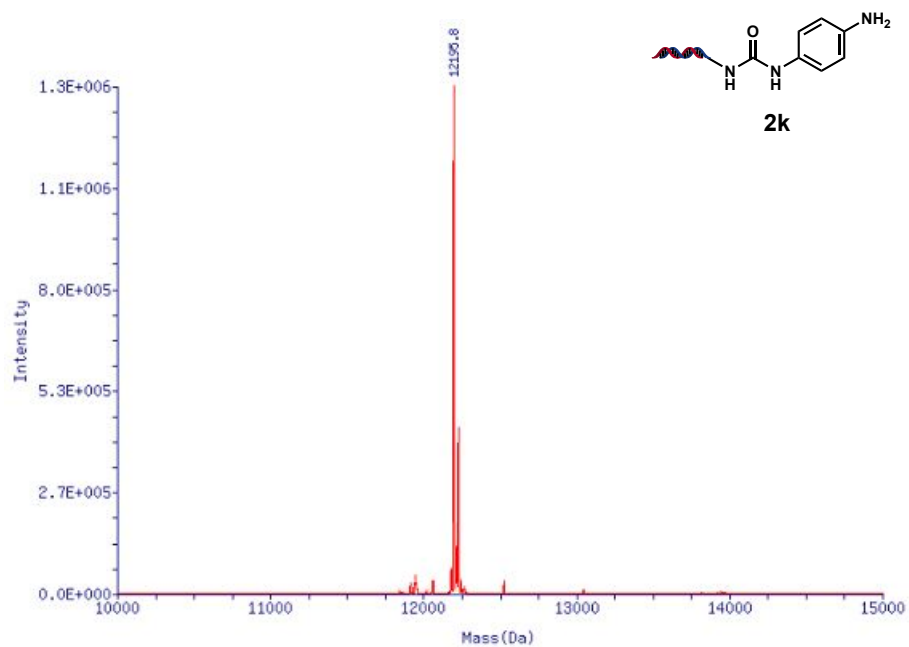


Figure S25. Deconvoluted mass spectrum of compound **2k**, expected: 12194.1; observed 12195.8.

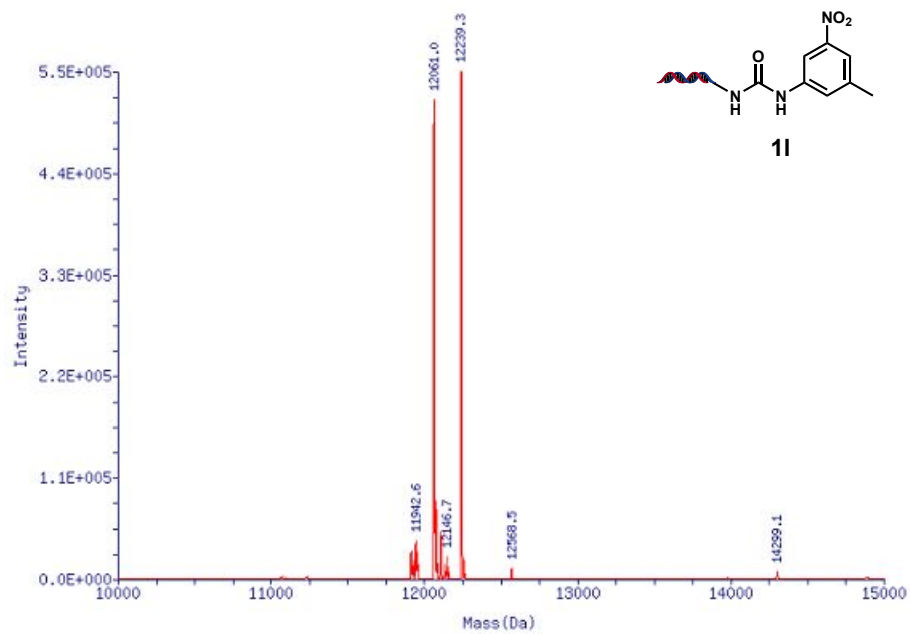


Figure S26. Deconvoluted mass spectrum of compound **1I**, expected: 12238.2; observed 12239.3.

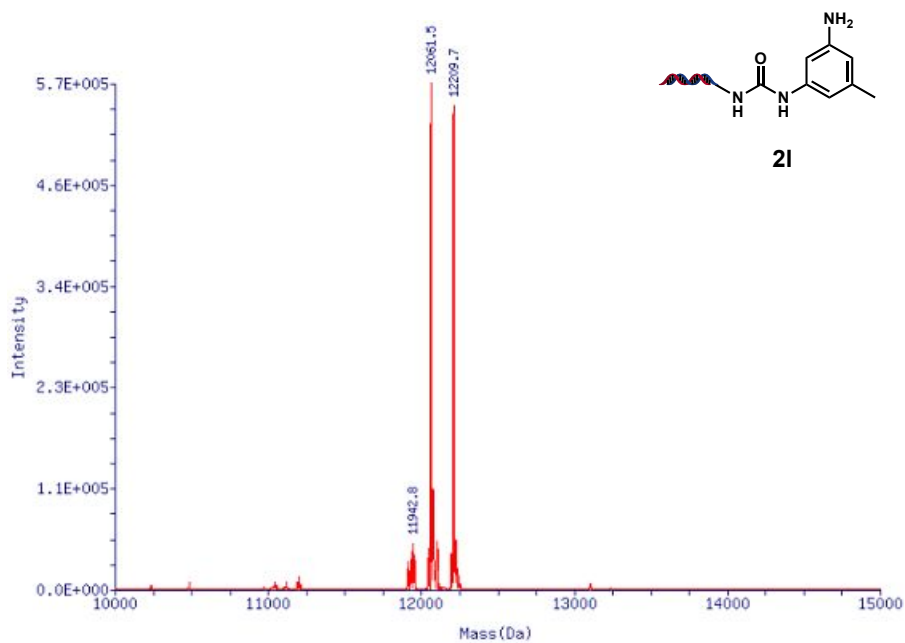


Figure S27. Deconvoluted mass spectrum of compound **2I**, expected: 12208.2; observed 12209.7.

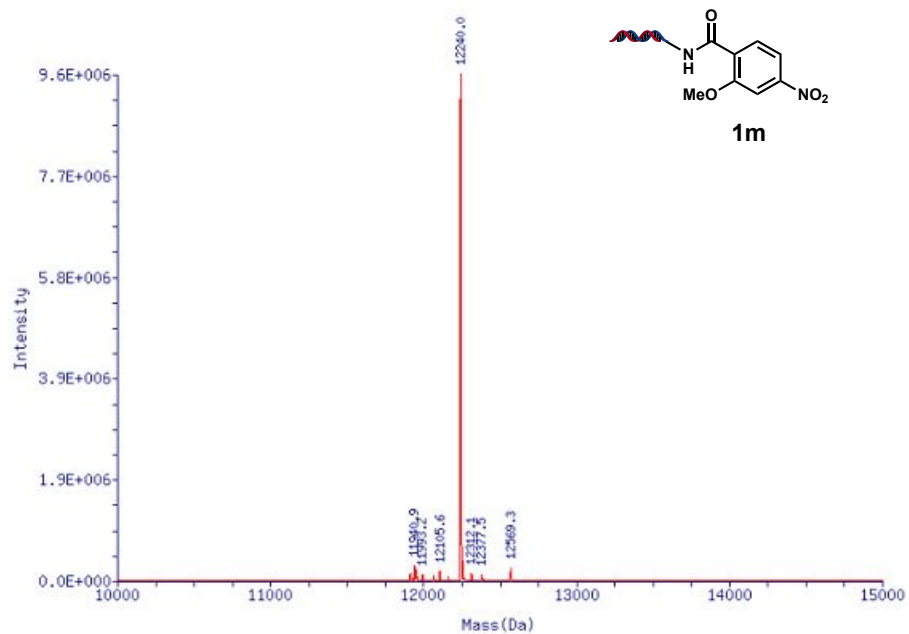


Figure S28. Deconvoluted mass spectrum of compound **1m**, expected: 12239.1; observed 12240.0.

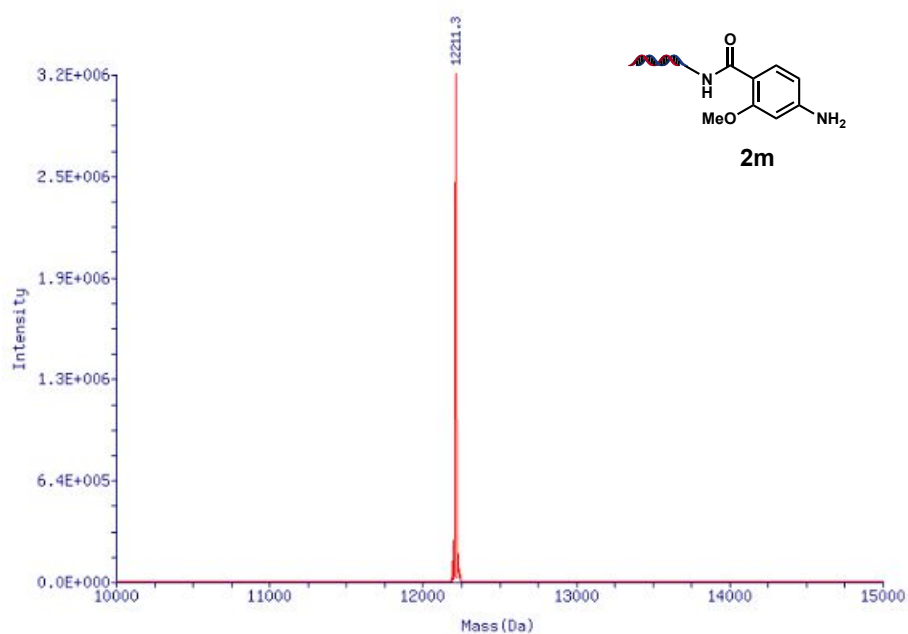


Figure S29. Deconvoluted mass spectrum of compound **2m**, expected: 12209.1; observed 12211.3.

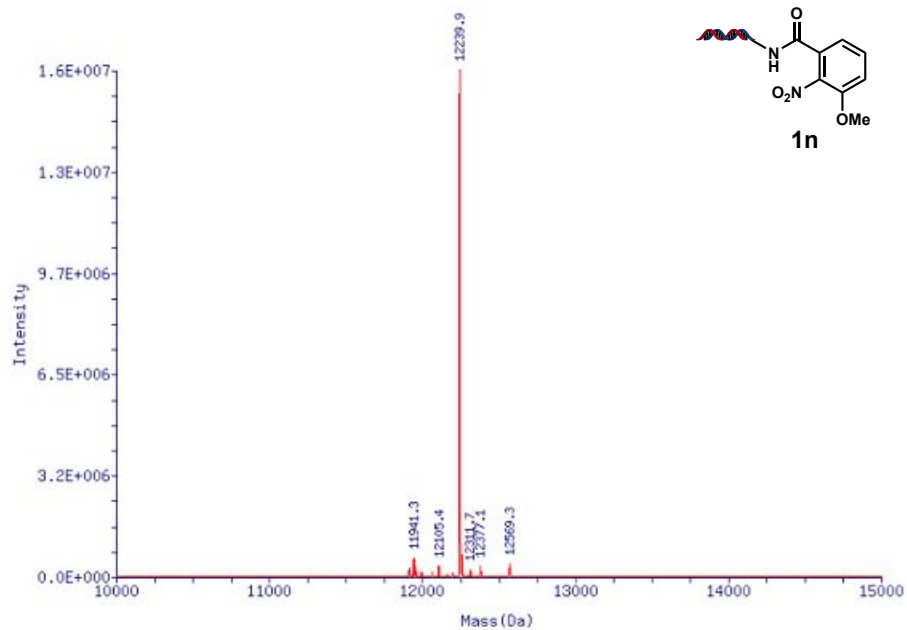


Figure S30. Deconvoluted mass spectrum of compound **1n**, expected: 12239.1; observed 12239.9.

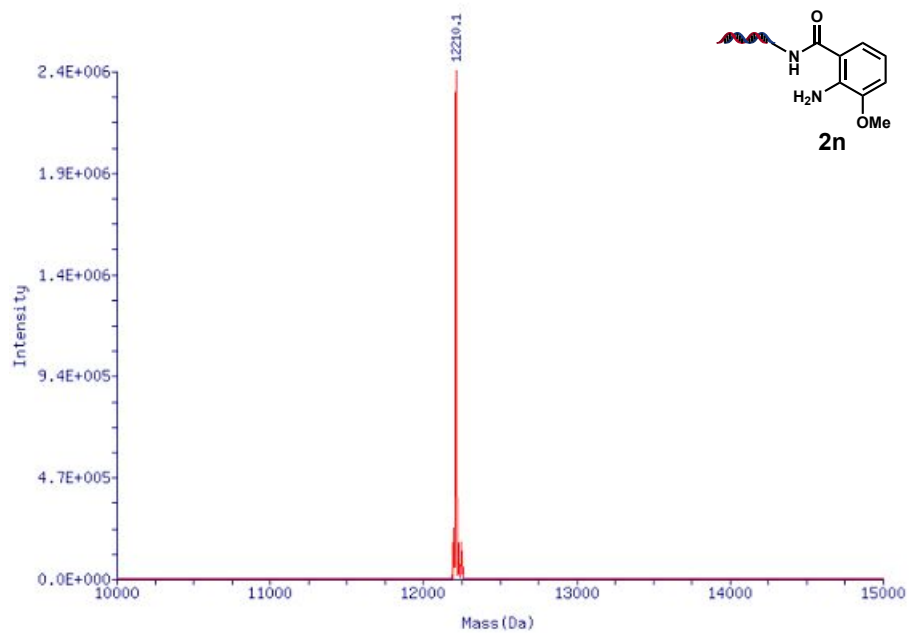


Figure S31. Deconvoluted mass spectrum of compound **2n**, expected: 12209.1; observed 12210.1.

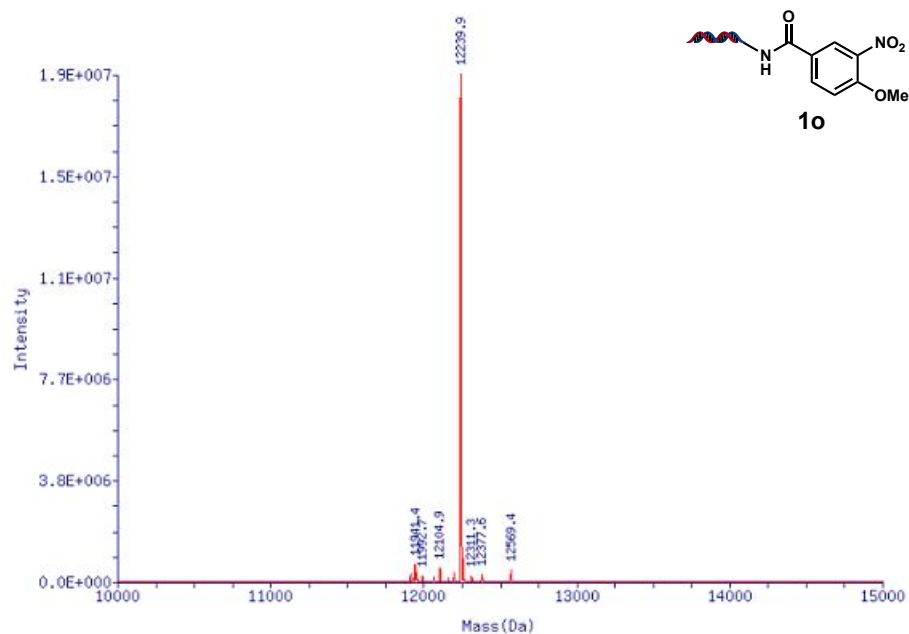


Figure S32. Deconvoluted mass spectrum of compound **1o**, expected: 12239.1; observed 12239.9.

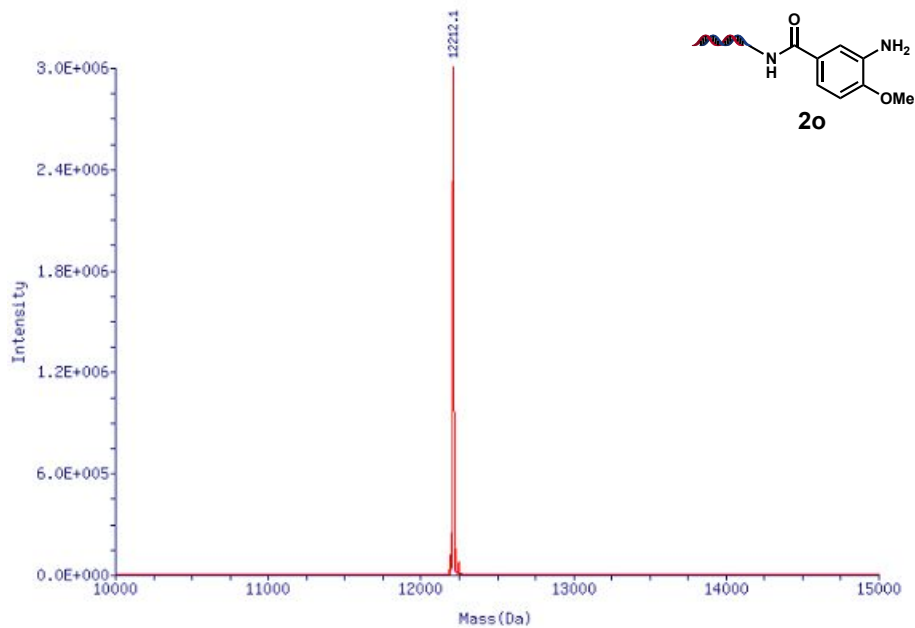


Figure S33. Deconvoluted mass spectrum of compound **2o**, expected: 12209.1; observed 12212.1.

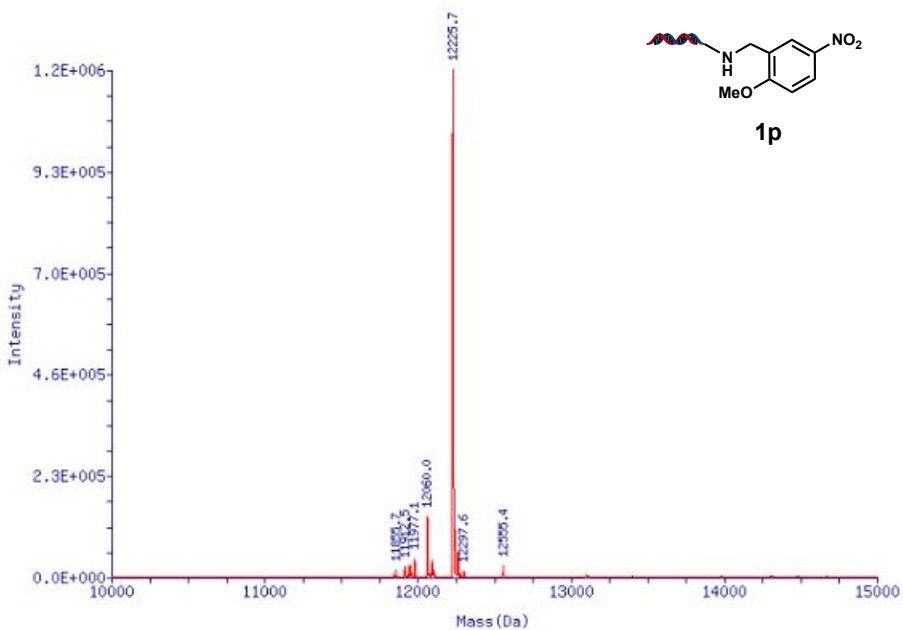


Figure S34. Deconvoluted mass spectrum of compound **1p**, expected: 12225.2; observed 12225.7.

