

SUPPORTING INFORMATION

The *R*-Diastereomer of 6'-*O*-Toluoyl-carba-LNA Modification in the Core Region of siRNA Leads to 24-times Improved RNA Silencing Potency Against the HIV-1 Compared to its *S*-counterpart

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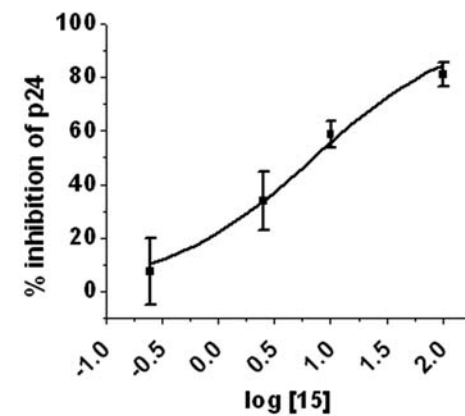
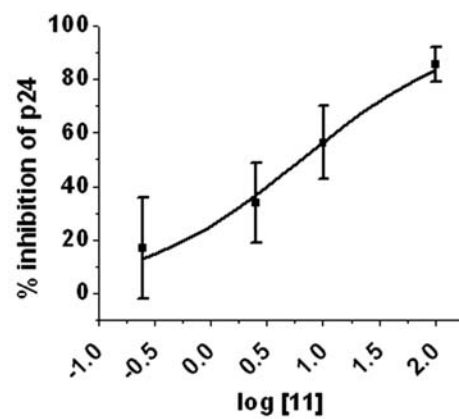
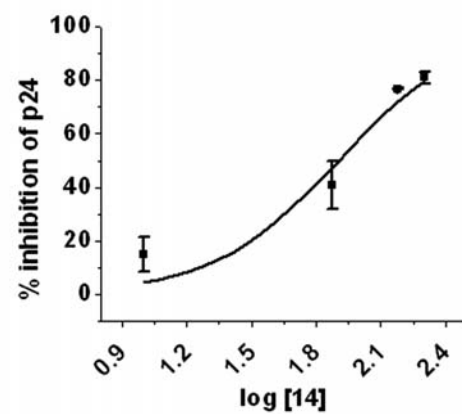
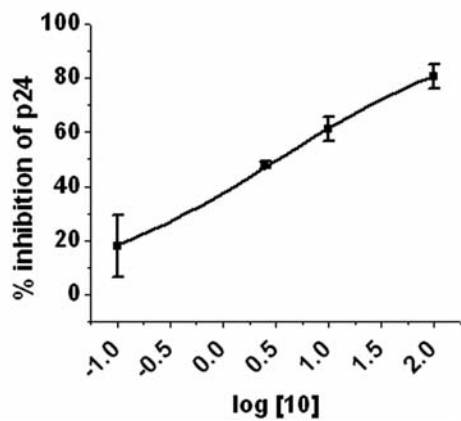
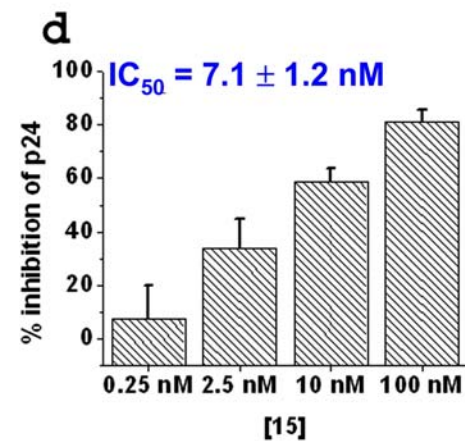
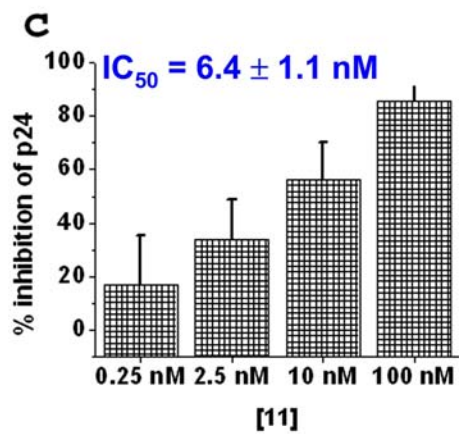
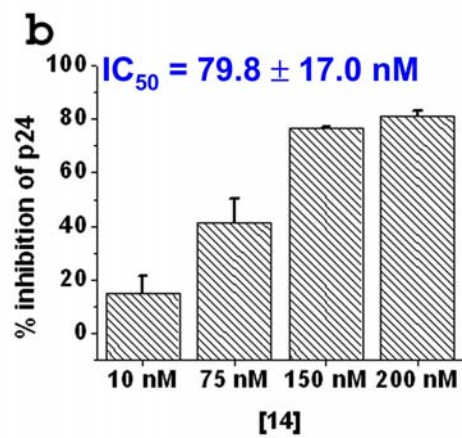
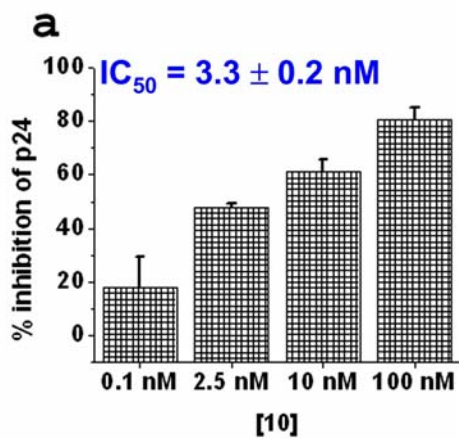
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5'-UAGCCAGAGAGCT¹³CCCAGGUU-3'

5'-T¹AGCCAGAGAGCUCCCAGGUU-3'



5'- UAGCCAGAGAGCUCCCAGGT20U -3'

5'- T1AGCCAGAGAGCUCCCAGGT20U -3'

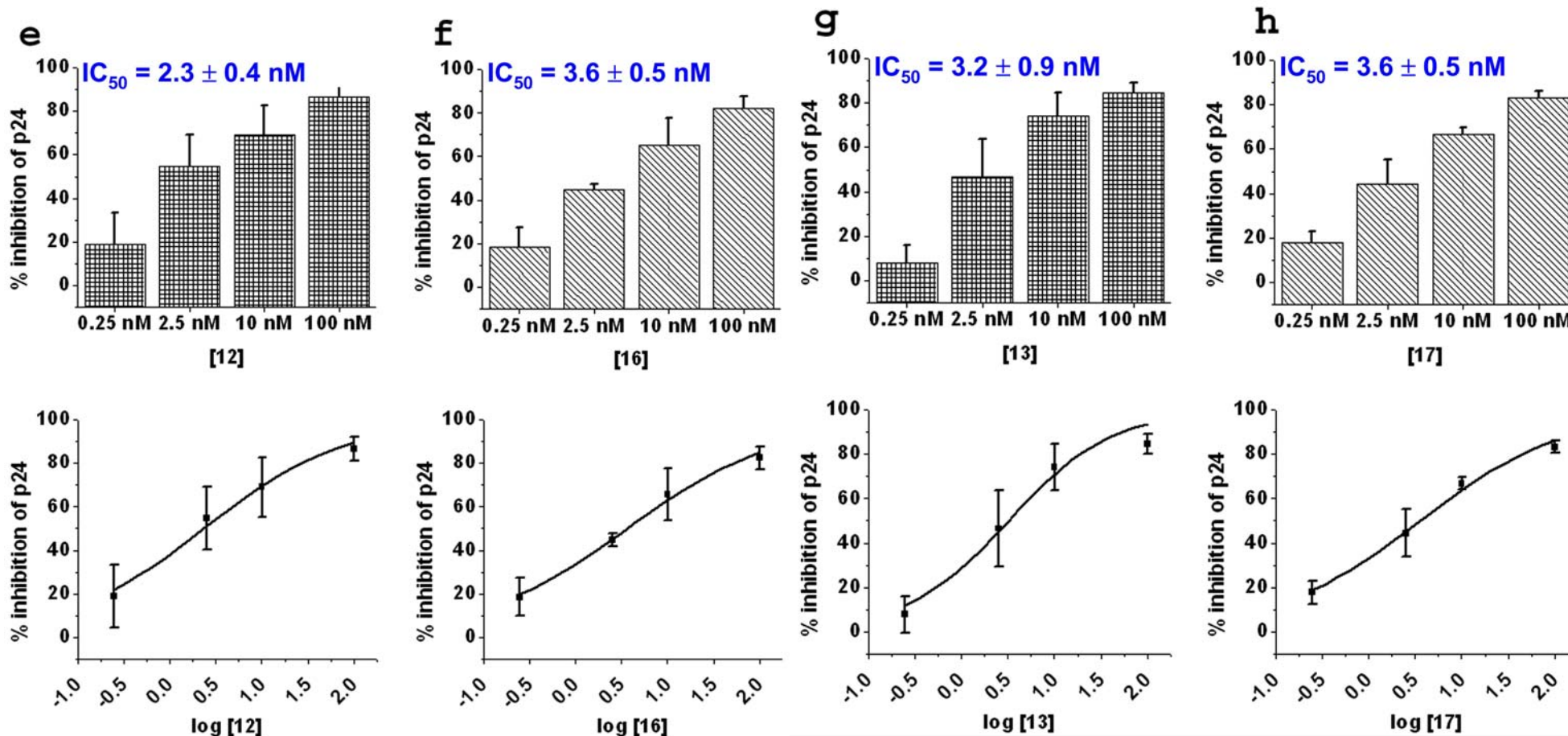


Figure S1. Dose response studies using p24 ELISA for [6'(R), 7'(S); 6'(S), 7'(S)]-jcLNA modified siRNA (as in **IVa** or **IVb** in Fig 1) showing % inhibition of viral replication in comparison to the virus control at various doses. Left panels represent 6'R/7'S diastereomer **IVa** modified siRNAs **10-13**, and right panels represent 6'S/7'S diastereomer **IVb** modified siRNAs **14-17** respectively. Panels are marked as following: **a**: siRNA **10**; **b**: siRNA **14**; **c**: siRNA **11**; **d**: siRNA **15**; **e**: siRNA **12**; **f**: siRNA **16**; **g**: siRNA **13** and **h**: siRNA **17**. The siRNA sequences containing corresponding diastereomers are shown above the bar plots with the position(s) of modification highlighted. Respective dose titration sigmoidal plots are placed below each bar plot. Results are cumulative of at least three independent experiments. Error bars represent \pm SD from the mean value. p24 ELISA has been carried out with culture supernatants 48 h post-co-transfection of pNL4-3 and varying doses of siRNA. The determination of IC_{50} values using Western blot and RT-PCR.

