Supplementary Information for

Interrogating a single light-driven rotary molecular motor with force

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1. Synthesis

1.1 Materials and general methods

Commercial reagents and solvents: All chemicals and solvents were purchased from commercial suppliers unless otherwise stated. Anhydrous solvents were obtained using a MBraun SPS 800 system and stored under N_2.

Synthesized reagents: 2-(±)Methyl-2,3-dihydro-1*H*-cyclopenta[a]-(6-bromonaphthalen)-1-one[1], Dimethyl 3,3'-(10-hydrazono-9,10-dihydroanthracene-9,9-diyl)dipropanoate[2,3] and 3-(Triisopropylsilyl)prop-2-yn-1-ol[4] were synthesized according to reported literature procedures and characterized using routine characterization techniques.

Synthesis and purification: Standard Schlenk techniques were used, employing nitrogen or argon as the inert gas. If they were not performed at room temperature, the reaction temperatures refer to the temperature of the heating/cooling bath or heating block.

Flash column chromatography was performed on a Biotage Selekt system using the indicated solvents. TLC analysis was done on Merck silica gel 60 F_{254} aluminum sheets, and compounds were visualized with a UV lamp (254 nm or 365 nm).

Analysis:

NMR:

Full characterization of the newly synthesized compounds (including ^{1}H, ^{13}C, ^{29}Si and 2D NMR experiments) was performed using a Bruker Avance Neo 600 (600 MHz) spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to TMS, using the solvent residual peak as internal standard ($CDCl_3$: δ = 7.26 for ^{1}H, δ = 77.16 for ^{13}C; CD_2Cl_2: δ = 5.32 for ^{1}H, δ = 53.84 for ^{13}C). Data is reported as follows: chemical shifts (δ) in ppm, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, td = triplet of doublets, t = triplet, q = quartet, p = quintet, br. = broad, m = multiplet), coupling constants *J* (Hz), and integration. Signals were assigned with the help of 2D NMR experiments.

High-resolution mass (HMRS) spectra were recorded on a Thermofisher LTQ Orbitrap XL.

1.2 Synthetic procedures

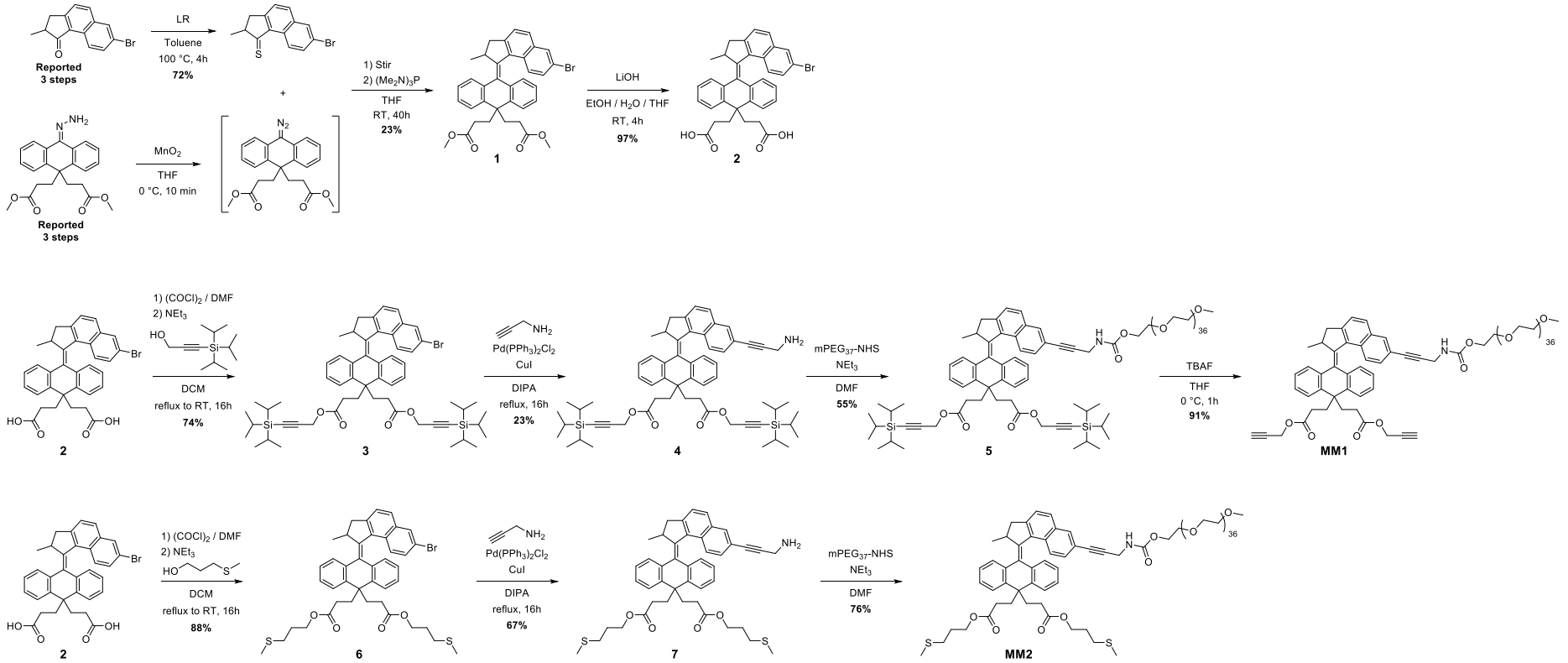
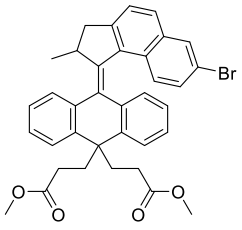


Figure S1: Overview of the synthesis of molecular motors **MM1** and **MM2**

Molecular motor 1

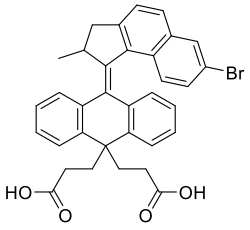


2-(±)Methyl-2,3-dihydro-1*H*-cyclopenta[a]-(6-bromonaphthalen)-1-one[1] (900 mg, 3.27 mmol, 1 equiv.) and Lawesson's Reagent (3.97 g, 9.81 mmol, 3 equiv.) were suspended in degassed anhydrous toluene (35 mL). The mixture was stirred at 100 °C for 4 h. After cooling down to room temperature, the suspension was directly transferred to a short chromatography column (SiO_2, CH_2Cl_2/Pentane 0:100 to 20:90). The red fraction was collected. Solvents were removed, affording 2-(±)Methyl-2,3-dihydro-1*H*-cyclopenta[a]-(6-bromonaphthalen)-1-thione in 72% yield (687 mg, 2.36 mmol) which was immediately used without any further purification for the next step.

Dimethyl 3,3'-(10-hydrazono-9,10-dihydroanthracene-9,9-diyl)dipropanoate[2,3] (1.01 g, 2.65 mmol, 1.4 equiv.) was dissolved in anhydrous tetrahydrofuran (38 mL). Anhydrous sodium sulfate (2.69 g, 18.96 mmol, 10 equiv.) was added. The suspension was degassed by bubbling argon for 15 min and the reaction mixture was cooled down to 0 °C and placed in the dark. MnO_2 (824 mg, 9.48 mmol, 5 equiv.) was added and the suspension was stirred at 0 °C for 10 min. The *in-situ* generated diazo compound was then filtered and transferred to another Schlenk flask containing 2-(±)methyl-2,3-dihydro-1*H*-cyclopenta[a]-(6-bromonaphthalen)-1-thione (687 mg, 2.36 mmol, 1 equiv.) by cannula filtration. An extra 8 mL of THF was used for rinsing. The resulting solution was then stirred at room temperature for 24 h in the dark before tris(dimethylamino)phosphine (1.0 mL, 5.69 mmol, 3 equiv.) was added. The reaction mixture was stirred for a further 16 h and the volatiles were removed in vacuo. The crude product was adsorbed on silica and purified by column chromatography (SiO_2, CH_2Cl_2/pentane, 5:95) to yield pure molecular motor **1** a white solid in a 23% yield (261 mg, 0.43 mmol).

R_f = 0.45 (SiO_2, EtOAc/pentane 10:90). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C): 7.90 (dd, *J* = 5.8, 3.3 Hz, 1H), 7.85 (d, *J* = 2.2 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.48 – 7.43 (m, 3H), 7.30 (dd, *J* = 5.8, 3.3 Hz, 2H), 7.15 (td, *J* = 7.6, 1.6 Hz, 1H), 6.84 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.81 (dd, *J* = 9.1, 2.2 Hz, 1H), 6.68 (td, *J* = 7.5, 1.4 Hz, 1H), 6.52 (d, *J* = 9.1 Hz, 1H), 4.46 (p, *J* = 6.8 Hz, 1H), 3.71 (s, 3H), 3.61 (dd, *J* = 15.3, 6.1 Hz, 1H), 3.47 (s, 3H), 2.87 – 2.75 (m, 2H), 2.58 (d, *J* = 15.3 Hz, 1H), 2.51 – 2.38 (m, 4H), 2.19 (dd, *J* = 9.1, 7.3 Hz, 2H), 0.73 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, $CDCl_3$, 25 °C): δ = 174.5, 173.5, 146.8, 144.6, 141.3, 140.2, 140.0, 139.5, 136.3, 134.5, 130.0, 129.1, 128.4, 128.3, 128.3, 127.7, 127.6, 127.4, 126.9, 126.5, 126.4, 126.0, 125.9, 125.6, 125.3, 118.2, 51.9, 51.7, 47.1, 39.7, 37.9, 35.9, 30.6, 29.6, 27.6, 18.8 ppm. **HR-MS** (ESI+): calcd. for $C_{36}H_{34}BrO_4$ $[M+H]^+$: 609.16350, found 609.16385.

Molecular motor 2

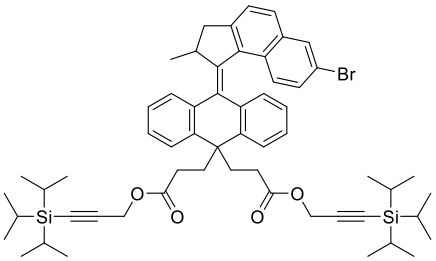


Molecular motor 1 (100 mg, 0.16 mmol, 1 equiv.) was dissolved in THF (1 mL). A solution of LiOH (31 mg, 1.29 mmol, 8 equiv.) dissolved in a H_2O (1 mL) / EtOH (1 mL) mixture was added. The resulting mixture was stirred for 5 h under an inert atmosphere, acidified to pH 2 with 1M aqueous HCl and extracted with EtOAc (3x10 mL). The organic layer was washed with water (10 mL), brine (10 mL), dried over anhydrous $MgSO_4$ and the solvents were removed in vacuo to yield molecular motor **2** as a white solid in 97% yield (92 mg, 0.16 mmol).

^{1}H NMR (600 MHz, $CDCl_3$/CD_3OD 4:1, 25 °C): 7.88 (dd, *J* = 5.9, 3.1 Hz, 1H), 7.81 (d, *J* = 2.2 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.28 (dd, *J* = 5.9, 3.2 Hz, 2H), 7.12 (td, *J* = 7.6, 1.5 Hz, 1H), 6.83 – 6.74 (m, 2H), 6.64 (t, *J* = 7.5 Hz, 1H), 6.53 (d, *J* = 9.2 Hz, 1H), 4.44 (p, *J* = 6.7 Hz, 1H), 3.58 (dd, *J* = 15.3, 6.2 Hz, 1H), 2.89 – 2.70 (m, 2H), 2.54 (d, *J* = 15.4 Hz, 1H), 2.49 – 2.33 (m, 4H), 2.13 (t, *J* = 8.2 Hz, 2H), 0.69 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, $CDCl_3$/CD_3OD 4:1, 25 °C): 176.2, 175.5, 146.5, 144.1, 141.2, 139.9, 139.9, 139.1, 135.9, 134.2, 129.6, 128.7, 128.1, 128.0, 128.0, 127.4, 127.2, 127.0, 126.5, 126.1, 125.9, 125.8, 125.5, 125.3, 125.0, 117.7, 46.8, 39.3, 37.5, 35.6, 30.2, 29.3, 27.2, 18.3 ppm. **HR-MS** (ESI+): calcd. for $C_{34}H_{30}BrO_4$ $[M+H]^+$: 581.13220, found 581.13244.

R_f could not be reliably measured due to smearing.

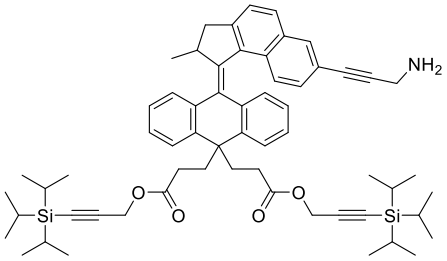
Molecular motor 3



Oxalyl chloride (59 µL, 0.69 mmol, 8 equiv.) was added to a solution of molecular motor **2** (50 mg, 86 µmol, 1 equiv.) in anhydrous CH_2Cl_2 (1 mL). A drop of DMF (~10-20 µL) was added and the mixture was heated to reflux for 3 h. The solvents were removed in vacuo and the residue was redissolved in anhydrous CH_2Cl_2 (1 mL). The solvents were removed in vacuo again, and the residue was redissolved in anhydrous CH_2Cl_2 (1 mL). 3-(Triisopropylsilyl)prop-2-yn-1-ol[4] (274 mg, 1.29 mmol, 15 equiv.) and triethylamine (48 µL, 0.34 mmol, 4 equiv.) were added, and the solution was stirred at room temperature for 8 hours. The solvents were removed and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2/pentane, 10:90) to yield pure molecular motor **3** a dark reddish oil in 74% yield (61 mg, 63 µmol).

R_f = 0.38 (SiO_2, CH_2Cl_2/pentane 30:70). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C): 7.91 – 7.88 (m, 1H), 7.84 (d, *J* = 2.2 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.50 – 7.42 (m, 3H), 7.32 – 7.29 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.80 (dd, *J* = 9.1, 2.2 Hz, 1H), 6.68 (t, *J* = 7.5 Hz, 1H), 6.51 (d, *J* = 9.1 Hz, 1H), 4.75 (s, 2H), 4.51 (q, *J* = 15.9 Hz, 2H), 4.46 – 4.42 (m, 1H), 3.60 (dd, *J* = 15.3, 6.1 Hz, 1H), 2.82 (t, *J* = 8.2 Hz, 2H), 2.57 (d, *J* = 15.3 Hz, 1H), 2.54 – 2.33 (m, 4H), 2.22 (td, *J* = 7.0, 2.5 Hz, 2H), 1.08 (br. s, 21H), 0.96 (br. s, 21H), 0.73 (d, *J* = 6.8 Hz, 3Hppm. **^{13}C{^{1}H} NMR** (151 MHz, $CDCl_3$, 25 °C): δ = 13C NMR (151 MHz, CDCl3) δ 173.2, 172.2, 146.8, 144.7, 141.1, 140.2, 140.1, 139.6, 136.2, 134.5, 130.0, 129.1, 128.4, 128.2, 128.2, 127.7, 127.6, 127.5, 127.0, 126.5, 126.4, 125.9, 125.9, 125.6, 125.2, 118.2, 101.1, 100.8, 88.6, 88.4, 53.1, 52.9, 47.0, 39.7, 37.9, 35.8, 30.6, 29.7, 27.6, 18.9, 18.7 (6 C, CH_3-TIPS), 18.6 (6 C, CH_3-TIPS), 11.2 (3 C, CH-TIPS), 11.2 (3 C, CH-TIPS) ppm. **HR-MS** (APCI+): calcd. for $C_{58}H_{74}BrO_4Si_2$ $[M+H]^+$: 969.43035, found 969.42744.

