

Rapid Determination of RNA Modifications in Consensus Motifs by Nuclease Protection with Ion-Tagged Oligonucleotide Probes and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

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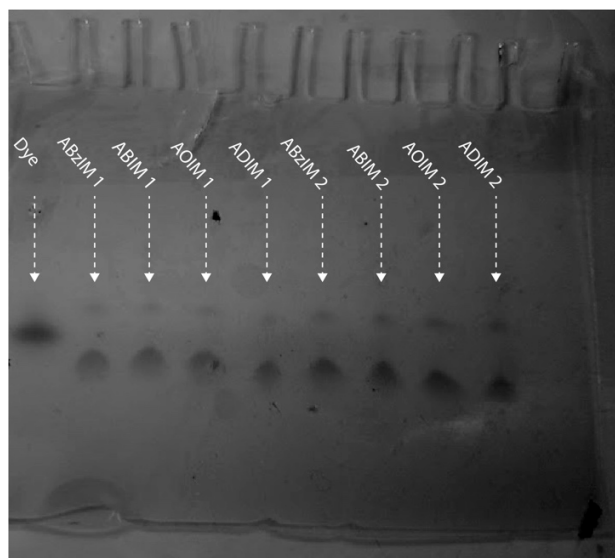


Figure S1. Photograph of a denaturing gel after the electrophoretic separation of a series of ITO probes from their unreacted thiolated oligonucleotide counterparts. The top band in each lane is the ITO while the bottom band in unreacted thiolated oligonucleotide. ABzIM, ABIM, AOIM, and ADIM ITOs were generated from allylbenzylimidazolium, allylbutylimidazolium, allyloctylimidazolium, and allyldecylimidazolium salts, respectively.

Table S1. The RNA standard sequences used in this study with the target sequence underlined. Theoretical and observed m/z values for the liberated target sequence are shown, as well as RNA fragments derived from nuclease digestion/unsuccessful protection of the GGACU motif.

Sequence Name	Sequence (5' to 3')	m/z of target sequence (theoretical)	m/z of target sequence (observed)	m/z MBN degradation products (sequence, theoretical m/z , observed m/z)
15mer RNA Internal Target	AUA AUG <u>GAC</u> UAA AAU	1649.230	1649.383	pAC U, 959.136, 959.148 pGAC, 998.158, 998.727 pAUG, 999.142 pUGG, 1015.137, 1015.501 pG GA, 1038.164, 1037.851 pACUA, 1288.188, 1288.039 pGACU, 1304.183, 1304.134 pG GAC, 1343.205, 1343.612 pAUGG, 1344.189, 1344.323 pGACUA, 1633.235 pAUGGA, 1673.241
15mer RNA Terminal Target	<u>GGA</u> CUA UAA UAA AAU	1569.264	1569.204	GGAC, 1263.239, 1262.996 GGA, 958.198, 958.070 pCUA/pACU, 959.136, 958.994 pGAC, 998.158, pCUAU, 1265.161, 12 pACUA, 1288.188, pGACU, 1304.183, 1304.605 pGACUA, 1633.235, 1632.832
15mer RNA Modified Terminal Target	<u>GGm₆A</u> CUA UAA UAA AAU	1583.291	1583.022	GGm6AC, 1277.266, 1277.403 GGm6A, 972.225, 972.483 pCUm6A, 959.136, 959.041 pm6ACU, 973.163, 972.884 pGm6AC, 1012.185, pCUAU, 1265.161 pm6ACUA, 1302.215 pGm6ACU, 1318.21, 1318.490 pGm6ACUA, 1647.262, 1647.432

Table S2. Chemical structures and abbreviations of the un-tagged probe and ITOs used in this study as well as corresponding theoretical and observed m/z values.

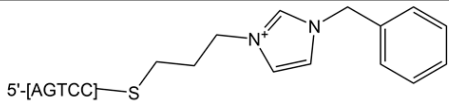
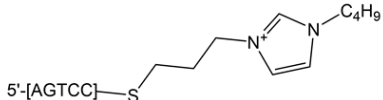
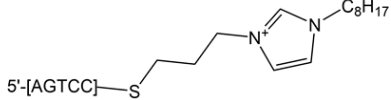
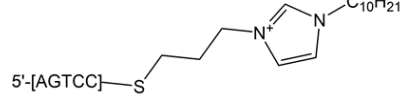
ITO Probe	Structure	Theoretical m/z	Observed m/z
Unmodified AGTCC	5'-AGTCC-3'	1463.30	1463.207
ABzIM	 5'-[AGTCC]-S-CH ₂ -(4-phenyl-1-methylpyrimidin-2-yl)	1815.40	1815.564
ABIM	 5'-[AGTCC]-S-CH ₂ -(4-butyl-1-methylpyrimidin-2-yl)	1781.42	1781.265
AOIM	 5'-[AGTCC]-S-CH ₂ -(4-octadecyl-1-methylpyrimidin-2-yl)	1837.48	1837.407
ADIM	 5'-[AGTCC]-S-CH ₂ -(4-dodecyl-1-methylpyrimidin-2-yl)	1865.51	1865.187

Table S3. Stability ratios for nuclease protection assays using different alkylimidazolium or benzylimidazolium ITO probes compared to a standard DNA oligo probe and no probe samples.

probe	stability ratio (avg)	standard deviation
no probe	-0.281	0.620
no tag	-0.192	0.335
ABzIM-ITO	0.409	0.222
ABIM-ITO	-0.016	0.343
AOIM-ITO	-0.320	0.363
ADIM-ITO	0.044	0.318

Table S4. Figures of merit for the nuclease protection method using 3' ABzIM-ITO and MALDI-MS detection for characterization of modified or unmodified consensus motifs in a 15-mer RNA standard.

RNA target ^a	amount (pmol)	average signal-to-noise (n = 3)	average intensity (n = 3)	standard deviation (n = 3)	relative standard deviation (% , n = 3)
GGACU	10	13.7	630.1	130.8	20.8
GG(m ⁶ A)CU	10	16.5	751.7	178.2	23.7

^a 15-mer RNA standard with 5' consensus motif either modified or unmodified with m⁶A.