

## Application of “click” chemistry to the production of DNA microarrays

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#### (1) General remarks:

All reagents (analytical grade) were obtained from commercial suppliers and used without further purification. Anhydrous benzene, toluene and CH<sub>2</sub>Cl<sub>2</sub> were freshly distilled from P<sub>2</sub>O<sub>5</sub> and CaH<sub>2</sub>, respectively. All other anhydrous solvents and liquid reagents were dried through storage over activated 4Å (EtOH, Et<sub>3</sub>N, pyridine,) or 3Å (MeOH, MeCN) molecular sieves.

Progress of reaction was monitored by Thin Layer Chromatography and <sup>31</sup>P NMR spectra while purification was effected by column chromatography, using silica gel (60-120 mesh).

<sup>1</sup>H, <sup>13</sup>C, <sup>29</sup>Si NMR. The NMR spectra were recorded at 298 K on a Bruker Advance DRX 400 spectrometer operating at frequencies 400.13201 MHz (<sup>1</sup>H), 100.62281 MHz (<sup>13</sup>C) and 79.45750 MHz (<sup>29</sup>Si). <sup>31</sup>P NMR spectra were recorded at on Varian Unity 300 spectrometer operating at frequencies 121 MHz; 5% H<sub>3</sub>PO<sub>4</sub> in D<sub>2</sub>O as an external

In all spectra for **1** CDCl<sub>3</sub> was used and for **2** DMSO was used as the solvent.

FT-IR spectra were recorded on a Bruker Tensor 27 Fourier transform spectrometer equipped with a SPECAC Golden Gate diamond ATR unit. In all cases, 16 scans at a resolution of 2 cm<sup>-1</sup> at RT were collected for the spectrum.

Mass spectra of analyzed compound **1** was collected on Varian 4000 GC/MS chromatographic system equipped with Varian VF Factor-Four – 5ms, 30 m long capillary column, split/splitless type injection chamber, MS (ion trap) detector and autosampler. Mass spectra was recorded in a range 50 to 1000 m/z and electron impact (EI) ionization technique was used.

