Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

A Solid-phase Method for Peptide-siRNA Covalent Conjugates Based on Click Chemistry

Supporting Information

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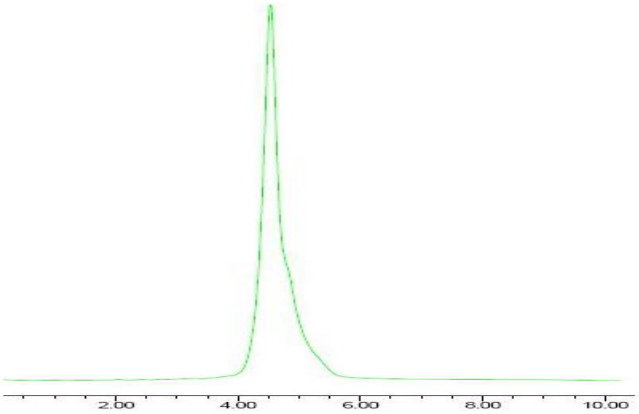
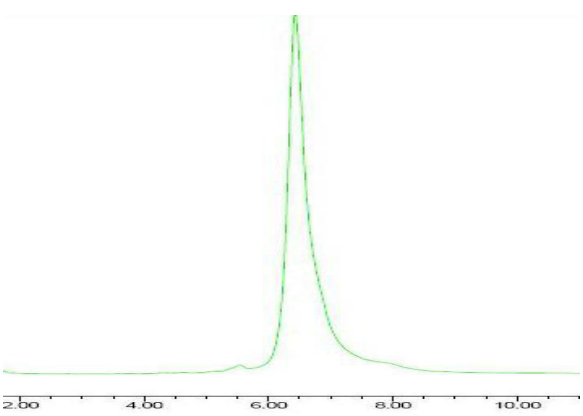
General method for HPLC analysis and HPLC traces

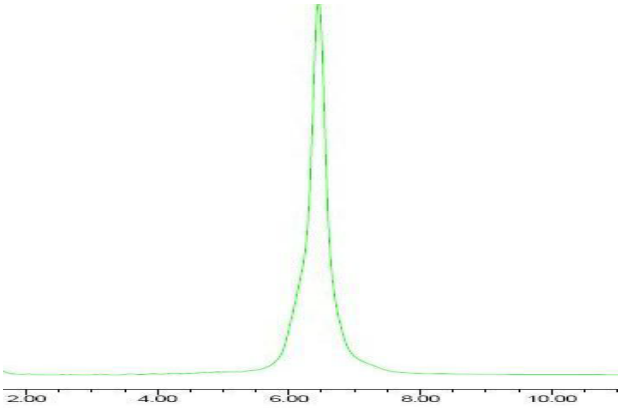
The final molecules were analyzed by HPLC using Oligo-WAX column (Phenomenex, 300Å, 10 µm, 100 × 4.6 mm). The gradient was 0-50% B in 10min with 2.2 ml/min flow, the mobile phase were the same as above. The desired conjugates prepared as described were >95% purity following HPLC analysis.



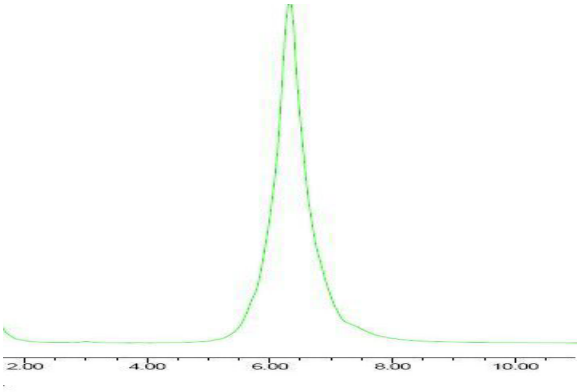
a (5'-dT$_9$dU-3'-LALLAK)



