

Supplementary Information

Assessment of H₂S *in vivo* using the recently developed mitochondria-targeted mass spectrometry probe MitoA

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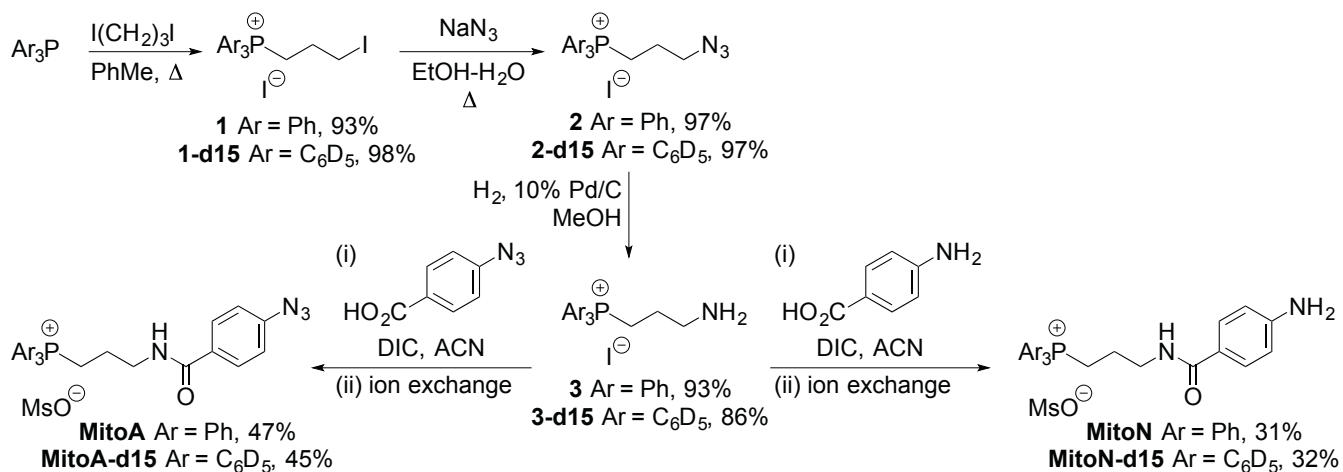
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A



B

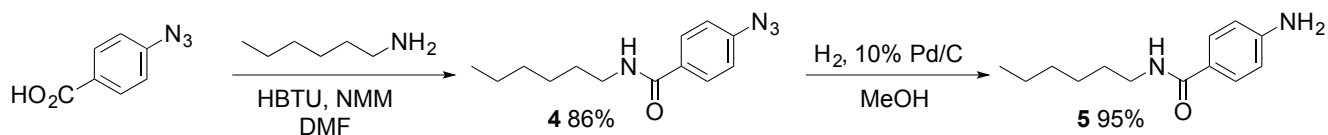


Fig. S1 (A) Synthesis of MitoA and MitoN. ACN = acetonitrile, DIC = *N,N'*-diisopropylcarbodiimide. (B) Control compounds **4** and **5** lacking the TPP targeting group were also prepared. Coupling of hexylamine with 4-azidobenzoic acid was achieved using *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) to give amide **4**, which was then hydrogenated to give the amine **5**.

