

# Antisense Oligonucleotides Conjugated with Lipophilic Compounds: Synthesis and in Vitro Evaluation of Exon Skipping in Duchenne Muscular Dystrophy

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**Table S1.** L-ASO conjugates: reaction conditions for formation of amide bond and ESI-MS analyses

L (ASO 51) <sup>a</sup>	Conditions <sup>b</sup>	Efficiency <sup>c</sup> (%) <sup>c</sup>	Molecular Mass, Da <sup>d</sup>	
			Calc.[M]	Exp. [M]
5'-UDC	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	83	7633.66	7631.84
5'-HDC	2 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	81	7633.66	7632.64
5'-DH	3 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min	52	7570.35	7566.24
5'-EP	4 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min	53	7544.46	7547.91
5'-N-UDC	8 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	20	7733.70	7735.95
5'-DH-N-UDC	10 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	15	7943.50	7946.4
5'-N-TUDCA	i) Succinic acid (20 eq.), HBTU (20 eq.), DIPEA (40 eq.) in DMF (1 mL), 40 min.; ii) 12 (20 eq.), HBTU (20 eq.), DIPEA (40 eq.) in DMF (1 mL), 40 min.	62	7840.90	7839.01
5'-UDC 68% PS	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	80	7521.10	7521.08
5'-UDC 53% PS	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	79	7489.35	7487.60
5'-UDC 42% end-PS	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	81	7457.25	7457.78
5'-UDC 42% PS	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	80	7457.25	7459.34
5'-UDC 32% PS	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	78	7425.15	7427.92
5',3'-bis-UDC	1 (10 eq.), DIPEA (40 eq.) in DMSO (1 mL), 60 min.	68	8203.41	8202.64

<sup>a</sup> All synthesized ASO 51 were full-length 2'-OMe and full-length PS unless stated otherwise and contained the following sequence: 5'-UCAAGGAAGAUGGCAUUUCU-3'. <sup>b</sup> All reactions were performed at 7  $\mu$ mol scale and room temperature by using ssH-ASO 51-S or ss-H-ASO 51 3'-UDC (in the case of 5',3'-bis-UDC ASO 51). <sup>c</sup> Calculated on UV areas measured at wavelength of 260 nm, of conjugated full-length sequences versus unconjugated ones including failures. <sup>d</sup> According to ESI-MS in the negative ions mode.

