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

Paus, L.V. de

Chapter 4

Discovery of a Cannabinoid CB_2 Receptor Fluorescent Probe Based on a Pyridin-2-yl-benzyl-imidazolidine-2,4-dione Scaffold

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Discovery of a Cannabinoid CB_2 Receptor Fluorescent Probe Based on a Pyridin-2-yl-benzyl-imidazolidine-2,4-dione Scaffold

Introduction

The cannabinoid receptor type 2 (CB_2R) is a class A G protein-coupled receptor (GPCR) with a role in inflammation and neurodegenerative diseases, making it an interesting target for drug discovery for multiple applications.[1] Confirming target engagement and understanding the biological cascades that lead to the therapeutic effect of a therapeutic agent are necessary for the successful translation of compounds into the clinic. At the moment no CB_2R-selective drugs have yet reached the market.[2] Fluorescent probes are ideal for detecting CB_2R in relevant cell types, target engagement studies and explore the roles of CB_2R in healthy and pathological systems.[3] To this end, several fluorescent probes have been reported.[2–14] Leiden university has also contributed to this field and reported the 5-fluoropyridin-2-yl-benzyl-imidazolidine-2,4-dione LEI-121 (Figure 4.1A) as a CB_2R selective bifunctional probe that captured the CB_2R upon photoactivation.[15] An incorporated alkyne served as a ligation handle for the introduction of fluorescent reporter groups. LEI-121 enabled target engagement studies and visualization of endogenously expressed CB_2R in HL-60 and primary human immune cells using flow cytometry.[15] However, LEI-121 is a two-step probe that requires copper-catalysed azide-alkyne cycloaddition (click-reaction) with a fluorophore to visualize the receptor. For live imaging purposes, it would be beneficial to avoid the copper-mediated click reaction, which is toxic to cells, and to have a one-step fluorescent probe in which the fluorophore is incorporated into the structure of the ligand.

Previously, Leiden university reported the three-dimensional structure of CB_2R in complex with the 5-fluoropyridin-2-yl-benzyl-imidazolidine-2,4-dione analogue LEI-102 using cryogenic electron microscopy (Figure 4.1).[16] This provided an excellent opportunity for the structure-based design of novel one-step fluorescent CB_2R probes. To validate this reasoning, novel one-step fluorescent CB_2R probes based on the 5-fluoropyridin-2-yl-benzyl-imidazolidine-2,4-dione series were designed, synthesized and characterized.

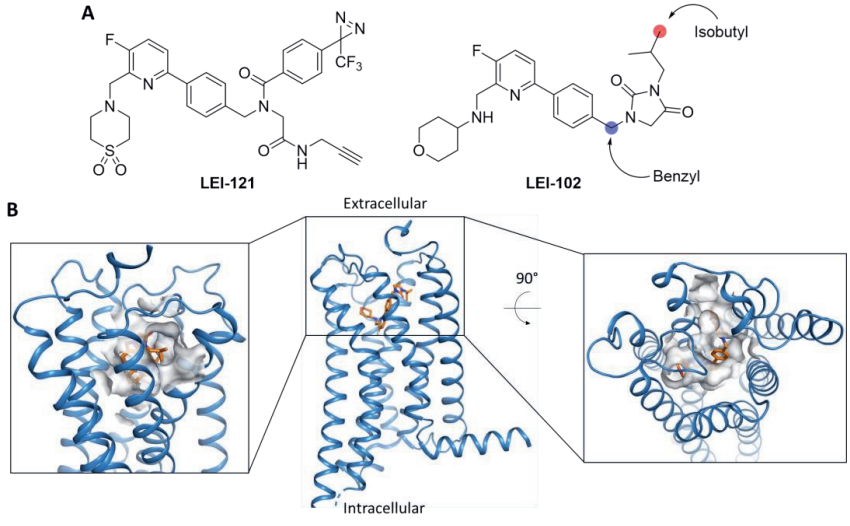


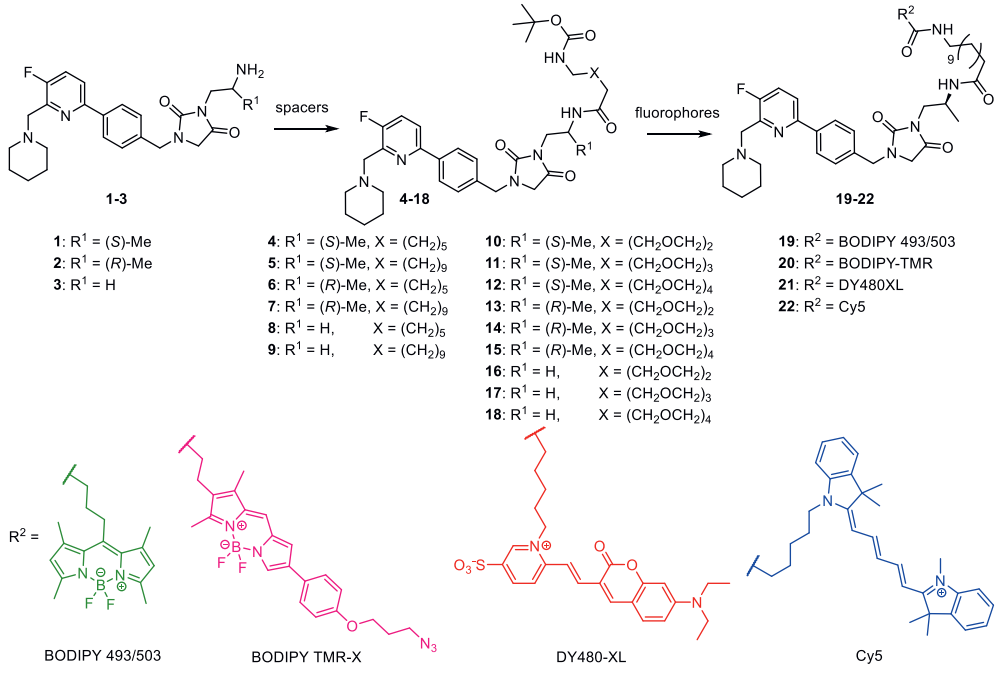
Figure 4.1 LEI-121 and LEI-102 and the resolved cryo-EM structure of hCB_2R with LEI-102. (A) The chemical structures of CB_2R probe LEI-121 and CB_2R agonist LEI-102. The optimal positions to attach a spacer have been marked in the structure of

LEI-102. (B) Cryo-EM structures of CB_2R (sky blue, PDB: 8GUT) in complex with LEI-102 (orange) at two different angles, with surface representation of receptor atoms within 4 Å. Figure generated with Open Source PyMOL Molecular Viewer v2.4.[16]

Results & Discussion

Design

The cryo-EM structure of the human CB_2R in complex with LEI-102 was inspected to find the best exit vector on the scaffold for the introduction of a spacer that could be coupled to various fluorophores. The isobutyl and benzylic position were positioned equally well to serve as an attachment point for spacer plus fluorophore. For synthetic reasons the isobutyl position was chosen to introduce an optionally methyl substituted ethylamine (compound **1**-**3**) as attachment point for various alkyl (compound **4**-**9**) or polyethylene glycol (PEG) (compound **10**-**18**) spacers. The most promising scaffold spacer compound (**5**) was coupled to four different fluorophores, *i.e.* BODIPY 493/503, BODIPY-TMR-X, DY480-XL and Cy-5, resulting in compound **19**-**22** (Scheme 4.1). BODIPY dyes have the advantages of high quantum yield, high molar extinction coefficients an brightness, as well as being relatively insensitive to the pH of their environment.[17,18] Cy5 is frequently used and emits in the near infrared range which is not affected by biological auto-fluorescence, shows high stability, moderate insensitivity to solvent polarity, is highly water soluble, and has one of the highest molar extinction coefficients.[19–21] DY 480XL has a large stokes shift which increases the signal to noise by lowering chance of self-quenching, self-absorption and excitation source cross-talk.[22]

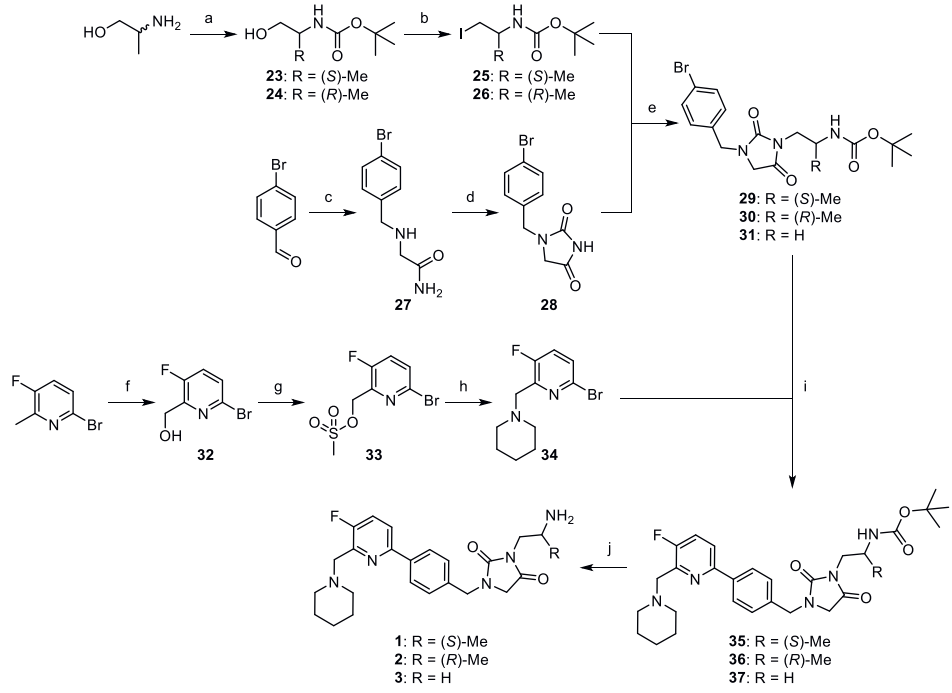


Scheme 4.1 The synthesis plan of the LEI-102-derived fluorescent probes. The scaffold contains an amine conjugation site which is either the (*S*)- or (*R*)- 2-aminopropyl enantiomer or achiral 2-aminoethyl moiety. To all three scaffolds a selection of five spacers was conjugated: C_8, C_{12}, PEG2, PEG3, or PEG4. **5** exhibited the highest CB_2R affinity and was conjugated to the four fluorophores. The Cy5 conjugate **22** showed the best biochemical properties.

Synthesis

The synthesis of the CB_2R selective fluorescent probes based on LEI-102 **19**-**22** started with the construction of the pyridinylbenzylimidazolidine-2,4-dione intermediates **1**-**18**. Commercially available

alaninol (D/L, Scheme 4.2) was carbamate protected (**23**, **24**), then subsequent iodination gave iodide derivatives **25** and **26**, respectively. Meanwhile reductive amination of 4-bromobenzaldehyde and aminoacetamide led to compound **27**. After cyclization the formed imidazolidinedione **28** was alkylated with tert-butyl-(2-bromoethyl)carbamate, **25**, or **26** which afforded compounds **29**-**31**. Next, oxidation of 6-bromo-3-fluoro-2-methylpyridine with *m*-CPBA followed by a Boekelheide rearrangement led to **32**. After mesylation of the primary alcohol, the mesyl **33** was substituted with piperidine (**34**). Subsequently, borylation of **29**-**31** with pinacolboronic ester and conjugation to **34** via a Suzuki coupling (**35**-**37**) and finally acidolysis gained pharmacophores **1**-**3** (Scheme 4.2).

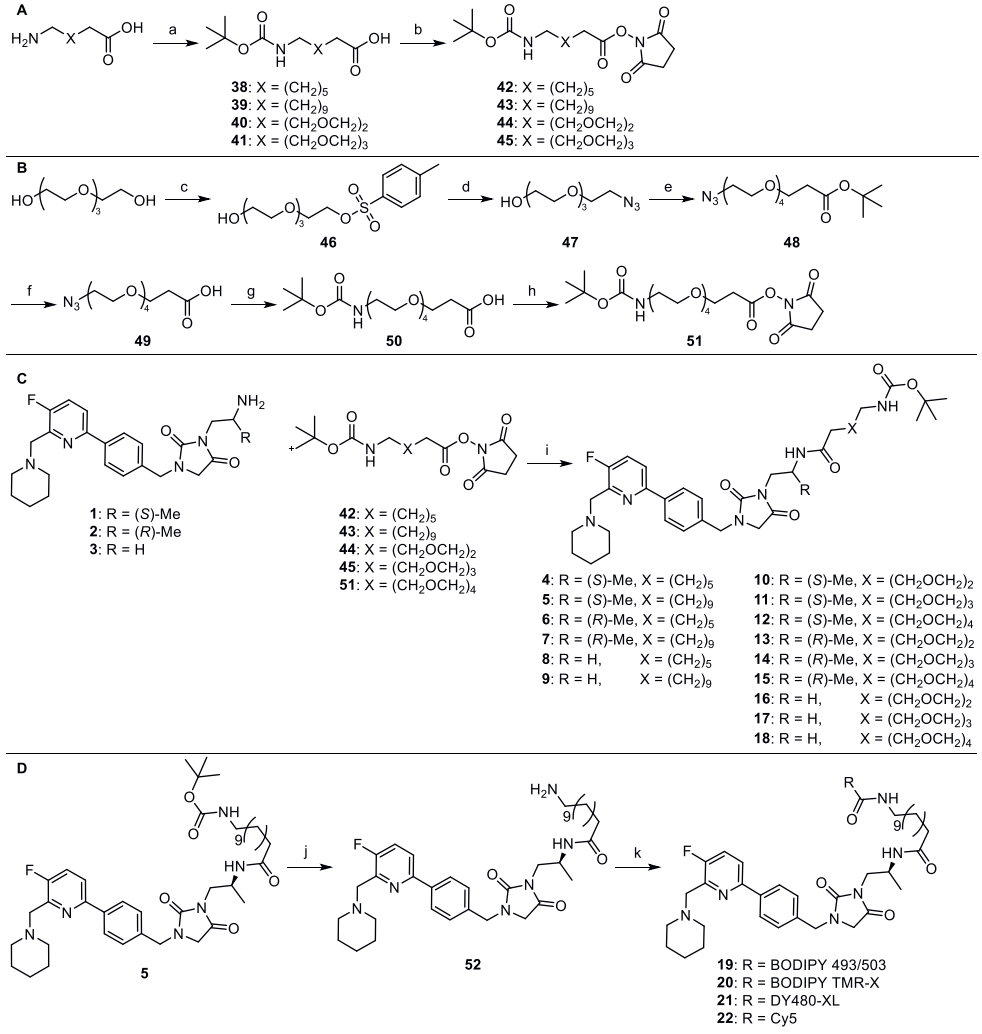


Scheme 4.2 The synthesis of the three scaffold intermediates **1**-**3**. Reagents and conditions: a) Boc_2O (1-2 eq), NaOH (1 M, 2 eq), 1,4 dioxane, RT, 3 h, 97-99%; b) PPh_3 (1.2-1.5 eq), imidazole (1.4-1.8 eq), iodine (1.3-1.7 eq), ACN:Et_2O (3:10, v/v), RT, 16 h, 53-62%; c) step 1: 2-aminoacetamide hydrochloride (1.0 eq), NaOH (1.1 eq), MeOH:H_2O (5:1), RT, 18 h; Step 2: $NaBH_4$ (2.1 eq), 18 h, 91% (two steps); d) CDI (2.1 eq), DMAP (2.1 eq), ACN, 60 °C, 70 h, 37%; e) *tert*-butyl-(2-bromoethyl)carbamate for **31**/ **25** for **29**/ **26** for **30** (2 eq), 1-(4-bromobenzyl)imidazolidine-2,4-dione (1 eq), K_2CO_3 (6 eq), 18-crown-6 (0.2 eq), DMF (0.2 M), 50 °C, 16 h, 62-88%; f) step 1: *m*-CPBA (1.8 eq), 0 °C-RT, DCM, 4 days; step 2: TFAA (2.2 eq), 55 °C, 3 h; step 3: K_2CO_3 (2.3 eq), THF:MeOH (20:1), 17 h, 35% (three steps); g) Et_3N (2.3 eq), MsCl (1.7 eq), THF, 0 °C-RT, 1 h, 75%; h) K_2CO_3 (2.2 eq), piperidine (1.2 eq), ACN (0.2 M), 50 °C, 1.5 h, 93%; i) step 1: KOAc (4-6 eq), bis(pinacolato)diboron (1.5-2.2 eq), $Pd(dppf)Cl_2$ (0.05-0.08 eq), DMF (degassed, 0.2 M), 75 °C, 16 h; step 2: 34 (1 eq), K_2CO_3 (4-8 eq), $Pd(PPh_3)_4$ (0.05-0.2 eq), toluene:EtOH (degassed, 4:1, v/v, 0.2 M), 75 °C, 16 h, 36-76% (two steps); j) 4 M HCl (1,4 dioxane, 4 eq), acetonitrile (0.5 M), 80 °C, 2 h, 62-86%.

