Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Synthesis of 5-substituted Tetrazoles *via* DNA-conjugated Nitriles

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1. General Information

In this study, general materials, equipment and procedures are adapted from previous reports[1-6] by our groups and other DNA-encoded library publications.[7-8]

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), MES 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).

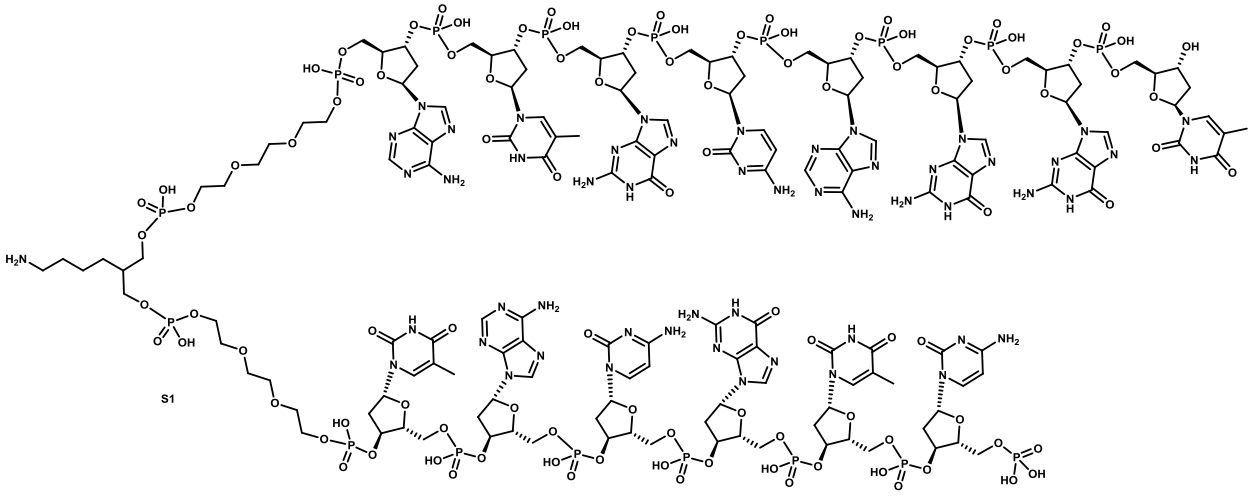


Figure S1. Structure of "DTSU" **S1** (5'-Phos-CTGCAT-Spacer 9-Amino C7-Spacer 9-ATGCAGGT 3').

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: 0.40 mL/min
Run time: 2.5 mins
Solvent gradient: 0.0 min (98%A / 2%B), 0.3 min (100% B), 0.31 min (100%C), 1.70 min (100%C), 1.71-2.50 min (98%A / 2%B, flow 0.65 mL/min)
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 **7** (1 nmol, 5.0 µL, 1.0 equiv) was added DNA_1 (5'-ACACTTGCTGGT-3', 2.5 nmol, 2.5 µL, 2.5 equiv), DNA_2 (5'-CAGCAAGTGTGA-3', 2.5 nmol, 2.5 µL, 2.5 equiv), and nuclease-free water (7.0 µL). The solution was heated at 95 ^{o}C for 3min, and then cooled to room temperature prior to the addition of 10× HEPES buffer (2 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 120 V for 40 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 **7** 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 with primary amine on DNA: 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 DMA, 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.

Acylation with carboxylic acid on DNA: A cyano amine building block (1000 nmol, 5 μL, 200 mM in MeCN, 100 equiv) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM, 1000 nmol, 5 μL, 200 mM in H_2O, 100 equiv) were added to a solution of DNA (10 nmol, 10 μL, 1.0 mM, 1 equiv) in H_2O with pH 5.8 MES buffer (4000 nmol, 16 μL, 250 mM, 400 equiv), which was allowed to sit at room temperature for overnight 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 2 h before being quenched by EtOH precipitation.

2. Generation of 5-substituted Tetrazoles

General procedure for 5-substituted tetrazoles

DNA-conjugated nitriles were reconstituted to the concentration of 1.0 mM after attaching procedure. pH 5.8 Mes 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 1,4-dioxane (18 μL), NaN_3(2000 nmol, 5 μL, 400 mM in H_2O, 200 equiv), and $ZnBr_2$ (500 nmol, 2 μL, 250 mM in H_2O, 50 equiv). The reaction mixture was heated at 80 ^oC for 16 h. After the reaction mixture was cooled down to room temperature, sodium cysteinate (1000 nmol, 5 μL, 200 mM in H_2O, 100 equiv) was added and then heated at 80 ^oC for 15 min before being quenched by EtOH precipitation. During the DNA reconstitution step, insoluble salt was remained in the bottom of tube and DNA can be aspirated out with a pipette.

3. UV, TIC, RSI, and Deconvoluted Mass Spectra of DNA-Conjugates

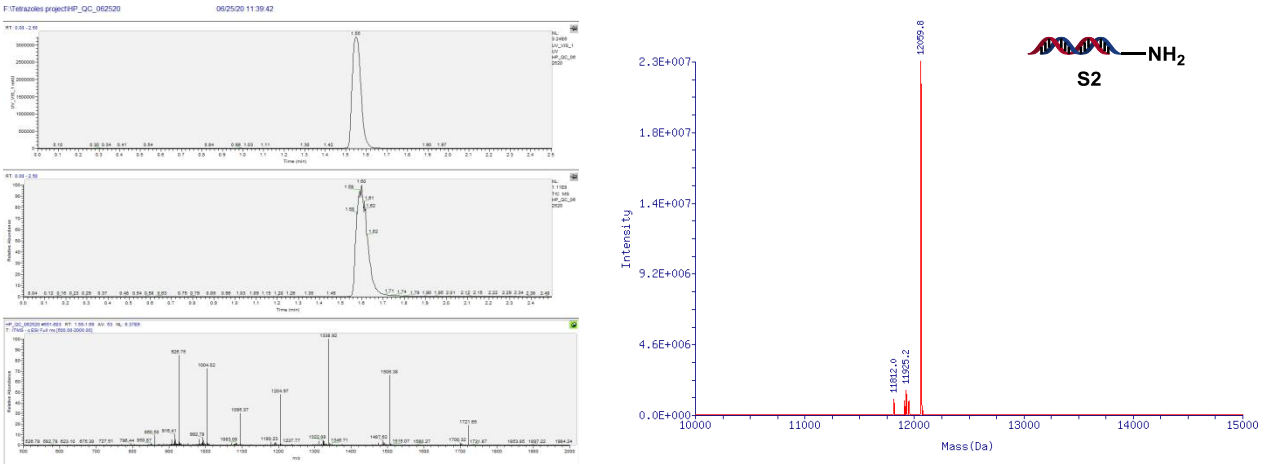


Figure S2. UV, TIC, ESI m/z, and deconvoluted mass spectra of DNA **S2**, expected: 12060.0; observed 12059.8

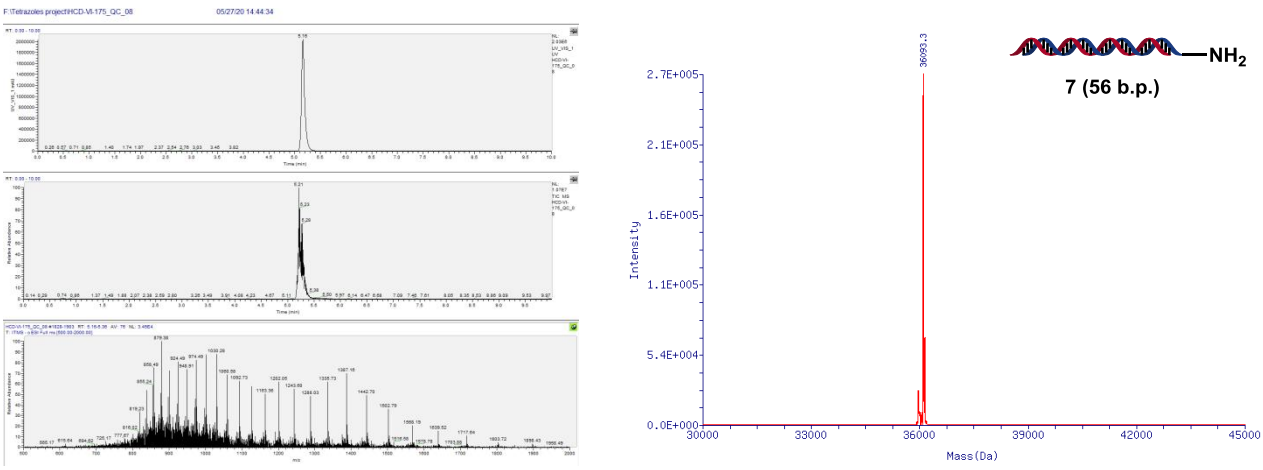


Figure S3. UV, TIC, ESI m/z, and deconvoluted mass spectra of the 56 b.p. DNA **7**, expected: 36088.7; observed 36093.3

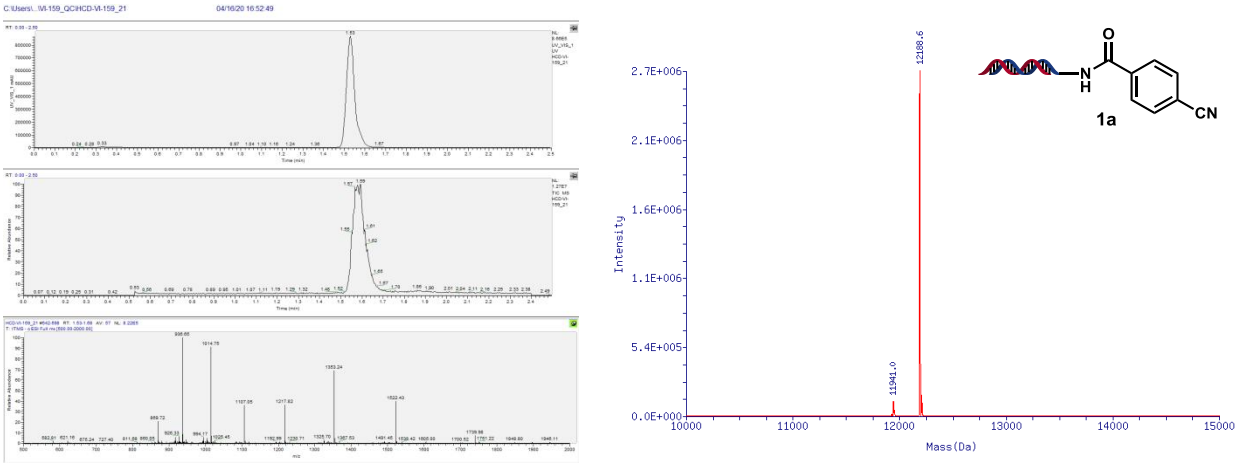


Figure S4. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1a**, expected: 12189.1; observed 12188.6

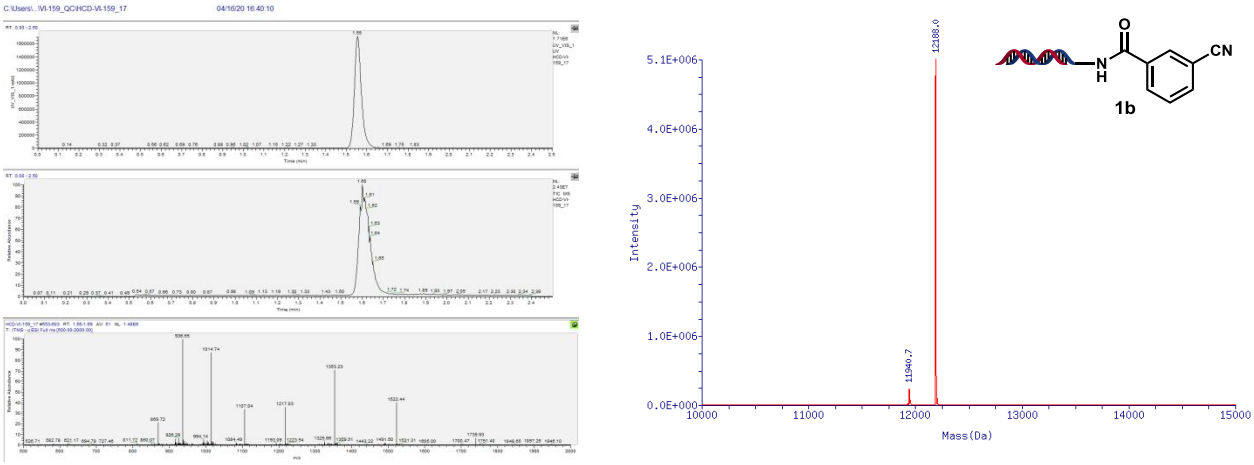


Figure S5. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1b**, expected: 12189.1; observed 12188.0

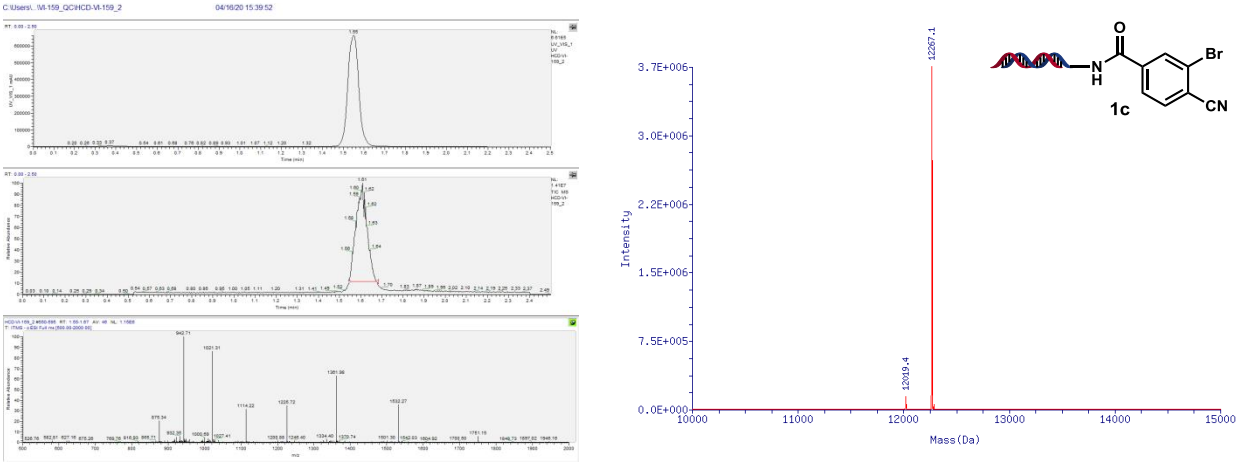


Figure S6. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1c**, expected: 12268.0; observed 12267.1

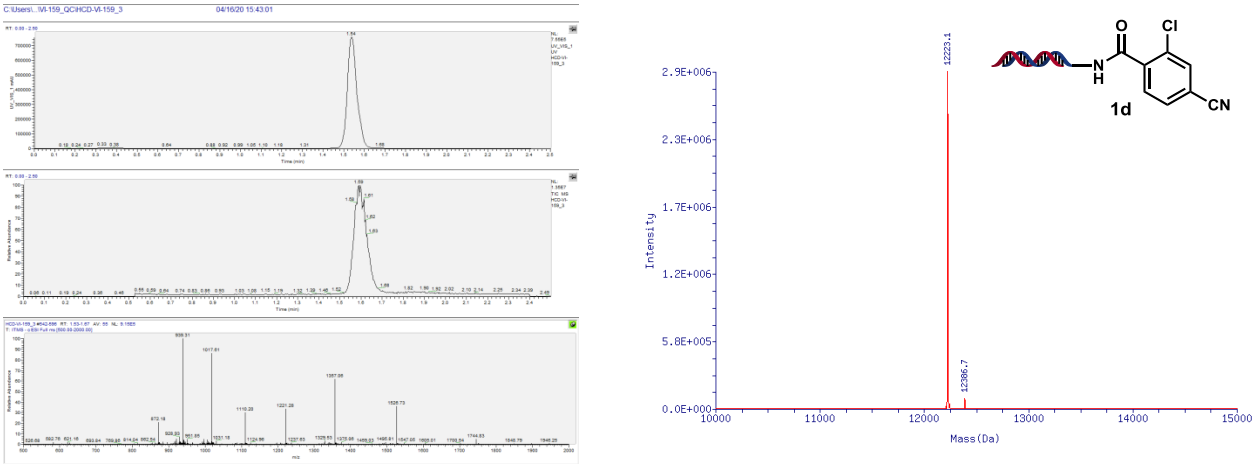


Figure S7. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1d**, expected: 12223.6; observed 12223.1

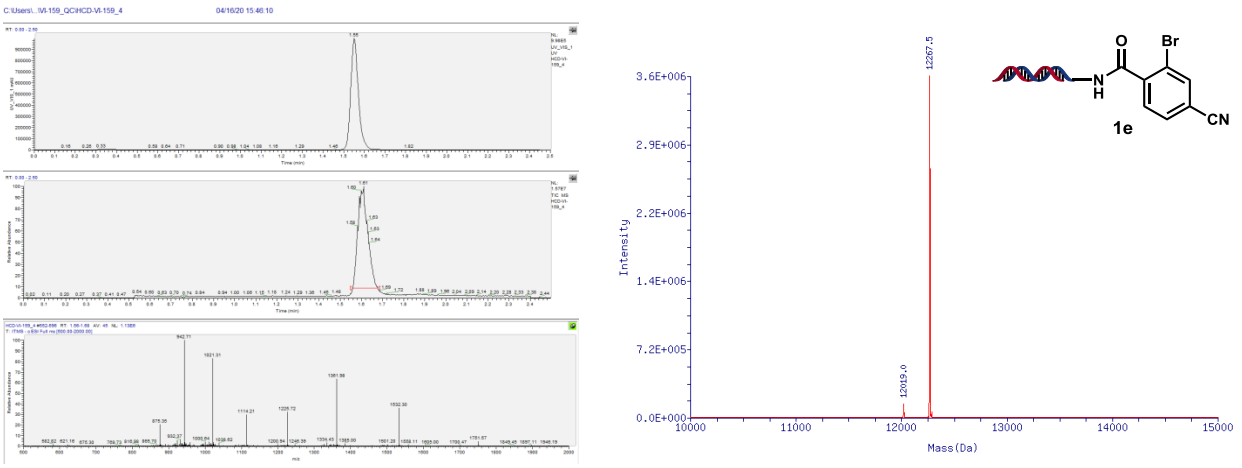


Figure S8. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1e**, expected: 12268.0; observed 12267.5

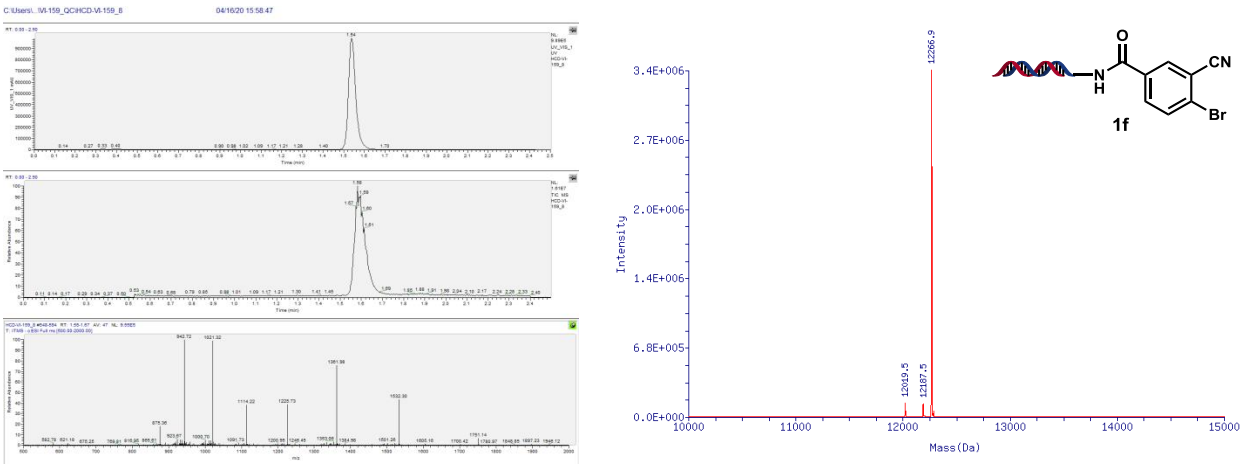


Figure S9. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1f**, expected: 12268.0; observed 12266.9

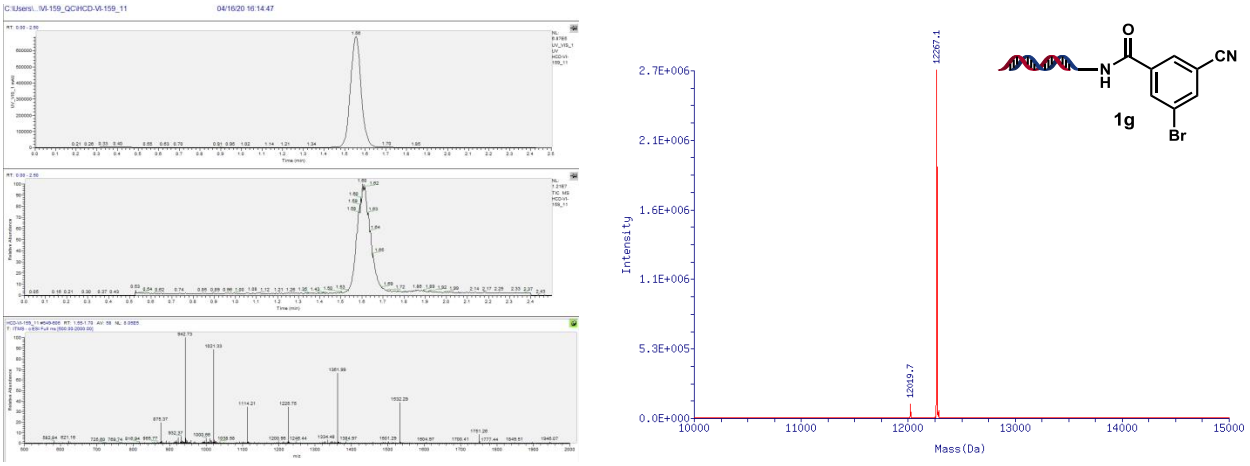


Figure S10. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1g**, expected: 12268.0; observed 12267.1

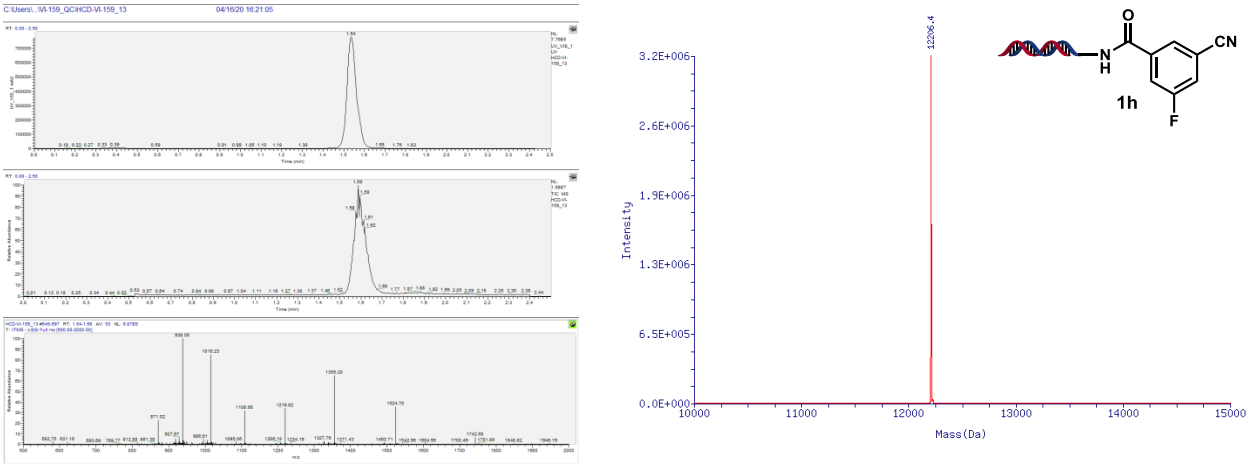
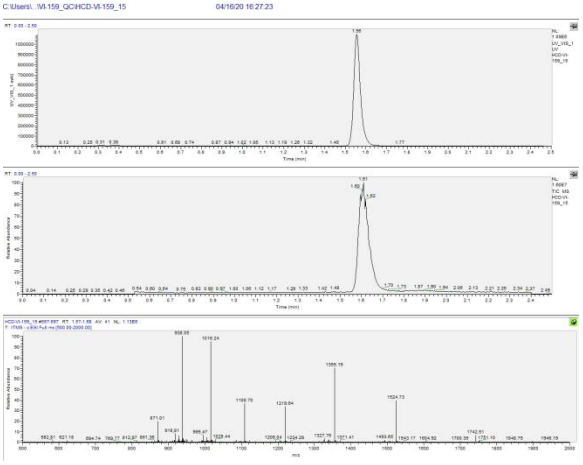


Figure S11. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1h**, expected: 12207.1; observed 12206.4



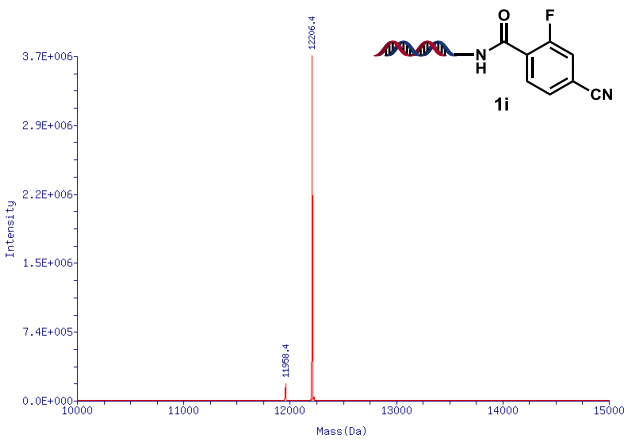
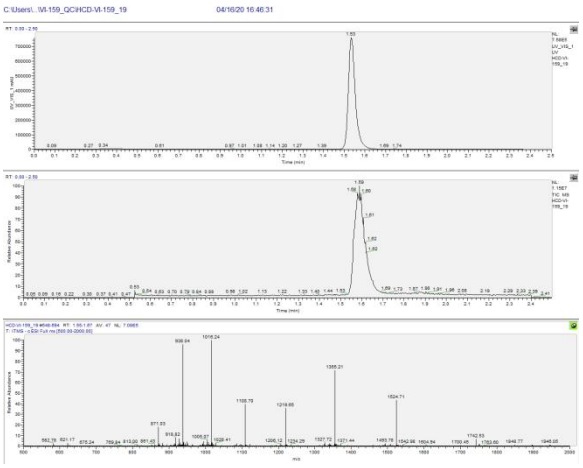


Figure S12. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1i**, expected: 12207.1; observed 12206.4



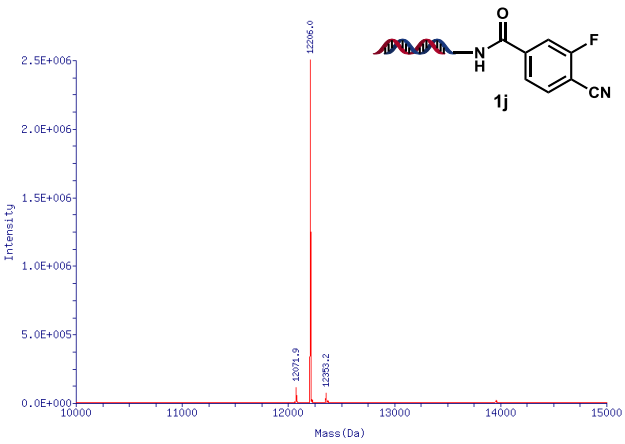


Figure S13. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1j**, expected: 12207.1; observed 12206.0

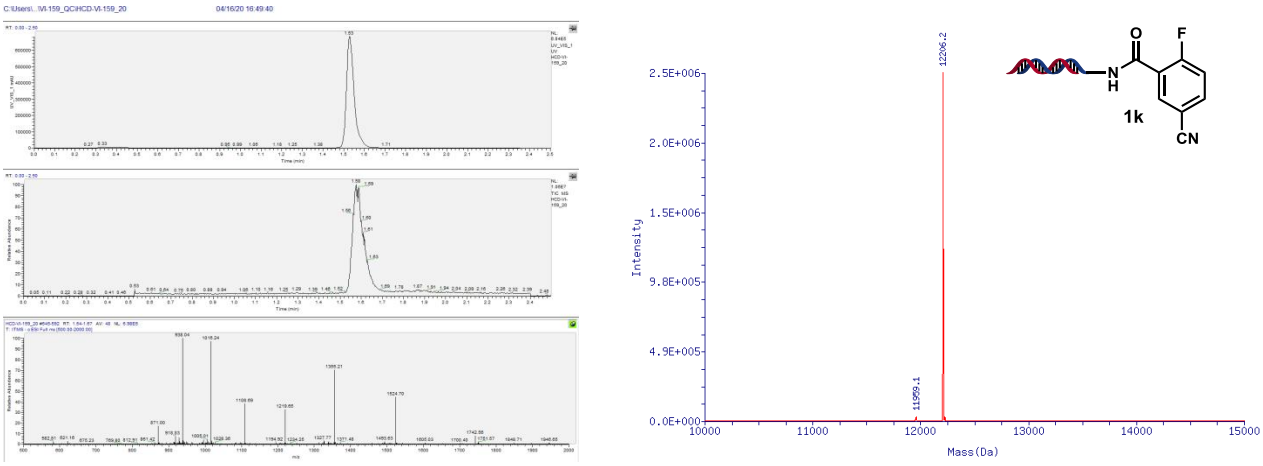


Figure S14. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1k**, expected: 12207.1; observed 12206.2

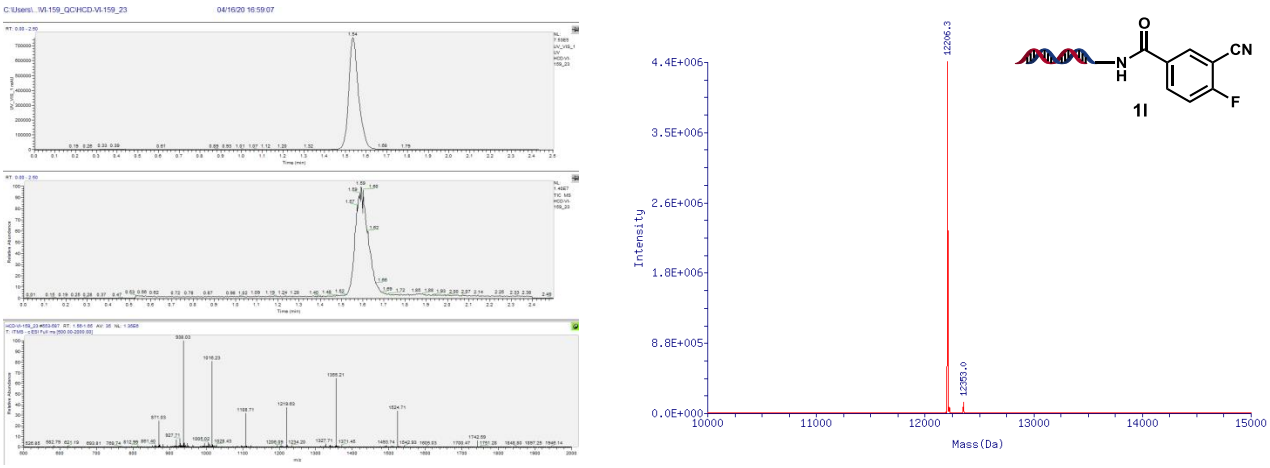


Figure S15. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1l**, expected: 12207.1; observed 12206.3

