A modular click ligand-directed approach to label endogenous dopamine D_1 receptors in live cells

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SUPPLEMENTARY INFORMATION

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1. Supplementary Figures

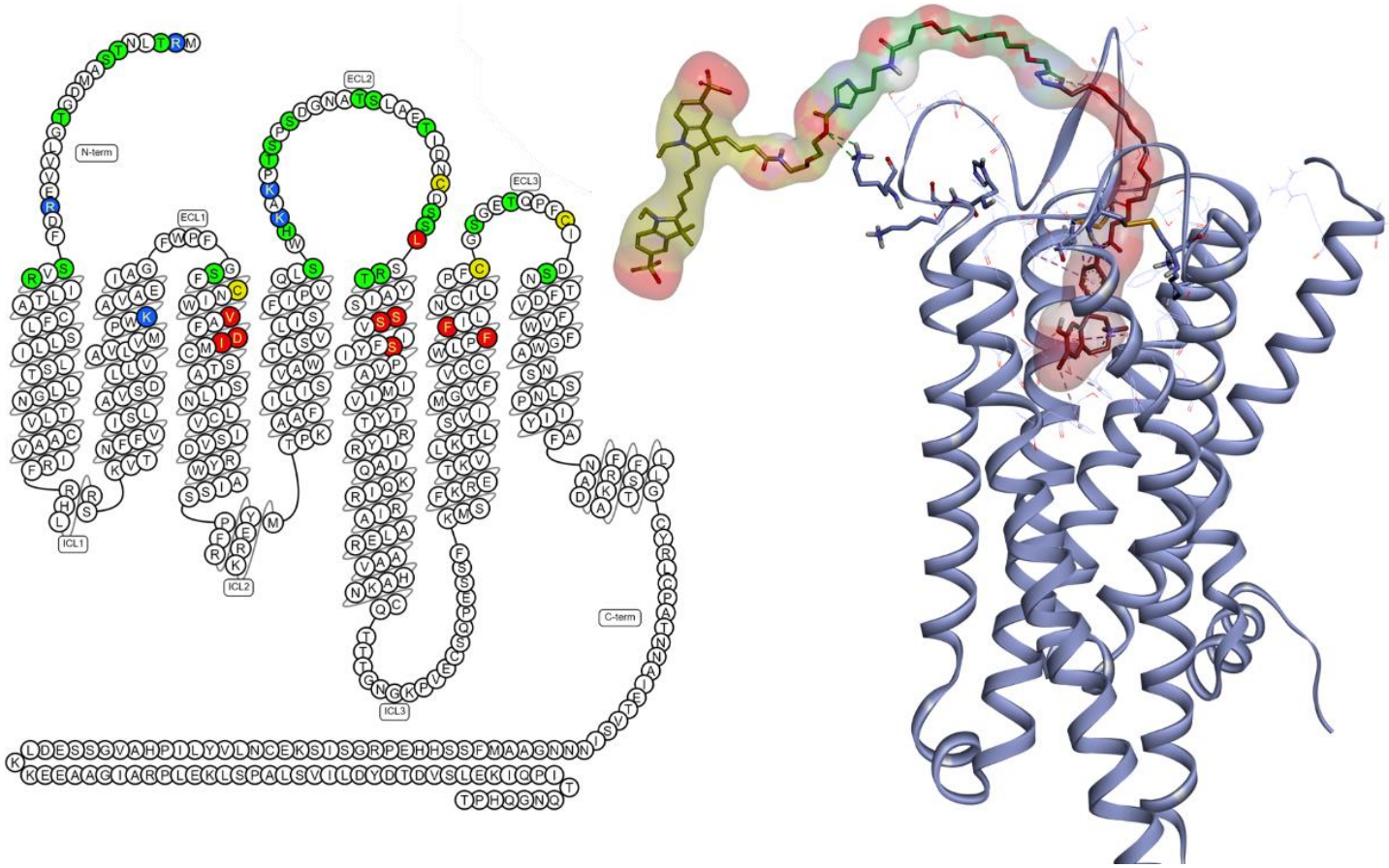


Figure S1: Structural features of human D_1 and hypothesis of labelling residues with CLD technology. (*Left*) Snakeplot of human D_1 receptor. The residues involving the binding of D_1 ligands from the benzoazepine family are marked in red. Extracellular positively charged residues (i.e. lysines (K) and arginines (R)) are marked in blue. Only solvent exposed lysines K165 and K167 on extracellular loop 2 (ECL2) are hypothised to react with CLD probes. Extracellular cysteines form disulfide bonds are marked in yellow and additionally extracellular residues hypothesised to be able to react with the CLD probes with lower extent are marked in green. Figure obtained from GPCRdb.[1,2] (Right) Model of binding of CLD probe 5h positioning the acyl imidazole (reactive moiety) towards the amine of lysine K167 on ECL2, revealing the need of a linkers with a considerable length to reach the desired lysine residues, The model has been obtained from GPCRdb[1,2] and the binding pose of the ligand unit has been adapted from the binding pose of SKF81297 in a cryoEM structure (PDB7JV5).[3]

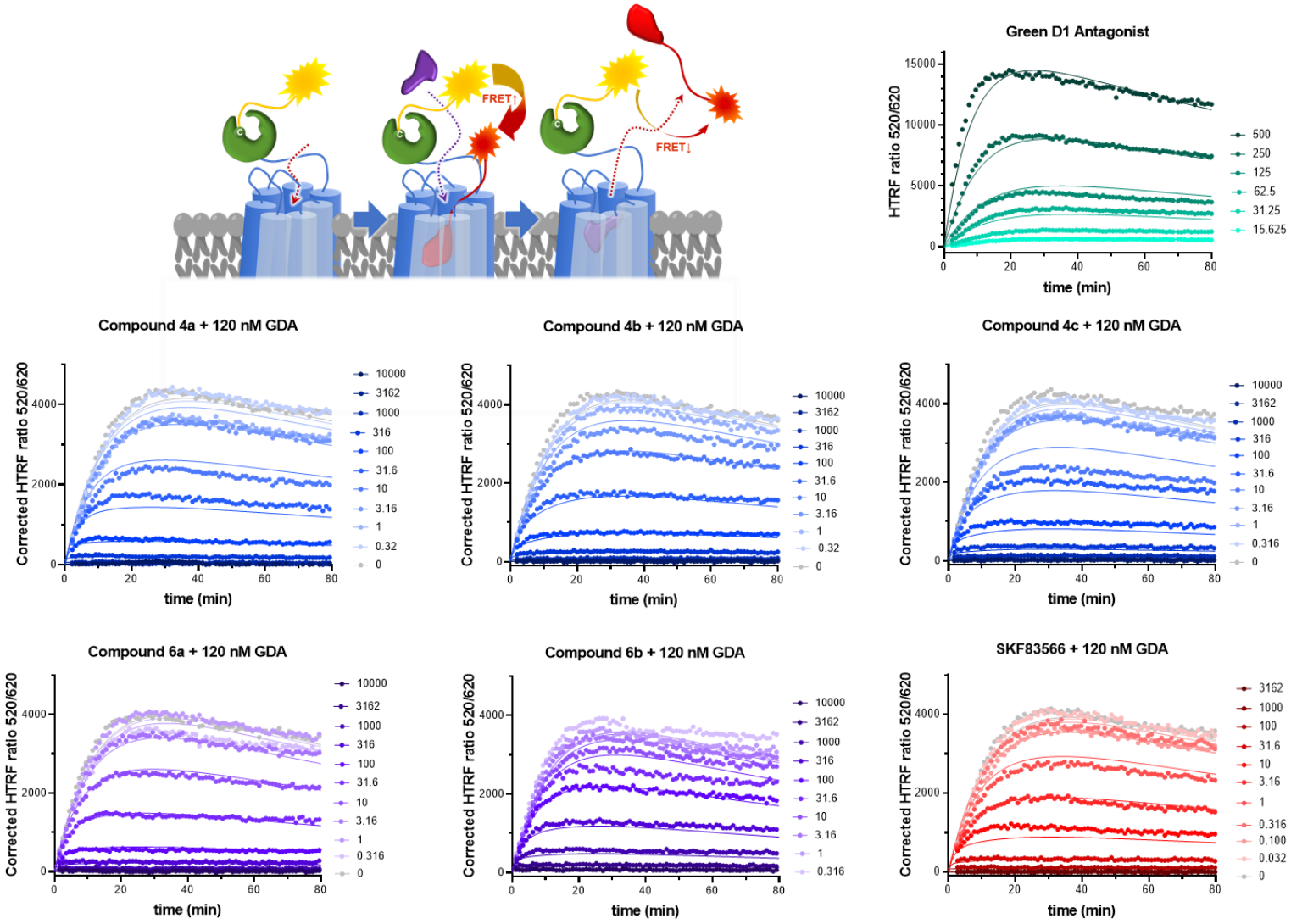


Figure S2: Traces of kinetic binding experiments using tag-lite® technology. Representative examples of traces of kinetic association binding experiments (for GDA) and kinetic association-competition experiments for compounds 4a-c, 6a-b and SKF83566. A drift on the TR-FRET was observed and was included in the fitting function. The affinity obtained for GDA (K_d = 294±85) is in line with the manufacturer (PerkinElmer) and has a residence time of 10.8±1.5 minutes. Residence time for the antagonists are similar or slightly lower. Minimum, 3 independent replicates were perfomed.

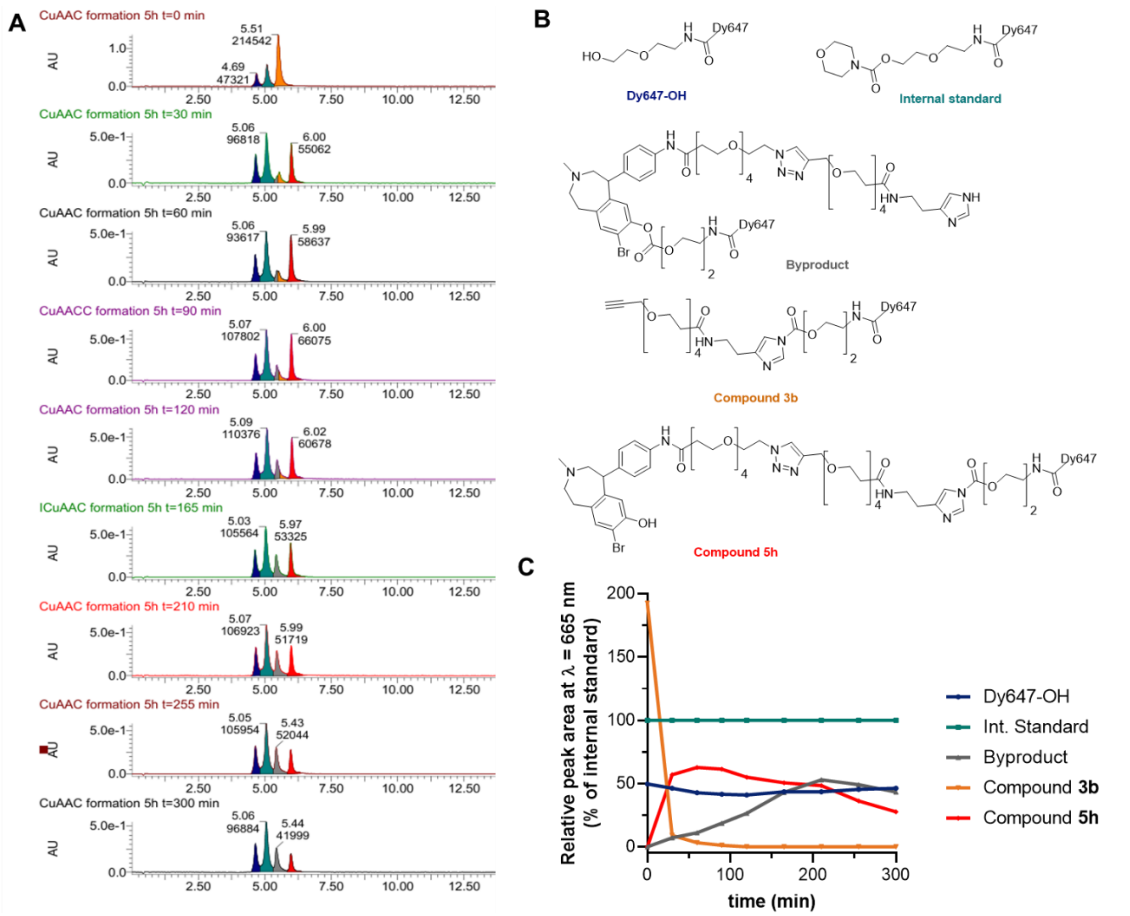


Figure S3: Monitoring by LC/PDA/MS of a click reaction between 3b and 4c to obtain 5h. (A) Absorbance chromatograms at λ = 650 nm of samples taken at several reaction time points. Coloured peaks correspond to the compounds shown in panel B and the integration of the areas revealed a rapid formation of **5h** but also progressive degradation over time (C). LC/PDA/MS Waters 2795 Alliance separation module coupled to a diode array detector (Agilent 1100) scanning at a wavelength range of 210-700 nm and an ESI Quattro Micro MS detector (Waters) in positive mode with mass range (m/z) of 150-1500. A column ZORBAX Extend-C18 3.5 µm 2.1x50mm (Agilent) at 35°C was used with a mixture of A = H_2O + 0.05% formic acid and B = MeCN + 0.05% formic acid as mobile phase and the method as follows: flow 0.5 mL/min, Gradient 0-1min 10%B 1-14min 10-50%B 14-16min 50-100%B 16-18 100%B 18-19min 10%B 10B 19-20 10%B total runtime 20 min.

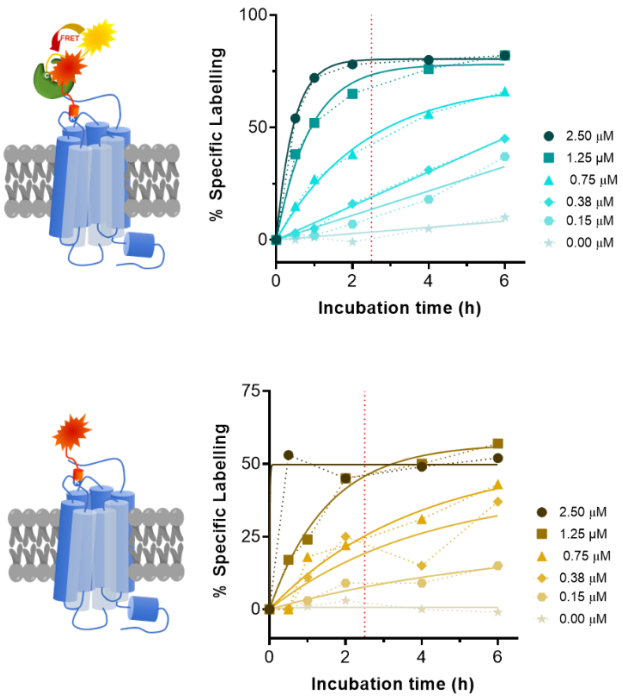


Figure S4: Optimisation of the incubation to label D_1 receptors. Plot representing the % of specific labelling (Specific/non-specific x100) of CLD probe **5e** in F-ST-D_1 reading TR-FRET 337/665 (upper) and HA-D_1 reading HA- D_1 reading fluorescence 640/680 (lower). The specific labelling improved with time and **5e** concertation. We considered that 2.5 h at micromolar concentration was close to the maximal specific labelling.

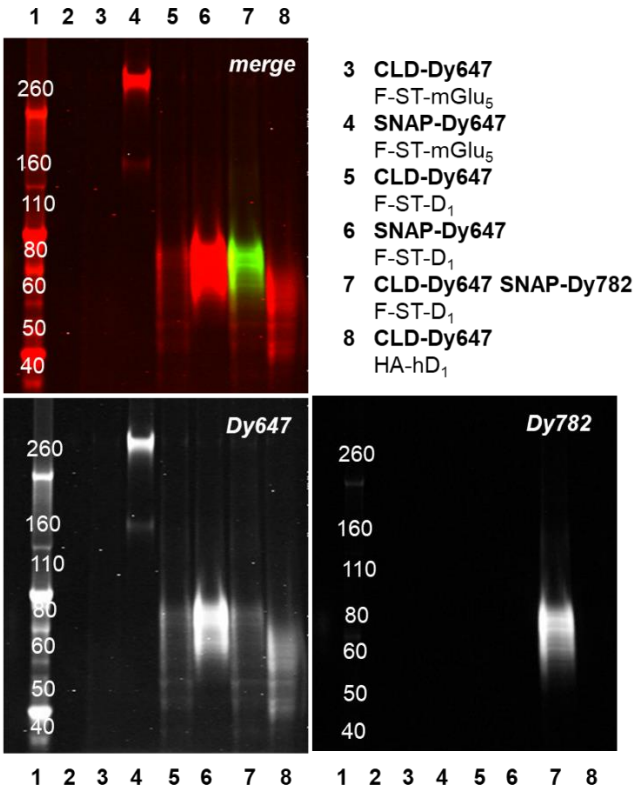


Figure S5: Gel fluorescence analysis on SDS-PAGE of D1 labelling of 5e. Our CLD probe was able to label F-ST D1 and HA-D1 receptors but not F-ST-$mGlu_5$. SNAP-tagged versions of D_1 and $mGlu_5$ receptors were able to be labelled with SNAP-surface-647. F-ST D_1 was able to be labelled simultaneously both CLD probe (Dy647) and SNAP-782 and being fluorescent at both 700- and 800-nm channel.

