Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Cell-resistant wavelength-shifting molecular beacons made of L-DNA and a clickable L-configured uridine

Fabian Lang, Franziska Rönicke and Hans-Achim Wagenknecht

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

Supporting Information

Content

1. General materials and methods

All chemicals were purchased from Abovchem, Aldrich, Alfa Aesar, ABCR, Fluka, TCI. The unmodified and Atto550-modified DNA were purchased from metabion. Thin layer chromatogarphy was performed on Fluka silica gel 60 F254 coated aluminium foil. Flash chromatography was carried out on silica gel 60 from Aldrich (43-60 µm). The dye azides were purchased from the companies Lumiprobe, Jena Bioscience and ATTO-TEC. The solvents used for synthesis were of at least the quality level "per analysis" (p.a.). Dry solvents were purchased from Acros Organics and stored via molecular sieve and in an argon atmosphere. For the purification on the RP-HPLC, high-purity organic solvents (HPLC grade) were solvents (HPLC grade) from the Fisher Scientific and deionized water from a Merck Milli-Q Direct 8 desalination system were used. Deuterated solvents were purchased from Eurisotop purchased.

NMR spectroscopy. ^{1}H NMR (400 MHz), ^{13}C NMR spectra (101 MHz) and ^{31}P NMR spectra (162 MHz) were measured on a Bruker Advance DRX 500. ^{1}H NMR (500 MHz), ^{13}C NMR-spectra (126 MHz) and ^{31}P NMR spectra (202 MHz) were measured on a Bruker Advance 500. The chemical shifts in the ^{1}H and ^{13}C NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. The chemical shifts in the ^{31}P NMR spectra are reported also in ppm relative to the deuterated solvent. The coupling constant (J) are given in Hertz (Hz), and the multiplicity of signals are reported as followed: s (singlet), d (doublet), t (triplet), q (quadruplet), quint (quintet), sext (sextet), m (multiplet), br. s (broad singlet), dt (doublet of triplets), and td (triplet of doublets).

Mass spectrometry. Mass spectrometry was performed on a Finnigan MAT 95 with FAB and EI as ionization methods. ESI mass spectrometry was performed on a Thermo Fisher Scientific Q Exactive (Orbitrap). Oligonucleotides were identified by MALDI mass spectrometry on an AXIMA Confidence spectrometer from Shimadzu.

Optical spectroscopy. Absorption spectra and melting temperatures (2.5 µM DNA, 250 mM NaCl, 10 mM Na-P_i buffer, 10 – 90 °C, 0.7 °C/min, step width 0.5 °C) were recorded with a Cary 100 Bio Varian UV/Vis spectrometer and a Cary.3500 UV/Vis Multicell Peltier spectrophotometer. All emission spectra were measured on a JOBIN YVON Fluoromax-3 with Peltier element (LFI-3751) or a Fluoromax-4. The spectra were corrected by Raman scattering of the pure solvent. The following parameters were used for the measurements: Step size 1.0 nm, increment time 0.2 s, integration time 0.l s, slit widths 2 nm, 3 nm and 9 nm, respectively.

2. Synthetic procedures

Compound 4. 670 mg L-uridine (2.75 mmol, 1.00 equiv.) was dissolved in 10 mL dry pyridine under argon atmosphere. 1.07 mL TIPDSiCl_2 (1.05 g, 3.32 mmol, 1.20 equiv.) was added under cooling in an ice bath. The reaction mixture was stirred at 0°C for 2 h and then at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2:EtOAc=2:1). Product **4** (1.07 g, 2.20 mmol, 80%) was obtained as a white solid.

TIc (CH_2Cl_2:EtOAc=5:1): R_f = 0.6.

^{1}H NMR (500 MHz, DMSO-d_6): δ(ppm) = 11.36 (d, *J* = 2.1 Hz, 1H, NH), 7.68 (d, *J* = 8.1 Hz, 1H, H-6), 5.58 (d, *J* = 4.7 Hz, 1H, OH-2‘), 5.56 – 5.47 (m, 2H, H-1‘, H-5), 4.18 – 4.09 (m, 3H, H-2‘, H-3‘, H-5‘), 3.97 (dt, *J* = 8.7 Hz, 2.5 Hz, 1H, H-4‘), 3.91 (dd, *J* = 13.2 Hz, 2.7 Hz, 1H, H-5‘), 1.13 – 0.91 (m, 28H, CH_3, CH).

^{13}C NMR (126 MHz, DMSO-d_6): δ(ppm) = 163.2 (C-4), 150.1 (C-2), 139.8 (C-6), 100.9 (C-5), 90.5 (C-1‘), 80.9 (C-4‘), 73.5 (C-2‘), 68.8 (C-3‘), 60.2 (C-5‘), 17.6 – 16.5 (CH_3), 12.9 – 11.7 (CH).

HR-MS (FAB) m/z: calcd. $[M+H]^+$: 487.2290, found 487.2292.

Compound 5. 730 mg **4** (1.50 mmol, 1.00 equiv.) were dissolved in 20 mL dry THF under argon atmosphere and cooled to -10 °C. Subsequently, 120.0 mg sodium hydride (60% in paraffine) (3.00 mmol, 2.00 equiv.) was added in three portions at 15 min intervals and then stirred for another 15 min. The reaction mixture was slowly warmed to room temperature, 0.67 mL of propargyl bromide (80% in toluene) (714 mg, 6.00 mmol, 4.00 equiv.) was slowly added over 15 min and stirred overnight at room temperature. The reaction was stopped by adding 5 mL of water and then extracted with EtOAc (2 x 50 mL). The combined organic phases were washed with approx. 100 mL saturated $NaHCO_3$ solution and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane:EtOAc=3:2). Product **5** (425 mg, 0.81 mmol, 54%) was obtained as a white solid.

TLC (hexane:EtOAc=1:1): R_f = 0.6.

^{1}H NMR (500 MHz, $CDCl_3$): δ(ppm) = 8.24 (s, 1H, NH), 7.89 (d, *J* = 8.1 Hz, 1H, H-6), 5.74 (s, 1H, H-1‘), 5.68 (dd, *J* = 8.2 Hz, 2.3 Hz, 1H, H-5), 4.60 – 4.51 (m, 2H, OCH_2), 4.27 – 4.22 (m, 2H, H-2’, H-3’), 4.16 – 4.10 (m, 2H, H-4’, H-5’), 3.97 (dd, *J* = 13.6 Hz, 2.4 Hz, 1H, H-5‘), 2.46 (t, *J* = 2.4 Hz, 1H, C≡CH), 1.13 – 0.94 (m, 28H, CH_3, CH).

^{13}C NMR (126 MHz, $CDCl_3$): δ(ppm) = 163.0 (C-4), 149.7 (C-2), 139.6 (C-6), 101.7 (C-5), 89.3 (C-1‘), 81.9 (C-4‘), 80.5 (C-2‘), 79.4 (C≡CH), 75.4 (C≡CH), 68.3 (C-3‘), 59.5 (C-5‘), 58.2 (OCH_2), 18.4 – 16.4 (CH_3), 13.8 – 11.8 (CH).

HR-MS (FAB) m/z: calcd.$[M+H]^+$: 525.2447, found 525.2445.

Compound 1. 420 mg **5** (0.80 mmol, 1.00 equiv.) was dissolved in 16 mL dry THF and added with 1.00 mL TBAF solution (1 M in THF) (523 mg, 2.00 mmol, 2.50 equiv.). The reaction solution was stirred for 5 min at room temperature, then transferred to a short silica gel column and eluted with CH_2Cl_2:MeOH=5:1. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (CH_2Cl_2:MeOH=10:1). The product **1** (226 mg, 0.80 mmol, quant.) was obtained as a white solid.

TLC (CH_2Cl_2:MeOH=9:1): R_f = 0.3.

^{1}H NMR (500 MHz, DMSO-d_6): δ(ppm) = 11.36 (s, 1H, NH), 7.93 (d, *J* = 8.1 Hz, 1H, H-6), 5.88 (d, *J* = 5.3 Hz, 1H, H-1‘), 5.66 (d, *J* = 8.1 Hz, 1H, H-5), 5.25 (d, *J* = 5.6 Hz, 1H, OH-3‘), 5.17 (t, *J* = 5.0 Hz, 1H, OH-5‘), 4.30 (dd, *J* = 15.9 Hz, 2.4 Hz, 1H, O-CH_2), 4.23 (dd, *J* = 16.0 Hz, 2.4 Hz, 1H, O-CH_2), 4.15 – 4.12 (m, 1H, H-3’), 4.09 (t, *J* = 5.2 Hz, 1H, H-2’), 3.87 (q, *J* = 3.2 Hz, 1H, H-4’), 3.63 (ddd, *J* = 12.1 Hz, 5.1 Hz, 3.0 Hz, 1H, H-5), 3.56 (ddd, *J* = 12.1 Hz, 4.9 Hz, 3.0 Hz, 1H, H-5‘), 3.44 (t, *J* = 2.2 Hz, 1H, C≡CH).

^{13}C NMR (126 MHz, DMSO-d_6): δ(ppm) = 163.1 (C-4), 150.6 (C-2), 140.5 (C-6), 102.0 (C-5), 85.8 (C-1‘), 85.4 (C-4‘), 79.9 (C-2‘), 79.8 (C≡CH), 77.6 (C≡CH), 68.4 (C-3‘), 60.6 (C-5‘), 56.9 (OCH_2).

HR-MS (FAB) m/z: cald. $[M+H]^+$: 283.0925, found 283.0923.

Compound 6. 85 mg **1** (0.30 mmol, 1.00 equiv.) was dissolved in 5 mL dry pyridine, followed by addition of 132 mg 4,4'-dimethoxytrityl chloride (0.39 mmol, 1.30 equiv.) and stirred overnight at room temperature. 2 mL MeOH was added to stop the reaction and the solvent was removed under reduced pressure. The residue was purified by column chromatography

(CH_2Cl_2:MeOH=99:1 + 0.1 % NEt3). Product **6** (140 mg, 0.24 mmol, 80 %) was obtained as a white solid.

TLC (CH_2Cl_2:MeOH=50:1 + 0.1% NEt_3): R_f = 0.3.

^{1}H NMR (500 MHz, MeOD-d_4): δ(ppm) = 8.02 (d, *J* = 8.0 Hz, 1H, H-6), 7.46 – 7.21 (m, 9H, DMTr), 6:92 – 6.85 (m, 4H, DMTr), 5.97 (d, *J* = 3.5 Hz, 1H, H-1‘), 5.23 (d, *J* = 8.1 Hz, 1H, H-5), 4.48 (t, *J* = 6.2 Hz, 1H, H-3‘), 4.44 (s, 1H, O-CH_2), 4.43 (s, 1H, O-CH_2), 4.29 (dd, *J* = 5.2 Hz, 3.5 Hz, 1H, H-2’), 4.11 – 4.05 (m, 1H, H-4’), 3.79 (s, 6H, OCH_2), 3.52 – 3.43 (m, 2H, H-5‘), 2.92 (t, *J* = 2.4 Hz, 1H, C≡CH).

^{13}C NMR (126 MHz, MeOD-d_4): δ(ppm) = 166.1 (C-4), 160.4 (C_{arom}), 152.0 (C-2), 146.0 (C_{arom}), 142.4 (C-6), 142.4 (C_{arom}), 136.8 (C_{arom}), 136.5 (C_{arom}), 131.5 (C_{arom}), 131.4 (C_{arom}), 129.3 (C_{arom}), 129.9 (C_{arom}), 128.1 (C_{arom}), 114.3 (C_{arom}), 102.5 (C-5), 88.8 (C-1‘), 88.4 (C-4‘), 84.9 (C-2‘), 76.7 (C≡CH), 70.2 (C≡CH), 63.4 (C-3‘), 58.6 (C-5‘), 55.8 (OCH_2).

HR-MS (FAB) m/z: cald. $[M]^+$: 584.2153, found 584.2152.

Compound 2. 105 mg **6** (0.18 mmol, 1.00 equiv.) was dissolved in 5 mL of dry CH_2Cl_2 under argon atmosphere followed by addition of 95.0 µL of *N*,*N*-diisopropylethylamine (70.0 mg, 0.54 mmol, 3.00 equiv.) and 60.0 µL of 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (63.6 mg, 0.27 mmol, 1.50 equiv.). The reaction mixture was stirred for 3 h at room temperature and then purified directly, without removing the solvent, by column chromatography (CH_2Cl_2:acetone=5:1 + 0.1% NEt3). Product **2** (120 mg, 0.15 mmol, 85 %) was obtained as a white solid.

DC (CH_2Cl_2:acetone=5:1 + 0.1% NEt_3): R_f = 0.7

^{31}P NMR (202 MHz, CD_2Cl_2): δ(ppm) = 150.5.

MS (MALDI) m/z: 783.37 $[MH]^+$.

3. Oligonucleotide synthesis

The oligonucleotides were synthesized under an argon atmosphere on an H-6 DNA/RNA synthesizer from K&A LABORGERÄTE. Controlled pore glass (CPG) with 1 µmol (500 Å) was used as the solid phase. All reagents, phosphoramidites, solvents and CPG columns were obtained from GlenResearch and Sigma Aldrich.

After automated DNA synthesis of the first part of the sequence of the molecular beacons, the CPG column was dried under vacuum. For the first click modification (on solid phase), he click reagent, consisting of 228 µL Cy3-azide solution (10 mM in DMSO:*t*-BuOH=3:1), 34 µL tetrakis(acetonitril)copper(I)-hexafluorophosphate solution (100 mM in DMSO:*t*-BuOH=3:1), 50 µL sodium ascorbate solution (400 mM in H_2O) und 130 µL MeCN, was applied to the CPG columns using syringes. The reaction vessel was heated to 60 °C for 2 h. After cooling to r.t., the reagent was removed from the CPG column. The CPG column was rinsed using 10 mL MeCN until a colorless solution came out of it, and plugged into the DNA synthesizer. The second part of the sequence was synthesized automatically. After synthesis the CPG columns were dried under high vacuum, opened and the CPG transferred to an Eppendorf vial equipped with a safe-lock lid. 750 µl NH_4OH solution (>25%) were added and the mixture was incubated overnight at 55 °C. The solvent was removed using a Christ Alpha RVC vacuum concentrator and the samples were *lyophilized using a Christ* Alpha 1-2 LD Plus lyophilizer. The oligonucleotide was prepurified by Glen-Pak™ DNA Purification Cartridges. For the second

click modification (in solution), 100 µl H_20 and 114 µl Cy5-azide solution (10 mM in DMSO:*t*-BuOH=3:1), 34 µL TBTA solution (100 mM in DMSO:*t*-BuOH=3:1, 17 µL tetrakis(acetonitril)copper(I)-hexafluorophosphate solution (100 mM in DMSO:*t*-BuOH=3:1), 25 µL sodium ascorbate solution (400 mM in ddH_2O) were added. The reaction mixture was heated to 60 °C for 2 h. After cooling to r.t., the reaction mixture was transferred to Falcon tube. The oligonucleotide was precipitated by adding 150 µL Na_2EDTA solution (40 mM in H_2O), 450 µL sodium ascorbate solution (300 mM in H_2O) and 8 mL EtOH and storing overnight at -28 °C. The precipitate was centrifuged, the pellet was washed two times with 1 mL 80% EtOH solution and dried under high vacuum.

Table S1. Protocol for coupling of the L-configured DNA building blocks including **2**.