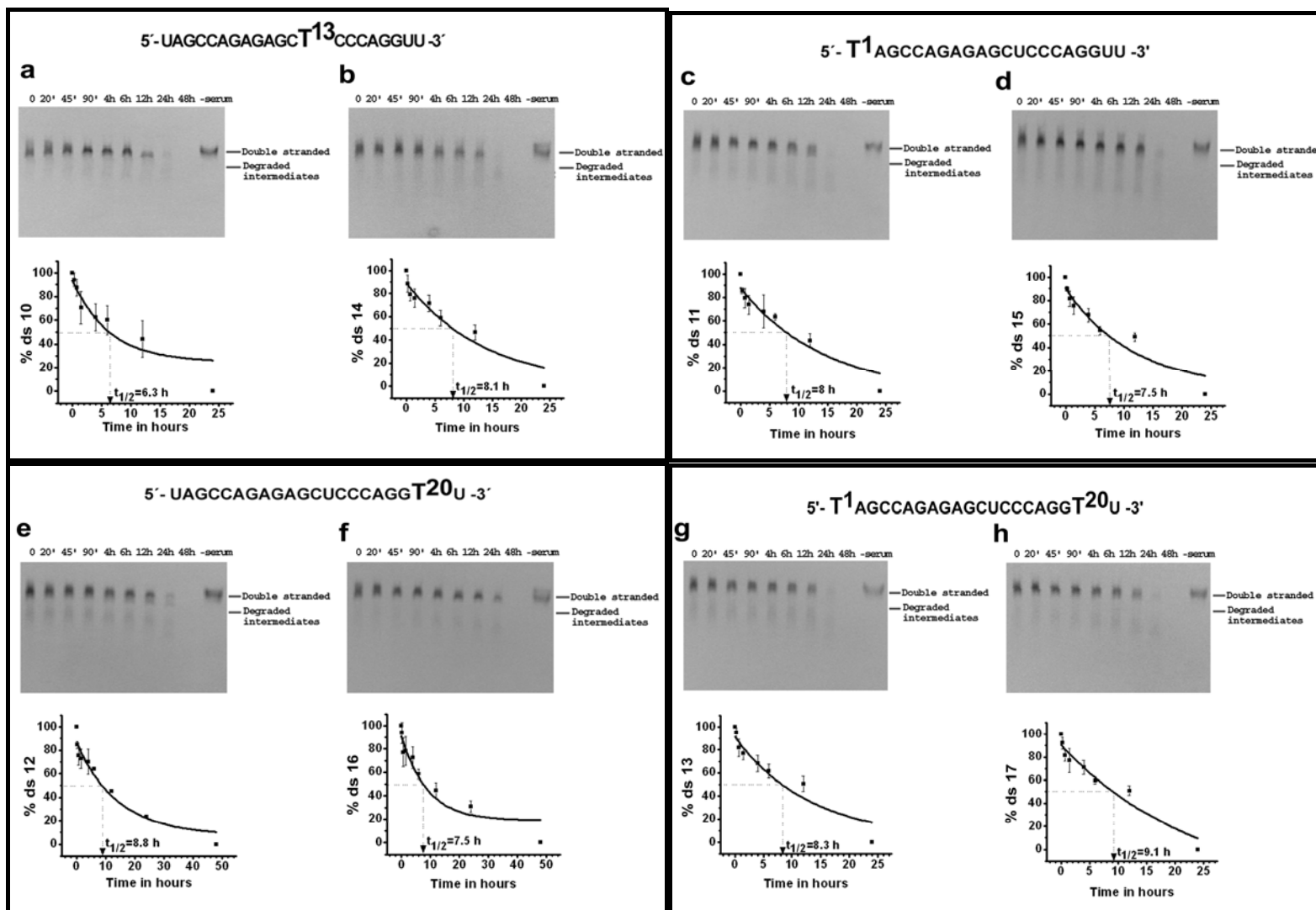


Figure S2. Serum stability of chemically modified double-stranded 6'(R)-O-Tol-jcLNA (**10-13**) and 6'(S)-O-Tol-jcLNA (**14-17**) containing siRNAs targeting HIV1 TAR1 at different time points. Left panels represent 6'(R), 7'(S) diastereomer **IVa** modified siRNAs **10-13**, and right panels represent 6'(S), 7'(S) diastereomer **IVb** modified siRNAs **14-17** respectively. Panels are marked as following: **a**: siRNA **10**; **b**: siRNA **14**; **c**: siRNA **11**; **d**: siRNA **15**; **e**: siRNA **12**; **f**: siRNA **16**; **g**: siRNA **13** and **h**: siRNA **17**. The sequence for a particular isomeric pair is represented above the gels with the position(s) of modification highlighted. Respective best fit exponential decay curve of the double stranded form showing the $t_{1/2}$ value is represented below the gels. Gels and curves are representative of at least three experimental repeats. Error bars represent \pm SD from the mean value.

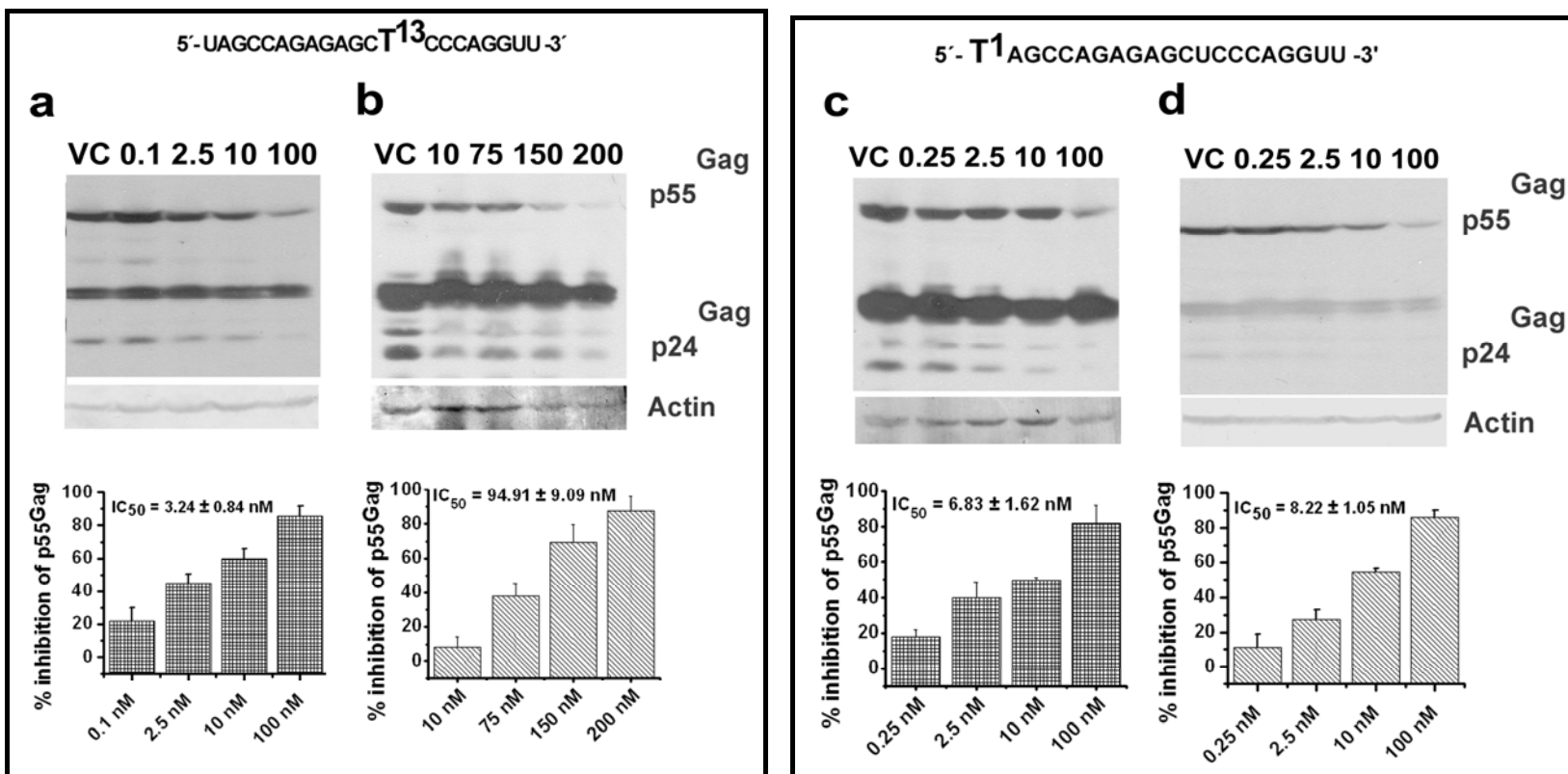


Figure S3

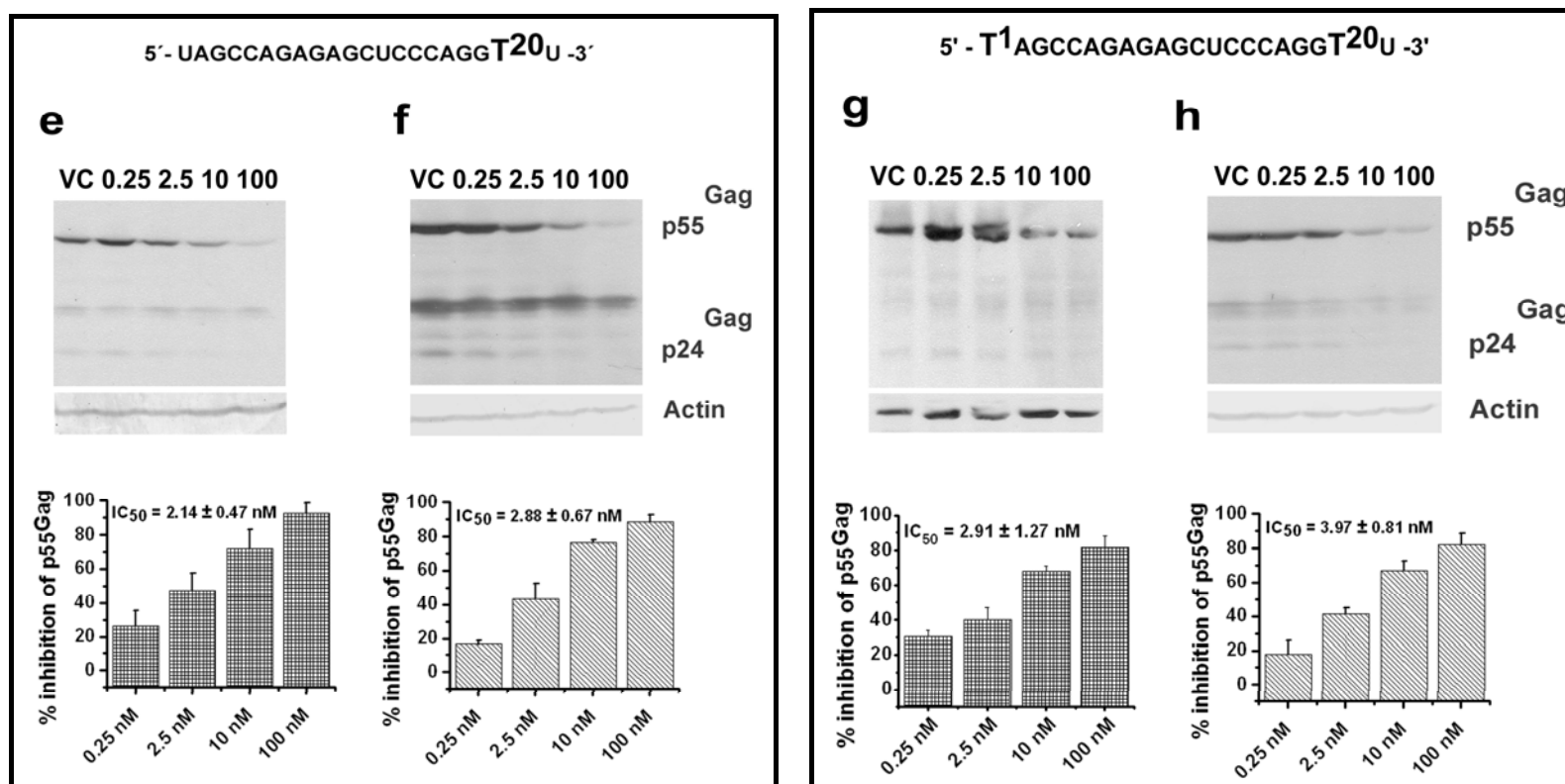


Figure S3: Dose response studies using Western blot for [C6'-(R/S)-O-Tol/7'S]-jcLNA modified siRNAs targeting HIV-1 TAR region. Left panels represent C6'-(R)-O-Tol isomer and the right panels represent C6'-(S)-O-Tol isomer. a: siRNA 10, b: siRNA 14, c: siRNA 11, d: siRNA 15, e: siRNA 12, f: siRNA 16, g: siRNA 13, h: siRNA 17. The sequence for a particular isomer pair is represented above the blot with the position(s) of modification highlighted. Concentrations in nM are indicated above the gels. Results are cumulative of at least three independent experiments. Error bars represent ±SD from mean. Western blot was performed with 40 ug of total protein isolated from cells 48 h post co-transfection of pNL4-3 and varying doses of siRNA. The blots were probed with HIV-1 α -p24 and α -Actin antibodies. Band intensities of p55^{Gag} were calculated and normalized with that of actin intensity for each sample using ImageJ software. % inhibition was calculated from the normalized intensity over virus control (VC).

