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# UCC

**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh

## **Binary mixtures of flufenamic acid and its chloro, difluoromethyl and fluoromethyl analogues; incorporation and distribution in flufenamic acid crystals.**

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## Synthesis and Characterisation

*Infra-red spectra* were recorded on a Perkin Elmer Spectrum 100 FT-IR Spectrometer using thin films between sodium chloride discs following evaporation of a solution of a small amount of the solid sample in a volatile solvent.

*Nuclear Magnetic Resonance* spectra were recorded, for  $^1\text{H}$  NMR, at 300 MHz, and for  $^{13}\text{C}$  NMR at 75.5 MHz, on a Bruker AVANCE 300 NMR spectrometer.

*Mass spectrometry:* Nominal mass spectra were recorded on a Waters Quattro Micro Triple Quadrupole Spectrometer (QAA1202) in ESI mode using 50% acetonitrile / water containing 0.1% formic acid as an eluent. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier Time of Flight spectrometer (KD160) or a Waters Vion IMS Mass Spectrometer (SAA0 55K) in ESI mode using 50% acetonitrile / water containing 0.1% formic acid as an eluent. Mass spectrometry samples were prepared in acetonitrile.

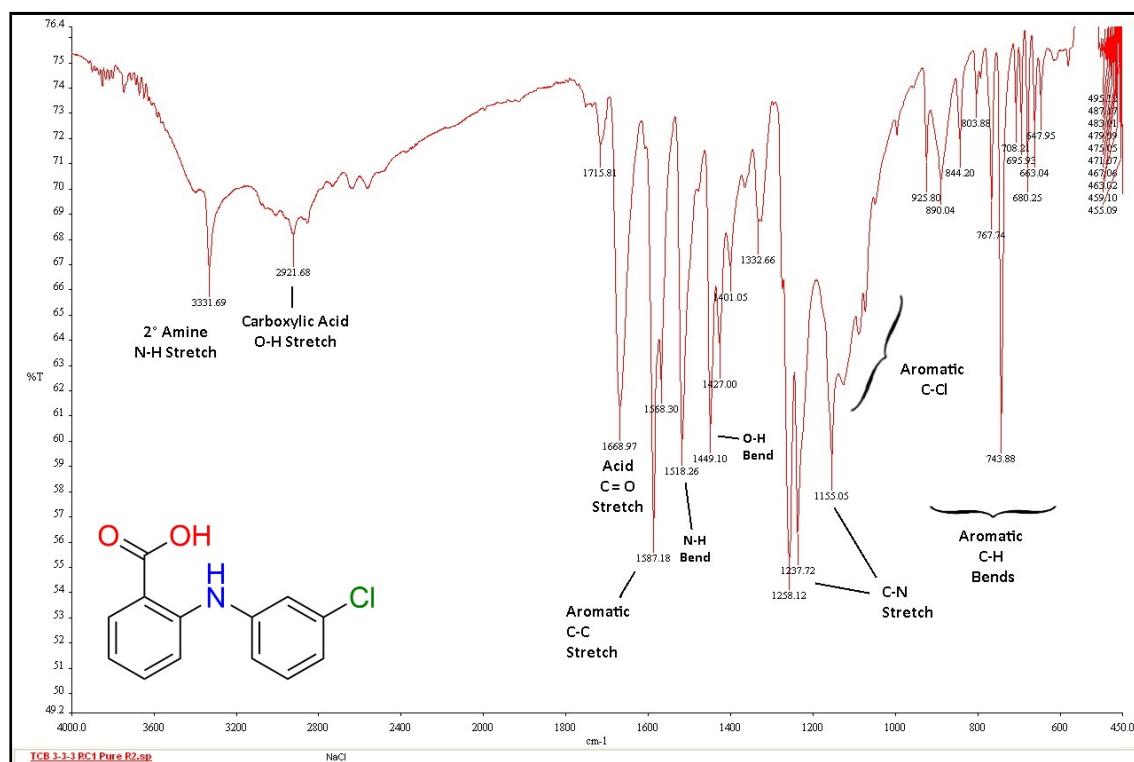


Fig. S1 IR spectrum of CIFA.

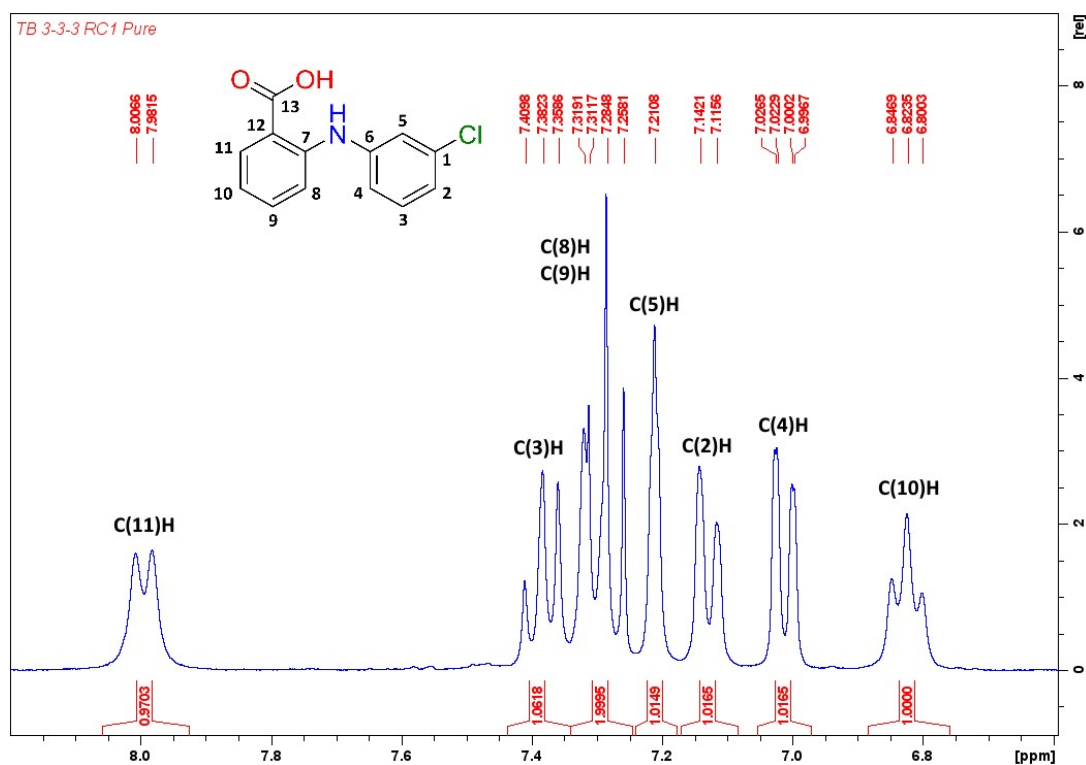


Fig. S2. Aromatic region of the  $^1\text{H}$  NMR spectrum of ClFA.

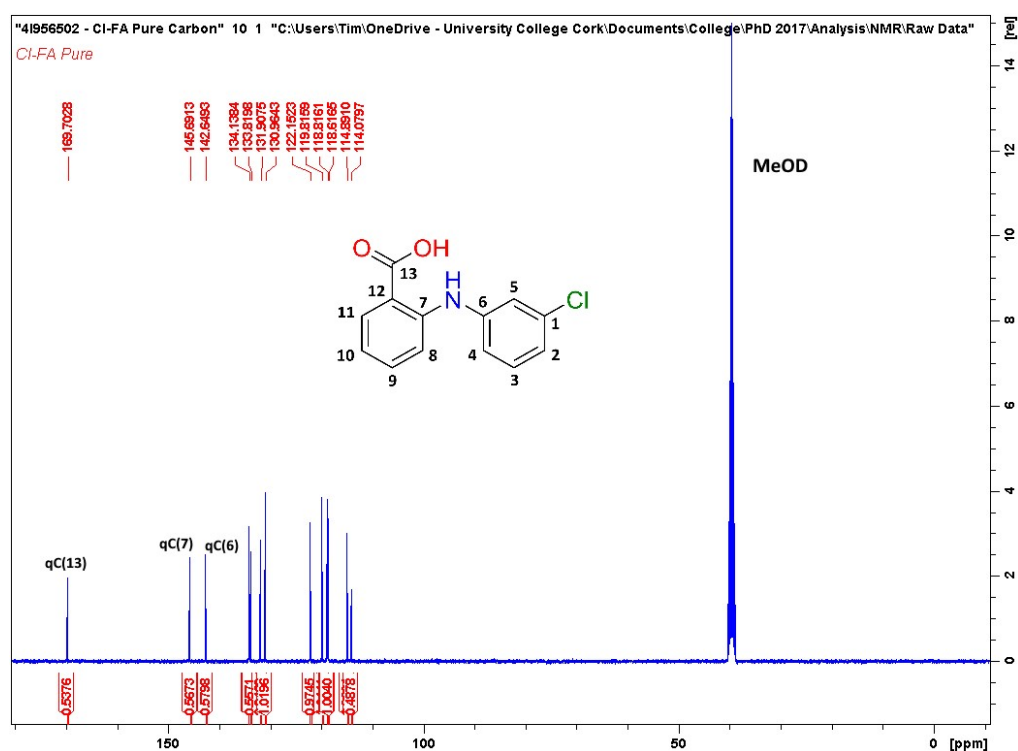


Fig. S3. Full  $^{13}\text{C}$  NMR spectrum of ClFA.

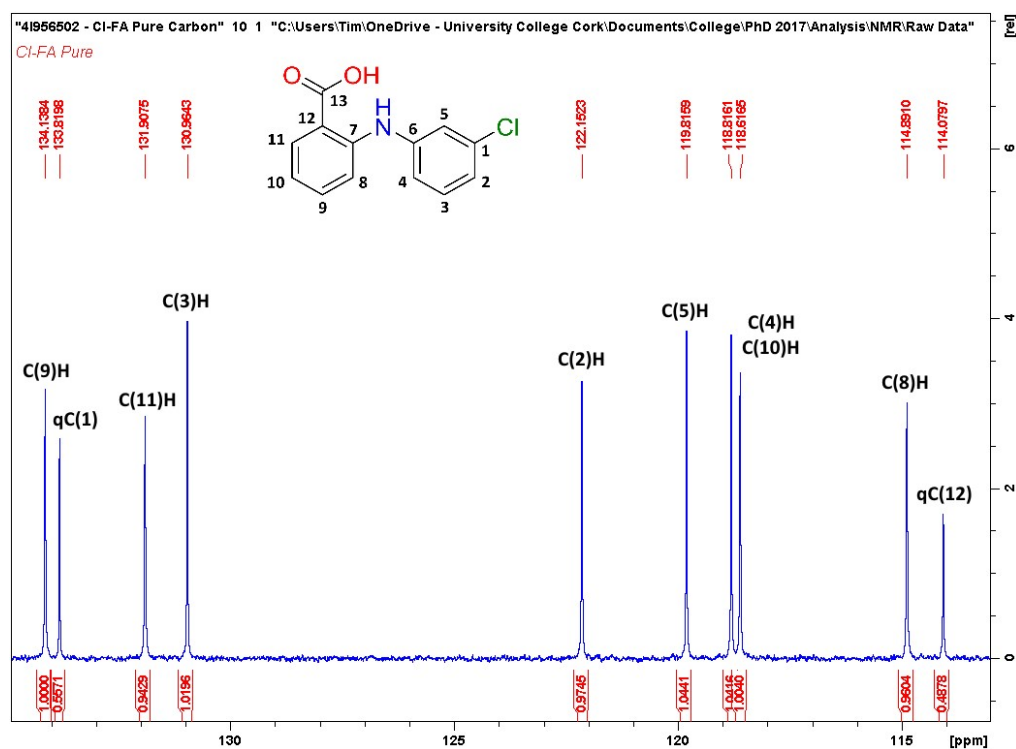


Fig. S4. Aromatic region of the  $^{13}\text{C}$  NMR spectrum of Cl-FA.

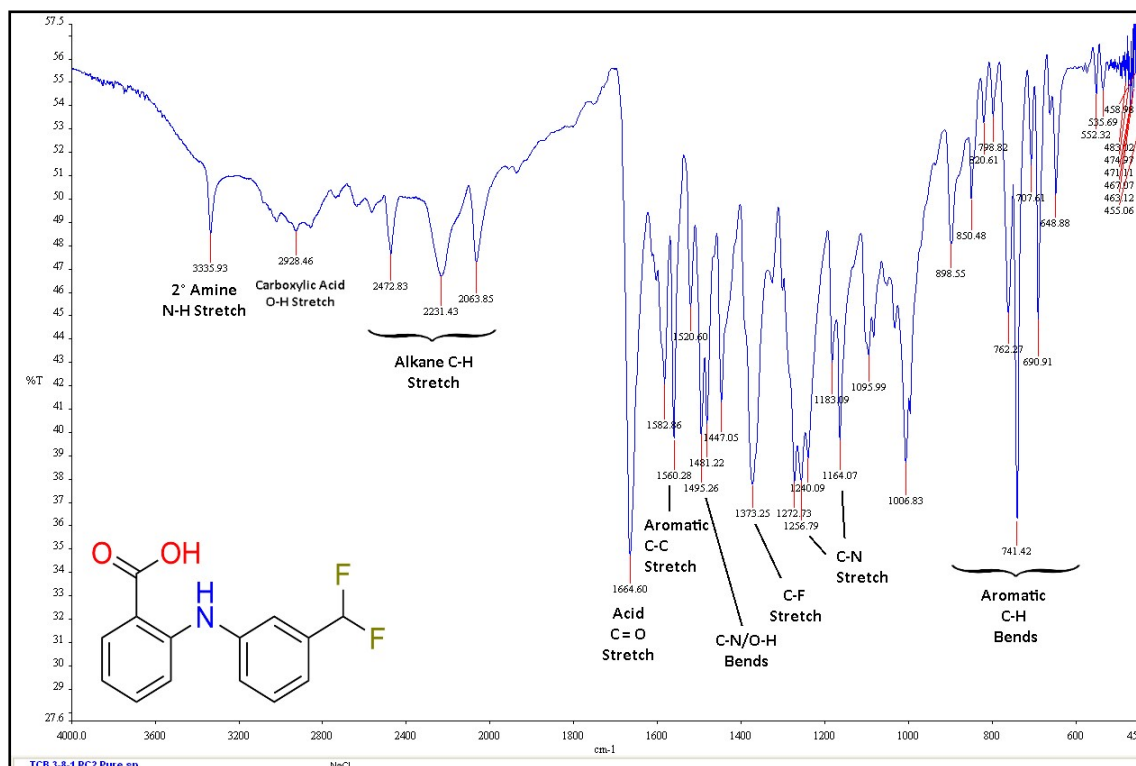


Fig. S5. IR spectrum of HF<sub>2</sub>FA.

