Synthesis of fluorinated curcumin derivatives for detecting amyloid plaques by ^{19}F-MRI

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1. General methods

1.1. NMR spectroscopy

^{1}H-NMR and heterocorrelated 2D ^{1}H,^{13}C NMR spectra were acquired with a Bruker Avance spectrometer operating at 14 T (corresponding to Larmor frequencies of 600 and 150 MHz for ^{1}H and ^{13}C, respectively), equipped with an inverse Z-gradient 5 mm BBI probe. The sample temperature was set to 300 K and controlled within ±0.1K. Samples were dissolved in 600 µL of dmso-d_6. ^{1}H and ^{13}C-NMR resonance assignment was carried out by the analysis of 2D-COSY, 2D-ROESY, 2D ^{1}H,^{13}C HSQC and 2D ^{1}H,^{13}C HMBC NMR spectra. 2D-COSY NMR spectra were acquired in the phase insensitive mode with gradient selection (Bruker pulse program cosygpqf), with 1.5 s relaxation delay, 8 scans, 16 dummy scans, and 2048×200 time data points. Data were treated with sine window functions (both along F2 and F1) prior to FT in magnitude mode. 2D-ROESY NMR spectra were acquired in the phase-sensitive mode according to the States-TPPI scheme (Bruker pulse program roesyph.2) with 2 s relaxation delay, 32 scans, 16 dummy scans, 2048×256 data points, and a 300 µs pulse for ROESY spinlock. Data were treated with squared cosine window functions (both along F2 and F1) prior to FT and phase correction. 2D-^{1}H,^{13}C-HSQC NMR spectra were acquired in the phase sensitive mode using echo/antiecho-TPPI gradient selection, with decoupling during acquisition (Bruker pulse program hsqcetgp). Acquisition parameters included 1.5 s relaxation delay, 32 scans, 16 dummy scans, 1024×256 data points, and 145 Hz for $^{1}J_{CH}$. Data were treated with squared cosine window functions (both along F2 and F1) prior to FT and phase correction. 2D ^{1}H,^{13}C HMBC NMR spectra were acquired in the phase insensitive mode using gradients for selection, with low-pass J-filter to suppress one-bond correlations and no decoupling during acquisition (Bruker pulse program hmbcgplpndqf). Acquisition parameters included 1.5 s relaxation delay, 32 scans, 16 dummy scans, 2048×128 data points, 145 Hz for $^{1}J_{CH}$ and 8 Hz for long range couplings. Data were treated with sine window functions (both along F2 and F1) prior to FT in the magnitude mode. ^{19}F-NMR spectra were acquired with a Bruker Avance Neo 500 MHz spectrometer operating at 11.7 T (corresponding to a ^{19}F Larmor frequency of 470 MHz) equipped with a SmartProbe, at a temperature of 298±0.1 K. Samples were

dissolved in a mixture of 550 μL of ethanol and 50 μL dmso-d_6. All NMR spectra were processed by the Bruker Topspin 4.0.7 software package.

1.2. qNMR

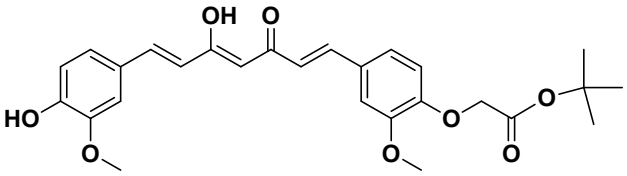
For in vitro plaque binding assays, stock solutions of the ^{19}F-labelled curcumin probes were prepared by dissolving the required mass of the probe into 5-6 mL of analytical grade ethanol to obtain a theoretical concentration in the 0.5-3.0 mM range. The actual concentration of the probe in these stock solutions was checked by ^{19}F qNMR. An analytical standard sample was prepared by dissolving an accurately weighted mass of ultrapure trifluoroethanol (TFE) into ethanol, to have an 8.92 mM stock solution. Three-hundred μL of such stock solution were mixed with 300 μL of diluent (ethanol/dmso-d6 5:1 volume ratio) and transferred into a 5 mm NMR tube. Likewise, 300 μL of the stock solution of each of the ^{19}F-labelled curcumin probes were mixed with 300 μL of the diluent. Quantification was performed according to the PULCON principle (Wider G. & Dreier L., J. Am. Chem. Soc., 2006, 128, 2571-2576) as implemented by the ERETIC2 module of the Bruker TopSpin 4.0.7 software. ^{19}F qNMR spectra were acquired on a Bruker Avance Neo 500 NMR spectrometer (see above). Acquisition parameters included: 90° pulse, relaxation delay 30 s, 65536 time domain complex data points, and 4 scans for signal averaging. Exponential multiplication with a line broadening factor of 0.3 Hz was performed prior to Fourier transform and baseline correction.

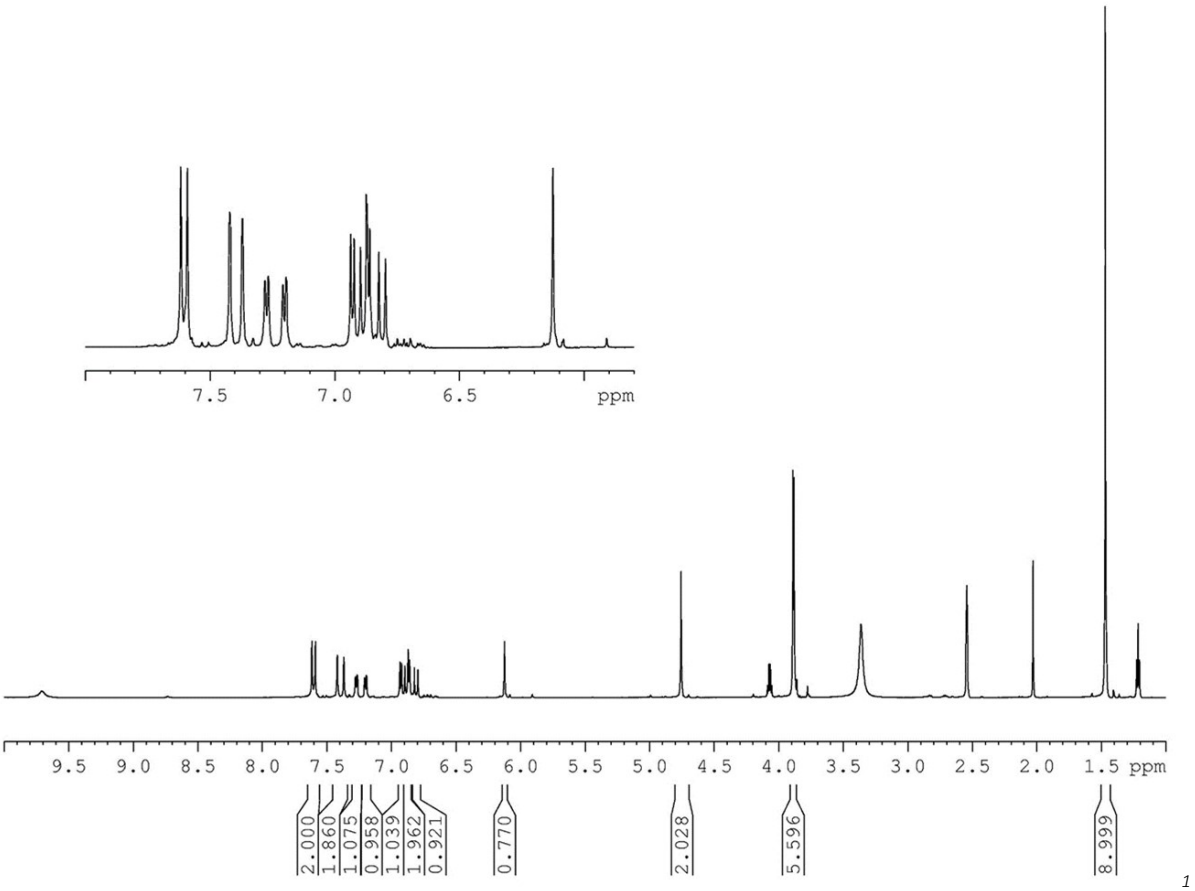
1.3. UPLC-UV-MS

UPLC analysis was performed using a UPLC Acquity H-Class coupled with the QDa and TUV detectors, using Kinetex® F5 column, 1.7 μm, 2.1×100 mm, applying a gradient of CH_3CN (0.05% TFA) in H_2O (0.05% TFA) from 50% to 100% in 8 min and 100% of B in 4 min (0.2 mL min-1), peak area revealed at 210 nm and 430 nm (**method 1**).

2. Characterization of products and intermediates

2.1. Compound 1a.

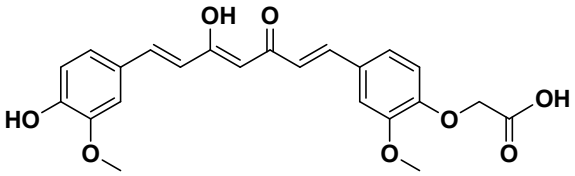


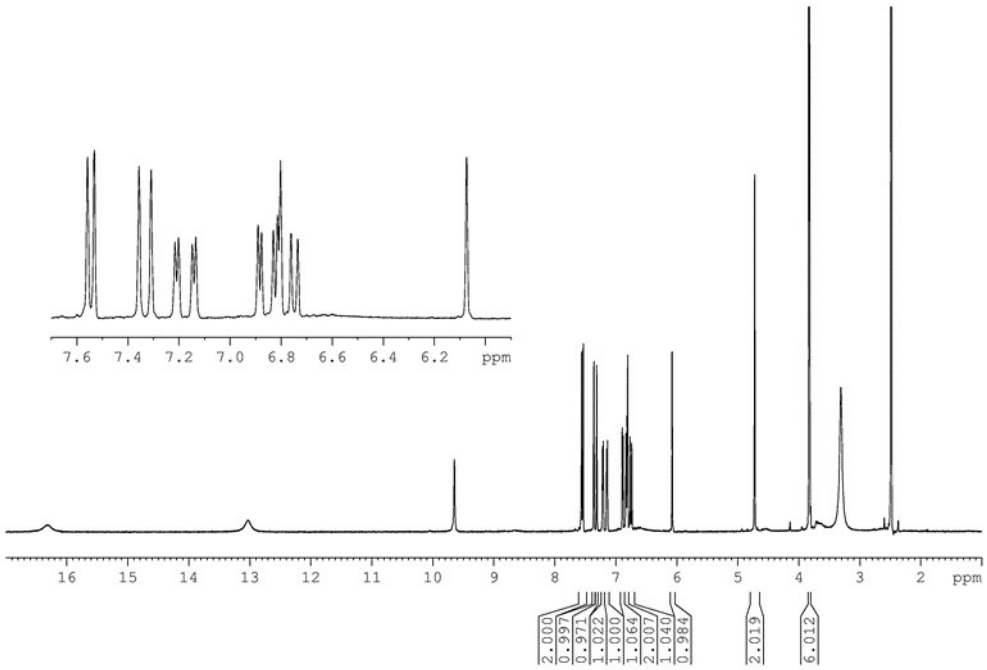


1

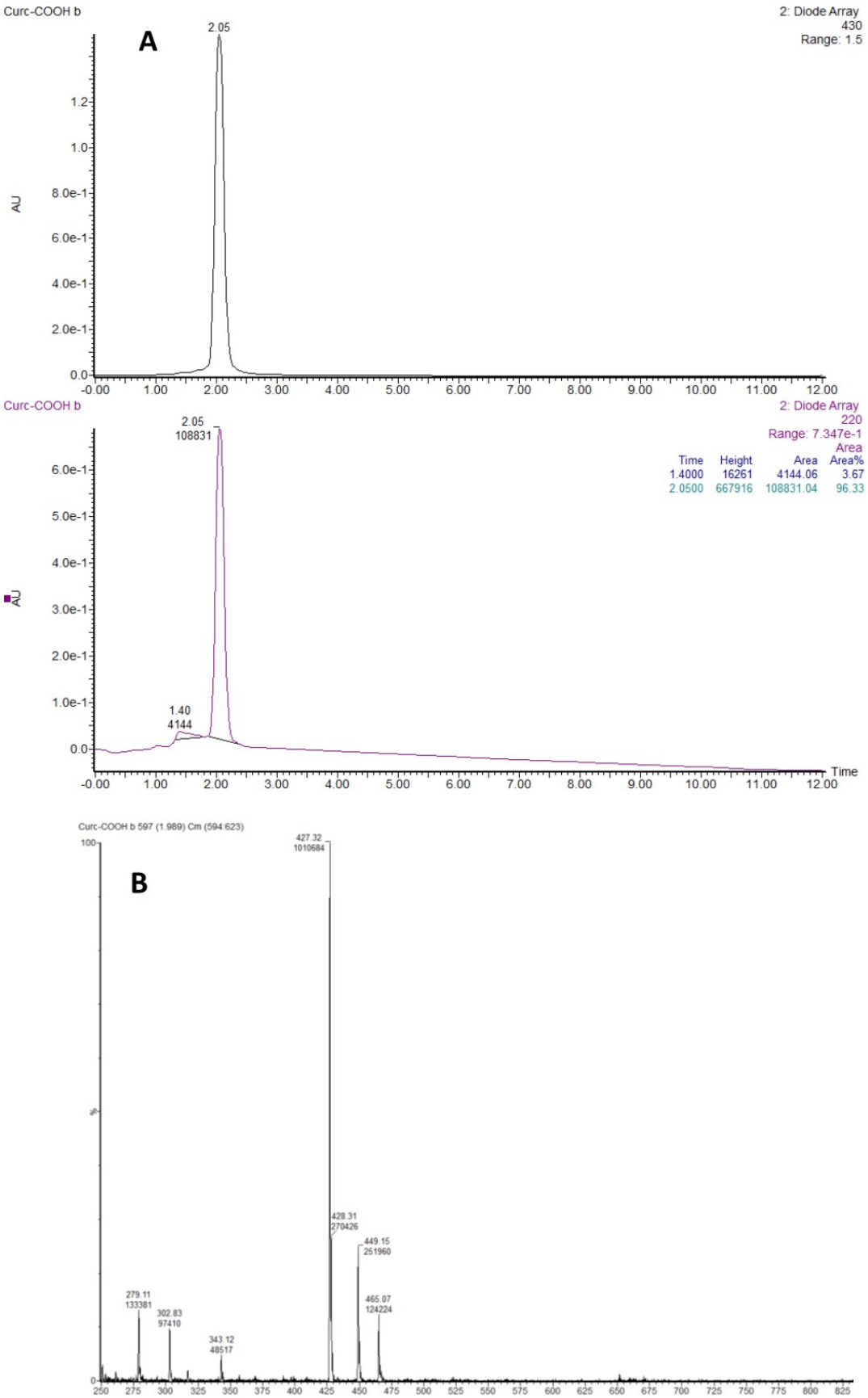
*H-NMR spectrum of compound **1a** Curc-mono-COOtBu (dmso-d_6, 310 K)*