The various alkyl and ethyleneglycol based spacers (**42**-**45**, **51**) were synthesized according to the generic synthesis depicted in Scheme 4.3A-B. Alkyl spacers C8 and C12 were synthesized from 8 aminooctanoic acid and 12 aminododeanoic acid respectively. The glycol spacers PEG2 and PEG3 were synthesized from 3-(2-(2-aminoethoxy)ethoxy)propanoic acid and 3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanoic acid respectively. The amino acids were *N*-Boc protected (**38**-**41**) and subsequently converted to the O-Su active esters **42**-**45**. The PEG4 glycol spacer required several additional steps (Scheme 4.3B). Tosylation of tetraethylene glycol (**46**) was followed by azide substitution (**47**) of the sulfonate ester. This allowed a Michael addition to tert-butyl acrylate under

TBAF conditions to give **48**. Acidolysis of the ester (**49**) was followed by reduction of the azide and simultaneous *N*-Boc protection gained acid **50**. The last step introduced the *N*-succinimide ester to give **51**.

The three pharmacophores (**1**-**3**) were under basic conditions conjugated to the five spacers (**42**-**45**, **51**) to yield the fifteen CB_2R probe precursors, **4**-**18** (Scheme 4.3C). The most potent of theses series, *i.e.* compound **5**, was *N*-Boc deprotected (**52**) and thereafter conjugated to the four fluorophores (Scheme 4.3D): affording the BODIPY 493/503 (**19**), BODIPY TMR X (**20**), DY480 XL (**21**) and Cy5 (**22**) LEI-102 based probes.



Scheme 4.3 The synthesis of the fifteen intermediates followed by the synthesis of the fluorescent probes. Reagents and conditions: a) *Alkyl*: Et_3N (2 eq), Boc_2O (1.1 eq), acetone:H_2O (1:1, v/v, 0.5 M), RT, 16 h, 95-99%; *PEG*: K_2CO_3 (3 eq), Boc_2O (1.3 eq), H_2O:THF (1:1, v/v, 0.1 M), RT, 16 h, 40-81%; b) *Alkyl*: EDC.HCl (0.8-0.9 eq), NHS (1.7-2.8 eq), DCM (0.3 M), RT, 16-72 h, 36-40%; *PEG*: EDC.HCl (3 eq), NHS (1.5 eq), Et_3N (3 eq), DCM (0.2 M), RT, 16 h, 46-58%; c) *p*-TsCl (1 eq), NaOH (2 M, 1.6 eq), THF (0.6 M), 0 °C, 4 h, 90%; d) NaN_3 (1.5 eq), ACN (0.4 M), 80 °C, 8 h, 94%; e) *tert*-butyl acrylate (1 eq), TBAF (0.4 eq), NaOH (25 wt% in H_2O, 2.6 eq), DCM, RT, 8 h, 75%; f) TFA (50 eq), DCM, RT, 4 h, 65%; g) step 1: 10% Pd/C (0.1 eq), H_2 (g), EtOH (0.3 M), RT, 16 h; step 2: K_2CO_3 (3 eq), Boc_2O (1.3 eq), H_2O:THF (1:1, v/v, 0.1 M), RT, 16 h, 55% (two steps); h) EDC.HCl (1.2 eq),

NHS (1.1 eq), DCM (0.2 M), RT, 16 h, 84% ; i) Et_3N (6 eq), DCM (0.3 M), RT, 1-3 h, 13-65%. j) TFA (110 eq), DCM, RT, 2 h, quant.; k) **19**-**21**: Et_3N (1 eq), fluorophore-NHS ester (1 eq), DCM (0.3 M), RT, 13 h, 64-100%; **22**: HOBt (1.2 eq), DIPEA (2.5 eq), EDC.HCl (1.3 eq), Cyanine 5 carboxylic acid (1.1 eq), DMF (0.007 M), RT, 16 h, quant.).

Molecular Pharmacology

Compounds **1**-**22** were tested at 1 μM in a [^{3}H]CP-55,940 radioligand displacement assay to determine their affinity for the CB_2R and CB_1R. Compounds with less than 50% displacement were considered inactive. The results are shown in Table 4.1.

Table 4.1 hCB_1R and hCB_2R binding affinity and potency of compounds **1**-**22**.

		CB_2R			CB_1R
Compound	**Displacement (% ± SEM)**	**pKi ± SEM**	**pEC_{50} ± SEM**	**E_{max} (% ± SEM)**	**Displacement (% ± SEM)**
LEI-102	84 ± 04	8.6 ± 0.3	7.3 ± 0.4	46 ± 17	-20 ± 08
1	35 ± 10	< 5	6.4 ± 0.1	28 ± 04	-20 ± 27
2	-4 ± 10	n.d.	n.d.	n.d.	-20 ± 17
3	0 ± 13	n.d.	n.d.	n.d.	-2 ± 18
4	25 ± 09	n.d.	n.d.	n.d.	28 ± 13
5	63 ± 05	6.5 ± 0.1	6.3 ± 0.2	-73 ± 04	36 ± 03
6	22 ± 07	n.d.	n.d.	n.d.	-16 ± 29
7	19 ± 08	n.d.	n.d.	n.d.	30 ± 02
8	27 ± 10	n.d.	n.d.	n.d.	-15 ± 28
9	43 ±4	n.d.	n.d.	n.d.	32 ± 20
10	-13 ± 22	n.d.	n.d.	n.d.	-17 ± 24
11	-4 ± 04	n.d.	n.d.	n.d.	-22 ± 14
12	11 ± 21	n.d.	n.d.	n.d.	1 ± 12
13	-12 ± 15	n.d.	n.d.	n.d.	5 ± 10
14	3 ± 01	n.d.	n.d.	n.d.	-3 ± 15
15	1 ± 19	n.d.	n.d.	n.d.	-2 ± 10
16	-7 ± 16	n.d.	n.d.	n.d.	0 ± 16
17	1 ±10	n.d.	n.d.	n.d.	5 ± 19
18	15 ± 14	n.d.	n.d.	n.d.	7 ± 16
19	31 ± 03	< 5	n.d.	n.d.	-12 ± 06
20	16 ± 04	< 5	n.d.	n.d.	-63 ± 37
21	17 ± 14	< 5	n.d.	n.d.	-16 ± 19
22	39 ± 05	6.2 ± 0.6	5.3 ± 0.1	-63 ± 06	10 ± 18

Binding affinities were determined either as displacement (%) or pK_i using a [^{3}H]CP-55,940 radioligand displacement assay on CHO cells stably over expressing either hCB_2R_bgal or hCB_1R_bgal. The displacement percentages represent the percentage of radioligand displaced from the receptor at 1 μM compound. Total binding at vehicle concentration was set at 0%, while non-specific binding was set at 100%. Potency values (pEC_{50}) were obtained for compounds with displacement ≥ 35% using a [^{35}S]GTPγS assay on CHO cells stably over expressing hCB_2R_bgal. The maximum effect (E_{max} in %) was normalized to reference full agonist CP-55,940. All values are presented as the mean ± SEM of at least two independent experiments performed in triplicate. N.d. not determined.

Compound **5** was the only scaffold that was active on hCB_2R (63% ± 5 at 1 μM), but not hCB_1R (pK_i < 5), therefore the four fluorophores were only conjugated to this scaffold. Subsequently, the binding affinity (pK_i), potency (pEC_{50}) and efficacy (E_{max}) of compounds **19**-**22** was determined in a functional [^{35}S]GTPγS assay (Table 4.1 and Figure 4.2). Compound **22** displayed a pK_i (CB_2R) of 6.2 ± 0.6 and was inactive on CB_1R. The other fluorescent probes were inactive at CB_2R. In contrast to LEI-102 and the parent compound **1**, compound **22** was an inverse agonist with a pEC_{50} of 5.3 ± 0.1 and E_{max} of -63% ± 6. Previous research on LEI-121 showed that very small structural changes in a compound may change the functional behaviour of the CB2R.[15] It is not clear from a structural point of view what is the cause of this switch in functionality. However, it could be presumed that the alkyl linker may prevent the

conformational change needed by one or more of the seven transmembrane helices in the binding site to activate the receptor.

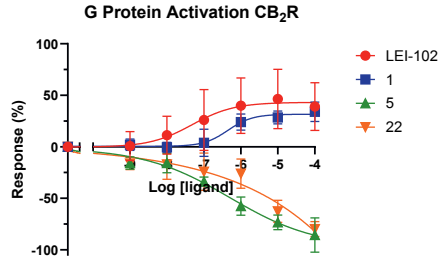


Figure 4.2 G protein activation levels on CB_2R were determined with a [^{35}S]GTPγS assay. Basal activity in presence of vehicle was set to 0%, whereas full G protein activation was determined using 10 µM of full agonist CP-55,940 and was set as 100%. Data are expressed as mean ± SEM from three experiments performed in triplicate.

Conclusion

A structure-based approach in combination with rational design was used to develop a one-step fluorescent probe for the cannabinoid CB_2 receptor based on a pyridin-2-yl-benzyl-imidazolidine-2,4-dione scaffold. Ultimately probe **22** was synthesized with an alkyl spacer and Cy5 fluorescent dye. Probe **22** demonstrated reasonable CB_2R affinity (pK_i 6.2 ± 0.6), was selective over CB_1R and behaved as an inverse agonist (EC_{50} 5.3 ± 0.1, E_{max} -63% ± 6). It is envisioned that probe **22** can be used to visualize CB_2R in cells without the need for copper-assisted click reactions.

Experimental

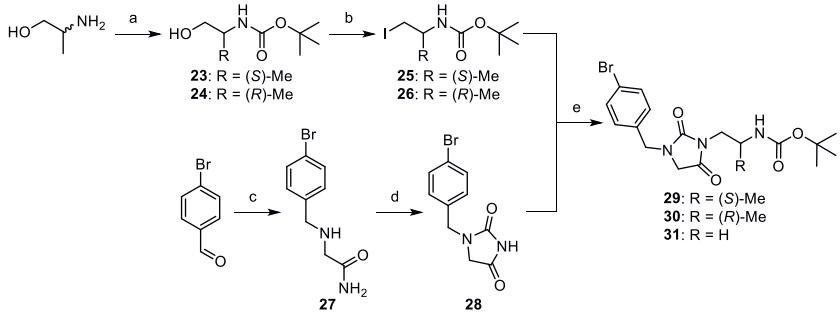
Chemistry

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 (λ254) 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 $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (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, 13C, 1H-COSY and HSQC Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AV 300 (300/75 MHz), AV 400 (400/100 MHz), AV 500 (500/125 MHz) or AV 850 (850/214 MHz) spectrometer at ambient temperature using CDCl3 as solvent. Chemical shifts (δ) are referenced in parts per million (ppm) with tetramethylsilane (TMS) or CDCl3 resonance as the internal standard peak (CDCl3/TMS, δ 0.00 for 1H (TMS), δ 77.16 for 13C (CDCl3)). Multiplicity is reported as s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, p = quintet, hept = heptet, m = multiplet. Coupling-constants (*J*) are reported in Hertz (Hz).

Synthesis of the imidazolidine building block:



Scheme 4.4 Reagents and conditions: a) Boc_2O (1-2 eq), NaOH (1 M, 2 eq), 1,4-dioxane, RT, 3 h, 97-99%; b) PPh_3 (1.2-1.5 eq), imidazole (1.4-1.8 eq), iodine (1.3-1.7 eq), ACN:Et_2O (3:10, v/v), RT, 16 h, 53-62%; c) step 1: 2-aminoacetamide hydrochloride (1.0 eq), NaOH (1.1 eq), MeOH:H_2O (5:1), RT, 18 h; Step 2: $NaBH_4$ (2.1 eq), 18 h, 91% (two steps); d) CDI (2.1 eq), DMAP (2.1 eq), ACN, 60 °C, 70 h, 37%; e) tert-butyl-(2-bromoethyl)carbamate for **31**/ **25** for **29**/ **26** for **30** (2 eq), 1-(4-bromobenzyl)imidazolidine-2,4-dione (1 eq), K_2CO_3 (6 eq), 18-crown-6 (0.2 eq), DMF (0.2 M), 50 °C, 16 h, 62-88%.

***tert*-Butyl (*S*)-(1-hydroxypropan-2-yl)carbamate (23):** To a cooled (0 °C) and stirred mixture of L-alaninol (0.38 g, 5.0 mmol, 1 eq) in 1 M NaOH (10 mL) was added Boc_2O (2.20 g, 10.0 mmol, 2 eq) dissolved in 1,4-dioxane (10 mL). After stirring for 3 h at RT, the organic solvent was evaporated under reduced pressure and the aqueous layer was acidified to pH = 8 with sat. NH_4Cl (aq). The aqueous layer was extracted thrice with EtOAc. The combined organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 33% EtOAc in pentane) to yield a colourless oil (0.87 g, 5.0 mmol, 99%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.79 (s, 1H), 3.74 (s, 1H), 3.64 – 3.56 (m, 1H), 3.52 – 3.43 (m, 1H), 3.10 (bs, 1H), 1.42 (s, 9H), 1.12 (d, *J* = 7.3 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 156.48, 79.78, 67.35, 48.66, 29.61, 17.42. LC-MS (ESI, 10-90): t_R = 4.25 min; m/z = 175.60 $[M]^+$.