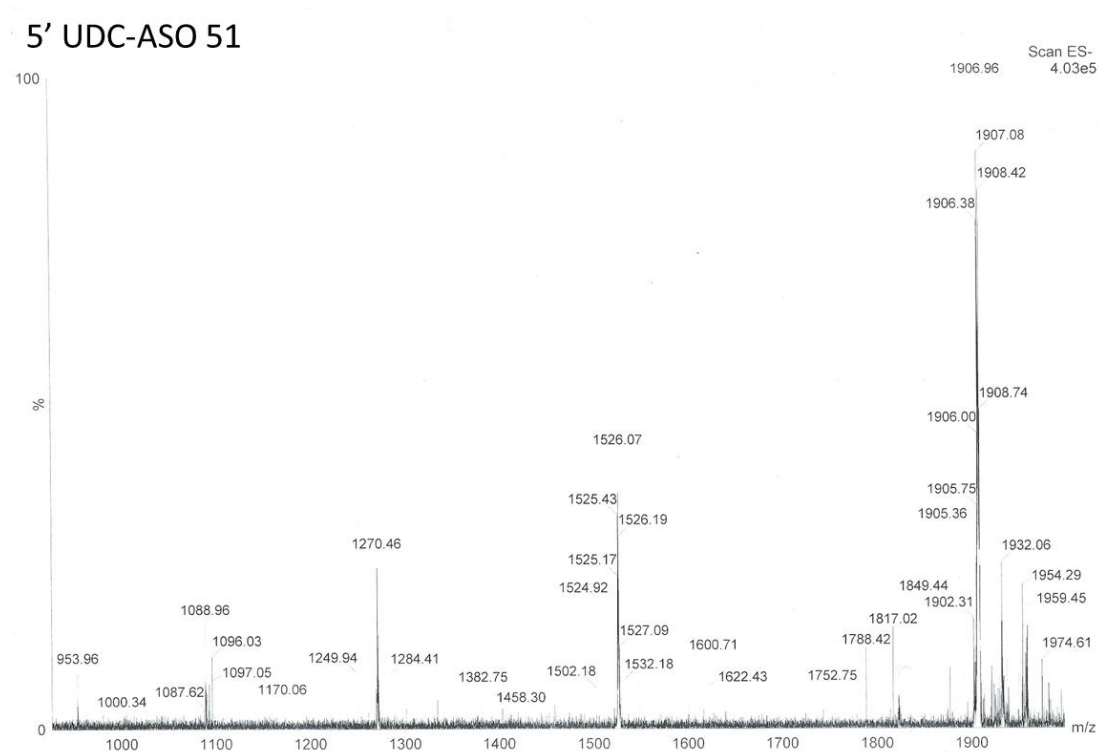


Figure S1: 5'-UDC-ASO 51 ESI-MS spectrum

