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Thermosensitive Dendrimer Formulation for Drug Delivery at Physiologically Relevant Temperatures

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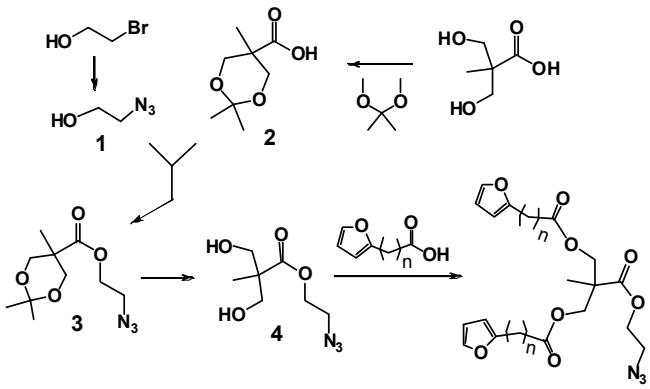
Synthesis and Characterization

All starting reagents have been obtained from commercial vendors while **G0-Ac$_4$**,[1] **5**,[2] **6**,[3] **7**[4] and **8**[4] have been prepared according to literature procedures. **AzFu$_2$ (n = 0, 2)** have been prepared as previously reported,[5] as follows. For the full characterization of compounds **1**-**4**, see ref 6. NMR spectra were recorded on Varian instruments (200-500 MHz).

Building Blocks



AzFu$_2$ (n = 0, 2)

1. Convenient pathways for the preparation of that compound were published elsewhere but the procedure that we report here demonstrates that the use of a phase-transfer catalyst is not necessary. A solution of 2-bromoethanol (15.00 g, 120 mmol) and sodium azide (15.61 g, 240 mmol) was heated in water (25 mL) at 65 °C for 16 h. The final mixture was extracted with dichloromethane (3 x 100 mL), the solution was dried with $MgSO_4$ and filtered. Compound **1** was obtained as a volatile oil (7.37 g, 71 %) after evaporation of the dichloromethane with the use of a rotavap (water tap vacuum) at 40 °C. *Caution : because of the explosive nature of azides, special care should be taken and the reaction temperature should not be further increased.*

2. 2,2-methoxypropane (5.82 g, 55.9 mmol), *p*-toluenesulfonic acid (0.355 g, 2.0 mmol) and magnesium sulfate (0.449 g, 3.7 mmol) were added to a solution of dimethylolpropionic acid (5.00 g, 37.3 mmol) in acetone (20 mL). The mixture was stirred under nitrogen for 16 h after ammonia (3.72 mL of a 0.5 M solution in dioxane, 1.9 mmol) was added. The mixture was filtered and the precipitate washed with dichloromethane. Compound **2** (5.93 g, 91 %) was obtained as a white solid after evaporation of the volatiles.

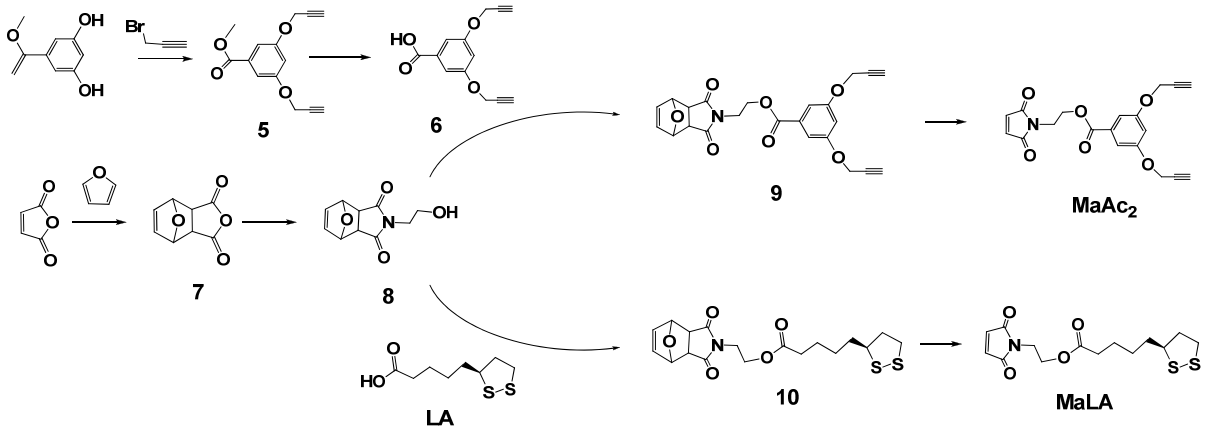
3. 4-dimethylaminopyridine (2.860 g, 23.4 mmol) was added to a solution of compounds **1** (3.580 g, 41.1 mmol) and **2** (8.596 g, 49.4 mmol) in dry dichloromethane (80 mL). After 10 min, N-N'-dicyclohexylcarbodiimide (11.570 g, 56.1 mmol) was added and the resulting mixture was stirred for 16 h under nitrogen. The precipitate was then filtered and the solvent evaporated. Compound **3** was obtained as an oil (6.600 g, 66%) after a purification of the resultant residue by column chromatography (1:7, ethyl acetate:hexanes).

4. To a solution of compound **3** (2.470 g, 10.2 mmol) in methanol (50 mL) was added a spoon (approximately 1,000 g) of the cationic resin *Dowex Marathon C*. After 48 h, the reaction mixture was filtered. Compound **4** was obtained as a colorless oil (1.700 g, 80%) after evaporation of the filtrate.

$AzFu_2$ (n = 0). 4-dimethylaminopyridine (0.800 g, 6.5 mmol) was added to a solution of **4** (2.670 g, 13.1 mmol) and furoic acid (3.220 g, 28.8 mmol) in dry dichloromethane (25 mL) and the mixture was allowed to react for 5 min. N-N'-dicyclohexylcarbodiimide (3.240 g, 15.7 mmol) was then added and the reaction mixture was stirred for 16 h. The resulting precipitate was filtered and washed with dichloromethane. The filtrate was concentrated and compound **$AzFu_2$ (n = 0)** was obtained as a yellow oil (0.920 g, 18%) after purification by column chromatography eluting with a methanol:dichloromethane (2.5:100) mixture. ^{1}H NMR ($CDCl_3$) : 1.45 (s, 3H, CH_3), 3.49 (m, 2H, N_3CH_2), 4.33 (m, 2H, N_3CH_2), 4.53 (d, *J* = 11, 2H, CCH_2O), 4.58 (d, *J* = 11, 2H, CCH_2O), 6.51 (dd, *J* = 3, 2, 2H, CH_{fur}), 7.18 (dd, *J* = 4, 1 Hz, CH_{fur}, 2H), 7.58 (dd, *J* = 2, 1 Hz,

C*H*$_{fur}$, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) : 17.9, 46.9, 49.8, 64.0, 65.7, 112.1, 118.6, 144.1, 146.9, 158.2, 172.4; ESI MS (+) : *m/z* 414.1 $[M + Na]^+$, 804.7 $[2M + Na]^+$.