***tert*-Butyl (*R*)-(1-hydroxypropan-2-yl)carbamate (24):** To a cooled (0 °C) and stirred mixture of D-alaninol (0.38 g, 5.0 mmol, 1 eq) in 1 M NaOH (10 mL) was added Boc_2O (1.20 g, 5.5 mmol, 1.1 eq) dissolved in 1,4-dioxane (5.5 mL). After stirring for 3 h at RT, the organic solvent was evaporated under reduced pressure and the aqueous layer was acidified to pH = 8 with sat. NH_4Cl (aq). The aqueous layer was extracted thrice with EtOAc. The combined organic layer was washed with H_2O and brine, then dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 33% EtOAc in pentane) to yield a colourless oil (0.85 g, 4.9 mmol, 97%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 1H), 3.80 – 3.72 (m, 1H), 3.62 (dd, *J* = 10.9, 3.8 Hz, 1H), 3.49 (dd, *J* = 10.9, 6.2 Hz, 1H), 2.75 (bs, 1H) 1.44 (s, 9H), 1.13 (d, *J* = 6.8 Hz, 3H). ^{13}C-NMR

(101 MHz, $CDCl_3$) δ 79.85, 67.60, 48.74, 28.51, 17.44. LC-MS (ESI, 10-90): t_R = 4.20 min; m/z = 175.67 $[M]^+$.

(*S*)-2-((*tert*-Butoxycarbonyl)amino)propyl iodide (25): To a cooled (0 °C) and stirred mixture of **23** (2.00 g, 11.4 mmol, 1 eq), imidazole (1.09 g, 16.0 mmol, 1.4 eq) and PPh_3 (3.59 g, 13.7 mmol, 1.2 eq) in Et_2O:ACN (65 mL, 10:3 v/v) was added I_2 (3.77 g, 14.8 mmol, 1.3 eq). After stirring overnight at RT, the mixture was diluted with EtOAc and quenched with sat. $Na_2S_2O_3$ (aq). After stirring 0.5 h at RT the layers were separated and the aqueous layer extracted twice with EtOAc. The combined organic layer was washed with H_2O and 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 oil (2.02 g, 7.1 mmol, 62%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.63 (s, 1H), 3.53 (s, 1H), 3.41 (s, 1H), 3.30 (dd, *J* = 9.8, 3.7 Hz, 1H), 1.45 (s, 9H), 1.20 (d, *J* = 6.6 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 154.94, 79.83, 46.00, 28.50, 21.34, 16.21. LC-MS (ESI, 10-90): t_R = 3.77 min; m/z = 285.27 $[M+H]^+$.

(*R*)-2-((*tert*-Butoxycarbonyl)amino)propyl iodide (26): To a cooled (0 °C) and stirred mixture of **24** (7.25 g, 41.4 mmol, 1 eq), imidazole (5.07 g, 74.5 mmol, 1.8 eq) and 16.28 g PPh_3 (62.1 mmol, 1.5 eq) in Et_2O:ACN (250mL, 10:3, v/v) was added I_2 (17.85 g, 70.3 mmol, 1.7 eq). After stirring overnight at RT, the mixture was diluted with EtOAc and quenched with sat. $Na_2S_2O_3$ (aq). After stirring 0.5 h at RT the layers were separated and the aqueous layer extracted twice with EtOAc. The combined organic layer was washed with H_2O and brine, dried over $MgSO_4$, 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 oil (6.28 g, 21.9 mmol, 53%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.62 (s, 1H), 3.56 – 3.45 (m, 1H), 3.39 (dd, *J* = 9.9, 3.7 Hz, 1H), 3.27 (dd, *J* = 9.9, 3.7 Hz, 1H), 1.43 (s, 9H), 1.18 (d, *J* = 6.6 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 154.91, 79.78, 46.41, 28.47, 20.94, 16.15.

2-((4-Bromobenzyl)amino)acetamide (27): To a stirred mixture of 4-bromobenzaldehyde (9.2 g (49.7 mmol, 1.1 eq) and 2-aminoacetamide hydrochloride (5.06 g, 45.8 mmol, 1.0 eq) in MeOH:H_2O (170 mL, 5:1, v/v) was added NaOH (2.06 g, 51.5 mmol, 1.1 eq). After stirring at RT overnight, $NaBH_4$ (3.6 g, 95.2 mmol, 2.1 eq) was added and the solution was stirred overnight at RT. The solution was acidified to pH 3 with 2 M HCl, then neutralized with sat. $NaHCO_3$ (aq). Methanol was evaporated under reduced pressure and the resulting slurry was filtered to yield a white solid (11.0 g, 45.2 mmol, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.43 (m, 2H), 7.22 – 7.16 (m, 2H), 6.93 (s, 1H), 5.85 (s, 1H), 3.75 (s, 2H), 3.29 (s, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 174.42, 138.40, 131.80, 129.89, 121.32, 53.29, 51.78.

1-(4-Bromobenzyl)imidazolidine-2,4-dione (28): To stirred suspension of **27** (10.0 g, 40.1 mmol, 1.0 eq) in acetonitrile (300 mL) were added CDI (13.86 g, 85.5 mmol, 2.1 eq) and DMAP (10.2 g, 83.5 mmol, 2.1 eq). The mixture was heated (60 °C) under inert atmosphere for 70 h. 1 M HCl (aq, 250 mL) was added and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography with dry loading over Celite (5-10% acetone in DCM) to yield a yellow solid (3.95 g, 14.7 mmol, 37%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 4.49 (s, 2H), 3.79 (s, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 174.26, 132.41, 129.37, 51.17, 46.78.

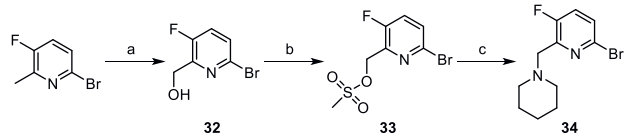
***tert*-Butyl (*S*)-(1-(3-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)carbamate (29):** A mixture of **28** (2.70 g, 10.0 mmol, 1 eq), **25** (5.72 g, 20.1 mmol, 2 eq), K_2CO_3 (8.32 g, 60.2 mmol, 6 eq) and 18-crown-6 (0.53 g, 2.0 mmol, 0.2 eq) in DMF (55 mL) was heated (50 °C) overnight. After cooling

to RT the mixture was diluted with H_2O (40 mL) and Et_2O (40 mL). The layers were separated and the aqueous layer extracted thrice with Et_2O. The combined organic layer was washed five times with H_2O 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, 20-50% EtOAc in pentane) to yield a yellow solid (2.67 g, 6.3 mmol, 62%). ^{1}H-NMR (850 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 8.1 Hz, 2H), 4.71 – 4.67 (m, 2H), 4.34 (d, *J* = 15.3 Hz, 1H), 4.06 (hept, *J* = 6.5 Hz, 1H), 3.72 (d, *J* = 17.1 Hz, 1H), 3.66 (d, *J* = 17.2 Hz, 1H), 3.51 (d, *J* = 7.9 Hz, 2H), 1.37 (s, 9H), 1.17 (d, *J* = 6.8 Hz, 3H). ^{13}C-NMR (214 MHz, $CDCl_3$) δ 170.11, 157.03, 155.73, 134.58, 132.24, 129.93, 122.26, 79.32, 49.10, 46.16, 45.52, 44.97, 28.41, 18.63. LC-MS (ESI, 10-90): t_R = 7.64 min; m/z = 425.53 $[M + H]^+$.

***tert*-Butyl (*R*)-(1-(3-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)carbamate (30):** A mixture of **28** (2.58 g, 9.6 mmol, 1 eq), **26** (6.28 g, 22.0 mmol, 2.3 eq), K_2CO_3 (7.95 g, 57.5 mmol, 6 eq) and 18-crown-6 (0.51 g, 1.9 mmol, 0.2 eq) in DMF (55 mL) was heated (50 °C) overnight. After cooling to RT the mixture was diluted with H_2O (40 mL) and Et_2O (40 mL). The layers were separated and the aqueous layer extracted thrice with Et_2O. The combined organic layer was washed five times with H_2O 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, 20-50% EtOAc in pentane) to yield a yellow solid (2.69 g, 6.3 mmol, 66%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 2H), 4.75 – 4.64 (m, 2H), 4.35 (d, *J* = 15.3 Hz, 1H), 4.06 (hept, *J* = 7.1 Hz, 1H), 3.69 (d, *J* = 7.0 Hz, 2H), 3.51 (d, *J* = 7.1 Hz, 2H), 1.38 (s, 9H), 1.17 (d, *J* = 6.8 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.10, 157.03, 155.74, 134.58, 132.25, 129.93, 122.23, 80.50, 49.11, 46.18, 45.52, 44.96, 28.41, 18.63. LC-MS (ESI, 10-90): t_R = 7.60 min; m/z = 425.67 $[M + H]^+$.

***tert*-Butyl (2-(3-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)ethyl)carbamate (31):** A mixture of **28** (1.74 g, 6.5 mmol, 1 eq), *tert*-butyl-(2-bromoethyl)carbamate (2.90 g, 12.9 mmol, 2 eq), K_2CO_3 (5.36 g, 38.8 mmol, 6 eq) and 18-crown-6 (0.34 g, 1.3 mmol, 0.2 eq) was heated (50 °C) overnight. After cooling to RT the mixture was diluted with H_2O (40 mL) and Et_2O (40 mL). The layers were separated and the aqueous layer extracted thrice with Et_2O. The combined organic layer was washed five times with H_2O 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, 20-50% EtOAc in pentane) to yield a yellow solid (2.35 g, 5.7 mmol, 88%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 4.94 (t, *J* = 6.2 Hz, 1H), 4.51 (s, 2H), 3.70 (s, 2H), 3.68 – 3.62 (m, 2H), 3.37 (q, *J* = 5.8 Hz, 2H), 1.38 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 13C NMR (101 MHz, CDCl3) δ 170.05, 156.89, 156.25, 134.53, 132.24, 129.91, 122.27, 79.46, 49.15, 46.17, 39.58, 39.33, 28.41. LC-MS (ESI, 10-90): t_R = 7.21 min; m/z = 413.47 $[M + H]^+$.

Synthesis of the pyridinylbenzyl building block:



Scheme 4.5 Reagents and conditions: a) step 1: m-CPBA (1.8 eq), 0 °C-RT, DCM, 4 days; step 2: TFAA (2.2 eq), 55 °C, 3 h; step 3: K_2CO_3 (2.3 eq), THF:MeOH (20:1), 17 h, 35% (three steps); b) Et_3N (2.3 eq), MsCl (1.7 eq), THF, 0 °C-RT, 1 h, 75%; c) K_2CO_3 (2.2 eq), piperidine (1.2 eq), ACN (0.2 M), 50 °C, 1.5 h, 93%.

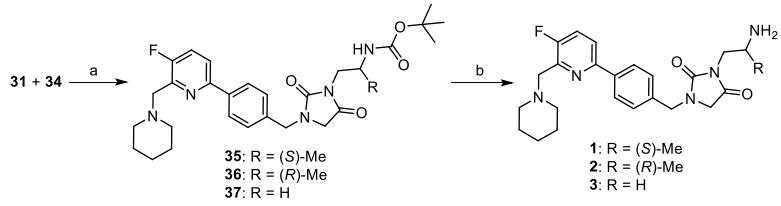
(6-Bromo-3-fluoropyridin-2-yl)methanol (32): To a stirred and cooled (0 °C) mixture under inert atmosphere of 6-bromo-3-fluoro-2-methylpyridine (10.7 g, 56.3 mmol, 1 eq) in DCM (370 mL) was added portion-wise *m*-CPBA (23.6 g, 70-75%, 100 mmol, 1.8 eq). The reaction mixture was stirred at room temperature (RT) for 4 days. Sat. $NaHCO_3$ (aq) and sat. $Na_2S_2O_3$ (aq) was added (1:1, v/v) and the

layers were separated. The aqueous layer was extracted thrice with DCM. The combined organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. To the residue was added TFAA (17 mL, 122 mmol, 2.2 eq) at 0 °C. After 15 minutes the temperature was increased to 55 °C for 3 h. The mixture was concentrated under reduced pressure, redissolved in DCM and sat. Na_2CO_3 (aq) was added. The layers were separated and the organic layer was washed with sat. $NaHCO_3$ (aq). The solvent was evaporated and the residue was dissolved in THF:MeOH (20:1, v/v) and K_2CO_3 (18.2 g, 132 mmol, 2.3 eq) was added. After 17 h H_2O was added and the layers were separated. The aqueous layer was extracted thrice with EtOAc. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (10-20% EtOAc in pentane) to yield a white solid (5.79 g, 19.7 mmol, 35%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 8.3, 3.5 Hz, 1H), 7.29 (t, *J* = 8.5 Hz, 1H), 4.80 (d, *J* = 2.0 Hz, 2H), 3.36 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 156.10 (d, *J* = 256.6 Hz), 148.84, 135.01 (d, *J* = 2.8 Hz), 128.17 (d, *J* = 4.1 Hz), 126.09 (d, *J* = 20.0 Hz), 59.08.

(6-Bromo-3-fluoropyridin-2-yl)methyl methanesulfonate (33): To a cooled (0 °C) mixture of **32** (1.6 g, 7.8 mmol, 1 eq) and Et_3N (2.5 mL, 17.9 mmol, 2.3 eq) in dry THF (40 mL) was added dropwise MsCl (1.0 mL, 12.9 mmol, 1.7 eq). After stirring at RT for 1 h the solution was concentrated under reduced pressure. DCM and H_2O were added and the layers were separated. The aqueous layer was extracted thrice with DCM. The combined organic layer was washed with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure to yield a yellow solid (1.65 g, 5.8 mmol, 75%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.53 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.38 (t, *J* = 8.5 Hz, 1H), 5.34 (d, *J* = 2.1 Hz, 2H), 3.14 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 157.85 (d, *J* = 261.4 Hz), 142.22 (d, *J* = 16.4 Hz), 135.64 (d, *J* = 3.1 Hz), 130.75 (d, *J* = 4.6 Hz), 127.06 (d, *J* = 20.5 Hz), 65.50, 38.46.

6-Bromo-3-fluoro-2-(piperidin-1-ylmethyl)pyridine (34): A mixture of **33** (4.68 g, 16.5 mmol, 1 eq), K_2CO_3 (4.98 g, 36.2 mmol, 2.2 eq) and piperidine (1.95 mL, 19.8 mmol, 1.2 eq) in ACN (80 mL) was heated (50 °C) for 1.5 h. After adding H_2O (100 mL) and DCM (100 mL) the layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 20-50% EtOAc in pentane) to yield a yellow solid (4.18 g, 15.3 mmol, 93%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 8.5, 3.5 Hz, 1H), 7.24 (t, *J* = 8.5 Hz, 1H), 3.67 (d, *J* = 2.7 Hz, 2H), 2.48 (t, *J* = 5.3 Hz, 4H), 1.56 (p, *J* = 5.8 Hz, 4H), 1.44 – 1.35 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 158.24 (d, *J* = 257.9 Hz), 147.66 (d, *J* = 16.7 Hz), 134.85 (d, *J* = 4.1 Hz), 128.16 (d, *J* = 4.3 Hz), 126.02 (d, *J* = 21.8 Hz), 58.19 (d, *J* = 3.1 Hz), 54.45, 25.97, 24.16. LC-MS (ESI, 10-90): t_R = 1.32 min; m/z = 273.07 $[M + H]^+$.