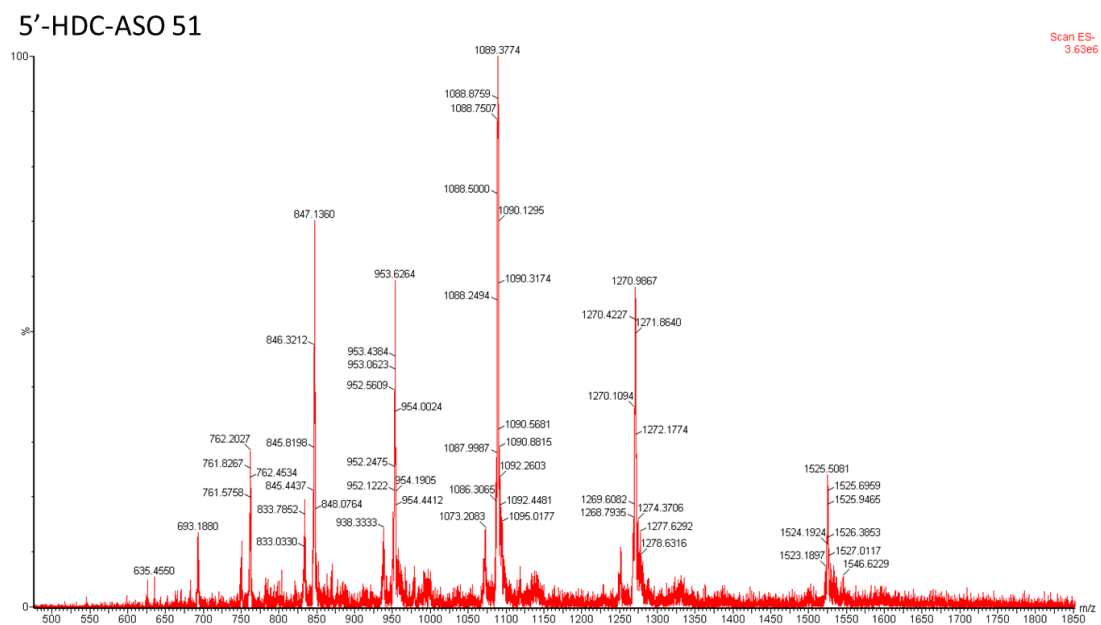
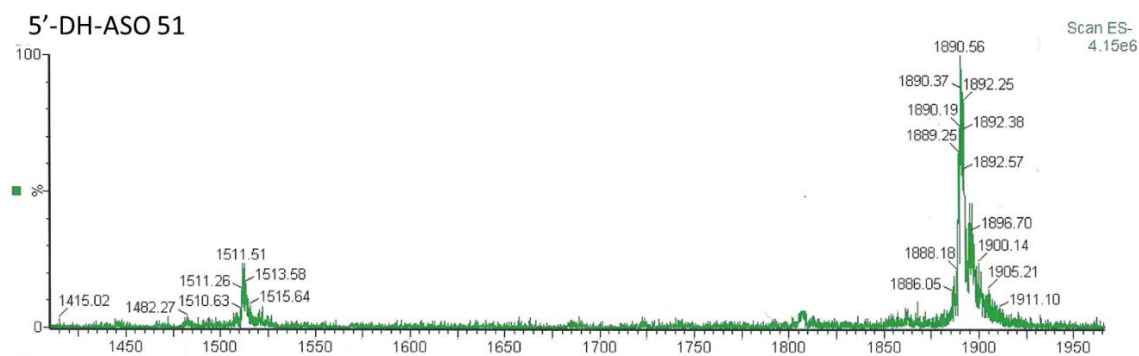
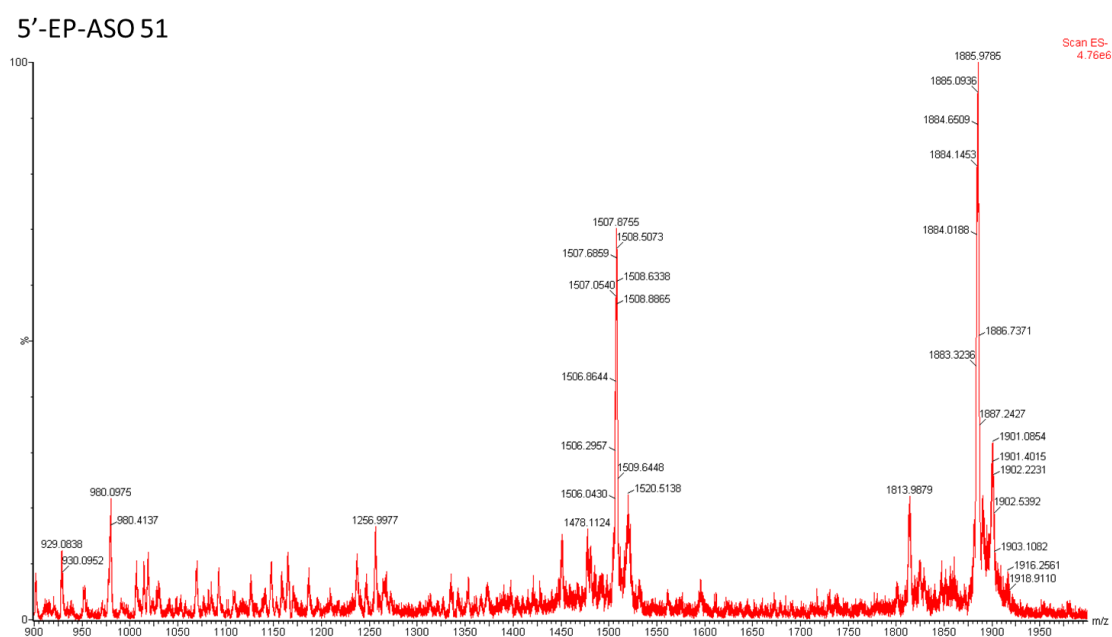


