Supporting information

EPR Imaging of Sinapyl Alcohol and its Application to Visualization of Plant Cell Wall Lignification

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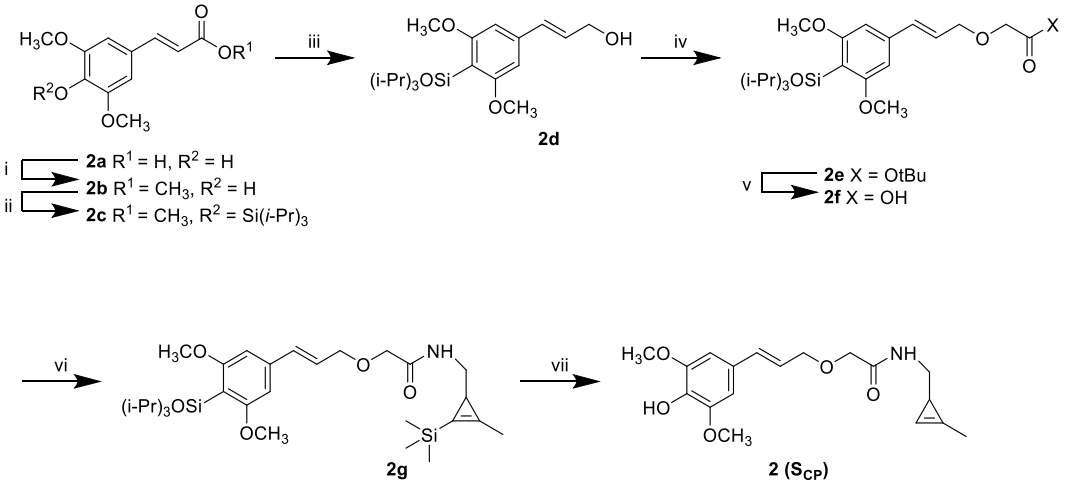
I. General methods and materials

Tetrazine-NHS esters were purchased from Click Chemistry Tools. Tetrazine-Cy5 was purchased from Jena Bioscience. Other chemical reagents were purchased from Sigma Aldrich, TCI and Fluorochem and were used with no further purification. Unless otherwise stated, all solvents used were at least of HPLC grade, except in reactions that required an inert atmosphere, in which case anhydrous solvents were used. Reactions were monitored by TLC, using pre-coated 0.20 mm ALUGRAM XtraSil G UV254 sheets from Macherey-Nagel. Detection was done under UV light at 254 nm and by chemical revelation of the plates with solutions of phosphomolybdic acid or vanillin. Flash chromatography purifications were performed on an Interchim Puriflash 430 equipped with an evaporative light-scattering detector and a UV detector. 30 μm silica columns were purchased from Interchim. HPLC analyses were carried out on a Thermo Finnigan SCM1000 system equipped with a Grace 5 μm Alltima C_{18} column and a UV6000LP UV detector (compounds **2**, **3**) or on a Dionex UltiMate 3000 HPLC equipped with a Restek UltraC18 150 mm x 3 mm column (compounds **4**, **5**). Nuclear magnetic resonance analyses (^{1}H and ^{13}C NMR (1D, 2D)) were recorded on a Brüker 300 MHz SB Advance equipped with a 5 mm TBI probe ($^{1}H/^{13}C/BB/^{2}H$). NMR spectra were recorded in chloroform-d or acetone-d6 purchased from Eurisotop. Chemical shifts are reported in ppm relative to TMS, *J* coupling constants are reported in Hz. MALDI-ToF mass spectra were acquired on MALDI ToF/ToF 4800 Proteomics Analyzer mass spectrometer (Applied Biosystems, Framingham, MA) in reflectron positive mode and using 2,5-dihydroxybenzoic acid as matrix. All spectra and chromatograms are provided in the final section of the present document. CW-EPR and EPR imaging experiments were carried out using a conventional X-band Bruker Elexsys E580 spectrometer operating at around 9.6 GHz and room temperature. The microwave power supplied into the resonator was set to 15 mW for biological sample and 0.6 mW for solution measurements. The modulation frequency and amplitude were set to 100 kHz and 2 G, respectively. Conversion time and time constant were set to 40.96 ms and 20.48 ms respectively. All spectra were acquired using the same conditions and normalized. The relative weights of all identified species were directly calculated from simulations performed with the EasySpin toolbox: each population carries a weight; from the total spectrum integral, the integrals of each population are weighted by their contributions. Molar concentrations were then calculated using our standard curve. The spatial-spatial images were recorded with a field-of-view of 5 mm and gradient strength of 175 G/cm. The size of spatial-spatial images was 512 × 512 pixels resulting in a pixel size of 13.7 μm. In order to increase the resolution inside a pixel, 1608 projections instead of 402 were collected. The high resolution spatial-spatial EPR images were obtained by deconvolution of the acquired signal from the signal without gradient followed by filtered back-projection algorithm.

II. Synthetic procedures

II.1. Synthesis of cyclopropenyl-tagged sinapyl alcohol surrogate S_{CP} **2**



Scheme S1. Synthesis of $\mathbf{S_{CP}}$ **2**. (i) H_2SO_4, MeOH, reflux, 2h - 95% ; (ii) (*i*-Pr)$_3$SiCl, imidazole, DMF, 0°C to rt, 3h - 96% ; (iii) DIBAL-H, THF, 0°C, 2h - 77% ; (iv) *tert*-butyl bromoacetate, NaOH, TBAB, H_2O-toluene, rt, 15h - 75% ; (v) LiOH, H_2O-EtOH, 0°C to rt, 4h - 95% ; (vi) [2-methyl-3-(trimethylsilyl)cycloprop-2-en-1-yl]methanamine, PyBOP, DIEA, DMF-CH_2Cl_2, 0°C to rt, 24h - 67% ; (vii) TBAF, THF, 0°C to rt, 1h - 35%.

(*E*)-methyl 3-(4-hydroxy-3,5-dimethoxyphenyl)acrylate 2b. Concentrated sulfuric acid (2 mL) was added to a solution of sinapic acid **2a** (0.972 g, 4.3 mmol) in methanol (60 mL). The reaction mixture was heated to reflux over 2 hours and monitored by TLC (cyclohexane/ethyl acetate 7:3). It was then cooled down to room temperature, and the solvent was removed *in vacuo*. The residue was taken up into ethyl acetate (60 mL) and the organic phase was washed with saturated aqueous sodium bicarbonate (3 x 40 mL) then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to yield compound **2b** as an orange solid (0.970 g, 4.1 mmol, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, *J* = 15.9 Hz, 1H), 6.77 (s, 2H), 6.30 (d, *J* = 15.9 Hz, 1H), 5.76 (s, 1H), 3.92 (s, 6H), 3.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.70, 147.39, 145.26, 137.34, 126.05, 115.73, 105.26, 56.50, 51.75. MALDI-ToF : m/z $[M+H]^+$ calcd for $C_{12}H_{15}O_5$: 239.09; found: 239.05. m/z $[M+Na]^+$ calcd for $C_{12}H_{14}NaO_5$: 261.07; found: 261.04. m/z $[M+K]^+$ calcd for $C_{12}H_{14}KO_5$: 277.05; found: 276.99.

(*E*)-methyl 3-(3,5-dimethoxy-4-((triisopropylsilyl)oxy)phenyl)acrylate 2c. Under a nitrogen atmosphere, compound **2b** (0.970 g, 4.1 mmol) was dissolved in anhydrous dimethylformamide (6 mL). Imidazole (0.554 g, 8.1 mmol) was added at room temperature then the reaction mixture was cooled down to 0°C and triisopropylsilyl chloride (0.945 g, 4.9 mmol) was added dropwise. The solution was left to stir at 0°C for 15 minutes then at room temperature for a further 3 hours and monitored by TLC (cyclohexane/ethyl acetate 7:3). Upon completion, the reaction was quenched by addition of methanol (1 mL), diluted with water (20 mL) and extracted with diethyl ether (3 x 20 mL). The organic phases were combined, washed with saturated aqueous ammonium chloride (3 x 20 mL) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to yield compound **2c** as a yellow solid (1.55 g, 3.9 mmol, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, *J* = 15.9 Hz, 1H), 6.71 (s, 2H), 6.29 (d, *J* = 15.9 Hz, 1H), 3.81 (s, 6H), 1.33 – 1.17 (m, 3H), 1.08 (d, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.82, 151.69, 145.58, 133.76, 126.75, 115.57, 105.31, 55.72, 51.71, 18.06, 13.51. MALDI-ToF : m/z

$[M+H]^+$ calcd for $C_{21}H_{35}O_5Si$: 395.22; found: 395.17. m/z $[M+Na]^+$ calcd for $C_{21}H_{34}NaO_5Si$: 417.21; found: 417.13.

(*E*)-3-(3,5-dimethoxy-4-((triisopropylsilyl)oxy)phenyl)prop-2-en-1-ol 2d. Compound **2c** (0.987 g, 2.5 mmol) was dissolved in anhydrous tetrahydrofuran (20 mL) under a nitrogen atmosphere and the mixture was cooled down to 0°C. A 1M solution of diisobutylaluminium hydride in tetrahydrofuran (10 mL) was added dropwise over a period of 1 hour with the help of a syringe-driver. The reaction was stirred for a further 1 hour at 0°C and methanol (5 mL) was added slowly. After another 30 minutes, the gel was treated with a biphasic mixture of ethyl acetate (30 mL) and water (30 mL) and filtered over a sintered glass funnel. The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 30 mL). The organic phases were combined, washed with brine (3 x 20 mL) and dried over anhydrous sodium sulfate. The residue was purified by flash chromatography (cyclohexane/ethyl acetate gradient, 90:10 to 70:30) to afford compound **2d** as a white solid (0.705 g, 1.9 mmol, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (s, 2H), 6.52 (d, J = 15.8 Hz, 1H), 6.24 (dt, J = 15.8, 5.9 Hz, 1H), 4.30 (d, J = 5.8, 2H), 3.79 (s, 6H), 1.31 – 1.17 (m, 3H), 1.07 (d, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.58, 135.27, 131.92, 129.15, 126.60, 103.62, 63.92, 55.66, 18.07, 13.44. MALDI-ToF : m/z $[M+H]^+$ calcd for $C_{20}H_{35}O_4Si$: 367.23; found: 367.18. m/z $[M+Na]^+$ calcd for $C_{20}H_{34}NaO_4Si$: 389.21; found: 389.18.

(*E*)-tert-butyl 2-((3-(3,5-dimethoxy-4-((triisopropylsilyl)oxy)phenyl)allyl)oxy)acetate 2e. Compound **2d** (700 mg, 1.9 mmol) and *tert*-butyl bromoacetate (846 µL, 5.7 mmol) were dissolved in a biphasic solution of toluene (6 mL) and sodium hydroxide aq. (50%, w/w, 4 mL). Tetrabutylammonium bromide (31 mg, 0.01 mmol) was then added at room temperature. The solution was left to stir overnight (~15 h) and monitored by TLC (cyclohexane/ethyl acetate 7:3). The reaction mixture was then extracted with ethyl acetate (3 x 20 mL), washed with saturated aqueous ammonium chloride (3 x 50 mL), dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/ethyl acetate, 80:20) to afford compound **2e** as a beige oil (0.685 g, 1.4 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (s, 2H), 6.51 (d, J = 15.8 Hz, 1H), 6.16 (dt, J = 15.8, 6.4 Hz, 1H), 4.22 (dd, J = 6.4, 1.2 Hz, 2H), 4.00 (s, 2H), 3.78 (s, 6H), 1.53 – 1.44 (m, 9H), 1.32 – 1.16 (m, 3H), 1.07 (d, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.85, 151.57, 135.39, 134.11, 128.99, 123.26, 103.73, 81.70, 72.17, 67.75, 55.68, 28.27, 18.08, 13.46. MALDI-ToF : m/z $[M+Na]^+$ calcd for $C_{26}H_{44}NaO_6Si$: 503.28; found: 503.22.

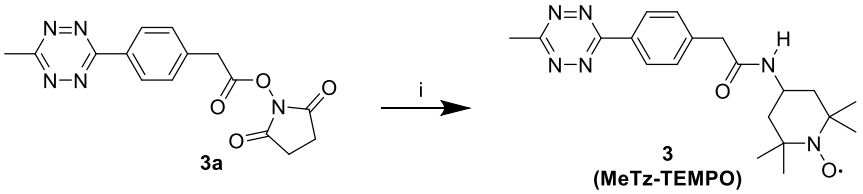
(*E*)-2-((3-(3,5-dimethoxy-4-((triisopropylsilyl)oxy)phenyl)allyl)oxy)acetic acid 2f. Lithium hydroxide monohydrate (63 mg, 1.5 mmol) in water (1 mL) was added dropwise at 0 °C to a solution of compound **2e** (360 mg, 0.7 mmol) in ethanol (6 mL). The solution was left to stir 4 h at room temperature and monitored by TLC (cyclohexane/ethyl acetate 8:2). The reaction mixture was then slowly acidified with 1M aqueous hydrochloric acid (5 mL) at 0 °C, further stirred at that temperature for 10 min, and then extracted with ethyl acetate (3 x 30 mL). The organic layer was washed with brine (3 x 30 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to yield compound **2f** as a white powder (0.303 g, 0.7 mmol, 95%). ^{1}H NMR (300 MHz,

$CDCl_3$) δ 6.56 (s, 2H), 6.50 (d, *J* = 15.8 Hz, 1H), 6.11 (dt, *J* = 15.7, 6.4 Hz, 1H), 4.23 (d, *J* = 6.3 Hz, 2H), 4.11 (s, 2H), 3.78 (s, 6H), 1.30 – 1.16 (m, 3H), 1.07 (d, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.82, 151.62, 135.65, 135.08, 128.59, 122.15, 103.81, 72.30, 66.97, 55.70, 18.09, 13.48. MALDI-ToF : m/z $[M+Na]^+$ calcd for $C_{22}H_{36}NaO_6Si$: 447.22; found: 447.12. m/z $[M+K]^+$ calcd for $C_{22}H_{36}KO_6Si$: 463.19; found: 463.11.

(*E*)-2-((3-(3,5-dimethoxy-4-((triisopropylsilyl)oxy)phenyl)allyl)oxy)-*N*-((2-methyl-3-(trimethylsilyl)cycloprop-2-en-1-yl)methyl)acetamide 2g. Compound **2f** (215 mg, 0.5 mmol), commercially available 2-methyl-3-(trimethylsilyl)cycloprop-2-en-1-yl]methanamine (79 mg, 0.5 mmol), and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (263 mg, 0.5 mmol) were dissolved in a mixture of anhydrous *N*,*N*-dimethylformamide (2 mL) and dichloromethane (3 mL). *N,N*-diisopropylethylamine (DIEA) (441 µL, 2,5 mmol) was added dropwise at 0 °C. The reaction was cooled down to room temperature and monitored by TLC (cyclohexane/ethyl acetate 7:3). After being stirred 24 h, the dichloromethane was evaporated. The resulting solution was extracted with ethyl acetate (3 x 20 mL), washed with saturated ammonium chloride aqueous solution (3 x 20 mL) and brine (3 x 20 mL), dried over sodium sulfate. The residue was purified by flash chromatography (cyclohexane/ethyl acetate gradient, 90:10 to 80:20) yielded to afford compound **2g** as white solid (0.191 g, 0.3 mmol, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (s, 2H), 6.52 (dt, *J* = 15.8, 1.3 Hz, 1H), 6.49 (bs, 1H, NH), 6.11 (dt, *J* = 15.8, 6.3 Hz, 1H), 4.18 (dd, *J* = 6.3, 1.2 Hz, 2H), 3.98 (s, 2H), 3.79 (s, 6H), 3.26 (ddd, *J* = 13.5, 5.7, 4.5 Hz, 1H), 3.15 (ddd, *J* = 13.5, 5.5, 4.8 Hz, 1H), 2.18 (s, 3H), 1.48 (t, *J* = 4.7 Hz, 1H), 1.32 – 1.17 (m, 3H), 1.07 (d, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.43, 151.63, 135.93, 135.60, 134.48, 128.73, 122.56, 111.91, 103.74, 72.24, 69.50, 55.70, 45.54, 19.36, 18.08, 13.46, 13.26, -0.97. MALDI-ToF : m/z $[M+Na]^+$ calcd for $C_{30}H_{51}NNaO_5Si_2$: 584.32; found: 584.17. m/z $[M+K]^+$ calcd for $C_{30}H_{51}KNO_5Si_2$: 600.29; found: 600.13.

(*E*)-2-((3-(4-hydroxy-3,5-dimethoxyphenyl)allyl)oxy)-*N*-((2-methylcycloprop-2-en-1-yl)methyl)acetamide 2. Compound **2g** (191 mg, 0.3 mmol) was dissolved in dry THF (7 mL). TBAF (952 µL, 1.0 M in THF) was added dropwise at 0 °C. After 1 h of stirring at room temperature, the reaction was diluted with 6 mL of H_2O. The reaction mixture was extracted with diethyl ether (3 x 20 mL). The combined organic layers were washed with H_2O (3 x 20 mL) and brine (2 x 20 mL) then dried over anhydrous sodium sulfate. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 4:6) yielded to afford compound **2** (**S_{CP}**) as a brown oil (0.073 g, 0.2 mmol, 35%). The purity of **2** was confirmed by reverse phase HPLC (see analytical data). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.63 (s, 2H), 6.59 (m, 1H), 6.53 (dt, *J* = 15.8, 1.3 Hz, 1H), 6.52 (1H, NH), 6.12 (dt, *J* = 15.8, 6.3 Hz, 1H), 5.61 (1H, OH), 4.19 (dd, *J* = 6.3, 1.3 Hz, 2H), 3.98 (s, 2H), 3.90 (s, 6H), 3.31 (ddd, *J* = 13.7, 6.0, 4.2 Hz, 1H), 3.18 (ddd, *J* = 13.7, 5.7, 4.5 Hz, 1H), 2.12 (d, *J* = 1.1 Hz, 3H), 1.58 (td, *J* = 4.35, 1.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.56, 147.29, 135.26, 134.10, 127.82, 122.65, 121.51, 103.61, 102.96, 72.16, 69.48, 56.39, 44.72, 17.95, 11.65. MALDI-ToF : m/z $[M+Na]^+$ calcd for $C_{18}H_{23}NNaO_5$: 356.15; found: 355.90. m/z $[M+K]^+$ calcd for $C_{18}H_{23}KNO_5$: 372.26; found: 371.84.

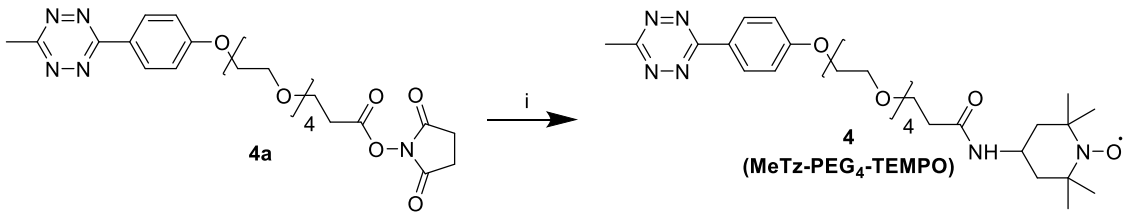
II.2. Synthesis of MeTz-TEMPO **3**



Scheme S2. Synthesis of **MeTz-TEMPO 3**. (i) 4-amino-TEMPO, DIEA, THF-DCM, 0°C to rt, 3h - 83%.

(2,2,6,6-tetramethyl-4-{2-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]acetamido}piperidin-1-yl)oxidanyl 3. 4-amino-TEMPO (32 mg, 0.18 mmol) and *N*,*N*-diisopropyethylamine (0.054 mL, 0.31 mmol) were dissolved in THF (5 mL). To this solution was dropwise added the methyltetrazine-NHS ester **3a** (50 mg, 0.15 mmol) in CH_2Cl_2 (2 mL) at 0°C. The reaction mixture was stirred for 3 h, at which the reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (10 mL), the pH was adjusted at 5 with an aqueous solution of HCl 1M. The mixture was extracted by ethyl acetate (3 x 10 mL). The organic phases were combined, washed with brine (3 x 20 mL), dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford compound **3** (**MeTz-TEMPO)** as a purple solid (48 mg, 0.13 mmol, 83 %). The purity of **3** was further confirmed by reverse-phase HPLC, see analytical data. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (bs, 2H), 7.61 (bs, 2H), 3.75 (bs, 2H), 3.19 (bs, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.25, 165.88, 162.32, 137.93, 129.63, 129.09, 127.16, 19.85. Not all signals were observable due to paramagnetic broadening by the nitroxide radical. MALDI-ToF m/z $[M+H]^+$ calcd for $C_{20}H_{29}N_6O_2$: 384.23; found: 384.38. m/z $[M+Na]^+$ calcd for $C_{20}H_{28}N_6NaO_2$: 406.21; found: 406.31. m/z $[M+K]^+$ calcd for $C_{20}H_{28}KN_6O_2$: 422.18; found: 422.18. The NMR tube was submitted to phenylhydrazine reduction in situ by addition of 2 drops of 1:1 v/v phenylhydrazine/$CDCl_3$ to prevent paramagnetic broadening and restore TEMPO signals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 3.47 (s, 2H), 3.38 (bs, 1H), 2.98 (s, 3H), 1.72 (m, 2H), 1.23 (m, 2H), 1.10 (s, 6H), 1.08 (s, 6H).

II.3. Synthesis of MeTz-PEG_4-TEMPO **4**

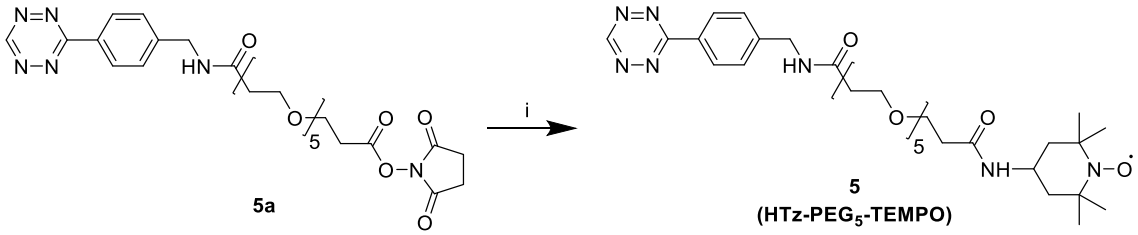


Scheme S3. Synthesis of **MeTz-PEG_4-TEMPO** (**4**). (i) 4-amino-TEMPO, DIEA, THF-DCM, 0°C to rt, 3h - 89%.