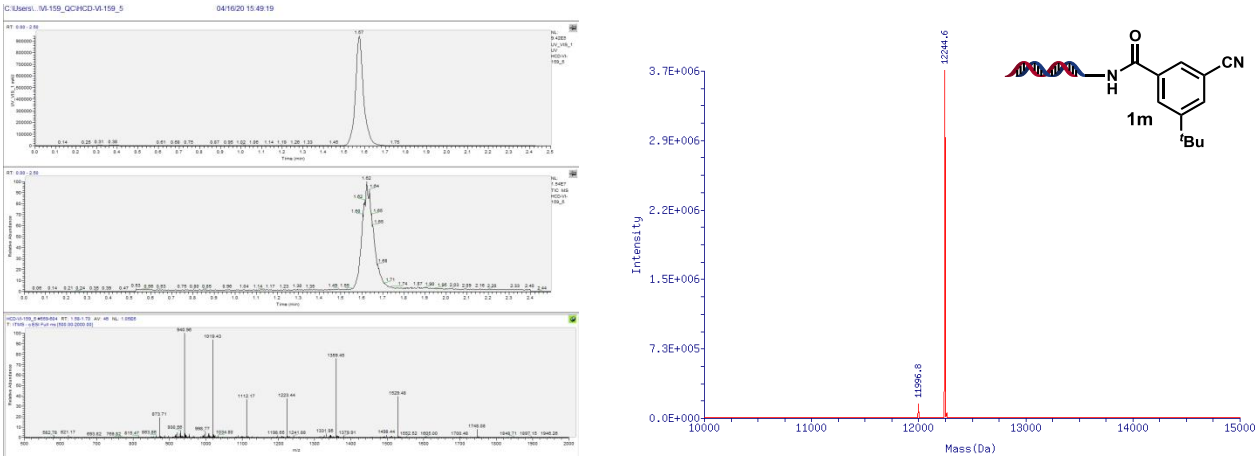


Figure S16. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1m**, expected: 12245.2; observed 12244.6

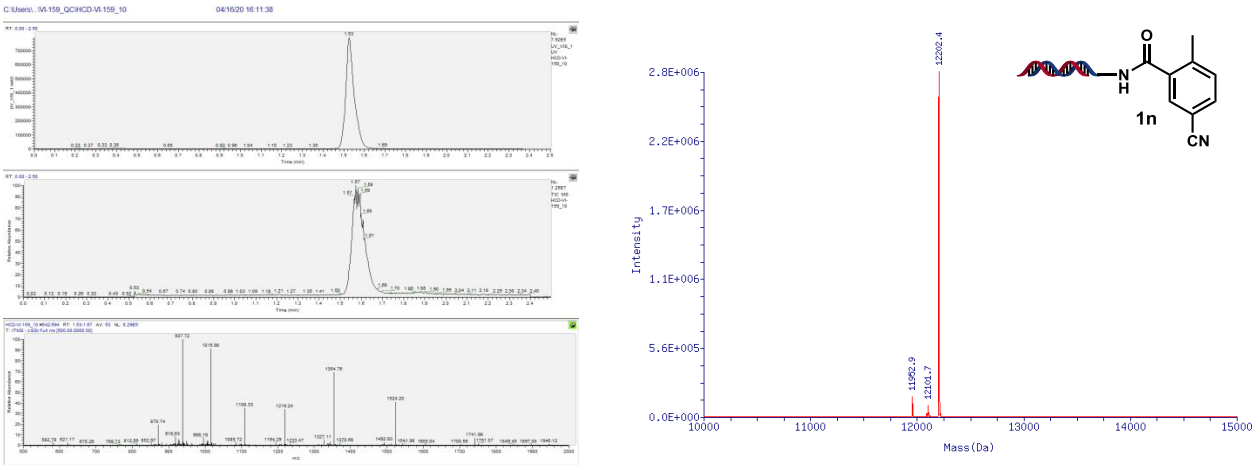


Figure S17. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1n**, expected: 12203.1; observed 12202.4

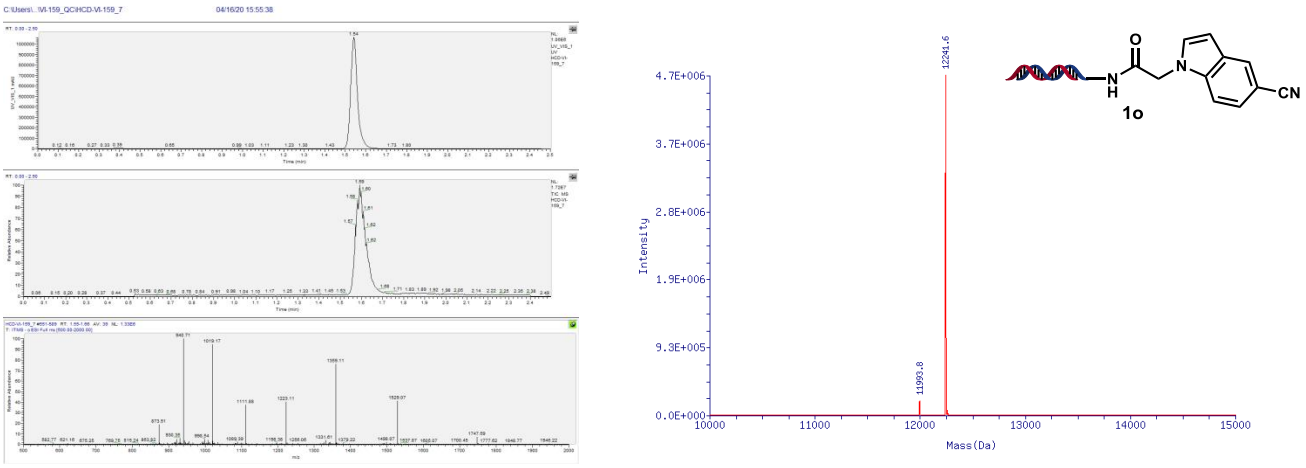


Figure S18. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1o**, expected: 12242.2; observed 12241.6

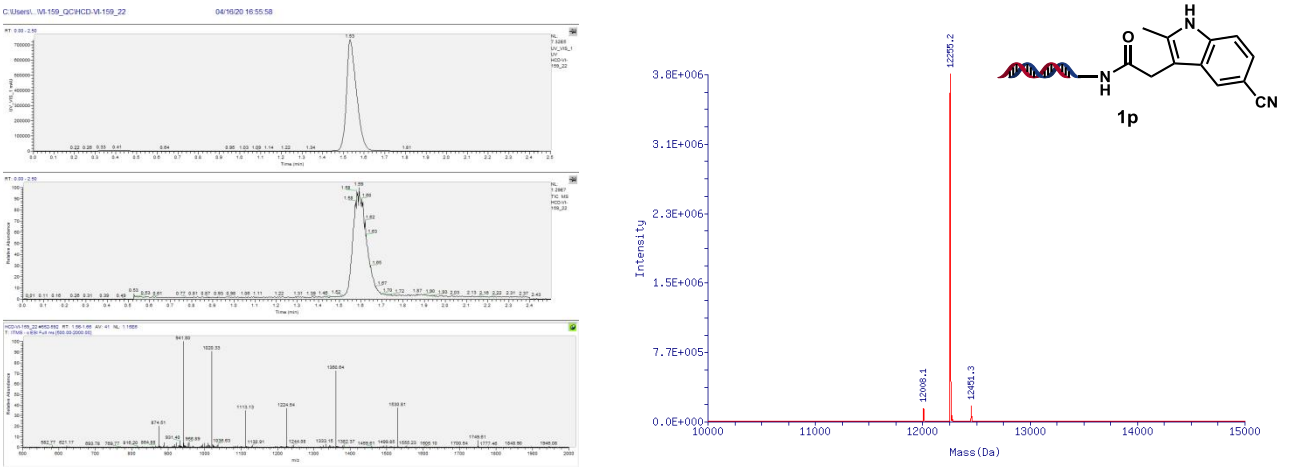


Figure S19. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1p**, expected: 12256.2; observed 12255.2

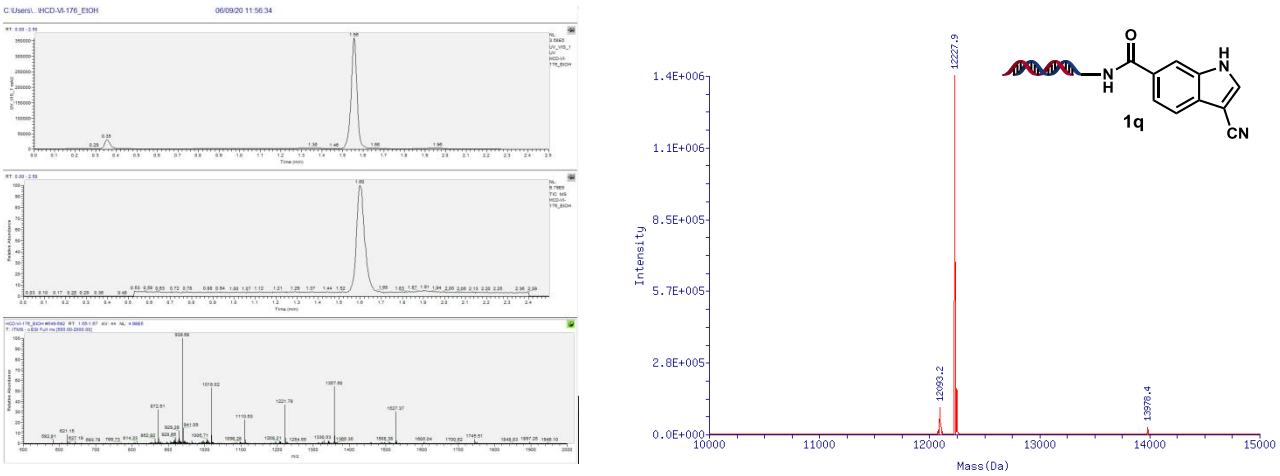


Figure S20. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1q**, expected: 12228.2; observed 12227.9

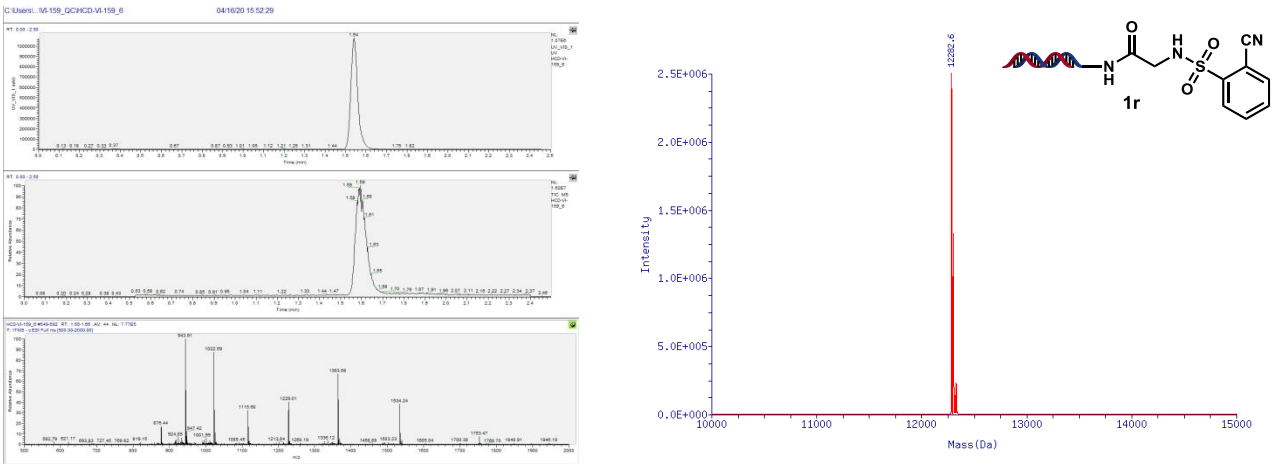


Figure S21. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1r**, expected: 12282.2; observed 12282.6

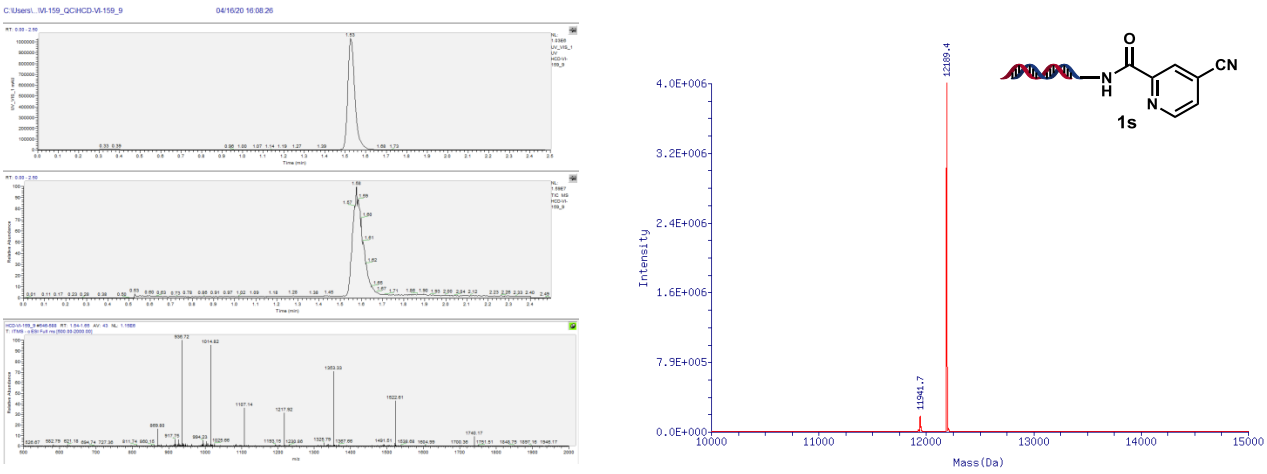


Figure S22. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1s**, expected: 12190.2; observed 12189.4

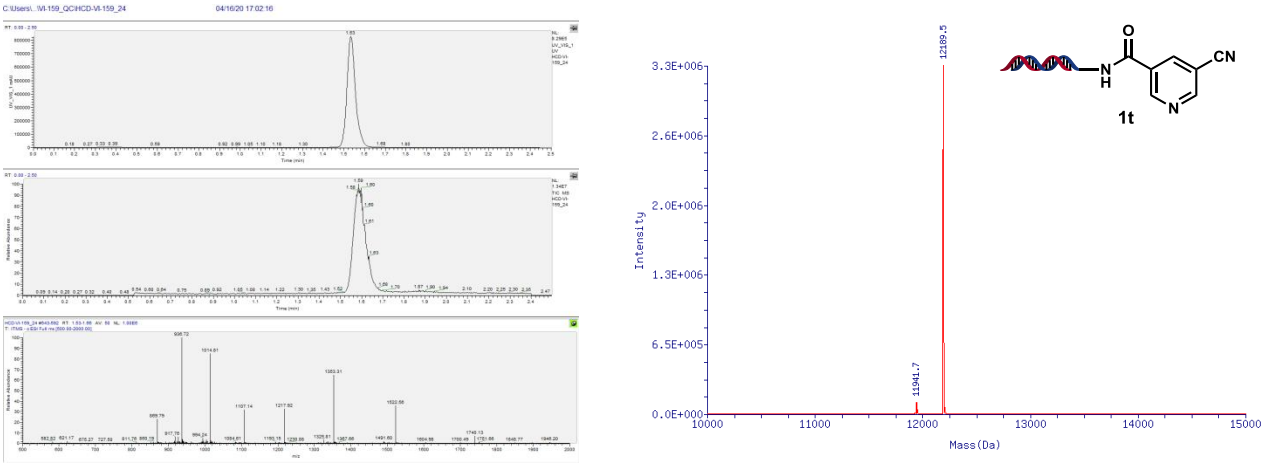


Figure S23. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1t**, expected: 12190.1; observed 12189.5

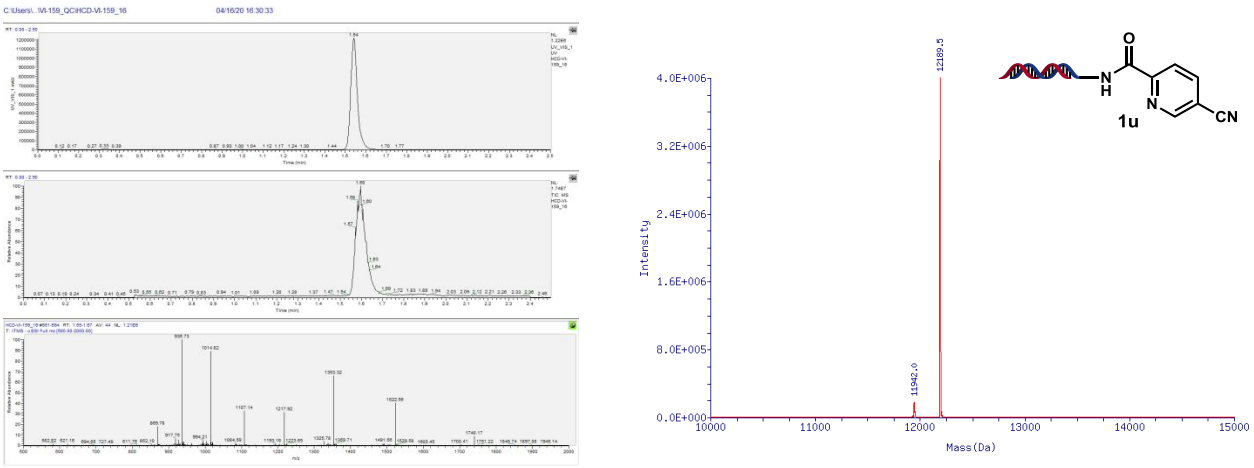


Figure S24. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1u**, expected: 12190.1; observed 12189.5

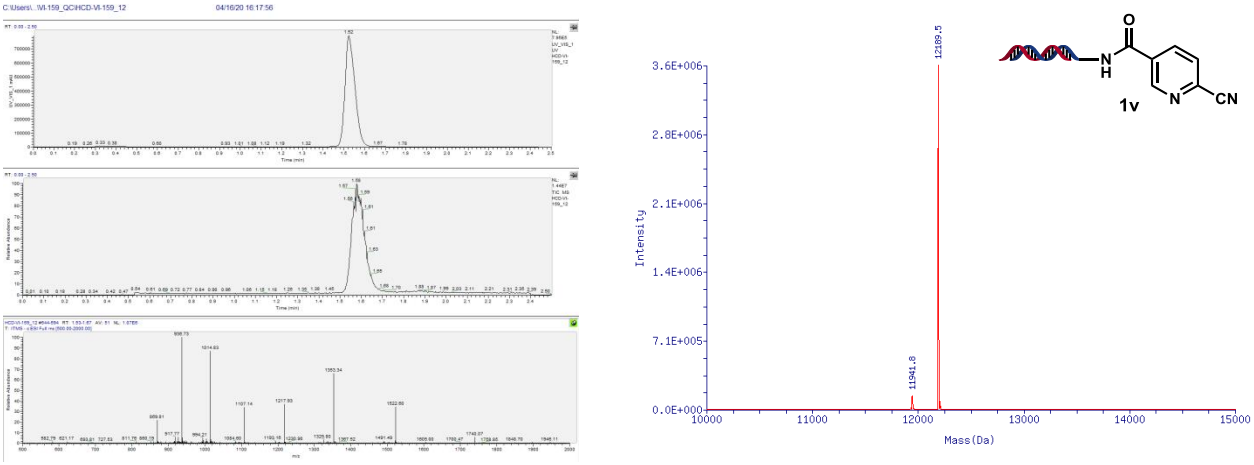


Figure S25. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1v**, expected: 12190.1; observed 12189.5

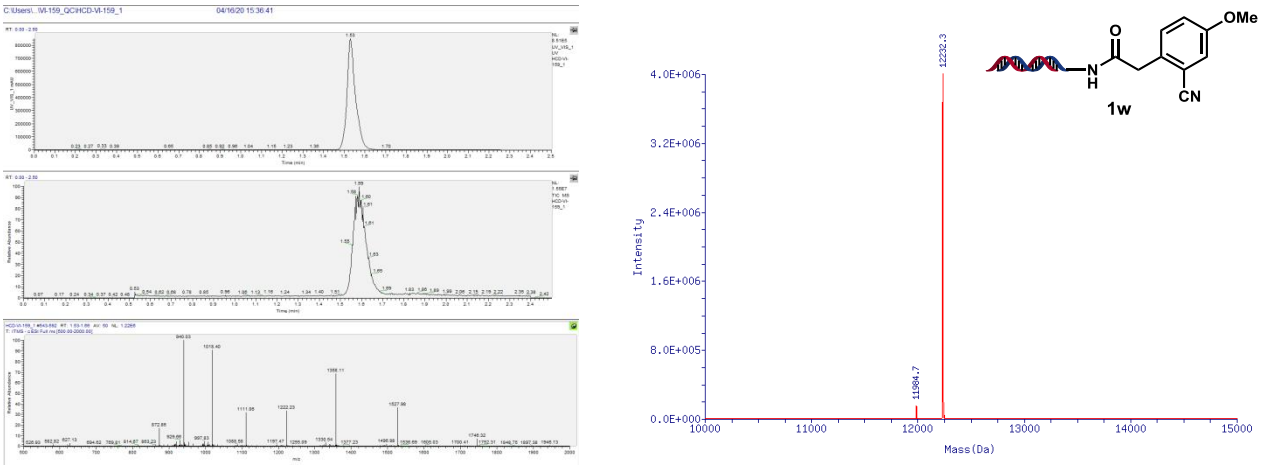


Figure S26. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1w**, expected: 12233.2; observed 12232.3

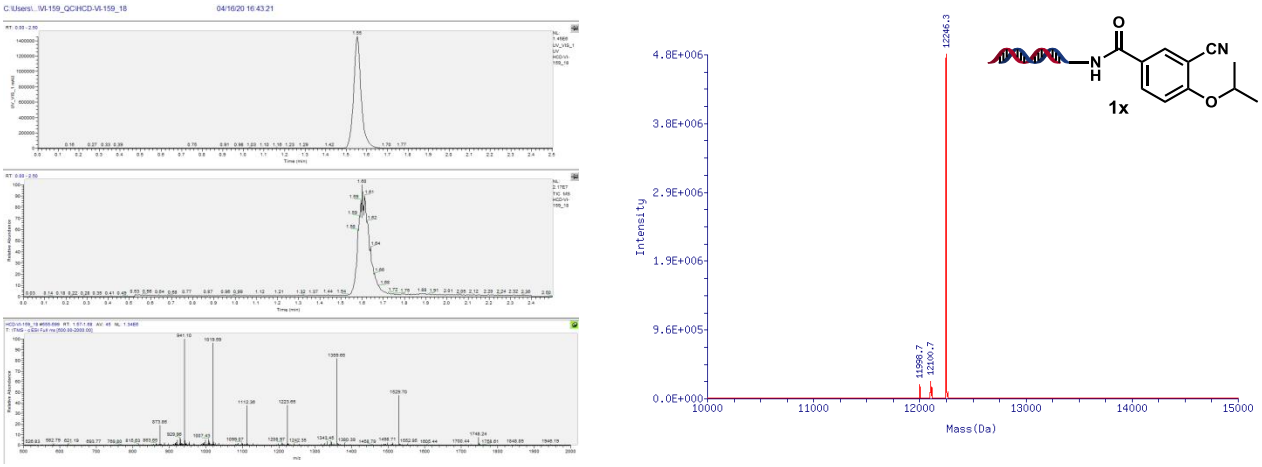


Figure S27. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1x**, expected: 12247.2; observed 12246.3

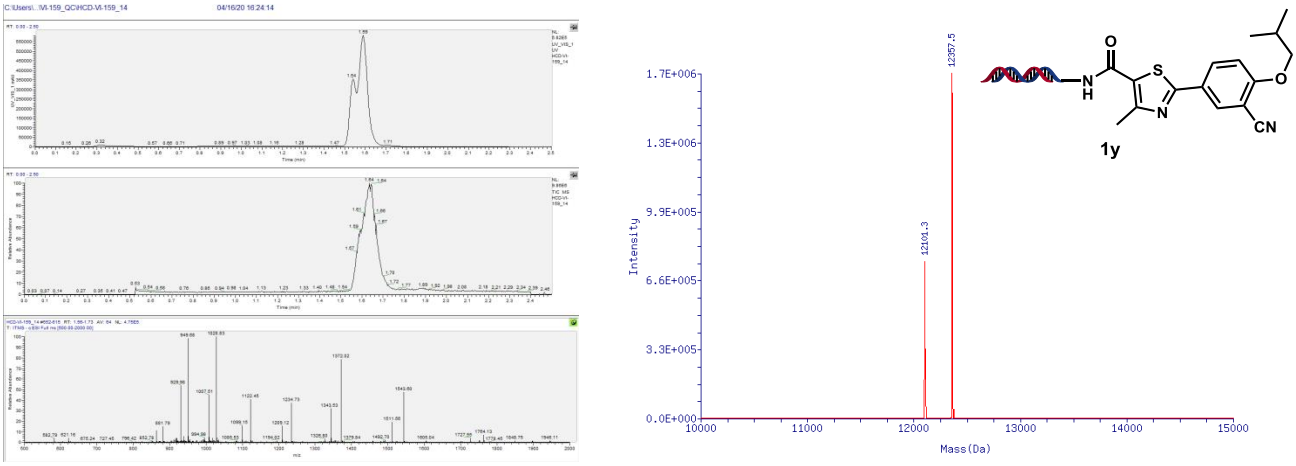


Figure S28. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1y**, expected: 12358.4; observed 12357.5

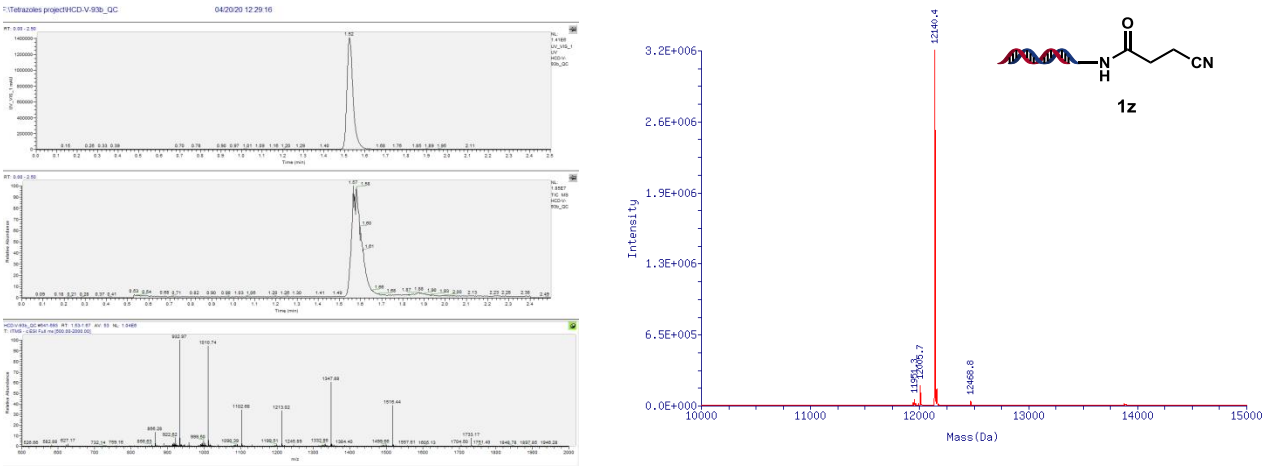
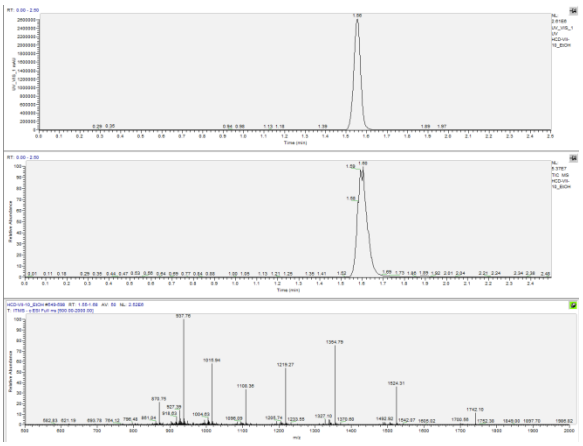


Figure S29. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1z**, expected: 12141.1; observed 12140.4



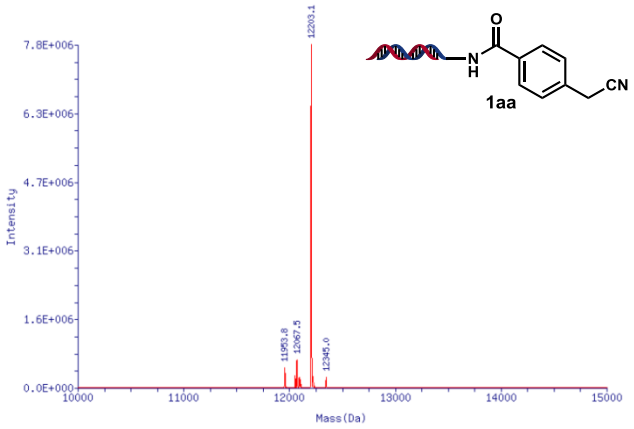
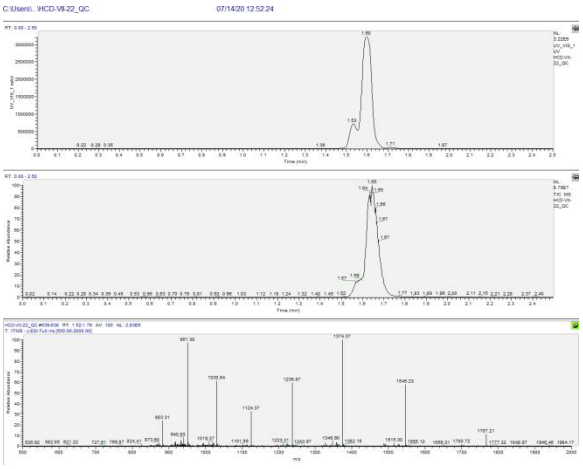


Figure S30. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1aa**, expected: 12203.1; observed 12203.1



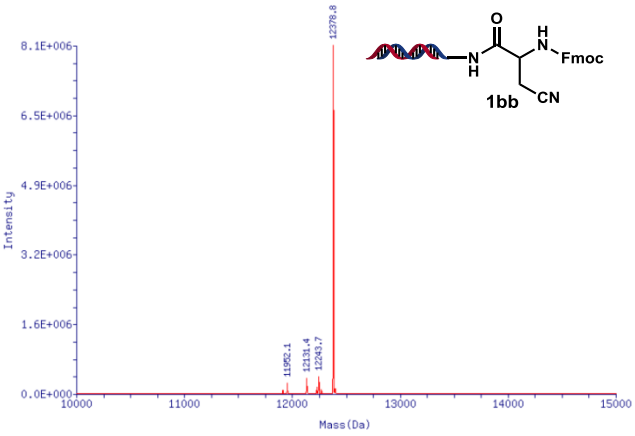


Figure S31. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **1bb**, expected: 12378.3; observed 12378.8

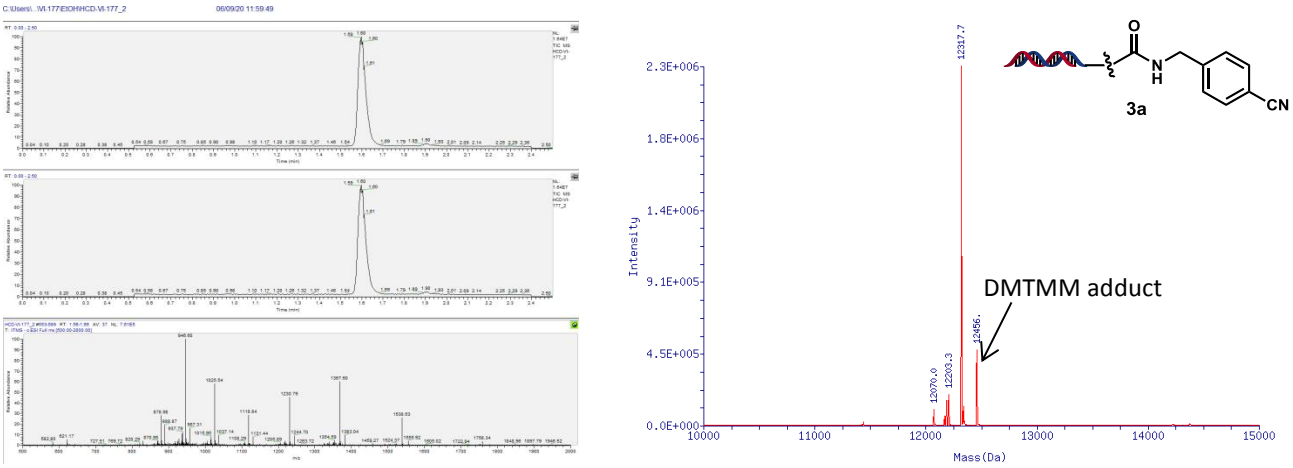


Figure S32. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3a**, expected: 12318.2; observed 12317.7