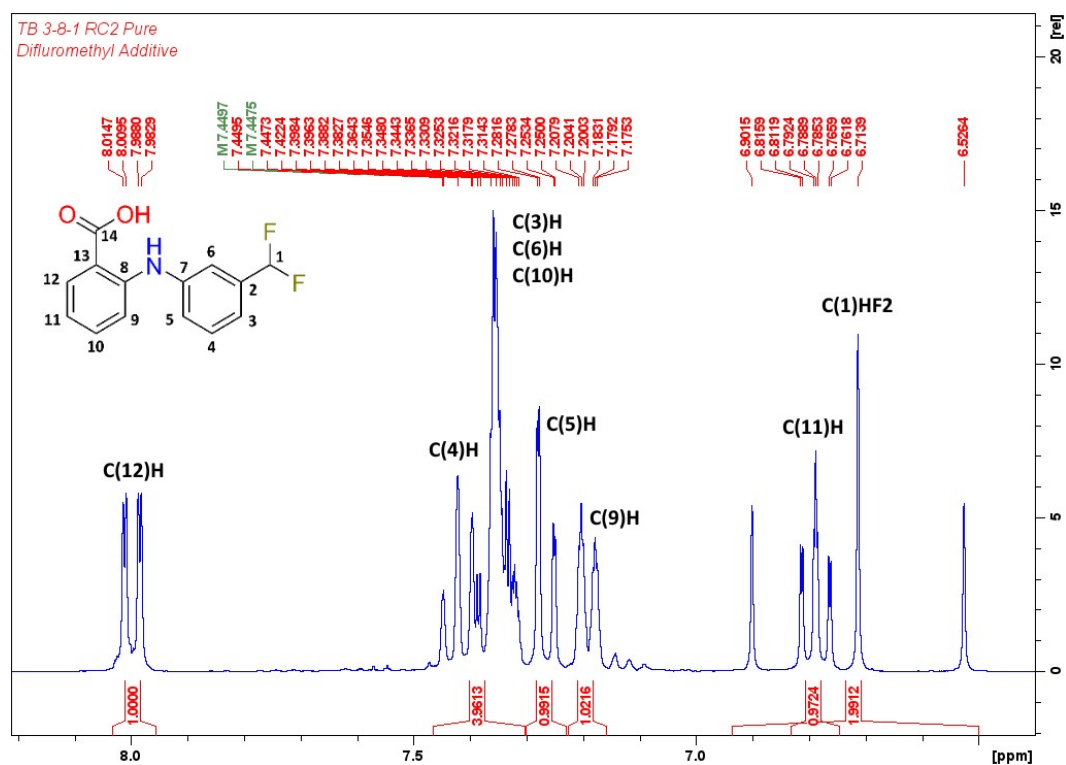


Fig. S6. Aromatic region of the <sup>1</sup>H NMR spectrum of HF<sub>2</sub>FA.

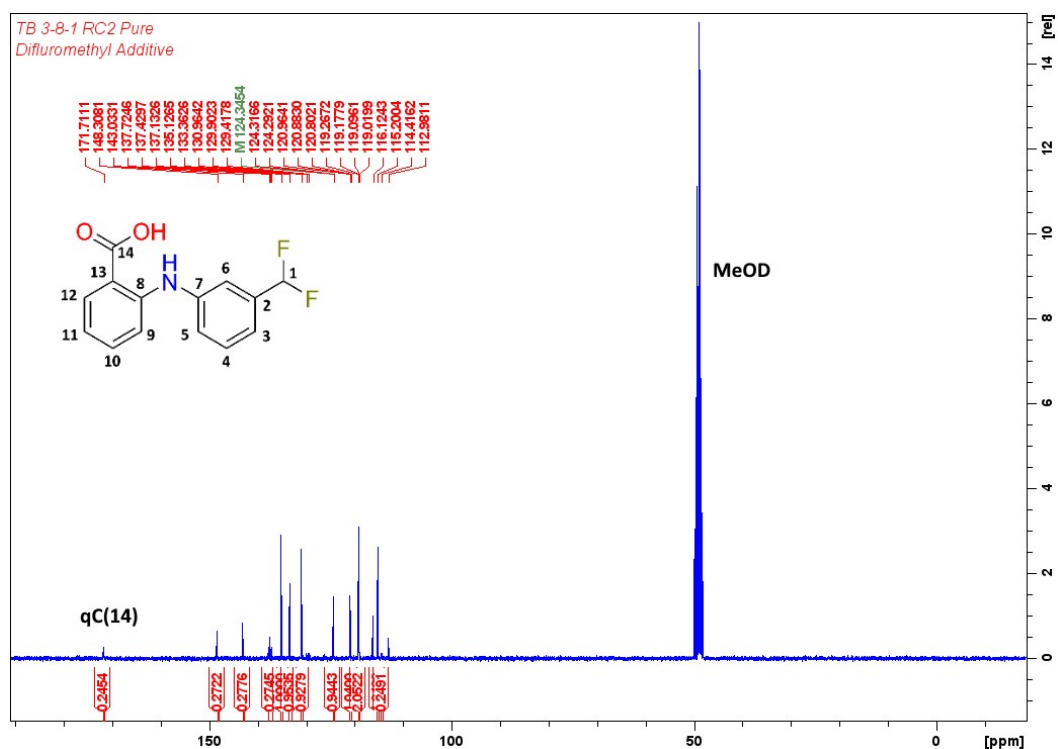


Fig. S7. Full <sup>13</sup>C NMR spectrum of HF<sub>2</sub>FA.

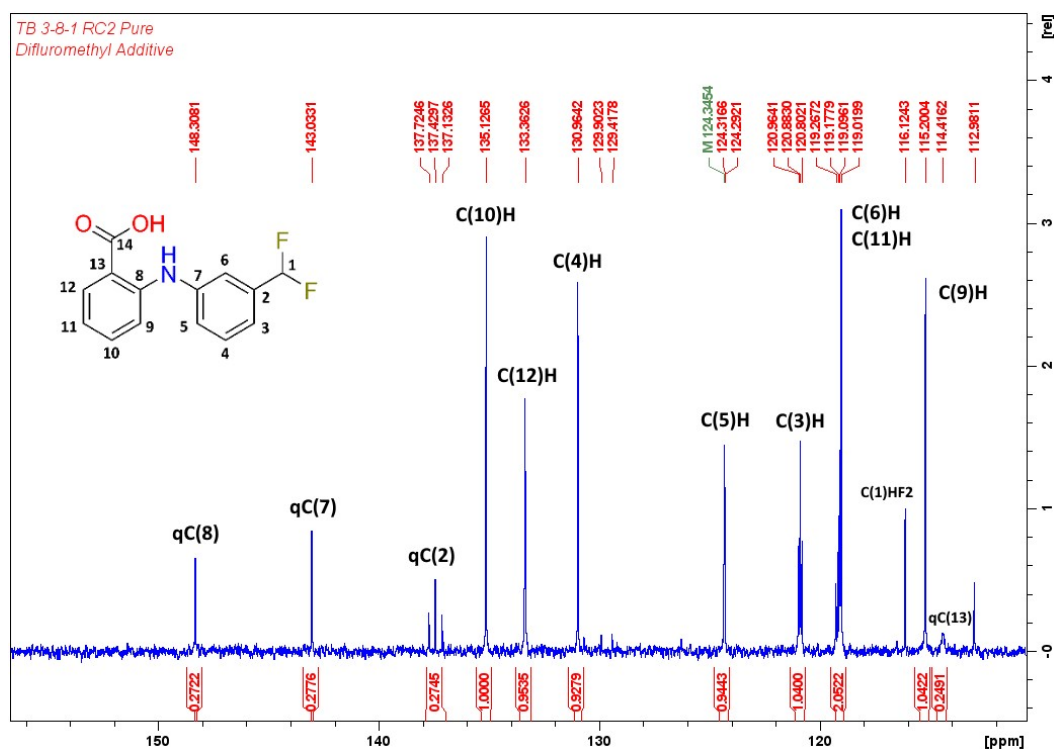


Fig. S8. Aromatic region of the <sup>13</sup>C NMR spectrum of HF<sub>2</sub>FA.

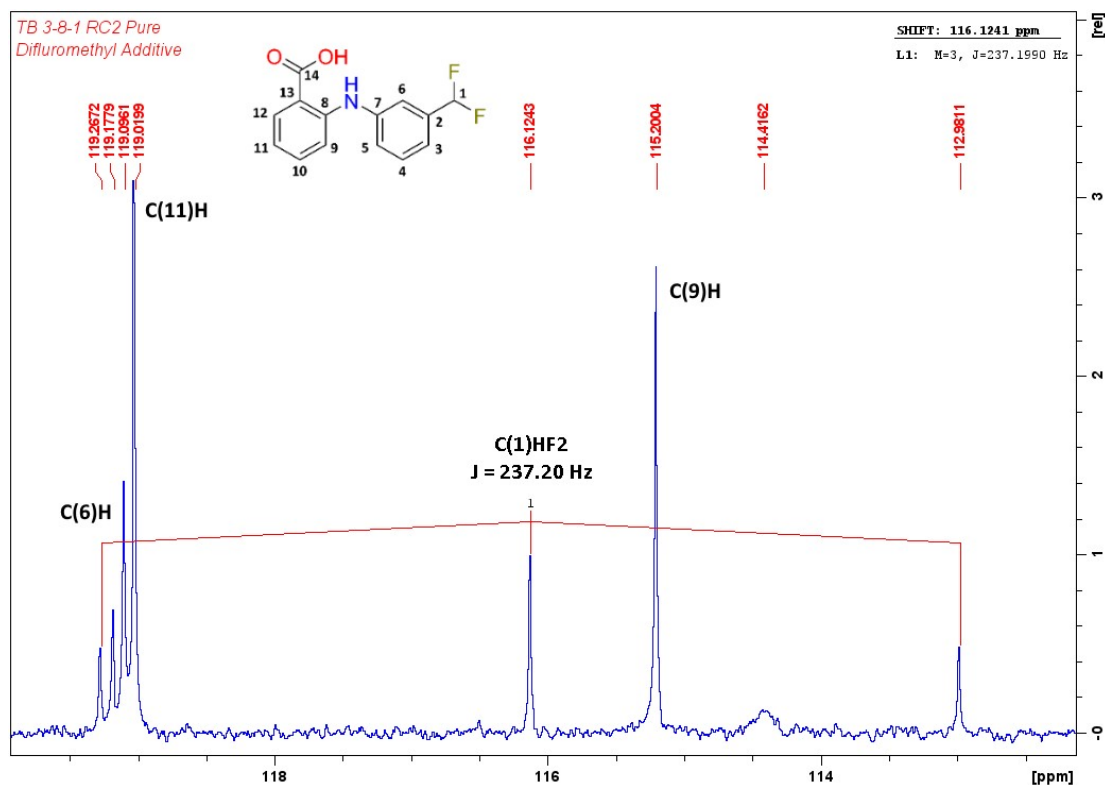


Fig. S9. A portion of the aromatic region of the <sup>13</sup>C NMR spectrum of HF<sub>2</sub>FA, illustrating the large splitting of the C<sup>1</sup> carbon peak due to coupling with two geminal fluorine atoms.

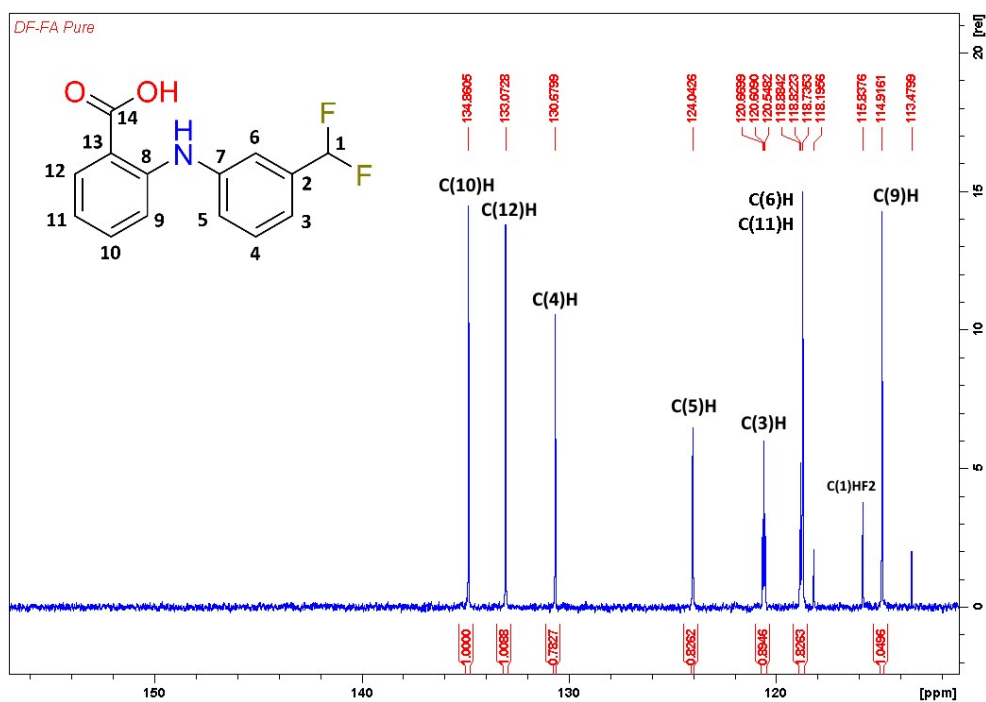


Fig. S10. Aromatic region of the DEPT 90 NMR spectrum of HF<sub>2</sub>FA.

