# Supporting Information

## Chemical Labeling and Affinity Capture of Inosine-Containing RNAs Using Acrylamidofluorescein

Steve D. Knutson, Tewoderos M. Ayele, and Jennifer M. Heemstra*

Department of Chemistry, Emory University, Atlanta, GA 30302, United States

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**General**

Unless otherwise noted, all starting materials were obtained from Sigma Aldrich Corporation (St. Louis, MO) and were used without further purification. Flash column chromatography was carried out using silica gel 60 (230–400 mesh). $^{1}$H NMR chemical shifts are expressed in parts per million (δ). Mass spectra were obtained through the Mass Spectrometry Core Facility at Emory University.

**Synthesis of Acrylamidofluorescein**

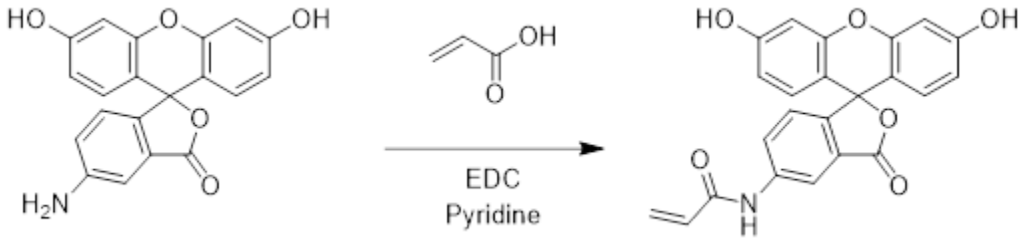



**Figure S1.** Synthetic scheme for acrylamidofluorescein.

To a solution of 5-aminofluorescein (1.00 g, 2.88 mmol) in pyridine (8.00 ml, 98.9 mmol), *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (828 mg, 4.32 mmol) and acrylic acid (0.390 ml, 5.76 mmol) were added and left to stir at room temperature overnight. Once the 5-aminofluorescein was consumed, as determined by TLC, the reaction was dried under reduced pressure to form a crude oil. The crude oil was added into 20 mL of 10% sodium hydroxide and extracted using dichloromethane. The organic layer was collected and acidified by adding concentrated hydrochloric acid until orange precipitates formed. The product was then vacuum filtered and dried to yield 0.930 g (80.7%) of orange powder. $^{1}$H NMR (400 MHz, DMSO-d6) δ 10.93 (s, 1H), 8.47 (s, 1H), 7.96 (d, *J* = 8.6, 1H), 7.22 (d, *J* = 8.2, 1H), 6.77 (s, 2H), 6.53-6.68 (m, 5H), 6.35 (d, *J* = 16, 1H), 5.84 (d, *J* = 11.3, 1H). $^{13}$C NMR (400 MHz, DMSO-d6) δ 168.9, 164.2, 160.9, 152.8, 141.2, 132.0, 130.0, 128.2, 127.7, 126.7, 125.4, 114.8, 113.7, 110.6, 102.7. HRMS m/z (ESI) calcd for $C_{23}H_{15}NO_6$ $(M+H)^+$ 402.09776, found 402.09658.

