Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information for

Synthesis and EPR studies of the first water-soluble N@C_{60} derivative

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Contents

1. Synthesis and Characterisation

1.1 General Remarks

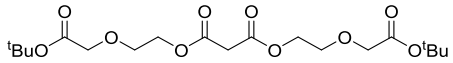
All commercial solvents and reagents were used as purchased, unless otherwise stated. Anhydrous solvents were degassed with N_2 and dried by passing them through an MBraun-800 column. Water was distilled and microfiltered using a Milli-Q Millipore machine. Triethylamine was distilled and stored over KOH pellets. Chromatography was undertaken using silica gel (particle size: 40-63 µm) or preparative TLC plates (20 x 20 cm, 1 cm silica thickness).

The $N@C_{60}/C_{60}$ sample used to synthesize **CD-N@C$_{60}$** was prepared using an optimized ion implantation process,[1] followed by recycling HPLC to enrich the $N@C_{60}$ content from ~100 ppm to ~2000 ppm.[2–4] The $N@C_{60}$ percentage of the sample was determined using a combination of TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl) calibrated cw-EPR spectroscopy and C_{60} calibrated UV/vis spectroscopy.

NMR spectra were recorded using Bruker AVIII400 and Bruker AVII 500 spectrometers at 298 K. ESI mass spectra were recorded on a Waters LCT Premier instrument or an Agilent HP1100 instrument. MALDI mass spectra were recorded on a Bruker Microflex LT spectrometer using dithranol as the matrix. DLS measurements were performed with a Malvern Zetasizer Nano ZS instrument, using a 0.25 mM sample concentration and with the refractive index set to 2.2. X-Band cw-EPR measurements were performed on Magnettech Miniscope MS200 and Bruker EMX spectrometers. Simulations of cw-EPR spectra were performed using the EASYSPIN software package.[5]

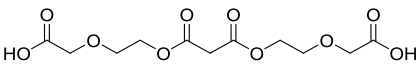
1.2 Synthetic Procedures

Bis-ester 2



To a mixture of alcohol **1** (217 mg, 1.23 mmol) and trimethylamine (125 mg, 1.23 mL) in dry CH_2Cl_2 (15 mL) was added a solution of malonyl dichloride (79 mg, 0.56 mmol) in dry CH_2Cl_2 (10 mL) dropwise. The mixture was stirred at room temperature for 15 h after which the solvent was removed under vacuum. The crude material was purified by silica gel column chromatography (EtOAc/hexane 2:3) to afford bis-ester **2** as a colourless oil (165 mg, 0.39 mmol, 70%). **^{1}H NMR** (400 MHz, Chloroform-*d*, 25°C): δ = 4.38 – 4.31 (m, 4H; COOC*H*$_2$), 4.01 (s, 4H; OC(O)C*H*$_2$), 3.81 – 3.76 (m, 4H; COOCH$_2$C*H*$_2$O), 3.48 (s, 2H; C(O)C*H*$_2$C(O)), 1.48 (s, 18H; ^{t}Bu). **^{13}C NMR** (101 MHz, Chloroform-*d*, 25°C): δ = 169.45, 166.59, 81.97, 69.18, 69.04, 64.69, 41.40, 28.26. **ESI-MS** (positive): *m/z*: 443.2 $[M + Na]^+$ ($C_{19}H_{32}O_{10}Na$ calc. 443.2).

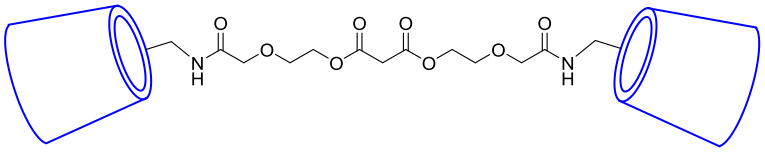
Bis-acid 3



Bis-ester **2** (82 mg, 0.195 mmol) was dissolved in CH_2Cl_2 (4 mL) and trifluoroacetic acid (1 mL) was added. The solution was stirred at room temperature for 4 h after which, the residual acid and solvent were removed under vacuum to afford bis-acid **3** as a colourless oil in quantitative yield. **^{1}H NMR** (400 MHz, Acetone-d_6, 25°C): δ = 4.33 – 4.24 (m, 4H; COOC*H*$_2$), 4.14 (s, 4H; OC(O)C*H*$_2$), 3.84 – 3.75 (m, 4H;

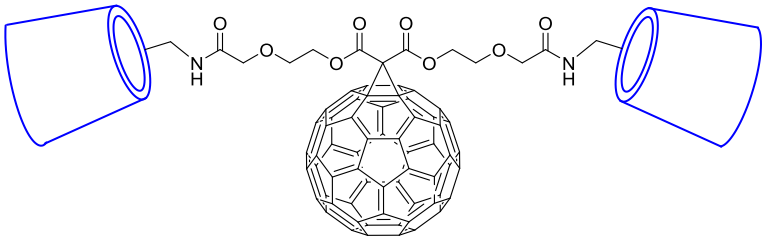
$COOCH_2CH_2O$), 3.47 (s, 2H; $C(O)CH_2C(O)$). **ESI-MS** (positive): *m/z*: 331.0 $[M + Na]^+$ ($C_{11}H_{16}O_{10}Na$ calc. 331.1).

