Supplementary Information

Sub-stoichiometric inhibition of IAPP aggregation: A peptidomimetic approach to anti-amyloid agents

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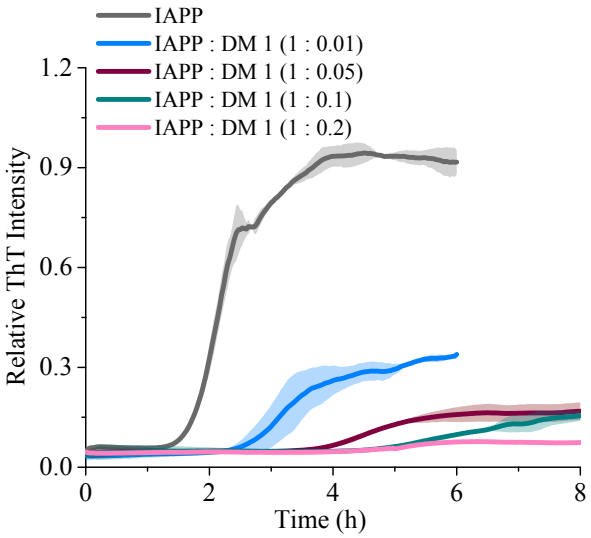


Fig. S1 ThT fluorescence based kinetic profiles of 25 μM IAPP aggregation in the absence and presence of DM 1 at the indicated substoichiometric ratios in the phosphate buffer (50 mM NaPi, 150 mM KCl, pH 7.4) [ThT] = 12.5 μM. Solid curves represent the average of three independent trials while the shaded regions represent the standard deviations of those measurements.

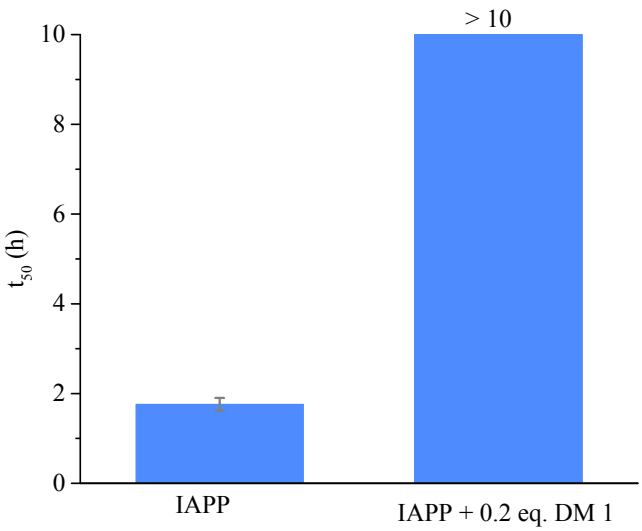


Fig. S2 Statistical analysis of IAPP aggregation (15 μM) in the form of t_{50} in the absence and presence of 0.2 eq. DM 1 under lipid catalysed (LUVs, DOPG : DOPC, 1 : 1, 30% cholesterol, d = 100 nm) condition.

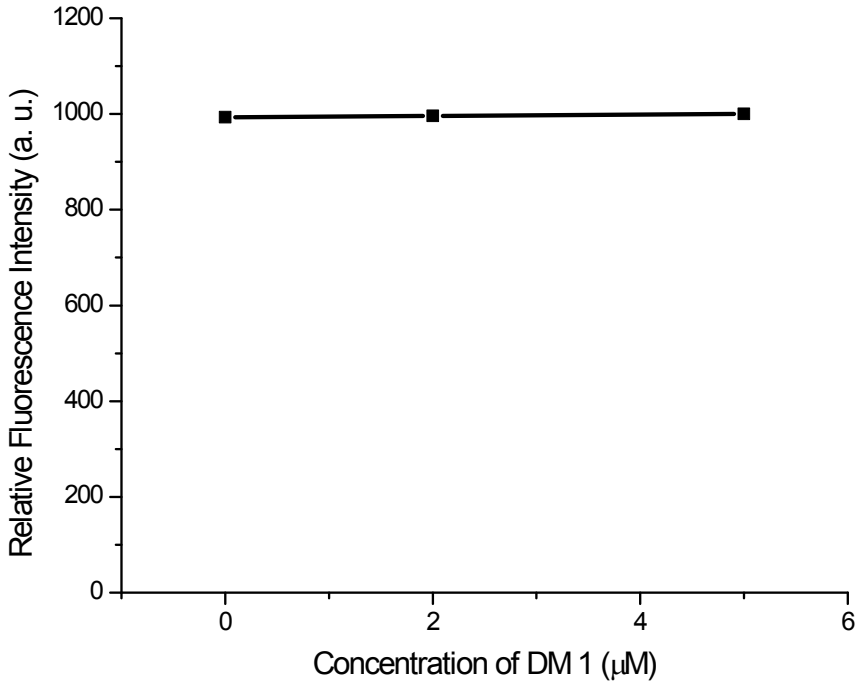


Fig. S3 Fluorescence intensity at 485 nm of ThT (10 μM) upon addition of DM 1 in 50 mM NaPi, 150 mM KCl, pH 7.4; 750 μM, LUVs, DOPG : DOPC, 3 : 7, d = 100 nm. Excitation: 450 nm.

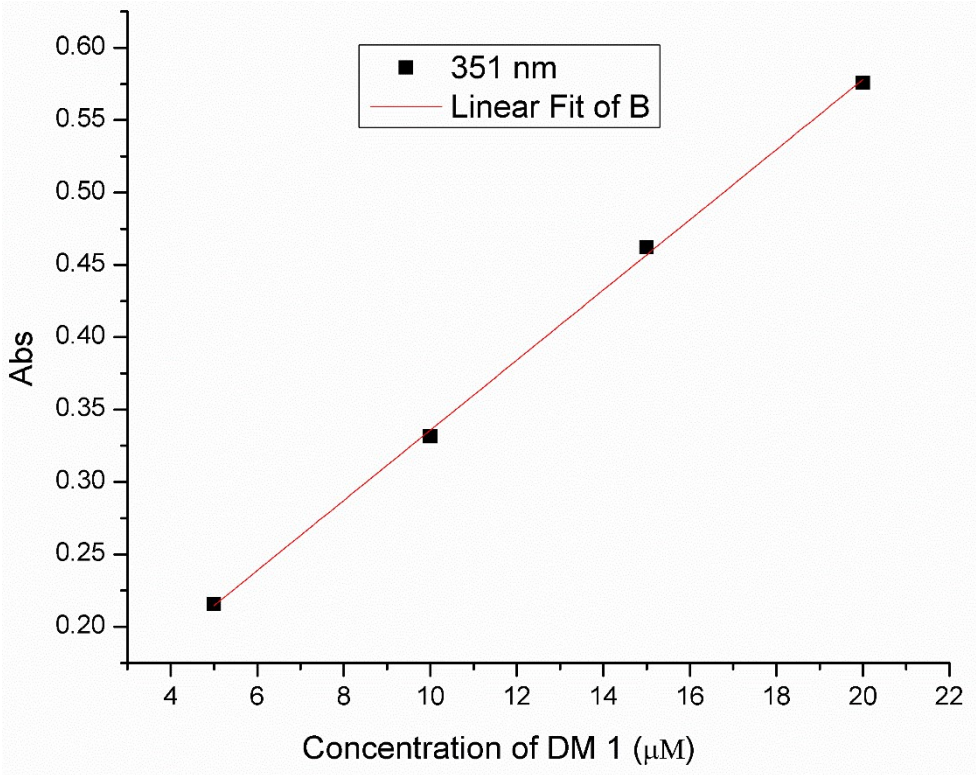


Fig. S4 The plot of absorption values with increasing concentrations of DM 1 in phosphate buffer (50 mM NaPi, 150 mM KCl, pH 7.4)

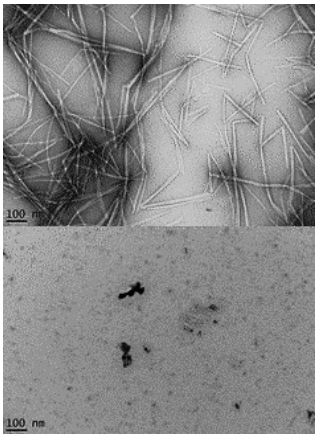


Fig. S5 TEM images of 15 μM IAPP (top) and in presence of DM 1 (3 μM) after 8 h (bottom) under non-lipid condition.

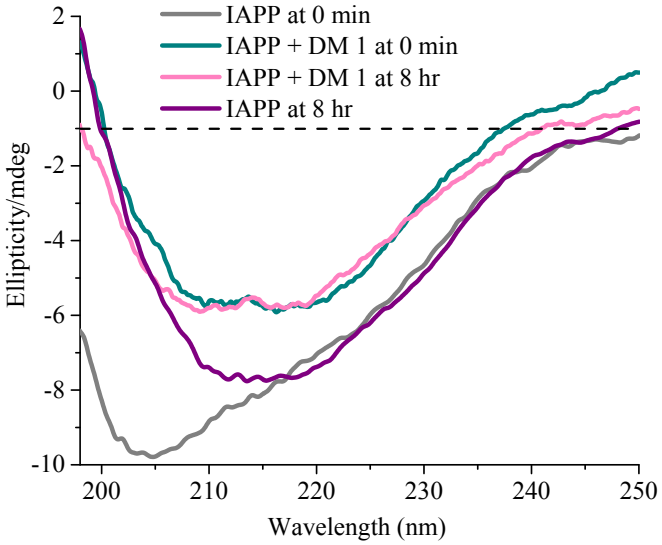


Fig. S6 Circular dichroism spectra of 20 μM IAPP in the absence (gray and purple curves) and presence (green and pink curves) of DM 1 in phosphate buffer (50 mM NaPi, 150 mM KCl, pH 7.4) at different time intervals depicted in the figure.