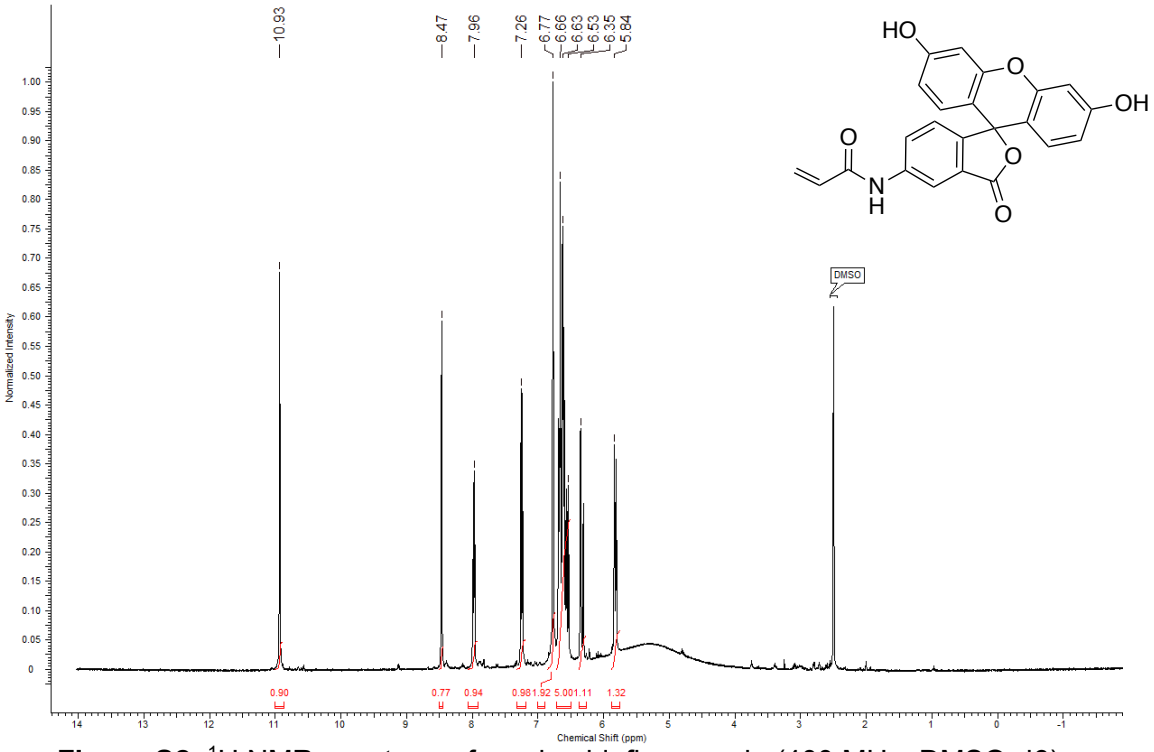



**Figure S2.** $^{1}$H NMR spectrum of acrylamidofluorescein (400 MHz, DMSO-d6).

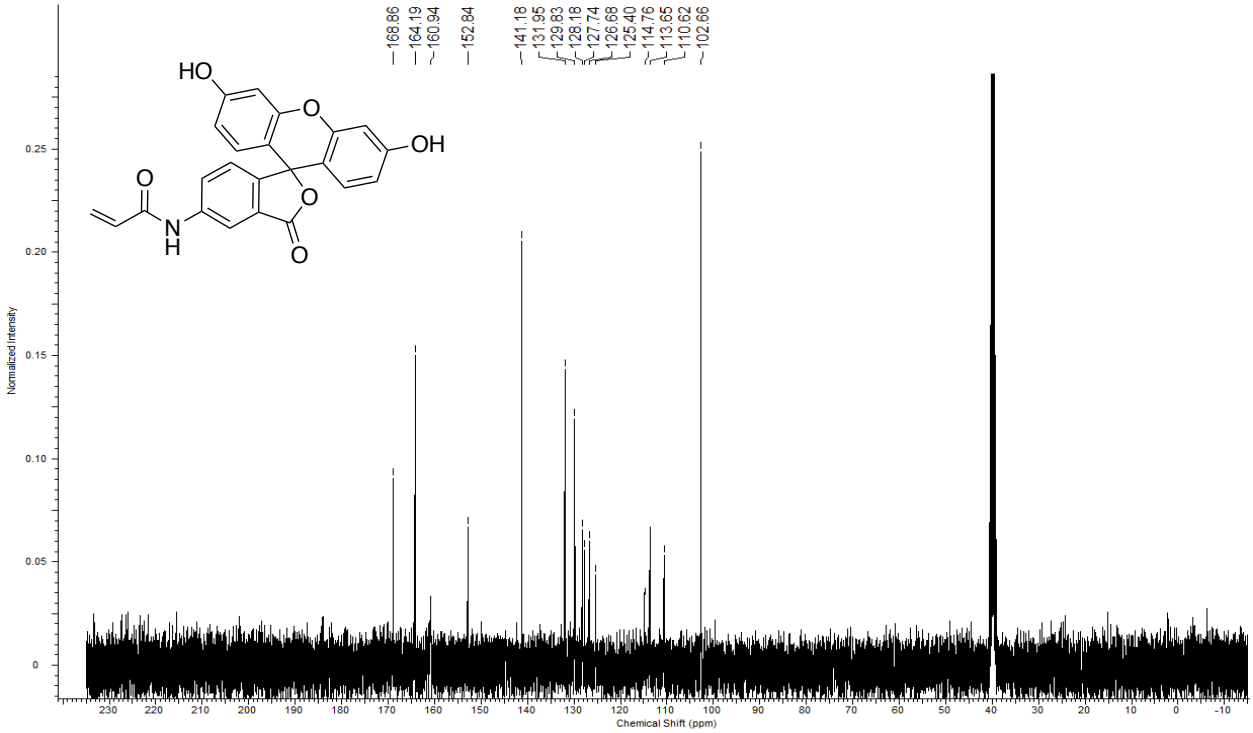



**Figure S3.** $^{13}$C NMR (spectrum of acrylamidofluorescein 400 MHz, DMSO-d6).

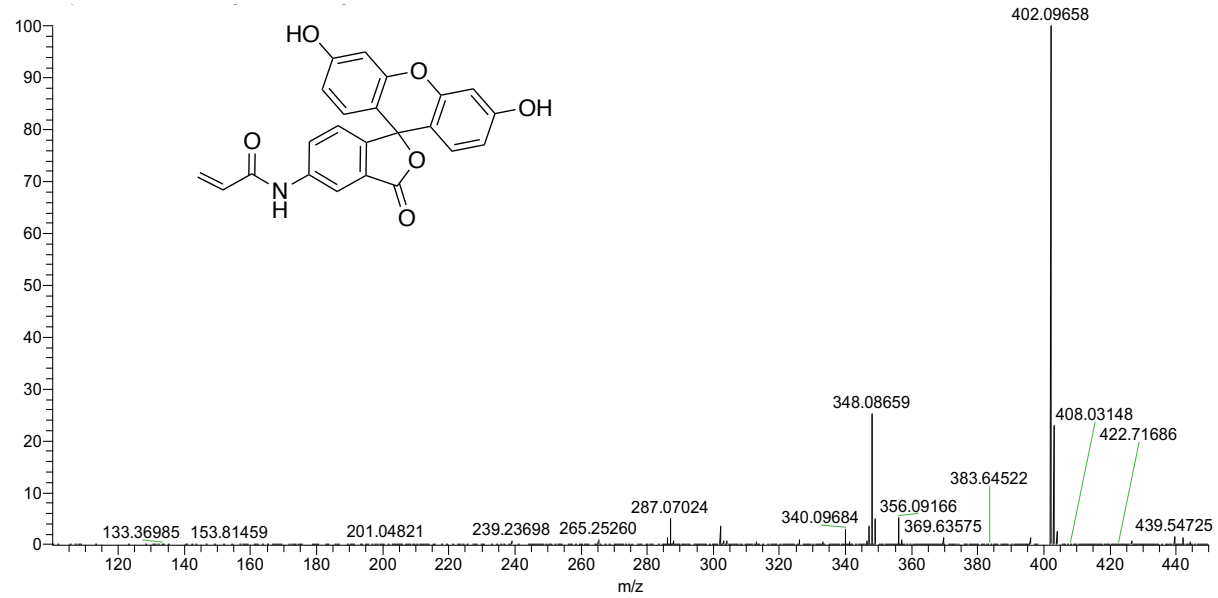


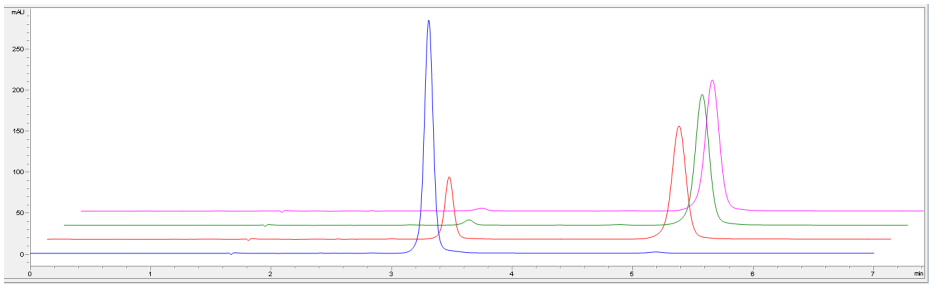

**Figure S4.** ESI-MS spectrum of acrylamidofluorescein. Calculated $(M+H)^+$ for $C_{23}H_{15}NO_6$ 402.09776

## Ribonucleoside Labeling and HPLC Analysis

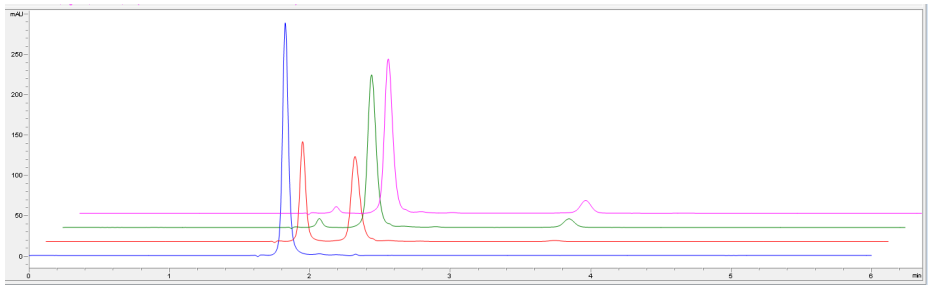
Ribonucleosides inosine, guanosine, adenosine, cytidine and uridine were purchased from Sigma Aldrich Corporation (St. Louis, MO). Pseudouridine was obtained from MP Biomedicals LLC (Santa Ana, CA). Labeling reaction mixtures were comprised of 50 mM ribonucleoside and 250 mM reagent (acrylonitrile or acrylamidofluorescein) in 50:50 EtOH:reaction buffer. Phosphate buffered saline (PBS) was used for reactions from pH 6.5-7.5 and 1M triethylammonium acetate (TEAA) for pH 8.0-10.5. Reactions were incubated at 70 °C for the time periods indicated. Reversed-phase HPLC analysis was performed on an Agilent 1260 Infinity II system using a 4 µm, 150 x 4.6 mm Phenomenex Synergi Fusion-RP 80A C18 column. Samples were prepared in a stationary phase solution of 5% acetonitrile in PBS. Acrylonitrile reactions were analyzed using an isocratic mobile phase of 5:95 acetonitrile:water. Acrylamidofluorescein reactions were analyzed using a linear mobile phase gradient from 5% to 45% acetonitrile in water over 25 minutes. All mobile phases contained 0.1% trifluoracetic acid.