Malonate 5



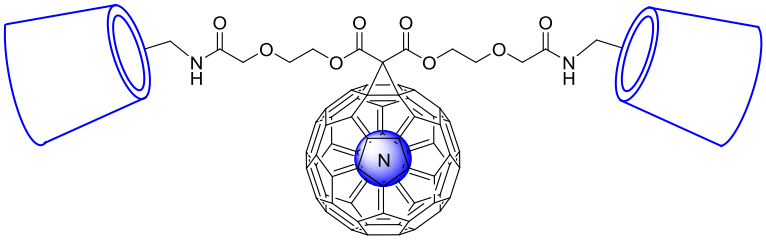
To a solution of bis-acid **3** (17.0 mg, 0.055 mmol) and 6^A-amino-permethylated-β-cyclodextrin **4** (194 mg, 0.137 mmol) in dry CH_2Cl_2 (7.5 mL) was added EDC·HCl (26.3 mg, 0.137 mmol) and DMAP (3.3 mg, 0.027 mmol). The resulting mixture was stirred at room temperature for 15 h after which the solvent was removed under vacuum. The crude material was then purified by column chromatography (CH_2Cl_2/MeOH 93:7) to afford malonate **5** as an off-white glassy solid (136 mg, 0.044 mmol, 80%). **^{1}H NMR** (400 MHz, Chloroform-*d*, 25°C): δ = 6.86 (t, ^3J(H,H) = 5.7 Hz, 2H; NH), 5.23 – 5.07 (m, 14H; CD 1-H), 4.39 – 4.31 (m, 4H; $COOCH_2$), 4.07 – 3.95 (m, 4H), 3.95 – 3.73 (m, 32H), 3.73 – 3.31 (m, 164H), 3.26 – 3.12 (m, 14H; CD 2-H). **^{13}C NMR** (101 MHz, Chloroform-*d*, 25°C): δ = 169.30, 166.35, 99.48, 99.19, 99.07, 98.95, 98.87, 98.68, 82.28, 82.22, 82.12, 82.05, 81.95, 81.87, 81.78, 81.63, 81.38, 80.56, 80.43, 80.29, 80.21, 79.82, 77.48, 77.36, 77.16, 76.84, 71.69, 71.62, 71.57, 71.43, 71.37, 71.12, 71.06, 70.86, 69.90, 69.38, 64.35, 61.69, 61.60, 61.52, 61.50, 59.32, 59.18, 59.13, 59.09, 58.76, 58.73, 58.65, 58.60, 58.56, 58.53. **MALDI-MS** (positive): *m/z*: 3123.496 $[M + Na]^+$ ($C_{135}H_{234}N_2O_{76}Na$ calc. 3123.443).

CD-C_{60}



C_{60} (7.0 mg, 0.0097 mmol), malonate **5** (30 mg, 0.0097 mmol), iodine (3.7 mg, 0.015 mmol) and DBU (3.7 mg, 0.024 mmol) were dissolve in dry, degassed toluene (7 mL) and the mixture was stirred at room temperature for 15 h. The solvent was removed under vacuum and the crude material was purified using preparative thin layer chromatography (acetone/cyclcohexane 1:1) to afford **CD-C_{60}** as a brown solid (9.3 mg, 0.0024 mmol, 25%). **^{1}H NMR** (400 MHz, Chloroform-*d*, 25°C): δ = 6.85 (t, ^3J(H,H) = 5.8 Hz, 2H; NH), 5.24 – 5.06 (m, 14H; CD 1-H), 4.77 – 4.63 (m, 4H; $COOCH_2$), 4.11 – 3.98 (m, 4H), 3.95 – 3.72 (m, 36H), 3.70 – 3.30 (m, 158H), 3.25 – 3.10 (m, 14H; CD 2-H). **^{13}C NMR** (126 MHz, Chloroform-*d*, 25°C): δ = 169.01, 163.53, 145.50, 145.41, 145.14, 145.11, 145.09, 144.87, 144.66, 144.03, 143.32, 143.26, 143.13, 142.32, 141.87, 141.16, 139.09, 99.36, 99.20, 99.05, 98.90, 98.85, 98.62, 82.38, 82.25, 82.23, 82.19, 82.08, 82.04, 81.97, 81.91, 81.85, 81.79, 81.60, 81.54, 80.52, 80.32, 80.16, 79.51, 71.68, 71.64, 71.54, 71.38, 71.35, 71.14, 71.08, 71.05, 70.99, 70.91, 69.93, 69.65, 69.33, 65.82, 61.70, 61.64, 61.59, 61.57, 61.55, 61.51, 59.41, 59.21, 59.19, 59.15, 59.10, 58.78, 58.76, 58.67, 58.59, 58.57, 58.54, 39.80, 31.89. **MALDI-MS** (negative): *m/z*: 3818.562 $[M – H]^-$ ($C_{195}H_{231}N_2O_{76}$ calc. 3818.434).

CD-N@C_{60}



To a solution of N@C_{60} (0.2% purity, 4.9 mg, 0.0068 mmol), malonate **5** (21.1 mg, 0.0068 mmol) and iodine (2.6 mg, 0.0102 mmol) in dry, degassed toluene (5 mL) was added a solution of DBU (2.6 mg, 0.017 mmol) in toluene (1 mL) dropwise. The resultant mixture was stirred at room temperature, in the dark, for 15 h. The solvent was then removed under vacuum and the crude material was purified by preparative thin layer chromatography (acetone/cyclohexane 1:1) to afford **CD-N@C_{60}** as a brown solid (8.2 mg, 0.0022 mmol, 32%).