# <sup>1</sup>H NMR of compound 1

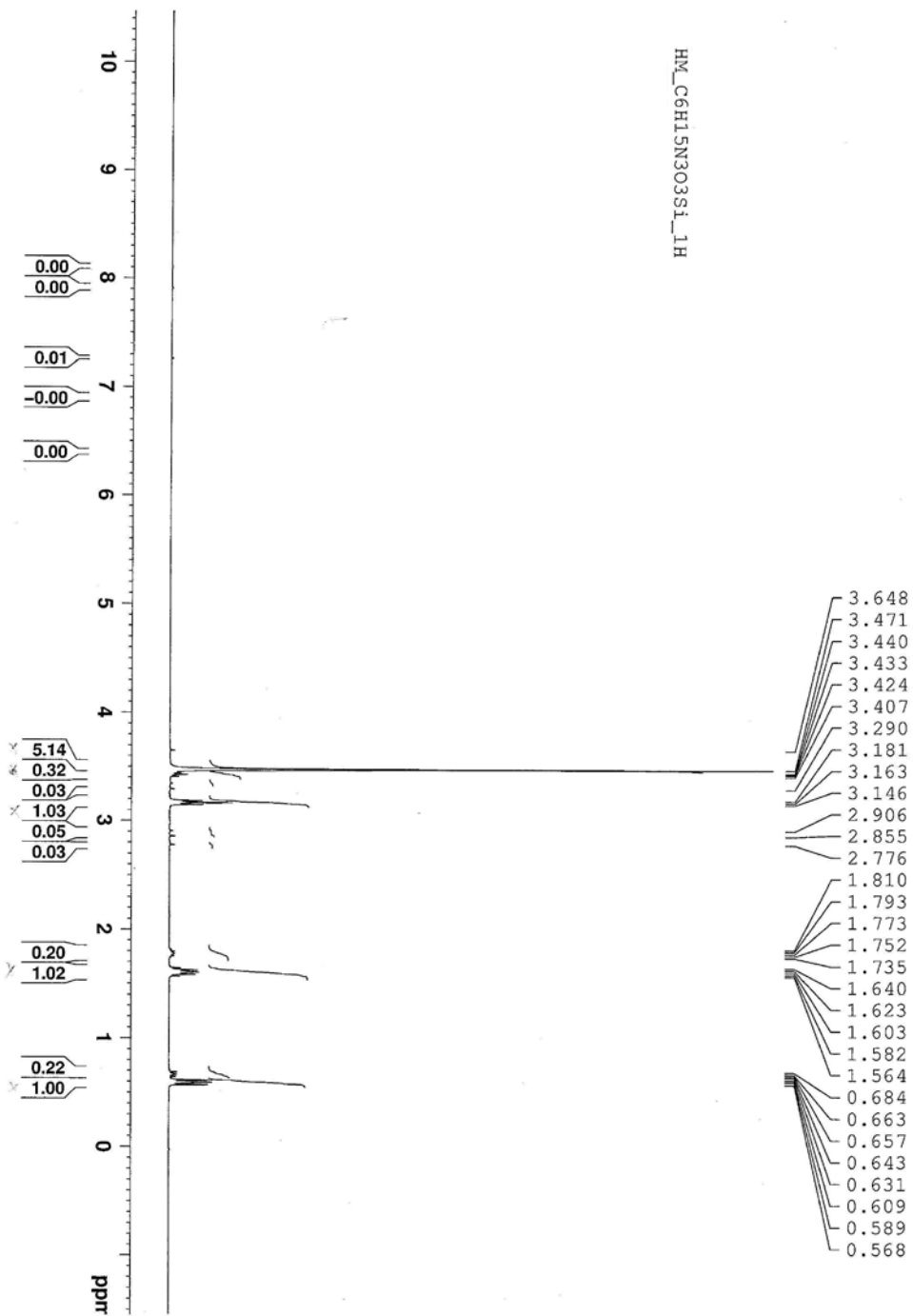


Fig  $^{13}\text{C}$  NMR of compound 1

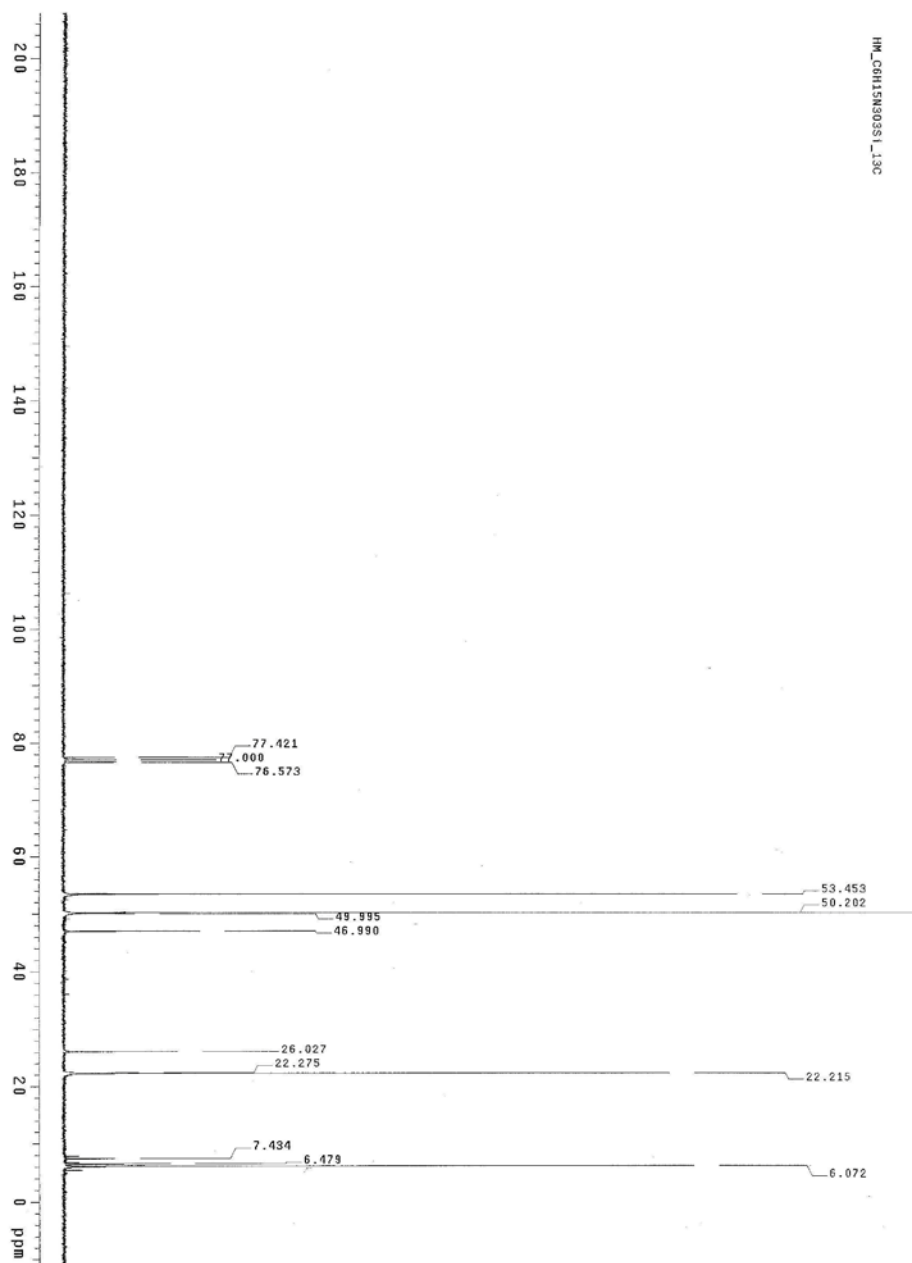


Fig  $^{29}\text{Si}$  NMR of compound **1**

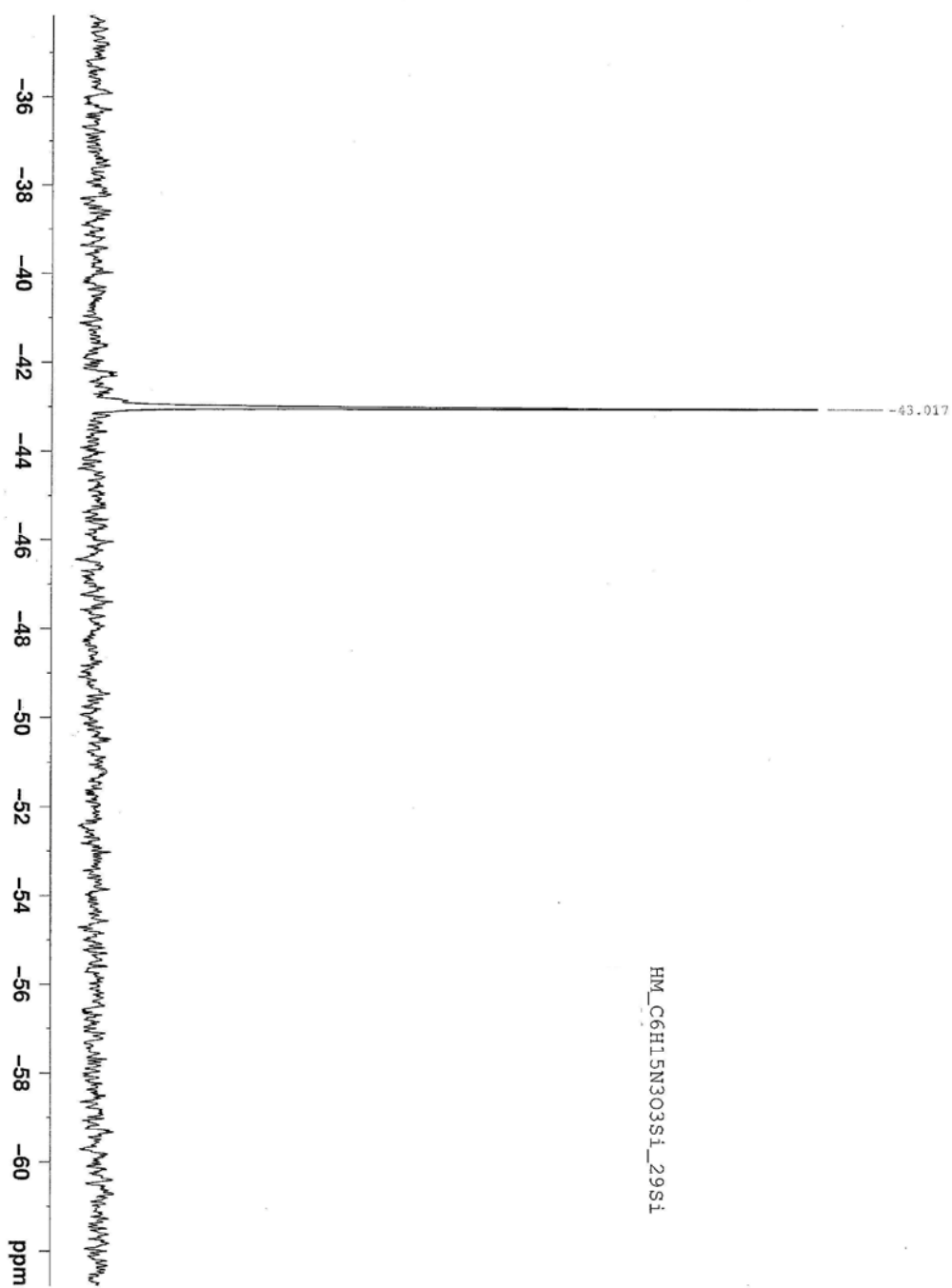


Fig FT-IR of compound 1

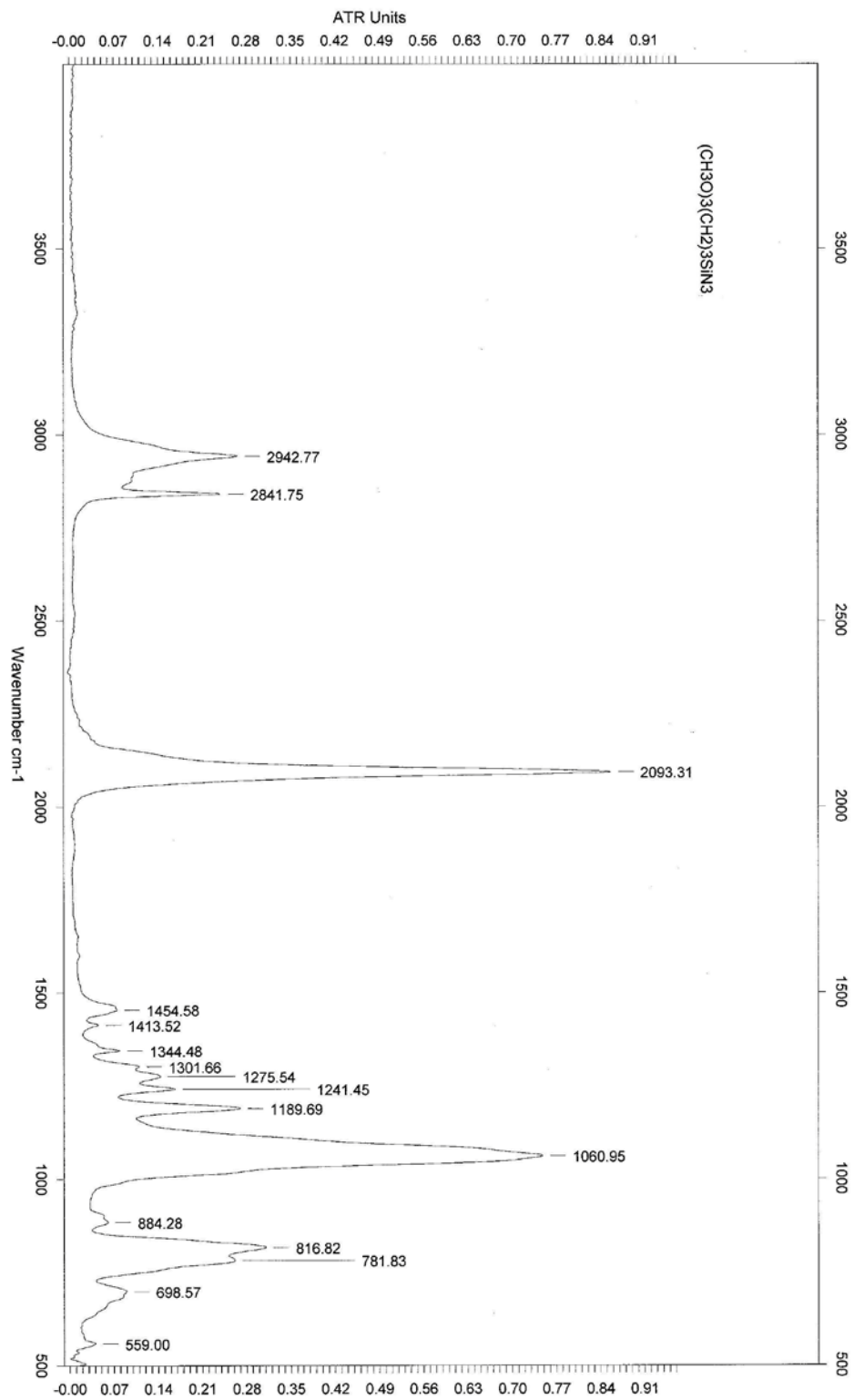