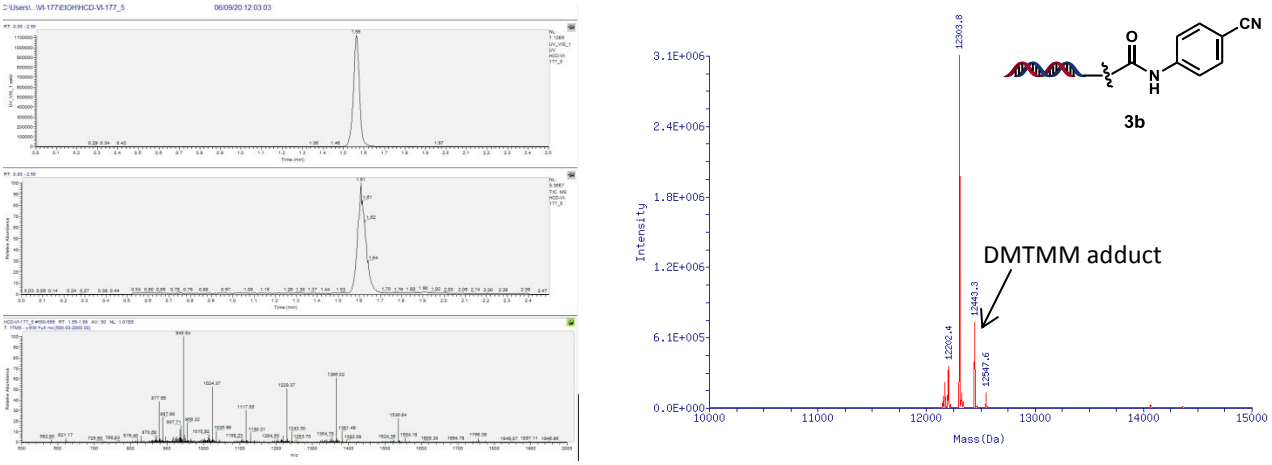


Figure S33. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3b**, expected: 12304.1; observed 12303.8

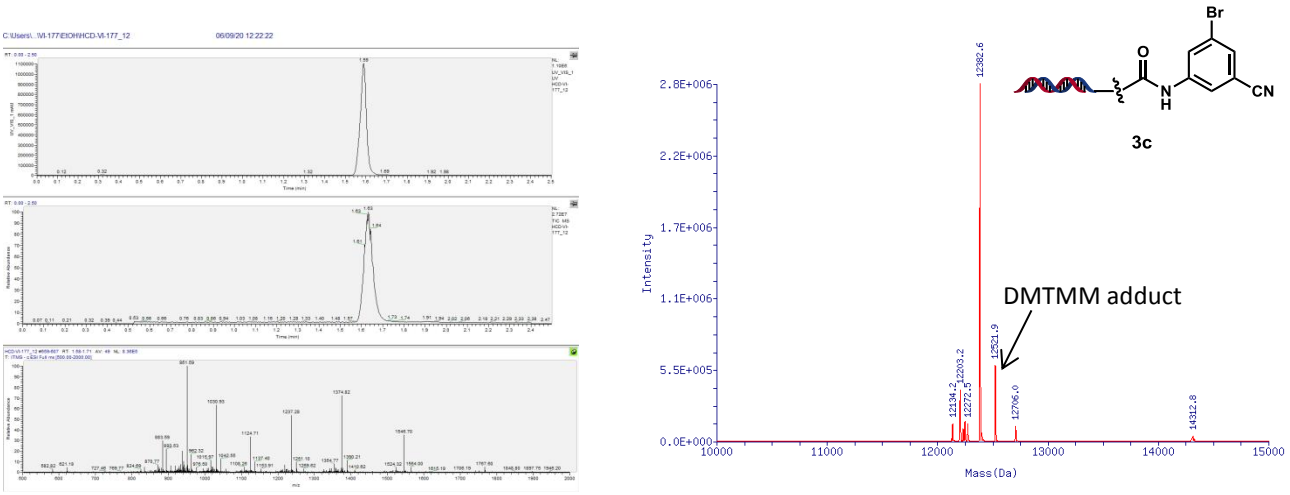


Figure S34. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3c**, expected: 12383.0; observed 12382.6

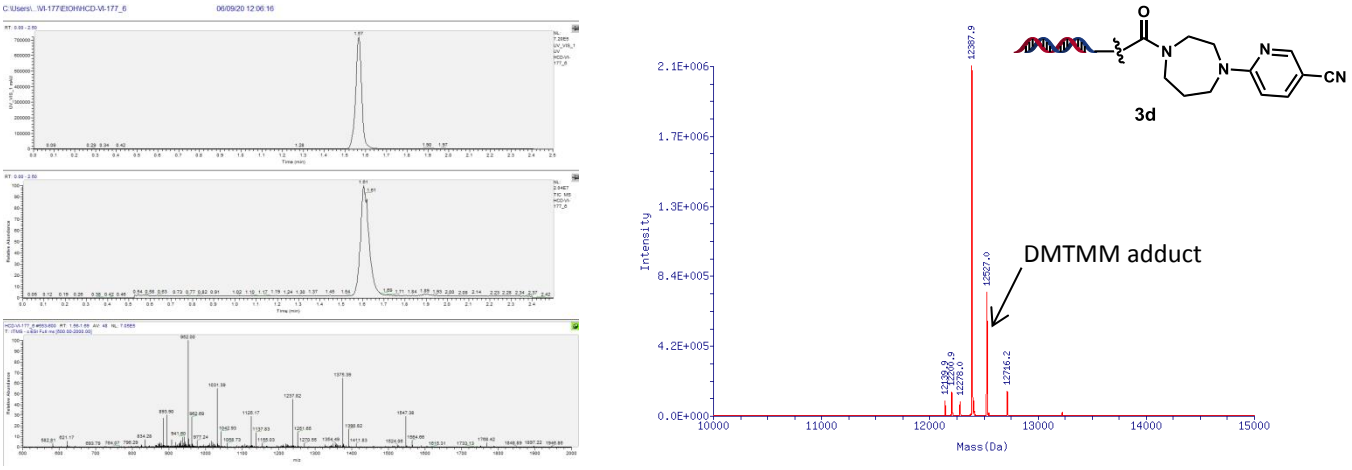


Figure S35. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3d**, expected: 12388.3; observed 12387.9

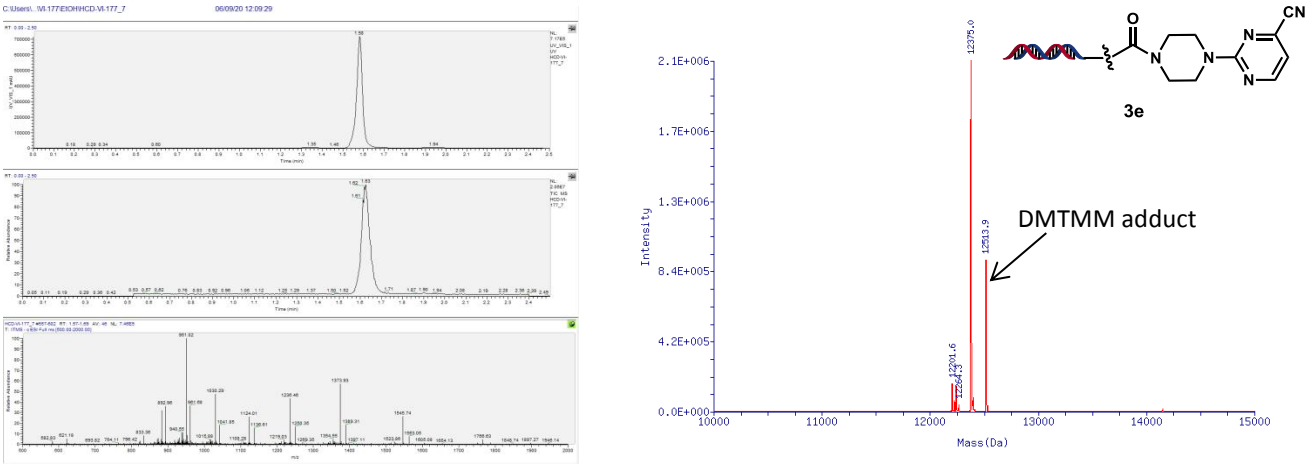


Figure S36. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3e**, expected: 12375.2; observed 12375.0

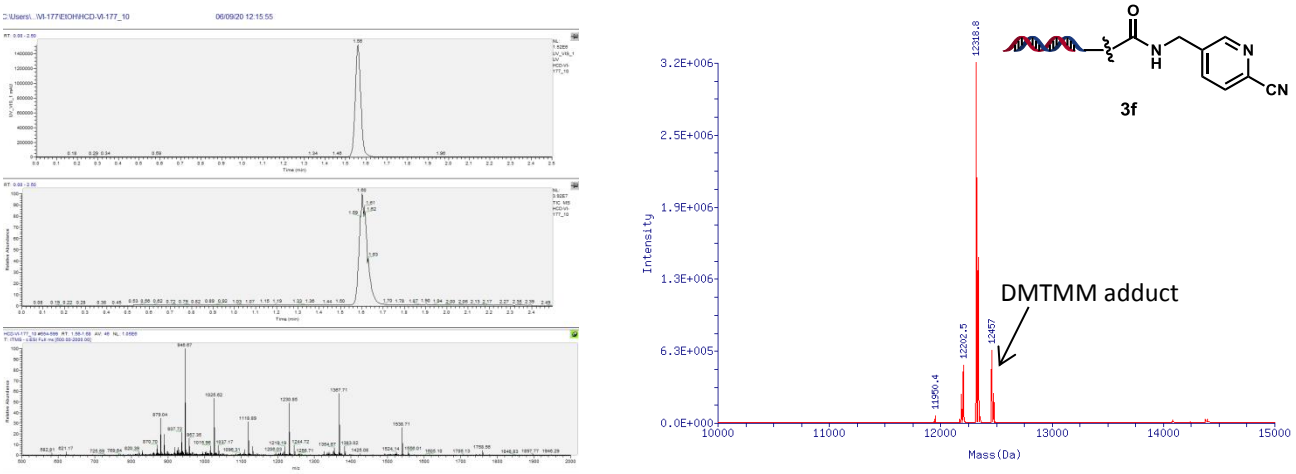


Figure S37. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3f**, expected: 12319.2; observed 12318.8

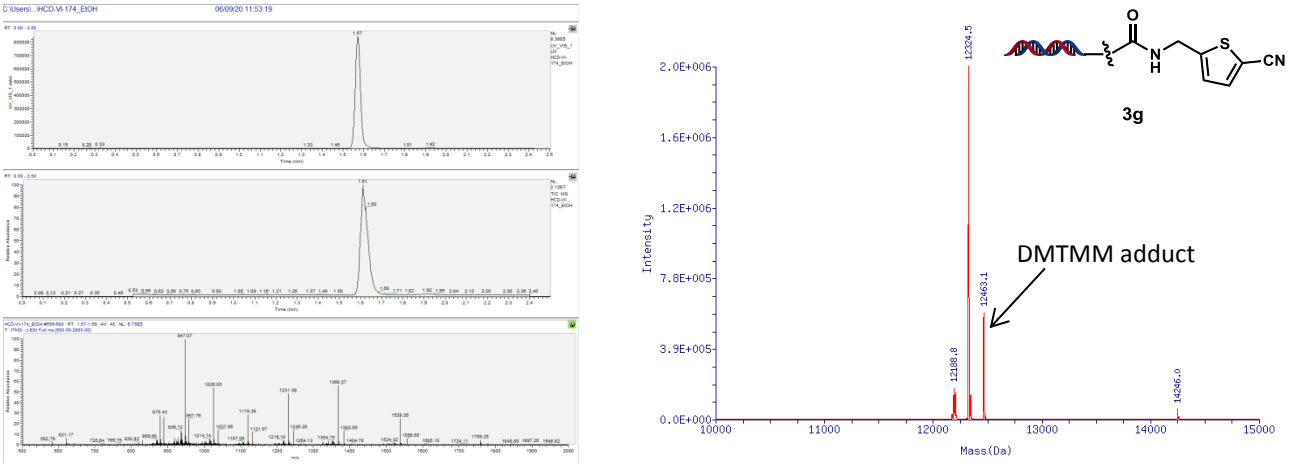


Figure S38. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3g**, expected: 12324.3; observed 12324.5

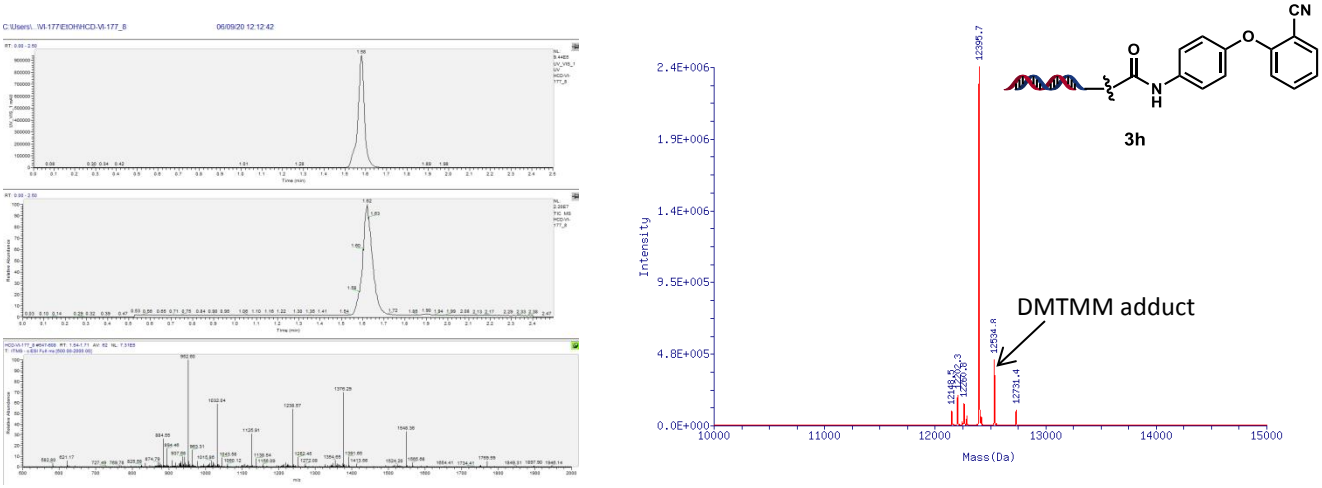


Figure S39. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3h**, expected: 12396.2; observed 12395.7

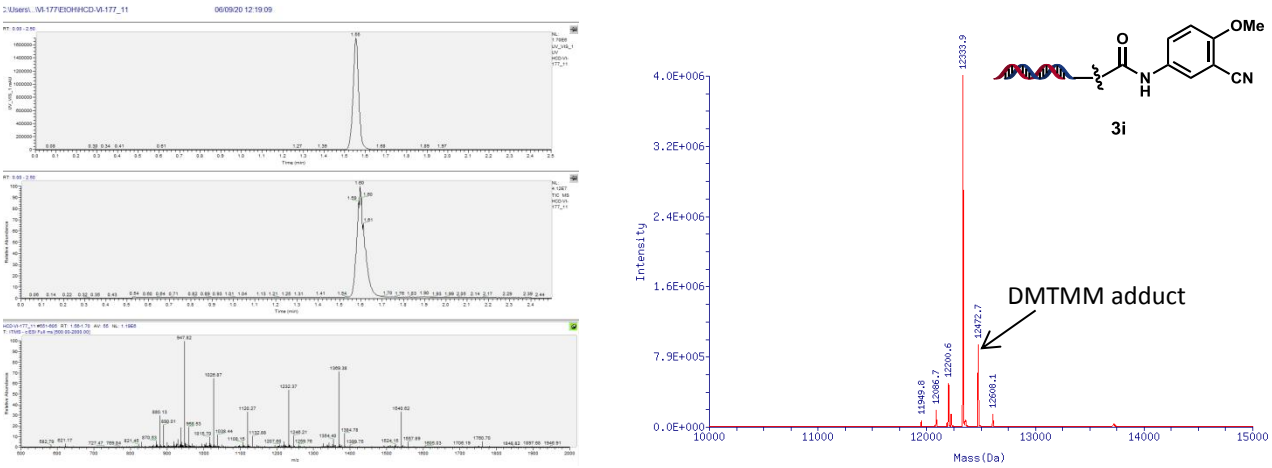


Figure S40. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3i**, expected: 12334.2; observed 12333.9

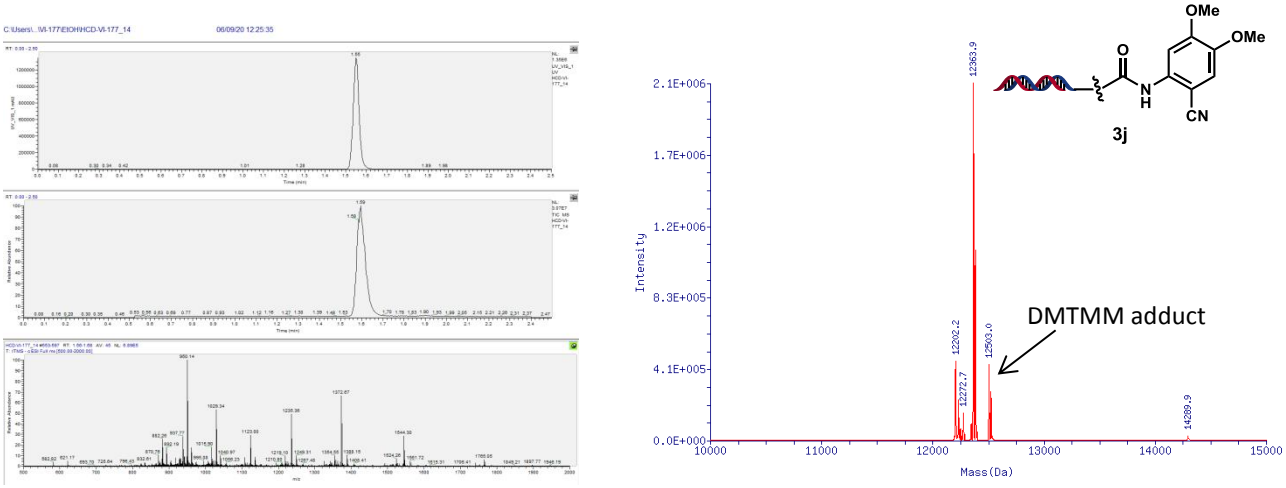


Figure S41. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3j**, expected: 12364.2; observed 12363.9

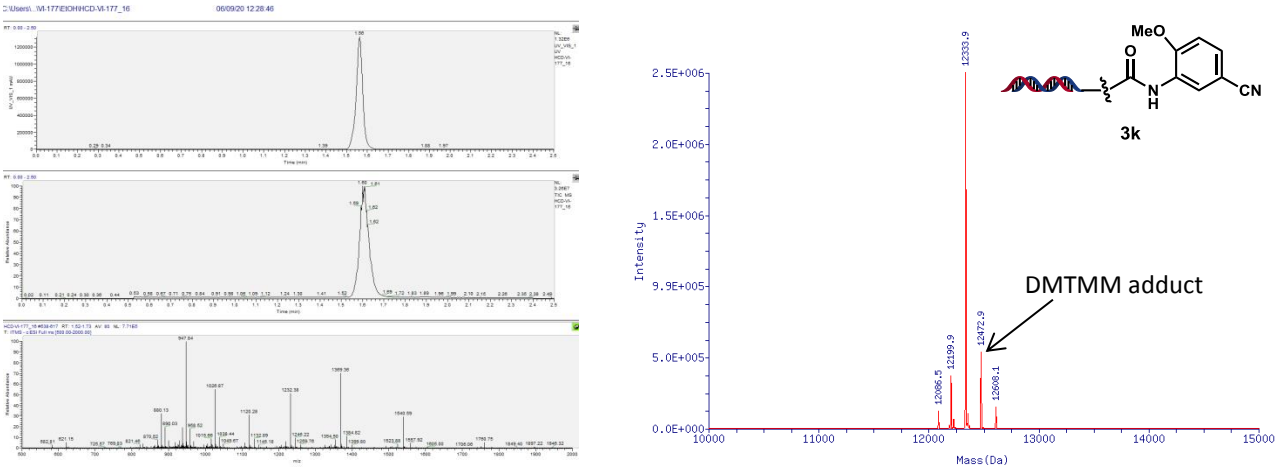


Figure S42. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3k**, expected: 12334.2; observed 12333.9

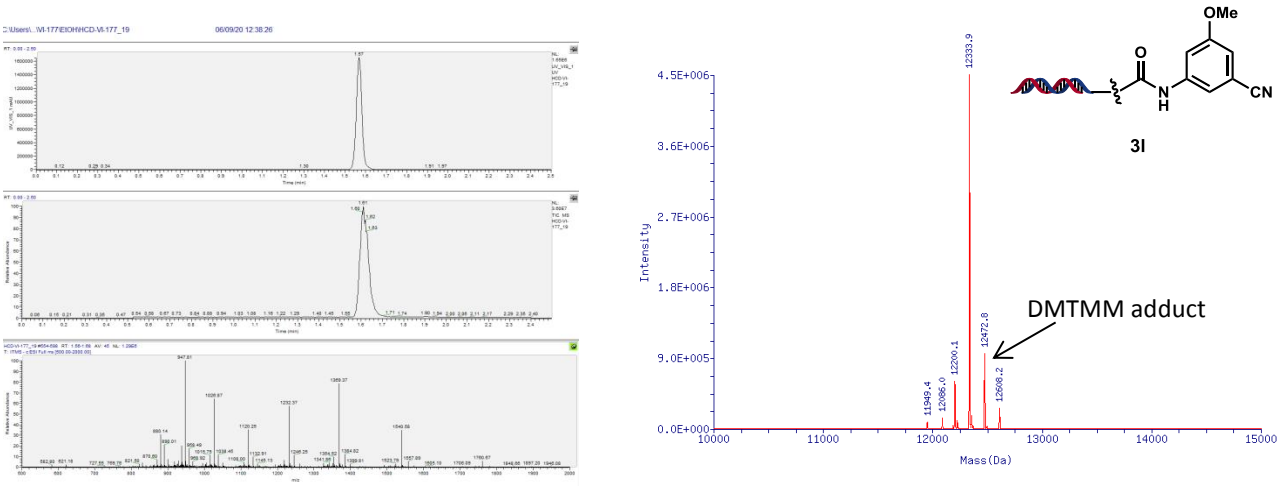


Figure S43. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3l**, expected: 12334.2; observed 12333.9

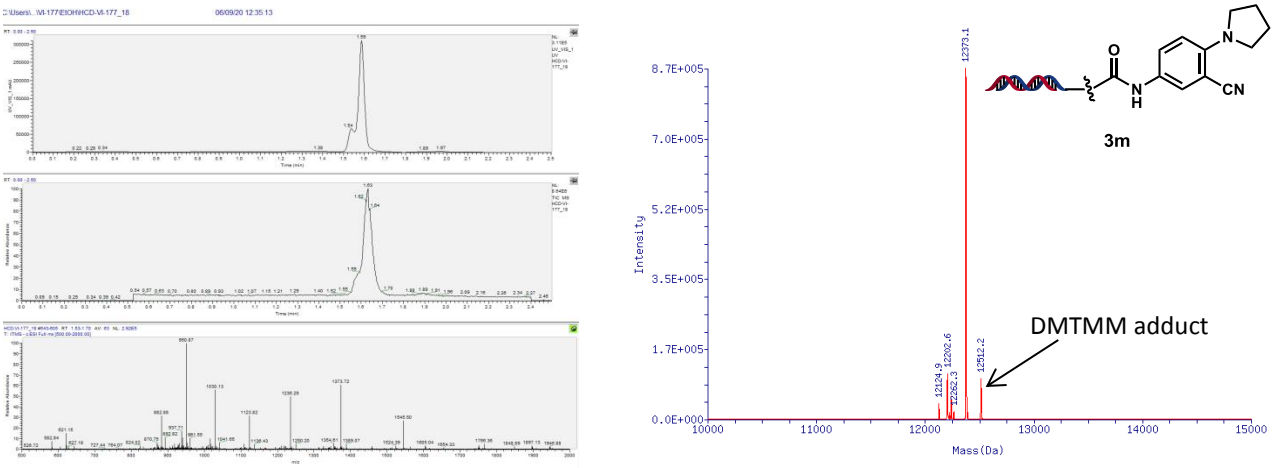


Figure S44. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **3m**, expected: 12373.2; observed 12373.1

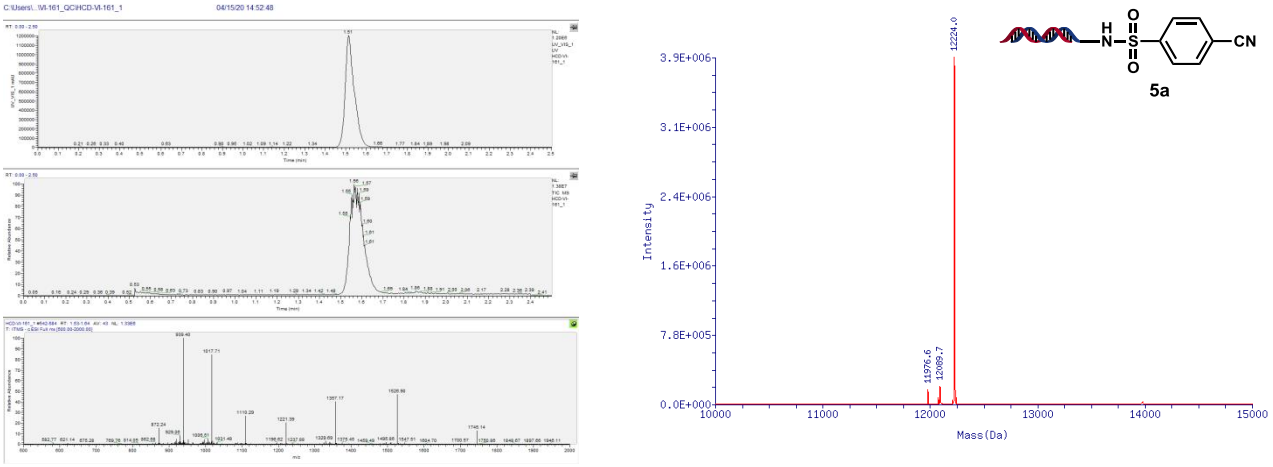


Figure S45. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5a**, expected: 12225.1; observed 12224.0

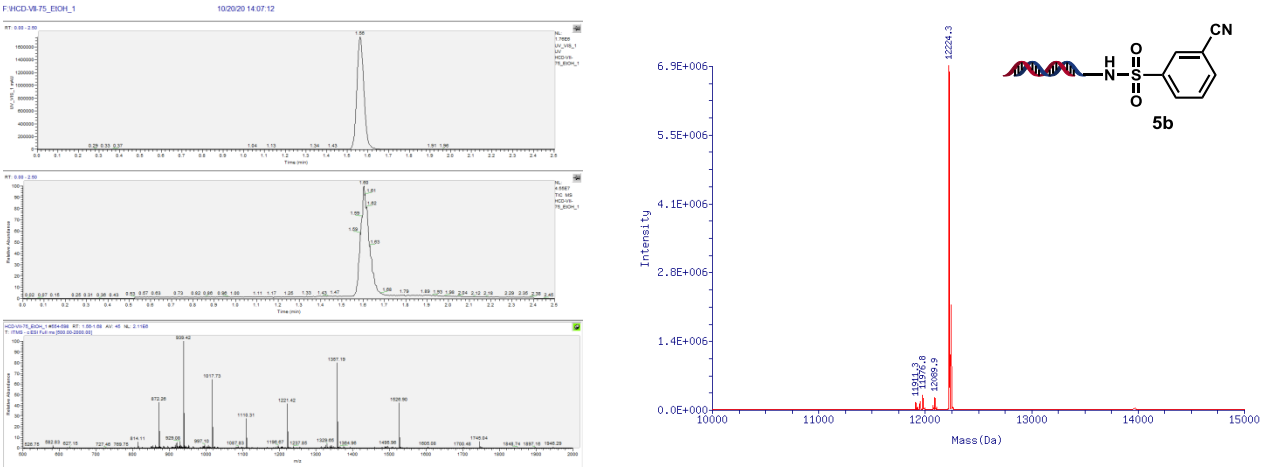


Figure S46. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5b**, expected: 12225.1; observed 12224.3

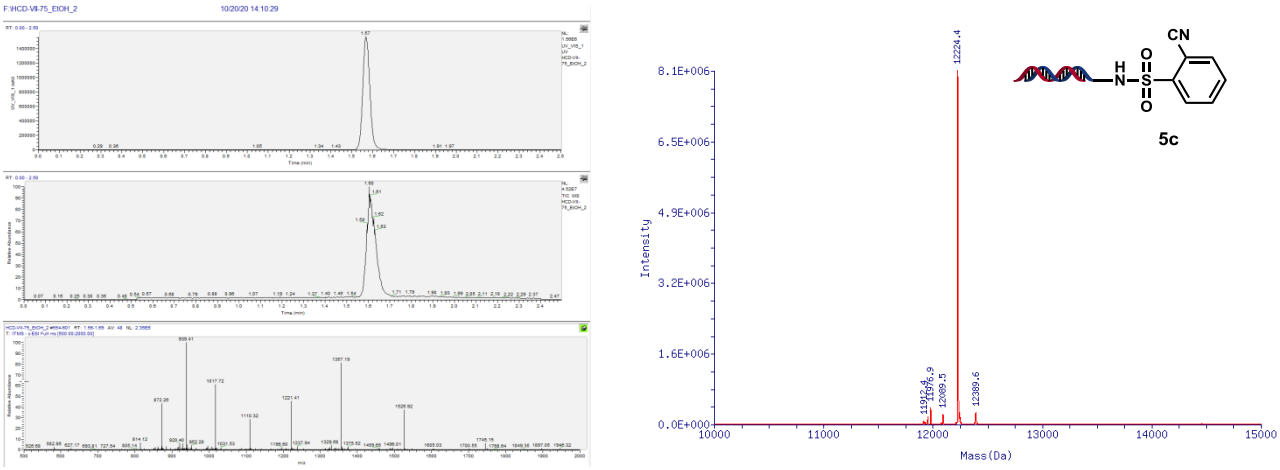


Figure S47. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5c**, expected: 12225.1; observed 12224.4