1.3 Spectral Characterisation

Bis-ester 2

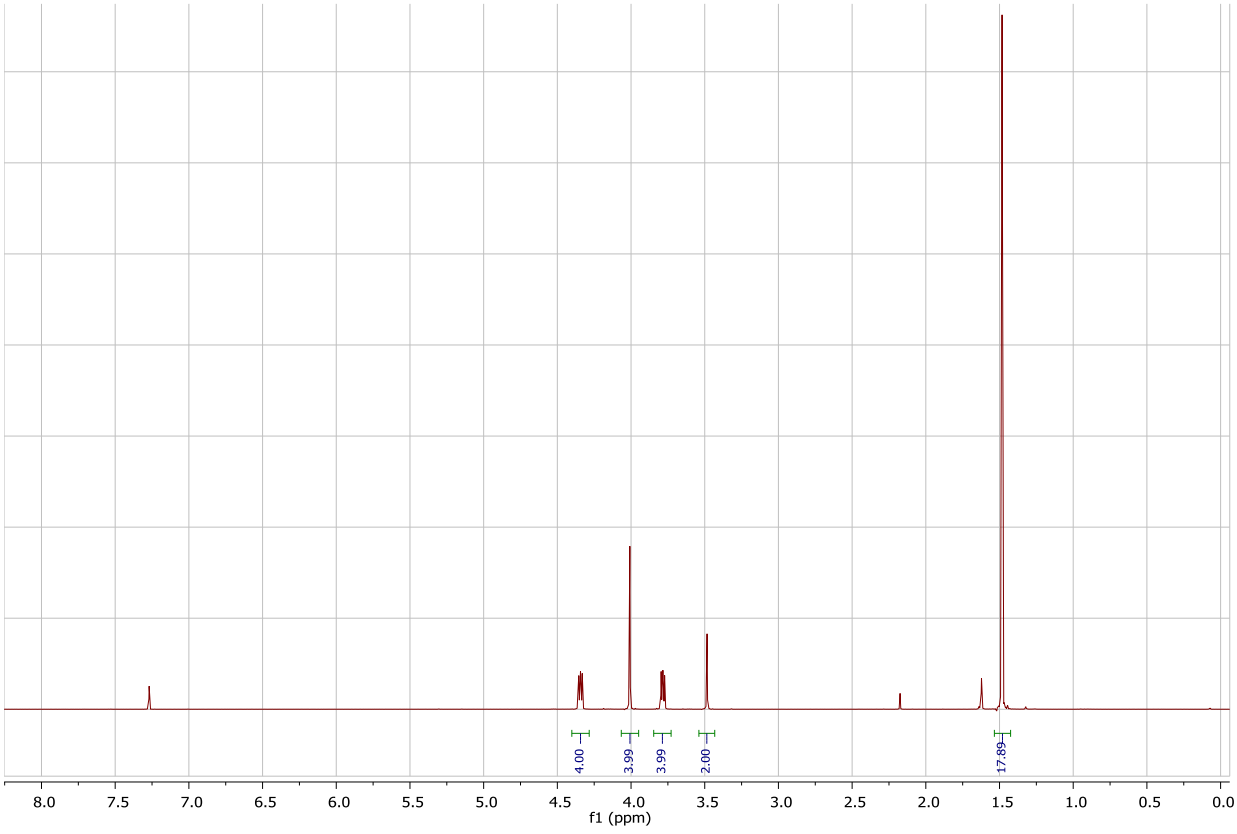


Figure S1: ^{1}H NMR of bis-ester **2** in $CDCl_3$ at 298 K (400 MHz).

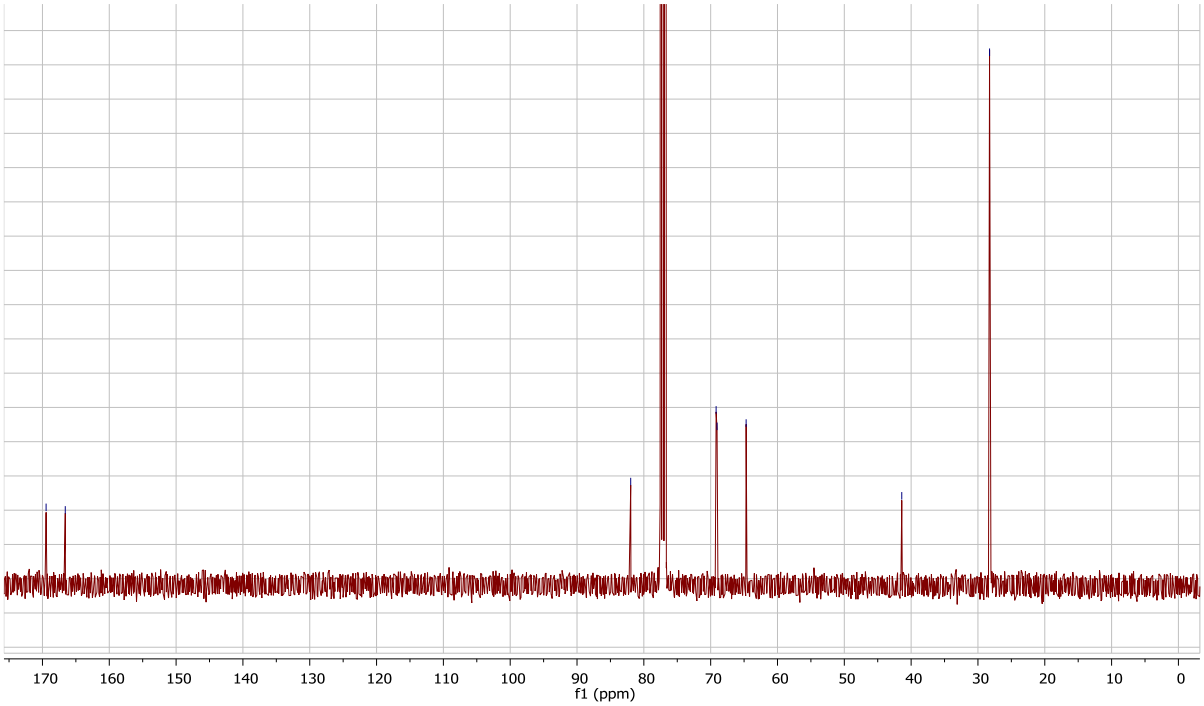


Figure S2: ^{13}C NMR of bis-ester **2** in $CDCl_3$ at 298 K (101 MHz).