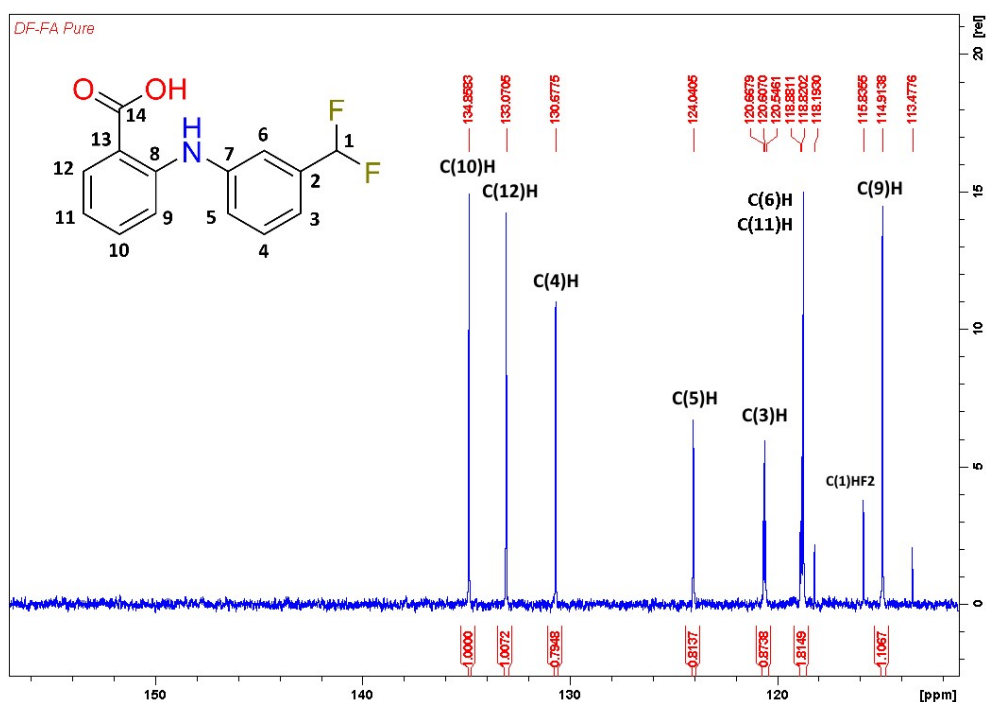


Fig. S11. Aromatic region of the DEPT 135 NMR spectrum of HF<sub>2</sub>FA.

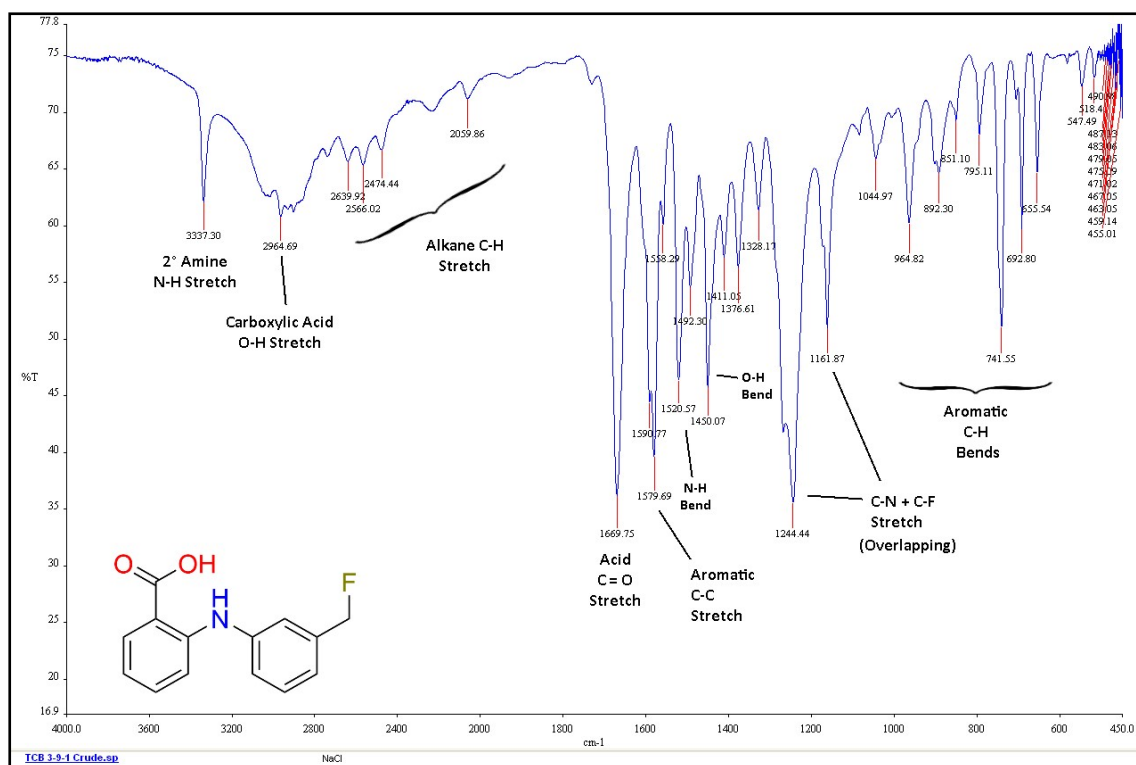


Fig. S12. IR spectrum of H<sub>2</sub>FFA.

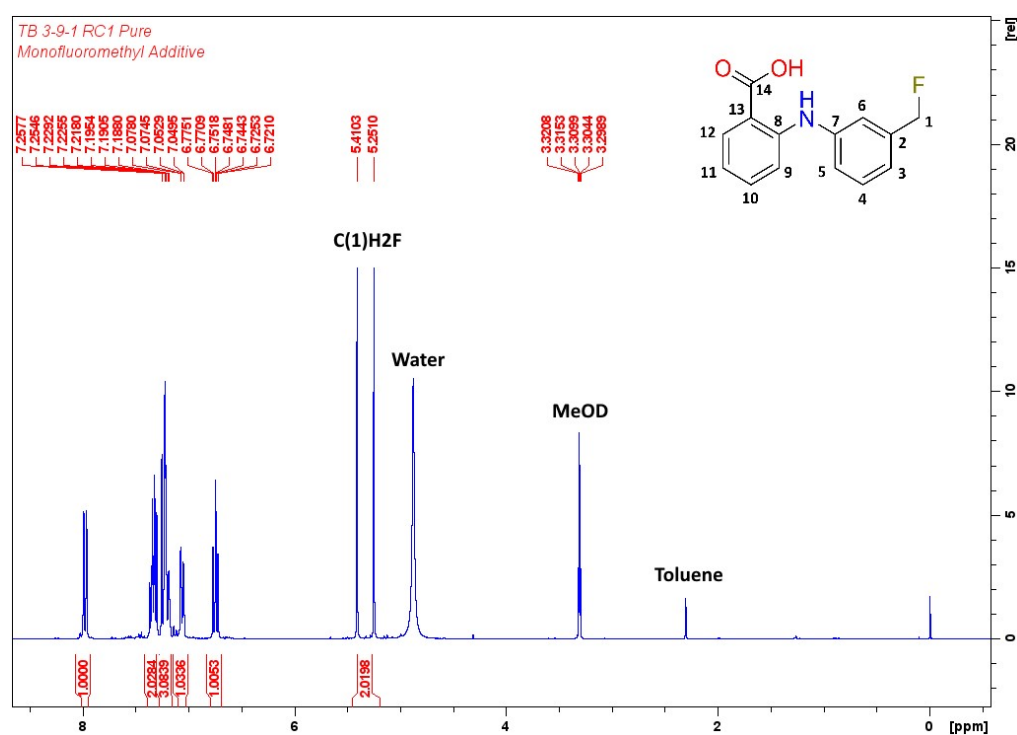


Fig. S13. Full <sup>1</sup>H NMR spectrum of H<sub>2</sub>FFA showing the position of the C<sup>1</sup>H<sub>2</sub>F doublet signal outside the aromatic region.

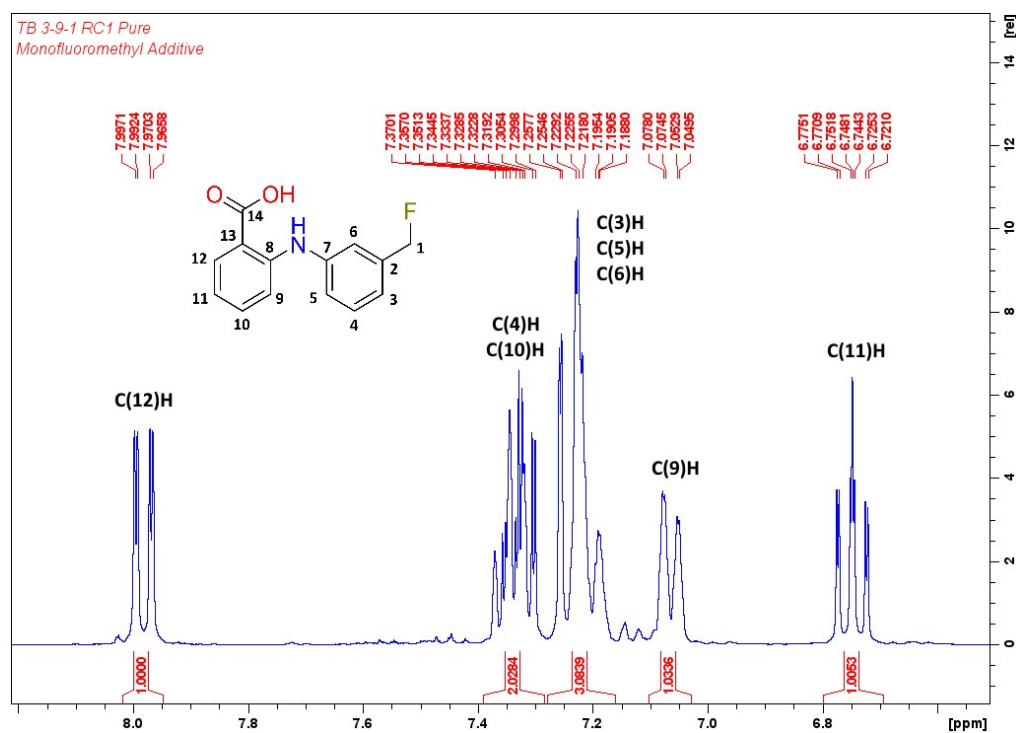


Fig. S14. Aromatic region of the  $^1\text{H}$  NMR spectrum of  $\text{H}_2\text{FFA}$ .

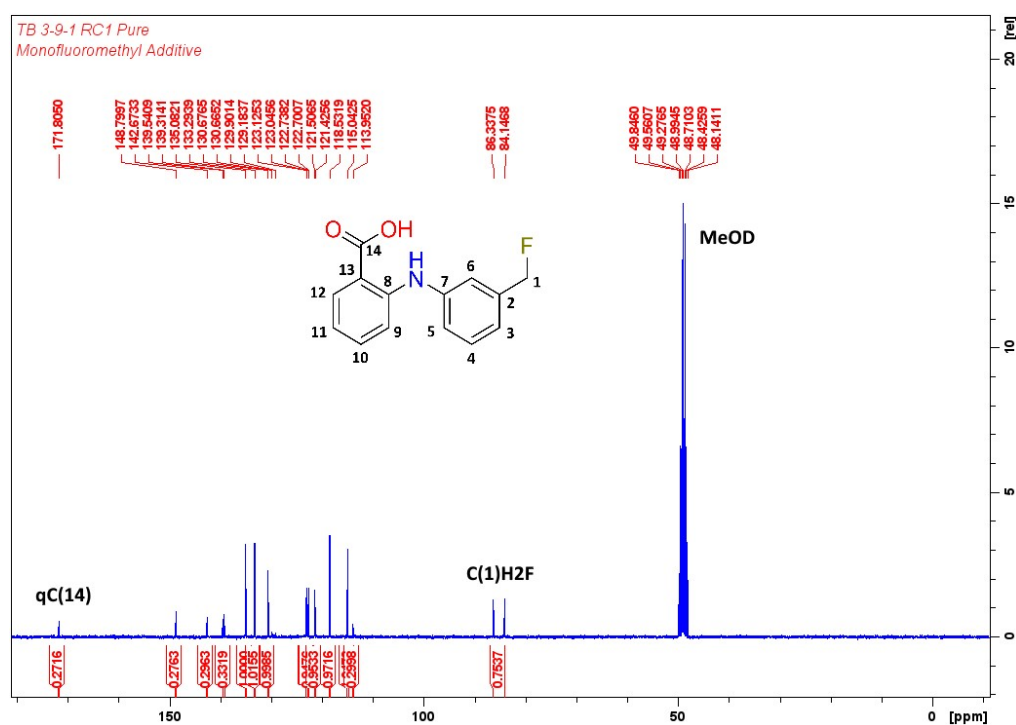


Fig. S15. Full  $^{13}\text{C}$  NMR spectrum of  $\text{H}_2\text{FFA}$  showing the position of one quaternary carbon signal as well as the  $\text{C}^1\text{H}_2\text{F}$  doublet signal outside the aromatic region.

