Development of new chemical tools to study the cannabinoid receptor type 2

Paus, L.V. de

Chapter 5

Ligand-Directed Targeting of Mitochondrial CB_1 Receptors

Ligand-Directed Targeting of Mitochondrial CB_1 Receptors

Introduction

Cannabinoid Receptor 1 (CB_1R) was cloned in 1990, which proved that Δ^9-tetrahydrocannabinol (THC), the psychoactive cannabinoid in marijuana, acts by interaction with a specific receptor to elicit central nervous system (CNS) effects, rather than through membrane disruption.[1] CB_1R is a $G_{i/o}$ protein-coupled receptor that suppresses transmitter release at synapses in neurons.[2] During both short- and long-term plasticity the G protein-dependent pathway inhibits Ca^{2+} influx by acting on voltage-gated Ca^{2+} channels. Additionally CB_1R engagement leads to inhibition of adenyl cyclase and downregulation of the cAMP/PKA pathway.[3] CB_1R is implied in many brain functions, including mood, nociception, appetite, and motor function.[4] Furthermore, dysfunction of the CB_1R has been linked to epilepsy[5], depression[3] and several neurological disorders including Alzheimer's, Parkinson's and Huntington's.[6]

A link between cannabinoids and mitochondria was established in the 1970's, prior to the discovery of CB_1R.[1,7–10] It was shown that THC and other cannabinoids can inhibit monoamine oxidase (MAO) and affect mitochondrial respiration.[11,12] This effect was later shown to be CB_1R-dependent, as inhibition of mitochondria respiration by cannabinoids could be reversed by antagonist AM251.[13] Additionally, fatty-acid amide hydrolase 1 (FAAH) and monoacylglycerol lipase (MAGL), enzymes which are responsible for the metabolism of the endogenous cannabinoids, were also found present in mitochondria.[14] In 2012 it was demonstrated that approximately 15% of CB_1R is located on mitochondria using electron immune-gold detection assays.[14] Recently, the presence of mitochondrial CB_1R ($mtCB_1R$) has been detected in spermatozoa[15], skeletal muscles[16] and several brain cell types including astrocytes.[17] $MtCB_1R$-induced ATP reduction affects learning and memory by modulating synaptic plasticity as well as feeding behavior.[18–22]

Most studies on $mtCB_1R$ utilize agonists that act both on the CB_1R in the plasma membrane and mitochondria. Differentiation between CB_1R subgroups is done by using membrane (im)permeable CB_1R ligands, manipulation of membrane permeability, or $mtCB_1R$ KO cells to enable or disable $mtCB_1R$ signalling. Another potential way to study the $mtCB_1R$ is to target ligands directly to mitochondria, such as lipophilic, cationic small molecules or peptides, nanocarriers.[23] The most common mitochondrial carrier is triphenyl phosphonium (TPP^+). TPP^+ easily passes through hydrophobic membranes due to the large, diffuse surface area of the cation, limiting its polarizing ability.[24,25] The negative membrane potential of mitochondria allows lipophilic cations to migrate towards the mitochondria.[25] While TPP^+ is commonly used, it has been shown to uncouple the oxidative phosphorylation (OXPHOS) system. *Para*-trifluoromethyl phenyl in tris(4-trifluormethyl)phenyl phosphonium ($TFPP^+$) has a decreased electron density of the phosphonium cation and consequently a reduced effect on mitochondrial membrane potential and uncoupling.[26]

The aim of this chapter is to design and synthesize mitochondria-targeted CB_1R agonists. To this end, TPP^+ and $TFPP^+$ were introduced into the scaffold of the non-selective CBR agonist ORG28611, which is a water-soluble, potent, high-efficacy CB_1R agonist (pK_i = 8.9, pEC_{50} = 7.6), which was evaluated in clinical trials as an intravenous analgesic agent.[27–29] Compounds (**1**-**4**) were designed as CB_1R agonists with an alkyl (**1**,**2**) or poly-glycol (**3**,**4**) spacer conjugated TPP^+ (**1**,**3**) or $TFPP^+$ (**2**,**4**) (Figure 5.1). Their binding activity and functional effect on CB_1R was tested. While these compounds represent the first $T(F)PP^+$-ligands capable of binding the CB_1R, further optimization of their potency and efficacy is required to study the biological role of $mtCB_1R$.

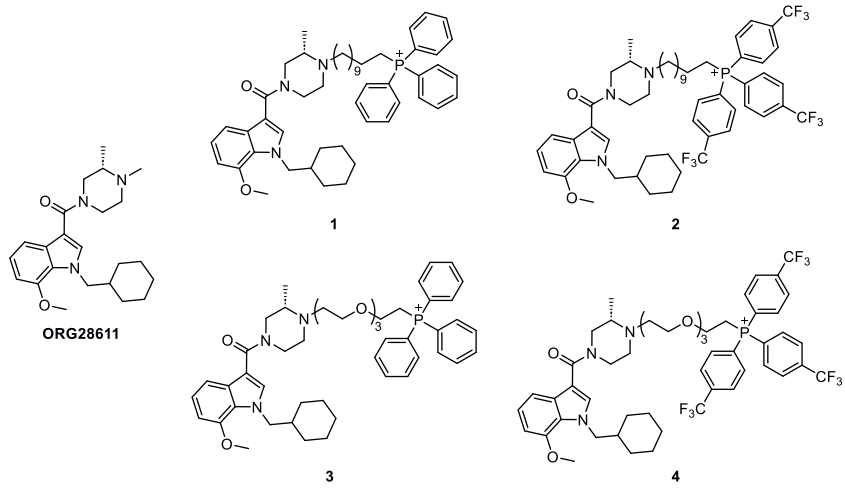


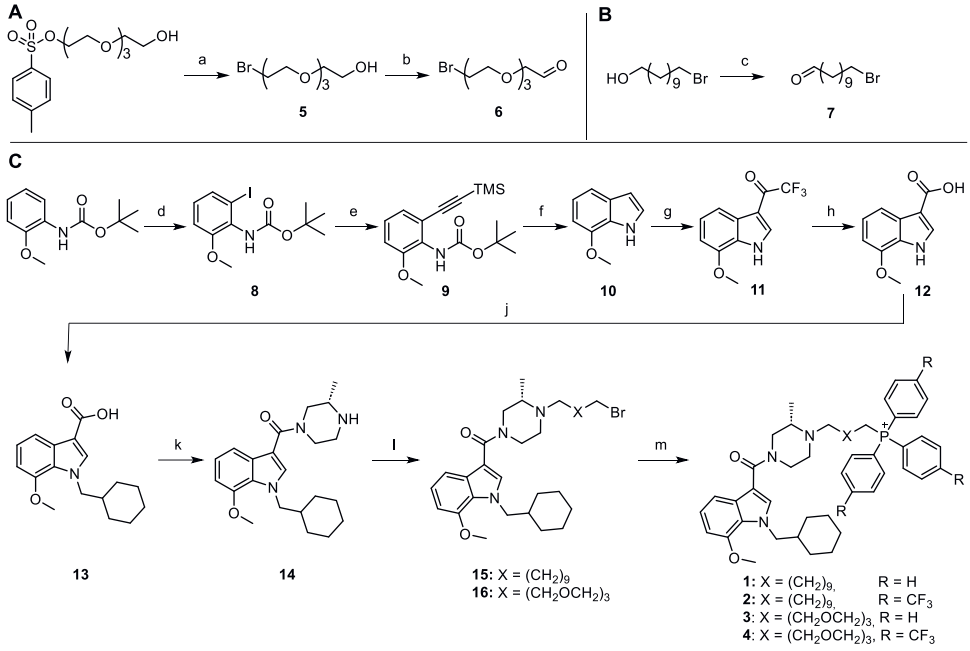
Figure 5.1 ORG28611 and the four mitochondrial-directed agonists **1**-**4** based on the indole-3-carboxamide scaffold of ORG28611. In **1** the scaffold is connected to TPP^+ with a C_{11} alkyl spacer. **2** has the C_{11} alkyl spacer and $TFPP^+$. In **3** the scaffold is connected to TPP^+ with a PEG3 spacer. **4** has the PEG3 spacer and $TFPP^+$.