	Time [0.1 s]	Reagent 1	Reagent 2	Target	*S.Col.Ptr.*	Time [s]	Branch
				BRANCH			
1	4	TET		COL	ON		
2	4	AMD	TET	COL			
3					ON		
4						99	
5						99	
6	4	AMD	TET	COL	ON		
7					ON		
8						99	
9						99	
10	10	ACN		M_W			
11	10	GAS		M_W			

High Performance Liquid Chromatography (HPLC). The synthesized oligonucleotides were purified on a ThermoFisher system (Dionex UltiMate3000 with autosampler, pump module, column oven, multidiode array, RS fluorescence detector, fraction collector) using the Chromeleon 7 software). For the semi-preparative separation, a reversed-phase Supelco Discovery® BIO Wide Pore C18 column (250 x 10 mm, 5 µm) was used with a flow rate of 2.5 mL/min. A VDSpher OptiBio PUR 300 S18-SE column (250 x 4.6 mm, 5 µm) with a flow rate of 1.0 mL/min was used for analytical chromatography. A 100 mM ammonium bicarbonate buffer (component A, RNase-free, DEPC-treated) was used as the mobile phase for the purification. The respective sample could be eluted with an increasing acetonitrile gradient (0-50% B in A over 40 min, 40 °C). Detection was at a wavelength of λ = 260 nm, 290 nm, 555 nm and 646 nm.

MALDI-TOF MS. Mass spectrometry was performed using a Shimadzu Axima Confidence Spectrometer using the following matrix: 3-hydroxypicolinic acid (saturated in MeCN:H_2O=1:1) / diammoniumhydrogencitrat (0.44 M in H_2O) = 9:1.

Optical Spectroscopy. The optical-spectroscopic characterization of the synthesized RNA was carried out in Starna quartz glass cuvettes (path length 1 cm, sample volume 1 mL) at a temperature of 20 °C. The spectra were baseline corrected using the respective solvent. UV/Vis absorption was measured on a Varian Cary 100 Bio spectrometer with a temperature-controlled cell holder (Cary 100 temperature controller) using the Cary WinUV Scan Application software. The following parameters were selected: averaging time 0.1 s, data interval 1.0 nm, scanning speed 600 nm/min, lamp change 350 nm. The melting temperatures of oligonucleotide duplexes were measured on the same device using the Thermal program as software. The change in absorption of the oligonucleotides was detected at 260 nm in a temperature range of 10-90 °C (heating or cooling rate 0.7 °C/min, recording of the

measurement data per 0.5 °C). The temperature at the start and end point was maintained for 10 min each. Fluorescence was measured on a Horiba Fluoromax-4. The spectra were corrected for the Raman scattering of the pure solvent. The following parameters were used for the measurements: step size 1.0 nm, increment time 0.2 s, integration time 0.1 s, slit widths 2 nm, 3 nm or 9 nm.

The synthesized oligonucleotides were quantified by their UV/Vis absorbance according to the following equation

$$\varepsilon_{260} = (\mathrm{A} \cdot \varepsilon_{\mathrm{A}} + \mathrm{C} \cdot \varepsilon_{\mathrm{C}} + \mathrm{G} \cdot \varepsilon_{\mathrm{G}} + \mathrm{T} \cdot \varepsilon_{\mathrm{T}} + \mathrm{cU} \cdot \varepsilon_{\mathrm{cU}}) \cdot \ 0.9 \qquad (4)$$

$$\varepsilon_{\mathrm{A}} = 15.4\ \mathrm{mM^{-1}cm^{-1}}\ \ \varepsilon_{\mathrm{C}} = 7.3\ \mathrm{mM^{-1}cm^{-1}}$$

$$\varepsilon_{\mathrm{G}} = 11.7\ \mathrm{mM^{-1}cm^{-1}}\ \ \varepsilon_{\mathrm{T}} = 8.8\ \mathrm{mM^{-1}cm^{-1}}$$

and the following extinction coefficients (Table S2).

Table S2. Exctinction coefficients ε for modified building blocks at λ = 260 nm.

Modification	ε_{260} [$mM^{-1}cm^{-1}$]
1/2	10.1
Cy3	4.9
Cy5	10.0
AF488	21.3

The synthesized oligonucleotides were identified by MALDI-TOF mass spectrometry (Table S3).

Table S3. Extinction coefficients ε_{260} and MALDI-TOF MS analyses of **LMB1-LMB4**, **DMB1** and **DMB4**. The small differences between calcd. and found m/z data is due to the inaccuracy of the MALDI-TOF-MS instrument.

DNA	ε_{260} [$mM^{-1}cm^{-1}$]	m/z calcd. [$gmol^{-1}$]	m/z found [$gmol^{-1}$]
LMB1	336.2	11241.2	11242.2
LMB2	362.7	11857.3	11859.4
LMB3	353.3	11859.3	11862.4
LMB4	379.8	12475.4	12477.4
DMB1	336.2	11241.2	11243.5
DMB4	379.8	12475.4	12477.6

4. Images of NMR spectra

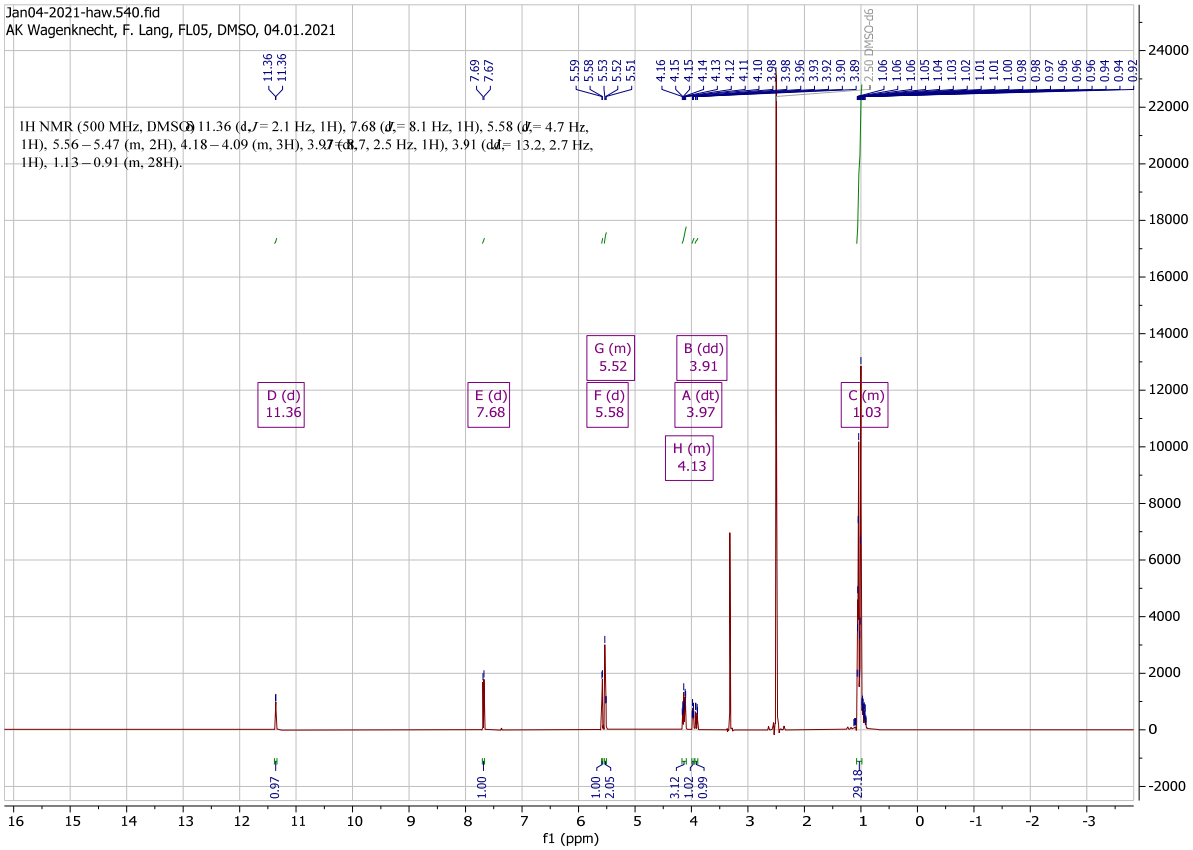


Figure S1. ^{1}H NMR spectrum of compound **4**.

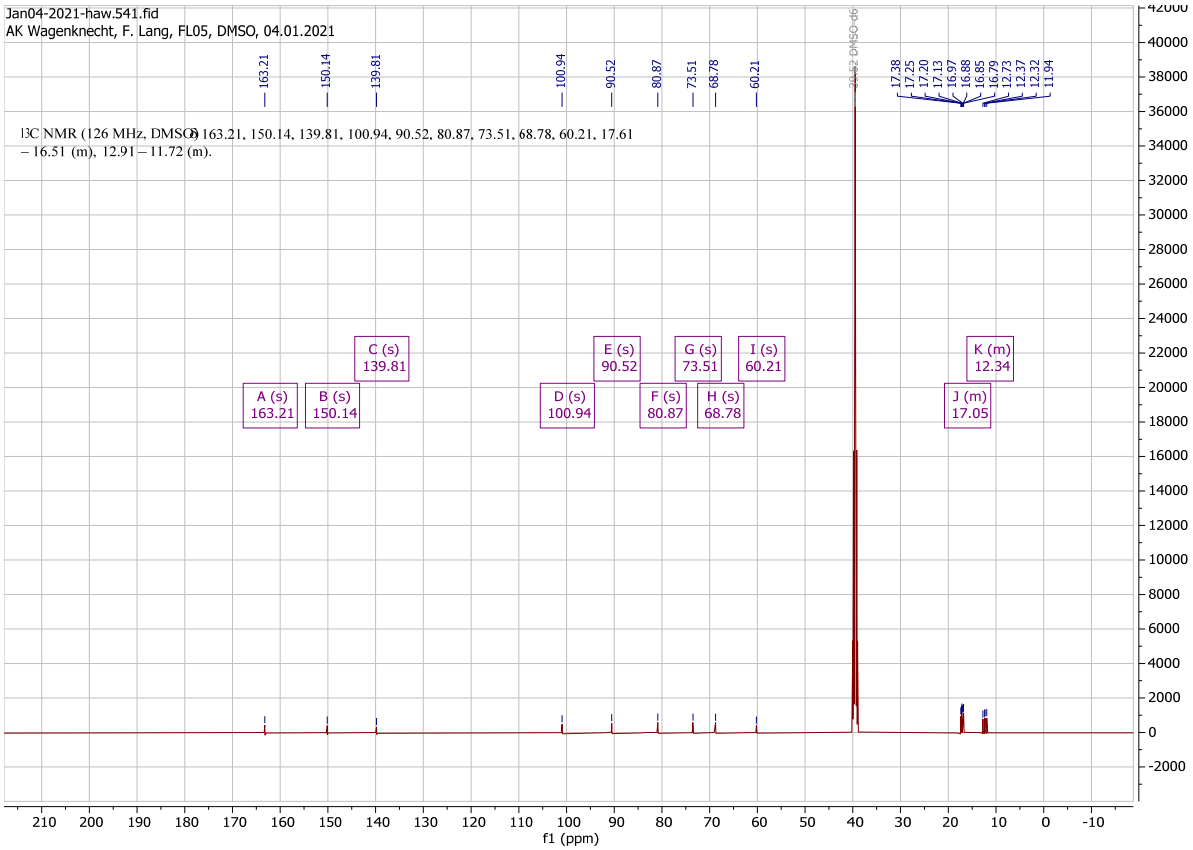


Figure S2. ^{13}C NMR spectrum of compound **4**.

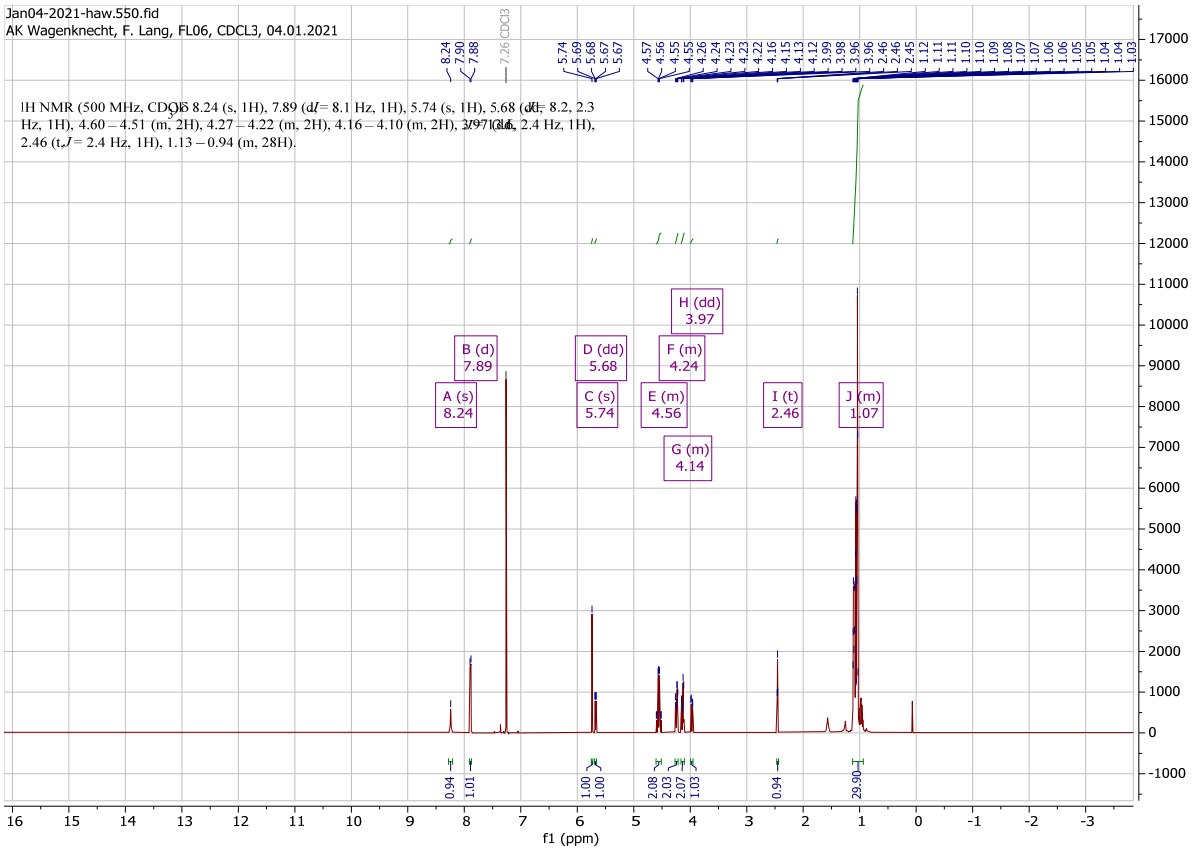


Figure S3. ^{1}H NMR spectrum of compound **5**.

