

Supplementary Material

A universal cannabinoid CB1 and CB2 receptor TR-FRET kinetic ligand binding assay.

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Supplementary Methods

Receptor concentration estimation using a terbium cryptate standard curve

A standard curve with known terbium cryptate concentrations (5 pM to 5 nM) was used to quantify receptor numbers in the membrane preparation. Terbium cryptate was serially diluted in assay buffer and added to the 384 well Optiplates. HTRF measurements were made precisely as described in 'Common procedures used in TR-FRET experiments' in the main text.

Confocal microscopy

HEK293T cells were seeded into 6-well plates containing a poly-D-lysine coated glass coverslip. 24h later cells were transfected with 1 μ g/well of pcDNA4/TO plasmids encoding either full length human CB1R or truncated (CB1R₉₁₋₄₇₂) receptors containing an N-terminal SNAP tag, using PEI transfection and a 1:6 ratio of DNA:PEI. 24 hours post transfection, cells were washed in PBS and labeled with SNAP-Surface® Alexa Fluor® 647 (100 nM; NEBiolabs) for 30 minutes in growth medium, washed 3 times with PBS to remove unreacted dye, and fixed using 3% paraformaldehyde for 20 min, and washed twice with PBS. Coverslips were then mounted onto glass slides for confocal imaging. Images were recorded using a Zeiss LSM 710 laser scanning confocal microscope fitted with a Zeiss Plan-Apochromat 63x/1.40 NA oil immersion objective.

Radioligand binding assays

All radioligand binding assays were carried out at 25°C in a 100 μ L reaction volume containing assay buffer (50 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 0.1% (w/v) bovine serum albumin (BSA)) and 1.5 μ g membrane protein from T-RexTM-293 cells expressing an N-terminally SNAP tagged CB1R or CB1R₉₁₋₄₇₂ in the presence of 1.5 nM [³H]CP 55,940 (Specific activity 101 Ci/mmol, Revvity, Waltham, MA, USA). Nonspecific binding was determined using 10 μ M SR141716A (rimonabant) and DMSO concentrations were < 1%. In all cases, total radioligand binding (TB) did not exceed 10% of the total radioligand added to avoid ligand depletion.

Displacement assays were carried out on membrane aliquots using radioligand and increasing concentrations of competing ligands CP 55,940 or SR141716A. The reaction mixture was incubated for 120 minutes after which receptor-bound radioactivity was measured. For association assays, T-RexTM-293 CB1R WT or T-RexTM-293 CB1R₉₁₋₄₇₂ membranes were added to the reaction mixture at eleven different time points over a total incubation time of 120 minutes. In dissociation assays, membranes were first incubated with radioligand for 120 minutes to reach equilibrium. Dissociation was initiated by addition of 5 μ L 10 μ M SR141716A (final concentration) at ten different time points. Final DMSO concentrations were increased to 1.5% to improve solubility.

For all assays, incubations were terminated by rapid vacuum filtration over 96-well Whatman GF/C filter plates using a PerkinElmer Filtermate harvester (Revvity, Waltham, MA, USA).

Subsequently, filters were washed 20 times using ice-cold wash buffer (50 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 0.1% (w/v) BSA) to reduce non-specific binding. Filter-bound radioactivity was determined in a 2450 Microbeta2 plate counter after addition of 25 µL MicroScint scintillation cocktail per well (Revvity).

All radioligand binding data were analyzed using Prism 9.0 (GraphPad Software, San Diego, California, USA). The pIC₅₀ values were obtained from nonlinear regression analysis of competition displacement assays. Apparent association rate constants (k_{obs}) were determined by fitting the association data to a one-phase exponential association function. Dissociation rate constants (k_{off}) were determined by fitting the data to the statistically preferred biphasic ($k_{off, fast}$ and $k_{off, slow}$) exponential decay function. Data are shown as mean ± SEM of three individual experiments performed in duplicate. To compare differences in kinetic parameters, an unpaired, two-tailed Student's t-test was performed ($p < 0.05$ *).