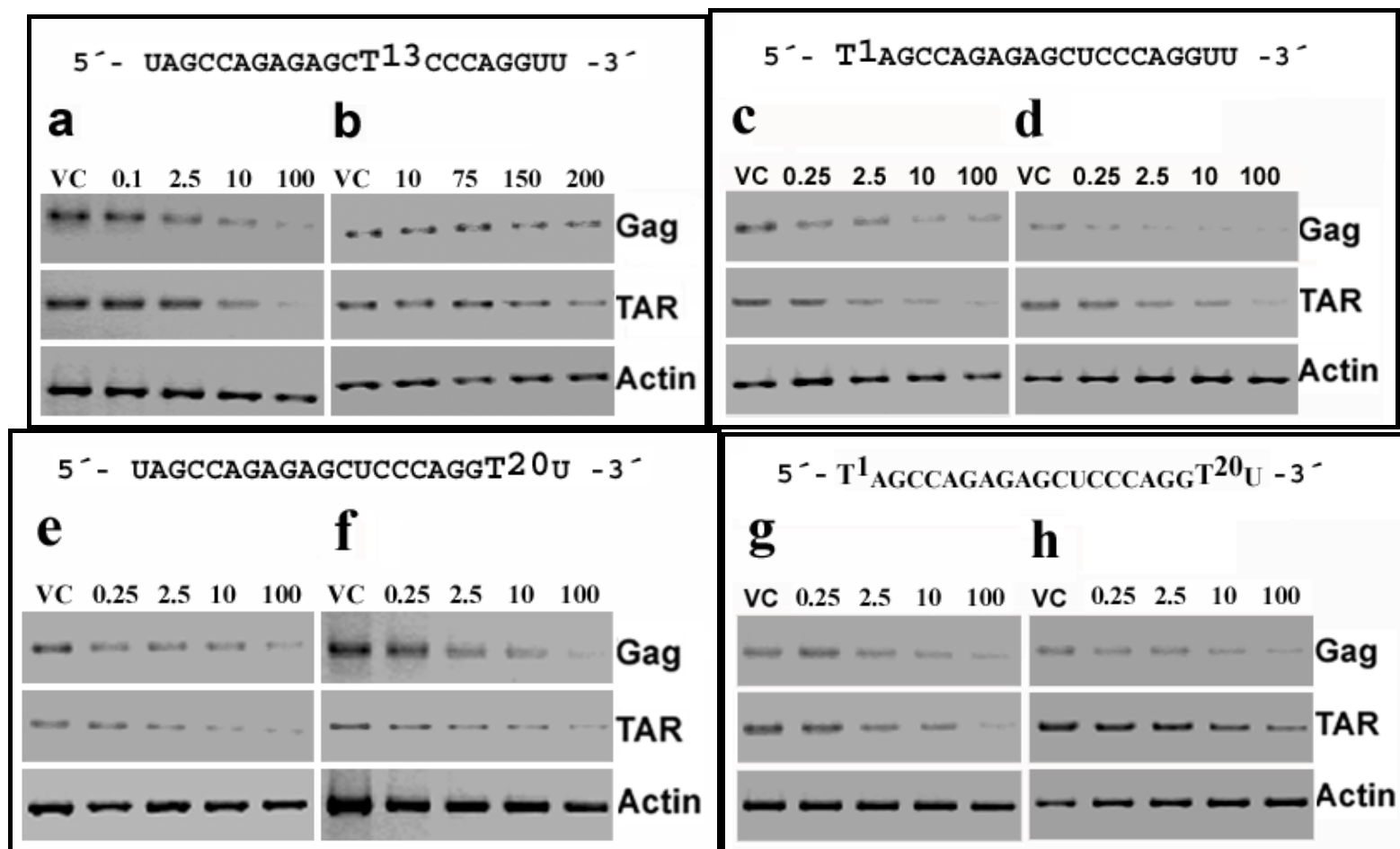


Figure S4: Dose response studies using RTPCR for [C6'-(*R/S*)-OTol/7'S]-jcLNA modified siRNAs targeting HIV-1 TAR region. Left panels represent C6'-(*R*)-*O*-Tol isomer and the right panels represent C6'-(*S*)-*O*-Tol isomer. a: siRNA 10, b: siRNA 14, c: siRNA 11, d: siRNA 15, e: siRNA 12, f: siRNA 16, g: siRNA 13, h: siRNA 17. The sequence for a particular C6'-achiral-jcLNA/LNA pair is represented above the gels with the position(s) of modification highlighted. Concentrations in nM are indicated above the gels. Gels are representative of at least two independent experiments. RTPCR was performed with 1 μ g of RNA isolated from cells 48 h post co-transfection of pNL4-3 and varying doses of siRNA. The PCR was performed with HIV-1 Gag and TAR along with human actin specific primers.

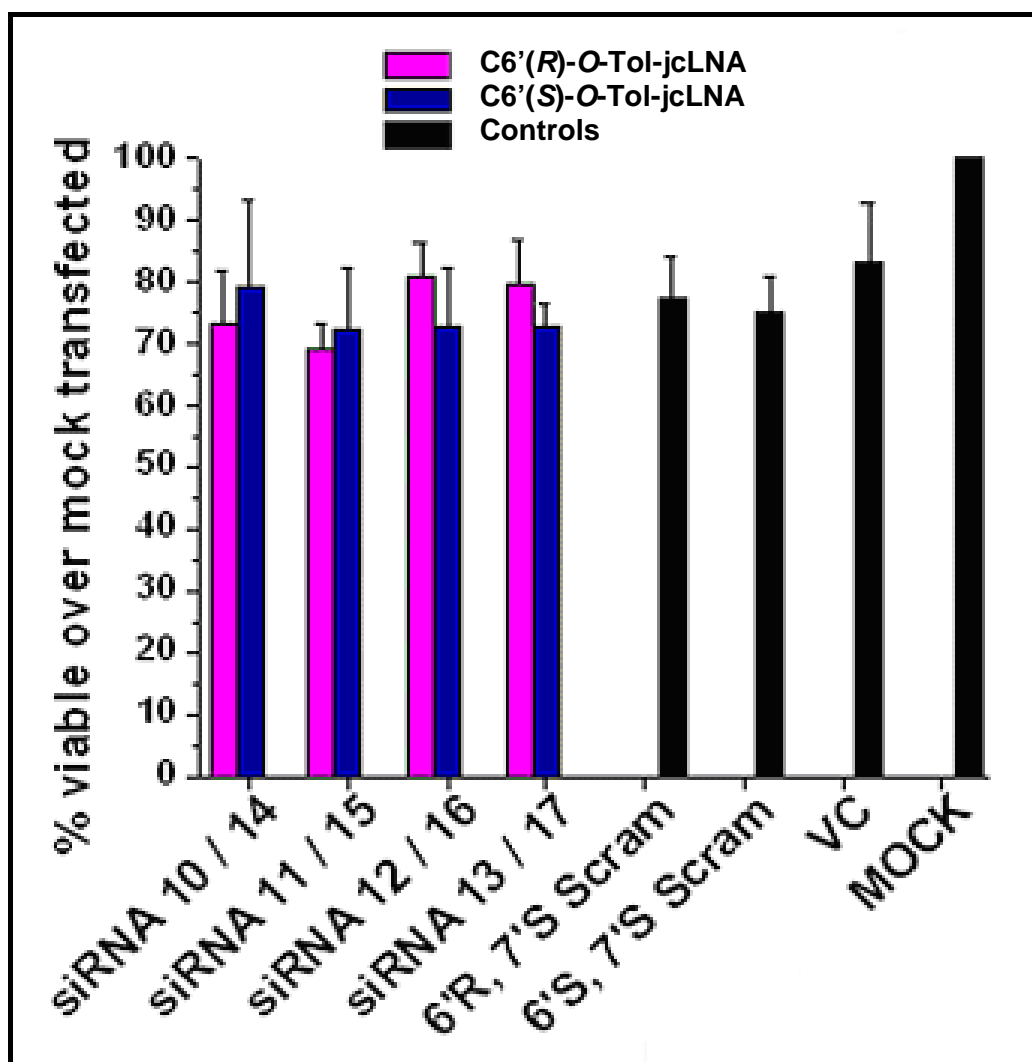


Figure S5. Cell viability assay (MTT assay) for [C6'-(R/S)-O-Tol/7'S]-jcLNA modified siRNAs. 48 h post transfection 10^4 cells were subjected to MTT assay in a 96 well plate. Cells having the highest dose of each siRNA (100 nM) were used. Error bars represent \pm SD from the mean values. Viability was measured over that of mock transfected cells. Results are cumulative of at least three independent experiments.

Table S1. Atomic charges, names and types of the C6'-(R)-O-Tol-jcLNA and C6'-(S)-O-Tol-jcLNA nucleotides used as parameters of the MM minimization. The charges have been obtained from the 6-31G** *ab initio* geometry optimizations performed using GAUSSIAN 03⁵ and converted employing two-stage Resp fitting as implemented in the Antechamber set of auxiliary programs of Amber10¹.

C6'-(R)-O-To-jcLNA			C6'-(S)-O-To-jcLNA		
<i>Atom name</i>	<i>Atom type</i>	<i>Atom name</i>	<i>Atom type</i>	<i>Atom name</i>	<i>Atom type</i>
"P"	P	1.1659	"P"	P	1.1659
"O1P"	O2	-0.7761	O1P	O2	-0.7761
"O2P"	O2	-0.7761	O2P	O2	-0.7761
"O5*"	OS	-0.3895	O5'	OS	-0.3895
"C5*"	CT	0.0222	C5'	CT	0.0431
"H5*1"	H1	0.2304	H5'1	H1	0.1783
"H5*2"	H1	0.1437	H5'2	H1	0.1584
"C4*"	CT	0.1923	C4'	CT	0.2149
"CH4"	CT	0.2248	CH4	CT	0.2043
"H66"	H1	0.1935	H66	H1	0.2109
"O4*"	OS	-0.6977	O4'	OS	-0.6934
"C1*"	CT	0.4373	C1'	CT	0.4371
"H1*"	H2	0.2109	H1'	H2	0.2448
"N1"	N*	-0.8283	N1	N*	-0.8276
"C6"	CM	0.1288	C6	CM	0.1321
"H6"	H4	0.3442	H6	H4	0.3341
"C5"	CM	-0.2188	C5	CM	-0.2177
"C5M"	CT	-0.5066	C5M	CT	-0.5010
"H5M1"	HC	0.1710	H5M1	HC	0.1737
"H5M2"	HC	0.1497	H5M2	HC	0.1528
"H5M3"	HC	0.2620	H5M3	HC	0.2468
"C4"	C	0.8260	C4	C	0.8268
"O4"	O	-0.6191	O4	O	0.6209
"N3"	NA	-0.9377	N3	NA	-0.9383
"H3"	H	0.4065	H3	H	0.4064
"C2"	C	1.0767	C2	C	1.0764
"O2"	O	-0.6574	O2	O	0.6515
"C3*"	CT	0.1928	C3'	CT	0.1516
"H3*"	H1	0.2089	H3'	H1	0.2147
"C2*"	CT	-0.2501	C2'	CT	-0.2454
"H2*1"	HC	0.2101	H2'1	HC	0.2075
"C2H"	CT	-0.2069	C2H	CT	-0.2077
"H7"	HC	0.2216	H7	HC	0.2100
"O3*"	OS	-0.7242	O3'	OS	-0.7354
"C8M"	CT	-0.4743	C8M	CT	-0.4823
"H8M1"	HC	0.1574	H8M1	HC	0.2111
"H8M2"	HC	0.1690	H8M2	HC	0.1547
"H8M3"	HC	0.1557	H8M3	HC	0.1459
"O6R"	OS	-0.7074	O6S	OS	-0.7078
"C6O"	C	0.8597	C6O	C	0.8917
"O6O"	O	-0.5499	O6O	O	-0.5891
"C1"	CA	-0.1637	C1	CA	-0.1639

"C21"	CA	-0.1692	C21	CA	-0.1675
"HC21"	HA	0.2550	HC21	HA	0.2447
"C31"	CA	-0.2357	C31	CA	-0.2369
"HC31"	HA	0.1936	HC31	HA	0.1919
"C32"	CA	-0.2450	C32	CA	-0.2429
"HC32"	HA	0.1897	HC32	HA	0.1938
"C9"	CA	-0.1700	C22	CA	-0.1668
"HC22"	HA	0.2332	HC22	HA	0.2396
"C7"	CA	0.0580	C7	CA	0.0581
"CBM"	CT	-0.5085	CBM	CT	-0.5079
"HBM1"	HC	0.1757	HBM1	HC	0.1715
"HBM2"	HC	0.1762	HBM2	HC	0.1807
"HBM3"	HC	0.1697	HBM3	HC	0.1714