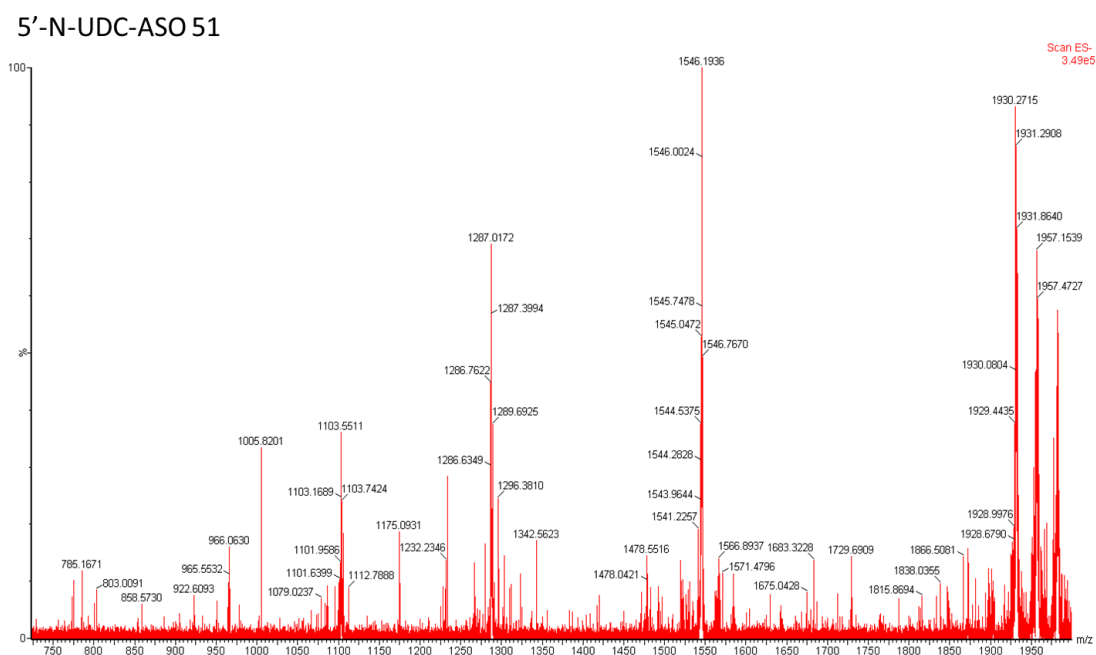
Figure S2: 5'-HDC-ASO 51 ESI-MS spectrum