Bis-acid 3

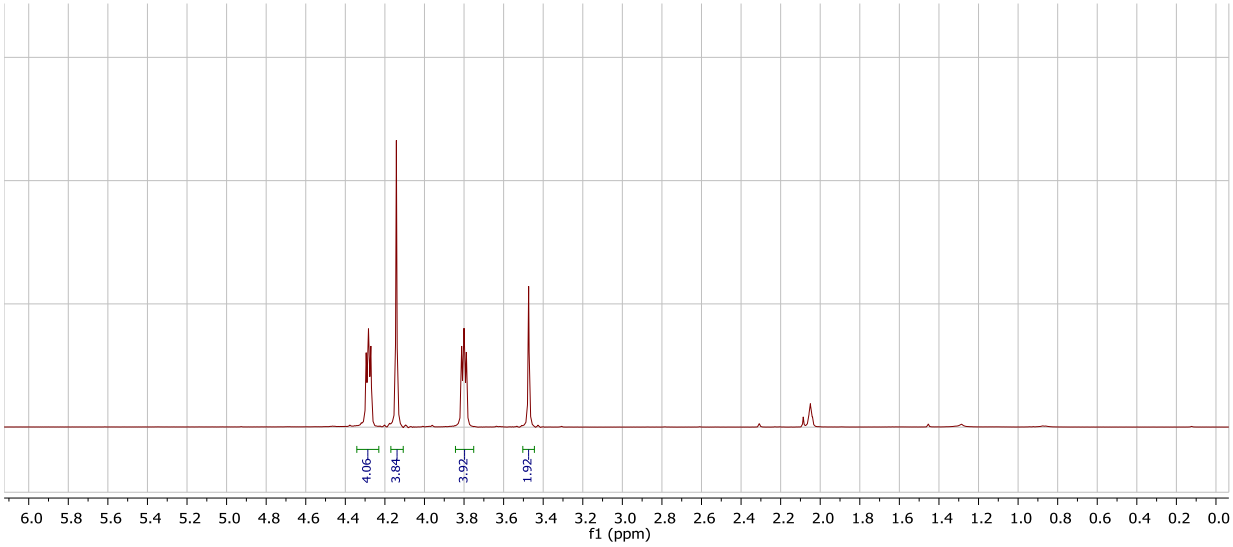


Figure S3: ^{1}H NMR of bis-acid **3** in d_6-acetone at 298 K (400 MHz).

Malonate 5

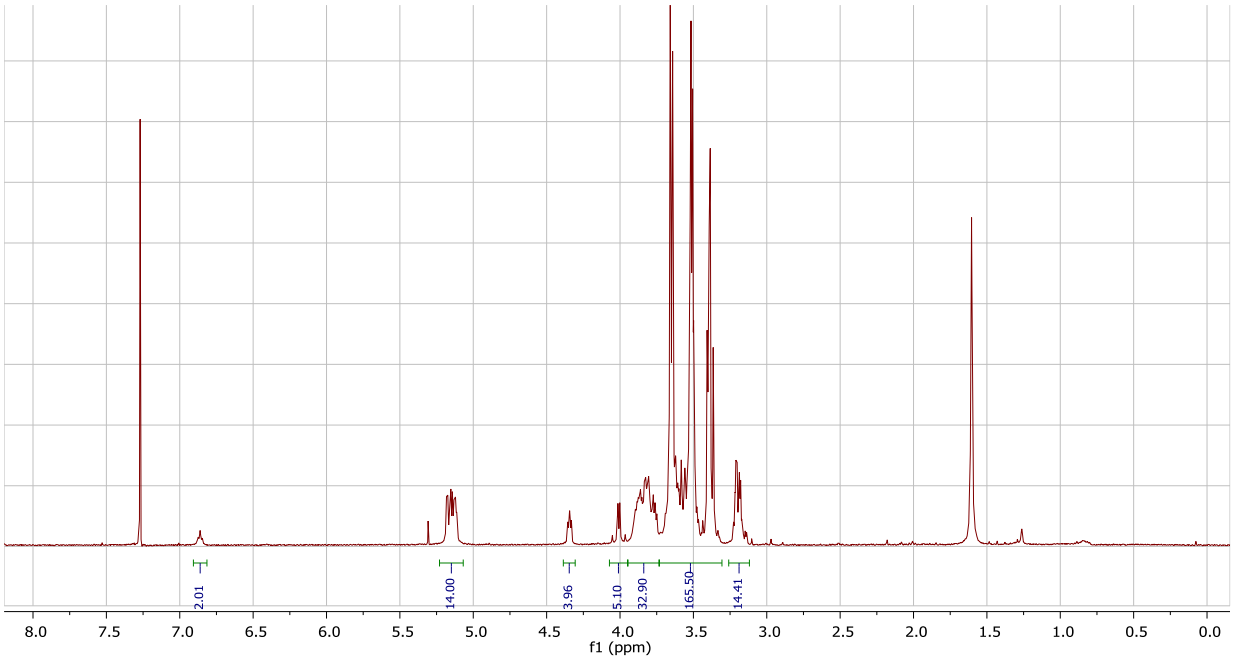


Figure S4: ^{1}H NMR of malonate **5** in $CDCl_3$ at 298 K (400 MHz).