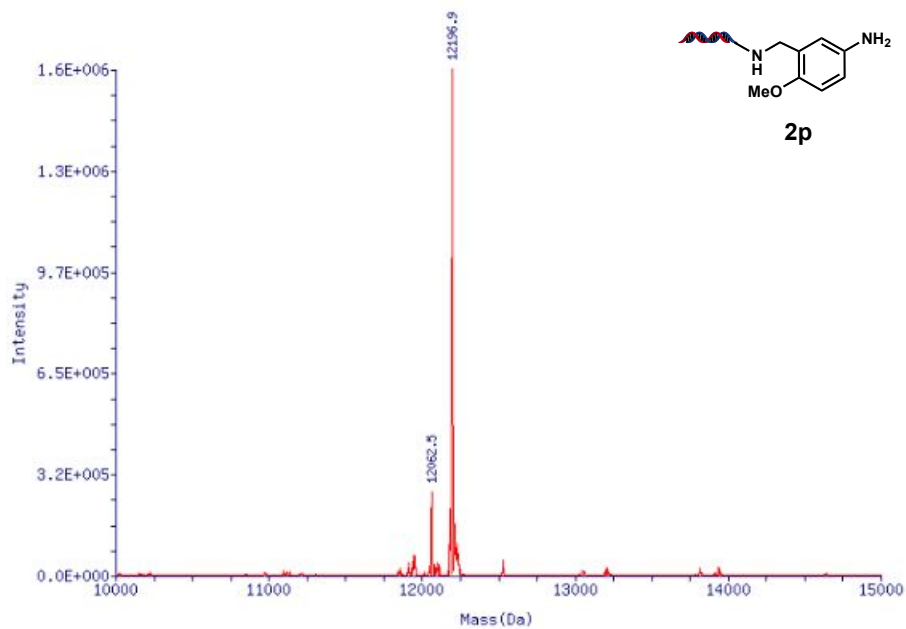


Figure S35. Deconvoluted mass spectrum of compound **2p**, expected: 12195.2; observed 12196.9.

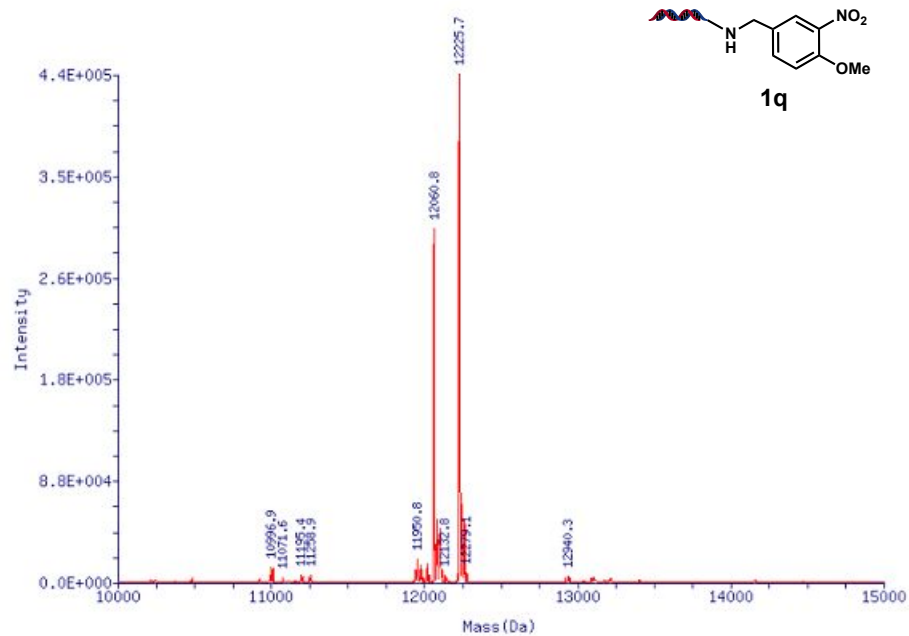


Figure S36. Deconvoluted mass spectrum of compound **1q**, expected: 12225.2; observed 12225.7.

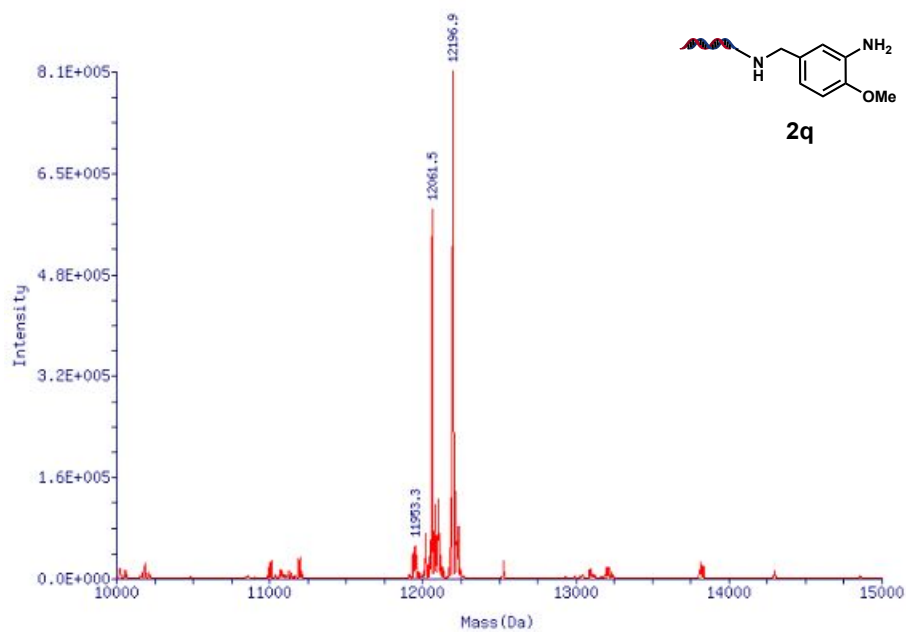


Figure S37. Deconvoluted mass spectrum of compound **2q**, expected: 12195.2; observed 12196.9.

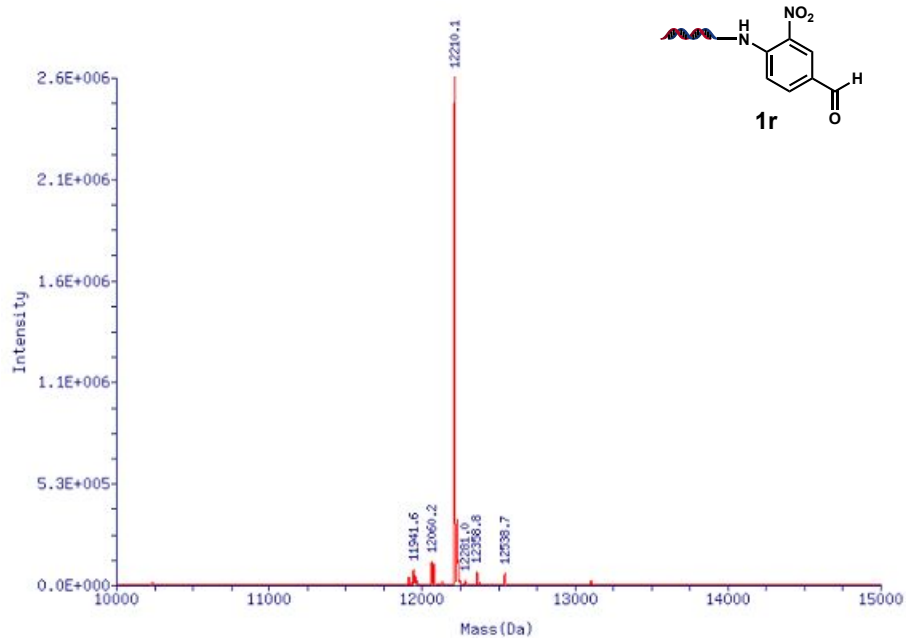


Figure S38. Deconvoluted mass spectrum of compound **1r**, expected: 12209.1; observed 12210.1.

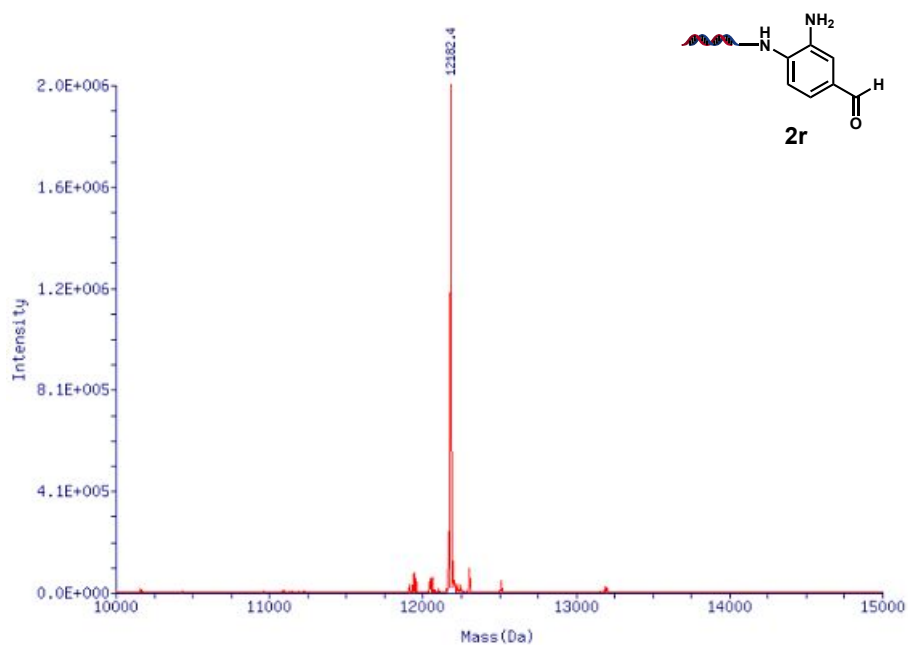


Figure S39. Deconvoluted mass spectrum of compound **2r**, expected: 12179.1; observed 12182.4.

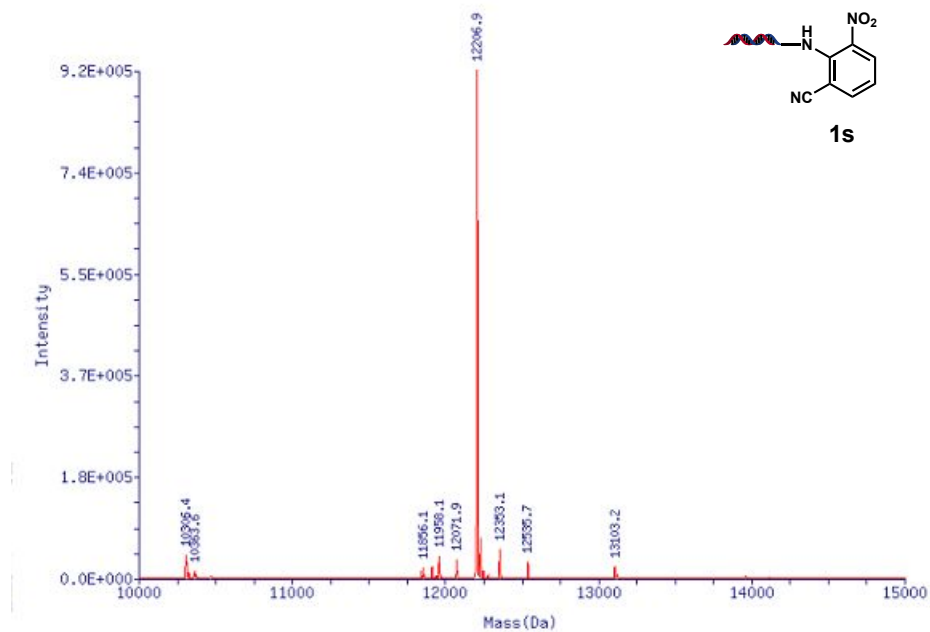


Figure S40. Deconvoluted mass spectrum of compound **1s**, expected: 12206.1; observed 12206.9.

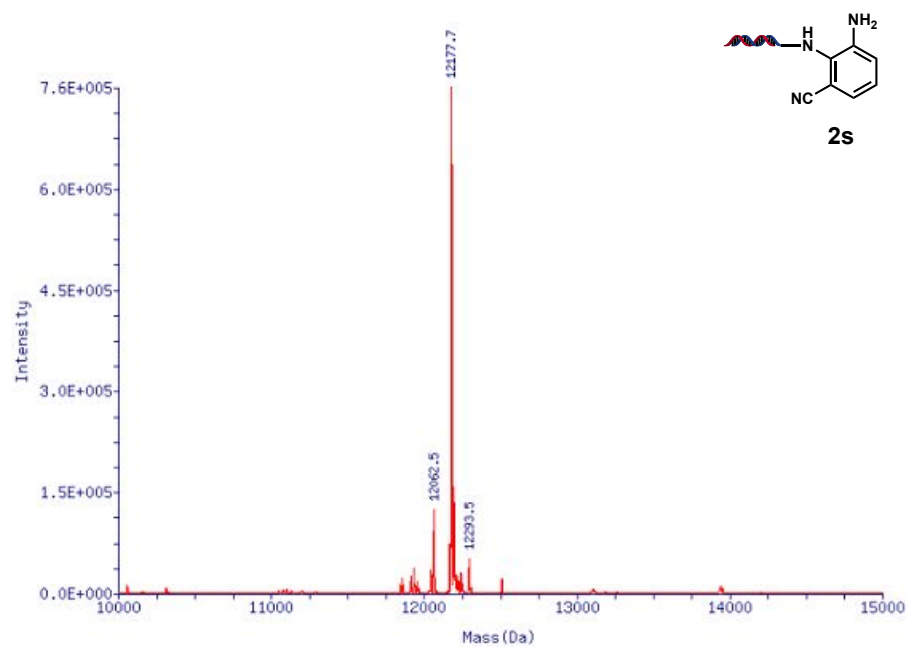


Figure S41. Deconvoluted mass spectrum of compound **2s**, expected: 12176.1; observed 12177.7.

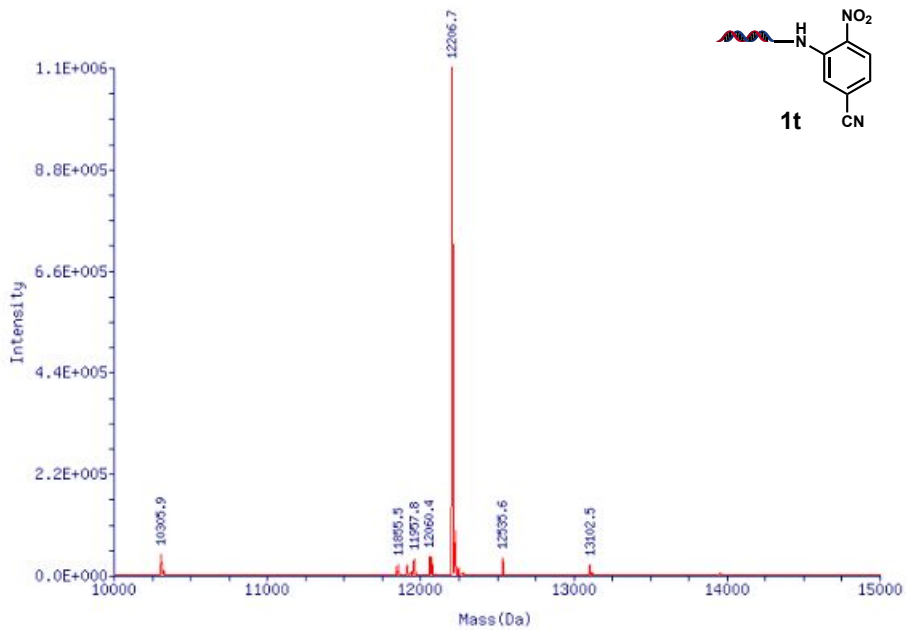


Figure S42. Deconvoluted mass spectrum of compound **1t**, expected: 12206.1; observed 12206.7.

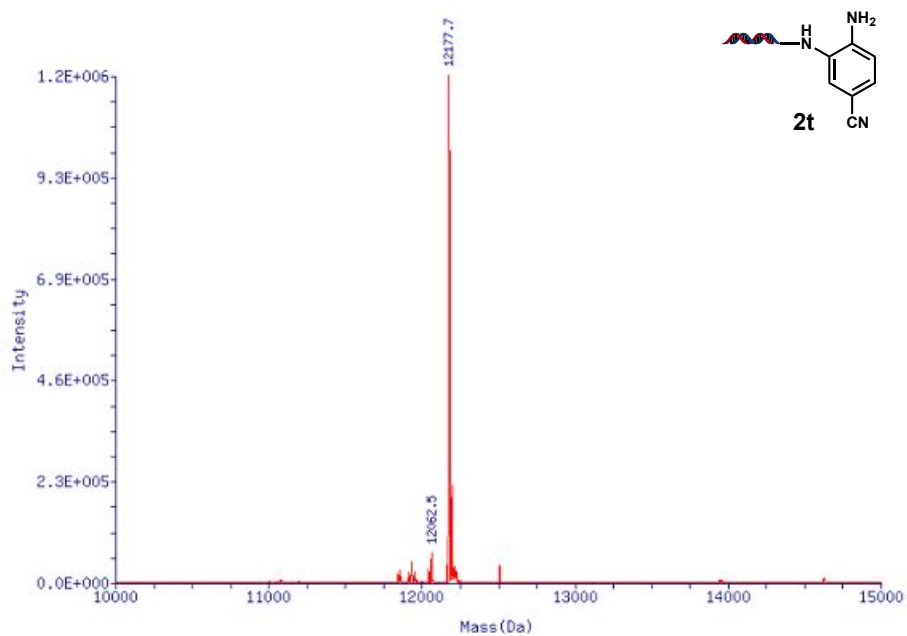


Figure S43. Deconvoluted mass spectrum of compound **2t**, expected: 12176.1; observed 12177.7.

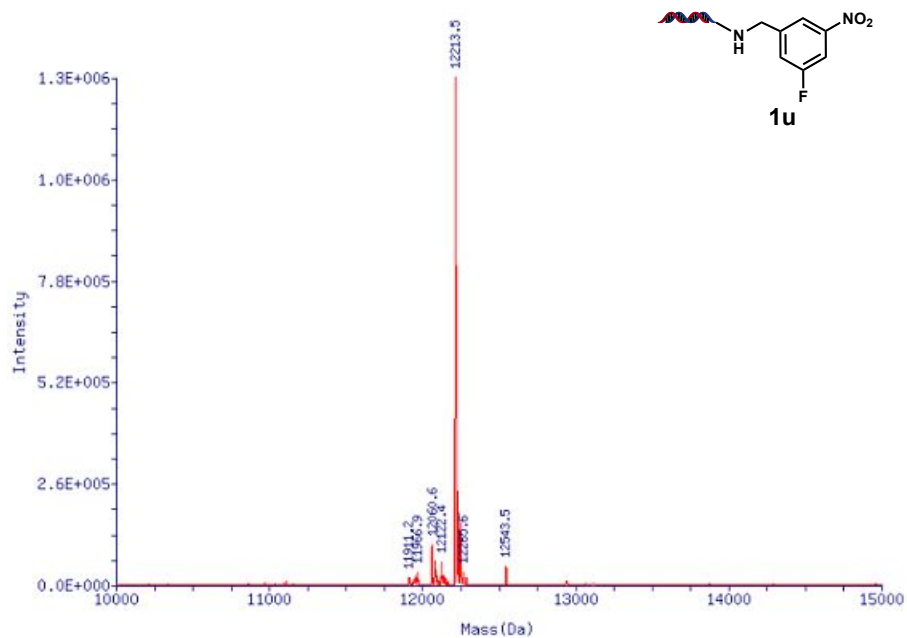


Figure S44. Deconvoluted mass spectrum of compound **1u**, expected: 12213.1; observed 12213.5.

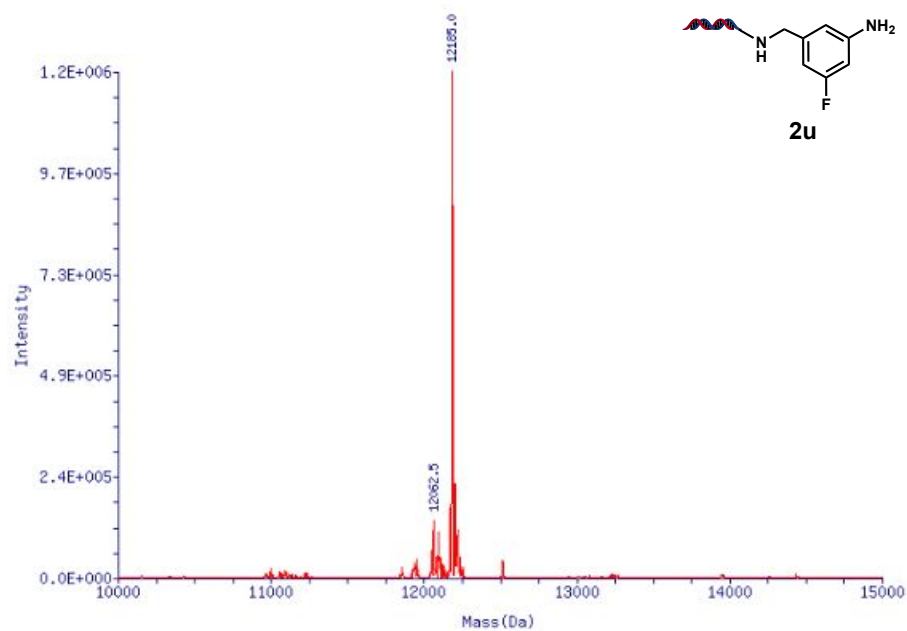


Figure S45. Deconvoluted mass spectrum of compound **2u**, expected: 12183.1; observed 12185.0.