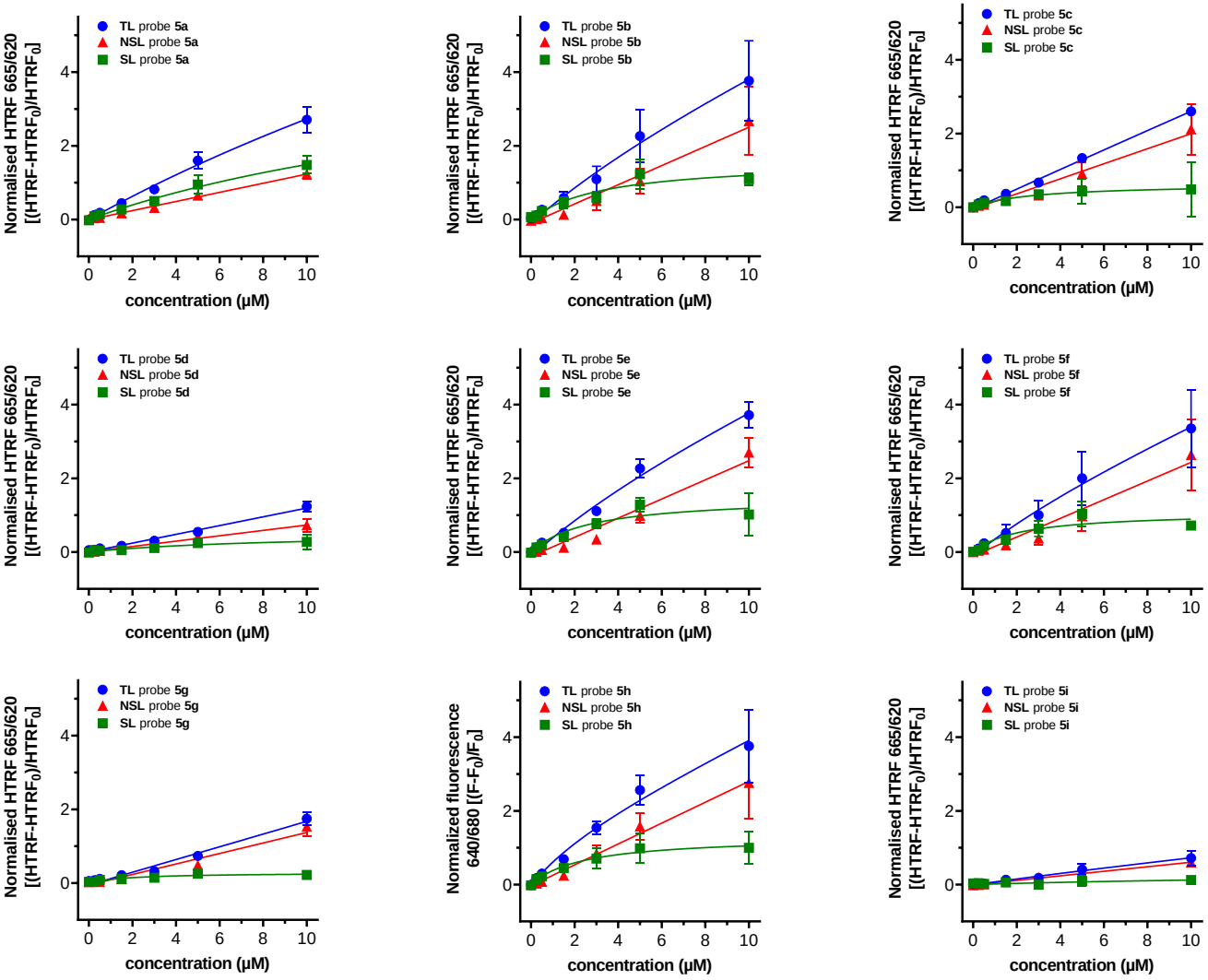


Figure S6: Labelling of F-ST-D$_1$ receptor with CLD probe 5a-i. Normalised saturation labelling curves of CLD probe **5a-i** using the human F-ST-D$_1$ with the ST labelled with Lumi4Tb and reading HTRF between SNAP-Lumi4Tb and -CLD-Dy647. The curves include total (TL, blue), non-specific (NS, red) and specific labeling (SL, green) A minimum of two independent experiments were performed in duplicate and points correspond to means with SEM as error bars).

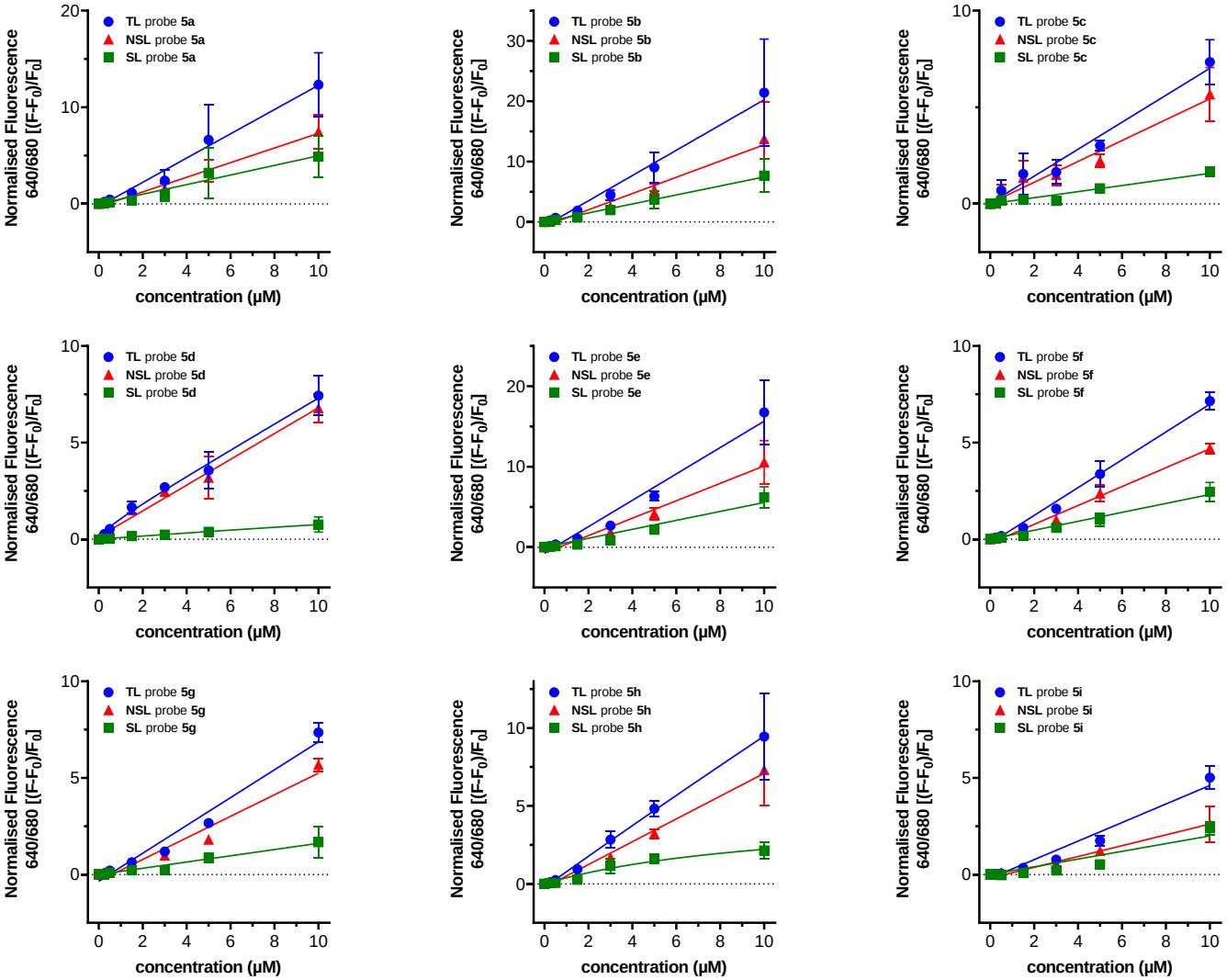


Figure S7: Labelling of HA-D_1 receptor with CLD probe 5a-i. Normalised saturation labelling curves of CLD probe **5a-i** using the human HA-D_1 reading Dy647 fluorescence. The curves include total (TL, blue), non-specific (NS, red) and specific labeling (SL, green) A minimum of two independent experiments were performed in duplicate and points correspond to means with SEM as error bars). Only probe **5h** showed a saturation profile on the specific labelling curve.

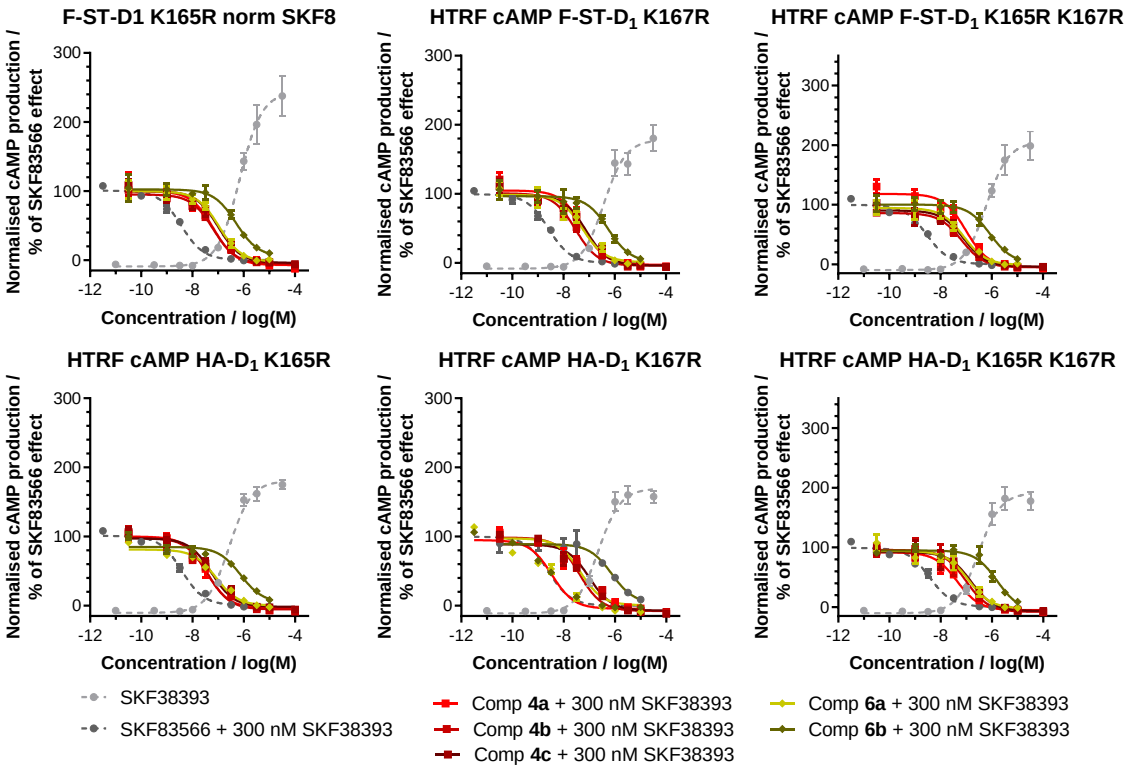


Figure S8: Functional concentration-response curves of agonist SKF38393 and antagonists SKF833566, 4a-c and 6a-b in competition with 300 nm of SKF38393 using HTRF® cAMP Gs Dynamic assay. Colour-code of the compounds matches Figure 2 and Table S2. Dots correspond to the means of minimum 3 independent replicates with the corresponding standard error of the mean (SEM) as error bars

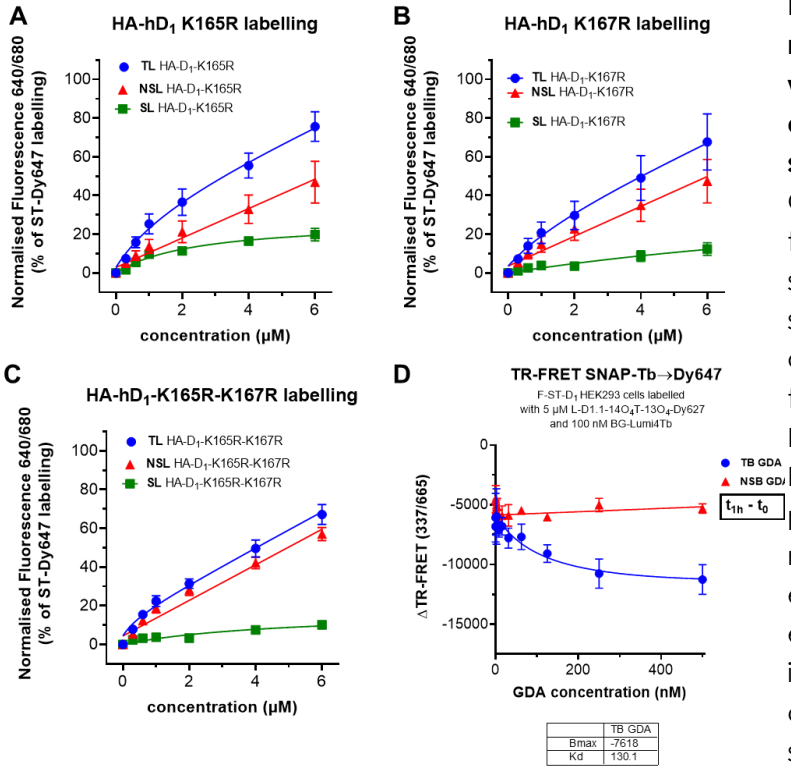


Figure S9: Labelling of mutants of D_1 receptors with CLD probe 5h and availability of the binding site after labelling. Graphics representing the total (blue), non-specific (red) and specific labelling (green) of CLD probe **5h** using the human D_1 mutants K165R (A), L167R (B) and K165R-K167R (C). Data points correspond to the mean of 3 independent experiments with SEM as error bars. (D) Second independent replicate of the experiment showed in figure 3D.

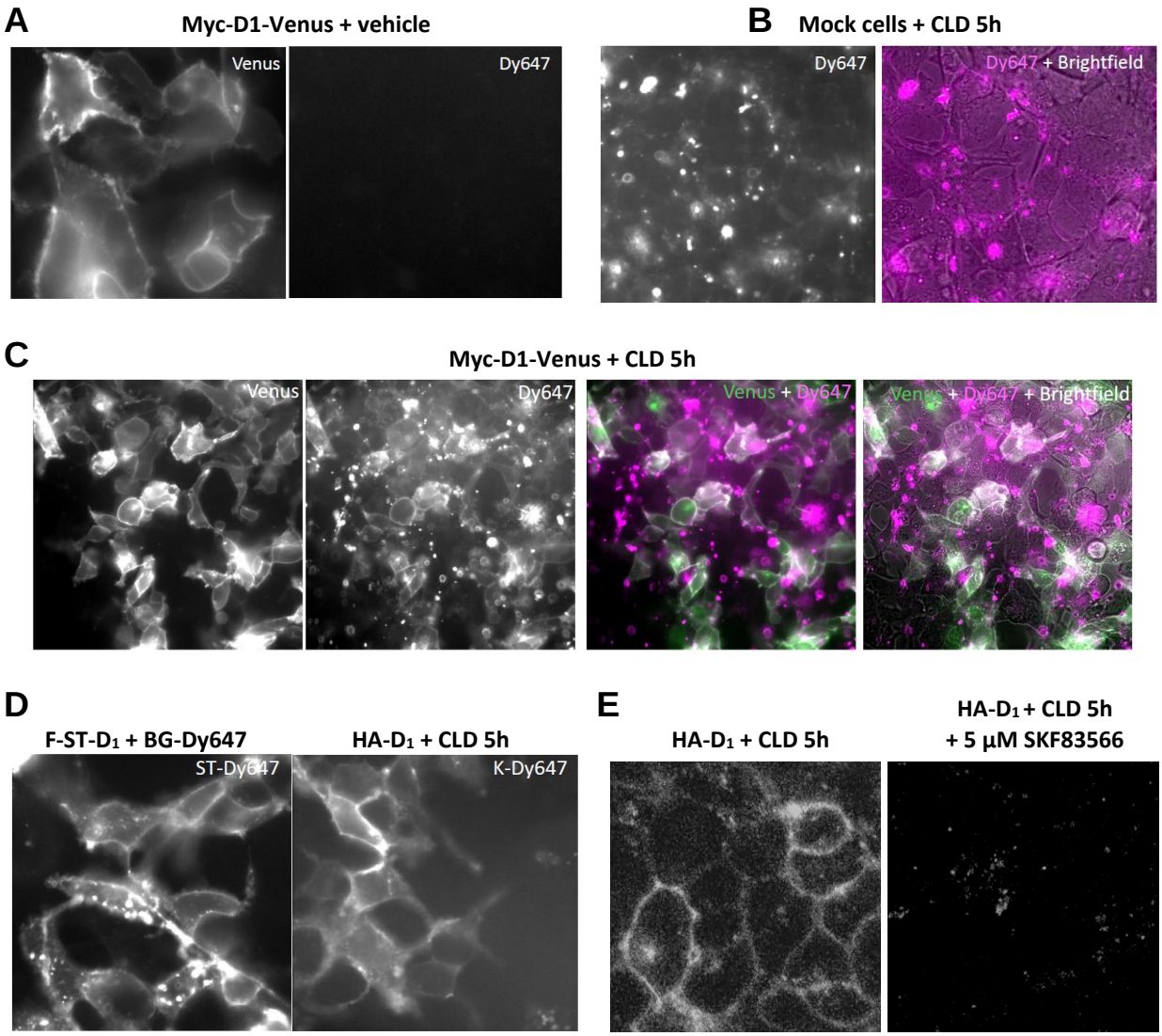


Figure S10: Microscopy supporting images. (A) Widefield microscopy images of live HEK293 cells expressing Myc-D_1-Venus and treated with a mix of the click reaction excluding the fluorescent molecular module **3b**. We did not observe fluorescence at the Dy647 channel, but showed fluorescence at the channel of Venus. (B) Widefield microscopy images of live HEK293 cells transfected with an empty vector (pRk6) and treated with a mix of the click reaction **5h**. with no filtration step. A dispersion of fluorescent aggregates was observed but no membrane labelling. (C) Widefield microscopy images of live HEK293 cells tr expressing Myc-D_1-Venus and treated with a mix of the click reaction **5h**. with no filtration step. Membrane labelling and a dispersion of fluorescent aggregates were observed (D) Widefield microscopy images showing that the labelling of the SNAP-tag of F-ST-D_1 with BG-Dy647 and labelling of the K167 with CLD probe **5h** produce similar labelling at the surface of the HEK293 cells transiently transfected with the corresponding receptors. (E) Confocal microscopy images showing that the labelling of HA-D_1 with CLD probe **5h** is prevented by a competition of an excess of D_1 selective antagonist SKF83566.

