Imaging of RNA delivery to cells by thiazole orange as an artificial RNA base

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Supporting Information

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Materials and Methods. Chemicals were purchased from Aldrich, Alfa Aesar and Merck. Thiazole orange was synthesized as published previously.[1] Spectroscopic measurements were recorded in Na-P_i buffer solution (10 mM) using quartz glass cuvettes (10 mm). Absorption spectra and the melting temperatures (2.5 µM DNA, 250 mM NaCl, 10-90 °C, 0.7 °C/min, step width 1 °C) were recorded on a Varian Cary 100 spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 2 nm and are corrected for Raman emission from the buffer solution.

Preparation of RNA. Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using fast deprotection phosphoramidite chemistry. Reagents and CPG (1 µmol) were purchased from Proligo. After preparation (activator: 5-(Benzylthio)-1H-tetrazole, fast deprotection Cap A: Tac_2O) the trityl-off oligonucleotide was cleaved from the resin and deprotected by treatment with conc. NH_4OH and methylamine (1:1 v/v) at 65 °C for 20 minutes. The oligonucleotide was washed twice with water and ethanol 1:1 v/v and dried afterwards. After a third washing step with pure ethanol the oligonucleotide was reduced to dryness. After addition of DMSO and HF•NEt_3 and standing at 65°C for 2.5 hours the oligonucleotide was slowly cooled to room temperature. HF was quenched by addition of trimethyl(propoxy)silane while shaking the solution for 10 minutes. The precipitate was washed with ethanol twice and dried in vacuo. The oligonucleotide was purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = acetonitrile, gradient 0-15 % B over 70 min., flow rate 2.5 mL/min, UV detection at 260 and 290 nm. The oligonuclotides were lyophilized and quantified by their absorbance at 260 nm on a Varian Cary 100 spectrometer. The synthesis of thiazole orange-modified RNA oligonucleotides was performed using a modified protocol. Activator solution was pumped simultaneously with the building block (0.1 M in acetonitrile). The coupling time was extended to 61 minutes with an intervening step after 30.8 min for washing and refreshing the activator/phosphoramidite solution in the CPG vial. The CPG vial was flushed with dry acetonitrile after the coupling. After preparation, the trityl-off oligonucleotide was cleaved from the resin as described above. The modified oligonucleotides were purified by HPLC on a semipreparative RP-C18 column (300Å, Supelco) using the following conditions: A= NH4OAc buffer (50 mM), pH = 6.5; B= acetonitrile; gradient 0-20% B over 70 min, flow rate 2.5 mL/min, UV/Vis detection at 260 and 512 nm. The oligonucleotides were lyophilized and quantified by their absorbance in 10 mM Na-P_i buffer at 260 nm on a Varian Cary 100 spectrometer. Duplexes were formed by heating to 90 °C (15 min.) followed by slow cooling to room temperature.

Microinjection into cells. CHO-K1 cells were seeded one day prior microinjection on glass coverslips. Glass capillaries were loaded with 50 µM RNA solutions. The microinjection was performed using an InjectMan in combination with a microinjector FemtoJet (Eppendorf) coupled to a Axiovert 200 fluorescence microscope (Carl Zeiss). The injection pressure was ~ 120 hPa and the injection time per cell was 0.2 s. After injection, the culture medium (Ham´s F12 containing 10% serum) was changed and the fluorescence of cells was imaged from 500 to 550 nm (green channel) and from 570 to 640 nm (orange channel).

Confocal microscopy. A Zeiss Axiovert 200 M microscope coupled to a Zeiss LSM 510 scanning device (Carl Zeiss) was used to observe the cellular uptake of RNA-Lipofectamine^{TM}2000 particles. CHO-K1 cells were plated in an 8-well Lab-Tek^{TM} Chambered Coverglass at an initial density of 40,000 cells per chamber. After 20 hours, RNA-Lipofectamine^{TM}2000 particles were added at a final concentration of 100 nM, and imaging commenced after 2 hours in each well at 37 °C. Fluorescent and transmitted light images were taken simultaneously using a 488 nm argon laser. The fluorescence was detected using a 505-530 nm band-pass filter and a 560 nm longpass filter. The pinhole was set to one Airy Unit.

