Supplementary Material (ESI) for Chemical Communications

Supporting Information

Building Addressable libraries: A site-selective click reaction strategy for rapidly assembling mass spec cleavable linkers

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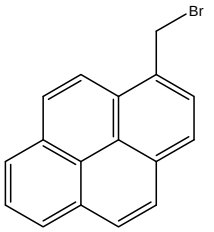
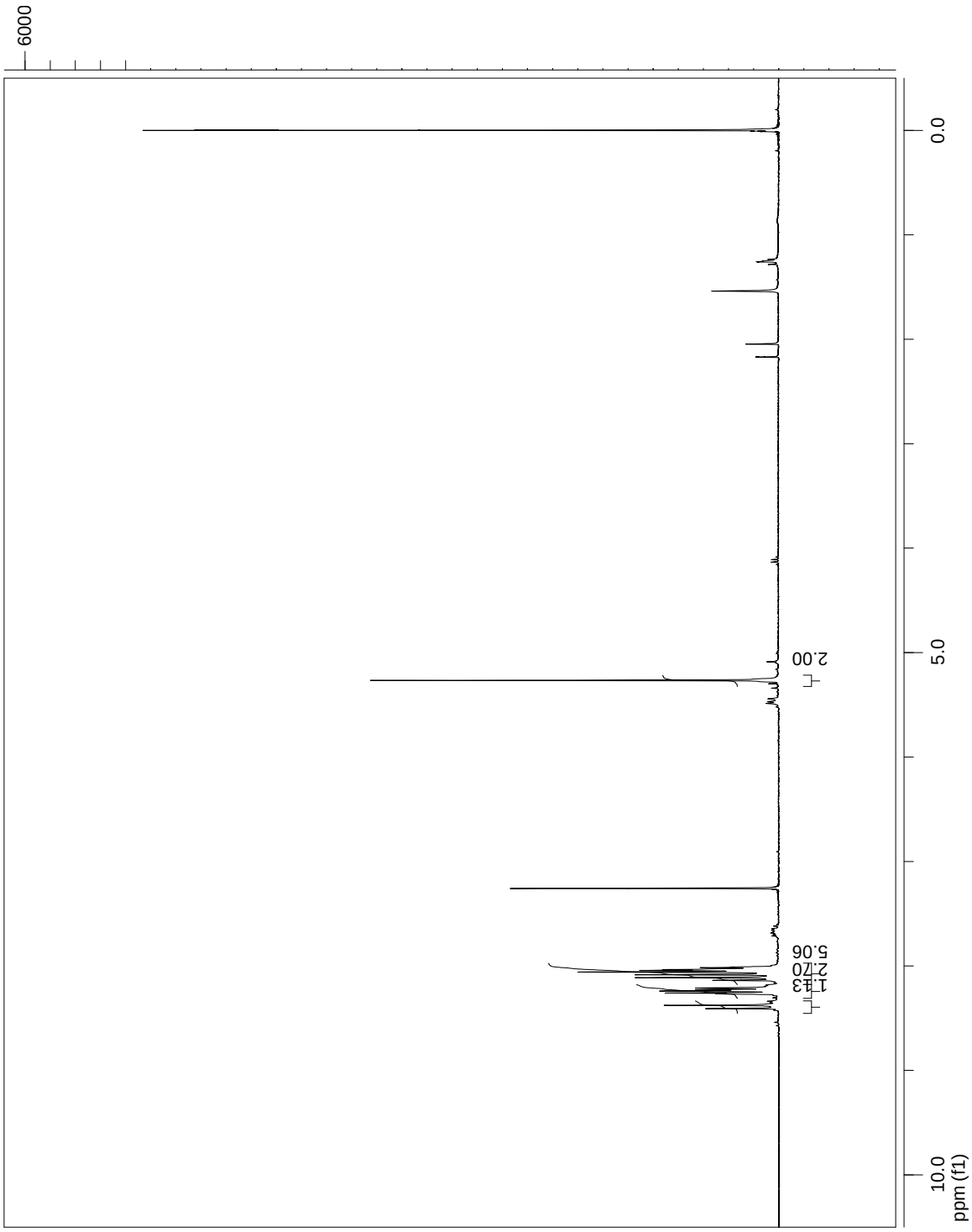
Synthesis of compounds -