Results & Discussion

Synthesis

The synthesis of the mitochondrial directing ORG28611-derived ligands commenced with the individual construction of the alkyl- and glycol-spacers and the indole-3-carboximide scaffold prior to conjugation and completed with the attachment of the phosphonium group. Thus, the glycol spacer (Scheme 5.1A) 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl benzenesulfonate (**Chapter 4**) was substituted with lithium bromide (**5**) and the alcohol subsequently oxidized to an aldehyde (**6**). For the alkyl spacer (Scheme 6.1B) only an oxidation of 11-bromoundecanol (**7**) was required.

Synthesis of the indole-3-carboximide scaffold (**14**) (Scheme 5.1C) started with a reaction of iodine with the *in-situ* generated C6 lithium species of *tert*-butyl(2-methyloxyphenyl)carbamate (**8**) followed by a Sonogashira coupling with TMS-acetylene to give **9**. Basic induced indole formation (**9** → **10**) followed by TFAA mediated trifluoroketone introduction (**10** → **11**), and subsequent nucleophilic acyl substitution by hydroxide gained acid **12**. *N*-Alkylation with bromo-methylcyclohexane followed by HATU mediated peptide coupling introduced (*S*)-2-methylpiperazine in an region selective manner to give **14**. The glycol spacer **6** and alkyl spacer **7** were introduced (**15** and **16** respectively), directly followed by the conjugation to either TPP^+ and $TFPP^+$ to complete synthesis of the four ligands **1**-**4**.



Scheme 5.1 Total synthesis of the four mitochondrial directing agonists. Reagents and conditions: a) LiBr, acetone, RT, 16 h, 93%; b) PCC, DCM, RT, 17 h, 36%; c) PCC, DCM, RT, 17 h, 43%; d) Step 1: *tert*-BuLi, Et_2O, -20 °C, 2 h; Step 2: I_2, -100 °C-RT, 17 h, 48%; e) Et_3N, $Pd(PPh_3)_4$, TMS-acetylene, 65 °C, 17 h, 57%; f) potassium *tert*-butoxide, *tert*-butanol, 85 °C, 4 h, 86%; g) TFAA, DMF, RT, 1 h, 96%; h) NaOH, H_2O, 100 °C, 2 h, 63%; j) NaH, bromo-methylcyclohexane, DMF, 60 °C, 2 h, 78%; k) Step 1: HATU, DiPEA, DMF, 0 °C, 1 h; Step 2: (*S*)-2-methylpiperazine, RT, 17 h, quant.; l) **6**/**7**, sodium triacetoxyborohydride. DCM, RT, 17 h, 19-77%; m) Step 1: PPh_3 (**1**/**3**) or tris(4-trifluoromethylphenyl)phosphine (**2**/**4**), ACN, 85 °C, 96 h, Step 2: 120-180 °C microwave, 0-14 h, 8-46%.

Molecular Pharmacology

After the synthesis of compounds **1**-**4** was completed, the ligands were evaluated for affinity (pK_i) in a [^{3}H]CP-55,940 radioligand displacement assay on membranes derived from CHO cells overexpressing hCB_1R or hCB_2R and compared to the parent compound ORG28611. Moreover, the potency (pEC_{50}) and maximal efficacy (E_{max}) were evaluated in a GTPγS assay (relative to CP-55,940, E_{max} = 100%) using the same membranes. The results are summarized in Table 5.1.

Table 5.1 The Pharmacochemical properties of the four ORG28611-based mitochondrial directing ligands **1**-**4**.

	CB_1R			CB_2R		
Compound	**Affinity pK_i ± SEM**	**Potency pEC_{50} ± SEM**	**E_{max} ± SEM**	**Affinity pK_i ± SEM**	**Potency pEC_{50} ± SEM**	**E_{max} ± SEM**
1	5.90 ± 0.17	< 5	-17 ± 1.13	6.33 ± 0.16	< 5	-22 ± 1.19
2	5.59 ± 0.23	< 5	-18 ± 0.52	6.13 ± 0.16	< 5	-16 ± 4.98
3	5.80 ± 0.26	5.90 ± 0.30	25 ± 1.56	6.19 ±0.18	< 5	9 ± 2.08
4	5.16 ± 0.57	5.70 ± 0.20	63 ± 2.28	5.79 ± 0.20	< 5	24 ± 1.55
ORG28611	8.61 ± 0.11	7.6[27]	77[27]	8.83 ± 0.11	-	-
CP-55,940	-	8.38 ± 0.06	105 ± 1.9	-	8.40 ± 0.09	96 ± 3.02

Binding affinities (pK_i) and potency (pEC_{50}) were determined as described previously (Chapter 2) on CBR-overexpressing CHO membranes. Efficacy (E_{max}) was normalized to the effect of 10 μM CP-55,940. Data are presented as the mean ± SEM from at least three (two for pEC_{50} of CB_2R) independent experiments performed in triplicate.

All compounds **1**-**4** have significantly decreased binding affinity (500- and 2800-fold) on both CB_1R and CB_2R compared to ORG28611. TPP^+ containing ligands **1** and **3** are slightly more potent than the $TFPP^+$ derivatives **2** and **4**.

Next, the potencies in the G protein activation assay for the CB_1R and CB_2R (Figure 5.2) were determined. Compounds **1** and **2** do not show any G protein activation at 1 µM, while compounds **3** and **4** activated CB_1R with potencies in a similar range as their binding affinities. This suggests that a PEG3 spacer is somewhat better tolerated than an alkyl spacer. While all compounds have moderate affinity for CB_2R, none showed significant G protein activation via CB_2R.

Interestingly, it seems that the functionality of compounds with a C_{11} spacer is switched from an agonist to an inverse agonist in both CB_1R and CB_2R, i.e. compare **3** and **4** to **1** and **2**, respectively. Compounds **1** and **2** have a negative maximal efficacy at 10 µM, signifying a decrease of basal receptor activity. This observation is in line with the hypothesis introduced in Chapter 4, that some hydrophobic interaction will stabilize the inactive state of the receptor. However, further investigation in this phenomenon was outside the scope of this chapter.