2.2. Compound 2a.



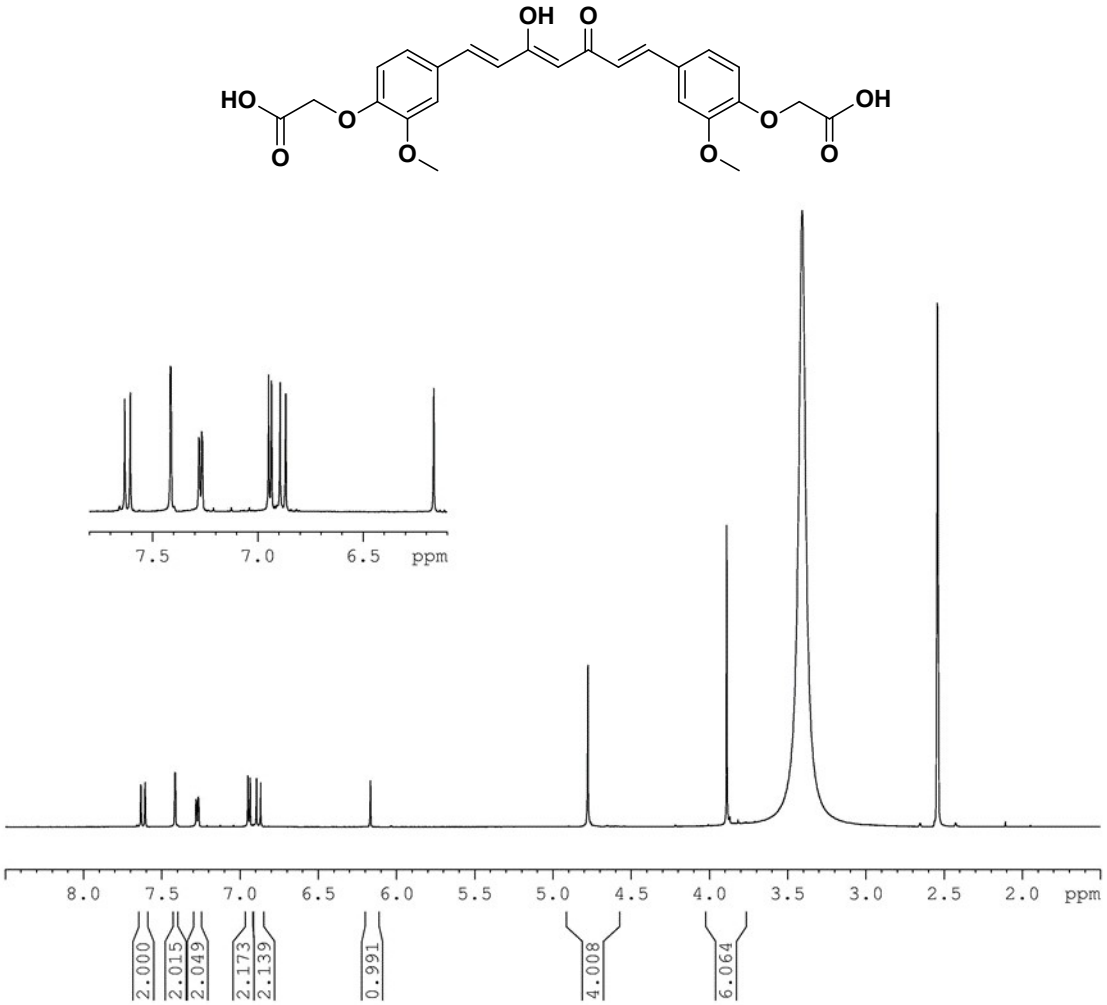


*^{1}H-NMR spectrum of compound **2a** (dmso-d_6, 298 K)*

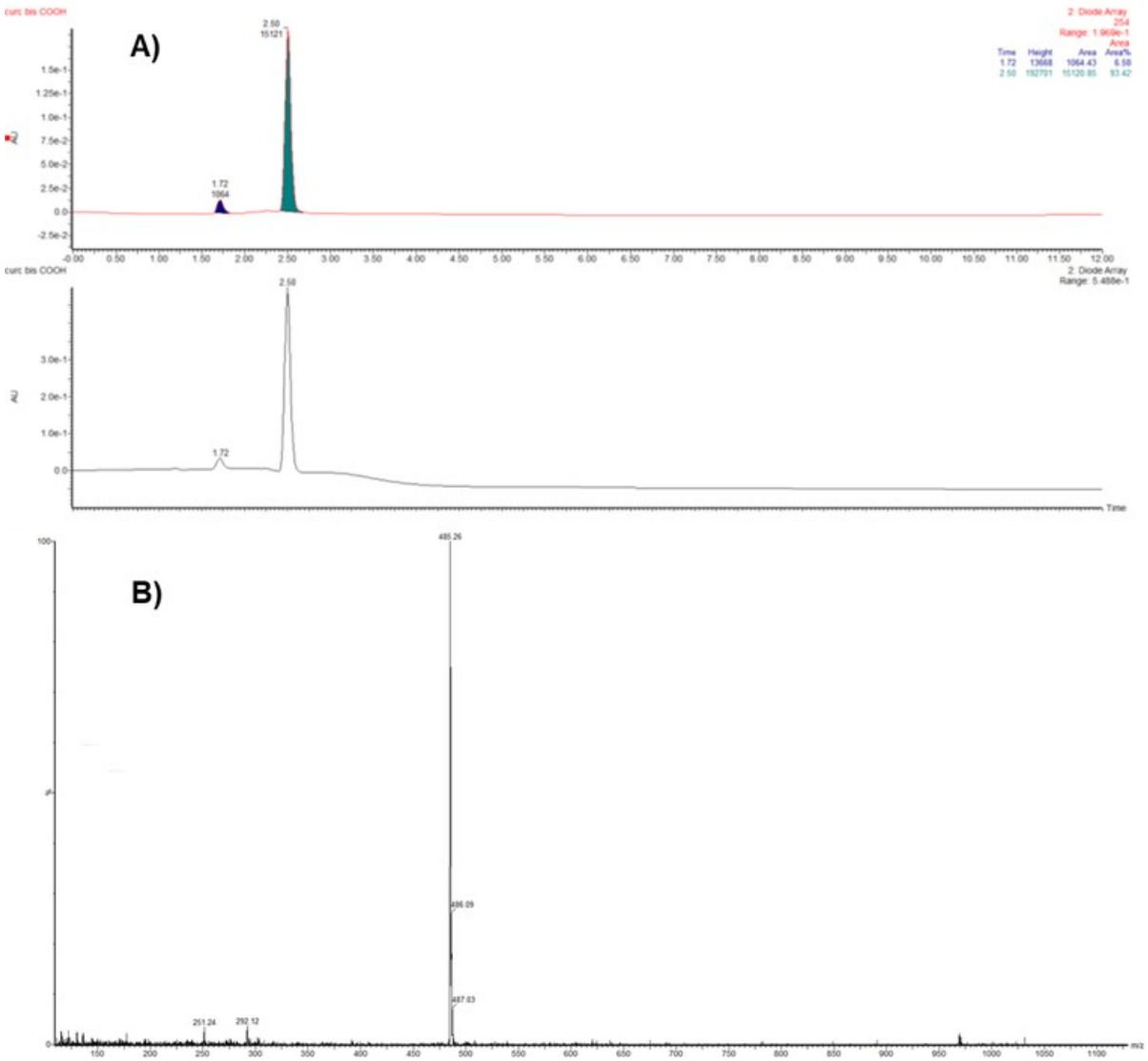


*A) UV chromatogram of compound **2a** recorded at 220 nm and 430 nm (retention time of 2.05 min and purity of 96%); B) Mass spectrum (ESI+) of the peak at 2.05 min*

2.3 Compound 2b.

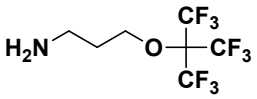


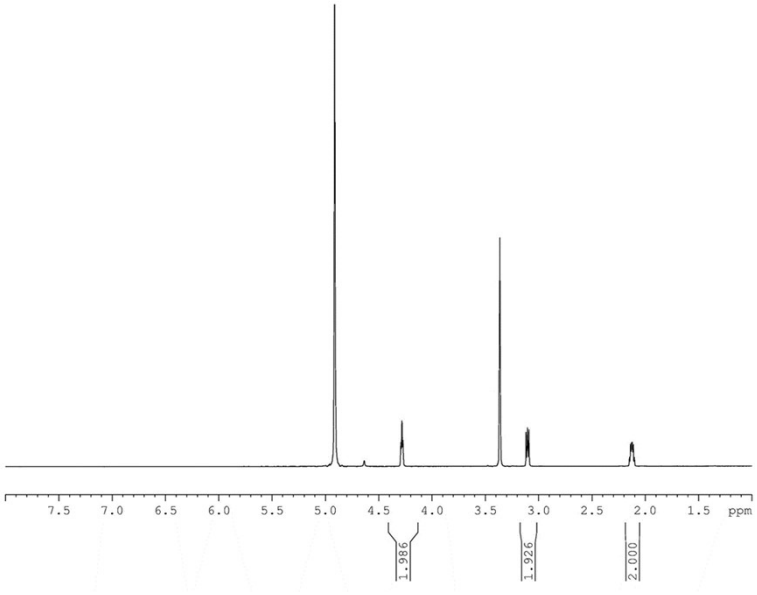
*^{1}H-NMR spectrum of compound **2b** (dmso-d_6, 298 K)*



*A) UV chromatogram of compound **2b** recorded at 220 nm and 254 nm (retention time of 2.50 min and purity of 93%); B) Mass spectrum (ESI+) of the peak at 2.50 min*

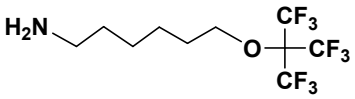
2.4. Compound F9-C_3-NH_2.

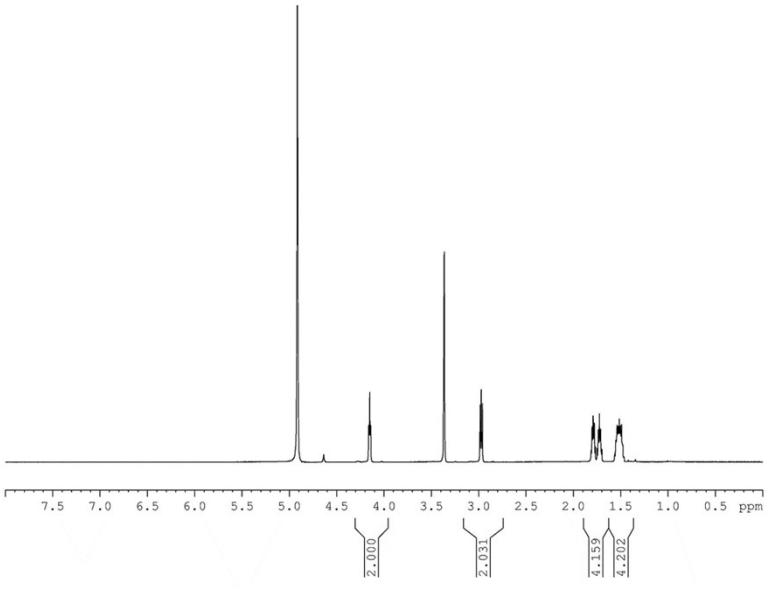




*[1]H-NMR spectrum of compound **F9-C_3-NH_2** (MeOD, 298 K)*

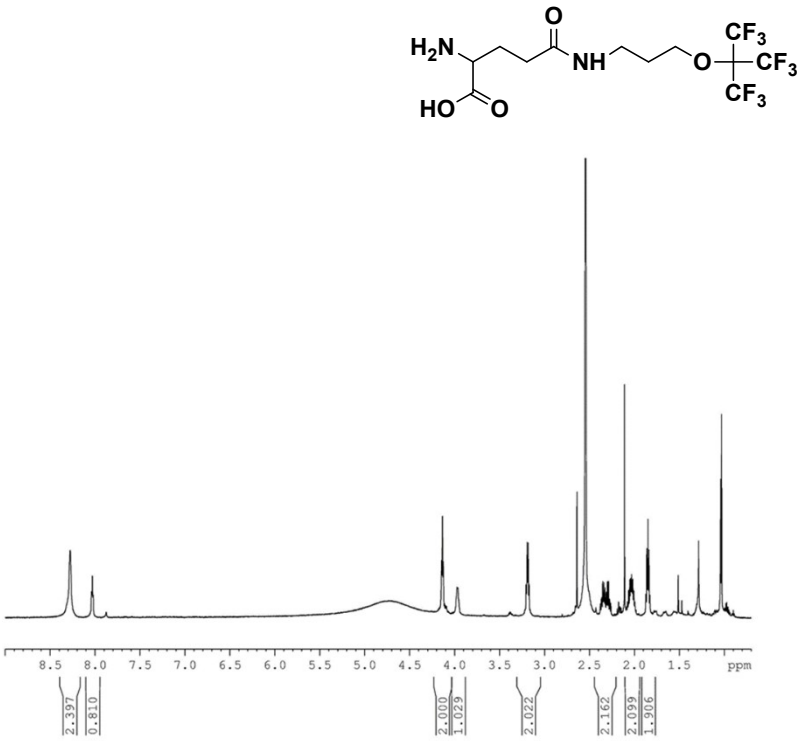
2.5. Compound F9-C_6-NH_2.





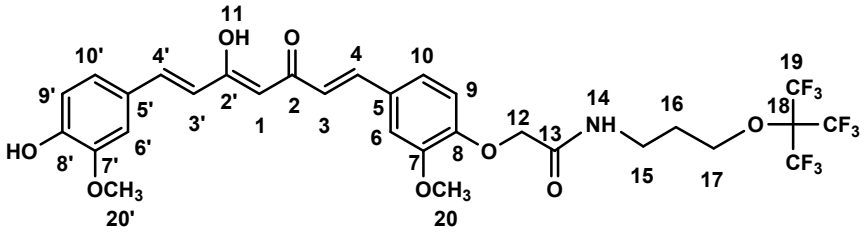
*[1]H-NMR spectrum of compound **F9-C_6-NH_2** (MeOD, 298 K)*

2.6. Compound F9-Glu-NH_2



[1]H-NMR spectrum of compound ***F9-Glu-NH_2*** *(MeOD, 298 K)*

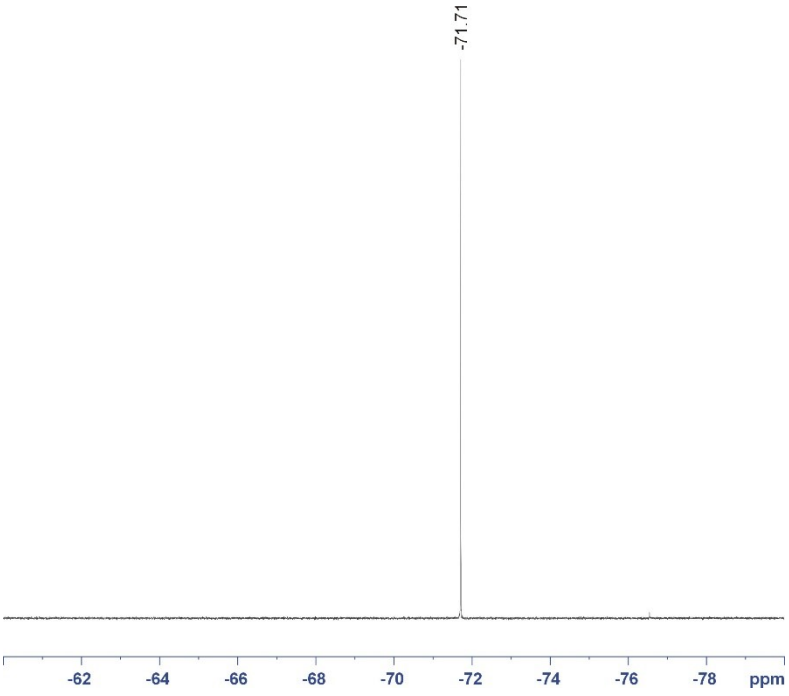
2.7. Compound Curc-C_3-F9



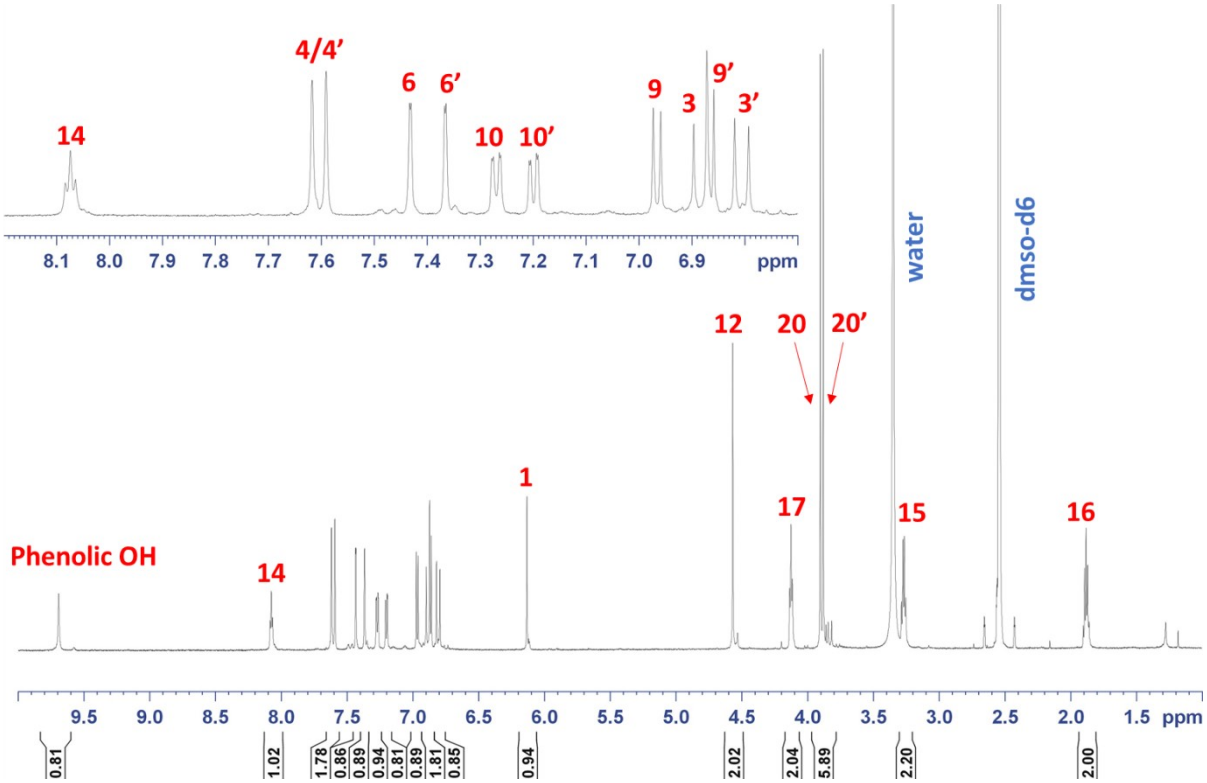
[1]H-NMR (600 MHz, dmso-d_6, 300 K), δ ppm: 9.69 (s, 1H, phenolic-OH), 8.08 (t, *J*=5.6 Hz, 1H, H14), 7.61 (d, *J*=15.9 Hz, 2H, overlapping H4/H4'), 7.43 (d, *J*=1.9 Hz, 1H, H6), 7.37 (d, *J*=1.9 Hz, 1H, H6'), 7.27 (dd, *J*=8.3 and 1.9 Hz, 1H, H10), 7.20 (dd, *J*=8.2 and 1.9 Hz, 1H, H10'), 6.97 (d, *J*=8.3 Hz, 1H, H9), 6.88 (d, 15.9 Hz, 1H, H3), 6.87 (d, *J*=8.2 Hz, 1H, H9'), 6.81 (d, *J*=15.9 Hz, 1H, H3'), 6.13 (s, 1H, H1), 4.57 (s, 2H, H12), 4.13 (t, *J*=6.2 Hz, 2H, H17), 3.90 (s, 3H, H20), 3.88 (s, 3H, H20'), 3.27 (q, *J*=6.2 Hz, 2H, H15), 1.88 (m, *J*=6.2 Hz, 2H, H16).