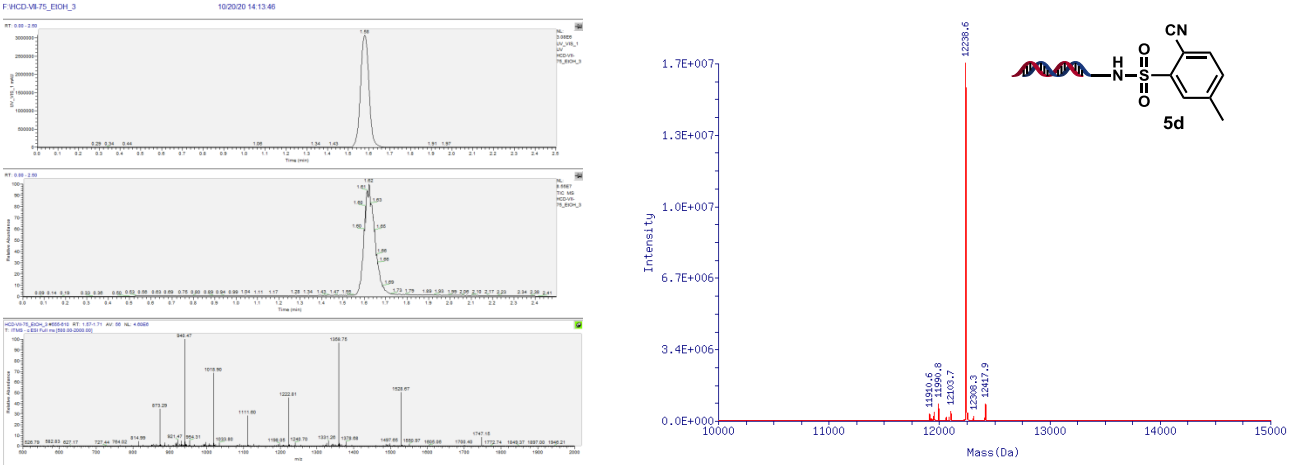


Figure S48. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5d**, expected: 12239.2; observed 12238.6

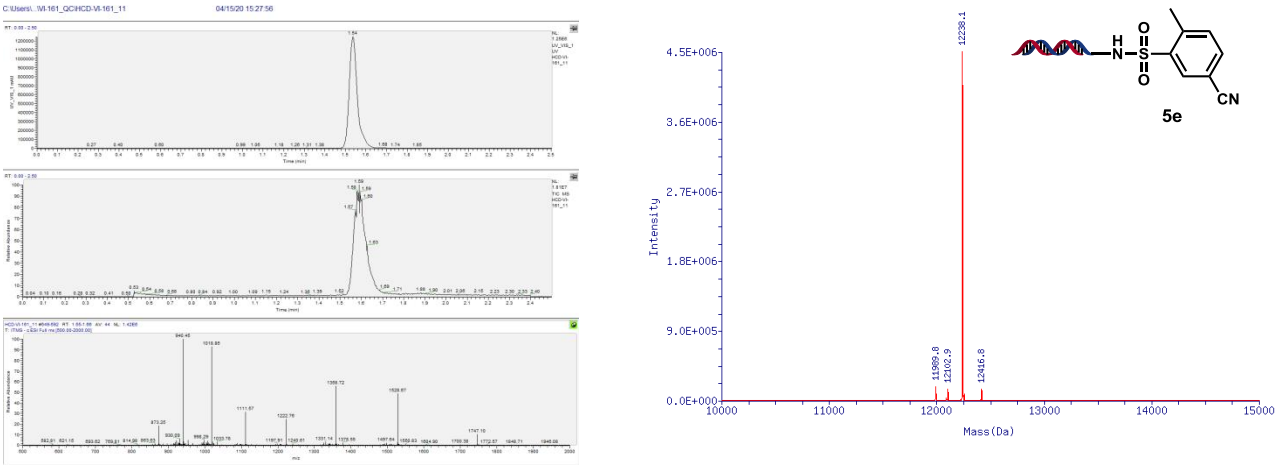


Figure S49. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5e**, expected: 12239.2; observed 12238.1

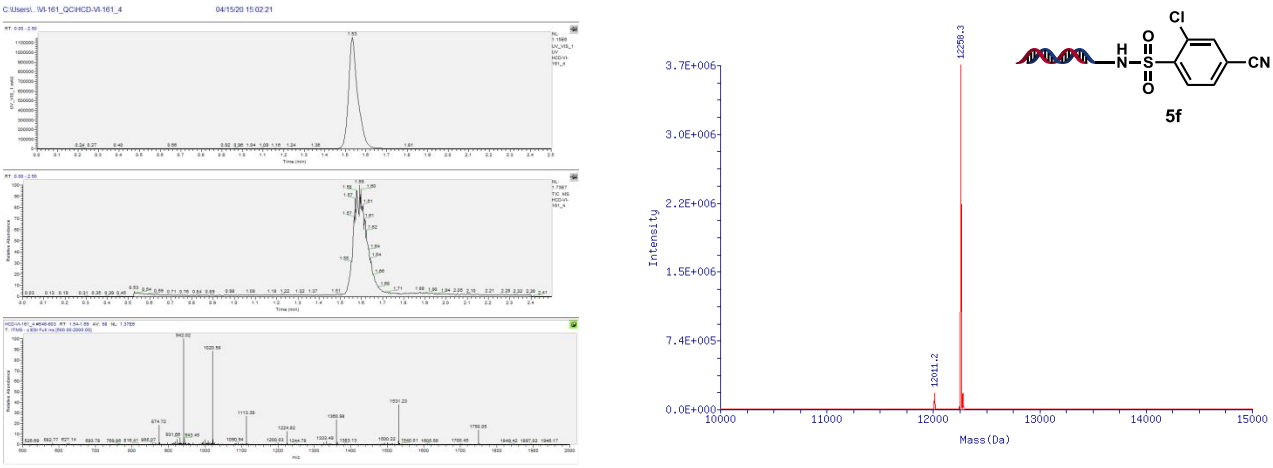


Figure S50. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5f**, expected: 12259.6; observed 12258.3

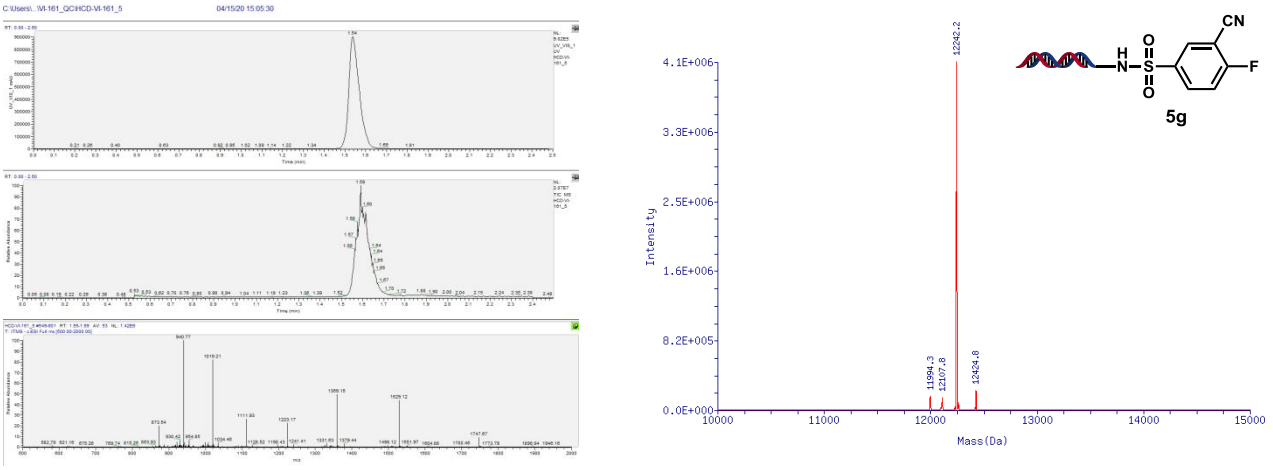


Figure S51. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5g**, expected: 12243.1; observed 12242.2

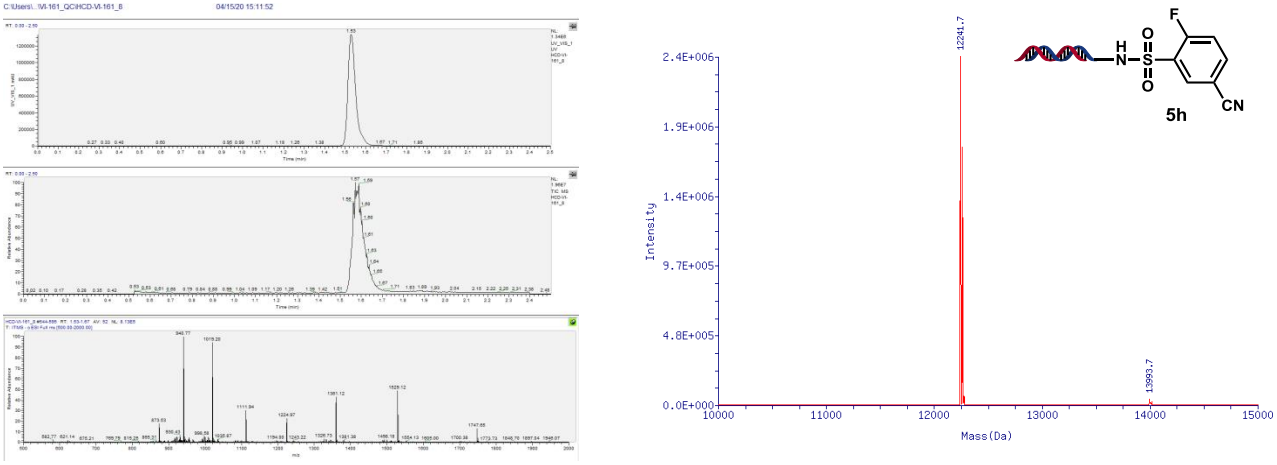


Figure S52. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5h**, expected: 12243.1; observed 12241.7

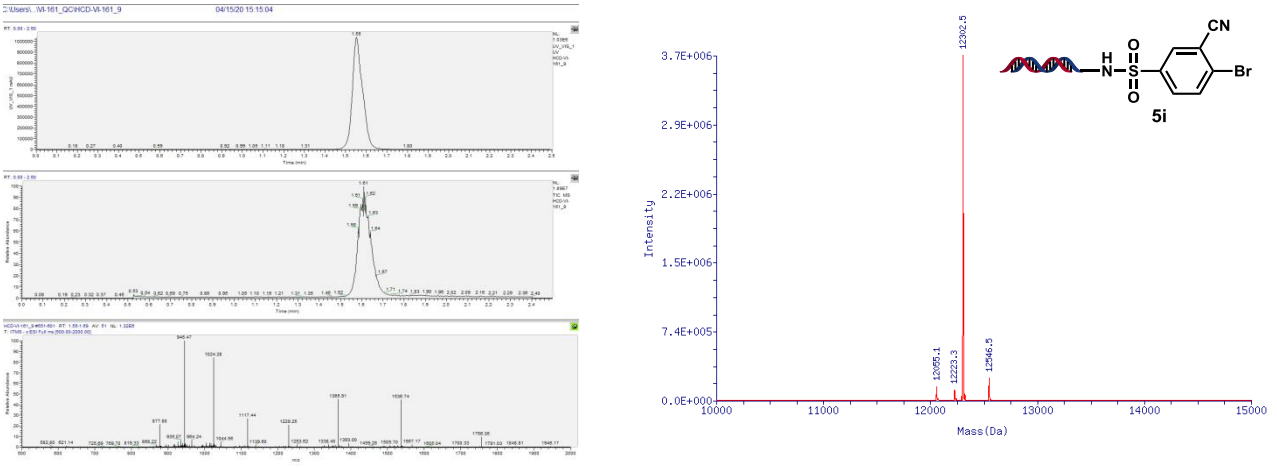
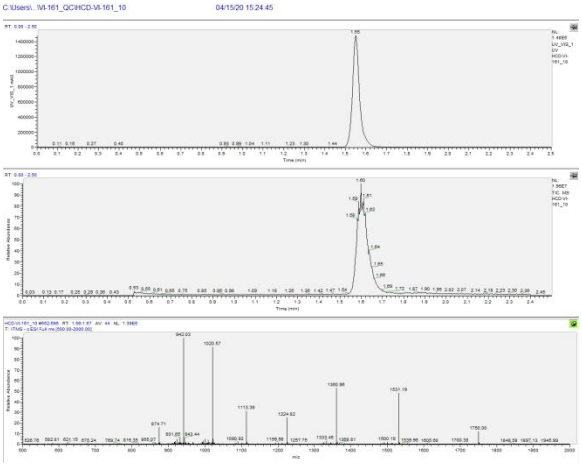


Figure S53. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5i**, expected: 12304.0; observed 12302.5



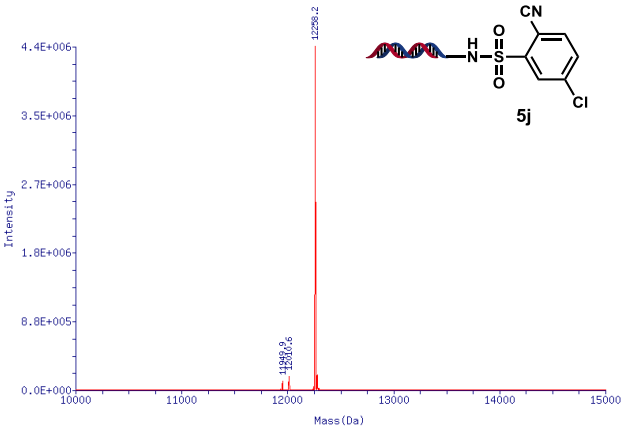
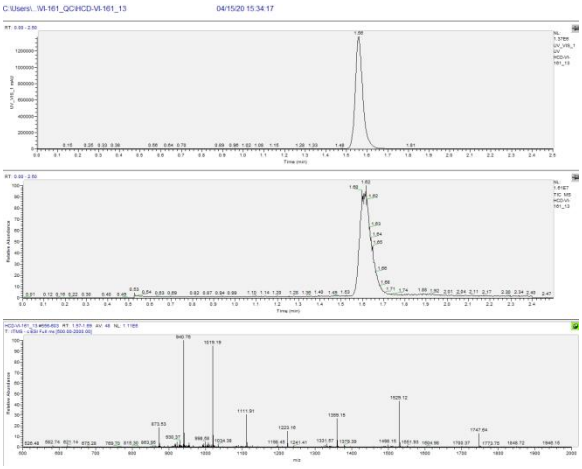


Figure S54. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5j**, expected: 12259.6; observed 12258.2



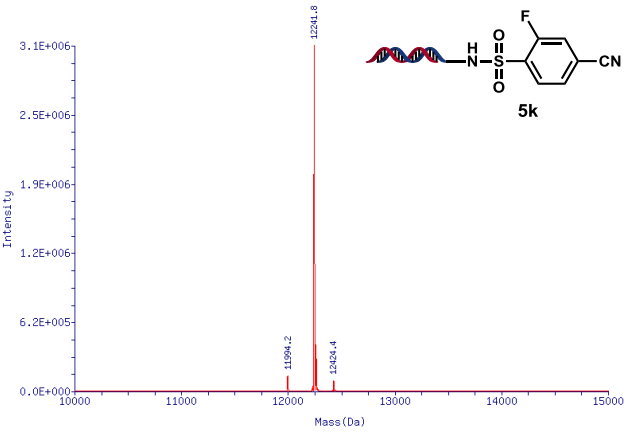


Figure S55. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5k**, expected: 12243.1; observed 12241.8

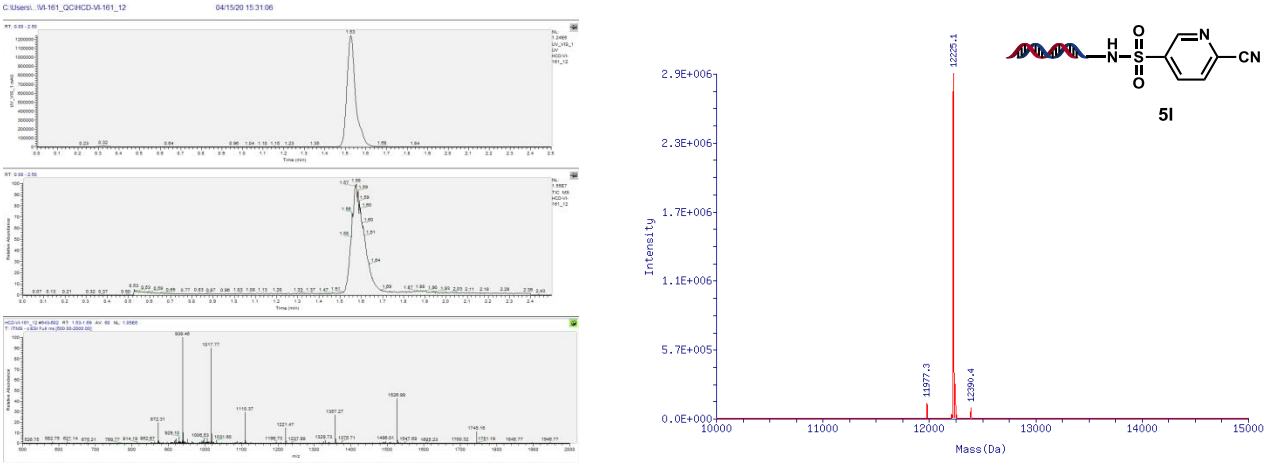


Figure S56. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5l**, expected: 12226.1; observed 12225.1

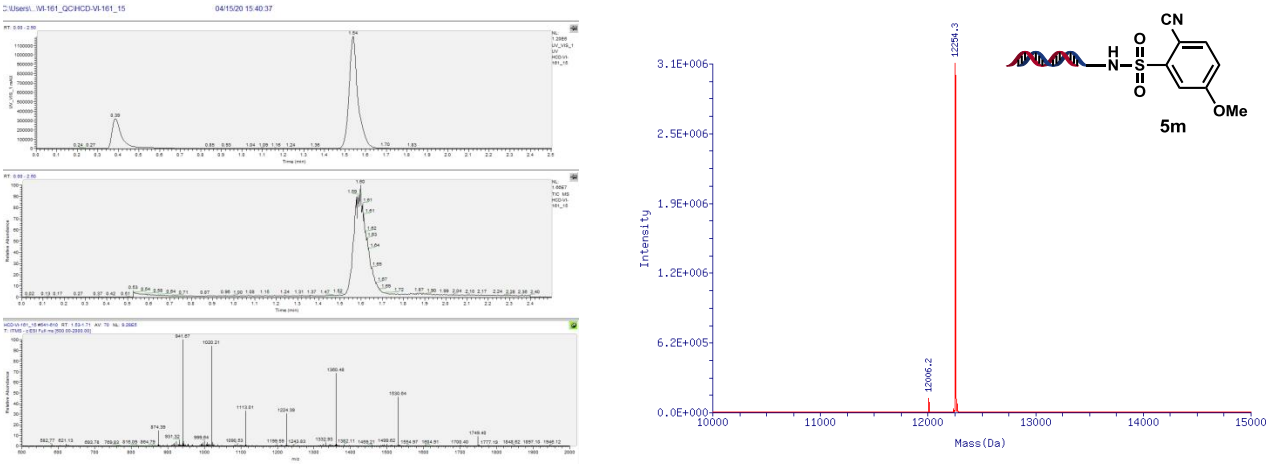
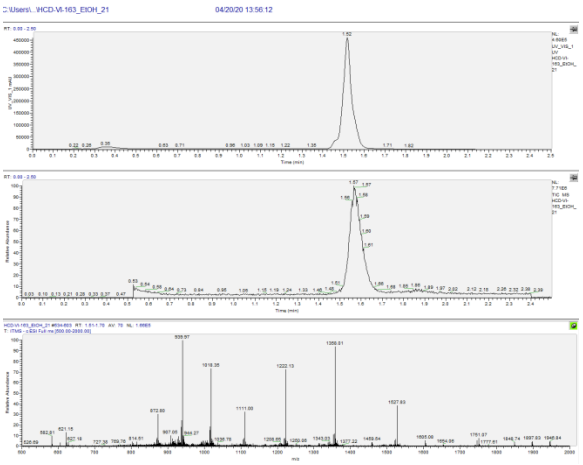


Figure S57. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **5m**, expected: 12255.2; observed 12254.3



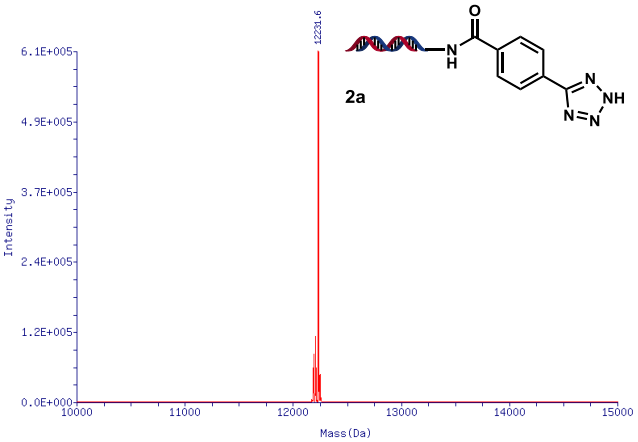
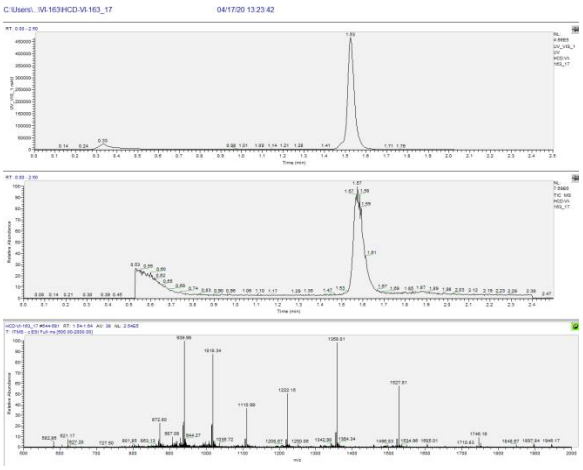


Figure S58. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2a**, expected: 12232.1; observed 12231.6



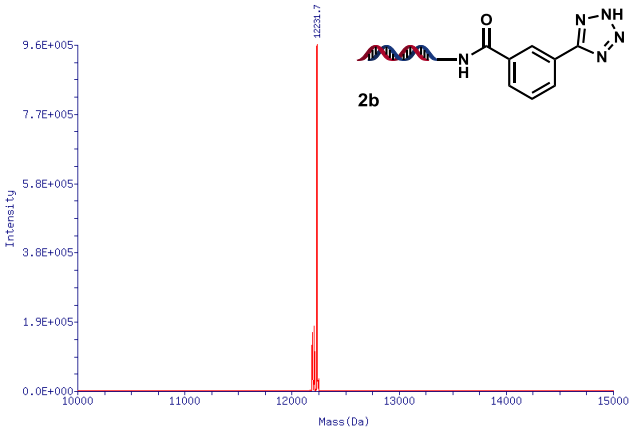


Figure S59. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2b**, expected: 12232.1; observed 12231.7

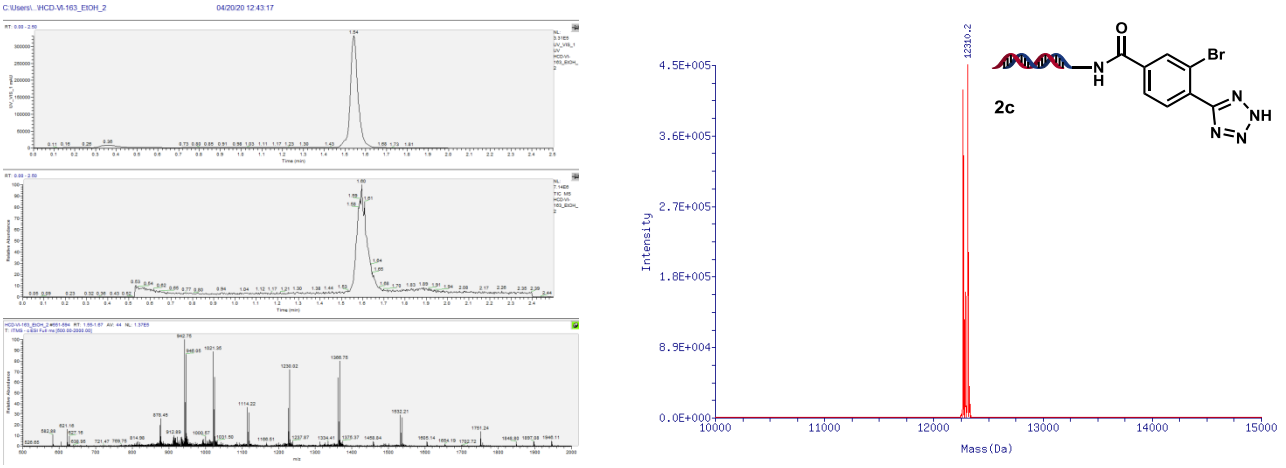


Figure S60. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2c**, expected: 12311.0; observed 12310.2

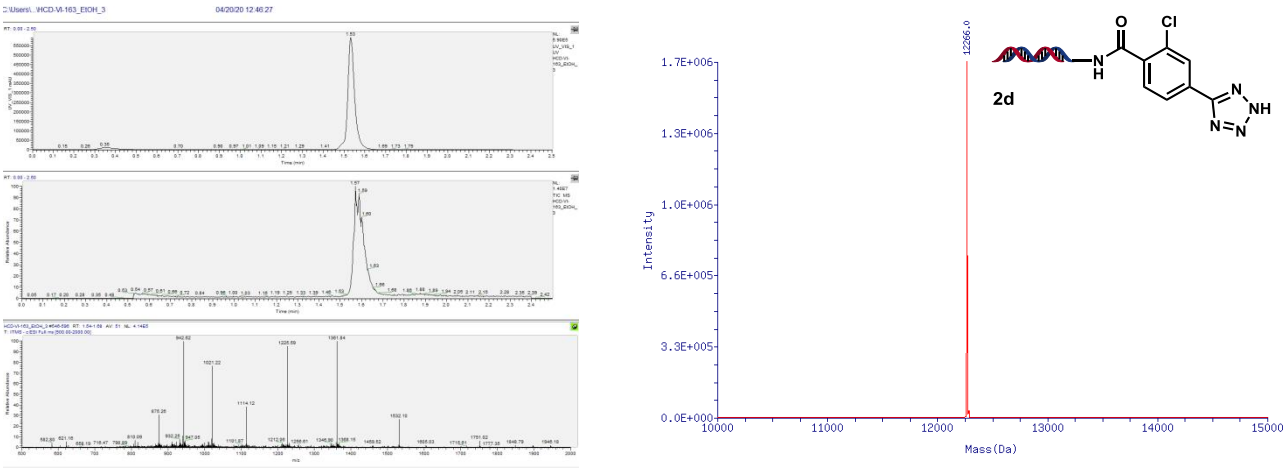


Figure S61. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2d**, expected: 12266.6; observed 12266.0

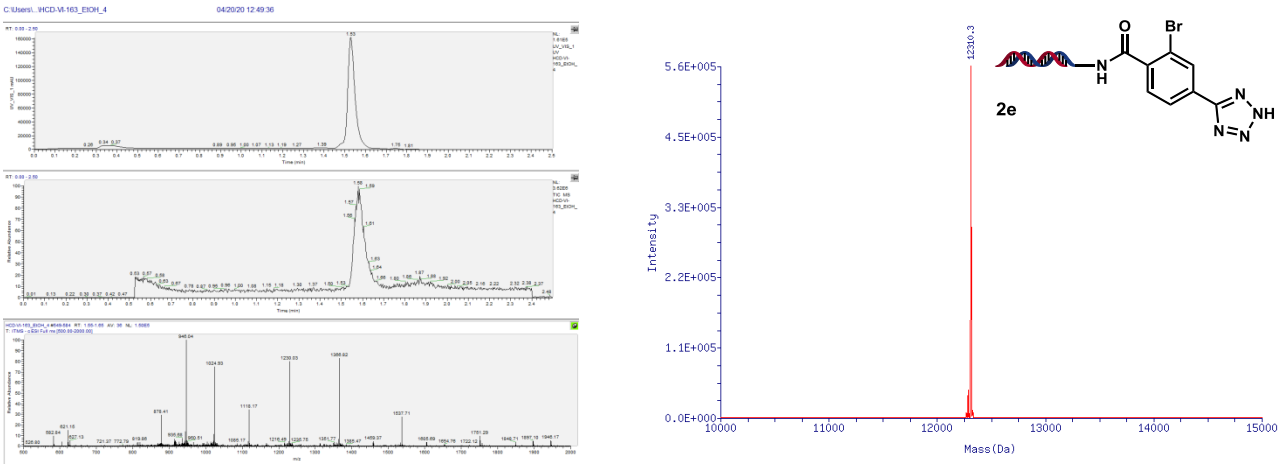


Figure S62. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2e**, expected: 12311.0; observed 12310.3

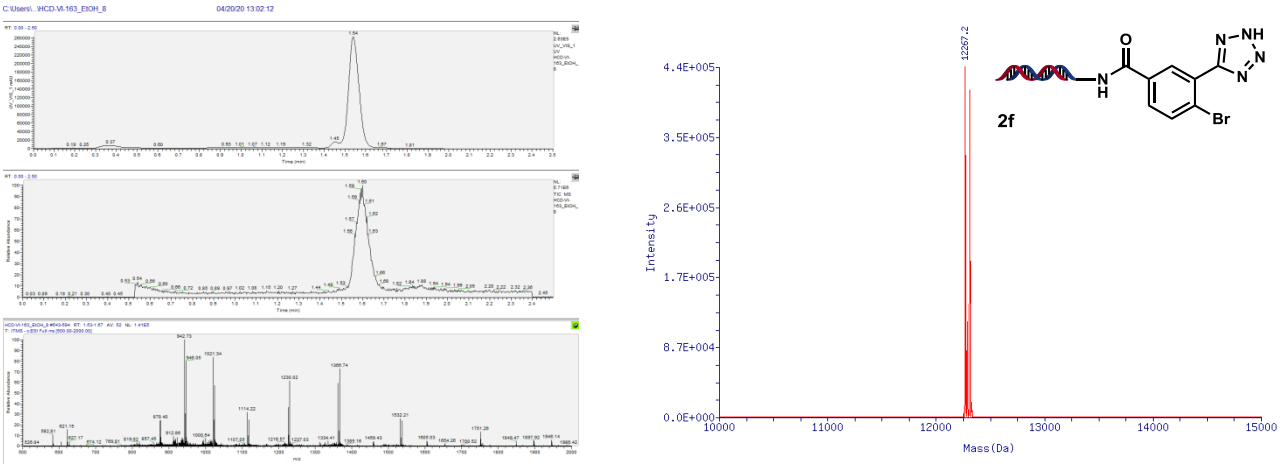


Figure S63. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2f**, expected: 12311.0; observed 12310.2

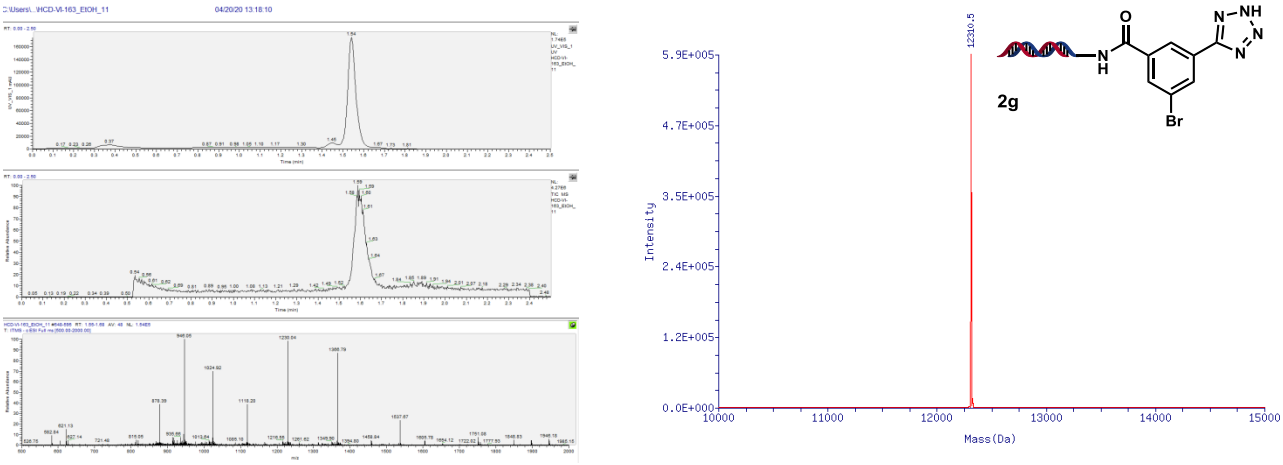


Figure S64. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2g**, expected: 12311.0; observed 12310.5