Synthesis of the pyridinylbenzylimidazolidine-2,4-dione scaffolds:



Scheme 4.6 Reagents and conditions: a) step 1: KOAc (4-6 eq), bis(pinacolato)diboron (1.5-2.2 eq), $Pd(dppf)Cl_2$ (0.05-0.08 eq), DMF (degassed, 0.2 M), 75 °C, 16 h; step 2: 34 (1 eq), K_2CO_3 (4-8 eq), $Pd(PPh_3)_4$ (0.05-0.2 eq), toluene:EtOH (degassed, 4:1, v/v, 0.2 M), 75 °C, 16 h, 36-76% (two steps); b) 4 M HCl (1,4-dioxane, 4 eq), acetonitrile (0.5 M), 80 °C, 2 h, 62-86%.

***tert*-Butyl (*S*)-(1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)carbamate (35):** A stirred and degassed mixture of **29** (2.67 g, 6.3 mmol, 1.1 eq), $Pd(dppf)Cl_2$ (0.24 g, 0.3 mmol, 0.05 eq), bis(pinacolato)diboron (2.40 g, 9.5 mmol, 1.5 eq) and KOAc (2.70 g, 27.5 mmol, 4.4 eq) in DMF (30 mL) was heated (75 °C) overnight. The reaction was diluted at RT with H_2O and EtOAc. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with sat. $NaHCO_3$ (aq), H_2O, and brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude residue was used without further purification. The crude residue was co-evaporated thrice with chloroform and re-dissolved in degassed toluene:EtOH (30 mL, 4:1, v/v). To the stirred mixture was added **34** (1.64 g, 6.0 mmol, 1 eq), K_2CO_3 (3.46 g, 25.0 mmol, 4 eq) and $Pd(PPh_3)_4$ (0.50 g, 0.4 mmol, 0.06 eq). After heating (75 °C) overnight the reaction was diluted at RT with H_2O and EtOAc. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with H_2O and brine, dried ($MgsO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-4% MeOH in DCM) to yield a brown oil (1.40 g, 2.6 mmol, 42%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.3 Hz, 2H), 7.60 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 4.82 – 4.68 (m, 2H), 4.47 (d, *J* = 15.2 Hz, 1H), 4.05 (p, *J* = 7.1 Hz, 1H), 3.82 (d, *J* = 2.7 Hz, 2H), 3.72 (d, *J* = 8.8 Hz, 2H), 3.52 (d, *J* = 6.6 Hz, 2H), 2.62 – 2.55 (m, 4H), 1.59 (p, *J* = 5.7 Hz, 4H), 1.38 (s, 11H), 1.17 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.27, 157.94 (d, *J* = 258.1 Hz), 157.06, 155.71, 151.88 (d, *J* = 4.9 Hz), 145.63 (d, *J* = 15.1 Hz), 138.62, 135.95, 128.59, 127.61, 127.55, 123.70 (d, *J* = 20.4 Hz), 120.39 (d, *J* = 4.1 Hz), 79.26, 58.44 (d, *J* = 3.0 Hz), 54.36, 49.10, 46.49, 45.60, 44.80, 28.43, 25.98, 24.18, 18.62. LC-MS (ESI, 10-90): t_R = 5.47 min; m/z = 540.20 $[M + H]^+$.

***tert*-Butyl (*R*)-(1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)carbamate (36):** A stirred and degassed mixture of **30** (2.69 g, 6.3 mmol, 1 eq), $Pd(dppf)Cl_2$ (0.26 g, 0.4 mmol, 0.06 eq), bis(pinacolato)diboron (2.40 g, 9.5 mmol, 1.5 eq) and KOAc (2.72 g, 27.8 mmol, 4.4 eq) in DMF (30 mL) was heated (75 °C) overnight. The reaction was diluted at RT with H_2O and EtOAc. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with sat. $NaHCO_3$ (aq), H_2O, and brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude residue was used without further purification. The crude residue was co-evaporated thrice with chloroform and re-dissolved in degassed toluene:EtOH (30 mL, 4:1, v/v). To the stirred mixture was added **34** (1.67 g, 6.1 mmol, 1 eq), K_2CO_3 (5.05 g, 36.5 mmol, 5.8 eq) and $Pd(PPh_3)_4$ (0.73 g, 0.6 mmol, 0.1 eq). After heating (75 °C) overnight the reaction was diluted at RT with H_2O and EtOAc. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with H_2O and brine, dried ($MgsO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-4% MeOH in DCM) to yield an orange oil (2.57 g, 4.8 mmol, 76%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.60 (dd, *J* = 8.6, 3.6 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.36 (d, *J* = 8.2 Hz, 2H), 4.81 – 4.69 (m, 1H), 4.48 (d, *J* = 15.2 Hz, 1H), 4.06 (p, *J* = 7.0 Hz, 1H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.72 (d, *J* = 5.0 Hz, 2H), 3.53 (d, *J* = 6.6 Hz, 2H), 2.58 (t, *J* = 5.4 Hz, 4H), 1.66 – 1.52 (m, 4H), 1.39 (s, 11H), 1.17 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.24, 158.36 (d, *J* = 195.0 Hz), 156.24, 155.69, 151.84 (d, *J* = 4.9 Hz), 145.94 (d, *J* = 16.0 Hz), 138.71, 135.94, 128.60, 127.57, 123.66 (d, *J* = 20.4 Hz), 120.31 (d, *J* = 4.2 Hz), 79.27, 58.64 (d, *J* = 3.2 Hz), 54.47, 53.56, 49.12, 46.53, 45.65, 44.80, 28.45, 26.13, 24.28, 18.65.LC-MS (ESI, 10-90): t_R = 5.51 min; m/z = 540.20 $[M + H]^+$.

***tert*-Butyl (2-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)ethyl)carbamate (37)**: A stirred and degassed mixture of **31** (2.35 g, 5.5 mmol, 1.4 eq), $Pd(dppf)Cl_2$ (0.23 g, 0.3 mmol, 0.08 eq), bis(pinacolato)diboron (2.10 g, 8.3 mmol, 2.2 eq) and KOAc (2.38 g, 24.3 mmol, 6.4 eq) in DMF (30 mL) was heated (75 °C) overnight. The reaction was diluted at RT with

H_2O and EtOAc. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with sat. $NaHCO_3$ (aq), H_2O, and brine, dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure. The crude residue was used without further purification. The crude residue was co-evaporated thrice with chloroform and re-dissolved in degassed toluene:EtOH (30 mL, 4:1, v/v). To the stirred mixture was added **34** (1.04 g, 3.8 mmol, 1 eq), K_2CO_3 (4.57 g, 33.1 mmol, 8.7 eq) and $Pd(PPh_3)_4$ (0.64 g, 0.6 mmol, 0.2 eq). After heating (75 °C) overnight the reaction was diluted at RT with H_2O and EtOAc. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with H_2O and brine, dried ($MgsO_4$), filtered and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-4% MeOH in DCM) to yield an orange oil (1.04 g, 2.0 mmol, 36%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.6 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 4.98 (t, *J* = 6.2 Hz, 1H), 4.60 (s, 2H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.73 (s, 2H), 3.71 – 3.64 (m, 2H), 3.39 (q, *J* = 5.7 Hz, 2H), 2.58 (t, *J* = 5.4 Hz, 4H), 1.59 (p, *J* = 5.6 Hz, 4H), 1.41 (s, 11H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.11, 157.84 (d, *J* = 258.0 Hz), 156.84, 156.13, 151.75 (d, *J* = 4.8 Hz), 138.62, 135.78, 128.53, 127.49, 123.60 (d, *J* = 20.5 Hz), 120.27 (d, *J* = 4.2 Hz), 79.38, 58.48 (d, *J* = 3.0 Hz), 54.35, 49.06, 46.42, 39.37, 28.35, 27.43, 25.98, 24.15. LC-MS (ESI, 10-90): t_R = 5.33 min; m/z = 526.13 $[M + H]^+$.

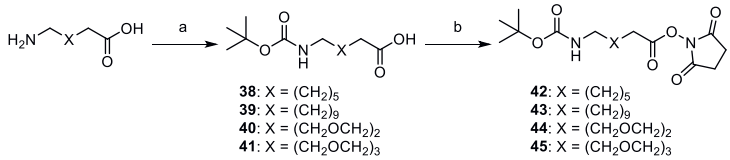
(*S*)-3-(2-Aminopropyl)-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)imidazolidine-2,4-dione (1): To a solution of **35** (1.40 g, 2.6 mmol, 1 eq) in ACN (5 mL) was added 4 M HCl in 1,4-dioxane (2.7 mL, 10.8 mmol, 4.1 eq). After the reaction was heated (80 °C) for 2 h, the ACN was evaporated under reduced pressure. The mixture was basified with 1M NaOH (aq) until pH = 10. The aqueous layer was extracted with $CHCl_3$:MeOH (7:1, v/v). NaCl was added for increased separation. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-4% MeOH in DCM with 2% Et_3N (v/v)) to yield an orange oil (0.98 g, 2.2 mmol, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.2 Hz, 2H), 7.62 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 4.61 (s, 2H), 3.83 (d, *J* = 2.7 Hz, 2H), 3.78 (s, 2H), 3.54 – 3.38 (m, 2H), 3.32 – 3.19 (m, 1H), 2.59 (s, 4H), 1.60 (p, *J* = 5.6 Hz, 6H), 1.46 – 1.36 (m, 2H), 1.13 (d, *J* = 6.4 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.35, 157.98 (d, *J* = 258.1 Hz), 157.25, 151.84 (d, *J* = 4.9 Hz), 145.80 (d, *J* = 15.0 Hz), 138.79, 135.92, 128.66, 127.63, 123.73 (d, *J* = 20.4 Hz), 120.43 (d, *J* = 4.2 Hz), 58.55 (d, *J* = 3.2 Hz), 54.45, 49.15, 47.19, 46.61, 46.21, 26.06, 24.23, 22.10. LC-MS (ESI, 10-90): t_R = 3.74 min; m/z = 440.33 $[M + H]^+$. HRMS $[C_{24}H_{30}FN_5O_2 + H]^+$: 440.24563 calculated, 440.24533 found.

(*R*)-3-(2-Aminopropyl)-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)imidazolidine-2,4-dione (2): To a solution of **36** (2.57 g, 4.8 mmol, 1 eq) in ACN (10 mL) was added 4 M HCl in 1,4-dioxane (4.9 mL, 19.8 mmol, 4.1 eq). After the reaction was heated (80 °C) for 2 h, the ACN was evaporated under reduced pressure. The mixture was basified with 1 M NaOH (aq) until pH = 10. The aqueous layer was extracted with $CHCl_3$:MeOH (7:1, v/v). NaCl was added for increased separation. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-4% MeOH in DCM with 2% Et_3N (v/v)) to yield an orange oil (1.70 g, 3.8 mmol, 81%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.3 Hz, 2H), 7.60 (dd, *J* = 8.6, 3.6 Hz, 1H), 7.40 (t, *J* = 8.7 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 4.60 (s, 2H), 3.80 (d, *J* = 2.6 Hz, 2H), 3.76 (s, 2H), 3.52 – 3.36 (m, 2H), 3.24 (h, *J* = 6.5 Hz, 1H), 2.57 (t, *J* = 5.4 Hz, 4H), 1.58 (p, *J* = 5.6 Hz, 4H), 1.52 – 1.46 (m, 2H), 1.44 – 1.34 (m, 2H), 1.11 (d, *J* = 6.4 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.29, 157.92 (d, *J* = 258.2 Hz), 157.20, 151.76 (d, *J* = 4.9 Hz), 145.90 (d, *J* = 15.0 Hz), 138.75, 135.87, 128.61, 127.58, 123.66 (d, *J* = 20.4 Hz), 120.33 (d, *J* = 4.1 Hz), 58.59 (d, *J* = 3.1 Hz), 54.43, 49.09,

47.18, 46.55, 46.15, 26.07, 24.23, 22.08. LC-MS (ESI, 10-90): t_R = 3.72 min; m/z = 440.20 $[M + H]^+$. HRMS $[C_{24}H_{30}FN_5O_2 + H]^+$: 440.24563 calculated, 440.24530 found.

3-(2-Aminoethyl)-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)imidazolidine-2,4-dione (3): To a solution of **37** (1.04 g, 2.0 mmol, 1 eq) in ACN (4 mL) was added 4 M HCl in 1,4-dioxane (2.1 mL, 8.2 mmol, 4.1 eq). After the reaction was heated (80 °C) for 2 h, the ACN was evaporated under reduced pressure. The mixture was basified with 1 M NaOH (aq) until pH = 10. The aqueous layer was extracted with $CHCl_3$:MeOH (7:1, v/v). NaCl was added for increased separation. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 0-4% MeOH in DCM with 2% Et_3N (v/v)) to yield an orange oil (0.52 g, 1.2 mmol, 62%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.6 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 4.61 (s, 2H), 3.82 (d, *J* = 2.7 Hz, 2H), 3.77 (s, 2H), 3.61 (t, *J* = 6.3 Hz, 2H), 2.95 (t, *J* = 6.3 Hz, 2H), 2.65 – 2.54 (m, 4H), 1.59 (p, *J* = 5.6 Hz, 4H), 1.45 – 1.35 (m, 2H), 1.07 (t, *J* = 7.2 Hz, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.24, 157.97 (d, *J* = 257.9 Hz), 157.11, 151.79 (d, *J* = 5.0 Hz), 146.00 (d, *J* = 15.1 Hz), 138.83, 135.87, 128.66, 127.63, 123.69 (d, *J* = 20.4 Hz), 120.36 (d, *J* = 4.2 Hz), 58.65 (d, *J* = 3.1 Hz), 54.48, 49.17, 46.59, 42.25, 40.53, 26.14, 24.29. LC-MS (ESI, 10-90): t_R = 3.70 min; m/z = 426.07 $[M + H]^+$. HRMS $[C_{23}H_{28}FN_5O_2 + H]^+$: 426.22998 calculated, 426.23010 found.

Synthesis of the alkyl and glycol (PEG2/3) spacers:



Scheme 4.7 Reagents and conditions: a) Alkyl: Et_3N (2 eq), Boc_2O (1.1 eq), acetone:H_2O (1:1, v/v, 0.5 M), RT, 16 h, 95-99%; PEG: K_2CO_3 (3 eq), Boc_2O (1.3 eq), H_2O:THF (1:1, v/v, 0.1 M), RT, 16 h, 40-81%; b) Alkyl: EDC.HCl (0.8-0.9 eq), NHS (1.7-2.8 eq), DCM (0.3 M), RT, 16-72 h, 36-40%; PEG: EDC.HCl (3 eq), NHS (1.5 eq), Et_3N (3 eq), DCM (0.2 M), RT, 16 h, 46-58%.

