Tryptophan trimers and tetramers inhibit dengue and zika virus replication by interfering with viral attachment processes

Antonios Fikatas[a], Peter Vervaeke[a], Belén Martínez-Gualda[b¥], Olaia Martí-Marí[b] Sam Noppen[a], Eef Meyen[a], María-José Camarasa[b], Ana San-Félix[b], Christophe Pannecouque[a], Dominique Schols[a]

[a] *KU Leuven-University of Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000, Leuven, 7 Belgium*

[b] *Instituto de Química Médica (IQM-CSIC), Juan de la Cierva 3, 28006, Madrid, Spain*

[¥]*Present address: Medicinal Chemistry, Rega Institute of Medical Research, KU Leuven, 10 Herestraat 49, 3000, Leuven, Belgium*

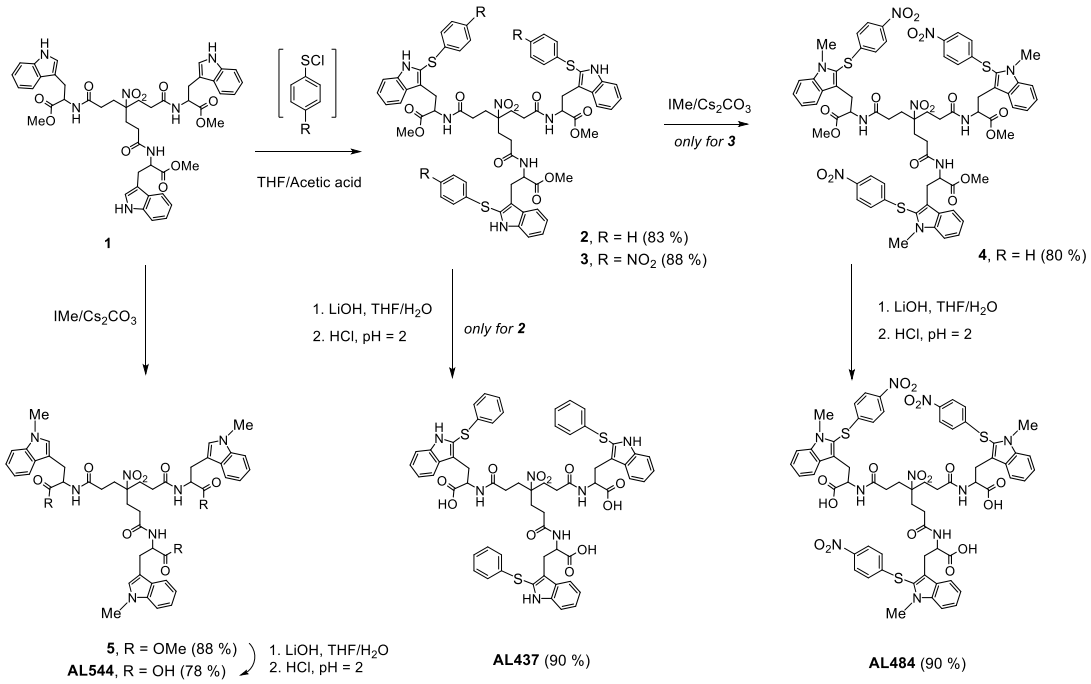
Includes:

1. CHEMISTRY

1.1. Synthetic Schemes

First, the corresponding sulfenylating agent, phenylsulfenyl chloride (PS-Cl) or *p*-nitro-benzenesulfenyl chloride (*p*NPS-Cl) were obtained following reported procedures.[1,2] Reaction of these sulfenyl halides, with the methyl protected tryptophan trimer **1**[3] in dry THF/acetic acid afforded the C2-sulfenyltryptophan intermediates **2** and **3** in 83 and 88 yield respectively (Scheme 1). Reaction of intermediate **3** with methyl iodide, in the

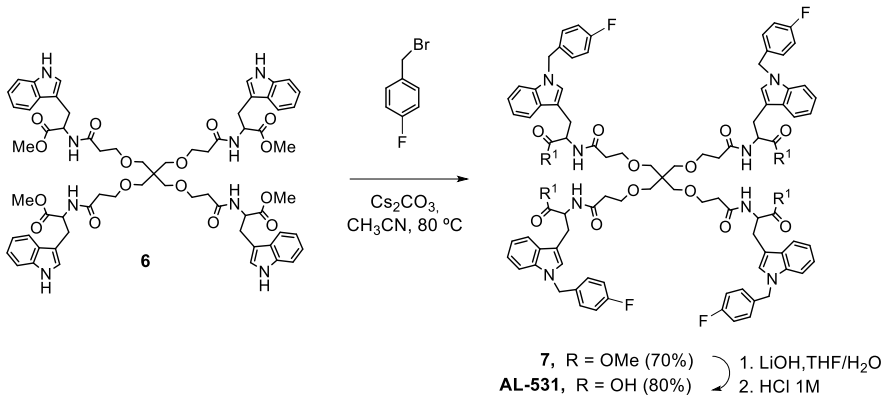
presence of cesium carbonate (Cs_2CO_3) at 80 ºC, followed by deprotection ($LiOH/H_2O$) of intermediate **4** afforded the N1 methyl Trp derivative **AL-484** in 90% yield (Scheme 1). In addition, the N1 unsubstituted Trp derivative **AL437** was prepared in 90% yield by deprotection ($LiOH/H_2O$) of the sulfenyl intermediate **2** (Scheme 1).



Scheme 1. Synthesis of the 2-sulfenyltryptophan **AL437**, **AL484** and N^{α} methyl tryptophan **AL-544** trimers

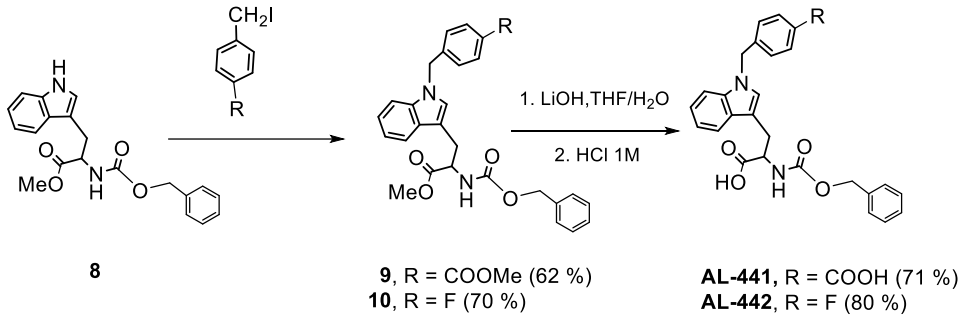
In addition, reaction of trimer **1** with methyl iodide in the presence of cesium carbonate (Cs_2CO_3) at 80 ºC, followed by subsequent deprotection ($LiOH/H_2O$) of intermediate **5** afforded compound **AL-544** in 78% yield (Scheme 1).

Next, reaction of tetramer **6**[3] with *p*-fluoro benzyl bromide, in the presence of Cs_2CO_3 at 80 ºC, followed by subsequent deprotection ($LiOH/H_2O$) of intermediate **7** afforded tetramer **AL-531** in 80% yield (Scheme 2).



Scheme 2. Synthesis of the N1-benzyl tryptophan tetramer **AL531**

The synthesis of the N1 substituted monopodal derivatives **AL441** and **AL442** started with the commercially available scaffold **8** (Scheme 3). Reaction of **8** with the corresponding benzyl iodide, in the presence of cesium carbonate (Cs_2CO_3) at 80 ºC, followed by subsequent deprotection ($LiOH/H_2O$) of intermediates **9** and **10** afforded compounds **AL-441** and **AL-442** in 71 and 80 % yield respectively (Scheme 3).



Scheme 3. Synthesis of the N1-benzyl tryptophan monomers **AL441** and **AL442**

1.2. Chemistry procedures and characterization of the compounds

General Chemical Procedures. Commercial reagents and solvents were used as received from the suppliers without further purification unless otherwise stated. The solvents used in some reactions were dried prior to use. DMF dry was commercially available (Aldrich).

Analytical thin-layer chromatography (TLC) was performed on aluminum plates pre-coated with silica gel 60 (F_{254}, 0.20 mm). Products were visualized using an ultraviolet

lamp (254 nm and 365 nm) or by heating after treatment with a 5% solution of phosphomolybdic acid (PMA) or vanillin in ethanol.

The compounds were purified by: a) high performance flash chromatography (HPFC) with a system “Isolera One” (Biotage) in reverse phase using water/acetonitrile (100:0 to 0:100) as eluent, b) preparative centrifugal circular thin layer chromatography (CCTLC) on a chromatotron® (Kiesegel 60 PF254 gipshaltig, Merck) layer thickness 1 mm, flow rate 2–4 mL/min.

For HPLC analysis an Agilent Technologies 1120 Compact LC with a reverse phase column ACE 5 C18-300 (4.6 mm × 150 mm, 3.5 µm) equipped with a PDA (Photo Diode Array) detector was used. Acetonitrile was used as mobile phase A, and water 0.05% of TFA was used as mobile phase B with at a flow rate of 1 $mL \cdot min^{-1}$. All retention times are quoted in minutes and the gradients are specified for each compound in the experimental data.

High resolution mass spectrometry (HRMS) was recorded on an Agilent 6520 Accurate Mass QTOF (quadrupole time of flight) coupled with LC/MS using an electrospray interface (ESI) working in the positive-ion (ESI^+) and negative-ion (ESI^-) mode.

NMR spectra (^1H, ^{13}C NMR) were recorded on a Varian UNIT INOVA-300 (300 MHz), Bruker AVANCE 300 (300 and 75 MHz), Varian INOVA-400 (400 and 100 MHz), Varian MERCURY-400 (400 and 100 MHz) and Varian-500 (500 and 125 MHz) spectrometers, using $(CD_3)_2SO$ and $CDCl_3$ as solvents. Chemical shift (δ) values are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in ^1H and $CDCl_3$ (δ = 77.0) in ^{13}C NMR. Coupling constant (*J* values) are reported in hertz (Hz) and multiplicities of signals are indicated by the following symbol: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and bs (broad singlet).

Final compounds were lyophilized using a Telstar 6-80 system.

Trp(PS)-OMe trimer (2). Phenylsulfenyl chloride was prepared from phenyl thiol according to literature procedures.[1,2] Thus, sulfuryl chloride (92.2 mg, 0.055 mL, 12 equiv) was added dropwise over a period of 1 h. to a cold solution (0 ºC, ice-bath) of phenyl thiol (37.7 mg, 0.035 mL, 6 equiv) in THF (5 mL). After an additional hour of stirring, the yellow phenyl chloride[1,2] (PS-Cl) solution, generated *in situ*, was added to a cold (0 ºC, ice-bath) solution of trimer **1**[3] (50 mg,0.06 mmol) in acetic acid (10 mL), and the mixture was stirred at room temperature until the starting material disappeared (30 min approximately). Removal of the solvent left a residue which was purified by CCTLC using dichloromethane:methanol (20:1) as eluent to give 113.7 mg (83%) of **2** as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) *δ*: 8.60 (s, 3H, 3H, NH-Trp), 7.52 (d, *J* = 7.9 Hz, 3H, Ar), 7.19 – 7.14 (m, 11H), 7.13 – 7.07 (m, 5H, Ar), 6.99 (m, 5H, Ar), 6.21 (d, *J* = 7.8 Hz, 3H, Ar), 4.91 (m, 3H, α-CHTrp), 3.69 (s, 9H, OCH_3), 3.45 (dd, *J* = 14.5, 5.5 Hz, 3H, β-CH_2Trp), 3.31 (dd, *J* = 14.5, 5.5 Hz, 3H, β-CH_2Trp), 2.04 – 1.88 (m, 6H, CH_2), 1.85 – 1.70 (m, 6H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$-*d*) *δ*: 172.4, 170.7, 137.1, 136.2, 129.5, 127.9, 127.1, 126.5, 123.9, 123.6, 120.3, 119.1, 117.3, 111.5, 92.3, 52.9, 52.7, 30.5, 30.1, 27.3. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 6.859 min. HRMS (ESI$^+$) m/z*:* calculated for $C_{64}H_{63}N_7O_{11}S_3$. 1201.3747; found 1201.3717.