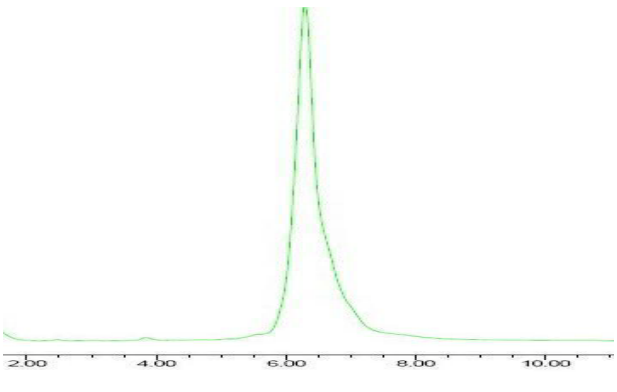
b (5'-UCG GGA AAU UUC UCU AUU AUdU-3'-LALLAK)



c (5'-UAA UAG AGA AAU UUC CCG AUdU-3'-LALLAK)



d (5'-UAA UAG AGA AAU UUC CCG AUU-3')



e (5'-UCG GGA AAU UUC UCU AUU AUU-3')

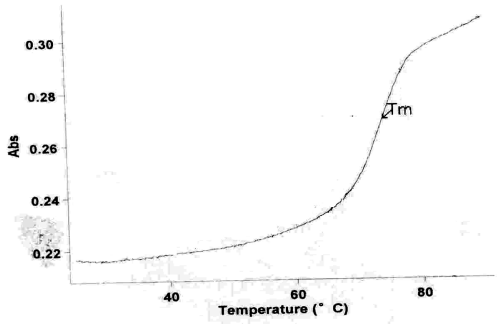
Fig. 1 HPLC analysis of oligonucleotide products (UV absorbance at 260 nm vs time).

Duplexes annealing

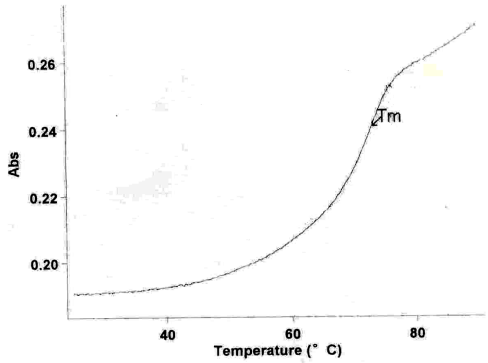
The concentrations of single sense (or peptide conjugated) strand and antisense (or peptide conjugated) strand were determined by NanoDrop 2000 (Thermo Fisher Scientific). Two complementary single strands were mixed in DEPC water at equimolar concentrations, to give a final siRNA concentration of 1μM. Annealing was performed by heating to 95°C for 5min on a Peltier element thermal cycler block followed by slow cooling over a period of 1h.

Melting temperature determinations

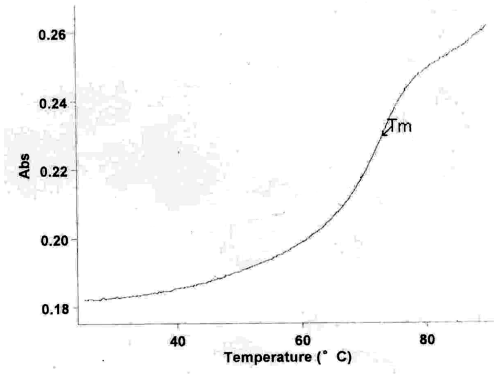
Normal and peptide-siRNAs **I-III** were annealed by **e/d**, **b/d** and **e/c** separately. Melting temperatures were determined using UV-spectroscopy (Cary300 Scan) measuring the absorbance at 260 nm of siRNA (1μM of each annealed siRNA in 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, pH=7.4) every 0.25 °C between 25 and 90 °C, using a gradient of 0.5 °C/min and this was repeated at least two times.