**Figure S3: 5'-DH-ASO 51 ESI-MS spectrum**



**Figure S4: 5'-EP-ASO 51 ESI-MS spectrum**



**Figure S5: 5'-N-UDC-ASO 51 ESI-MS spectrum**

5'-DH-N-UDC-ASO 51

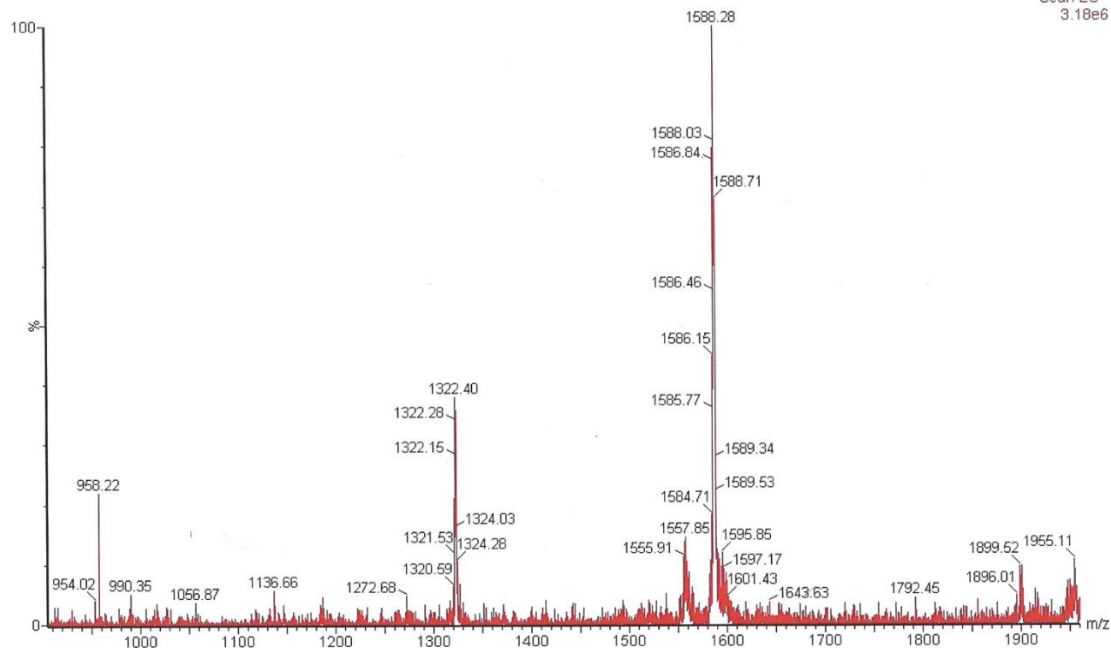


Figure S6: 5'-DH-N-UDC-ASO 51 ESI-MS spectrum

5'-N-TUDCA-ASO 51

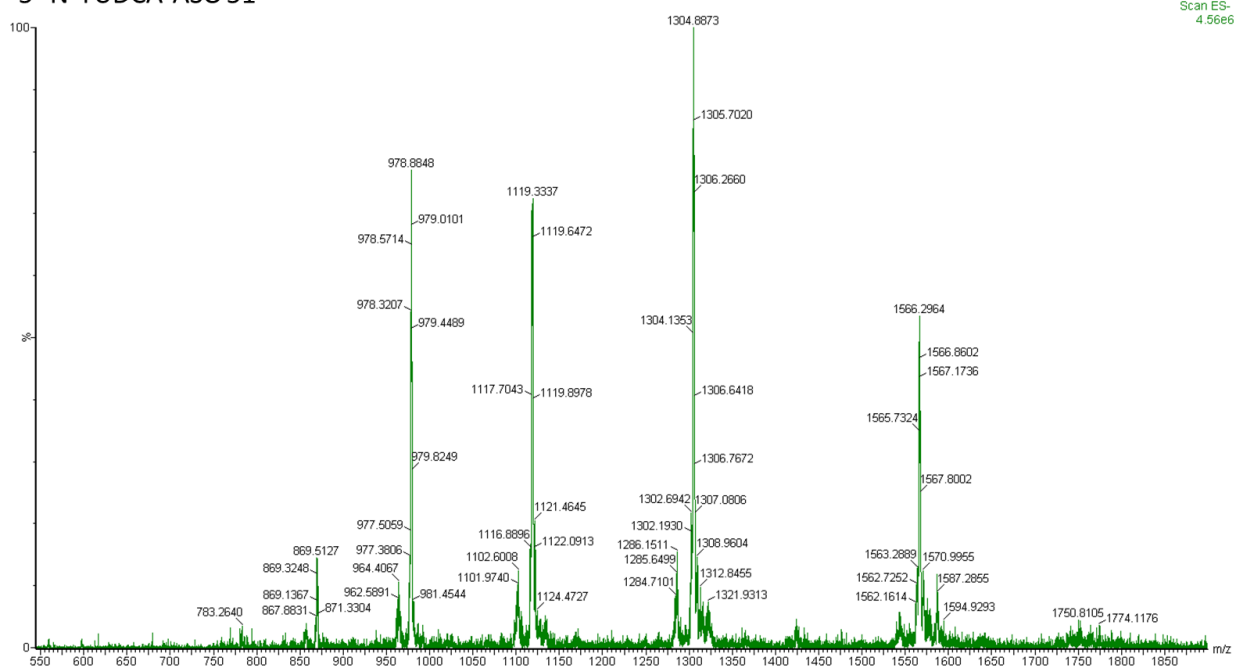


Figure S7: 5'-N-TUDCA-ASO 51 ESI-MS spectrum