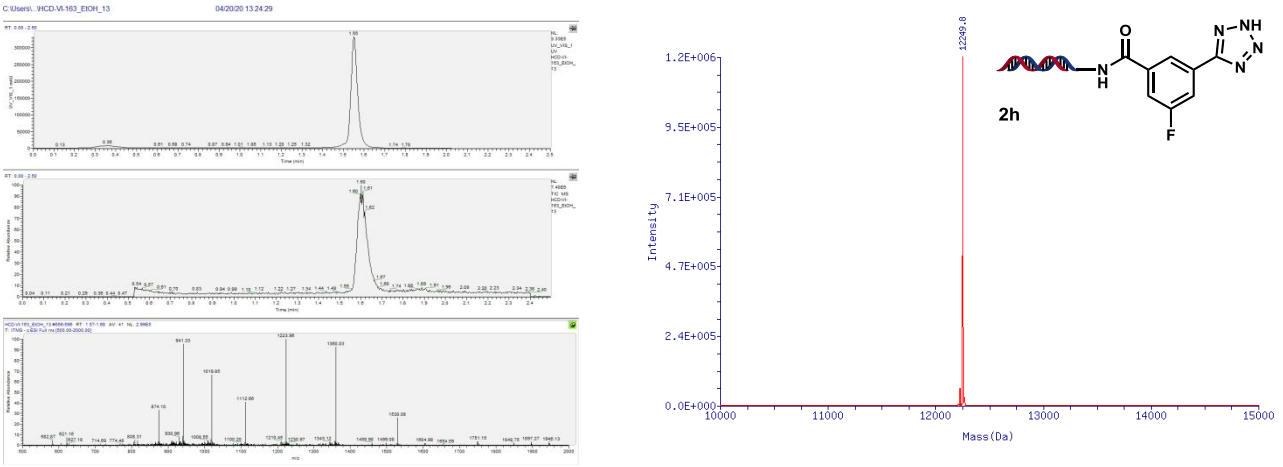


Figure S65. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2h**, expected: 12250.1; observed 12249.8

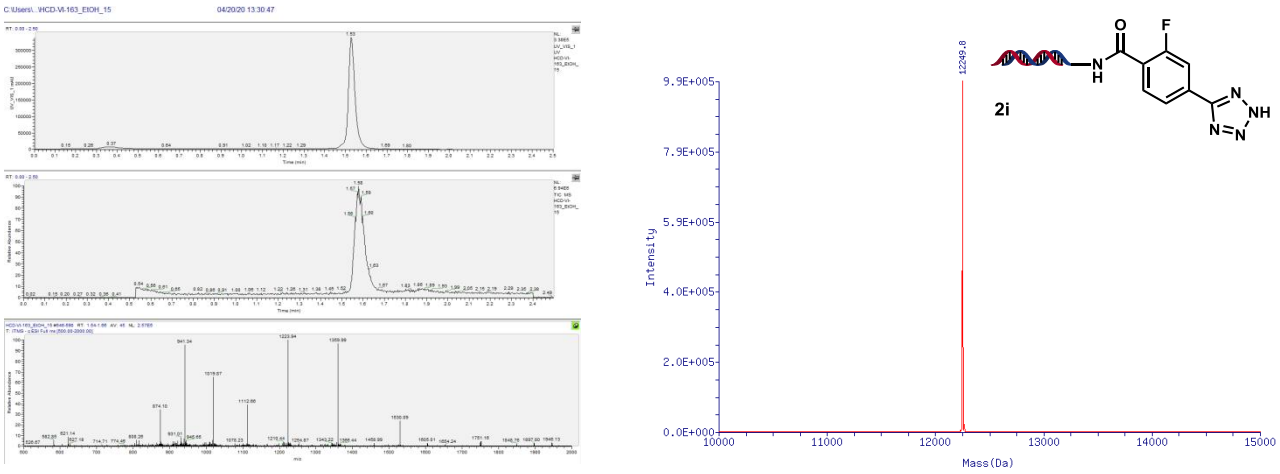


Figure S66. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2i**, expected: 12250.1; observed 12249.8

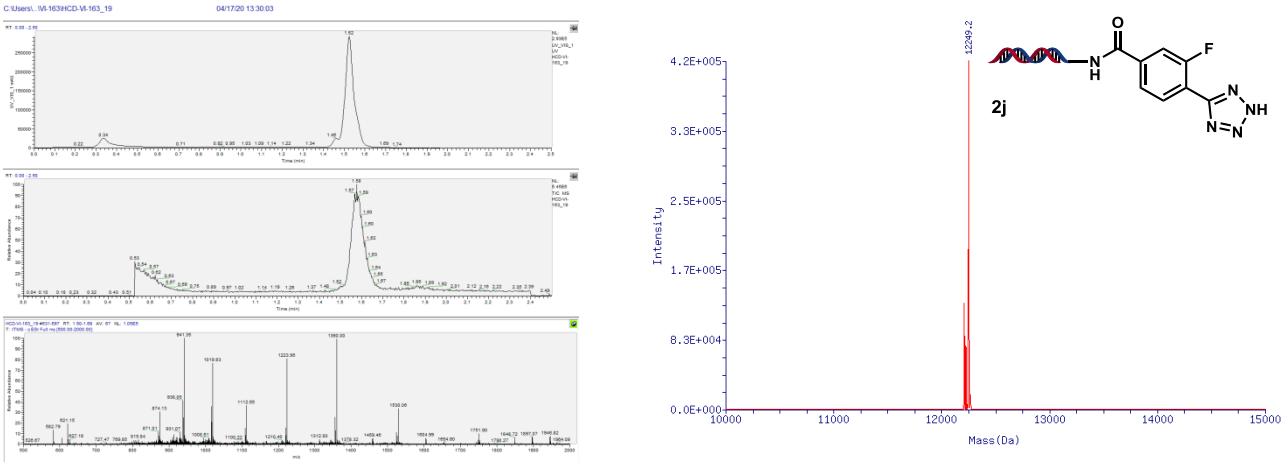
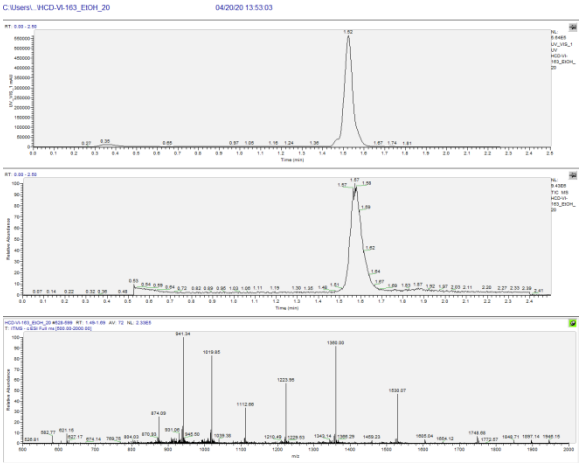


Figure S67. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2j**, expected: 12250.1; observed 12249.2



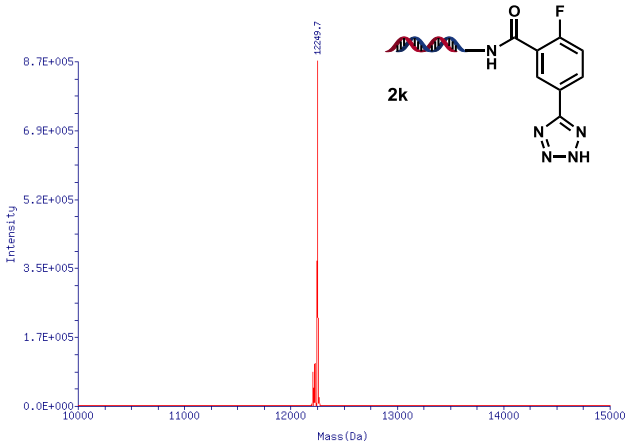
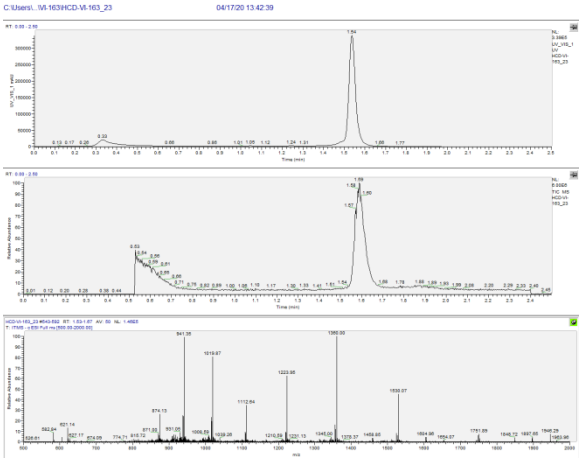


Figure S68. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2k**, expected: 12250.1; observed 12249.7



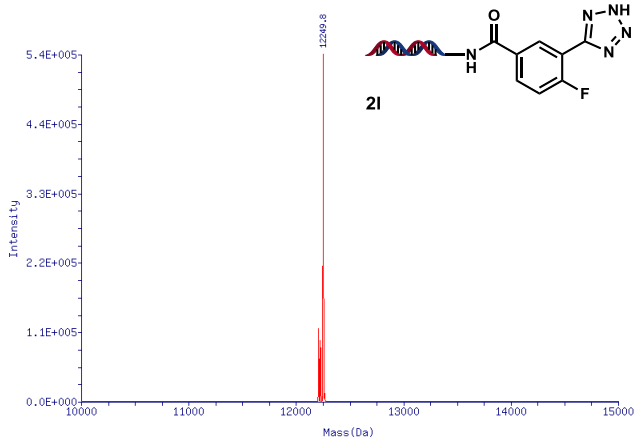


Figure S69. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2l**, expected: 12250.1; observed 12249.8

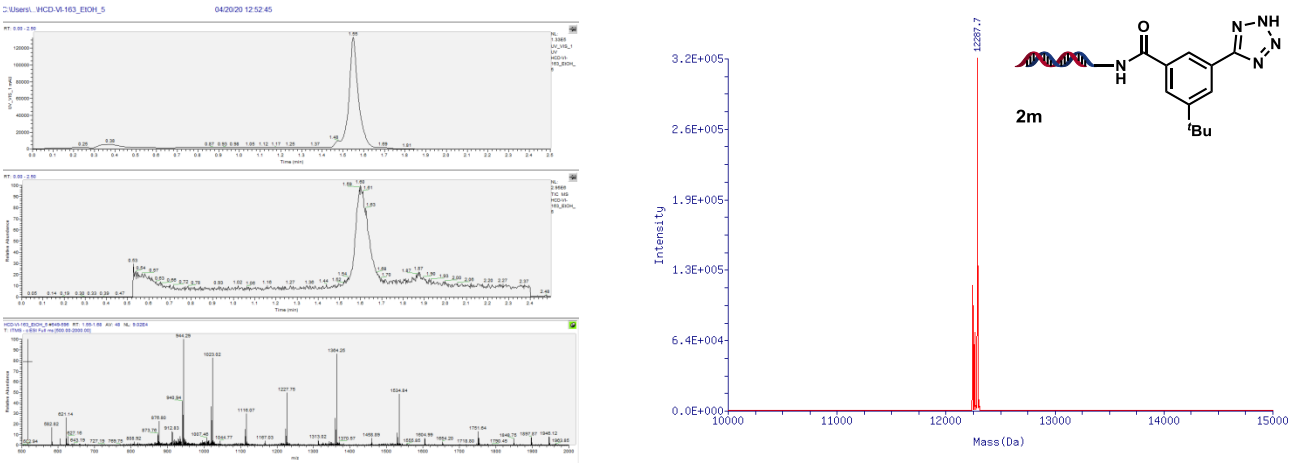


Figure S70. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2m**, expected: 12288.2; observed 12287.7

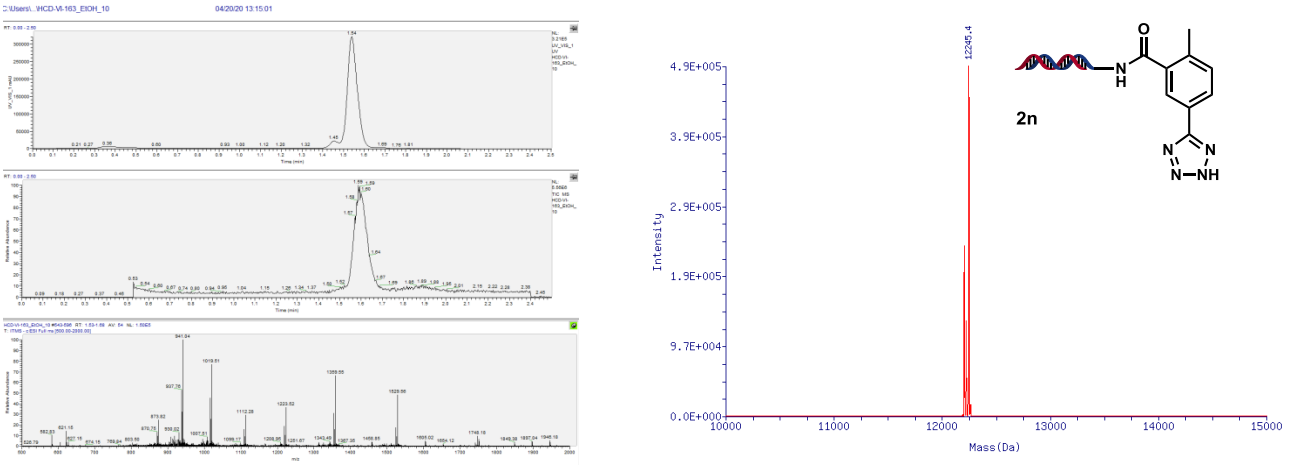


Figure S71. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2n**, expected: 12246.2; observed 12245.4

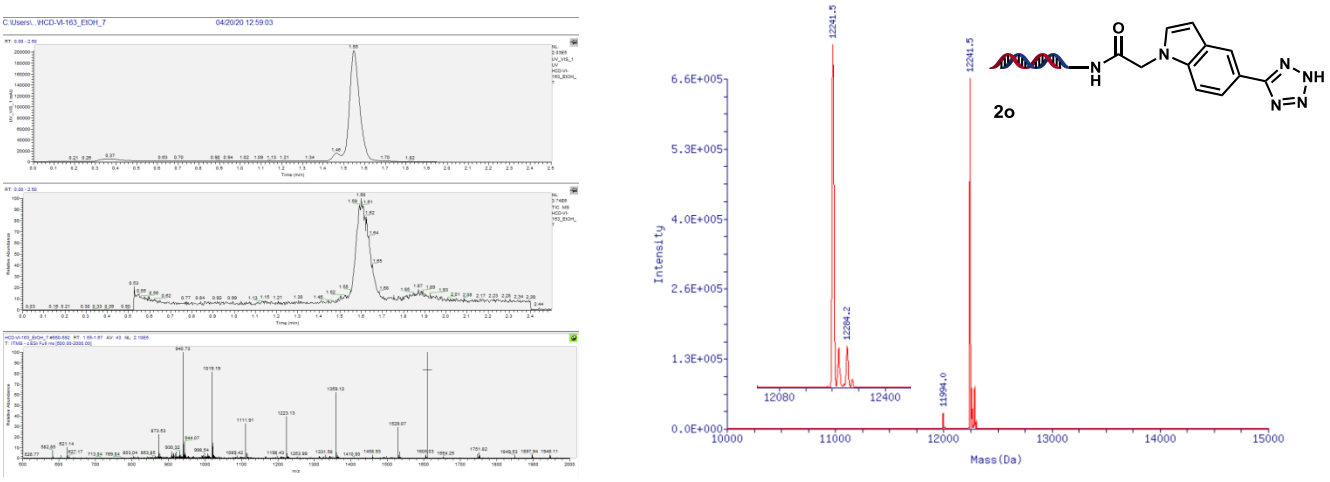


Figure S72. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2o**, expected: 12285.2; observed 12284.2

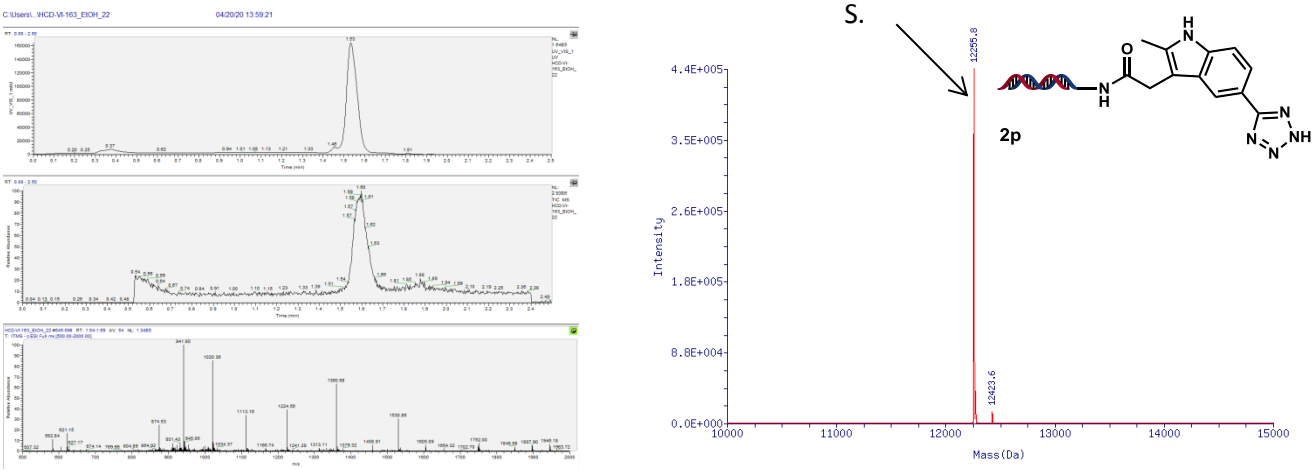


Figure S73. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2p**, expected: 12299.2; observed N/A

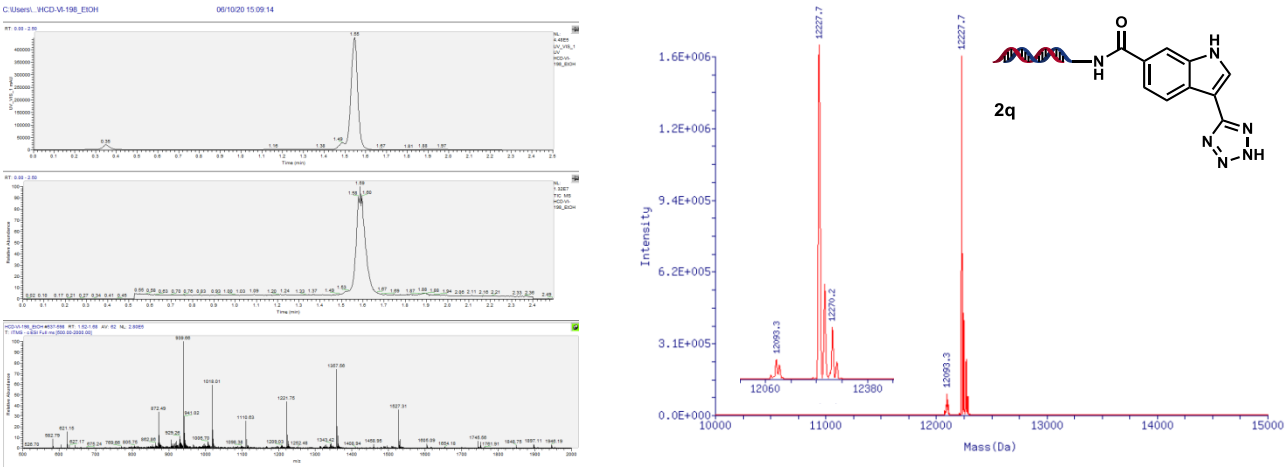


Figure S74. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2q**, expected: 12271.2; observed 12270.2

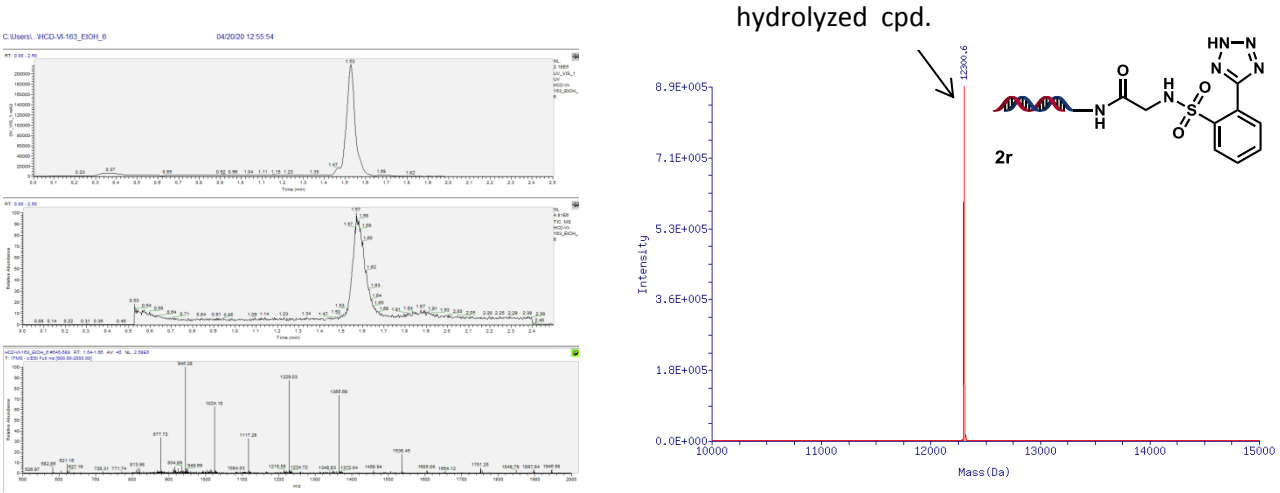


Figure S75. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2r**, expected: 12325.2; observed N/A

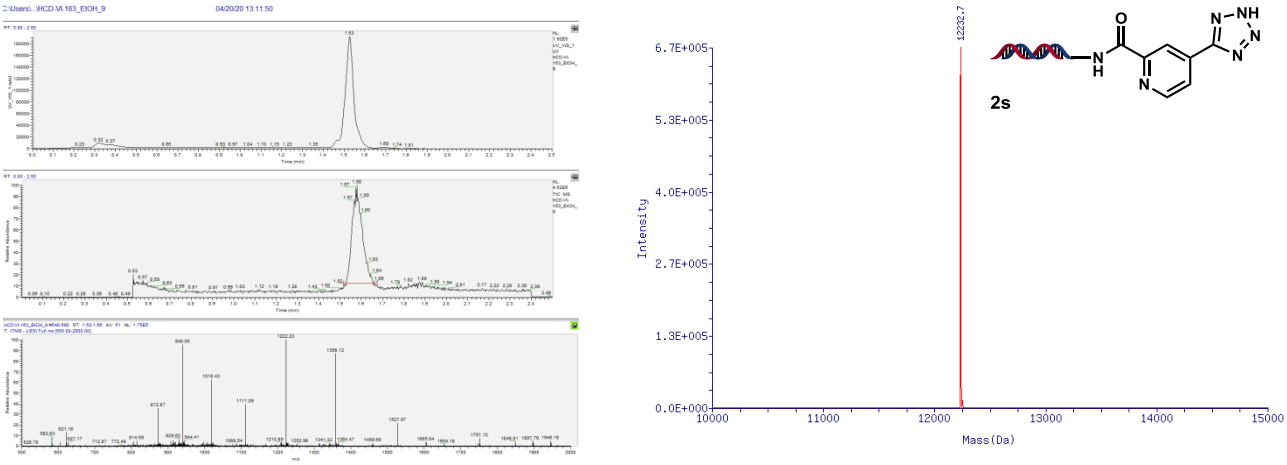


Figure S76. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2s**, expected: 12233.1; observed 12232.7

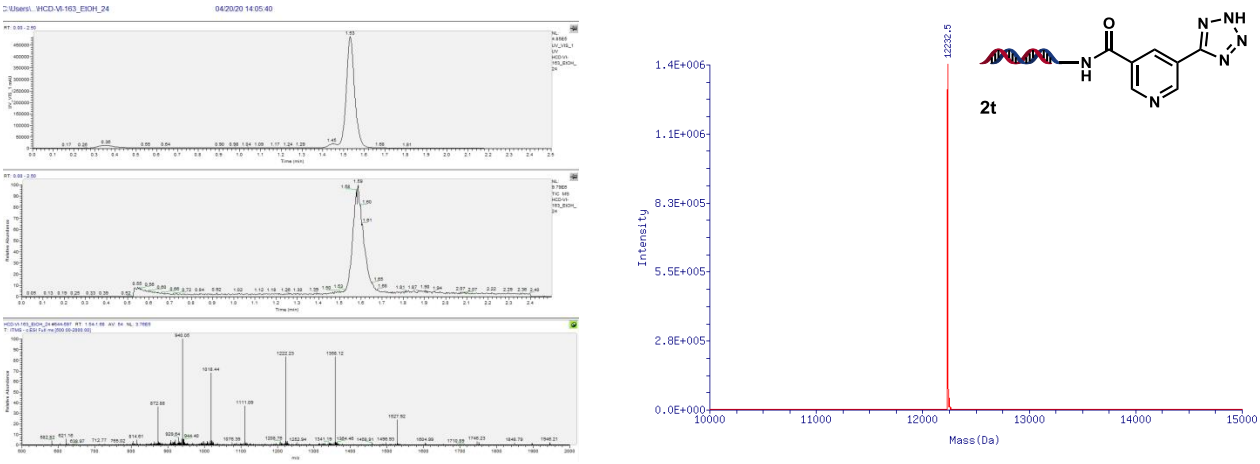


Figure S77. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2t**, expected: 12233.1; observed 12232.5

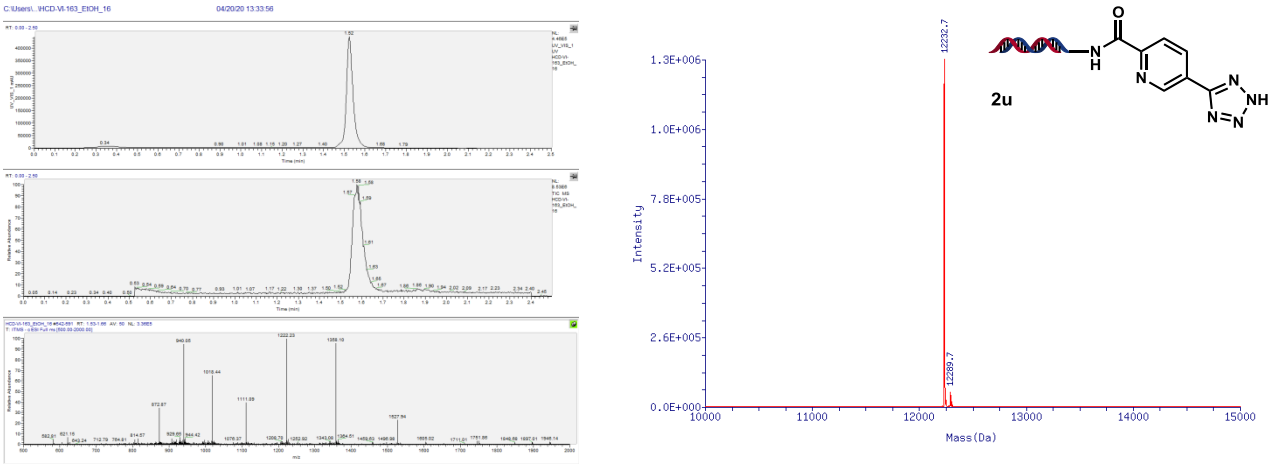


Figure S78. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2u**, expected: 12233.1; observed 12232.7

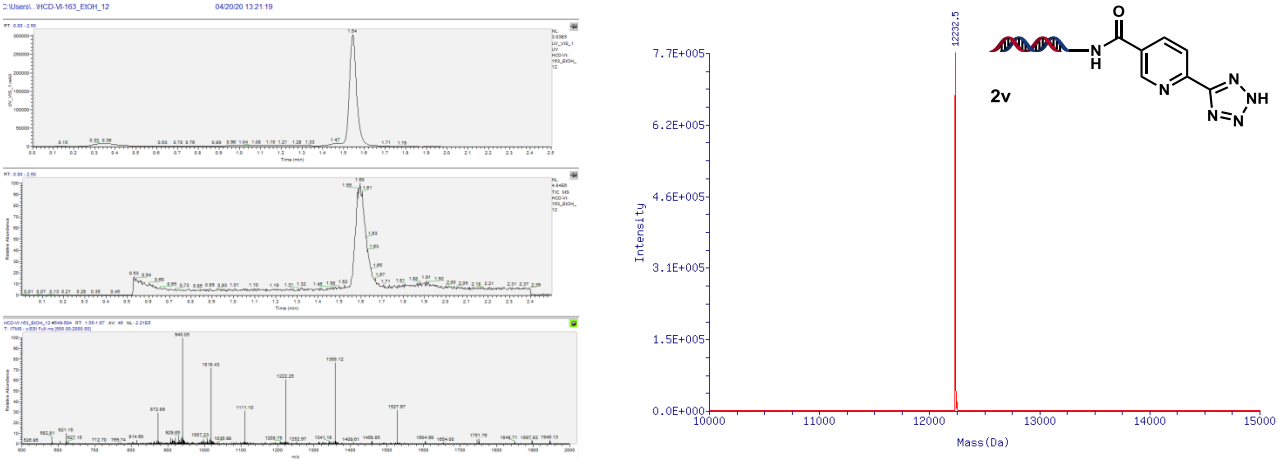


Figure S79. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2v**, expected: 12233.1; observed 12232.5

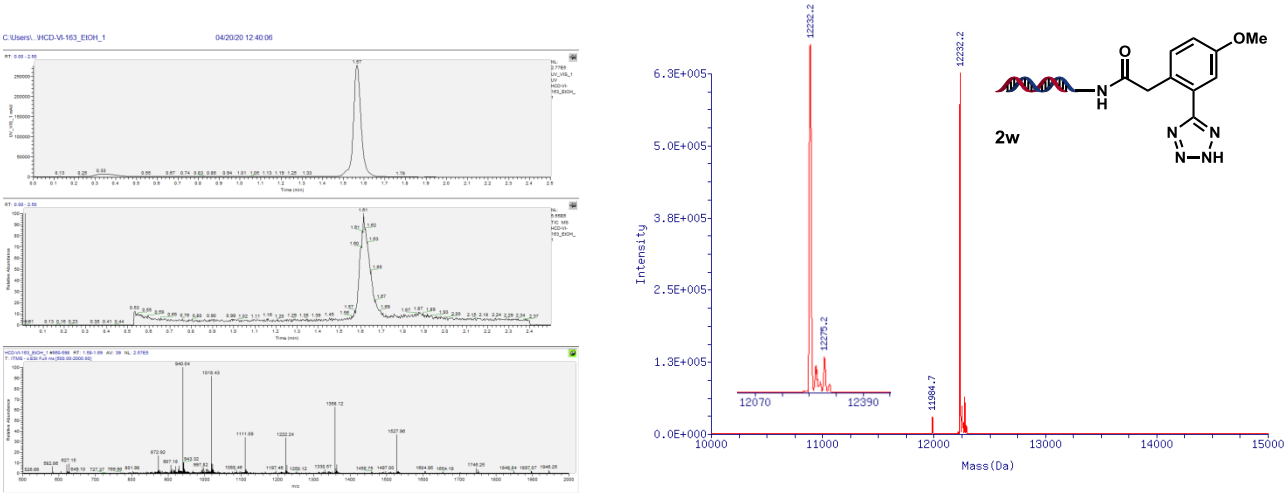


Figure S80. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2w**, expected: 12276.2; observed 12275.2