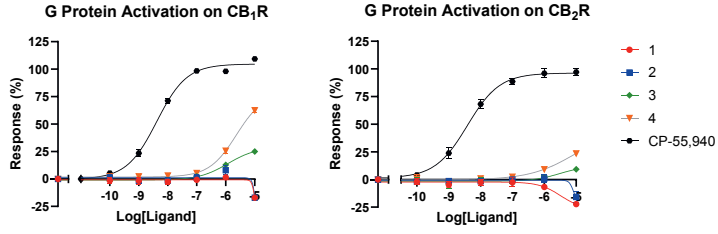


Figure 5.2 G protein activation levels were determined with a [^{35}S]GTPγS functionality assay. Basal receptor activity was set to 0%. G protein activation with 10 µM concentration of full agonist CP-55,940 was set as 100%. Data are expressed as mean ± SEM from at least three (two for CB_2R) independent experiments performed in triplicate.

Conclusion

Compounds **1**-**4** were successfully synthesized and profiled in molecular pharmacology assays. They represent the first representatives of $T(F)PP^+$-ligands that bind the CB_1R. Unfortunately, the compounds displayed weak affinity for the CB_1R and no selectivity over CB_2R. Additionally, compounds **1** and **2** behaved as inverse agonists, as opposed to parent compound ORG28611. Of note, the assays described in this chapter do not distinguish between plasmalemmal CB_1R and mitochondrial CB_1R. Hence, new compounds have to be designed and additional assays will need to be developed to assure the efficacy of the mitochondrial directing capability of the phosphine group.

Experimental Section

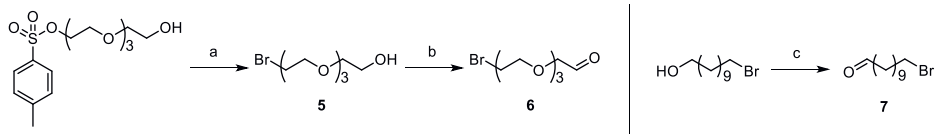
Chemistry

General Remarks

All reagents and solvents were purchased from commercial sources and were of analytical grade (Sigma-Aldrich, BroadPharm®). Reagents and solvents were not further purified before use. All moisture sensitive reactions were performed under inert atmosphere. Solvents were dried using 4 Å molecular sieves prior to use when anhydrous conditions were required. Water used in reactions was always demineralized. Analytical Thin-layer Chromatography (TLC) was routinely performed to monitor the progression of a reaction and was conducted on Merck Silica gel 60 F254 plates. Reaction compounds on the TLC plates were visualized by UV irradiation (λ_{254}) and/or spraying with potassium permanganate solution (K_2CO_3 (40 g), $KMnO_4$ (6 g), and H_2O (600 mL)), ninhydrin solution (ninhydrin (1.5 g), n-butanol

(100 mL) and acetic acid (3.0 mL)) or molybdenum solution ($(NH_4)_6MO_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot H_2O$ (10 g/L) in sulfuric acid (10%)) followed by heating as appropriate. Purification by flash column chromatography was performed using Screening Devices B.V. silica gel 60 (40-63 µm, pore diameter of 60 Å). Solutions were concentrated using a Heidolph laborata W8 4000 efficient rotary evaporator with a Laboport vacuum pump. Analytical purity was determined with Liquid Chromatography-Mass Spectrometry (LC-MS) using a Finnigan LCQ Advantage MAX apparatus with electrospray ionization (ESI), equipped with a Phenomenex Gemini 3 µm NX-C18 110Å column (50x4.6mm), measuring absorbance at 254 nm using a Waters 2998 PDA UV detector and the m/z ratio by using an Acquity Single Quad (Q1) detector. Injection was with the Finnigan Surveyor Autosampler Plus and pumped through the column with the Finnigan Surveyor LC pump plus to be analysed with the Finnigan Surveyor PDA plus detector. Samples were analysed using eluent gradient 10% → 90% ACN in MilliQ water (+ 0.1% TFA (v/v)). For purification by mass guided preparative High-Performance Liquid Chromatography (Prep-HPLC) was performed on a Waters AutoPurification HPLC/MS apparatus with a Gemini prep column 5 µm 18C 110 Å (150x21.2mm), Waters 2767 Sample manager, Waters 2545 Binary gradient module, Waters SFO System fluidics organizer, Waters 515 HPLC pump M, Waters 515 HPLC pump L attached to a Waters SQ detector Acquity Ultra performance LC. A five column volume purification protocol was applied with the eluents A: 0.2% aq. TFA, B: ACN, flow 25 mL/min, with a minimum start gradients of 0% to maximum end gradient of 100% of B. ^1H, ^{13}C, ^1H-COSY and HSQC Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AV 300 (300/75 MHz), AV 400 (400/100 MHz or AV850 (850/216 MHz) spectrometer at ambient temperature using $CDCl_3$ or MeOD as solvent. Chemical shifts (δ) are referenced in parts per million (ppm) with tetramethylsilane (TMS) or $CDCl_3$ resonance as the internal standard peak ($CDCl_3$/TMS, δ 0.00/7.26 for ^1H (TMS/$CDCl_3$), δ 77.16 for ^{13}C ($CDCl_3$)) or MeOD resonance internal standard peak (δ 3.31 (^1H) and δ 49.00 (^{13}C)). Multiplicity is reported as bs = broad singlet, s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, qd = quartet of doublets, p = quintet, hept = heptet, m = multiplet. Coupling-constants (*J*) are reported in Hertz (Hz).

Synthesis of the spacers:



Scheme 5.2 Synthesis of the glycol (PEG3) and alkyl (C_{11}) spacers. Reagents and conditions: a) LiBr, acetone, RT, 16 h, 93%; b) PCC, DCM, RT, 17 h, 36%; c) PCC, DCM, RT, 17 h, 43%;

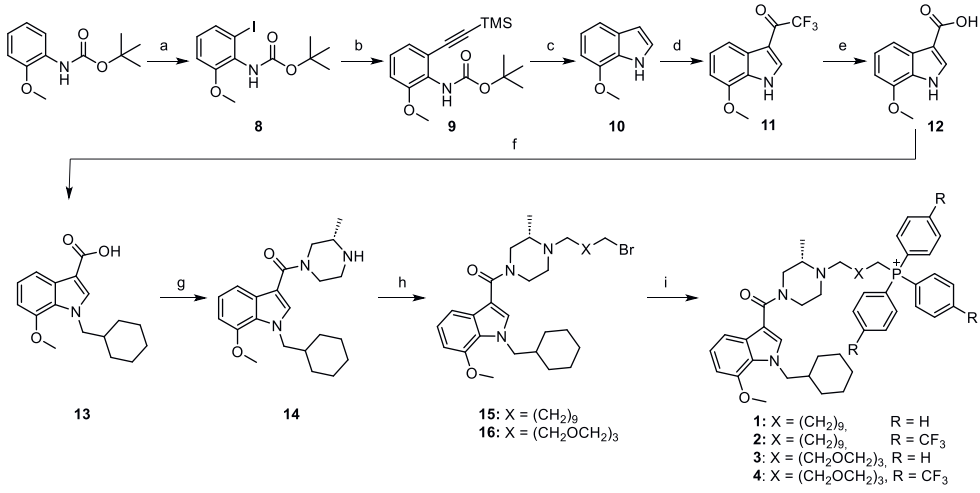
2-(2-(2-(2-Bromoethoxy)ethoxy)ethoxy)ethan-1-ol (5): To a stirred solution of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (2.00 g, 5.8 mmol, 1 eq) in acetone (50 mL) was added LiBr (1.33 g, 11.5 mmol, 2 eq). After stirring at RT for 16 h the solvent was evaporated under reduced pressure. The crude product was redissolved in $CHCl_3$ (50 mL) and the organic layer was washed four times with brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to yield a colourless oil (1.38 g, 5.4 mmol, 93%). ^1H-NMR (400 MHz, $CDCl_3$) δ 3.49 (t, *J* = 5.5 Hz, 2H), 3.40 (t, *J* = 6.2 Hz, 2H), 3.31 – 3.20 (m, 8H), 3.18 – 3.15 (m, 2H), 3.11 (t, *J* = 6.2 Hz, 2H). *No C-NMR Available*.