(2,2,6,6-tetramethyl-4-{1-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy]-3,6,9,12-tetraoxapentadecan-15-amido}piperidin-1-yl)oxidanyl 4. 4-amino-TEMPO (19 mg, 0.11 mmol) and

N,*N*-diisopropyethylamine (0.032 mL, 0.19 mmol) were dissolved in THF (3 mL). To this solution was dropwise added the methyltetrazine-PEG$_4$-NHS ester **4a** (48 mg, 0.09 mmol) in CH_2Cl_2 (3 mL) at 0°C. The reaction mixture was stirred for 3 h, at which the reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (10 mL), the pH was adjusted at 5 with an aqueous solution of HCl 1M. The mixture was extracted by ethyl acetate (3 x 10 mL). The organic phases were combined, washed with brine (3 x 20 mL), dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford compound **4** (**MeTz-PEG$_4$-TEMPO)** as a purple oil (48 mg, 0.08 mmol, 89 %). The purity of 4 was further confirmed by reverse-phase HPLC, see analytical data. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (bs, 2H), 7.17 (bs, 2H), 4.32 (bs, 2H), 3.98 (bs, 2H), 3.86 – 3.51 (bs, 14H), 3.14 (s, 3H), 2.55 (bs, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.63, 165.11, 162.23, 160.99, 128.30, 122.93, 114.04, 69.96, 69.81, 69.58, 69.47, 69.17, 68.95, 68.86, 66.98, 65.70, 37.95, 19.75. Signals of the TEMPO ring are not observed due to paramagnetic broadening by the nitroxide radical. MALDI-ToF m/z $[M+Na]^+$ calcd for $C_{29}H_{46}N_6NaO_7^+$: 612.32; found: 612.56. m/z $[M+K]^+$ calcd for $C_{29}H_{46}KN_6O_7^+$: 628.30; found: 628.51.

II.4. Synthesis of HTz-PEG$_5$-TEMPO **5**

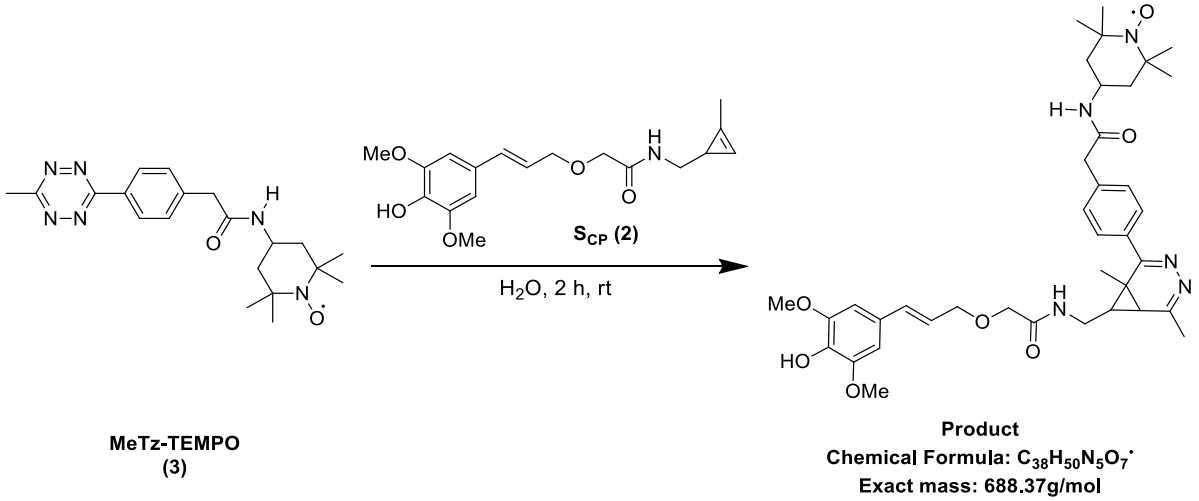


Scheme S4. Synthesis of **HTz-PEG$_5$-TEMPO 5**. (i) 4-amino-TEMPO, DIEA, THF-DCM, 0°C to rt, 4h - 99%.

{2,2,6,6-tetramethyl-4-[1-({[4-(1,2,4,5-tetrazin-3-yl)phenyl]methyl}carbamoyl)-3,6,9,12,15-pentaoxaoctadecan-18-amido]piperidin-1-yl}oxidanyl 5. 4-amino-TEMPO (17 mg, 0.10 mmol) and *N*,*N*-diisopropyethylamine (0.028 mL, 0.17 mmol) were dissolved in THF (3 mL). To this solution was dropwise added the tetrazine-PEG$_5$-NHS ester **5a** (50 mg, 0.08 mmol) in CH_2Cl_2 (1.5 mL) at 0°C. The reaction mixture was stirred for 4 h, at which the reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (10 mL), the pH was adjusted at 5 with an aqueous solution of HCl 1M. The mixture was extracted by ethyl acetate (3 x 10 mL). The organic phases were combined, washed with brine (3 x 20 mL), dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford compound **5** (**HTz-PEG$_5$-TEMPO)** as a purple oil (54 mg, 0.08 mmol, 99 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.36 (bs, 1H), 8.75 (bs, 2H), 7.72 (bs, 2H), 4.90 (bs, 2H), 4.25 – 3.50 (m, 20H), 2.99 – 2.41 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.18, 167.30, 162.55, 155.15, 140.98, 126.72, 124.97 (2C), 68.36, 67.51, 67.32 (2C), 67.16, 67.13, 66.80, 66.64, 64.11, 63.66, 39.79, 36.12, 34.29. Signals of the TEMPO ring were observable due to paramagnetic broadening by the nitroxide radicals. MALDI-ToF m/z $[M+Na]^+$ calcd for $C_{32}H_{51}N_7NaO_8^+$: 683.36; found: 683.53. m/z $[M+K]^+$ calcd for $C_{32}H_{51}KN_7O_8^+$: 699.34; found: 699.48.

III. Monitoring of DAR_{inv} ligation in solution

III.1. Identification of cycloadduct by mass spectrometry



Inverse electronic demand Diels-Alder (DAinv) reaction of S_{CP} **2** (50 µM)and MeTz-TEMPO **3** (100 µM) was carried out in ultrapure water at room temperature in the dark. After 2 hours of reaction, the formed product was characterized by MALDI-ToF mass spectrometry using dihydroxybenzoïc acid as matrix (see **figure S1A**): m/z $[M+H]^+$ calcd: 689.38; found: 689.60. m/z $[M+Na]^+$ calcd: 711.36; found: 711.58.

III.2. Monitoring of the DAinv ligation between **2** and **3** in solution.

The disappearance of probe MeTz-TEMPO **3** (200 µM) was monitored by HPLC-UV when in presence of a 5-fold excess of reporter S_{CP} **2** (1 mM). The reaction was carried out at room temperature in ultrapure water in the dark and samples were prepared from stock solutions of **2** and **3** in 20% DMSO. 20 µL injections were carried out at different time points (t_0, t_0 + 1h, t_0 + 2h, t_0 + 3h) on a Thermo Finnigan SCM1000 system equipped with a Grace 5 µm Alltima C_{18} column and a UV6000LP detector. Data were processed in the ChromQuest software.

Eluant A: H_2O + 0.1% TFA Eluant B: MeCN/H_2O (90:10) + 0.1% TFA

Elution method: t(0 – 20 min) : gradient A/B 95:5 à A/B 5:95 ; t(20 – 40 min) : isocratic A/B 10:90

In the presence of an excess of **2**, probe **3** is consumed and the formation of 4 peaks of cycloadducts can be observed (see **figure S1B**). This is consistent with the reaction mechanism that leads to a mixture of 4 isomers, namely 4,5-dihydropyridazines (2 regioisomers) and 1,4-dihydropyridazines (2 regioisomers).

Note: the kinetic paramaters in solution and within the biological sample are not transposable. The ligation time was then optimized directly in plant samples and the reaction in plants exhibits faster kinetics than in solution as i) a large excess of probe **3** is used compared to **2**, and ii) the non-soluble nature of the tagged lignin products presumably drives the reaction.

IV. EPR experiments

IV.1. Plant Material

2-month-old flax (*Linum usitatissimum* L.) were grown from seeds sown in compost (Neuhaus) and cultivated in growth chambers (Angelantoni Life Sciences) at 22°C with a photoperiod of 16hr/8hr day/night.

IV.2. S_{CP} incorporation and labeling *in planta*

IV.2.1. Chemical reporter incorporation

Each experiment consisted of 3 biological replicates (*i.e., 3 separate plants grown at the same time period*). For each biological replicate of a given experiment, 9 technical replicates (*i.e., 9 cross-sections taken from the same plant at the same stem-height*) were measured. Each experiment was reproduced 3 times over a period of several weeks, each time on a newly grown plant batch.

Vibratome cross-sections (120 µm thick) of flax stems were made at 10 cm above soil level and stored in liquid sterile ½ MS (Murashige and Skoog[1]) prior to monolignol incubation. Sections were then incubated in 300 µL ½ MS containing 10 µM methylcyclopropene tagged sinapyl alcohol **2** (**S_{CP}**) for 20 h in the light at 20°C (Grobank growth chamber). Control samples were incubated in 300 µL ½ MS without **S_{CP} 2** under identical conditions. After incorporation, samples were washed with ½ MS (4 x 5 min), fixed with 4 % paraformaldehyde for 1 hour at ambient temperature and washed with water (2 x 1 min) prior to DAinv spin labeling.

IV.2.2. DAinv spin labeling

Paramagnetic probes are prepared as 5 mM stock solutions in 100 % DMSO for MeTz-TEMPO **3** and in 20 % DMSO for MeTz-PEG_4-TEMPO **4** and HTz-PEG_5-TEMPO **5**.

For spin labeling of **S_{CP}**, DAR_{inv} was used: plant sections were incubated with 300 µL of a click labeling solution containing 100 µM paramagnetic probe of interest (MeTz-TEMPO **3**, MeTz-PEG_4-TEMPO **4** or HTz-PEG_5-TEMPO **5**) in ultrapure water for 2 h in the dark. Sections were then washed in the dark according to an optimized procedure (water, 2 x 5 min; MeOH 70 %, 1 x 15 min; water, 4 x 5 min) to maximize removal of unreacted paramagnetic probes. Sections were then stored in the dark at 4°C in a mixture of glycerol/water (1:1, *v/v*). In order to minimize evolution of the paramagnetic species, CW-EPR spectroscopic analyses were carried out the same day or the next morning.

IV.3. EPR spectroscopy

CW-EPR experiments were performed with a X-band Bruker Elexsys E580 spectrometer operating at 9.6 GHz at room temperature, with a modulation frequency of 100 kHz and modulation amplitude of 2G. Quality factor Q was in the range 4200-4400.

- For measurement of probes in solution, a solution of 100 µM of the paramagnetic probe of interest (MeTz-TEMPO **3**, MeTz-PEG$_4$-TEMPO **4** or HTz-PEG$_5$-TEMPO **5**) in ultrapure water was filled into a glass capillary of 50 µL, which was in turn, placed in 4 mm quartz EPR tubes before EPR analysis. The microwave power supplied into the resonator was set at 0.6 mW to avoid saturation.
- For measurement in biological samples, 9 plant cross-sections were deposited on the wall of 4 mm quartz EPR tubes. CW-spectra were recorded with the same modulation amplitude using 15 mW of microwave power.

IV.4. EPR Simulation and relative contribution of radical species

EPR spectra of paramagnetic probes on plant tissues was simulated in MATLAB with homewritten scripts based on the EasySpin EPR simulation package.[2] The chili function was used to compute the rotation correlation time.

The spectra were fitted to compute the weight of each component. Relative weights (%w) for each population were directly calculated from simulations performed with the EasySpin toolbox. From the total spectrum integral I_{total}, the integral of each population ($I_{(IIIa)}$, $I_{(IIIb)}$, etc.) was determined by their contribution. The molar concentration can then be inferred using our calibration curve (see **figure S5**, equation I_{total} = 20581 x [**3**] determined by linear regression).

EPR spectra intensities were normalized. Variation of acquisition parameters between solution samples and plant samples was accounted for by applying a correction factor (calculated factor 12.2). Each EPR spectrum was acquired on a tube containing 9 plant cross-sections of equal volume and weight (cut precisely with a vibratome), which was also taken into account to derive the concentration of a species per cross-section

$$[\mathbf{3}(\mathrm{IIIa})] = \frac{\%w(IIIa) * \boldsymbol{I}total}{2259794}$$

IV.5. EPR imaging

Images were acquired at room temperature using a Bruker E580 ELEXSYS system (Bruker BioSpin GmBh, Karlsruhe, Germany). The system was operated in X-band mode at 9.6 GHz and modulation frequency of 100 kHz. For imaging, the system was equipped with water-cooling gradient allowing a magnetic field gradient up to 20 mT/cm along the Z-, and Y-plan.

Nine flax stem cross-sections were pasted side by side into an EPR tube for imaging measurements. The tube was positioned at the center of the microwave cavity.

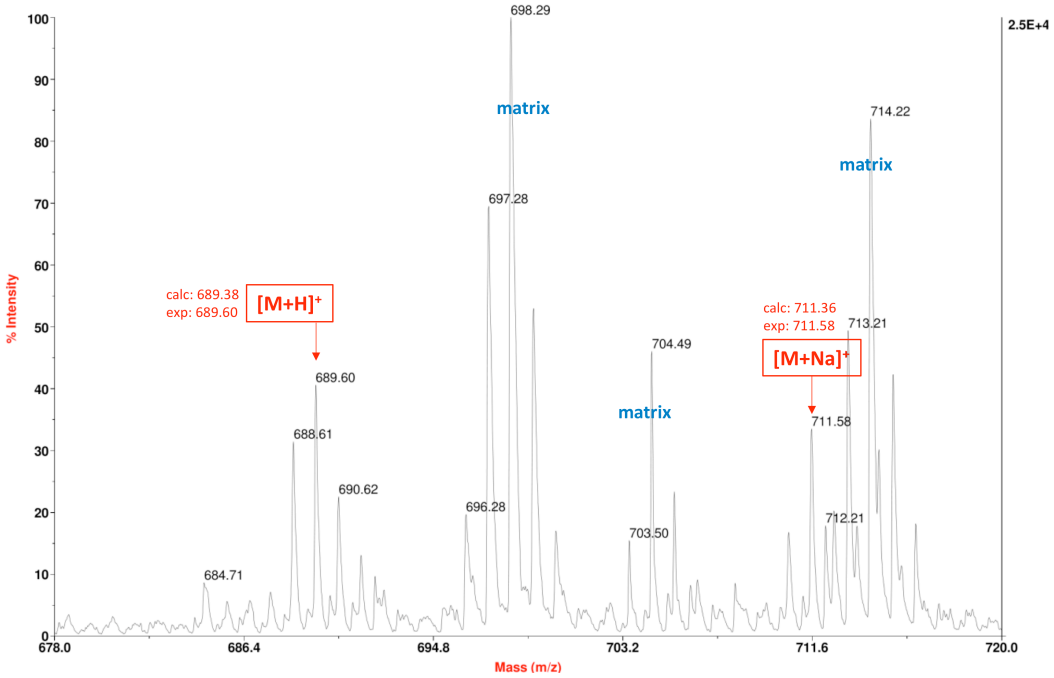
The signals were acquired with a field-of-view of 5 mm and gradient strength of 175 G cm^{-1}. The two-dimensional (2D) images were acquired with a size of 512 x 512 pixels resulting in a pixel size of 20 µm. In order to increase the resolution inside a pixel, 1608 projections instead of 402 were collected. The high resolution spatial-spatial EPR images were obtained by deconvolution of the acquired signal from the signal without gradient followed by filtered back-projection algorithm. The image processing involves deconvoluting the whole signal acquired under a magnetic field gradient from the reference signal collected without gradient. Both signals with and without gradient were computed and back-projected using Fourier Transform, giving the spatial distribution of MeTz-TEMPO **3** probe.

V. S_{CP} fluorescence imaging

For detection of incorporated $\mathbf{S_{CP}}$ **2** by fluorescence imaging (figure S3), flax cross-sections were made as described above. Sections were then incubated in 300 µL ½ MS containing 0 or 10 µM $\mathbf{S_{CP}}$ for 20 h in the light at 20°C. After incorporation, samples were washed with ½ MS (4 x 5 min). Sections were then incubated with 300 µL DARinv labeling solution containing 5 µM 3-(*p*-Benzylamino)-1,2,4,5-tetrazine-Cy5 (tetrazine-Cy5, λ_{exc} 649/λ_{em} 670 nm) in ½ MS for 1 h in the dark. Sections were then washed according to an optimized procedure (2 x ½ MS, 5 min, 1 x MeOH 70 %, 60 min, 1 x ½ MS, 5 min and 3 x ½ MS, 10 min). Sections were then mounted on slides in Fluoromount-G® mounting medium and observed by confocal microscopy (Nikon A1R) using x20 (NA 0.8) with the following excitation wavelengths and adapted bandpass filters for tetrazine-Cy5: exc: 561 nm, em: 700/75. Microscope settings (gain, laser intensity, pinhole size) were kept constant throughout a given set of experiments.

VI. Supplementary Figures

A



B

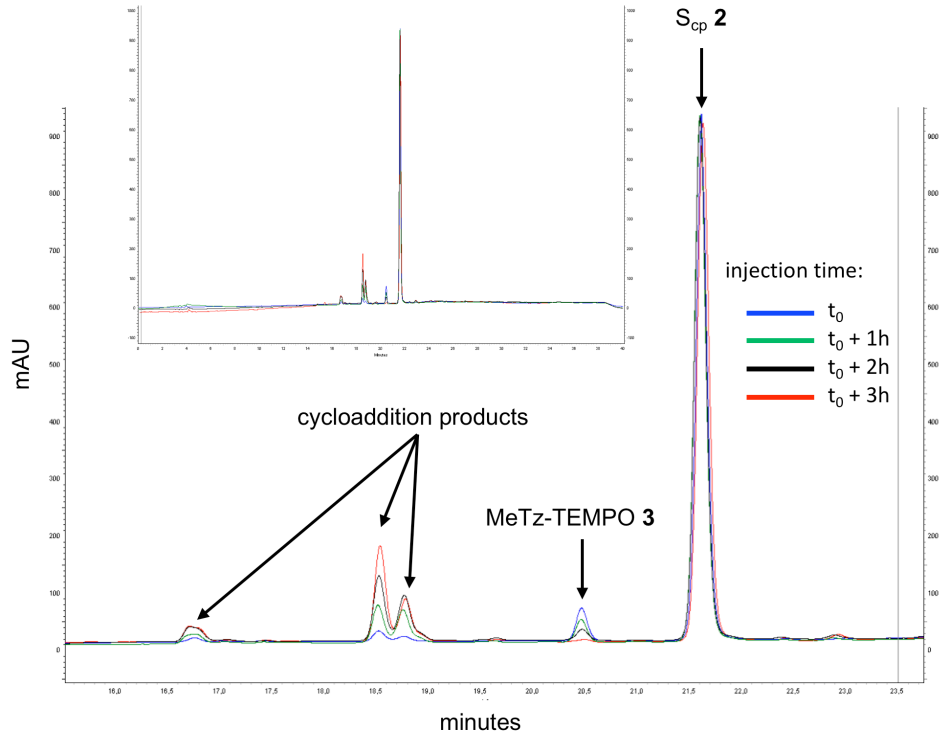


Figure S1. Validation of the DARinv ligation between MeTz-TEMPO **3** and S_{CP} **2** in solution. **(A)** MALDI-ToF mass spectrometry of the DAR_{inv} cycloadduct. **(B)** HPLC-UV monitoring of the DARinv reaction between **2** (1 mM, 5 eq) and **3** (200 µM, 1 eq). As expected from the alkene-tetrazine reaction mechanism, formation of 4 isomers of the cycloadduct can be observed (2 regioisomers of the 4,5-dihydropyridazine cycloadducts and 2 regioisomers of the 1,4-dihydropyridazine).

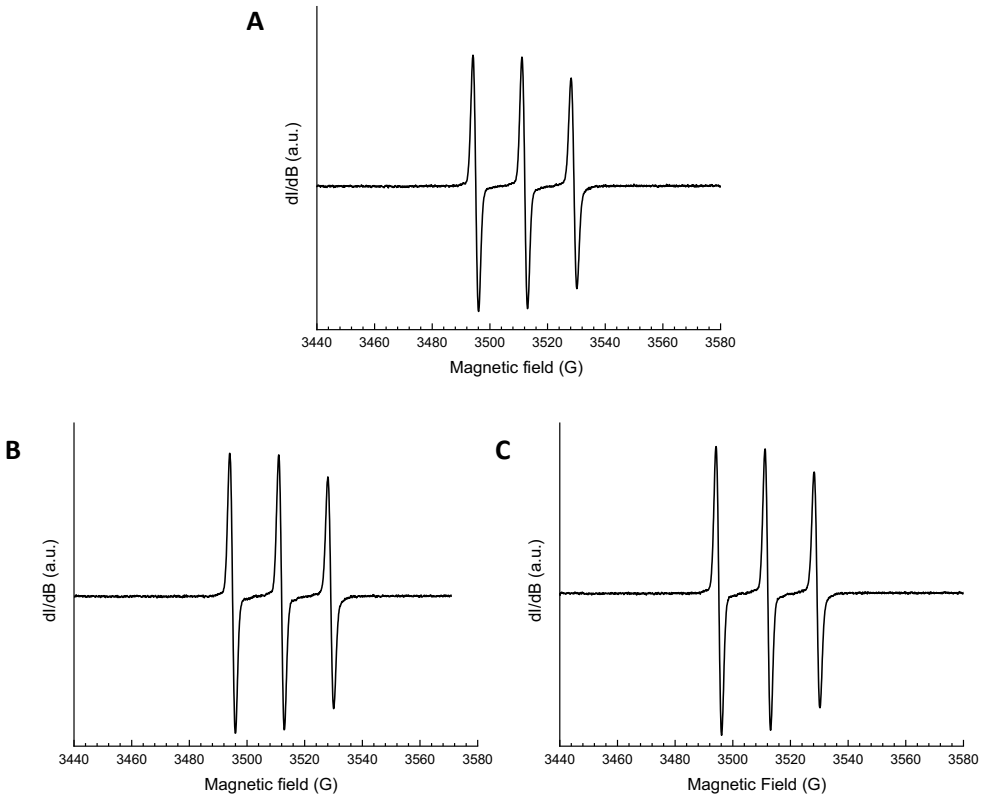


Figure S2. CW-EPR spectra recorded at room temperature in the X-band of each paramagnetic probe in solution (100 µM in ultrapure water). (A) MeTz-TEMPO **3**, hyperfine coupling constant = 16.99 G, g factor = 2.0056; (B) MeTz-PEG$_4$-TEMPO **4**, hyperfine coupling constant = 17.14 G, g factor = 2.0056; (C) HTz-PEG$_5$-TEMPO **5**, hyperfine coupling constant = 17.00 G, g factor = 2.0056. Acquisition parameters: modulation amplitude = 2 G, microwave power = 0.6 mW.