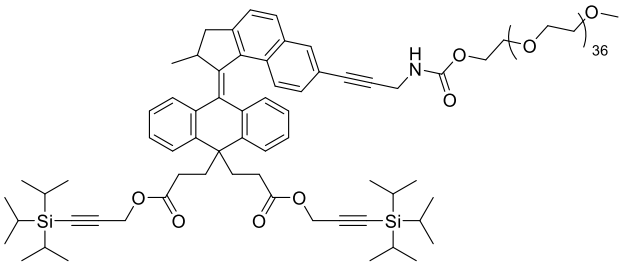
Molecular motor 4



Molecular motor **3** (50 mg, 52 µmol, 1 equiv.) and diisopropylamine (2.3 mL) were placed in a Schlenk flask. The solution was degassed by bubbling argon for 2 min and propargylamine (28 mg, 515 µmol, 10 equiv.) was added. The mixture was degassed by a freeze-pump-thaw cycle. Copper(I) iodide (0.5 mg, 2.6 µmol, 5 mol%) and $Pd(PPh_3)_2Cl_2$ (7.0 mg, 10.4 µmol, 20 mol%) were then added under a flow of nitrogen. The suspension was submitted to two additional freeze-pump-thaw cycles and heated to reflux under an argon atmosphere in the dark for 24 h. After cooling to room temperature, the reaction mixture was filtered over a pad of silica (eluted with EtOAc). The solvents were removed, and the crude residue was purified by column chromatography (SiO_2, MeOH/EtOAc, 0:100 to 10:90) to yield molecular motor **4** a brown oil in 27% yield (13 mg, 14 µmol). The samples obtained using this method were found to contain ca. 0.6 equiv. of triphenylphosphine oxide (NMR) resulting from catalyst decomposition. Taking in to account the presence of this impurity, the yield in pure motor **4** can be corrected to 23% (11.7 µmol). In reason of the relatively low stability of the propargylamine moiety, it was chosen to proceed without further purification and remove the leftover triphenylphosphine oxide at the next step.

R_f = 0.35 (SiO_2, EtOAc). **^{1}H NMR** (600 MHz, CD_2Cl_2, 25 °C): 7.96 – 7.90 (m, 1H), 7.78 (br. s, 1H), 7.68 – 7.64 (m, 1H), 7.49 – 7.44 (m, 3H), 7.36 – 7.29 (m, 2H), 7.15 (td, *J* = 7.5, 1.5 Hz, 1H), 6.82 (dd, *J* = 7.6, 1.6 Hz, 1H), 6.71 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.68 – 6.63 (m, 1H), 6.58 (d, *J* = 8.8 Hz, 1H), 4.74 (s, 2H), 4.54 – 4.43 (m, 3H), 3.64 (dd, *J* = 15.3, 6.2 Hz, 2H), 2.83 (q, *J* = 7.2 Hz, 2H), 2.60 (d, *J* = 15.5 Hz, 1H), 2.55 – 2.35 (m, 5H), 2.19 (t, *J* = 8.1 Hz, 2H), 1.08 (br. s, 21H), 0.98 – 0.94 (m, 21H), 0.72 (d, *J* = 6.9 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, CD_2Cl_2, 25 °C): δ = 173.1, 172.4, 147.7, 145.1, 141.5, 140.7, 140.4, 139.9, 136.3, 133.2, 131.6, 130.0, 128.8, 128.5, 128.3, 127.6, 127.1, 127.1, 126.8, 126.7, 126.4, 126.4, 126.2, 126.0, 125.2, 119.1, 101.7, 101.5, 88.5, 88.2, 82.6, 53.2, 53.0, 47.4, 40.0, 38.2, 36.0, 30.8, 29.9, 27.8, 18.9, 18.7, 18.6, 11.5, 11.4 ppm. **HR-MS** (ESI+): calcd. for $C_{61}H_{78}NO_4Si_2$ $[M+H]^+$: 944.54639, found 944.54690.

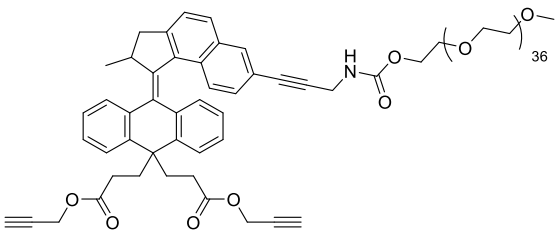
Molecular motor 5



Molecular motor **4** (5 mg, 5.3 µmol, 1 equiv.) and commercial monodisperse PEG chain mPEG37-NHS ester (9.5 mg, 5.3 µmol, 1 equiv.) were dissolved in anhydrous DMF (0.5 mL) and triethylamine (5.4 mg, 53 µmol, 10 equiv.) was added. The solution was stirred at room temperature under an argon atmosphere for 24 h and the solvents were removed in vacuo. The crude residue was purified by column chromatography (SiO_2, NEt_3/MeOH/CH_2Cl_2, 1:1:98) to yield pure molecular motor **5** a viscous brown solid in 55% yield (8 mg, 2.9 µmol).

***R*f** = 0.23 (SiO_2, NEt_3/MeOH/CH_2Cl_2 1:1:98). **^{1}H NMR** (600 MHz, CD_2Cl_2, 25 °C): 7.94 – 7.91 (m, 1H), 7.80 (d, *J* = 1.9 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.52 – 7.44 (m, 3H), 7.35 – 7.28 (m, 2H), 7.15 (td, *J* = 7.4, 1.5 Hz, 1H), 6.82 (m, 1H), 6.81 (dd, *J* = 7.6, 1.5 Hz, 1H), 6.72 (dd, *J* = 8.8, 1.7 Hz, 1H), 6.65 (td, *J* = 7.4, 1.2 Hz, 1H), 6.59 (d, *J* = 8.8 Hz, 1H), 4.74 (br. s, 2H), 4.50 (d, *J* = 2.5 Hz, 2H), 4.47 – 4.44 (m, 1H), 4.24 (d, *J* = 5.4 Hz, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 3.73 – 3.70 (m, 2H), 3.66 – 3.57 (m, 144H), 3.51 – 3.49 (m, 2H), 3.34 (s, 3H), 2.82 (dd, *J* = 9.8, 6.6 Hz, 2H), 2.60 (d, *J* = 15.5 Hz, 1H), 2.58 – 2.35 (m, 6H), 2.21 – 2.16 (m, 2H), 1.08 (m, 21H), 0.98 – 0.94 (m, 21H), 0.72 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, CD_2Cl_2, 25 °C): δ = 73.1, 172.3, 171.3, 147.8, 145.0, 141.4, 140.6, 140.5, 139.9, 136.3, 133.1, 132.0, 130.0, 128.9, 128.5, 128.4, 127.6, 127.1, 127.0, 126.9, 126.7, 126.4, 126.3, 126.2, 126.0, 125.2, 118.6, 101.7, 101.5, 88.5, 88.2, 86.0, 83.0, 72.3, 70.9, 70.8, 70.7, 67.4, 59.0, 53.2, 53.0, 47.4, 40.0, 38.1, 37.1, 36.1, 30.8, 30.2, 30.1, 30.1, 30.1, 29.9, 29.9, 29.8, 27.7, 18.9, 18.7, 18.6, 11.5, 11.4 ppm. **HR-MS** (ESI+): calcd. for $C_{137}H_{228}NO_{42}Si_2$ $[M+H]^+$: 2616.53025, found 2616.51702.

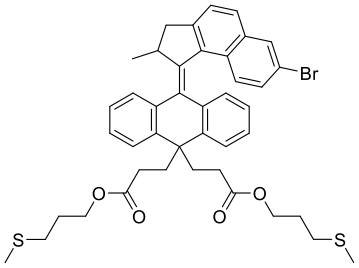
Molecular motor MM1



Molecular motor **5** (10 mg, 3.8 µmol, 1 equiv.) was dissolved in anhydrous THF (1 mL) under an inert atmosphere. The mixture was cooled down to 0 °C and TBAF (1.0 M in THF, 11 µL, 11.5 µmol, 3 equiv.) was added. The solution was stirred at this temperature for 1 h, warmed up to room temperature and filtered through a plug of SiO_2 (eluted with EtOAc). The solvents were removed in vacuo, and the crude product was purified by two successive column chromatographies (SiO_2, MeOH/CH_2Cl_2 0:100 to 20:80 and NEt_3/MeOH/CH_2Cl_2 98:1:1) to afford pure molecular motor **MM1** as a light-brown oil in 91% yield (8 mg, 3.5 µmol).

R_f = 0.72 (SiO_2, NEt_3/MeOH/CH_2Cl_2 1:1:98). **^{1}H NMR** (600 MHz, CD_2Cl_2, 25 °C): 7.97 – 7.92 (m, 1H), 7.80 (d, *J* = 1.7 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.49 – 7.45 (m, 3H), 7.37 – 7.30 (m, 2H), 7.16 (td, *J* = 7.6, 1.4 Hz, 1H), 6.87 – 6.83 (m, 1H), 6.82 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.73 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.66 (td, *J* = 7.5, 1.1 Hz, 1H), 6.57 (d, *J* = 8.8 Hz, 1H), 4.71 (d, *J* = 2.5 Hz, 2H), 4.49 – 4.41 (m, 3H), 4.23 (d, *J* = 5.5 Hz, 2H), 3.77 – 3.69 (m, 3H), 3.67 – 3.56 (m, 139H), 3.52 – 3.46 (m, 4H), 3.34 (s, 3H), 3.05 (m, 1H), 2.90 – 2.75 (m, 2H), 2.60 (d, *J* = 15.4 Hz, 1H), 2.55 – 2.43 (m, 6H), 2.36 (t, *J* = 2.5 Hz, 1H), 2.22 – 2.17 (m, 2H), 1.36 (t, *J* = 7.3 Hz, 1H), 0.72 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, CD_2Cl_2, 25 °C): δ = 173.2, 172.3, 171.4, 147.9, 145.0, 141.7, 140.7, 140.1, 139.8, 136.3, 133.1, 132.0, 130.0, 128.9, 128.5, 128.4, 127.6, 127.1, 127.1, 126.8, 126.7, 126.5, 126.2, 125.8, 125.3, 118.6, 86.1, 82.9, 75.0, 75.0, 72.3, 70.9, 70.8, 70.8, 70.7, 67.4, 65.0, 59.0, 52.3, 52.1, 47.4, 46.2, 40.0, 38.2, 37.2, 35.9, 30.7, 29.9, 29.8, 27.6, 18.8, 8.8 ppm. **HR-MS** (ESI+): calcd. for $C_{119}H_{187}NO_{42}Na$ $[M+Na]^+$: 2326.24644, found 2326.23722.

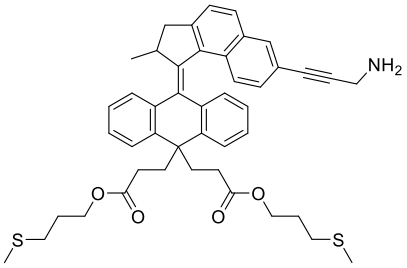
Molecular motor 6



Oxalyl chloride (59 µL, 0.69 mmol, 8 equiv.) was added to a solution of molecular motor **2** (50 mg, 86 µmol, 1 equiv.) in anhydrous CH_2Cl_2 (1 mL). A drop of DMF (~10-20 µL) was added and the mixture was heated to reflux for 3 h. The solvents were removed in vacuo and the residue was redissolved in anhydrous CH_2Cl_2 (1 mL). The solvents were removed in vacuo again, and the residue was redissolved in anhydrous CH_2Cl_2 (1 mL). Methionol (3-(Methylthio)-1-propanol) (137 mg, 1.29 mmol, 15 equiv.) and triethylamine (48 µL, 0.34 mmol, 4 equiv.) were added, and the solution was stirred at room temperature for 8 hours. The solvents were removed and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2/pentane, 10:90) to yield pure molecular motor **6** a light-yellow solid in 88% yield (57 mg, 63 µmol).

R_f = 0.48 (SiO_2, EtOAc/pentane 10:90). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C): 7.90 (dd, *J* = 5.8, 3.3 Hz, 1H), 7.85 (d, *J* = 2.1 Hz, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.48 – 7.42 (m, 3H), 7.33 – 7.29 (m, 2H), 7.16 (td, *J* = 7.6, 1.4 Hz, 1H), 6.84 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.80 (dd, *J* = 9.1, 2.1 Hz, 1H), 6.69 (td, *J* = 7.4, 1.1 Hz, 1H), 6.52 (d, *J* = 9.0 Hz, 1H), 4.46 (p, *J* = 6.7 Hz, 1H), 4.21 (t, *J* = 6.3 Hz, 2H), 4.00 – 3.91 (m, 2H), 3.61 (dd, *J* = 15.3, 6.1 Hz, 1H), 2.89 – 2.74 (m, 2H), 2.58 (d, *J* = 15.3 Hz, 1H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.49 – 2.41 (m, 4H), 2.28 (t, *J* = 7.2 Hz, 2H), 2.21 – 2.15 (m, 2H), 2.10 (s, 3H), 1.92 (p, *J* = 6.5 Hz, 2H), 1.89 (s, 3H), 1.70 – 1.64 (m, 2H), 0.72 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, $CDCl_3$, 25 °C): δ = 174.0, 173.0, 146.8, 144.5, 141.5, 140.2, 139.9, 139.5, 136.2, 134.5, 130.0, 129.1, 128.4, 128.3, 128.2, 127.7, 127.6, 127.4, 126.9, 126.5, 126.4, 126.1, 125.9, 125.5, 125.3, 118.2, 77.4, 77.2, 77.0, 63.3, 63.2, 47.1, 39.7, 37.9, 35.9, 30.9, 30.8, 30.5, 29.8, 28.3, 28.1, 27.5, 18.9, 15.7, 15.4 ppm. **HR-MS** (ESI+): calcd. for $C_{42}H_{45}BrO_4S_2Na$ $[M+Na]^+$: 779.1835, found 779.1833.

Molecular motor 7

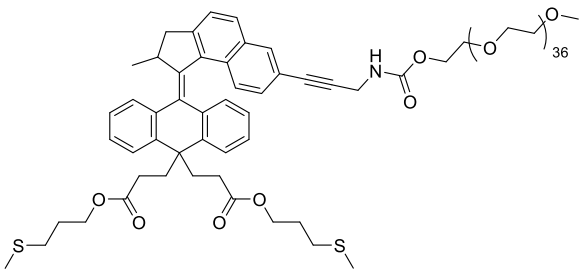


Molecular motor **3** (50 mg, 66 µmol, 1 equiv.) and diisopropylamine (2.8 mL) were placed in a Schlenk flask. The solution was degassed by bubbling argon for 2 min and propargylamine (36 mg, 660 µmol, 10 equiv.) was added. The mixture was degassed by a freeze-pump-thaw cycle. Copper(I) iodide (0.6 mg, 3.3 µmol, 5 mol%) and $Pd(PPh_3)_2Cl_2$ (4.6 mg, 6.6 µmol, 10 mol%) were then added under a flow of nitrogen. The suspension was submitted to two additional freeze-pump-thaw cycles and heated to reflux under an argon atmosphere in the dark for 17 h. After cooling to room temperature, the reaction mixture was filtered over a pad of silica (eluted with EtOAc). The solvents were removed, and the crude residue was purified by column chromatography (SiO_2, MeOH/EtOAc, 0:100 to 5:95) to yield pure molecular motor **7** an amorphous brown solid in 67% yield (33 mg, 44 µmol).

Molecular motor **7** was found to be relatively unstable under ambient conditions. Solvent should be removed as quick as possible to limit degradation and the product was stored in a freezer under an argon atmosphere.