[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K), δ ppm (relative to TFA -76.55 ppm): -71.71 (s)

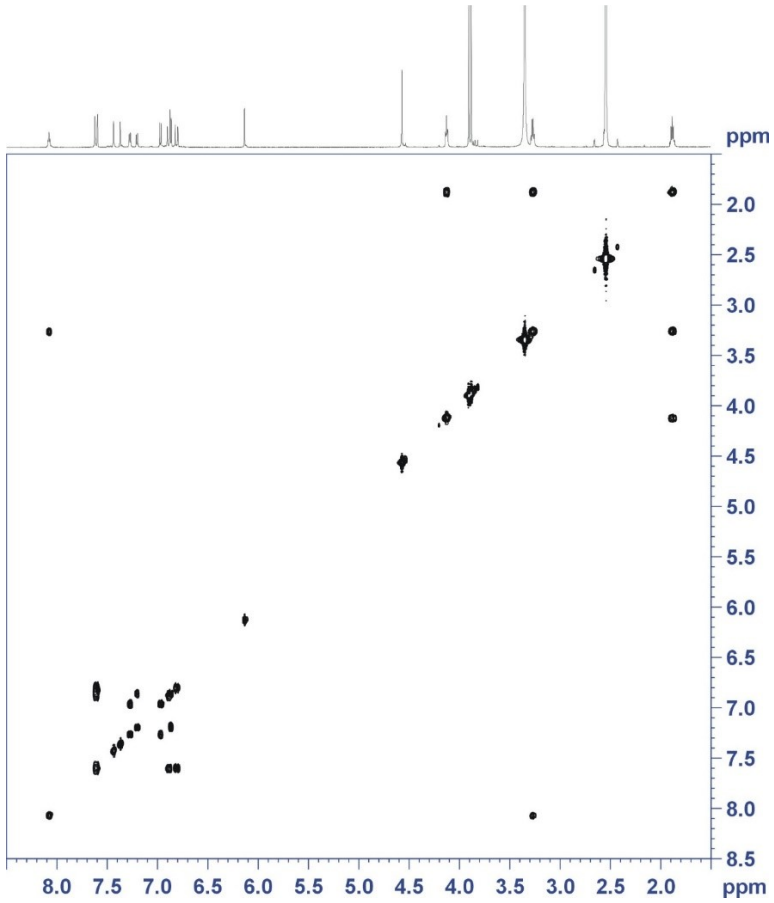
[13]C-NMR (150 MHz, δ ppm from 2D HSQC and 2D HMBC, dmso-d_6, 300 K): 184.4 (C2'), 182.8 (C2), 168.0 (C13), 150.0 (overlapping C7, C8, C8'), 148.5 (C7'), 141.4 (C4'), 140.3 (C4), 129.2 (C5), 126.7 (C5'), 123.7 (C10'), 123.0 (C3), 122.7 (C10), 121.6 (C3'), 116.3 (C9'), 114.3 (C9), 111.8 (C6'), 111.5 (C6), 101.3 (C1), 68.8 (C17), 68.6 (C12), 56.2 (overlapping C20, C20'), 35.2 (C15), 29.8 (C16).



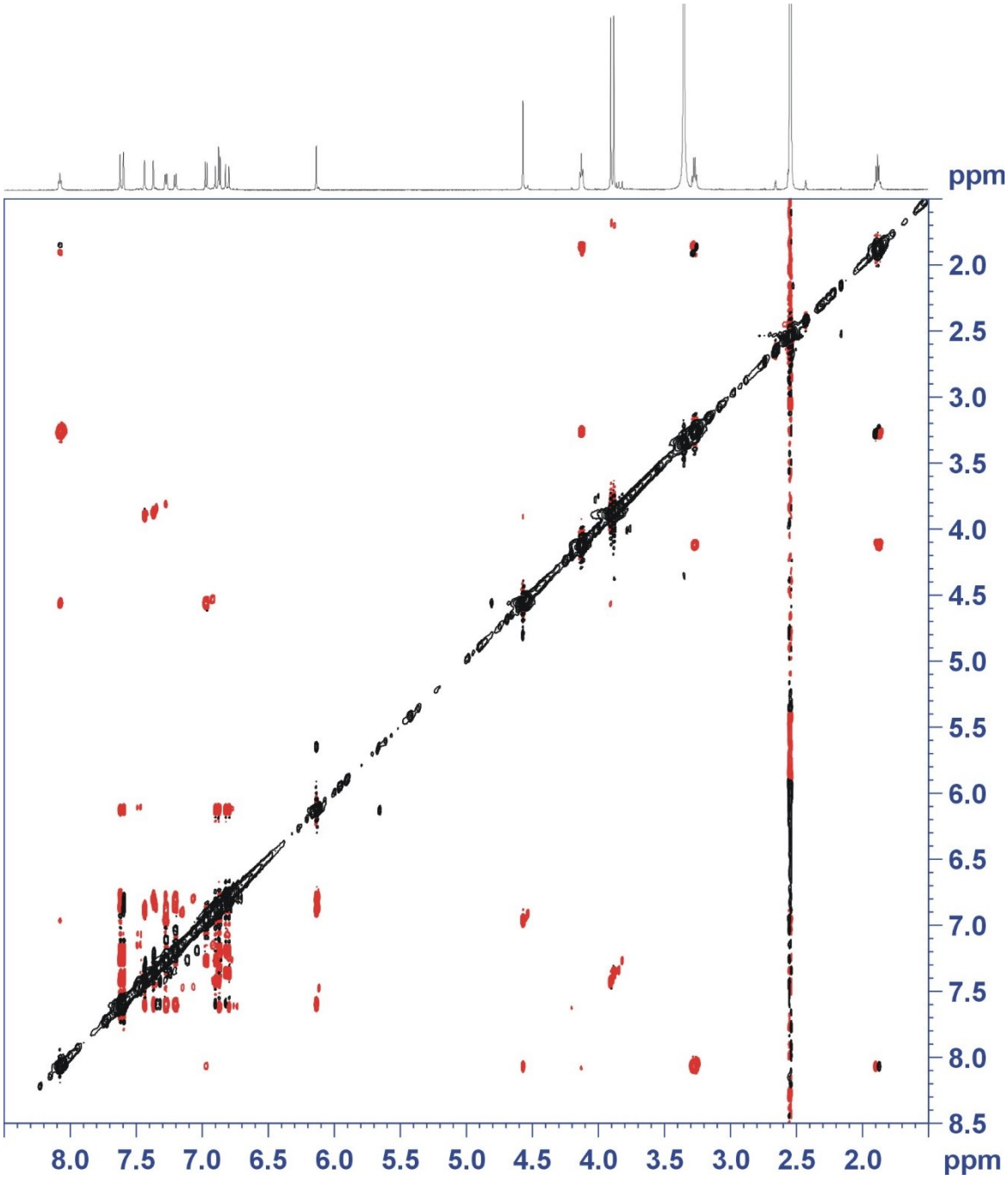
[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K)



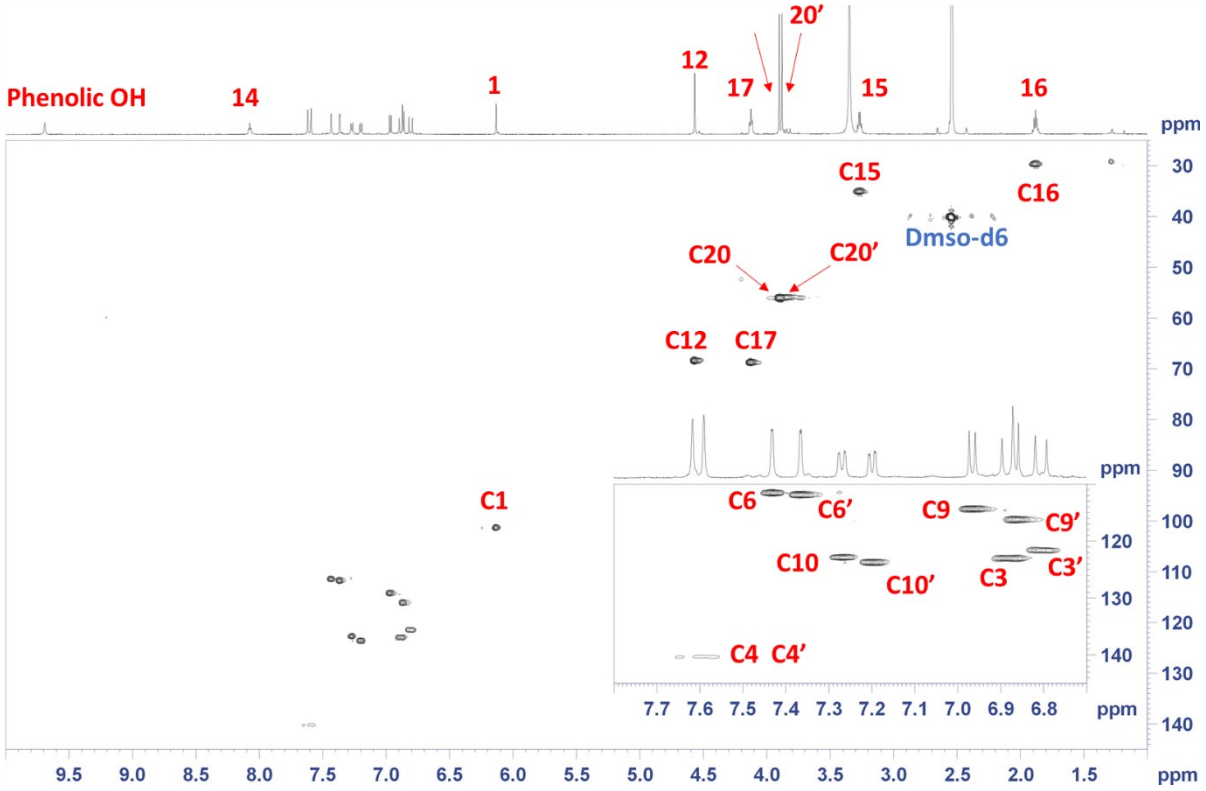
^{1}H-NMR (600 MHz, dmso-d_6, 300 K)



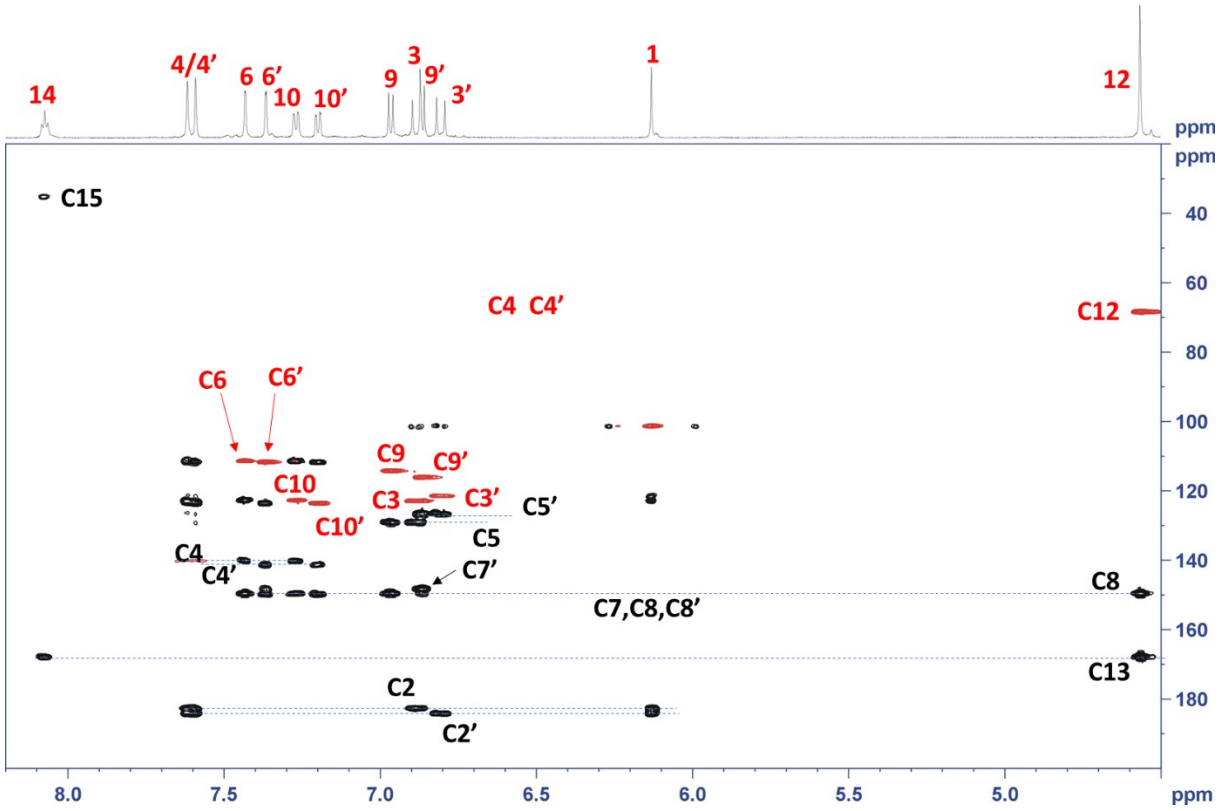
2D ^{1}H,^{1}H-COSY NMR (600 MHz, dmso-d_6, 300 K)



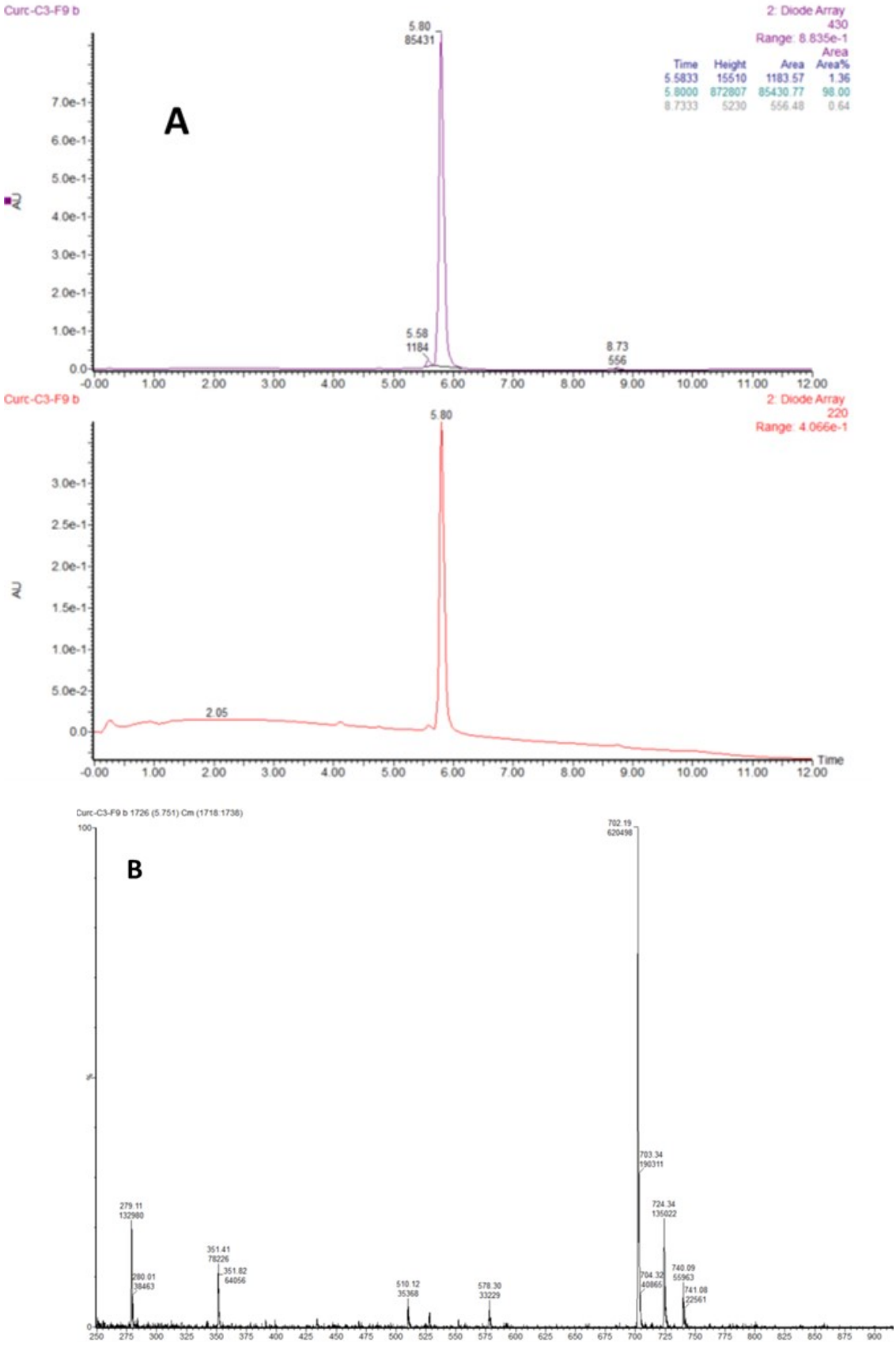
2D $^{1}H,^{1}H$-ROESY NMR (600 MHz, dmso-d_6, 300 K)



2D $^{1}H,^{13}C$-HSQC NMR (600 MHz, dmso-d6, 300 K) with ^{13}C assignment of non-quaternary carbons.