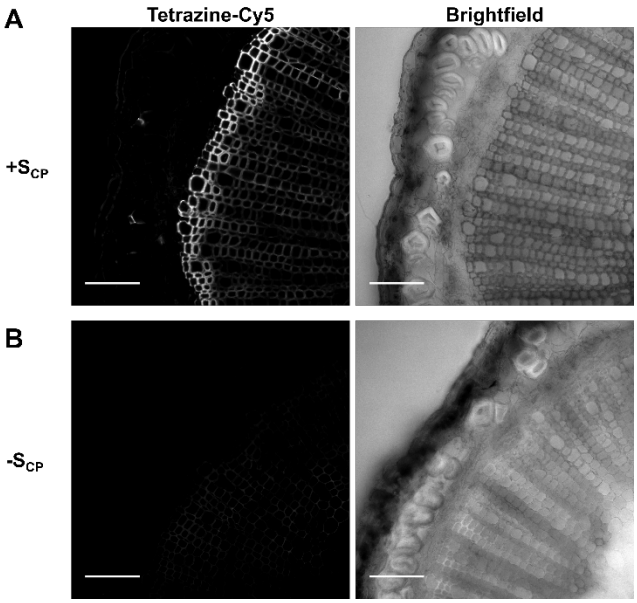


Figure S3. Efficiency of S_{CP} incorporation into flax stem cell wall. 2-month-old flax stem sections incubated in ½ MS containing (A) 10 µM S_{CP}, or (B) no S_{CP} for 20 h before performing DARinv fluorophore ligation. Tetrazine-Cy5 fluorescence channel and brightfield are shown. Scale bar = 100 µm.

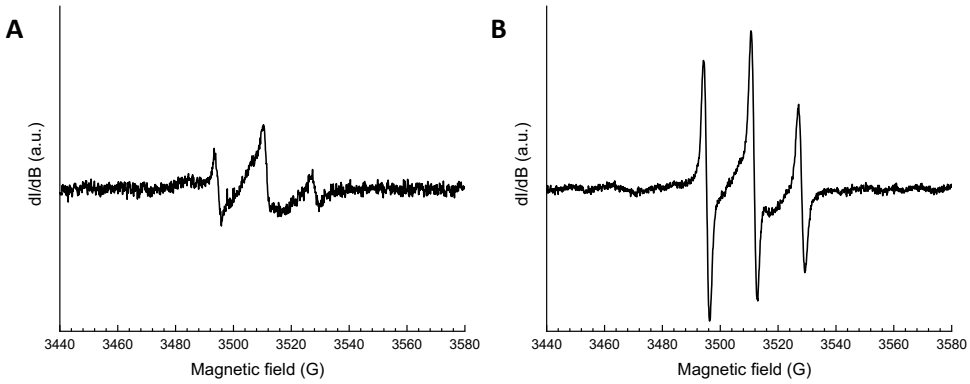


Figure S4. CW-EPR spectra recorded at room temperature of MeTz-TEMPO in flax cross-sections after the chemical reporter strategy with (A) or without (B) paraformaldehyde fixation after metabolic incorporation of S_{CP} and prior DARinv ligation. Acquisition parameters: modulation amplitude = 2 G, microwave power = 15 mW.

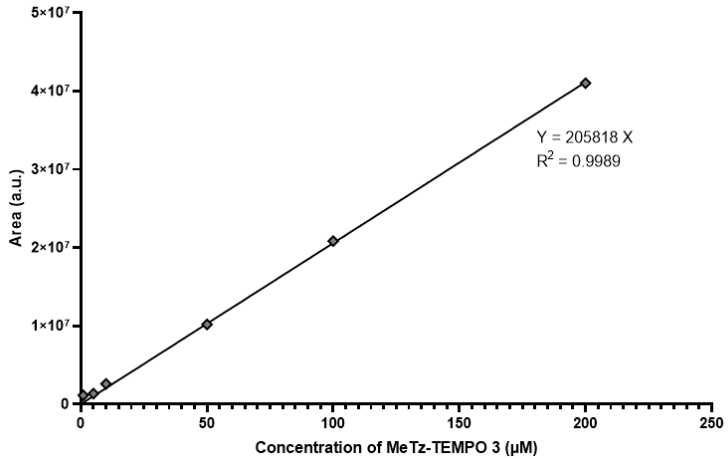


Figure S5. Calibration curve of the paramagnetic probe MeTz-TEMPO **3**. Area of the EPR spectrum of MeTz-TEMPO depending on its concentration (0-200 µM).

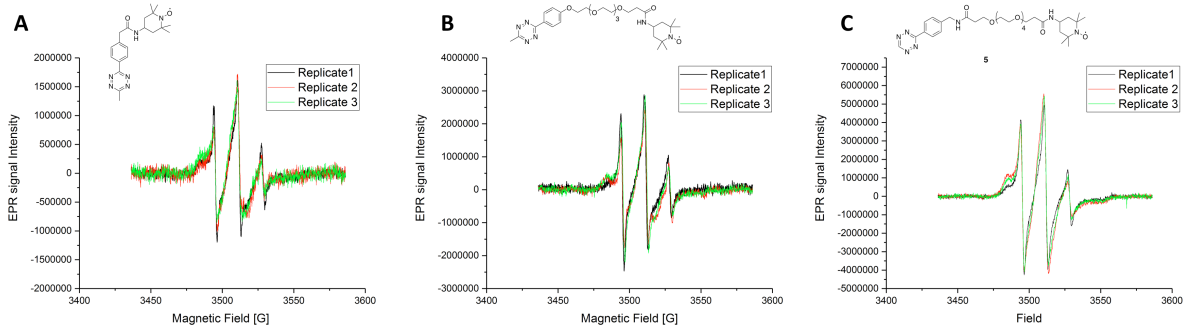


Figure S6. Illustration of the low biological variability of the used samples. CW-EPR spectra of 3 biological replicates (carried out on different plant batches at different dates over several weeks) are superimposed. S_{CP} **2** was incorporated into plant cross-sections as described above, then the samples were fixed with paraformaldehyde and submitted to DAinv ligation with MeTz-TEMPO **3** (A), MeTz-PEG4-TEMPO **4** (B) or HTz-PEG5-TEMPO **5** (C). Relative weights of each species were quantified as previously described. **A**: for **3**, SD <5%. **B**: for **4**, SD <8%. **C**: for **5**, SD <20%. The high variability of **5** is likely due to instability of the H-tetrazine leading to non-specific covalent binding in planta and not to biological factors. Low variability was obtained for **3** and **4**.

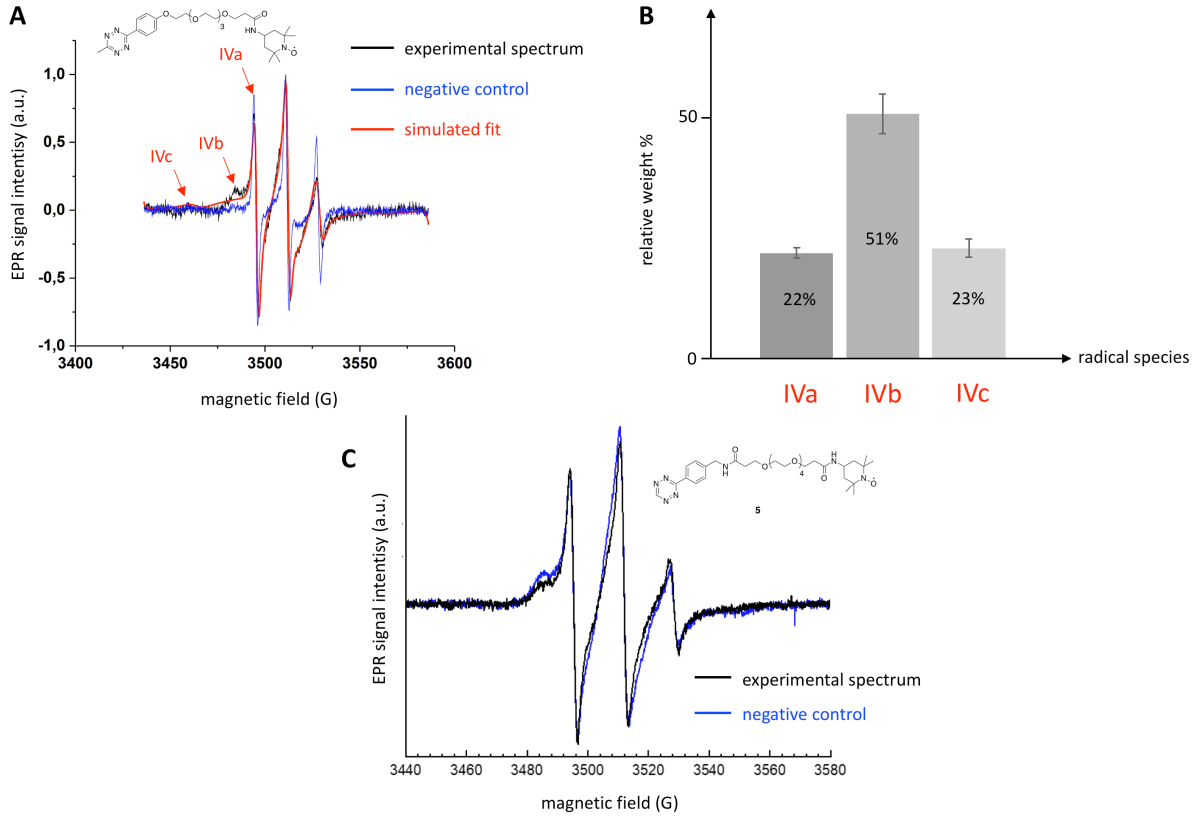


Figure S7. CW-EPR spectra recorded at room temperature on flax cross sections using MeTz-PEG$_4$-TEMPO **4** (A, B) or HTz-PEG$_5$-TEMPO **5** (C) as the spin-probe for DARinv ligation. Experiments were conducted on samples previously incubated with reporter S$_{CP}$ **2** (black spectra) or without reporter S$_{CP}$ **2** (blue spectra, negative control). Acquisition parameters: modulation amplitude = 2 G, microwave power = 15 mW. A,B: for **4**, significant differences were detected and the EPR spectra were fitted with the EasySpin toolbox (red trace) to calculate the relative contribution of each species. IVa represents mobile free probe remaining in the sample after washes, while IVb and IVc shows a much more constrained environment. C: for **5**, no significant difference was detected between samples and negative controls. The presence of free probe and immobilized species can be observed in both samples and negative controls, suggesting that the poor stability of **5** led to strong non-specific binding within the cell walls.

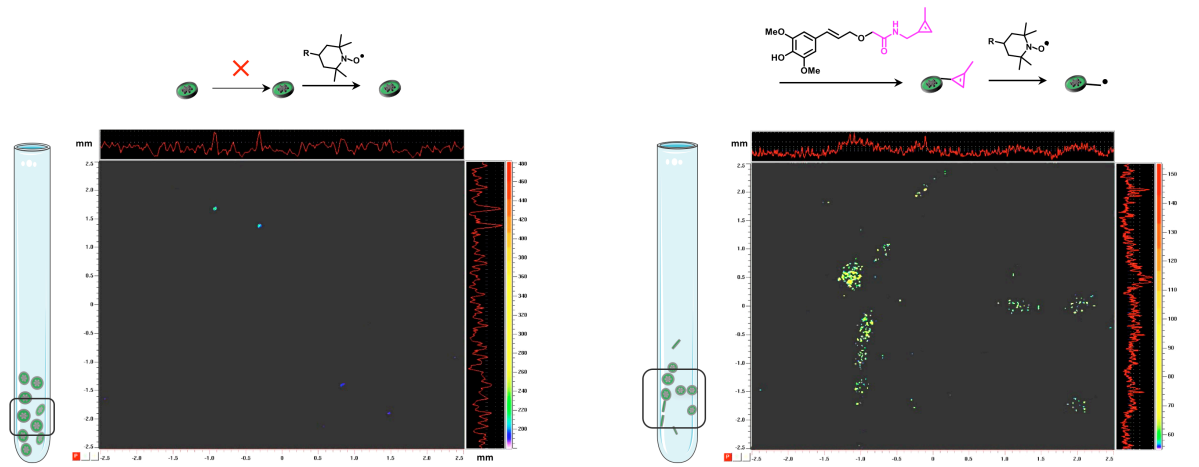
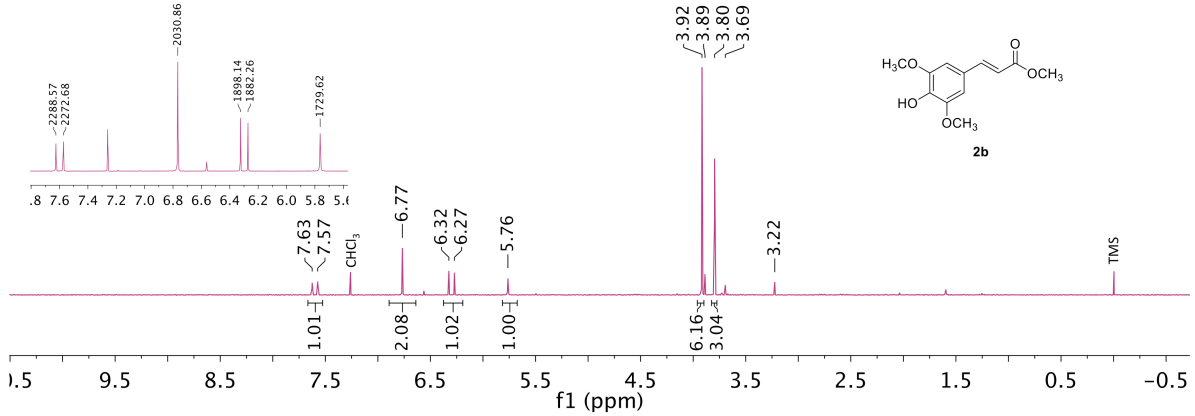


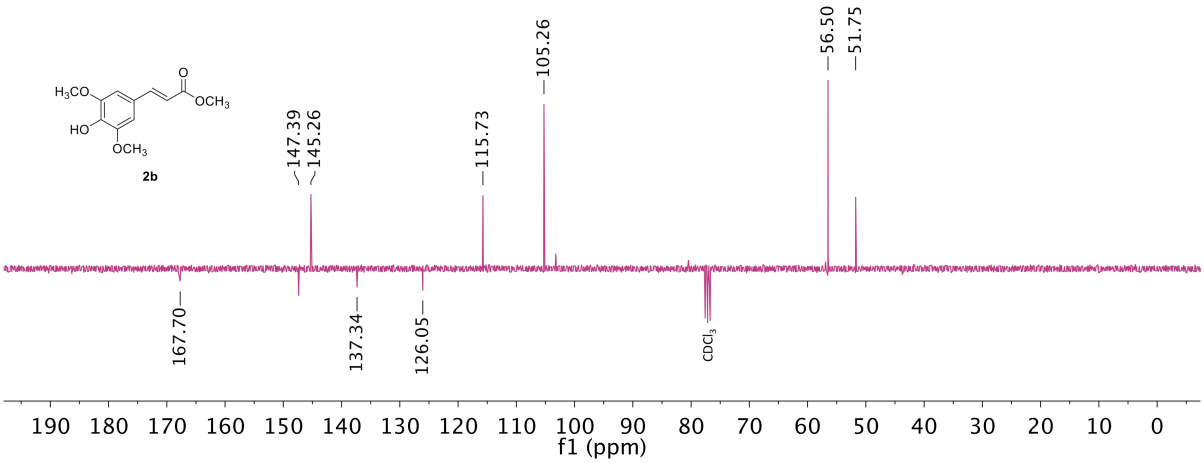
Figure S8. EPR imaging data after DARinv ligation with probe **3** on black background with signal intensity scale legend. Sample without priori incubation with SCP 2 (negative control, left panel) and with prior incubation with 2 (right panel). 9 flax stem sections were placed side-by-side on the interior surface of an EPR tube.

VII. Spectroscopic and analytical data (NMR, MS, HPLC)

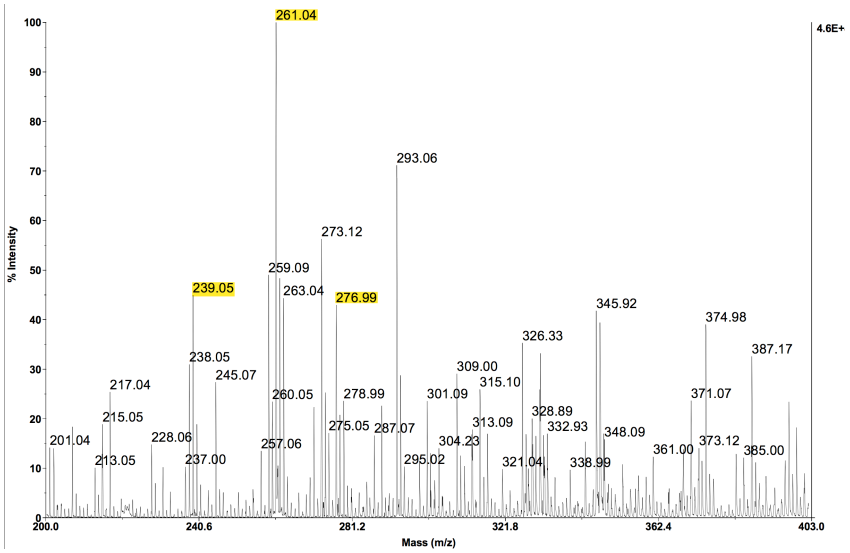
Intermediate compound **2b** – ^{1}H NMR in $CDCl_3$



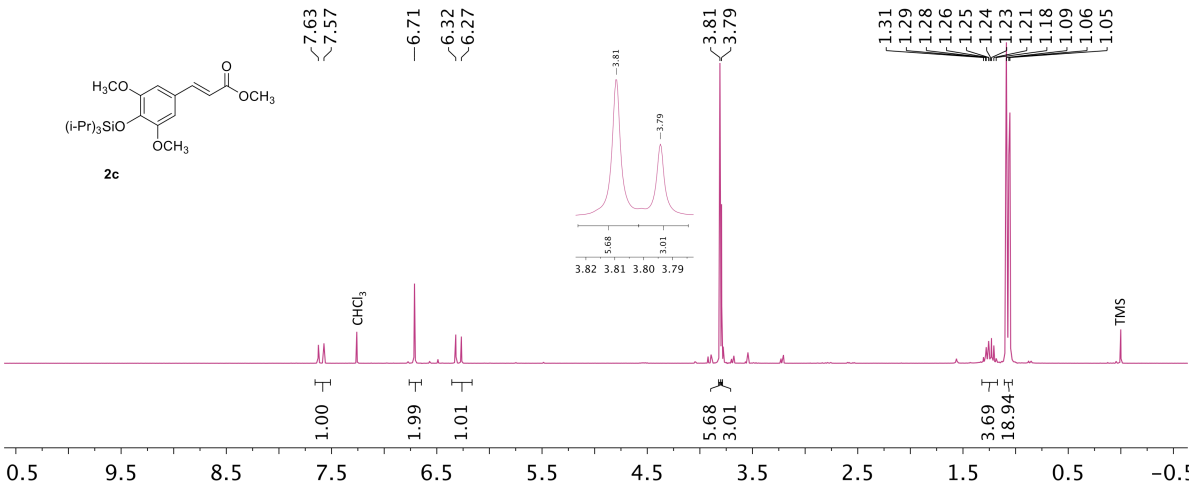
Intermediate compound **2b** – ^{13}C APT NMR in $CDCl_3$



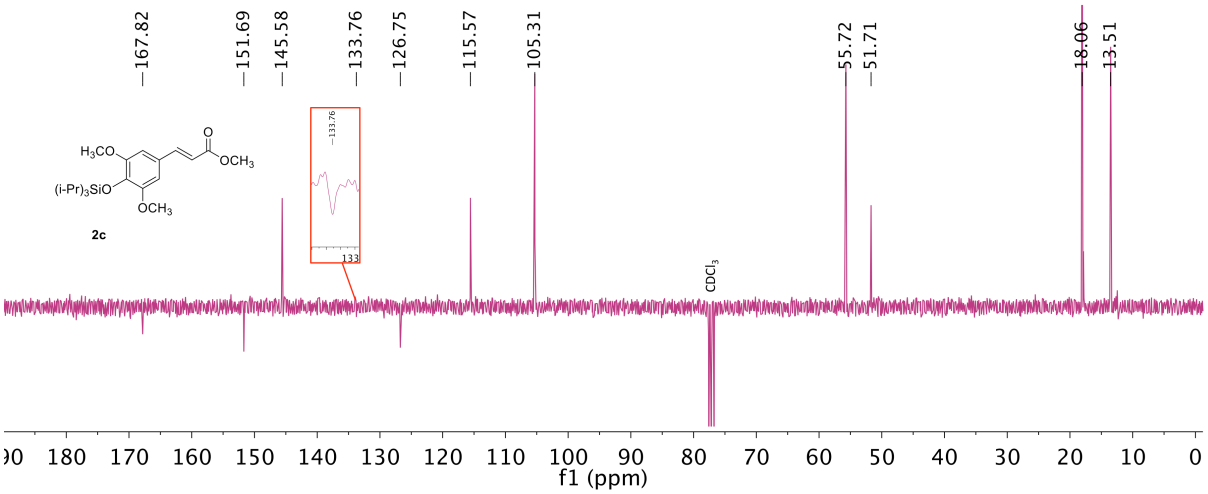
Intermediate compound **2b** – MALDI-ToF MS



