# Supporting Information

## Multistep Synthesis of 1,2,4-Oxadiazoles *via* DNA-Conjugated Aryl Nitrile Substrates

Huang-Chi Du*, Madison Bangs, Nicholas Simmons, and Martin Matzuk

*Center for Drug Discovery, Department of Pathology and Immunology*

*Baylor College of Medicine, Houston, TX 77030*

*to whom correspondence should be addressed:

huang-chi.du@bcm.edu

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. Generation of 3, 5-disubstituted 1,2,4-oxadiazoles
- 2a) General procedure for amidoxime formation ..... 4
- 2b) General procedure for *O*-acylamidoxime ..... 5
- 2c) General procedure for cyclodehydration of the *O*-acylamidoxime ..... 5

3. Deconvoluted mass spectra ..... 6
4. Preparation of substrates ..... 67
5. DNA stability and ligation test ..... 68
6. References ..... 74

# 1. General Information

In this study, general materials, equipment and procedures are adapted from previous reports[1-4] by our groups and other DNA-encoded library publications.[5-6]

**1a. Materials and equipment used for the synthesis and analysis of oligonucleotides and DNA-encoded chemical libraries.** The DEC-Tec starting unit (DTSU) DNA oligonucleotide (**S1**, Figure S1) and 5'-phosphorylated oligonucleotides were purchased from LGC Biosearch Technologies. The purities of all DNA oligonucleotides were assessed through the general analytical procedure. High-concentration T4 DNA ligase was obtained from Enzymatics (Qiagen) and its activity was determined through test DNA oligomer ligations on DTSU. All reagents were purchased from various vendors and used without further purification. Generally reagents were dissolved in acetonitrile or mixed aqueous acetonitrile solutions. All buffers and ionic solutions, including HEPES 10X ligation buffer, aq. NaCl (5M), phosphate buffer (pH 8.0), and basic borate buffer (pH 9.5 and pH 8.2), were freshly prepared in-house. The 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), and reactions were generally performed in polypropylene Eppendorf tubes (various brands). Heated reactions were performed in ep384 Mastercyclers (Eppendorf). Solutions were centrifuged in either Avanti J-30I or Allegra X-15R centrifuges (Beckman-Coulter). DNA solution concentration was measured using Biophotometer (Eppendorf). LC/MS analysis of oligonucleotides was accessed using a Vanquish UHPLC system integrated with LTQ XL ion trap mass spectrometer (ThermoFisher Scientific).

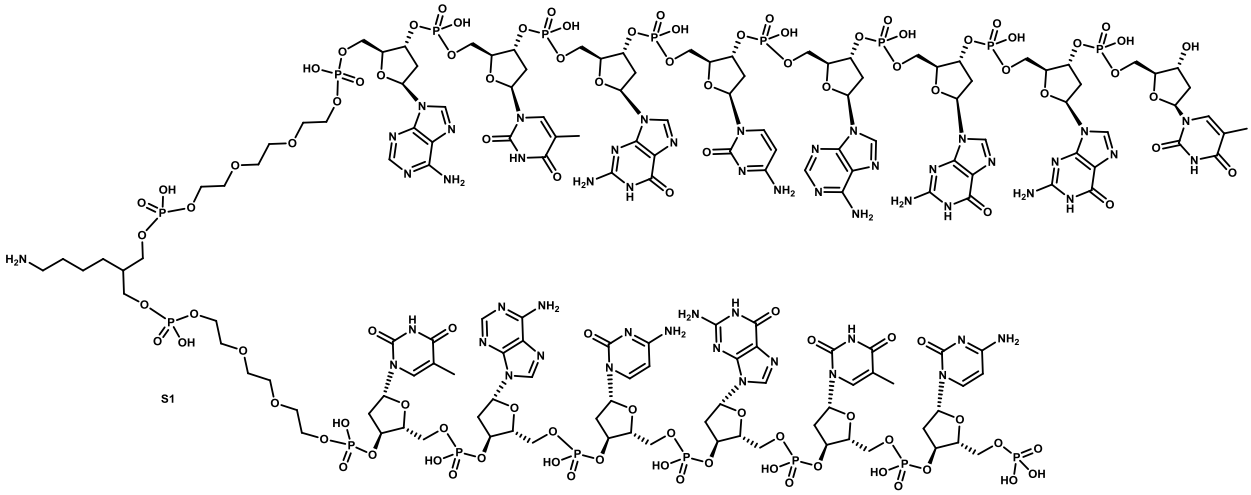



**Figure S1.** Structure of "DTSU" **S1** (5'-Phos-CTGCAT-Spacer 9-Amino C7-Spacer 9-ATGCAGGT 3').

**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, 4µm)
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
Solvent gradient: 0.0 min (98%A / 2%B), 0.8 min (100% B), 1.0 min (100%C), 1.15 min (98%A / 2%B)
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, 4µm) 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 and 70 % aq. ethanol then was added to the pellet before centrifuged again. The DNA pellet was dried in air or under gentle vacuum. Water was added to reconstitute the DNA to the concentration of 0.5–1 mM. Ethanol precipitation was generally performed after each chemical reaction.

**1d. Representative general procedure for DNA ligation.**
To DNA conjugate **6b** (1.5 nmol, 5 µL, 1.0 equiv) was added DNA_1 (5'-ACACTTGCTGGT-3', 1.95 nmol, 1.95 µL, 1.3 equiv), DNA_2 (5'-CAGCAAGTGTGA-3', 1.95 nmol, 1.95 µL, 1.3 equiv), and nuclease-free water (3.6 µL), followed by the addition of 10× 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. Gel electrophoresis was executed using precast 10% TBE acrylamide gel from Invitrogen (12 wells). The gel box was filled with 1× 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× 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 for substrate preparation.**

This elaborated DNA, "HP" **S2**, was prepared through ligation of two duplexed 11-mer oligonucleotides with DTSU **S1** 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, 56 b.p DNA oligonucleotide **5** for chemistry validation and ligation tests was prepared by ligating two 39-mer duplexed oligonucleotides with **S2**. (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 cyano carboxylic acid building block (1000 nmol, 5 µL, 200 mM in MeCN, 100 equiv), *N,N*-diisopropyl-ethylamine (DIPEA, 1000 nmol, 5 µL, 200 mM in MeCN, 100 equiv), and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU, 1000 nmol, 5 µL, 200 mM in MeCN, 100 equiv) were premixed for 10 min. The mixture was then added to a solution of DNA (10 nmol, 10 µL, 1.0 mM, 1 equiv) in $H_2O$ with pH 9.5 borate buffer (4000 nmol, 16 µL, 250 mM, 400 equiv), which was allowed to sit at room temperature for 2–4 h before being quenched by EtOH precipitation.

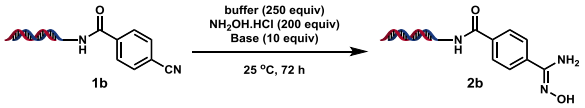
**Sulfonylation**: A cyano sulfonyl chloride building block (1000 nmol, 5 µL, 200 mM in MeCN, 100 equiv) was added to a solution of DNA (10 nmol, 10 µL, 1.0 mM, 1 equiv) in $H_2O$ with pH 9.5 borate buffer (4000 nmol, 16 µL, 250 mM, 400 equiv), which was allowed to sit at room temperature for 8 h before being quenched by EtOH precipitation.

**Nucleophilic Aromatic Substitution:** An electrophilic cyano aryl fluoride building block (1000 nmol, 5 µL, 200 mM in MeCN, 100 equiv) was added to a solution of DNA (10 nmol, 10 µL, 1.0 mM, 1 equiv) in $H_2O$ with pH 9.5 borate buffer (4000 nmol, 16 µL, 250 mM, 400 equiv) and the reaction was heated to 60 °C for 12 h before being quenched by EtOH precipitation.

## 2. Generation of 3, 5-disubstituted 1,2,4-oxadiazoles

**2a) General procedure for amidoxime formation** DNA-conjugated nitrile substrates were reconstituted to the concentration of 1.0 mM after attaching procedure. pH 8.2 borate buffer (2500 nmol, 10 µL, 250 mM, 250 equiv) was added to DNA conjugates (10 nmol, 10 µL, 1.0 mM in $H_2O$, 1 equiv) followed by the addition of $NH_2OH{\cdot}HCl$ (2000 nmol, 10 µL, 200 mM in $H_2O$, 200 equiv) and $Na_2CO_3$ (100 nmol, 0.5 µL, 200 mM in $H_2O$, 10 equiv). The reaction mixture was allowed to sit at room temperature for 48–72 h before being quenched by EtOH precipitation.

**Table S1**. Optimization of amidoxime formation.




[a]conversions determined by LC-MS. [b]Hydrolysis of nitrile to acid was observed.

| | Buffer | Base | 2b[a](%) |
|---|---|---|---|
| 1 | pH 9.5 borate[b] | $Cs_2CO_3$ | 91% |
| 2 | pH 9.5 borate[b] | NaOH | 93% |
| 3 | pH 8.2 borate | None | 87% |
| 4 | pH 8.2 borate | NaOAc | 87% |
| 5 | pH 8.2 borate | $Cs_2CO_3$ | 94% |
| 6 | pH 8.2 borate | $Na_2CO_3$ | 97% |
| 7 | pH 8.2 borate | DIPEA | 85% |
| 8 | pH 8.2 borate | TEA | 82% |
| 9 | pH 7.0 phosphate | $Na_2CO_3$ | 88% |
| 10 | pH 5.8 Mes | $Na_2CO_3$ | 67% |

**2b) General procedure for *O*-acylamidoxime.** To a solution of DNA-conjugate amidoxime (10 nmol, 10 µL, 1.0 mM, 1 equiv) in $H_2O$ was added pH 8.0 phosphate buffer (4000 nmol, 20 µL, 200 mM, 400 equiv), aromatic or aliphatic carboxylic acids (2000 nmol, 10 µL, 200 mM in $CH_3CN$, 200 equiv), and 7-Azabenzotriazol-1-yloxy) tripyrrolidino-phosphonium hexafluorophosphate (PyAOP, 2000 nmol, 10 µL, 200 mM in DMA, 200 equiv), which was incubated at room temperature for 16 h prior to EtOH precipitation.

**2c) General procedure for cyclodehydration of the *O*-acylamidoxime.** To a solution of DNA-conjugate *O*-acylamidoxime (5 nmol, 5 µL, 1.0 mM, 1 equiv) in $H_2O$ was added pH 9.5 borate buffer (2500 nmol, 10 µL, 250 mM, 500 equiv), *N,N*-diisopropylethylamine (DIPEA, 500 nmol, 2.5 µL, 200 mM in MeCN, 100 equiv), and $CH_3CN$ (10 µL, 40% v/v). The reaction was heated at 90 °C for 1.5 h before being quenched by EtOH precipitation.

