

Fluorogenic “photoclick” labelling of DNA using a Cy3 dye

Benjamin Lehmann and Hans-Achim Wagenknecht*

Supporting Information

Institute of Organic Chemistry
Karlsruhe Institute of Technology (KIT)
Fritz-Haber-Weg 6
76131 Karlsruhe, Germany,
E-mail: Wagenknecht@kit.edu

List of contents

1. Materials and Methods.....	2
2. Synthetic procedures	3
3. DNA synthesis	13
4. “Photoclick” experiments	14
5. Kinetic measurements	18
6. Images of NMR and mass spectra.....	21
7. References	41

1. Materials and Methods

All chemicals and solvents were purchased from *ABCR, Acros Organics, Alfa Aesar, Fisher, Fluka, Sigma Aldrich, TCI Chemicals* and *VWR* and used without further purification according to suppliers' procedures. The sulfonated Cy3 dye was purchased from *lumiprobe*. Water for HPLC-separations and DNA experiments was deionized and ultra-filtrated by a *Millipore Q8* water purification system.

Moisture and air sensitive reactions were performed in vacuum-dried round-bottom flasks under dry argon atmosphere. Solvents were removed under reduced pressure and 40 °C. Light sensitive reactions were handled in aluminium foil wrapped or brown glassware.

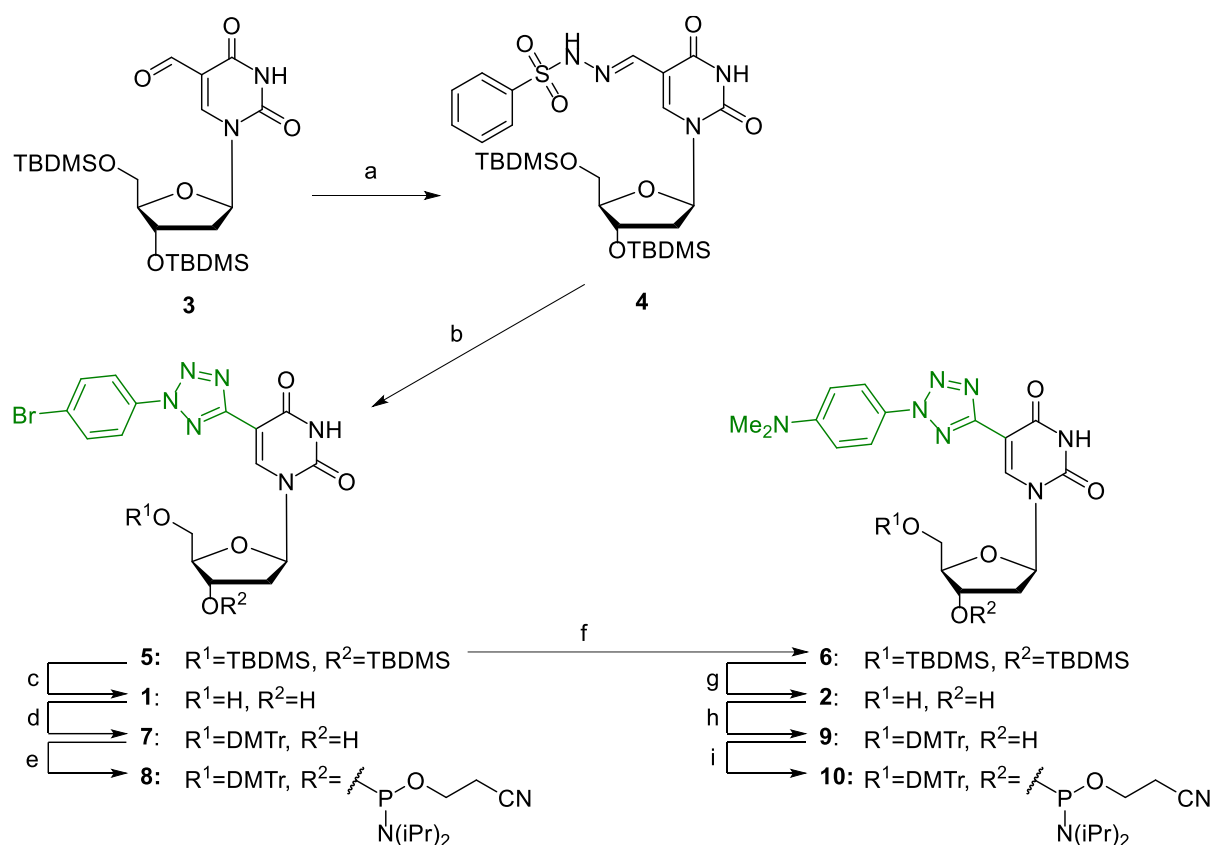
Reaction progress was monitored by TLC on silica gel coated aluminium plates (*Merck*, silica gel 60, thickness 0.2 mm, F254). TLC plates were analyzed by irradiation with UV light ($\lambda_{\text{exc}} = 254 \text{ nm}$) and staining with 5% H_2SO_4 in MeOH. Flash chromatography was carried out with silica gel 60 from *Sigma Aldrich* (43 – 60 μm).

Spectroscopic measurements were recorded in 10 mM Na- P_i buffer solution containing 250 mM NaCl and 2.5 μM DNA in 10 mm quartz glass cuvettes at 20 °C. Absorption spectra were recorded with a *Perkin Elmer Lambda 750 UV/Vis/NIR spectrophotometer* and baseline corrected. Fluorescence was measured with a *Horiba Scientific Fluoromax-4* spectrofluorometer (increment: 0.1 nm, increment time: 0.2s, slits: 7 nm) and corrected against the raman peak of pure water ($\lambda = 397 \text{ nm}$) and the raman scattering of the pure solvent.

NMR spectra were recorded in deuterated DMSO-d_6 at a *Bruker Avance 400* (400 MHz ^1H -NMR, 101 MHz ^{13}C -NMR) or *Bruker Avance 500* (500 MHz ^1H -NMR, 126 MHz ^{13}C -NMR, 202 MHz ^{31}P -NMR) and calibrated to the solvent signal at $\delta = 2.50 \text{ ppm}$ (^1H) and $\delta = 39.52 \text{ ppm}$ (^{13}C). Coupling patterns were described as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = doublet of doublet, m = multiplet.

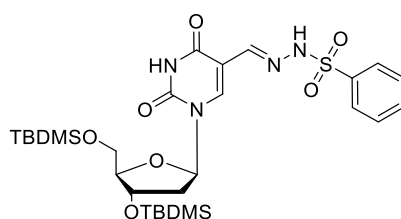
Molecular mass was measured by *fast atom bombardment* (FAB, Finnigan MAT 95 mass spectrometer), *matrix assisted laser desorption ionization* (MALDI, *Shimadzu AXIMA Confidence*) with 2,4,6-trihydroxyacetophenon (THAP, 0.3 M in ethanol) as matrix. DNA masses were analyzed via MALDI-TOF with 3-Hydroxypicolinic acid (HPA) and ESI (*LTQ-XL Orbitrap spectrometer, Thermo Scientific*).

2. Synthetic procedures



Scheme S1. Synthesis of nucleosides **1** and **2** and their phosphoramidites **8** and **10** as DNA building blocks. a) benzenesulfonyl hydrazide, EtOH, r.t., 1 h, 84%; b) 4-bromobenzenediazonium tetrafluoroborate, pyridine, -10 °C – r.t., 16 h, 58%; c) TBAF, THF, 15 min, 86%; d) DMTrCl, pyridine, 2 h, 89%; (e) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA, CH₂Cl₂, 2 h, 78%; f) HNMe₂·HCl, NaOtBu, Pd(dba)₂, JohnPhos, toluene, 40 °C, 18 h, 29%; g) TBAF, THF, 10 min, 74%; h) DMTrCl, pyridine, 2 h, 93%; i) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, DIPEA, CH₂Cl₂, 2 h, 40%.

Compound 4



Chemical Formula: $C_{28}H_{46}N_4O_7SSi_2$

Molecular Weight: 638,93

Benzenesulfonyl hydrazide (1.00 eq, 1.10 g, 6.38 mmol) was added to a solution of 2'-deoxy-5-formyluridine **3** (1.00 eq, 3.10 g, 6.38 mmol) in EtOH and stirred for 1 hour at room temperature. After filtration, the precipitate was washed with cold EtOH and dried in vacuum to give compound **4** as colorless solid (3.42 g, 5.36 mmol, 84% yield).

TLC (hexane/ethyl acetate = 2/1): $R_f = 0.21$.

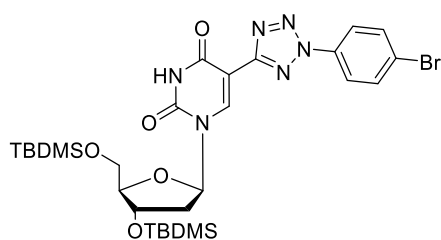
1H NMR (500 MHz, DMSO) δ (ppm) = 11.65 (s, 1H), 11.41 (s, 1H), 7.87 – 7.79 (m, 3H), 7.77 (s, 1H), 7.70 – 7.57 (m, 3H), 6.00 (t, $J = 6.5$ Hz, 1H), 4.37 – 4.31 (m, 1H), 3.93 – 3.87 (m, 1H), 3.72 – 3.64 (m, 2H), 2.23 (t, $J = 5.8$ Hz, 2H), 0.85 (d, $J = 26.0$ Hz, 18H), 0.12 – 0.00 (m, 12H).

^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) = 161.7, 149.5, 140.2, 139.2, 136.9, 133.1, 129.2, 127.0, 107.2, 87.5, 86.1, 72.3, 62.9, 25.7, 25.7, 17.9, 17.7, -4.8, -4.9, -5.5, -5.6.

FAB MS: m/z (%): 639.2 (18) [MH^+].

HRMS ($C_{28}H_{47}N_4O_7SSi_2$): calc. 639.2699 [MH^+], found 639.2699.

Compound 5



Chemical Formula: $C_{28}H_{43}BrN_6O_5Si_2$
Molecular Weight: 679,76

A solution of **4** (1.00 eq, 2.45 g, 3.83 mmol) in 30 mL pyridine was degassed and cooled down to $-10^{\circ}C$. Bromobenzene diazonium tetrafluoroborate (1.20 eq, 1.25 g, 4.60 mmol) was added in portions and the mixture stirred for another 30 minutes at $-10^{\circ}C$. The mixture was slowly warmed to room temperature and stirred for another 16 hours. After dilution with 100 mL ethyl acetate, the solution was washed 2 times with 80 mL of 1M hydrochloric acid and 80 mL of saturated sodium bicarbonate solution. The combined organic phases were dried over Na_2SO_4 and solvents were removed under reduced pressure. Flash chromatography (SiO_2 , hexane/ethyl acetate, 4/1) yields **5** a red foam (1.51 g, 2.22 mmol, 58% yield).

TLC (hexane/ethyl acetate = 2/1): $R_f = 0.38$.

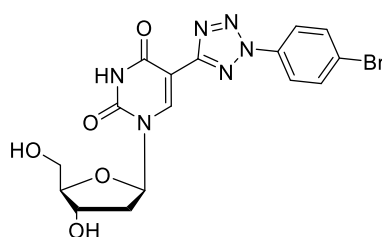
1H NMR (400 MHz, DMSO) δ (ppm) = 11.78 (s, 1H), 8.27 (s, 1H), 7.99 – 7.93 (m, 2H), 7.84 – 7.78 (m, 2H), 6.07 (t, $J = 6.5$ Hz, 1H), 4.33 – 4.26 (m, 1H), 3.82 (q, $J = 3.5$ Hz, 1H), 3.72 (dd, $J = 11.5, 3.6$ Hz, 1H), 3.64 (dd, $J = 11.5, 3.6$ Hz, 1H), 2.26 – 2.13 (m, 2H), 0.79 (s, 8H), 0.63 (s, 8H), 0.00 (d, $J = 2.3$ Hz, 6H), -0.13 (d, $J = 15.2$ Hz, 6H).

^{13}C NMR (101 MHz, DMSO- d_6) δ (ppm) = 160.0, 159.8, 149.6, 141.7, 135.2, 133.2, 123.1, 121.8, 101.5, 87.3, 85.4, 71.7, 62.4, 25.7, 25.6, 17.9, 17.7, -4.8, -5.0, -5.7, -5.7.

FAB MS: m/z (%): 681.2 (100) [MH^+].

HRMS ($C_{28}H_{44}BrN_6O_5Si_2$): calc. 679.2090 [MH^+], found 679.2089.

Compound 1



Chemical Formula: $C_{16}H_{15}BrN_6O_5$
Molecular Weight: 451,24

TBAF (2.50 eq, 2.76 mL, 2.76 mmol, 1M in THF) was added to a solution of **5** (1.00 eq, 750 mg, 1.10 mmol) in 18 mL THF. After 15 minutes, a spatula of silica gel was added and the solvents were removed under reduced pressure. Flash chromatography (SiO_2 , DCM/MeOH, 9/1) yields **1** as a colorless foam (427 mg, 0.946 mmol, 86% yield).

1H NMR (500 MHz, DMSO) δ (ppm) = 11.81 (s, 1H), 8.80 (s, 1H), 8.05 (d, J = 8.4 Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H), 6.21 (t, J = 6.3 Hz, 1H), 5.30 (d, J = 4.3 Hz, 1H), 5.08 (t, J = 4.7 Hz, 1H), 4.34 – 4.25 (m, 1H), 3.86 (d, J = 3.3 Hz, 1H), 3.61 (m, 2H), 3.16 (d, J = 4.8 Hz, 1H), 2.23 (t, J = 5.6 Hz, 2H).

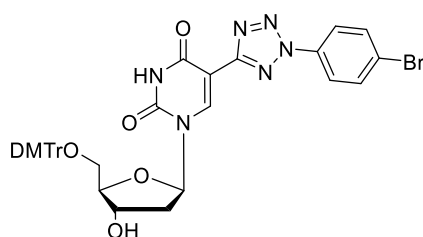
^{13}C NMR (126 MHz, DMSO- d_6) δ (ppm) = 160.0, 159.9, 149.7, 142.5, 135.3, 133.1, 123.0, 121.8, 101.5, 87.8, 85.2, 70.2, 60.9, 48.6.

FAB MS: m/z (%): 451.1 (25) [MH^+].

HRMS ($C_{16}H_{16}BrN_6O_5$): calc. 451.0366 [MH^+], found 451.0364.

UV/Vis: ϵ_{260nm} = 13370 $M^{-1}cm^{-1}$, ϵ_{287nm} = 22480 $M^{-1}cm^{-1}$.

Compound 7



Chemical Formula: $C_{37}H_{33}BrN_6O_7$
Molecular Weight: 753,61

1 (1.00 eq, 500 mg, 1.11 mmol) was coevaporated three times with dry pyridine and solved in 15 mL of dry pyridine. 4,4'-Dimethoxytrityl chloride (1.10 eq, 413 mg, 1.22 mmol) was added in portions and the mixture stirred for another 2 hours at room temperature. After stopping the reaction with MeOH, the solvents were removed under reduced pressure. Flash chromatography (SiO_2 , DCM/MeOH, 50/1 + 0.1% Et_3N) yielded **7** as a colorless foam (745 mg, 0.988 mmol, 89% yield).

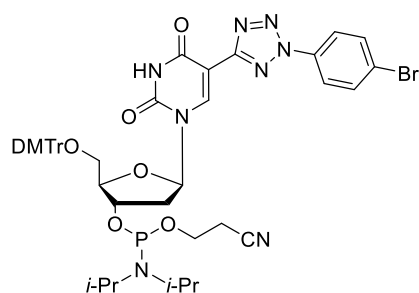
1H NMR (500 MHz, DMSO) δ (ppm) = 11.88 (s, 1H), 8.46 (s, 1H), 7.72 (d, J = 4.0 Hz, 2H), 7.37 (s, 2H), 7.33 (d, J = 7.8 Hz, 2H), 7.24 – 7.15 (m, 6H), 7.09 – 7.06 (m, 2H), 6.87 – 6.83 (m, 2H), 6.72 (d, J = 8.6 Hz, 4H), 6.19 (t, J = 6.2 Hz, 1H), 5.39 (d, J = 4.5 Hz, 1H), 4.31 (quin, J = 4.8 Hz, 1H), 3.96 (q, J = 3.7 Hz, 1H), 3.65 (s, 6H), 2.38 – 2.31 (m, 2H).

^{13}C NMR (126 MHz, DMSO) δ (ppm) = 160.0, 159.5, 157.9, 157.8, 149.6, 148.4, 144.5, 140.2, 135.5, 135.3, 135.0, 132.8, 129.6, 129.5, 128.9, 128.3, 127.7, 127.6, 127.6, 127.4, 126.4, 122.6, 121.5, 113.0, 112.9, 112.8, 101.6, 85.8, 85.8, 85.5, 79.9, 69.7, 55.0, 54.5.

FAB MS: m/z (%): 754.2 (100) [MH^+].

HRMS ($C_{37}H_{34}BrN_6O_7$): calc. 752.1594 [MH^+], found 752.1596.

Compound 8



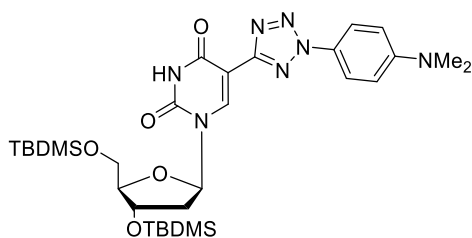
Chemical Formula: $C_{46}H_{50}BrN_8O_8P$
Molecular Weight: 953,83

DIPEA (3.00 eq, 168 μ L, 0.987 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (1.50 eq, 110 μ L, 0.494 mmol) were added to a solution of **7** (1.00 eq, 248 mg, 0.329 mmol) in dry DCM. After stirring for 2 hours at room temperature, the mixture was directly transferred to a flash column. Flash chromatography (SiO_2 , DCM/MeOH, 20/1 + 0.1% Et_3N) yielded phosphoramidite building block **8** as a colorless foam (245 mg, 0.257 mmol, 78% yield).

^{31}P NMR (202 MHz, $DMSO-d_6$) δ (ppm) = 147.62, 147.32.