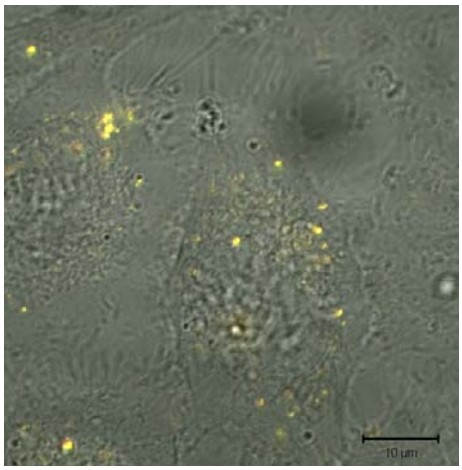
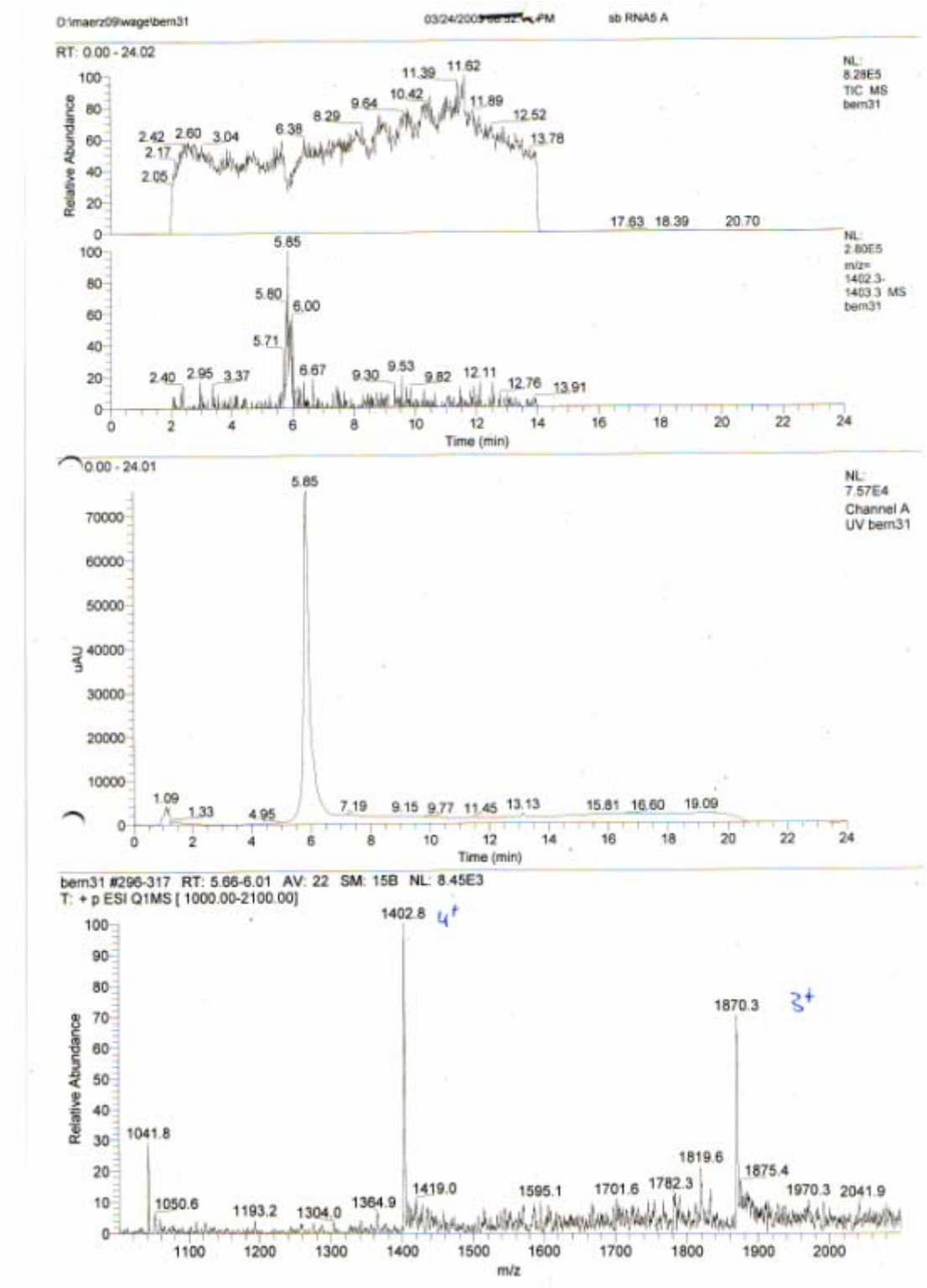