## 3. Deconvoluted Mass Spectra of DNA-Conjugates

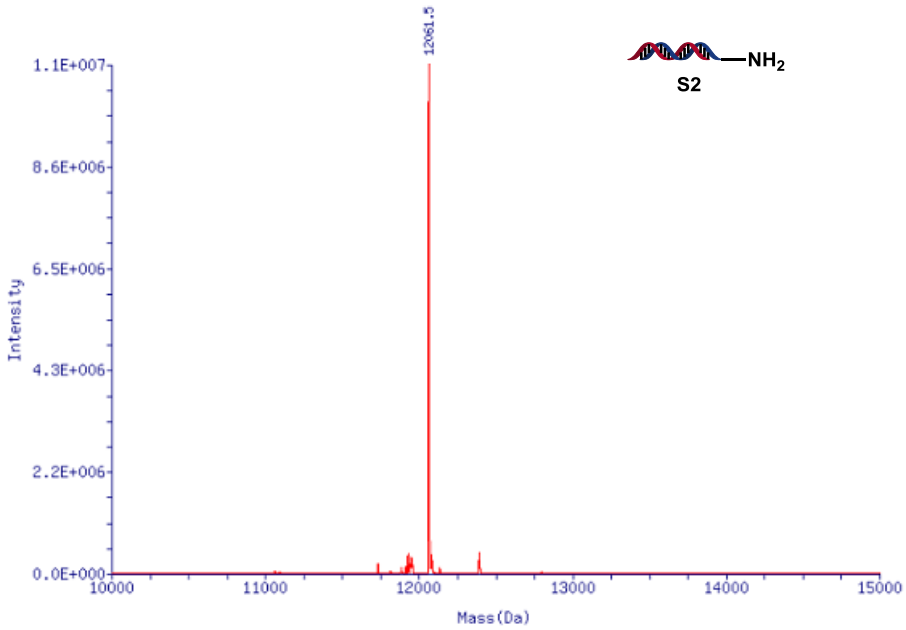



**Figure S2.** Deconvoluted mass spectrum of DNA **S2**, expected: 12060.0; observed 12061.5.

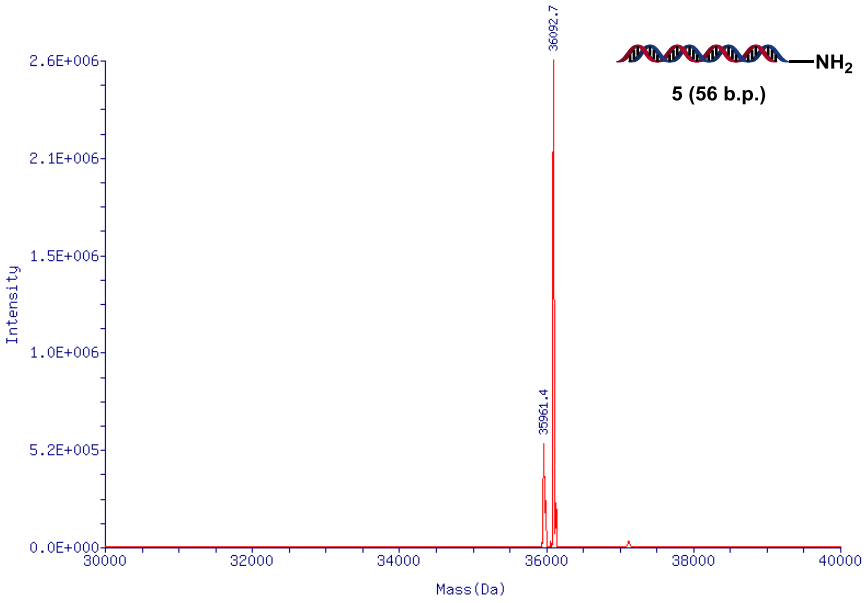



**Figure S3.** Deconvoluted mass spectrum of the 56 b.p. DNA **5**, expected: 36088.7; observed 36092.7.

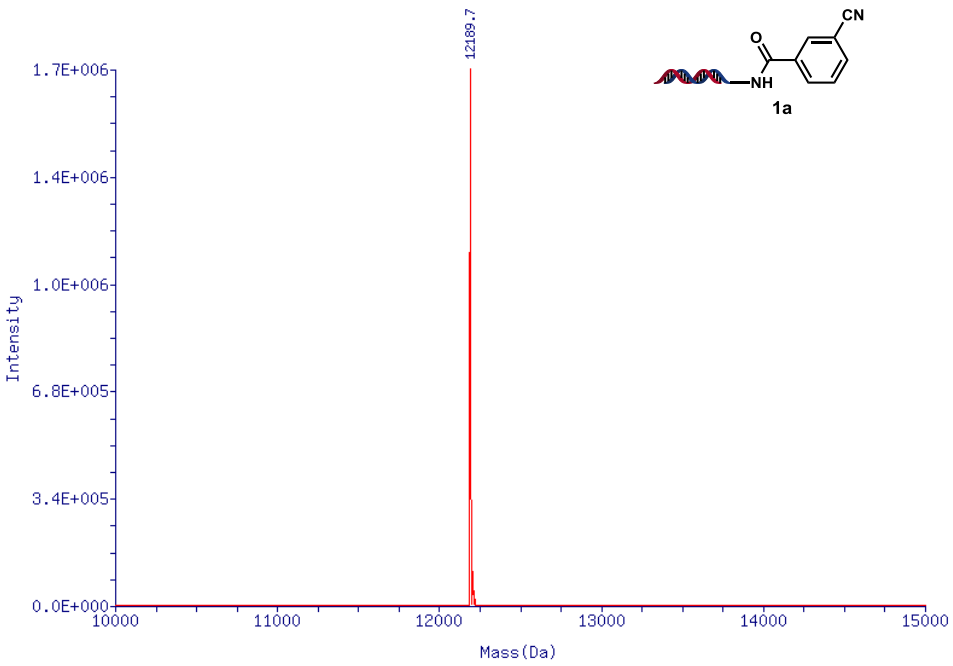



**Figure S4.** Deconvoluted mass spectrum of compound **1a**, expected: 12189.1; observed 12189.7.

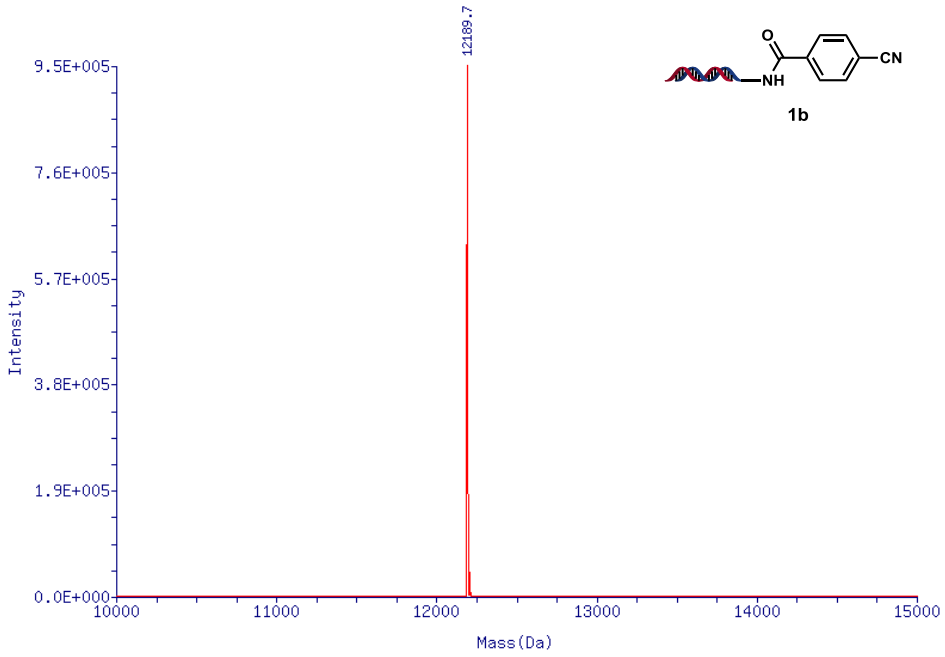



**Figure S5.** Deconvoluted mass spectrum of compound **1b**, expected: 12189.1; observed 12189.7.

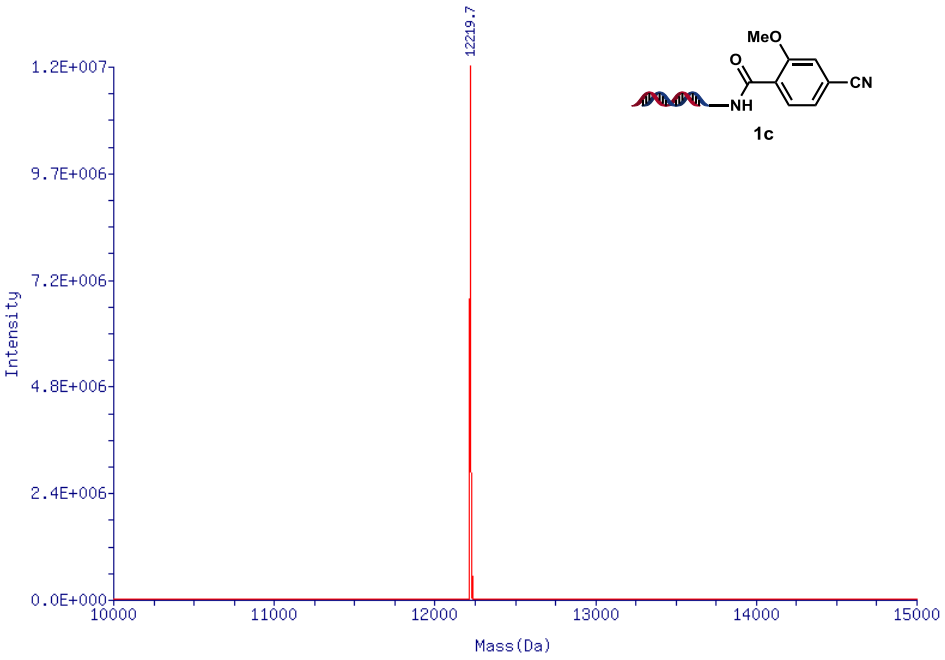



**Figure S6.** Deconvoluted mass spectrum of compound **1c**, expected: 12219.1; observed 12219.7.

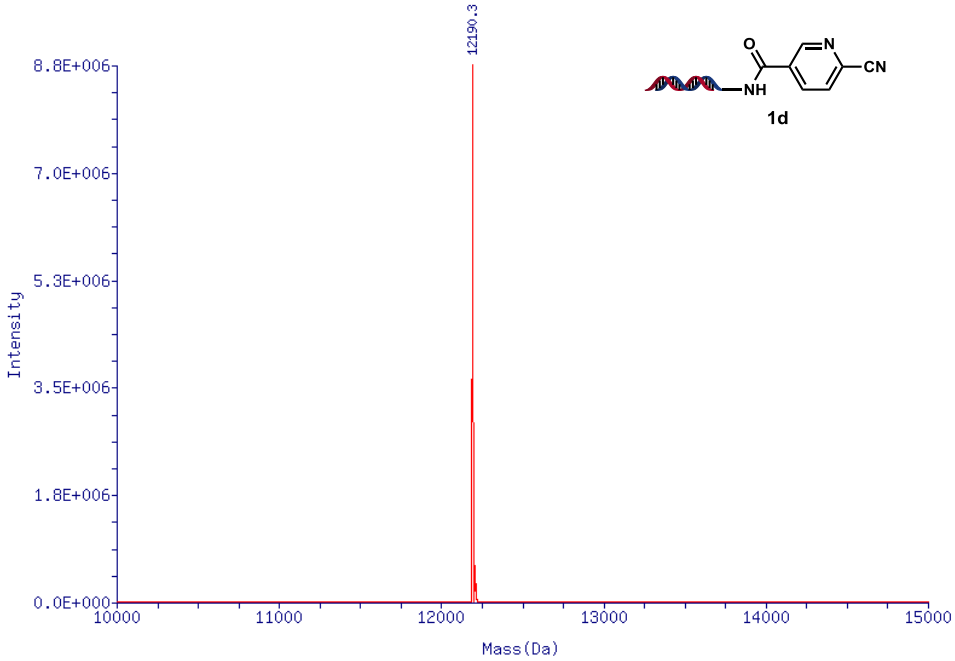



**Figure S7.** Deconvoluted mass spectrum of compound **1d**, expected: 12190.1; observed 12190.3.

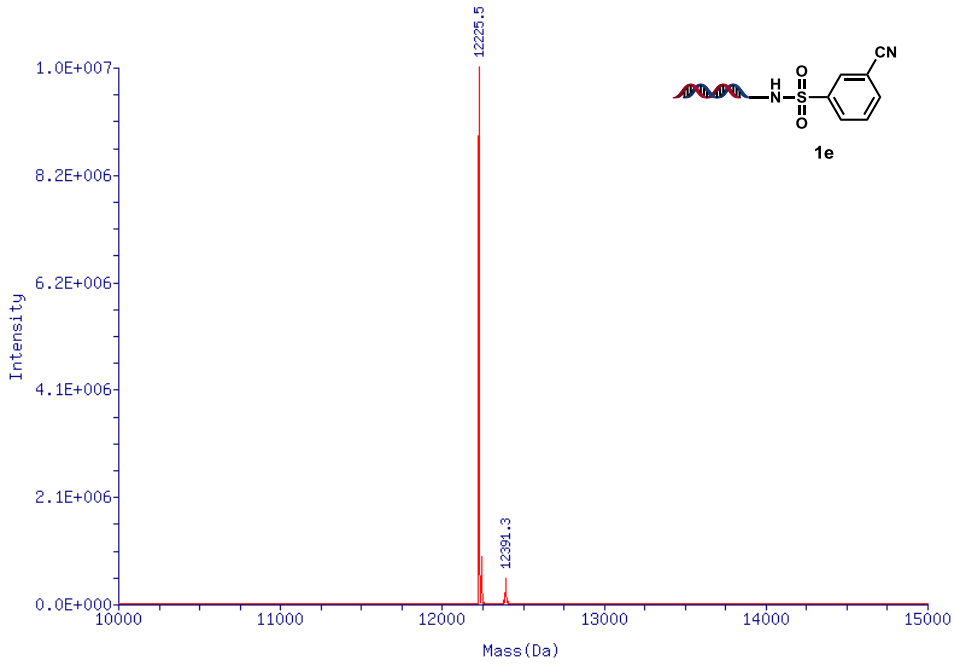



**Figure S8.** Deconvoluted mass spectrum of compound **1e**, expected: 12225.2; observed 12225.5.

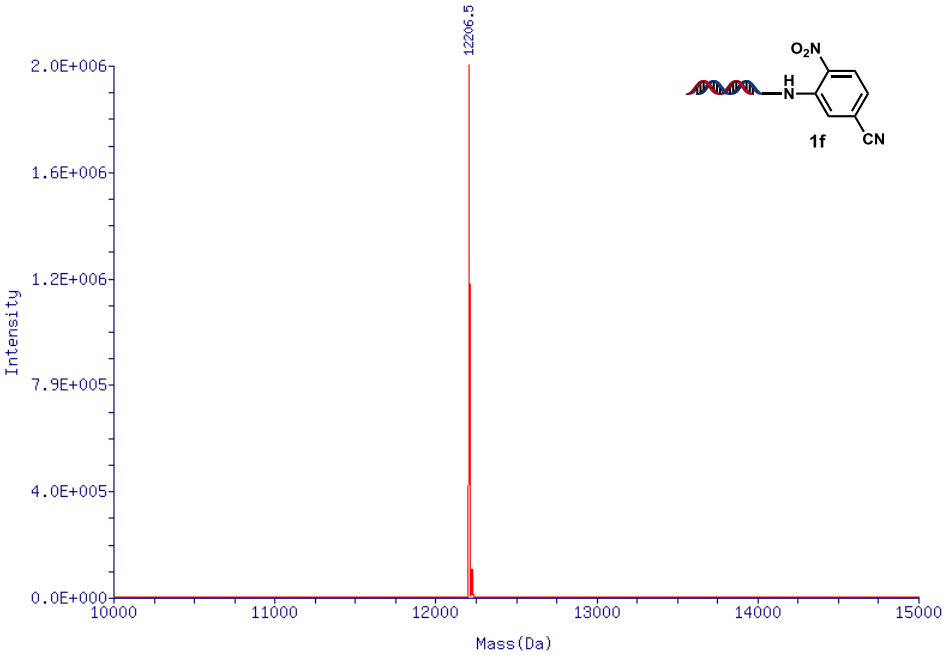



**Figure S9.** Deconvoluted mass spectrum of compound **1f**, expected: 12206.1; observed 12206.5.

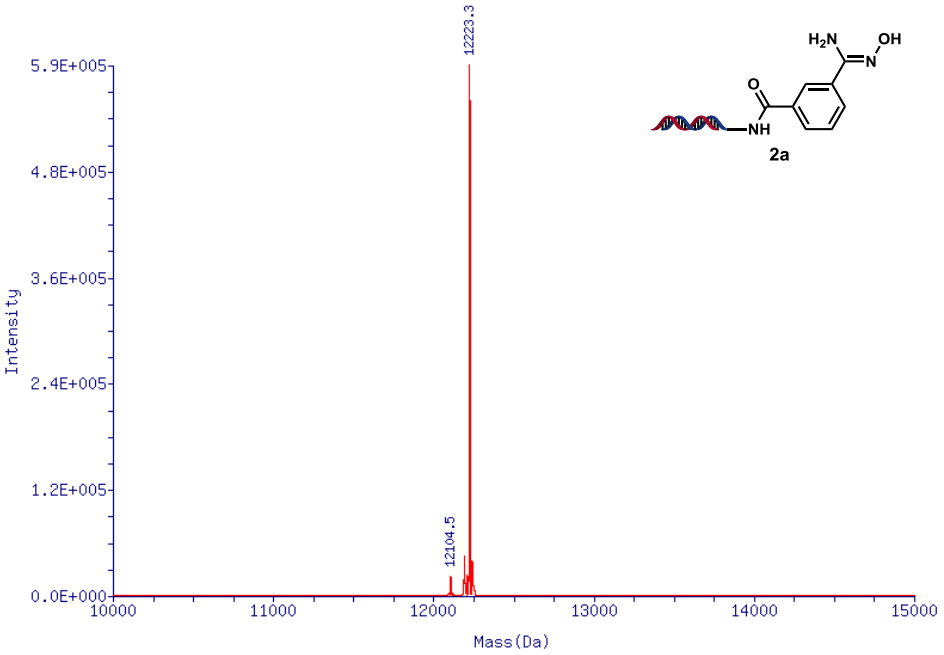



**Figure S10.** Deconvoluted mass spectrum of compound **2a**, expected: 12222.1; observed 12223.3.

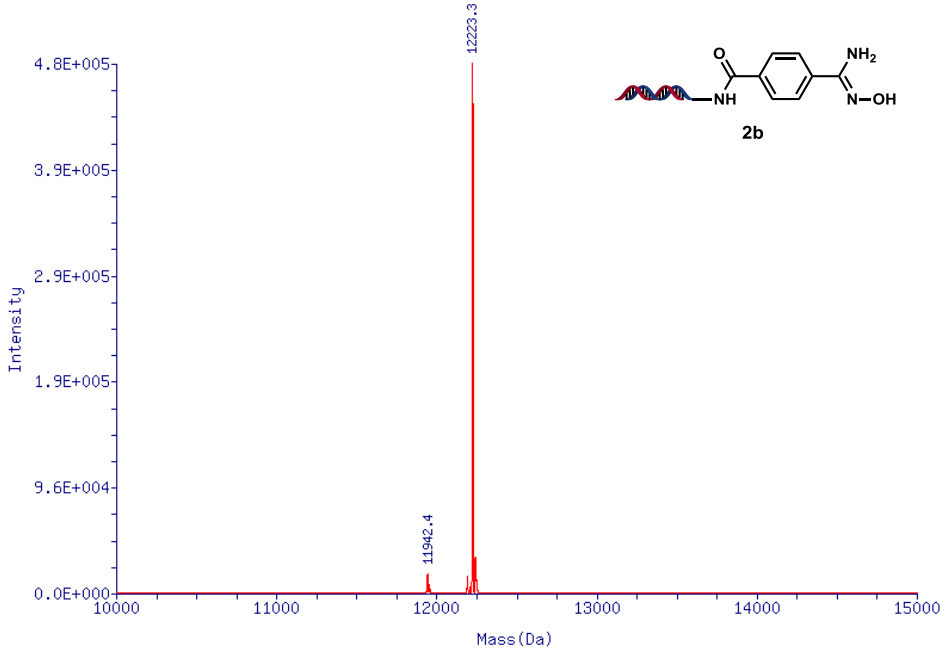



**Figure S11.** Deconvoluted mass spectrum of compound **2b**, expected: 12222.1; observed 12222.3.

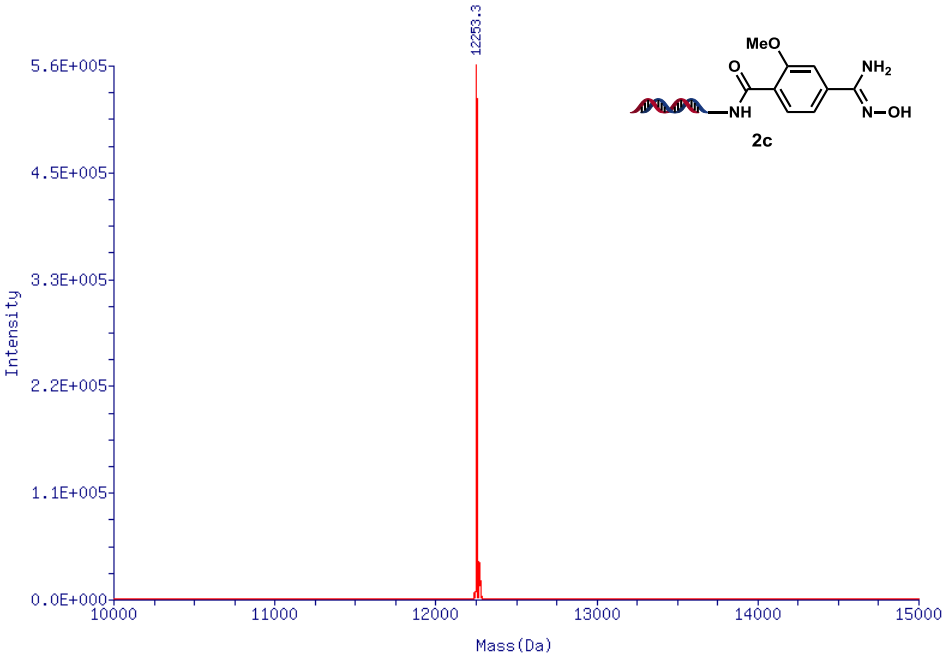



**Figure S12.** Deconvoluted mass spectrum of compound **2c**, expected: 12252.2; observed 12253.3.

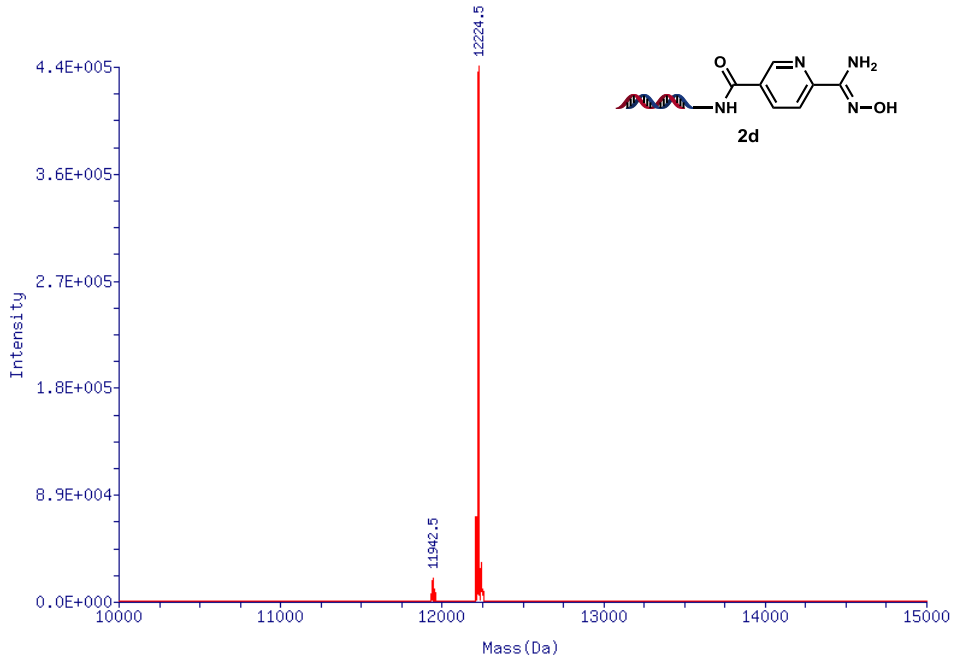



**Figure S13.** Deconvoluted mass spectrum of compound **2d**, expected: 12223.1; observed 12224.5.

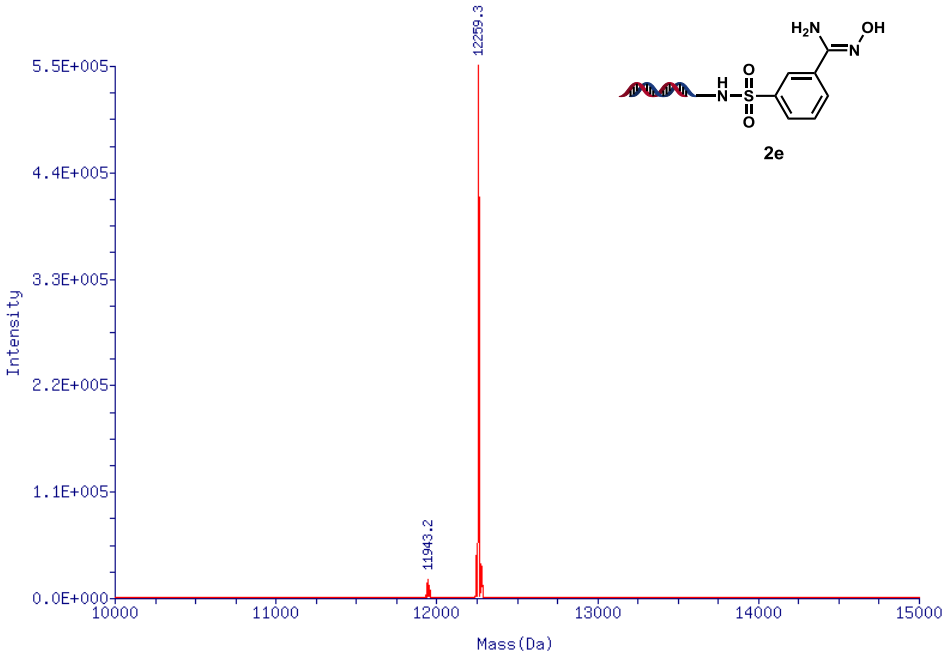



**Figure S14.** Deconvoluted mass spectrum of compound **2e**, expected: 12258.2; observed 12259.3.

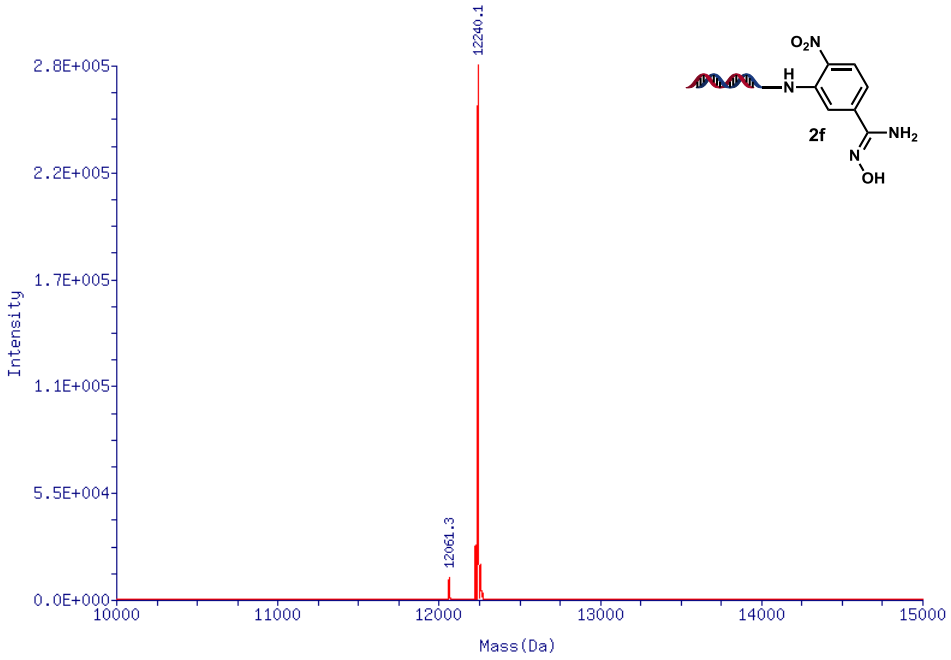



**Figure S15.** Deconvoluted mass spectrum of compound **2f**, expected: 12239.1; observed 12240.1.

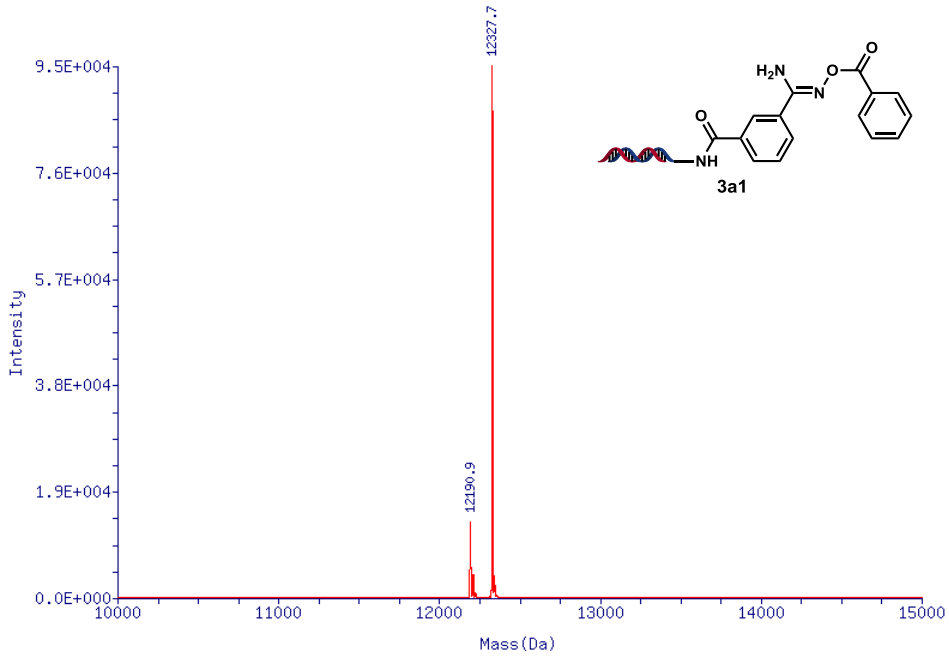



**Figure S16.** Deconvoluted mass spectrum of compound **3a1**, expected: 12326.3; observed 12327.7.

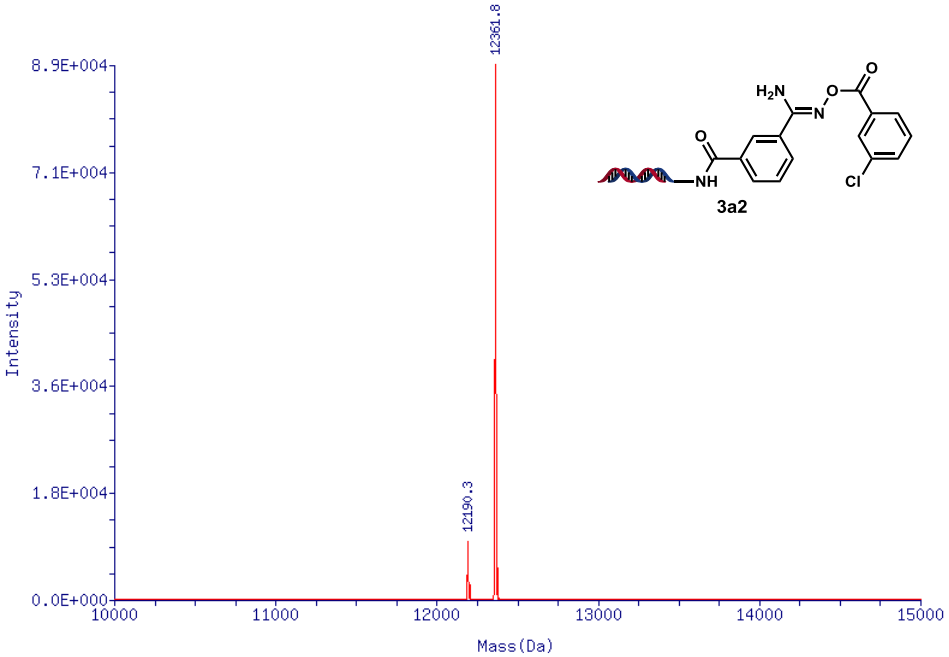



**Figure S17.** Deconvoluted mass spectrum of compound **3a2**, expected: 12360.7; observed 12361.8.

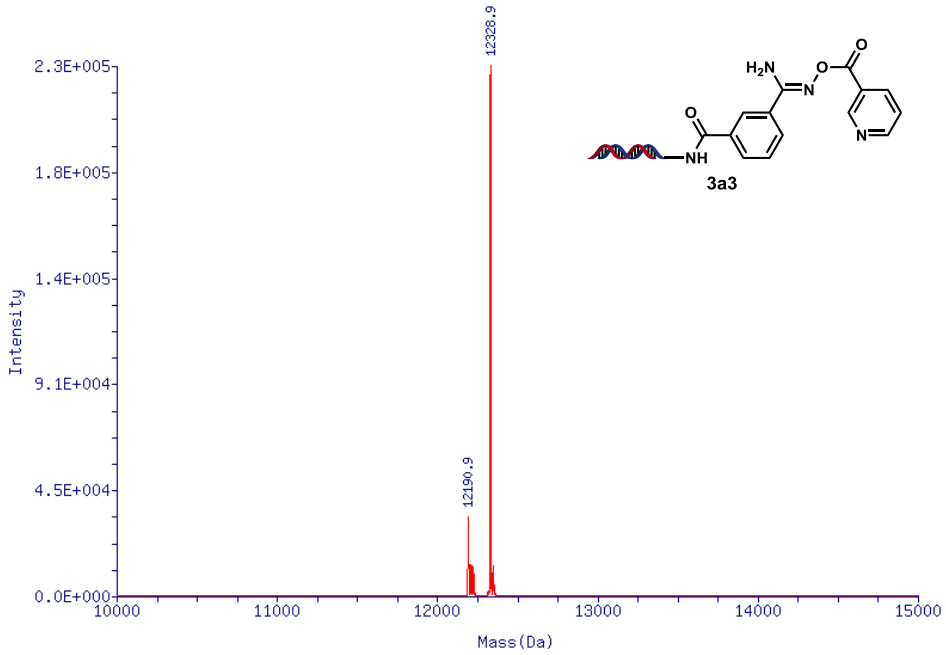



**Figure S18.** Deconvoluted mass spectrum of compound **3a3**, expected: 12327.2; observed 12328.9.

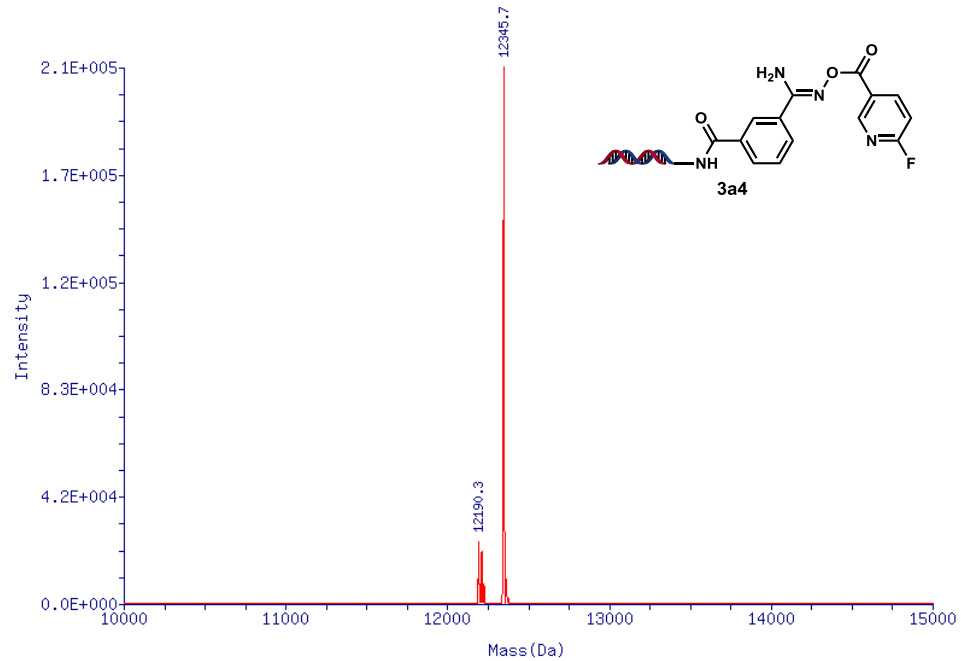



**Figure S19.** Deconvoluted mass spectrum of compound **3a4**, expected: 12345.2; observed 12345.7.

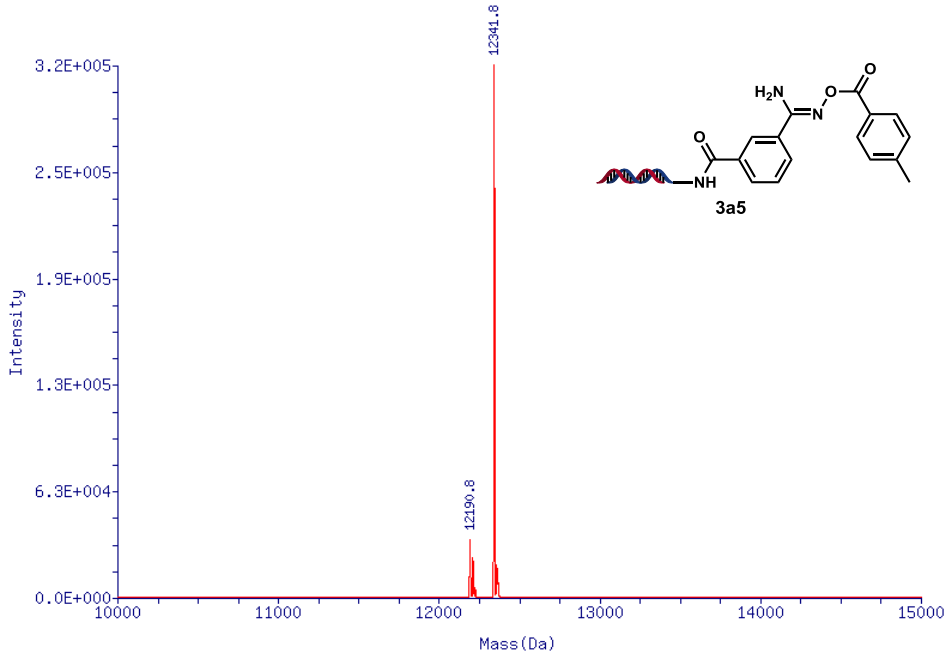



**Figure S20.** Deconvoluted mass spectrum of compound **3a5**, expected: 12340.3; observed 12341.8.

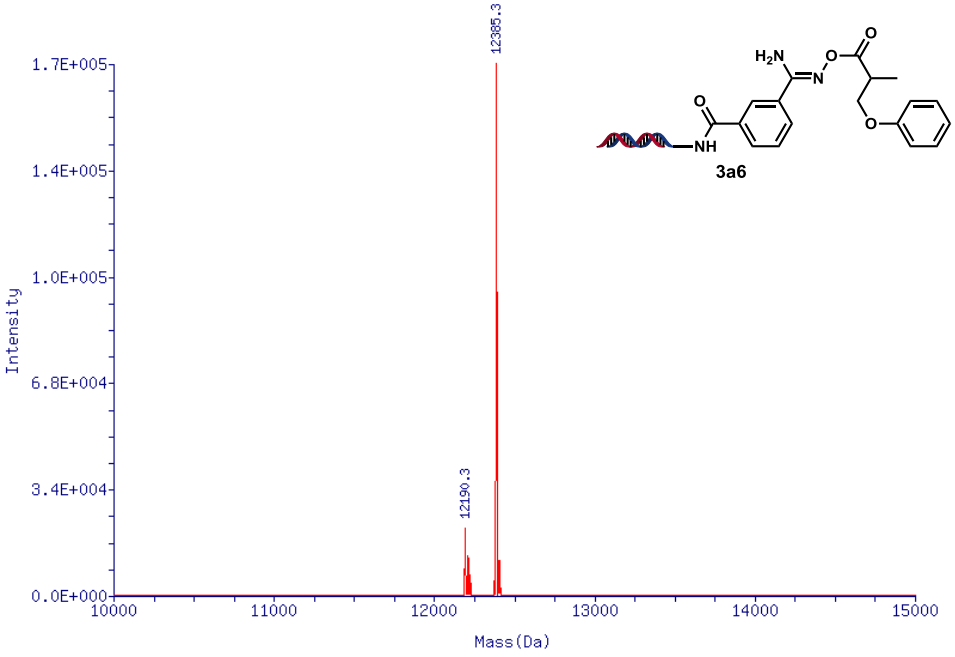



**Figure S21.** Deconvoluted mass spectrum of compound **3a6**, expected: 12384.3; observed 12385.3.

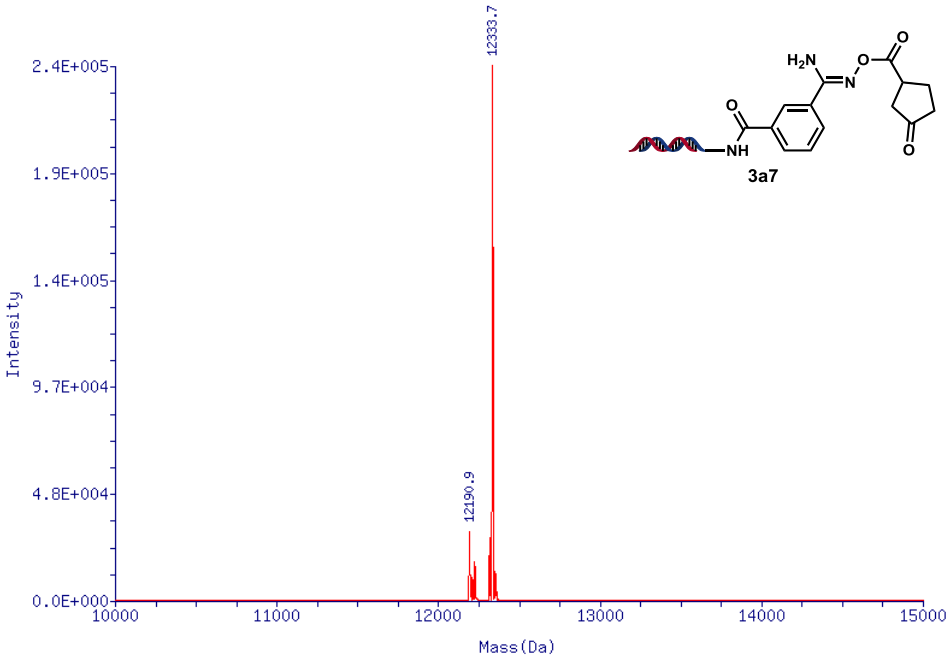



**Figure S22.** Deconvoluted mass spectrum of compound **3a7**, expected: 12332.3; observed 12333.7.

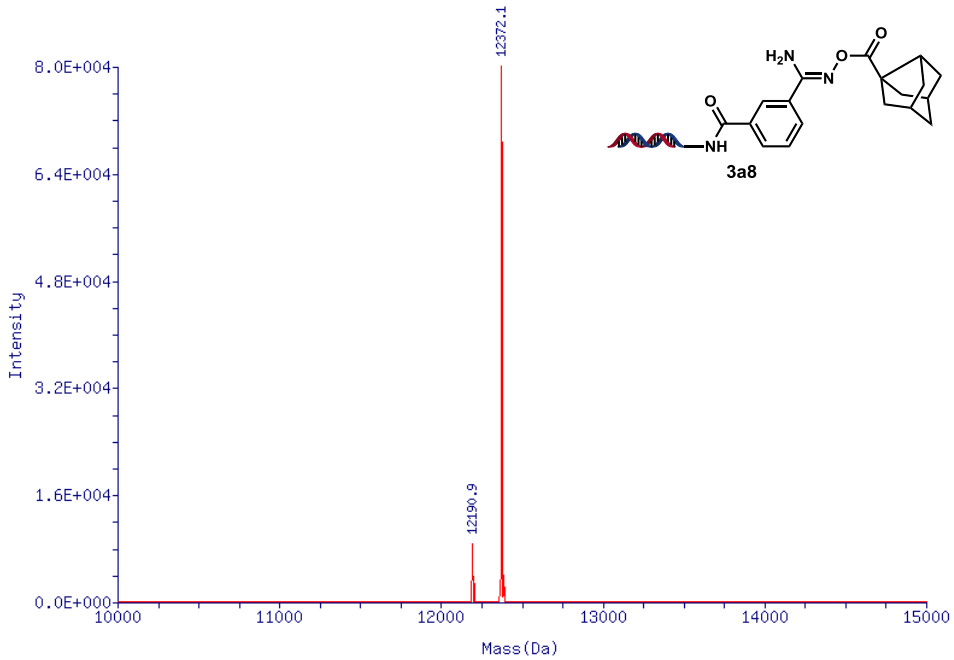