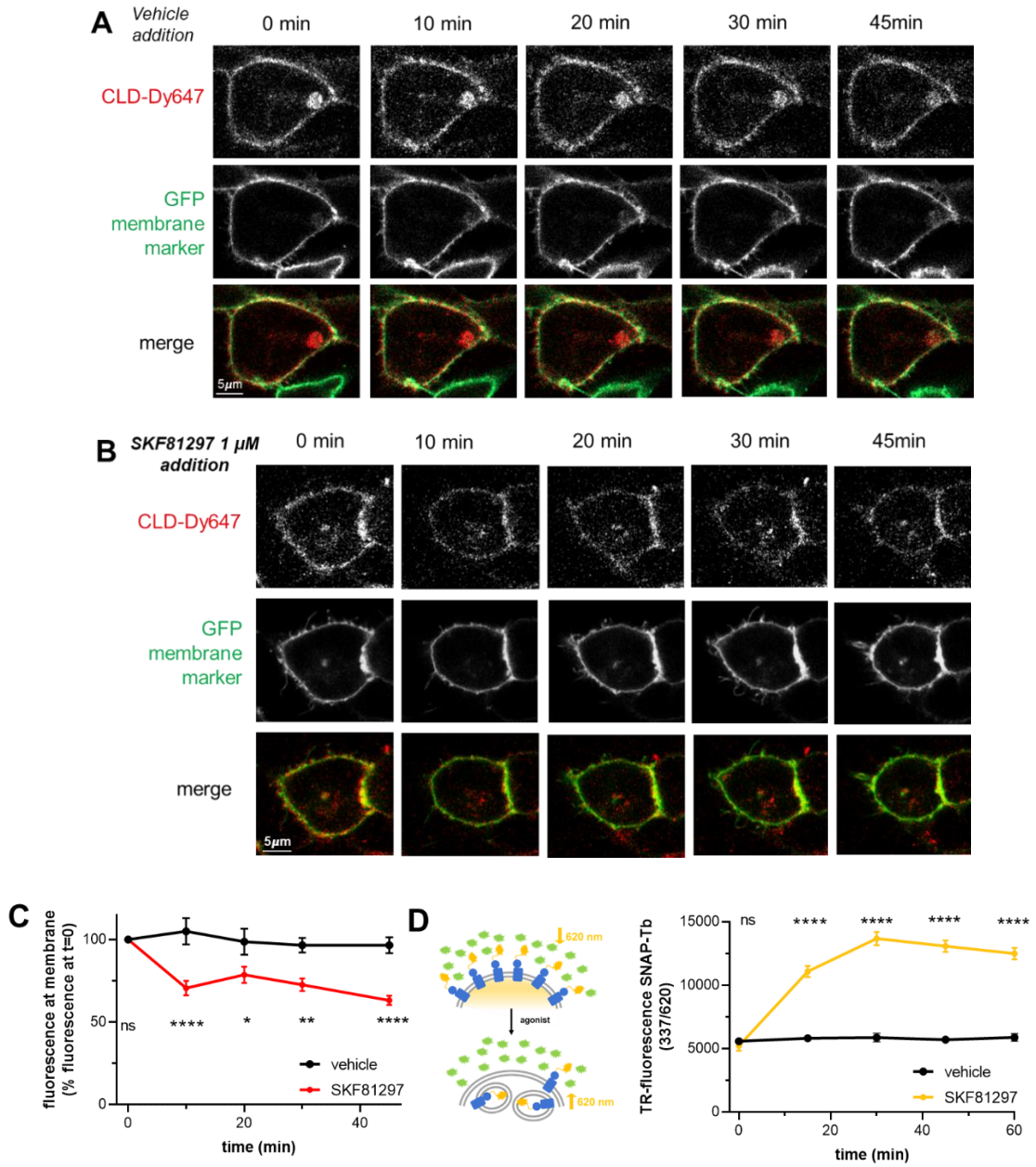


Figure S11: Agonist induced D_1 receptor trafficking. Confocal imaging of live HEK293 cells expressing a membrane marker fused to GLP and HA-D_1 labelled with the CLD probe **5h**, which were treated with vehicle (A) or SKF81297 1µM (B). The addition of the agonist SKF81297 induced a decrease of the colocalization GFP and CLD fluorescence, in contrast to the vehicle addition, that did not affect to such co-localization. (C) Evolution of the membrane fluorescence of cells expressing HA-hD_1R labelled with the CLD probe under vehicle condition, or after agonist SKF81297 stimulation. Analysis of the images of 9 cells confirmed this tendency, resulting in significant differences of fluorescence at the plasma membrane between cells treated with D_1 agonists and with vehicle. (D) Tb fluorescence of cell expressing the ST-D1R in the presence of fluorescein (quenching the extracellular Tb-fluorescence) under control condition or after addition of the agonist. The increase of Lumi4Tb fluorescence was in line with the decrease of 680-nm fluorescence (C). Data analysis show significant differences between agonist and vehicle treated cells at time using two-way ANOVA with Sidak correction for multiple comparisons (ns non-significant, * 0.05<p<0.01,** 0.01<p<0.001, **** p<0.0001)

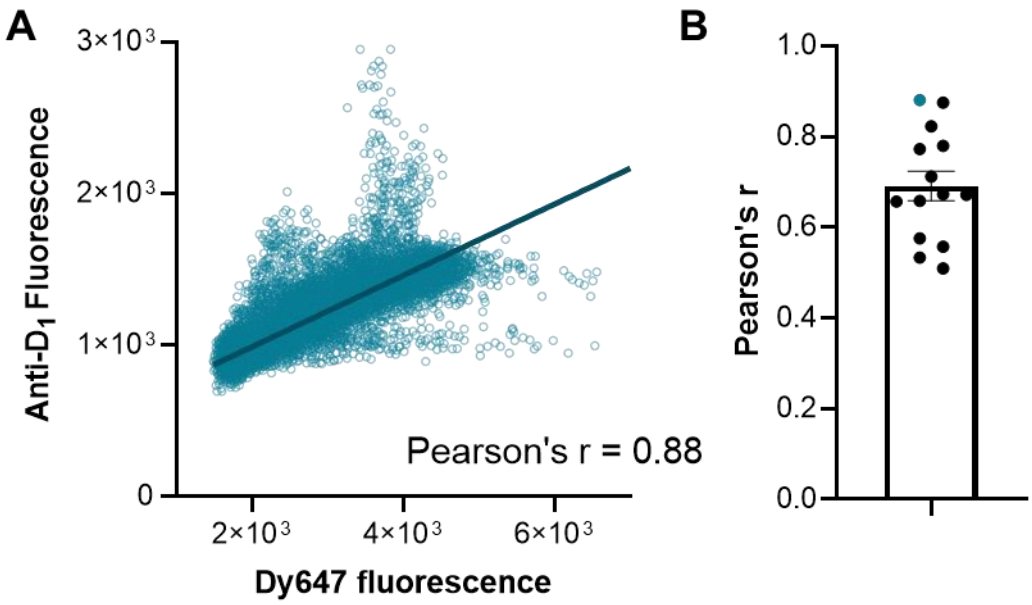


Figure S12: Quantification of the fluorescence colocalisation observed after labelling SH-SY5Y cells differentiated to a neuron-like cells with 5h and immunostaining. (A) *JACoP analysis* on the illustrated cell *in Figure 4E. ImageJ according* to Bolte & Cordelières.[4] (B) Representation of Person's rs calculated on 14 imaged cells (r = 0.69 ± 0.03).Value observed in panel A (0.88) is marked in turquoise.

2. Supplementary Tables

Table S1: Labelling of F-ST-D1 and HA-D1 receptor with CLD probe 5a-i. Values of normalised specific labelling corresponding to saturation labelling curves of CLD probe **5a-i** using F-ST-D1 with the ST labelled with Lumi4Tb and reading HTRF between SNAP-Lumi4Tb and CLD-Dy647 and HA-D1 reading Dy647 fluorescence . A minimum of two independent experiments were performed in duplicate and points correspond to means with SEM as error bars.

F-ST-D_1 Specific labelling (fluorescence 640/680)

	Probe 5a		Probe 5b		Probe 5c		Probe 5d		Probe 5e	
Conc	**Mean**	**± SEM**	**Mean**	**± SEM**	**Mean**	**± SEM**	**Mean**	**± SEM**	**Mean**	**± SEM**
10 µM	1.48	± 0.24	1.09	± 0.17	0.48	± 0.74	0.27	± 0.20	1.02	± 0.57
5 µM	0.95	± 0.24	1.23	± 0.39	0.43	± 0.34	0.24	± 0.07	1.28	± 0.20
3 µM	0.50	± 0.02	0.59	± 0.13	0.35	± 0.07	0.11	± 0.04	0.77	± 0.14
1.5 µM	0.28	± 0.04	0.46	± 0.15	0.17	± 0.02	0.05	± 0.03	0.41	± 0.03
0.5 µM	0.14	± 0.06	0.23	± 0.08	0.12	± 0.05	0.04	± 0.04	0.20	± 0.00
0.25 µM	0.09	± 0.00	0.11	± 0.04	0.07	± 0.07	0.05	± 0.03	0.12	± 0.03
0 µM	-0.02	± 0.02	0.07	± 0.02	0.00	± 0.04	-0.01	± 0.02	-0.02	± 0.04

	Probe 5f		Probe 5g		Probe 5h		Probe 5i	
conc	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
10 µM	0.72	± 0.11	0.22	± 0.07	1.00	± 0.45	0.12	± 0.10
5 µM	1.04	± 0.33	0.26	± 0.03	0.99	± 0.40	0.09	± 0.14
3 µM	0.63	± 0.21	0.14	± 0.04	0.70	± 0.28	-0.01	± 0.11
1.5 µM	0.33	± 0.11	0.11	± 0.06	0.45	± 0.05	0.06	± 0.02
0.5 µM	0.17	± 0.10	0.09	± 0.00	0.22	± 0.02	0.02	± 0.03
0.25 µM	0.06	± 0.03	0.03	± 0.01	0.15	± 0.04	0.04	± 0.02
0 µM	0.00	0.01	0.03	± 0.00	-0.02	0.03	0.03	0.01

HA-D1 Specific labelling (HTRF 665/620)

	Probe 5a		Probe 5b		Probe 5c		Probe 5d		Probe 5e	
conc	**Mean**	**± SEM**	**Mean**	**± SEM**	**Mean**	**± SEM**	**Mean**	**± SEM**	**Mean**	**± SEM**
10 µM	4.88	± 2.16	7.67	± 2.79	1.67	± 0.24	0.76	± 0.39	6.20	± 1.31
5 µM	3.20	± 2.59	3.73	± 2.18	0.80	± 0.07	0.38	± 0.12	2.17	± 0.31
3 µM	0.86	± 0.60	1.95	± 0.52	0.15	± 0.10	0.24	± 0.05	0.83	± 0.07
1.5 µM	0.33	± 0.11	0.72	± 0.18	0.20	± 0.18	0.15	± 0.08	0.32	± 0.07
0.5 µM	0.17	± 0.08	0.35	± 0.15	0.18	± 0.09	0.02	± 0.02	0.12	± 0.02
0.25 µM	0.06	± 0.02	0.09	± 0.05	0.03	± 0.00	0.03	± 0.01	0.08	± 0.00
0 µM	0.01	± 0.01	-0.01	± 0.02	0.00	± 0.00	-0.02	± 0.02	0.00	± 0.00

	Probe 5f		Probe 5g		Probe 5h		Probe 5i	
conc	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
10 µM	2.45	± 0.48	1.67	± 0.81	2.14	± 0.54	2.39	± 0.34
5 µM	1.00	± 0.33	0.86	± 0.24	1.60	± 0.31	0.53	± 0.03
3 µM	0.58	± 0.08	0.22	± 0.10	1.15	± 0.49	0.21	± 0.04
1.5 µM	0.17	± 0.04	0.22	± 0.05	0.27	± 0.15	0.07	± 0.08
0.5 µM	0.08	± 0.05	0.10	± 0.01	0.07	± 0.01	-0.04	± 0.02
0.25 µM	0.05	± 0.01	-0.01	± 0.01	0.04	± 0.01	0.00	± 0.02
0 µM	0.02	± 0.02	0.02	± 0.02	0.00	± 0.01	0.01	± 0.03

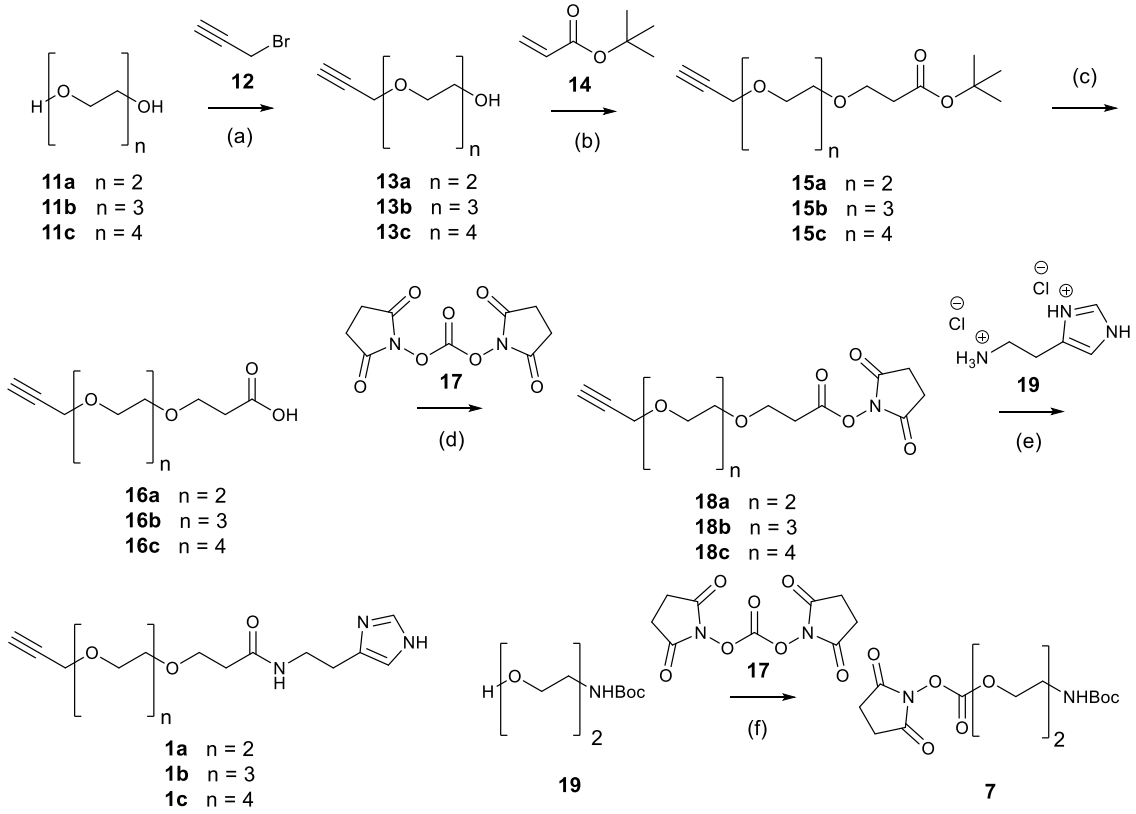
Table S2: Functional data of antagonists SKF833566, 4a-c and 6a-b in hD_1 mutants. Functional affinities (pK_i) extracted from concentration-response curves using HTRF® cAMP Gs Dynamic assay in competition with 300 nm of SKF38393. IC_{50}-values were transformed to K_i constants with Cheng-Prussoff equation. Mutations K165R, K167R and K165R-K167R of human D_1 receptor do not affect to the functional properties of the antagonists SKF83566, **4a-c** and **6a-b.** pKi values are the mean of minimum 3 independent replicates with the corresponding standard error of the mean (SEM).

	F-ST-hD$_1$ K165R		***F-ST- hD$_1$ K167R***		***F-ST- hD$_1$ K165R K167R***	
	pKi	***± SEM***	***pKi***	***± SEM***	***pKi***	***± SEM***
SKF83566	8.61	± 0.13	8.87	± 0.06	8.74	± 0.13
4a	7.34	± 0.09	7.49	± 0.16	7.22	± 0.15
4b	7.33	± 0.10	7.91	± 0.15	7.38	± 0.12
4c	7.14	± 0.18	7.54	± 0.08	7.27	± 0.24
6a	7.12	± 0.11	7.66	± 0.19	7.27	± 0.05
6b	6.43	± 0.13	6.66	± 0.18	6.26	± 0.03

	HA- hD$_1$ K165R		***HA- hD$_1$ K167R***		***HA- hD$_1$ K165R pK167R***	
	pKi	***± SEM***	***pKi***	***± SEM***	***pKi***	***± SEM***
SKF83566	8.73	± 0.08	8.99	± 0.07	8.73	± 0.12
4a	7.78	± 0.11	7.78	± 0.16	7.69	± 0.12
4b	7.69	± 0.14	7.71	± 0.16	7.40	± 0.16
4c	7.45	± 0.23	7.40	± 0.21	7.18	± 0.15
6a	7.39	± 0.11	7.70	± 0.28	7.25	± 0.16
6b	6.41	± 0.14	6.41	± 0.18	6.15	± 0.15

3. Supplementary Note S1: Synthetic strategies

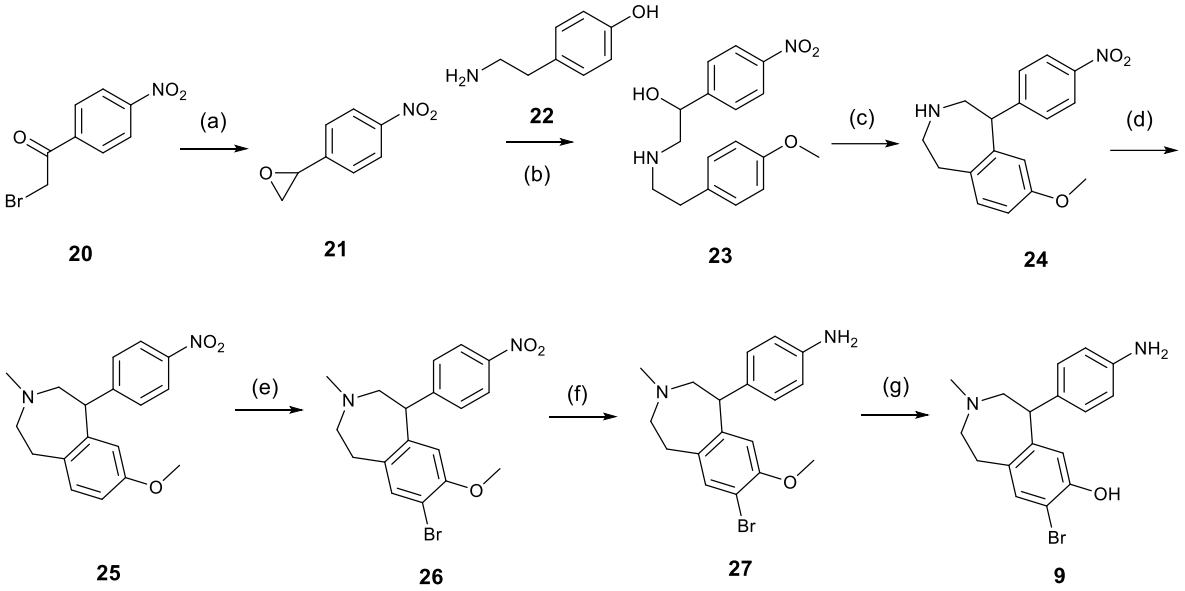
The synthesis of the alkyne clickable imidazole linkers**1a-c** is depicted in Scheme S1 and started with polyethylene glycols with different lengths (di-, tri- and tetra-ethylene glycol **11a-c**). First, a nucleophilic substitution of one of alcohol groups of each glycol on propargyl bromide (**12**) gave the alkyl alcohols **13a-c** with high yields (65-86%). Following Michael addition of alcohols **9a-c** to *tert*-butyl acrylate (**14**) with sodium catalysis yielded the *tert*-butyl esters **15a-c** (52-63%). The tert-butyl groups from the esters **15a-c** were cleaved with TFA/DCM to give the corresponding carboxylic acid **16a-c,** which were activated with disuccinimidyl carbonate (DSC, **17**) to give the NHS-esters **18a-c** with moderate yields. The activated NHS-esters **18a-c** were used to acylate histamine hydrochloride (**19**) and give the advanced intermediate imidazole clickable linkers **1a-c** with moderate to high yields (42-78%). Additionally, the NHS-activated carbonate **7** was prepared from the Boc-protected amino-alcohol **19** with DSC (**13**) with moderate yields (39%).