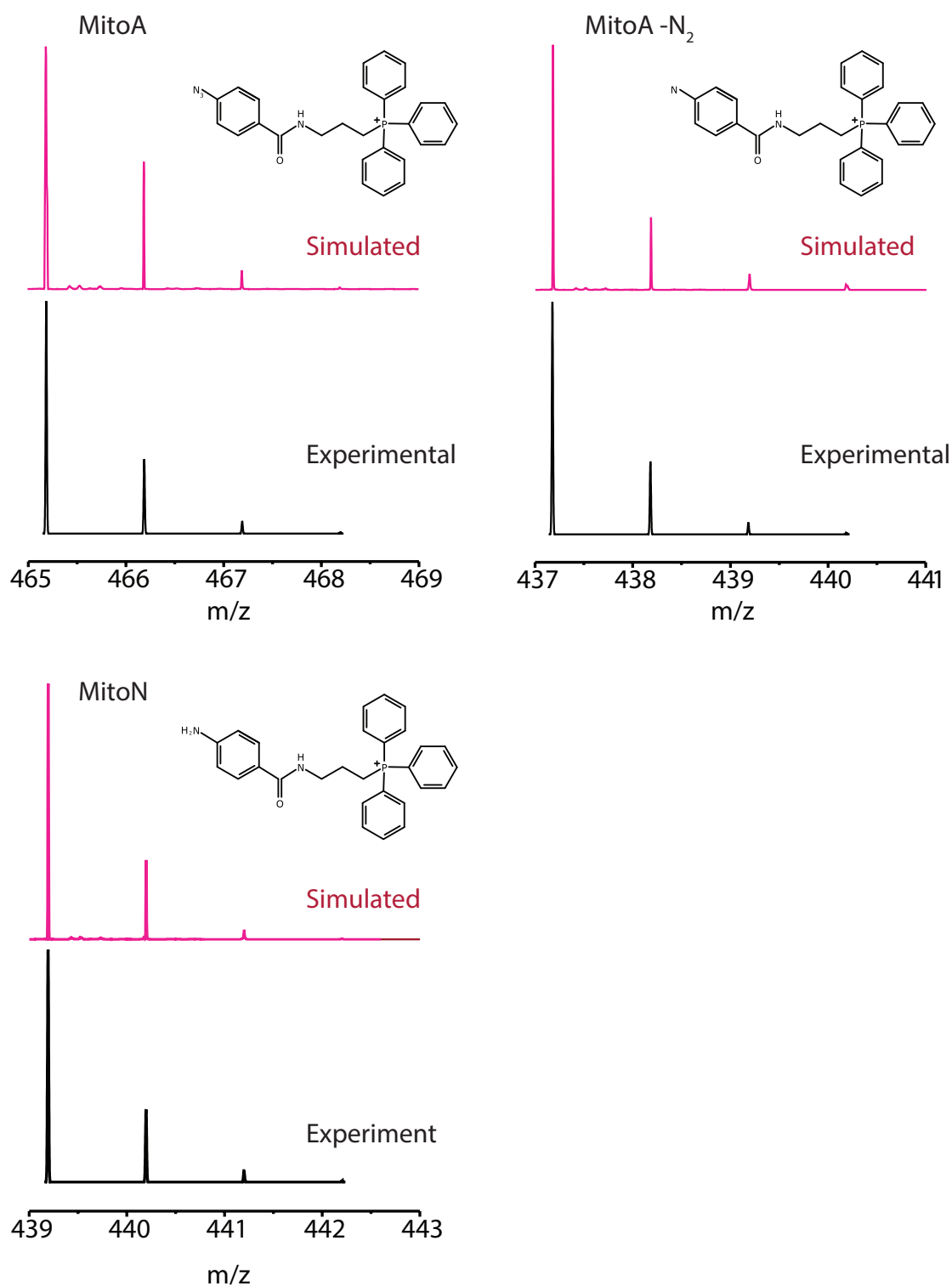


Fig. S2 Mass spectra of MitoA and MitoN. Observed (black) and simulated peaks (pink) for: MitoA, $[C_{28}H_{26}N_4OP]^+$ predicted m/z 465.1839. MitoA nitrene decomposition product from N_2 neutral loss $[C_{28}H_{26}N_2OP]^+$ predicted m/z 437.1782 MitoN, $[C_{28}H_{29}N_2OP]^+$ predicted m/z 439.1934. Spectra were obtained using an ultra-high resolution electron spray time of flight mass spectrometer (maXis Bruker Daltonics).

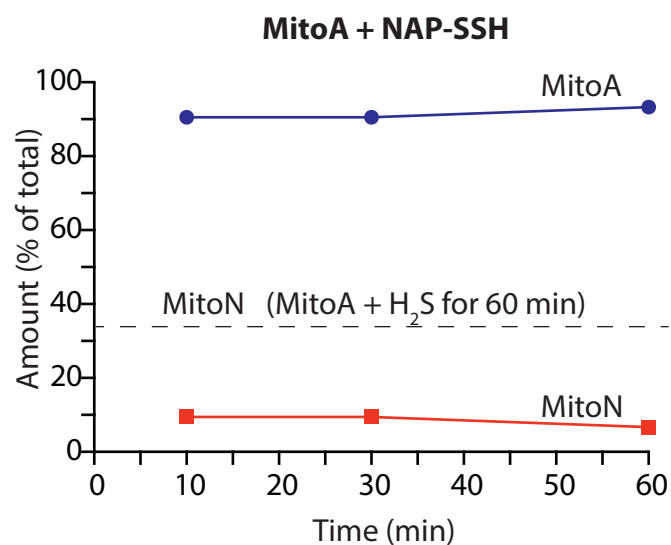


Fig. S3 Reactivity of MitoA with a persulfide. MitoA (10 μM) was mixed with NAP-SSH (25 μM) in KCl buffer, pH 7.2 at 37 $^{\circ}\text{C}$. Samples were taken at the indicated times and analysed by RP-HPLC. The amounts of MitoA and MitoN were determined by RP-HPLC from standard curves and used to calculate the amount of that compound as a percentage of the sum of MitoA and MitoN. The dashed line shows the amount of MitoN formed by incubating MitoA (10 μM) NaS₂ (25 μM) in KCl buffer, pH 7.2 at 37 $^{\circ}\text{C}$ for 60 min.