**Figure S5 (Below).** Representative HPLC traces of ribonucleoside reactivity with acrylonitrile. All chromatograms were monitored at 254nm. Blue = 0 hours, red = 1 hour, green = 8 hours, pink = 24 hours.

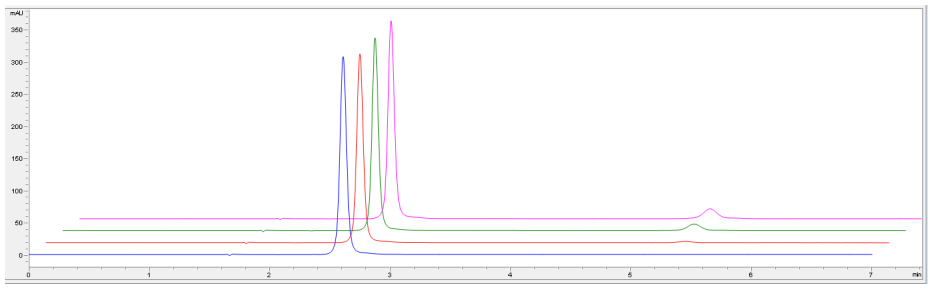
a) Inosine



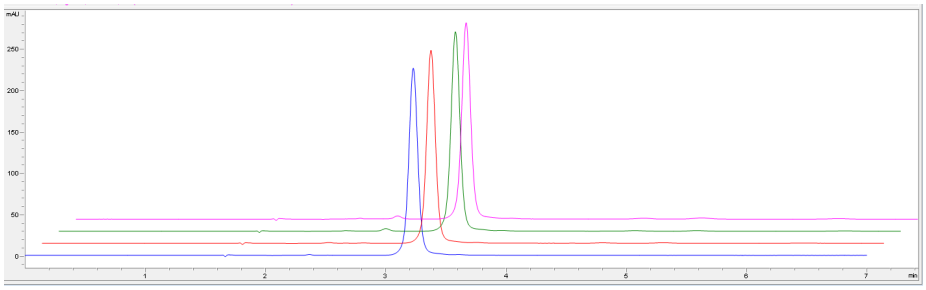
b) Pseudouridine



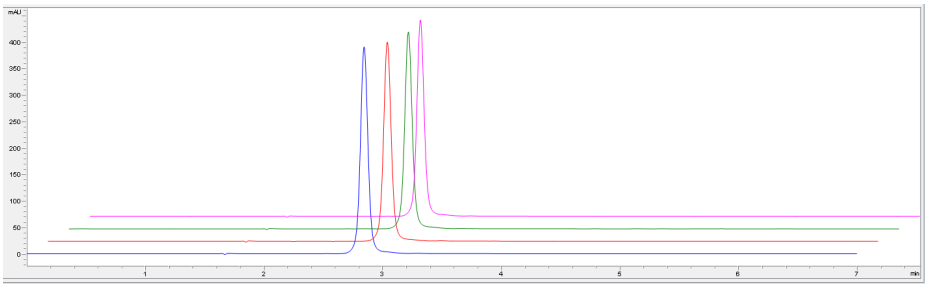
c) Uridine



d) Guanosine



e) Adenosine



f) Cytidine

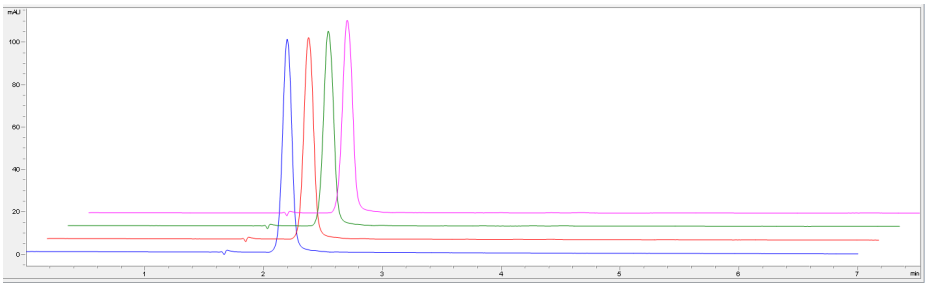
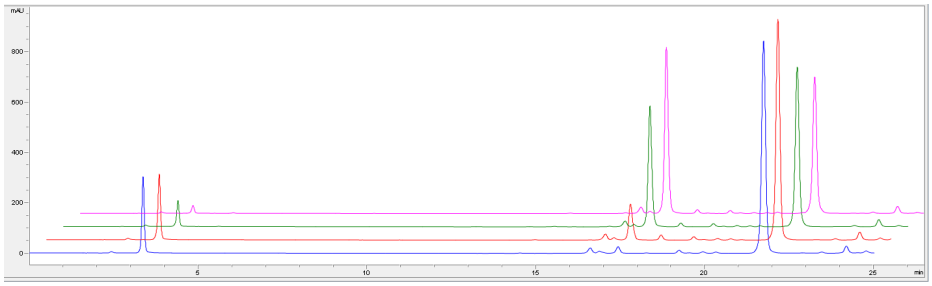
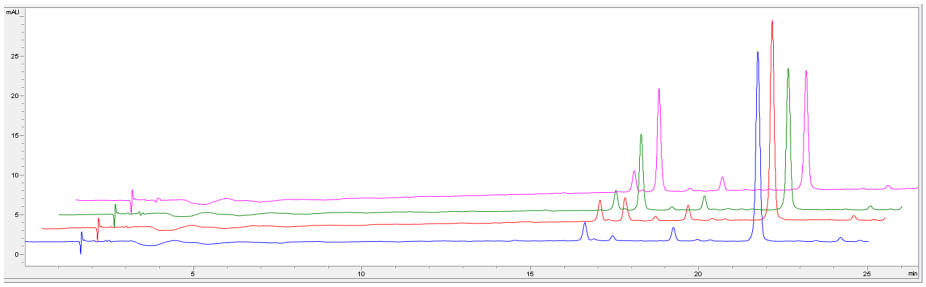


**Figure S6 (Below).** Representative HPLC traces of ribonucleoside reactivity with acrylamidofluorescein. Chromatograms were monitored at 254 and 494 nm, as indicated. Blue = 0 hours, red = 1 hour, green = 8 hours, pink = 24 hours.