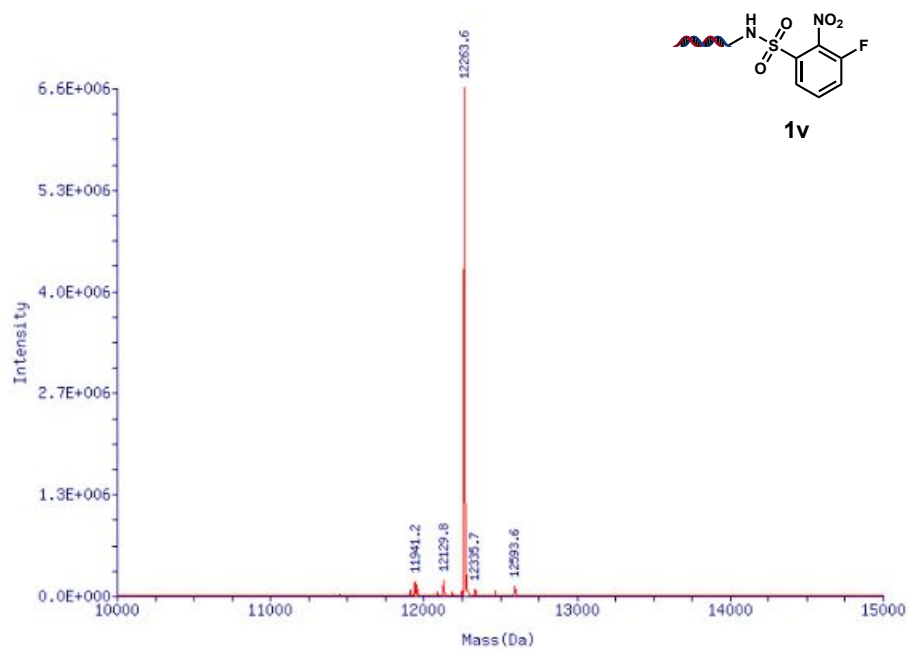


Figure S46. Deconvoluted mass spectrum of compound **1v**, expected: 12263.2; observed 12263.6.

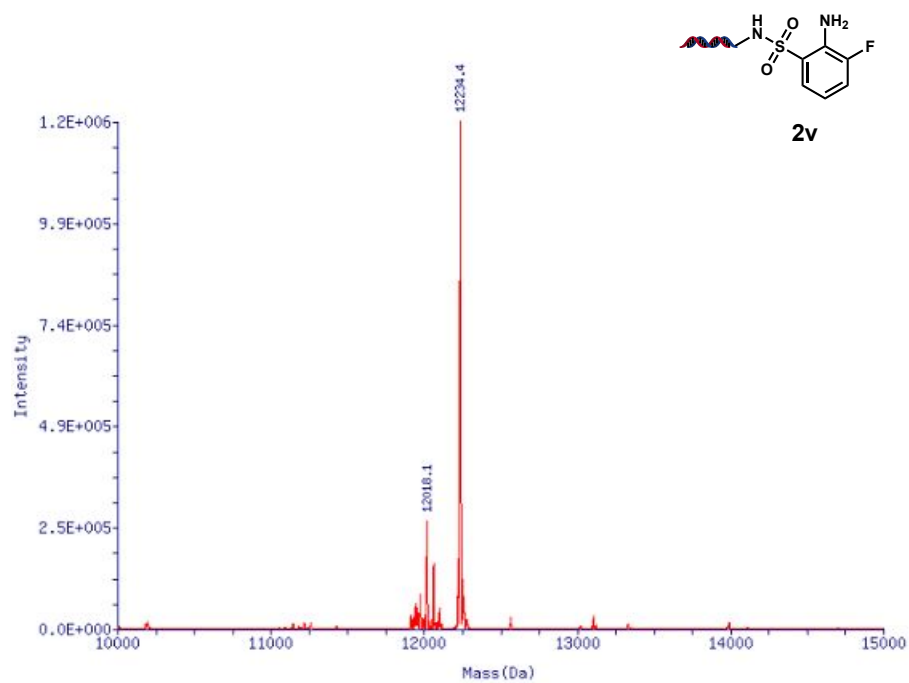


Figure S47. Deconvoluted mass spectrum of compound **2v**, expected: 12233.2; observed 12234.4.

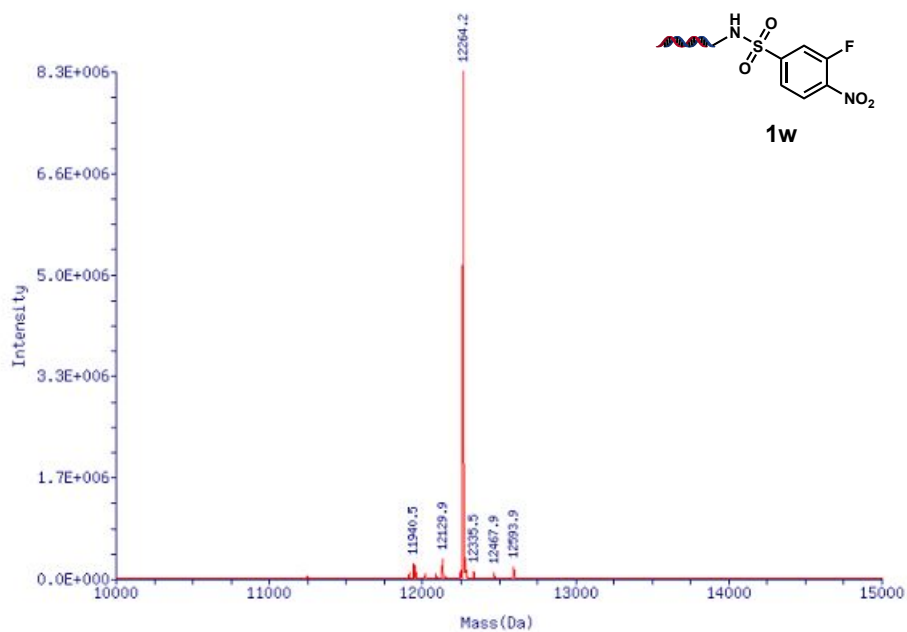


Figure S48. Deconvoluted mass spectrum of compound **1w**, expected: 12263.2; observed 12264.2.

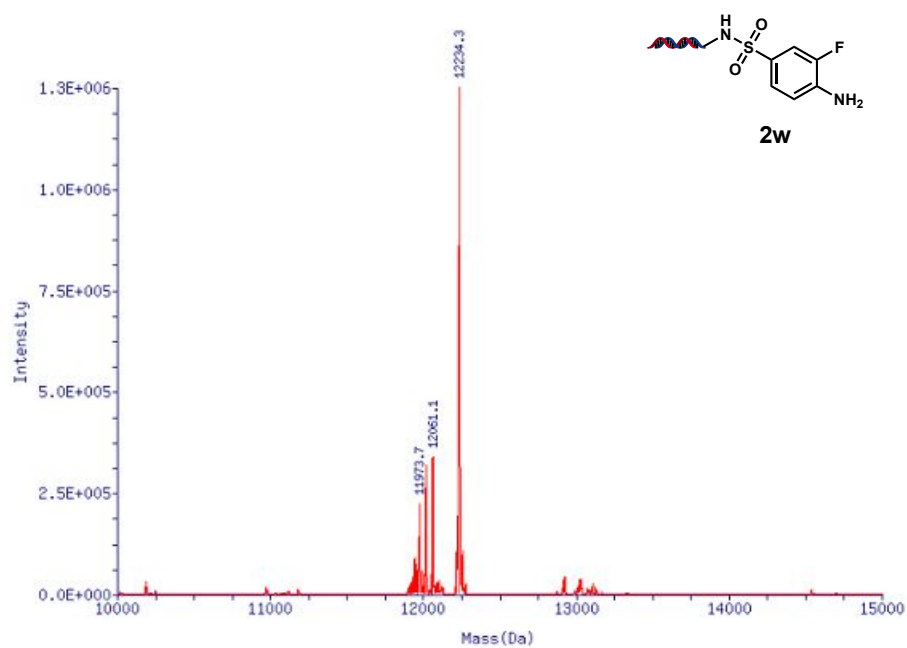


Figure S49. Deconvoluted mass spectrum of compound **2w**, expected: 12233.2; observed 12234.3.

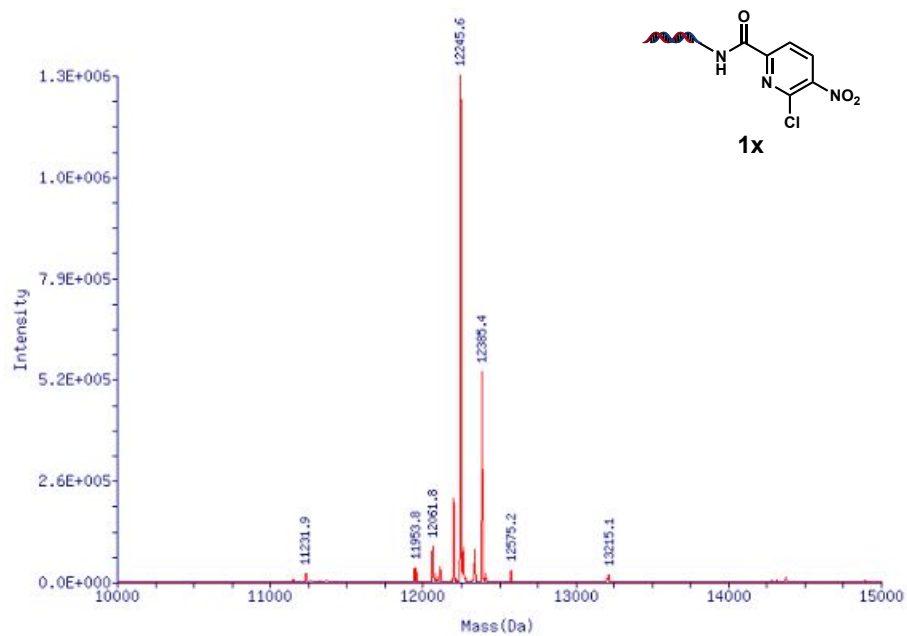


Figure S50. Deconvoluted mass spectrum of compound **1x**, expected: 12244.5; observed 12245.6.

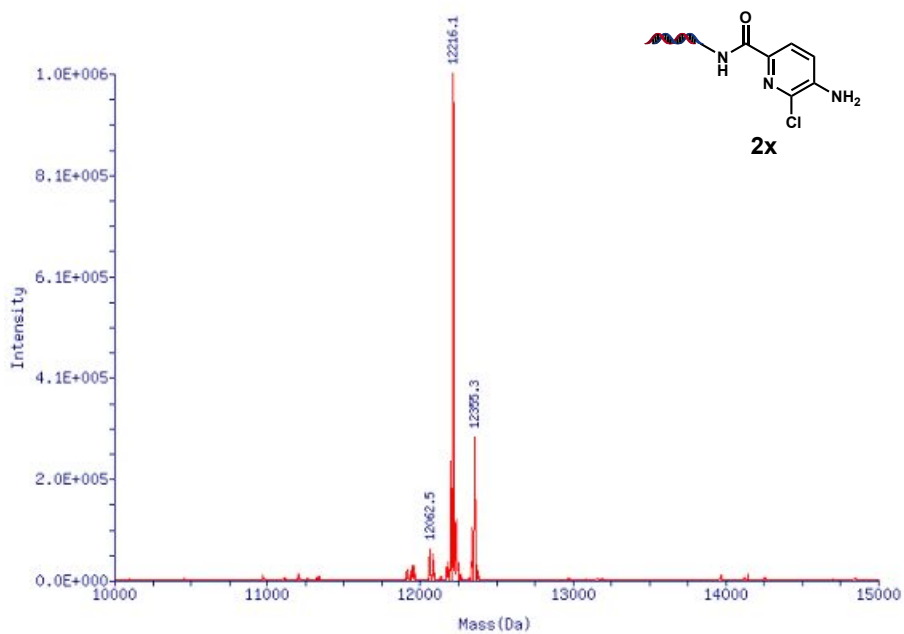


Figure S51. Deconvoluted mass spectrum of compound **2x**, expected: 12214.5; observed 12216.1.

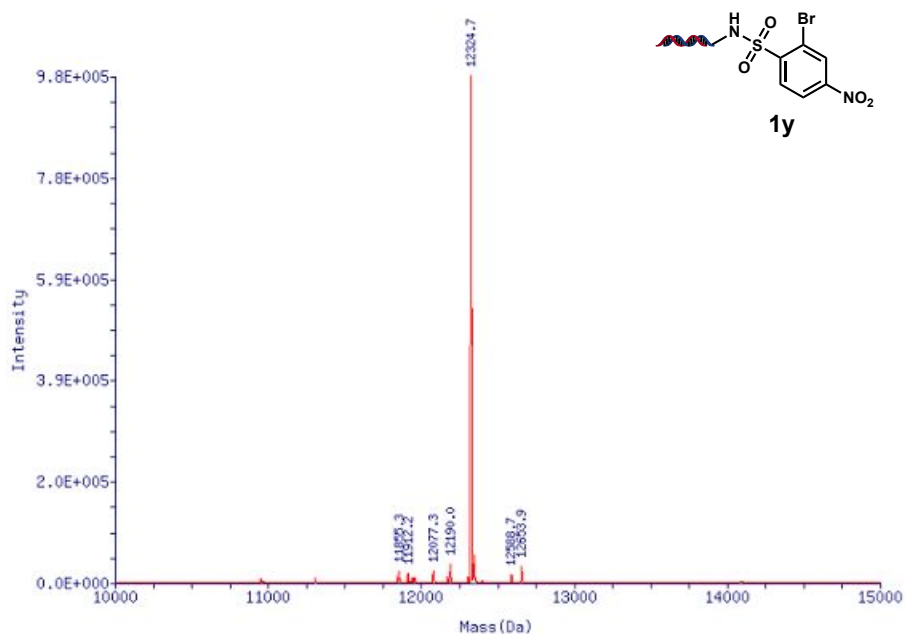


Figure S52. Deconvoluted mass spectrum of compound **1y**, expected: 12324.1; observed 12324.7.

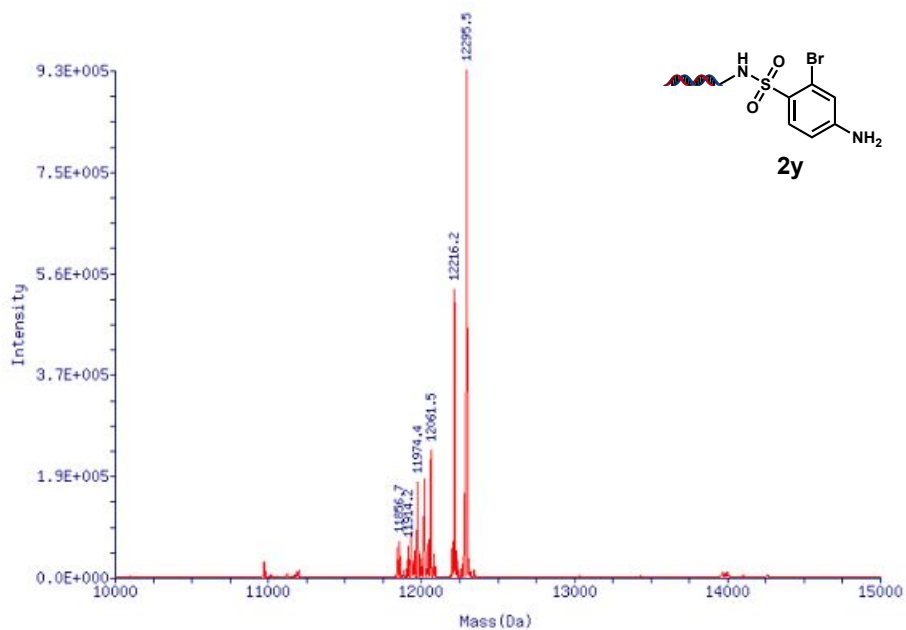


Figure S53. Deconvoluted mass spectrum of compound **2y**, expected: 12294.1; observed 12295.5.

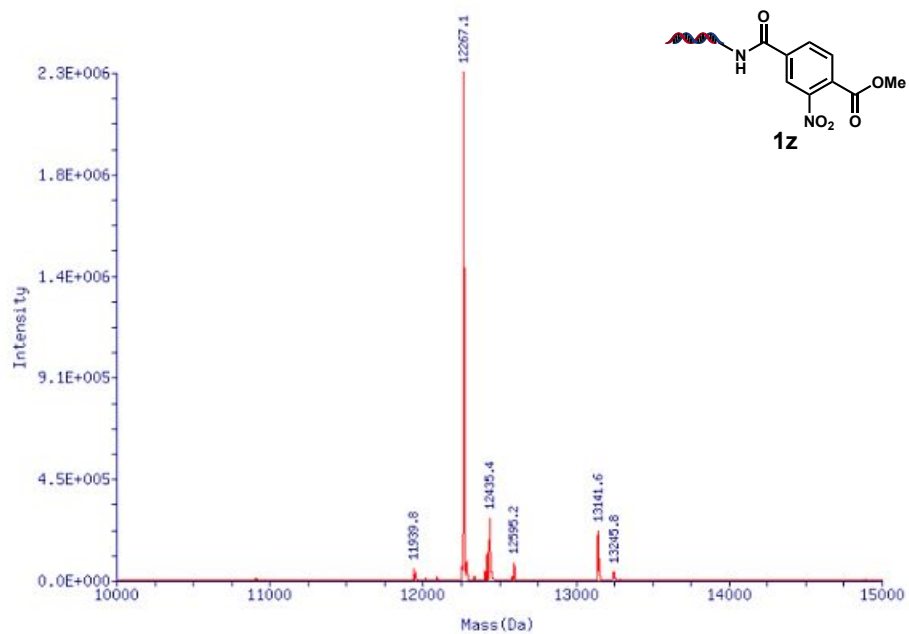


Figure S54. Deconvoluted mass spectrum of compound **1z**, expected: 12267.1; observed 12267.1.

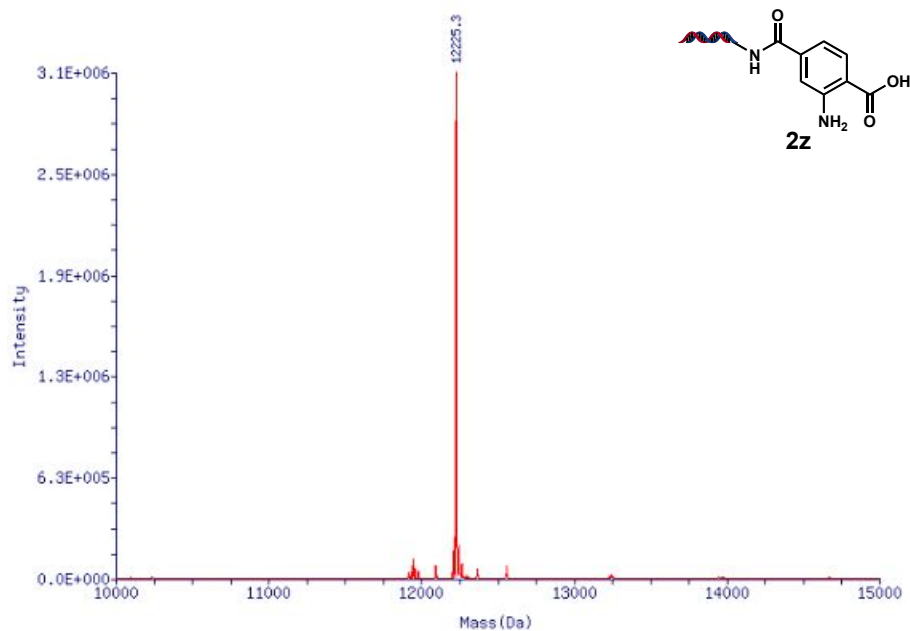


Figure S55. Deconvoluted mass spectrum of compound **2z**, expected: 12223.1; observed 12225.3. (hydrolysis product).