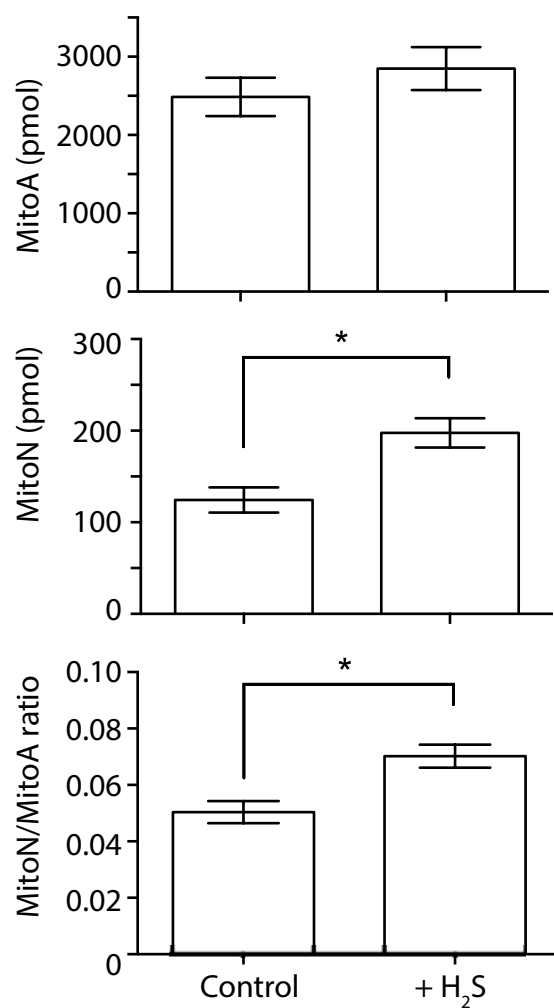


Fig. S4 Reactivity of MitoA with H₂S during extraction for mass spectrometry MitoA (10 μ M) was incubated with NaHS (10 μ M) for 4 h in 95 % ACN. After the incubation, samples were dried under vacuum, resuspended in 20 % ACN, 0.1 % FA with internal standards and the amounts of MitoA, MitoN and the MitoN/MitoA ratio were quantified by LC-MS/MS. Data are means \pm SEM, n = 4. *p < 0.05; two-tailed t-test.

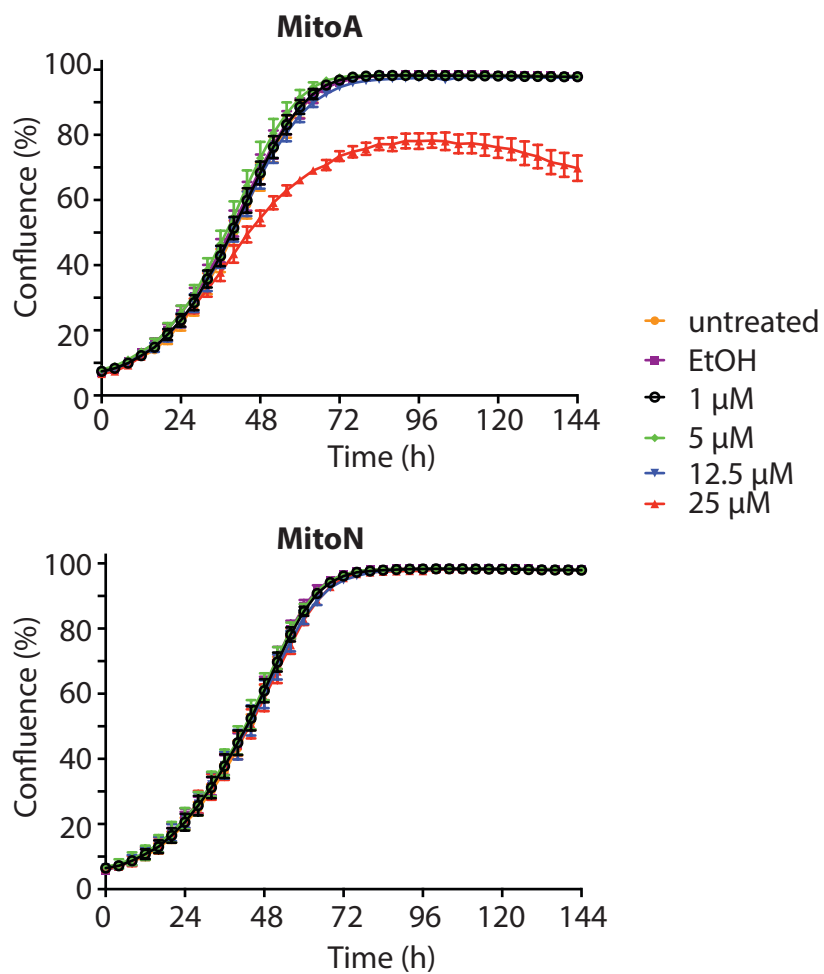


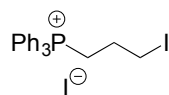
Fig. S5 Effect of MitoA and MitoN on cell proliferation. C2C12 cells were plated at 10,000 cells per well in 6-well plates ($\sim 1\,000$ cells per cm^2) and treating with MitoA or MitoN. Cell proliferation was then monitored by measuring confluency via automated bright field imaging (IncuCyte HD, EssenBioScience) for 144 h. Data are means \pm SEM, $n = 3$.

Supplementary Experimental Procedures

Chemical syntheses

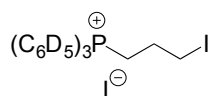
All reactions under an inert atmosphere were carried out using oven-dried or flame-dried glassware and solvents were added via syringe. Reagents were obtained from commercial suppliers and used without further purification. Dry solvents were collected from a Puresolv solvent purification system, obtained from commercial suppliers or dried in the laboratory. Ethanol was distilled from Mg turnings activated with iodine. ^1H NMR spectra were obtained using Bruker DPX/400 and Bruker-Avance III spectrometers operating at 400 and 500 MHz, ^{13}C NMR spectra at 101 and 126 MHz respectively. Signal splitting patterns were described as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad singlet (s, broad), or any combination of the above. All coupling constants were recorded in Hz. DEPT was used to assign the signals in ^{13}C NMR spectra as C, CH, CH_2 and CH_3 .