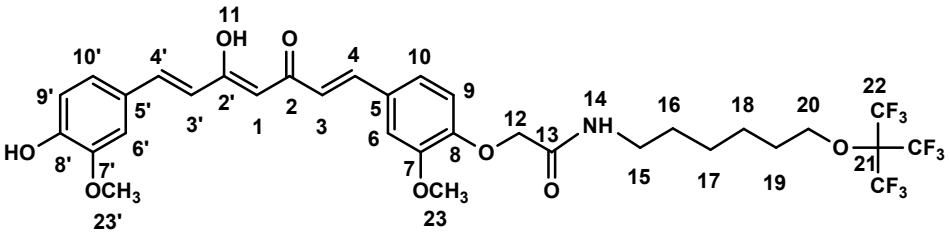


2D $^{1}H,^{13}C$-HMBC NMR (600 MHz, dmso-d_6, 300 K). A zoomed region of the 2D-HMBC NMR spectrum (black) showing the ^{13}C assignment of quaternary carbons is overlaid with the 2D-HSQC NMR spectrum (red).



A) UV chromatogram of compound ***Curc-C_3-F9*** *recorded at 220 nm and 430 nm (retention time of 5.80 min and purity of 98%); B) Mass spectrum (ESI+) of the peak at 5. 80 min*

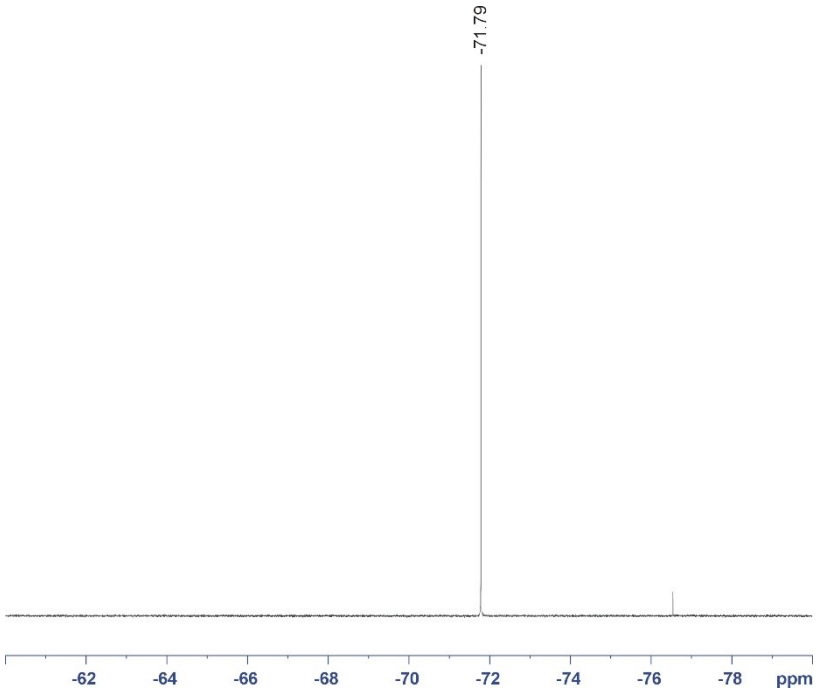
2.8. Compound Curc-C_6-F9



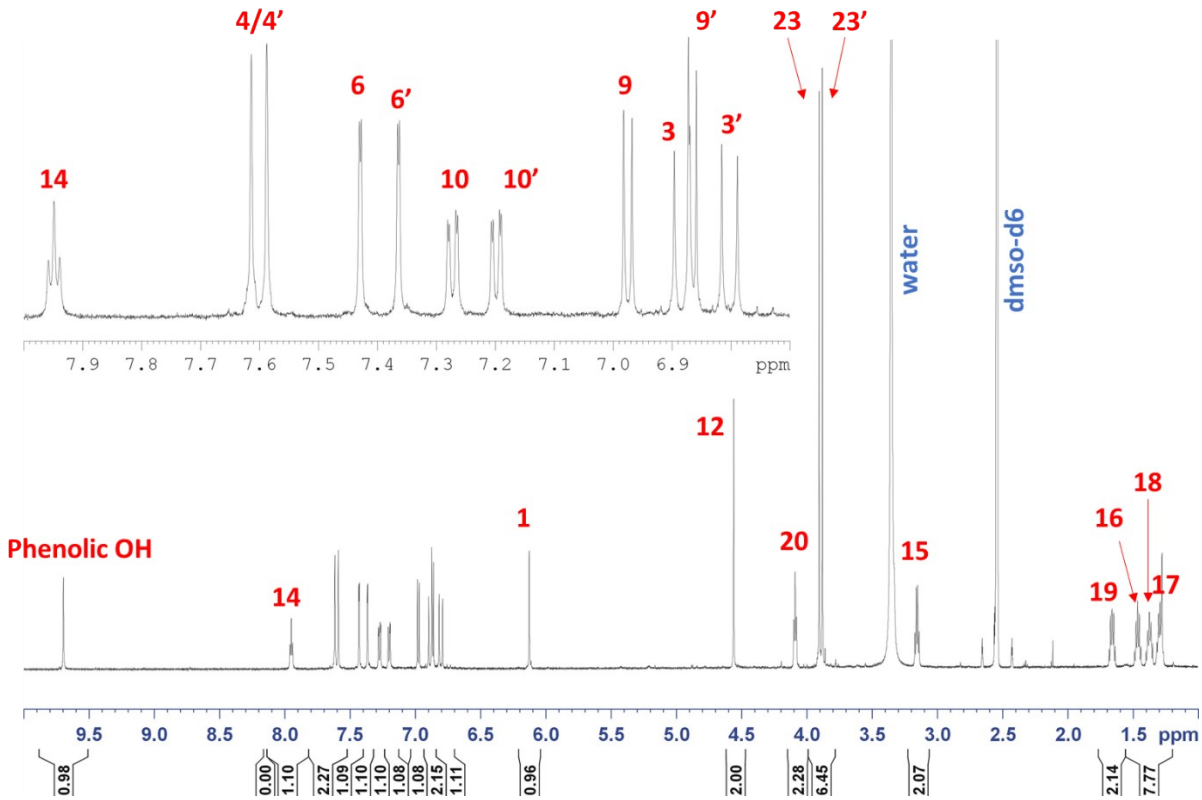
[1]H-NMR (600 MHz, dmso-d_6, 300 K), δ ppm: 9.70 (s, 1H, phenolic-OH), 7.95 (t, *J*=5.7 Hz, 1H, H14), 7.60 (d, *J*=15.8 Hz, 2H, overlapping H4/H4'), 7.43 (d, *J*=1.9 Hz, 1H, H6), 7.36 (d, *J*=1.9 Hz, 1H, H6'), 7.27 (dd, *J*= 8.4 and 1.9 Hz, 1H, H10), 7.20 (dd, *J*= 8.4 and 1.9 Hz, 1H, H10'), 6.97 (d, *J*= 8.4 Hz, 1H, H9), 6.88 (d, *J*= 15.8 Hz, 1H, H3), 6.86 (d, *J*= 8.4 Hz, 1H, H9'), 6.80 (d, *J*= 15.8 Hz, 1H, H3'), 6.12 (s, 1H, H1), 4.56 (s, 2H, H12), 4.09 (t, *J*= 6.2 Hz, 2H, H20), 3.90 (s, 3H, H23), 3.88 (s, 3H, H23'), 3.15 (q, *J*= 6.2 Hz, 2H, H15), 1.66 (m, *J*= 6.4 Hz, 2H, H19), 1.46 (m, *J*= 7.4 Hz, 2H, H16), 1.37 (m, *J*= 7.4 Hz, 2H, H18), 1.295 (m, *J*= 7.4 Hz, 2H, H17).

[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K), δ ppm (relative to TFA -76.55 ppm): -71.79 (s)

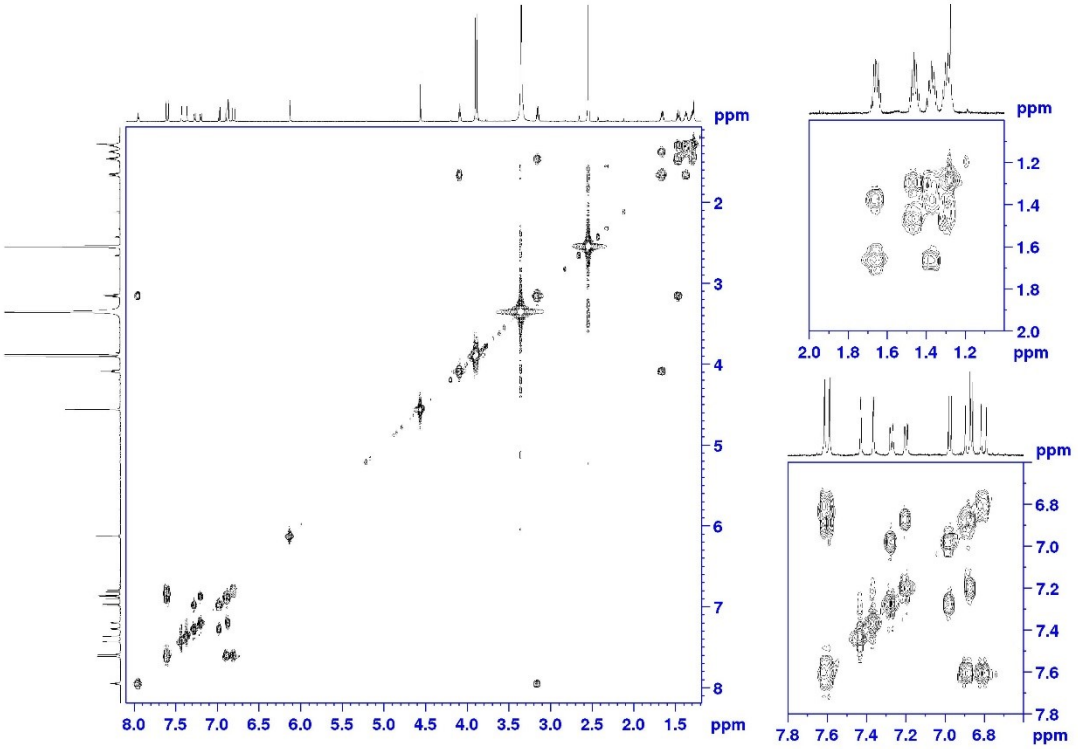
[13]C-NMR (150 MHz, δ ppm from 2D HSQC and 2D HMBC, dmso-d_6, 300 K): 184.4 (C2'), 182.7 (C2), 167.7 (C13), 149.8 (overlapping C7, C8, C8'), 148.7 (C7'), 141.5 (C4'), 140.2 (C4), 129.2 (C5), 126.7 (C5'), 123.7 (C10'), 123.0 (C3), 122.9 (C10), 121.6 (C3'), 116.2 (C9'), 114.4 (C9), 111.8 (C6'), 111.5 (C6), 101.4 (C1), 70.7 (C20), 68.5 (C12), 56.15 (overlapping C23, C23'), 38.6 (C15), 29.55 (C19), 29.3 (C16), 26.3 (C17), 24.9 (C16).



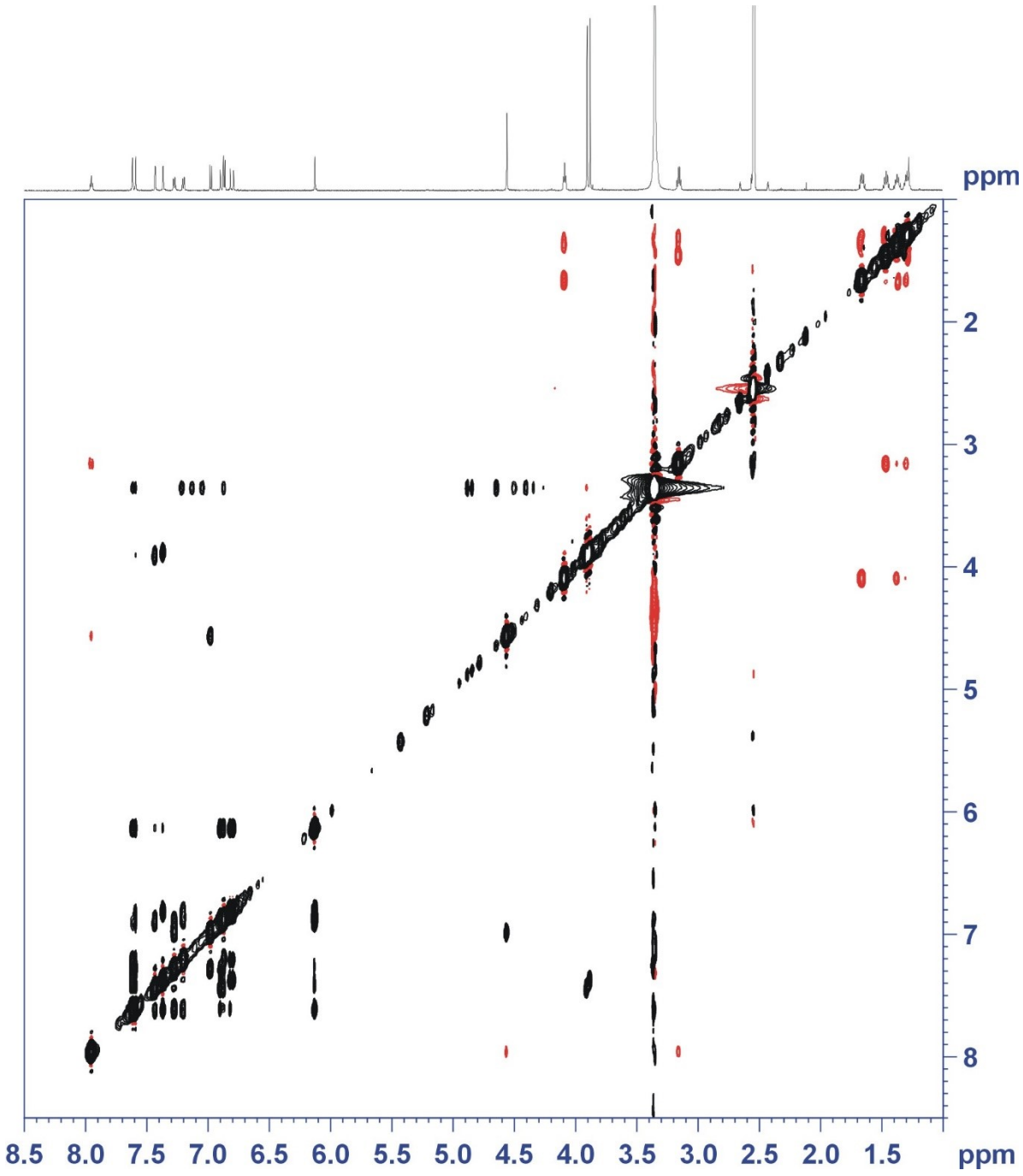
[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K)