Fig GC-MS of compound 1

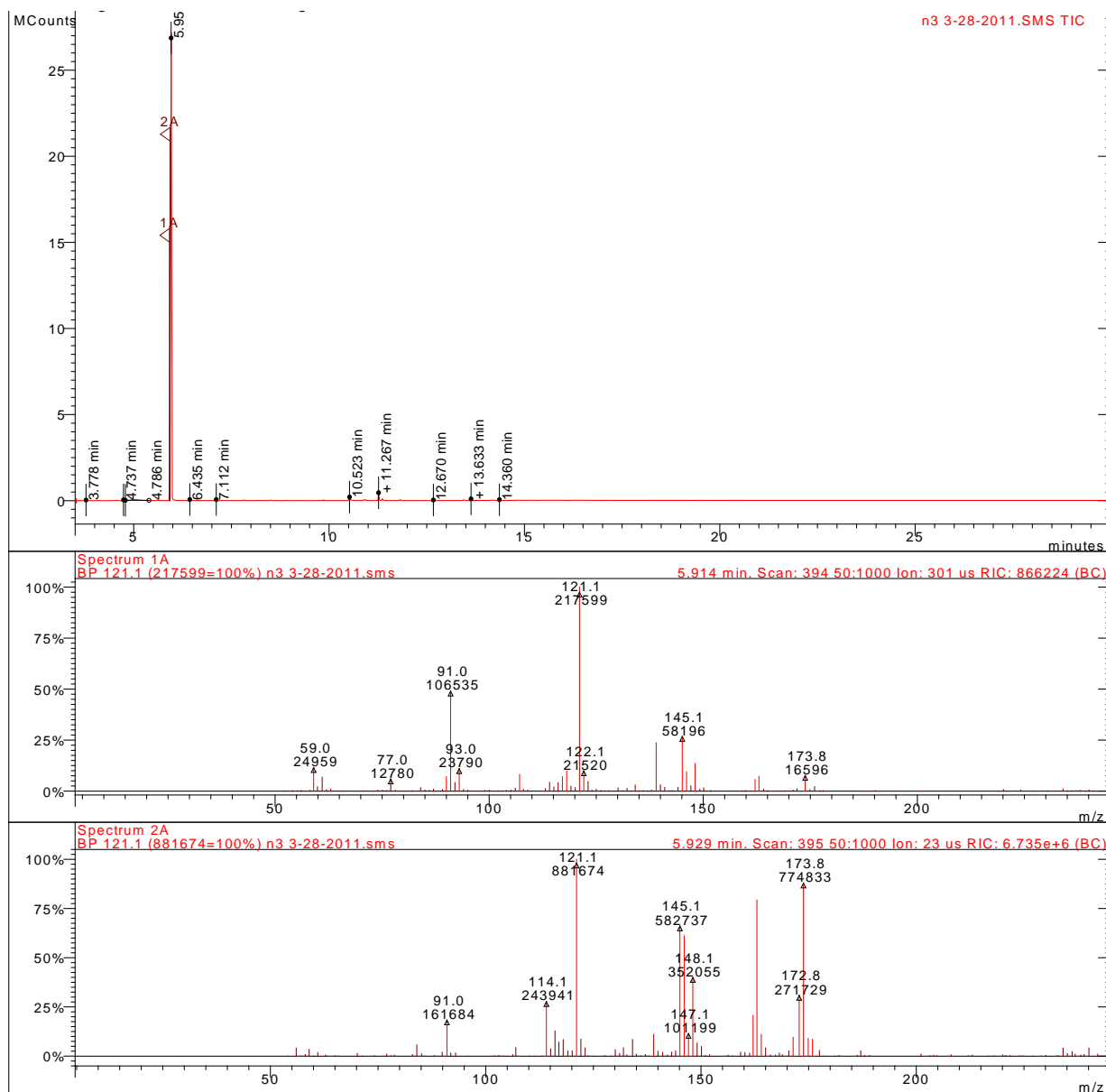


Fig  $^1\text{H}$  NMR of compound 2





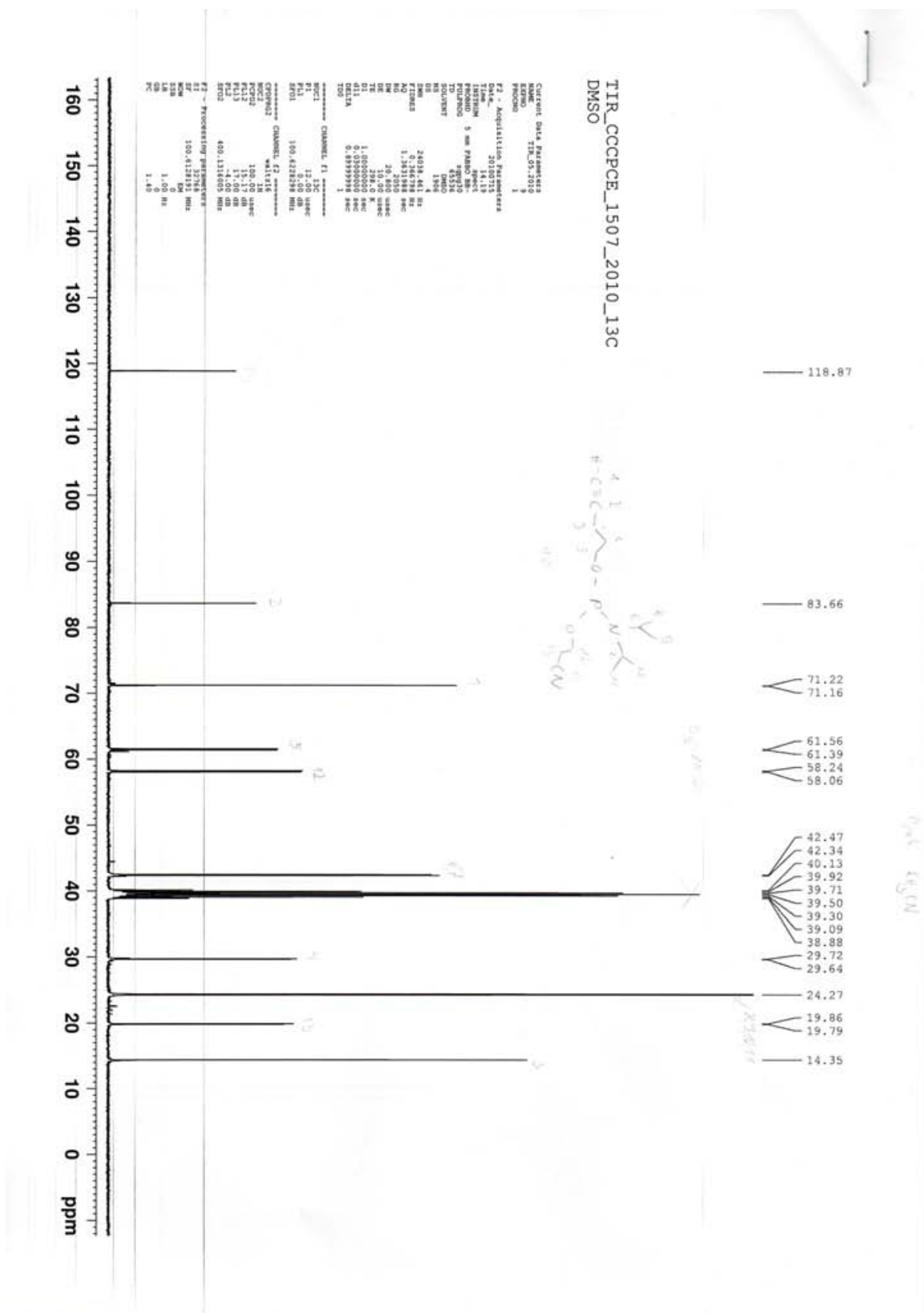


Fig uncoupled <sup>31</sup>P NMR a of compound 2

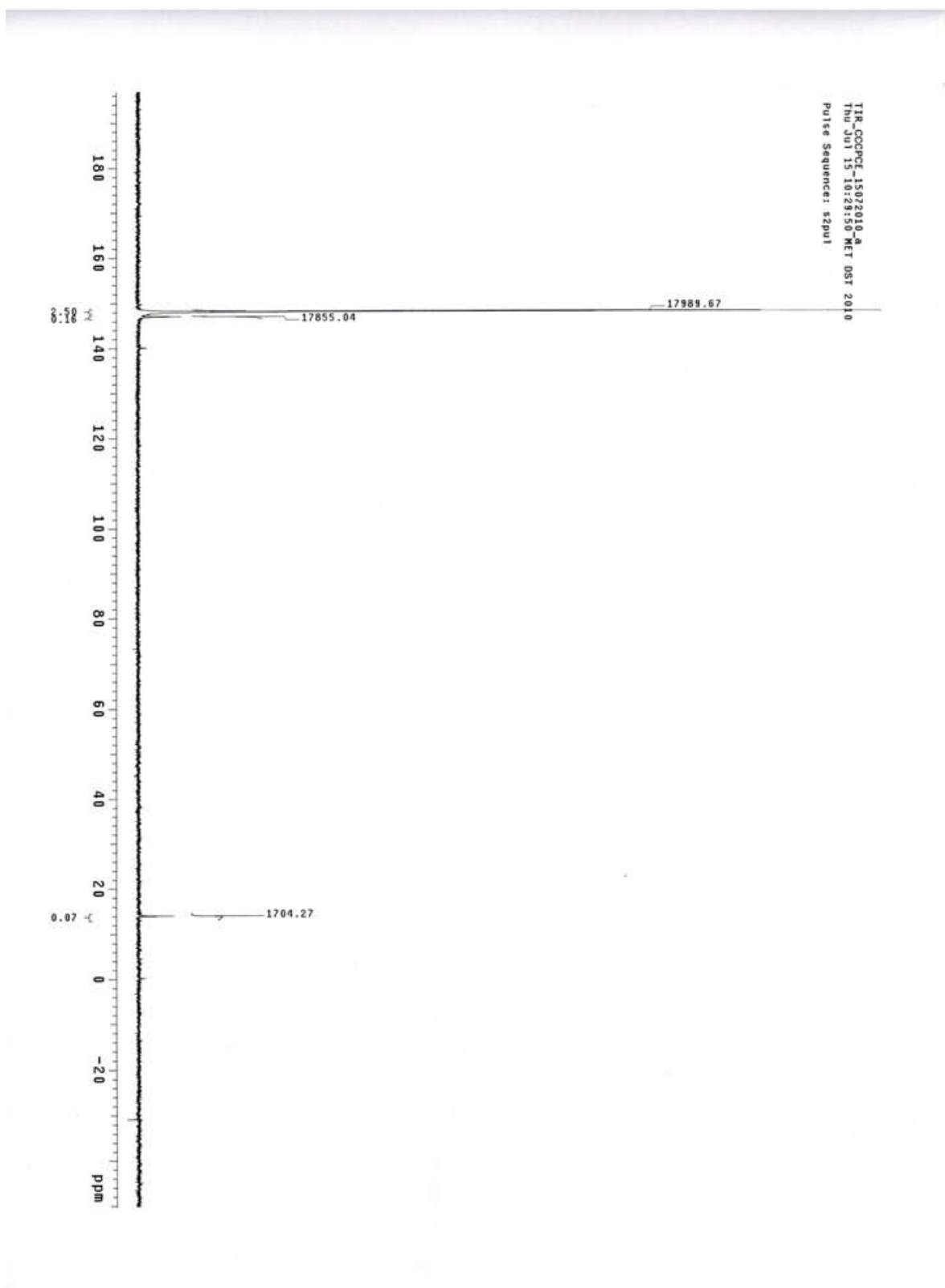


Fig  $^{31}\text{P}$  NMR couple with hydrogen of compound 2