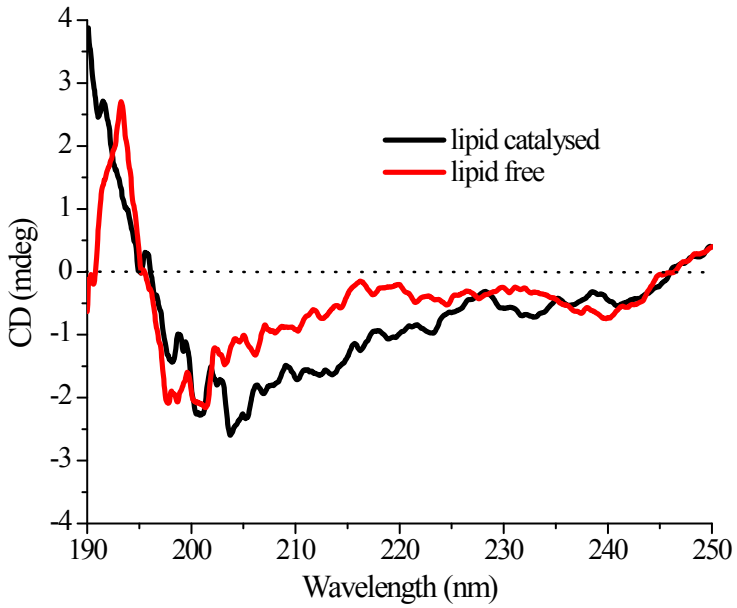


Fig. S7 Circular dichroism spectra of 20 μM DM 1 in lipid catalysed and lipid free conditions.

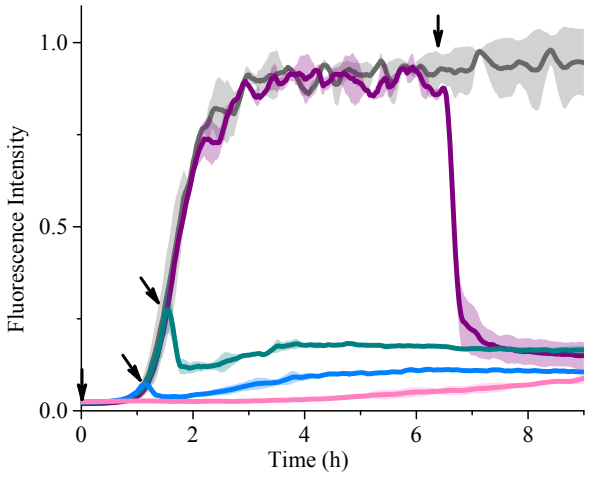


Fig. S8 The effect of DM 1 on the preformed IAPP amyloid fibrils. Conditions: 50 mM NaPi, 150 mM KCl, pH 7.4; 750 μM, LUVs, DOPG : DOPC, 3 : 7, d = 100 nm; ThT = 7.5 μM. ThT fluorescence based kinetic profiles of 15 μM IAPP (gray) alone. 0.2 equivalent of DM 1 added to respective IAPP sample at different time indicated by small black arrow. Solid curves represent the average of three independent trials while the shaded regions represent the standard deviations of those measurements.

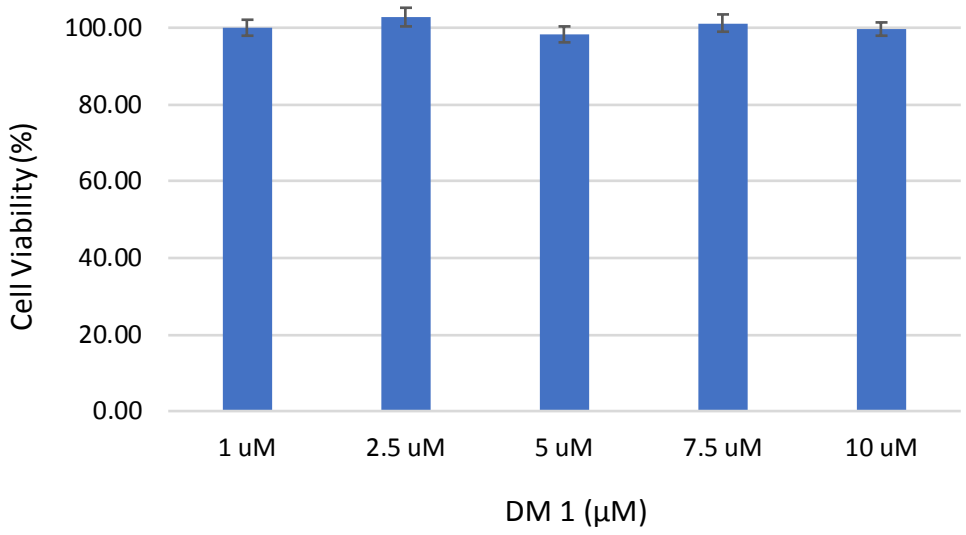
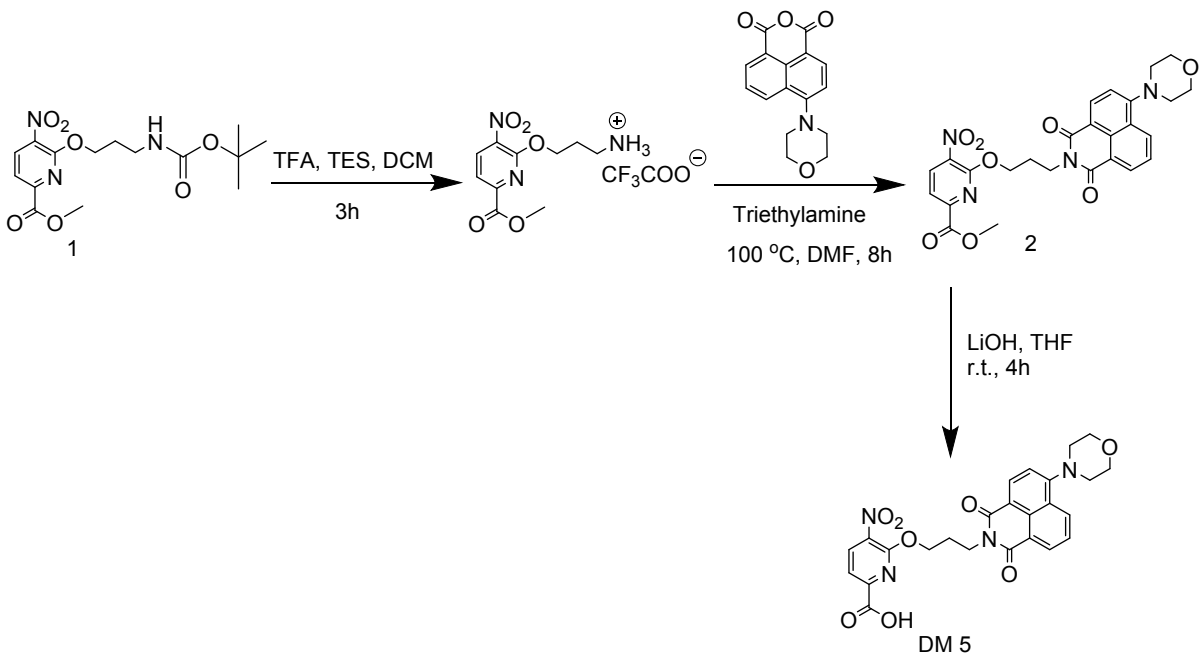


Fig. S9 The cell viability of DM 1 at different concentration. The experimental conditions were similar to the cell-viability assay presented in the main text except that no IAPP was added to the cells. Error bars reflect variability across 4–8 technical replicates within a single execution of the assay.