General: 1-Pyrenemethanol, sodium azide, N-hydroxysuccinimide, phosphorus tribromide (1.0 M in CH_2Cl_2), 4-pentynoic acid, copper (II) sulfate (anhydrous powder), dimethylaminopyridine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), vitamin B_{12} (cyanocobalamin), tetramethylammonium nitrate, tetrabutylammonium bromide, anhydrous DMF, MeOH, and MeCN were purchased from Aldrich and used as received. Methylene chloride was dried over calcium hydride. Sulfonated bathophenanthroline (sodium salt) was purchased from GFS Chemicals and used as received. ^{1}H and ^{13}C NMR were obtained on a Varian Mercury (300 MHz). Fluorescence images in Figure 2 were taken with an Olympus IX70 epifluorescence microscope (pyrene filter excitation wavelengths 320-400 nm, emission 420-540 nm and red excitation filter wavelengths 510-550 nm, emission 590 nm. Microelectrode arrays were spin coated with a spin coater spin-coater MODEL WS-400B-6NPP/LITE. The time of flight secondary ion mass spectra was obtained using a TOF-SIMS IV (ION-TOF Inc).

Compound 1



1-(bromomethyl)pyrene (1)- 1-Pyrenemethanol (500 mg, 2.15 mmol) was added to a flame dried round bottom flask under argon. It was dissolved in 14 mL CH_2Cl_2. Phosphorus tribromide (3.6 mL, 3.6 mmol) was added to the reaction over a 30 minute period. After addition, the reaction mixture was allowed to stir at room temperature for 4 hours. The mixture was then poured over 50 g of ice and extracted with CH_2Cl_2. Organic extractions were combined, dried ($MgSO_4$) and concentrated. The crude residue was purified via silica gel chromatography (4:1:0.5 hexane/ EtOAc/MeOH) to provide the desired compound as a light yellow solid (The compound was used as is despite minor impurities seen in NMR.) (537.9 mg, 85% yield). IR (thin film, KBr plate): 3041, 2918, 1588, 1203, 842 636 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 8.39 (d, J= 10 Hz, 1H), 8.20-8.27 (m, 3H), 8.00-8.14 (m, 5H), 5.20 (s, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 131.7, 131.0, 130.6, 130.2, 128.0, 127.8, 127.5, 127.1, 126.1, 125.4, 124.9, 124.6, 124.7, 124.4, 122.6, 32.1 ppm; HRMS (FAB): *m/z* calculated for $C_{17}H_{11}Br$: 294.0044, measured: [M-Br] : 159.455.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10.0
ppm (f1)
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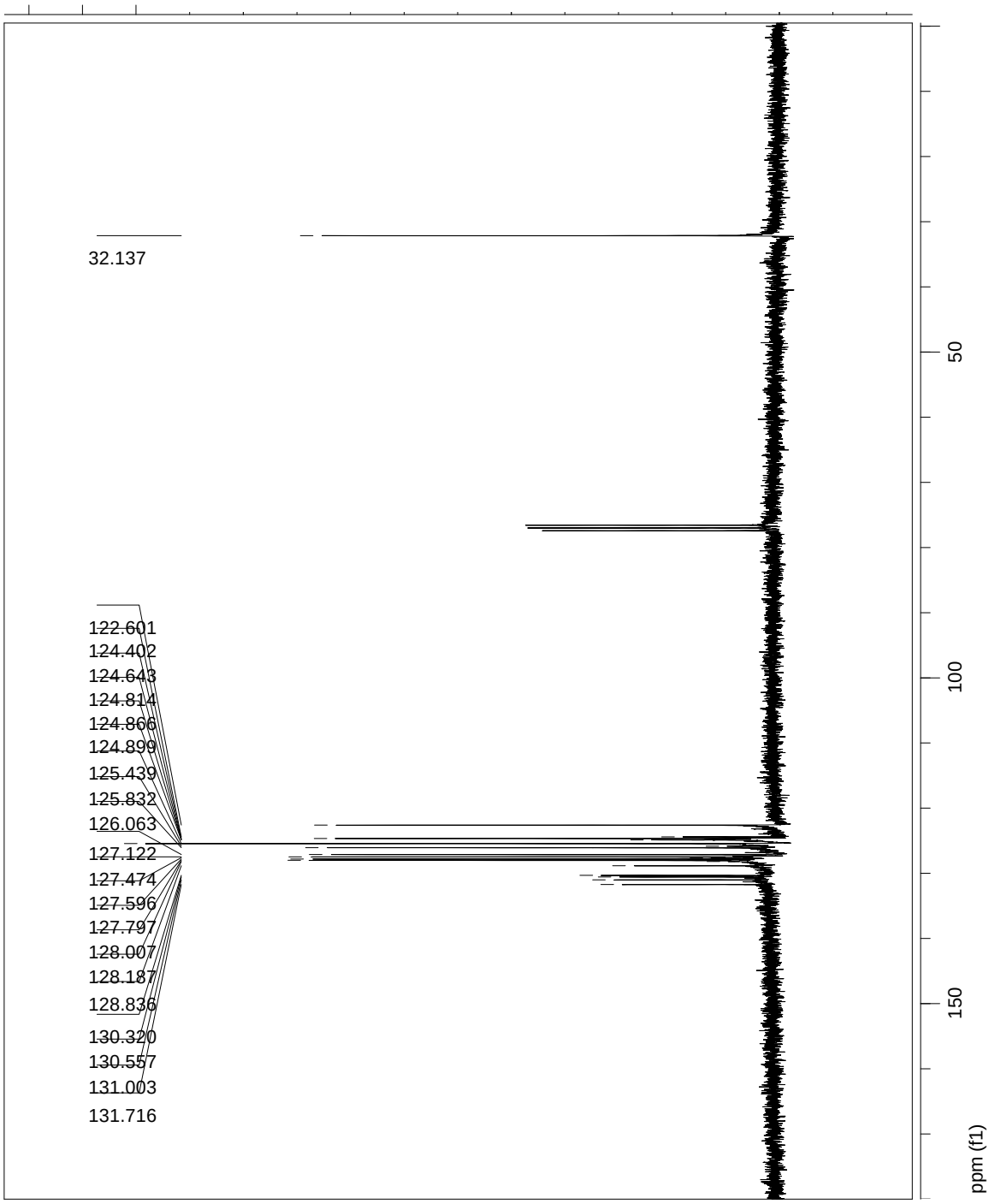
1.43
2.20
5.06