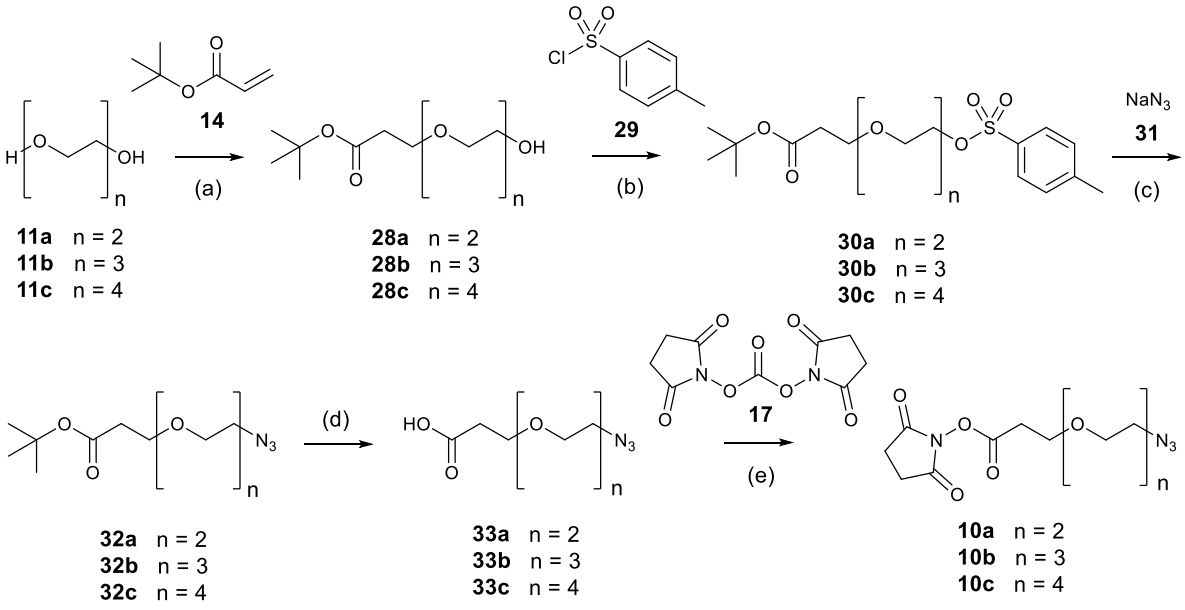
Scheme S1: Synthesis of alkylyl-PEGs-imidazole 1a-c and NHS-activated carbonate 7. Reagents and conditions: (a) (i)**11** 2.0 eq, *t*-BuOK 1.0 eq, 0 °C, 30 min (ii) **12** 1.0eq, THF, rt, 16 h, 65-86%; (b) (i) Na cat., THF, rt, 1-4 h, (ii) **14**, 1.0 eq, rt, 20 h, (iii) HCl (0.1 eq), 52-63%; (c) TFA/DCM, rt, 2-4h, 66-100%; (d) **17** 1.5eq, Pyr 3 eq, MeCN, rt, 16 h, 60%; (e) **19** 1.5eq, TEA 3.0 eq, MeCN, 50 °C, 20-50h, 42-78%; (f) **19** 1.0 eq, **17** 2.0eq, TEA 2.0 eq, DMF, 40 °C, 1.5 h, 39%;

To synthesise azido clickable D_1 antagonists **4a-c**, the common intermediate SKF83566-NH_2 (**9**) was synthesised before, using a similar synthetic strategy as reported before,[5] which is depicted is Scheme S3. The reduction of the bromoketone **20** and following cyclisation gave the epoxide **21** in good yield (97%), which was opened with the amine **22** to give the secondary amine **23** in good yields (64%). Following cyclisation with polyphosphoric acid gave the benzoazepine **24** in moderate yields (46%) and was methylated with Eschweiler–Clarke reaction with moderate to good yields (77%) to give the heterocyclic compound **25**. The *ortho* position in respect to the methoxide of compound **25** was selectively brominated with good conversion (96%) to give compound **26**. Since purification of compound **26** proved to be difficult, we opted to continue the synthesis without further purification. The nitro group from compound **26** was reduced with

hydrazine and nickel-Raney to give aniline **27** with very good yields (97%) and the methoxy group could be de-protected to give the advanced intermediate phenol compound **9** in good yields (75%).



Scheme S2: Synthesis of SKF83566-NH_2 (19). Reagents and conditions: (a) (i) $NaHBH_4$ 1.1 eq, MeOH, 0-5 °C, 2 h, (ii) K_2CO_3 1.1 eq, 0-5°C, 36 h, 97%; (b) **21** 1.0 eq, THF, 66°C, 29h, rt, 55h, 64%; (c) polyphosphoric acid (PPA), 110 °C, 3h, 46%; (d) CH_2O/HCO_2H 3 eq, H_2O, 80°C, 5.5h, 77%; (e) Br_2 4.5eq, AcOH, 60°C, 4 h, rt, 16h, 96%; (f) Ni-Ra 1 eq, N_2H_4 5 eq, EtOH, 50 °C, 2h, 97%; (g) BBr_3 25eq, DCM, -78 °C, 1 h, rt, 3 h 75%.



Scheme S3: Synthesis of SKF83566-PEGs-N_3 (4a-c). Reagents and conditions: (a) (i) **7** 2.9 eq, Na 0.07eq, THF, rt, 1-4 h, (ii) **10**, 1.0 eq, rt, 20 h (iii) HCl 0.1 eq, 30-97%; (b) **29** 1.0 eq, TEA 2.0 eq, DCM, rt, 20h, 78-83%; (c) **31** 3.3-3.5eq, 50 °C, 20 h, 99-100%; (d) TFA/DCM, rt, 2-4h, 74-97%; (e) **17** 1.5 eq MeCN, rt, 16 h, 26-72%;

The synthesis of the azido-NHS-ester PEG-linkers **10a-c** is depicted in Scheme S4 and started with a Michael addition of one of the alcohols polyethylene glycols with different lengths (di-, tri- and tetraethylene glycol, **11a-c**) on *tert*-butyl acrylate (**14**) to give the *tert*-butyl esters **28a-c** with moderate to good yields (90-97%). The resting alcohol of compounds **28a-c** was tosylated to give compounds **30a-c** with good yields (78-83%) and the following nucleophilic attack with sodium azide (**31**) gave the azido tert-butyl esters **32a-c** with high yields. Following *tert*-butyl

cleavage with TFA yielded the corresponding carboxylic acid **33a-c** (74-97%), which was subsequently activated with DSC (**13**) to give the compounds **10a-c** with moderate to good yields (26-72%).

Synthetic procedures

General procedure A for synthesis of compounds **13a**, **13b** and **13c**

The corresponding glycol **11** (2.0 eq) was added slowly to a suspension of *t*-BuOK (1.0 eq) in THF (0.72/0.37M) at 0-5 °C and it was stirred at 0-5 °C for 30 min. The resulting suspension was warmed up to rt and a solution of propargyl bromide (**12**, 1.0 eq) in THF (1.8 M) was added drop wise. The resulting brown suspension was stirred at rt for 16 h. The reaction mixture was diluted with THF, filtered through celite and the solvent was removed in vacuo to give a brown oil. The brown oil was purified with a silica flash column (Hex/EtOAc 1:4 to 1:9) for compound **13a** and **13b** to give the corresponding product **13** as a yellow oil. For compound **13c**, the crude oil was re-dissolved with brine (50 mL) and AcOEt (50 mL), the layers were separated and the aqueous layer was extracted with AcOEt (2 x 25 mL). Combined the organic layers were wash with brine (35 mL), dried over anh. $MgSO_4$, filtered and concentrated to give an amber oil that corresponded to the product **13c**.

General procedure B for synthesis of compounds **15a**, **15b**, **15c**, **28a**, **28b** and **28c**

Sodium was added (0.03-0.08 eq) to a colourless solution of the alcohol **13a-c** or glycol **11a-c** (1.0-2.9 eq) in THF (0.66-1.9 M) and it was stirred at rt until the sodium was dissolved *tert*-butyl acrylate (**14**, 1.0 eq) was added dropwise and stirred at rt for 20 h. HCl 1M (0.08 eq) was added and the solvents were removed in vacuo. The resulting reside was diluted with brine (50 mL) and was extracted with EtOAc (3 x 25 mL). Combined the organic layers and wash with brine (1 x 20 mL). The organic was dried over $MgSO_4$, filtered and concentrated to give a colourless oil that was optionally purified with a silica flash column (EtOAc/Hex) to give the *tert*-butyl esters **15a**, **15b**, **15c**, **28a**, **28b** or **29c** as colourless oils.

General procedure C for synthesis of compounds **16a**, **16b**, **16c**, **33a**, **33b** and **33c**

Trifluoroacetic acid (TFA) was added (0.18-0.80 mL/mmol) to a solution of the corresponding *tert*-butyl ester **15a-c** or **32a-c** (1.0 eq) in DCM (0.6-1.8 M) and the resulting solution was stirred at rt for 2-4 h. The solvent was removed and dried in vacuo to get rid of TFA traces and give the carboxylic acids **16a-c** or **32a-c**.

General procedure D for synthesis of compounds **18a**, **18b**, **18c**, **10a**, **10b** and **10c**

Pyridine (3 eq) was added on a solution corresponding carboxylic acids **16a-c** or **33a-c** (1.0 eq) and DSC (**17**, 1.5 eq) in MeCN (0.18-0.50 M) and it was stirred at rt for 16h.The reaction mixture was diluted with DCM (70 ml) and washed with 1M HCl (2x20 mL), sat Na_2CO_3 (1x20 mL), sat $NaHCO_3$ (1x20 mL), and sat. NaCl (1x 30 mL), was dried over anh. Na_2SO_4, filtered and concentrated in vacuo to give the corresponding activated carboxylic acids **18a-c** or **10a-c** as an oil that was used without further purification.

General procedure E for synthesis of compounds **8a**, **8b** and **8c**

Anh. pyridine (2.0 eq) was added to a solution of the imidazole clickable linker **1a-c** and the NHS-activated carbonate **7** (1.0 eq) in anh. DMF (80-180 mM) and the resulting solution was stirred at 25°C at 1400 rpm for 3 h. The solvent was removed in vacuo and the residue was re-

dissolved in DCM (20 ml) and washed with water (10 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give a yellow oil. The residue was purified with automated silica flash column chromatography (Grace 40 g column, DCM/MeOH 3-10%) to obtain the compounds **8a-c** as colourless oils.

General procedure F for synthesis of compounds **3a**, **3b**, and **3c**

A solution of the Boc-protected amine **8a-c** (1.0 eq) in TFA (10000 eq) was stirred at 25°C at 1200 rpm for 0.5 h. The solvent was removed in vacuo and the traces of TFA were co-evaporated with toluene. The resulting oil containing the ammonium salt was dissolved in anh. DMSO (1.3-3.1 mM) and Dy647-NHS (**2**) 2.6-11.0 mM in DMSO (1.1eq) and anh. DIPEA 0.2 M in DMSO (5.0eq) were added. The resulting solution was stirred at 25°C at 1400 rpm for 1 h and was directly purified with preparative HPLC (TEAAc25mM pH = 6-7 MeCN) 20 mL/min 19 minutes 15-45% MeCN to give the expected products **3a-c** as blue residues.

General procedure G for synthesis of compounds **30a**, **30b** and **30c**

A solution of triethylamine (0.84 ml, 6.0 mmol) and tosyl chloride (**29**, 1.14 g, 6.0 mmol) in DCM (8 mL) was added to a solution of the alcohol **28a-c** (0.70 g, 3.0mmol) in DCM (8 ml) at to 0-5°C. The resulting solution was stirred at 0-5°C for20 h and it partitioned in DCM / sat. NaCl 1:1 (150 mL). The aqueous layer was back extracted with DCM (2 x 25 mL) and the combined the organic layers were washed with sat. NaCl (3 x 50 mL), dried over anh. Na_2SO_4, filtered and concentrated in vacuo to give a colourless oil. The residue was purified with automated silica flash column chromatography (SNAP KP-Sil-HS 25g column, Hex/EtOAc) to give the expected products **30a-c** as colourless oils.

General procedure H for synthesis of compounds **32a**, **32b** and **32c**.

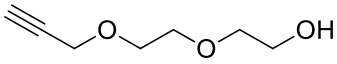
A white suspension of the tosylate **30a-c** (1.0 eq) and sodium azide (**31**, 3.3-3.5 eq) in DMF (0.9M) was stirred at 50°C for 20h. The reaction mixture was diluted with EtOAc (75 ml) and was washed with sat. NaCl (4x25 mL), dried over $MgSO_4$, and concentrated in vacuo to give the expected azide product **32a-c** as colourless oils.

General procedure I for synthesis of compounds **4a**, **4b** and **4c**.

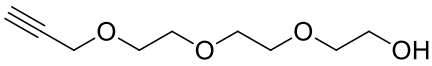
A suspension of the NHS-activated ester **10a-c** (1.0 eq) and SKF83566-NH_2 (**9**, 1.0 eq) in dry THF (50 mM) or a mixture of Dry THF/DCM 1M (36 mM) to give an orange suspension that was stirred at 60°C for 20 to 48h and the solvent was removed in vacuo. The residue was purified with automated silica/C18 flash column chromatography to obtain the compounds **4a-c** as colourless or pale-yellow oils.

General procedure J for synthesis of compounds **6a** and **6b**.

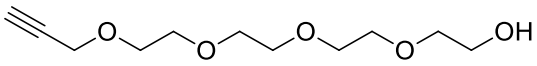
The alkylyl imidazole linker **1b-c** 0.25-0.50 M in THF (1.0 eq) and the azido ligand **4b-c** 0.25-0.50 M in THF (1.0 eq) were added to a suspension copper(I) oxide (0.25 eq), benzoic acid (4 eq) in water (31-63 mM) to give a reddish suspension, which was stirred at rt for 0.5-1.5 h. The resulting mixture was filtered and the solvents were removed in vacuo. After purification the compounds **6a** and **6b** were obtained as colourless oils.



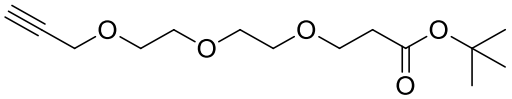
2-(2-(prop-2-yn-1-yloxy)ethoxy)ethanol (13a): The general procedure A was used with the glycol **11a** (1.37 mL, 14.4 mmol) and *t*-BuOK (0.82 g, 7.3mmol) in THF (20 mL) and propargyl bromide (**12**, 0.80 mL, 7.2 mmol) in THF (4 ml) to obtain the alcohol **13a** with a 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (d, *J* = 2.4 Hz, 2H), 3.75 – 3.65 (m, 6H), 3.59 (dd, *J* = 5.2, 3.9 Hz, 3H), 2.55 (s, 1H), 2.44 (t, *J* = 2.4 Hz, 1H).^{1}H NMR matches previously described in literature.[6]



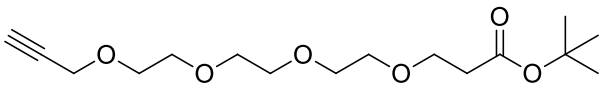
2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethanol (13b): The general procedure A was used with the glycol **11b** (1.92 mL, 14.4 mmol) and *t*-BuOK (0.82 g, 7.3mmol) in THF (20 mL) and propargyl bromide (**12**, 0.80 mL, 7.2 mmol) in THF (4 ml) to obtain the alcohol **13a** with a 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (d, *J* = 2.4 Hz, 3H), 3.74 – 3.61 (m, 10H), 3.58 (dd, *J* = 5.3, 3.8 Hz, 2H), 2.70 (br, 1H), 2.42 (t, *J* = 2.4 Hz, 1H).^{1}H NMR matches previously described in literature.[7]



3,6,9,12-tetraoxapentadec-14-yn-1-ol (13c): The general procedure A was used with the glycol **11c** (2.8 g, 14.4 mmol) and *t*-BuOK (0.82 g, 7.3mmol) in THF (20 mL) and propargyl bromide (**12**, 0.80 mL, 7.2 mmol) in THF (4 ml) to obtain the alcohol **13c** with a 86% yield.^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (d, *J* = 2.2 Hz, 2H), 3.76 – 3.63 (m, 14H), 3.60 (dd, *J* = 5.2, 3.8 Hz, 2H), 2.72 (br, 1H), 2.43 (t, *J* = 2.4 Hz, 1H).^{1}H NMR matches previously described in literature.[7]

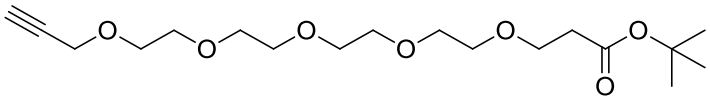


***Tert*-butyl 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoate (15a):** The general procedure B was used with the alcohol **13a** (360 mg, 2.5 mmol),sodium (1.7 mg, 0.08 mmol),*tert*-butyl acrylate **14** (0.36 mL, 2.5 mmol), HCl (0.25 mL)and THF (3.8 ml)and the purification was performed with a silica flash column (EtOAc/Hex 35:65) to obtain the tert-butyl ester **15a** (355 mg, 52%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (d, *J* = 2.4 Hz, 2H), 3.71 – 3.62 (m, 6H), 3.61 – 3.58 (m, 4H), 2.46 (t, *J* = 6.6 Hz, 2H), 1.46 – 1.37 (m, 9H), 2.40 (t, *J* = 2.4 Hz, 1H), 1.41 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 80.6, 79.7, 74.6, 70.6, 70.5, 70.4, 69.2, 67.0, 58.5, 36.3, 28.2. LC/MS (*LC1*) TIC RT = 3.48 min $[M+Na]^+$ (m/z) 295

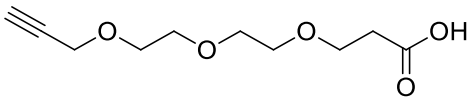


***Tert*-butyl 4,7,10,13-tetraoxahexadec-15-yn-1-oate (15b):** The general procedure B was used with the alcohol **13b** (471 mg, 2.5 mmol), sodium (1.7 mg, 0.08 mmol),*tert*-butyl acrylate **14** (0.36 mL, 2.5 mmol), HCl (0.25 mL)and THF (3.8 ml) and the purification was performed with a silica flash column (EtOAc/Hex 40:60) to obtain the *tert*-butyl ester **15b** with a (497 mg, 63%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (d, *J* = 2.4 Hz, 2H), 3.71 – 3.64 (m, 6H), 3.63 (s, 4H), 3.62 – 3.56 (m, 4H), 2.48 (t, *J* = 6.5 Hz, 2H), 2.41 (t, *J* = 2.4 Hz, 1H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 80.6, 79.8,

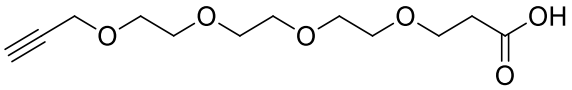
74.6, 70.7, 70.7, 70.6, 70.5, 70.5, 69.2, 67.0, 58.5, 36.4, 28.2. ^{1}H NMR matches previously described in literature.[6]



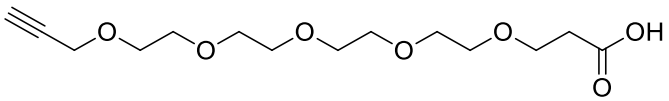
***Tert*-butyl 4,7,10,13,16-pentaoxanonadec-18-yn-1-oate (15c):** The general procedure B was used with the alcohol **13c** (581 mg, 2.5 mmol), sodium (1.7 mg, 0.08 mmol),*tert*-butyl acrylate **14** (0.36 mL, 2.5 mmol), HCl (0.25 mL)and THF (3.8 ml) and the purification was performed with a silica flash column (EtOAc/Hex 45:55) to obtain the *tert*-butyl ester **15c** with a (528 mg, 59%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (d, *J* = 2.4 Hz, 2H), 3.73 – 3.55 (m, 18H), 2.48 (t, *J* = 6.6 Hz, 2H), 2.42 (t, *J* = 2.4 Hz, 1H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 80.6, 79.8, 74.6, 70.7, 70.7, 70.7, 70.6, 70.5, 70.5, 69.2, 67.0, 58.5, 36.4, 28.2. ^{1}H NMR and ^{13}C NMR match previously described in literature.[8] LC/MS (*LC1*) TIC RT = 3.44 min $[M+Na]^+$ (m/z) 383



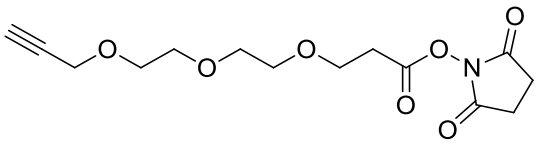
3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoic acid (16a): The general procedure C was used with the *tert*-butyl ester **15a** (1.31 g, 4.8 mmol), TFA (1.9 mL) and DCM (8 mL) to give the carboxylic acid **16a** as a yellow oil (1.11 g, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (br, 1H), 4.21 (d, *J* = 2.4 Hz, 2H), 3.78 (t, *J* = 6.2 Hz, 2H), 3.72 – 3.63 (m, 8H), 2.65 (t, *J* = 6.2 Hz, 2H), 2.43 (t, *J* = 2.5 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 175.2, 79.8, 79.7, 74.7, 74.7, 70.6, 70.6, 70.5, 69.3, 66.5, 58.6, 34.8. LC/MS (*LC1*) TIC RT = 2.49 min $[M+H]^+$ (m/z) 217.