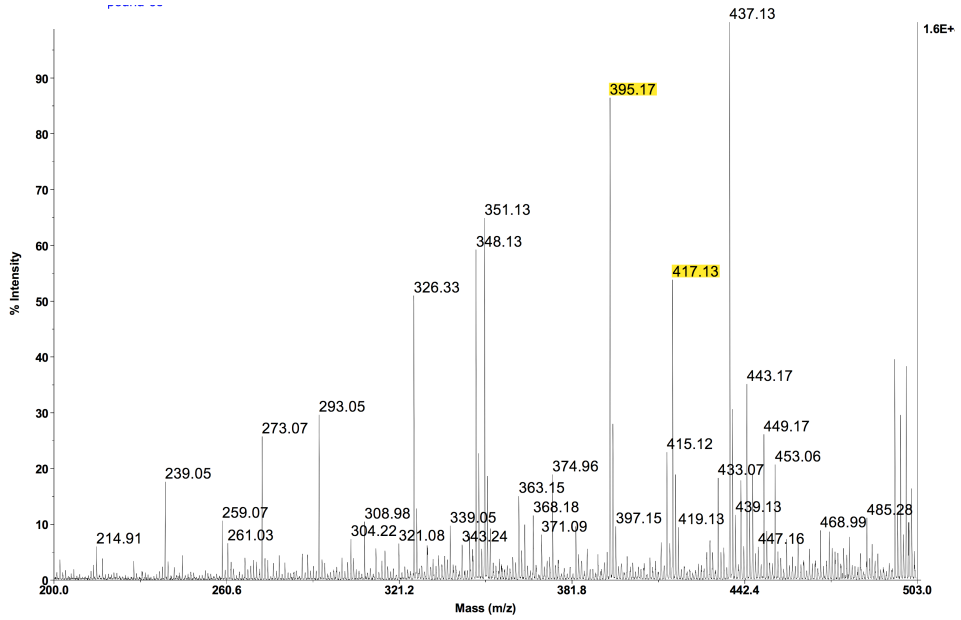
Intermediate compound **2c** – ^{1}H NMR in $CDCl_3$



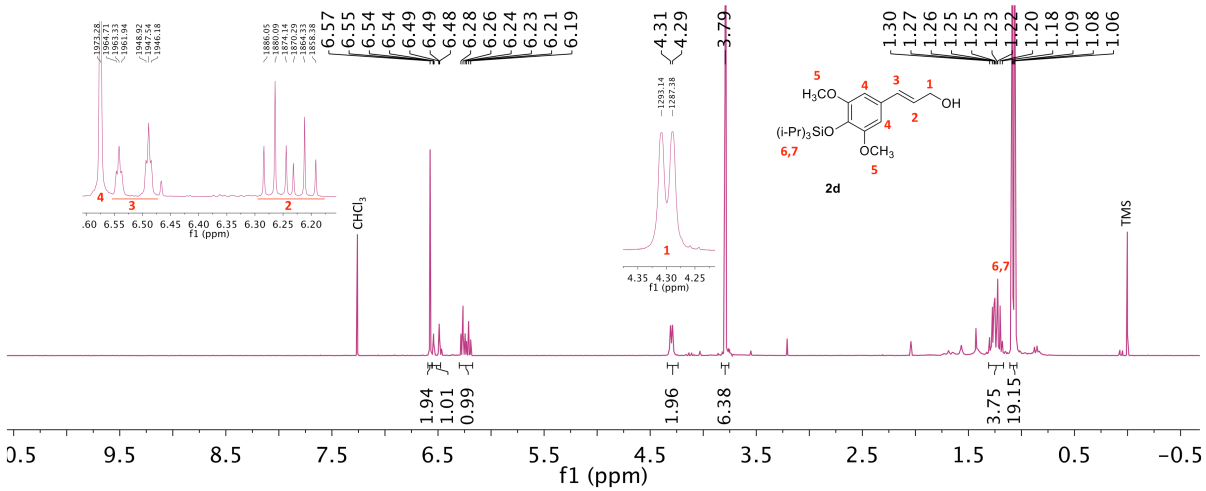
Intermediate compound **2c** – ^{13}C APT NMR in $CDCl_3$



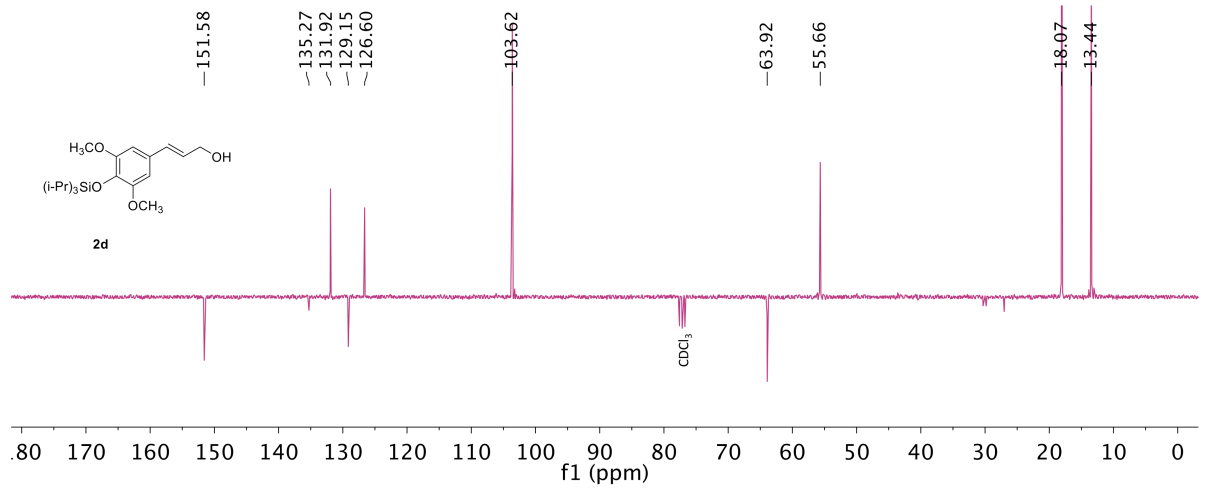
Intermediate compound **2c** – MALDI-ToF MS



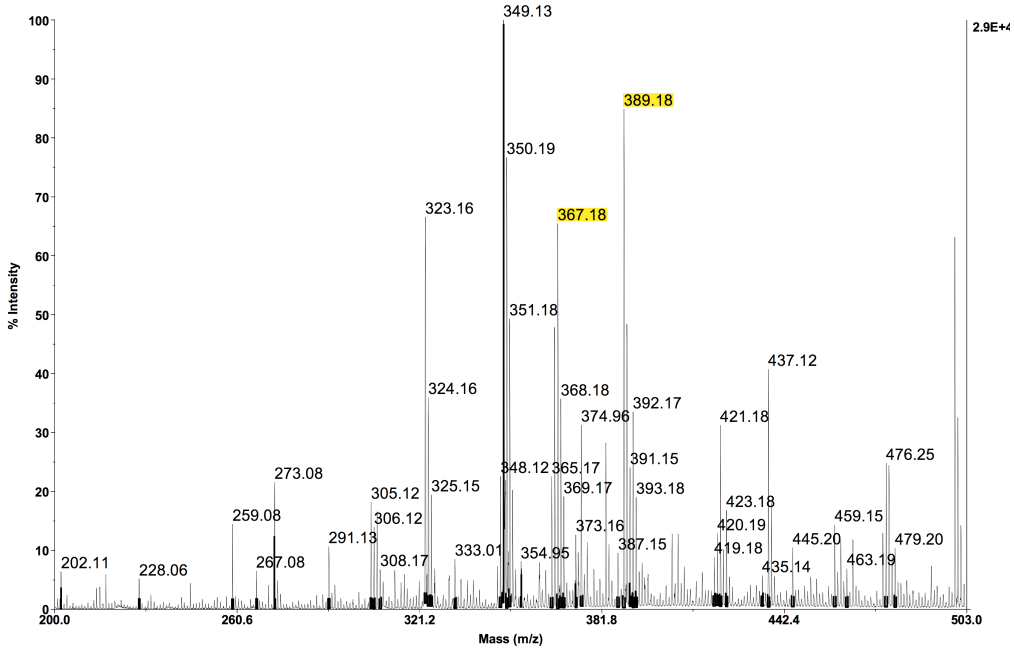
Intermediate compound **2d** – ^{1}H NMR in $CDCl_3$



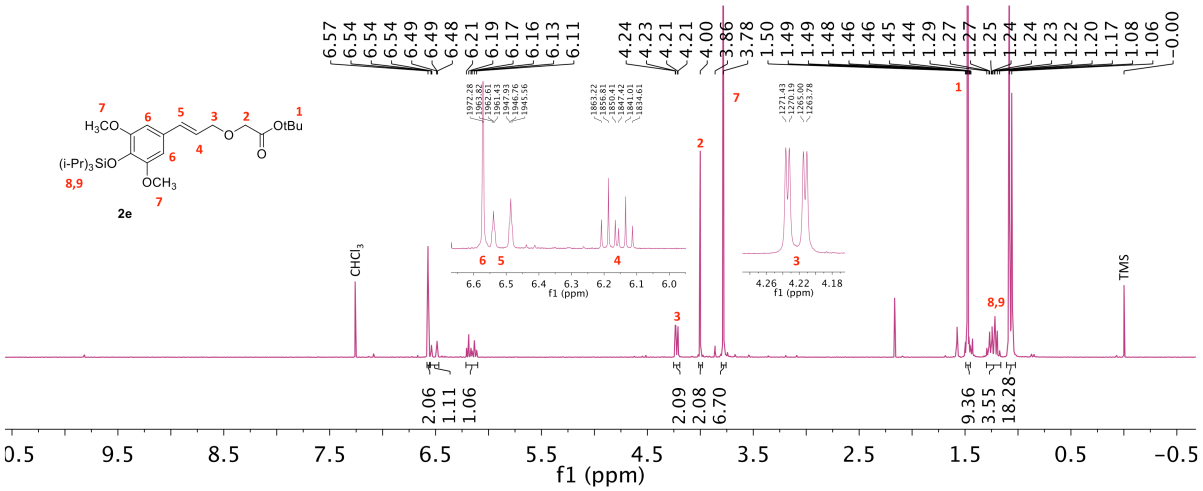
Intermediate compound **2d** – ^{13}C APT NMR in $CDCl_3$



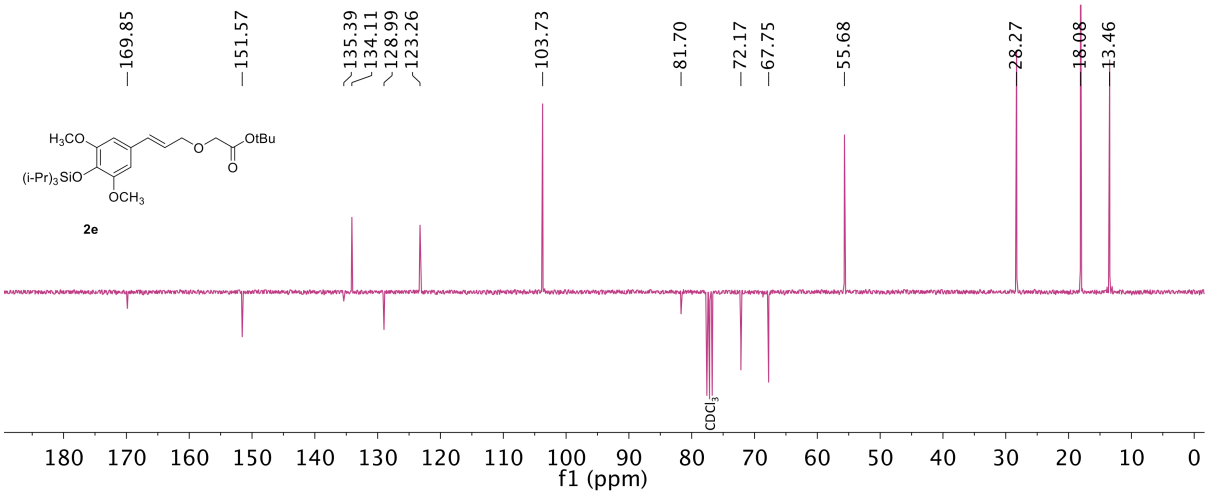
Intermediate compound **2d** – MALDI-ToF MS



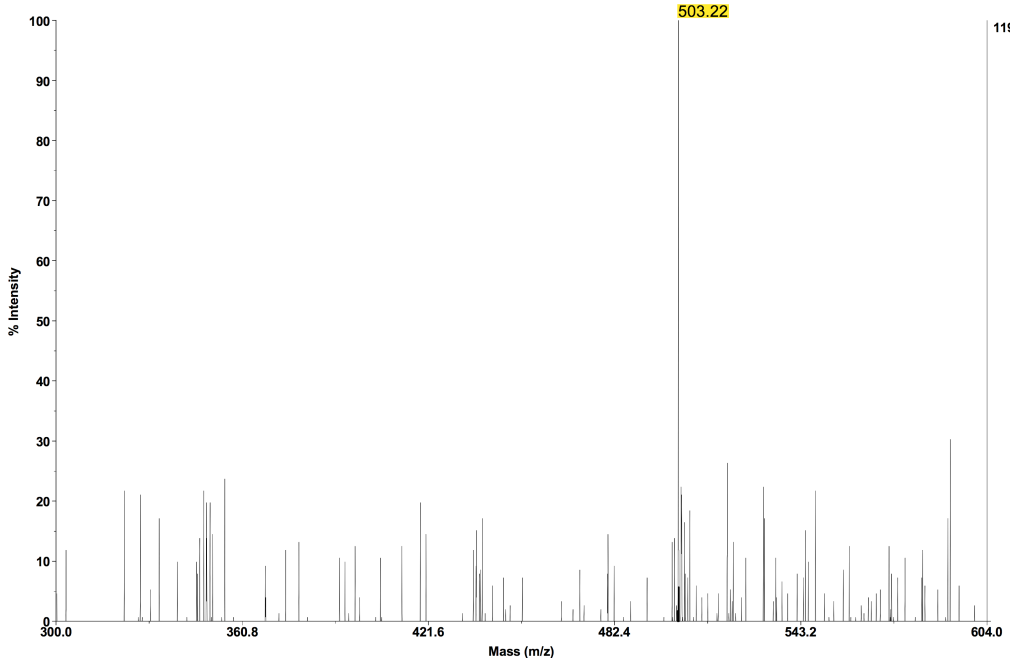
Intermediate compound **2e** – ^{1}H NMR in $CDCl_3$



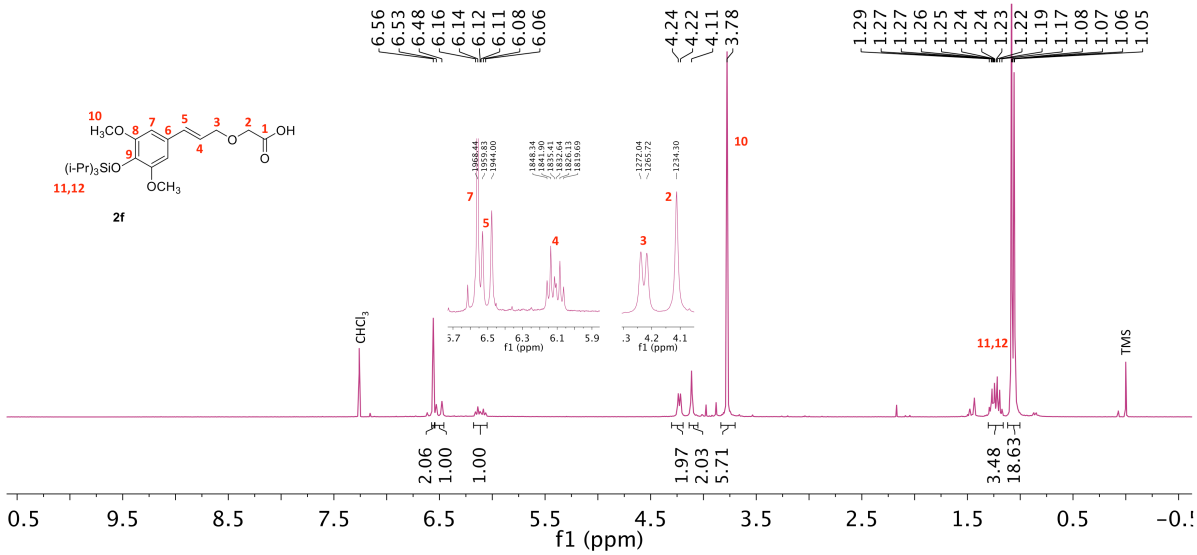
Intermediate compound **2e** – ^{13}C APT NMR in $CDCl_3$



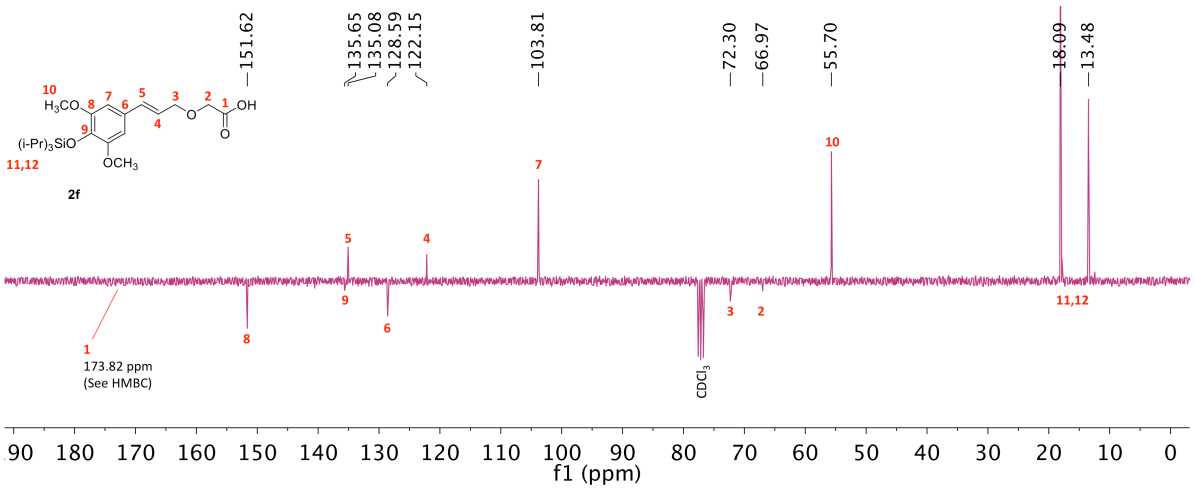
Intermediate compound **2e** – MALDI-ToF MS



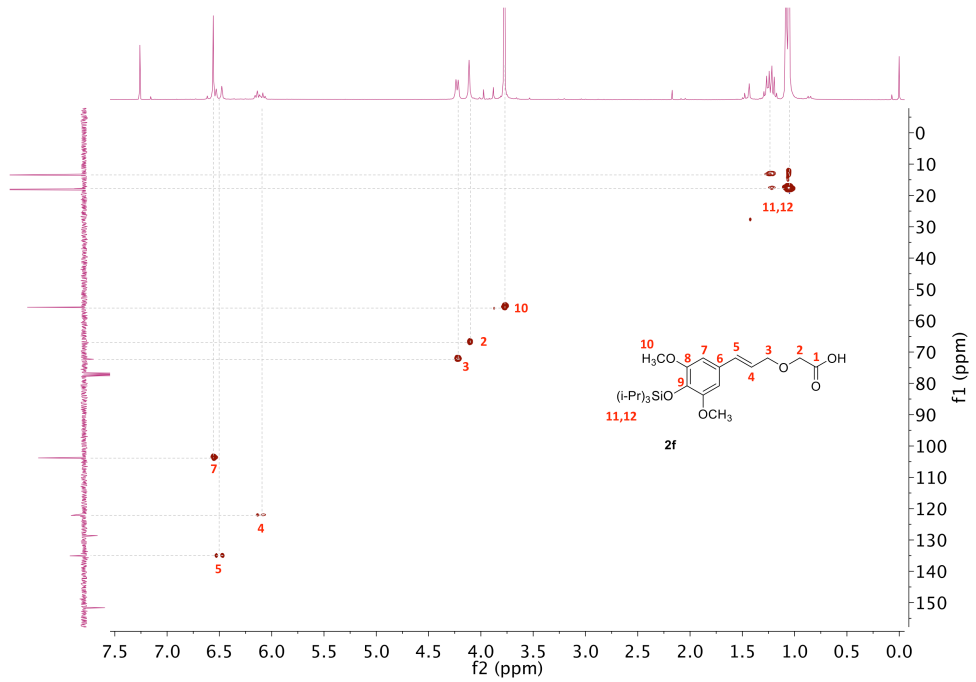
Intermediate compound **2f** – ^{1}H NMR in $CDCl_3$