a) Inosine

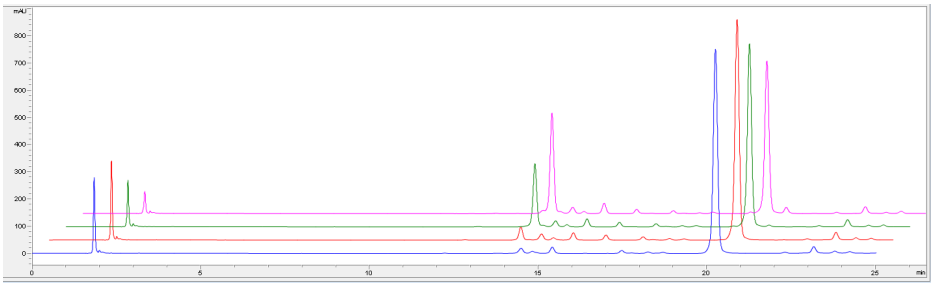


254nm

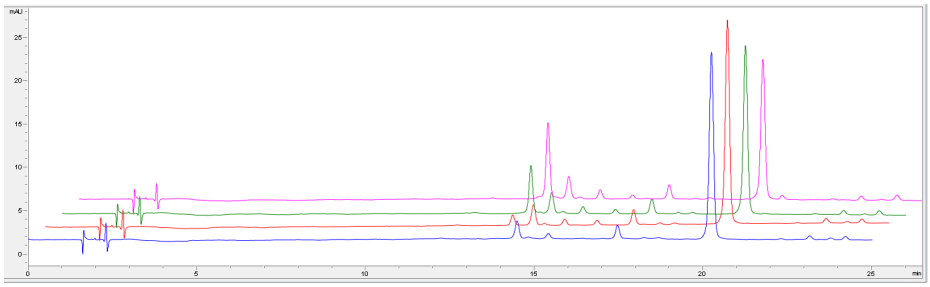


494nm

b) Pseudouridine

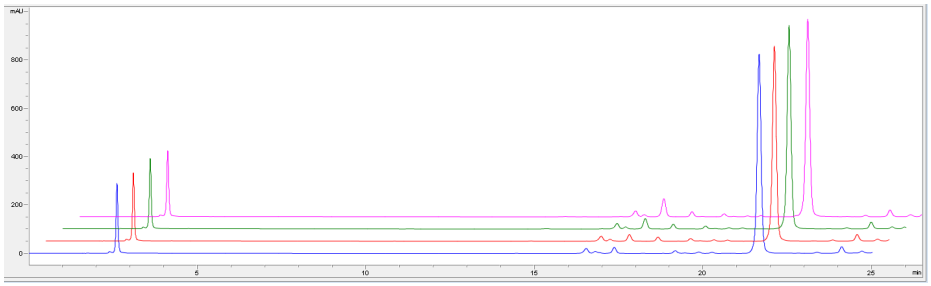


254nm

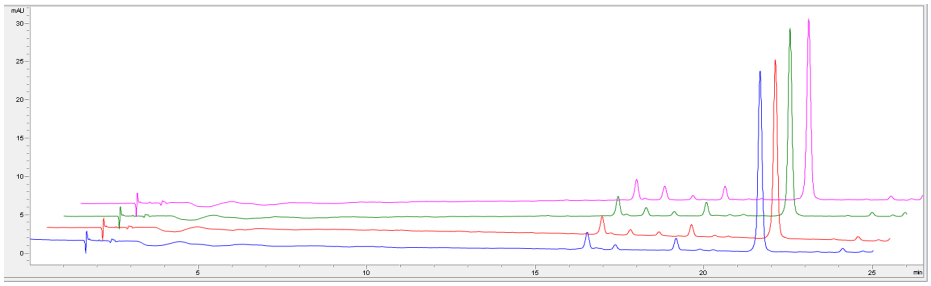


494nm

c) Uridine

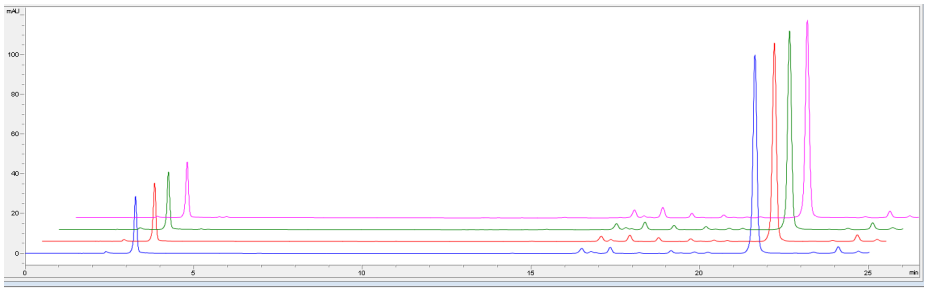


254nm

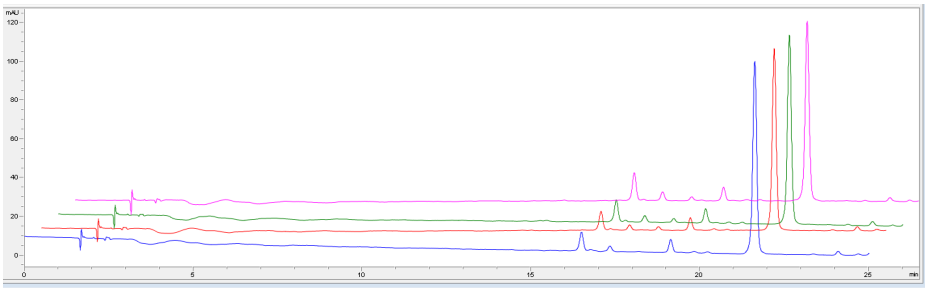


494nm

d) Guanosine

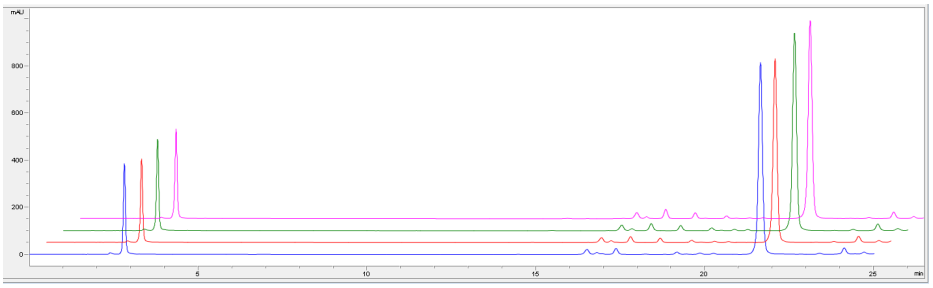


254nm

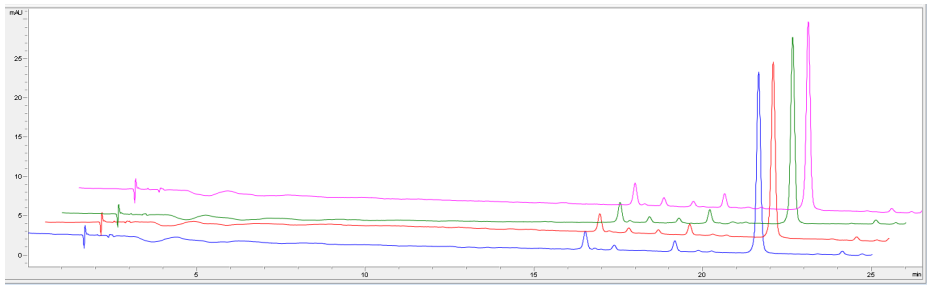


494nm