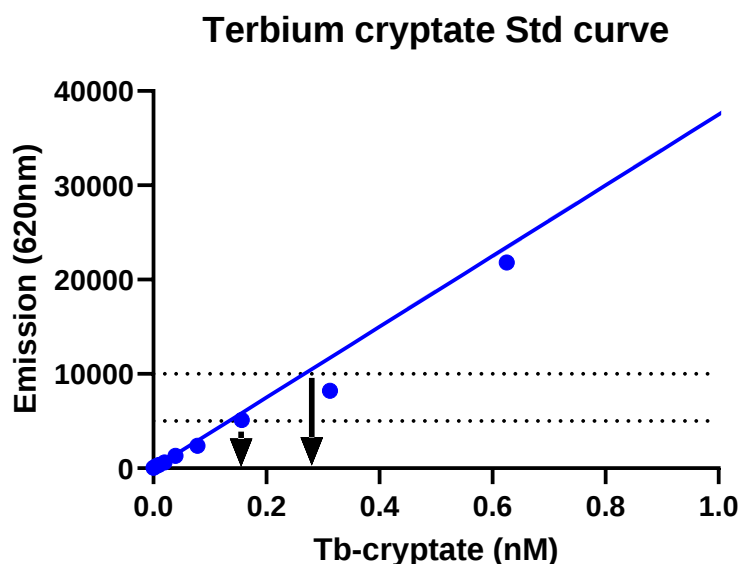
Gi-CASE cellular Nano-BRET assay

Receptor activation of CB1R and truncated CB1R₉₁₋₄₇₂-expressing T-RexTM-293 cells with the Gi-CASE biosensor(Schihada et al. 2021) was achieved as follows. Cells were plated in a white, 96-well clear-bottomed previously coated in poly-D-lysine (5 µg/mL in PBS). After 48-72-hour incubation with tetracycline to induce CB1R expression, cell culture media was aspirated and the cells were then washed (100 µl/well) with assay buffer, Hank's balanced salt solution (HBSS) containing 0.5% BSA, 5 mM HEPES. Assay buffer (90 µl/well) containing the NanoLuciferase substrate furimazine (10 µM) was dispensed into the wells and the plate was incubated at 37°C for 15 minutes. The assay plate was transferred to the PHERAstar FSX and three BRET cycles were collected every minute, before adding 10 µl of a 10x stock of compound. Dilutions of the synthetic CBR agonist HU-210 and endocannabinoids 2-AG and AEA were prepared as follows. Compounds were initially serially diluted in DMSO, and then diluted 1/10 in assay buffer before the addition to the assay plate. The final DMSO concentration in the cell plate was 1% after compound addition. Data from compound and vehicle treated cells were collected with 1 min intervals during 40 cycles after compound addition using the BRET1 plus module (535-30LP/475-30BP). Normalized data was fit to a four-parameter log(agonist) vs. response model in GraphPad Prism.

Supplementary Results

Receptor concentration estimation using a Terbium cryptate standard curve

[Supplementary Figure 1](#) shows the range in terbium levels (150-300pM) observed for CB1 and CB2R membranes indicated by the black arrows. Ligand depletion is considered significant when more than 10% of the ligand is bound, and that would mean having nmol range receptor amount per ug of protein. That range is far from the GPCR yields that overexpression systems afford in mammalian cells such as HEK293, even if we account for the enrichment in receptor concentration that happens when membranes are prepared from whole lysates. Nevertheless, this concentration of the receptor could be a limitation when working with high affinity ligands.