AzFu$_2$ (n = 2). 4-dimethylaminopyridine (0.60 g, 4.9 mmol) was added to a dry dichloromethane (20 mL) solution of **4** (1.00 g, 4.9 mmol) and 3-(2-furyl)propionic acid (1.52 g, 10.8 mmol), and the mixture was allowed to react for 5 min. N-N'-dicylohexylcarbodiimide (2.44 g, 11.8 mmol) was then added and the reaction mixture was stirred for 40 h. The resulting precipitate was filtered and washed with dichloromethane. The filtrate was concentrated and compound **AzFu$_2$ (n = 2)** was obtained as a yellow oil (1.91 g, 87%) after purification by column chromatography eluting with a methanol:dichloromethane (1:99) mixture. ^1H NMR ($CDCl_3$) : 1.23 (s, 3H, C*H*$_3$), 2.67 (t, *J* = 8 Hz, 4H, C*H*$_2$C*H*$_2$fur), 2.95 (t, *J* = 7 Hz, 4H, C*H*$_2$C*H*$_2$fur), 3.46 (m, 2H, N_3C*H*$_2$), 4.20-4.30 (m, 6H, N_3CH_2C*H*$_2$ + CC*H*$_2$O), 6.01 (dd, *J* = 3, 1 Hz, 2H, C*H*$_{fur}$), 6.26 (dd, *J* = 3, 2 Hz, C*H*$_{fur}$, 2H), 7.29 (dd, *J* = 2, 1 Hz, C*H*$_{fur}$, 2H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) : 17.8, 23.4, 32.6, 46.5, 49.7, 63.9, 65.5, 105.5, 110.3, 141.4, 153.9, 172.0, 172.6; ESI MS (+) : *m/z* 470.2 $[M + Na]^+$.



9. 4-dimethylaminopyridine (2.070 g, 17.0 mmol) was added to a dry dichloromethane (300 mL) solution of **6** (7.800 g, 33.9 mmol) and **8** (8.500 g, 40.7 mmol) and the mixture was allowed to react for 5 min. N-N'-dicylohexylcarbodiimide (8.400 g, 40.7 mmol) was then added and the reaction mixture was stirred for 16 h. The resulting precipitate was filtered and washed with

dichloromethane. The filtrate was concentrated and compound **9** was obtained as a yellow oil (14.000 g, 98%) after purification by column chromatography, eluting with a acetone:dichloromethane (1:3) mixture. ^{1}H NMR ($CDCl_3$) : 2.54 (t, *J* = 2 Hz, 2H, C≡*H*), 2.87 (s, 2H, NCOC*H*), 3.89 (m, 2H NCH_2CH_2), 4.43 (m, 2H, NCH_2CH_2), 4.72 (d, *J* = 2 Hz, 4H, CH_2C≡H), 5.26 (d, *J* = 1 Hz, 2H, C*H*CH=CH), 6.50 (d, *J* = 1 Hz, 2H, C*H*=C*H*), 6.81 (t, *J* = 2 Hz, 1H, CH_{Ar}), 7.25 (d, *J* = 2 Hz, 2H, CH_{Ar}); ^{13}C{^{1}H} NMR ($CDCl_3$) : 37.9, 47.7, 56.3, 61.8, 76.3, 78.4, 81.1, 107.9, 109.1, 131.9, 136.8, 158.6, 165.7, 176.2; ESI MS (+) : *m/z* 444.05 $[M+Na]^+$, 864.79 $[2M+Na]^+$.

MaAc$_2$. Compound **9** (1.680 g, 3.98 mmol) was dissolved in toluene (8 mL) and the solution was refluxed for 48 h. **MaAc$_2$** was obtained as a white solid (0.980 g, 70%) after purification by column chromatography eluting with dichloromethane. ^{1}H NMR ($CDCl_3$) : 2.53 (t, *J* = 2 Hz, 2H, C≡*H*), 3.95 (t, *J* = 5 Hz, 2H, NCH_2CH_2), 4.42 (t, *J* = 5 Hz, 2H, NCH_2CH_2), 4.73 (d, *J* = 2 Hz, 4H, CH_2C≡H), 6.74 (s, 2H, C*H*=C*H*), 6.81 (t, *J* = 2 Hz, 1H, CH_{Ar}), 7.25 (d, *J* = 2 Hz, 2H, CH_{Ar}); ^{13}C{^{1}H} NMR ($CDCl_3$) : 37.0, 56.3, 62.8, 76.1, 78.3, 108.2, 109.1, 131.8, 134.5, 158.7, 165.9, 170.6; ESI MS (+) : *m/z* 376.39 $[M+Na]^+$, 729.11 $[2M+Na]^+$.

10. 4-dimethylaminopyridine (0.204 g, 1.67 mmol) was added to a dry dichloromethane (50 mL) solution of **8** (0.700 g, 3.35 mmol) and (±)-α-lipoic acid (0.828 g, 4.02 mmol), and the mixture was allowed to react for 5 min. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide methiodide (1.381 g, 4.65 mmol) was then added and the reaction mixture which was stirred for 12 h. The resulting precipitate was filtered and washed with dichloromethane. The filtrate was concentrated and compound **10** was obtained as an oil (1.330 g, quantitative yield) after purification by column chromatography eluting with dichloromethane followed by an acetone:dichloromethane (1:4) mixture. ^{1}H NMR ($CDCl_3$) : 1.41 (m, 2H), 1.50-1.70 (m, 4H), 1.88 (m, 1H), 2.25 (t, *J* = 7 Hz, 2H), 2.42 (m, 1H), 2.85 (s, 2H, NCOC*H*), 3.00-3.20 (m, 2H), 3.54 (m, 1H), 3.72 (m, 2H, NCH_2CH_2), 4.19 (m, 2H, NCH_2CH_2), 5.23 (s, 2H, C*H*CH=CH), 6.49 (s, 2H,

C*H*=C*H*); $^{13}C\{^1H\}$ NMR ($CDCl_3$) : 23.9, 28.2, 33.3, 34.1, 37.4, 38.1, 39.8, 47.1, 55.9, 60.0, 80.5, 136.2, 172.6, 175.6; ESI MS (+) : *m/z* 419.9 $[M + Na]^+$, 816.6 $[2M + Na]^+$.