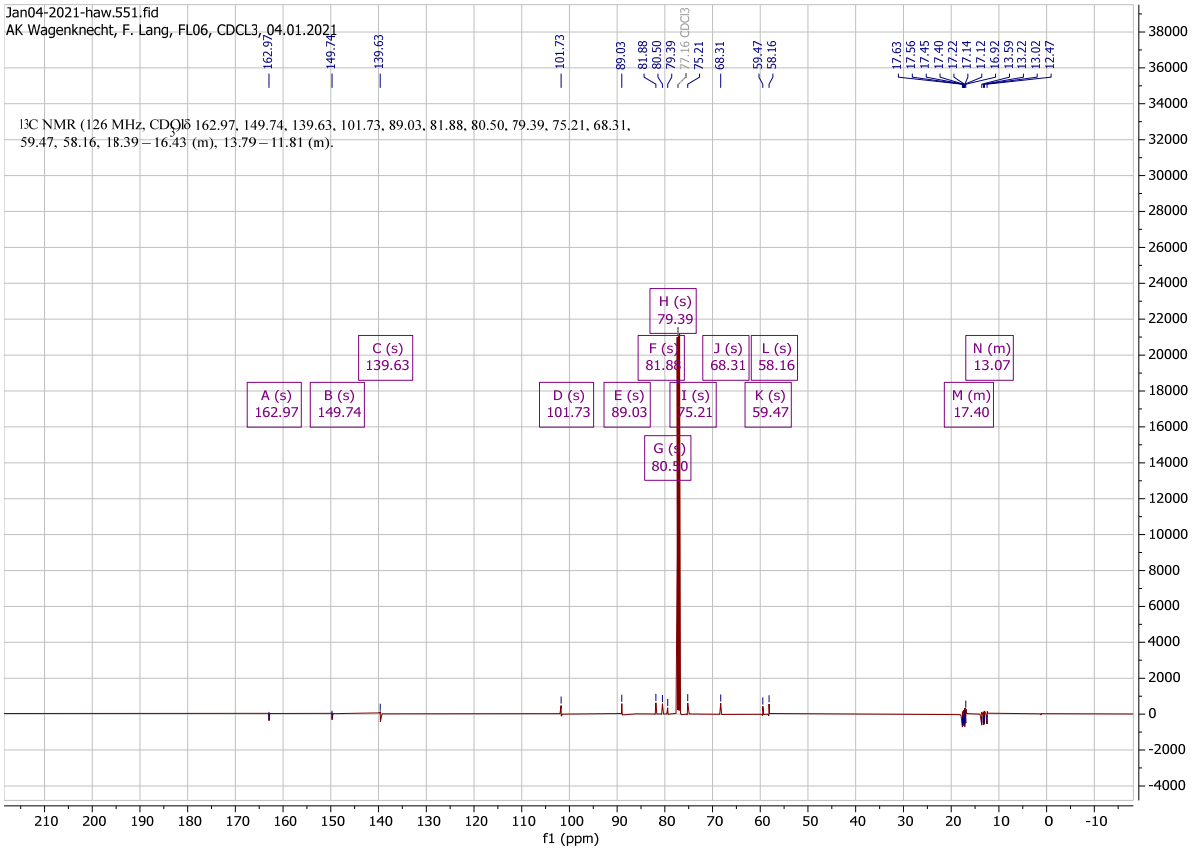


Figure S4. ^{13}C NMR spectrum of compound **5**.

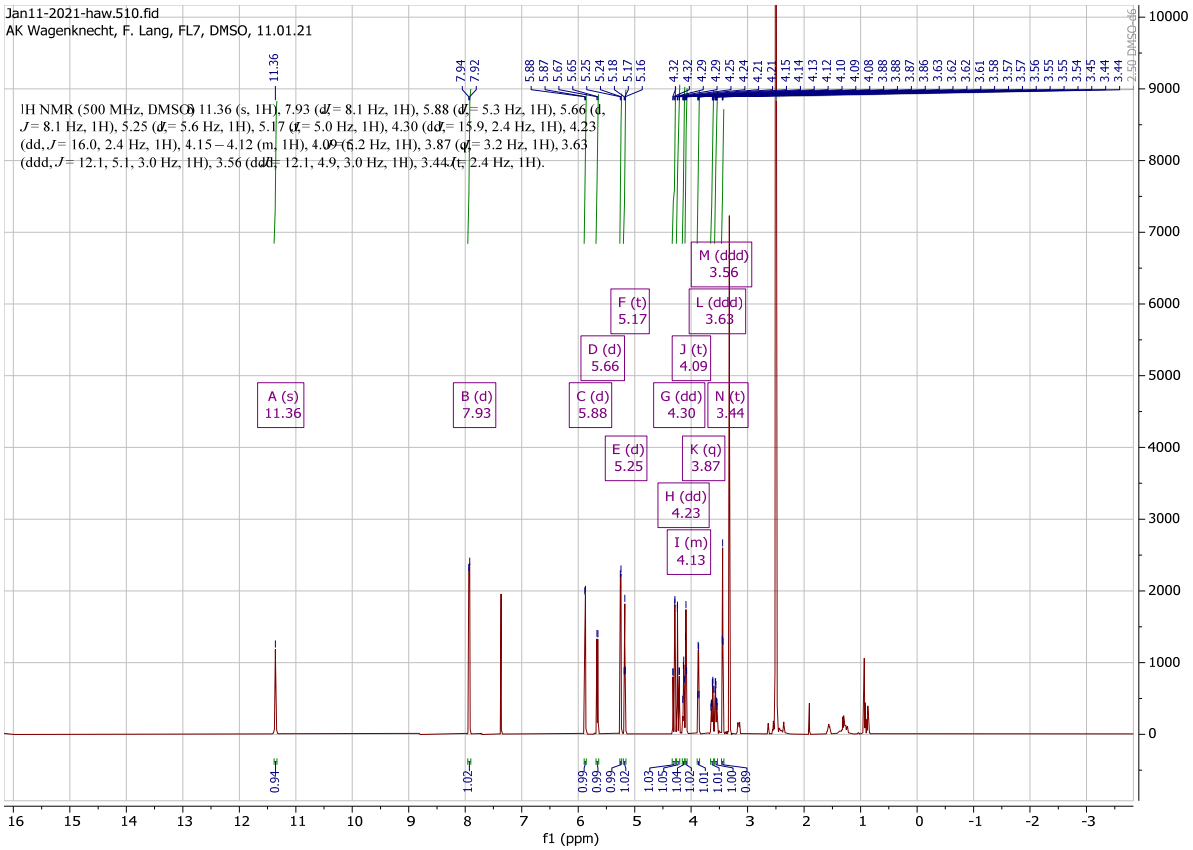


Figure S5. ^{1}H NMR spectrum of compound **1**.

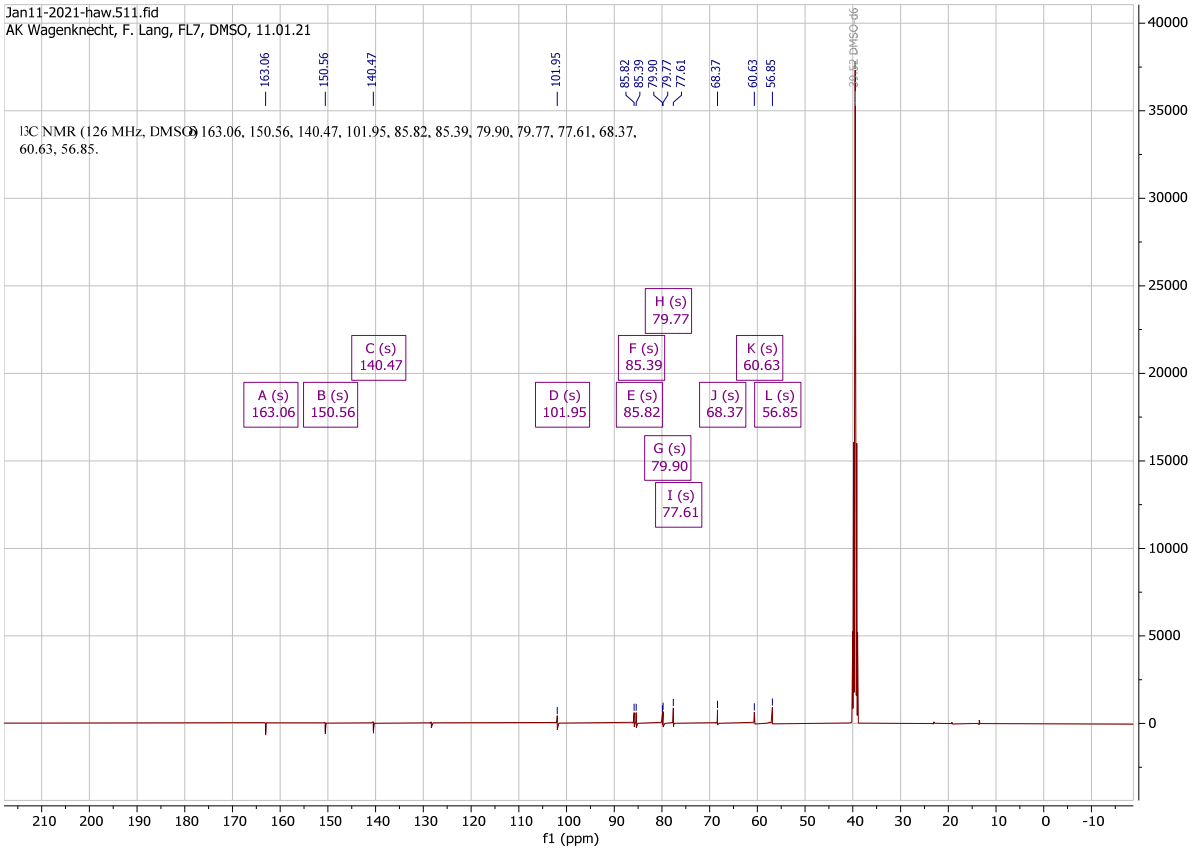


Figure S6. ^{13}C NMR spectrum of compound **1**.

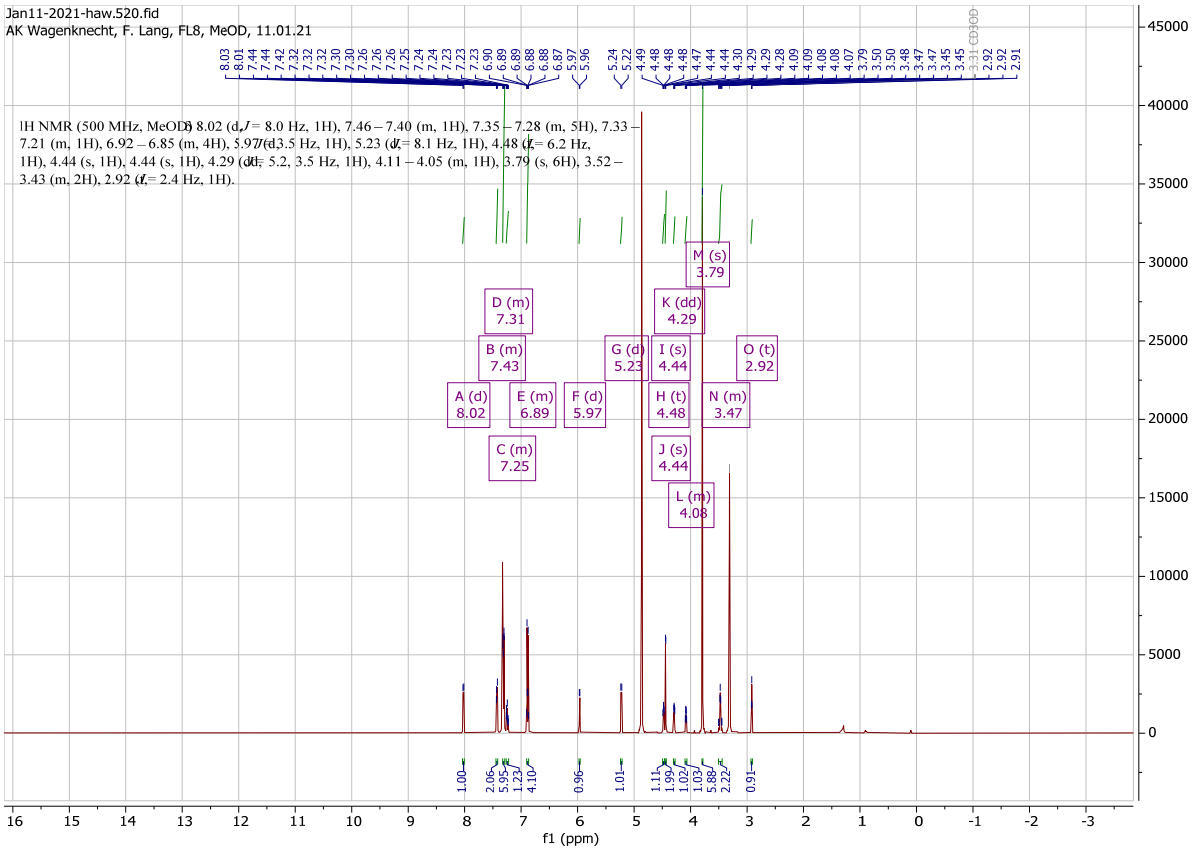


Figure S7. ^{1}H NMR spectrum of compound **6**.

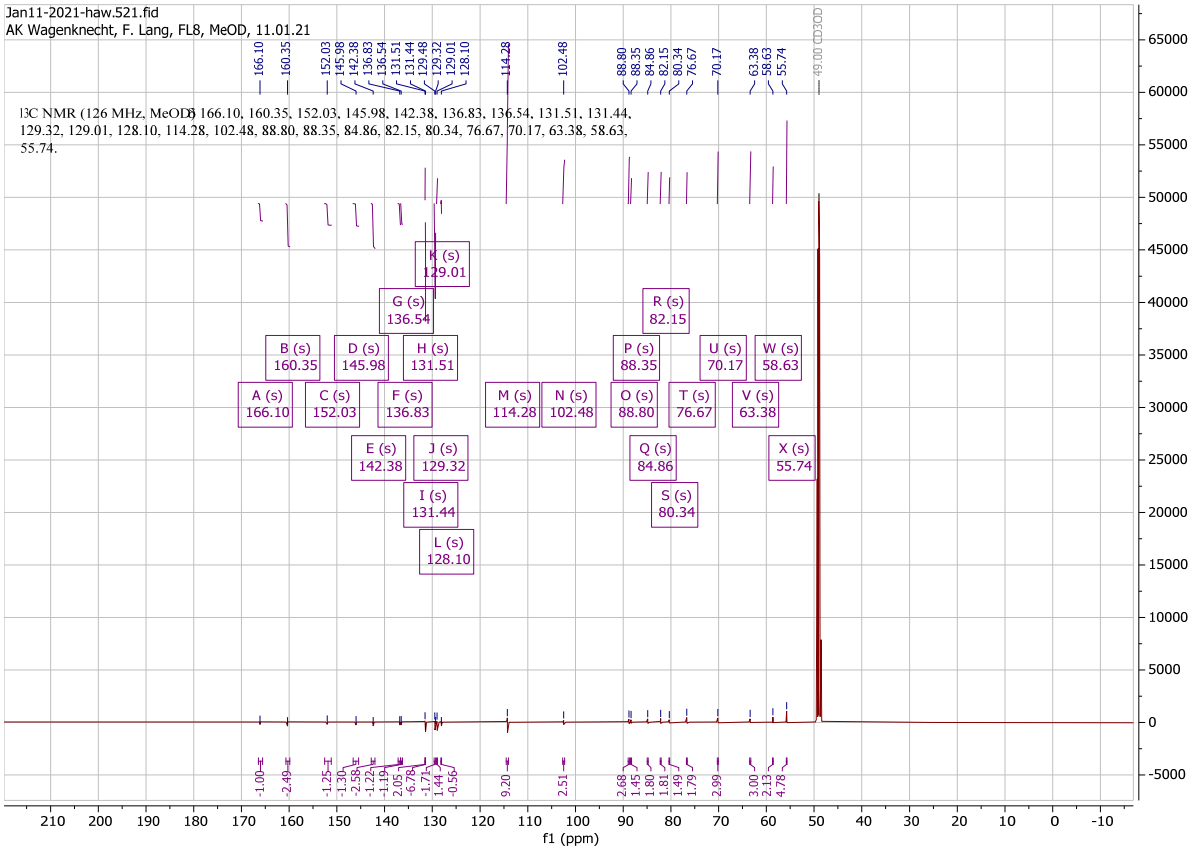


Figure S8. ^{13}C NMR spectrum of compound **6**.