MALDI MS: m/z: calc. for $C_{46}H_{51}BrN_6O_8P$ [MH^+-N_2] 925.27, found 927.60 [MH^+-N_2],

Compound 6



Chemical Formula: C₃₀H₄₉N₇O₅Si₂
Molecular Weight: 643,94

5 (1.00 eq, 250 mg, 0.368 mmol) was lyophilized in a Schlenk flask in benzene overnight. Pd(dba)₂ (0.05 eq, 10.6 mg, 0.0184 mmol) and JohnPhos (0.10 eq, 17.5 mg, 0.0368 mmol) were added and the flask was repeatedly decompressed and refilled with argon. Dimethylamine hydrochloride (5.00 eq, 149.9 mg, 1.84 mmol) and sodium tert-butoxide (6.00 eq, 369.2 mg, 2.21 mmol) were added and the flask sealed. After addition of 5 mL dry toluene the suspension was stirred vigorously for 16 hours at 40 °C. The resulting violet mixture was diluted with twice the volume of diethyl ether and poured into saturated NH₄Cl solution. The aqueous phase was extracted three times with 20 mL of diethyl ether and the organic phases were combined and dried over anhydrous Na₂SO₄. After removal of the solvents under reduced pressure flash chromatography (SiO₂, hexane/ethyl acetate, 5/1) yields **6** as a purple foam (68.7 mg, 0.107 mmol, 29% yield).

TLC (hexane/ethyl acetate = 1/1): R_f = 0.30.

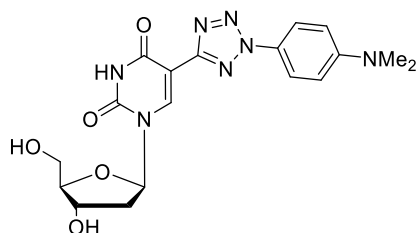
¹H NMR (500 MHz, Chloroform-*d*) δ (ppm) = 8.53 (s, 1H), 7.97 (d, *J* = 9.1 Hz, 2H), 6.76 (d, *J* = 9.2 Hz, 2H), 6.33 (dd, *J* = 7.8, 5.8 Hz, 1H), 4.44 (dt, *J* = 5.2, 2.4 Hz, 1H), 4.04 (q, *J* = 2.9 Hz, 1H), 3.87 (dd, *J* = 11.3, 3.3 Hz, 1H), 3.78 (dd, *J* = 11.3, 3.0 Hz, 1H), 3.04 (s, 6H), 2.46 – 2.39 (m, 1H), 2.18 – 2.07 (m, 1H), 0.90 (s, 9H), 0.78 (s, 9H), 0.09 (d, *J* = 5.5 Hz, 6H), 0.02 (d, *J* = 27.6 Hz, 6H).

¹³C NMR (126 MHz, CDCl₃) δ (ppm) = 160.0, 158.7, 151.2, 149.5, 141.3, 129.3, 128.6, 126.7, 121.5, 120.3, 113.3, 111.9, 103.7, 88.8, 86.6, 72.8, 63.2, 42.0, 40.5, 29.8, 26.1, 26.0, 25.9, 18.5, 18.2, 1.2, -4.5, -4.9, -5.4, -5.5.

FAB MS: m/z (%): 644.4 (12) [MH⁺].

HRMS (C₃₀H₅₀N₇O₅Si₂): calc. 664.3407 [MH⁺], found 664.3404.

Compound 2



Chemical Formula: $C_{18}H_{21}N_7O_5$
Molecular Weight: 415,41

TBAF (2.50 eq, 1.18 mL, 1.18 mmol, 1M in THF) was added to a solution of **6** (1.00 eq, 305 mg, 0.474 mmol) in 7 mL THF. After 10 minutes, a spatula of silica gel was added and the solvents were removed under reduced pressure. Flash chromatography (SiO_2 , DCM/MeOH, 9/1) yields **2** as a colorless foam (146 mg, 0.351 mmol, 74% yield).

TLC (DCM/MeOH, 20/1): $R_f = 0.12$.

1H NMR (500 MHz, $DMSO-d_6$) δ (ppm) = 11.76 (s, 1H), 8.69 (s, 1H), 7.87 (d, $J = 9.1$ Hz, 2H), 6.90 (d, $J = 9.3$ Hz, 2H), 6.21 (t, $J = 6.5$ Hz, 1H), 5.29 (d, $J = 4.3$ Hz, 1H), 5.06 (t, $J = 4.9$ Hz, 1H), 4.29 – 4.23 (m, 1H), 3.85 (q, $J = 3.4$ Hz, 1H), 3.59 (qt, $J = 11.7, 4.1$ Hz, 2H), 3.01 (s, 6H), 2.24 – 2.19 (m, 2H).

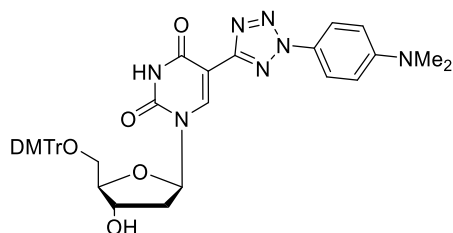
^{13}C NMR (126 MHz, DMSO) δ (ppm) = 160.1, 159.1, 151.1, 149.8, 142.0, 125.5, 121.0, 112.1, 102.1, 87.7, 85.1, 70.2, 61.0.

FAB MS: m/z (%): 416.2 (35) [MH^+].

HRMS ($C_{18}H_{22}N_7O_5$): calc. 416.1682 [MH^+], found 416.1681.

UV/Vis: $\epsilon_{260nm} = 5790 M^{-1}cm^{-1}$, $\epsilon_{320nm} = 10860 M^{-1}cm^{-1}$.

Compound 9



Chemical Formula: C₃₉H₃₉N₇O₇
Molecular Weight: 717,78

2 (1.00 eq, 500 mg, 1.20 mmol) was coevaporated three times with dry pyridine and solved in 15 mL of dry pyridine. 4,4'-Dimethoxytrityl chloride (1.20 eq, 489 mg, 1.44 mmol) was added in portions and the mixture stirred for another 2 hours at room temperature. After stopping the reaction with MeOH, the solvents were removed under reduced pressure. Flash chromatography (SiO₂, DCM/MeOH, 50/1 + 0.1% Et₃N) yielded **9** as a colorless foam (801 mg, 1.12 mmol, 93% yield).

TLC (DCM/MeOH, 20/1): R_f = 0.35.

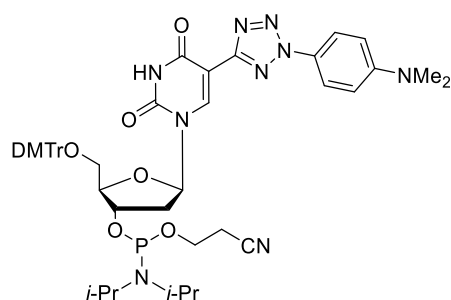
¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm) = 11.83 (s, 1H), 8.34 (s, 1H), 7.64 (d, *J* = 9.1 Hz, 2H), 7.37 (s, 2H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.24 – 7.12 (m, 6H), 7.08 (t, *J* = 7.3 Hz, 1H), 6.79 (d, *J* = 9.2 Hz, 2H), 6.75 (dd, *J* = 8.9, 3.7 Hz, 4H), 6.17 (t, *J* = 6.3 Hz, 1H), 5.37 (d, *J* = 4.6 Hz, 1H), 4.26 (quin, *J* = 4.8 Hz, 1H), 3.95 (q, *J* = 3.9 Hz, 1H), 3.65 (s, 6H), 3.00 (s, 6H), 2.36 – 2.26 (m, 2H).

¹³C NMR (126 MHz, DMSO) δ (ppm) = 160.6, 159.1, 158.3, 151.3, 150.1, 145.1, 141.5, 135.9, 135.7, 130.1, 130.0, 128.8, 128.1, 128.0, 127.0, 125.9, 121.3, 113.5, 113.5, 112.3, 102.6, 100.0, 86.3, 86.2, 85.9, 70.5, 55.4.

FAB MS: *m/z* (%): 718.3 (43) [MH⁺].

HRMS (C₃₉H₄₀N₇O₇): calc. 718.2989[MH⁺], found 718.2989.

Compound 10



Chemical Formula: $C_{48}H_{56}N_9O_8P$
Molecular Weight: 918,00

DIPEA (3.00 eq, 32 μ L, 0.188 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (1.50 eq, 21 μ L, 0.0940 mmol) were added to a solution of **9** (1.00 eq, 45 mg, 0.0627 mmol) in dry DCM. After stirring for 2 hours at room temperature, the mixture was directly transferred to a flash column. Flash chromatography (SiO_2 , DCM/MeOH, 20/1 + 0.1% Et_3N) yielded phosphoramidite building block **10** as a colorless foam (23 mg, 0.0251 mmol, 40% yield).

TLC (DCM/MeOH, 20/1): $R_f = 0.41$.

^{31}P NMR (202 MHz, $DMSO-d_6$) δ (ppm) = 147.57, 147.26.

MALDI MS: m/z : calc. for $C_{48}H_{57}N_7O_8P$ [$MH^+ - N_2$] 890.40, found 890.35 [$MH^+ - N_2$].

3. DNA synthesis

Synthesis of oligonucleotides was performed on solid phase on a DNA synthesizer *H-6* from *K&A Laborgeräte*. Reagents and CPG columns (1 μ mol) were purchased from *Glen Research*, *Alfa Aesar* and *Sigma Aldrich*. Coupling times for the tetrazole building blocks were increased from 56 seconds to 24 minutes in comparison to the natural phosphoramidites.

After synthesis the trityl-off oligonucleotide was cleaved off and deprotected by incubation in concentrated ammonium hydroxide (25% in water) for 24 h and 35 °C. All oligonucleotides were purified by semipreparative HPLC (RP-C18 column, A = NH₄OAc buffer, B = acetonitrile, flow rate 2.5 mL/min, UV/Vis detection at 260 nm and 290 nm or 320 nm respectively), identified by MALDI-TOF mass spectrometry and quantified by UV/Vis absorption at 260 nm on a *Nanodrop ND-1000* spectrophotometer.

Table S1. Molar extinction coefficients and MS characterization for the synthesized DNA strands.

DNA	Mass calc. [Da]	Mass found [Da]	ϵ_{260} [mM ⁻¹ cm ⁻¹]	Modification
DNA 1A	5356.8	5357.6	160.3	1
DNA 2A	5356.8	5356.1	160.3	1
DNA 1B	5321.9	-	152.7	2
	5159.9 (nitrile)	5152.0 (nitrile)		
DNA 2B	5321.9	5320.5	152.7	2

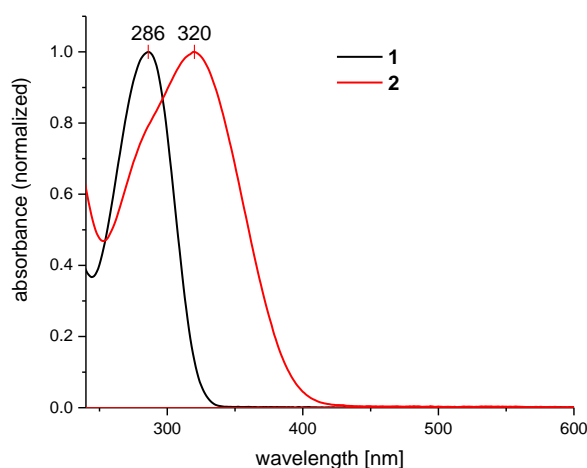


Figure S1. UV/Vis absorption of nucleosides **1** and **2**.

4. "Photoclick" experiments

For the postsynthetic modification of tetrazole-modified **DNA1A/B** and **DNA2A/B** the reaction mixture (containing 2.5 μM DNA, 10 mM Na-P_i buffer (pH = 7), 250 mM NaCl, 1.5 eq Sulfo-Cyanine3 maleimide) was transferred into 10 mm quartz glass cuvettes. These were irradiated for defined intervals using LEDs with 300 nm, 365 nm, 385 nm or 405 nm respectively. Afterwards a defined volume was lyophilized and analysed by reversed phase HPLC (RP-C18 column, A = NH₄OAc buffer, B = acetonitrile, flow rate 1.0 mL/min, UV/Vis detection at 260 nm, 358 nm or 367 nm respectively, 548 nm). Product concentrations were determined by its absorption at 260 nm and integration.

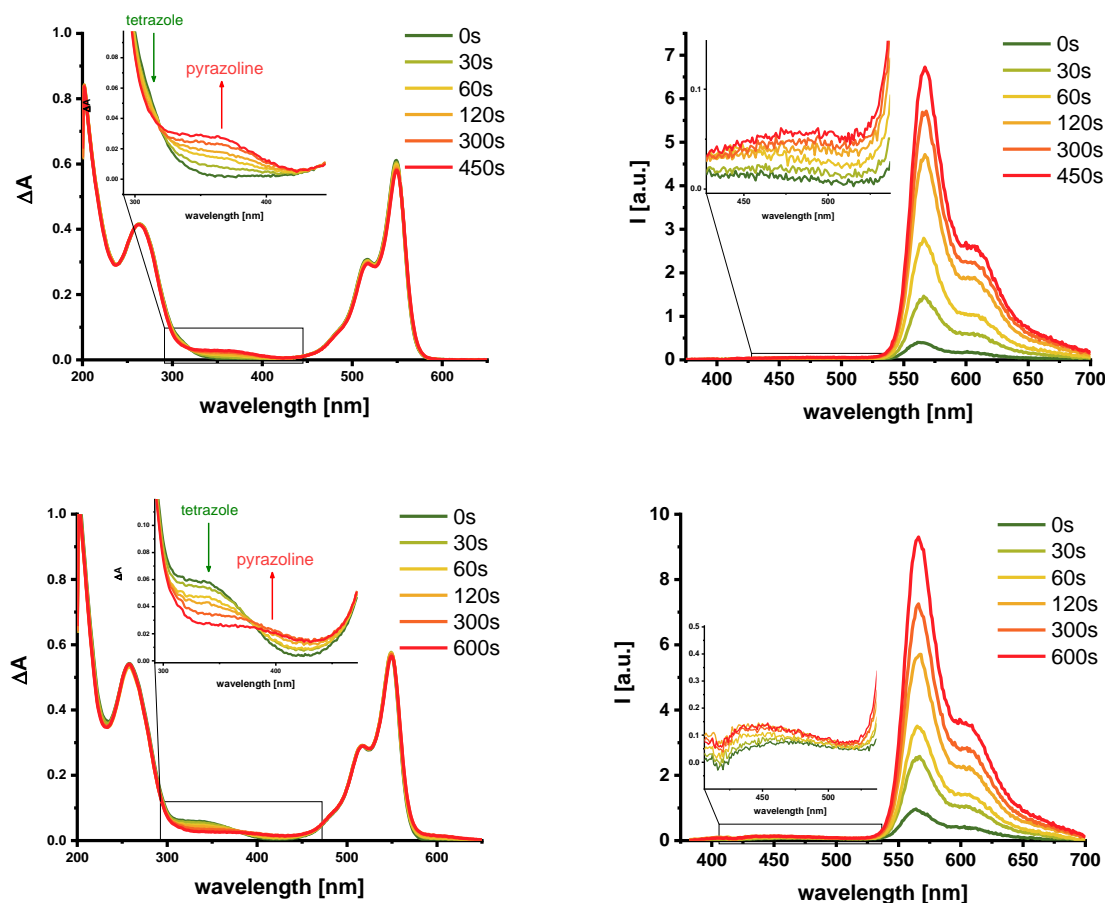


Figure S2. Spectroscopic snapshots of the development during "photoclick" reaction of **DNA2A** (upper row) and **DNA2B** (bottom row) with Sulfo-Cyanine3 maleimide (2.5 μM DNA, 10 mM Na-P_i buffer, 250 mM NaCl, pH = 7, 20 °C). The absorption spectrum (left) shows the decrease of tetrazole absorption while concomitantly the pyrazoline absorption ($\lambda_{\text{max}} = 358$ nm for **DNA2A**, $\lambda_{\text{max}} = 367$ nm for **DNA2B**) rises. The fluorescence spectrum (right) shows nearly no increase of pyrazoline

emission while the emission of the Cy3 dye increases drastically (17-fold for **DNA2A**, 10-fold for **DNA2B**) due to energy transfer from the pyrazoline moiety.

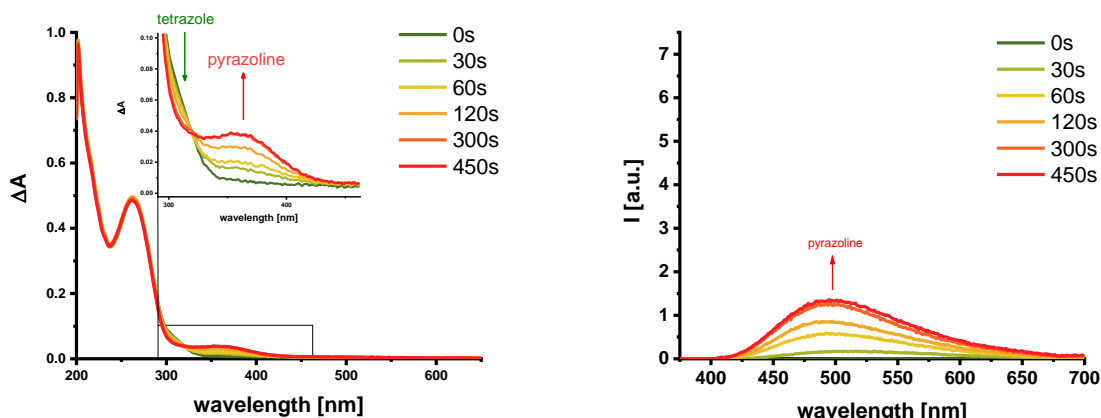


Figure S3. Spectroscopic snapshots of the development during “photoclick” reaction of **DNA2A** with N-Methylmaleimide (2.5 μM DNA, 10 mM Na- P_i buffer, 250 mM NaCl, pH = 7, 20 $^\circ\text{C}$). The absorption spectrum (left) shows the decrease of tetrazole absorption while concomitantly the pyrazoline absorption ($\lambda_{\text{max}} = 358$ nm) rises. The fluorescence spectrum (right) shows increase of pyrazoline emission (8-fold) when no suitable acceptor is available.