MaLA. Compound **10** (1.260 g, 3.17 mmol) was dissolved in toluene (30 mL) and the solution was refluxed for 72 h. **MaLA** was obtained as a yellow solid (0.910 g, 87%) after evaporation of the volatiles. ^1H NMR ($CDCl_3$) : 1.44 (m, 2H), 1.63 (m, 4H), 1.91 (m, 1H), 2.27 (t, *J* = 7 Hz, 2H), 2.44 (m, 1H), 3.14 (m, 2H), 3.56 (m, 1H), 3.78 (t, *J* = 5 Hz, 2H, NCH_2CH_2), 4.22 (t, *J* = 5 Hz, 2H, NCH_2CH_2), 6.72 (s, 2H, C*H*=C*H*); $^{13}C\{^1H\}$ NMR ($CDCl_3$) : 24.5, 28.8, 33.8, 34.7, 37.1, 38.6, 40.3, 56.4, 61.4, 134.3, 170.5, 173.3; EI MS (+) : *m/z* 329 $[M + Na]^{\cdot+}$. Anal. Calcd for $C_{14}H_{19}N_1O_4S_2$: C, 51.04; H, 5.81; N, 4.25; S, 19.47. Found: C, 50.93; H, 5.90; N, 4.25; S, 18.86.

Dendrimers

G1-Fu$_8$ (n = 0). Sodium ascorbate (0.055 g, 0.28 mmol) was added to a solution of **G0-Ac$_4$** (0.100 g, 0.35 mmol) and **AzFu$_2$ (n = 0)** (0.651 g, 1.67 mmol) in THF (2 mL). $CuSO_4{\cdot}5H_2O$ (0.034 g, 0.14 mmol) was dissolved in H_2O (1mL) and the solution was added to the mixture, which was stirred at room temperature for 16 h. The volatiles were then evaporated and the crude product was purified by column chromatography, eluting with an ethyl acetate and dichloromethane (1:4) mixture and finally with a methanol and dichloromethane (1:4) mixture to give **G1-Fu$_8$ (n = 0)** as a sticky oil (0.514 g, 80%). *Unfortunately, this compound did not lead to the formation of a second generation dendrimer when reacted with an excess of **MaAc$_2$** under various conditions. This result prompted us to prepare another first generation dendrimer with more electron rich peripherial furan rings (n = 2).* ^1H NMR ($CDCl_3$) : 1.31 (s, 12H, CH_3), 3.39 (s, 8H, CCH_2O), 4.40-4.53 (m, 24H, CCH_2O), 4.56 (m, 8H, NCH_2CH_2), 4.65 (m, 8H, NCH_2CH_2), 6.49 (s, 8H, CH_{fur}), 7.13 (s, 8H, CH_{fur}), 7.56 (s, 8H, OCH_{fur}), 7.74 (s, 4H, NC*H*).

G1-Fu$_8$ (n = 2). Sodium ascorbate (0.014 g, 0.07 mmol) was added to a solution of **G0-Ac$_4$** (0.026 g, 0.09 mmol) and **AzFu$_2$ (n = 2)** (0.200 g, 0.43 mmol) in THF (1 mL). $CuSO_4{\cdot}5H_2O$ (0.009 g, 0.04 mmol) was dissolved in H_2O (1 mL) and the solution was added to the