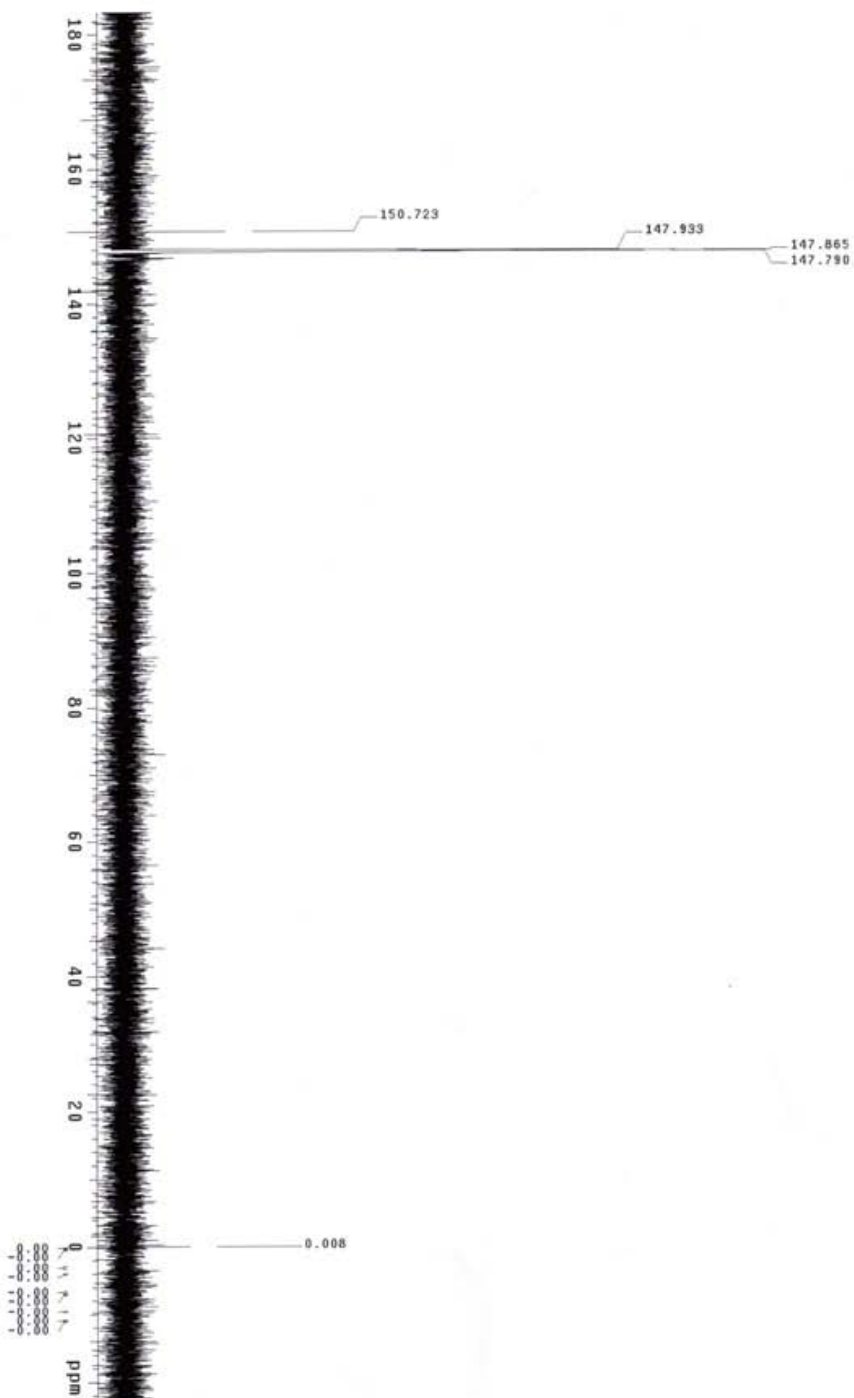


Image microarrays after printing

Fig 0.5% concentration of azidofunctional silane

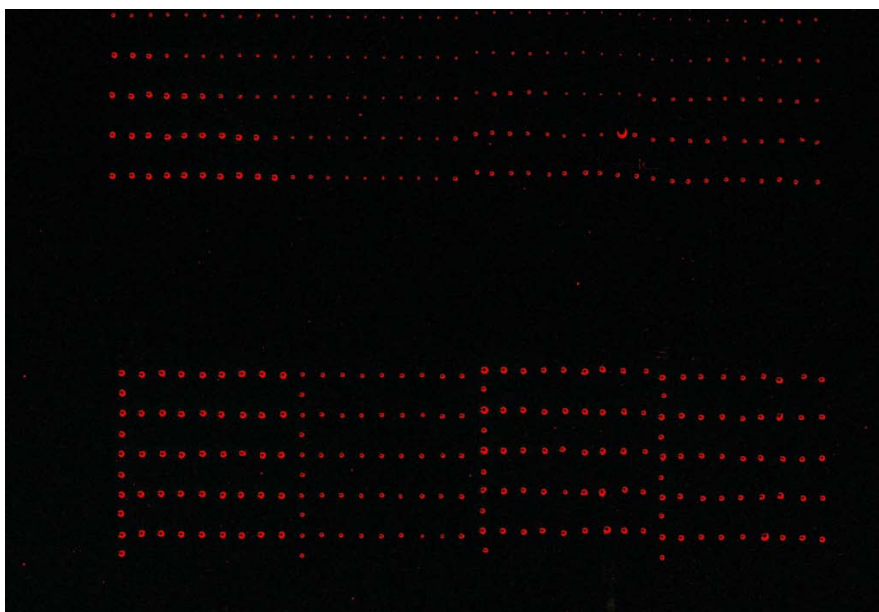
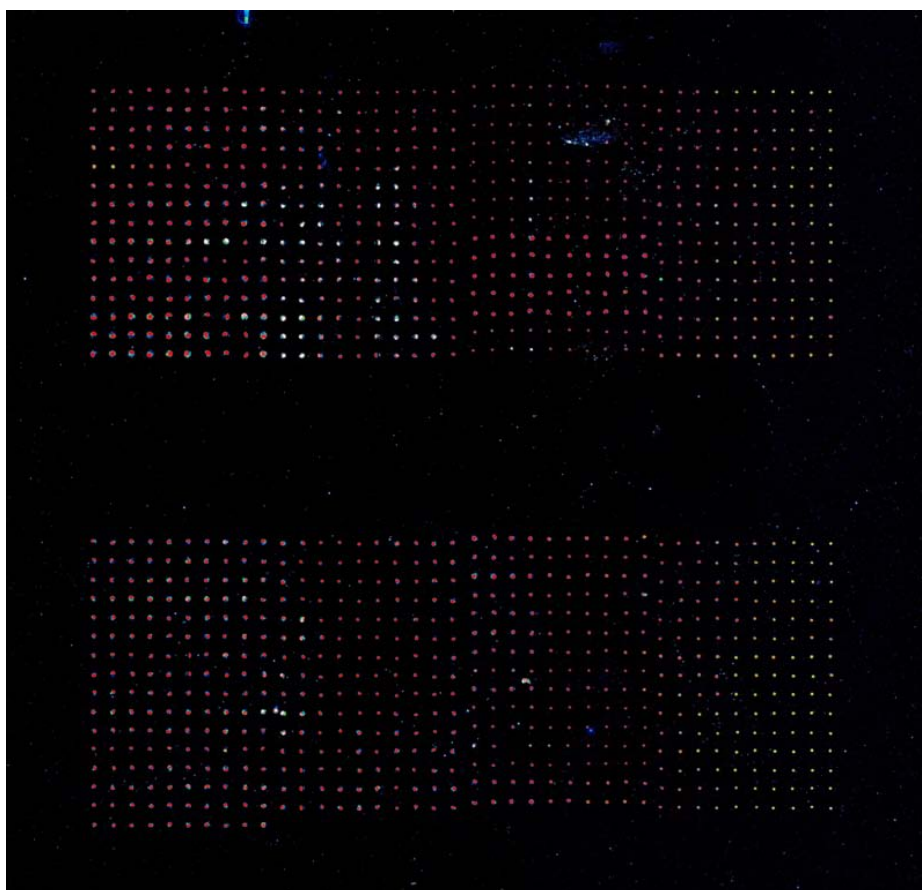
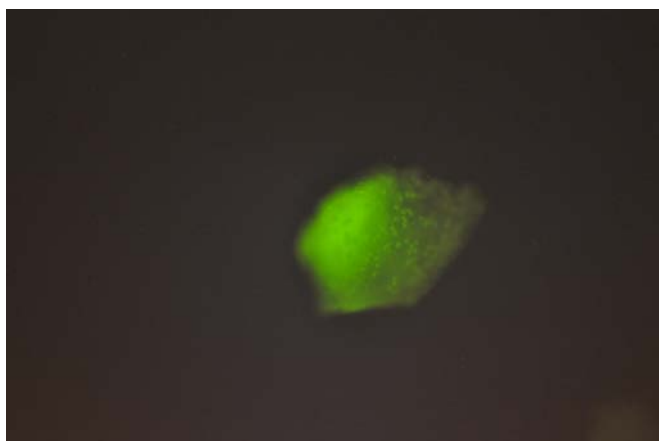
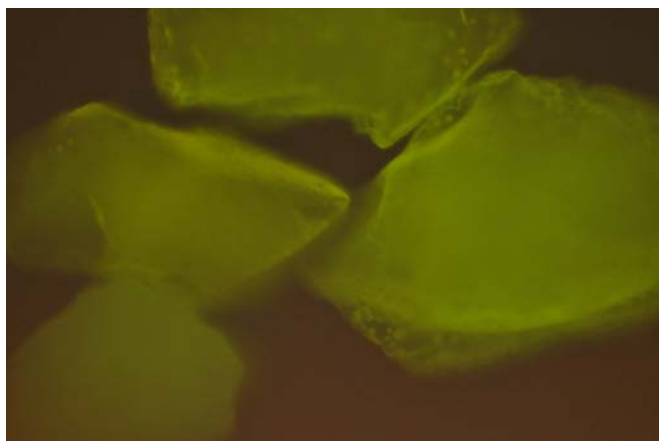
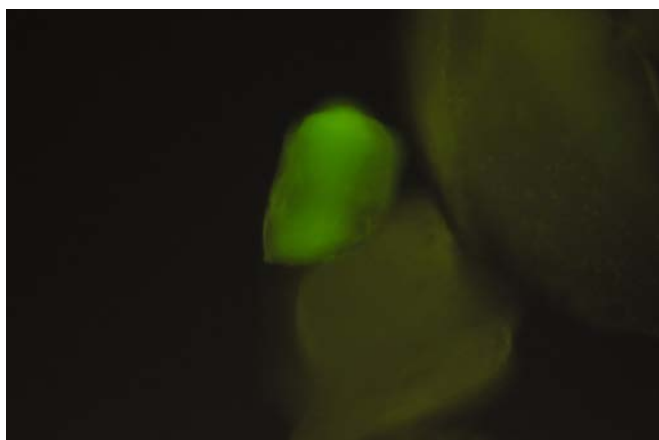