Synthetic details

DM 2 and DM 3 were synthesized by previous procedure.[1, 2]

Synthesis of DM 5

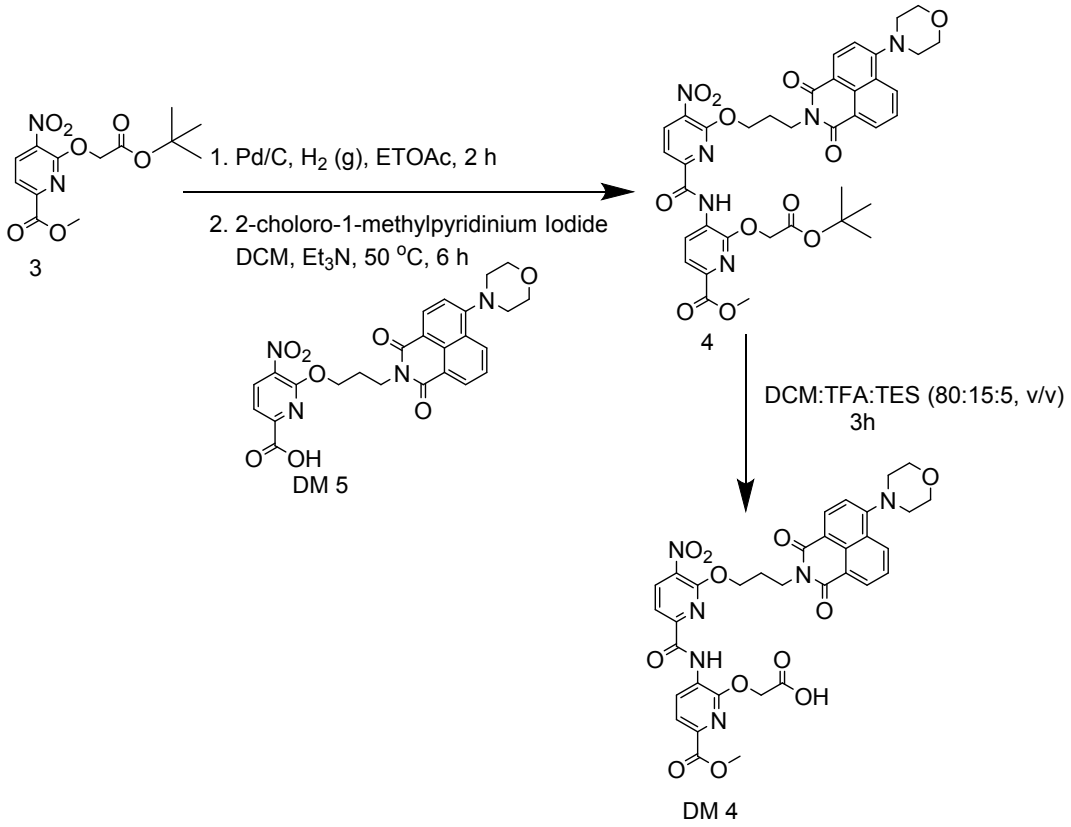


Compound 1 was synthesized according to previous protocol.[3] Compound 1 (100 mg, 0.28 mmol) was deprotected in a mixture of 5 mL dichloromethane (DCM), 250 µL triethylsilane (TES) and 500 µL trifluoroacetic acid (TFA). The reaction mixture was stirred constantly for 3 h. The reaction mixture was dried under rotovap which resulted in a thick oil. The compound was washed with cold diethyl ether (3 × 5 mL) to afford the product as a yellow solid. This product was used for next step without further purification. Deprotected product was mixed with 4-morpholine-1,8-naphthalic anhydride[4] (72 mg, 0.25 mmol) in 5 mL dimethylformamide (DMF) solvent. Triethylamine (87 µL, 0.63 mmol) was added to the mixture and heated for overnight at 100 ºC. Then DMF was evaporated under rotovap which resulted yellow oil. It was dissolved in 5 mL DCM and washed with brine solution (3 × 20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography (50 to 70% Ethylacetate in hexane, v/v) afforded the desired product (2) as orange coloured solid (123.5 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) 2.29-2.36 (m, 2H), 3.27 (t, J = 4.5 Hz, 4H), 3.94 (s, 3H), 4.02 (t, J = 4.6 Hz, 4H), 4.41 (t, J = 6.9 Hz, 2H), 4.73 (t, J = 6.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 1H), 7.69 (t, J = 7.4 Hz, 1H), 7.76 (d, J = 8 Hz, 1H), 8.27 (d, J = 8 Hz, 1H), 8.41 (dd, J = 7.5 Hz, 1 Hz, 1H), 8.47 (d, J = 8 Hz, 1H), 8.53 (d, J = 7 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) 27.9, 37.4, 52.7, 53.4, 66.9, 68.3, 114.9, 115.6, 117.0, 121.2, 123.2, 125.3, 126.1, 129.9, 130.1, 131.2, 132.5, 134.8, 142.3, 151.9, 155.7, 164.0, 164.5, 165.2 ppm.

To a solution of compound 2 (120 mg, 0.23 mmol) in 15 mL tetrahydrofuran (THF), 10 mL of 0.2 N Lithium hydroxide (LiOH) was added and the reaction was stirred for 6 h at room

temperature. The pH of the reaction solution was adjusted to 4 by adding 0.1 M HCl. The aqueous layer was extracted with EtOAc (2×30 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated on rotovap to afford the desired product DM 5 as orange solid (109 mg, 94%). ^{1}H NMR (400 MHz, DMSO-d_6) 2.16 (t, *J* = 6.2 Hz, 2H), 3.22 (t, *J* = 4.2 Hz, 4H), 3.91 (t, *J* = 4.2 Hz, 4H), 4.23 (t, *J* = 6.7 Hz, 2H), 4.58 (t, *J* = 5.9 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.69 (d, *J* = 8 Hz, 1H), 7.79 (dd, *J* = 7.4 Hz, 0.9 Hz, 1H), 8.35 (d, *J* = 8 Hz, 1H), 8.41-8.44 (m, 2H), 8.48 (d, *J* = 8.4 Hz, 1H), 13.7 (br, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) 27.8, 37.3, 53.4, 66.6, 68.3, 115.1, 115.6, 116.2, 121.5, 122.9, 125.5, 126.2, 129.4, 130.5, 130.7, 132.2, 136.3, 142.0, 151.4, 155.6, 163.4, 163.9, 166.2 ppm. HRMS (m/z): calculated for $C_{25}H_{22}N_4O_8$ $(M + H)^+$: 507.1510, found 507.1515.

Synthesis of DM 4

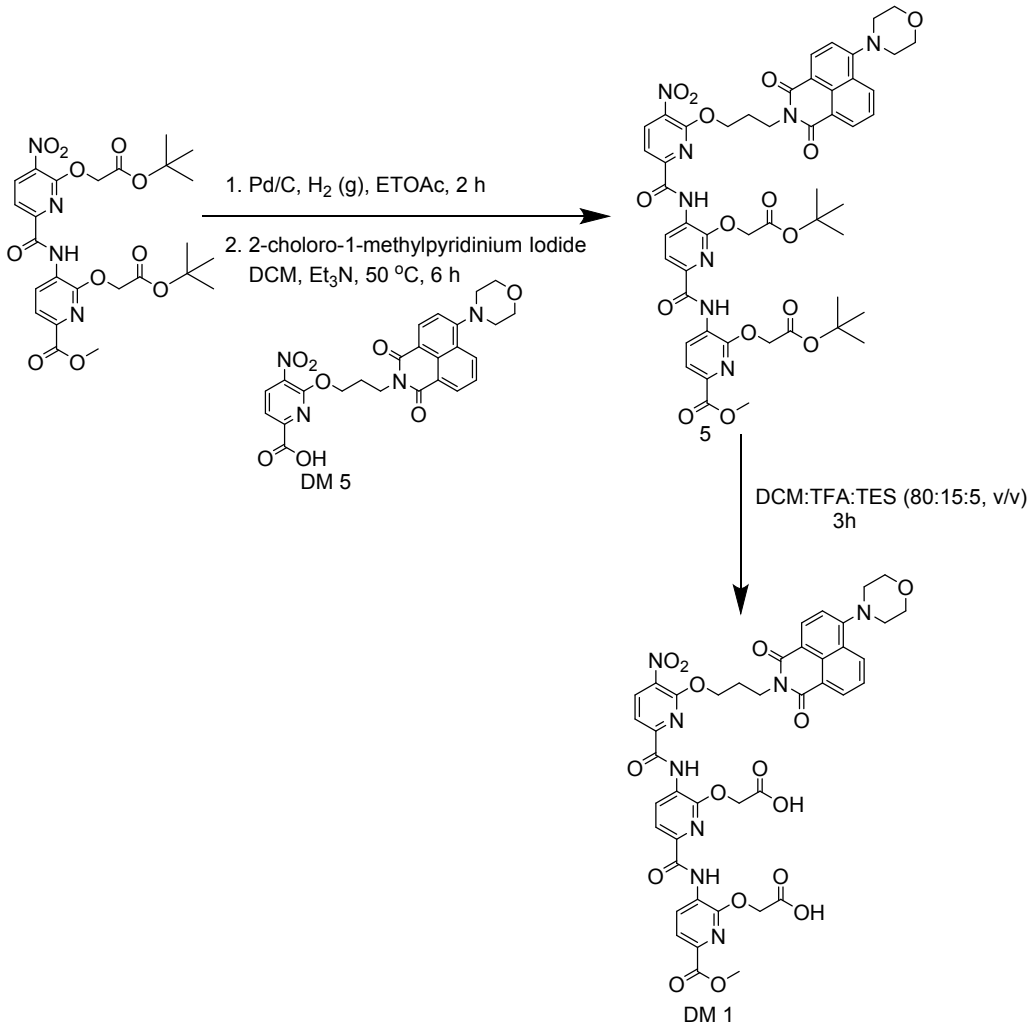


Compound 3 was synthesized according to previous protocol.[5]

To a solution of compound 3 (100 mg, 0.32 mmol) in EtOAc (10 mL), Pd/C (10% by wt.) was added and the reaction mixture was stirred in the atmosphere of H_2 (g) at room temperature for 2h. The progress of the reaction was monitored using TLC. The disappearance of the starting material confirms the completion of the reaction. The reaction mixture was filtered, and the filtrate was dried over rotovap to afford the desired product as a yellow solid, which is used in next step without further characterization.