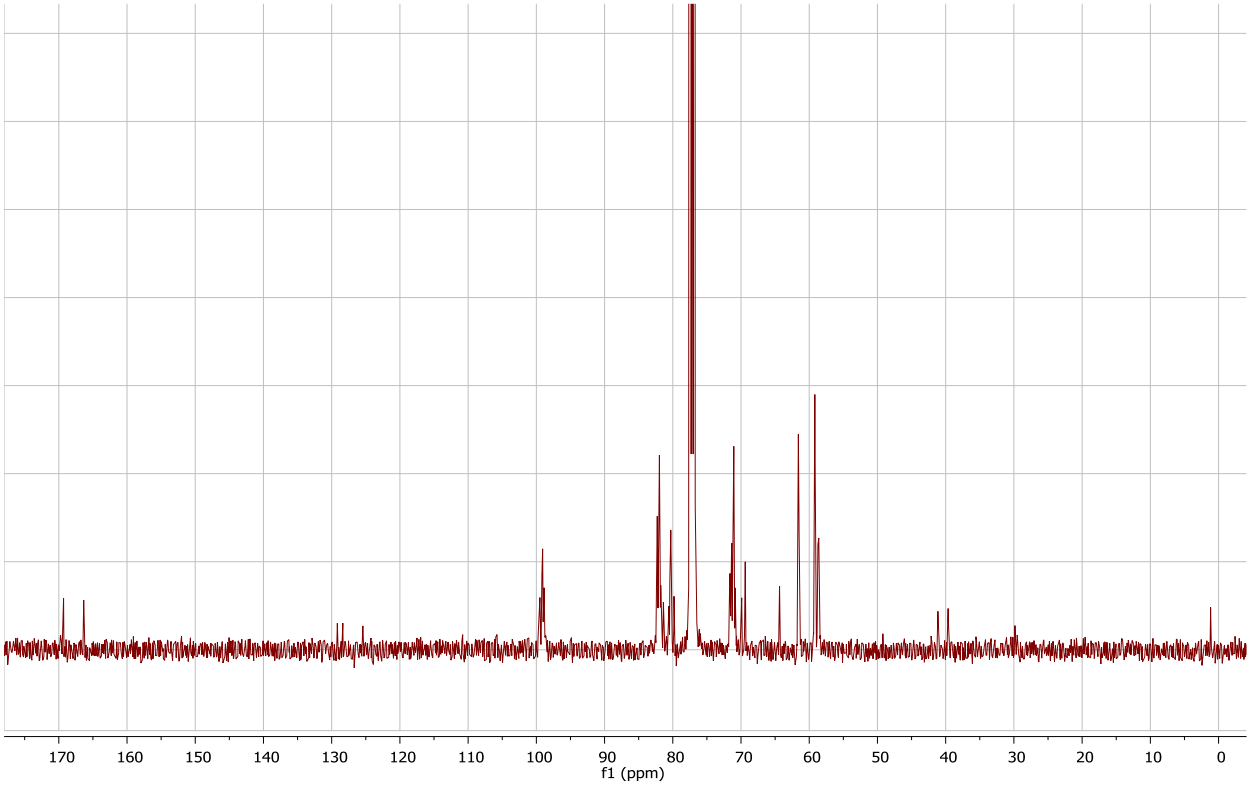


Figure S5: ^{13}C NMR of malonate **5** in $CDCl_3$ at 298 K (101 MHz).

CD-C_{60}

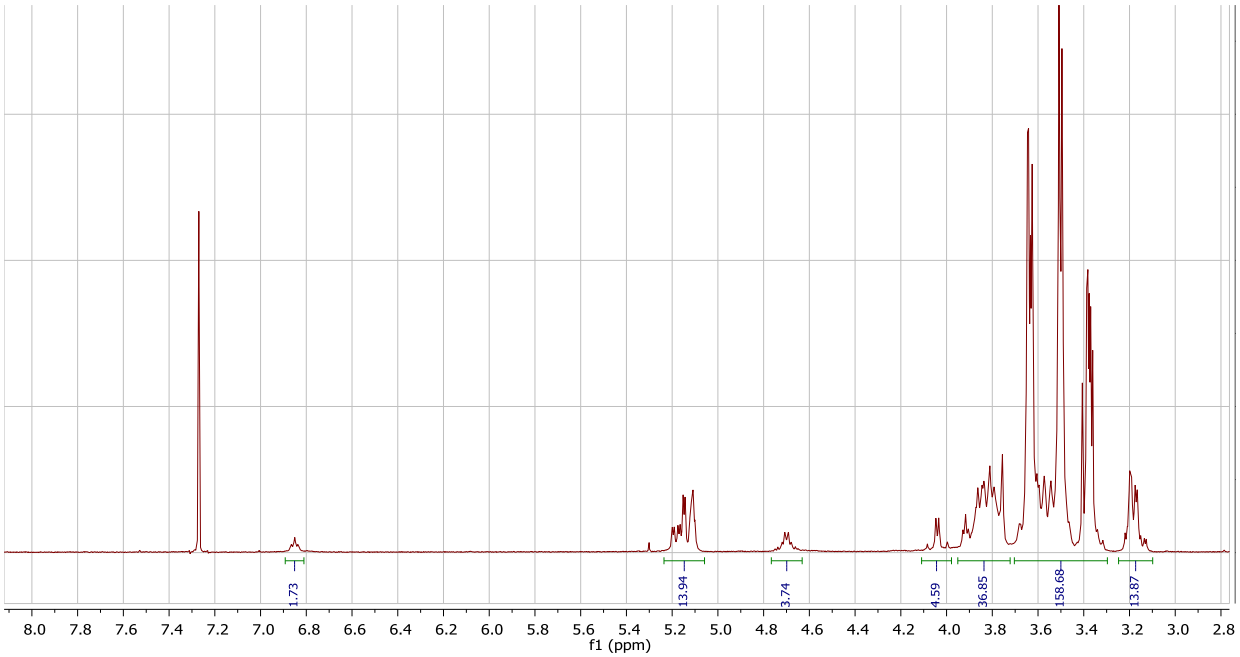


Figure S6: ^{1}H NMR of **CD-C_{60}** in $CDCl_3$ at 298 K (400 MHz).