2-(2-(2-(2-Bromoethoxy)ethoxy)ethoxy)acetaldehyde (6): A mixture of **5** (1.05 g, 4.1 mmol, 1 eq) and PCC (0.88 g, 4.1 mmol, 1 eq) in DCM (15 mL) was stirred at RT for 17 h. The solvent was evaporated under reduced pressure and the crude product re-dissolved in $CHCl_3$ (35 mL). The organic layer was washed twice with 2 M HCl (aq) and once with sat. $NaHCO_3$ (aq), dried ($MgSO_4$), filtered and the solved

evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-2% MeOH in DCM) to yield a colourless oil (0.37 g, 1.5 mmol, 36%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 3.72 (t, *J* = 6.3 Hz, 2H), 3.65 – 3.61 (m, 2H), 3.58 (s, 6H), 3.51 (t, *J* = 4.3 Hz, 2H), 3.39 (t, *J* = 6.2 Hz, 2H). *No C-NMR Available*.

11-Bromoundecanal (7): A mixture of 11-bromoundecanol (0.61 g, 2.4 mmol, 1 eq) and PCC (0.53 g. 2.4 mmol, 1 eq) in DCM (10 mL) was stirred at RT for 17 h. The solvent was evaporated under reduced pressure and the crude re-dissolved in Et_2O (20 mL). The organic layer was washed thrice with 2 M HCl (aq), four times with sat. $NaHCO_3$ (aq) and once with brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 5-15% EtOAc in pentane) to yield a colourless oil (0.26 g, 1.04 mmol, 43%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.8 Hz, 1H), 3.40 (t, *J* = 6.9 Hz, 2H), 2.43 (td, *J* = 7.3, 1.8 Hz, 2H), 1.85 (p, *J* = 7.0 Hz, 2H), 1.62 (p, *J* = 7.3 Hz, 2H), 1.42 (p, *J* = 6.9 Hz, 2H), 1.30 (s, 10H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 202.63, 43.78, 33.90, 32.72, 29.26, 29.21, 29.12, 29.03, 28.63, 28.05, 21.96.

Synthesis of mitochondrial directing ligands 1-4



Scheme 5.3 Synthesis of the scaffold and the mitochondrial directing ligands **1-4**. Reagents and conditions: a) Step 1: *tert*-BuLi, Et_2O, -20 °C, 2 h; Step 2: I_2, -100 °C-RT, 17 h, 48%; b) Et_3N, $Pd(PPh_3)_4$, TMS-acetylene, 65 °C, 17 h, 57%; c) potassium *tert*-butoxide, *tert*-butanol, 85 °C, 4 h, 86%; d) TFAA, DMF, RT, 1 h, 96%; e) NaOH, H_2O, 100 °C, 2 h, 63%; f) NaH, bromomethylcyclohexane, DMF, 60 °C, 2 h, 78%; g) Step 1: HATU, DiPEA, DMF, 0 °C, 1 h; Step 2: (*S*)-2-methylpiperazine, RT, 17 h, quant.; h) **6**/**7**, sodium triacetoxyborohydride. DCM, RT, 17 h, 19-77%; j) Step 1: PPh_3 (**1**/**3**) or tris(4-trifluoromethylphenyl)phosphine (**2**/**4**), ACN, 85 °C, 96 h, ii) 120-180 °C microwave, 0-14 h, 8-46%.

tert-Butyl(2-iodo-6-methoxyphenyl)carbamate (8): To a cooled (-20 °C) and stirred mixture of *tert*-butyl(2-methyloxyphenyl)carbamate (2.00 g, 9.0 mmol, 1 eq) in anhydrous Et_2O (10 mL) under inert atmosphere was added dropwise *tert*-butyllithium (12 mL, 1.7 M in pentane, 20.4 mmol, 2.3 eq). After 2 h the mixture was cooled (-100 °C) further and iodine (2.73 g. 10.8 mmol, 1.2 eq) was added dropwise. The mixture was stirred at RT for 17 h. The reaction was quenched with sat. $Na_2S_2O_3$ (aq) (120 mL) and stirred for 45 minutes. After addition of Et_2O the layers were separated and the organic layer washed five times with sat. $Na_2S_2O_3$ (aq) and thrice with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 2-20% EtOAc in pentane) to yield a yellow solid (1.55 g, 4.4 mmol, 48%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 7.6, 1.3 Hz, 1H), 6.92 – 6.77 (m, 2H), 6.29 (s, 1H), 3.72 (s, 3H),

1.47 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 155.14, 153.12, 130.28, 128.71, 128.26, 111.17, 100.27, 79.77, 55.65, 28.04.[30]

tert-Butyl(2-methoxy-6-((trimethylsilyl)ethynyl)phenyl)carbamate (9): To a stirred mixture of **8** (1.55 g, 4.4 mmol, 1 eq), Et_3N (0.72 mL, 0.2 mmol, 0.05 eq) in THF (20 mL) under inert atmosphere was added $Pd(PPh_3)_4$ (0.10 g, 0.1 mmol, 0.02 eq) and trimethylsilylacetylene (0.75 mL, 5.4 mmol, 1.3 eq). After heating (70 °C) for 17 h H_2O and Et_2O were added and the mixture filtered over a Celite™ pad. The layers were separated and the organic layer was washed thrice with brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 3-15% EtOAc in pentane) to yield a yellow oil (0.79 g, 2.5 mmol, 57%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.00 – 6.95 (m, 2H), 6.81 – 6.72 (m, 1H), 6.12 (s, 1H), 3.72 (s, 3H), 1.41 (s, 9H), 0.15 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 153.85, 153.28, 128.01, 126.31, 124.79, 121.37, 111.87, 101.78, 99.09, 80.04, 55.87, 28.33, 0.04.