R_f = 0.33 (SiO_2, MeOH/EtOAc 5:95). **^{1}H NMR** (600 MHz, $CDCl_3$, 25 °C): 7.91 (dd, *J* = 5.8, 3.4 Hz, 1H), 7.79 (d, *J* = 1.8 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.52 – 7.38 (m, 3H), 7.30 (dd, *J* = 5.8, 3.3 Hz, 2H), 7.14 (td, *J* = 7.6, 1.4 Hz, 1H), 6.83 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.73 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.65 (td, *J* = 7.5, 1.1 Hz, 1H), 6.57 (d, *J* = 8.8 Hz, 1H), 4.46 (p, *J* = 6.7 Hz, 1H), 4.21 (t, *J* = 6.4 Hz, 2H), 3.99 – 3.90 (m, 2H), 3.70 (s, 2H), 3.63 (dd, *J* = 15.4, 6.1 Hz, 1H), 2.90 – 2.72 (m, 2H), 2.59 (d, *J* = 15.4 Hz, 1H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.51 – 2.39 (m, 4H), 2.27 (t, *J* = 7.3 Hz, 2H), 2.22 – 2.15 (m, 4H), 2.10 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.87 (s, 3H), 1.70 – 1.62 (m, 2H), 0.73 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, $CDCl_3$, 25 °C): δ = 174.0, 173.1, 147.3, 144.6, 141.6, 140.3, 139.7, 139.5, 136.0, 132.8, 131.7, 129.8, 128.5, 128.4, 128.2, 127.4, 126.9, 126.8, 126.5, 126.4, 126.4, 126.1, 125.8, 125.5, 124.9, 118.5, 89.1, 83.5, 63.3, 63.2, 47.1, 39.8, 37.9, 35.9, 32.2, 30.9, 30.8, 30.5, 29.8, 28.3, 28.2, 27.5, 18.9, 15.7, 15.4 ppm. **HR-MS** (ESI+): calcd. for $C_{45}H_{50}NO_4S_2$ $[M+H]^+$: 732.31758, found 732.31733.

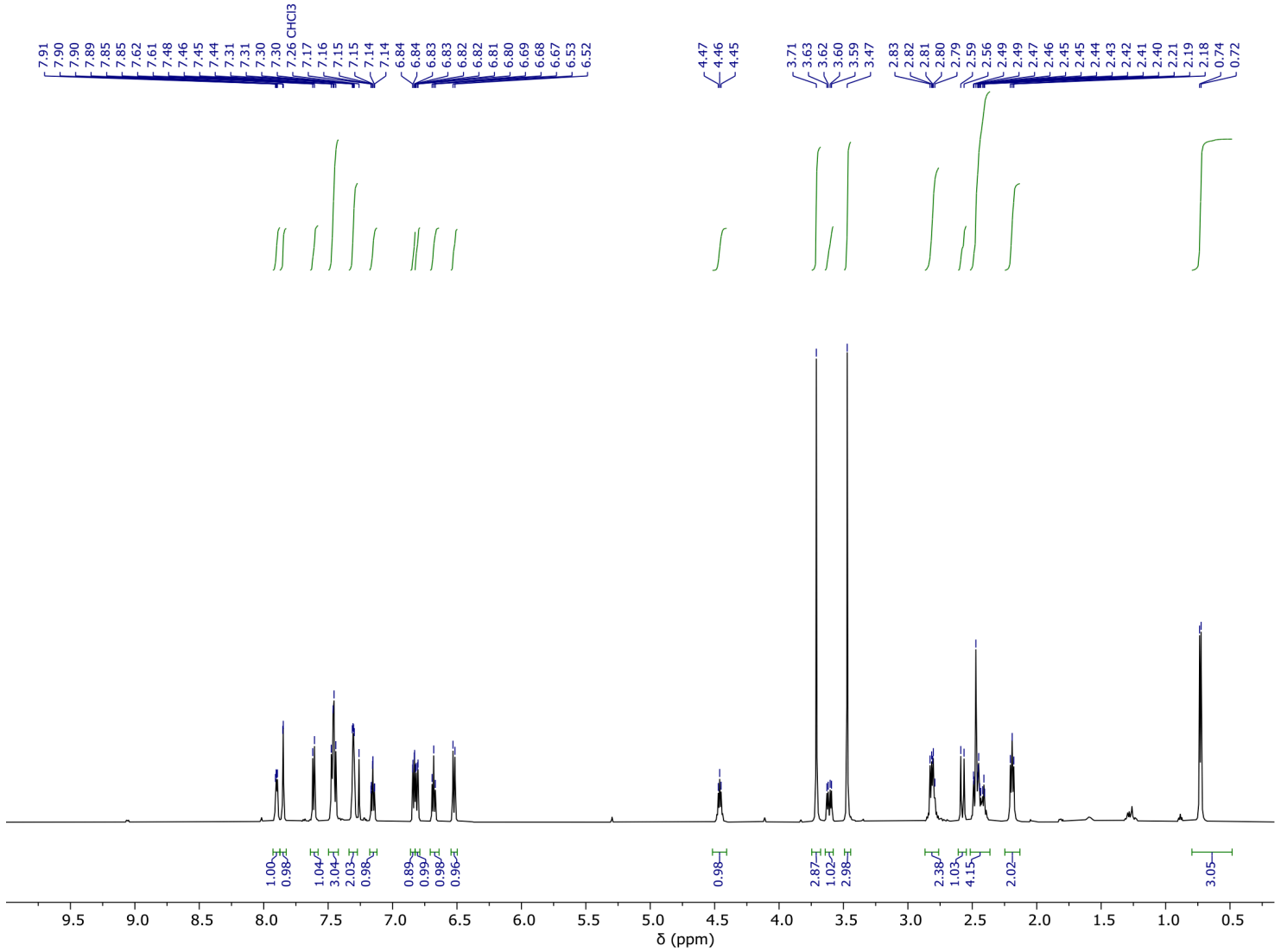
Molecular motor MM2



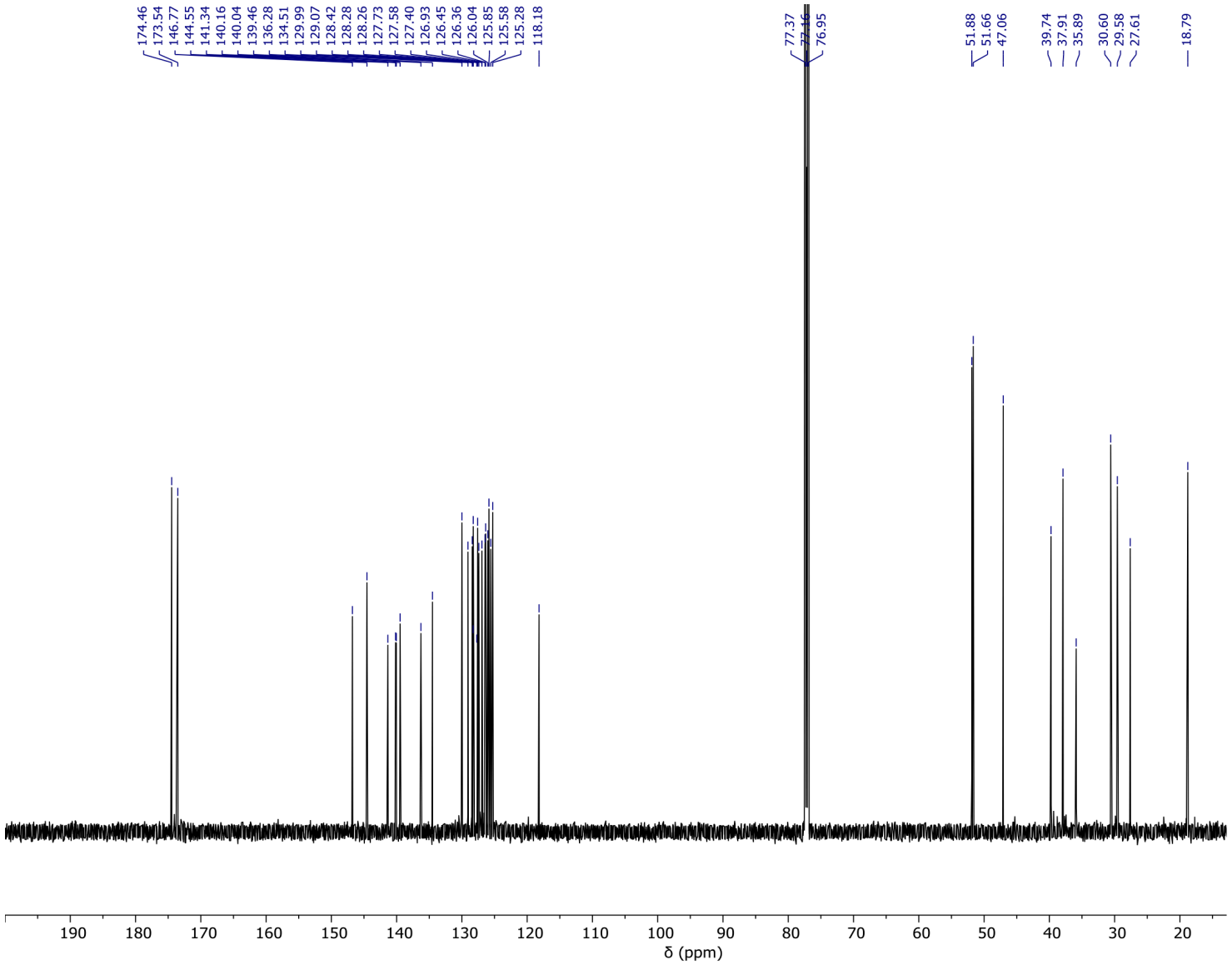
Molecular motor **7** (5 mg, 6.8 µmol, 1 equiv.) and commercial monodisperse PEG chain mPEG37-NHS ester (12 mg, 6.8 µmol, 1 equiv.) were dissolved in anhydrous DMF (0.5 mL) and triethylamine (6.9 mg, 68 µmol, 10 equiv.) was added. The solution was stirred at room temperature under an argon atmosphere for 24 h and the solvents were removed in vacuo. The crude residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2 5:95 to flush impurities out, followed by MeOH/acetone 20:80) to yield pure molecular motor **MM2** an amorphous brown solid in 76% yield (12 mg, 5.2 µmol).

***R*f** = 0.26 (SiO_2, NEt_3/MeOH/CH_2Cl_2 1:1:98). **^{1}H NMR** (600 MHz, CD_2Cl_2, 25 °C): 7.95 – 7.91 (m, 1H), 7.80 (d, *J* = 1.9 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 3H), 7.37 – 7.31 (m, 2H), 7.18 – 7.13 (m, 1H), 6.83 (m, 1H), 6.81 (dd, *J* = 7.6, 1.4 Hz, 1H), 6.73 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.65 (td, *J* = 7.4, 1.1 Hz, 1H), 6.59 (d, *J* = 8.8 Hz, 1H), 4.47 (p, *J* = 6.7 Hz, 1H), 4.24 (d, *J* = 5.5 Hz, 1H), 4.18 (t, *J* = 6.4 Hz, 2H), 3.94 – 3.90 (m, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 3.60 (d, *J* = 1.7 Hz, 147H), 3.34 (s, 3H), 2.90 – 2.75 (m, 3H), 2.60 (d, *J* = 15.5 Hz, 1H), 2.55 – 2.51 (m, 2H), 2.50 – 2.41 (m, 5H), 2.27 (t, *J* = 7.3 Hz, 2H), 2.14 (ddd, *J* = 9.3, 6.4, 2.2 Hz, 2H), 2.08 (s, 3H), 1.94 – 1.88 (m, 2H), 1.86 (s, 3H), 1.68 – 1.62 (m, 1H), 0.71 (d, *J* = 6.8 Hz, 3H) ppm. **^{13}C{^{1}H} NMR** (151 MHz, CD_2Cl_2, 25 °C): δ = 173.9, 173.1, 171.3, 147.9, 144.9, 142.0, 140.7, 140.3, 139.8, 136.3, 133.1, 132.0, 130.0, 128.8, 128.5, 127.5, 127.0, 127.0, 126.9, 126.7, 126.6, 126.4, 126.1, 125.9, 125.3, 118.5, 86.1, 82.9, 72.3, 71.0, 70.9, 70.8, 70.8, 70.7, 67.4, 66.1, 63.5, 63.3, 59.0, 47.5, 40.0, 38.2, 37.2, 36.2, 32.6, 31.1, 30.9, 30.7, 30.0, 29.9, 28.6, 28.4, 27.6, 26.0, 18.8, 15.6, 15.3 ppm. **HR-MS** (ESI+): calcd. for $C_{121}H_{200}NO_{42}S_2$ $[M+H]^+$: 2404.30144, found 2404.29784.