e) Adenosine

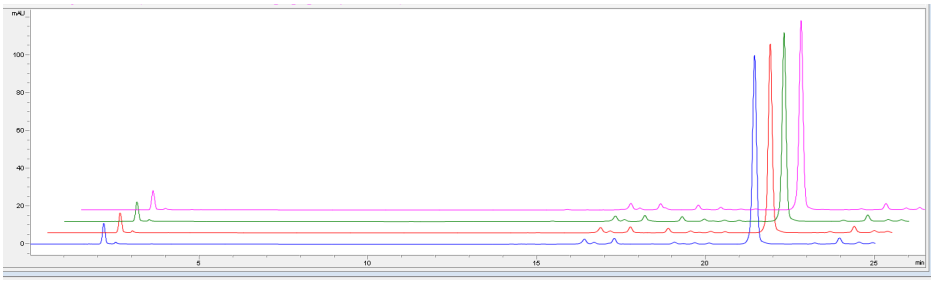


254nm

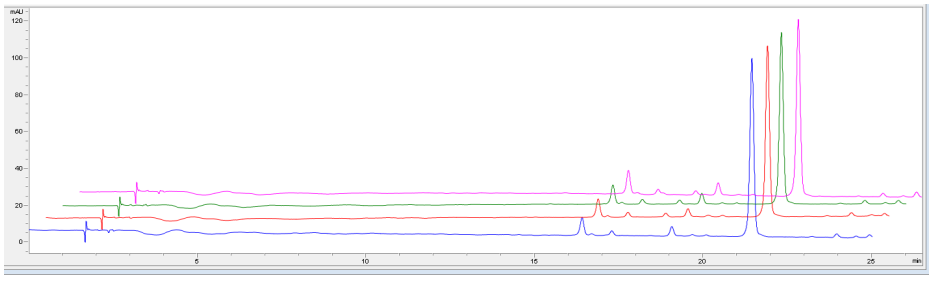


494nm

f) Cytidine

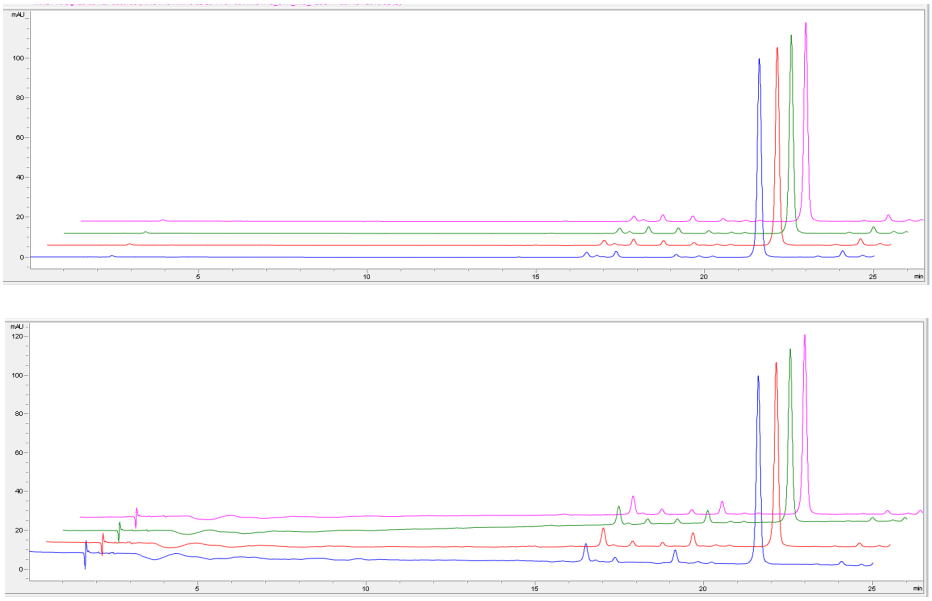


254nm



494nm

g) Acrylamidofluorescein alone (no ribonucleoside)



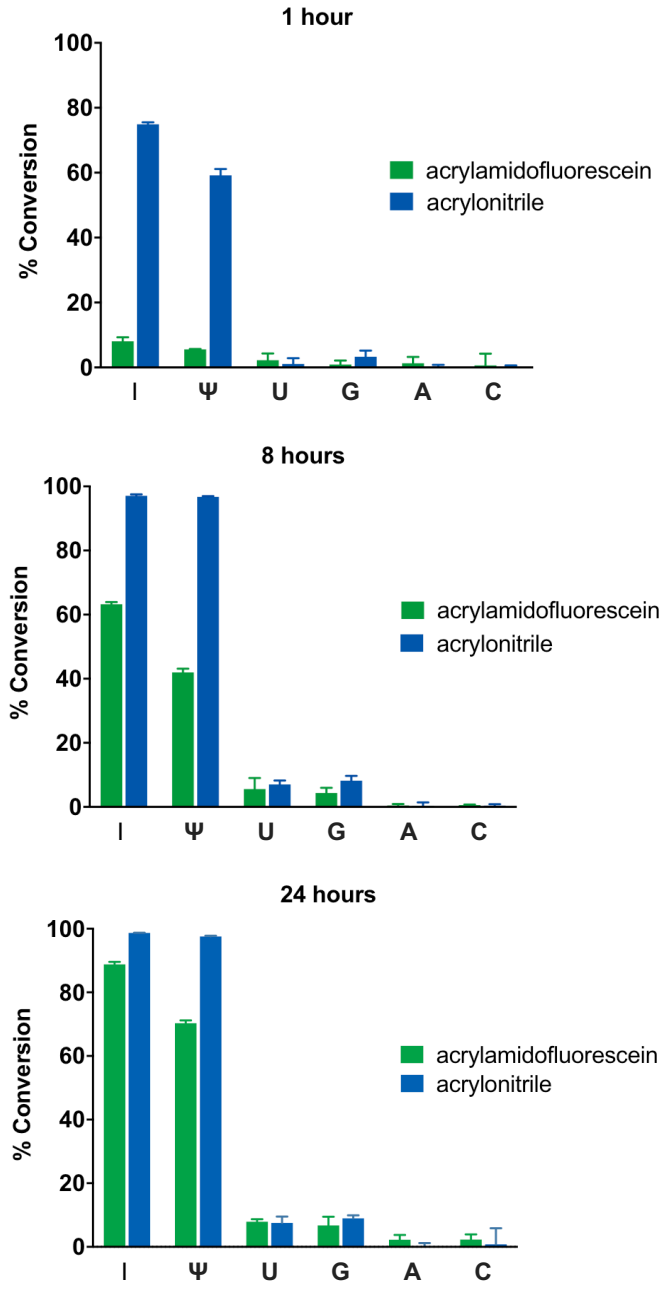



**Figure S7.** Percent conversion of ribonucleosides when reacted with acrylamidofluorescein (green bars) or acrylonitrile (blue bars) after 1, 8 and 24 hours at 70 °C, pH 8.6.