a



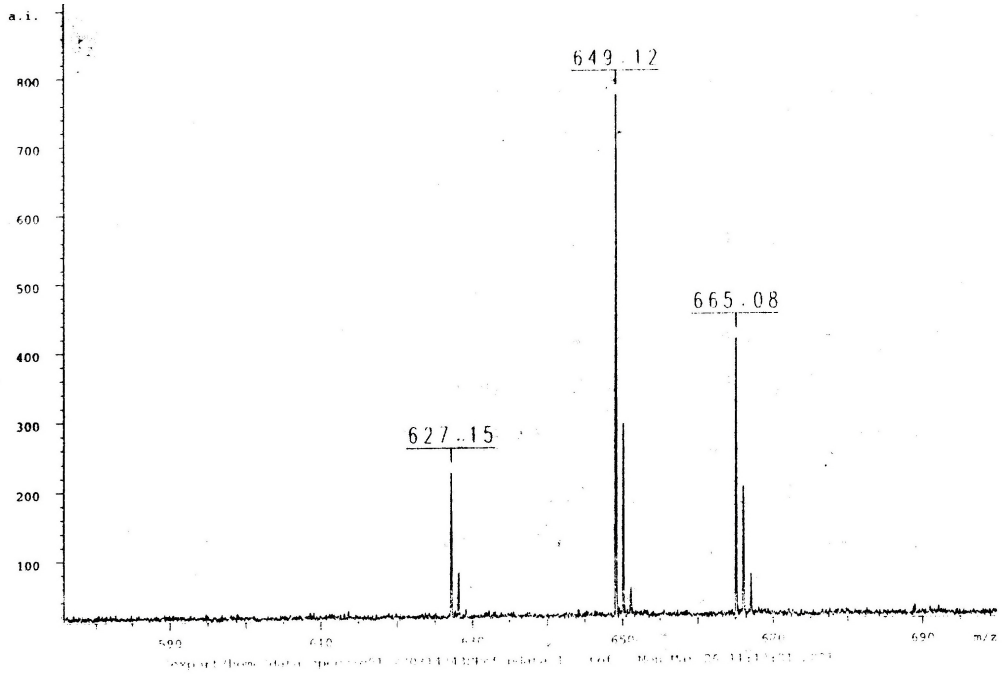
b



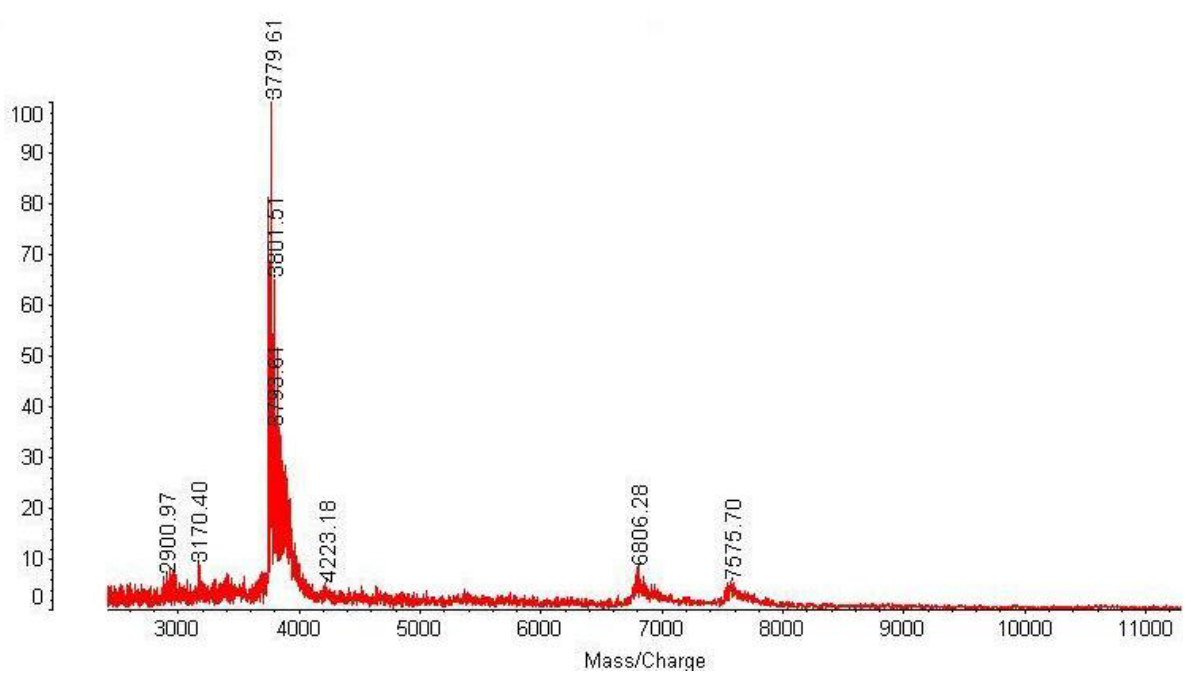
c

Fig. 2 Normalized melting curves of siRNA products. a. **I**; b. **II**; c. **III**.