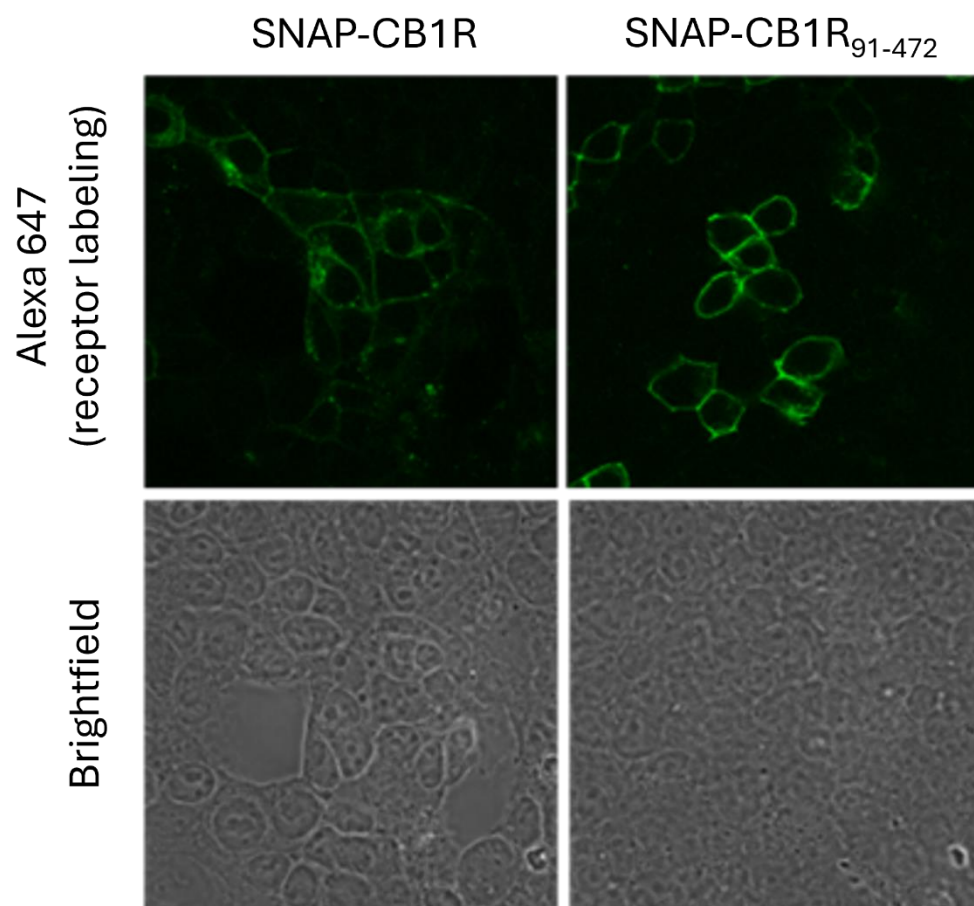


Supplementary Figure 1. A terbium cryptate standard curve (5pM to 5nM) was constructed in 384 well Optiplates in assay buffer to determine the levels of receptor present in the wells following the addition of membranes. Based on the 620 nm emission signal in our assays, indicated by the dotted lines, the receptor concentration was estimated to be in the range of 150–300 pM. This quantification assumes 100% labelling efficiency. (Miljus, Sykes et al. 2020).

Confocal imaging to determine cell surface localisation

Removing part of the CB1R at its N-terminus meant removing amino acid residues which are likely to be glycosylated. As NT glycosylation is implicated in receptor maturation and trafficking for many receptors (Schjoldager et al. 2020), it was important to check the localisation of the truncated receptor to ensure appropriate expression, folding, and cell surface trafficking. In transiently transfected HEK293T cells, there was clear evidence of cell surface localisation of both the SNAP-tagged full length CB1R and CB1R₉₁₋₄₇₂ receptors when

the SNAP-tag was labelled with Alexa Fluor 647 and visualised by confocal microscopy (see [Supplementary Figure 2](#)).



Supplementary Figure 2. Cell surface localization of SNAP-tagged full-length CB1R and truncated CB1R₉₁₋₄₇₂ receptors by confocal microscopy.

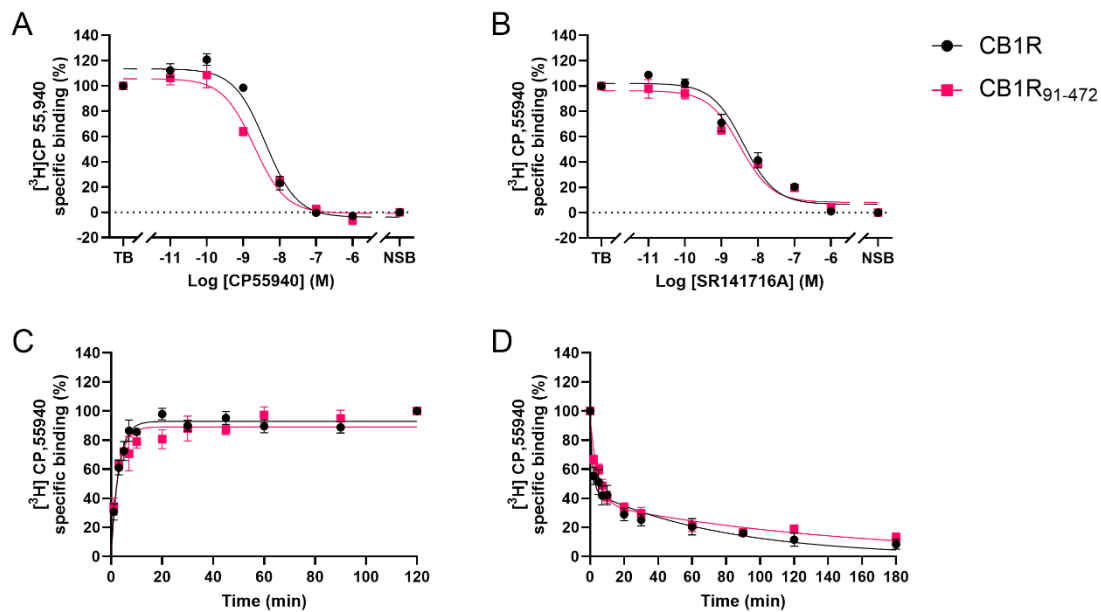
Detailed binding and functional comparison of the full length CB1R WT and truncated CB1R₉₁₋₄₇₂

The precise role of the long extracellular N-terminus of CB1R is still not fully understood. It has been suggested that splice variants of CB1R with similarly truncated NT regions might have disrupted the binding or signalling potency of the endogenous agonists such as anandamide (AEA) (Ryberg et al. 2005). However, a more recent study contradicted these findings, suggesting no effect on the pharmacology of recombinantly expressed CB1R splice variants (Xiao et al. 2008). It was therefore important to determine any potential deleterious effect of CB1R NT truncation in the CB1R₉₁₋₄₇₂ containing cell line.