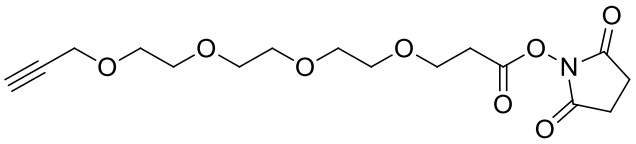
4,7,10,13-tetraoxahexadec-15-yn-1-oic acid (16b): The general procedure C was used with the *tert*-butyl ester **15b** (1.77 g, 5.6 mmol), TFA (1 mL) and DCM (3 mL) to give the carboxylic acid **16b** as a yellow oil (1.45 g, quantitative).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (d, *J* = 2.3 Hz, 2H), 3.76 (t, *J* = 6.2 Hz, 2H), 3.72 – 3.59 (m, 12H), 2.63 (t, *J* = 6.2 Hz, 2H), 2.43 (t, *J* = 2.4 Hz, 1H).^{1}H NMR matches previously described in literature.[6]



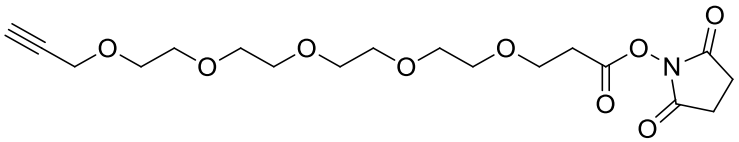
4,7,10,13,16-pentaoxanonadec-18-yn-1-oic acid (16c): The general procedure C was used with the *tert*-butyl ester **15c** (3.34 g, 9.3 mmol), TFA (3.6 mL) and DCM (15 mL) to give a tan oil, which was purified with a silica flash column (EtOAc + 0.05% AcOH) to obtain the acid **16c** as a colourless oil (1.86 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (d, *J* = 2.4 Hz, 2H), 3.78 (t, *J* = 6.0 Hz, 2H), 3.75 – 3.59 (m, 16H), 2.63 (t, *J* = 5.8 Hz, 2H), 2.43 (t, *J* = 2.4 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 79.7, 74.7, 70.8, 70.8, 70.7, 70.6, 70.6, 70.4, 70.3, 69.2, 66.7, 58.5, 35.1. LC/MS (*LC1*) TIC RT = 2.66 min $[M+H]^+$(m/z) 305.



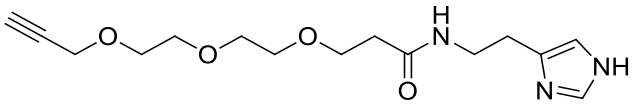
2,5-dioxopyrrolidin-1-yl 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoate (18a): The general procedure D was used with the carboxylic acid **16a** (1.15 g, 5.3mmol), DSC (**17**, 2.04 g, 8.0mmol), pyridine (1.3 mL, 16.0mmol) and MeCN (11 mL) to give the activated carboxylic acid **18b** as a tan oil (1.51 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (d, *J* = 2.4 Hz, 2H), 3.85 (t, *J* = 6.5 Hz, 2H), 3.74 – 3.63 (m, 8H), 2.91 (t, *J* = 6.5 Hz, 2H), 2.83 (br, 4H), 2.43 (t, *J* = 2.3 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.1, 166.9, 79.9, 74.6, 70.9, 70.7, 70.6, 69.3, 65.9, 58.6, 32.3, 25.7. LC/MS (*LC2*) TIC RT = 1.86 min, $[M+H]^+$(m/z) 314, $[M+NH_4]^+$ (m/z) 331, $[M+Na]^+$ (m/z) 336



2,5-dioxopyrrolidin-1-yl 4,7,10,13-tetraoxahexadec-15-yn-1-oate (18b): The general procedure D was used with the carboxylic acid **16b** (150mg, 0.58 mmol), DSC (**17**, 221 mg, 0.86 mmol), pyridine (140 µL, 1.73 mmol) and MeCN (5 mL) to give the activated carboxylic acid **18b** as a yellow oil (123mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (d, *J* = 2.5 Hz, 2H), 3.85 (t, *J* = 6.5 Hz, 2H), 3.73 – 3.61 (m, 12H), 2.90 (t, *J* = 6.5 Hz, 2H), 2.83 (br, 4H), 2.43 (t, *J* = 2.4 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 166.9, 79.8, 74.6, 70.9, 70.8, 70.8, 70.7, 70.5, 69.3, 65.9, 58.5, 32.3, 25.7. LC/MS (*LC1*) TIC RT = 2.91 min $[M+Na]^+$(m/z) 380

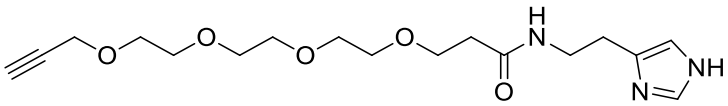


2,5-dioxocyclopentyl 4,7,10,13,16-pentaoxanonadec-18-yn-1-oate (18c): The general procedure C was used with the carboxylic acid **16a** (1.86 g, 6.1mmol), DSC (**17**, 2.34 g, 9.1mmol), pyridine (1.5 mL, 18.3mmol) and MeCN (12 mL) to give the activated carboxylic acid **18c** as a tan oil (1.52 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (d, *J* = 2.4 Hz, 2H), 3.85 (t, *J* = 6.4 Hz, 2H), 3.74 – 3.62 (m, 16H), 2.90 (t, *J* = 6.5 Hz, 2H), 2.84 (br, 4H), 2.43 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.1, 166.9, 79.8, 74.7, 70.9, 70.8, 70.8, 70.7, 70.7, 70.7, 70.6, 69.3, 65.9, 58.6, 32.3, 25.7. LC/MS (*LC2*) TIC RT = 1.95 min, $[M+H]^+$ (m/z) 402, $[M+NH_4]^+$ (m/z) 419, $[M+Na]^+$ (m/z) 424

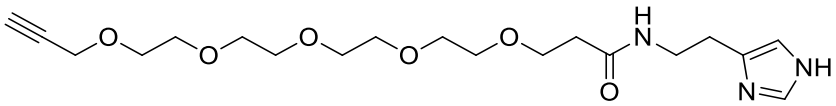


***N*-(2-(1*H*-imidazol-4-yl)ethyl)-3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanamide (1a, MCS0388):** Triethylamine(1.2 ml, 8.6mmol) was added to a colourless suspension of histamine dihydrochloride (**19**, 594mg, 3.2mmol) and activated carboxylic acid **18a** (674mg, 2.2mmol) in DMF (11 mL) to give a colourless suspension. The resulting mixture was stirred at 50 °C for 60h. The reaction mixture was diluted in DCM (100 mL) and was extracted with sat. $NaHCO_3$(70 mL). The aqueous solution was back-extracted with DCM (5x25 mL) and the combined organic layers were concentrated in vacuo to give an orange residue. The residue was purified with

automated C18 flash column chromatography (KP-C18-HS 120 g, H_2O/MeOH) and silica flash column chromatography (EtOAc/MeOH 4:1) to obtain the imidazole clickable linker **1a** (**MCS0388**) as a pale-yellow oil (369 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.62 (s, 1H), 6.87 (s, 1H), 6.82 (t, *J* = 5.6 Hz, 1H), 4.18 (d, *J* = 2.4 Hz, 2H), 3.97 (br, 2H), 3.73 – 3.65 (m, 6H), 3.63 – 3.57 (m, 4H), 3.52 (app. q, *J* = 6.2 Hz, 2H), 2.86 (t, *J* = 6.3 Hz, 2H), 2.48 – 2.41 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.98, 134.60, 133.42, 118.52, 69.08, 67.58, 58.52, 38.96, 37.11, 26.24.COSY, HSQC. LC/MS (*LC2*) TIC RT = 0.25 min, $[M+H]^+$ (m/z) 310. HRMS $[M+H]^+$(m/z) calculated for $C_{15}H_{24}N_3O_4$310.1767, found 310.1786.

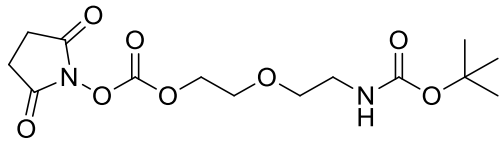


***N*-(2-(1H-imidazol-4-yl)ethyl)-4,7,10,13-tetraoxahexadec-15-yn-1-amide (1b, MCS0389):** Triethylamine (100 µl, 0.72mmol) was added to a colourless suspension of histamine dihydrochloride(**19**, 93 mg, 0.50 mmol) and activated carboxylic acid **18b** (120 mg, 0.34mmol) in DMF (3 mL) to give a colourless suspension. The resulting mixture was stirred at 50 °C for 20h and at 5 °C for 48h. The reaction mixture was diluted in EtOAc(50 mL) and was washed with sat. $NaHCO_3$ (2x30 mL), H_2O (30 mL) and sat. NaC l(30 mL), dried over $MgSO_4$, filtered and concentrated to give a residue which did not correspond to the expected product **1b**. The combined $NaHCO_3$ solutions were extracted with DCM (4X30 mL) and the solvent was removed in vacuo to give a colourless oil, which was purified with automated C18 flash column chromatography (SNAP KP-C18 12g column, water/MeOH;). to give the expected imidazole clickable linker **1b** (**MCS0389**) as a colourless oil (50 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.74 (s, 1H), 7.02 (t, *J* = 5.8 Hz, 1H), 6.90 (s, 1H), 6.69 (br, 1H), 4.16 (d, *J* = 2.2 Hz, 2H), 3.73 – 3.47 (m, 14H), 3.52 (app. q, *J* = 6.0 Hz, 2H), 2.87 (t, *J* = 6.3 Hz, 2H), 2.51 – 2.37 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 134.6, 133.4, 118.5, 79.4, 75.1, 70.7, 70.5, 70.4, 70.4, 70.3, 69.1, 67.6, 58.5, 39.0, 37.1, 26.2. COSY, HSQC, HMBC. LC/MS (*LC1*) TIC RT = 2.13 min, $[M+H]^+$(m/z) 354. HRMS $[M+H]^+$(m/z) calculated for $C_{17}H_{28}N_3O_5$ 354.2029, found 354.2027.

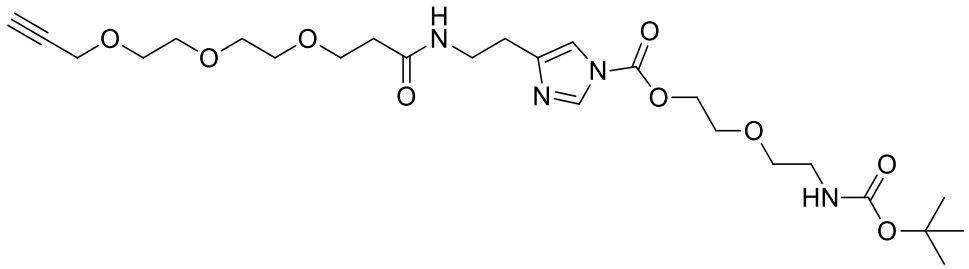


N-(2-(1H-imidazol-4-yl)ethyl)-4,7,10,13,16-pentaoxanonadec-18-yn-1-amide (1c, MCS0390): Triethylamine (1.03 ml, 7.4mmol) was added to a colourless suspension of histamine dihydrochloride(**19**, 510mg, 2.8mmol) and activated carboxylic acid **18c** (741mg, 1.8 mmol) in DMF (9 mL) to give a colourless suspension. The resulting mixture was stirred at 50 °C for 36h. The reaction mixture was diluted in DCM (100 mL) and was washed with sat. $NaHCO_3$ (2x50 mL), H_2O (50 mL) and sat. NaCl(50 mL), dried over Na_2SO_4, filtered and concentrated to give an orange oil which did not correspond to the expected product **1c**. The combined aqueous layers were concentrated and the obtained residue was stirred in chloroform (100 ml) during 50 h, filtered and concentrated in vacuo to give a tan oil that was purified with automated C18 flash column chromatography (SNAP KP-C18 120 g column, water/MeOH) to give the expected imidazole clickable linker **1c** (**MCS0390**) as a colourless oil (576 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.60 (d, *J* = 1.1 Hz, 1H), 6.89 (t, *J* = 5.6 Hz, 1H), 6.86 (d, *J* = 1.1 Hz, 1H), 4.15 (d, *J* = 2.4 Hz, 2H), 3.71 – 3.56 (m, 14H), 3.56 – 3.48 (m, 6H), 2.83 (t, *J* = 6.2 Hz, 2H), 2.47 – 2.41 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 135.1, 118.8, 104.2, 79.5, 77.5, 74.9, 70.8, 70.6, 70.6, 70.6, 70.4, 70.4, 70.4, 69.1, 67.6, 58.5, 39.1, 37.2, 26.6. COSY, HSQC. LC/MS (*LC2*) TIC RT = 0.26 min, $[M+H]^+$(m/z) 398. HRMS $[M+H]^+$(m/z)

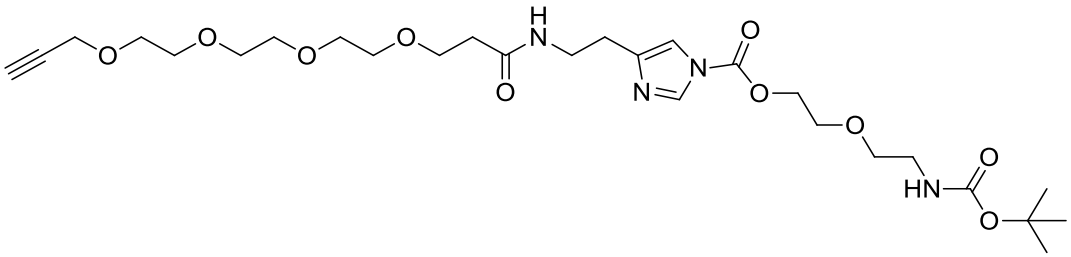
calculated for $C_{19}H_{32}N_3O_6$ 398.2291, found 398.2287; $[M+Na]^+$(m/z) calculated for $C_{19}H_{31}N_3O_6Na$ 40.2111, found 420.2108.



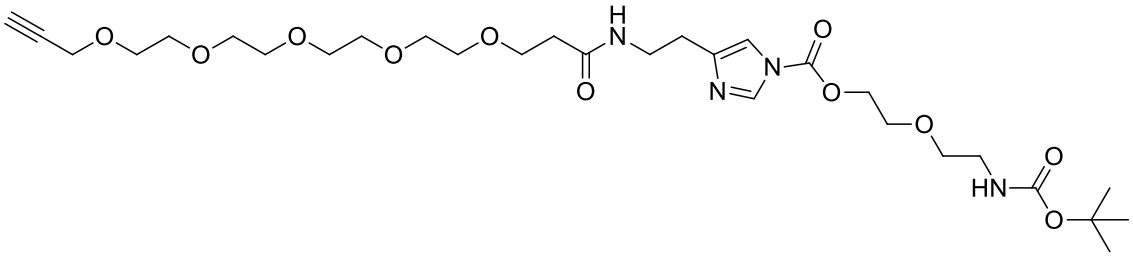
***Tert*-butyl (2-(2-((((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)oxy)ethoxy)ethyl)carbamate (7):** Triethylamine (1.4 ml, 9.7mmol) was added to a solution of DSC (**17**, 2.5 g, 9.7mmol) in MeCN (35 ml). Subsequently *tert*-butyl (2-(2-hydroxyethoxy)ethyl)carbamate (**19**, 0.94 ml, 4.9 mmol) was added drop wise and the resulting solution was stirred at 50 °C for 1.5 h. The solvent was removed in vacuo and the resulting residue was re-dissolved in EtOAc (100 ml) and washed with sat $NaHCO_3$ (3x30mL), dried over anh. Na_2SO_4, filtered and concentrated in vacuo to give a colourless oil. The residue was residue was purified with automated silica column chromatography (Grace 40 g column, DCM/MeOH 2-5%) to give the expected product **7** as a colourless oil (0.65 g, 39%).^{1}H NMR (400 MHz, $CDCl_3$)δ 5.03 (br, 1H), 4.48 – 4.38 (m, 2H), 3.75 – 3.67 (m, 2H), 3.52 (t, *J* = 5.1 Hz, 2H), 3.28 (q, *J* = 5.4 Hz, 2H), 2.81 (s, 4H), 1.41 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.7, 156.1, 151.7, 79.3, 70.6, 70.0, 68.2, 40.4, 28.5, 25.5. LC/MS (*LC3*) TIC RT = 2.77 min, $[M+2H\text{-}Boc]^+$ (m/z) 247.78.