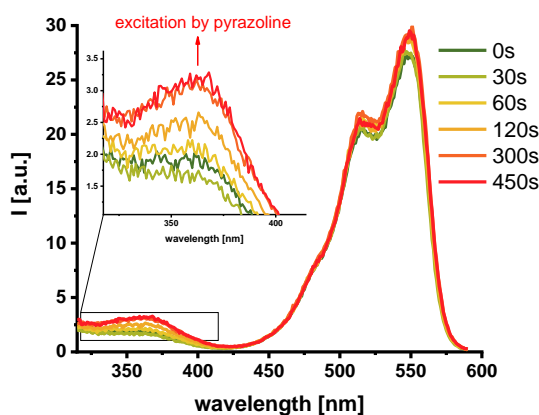
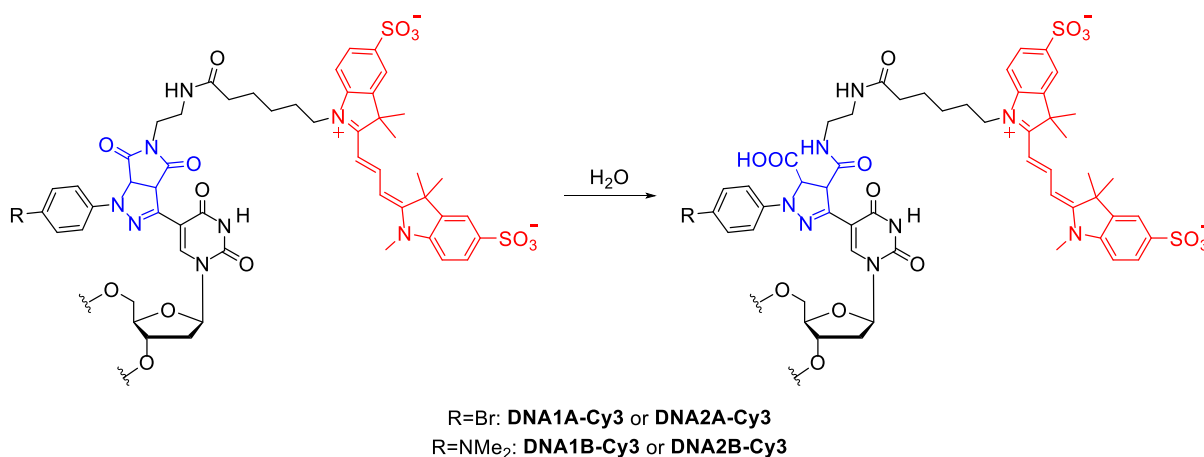


Figure S4. Excitation spectra as snapshots of the development during “photoclick” reaction of **DNA2A** with Sulfo-Cyanine3 maleimide (2.5 μM DNA, 10 mM Na- P_i buffer, 250 mM NaCl, pH = 7, 20 $^\circ\text{C}$). The excitation by the pyrazoline moiety rises more than 2-fold during the reaction while the value of direct excitation changes only slightly.

Table S2. Characterization of “photoclick” products by mass spectrometry.

DNA	Dipolarophile	product	Mass calc. [Da]	Mass found [Da]
DNA2A	Cy3	pyrazoline	6066.05	6066.1
		hydrolyzed pyrazoline ^a	6084.06	6084.0
DNA2B	Cy3	pyrazoline	6031.18	
		hydrolyzed pyrazoline ^a	6049.19	6072.1 (+Na)

^aThe succinimide is prone to nucleophilic ring opening:



We performed additional experiments with two commercially available maleimide-modified dyes SulfoCy5 (Figures S5, S6) and Alexa Fluor 488 (Figure S5, S7). These experiments show that the energy transfer also works with these two dyes.

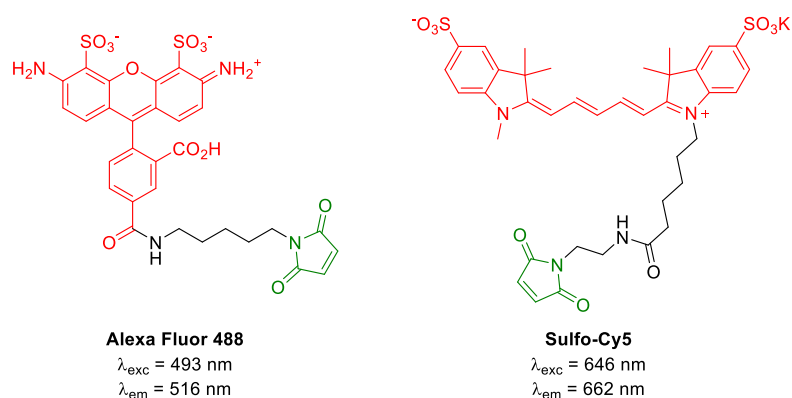


Figure S5: Alexa Fluor 488 and sulfo-Cy5 as additional acceptor dyes for the energy transfer from the pyrazoline-DNA.

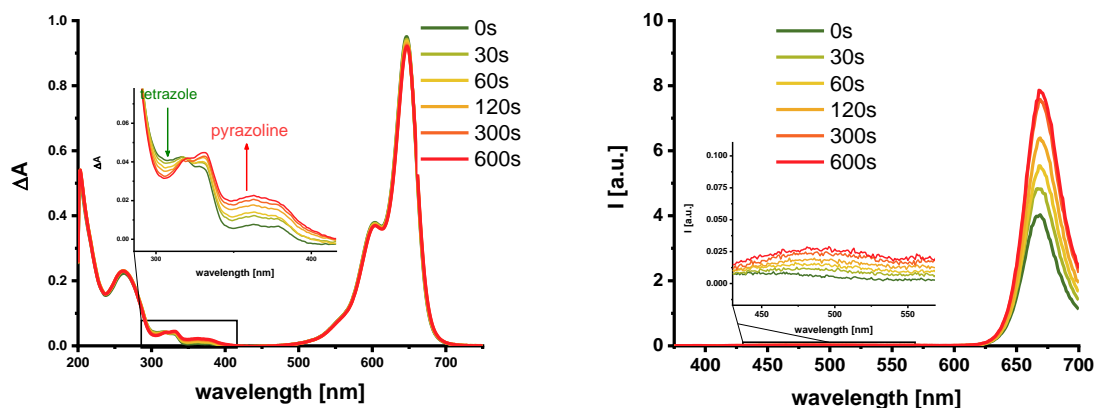


Figure S6: Spectroscopic snapshots of the development during “photoclick” reaction of **DNA2A** with 1.5 equivalents of sulfo-Cy5 maleimide (2.5 μM DNA, 10 mM Na-P_i buffer, 250 mM NaCl, pH = 7, 20 °C). The absorption spectrum (left) shows the decrease of tetrazole absorption while concomitantly the pyrazoline absorption ($\lambda_{\text{max}} = 358$ nm) rises. The fluorescence spectrum (right) shows nearly no increase of pyrazoline emission while the emission of the Cy5 dye increases 2-fold due to energy transfer from the pyrazoline moiety.

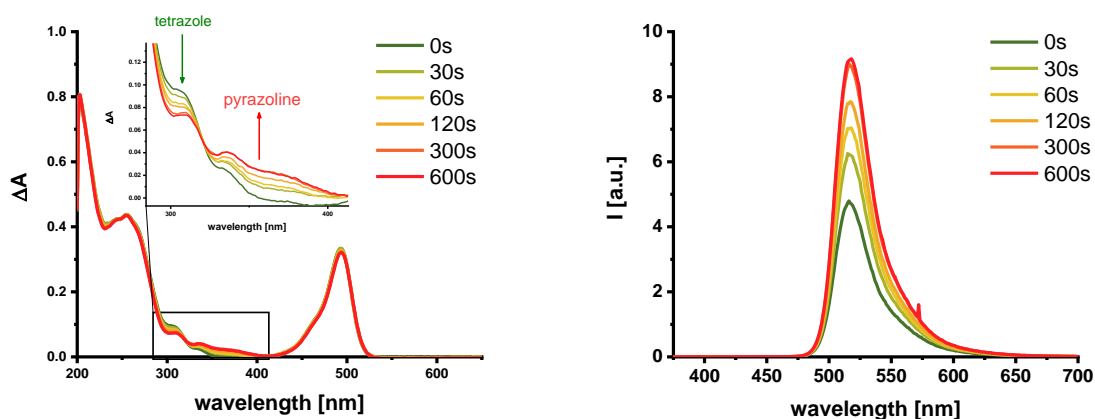


Figure S7: Spectroscopic snapshots of the development during “photoclick” reaction of **DNA2A** with 1.5 equivalents of Alexa Fluor 488 maleimide (2.5 μM DNA, 10 mM Na-P_i buffer, 250 mM NaCl, pH = 7, 20 °C). The absorption spectrum (left) shows the decrease of tetrazole absorption while concomitantly the pyrazoline absorption ($\lambda_{\text{max}} = 358$ nm) rises. The fluorescence spectrum (right) shows nearly no increase of pyrazoline emission while the emission of the Alexa Fluor 488 dye increases 1.9-fold due to energy transfer from the pyrazoline moiety.

5. Kinetic measurements

For the calculation of the second-order rate constants, samples of **DNA2A/B** were irradiated with 5 and 10 eq of Sulfo-Cyanine3 maleimide for 15 min. After each minute, a defined volume was lyophilized, desalted and separated by reversed phase HPLC. Product concentrations were determined by its absorption at 260 nm and integration. As described before^[1] the rate law can be treated as pseudo first-order, which allows us to plot the data according to the following equation:

$$\ln\left(\frac{[A]_0}{[A]_0 - [P]_t}\right) = k_{obs}t$$

Equation S1. Integrated form of pseudo first-order rate law.

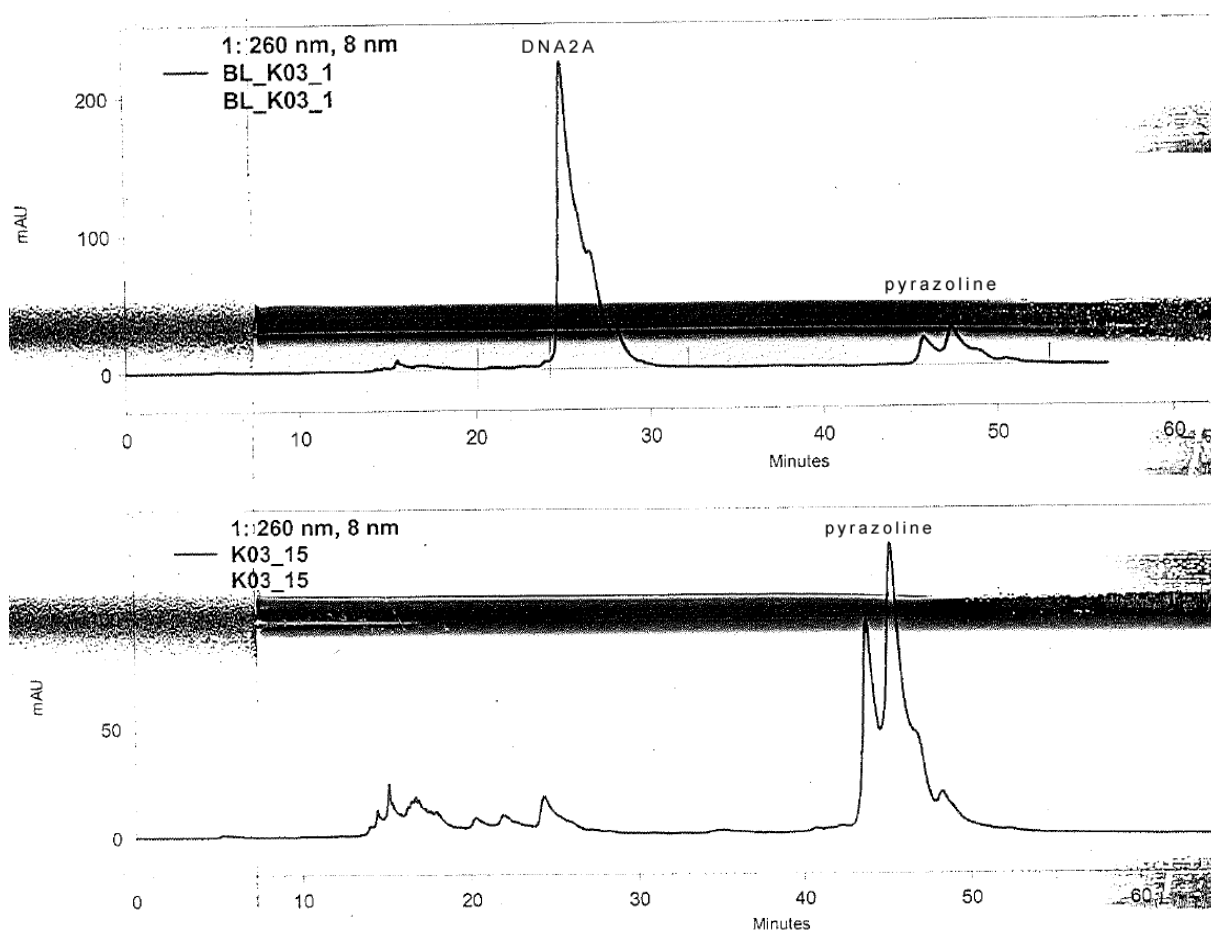


Figure S8. Chromatogram after 1 (top) and 15 minutes (bottom) of irradiation of **DNA2A** (2.5 μ M DNA, 25 μ M Cy3, 10 mM Na-P_i buffer, 250 mM NaCl, pH = 7, 20 °C) with 300 nm.

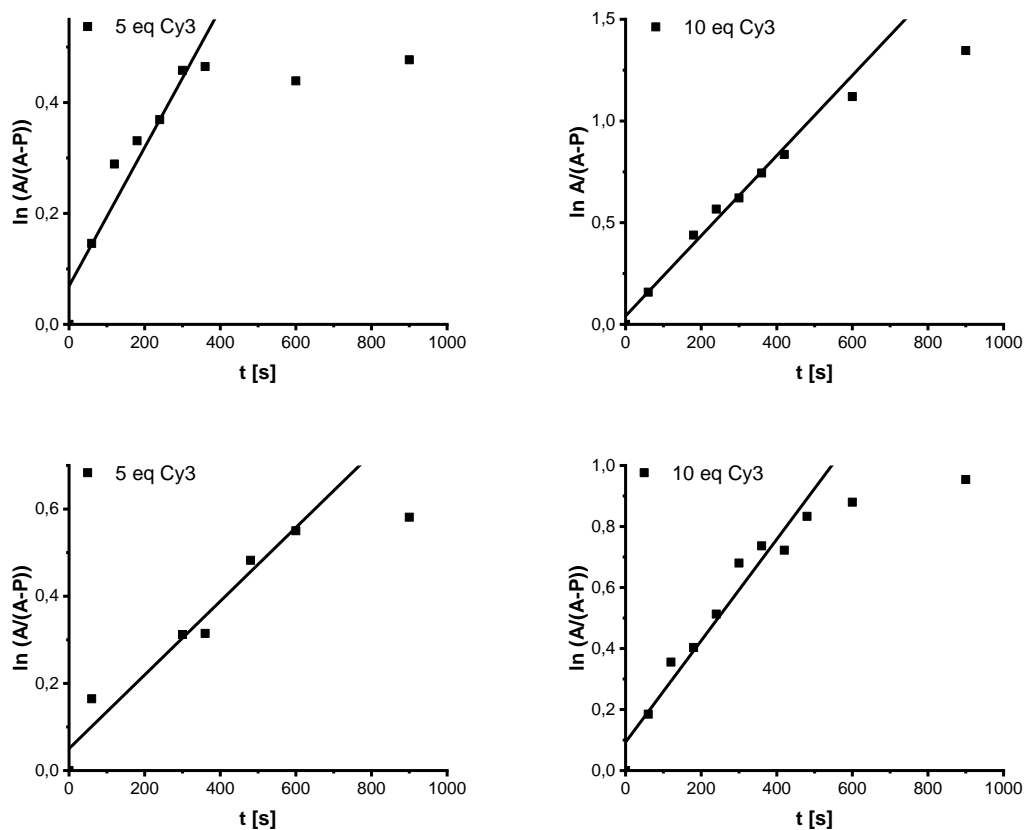


Figure S9. Product concentrations determined by HPLC analysis plotted against time according to rate law S1. Linear parts were fitted to obtain the rate constants. Top row: **DNA2A**, bottom row: **DNA2B**

DNA2A: $k_{obs,5eq,DNA2A} = 0.00125 \text{ s}^{-1}$ and $k_{obs,10eq,DNA2A} = 0.00197 \text{ s}^{-1}$

DNA2B: $k_{obs,5eq,DNA2B} = 8.45 \cdot 10^{-4} \text{ s}^{-1}$ and $k_{obs,10eq,DNA2B} = 0.00166 \text{ s}^{-1}$

To determine the second-order rate constant the concentration of the dipolarophile must be taken into consideration by using the following equation:

$$k_{obs} = k_{cycloaddition} \cdot [dipolarophile]$$

Equation S2. Equation for the determination of the second-order rate constant.