In dichloromethane (10 mL, anhydrous), DM 5 (194 mg, 0.38 mmol), triethylamine (89 µL, 0.64 mmol) and 2-chloro-1-methylpyridinium iodide (97 mg, 0.38 mmol) were added and the reaction stirred for 20 min. at 60 °C. Then above reduced product in dichloromethane (10 mL, anhydrous) was added and the reaction mixture was stirred at 60 °C for 8 h in the atm. of argon. The volatiles were removed on rotovap. Column chromatography (40 to 60% ethyl acetate in hexane, v/v) afforded the desired product 4 as orange solid (209 mg, 85%). Then the product 4 was subjected to deprotection by stirring in 10 mL of DCM:TFA:TES (80:15:5, v/v) cocktail

for 3h. The reaction mixture was dried under rotovap which resulted in oily product. The compound was washed with cold diethyl ether (3 × 5 mL) to afford the pure product DM 4 as orange solid (192 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) 2.29 (t, J = 5.7 Hz, 2H), 3.11 (t, J = 4 Hz, 4H), 3.85 (m, 7 Hz), 4.24 (t, J = 6.1 Hz, 2H), 4.62 (s, 2H), 4.77 (t, J = 5.3 Hz, 2H), 7.18 (d, J = 8.1 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.89 (d, J = 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1H), 8.29 (t, J = 7.7 Hz, 2H), 8.62 (d, J = 8 Hz, 1H), 8.74 (d, J = 8 Hz, 1H), 10.03 (s, 1H), 13.02 (br, 1H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) 27.2, 36.7, 52.8, 53.4, 63.1, 65.7, 66.6, 115.2, 115.9, 116.3, 121.1, 123.0, 125.4, 125.5, 126.1, 126.3, 129.5, 130.7, 130.9, 132.4, 136.7, 138.0, 138.3, 148.8, 151.3, 154.9, 155.7, 160.7, 163.6, 164.1, 164.6, 169.3 ppm. HRMS (m/z): calculated for $C_{34}H_{30}N_6O_{12}$ $(M + H)^+$: 715.1994, found 715.1992.



Synthesis of DM 1

To a solution of acid functionality protected dipyridylamide (100 mg, 0.18 mmol) in EtOAc (10 mL), Pd/C (10% by wt.) was added and the reaction started with constant stirring in the atmosphere of H_2 (g) at room temperature for 2h. The progress of the reaction was monitored using TLC. The disappearance of the starting material confirms the completion of the reaction. The reaction mixture was filtered, and the filtrate was dried over rotovap to afford the desired product as a yellow solid, which is used in next step without further characterization.

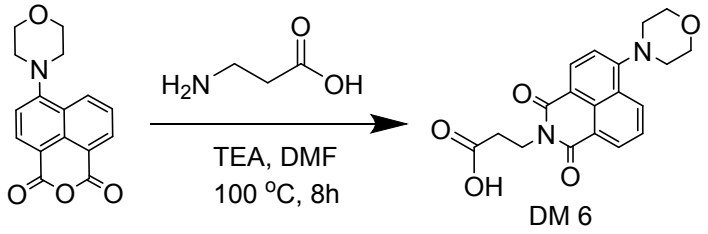
In dichloromethane (10 mL, anhydrous), DM 5 (109 mg, 0.21 mmol), triethylamine (50 µL, 0.36 mmol) and 2-chloro-1-methylpyridinium iodide (54 mg, 0.21 mmol) were added and the reaction stirred for 20 min. at 60 °C. Then above reduced product in dichloromethane (10 mL, anhydrous) was added and the reaction mixture was stirred at 60 °C for 8 h in the atm. of argon. The volatiles were removed on rotovap. Column chromatography (60 to 80% ethyl acetate in

hexane, v/v) afforded the desired product 5 as orange solid (138 mg, 75%). Then the product 5 was subjected to deprotection by stirring in 10 mL of DCM:TFA:TES (80:15:5, v/v) cocktail for 3h. The reaction mixture was dried under rotovap which resulted in oily product. The compound was washed with cold diethyl ether (3 × 5 mL) to afford the pure product DM 1 as orange solid (121 mg, 99%).

Product 5: ^{1}H NMR (400 MHz, $CDCl_3$) 1.41 (s, 9H), 1.51 (s, 9H), 2.45 (t, *J* = 5.6 Hz, 2H), 3.18 (m, 4H), 3.94 (m, 7H), 4.46 (t, *J* = 5.9 Hz, 2H), 4.87 (m, 4H), 5.04 (s, 2H), 7.12 (d, *J* = 8 Hz, 1H), 7.64 (dd, *J* = 7.3 Hz, 0.8 Hz, 1H), 7.86 (d, *J* = 8 Hz, 1H), 7.98 (dd, *J* = 8 Hz, 2.6 Hz, 2H), 8.32 (d, *J* = 8 Hz, 1H), 8.39 (t, *J* = 7.9 Hz, 2H), 8.47 (d, *J* = 6.3 Hz, 1H), 8.92 (d, *J* = 8 Hz, 1H), 8.99 (d, *J* = 8 Hz, 1H), 10.22 (s, 1H), 10.34 (s, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) 27.4, 28.0, 28.1, 37.2, 52.4, 53.4, 63.6, 63.7, 66.2, 66.9, 82.2, 82.5, 114.7, 115.3, 116.8, 118.1, 121.3, 123.1, 125.6, 125.7, 125.7, 125.9, 126.2, 127.0, 129.8, 130.1, 131.0, 132.4, 136.2, 136.8, 137.9, 139.7, 149.3, 150.4, 151.2, 155.4, 155.6, 160.6, 162.1, 163.9, 164.4, 165.0, 166.7 ppm.

DM 1: ^{1}H NMR (400 MHz, $CDCl_3$) 2.31 (t, J = 5.7 Hz, 2H), 3.04 (m, 4H), 3.75 (t, J = 3.7 Hz, 4H), 3.85 (s, 3H), 4.24 (t, J = 6 Hz, 2H), 4.59 (s, 2H), 4.79 (t, J = 5.3 Hz, 2H), 5.03 (s, 2H), 7.11 (d, J = 8.16, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.90-7.93 (m, 2H), 8.16 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.30 (d, J = 7.2 Hz, 1H), 8.65 (d, J = 8 Hz, 1H), 8.79-8.83 (m, 2H), 10.0 (s, 1H), 10.20 (s, 1H), 13.24-13.26 (br, 2H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) 27.1, 36.5, 52.8, 53.4, 63.1, 63.4, 65.2, 66.5, 115.1, 115.9, 116.2, 118.2, 121.3, 123.0, 125.4, 125.6, 125.8, 126.1, 126.3, 126.9, 129.5, 130.7, 130.9, 132.4, 136.7, 137.9, 139.2, 148.6, 150.5, 151.7, 154.9, 155.6, 160.6, 161.9, 163.6, 164.1, 164.6, 169.3, 169.9 ppm. HRMS (m/z): calculated for $C_{42}H_{37}N_8O_{16}$ ($M + H^+$): 909.2322, found 909.2303.