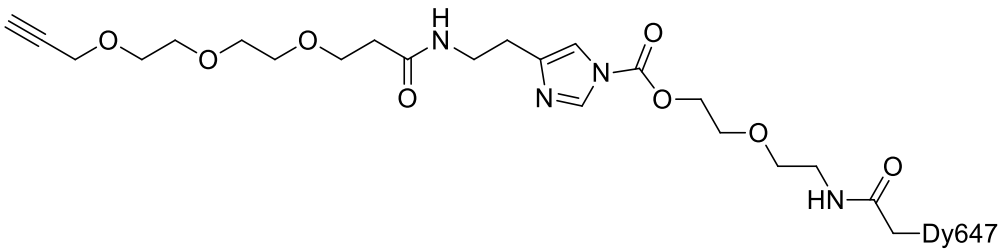
2-(2-((*tert*-butoxycarbonyl)amino)ethoxy)ethyl 4-(4-oxo-7,10,13-trioxa-3-azahexadec-15-yn-1-yl)-1*H*-imidazole-1-carboxylate (8a): The general procedure D was used with pyridine (29 µl, 0.36 mmol), the imidazole clickable linker **1a** (**MCS0388**) (42 mg, 0.12 mmol), the activated carbonic acid **7** 0.58M in DMF (309 µL, 0.18 mmol) and DMF (1.5 mL) to give the compound **8a** (19 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$)) δ 8.08 (d, *J* = 1.4 Hz, 1H), 7.22 (d, *J* = 1.3 Hz, 1H), 6.81 (br, 1H), 4.91 (br, 1H), 4.56 – 4.49 (m, 2H), 4.18 (d, *J* = 2.3 Hz, 2H), 3.83 – 3.75 (m, 2H), 3.75 – 3.63 (m, 6H), 3.61 (app. s, 4H), 3.54 (p, *J* = 6.4, 5.8 Hz, 4H), 3.31 (app. q, *J* = 5.5 Hz, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 2.50 – 2.41 (m, 3H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 156.0, 148.7, 141.9, 137.0, 113.8, 79.7, 79.6, 74.8, 70.5, 70.4, 69.2, 68.5, 67.4, 67.0, 58.5, 40.4, 38.7, 37.2, 28.5, 28.0. LC/MS (*LC3*) TIC RT = 2.71 min, $[M+H]^+$ (m/z) 540.06.



2-(2-((*tert*-butoxycarbonyl)amino)ethoxy)ethyl 4-(4-oxo-7,10,13,16-tetraoxa-3-azanonadec-18-yn-1-yl)-1*H*-imidazole-1-carboxylate (8b): The general procedure D was used with pyridine (26 µl, 0.32 mmol), the imidazole clickable linker **1b** (**MCS0389**) (33 mg, 0.11 mmol), the activated carbonic acid **7** 0.58 M in DMF (280 µL, 0.16 mmol) and DMF (1.5 mL) to give the compound **8b** (31 mg, 54%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.23 (s, 1H), 6.81 (br, 1H), 4.90 (br, 1H), 4.59 – 4.49 (m, 2H), 4.19 (dd, *J* = 2.4, 0.9 Hz, 2H), 3.82 – 3.76 (m, 2H), 3.75 – 3.59 (m, 14H), 3.59 – 3.49 (m, 4H), 3.32 (app. q, *J* = 5.5 Hz, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 2.46 (t, *J* = 5.9 Hz, 2H), 2.43 (t, *J* = 2.4 Hz, 1H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 156.1, 148.7, 142.0, 137.0, 113.8, 79.8, 79.7, 74.7, 70.7, 70.7, 70.5, 70.5, 70.5, 70.4, 69.2, 68.5, 67.4, 67.0, 58.5, 40.4, 38.7, 37.2, 28.5, 28.0. LC/MS (*LC3*) TIC RT = 2.76 min, $[M+H]^+$ (m/z) 584.09.

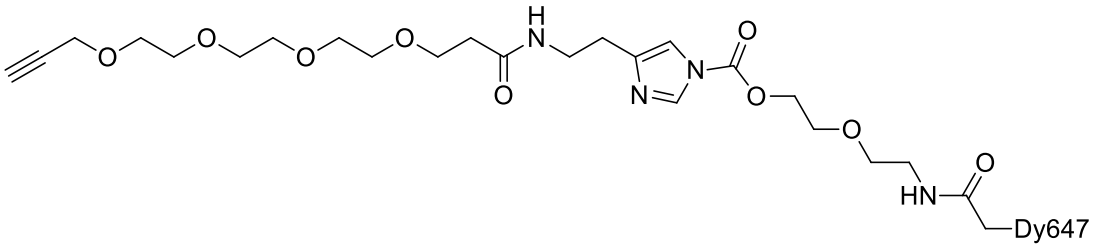


2-(2-((*tert*-butoxycarbonyl)amino)ethoxy)ethyl 4-(4-oxo-7,10,13,16,19-pentaoxa-3-azadocos-21-yn-1-yl)-1*H*-imidazole-1-carboxylate (8c): The general procedure D was used with pyridine (40 µl, 0.50 mmol), the imidazole clickable linker **1c** (**MCS0390**) (67 mg, 0.17 mmol), the activated carbonic acid **7** (85 mg, 0.25 mmol) and DMF (1.5 mL) to give the compound **8c** (84 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) 8.07 (d, J = 1.2 Hz, 1H), 7.22 (d, J = 1.3 Hz, 1H), 6.90 – 6.79 (m, 1H), 4.92 (s, 1H), 4.57 – 4.47 (m, 2H), 4.18 (d, J = 2.4 Hz, 2H), 3.84 – 3.72 (m, 2H), 3.75 – 3.56 (m, 20H), 3.60 – 3.46 (m, 4H), 3.31 (app. q, J = 5.5 Hz, 2H), 2.75 (t, J = 6.7 Hz, 2H), 2.49 – 2.38 (m, 3H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 156.1, 148.7, 141.9, 137.0, 113.8, 79.8, 79.6, 74.7, 70.7, 70.6, 70.6, 70.5, 70.5, 70.4, 70.4, 69.2, 68.5, 67.4, 67.0, 58.5, 40.4, 38.7, 37.2, 28.5, 28.0. LC/MS (*LC3*) TIC RT = 2.80 min, $[M+H]^+$ (m/z) 630.35

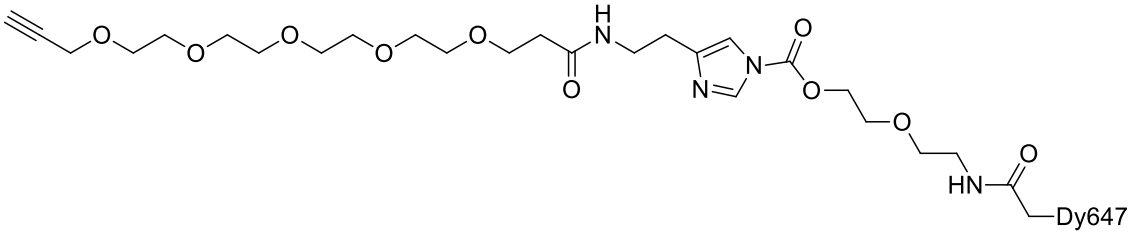


Alkyne clickable activated Dy647 3a: The general procedure F was used with the Boc-protected amine linker **18a** (0.66 mg, 1.22 µmol), TFA (941 µL, 12.2 mmol), anh. DMSO (400 µL), Dy647-NHS (**2**) in DMSO 11 mM (122 µL, 1.34 µmol) and DIPEA 200 mM (31 µL, 6.10 µmol) and preparative HPLC purification with "acc7" C18 column and mobile phase TEAOAc 25 mM pH 7MeCN 5-45%

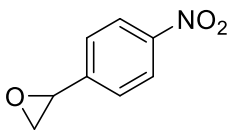
20 mL/min, 19 min runtime and TEAOAc 25 mM pH 7MeCN15-40% 20 mL/min, 19 min runtime to give the expected blue product **3a** (0.31 µmol, 25%). LC/MS (*LC3*) DAD RT = 3.38 purity 96.8% (254 nm), TIC RT = 3.39 min, $[M+H]^+$(m/z) 1066.82, $[M+2H]^{2+}$(m/z) 534.33, $[M-H]^-$(m/z) 1064.82, $[M-2H]^{2-}$(m/z) 532.06. UV-Vis λ_{max}= 648 nm.



Alkyne clickable activated Dy647 3b: The general procedure F was used with the Boc-protected amine linker **18b** (0.64 mg, 1.10 µmol), TFA (843 µL, 10.9mmol), anh. DMSO (400 µL), Dy647-NHS (**2**) in DMSO 11 mM (109 µL, 1.20 µmol) and DIPEA 200 mM (27 µL, 5.47 µmol) and preparative HPLC purification with "acc7" C18 column and mobile phase TEAOAc 25 mM pH 7 MeCN 15-40% 20 mL/min, 19 min runtime to give the expected blue product **3b** (0.23 µmol, 21%). LC/MS (*LC3*) DAD RT = 3.46 purity 95.0% (254 nm), TIC RT = 3.46 min, $[M+H]^+$ (m/z) 1111.24, $[M+2H]^{2+}$(m/z) 556.36, $[M-H]^-$(m/z) 1108.58, $[M-2H]^{2-}$(m/z) 554.37. UV-Vis λ_{max}= 648 nm.

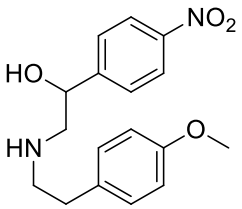


Alkyne clickable activated Dy647 3c: The general procedure F was used with the Boc-protected amine linker **18c** (0.52 mg, 0.83 µmol), TFA (637 µL, 8.3mmol), anh. DMSO (400 µL), Dy647-NHS (**2**) in DMSO 11 mM (83 µL, 0.91 µmol) and DIPEA 200 mM (21 µL, 4.14 µmol) and preparative HPLC purification with "acc7" C18 column and mobile phase TEAOAc 25 mM pH 7 MeCN15-40% 20 mL/min, 19 min runtime to give the expected blue product **3c** (0.17 µmol, 20%). LC/MS (*LC3*) DAD RT = 3.53 purity 97.9% (254 nm), TIC RT = 3.54 min, $[M+H]^+$(m/z) 1155.03, $[M+2H]^{2+}$(m/z) 578.46, $[M-H]^-$(m/z) 1153.14, $[M-2H]^{2-}$(m/z) 576.37. UV-Vis λ_{max}= 648 nm.

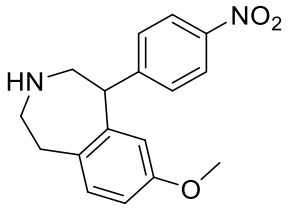


2-(4-nitrophenyl)oxirane (21, MCS0259): $NaBH_4$ (2.2 g, 57 mmol) was portion wise added to a colourless solution of 2-bromo-1-(4-nitrophenyl)ethanone (**20**, 12.5 g, 51 mmol) in MeOH (130 ml) at 0-5°C and the resulting suspension was stirred 2 h at 0-5°C. K_2CO_3 (7.4 g, 54mmol) was added portion wise and the resulting white suspension was left stirring 36 h at rt. Sat. NaCl (260 mL) was added and the suspension was extracted with Et_2O (130 mL + 3x70 mL). The organic layers were combined, dried with anh. $MgSO_4$ and concentrated in vacuo till a precipitate appeared. The precipitate was filtered and washed with cold MeOH to give a yellow solid. The filtering waters

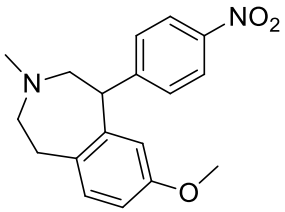
were re-crystallised, and filtered to give another batch the yellow solid. Both batches were joined to obtain a yellow solid that corresponds to the product **21** with high purity (8.3 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 3.96 (dd, *J* = 4.1, 2.5 Hz, 1H), 3.23 (dd, *J* = 5.5, 4.2 Hz, 1H), 2.78 (dd, *J* = 5.5, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.9, 145.4, 126.3, 123.9, 51.8, 51.5. LC/MS (*LC1*) TR = 2.30, 99.0% purity (254 nm), TIC RT = 3.38 min, $[M+H]^+$(m/z) 166, $[2M+H]^+$(m/z) 331. ^{1}H NMR matches that previously described in literature.[9]



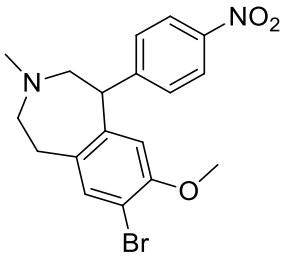
2-((4-methoxyphenethyl)amino)-1-(4-nitrophenyl)ethanol (23, MCS0260): 2-(4-methoxyphenyl)ethanamine **22** (6.0 ml, 41mmol) was added to a yellow solution of the epoxide **21** (6.7 g, 41mmol) in THF (73 ml). The resulting solution was heated to reflux for29 hours and stirred at rt for 2 d. The solvent was removed in vacuo to give a yellow solid that was digested with MTBE at reflux, cooled down to 0-5ºC for 16h. The solid was filtered and washed with cold MTBE to give compound **23** as a white solid (8.3 g, 66%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.77 (dd, *J* = 9.2, 3.6 Hz, 1H), 3.79 (s, 3H), 3.00 – 2.90 (m, 2H), 2.86 (dd, *J* = 11.7, 6.7 Hz, 1H), 2.79 – 2.73 (m, 2H), 2.63 (dd, *J* = 12.3, 9.2 Hz, 1H), 2.51 (br, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 158.4, 150.0, 147.5, 131.3, 129.7, 126.6, 123.8, 114.2, 70.5, 56.6, 55.4, 50.7, 35.4. LC/MS (*LC1*) TR = 2.82, 99.0% purity (254 nm), TIC RT = 2.90 min, $[M+H]^+$(m/z) 317.



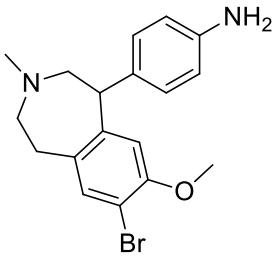
8-methoxy-1-(4-nitrophenyl)-2,3,4,5-tetrahydro-1H-benzo[d]azepine (24, MCS0261): A white suspension of 2-((4-methoxyphenethyl)amino)-1-(4-nitrophenyl)ethanol **23** (5.0 g, 15.8 mmol) in PPA (45 ml) was heated to 110 °C and mechanically stirred for 3 hours. Cold water was slowly added (100 mL) and the resulting dark suspension was basified with ammonium hydroxide solution 30% (100 mL). The suspension was extracted with DCM (150 x 3) and the joined organic layers were washed with sat. Na_2CO_3 (3x75 ml), dried over anh. Na_2SO_4, filtered, concentrated in vacuo to give a dark residue corresponding to the compound **24** that was used without further purification. (2.2 g, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.31 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.2 Hz, 1H), 6.72 (dd, *J* = 8.2, 2.6 Hz, 1H), 6.41 (d, *J* = 2.1 Hz, 1H), 4.31 (d, *J* = 6.7 Hz, 1H), 3.73 (s, 3H), 3.62 (dd, *J* = 13.7, 6.9 Hz, 1H), 3.37 (dd, *J* = 13.6, 1.8 Hz, 1H), 3.04 – 2.91 (m, 2H), 2.90 – 2.81 (m, 1H), 2.75 (ddd, *J* = 15.0, 7.9, 3.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.8, 149.6, 146.0, 142.9, 133.4, 131.2, 128.8, 123.4, 116.0, 110.7, 54.8, 53.2, 52.3, 48.1, 38.5 LC/MS (*LC1*) TR = 2.61, 98.4% purity (254 nm), TIC RT = 2.69 min, $[M+H]^+$(m/z) 299.



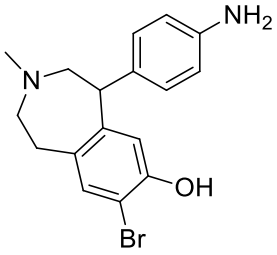
8-methoxy-3-methyl-1-(4-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (25, MCS0262): Formaldehyde (1.46 ml, 19.6 mmol) was added to a solution of 8-methoxy-1-(4-nitrophenyl)-2,3,4,5-tetrahydro-1H-benzo[d]azepine **24** (2.0 g, 6.5 mmol) in formic acid (0.78 ml, 19.6 mmol) to give a black suspension that was stirred 5.5 h at 80°C. The resulting suspension was cooled down, basified with Na_2CO_3 and extracted with DCM (200 mL + 3x20 mL). The organic layers were joined and washed with sat Na_2CO_3 (40 mL), dried over anh. Na_2SO_4 and the solvent was removed in vacuo to give the title compound as a dark oil (2.1 g). The residue was purified with automated flash column (Hex/EtOAc; SNAP KP-Sil 80 g column) to give a pale solid that corresponds to the product **25** (1.6 g, 77%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.6 Hz, 2H), 7.35 (d, *J* = 8.6 Hz, 2H), 7.08 (d, *J* = 8.2 Hz, 1H), 6.70 (dd, *J* = 8.2, 2.8 Hz, 1H), 6.30 (d, *J* = 2.3 Hz, 1H), 4.36 (d, *J* = 7.4 Hz, 1H), 3.71 (s, 3H), 3.12 (dd, *J* = 12.5, 7.7 Hz, 1H), 3.00 – 2.85 (m, 2H), 2.80 – 2.61 (m, 2H), 2.58 – 2.48 (m, 1H), 2.39 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 150.6, 146.6, 143.8, 133.6, 131.1, 129.3, 123.8, 115.7, 110.9, 61.9, 57.8, 55.3, 50.4, 48.0, 35.5. LC/MS (*LC1*) RT 2.63 min, purity 99% (254 nm), M+H$^+$ (m/z) 313.



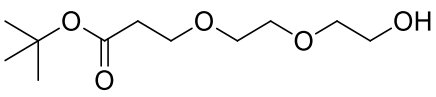
7-bromo-8-methoxy-3-methyl-1-(4-nitrophenyl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (26, MCS0263): A solution of bromine (355 µl, 6.9mmol) in AcOH (15 mL) was added dropwise to a solution of the benzoazepine **25** (1.42 g, 4,55 mmol) in AcOH(76 mL) at 60 °C during 0.5 h. The resulting orange solution was stirred at 60°C for 4 h and at rt for 16 h. Sat.$Na_2S_2O_3$(50 ml) and water (25 mL) were added and the resulting suspension was filtered to remove the insoluble salts. The solution was added over a saturated solution of Na_2CO_3(200 mL) and EtOAc(200 mL) was added. The resulting mixture was filtered and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with Na_2CO_3 (200 mL), dried $MgSO_4$, filtered and concentrated to give a dark red thick oil that corresponded to the expected product **26** and was used without further purification (1.70 g, 96%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 8.7 Hz, 2H), 7.33 (s, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 6.35 (s, 1H), 4.33 (d, *J* = 7.2 Hz, 1H), 3.72 (s, 3H), 3.19 (dd, *J* = 12.7, 7.4 Hz, 1H), 2.89 (dd, *J* = 12.6, 1.3 Hz, 1H), 2.82 (dt, *J* = 15.2, 4.7 Hz, 1H), 2.75 – 2.65 (m, 1H), 2.60 – 2.52 (m, 2H), 2.38 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.4, 150.0, 146.6, 142.8, 135.0, 134.7, 129.1, 123.8, 113.3, 109.5, 61.4, 57.4, 56.3, 50.5, 48.0, 35.0. LC/MS (*LC1*) TR = 2.93, 89.7% purity (254 nm), TIC RT = 3.01 min, [M+H]$^+$(m/z) 391, 393.