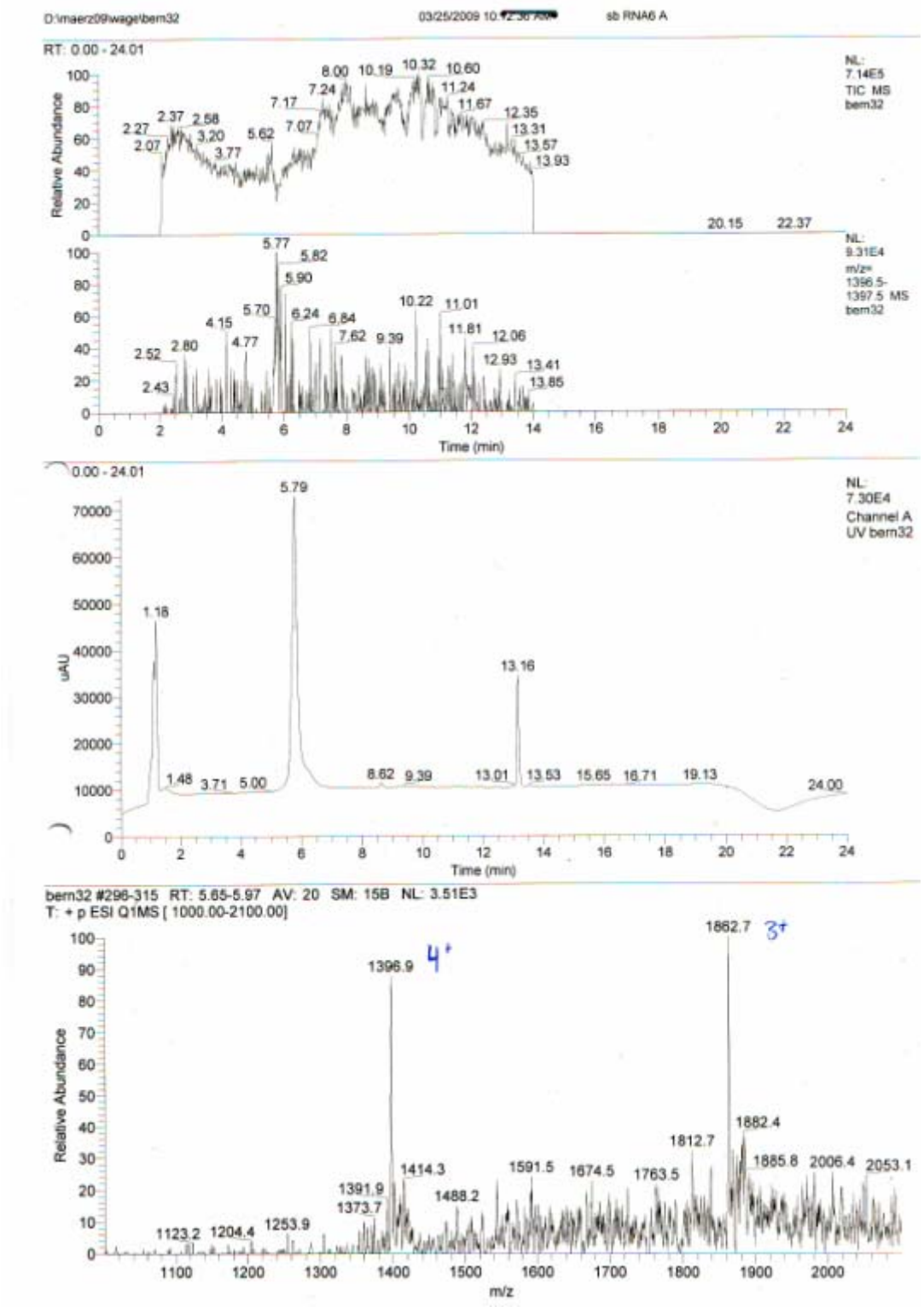