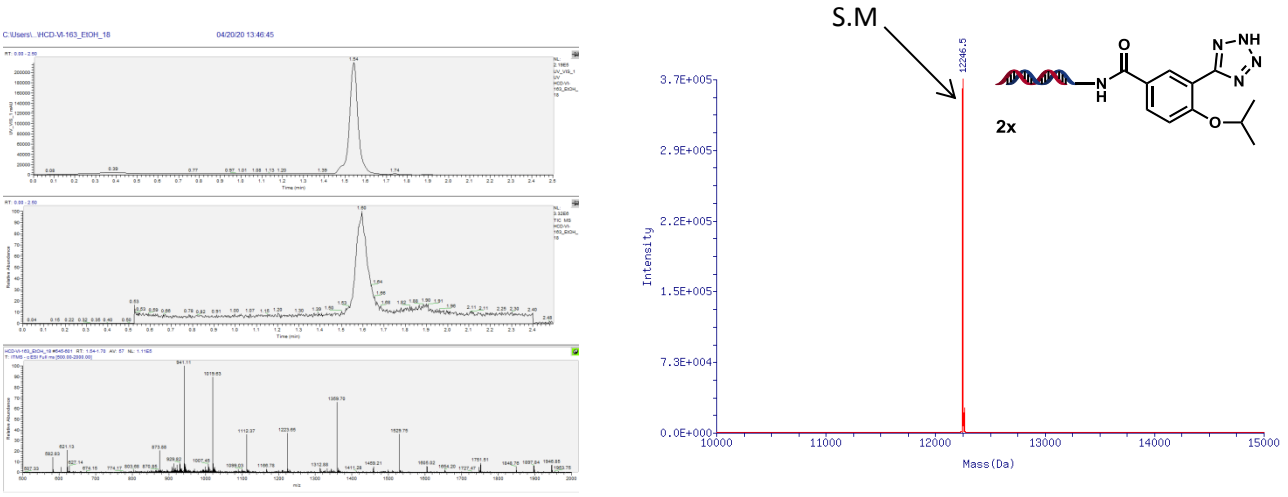


Figure S81. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2x**, expected: 12290.2; observed N/A

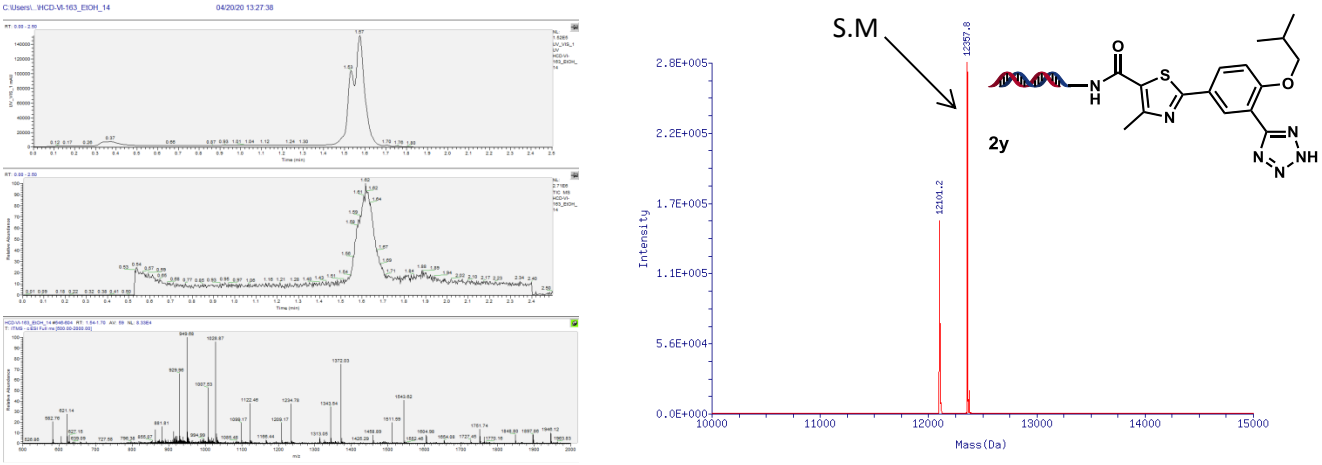


Figure S82. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2y**, expected: 12401.4; observed N/A

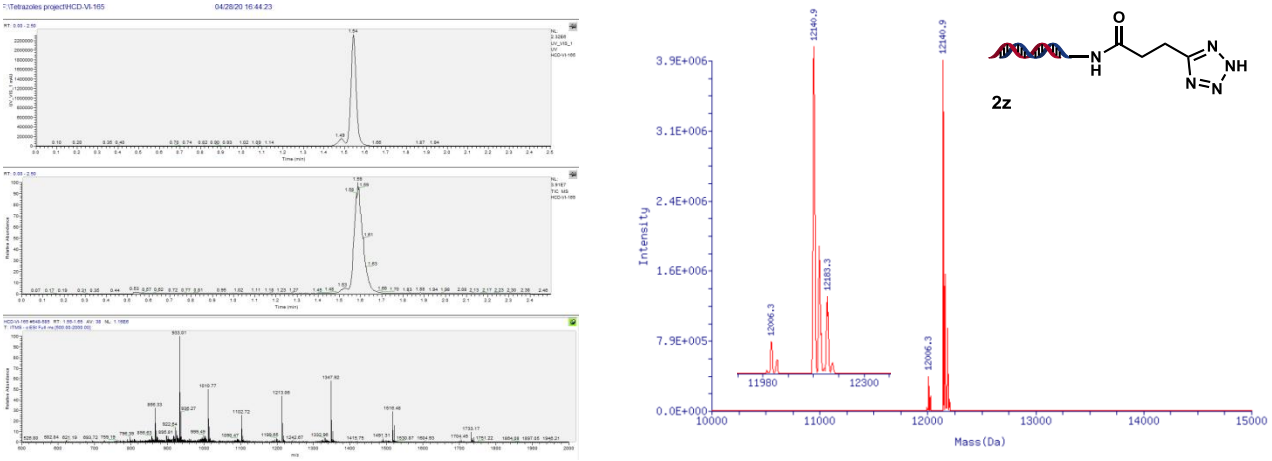


Figure S83. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2z**, expected: 12184.1; observed 12183.3

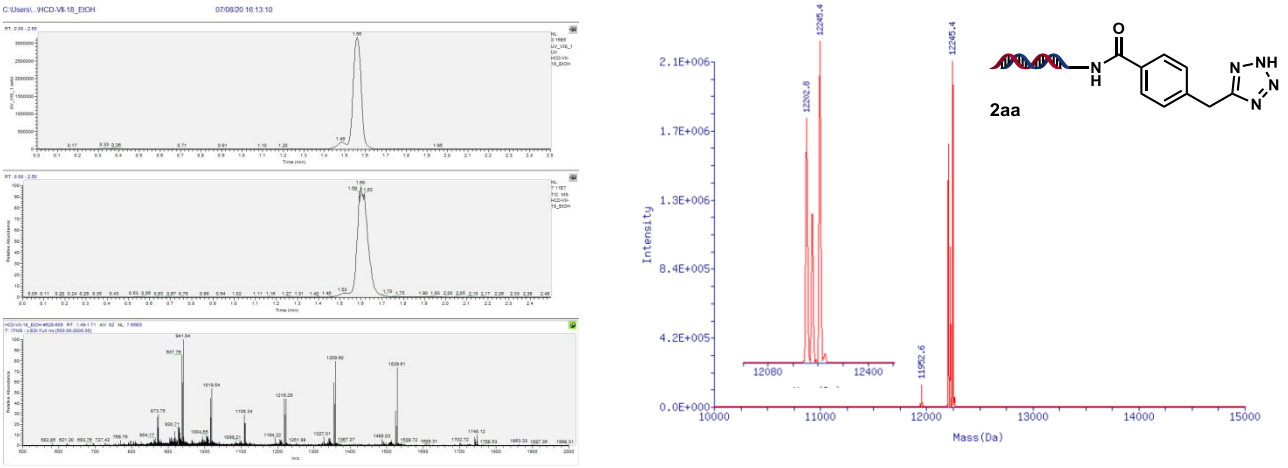


Figure S84. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2aa**, expected: 12246.2; observed 12245.4

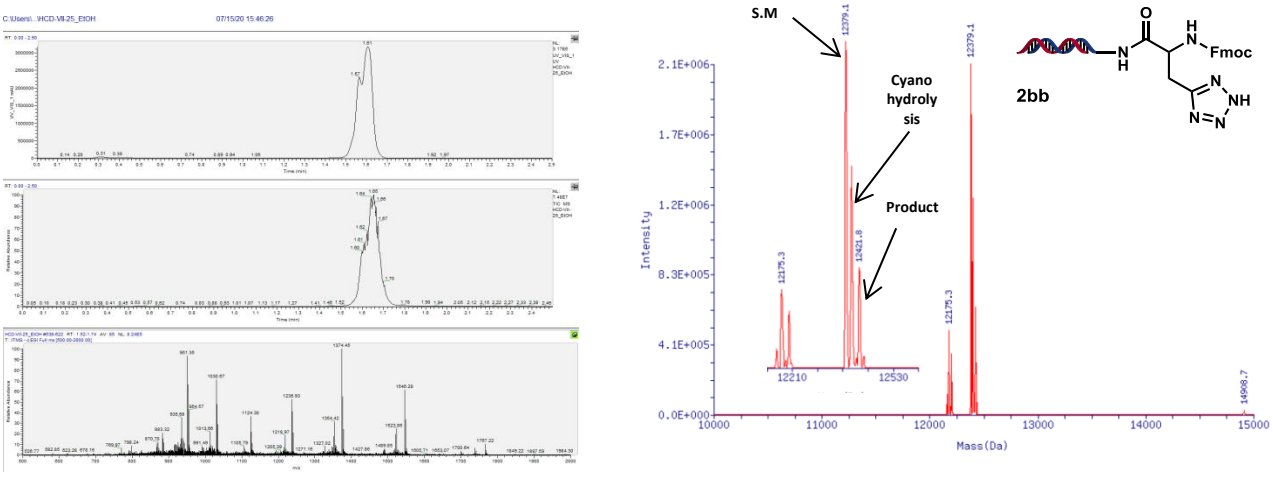


Figure S85. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **2bb**, expected: 12421.4; observed 12421.8

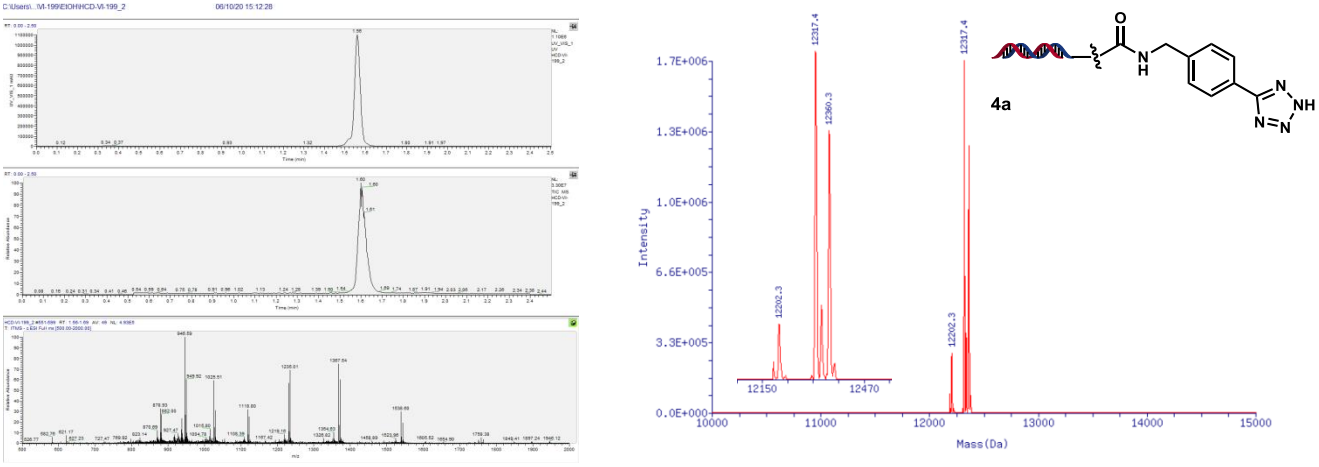


Figure S86. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4a**, expected: 12361.2; observed 12360.3

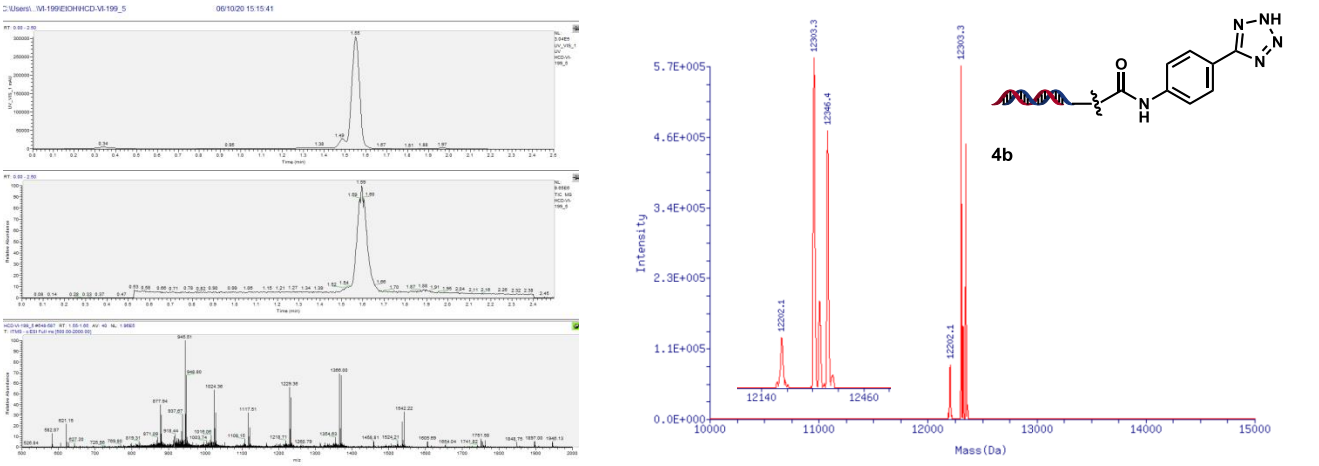


Figure S87. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4b**, expected: 12347.2; observed 12346.4

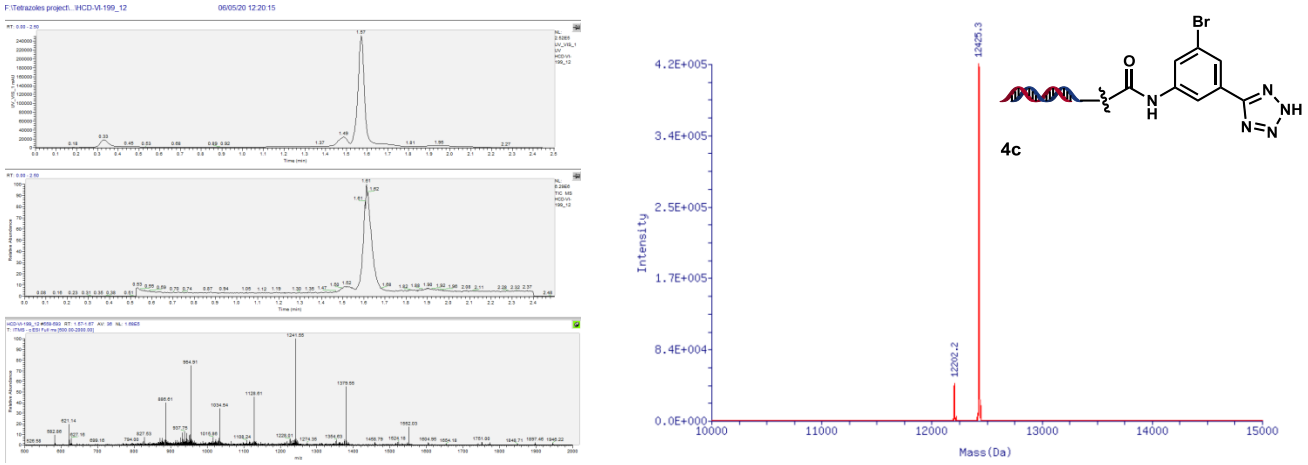


Figure S88. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4c**, expected: 12426.0; observed 12425.3

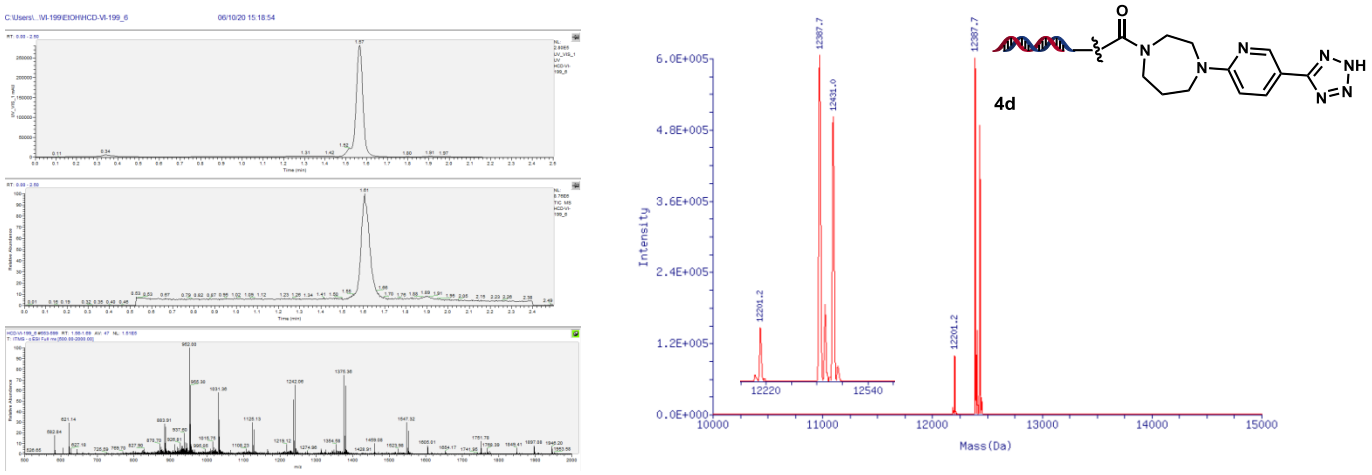


Figure S89. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4d**, expected: 12431.3; observed 12431.0

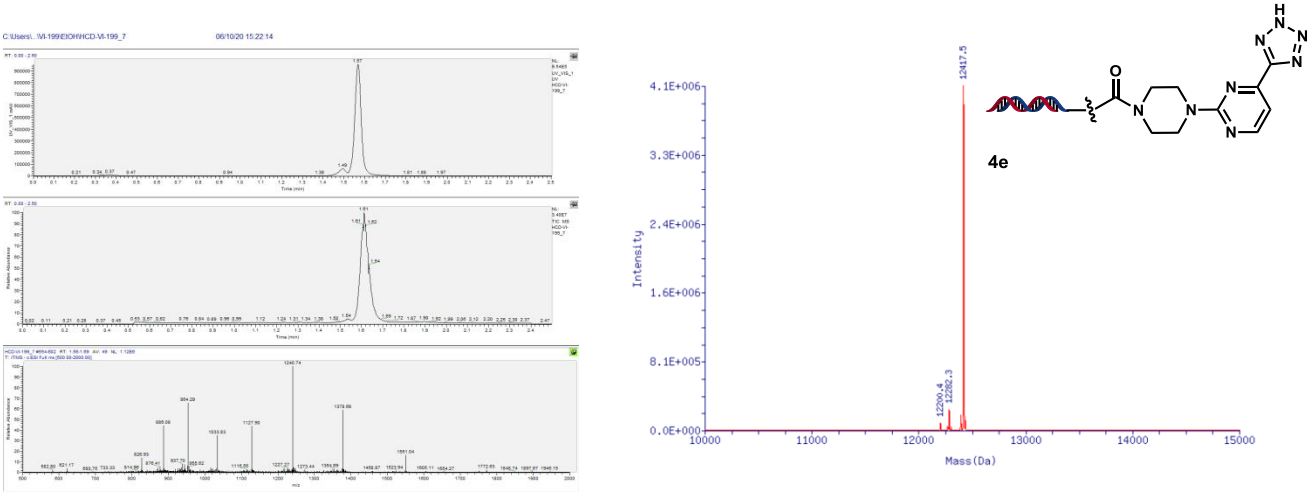


Figure S90. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4e**, expected: 12418.2; observed 12417.5

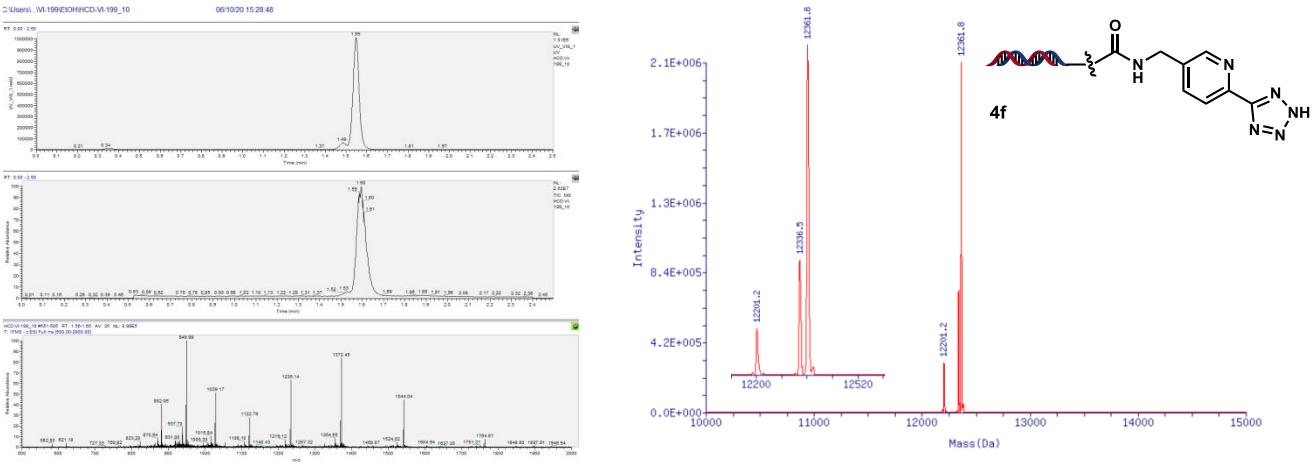


Figure S91. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4f**, expected: 12362.2; observed 12361.8

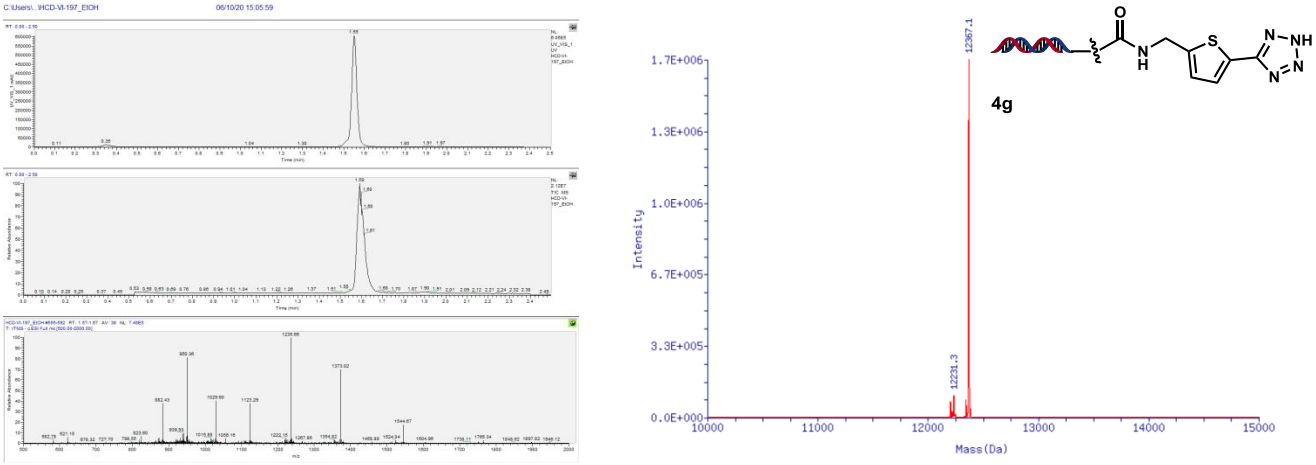


Figure S92. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4g**, expected: 12367.3; observed 12367.1

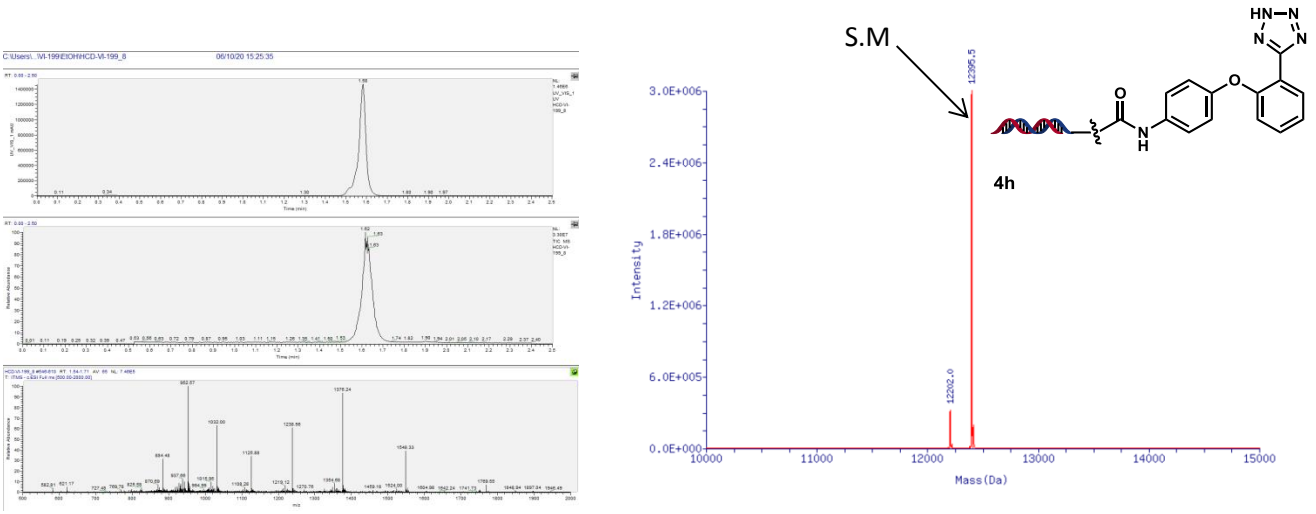


Figure S93. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4h**, expected: 12439.2; observed N/A

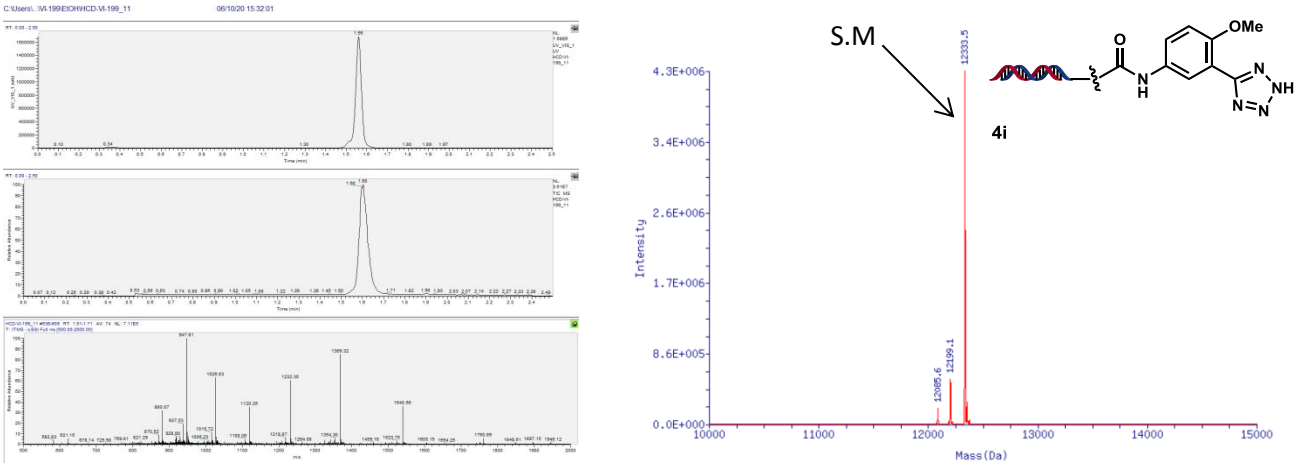


Figure S94. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4i**, expected: 12377.2; observed N/A

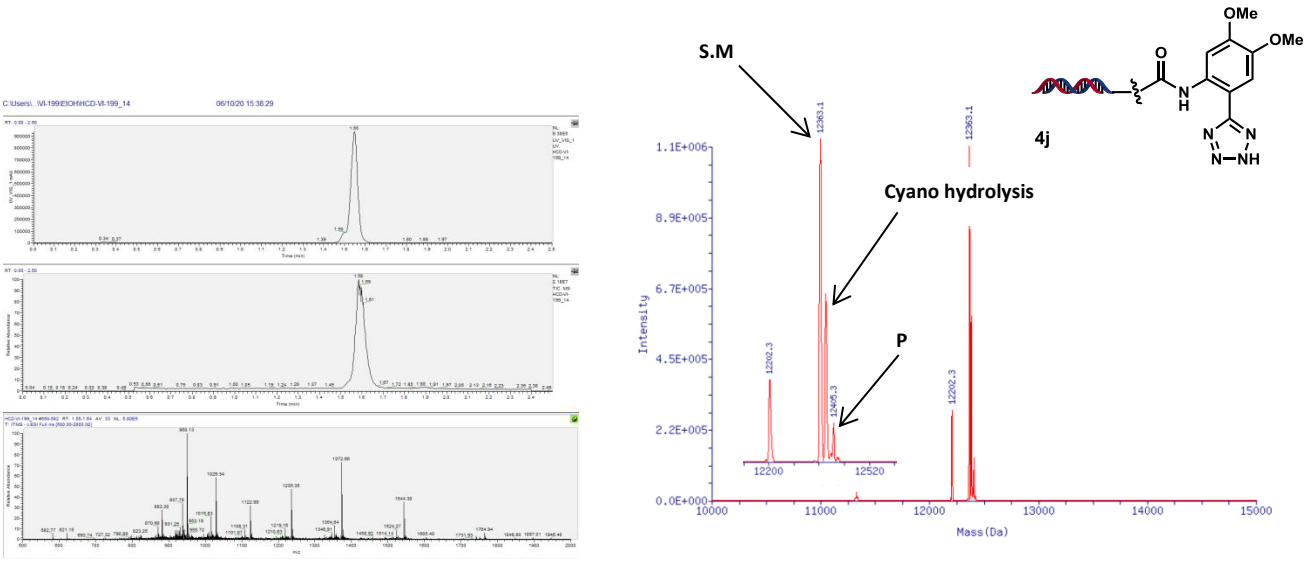


Figure S95. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4j**, expected: 12407.2; observed 12405.3

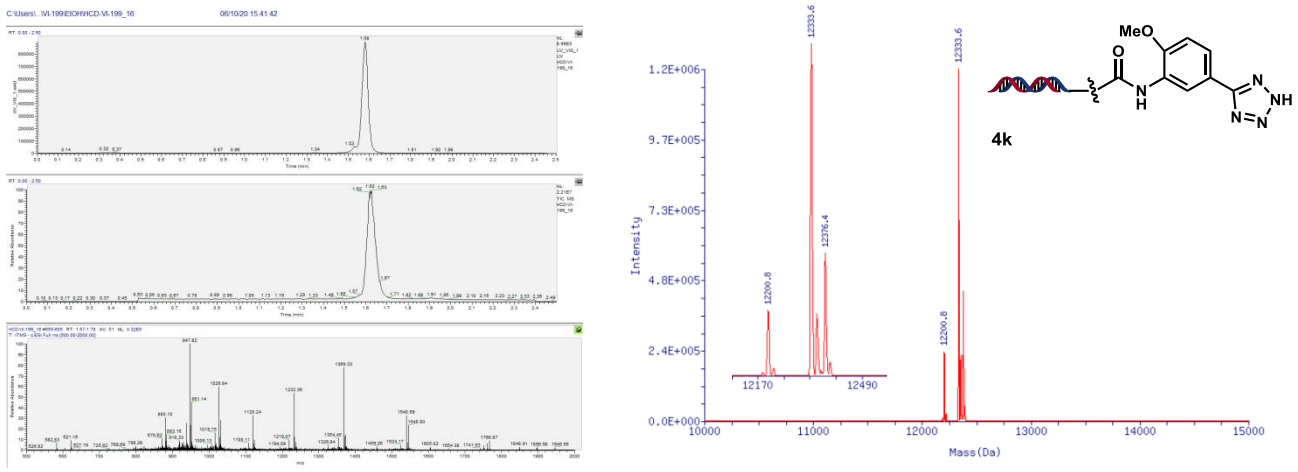


Figure S96. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4k**, expected: 12377.2; observed 12376.4