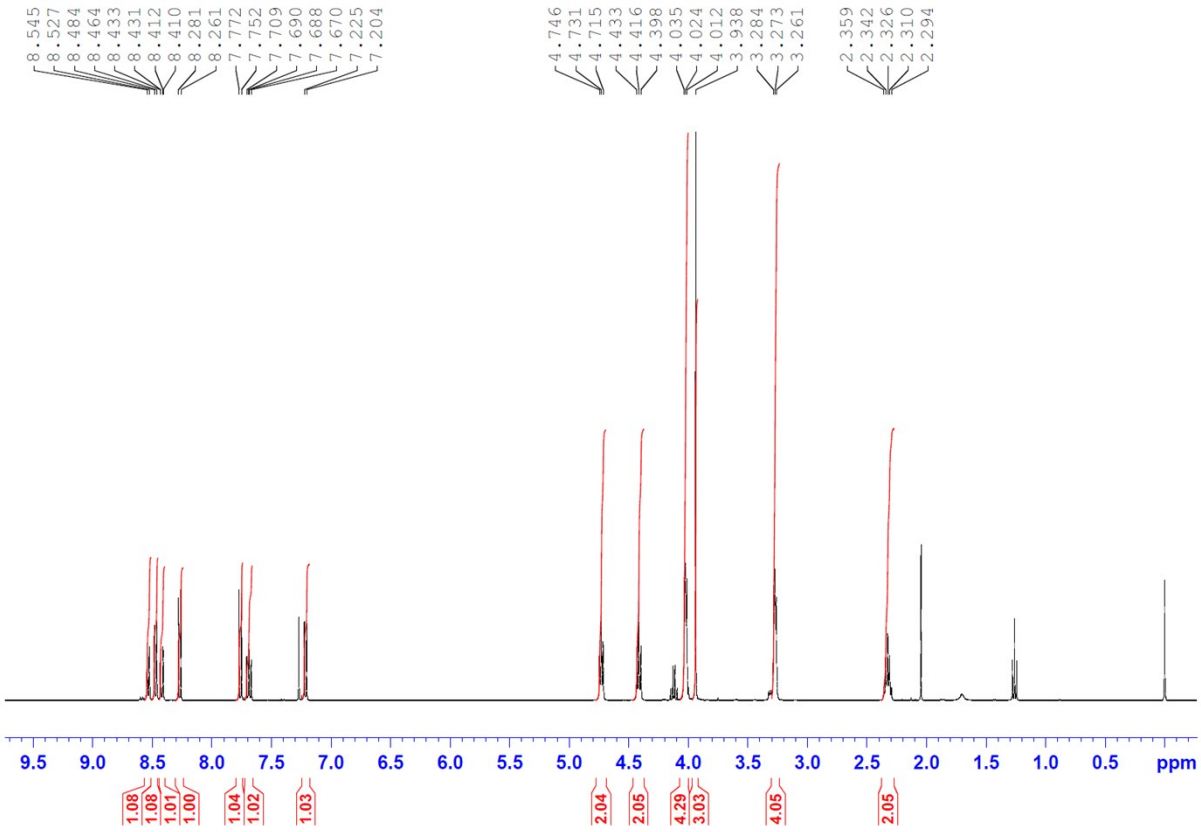
Synthesis of DM 6

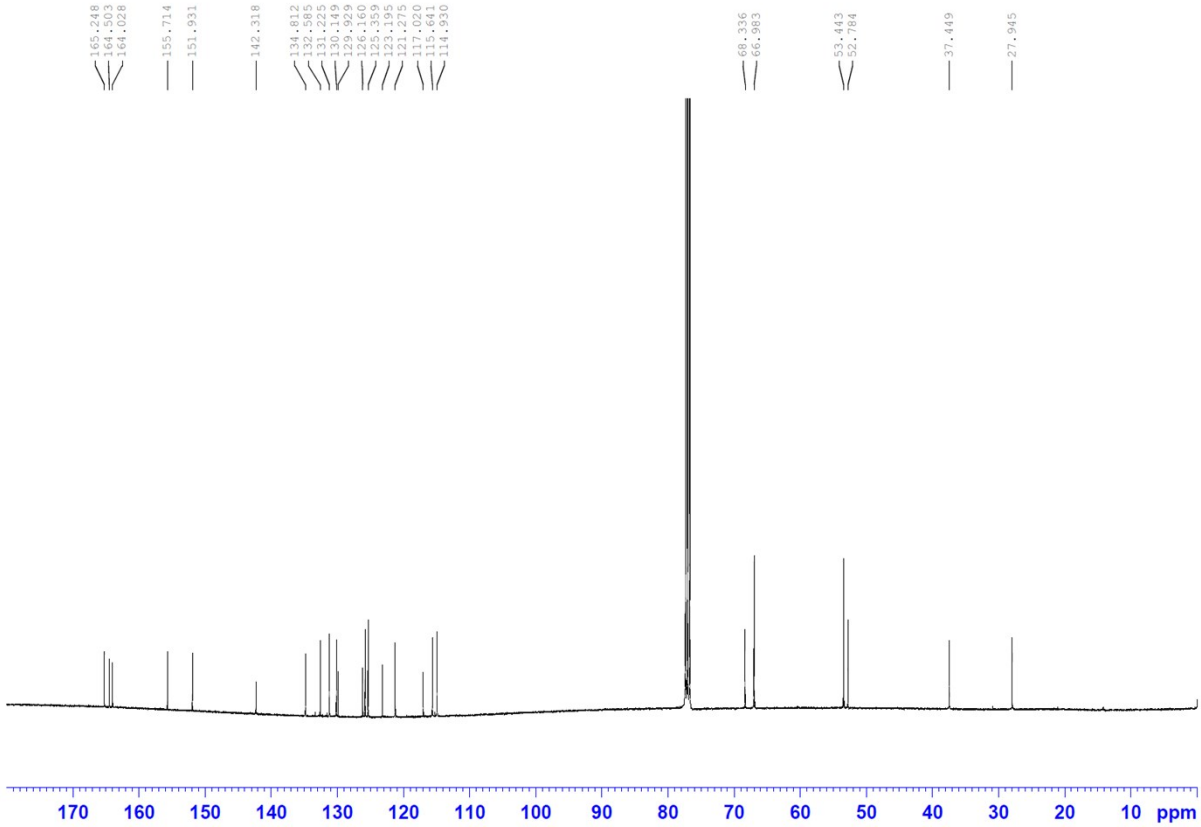


DM 6

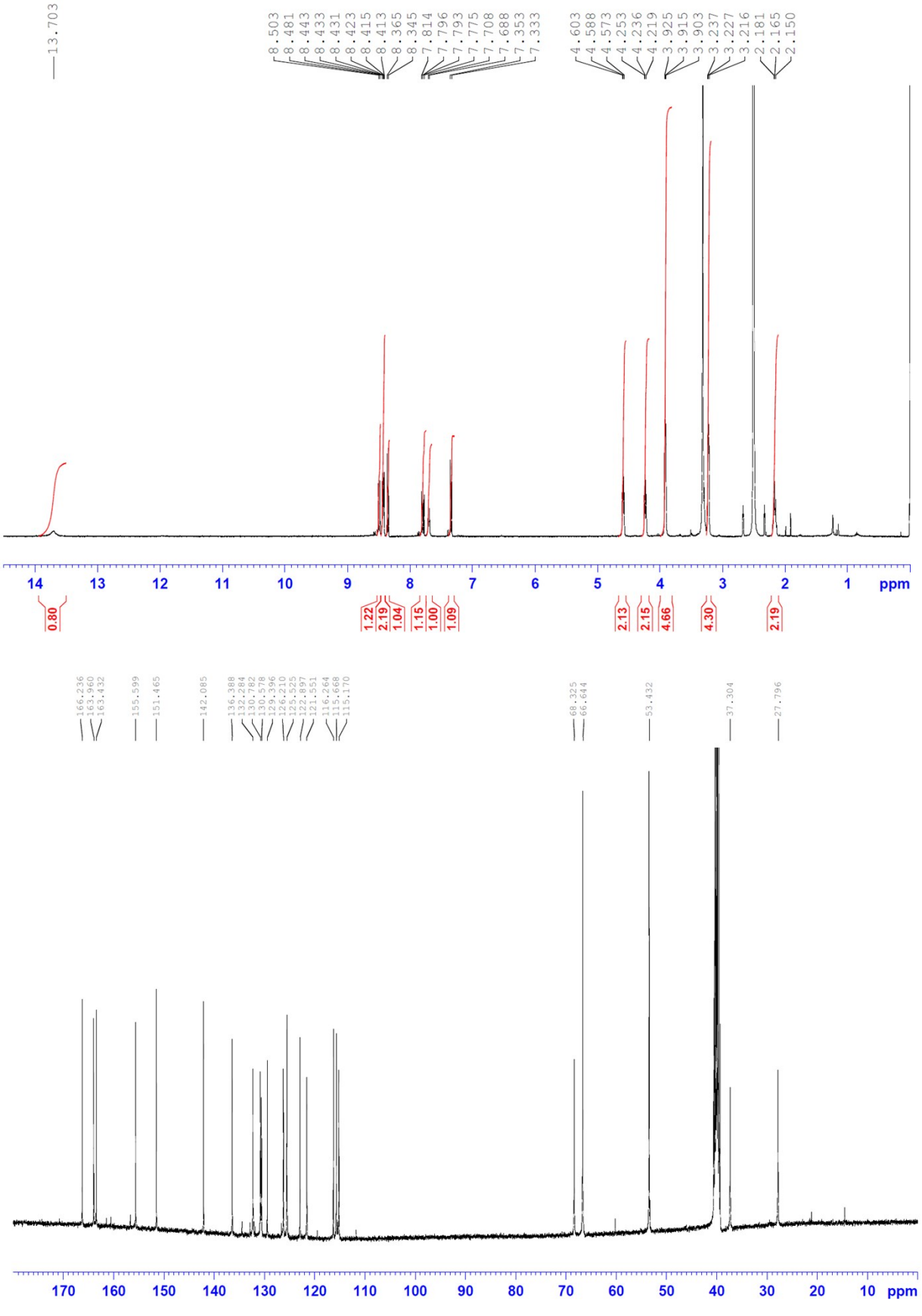
4-morpholine-1,8-naphthalic anhydride (100 mg, 0.35 mmol), β-alanine (47 mg, 0.53 mmol) and triethylamine (200 µL, 1.43 mmol) were stirred at 100 ºC in 5 mL dimethylformamide (DMF) solvent for overnight. Then DMF was evaporated under rotovap which resulted yellow oil. It was dissolved in 5 mL DCM and washed with brine solution (3 × 20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography (0 to 10% methanol in DCM, v/v) afforded the desired product DM 6 as orange coloured solid (121.5 mg, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) 2.57 (t, J = 7.8 Hz, 2H), 3.23 (t, J = 4.4 Hz, 4H), 3.91 (t, J = 4.2 Hz, 4H), 4.25 (t, J = 7.6 Hz, 2H), 7.37 (d, J = 8.1 Hz, 1H), 7.83 (t, J = 7.4 Hz, 1H), 8.42 (d, J = 8 Hz, 1H), 8.50 (t, J = 8.2 Hz, 2H), 12.33 (s, 1H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) 32.7, 36.0, 53.4, 66.6, 115.4, 116.1, 122.8, 125.5, 126.4, 129.4, 130.9, 131.0, 132.5, 155.8, 163.2, 163.7, 172.9 ppm. HRMS (m/z): calculated for $C_{19}H_{19}N_2O_5$ ($M + H^+$): 355.1288, found 355.1287.