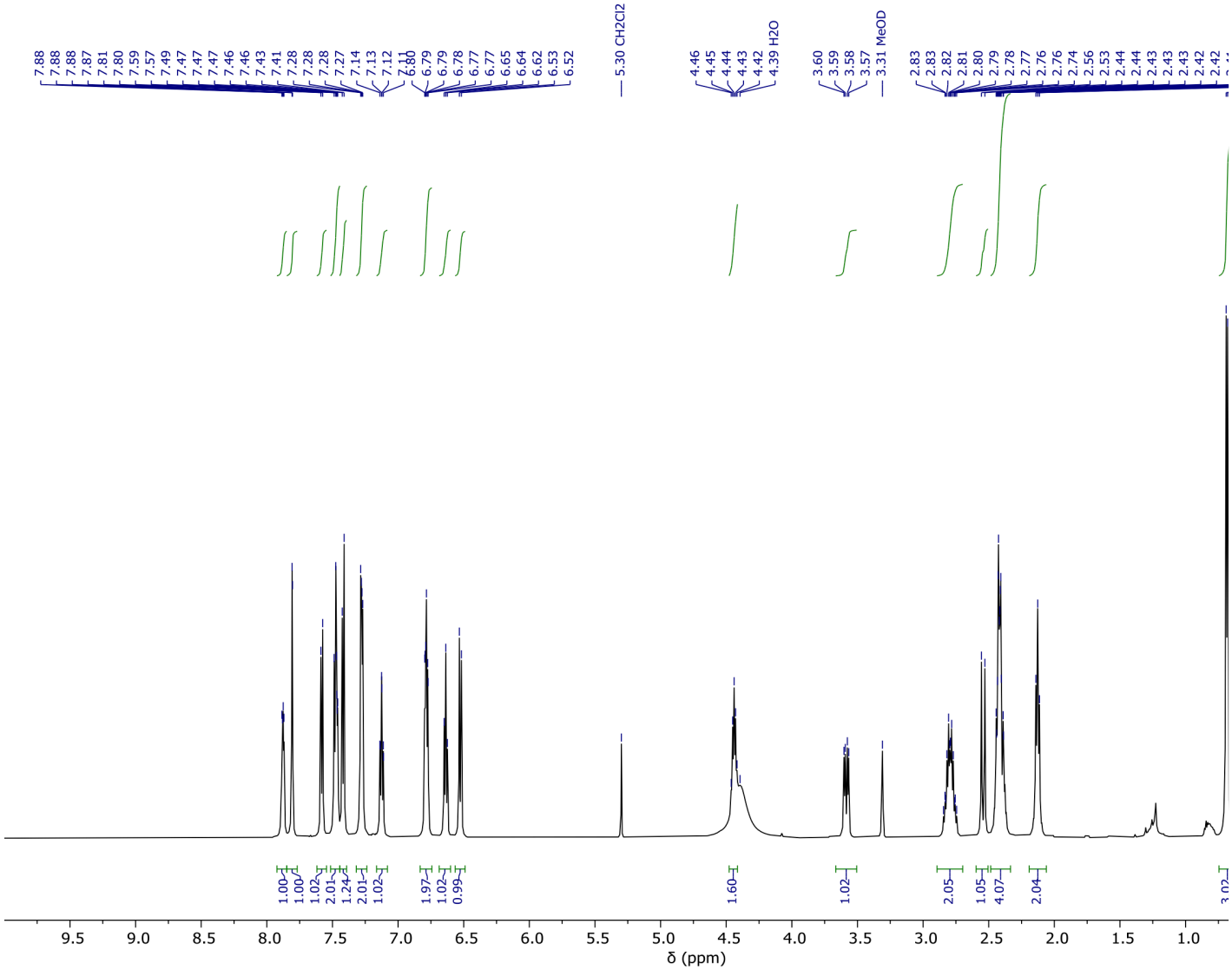
1.3 NMR spectra of new compounds



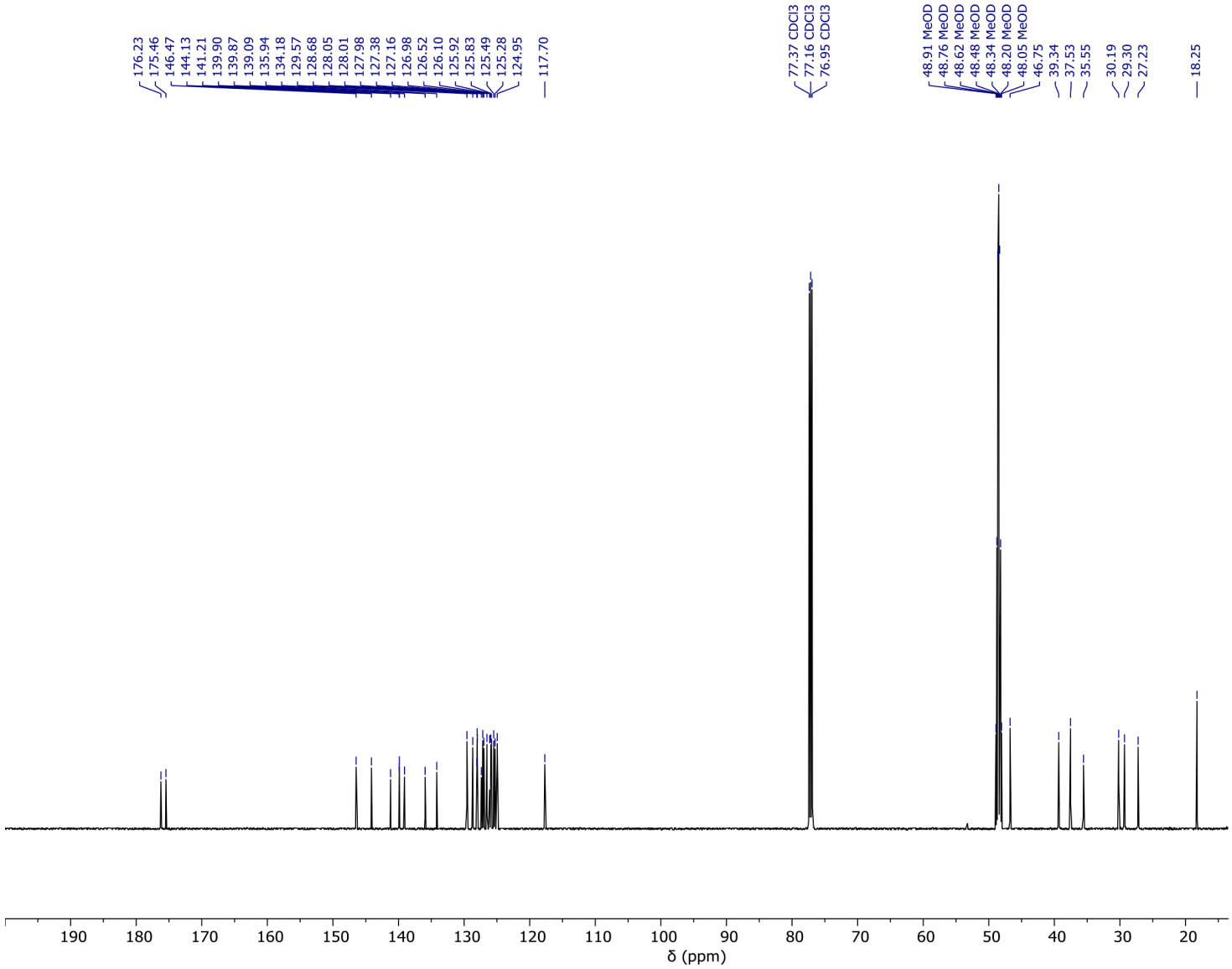
^{1}H-NMR of molecular motor **1** (600 MHz, $CDCl_3$, 25 °C).



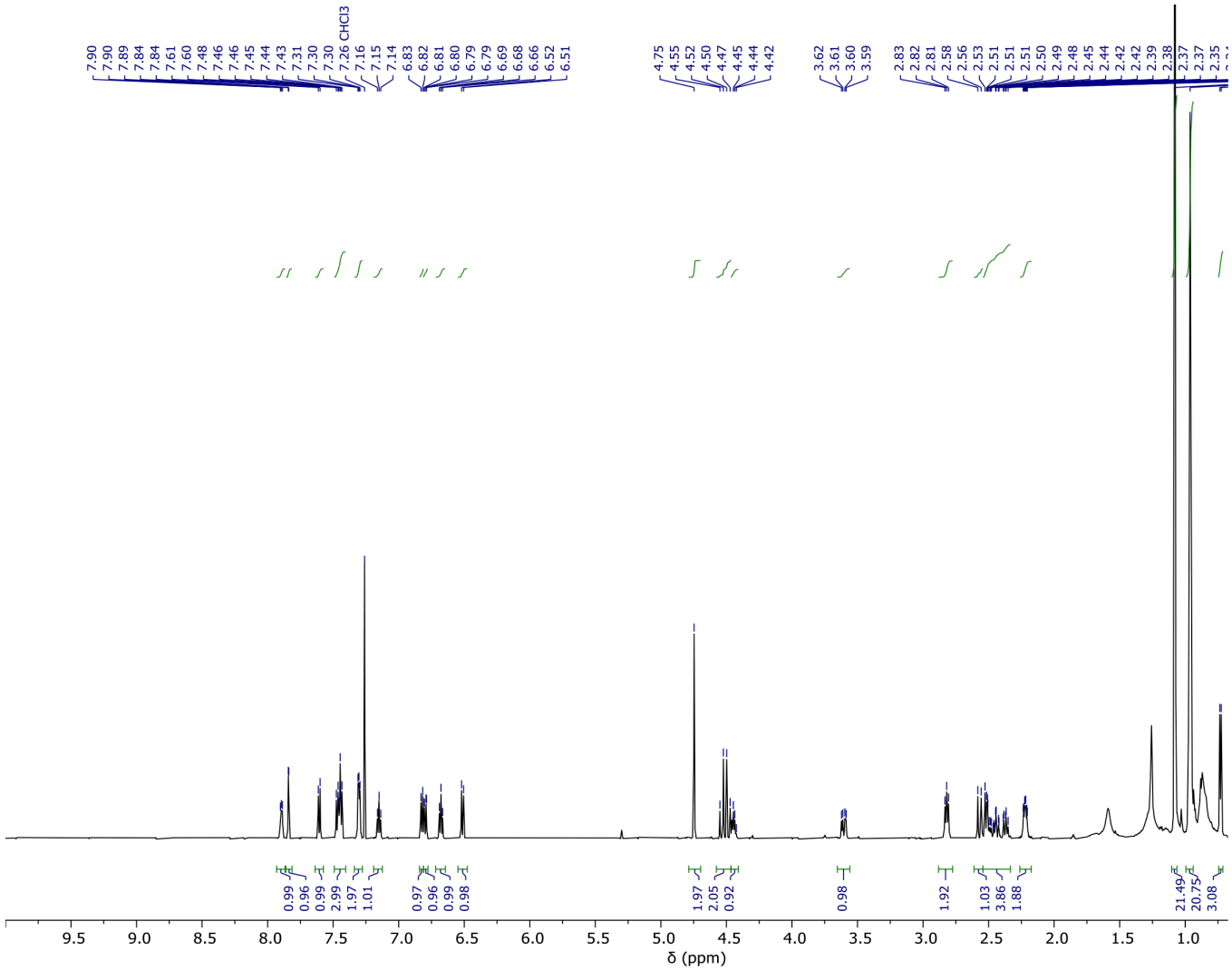
$^{13}C\{^{1}H\}$-NMR of molecular motor **1** (151 MHz, $CDCl_3$, 25 °C).



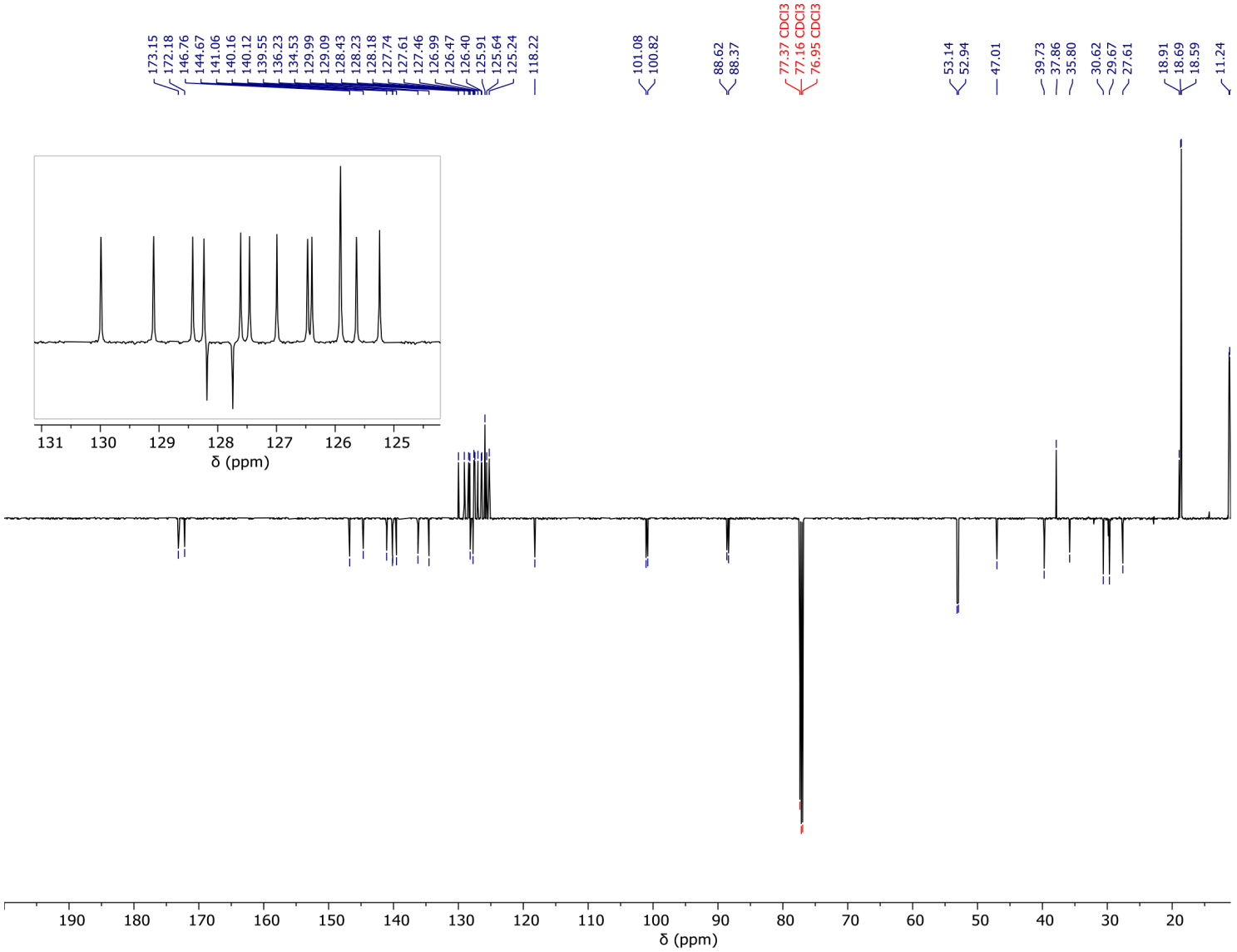
^{1}H-NMR of molecular motor **2** (600 MHz, $CDCl_3$/CD_3OD 4:1, 25 °C).



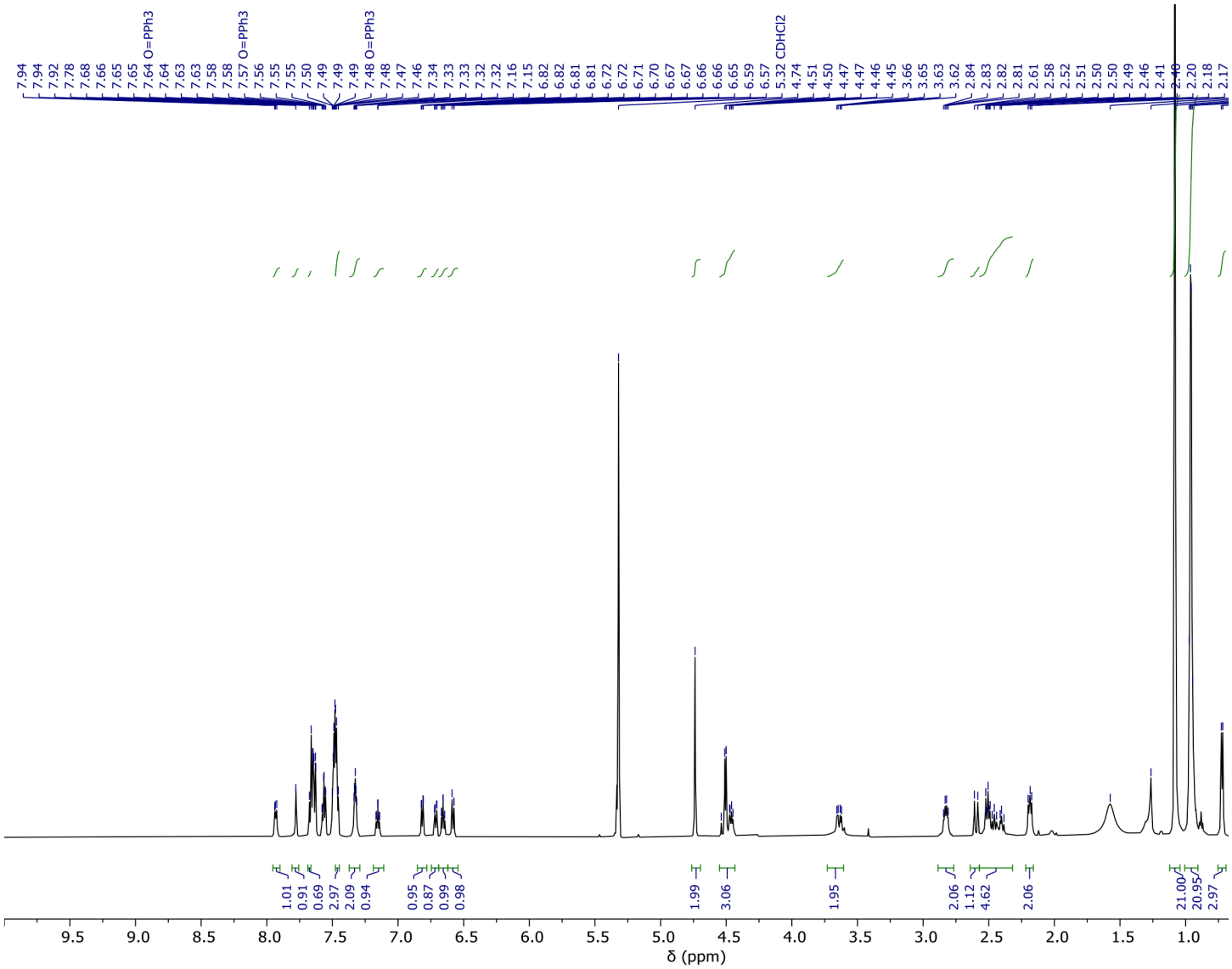
$^{13}C\{^{1}H\}$-NMR of molecular motor **2** (151 MHz, $CDCl_3/CD_3OD$ 4:1, 25 °C).