7-Methoxyindole (10): To a stirred solution of **9** (0.20 g, 0.6 mmol, 1 eq) in *tert*-butanol (5 mL) under inert atmosphere was added potassium *tert*-butoxide (0.34 g, 3.0 mmol, 4.9 eq) in several portions. After heating (85 °C) for 4 h the mixture was diluted with H_2O and DCM. The layers were separated and the aqueous layer was extracted thrice with DCM. The combination of organic layers was washed thrice with brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 2.5-5% EtOAc in pentane) to yield a brown oil (0.08 g, 0.54 mmol, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 2.8 Hz, 1H), 7.03 (t, *J* = 7.8 Hz, 1H), 6.63 (d, *J* = 7.0 Hz, 1H), 6.52 (dd, *J* = 3.1, 2.2 Hz, 1H), 3.93 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 146.26, 129.28, 126.53, 123.80, 120.23, 113.53, 102.93, 101.80, 55.39.[30]

2,2,2-Trifluoro-1-(7-methoxy-1*H*-indol-3-yl)ethan-1-one (11): To a cooled (0 °C) and stirred mixture of **10** (0.82 g, 5.6 mmol, 1 eq) in DMF (10 mL) was added dropwise trifluoroacetic anhydride (1.3 mL, 8.8 mmol, 1.3 eq). After stirring at RT for 1 h the reaction was quenched with H_2O (100 mL) and the precipitate filtered. The precipitate was dissolved in EtOAc and the organic layer was washed twice with sat. $NaHCO_3$ (aq) and twice with brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to yield a beige solid (1.31 g, 5.4 mmol, 96%). ^{1}H-NMR (400 MHz, MeOD) δ 8.03 (q, *J* = 1.9 Hz, 1H), 7.82 (d, *J* = 7.3 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 7.1 Hz, 1H), 3.96 (s, 3H). ^{13}C-NMR (101 MHz, MeOD) δ 174.96 (q, *J* = 34.4 Hz), 146.32, 134.85 (q, *J* = 4.8 Hz), 127.29, 126.60, 123.87, 116.79 (q, *J* = 290.5 Hz), 113.56, 109.96, 104.09, 54.63. LCMS (10-90%): t_r = 7.31 min, 244.0 m/z $[C_{11}H_8F_3NO_2+H]^+$.

7-Methoxy-1*H*-indole-3-carboxylic acid (12): A stirred solution of **11** (1.32 g, 5.4 mmol, 1 eq) in 4 M NaOH (aq) (50 mL) was heated (100 °C) for 2 h. The solution was diluted with Et_2O and the layers separated. The aqueous layer was washed once with Et_2O, then acidified to pH ~1 with 3 M HCl (aq). The formed precipitate was filtered to yield a beige solid (0.64 g, 3.4 mmol, 63%). ^{1}H-NMR (400 MHz, DMSO) δ 11.98 (s, 1H), 11.93 (s, 1H), 7.82 (d, *J* = 3.0 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.07 (t, *J* = 7.9 Hz, 1H), 6.75 (d, *J* = 6.9 Hz, 1H), 3.92 (s, 3H). ^{13}C-NMR (101 MHz, DMSO) δ 165.93, 146.33, 131.40, 127.56, 126.46, 121.71, 113.21, 107.96, 102.70, 55.25 LCMS (10→90%): t_r = 4.86 min, 192.07 m/z $[C_{10}H_9NO_3+H]^+$.[31]

1-(Cyclohexylmethyl)-7-methoxy-1*H*-indole-3-carboxylic acid (13): To a stirred (1 h) mixture of **12** (1.82 g, 9.5 mmol, 1 eq) and NaH (60% in mineral oil, 1.04 g, 23.8 mmol, 2.5 eq) in DMF (40 mL) under inert atmosphere was added dropwise bromo-methylcyclohexane (2.7 mL, 19.0 mmol, 2 eq). After heating (60 °C) for 2 h H_2O (120 mL) acidified to pH 1 with 3 M HCl and EtOAc (50 mL) were added. The layers were separated and the aqueous layer was extracted twice with EtOAc and twice with Et_2O. The

combination of organic layers was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 2.5-10% MeOH in DCM) to yield a white solid (2.13 g, 7.4 mmol, 78%). ^{1}H-NMR (400 MHz, MeOD:$CDCl_3$ 1:1) δ 7.73 – 7.64 (m, 2H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.68 (d, *J* = 7.9 Hz, 1H), 4.17 (d, *J* = 7.3 Hz, 2H), 3.91 (s, 3H), 1.79 (hept, *J* = 3.5 Hz, 1H), 1.73 – 1.59 (m, 3H), 1.55 (d, *J* = 12.7 Hz, 2H), 1.15 (s, 3H), 0.96 (q, *J* = 11.5 Hz, 2H). ^{13}C-NMR (101 MHz, MeOD:$CDCl_3$ 1:1) δ 167.30, 147.19, 136.04, 128.90, 125.88, 121.76, 113.41, 105.63, 103.04, 55.89, 54.51, 39.32, 29.95, 25.76, 25.19; LCMS (10-90%): t_r = 8.30 min, 288.07 m/z $[C_{17}H_{21}NO_3+H]^+$.

(*S*)-(1-(Cyclohexylmethyl)-7-methoxy-1*H*-indol-3-yl)(3-methylpiperazin-1-yl)methanone (14): To a cooled (0 °C) and stirred (1 h) mixture of **13** (0.50 g, 1.7 mmol, 1 eq), HATU (1.02 g, 2.7 mmol, 1.5 eq) and DiPEA (1.2 mL, 7.0 mmol, 4 eq) in DMF (20 mL) was added (*S*)-2-methylpiperazine (0.35 g, 3.5 mmol, 2 eq). After stirring at RT for 17 h the mixture was diluted with EtOAc. The organic layer was washed thrice with 4 M NaOH (aq). The combination of aqueous layers was extracted twice with EtOAc. The combination of organic layers was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 1-15% MeOH in DCM) to yield a white solid (0.64 g, 1.7 mmol, quant.). ^{1}H-NMR (400 MHz, MeOD) δ 7.24 (s, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.82 (t, *J* = 8.0 Hz, 1H), 6.44 (d, *J* = 7.9 Hz, 1H), 4.11 (d, *J* = 13.6 Hz, 2H), 3.94 (d, *J* = 7.1 Hz, 2H), 3.61 (s, 3H), 3.11 – 2.93 (m, 3H), 2.87 – 2.77 (m, 2H), 1.51 (hept, *J* = 3.5 Hz, 1H), 1.41 – 1.26 (m, 3H), 1.22 (d, *J* = 11.6 Hz, 2H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.90 – 0.66 (m, 5H). ^{13}C-NMR (101 MHz, MeOD) δ 168.60, 148.89, 133.66, 129.91, 126.89, 122.99, 113.51, 107.93, 104.62, 56.56, 55.80, 52.17, 44.13, 43.46, 40.89, 31.12, 27.14, 26.55, 15.94. LCMS (10-90%): t_r = 5.99 min, 370.0 m/z $[C_{22}H_{31}N_3O_2+H]^+$.