32.137
122.601
124.402
124.643
124.814
124.866
124.899
125.439
125.832
126.063
127.122
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127.596
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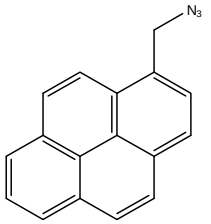
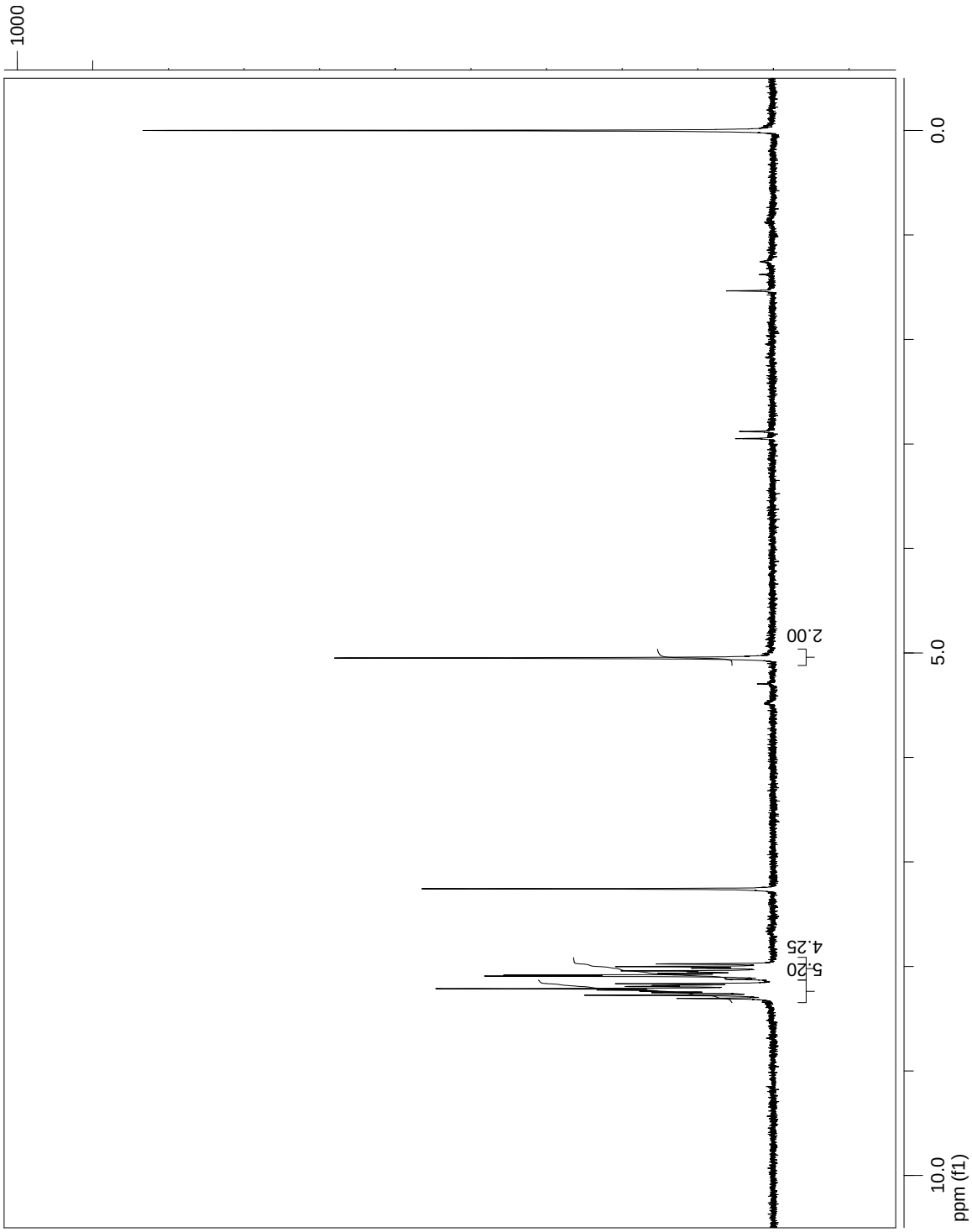
131.716
50
100
150
ppm (f1)