Radioligand binding

The binding affinity of CP 55,940 and SR141716A (rimonabant) was measured using equilibrium radioligand displacement experiments in order to compare the full length CB1R WT and the truncated CB1R₉₁₋₄₇₂ receptor (see [Supplementary Figure 3A and B](#)). CP 55,940's IC₅₀ for CB1R₉₁₋₄₇₂ was increased by 0.3 log units compared to WT, which represents a small change from the pharmacological perspective. The affinity of SR141716A was unaffected (see [Supplementary Table 1](#)). It is possible that the kinetics (i.e., k_{off} and k_{on}) of ligand binding may be different even when equilibrium measures of affinity appear the same. Hence, we studied the kinetics of [3H]CP 55,940 binding to both the CB1 WT and the truncated CB1R₉₁₋₄₇₂. The association and dissociation rates of [3H]CP 55,940 binding to the CB1R WT and truncated CB1R₉₁₋₄₇₂ were very similar ([Supplementary Figure 3C and D](#)) and their observed association rate (k_{obs}) and constant dissociation rate showed no statistically significant differences (see [Supplementary Table 1](#)).

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Supplementary Figure 3. Equilibrium binding and binding kinetics on CB1R and CB1₉₁₋₄₇₂ membranes. Displacement of [³H]CP 55,940 from CB1R and CB1R₉₁₋₄₇₂ in the presence of increasing concentration of CP 55,940 (**A**) and SR141716A (**B**). Association (**C**) and dissociation (**D**) of [³H]CP 55,940 from CB1R WT and CB1R₉₁₋₄₇₂. Results are presented as mean ± SEM of three independent experiments performed in duplicate.

Supplementary Table 1. Affinity and binding kinetic parameters on CB1R and CB1R₉₁₋₄₇₂ membranes determined in [³H]CP 55,940 binding assays.

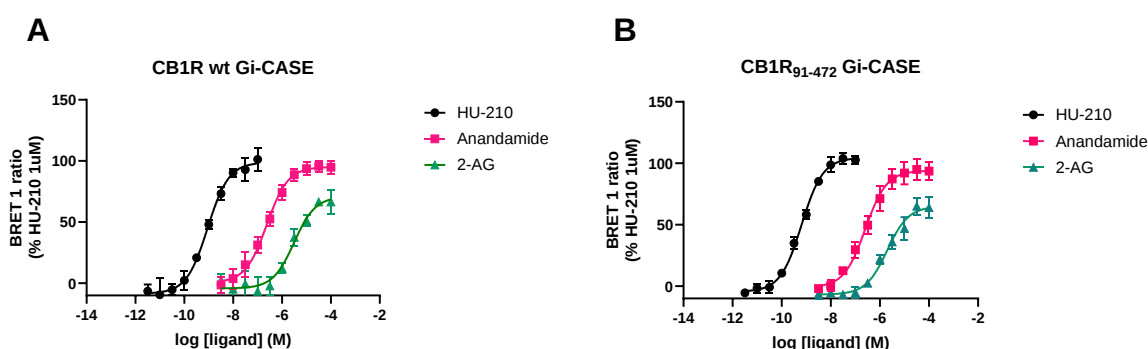
	pIC ₅₀ ± SEM (IC ₅₀ (nM)) CP55940	pIC ₅₀ ± SEM (IC ₅₀ (nM)) SR141716A	k _{obs} (min ⁻¹)	k _{off, fast} (min ⁻¹)	k _{off, slow} (min ⁻¹)	% fast
CB1R	8.38 ± 0.04 (4)	8.40 ± 0.22 (4)	0.37 ± 0.09	0.56 ± 0.19	0.012 ± 0.003	59 ± 5
CB1R₉₁₋₄₇₂	8.69 ± 0.06 (2)	8.47 ± 0.12 (3)	0.36 ± 0.05	0.23 ± 0.01	0.007 ± 0.002	62 ± 5

Values represent mean ± SEM of three independent experiments performed in duplicate.

Differences in kinetic parameter values (k_{obs}, k_{off, fast}, k_{off, slow}) were analyzed using a two-tailed Student's t-test with Welch's correction and no significant differences were found between CB1R and CB1R₉₁₋₄₇₂.

Gi-CASE cellular assay

HU-210, a high affinity non-selective synthetic cannabinoid agonist, and the two endogenous cannabinoids 2-AG and AEA were tested for their ability to bind CB1R and induce an active receptor conformation. In the BRET-based Gi-CASE assay agonist stimulation of the receptors leads to the dissociation of the $G\alpha$ subunit (tagged with Nluc) from $\beta\gamma$ (the γ subunit tagged with Venus) and a decrease in the BRET signal. There was no discernible difference in the agonist potencies comparing the full length CB1R and the truncated CB1R₉₁₋₄₇₂ (see [Supplementary 4A and B](#), and [Supplementary Table 2](#)).