^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of compound 2.

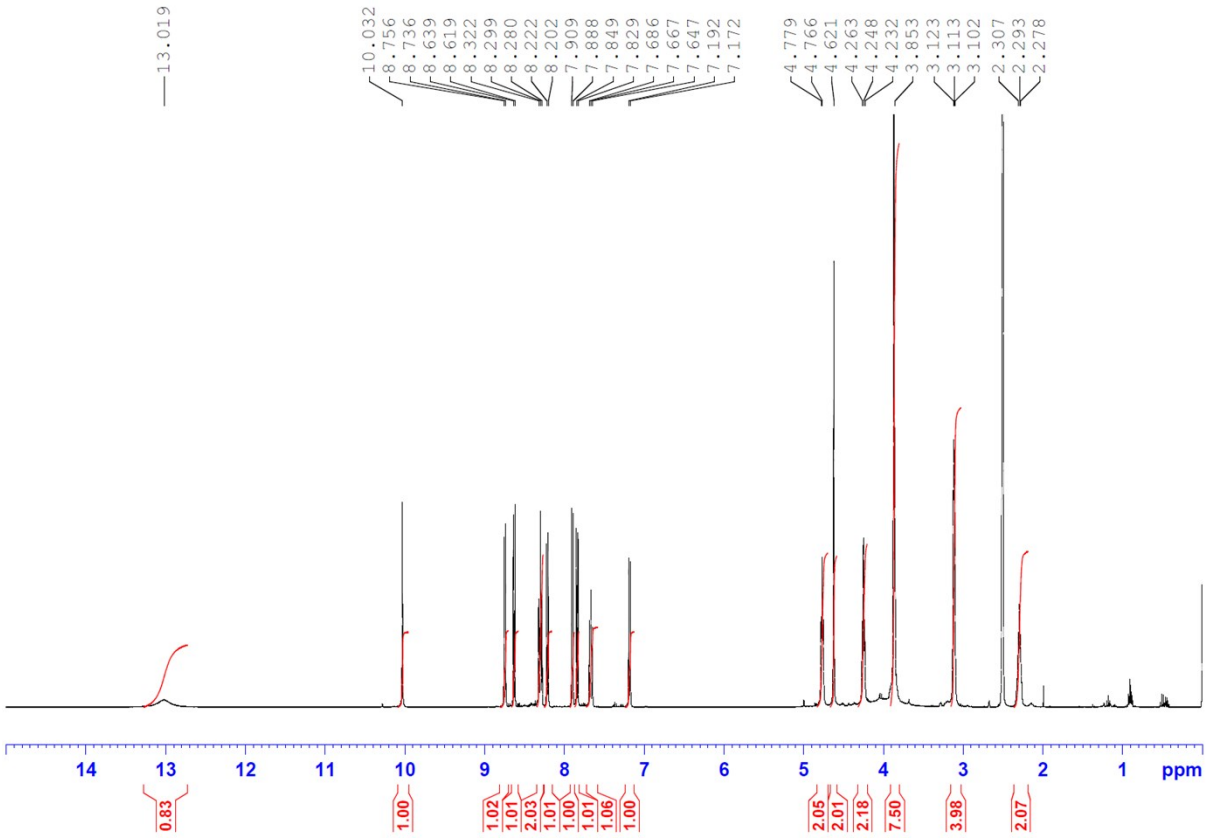




^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of compound DM 5.