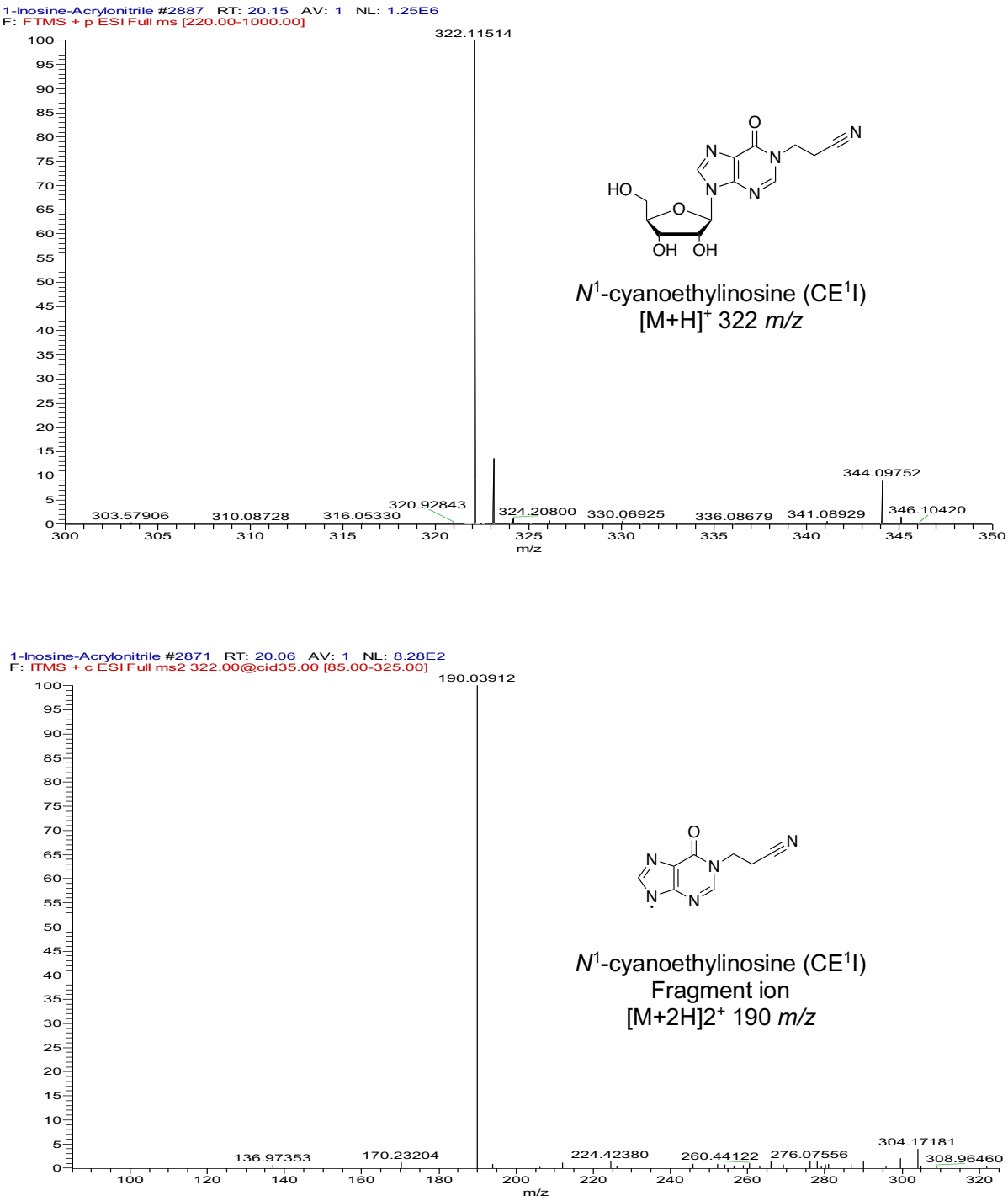
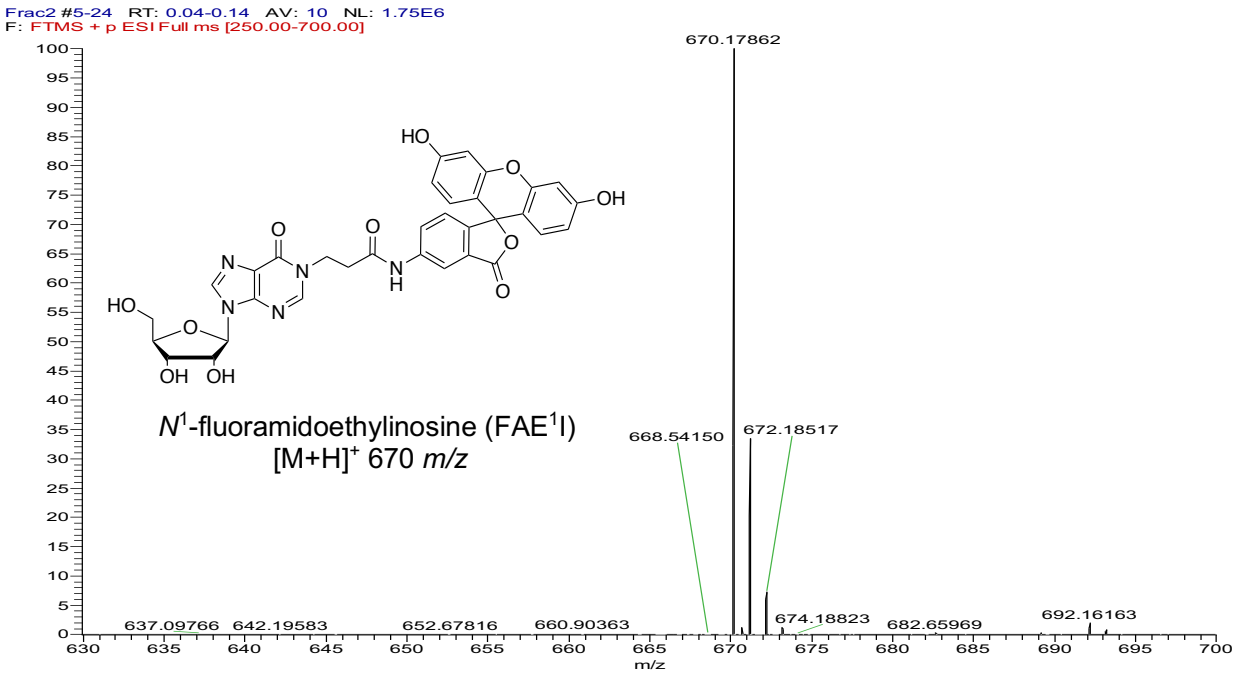