4-(7-bromo-8-methoxy-3-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-1-yl)aniline (27, MCS0264): Hydrazine hydrate (1.18 mL, 24.3mmol) was added to a dark red suspension of the nitrocompound **26** (1.90 g, 4.9mmol) and Ni-Ra (285 mg, 4.9 mg) in EtOH (97 mL) and the resulting mixture was stirred 2h at 50ºC. The mixture was filtered over Celite and then concentrated in vacuo to give a white solid which corresponded to the expected aniline **27** (1.71 mg, 97%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 6.96 (d, *J* = 8.3 Hz, 2H), 6.69 (d, *J* = 8.3 Hz, 2H), 6.31 (s, 1H), 4.22 (d, *J* = 8.5 Hz, 1H), 3.62 (s, 3H), 3.13 – 2.99 (m, 2H), 2.92 – 2.70 (m, 3H), 2.39 (s, 3H), 2.44 – 2.29 (m, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 154.1, 145.7, 145.0, 134.8, 133.9, 132.6, 129.2, 115.6, 113.0, 108.4, 63.1, 57.3, 56.2, 49.0, 47.7, 35.0. LC/MS (*LC1*) TR = 2.42, 74.2% purity (254 nm), TIC RT = 2.49 min, $[M+H]^+$(m/z) 361, 363.

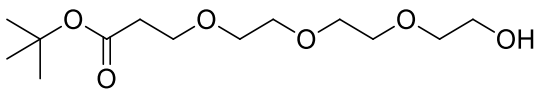


5-(4-aminophenyl)-8-bromo-3-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-ol (SKF83566-NH_2, MCS0265, 9): Boron tribromide 1M in DCM (49 mL, 49 mmol) was added dropwise into a yellow solution of the benzoazepine **27** (710 mg, 2.0mmol) in DCM (250 mL) at - 78ºC and the resulting solution was stirred at-78ºC for 1 h and was warmed up to rt and stirred for 3 h. The solution was cooled down to -78 ºC and quenched with methanol (250 mL). The solvents were removed in vacuo to give a residue that was suspended in a mixture of DCM/sat. NaHCO3 1:1 (600 mL). The layers were separated and the aqueous layer was extracted with DCM (10x50 mL). The combined organic layers were dried over anh. Na_2SO_4 and the solvents were removed under reduced pressure to give a pale solid which corresponded to the advanced intermediate product **9**, which was used without further purification.^{1}H NMR (400 MHz, CD_3OD)δ 7.20 (s, 1H), 6.93 (d, *J* = 8.3 Hz, 2H), 6.76 (d, *J* = 8.3 Hz, 2H), 6.22 (s, 1H), 4.14 (d, *J* = 9.1 Hz, 1H), 3.13 (d, *J* = 11.8 Hz, 1H), 3.10 – 3.03 (m, 1H), 3.03 – 2.93 (m, 1H), 2.80 – 2.65 (m, 2H), 2.36 (s, 3H), 2.23 (t, *J* = 11.4 Hz, 1H).).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 7.21 (s, 1H), 6.81 (d, *J* = 8.3 Hz, 2H), 6.55 (d, *J* = 8.3 Hz, 2H), 6.31 (s, 1H), 4.94 (br, 2H), 4.02 (d, *J*= 8.3 Hz, 1H), 2.97 – 2.80 (m, 2H), 2.80 – 2.60 (m, 3H), 2.25 (s, 3H), 2.16 (t, *J* = 10.8 Hz, 1H).^{13}C NMR (101 MHz, DMSO) δ 151.8, 146.8, 146.2, 133.3, 132.7, 129.9, 128.6, 116.3, 114.1, 105.5, 62.9, 56.9, 47.8, 47.1, 34.0. LC/MS (*LC1*) TR = 1.26, 91.7% purity (254 nm), TIC RT = 1.33 min, $[M+H]^+$(m/z) 347, 349.

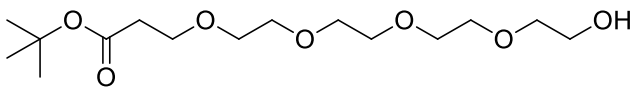


***Tert*-butyl 3-(2-(2-hydroxyethoxy)ethoxy)propanoate (28a):**The general procedure B was used

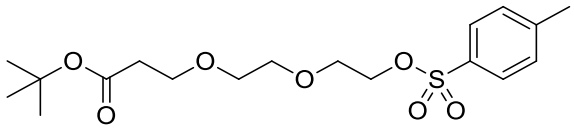
with the diethylene glycol (**11a**, 6.9mL, 73mmol), sodium (15 mg, 0.65 mmol), *tert*-butyl acrylate **14** (3.6mL, 25mmol), HCl (2.5mL, 2.5mmol)and THF (38 ml) to obtain the *tert*-butyl ester **18a** (1.8 g, 30%).^{1}H NMR (400 MHz, $CDCl_3$)δ 3.73 – 3.67 (m, 4H), 3.65 – 3.55 (m, 6H), 2.68 (br, 1H), 2.49 (t, *J* = 6.3 Hz, 2H), 1.42 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 80.8, 72.6, 70.5, 70.4, 66.9, 61.8, 36.3, 28.2. ^{1}H NMR and ^{13}C NMR match previously described in literature.[10]



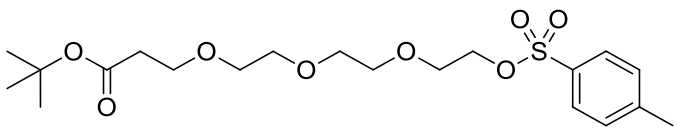
***Tert*-butyl 3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)propanoate (28b):** The general procedure B was used with diethylene glycol (**11b**, 9.7 mL, 73mmol), sodium (15 mg, 0.65 mmol),*tert*-butyl acrylate **14** (3.6mL, 25mmol), HCl (2.5 mL, 2.5 mmol)and THF (38 ml) to obtain the *tert*-butyl ester **28b** (6.7 g, 96%).^{1}H NMR (400 MHz, $CDCl_3$)δ 3.68 – 3.61 (m, 4H), 3.61 – 3.58 (m, 4H), 3.58 – 3.50 (m, 6H), 2.80 (br, 1H), 2.43 (t, J = 6.6 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 80.6, 72.6, 70.6, 70.5, 70.4, 70.4, 66.9, 61.7, 36.2, 28.1. ^{1}H NMR and ^{13}C NMR match previously described in literature.[11]



***Tert*-butyl 1-hydroxy-3,6,9,12-tetraoxapentadecan-15-oate (28c):** The general procedure B was used with tetraethylene glycol (**11c**, 14.1 g, 73mmol), sodium (15 mg, 0.65mmol),*tert*-butyl acrylate **14** (3.6mL, 25mmol), HCl (2 mL, 2 mmol)and THF (38 ml) to obtain the *tert*-butyl ester **28c** (7.8 g, 97%).^{1}H NMR (400 MHz, $CDCl_3$)δ 3.69 – 3.64 (m, 4H), 3.63 – 3.54 (m, 14H), 2.82 (br, 1H), 2.45 (t, *J*= 6.6 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 80.5, 72.6, 70.6, 70.6, 70.5, 70.4, 70.4, 66.9, 61.7, 36.3, 28.1. ^{1}H NMR and ^{13}C NMR match previously described in literature.[10]

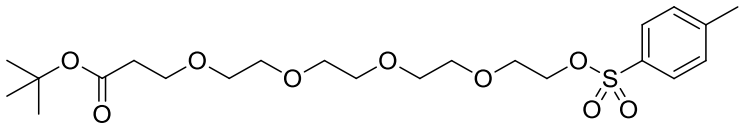


***Tert*-butyl 3-(2-(2-(tosyloxy)ethoxy)ethoxy)propanoate (30a):**The general procedure G was used with triethylamine(0.84 ml, 6.0 mmol) and tosyl chloride (**29**, 1.14 g, 6.0 mmol) in DCM (3 mL) and the alcohol **28a** (0.70 g, 3.0mmol) in DCM (8 ml) to give the expected product **39a** as a colourless oil (0.97 g, 83%).^{1}H NMR (400 MHz, $CDCl_3$)δ 7.72 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 4.12 – 4.04 (m, 2H), 3.64 – 3.56 (m, 4H), 3.51 – 3.44 (m, 4H), 2.41 (t, *J* = 6.5 Hz, 2H), 2.38 (s, 3H), 1.37 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$)δ 170.7, 144.7, 132.9, 129.8, 127.9, 80.4, 70.5, 70.2, 69.2, 68.6, 66.8, 36.2, 28.0, 21.5. LC/MS (*LC2*) TIC RT = 2.99 min $[M+Na]^+$(m/z) 411,$[M+NH_4]^+$(m/z) 406, [M-*t*Bu+2H]$^+$(m/z) 331.

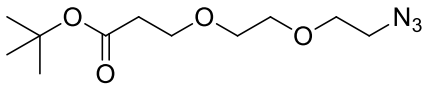


***Tert*-butyl 3-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)propanoate (30b):** The general procedure G was used with triethylamine(0.84 ml, 6.0 mmol) and tosyl chloride (**29**, 1.14 g, 6.0 mmol) in DCM

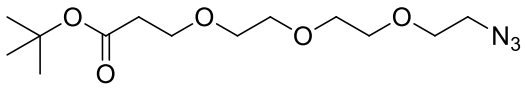
(3 mL) and the alcohol **28b** (0.84 g, 3.0mmol) in DCM (8 ml) to give the expected product **30b** as a colourless oil (1.04 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.74 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 4.14 – 4.04 (m, 2H), 3.67 – 3.60 (m, 4H), 3.53 (app. s, 4H), 3.52 (app. s, 4H), 2.43 (t, *J* = 6.5 Hz, 2H), 2.39 (s, 3H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 144.8, 133.0, 129.8, 128.0, 80.5, 70.7, 70.5, 70.5, 70.3, 69.3, 68.7, 66.9, 36.3, 28.1, 21.6. ^{1}H NMR and ^{13}C NMR match previously described in literature. [12]



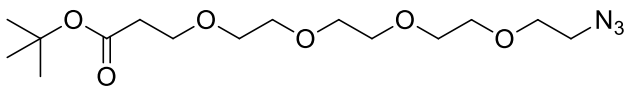
***Tert*-butyl 1-(tosyloxy)-3,6,9,12-tetraoxapentadecan-15-oate (30c):** The general procedure G was used with triethylamine(0.84 ml, 6.0 mmol) and tosyl chloride (**29**, 1.14 g, 6.0 mmol) in DCM (3 mL) and the alcohol **28c** (0.97 g, 3.0mmol) in DCM (8 ml) to give the expected product **30c** as a colourless oil (1.14 g, 79%).^{1}H NMR (400 MHz, $CDCl_3$)δ 7.78 (d, *J* = 8.2 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 4.18 – 4.11 (m, 2H), 3.73 – 3.63 (m, 4H), 3.65 – 3.57 (m, 8H), 3.57 (app. s, 4H), 2.48 (t, *J* = 6.6 Hz, 2H), 2.43 (s, 3H), 1.43 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$)δ 170.8, 144.8, 133.0, 129.8, 127.9, 80.4, 70.7, 70.6, 70.5, 70.5, 70.4, 70.3, 69.2, 68.6, 66.8, 36.2, 28.1, 21.6. LC/MS (thermo) TIC RT = 2.99 min $[M+Na]^+$ (m/z) 499, $[M+NH_4]^+$(m/z) 494, [M-*t*Bu+2H]$^+$(m/z) 421,



***Tert*-butyl 3-(2-(2-azidoethoxy)ethoxy)propanoate (32a):** The general procedure H was used with the tosylate **30a** (0.87 g, 2.3mmol) and sodium azide (**31**, 0.51 g, 7.9mmol) and DMF (2.6 ml) to give the expected azide product **32a** (0.58 g, 99%).^{1}H NMR (400 MHz, $CDCl_3$)δ 3.70 (t, *J* = 6.5 Hz, 2H), 3.67 – 3.63 (m, 2H), 3.63 – 3.56 (m, 4H), 3.36 (t, *J* = 5.1 Hz, 2H), 2.52 – 2.43 (m, 2H), 1.42 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$)δ 171.0, 80.6, 70.7, 70.5, 70.1, 67.0, 50.8, 36.4, 28.2. LC/MS (*LC1*) TIC RT = 3.66 min $[M+Na]^+$(m/z) 282, [M-*t*Bu+Na]$^+$(m/z) 226, [M-*t*Bu+2H]$^+$(m/z) 204,

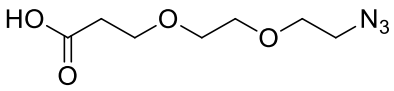


***Tert*-butyl 3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)propanoate (32b):** The general procedure H was used with the tosylate **30b** (0.96 g, 2.3mmol) and sodium azide (**31**, 0.49 g, 7.5mmol) and DMF (2.5 ml) to give the expected azide product **32b** (0.67 g, 99%).^{1}H NMR (400 MHz, $CDCl_3$)δ 3.68 (t, *J* = 6.6 Hz, 2H), 3.66 – 3.62 (m, 6H), 3.62 – 3.54 (m, 4H), 3.35 (t, *J* = 5.1 Hz, 2H), 2.46 (t, *J* = 6.6 Hz, 2H), 1.41 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$)δ 170.9, 80.5, 70.8, 70.7, 70.6, 70.4, 70.1, 66.9, 50.7, 36.3, 28.1. LC/MS (*LC1*) TIC RT = 3.64 min $[M+Na]^+$(m/z) 326, [M-*t*Bu+Na]$^+$(m/z) 270, [M-*t*Bu+2H]$^+$(m/z) 248.

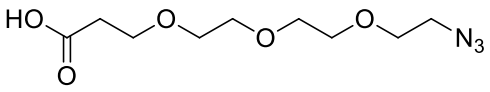


***Tert*-butyl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate (32c):**The general procedure H was used with the tosylate **30c** (0.94 g, 2.0mmol) and sodium azide (**31** 0.45 g, 6.9mmol) and DMF (2.2

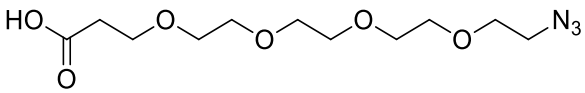
ml) to give the expected azide product **32c** (0.68 g, quantitative).^{1}H NMR (400 MHz, $CDCl_3$)δ 3.67 (t, *J* = 6.7 Hz, 2H), 3.66 – 3.61 (m, 10H), 3.61 – 3.55 (m, 4H), 3.35 (t, *J* = 5.1 Hz, 2H), 2.51 – 2.42 (m, 2H), 1.41 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$)δ 170.9, 80.5, 70.7, 70.7, 70.7, 70.7, 70.5, 70.4, 70.1, 66.9, 50.7, 36.3, 28.1. LC/MS (*LC1*) TIC RT = 3.62 min $[M+Na]^+$(m/z) 370, $[M+NH_4]^+$(m/z) 365, [M-*t*Bu+2H]$^+$(m/z) 292.



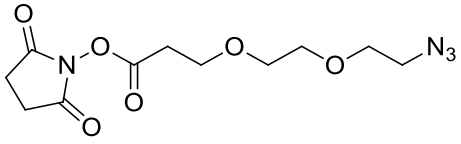
3-(2-(2-azidoethoxy)ethoxy)propanoic acid (33a): The general procedure C was used with the *tert*-butyl ester **32a** (4.1 g, 16 mmol), TFA (6.1 mL) and DCM (27 mL) to give a tan oil, which was purified twice with a silica flash column (DCM/MeOH4:1 and AcOEt/MeOH 19:1) to obtain the carboxylic acid **33a** as a yellow oil (2.6 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$)δ 10.25 (br, 1H), 3.76 (t, *J* = 6.3 Hz, 2H), 3.68 – 3.61 (m, 6H), 3.36 (t, *J* = 5.1 Hz, 2H), 2.63 (t, *J* = 6.2 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$δ 175.9, 70.6, 70.6, 70.2, 66.5, 50.8, 34.8. LC/MS (*LC2*) TIC RT = 1.50 min $[M+Na]^+$(m/z) 226.



3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)propanoic acid (33b): The general procedure C was used with the *tert*-butylester **32b** (0.64 g, 2.1mmol), TFA (1.6mL) and DCM (4.8mL) to give the carboxylic acid **33b** as an oil (0.39 g, 74%). δ 3.77 (t, *J* = 6.3 Hz, 2H), 3.70 – 3.62 (m, 10H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.64 (t, *J* = 6.3 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$)δ 176.3, 70.8, 70.8, 70.6, 70.6, 70.2, 66.4, 50.8, 34.9. LC/MS (*LC1*) TIC RT = 2.60 min $[M-H]^-$(m/z) 246,$[M+Na]^+$(m/z) 270.

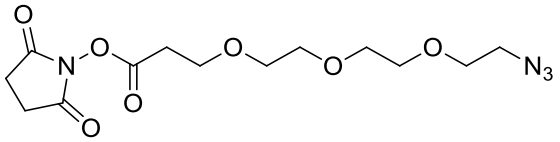


1-azido-3,6,9,12-tetraoxapentadecan-15-oic acid (33c): The general procedure C was used with the *tert*-butyl ester **32c** (4.1 g, 16 mmol), TFA (4.6 mL) and DCM (20 mL) to give a tan oil, which was purified twice with a silica flash column (DCM/MeOH9:1 and AcOEt/MeOH 4:1) to obtain the carboxylic acid **33c** as a yellow oil (3.3 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42 (br, 2H), 3.77 (t, *J* = 6.0 Hz, 2H), 3.71 – 3.62 (m, 14H), 3.40 (t, *J* = 5.1 Hz, 2H), 2.63 (t, *J* = 6.0 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 70.7, 70.7, 70.7, 70.6, 70.5, 70.3, 70.1, 66.5, 50.8, 34.9. LC/MS (*LC2*) TIC RT = 1.73 min $[M-H]^-$ (m/z) 290.

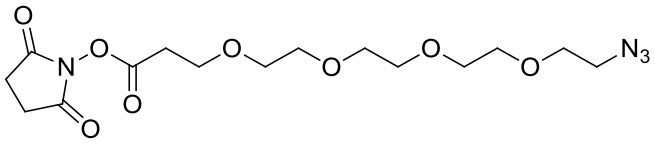


2,5-dioxopyrrolidin-1-yl 3-(2-(2-azidoethoxy)ethoxy)propanoate (10a): The general procedure D was used with the carboxylic acid **33a** (2.6 g, 13 mmol), DSC (**17**, 4.9 g, 19 mmol), pyridine (3.1 mL, 38 mmol) and MeCN (26 mL) to give the activated carboxylic acid **10a** as a yellow oil (1.0 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (t, *J* = 6.3 Hz, 2H), 3.72 – 3.61 (m, 6H), 3.39 (t, *J* = 5.1 Hz,

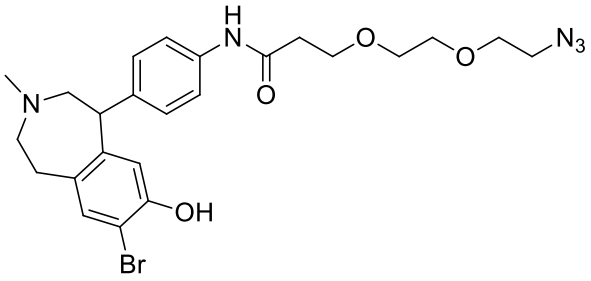
2H), 2.90 (t, *J* = 6.3 Hz, 2H), 2.84 (br, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.1, 166.9, 71.0, 70.7, 70.2, 65.9, 50.9, 32.4, 25.7. LC/MS (*LC2*) TIC RT = 1.92 min, $[M+H]^+$(m/z) 301, $[M+NH_4]^+$(m/z) 318, $[M+Na]^+$ (m/z) 323.



