Electronic Supplementary Information (ESI)

Design and synthesis of multifunctional traceable dendrimers for visualizing drug delivery

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Table S1. Photophysical properties of free TIF, TIF conjugated traceable dendrimers, and linear counterparts.

Sr. No.	Name/ Compound No.	Absorption (nm)	Emission (nm)	Quantum[a] yield (%)
1.	TIF_Mono_Acet (4)	540	560	16
2.	TIF_6_Acet (7)	540	559	16
3.	TIF_6_OH (8)	540	560	17
4.	TIF_6_LA (9)	538	558	15
5.	TIF_12_OH (12)	540	560	7
6.	TIF_Mono_OH (13)	539	561	15

[a] Average of 5 readings.

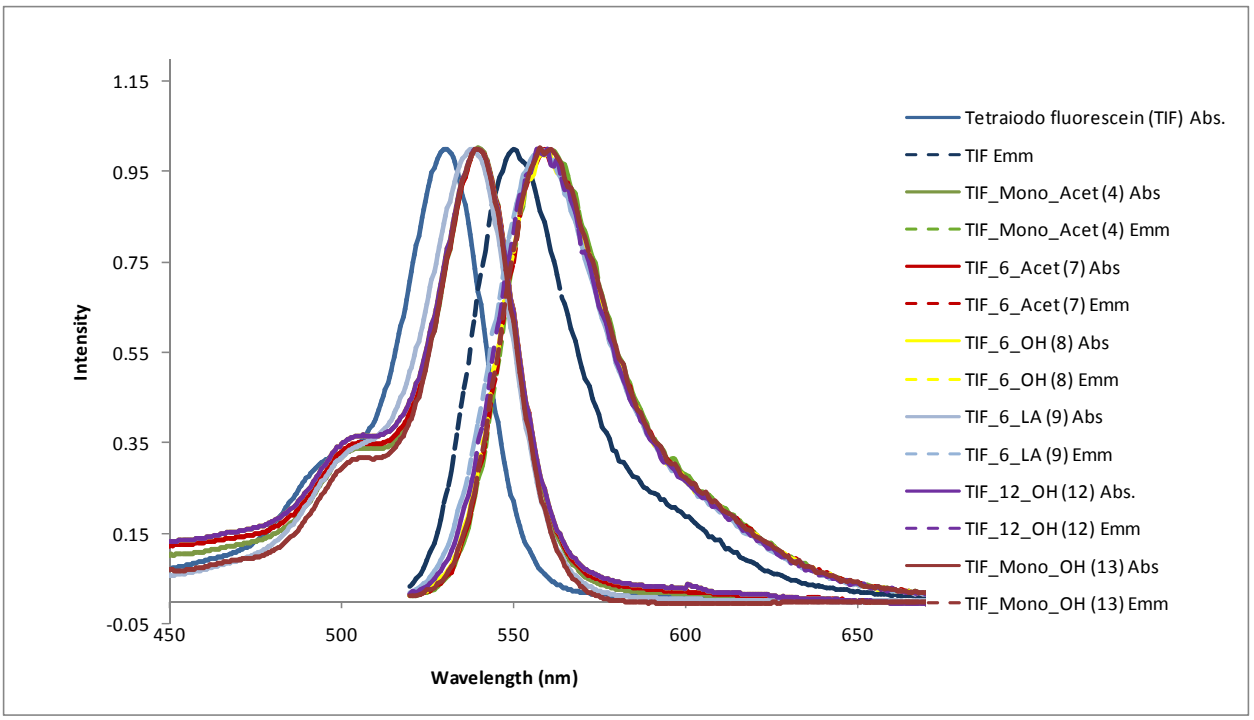


Figure S1. Absorption and emission spectra of intermediates, fluorescent dendrimers and linear counterparts.

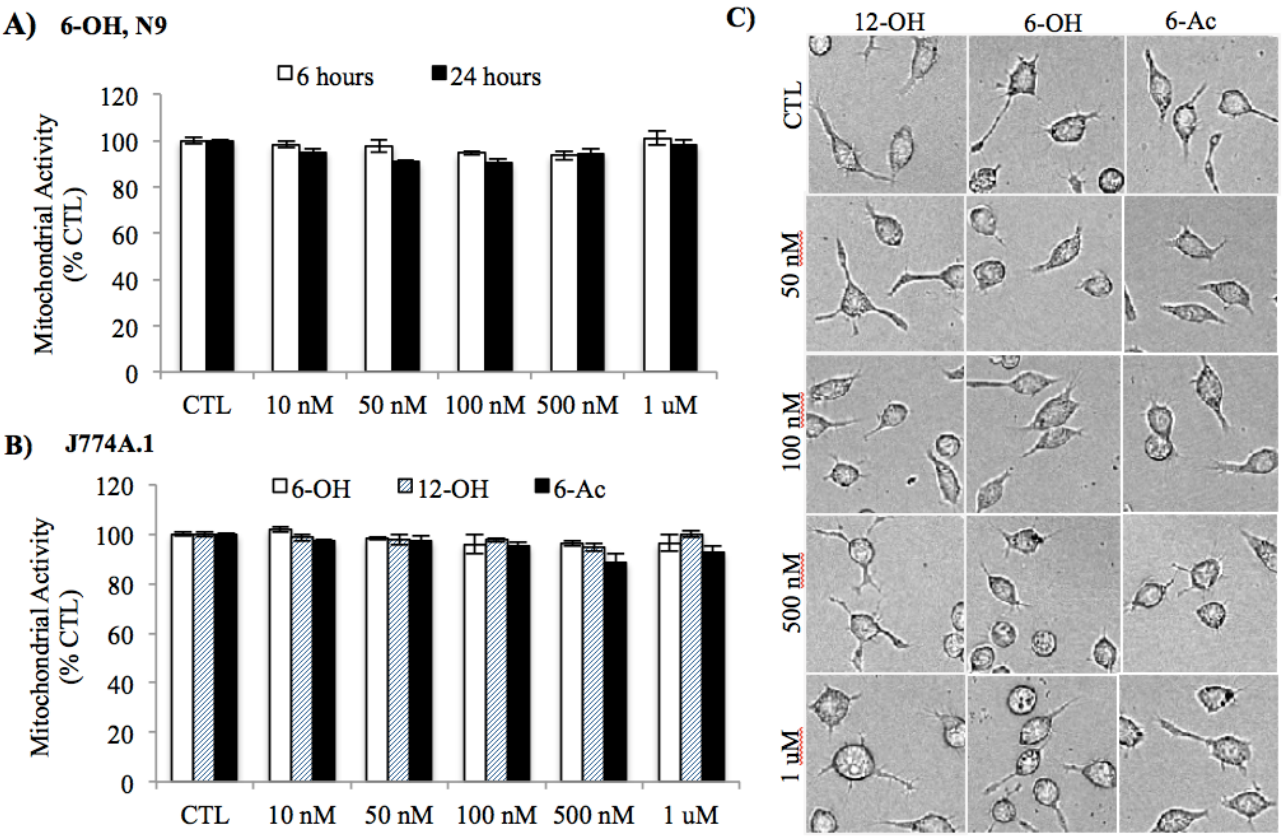


Figure S2. Fluorescent dendrimers do not affect N9 or J774.2 cell viability. Microglia and macrophages were assayed for cell viability as measured by the extent of mitochondrial activity with an MTT assay. **A)**N9 microglia were treated with 6-OH dendrimer for 6 and 24 hours. **B)**Macrophages were treated with dendrimers for 24 hours**.** At treatment end-time point, MTT was added to cells and formazan crystal formation was measured using a spectrophotometer at 595 nm. Data is represented as mean ± standard deviation (n=3). **C)**Representative bright field images were acquired for microglia treated for 90 minutes with fluorescent dendrimers.