Intermediate compound **2f** – ^{13}C APT NMR in $CDCl_3$



Intermediate compound **2f** – ^{1}H-^{13}C HSQC NMR in $CDCl_3$



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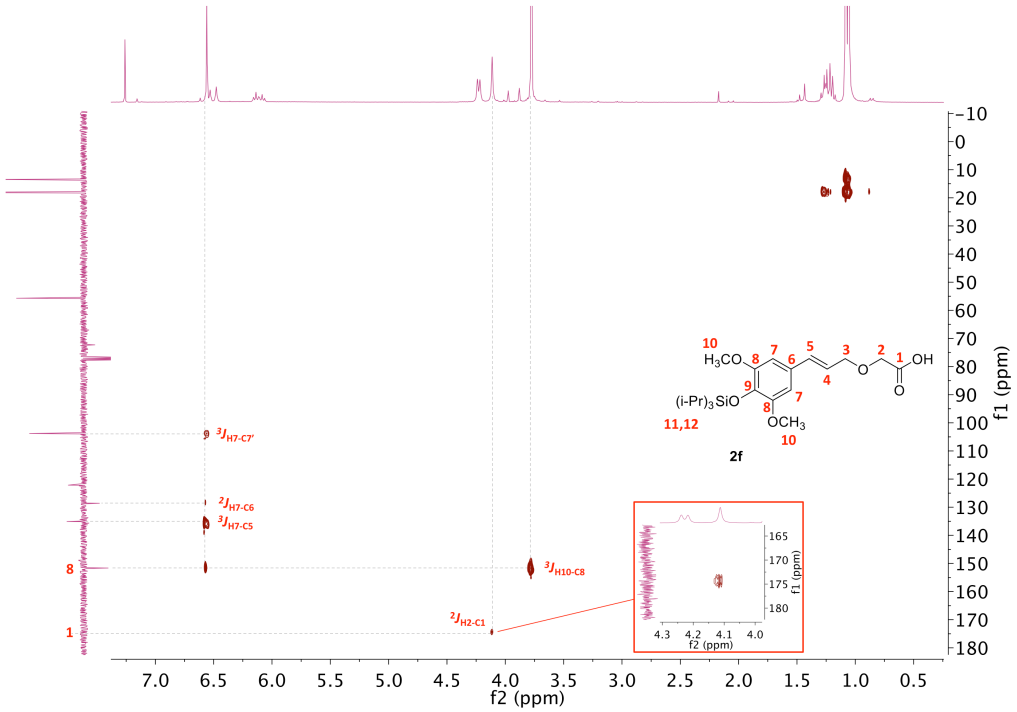
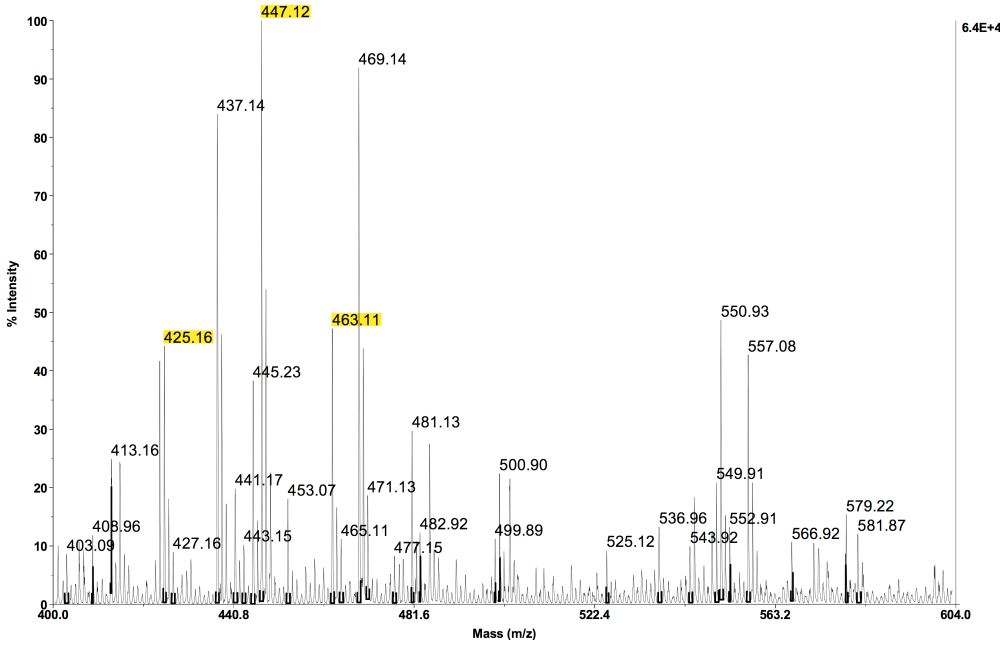
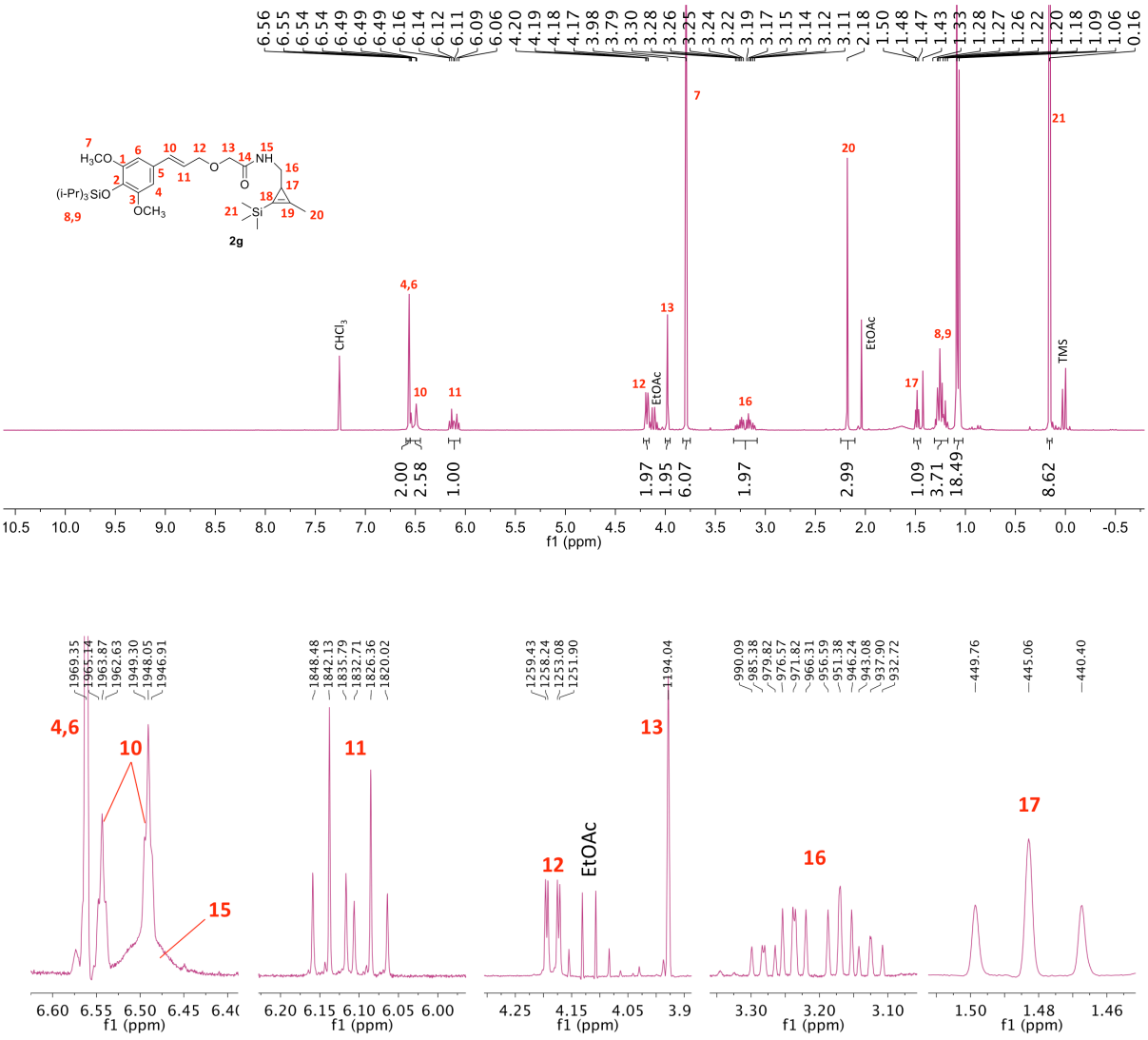


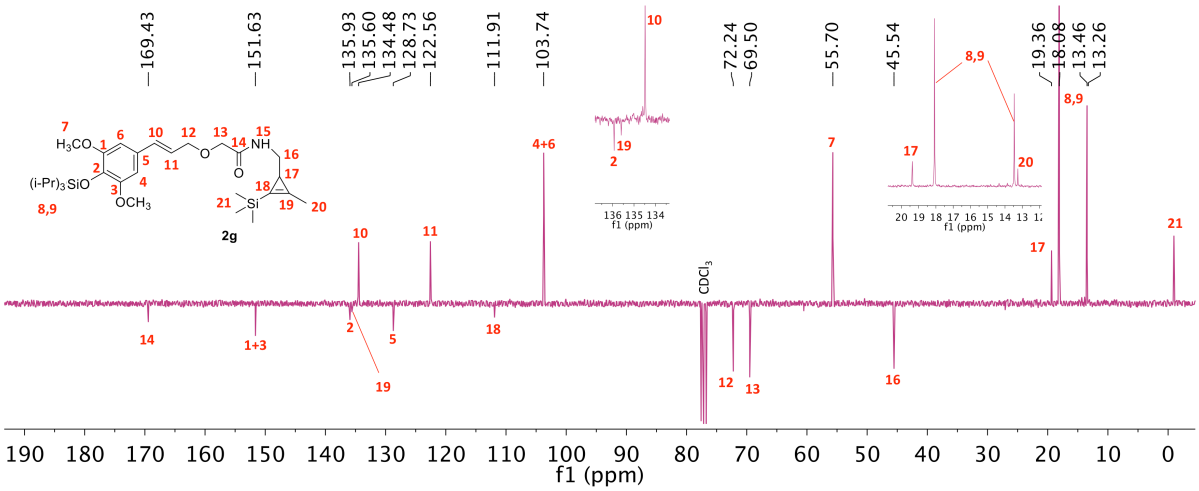
Intermediate compound **2f** – MALDI-ToF MS



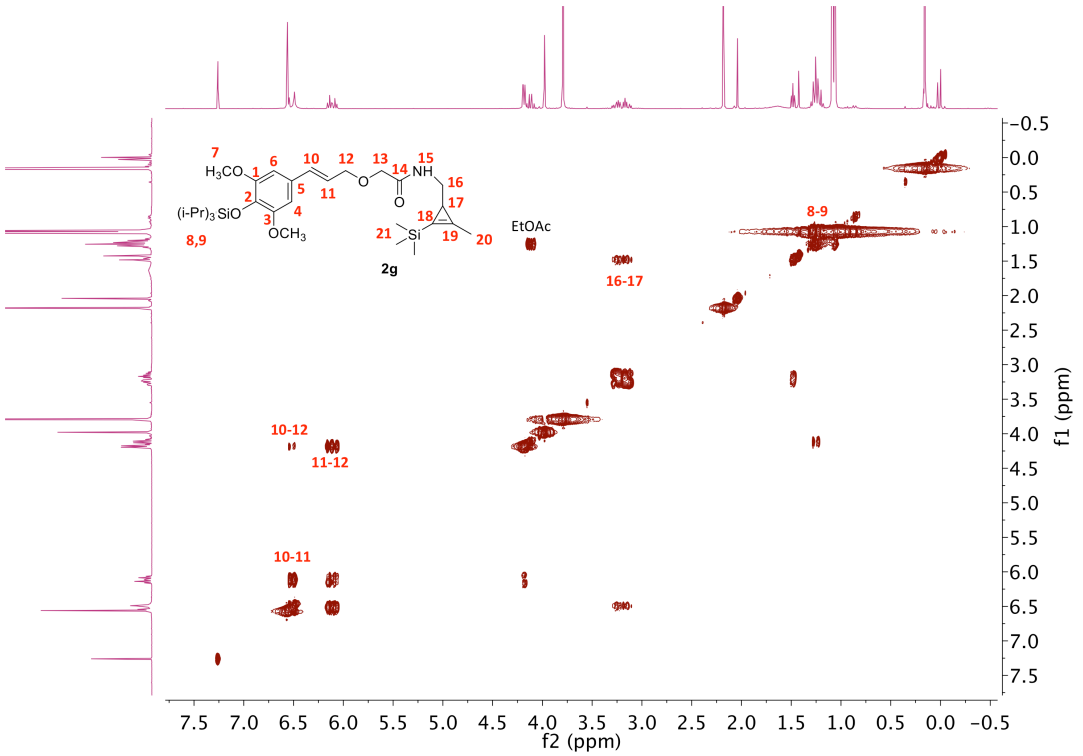
Intermediate compound **2g** – ^{1}H NMR in $CDCl_3$



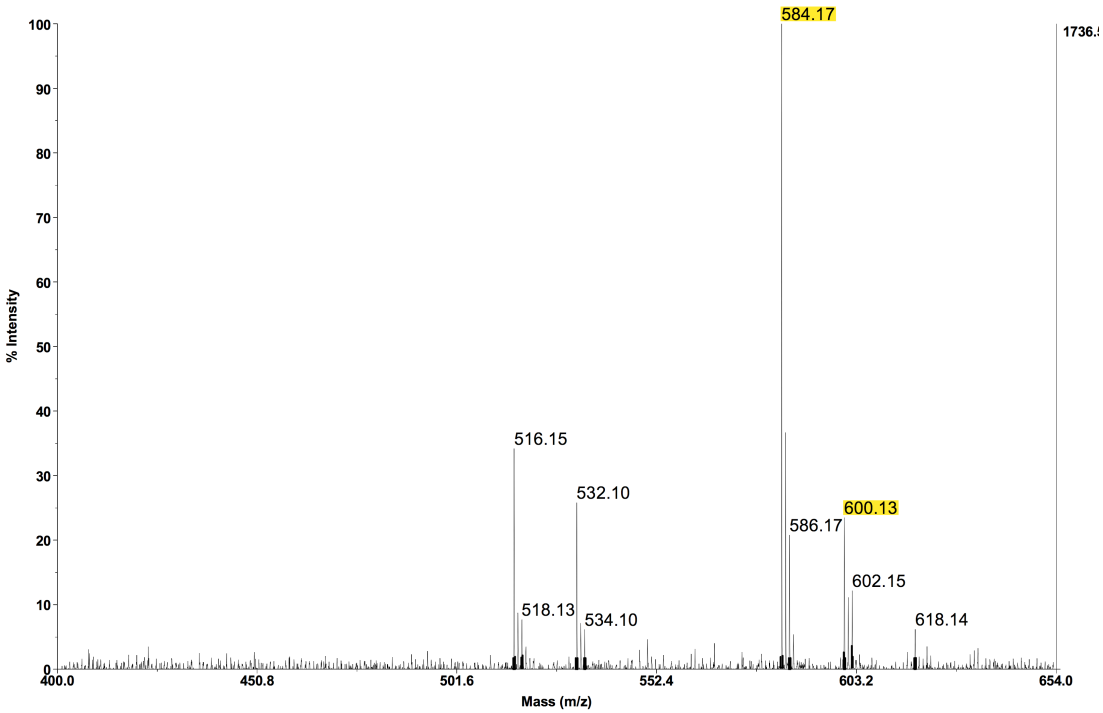
Intermediate compound **2g** – ^{13}C APT NMR in $CDCl_3$



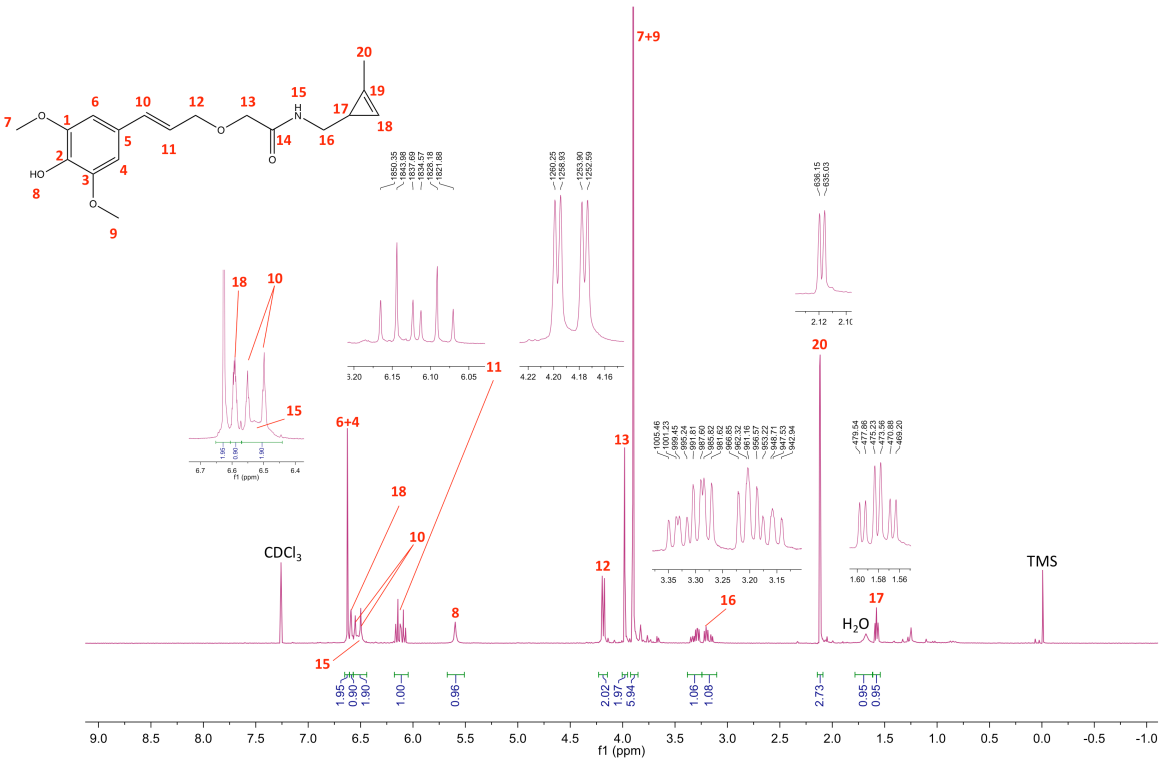
Intermediate compound **2g** – ^{1}H-^{1}H COSY NMR in $CDCl_3$



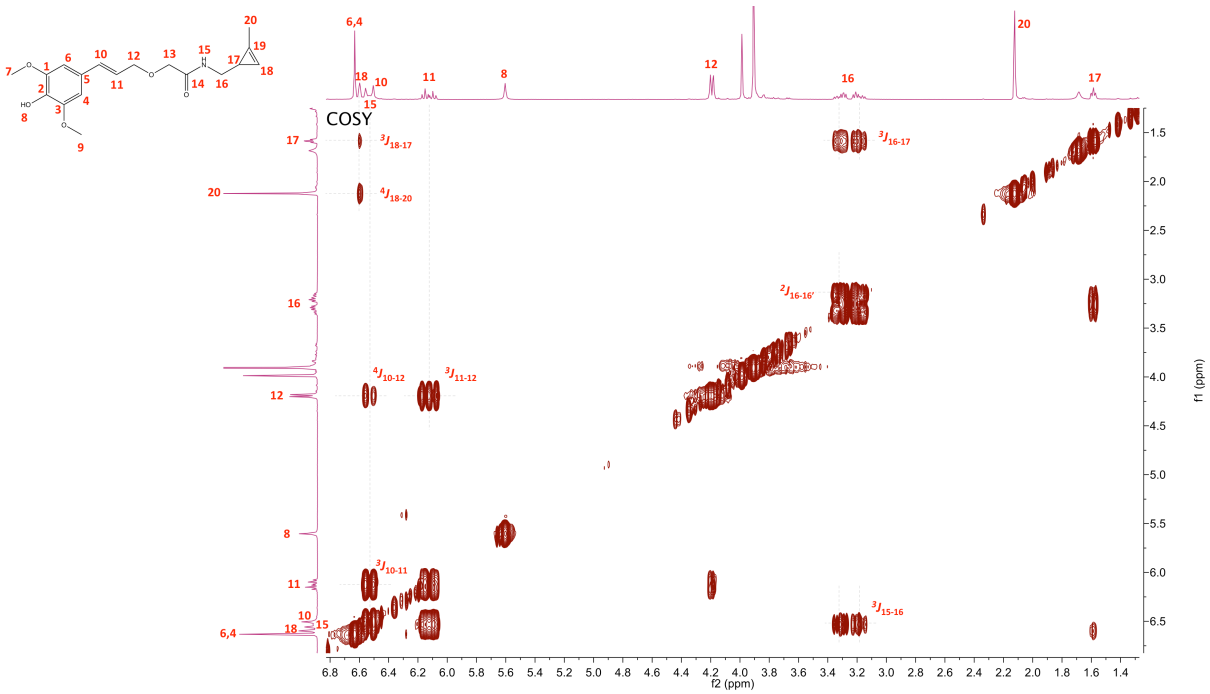
Intermediate compound **2g** – MALDI-ToF MS



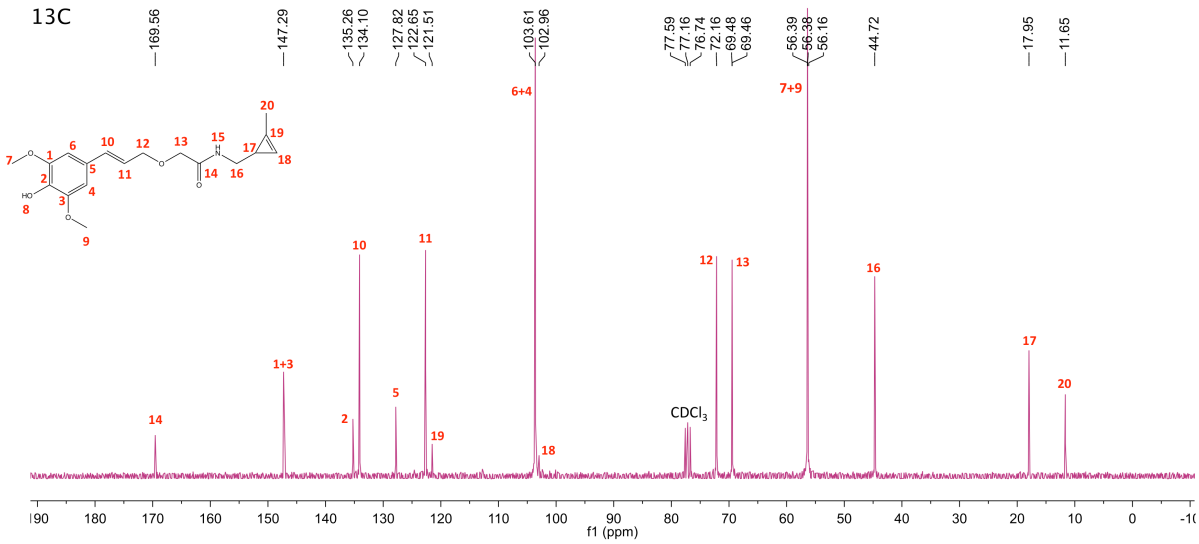
S_{CP} (**2**) – ^{1}H NMR spectrum in $CDCl_3$