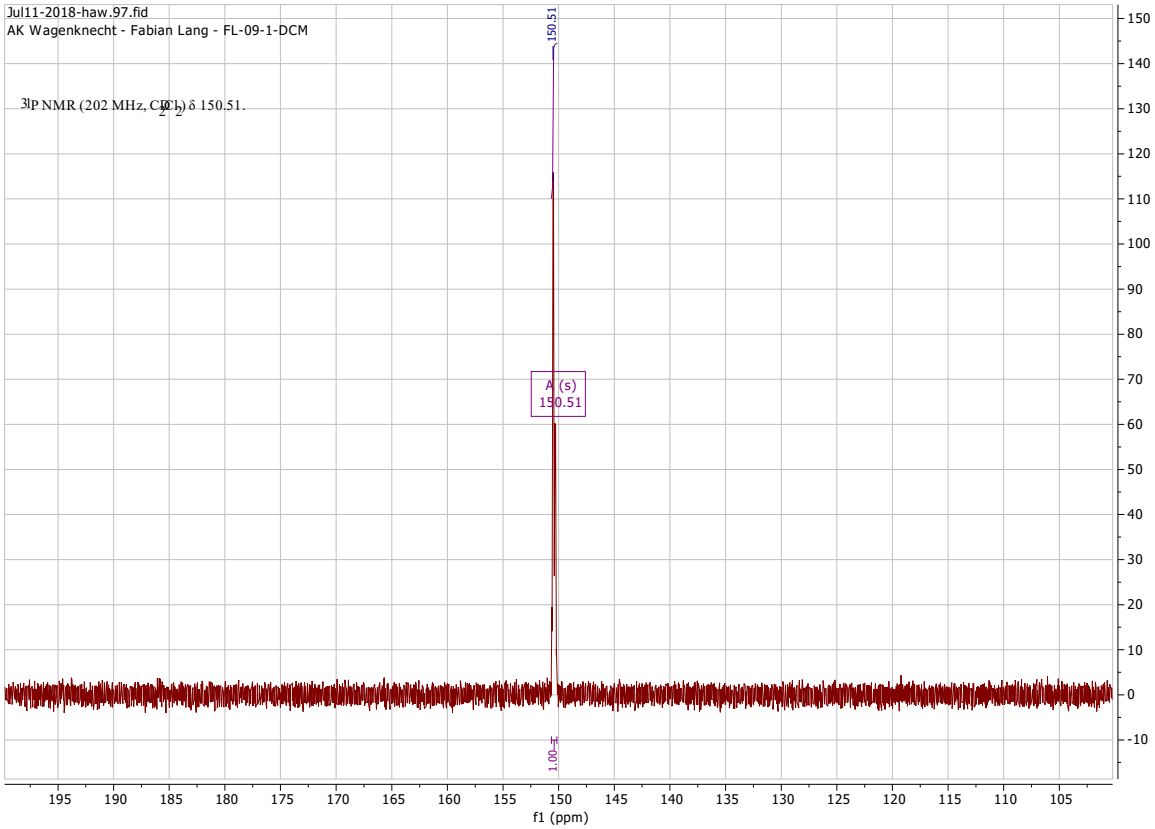


Figure S9. ^{31}P NMR spectrum of compound **2**.

5. Images of MS analyses

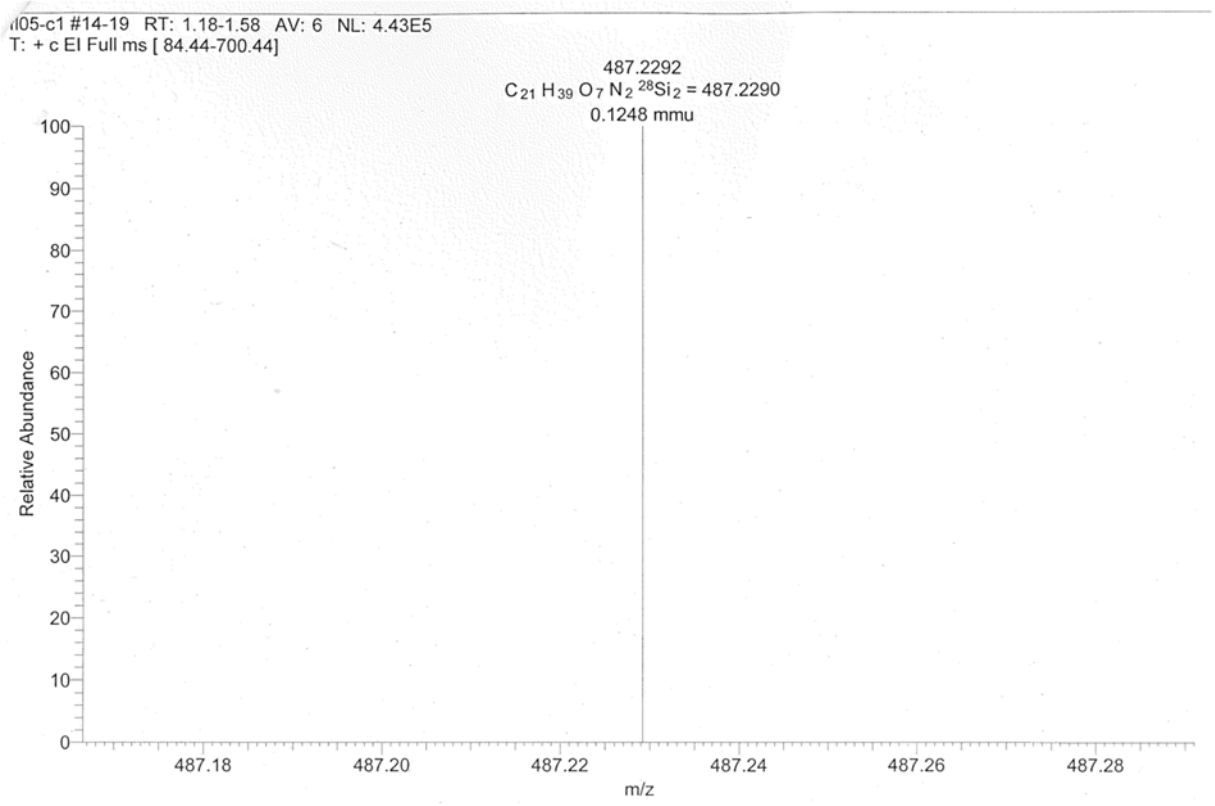


Figure S10. HR-MS (FAB) of compound **4**.

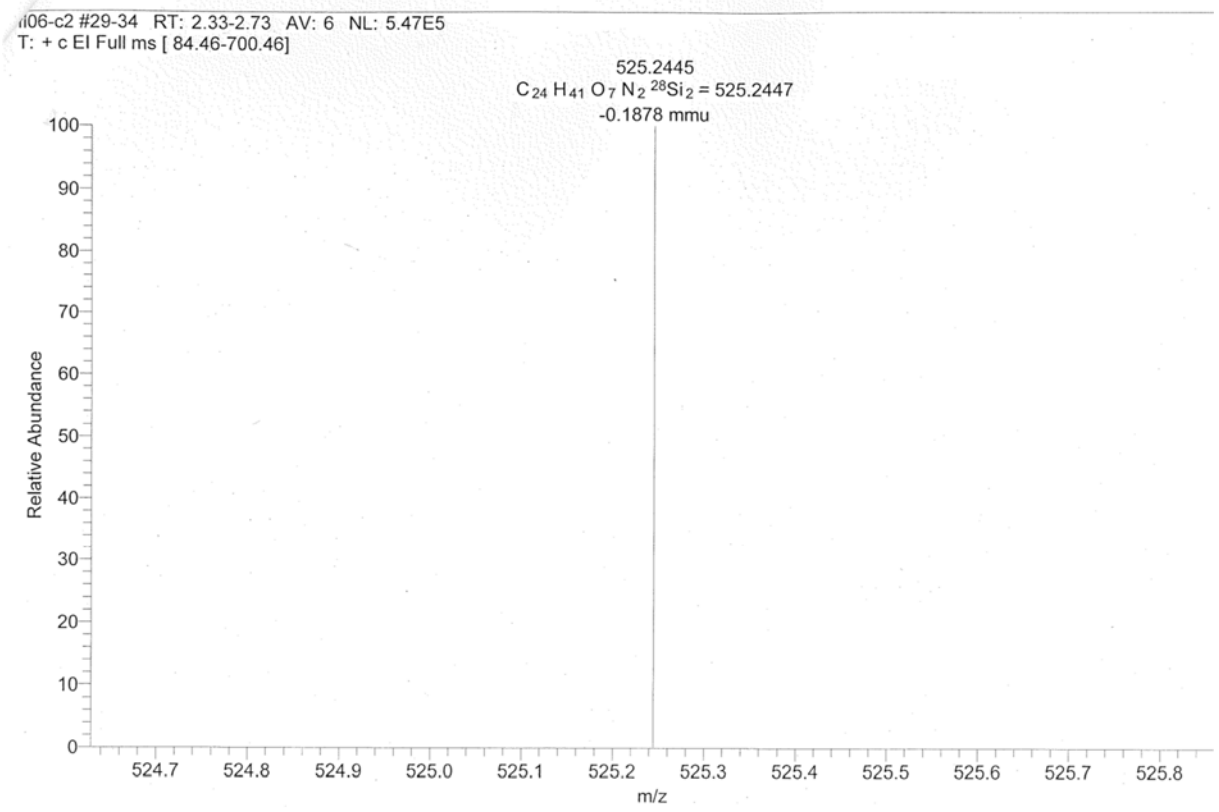


Figure S11. HR-MS (FAB) of compound **5**.

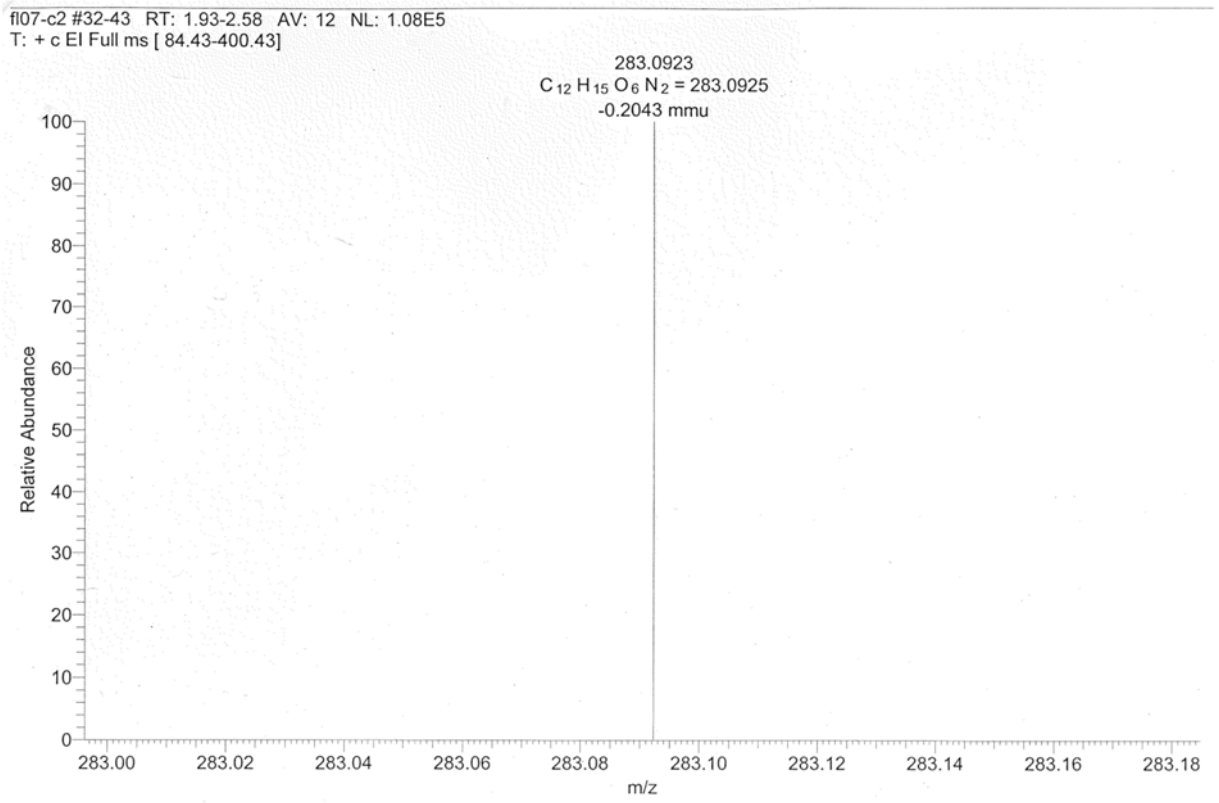


Figure S12. HR-MS (FAB) of compound **1**.

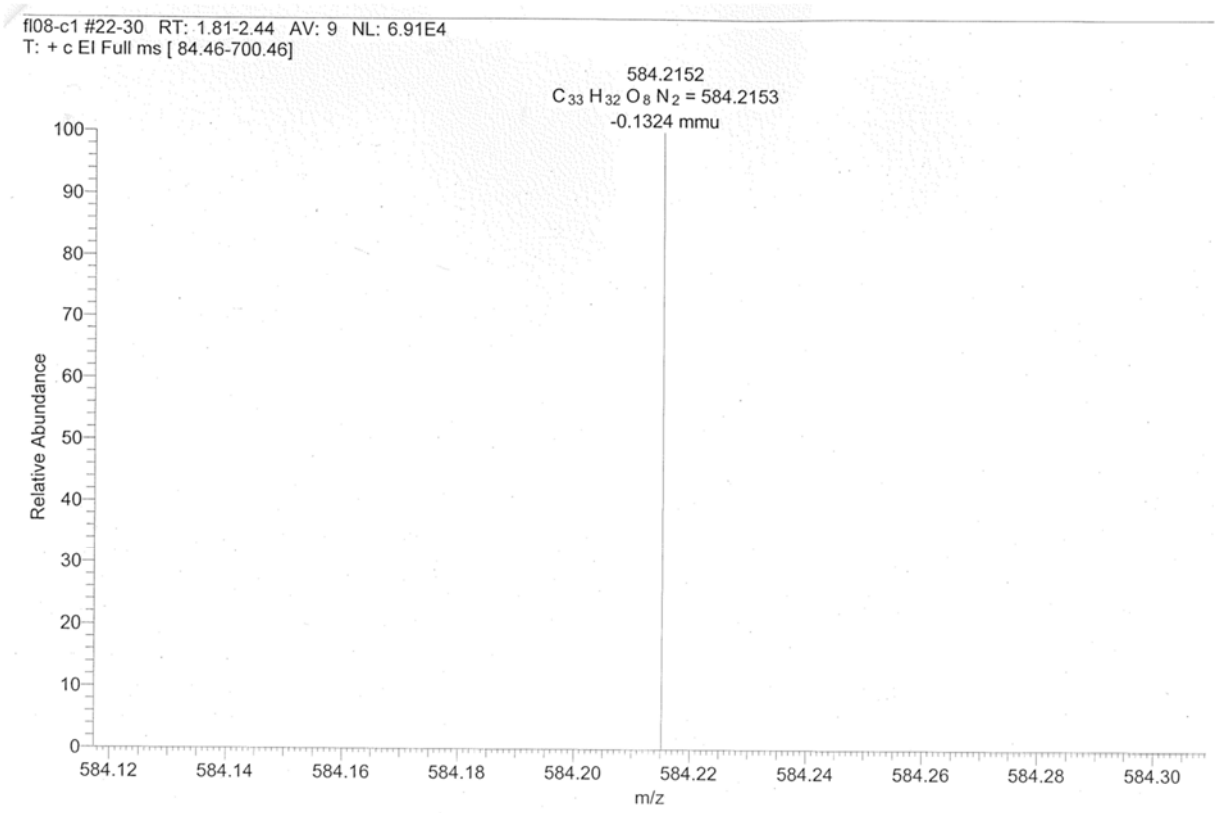


Figure S13. HR-MS (FAB) of compound **6**.