Trp(*p*NPS)-OMe trimer (3). *p*-Nitrobenzenesulfenyl chloride was prepared from *p*-nitrophenyl thiol according to literature procedures.[2] Thus, a stream of chlorine, dried by bubbling through concentrated sulfuric acid, was added to a suspension of 30 g (0.1 mol) of 4,4-dinitrodiphenyl disulfide in dry carbon tetrachloride (200 mL). The rate of flow of chlorine was regulated so that 10 g reacted over a period of 2-2.5 hr at a temperature maintained at 50-60 °C. The solid 4,4-dinitrodiphenyl disulfide gradually disappeared and at the end of the reaction time a dark yellow solution was obtained. The warm solution was filtered through a hot Buchner funnel. The excess of chlorine and the solvent were evaporated, leaving an orange-red residue. Recrystallization from carbon tetrachloride afforded *p*-nitrobenzenesulfenyl chloride, mp 52° (lit.[2] mp 50-52°). A solution of *p*-

nitrobenzenesulfenyl chloride (259 mg, 1.37 mmol) in dioxane (8 mL) was added to a solution of trimer **1**[3] (400 mg,0.4 mmol) in acetic acid (2 mL), and the mixture was stirred at room temperature for 1 h. A solution of $NaHCO_3$ was added to reach pH = 7. Elimination of the solvent left a residue which was dissolved in ethyl acetate (30 mL) and washed successively with saturated solutions of $NaHCO_3$ (3 x 20 mL) and brine (1 x 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was concentrated and purified by precipitation using dichloromethane:diethyl ether to give 539 mg (88%) of **3** as a yellow solid. m.p 138-141 ºC.^{1}H NMR (400 MHz, $CDCl_3$-*d*) *δ*: 8.86 (s, 3H, NH-Trp), 7.95 (d, *J* = 8.8 Hz, 6H, Ar), 7.53 (d, *J* = 8.0 Hz, 3H, Ar), 7.27 – 7.16 (m, 6H, Ar), 7.11 (t, *J* = 7.3 Hz, 4H, Ar), 6.97 (d, *J* = 8.8 Hz, 6H, Ar), 6.30 (d, *J* = 8.0 Hz, 3H, NHCO), 4.86 (m, 3H, α-CHTrp), 3.63 (s, 9H, OCH_3), 3.37 (dd, *J* = 14.4, 6.0 Hz, 3H, β-CH_2Trp), 3.23 (dd, *J* = 14.4, 6.0 Hz, 3H, β-CH_2Trp), 2.01 – 1.74 (m, 12H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 171.8, 170.5, 147.3, 145.0, 137.5, 127.2, 125.8, 124.3, 123.4, 119.7, 119.4, 119.3, 118.5, 111.7, 93.0, 53.1, 51.9, 30.4, 29.2, 27.0. HPLC [gradient: H_2O:MeCN, 30-100% of A in 10 min]: 9.149 min. HRMS (ESI-) m/z: calculated for $C_{64}H_{60}N_{10}O_{17}S_3$ 1336.3300; found 1336.3289.

NMe-Trp(*p*NPS)-OMe trimer (4). To a solution containing trimer **3** (50 mg, 0.04 mmol) in anhydrous DMF (5 mL), Cs_2CO_3 (54.2 mg, 0.16 mmol) was added and the mixture was stirred at room temperature for 15 minutes. Then, methyl iodide (15.76 mg, 7 µL, 0.11 mmol) was added and the reaction mixture was stirred in a sealed tube at 50 ºC overnight. The mixture was evaporated to dryness, dissolved in dichloromethane (20 mL) and washed successively with citric acid (3 x 20 mL) and brine (3 x 20 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by CCTLC using dichloromethane:methanol (20:1) as eluent to give 41.22 mg (80%) of **4** as a yellow solid. m.p 133-135 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.03 (d, *J* = 8.9 Hz, 6H), 7.60 (d, *J* = 8.0 Hz, 3H, Ar), 7.38 – 7.27 (m, 6H, Ar), 7.17 (t, *J* = 8.1, 6.1 Hz, 3H, Ar), 6.99 (d, *J* = 8.9 Hz, 6H, Ar), 6.15 (d, *J* = 7.9 Hz, 3H, NHCO), 4.83 (m, 3H, α-

CHTrp), 3.66 (s, 9H, OCH_3), 3.63 (s, 9H, OCH_3), 3.41 (dd, *J* = 14.4, 6.0 Hz, 3H, β-CH_2Trp), 3.31 (dd, *J* = 14.4, 6.0 Hz, 3H, β-CH_2Trp), 2.08 – 1.98 (m, 6H, CH_2), 1.92 (m, 6H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 171.9, 170.5, 146.6, 145.9, 138.6, 127.1, 125.6, 124.6, 124.4, 122.9, 120.5, 119.6, 118.8, 110.4, 92.8, 53.1, 52.7, 30.4, 28.1. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 7.981 min. HRMS (ESI-) m/z: calculated for $C_{67}H_{66}N_{10}O_{17}S_3$ 1378.3770; found 1378.3764.

NMe-Trp OMe trimer (5). To a solution containing trimer **1**[3] (400 mg, 0.45 mmol) in anhydrous DMF (10 mL), Cs_2CO_3 (668.0 mg, 2.05 mmol) was added and the mixture was stirred at room temperature for 15 minutes. Then methyl iodide (232.8 mg, 102 µL, 1.64 mmol) was added and the reaction mixture was treated as it was described for **4**. The residue was purified by CCTLC using dichloromethane:methanol (20:1) as eluent to give 370 mg (88%) of **5** as a white solid. m.p 135-137 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.47 (dt, *J* = 7.9, 1.0 Hz, 3H, Ar), 7.24 (m, 3H, Ar), 7.19 (t, *J* = 7.5 Hz, 3H), 7.08 (t, *J* = 7.5 Hz, 3H, Ar), 6.86 (s, 3H, Ar), 6.33 (s, 3H, NHCO), 4.84 (m, 3H, *α*-CHTrp), 3.73 (s, 9H, CH_3), 3.69 (s, 9H, CH_3), 3.29 (dd, *J* = 14.8, 5.6 Hz, 3H, *β*-CH_2Trp), 3.23 (dd, *J* = 14.8, 5.6 Hz, 3H, *β*-CH_2Trp), 2.10 – 1.98 (m, 12H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 172.2, 171.2, 137.1, 137.0, 128.2, 127.7, 122.0, 119.4, 118.7, 109.6, 108.3, 93.0, 53.3, 52.6, 32.9, 30.6, 30.4, 27.5. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 9.724 min. HRMS (ESI-) m/z: calculated for $C_{49}H_{57}N_7O_{11}$ 919.4116; found 919.4138.

N$^{\alpha}$-(4-fluorobenzyl)-L-Trp-OMe tetramer (7). To a solution containing the tetrapodal L-tryptophan methyl ester **6**[3] (100 mg, 0.08 mmol) in dry acetonitrile (1 mL) at 0 ºC (ice-bath), Cs_2CO_3 (159.45 mg, 0.49 mmol) was added and the mixture was stirred at this temperature for 15 minutes. Then a solution of 4-fluorobenzyl bromide (73 mg, 48 µL, 0.38 mmol) in DMF (1 mL) was added dropwise and the reaction mixture was stirred at 80 ºC overnight. Ammonium chloride was added and the mixture was evaporated to dryness. The residue was dissolved in dichloromethane (20 mL) and washed successively with citric acid (3 x 20 mL) and brine (3 x 20 mL). The organic phase was

dried over anhydrous Na_2SO_4 and evaporated to dryness and the residue was purified on a Biotage HPFC (High Performance Flash Chromatography) purification system on reverse phase using water/acetonitrile (100:0 to 0:100) as eluent to afford 93 mg (70%) of **7** as an amorphous solid of white color. ^{1}H NMR (400 MHz, $CDCl_3$) *δ:* 7.53 (d, *J* = 7.7 Hz, 4H, Ar), 7.18 - 6.89 (m, 32H, Ar), 6.82 (d, *J* = 7.82 Hz, 4H, NHCO), 5.18 (s, 8H, CH_2Bn), 4.91 (m, 4H, α-CHTrp), 3.55 (s, 12H, OCH_3), 3.43 (m, 8H, OCH_2), 3.28 (dd, *J* = 14.8, 5.9 Hz, 4H, β-CH_2Trp), 3.21 (dd, *J* = 14.8, 5.9 Hz, 4H, β-CH_2Trp), 3,11 (m, 8H, OCH_2), 2.31 (m, 8H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 172.5, 171.3, 163.5, 161.1, 136.5, 133.4, 133.3, 128.5, 128.5, 128.4, 126.8, 122.2, 119.6, 119.1, 115.9, 115.6, 109.9, 109.8, 69.3, 67.1, 53.3, 52.3, 49.3, 45.0, 36.7, 28.1, 1.2. HRMS (ESI-) m/z: calculated for $C_{61}H_{57}N_7O_{11}S_3$ 1656.6880; found 1656.6834.

N$^{\alpha}$-(4-methoxycarbonylbenzyl)-*N*-Cbz-L-tryptophan methyl ester (9). To a solution containing Cbz-L-tryptophan methyl ester **8** (100 mg, 0.28 mmol) in dry acetonitrile (1 mL) at 0 ºC (ice-bath), Cs_2CO_3 (184 mg, 0.56 mmol) was added and the mixture was stirred at this temperature for 15 minutes. Then a solution of 4-methoxycarbonylbenzylbromide (128.26 mg, 0.56 mmol) in DMF (1 mL) was added dropwise and the reaction mixture was stirred at 80 ºC overnight and treated as described for **7** to afford 88 mg (62%) of **9** as an amorphous solid of cream color. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.95 (d, *J* = 7.9 Hz, 2H, Ar), 7.54 (d, *J* = 7.9 Hz, 1H, Ar), 7.34 (m, 5H, Ar), 7.17 (m, 2H, Ar), 7.17 (m, 2H, Ar), 7.09 (m, 3H, Ar), 6.89 (s, 1H, Ar), 5.36 (d, *J* = 8.2 Hz, 1H, NHCO), 5.30 (s, 2H, CH_2Ph), 5.12 (m, 2H, CH_2Ph), 4.74 (m, 1H, *α*-CHTrp), 3.89 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.33 (m, 2H, *β*-CH_2Trp). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 172.4, 166.8, 155.8, 142.7, 136.6, 130.2, 129.7, 128.6, 128.5, 128.3, 128.3, 126.9, 126.6, 122.4, 119.8, 119.1, 109.8, 67.0, 54.8, 52.5, 52.3, 49.8, 28.1. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 6.238 min. HRMS (ESI-) m/z: calculated for $C_{29}H_{28}N_2O_6$ 500.1947; found 500.1952.

N$^{\alpha}$-(4-fluorobenzyl)-*N*-Cbz-L-tryptophan methyl ester (10). Following the above mentioned procedure, Cbz-L-tryptophan methyl ester **8** (100 mg, 0.28 mmol), Cs_2CO_3

(184 mg, 0.56 mmol) and 4-fluorobenzyl bromide (106 mg, 70 µL, 0.56 mmol) afforded 130 mg (70%) of **10** as an amorphous solid of cream color that was used inmediately for deprotection.

General procedure for the deprotection of the methyl esters. Synthesis of AL437, AL484, AL544, AL531, AL441 and AL442. To a solution containing the corresponding methyl ester derivative (1.0 mmol) in THF (10 mL) at 0 ºC (ice-bath), a solution of $LiOH{\cdot}H_2O$ (2 eq for each methyl ester group) in water (2 mL) was added, and the mixture was stirred at room temperature overnight. Then 1 N hydrochloric acid aqueous solution was added to reach pH = 2, and volatiles were evaporated to dryness. The residue was dissolved in ethyl acetate (15 mL) and washed with H_2O (3 x 10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The compounds were precipitated using a mixture of ethyl acetate and diethyl ether to afford the pure deprotected derivatives.