S_{CP} (**2**) – ^{1}H-^{1}H COSY NMR spectrum in $CDCl_3$



S_{CP} (**2**) – ^{13}C NMR spectrum in $CDCl_3$



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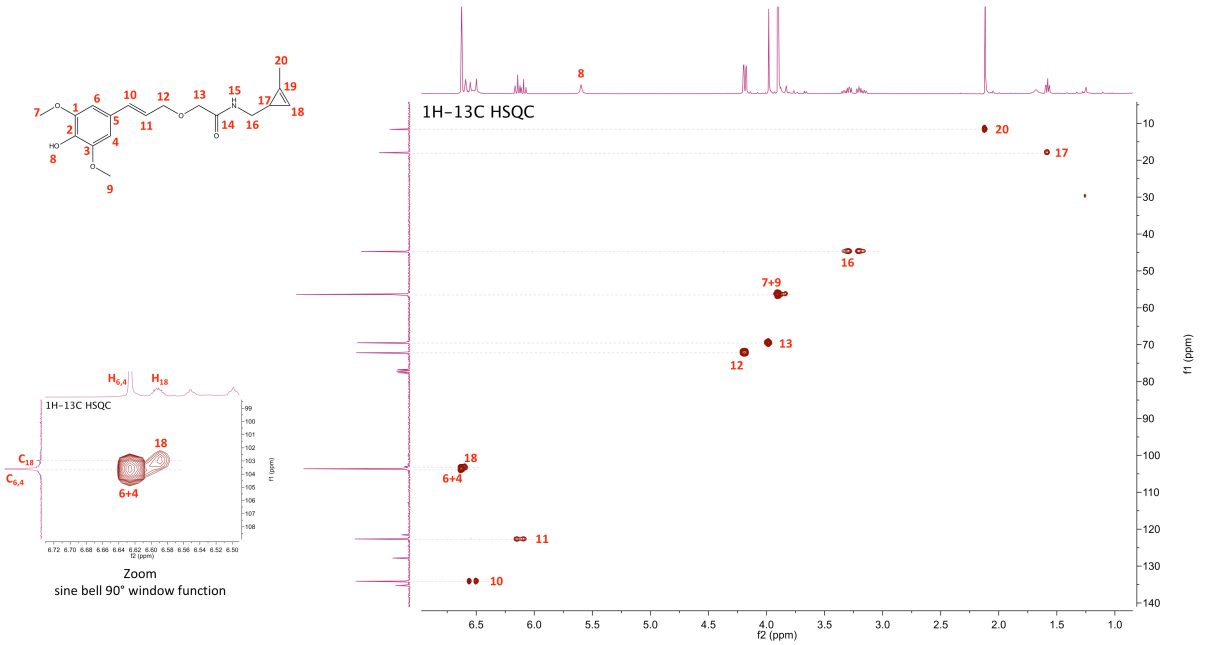
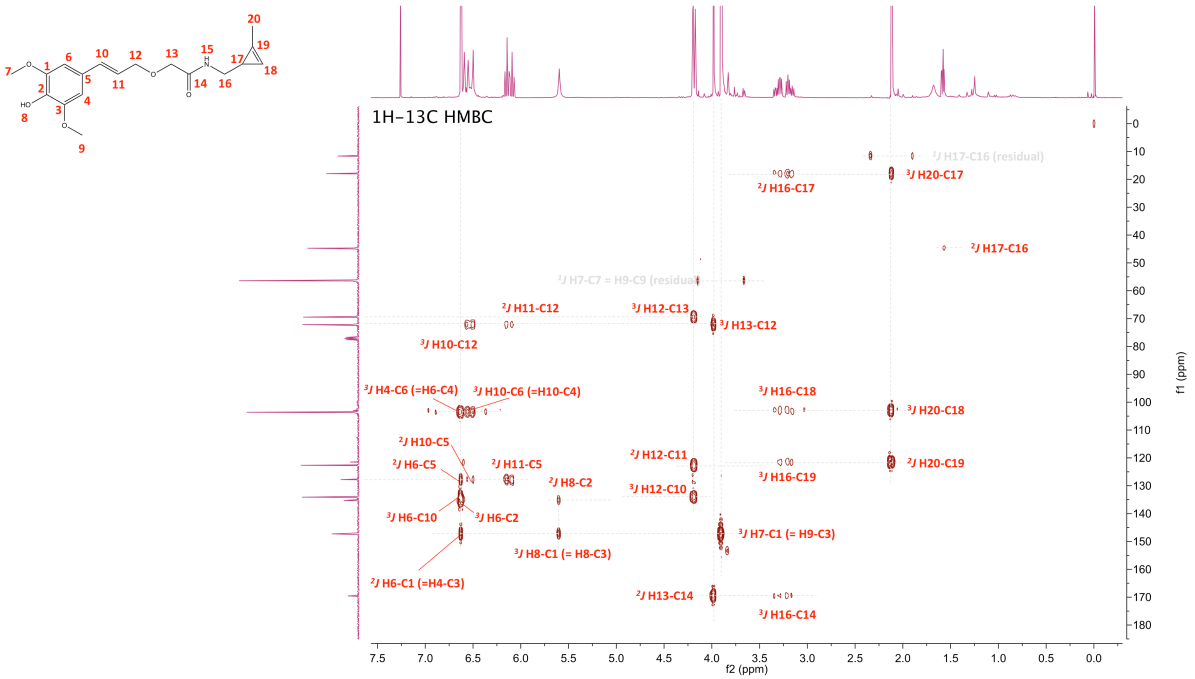
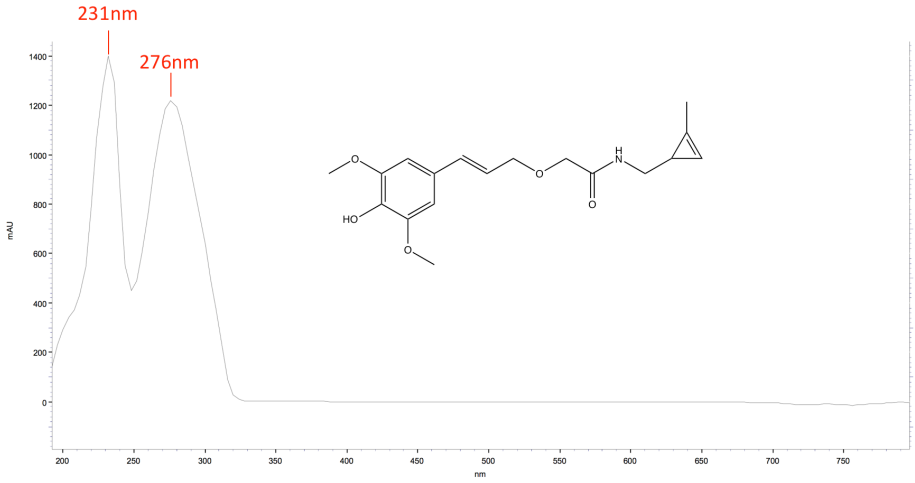


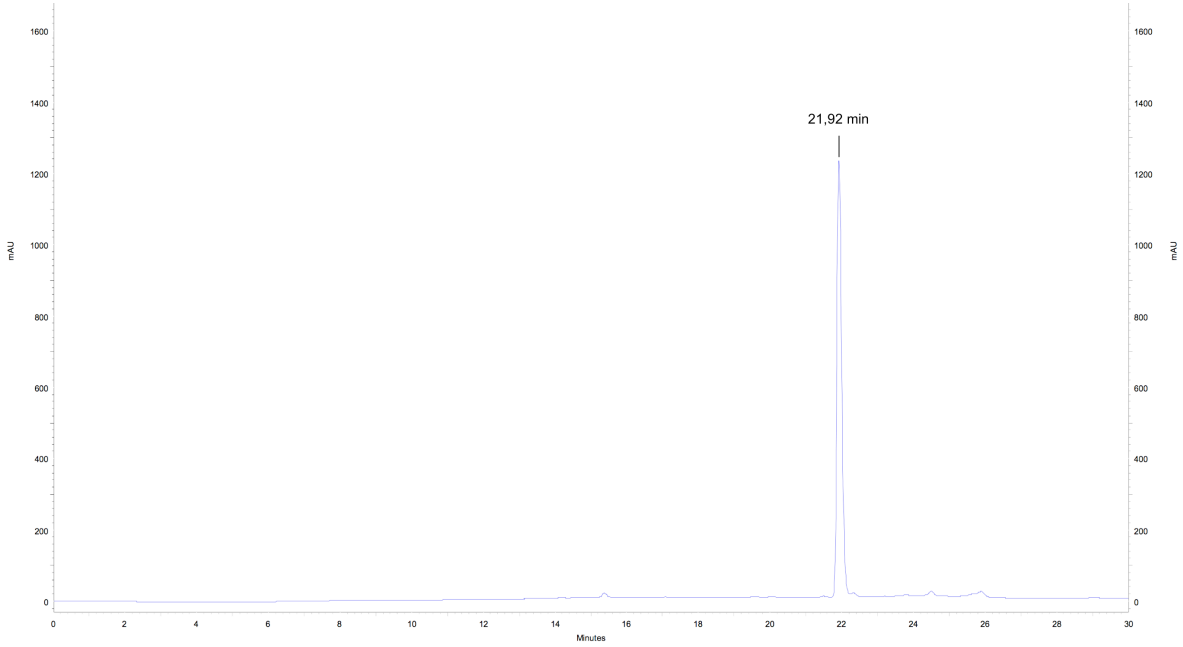
S_{CP} (**2**) – ^{1}H-^{13}C HMBC NMR spectrum in $CDCl_3$



S_{CP} (**2**) – UV spectrum (5mM solution in H_2O/MeOH 9:1)



S_{CP} (**2**) – HPLC-UV chromatogram (detection: 254 nm)

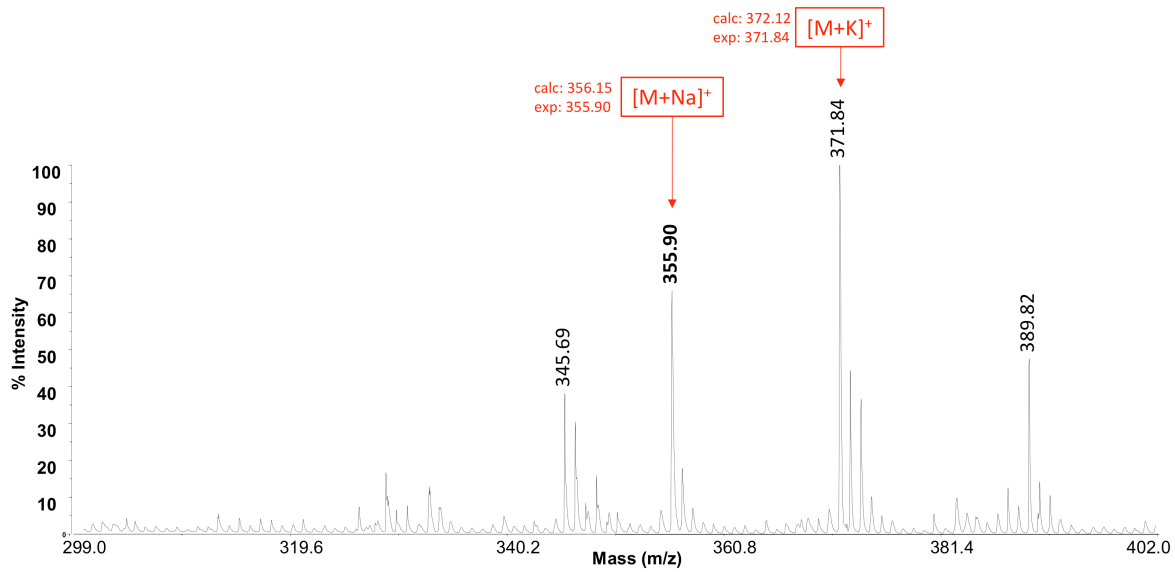


20 µL of a 5 mM solution of **2** in H_2O/MeOH 9:1 were injected in a Thermo Finnigan SCM1000 system equipped with a Grace 5 µm Alltima C_{18} column and a UV6000LP detector. Data were processed in the ChromQuest software.

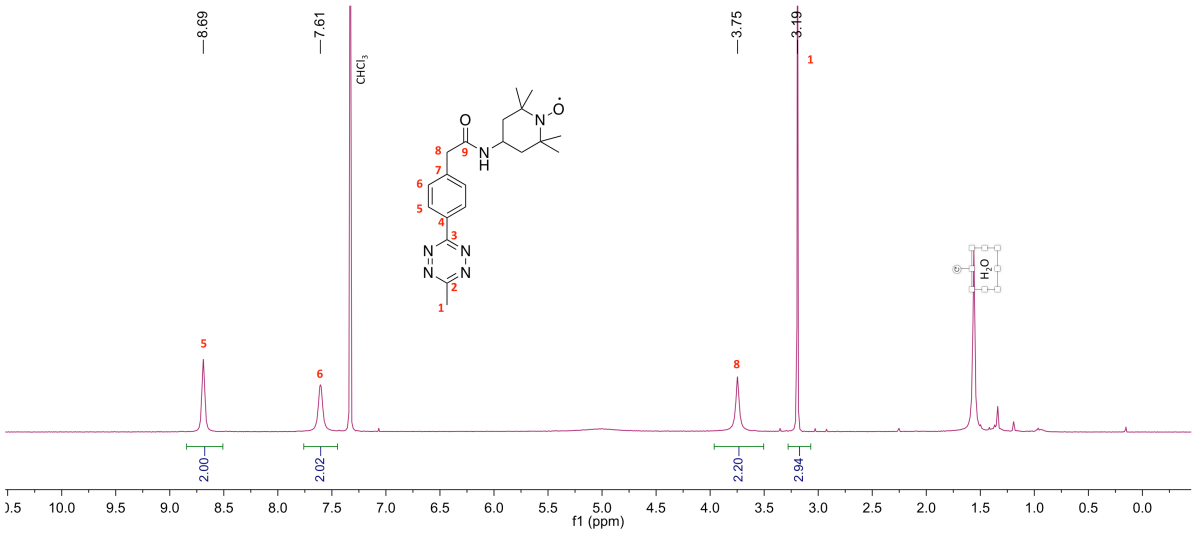
Eluant A: H_2O + 0.1% TFA Eluant B: MeOH/H_2O (90:10) + 0.1% TFA

Elution method : t(0 – 20 min) : gradient A/B 90:10 à A/B 10:90 ; t(20 – 30 min) : isocratic A/B 10:90

S_{CP} (2) MALDI-Tof MS (matrix: 2,5-dihydroxybenzoic acid)

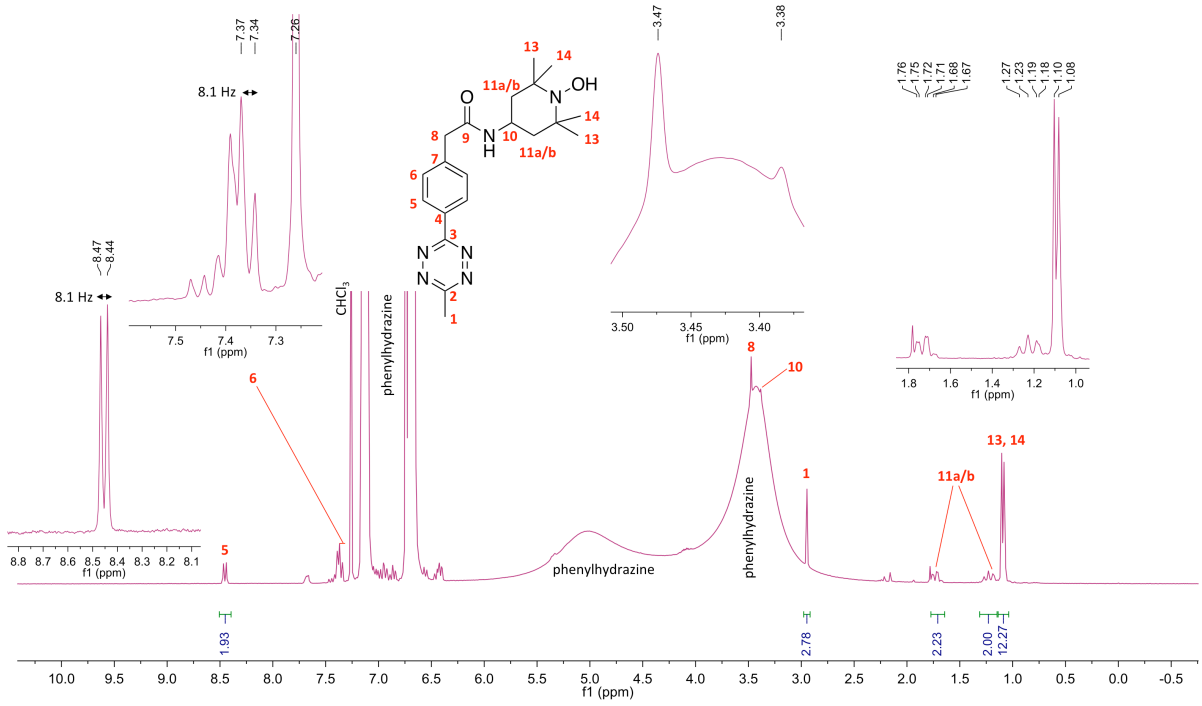


MeTz-TEMPO **3** – ^{1}H NMR in $CDCl_3$ *



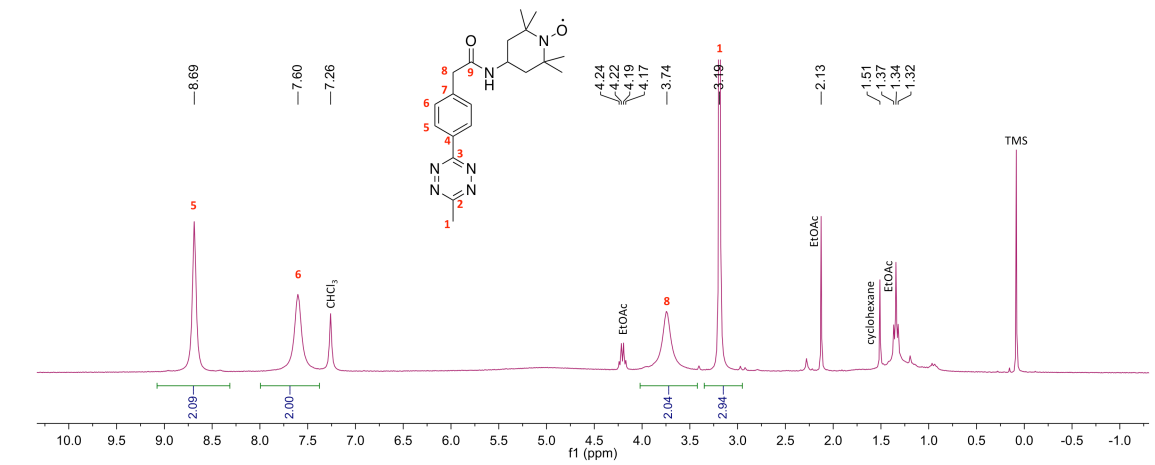
* *Paramagnetic broadening of the NMR signals occurs, due to the nitroxide free radical. Signals corresponding to hydrogens that are close to the radical centre (a few Angströms, TEMPO ring) cannot be observed while hydrogens that are further away are less broadened and can be detected. See ^{13}C and 2D data on the following page for full interpretation.*

MeTz-TEMPO **3** reduced *in situ* by a phenylhydrazine excess – ^{1}H NMR **

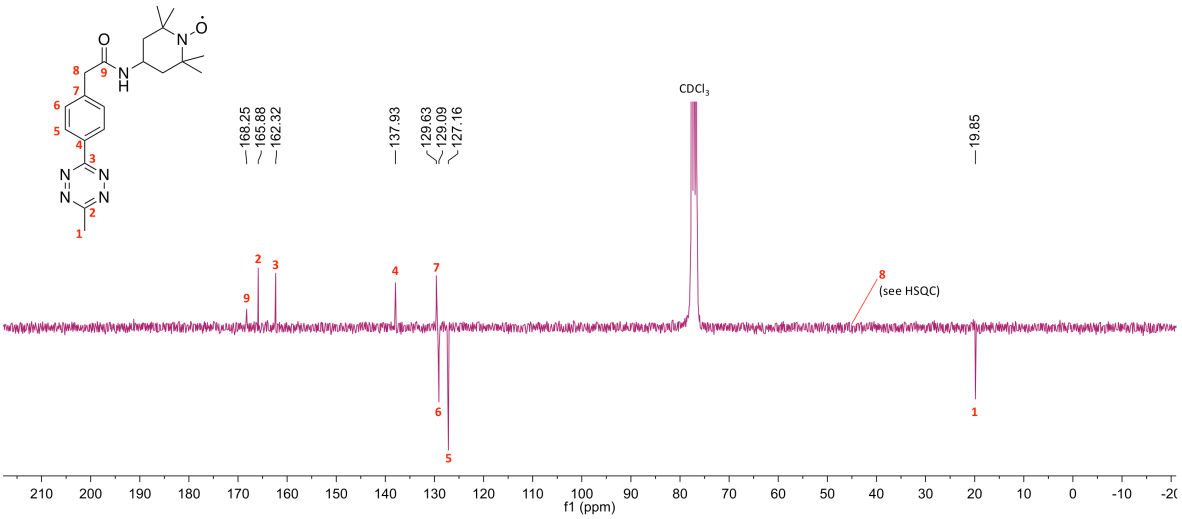


** *Reduction of the nitroxide radical to its hydroxylamine derivative suppresses paramagnetic broadening and restores signals 10, 11a/b, 13 and 14 of the TEMPO ring (which were not observed in the spectra of radical **3** because of the radical). $^{3}J_{ortho\text{-}ortho}$ coupling of the AA'BB' para-substituted phenyl system (5, 6) can now also be measured, even though signal 6 is partially overlapped with ^{13}C satellites peaks of phenylhydrazine. Excess phenylhydrazine was used in situ.*