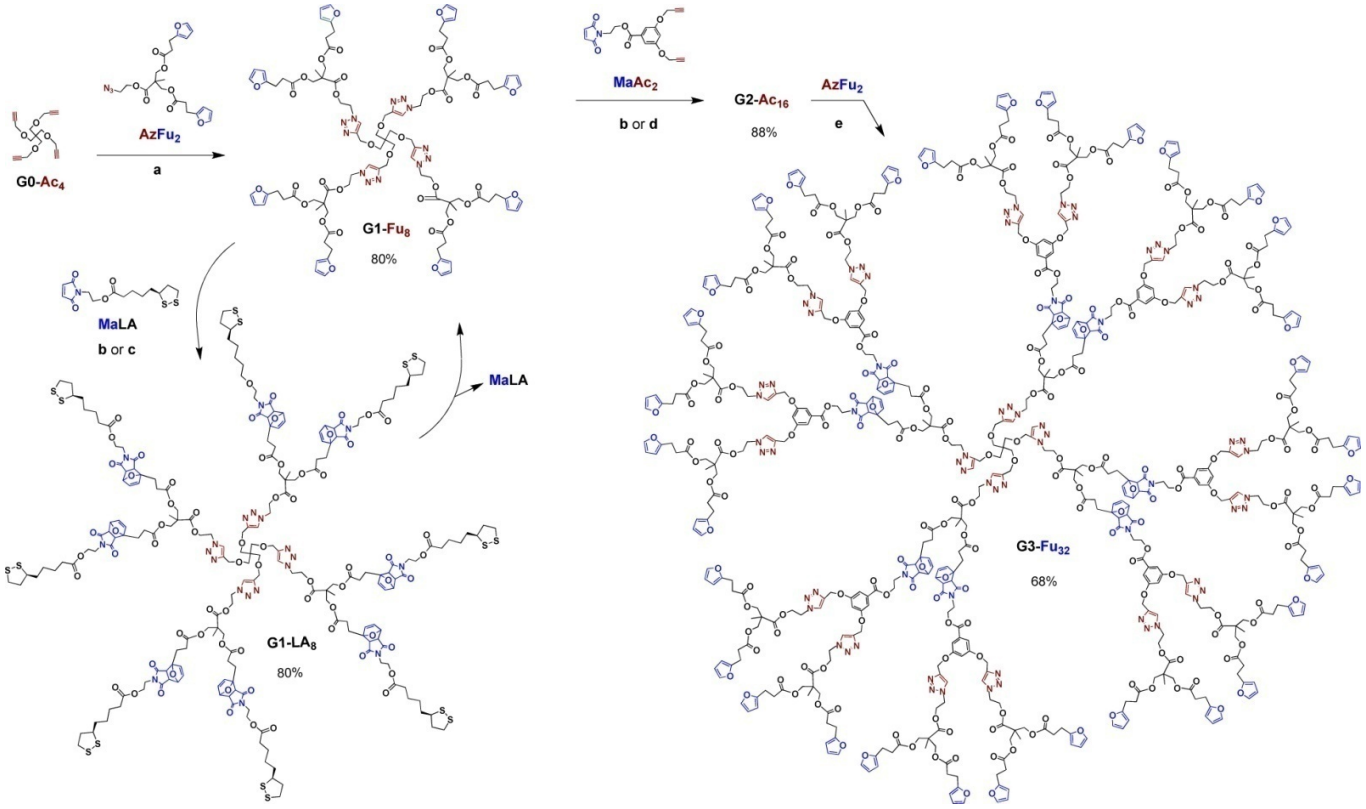
mixture, which was stirred at room temperature for 2 h. The volatiles were then evaporated and the crude product was purified by column chromatography eluting with dichloromethane, followed by an acetone:dichloromethane (1:3) mixture and finally with acetone, to give **G1-Fu$_8$ (n = 2)** as a sticky oil (0.150 g, 80%). *The same reaction was also performed in a microwave (MW) reactor at 65 ºC for 30 min and* ***G1-Fu$_8$ (n = 2)*** *was obtained with 64% yield. The lower yield obtained could be explained by the occurance of side reactions, under these conditions, since the yield was found to be even lower (32%) when the reaction was left in the MW reactor for a longer period of time (1 h). Nevertheless, this demonstrates that the synthesis can be performed in a short period of time when MWs are used.* ^{1}H NMR ($CDCl_3$) : 1.13 (s, 12H, CH_3), 2.62 (t, *J* = 8 Hz, 16H, CH_2CH_2Fu), 2.91 (t, *J* = 8 Hz, 16H, CH_2CH_2Fu), 3.44 (s, 8H, CCH_2O), 4.15 (t, *J* = 12 Hz, 8H, NCH_2CH_2), 4.17 (t, *J* = 12 Hz, 8H, NCH_2CH_2), 4.45-4.65 (m, 24H, CCH_2O), 5.99 (s, 8H, CH_{fur}), 6.24 (s, 8H, CH_{fur}), 7.26 (s, 8H, OCH_{fur}), 7.69 (s, 4H, NC*H*); ^{13}C{^{1}H} NMR ($CDCl_3$) : 17.7, 23.4, 32.5, 44.4, 45.3, 48.8, 63.1, 64.9, 65.3 69.1, 105.5, 110.3, 123.2, 141.3, 145.5, 153.8, 171.9, 172.2; ESI MS (+) : *m/z* 2099.8 $[(M_{G1\text{-}Fu8\ (n\ =\ 2)} + Na)]^+$.

G2-Ac$_{16}$. *Method A.* **MaAc$_2$** (0.372 g, 1.05 mmol) was added to a solution of **G1-Fu$_8$ (n = 2)** (0.080 g, 0.038 mmol) in ethyl acetate (3 mL) and the mixture was stirred at 55°C for 3 days. The solvent was then evaporated and **G2-Ac$_{16}$** was obtained after purification by column chromatography, eluting with an ethyl acetate: dichloromethane (1:2) mixture followed with acetone. When the reaction did not go to completion, **MaAc$_2$** (0.372 g, 1.05 mmol) was added to a solution of the resultant defected dendrimer in chloroform (3 mL) and the mixture was stirred at room temperature for 24 h. **G2-Ac$_{16}$** was obtained (0.166 g, 88%, 10% *endo*) after purification by column chromatography, using the same conditions. *Method B.* **MaAc$_2$** (1.840 g, 5.21 mmol) was added to a solution of **G1-Fu$_8$ (n = 2)** (0.108 g, 0.052 mmol) in chloroform (6-8 mL) and the mixture was stirred at room temperature for 6-11 days. The solvent was then evaporated and **G2-Ac$_{16}$** was obtained (50% *endo*) after purification by column chromatography (same conditions as for *method A*). ^{1}H NMR ($CDCl_3$, exo isomer) : 1.14 (s, 12H, CH_3), 2.20-2.60 (m,

32H, CH_2CH_2fur), 2.56 (s, 16H, CCH), 2.78 (d, J = 6 Hz, 8H, NCOCH), 2.96 (d, J = 6 Hz, 8H, NCOCH), 3.43 (s, 8H, CCH_2OCH_2C), 3.85 (m, 16H, OCNCH_2CH_2), 4.14 (m, 16H, CCH_2O), 4.40 (m, 16H, OCNCH_2CH_2), 4.52 (m, 16H, CHNCH_2CH_2 + CCH_2OCH_2C), 4.64 (m, 8H, CHNCH_2CH_2), 4.70 (s, 32H, OCH_2CCH), 5.13 (s, 8H, CH_{fur}O), 6.30 (br d, 8H, CH_{fur}), 6.47 (br d, 8H, CH_{fur}), 6.77 (s, 8H, ArH), 7.21 (s, 16H, ArH), 7.73 (s, 4H, NCH); $^{13}C\{^1H\}$ NMR ($CDCl_3$, exo isomer) : 17.8, 24.7, 29.4, 29.9, 37.9, 46.5, 48.9, 49.2, 50.7, 56.2, 61.7, 63.2, 64.9, 65.4, 69.2, 76.2, 78.3, 80.7, 90.9, 107.8, 109.1, 123.4, 131.8, 137.8, 138.4, 145.5, 158.6, 165.7, 172.2, 172.3, 174.6, 175.9; MALDI-TOF MS (+) : *m/z* 2099.6 $[M_{G1\text{-}Fu8\ (n\ =\ 2)} + Na]^+$, 2115.6 $[M_{G1\text{-}Fu8\ (n\ =\ 2)} + K]^+$; ESI MS (+) : *m/z* 2514 $[M_{G2\text{-}Ac16} + Cu]^{2+}$.