^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of compound DM 4.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4.248
4.232
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3.123
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3.102
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2.278
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1.02
1.01
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2.01
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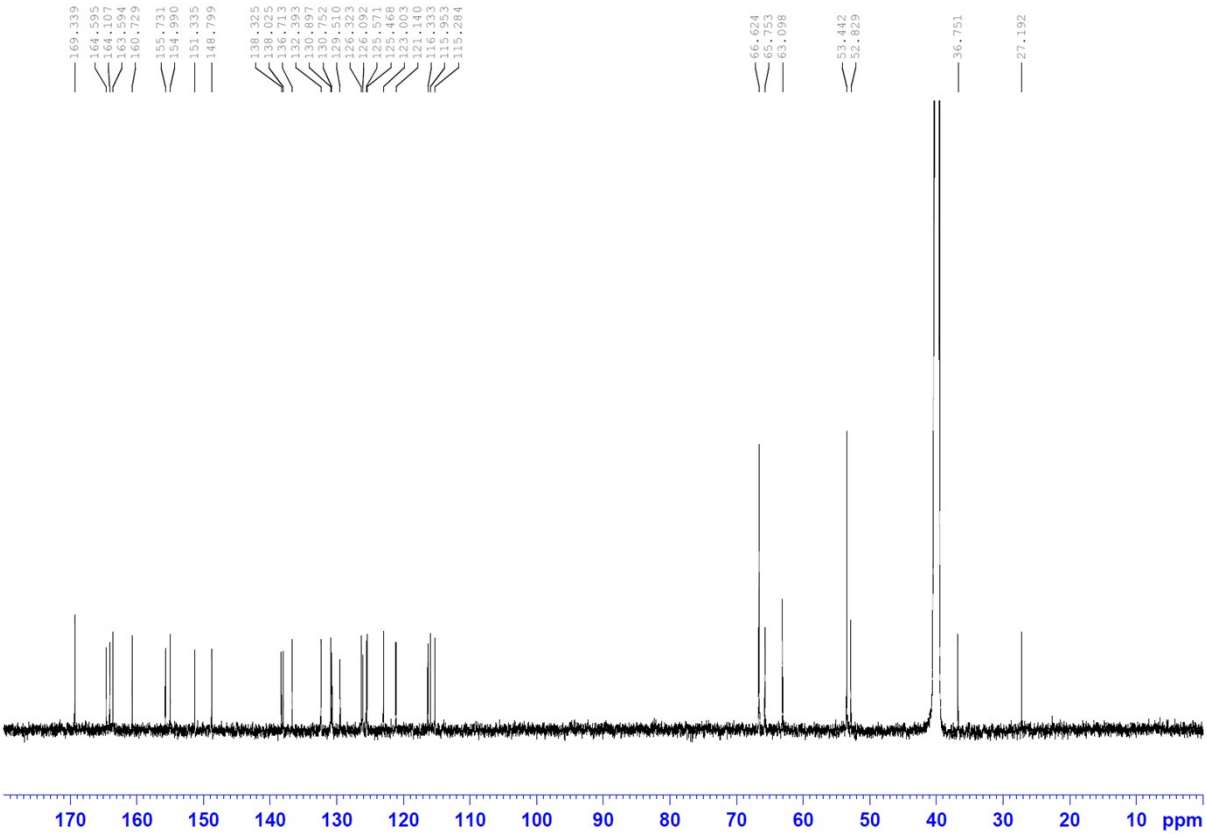
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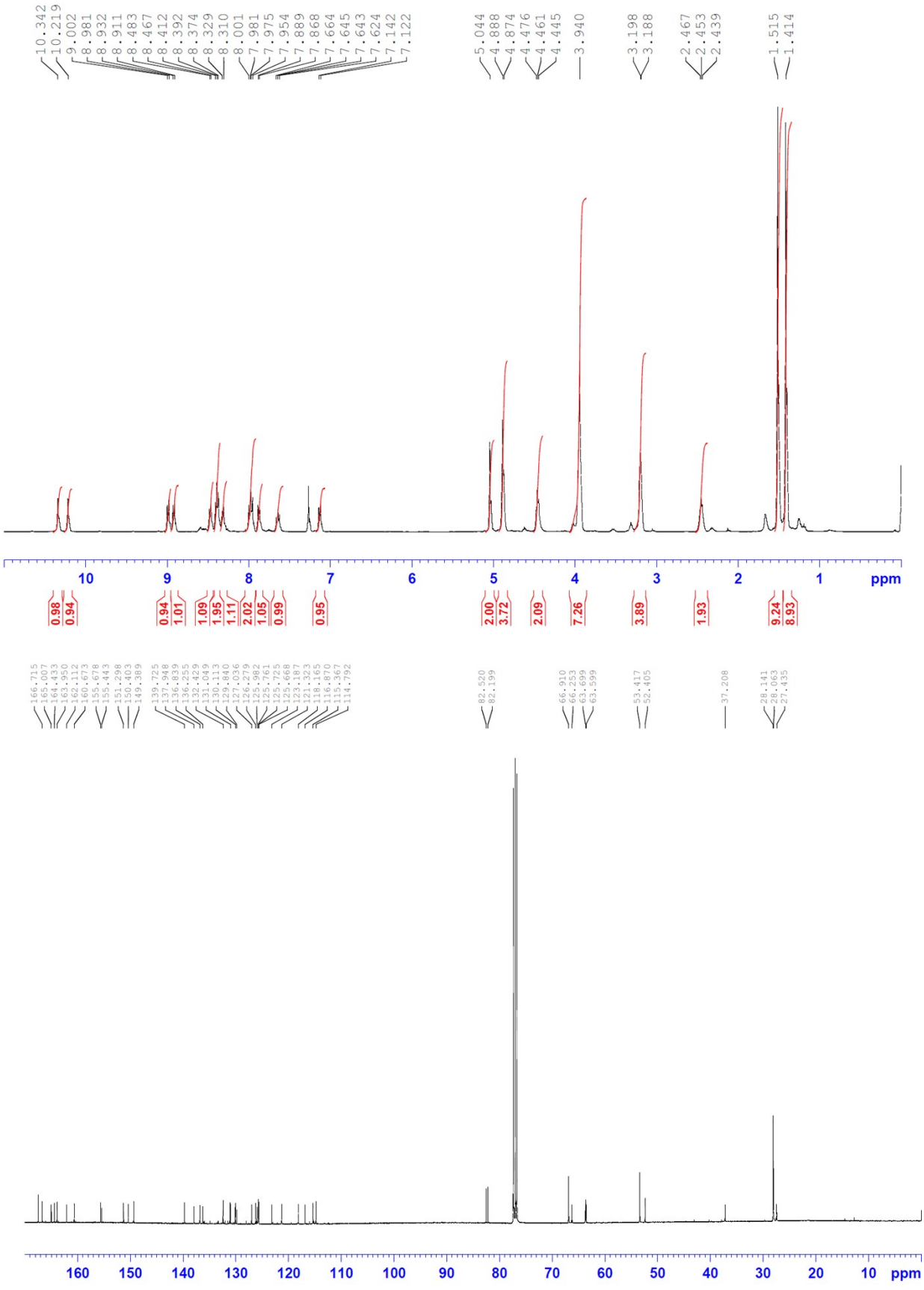
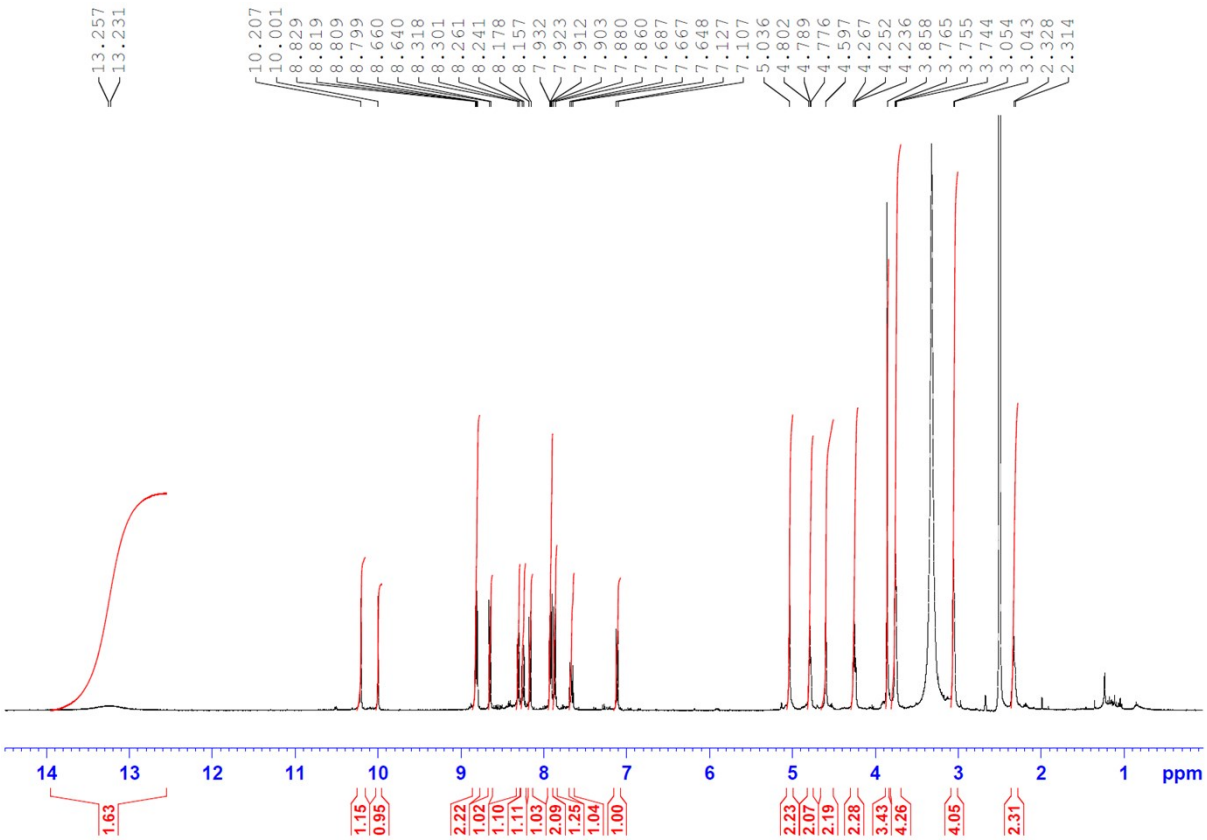
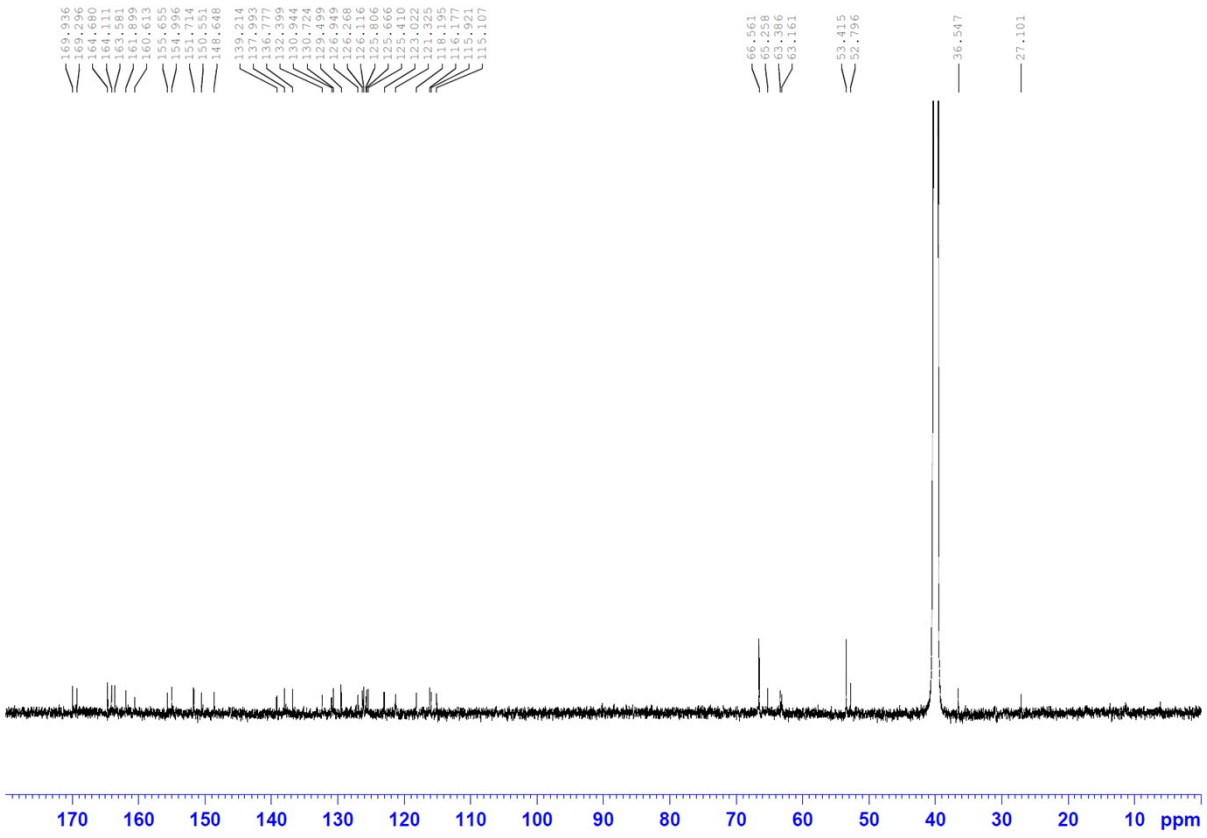
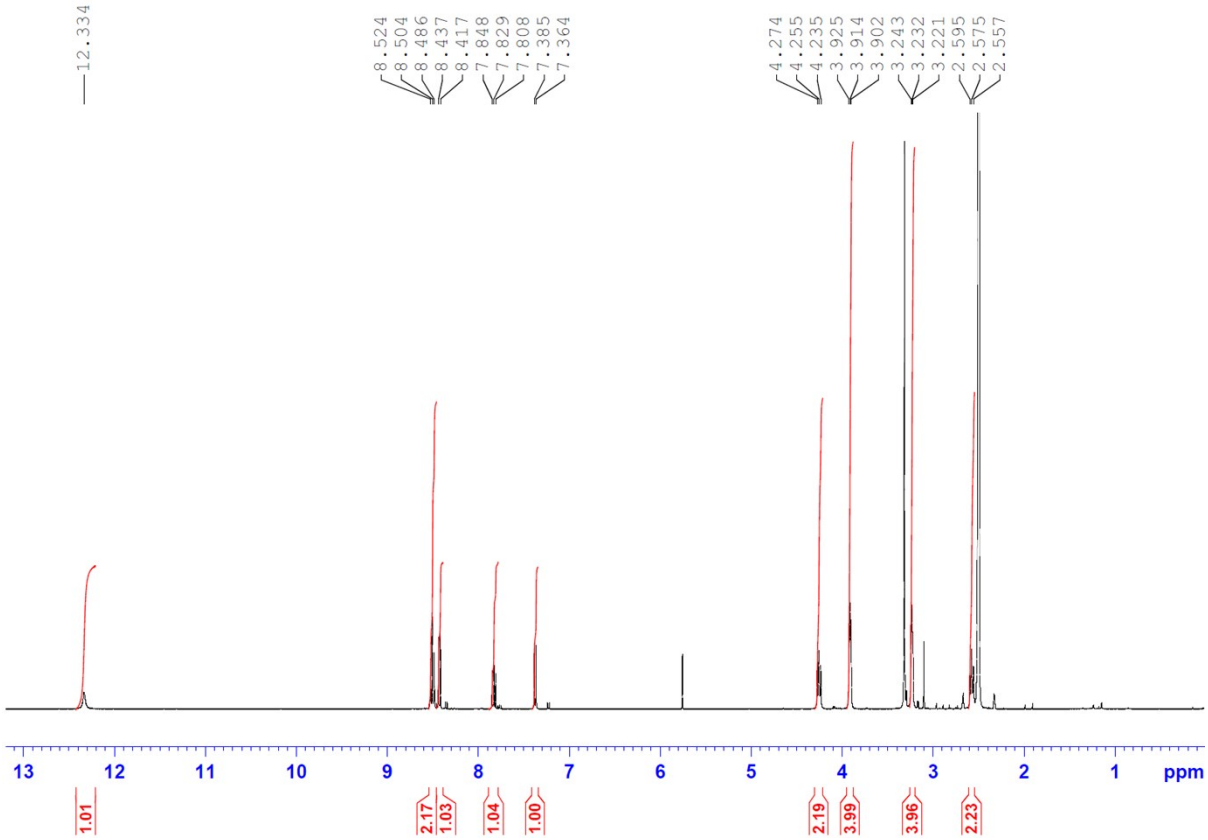
^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of compound 5.