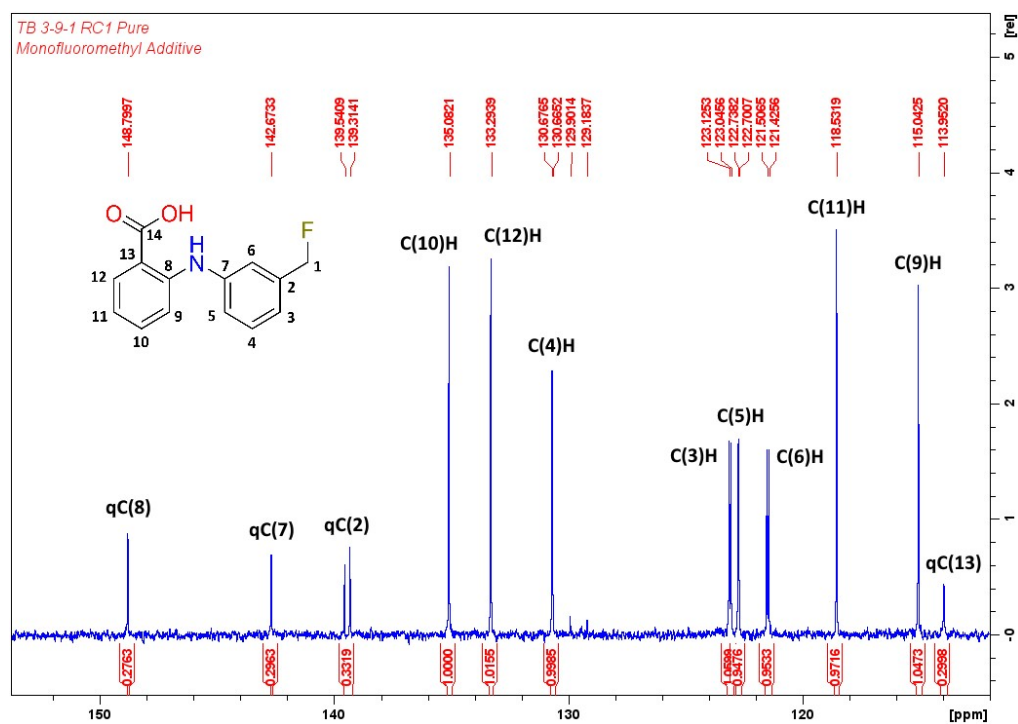


Fig. S16. Aromatic region of the  $^{13}\text{C}$  NMR spectrum of  $\text{H}_2\text{FFA}$ .

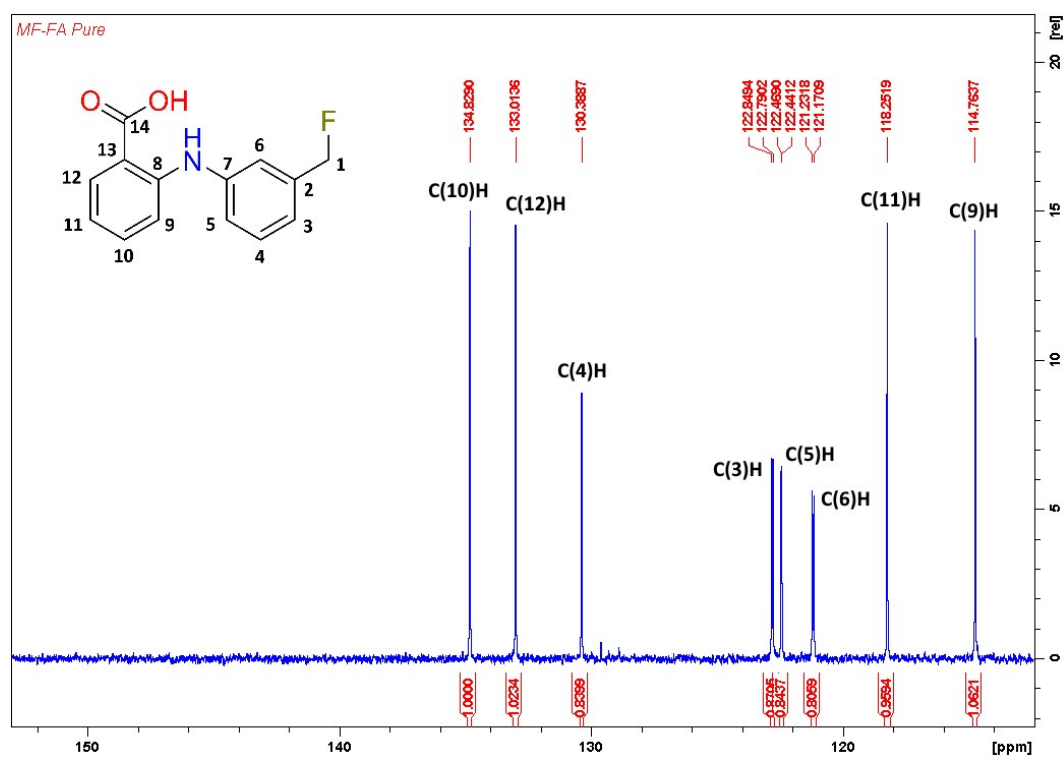


Fig. S17. Aromatic region of the DEPT 90 NMR spectrum of  $\text{H}_2\text{FFA}$ .

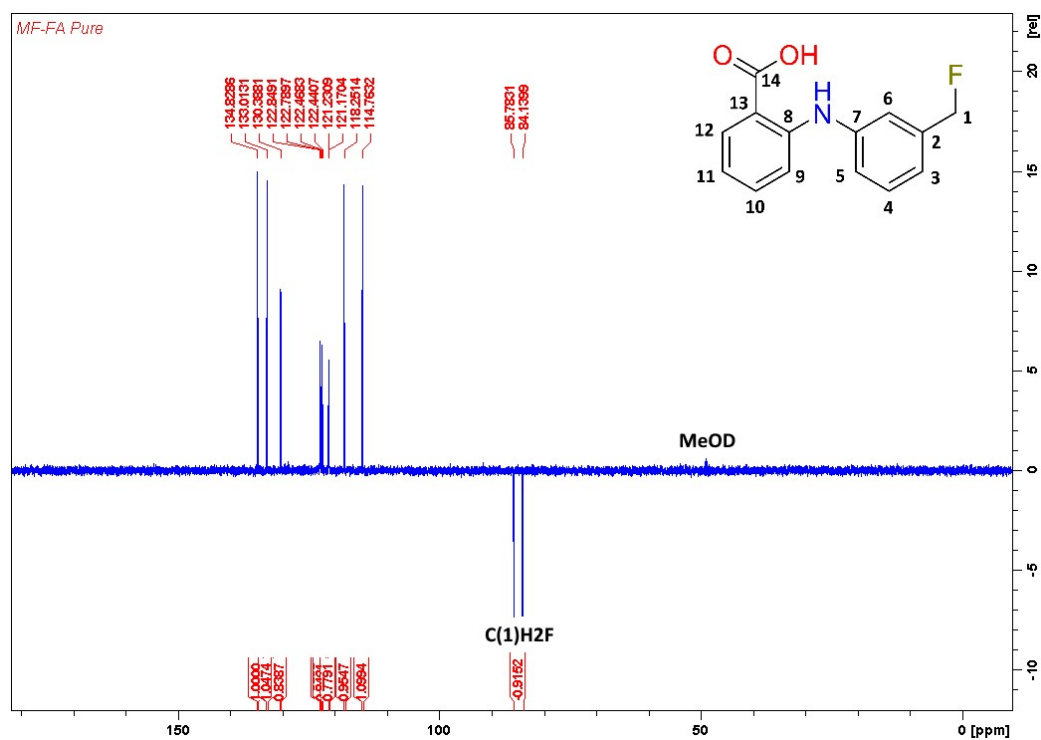


Fig. S18. Full DEPT 135 NMR spectrum of  $\text{H}_2\text{FFA}$ , with labelled peaks.

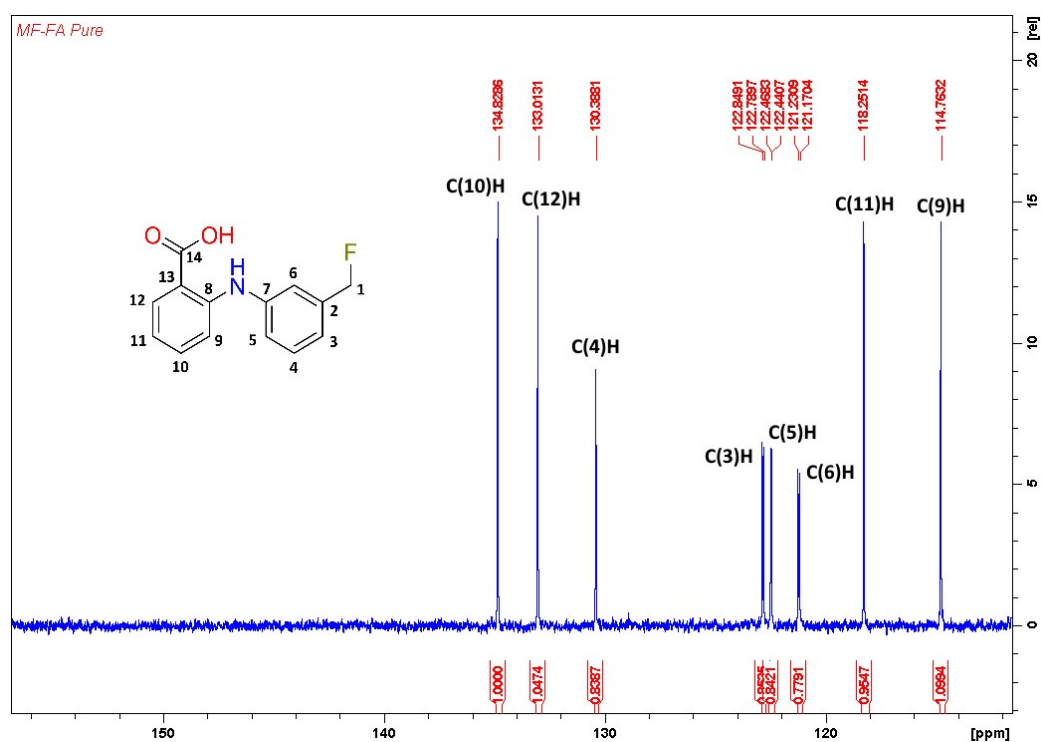
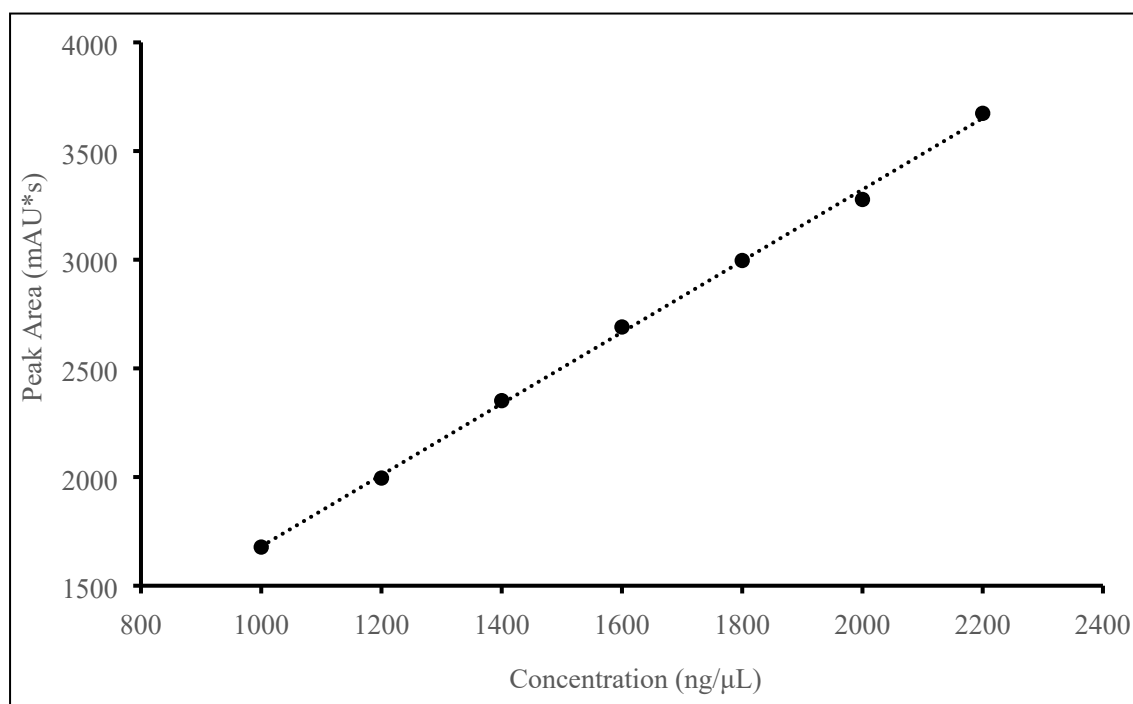


Fig. S19. Aromatic region of the DEPT 135 NMR spectrum of  $\text{H}_2\text{FFA}$ .

## HPLC

### Calibration curves

Four to seven standard solutions were prepared for each calibration curve. Standard solutions were produced by dissolving an appropriate mass of the material of interest in 100 mL of MeCN with thorough mixing, so that the concentrations of each standard fell between 0.25 and 2.20 mg mL<sup>-1</sup>. Three aliquots were removed from each standard for analysis by HPLC. Each aliquot was analysed in triplicate by HPLC. The resulting calibration curves and statistics are shown in Figs. S20 to S23 and Tables S1 to S8 below. Examples of HPLC traces of solution of **FFA** containing quantities of either **CIFA**, **HF<sub>2</sub>FA** or **H<sub>2</sub>FFA** are shown in Figs. S24 to S26 below.



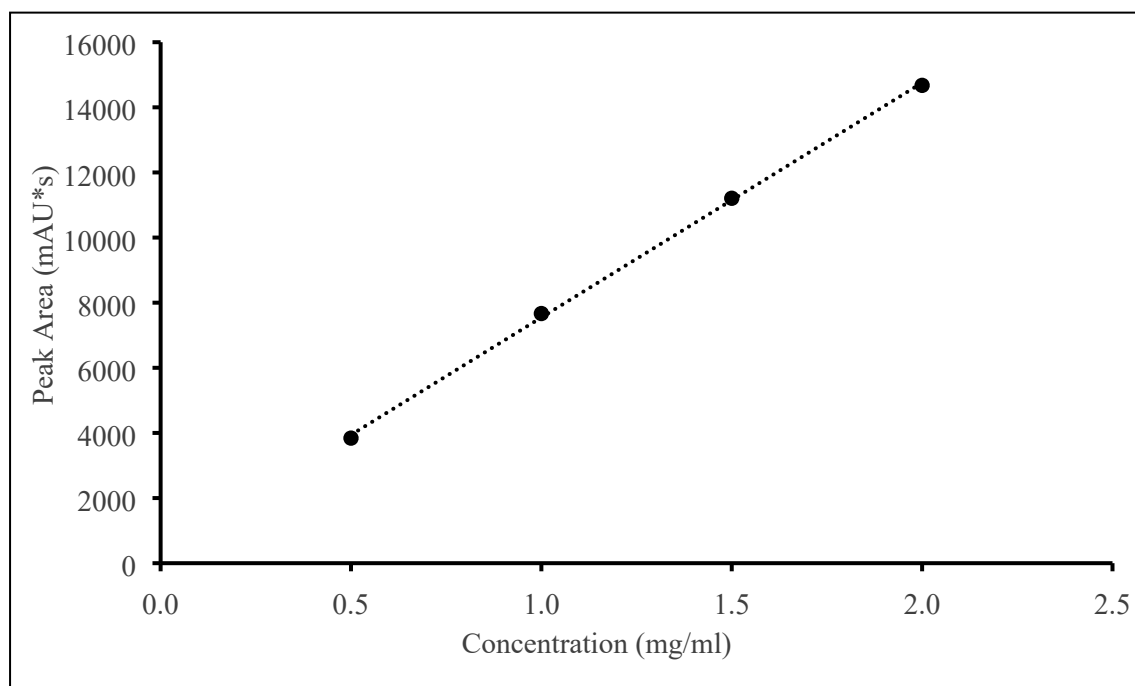
**Fig. S20.** Linear regression curve fitted to data points for standard solution of commercial **FFA** and the equation of that curve.