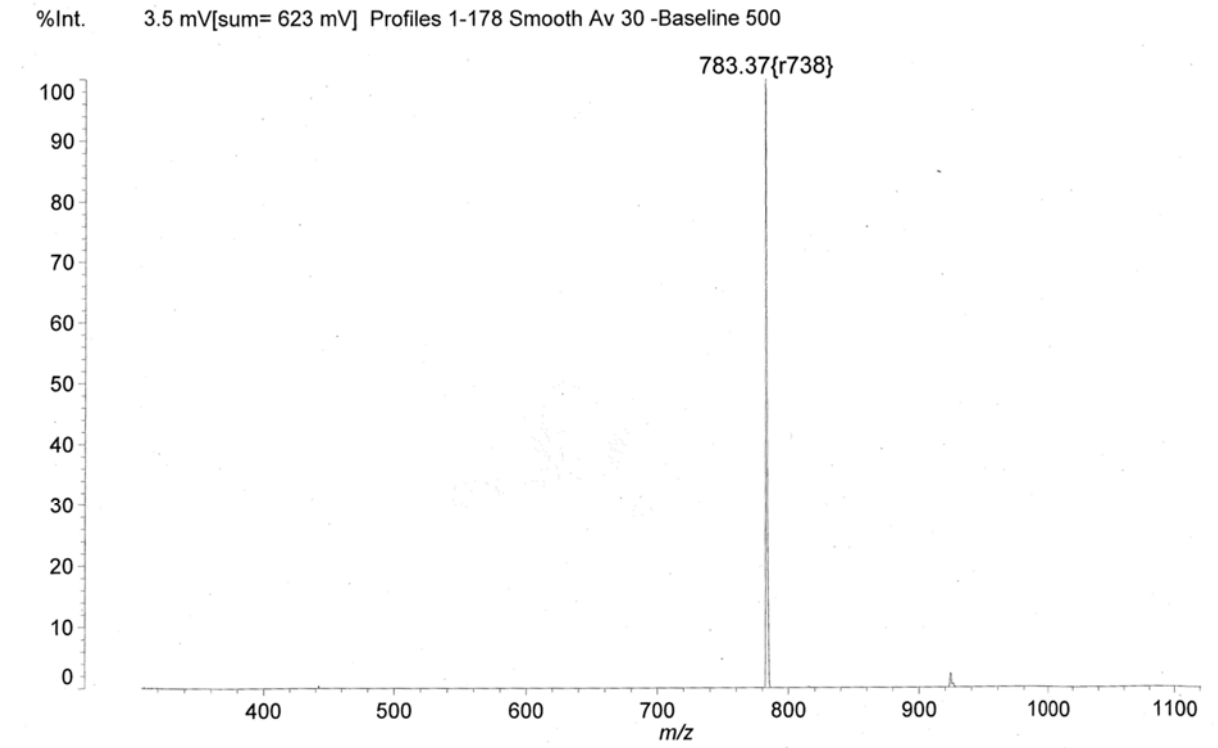


Figure S14. MS (MALDI-TOF) of compound **2**.

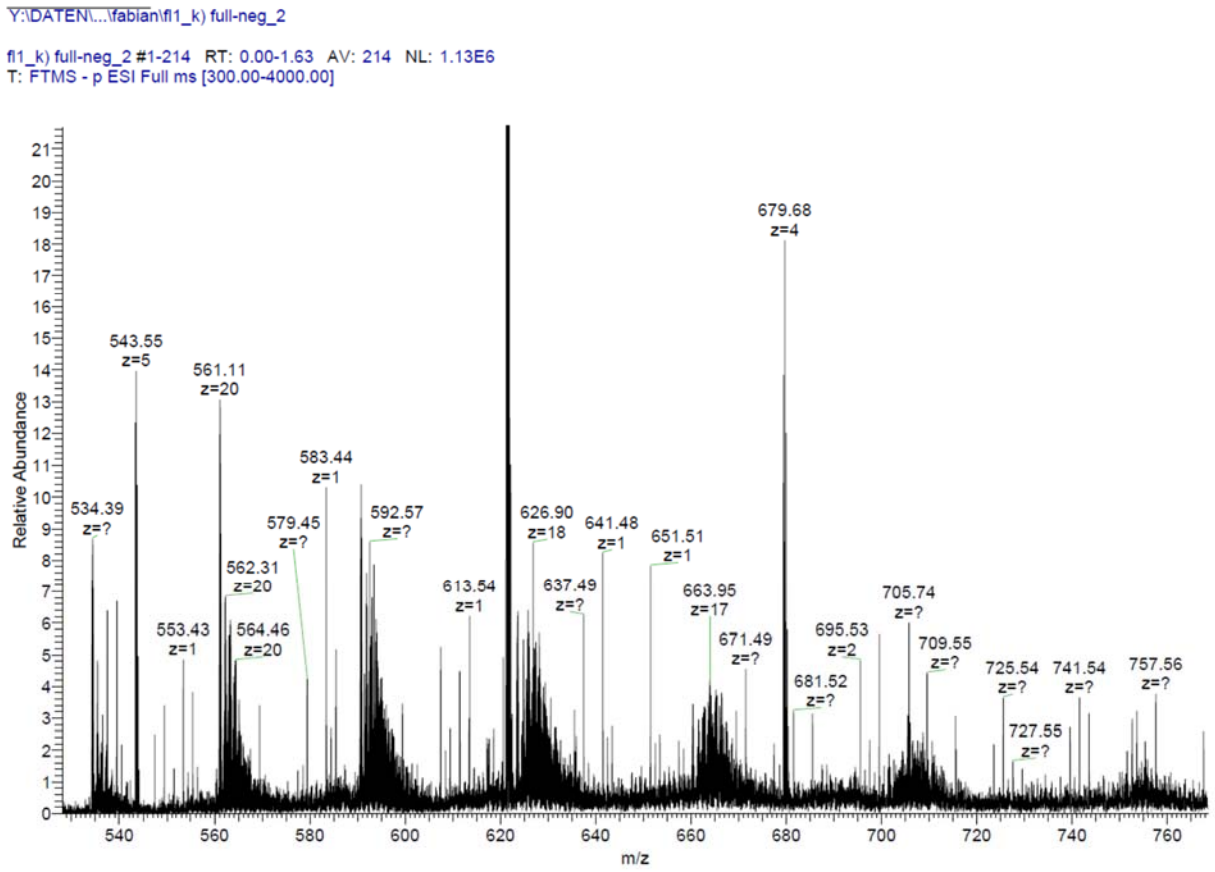


Figure S15. MS (ESI) of **LMB1** (m/z=561.11·20 + 20 = 11242.2).

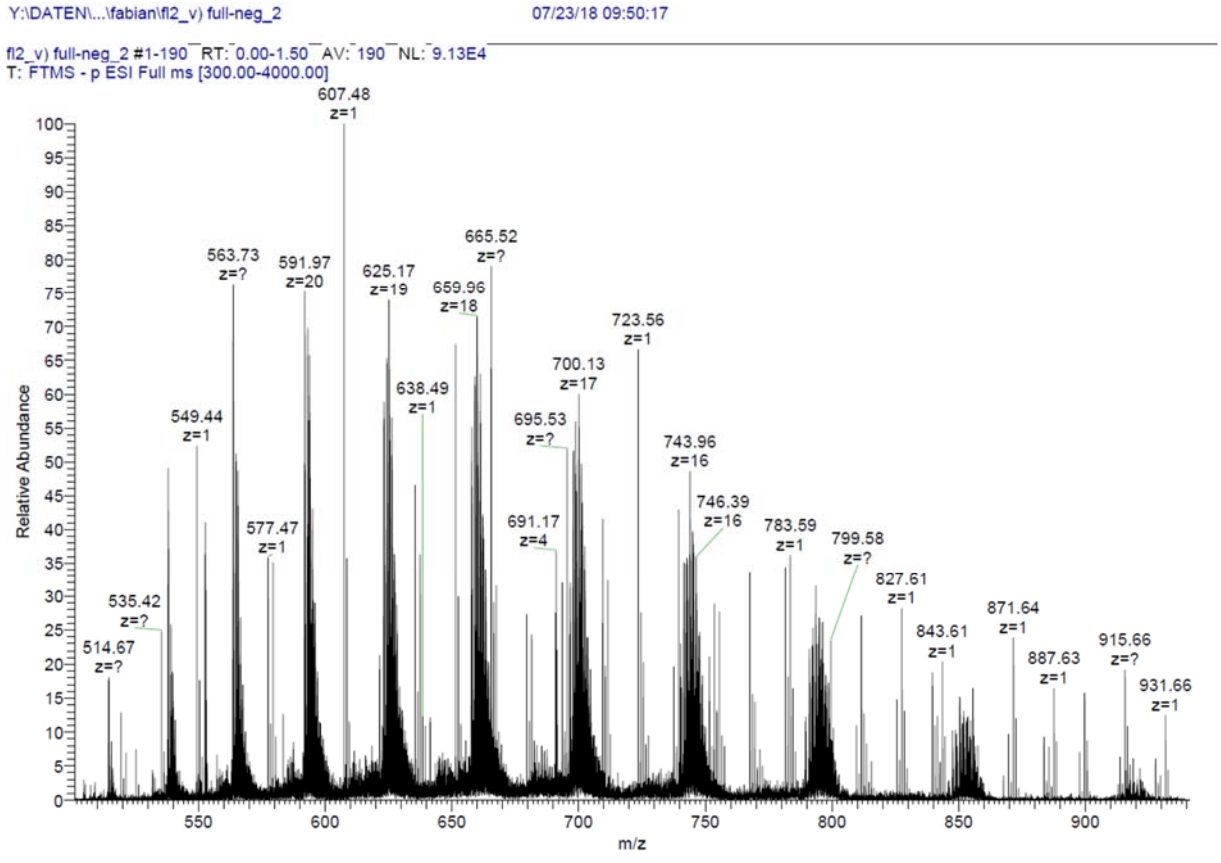


Figure S16. MS (ESI) of **LMB2** (m/z=591.97·20 + 20 = 11859.4).

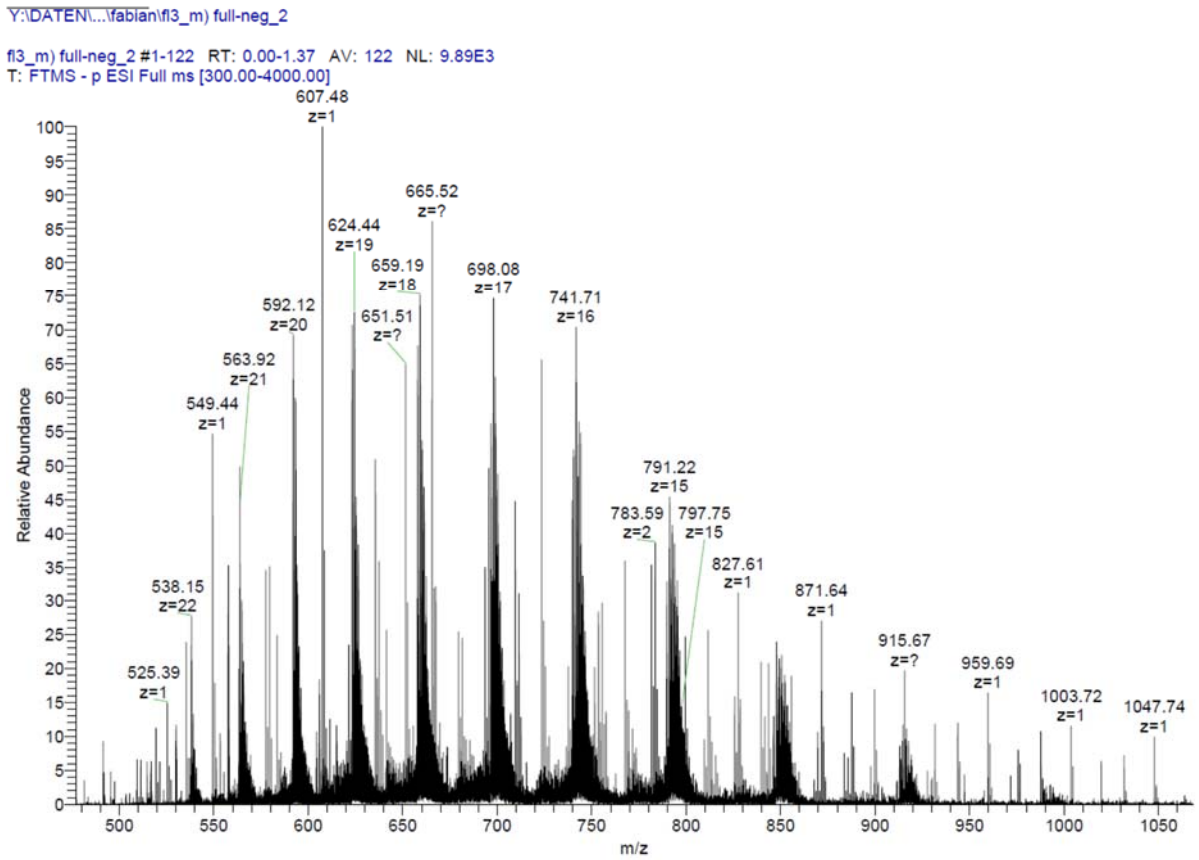


Figure S17. MS (ESI) of **LMB3** (m/z=592.12·20 + 20 = 11862.4).

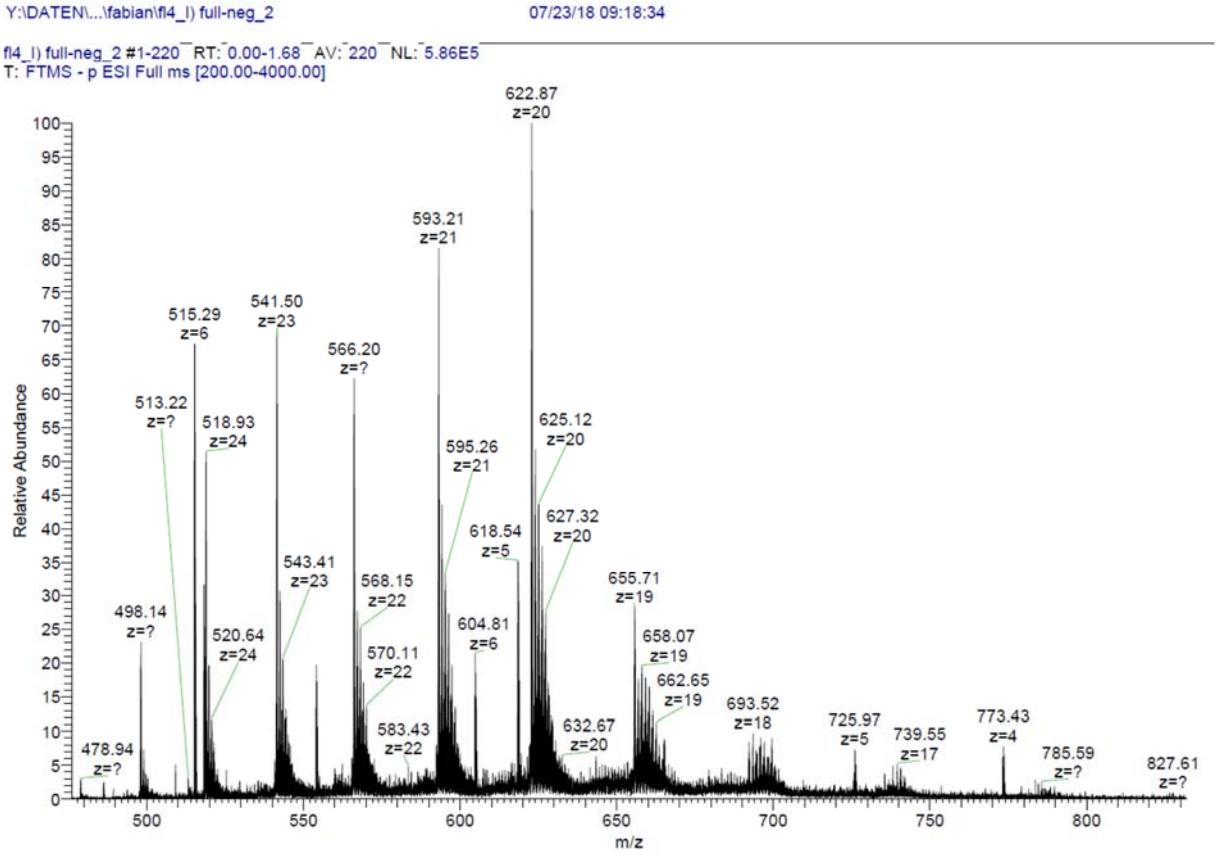


Figure S18. MS (ESI) of **LMB4** (m/z=622.87·20 + 20 = 12477.4).