**Figure S23.** Deconvoluted mass spectrum of compound **3a8**, expected: 12370.4; observed 12372.1.

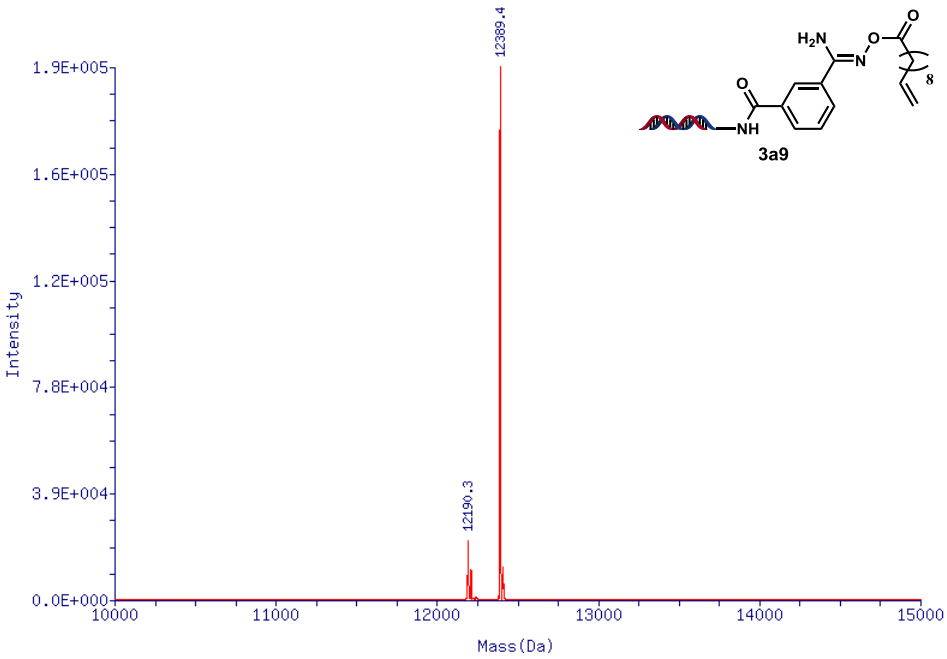



**Figure S24.** Deconvoluted mass spectrum of compound **3a9**, expected: 12388.4; observed 12389.4.

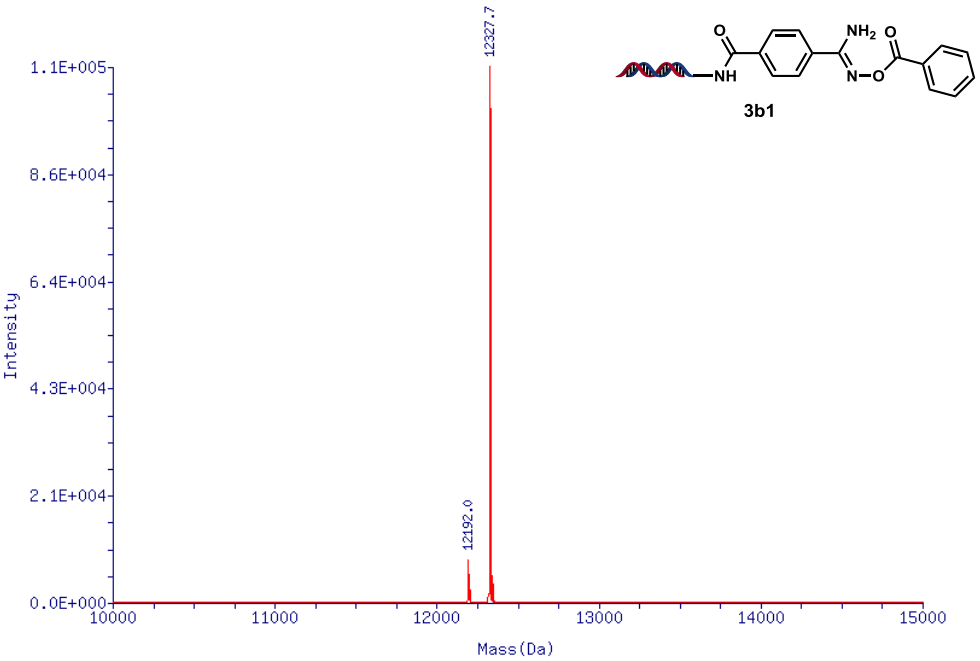



**Figure S25.** Deconvoluted mass spectrum of compound **3b1**, expected: 12326.3; observed 12327.7.

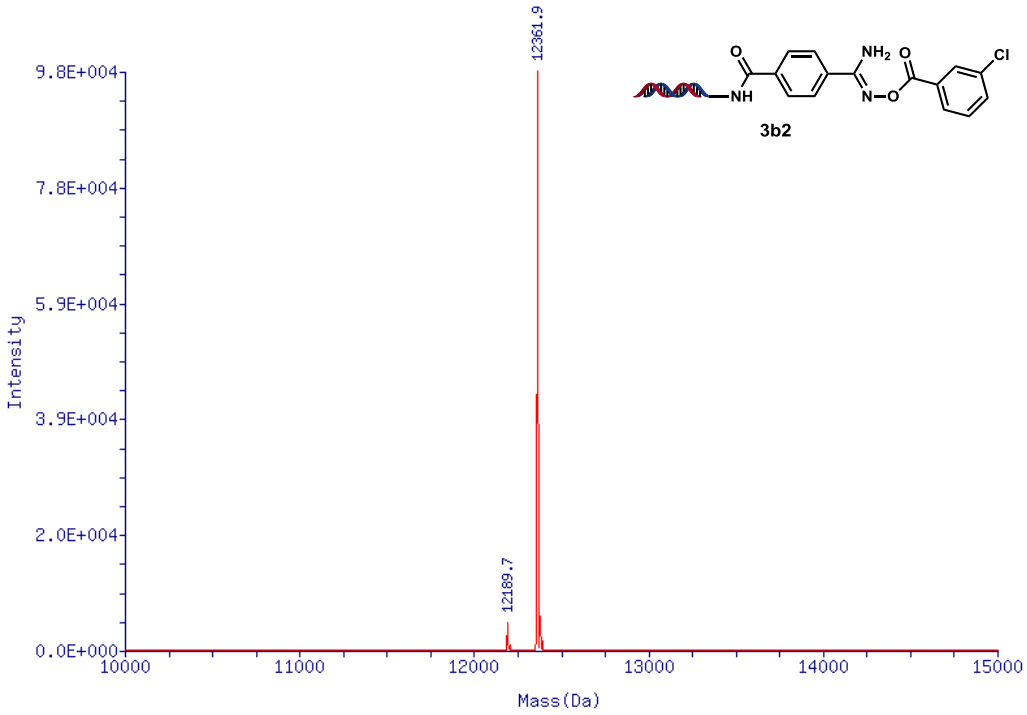



**Figure S26.** Deconvoluted mass spectrum of compound **3b2**, expected: 12360.7; observed 12361.9.

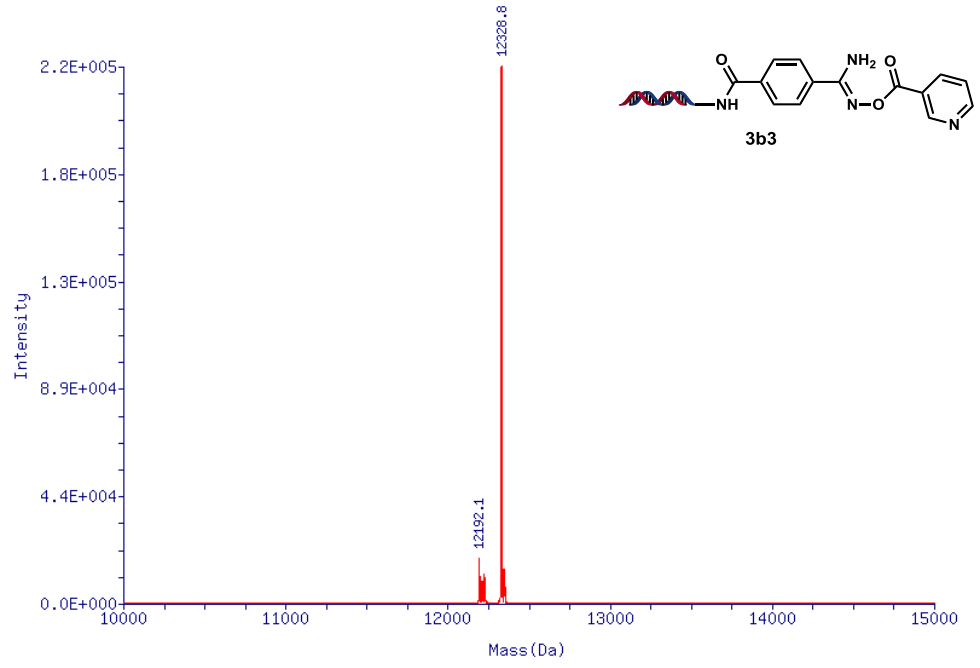



**Figure S27.** Deconvoluted mass spectrum of compound **3b3**, expected: 12327.2; observed 12328.8.

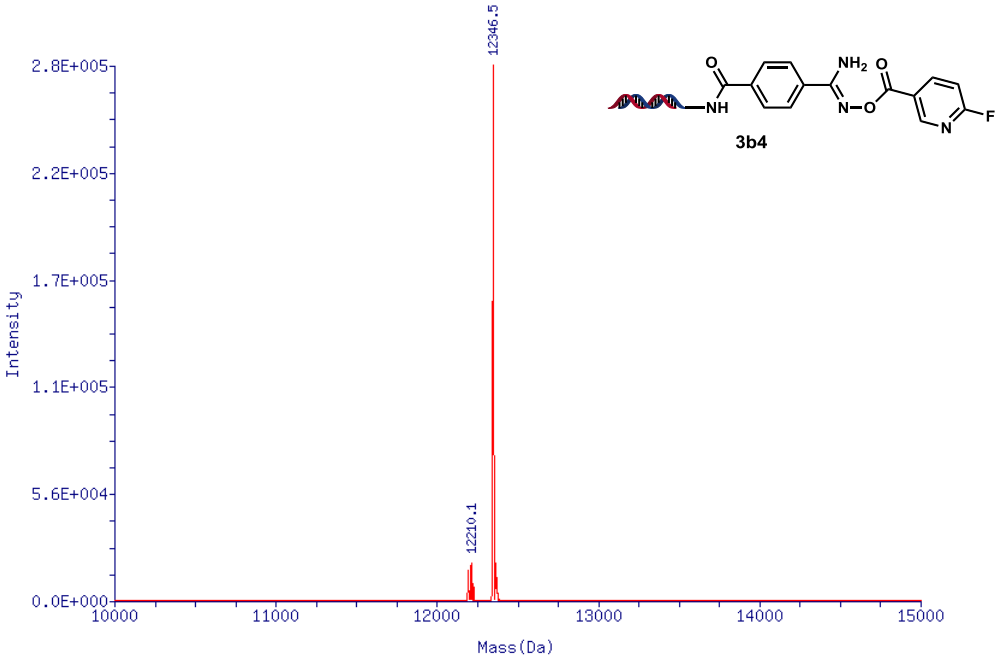



**Figure S28.** Deconvoluted mass spectrum of compound **3b4**, expected: 12345.2; observed 12346.5.

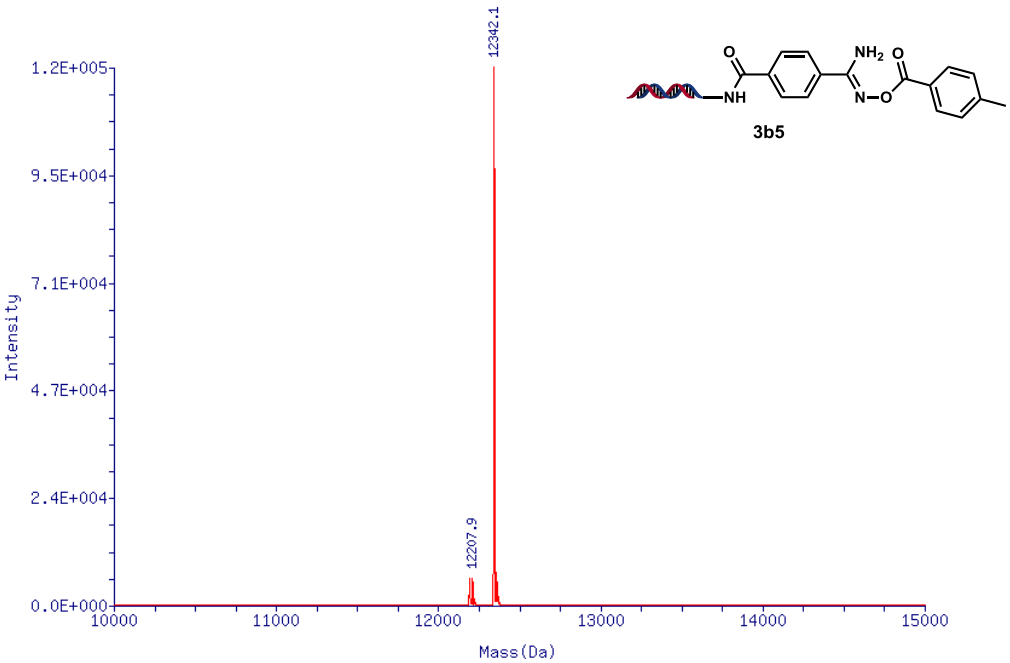



**Figure S29.** Deconvoluted mass spectrum of compound **3b5**, expected: 12340.3; observed 12342.1.

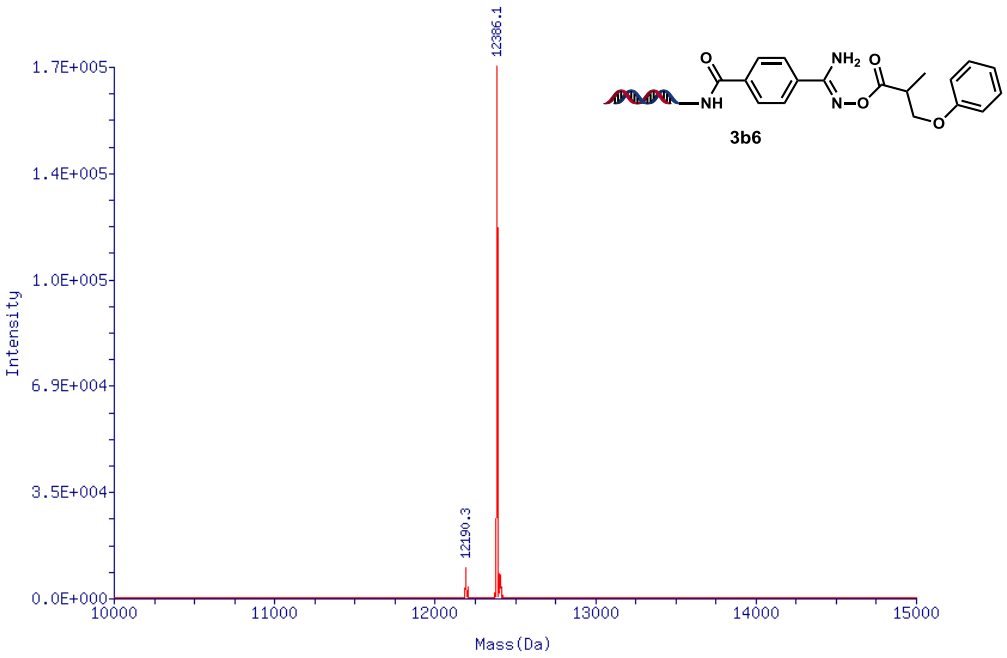



**Figure S30.** Deconvoluted mass spectrum of compound **3b6**, expected: 12384.3; observed 12386.1.

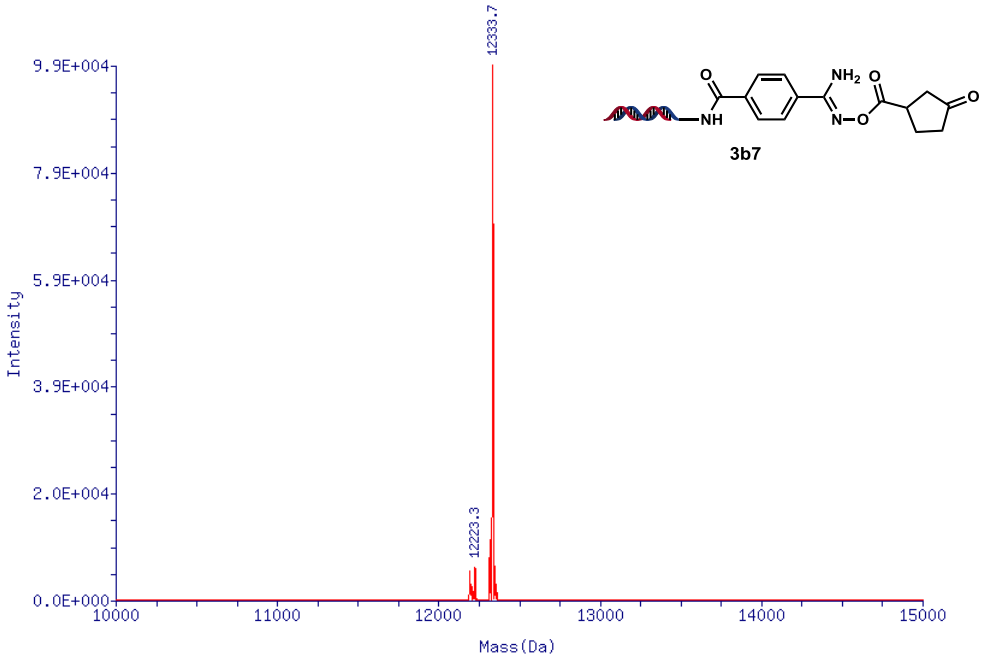



**Figure S31.** Deconvoluted mass spectrum of compound **3b7**, expected: 12332.3; observed 12333.7.

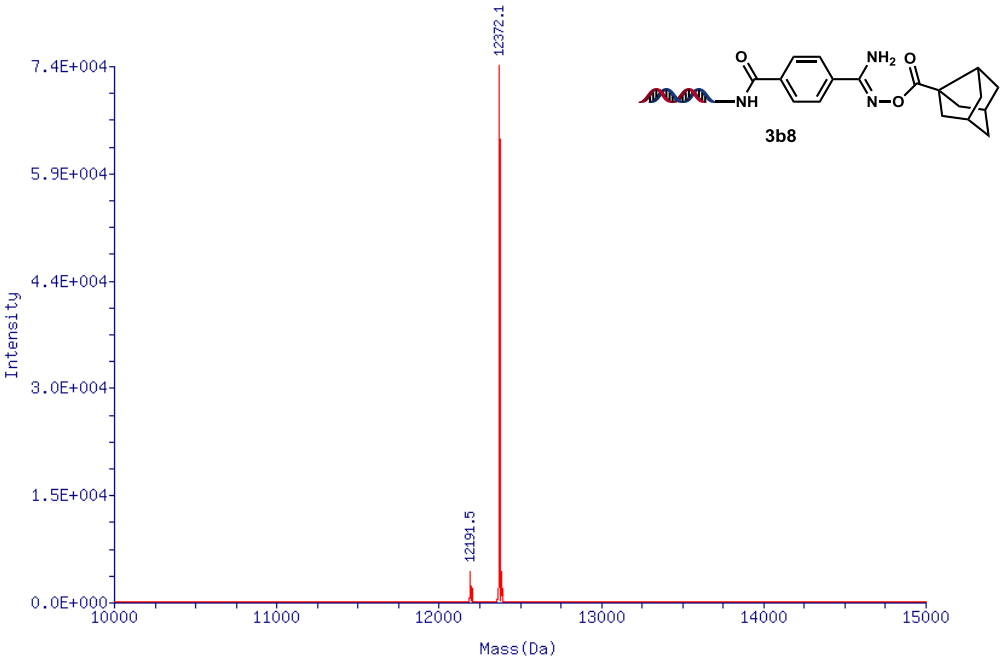



**Figure S32.** Deconvoluted mass spectrum of compound **3b8**, expected: 12370.4; observed 12372.1.

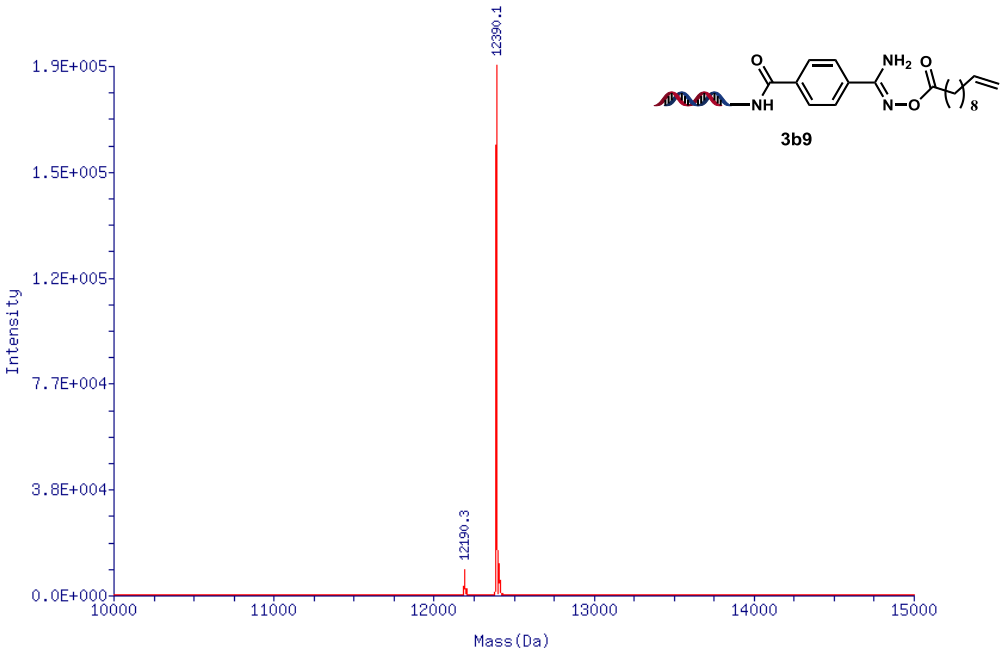



**Figure S33.** Deconvoluted mass spectrum of compound **3b9**, expected: 12388.4; observed 12390.1.

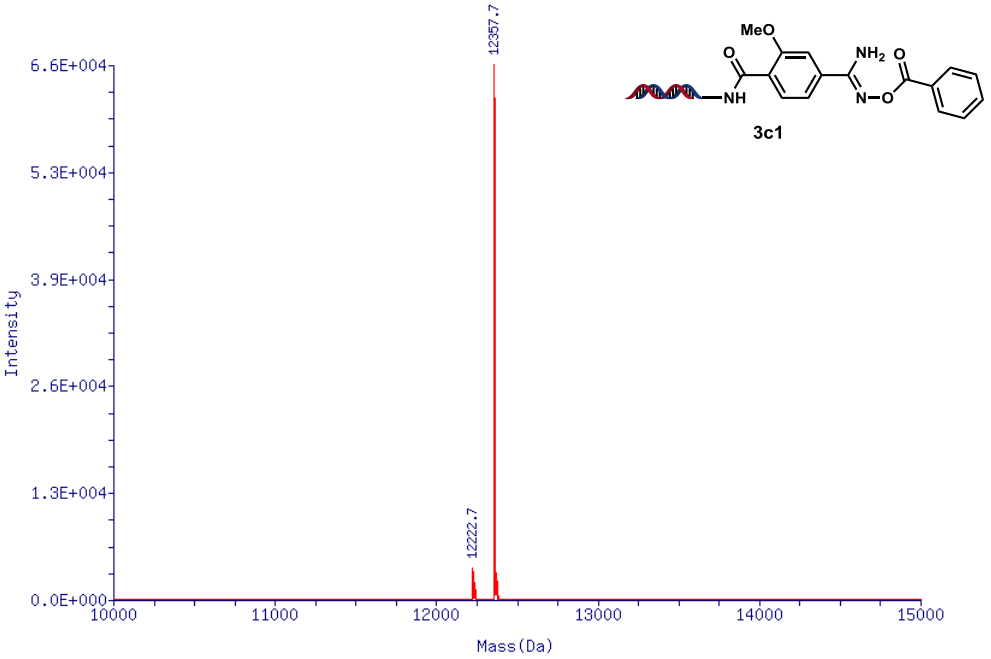



**Figure S34.** Deconvoluted mass spectrum of compound **3c1**, expected: 12356.3; observed 12357.7.

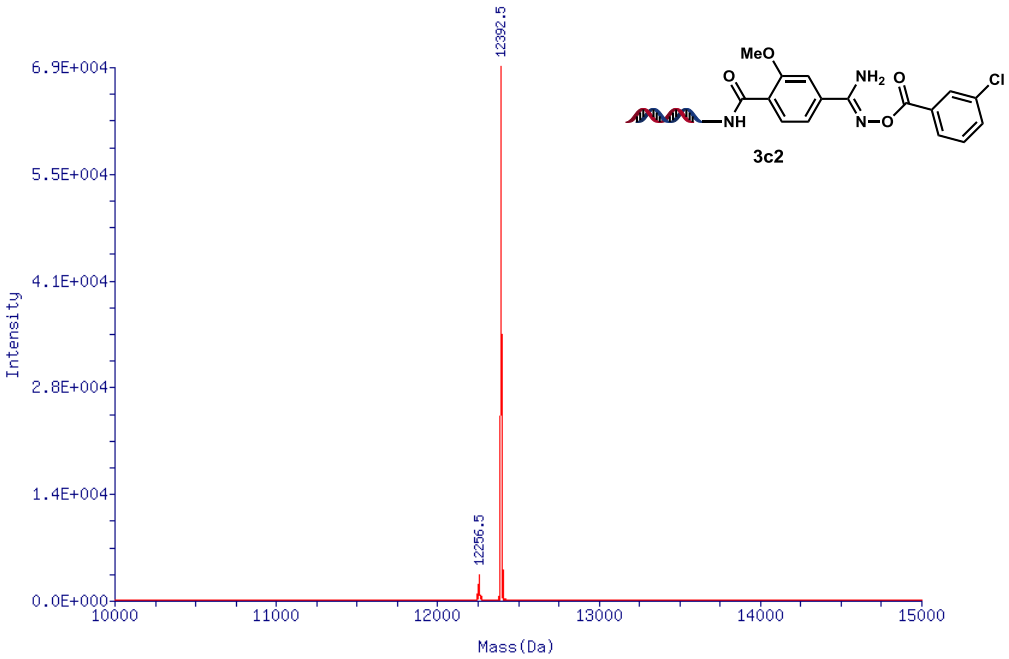



**Figure S35.** Deconvoluted mass spectrum of compound **3c2**, expected: 12390.7; observed 12392.5.

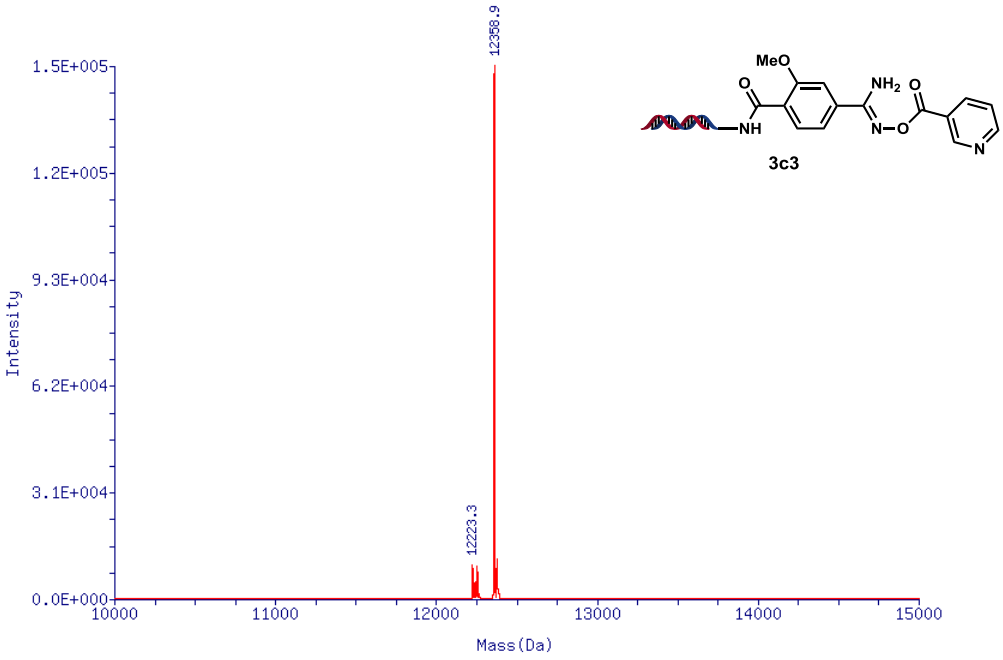



**Figure S36.** Deconvoluted mass spectrum of compound **3c3**, expected: 12357.3; observed 12358.9.

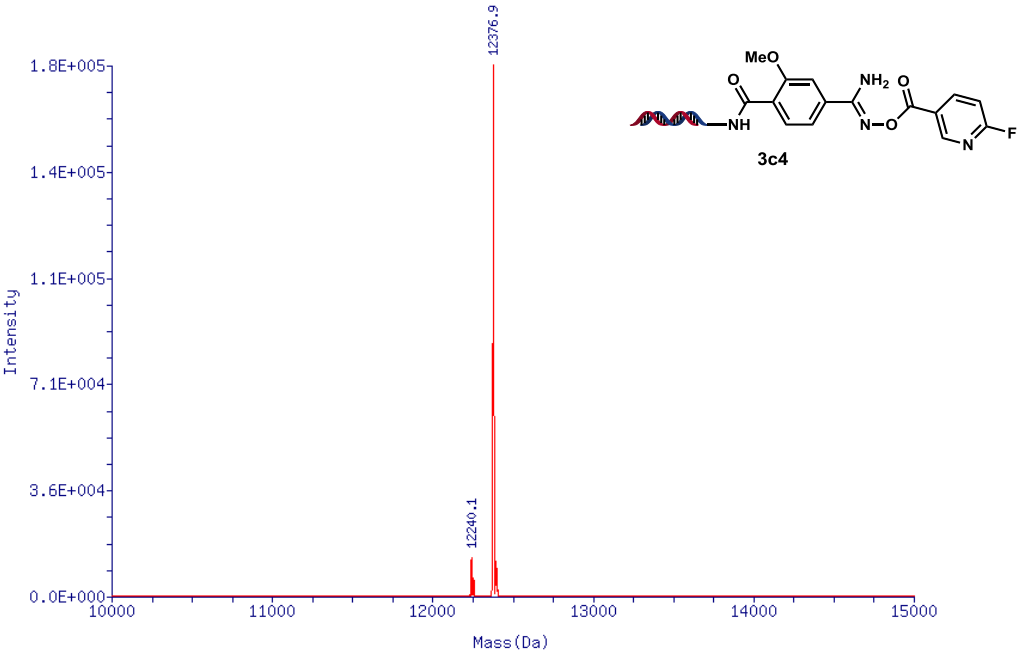



**Figure S37.** Deconvoluted mass spectrum of compound **3c4**, expected: 12375.3; observed 12376.9.

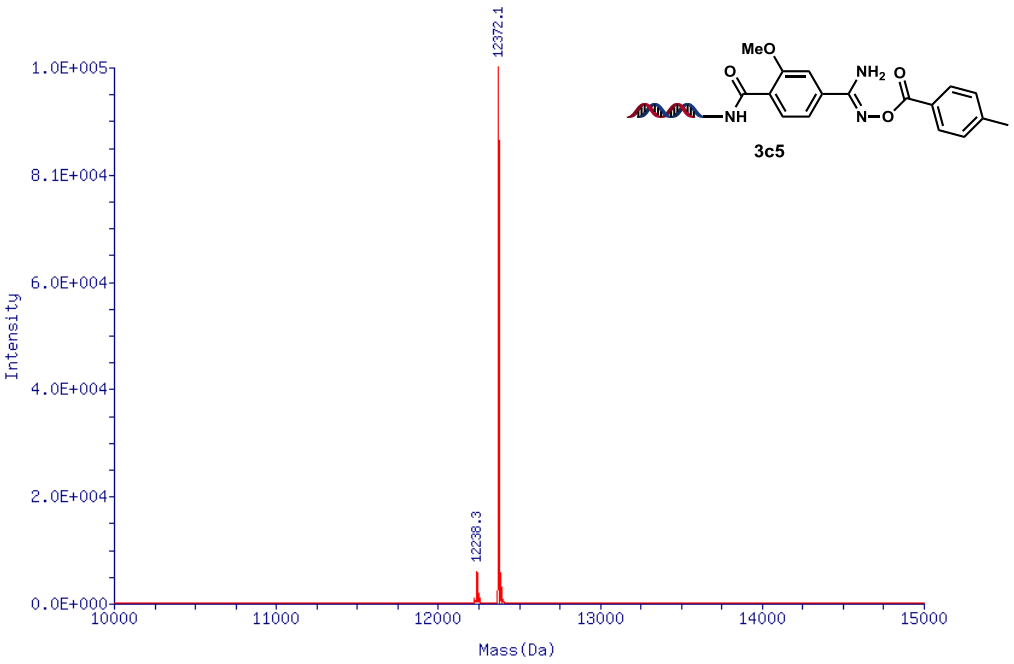



**Figure S38.** Deconvoluted mass spectrum of compound **3c5**, expected: 12370.3; observed 12372.1.

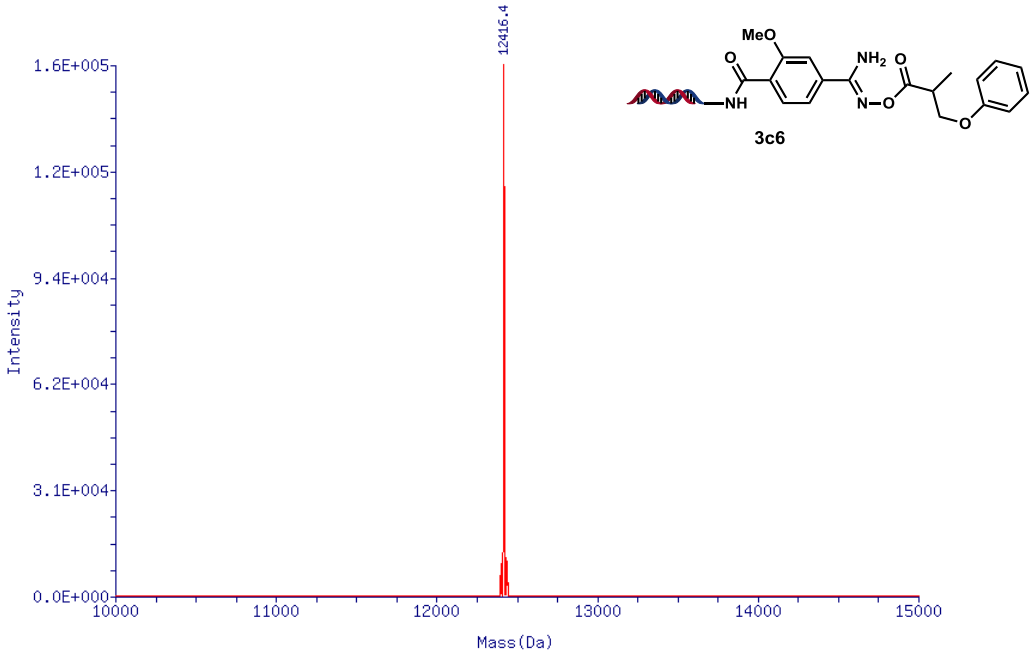



**Figure S39.** Deconvoluted mass spectrum of compound **3c6**, expected: 12414.4; observed 12416.4.

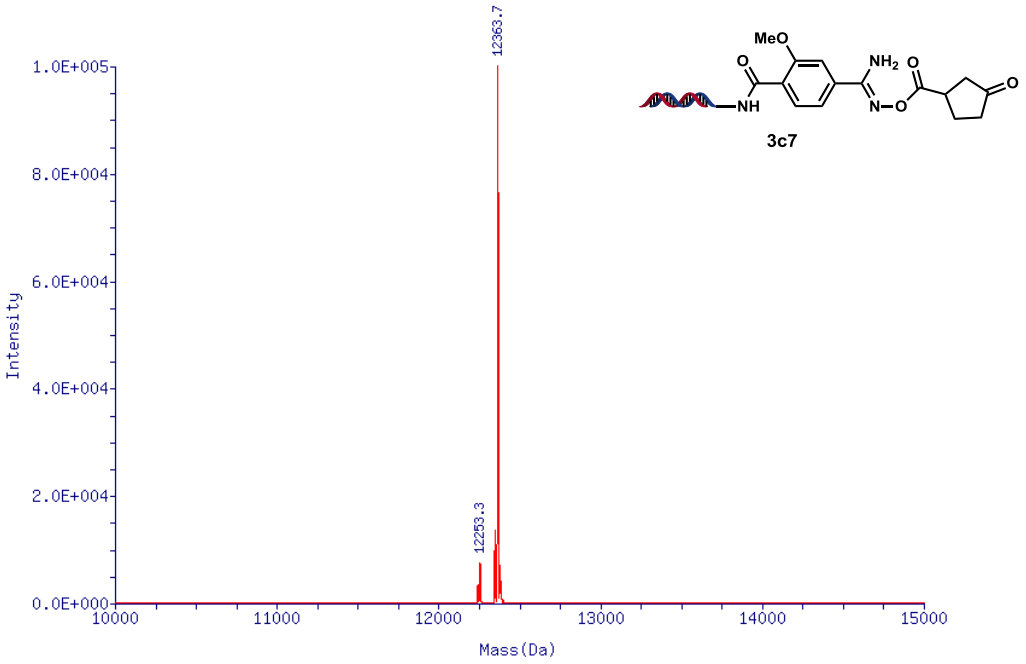



**Figure S40.** Deconvoluted mass spectrum of compound **3c7**, expected: 12362.3; observed 12363.7.

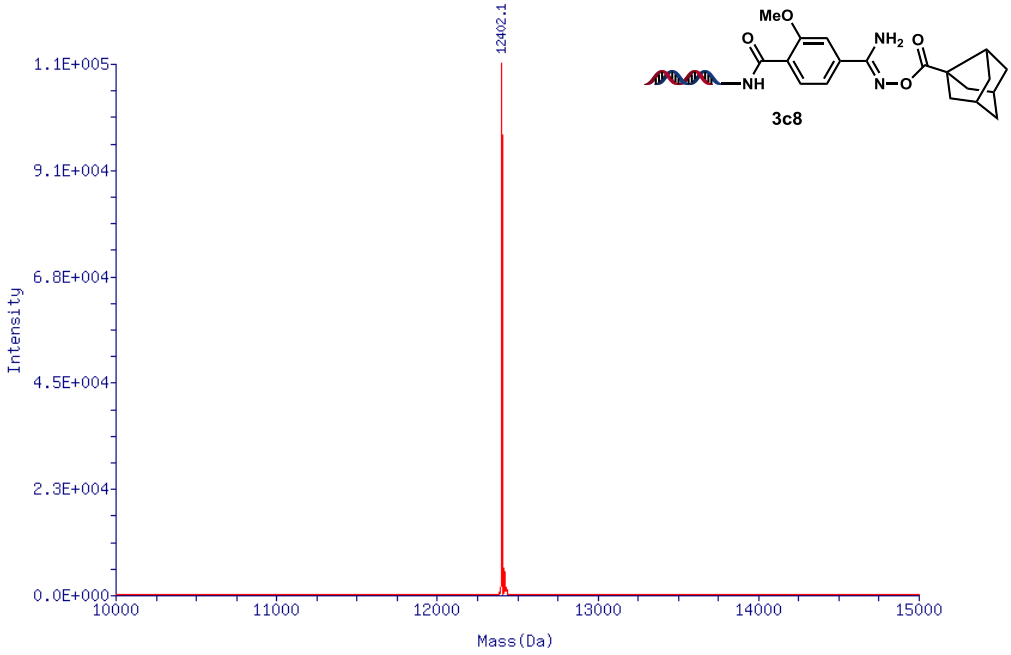



**Figure S41.** Deconvoluted mass spectrum of compound **3c8**, expected: 12400.4; observed 12402.1.

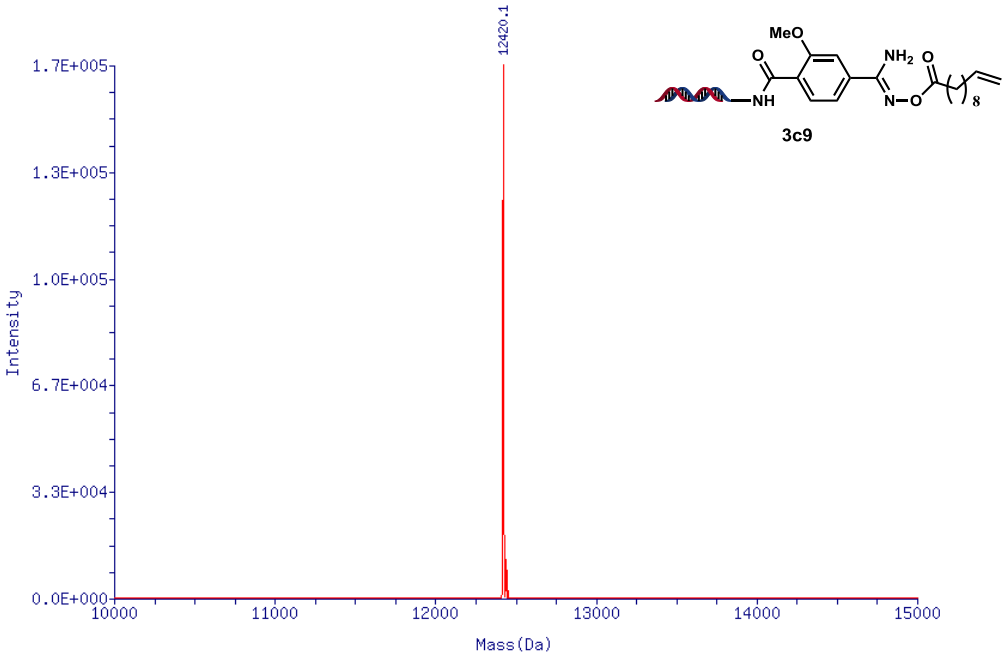



**Figure S42.** Deconvoluted mass spectrum of compound **3c9**, expected: 12418.4; observed 12420.1.

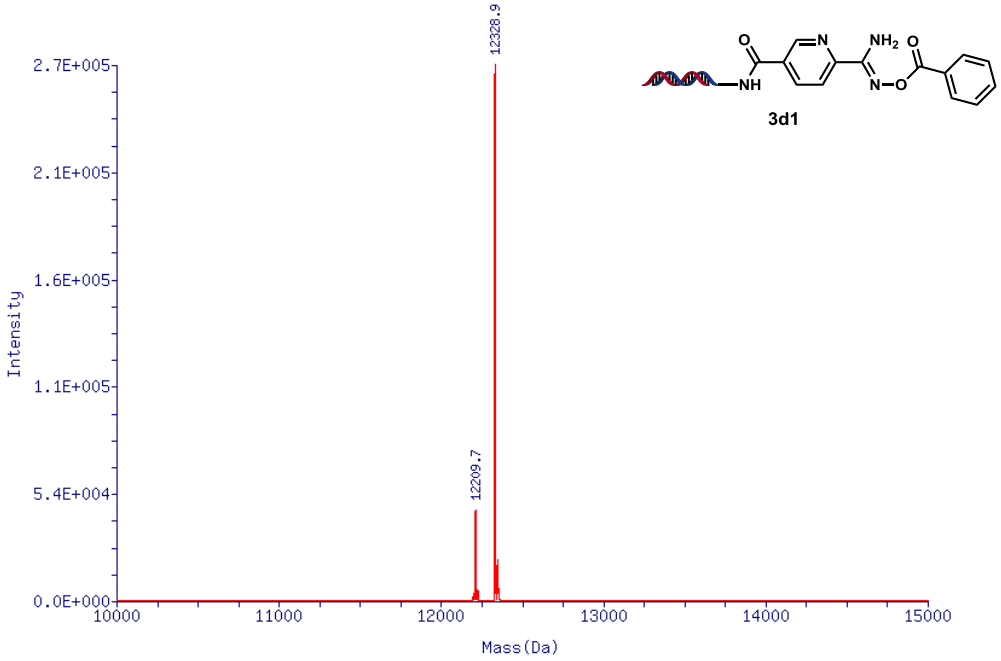



**Figure S43.** Deconvoluted mass spectrum of compound **3d1**, expected: 12327.3; observed 12328.9.

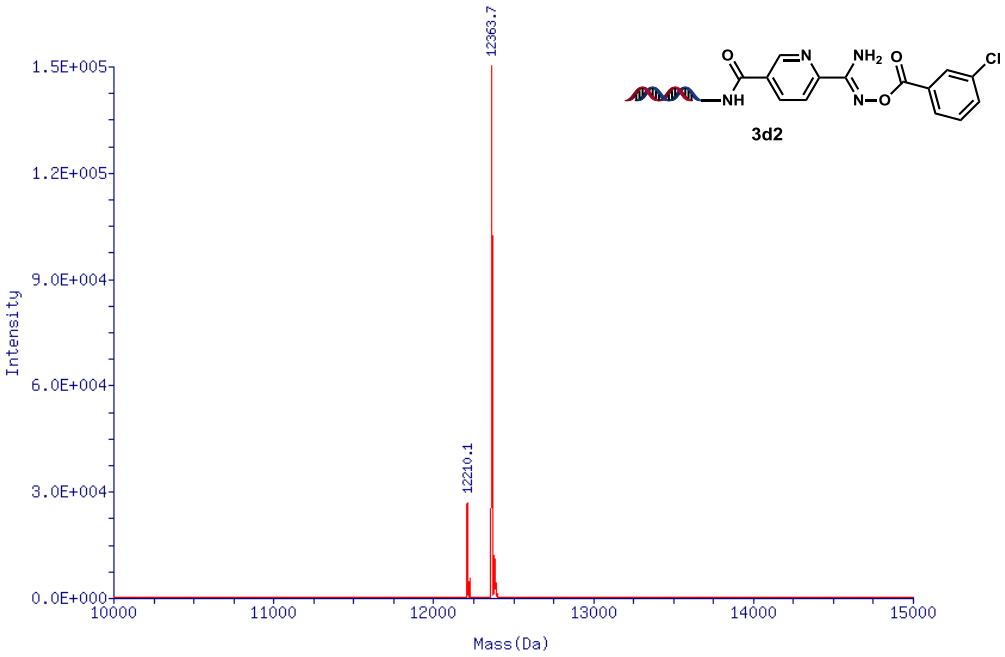



**Figure S44.** Deconvoluted mass spectrum of compound **3d2**, expected: 12361.7; observed 12363.7.

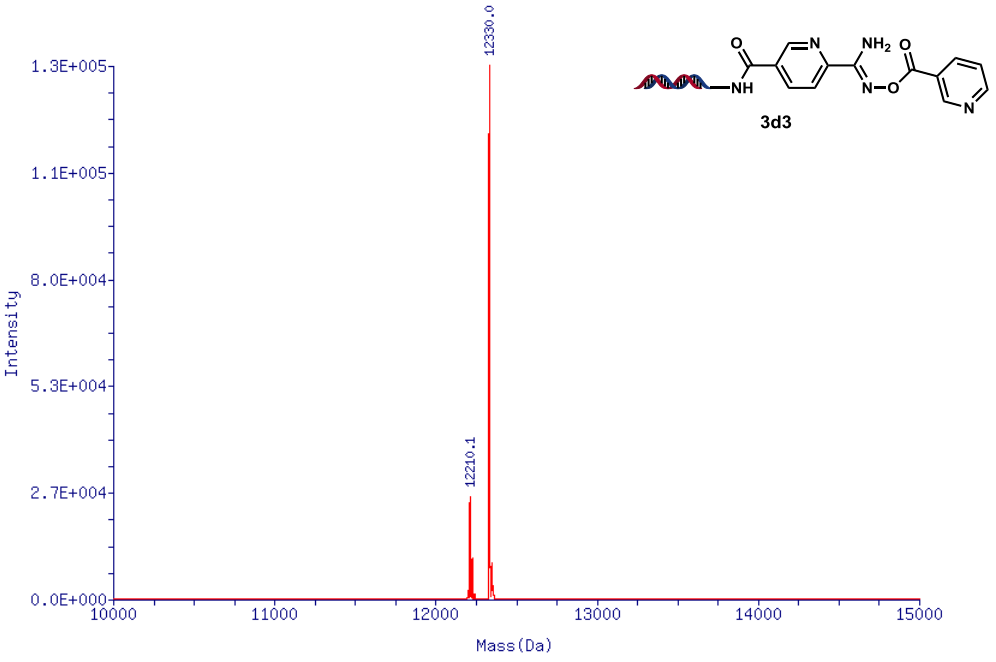