**Figure S8.** ESI-MS and MS/MS spectra of isolated product fraction for the reaction of inosine and acrylonitrile.



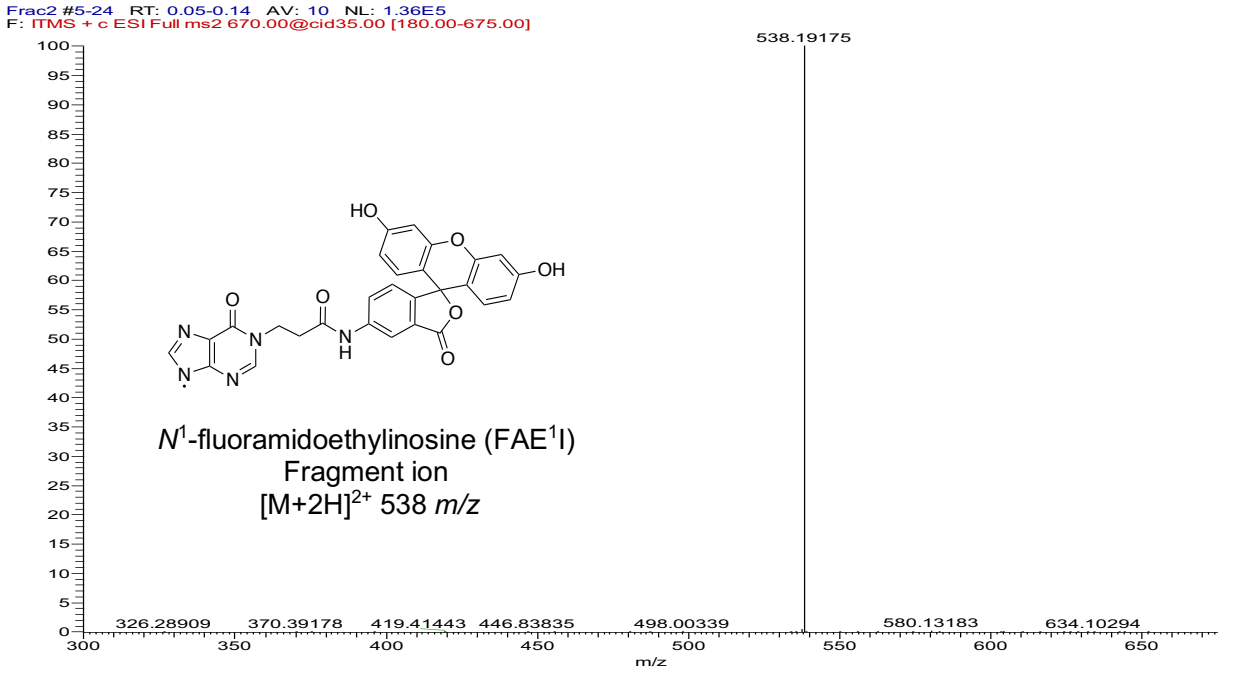




**Figure S9.** ESI-MS and MS/MS spectra of isolated product fraction for the reaction of inosine and acrylamidofluorescein.

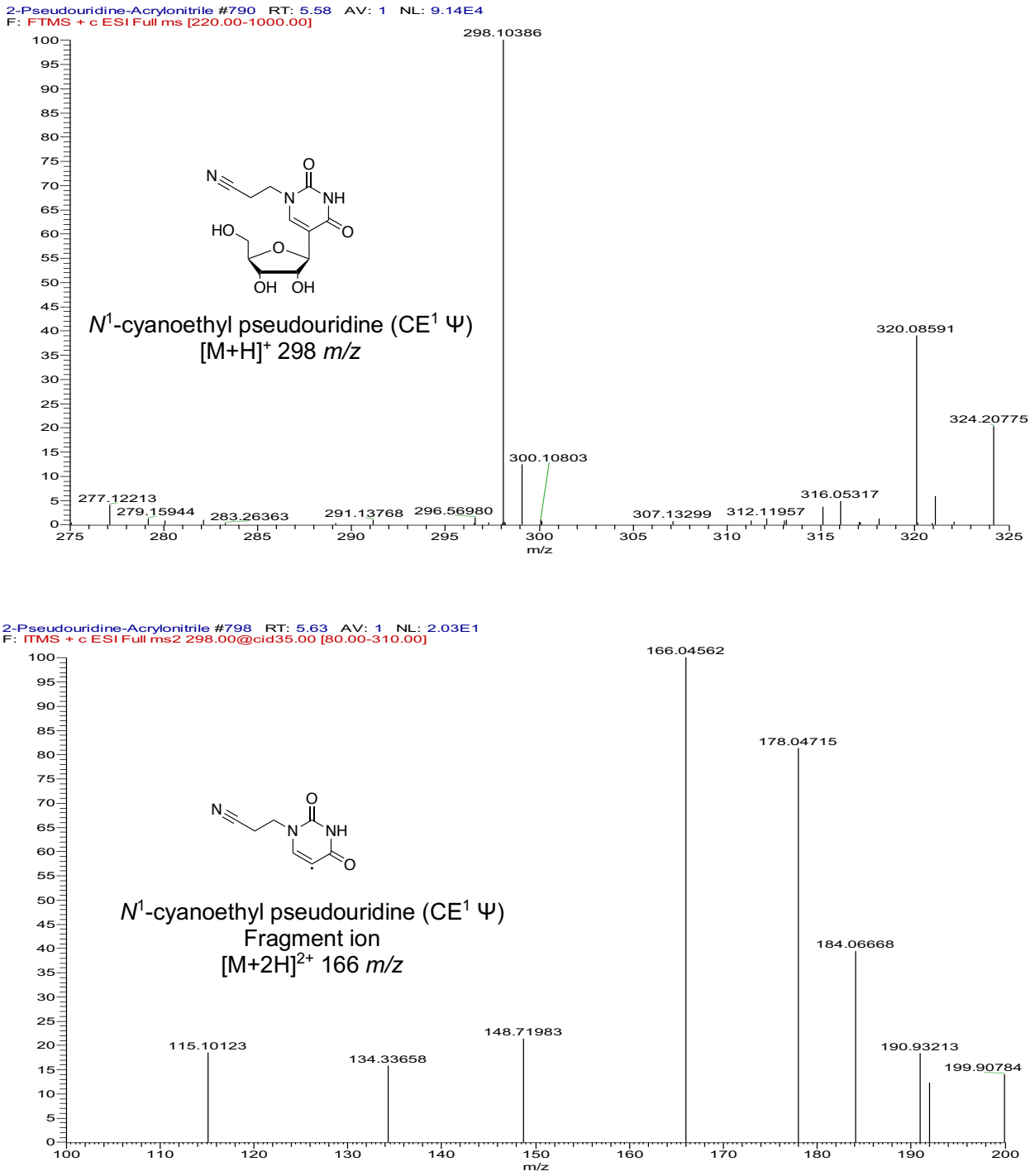



**Figure S10.** ESI-MS and MS/MS spectra of isolated product fraction for the reaction of pseudouridine and acrylonitrile.