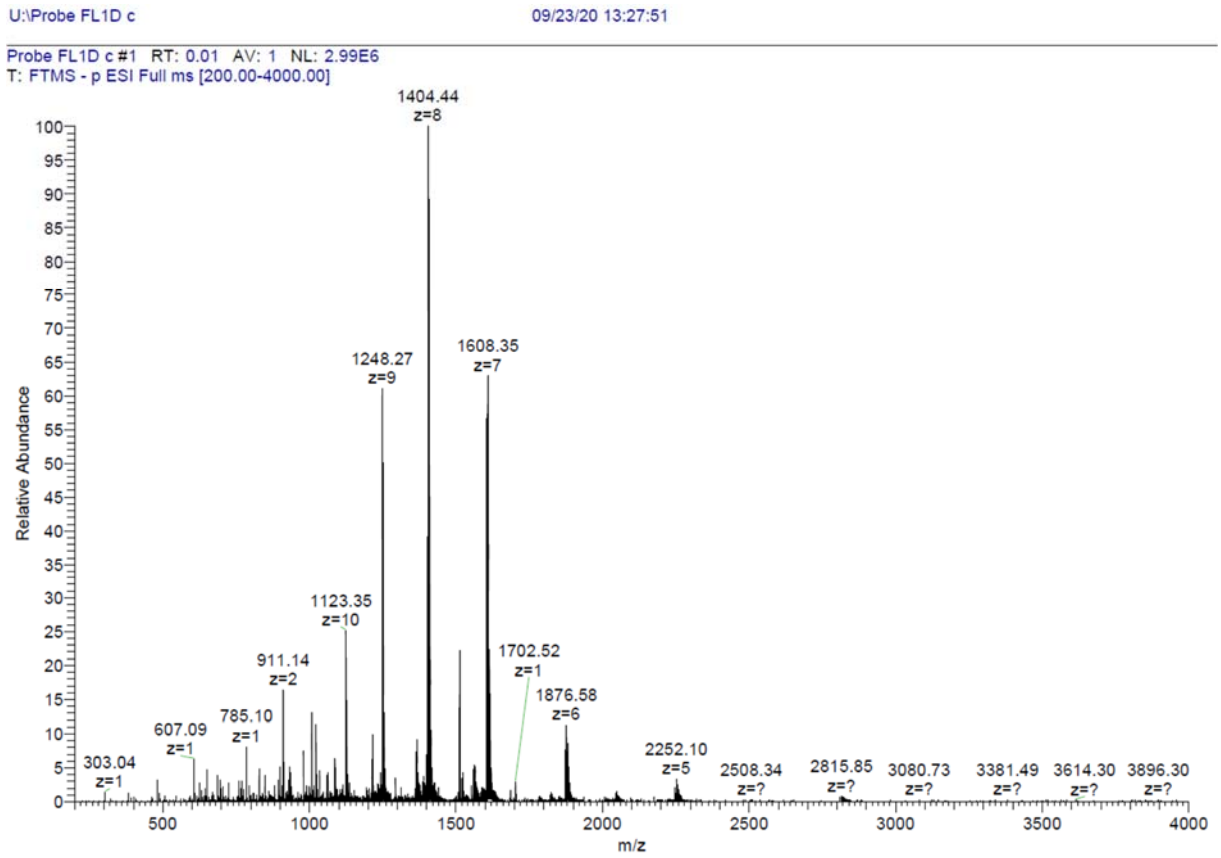


Figure S19. MS (ESI) of **DMB1** (m/z=1401.44·8 + 8 = 11243.5).

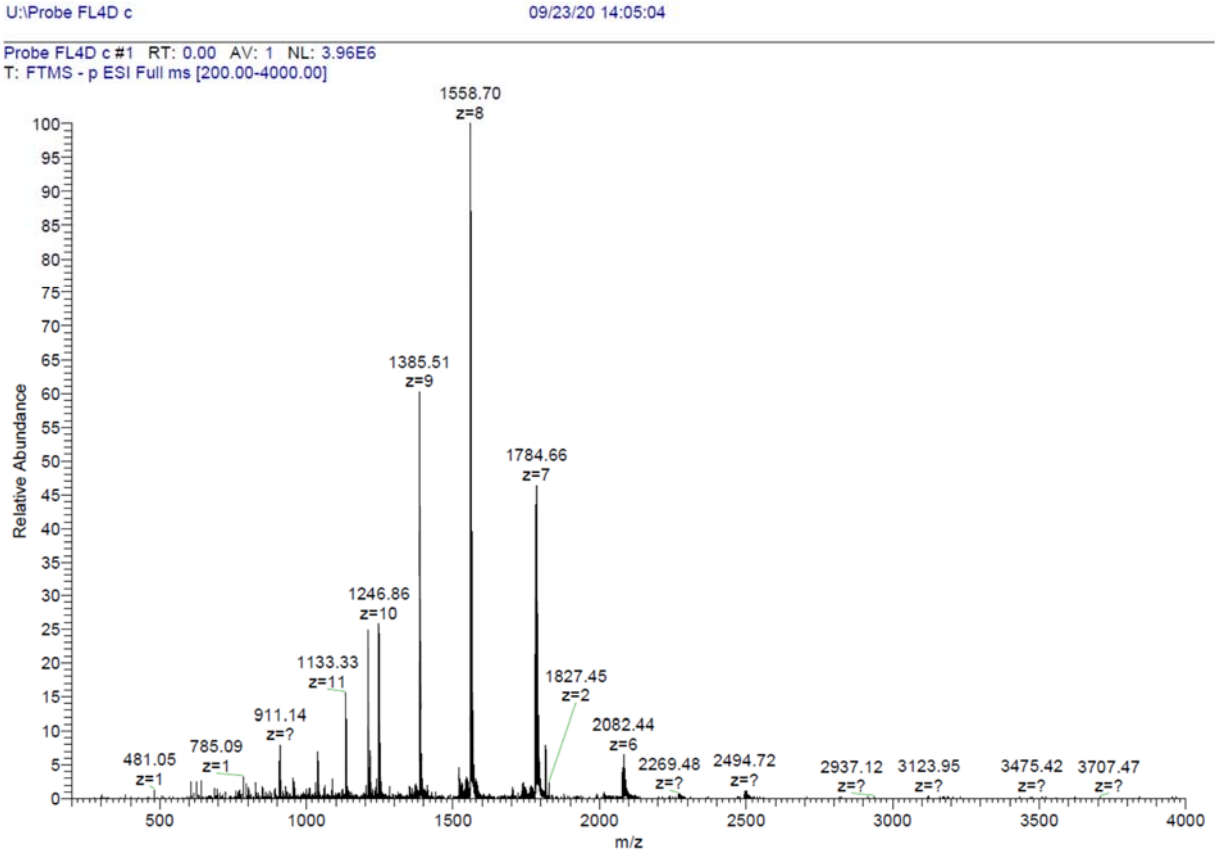


Figure S20. MS (ESI) of **DMB4** (m/z=1558.70·8 + 8 = 12477.6).

6. Images of HPLC analyses

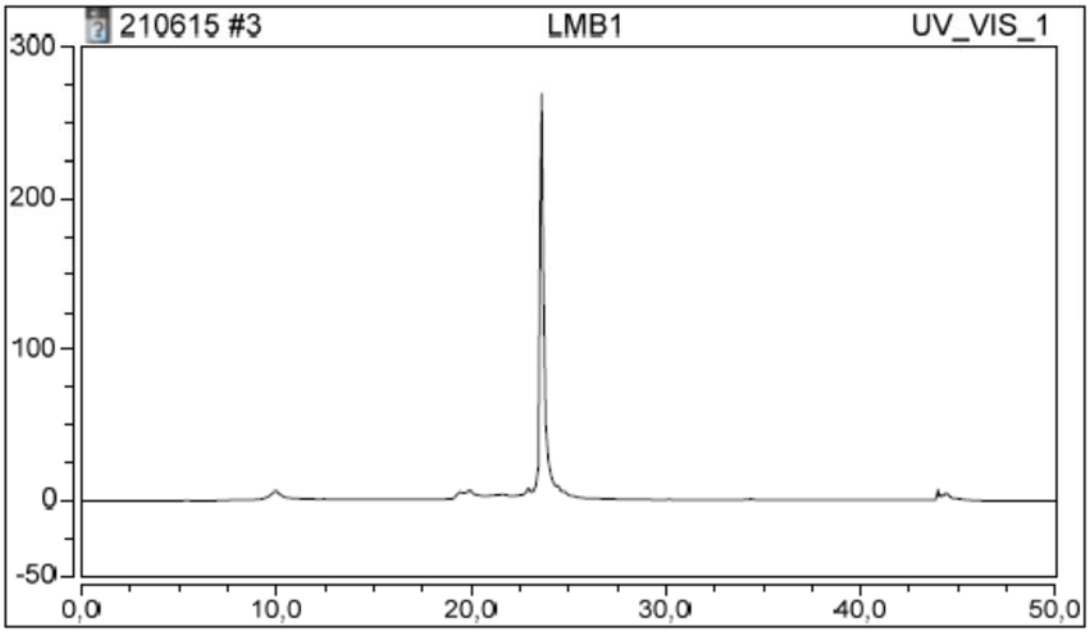


Figure S21. HPLC analysis of **LMB1**.

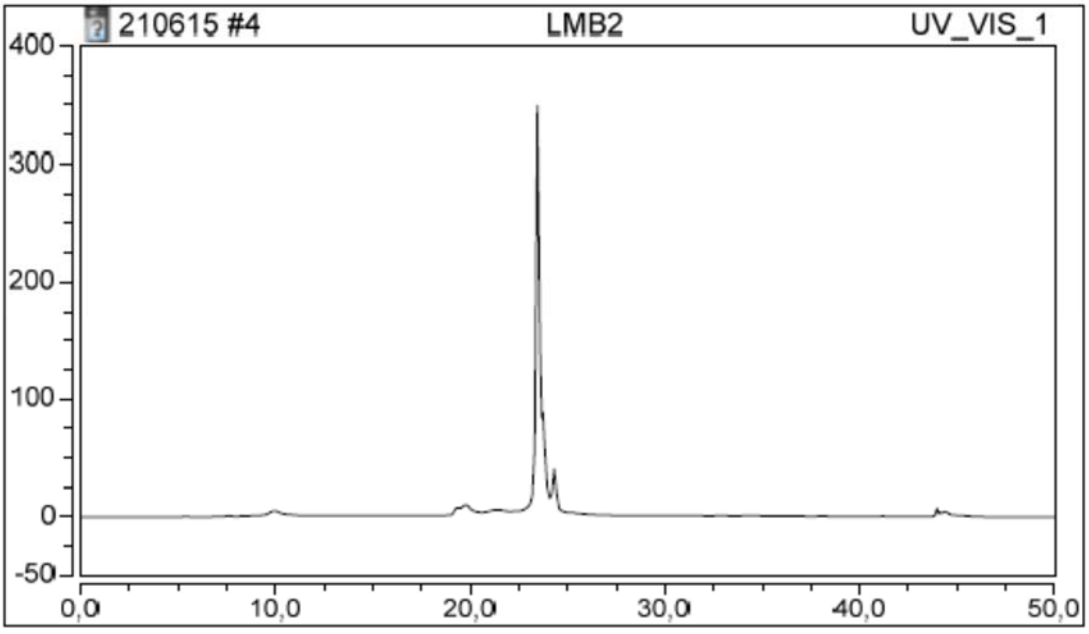


Figure S22. HPLC analysis of **LMB2**.

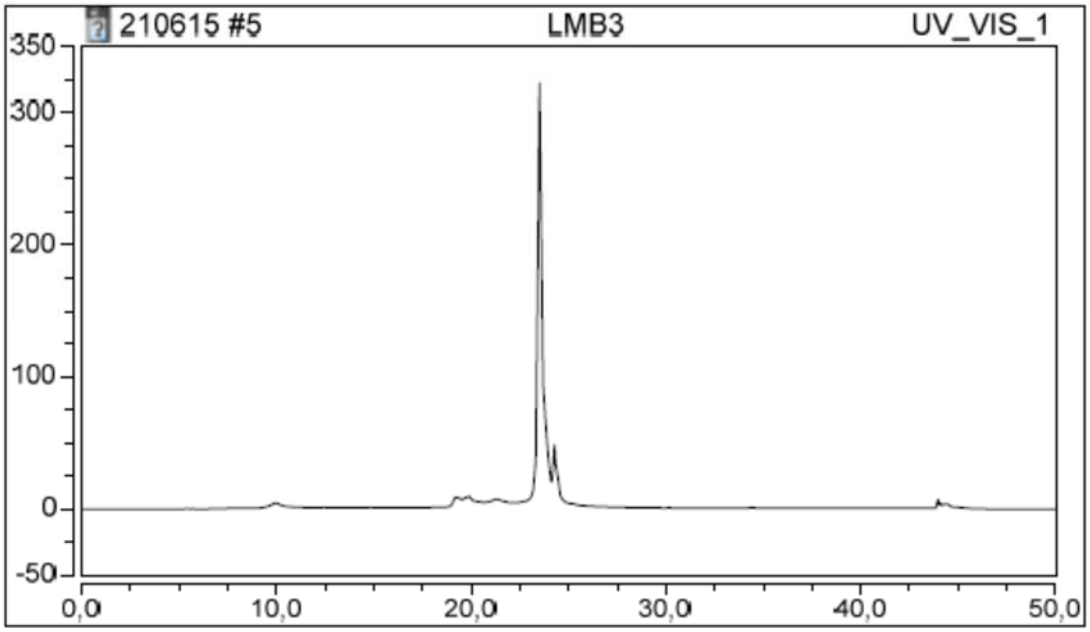


Figure S23. HPLC analysis of **LMB3**.