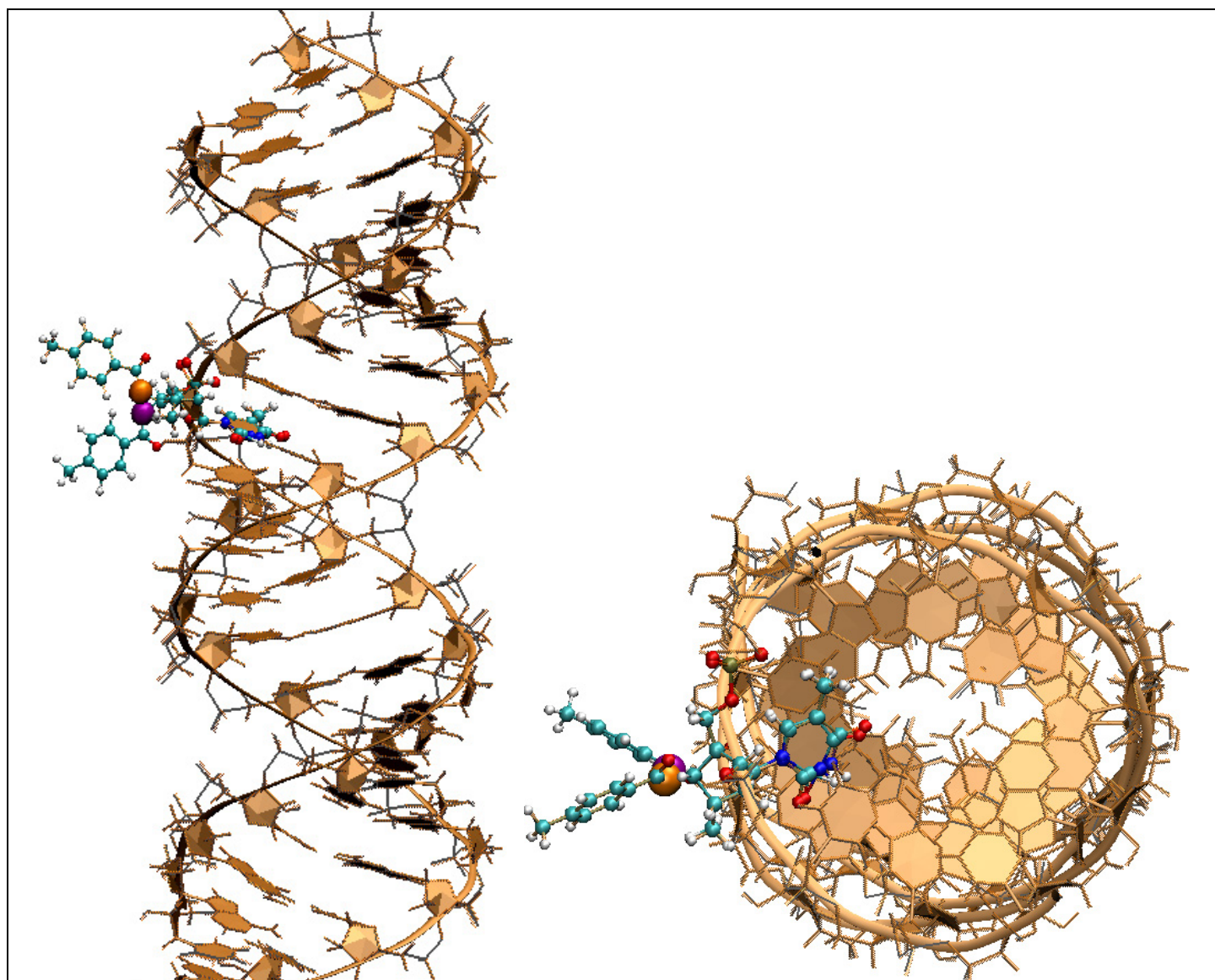


Figure S6. Overlap of molecular structure of the C6'-(R)-O-Tol and C6'-(S)-O-Tol-jcLNA T13 modified-siRNAs duplexes (**10** and **14** in Table 1) with the target RNA (top and side view). It is clearly visible that the C6'-(S)-O-Tol is partially located hindering the entrance to the minor groove of the duplex while C6'-(R)-O-Tol is pointing out (for details of the calculations see the main text). Visualized using VMD.⁶

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1. Case, D. A., *et al.*, *AMBER 7*. University of California: San Francisco, 2002.
2. Pérez, A.; Marchán, I.; Svozil, D.; Sponer, J.; Cheatham III, T. E.; Laughton, C. A.; Orozco, M. *Biophysical Journal* **2007**, 92 (11), 3817-3829.
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6. Humphrey, W.; Dalke, A.; Schulten, K. *Journal of Molecular Graphics* **1996**, 14, 33-38.

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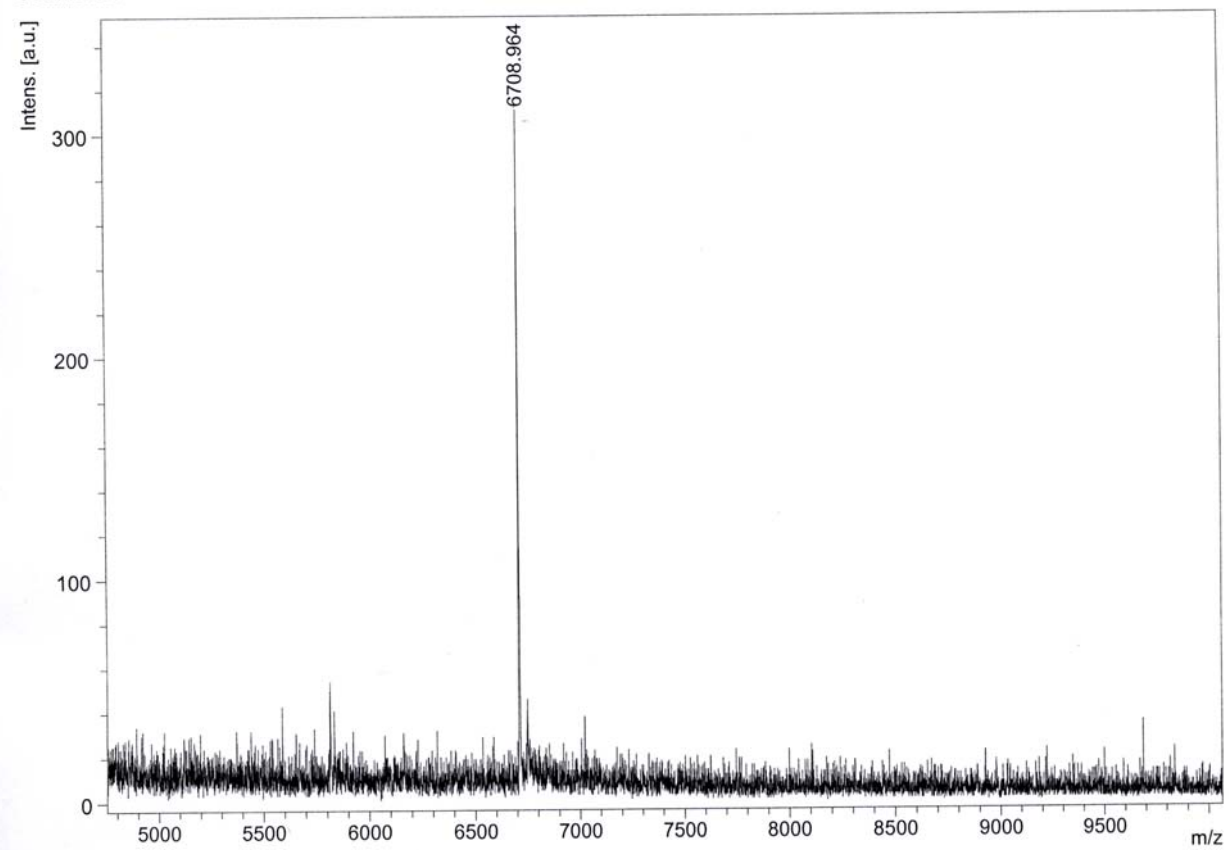


Figure S7. MALDI-MS spectrum for sequence 1, native

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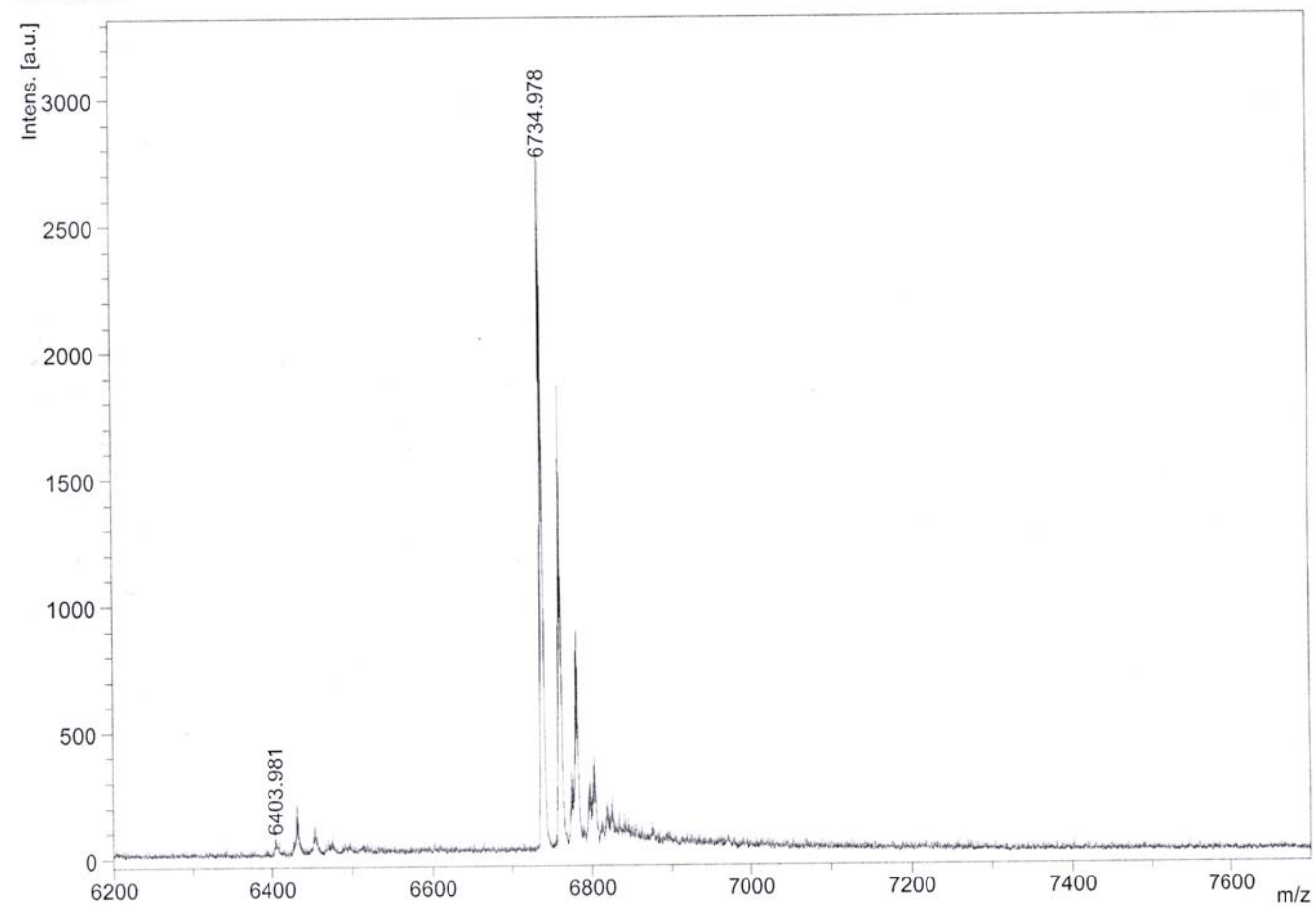


Figure S8: MALDI-MS spectrum for sequence 2, LNA-T modification at T¹³

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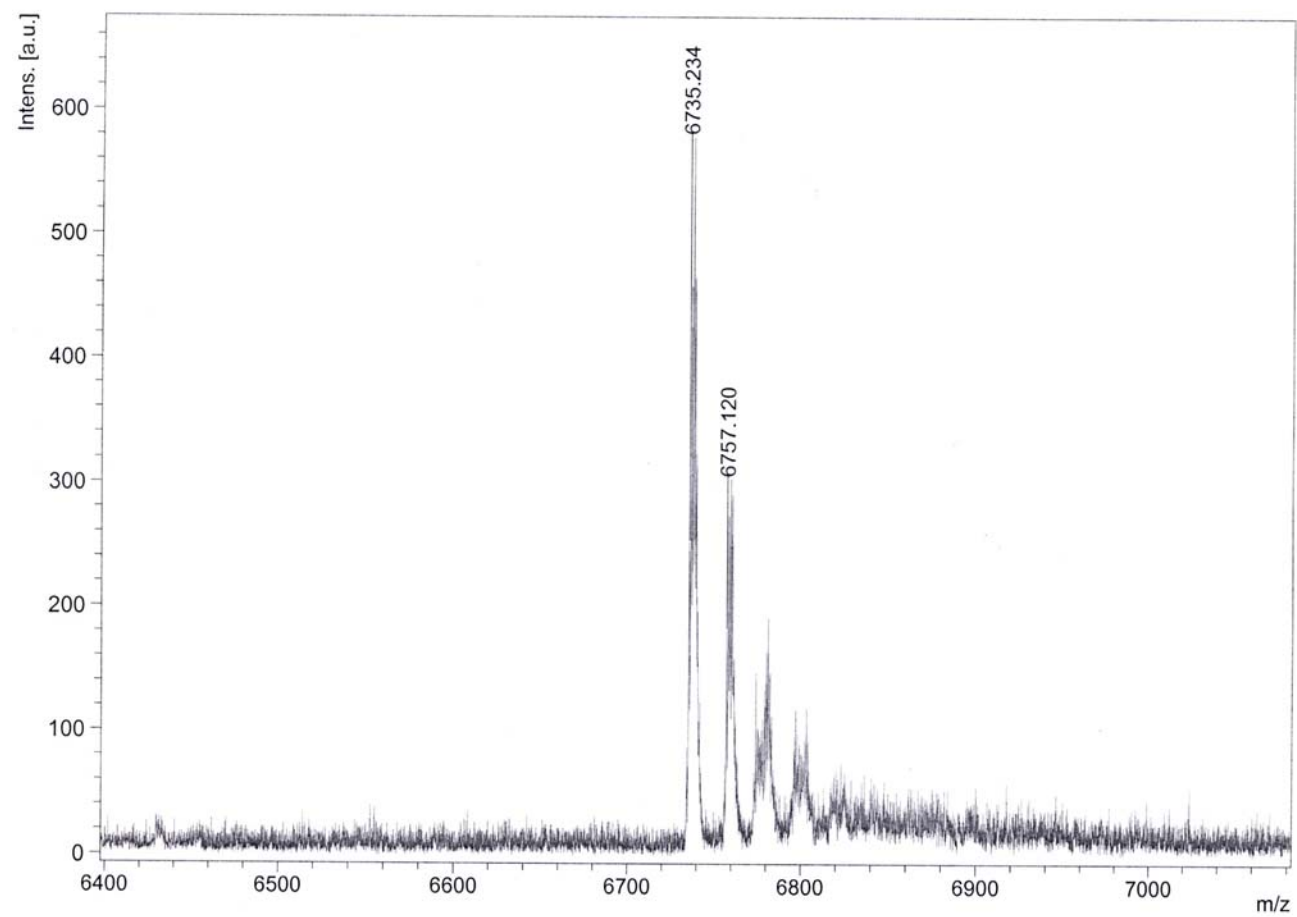