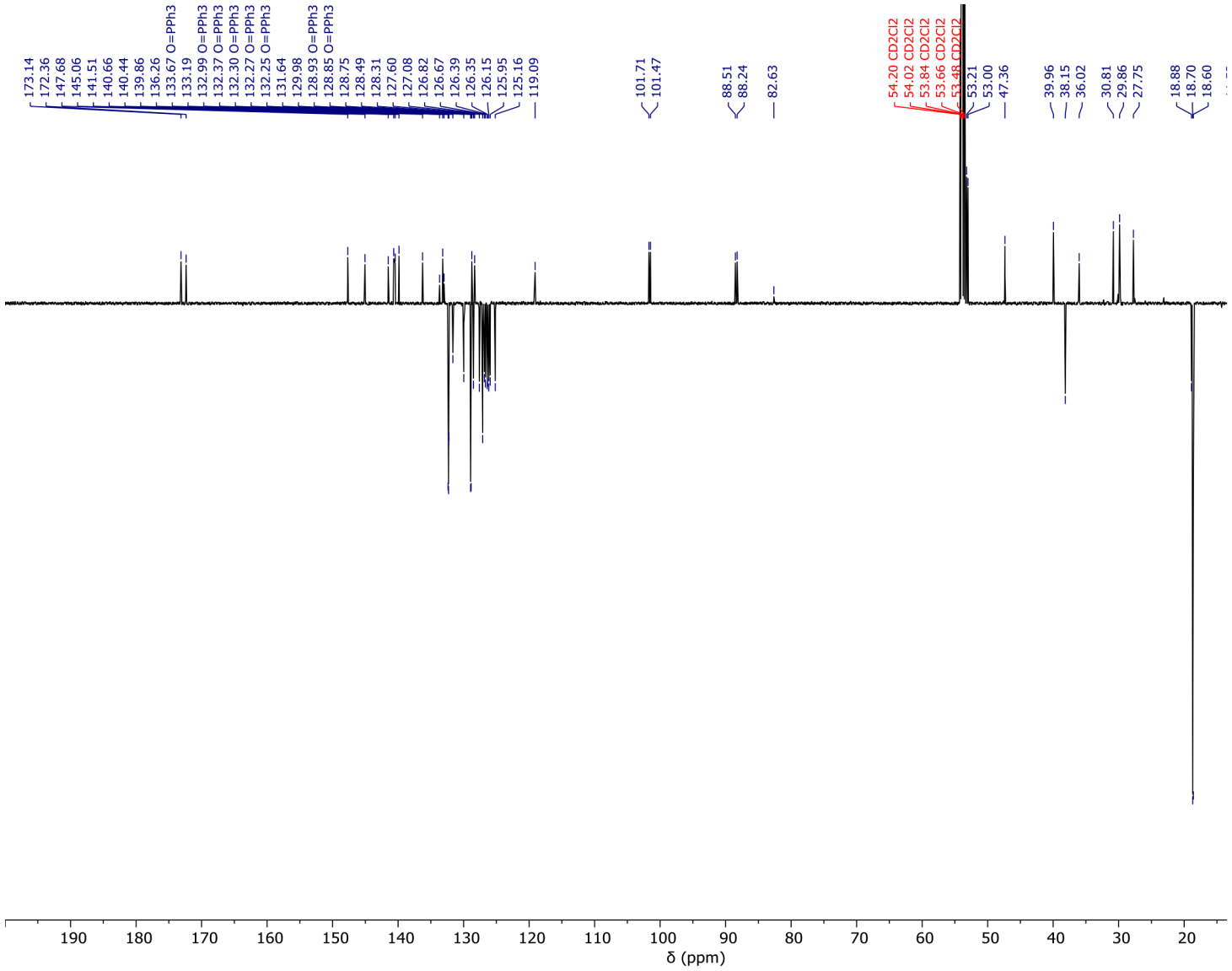
^{1}H-NMR of molecular motor **3** (600 MHz, $CDCl_3$, 25 °C).



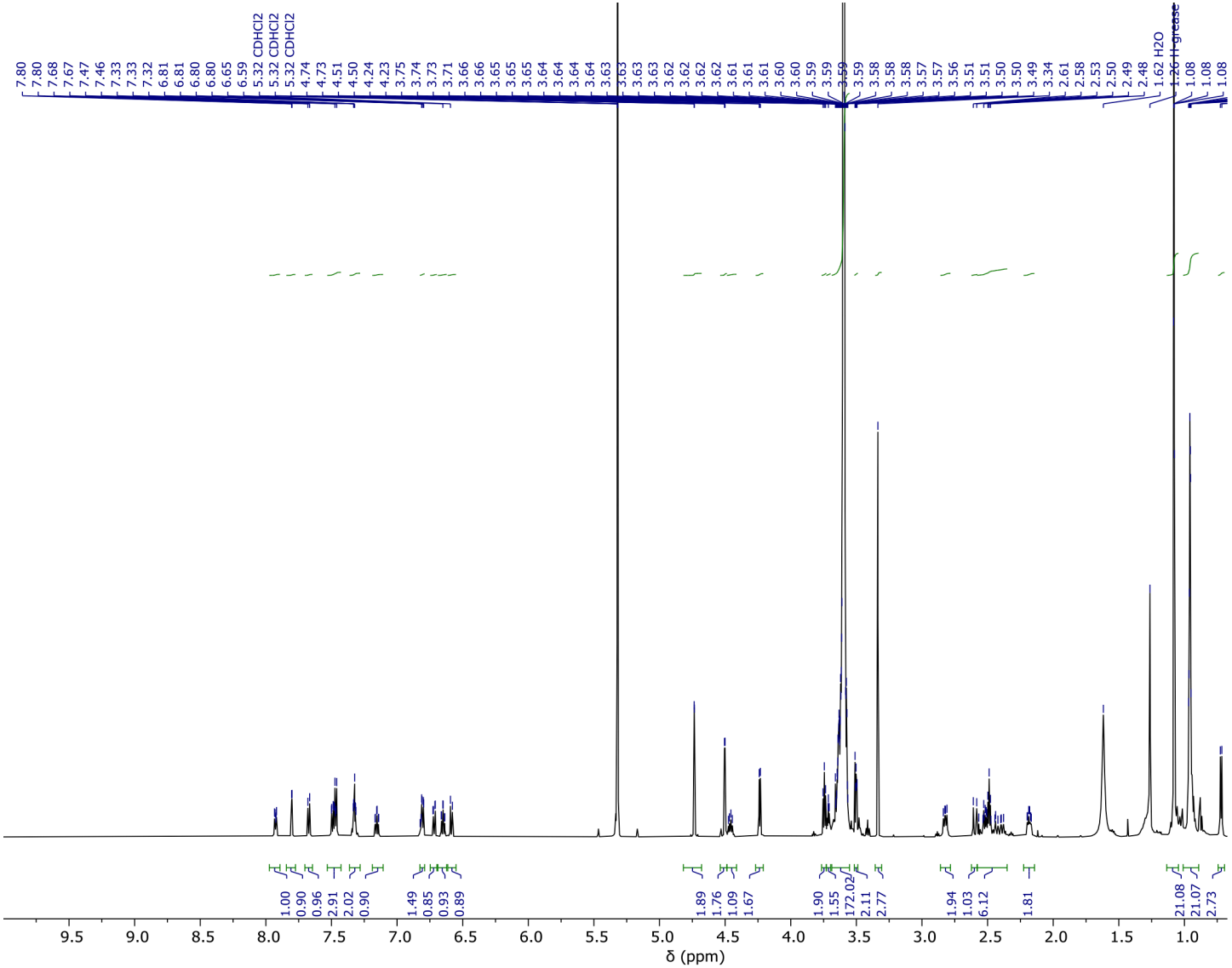
$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **3** (151 MHz, $CDCl_3$, 25 °C).



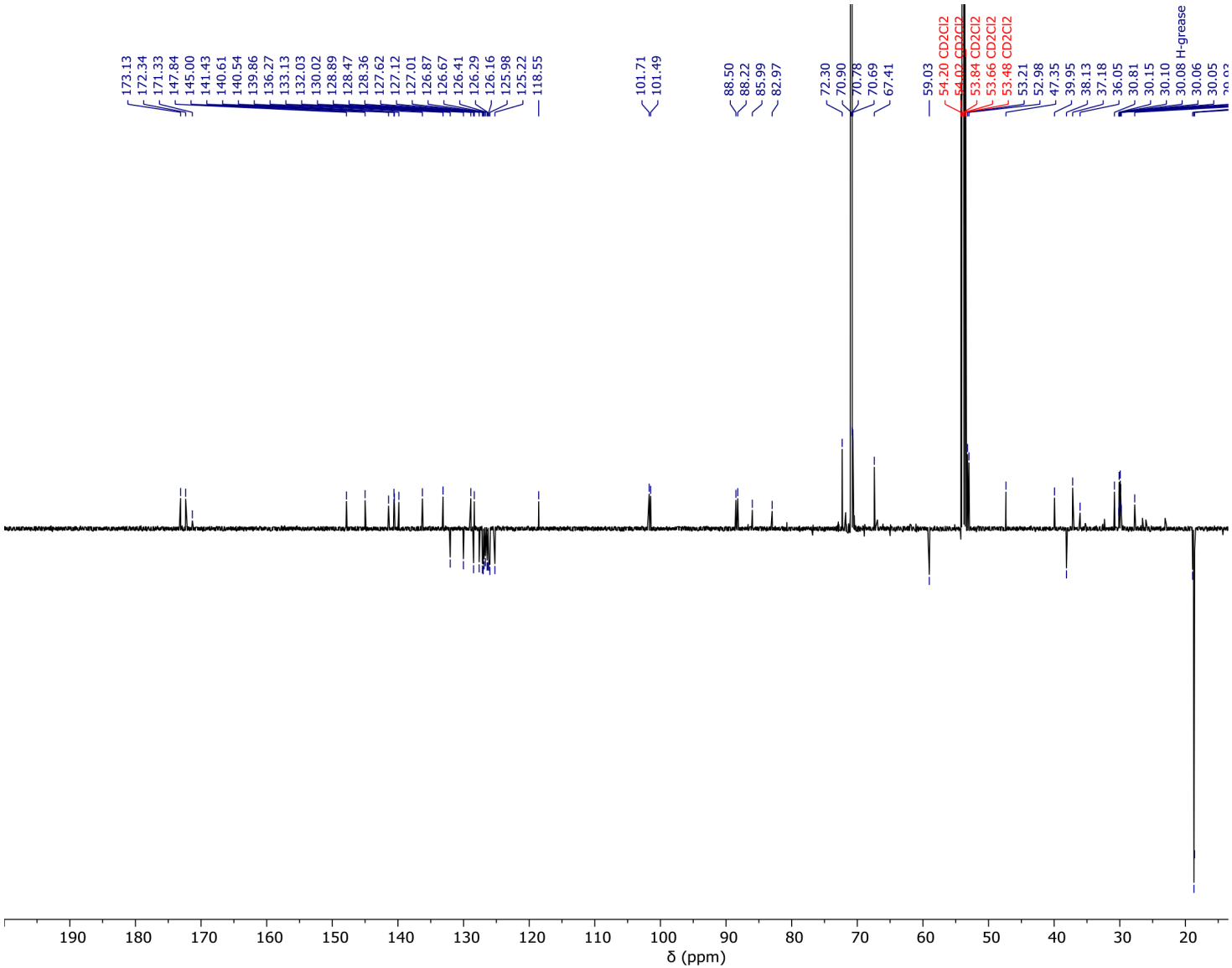
^{1}H-NMR of molecular motor **4** (600 MHz, CD_2Cl_2, 25 °C).



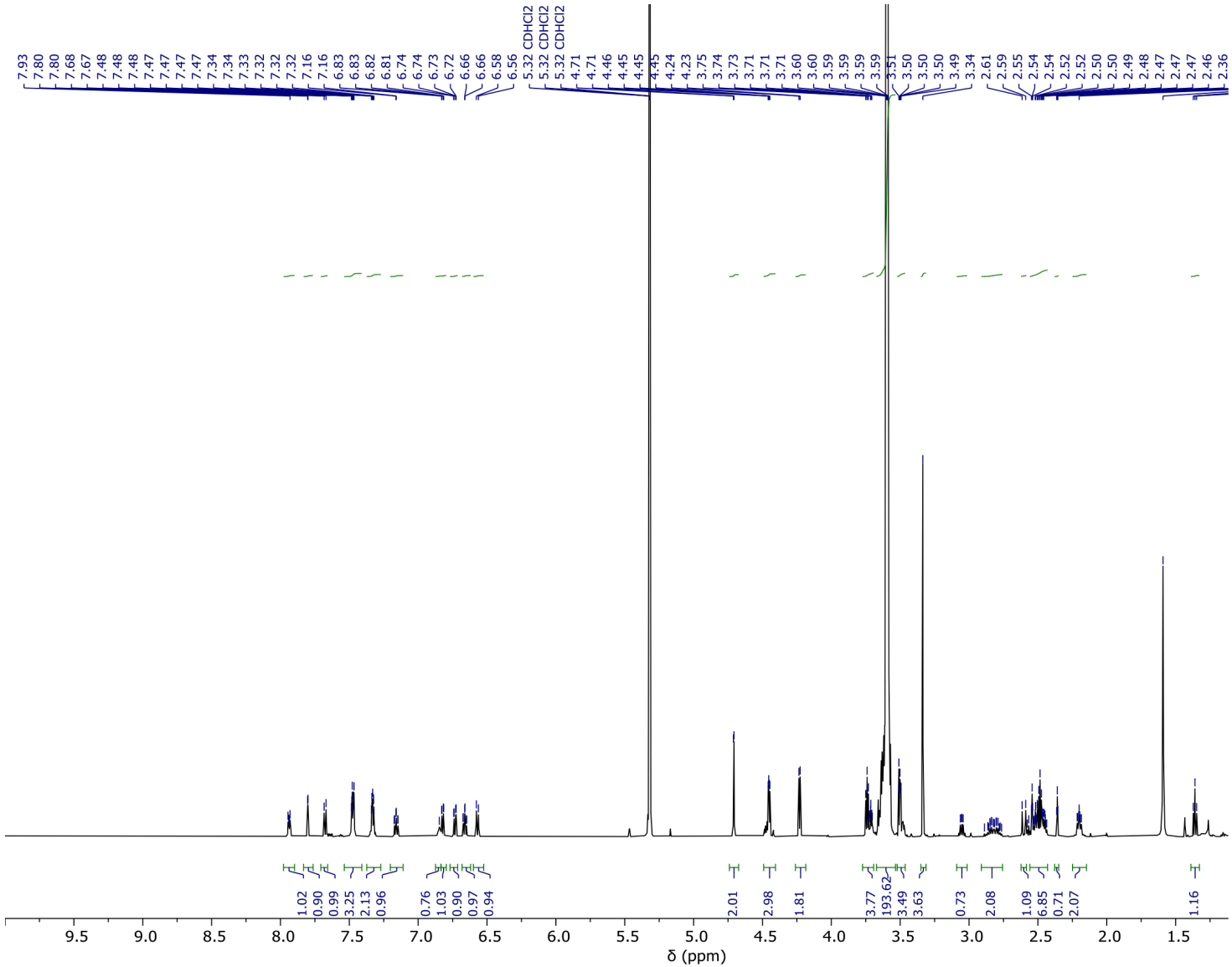
$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **4** (151 MHz, CD_2Cl_2, 25 °C).