Compound 5



1-(azidomethyl)pyrene (5)- (1) (537.9 mg, 1.83 mmol) and NaN_3 (616.3 mg, 9.48 mmol) were added to a flame dried round bottom flask under argon. 5.2 mL of DMF was added to the mixture. The reaction mixture was placed in an oil bath and heated at 80^o for 12 hours. It was then poured into 100 mL water and extracted with CH_2Cl_2 (3 x 25 mL). Combined organic extractions were dried ($MgSO_4$) and concentrated to yield a crude solid. The crude residue was purified via silica gel chromatography (9:1 hexane/EtOAc) to provide the desired compound as light orange solid (409 mg, 87 % yield). IR (thin film, KBr plate): 3041, 2918, 2099, 1231, 842 706 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 8.16-8.31 (m, 5H), 7.96-8.12 (m, 4H), 5.05 (s, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 131.4, 130.9, 130.4, 128.8, 128.0, 127.9, 127.5, 127.0, 125.8, 125.3, 125.2, 124.7, 124.3, 122.3, 52.8 ppm; HRMS (FAB): *m/z* calculated for $C_{17}H_{11}N_3$: 257.0953, measured: 257.0955.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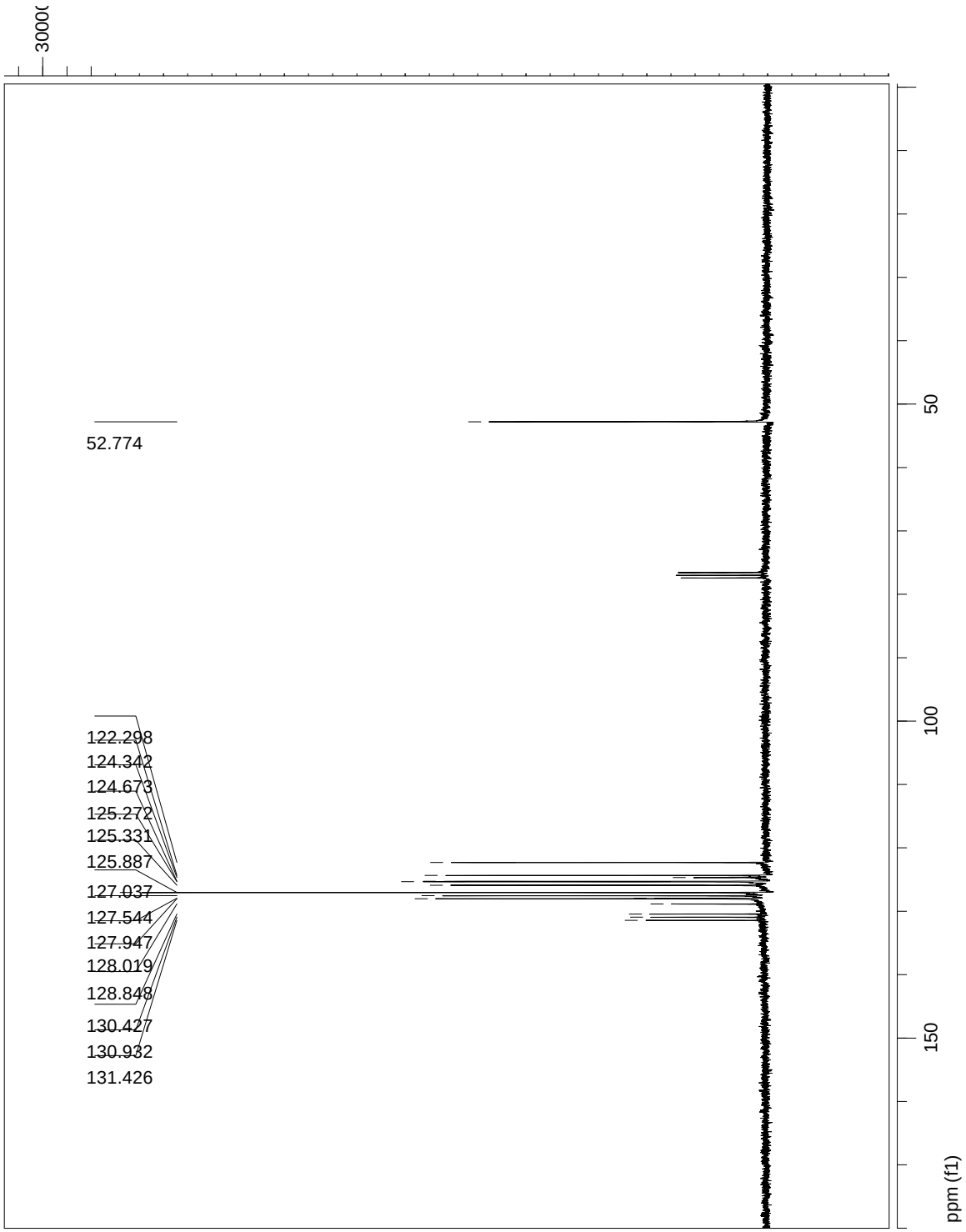
ppm (f1)
2.00
5.20
4.25