(3-Iodopropyl)triphenylphosphonium iodide (**1**)

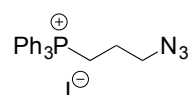


1,3-Diiodopropane (2.65 mL, 23 mmol) was added to a stirred solution of triphenylphosphine (3.01 g, 11.5 mmol) in toluene (25 mL) at 95 °C and the mixture was stirred for 20 h under reflux. After cooling to RT, the precipitate was collected by filtration and washed with Et_2O , after this, it was dried under reduced pressure to yield the alkyl iodide **1** as a white amorphous solid. (5.98 g, 93%). Mp: 172-174 °C. ν_{max} (ATR): 3056 (CH), 2916 (CH), 2849 (CH), 1587 (Ar). δ_{H} (CDCl_3 , 400 MHz): 7.81-7.76 (9H, m, 6 *o*-H PPh_3 , 3 *p*-H PPh_3), 7.71-7.66 (6H, m, 6 *m*-H PPh_3), 3.92-3.84 (2H, m, P- CH_2), 3.56 (2H, t, $J = 6.4$ Hz, I- CH_2), 2.19-2.10 (2H, m, CH_2 - CH_2 - CH_2). δ_{C} (CDCl_3 , 125 MHz): 135.61 (d, $J = 2.8$ Hz, CH), 134.00 (d, $J = 10.1$ Hz, CH), 130.93 (d, $J = 12.8$ Hz, CH), 118.01 (d, $J = 86.1$ Hz, C), 27.08 (d, $J = 1.8$ Hz, CH_2), 24.41 (d, $J = 51.3$ Hz, CH_2), 6.96 (d, $J = 20.1$ Hz, CH_2). LRMS (ESI^+) 431 [M^+ (phosphonium cation), 100%]. HRMS (ESI^+): 430.9137. $\text{C}_{21}\text{H}_{21}\text{IP}$ requires M^+ (phosphonium cation), 430.9117.

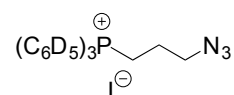
(3-Iodoprop-1-yl)-tri(pentadeuterophenyl)phosphonium iodide (**d₁₅-1**)



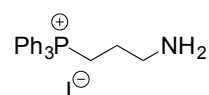
1,3-Diiodopropane (500 μL , 4.3 mmol) was added to a stirring solution of d-15 triphenylphosphine (500 mg, 1.8 mmol) in toluene (10 mL) at 95 °C and the mixture was set to react for 20 h under reflux conditions. After cooling to RT, the precipitate was collected by filtration and washed with Et_2O , after this, it was dried under reduced pressure to yield the alkyl iodide **d₁₅-1** as a white amorphous solid. (1.01 g, 98%). Mp: 173-175 °C. ν_{max} (ATR): 2859 (CH), 2343 (CD), 1559 (Ar). δ_{H} (CDCl_3 , 500 MHz): 3.97-3.91 (2H, m, P- CH_2), 3.60 (2H, t, $J = 6.5$, I- CH_2), 2.21-2.14 (2H, m, CH_2 - CH_2 - CH_2). δ_{C} (CDCl_3 , 125 MHz): 135.11-134.73 (m, CD), 133.65-133.16 (m, CD), 130.50-129.99 (m, CD), 117.2 (d, $J = 86.2$ Hz, C), 26.90 (d, $J = 2.0$ Hz, CH_2), 24.24 (d, $J = 51.2$ Hz, CH_2), 6.77 (d, $J = 20.0$ Hz, CH_2). LRMS (ESI^+) 446 [M^+ (phosphonium cation), 100%] HRMS (ESI^+): 446.1351. $\text{C}_{21}\text{H}_6\text{D}_{15}\text{IP}$ requires M^+ (phosphonium cation), 446.1362.

(3-Azidoprop-1-yl)triphenylphosphonium iodide (2)

A solution of (3-iodoprop-1-yl)triphenylphosphonium iodide **1** (5.02 g, 9.0 mmol) and NaN₃ (1.17 g, 18.0 mmol) in EtOH (15.0 mL) and H₂O (15.0 mL) was stirred and heated to 70 °C for 24 h. After cooling, an extraction was carried out with CHCl₃ (3 30 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to yield the alkyl azide **2** as an amorphous beige solid (4.13 g, 97%). MP: 151-153 °C δ_H (CDCl₃, 400 MHz): 7.85-7.79 (9H, m, 6 *o*-H PPh₃, 3 *p*-H PPh₃), 7.73-7.68 (6H, m, 6 *m*-H PPh₃), 3.95-3.87 (2H, m, P-CH₂), 3.82 (2H, t, *J* = 6.2 Hz, N₃-CH₂), 1.95-1.86 (2H, m, CH₂-CH₂-CH₂). δ_C (CDCl₃, 100 MHz): 135.57 (d, *J* = 2.9 Hz, CH), 133.95 (d, *J* = 10.3 Hz, CH), 130.92 (d, *J* = 12.5 Hz, CH), 118.05 (d, *J* = 86.6 Hz, C), 50.98 (d, *J* = 17.6 Hz, CH₂), 22.92 (d, *J* = 2.9 Hz, CH₂), 20.70 (d, *J* = 52.8 Hz, CH₂). ¹H and ¹³C NMR data agree with those for the bromide salt ¹.