G3-Fu$_{32}$. Sodium ascorbate (0.056 g, 0.28 mmol) was added to a solution of **G2-Ac$_{16}$** (0.256 g, 0.05 mmol) and **AzFu$_2$ (n = 2)** (0.740 g, 1.65 mmol) in THF (3 mL). $CuSO_4 \cdot 5H_2O$ (0.040 g, 0.16 mmol) was dissolved in H_2O (1 mL) and the solution was added to the reaction mixture. After 16 h, the volatiles were evaporated and **G3-Fu$_{32}$** was obtained (0.426 g, 68%) after a purification by column chromatography eluting with an acetone:dichloromethane (1:2) mixture, and finally with acetone. ^1H NMR ($CDCl_3$) : 1.11 (br s, 60H, CH_3), 2.20-2.60 (m, 32H, CH_2CH_2fur), 2.62 (t, J = 7 Hz, 64H, CH_2CH_2fur), 2.81 (d, J = 6 Hz, 8H, NCOCH), 2.89 (t, J = 7 Hz, 64H, CH_2CH_2fur), 2.98 (d, J = 6 Hz, 8H, NCOCH), 3.42 (br s, 8H, CCH_2O), 3.83 (br s, 16H, OCNCH_2CH_2), 4.15 (m, 80H, CCH_2O), 4.38 (br s, 16H, OCNCH_2CH_2), 4.40-4.70 (24H, CHNCH_2CH_2 + NCCH_2O), 4.48 (br s, 32H, CHNCH_2CH_2), 4.59 (br s, 32H, CHNCH_2CH_2), 5.10 (br s, 8H, CH_{fur}O), 5.18 (br s, 32H, ArOCH_2), 5.98 (br s, 32H, CH_{fur}), 6.22 (br s, 40H, CH_{fur}), 6.40 (br s, 8H, CH_{fur}), 6.78 (br s, 8H, ArH), 7.21 (s, 16H, ArH), 7.26 (br s, 32H, CH_{fur}O), 7.76 (br s, 20H, NCH); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz): 17.5, 23.2, 24.5, 24.9, 25.6, 29.7, 32.3, 33.9, 37.8, 46.3, 48.9, 50.5, 53.4, 61.6, 61.9, 62.9, 65.2, 80.7, 90.8, 105.4, 107.3, 108.5, 110.2, 123.7, 131.7, 141.2, 143.7, 153.7, 159.2, 165.6, 171.8, 172.1, 172.6, 174.5, 175.8. MALDI-TOF MS (+) : *m/z* 2099.6 $[M_{G1\text{-}Fu8\ (n\ =\ 2)} + Na]^+$, 1270.7 $[M_{MaFu4} + Na]^+$.

G1-LA$_8$. *Method A.* **MaLA** (0.477 g, 1.45 mmol) was added to a solution of **G1-Fu$_8$ (n = 2)** (0.050 g, 0.02 mmol) in ethyl acetate (3 mL) and the mixture was stirred at 55°C for 3 days. The solvent was then evaporated and **G1-LA$_8$** was obtained after purification by column chromatography, eluting with an acetone:dichloromethane (1:4) mixture, followed with acetone. When the reaction did not go to completion, **MaLA** (0.477 g, 1.45 mmol) was added to a solution of the resultant defected dendrimer in chloroform (3 mL) and the mixture was stirred at room temperature for 24 h. **G1-LA$_8$** was obtained (0.091 g, 80%, 6% *endo*) after purification by column chromatography, using the same conditions. *Method B.* **MaLA** (0.350 g, 1.06 mmol) was added to a solution of **G1-Fu$_8$ (n = 2)** (0.060 g, 0.03 mmol) in chloroform (2 mL) and the mixture was stirred at room temperature for 4 days. The solvent was then evaporated and **G1-LA$_8$** was obtained (50% *endo*) after purification by column chromatography (same conditions as for *method A*). ^{1}H NMR ($CDCl_3$, exo isomer) : 1.17 (s, 12H, C*H*$_3$), 1.46 (m, 16H, LA), 1.50-1.75 (m, 32H, LA),1.92 (m, 8H, LA), 2.27 (t, *J* = 7 Hz, 16H), 2.30-2.60 (m, 40H, C*H*$_2$C*H*$_2$fur + LA), 2.79 (d, *J* = 6 Hz, 8H, NCOC*H*), 2.99 (d, *J* = 6 Hz, 8H, NCOC*H*), 3.14 (m, 16H, LA), 3.45 (s, 8H, CC*H*$_2$OC*H*$_2$C), 3.56 (m, 8H, LA), 3.72 (m, 16H, OCNC*H*$_2$C*H*$_2$), 4.00-4.30 (m, 32H, CC*H*$_2$O + OCNC*H*$_2$C*H*$_2$), 4.54 (br s, 16H, CHNC*H*$_2$C*H*$_2$ + CC*H*$_2$OC*H*$_2$C), 4.65 (m, 8H, CHNC*H*$_2$C*H*$_2$), 5.17 (s, 8H, C*H*$_{fur}$*O*), 6.34 (br d, *J* = 6 Hz, 8H, C*H*$_{fur}$), 6.52 (br d, *J* = 5 Hz, 8H, C*H*$_{fur}$), 7.75 (s, 4H, NC*H*); ^{13}C{^{1}H} NMR ($CDCl_3$, exo isomer): 17.8, 24.5, 24.7, 28.8, 29.4, 29.9, 33.9, 34.7, 38.0, 38.6, 40.4, 46.5, 48.9, 49.2, 50.7, 56.5, 60.7, 63.2, 65.0, 65.4, 69.2, 80.7, 90.9, 123.4, 137.8, 138.3, 172.2, 172.3, 173.3, 174.6, 175.9; MALDI-TOF MS (+) : *m/z* 2099 $[(M_{G1\text{-}Fu8\ (n\ =\ 2)} + Na)]^+$, 2115 $[(M_{G1\text{-}Fu8\ (n\ =\ 2)} + K)]^+$; ESI MS (+) : *m/z* 2418 $[(M_{G1\text{-}La8} + Cu)]^{2+}$.



Scheme S1. Synthesis of the first generation dendrimer, **G1-Fu$_8$**, its conjugation with LA to give **G1-LA$_8$**, and the preparation of the second and third generation dendrimers, **G2-Ac$_{16}$** and **G3-Fu$_{32}$**. Conditions : a) $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, THF/H_2O, 22 °C, 2h; b) EtOAc, 55 °C, 72 h and then $CHCl_3$, 22 °C, 24 h; c) $CHCl_3$, 22 °C, 4 days; d) $CHCl_3$, 22 °C, 6-11 days; e) $CuSO_4{\cdot}5H_2O$, Sodium ascorbate, THF/H_2O, 22 °C, 16 h.