52.774
122.298
124.342
124.673
125.272
125.331
125.887
127.037
127.544
127.947
128.019
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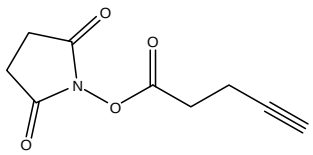
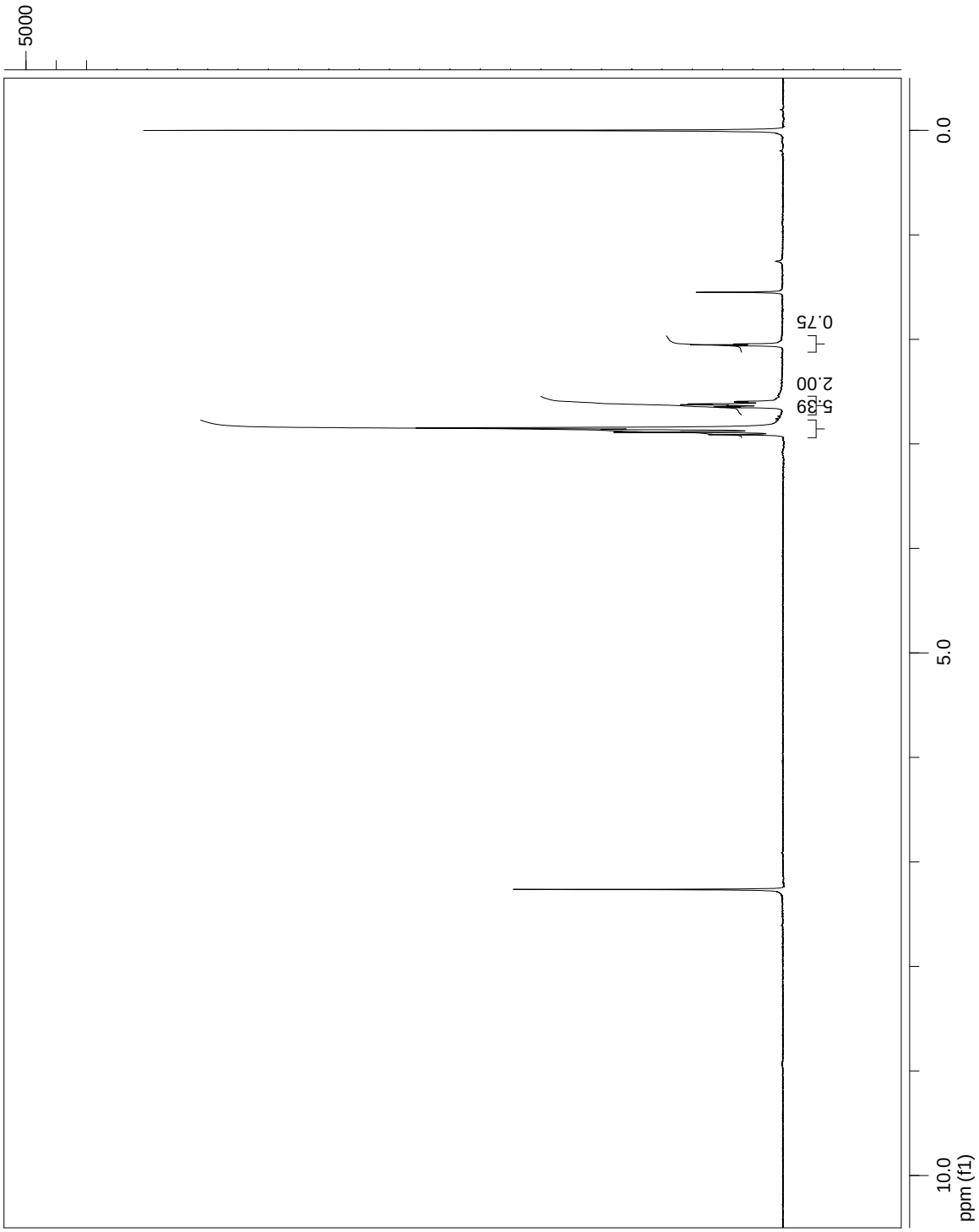
131.426
50
100
150
ppm (f1)