8-((*tert*-Butoxycarbonyl)amino)octanoic acid (38): To a cooled (0 °C) and stirred mixture of 8-aminooctanoic acid (2.00 g, 12.6 mmol, 1 eq) and Et_3N (3.5 mL, 25.1 mmol, 2 eq) in acetone:H_2O (25 mL, 1:1, v/v) was added dropwise Boc_2O (3.02 g, 13.8 mmol, 1.1 eq) in acetone (6 mL). After stirring at RT overnight the acetone was evaporated under reduced pressure. The aqueous layer was acidified with 1 M HCl to pH = 4 before being extracted thrice with EtOAc. The combined organic layer was washed with brine, dried ($MgSO_4$), and filtered. The solvent was evaporated under reduced pressure to yield a white solid (3.23 g, 12.5 mmol, 99%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (s, 1H), 3.10 (q, *J* = 6.8 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.62 (q, *J* = 7.2 Hz, 2H), 1.53 – 1.39 (m, 11H), 1.38 – 1.26 (m, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 179.34, 156.21, 79.27, 40.68, 34.12, 30.08, 29.08, 29.01, 28.56, 26.69, 24.73. LC-MS (ESI, 10-90): t_R = 6.48 min; m/z = 259.73 $[M]^+$.

12-((*tert*-Butoxycarbonyl)amino)dodecanoic acid (39): To a cooled (0 °C) and stirred mixture of 12-aminododecanoic acid (1.50 g, 7.0 mmol, 1 eq) and Et_3N (1.9 mL, 13.9 mmol, 2 eq) in acetone:H_2O (14 mL, 1:1, v/v) was added dropwise Boc_2O (1.67 g, 7.7 mmol, 1.1 eq) in acetone (4 mL). After stirring at RT overnight the acetone was evaporated under reduced pressure. The aqueous layer was acidified with 1 M HCl to pH = 4 before being extracted thrice with EtOAc. The combined organic layer was washed with brine, dried ($MgSO_4$), and filtered. The solvent was evaporated under reduced pressure to yield a white solid (2.09 g, 6.6 mmol, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (s, 1H), 3.10 (m, 2H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.63 (p, *J* = 7.4 Hz, 2H), 1.51 – 1.40 (m, 11H), 1.38 – 1.21 (m, 14H). ^{13}C-NMR (101

MHz, $CDCl_3$) δ 179.28, 156.27, 79.12, 40.64, 33.98, 30.02, 29.44, 29.42, 29.34, 29.24, 29.18, 29.02, 28.44, 26.78, 24.69. LC-MS (ESI, 10-90): t_R = 8.44 min; m/z = 315.60 $[M + H]^+$.

3-(2-(*tert*-butoxycarbonyl-2-aminoethoxy)ethoxy)propanoic acid (40): To a cooled (0 °C) and stirred mixture of 3-(2-(2-aminoethoxy)ethoxy)propanoic acid (0.50 g, 2.8 mmol, 1 eq) and K_2CO_3 (1.17 g, 8.5 mmol, 3 eq) in H_2O (15 mL) was added dropwise Boc_2O (0.74 g, 3.4 mmol, 1.2 eq) in THF (10 mL). After stirring at RT overnight the solution was acidified with 1 M HCl to pH = 4. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed twice with H_2O and once with brine, dried ($MgSO_4$), and filtered. The solvent was evaporated under reduced pressure to yield a colourless oil (0.31 g, 1.1 mmol, 40%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.11 (s, 1H), 3.78 (t, *J* = 6.2 Hz, 2H), 3.67 – 3.56 (m, 4H), 3.54 (t, *J* = 5.4 Hz, 2H), 3.30 (q, *J* = 5.4 Hz, 2H), 2.63 (t, *J* = 6.2 Hz, 2H), 1.45 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 175.89, 156.23, 79.54, 70.43, 70.32, 70.07, 66.50, 40.44, 34.89, 28.51. LC-MS (ESI, 10-90): t_R = 4.75 min; m/z = 277.84 $[M]^+$.

2,2-Dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oic acid (41): To a cooled (0 °C) and stirred solution of 3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanoic acid (1.00 g, 4.5 mmol, 1 eq) and K_2CO_3 (1.87 g, 13.6 mmol, 3 eq) in H_2O (20 mL) was added dropwise Boc_2O (1.28 g, 5.9 mmol, 1.3 eq) in THF (20 mL). After stirring at RT overnight the solution was acidified with 1 M HCl to pH = 4. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed twice with H_2O and once with brine, dried ($MgSO_4$), and filtered. The solvent was evaporated under reduced pressure to yield a colourless oil (1.18 g, 3.7 mmol, 81%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.19 (s, 1H), 3.78 (t, *J* = 6.5 Hz, 2H), 3.69 – 3.58 (m, 8H), 3.59 – 3.46 (m, 2H), 3.39 – 3.20 (m, 2H), 2.62 (d, *J* = 6.4 Hz, 2H), 1.45 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 175.44, 156.23, 79.35, 70.59, 70.54, 70.47, 70.42, 70.21, 66.46, 40.38, 34.84, 27.42. LC-MS (ESI, 10-90): t_R = 4.84 min; m/z = 321.80 $[M]^+$.

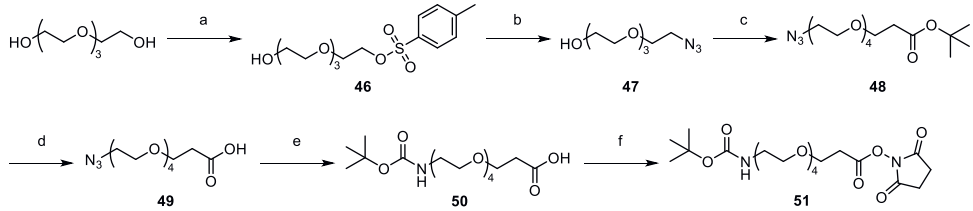
8-((*tert*-Butoxycarbonyl)amino)octanoic acid *N*-succinimidyl ester (42): To a stirred solution of **38** (3.23 g, 12.5 mmol, 1.1 eq) in DCM (40 mL) was added EDC.HCl (2.15 g, 11.2 mmol, 1 eq) and *N*-hydoxysuccinimide (2.15 g, 18.7 mmol, 1.7 eq). After stirring at RT for 3 days the reaction was quenched with sat. NH_4Cl (aq). The layers were separated and the aqueous layer was extracted once with DCM. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 10-40% EtOAc in pentane) to yield a white solid (1.40 g, 4.0 mmol, 36%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (s, 1H), 3.10 (q, *J* = 6.7 Hz, 2H), 2.84 (d, *J* = 4.2 Hz, 4H), 2.60 (t, *J* = 7.4 Hz, 2H), 1.74 (p, *J* = 7.4 Hz, 2H), 1.47 – 1.43 (m, 11H), 1.41 – 1.25 (m, 6H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 169.33, 168.76, 79.15, 40.64, 31.02, 30.03, 28.83, 28.74, 28.55, 26.61, 25.72, 24.60. LC-MS (ESI, 10-90): t_R = 7.28 min; m/z = 356.67 $[M]^+$.

12-((*tert*-Butoxycarbonyl)amino)dodecanoic acid *N*-succinimidyl ester (43): To a stirred solution of **39** (2.09 g, 6.6 mmol, 1.3 eq) in DCM (35 mL) was added EDC.HCl (0.99 g, 5.2 mmol, 1 eq) and *N*-hydroxysuccinimide (1.66 g, 14.4 mmol, 2.8 eq). After stirring at RT for 3 days the reaction was quenched with sat. NH_4Cl (aq). The layers were separated and the aqueous layer was extracted once with DCM. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (SiO_2, 10-40% EtOAc in pentane) to yield a white solid (0.88 g, 2.1 mmol, 40%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.54 (s, 1H), 3.12 (q, *J* = 6.8 Hz, 2H), 2.86 (d, *J* = 4.3 Hz, 4H), 2.62 (t, *J* = 7.5 Hz, 2H), 1.76 (p, *J* = 7.4 Hz, 2H), 1.50 – 1.42 (m, 11H), 1.35 – 1.25 (m, 14H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 169.34, 168.83, 165.27, 156.11, 79.12, 40.76, 31.07, 30.18, 29.61, 29.54, 29.41, 29.39, 29.17, 28.88, 28.56, 26.92, 25.72, 24.69. LC-MS (ESI, 10-90): t_R = 8.87 min; m/z = 412.60 $[M]^+$.

2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatetradecan-14-oate (44): To a stirred mixture of **40** (1.53 g, 5.5 mmol, 1 eq), Et_3N (2.3 mL, 16.6 mmol, 3 eq) and *N*-hydroxysuccinimide (0.95 g, 8.3 mmol, 1.5 eq) in DCM (30 mL) was added dropwise EDC.HCl (3.17 g, 16.6 mmol, 3 eq) in DCM (15 mL). After stirring at RT overnight the reaction was quenched with H_2O and DCM. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed thrice with H_2O 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, 50-70% EtOAc in pentane) to yield a yellow oil (1.19 g, 3.2 mmol, 58%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.11 (s, 1H), 3.86 (t, *J* = 6.3 Hz, 2H), 3.69 – 3.59 (m, 4H), 3.55 (t, *J* = 5.2 Hz, 2H), 3.31 (q, *J* = 5.4 Hz, 2H), 2.91 (t, *J* = 6.3 Hz, 2H), 2.85 (d, *J* = 3.4 Hz, 4H), 1.44 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.97, 169.16, 166.84, 156.24, 79.32, 70.72, 70.31, 70.18, 65.83, 40.42, 32.26, 28.49, 25.66, 25.46. LC-MS (ESI, 10-90): t_R = 5.66 min; m/z = 374.80 $[M]^+$.

2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azaheptadecan-17-oate (45): To a stirred mixture of **41** (1.18 g, 3.7 mmol, 1 eq), Et_3N (1.5 mL, 11.0 mmol, 3 eq) and *N*-hydroxysuccinimide (0.63 g, 5.5 mmol, 1.5 eq) in DCM (18 mL) was added dropwise EDC.HCl (2.11 g, 11.0 mmol, 3 eq) in DCM (10 mL). After stirring at RT overnight the reaction was quenched with H_2O and DCM. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed thrice with H_2O 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, 50-70% EtOAc in pentane) to yield a yellow oil (0.70 g, 1.7 mmol, 46%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.09 (s, 1H), 3.86 (t, *J* = 6.4 Hz, 2H), 3.69 – 3.58 (m, 8H), 3.54 (t, *J* = 5.1 Hz, 2H), 3.31 (q, *J* = 5.4 Hz, 2H), 2.91 (t, *J* = 6.4 Hz, 2H), 2.84 (d, *J* = 3.0 Hz, 4H), 1.44 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 169.10, 166.84, 156.14, 79.24, 70.82, 70.64, 70.56, 70.30, 70.25, 65.80, 40.42, 32.22, 28.50, 25.65, 25.47. LC-MS (ESI, 10-90): t_R = 5.63 min; m/z = 418.80 $[M]^+$.

Synthesis of the PEG4 spacer:



Scheme 4.8 Reagents and conditions: a) *p*-TsCl (1 eq), NaOH (2 M, 1.6 eq), THF (0.6 M), 0 °C, 4 h, 90%; b) NaN_3 (1.5 eq), ACN (0.4 M), 80 °C, 8 h, 94%; c) tert-butyl acrylate (1 eq), TBAF (0.4 eq), NaOH (25 wt% in H_2O, 2.6 eq), DCM, RT, 8 h, 75%; d) TFA (50 eq), DCM, RT, 4 h, 65%; e) step 1: 10% Pd/C (0.1 eq), H_2 (g), EtOH (0.3 M), RT, 16 h; step 2: K_2CO_3 (3 eq), Boc_2O (1.3 eq), H_2O:THF (1:1, v/v, 0.1 M), RT, 16 h, 55% (two steps); f) EDC.HCl (1.2 eq), NHS (1.1 eq), DCM (0.2 M), RT, 16 h, 84%.

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (46): To a stirred and cooled (0 °C) solution of tetraethylene glycol (23.00 g, 118.0 mmol, 8 eq) in THF (26 mL) was added 2 M NaOH (12 mL). After 15 minutes additionally *p*-TsCl (2.81 g, 14.7 mmol, 1 eq) was added. After stirring at 0 °C for 4 h the reaction was quenched with ice cold H_2O (200 mL) and stirred 15 minutes at RT. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed thrice with H_2O and once with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure to yield a colourless oil (4.63 g, 13.3 mmol, 90%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 7.8 Hz, 2H), 4.12 (t, *J* = 4.8 Hz, 2H), 3.68 – 3.63 (m, 4H), 3.63 – 3.57 (m, 4H), 3.57 – 3.53 (m, 6H), 2.40 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 144.87, 132.89, 129.86, 128.92,

72.47, 70.69, 70.62, 70.51, 70.43, 70.29, 69.30, 68.66, 61.66, 21.64. LC-MS (ESI, 10-90): t_R = 5.40 min; m/z = 348.41 $[M]^+$.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol (47): A stirred mixture of **46** (5.40 g, 15.5 mmol, 1 eq) and NaN_3 (1.51 g, 23.2 mmol, 1.5 eq) in acetonitrile (43 mL) was refluxed for 8 h. After cooling down the reaction was diluted with H_2O (40 mL). The aqueous layer was extracted thrice with DCM. The combined organic layer was dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure to yield a yellow oil (3.09 g. 14.5 mmol, 94%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.73 – 3.68 (m, 2H), 3.68 – 3.64 (m, 10H), 3.61 – 3.57 (m, 2H), 3.38 (t, *J* = 5.3 Hz, 2H), 2.61 (t, *J* = 6.2 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 72.55, 70.78, 70.74, 70.68, 70.43, 70.14, 61.81, 50.73. LC-MS (ESI, 10-90): t_R = 1.35 min; m/z = 219.87 $[M]^+$.

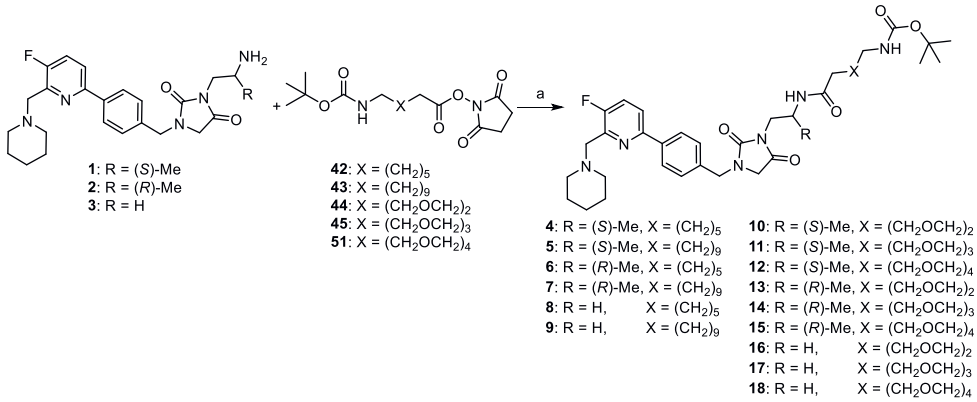
***tert*-Butyl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate (48):** A stirred mixture of **47** (2.70 g, 12.3 mmol, 1 eq), *tert*-butyl acrylate (1.78 mL, 12.2 mmol, 1 eq), TBAF (1.29 g, 4.9 mmol, 0.4 eq) and NaOH (5 mL, 25 wt% in H_2O) in DCM (30 mL) was stirred at RT for 8 h. The mixture was diluted with H_2O (40 mL) and the layers separated. The aqueous layer was extracted thrice with DCM. The combined organic layer was wased twice with H_2O and once with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (0-100% EtOAc in pentane) to yield a colourless oil (3.22 g, 9.3 mmol, 75%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.71 (t, *J* = 6.6 Hz, 2H), 3.66 (d, *J* = 3.6 Hz, 10H), 3.64 – 3.60 (m, 4H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.51 (t, *J* = 6.6 Hz, 2H), 1.45 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.01, 80.60, 70.80, 70.78, 70.74, 70.71, 70.60, 70.47, 70.14, 67.00, 50.78, 36.36, 28.19. LC-MS (ESI, 10-90): t_R = 7.01 min; m/z = 347.67 $[M]^+$.