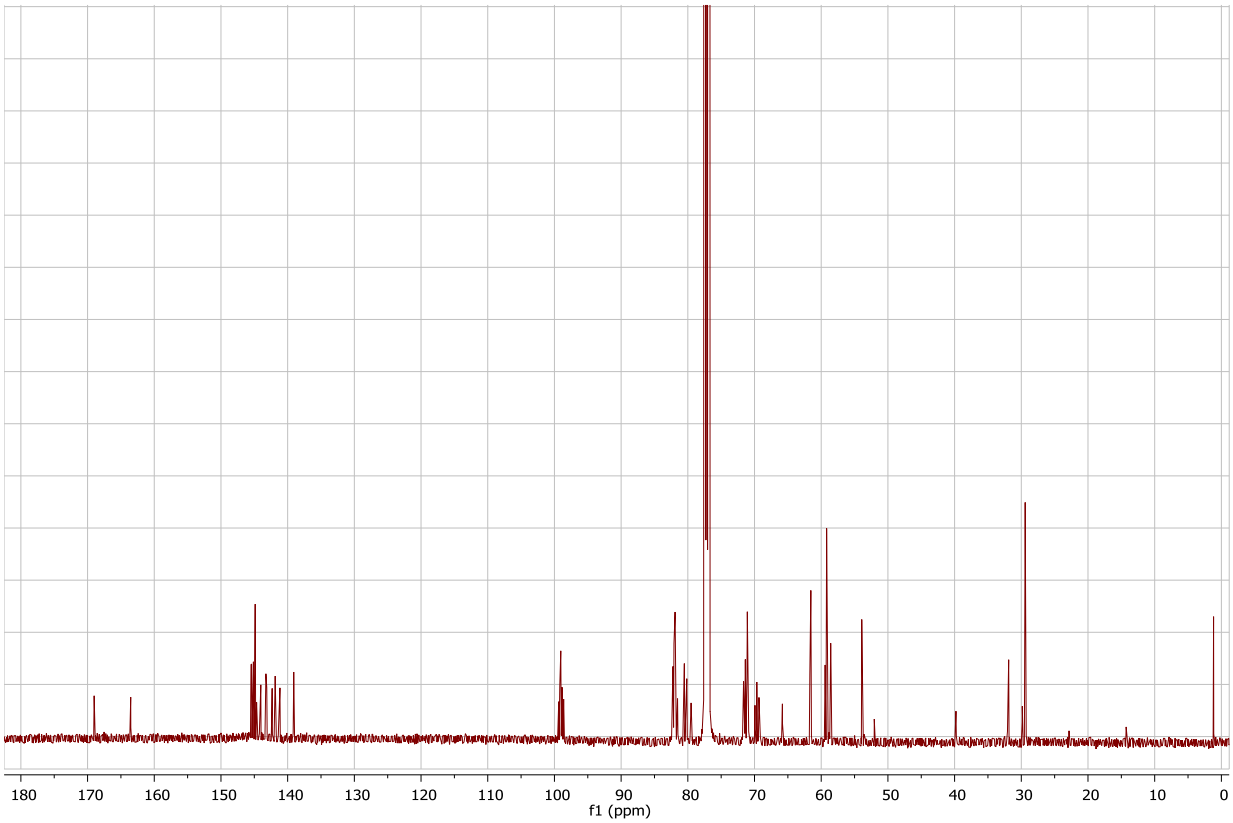


Figure S7: ^{13}C NMR of **CD-C_{60}** in $CDCl_3$ at 298 K (126 MHz).

CD-N@C_{60}

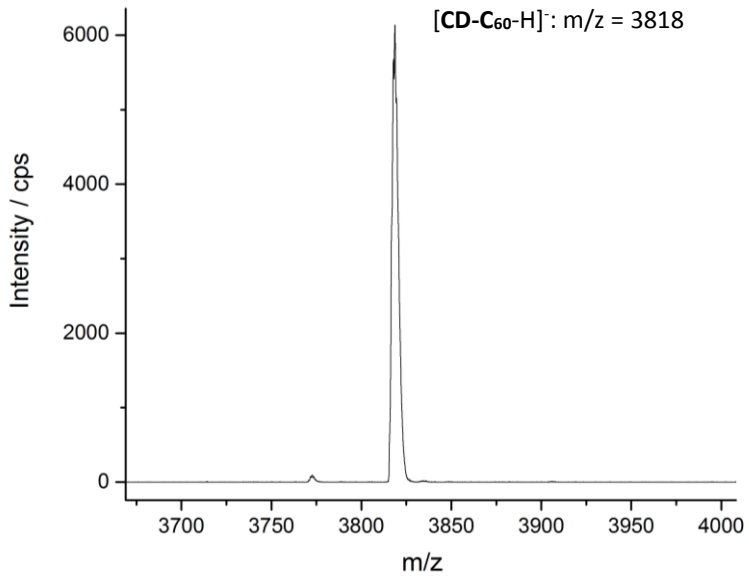


Figure S8: MALDI mass spectrum of **CD-N@C_{60}** (negative mode).