For **DNA2A** this results in the following second-order rate constant:

$$k_{cycloaddition}(5eq) = 100 \pm 13 s^{-1}M^{-1}$$

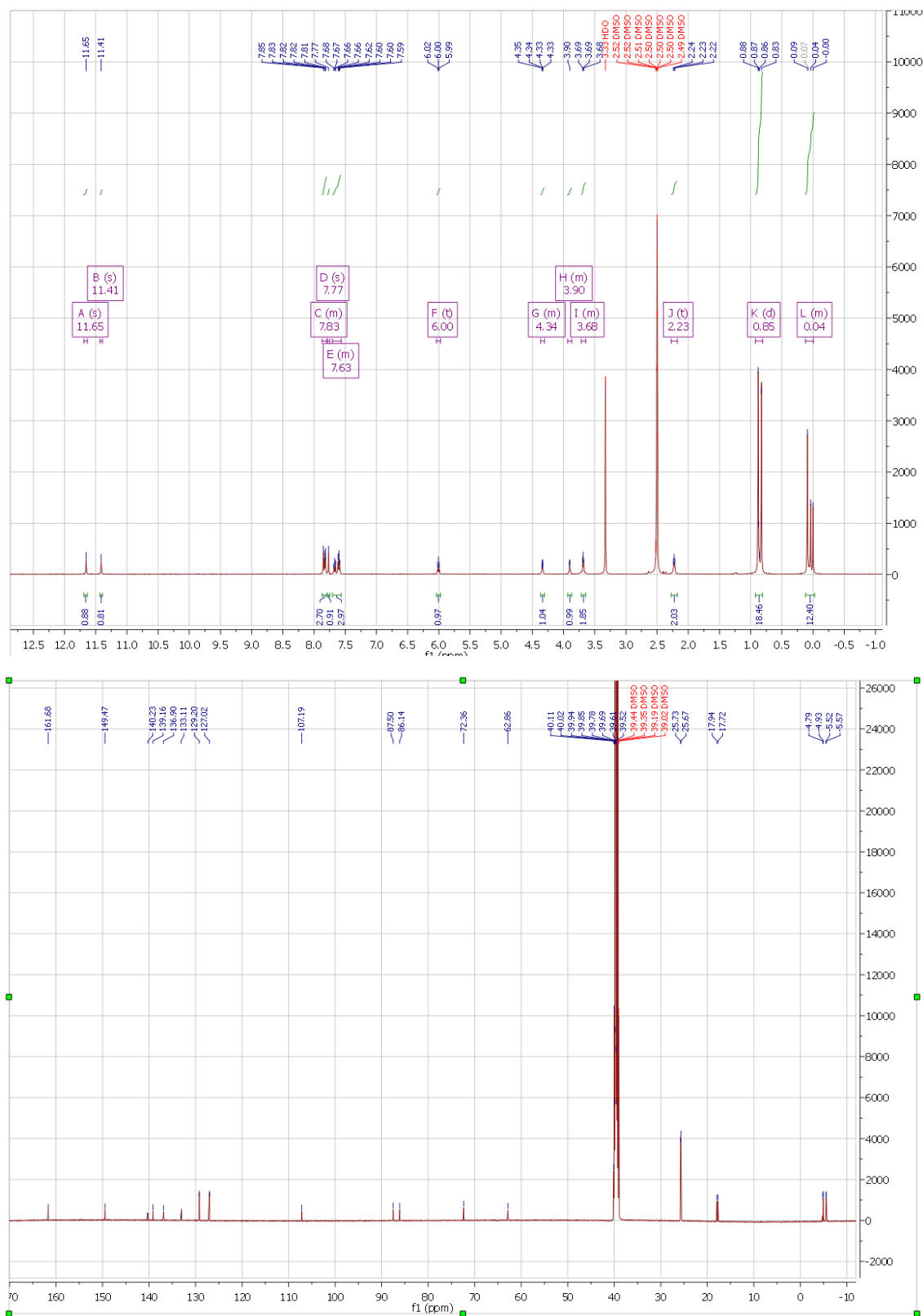
$$k_{cycloaddition}(10eq) = 79 \pm 4 s^{-1}M^{-1}$$

The average rate constant for **DNA2A** is $k = 89 \pm 13 s^{-1}M^{-1}$. The average rate constant for **DNA2B** was determined in the same way and is $k = 67 \pm 6 s^{-1}M^{-1}$.

The error estimates were determined by using the maximum curve fit errors for each DNA from Figure S9 and Equation S2.

6. Images of NMR and mass spectra

Compound 4



MAT 95 +FAB
Ro

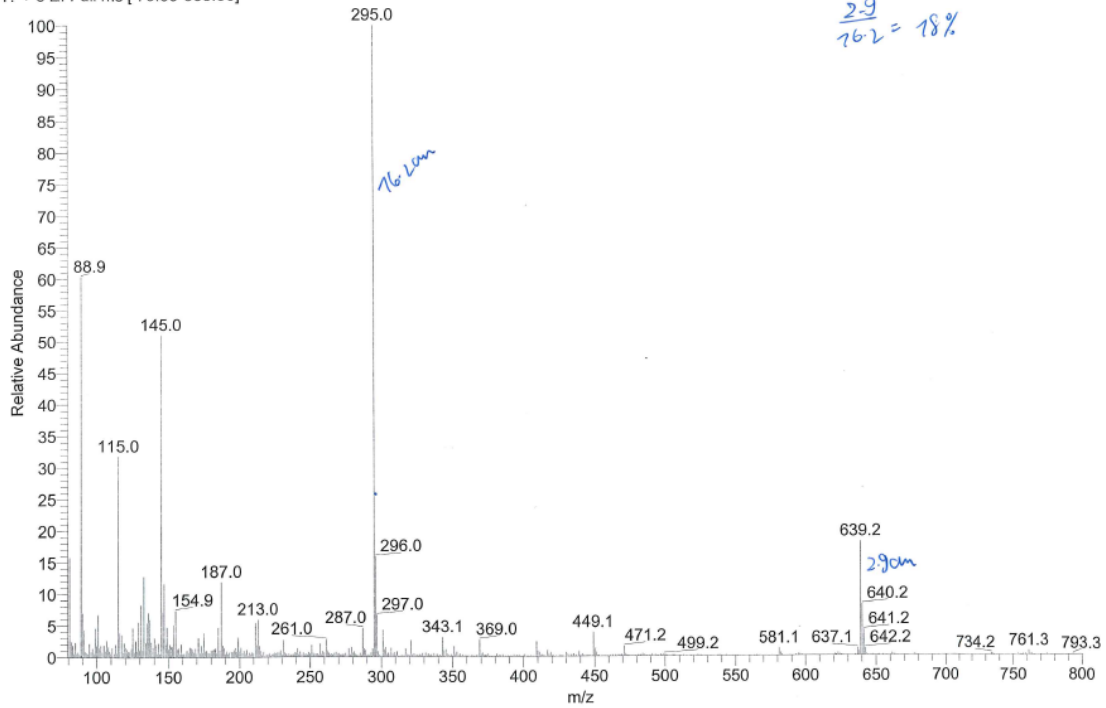
3/16/2016 5:05:25 PM
B. Lehman

BL042-3-NBA
AK Wagenknecht

$EM+HJ^+ = 639.2$

bl042 #11 RT: 0.98 AV: 1 NL: 1.93E6
T: + c EI Full ms [79.50-800.50]

$\frac{2.9}{16.2} = 18\%$



3/16/2016 5:09:55 PM

File recalibrated by CMass.

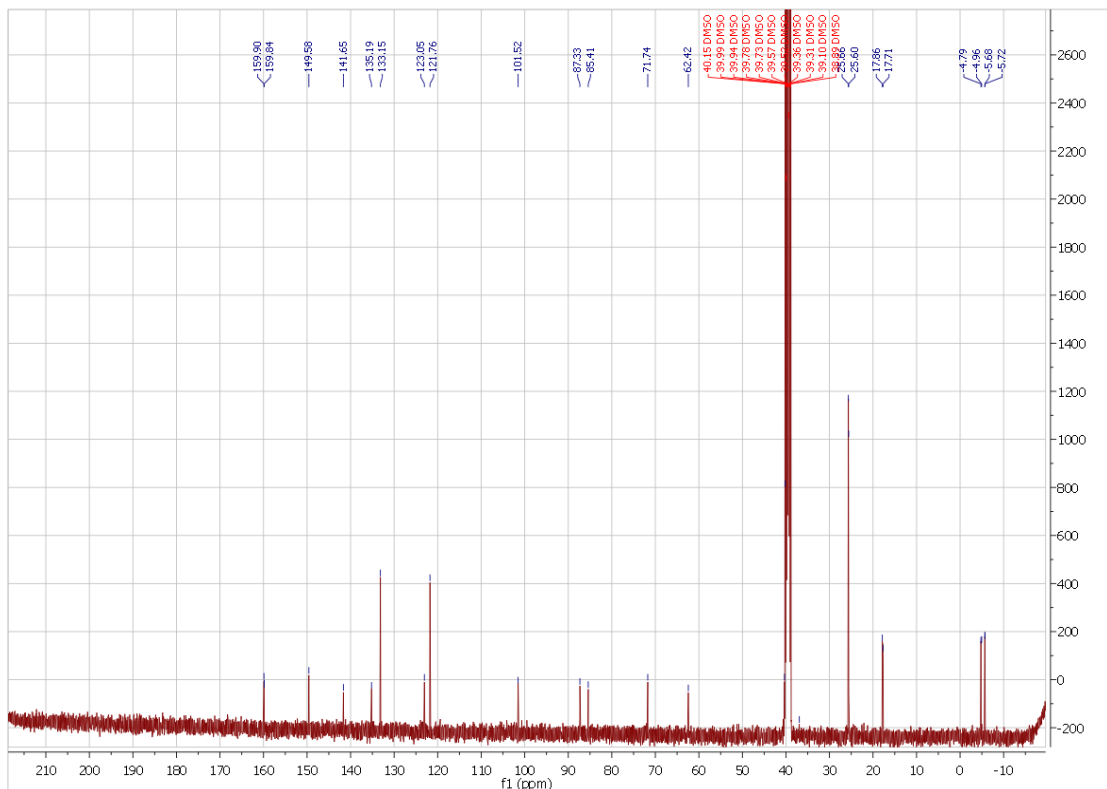
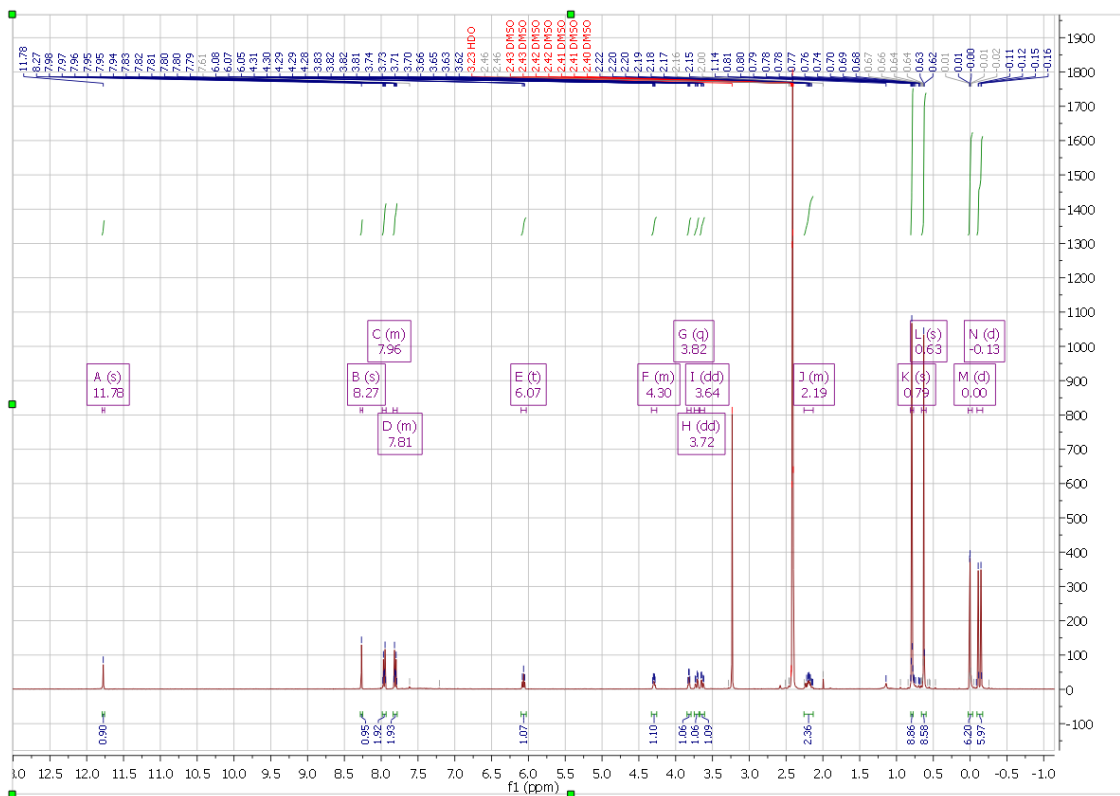
bl042-c3#3 RT: 0.29

T: + c EI Full ms [79.50-800.50]

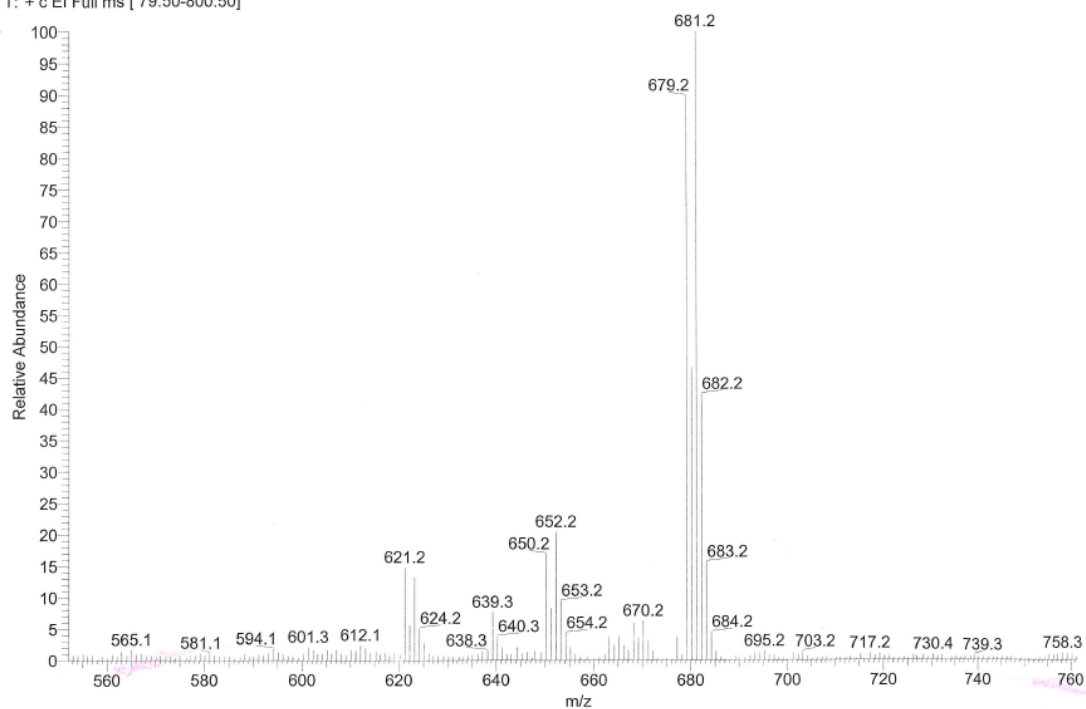
m/z= 639.0429-639.9077

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
639.2699	466732.0	100.00	639.2699	0.08	C ₂₈ H ₄₇ O ₇ N ₄ ³² S ₁ ²⁸ Si ₂

Compound 5



MA⁺ FAB
 Ro 4/15/2016 9:38:10 AM BL045_3-NBA
 B. Lehmann AK Wagenknecht
 bl045 #20-22 RT: 1.75-1.93 AV: 3 NL: 6.97E5
 T: + c EI Full ms [79.50-800.50]



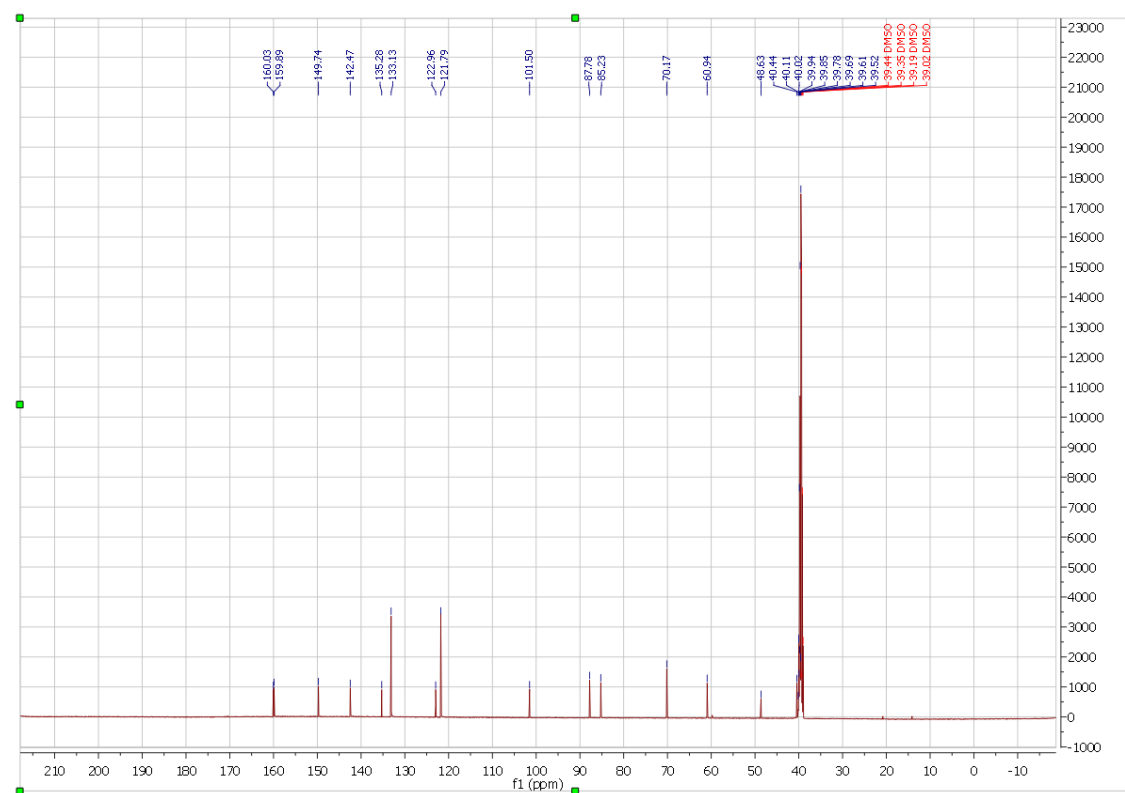
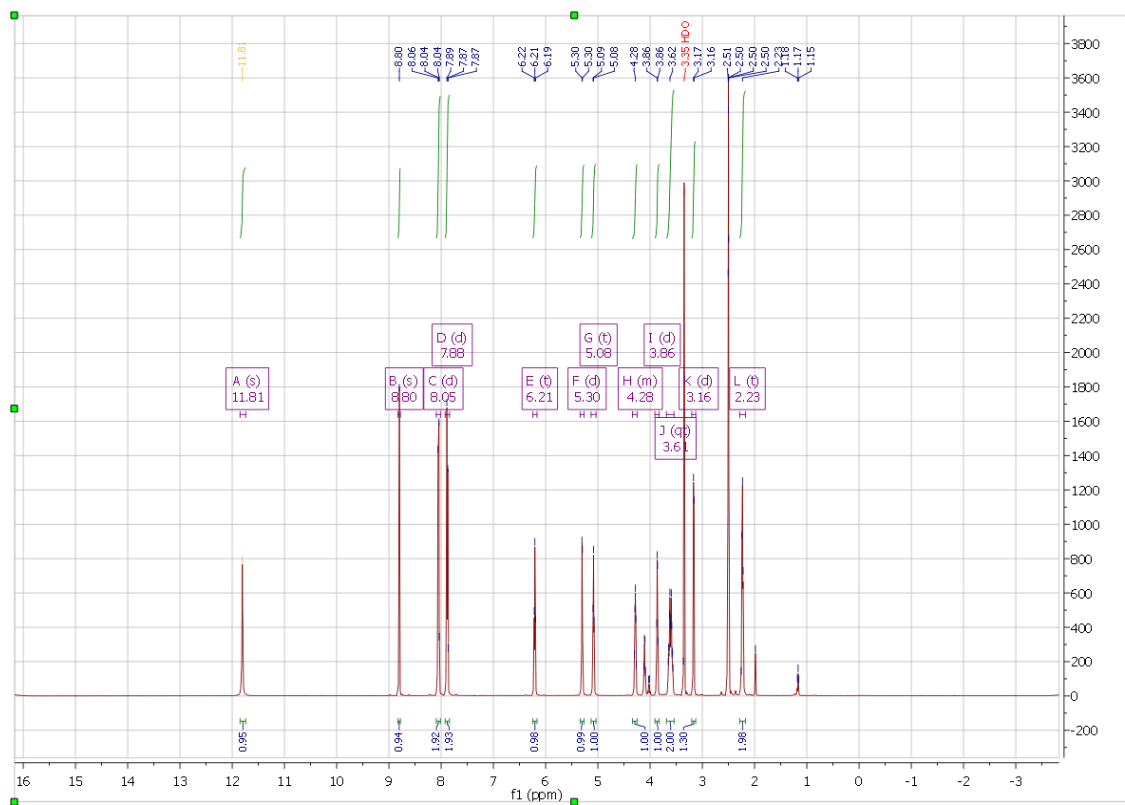
4/15/2016 9:48:10 AM

File recalibrated by CMass.