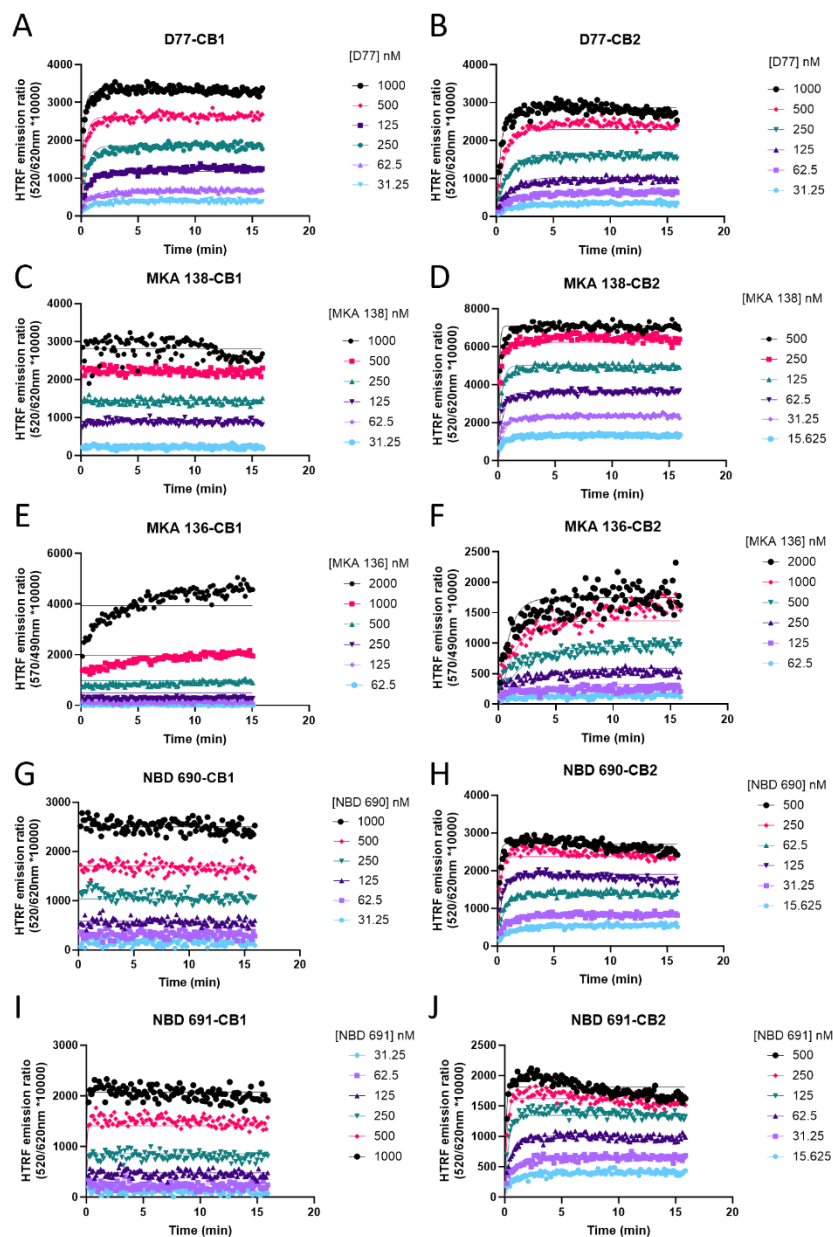
Supplementary Figure 4. Gi-CASE activation at the full-length or truncated CB1R₉₁₋₄₇₂ receptors when stimulated with HU-210 and the endocannabinoids 2-AG and anandamide at 37°C. Concentration-response curves for HU-210, 2-AG and anandamide at the full-length receptor (**A**) or truncated receptor (**B**). Data points and error bars represent the mean $\pm$ SEM of ≥ 3 independent experiments performed with duplicate technical replicates.

Supplementary Table 2. Gi-CASE cellular assay. Compound potency values expressed as pEC_{50} for full length CB1R WT and truncated CB1R₉₁₋₄₇₂ at 37°C. Values were fitted from [Supplementary Figure 4](#). Values represent mean $\pm$ SEM of ≥ 3 independent experiments.