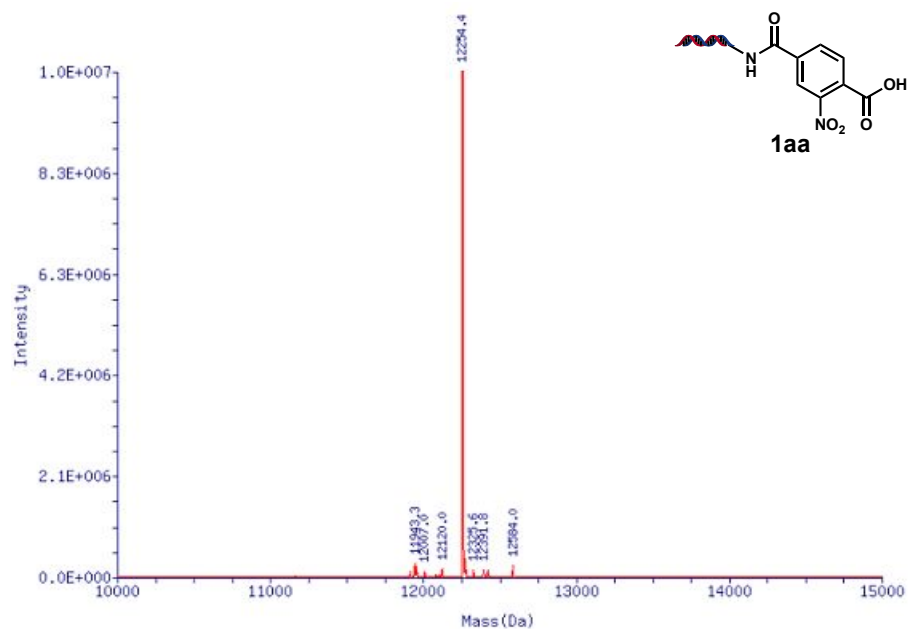


Figure S56. Deconvoluted mass spectrum of compound **1aa**, expected: 12253.1; observed 12254.4.

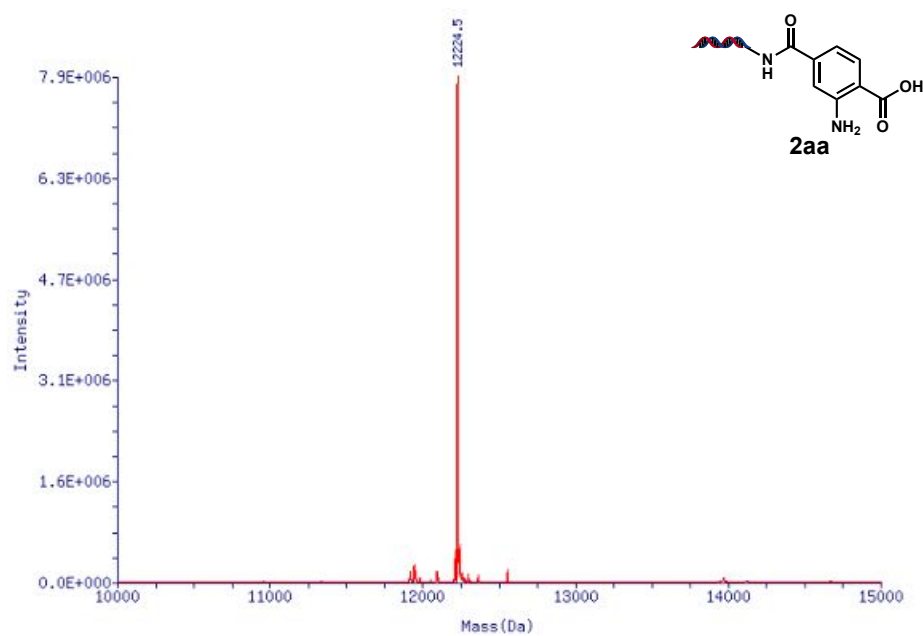


Figure S57. Deconvoluted mass spectrum of compound **2aa**, expected: 12223.1; observed 12224.5.

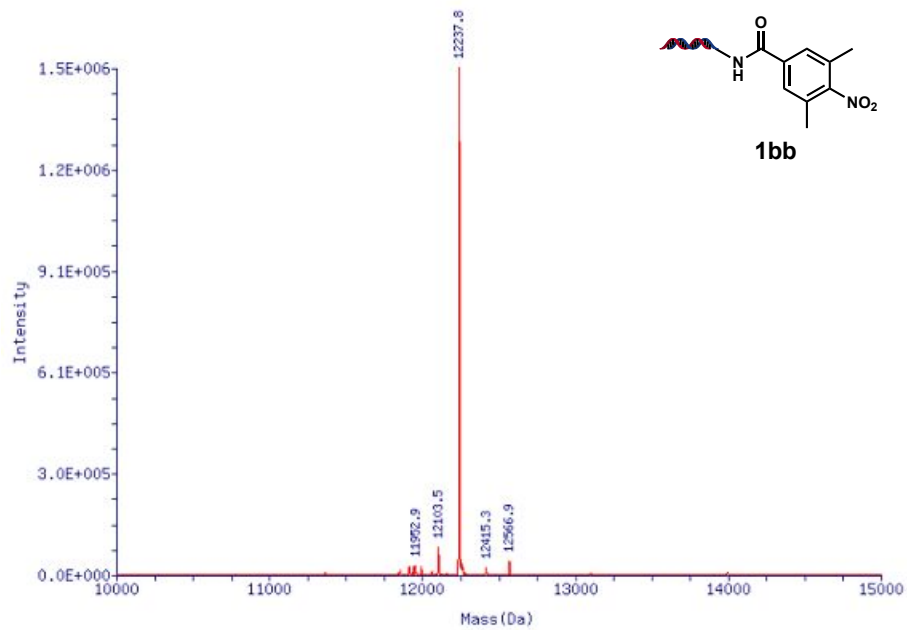


Figure S58. Deconvoluted mass spectrum of compound **1bb**, expected: 12237.2; observed 12237.8.

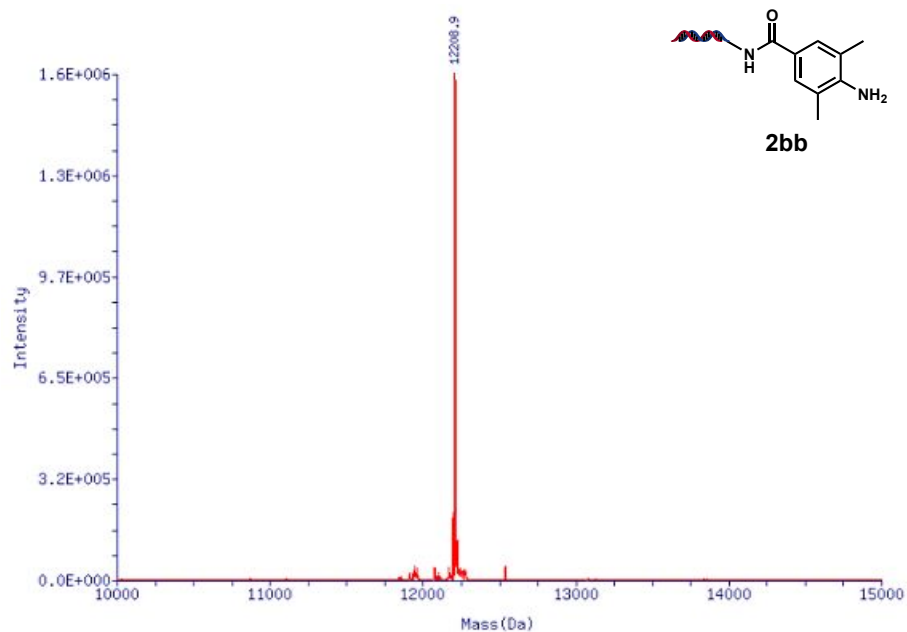


Figure S59. Deconvoluted mass spectrum of compound **2bb**, expected: 12207.2; observed 12208.2.

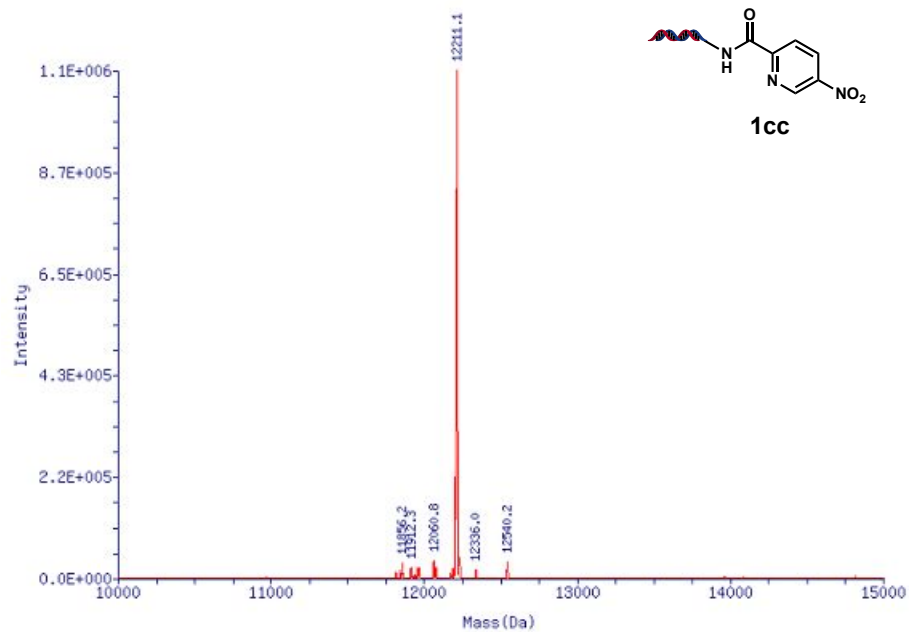


Figure S60. Deconvoluted mass spectrum of compound **1cc**, expected: 12210.1; observed 12211.1.

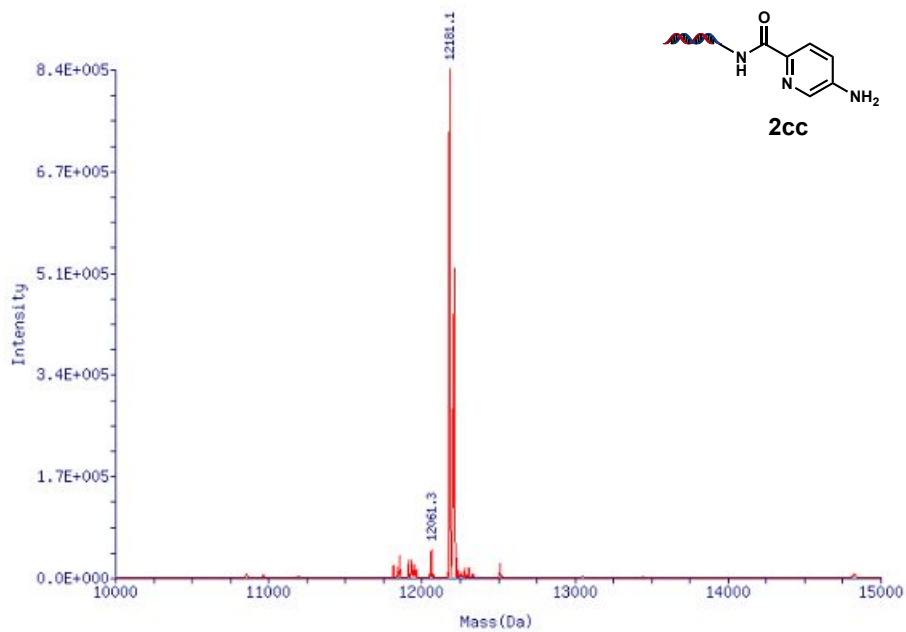


Figure S61. Deconvoluted mass spectrum of compound **2cc**, expected: 12180.1; observed 12181.1.

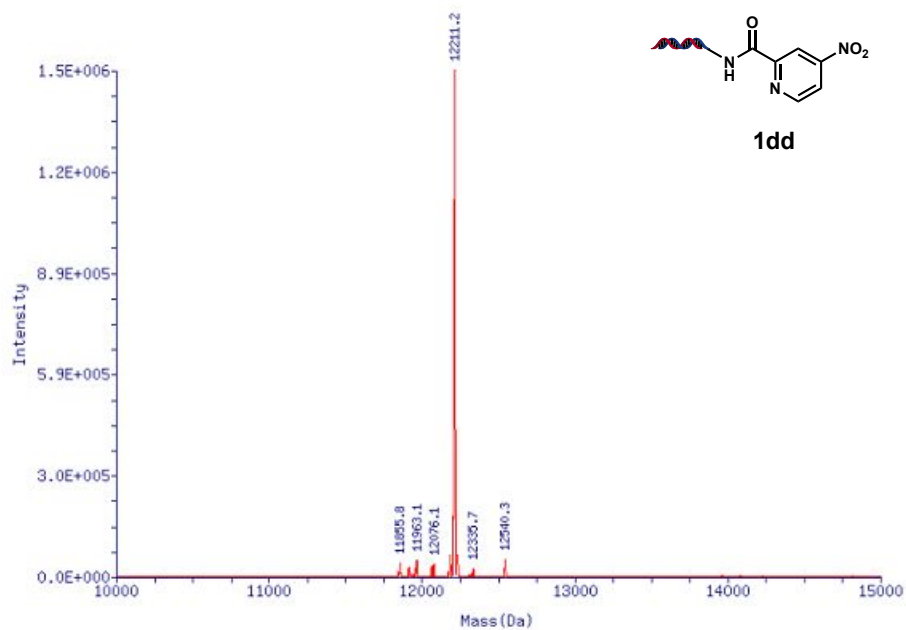


Figure S62. Deconvoluted mass spectrum of compound **1dd**, expected: 12210.1; observed 12211.2.

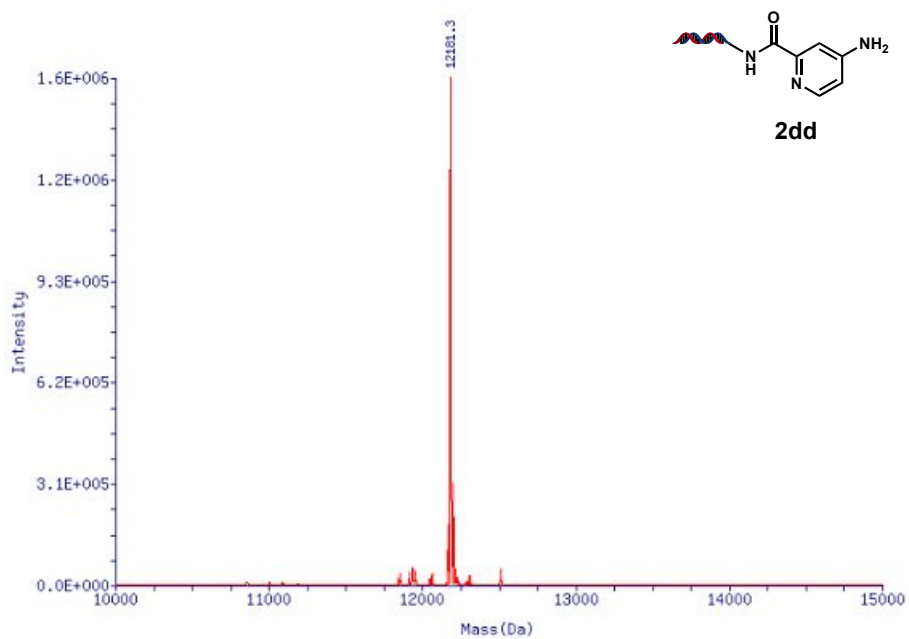


Figure S63. Deconvoluted mass spectrum of compound **2dd**, expected: 12180.1; observed 12181.3.

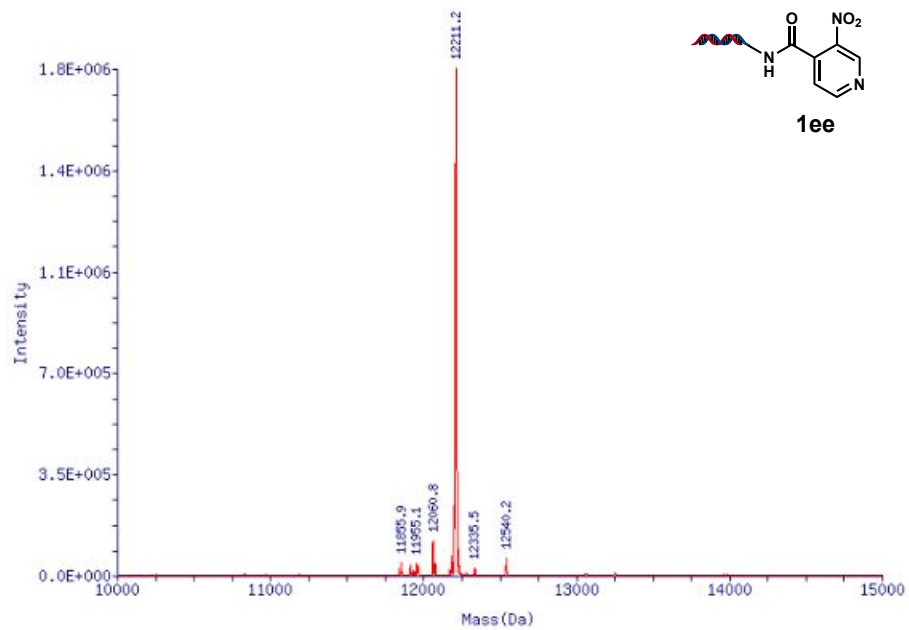


Figure S64. Deconvoluted mass spectrum of compound **1ee**, expected: 12210.1; observed 12211.2.

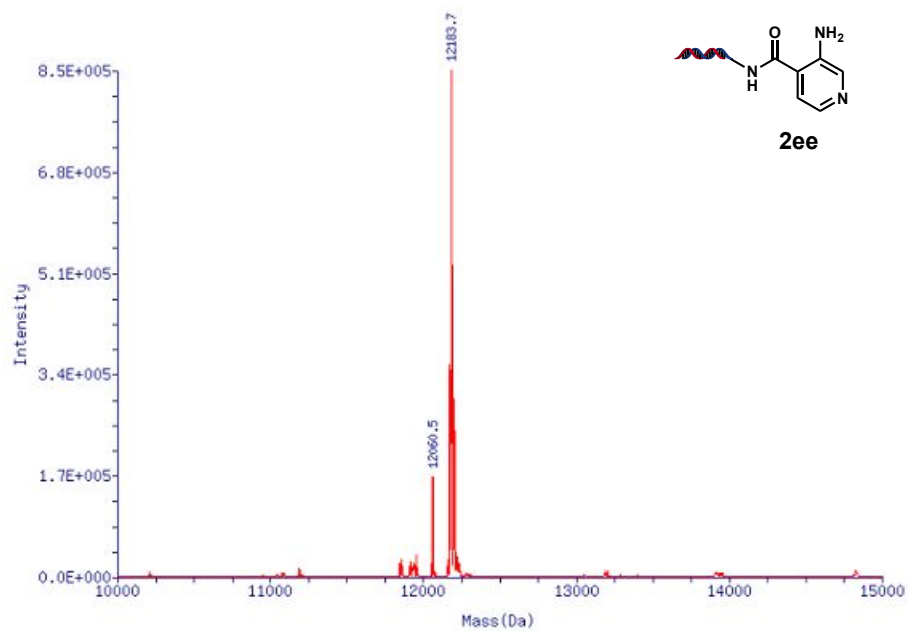


Figure S65. Deconvoluted mass spectrum of compound **2ee**, expected: 12180.1; observed 12183.7.