Trp(PS)-OH trimer (AL437). Following the general procedure for the deprotection of methyl esters, the methyl ester derivative **2** (100 mg, 0.08 mmol) in THF (8 mL) at 0 ºC (ice-bath), was treated with a solution of $LiOH{\cdot}H_2O$ (21 mg, 0.5 mmol) in water (2 mL) to afford 87.2 mg (90 %) of **AL437** as a solid of cream color. m.p 147-149 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.42 (s, 3H, NH-Trp), 8.21 (d, J = 8.1 Hz, 3H, Ar), 7.62 (d, J = 8.1 Hz, 3H, Ar), 7.27 – 7.18 (m, 8H, Ar), 7.16 – 7.04 (m, 5H, Ar), 7.01 (m, 8H, Ar), 4.43 (m, 3H, α-CHTrp), 3.25 (dd, J = 14.0 Hz, 7.5 Hz, 3H, β-CH_2Trp), 3.08 (dd, J = 14.0, 7.5 Hz, 3H, β-CH_2Trp), 1.92 – 1.66 (m, 12H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 173.1, 170.4, 137.2, 137.1, 129.3, 127.4, 126.3, 125.8, 122.8, 122.3, 119.3, 119.1, 118.1, 111.3, 93.0, 53.3, 30.6, 30.5, 29.3, 27.3. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 9.651 min. HRMS (ESI-) m/z: calculated for $C_{61}H_{57}N_7O_{11}S_3$ 1159.3278; found 1159.3269.

NMe-Trp(*p*NPS)-OH trimer (AL484). Following the general procedure for the deprotection of methyl esters, the methyl ester derivative **4** (116 mg, 0.08 mmol) in THF (6 mL) at 0 ºC (ice-bath) was treated with a solution of $LiOH{\cdot}H_2O$ (21 mg, 0.5 mmol) in

water (2 mL) to afford 101.21 mg (90%) of **AL484** as an amorphous yellow solid. m.p 160-163 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.18 (m, 3H, NHCO), 8.08 – 8.02 (m, 6H, Ar), 7.75 (d, J = 8.0 Hz, 3H, Ar), 7.48 (d, J = 8.0 Hz, 3H, Ar), 7.25 (t, J = 7.7 Hz, 3H, Ar), 7.12 (m, 9H, Ar), 4.44 (m, 3H, α-CHTrp), 3.62 (s, 9H, N-CH_3), 3.29 (dd, J = 14.0, 7.2 Hz, 3H, β-CH_2Trp), 3.15 (dd, J = 14.0, 7.2 Hz, 3H, β-CH_2Trp), 1.83 (d, J = 9.0 Hz, 12H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 172.9, 170.3, 146.6, 145.1, 138.1, 126.6, 125.7, 124.4, 123.5, 122.1, 120.1, 119.8, 119.6, 110.5, 93.2, 53.4, 30.3, 29.9, 29.4, 27.7. HRMS (ESI$^+$) m/z*:* calculated for $C_{64}H_{60}N_{10}O_{17}S_3$ 1336.3300; found 1336.3295.

NMe-L-Trp-OH trimer (AL544). Following the general procedure for the deprotection of methyl esters, the methyl ester derivative **5** (50 mg, 0.05 mmol) in THF (5 mL) was treated with a solution of LiOH·H_2O (14 mg, 0.33 mmol) in water (2 mL) to afford 37 mg (78%) of **AL544** as an amorphous white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.22 (d, J = 7.8 Hz, 3H, NHCO), 7.53 (dt, J = 8.1, 1.0 Hz, 3H, Ar), 7.33 (dt, J = 8.1, 1.0 Hz, 3H, Ar), 7.10 (t, J = 7.6 Hz, 3H, Ar), 7.08 (s, 3H, Ar), 7.00 (t, J = 7.0 Hz, 3H, Ar), 4.44 (m, 3H, α-CHTrp), 3.68 (s, 9H, CH_3), 3.14 (dd, J = 14.6, 5.6 Hz, 3H, β-CH_2Trp), 2.98 (dd, J = 14.6, 5.6 Hz, 3H, β-CH_2Trp), 2.04 – 1.95 (m, 12H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ*:* 173.3, 170.6, 136.5, 127.9, 127.5, 121.0, 118.5, 118.4, 109.5, 109.2, 93.2, 53.1, 32.2, 30.6, 29.3, 27.0. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 8.076 min. HRMS (ESI-) m/z: calculated for $C_{46}H_{51}N_7O_{11}$ 877.3646; found 877.3655.

N$^{\alpha}$-(4-fluorobenzyl)-L-Trp-OH tetramer (AL531). Following the general procedure for the deprotection of methyl esters, the methyl ester derivative **7** (32.2 mg, 0.019 mmol) in THF (6 mL) was treated with a solution of LiOH·H_2O (6.52 mg, 0.15 mmol) in water (2.5 mL) to afford 24 mg (80 %) of **AL531** as an amorphous white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ*:* 8.24 (d, J = 7.7 Hz, 4H, NHCO), 7.54 (d, J = 7.9 Hz, 4H, Ar), 7.34 (d, J = 8.2 Hz, 4H, Ar), 7.25 (s, 4H, Ar), 7.16 (m, 8H, Ar), 7.05 (m, 12H, Ar), 6.97 (t, J = 7.3 Hz, 4H, Ar), 5.29 (s, 8H, CH_2Bn), 4.50 (m, 4H, α-CHTrp), 3.38 (m, 8H, OCH_2), 3.14 (m, 12H, OCH_2, β-CH_2Trp), 3.00 (dd, J = 14.5, 7.9 Hz, 4H, β-CH_2Trp), 2.24 (m, 8H, CH_2). ^{13}C

NMR (101 MHz, $CDCl_3$) δ 173.6, 170.0, 162.3, 160.3, 135.8, 134.5, 134.5, 128.9, 128.9, 128.0, 127.3, 121.2, 118.7, 115.3, 115.1, 110.3, 110.0, 68.9, 67.0, 53.4, 48.1, 44.7, 35.8, 27.3. HRMS (ESI^+) m/z*:* calculated for $C_{89}H_{88}F_4N_8O_{16}$ 1600.6254; found 1600.6334.

N^{α}-(4-carboxybenzyl)-*N*-Cbz-Trp-OH (AL441). Following the general procedure for the deprotection of methyl esters, the methyl ester derivative **9** (60 mg, 0.12 mmol) in THF (6 mL) was treated with a solution of $LiOH{\cdot}H_2O$ (10 mg, 0.25 mmol) in water (2 mL) to afford 40 mg (71%) of **AL441** as an amorphous white solid. ^1H NMR (400 MHz, $CDCl_3$) *δ*: 7.88 (dd, *J* = 8.3, 3.9 Hz, 2H, Ar), 7.58 (d, *J* = 8.5 Hz, 1H, Ar), 7.39 (m, 5H, Ar), 7.16 (m, 2H, Ar), 7.08 (qd, *J* = 4.9, 2.4 Hz, 1H, Ar), 7.03 – 6.97 (m, 1H, Ar), 6.84 (t, *J* = 7.4 Hz, 2H, Ar), 5.52 (d, *J* = 8.5 Hz, 1H, NCH_2-Ph), 5.43 (m, 1H, NHCO), 5.32 – 5.28 (m, 1H, OCH_2-Ph), 5.28 – 5.24 (m, 1H, OCH_2-Ph), 5.14 (d, *J* = 8.5 Hz, 1H, NCH_2-Ph), 4.95 (m, 1H, α-CHTrp), 3.48 – 3.40 (m, 2H, β-CH_2Trp). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 178.6, 172.3, 155.8, 144.6, 136.5, 131.0, 128.7, 128.4, 128.3, 127.7, 127.2, 125.3, 122.5, 120.1, 119.6, 109.9, 109.4, 67.2, 56.0, 48.9, 30.5. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 5.438 min. HRMS (ESI^+) m/z*:* calculated for $C_{27}H_{24}N_2O_6$ 472.1634; found 472.1594.

N^{α}-(4-fluorobenzyl)-*N*-Cbz-Trp-OH (AL442). Following the general procedure for the deprotection of methyl esters, the methyl ester derivative **10** (57 mg, 0.12 mmol) in THF (6 mL) was treated with a solution of $LiOH{\cdot}H_2O$ (10 mg, 0.25 mmol) in water (1 mL) to afford 44 mg (80%) of **AL442** as an amorphous white solid**.** ^1H NMR (400 MHz, $CDCl_3$-*d*) *δ*: 7.57 (d, *J* = 7.9 Hz, 1H, Ar), 7.32 (m, 3H, Ar), 7.18 (m, 3H, Ar), 7.07 (t, *J* = 7.0 Hz, 1H, Ar), 7.00 – 6.87 (m, 6H, Ar), 5.28 (d, *J* = 8.0 Hz, 1H, CH_2Ph), 5.17 (s, 2H, CH_2Ph), 5.09 (q, *J* = 12.3 Hz, 2H, CH_2Ph), 4.72 (m, 1H, *α*-CHTrp), 3.38 (dd, *J* = 14.8, 5.4 Hz, 1H, *β*-CH_2Trp), 3.29 (dd, J = 14.8, 5.4 Hz, 1H, *β*-CH_2Trp). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8, 162.3 ($J_{C\text{-}F}$ = 247 Hz), 156.1, 136.5, 136.2, 133.2, 128.7, 128.4, 128.4, 128.3, 127.1, 122.3, 119.8, 119.1, 115.8 ($J_{C\text{-}F}$ = 21.5 Hz) 109.9, 109.1, 77.2, 67.2, 54.7, 49.4,

27.8. HPLC [gradient: H_2O:MeCN, 10-100% of A in 10 min]: 6.612 min. HRMS (ESI^+) m/z*:* calculated for $C_{26}H_{23}FN_2O_4$ 446.1642; found 446.1655.