2. EPR data

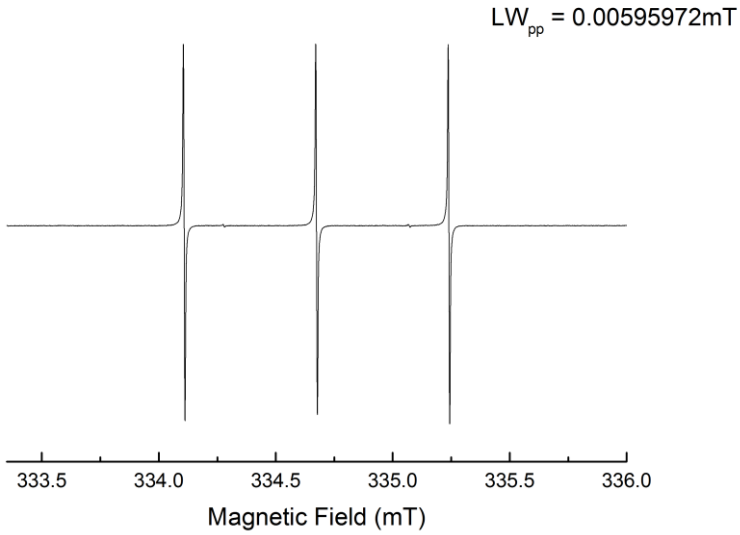


Figure S9: X-band cw-EPR spectrum of **CD-N@C_{60}** (toluene, 298 K).

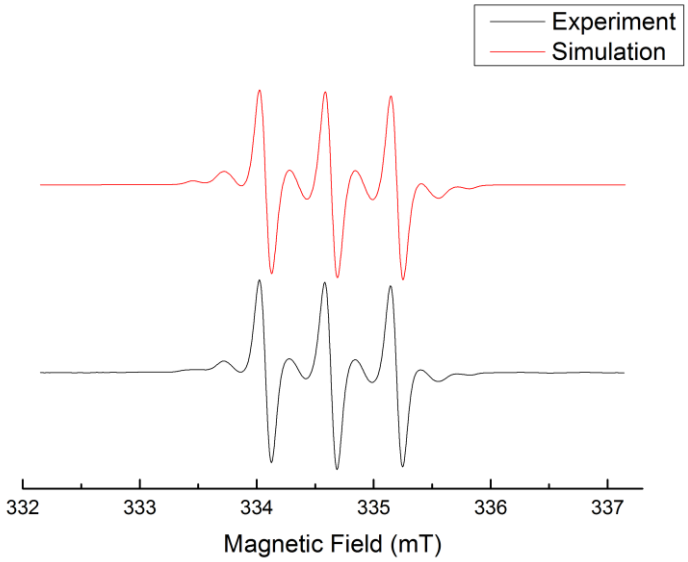


Figure S10: X-band cw-EPR spectrum of **CD-N@C_{60}** (water, 100 K); experimental data (black line), Easyspin simulation (red line).

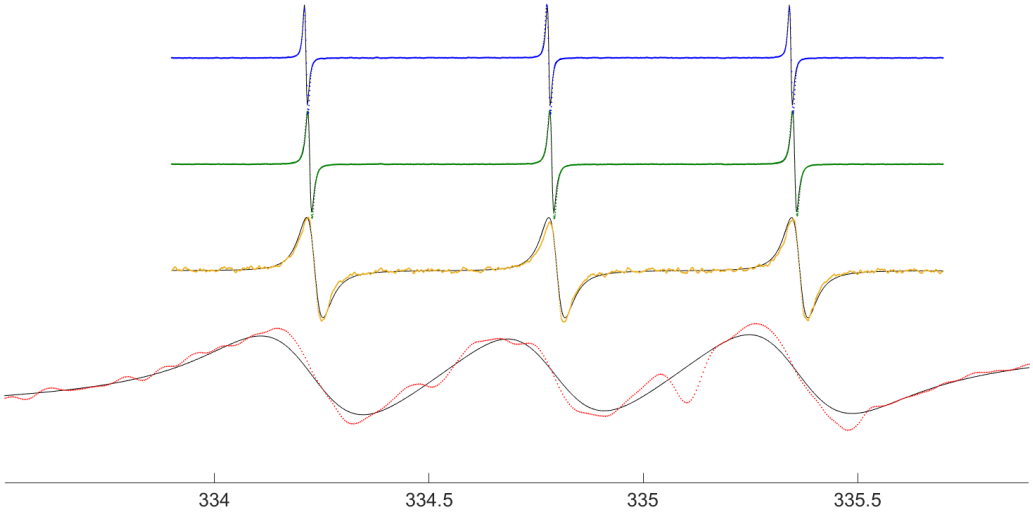


Figure S11: X-band cw-EPR spectra of acetone solutions of **CD-N@C_{60}** containing various concentrations of $Cu(ClO_4)_2 \cdot 6H_2O$; 0 mM (blue), 1 mM (green), 10 mM (yellow) and 100 mM (red). Easyspin simulations shown as black line.