Figure S1. Confocal laser scanning microscopy image of CHO-K1 cells after 2 hours of incubation with RNA1-2 -Lipofectamine^{TM}2000 particles. The picture is an overlay of transmitted light and fluorescence image. The yellow color of the RNA1-2 -Lipofectamine^{TM}2000 particles is due to the fluorescence emission of RNA1-2 in both detection channels. Scale bar: 10µm.

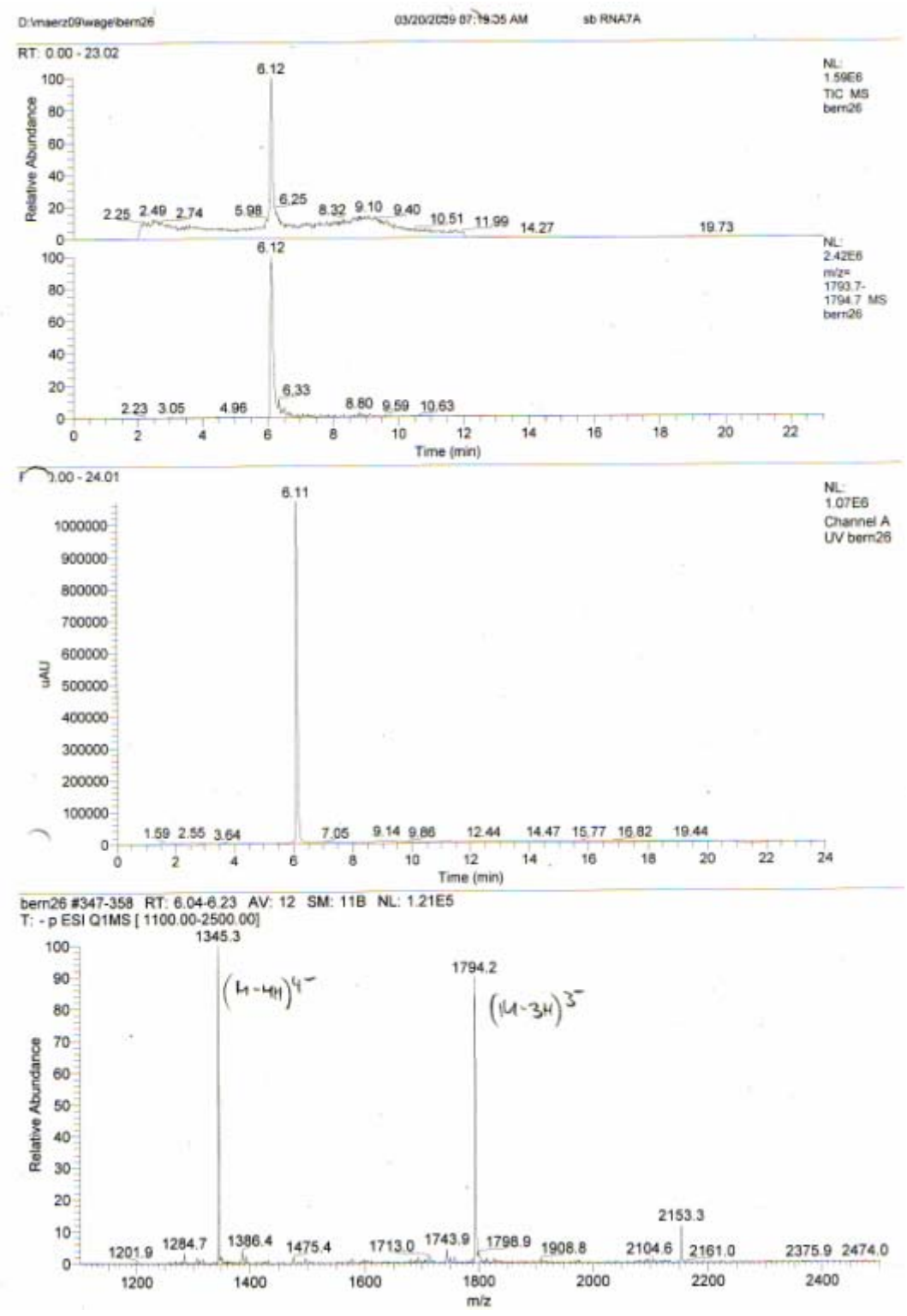
RNA1: HPLC and ESI MS



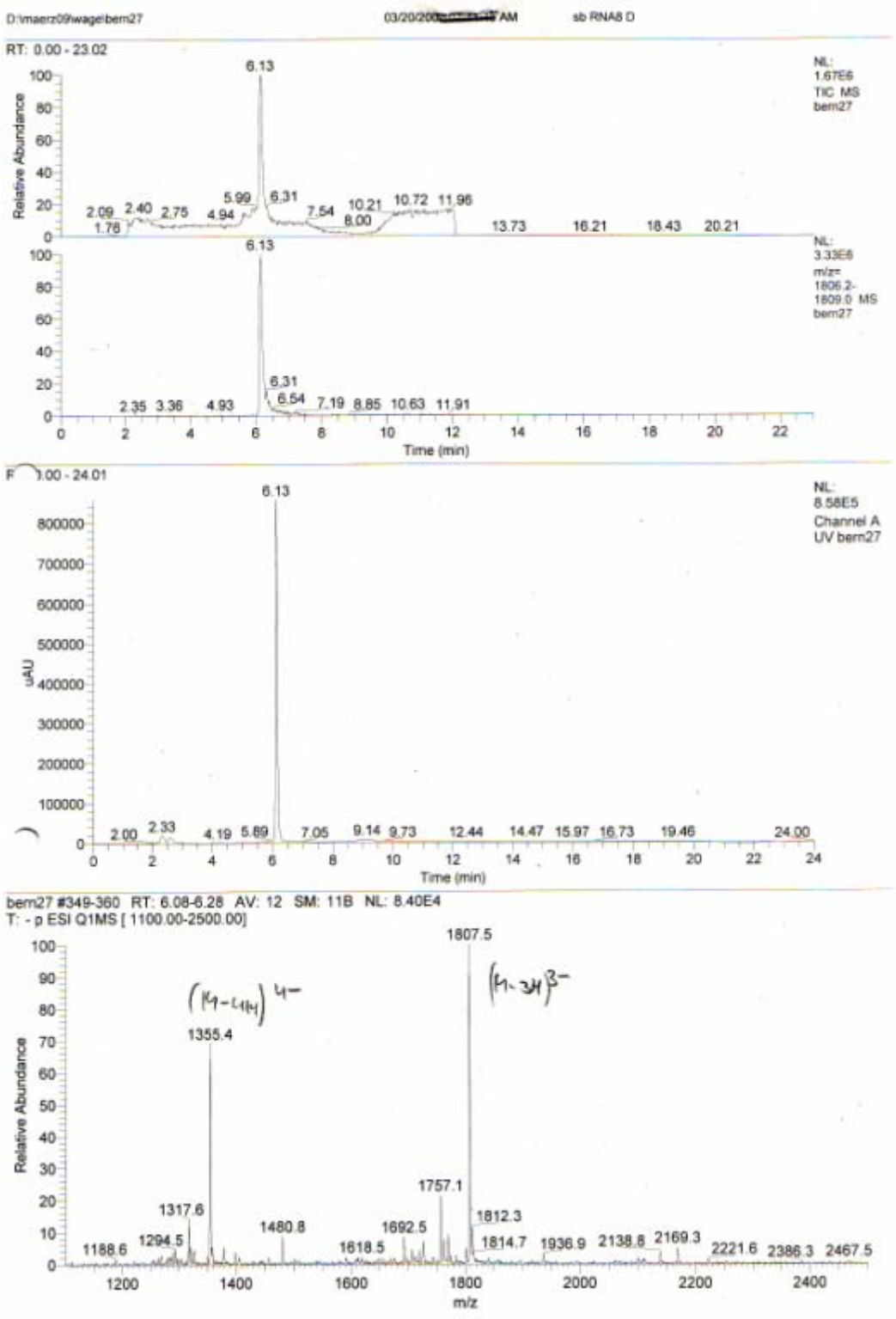
RNA2: HPLC and ESI MS



RNA3: HPLC and ESI MS



RNA4: HPLC and ESI MS



References

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[2] Breunig, M.; Hozsa, C.; Lungwitz, U.; Watanabe, K.; Umeda, I.; Kato, H.; Goepferich, A. *J Control Release* **2008,** *130*, 57-63.