Figure S9: MALDI-MS spectrum for sequence 3, LNA-T modification at T¹

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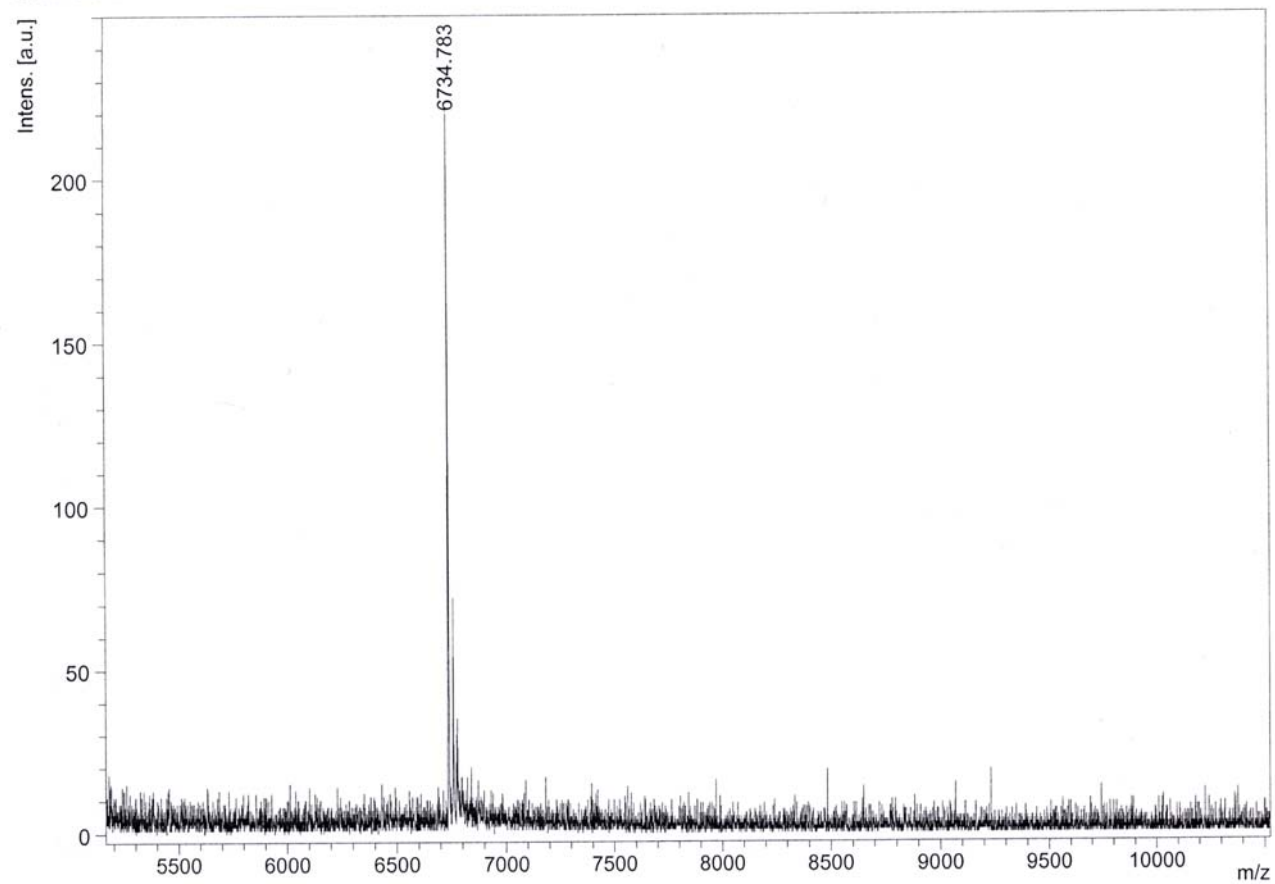


Figure S10: MALDI-MS spectrum for sequence 4, LNA-T modification at T²⁰

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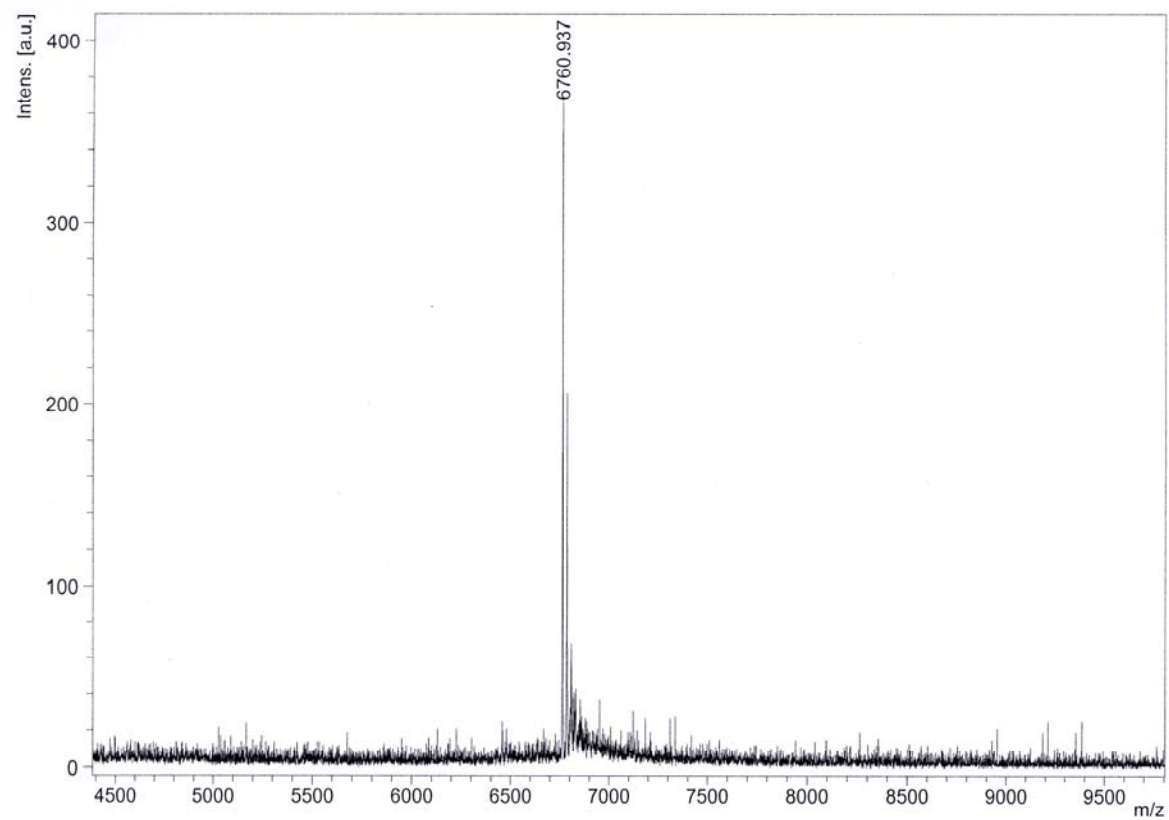


Figure S11: MALDI-MS spectrum for sequence 5, LNA-T modification at T¹+T²⁰

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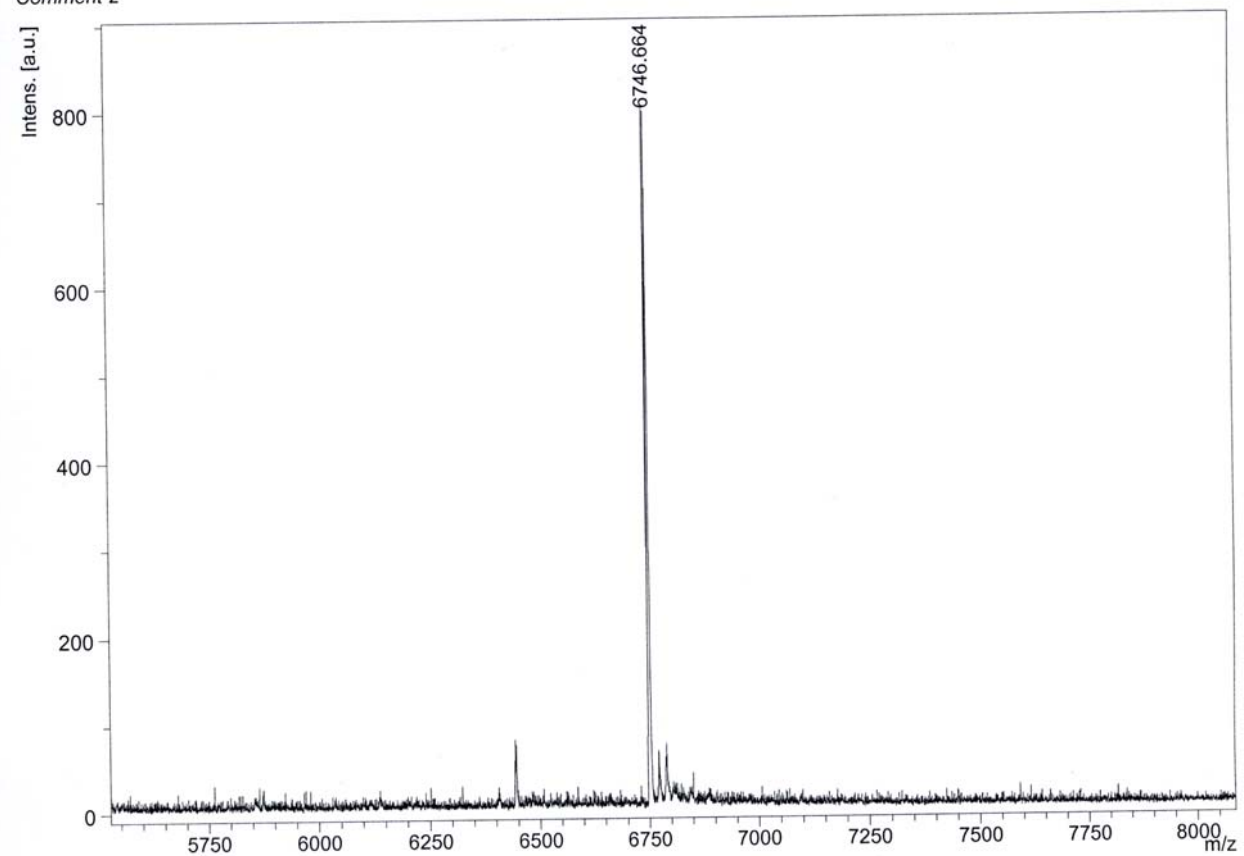


Figure S12: MALDI-MS spectrum for sequence 6, jcLNA-T modification at T¹³

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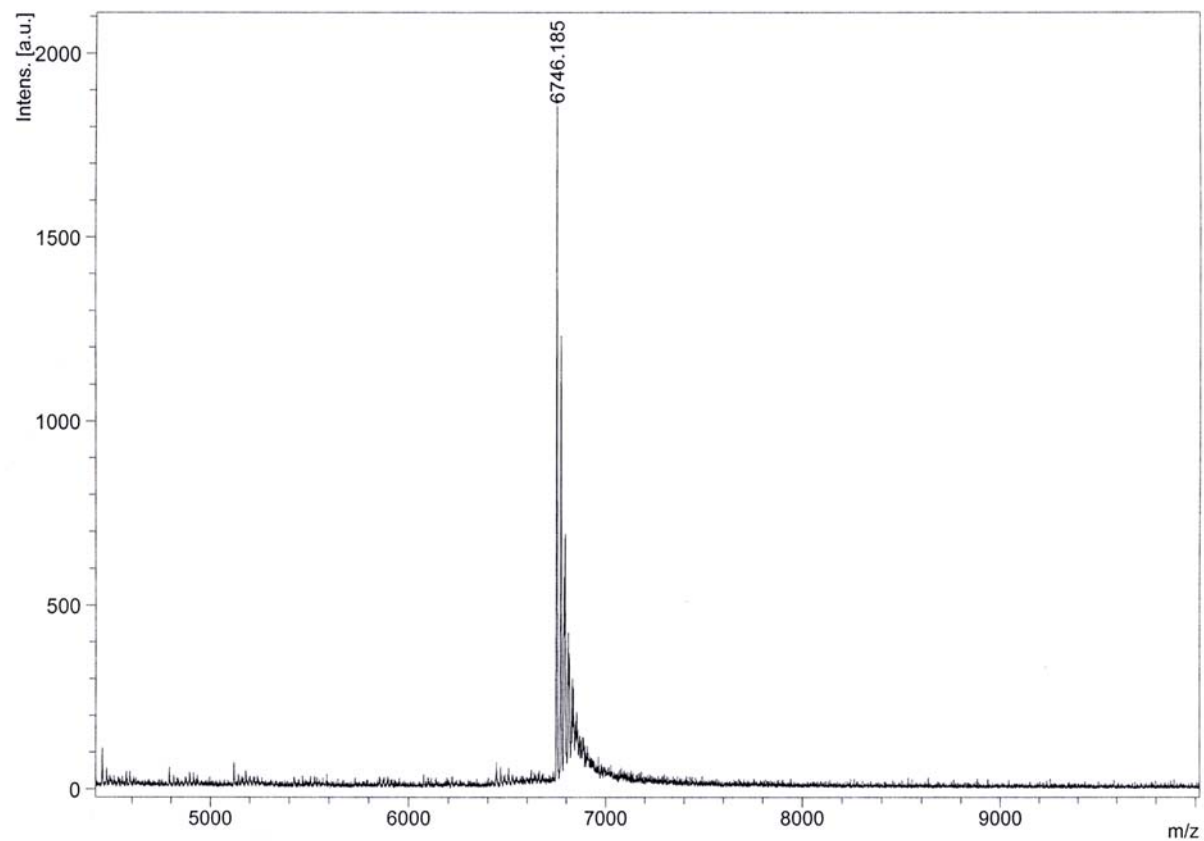