**Figure S45.** Deconvoluted mass spectrum of compound **3d3**, expected: 12328.2; observed 12330.0.

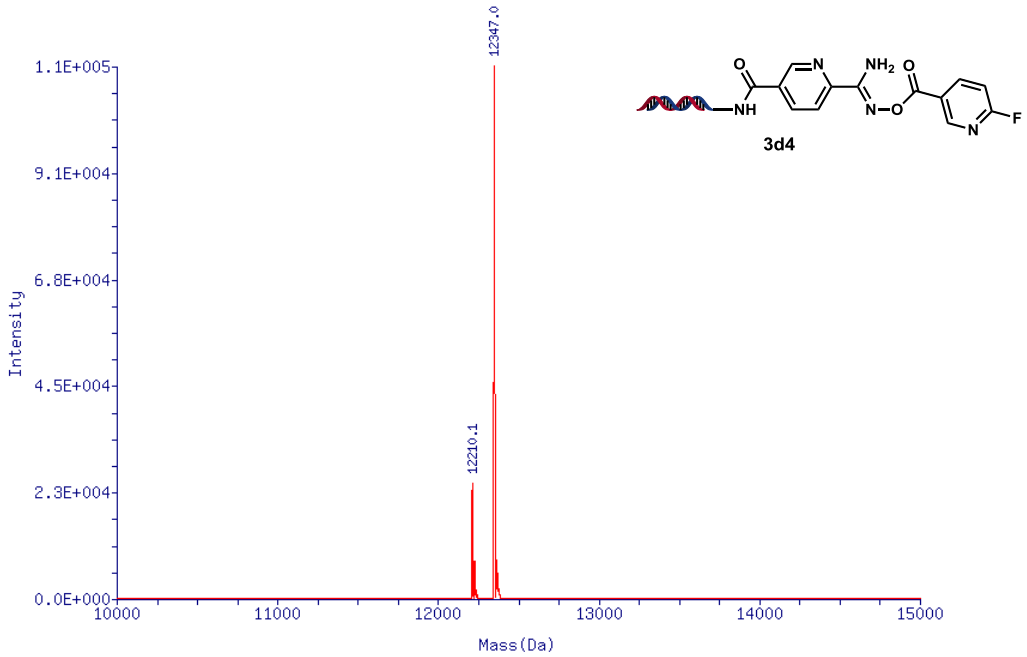



**Figure S46.** Deconvoluted mass spectrum of compound **3d4**, expected: 12346.2; observed 12347.0.

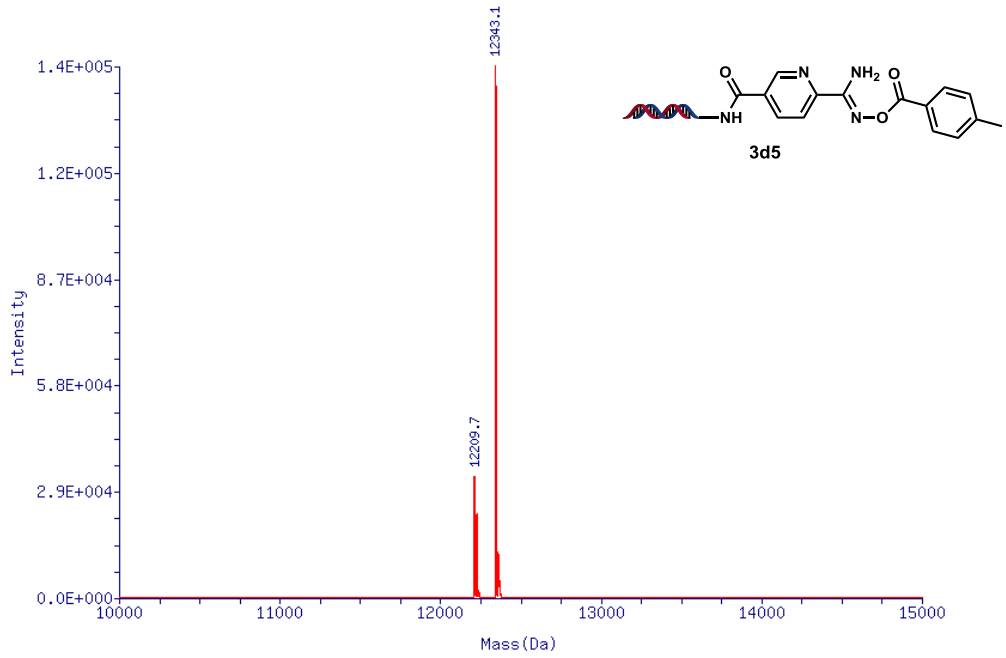



**Figure S47.** Deconvoluted mass spectrum of compound **3d5**, expected: 12341.3; observed 12343.1.

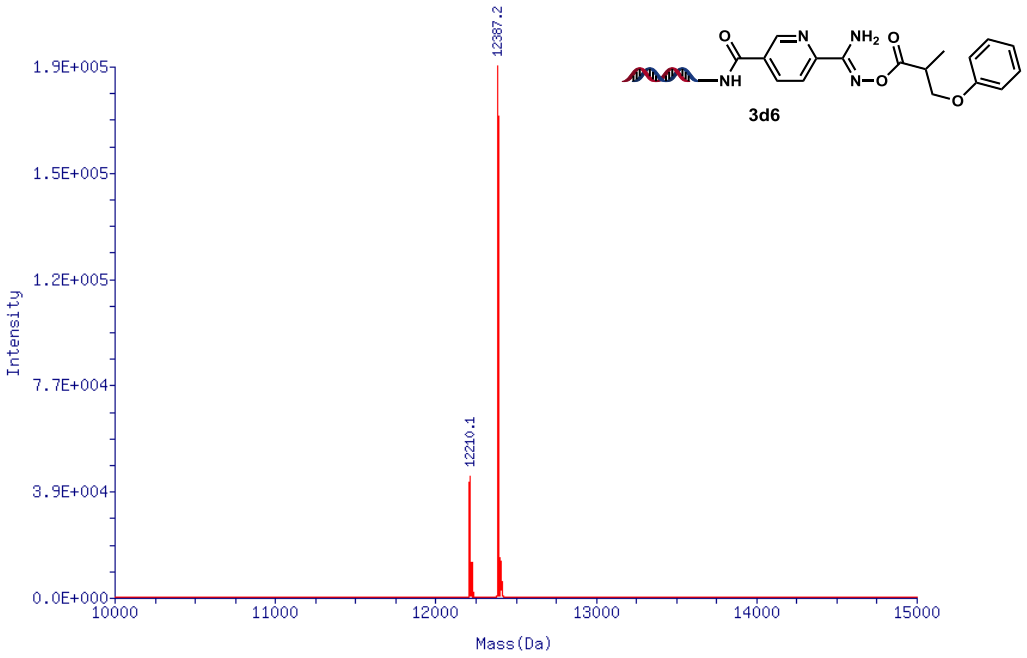



**Figure S48.** Deconvoluted mass spectrum of compound **3d6**, expected: 12385.3; observed 12387.2.

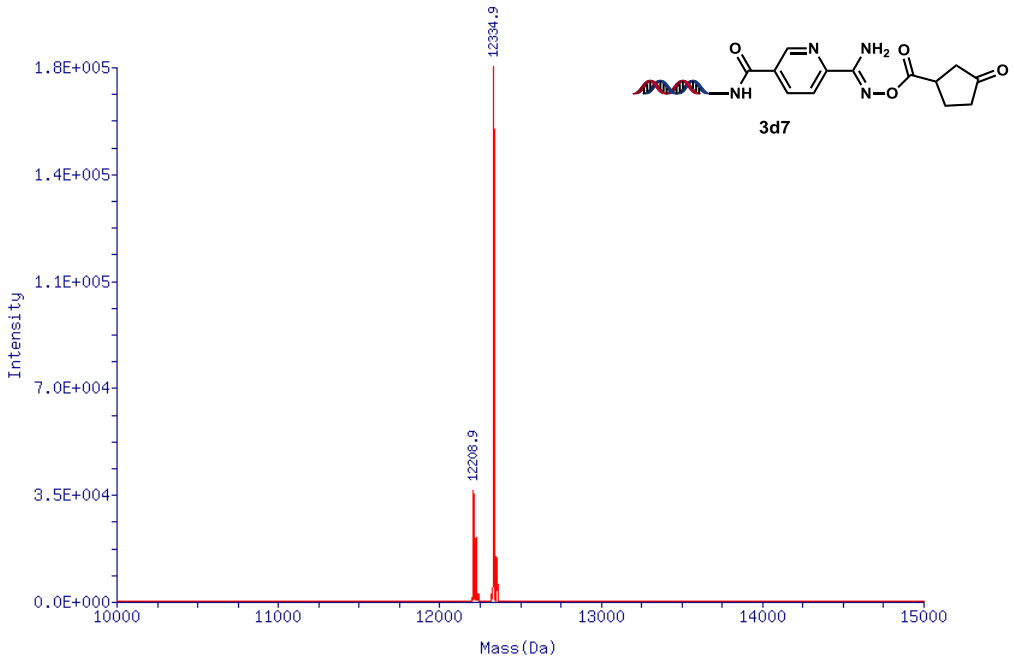



**Figure S49.** Deconvoluted mass spectrum of compound **3d7**, expected: 12333.3; observed 12334.9.

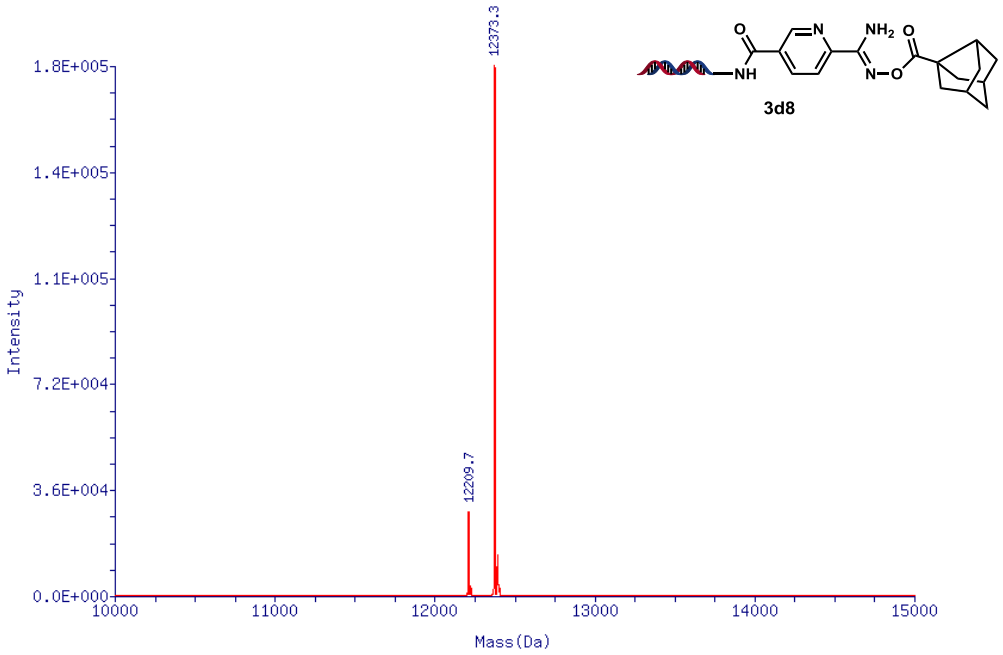



**Figure S50.** Deconvoluted mass spectrum of compound **3d8**, expected: 12371.4; observed 12373.3.

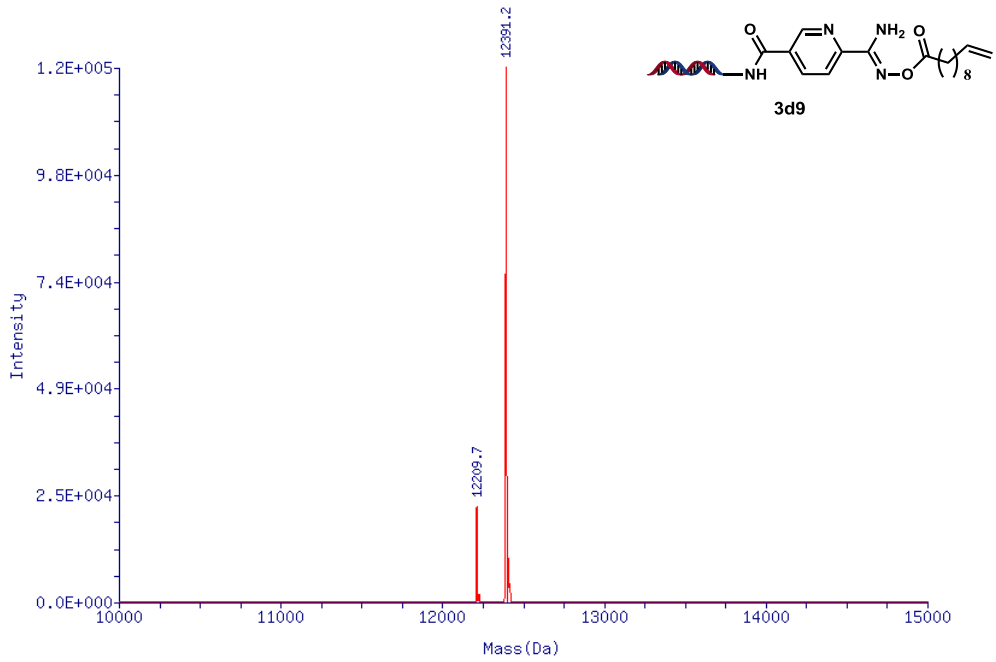



**Figure S51.** Deconvoluted mass spectrum of compound **3d9**, expected: 12389.4; observed 12391.2.

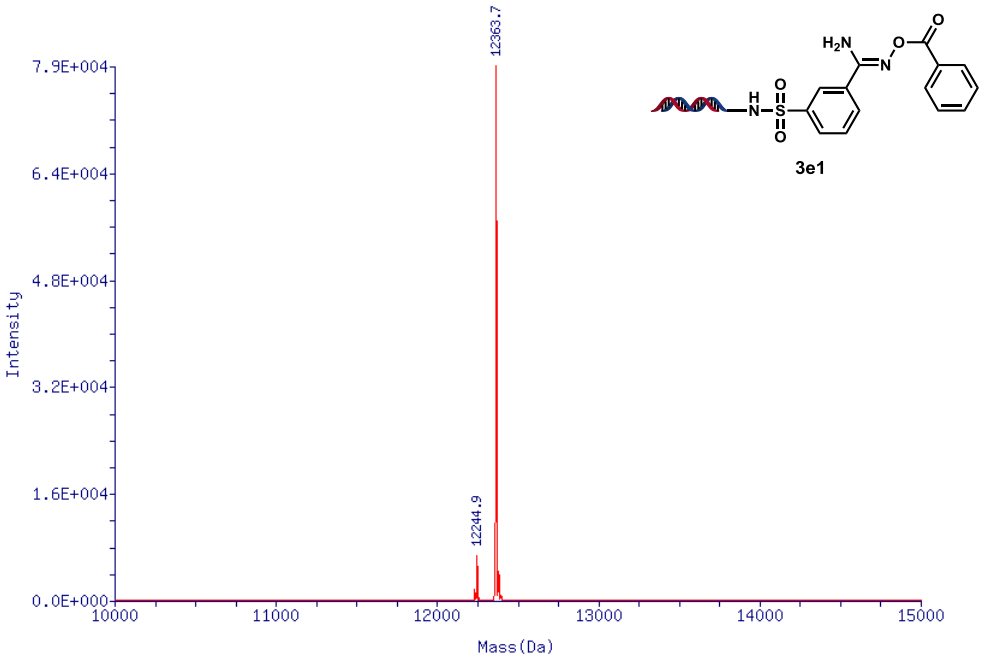



**Figure S52.** Deconvoluted mass spectrum of compound **3e1**, expected: 12362.3; observed 12363.7.

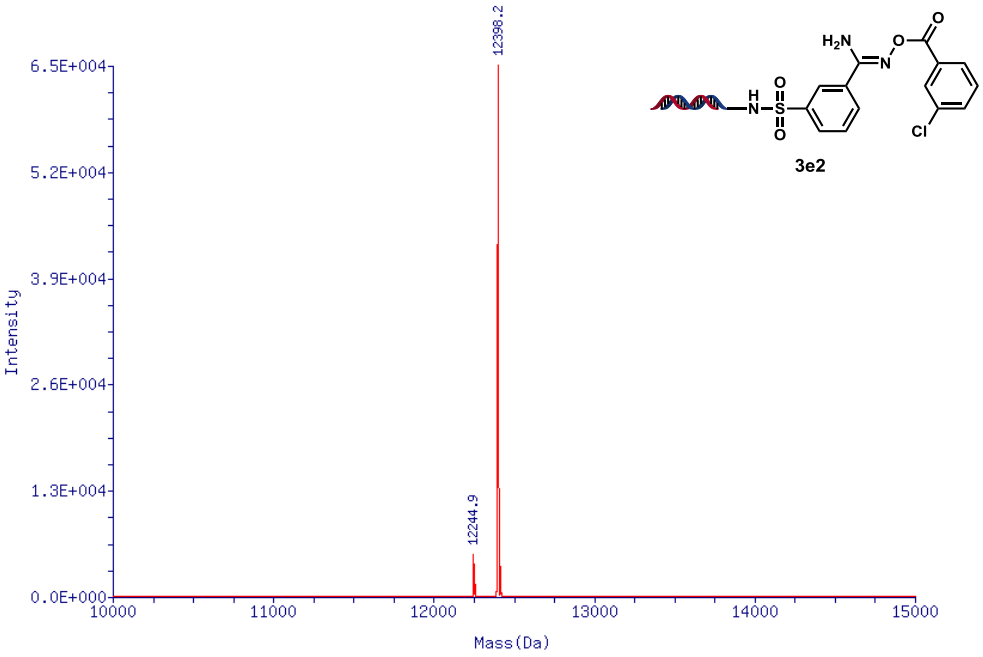



**Figure S53.** Deconvoluted mass spectrum of compound **3e2**, expected: 12396.7; observed 12398.2.

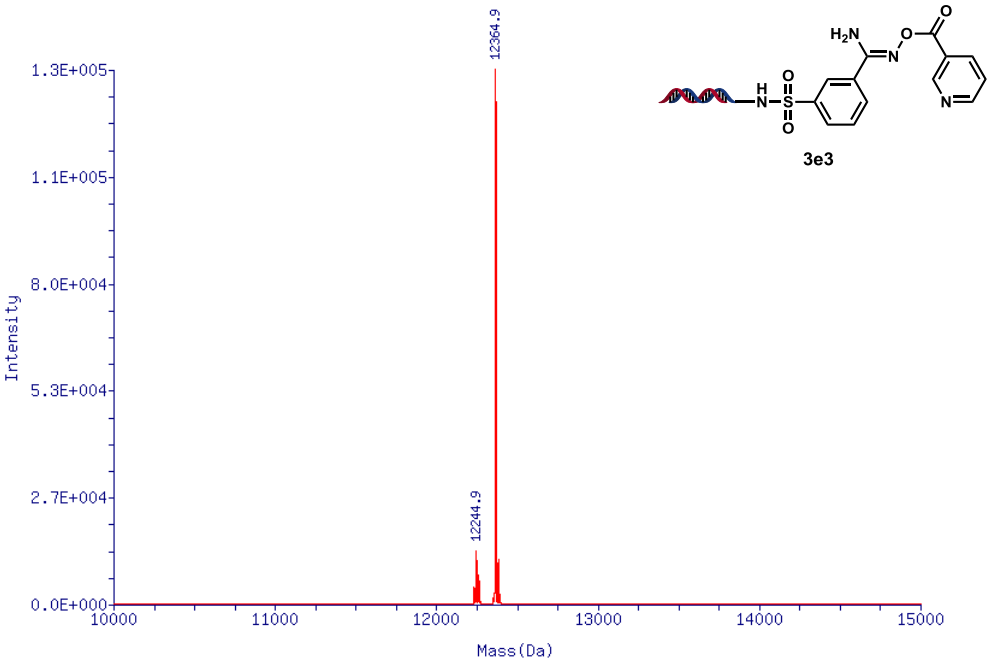



**Figure S54.** Deconvoluted mass spectrum of compound **3e3**, expected: 12363.3; observed 12364.9.

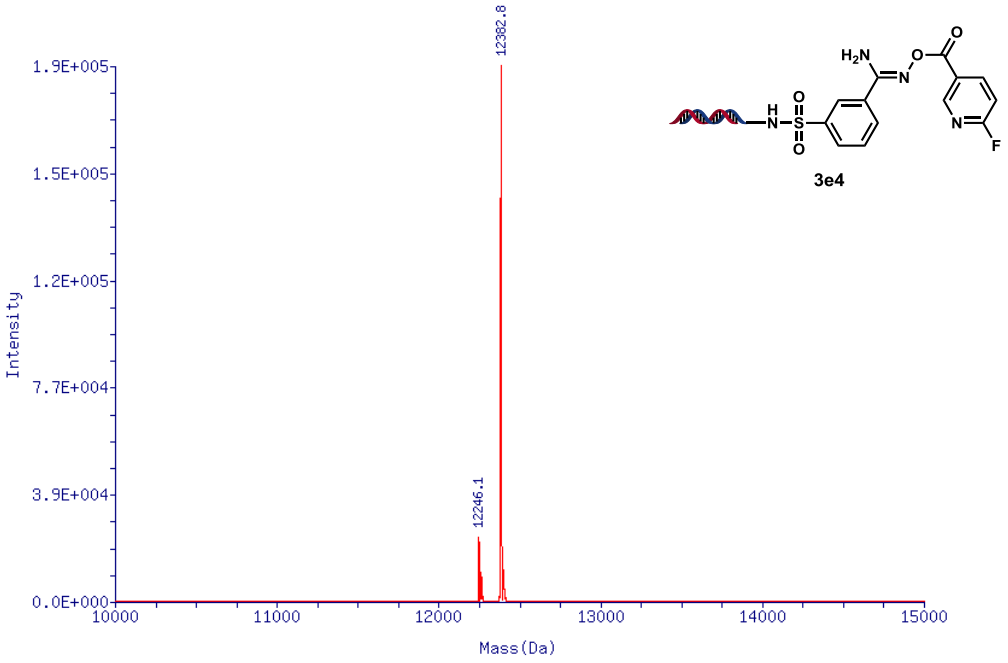



**Figure S55.** Deconvoluted mass spectrum of compound **3e4**, expected: 12381.3; observed 12382.8.

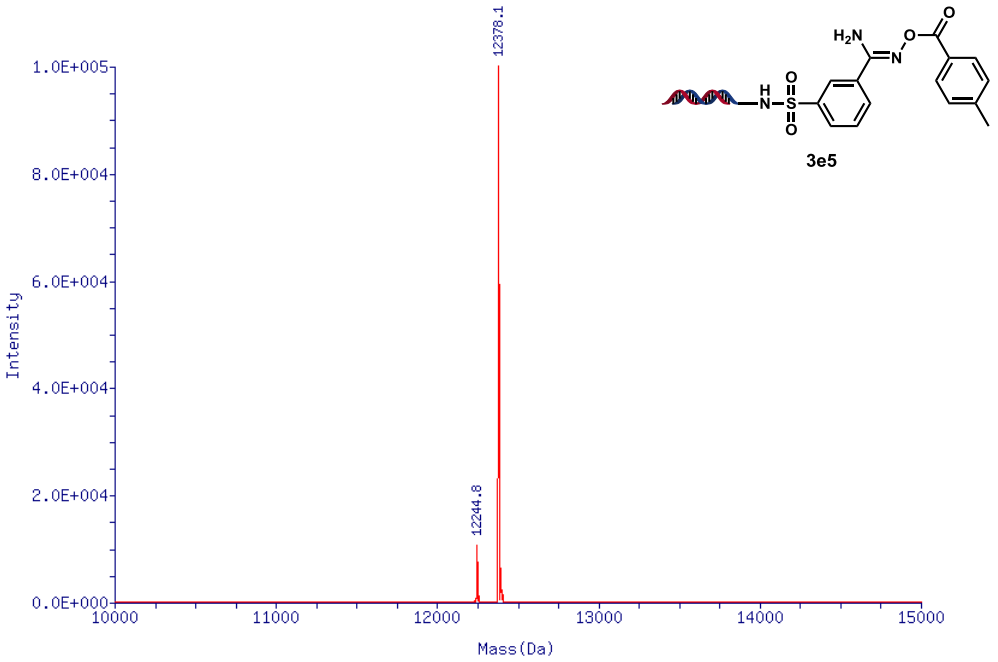



**Figure S56.** Deconvoluted mass spectrum of compound **3e5**, expected: 12376.3; observed 12378.1.

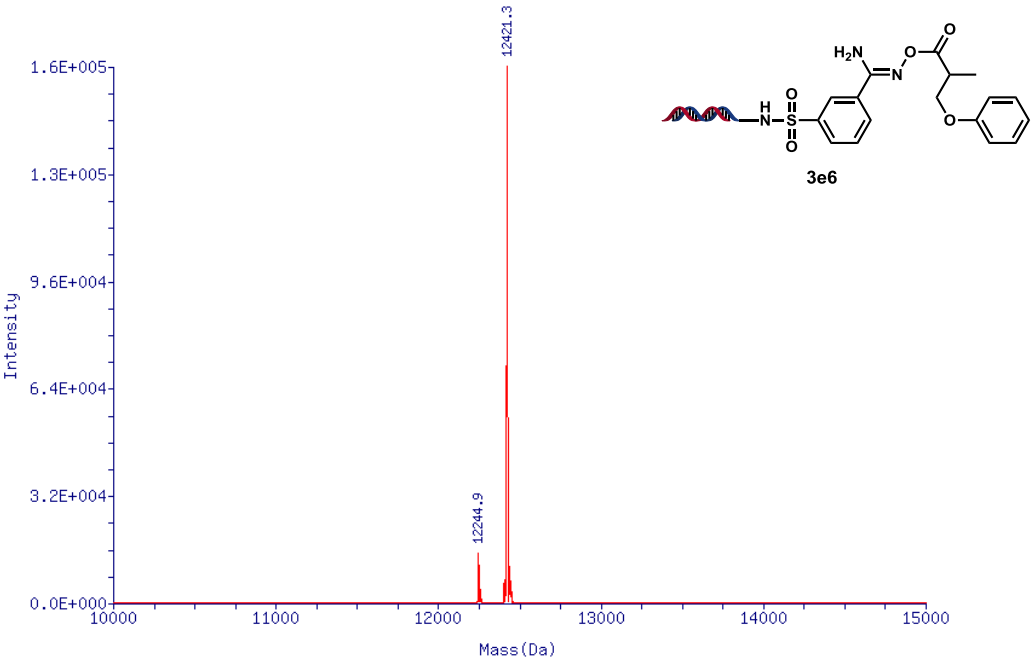



**Figure S57.** Deconvoluted mass spectrum of compound **3e6**, expected: 12420.4; observed 12421.3.

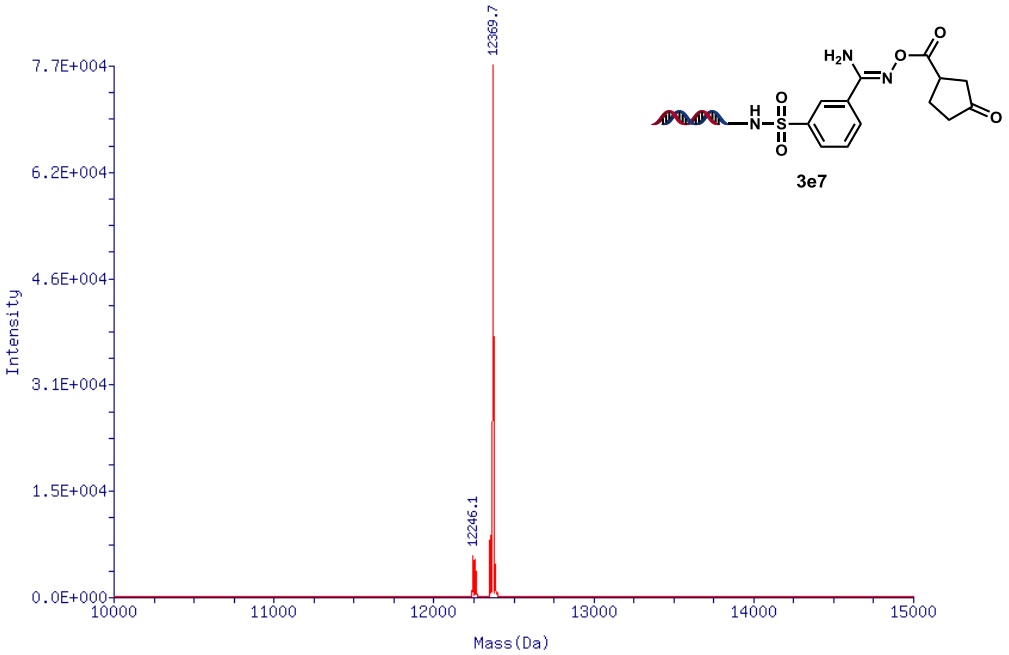



**Figure S58.** Deconvoluted mass spectrum of compound **3e7**, expected: 12368.3; observed 12369.7.

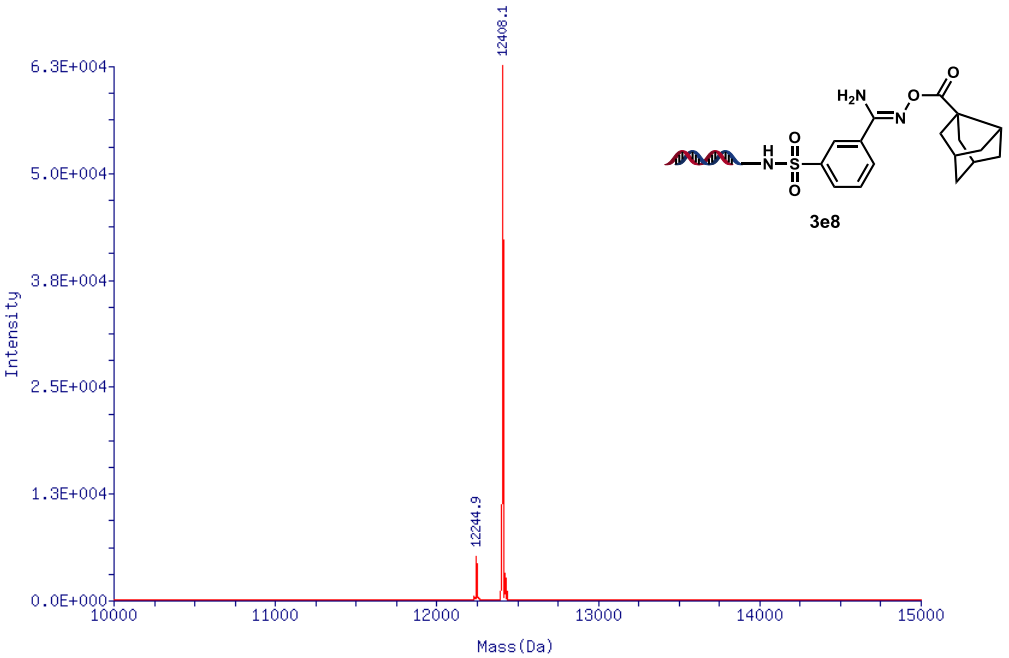



**Figure S59.** Deconvoluted mass spectrum of compound **3e8**, expected: 12406.4; observed 12408.1.

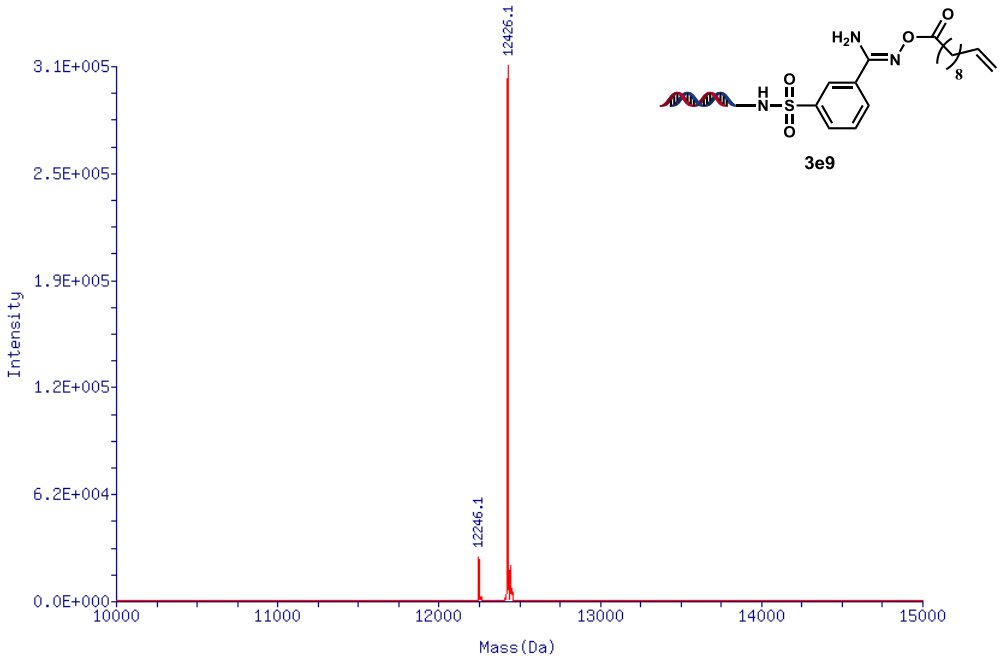



**Figure S60.** Deconvoluted mass spectrum of compound **3e9**, expected: 12424.5; observed 12426.1.

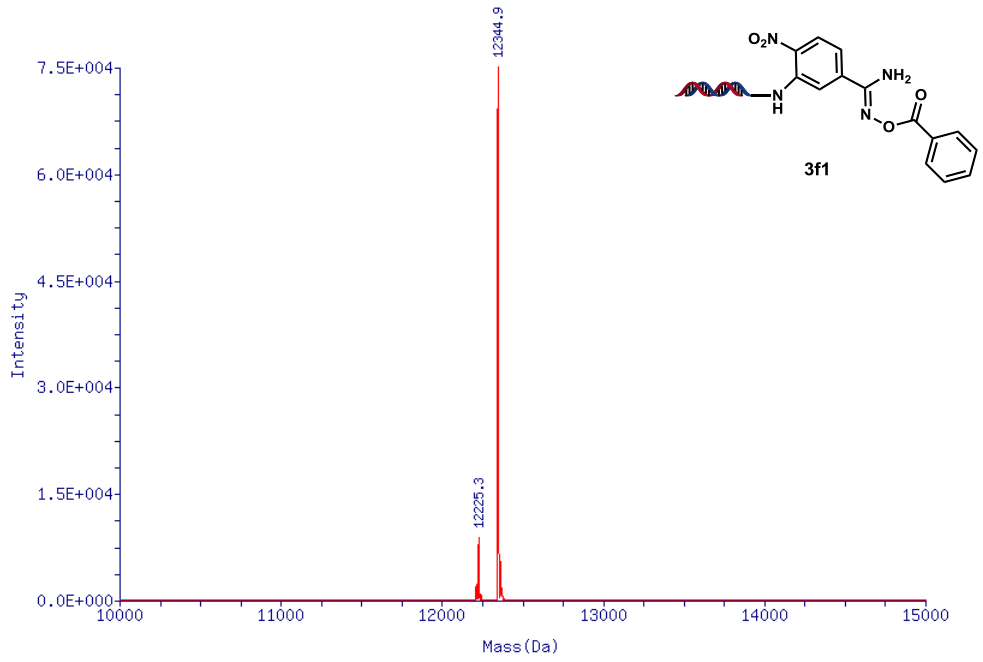



**Figure S61.** Deconvoluted mass spectrum of compound **3f1**, expected: 12343.2; observed 12344.9.

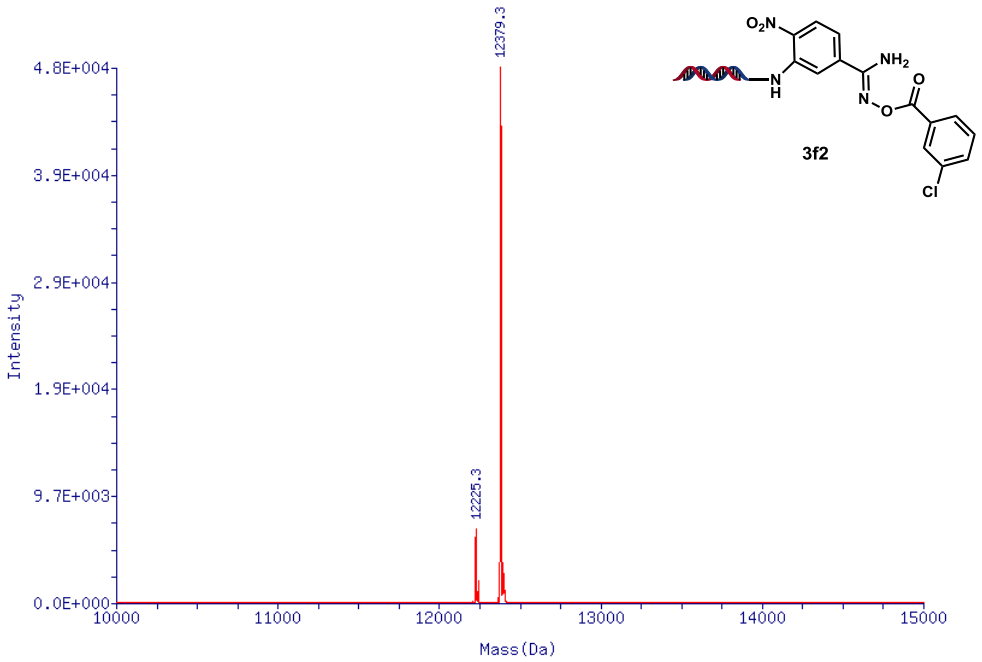



**Figure S62.** Deconvoluted mass spectrum of compound **3f2**, expected: 12377.7; observed 12379.3.

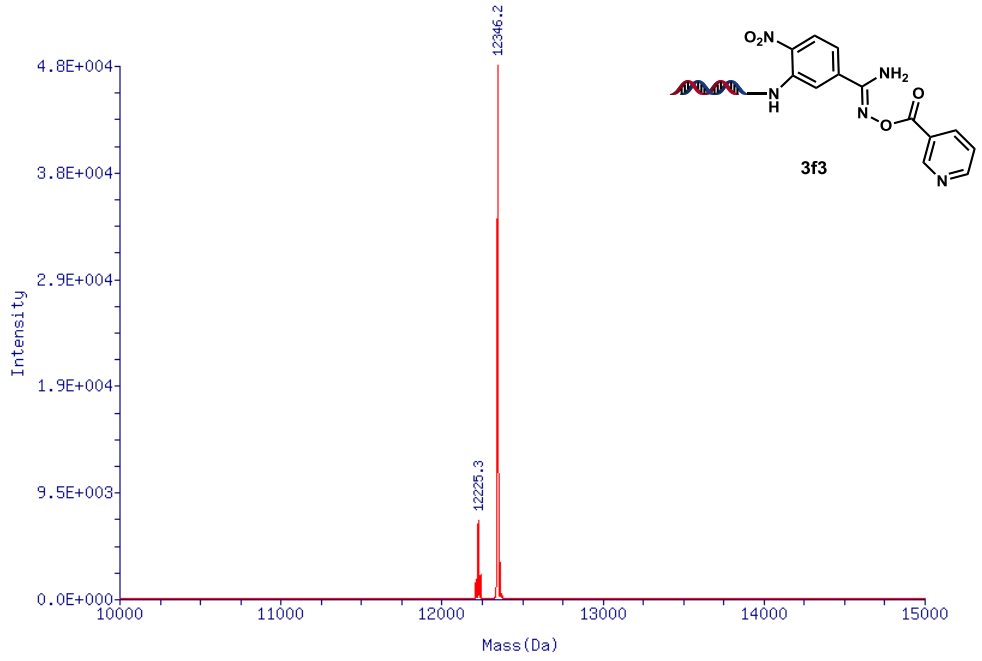



**Figure S63.** Deconvoluted mass spectrum of compound **3f3**, expected: 12344.2; observed 12346.2.

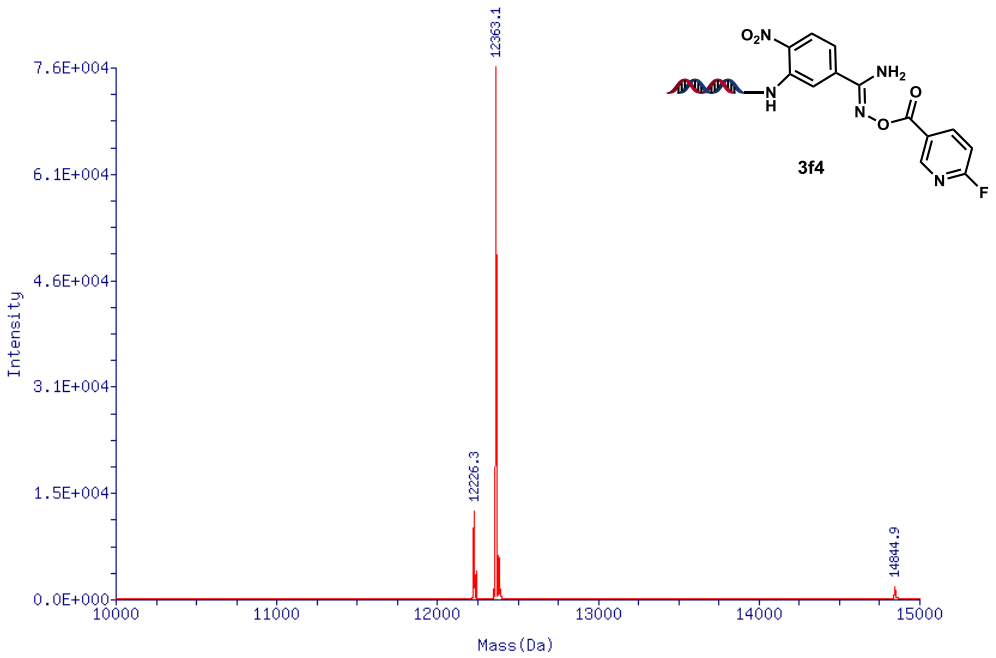



**Figure S64.** Deconvoluted mass spectrum of compound **3f4**, expected: 12362.2; observed 12363.1.

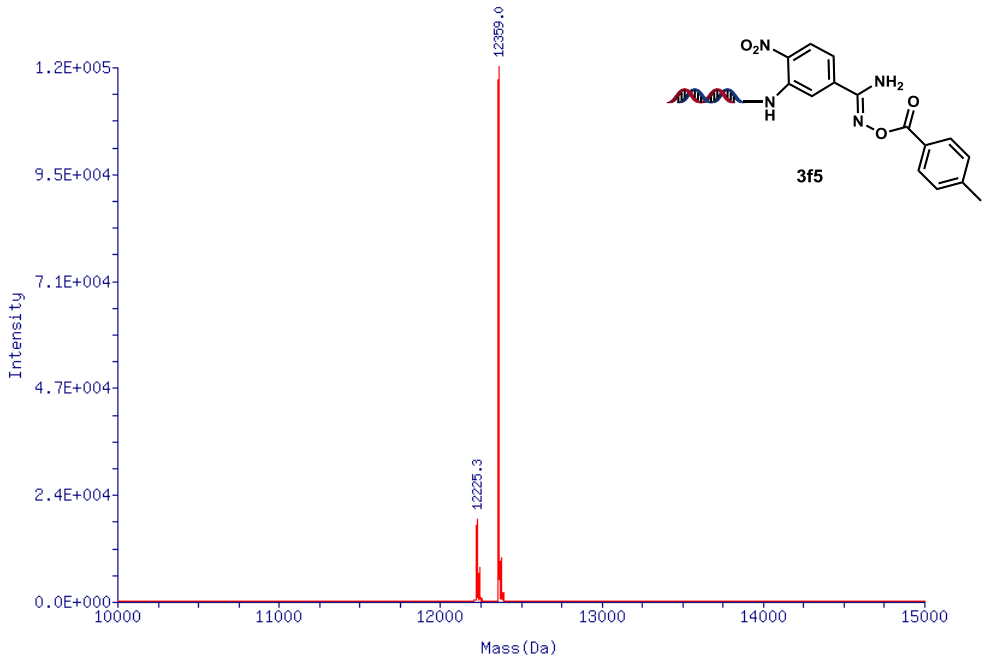



**Figure S65.** Deconvoluted mass spectrum of compound **3f5**, expected: 12357.3; observed 12359.0.

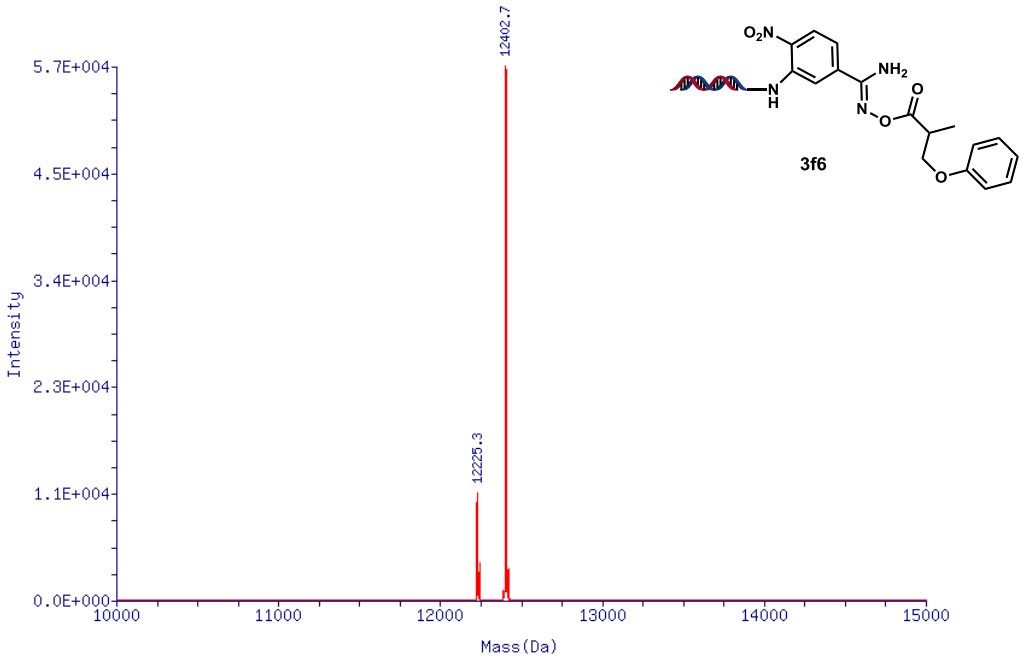



**Figure S66.** Deconvoluted mass spectrum of compound **3f6**, expected: 12401.3; observed 12402.7.

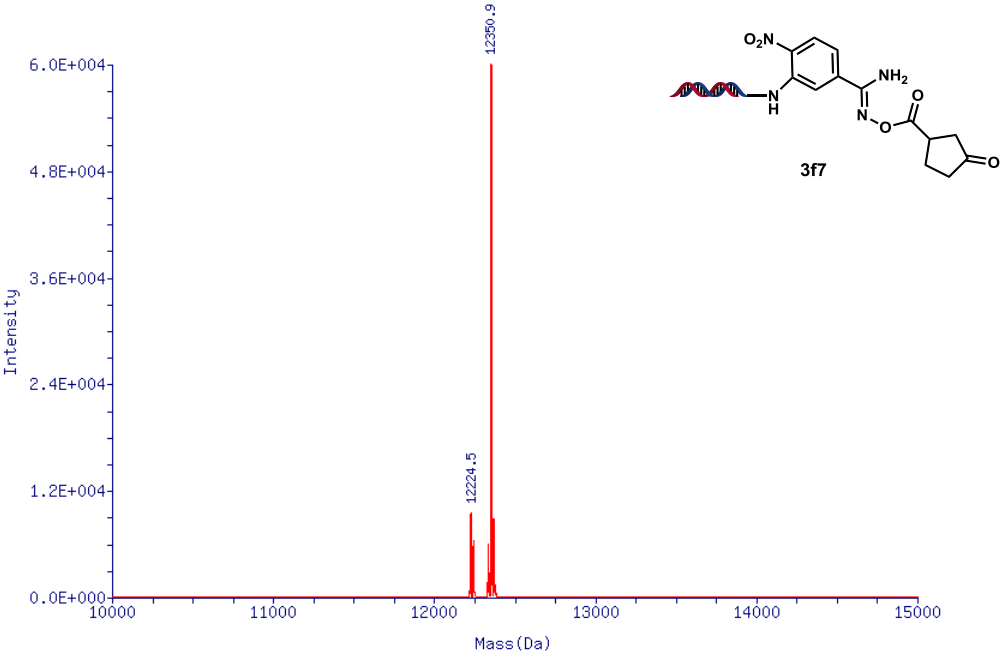