Fig 0.2% concentration of azidofunctional silane



**Image CPG from fluorescent microscope**

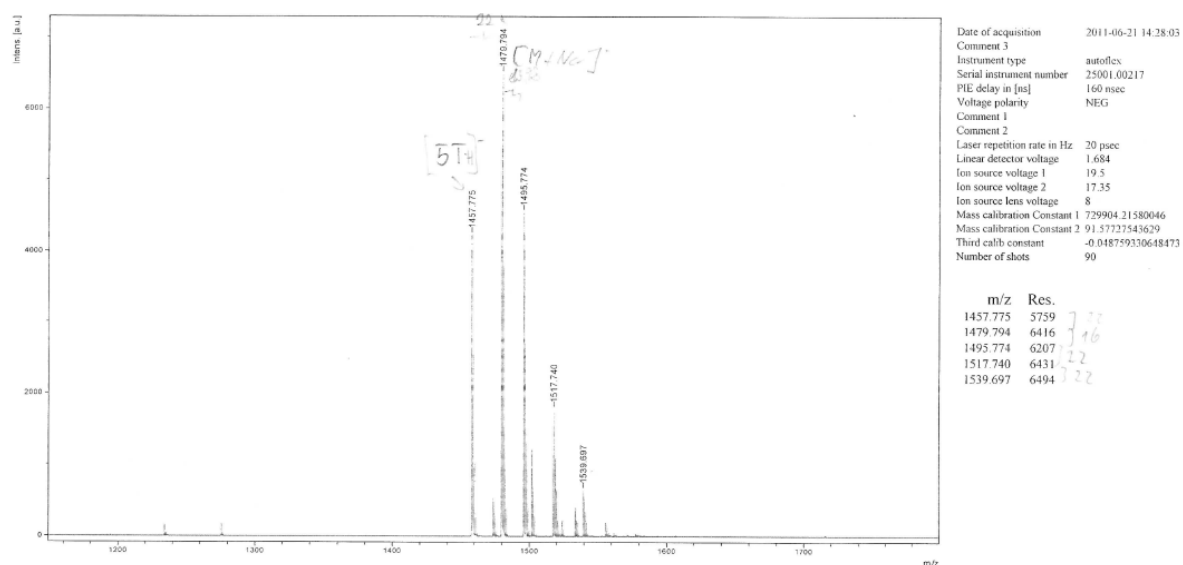
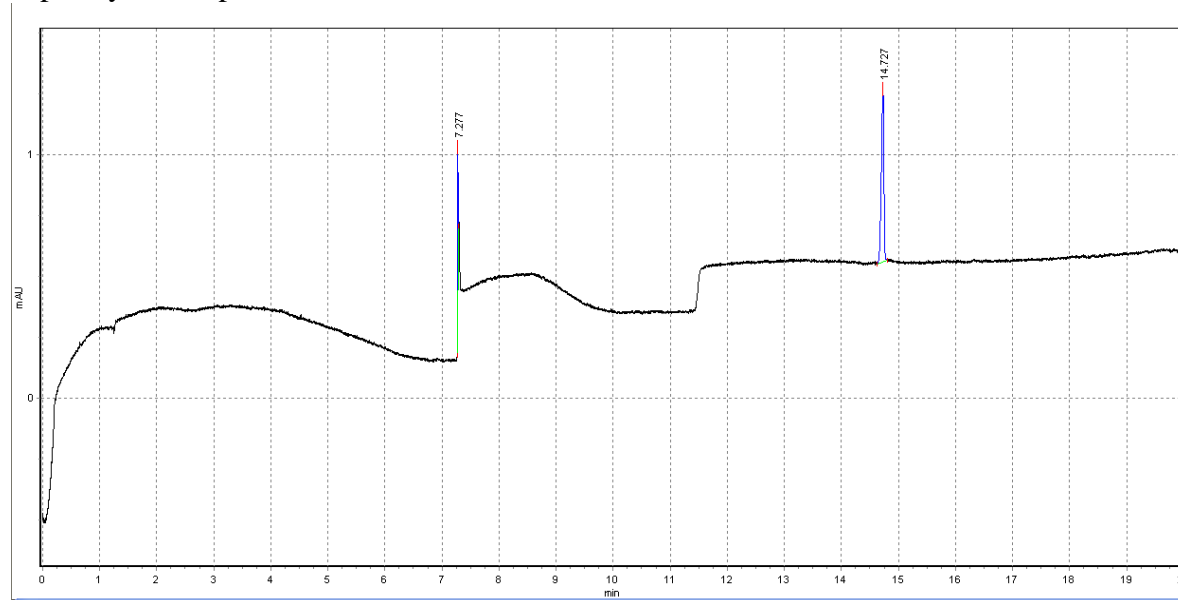
Fig . Effect of treatment azide-functionalized CPG with ODN-3 and labeled oligomer MIX-F  
The green fluorescence was visualized by fluorescent microscope