Compound 3



N-succinimidyl pent-4-ynoate (3)- 4-Pentynoic acid (981 mg, 10 mmol), N-hydroxysuccinimide (1.15 g, 10 mmol), and DMAP (small crystal) were added to a flame dried round bottom flask under argon. The reaction mixture was dissolved into 13 mL of CH_2Cl_2 and cooled to 0^o. Once cooled, EDC (1.8 mL, 10.2 mmol) was added. The reaction stirred at 0^o for 10 minutes then stirred at room temperature overnight. The reaction was diluted with CH_2Cl_2 (20 mL) and washed with 0.5 M HCl (25 mL), saturated $NaHCO_3$ (25 mL), dried ($MgSO_4$) and concentrated. The crude residue was purified by silica gel chromatography (1:1 hexane/ EtOAc) to provide the desired compound as a white, crystalline solid (742.3 mg, 83 % yield). IR (thin film, KBr plate): 3282, 2924, 1814, 1784, 1734, 1372, 1206, 1069, 647 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 2.82-2.91(m, 6H), 2.62 (td, J = 7 Hz, J = 3 Hz, 2H), 2.5 (t, J = 3 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 168.8, 166.9, 80.8, 69.9, 30.5, 25.5, 14.0 ppm; HRMS (FAB): *m/z* calculated for $C_9H_9NO_4$: 195.0532, measured [M+Na]+: 218.0424.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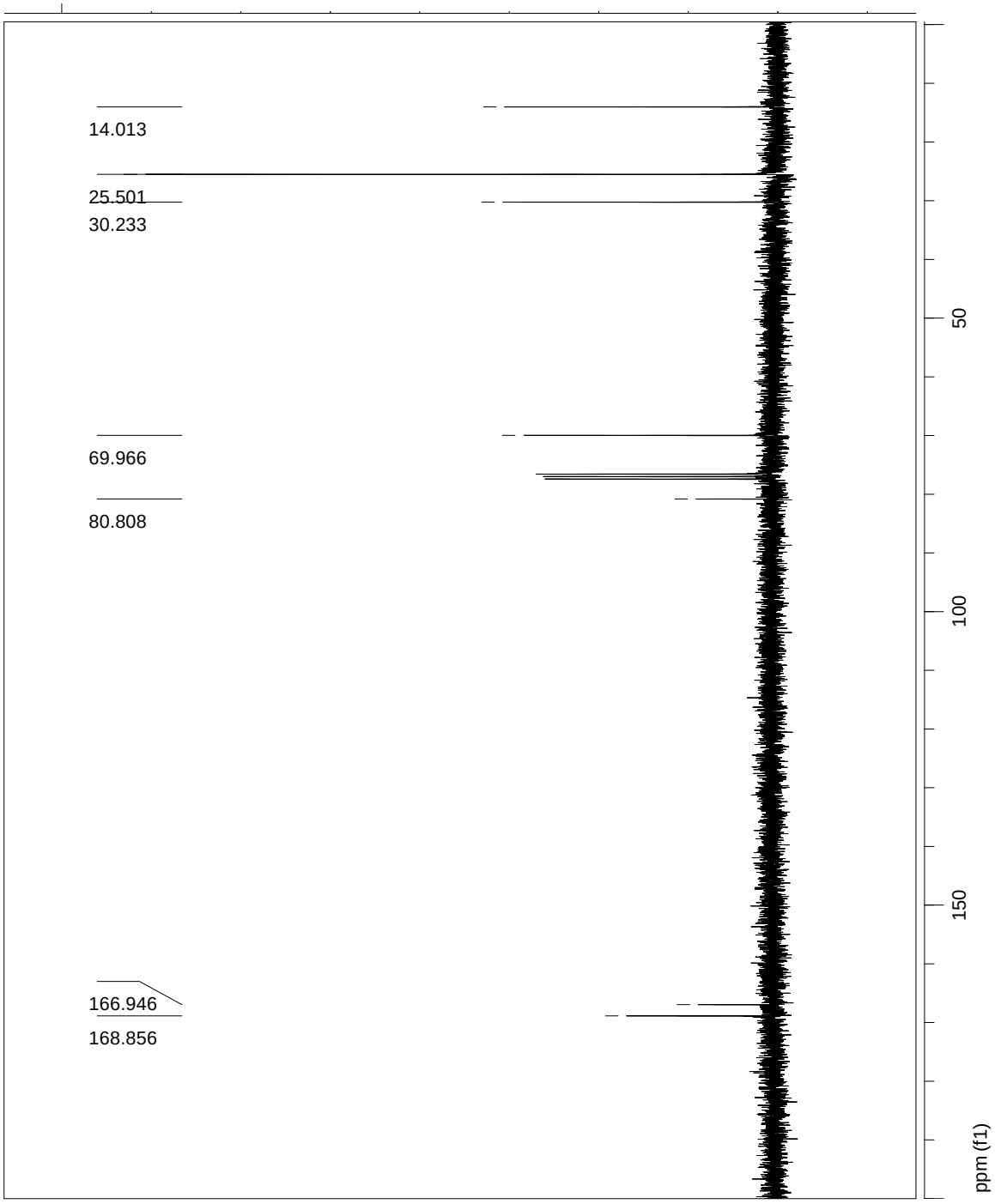
5.89