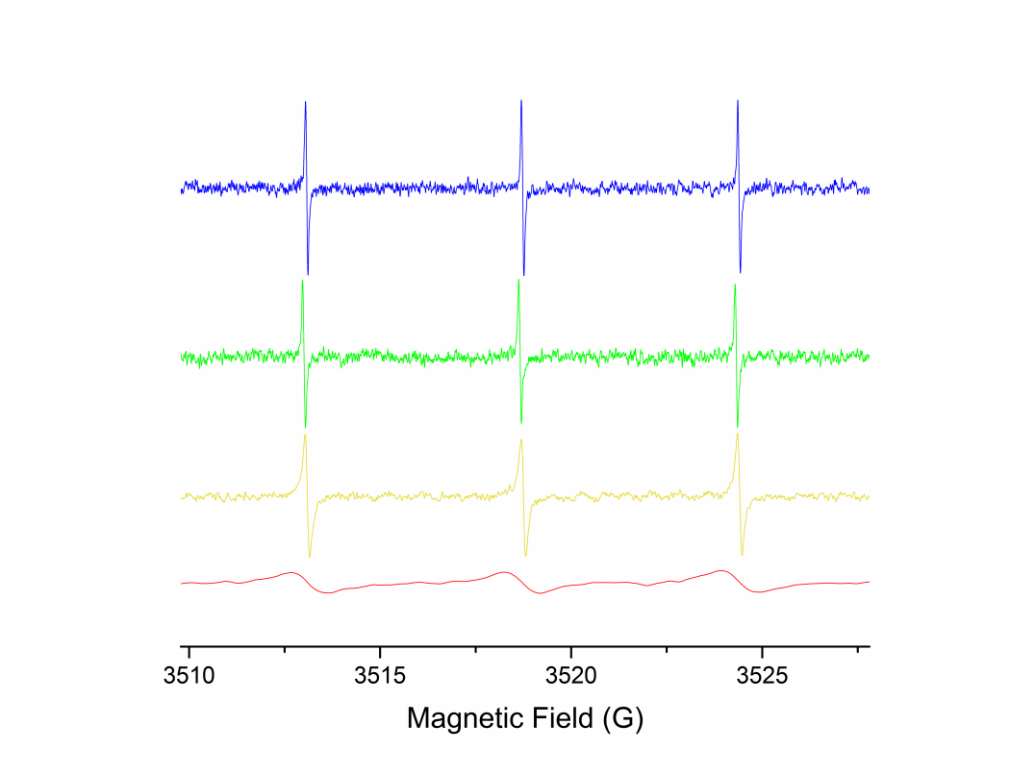


Figure S12: X-band cw-EPR spectra of ethanol solutions of **CD-N@C_{60}** containing various concentrations of $Cu(ClO_4)_2 \cdot 6H_2O$; 0 mM (blue), 1 mM (green), 10 mM (yellow) and 100 mM (red).

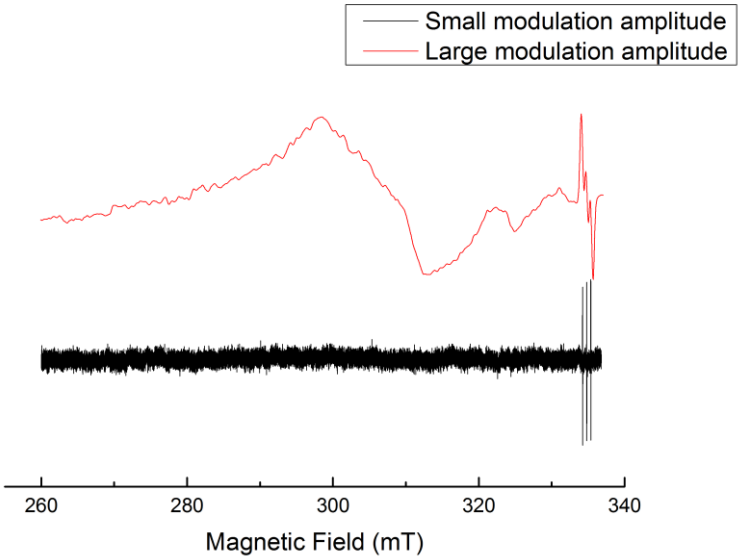


Figure S13: X-band cw-EPR spectra of an acetone solution of $Cu(ClO_4)_2 \cdot 6H_20$ (1mM) and **CD-N@C_{60}** with a large modulation amplitude applied (red line) and a small modulation amplitude applied (black line) (298 K).

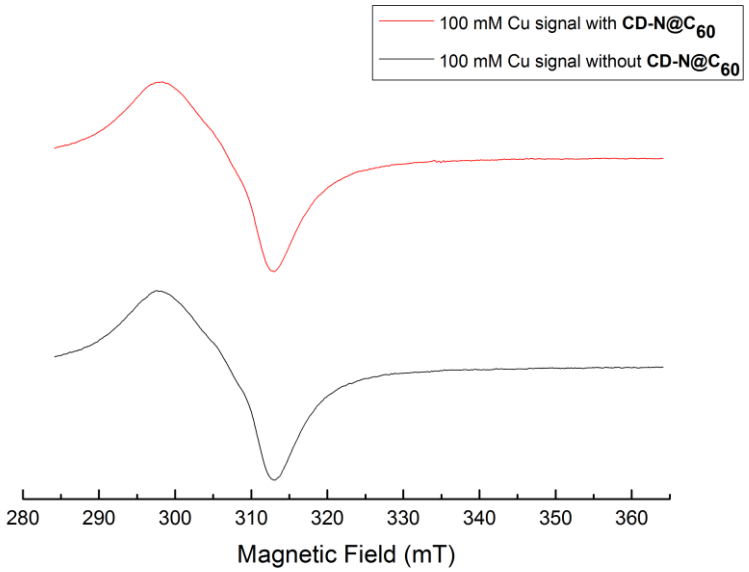


Figure S14: X-band cw-EPR spectra of an acetone solution of $Cu(ClO_4)_2 \cdot 6H_20$ (100 mM) in the presence of **CD-N@C_{60}** (red line) and in the absence of **CD-N@C_{60}** (black line) (298 K). The identical Cu(II) signal in both spectra suggest that the Cu(II) ions do not coordinate with the cyclodextrin units of **CD-N@C_{60}**.

3. References

[1] T. Almeida Murphy, T. Pawlik, A. Weidinger, M. Höhne, R. Alcala, J.-M. Spaeth, *Phys. Rev. Lett.* **1996**, *77*, 1075–1078.

[2] P. Jakes, K.-P. Dinse, C. Meyer, W. Harneit, A. Weidinger, *Phys. Chem. Chem. Phys.* **2003**, *5*, 4080–4083.

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[5] S. Stoll, A. Schweiger, *J. Magn. Reson.* **2006**, *178*, 42–55.