Figure S13: MALDI-MS spectrum for sequence 7, jcLNA-T modification at T¹

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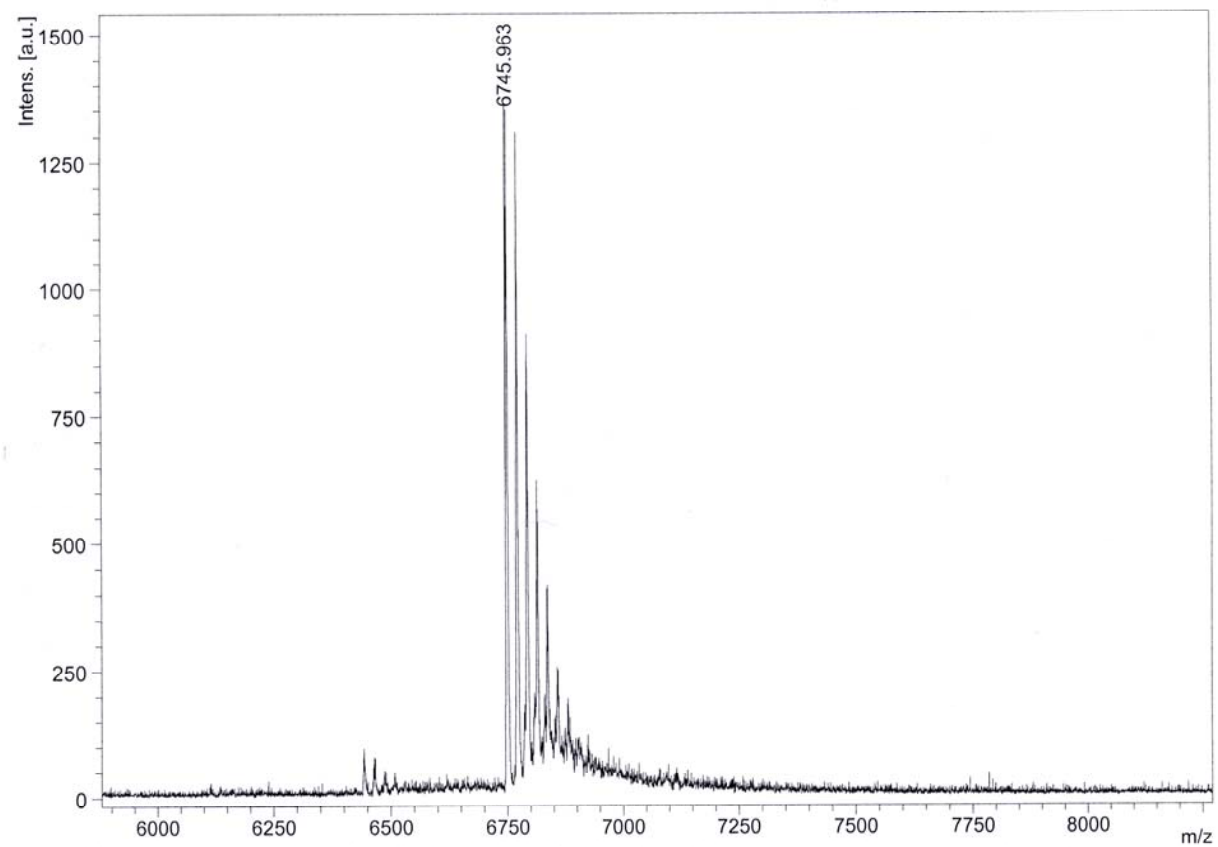


Figure S14: MALDI-MS spectrum for sequence 8, jcLNA-T modification at T²⁰

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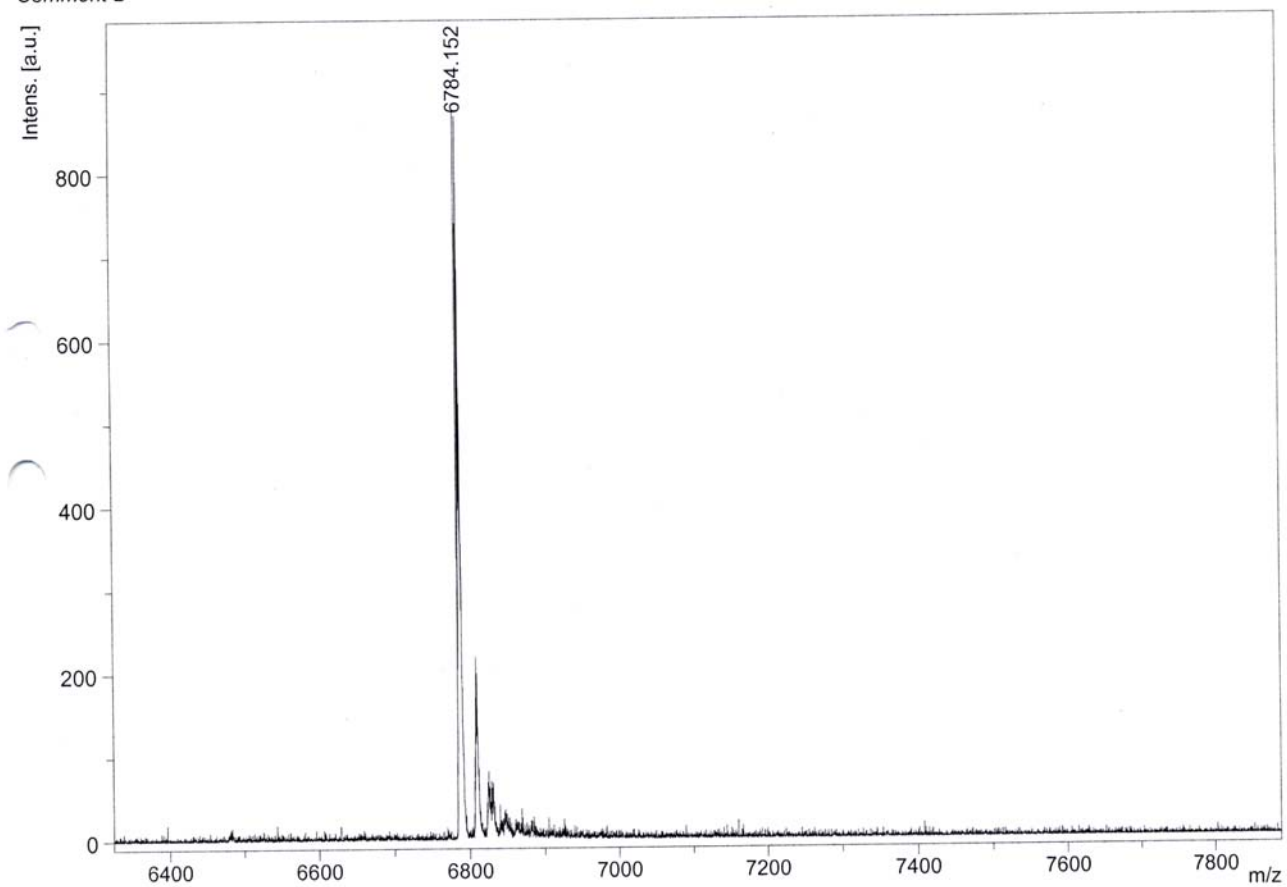


Figure S15: MALDI-MS spectrum for sequence 9, jcLNA modification at T¹+T²⁰

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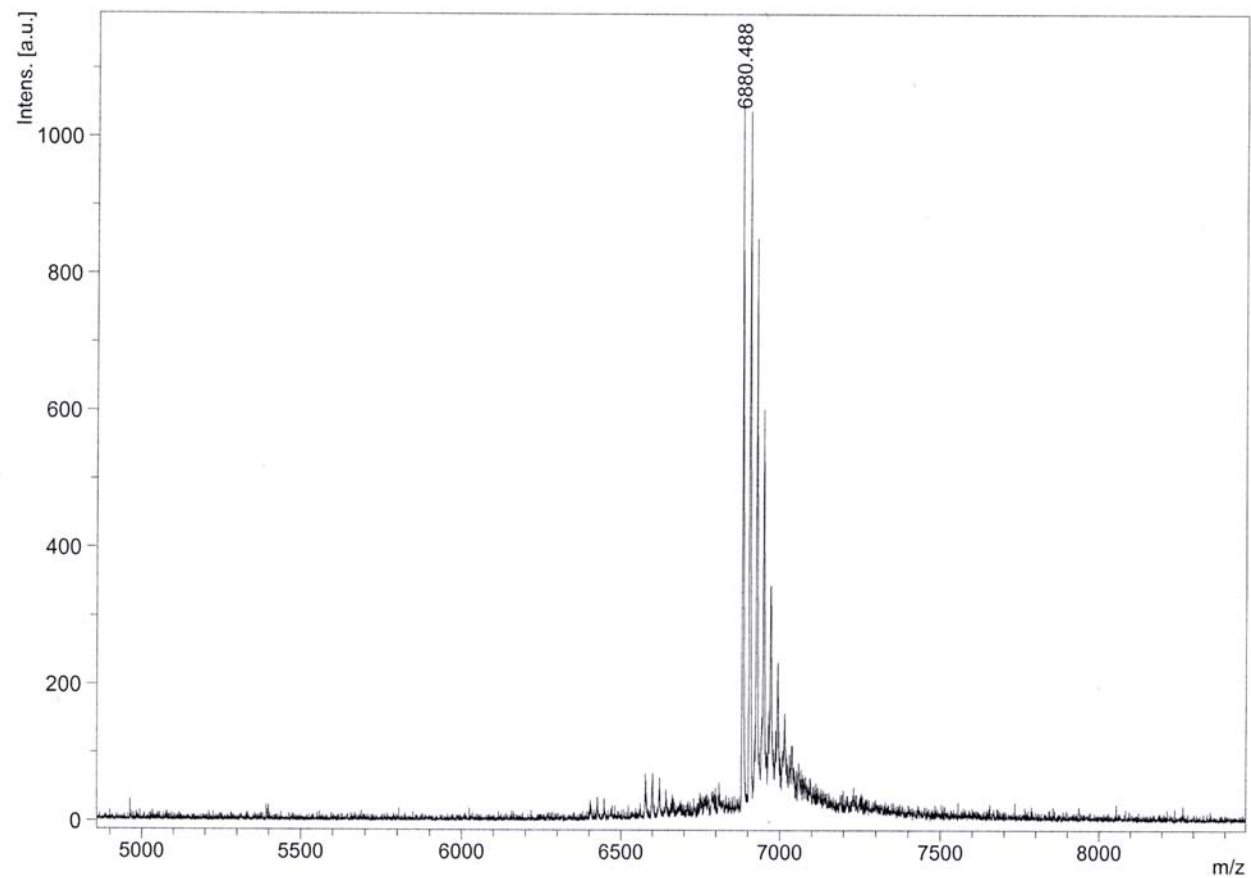


Figure S16: MALDI-MS spectrum for sequence 10, 6'(R) -O-Tol-jcLNA-T modification at T¹³ (6'R, 7'S)