(3-Azidoprop-1-yl)-tri(pentadeuterophenyl)phosphonium iodide (d₁₅-2)

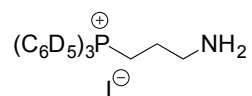
A solution of alkyl iodide **d₁₅-1** (1.00 g, 1.74 mmol) and NaN₃ (260 mg, 4.0 mmol) in EtOH (5.0 mL) and H₂O (5.0 mL) was stirred and heated to 70 °C for 24 h. After cooling, an extraction was carried out with DCM (3 30 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to yield the alkyl azide **d₁₅-2** as an amorphous beige solid (825 mg, 97%). Mp: 151-153 °C. ν_{max} (ATR): 2888 (CH), 2796 (CH), 2362 (CD), 2110 (N₃), 1546 (Ar). δ_H (CDCl₃, 500 MHz): 3.83-3.76 (4H, m, P-CH₂, N₃-CH₂), 1.91-1.83 (2H, m, CH₂-CH₂-CH₂). δ_C (CDCl₃, 125 MHz): 134.99-134.60 (m, CD), 133.47-132.98 (m, CD), 130.40-129.91 (m, CD), 117.40 (d, *J* = 86.2 Hz, C), 50.71 (d, *J* = 17.5 Hz, CH₂), 22.63 (d, *J* = 2.6 Hz, CH₂), 20.33 (d, *J* = 52.9 Hz, CH₂). LRMS (ESI⁺) 361 [M⁺ (phosphonium cation), 34%], 333 [M⁺ (phosphonium cation) - N₂, 100%]. HRMS (ESI⁺): 361.2395. C₂₁H₆D₁₅N₃P requires M⁺ (phosphonium cation), 361.2409.

(3-Aminoprop-1-yl)triphenylphosphonium iodide (3)

A solution of alkyl azide **2** (4.10 g, 8.7 mmol) and 10% Pd/C (425 mg, 4.0 mmol) in MeOH (40 mL) was stirred at RT under a H₂ (1 atm) atmosphere for 6 h. After this time, the mixture was filtered through Celite®. Then, the mixture was concentrated under reduced pressure to yield the amine **3** as an amorphous beige solid (3.62 g, 93%). Mp: 212-214 °C. ν_{max} (ATR): 3050 (NH), 2887 (CH), 2789 (CH), 1586 (Ar). δ_H (CDCl₃, 400 MHz): 7.83-7.76 (9H, m, 6 *o*-H PPh₃, 3 *p*-H PPh₃), 7.71-7.66 (6H, m, 6 *m*-H PPh₃), 3.78-3.71 (2H, m, P-CH₂), 3.01 (2H, t, *J* = 6.2 Hz, NH₂-CH₂), 1.88 (2H, s, NH₂), 1.82-1.72 (2H, m, CH₂-CH₂-CH₂). δ_C (CDCl₃, 100 MHz): 135.38 (d, *J* = 2.9 Hz, CH), 133.99 (d, *J* = 9.7 Hz, CH), 130.84 (d, *J* = 12.6 Hz, CH), 118.48 (d, *J* = 85.5 Hz, C), 41.74 (d, *J* = 17.5 Hz, CH₂), 26.13 (d, *J* = 4.9 Hz, CH₂), 21.14 (d, *J* = 51.5 Hz,

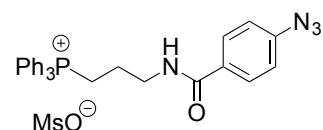
CH₂). LRMS (ESI⁺) 320 [M⁺ (phosphonium cation), 100%], HRMS (ESI⁺): 320.1553. C₂₁H₂₃NP requires M⁺ (phosphonium cation), 320.1563.

(3-Aminopropyl)-tri(pentadeuterophenyl)phosphonium iodide (d₁₅-3)



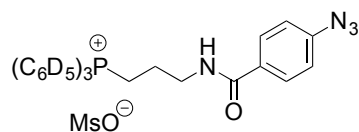
A solution of alkyl azide *d*₁₅-2 (820 mg, 1.68 mmol) and 10% Pd/C (100 mg, 0.94 mmol) in MeOH (10 mL) was stirred at RT under a H₂ (1 atm) atmosphere for 6 h. After this time, the mixture was filtered through Celite®. Then, the mixture was concentrated under reduced pressure to yield the amine *d*₁₅-3 as an amorphous beige solid (671 mg, 86%). Mp: 211-213 °C. ν_{max} (ATR): 2950 (NH), 2887 (CH), 2789 (CH), 2251 (CD), 1547 (Ar). δ_H (CDCl₃, 400 MHz): 3.79-3.72 (2H, m, P-CH₂), 3.01 (2H, t, *J* = 6.3 Hz, NH₂-CH₂), 1.80-1.71 (2H, m, CH₂-CH₂-CH₂), 1.48 (2H, s, NH₂). δ_C (CDCl₃, 100 MHz): 135.04-134.48 (m, CD), 133.70-133.09 (m, CD), 130.50-129.84 (m, CD), 118.15 (d, *J* = 85.7 Hz, C), 41.65 (d, *J* = 17.5 Hz, CH₂), 26.18 (d, *J* = 2.5 Hz, CH₂), 21.01 (d, *J* = 52.7 Hz, CH₂). LRMS (ESI⁺) 335 [M⁺ (phosphonium cation), 100%]. HRMS (ESI⁺): 335.2489. C₂₁H₈D₁₅NP requires M⁺ (phosphonium cation), 335.2504.