## Analysis of oligonucleotides by capillary electrophoresis and MALDI-TOF analysis

ODN-1

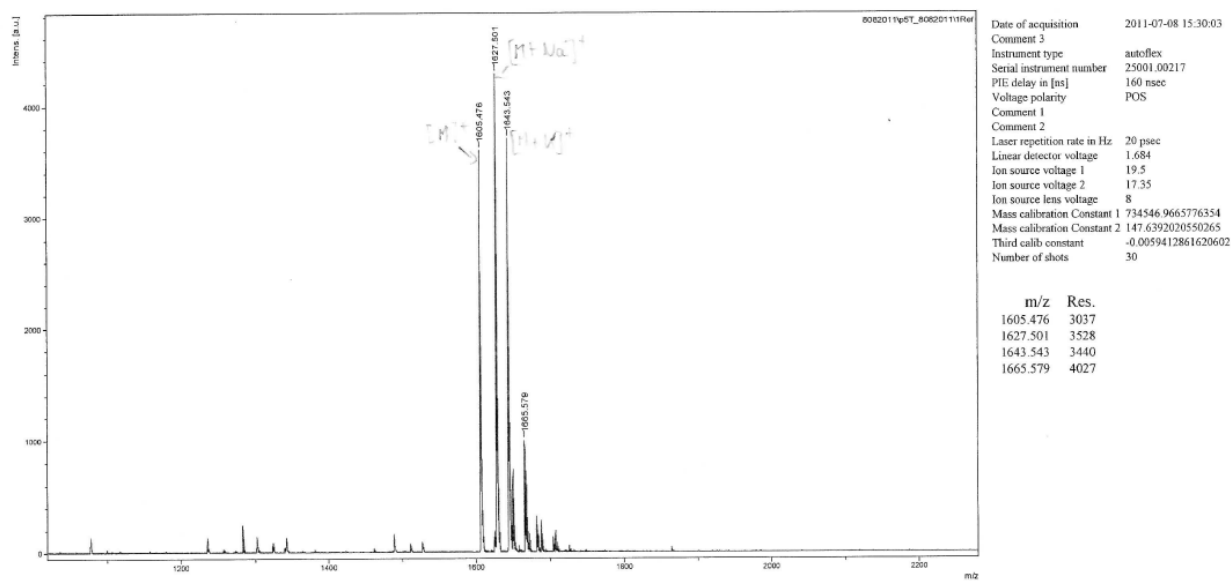
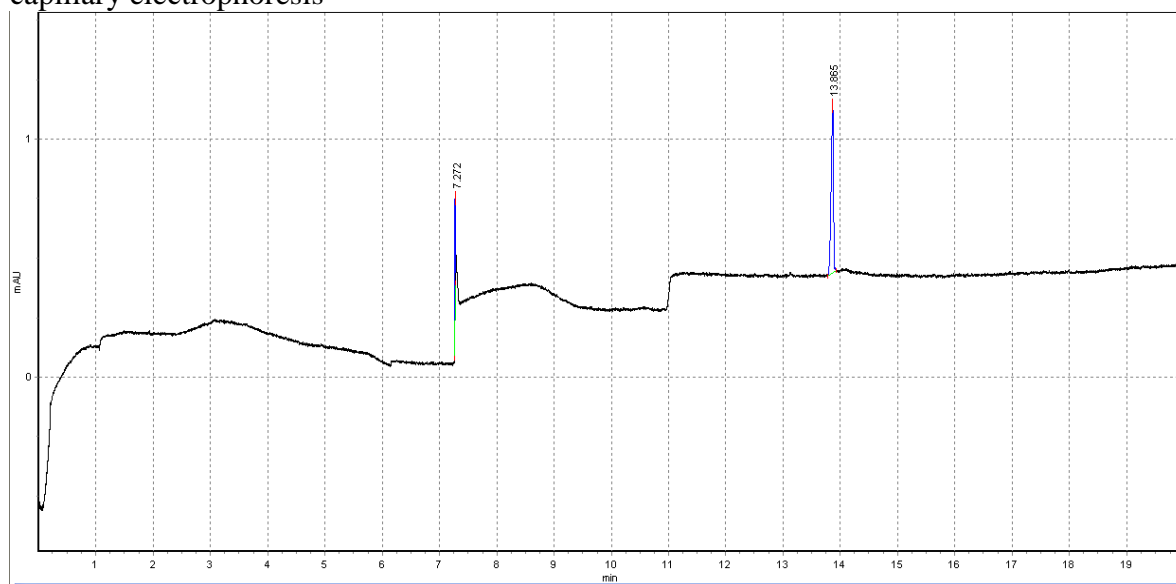
capillary electrophoresis



MS MALDI calculated mass 6661,467; found m/z M- 1457,775 m/z; 1479,794 [M+Na] m/z; 1495,774 m/z [M+K]

ODN-2

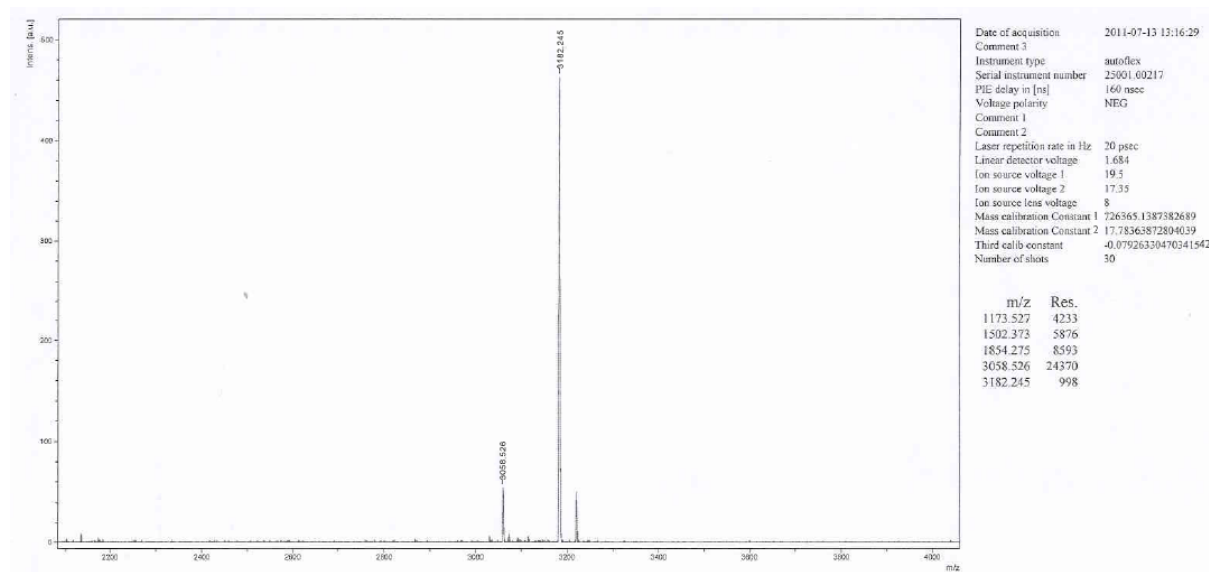
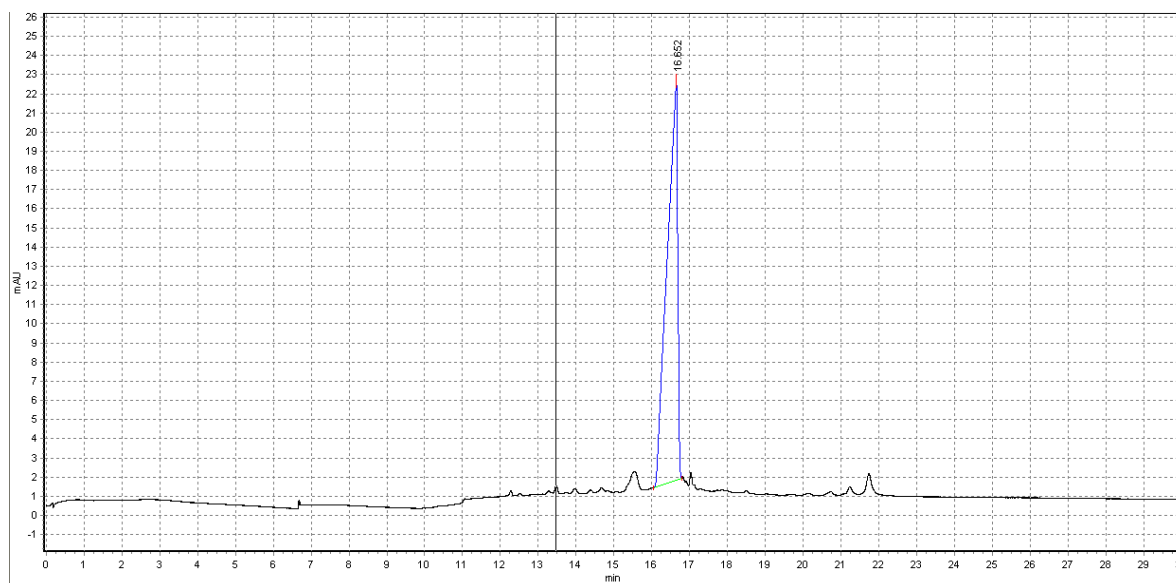
capillary electrophoresis



MS MALDI calculated mass 1605; found m/z 1605,476 m/z; 1627,501 m/z [M+Na]<sup>+</sup>;  
1643,543 m/z [M+K]<sup>+</sup>