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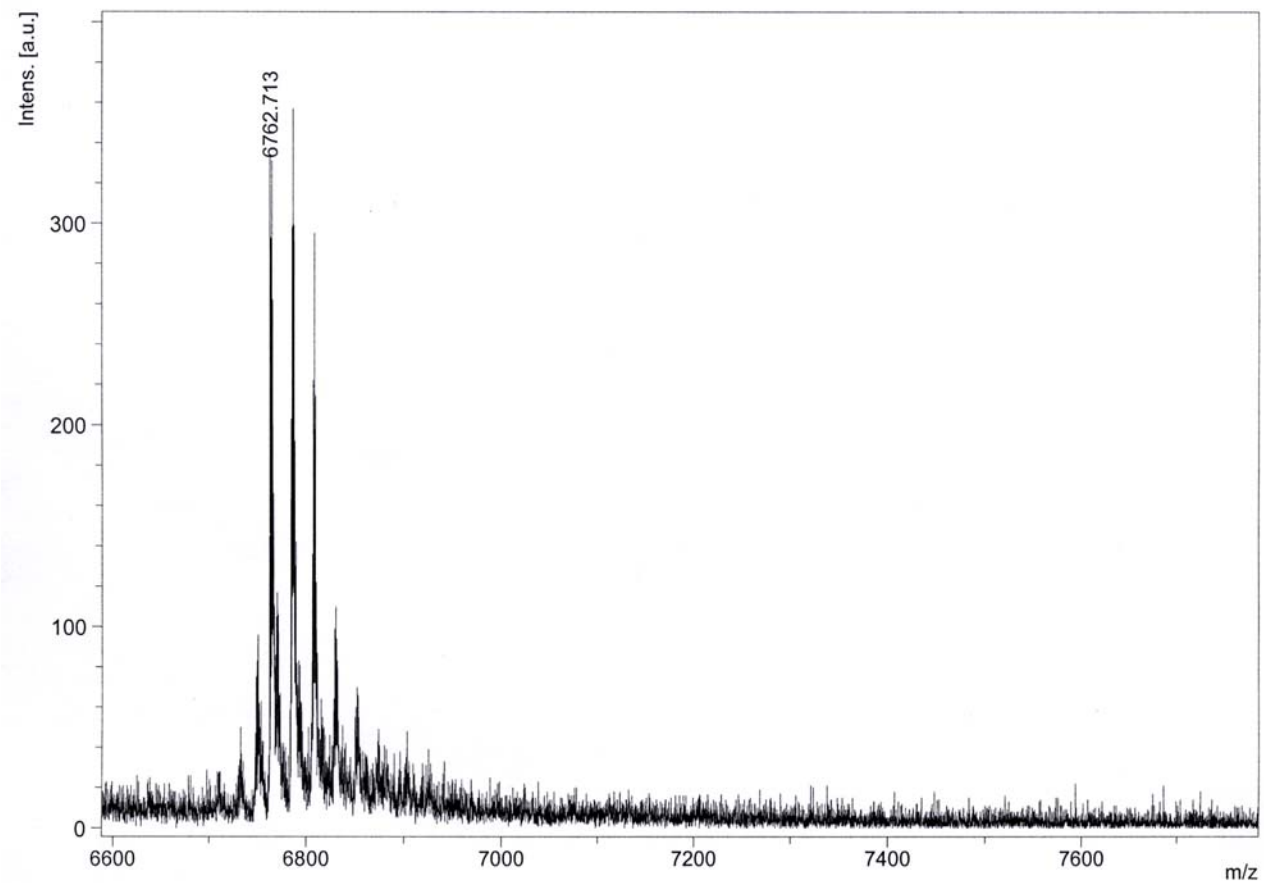


Figure S17: MALDI-MS spectrum for sequence 11, 6'(R)-O-Tol-jcLNA-T modification at T¹ (6'R,7'S)

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Comment 2

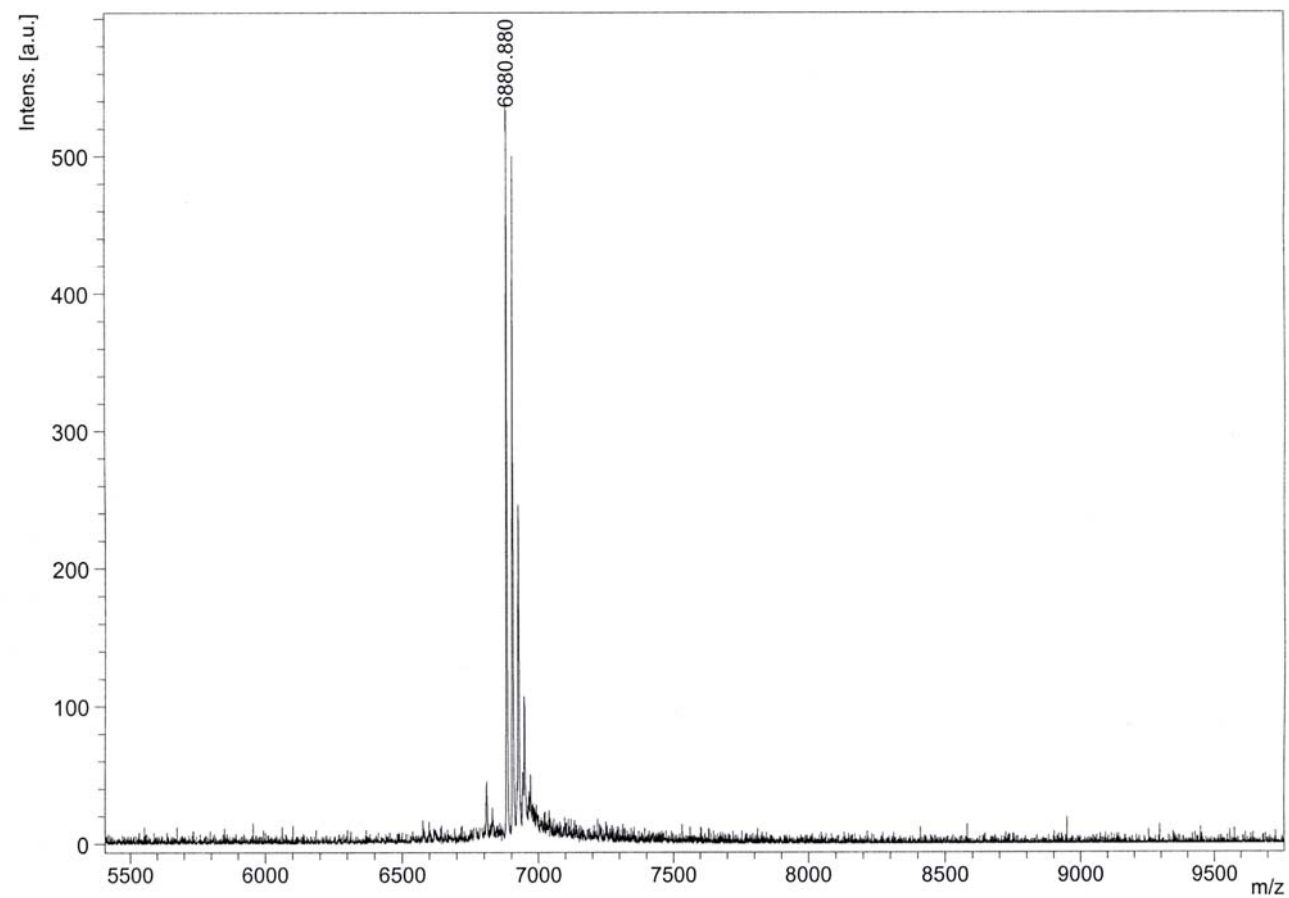


Figure S18: MALDI-MS spectrum for sequence 12, 6'(R)-O-Tol-jcLNA-T modification at T²⁰ (6'R, 7'S)

D:\Data\Ake\110420_joyti_LQ_Ram_antisense\13_50ul\0_L2\1

Comment 1

Comment 2

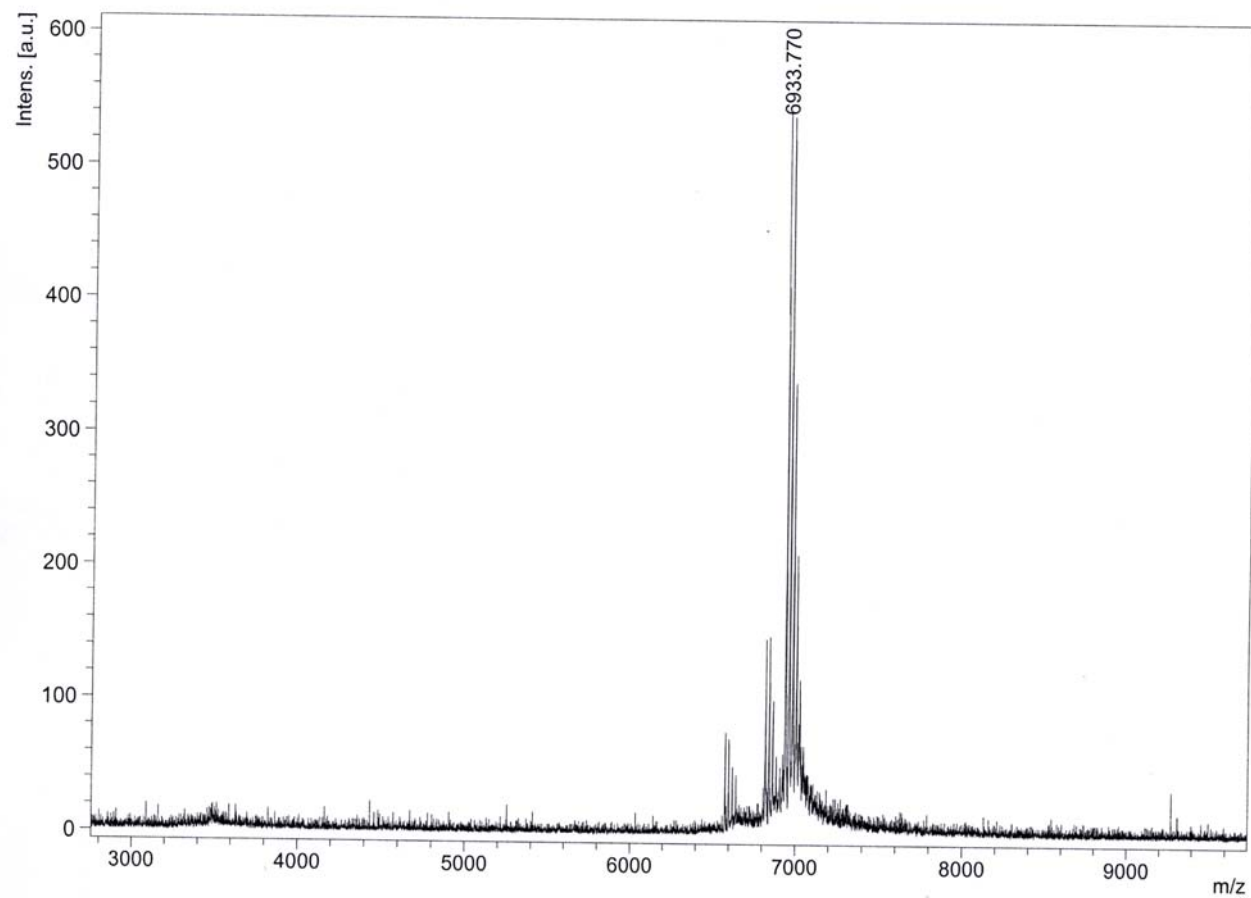


Figure S19: MALDI-MS spectrum for sequence 13, 6' (*R*) -*O*-Tol-jcLNA-T modification at T¹+T²⁰ (6'*R*,7'*S*)

D:\Data\Ake\110420_joyti_LQ_Ram_antisense\no14_50ul\0_L3\1

Comment 1

Comment 2

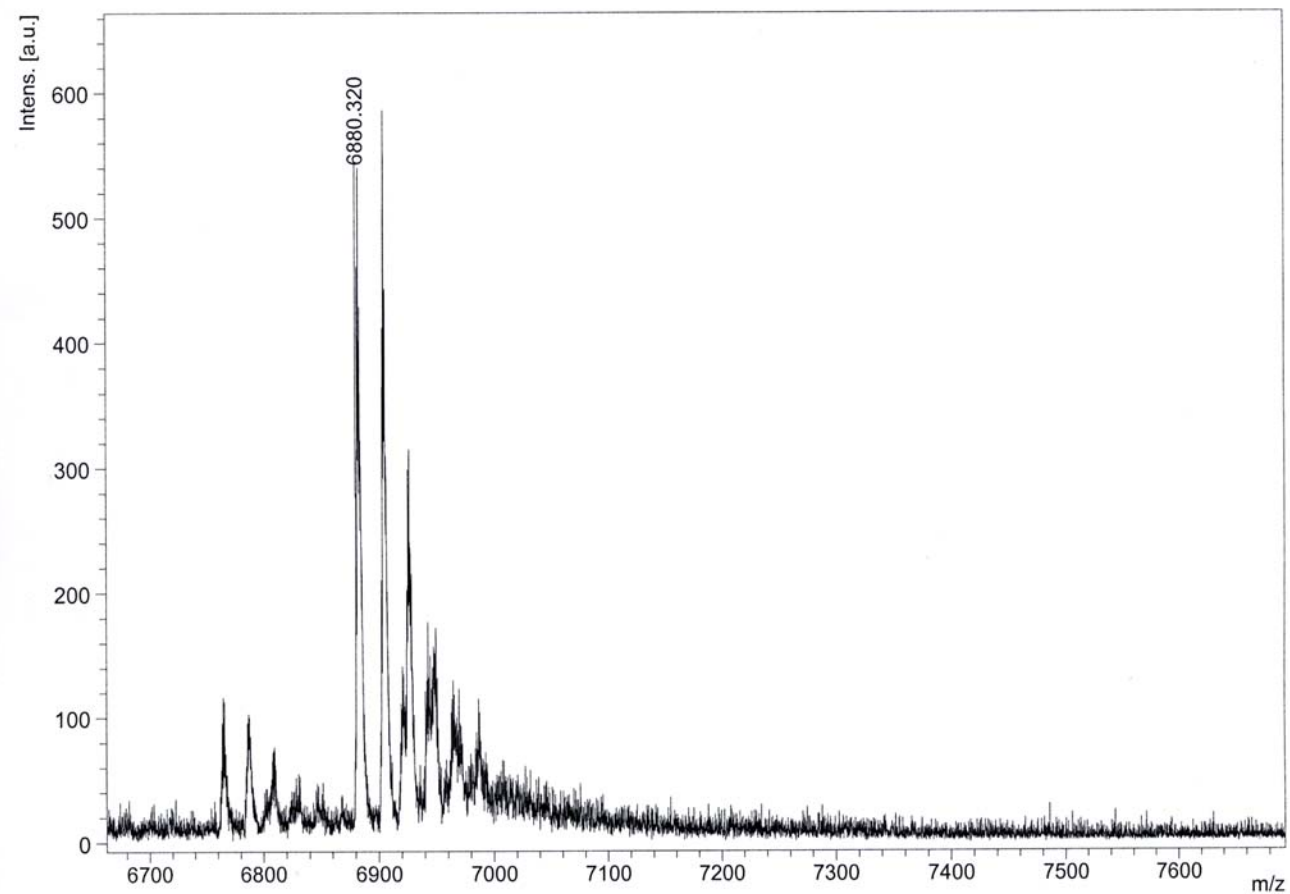


Figure S20: MALDI-MS spectrum for sequence 14, 6'(*S*)-*O*-Tol-jcLNA-T modification at T¹³ (6'*S*,7'*S*)