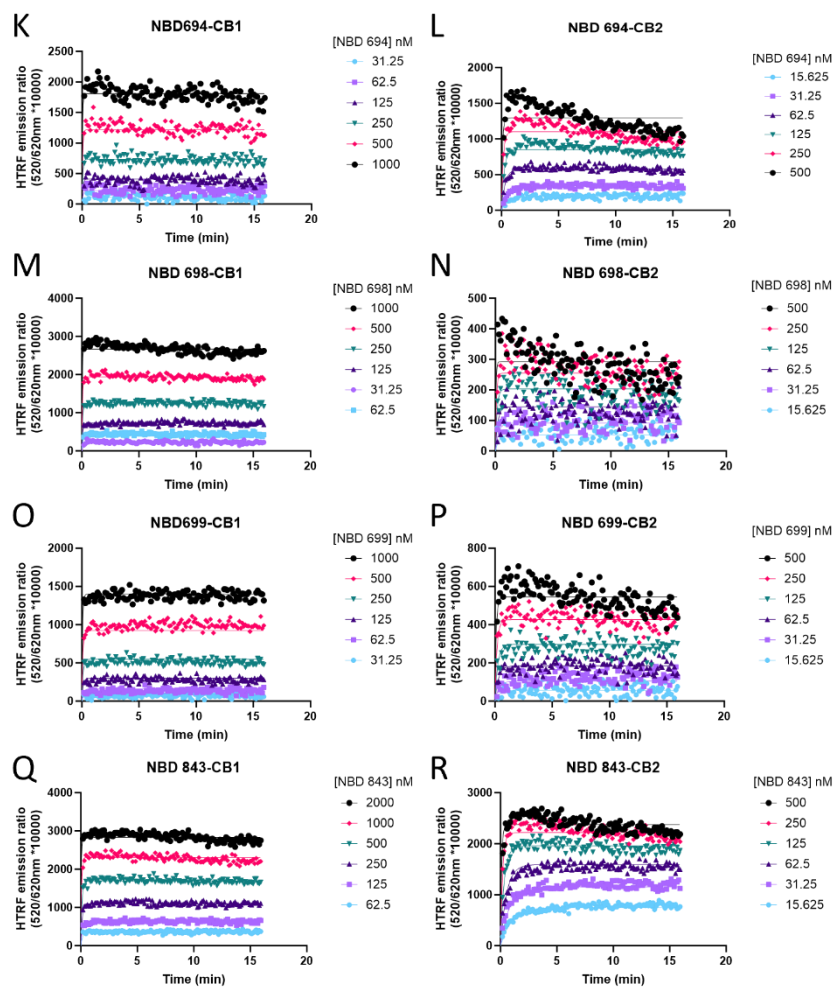
	CB1R		CB1R ₉₁₋₄₇₂	
	pEC_{50}	E_{max} (%)	pEC_{50}	E_{max} (%)
HU-210	8.97 ± 0.08	98.2 ± 6.8	9.15 ± 0.09	104.1 ± 4.7
AEA	6.63 ± 0.12	97.0 ± 6.6	6.55 ± 0.13	95.3 ± 9.7
2-AG	5.54 ± 0.20	68.5 ± 8.3	5.51 ± 0.12	66.8 ± 9.5

Differences in pEC_{50} and E_{max} parameters values were analyzed using a paired t-test with differences compared to WT. No significant differences were observed between the pEC_{50} or E_{max} values $p > 0.05$.

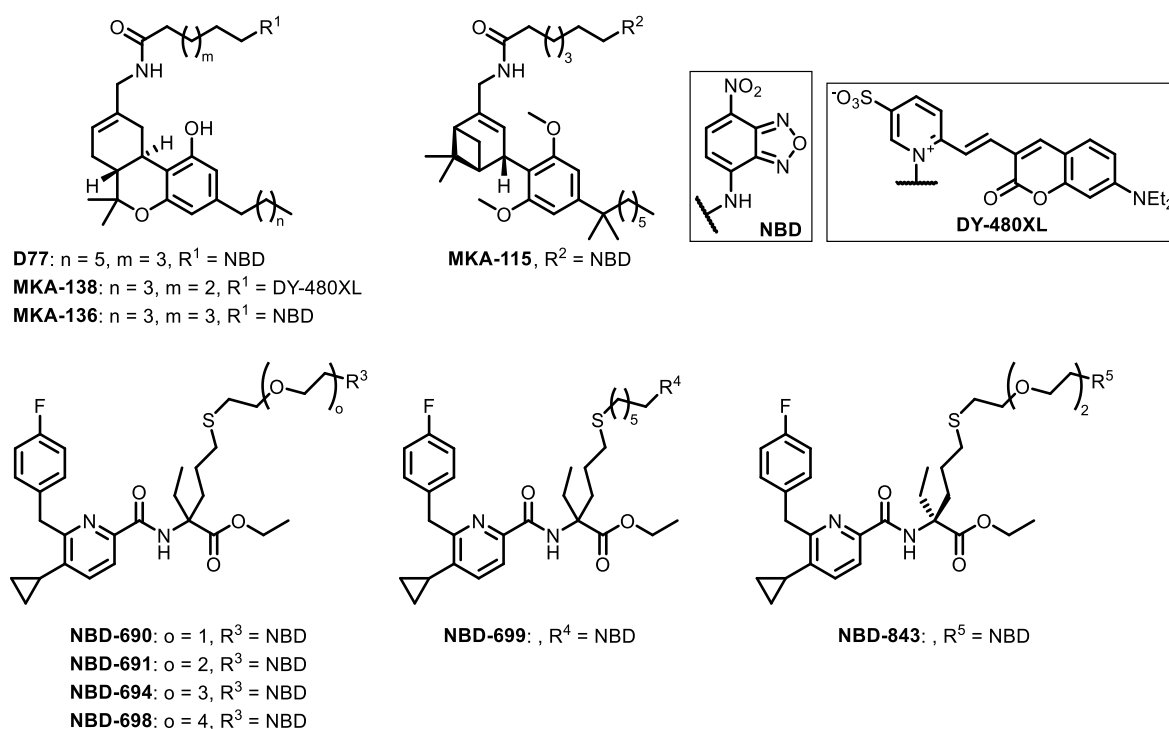
Kinetic characterization of fluorescent tracers binding to CB1R and CB2R