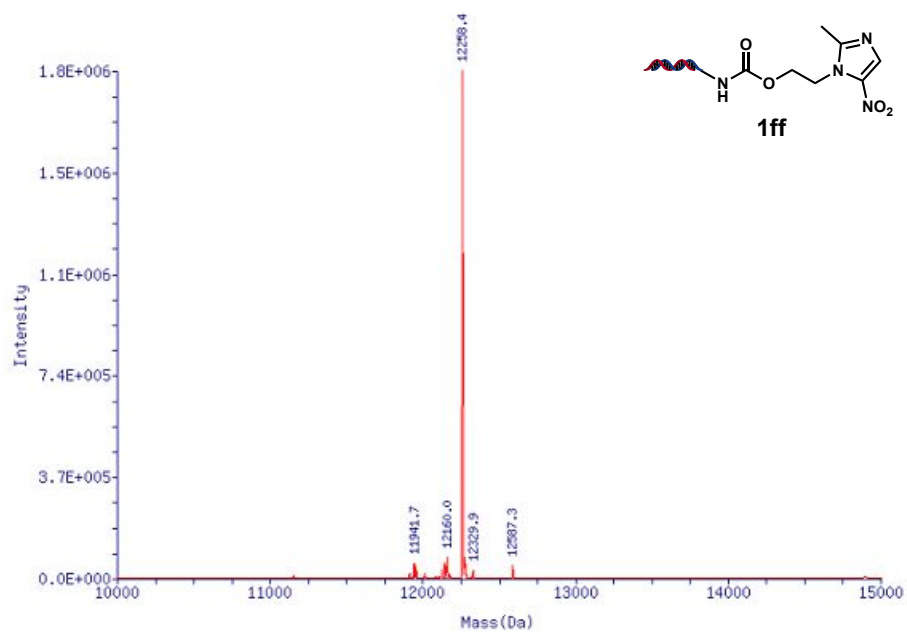


Figure S66. Deconvoluted mass spectrum of compound **1ff**, expected: 12257.2; observed 12258.4.

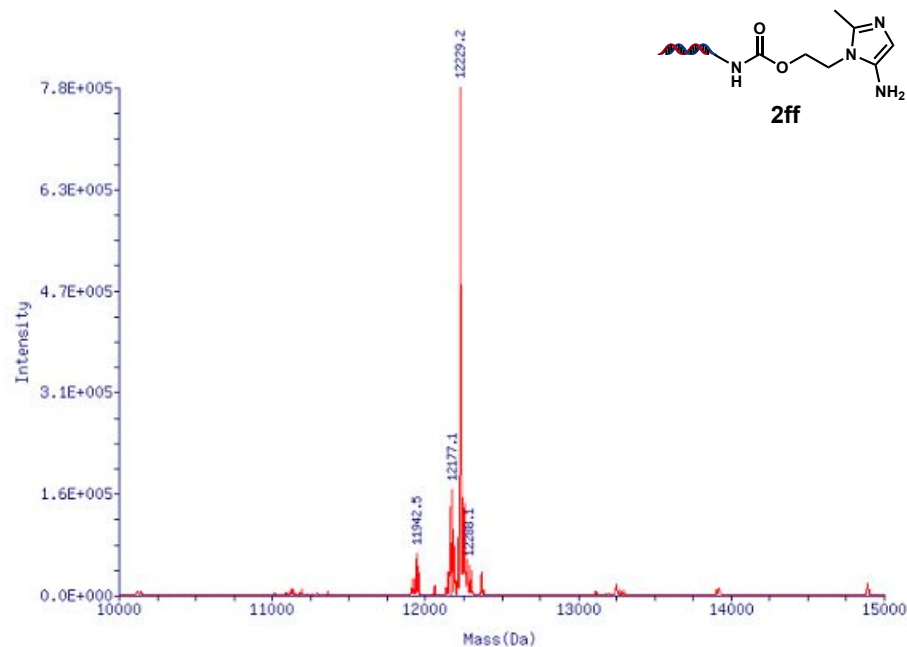


Figure S67. Deconvoluted mass spectrum of compound **2ff**, expected: 12227.2; observed 12229.2.

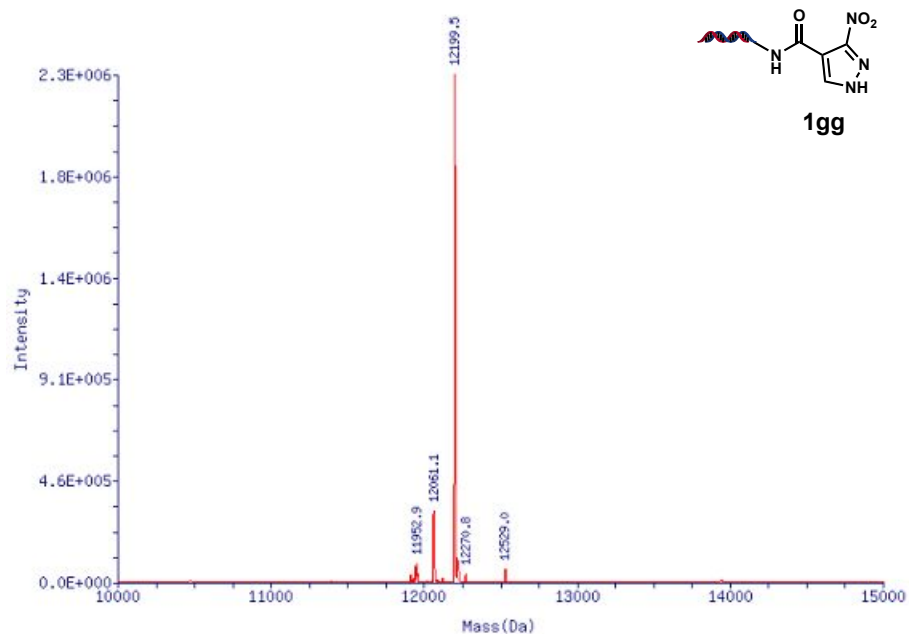


Figure S68. Deconvoluted mass spectrum of compound **1gg**, expected: 12199.1; observed 12199.5.

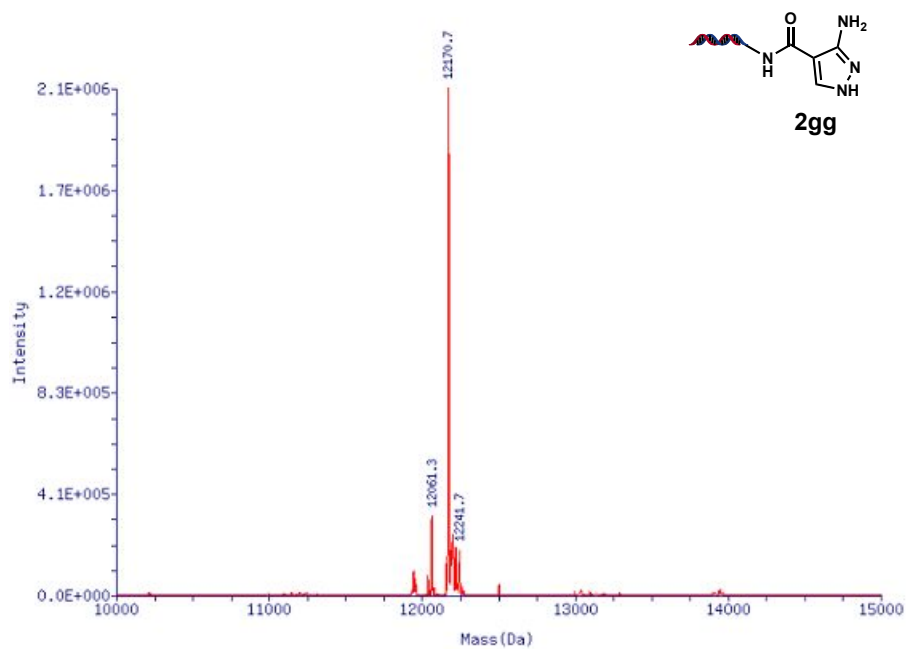


Figure S69. Deconvoluted mass spectrum of compound **2gg**, expected: 12169.1; observed 12170.7.

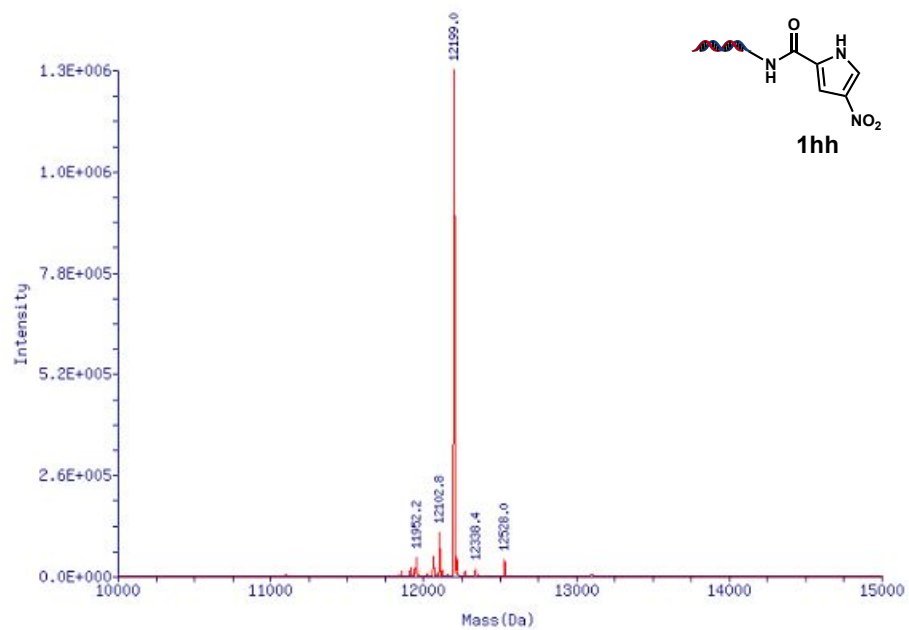


Figure S70. Deconvoluted mass spectrum of compound **1hh**, expected: 12198.1; observed 12199.0.

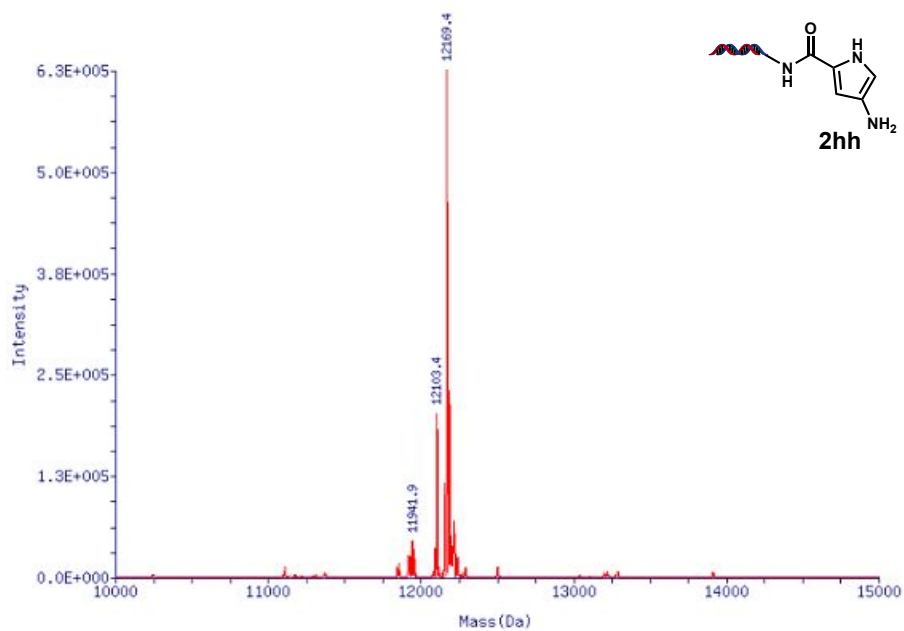


Figure S71. Deconvoluted mass spectrum of compound **2hh**, expected: 12168.1; observed 12169.4.

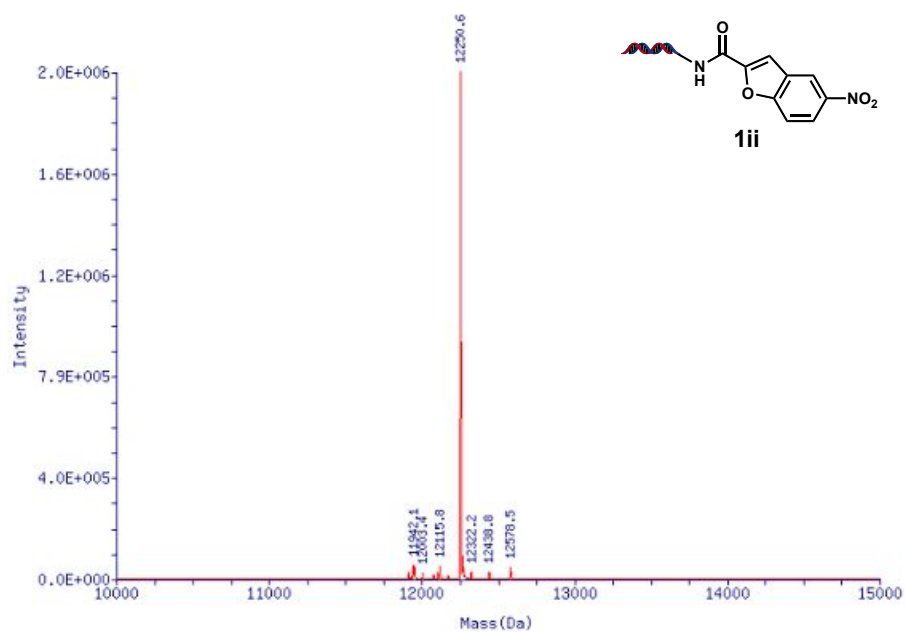


Figure S72. Deconvoluted mass spectrum of compound **1ii**, expected: 12249.1; observed 12250.6.

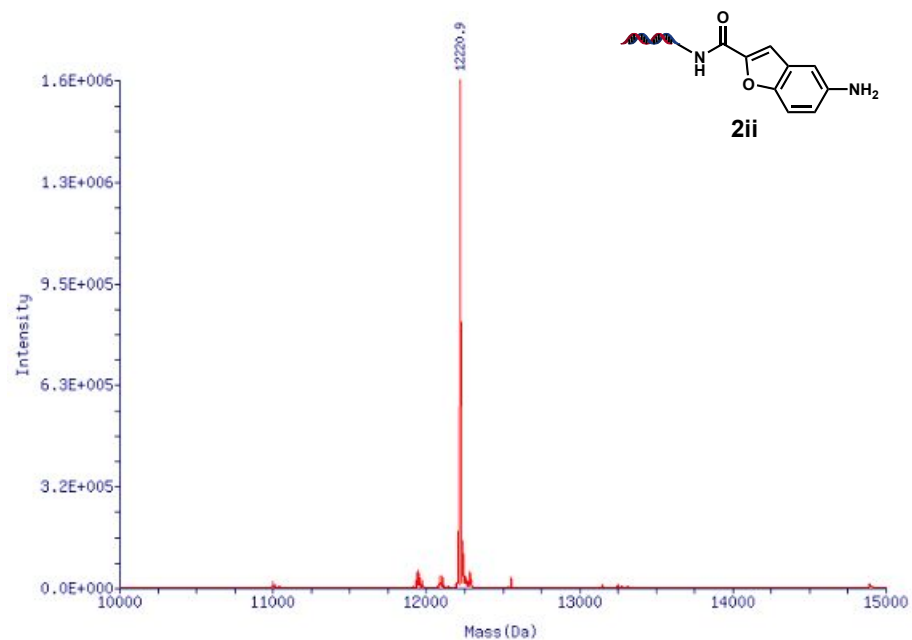


Figure S73. Deconvoluted mass spectrum of compound **2ii**, expected: 12219.1; observed 12220.9.

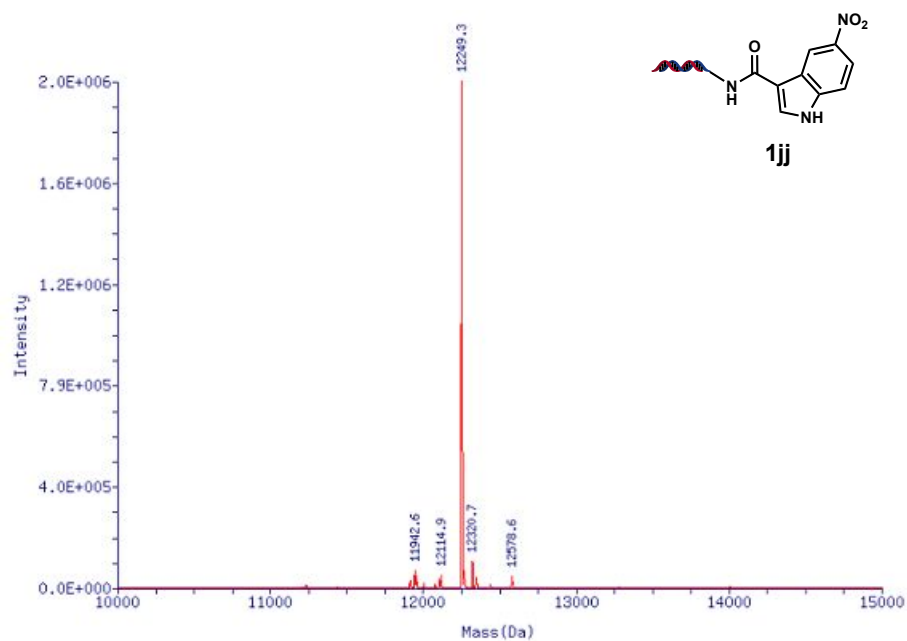


Figure S74. Deconvoluted mass spectrum of compound **1jj**, expected: 12248.2; observed 12249.3.

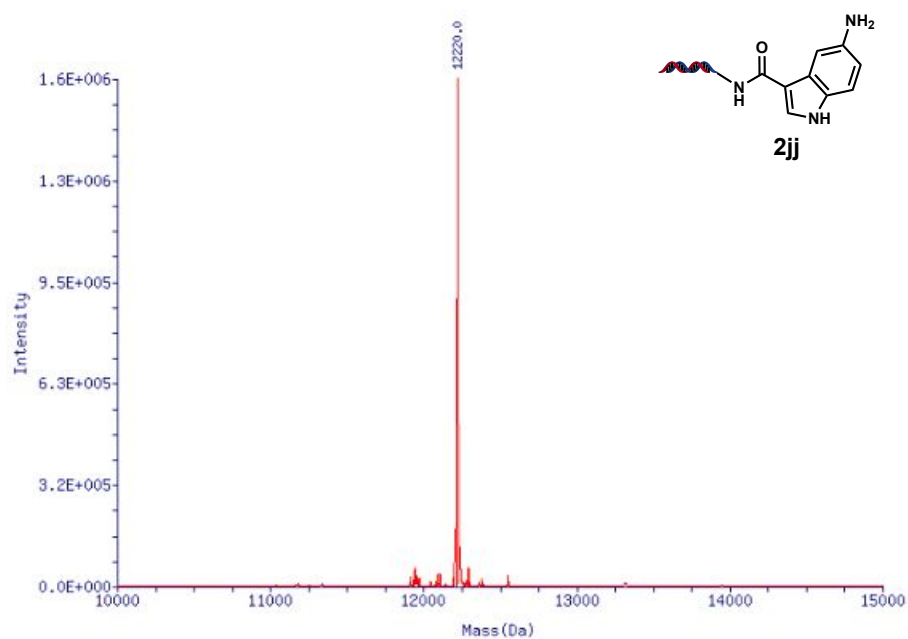


Figure S75. Deconvoluted mass spectrum of compound **2jj**, expected: 12218.2; observed 12220.0.

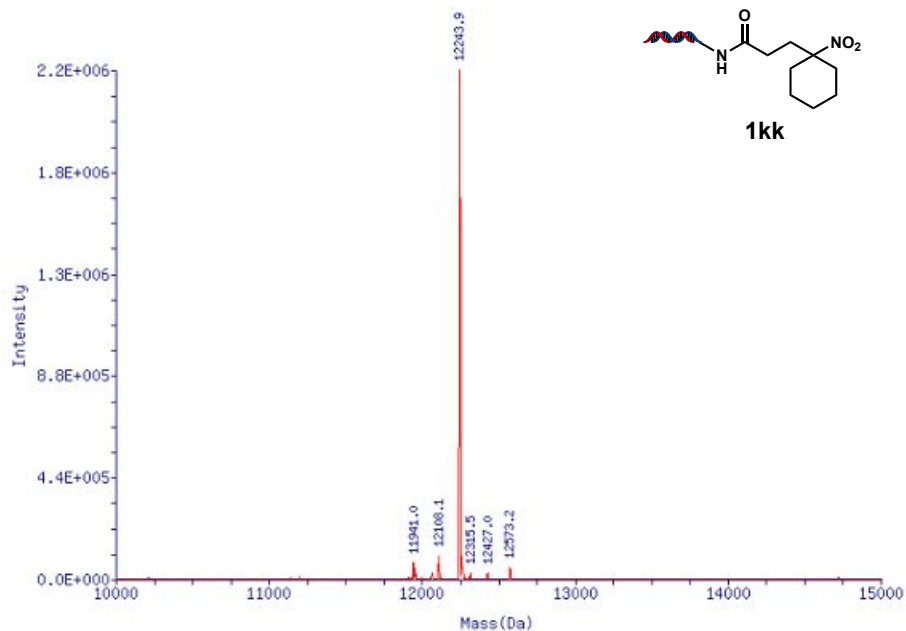


Figure S76. Deconvoluted mass spectrum of compound **1kk**, expected: 12243.2; observed 12243.9.