ODN-3

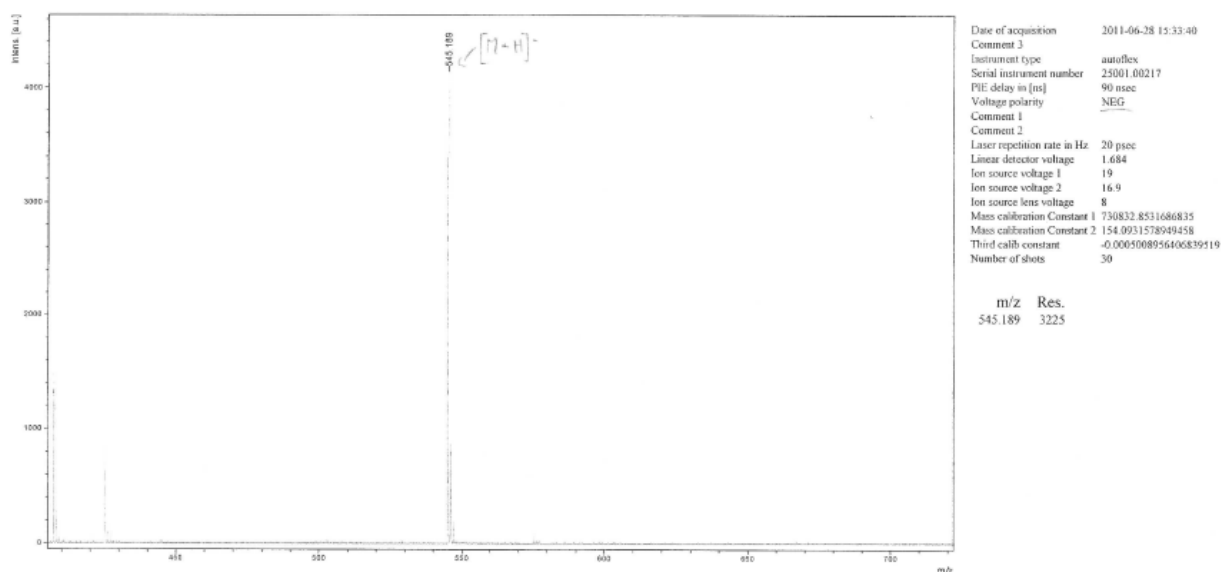
capillary electrophoresis



MS MALDI calculated mass 3182,2; found m/z 3182,081 m/z;



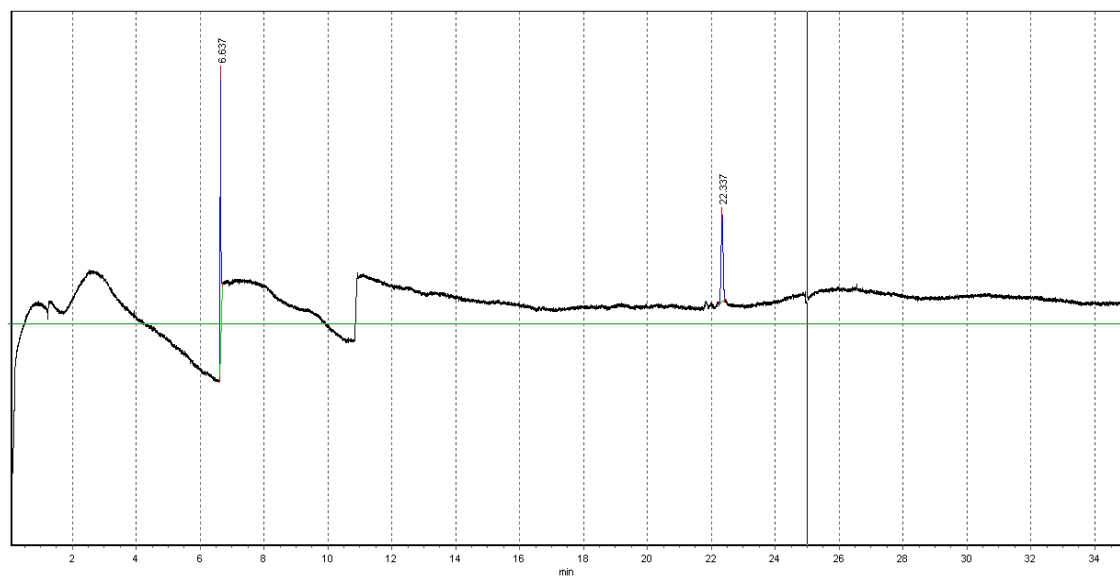
## ODN-4



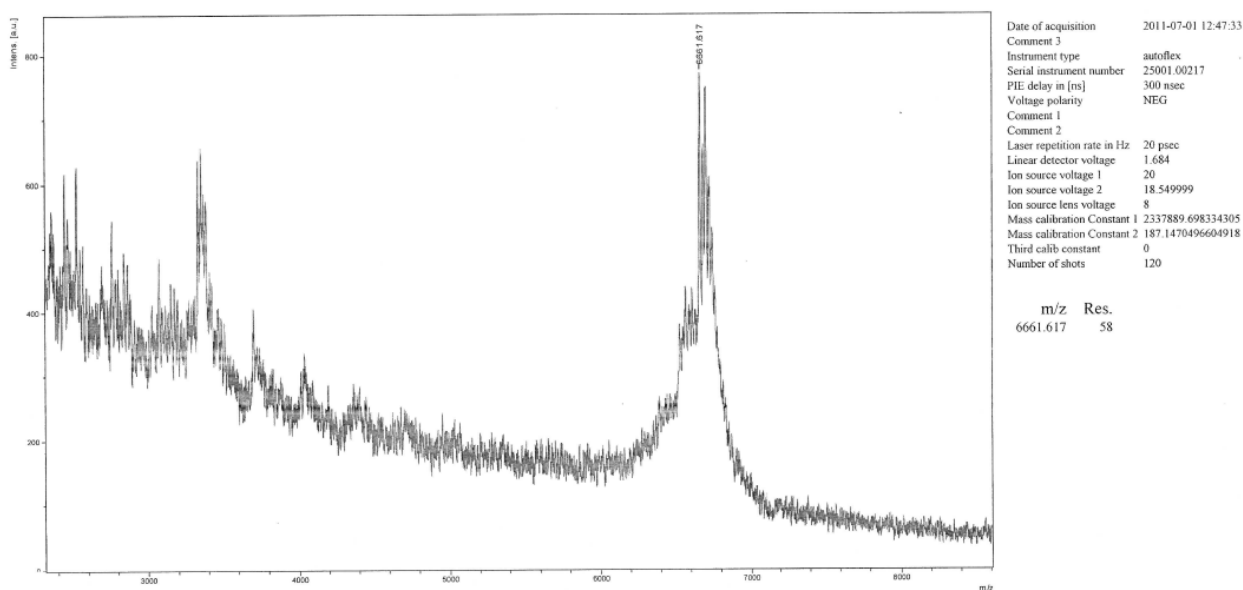
MS MALDI calculated mass 545.4; found m/z 545,189 m/z;