Gel permeation chromatography (GPC)

GPC was performed in DMF at 30 °C at a rate of 1 mL/min on a Viscogel G-MBLMW-3078 column equipped with a Waters 410 refractive index detector that was calibrated with polyethylene glycol standards. GPC traces of all the dendrimers prepared are illustrated in Fig. **S1**. As noted, a small peak of lower mass than **G1-Fu$_8$ (n = 2)** (retention time ~9.5 min, PDI = 1.09) was observed in the chromatogram of **G2-Ac$_{16}$**. **MaAc$_2$** was ruled out as being the species

responsible for the peak observed at ~ 9.5 min since a pure sample of **$MaAc_2$** displayed a retention time higher than 10 min. Oligomerization of the maleimide or the acetylene units of **$MaAc_2$** could be a possible explanation for this small peak.

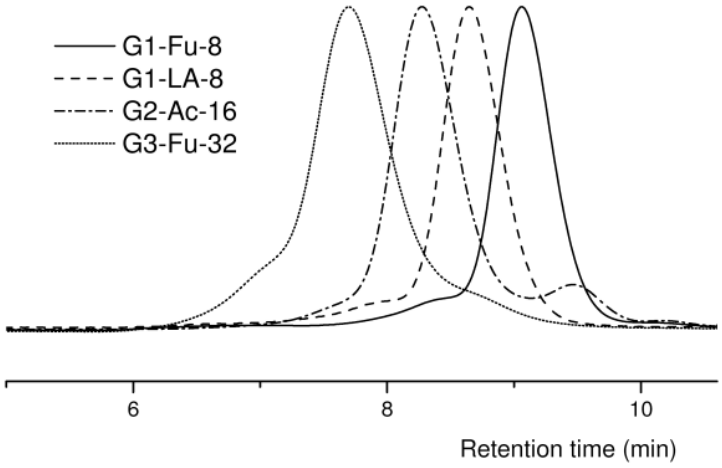


Figure S1. GPC analyses of **$G1\text{-}Fu_8$ (n = 2)** (PDI = 1.12), **$G1\text{-}LA_8$** (PDI = 1.04), **$G2\text{-}Ac_{16}$** (PDI = 1.06) and **$G3\text{-}Ac_{32}$** (PDI = 1.23).

Time dependence MaLA release at different temperatures

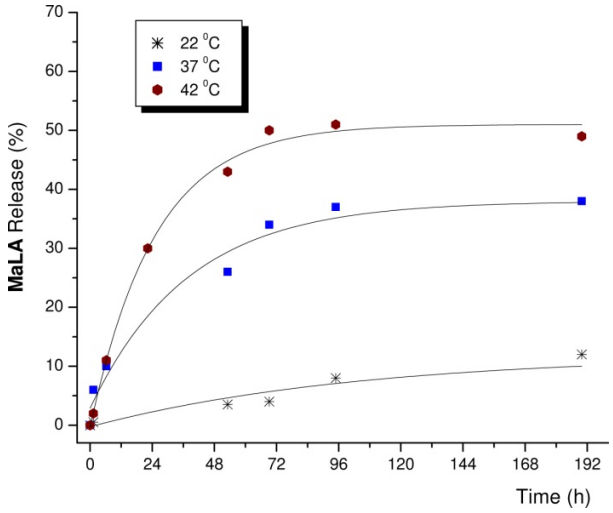


Figure S2. Time dependance of the MaLA release (% ± 5 %) from **$G1\text{-}LA_8$** at different temperatures (22 ºC, 37 ºC and 42 ºC), as evaluated by ^{1}H NMR (3 mM dmso-d_6 solutions, based on the integration of the peaks corresponding to the DA and rDA furan protons).

Materials and Methods for Biology Experiments

Cell Culture and Media

Murine microglia (N9) cells obtained from ATCC were seeded in Iscove's Modified Dulbecoo's Medium (IMDM) (Gibco) containing 5% of fetal bovine serum (Gibco) and1% penicillin–streptomycin (Gibco). For all experiments, unless otherwise stated, cells were seeded in 96-well plate (Costar) at a density of $2.5x10^4$ cells/well maintained at 37 °C, 5% CO_2 in a humidified atmosphere and were grown in serum containing media for 24 hours before cell treatments to attain confluency. Cells were used between 10 and 30 passages.

Cells Viability

Mitochondrial metabolic activity of cells was measured using MTT assay. Media was aspirated and cells were treated in serum-free media with **G1-LA$_8$** or **G1-Fu$_8$** (1.56, 3.125, 6.25, 12.5, 18.75, 25, 37.5 and 50 μM) in equimolar concentration with respect to (±)-α-Lipoic acid (LA) (Sigma) (12.5, 25, 50, 100, 150, 200, 300 and 400 μM) for 24 h.

Following treatment media was removed and replaced with fresh serum-free media (200 μl/well). MTT solution (0.5 mg/ml) (Sigma) was added to each well and incubated for 30 min at 37° C. Formazan crystals were dissolved by adding dimethylsulphoxide (DMSO) (Sigma) and quantified by measuring the absorbance of the solution at 595 nm using Benchmark microplate reader (Bio-Rad, Canada). The extent of formazan conversion is expressed in percentages relative to the untreated control. Results are expressed as mean ± SEM obtained from at least three independent experiments performed in triplicate.

The copper compounds used in the "click" chemistry based synthesis of dendrimers are in catalytic amounts, and the copper content in the "clicked" products, specifically those related to the pharmaceutical industry, has been widely discussed (*Pharm Res*. **2008**, *25*, 2216–2230). It has been demonstrated by many researchers that the "clicked" products contain

nonmeasureable amounts of copper (*Chem. Phys. Lipids*, **2003**, *126*, 95-110). In addition, nanoconjugates reported here were found to be noncytotoxic.

Nitric Oxide release

Nitric oxide released from cells was measured using Griess reagent (Sigma). Briefly, cells were seeded in 24-well plates (Sarstedt) at a density of $2x10^5$ cells/well for 24 h. Cells were co-treated with LPS (10 µg/ml) (Sigma) and **G1-LA$_8$** (12.5 µM), **G1-Fu$_8$** (12.5 µM) or equimolar concentration of LA (100 µM) for 24 h. Following treatment, 50 µl of cells supernatant from each well were collected and transferred to another new plate. 50 µl of Griess reagent was added to each well and incubated at room temperature for 15 min. Absorbance of the produced nitrite was measured using spectrophotometer at 540 nm. Results are expressed as mean ± SEM obtained from at least three independent experiments performed in triplicate.