### 5'-UDC-ASO 51-68% PS

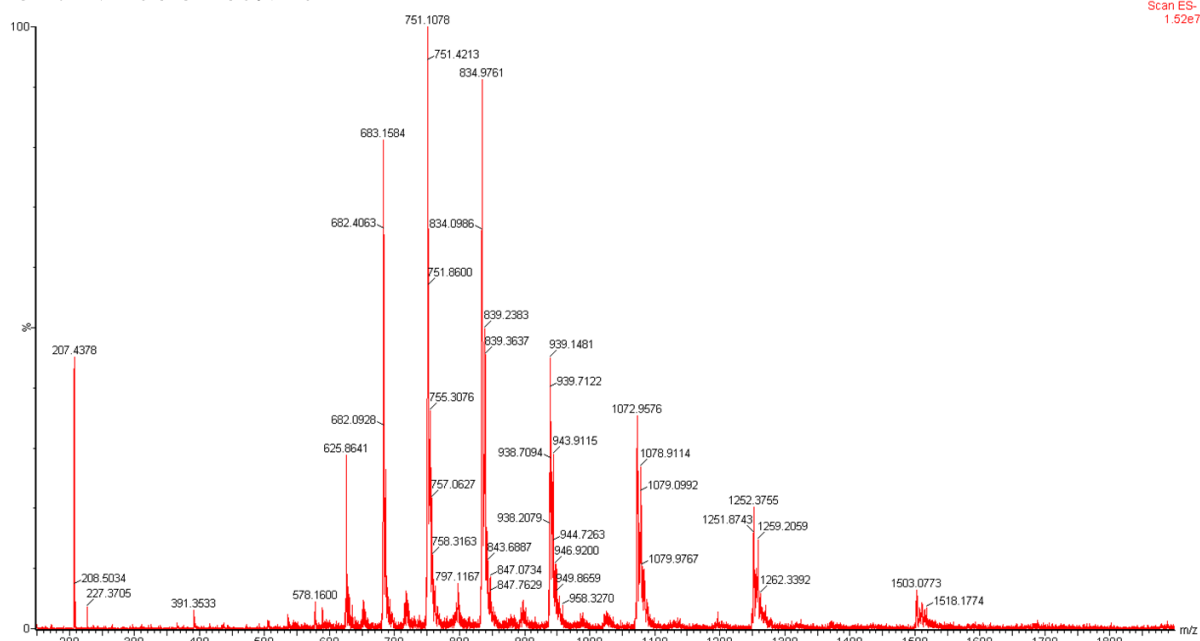


Figure S8: 5'-UDC-ASO 51-68% PS ESI-MS spectrum

### 5'-UDC-ASO 51-53% PS

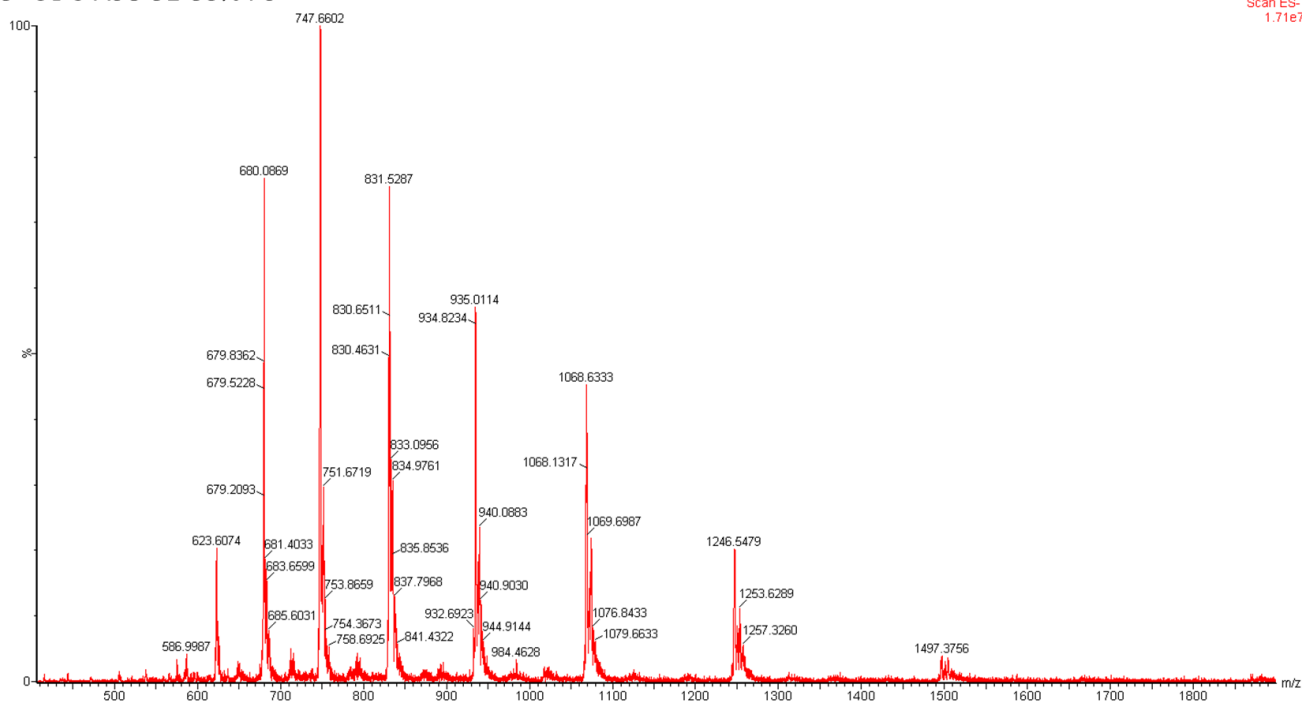


Figure S9: 5'-UDC-ASO 51-53% PS ESI-MS spectrum

# 5'-UDC-ASO 51-42% end-PS

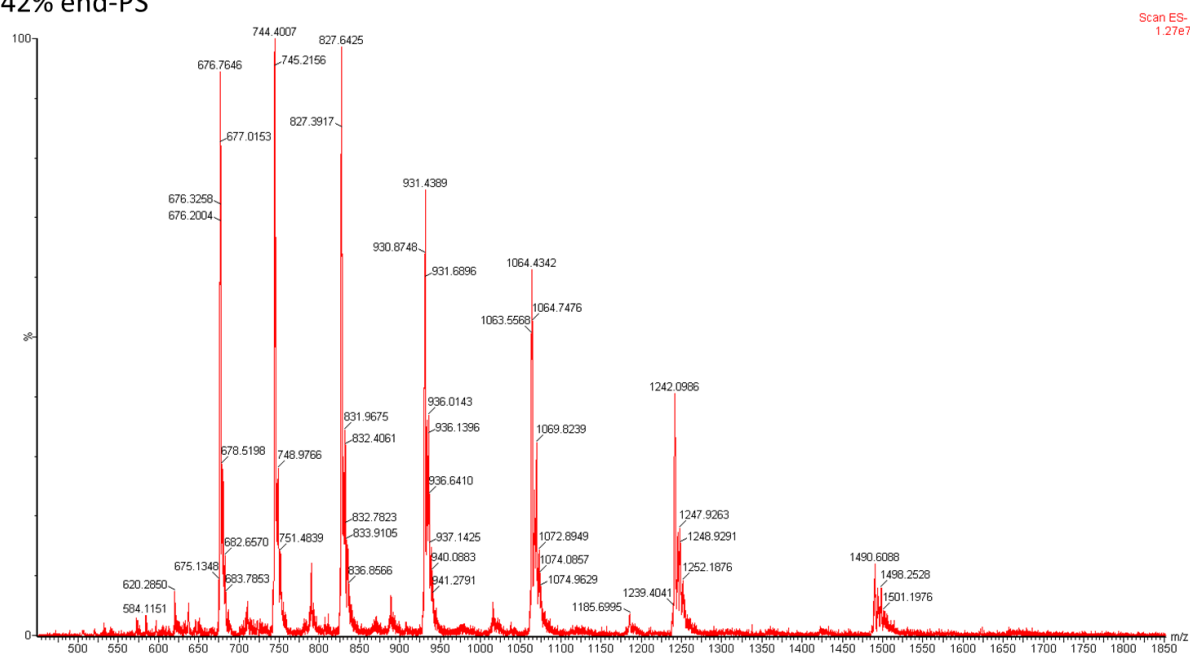


Figure S10: 5'-UDC-ASO 51-42% end-PS ESI-MS spectrum