Supplementary Figure 5. Determination of tracer kinetic binding parameters. Kinetic association binding curves of fluorescent cannabinoid ligands CB1R (left column) and CB2R (right column) expressing membranes at 25°C. Representative graphs show specific binding expressed as HTRF emission ratio for the tracer molecule D77 (**A**) and (**B**), MKA 138 (**C**) and (**D**), MKA 136 (**E**) and (**F**), NBD 690 (**G**) and (**H**), NBD 691 (**I**) and (**J**), NBD 694 (**K**) and (**L**), NBD 698 (**M**) and (**N**), NBD 699 (**O**) and (**P**), and NBD 843 (**Q**) and (**R**), at CB1R and CB2R respectively.



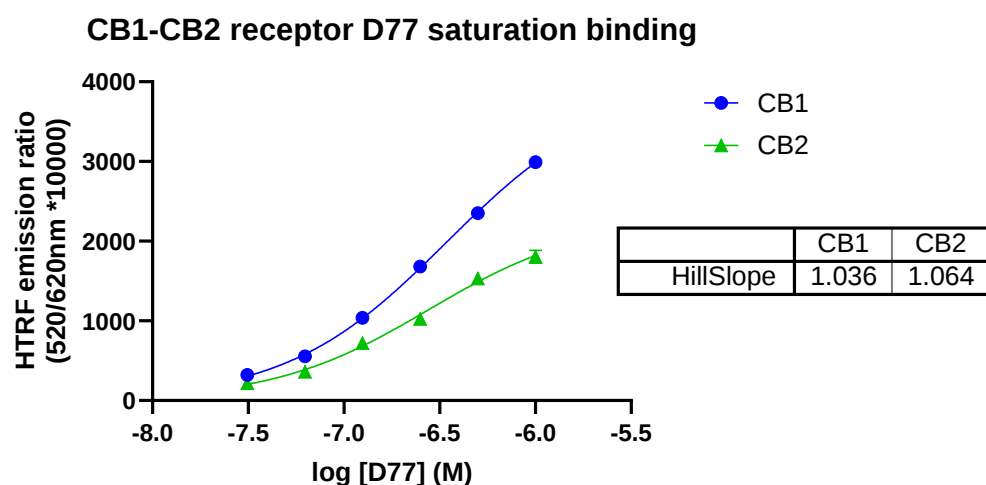
Supplementary Figure 5 (continued)



Supplementary Figure 6. Structures of TR-FRET tracers employed in the study.: D77; MKA-115; MKA-138; MKA-136; NBD-690; NBD-691; NBD-694; NBD-698; NBD-699; NBD-843.

Assessing the potential for ligand depletion

The saturation data obtained for CB1 and CB2 receptor shown in Figure 4 of the main text was replotted on a log scale to assess the potential for ligand depletion (see [Supplementary Figure 7](#)). A steep Hill coefficient (or slope) significantly greater than one would be indicative of ligand depletion. The resulting curves fitted to a 4-parameter logistic equation suggest that no depletion was observed with D77 either at CB1 or CB2R.



Supplementary Figure 7. D77 CB1 and CB2R saturation binding plots were plotted on a log scale to determine the Hill slope. A Hill coefficient (or slope) close to one indicates that the binding of the fluorescent ligand is not subject to the phenomenon of ligand depletion.

Supplementary Table 3. Affinity and binding kinetic parameters of fluorescent tracers binding to CB1R and CB2R. Data are expressed as mean $\pm$ SEM of ≥ 3 independent experiments conducted at 25°C. ND = Not determined.