**Table S1** Regression statistics for the commercial **FFA** calibration curve, including *r* values.

|                   |            |
|-------------------|------------|
| Multiple R        | 0.9994083  |
| R Square          | 0.9988169  |
| Adjusted R Square | 0.9985802  |
| Standard Error    | 26.7487195 |
| Observations      | 7          |

**Table S2.** Regression coefficient statistics for the commercial **FFA** calibration curve.

|                                        | Coefficients | Standard Error | t Stat | P-value  | Lower 95.0% | Upper 95.0% |
|----------------------------------------|--------------|----------------|--------|----------|-------------|-------------|
| Intercept                              | 38.661       | 41.685         | 0.927  | 0.396    | -68.494     | 145.81      |
| Concentration (ng $\mu\text{L}^{-1}$ ) | 1.642        | 0.025          | 64.970 | 1.64E-08 | 1.577       | 1.707       |



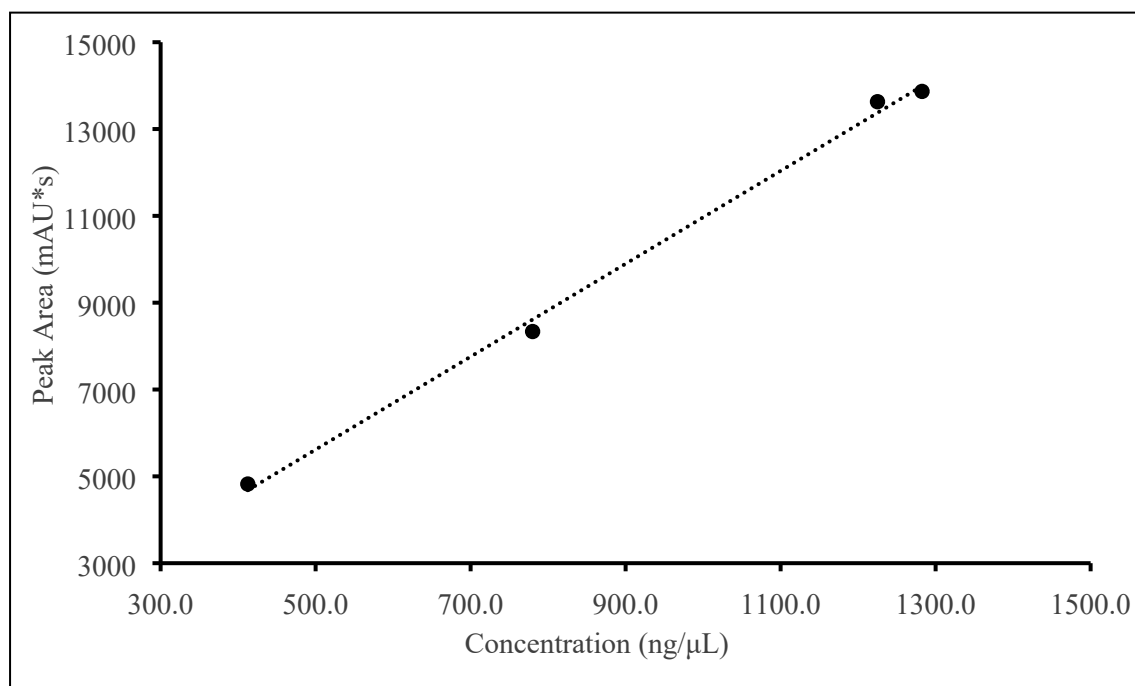
**Fig. S21.** Calibration Curve for standard solutions of **CIFA** with linear regression curve equation.

**Table S3.** Residual Statistics for **CIFA** Calibration Curve.

|                   |             |
|-------------------|-------------|
| Multiple R        | 0.9997352   |
| R Square          | 0.9994705   |
| Adjusted R Square | 0.9992057   |
| Standard Error    | 131.1707562 |
| Observations      | 4           |

**Table S4.** Residual Coefficients Statistics for CIFA calibration curve.

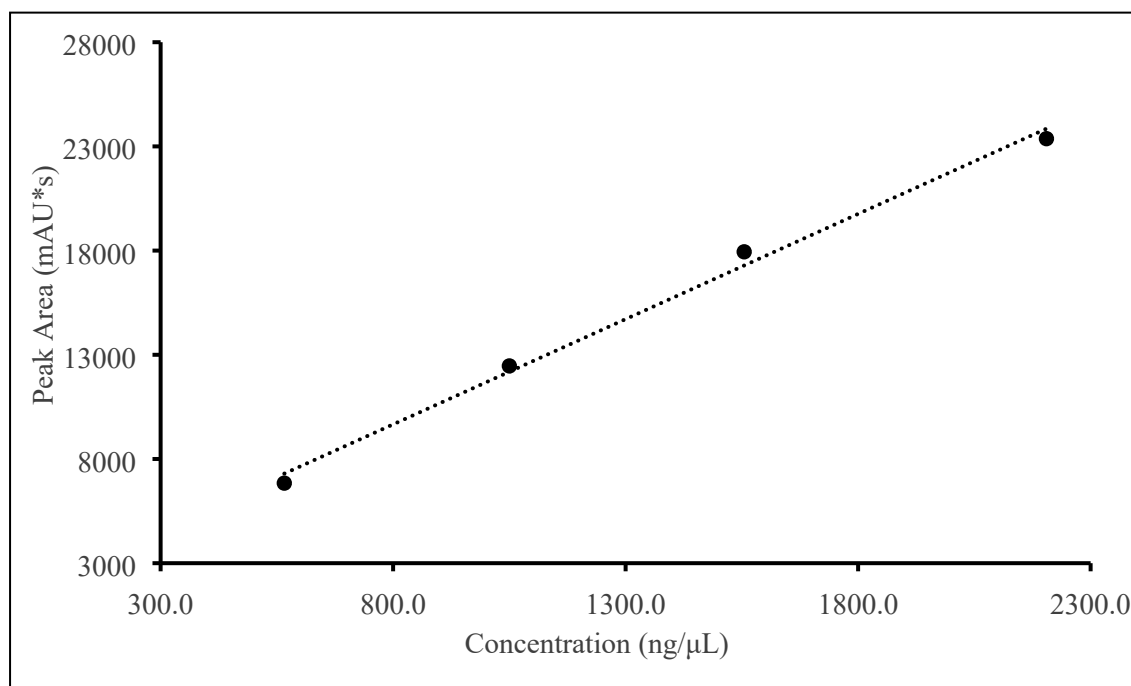
|                       | Coefficients | Standard Error | t Stat | P-value   | Lower 95.0% | Upper 95.0% |
|-----------------------|--------------|----------------|--------|-----------|-------------|-------------|
| Intercept             | 337.065      | 160.651        | 2.098  | 0.171     | -354.159    | 1028.289    |
| Concentration (mg/mL) | 7208.245     | 117.323        | 61.439 | 2.648E-04 | 6703.446    | 7713.044    |

**Fig. S22.** Calibration curve for **HF<sub>2</sub>FA** with linear regression curve equation.**Table S5.** Residual Statistics for **HF<sub>2</sub>FA** calibration curve.

|                   |             |
|-------------------|-------------|
| Multiple R        | 0.9984595   |
| R Square          | 0.9969214   |
| Adjusted R Square | 0.9953820   |
| Standard Error    | 297.7301230 |
| Observations      | 4           |

**Table S6.** Residual Coefficients Statistics for **HF<sub>2</sub>FA** Calibration Curve.

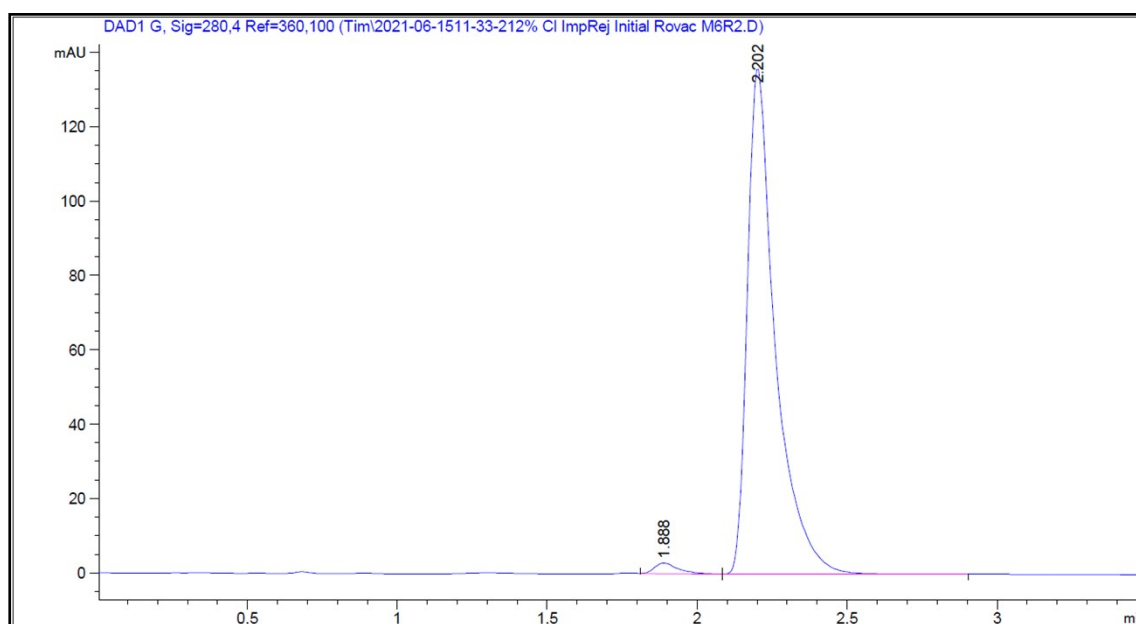
|                             | Coefficients | Standard Error | t Stat | P-value | Lower 95.0% | Upper 95.0% |
|-----------------------------|--------------|----------------|--------|---------|-------------|-------------|
| Intercept                   | 266.827      | 416.415        | 0.641  | 0.587   | -1524.863   | 2058.517    |
| Concentration (ng/ $\mu$ L) | 10.699       | 0.420          | 25.449 | 0.002   | 8.890       | 12.508      |

**Fig. S23.** Calibration curve for **H<sub>2</sub>FFA** with linear regression curve equation.**Table S7.** Residual Statistics for **H<sub>2</sub>FFA** Calibration Curve.

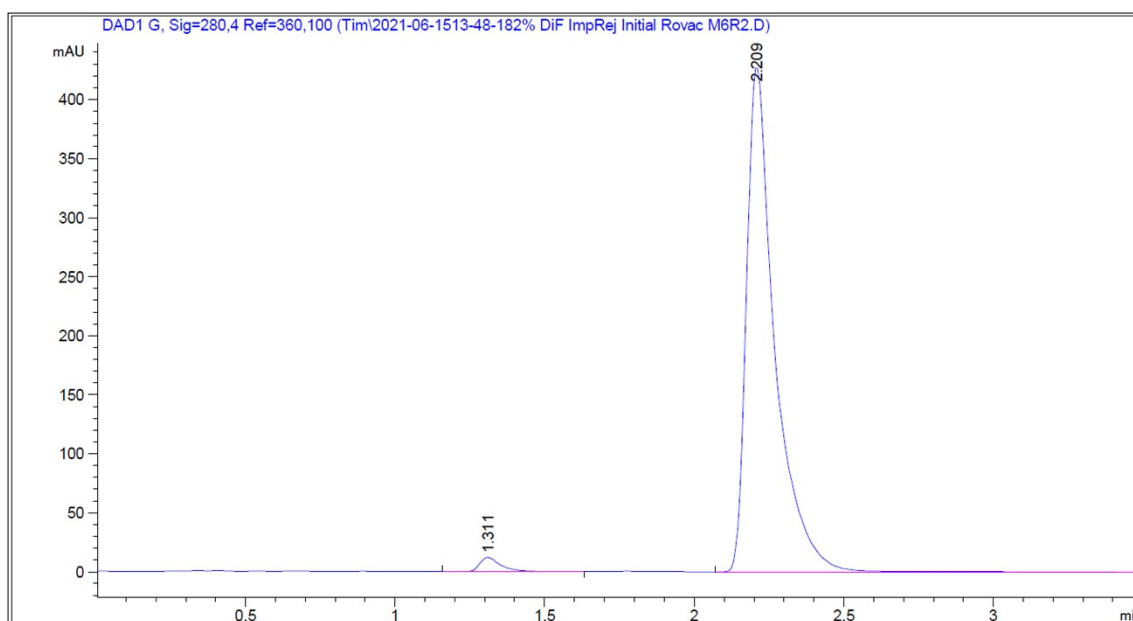
|                   |             |
|-------------------|-------------|
| Multiple R        | 0.9968719   |
| R Square          | 0.9937536   |
| Adjusted R Square | 0.9906304   |
| Standard Error    | 688.1365401 |
| Observations      | 4           |