References

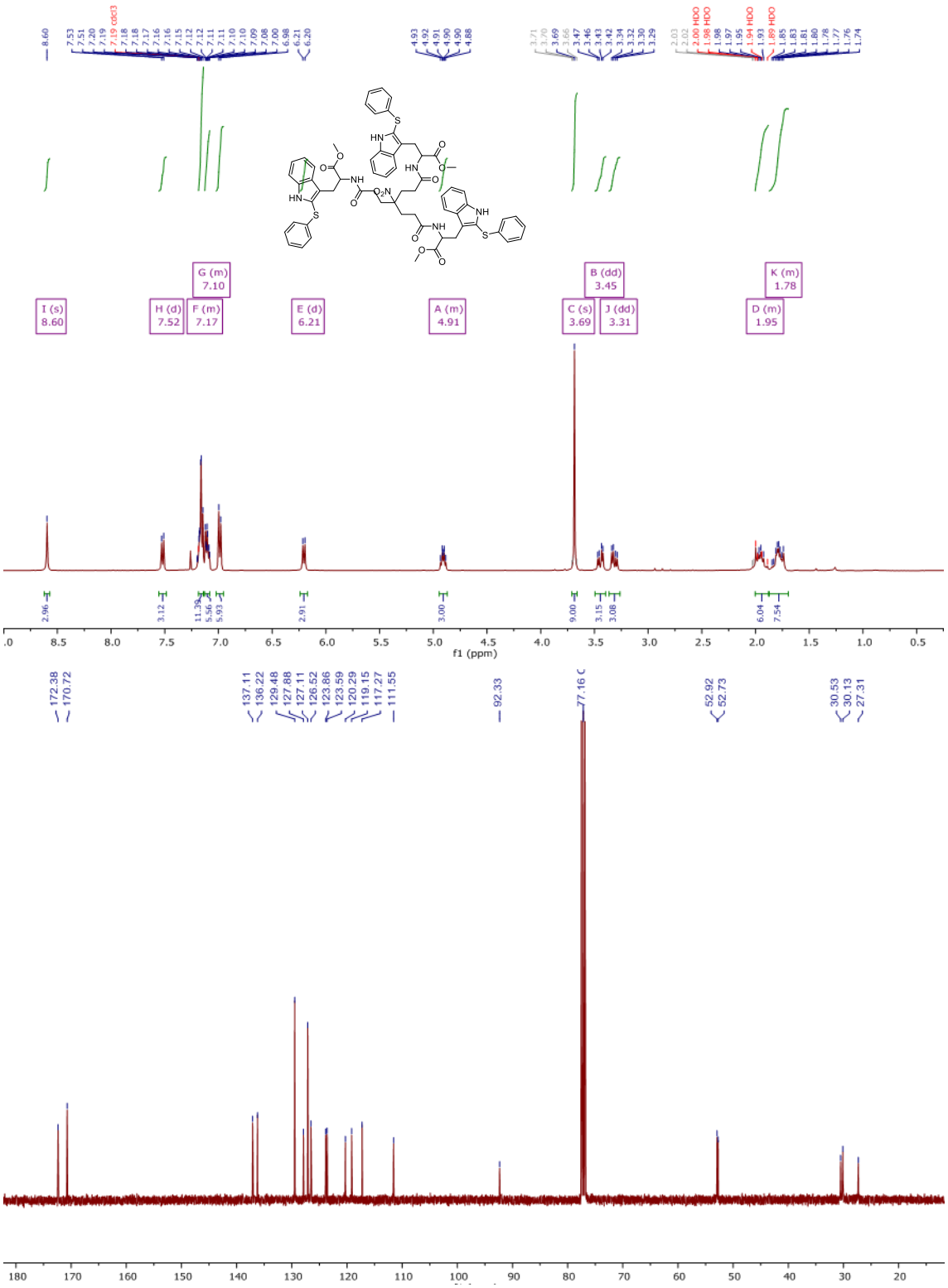
1.-Mueller WH, Butter PE. 1968. Factors influencing the nature of the episulfonium ion in sulfenyl chloride addition to terminal olefins. J Am Chem Soc 90, 2075-2081.

2.- Raban M., Jones FB Jr. 1971. Stereochemistry at trivalent nitrogen. XI. Effect of polar substituents on the barrier to rotation about the sulfenyl sulfur-nitrogen bond in N-alkyl-N-arenesulfonylarenesulfenamides. J Am Chem Soc, 93, 2692- 2699.

3.- Martínez-Gualda B, Sun L, Martí-Marí O, Noppen, S, Abdelnabi R., Bator C.M., Quesada E, Delang L, Mirabelli C, Lee H, Schols D, Hafenstein S, Camarasa M.J, Gago F, San-Félix A. J. Med. Chem 2019 in press, PubMed ID: 3180904, DOI: 10.1021/acs.jmedchem.9b01737.

1.3. ^{1}HNMR and ^{13}CNMR of the synthesized compounds

Trimer 2



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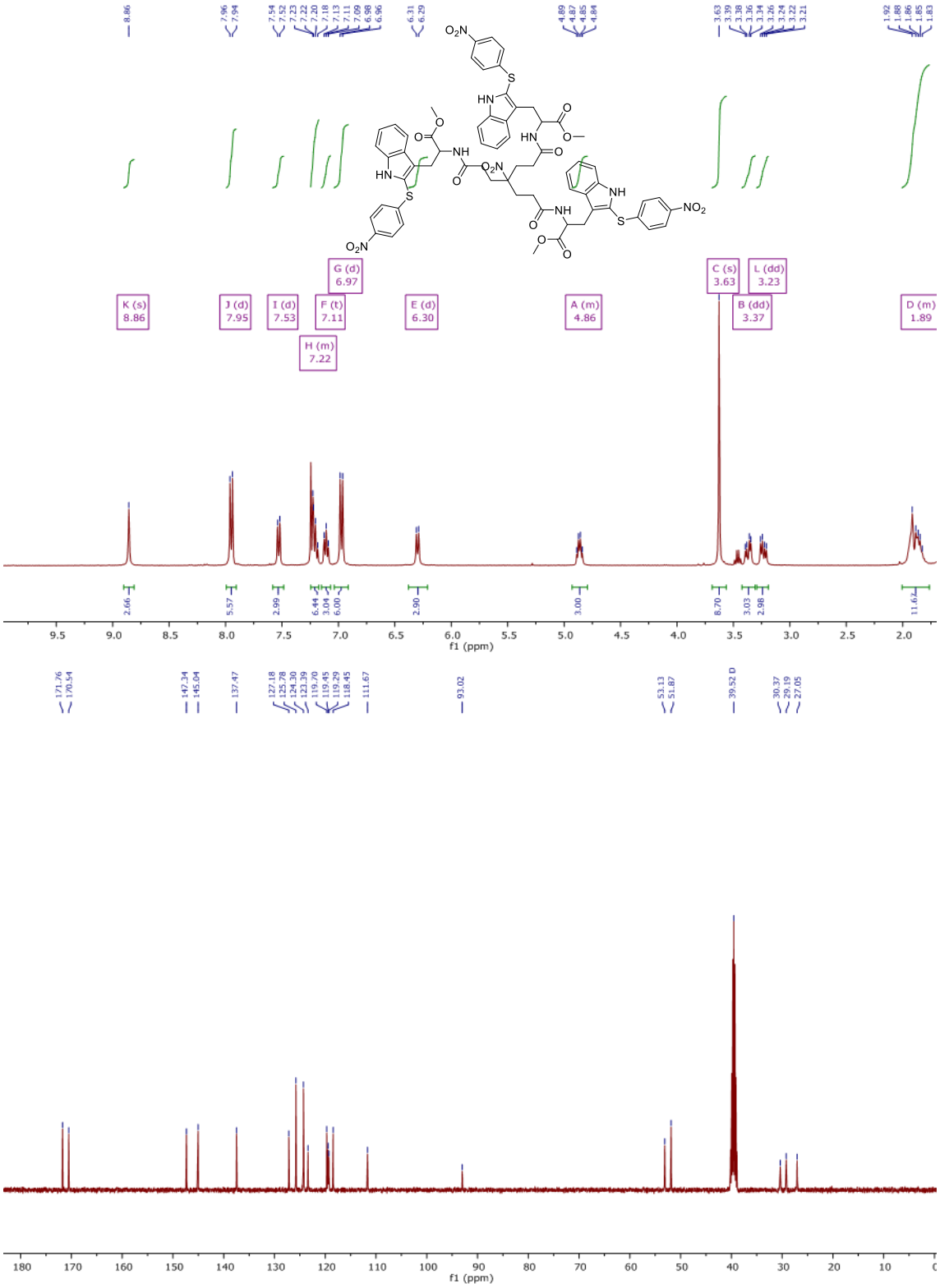
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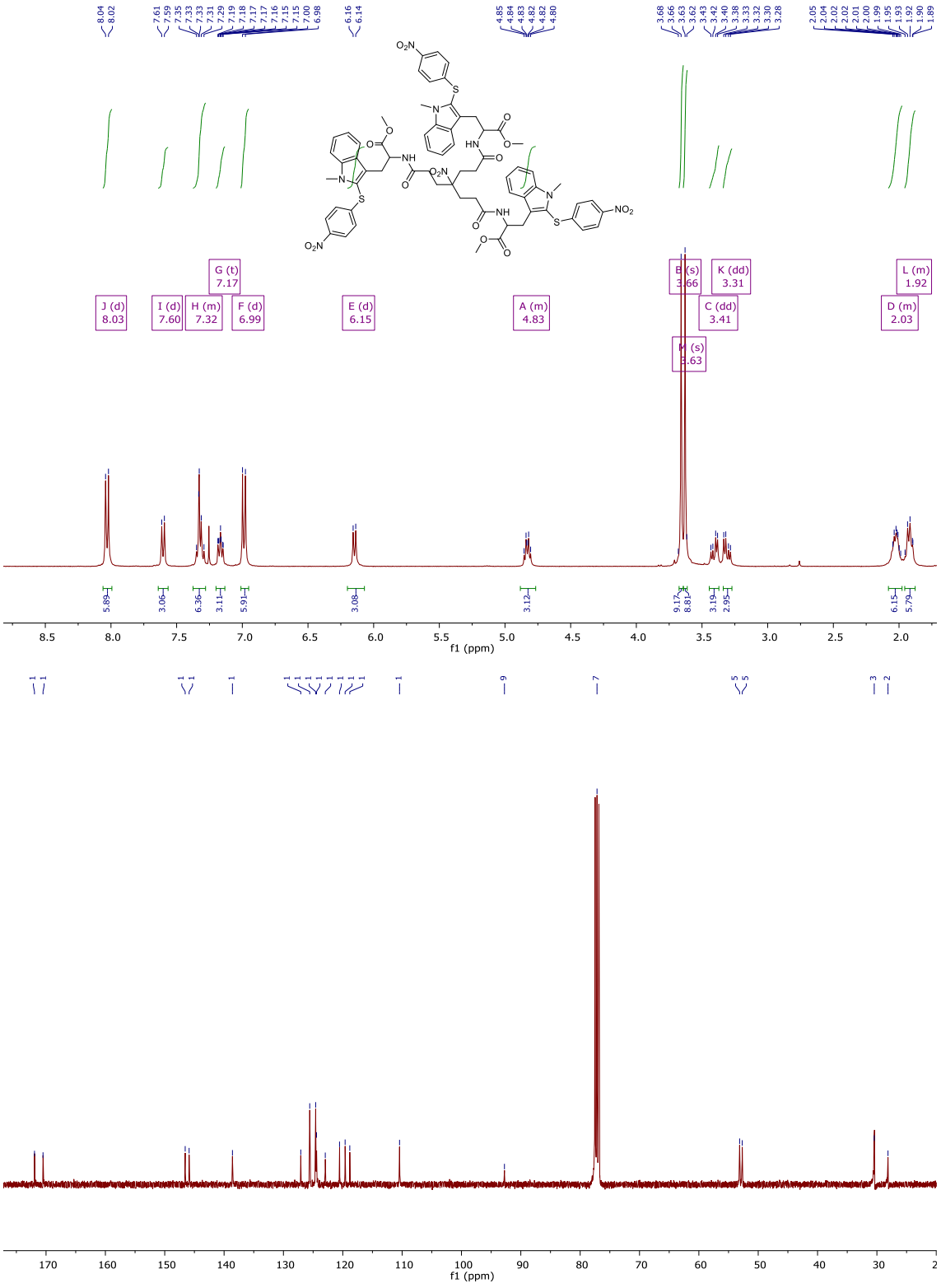
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M (s) 3.63
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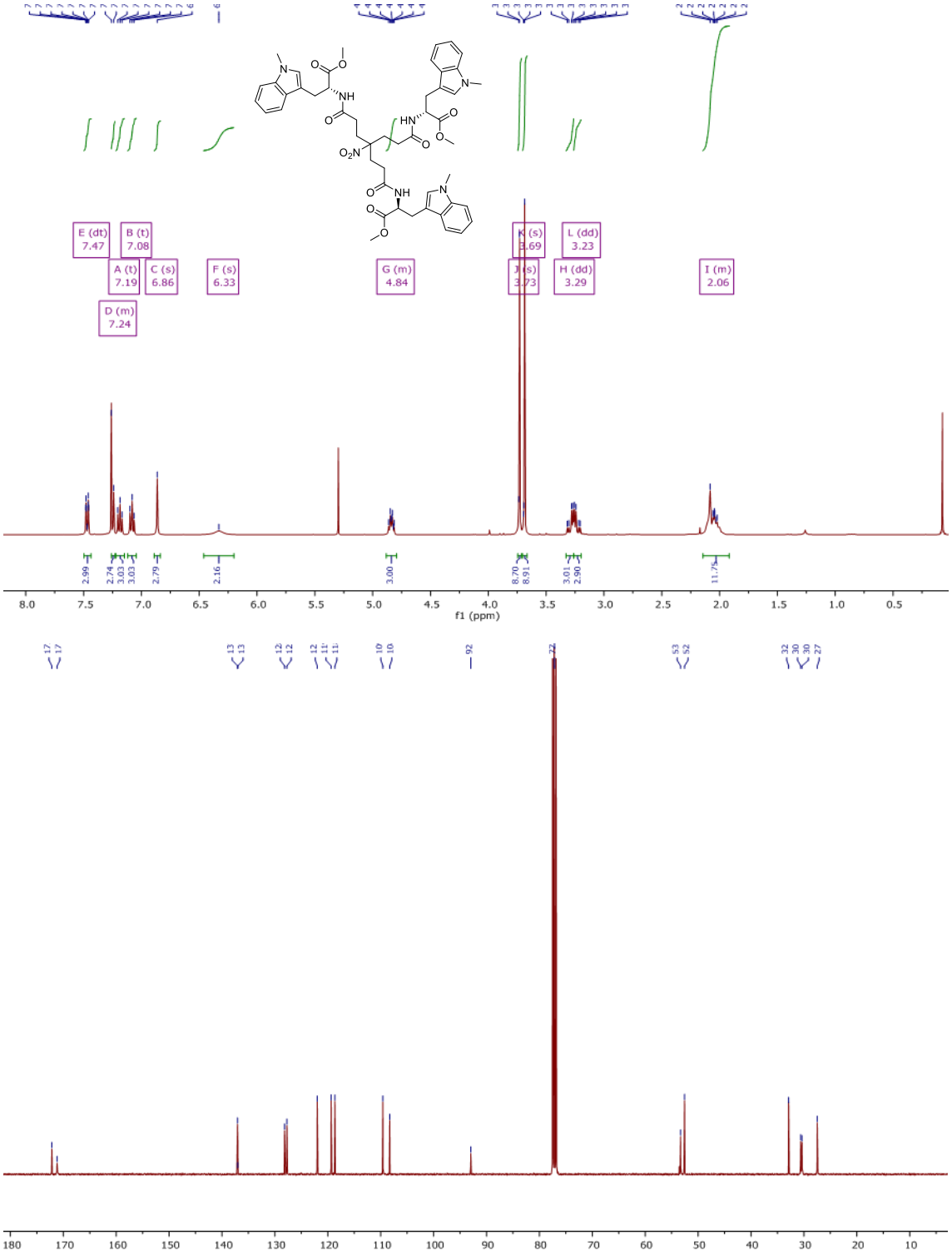
D (m) 2.03
f1 (ppm)