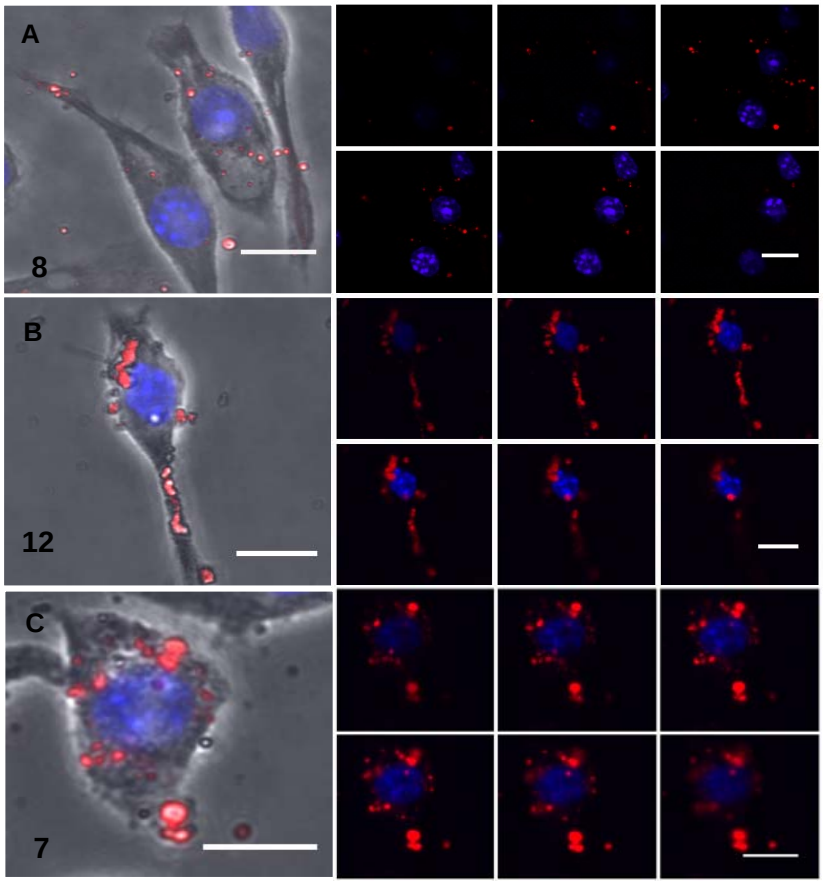


Figure S3. Dendrimer internalization by microglial cells was investigated. N9 microglia were treated with **TIF_6_OH (8, A), TIF-12-OH (12, B),** and **TIF_6_Acet (7, C)** for 1 hour. Internalization was confirmed through z-stack confocal microscopy. Scale bars represent 20 µm.

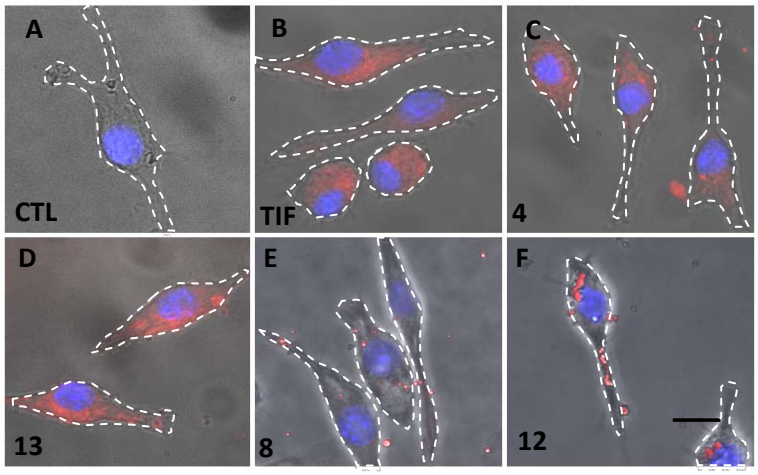
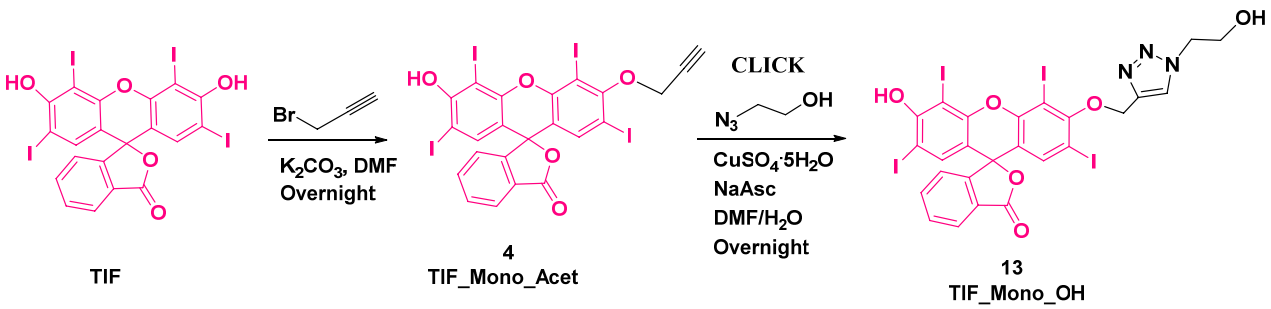


Figure S4. Representative images of microglia treated with **A) Control, B) TIF, C) TIF_Mono_Acet (4), D) TIF_Mono_OH (13), E) TIF_6_OH (8)**, and **F) TIF-12-OH (12)** (5 µM, 90 min) are shown. Note the fluorescence in the soma and the cell extensions. Scale bar represents 20nm.



Scheme S1. Synthesis of linear fluorescent analogs **4** and **13.**

Experimental Section

Materials and methods

Tetraiodofluorescein was purchased from TCI (Tokyo Chemical Industry Co., Ltd. and used as received. Copper (II) sulfatepentahydrate ($CuSO_4.5H_2O$) (>98.0%), sodium ascorbate (NaAsc) (crystalline) (98%), 6-bromohexanoic acid (97%), 2-bromo-ethanol (98%), α-lipoic acid (>99%), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimidemethiodide (EDC), 4(dimethylamino)pyridine (DMAP) (99%), and sodium azide (NaN_3) (>99.5%) were purchased from Sigma-Aldrich Canada, and used as received. All other solvents were used as received in their anhydrous forms. NMR spectra were recorded on a 200 MHz, 400MHz or 500MHz (as specified) spectrometer at ambient temperatures. The chemical shifts in ppm are reported relative to tetramethylsilane as an internal standard for ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra. The high resolution and high accuracy mass spectra (ESI-MS) were obtained using an ExactiveOrbitrap spectrometer from ThermoFisher Scientific, and MALDI-TOF spectra on Autoflex III Mass Spectrometer (Bruker) using α-Cyano-4-hydroxycinnamic acid (HCCA) as the matrix as specified. Absorption spectra were

recorded with Jasco V-670 spectrophotometer and the fluorescence was recorded on Cary Eclipse fluorimeter in methanol.

Synthesis and Characterization

The following compounds were synthesized by adaptation of the previously published procedures: Compound **1**[1], 2-azido ethanol[2], 6-azido hexanoic acid[3], tripropargylatedpentaerythritol **6**[4], and monopropargylated tetraiodofluorescein **4**[5, 6].

Synthesis of compound 2: Compound **1** (200 mg, 0.913 mmol) and 6-azido-hexanoic acid (430 mg, 2.739 mmol) were dissolved in 3 mL of tetrahydrofuran (THF), followed by the addition of sodium ascorbate (36.1 mg, 0.182 mmol). An aqueous solution (1 mL) of $CuSO_4.5H_2O$ (22.1 mg, 0.091 mmol) was added dropwise to the solution. The reaction mixture was left to stir overnight at room temperature. THF was then evaporated, water (10 mL) was added, and the solution was extracted with DCM (3x20 mL). The organic layer was washed with ethylenediaminetetraacetate (EDTA) solution and brine (3x50 mL). It was dried over $MgSO_4$, and the solvent was evaporated. The residue was washed several times with ether to afford the product as white solid (405 mg, 83%). ^{1}H NMR (400 MHz, DMSO): δ (ppm) 1.24-1.30 (m, 4H, -CH_2), 1.49-1.56 (m, 4H, -CH_2), 1.83-1.91 (m, 4H, -CH_2), 2.19 (t, 4H, -CH_2), 4.40 (t, 4H, -CH_2), 4.79 (s, 2H, -CH_2Br), 7.87 (s, 2H, ArH), 8.24 (s, 1H, ArH), 8.68 (s, 2H, triazoleH), and 11.99 (s, 2H, -COOH). ^{13}C{^{1}H} NMR (125 MHz, DMSO): δ (ppm) 24.34, 25.86, 29.75, 33.86, 34.54, 49.92, 122.06, 122.20, 125.63, 132.37, 139.93, and 146.03. ESI-MS: m/z Theoretical 531.14 [$(M-H)^-$], found 531.13 [$(M-H)^-$].