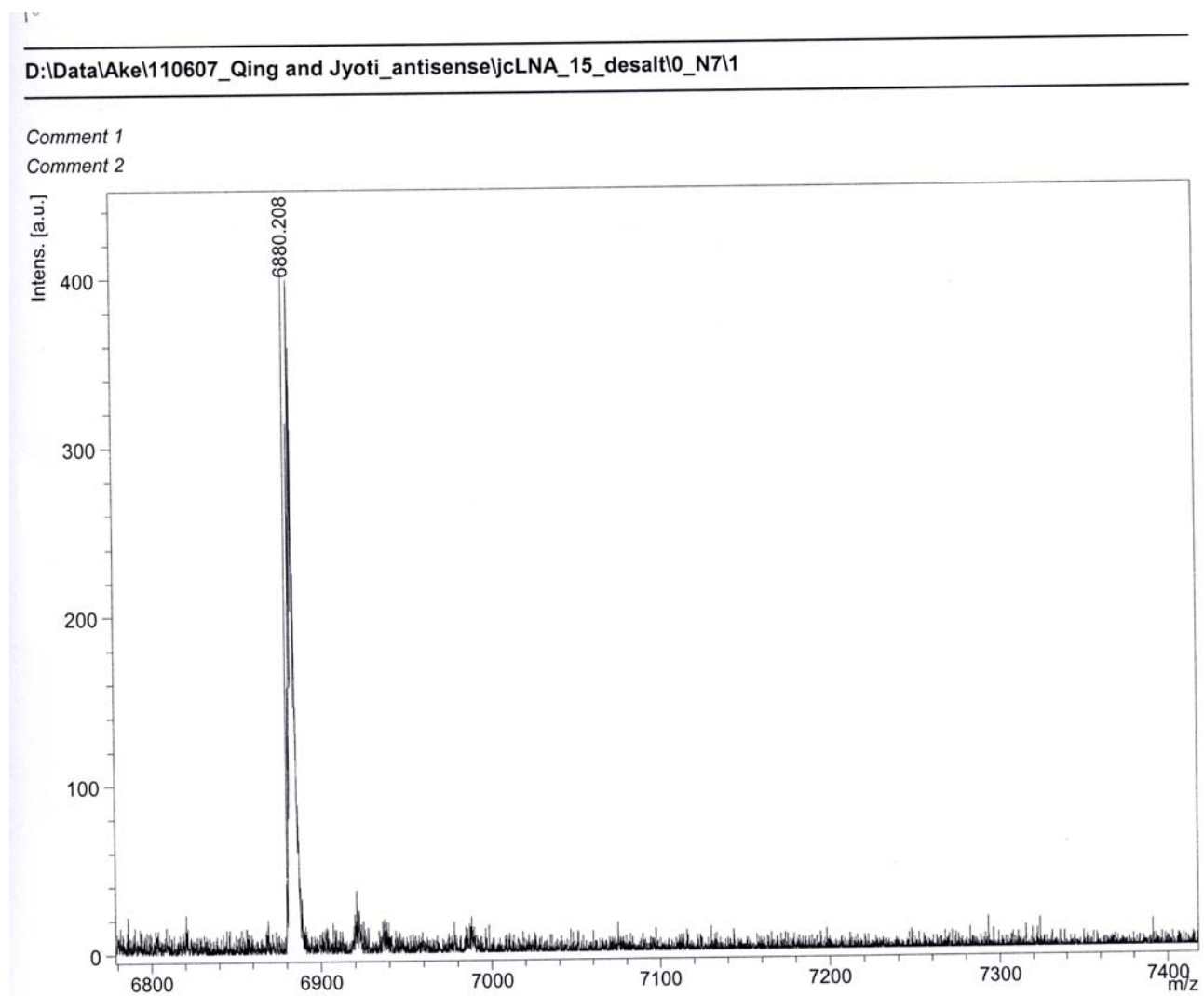


Figure S21: MALDI-MS spectrum for sequence 15, 6'(S)-O-Tol-jcLNA-T modification at T¹ (6'S,7'S)

D:\Data\Ake\110607_Qing and Jyoti_antisense\jcLNA16_desalt\0_D9\1

Comment 1

Comment 2

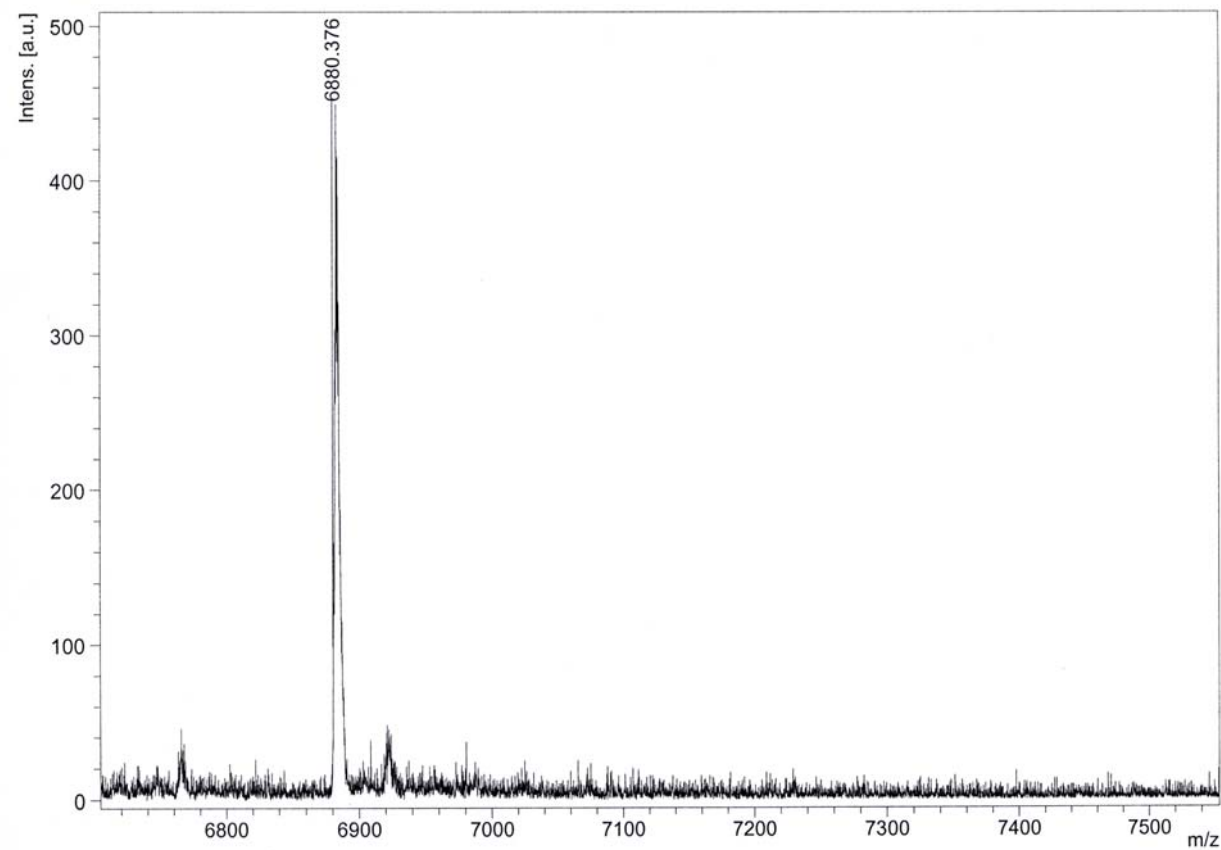


Figure S22: MALDI-MS spectrum for sequence 16, 6'(R)-O-Tol-jcLNA-T modification at T²⁰ (6'S,7'S)

D:\Data\Ake\110607_Qing and Jyoti_antisense\jcLNA17_desalt\0_D10\1

Comment 1

Comment 2

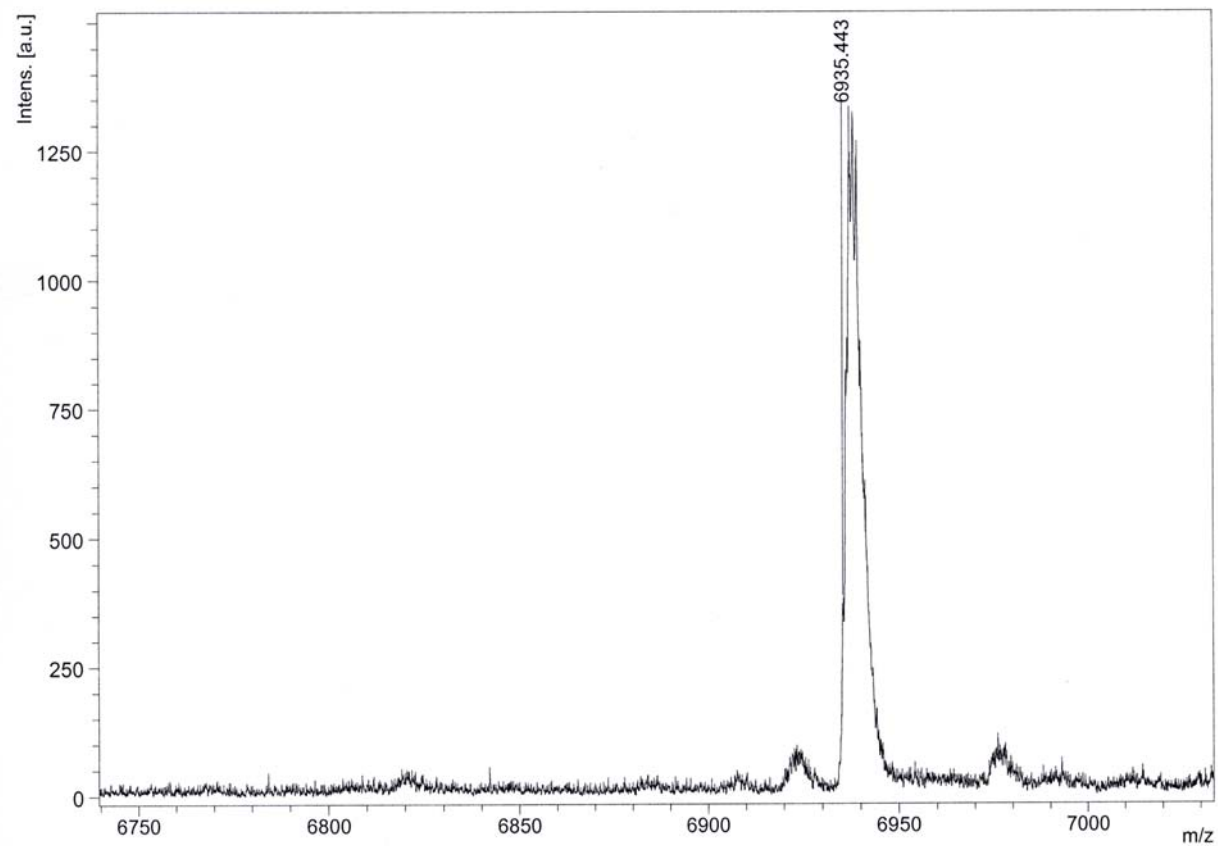


Figure S23: MALDI-MS spectrum for sequence 17, 6'(S)-O-Tol-jcLNA-T modification at T¹+T²⁰ (6'S,7'S)