(*S*)-(4-(11-Bromoundecyl)-3-methylpiperazin-1-yl)(1-(cyclohexylmethyl)-7-methoxy-1Hindol-3-yl)methanone (15): A mixture of **14** (0.17 g, 0.5 mmol, 1 eq), **7** (0.26 g, 1.0 mmol, 2.3 eq) and $NaHB(OAc)_3$ (0.15 g, 0.7 mmol, 1.6 eq) in DCM (5 mL) under inert atmosphere was stirred at RT for 17 h. The reaction was quenched with sat. $NaHCO_3$ (aq) (20 mL), and the layers separated. The organic layer was washed twice with sat. $NaHCO_3$ (aq) and twice with brine. The aqueous layer was extracted twice with DCM. The combination of organic layers was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-30% acetone in DCM) to yield a white solid (0.21 g, 0.4 mmol, 77%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.27 (m, 2H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.64 (d, *J* = 7.5 Hz, 1H), 4.17 (dd, *J* = 7.0, 1.5 Hz, 2H), 3.91 (s, 3H), 3.39 (t, *J* = 6.9 Hz, 2H), 3.05 (dd, *J* = 12.7, 9.3 Hz, 2H), 2.86 (d, *J* = 11.6 Hz, 1H), 2.77 – 2.65 (m, 1H), 2.55 – 2.47 (m, 2H), 2.39 – 2.29 (m, 3H), 1.83 (p, *J* = 6.9 Hz, 2H), 1.72 – 1.56 (m, 7H), 1.49 – 1.37 (m, 4H), 1.27 (s, 12H), 1.16 (t, *J* = 7.3 Hz, 2H), 1.05 (d, J = 5.5 Hz, 3H), 0.97 (qd, *J* = 11.5, 2.5 Hz, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 166.43, 147.52, 132.02, 128.50, 125.52, 121.10, 113.22, 109.63, 102.91, 56.06, 55.26, 54.97, 53.36, 50.65, 39.89, 33.97, 32.73, 30.59, 29.45, 29.43, 29.38, 29.33, 28.67, 28.07, 27.50, 26.30, 25.70, 25.28, 15.19. LCMS (10-90%): t_r = 8.72 min, 602.2 m/z. HRMS $[C_{33}H_{52}BrN_3O_2+H]^+$: 602.32429 calculated, 602.33133 found.

(*S*)-(4-(2-(2-(2-(2-Bromoethoxy)ethoxy)ethoxy)ethyl)-3-methylpiperazin-1-yl)(1- (cyclohexylmethyl)-7-methoxy-1*H*-indol-3-yl)methanone (16): A mixture of **14** (0.16 g, 0.4 mmol, 1eq), **6** (0.22 g, 0.9 mmol, 2 eq) and $NaHB(OAc)_3$ (0.16 g, 0.8 mmol, 1.8 eq) was stirred at RT for 17 h. The reaction was quenched with sat. $NaHCO_3$ (aq) (20 mL), and the layers separated. The organic layer was washed twice with sat. $NaHCO_3$ (aq) and twice with brine. The aqueous layer was extracted twice with DCM. The combination of organic layers was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-20% 2-propanol in DCM) to yield a white solid (48.4 mg, 0.1 mmol, 19%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 8.1,

0.9 Hz, 1H), 7.23 (s, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.63 (d, *J* = 7.0 Hz, 1H), 4.16 (dd, *J* = 7.0, 1.7 Hz, 2H), 4.07 (bs, 2H), 3.91 (s, 3H), 3.77 (t, *J* = 6.3 Hz, 2H), 3.69 – 3.54 (m, 10H), 3.44 (t, *J* = 6.3 Hz, 2H), 3.39 – 3.28 (m, 1H), 3.04 – 2.84 (m, 3H), 2.59 – 2.36 (m, 3H), 1.79 (hept, *J* = 3.8 Hz, 1H), 1.72 – 1.53 (m, 5H), 1.20 – 1.09 (m, 4H), 1.04 (d, *J* = 6.1 Hz, 3H), 0.95 (q, *J* = 10.6, 9.1 Hz, 1H). ^{13}C-NMR (101 MHz, CDCl3) δ 166.56, 147.66, 132.13, 128.60, 125.65, 121.20, 113.34, 109.82, 103.03, 71.23, 70.74, 70.65, 70.58, 70.49, 68.95, 61.76, 56.21, 55.52, 55.39, 53.53, 51.88, 40.03, 30.73, 30.39, 26.42, 25.82, 15.74. LCMS (10-90%): t_r = 6.87 min, 610.0 m/z. HRMS $[C_{30}H_{46}BrN_3O_5+H]^+$: 608.26208 calculated, 608.26922 found.

(*S*)-(11-(4-(1-(Cyclohexylmethyl)-7-methoxy-1H-indole-3-carbonyl)-2-methylpiperazin-1-yl)undecyl)triphenylphosphonium TFA-salt (1): A stirred mixture of **15** (62.8 mg, 0.10 mmol, 1 eq) and PPh_3 (27.3 mg, 0.10 mmol, 1eq) in ACN (2.5 mL) was heated (85 °C) for 96 h. The crude product was purified with preparative HPLC to yield a white solid (37.5 mg, 0.05 mmol, 46%). ^{1}H-NMR (300 MHz, MeOD) δ 7.95 – 7.82 (m, 3H), 7.86 – 7.68 (m, 12H), 7.55 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.10 (t, *J* = 7.9 Hz, 1H), 6.76 (d, *J* = 7.9 Hz, 1H), 4.47 (s, 2H), 4.26 (d, *J* = 7.0 Hz, 2H), 3.95 (s, 3H), 3.46 – 3.32 (m, 3H), 3.11 (t, *J* = 8.4 Hz, 2H), 1.83 (hept, *J* = 3.5 Hz, 1H), 1.75 – 1.59 (m, 6H), 1.60 – 1.46 (m, 4H), 1.36 (s, 11H), 1.29 (s, 10H), 1.19 (t, *J* = 7.0 Hz, 2H), 1.03 (q, *J* = 11.6 Hz, 2H). ^{13}C-NMR (75 MHz, MeOD) δ 169.29, 149.22, 136.29, 136.25, 134.84, 134.71, 131.59, 131.43, 129.99, 123.17, 120.56, 119.41, 113.84, 108.46, 104.82, 57.03, 55.95, 54.00, 41.35, 31.76, 31.58, 31.55, 30.46, 30.43, 30.41, 30.19, 29.94, 27.60, 27.47, 26.90, 23.59, 23.54, 23.00, 22.32. LCMS (10-90%): t_r = 7.40 min, 784.6 m/z. HRMS $[C_{51}H_{67}N_3O_2P]^+$: 784.49654 calculated, 784.49600 found.