Synthesis of compound 3: To the solution of compound **2** (1.05 g, 1.969 mmol) in *N*,*N*-dimethyl formamide (DMF), NaN_3 (320 mg, 4.921 mmol) was added. The reaction mixture was

left to stir at room temperature for overnight. The solution was diluted with water and extracted with DCM (3x20 mL). The organic phase was washed with brine (3x70 mL). It was dried over $MgSO_4$ and followed by removal of the solvent. The residue was washed with ether and the product was obtained as a white solid (915 mg, 94%). ^{1}H NMR (400 MHz, DMSO): δ (ppm) 1.22-1.30 (m, 4H, -CH_2), 1.49-1.56 (m, 4H, -CH_2), 1.83-1.91 (m, 4H, -CH_2), 2.19 (t, 4H, -CH_2), 4.40 (t, 4H, -CH_2), 4.57 (s, 2H, -CH_2N_3), 7.79 (s, 2H, ArH), 8.28 (s, 1H, ArH), 8.69 (s, 2H, triazoleH), and 12.02 (s, 2H, -COOH). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO): 24.37, 25.87, 29.77, 33.91, 36.21, 49.92, 53.93, 121.88, 122.19, 124.64, 132.38, 137.64, 146.16, and 174.78. ESI-MS: m/z Theoretical 494.23[$(M-H)^-$], found 494.22 [$(M-H)^-$].

Synthesis of compound 4 (TIF_Mono_Acet): To a stirring solution of tetraiodofluorescein (500 mg, 0.598 mmoles) in DMF (2 mL) was added K_2CO_3 (450 mg) followed by the addition of propargyl bromide; 80% in toluene (0.2 mL). The reaction mixture was stirred at room temperature for overnight. On completion, the reaction mixture was washed with ether to remove DMF. The residue was then precipitated using ice and cold water, and filtered to afford a red solid (420 mg, 80%). ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 2.61 (t, 1H, AcetyleneH), 4.60 (d, 2H, -OCH_2), 7.42 (s, 2H, ArH), 7.46 (dd, 1H, ArH), 7.80-7.86 (m, 2H, ArH), and 8.27 (dd, 1H, ArH). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO): 31.22, 36.24, 53.16, 76.26, 78.00, 78.18, 96.64, 111.68, 129.55, 130.54, 131.06, 131.27, 133.75, 134.41, 136.72, and 148.67. ESI-MS: m/z Theoretical 872.67 [$(M-H)^-$], found 872.66 [$(M-H)^-$].

Synthesis of 5: TIF-Mono_Acet **(4)** (176.8 mg, 0.202 mmol) and compound **3** (100 mg, 0.202 mmol) were dissolved in 3 mL of DMF, followed by the addition of sodium ascorbate (4 mg, 0.020 mmol). An aqueous solution (1 mL) of $CuSO_4.5H_2O$ (2.5 mg, 0. 010 mmol) was added dropwise to the solution. The reaction mixture was left to stir for 18 hours at room temperature.

On completion, the reaction mixture was washed with ether to remove DMF. The residue was then precipitated using ice and cold water, and filtered to afford a red solid (200 mg, 72%). ^{1}H NMR (400 MHz, DMSO): δ (ppm) 1.22-1.30 (m, 4H, -CH_2), 1.49-1.56 (m, 4H, -CH_2), 1.83-1.91 (m, 4H, -CH_2), 2.17-2.22 (m, 4H, -CH_2), 4.39 (t, 4H, -CH_2), 5.09 (s, 2H, -CH_2), 5.62 (s, 2H, -CH_2), 7.15 (s, 2H, ArH), 7.45 (d, 1H, ArH), 7.73 (s, 2H, ArH), 7.77-7.86 (m, 2H, ArH), 7.89 (s, 1H, ArH), 8.12 (d, 1H, ArH), 8.27 (s, 1H, triazoleH), 8.66 (s, 2H, triazoleH), and 11.97 (brs, 2H, -COOH). ^{13}C{^{1}H} NMR (125 MHz, DMSO): 14.42, 15.61, 22.50, 24.58, 25.99, 29.8, 31.40, 49.93, 53.16, 53.60, 58.70, 65.35, 65.63, 78.00, 78.18, 111.96, 121.97, 122.31, 124.68, 129.53, 130.01, 130.47, 131.01, 131.19, 132.13, 132.46, 133.48, 133.74, 134.14, 134.42, 136.74, 137.53, 146.04, and 148.55. ESI-MS: m/z Theoretical 1368.90 [$(M-H)^-$], found 1367.89 [$(M-H)^-$].

Synthesis of compound 7 (TIF_6_Acet): To a stirring solution of compound **5** (100 mg, 0.073 mmol), tripropargylated pentaerythritol **6** (55 mg, 0.219 mmoles), and DMAP (13.3 mg. 0.109 mmoles) in DMF (3 mL), was added EDC (42 mg, 0.219 mmoles), and the solution was stirred at room temperature for 18 hours. On completion, the reaction mixture was washed with ether to remove DMF. The residue was then precipitated using ice and cold water, and filtered to afford a red solid. The solid was washed several times with diethyl ether to remove impurities and yield compound **7** (110 mg, 83%). ^{1}H NMR (400 MHz, DMSO): δ (ppm) 1.24-1.30 (m, 4H, -CH_2), 1.55-1.58 (m, 4H, -CH_2), 1.84-1.89 (m, 4H, -CH_2), 2.28-2.31 (m, 4H, -CH_2), 3.39 (s, 12H, -CH_2), 3.94 (s, 4H, -$COOCH_2$), 4.08 (s, 12H, -CH_2), 4.39 (t, 4H, -CH_2), 5.09 (s, 2H, -CH_2), 5.61 (s, 2H, -CH_2), 7.12 (s, 2H, ArH), 7.44-7.46 (m, 1H, ArH), 7.73 (s, 2H, ArH), 7.81-7.86 (m, 2H, ArH), 8.10-8.12 (m, 2H, ArH), 8.28 (s, 1H, triazoleH), and 8.66 (s, 2H, triazoleH). ^{13}C{^{1}H} NMR (125 MHz, DMSO): 24.27, 25.82, 29.74, 31.40, 33.75, 43.85, 49.91, 53.61, 58.56, 63.18, 68.82, 77.70, 80.57, 110.00, 121.97, 122.28, 124.71, 130.03, 130.45, 131.01, 131.21, 132.45, 133.47,

134.13, 136.79, 137.51, 145.99, 157.52, 165.10, and 172.94. ESI-MS: m/z Theoretical 1832.12 [$(M-H)^-$], found 1832.11 [$(M-H)^-$].