Superoxide anion and reactive oxygen species detection

Superoxide anion and reactive oxygen species were detected using dihydroethidium (DHE) (Molecular probes) and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Molecular probes) respectively. Briefly, Media was aspirated and cells were treated in fresh serum-free media with **G1-LA$_8$**, **G1-Fu$_8$** (12.5 µM) in equimolar concentration with respect to LA (100 µM) for 24 h. 3 h prior to time-end point treatment, cells were stressed with methyl viologen dichloride (Paraquat, 500 µM) (Sigma), or hydrogen superoxide (H_2O_2) (200 µM) (EMD). Following treatment, media was replaced with fresh media containing DHE (20 µM) or DCFH-DA (20 µM) and incubated for 30 min at 37°C. After which, cells were washed once with media (200 µl/well) and fresh serum free media was added. Fluorescence of ethidium and 2′,7′-dichlorodihydrofluorescein (DCF) was determined with Fluostar Optima spectrofluorometer (BMG, LabTech) using excitation/emission wavelengths= 544/590 nm and 485/520 respectively. Fluorescent images of cells were acquired at 40x with a Leica DFC350FX monochrome digital camera connected to a Leica DMI4000B inverted fluorescence microscope.

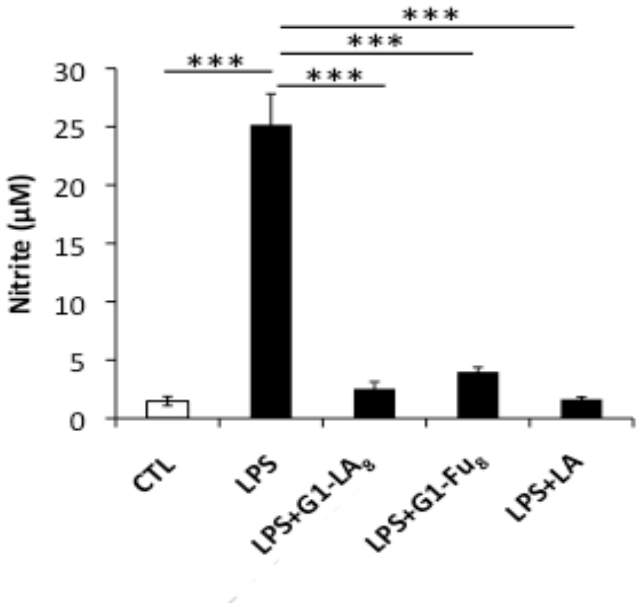


Figure S3. Reduction of nitrite released in microglia following LPS exposure by **G1-LA$_8$**, **G1-Fu$_8$** or LA. Nitrite released from microglia co-treated with **G1-LA$_8$** (12.5 μM), ¸**G1-Fu$_8$** (12.5 μM) or LA in equimolar concentration (100 μM) and LPS 10 μg/ml for 24 h is measured using Griess reagent (n=9). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates. Statistically significant differences are indicated by p*** <0.001.

Intracellular glutathione measurement

Intracellular glutathione was measured using monochlorobimane (mCBI) (Calbiochem). Media was aspirated and cells were treated in fresh serum-free media with **G1-LA$_8$** (12.5 μM) in equimolar concentration with respect to LA (100 μM) for 24 h. Following treatment, media was replaced with fresh media containing mCBI (50 μM) and incubated for 1 h at 37°C. Cells were washed once with IMDM (200 μl/well) and kept in a fresh serum free medium and fluorescence of mCBI-GSH adduct was determined with Fluostar Optima spectrofluorometer (BMG, LabTech) using excitation/emission wavelengths= 380/460 nm. Fluorescent images of cells were acquired at 40x with a Leica DFC350FX monochrome digital camera connected to a Leica DMI4000B

inverted fluorescence microscope.

GSH reacts specifically with mCBi, a non-fluorescent membrane permeable dye, to form a fluorescent adduct (mCBi-GSH). MCBi-GSH binding was determined in non-stressed cells following the treatment with **G1-LA$_8$** and unconjugated LA. Fluorescence micrographs clearly showed a significant increase in mCBi fluorescence with unbound LA whereas **G1-LA$_8$** maintained GSH homeostasis comparable to the unstressed control cells (Fig. **S4**). Glutathione is considered to be a key regulator of the cellular redox homeostasis due to its low redox potential (240 mV at pH 7.0), high intracellular abundance (1–13 mM) and ubiquitous expression in eukaryotic cells.

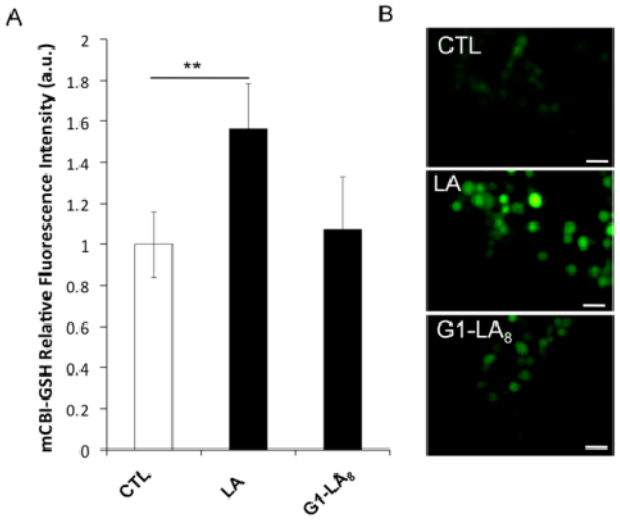


Figure S4. Intracellular glutathione level is not enhanced upon exposure to G1-LA$_8$. A) Spectrofluorometric detection and quantification of mCBi-GSH fluorescent intensity (arbitrary units). Controls (CTL) = untreated cells = 1) (n=9). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates. Statistically significant differences are indicated by p** <0.01. B) Fluorescent micrographs showing mCBi-GSH fluorescent adduct following LA or G1-LA$_8$ treatment. Scale bar, 20 *μm.*

Figure 1 from the Manuscript with expanded Figure caption:

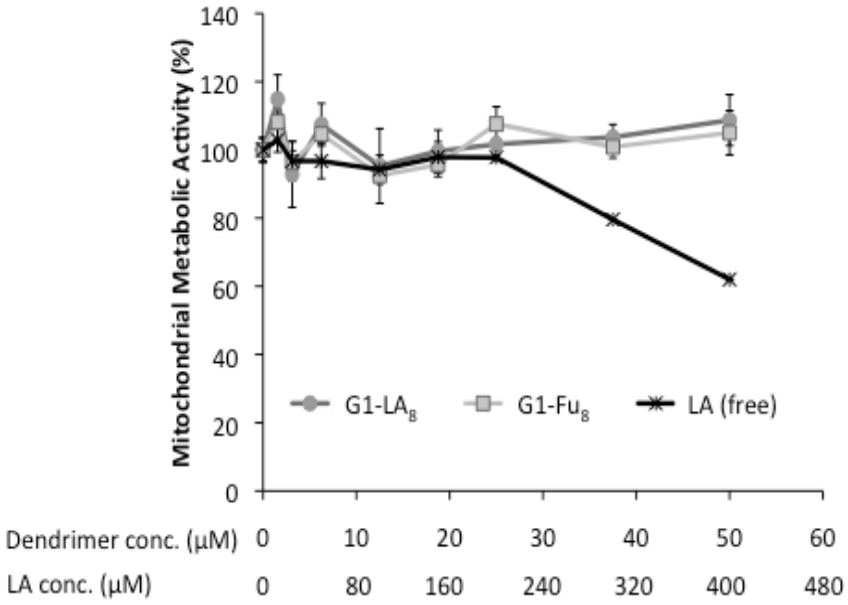


Figure 1. Mitochondrial metabolic activity determined by MTT assay, upon exposure to **G1-LA$_8$** (0-50 μM, dendrimer conc.), **G1-Fu$_8$** (0-50 μM, dendrimer conc.) or LA (0-400 μM, LA conc.) for 24 h. Note that LA is added in equimolar concentration to that of **G1-LA$_8$**. Metabolic activity (%) in treated cells was expressed relative to controls (untreated cells that were set to 100%) (n=9). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates.

Figure 2 from the Manuscript with expanded Figure caption:

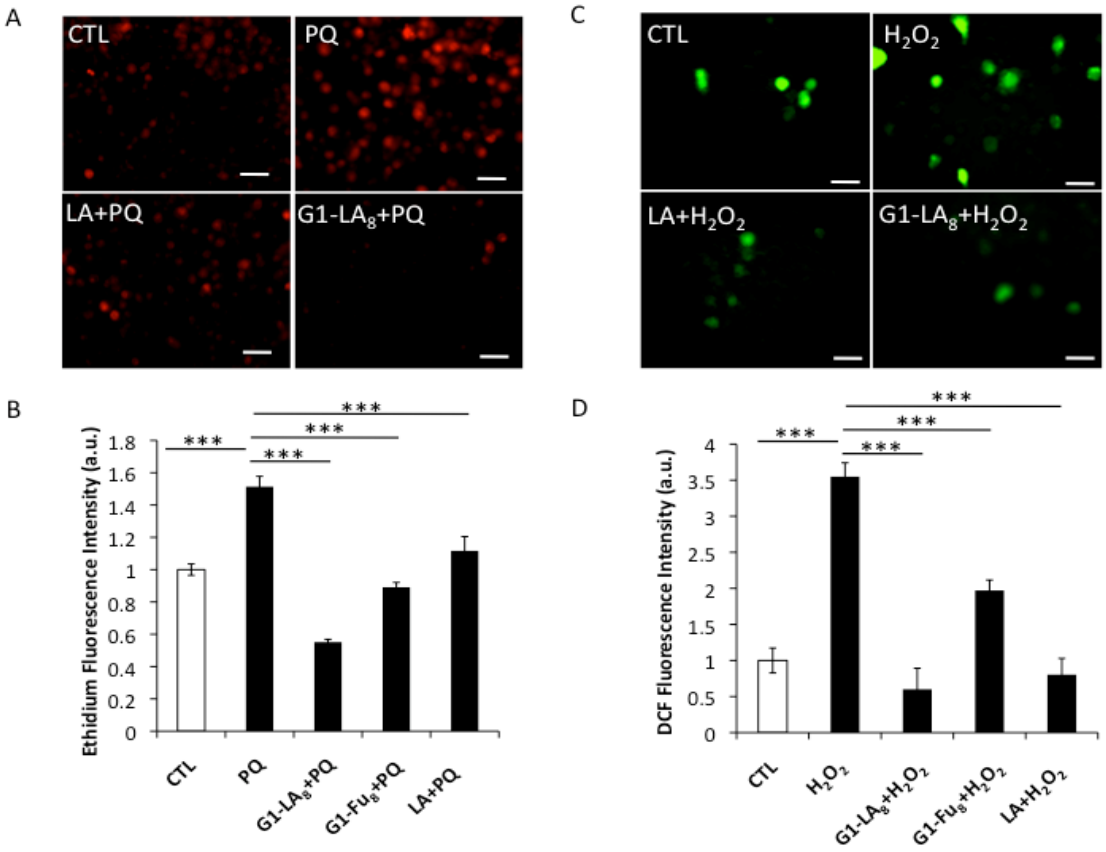


Figure 2. Generation of superoxide and reactive oxygen species (ROS) by paraquat (PQ) and H_2O_2 respectively in cells treated with **G1-LA$_8$**, **G1-Fu$_8$** or LA. Microglia were treated with **G1-LA$_8$** (12.5 μM), **G1-Fu$_8$** (12.5 μM) or LA in equimolar concentration (100 μM) for 24 h. A) Fluorescent micrographs showing superoxide anion (O_2^-) generation following PQ exposure (500 μM, 3 h) using dihydroethidium (DHE). Scale bar, 20 *μm.* B). Spectrofluorometric determination of ethidium fluorescence intensity (arbitrary units). Controls (CTL) = untreated cells = 1 (n=6). C) Fluorescent micrographs showing ROS generation following H_2O_2 exposure (200 μM, 3 h) using DCFH-DA. Scale bar, 20 *μm.* D) Spectrofluorometric determination of DCF fluorescence intensity (arbitrary units). Controls (CTL) = untreated cells = 1 (n=6). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates. Statistically significant differences are indicated by p*** <0.001.

Notes and References

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