1-Azido-3,6,9,12-tetraoxapentadecan-15-oic acid (49): A mixture of **48** (3.72 g, 10.7 mmol, 1 eq) and TFA (42 mL, 0.55 mol, 51 eq) in DCM (86 mL) was stirred at RT for 4 h. The volatile compounds were removed under reduced pressure. The crude product was co-evaporated four times with Et_2O and twice with MeOH to yield a colourless oil (2.04 g, 7.0 mmol, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.77 (t, *J* = 6.3 Hz, 2H), 3.70 – 3.64 (m, 14H), 3.40 (t, *J* = 5.1 Hz, 2H), 2.64 (t, *J* = 6.2 Hz, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 176.15, 70.78, 70.72, 70.67, 70.64, 70.52, 70.37, 70.10, 66.50, 50.76, 34.98. LC-MS (ESI, 10-90): t_R = 4.14 min; m/z = 291.80 $[M]^+$.

2,2-Dimethyl-4-oxo-3,8,11,14,17-pentaoxa-5-azaicosan-20-oic acid (50): A stirred mixture of **49** (1.55 g, 5.3 mmol, 1 eq) and 10% Pd/C (0.06 g, 0.4 mmol, 0.1 mmol) in absolute EtOH (20 mL) was purged with H_2 (g) for 15 minutes and kept under H_2 (g) atmosphere for an additional 16 h at RT. The mixture was filtered through a celite pad and the solvent evaporated under reduced pressure. The crude residue was used without further purification. To a cooled (0 °C) mixture of the crude residue re-dissolved in H_2O (25 mL) with K_2CO_3 (2.20 g, 15.9 mmol, 3 eq) was added dropwise Boc_2O (1.51 g, 6.9 mmol, 1.3 eq) in THF (25 mL). After stirring overnight at RT the reaction mixture was acidified with 1 M HCl to pH 4. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed twice with H_2O and once with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (2-4% MeOH in DCM with 3% AcOH) to yield a colourless oil (1.07 g, 2.9 mmol, 55%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.19 (s, 1H), 3.74 (t, *J* = 6.2 Hz, 2H), 3.67 – 3.58 (m, 14H), 3.53 (t, *J* = 5.2 Hz, 2H), 2.59 (t, *J* = 6.3 Hz, 2H), 1.42 (d, *J* = 3.5 Hz, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 175.41, 156.28, 79.52, 70.66, 70.64, 70.56, 70.50, 70.39, 70.38, 70.26, 66.61, 40.30, 35.06, 28.50. LC-MS (ESI, 10-90): t_R = 5.14 min; m/z = 365.93 $[M]^+$.

2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-4-oxo-3,8,11,14,17-pentaoxa-5-azaicosan-20-oate (51): A mixture of **50** (1.04 g, 2.9 mmol, 1 eq), EDC.HCl (0.66 g, 3.4 mmol, 1.2 eq) and *N*-hydroxysuccinimide (0.36 g, 3.1 mmol, 1.1 eq) in DCM (14 mL) was stirred at RT overnight. The reaction mixture was diluted with DCM (15 mL). The organic layer was washed thrice with H_2O, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with flash column chromatography (50-70% EtOAc in pentane) to yield a colourless oil (1.10 g, 2.4 mmol, 84%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.08 (s, 1H), 3.82 (t, *J* = 6.4 Hz, 2H), 3.65 – 3.57 (m, 12H), 3.51 (t, *J* = 5.2 Hz, 2H), 3.28 (q, *J* = 5.4 Hz, 2H), 2.88 (t, *J* = 6.4 Hz, 2H), 2.81 (m, 4H), 1.41 (s, 9H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.94, 169.11, 166.81, 156.16, 70.77, 70.68, 70.62, 70.54, 70.25, 65.77, 40.41, 32.19, 28.49, 25.64. LC-MS (ESI, 10-90): t_R = 5.81 min; m/z = 426.73 $[M]^+$.

Synthesis of key intermediates:



Scheme 4.9 Reagents and conditions: a) Et_3N (6 eq), DCM (0.3 M), RT, 1-3 h, 13-65%.

General procedure for the synthesis of key intermediates (4-18)

A mixture of **1**, **2 or 3** (1 eq), Et_3N (6 eq) and *O*-Su ester (**42**, **43**, **44**, **45** or **51,** 1 eq) in DCM (0.3 M) was stirred at RT for 1-3 h. The reaction mixture was diluted with H_2O and DCM. The layers were separated and the aqueous layer was extracted thrice with DCM. The combined organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified using preparative HPLC and freeze dried twice.

***tert*-Butyl (*S*)-(8-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-8-oxooctyl)carbamate (4):** The title compound was synthesized according to general procedure using **1** (29.0 mg, 66.1 µmol, 1 eq) and **42** (23.5 mg, 66.1 µmol, 1 eq). The product was obtained as a white solid (28.8 mg, 42.4 µmol, 64%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.62 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.43 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 5.99 (d, *J* = 8.1 Hz, 1H), 4.61 (s, 2H), 4.38 – 4.25 (m, 1H), 3.84 – 3.80 (m, 2H), 3.69 – 3.66 (m, 2H), 3.63 – 3.53 (m, 2H), 3.40 (q, *J* = 4.7 Hz, 1H), 3.06 (d, *J* = 6.8 Hz, 2H), 2.59 (s, 4H), 2.10 (t, *J* = 7.7 Hz, 2H), 1.63 – 1.52 (m, 6H), 1.43 (s, 10H), 1.29 – 1.24 (m, 9H), 1.18 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.10, 170.42, 158.00 (d, *J* = 258.0 Hz), 157.26, 138.82, 135.78, 128.57, 127.66, 123.74 (d, *J* = 20.3 Hz), 120.40, 70.87, 54.50, 49.23, 46.61, 45.12, 44.25, 36.92, 30.12, 29.85, 29.25, 29.10, 28.58, 26.75, 26.12, 25.55, 24.29, 18.45. LC-MS (ESI, 10-90): t_R = 5.99 min; m/z = 681.33 $[M + H]^+$. HRMS $[C_{37}H_{53}FN_6O_5 + H]^+$: 681.41342 calculated, 681.41326 found.

***tert*-Butyl (*S*)-(12-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-12-oxododecyl)carbamate (5):** The title compound was synthesized according to general procedure using **1** (92.0 mg, 21.0 µmol, 1 eq) and **43** (87.0 mg, 21.0 µmol, 1 eq). The product was obtained as a white solid (75.2 mg, 0.10 mmol, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 2H), 7.63 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.43 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 2H), 6.00 (d, *J* = 8.1 Hz, 1H), 4.62 (s, 2H), 4.54 (s, 1H), 4.39 – 4.24 (m, 1H), 3.86 (d, *J* = 2.5 Hz, 2H), 3.76 (q, *J* = 17.3 Hz, 2H), 3.64 – 3.51 (m, 2H), 3.09 (q, *J* = 6.7 Hz, 2H), 2.62 (s, 4H), 2.11 (t, *J* = 7.6 Hz, 2H), 1.66 – 1.54 (m, 6H), 1.44 (s, 13H), 1.34 – 1.23 (m, 14H), 1.19 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 173.25, 170.39, 158.01 (d, *J* = 258.3 Hz), 157.26, 151.82, 138.76, 135.80, 128.55, 127.65, 123.75 (d, *J* = 20.2 Hz), 120.46, 77.48, 77.16, 76.84, 58.34, 54.31, 49.22, 46.60, 45.07, 44.23, 40.77, 37.03, 30.19, 29.64, 29.58, 29.47, 29.41, 28.57, 26.93, 25.95, 25.70, 24.19, 18.45. LC-MS (ESI, 10-90): t_R = 7.10 min; m/z = 737.33 $[M + H]^+$. HRMS $[C_{41}H_{61}FN_6O_5 + H]^+$: 737.47602 calculated, 737.47562 found.

***tert*-Butyl (*R*)-(8-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-8-oxooctyl)carbamate (6):** The title compound was synthesized according to general procedure using **2** (38.2 mg, 87.0 µmol, 1.1 eq) and **42** (29.2 mg, 81.9 µmol, 1 eq). The product was obtained as a white solid (18.2 mg, 26.8 µmol, 31%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 5.99 (d, *J* = 7.8 Hz, 1H), 4.61 (s, 2H), 4.38 – 4.26 (m, 1H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.75 (d, *J* = 17.7 Hz, 2H), 3.71 – 3.67 (m, 1H), 3.60 – 3.57 (m, 2H), 3.06 (q, *J* = 6.7 Hz, 2H), 2.58 (s, 4H), 2.10 (t, *J* = 7.7 Hz, 2H), 1.59 (dt, *J* = 17.0, 8.5 Hz, 6H), 1.43 (s, 10H), 1.32 – 1.23 (m, 9H), 1.18 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.09, 170.41, 157.99 (d, *J* = 258.0 Hz), 157.26, 151.83, 151.80, 138.82, 135.77, 128.56, 127.66, 123.72 (d, *J* = 20.6 Hz), 120.38 (d, *J* = 4.2 Hz), 58.64, 54.50, 49.22, 46.60, 45.11, 44.25, 40.68, 36.91, 30.11, 29.84, 29.25, 29.09, 28.58, 26.13, 25.54, 24.29, 18.44. LC-MS (ESI, 10-90): t_R = 6.03 min; m/z = 681.27 $[M + H]^+$. HRMS $[C_{37}H_{53}FN_6O_5 + H]^+$: 681.41342 calculated, 681.41308 found.

***tert*-Butyl (*R*)-(12-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-12-oxododecyl)carbamate (7):** The title compound was synthesized according to general procedure using **2** (105.1 mg, 0.24 mmol, 1 eq) and **43** (98.5 mg, 0.24 mmol, 1 eq). The product was obtained as a white solid (85.6 mg, 0.12 mmol, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.62 (dd, *J* = 8.6, 3.6 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 5.98 (d, *J* = 8.1 Hz, 1H), 4.61 (s, 2H), 4.53 (s, 1H), 4.32 (m, 1H), 3.84 (d, *J* = 2.6 Hz, 2H), 3.82 – 3.68 (m, 2H), 3.64 – 3.50 (m, 2H), 3.08 (q, *J* = 6.7 Hz, 2H), 2.60 (s, 4H), 2.10 (t, *J* = 7.7 Hz, 2H), 1.59 (1.66 – 1.53, 6H), 1.43 (s, 9H), 1.32 – 1.20 (m, 18H), 1.18 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 173.24, 170.39, 158.00 (d, *J* = 258.9 Hz), 157.25, 151.87, 138.76, 135.79, 128.55, 127.64, 123.74 (d, *J* = 20.3 Hz), 120.44 (d, *J* = 4.2 Hz), 69.36, 58.39, 54.33, 49.21, 46.59, 45.06, 44.22, 40.75, 37.02, 30.18, 29.63, 29.58, 29.46, 29.40, 28.57, 26.93, 25.97, 25.69, 24.20, 18.45. LC-MS (ESI, 10-90): t_R = 7.10 min; m/z = 737.27 $[M + H]^+$. HRMS $[C_{41}H_{61}FN_6O_5 + H]^+$: 737.47602 calculated, 737.47552 found.

***tert*-Butyl (8-((2-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)ethyl)amino)-8-oxooctyl)carbamate (8):** The title compound was synthesized according to general procedure using **3** (22.5 mg, 53.0 µmol, 1 eq) and **42** (18.9 mg, 53.0 µmol, 1 eq). The product was obtained as a white solid (21.7 mg, 33.0 µmol, 61%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.62 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.7 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 2H), 6.13 (s, 1H), 4.61 (s, 2H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.77 (s, 2H), 3.75 – 3.70 (m, 2H), 3.55 – 3.46 (m, 2H), 3.25 – 3.18 (m, 1H), 3.14 – 3.02 (m, 4H), 2.59 (s, 4H), 2.14 (t, *J* = 7.6 Hz, 2H), 1.60 (p, *J* = 5.7 Hz, 6H), 1.43 (s, 9H), 1.32 – 1.26

(m, 8H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.71, 170.33, 158.00 (d, *J* = 258.2 Hz), 157.14, 151.84, 138.86, 136.40, 135.75, 128.64, 128.25, 127.67, 123.74 (d, *J* = 20.6 Hz), 120.40 (d, *J* = 4.1 Hz), 58.61 (d, *J* = 1.7 Hz), 54.50, 51.75, 50.63, 49.29, 46.64, 46.58, 39.19, 39.05, 36.72, 30.10, 29.85, 29.24, 29.05, 26.72, 26.12, 25.53, 24.29. LC-MS (ESI, 10-90): t_R = 5.90 min; m/z = 667.33 $[M + H]^+$. HRMS $[C_{36}H_{51}FN_6O_5 + H]^+$: 667.39777 calculated, 667.39749 found.

***tert*-Butyl (12-((2-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)ethyl)amino)-12-oxododecyl)carbamate (9):** The title compound was synthesized according to general procedure using **3** (21.6 mg, 51.0 µmol, 1 eq) and **43** (21.0 mg, 51.0 µmol, 1 eq). The product was obtained as a white solid (22.0 mg, 30.0 µmol, 60%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.2 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 6.11 (s, 1H), 4.61 (s, 2H), 4.52 (s, 1H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.76 (s, 2H), 3.75 – 3.69 (m, 2H), 3.54 – 3.47 (m, 2H), 3.08 (q, *J* = 7.2, 6.6 Hz, 2H), 2.58 (d, *J* = 5.6 Hz, 4H), 2.18 – 2.11 (m, 2H), 1.79 – 1.75 (m, 2H), 1.60 (p, *J* = 5.6 Hz, 6H), 1.43 (s, 9H), 1.32 – 1.21 (m, 16H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.81, 170.30, 158.00 (d, *J* = 258.2 Hz), 157.14, 151.81 (d, *J* = 5.0 Hz), 145.99 (d, *J* = 15.4 Hz), 138.87, 135.73, 128.62, 127.67, 123.71 (d, *J* = 20.5 Hz), 120.38 (d, *J* = 4.2 Hz), 58.64 (d, *J* = 3.0 Hz), 54.49, 49.28, 46.64, 40.77, 39.17, 39.02, 36.84, 30.30, 30.19, 29.84, 29.62, 29.56, 29.45, 29.43, 29.39, 28.58, 26.12, 25.68, 24.29. LC-MS (ESI, 10-90): t_R = 6.93 min; m/z = 723.33 $[M + H]^+$. HRMS $[C_{40}H_{59}FN_6O_5 + H]^+$: 723.46037 calculated, 723.46000 found.