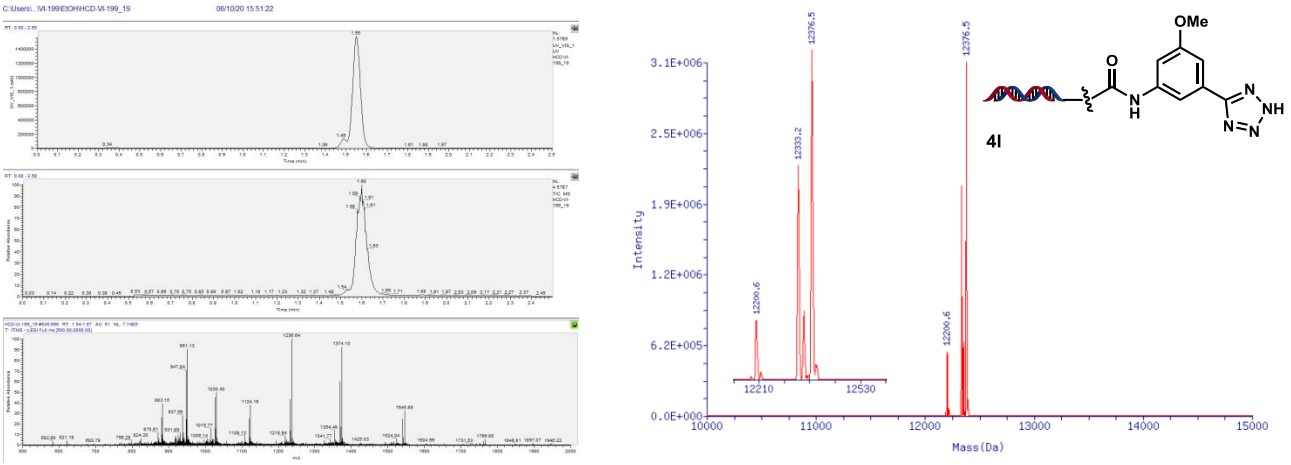


Figure S97. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4l**, expected: 12377.2; observed 12376.5

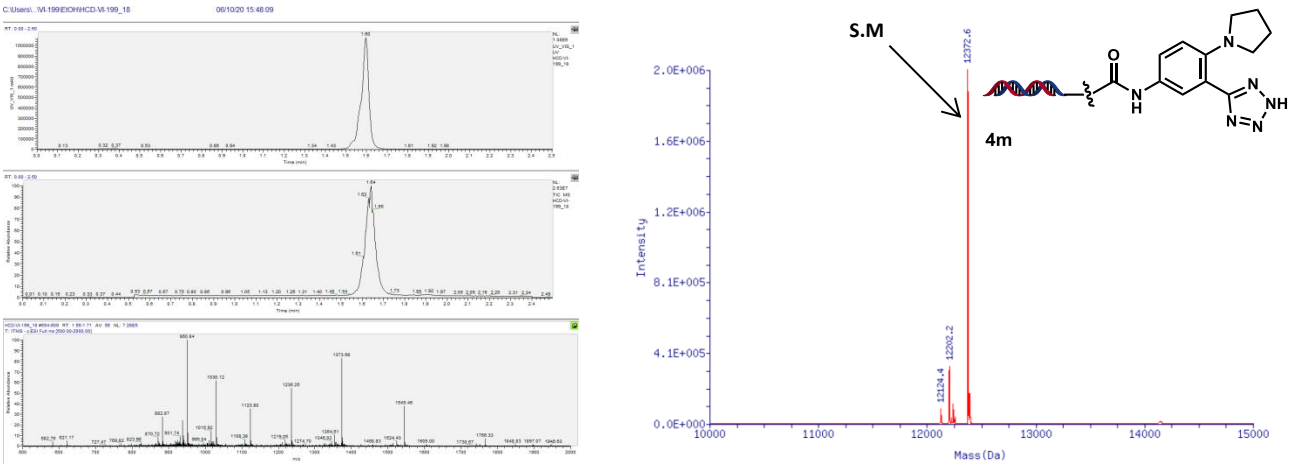


Figure S98. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **4m**, expected: 12416.2; observed N/A

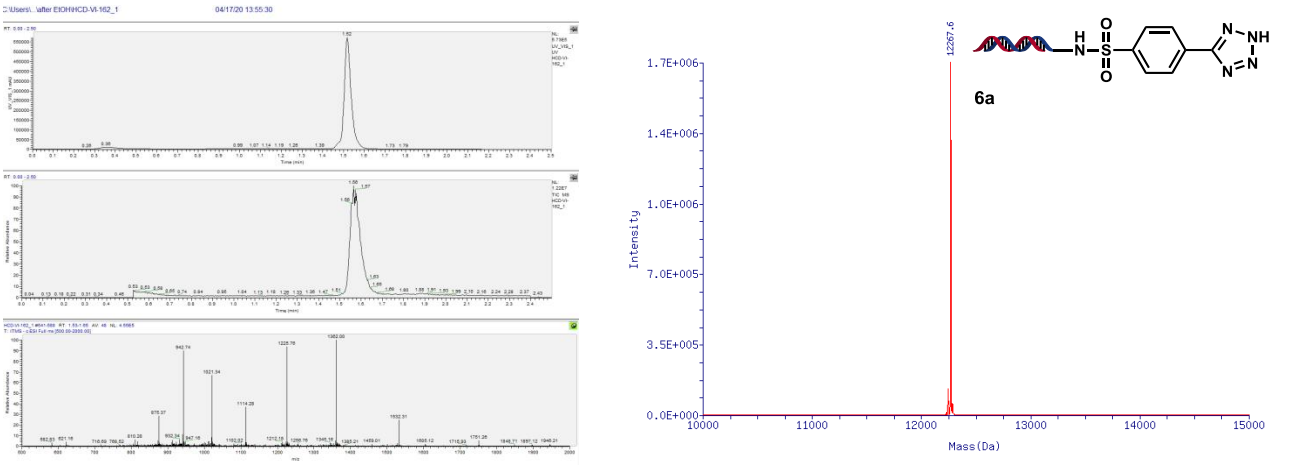


Figure S99. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6a**, expected: 12268.1; observed 12267.6

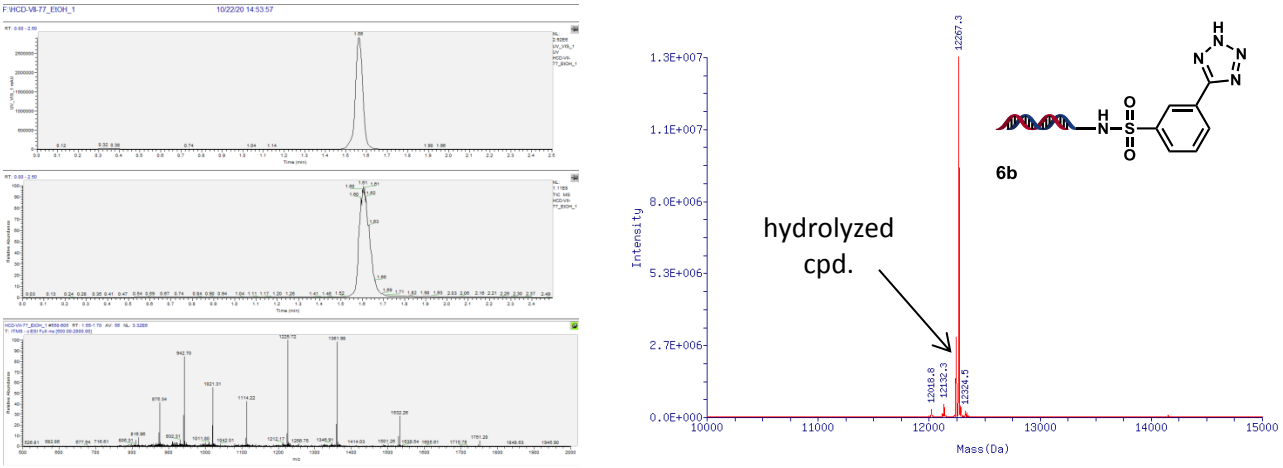


Figure S100. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6b**, expected: 12268.1; observed 12267.3

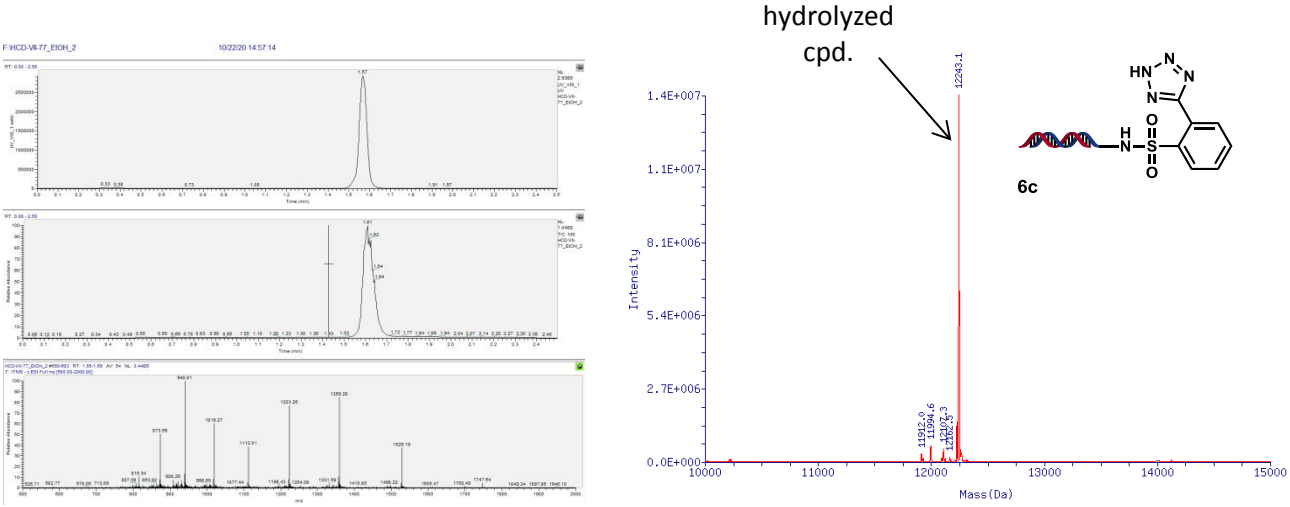


Figure S101. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6c**, expected: 12268.1; observed N/A

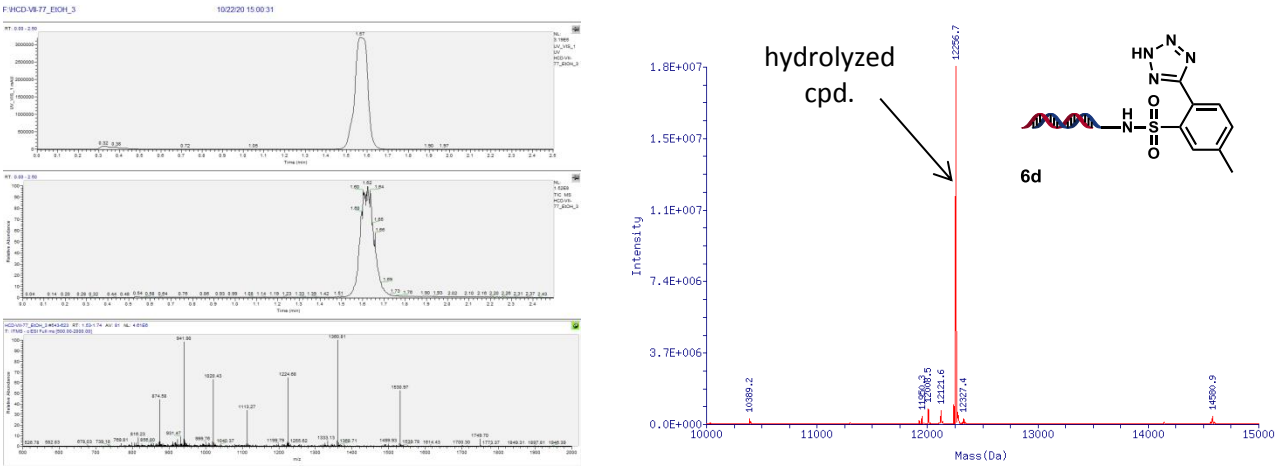


Figure S102. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6d**, expected: 12282.2; observed N/A

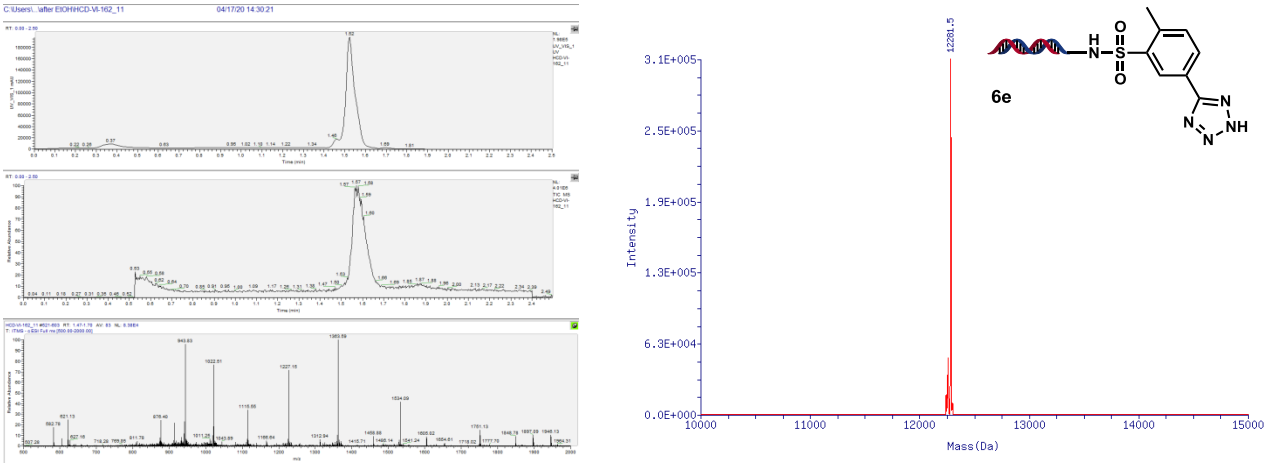


Figure S103. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6e**, expected: 12282.2; observed 12281.5

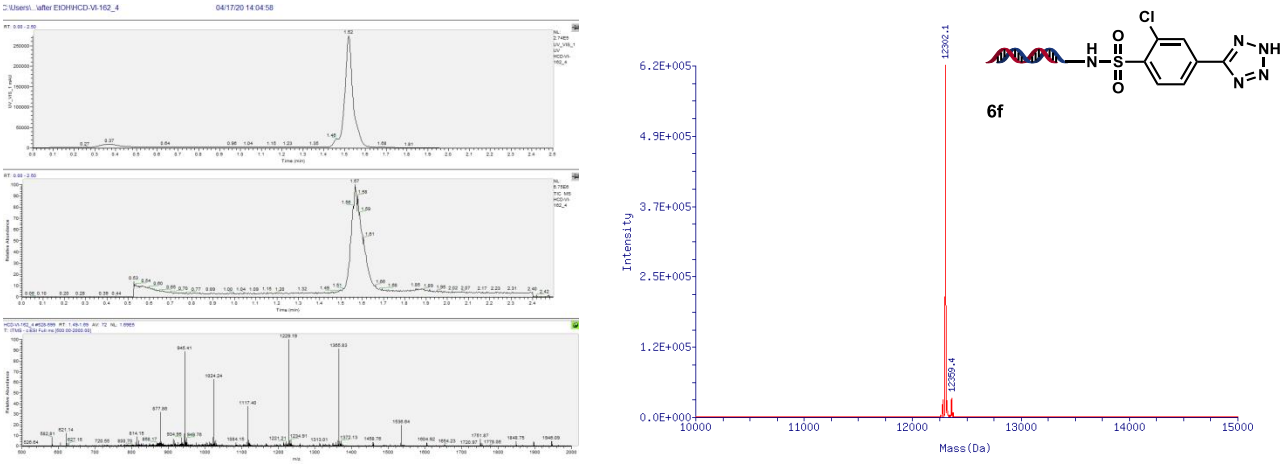


Figure S104. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6f**, expected: 12302.6; observed 12302.1

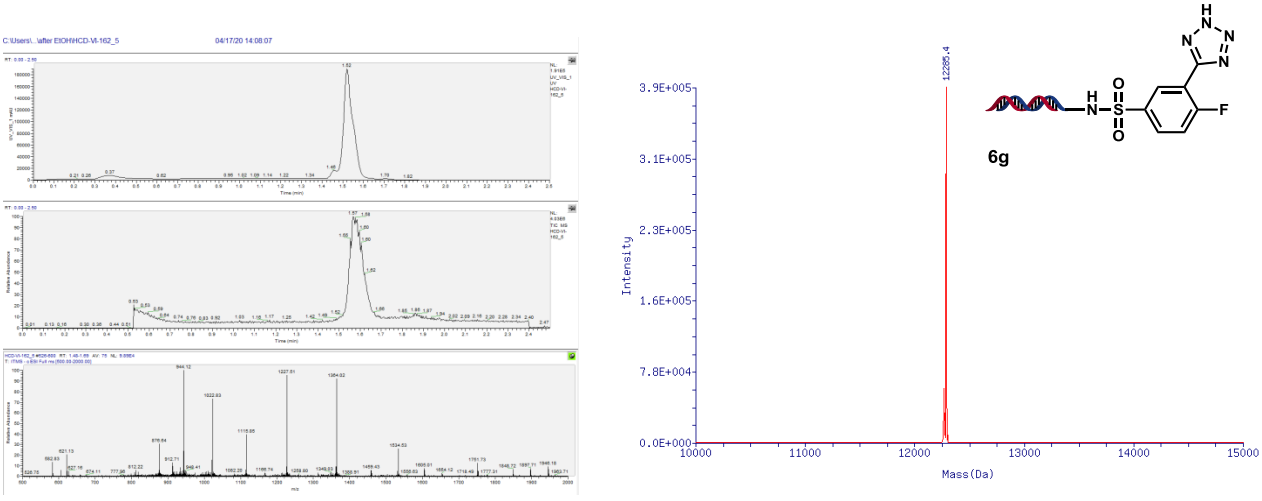


Figure S105. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6g**, expected: 12286.1; observed 12285.4

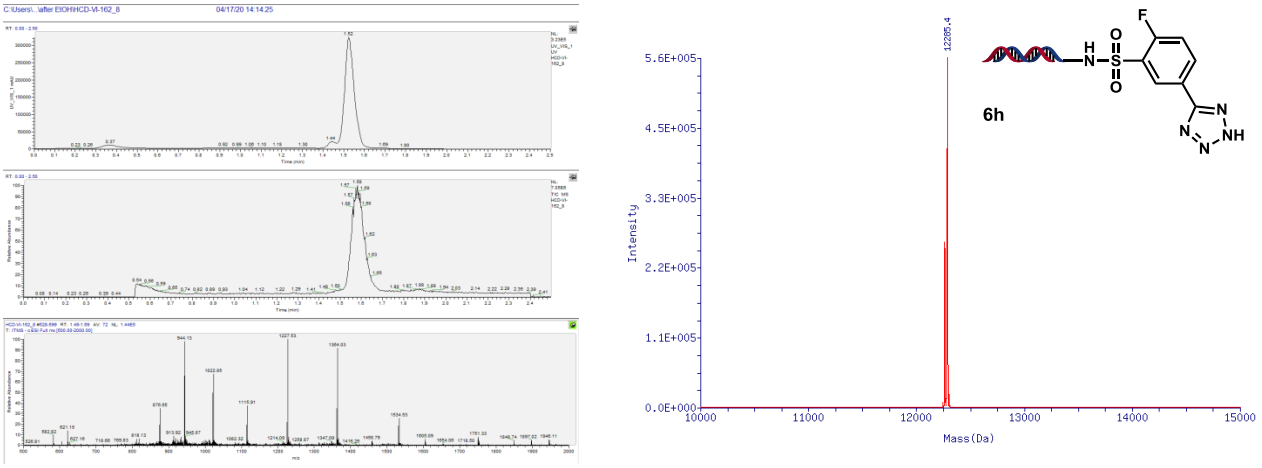


Figure S106. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6h**, expected: 12286.1; observed 12285.4

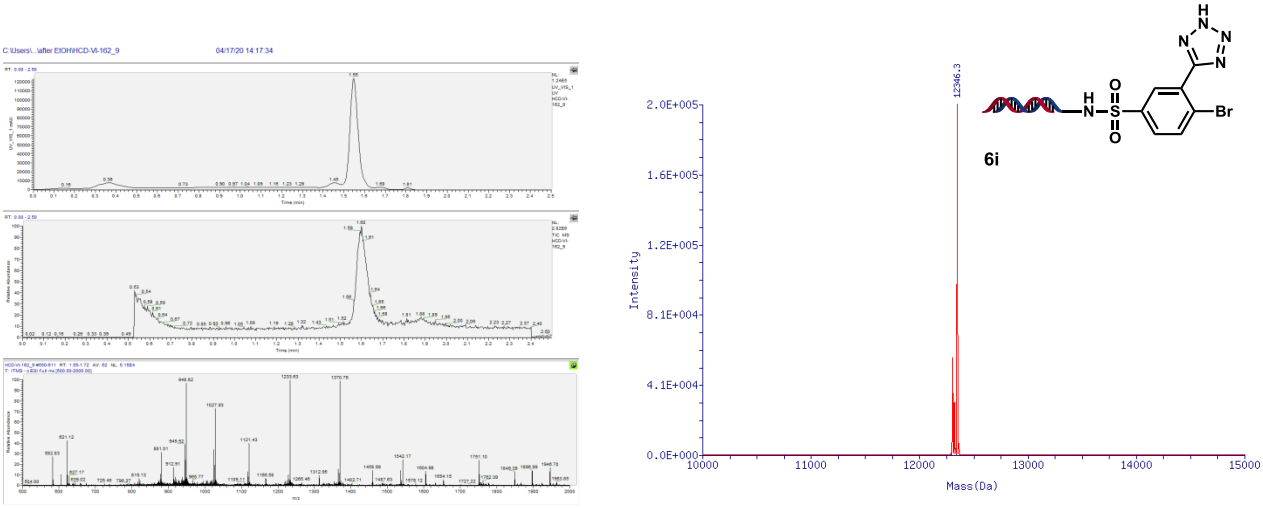


Figure S107. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6i**, expected: 12347.0; observed 12346.3

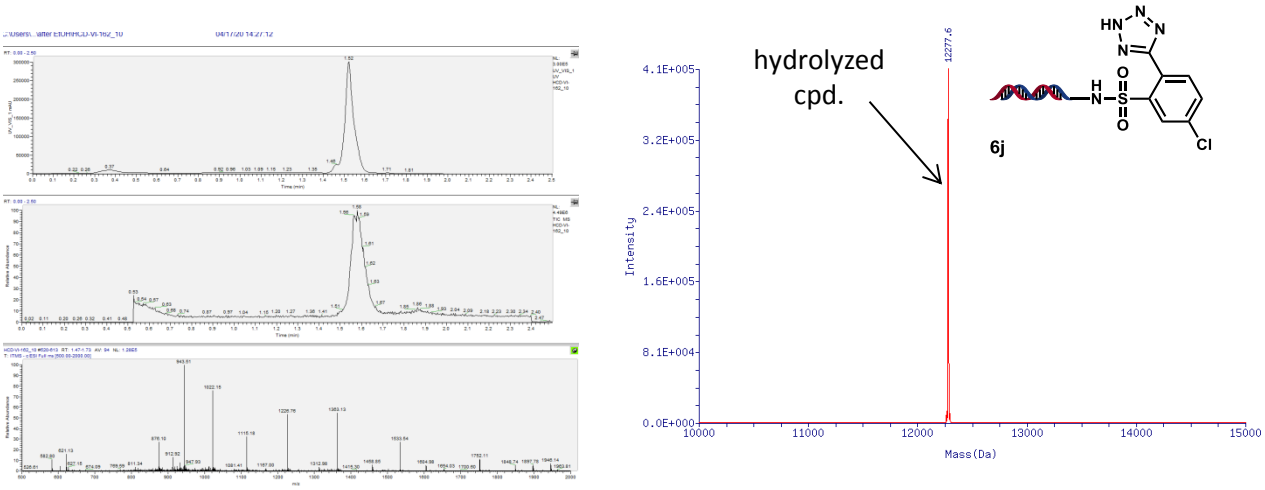


Figure S108. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6j**, expected: 12302.6; observed N/A

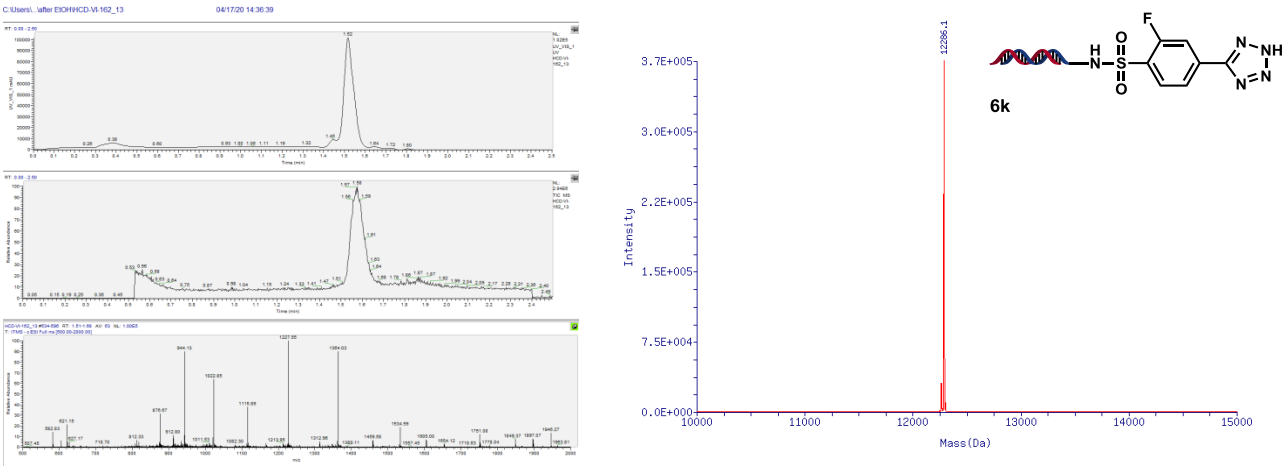


Figure S109. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6k**, expected: 12286.1; observed 12286.1

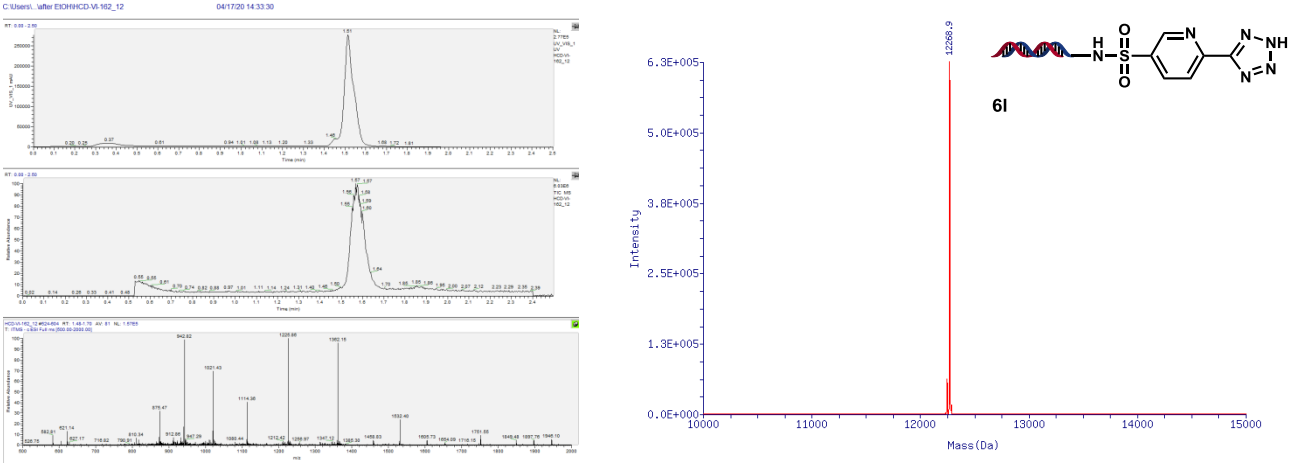


Figure S110. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6l**, expected: 12269.1; observed 12268.9

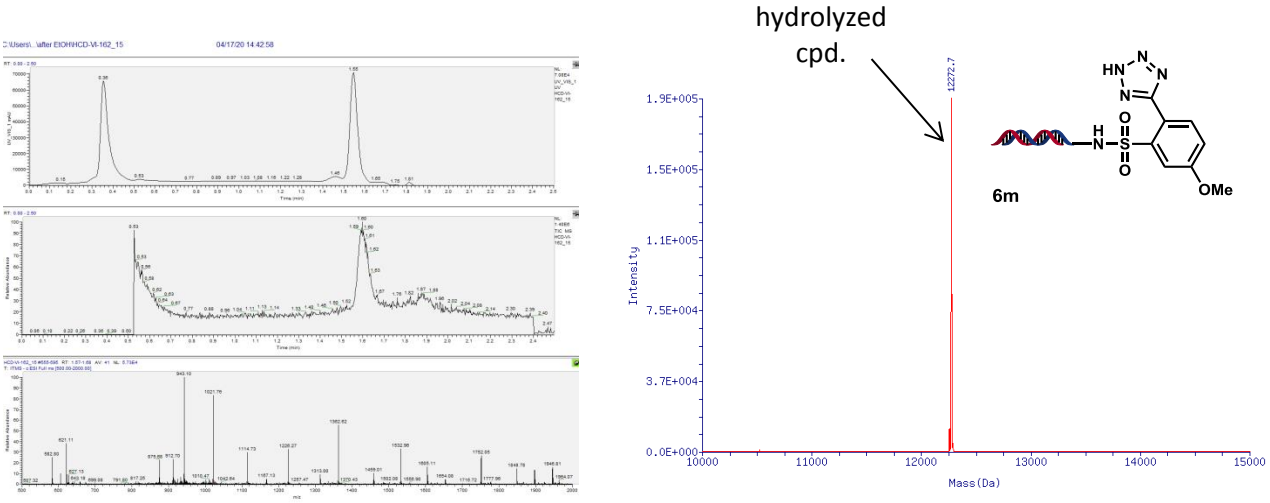
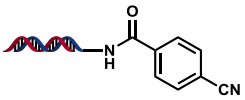
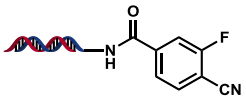
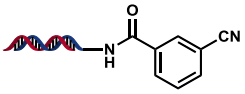
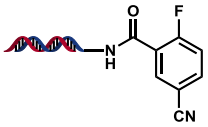
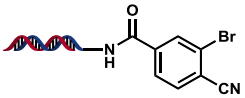
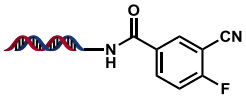
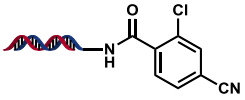
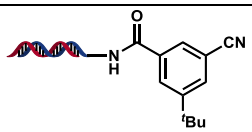
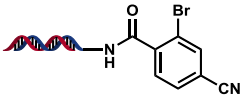
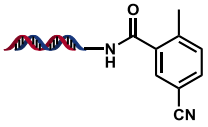
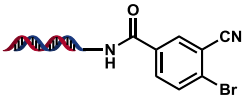
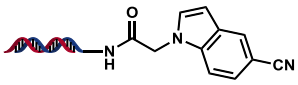
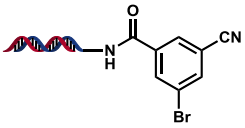
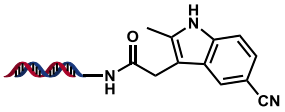
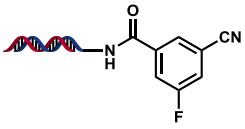
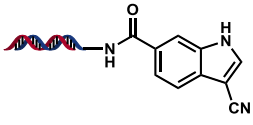
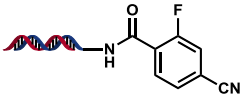
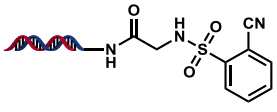