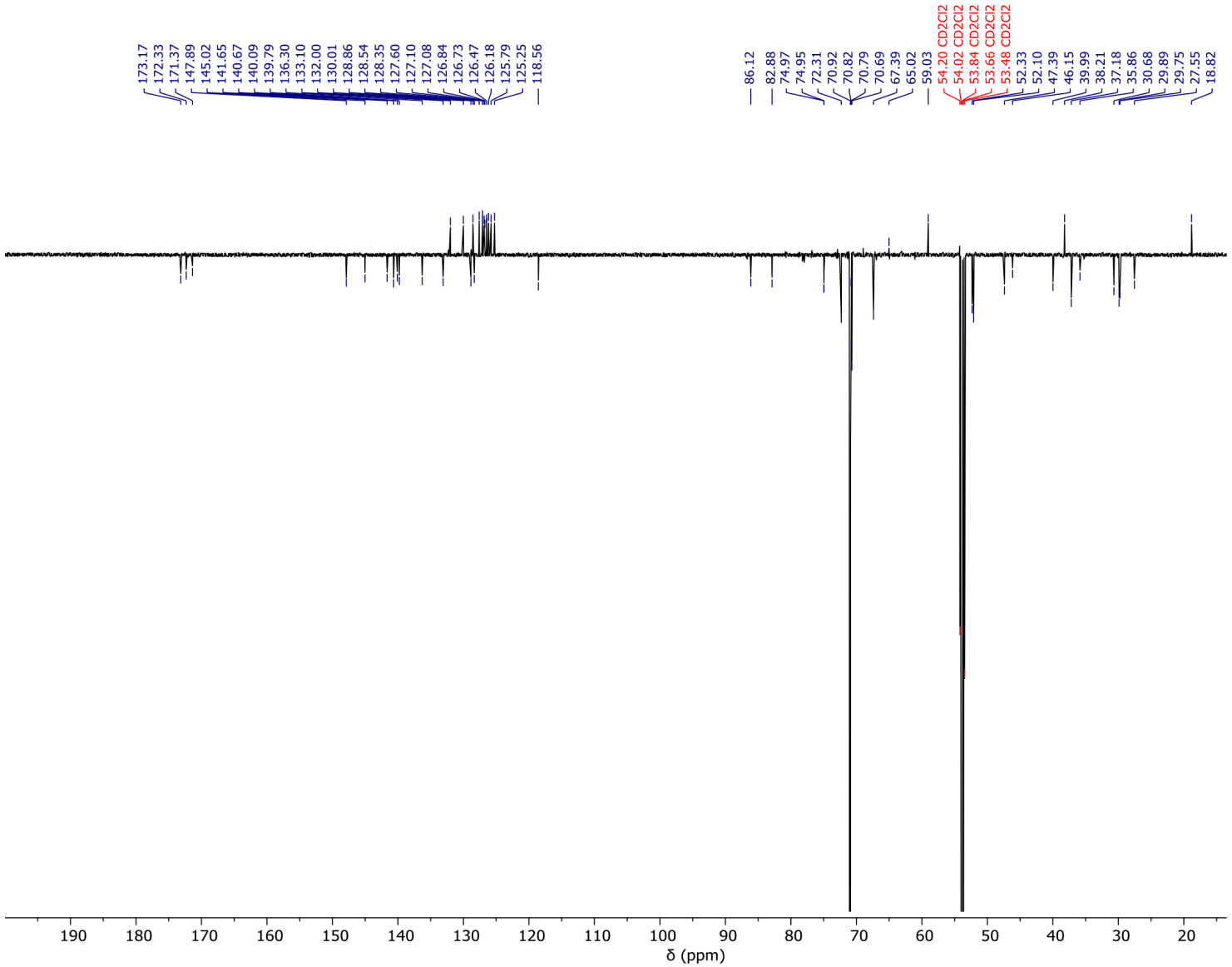
^{1}H-NMR of molecular motor **5** (600 MHz, CD_2Cl_2, 25 °C).



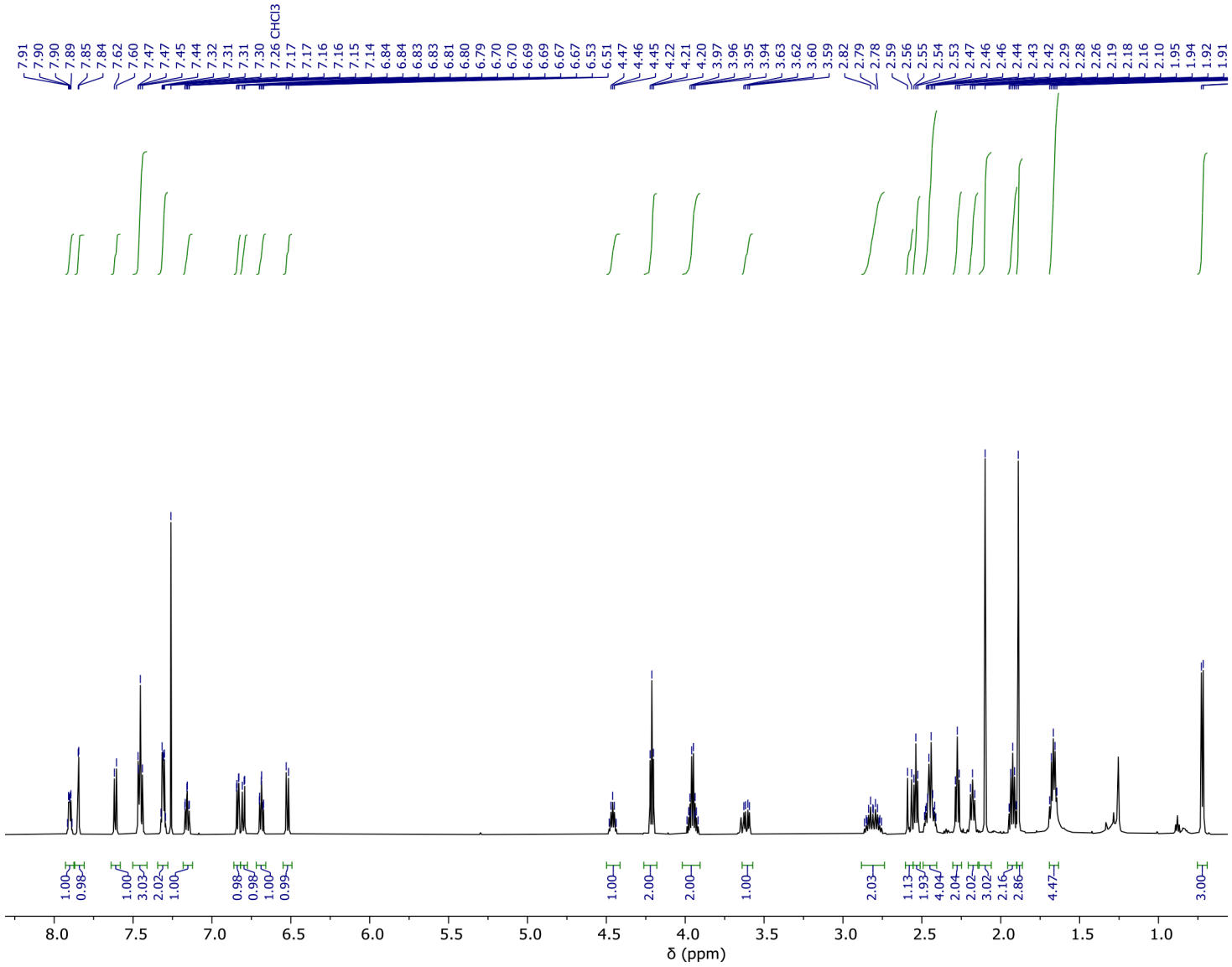
$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **5** (151 MHz, CD_2Cl_2, 25 °C).



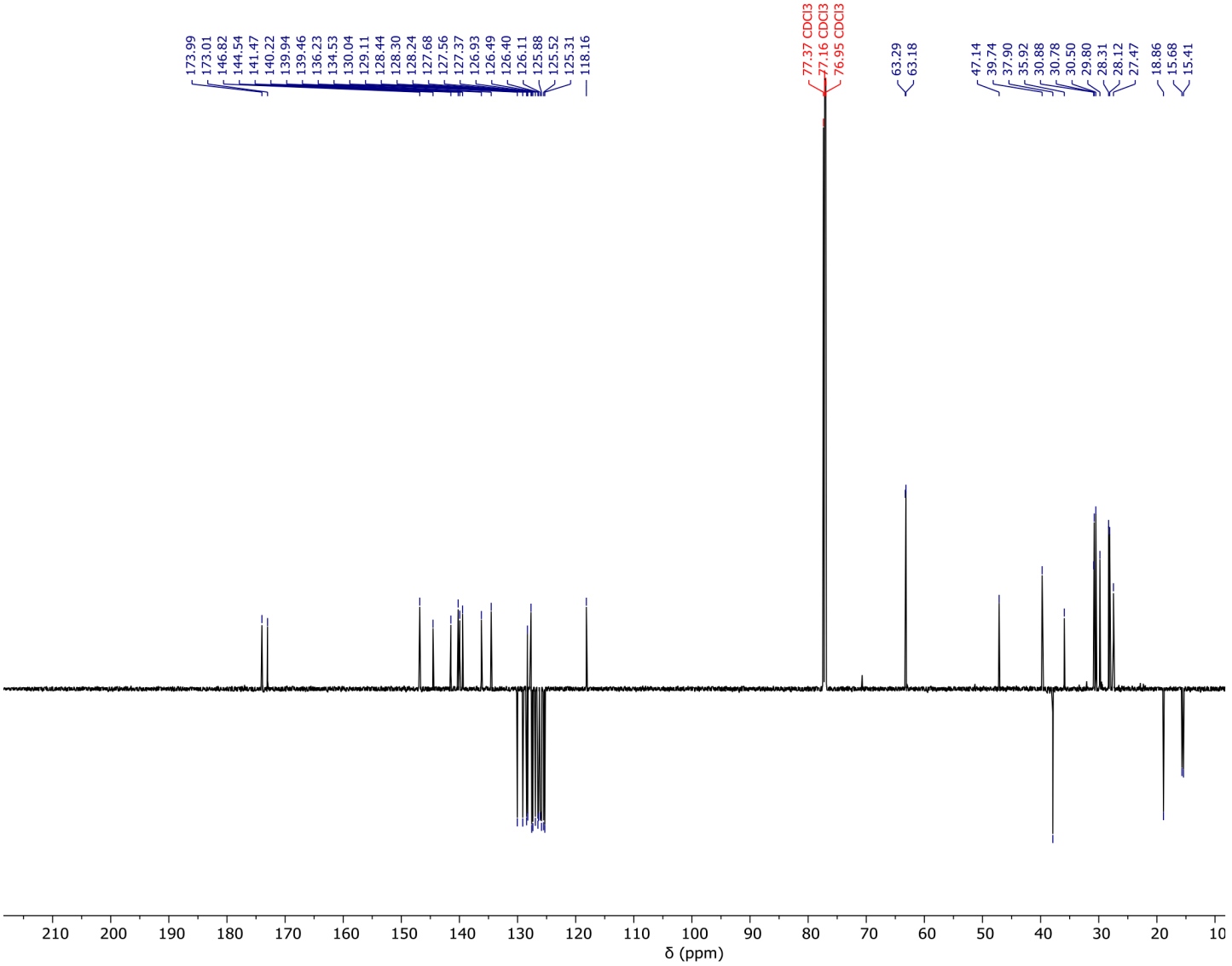
^{1}H-NMR of molecular motor **MM1** (600 MHz, CD_2Cl_2, 25 °C).



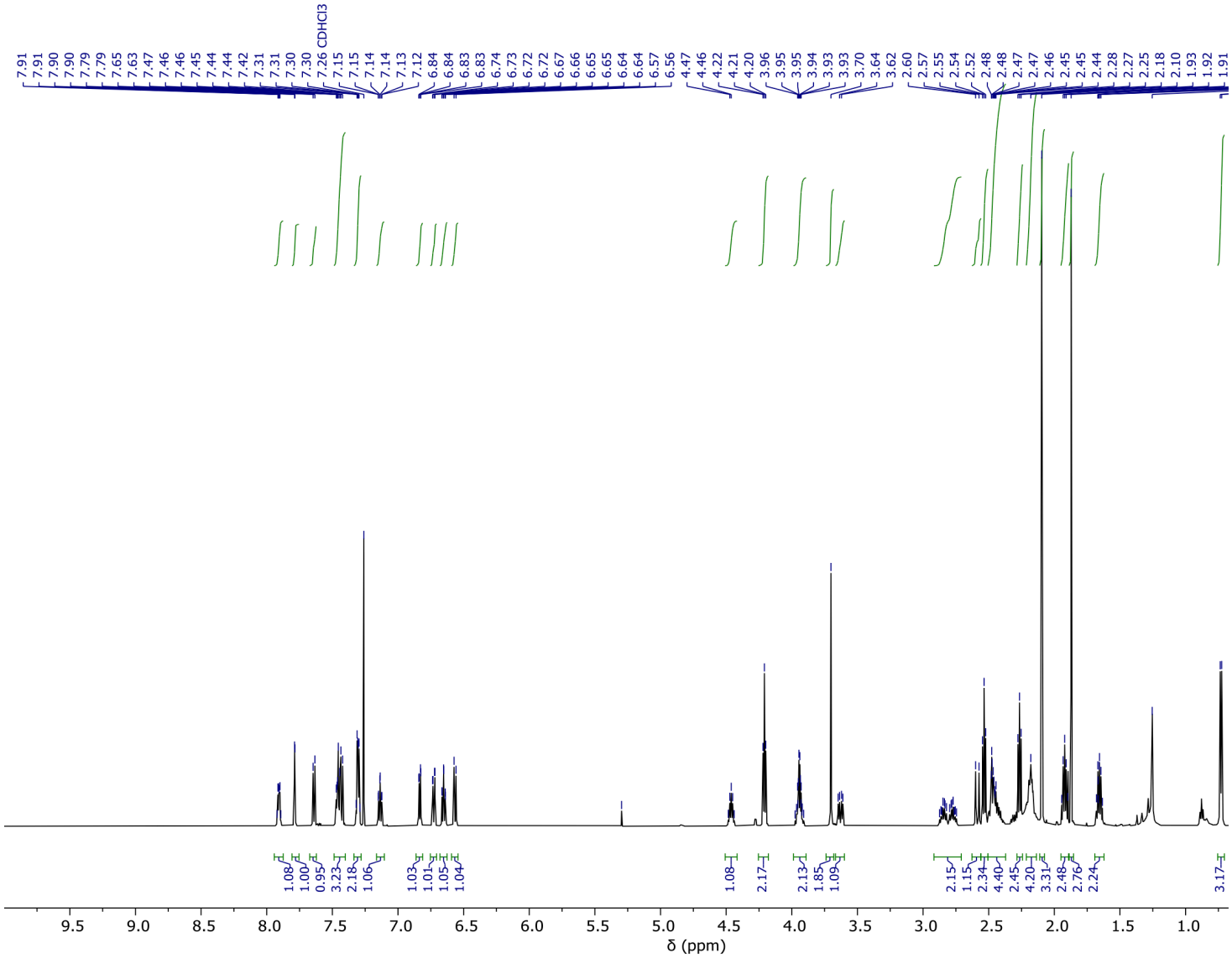
$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **MM1** (151 MHz, CD_2Cl_2, 25 °C).