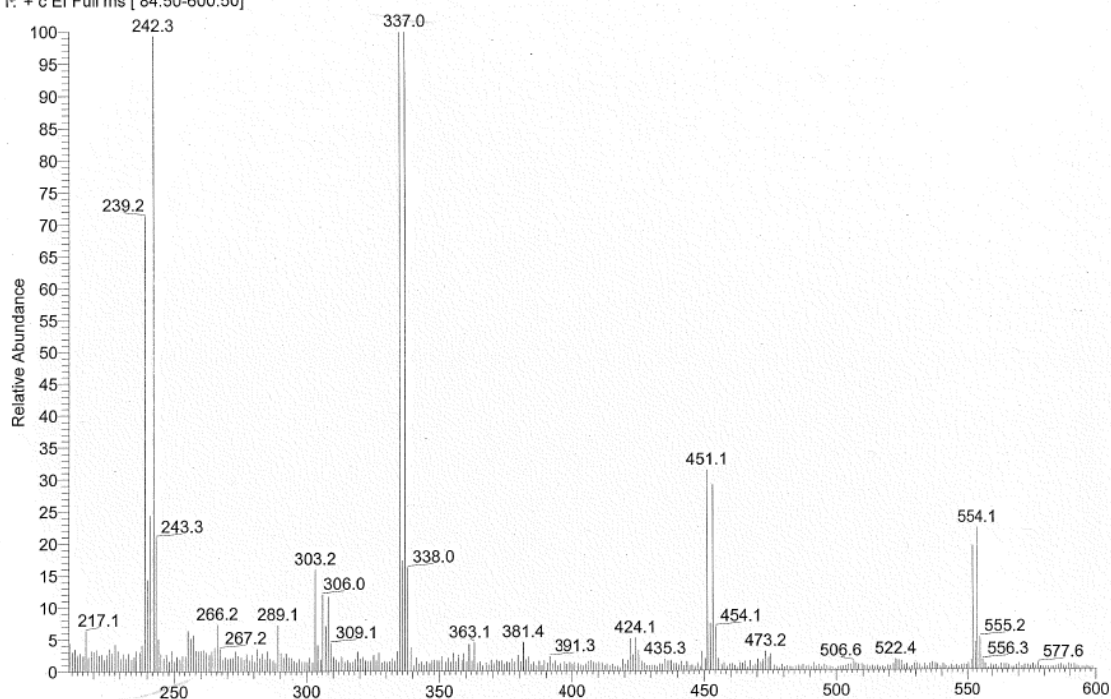
bl045-c2#10 RT: 0.88
 T: + c EI Full ms [79.53-800.53]
 m/z= 679.1350-679.3064

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
679.2089	69008.0	100.00	679.2090	-0.09	C ₂₈ H ₄₄ O ₅ N ₆ ⁷⁹ Br ₁ ²⁸ Si ₂

Compound 1



MAT 95,+FAB
 moë
 7/13/2018 2:19:20 PM
 B. Lehmann
 BL083,3-NBA
 AK Wagenknecht
 b1083_180713141920 #32-39 RT: 2.43-2.95 AV: 8 NL: 5.01E5
 T: + c EI Full ms [84.50-600.50]



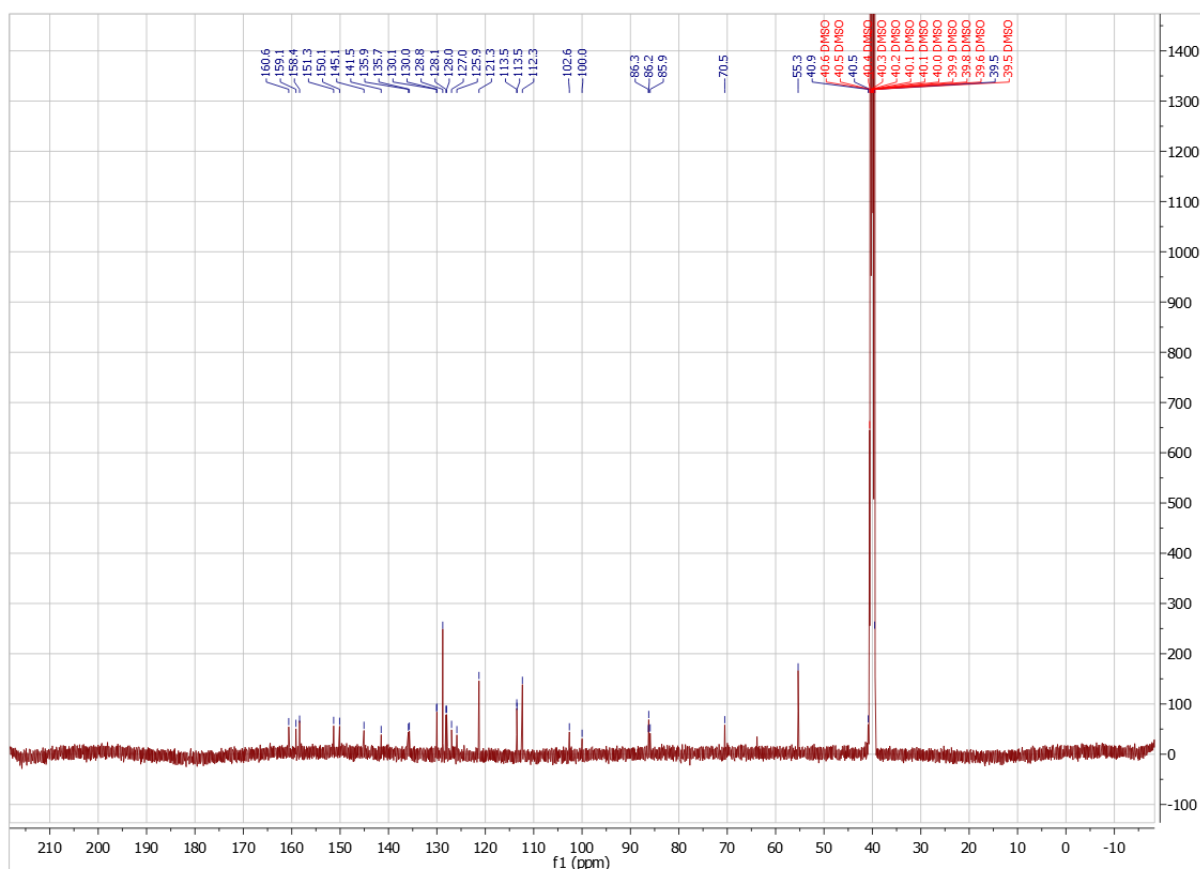
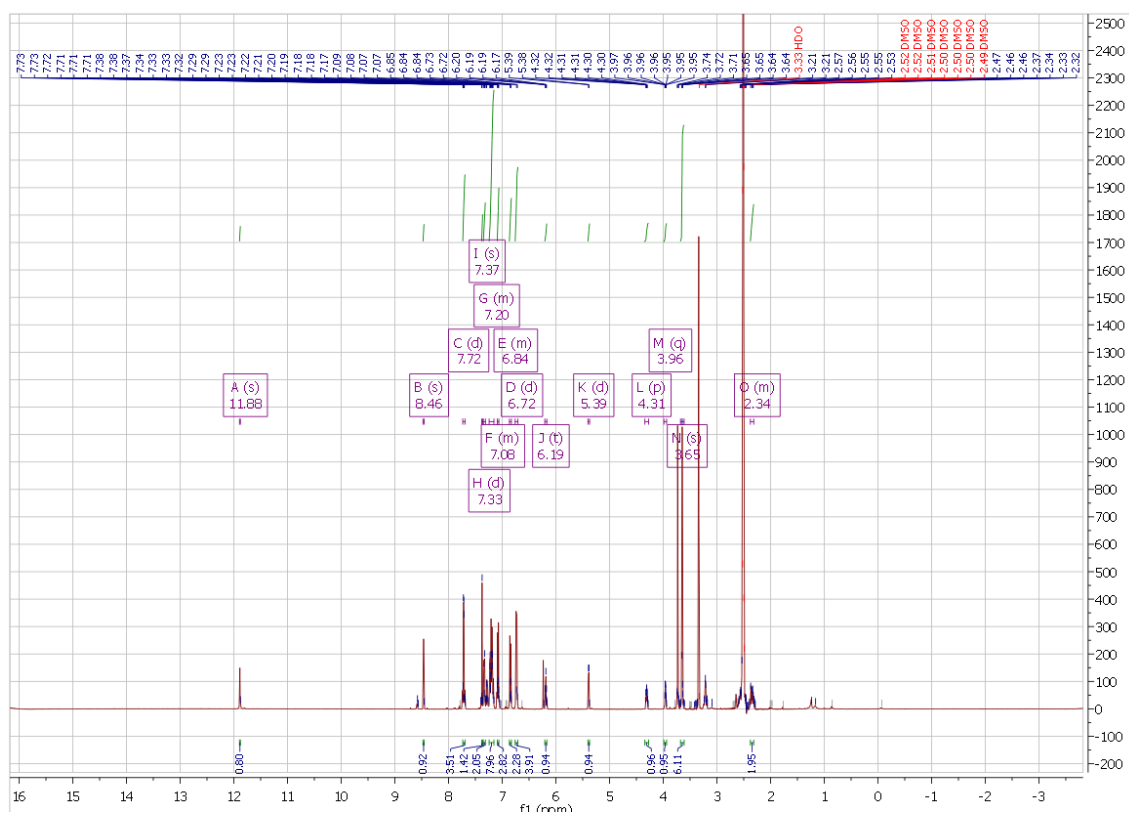
7/13/2018 2:25:48 PM

File recalibrated by CMass.

b1083_180713141920-c2#33 RT: 2.50
 T: + c EI Full ms [84.43-600.43]
 m/z= 451.0007-451.0697

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
451.0364	137002.0	100.00	451.0366	-0.14	C ₁₆ H ₁₆ O ₅ N ₆ ⁷⁹ Br ₁

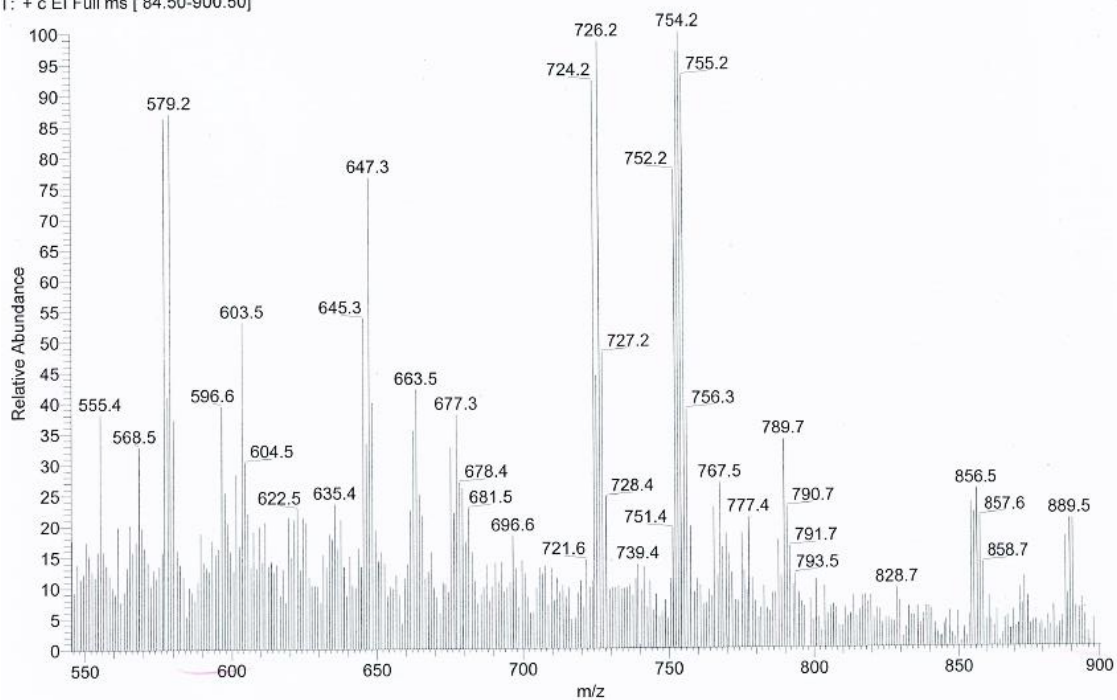
Compound 7



MAT 95,+FAB
 moe
 bl084 #38-46 RT: 3.42-4.13 AV: 9 NL: 2.51E4
 T: + c EI Full ms [84.50-900.50]

7/13/2018 2:47:45 PM
 B. Lehmann

BL084,3-NBA
 AK Wagenknecht

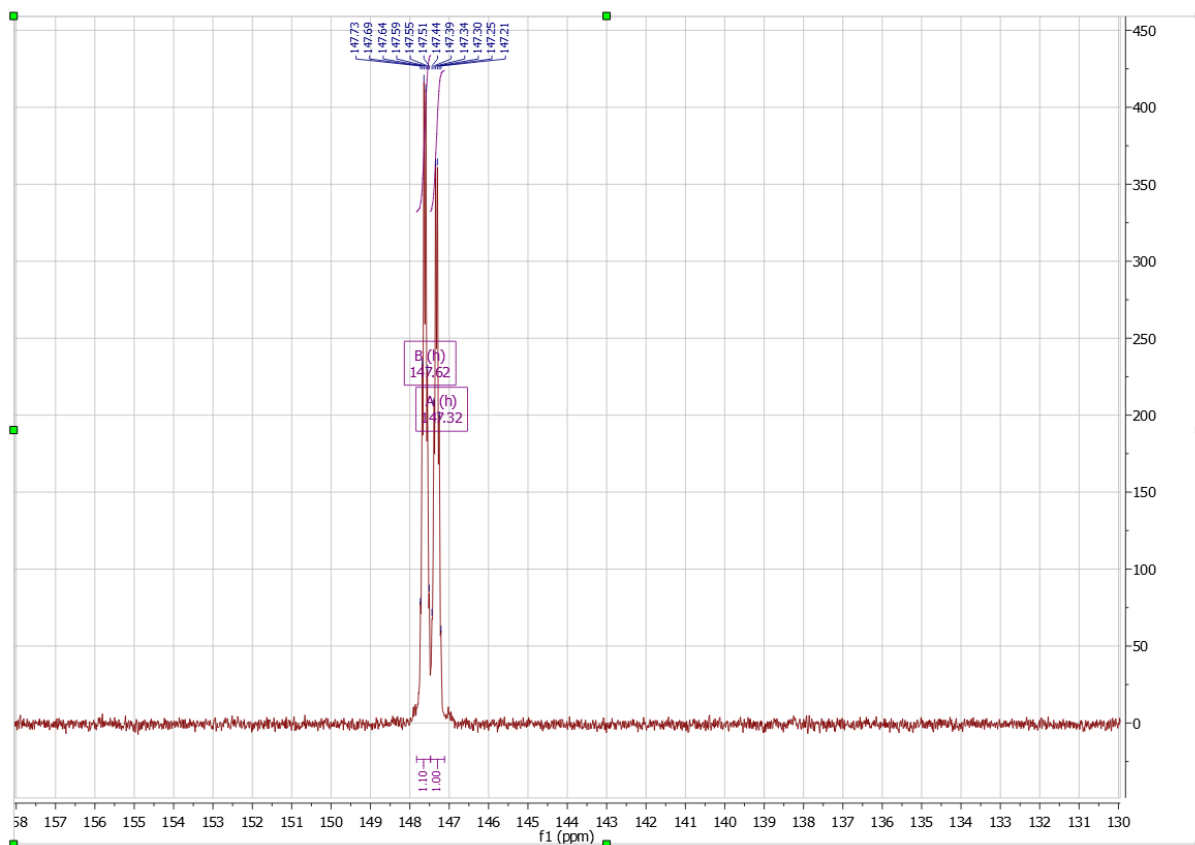


7/13/2018 2:56:30 PM File recalibrated by CMass.