# 5'-UDC-ASO 51-42% PS

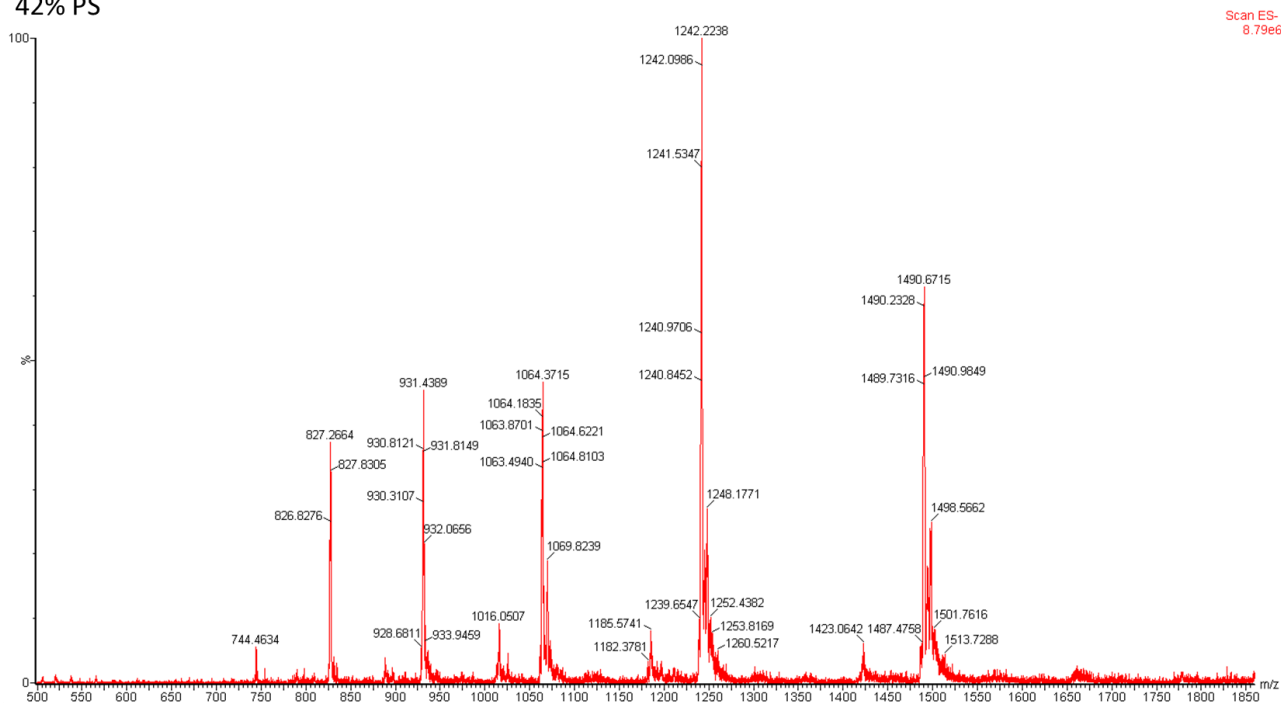


Figure S11: 5'-UDC-ASO 51-42% PS ESI-MS spectrum

Mass spectrum of compound 10. The x-axis represents the mass-to-charge ratio (m/z) from 500 to 1850, and the y-axis represents the relative intensity from 0 to 100%. The base peak is at m/z 824.4458. Other significant peaks are labeled with their m/z values.