2,5-dioxopyrrolidin-1-yl 3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)propanoate (10b): The general procedure D was used with the carboxylic acid **10b**(228mg, 0.92 mmol), DSC (**17**, 354 mg, 1.38 mmol), pyridine (224 µL, 2.77 mmol) and MeCN (5 mL) to give the activated carboxylic acid **10b** as a yellow oil (228 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (t, *J* = 6.4 Hz, 2H), 3.71 – 3.63 (m, 10H), 3.39 (t, *J* = 5.1 Hz, 2H), 2.90 (t, *J* = 6.4 Hz, 2H), 2.83 (br, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 166.8, 70.8, 70.8, 70.7, 70.7, 70.1, 65.8, 50.8, 32.2, 25.7.

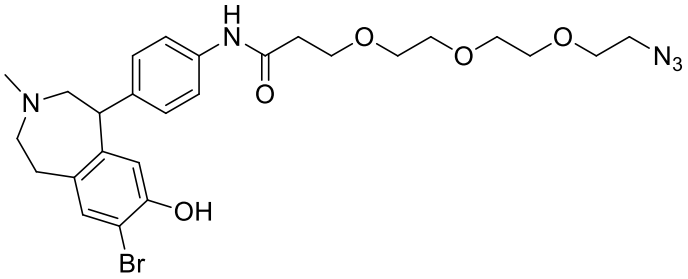


2,5-dioxopyrrolidin-1-yl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate (10c): The general procedure D was used with the carboxylic acid **33c** (3.3 g, 11 mmol), DSC (**17**, 4.2 g, 16 mmol), pyridine (2.6 mL, 33 mmol) and MeCN (22 mL) to give the activated carboxylic acid **10c** as a yellow oil (2.0 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (t, *J* = 6.5 Hz, 2H), 3.71 – 3.63 (m, 14H), 3.39 (t, *J* = 5.1 Hz, 2H), 2.90 (t, *J* = 6.5 Hz, 2H), 2.83 (br, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 166.9, 70.9, 70.9, 70.8, 70.8, 70.8, 70.7, 70.2, 65.9, 50.8, 32.3, 25.7. LC/MS (*LC2*) TIC RT = 2.02 min, $[M+H]^+$(m/z) 389, $[M+NH_4]^+$ (m/z) 406, $[M+Na]^+$ (m/z) 411.

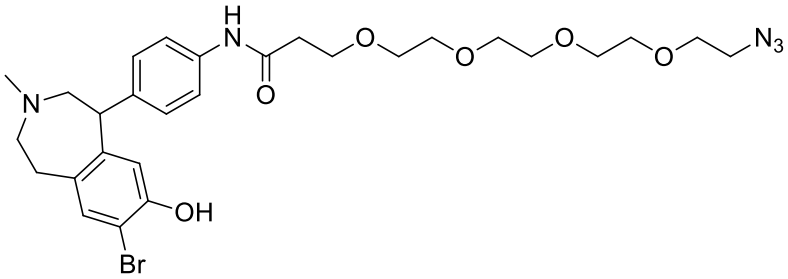


3-(2-(2-azidoethoxy)ethoxy)-N-(4-(7-bromo-8-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)phenyl)propanamide (4a, MSC0391): The general procedure I was used with activated azido-ester **10a** (44 mg, 0.14 mmol) and SKF83566-NH_2 (**9,** 49 mg, 0.14 mmol) in dry THF/DCM 1:1 (4 ml) to give a residue that was purified with automated flash silica column chromatography (SNAP KP-Sil-HS 10 g column, DCM/MeOH 5-10%) and with automated flash C18 column chromatography (SNAP KP-C18-HS 12 g column, H_2O/MeCN+ 0.05% HCO_2H) compound **4a** (**MCS0391**) as a colourless oil (15.5 mg, 21%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.1 Hz, 2H), 7.19 (s, 1H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.10 (s, 1H), 4.36 (d, *J* = 9.3 Hz, 1H), 3.80 (t, *J* = 5.8 Hz, 2H), 3.68 – 3.55 (m, 6H), 3.31 (t, 3H), 3.23 – 3.18 (m, 2H), 2.89 (t, *J* = 10.8 Hz, 1H), 2.74 (dd, *J* = 15.6, 6.5 Hz, 1H), 2.60 (t, *J* = 5.8 Hz, 2H), 2.50 (s, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 152.1, 144.4,

137.7, 136.7, 133.3, 132.3, 128.8, 121.2, 116.2, 107.1, 70.3, 70.3, 70.0, 67.2, 62.3, 56.9, 50.6, 46.8, 46.5, 37.6, 33.2. COSY, HSQC, HMBC. LC/MS (*LC1*) TR =2.82 min, 97.2% purity (254 nm), TIC RT = 2.89 min $[M+H]^+$(m/z) 532, 534. HRMS $[M+H]^+$(m/z) calculated for $C_{24}H_{31}N_5O_4Br$ 532.1554, found 532.1557.

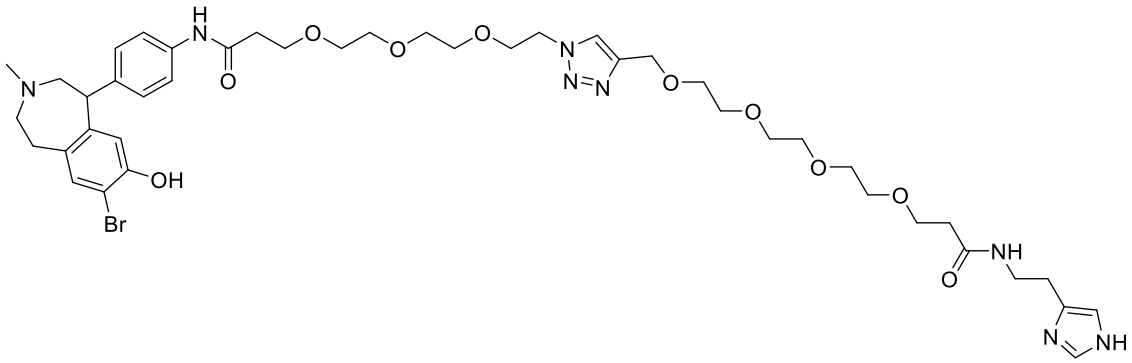


3-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-*N*-(4-(7-bromo-8-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-1-yl)phenyl)propanamide (4b, MCS0392): The general procedure I was used with activated azido-ester **10b** (179 mg, 0.52 mmol) and SKF83566-NH_2 **9** (181 g, 0.52 mmol) in dry THF (10 ml) to give compound **4b** (**MCS0392**) as a colourless oil (179 mg, 60%).^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (br, 1H), 8.38 (br, 1H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.21 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 2H), 6.14 (s, 1H), 4.41 (d, *J* = 9.5 Hz, 1H), 3.77 (t, *J* = 5.8 Hz, 2H), 3.68 – 3.56 (m, 10H), 3.41 – 3.27 (m, 5H), 2.93 (dd, *J* = 12.6, 9.3 Hz, 1H), 2.76 (dd, *J* = 15.8, 6.4 Hz, 1H), 2.59 (t, *J* = 5.7 Hz, 2H), 2.54 (d, *J* = 7.1 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 152.3, 144.2, 137.4, 136.6, 133.3, 131.8, 128.8, 120.7, 116.5, 107.6, 70.7, 70.6, 70.5, 70.4, 70.1, 67.1, 61.7, 56.4, 50.7, 46.0, 45.7, 37.8, 32.4. COSY, HSQC, HMBC.^{1}H NMR (400 MHz, DMSO) δ 9.96 (s, 1H), 9.79 (br, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.26 (s, 1H), 7.10 (d, *J* = 8.5 Hz, 2H), 6.25 (s, 1H), 4.21 (d, *J* = 8.0 Hz, 1H), 3.70 (t, *J* = 6.1 Hz, 2H), 3.57 (t, *J* = 4.4 Hz, 2H), 3.53 (s, 8H), 3.37 (t, *J* = 4.9 Hz, 2H), 3.01 – 2.87 (m, 3H), 2.80 (br, 1H), 2.74 – 2.65 (m, 1H), 2.55 (t, *J* = 6.1 Hz, 2H), 2.34 (br, 4H).^{13}C NMR (101 MHz, DMSO) δ 169.1, 152.0, 145.0, 137.5, 137.0, 133.0, 130.7, 128.4, 119.2, 116.3, 105.9, 69.8, 69.7, 69.7, 69.6, 69.2, 66.7, 61.8, 56.7, 50.0, 47.5, 46.8, 37.1, 33.4. COSY, HSQC, HMBC. LC/MS (*LC1*) TR = 2.62 min, 99.7% purity (254 nm), TIC RT = 2.68 min $[M+H]^+$ (m/z) 576, 578. HRMS $[M+H]^+$(m/z) calculated for $C_{26}H_{35}N_5O_5Br$576.1822, found 576.1812.

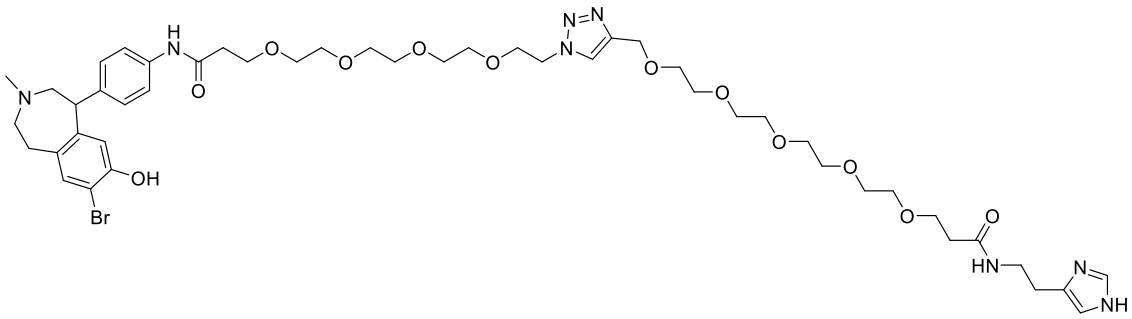


1-azido-N-(4-(7-bromo-8-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-benzo[d]azepin-1-yl)phenyl)-3,6,9,12-tetraoxapentadecan-15-amide (4c, MCS0393): The general procedure I was used with the NHS-activated azido-ester **10c** (55 mg, 0.14 mmol) and SKF83566-NH_2 **9** (50 mg, 0.14 mmol) in dry THF/DCM 1:1 (4 ml) to give a residue that was purified with automated flash silica column chromatography (SNAP KP-Sil-HS 10 g column, DCM/MeOH 5-10%) and with automated flash C18 column chromatography (SNAP KP-C18-HS 12 g column, H_2O/MeCN + 0.05% HCO_2H) compound **4c**(**MCS0393**) as a colourless oil (25 mg, 28%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.2 Hz, 2H), 7.16 (s, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.06 (s, 1H), 4.32 (d, *J* = 9.2 Hz, 1H), 3.75 (t, *J* = 5.7 Hz, 2H), 3.62 – 3.52 (m, 14H), 3.34 – 3.19 (m, 4H), 3.19 – 3.12 (m, 1H), 2.86 (dd, *J* = 11.7, 9.8 Hz, 1H), 2.70 (dd, *J* = 15.6, 6.3 Hz, 1H), 2.56 (t, *J* = 5.7 Hz, 2H), 2.47 (s, 3H), 2.41 (t, *J* = 11.8 Hz, 1H).^{13}C NMR

(101 MHz, $CDCl_3$) δ 170.9, 152.1, 144.3, 137.5, 136.9, 133.3, 132.1, 128.7, 121.1, 116.2, 107.0, 70.5, 70.4, 70.4, 70.2, 69.9, 67.1, 62.2, 56.8, 50.6, 46.6, 46.4, 37.6, 33.0.v. COSY, HSQC, HMBC. LC/MS (*LC1*) TR =2.88 min, 99.2% purity (254 nm), TIC RT = 1.96 min $[M+H]^+$(m/z) 620, 622. HRMS $[M+H]^+$(m/z) calculated for $C_{28}H_{39}N_5O_6Br$ 620.2084, found 620.2078.



***N*-(2-(1H-imidazol-4-yl)ethyl)-1-(1-(2-(2-(2-(3-((4-(7-bromo-8-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-1-yl)phenyl)amino)-3-oxopropoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)-2,5,8,11-tetraoxatetradecan-14-amide (6a, MCS0408):** The general procedure J was used with copper (I)oxide (1.07 mg, 7.5 µmol), benzoic acid (15 mg, 120 µmol), the alkylyl imidazole linker **1b** (**MCS0389**) 0.5 M in THF (60 µL, 30 µmol), the azido ligand **4b** (**MCS0392**) 0.5 M in THF (60 µL, 30 µmol) and water (480 µL) to give a residue, which was purified automated flash C18 column chromatography (SNAP KP-C18-HS 12 g column, H_2O/MeOH) to give the expected compound **6a** (**MCS0408**) as a colourless oil (7.1 mg, 26%). ^{1}H NMR (400 MHz, DMSO) δ 9.95 (s, 1H), 8.14 (br, 1H), 8.03 (s, 1H), 7.88 (t, J = 5.6 Hz, 1H), 7.58 (d, J = 8.2 Hz, 2H), 7.26 (s, 1H), 7.10 (d, J = 8.2 Hz, 2H), 6.79 (br, J = 9.5 Hz, 1H), 6.25 (s, 1H), 4.57 – 4.42 (m, 4H), 4.21 (d, J = 8.1 Hz, 1H), 3.79 (t, J = 5.2 Hz, 2H), 3.69 (t, J = 6.2 Hz, 2H), 3.57 (t, J = 6.5 Hz, 2H), 3.57 – 3.42 (m, 20H), 3.25 (app. q, J = 6.8, 6.6, 6.1 Hz, 2H), 3.01 – 2.89 (m, 3H), 2.82 (br, 1H), 2.74 – 2.65 (m, 2H), 2.62 (app. t, J = 7.4 Hz, 2H), 2.54 (t, J = 6.1 Hz, 2H), 2.35 (s, 3H), 2.34 – 2.31 (m, 1H), 2.28 (t, J = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) from HMBC δ 163.5 (CH), 133.5 (CH), 128.8 (CH), 124.7 (CH), 119.6 (CH), 116.8 (CH), 70.1 (CH_2), 67.3 (CH_2), 69.5 (CH_2), 69.1 (CH_2), 67.1 (CH_2), 63.9 (CH_2), 62.3 (CH_2), 57.1 (CH_2), 49.7 (CH_2), 47.9 (CH), 47.2 (CH_3), 39.1 (CH_2), 37.6 (CH_2), 36.6 (CH_2), 33.9 (CH_2), 33.7 (CH_2), 27.4 (CH_2). LC/MS TR = 2.54 min, 95.8% purity (254 nm), TIC RT = min $[M+H]^+$ (m/z) 929, 931, $[M+2H]^{2+}$ (m/z) 465, 466 HRMS $[M+H]^+$(m/z) calculated for $C_{43}H_{62}N_8O_{10}Br$ 929.3772, found 929.3748.



***N*-(2-(1*H*-imidazol-4-yl)ethyl)-1-(1-(15-((4-(7-bromo-8-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-1-yl)phenyl)amino)-15-oxo-3,6,9,12-tetraoxapentadecyl)-1H-1,2,3-triazol-4-yl)-2,5,8,11,14-pentaoxaheptadecan-17-amide**

(9b, MCS0462): The general procedure J was used with copper (I) oxide (0.36 mg, 2.5 µmol), benzoic acid (4.9 mg, 40 µmol), the alkylyl imidazole linker **1c** (**MCS0390**) 0.25 M in THF (40 µL, 10 µmol), the azido ligand **4c** (**MCS0393**) 0.25 M in THF (40 µL, 10 µmol) and water (320 µL) to give a

residue, which was purified automated flash C18 column chromatography (SNAP KP-C18-HS 12 g column, H_2O/MeCN + 0.05 HCO2H) to give the expected compound **6b** (**MCS0462**) as a colourless oil (8 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 7.76 (s, 1H), 7.50 (d, J = 7.9 Hz, 2H), 7.33 (br, 1H), 7.23 (s, 1H), 7.02 (d, J = 7.9 Hz, 2H), 6.72 (s, 1H), 6.19 (s, 1H), 4.58 (s, 2H), 4.46 (t, J = 5.1 Hz, 2H), 4.28 (d, J = 9.2 Hz, 1H), 3.86 (t, J = 5.6 Hz, 2H), 3.81 (t, J = 5.1 Hz, 2H), 3.71 – 3.49 (m, 32H), 3.42 (s, 2H), 3.22 (t, J = 11.8 Hz, 2H), 3.11 (q, J = 6.7 Hz, 1H), 2.80 – 2.69 (m, 3H), 2.68 (t, J = 5.6 Hz, 2H), 2.49 – 2.37 (m, 5H), 2.32 (t, J = 11.6 Hz, 1H). ^{1}H NMR (400 MHz, DMSO) δ 9.96 (s, 1H), 9.83 (br, 1H), 8.04 (s, 1H), 7.89 (t, J = 5.9 Hz, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.27 (s, 1H), 7.10 (d, J = 8.3 Hz, 2H), 6.80 (br, 1H), 6.25 (s, 1H), 4.54 – 4.46 (m, 4H), 4.23 (d, J = 7.8 Hz, 1H), 3.79 (t, J = 5.2 Hz, 2H), 3.69 (t, J = 6.2 Hz, 2H), 3.57 (t, J = 6.4 Hz, 3H), 3.54 – 3.45 (m, 28H), 3.25 (q, J = 6.9 Hz, 3H), 3.17 (d, J = 5.0 Hz, 1H), 3.07 – 2.85 (m, 3H), 2.61 (t, J = 7.4 Hz, 3H), 2.54 (t, J = 6.3 Hz, 2H), 2.38 (br, 3H), 2.31 – 2.24 (m, 3H). There was not enough quantity to obtain signals for a ^{13}C NMR. LC/MS TR = 2.38 min, 98.3% purity (254 nm), TIC RT = 2.69 min $[M+H]^+$ (m/z) 576, 578. LC/MS TR = 2.38 min, 99.4% purity (254 nm), TIC RT = 2.43 min $[M+2H]^{2+}$ (m/z) 509, 510. HRMS $[M+Na]^+$(m/z) calculated for $C_{47}H_{70}N_8O_{12}$ Br 1039.41111, found 1039.4091.

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