[3-(4'-Azidobenzamido)-prop-1-yl]triphenylphosphonium mesylate (MitoA, mesylate salt)



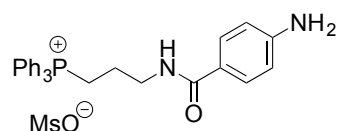
A solution of **3** (2.24 g, 5.0 mmol), 4-azidobenzoic acid (816 mg, 5.0 mmol), and *N,N'*-diisopropylcarbodiimide (800 μ l, 5.2 mmol) in dry ACN (20 mL) was stirred at RT under argon for 48 h. After this, the precipitate was washed with ACN (5 \times 10 mL). The precipitate was dried under high vacuum to remove the solvent, resulting in a brown powder. The powder was then treated for ion exchange with ion exchange resin Araldite® IRA-400 charged with mesylate ions giving a brown amorphous solid after solvent was removed. Column chromatography [SiO₂, DCM-MeOH (95:5)] yielded the mesylate salt of **MitoA** as a brown amorphous solid (1.34 g, 47%). *R_f* [SiO₂, DCM-MeOH (95:5)]: 0.25. Mp: 157-159 °C. ν_{max} (ATR): 3289 (NH), 3063 (CH), 2928 (CH), 2901 (CH), 2120 (N₃), 1653 (C=O), 1601 (Ar), 1530 (Ar), 1497 (Ar) cm⁻¹. δ_H (CDCl₃, 400 MHz): 9.28 (t, *J* = 5.9 Hz, NH), 8.15 (2H, d, *J* = 8.9 Hz, H-2', H-6'), 7.76-7.69 (9H, m, 3 \times *p*-H PPh₃, 6 \times *o*-H PPh₃), 7.64 - 7.59 (6H, m, 6 *m*-H PPh₃), 7.07 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 3.75-3.67 (4H, m, P-CH₂, NH-CH₂), 2.81 (3H, s, CH₃SO₃⁻), 1.98-1.90 (2H, m, CH₂-CH₂-CH₂). δ_C (CDCl₃, 100 MHz): 167.10 (C), 142.99 (C), 135.13 (d, *J* = 2.0 Hz, CH), 133.57 (d, *J* = 9.1 Hz, CH), 130.91 (C), 130.61 (d, *J* = 12.0 Hz, CH), 129.81 (CH), 118.92 (CH), 118.56 (d, *J* = 86.0 Hz, C), 39.78 (CH₃), 39.42 (d, *J* = 17.0 Hz, CH₂), 22.60 (d, *J* = 4.0 Hz, CH₂), 20.16 (d, *J* = 52.1 Hz, CH₂). LRMS (ESI⁺) 465 [M⁺ (phosphonium cation), 32%], 437 [M⁺ (phosphonium cation) - N₂, 100]. HRMS (ESI⁺): 465.1821. C₂₈H₂₆N₄OP requires M⁺ (phosphonium cation), 465.1839

[3-(4'-Azidobenzamido)propyl]tri(pentadeuterophenyl)phosphonium mesylate (**d₁₅-MitoA** mesylate salt)



A solution of amine **d₁₅-3** (100 mg, 0.22 mmol), 4-azidobenzoic acid (36 mg, 0.22 mmol), and *N, N'*-diisopropylcarbodiimide (150 μ l, 0.98 mmol) in dry ACN (2.5 mL) was stirred at RT under argon for 37 h. After this, the precipitate was washed with ACN (4 \times 10 mL). The precipitate was dried under high vacuum to remove the solvent, resulting in a brown powder. The powder was then treated for ion exchange with ion exchange resin Araldite® IRA-400 charged with mesylate ions giving a brown amorphous solid after solvent was removed. Column chromatography [SiO₂, DCM-MeOH (95:5)] yielded the target compound as a brown amorphous solid (57 mg). As the mesylate ion was under integrated in the NMR, the solid was treated with aqueous NaMeSO₃ (0.5 M) and extracted with DCM. The DCM was removed under reduced pressure to give the mesylate salt of **d₁₅-MitoA** as a hygroscopic thick yellow oil (55 mg, 45%). *R_f*[SiO₂, DCM-MeOH (95:5)]: 0.27. Mp: 156-158 °C. ν_{max} (ATR): 3283 (NH), 2935 (CH), 2906 (CH), 2823 (CH), 2269 (CD), 2120 (N₃), 1644 (C=O), 1603 (Ar), 1546 (Ar), 1500 (Ar). δ_H (CDCl₃, 400 MHz): 9.10-9.08 (1H, m, NH), 7.98 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 6.86 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 3.53-3.46 (4H, m, P-CH₂, NH-CH₂), 2.57 (3H, s, CH₃SO₃⁻), 1.83-1.81 (2H, m, CH₂-CH₂-CH₂). δ (CDCl₃, 100 MHz): 166.40 (C), 142.34 (C), 134.51-134.04 (m, CD), 132.96-132.36 (m, CD), 130.41 (C), 130.01-129.41 (m, CD), 129.31 (CH), 118.40 (CH), 117.66 (d, *J* = 86.1 Hz, C), 39.29 (CH₃), 39.06 (d, *J* = 17.0 Hz, CH₂), 22.14 (d, *J* = 4.1 Hz, CH₂), 19.66 (d, *J* = 52.3 Hz, CH₂). LRMS (ESI⁺) 480 [M⁺ (phosphonium cation), 30%], 452 [M⁺ (phosphonium cation) - N₂, 100]. HRMS (ESI⁺): 480.2760. C₂₈H₁₁D₁₅N₄OP⁺ requires M⁺ (phosphonium cation), 480.2780