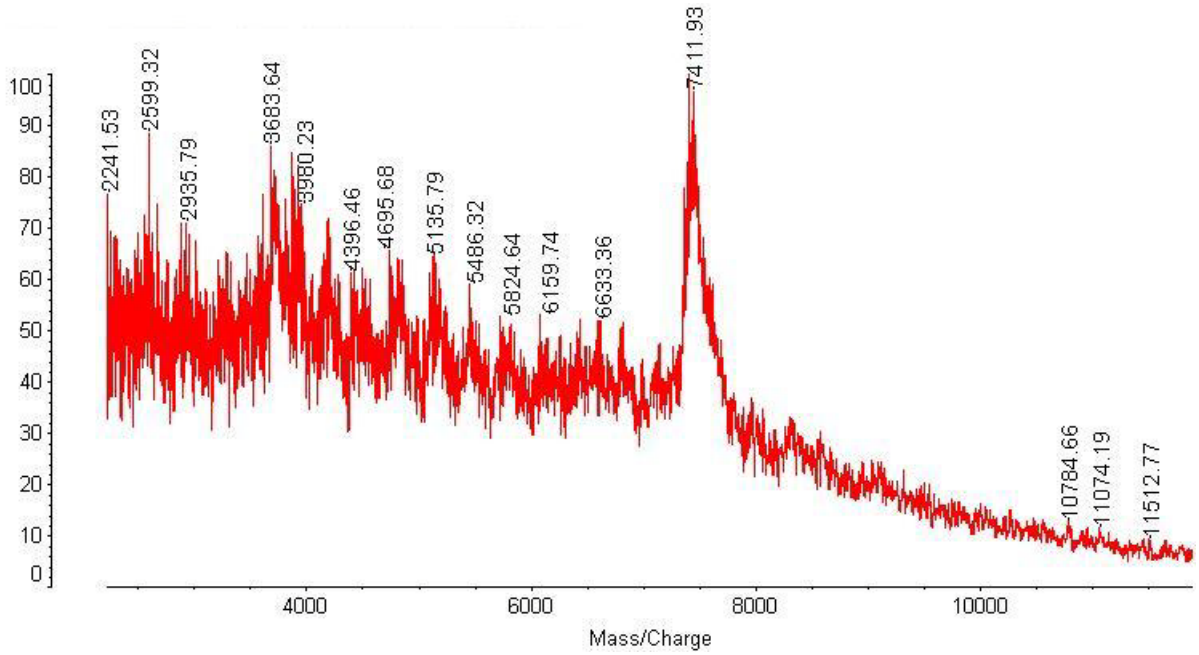
MALDI-TOF-MS data of a-e and peptide (LALLAK)