(*S*)-(11-(4-(1-(Cyclohexylmethyl)-7-methoxy-1*H*-indole-3-carbonyl)-2-methylpiperazin-1-yl)undecyl)tris(4-(trifluoromethyl)phenyl)phosphonium TFA-salt (2): A stirred mixture of **15** (103.0 mg, 0.13 mmol, 1 eq) and tris(4-trifluoromethylphenyl)phosphine (186.0 mg, 0.40 mmol, 3eq) in ACN (0.5 mL) was heated (85 °C) for 96 h. The mixture was transferred to the microwave and heated (180 °C) for an additional 4 h. The crude product was purified with preparative HPLC to yield a white solid (39.2 mg, 0.04 mmol, 31%). ^{1}H-NMR (400 MHz, MeOD) δ 8.13 – 8.04 (m, 12H), 7.55 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.10 (t, *J* = 7.9 Hz, 1H), 6.77 (d, *J* = 7.0 Hz, 1H), 4.48 (bs, 2H), 4.26 (d, *J* = 7.1 Hz, 2H), 3.95 (s, 3H), 3.68 – 3.56 (m, 4H), 3.41 (s, 1H), 3.16 – 3.03 (m, 2H), 1.83 (hept, *J* = 3.7 Hz, 1H), 1.75 – 1.63 (m, 6H), 1.63 – 1.52 (m, 4H), 1.43 – 1.25 (m, 19H), 1.20 (t, *J* = 5.8 Hz, 2H), 1.03 (q, *J* = 10.5 Hz, 2H). ^{13}C-NMR (101 MHz, MeOD) δ 170.69, 149.22, 137.76 (dd, *J* = 33.3, 3.2 Hz), 136.18 (d, J = 10.8 Hz), 134.40, 129.99, 128.44 (dq, *J* = 13.1, 3.8 Hz), 127.22, 125.90 (d, *J* = 1.4 Hz), 123.57 (d, *J* = 85.0 Hz), 123.18, 113.83, 108.44, 104.80, 60.62, 57.05, 55.93, 54.29, 52.03, 51.56, 47.34, 41.35, 31.78, 31.59, 30.49, 30.46, 30.22, 29.87, 27.61, 27.47, 26.91, 23.36, 23.32, 22.28, 21.80, 14.68. LCMS (10-90%): t_r = 8.10 min, 988.5 m/z. HRMS $[C_{54}H_{64}F_9N_3O_2P]^+$: 988.45870 calculated, 988.45810 found.

(*S*)-(2-(2-(2-(2-(4-(1-(Cyclohexylmethyl)-7-methoxy-1*H*-indole-3-carbonyl)-2-methylpiperazin-1-yl)ethoxy)ethoxy)ethoxy)ethyl)triphenylphosphonium TFA-salt (3): A stirred mixture of **16** (24.0 mg, 0.04 mmol, 1 eq) and PPh_3 (33.0 mg, 0.12 mmol, 3 eq) in ACN (0.5 mL) was heated (85 °C) for 96 h. The mixture was transferred to the microwave and heated (180 °C) for an additional 2 h. The crude product was purified with preparative HPLC to yield a white solid (8.9 mg, 0.01 mmol, 28%). ^{1}H-NMR (400 MHz, MeOD) δ 7.90 – 7.75 (m, 9H), 7.76 – 7.67 (m, 6H), 7.55 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 4.26 (d, *J* = 7.1 Hz, 2H), 3.95 (s, 3H), 3.84 (d, *J* = 3.3 Hz, 2H), 3.83 – 3.76 (m, 2H), 3.77 – 3.68 (m, 5H), 3.62 – 3.56 (m, 3H), 3.49 – 3.42 (m, 3H), 3.39 – 3.30 (m, 8H), 1.82 (hept, *J* = 3.6 Hz, 1H), 1.74 – 1.63 (m, 2H), 1.55 (d, *J* = 11.8 Hz, 2H), 1.37 (s, 2H), 1.18 (t, *J* = 8.9 Hz, 3H), 1.10 (s, 3H), 1.03 (q, *J* = 11.8 Hz, 1H). ^{13}C-NMR (101 MHz, MeOD) δ 168.40, 149.25, 136.09, 136.06, 135.09 (d, *J* = 10.3 Hz), 131.23 (d, *J* = 12.8 Hz), 129.99, 127.23, 123.24, 120.85, 119.98, 113.83, 104.84, 71.56, 71.34, 71.17, 71.08, 64.79, 64.72, 57.06, 55.97, 41.37, 31.59, 27.47, 26.92, 26.67. LCMS (10-90%): t_r = 6.60 min, 790.4 m/z. HRMS $[C_{48}H_{61}N_3O_5P]^+$: 790.43434 calculated, 790.43427 found.

(*S*)-(2-(2-(2-(2-(4-(1-(Cyclohexylmethyl)-7-methoxy-1*H*-indole-3-carbonyl)-2-methylpiperazin-1-yl)ethoxy)ethoxy)ethoxy)ethyl)tris(4-(trifluoromethyl)phenyl)phosphonium TFA-salt (4): A stirred mixture of **16** (24.0mg, 0.04 mmol, 1 eq) and tris(4-trifluoromethylphenyl)phosphine (114.0mg, 0.24mmol, 6 eq) in ACN (0.5 mL) was heated (85 °C) for 96 h. The mixture was transferred to the microwave and heated (120 °C) for an additional 14 h. The crude product was purified with preparative HPLC to yield a white solid (3.2mg, 3.0 µmol, 8%). ^{1}H-NMR (850 MHz, MeOD) δ 8.14 – 8.03 (m, 12H), 7.54 (s, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.77 (d, *J* = 7.7 Hz, 1H), 4.43 (br, 1H), 4.26 (d, *J* = 7.1 Hz, 2H), 4.03 – 3.98 (m, 2H), 3.95 (s, 3H), 3.87 – 3.79 (m, 4H), 3.65 (m, 2H), 3.60 – 3.56 (m, 2H), 3.48 (m, 1H), 3.44 – 3.41 (m, 2H), 3.37 – 3.33 (m, 5H), 1.82 (m, 1H), 1.70-1.65 (m, 3H), 1.54 (m, 2H), 1.37 (m, 3H), 1.31 (m, 3H), 1.19 (m, 3H), 1.10 (s, 1H), 1.03 (m, 2H). ^{13}C-NMR (214 MHz, MeOD) δ 163.08, 149.27, 137.51 (qd, *J* = 32.8, 32.3, 2.8 Hz), 136.40 (d, *J* = 11.2 Hz), 134.32, 130.00, 128.12 (dd, *J* = 13.4, 3.6 Hz), 127.26, 124.40, 123.99, 123.22, 113.80, 108.45, 104.86, 71.63, 71.31, 71.05, 71.03, 64.43, 64.39, 57.06, 55.96, 55.95, 49.38, 47.93, 36.95, 31.59, 27.46, 26.90, 24.92, 24.68, 9.20. LCMS (10-90%): t_r = 9.45 min, 994.4 m/z. HRMS $[C_{51}H_{58}F_9N_3O_5P]^+$: 994.39649 calculated, 994.39586 found.

Biology

All biologic assays have been previously described. “General remarks”, “Quantification and statistical analysis”, “Cell culture”, “Membrane preparation”, “[^{3}H]CP-55,940 Heterologous Displacement Assays” and “[^{35}S]GTPγS Binding Assays” can be referenced in **Chapter 2.**

References

1. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*. 1990;346(6284):561-564. doi:10.1038/346561a0

2. Katona I, Freund TF. Multiple functions of endocannabinoid signaling in the brain. *Annu Rev Neurosci*. 2012;35:529-558. doi:10.1146/annurev-neuro-062111-150420

3. Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y. Endocannabinoid Signaling and Synaptic Function. *Neuron*. 2012;76(1):70-81. doi:10.1016/j.neuron.2012.09.020

4. Mechoulam R, Parker LA. The endocannabinoid system and the brain. *Annu Rev Psychol*. 2013;64:21-47. doi:10.1146/annurev-psych-113011-143739

5. Kendall DA, Yudowski GA. Cannabinoid Receptors in the Central Nervous System: Their Signaling and Roles in Disease. *Front Cell Neurosci*. 2017;10. doi:10.3389/fncel.2016.00294