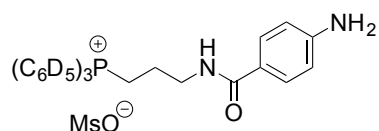
[3-(4'-Aminobenzamido)prop-1-yl]triphenylphosphonium mesylate (**MitoN** mesylate salt).



A solution of amine **3** (1.12 g, 2.50 mmol), 4-aminobenzoic acid (340 mg, 2.47 mmol), and *N, N'*-diisopropylcarbodiimide (400 μ l, 2.68 mmol) in dry ACN (10 mL) was stirred at RT under argon for 48 h. After this, the precipitate was washed with ACN (5 \times 10 mL). The precipitate was dried under high vacuum to remove the solvent, resulting in a beige powder. The powder was then treated for ion exchange with ion exchange resin Araldite® IRA-400 charged with mesylate ions giving a beige amorphous solid after solvent was removed. Column chromatography [SiO₂, DCM-MeOH (9:1)] yielded the mesylate salt of **MitoN** as a beige amorphous solid (410 mg, 31%). *R_f*[SiO₂, DCM-MeOH (9:1)]: 0.25. Mp: 206-208 °C. ν_{max} (ATR): 3333 (NH), 3225 (CH), 3059 (CH), 2931 (CH), 2904 (CH), 1628 (CO), 1603 (Ar), 1545 (Ar), 1508 (Ar). δ_H (CDCl₃, 400 MHz): 8.92 (t, *J* = 5.9 Hz, NH), 7.93 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 7.76-7.68 (9H, m, 3 \times *p*-H PPh₃, 6 \times *o*-H PPh₃), 7.63 - 7.59 (6H, m, 6 \times *m*-H PPh₃), 6.66 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 4.07 (2H, s, NH₂) 3.74-3.67 (4H, m, P-CH₂, NH-CH₂), 2.83 (3H, s, CH₃SO₃⁻), 1.97-1.88 (2H, m, CH₂-CH₂-

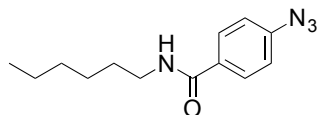
CH₂). δ_C (CDCl₃, 100 MHz): 167.74 (C), 149.59 (C), 134.92 (d, $J = 2.3$ Hz, CH), 133.41 (d, $J = 9.0$ Hz, CH), 130.42 (d, $J = 12.5$ Hz, CH), 129.47 (C), 123.71 (CH), 118.46 (d, $J = 86.0$ Hz, C), 114.07 (CH), 39.65 (CH₃), 39.06 (d, $J = 17.0$ Hz, CH₂), 22.64 (d, $J = 3.0$ Hz, CH₂), 19.79 (d, $J = 52.0$ Hz, CH₂). LRMS (ESI⁺) 439 [M⁺ (phosphonium cation), 100%]. HRMS (ESI⁺): 439.1915. C₂₈H₂₈N₂OP requires M⁺ (phosphonium cation), 439.1934.

[3-(4'-Aminobenzamido)prop-1-yl]tri(pentadeuterophenyl)phosphonium mesylate (*d*₁₅-MitoN mesylate salt)



A solution of amine *d*₁₅-**3** (100 mg, 0.22 mmol), 4-aminobenzoic acid (30 mg, 0.21 mmol), and *N,N'*-diisopropylcarbodiimide (150 μ l, 0.98 mmol) in dry ACN (2.5 mL) was stirred at RT under argon for 48 h. After this, the precipitate was washed with ACN (5 \times 10 mL). The precipitate was dried under high vacuum to remove the solvent, resulting in a beige powder. The powder was then treated for ion exchange with ion exchange resin Araldite® IRA-400 charged with mesylate ions giving a beige amorphous solid after solvent was removed. Column chromatography [SiO₂, DCM-MeOH (9:1)] yielded the amide *d*₁₅-MitoN as a beige amorphous solid (50 mg, 42%). As the mesylate ion was under integrated in the NMR, the solid was treated with aqueous NaMeSO₃ (0.5 M) and extracted with DCM. The DCM was removed under reduced pressure to give the mesylate salt of *d*₁₅-MitoN as a beige solid (38 mg, 32%). *R*_f[SiO₂, DCM-MeOH (9:1)]: 0.25. Mp: 207-209 °C. ν_{max} (ATR): 3335 (NH), 3228 (CH), 2931 (CH), 2902 (CH), 2281 (CD), 1623 (CO), 1599 (Ar), 1549 (Ar), 1509 (Ar). δ_H (CD₃OD, 400 MHz): 7.56 (2H, d, $J = 8.8$ Hz, H-2', H-6'), 6.62 (2H, d, $J = 8.8$ Hz, H-3', H-5'), 3.50-3.38 (4H, m, P-CH₂, NH-CH₂), 2.65 (3H, s, CH₃SO₃), 1.96-1.86 (2H, m, CH₂-CH₂-CH₂). δ_C CD₃OD, (100 MHz): 170.54 (C), 153.45 (C), 136.06-135.57 (m, CD), 134.64-134.05 (m, CD), 131.37-130.74 (m, CD), 130.02 (C), 122.54 (CH), 119.54 (d, $J = 86.4$ Hz, C), 114.65 (CH), 40.78 (d, $J = 19.0$ Hz, CH₂), 39.52 (CH₃), 23.99 (d, $J = 3.7$ Hz, CH₂), 20.68 (d, $J = 53.0$ Hz, CH₂). LRMS (ESI⁺) 454 [M⁺ (phosphonium cation), 100%]. HRMS (ESI): 454.2855. C₂₈H₁₃D₁₅N₂OP requires M⁺ (phosphonium cation), 454.2875