**Table S8.** Residual Coefficients Statistics for **H<sub>2</sub>FFA** Calibration Curve.

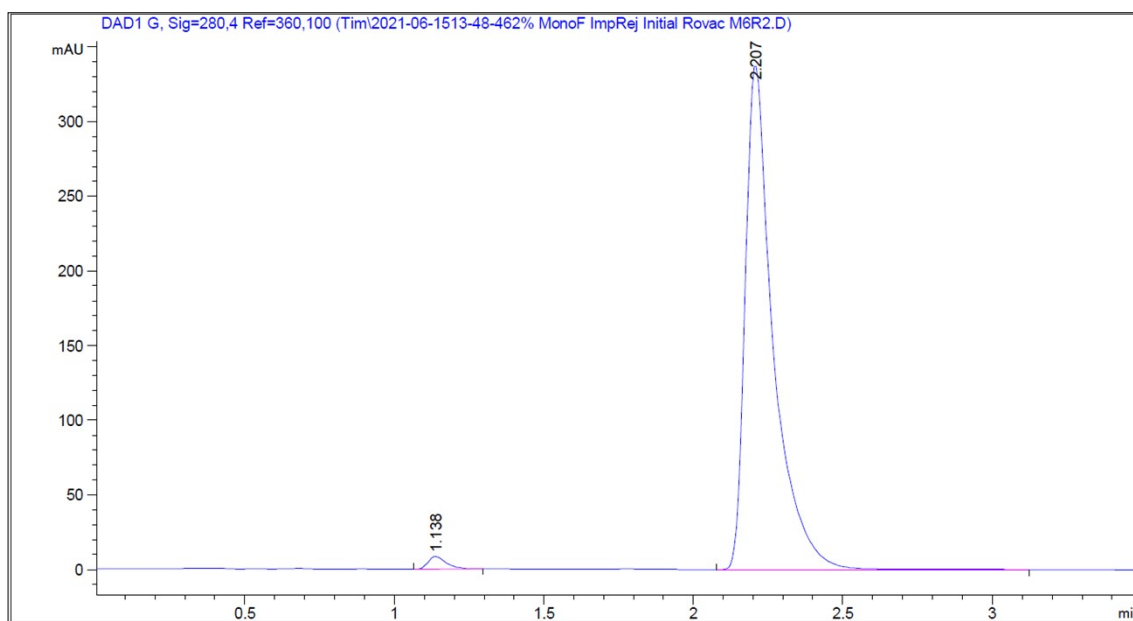
|                             | Coefficients | Standard Error | t Stat | P-value | Lower 95.0% | Upper 95.0% |
|-----------------------------|--------------|----------------|--------|---------|-------------|-------------|
| Intercept                   | 1582.955     | 835.034        | 1.896  | 0.198   | -2009.905   | 5175.814    |
| Concentration (ng/ $\mu$ L) | 10.098       | 0.566          | 17.838 | 0.003   | 7.662       | 12.534      |



**Fig. S24.** HPLC trace of a solution of **FFA** (right, 2.202 min) in acetonitrile containing approx. 2 mol% **CIFA** (left, 1.888 min).

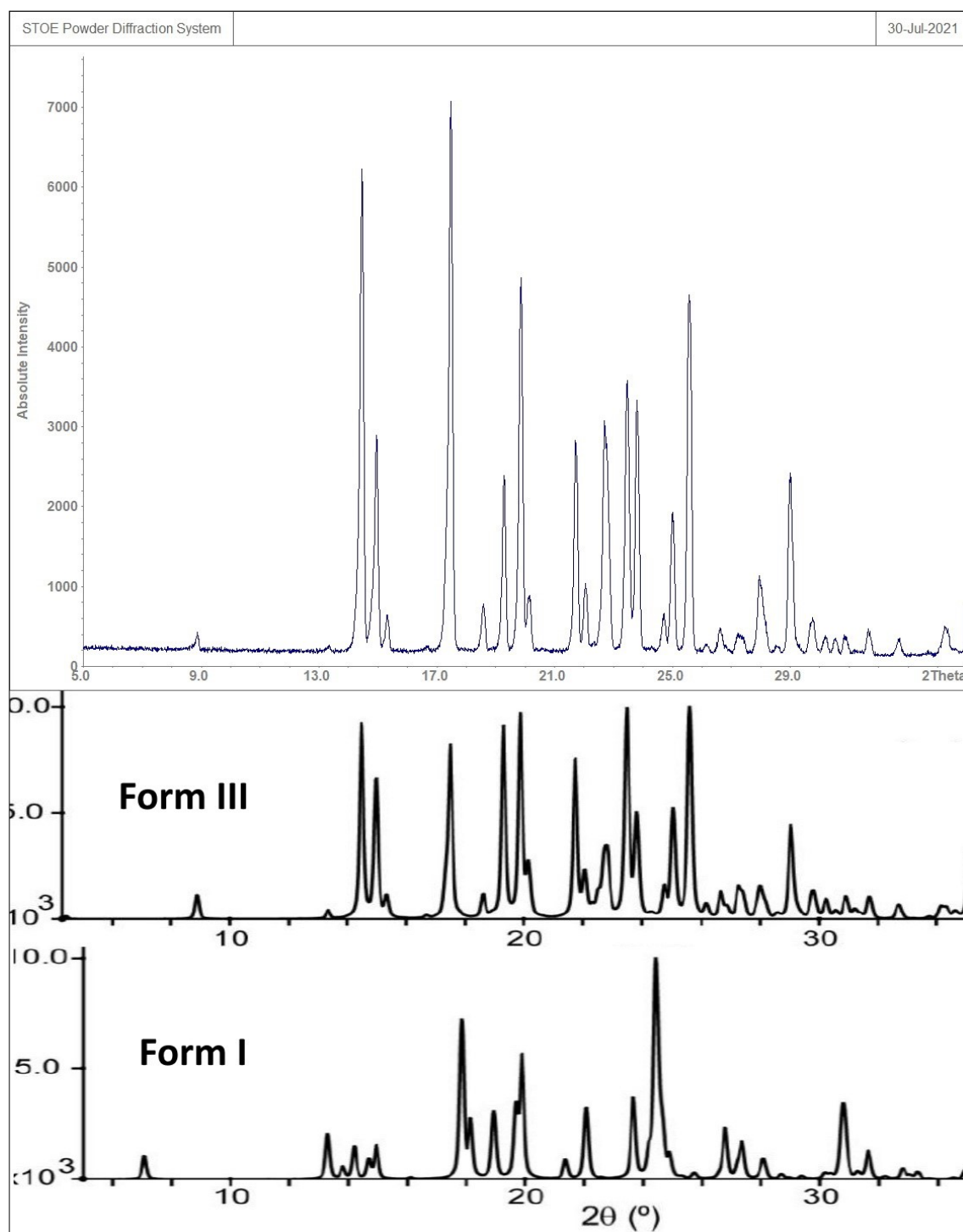


**Fig. S25.** HPLC trace of a solution of **FFA** (right, 2.209 min) in acetonitrile containing approx. 2 mol% **H<sub>2</sub>FFA** (left, 1.311 min).



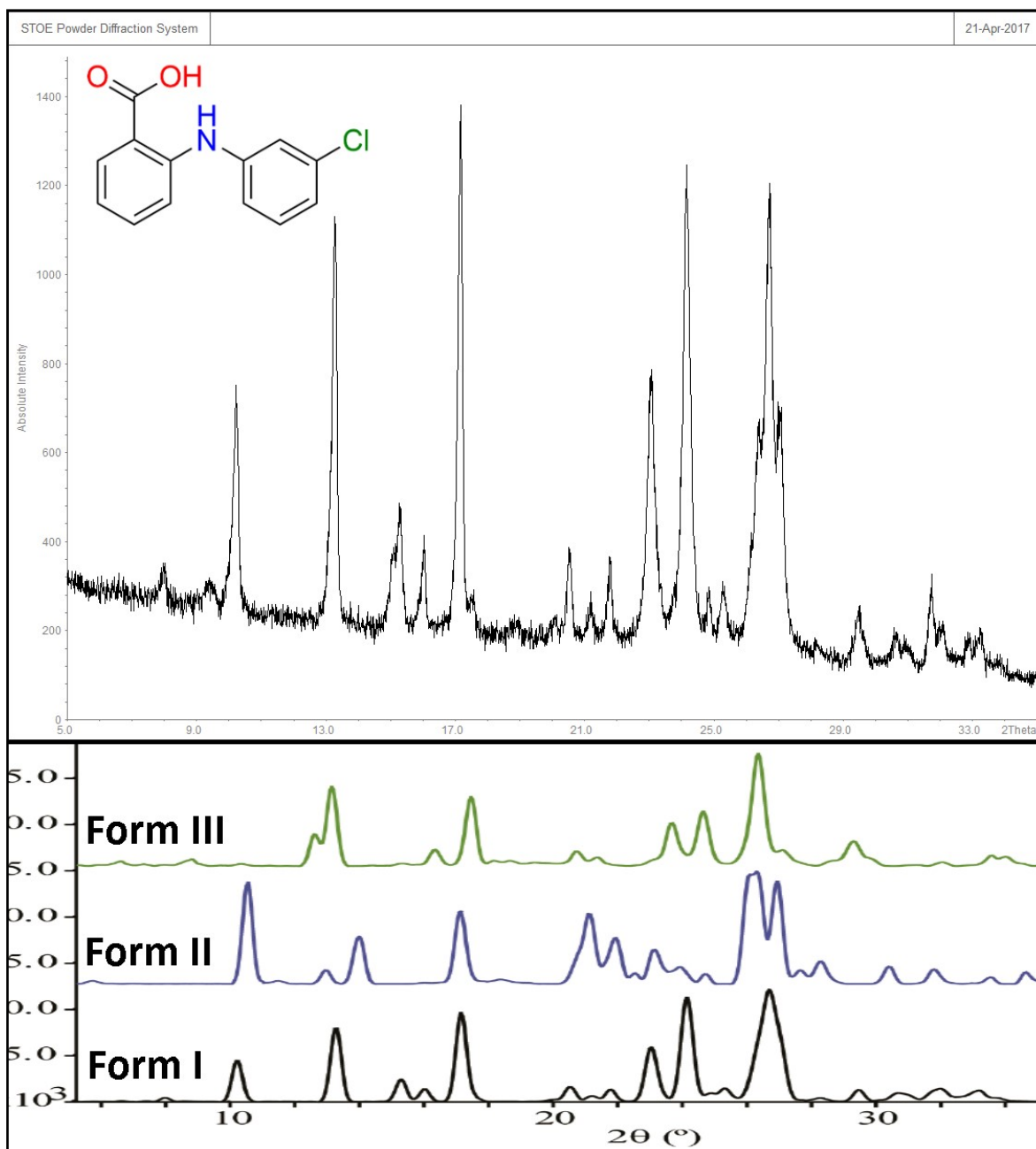
**Fig. S26** HPLC trace of a solution of **FFA** (right, 2.207 min) in acetonitrile containing approx. 2 mol% **HF<sub>2</sub>FA** (left, 1.138 min).

## PXRD



**Fig. S27.** Measured PXRD pattern of FFA recrystallised from EtOH (top) vs. literature patterns for Form III and Form I (bottom).<sup>1</sup>

1. V. López-Mejías, J. W. Kampf and A. J. Matzger, *J. Am. Chem. Soc.*, 2012, **134**, 9872-9875.



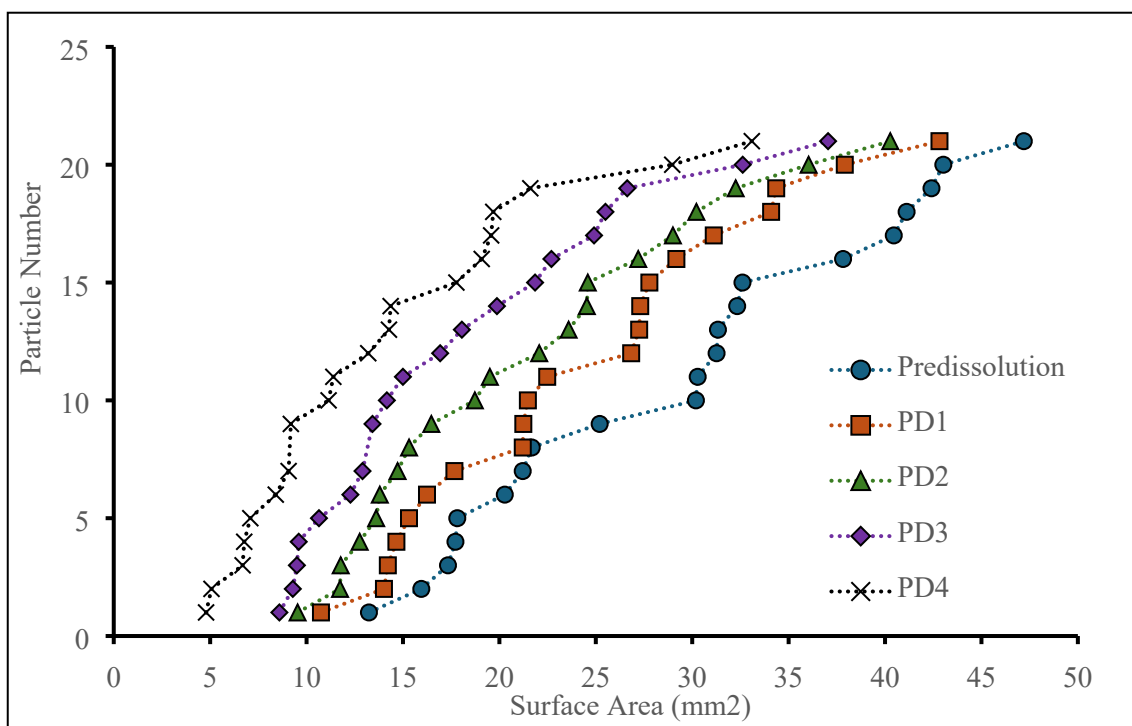
**Fig. S28.** Top: Measured PXR pattern of synthesised CIFA, bottom: literature patterns of CIFA Form I, Form II, and Form III.<sup>2</sup>