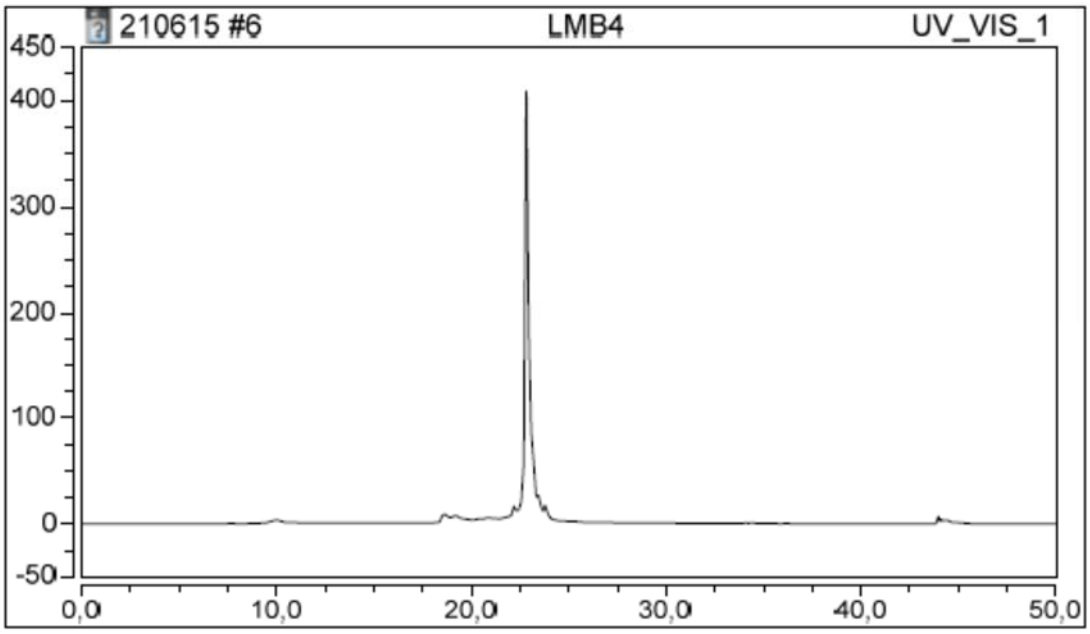


Figure S24. HPLC analysis of **LMB4**.

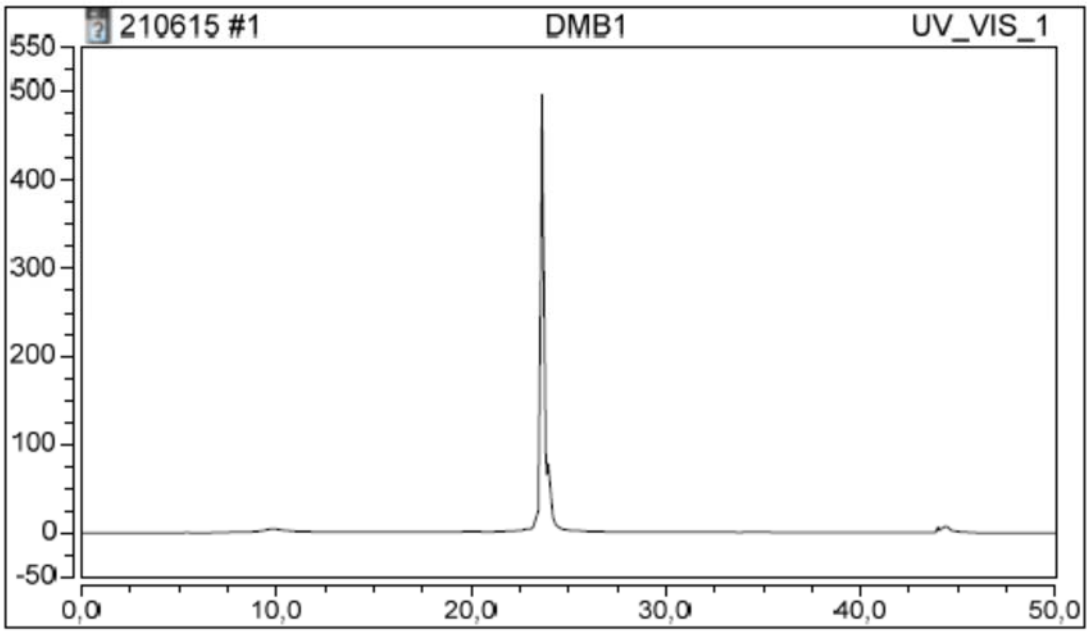


Figure S25. HPLC analysis of **DMB1**.

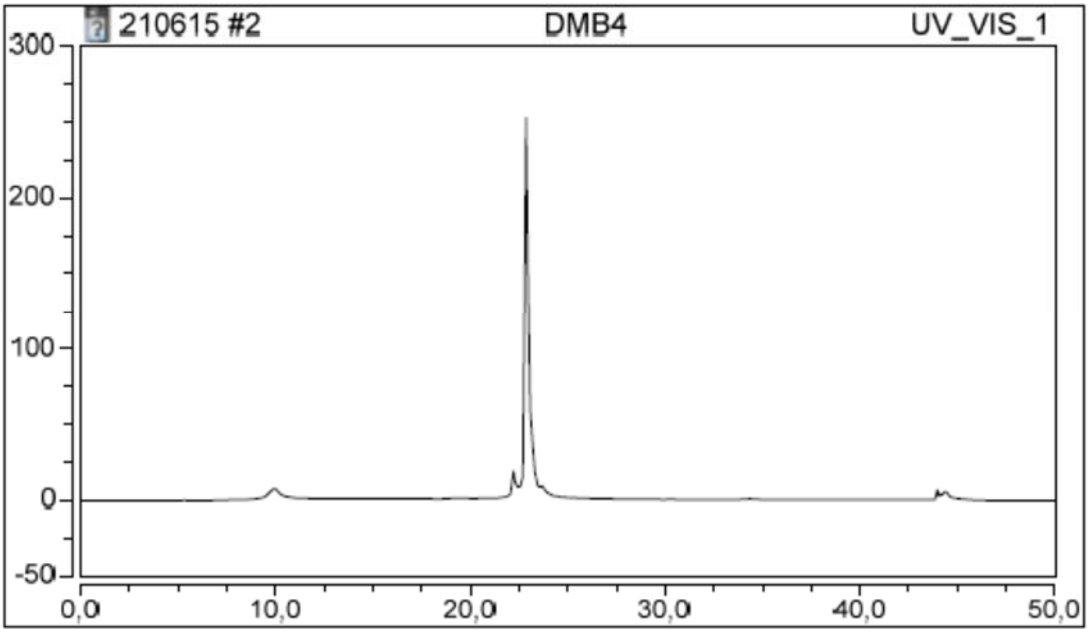


Figure S26. HPLC analysis of **DMB4**.

7. Additional optical spectra

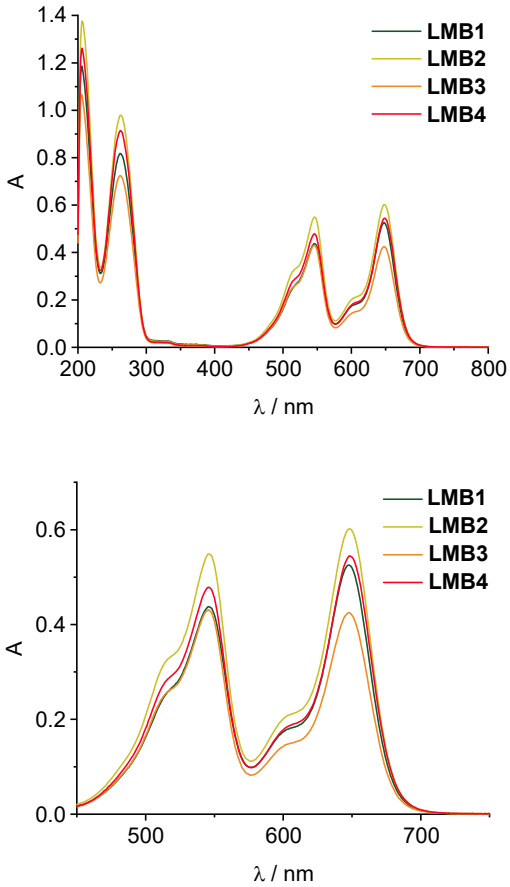


Figure S27. UV/Vis absorption of **LMB1** - **LMB4**; 2.5 µM DNA, 10 mM NaP_i buffer, 250 mM NaCl, pH 7, 20 °C.

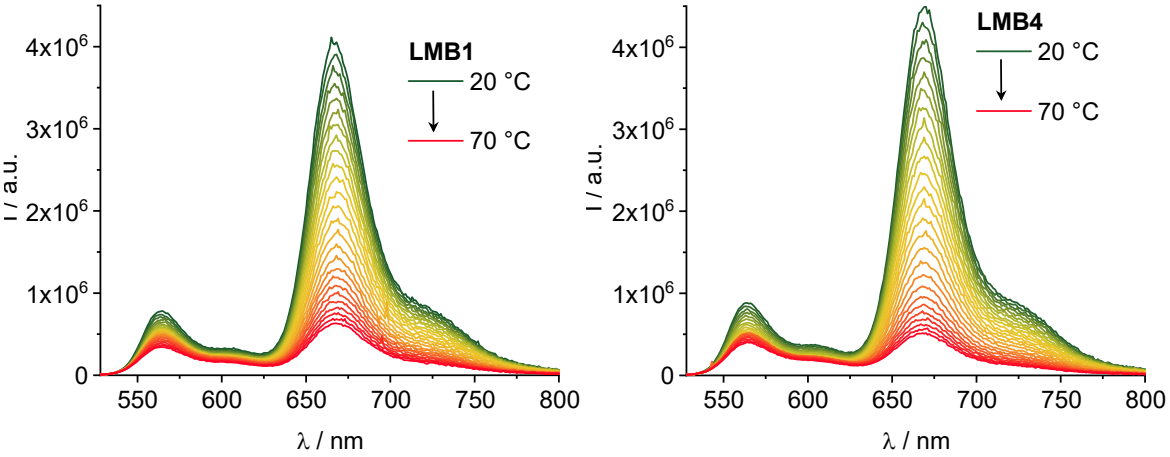


Figure S28. Temperature-dependent fluorescence of **LMB1** und **LMB4**; 2.5 µM DNA, 10 mM NaP_i buffer, 250 mM NaCl, pH 7, λ_{exc} = 518 nm.

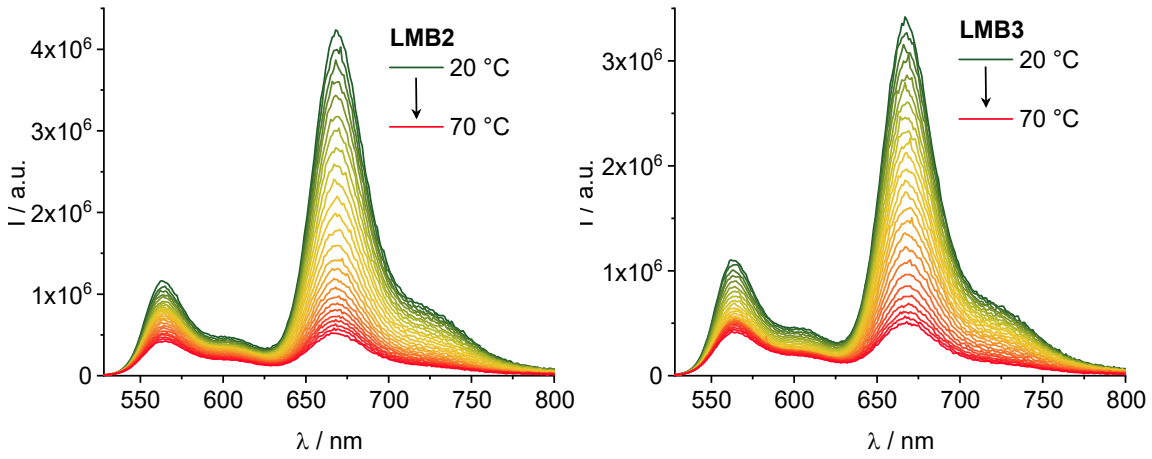


Figure S29. Temperature-dependent fluorescence of **LMB2** und **LMB3**; 2.5 µM DNA, 10 mM NaP_i buffer, 250 mM NaCl, pH 7, λ_{exc} = 518 nm.

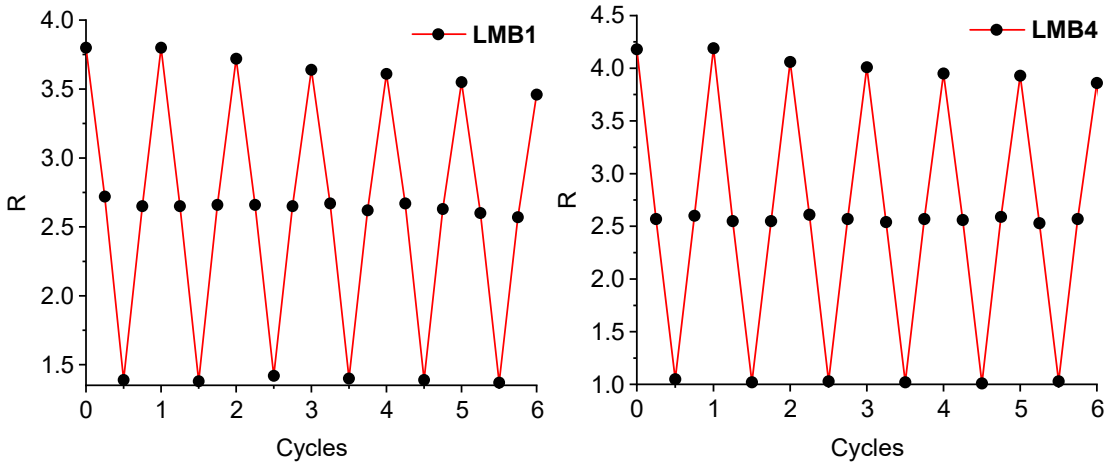
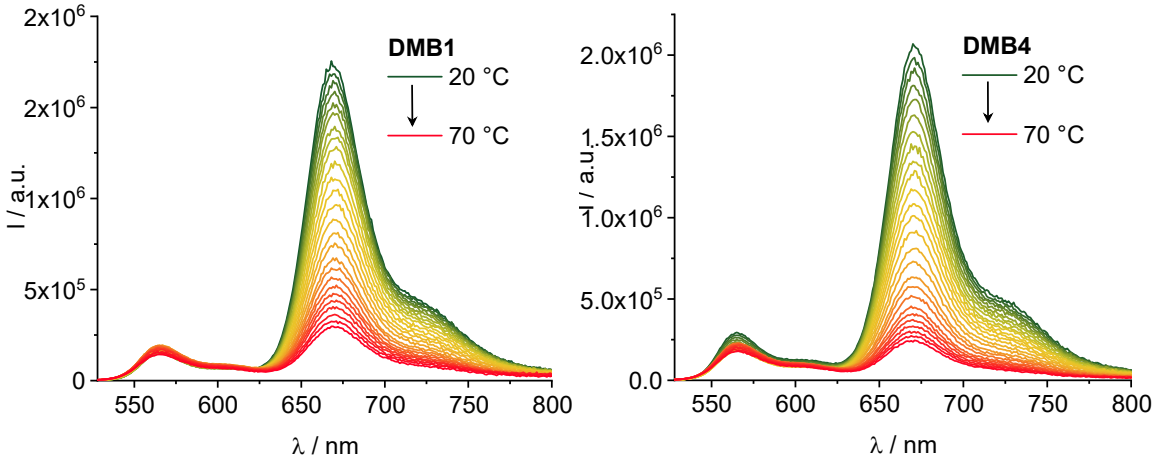


Figure S30. R observed during 6 cycles of heating and cooling of **LMB1** (left) and **LMB4** (right).



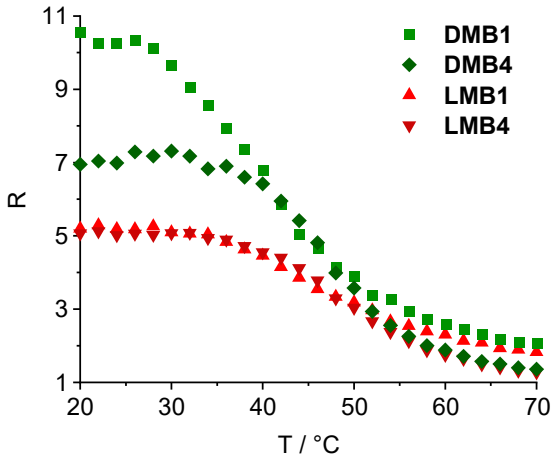


Figure S31. Temperature-dependent fluorescence of **DMB1** und **DMB4** and R of **DMB1, DMB4, LMB1** and **LMB4**; 2.5 µM DNA, 10 mM NaP_i buffer, 250 mM NaCl, pH 7, λ_{exc} = 518 nm.