**Figure S67.** Deconvoluted mass spectrum of compound **3f7**, expected: 12349.2; observed 12350.9.

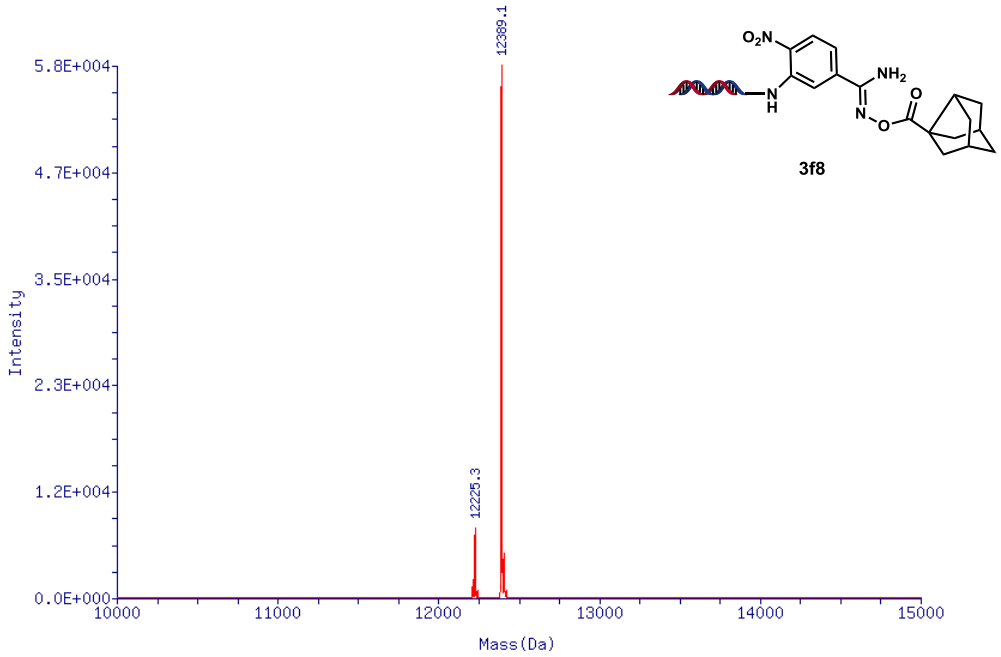



**Figure S68.** Deconvoluted mass spectrum of compound **3f8**, expected: 12387.3; observed 12389.1.

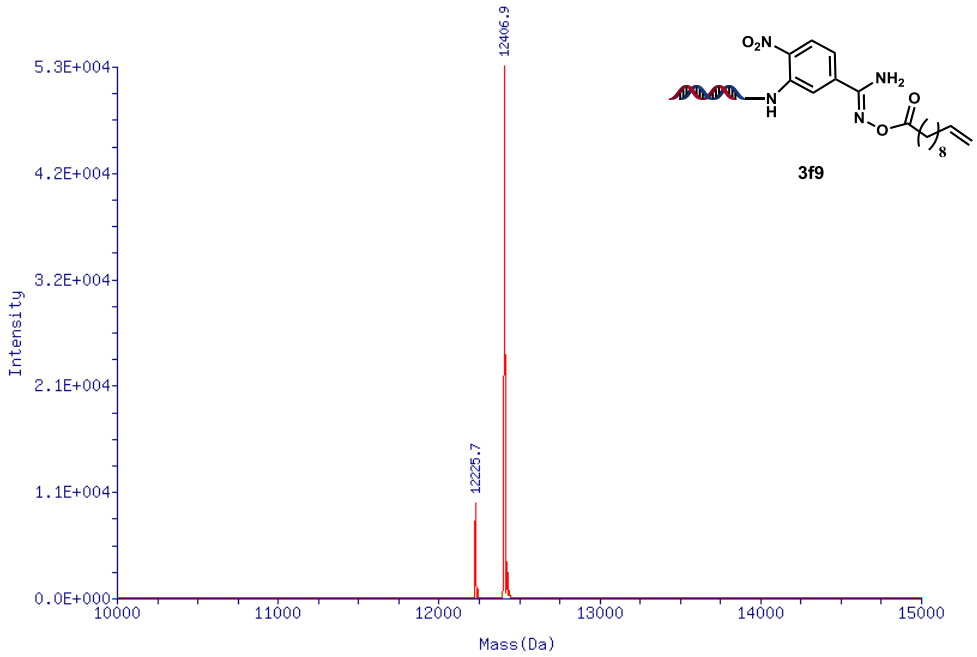



**Figure S69.** Deconvoluted mass spectrum of compound **3f9**, expected: 12405.4; observed 12406.9.

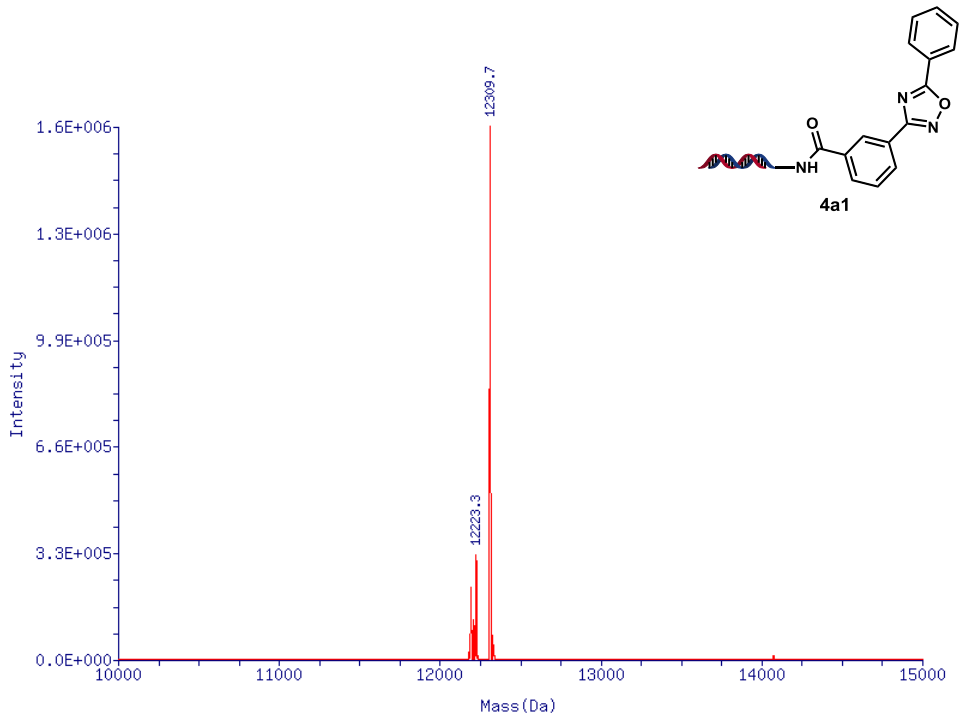



**Figure S70.** Deconvoluted mass spectrum of compound **4a1**, expected: 12308.2; observed 12309.7.

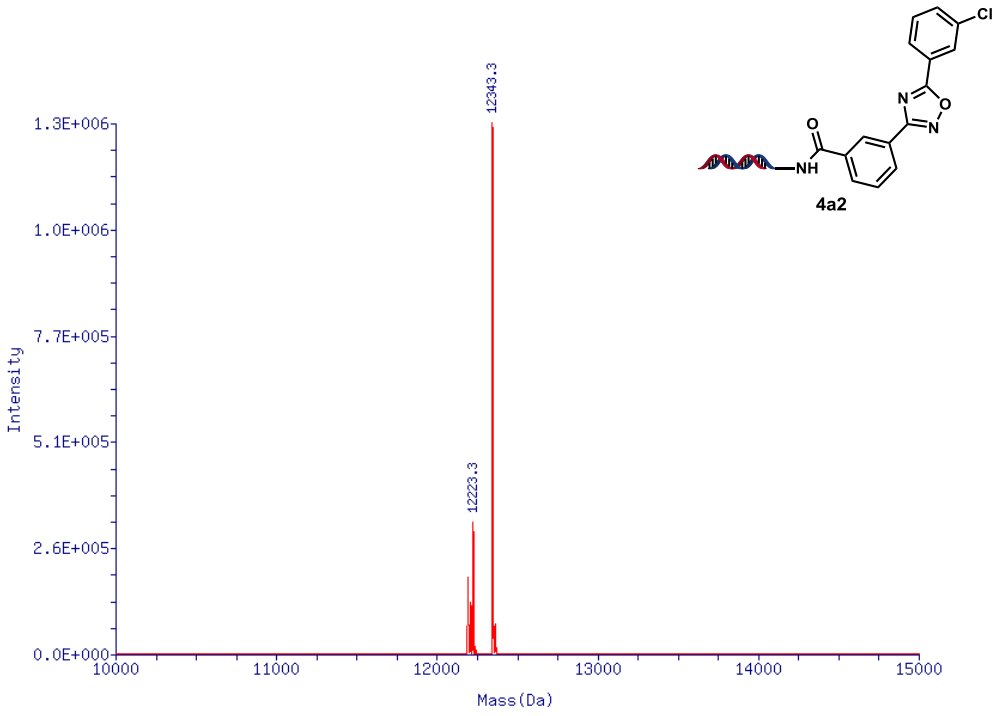



**Figure S71.** Deconvoluted mass spectrum of compound **4a2**, expected: 12342.7; observed 12343.3.

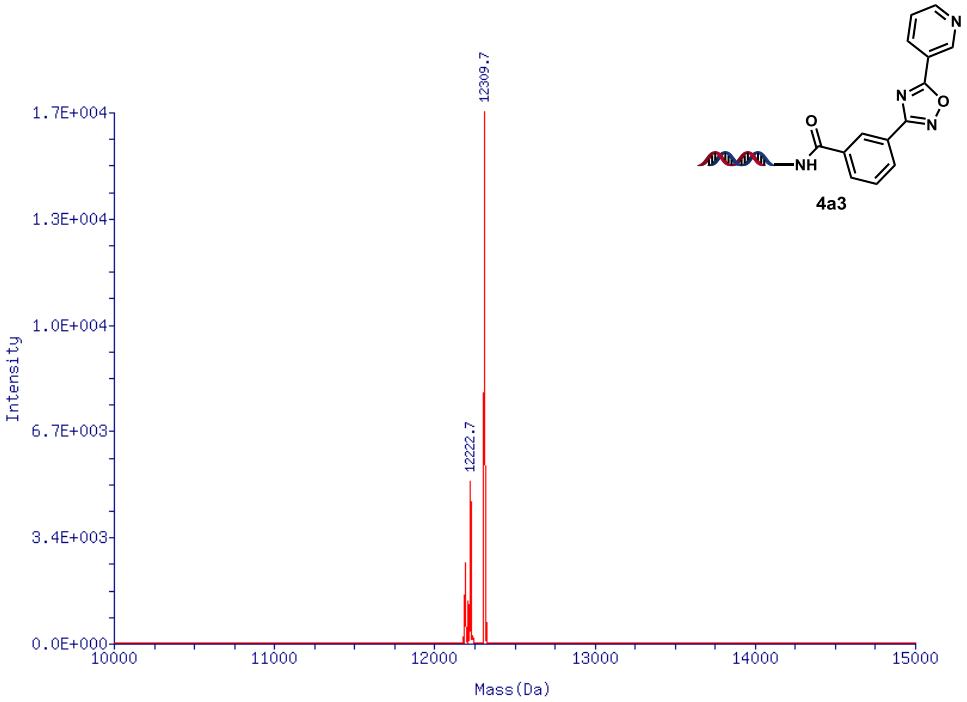



**Figure S72.** Deconvoluted mass spectrum of compound **4a3**, expected: 12309.2; observed 12309.7.

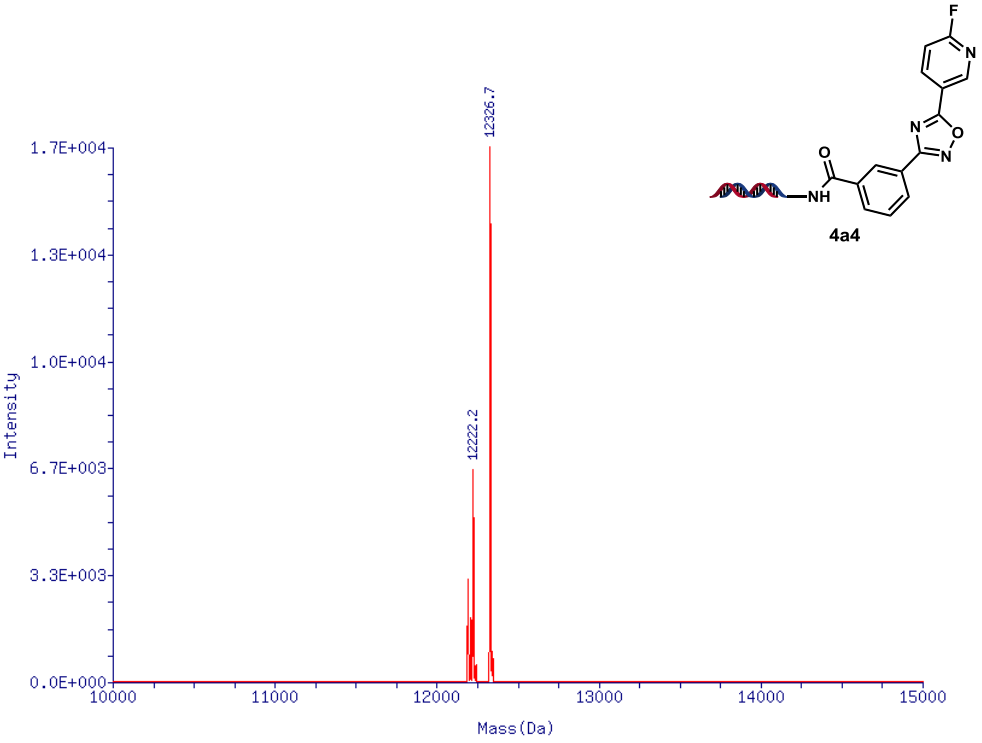



**Figure S73.** Deconvoluted mass spectrum of compound **4a4**, expected: 12327.2; observed 12326.7.

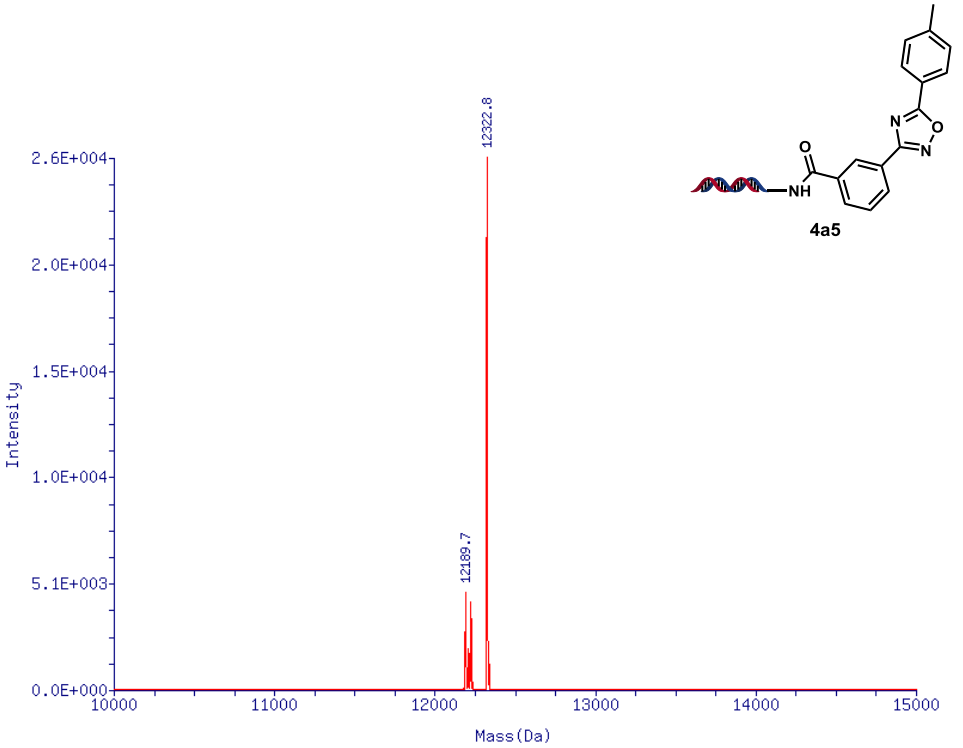



**Figure S74.** Deconvoluted mass spectrum of compound **4a5**, expected: 12322.3; observed 12322.8.

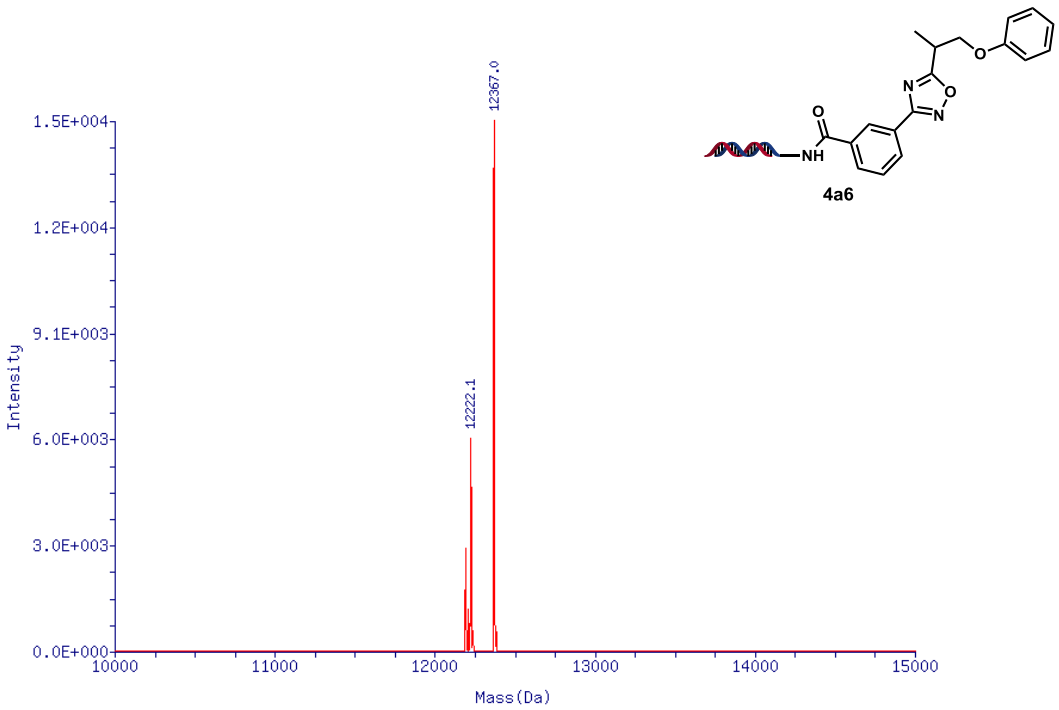



**Figure S75.** Deconvoluted mass spectrum of compound **4a6**, expected: 12366.3; observed 12367.0.

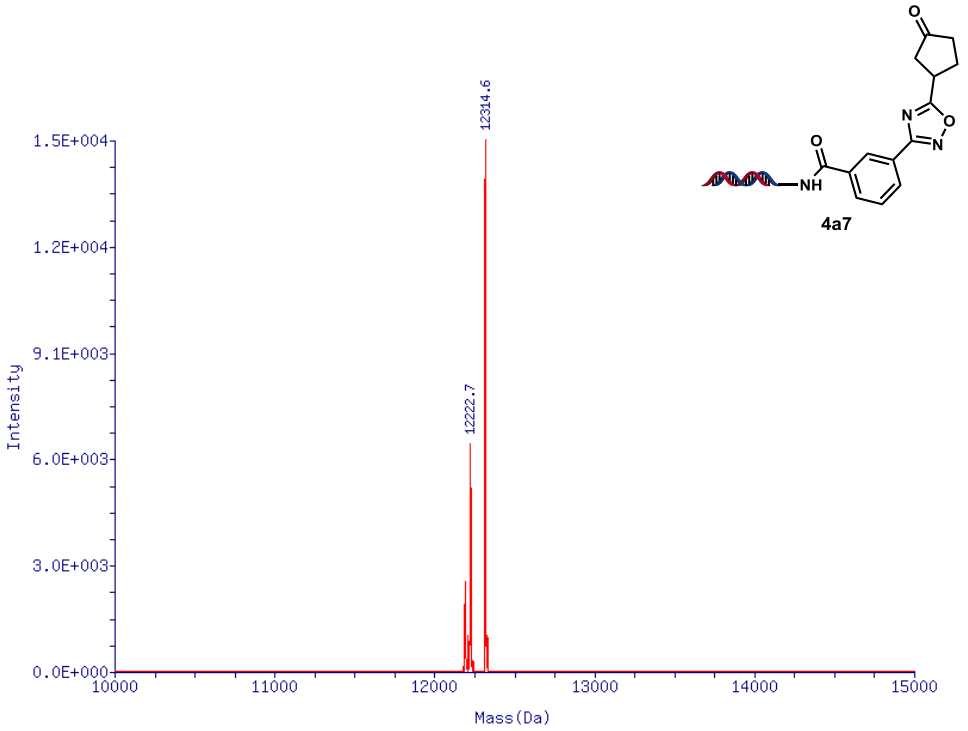



**Figure S76.** Deconvoluted mass spectrum of compound **4a7**, expected: 12314.3; observed 12314.6.

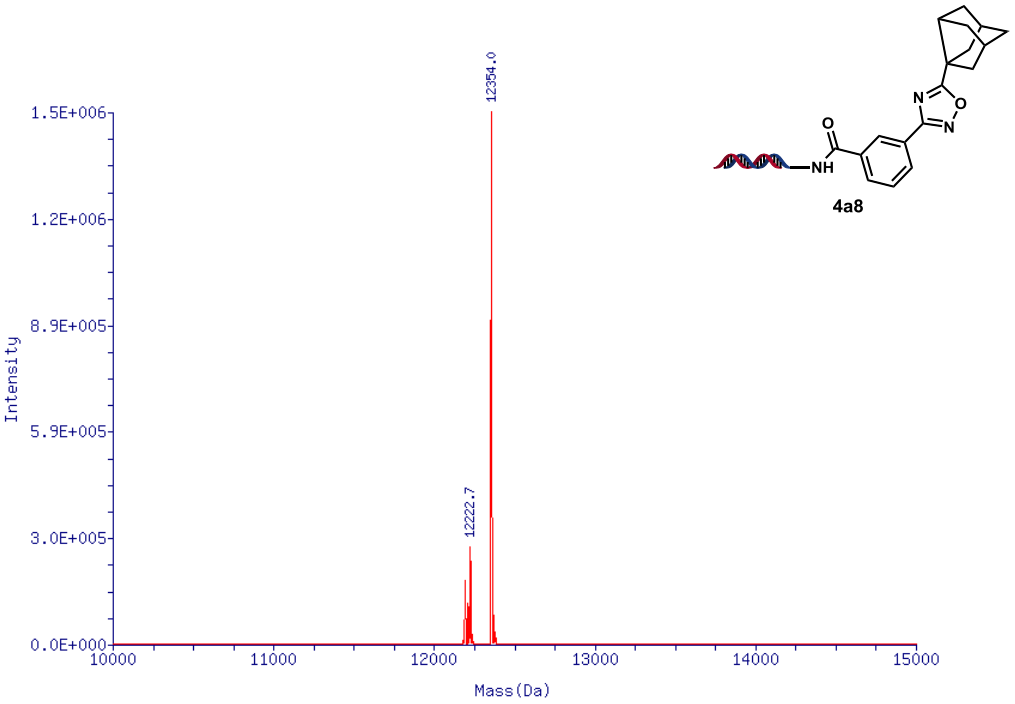



**Figure S77.** Deconvoluted mass spectrum of compound **4a8**, expected: 12352.4; observed 12354.0.

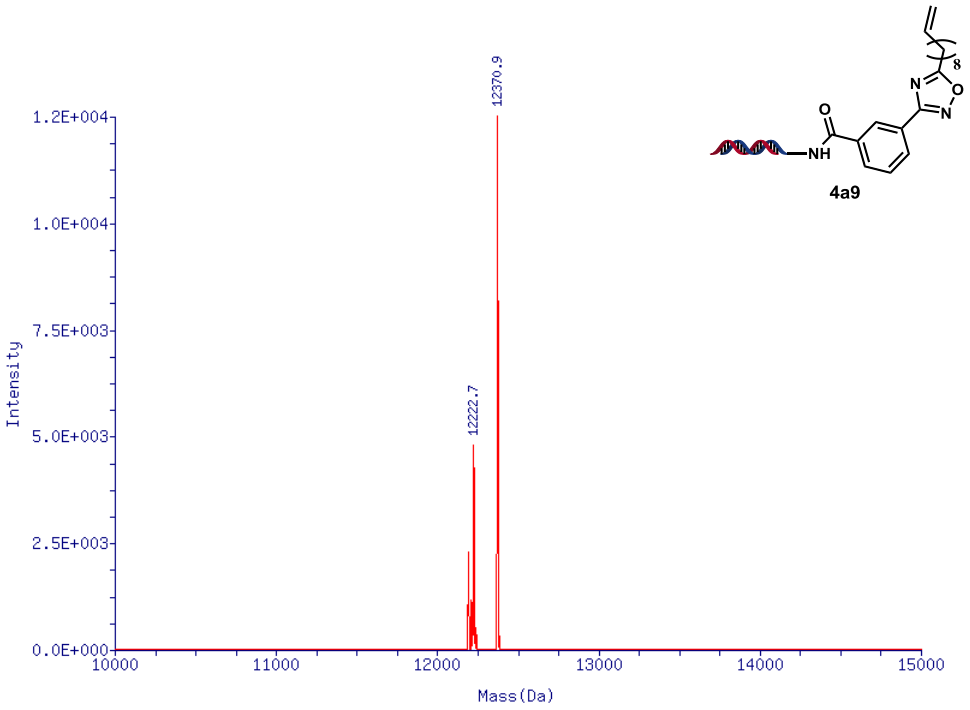



**Figure S78.** Deconvoluted mass spectrum of compound **4a9**, expected: 12370.4; observed 12370.9.

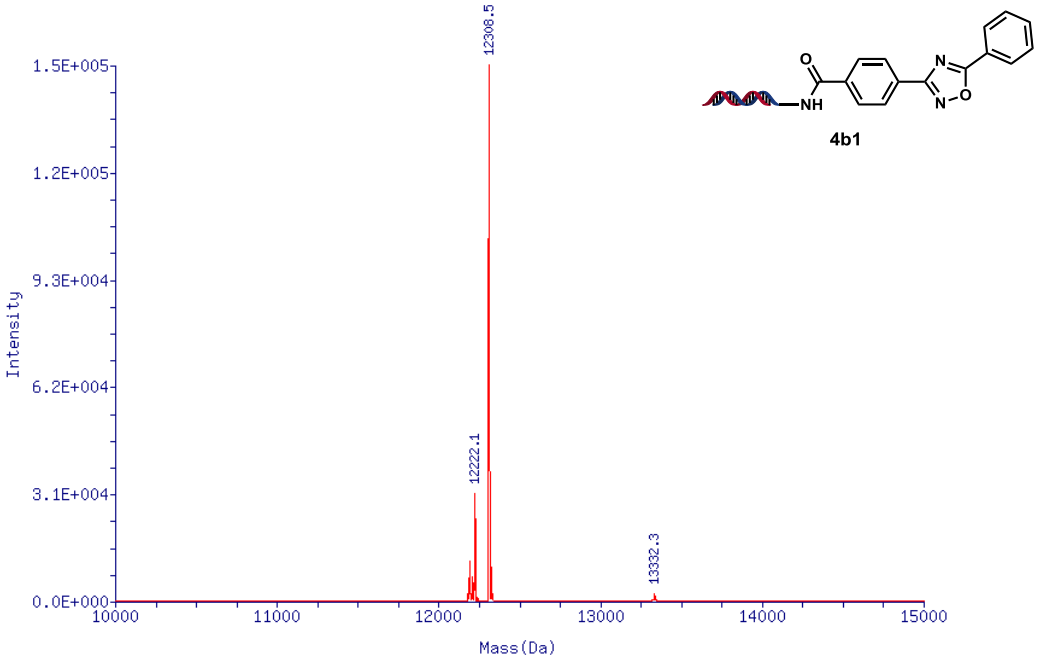



**Figure S79.** Deconvoluted mass spectrum of compound **4b1**, expected: 12308.2; observed 12308.5.

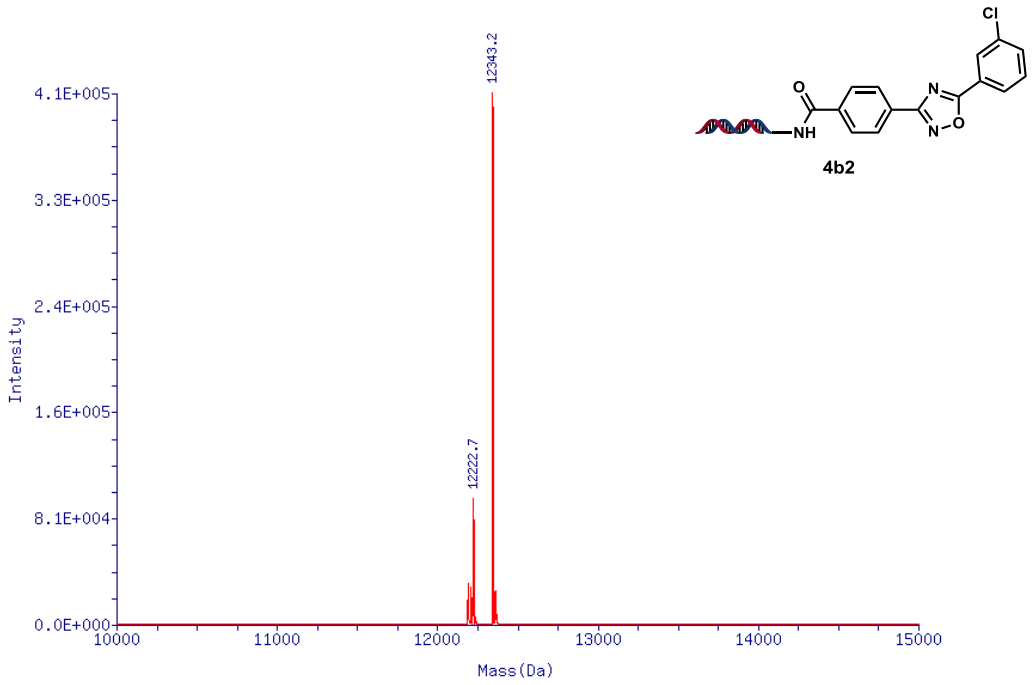



**Figure S80.** Deconvoluted mass spectrum of compound **4b2**, expected: 12342.7; observed 12343.2.

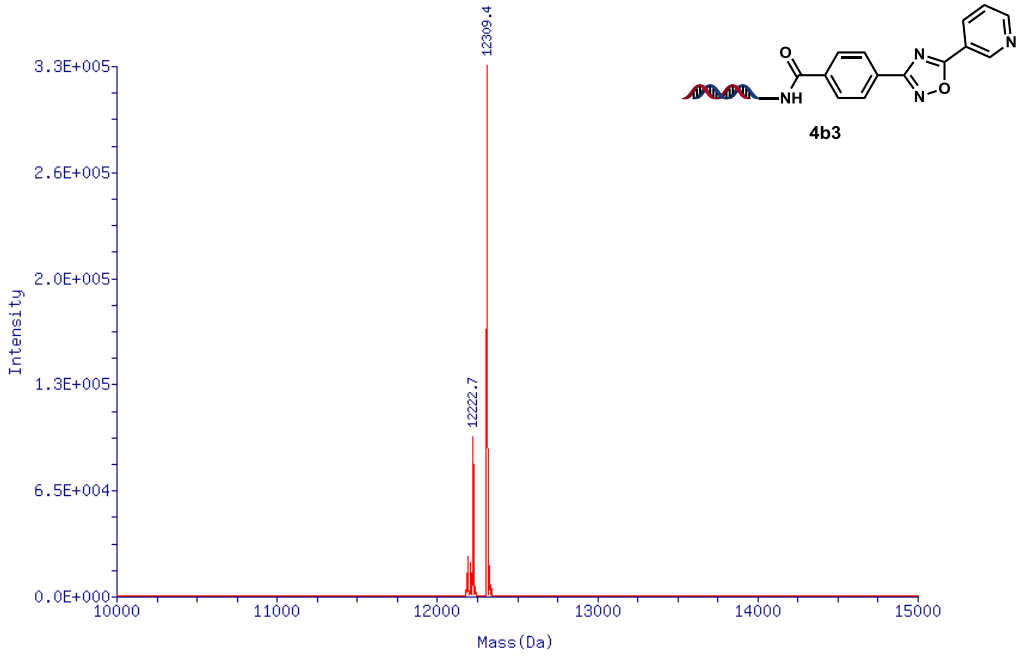



**Figure S81.** Deconvoluted mass spectrum of compound **4b3**, expected: 12309.2; observed 12309.4.

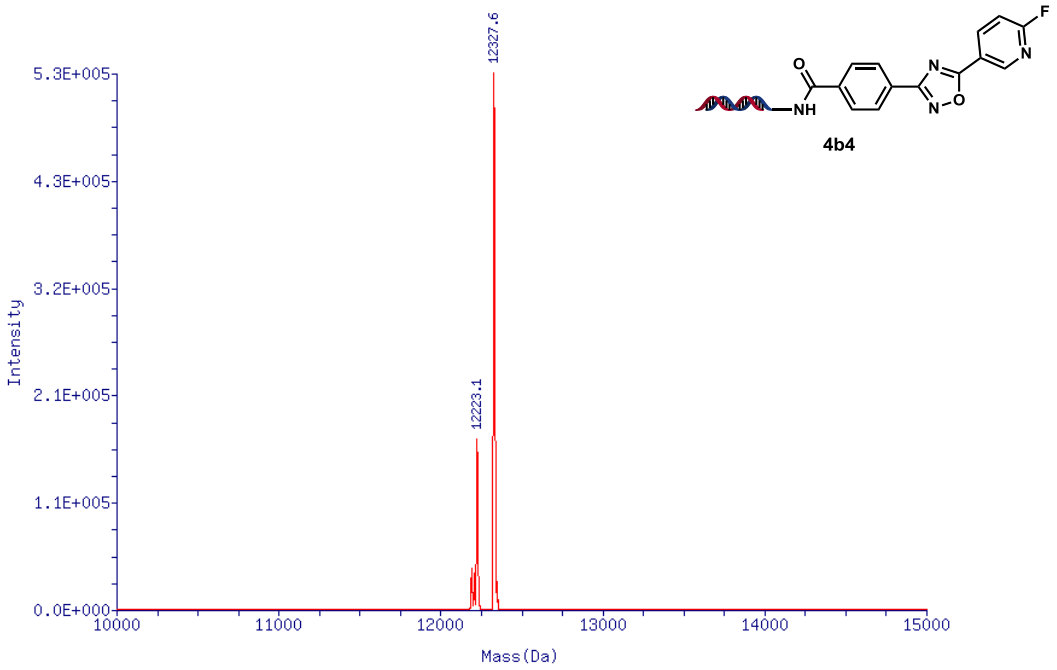



**Figure S82.** Deconvoluted mass spectrum of compound **4b4**, expected: 12372.2; observed 12327.6.

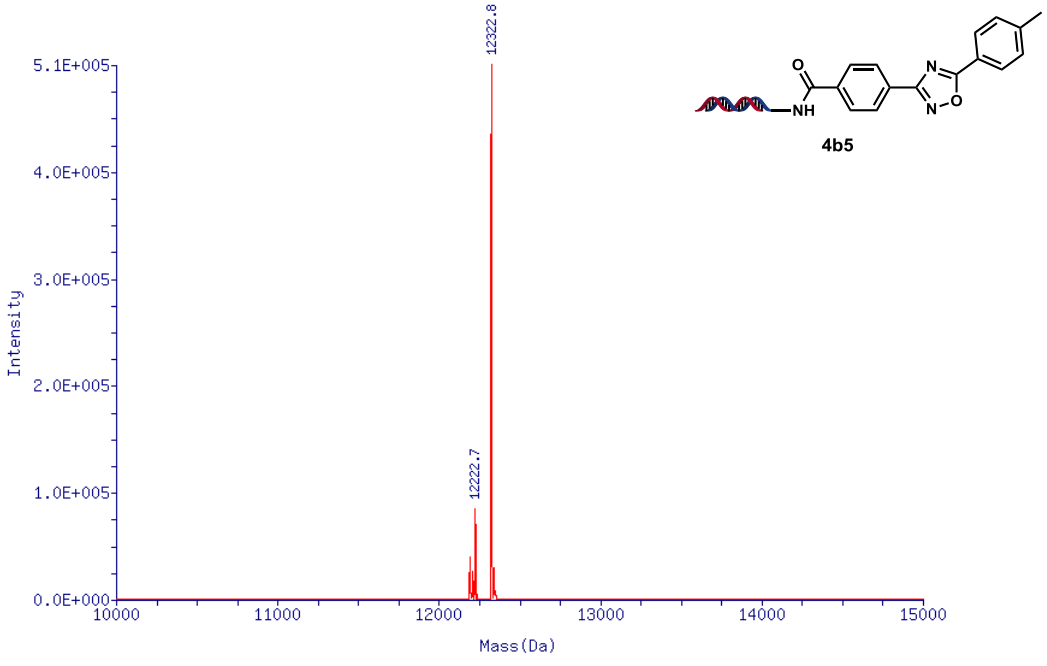



**Figure S83.** Deconvoluted mass spectrum of compound **4b5**, expected: 12322.3; observed 12322.8.

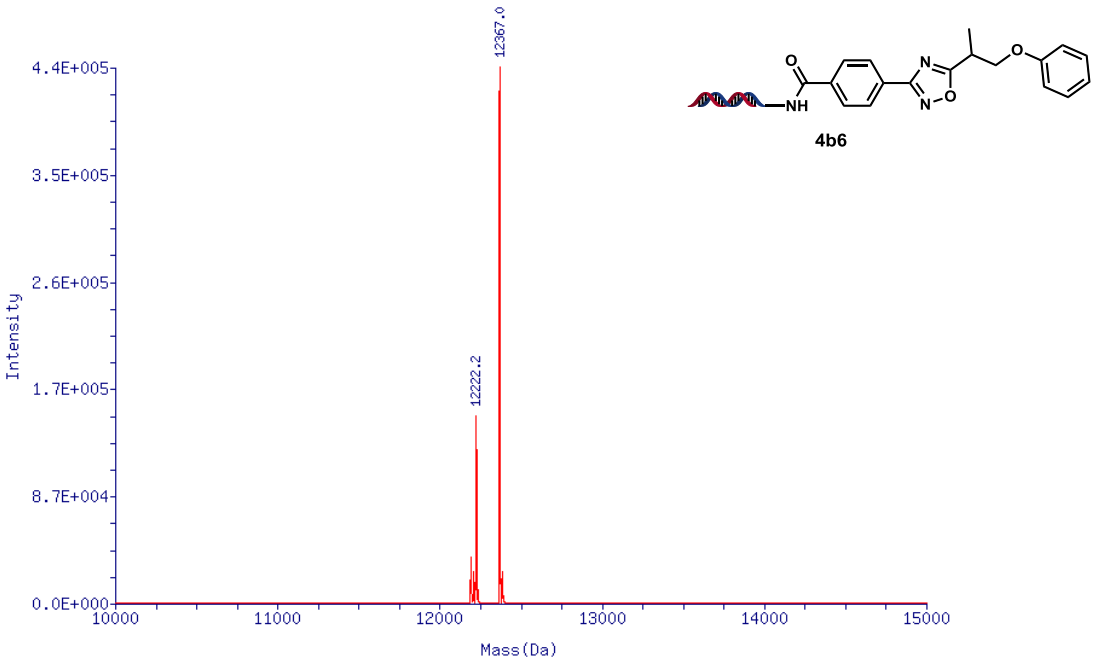



**Figure S84.** Deconvoluted mass spectrum of compound **4b6**, expected: 12366.3; observed 12367.0.

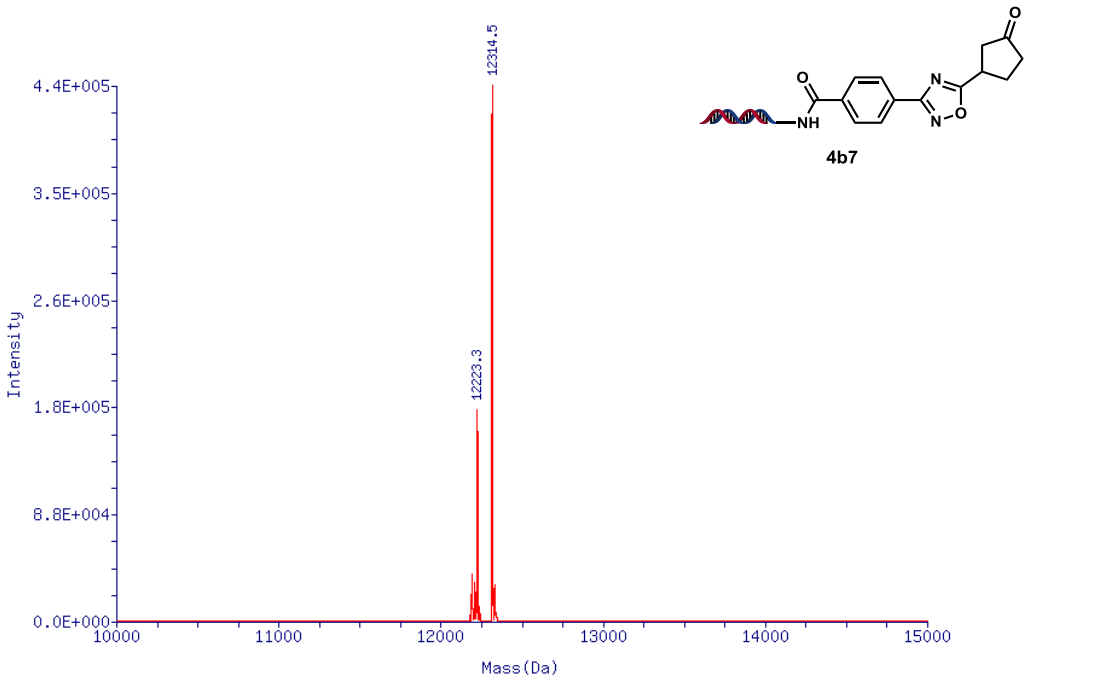



**Figure S85.** Deconvoluted mass spectrum of compound **4b7**, expected: 12314.3; observed 12314.5.

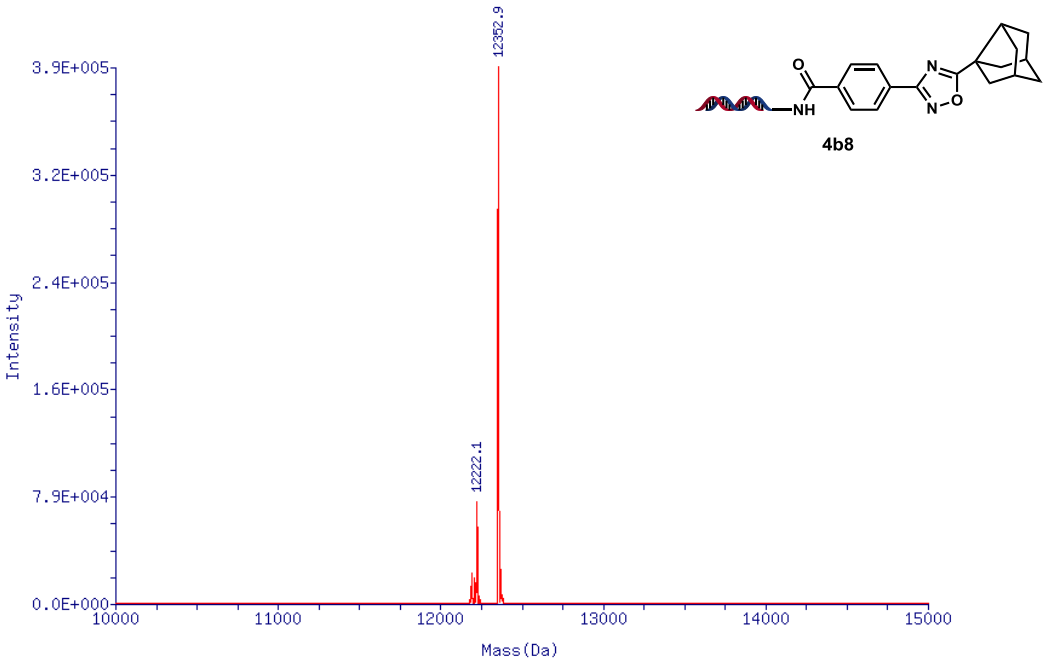



**Figure S86.** Deconvoluted mass spectrum of compound **4b8**, expected: 12352.4; observed 12352.9.

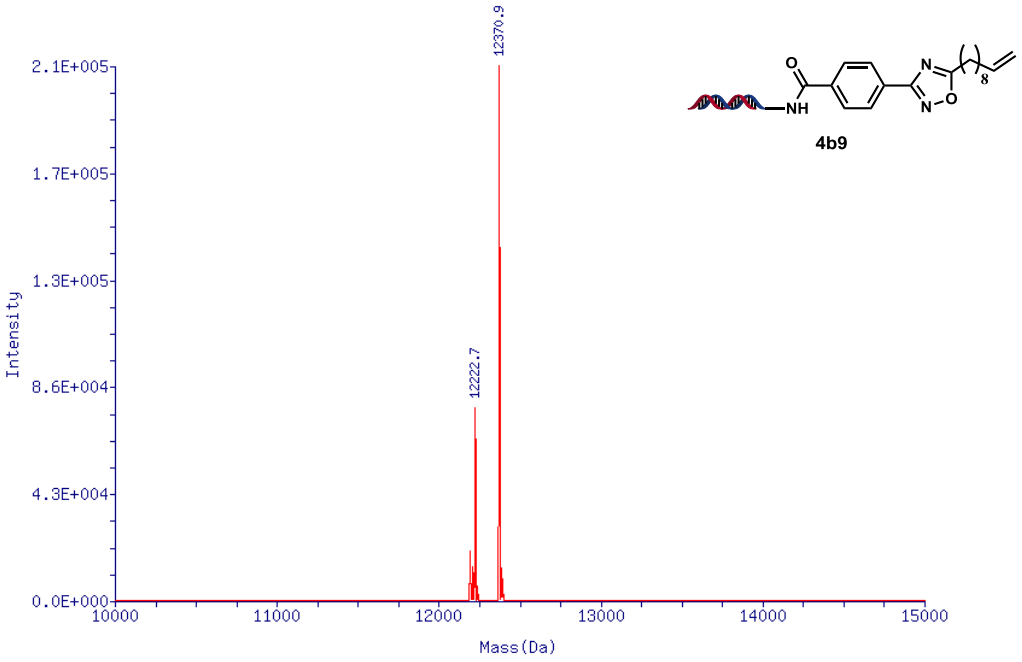



**Figure S87.** Deconvoluted mass spectrum of compound **4b9**, expected: 12370.4; observed 12370.9.

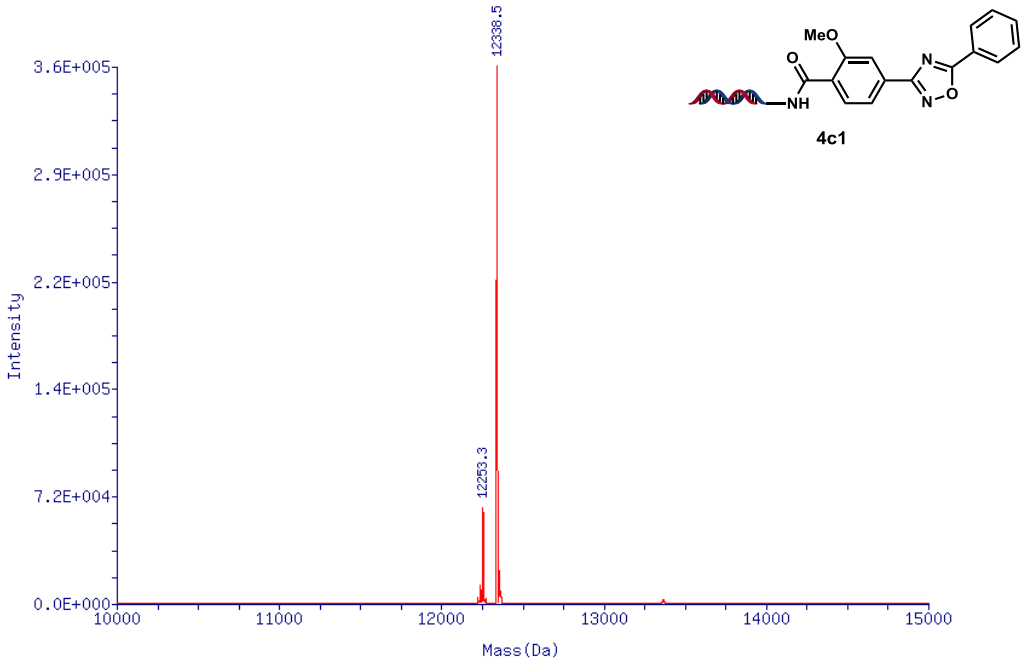



**Figure S88.** Deconvoluted mass spectrum of compound **4c1**, expected: 12338.3; observed 12338.5.

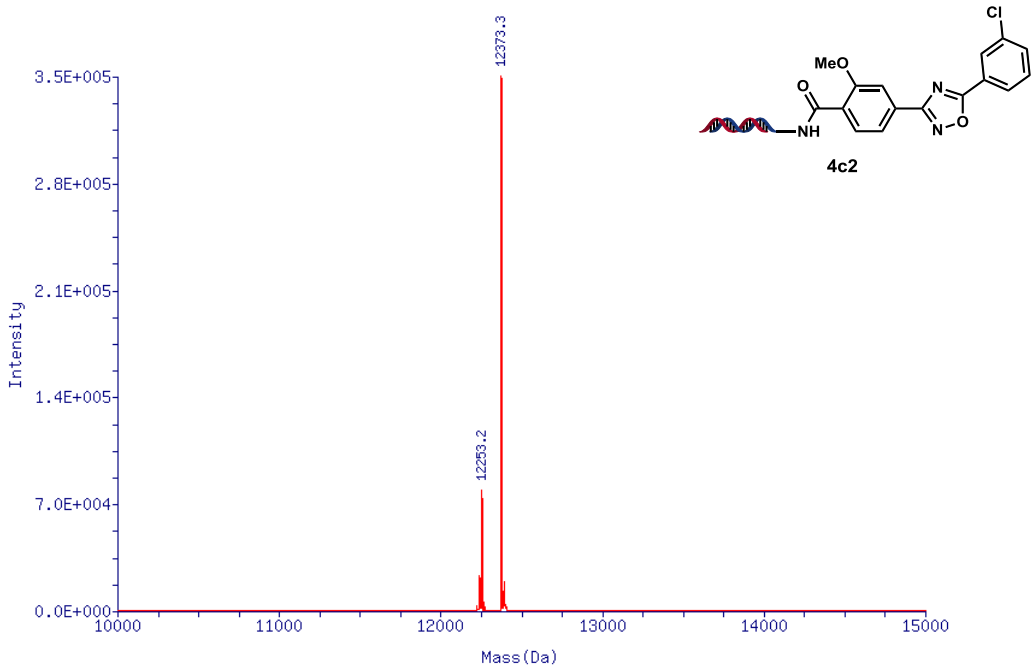