2. V. López-Mejías, A. J. Matzger, *Cryst. Growth Des.*, 2015, **15**, 3955–3962.

## Crystallisations / Partial Dissolutions

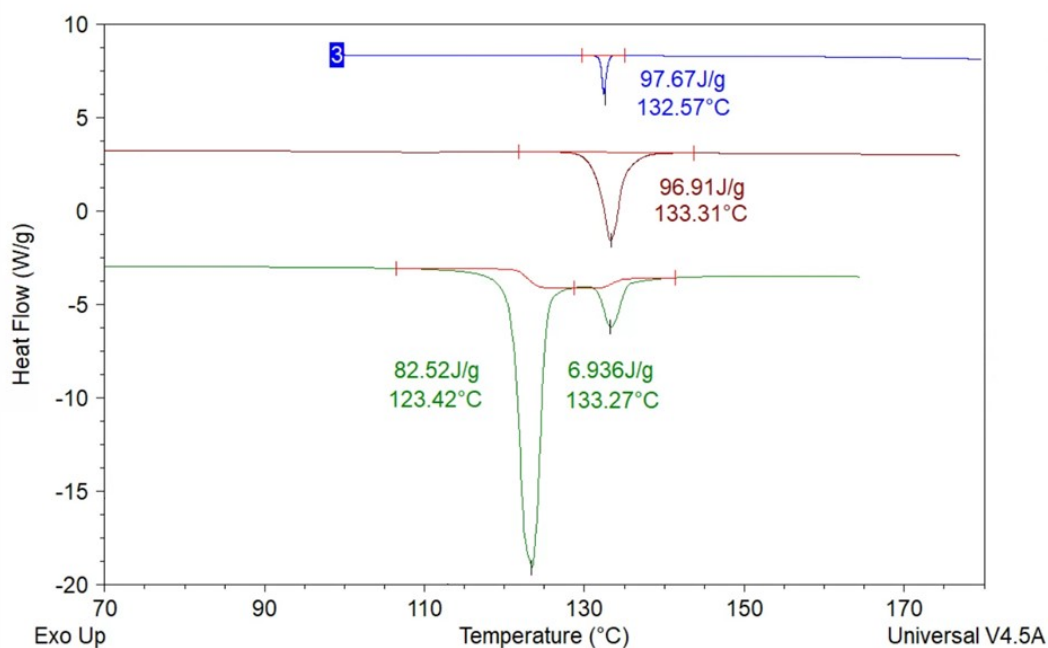


**Fig S29.** Example of FFA crystals grown by the slow evaporation from methanol solution of FFA.

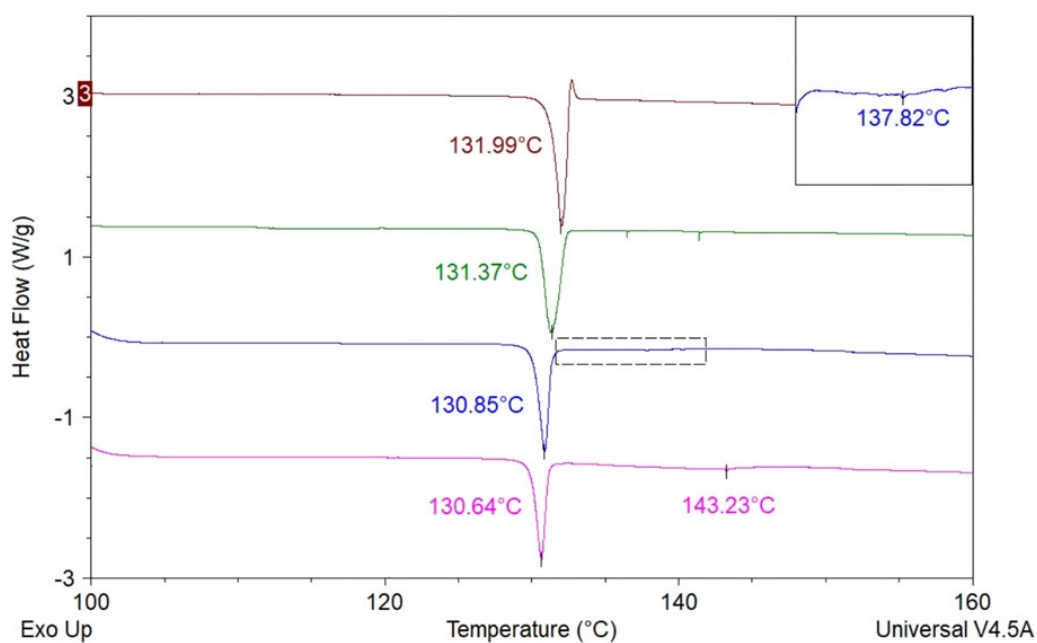


**Fig. S30.** Surface area distributions for a sample of 20 FFA crystals, grown from MeOH solutions containing 2%  $\text{HF}_2\text{FA}$ , for the initial sample and for the sample after each of a series of four partial dissolutions.

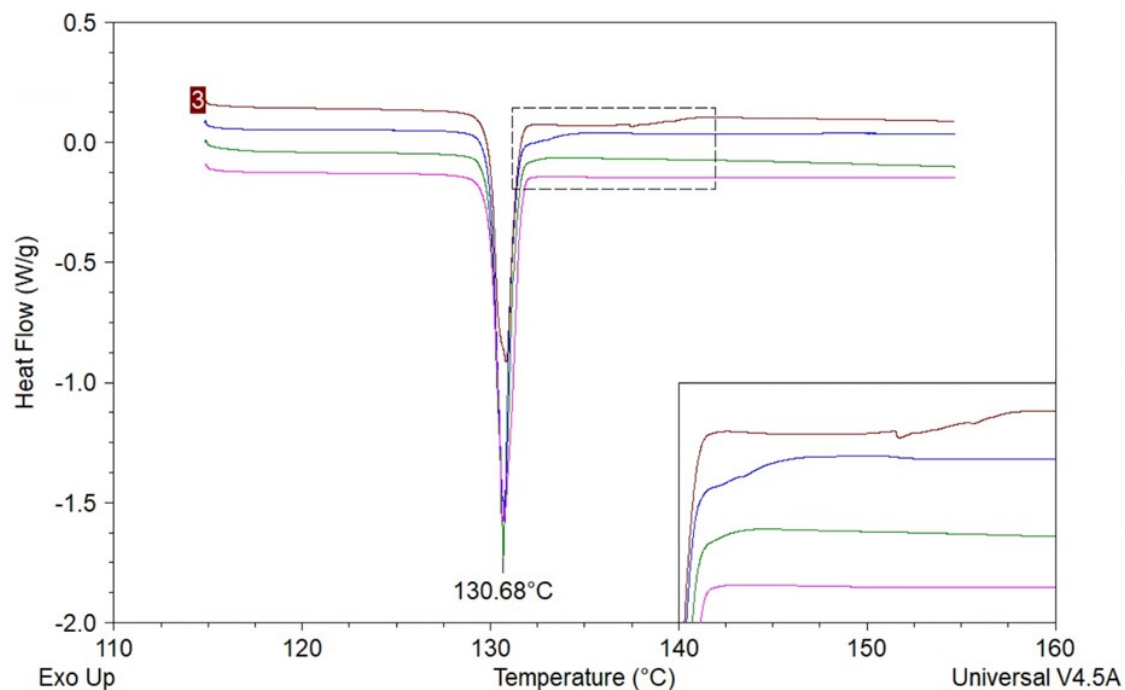
## DSC



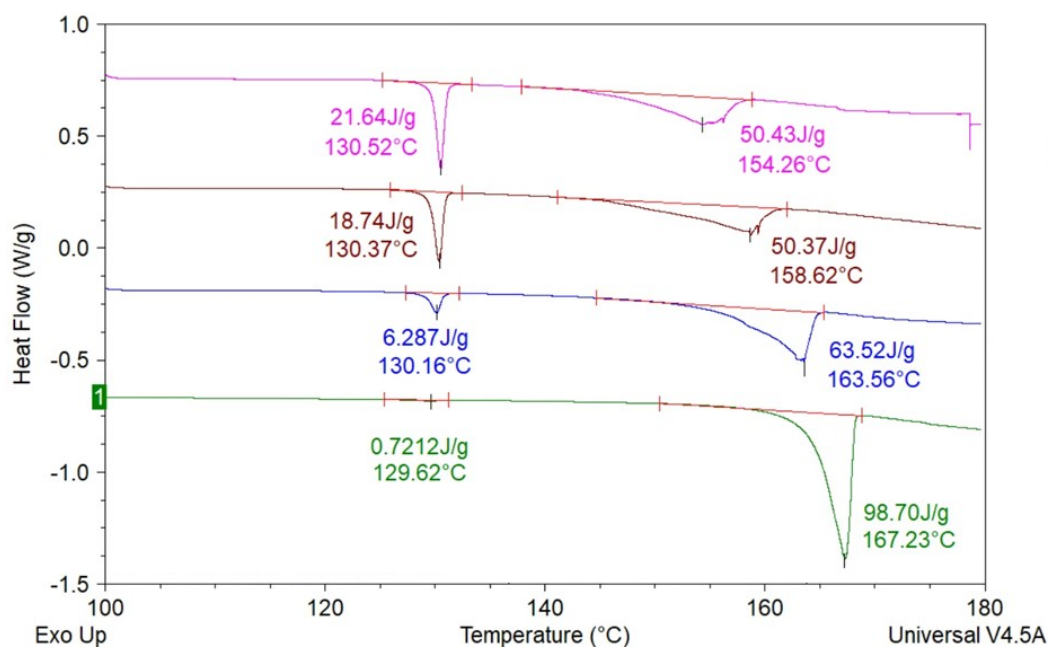
**Fig. S31.** Top to bottom, DSC curves of **FFA** crystallised with 10 % **CIFA**, collected at heating rates of 1, 10, and 50 °C min<sup>-1</sup> respectively.



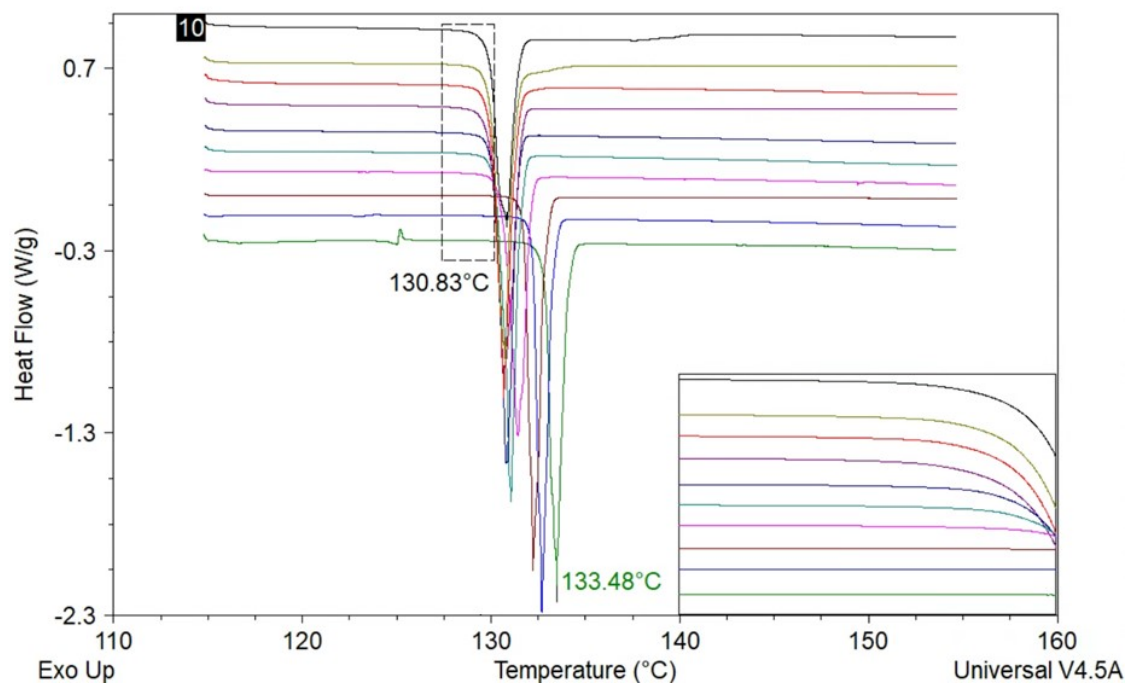
**Fig. S32.** Top to bottom, DSC curves of **FFA** crystallised with 10%, 20%, 30% and 40% **CIFA**, respectively, collected at a heating rate of 1 min<sup>-1</sup>. The inset in the top right shows the emergence of the higher melting endotherm.



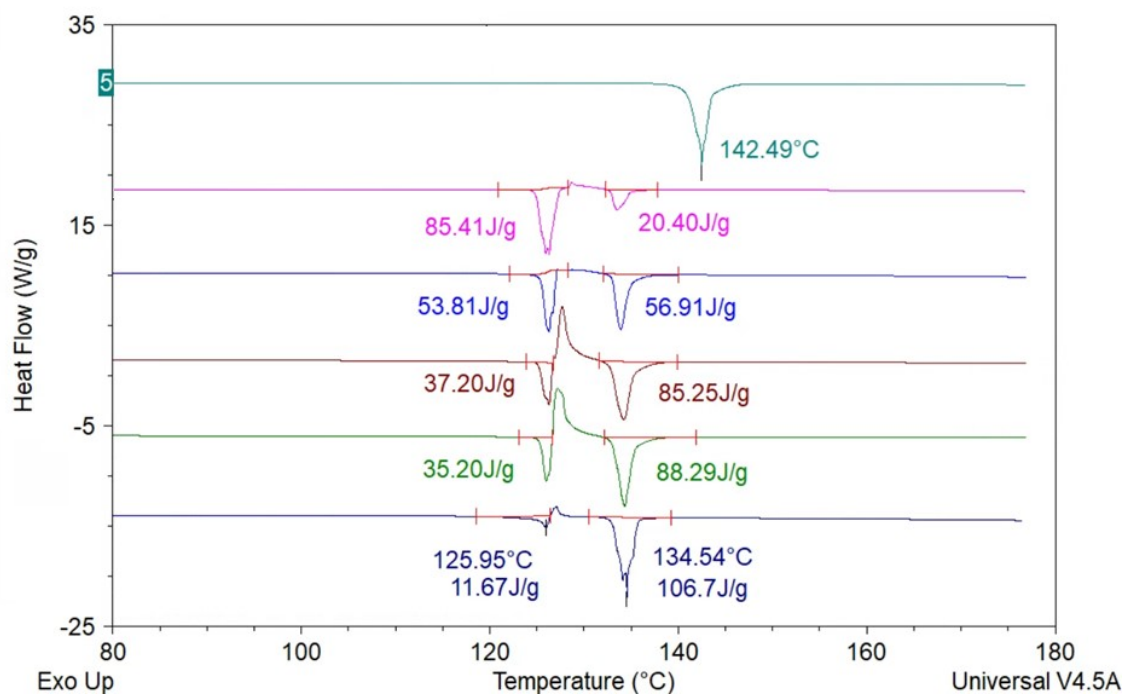
**Fig. S33.** Bottom to top, DSC curves of **FFA** crystallised with 24%, 26%, 28% and 30% **CIFA**, respectively, collected at a heating rate of  $1\text{ }^{\circ}\text{C min}^{-1}$ . The insert on bottom right shows the emergence of the higher melting endotherm.