Trimer 5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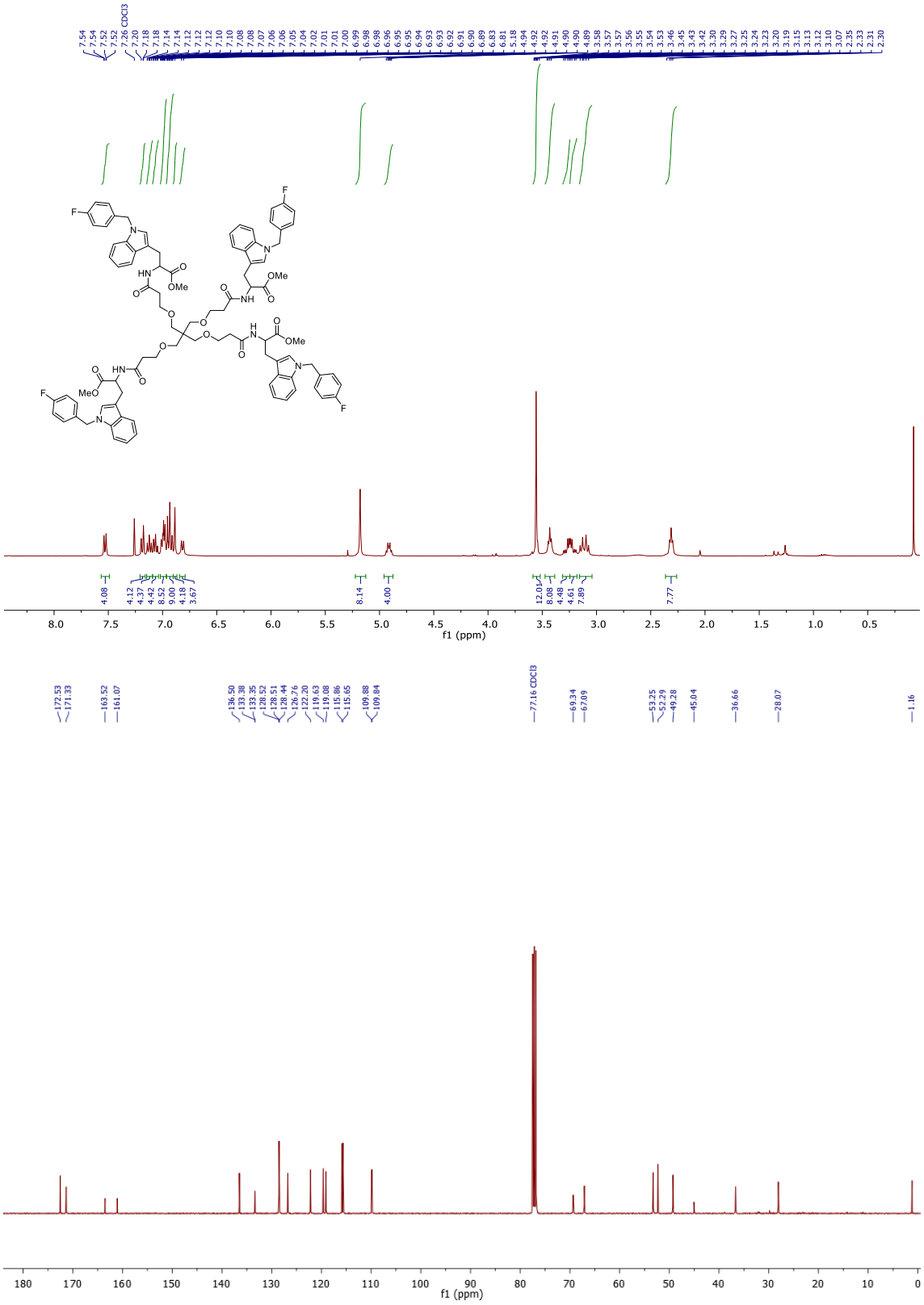
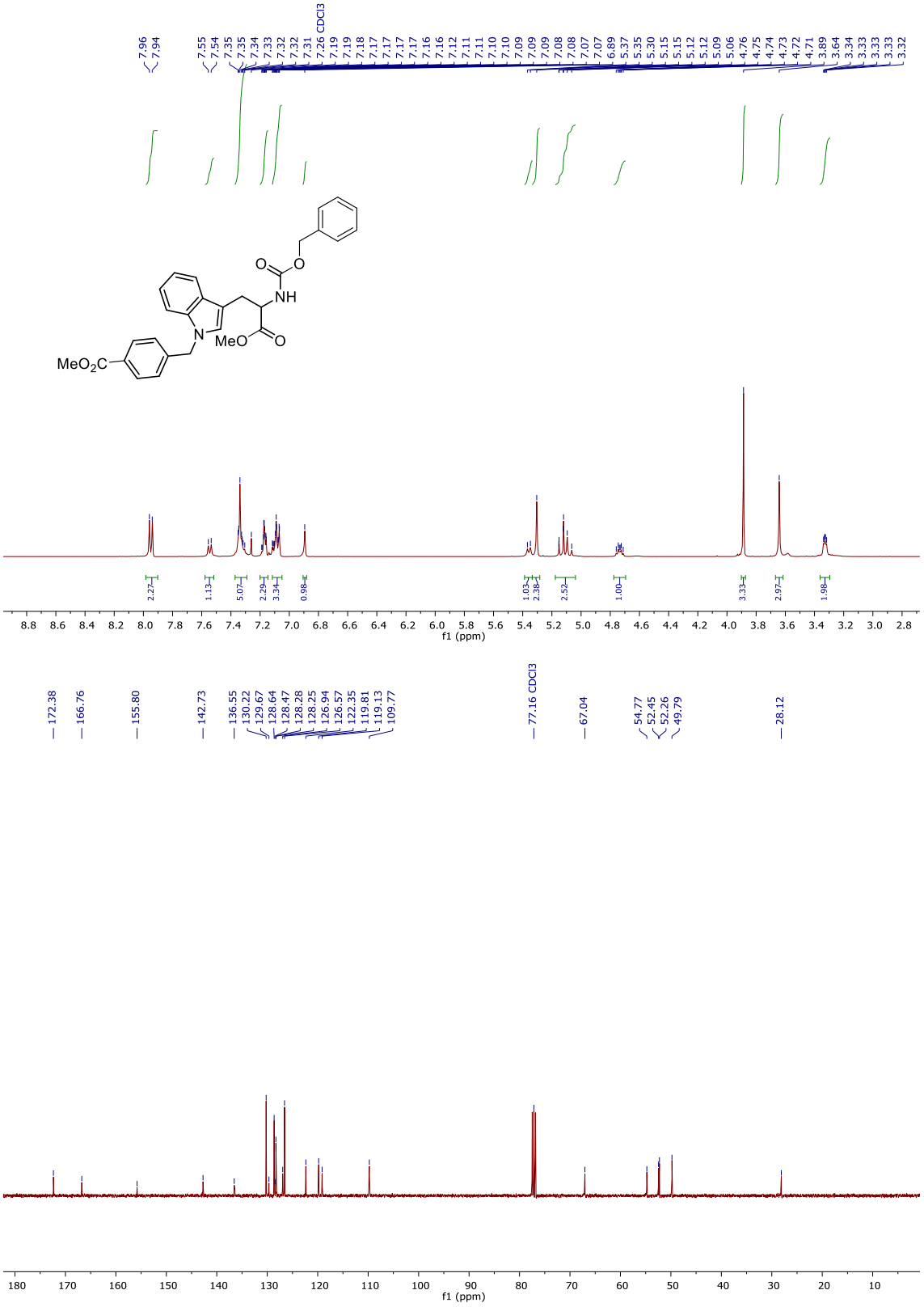
f1 (ppm)