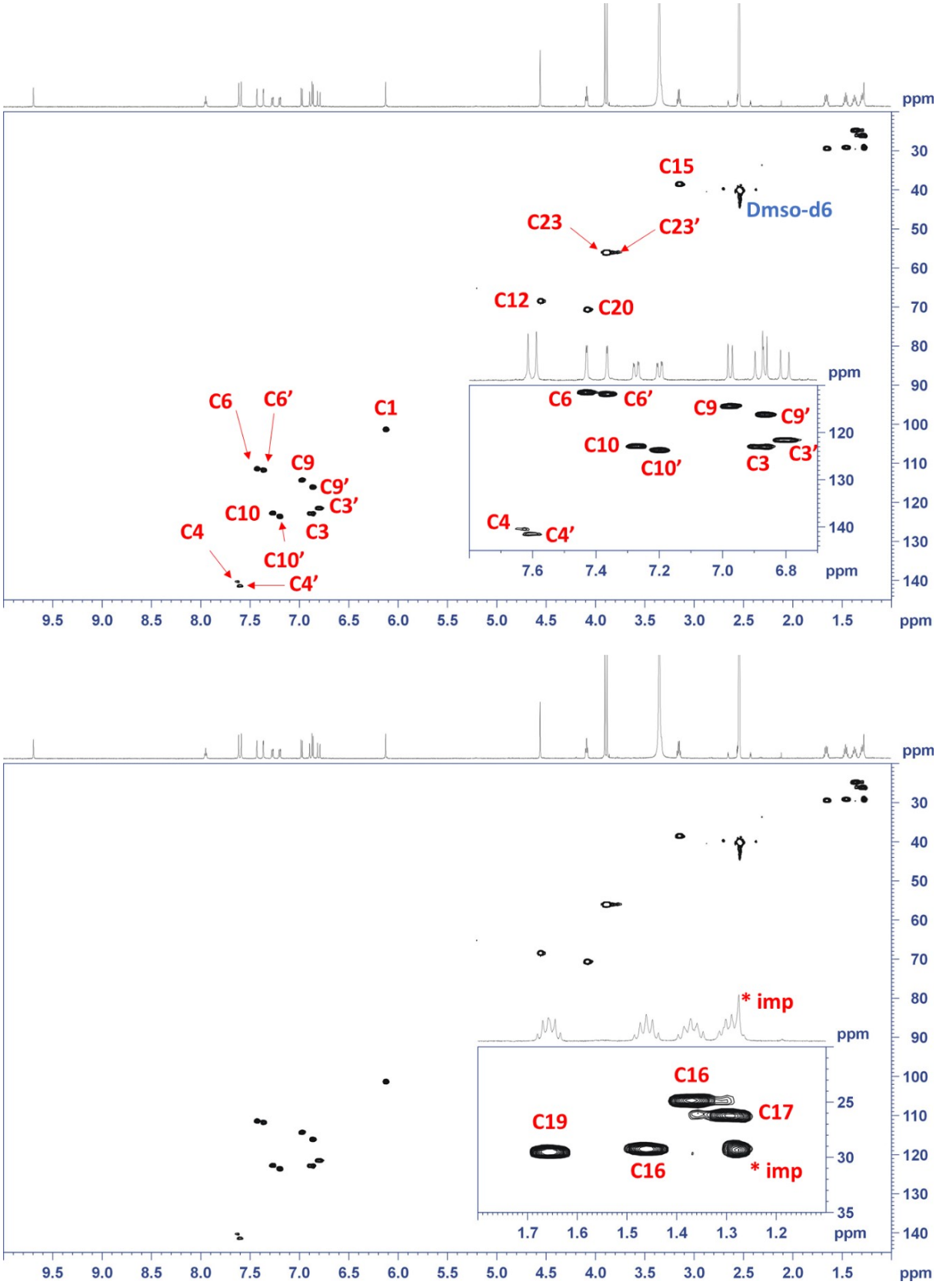
^{1}H-NMR (600 MHz, dmso-d_6, 300 K)



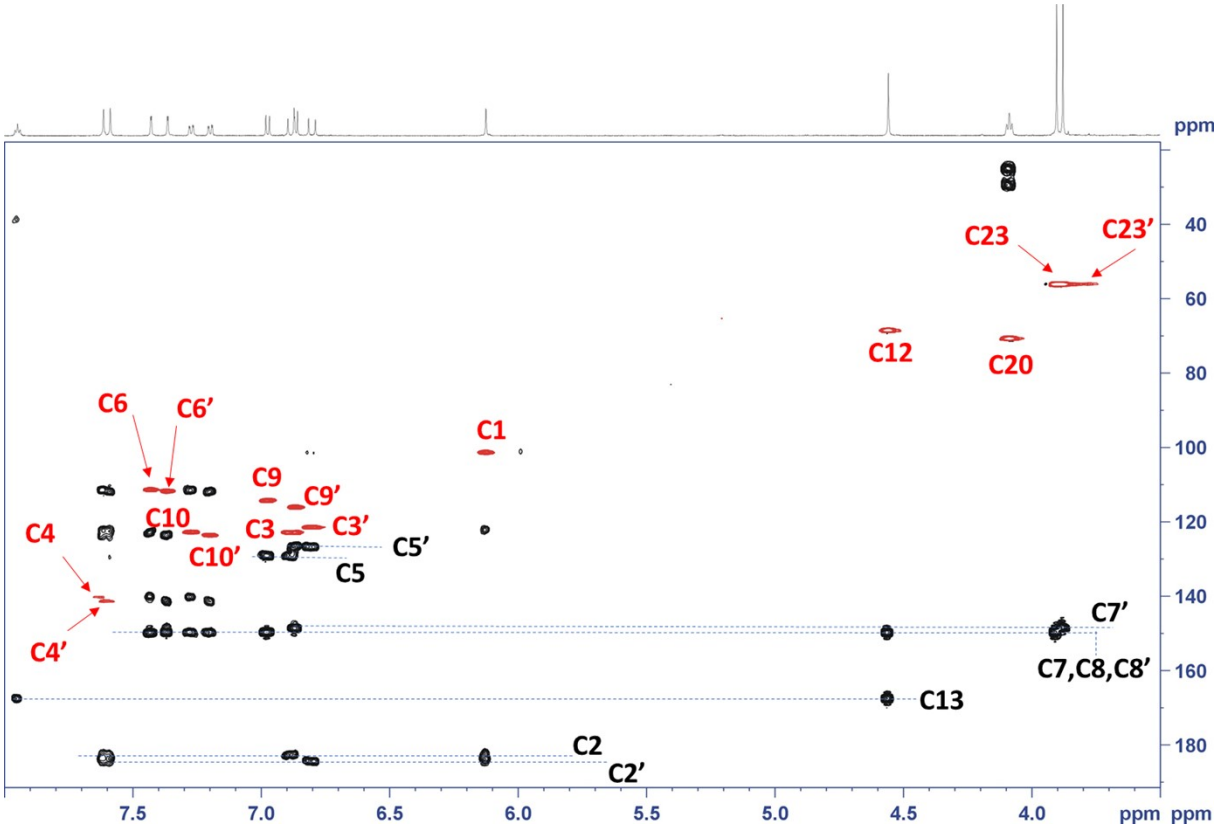
2D ^{1}H,^{1}H-COSY NMR (600 MHz, dmso-d_6, 300 K)



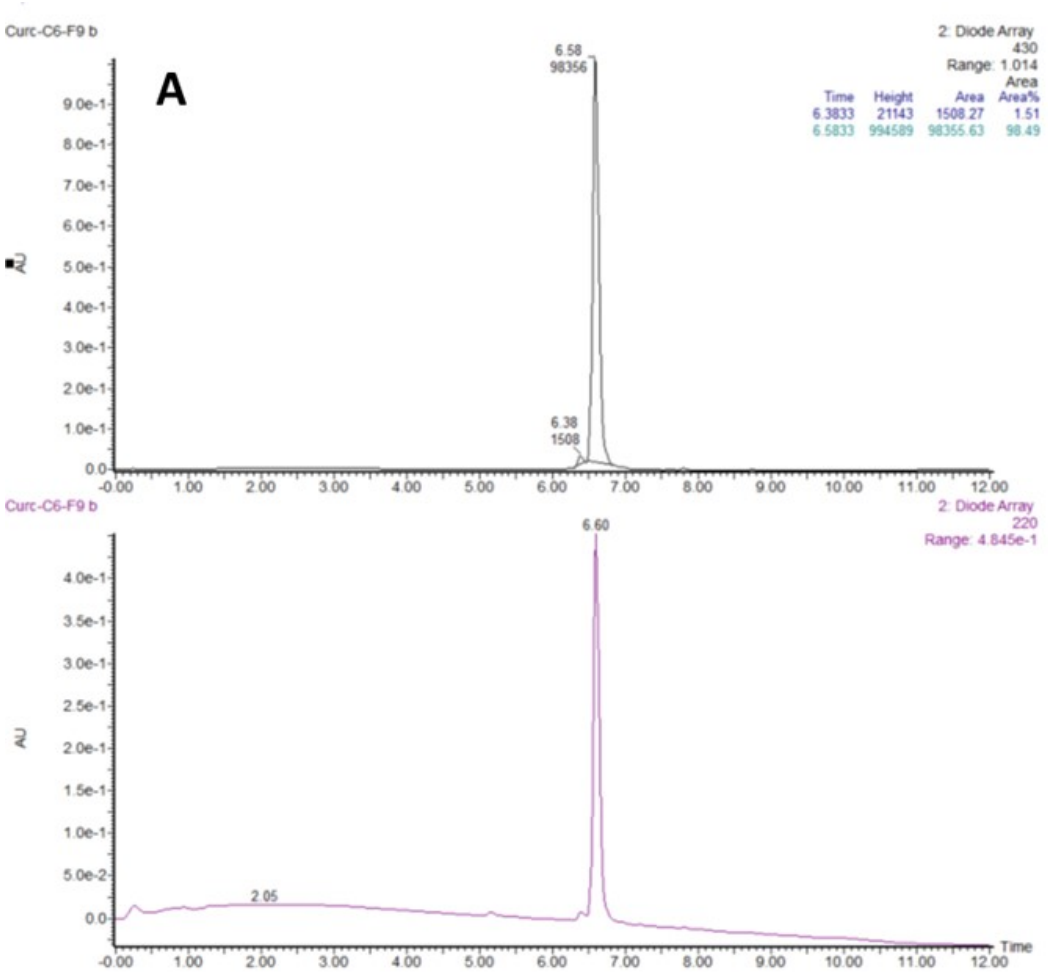
2D $^{1}H,^{1}H$-NOESY NMR (600 MHz, dmso-d_6, 300 K)

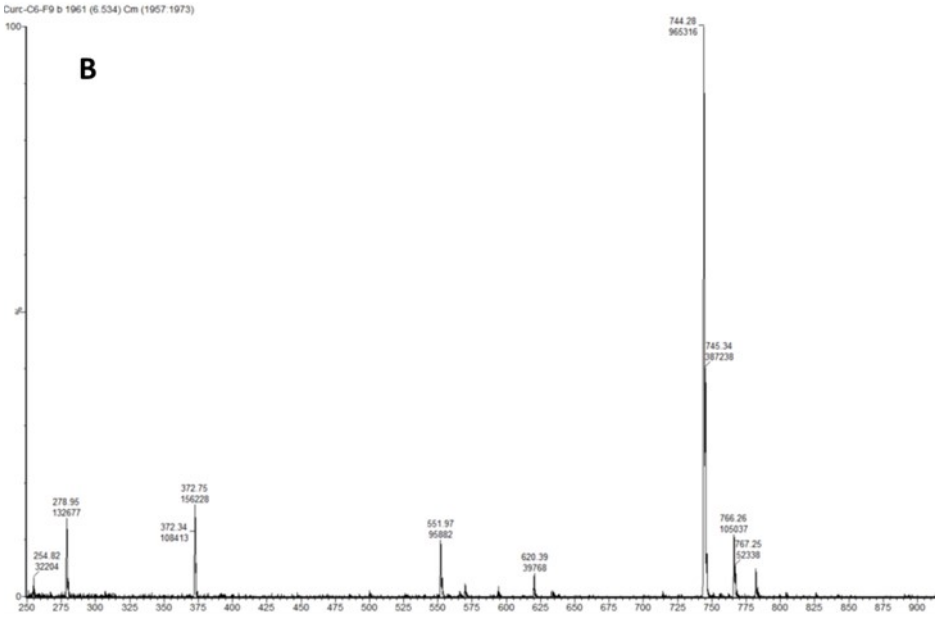


2D $^{1}H,^{13}C$-HSQC NMR (600 MHz, dmso-d6, 300 K) with ^{13}C assignment of non-quaternary carbons. The inserts show the magnification of the aromatic (top) and the aliphatic region (bottom).



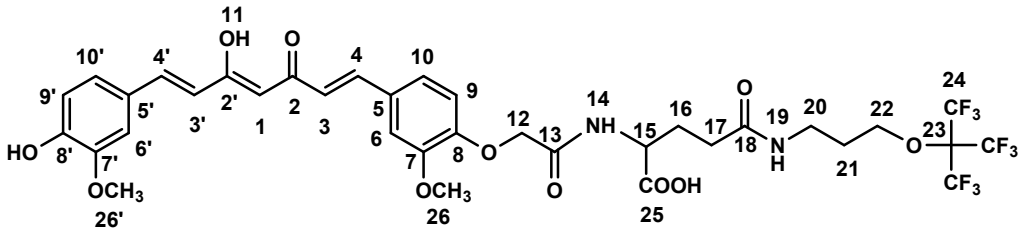
2D $^{1}H,^{13}C$-HMBC NMR (600 MHz, dmso-d_6, 300 K). A zoomed region of the 2D-HMBC NMR spectrum (black) showing the ^{13}C assignment of quaternary carbons is overlaid with the 2D-HSQC NMR spectrum (red).





A) UV chromatogram of compound ***Curc-C_6-F9*** *recorded at 220 nm and 430 nm (retention time of 6.58 min and purity of 98%); B) Mass spectrum (ESI+) of the peak at 6.58 min.*