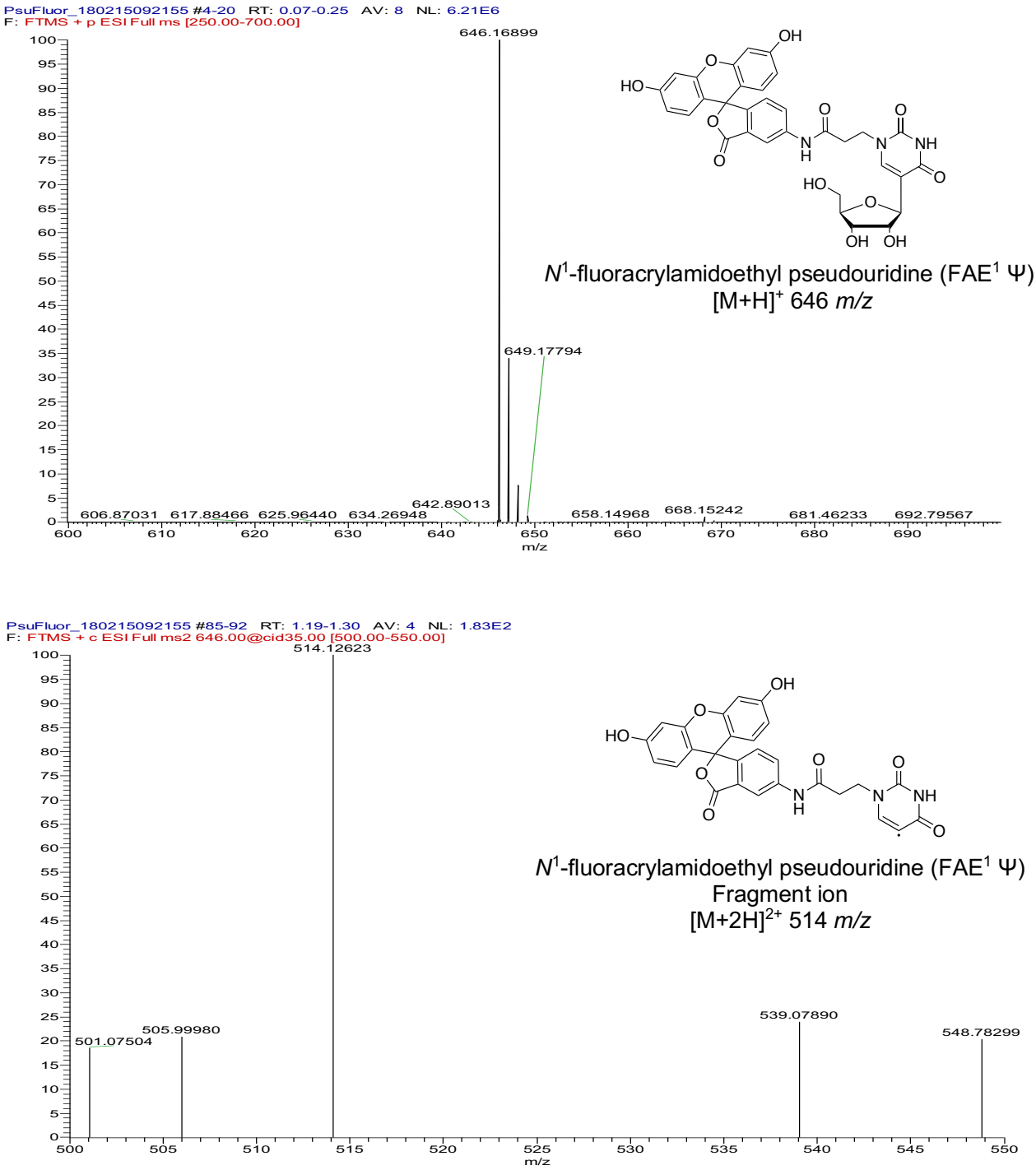



**Figure S11.** ESI-MS and MS/MS spectra of isolated product fraction for the reaction of pseudouridine and acrylamidofluorescein.

**RNA Oligoribonucleotides**

RNA oligoribonucleotides were custom designed and synthesized from Integrated DNA Technologies (Skokie, IL). Edited and non-edited controls were synthesized with either Cy5 or Cy3 at the 5' terminus as shown below.

RNA-I-Cy5 5' Cy5/GACACAUCCGC**I**CAGCAACGAG 3'

RNA-A-Cy3 5' Cy3/GACACAUCCGC**A**CAGCAACGAG 3'

RNA-A-Cy5 5' Cy5/GACACAUCCGC**A**CAGCAACGAG 3'

**Oligoribonucleotide Labeling and PAGE Analysis**

In triplicate, 1000 pmol of either RNA-A-Cy5 or RNA-I-Cy5 was added to a 0.1 mL solution of 250 mM acrylamidofluorescein in 50:50 EtOH:TEAA buffer and adjusted to pH 8.6. Reactions were incubated at 70°C. At indicated time points, crude reaction mixture was diluted 1:200 in tris-EDTA pH 7.5 buffer and ethanol precipitated. Samples were resuspended in Tris-EDTA buffer and quantified via Cy5 fluorescence. 1 pmol of each purified sample was loaded into each well, resolved on a 10% denaturing polyacrylamide gel, and imaged with a GE Amersham Typhoon. Densitometric quantification of bands was performed using ImageJ software. Each sample was normalized by comparing intensity of purified reaction bands to known amounts of RNA-I-Cy5 and a fluorescein labeled DNA oligonucleotide. Percent conversion was defined as the molar ratio of fluorescein to Cy5 for each well. All reactions were analyzed in triplicate.

**Oligoribonucleotide Labeling and Immunoprecipitation Pulldown**

In triplicate, varying mixtures of RNA-I-Cy5 and RNA-A-Cy3 were prepared in a 0.1 mL solution of 250 mM acrylamidofluorescein in 50:50 EtOH:TEAA buffer, adjusted to pH 8.6, and incubated at 70 °C for 24 hours. Mixtures were defined as follows:

| Input Ratio (RNA-I-Cy5:RNA-A-Cy3) | pmol RNA-I-Cy5 | pmol RNA-A-Cy3 |
|---|---|---|
| 1:1 | 500 | 500 |
| 1:10 | 100 | 1000 |
| 1:100 | 10 | 1000 |
| $1:10^4$ | 1 | 1000 |
| $1:10^5$ | 0.1 | 1000 |

After incubation, crude reaction mixtures were diluted 1:10 in tris-EDTA pH 7.5 buffer and ethanol precipitated. Samples were resuspended in 0.5 mL PBS + 0.05% tween 20 (PBST). An excess of monoclonal mouse anti-fluorescein antibody (MIF2901, Thermo Fisher Scientific, Rockford, IL) was added to each tube and incubated with end over end rotation for 2 hours at 4 °C. 0.02 mL of Protein A/G magnetic agarose beads (Thermo Fisher Scientific, Rockford, IL) was then added to each tube and incubated with end over end rotation for an additional 2 hours at 4 °C. Beads were then washed extensively with PBST, and bound oligoribonucleotides were eluted by heating to

95 °C for 20 minutes. Eluates were analyzed on a BioTek Cytation 5 spectrophotometer, and Cy5 and Cy3 concentrations were determined by correlating to a standard curve of RNA-I-Cy5 and RNA-Cy3. Fold-enrichment was defined as $\frac{[RNA-I-Cy5]_{final}/[RNA-A-Cy3]_{final}}{[RNA-I-Cy5]_{initial}/[RNA-A-Cy3]_{initial}}$.