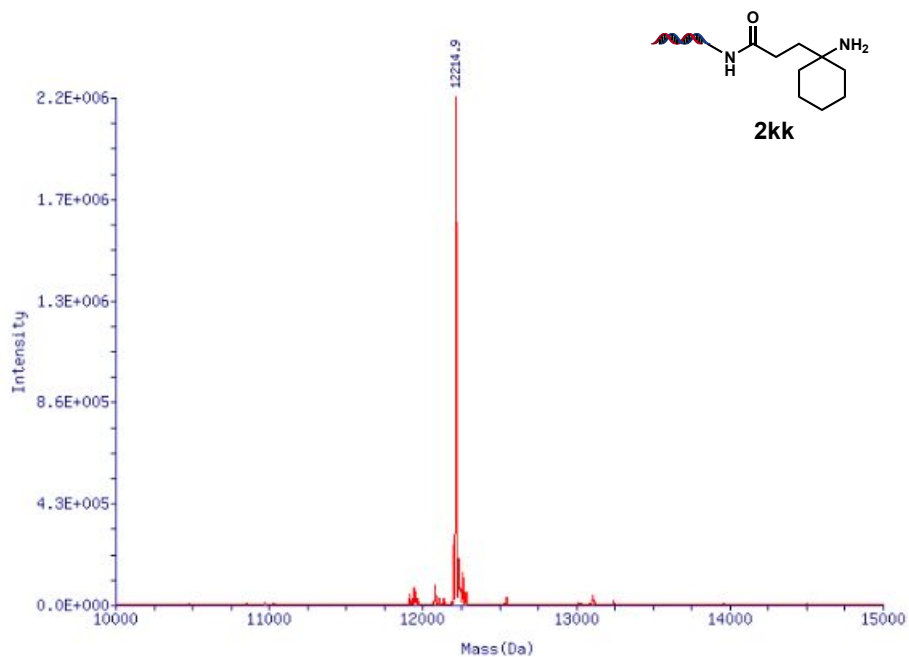


Figure S77. Deconvoluted mass spectrum of compound **2kk**, expected: 12213.2; observed 12214.9.

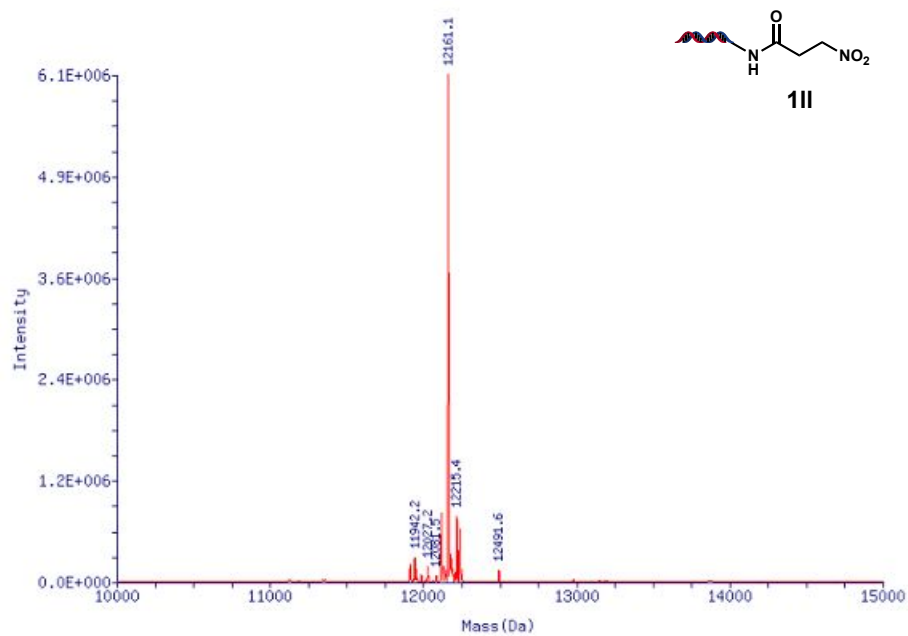


Figure S78. Deconvoluted mass spectrum of compound **1II**, expected: 12161.1; observed 12161.1.

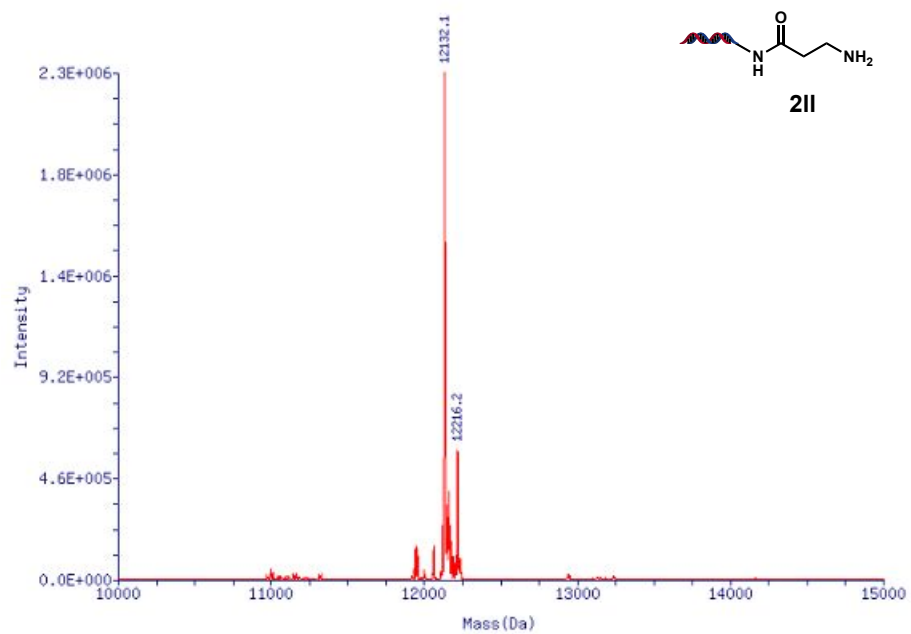
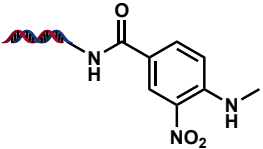
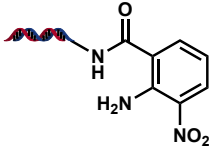
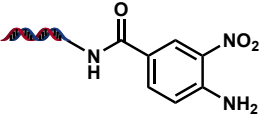
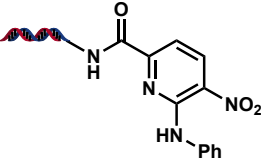
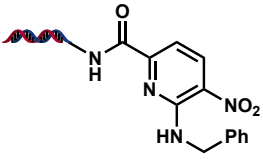


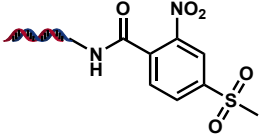
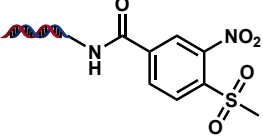
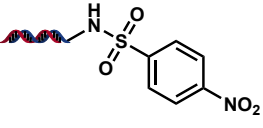
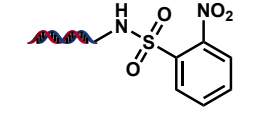
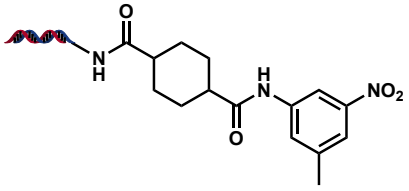
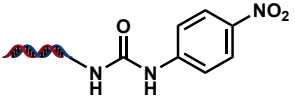
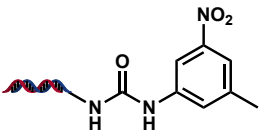
Figure S79. Deconvoluted mass spectrum of compound **2II**, expected: 12131.1; observed 12132.1.

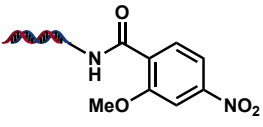
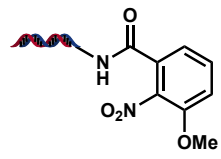
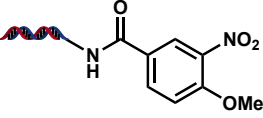
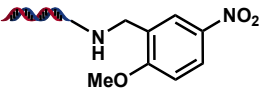
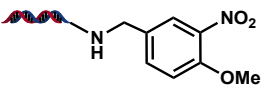
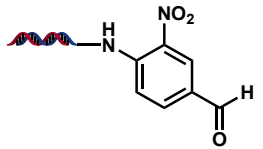
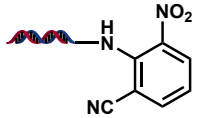
4. Preparation of Substrates

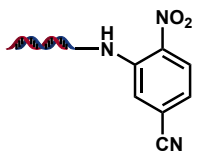
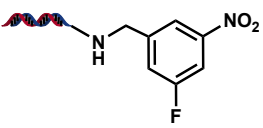
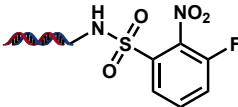
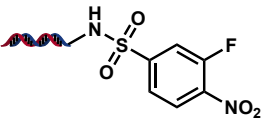
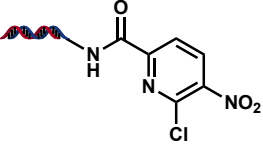
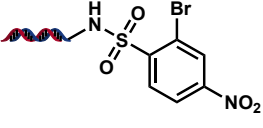
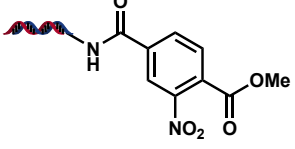
Test substrates were conjugated on DNA *via* acylation, sulfonylation, nucleophilic aromatic substitution, reductive alkylation, carbamoylation or carbamylation.

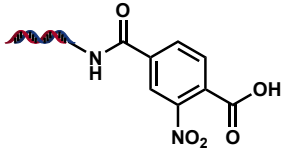
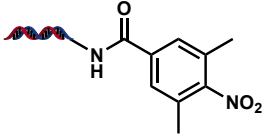
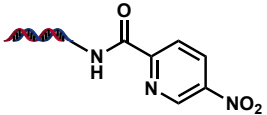
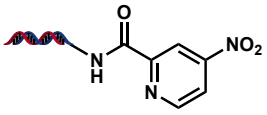
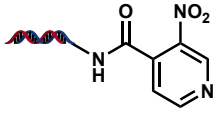
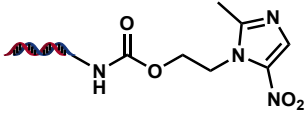
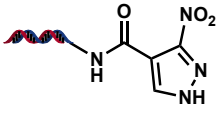
Table S1. Data for starting material preparation

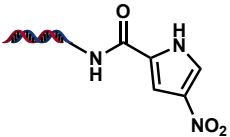
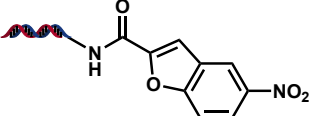
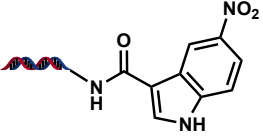
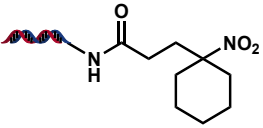
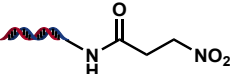
Products	Structure	Expected MW	Observed MW	Conversion
1a		12238.2	12238.7	91%
1b		12224.1	12224.7	91%
1c		12224.1	12224.2	83%
1d		12301.2	12301.6	73%
1e		12315.2	12314.5	84%

1f		12287.2	12288.1	63%
1g		12287.2	12288.2	> 95%
1h		12245.2	12245.7	> 95%
1i		12245.2	12245.9	> 95%
1j		12348.3	12349.0	88%
1k		12224.1	12225.3	> 95%
1l		12238.2	12239.3	48%

1m		12239.1	12240.0	> 95%
1n		12239.1	12239.9	> 95%
1o		12239.1	12239.9	> 95%
1p		12225.2	12225.7	83%
1q		12225.2	12225.7	45%
1r		12209.1	12210.1	88%
1s		12206.1	12206.9	93%

1t		12206.1	12206.7	92%
1u		12213.1	12213.5	77%
1v		12263.2	12263.6	> 95%
1w		12263.2	12264.2	> 95%
1x		12244.5	12245.6	77%
1y		12324.1	12324.7	> 95%
1z		12267.1	12267.1	85%

1aa		12253.1	12254.4	94%
1bb		12237.2	12237.8	> 95%
1cc		12210.1	12211.1	> 95%
1dd		12210.1	12211.2	> 95%
1ee		12210.1	12211.2	> 95%
1ff		12257.2	12258.4	87%
1gg		12199.1	12199.5	88%

1hh		12198.1	12199.0	92%
1ii		12249.1	12250.6	> 95%
1jj		12248.2	12249.3	> 95%
1kk		12243.2	12243.9	> 95%
1ll		12161.1	12161.1	74%

5. DNA stability and ligation test

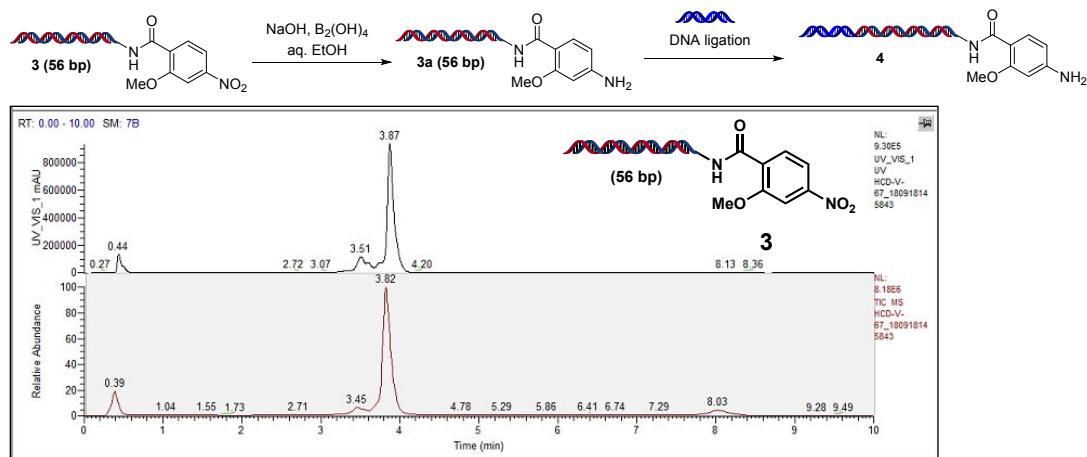


Figure S84. LC-MS spectrum of compound 3

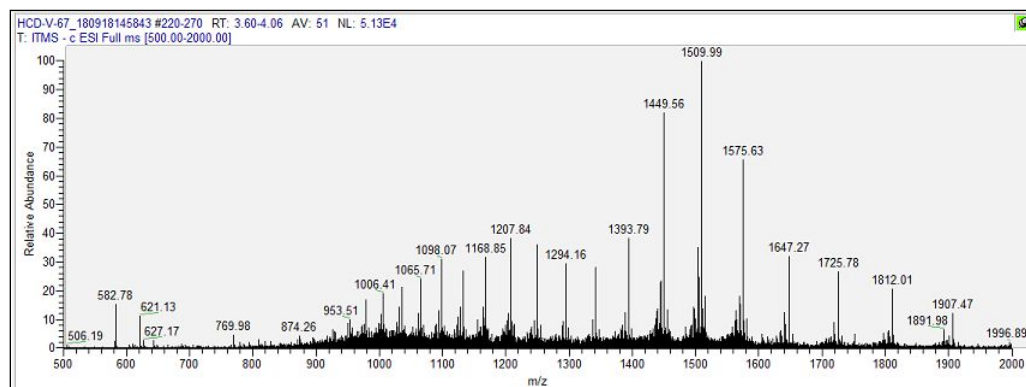


Figure S85. ESI Full MS spectrum of compound 3

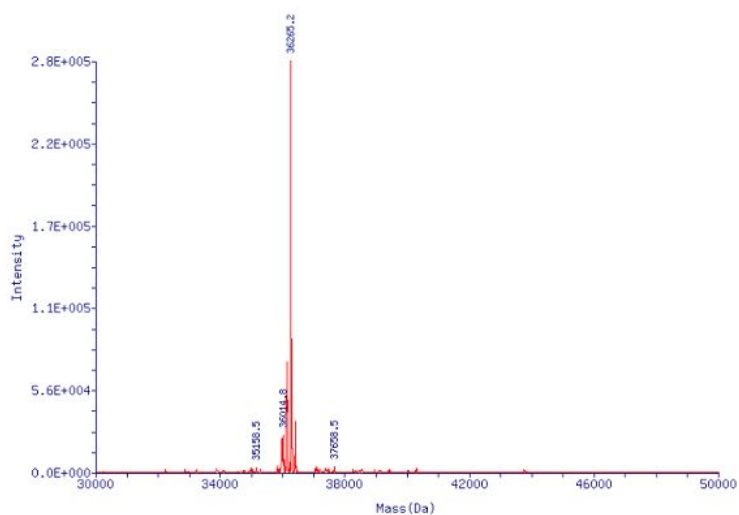


Figure S86. Deconvoluted mass spectrum of compound 3, expected: 36267.8; observed 36265.2.

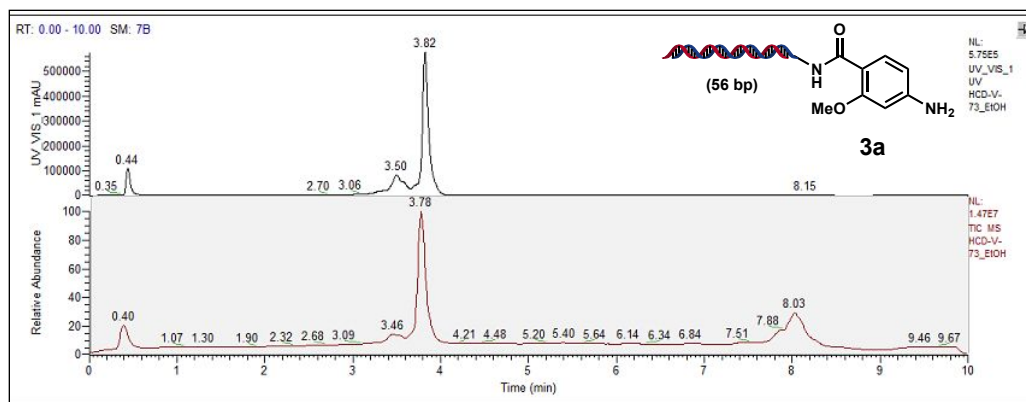


Figure S87. LC-MS spectrum of compound **3a**

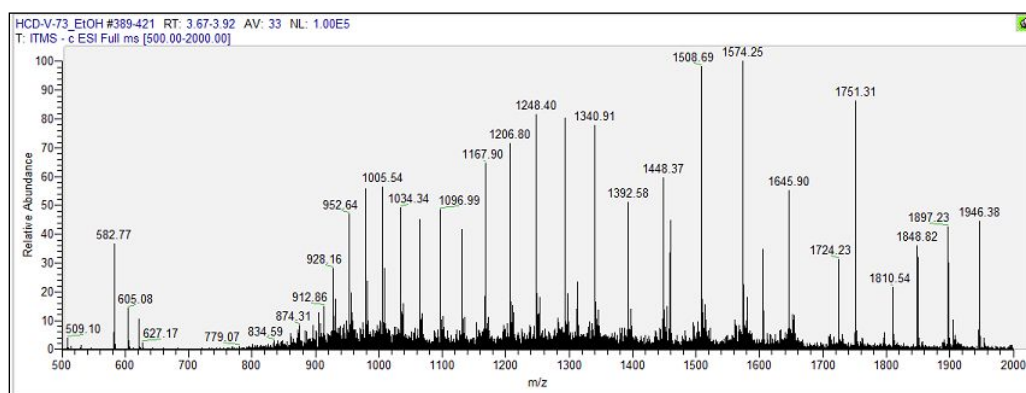


Figure S88. ESI Full MS spectrum of compound **3a**

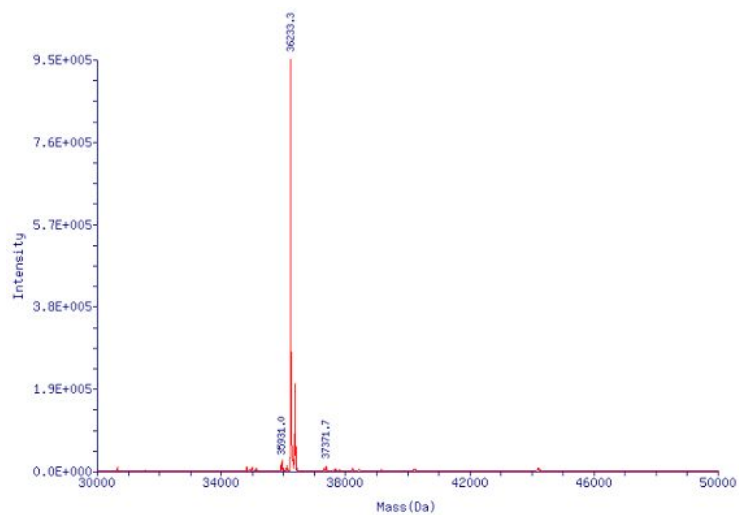


Figure S89. Deconvoluted mass spectrum of compound **3a**, expected: 36237.8; observed 36233.3.