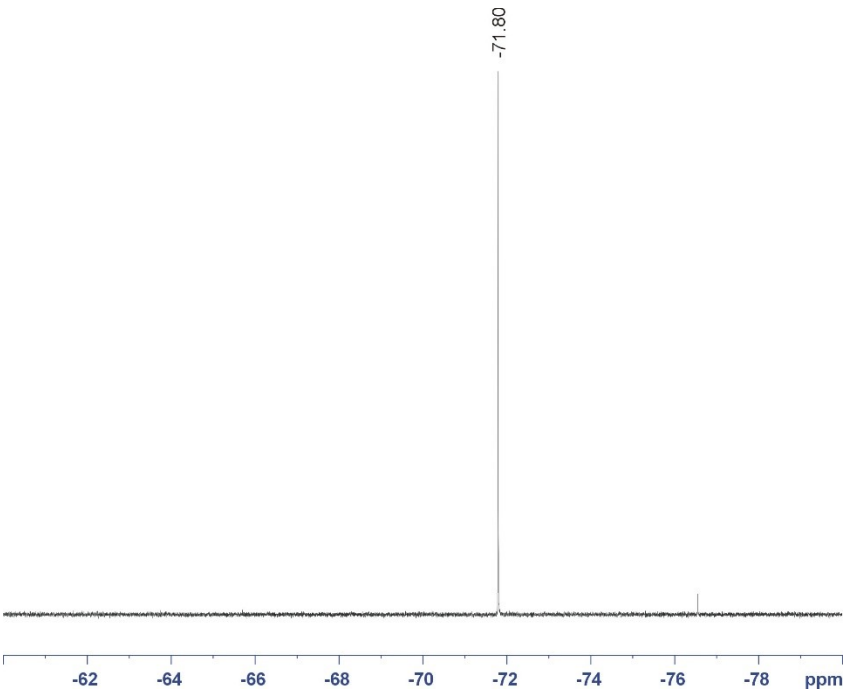
2.9. Compound Curc-Glu-F9



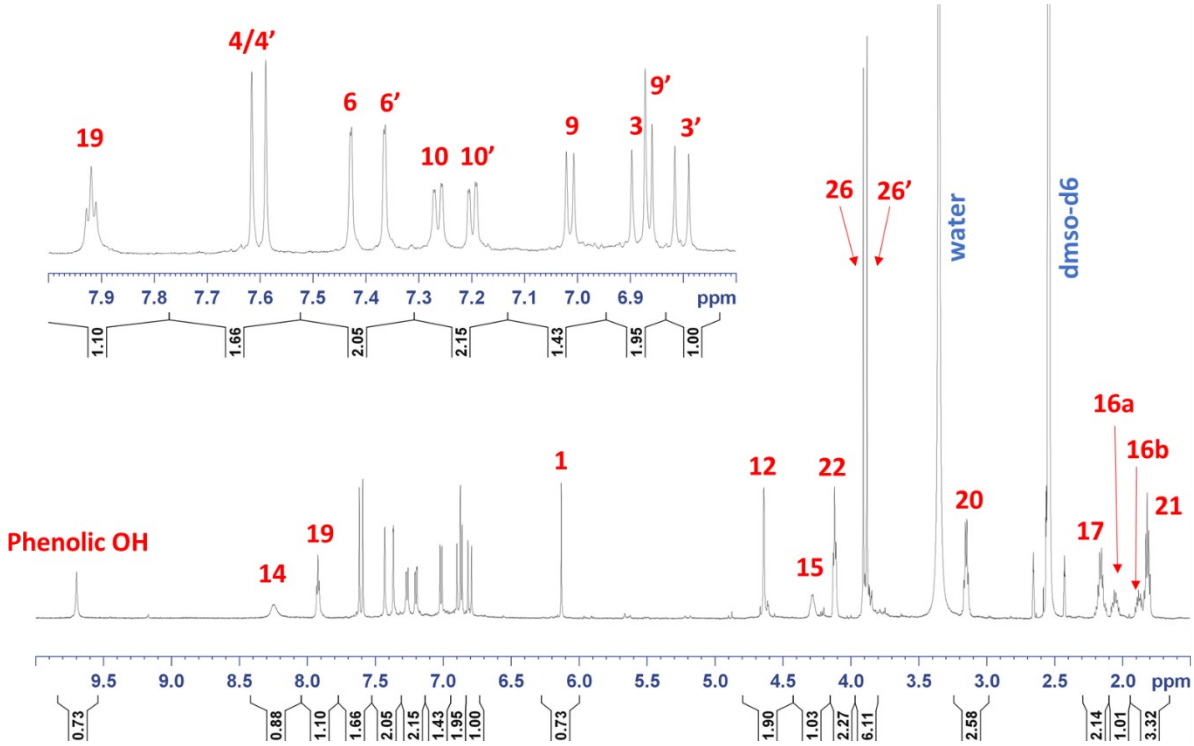
[1]H-NMR (600 MHz, dmso-d_6, 300 K), δ ppm: 9.70 (s, 1H, phenolic-OH), 8.24 (br, 1H, H14), 7.92 (t, *J*=5.6 Hz, 1H, H19), 7.60 (d, *J*=15.8 Hz, 2H, overlapping H4/H4'), 7.43 (d, *J*=1.6 Hz, 1H, H6), 7.36 (d, *J*=1.6 Hz, 1H, H6'), 7.26 (dd, *J*=8.4 and 1.6 Hz, 1H, H10), 7.20 (dd, *J*=8.4 and 1.6 Hz, 1H, H10'), 7.01 (d, *J*=8.3 Hz, 1H, H9), 6.88 (d, *J*=15.8 Hz, 1H, H3), 6.86 (d, *J*=8.4 Hz, 1H, H9'), 6.80 (d, *J*=15.8 Hz, 1H, H3'), 6.13 (s, 1H, H1), 4.64 (AB system, 2H, H12), 4.28 (m, br, 1H, H15), 4.11 (t, *J*=6.2 Hz, 2H, H22), 3.90 (s, 3H, H26), 3.88 (s, 3H, H26'), 3.15 (q, *J*=6.6 Hz, 2H, H20), 2.16 (m, 2H, H17), 2.06 (m, 1H, H16a), 1.87 (m, 1H, H16b), 1.81 (m, *J*=6.5 Hz, 2H, H21).

[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K), δ ppm (relative to TFA -76.55 ppm): -71.80 (s)

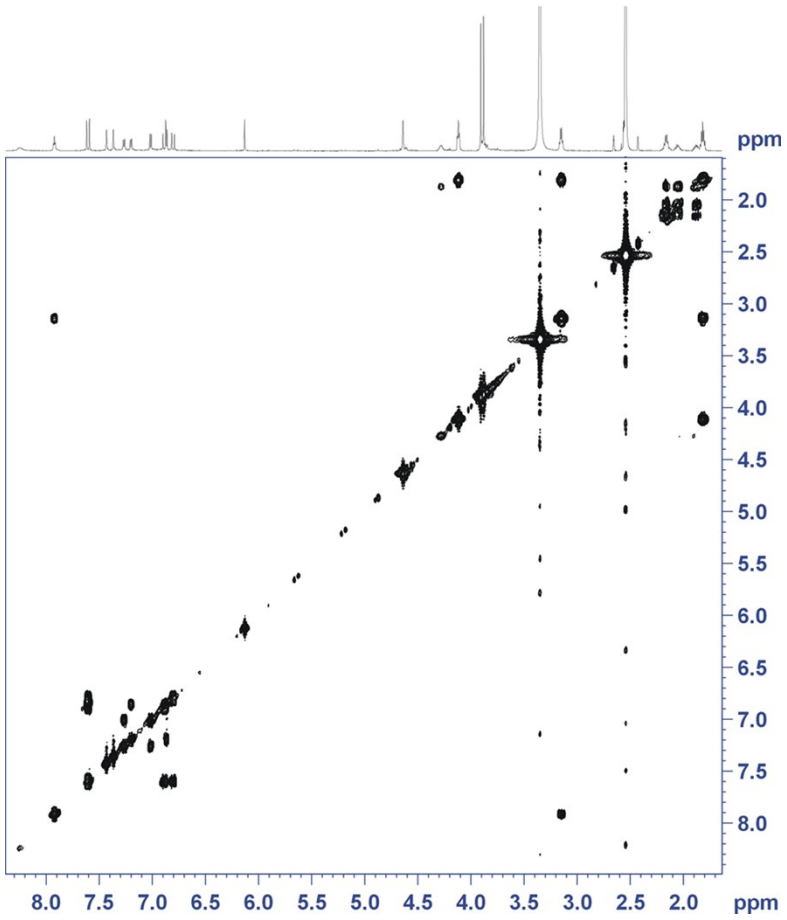
[13]C-NMR (150 MHz, δ ppm from 2D HSQC and 2D HMBC, dmso-d_6, 300 K): 184.3 (C2'), 182.8 (C2), 171.7 (C18), 168.0 (C13), 149.8 (overlapping C7, C8, C8'), 148.5 (C7'), 141.4 (C4'), 140.2 (C4), 129.4 (C5), 126.8 (C5'), 123.8 (C10'), 123.1 (C3), 122.9 (C10), 121.6 (C3'), 116.2 (C9'), 114.5 (C9), 111.8 (C6'), 111.6 (C6), 101.3 (C1), 68.9 (C22), 68.2 (C12), 56.3 (overlapping C26, C26'), 52.3 (C15), 35.3 (C20), 32.03 (C17), 29.9 (C21), 27.7 (C16).



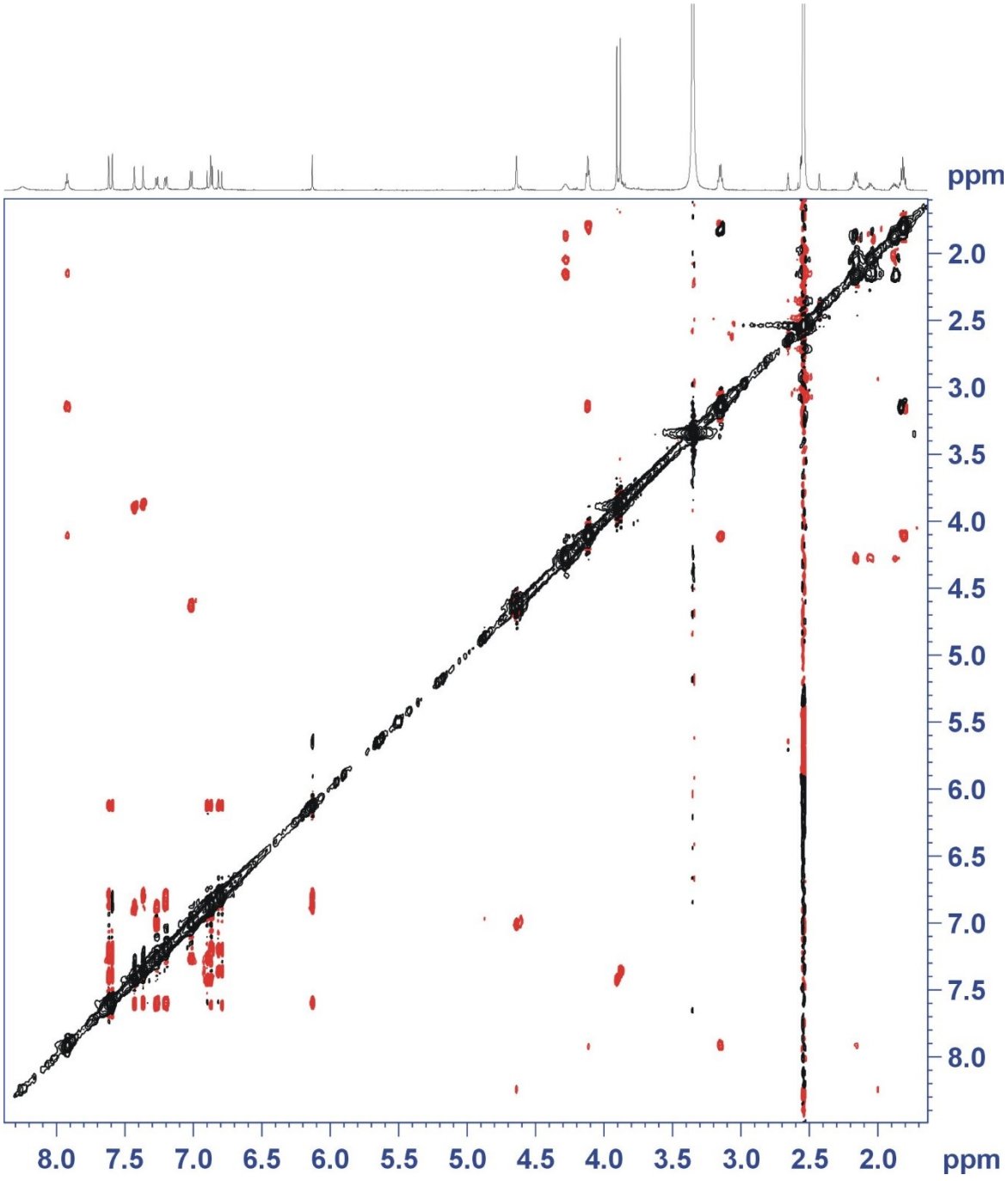
[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K)



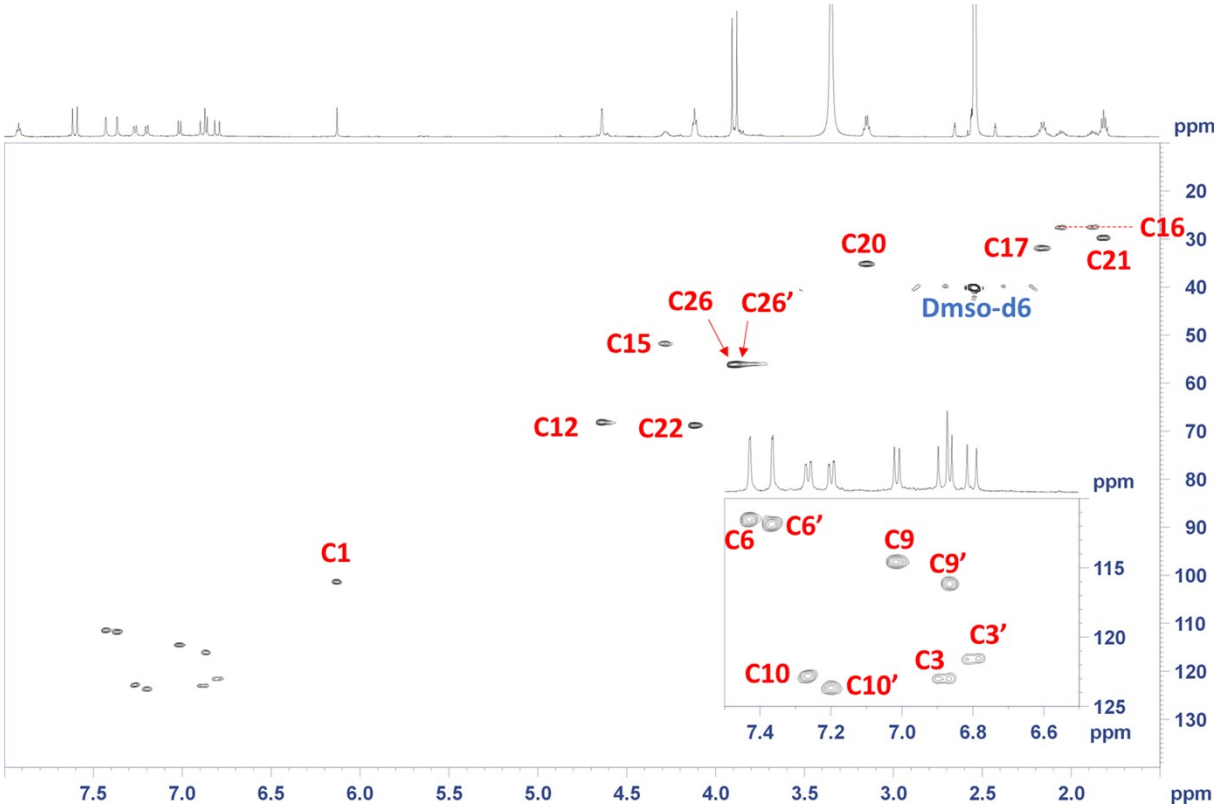
^{1}H-NMR (600 MHz, dmso-d_6, 300 K)



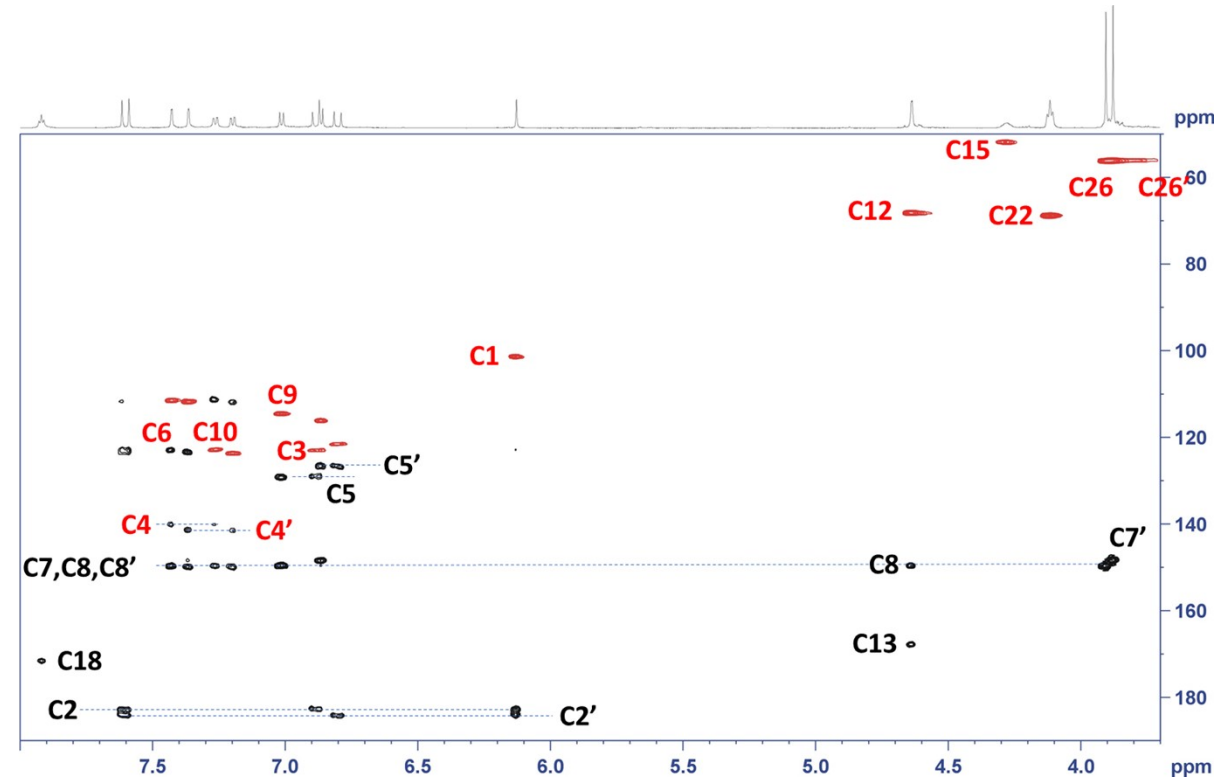
2D ^{1}H,^{1}H-COSY NMR (600 MHz, dmso-d_6, 300 K)