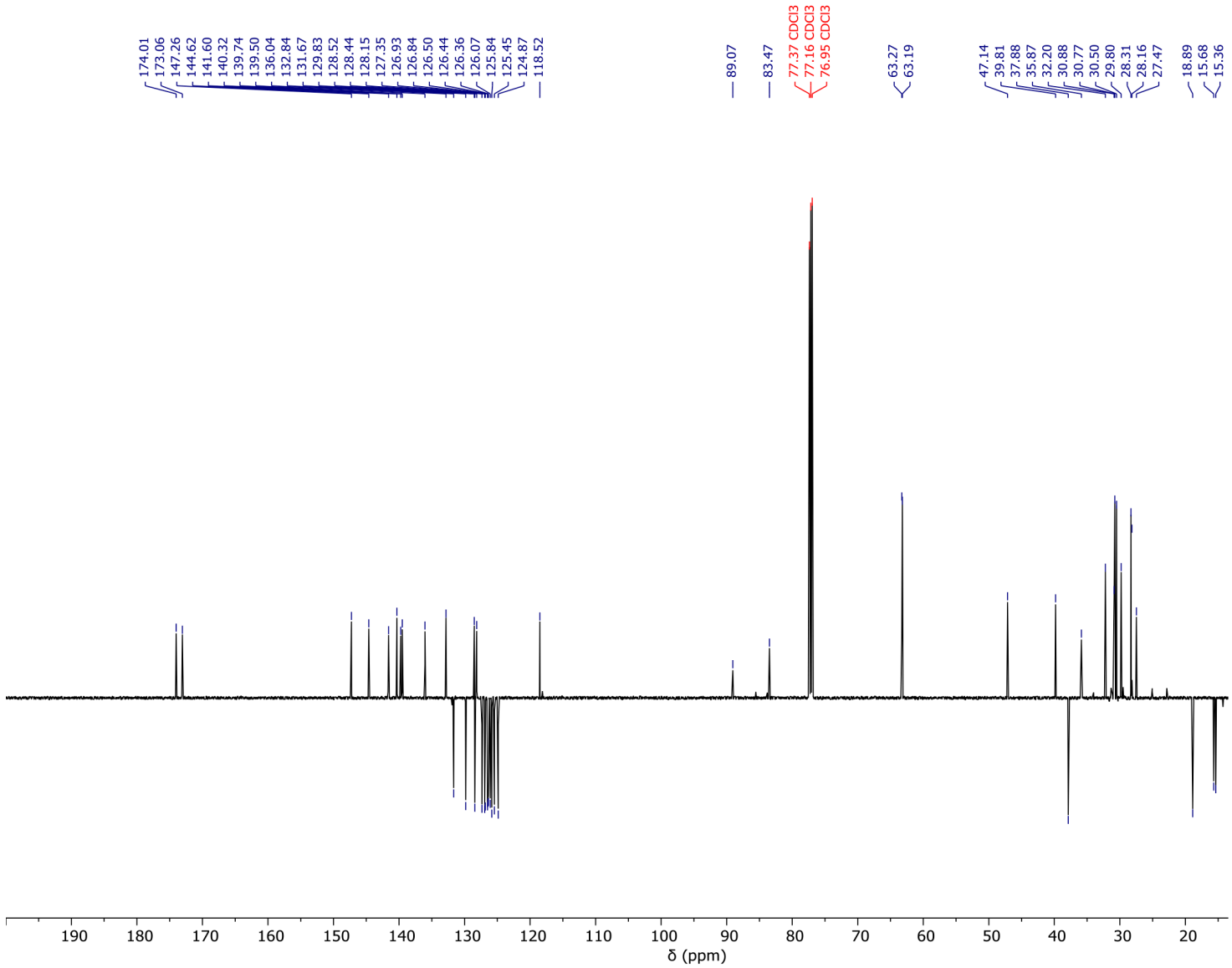
^{1}H-NMR of molecular motor **6** (600 MHz, $CDCl_3$, 25 °C).



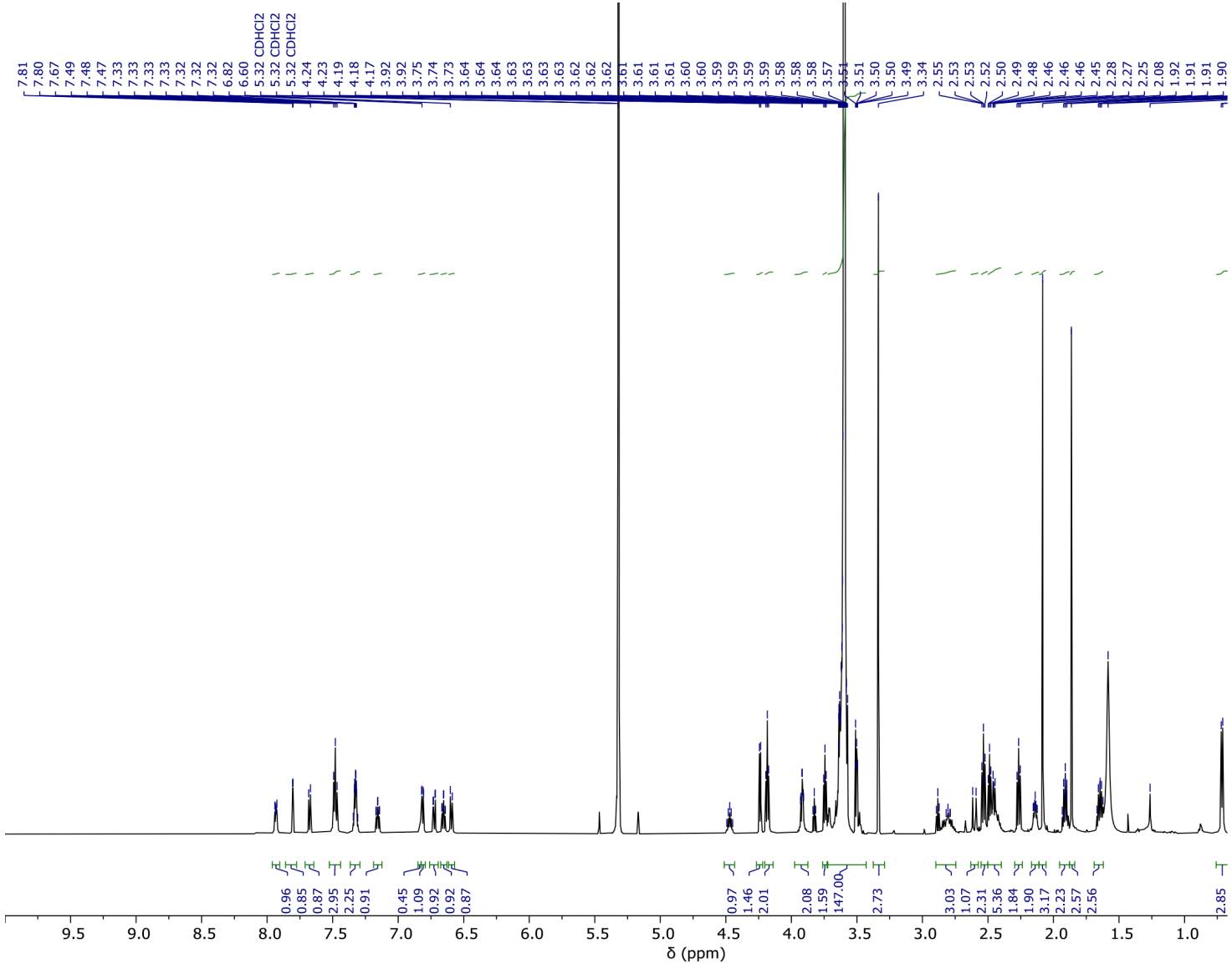
$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **6** (151 MHz, $CDCl_3$, 25 °C).



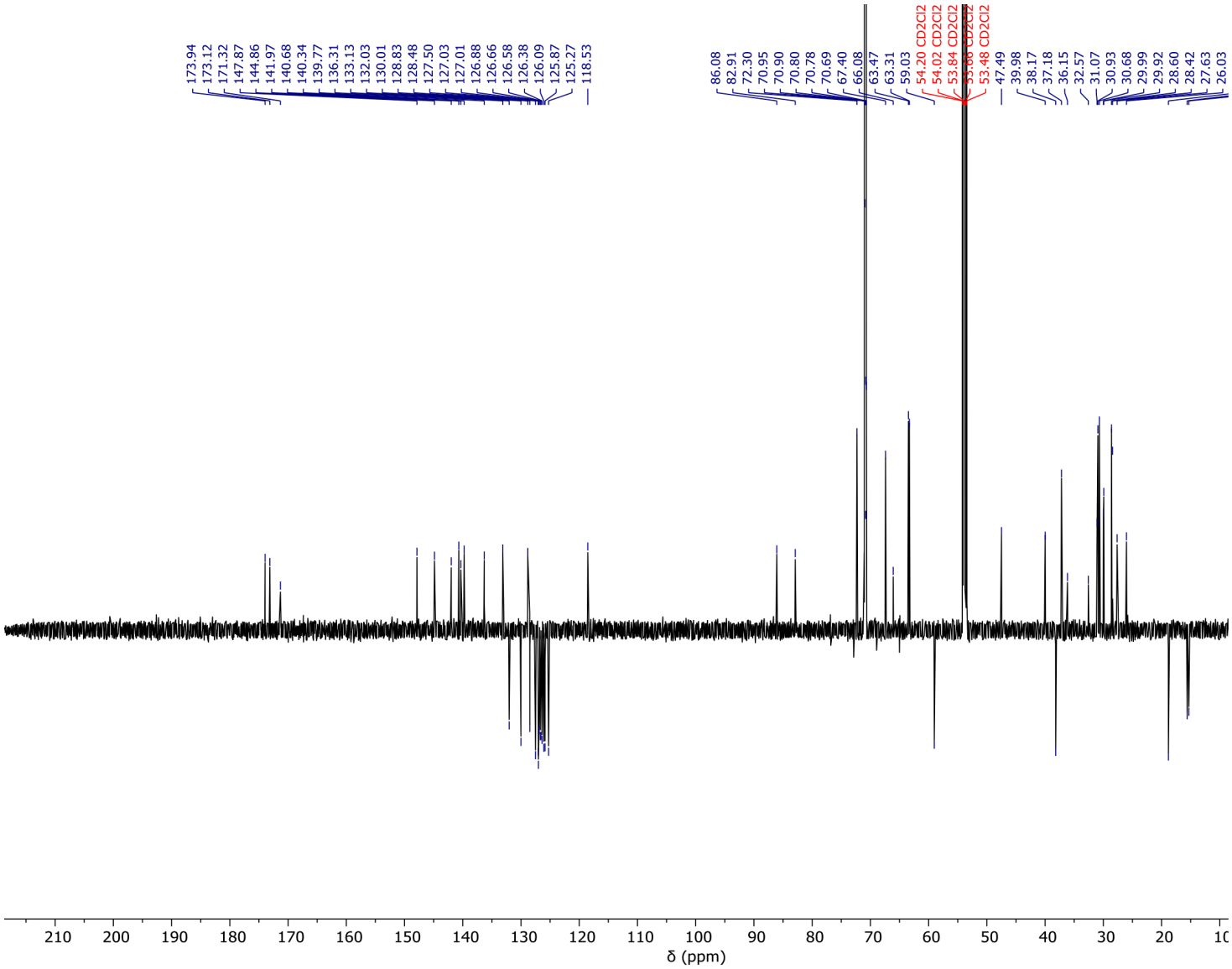
^{1}H-NMR of molecular motor **7** (600 MHz, $CDCl_3$, 25 °C).



$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **7** (151 MHz, $CDCl_3$, 25 °C).



^{1}H-NMR of molecular motor **MM2** (600 MHz, CD_2Cl_2, 25 °C).



$^{13}C\{^{1}H\}$-JMOD NMR of molecular motor **MM2** (151 MHz, CD_2Cl_2, 25 °C).

2. Grafting procedures

All chemicals and solvents were purchased from commercial suppliers unless otherwise stated. Glass surfaces were purchased from Oxford Instruments and gold surfaces were purchased from Sigma-Aldrich.

2.1 On glass

The grafting procedure on glass has been adapted from reference 5.

11-azidoundecyltrimethoxy silane (0.04 g) was dissolved in a mixture of milliQ water (31 µL), aqueous HCl 37% (4 µL) and THF (6 mL). 1.25 mL of this solution were added to 25 mL of cyclohexane. Glass surfaces were pre-cleaned in piranha solution (65°C) H_2SO_4/H_2O_2 (3:1) for 15 min, rinsed with ethanol and immersed in the solution overnight. The monolayered surfaces were sonicated in DMF, toluene and MeOH for 2 min each then dried under N_2 flow. Afterwards, the functionalized surfaces were immersed overnight in a DMF grafting solution containing molecular motor **MM1** (0.5 mM), $CuSO_4.5H_2O$ (1 mol%) and sodium ascorbate (5 mol%). The grafted surfaces were then sonicated in DMF, milliQ water and in MeOH for 2 min each and dried under N_2 flow.

2.2 On gold

The grafting procedure on gold has been adapted from reference 6.

A stock solution of dodecyl sulfide (DDS) was prepared dissolving DDS (8.9 mg) in dichloromethane (5 mL). 100 µL of this stock solution was added to molecular motor **MM2** (0.2048 mg) and evaporated under N_2 flow. The grafting solution was prepared by dissolving both solids in DMF (4.26 mL). Gold silica surfaces (Au/Si) were cleaned with isopropanol and UV-ozone (UV-Ozone cleaner, Ossila) for 15 min before being immersed in grafting solution for 1h. Grafted surfaces were then immersed three times in fresh DMF and dried under N_2 flow.

3. Computational details

All calculations were performed using the Orca 6.0.0 package[7]. Geometries were optimized with the composite functional r^{2}SCAN-3c[8], in the gas phase. The displayed geometries had no imaginary frequency and thus correspond to energy minima. As these calculations were designed to estimate the radius of the most rigid part of the motors, the geometry of analogues of **MM1** and **MM2** featuring shorter PEG chain fragments were optimised.

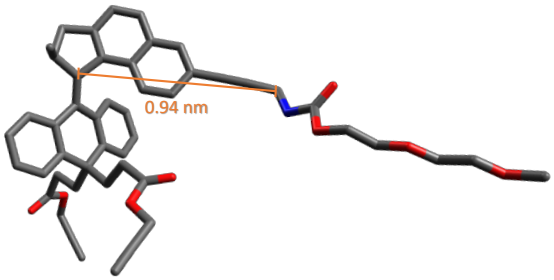


Figure S2: Calculated structure for a simplified analogue of **MM1** featuring a shorter PEG chain optimised at the r^{2}SCAN-3c level of theory. Hydrogen atoms were omitted for clarity. The length scale bar represents the coordinates used to estimate the rigid radius of **MM1**.

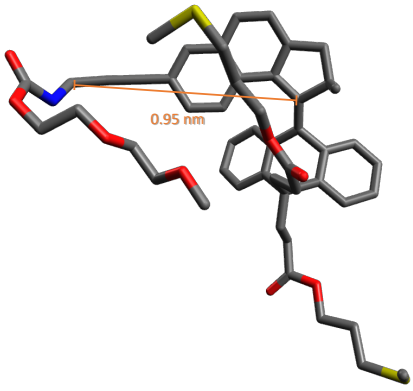


Figure S3: Calculated structure for a simplified analogue of **MM1** featuring a shorter PEG chain optimised at the r^{2}SCAN-3c level of theory. Hydrogen atoms were omitted for clarity. The length scale bar represents the coordinates used to estimate the rigid radius of **MM1**.