Tetramer 7



Monomer 9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7.94
7.55
7.54
7.35
7.35
7.34
7.33
7.32
7.32
7.31
7.26 CDCl3
7.19
7.19
7.18
7.17
7.17
7.17
7.17
7.16
7.16
7.12
7.11
7.11
7.10
7.10
7.09
7.09
7.09
7.08
7.08
7.07
7.07
6.89
5.37
5.35
5.30
5.15
5.15
5.12
5.12
5.09
5.06
4.76
4.75
4.74
4.73
4.72
4.71
3.89
3.64
3.34
3.33
3.33
3.33
3.32
O
O
NH
N
MeO
O
MeO2C
2.27
1.13
5.07
2.29
3.34
0.98
1.03
2.38
2.52
1.00
3.33
2.97
1.98
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155.80
142.73
136.55
130.22
129.67
128.64
128.47
128.28
128.25
126.94
126.57
122.35
119.81
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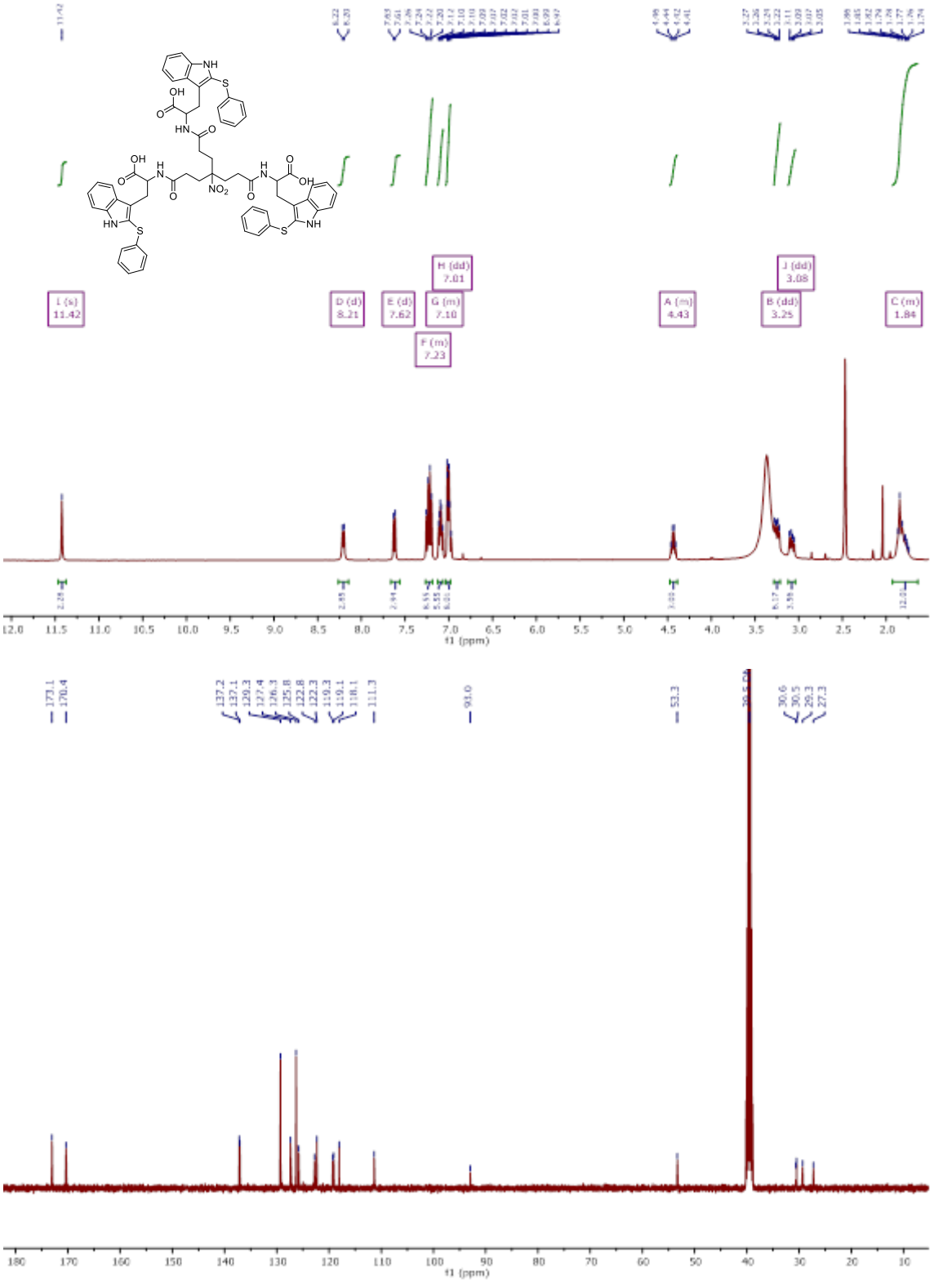
f1 (ppm)

AL437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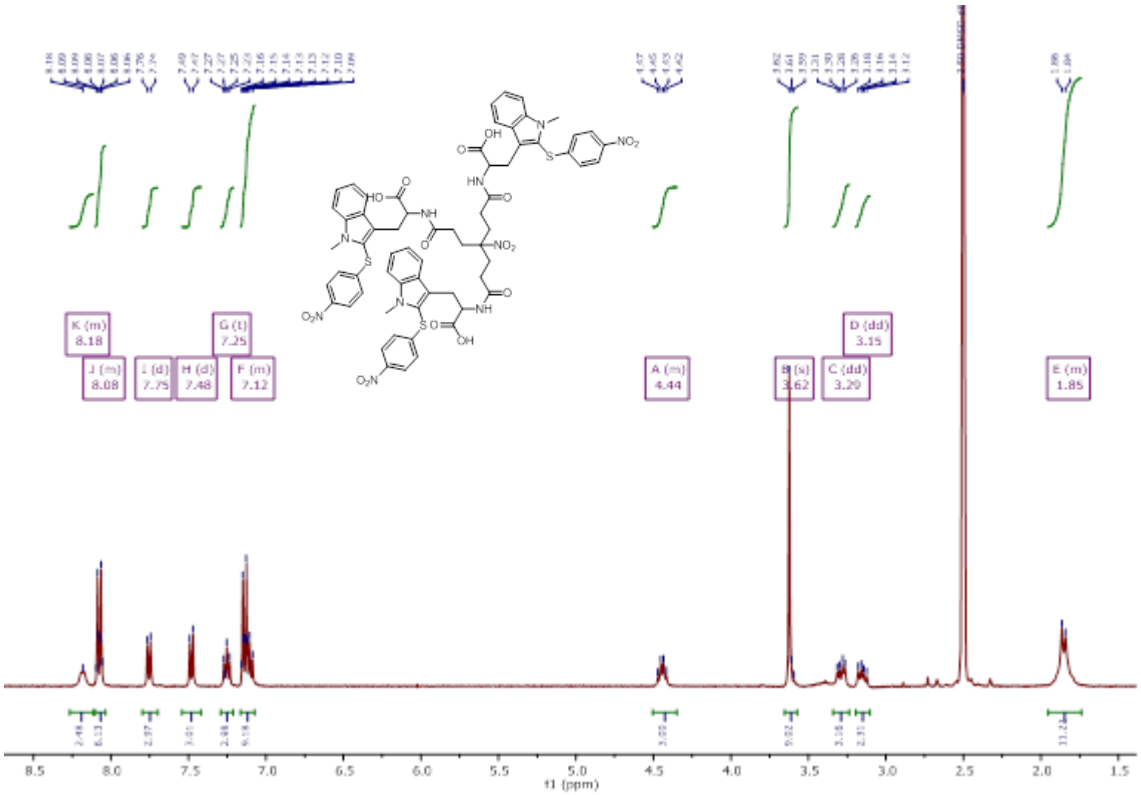
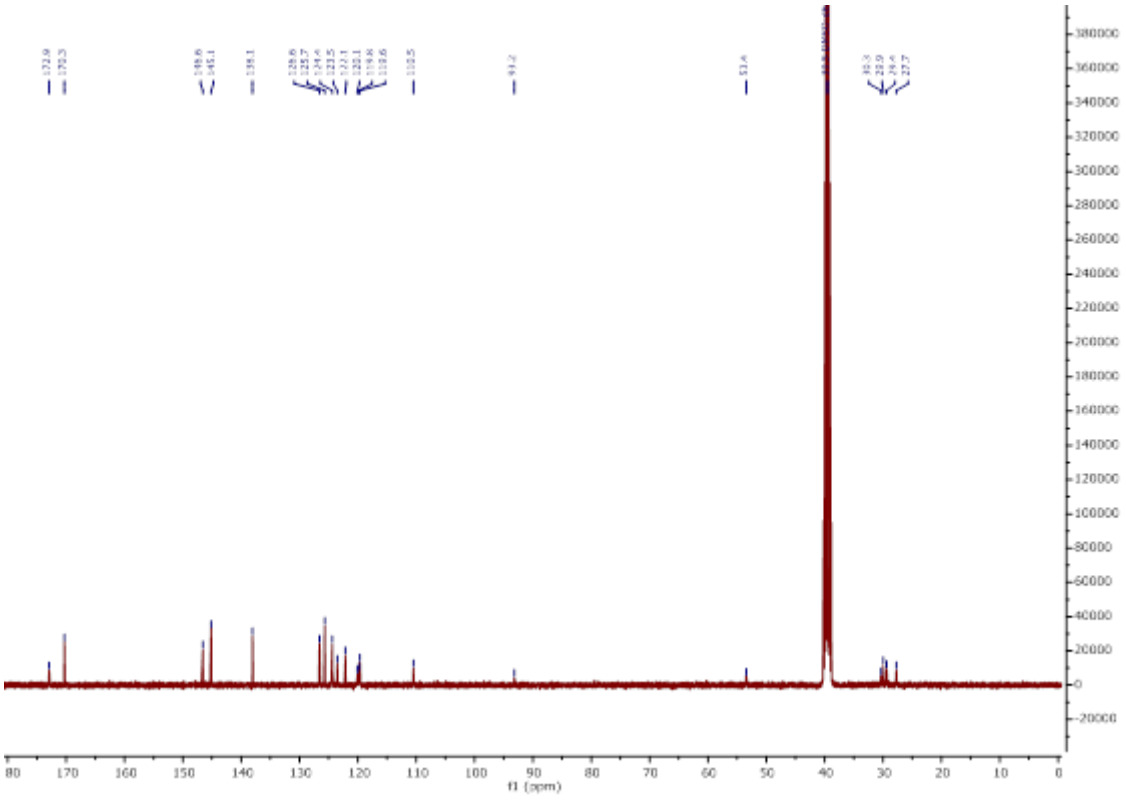
f1 (ppm)

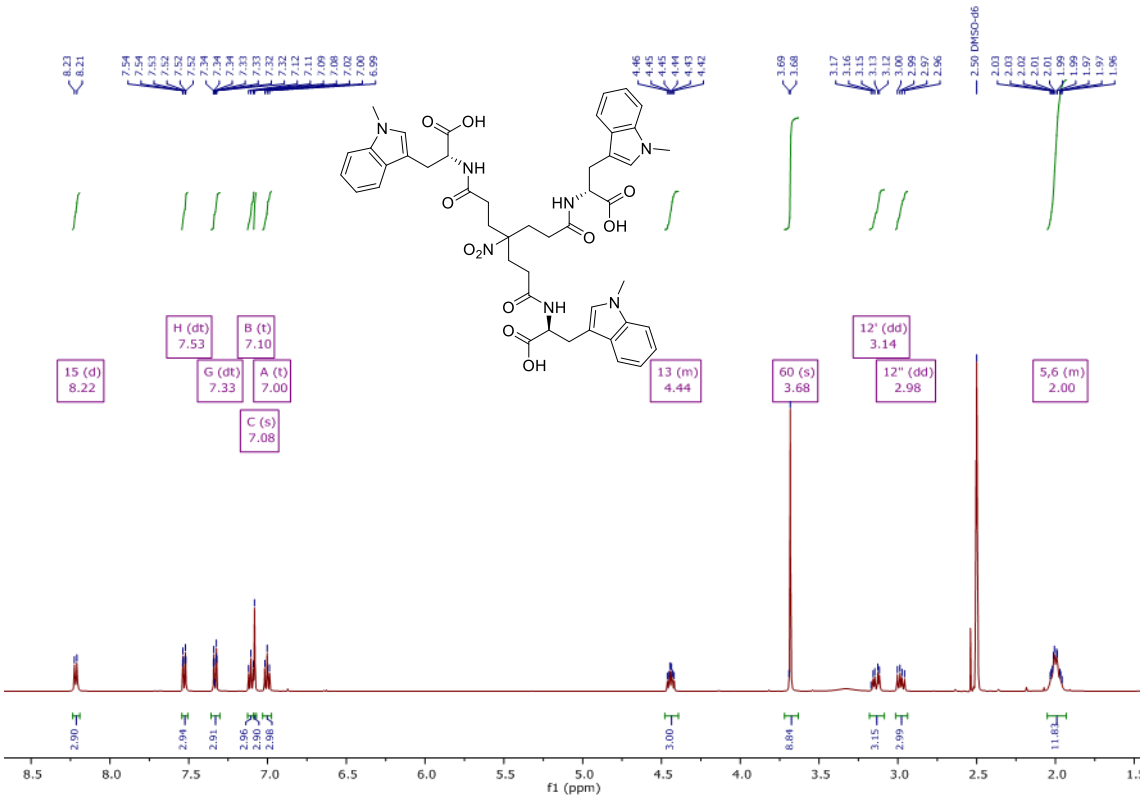
AL484



^{13}C NMR


f1 (ppm)