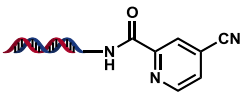
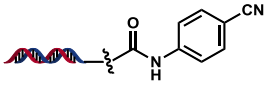
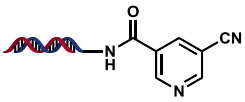
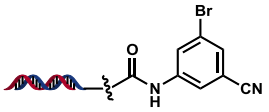
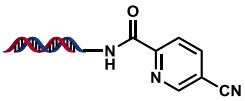
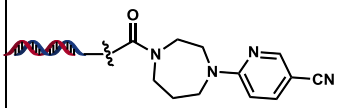
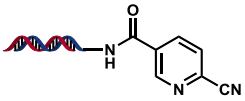
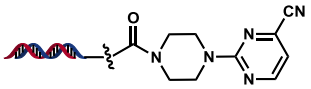
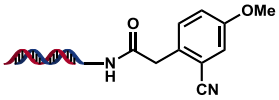
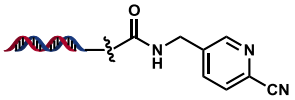
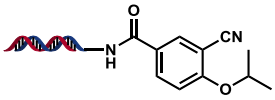
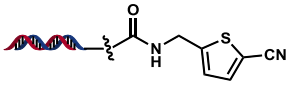
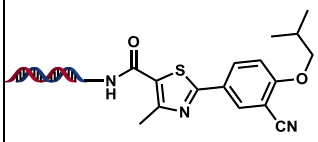
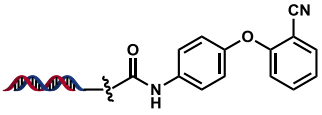
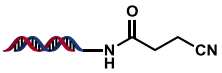
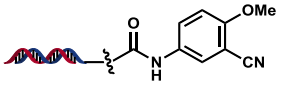
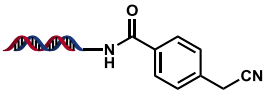
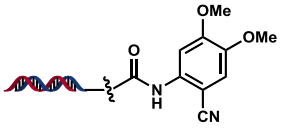
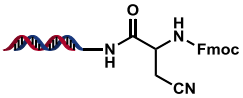
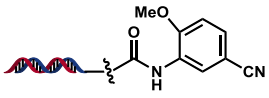
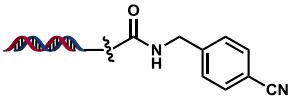
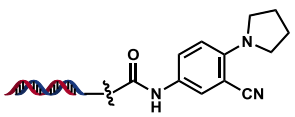
Figure S111. UV, TIC, ESI m/z, and deconvoluted mass spectra of compound **6m**, expected: 12298.2; observed N/A

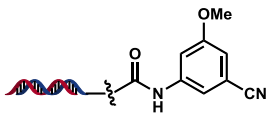
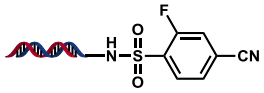
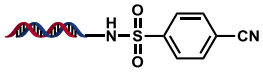
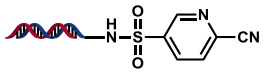
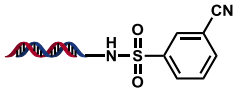
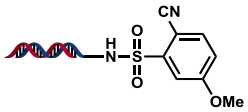
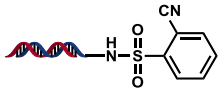
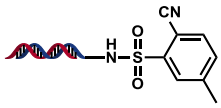
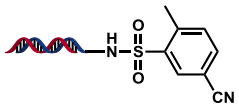
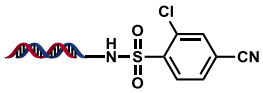
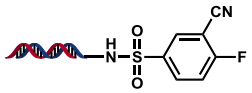
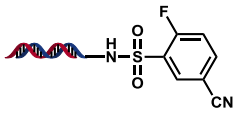
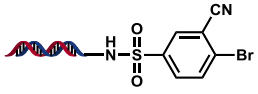
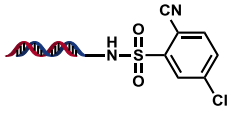
4. Preparation of Substrates

Testing substrates were conjugated on DNA via acylation and sulfonylation

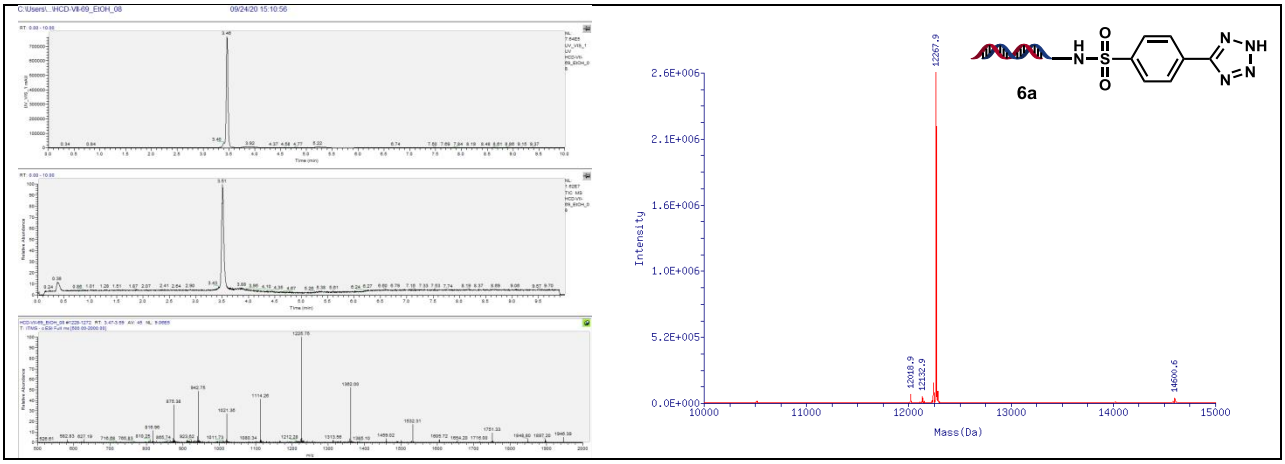
Table S1. Data for starting material preparation

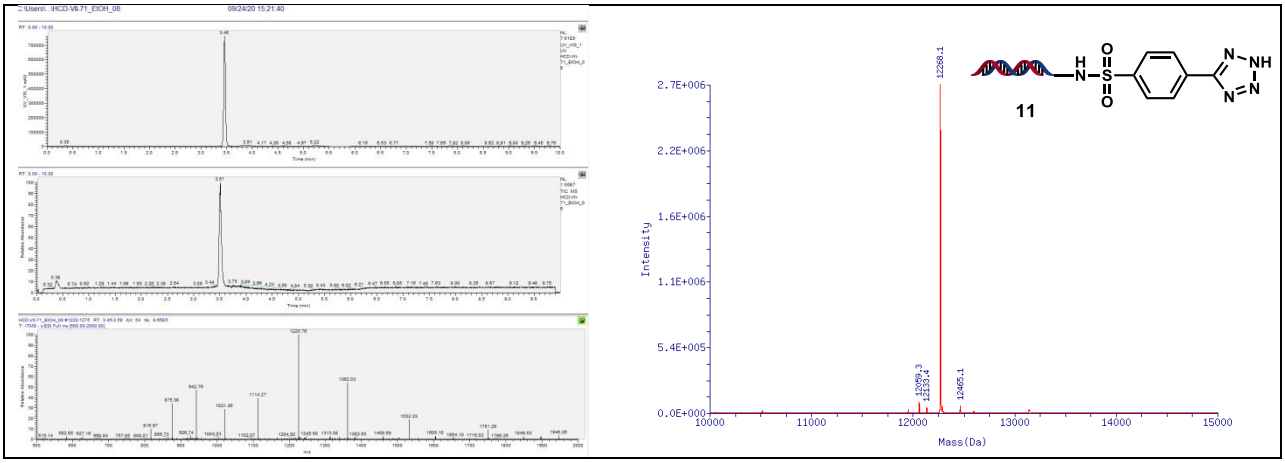
Substrate	Structure	Conversion		Substrate	Structure	Conversion
1a		94%		**1j**		> 95%
1b		> 95%		**1k**		> 95%
1c		> 95%		**1l**		> 95%
1d		> 95%		**1m**		> 95%
1e		> 95%		**1n**		95%
1f		> 95%		**1o**		> 95%
1g		>95%		**1p**		> 95%
1h		> 95%		**1q**		83%
1i		95%		**1r**		62%

1s		> 95%		**3b**		87%
1t		> 95%		**3c**		90%
1u		> 95%		**3d**		91%
1v		> 95%		**3e**		91%
1w		> 95%		**3f**		61%
1x		95%		**3g**		85%
1y		69%		**3h**		89%
1z		> 95%		**3i**		91%
1aa		93%		**3j**		52%
1bb		> 95%		**3k**		85%
3a		89%		**3l**		85%

3m		86%		**5k**		88%
5a		> 95%		**5l**		91%
5b		75%		**5m**		> 95%
5c		> 95%				
5d		89%				
5e		> 95%				
5f		> 95%				
5g		95%				
5h		57%				
5i		94%				
5j		> 95%				

5. Co-injection experiment by LC-MS





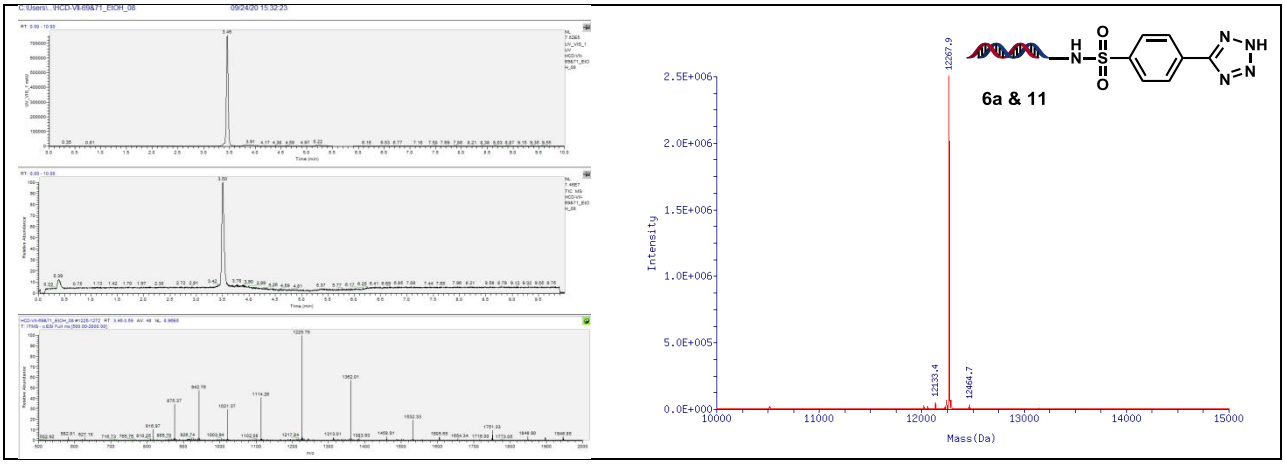


Figure S112. LC-MS analysis between **6a** and **11**

6. DNA integrity Evaluation

DNA integrity after chemical reactions was evaluated by LC-MS and by performing ligation and qPCR experiments. DNA headpiece without any chemical reaction was served as a control.

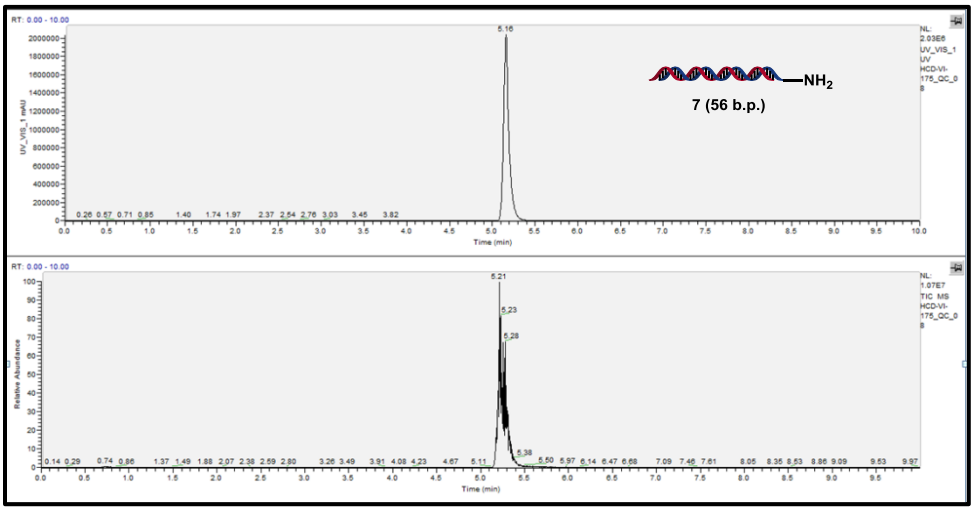


Figure S113. LC-MS spectrum of compound **7**

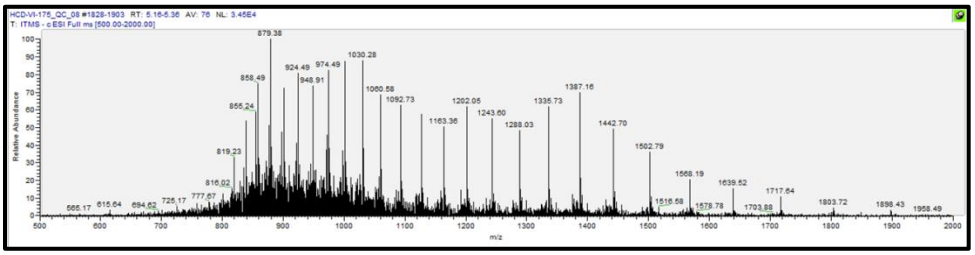


Figure S114. ESI Full MS spectrum of compound **7**

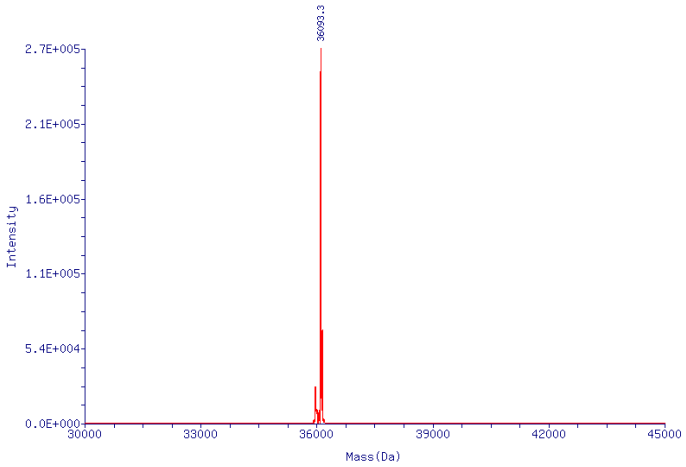


Figure S115. Deconvoluted mass spectrum of compound **7**, expected: 36088.7; observed 36093.3

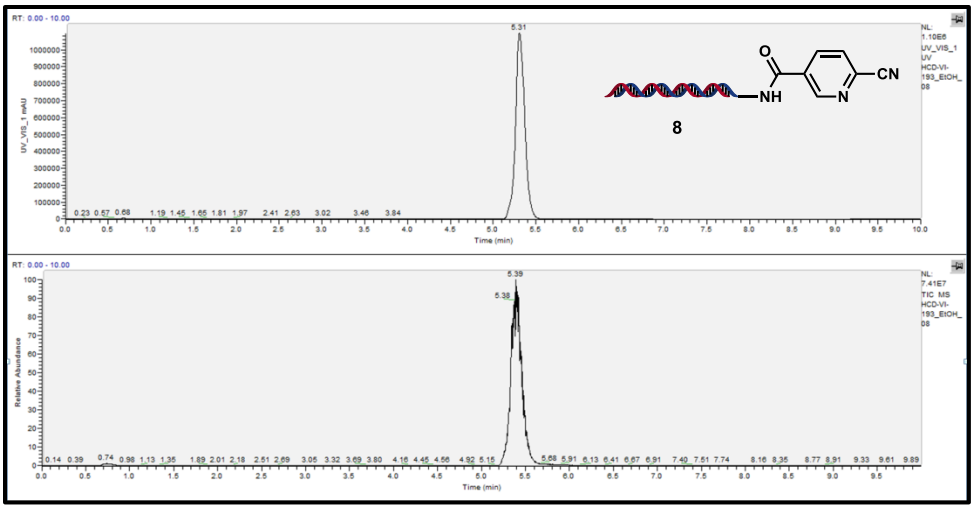


Figure S116. LC-MS spectrum of compound **8**

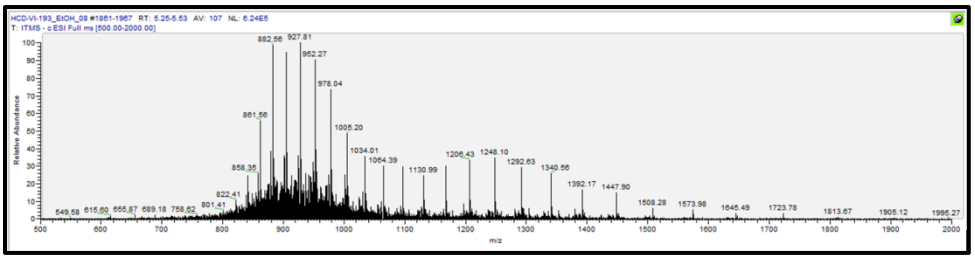


Figure S117. ESI Full MS spectrum of compound **8**

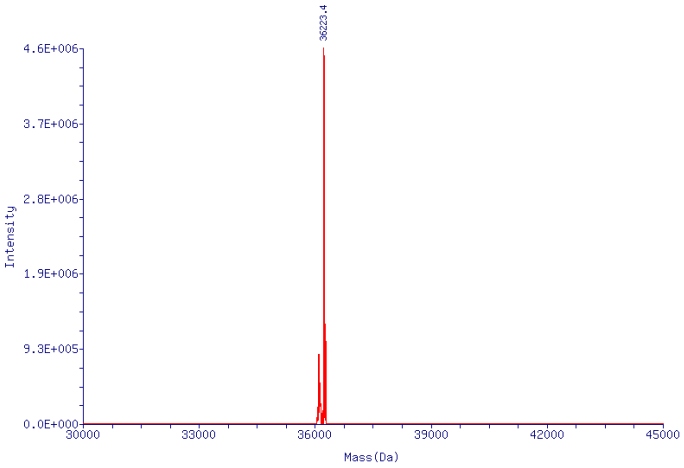


Figure S118. Deconvoluted mass spectrum of compound **8**, expected: 36218.8; observed 36223.4

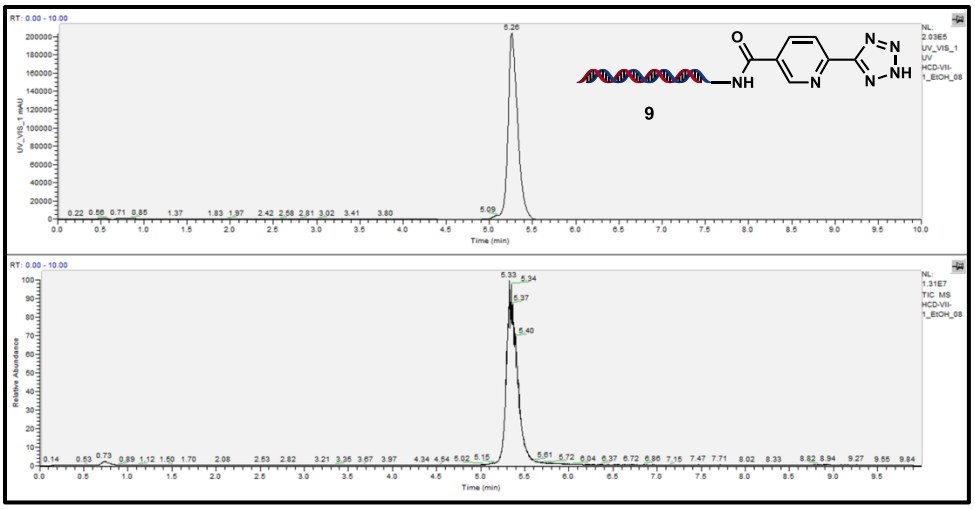


Figure S119. LC-MS spectrum of compound **9**

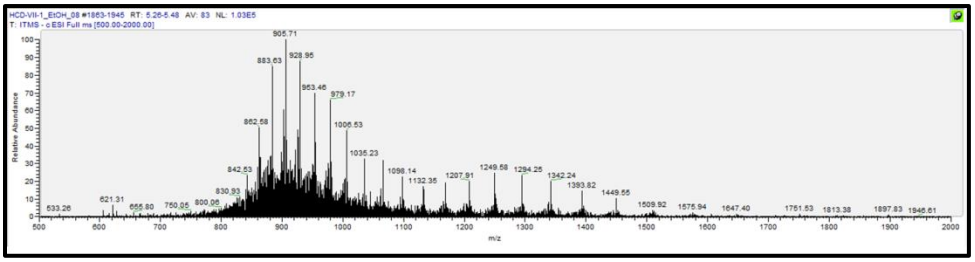


Figure S120. ESI Full MS spectrum of compound **9**

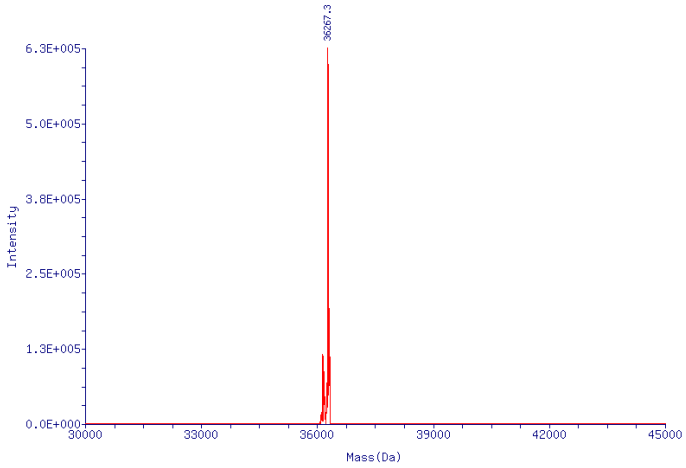


Figure S121. Deconvoluted mass spectrum of compound **9**, expected: 36261.8; observed 36267.3

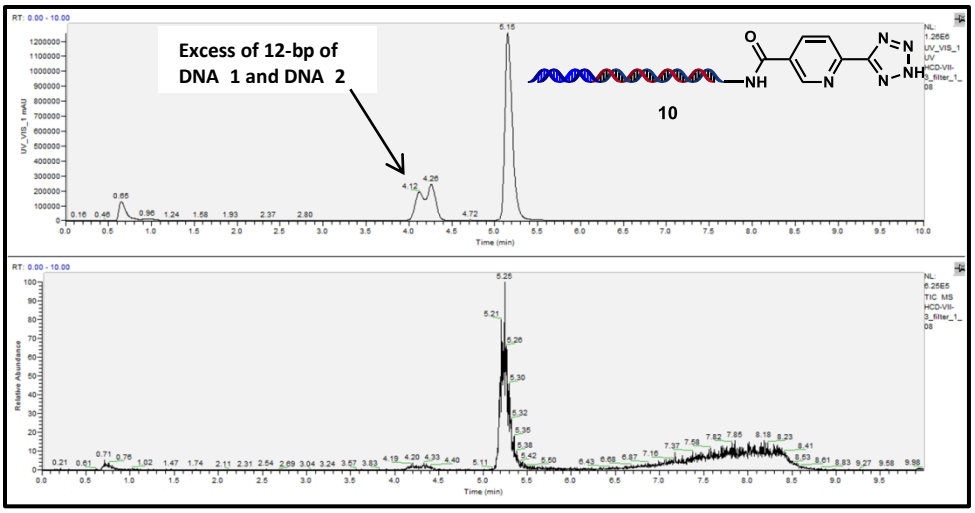


Figure S122. LC-MS spectrum of compound **10**

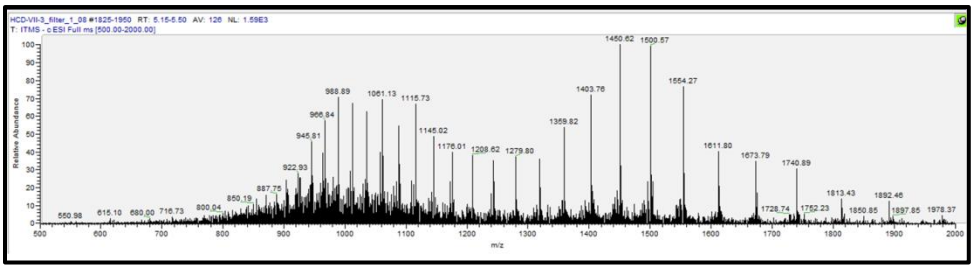


Figure S123. ESI Full MS spectrum of compound **10**

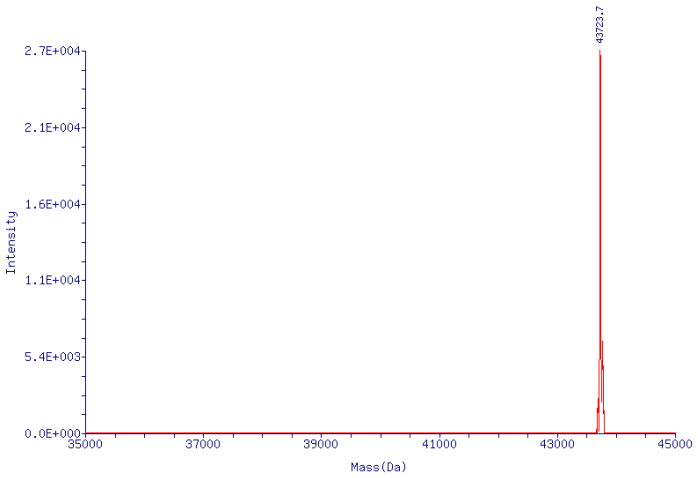
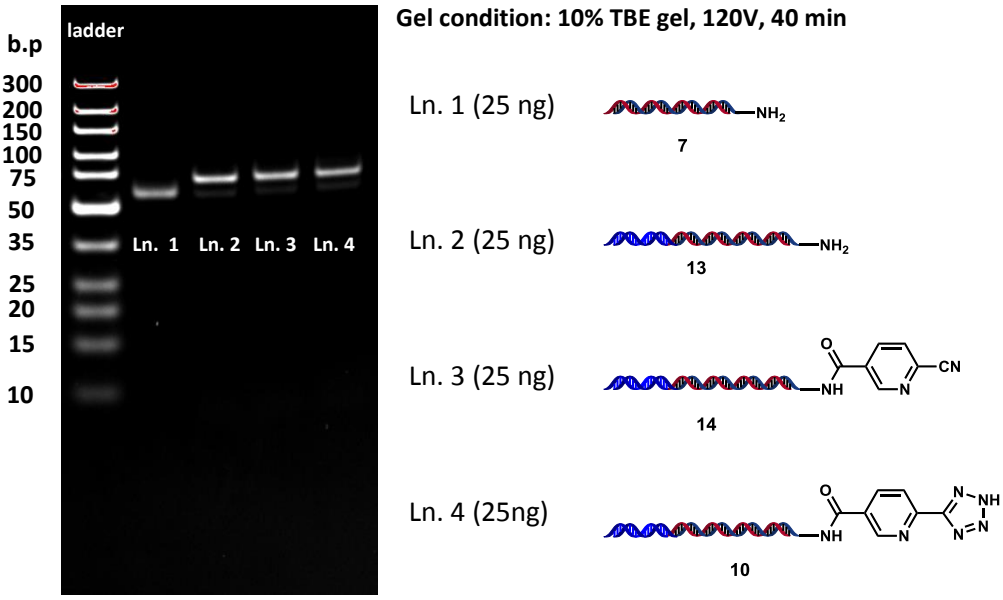


Figure S124. Deconvoluted mass spectrum of compound **10**, expected: 43716.6; observed 43723.7

LC setting for compounds **7**, **10**, **13**, and **14**

Column: Thermo DNAPac RP (3.1 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 / 3%B), 5.0 min (10%A / 90%B), 6.5 min (98%A / 3%B)
Column temperature: 100 °C (post column cooler at 40 °C)



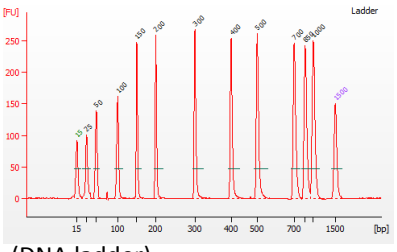
Loading unit "ng" is based on the O.D measurement

Entry	Sample	Ligation conversion
1	**13**	85 %
2	**14**	87 %
3	**10**	84 %

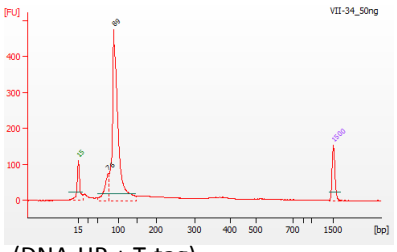
Figure S125. Gel electrophoresis image comparisons between starting material **7**, DNA-conjugate **10** and **13**, and Control **14**.

Total DNA quantification by Bioanalyzer

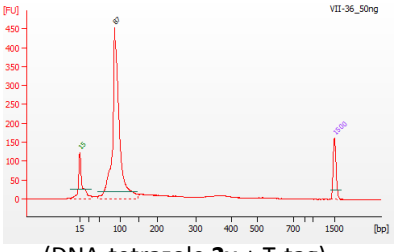
DNA-conjugated tetrazole **2v** was ligated with a 58-bp dsDNA fragment (T-tag) before performing Bioanalyzer and qPCR experiment. Following the manufacturer's instructions (Agilent 2100 Bioanalyzer), samples (1 µL of diluted samples, 25~50 ng estimated by O.D) are loaded in DNA 1000 chip with internal markers and DNA ladder prior to inserting the chip in the Agilent 2100 Bioanalyzer. DNA quantities were calculated by the concentrations from the Bioanalyzer output.



(DNA ladder)



(DNA-HP + T-tag)



(DNA-tetrazole **2v** + T-tag)

q-PCR test (Amplifiable DNA quantification)

The DNA samples were quantified by qPCR (quantitative polymerase chain reaction) using a FastStart Universal Probe Master (ROX) (Roche 04914058001) and a CFX 384 Real-Time PCR Detection system, C1000 Thermal Touch (Bio-Rad) Following the manufacturer's guidelines, samples were diluted in 4 series and run in parallel with T-tag 80mer dsDNA standards. All samples and standards were run in duplicates and subjected to PCR cycles as follows: 50 °C heat activation for 2 min and then 95 °C heated for 10 min followed by 40 cycles of 95 °C denaturation for 15 seconds, 60 °C annealing for 1 min. The standard curve was used to calculate the PCR efficiency and subsequently the DNA concentrations.

qPCR measurement and DNA damage evaluation	**Quantification by Bioanaylzer** (nmol/50 µL)	**Quantification by qPCR** (nmol/50 µL)	**Amplifiable DNA ratio**
DNA-HP	0.530	0.487	92%
DNA-Conjugated 2v	0.579	0.539	93%

7. References

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