***tert*-Butyl (*S*)-(2-(2-(3-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-3-oxopropoxy)ethoxy)ethyl)carbamate (10):** The title compound was synthesized according to general procedure using **1** (27.0 mg, 62 µmol, 1 eq) and **44** (23.1 mg, 62.0 µmol, 1 eq). The product was obtained as a white solid (6.9 mg, 9.9 µmol, 16%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 7.1 Hz, 2H), 4.61 (q, *J* = 15.1 Hz, 2H), 4.41 – 4.31 (m, 1H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.80 – 3.71 (m, 2H), 3.71 – 3.67 (m, 2H), 3.67 – 3.58 (m, 6H), 3.58 – 3.54 (m, 2H), 3.55 – 3.49 (m, 2H), 3.30 (q, *J* = 5.7 Hz, 1H), 2.87 (t, *J* = 5.1 Hz, 1H), 2.57 (s, 4H), 2.48 – 2.33 (m, 2H), 1.82 (s, 2H), 1.59 (p, *J* = 5.6 Hz, 4H), 1.43 (s, 9H), 1.19 (dd, *J* = 6.8, 2.5 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.45, 170.42, 157.97 (d, *J* = 258.1 Hz), 157.18, 151.86 (d, *J* = 4.9 Hz), 145.99 (d, *J* = 15.1 Hz), 138.73, 135.96, 128.52, 127.61, 123.69 (d, *J* = 20.4 Hz), 120.37 (d, *J* = 4.1 Hz), 73.30, 70.61, 70.52, 70.35, 67.17, 58.67 (d, *J* = 3.1 Hz), 54.49, 49.22, 46.57, 44.53, 44.42, 41.81, 37.09, 28.56, 26.15, 24.30, 18.31. LC-MS (ESI, 10-90): t_R = 5.35 min; m/z = 699.13 $[M + H]^+$. HRMS $[C_{36}H_{51}FN_6O_7 + H]^+$: 699.38760 calculated, 699.38718 found.

***tert*-Butyl (*S*)-(15-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-14-methyl-12-oxo-3,6,9-trioxa-13-azapentadecyl)carbamate (11):** The title compound was synthesized according to general procedure using **1** (25.7 mg, 59.0 µmol, 1 eq) and **45** (24.5 mg, 59.0 µmol, 1 eq). The product was obtained as a white solid (5.8 mg, 7.8 µmol, 13%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 2H), 4.67 – 4.55 (m, 2H), 4.42 – 4.31 (m, 1H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.80 – 3.72 (m, 2H), 3.71 – 3.67 (m, 2H), 3.67 – 3.58 (m, 10H), 3.58 – 3.55 (m, 2H), 3.55 – 3.50 (m, 2H), 3.30 (d, *J* = 5.6 Hz, 1H), 2.87 (t, *J* = 5.4 Hz, 1H), 2.58 (s, 4H), 2.49 – 2.34 (m, 2H), 1.79 (s, 2H), 1.60 (p, *J* = 5.6 Hz, 4H), 1.43 (s, 9H), 1.19 (dd, *J* = 6.8, 2.8 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.46, 171.40, 170.40, 157.97 (d, *J* = 258.1 Hz), 157.18, 151.87 (d, *J* = 4.7 Hz), 145.99 (d, *J* = 15.1 Hz), 138.74, 135.96, 128.53, 127.61, 123.69 (d, *J* = 20.4 Hz), 120.37 (d, *J* = 4.2 Hz), 73.28, 70.61, 70.59, 70.52, 70.50, 70.35, 67.17, 58.66 (d, *J* = 3.1 Hz), 54.50, 49.23, 46.57, 44.53, 44.49, 41.80, 37.10, 28.57, 26.15, 24.30, 18.31. LC-MS (ESI, 10-90): t_R = 5.35 min; m/z = 743.27 $[M + H]^+$. HRMS $[C_{38}H_{55}FN_6O_8 + H]^+$: 743.41382 calculated, 743.41351 found.

***tert*-Butyl (*S*)-(18-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-17-methyl-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecyl)carbamate (12):** The title compound was synthesized according to general procedure using **1** (32.5 mg, 74.0 µmol, 1 eq) and **51** (34.2 mg, 74.0 µmol, 1 eq). The product was obtained as a white solid (38.0 mg, 48.0 µmol, 65%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 2H), 4.67 – 4.55 (m, 2H), 4.41 – 4.31 (m, 1H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.74 (d, *J* = 13.5 Hz, 2H), 3.68 (t, *J* = 5.6 Hz, 2H), 3.66 – 3.58 (m, 14H), 3.58 – 3.54 (m, 2H), 3.54 – 3.51 (m, 2H), 3.30 (q, *J* = 5.4 Hz, 1H), 2.93 (td, *J* = 5.0, 1.9 Hz, 1H), 2.58 (s, 4H), 2.41 (t, *J* = 6.9 Hz, 2H), 1.98 (bs, 2H), 1.60 (p, *J* = 5.6 Hz, 4H), 1.43 (s, 9H), 1.18 (d, *J* = 6.8 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.44, 170.52, 170.41, 157.98 (d, *J* = 257.9 Hz), 157.17, 151.89 (d, *J* = 4.7 Hz), 145.94 (d, *J* = 14.9 Hz), 138.72, 136.03, 135.98, 128.53, 127.61, 123.70 (d, *J* = 20.4 Hz), 120.40 (d, *J* = 4.2 Hz), 70.73, 70.64, 70.59, 70.52, 70.42, 70.40, 70.34, 70.31, 67.15, 58.63 (d, *J* = 3.0 Hz), 54.49, 49.23, 46.57, 44.55, 44.44, 37.08, 28.57, 26.12, 24.29, 18.31. LC-MS (ESI, 10-90): t_R = 5.41 min; m/z = 787.27 $[M + H]^+$. HRMS $[C_{40}H_{59}FN_6O_9 + H]^+$: 787.44003 calculated, 787.43948 found.

***tert*-Butyl (*R*)-(2-(2-(3-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-3-oxopropoxy)ethoxy)ethyl)carbamate (13):** The title compound was synthesized according to general procedure using **2** (27.4 mg, 62.5 µmol, 1 eq) and **44** (23.3 mg, 62.2 µmol, 1 eq). The product was obtained as a white solid (5.8 mg, 8.3 µmol, 13%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.2 Hz, 2H), 6.53 (d, *J* = 8.5 Hz, 1H), 5.16 (s, 1H), 4.60 (q, *J* = 14.9 Hz, 2H), 4.41 – 4.31 (m, 1H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.74 (q, *J* = 14.4 Hz, 2H), 3.70 – 3.66 (m, 2H), 3.64 – 3.57 (m, 4H), 3.58 – 3.49 (m, 4H), 3.31 (q, *J* = 5.5 Hz, 1H), 2.86 (t, *J* = 4.9 Hz, 1H), 2.58 (t, *J* = 5.5 Hz, 4H), 2.47 – 2.35 (m, 2H), 1.73 (s, 2H), 1.59 (p, *J* = 5.6 Hz, 4H), 1.42 (s, 9H), 1.18 (dd, *J* = 6.8, 2.9 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.38, 170.38, 157.97 (d, *J* = 258.1 Hz), 157.16, 156.15, 151.85 (d, *J* = 5.0 Hz), 145.99 (d, *J* = 15.0 Hz), 138.74, 135.93, 128.52, 127.61, 123.69 (d, *J* = 20.5 Hz), 120.36 (d, *J* = 4.2 Hz), 73.51, 70.37, 70.31, 70.22, 67.15, 58.66 (d, *J* = 3.0 Hz), 54.49, 49.22, 46.56, 44.56, 44.40, 41.84, 37.09, 28.56, 26.14, 24.30, 18.34. LC-MS (ESI, 10-90): t_R = 5.34. min; m/z = 699.27 $[M + H]^+$. HRMS $[C_{36}H_{51}FN_6O_7 + H]^+$: 699.38760 calculated, 699.38720 found.

***tert*-Butyl (*R*)-(15-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-14-methyl-12-oxo-3,6,9-trioxa-13-azapentadecyl)carbamate (14):** The title compound was synthesized according to general procedure using **2** (25.3 mg, 58.0 µmol, 1 eq) and **45** (24.1 mg, 58.0 µmol, 1 eq). The product was obtained as a white solid (7.2 mg, 9.7 µmol, 17%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.62 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 6.52 (d, *J* = 8.5 Hz, 1H), 5.17 (s, 1H), 4.67 – 4.55 (m, 2H), 4.42 – 4.31 (m, 1H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.75 (d, *J* = 17.6 Hz, 2H), 3.70 – 3.59 (m, 6H), 3.58 – 3.52 (m, 8H), 3.32 (d, *J* = 5.7 Hz, 2H), 2.59 (s, 4H), 2.42 (q, *J* = 6.2 Hz, 2H), 1.64 – 1.57 (m, 6H), 1.43 (s, 9H), 1.19 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.46, 171.40, 170.42, 157.98 (d, *J* = 259.6 Hz), 157.18, 151.89, 146.06, 145.94, 138.75, 135.97, 128.53, 127.61, 123.70 (d, *J* = 21.4 Hz), 120.37 (d, *J* = 5.0 Hz), 73.31, 70.62, 70.60, 70.53, 70.51, 70.37, 67.17, 58.67 (d, *J* = 3.8 Hz), 54.50, 49.23, 46.58, 44.54, 44.43, 37.10, 28.57, 26.16, 24.31, 18.32. LC-MS (ESI, 10-90): t_R = 5.35 min; m/z = 743.27 $[M + H]^+$. HRMS $[C_{38}H_{55}FN_6O_8 + H]^+$: 743.41382 calculated, 743.41333 found.

***tert*-Butyl (*R*)-(18-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-17-methyl-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecyl)carbamate (15):** The title compound was synthesized according to general procedure using **2** (31.0 mg, 71.0 µmol, 1.1 eq) and **51** (30.0 mg, 65.0 µmol, 1 eq). The product was obtained as a white solid (35.1 mg, 45.0 µmol, 63%). ^{1}H-NMR (500

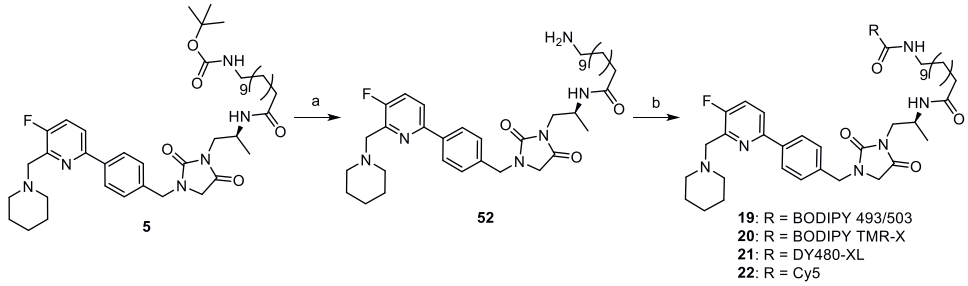
MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.2 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.35 (dd, *J* = 8.3, 3.0 Hz, 2H), 6.57 (d, *J* = 8.4 Hz, 1H), 5.12 (s, 1H), 4.61 (q, *J* = 15.3 Hz, 2H), 4.41 – 4.31 (m, 1H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.76 – 3.69 (m, 2H), 3.66 – 3.58 (m, 14H), 3.58 – 3.51 (m, 4H), 3.30 (q, *J* = 5.5 Hz, 2H), 2.58 (s, 2H), 2.48 – 2.35 (m, 2H), 1.97 (s, 2H), 1.60 (p, *J* = 5.6 Hz, 6H), 1.43 (s, 9H), 1.19 (t, *J* = 6.4 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 171.46, 170.41, 157.98 (d, *J* = 257.9 Hz), 157.17, 151.90 (d, *J* = 5.0 Hz), 145.93 (d, *J* = 14.9 Hz), 138.70, 135.99, 128.54, 127.60, 123.71 (d, *J* = 20.4 Hz), 120.41 (d, *J* = 3.9 Hz), 72.57, 70.73, 70.65, 70.64, 70.51, 70.41, 70.33, 67.15, 58.62 (d, *J* = 3.0 Hz), 54.49, 49.23, 46.56, 44.53, 44.44, 41.62, 36.98, 29.84, 28.57, 26.12, 24.29, 18.30. LC-MS (ESI, 10-90): t_R = 5.41 min; m/z = 787.27 $[M + H]^+$. HRMS $[C_{40}H_{59}FN_6O_9 + H]^+$: 787.44003 calculated, 787.43971 found.

***tert*-Butyl (2-(2-(3-((2-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)ethyl)amino)-3-oxopropoxy)ethoxy)ethyl)carbamate (16):** The title compound was synthesized according to general procedure using **3** (29.3 mg, 69.0 µmol, 1 eq) and **44** (25.8 mg, 69.0 µmol, 1 eq). The product was obtained as a white solid (12.9 mg, 19 µmol, 27%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.2 Hz, 2H), 7.65 (dd, *J* = 8.6, 3.4 Hz, 1H), 7.45 (t, *J* = 8.7 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 2H), 6.89 (s, 1H), 5.12 (s, 1H), 4.63 (s, 2H), 3.87 (t, J = 6.4 Hz, 2H), 3.77 (s, 2H), 3.73 (t, *J* = 5.7 Hz, 2H), 3.69 – 3.59 (m, 6H), 3.58 – 3.49 (m, 4H), 3.32 (d, *J* = 5.0 Hz, 4H), 2.63 (s, 2H), 2.47 (t, *J* = 5.8 Hz, 2H), 1.64 (s, 6H), 1.45 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 172.20, 170.32, 158.02 (d, *J* = 258.2 Hz), 157.06, 128.63, 127.63, 70.55, 70.38, 70.34, 70.22, 67.10, 54.38, 49.30, 46.60, 39.17, 38.39, 36.92, 28.57. LC-MS (ESI, 10-90): t_R = 5.16 min; m/z = 685.13 $[M + H]^+$. HRMS $[C_{35}H_{49}FN_6O_7 + H]^+$: 685.37195 calculated, 685.37165 found.

***tert*-Butyl (15-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-12-oxo-3,6,9-trioxa-13-azapentadecyl)carbamate (17):** The title compound was synthesized according to general procedure using **3** (28.8 mg, 68.0 µmol, 1 eq) and **45** (28.3 mg, 67.6 µmol, 1 eq). The product obtained as a white solid (6.3 mg, 8.7 µmol, 13%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 2H), 6.81 (s, 1H), 5.16 (s, 1H), 4.61 (s, 2H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.75 (s, 2H), 3.74 – 3.67 (m, 4H), 3.60 (s, 6H), 3.57 – 3.48 (m, 4H), 3.31 (d, *J* = 5.8 Hz, 2H), 2.58 (s, 4H), 2.45 (t, *J* = 5.8 Hz, 2H), 1.82 (s, 2H), 1.60 (p, *J* = 5.6 Hz, 6H), 1.42 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 172.08, 170.30, 157.98 (d, *J* = 258.1 Hz), 157.03, 156.18, 151.83 (d, *J* = 4.5 Hz), 146.00 (d, *J* = 14.9 Hz), 138.81, 135.86, 128.62, 127.63, 123.71 (d, *J* = 20.3 Hz), 120.37 (d, *J* = 4.1 Hz), 73.32, 70.34, 70.29, 70.22, 70.14, 70.02, 67.12, 58.65 (d, *J* = 3.1 Hz), 54.50, 49.27, 46.60, 41.79, 40.42, 39.17, 38.42, 36.93, 28.56, 26.14, 24.30. LC-MS (ESI, 10-90): t_R = 5.21 min; m/z = 729.27 $[M + H]^+$. HRMS $[C_{37}H_{53}FN_6O_8 + H]^+$: 729.39817 calculated, 729.39767 found.