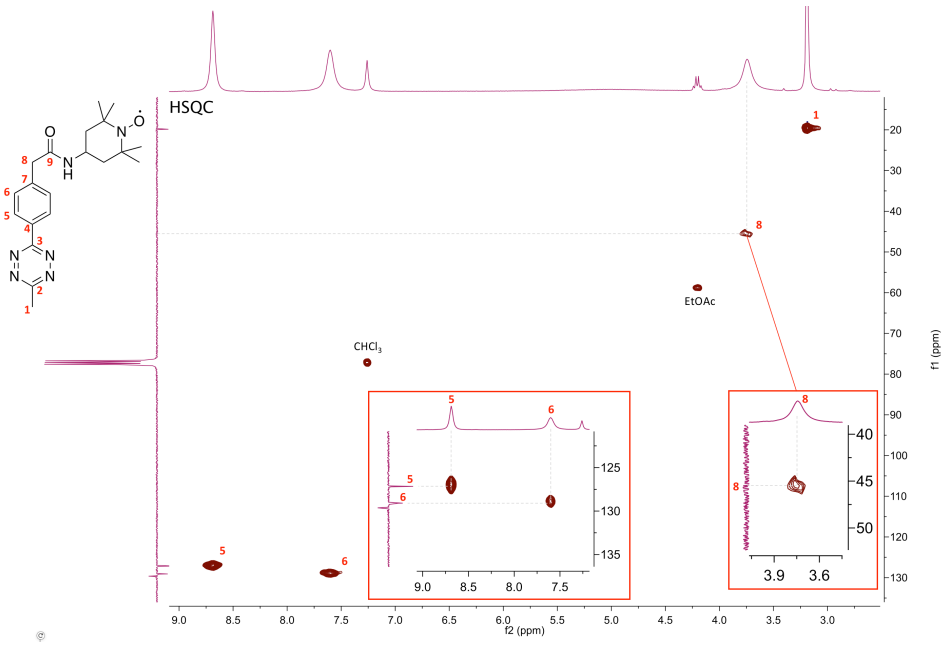
MeTz-TEMPO **3** – ^{1}H NMR in $CDCl_3$



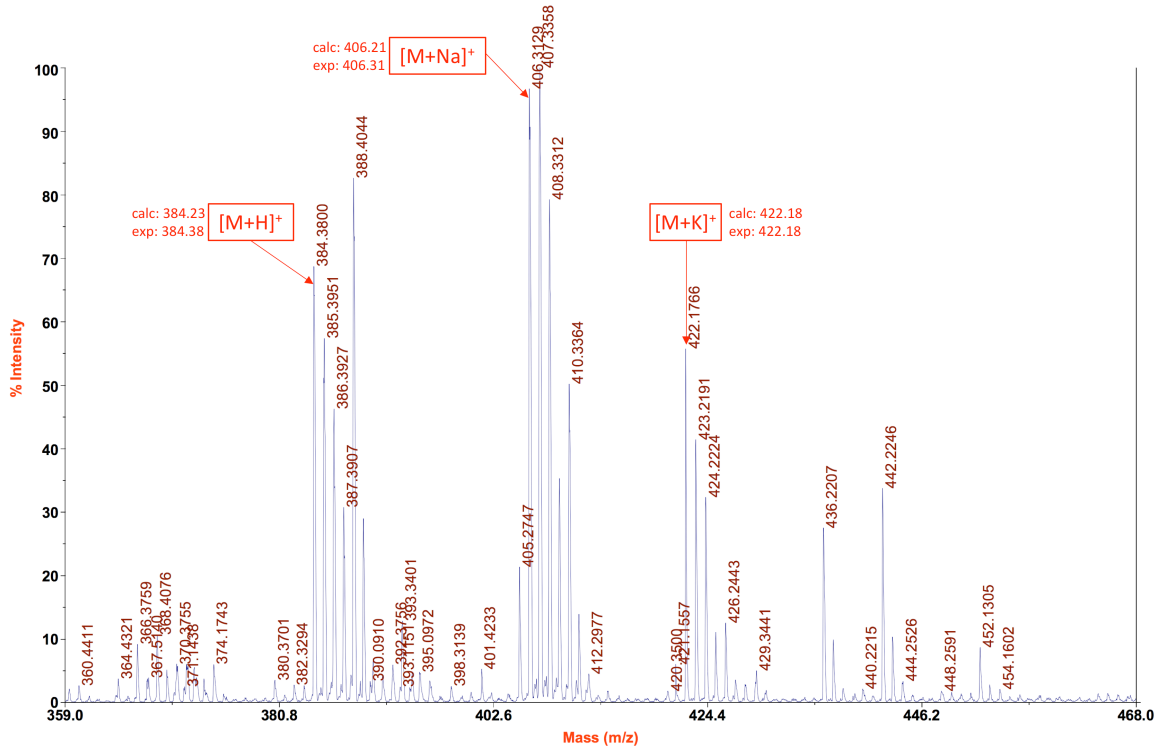
MeTz-TEMPO **3** – ^{13}C NMR in $CDCl_3$



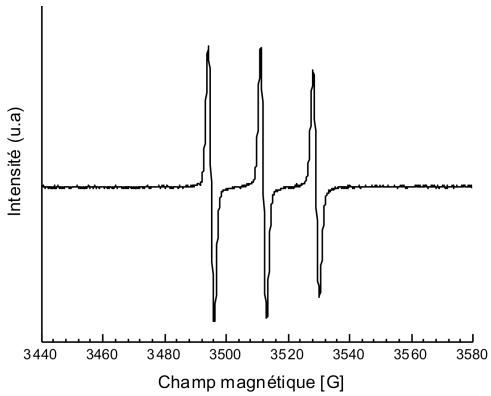
MeTz-TEMPO **3** – ^{1}H-^{13}C HSQC NMR in $CDCl_3$



MeTz-TEMPO **3** - MALDI-ToF MS



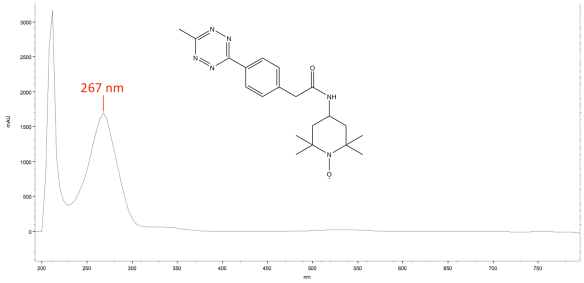
MeTz-TEMPO **3** – CW-EPR spectrum (power = 0.6325 mW ; amplitude modulation = 2G)



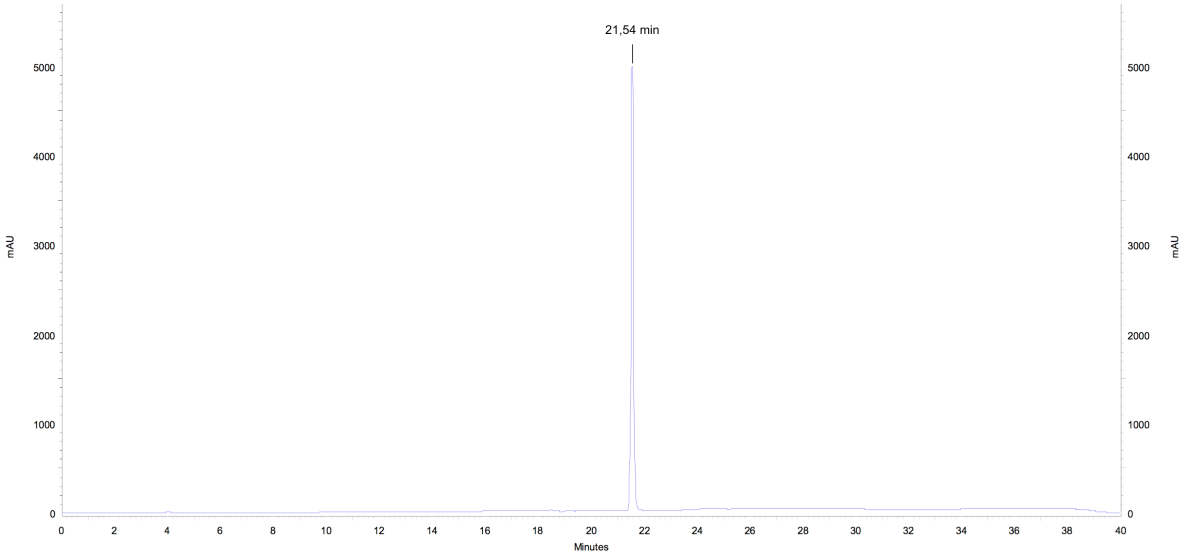
g = 2,0056

hyperfin A = 16,99

MeTz-TEMPO **3** – UV spectrum (5mM solution in H_2O/MeOH 9:1)



MeTz-TEMPO **3** – HPLC-UV chromatogram (detection 254 nm)



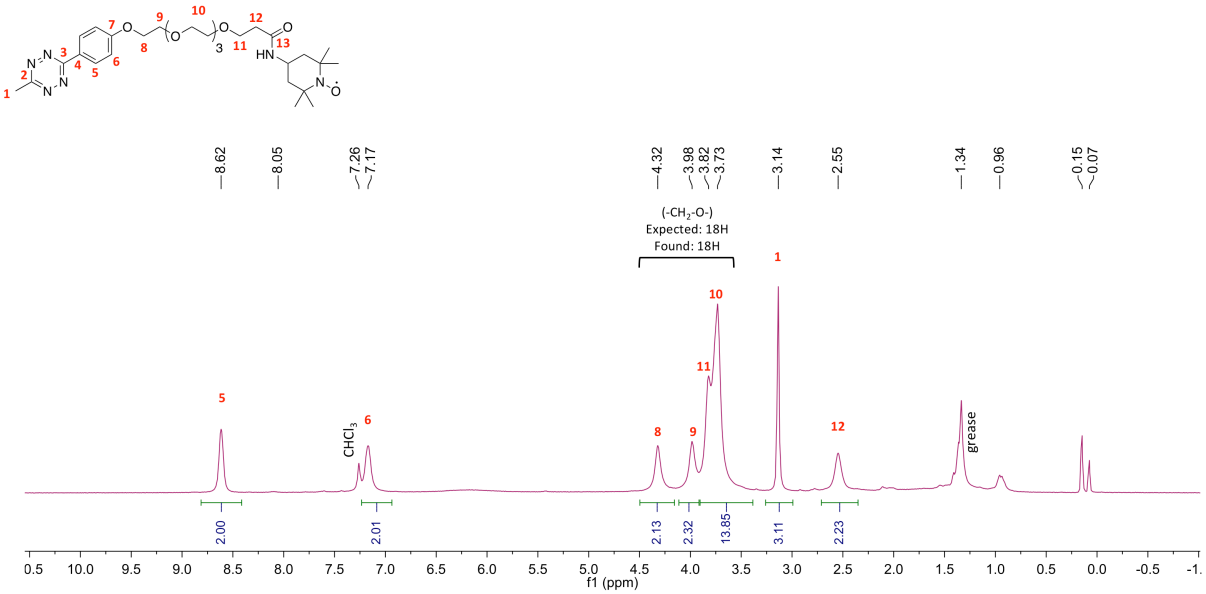
20 µL of a 5 mM solution of **3** in H_2O/MeOH 1:1 were injected in a Thermo Finnigan SCM1000 system equipped with a Grace 5 µm Alltima C_{18} column and a UV6000LP detector. Data were processed in the ChromQuest software.

Eluant A: MeOH/H_2O (10:90) + 0.1% TFA

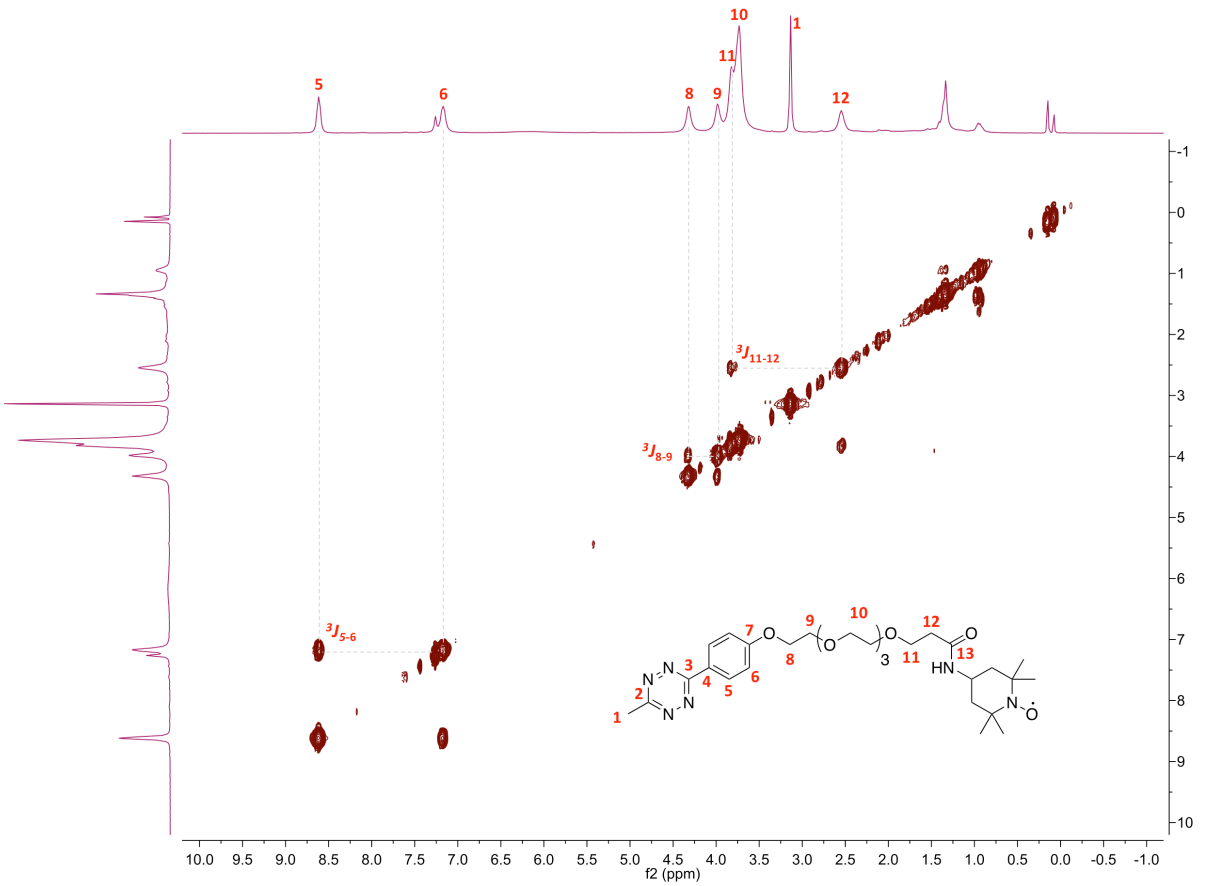
Eluant B: MeOH/H_2O (90:10) + 0.1% TFA

Elution method	t(0 – 20 min) :	gradient A/B 90:10 à A/B 10:90
	t(20 – 40 min) :	isocratic A/B 10:90

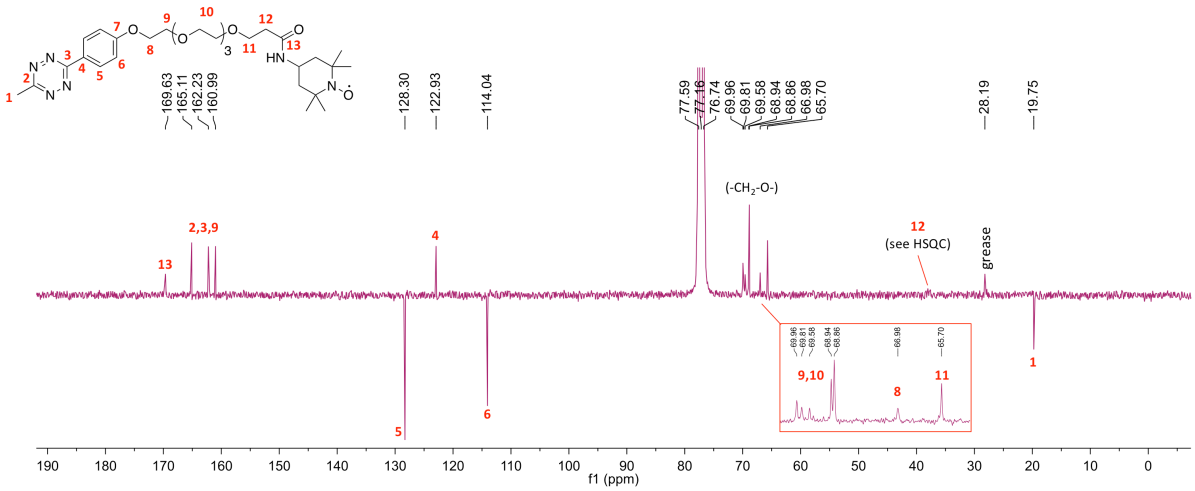
MeTz-PEG$_4$-TEMPO **4** – ^{1}H NMR in CDCl$_3$



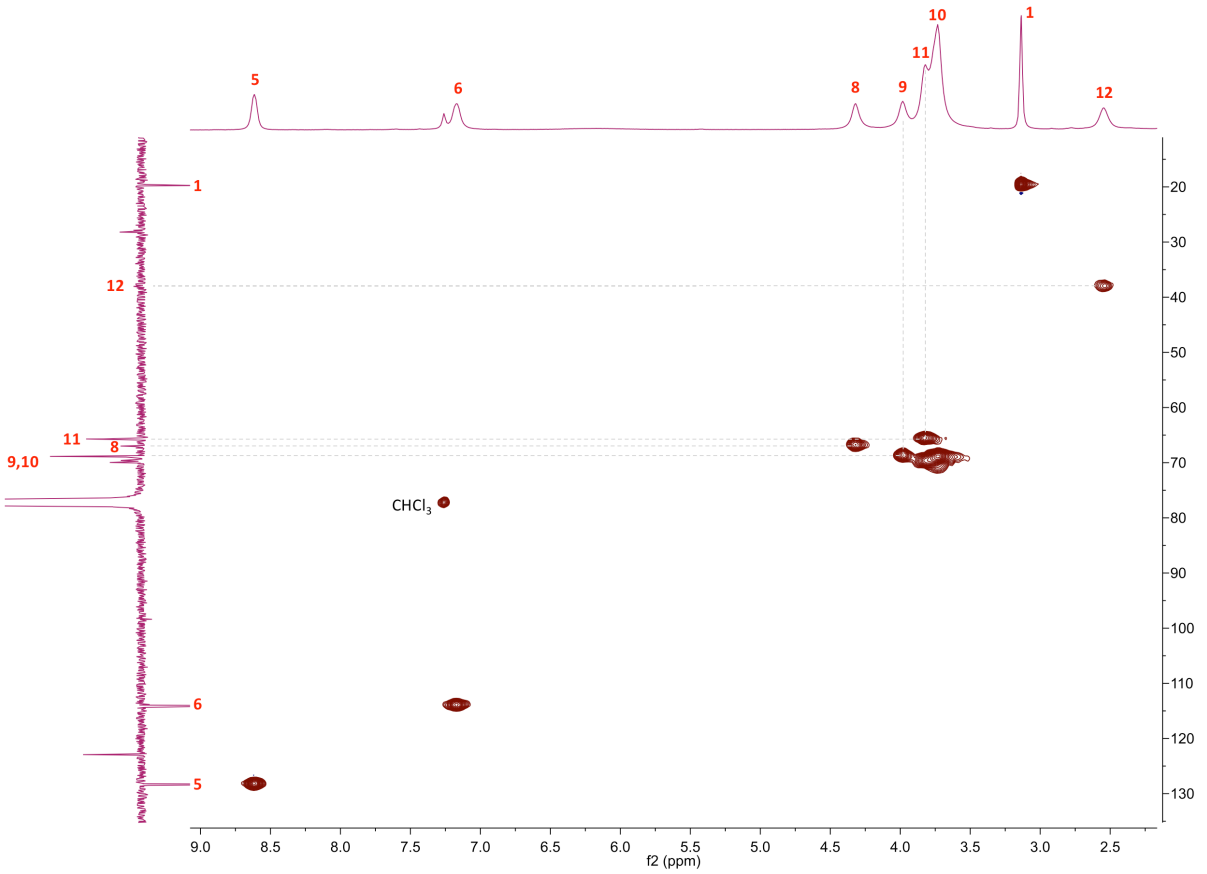
MeTz-PEG$_4$-TEMPO **4** – ^{1}H-^{1}H COSY NMR in CDCl$_3$



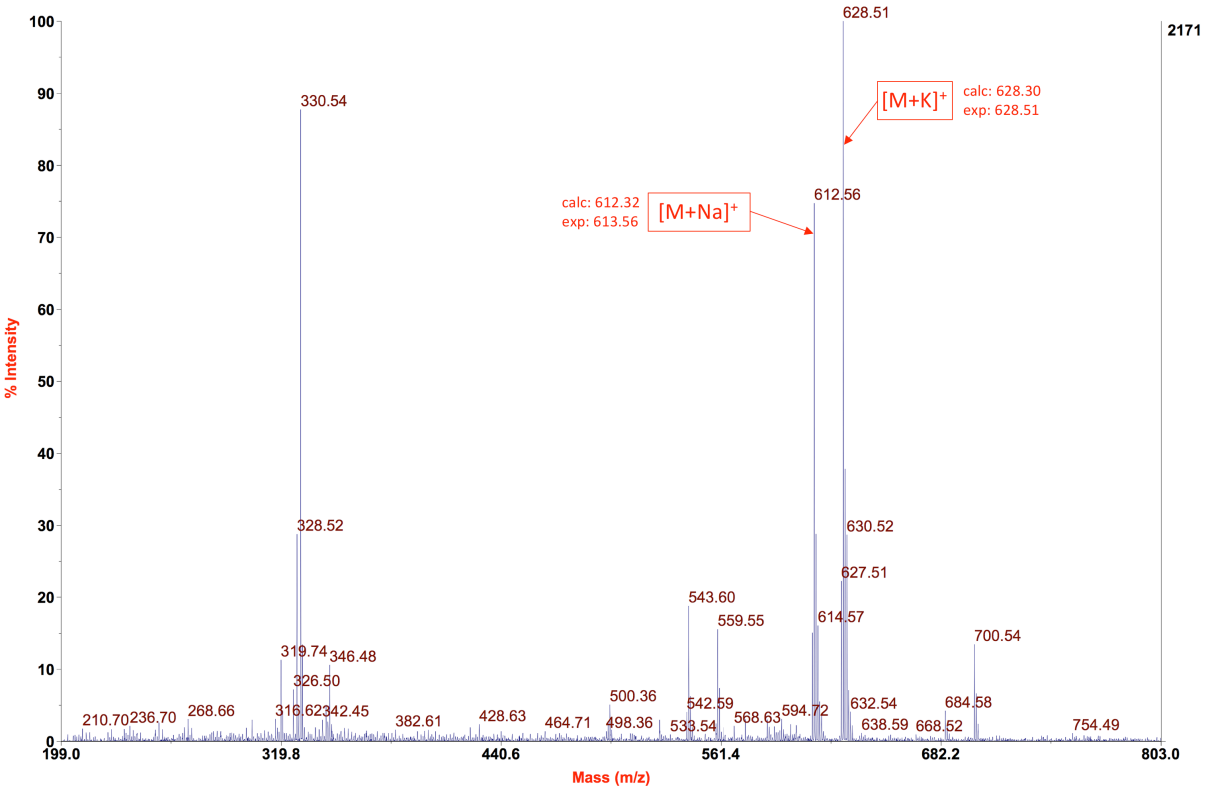
MeTz-PEG$_4$-TEMPO **4** – ^{13}C APT NMR in $CDCl_3$