Synthesis of compound 8 (TIF_6_OH): Compound **7** (105 mg, 0.057 mmol) and 2-azido-ethanol (49.8 mg, 0.572 mmol) were dissolved in 3 mL of DMF, followed by the addition of sodium ascorbate (6.8 mg, 0.034 mmol). An aqueous solution (1 mL) of $CuSO_4.5H_2O$ (4.3 mg, 0.017 mmol) was added dropwise to the solution. The reaction mixture was left to stir overnight at room temperature. On completion, the reaction mixture was washed with diethyl ether to remove DMF. The residue was then precipitated using ice and cold water, and filtered to afford a red solid. The solid was washed several times with EDTA solution and diethyl ether to remove impurities to yield compound **8** (98 mg, 73%). ^{1}H NMR (400 MHz, DMSO): δ (ppm) 1.22-1.30 (m, 4H, -CH_2), 1.49-1.54 (m, 4H, -CH_2), 1.83-1.91 (m, 4H, -CH_2), 2.17-2.20 (m, 4H, -CH_2), 3.36 (s, 12H, -CH_2), 3.74 (q, 12H, -CH_2OH), 3.91 (s, 4H, -COOCH_2), 4.34 (t, 12H, -CH_2), 4.35-4.41 (m, 4H, -CH_2), 4.42 (s, 12H, -CH_2), 4.99 (t, 6H, -OH), 5.09 (s, 2H, -CH_2), 5.61 (s, 2H, -CH_2), 7.15 (s, 2H, ArH), 7.45 (d, 1H, ArH), 7.70-7.75 (m, 3H, ArH), 7.80-7.84 (m, 2H, ArH), 7.98 (s, 6H, triazoleH), 8.11 (d, 1H, ArH), 8.28 (s, 1H, triazoleH), and 8.65 (s, 2H, triazoleH). ^{13}C{^{1}H} NMR (125 MHz, DMSO): 24.20, 25.79, 29.68, 31.22, 33.66, 36.23, 44.32, 49.97, 52.94, 53.68, 60.13, 63.07, 64.30, 69.07, 111.78, 122.38, 124.85, 125.30, 130.03, 131.00, 131.20, 132.38, 133.49, 134.07, 136.76, 146.03, 157.63, 162.76, 165.13, and 172.96. ESI-MS: m/z (+) Theoretical 2356.38[$(M+H)^+$], found 2357.40 [$(M+H)^+$].

Synthesis of compound 9 (TIF_6_LA): To a strirring solution of compound **8** (100 mg, 0.042 mmol), α-lipoic acid (70.05 mg, 0.339 mmoles), and DMAP (18.1 mg. 0.148 mmoles) in DMF (3 mL), was added EDC (56.9 mg, 0.297 mmoles), and the solution was stirred at room temperature for 12h. On completion, the reaction mixture was diluted with water and extracted

with DCM (3X10mL). The combined organic extracts were washed with water and brine. The organic layer was dried over sodium sulfate and concentrated. The residue was washed with diethylether and methanol to remove impurities and to afford compound 9 as red solid (95 mg, 65%). ^{1}H NMR (500 MHz, DMSO): δ (ppm) 1.19-1.27 (m, 16H, -CH_2), 1.38-1.60 (m, 28H, -CH_2**)**, 1.75-1.90 (m, 6H, -$SCHCH_2$ and 4H, -CH_2)), 2.18-2.23 (m, 12H, -CH_2COO- and 4H, -CH_2), 2.30-2.38 (m, 6H, -$SCHCH_2$-), 3.02-3.16 (m, 12H, -$SSCH_2$), 3.35 (s, 12 H, -CH_2), 3.49-3.53 (m, 6H, -SCH), 3.91 (s, 4H, -$COOCH_2$), 4.36-4.40 (t, 16H, -CH_2), 4.43 (s, 12H, -CH_2), 4.57 (t, 12H, -CH_2), 5.08 (s, 2H, -CH_2), 5.60 (s, 2H, -CH_2), 7.13 (s, 2H, ArH), 7.43 (d, 1H, ArH), 7.72 (s, 3H, ArH), 7.74-7.83 (m, 2H, ArH), 8.04 (s, 6H, triazoleH), 8.10 (d, 1H, ArH), 8.29 (s, 1H, triazoleH), and 8.65 (s, 2H, triazoleH). $^{13}C\{^{1}H\}$ NMR (125 MHz, DMSO): 24.23, 24.46, 25.84, 28.41, 29.77, 33.51, 33.66, 34.41, 38.52, 44.36, 48.94, 49.91, 56.43, 58.67, 62.58, 63.11, 64.61, 68.93, 111.81, 121.93, 122.27, 124.58, 130.04, 130.45, 131.01, 131.18, 132.46, 133.45, 134.12, 136.79, 137.48, 137.98, 144.48, 146.00, 148.63, 157.64, 165.11, and 172.82. ESI-MS: m/z (+) Theoretical 1742.79[$(M+H)^{2+}$], found 1742.80 [$(M+H)^{2+}$].

Synthesis of compound 10: Compound **1** (300 mg, 1.369 mmol) and 2-azido-ethanol (262 mg, 3.013 mmol) were dissolved in 3 mL of tetrahydrofuran (THF), followed by the addition of sodium ascorbate (54.2 mg, 0.273 mmol). An aqueous solution (1 mL) of $CuSO_4.5H_2O$ (34.1 mg, 0.136 mmol) was added dropwise to the solution. The reaction mixture was left to stir overnight at room temperature. THF was then evaporated, water (10 mL) was added, and the solution was extracted with DCM (3x20 mL). The organic layer was washed with EDTA solution and brine (3x50 mL). It was dried over $MgSO_4$, and the solvent was evaporated. The residue was washed several times with ether to afford the product as white solid (420 mg, 78%). ^{1}H NMR (200 MHz, DMSO): δ (ppm) 3.83 (q, 4H, -CH_2), 4.45 (t, 4H, -CH_2), 4.80 (s, 2H, -

CH_2Br), 5.09 (t, 2H, -OH), 7.89 (s, 2H, ArH), 8.26 (s, 1H, ArH), and 8.63 (s, 2H, triazoleH). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO): 34.58, 46.39, 52.97, 60.23, 122.01, 122.78, 125.08, 125.54, 132.41, 139.93, and 145.90. ESI-MS: m/z (+) Theoretical 393.06 $[(M+H)^+]$, found 393.06 $[(M+H)^+]$.

Synthesis of compound 11: To a solution of compound **10** (500 mg, 1.272 mmol) in N,N-dimethyl formamide (DMF), NaN_3 (206.7 mg, 3.180 mmol) was added. The reaction mixture was left to stir at room temperature for 18 hours. The solution was diluted with water and extracted with DCM (3x20 mL). The organic phase was washed with brine (3x70 mL). It was dried over $MgSO_4$ and followed by removal of the solvent. The residue was washed with ether to afford the product as a white solid (390 mg, 86%). ^1H NMR (400 MHz, DMSO): δ (ppm) 3.83 (q, 4H, $-CH_2$), 4.45 (t, 4H, $-CH_2$), 4.57 (s, 2H, $-CH_2N_3$), 5.10 (t, 2H, -OH), 7.82 (s, 2H, ArH), 8.31 (s, 1H, ArH), and 8.64 (s, 2H, triazoleH). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO): 13.93, 23.50, 52.96, 53.92, 60.24, 121.83, 122.78, 124.57, 132.42, 137.60, and 146.01. ESI-MS: m/z (+) Theoretical 356.15 $[(M+H)^+]$, found 356.15 $[(M+H)^+]$.

Synthesis of compound 12 (TIF_12_OH): Compound **7** (40 mg, 0.021 mmol) and compound **11** (54.2 mg, 0.152 mmol) were dissolved in 2 mL of DMF, followed by the addition of sodium ascorbate (2.6 mg, 0.013 mmol). An aqueous solution (1 mL) of $CuSO_4.5H_2O$ (1.6 mg, 0.006 mmol) was added dropwise to the solution. The reaction mixture was left to stir for 18 hours at room temperature. On completion, the reaction mixture was washed with diethyl ether to remove DMF. The residue was then precipitated using ice and cold water, and filtered to afford a red solid. The solid was washed several times with EDTA solution and diethyl ether to remove impurities to yield dendrimer **12** (55 mg, 64%). ^1H NMR (400 MHz, DMSO): δ (ppm) 1.08-1.21 (m, 4H, $-CH_2$), 1.30-1.60 (m, 4H, $-CH_2$), 1.70-1.90 (m, 4H, $-CH_2$), 2.08-2.16 (m, 4H, $-CH_2$), 3.36

(s, 12H, -CH_2), 3.78-3.84 (m, 24H, -CH_2), 3.88 (s, 4H, -$COOCH_2$), 4.30-4.33 (m, 4H, -CH_2), 4.20-4.45 (m, 24H+12H, -CH_2), 5.08 (t, 12H, -OH + s, 2H, -CH_2), 5.59 (s, 2H, -CH_2), 5.63 (s, 12H, -CH_2), 7.10 (s, 2H, ArH), 7.43 (d, 1H, ArH), 7.68-7.76 (m, 3H, ArH), 7.76 (s, 12H, ArH), 7.67-7.81 (m, 2H, ArH), 8.08-8.11 (m, 1H, ArH), 8.15 (s, 6H, ArH), 8.23 (s, 6H, triazoleH), 8.29 (s, 1H, triazoleH), 8.55 (s, 12H, triazoleH) and 8.62 (s, 2H, triazoleH).). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO): 13.93, 23.50, 24.10, 25.78, 29.73, 33.52, 44.33, 49.88, 52.93, 53.14, 60.23, 64.54, 69.00, 76.23, 96.55, 111.69, 121.99, 122.73, 124.37, 124.49, 132.45, 136.76, 137.85, 144.74, 145.89, 157.65, 172.03, and 172.90. MALDI: m/z Theoretical 3989.14 [$(M+Na)^+$], found 3989.27 [$(M+Na)^+$].