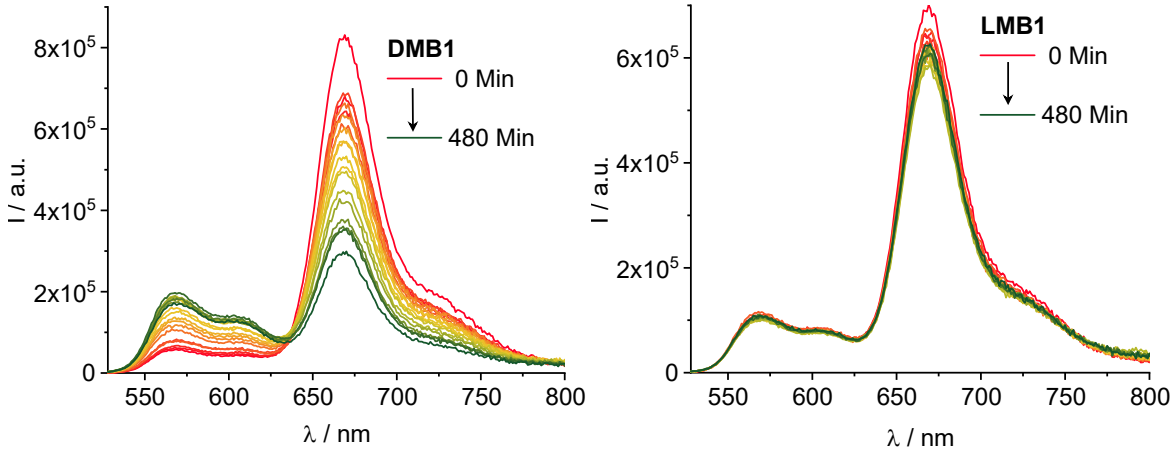


Figure S32. Time-dependent fluorescence of **DMB1** und **LMB1** in cell lysate; 2.5 µM DNA, 37 °C, λ_{exc} = 518 nm.

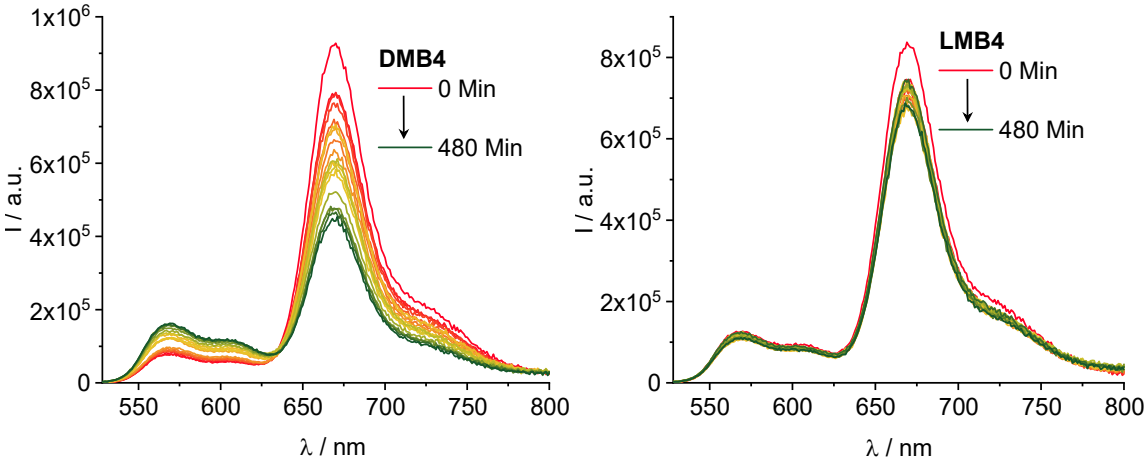


Figure S33. Time-dependent fluorescence of **DMB4** und **LMB4** in cell lysate; 2.5 µM DNA, 37 °C, λ_{exc} = 518 nm.

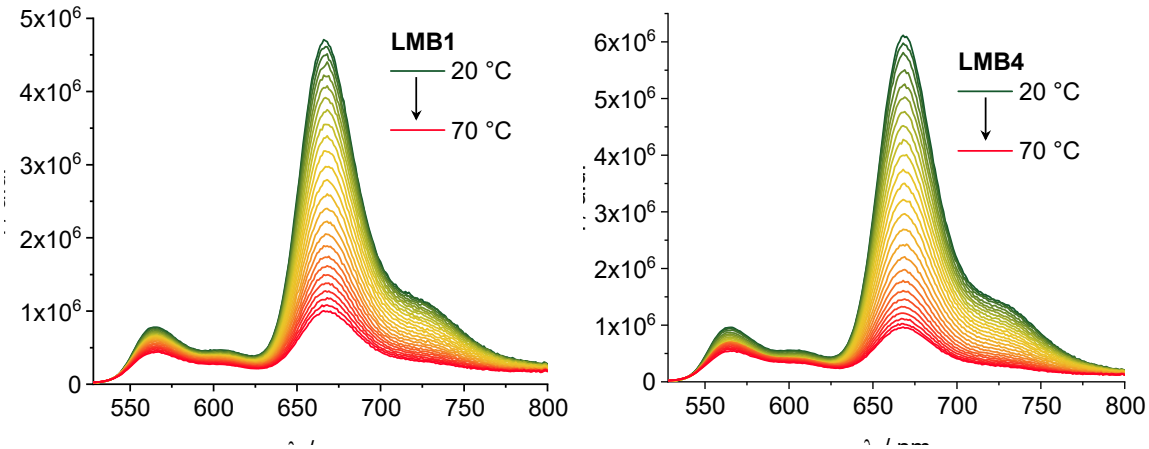


Figure S34. Temperature-dependent fluorescence of **LMB1** und **LMB4** in cell lysate; 2.5 µM DNA, 37 °C, λ_{exc} = 518 nm.

8. Cell biology and additional cell images

Human cervical carcinoma cells (HeLa wt) were cultured adherently under sterile conditions in Dulbecco's Modified Eagle Medium (DMEM medium) with 60 μg/mL penicilin, 100 □G/mL streptomycin and 10 % fetal calf serum (TCS, GIBCO) at 37 °C and 5 % CO_2 until 80 % confluence. The cells were washed with PBS for passaging, detached with a trypsin/EDTA solution (10 mL 0.25 % trypsin solution, 10 mL 5 mM EDTA solution, 30 mL PBS) and resuspended in DMEM medium. The number of cells was determined using a Neubauer counting chamber. 24 h before transfection, 4 x 104 HeLa cells per chamber were seeded in an 8-well cell culture slide (IBIDI® μ-Slide 8-well) in 200 μL Opti-MEM™ medium. 5 µL Opti-MEM™ medium was mixed with 0.30 µL Lipofectamine™ and 0.2 µg DNA was added to 10 µL Opti-MEM™ medium. Subsequently, 5 µL each of the two mixtures were combined and incubated for 15 min at room temperature. It was diluted with 200 μL Opti-MEM™ medium and the solution was added to the cells. A LEICA DMi8 TCS SPE microscope with HC PL APO CS2 63x/1.30 oil immersion objective was used for the confocal laser scanning fluorescence microscopy images. The donor dye Cy3 was excited at a wavelength of λ_{exc} = 488 nm with an argon ion laser and the fluorescence of the molecular beacons was recorded at λem = 545 - 585 nm for Cy3 and λem = 650 - 690 nm for Cy5 with an image resolution of 1024 x 1024 pixels. The first image was taken directly after adding the DNA-Lipofectamine™ solution to the cells. Subsequently, incubation at 37 °C and 5 % CO_2 was performed and a new image was taken every hour using the Leica LAS X software. The Leica LAS AF Lite software was used to analyze and process the cell images.

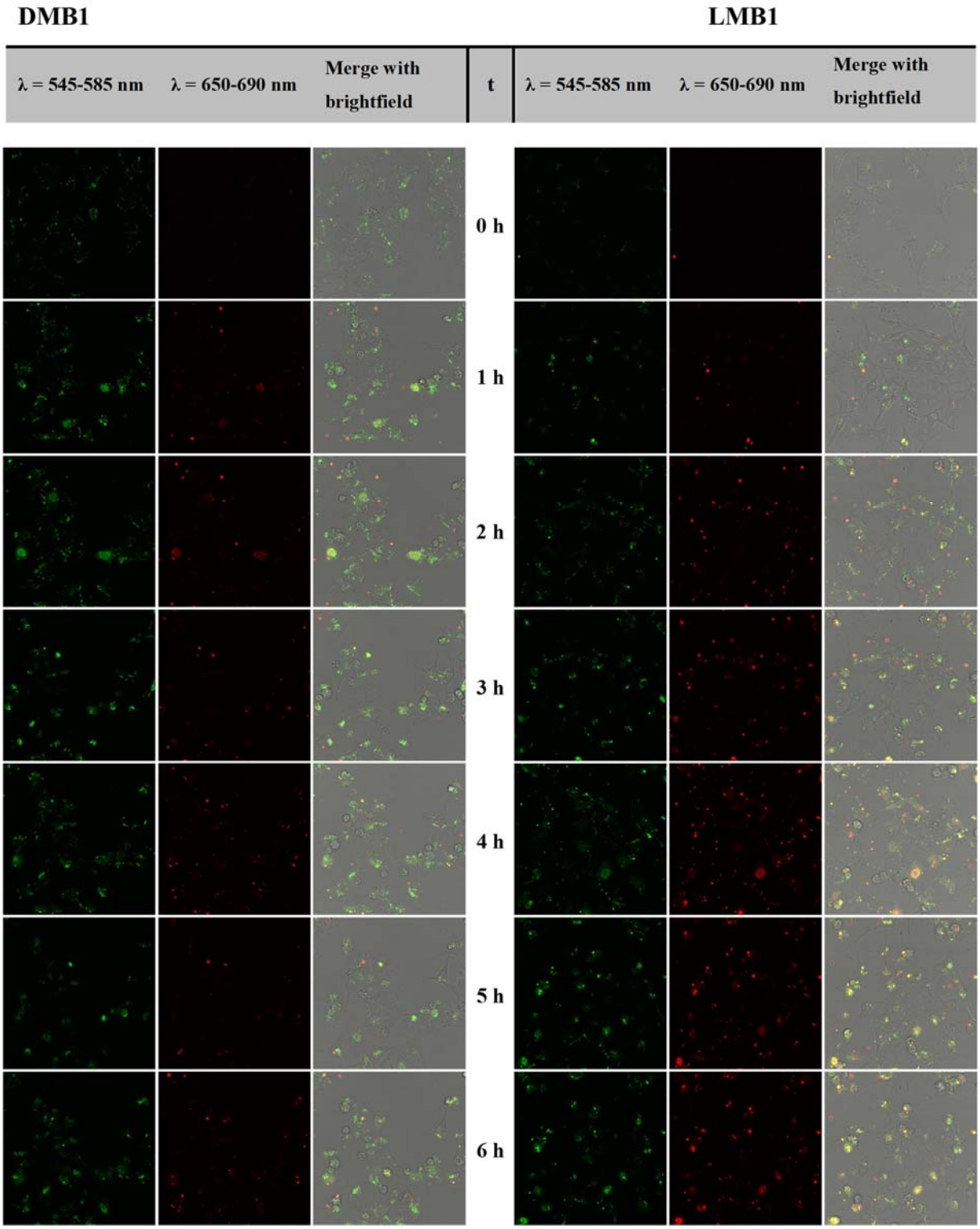


Figure 35: Confocal microscopy images after transfection with **DMB1** (100 ng, left) and **LMB1** (100 ng, right) and lipofectamine.

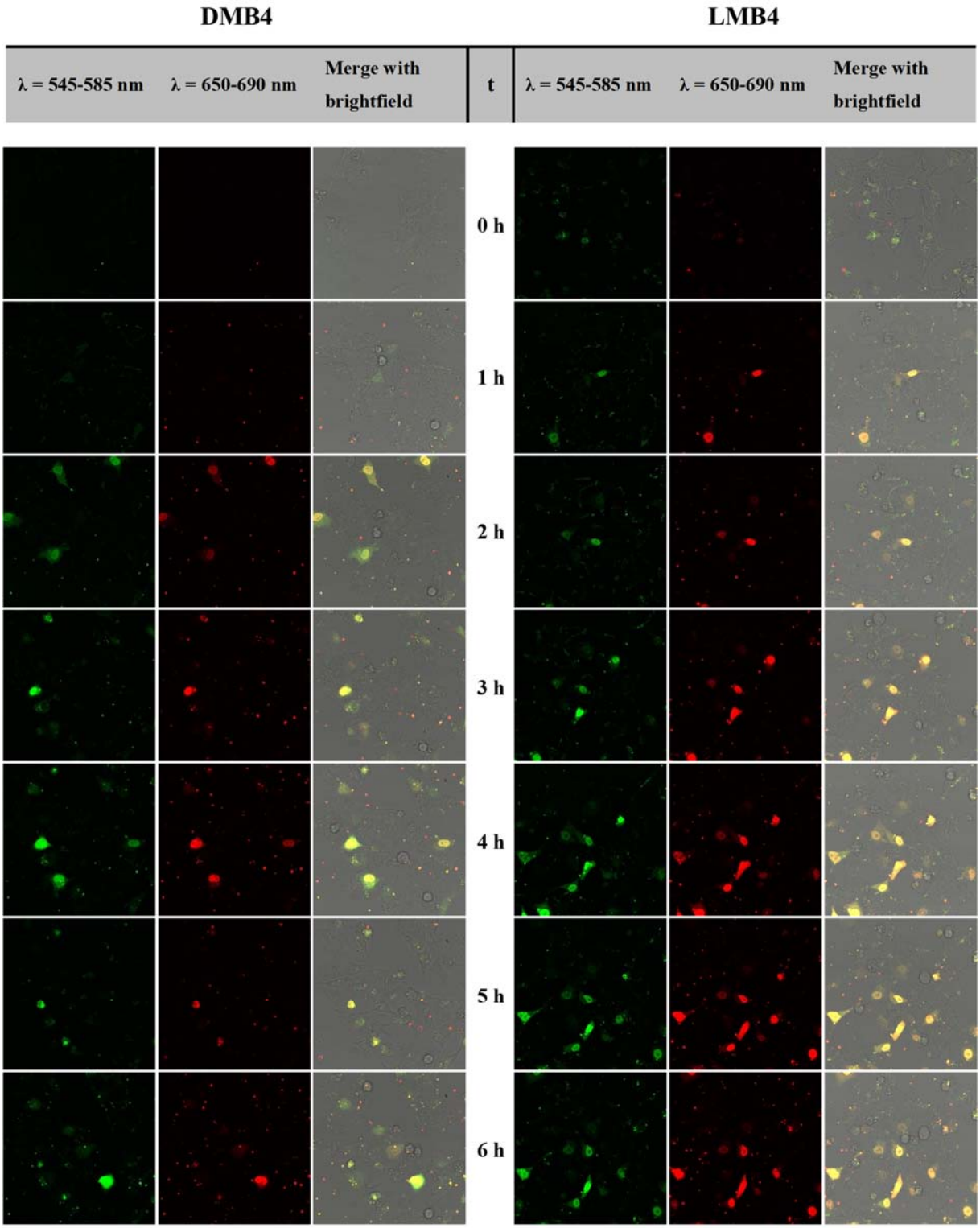


Figure 36. Confocal microscopy images after transfection with **DMB4** (100 ng, left) and **LMB4** (100 ng, right) and lipofectamine.