bl084-c3#32 RT: 2.88
 T: + c EI Full ms [84.43-900.43]
 m/z= 752.0559-752.2680

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
752.1596	3797.0	100.00	752.1594	0.15	C ₃₇ H ₃₃ O ₇ N ₆ ⁷⁹ Br ₁

Compound 8

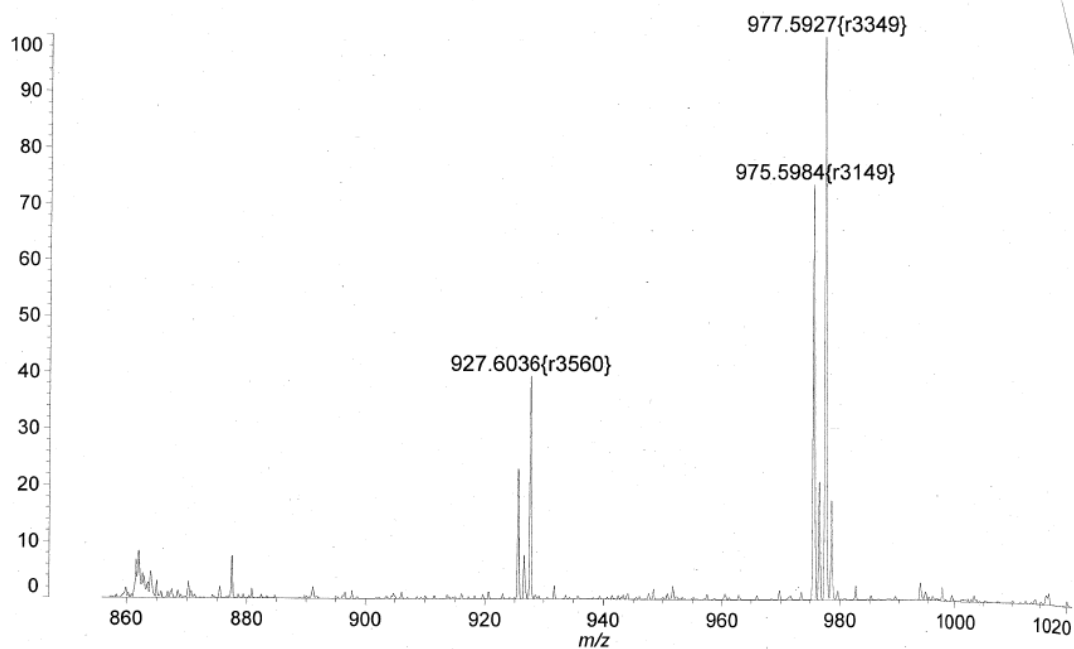


Confidence

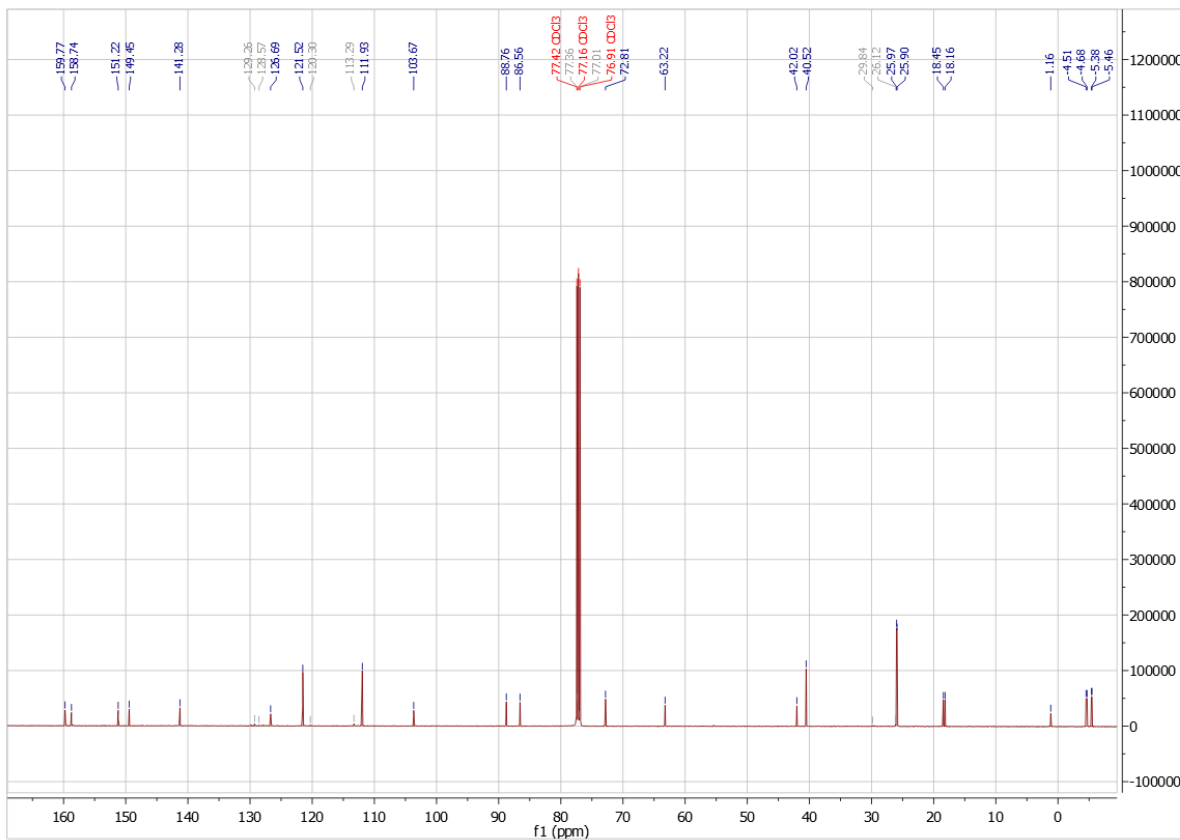
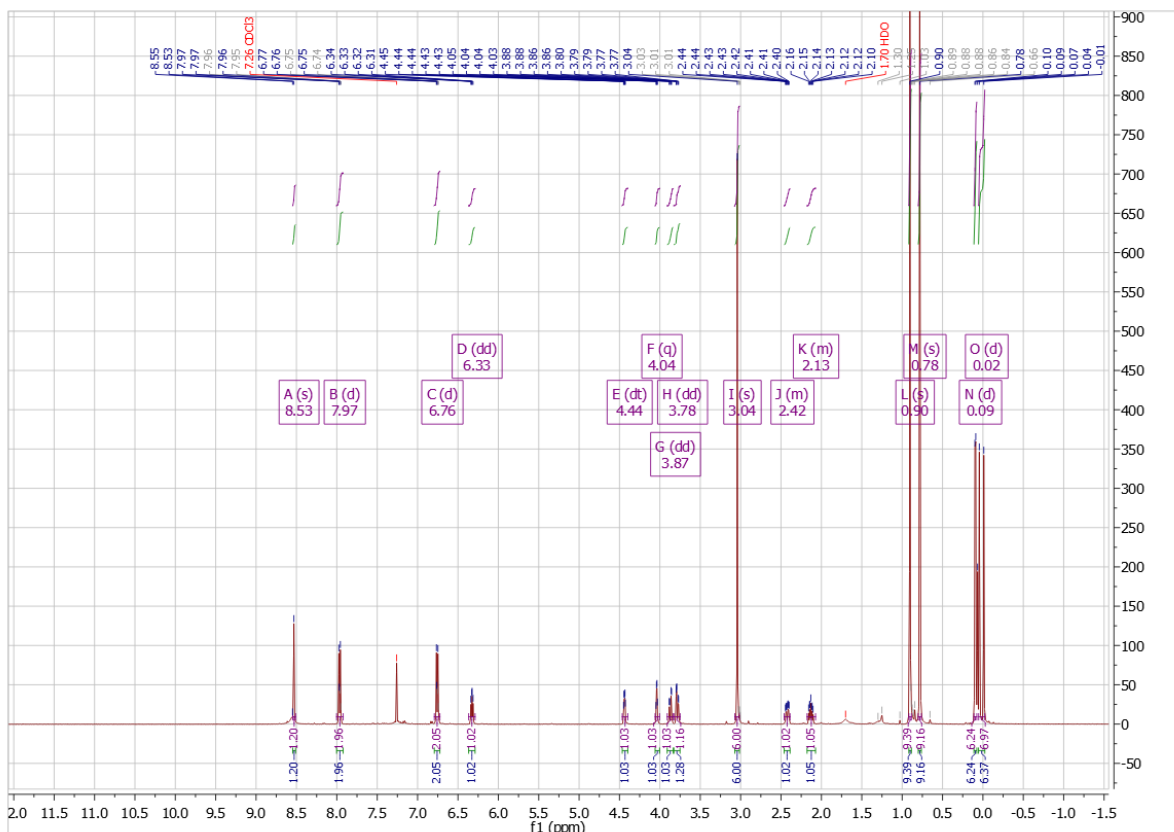
Data: BL085_10_THAP0001.K7[c] 21 Mar 2018 20:03 Cal: 20180303_CHCA 3 Mar 2018 12:16

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Reflectron, Power: 120, Blanked, P.Ext. @ 950 (bin 70)

%Int. 0.2 mV[sum= 73 mV] Profiles 1-349 Smooth Gauss 5 -Baseline 15



Compound 6



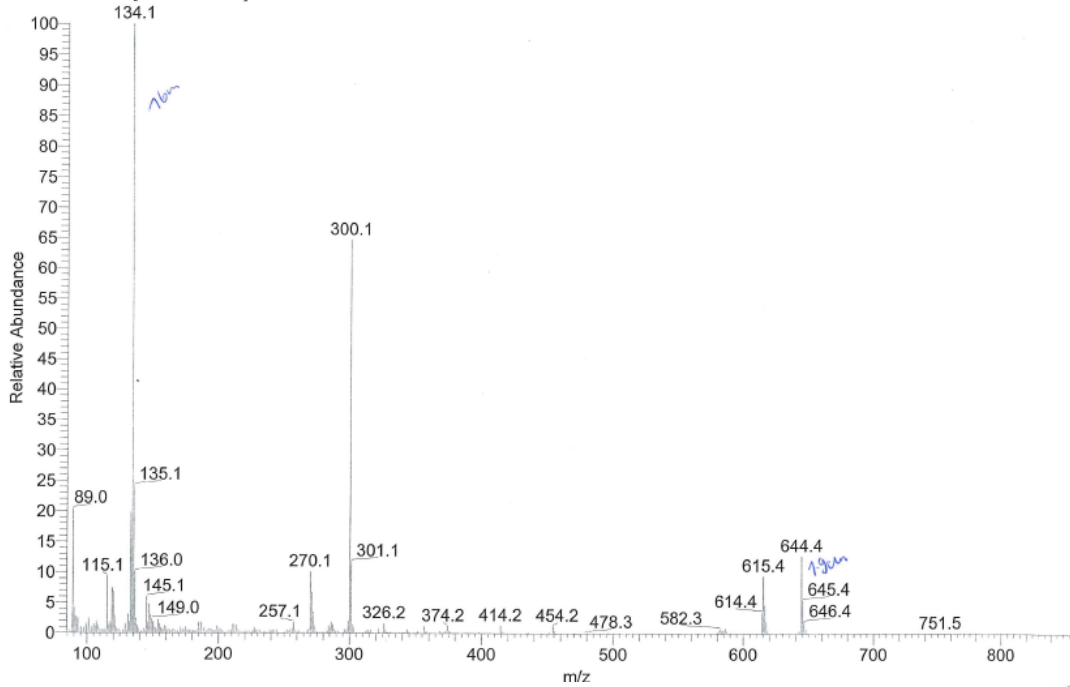
MAT 95 +FAB
moe

4/28/2016 5:22:14 PM
B. Lehmann

BLO44,3-NBA
AK Wagenknecht

b1044 #7-12 RT: 0.69-1.12 AV: 6 NL: 1.10E6

T: + c EI Full ms [84.50-860.50]



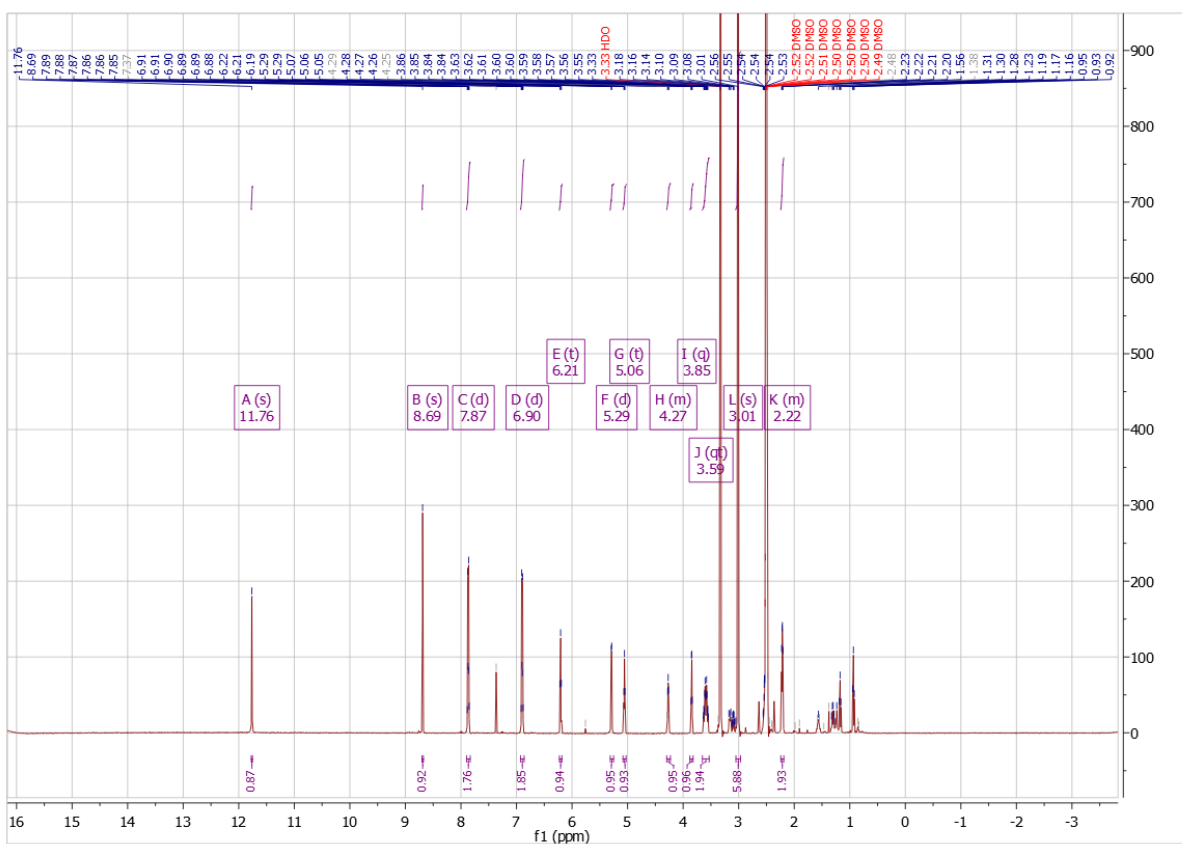
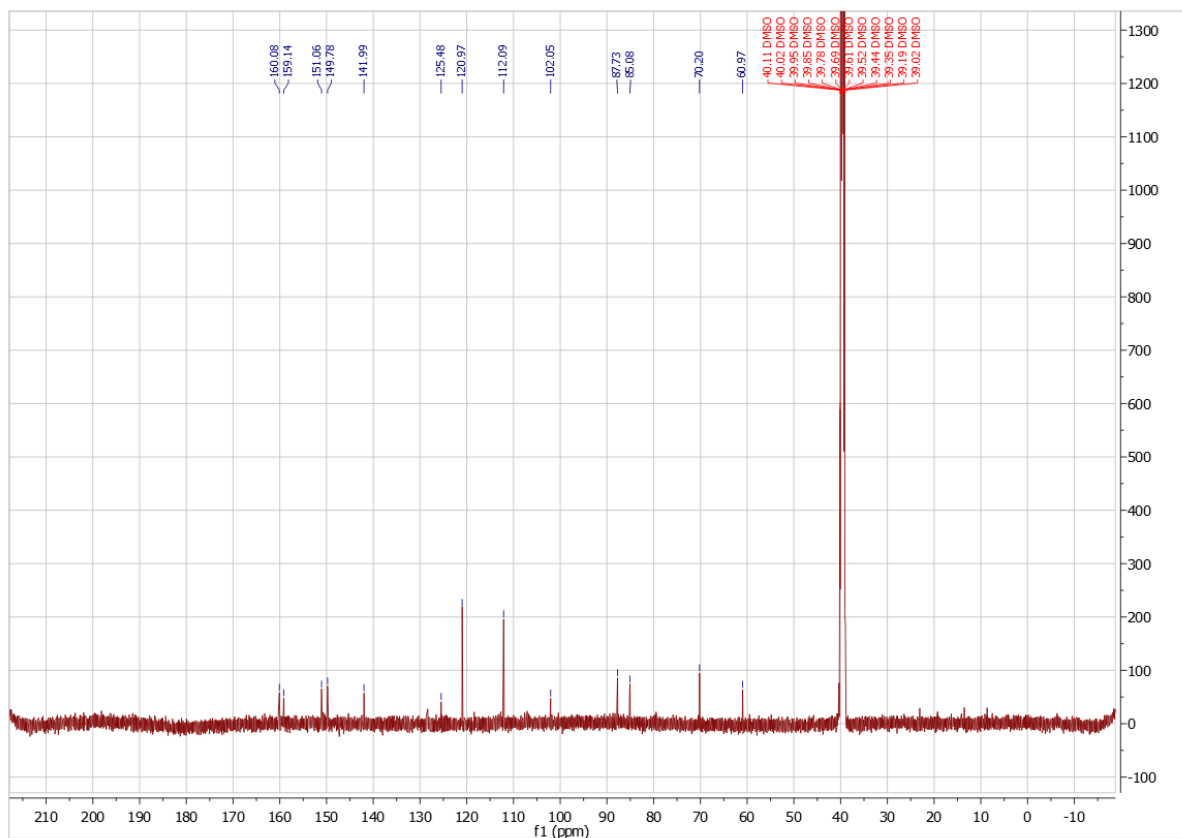
b1044-c3#4 RT: 0.43