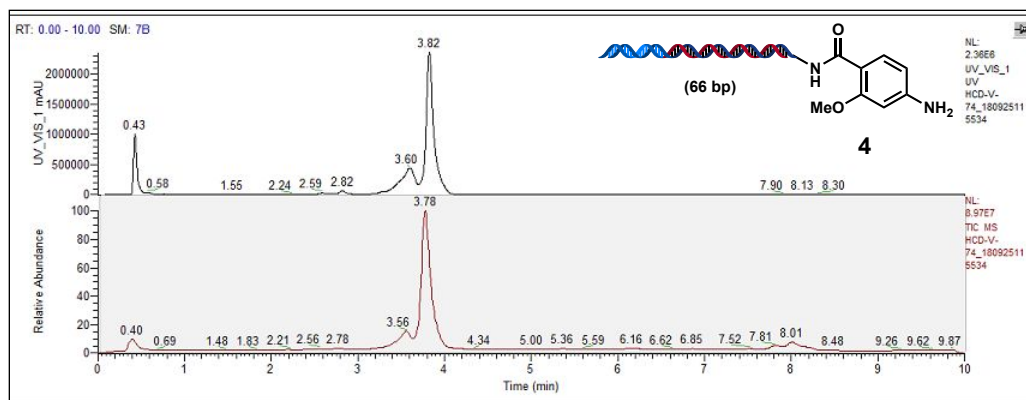


Figure S90. LC-MS spectrum of compound 4

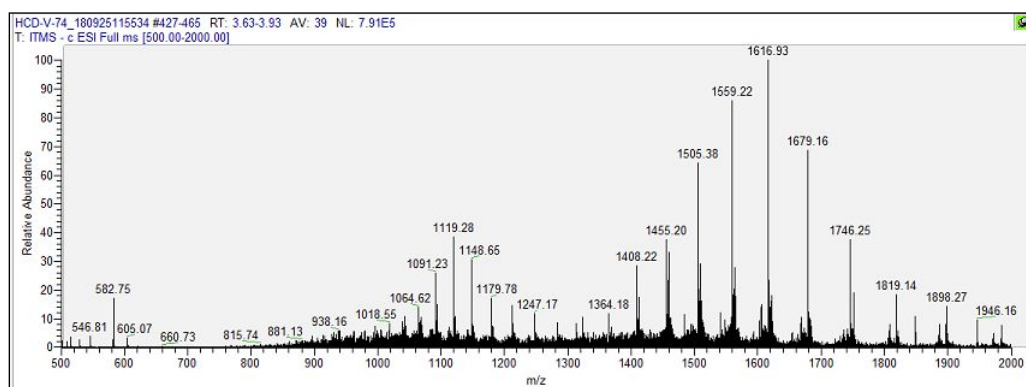


Figure S91. ESI Full MS spectrum of compound 4

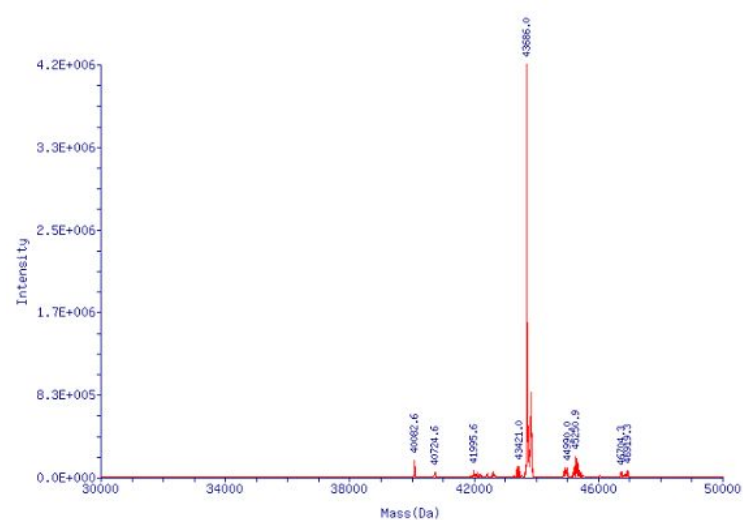


Figure S92. Deconvoluted mass spectrum of compound 4, expected: 43692.8; observed 43686.0.

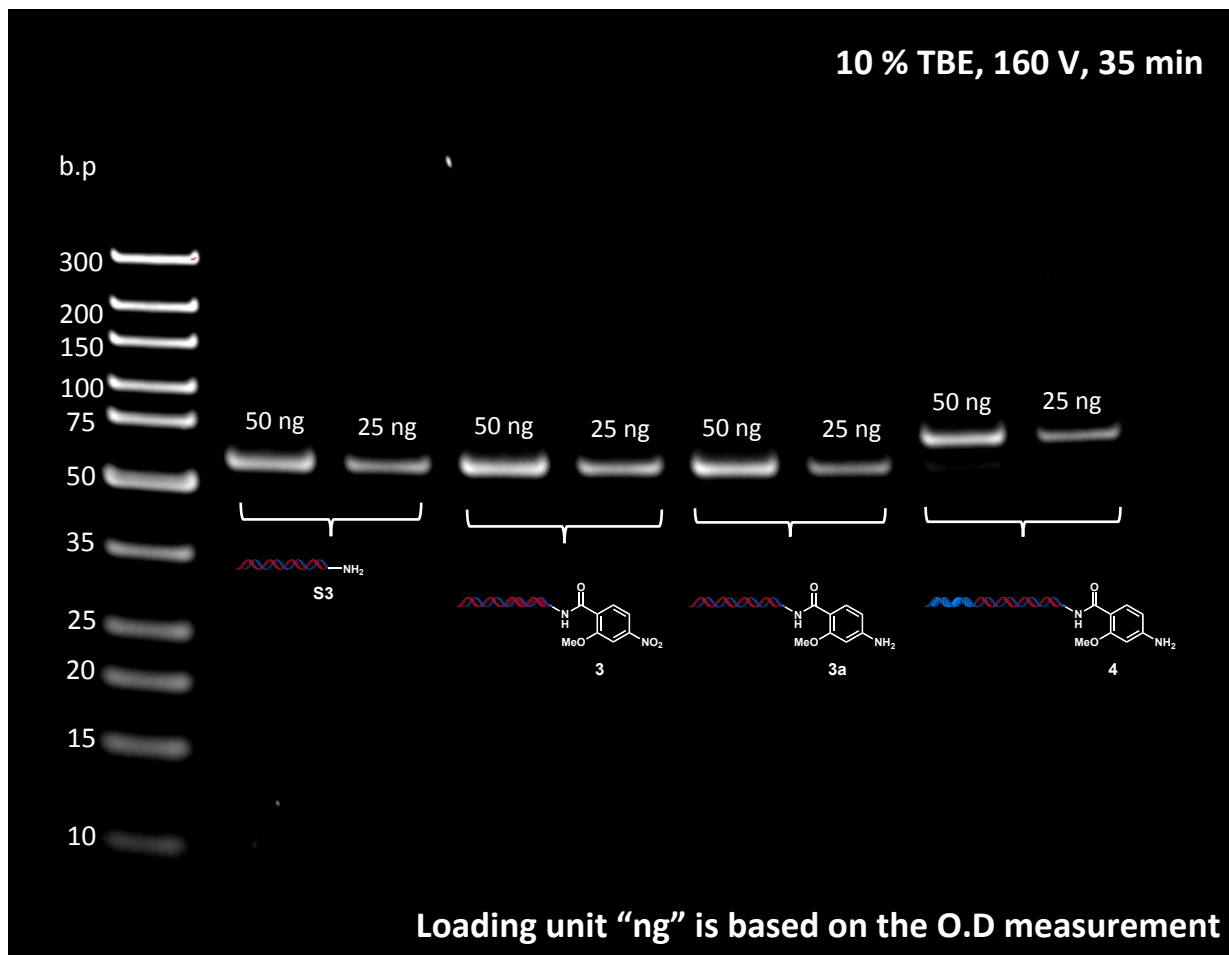
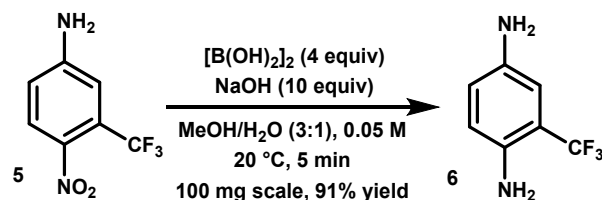
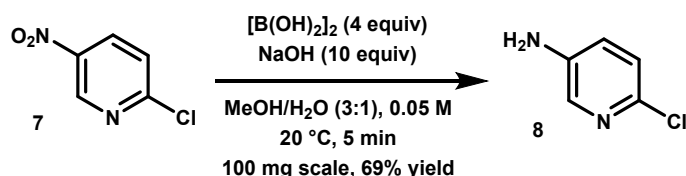


Figure S93. Gel electrophoresis image comparisons between starting material **S3**, compound **3**, reduction product **3a**, and ligation product **4**.

6. Benchtop test of nitro reduction



Aniline 6. To a flask charged with 4-nitro-3-trifluoromethylaniline (0.100 g, 0.485 mmol, 1 equiv) and hypodiboric acid (0.174 g, 1.94 mmol, 4 equiv), MeOH (7.28 mL) and H₂O (1.46 mL) were added and the flask was placed in a 20 °C water bath with vigorous stirring. Then an aq. soln. of NaOH (970 μ L, 5 M NaOH, 10 equiv) was added dropwise over 5 min, after which time the starting material was fully consumed. The reaction was poured into water (40 mL) and brine (10 mL) and extracted with CH₂Cl₂ (3 \times 10 mL) and EtOAc (3 \times 10 mL). The combined organics were dried (MgSO₄), filtered and concentrated. Purification of the crude by flash column chromatography (silica, 1:9 \rightarrow 3:7 EtOAc:hexanes) provided **6** (0.0775 g, 0.440 mmol, 91% yield). Characterization of **6** by ¹H NMR matched the previously reported spectra.³ ¹H NMR (600 MHz, CDCl₃) δ = 6.81 (d, J = 2.40 Hz, 1H), 6.72 (dd, J = 8.44, 2.40 Hz, 1H), 6.62 (d, J = 8.44 Hz, 1H), 3.71 (bs, 2H), 3.48 (bs, 2H) ppm; ¹H NMR (600 MHz, CD₃OD) δ = 6.86 (d, J = 2.63 Hz, 1H), 6.80 (dd, J = 8.62, 2.63 Hz, 1H), 6.72 (d, J = 8.62 Hz, 1H) ppm.



Aniline 8. To a flask charged with 2-chloro-5-nitropyridine (0.100 g, 0.633 mmol, 1 equiv) and hypodiboric acid (0.227 g, 2.532 mmol, 4 equiv), MeOH (9.50 mL) and H₂O (1.90 mL) were added and the flask was placed in a 20 °C water bath with vigorous stirring. Then an aq. soln. of NaOH (1.27 mL, 5 M NaOH, 10 equiv) was added dropwise over 5 min, after which time the starting material was fully consumed. The reaction was poured into water (40 mL) and brine (10 mL) and extracted with CH₂Cl₂ (3 \times 10 mL) and EtOAc (3 \times 10 mL). The combined organics were dried (MgSO₄), filtered and concentrated. Purification of the crude by flash column chromatography (silica, 1:9 \rightarrow 4:6 EtOAc:hexanes) provided **8** (0.0560 g, 0.435 mmol, 69% yield). Characterization of **8** by ¹H NMR matched the previously reported spectra.⁴ ¹H NMR (600 MHz, CDCl₃) δ = 7.85 (d, J = 3.00 Hz, 1H), 7.08 (d, J = 8.48 Hz, 1H), 6.96 (dd, J = 8.48, 3.0 Hz, 1H), 3.37 (bs, 2H) ppm; ¹H NMR (600 MHz, CD₃OD) δ = 7.73 (d, J = 2.84 Hz, 1H), 7.12–7.06 (m, 2H) ppm.