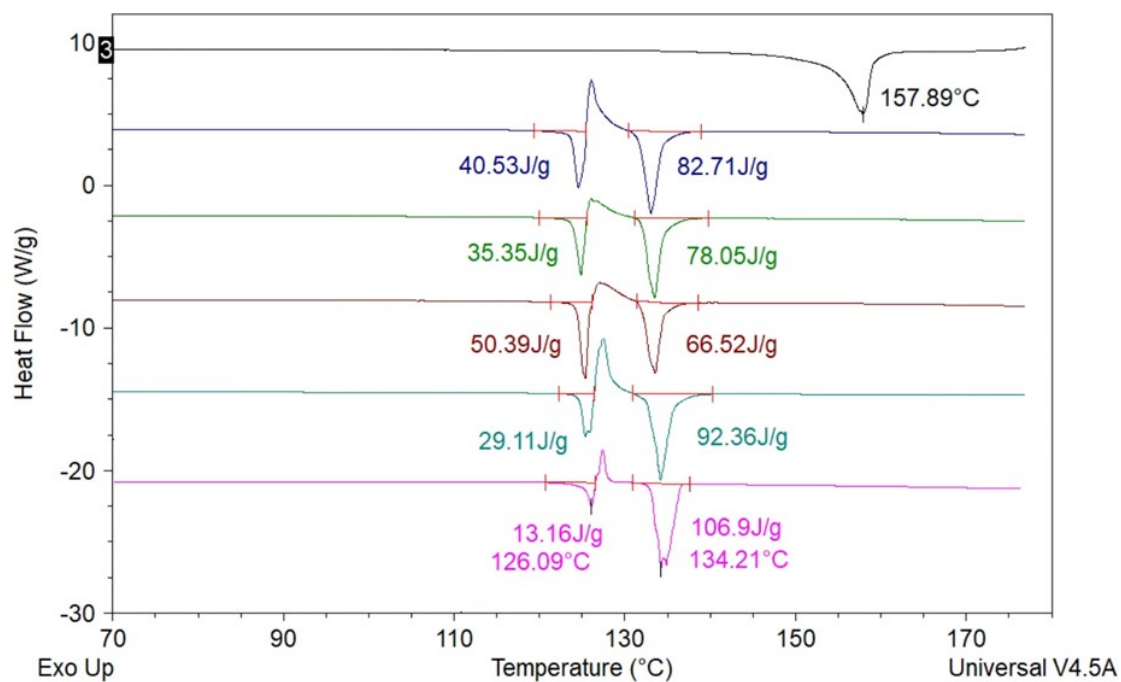
**Fig. S34.** Top to bottom, DSC curves of **FFA** crystallised with 60%, 70%, 80%, 90% **CIFA**, respectively, collected at a heating rate of  $1\text{ }^{\circ}\text{C min}^{-1}$ .



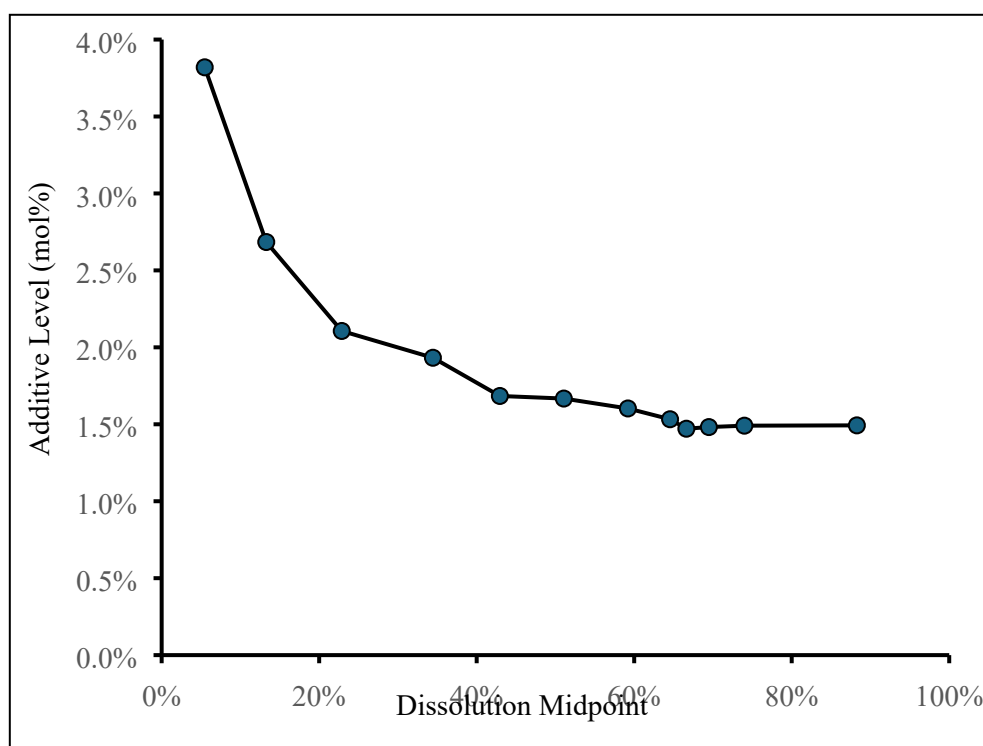
**Fig. S35.** Bottom to top, DSC curves of **FFA** crystallised with increasing levels of **ClFA**, from 2% to 30%, collected at a heating rates of  $1\text{ }^{\circ}\text{C min}^{-1}$ . The insert on the bottom right is an expansion of the region enclosed in dashed lines.



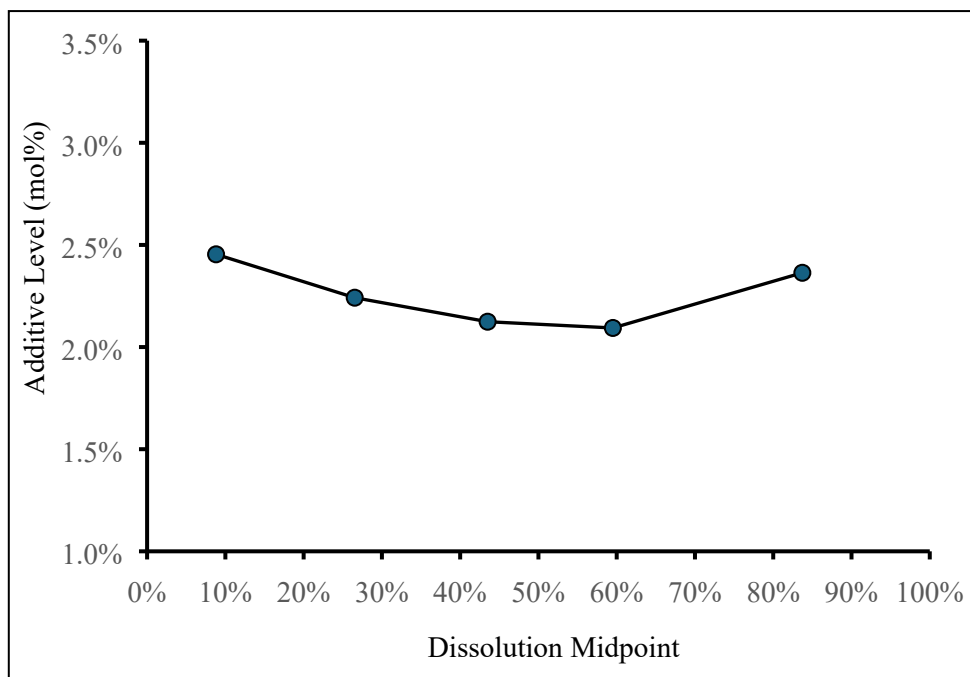
**Fig. S36.** DSC curves of **FFA** containing, bottom to top 0%, 2%, 3%, 4%, 5% and 100% **HF<sub>2</sub>FA**, collected at a heating rate of  $10\text{ }^{\circ}\text{C min}^{-1}$ .



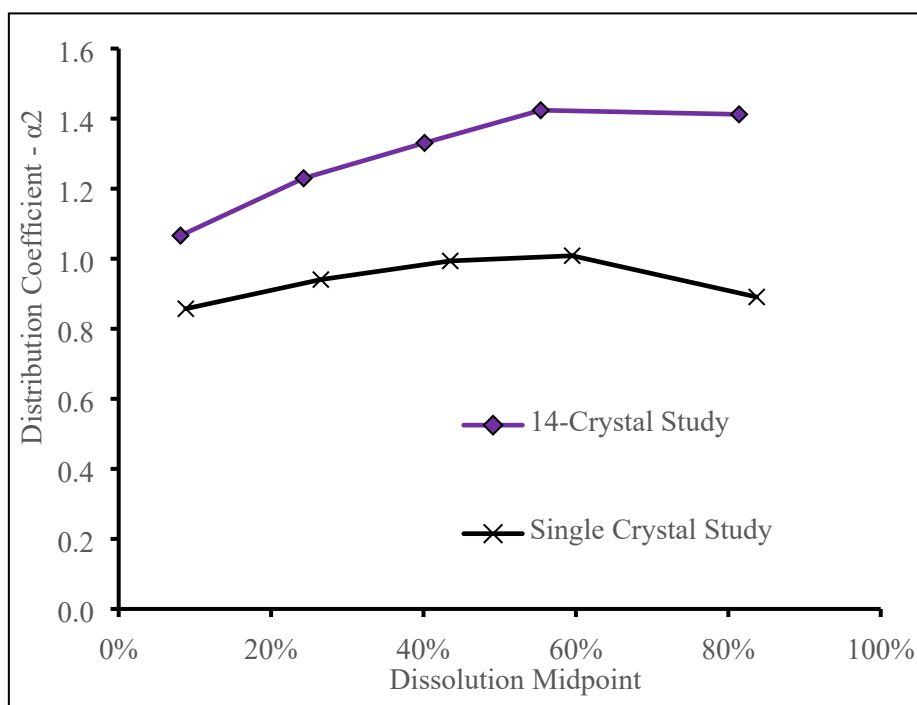
**Fig. S37.** DSC curves of FFA containing, bottom to top, 0%, 2%, 3%, 4%, 5 % and 100% **H<sub>2</sub>FFA**, collected at a heating rate of 10 °C min<sup>-1</sup>.



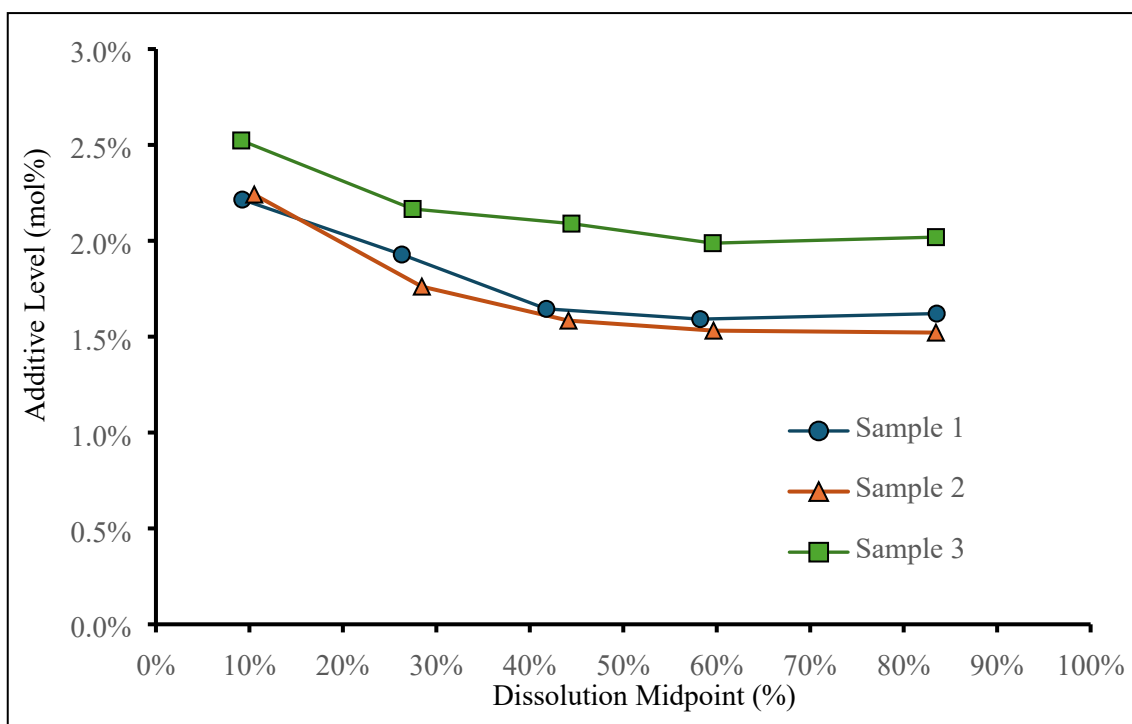
**Fig. S38.** Plot of **CIFA** level vs dissolution midpoint of a sample of 10 crystals of FFA grown from solution containing 2% **CIFA**.



**Fig. S39.** Plot of CIFA level vs dissolution midpoint of a large single crystal of FFA grown from solution containing 2% CIFA.



**Fig. S40.** Plot of  $\alpha_2$  value vs. dissolution midpoint a sample of 14 crystals or FFA, and also of a large single crystal of FFA, grown from solutions containing 2% CIFA.



**Fig. S41.** Plot of  $\text{HF}_2\text{FA}$  level vs dissolution midpoint of three separate samples of crystals of FFA grown from solutions containing 2%  $\text{HF}_2\text{FA}$ .