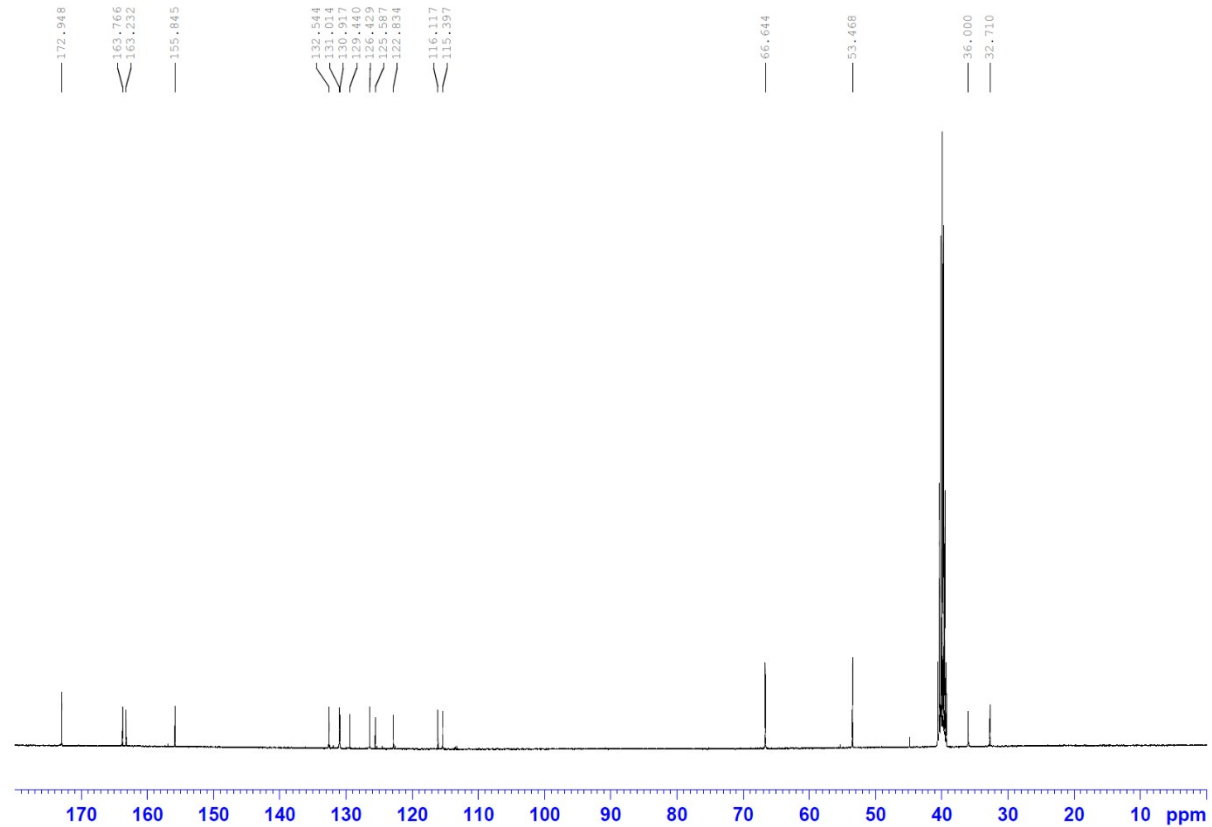


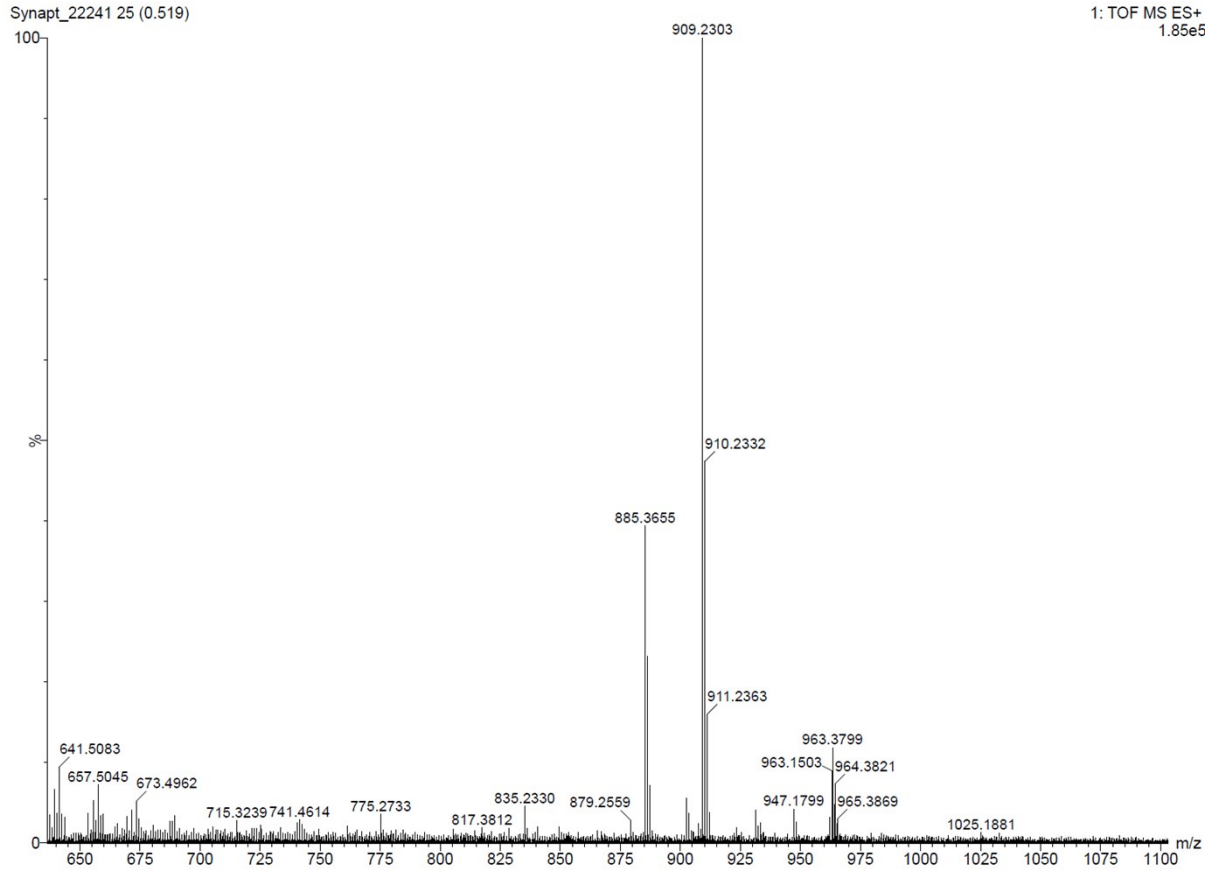
^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of compound DM 1.





^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of compound DM 6.





Mass spectra of DM 1:

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