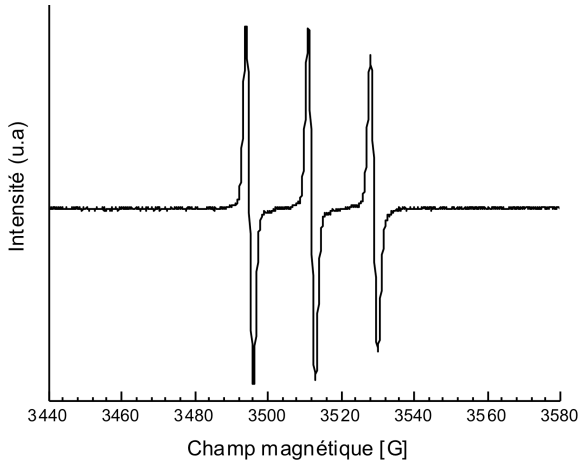
MeTz-PEG$_4$-TEMPO **4** – ^{1}H-^{13}C HSQC NMR in $CDCl_3$



MeTz-PEG$_4$-TEMPO **4** - MALDI-ToF MS



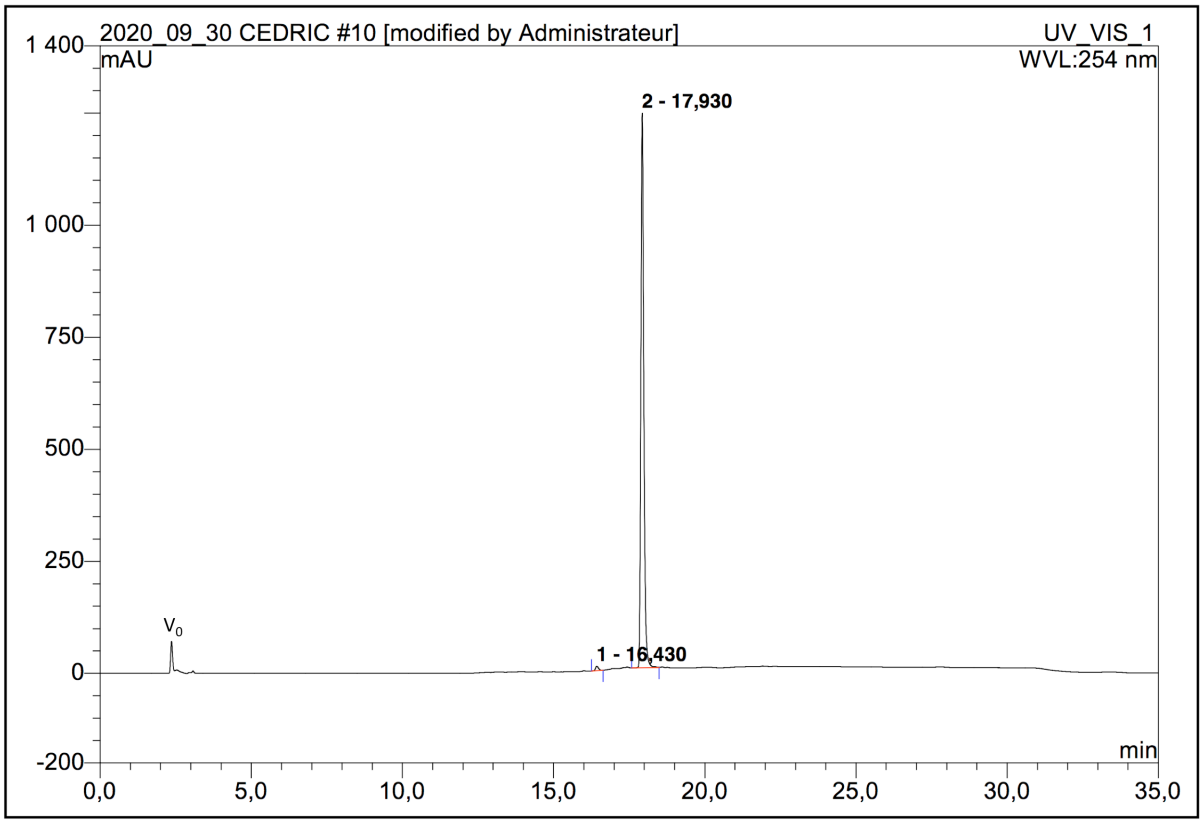
MeTz-PEG$_4$-TEMPO **4** – CW-EPR spectrum (power = 0.6325 mW ; amplitude modulation = 2G)



g = 2,0056

hyperfin A = 17,14

MeTz-PEG$_4$-TEMPO **4** – HPLC-UV chromatogram (detection 254 nm)



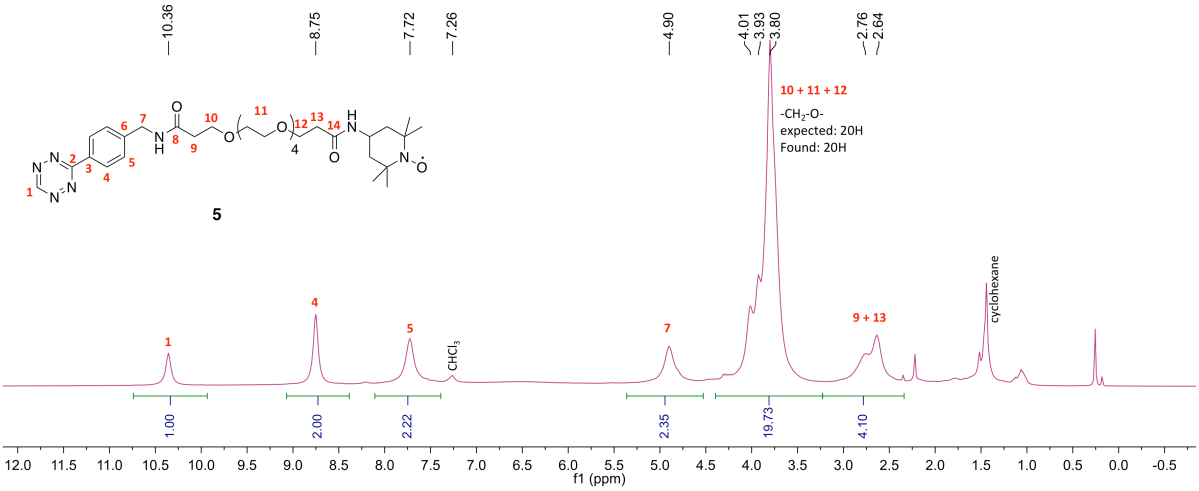
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	16,43	n.a.	9,790	1,121	0,77	n.a.	BMB
2	17,93	n.a.	1237,320	144,300	99,23	n.a.	BMB*
Total:			1247,109	145,421	100,00	0,000	

20 µL of a 4 mM solution of **4** in MeOH were injected in a Dionex UltiMate 3000 HPLC system equipped with a Restek UltraC18 150 mm x 3 mm, 3 µm beads C_{18} column and a DAD-3000 diode array detector. Data were processed in the chromeleon software suite.

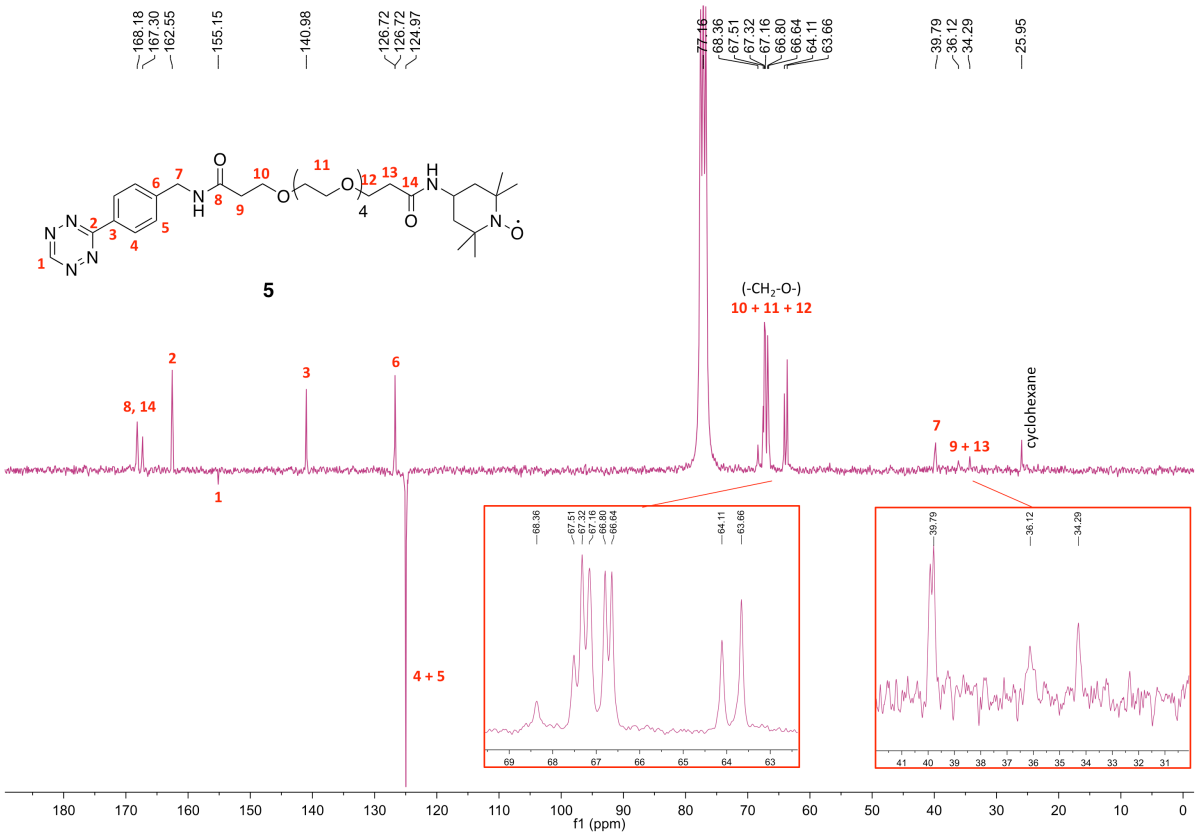
Eluant A: MeCN/H_2O (5:95) + 0.1% TFA Eluant B: MeCN/H_2O (95:5) + 0.1% TFA

Elution method: t(0 – 25 min) : gradient A/B 90:10 à A/B 10:90
t(25 – 35 min) : isocratic A/B 10:90

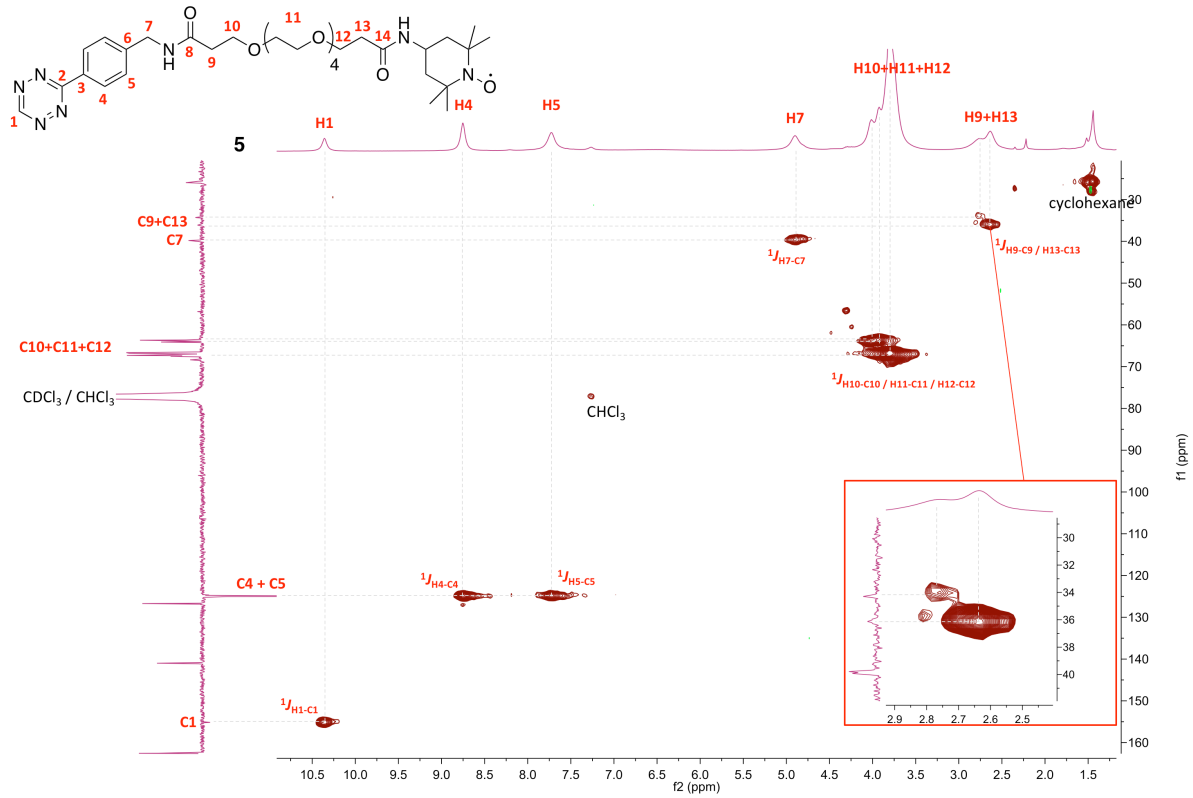
HTz-PEG$_5$-TEMPO **5** – ^{1}H NMR in CDCl$_3$



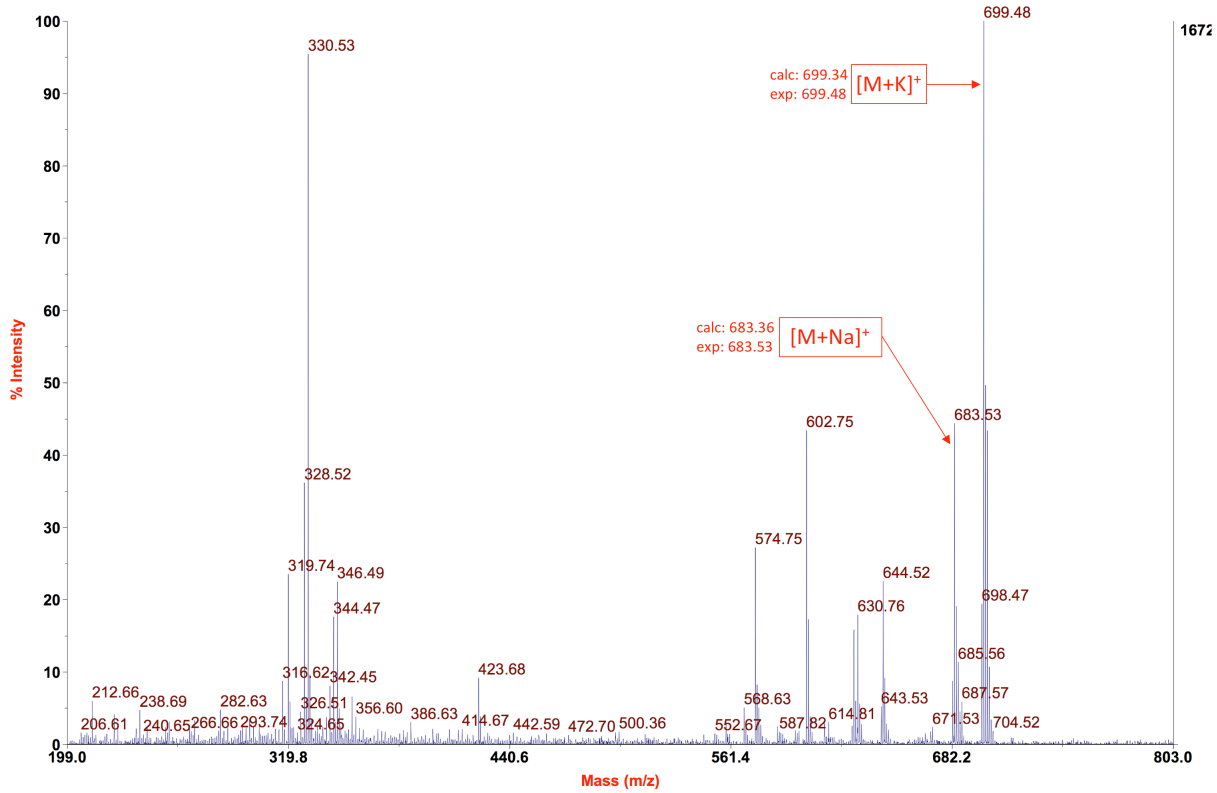
HTz-PEG$_5$-TEMPO **5** – ^{13}C APT NMR in CDCl$_3$



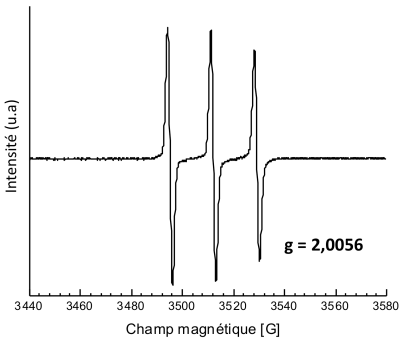
HTz-PEG$_5$-TEMPO **5** – ^{1}H-^{13}C HSQC NMR



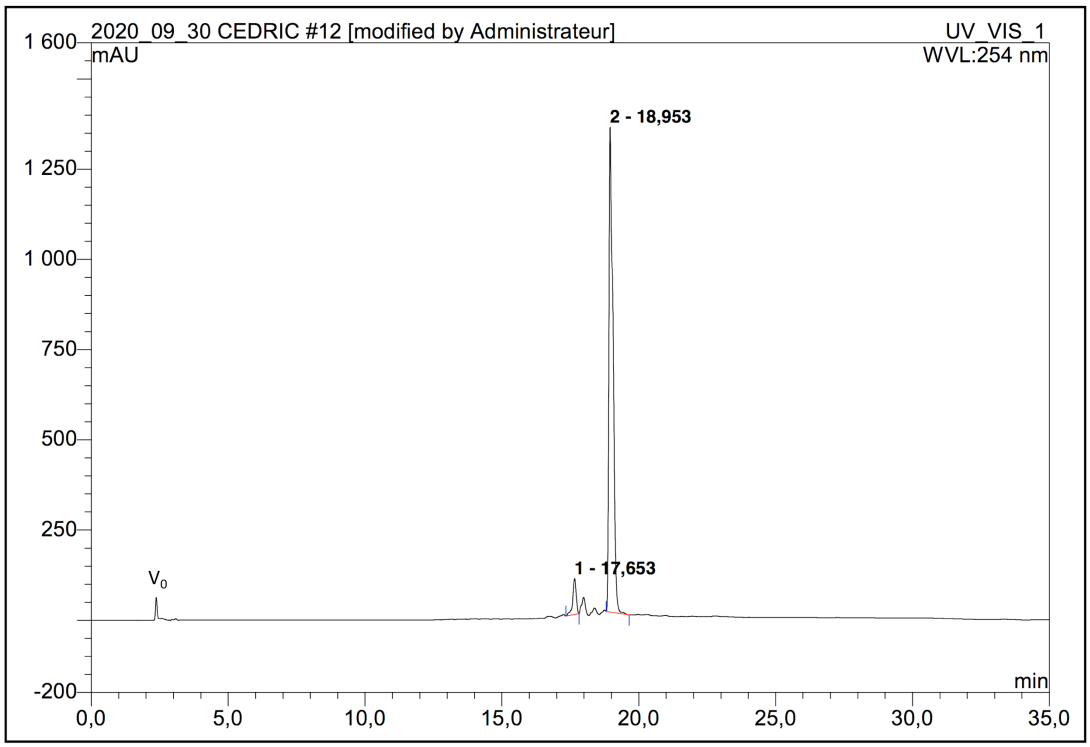
HTz-PEG$_5$-TEMPO **5** – MALDI-ToF MS



HTz-PEG$_5$-TEMPO **5** – CW-EPR spectrum (power = 0.6325 mW ; amplitude modulation = 2G)



HTz-PEG$_5$-TEMPO **5** – HPLC-UV chromatogram (detection 254 nm)



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	17,65	n.a.	99,768	14,719	5,93	n.a.	BMB*
2	18,95	n.a.	1343,593	233,668	94,07	n.a.	BMB*
Total:			1443,361	248,387	100,00	0,000	

20 µL of a 4 mM solution of **5** in MeOH were injected in a Dionex UltiMate 3000 HPLC system equipped with a Restek UltraC18 150 mm x 3 mm, 3 µm beads C_{18} column and a DAD-3000 diode array detector. Data were processed in the Chromeleon software suite.

Eluant A: MeCN/H_2O (5:95) + 0.1% TFA Eluant B: MeCN/H_2O (95:5) + 0.1% TFA

Elution method: t(0 – 25 min) : gradient A/B 90:10 à A/B 10:90
t(25 – 35 min) : isocratic A/B 10:90

References

1 T. Murashige and F. Skoog, *Physiol. Plant.*, 1962, **15**, 473–497.
2 S. Stoll and A. Schweiger, *J. Magn. Reson.*, 2006, **178**, 42–55.