T: + c EI Full ms [84.42-860.42]

m/z= 644.3050-644.3854

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
644.3404	77102.0	100.00	644.3407	-0.23	C ₃₀ H ₅₀ O ₅ N ₇ ²⁸ Si ₂

Compound 2



b1078-c3#5 RT: 0.38

T: + c EI Full ms [84.47-550.47]

m/z= 416.0250-416.3180

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
416.1681	11670.0	100.00	416.1682	-0.13	C ₁₈ H ₂₂ O ₅ N ₇

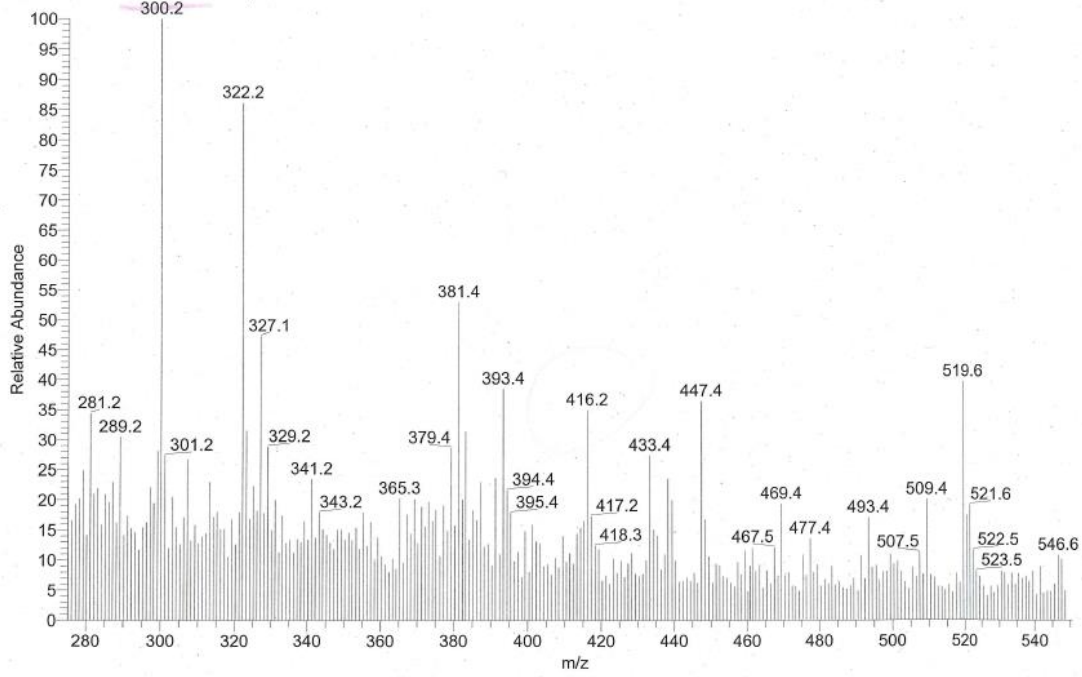
MAT 95, +FAB
moe

10/17/2017 3:35:36 PM
B. Lehmann

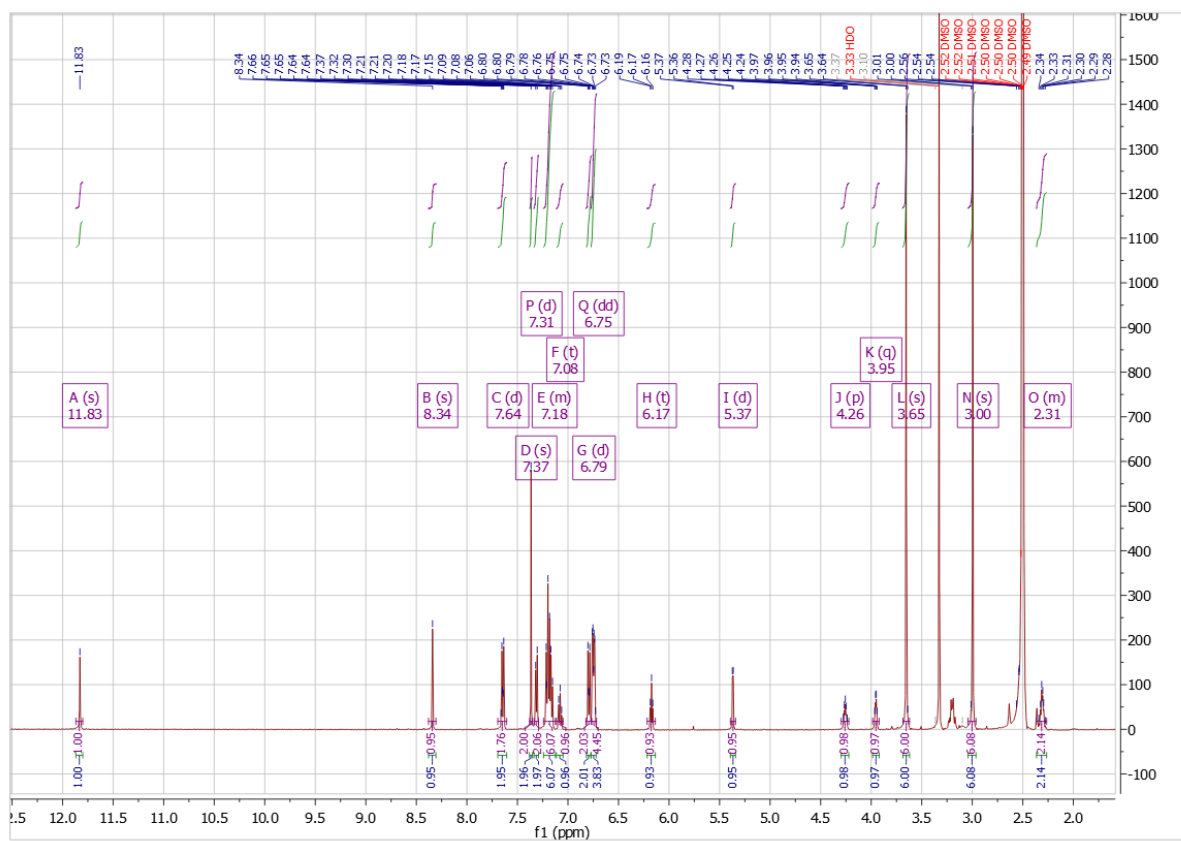
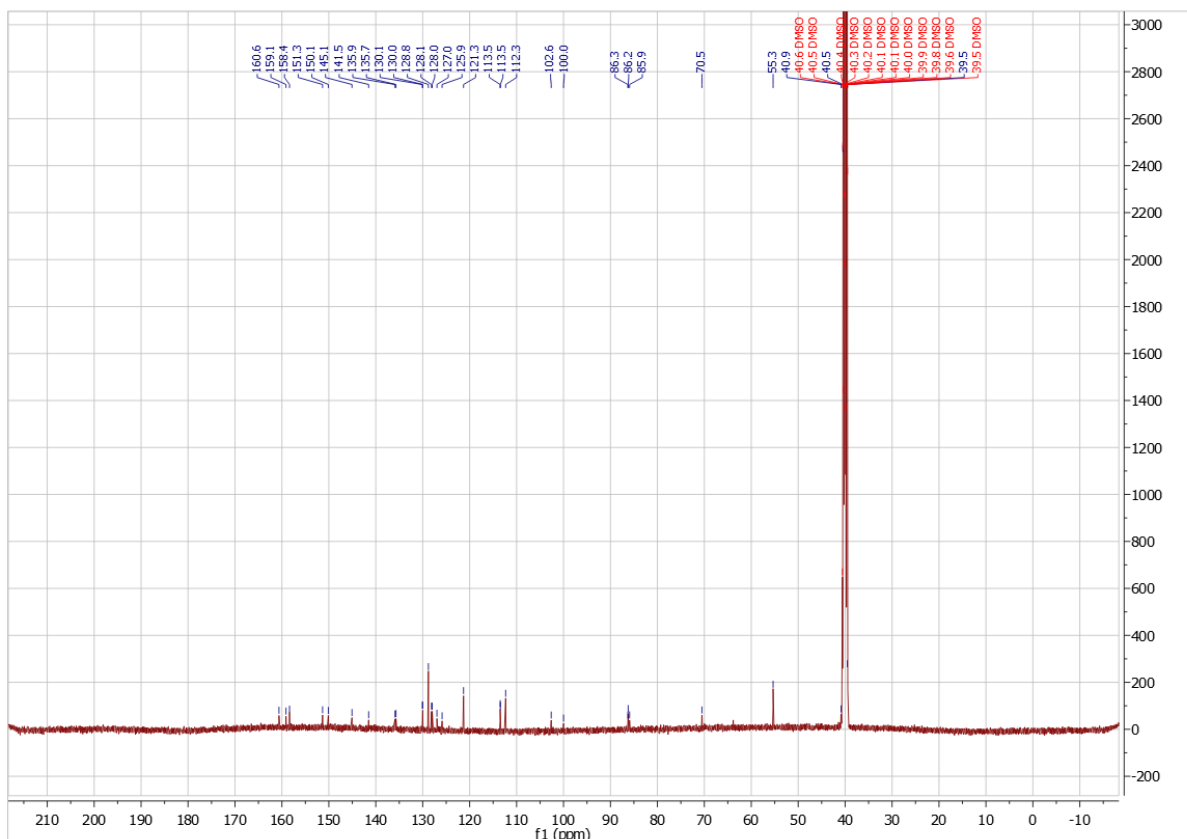
BL078,3-NBA
AK Wagenknecht

b1078 #33-40 RT: 2.37-2.86 AV: 8 NL: 1.09E5

T: + c EI Full ms [84.50-550.50]



Compound 9

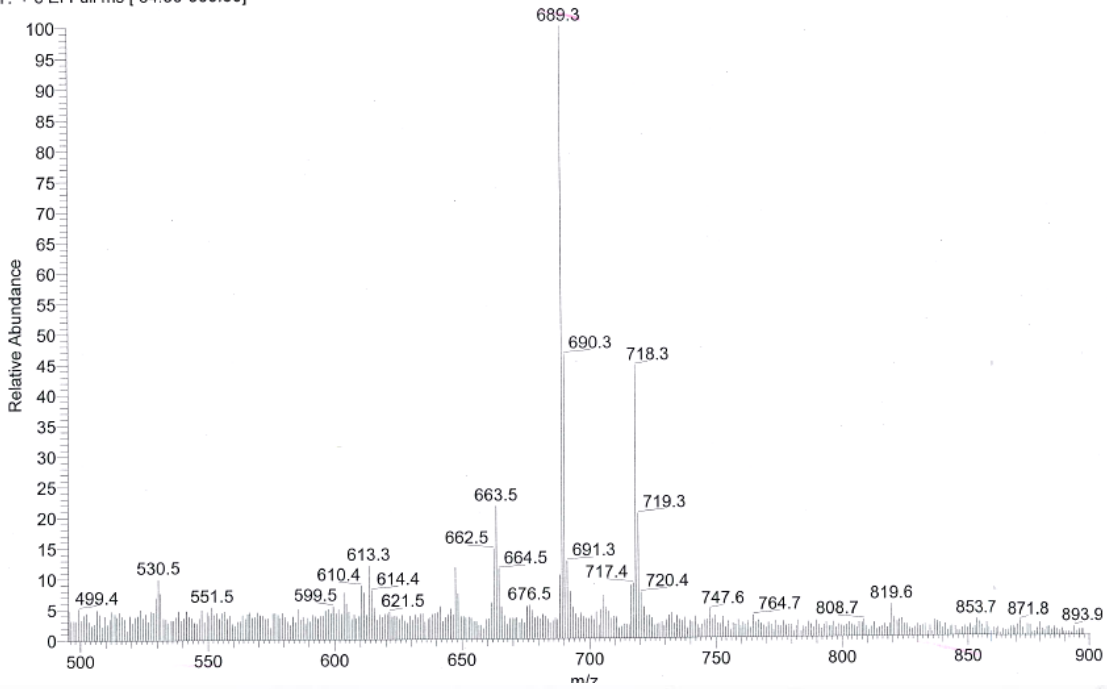


MAT 95,+FAB
moe

7/13/2018 2:32:29 PM
B. Lehmann

BL080,3-NBA
AK Wagenknecht

b1080 #30-40 RT: 2.72-3.60 AV: 11 NL: 9.69E4
T: + c EI Full ms [84.50-900.50]



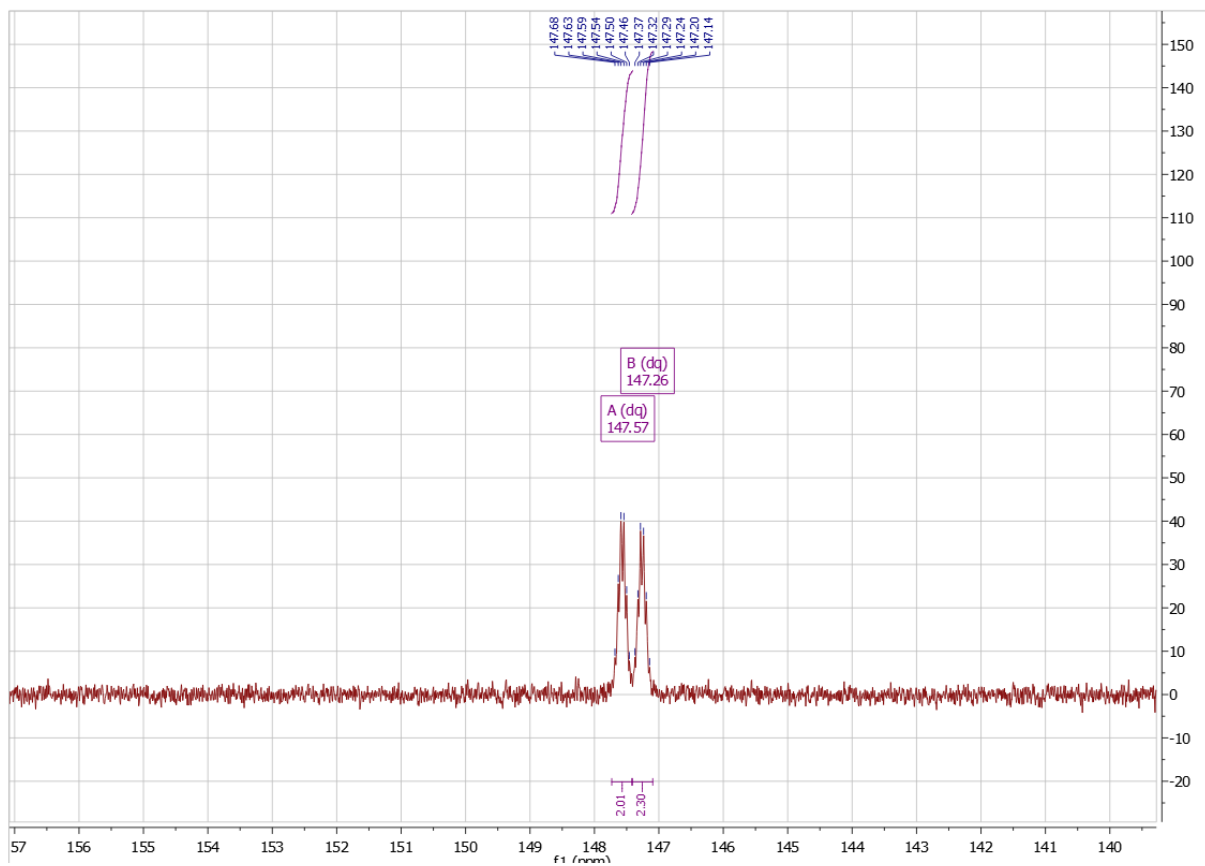
b1080-c5#41 RT: 3.69

T: + c EI Full ms [84.46-900.46]

m/z= 718.1873-718.4469

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
718.2989	46262.0	100.00	718.2989	0.02	C ₃₉ H ₄₀ O ₇ N ₇

Compound 10

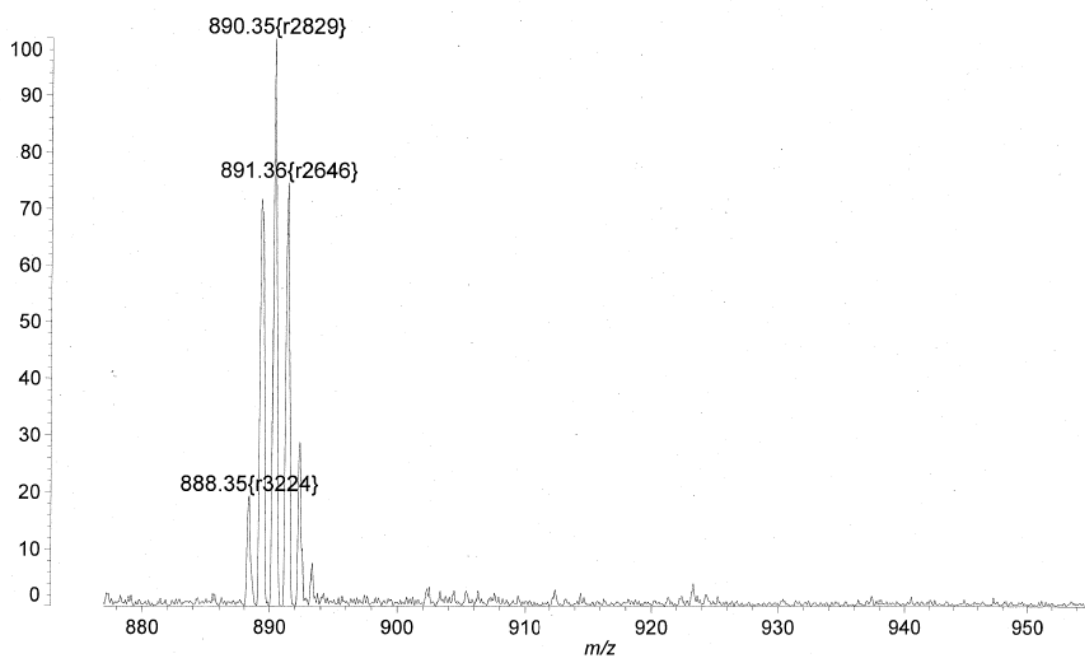


Confidence

Data: BL_82_ATT_ref20001.L17[c] 1 May 2018 9:04 Cal: AK Braese CHCA_Pep 15 May 2017 11:22