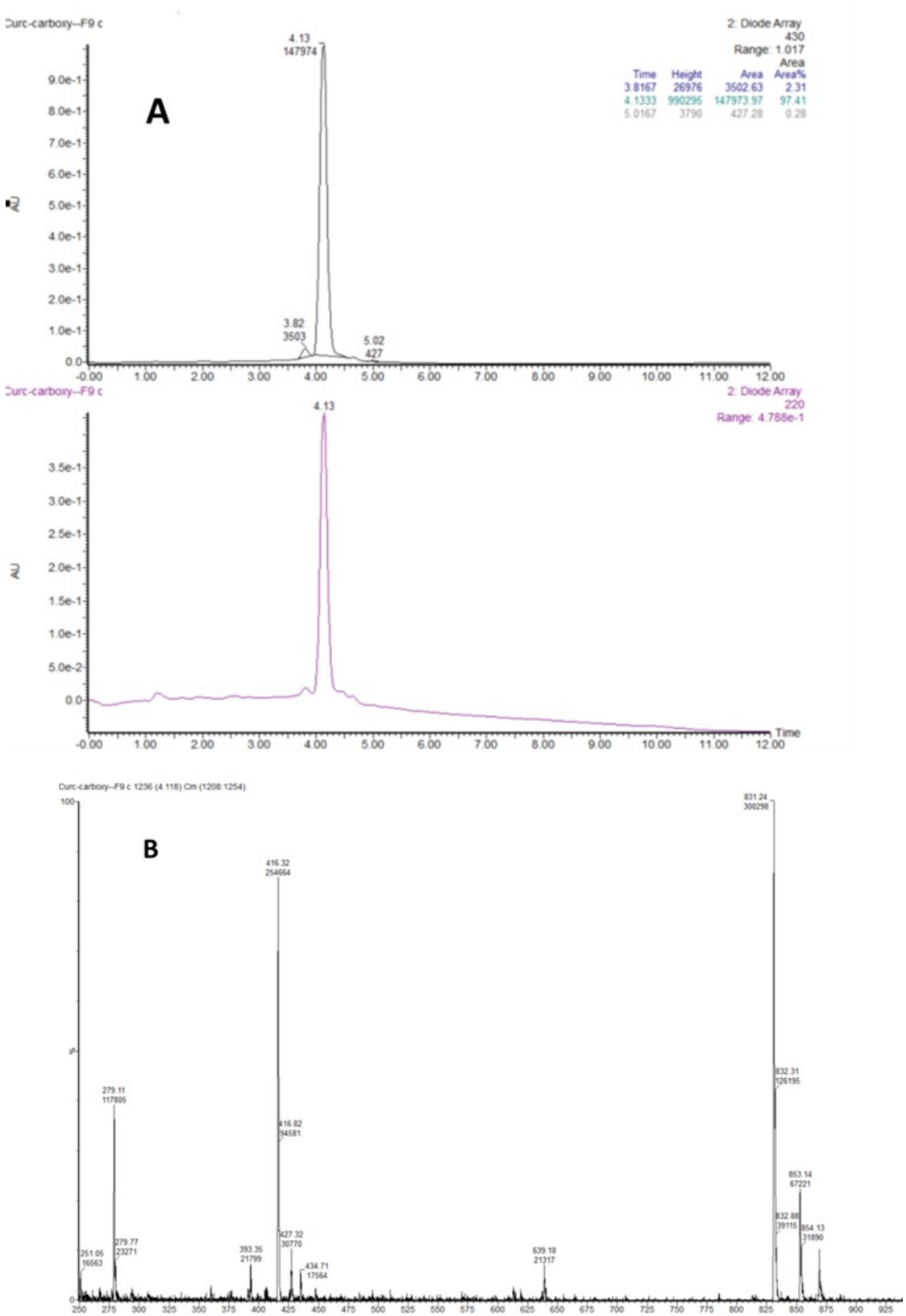
2D $^{1}H,^{1}H$-ROESY NMR (600 MHz, dmso-d_6, 300 K)



2D $^{1}H,^{13}C$-HSQC NMR (600 MHz, dmso-d6, 300 K) with ^{13}C assignment of non-quaternary carbons.

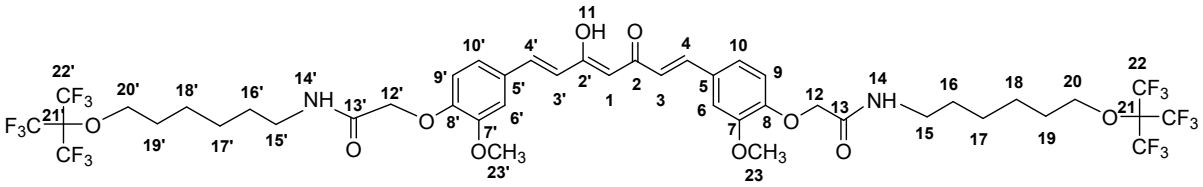


2D $^{1}H,^{13}C$-HMBC NMR (600 MHz, dmso-d_6, 300 K). A zoomed region of the 2D-HMBC NMR spectrum (black) showing the ^{13}C assignment of quaternary carbons is overlaid with the 2D-HSQC NMR spectrum (red).



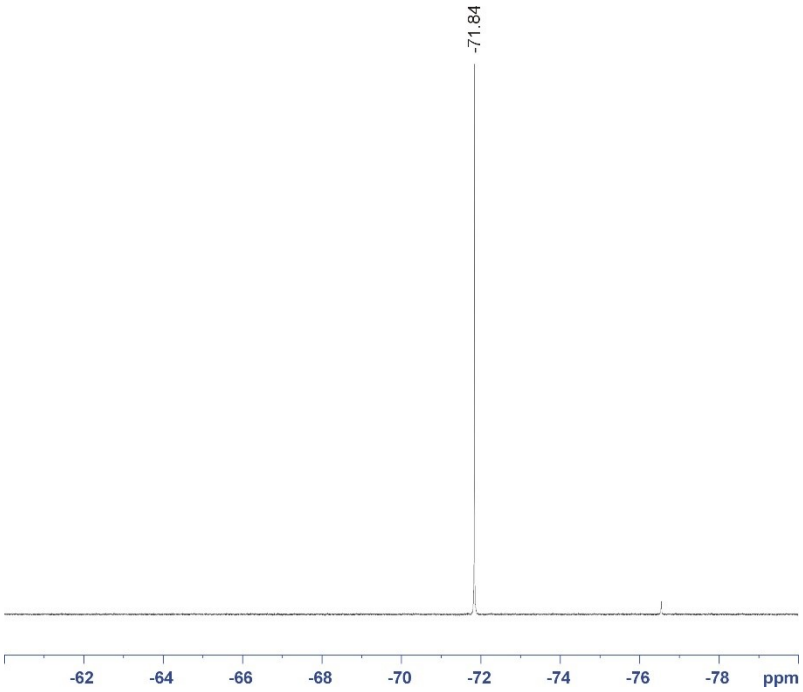
A) UV chromatogram of compound **Curc-Glu-F9** *recorded at 220 nm and 413 nm (retention time of 4.13 min and purity of 97%); B) Mass spectrum (ESI+) of the peak at 4.13 min.*

2.10. Compound Curc-C_6-F18

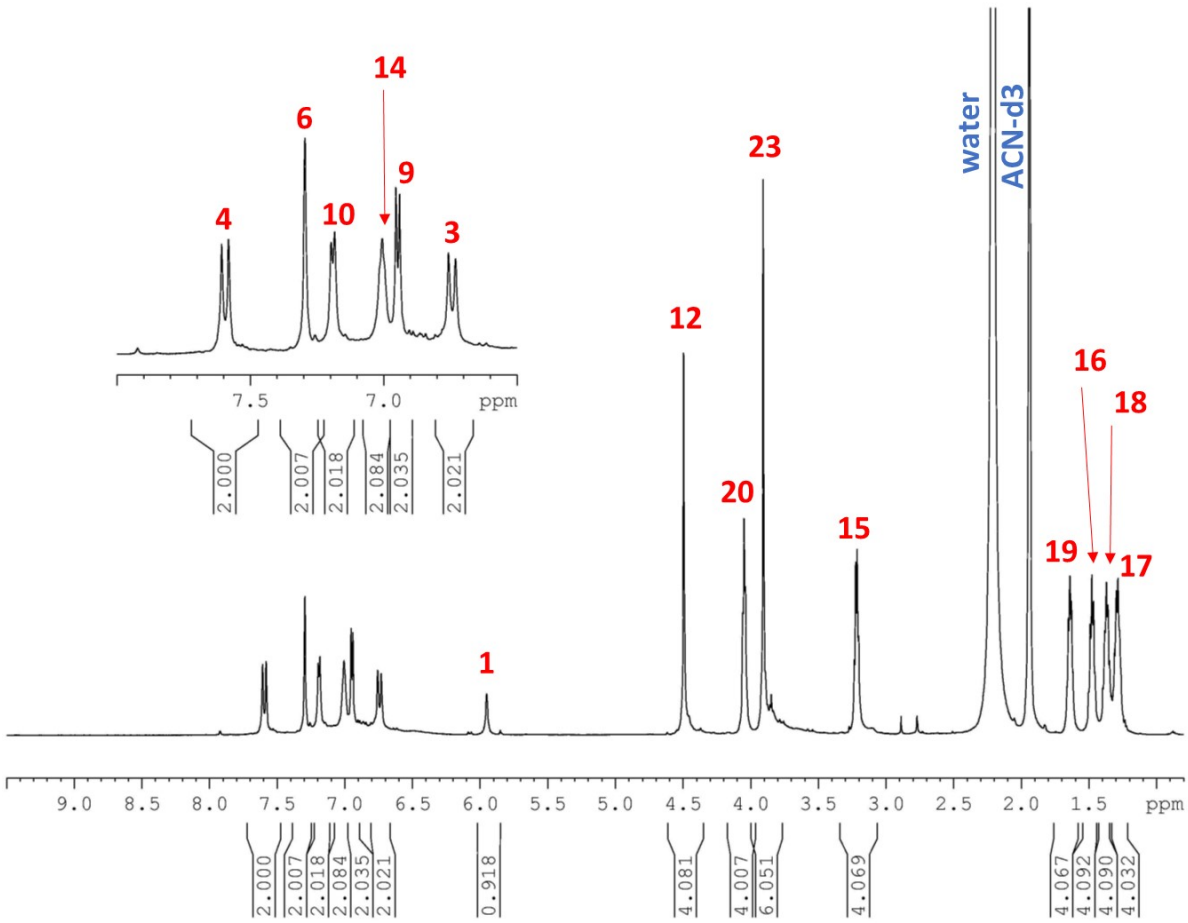


^{1}H-NMR (CD_3CN, 600 MHz, 310 K), δ ppm: 7.60 (d, *J*=15.9 Hz, 2H, H4), 7.30 (s, 2H, H6), 7.19 (d, *J*=8.0 Hz, 2H, H10), 7.01 (t, br, 2H, H14), 6.95 (d, *J*= 8.0 Hz, 2H, H9), 6.75 (d, *J*=15.9 Hz, 2H, H3), 5.95 (s, 1H, H1), 4.50 (s, 4H, H12), 4.05 (t, *J*= 5.6 Hz, 4H, H20), 3.91 (s, 6H, H23), 3.22 (q, *J*= 6.6 Hz, 4H, H15), 1.64 (m, 4H, H19), 1.48 (m, 4H, H16), 1.37 (m, 4H, H18), 1.29 (m, 4H, H17).

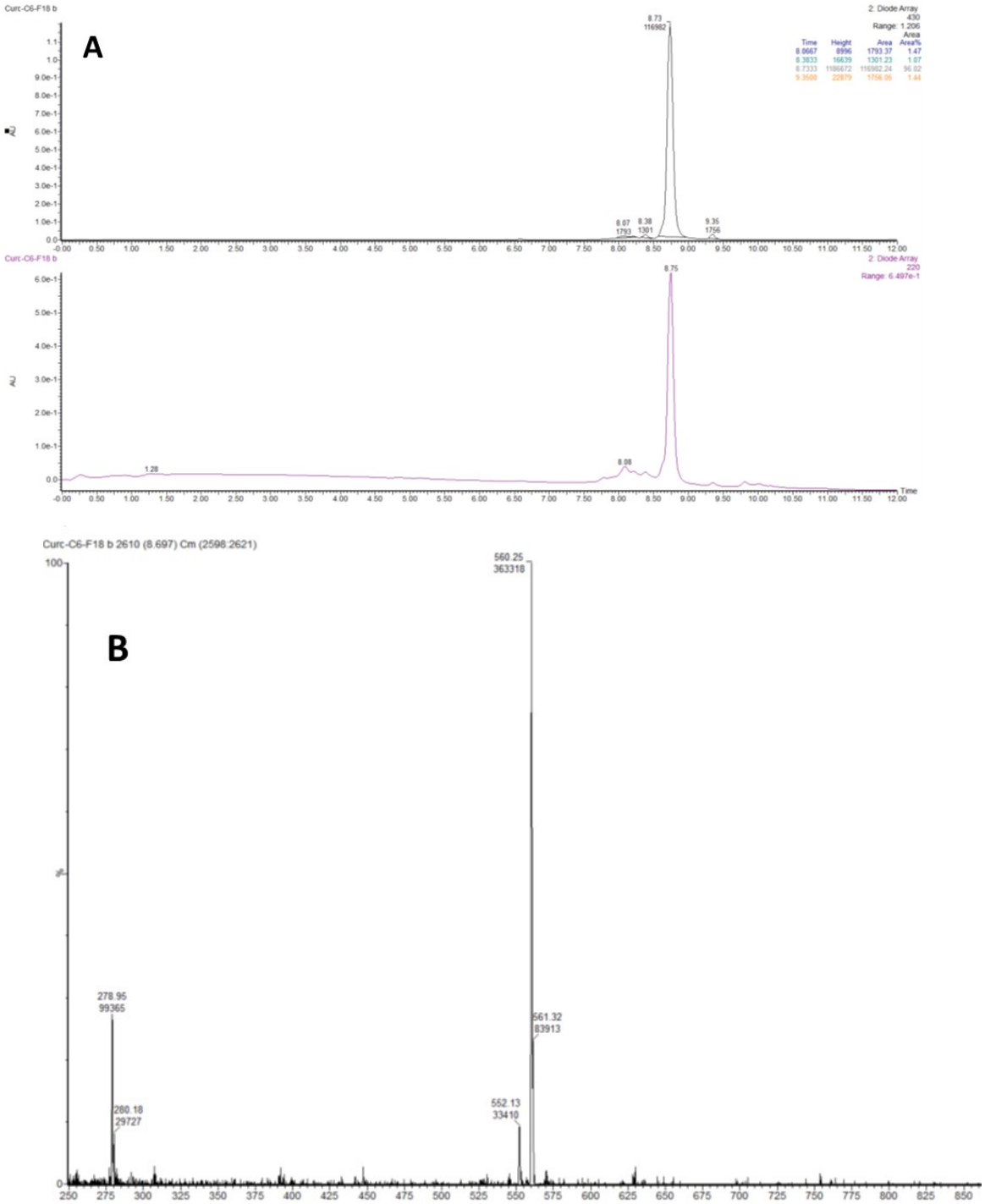
^{19}F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K), δ ppm (relative to TFA -76.55 ppm): -71.84 (s)



^{19}F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K)

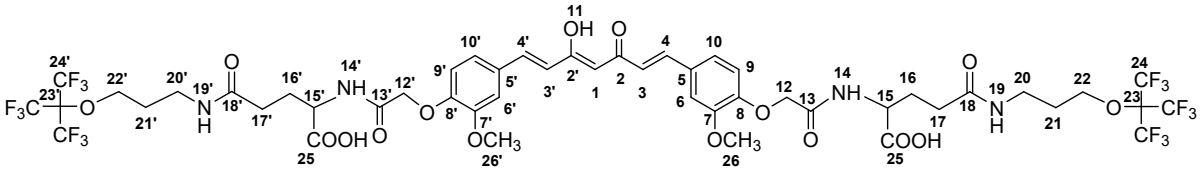


^{1}H-NMR (600 MHz, CD_3CN, 310 K)