Synthesis of compound 13 (TIF_Mono_OH): Compound **4** (200 mg, 0.228 mmol) and 2-azido-ethanol (23.8 mg, 0.274 mmol) were dissolved in 3 mL of DMF, followed by the addition of sodium ascorbate (4.5 mg, 0.022 mmol). An aqueous solution (1 ml) of $CuSO_4.5H_2O$ (2.8 mg, 0.011 mmol) was added dropwise to the solution. The reaction mixture was left to stir overnight at room temperature. On completion, the reaction mixture was washed with diethyl ether to remove DMF. The residue was then precipitated using ice and cold water, and filtered to afford a red solid. The solid was washed several times with EDTA solution and diethyl ether to remove impurities to yield compound **13** (160 mg, 73%). ^1H NMR (500 MHz, DMSO): δ (ppm) 3.70 (q, 2H, -CH_2OH), 4.32 (t, 2H, -CH_2), 4.94 (t, 1H, -OH), 5.09 (s, 2H, -OCH_2), 7.14 (s, 2H, ArH), 7.45 (d, 1H, ArH), 7.71 (s, 1H, triazoleH), 7.74 (t, 1H, ArH), 7.83 (t, 1H, ArH), and 8.12 (d, 1H, ArH). $^{13}C\{^1H\}$ NMR (125 MHz, DMSO): 52.97, 58.71, 60.16, 88.74, 112.04, 125.47, 127.84, 129.96, 130.45, 131.00, 131.18, 13.53, 134.24, 136.69, 157.57, 165.09, and 169.18. ESI-MS: m/z Theoretical 959.71 [$(M-H)^-$], found 959.70 [$(M-H)^-$].

Cell culture

Murine N9 microglia cells (Mouse embryonic brain primary cultures, ATCC Castagnoli, Italy) were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Gibco #12440-053) supplemented with 5% (v/v) fetal bovine serum (FBS, Gibco #26140-079) and 1% (v/v) Pen-Strep (Gibco #15140-122). Cells were seeded 24h prior to treatment. Treatment was performed in 1% serum-containing media for indicated amounts of time. Cell growth and treatment were performed at 37°C with 5% CO and >95% relative humidity. J774A.1 murine macrophages were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco #11995-073) containing 1% (v/v) Pen-Strep (Gibco #15140-122) in10% (v/v) fetal bovine serum(FBS, Gibco #26140-079) media solution.

Measurement of mitochondrial metabolic activity

N9 microglia cells were seeded in 24-well cell culture plate (Sarstedt) at a density of $2 \cdot 10^5$ cells/well in a final volume of 500 µl. Macrophages were seeded at a density of $1.75 \cdot 10^5$ cells/well. In both cases, cells were treated as indicated and at time-end point 50 µl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution in PBS (5 mg/ml) was added to the cells and incubated at 37°C with 5% CO and >95% relative humidity for 30-35 min. 500 µl of dimethyl sulfoxide (DMSO) was added to lyse the cells and dissolve the formazan crystals. Aliquots of 100 µl were collected from each well and transferred in triplicate to a 96 well plate (Sarstedt). The absorbance was measured at 595 nm using a Benchmark microplate reader (Bio-Rad, ON, Canada).

Spectrofluorometric measurements

To acquire fluorescence measurements for dyes and dendrimers, N9 cells were seeded in a 96 well black plate (Sarstedt) at a density of 15 000 cells/well and incubated for 24 hours at 37˚C. Phenol-free media was used for treatment with dyes or dendrimers. Cells were incubated for 90 minutes, or as stated and washed with PBS after treatment end-time. Cells were measured immediately using a BMG spectrofluorometer (ex 544 / em 590).

Confocal microscopy

Cells were grown on rat-tail collagen treated cover slips at a seeding density of 20 000 cells/well and incubated for 24 hours at 37˚C. Cells were treated for 90 minutes with dyes or dendrimers. At treatment end-time point, cells were washed with PBS and fixed with 4% PFA (parafomaldehyde). They were then stained with 10 μM Hoechst 33342 (Life Technologies Inc.) for 10 min at room temperature and washed at least once with PBS. The cells were imaged on microscope slides using a Leica DMI4000B inverted fluorescence microscope at 63×. Pictures were captured with the Leica DFC350FX digital camera through a UV filter (ex 350/ em 461 nm) and Cy3 filter (ex 543 / em 593) and analyzed using the Leica Application Suite software for image acquisition. To acquire z-stack images, a Zeiss microscope was used with a DAPI filter and Cy3 filter. Images were processed using ImageJ and Microsoft Office tools.

Data and Statistical analysis

Data was graphed and tabulated using Microsoft Excel®. Values in bar graphs are collected from triplicate samples from at least two independent experiments unless otherwise indicated. All data are presented as group means ± SEM. Student's t-test was used to analyze significant differences

between two group means. In all statistical tests, values are indicated by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

References:

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4. M. Schunack, M. Gragert, D. Döhler, P. Michael and W. H. Binder, *Macromolecular Chemistry and Physics*, 2012, **213**, 205-214.
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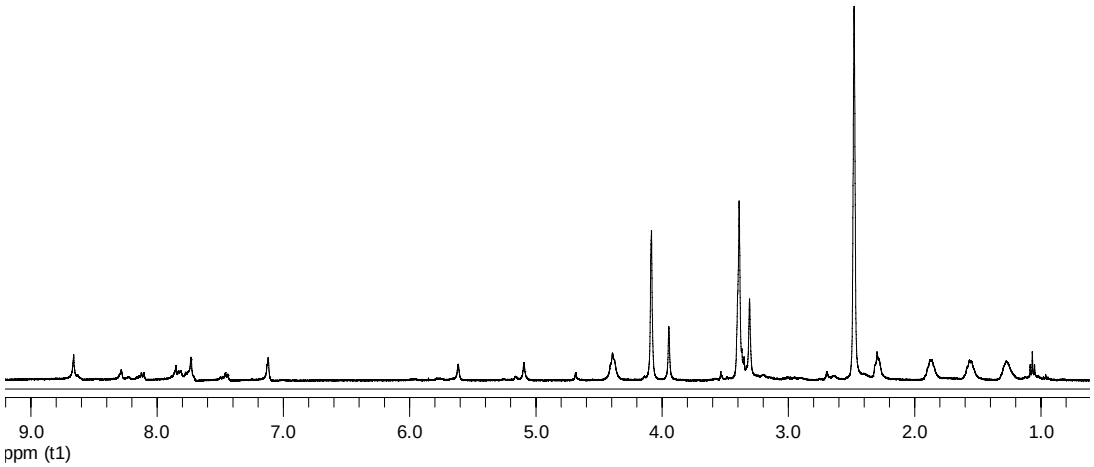
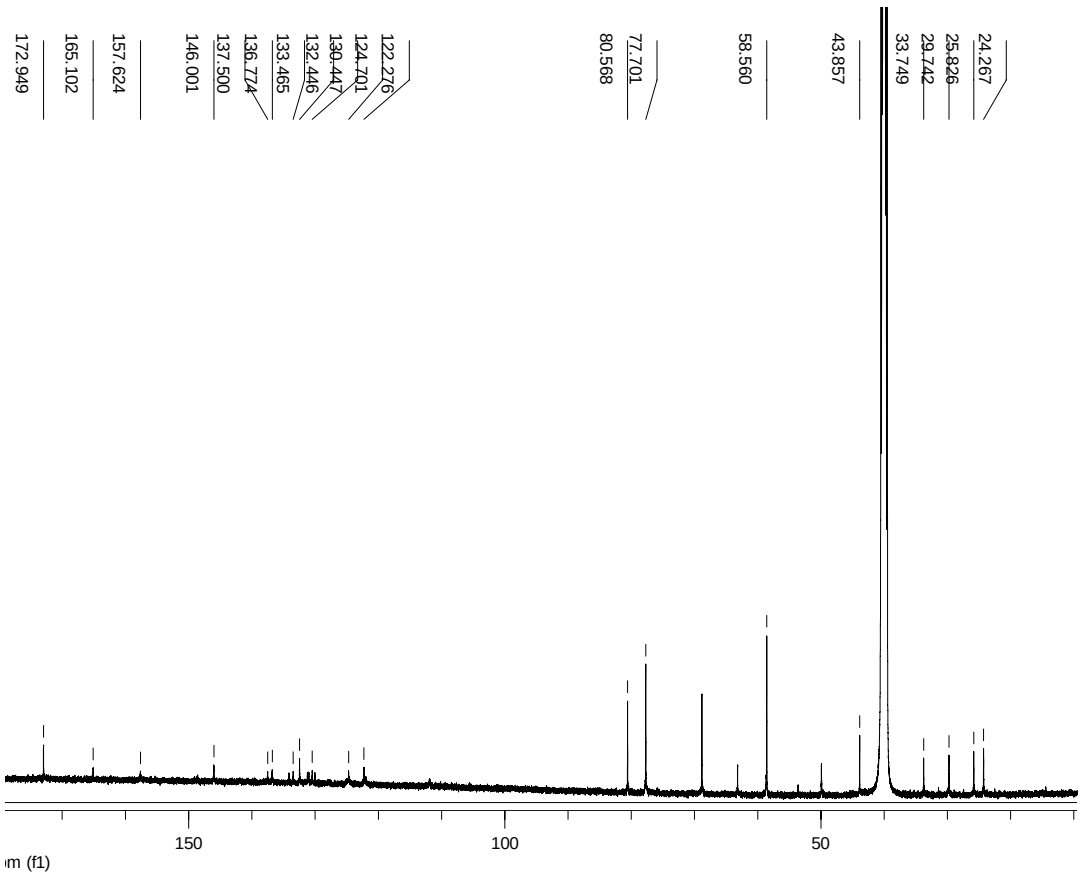
Compound 7 (TIF_6_Acet):