Figure S94. ^1H NMR spectrum (CDCl_3 , 600 MHz) of compound **6**

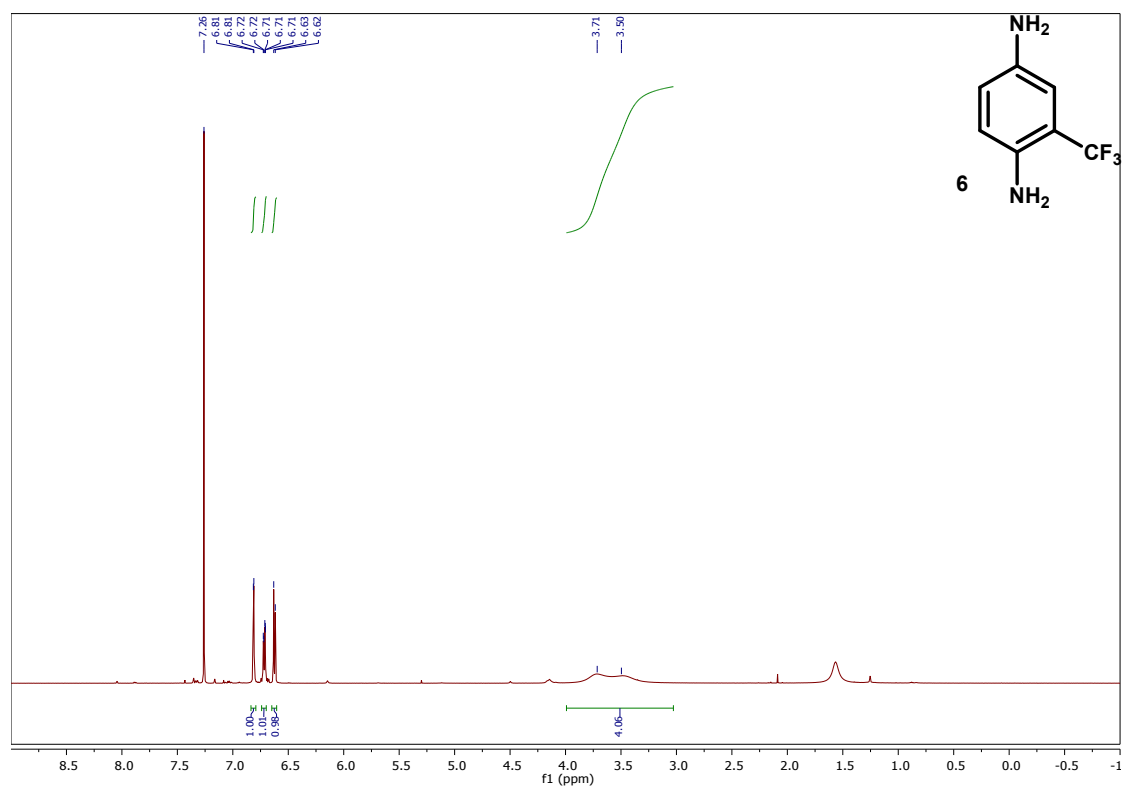


Figure S95. ^1H NMR spectrum (CD_3OD , 600 MHz) of compound **6**

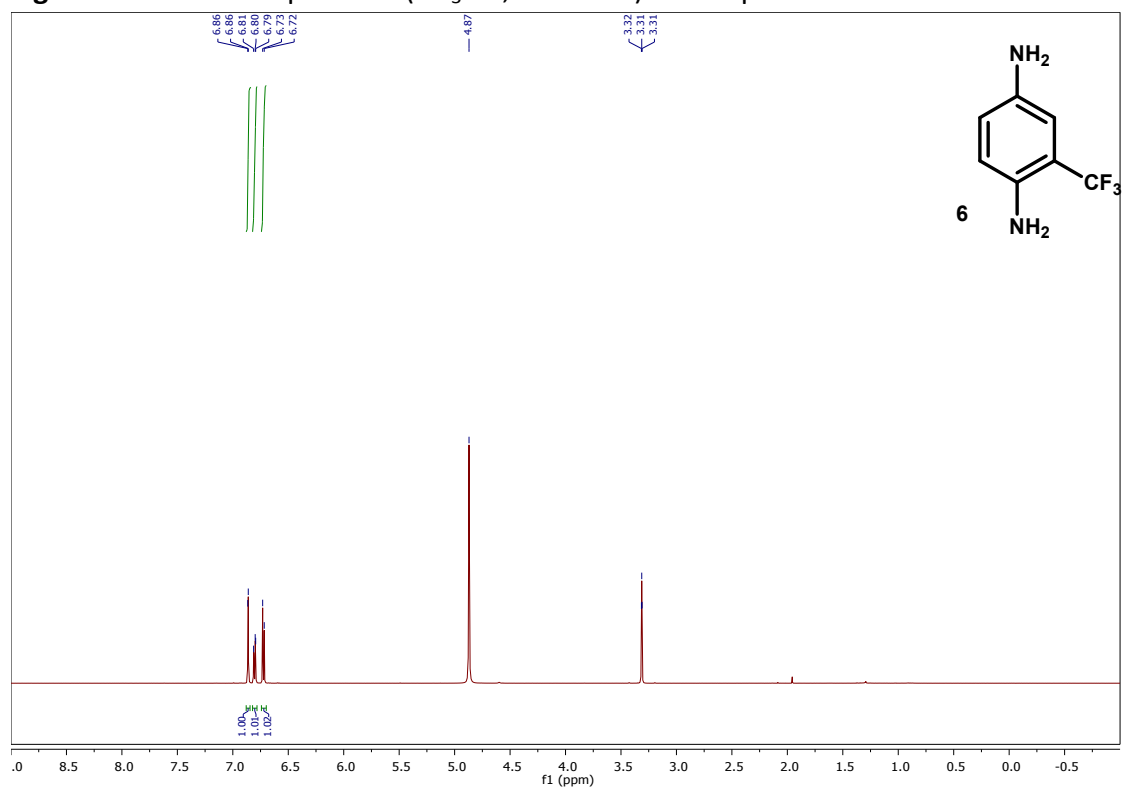


Figure S96. ^1H NMR spectrum (CDCl_3 , 600 MHz) of compound **8**

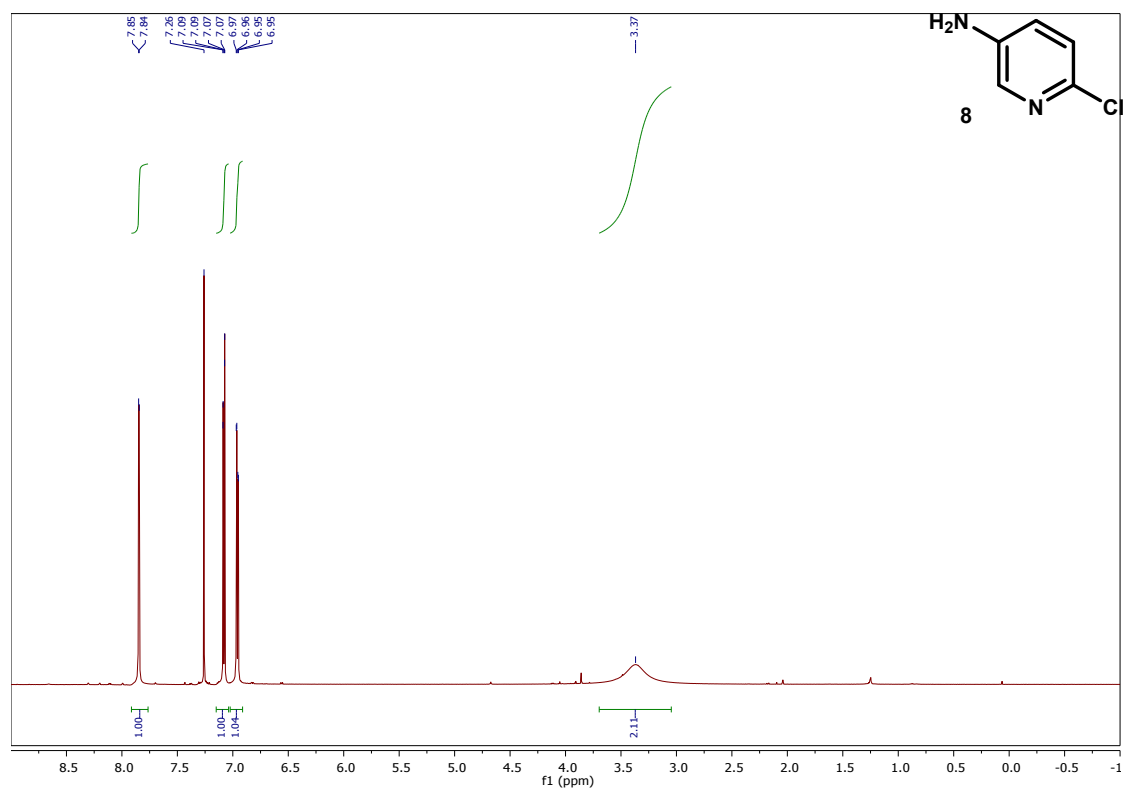
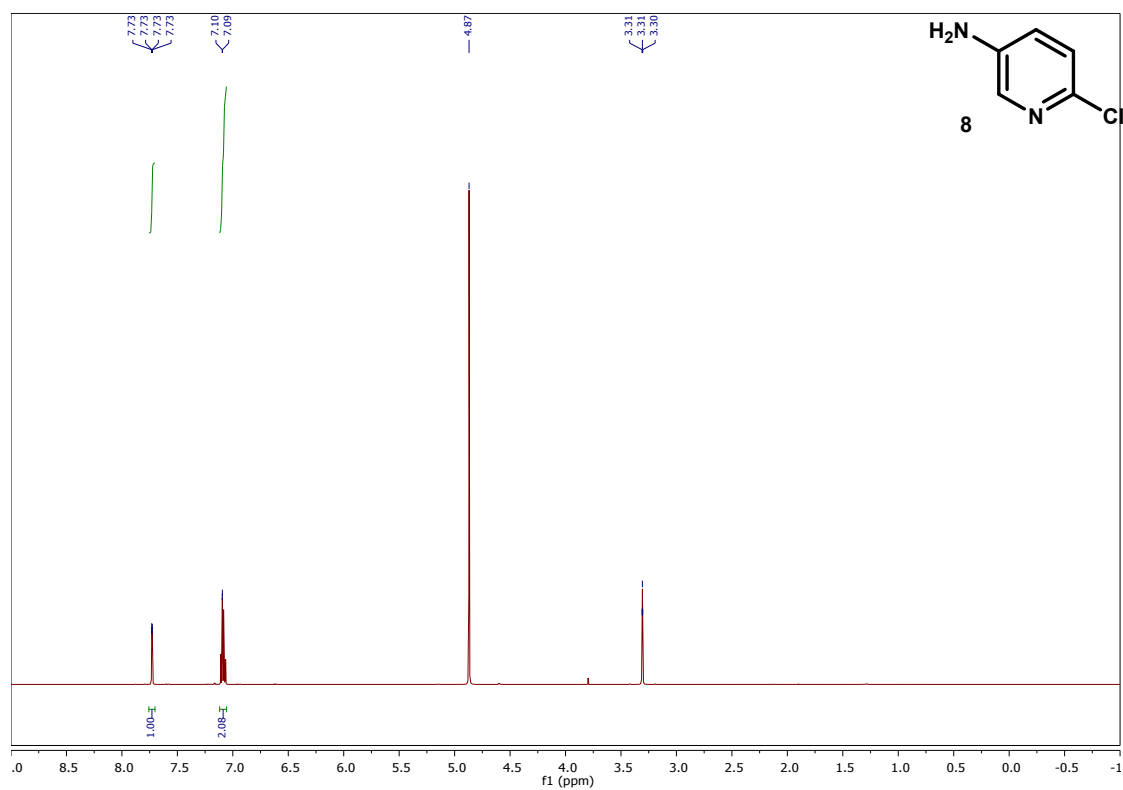


Figure S97. ^1H NMR spectrum (CD_3OD , 600 MHz) of compound **8**



7. Synthesis of a DNA-Encoded Chemical Library using Nitro reduction

7a. Architecture of the Main Library build. The DECL build described is a three-cycle library, produced through three iterative cycles each containing a chemical reaction phase, an encoding DNA oligonucleotide (codon) ligation phase with a final pooling phase. The library is constructed on “HP” **S2** (shown here as combination of DTSU, first overhang, forward primer unit, and second overhang) which had been further diversified on the “small molecule end” with a series of different amino- or carboxy-terminating linkers. Overhangs between codons are two base pairs and encoding regions within codons are eleven base pairs. Specific details and principles related to the overall oligonucleotide sequence design utilized in our DECL production pipeline have been discussed previously.^{1c}

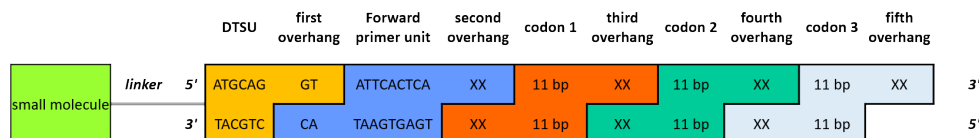


Figure S98. The structure of a completed Main Library Build. Separately assembled/ligated oligonucleotides (codons) are shown in alternate colors.

7b. General procedures utilized in the DECL build. The previously listed general information and procedures for materials, oligonucleotide analysis, ethanol precipitation, and ligation were utilized in the build of this library (see SI, sections 1a–1d). In addition, cycle 2 and cycle 3 reactions were monitored by use of a cholesterol-tagged DNA oligomer (“spike in”) with very different LC-MS retention to assess post-pool reaction completion. Other general procedures related to chemical transformations were performed:

“Reverse” Acylation of amines with on-DNA carboxylic acids: To carboxylic acid-terminated DNA (35 nmol, 32 μ L, 1 equiv, soln in H_2O) in pH 5.8 MES buffer (17500 nmol, 35 μ L, 500 equiv), additional water (29.25 μ L) and acetonitrile (43.75 μ L) were added, followed by an amino-building block (3500 nmol, 17.5 μ L, 100 equiv, 200 mM in CH_3CN) and DMTMM (3500 nmol, 17.5 μ L, 100 equiv, 200 mM in H_2O) and the solution was allowed to incubate overnight at room temperature. The reactions were then assessed for completion and/or precipitated by the general procedures.

Reductive amination of aldehydes with on-DNA amines with $NaBH_4$: To amino-terminated DNA (47.6 nmol, 50 μ L, 1 equiv, soln in H_2O) in pH 9.5 borate buffer (23810 nmol, 95 μ L, 500 equiv), additional acetonitrile (20 μ L) and an aldehyde building block (4762 nmol, 23.8 μ L, 100 equiv, 200 mM in CH_3CN) were added and the solution was allowed to sit at room temperature for 1 h. $NaBH_4$ (4762 nmol, 47.6 μ L, 100 equiv, 100 mM in CH_3CN) was added and the solution was incubated at room temperature overnight. The reactions were then assessed for completion and/or precipitated by the general procedures.

Nucleophilic aromatic substitution with DABCO: To amino-terminated DNA (47.72 nmol, 50 μ L, 1 equiv, soln in H_2O), NaOH (47620 nmol, 9.52 μ L, 1000 equiv, 5M in H_2O), and additional water (100 μ L) were added. Then an electrophilic dihaloarene (14286 nmol, 71 μ L, 300 equiv, 200 mM in CH_3CN) and DABCO (476 nmol, 4.76 μ L, 10 equiv, 100 mM in CH_3CN) were added and the solution was incubated at room temperature overnight. The reactions were then assessed for completion and/or precipitated by the general procedures.

Suzuki cross coupling: To halide containing DNA (10 nmol, 7.41 μ L, 1 equiv, soln in water), H₂O (2.59 μ L), CsOH (5000 nmol, 5 μ L, 500 equiv, 1M in H₂O) and 1-methoxy-2-propanol (10 μ L) were added. Then a boronic acid (2000 nmol, 10 μ L, 200 equiv, 200 mM in 1:1 dioxane:H₂O) and ssPhosG2 (50 nmol, 5 μ L, 5 equiv, 10 mM in 1-methoxy-2-propanol) were added and the solution carefully mixed by pipetting. The solution was heated to 95 °C for 25 min, and then cooled to room temperature. Cysteine (sodium salt, 1000 nmol, 5 μ L, 100 equiv, 200 mM in H₂O) was added and the solution was heated to 80 °C for 20 min. The reactions were then assessed for completion and/or precipitated by the general procedures.

Buchwald–Hartwig cross coupling: To halide containing DNA (10 nmol, 7.41 μ L, 1 equiv, soln in H₂O), H₂O (7.59 μ L), CsOH (5000 nmol, 5 μ L, 500 equiv, 1M in H₂O) and 1-methoxy-2-propanol (10 μ L) were added. Then an aniline (2000 nmol, 5 μ L, 200 equiv, 400 mM in 1-methoxy-2-propanol) and BrettPhosG3 (50 nmol, 5 μ L, 5 equiv, 10 mM in 1-methoxy-2-propanol) were added. The solution was then carefully pipette mixed, and heated to 95 °C for 25 min. After cooling, cysteine (sodium salt, 1000 nmol, 5 μ L, 100 equiv, 200 mM in H₂O) was added and the solution was heated to 80 °C for 20 min. The reactions were then assessed for completion and/or precipitated by the general procedures.

7c. Synthetic sequence of the library build.

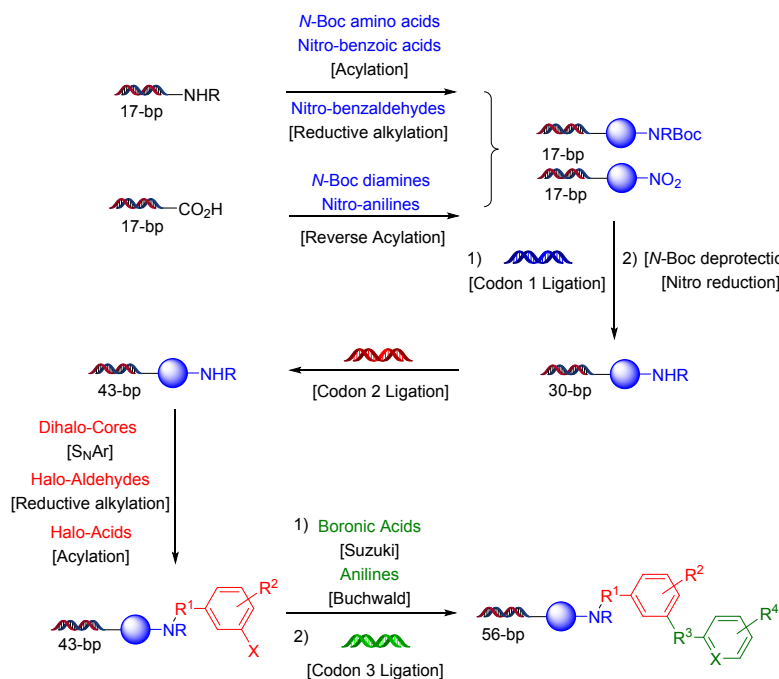


Figure S99. Synthetic sequence of the Main Build. Cycle 1 is shown in blue, cycle 2 is shown in red and cycle 3 is shown in green.

Procedure for Cycle 1. Several linker-functionalized **S2** (35 nmol/well) were plated individually into wells in 96-well plates. N-Boc diamines (183) and nitro anilines (23) were attached by “reverse” acylation onto two different carboxylic acid terminated DNA substrates in separate wells. N-Boc amino acids (57) and nitro benzoic acids (23) were acylated onto four different amine terminated DNA substrates in separate wells. Nitro benzaldehydes (14) were attached by reductive amination to four different amino terminated DNA substrates in separate wells. In addition, blank wells to encode the addition of no-building block or reagents were included. After precipitation, each chemical transformation was encoded by the ligation of a pair of 13-mer duplexed DNA oligonucleotides

(codon 1). Finally, the *N*-Boc carbamates and nitroarenes were deprotected in plate format using the general procedures for *N*-Boc deprotection or nitro reduction respectively, and each well was carefully analyzed by LC-MS. After pooling and additional ethanol precipitation, ~24 μmol of the cycle 1 library pool was recovered.

Procedure for Cycle 2. After splitting a portion of the cycle 1 pool into 336 wells (47.6 nmol/well), each well underwent a ligation with codon 2 by the general procedure. Then in individual wells, a series of electrophilic dihaloarenes (33) were attached through nucleophilic substitution using both the pH 9.5 heating and DABCO methods (encoded separately), a series of carboxyl aryl halides (99) were acylated using both the DEPBT and DMTMM methods (encoded separately), and a series of aldehyde aryl halides (33) were attached by reductive amination using both the NaCNBH_3 and NaBH_4 methods (encoded separately). In addition, blanks to encode the no reaction or reagent related side products were included. After pooling and additional precipitation, ~14.6 μmol of the Cycle 2 pool was recovered.

Procedure for Cycle 3. After splitting a portion of the cycle 2 pool into 606 wells (10 nmol/well), a series of boronic acids (378) were attached by Suzuki cross coupling and a series of anilines (222) were attached by Buchwald–Hartwig cross coupling. In addition, several additional wells to serve as no-building block or reagents only controls were included. After precipitation, each well was ligated with codon 3 to encode the chemical transformation. After pooling and additional precipitation, ~5.8 μmol of the Cycle 3 pool was recovered.

Preparation of amplifiable DECL samples (“shots”) for selection experiments. After completion of the main library builds, the entire library material was ligated with a duplexed pair of 12-bp oligonucleotides to encode the library structure/design. After precipitation, small portions of this material were further ligated with two oligonucleotides containing a region to encode experimental usage, a degenerate region as an amplification control, a segment to increase sequencing base diversity, and a reverse primer region to enable post-selection PCR amplification (the purposes/design of these components are discussed elsewhere).^{1c}

8. DECL sequencing and selection experiments

Naïve Amplification:

An amplifiable DECL shot was quantified by quantitative Real-Time PCR (qPCR) and then a total of 3×10^7 DNA copies were amplified by PCR with primers adaptors to add the sequences compatible with Illumina sequencing flowcells. Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific) PCR reagent was used for PCR amplification. A total of 13 PCR cycles were used for amplification and the following PCR conditions were used (Initial denaturation at 95 °C for 2.5 min, denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min). PCR library template was purified using Agencount AMPure XP SPRI beads according to the manufacturer’s instructions. The purified library was analyzed in Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) by using Agilent high sensitivity DNA kit to verify library size and concentration before clustering. Illumina NextSeq 500 was used for sequencing.

Selection against PLK1:

DEC-Tec selection was performed by incubating histidine-tagged human PLK1 full-length (Thermo Fisher Scientific, PR5304B) at 0.5 μM with the DNA encoded library where 1 million copies of each compound were present in a selection buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MgCl_2 , 2.5 mM DTT, 0.01 % Triton X-100, 10 mM imidazole, and 0.1 mg/ml sheared salmon sperm DNA (Invitrogen, 15632011). The incubation was allowed for 45 minutes at room temperature with continuous shaking. The non-targeting control (NTC) in the absence of protein was performed in parallel as a reference. Activated Ni-NTA magnetic beads (Thermo Fisher Scientific,

88831) were added to the incubation to instantaneously pull down the protein. Beads were washed 1 time with the aforementioned selection buffer without salmon sperm DNA by brief and vigorous vortex. The binding molecules were then eluted by heating the beads at 80 °C for 10 min in washing buffer. The resulting eluent was quantitated using qPCR and determined whether another round selection was needed. Totally 3 rounds of selection were performed for PLK1, and the oligonucleotides in the final eluent were PCR amplified for 18 cycles (NTC) and 17 cycles (PLK1) using Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, 10966026) with denaturation at 95 °C, annealing at 58 °C, and extension at 72 °C using primers that incorporate complementary sequences to the library headpiece or tailpiece along with the Illumina READ 1 or READ 2 sequences required for Illumina clustering and sequencing. The amplified DNA was cleaned by Agencourt AMPure XP beads and quantitated with Agilent high sensitivity DNA kit using a Bioanalyzer before the sequencing.

Sequencing analysis:

Raw DNA sequence reads (in the form of FASTQ files), quality metrics, and sequencing index-to-sample attribute value pairs were obtained from Illumina BaseSpace at the conclusion of sequencing. Samples were linked to their respective FASTQ files based on their sequencing index (DTSU) and were expanded into individual experiments if they were part of a larger pool. Individual samples were then decoded by perfectly matching individual oligonucleotide sub-structures without gaps and in the order defined by the known DNA encoding structure (Main Library Build). Valid DNA barcodes were annotated with the corresponding oligonucleotide sequence-to-building block lookup for each of the three codon cycles, which collectively represent a distinct small molecule within a specific DECL. The degenerate UMI (unique molecular identifier) portions of the DNA barcodes were accumulated into a list of UMIs for each unique codon tuple as a method to distinguish experimental vs. amplification events. Unique molecule counts were then evaluated using a directed-graph counting model as described previously.^{1c} The set of unique codon tuples with unique molecule counts was then aggregated across all possible combinations of codons (all *n*-synthons), and enrichment for each *n*-synthon was evaluated independently. Enrichment was evaluated with a normalized *z*-score metric which normalizes for sampling and library diversity.^{1c}

The sequencing of the naïve DECL resulted in 18,568,815 complete barcodes read and 17,166,316 unique molecules sampled. This corresponded to a sampling factor of 21674.6 for the cycle 1 codons of the DECL, enabling accurate statistics for the cycle 1 codon distribution of the library. The codon distribution was evaluated by normalizing observed c1 codon counts by the average cycle 1 codon count, and we report codon populations as percentage of the mean count.

The PLK1-selected library as well as a Non-Target Control (NTC) library sample were amplified, sequenced, and analyzed for enrichment of each *n*-synthon in the DECL. The enrichment of each *n*-synthon was measured using a normalized *z*-score metric^{1c} and the Agresti-Coull estimation interval for proportions.⁵ The resulting enrichment values (labeled as "AC_zscore_n") were then compared by plotting the enrichment in the target sample against enrichment in the NTC sample (**Figure S100**). DECL members with significant binding affinity for the target are detected by observing their component *n*-synthons as enriched on the Target axis but not the NTC axis. Figure S100 highlights *n*-synthon components of the PLK1 hit series described in the text. Two di-synthons related to the hit series are shown in blue, which correspond to two different syntheses of the cycle 1 component: N-Boc deprotection and Nitro reduction. Similarly, two tri-synthons in yellow and two mono-synthons in green correspond to components of the hit series arising from the two synthetic routes. Importantly, the measured enrichment of each pair of displayed *n*-synthons was comparable for the two synthetic routes in cycle 1.

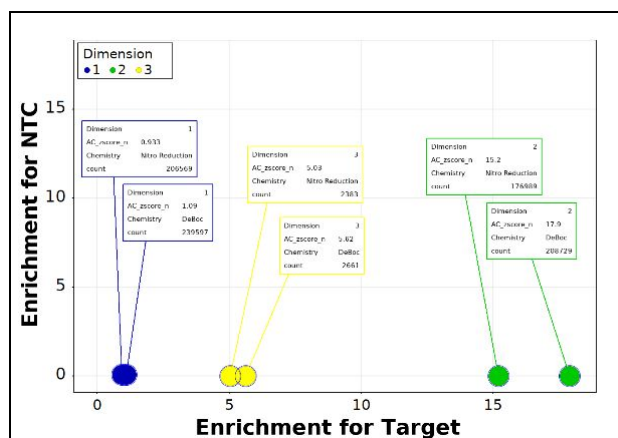


Figure S100. Analysis of enrichment of overlapping cycle 1 building blocks in the selection of kinase PLK1.

9. References

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