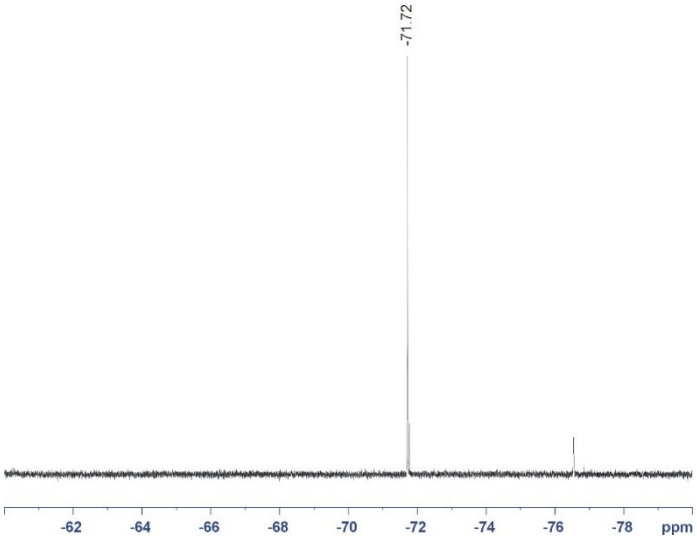
A) UV chromatogram of compound **Curc-C_6-F18** *recorded at 220 nm and 413 nm (retention time of 8.73 min and purity of 96%); B) Mass spectrum (ESI+) of the peak at 8.73 min.*

2.11. Compound Curc-Glu-F18

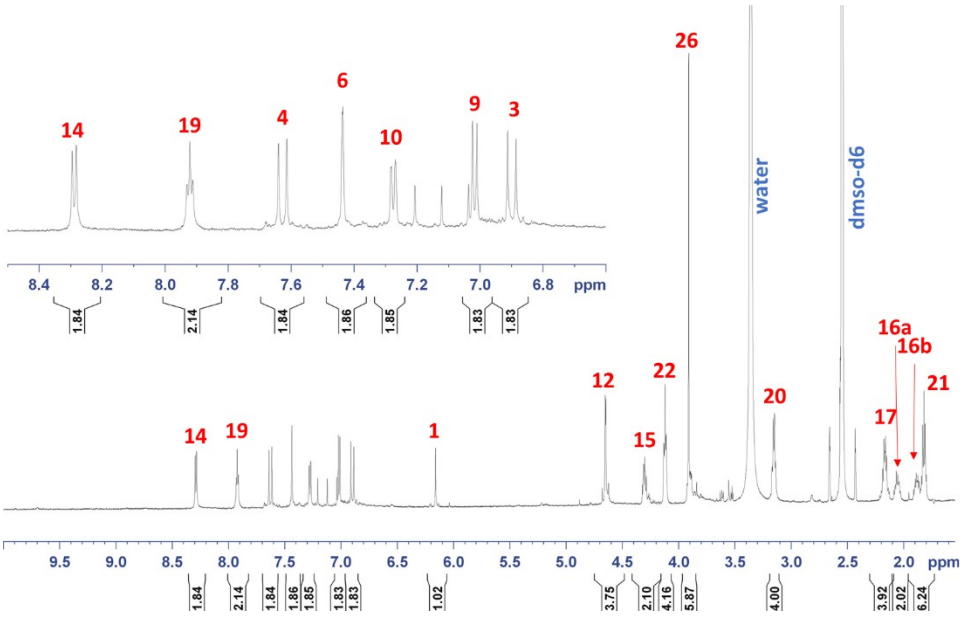


[1]H-NMR (600 MHz, dmso-d_6, 300 K), δ ppm: 8.29 (d, *J*=7.7 Hz, 2H, H14), 7.92 (t, *J*=5.6 Hz, 2H, H19), 7.62 (d, *J*=15.8 Hz, 2H, H4), 7.43 (d, *J*=1.5 Hz, 2H, H6), 7.27 (dd, *J*=8.4 and 1.5 Hz, 2H, H10), 7.01 (d, *J*=8.4 Hz, 2H, H9), 6.90 (d, *J*=15.8 Hz, 2H, H3), 6.15 (s, 1H, H1), 4.65 (AB system, 4H, H12), 4.30 (m, 2H, H15), 4.11 (t, *J*=6.2 Hz, 4H, H22), 3.91 (s, 6H, H26), 3.15 (m, 4H, H20), 2.17 (m, 4H, H17), 2.06 (m, 2H, H16a), 1.88 (m, 2H, H16b), 1.82 (m, *J*=6.5 Hz, 4H, H21).

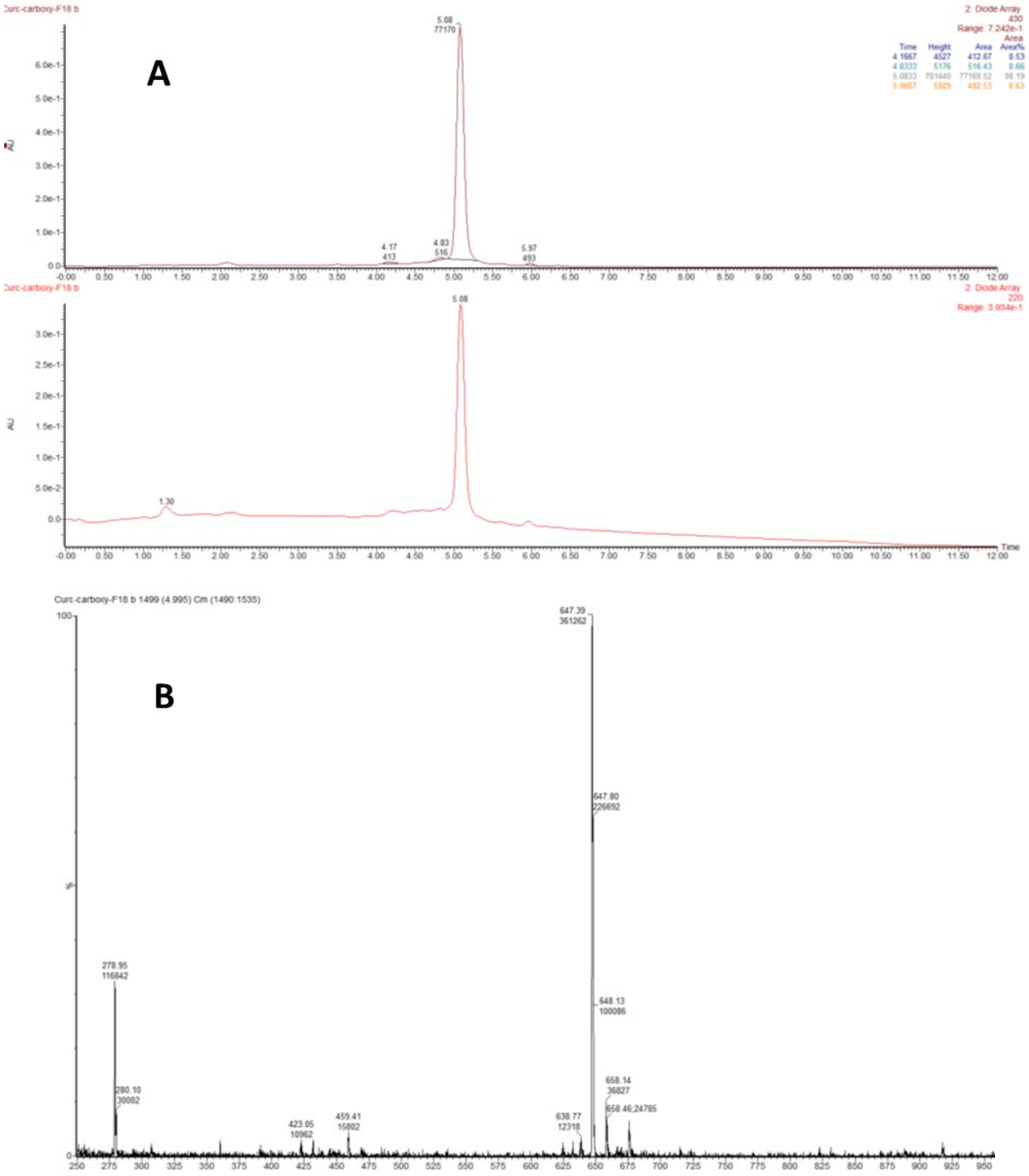
[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K), δ ppm (relative to TFA -76.55 ppm): -71.72 (s)



[19]F-NMR (470 MHz, ethanol/dmso-d_6 550:50, 298 K)

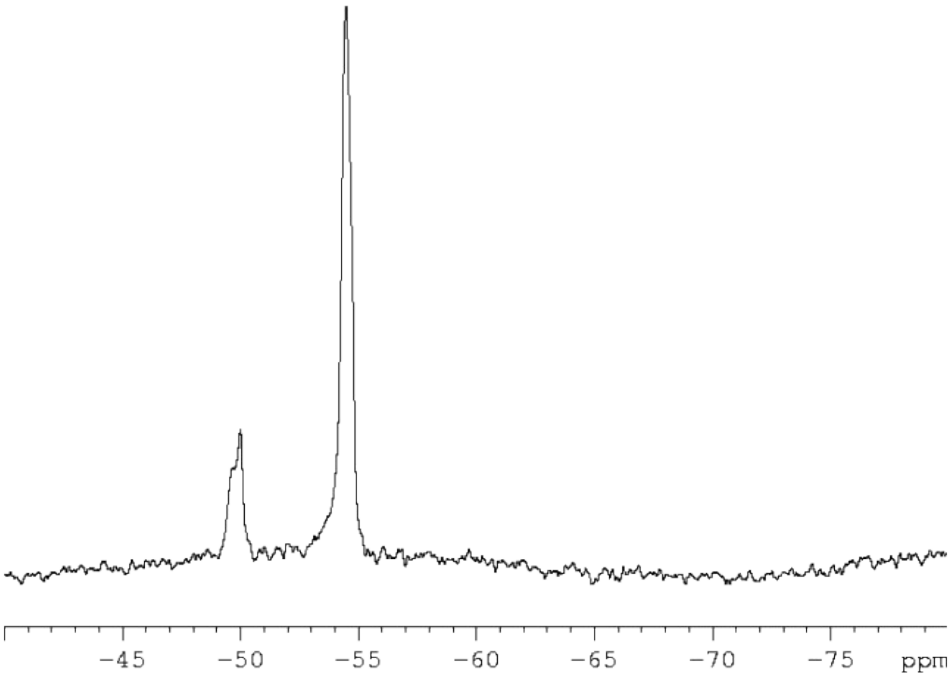


[1]H-NMR (600 MHz, dmso-d_6, 300 K)

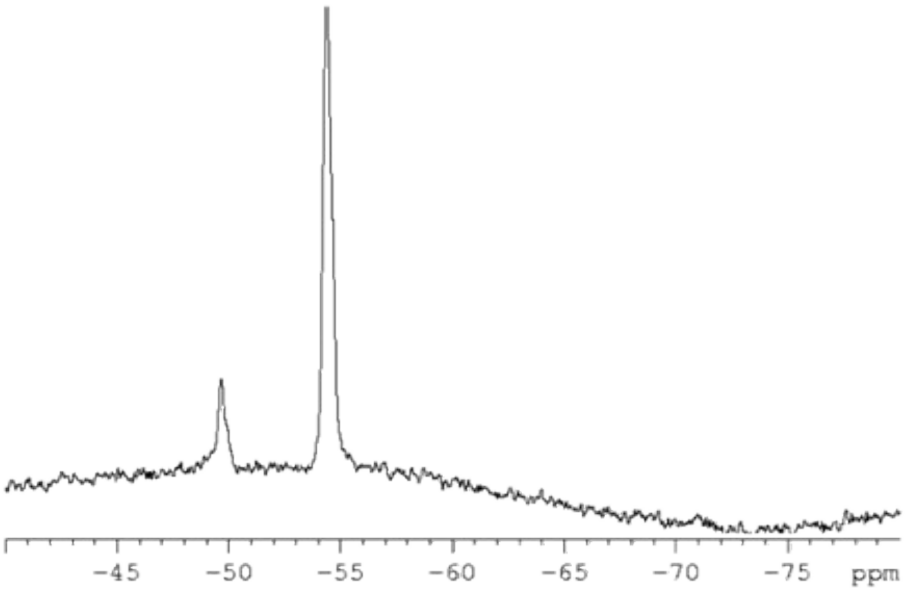


A) UV chromatogram of compound ***Curc-Glu-F18*** *recorded at 220 nm and 413 nm (retention time of 5.08 min and purity of 98%); B) Mass spectrum (ESI+) of the peak at 5.08 min.*

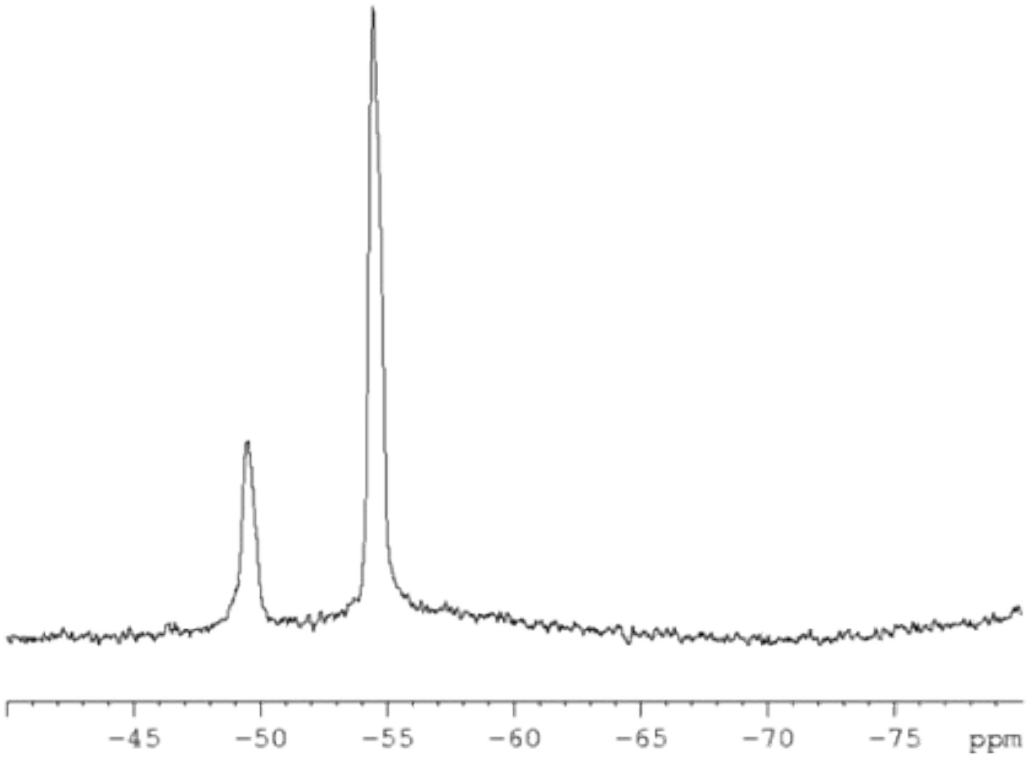
2.12. ^{19}F-NMR (300 MHz, 298 K)



^{19}F-NMR of Curc-C_6-F9 plus TFA

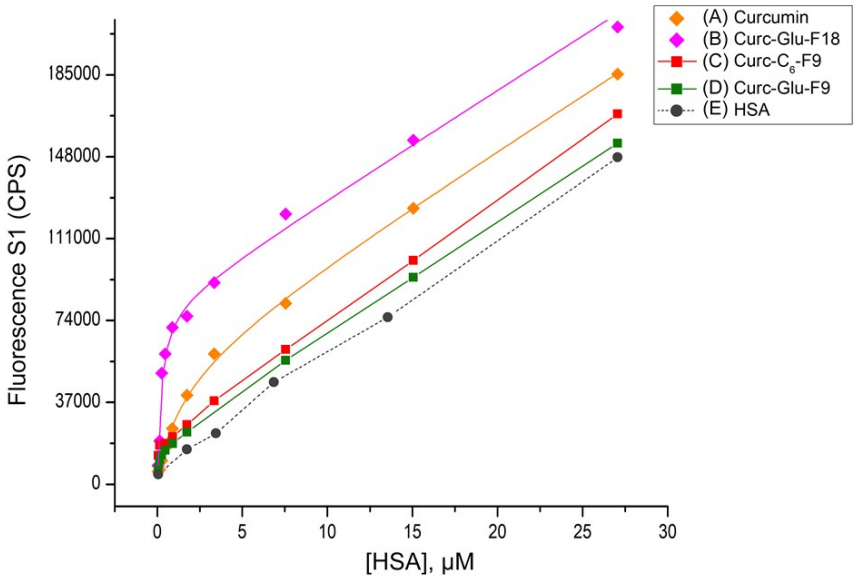


^{19}F-NMR of Curc-Glu-F9 plus TFA



^{19}F-NMR of Curc-Glu-F18 plus TFA

3. Binding assay to Human Serum Albumin (HSA)



Fluorescence emission (λex=430 nm, λem=485 nm) of Curcumin and its derivatives: (A) Curcumin R^2=0.999; (B) Curc-Glu-F18 R^2=0.991, (C) Curc-C_6-F9 (no plot); (D) Curc-Glu-F9 (no plot); (E) HSA.