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Reflectron, Power: 140, Blanked, P.Ext. @ 850 (bin 67)

%Int. 15 mV[sum= 1332 mV] Profiles 1-88 Smooth Gauss 2 -Baseline 6



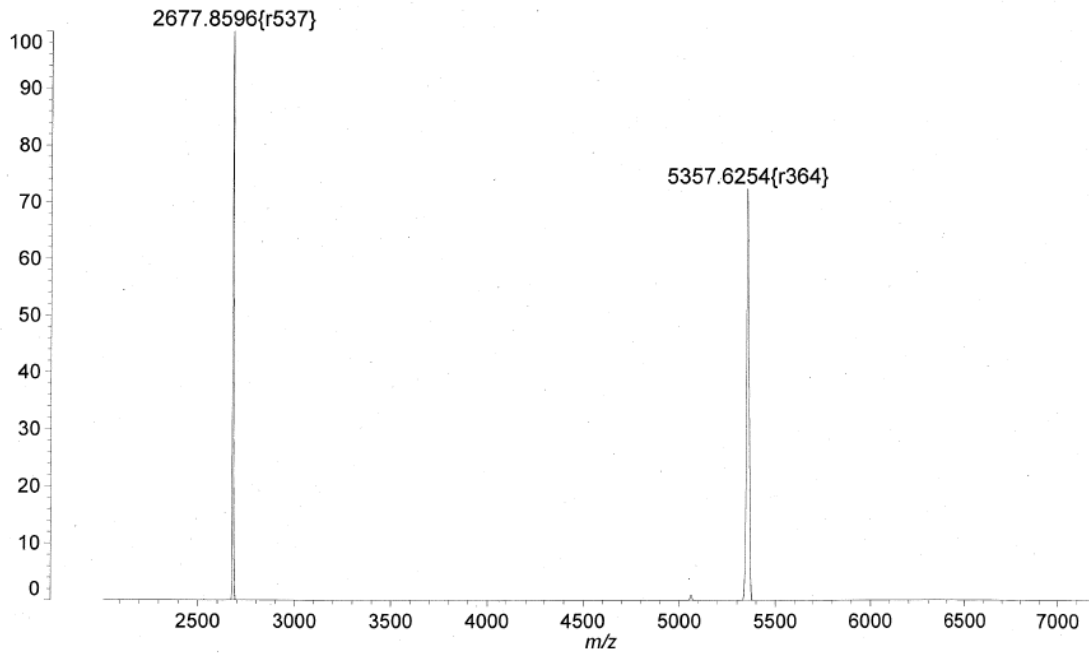
DNA1A

Confidence

Data: BL_1.7_23_HPA_0001.H7[c] 28 Mar 2018 10:34 Cal: 2-4kDa_HPA_inneg 13 Feb 2018 11:02

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Linear_neg, Power: 130, Blanked, P.Ext. @ 5500 (bin 121)

%Int. 145 mV[sum= 6100 mV] Profiles 1-42 Smooth Av 50 -Baseline 700



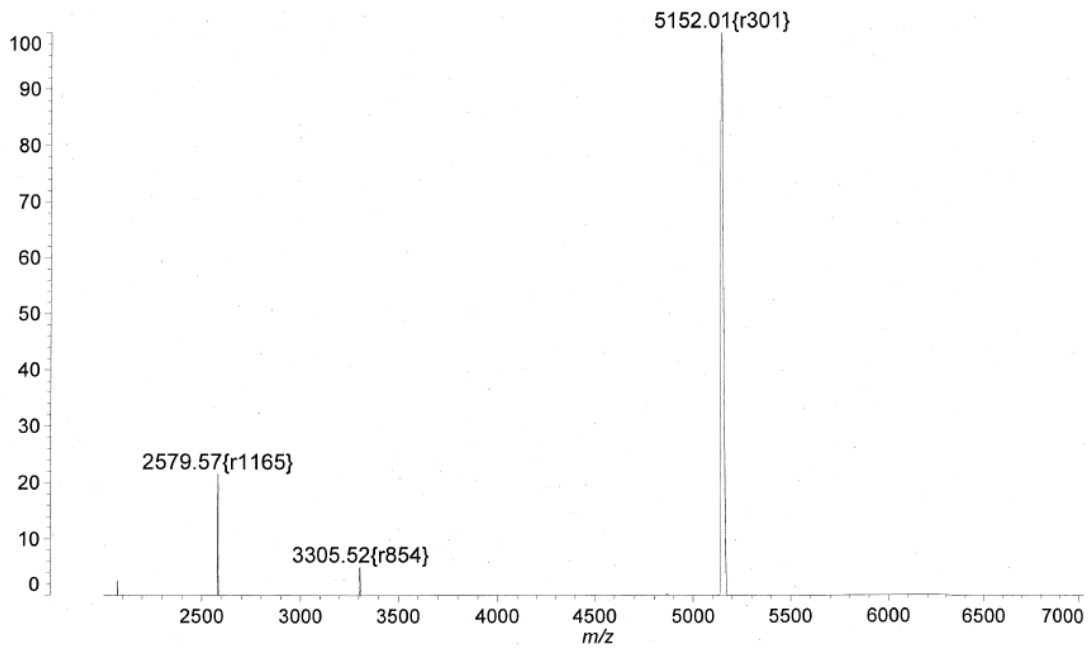
DNA1B

Confidence

Data: BL_DNA_4_21b_HPA_0001.J15[c] 6 Jun 2018 12:15 Cal: Big_DNA 14 Feb 2018 10:39

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Linear_neg, Power: 138, Blanked, P.Ext. @ 5000 (bin 116)

%Int. 0.2 mV[sum= 58 mV] Profiles 1-306 Smooth Av 30 -Baseline 400



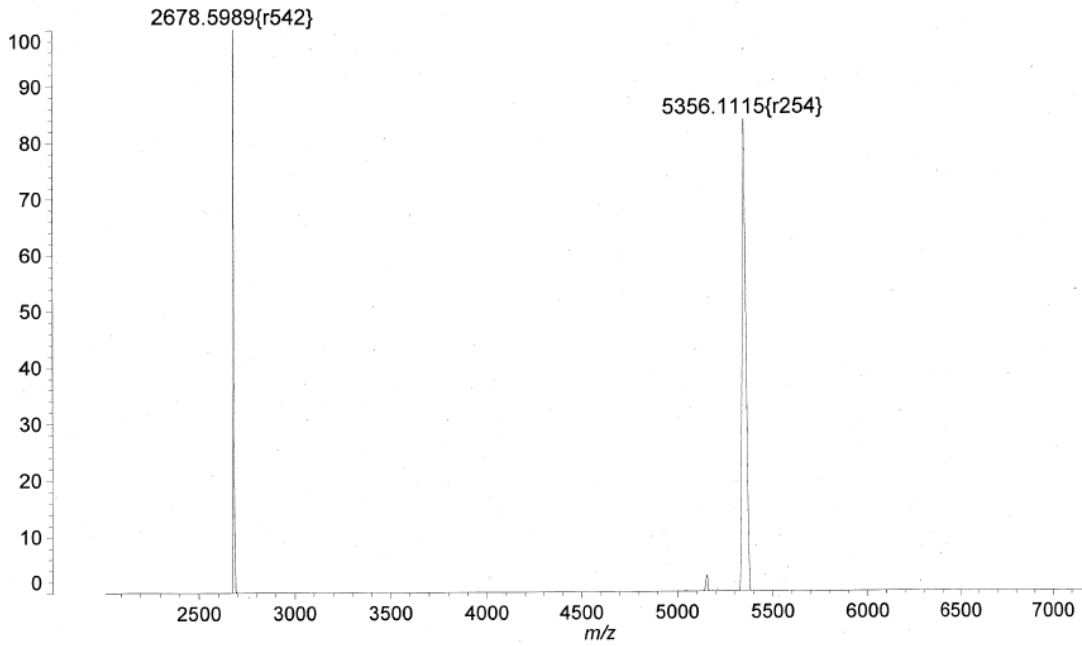
DNA2A

Confidence

Data: BL_3.2_22_HPA_0001.114[c] 28 Mar 2018 10:53 Cal: 2-4kDa_HPA_inneg 13 Feb 2018 11:02

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Linear_neg, Power: 130, Blanked, P.Ext. @ 5500 (bin 121)

%Int. 131 mV[sum= 4992 mV] Profiles 1-38 Smooth Av 40 -Baseline 500



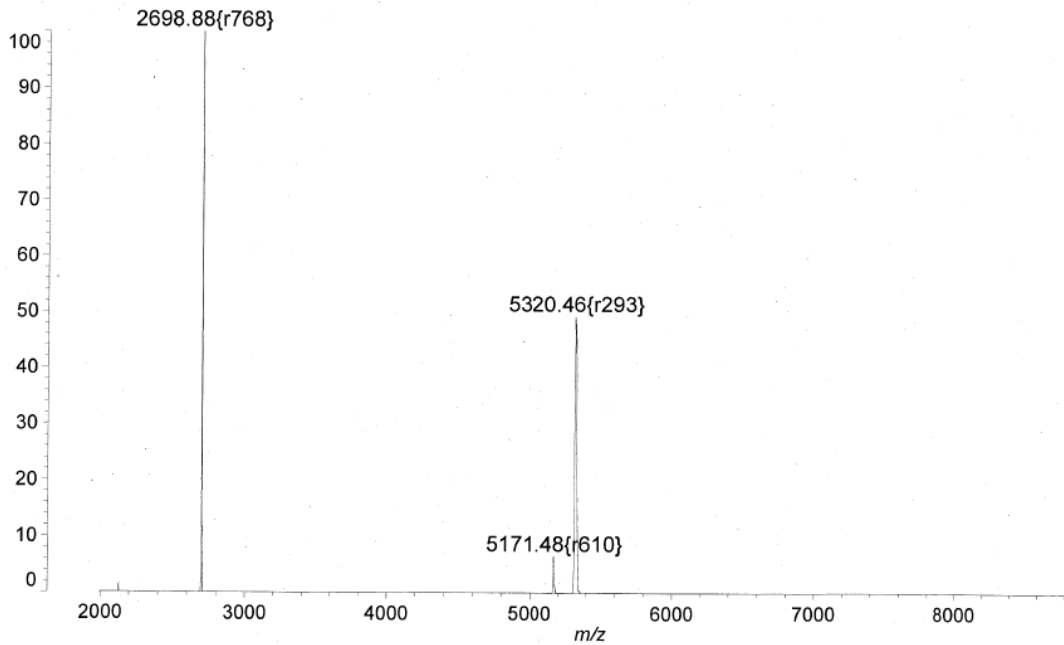
DNA2B

Confidence

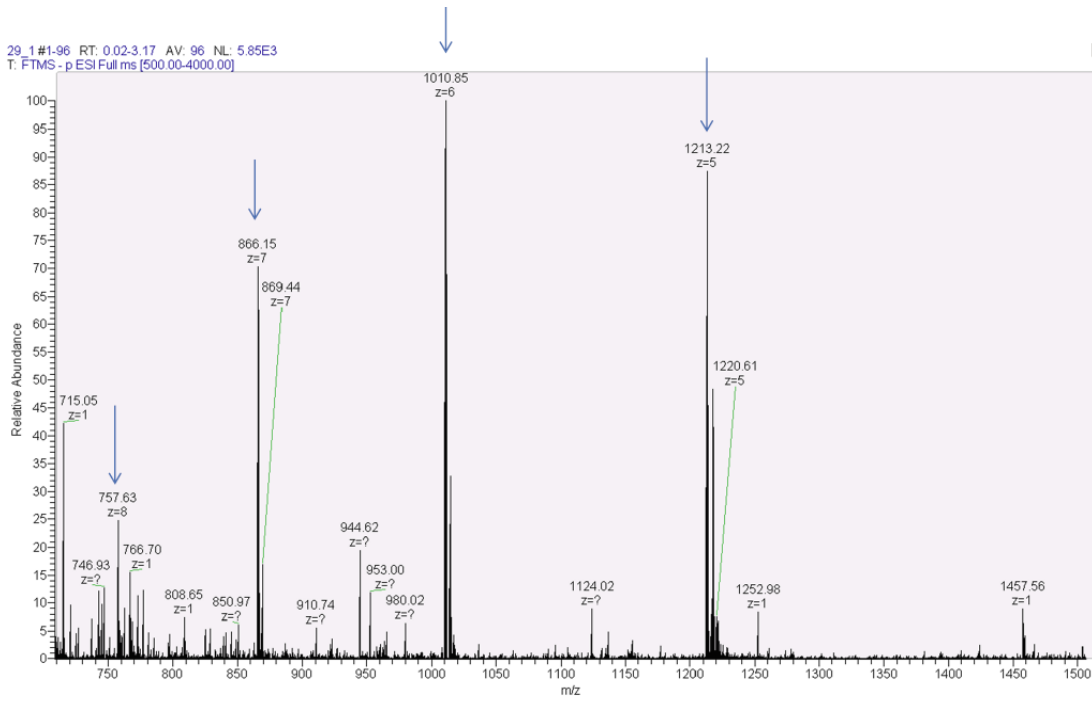
Data: BL_DNA_6_25_HPA_0002.J2[c] 6 Jun 2018 11:59 Cal: DNA_5_8_kDA 4 Oct 2017 11:12

Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Linear_neg, Power: 138, Blanked, P.Ext. @ 5000 (bin 116)

%Int. 0.2 mV[sum= 71 mV] Profiles 1-307 Smooth Av 30 -Baseline 400

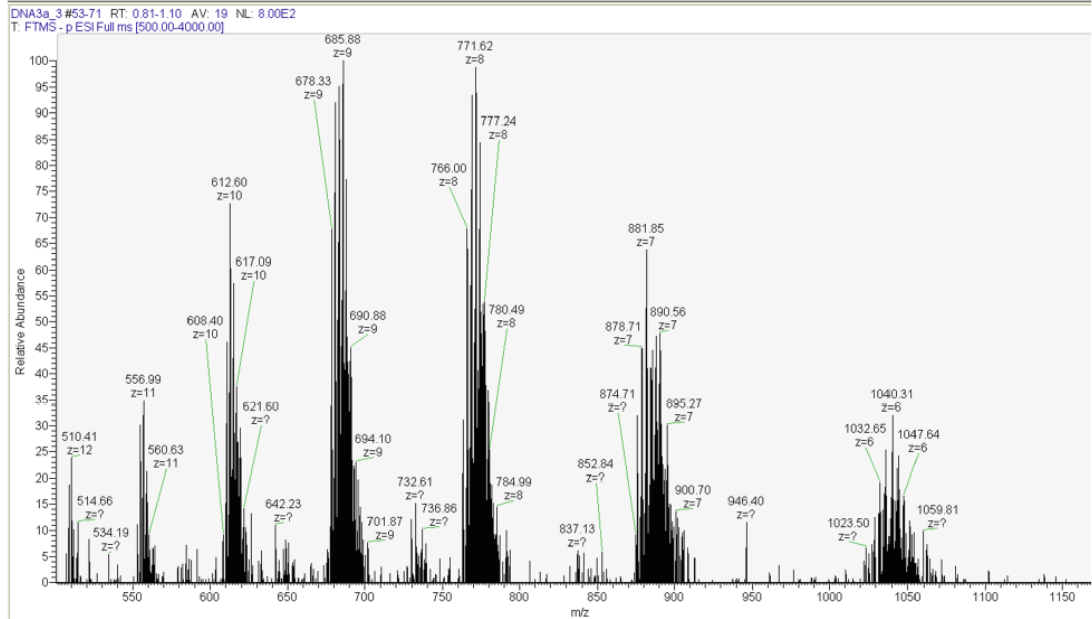


DNA2A+Cy3



Y:\DATEN\12018\180724_LehmannDNA3a_3

24.07.2018 16:38:36

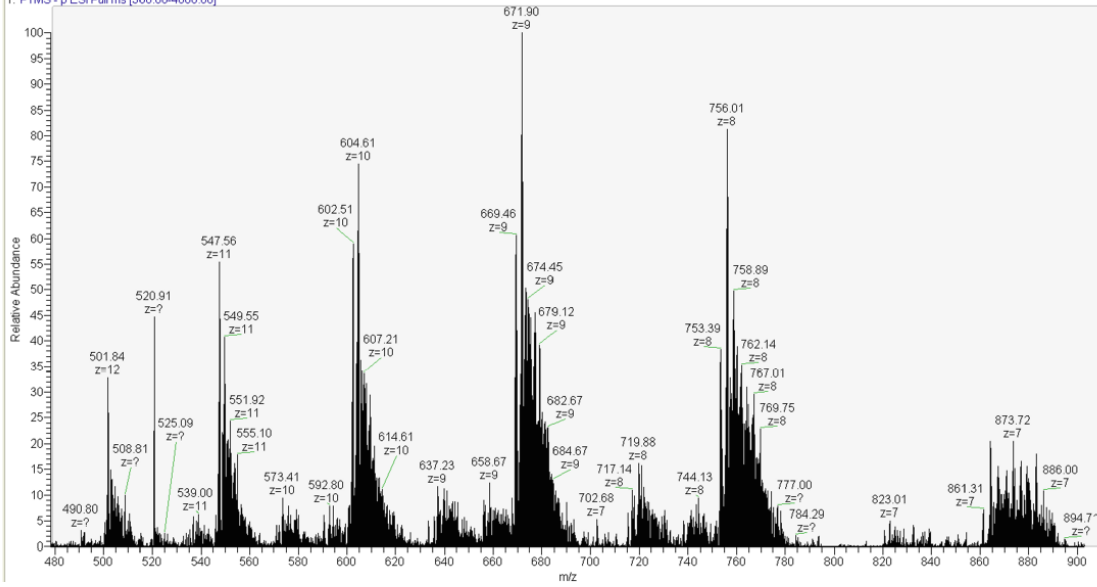


DNA2B+Cy3

Y:\DATEN_1\2018\180724_Lehmann\DNA6a_1

24.07.2018 17:00:06

DNA6a_1#46.155 RT: 0.64-2.24 AV: 110 NL: 2.92E3
T: FTMS - p ESI Full ms [300.00-4000.00]



7. References

- [1] W. Song, Y. Wang, J. Qu, Q. Lin, *Journal of the American Chemical Society* **2008**, *130*, 9654-9655.