AL544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8.22
H (dt)
7.53
G (dt)
7.33
B (t)
7.10
A (t)
7.00
C (s)
7.08
13 (m)
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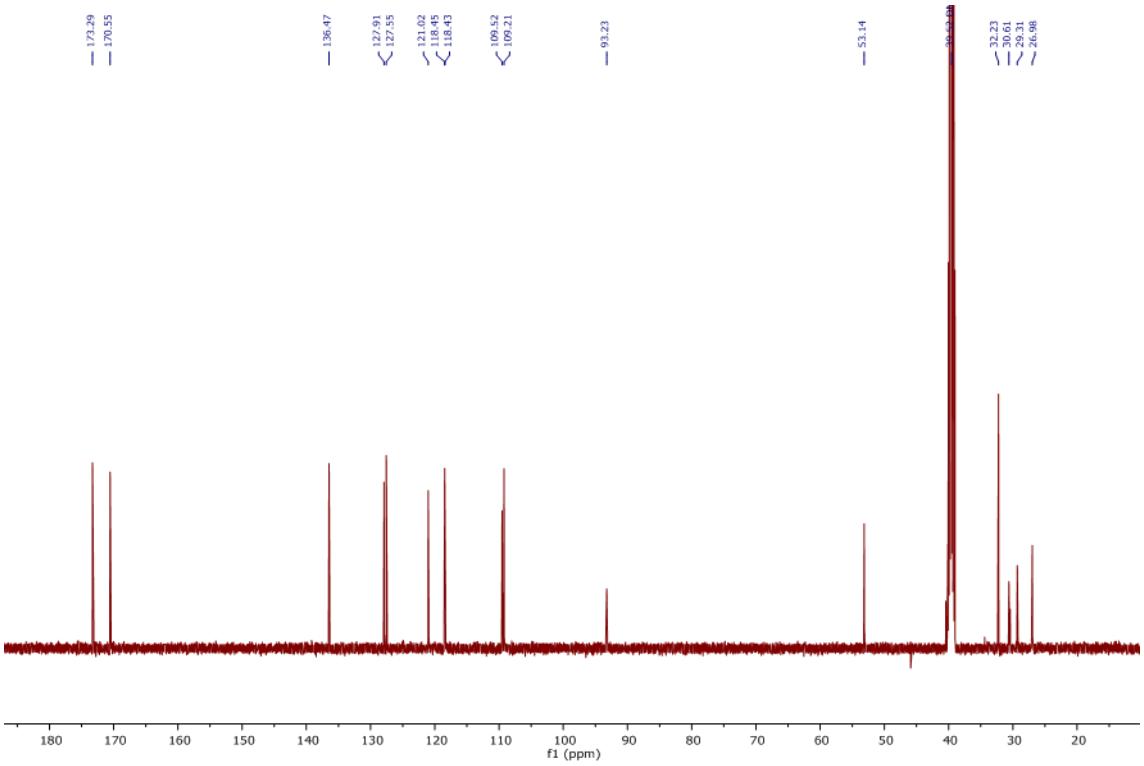
3.68
12' (dd)
3.14
12" (dd)
2.98
5,6 (m)
2.00
f1 (ppm)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127.55
121.02
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30.61
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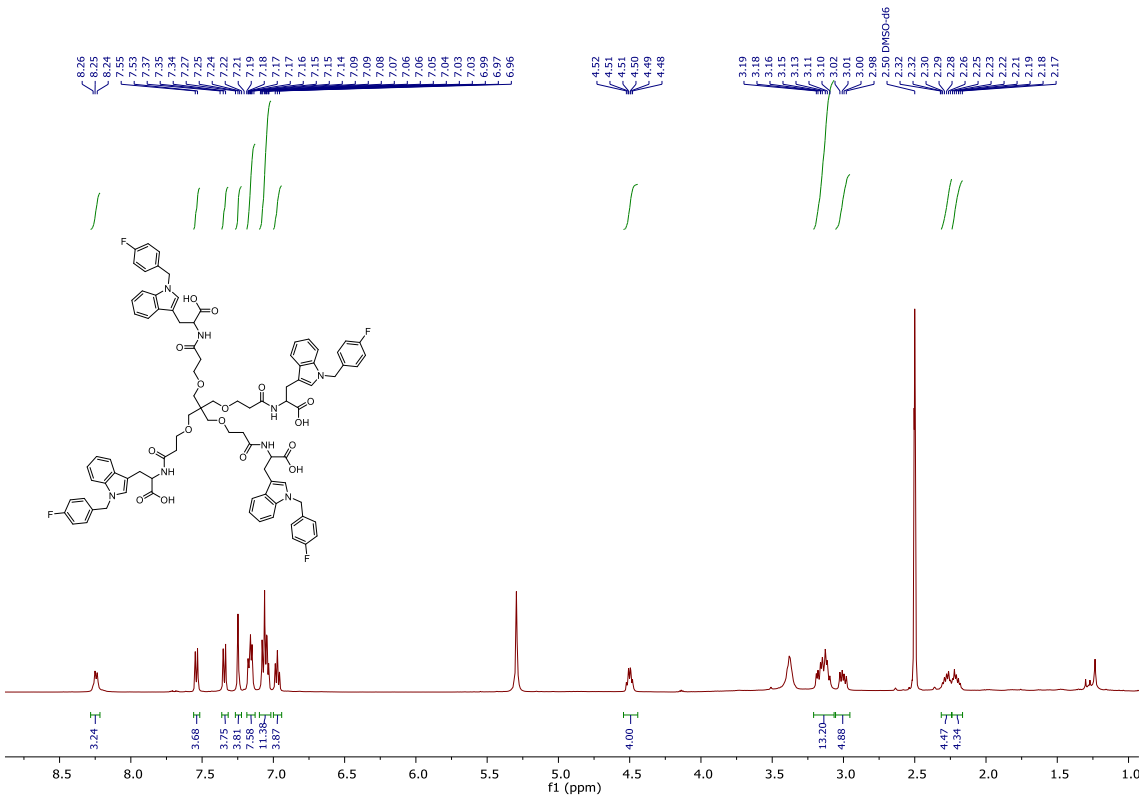
f1 (ppm)

AL531


DMSO-d6
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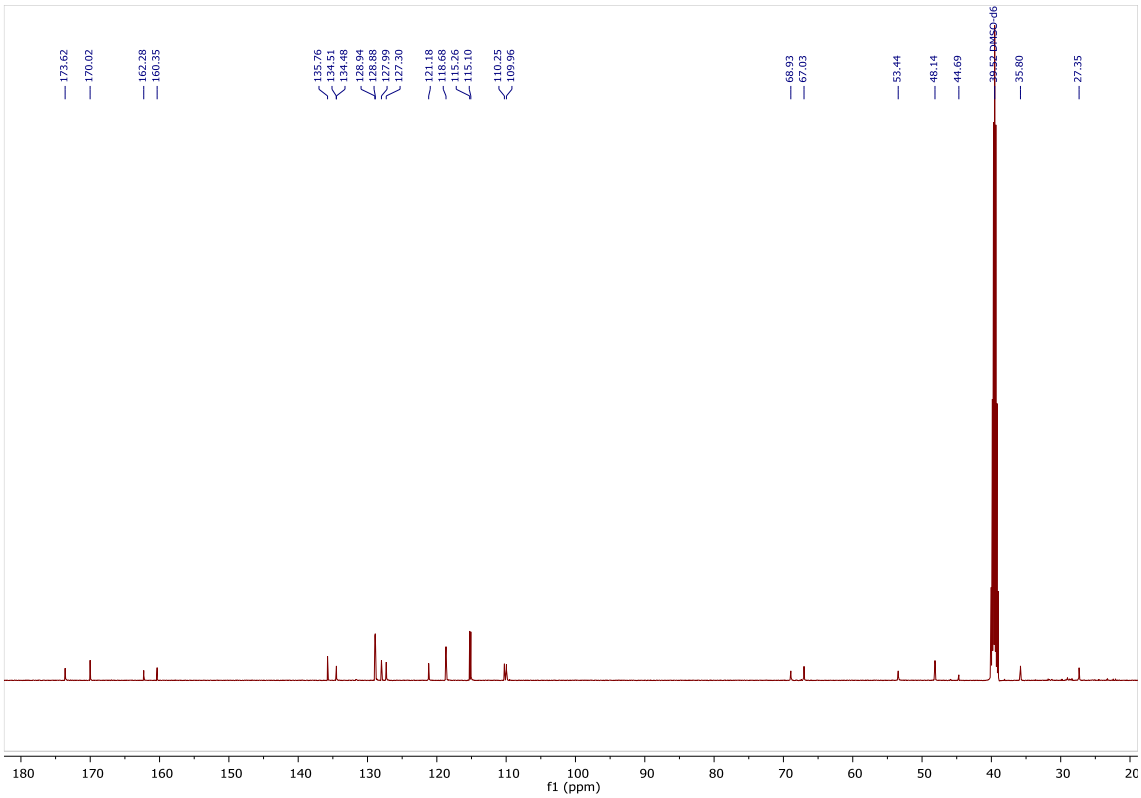
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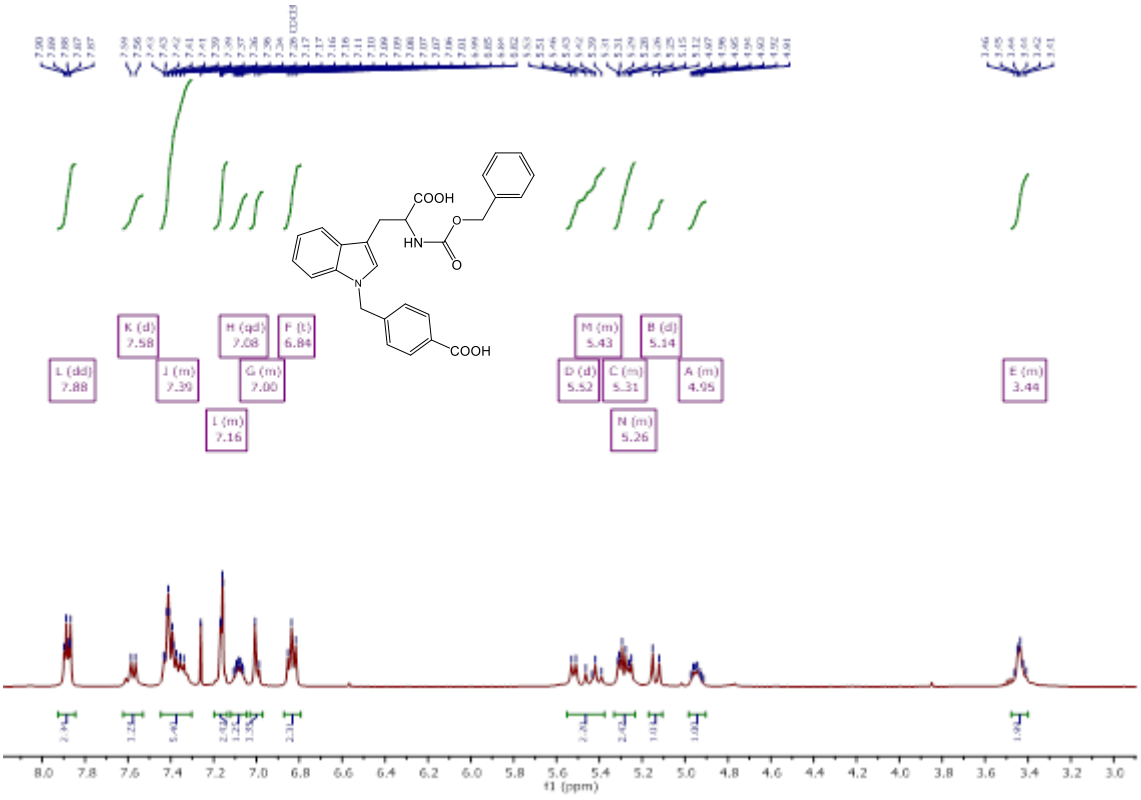
134.48
128.94
128.88
127.99
127.30
121.18
118.68
115.26
115.10
110.25
109.96
68.93
67.03
53.44
48.14
44.69
39.52 DMSO-d6
35.80
27.35
f1 (ppm)