**Figure S89.** Deconvoluted mass spectrum of compound **4c2**, expected: 12372.7; observed 12373.3.

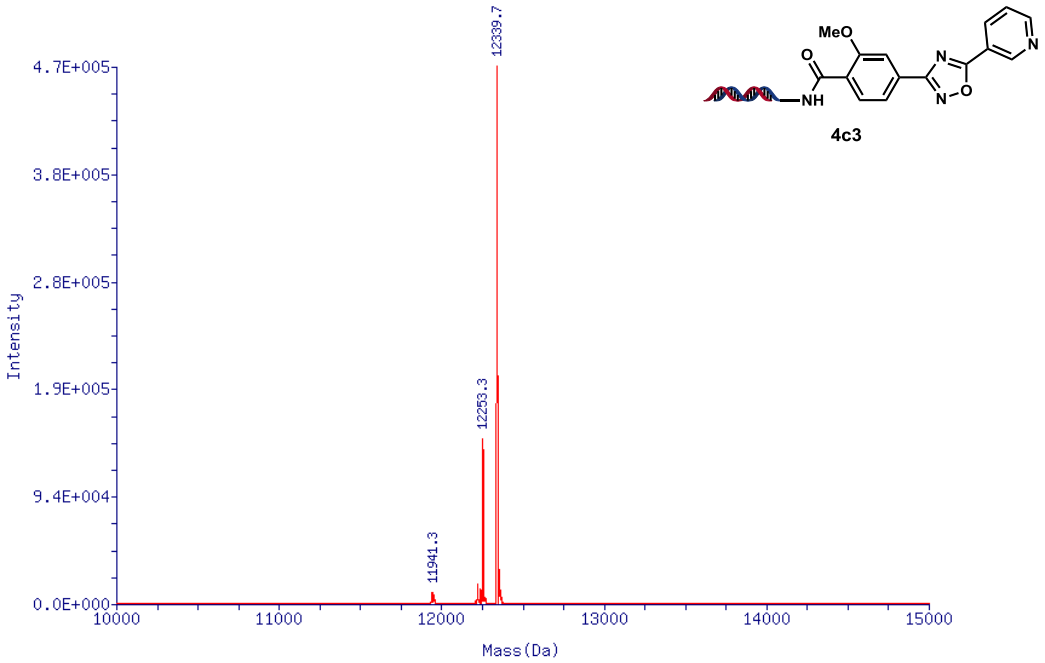



**Figure S90.** Deconvoluted mass spectrum of compound **4c3**, expected: 12339.3; observed 12339.7.

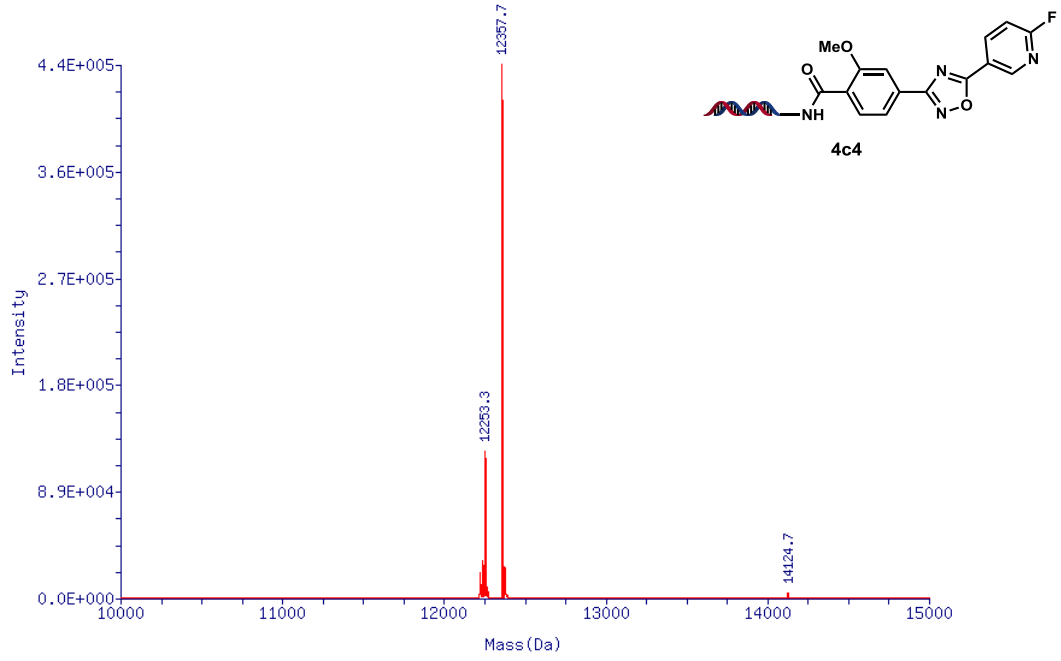



**Figure S91.** Deconvoluted mass spectrum of compound **4c4**, expected: 12357.3; observed 12357.7.

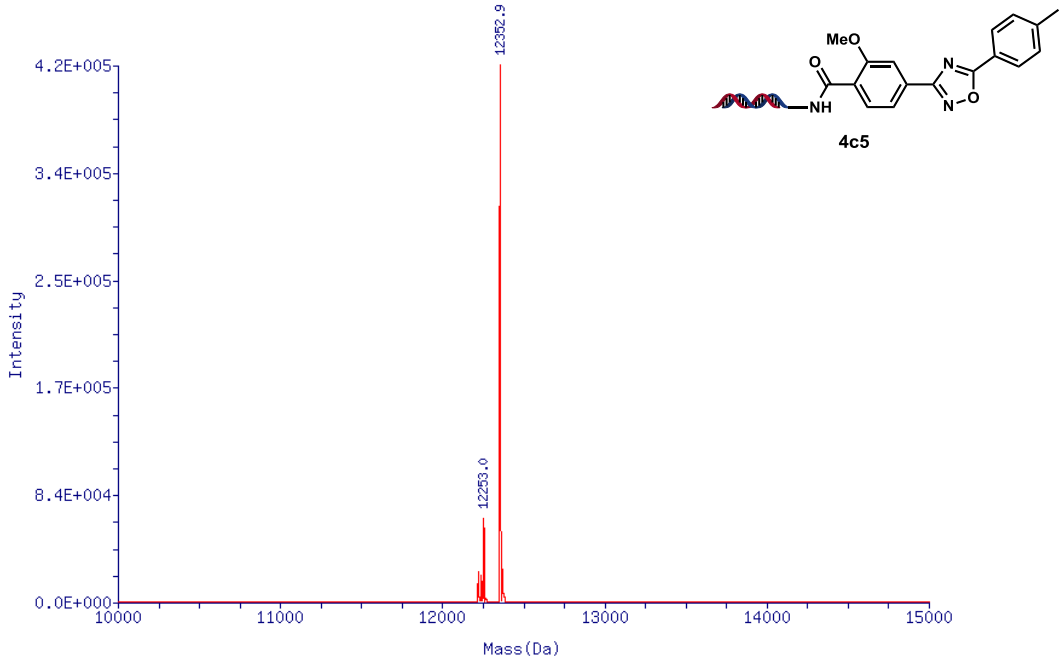



**Figure S92.** Deconvoluted mass spectrum of compound **4c5**, expected: 12352.3; observed 12352.9.

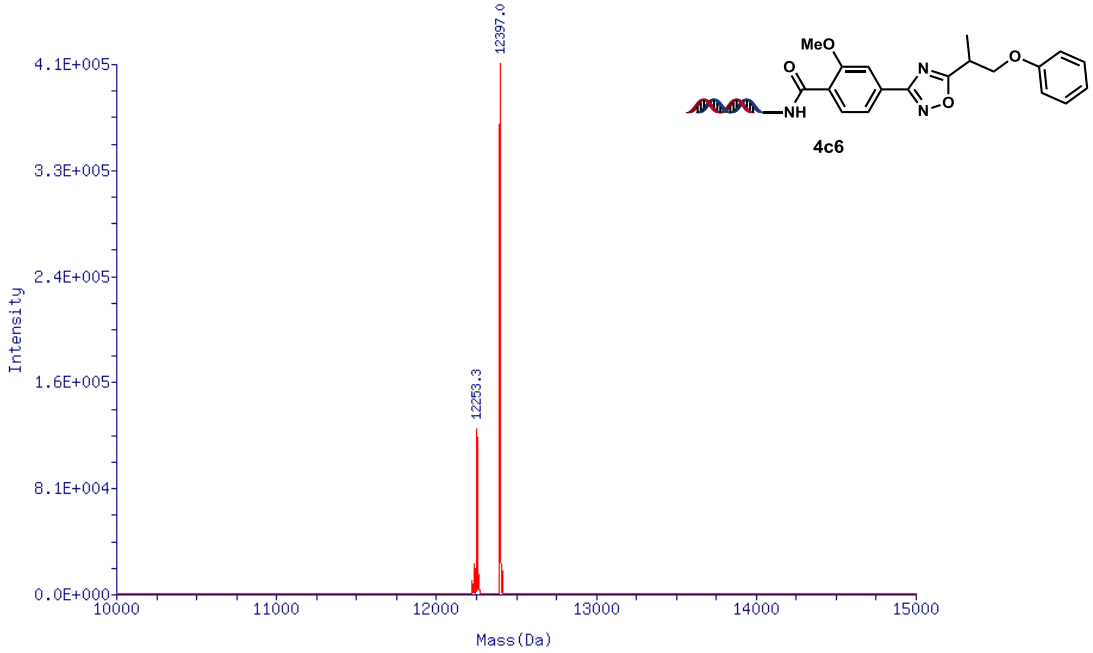



**Figure S93.** Deconvoluted mass spectrum of compound **4c6**, expected: 12396.4; observed 12397.0.

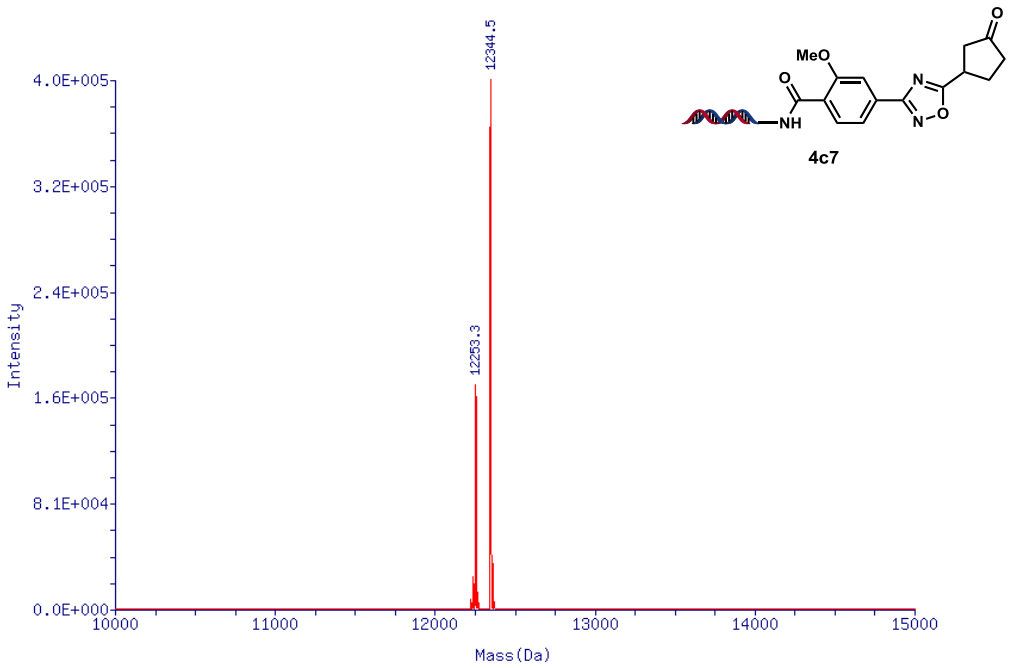



**Figure S94.** Deconvoluted mass spectrum of compound **4c7**, expected: 12344.3; observed 12344.5.

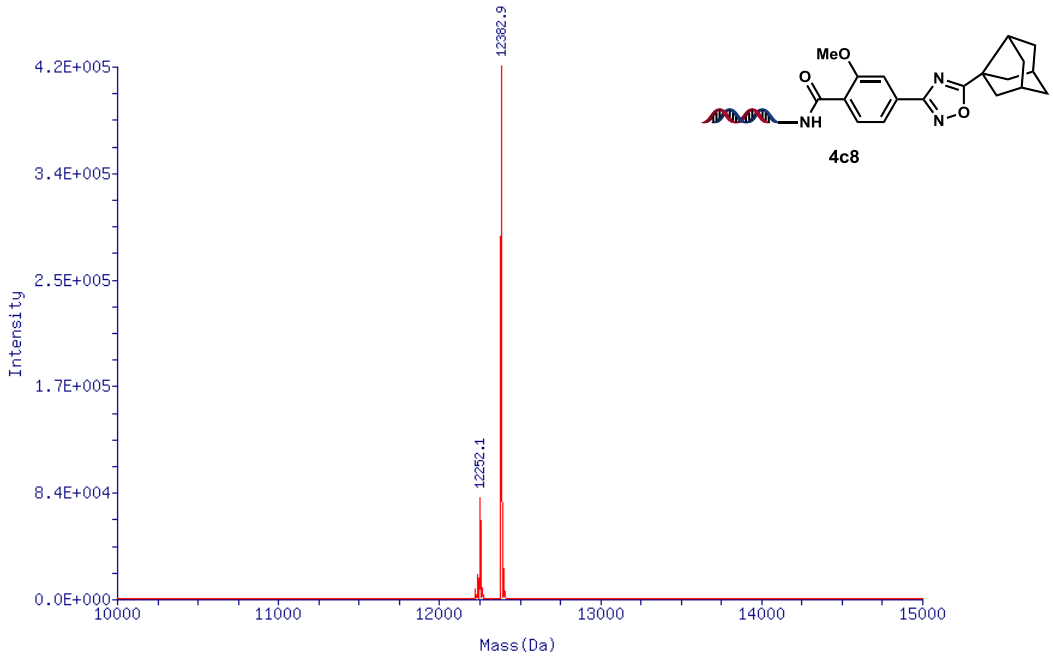



**Figure S95.** Deconvoluted mass spectrum of compound **4c8**, expected: 12382.4; observed 12382.9.

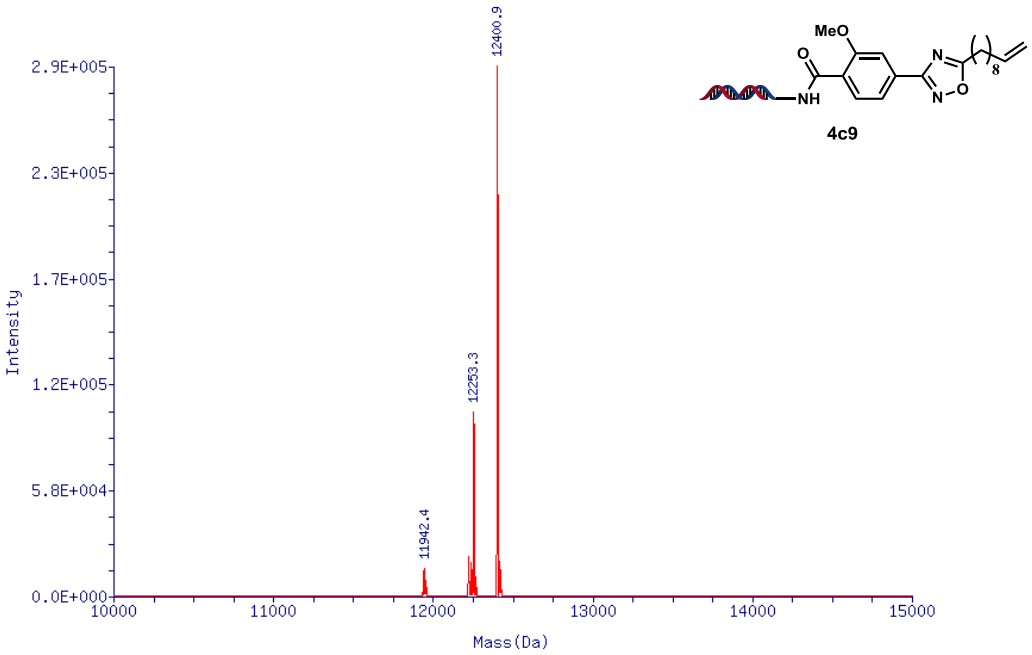



**Figure S96.** Deconvoluted mass spectrum of compound **4c9**, expected: 12400.4; observed 12400.9.

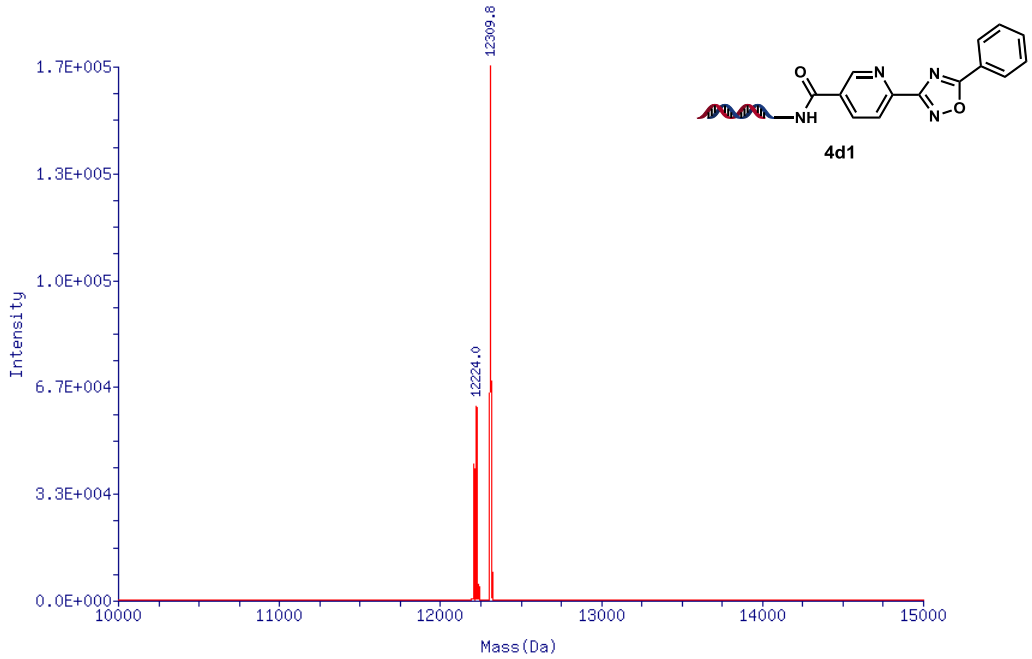



**Figure S97.** Deconvoluted mass spectrum of compound **4d1**, expected: 12309.2; observed 12309.8.

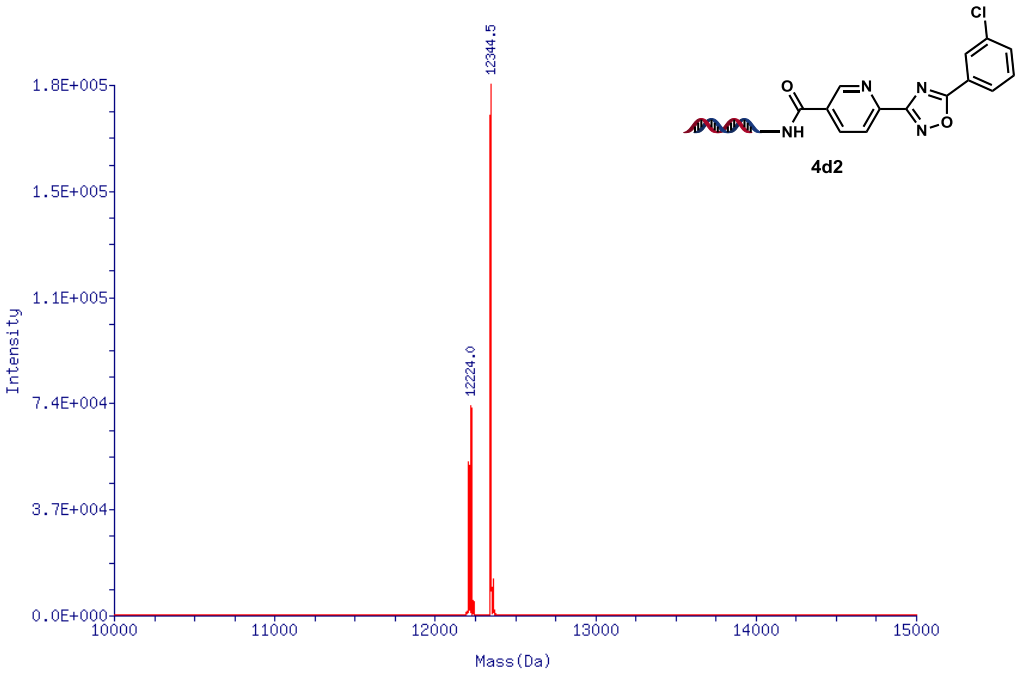



**Figure S98.** Deconvoluted mass spectrum of compound **4d2**, expected: 12343.7; observed 12344.5.

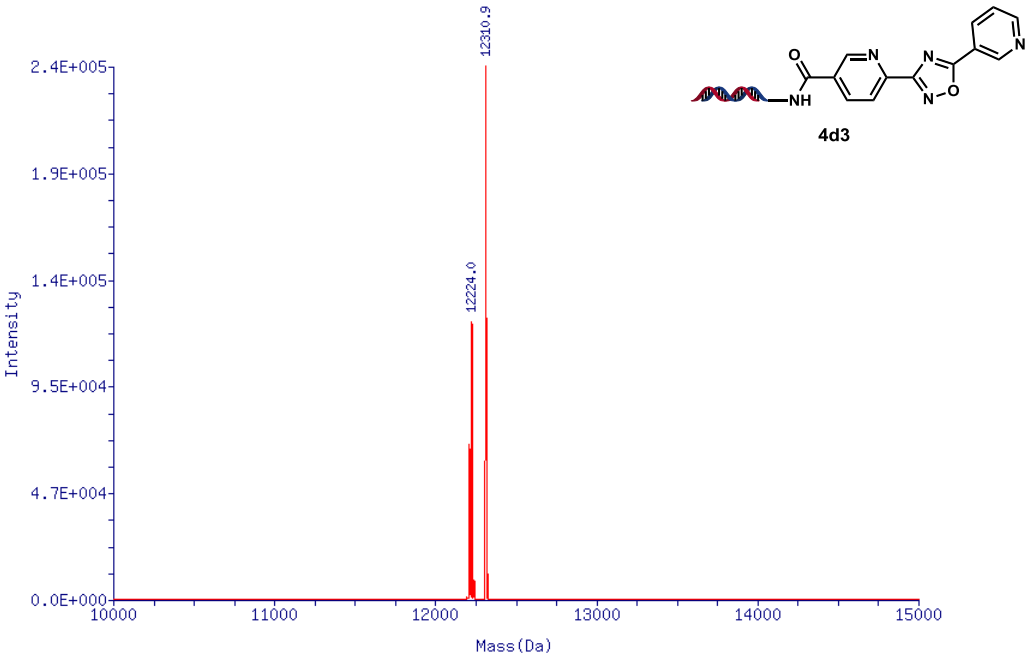



**Figure S99.** Deconvoluted mass spectrum of compound **4d3**, expected: 12310.2; observed 12310.9.

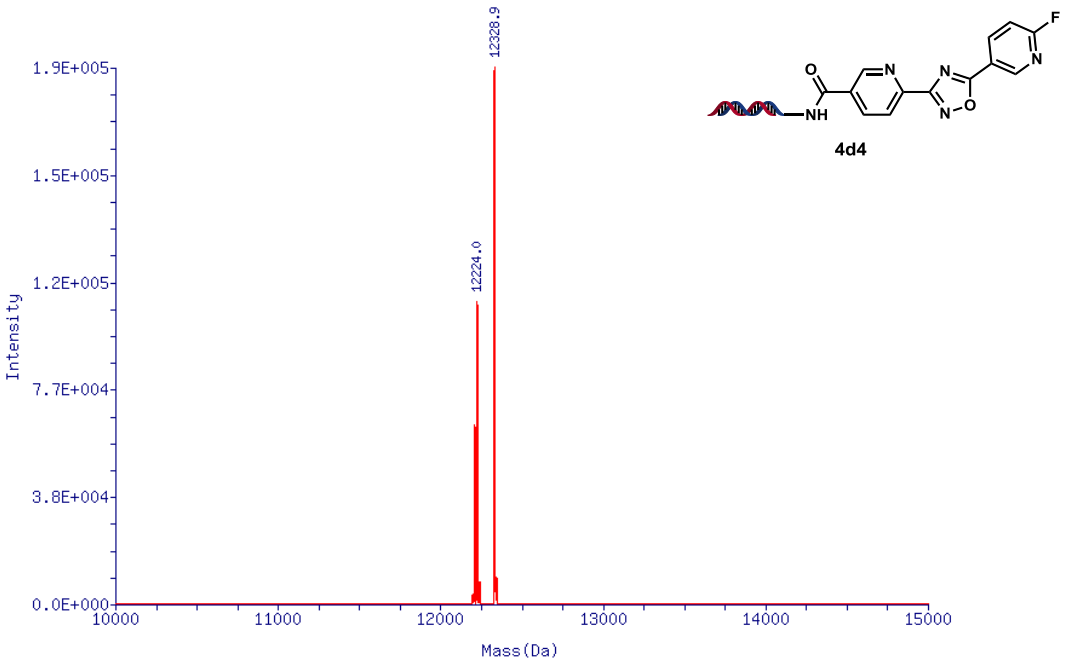



**Figure S100.** Deconvoluted mass spectrum of compound **4d4**, expected: 12328.2; observed 12328.9.

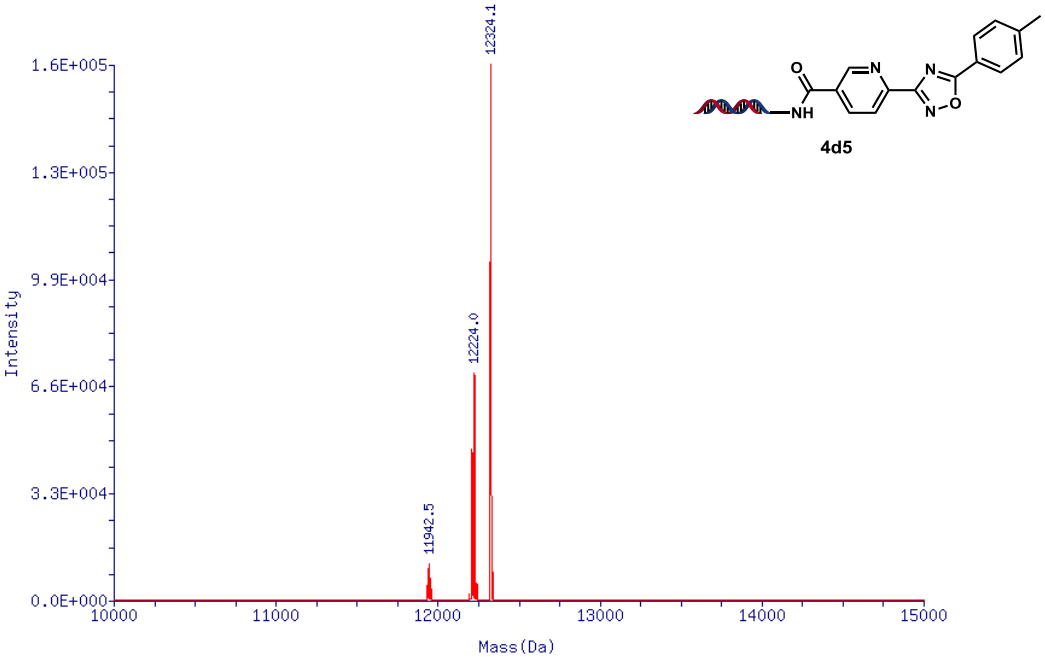



**Figure S101.** Deconvoluted mass spectrum of compound **4d5**, expected: 12323.3; observed 12324.1.

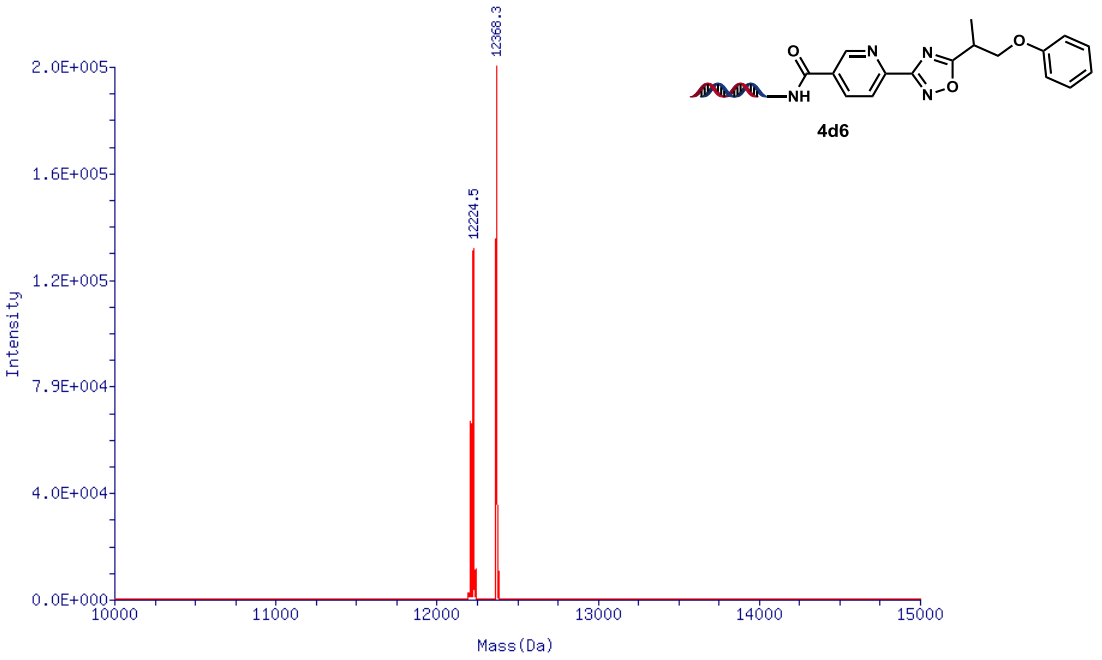



**Figure S102.** Deconvoluted mass spectrum of compound **4d6**, expected: 12367.3; observed 12368.3.

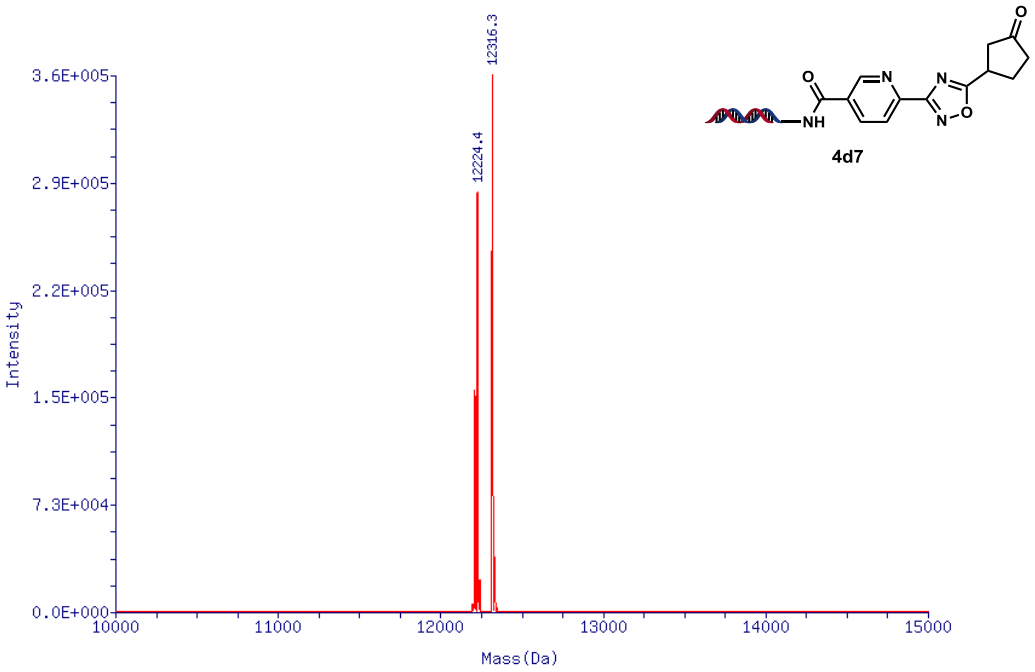



**Figure S103.** Deconvoluted mass spectrum of compound **4d7**, expected: 12315.3; observed 12316.3.

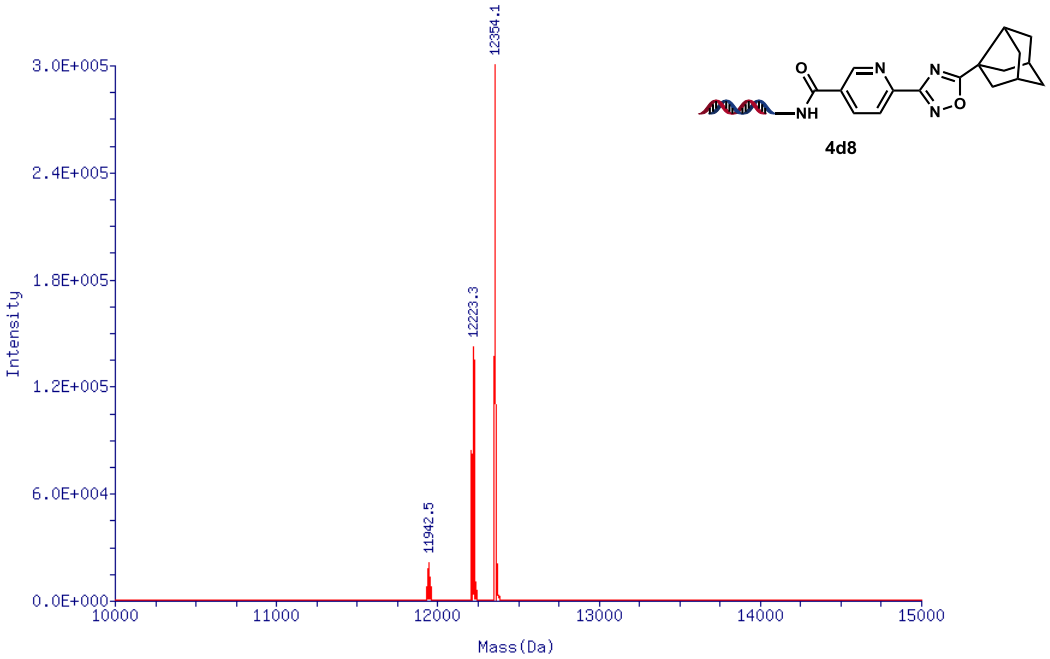



**Figure S104.** Deconvoluted mass spectrum of compound **4d8**, expected: 12353.4; observed 12354.1.

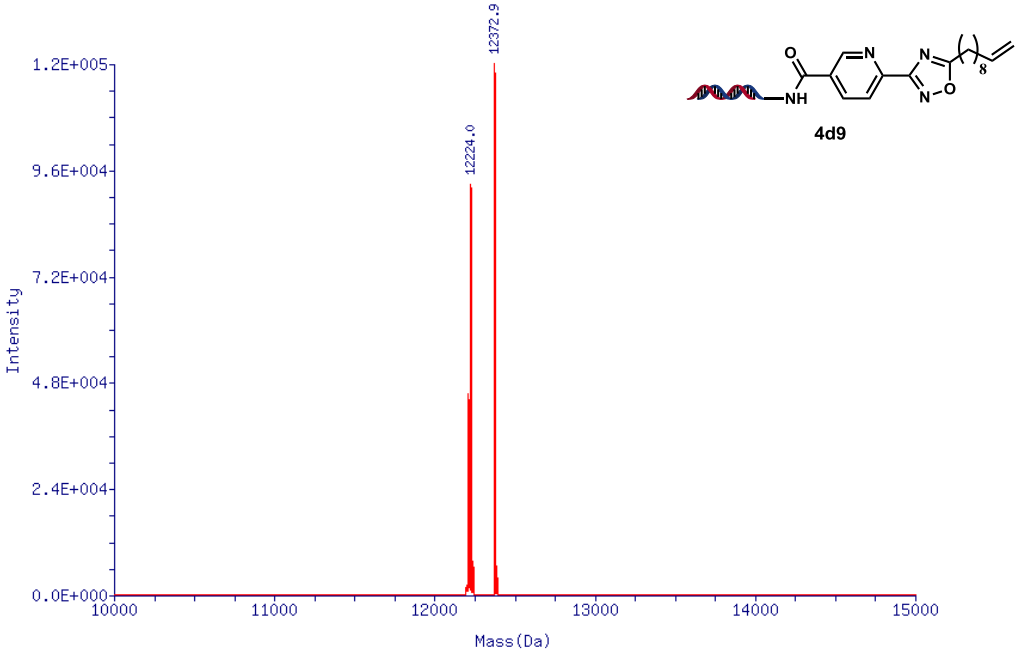



**Figure S105.** Deconvoluted mass spectrum of compound **4d9**, expected: 12371.4; observed 12372.9.

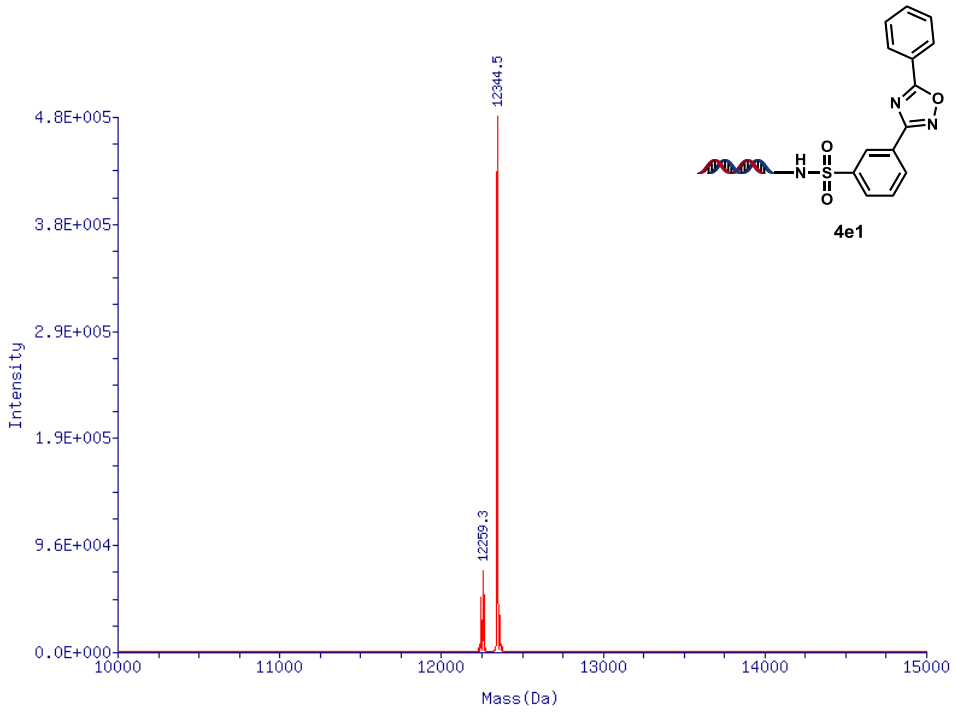



**Figure S106.** Deconvoluted mass spectrum of compound **4e1**, expected: 12344.3; observed 12344.5.

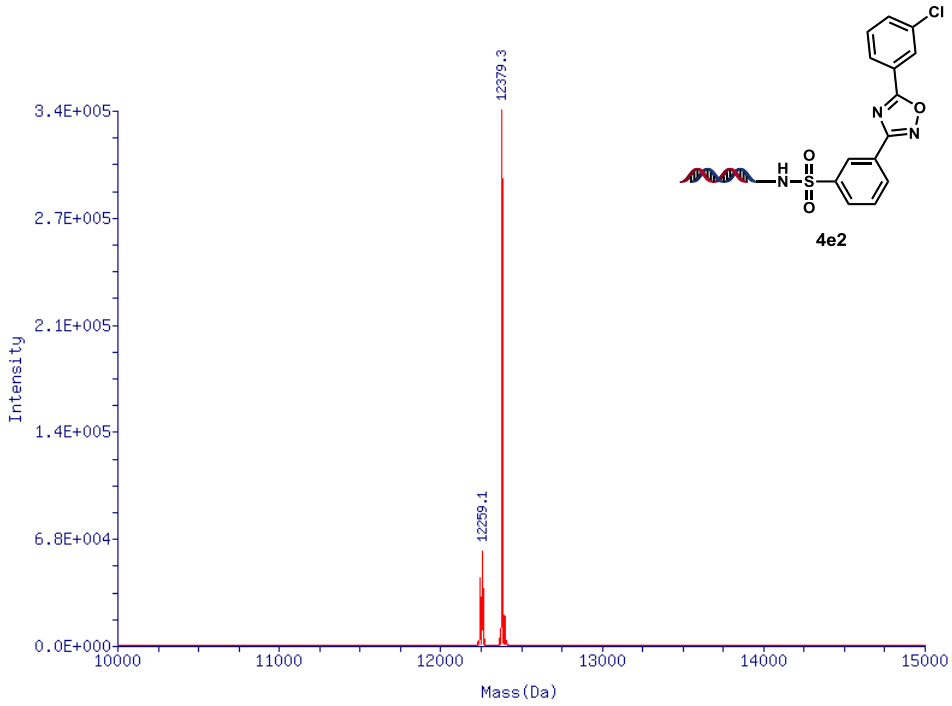



**Figure S107.** Deconvoluted mass spectrum of compound **4e2**, expected: 12378.7; observed 12379.3.

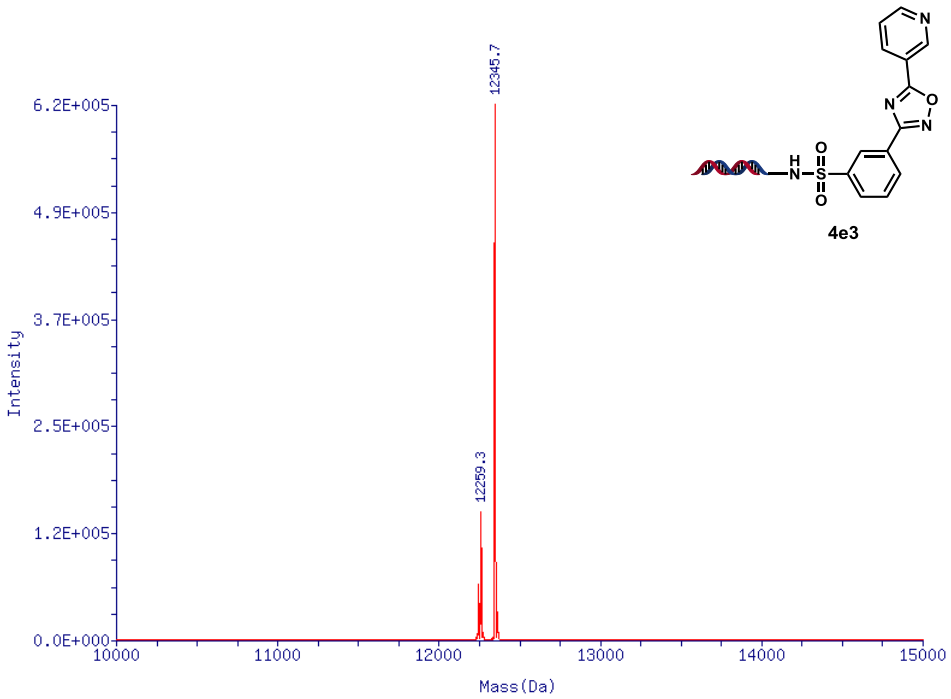



**Figure S108.** Deconvoluted mass spectrum of compound **4e3**, expected: 12345.3; observed 12345.7.

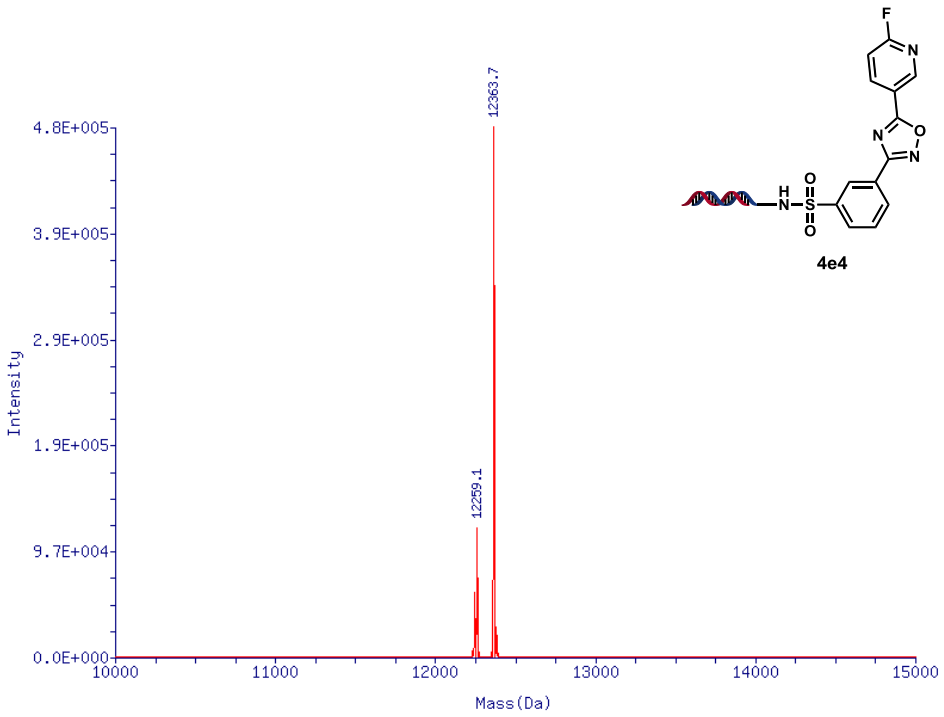



**Figure S109.** Deconvoluted mass spectrum of compound **4e4**, expected: 12363.3; observed 12363.7.

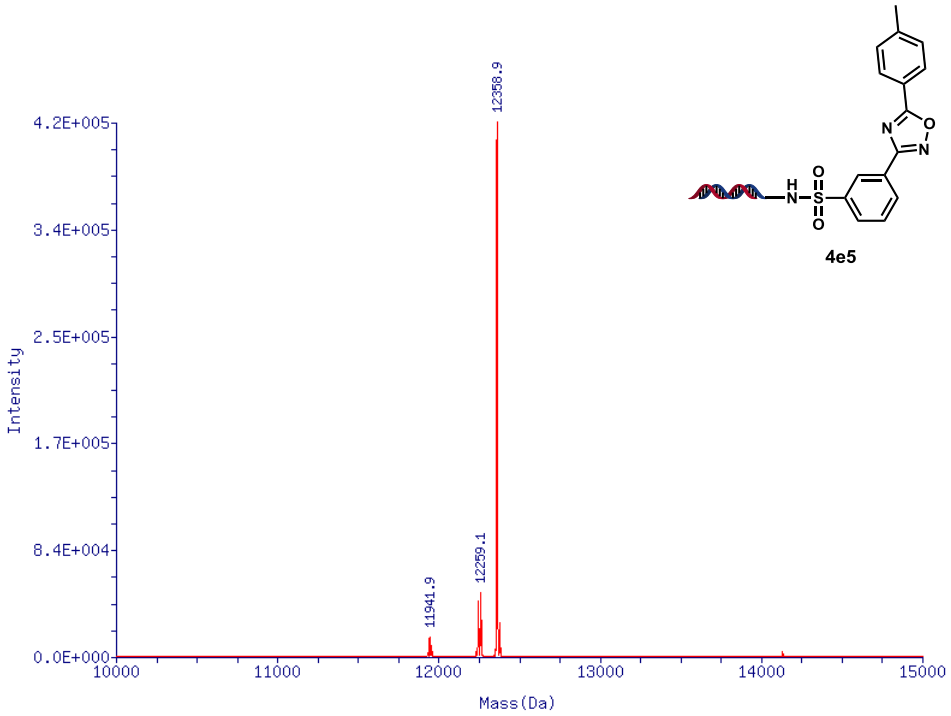



**Figure S110.** Deconvoluted mass spectrum of compound **4e5**, expected: 12358.3; observed 12358.9.

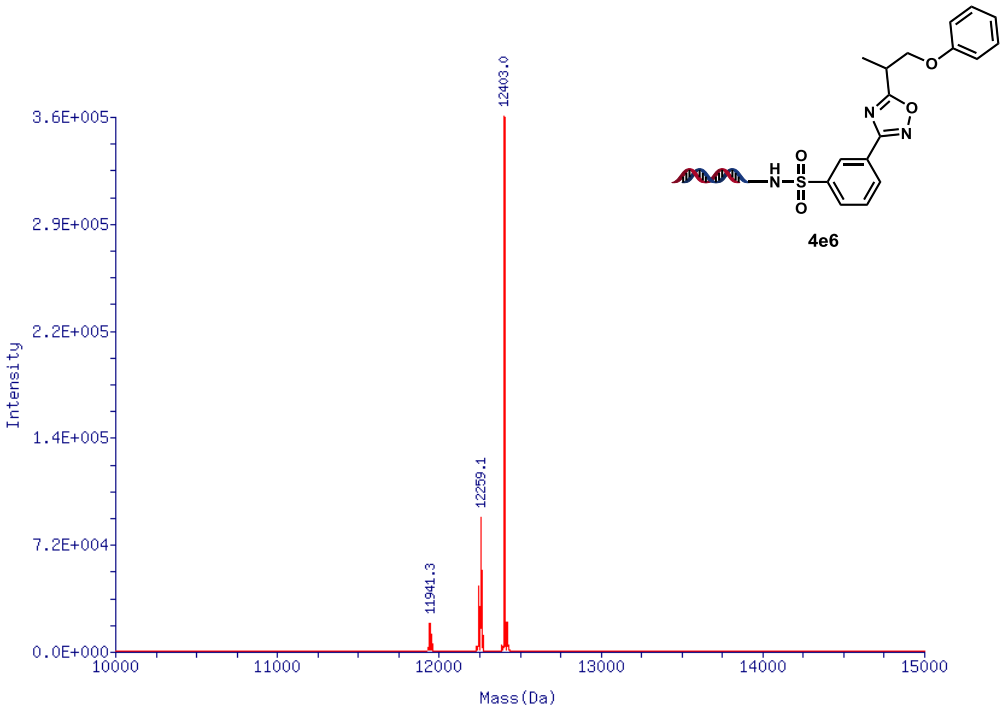