***tert*-Butyl (18-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecyl)carbamate (18):** The title compound was synthesized according to general procedure using **3** (28.5 mg, 65.0 µmol, 1 eq) and **51** (32.8 mg, 70.9 µmol, 1.1 eq). The product obtained as a white solid (22.6 mg, 28.8 µmol, 44%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.2 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 2H), 6.91 (s, 1H), 5.11 (s, 1H), 4.61 (s, 2H), 3.82 (d, *J* = 2.7 Hz, 2H), 3.76 (d, *J* = 5.0 Hz, 2H), 3.70 (t, *J* = 5.4 Hz, 2H), 3.67 – 3.58 (m, 12H), 3.56 – 3.48 (m, 4H), 3.30 (q, *J* = 5.2 Hz, 2H), 2.58 (s, 2H), 2.45 (t, *J* = 6.0 Hz, 2H), 1.84 (s, 4H), 1.60 (p, *J* = 5.6 Hz, 6H), 1.43 (s, 9H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 172.22, 170.33, 157.99 (d, *J* = 258.1 Hz), 157.13, 157.07, 151.87 (d, *J* = 4.8 Hz), 145.94 (d, *J* = 15.5 Hz), 138.77, 135.96, 128.60, 127.62, 123.71 (d, *J* = 20.4 Hz), 120.38 (d, *J* = 4.1 Hz), 70.72, 70.67, 70.63, 70.55, 70.41, 70.37, 70.32, 70.23, 67.24, 67.10, 58.66 (d, *J* = 3.0 Hz), 54.51, 49.29, 46.60, 39.22, 36.92, 29.84, 28.58, 26.15, 24.31. LC-MS (ESI, 10-90): t_R = 5.28 min; m/z = 773.27 $[M + H]^+$. HRMS $[C_{39}H_{57}FN_6O_9 + H]^+$: 773.42438 calculated, 773.42407 found.

Synthesis of the fluorescent probes:



Scheme 4.10 Reagent and conditions: a) TFA (110 eq), DCM, RT, 2 h, quant.; b) **19-21**: Et_3N (1 eq), fluorophore-NHS ester (1 eq), DCM (0.3 M), RT, 1-3 h, 64-100%; **22**: HOBt (1.2 eq), DiPEA (2.5 eq), EDC.HCl (1.3 eq), Cyanine 5 carboxylic acid (1.1 eq), DMF (0.007 M), RT, 16 h, quant.).

(*S*)-12-Amino-*N*-(1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)dodecanamide (52): A mixture of **5** (435 mg, 0.6 mmol, 1 eq) and TFA (5 mL, 64.9 mmol, 110 eq) in DCM (10 mL) was stirred at RT for 2 h. The volatile compounds were evaporated under reduced pressure. The crude was re-dissolved in DCM and 1 M (aq) NaOH was added until pH 10. The layers were separated and the aqueous layer extracted thrice with chloroform. The combined organic layer was dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to yield a yellow oil (376 mg, 0.59 mmol, quant.). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.3 Hz, 2H), 7.60 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 6.09 (d, *J* = 8.1 Hz, 1H), 4.60 (s, 2H), 4.35 – 4.25 (m, 1H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.75 (q, *J* = 17.5 Hz, 2H), 3.62 – 3.49 (m, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 2.58 (s, 4H), 2.10 (t, *J* = 7.5 Hz, 2H), 1.59 (p, *J* = 5.6 Hz, 4H), 1.56 – 1.46 (m, 2H), 1.44 – 1.37 (m, 2H), 1.29 – 1.18 (m, 16H), 1.17 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.48, 170.46, 162.32, 162.05, 157.93 (d, *J* = 258.3 Hz), 157.21, 156.90, 151.95 (d, *J* = 5.0 Hz), 145.52 (d, *J* = 15.1 Hz), 138.63, 135.82, 128.52, 127.58, 123.78 (d, *J* = 20.2 Hz), 120.56 (d, *J* = 5.0 Hz), 58.29 (d, *J* = 2.5 Hz), 54.37, 53.54, 50.57, 49.19, 46.49, 44.78, 44.18, 40.98, 36.86, 30.40, 29.40, 29.36, 29.27, 29.24, 29.18, 28.92, 28.82, 26.63, 25.88, 25.60, 24.13, 18.27. LC-MS (ESI, 10-90): t_R = 4.84 min; m/z = 637.53 $[M + H]^+$.

General procedure for the synthesis of final compounds (19-21)

A mixture of **52** (1 eq) Et_3N, (1 eq) and fluorophore-NHS-ester (1 eq) in DCM (0.3 M) was stirred at RT for 1-3 h. The reaction mixture was diluted with H_2O and DCM. The layers were separated and the aqueous layer extracted thrice with DCM. The combined organic layer was washed with H_2O and brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with preparative HPLC and freeze-dried twice.

(*S*)-12-(4-(5,5-Difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)butanamido)-*N*-(1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)dodecanamide (19): The title compound was synthesized according to general final compound procedure using **52** (5.52 mg, 8.7 µmol, 1 eq) and BODIPY 493/503-NHS-ester (3.74 mg, 8.7 µmol, 1 eq). The product was obtained as orange solid (5.3 mg, 5.6 µmol, 64%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.62 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.42 (t, *J* = 8.7 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 5.98 (d, *J* = 8.1 Hz, 1H), 5.53 (s, 1H), 5.37 – 5.32 (m, 1H), 4.60 (s, 2H), 4.36 – 4.27 (m, 1H), 3.84 (s, 2H), 3.75 (q, *J* = 17.4 Hz, 2H), 3.64 – 3.51 (m, 2H), 3.22 (q, *J* = 6.8 Hz, 2H), 3.04 – 2.97 (m, 2H), 2.60 (s, 4H), 2.51 (s, 3H), 2.42 (s, 6H), 2.31 (t, *J* = 7.1 Hz, 2H), 2.10 (t, *J* =

7.7 Hz, 2H), 2.04 – 1.92 (m, 2H), 1.65 – 1.53 (m, 6H), 1.50 – 1.39 (m, 2H), 1.34 – 1.20 (m, 18H), 1.18 (d, *J* = 6.7 Hz, 3H), 0.88 (t, *J* = 6.9 Hz, 2H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.23, 171.56, 170.38, 158.01 (d, *J* = 258.2 Hz), 157.27, 154.20, 145.53, 140.66, 138.74, 138.44, 138.10, 138.08, 135.83, 130.12 (d, *J* = 12.7 Hz), 129.96 (d, *J* = 19.0 Hz), 128.56, 127.65, 123.78 (d, *J* = 19.5 Hz), 121.90, 120.48, 115.27, 54.42, 49.23, 46.60, 45.07, 44.23, 39.83, 37.00, 36.50, 29.84, 29.74, 29.57, 29.55, 29.51, 29.46, 29.41, 29.36, 27.70, 27.62, 27.36, 27.03, 25.66, 22.83, 18.45, 16.57, 14.61, 14.26. LC-MS (ESI, 10-90): t_R = 7.47 min; m/z = 953.33 $[M + H]^+$. HRMS $[C_{53}H_{72}BF_3N_8O_4 + H]^+$: 953.57964 calculated, 953.57929 found.

(*S*)-12-(3-(8-(4-(3-azidopropoxy)phenyl)-5,5-difluoro-1,3-dimethyl-5*H*-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)propanamido)-*N*-(1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)dodecanamide (20): The title compound was synthesized according to general final compound procedure using **52** (10.0 mg, 15.7 µmol, 1 eq) and BODIPY TMR-X-NHS-ester (8.6 mg, 15.8 µmol, 1 eq). The product was obtained as orange solid (12.4 mg, 11.6 µmol, 74%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.3 Hz, 2H), 7.86 (d, *J* = 8.9 Hz, 2H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.08 (s, 1H), 6.97 (d, *J* = 2.1 Hz, 1H), 6.97 – 6.92 (m, 2H), 6.53 (d, *J* = 4.1 Hz, 1H), 5.98 (d, *J* = 8.1 Hz, 1H), 5.42 (t, *J* = 5.8 Hz, 1H), 4.60 (s, 2H), 4.35 – 4.27 (m, 1H), 4.10 (t, *J* = 5.9 Hz, 2H), 3.82 (d, *J* = 2.6 Hz, 2H), 3.74 (d, *J* = 17.5 Hz, 2H), 3.61 – 3.50 (m, 2H), 2.75 (d, *J* = 7.7 Hz, 2H), 2.59 (s, 4H), 2.52 (s, 3H), 2.25 (d, *J* = 7.2 Hz, 2H), 2.20 (s, 3H), 2.13 – 2.02 (m, 4H), 1.75 (s, 6H), 1.63 – 1.53 (m, 4H), 1.41 (q, *J* = 7.0, 6.3 Hz, 2H), 1.27 – 1.19 (m, 16H), 1.18 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 173.22, 171.59, 170.39, 159.60, 159.55, 158.00 (d, *J* = 258.2 Hz), 157.27, 155.65, 151.83, 139.96, 138.78, 135.81, 135.10, 134.54, 130.86 (t, *J* = 4.2 Hz), 130.77, 128.56, 127.99, 127.65, 125.90, 123.74 (d, *J* = 20.4 Hz), 122.97, 120.44 (d, *J* = 5.6 Hz), 118.43, 114.34, 64.60, 58.56, 54.47, 49.22, 48.39, 46.59, 45.07, 44.23, 39.85, 37.01, 36.65, 29.84, 29.67, 29.56, 29.52, 29.50, 29.39, 29.35, 28.92, 26.98, 26.07, 25.66, 24.25, 20.19, 18.45, 13.31, 9.76. LC-MS (ESI, 10-90): t_R = 7.91 min; m/z = 1086.40 $[M + H]^+$. HRMS $[C_{59}H_{75}BF_3N_{11}O_5 + H]^+$: 1086.60803 calculated, 1086.60667 found.

(*S,E*)-6-(2-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)vinyl)-1-(6-((12-((1-(3-(4-(5-fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-12-oxododecyl)amino)-6-oxohexyl)pyridin-1-ium-3-sulfonate (21): The title compound was synthesized according to general final compound procedure using **52** (1.04 mg, 1.41 µmol, 1 eq) and DY-480XL-NHS-ester (1.0 mg, 1.9 µmol, 1.4 eq). After Prep-HPLC, two product with the correct mass were obtained as red solids (1.8 mg, 1.6 µmol, quant.) and (0.9 mg, 0.8 µmol, 50%). *No NMR-data available*. LC-MS (ESI, 10-90): t_R = 6.41 min; m/z = 1133.60 $[M + H]^+$. HRMS $[C_{62}H_{83}FN_8O_9S + H]^+$: 1133.59040 calculated, 1133.59028 found.

2-((1*E*,3*E*)-5-((*E*)-1-(6-((12-(((*S*)-1-(3-(4-(5-Fluoro-6-(piperidin-1-ylmethyl)pyridin-2-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)propan-2-yl)amino)-12-oxododecyl)amino)-6-oxohexyl)-3,3-dimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-1,3,3-trimethyl-3*H*-indol-1-ium (22): To a stirred and cooled (0 °C) mixture of Cyanine5 carboxylic acid (23.4 g, 48.4 µmol, 1.1 eq) in DMF (7 mL) was added HOBt (8.09 mg, 52.8 µmol, 1.2 eq), **52** (26.94 mg, 42.4 µmol, 1 eq), DiPEA (18.4 µL, 105.6 µmol, 2.5 eq) and EDC.HCl (9.69 mg, 50.5 µmol, 1.2 eq). After stirring overnight at RT, H_2O (7 mL) and EtOAc (7 mL) was added. The layers were separated and the aqueous layer extracted thrice with EtOAc. The combined organic layer was washed with sat. (aq) $NaHCO_3$, five times with H_2O, and once with brine, dried ($MgSO_4$), filtered, and the solvent evaporated under reduced pressure. The crude product was purified with preparative HPLC and freeze-dried twice to yield a blue solid (50.4 mg, 45.8 µmol, quant.). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.88 (m, 3H), 7.67 (t, *J* = 5.7 Hz, 1H), 7.61 (dd, *J* = 8.6, 3.5 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.38 – 7.29 (m, 6H), 7.20 (dt, *J* = 11.9, 7.4 Hz, 2H), 7.08 (dd, *J* = 17.4, 7.9 Hz, 2H), 6.91 (t, *J*

= 12.5 Hz, 1H), 6.56 (d, *J* = 13.7 Hz, 1H), 6.27 (d, *J* = 13.5 Hz, 1H), 6.12 (d, *J* = 8.0 Hz, 1H), 4.60 (s, 2H), 4.35 – 4.23 (m, 1H), 4.07 (t, *J* = 7.7 Hz, 2H), 3.81 (d, *J* = 2.6 Hz, 2H), 3.73 (q, *J* = 17.4 Hz, 2H), 3.64 – 3.50 (m, 4H), 3.19 (q, *J* = 6.4 Hz, 2H), 2.57 (s, 4H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.09 (t, *J* = 7.8 Hz, 2H), 1.88 – 1.73 (m, 2H), 1.70 (s, 6H), 1.69 (s, 6H), 1.61 – 1.48 (m, 10H), 1.39 (q, *J* = 6.2 Hz, 2H), 1.29 – 1.19 (m, 18H), 1.16 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 173.51, 173.48, 173.25, 172.57, 170.39, 157.93 (d, *J* = 258.0 Hz), 157.22, 154.02, 152.97, 151.77 (d, *J* = 4.9 Hz), 145.79 (d, *J* = 15.0 Hz), 142.94, 142.03, 141.33, 140.76, 138.70, 135.81, 128.88, 128.73, 128.53, 127.59, 126.90, 125.49, 124.94, 123.69 (d, *J* = 20.4 Hz), 122.28, 122.20, 120.38 (d, *J* = 4.2 Hz), 111.03, 110.22, 104.93, 103.65, 58.55 (d, *J* = 3.1 Hz), 54.36, 49.50, 49.20, 49.05, 46.53, 45.01, 44.71, 44.16, 39.67, 36.96, 36.21, 29.80, 29.70, 29.65, 29.62, 29.54, 29.45, 29.40, 29.35, 28.16, 27.23, 27.10, 26.51, 26.06, 25.67, 25.34, 24.22, 18.39. LC-MS (ESI, 10-90): t_R = 7.36 min; m/z = 1101.73 $[M]^+$. HRMS $[C_{68}H_{90}FN_8O_4 + H]^+$: 1101.70636 calculated, 1101.70696 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.**

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