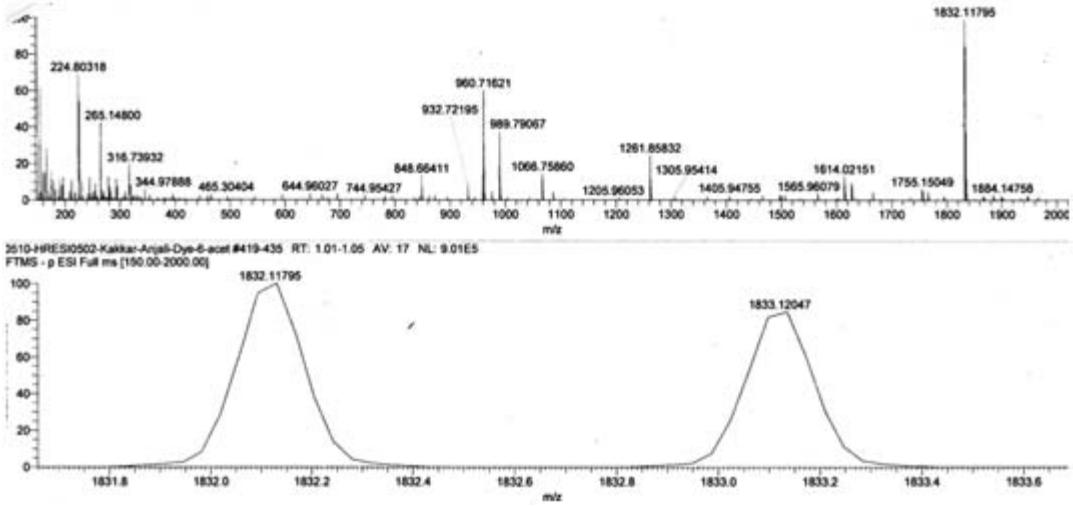
^{1}H NMR:



^{13}C NMR:

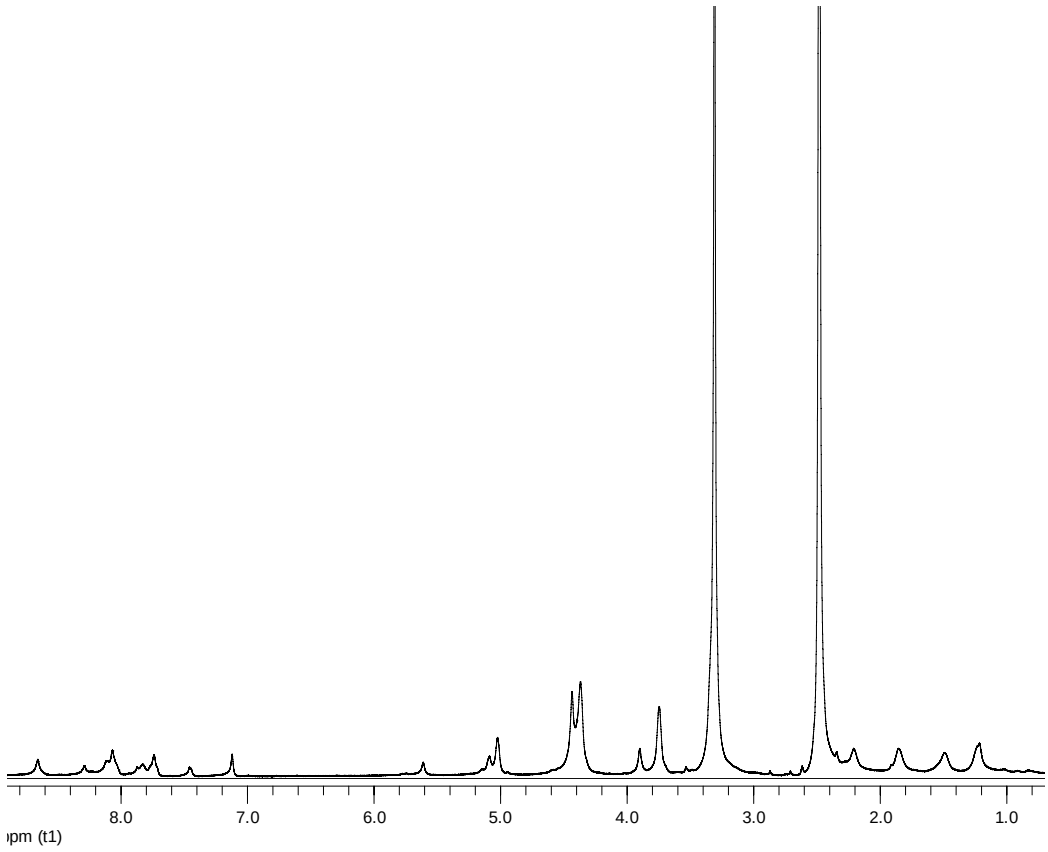


ESI-MS:

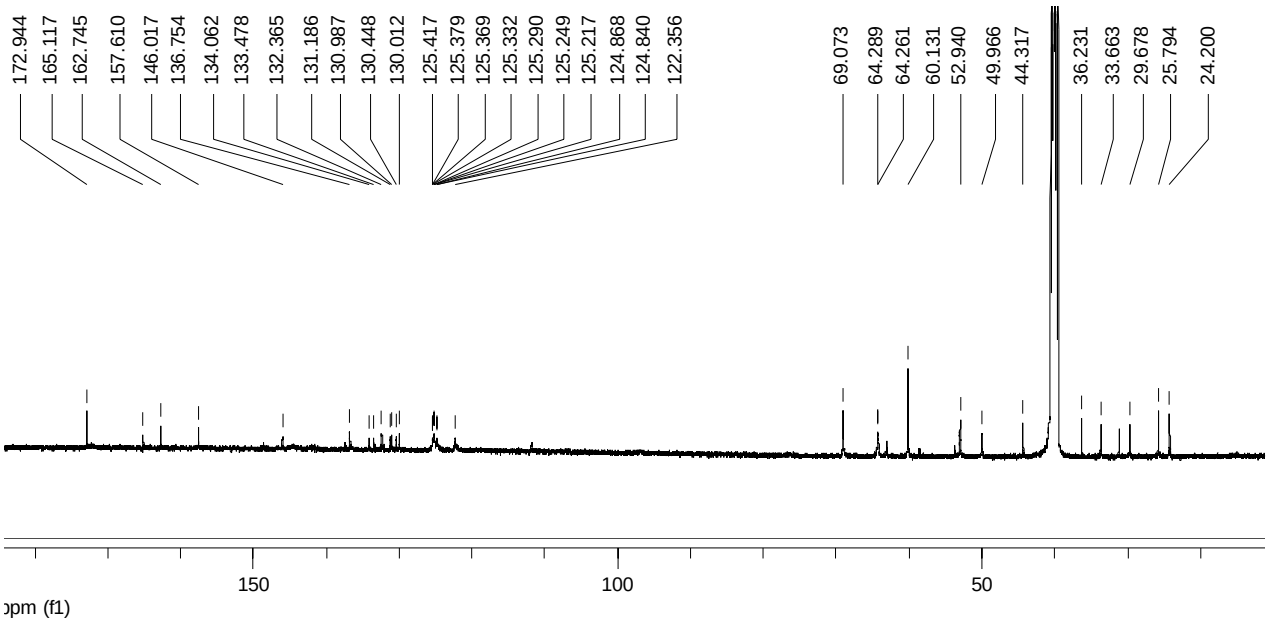

1832.11795
224.80318
960.71621
265.14800
932.72195
989.79067
316.73932
1261.85832
1066.75860
1305.95414
1614.02151
344.97888
465.30404
644.96027
744.95427
848.66411
1205.96053
1405.94755
1565.96079
1755.15049
1884.14758
m/z
2610-HRESI0502-Kakkar-Anjali-Dye-6-acet #419-435 RT: 1.01-1.05 AV: 17 NL: 9.01E5
FTMS - p ESI Full ms [150.00-2000.00]
1832.11795
1833.12047
m/z

Compound 8 (TIF_6_OH):

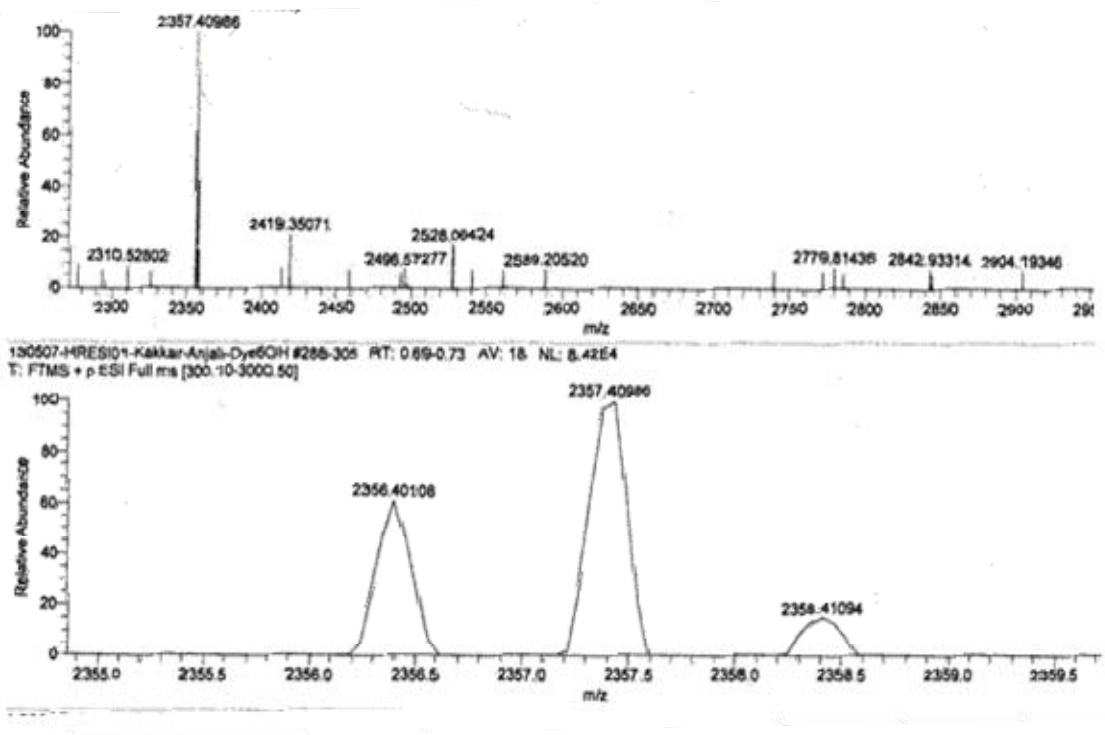
^{1}H NMR:



^{13}C NMR:

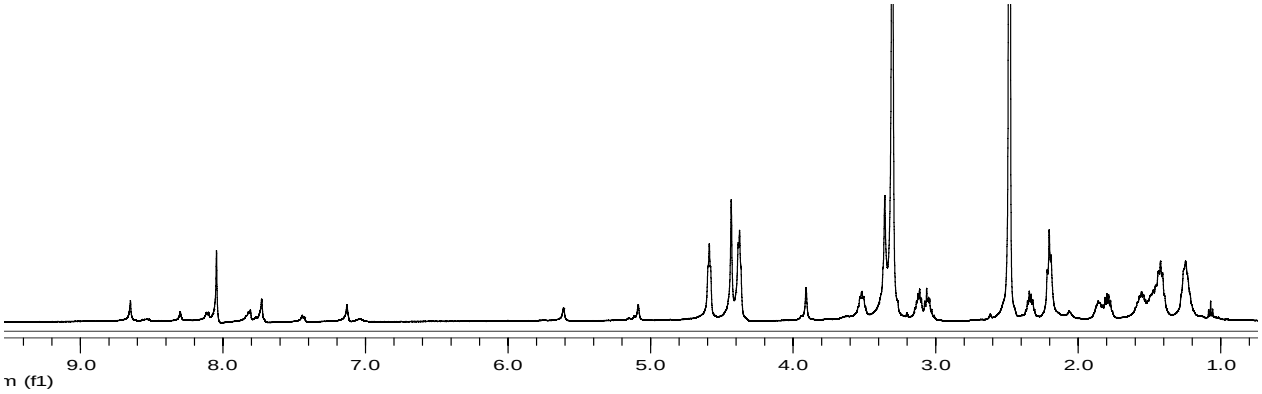


ESI-MS:



Compound 9 (TIF_6_LA):

^{1}H NMR:



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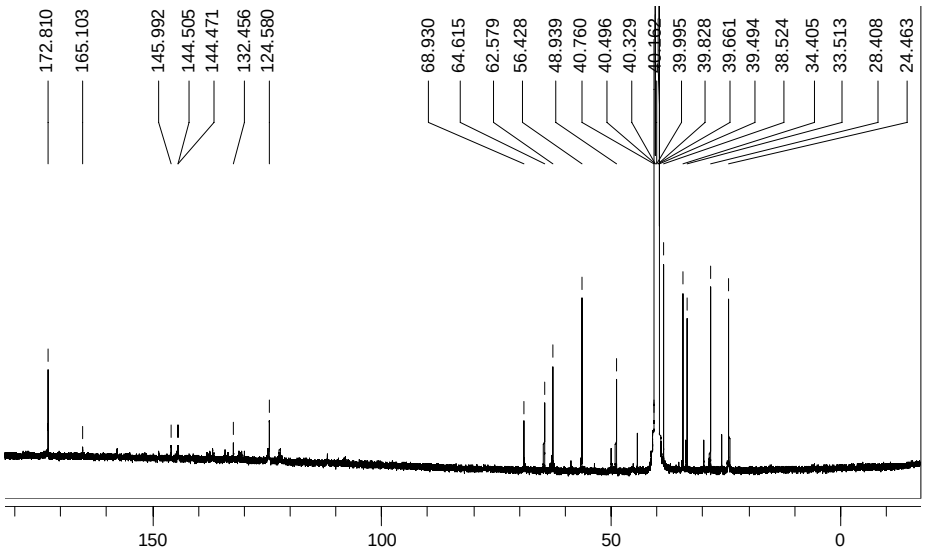