**Figure S111.** Deconvoluted mass spectrum of compound **4e6**, expected: 12402.4; observed 12403.0.

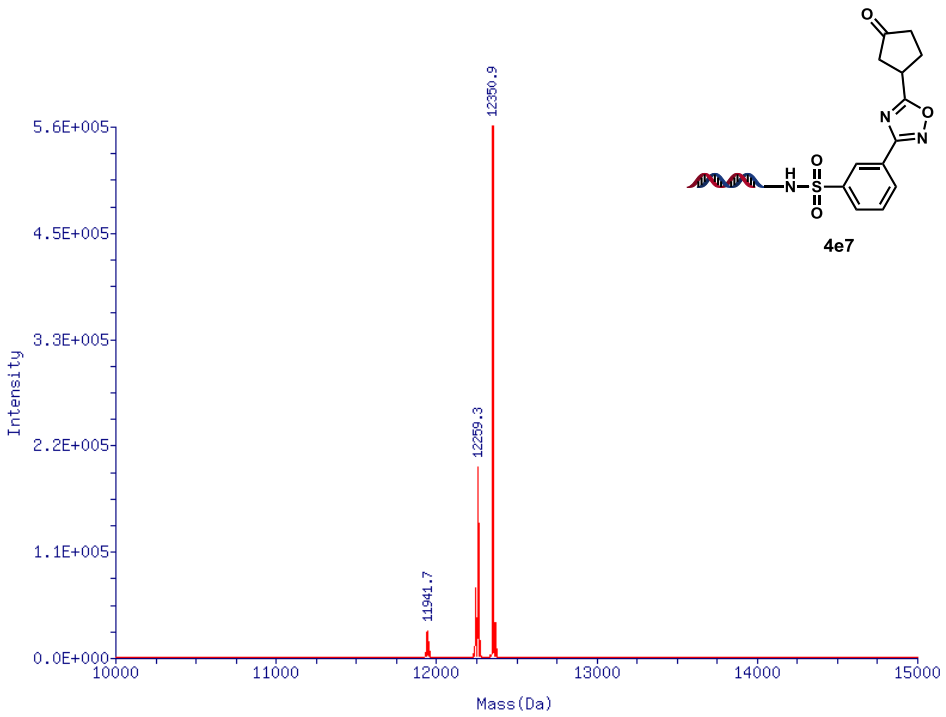



**Figure S112.** Deconvoluted mass spectrum of compound **4e7**, expected: 12350.3; observed 12350.9.

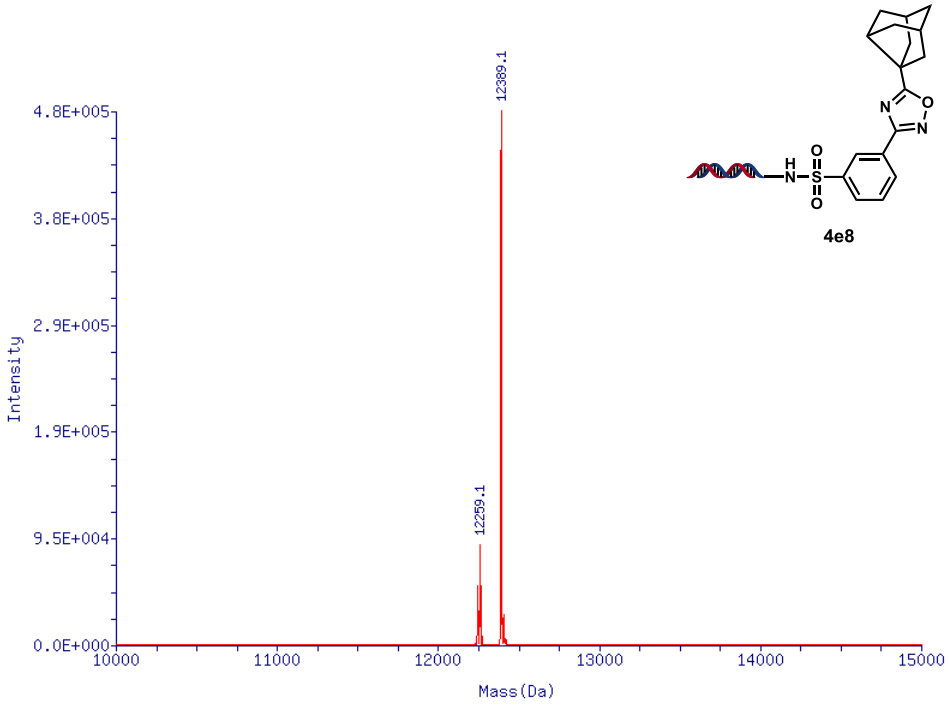



**Figure S113.** Deconvoluted mass spectrum of compound **4e8**, expected: 12388.4; observed 12389.1.

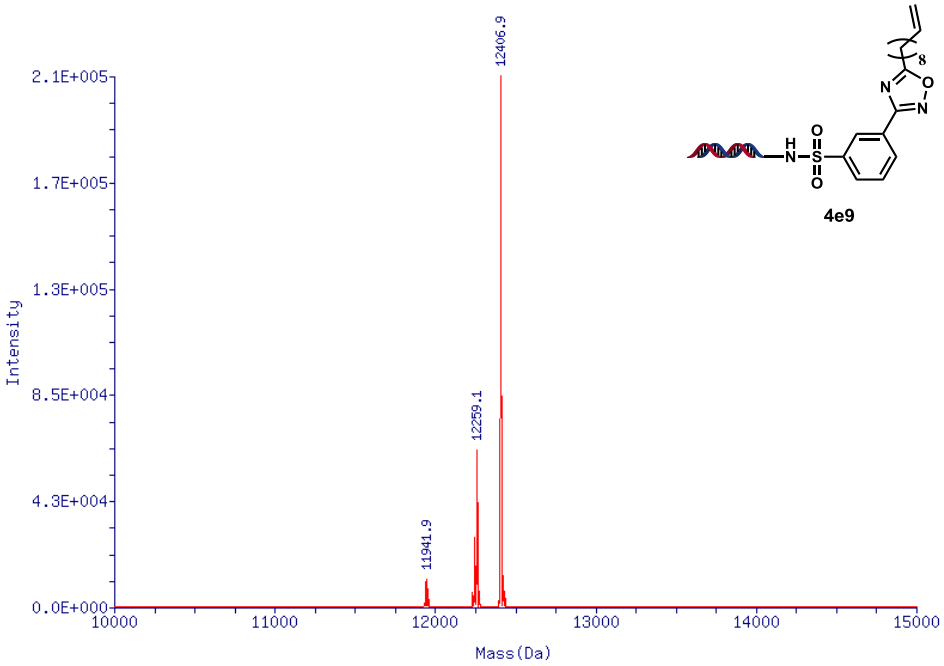



**Figure S114.** Deconvoluted mass spectrum of compound **4e9**, expected: 12406.5; observed 12406.9.

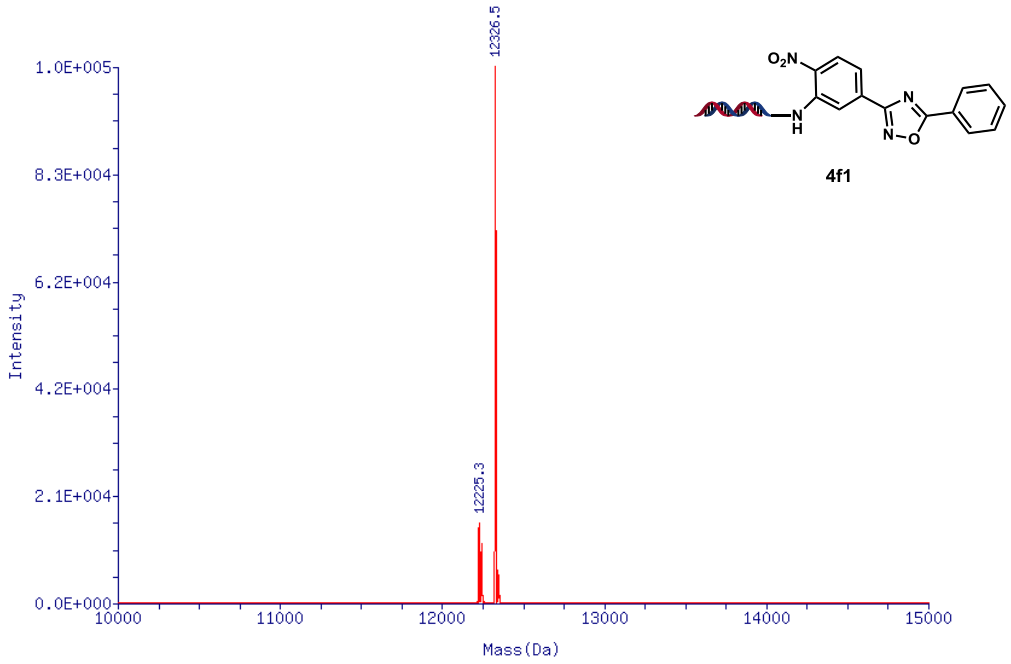



**Figure S115.** Deconvoluted mass spectrum of compound **4f1**, expected: 12325.2; observed 12326.5.

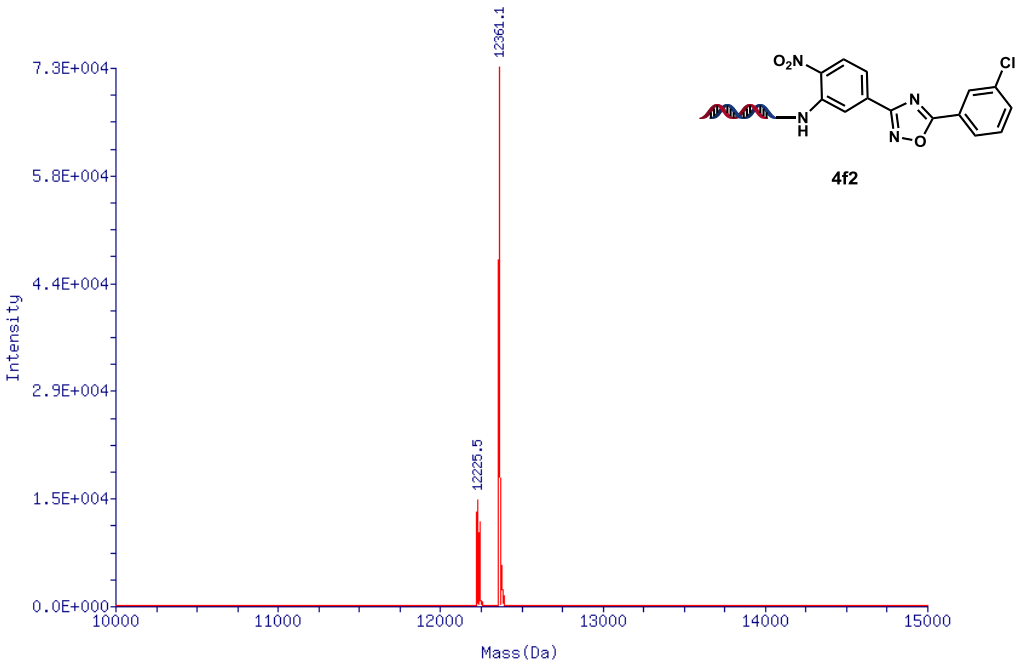



**Figure S116.** Deconvoluted mass spectrum of compound **4f2**, expected: 12359.7; observed 12361.1.

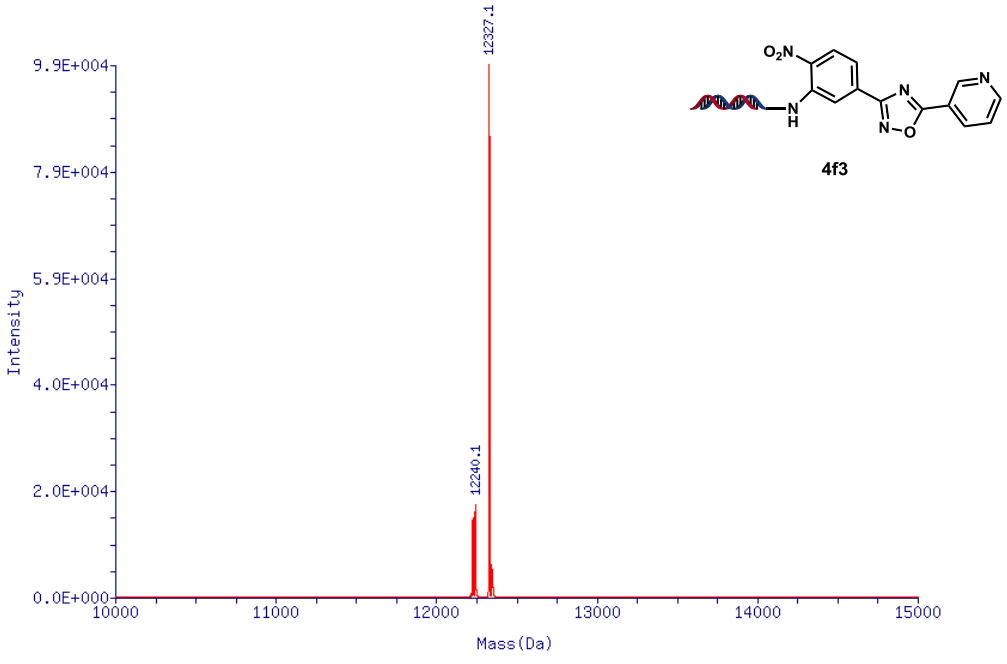



**Figure S117.** Deconvoluted mass spectrum of compound **4f3**, expected: 12326.2; observed 12327.1.

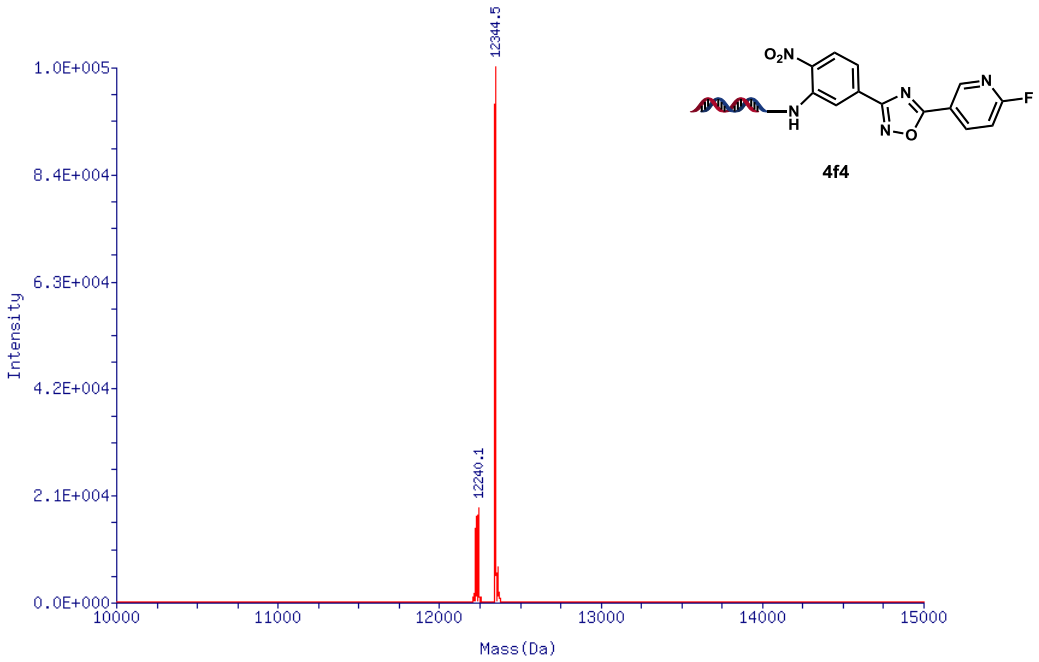



**Figure S118.** Deconvoluted mass spectrum of compound **4f4**, expected: 12344.2; observed 12344.5.

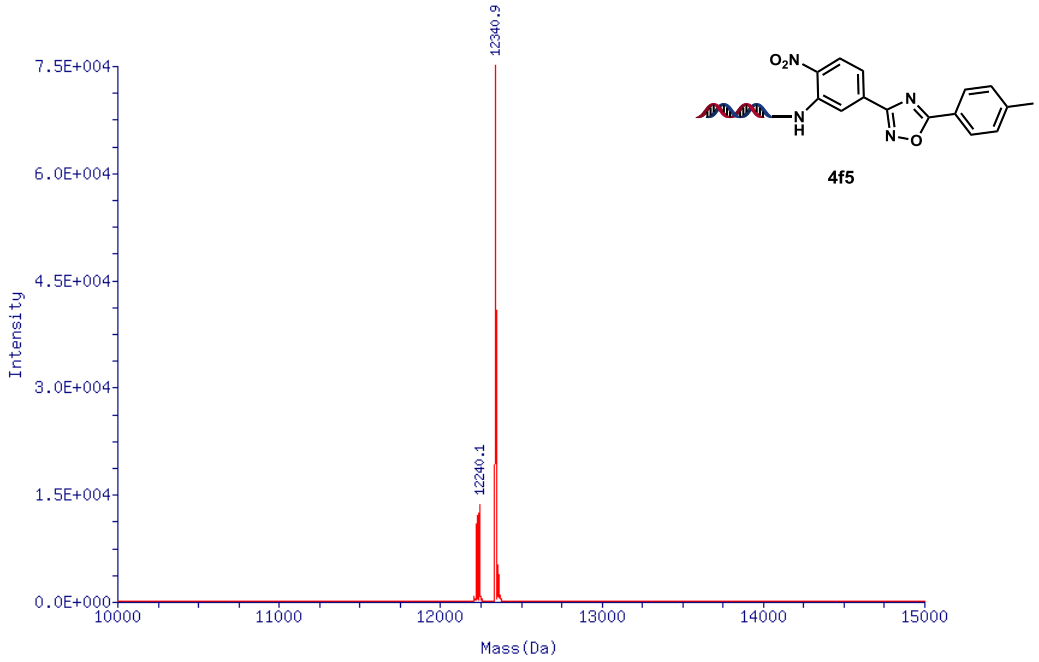



**Figure S110.** Deconvoluted mass spectrum of compound **4f5**, expected: 12339.3; observed 12340.9.

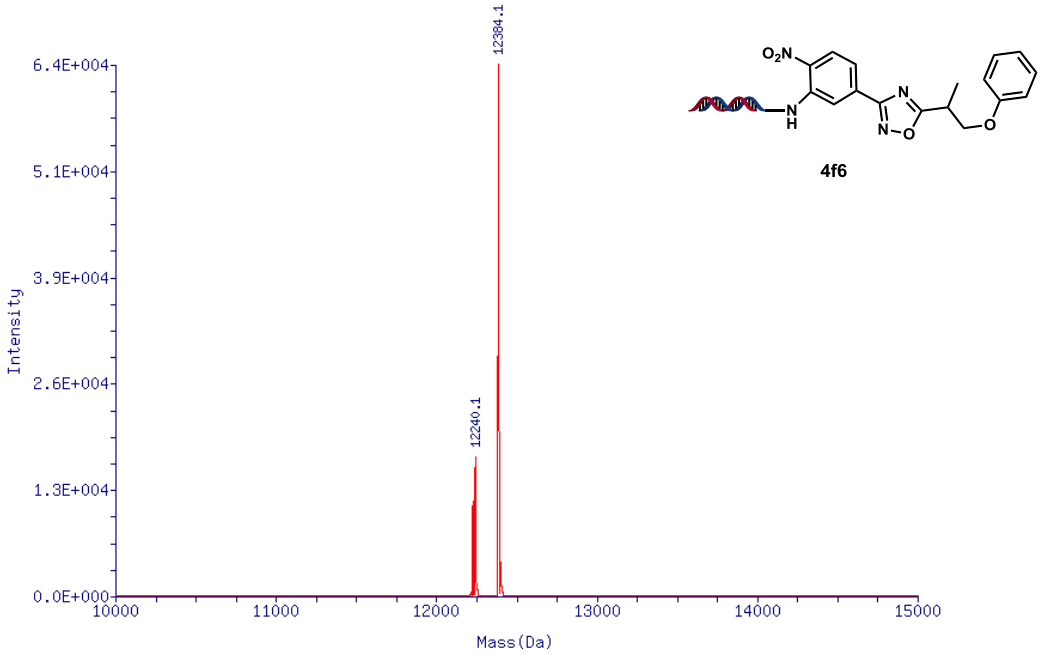



**Figure S120.** Deconvoluted mass spectrum of compound **4f6**, expected: 12383.3; observed 12384.1.

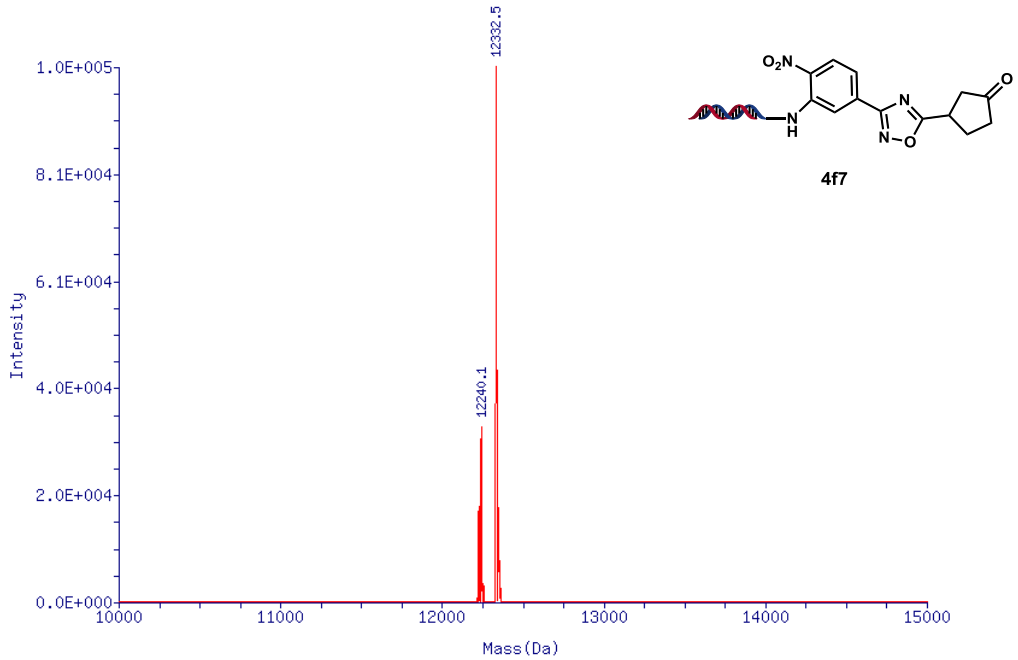



**Figure S121.** Deconvoluted mass spectrum of compound **4f7**, expected: 12331.2; observed 12332.5.

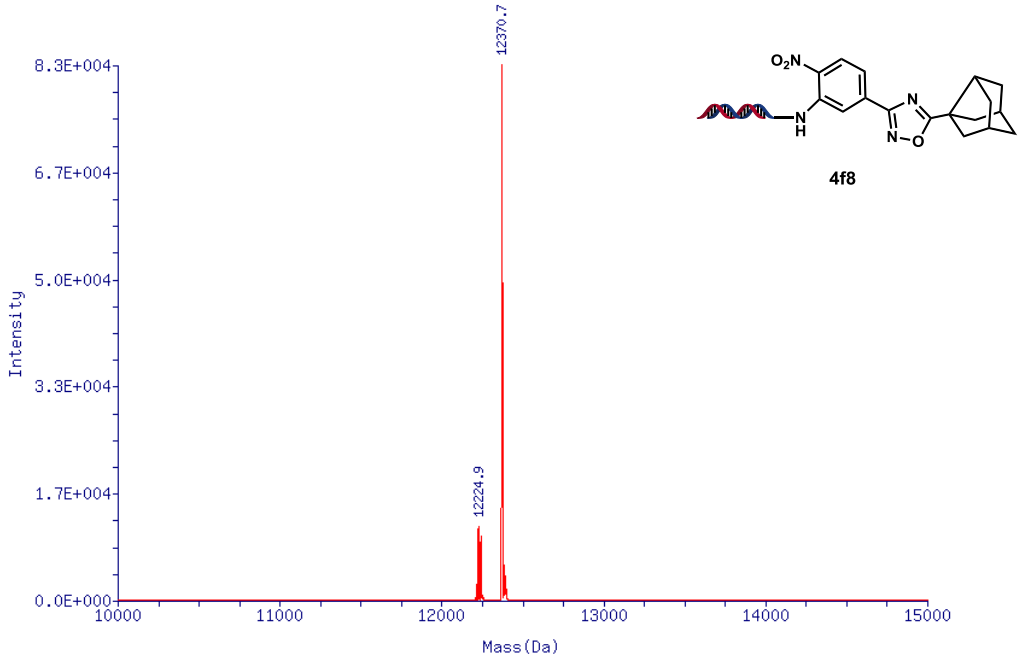



**Figure S122.** Deconvoluted mass spectrum of compound **4f8**, expected: 12369.3; observed 12370.7.

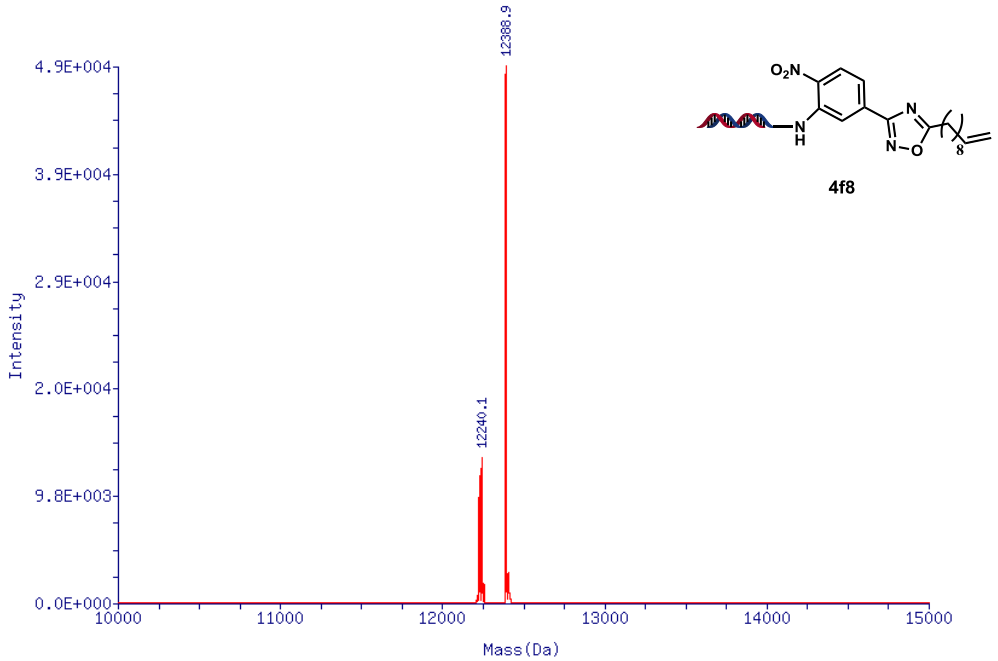
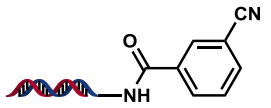
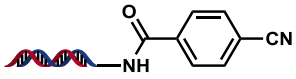
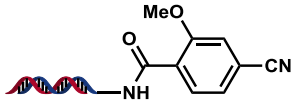
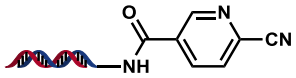
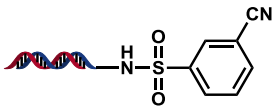
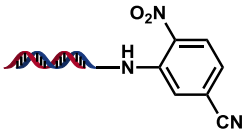



**Figure S123.** Deconvoluted mass spectrum of compound **4f9**, expected: 12387.4; observed 12388.9.

## 4. Preparation of Substrates

Testing substrates were conjugated on DNA via acylation, sulfonylation, or nucleophilic aromatic substitution ($S_NAr$).

**Table S2**. Data for starting material preparation

| Products | Structure | Expected MW | Observed MW | Conversion |
|---|---|---|---|---|
| **1a** |  | 12189.1 | 12189.7 | 99 % |
| **1b** |  | 12189.1 | 12189.7 | 99 % |
| **1c** |  | 12219.1 | 12219.7 | 99 % |
| **1d** |  | 12190.1 | 12190.3 | 99 % |
| **1e** |  | 12225.2 | 12225.5 | 95 % |
| **1f** |  | 12206.1 | 12206.5 | 99 % |

## 5. DNA stability and ligation test

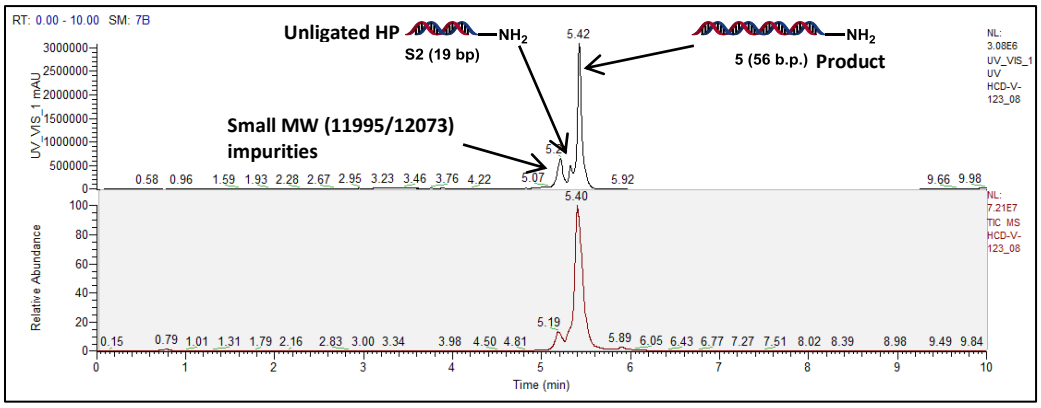



**Figure S124.** LC-MS spectrum of compound **5**

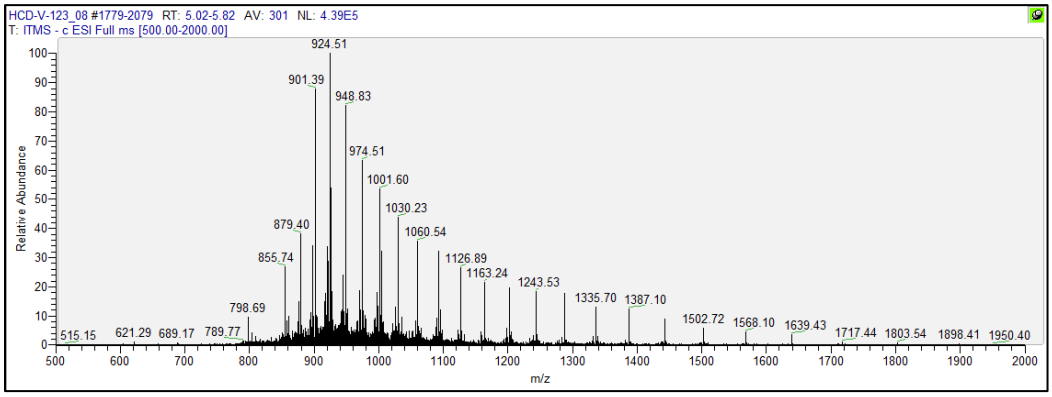



**Figure S125.** ESI Full MS spectrum of compound **5**

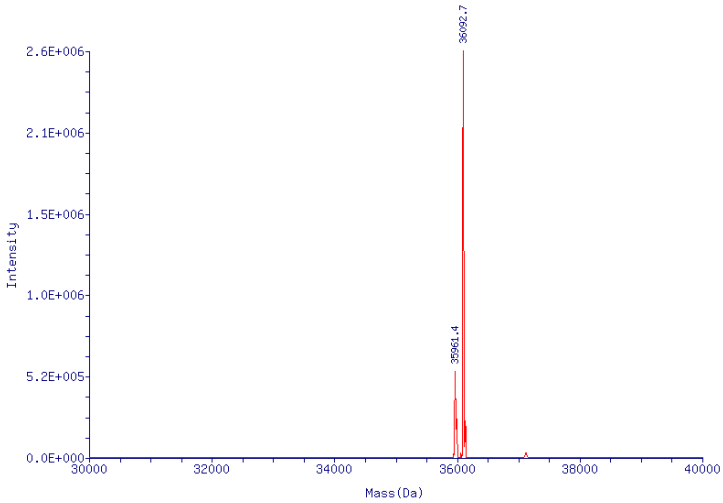



**Figure S126.** Deconvoluted mass spectrum of compound **5**, expected: 36088.7; observed 36092.7.

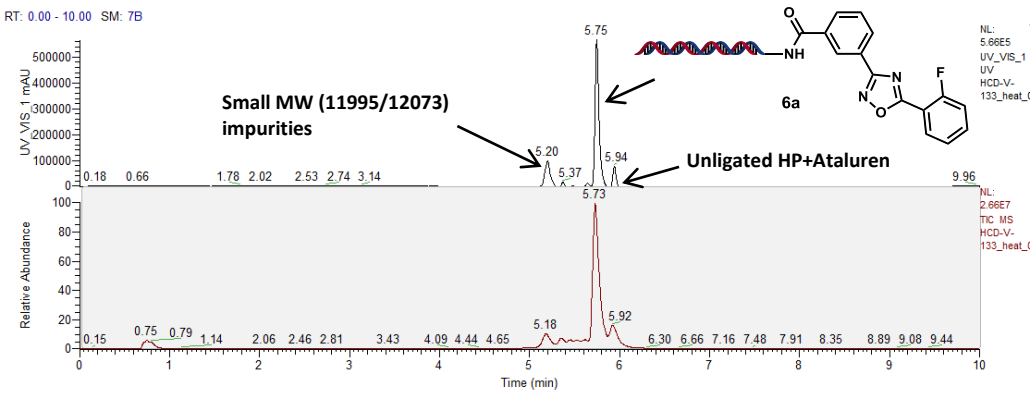



**Figure S127.** LC-MS spectrum of compound **6a**

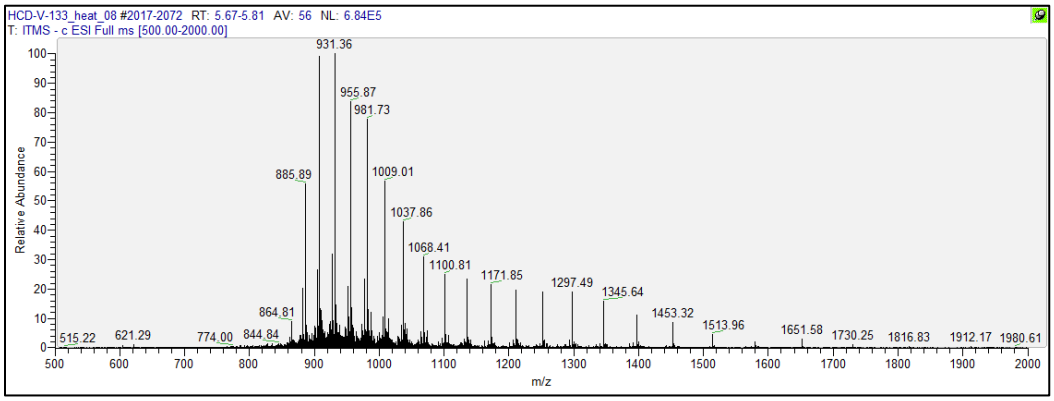