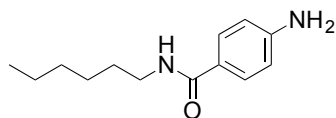
4'-Azido-*N*-(hex-1-yl)benzamide (**4**)



Hex-1-ylamine (320 μ L, 2.4 mmol), HBTU (835 mg, 2.2 mmol), and *N*-methylmorpholine (NMM, 440 μ L, 4 mmol) were added to a stirred solution of *para*-azidobenzoic acid (367 mg, 2.2 mmol) in anhydrous DMF (10 mL) at RT under argon. Stirring was continued for 18 h, and then water (15 mL) was added. The precipitate was collected by filtration and washed with water (3 \times 10 mL). The resulting solid was dried under high vacuum to give the amide **4** as an amorphous yellow solid (427 mg, 86%). Mp: 71-73 °C. ν_{max} (ATR): 3312 (NH), 3075 (CH), 2955 (CH), 2928 (CH), 2860 (CH), 2137 (N₃), 1629 (CO), 1605 (Ar), 1545 (Ar), 1499 (Ar). δ_H (CDCl₃, 500 MHz): 7.76 (2H, d, $J = 8.6$ Hz, H-2', H-6'), 7.05 (2H, d, $J = 8.6$ Hz, H-3', H-5'), 6.14 (1H, s, NH), 3.45-3.41 (2H, m, CH₂-1), 1.60 (2H, qn, $J = 7.0$

Hz, CH₂-2), 1.40-1.28 (6H, m, CH₂-3, CH₂-4, and CH₂-5), 0.91-0.87 (3H, t, $J = 7.0$ Hz, CH₃). δ_C (CDCl₃, 125 MHz): 166.57 (C), 143.30 (C), 131.53 (C), 128.78 (CH), 119.03 (CH), 40.32 (CH₂), 31.65 (CH₂), 29.78 (CH₂), 26.82 (CH₂), 22.69 (CH₂), 14.16 (CH₃). LRMS (EI⁺): 246 (M⁺, 47%), 218 (M⁺ - N₂, 100%), HRMS (EI⁺): 246.1483. C₁₃H₁₈N₄O requires M⁺, 246.1481.

4'-Amino-N-(hex-1-yl)benzamide (5)



A solution of azide **4** (200 mg, 0.81 mmol) and 10% Pd/C (20 mg, 0.19 mmol) in MeOH (5 mL) was stirred at RT under a H₂ (1 atm) atmosphere for 3 h. After this time, the mixture was filtered through Celite®. Then, the mixture was concentrated under reduced pressure to yield amine **5** as an amorphous beige solid (168 mg, 95%). Mp: 102-104 °C. ν_{max} (ATR): 3410 (NH), 3329 (NH), 3215 (CH), 2955 (CH), 2924 (CH), 2855 (CH), 1637 (C=O), 1601 (Ar), 1537 (Ar), 1504 (Ar). δ_H (CDCl₃, 400 MHz): 7.58 (2H, d, $J = 8.6$ Hz, H-2', H-6'), 6.60 (2H, d, $J = 8.6$ Hz, H-3', H-5'), 6.26 (1H, broad s, NH), 4.05 (2H, s, NH₂) 3.45-3.41 (2H, m, CH₂-1), 1.60 (2H, qn, $J = 7.0$ Hz, CH₂-2), 1.40-1.28 (6H, m, CH₂-3, CH₂-4 and CH₂-5), 0.91-0.87 (3H, t, $J = 7.0$ Hz, CH₃). δ_C (CDCl₃, 100 MHz): 166.57 (C), 152.43 (C), 133.65 (C), 128.88 (CH), 117.88 (CH), 40.27 (CH₂), 31.63 (CH₂), 29.75 (CH₂), 26.79 (CH₂), 22.67 (CH₂), 14.14 (CH₃). LRMS (ESI⁺) 243 [(M + Na)⁺, 100%] HRMS (ESI⁺): 243.1469. C₁₃H₂₀N₂ONa requires (M + Na)⁺, 243.1468. IR data in broad agreement with Lit. ².

Supplementary References

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