MM1 analogue **-** Cartesian coordinates:

O	-25.07167	-7.83155	-0.84199	C	-24.81144	1.32572	5.33149
C	-25.11973	-6.62659	-0.90159	C	-24.70132	-0.06528	5.18164
O	-25.59837	-5.94449	-1.98111	C	-25.39902	-1.13630	5.96315
C	-25.97700	-6.76951	-3.11169	C	-24.53725	-2.39577	5.67261
C	-24.82396	-7.14824	-3.90758	C	-23.42686	-2.54184	6.72143
C	-23.87098	-7.44481	-4.57950	H	-26.66150	-6.14809	-3.69607
C	-24.61529	-5.67747	0.14974	H	-26.50286	-7.66208	-2.75475
C	-23.10533	-5.48092	-0.04911	H	-23.02414	-7.73457	-5.15378
C	-22.47059	-4.50258	0.99013	H	-25.13169	-4.71521	0.07848
C	-22.70842	-5.08570	2.37982	H	-24.80776	-6.12313	1.13050
C	-23.42491	-4.42273	3.40611	H	-22.92175	-5.09913	-1.05938
C	-23.66846	-5.15144	4.58885	H	-22.60793	-6.45442	0.01409
C	-23.20523	-6.44007	4.78163	H	-24.24075	-4.71145	5.38745
C	-22.44861	-7.05812	3.79357	H	-23.43154	-6.95622	5.71012
C	-22.21733	-6.37581	2.61230	H	-22.05681	-8.06097	3.93260
C	-20.95395	-4.42737	0.67237	H	-21.64630	-6.87461	1.83594
C	-20.21093	-3.43036	1.56400	H	-20.82904	-4.13255	-0.37719
C	-18.73964	-3.34934	1.25604	H	-20.50505	-5.41971	0.77659
O	-18.13563	-4.55135	1.43259	H	-20.61410	-2.42706	1.41706
C	-16.71100	-4.55281	1.15227	H	-20.33638	-3.71481	2.61777
C	-16.21152	-5.89944	1.32788	H	-16.20885	-3.85266	1.83107
C	-15.76324	-7.00696	1.46482	H	-16.54258	-4.19804	0.12830
O	-18.14951	-2.35711	0.89346	H	-15.37186	-7.98809	1.58825
C	-23.12976	-3.14801	0.80308	H	-25.27105	-0.98075	2.28402
C	-23.81985	-2.50704	1.84290	H	-25.29631	-0.01510	0.02975
C	-24.64003	-1.41028	1.51439	H	-23.83399	-0.99233	-1.74735
C	-24.65924	-0.86779	0.24460	H	-22.55670	-3.02660	-1.27102
C	-23.85759	-1.42258	-0.75074	H	-21.74800	-1.38077	2.70250
C	-23.12731	-2.56540	-0.47049	H	-20.20924	0.11427	1.48778
C	-23.77157	-2.99093	3.23975	H	-18.45355	5.23438	0.43990
C	-23.98899	-2.10393	4.27348	H	-19.11432	4.48492	-1.01656
C	-23.86564	-0.63739	4.23074	H	-17.36344	2.59706	-0.32797
C	-22.95504	0.19387	3.51410	H	-13.27846	2.98205	0.68884
C	-21.89019	-0.30948	2.73812	H	-13.94017	4.56887	1.18968
C	-21.03393	0.52095	2.06616	H	-13.28205	3.75614	-1.69895
C	-21.20499	1.93168	2.12844	H	-13.87116	5.35856	-1.16071
C	-20.33242	2.77620	1.39532	H	-11.20770	4.84874	-2.47828
C	-19.57473	3.47416	0.76098	H	-11.82276	6.41995	-1.89748
C	-18.65644	4.26886	-0.03908	H	-9.35917	4.95431	-0.79281
N	-17.37164	3.59908	-0.21546	H	-9.96988	6.53243	-0.22753
C	-16.20919	4.20830	0.14773	H	-7.29331	6.02571	-1.53973
O	-15.18241	3.31143	0.07604	H	-7.90876	7.61001	-0.98128
C	-13.87642	3.84176	0.37492	H	-7.46424	7.37734	-2.69661
C	-13.27563	4.47949	-0.86509	H	-22.31502	3.53143	3.01862
O	-11.94995	4.85261	-0.52890	H	-24.12239	3.23190	4.63770
C	-11.28249	5.50715	-1.59638	H	-25.50094	1.74250	6.06128
C	-9.89459	5.86755	-1.10510	H	-26.42478	-1.28673	5.59681
O	-9.22295	6.50987	-2.17500	H	-25.45988	-0.90831	7.03381
C	-7.90714	6.89653	-1.82095	H	-25.19072	-3.27005	5.67322
O	-16.09127	5.37407	0.47095	H	-22.85472	-1.60950	6.78596
C	-22.20853	2.45409	2.92871	H	-22.73060	-3.34496	6.46336
C	-23.08095	1.61263	3.65149	H	-23.85159	-2.75393	7.70892
C	-24.04145	2.15174	4.54737				

MM2 analogue **-** Cartesian coordinates:

C	-1.87818	-1.85147	-1.83904	C	0.14746	-2.61455	2.78402
C	-1.30290	-0.45666	-1.56258	H	-2.96998	-1.80815	-1.91787
C	-1.55703	0.51169	-2.73468	H	-1.62014	-2.54082	-1.02799
C	-0.34096	0.32307	-3.59110	H	-1.47815	-2.26048	-2.77337
C	0.67872	-0.31823	-2.88968	H	-1.75043	-0.08042	-0.64538
C	1.84098	-0.74627	-3.60786	H	-1.62228	1.54833	-2.37390
C	2.82273	-1.63273	-3.10389	H	-2.48444	0.29395	-3.27769
C	3.91345	-2.00237	-3.84645	H	2.71699	-2.03795	-2.10887
C	4.09656	-1.51462	-5.16747	H	4.65683	-2.66993	-3.42169
C	5.24582	-1.87851	-5.91512	H	8.31551	-2.18846	-6.83197
C	6.22976	-2.17869	-6.55398	H	7.37781	-2.29539	-8.32766
C	7.41070	-2.62063	-7.28158	H	7.27050	-4.52677	-6.41427
N	7.50188	-4.08325	-7.28816	H	7.05461	-6.40781	-6.23749
C	7.31049	-4.77423	-8.45832	H	6.50500	-7.72922	-7.25790
O	7.05171	-6.11337	-8.33500	H	4.41695	-6.62897	-7.80456
C	6.46347	-6.64377	-7.13559	H	4.93555	-5.09244	-7.08370
C	5.00999	-6.18847	-6.99493	H	3.91546	-4.81735	-4.92699
O	4.47082	-6.64185	-5.76167	H	5.60292	-5.29788	-4.61962
C	4.57010	-5.67985	-4.71899	H	4.40928	-5.61978	-2.58874
C	4.18073	-6.32288	-3.41239	H	4.78424	-7.23465	-3.25900
O	2.80129	-6.63770	-3.42931	H	2.58322	-6.76111	-1.35526
C	2.41674	-7.35683	-2.26913	H	2.97879	-8.29960	-2.17875
O	7.41926	-4.28752	-9.56010	H	1.35140	-7.58152	-2.35394
C	3.11557	-0.70593	-5.71592	H	3.21182	-0.36820	-6.74383
C	1.97817	-0.32623	-4.97504	H	1.07620	0.70376	-6.64501
C	0.94919	0.41707	-5.60469	H	-1.02701	1.22136	-5.43284
C	-0.21021	0.70637	-4.93398	H	4.82878	-2.74988	1.00055
C	0.22396	-0.51227	-1.49203	H	6.23422	-0.99558	0.03103
C	0.91764	-0.69189	-0.33736	H	5.19956	0.98061	-1.06607
C	2.38488	-0.72299	-0.21199	H	2.72502	1.11998	-1.25265
C	2.95918	-1.80489	0.48200	H	3.51508	-4.34155	1.56598
C	4.34933	-1.89916	0.52709	H	1.98538	-4.56593	2.37127
C	5.15341	-0.90682	-0.03022	H	4.15359	-2.52457	3.09473
C	4.57547	0.19668	-0.64766	H	2.50138	-2.40276	3.71623
C	3.19342	0.28131	-0.74539	H	3.24162	-6.45101	5.50928
C	2.01645	-2.88122	1.03478	H	3.26883	-5.21239	6.77552
C	2.70138	-3.79785	2.06184	H	0.73131	-6.44448	5.56069
C	3.25060	-3.09406	3.31804	H	0.74705	-5.21564	6.82683
C	3.59613	-4.11198	4.37321	H	1.97926	-8.02228	7.11408
O	2.47009	-4.59730	4.95518	H	1.95606	-6.77625	8.36420
C	2.67088	-5.62650	5.95411	H	-1.71803	-9.18650	7.00616
C	1.29822	-6.07198	6.42224	H	-0.06621	-9.46192	6.41091
C	1.41618	-7.15738	7.48893	H	-1.01095	-8.04729	5.84425
S	-0.19255	-7.74574	8.14122	H	2.49340	-4.20378	-0.61643
C	-0.79200	-8.69450	6.69792	H	1.22414	-3.04432	-0.96263
O	4.70895	-4.48959	4.65853	H	0.85376	-5.63548	0.62291
C	1.59205	-3.73227	-0.20081	H	-0.33184	-4.34645	0.58897
C	0.50925	-4.78086	0.03001	H	0.02849	-6.12376	-3.77967
C	-0.08496	-5.35325	-1.24321	H	-1.34313	-5.01259	-3.56695
O	0.28853	-4.65159	-2.33026	H	1.43838	-4.24908	-4.61344
C	-0.24795	-5.07759	-3.60608	H	0.09796	-3.11531	-4.43322
C	0.34689	-4.15956	-4.65777	H	0.08279	-5.56556	-6.29553
C	-0.16729	-4.52601	-6.04574	H	-1.25968	-4.43384	-6.08904
S	0.45176	-3.44295	-7.38972	H	2.66298	-3.53353	-8.30184
C	2.20802	-3.94669	-7.39730	H	2.28779	-5.03829	-7.42617
O	-0.81869	-6.31571	-1.27922	H	2.74298	-3.55090	-6.52972
C	0.78587	-2.18061	1.62016	H	-1.05970	0.62668	1.08988
C	0.27180	-1.05419	0.94862	H	-2.14297	-0.14057	3.16990
C	-0.75301	-0.31105	1.54161	H	-1.39567	-2.26441	4.23577
C	-1.35079	-0.73298	2.72157	H	0.49319	-3.50348	3.30155
C	-0.92285	-1.90927	3.32503				

4. Theoretical considerations for the applicability of the microscopic reversibility

This section depicts the theoretical considerations for the applicability of the principle of microscopic reversibility to light-driven molecular motors. This development is based on the study of the transition $i \rightleftarrows i^*$ between the ground state of the molecular motor (i) and the excited state (i^*), where for simplicity we take the degeneracy of each state to be one. In his theory, Einstein considered three processes for absorption and emission of light[9]:

Absorption, $h\nu_i + i \xrightarrow{\rho(\nu_i)B_{i\rightarrow i^*}} i^*$;

Stimulated emission $h\nu_i + i^* \xrightarrow{\rho(\nu_i)B_{i^*\rightarrow i}} i + 2h\nu_i$;

Spontaneous emission $i^* \xrightarrow{A_{i^*\rightarrow i}} i + h\nu_i$.

In these processes, *A* and *B* are the Einstein coefficients, $\rho(\nu_i)$ the energy density, and $h\nu_i$ represents a quantum of energy (photon) equal the energy gap between the ground and excited states, i and i^*, which we will call ΔG_{ii^*}.

Given that $B_{i\rightarrow i^*} = B_{i^*\rightarrow i} = A_{i^*\rightarrow i}\frac{c^3}{8\pi\hbar\nu_i^3}$, the net transition rates can be written $i \underset{\omega_{i^*\rightarrow i}}{\overset{\omega_{i\rightarrow i^*}}{\rightleftarrows}} i^*$, where using Einstein's relations we find an expression for the ratio,

$$\frac{\omega_{i\rightarrow i^*}}{\omega_{i^*\rightarrow i}} = \frac{\rho(\nu_i)B_{i\rightarrow i^*}}{\rho(\nu_i)B_{i^*\rightarrow i} + A_{i^*\rightarrow i}} = \frac{\rho(\nu_i)}{\rho(\nu_i) + \frac{8\pi\hbar\nu_i^3}{c^3}}$$

In relatively bright irradiation conditions, such as our experimental setup, $\rho(\nu_i) \gg \frac{8\pi\hbar\nu_i^3}{c^3}$, and the ratio of transition rate constants (conditional probabilities) is unity. It is important to mention that when the molecule is excited to i^*, it almost immediately relaxes to the bottom of the well on the excited surface, so the equality between the transition constants does not imply that the quantum yield is less than 50%, and it could well be nearly 80%, as reported in previous works[10].

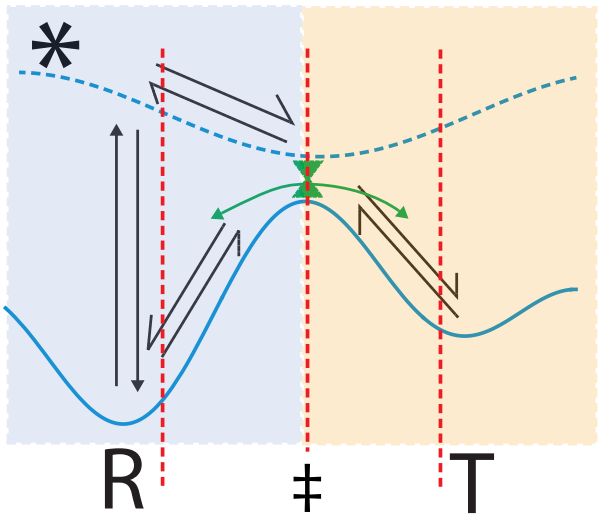


Figure S4: Detailed steps in the transition from the relaxed to the tensed state of the molecular motor.

Figure S4 depicts the ramifications of Einstein's theory in the context of a two state transition between a "relaxed" and "tensed" state form of a molecule. In this figure, the vertical arrows represent the light driven transition between the two energy surfaces at the UV frequency used in the experiment. These transition probabilities are governed by the Einstein relations, and for relatively bright light $\frac{\omega_{R\to R^*}}{\omega_{R^*\to R}} \approx 1$ (*vide supra*). The harpoons indicate equilibrium processes – overdamped sliding or up sliding – on a single energy surface and are governed by microscopic reversibility. The microscopic reversibility proves that the last-touch first-touch (LTFT) times on any single energy surface do not depend on direction, *i.e.* $\tau_{\mathrm{LTFT}}(a \to b) = \tau_{\mathrm{LTFT}}(b \to a)$, with the LTFT times for all transitions between two points on a single energy surface being equal. In the context of our experiment, one would thus expect that the number of data points, and the statistical distribution of that number, between any two forces would be independent on whether on starts at a small force to reach a large force, or starts at a large force to reach a smaller force.

With light-driven systems there are several trajectories leading between the relaxed and tensed states, one entirely on the ground state energy surface, and the other involving promotion to the excited states, thermal relaxation on the excited energy surface and intersystem crossing. The kinetic weighting of the optical transition coefficients in this case are the same. Therefore, the prediction of direction independence remains, as observed in Figure 4 and in the Extended Data Fig. 7.

To go further in details, of those trajectories that attain the mean force value in the relaxed state and reach the mean force value in the tensed state, the number of data points sampled at fixed sampling rate is equal to the number of data points sampled at the same rate in those trajectories that attain the mean force value in the tense state and reach the mean force value

in the relaxed state. A trajectory can be viewed as consisting of some stochastic waiting time in the relaxed state during which the motor diffuses in the vicinity of the bottom of an energy well, a quasi-deterministic 'run' to the tensed state, a stochastic waiting time in the tense state, and a quasi-deterministic 'run' to the relaxed state. The average run time, and hence speed during a run, from relaxed to tensed is the same as the average run time from the tensed to the relaxed state.

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