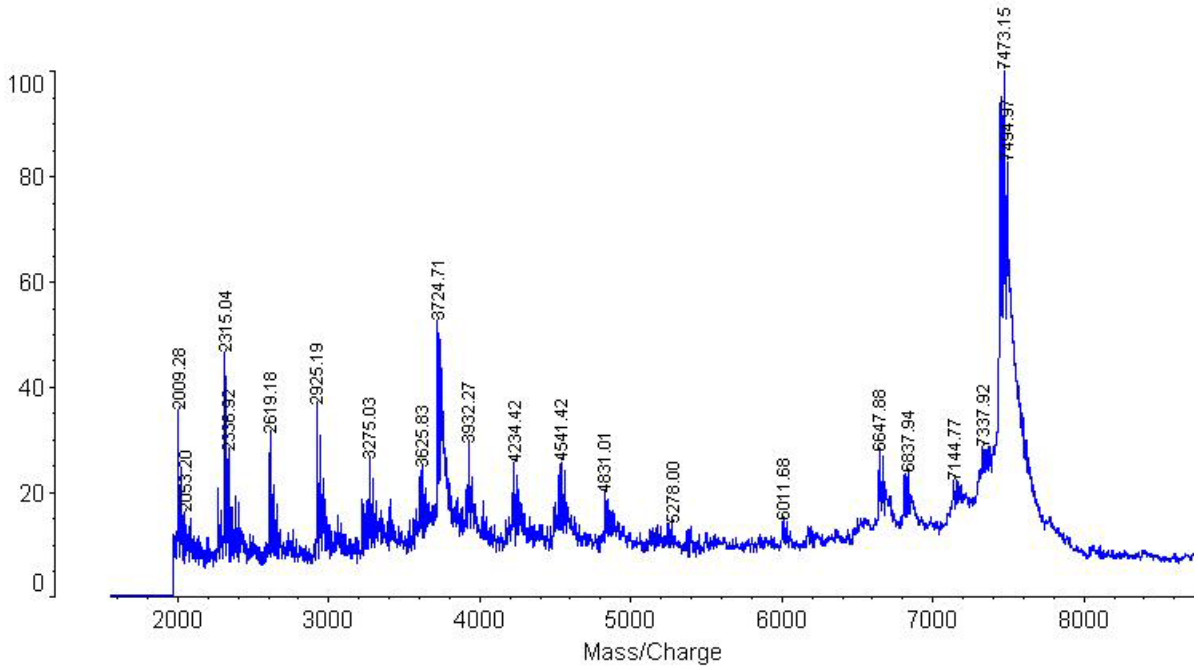
MALDI-TOF-MS data of **LALLAK**



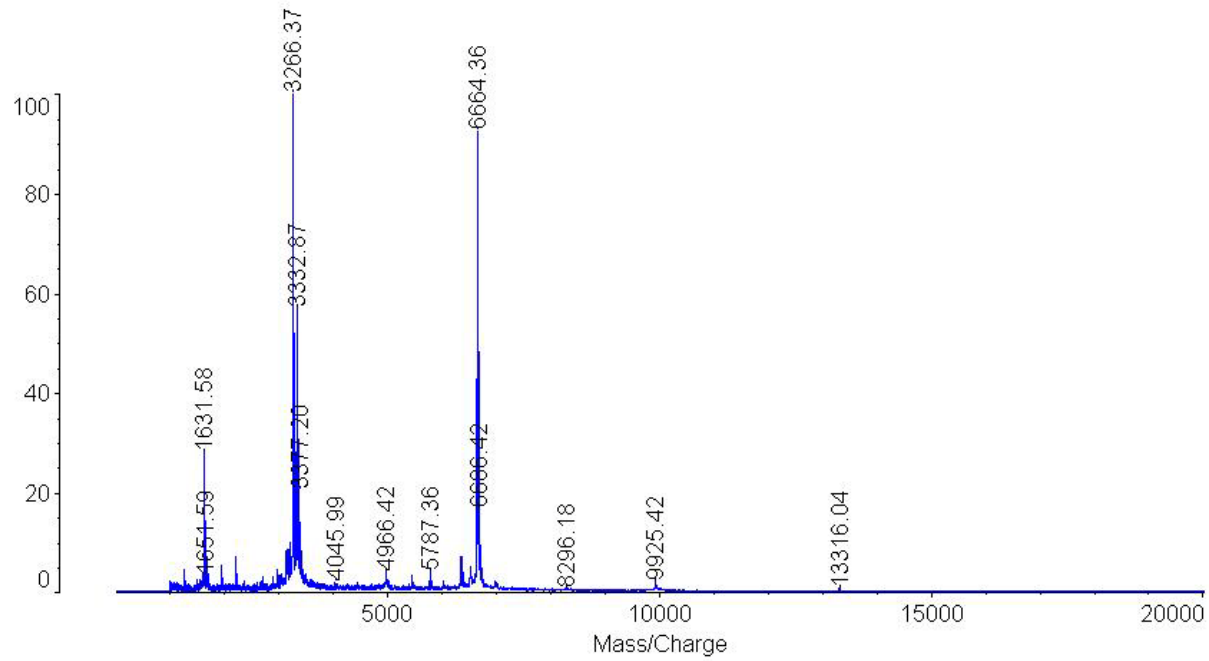
MALDI-TOF-MS of oligo **a**



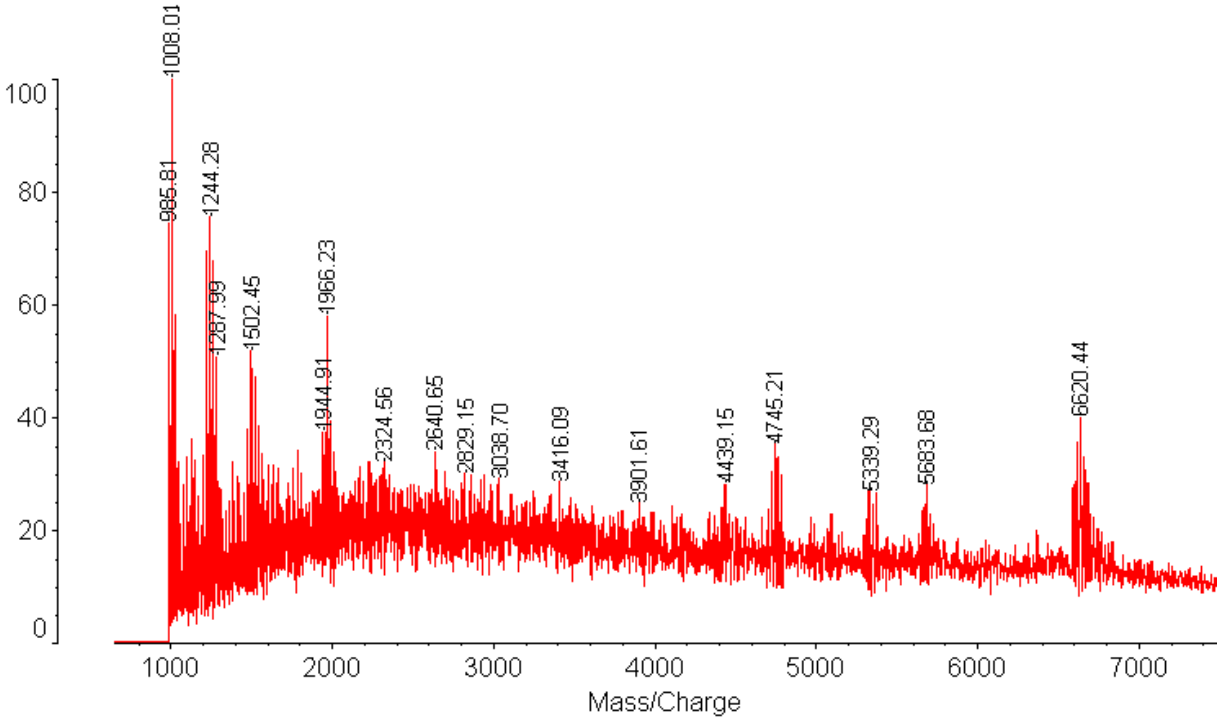
MALDI-TOF-MS of oligo **b**



MALDI-TOF-MS of oligo **c**



MALDI-TOF-MS of oligo **d**



MALDI-TOF-MS of oligo **e**

Gene silencing efficiency of I-III

The human embryonic kidney (HEK 293) cells were seeded into 24-well plates at a density of ~1×10^5cells/well one day before transfection. SiQuant vector (0.17μg/well) carrying the target gene was transfected into HEK 293 cells at approximately 50% confluence, together with pRL-TK control vector (0.017μg/well), with **I-III** (13 nM). The activity of both luciferases was determined by a fluorometer (Synergy HT, BioTek, USA) before the *firefly* luciferase activity was normalized to *renilla* luciferase for each well. Silencing efficiency of each siRNA was calculated by comparison with a sample without RNAi treatment. All experiments were performed in triplicate and repeated at least twice.