14.013
25.501
30.233
69.966
80.808
166.946
168.856
50
100
150
ppm (f1)

General procedure for chip based 'Click' reaction on 1K chip

- Agarose Coating on Chip:

A 1K microelectrode chip was spin coated (2000 rpm, 45s) with a 4% agarose solution (9:1 DMF/Water) and let to dry for 1 hour.

- Substrate Loading on Agarose Polymer:

8.0 mg of **(2)** was dissolved into 100 μL of DMF in an Eppendorf tube. 1.5 mL of MeOH containing 2.77 mg Vitamin B_{12} and 13.6 mg tetramethylammonium nitrate was added to the substrate and mixed. The chip was immediately incubated in this solution and all the electrodes were turned on at -2.4 V cycling 0.5 sec on and 0.1 sec off for 400 cycles. The chip was then washed with excess EtOH and let to dry.

- Cathodic Reduction:

8.0 mg each of **(4)** and tetrabutylammonium bromide were dissolved into 100 μL of DMF in an Eppendorf tube as well as 6 μL each of a 25 mM solution of $CuSO_4$ and 50 mM solution of sulfonated bathophenanthroline (Na salt). The DMF mixture was dissolved into 1.5 mL of a 7:2:1 mix of MeCN/DMF/Water. The chip prepared above was incubated in this solution. A checkerboard pattern was turned on and the chip was pulsed at -2.4 V relative to a remote Pt wire, cycling 0.5 sec on and 0.1 sec off for 400 cycles. The chip was then washed with EtOH and let to dry. The chips were visualized with a fluorescence microscope (either the pyrene blue or red filter could be used.

General procedure for chip based 'Click' reaction on a 12K slide:

-Agarose Coating on a slide:

A 12K microelectrode slide was spin coated (1500 rpm, 30s) with a 4% agarose solution and let to dry for 1 hour.

- Substrate Loading on Agarose Polymer:

The solution was prepared the same as for the 1K chip. The slide was then exposed to 100 µL of the reaction solution and a -1.5 V current was applied for 60 s. The slide was washed with EtOH and let to dry.

Cathodic Reduction:

The solution was prepared the same as for the 1K chip. The slide was exposed to 100 µL of the reaction solution and selected electrodes were grounded, while a potential of -2.4 V was applied to the auxiliary electrode for 90 s. The slide was washed with EtOH then visualized with a fluorescence microscope, either the pyrene blue or red filter could be used.