6. Pérez-Olives C, Rivas-Santisteban R, Lillo J, Navarro G, Franco R. Recent Advances in the Potential of Cannabinoids for Neuroprotection in Alzheimer's, Parkinson's, and Huntington's Diseases. *Adv Exp Med Biol*. 2021;1264:81-92. doi:10.1007/978-3-030-57369-0_6

7. Melis M. *Endocannabinoids and Lipid Mediators in Brain Functions*. (Melis M, ed.). Cham: Springer International Publishing; 2017. doi:10.1007/978-3-319-57371-7

8. Schurr A, Livne A. Differential inhibition of mitochondrial monoamine oxidase from brain by hashish components. *Biochem Pharmacol*. 1976;25(10):1201-1203. doi:10.1016/0006-2952(76)90369-5

9. Chari-Bitron A, Bino T. Effect of Δ l-tetrahydrocannabinol on ATPase activity of rat liver mitochondria. *Biochem Pharmacol*. 1971;20(2):473-475. doi:10.1016/0006-2952(71)90084-0

10. Sarafian TA, Kouyoumjian S, Khoshaghideh F, Tashkin DP, Roth MD. Δ 9 -Tetrahydrocannabinol disrupts mitochondrial function and cell energetics. *Am J Physiol Cell Mol Physiol*. 2003;284(2):L298-L306. doi:10.1152/ajplung.00157.2002

11. Cohen G, Kesler N. Monoamine oxidase and mitochondrial respiration. *J Neurochem*. 1999;73(6):2310-2315. doi:10.1046/j.1471-4159.1999.0732310.x

12. Shih JC, Chen K, Ridd MJ. MONOAMINE OXIDASE: From Genes to Behavior. *Annu Rev Neurosci*. 1999;22(1):197-217. doi:10.1146/annurev.neuro.22.1.197

13. Fišar Z, Singh N, Hroudová J. Cannabinoid-induced changes in respiration of brain mitochondria. *Toxicol Lett*. 2014;231(1):62-71. doi:10.1016/j.toxlet.2014.09.002

14. Bénard G, Massa F, Puente N, et al. Mitochondrial CB 1 receptors regulate neuronal energy metabolism. *Nat Neurosci*. 2012;15(4):558-564. doi:10.1038/nn.3053

15. Aquila S, Guido C, Santoro A, et al. Rimonabant (SR141716) induces metabolism and acquisition of fertilizing ability in human sperm. *Br J Pharmacol*. 2010;159(4):831-841. doi:10.1111/j.1476-5381.2009.00570.x

16. Arrabal S, Lucena MA, Canduela MJ, et al. Pharmacological Blockade of Cannabinoid CB1 Receptors in Diet-Induced Obesity Regulates Mitochondrial Dihydrolipoamide Dehydrogenase in Muscle. Appanna VD, ed. *PLoS One*. 2015;10(12):e0145244. doi:10.1371/journal.pone.0145244

17. Serrat R, Covelo A, Kouskoff V, et al. Astroglial ER-mitochondria calcium transfer mediates endocannabinoid-dependent synaptic integration. *Cell Rep*. 2021;37(12):110133. doi:10.1016/j.celrep.2021.110133

18. Koch M, Varela L, Kim JG, et al. Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature*. 2015;519(7541):45-50. doi:10.1038/nature14260

19. Hebert-Chatelain E, Desprez T, Serrat R, et al. A cannabinoid link between mitochondria and memory. *Nature*. 2016;539(7630):555-559. doi:10.1038/nature20127

20. Yin A, Wang F, Zhang X. Integrating endocannabinoid signaling in the regulation of anxiety and depression. *Acta Pharmacol Sin*. 2019;40(3):336-341. doi:10.1038/s41401-018-0051-5

21. Djeungoue-Petga M-A, Hebert-Chatelain E. Linking Mitochondria and Synaptic Transmission: The CB1 Receptor. *BioEssays*. 2017;39(12):1700126. doi:10.1002/bies.201700126

22. Pankratov Y V., Lalo U V., Krishtal OA. Role for P2X Receptors in Long-Term Potentiation. *J Neurosci*. 2002;22(19):8363-8369. doi:10.1523/JNEUROSCI.22-19-08363.2002

23. Jiménez-Uribe AP, Pedraza-Chaverri J. Promising Therapeutic Strategies Targeting Mitochondria in Kidney Diseases: From Small Molecules to Whole Mitochondria. *Futur Pharmacol*. 2022;2(3):256-275. doi:10.3390/futurepharmacol2030017

24. Ross MF, Kelso GF, Blaikie FH, et al. Lipophilic triphenylphosphonium cations as tools in mitochondrial bioenergetics and free radical biology. *Biochem*. 2005;70(2):222-230. doi:10.1007/s10541-005-0104-5

25. Murphy MP, Smith RAJ. Targeting Antioxidants to Mitochondria by Conjugation to Lipophilic Cations. *Annu Rev Pharmacol Toxicol*. 2007;47(1):629-656. doi:10.1146/annurev.pharmtox.47.120505.105110

26. Kulkarni CA, Fink BD, Gibbs BE, et al. A Novel Triphenylphosphonium Carrier to Target Mitochondria without Uncoupling Oxidative Phosphorylation. *J Med Chem*. 2021;64(1):662-676. doi:10.1021/acs.jmedchem.0c01671

27. Adam JM, Cairns J, Caulfield W, et al. Design, synthesis, and structure–activity relationships of indole-3-carboxamides as novel water soluble cannabinoid CB1 receptor agonists. *Medchemcomm*. 2010;1(1):54. doi:10.1039/c0md00022a

28. Kevin RC, Mirlohi S, Manning JJ, et al. Putative Synthetic Cannabinoids MEPIRAPIM, 5F-BEPIRAPIM (NNL-2), and Their Analogues Are T-Type Calcium Channel (Ca V 3) Inhibitors. *ACS Chem Neurosci*. 2022;13(9):1395-1409. doi:10.1021/acschemneuro.1c00822

29. Zuurman L, Passier P, de Kam M, Kleijn H, Cohen A, van Gerven J. Pharmacodynamic and pharmacokinetic effects of the intravenously administered CB1 receptor agonist Org 28611 in healthy male volunteers. *J Psychopharmacol*. 2009;23(6):633-644. doi:10.1177/0269881108091551

30. Kondo Y, Kojima S, Sakamoto T. General and Facile Synthesis of Indoles with Oxygen-Bearing Substituents at the Benzene Moiety. *J Org Chem*. 1997;62(19):6507-6511. doi:10.1021/jo970377w

31. Thakur G, Nikas S, Makriyannis A. CB1 Cannabinoid Receptor Ligands. *Mini-Reviews Med Chem*. 2005;5(7):631-640. doi:10.2174/1389557054368772

Acknowledgements

This work was supported by the Dutch Research Council (NWO, Navistroke #15851) (Mario van der Stelt[d], M.v.d.S.).

Author contributions: Conducting Experiments, L.d.B (Lorenzo de Bie), L.V.d.P. (Laura V. de Paus); Writing-Original Draft, L.V.d.P.; Writing Review & Editing, M.v.d.S., L.H.H. (Laura H. Heitman), R.J.B.H.N.v.d.B (Richard J. B. H. N. van den Berg); Supervision, R.J.B.H.N.v.d.B., M.v.d.S.