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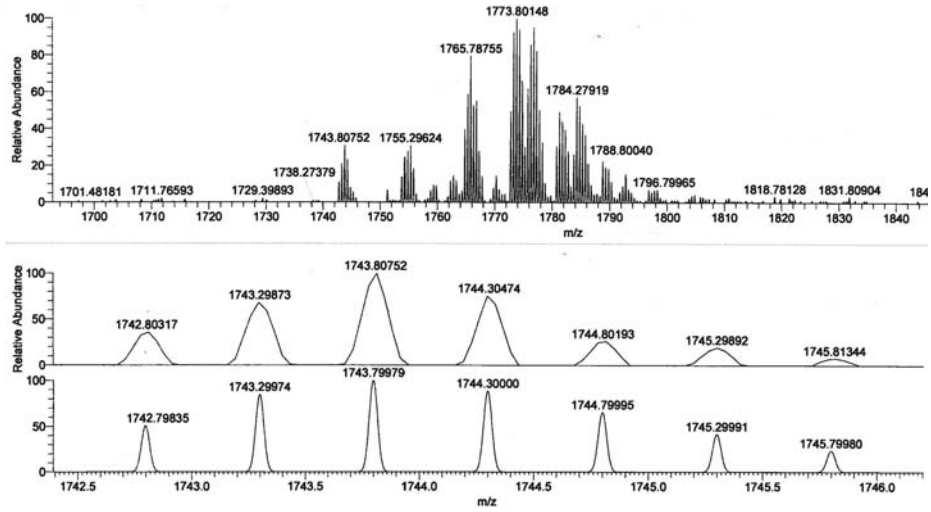
Relative Abundance
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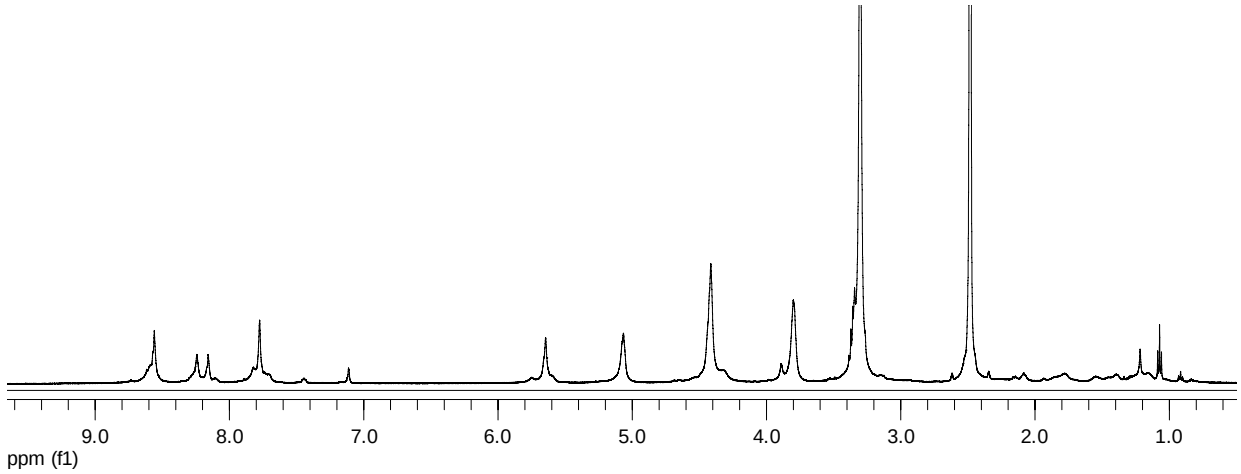
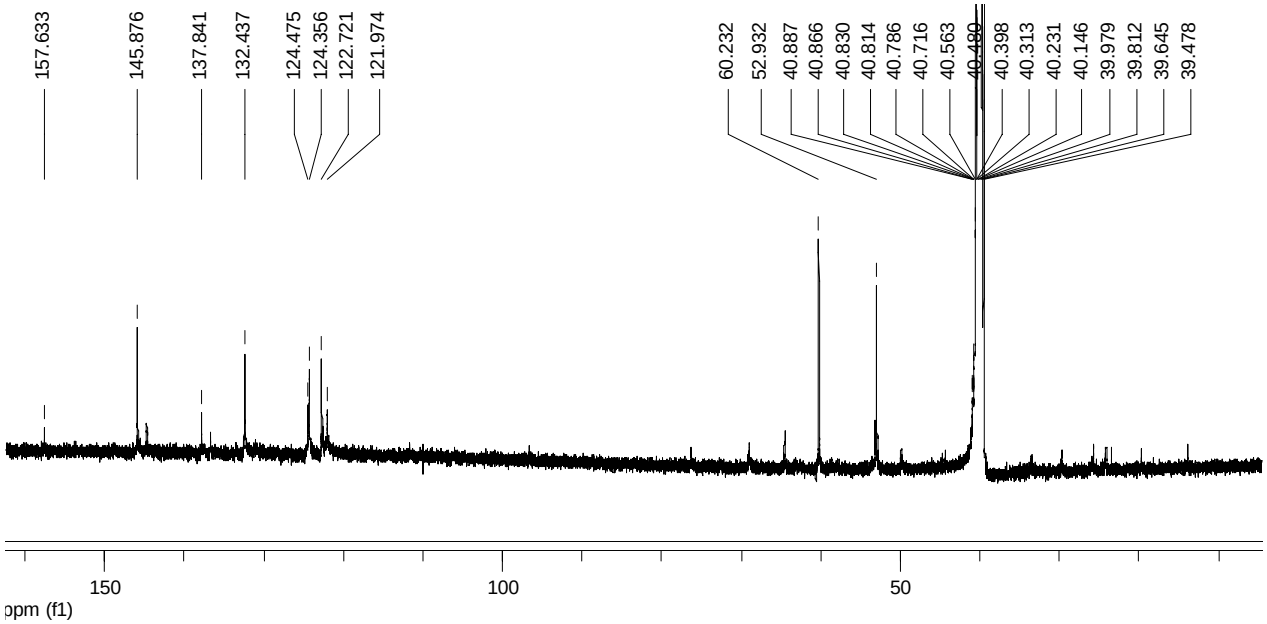
1784.27919
1743.80752
1755.29624
1788.80040
1738.27379
1796.79965
1701.48181
1711.76593
1729.39893
1818.78128
1831.80904
1743.80752
1743.29873
1744.30474
1742.80317
1744.80193
1745.29892
1745.81344
1743.79979
1743.29974
1744.30000
1742.79835
1744.79995
1745.29991
1745.79980

Compound 12 (TIF-12_OH):

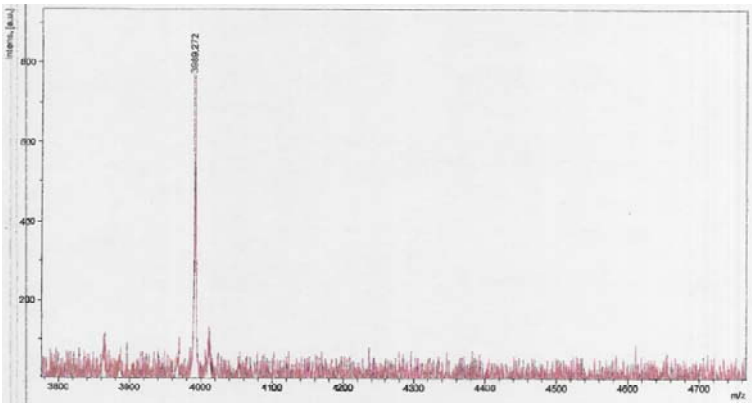
^{1}H NMR:



^{13}C NMR:



MALDI-TOF MS: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800
600
400
200
3800
3900
4000
4100
4200
4300
4400
4500
4600
4700
m/z