AL441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O
O
N
COOH
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F (t) 6.84
L (dd) 7.88
J (m) 7.39
G (m) 7.00
I (m) 7.16
M (m) 5.43
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D (d) 5.52
C (m) 5.31
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N (m) 5.26
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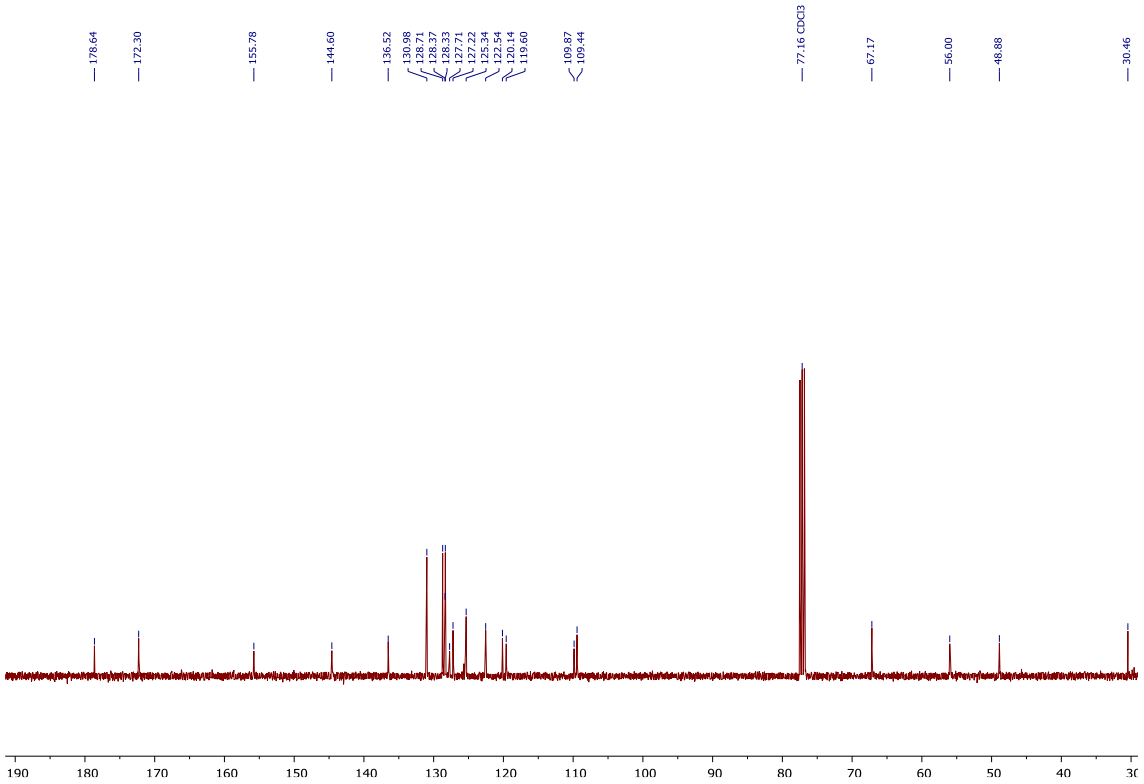
f1 (ppm)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130.98
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127.22
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109.44
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67.17
56.00
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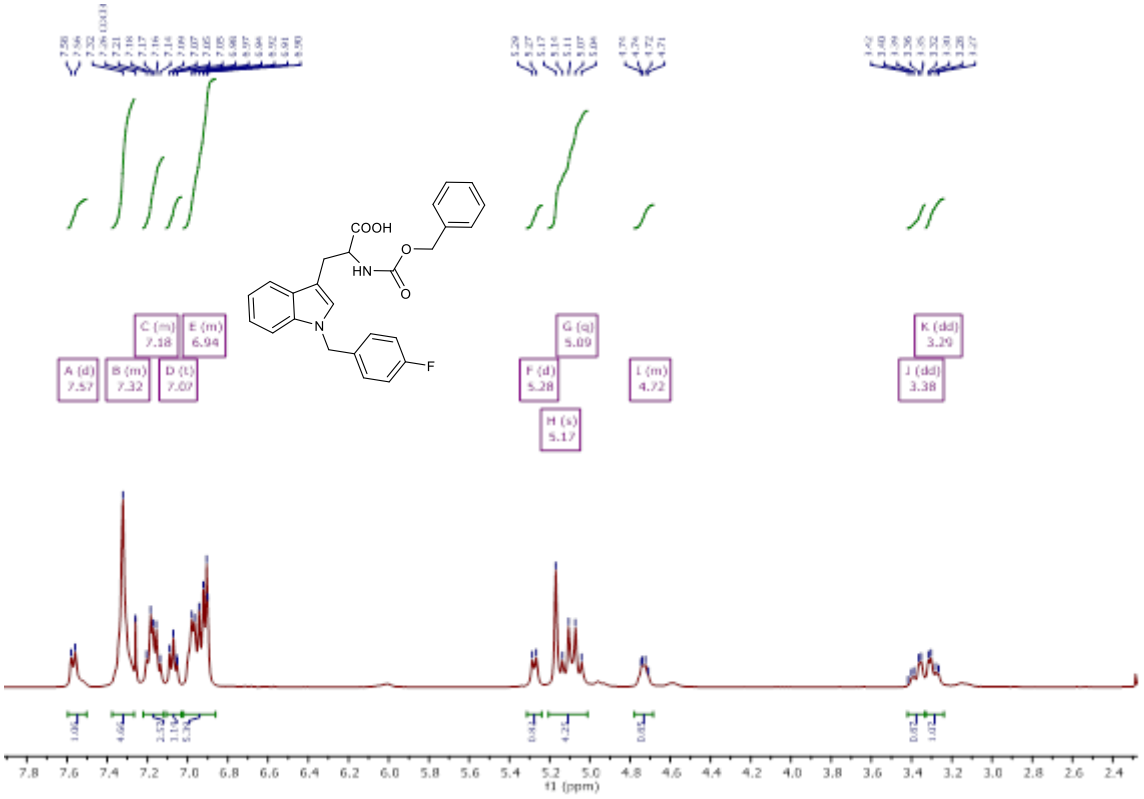
30.46

AL442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HN
O
O
N
F
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B (m) 7.32
C (m) 7.18
D (t) 7.07
E (m) 6.94
F (d) 5.28
G (q) 5.09
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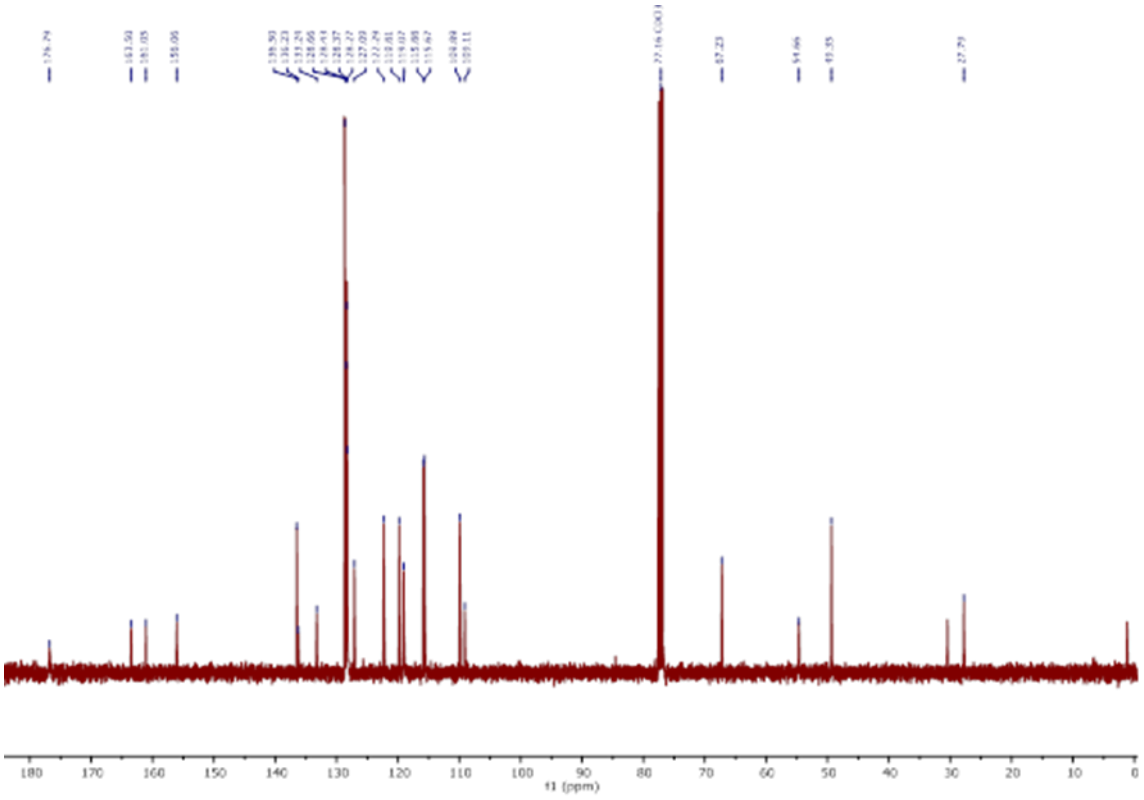
K (dd) 3.20
f1 (ppm)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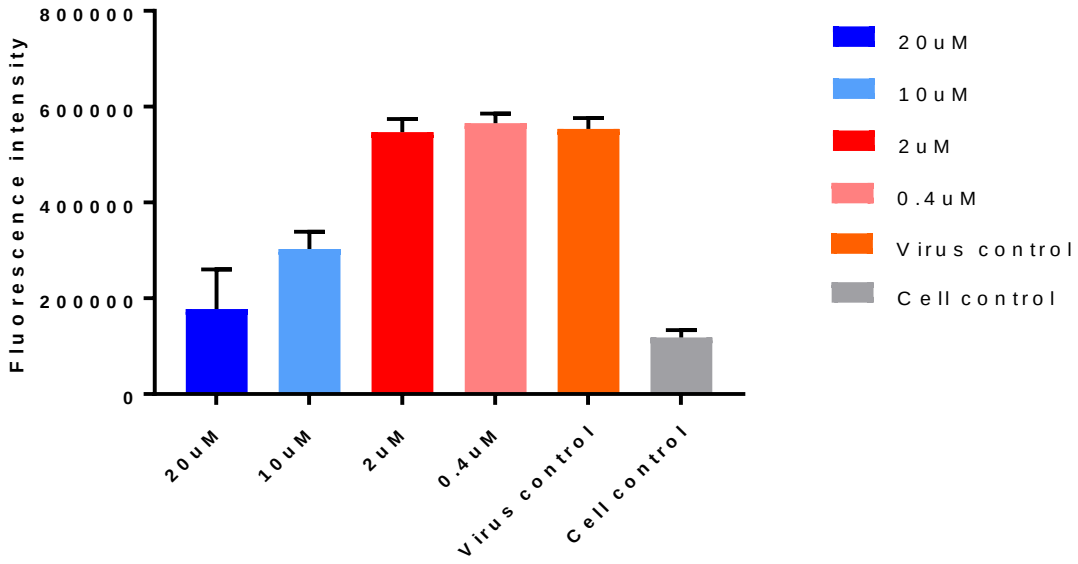

FigureS1: **Quantification data (total intensity) of immunofluorescence staining.** Different concentrations of AL440 were used and the fluorescence intensity signal is measured from 5 different spots/well. Images were processed by high content imaging (CellInsight CX5, Thermo Scientific) and the values are derived from 3 independent experiments. As shown in the Figure, the fluorescence intensity is decreased when the cells are treated with concentrations of AL440 higher than the EC_{50} values.