Time-of-Flight Secondary Ion Mass Spectrometry

Time-of-flight secondary ion mass spectra were obtained using a TOF-SIMS IV (ION-TOF Inc). The instrument consists of a loadlock, a preparation chamber and an analysis chamber, each separated by a gate valve. The preparation and analysis chambers were kept under ultra high vacuum (10^{-9} mbar). The primary ion beam was generated using a Bi+ liquid metal ion gun. The primary ions were mass selected using their flight time between two deflection plates. The energy of primary ion beam was 25 keV. The secondary ions generated were extracted into a time-of-flight mass spectrometer using an extraction voltage of 2000V. Before reaching the detector consisting of a multi channel plate/scintillator/photomultiplier tube, the secondary ions were reaccelerated to 10 keV energy. Analyzed sample areas were (500 x 500 μm^2) on chip surface. Positive secondary ion mass spectra were collected for each sample. The primary ion dose during data acquisition was less than 109 ions/cm^2, which corresponds to static SIMS. All the spectra were internally calibrated using H-, C-, CH-, CH2-, CH3- and C2- peaks.

Figure 4a.) Full ToF-SIMS for Agarose

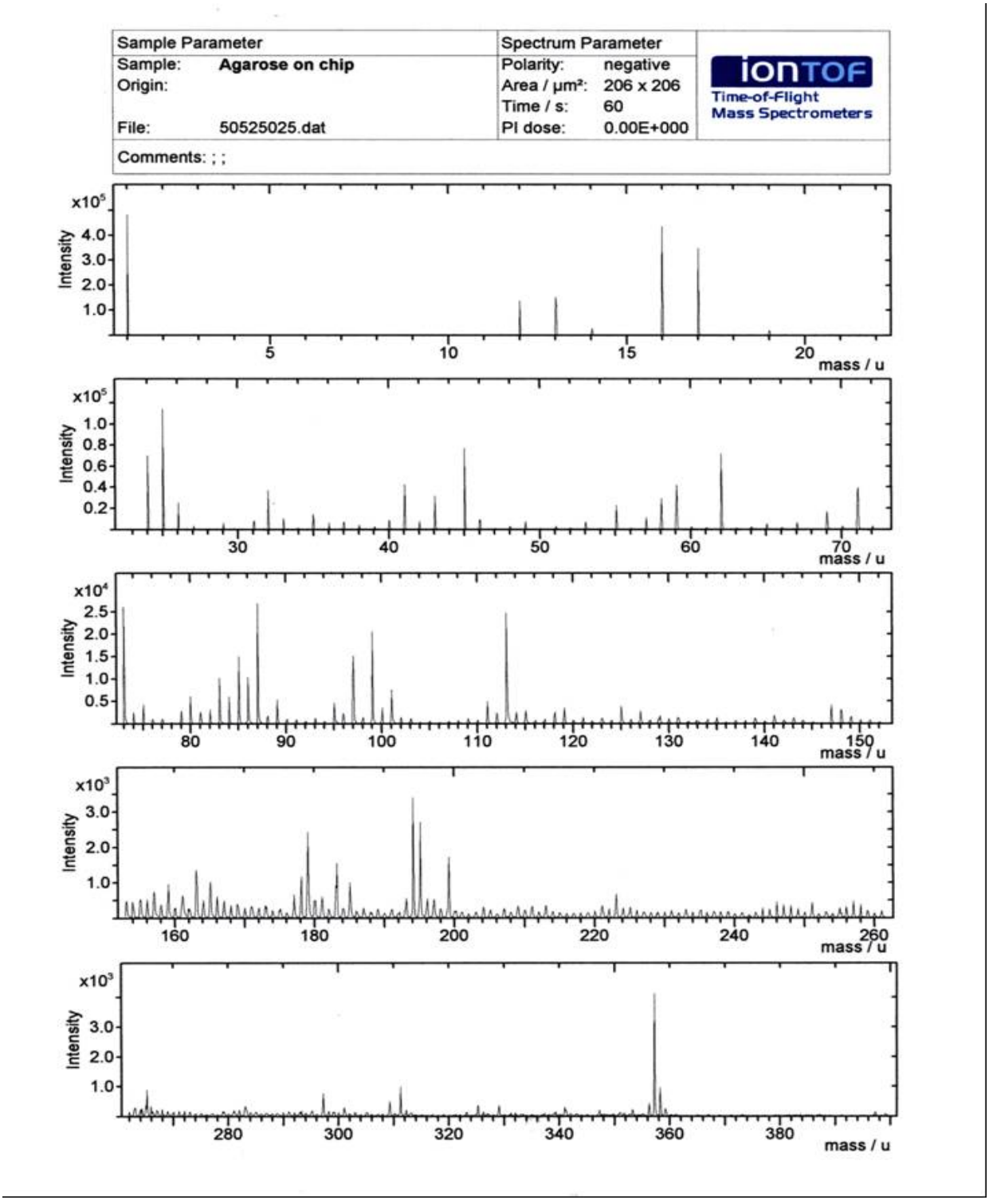


Figure 4b.) Full TOF-SIMS for agarose functionalized with the "click-reaction"