## ODN-5

capillary electrophoresis

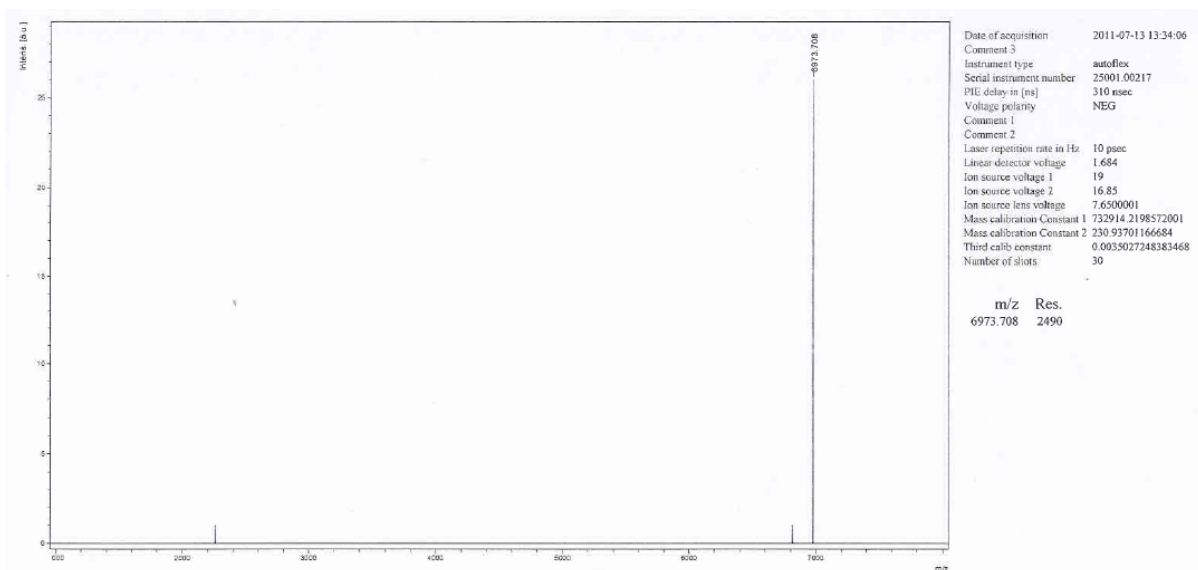


MALDI-TOF MS



MS MALDI calculated mass 6661,467; found m/z 6661,6172 m/z

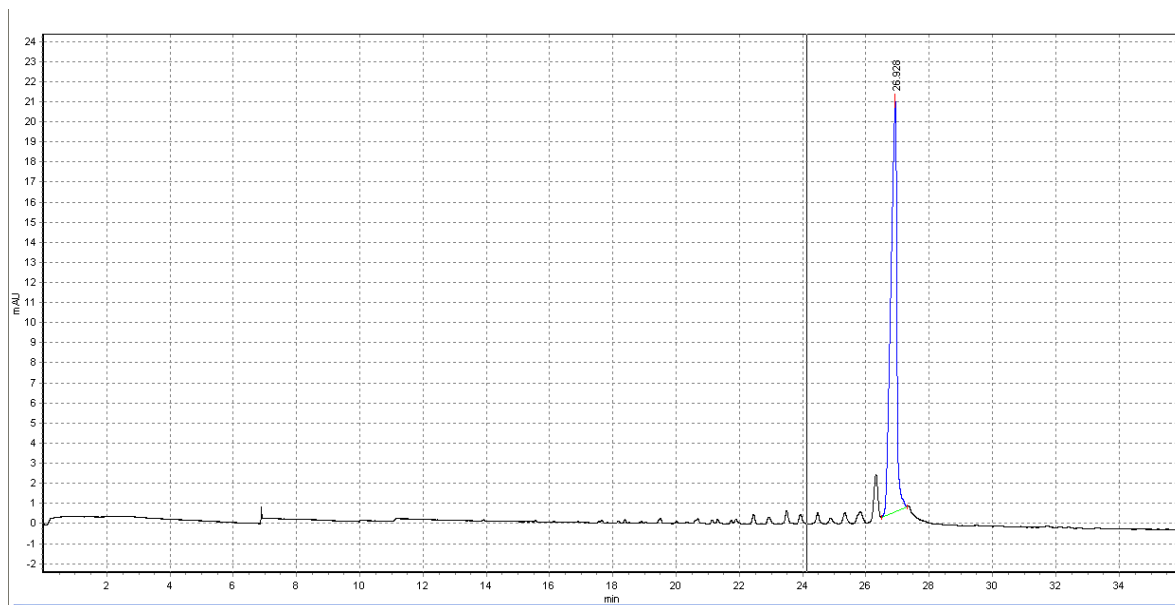
### ODN-6



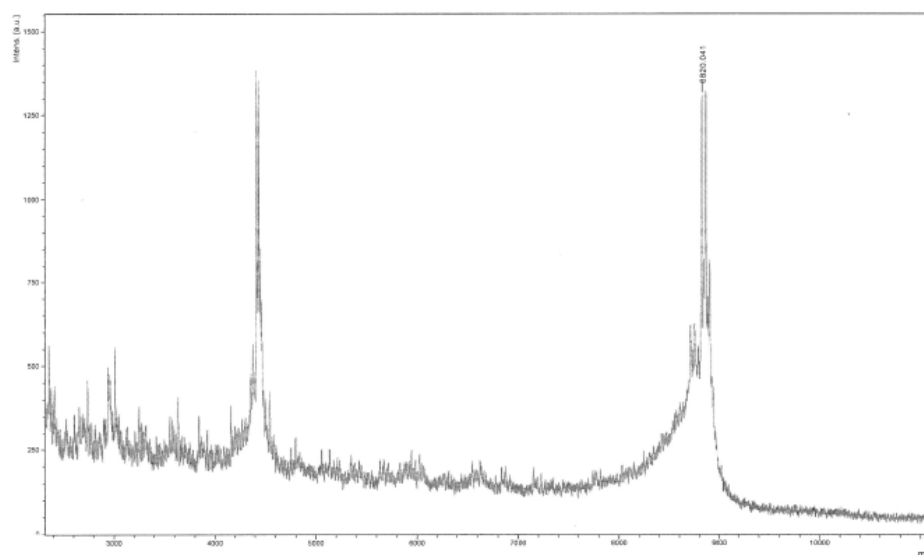
MS MALDI calculated mass 6972.959; found m/z 6973.708 m/z;

### ODN-7

capillary electrophoresis



### MALDI-TOF MS



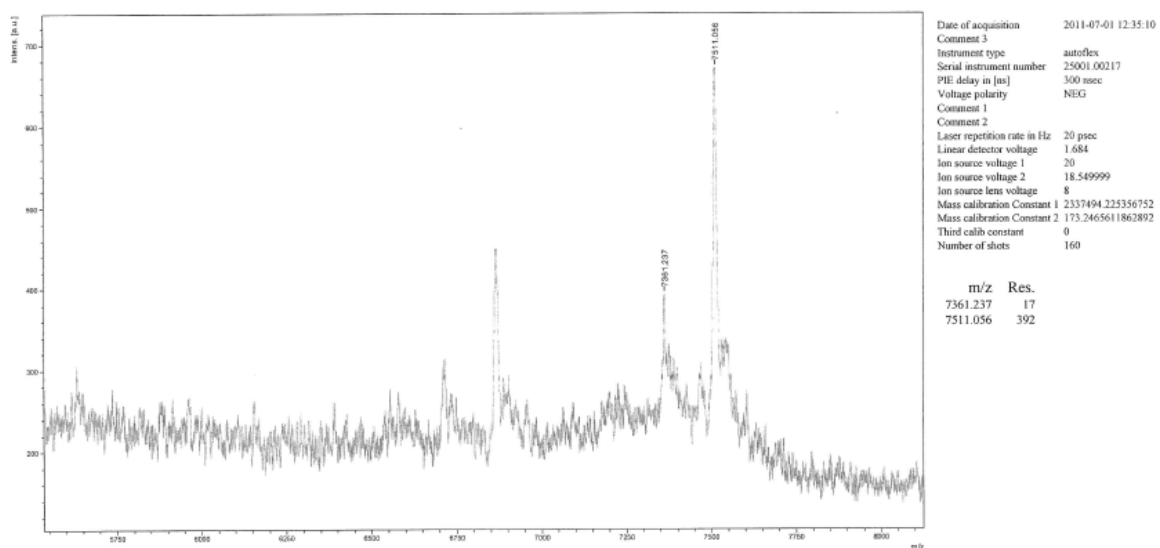
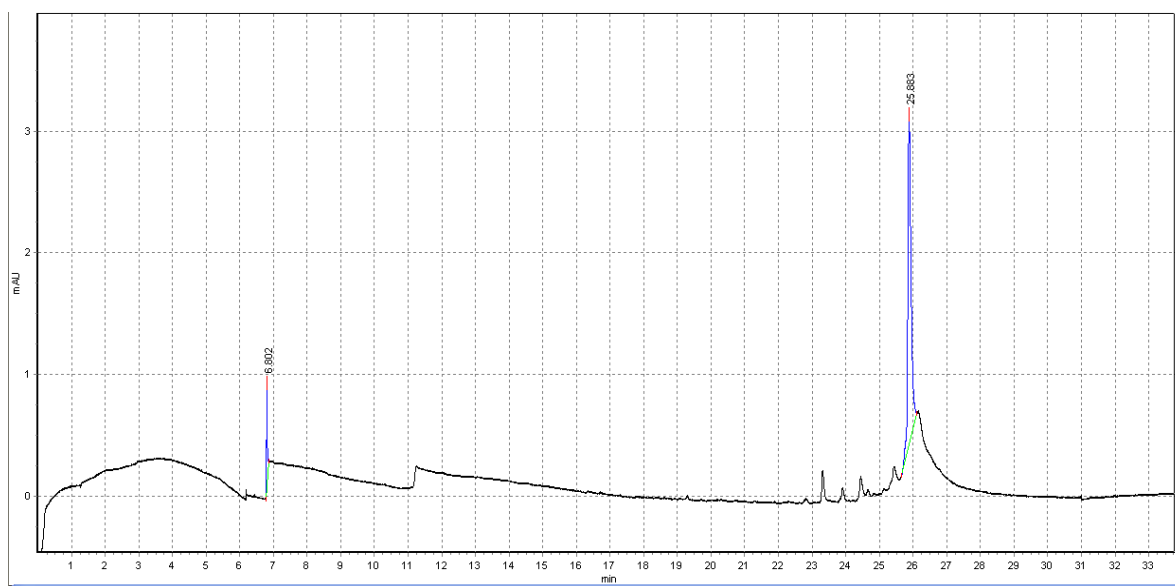
Date of acquisition 2011-07-01 12:48:13  
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Serial instrument number 25001.00217  
PIE delay in [ns] 300 nsec  
Voltage polarity NEG  
Comment 1  
Comment 2  
Laser repetition rate in Hz 20 psec  
Linear detector voltage 1.684  
Ion source voltage 1 20  
Ion source voltage 2 18.549999  
Ion source lens voltage 8  
Mass calibration Constant 1 2337889.698334305  
Mass calibration Constant 2 187.1470496604918  
Third calib constant 0  
Number of shots 80

m/z	Res.
8820.041	491

MS MALDI calculated mass 8820,717; found m/z 8820,041 m/z

OND – 8

capillary electrophoresis



MS MALDI calculated mass 7511,3; found  $m/z$  7511,056  $m/z$ ;

## Calculated the level of loading of CPG with functional groups

**Table 1.** The rate of loading calculated on the basis of UV measurements at 504 nm wavelength.

Support	Mass [mg]	1 attach	2 attach	Absolute loading [ $\mu\text{mol/g}$ ]	Background-corrected loading [ $\mu\text{mol/g}$ ]
1	6.00	1.36	1.24	32.48	27.21
2	7.84	1.73	1.57	31.45	27.42
3	7.22	1.63	1.43	32.33	27.96
blank	7.58	0.22	0.25	4.16	-
Average loading of support				31.96	27.31

**Table 2.** The rate of loading calculated on the basis of UV measurements.

Probe	Mass [mg]	Reaction time [h]	Absorbance	Loading of support [ $\mu\text{mol/g}$ ]
I	2.45	1	0.546	31.837
II	3.49	2	0.753	30.823
III	5.93	12	1.247	30.041

Microscope image of the CPG particles with length bar