m/z	Relative Intensity (%)
616.6493	~5
617.6522	~15
673.2543	~10
674.0692	~25
674.6334	~10
674.3825	~10
708.0442	~5
739.3234	~5
740.9532	~35
741.7681	~45
743.7112	~10
744.7769	~10
745.1530	~10
745.9678	~10
746.7200	~10
823.0040	~10
823.5682	~10
823.9443	~10
824.4458	100
828.3947	~10
828.7080	~10
830.5885	~10
830.7765	~10
831.2780	~10
832.0928	~10
832.3163	~10
832.5670	~10
832.9214	~10
833.0457	~10
925.0457	~10
926.1740	~10
926.8634	~40
926.5500	~10
927.4902	~55
931.6268	~10
932.1284	~10
932.0712	~10
932.3163	~10
932.5670	~10
932.9195	~10
935.5755	~10
1058.7311	~10
1058.8563	~10
1059.7965	~10
1065.8757	~10
1066.4480	~10
1068.0065	~10
1068.7585	~10
1069.0719	~10
1235.5188	~10
1236.2080	~10
1239.6547	~10
1243.1639	~10
1245.9839	~10
1246.6104	~10
1249.3049	~10
1236.5214	~15
1490.6088	~5
1491.6112	~10
1492.3630	~10

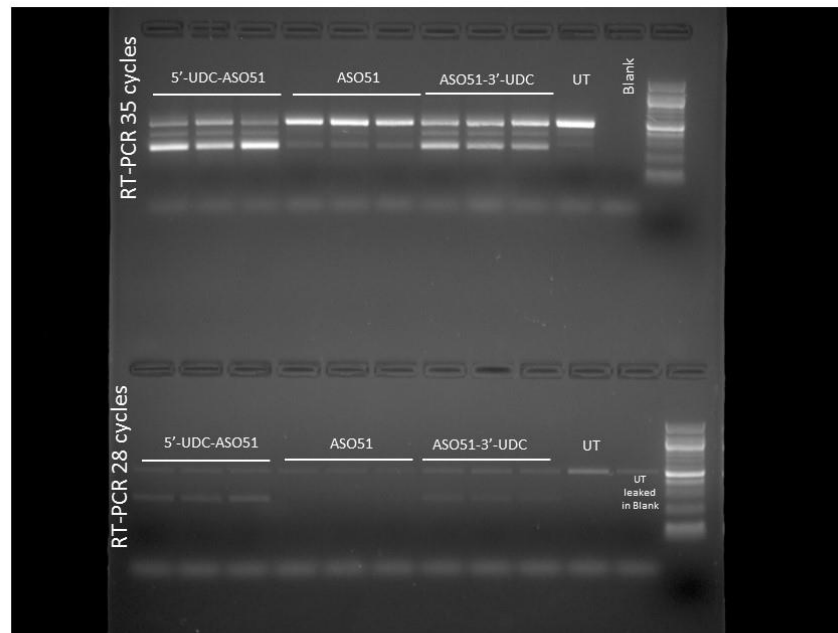
5',3'-bis-UDC-ASO 51

Scan ES-  
5.21e6

Mass spectrum showing relative intensity (%) versus mass-to-charge ratio ( $m/z$ ). The base peak is at  $m/z$  1170.8064. Other labeled peaks include:

- 784.3338
- 819.5353
- 820.1650
- 911.1558
- 1022.9808
- 1021.8475
- 1023.8623
- 1023.4215
- 1024.6179
- 1024.8069
- 1024.9957
- 1025.4993
- 1026.1919
- 1027.8289
- 1028.8475
- 1123.3380
- 1168.0907
- 1168.0995
- 1170.4918
- 1170.5111
- 1170.6178
- 1170.8064
- 1171.2472
- 1171.4360
- 1171.6879
- 1170.2400
- 1174.2062
- 1227.0238
- 1283.0500
- 1363.9362
- 1366.6450
- 1365.5121
- 1365.9525
- 1366.1414
- 1367.6523
- 1370.0441
- 1373.8210
- 1432.2974
- 1496.9410
- 1639.4437
- 1639.8844
- 1639.1289
- 1640.1991
- 1640.6395
- 1641.0172
- 1643.7239
- 1717.9973
- 1737.7618
- 1797.1199

**Figure S13: 5',3'-bis-UDC-ASO 51 ESI-MS spectrum**



**Figure S14:** Original images for RT-PCRs performed with primers DMDex50F and DMDex54R