**Figure S128.** ESI Full MS spectrum of compound **6a**

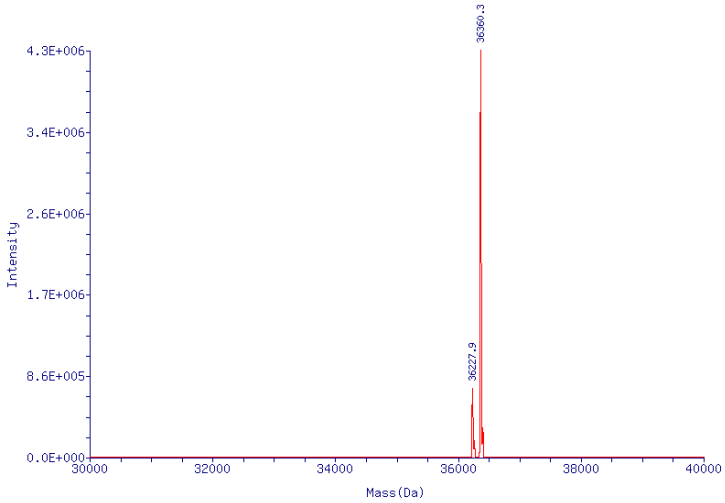



**Figure S129.** Deconvoluted mass spectrum of compound **6a**, expected: 36354.9; observed 36360.3.

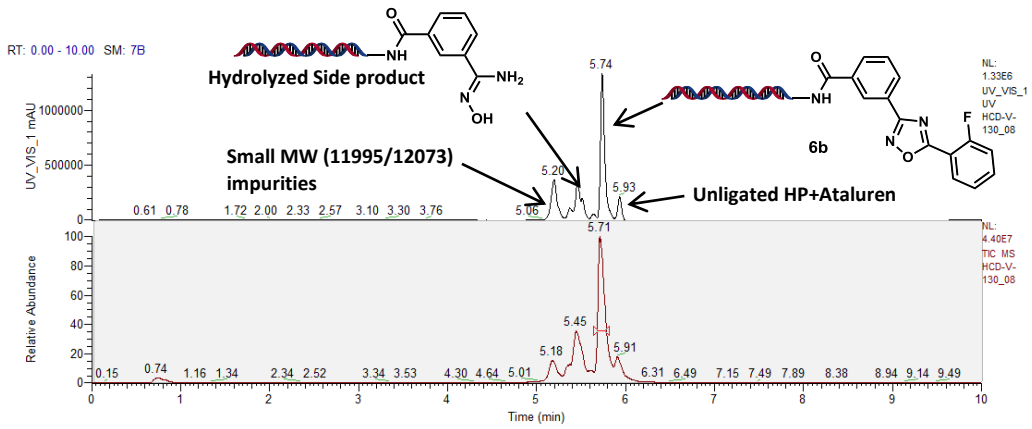



**Figure S130.** LC-MS spectrum of compound **6b**

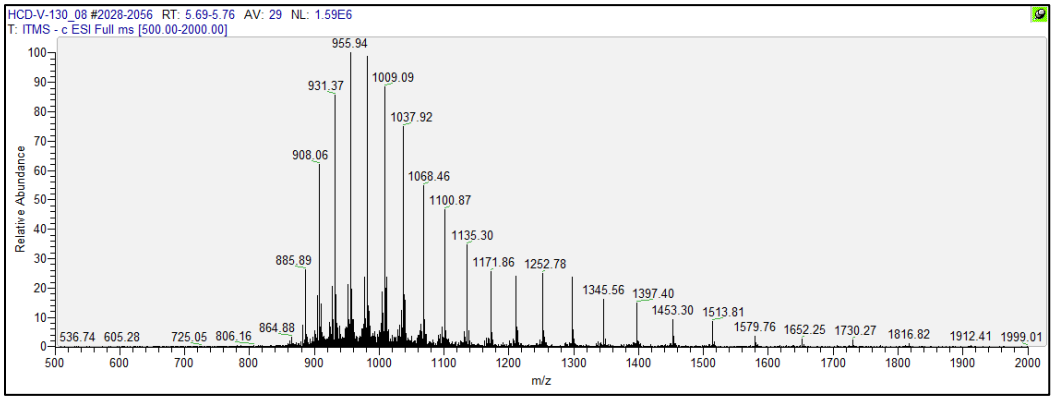



**Figure S131.** ESI Full MS spectrum of compound **6b**

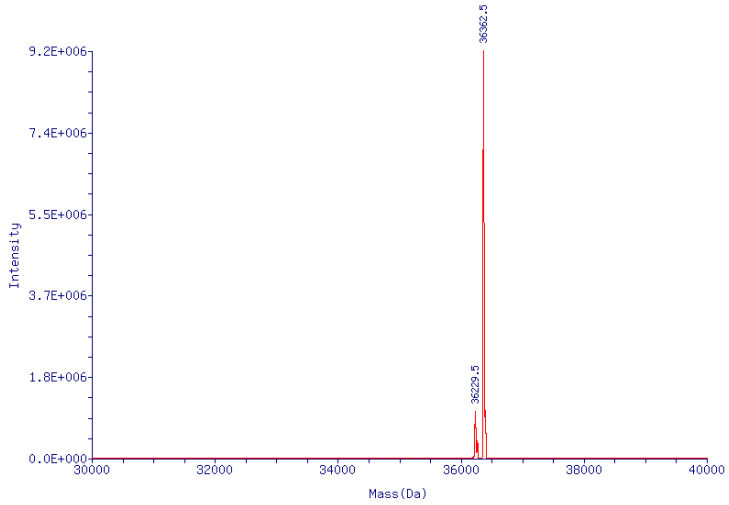



**Figure S132.** Deconvoluted mass spectrum of compound **6b**, expected: 36354.9; observed 36362.5.

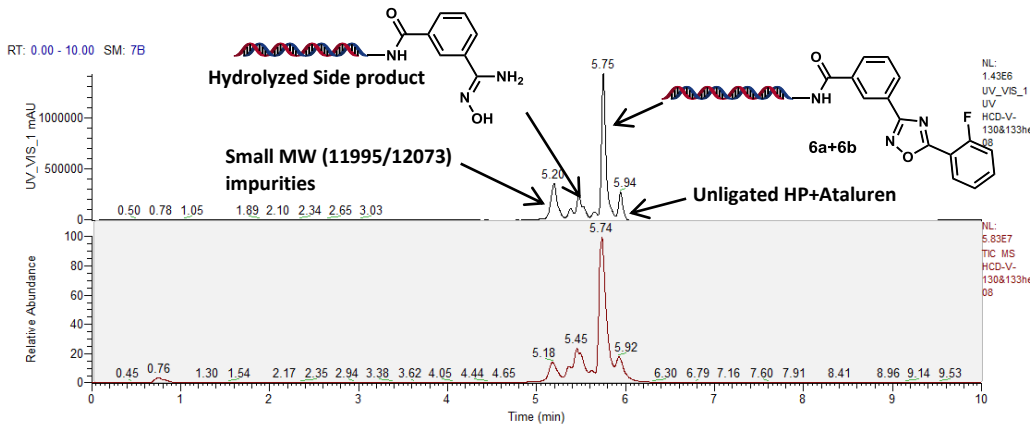



**Figure S133.** LC-MS spectrum of compound **6a**+**6b** (co-injection)

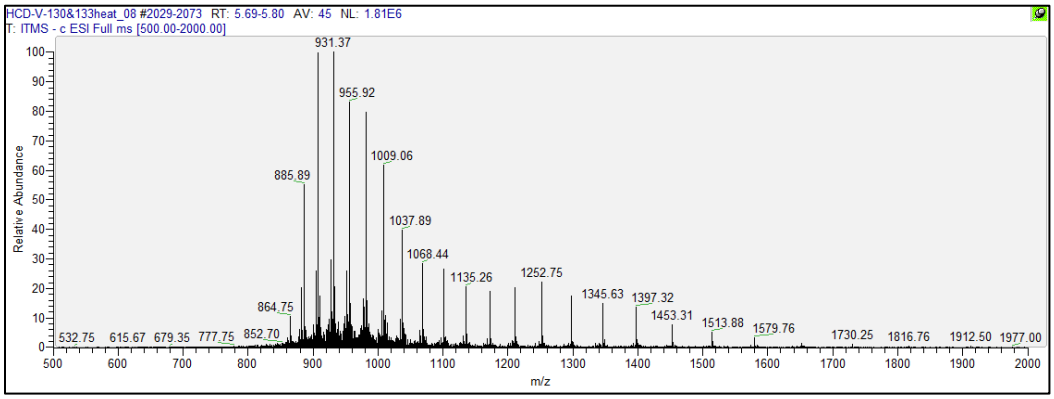



**Figure S134.** ESI Full MS spectrum of compound **6a**+**6b** (co-injection)

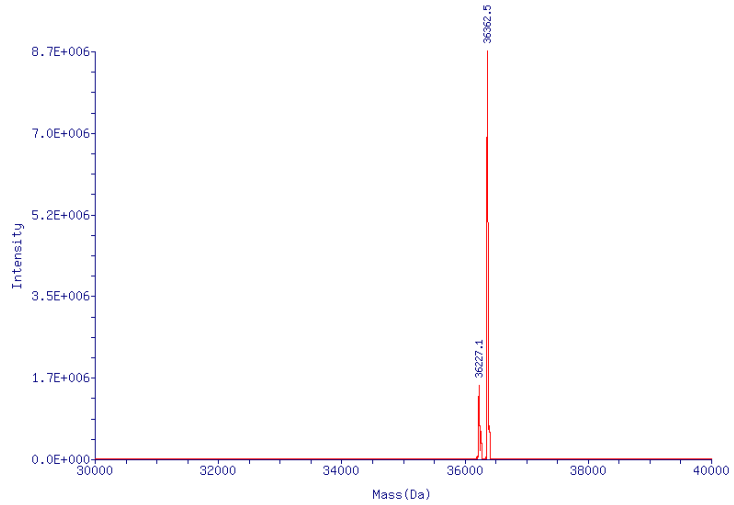



**Figure S135.** Deconvoluted mass spectrum of compound **6a**+**6b**, expected: 36354.9; observed 36362.5.

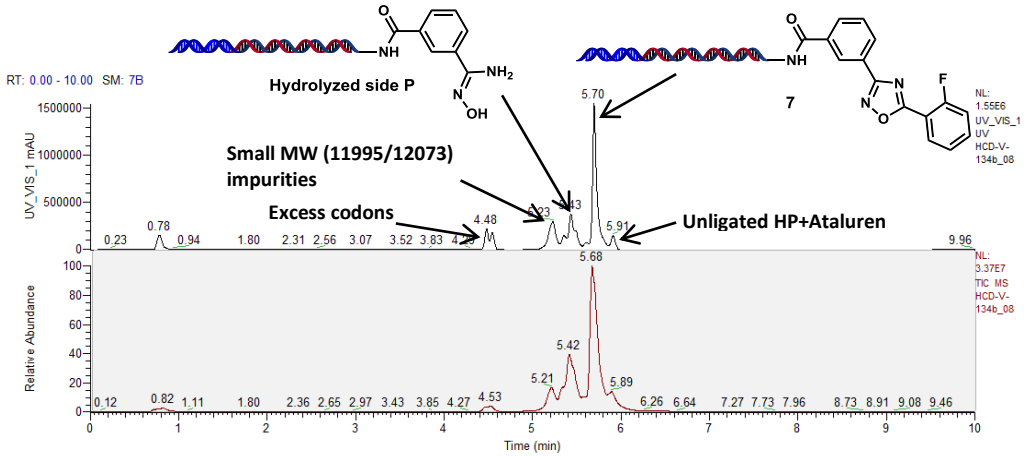



**Figure S136.** LC-MS spectrum of compound **7**

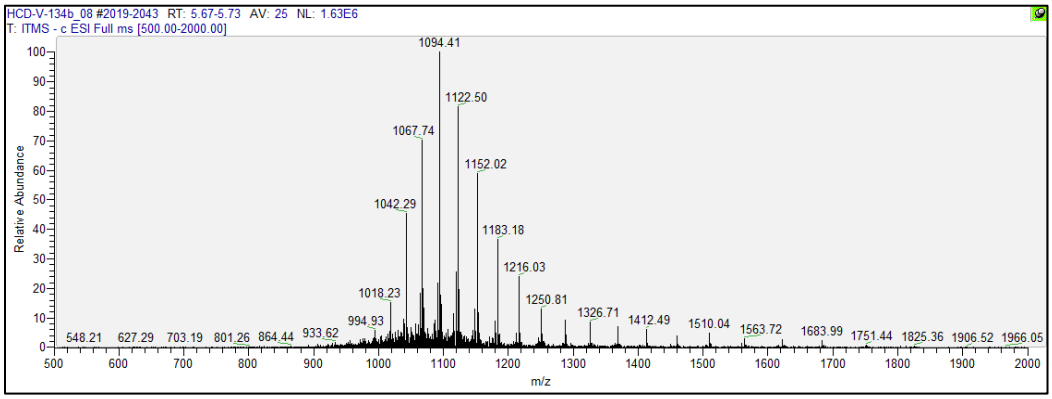



**Figure S137.** ESI Full MS spectrum of compound **7**

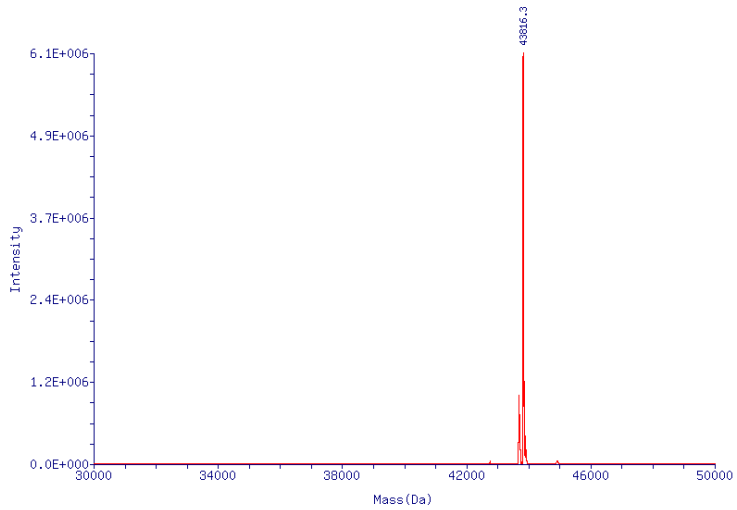



**Figure S138.** Deconvoluted mass spectrum of compound **7**, expected: 43809.7; observed 43816.3.

LC setting for compounds **5**, **6a**, **6b**, and **7**

Column: Thermo DNAPac RP (2.1 x 50 mm, 4µm)
Solvent A: 15mM triethylamine (TEA)/100mM hexafluoroisopropanol (HFIP) in water
Solvent B: 15mM TEA/100mM HFIP in 50% methanol
Flow rate: 0.65 mL/min
Run time: 8 mins
Solvent gradient: 0.0 min (98%A / 2%B), 5.0 min (10%A / 90%B), 6.5 min (98%A / 2%B)
Column temperature: 100 °C (post column cooler at 40 °C)

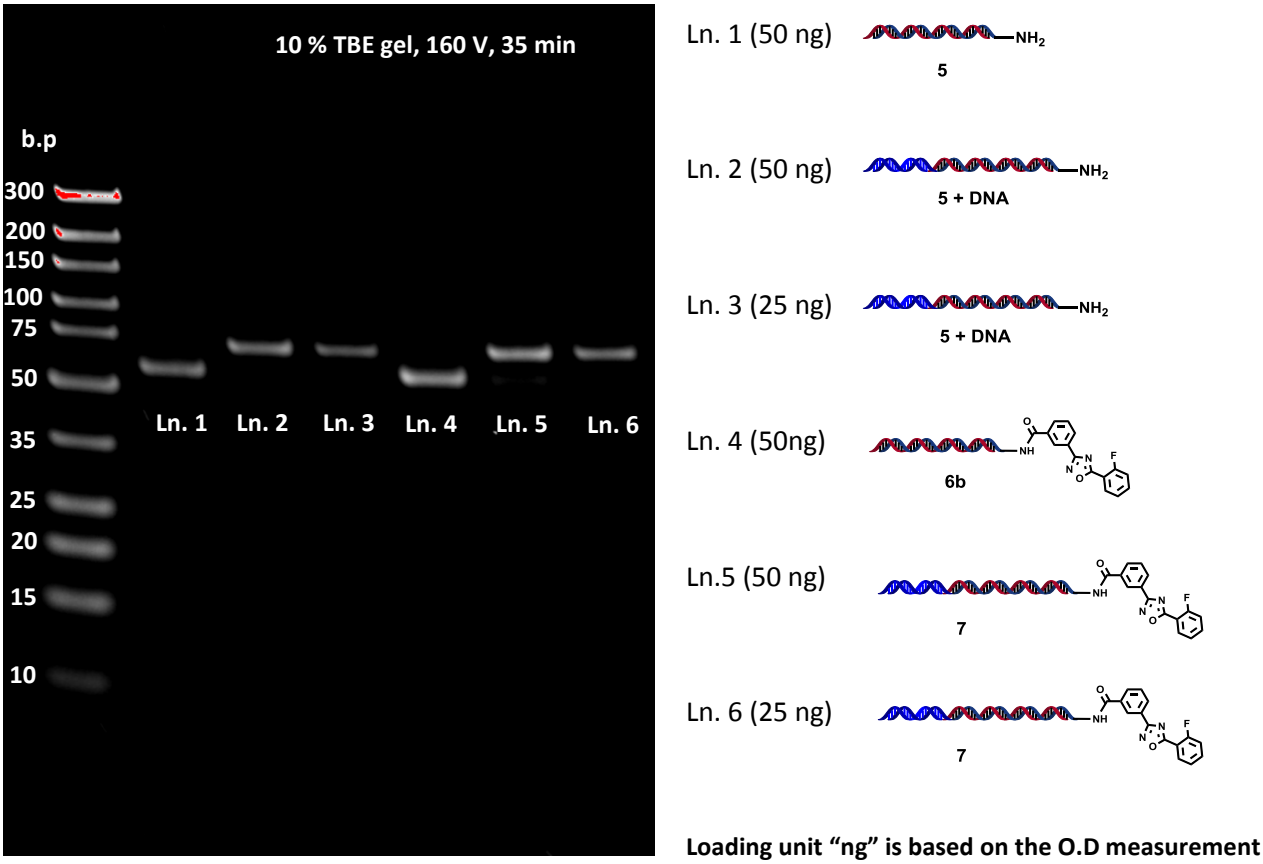



**Figure S139.** Gel electrophoresis image comparisons between starting material **5**, compound **6b**, and compound **7**.

## 6. References


1. Du, H.C.; Huang, H. DNA-Compatible Nitro Reduction and Synthesis of Benzimidazoles. *Bioconjugate Chem.* **2017**, *28*, 2575–2580.

2. Li, J.-Y.; Huang, H. Development of DNA-Compatible Suzuki-Miyaura Reaction in Aqueous Media. *Bioconjugate Chem.* **2018**, *29*, 3841–3846.

3. Faver, J. C.; Riehle, K.; Lancia, D. R.; Milbank, J. B. J.; Kollmann, C. S.; Simmons, N.; Yu, Z.; Matzuk, M. M. Quantitative Comparison of Enrichment from DNA-Encoded Chemical Library Selections. *ACS Comb. Sci.* **2019**, 21, 75–82.

4. Du, H.C.; Simmons, N.; Faver, J. C.; Yu, Z.; Palaniappan, M.; Riehle, K.; Matzuk, M. M. A mild, DNA-compatible nitro reduction using $B_2(OH)_4$. *Org. Lett.* **ASAP. DOI: 10.1021/acs.orglett.9b00497**

5. Clark, M.A.; Acharya, R.A.; Arico-Muendel, C.C.; Belyanskaya, S.; Benjamin, D.R.; Carlson, N.R.; Centrella, P.A.; Chiu, C.H.; Creaser, S.P. Cuozzo, J.W.; et al. Design, synthesis and selection of DNA-encoded small-molecule libraries. *Nat. Chem. Biol.* **2009**, *5*, 647–654.

6. Satz, A. L.; Cai, J.; Chen, Y.; Goodnow, R.;Gruber, F.; Kowalczyk, A.; Petersen, A.; Naderi-Oboodi, G.; Orzechowski, L.; Strebel, Q. DNA Compatible Multistep Synthesis and Applications to DNA Encoded Libraries. *Bioconjugate Chem.* **2015**, *26*, 1623–1632.