Fluorescent tracer	CB1R			CB2R		
	k_{on} ($M^{-1} min^{-1}$)	k_{off} (min^{-1})	pK_d	k_{on} ($M^{-1} min^{-1}$)	k_{off} (min^{-1})	pK_d
D77	$(3.64 \pm 0.08) \times 10^6$	1.13 ± 0.07	6.51 ± 0.03	$(3.26 \pm 0.26) \times 10^6$	0.52 ± 0.08	6.81 ± 0.04
MKA-138	$(1.91 \pm 0.49) \times 10^7$	9.00 ± 1.80	6.31 ± 0.05	$(9.42 \pm 2.08) \times 10^6$	0.92 ± 0.09	6.97 ± 0.08
MKA-136	ND	ND	ND	$(6.06 \pm 0.88) \times 10^5$	0.21 ± 0.07	6.71 ± 0.41
NBD-690	$(1.06 \pm 0.16) \times 10^7$	7.67 ± 0.44	6.13 ± 0.09	$(1.35 \pm 0.02) \times 10^7$	0.97 ± 0.05	7.15 ± 0.02
NBD-691	$(1.34 \pm 0.17) \times 10^7$	$(1.23 \pm 0.14) \times 10^1$	6.04 ± 0.01	$(2.00 \pm 0.12) \times 10^7$	1.25 ± 0.06	7.20 ± 0.01
NBD-694	$(1.19 \pm 0.02) \times 10^7$	$(1.14 \pm 0.03) \times 10^1$	6.02 ± 0.01	$(2.16 \pm 0.15) \times 10^7$	2.34 ± 0.22	6.97 ± 0.01
NBD-698	$(1.63 \pm 0.03) \times 10^7$	9.93 ± 0.24	6.22 ± 0.01	$(2.45 \pm 0.37) \times 10^7$	2.85 ± 0.40	6.93 ± 0.05
NBD-699	$(4.40 \pm 1.33) \times 10^6$	7.49 ± 0.60	5.74 ± 0.12	$(1.47 \pm 0.15) \times 10^7$	1.91 ± 0.47	6.94 ± 0.19
NBD-843	$(1.35 \pm 0.30) \times 10^7$	8.56 ± 1.12	6.18 ± 0.08	$(1.55 \pm 0.15) \times 10^7$	1.17 ± 0.63	7.24 ± 0.26

One of the key elements that will impact the fit of the Motulsky and Mahan model will be the binding kinetic characteristics of the tracer molecule, its association rate (k_{on}) and the dissociation rate (k_{off}). We performed a kinetic characterization of previously developed fluorescent tracers in order to characterise their kinetic binding properties towards CB1R and CB2R (see [Supplementary Figure 5 A-R](#)).

The ligand MKA 136 showed a slow k_{on} when binding to both CB1 and CB2, and equilibrium was not obtained in the 15 min data collection period, indicating that the time required for equilibrium was >15min. The slow association of MKA 136 makes it unsuitable for the use in the Motulsky and Mahan model, as a fast association is desired in order to observe significant competition with the unlabelled ligands at the very early time points. On the other hand, MKA 138, NBD 690, NBD 691, NBD 694, NBD 698, NBD-699 and NBD-843 show extremely fast association to CB1, although slightly slower to CB2 receptor. The fast observed rate of association at these receptors make it impractical to reliably measure the binding association phase, since binding equilibrium is reached almost instantly at the CB1R. Obtaining reliable data from the association phase of probe binding is crucial to apply the competition binding model, since the absence of early data points prior to reaching equilibrium leads to poorer kinetic parameters k_{on} ($K1$, in the Motulsky Mahan model) and k_{off} , ($K2$) estimations thus increasing the errors associated with cold compound kinetic determinations k_{on} ($K3$) and k_{off} , ($K4$). The kinetic profiles of MKA 138, NBD 690, NBD 691, NBD 694, NBD 698, NBD-699 and NBD-843 shown for their binding to CB2R could be considered suitable for the application of the Motulsky and Mahan modelling, as they exhibit moderately rapid association. However, a time-dependent decay of the HTRF ratio is observed for NBD 694, NBD 698 and NBD-699 binding to CB2R. And although less pronounced, the same signal decay can be observed for the NBD 690, NBD 691 and NBD-843 binding to CB2R. Structures of the probes tested are shown in [Supplementary Figure 6](#).

Kinetic parameters of unlabelled cannabinoid ligands at CB1R and CB2R at room temperature

The kinetic parameters of the cannabinoid compounds tested were also determined at room temperature (25 °C) following the Motulsky and Mahan approach and using the D77 fluorescent ligand as the tracer (600 nM and 900 nM for CB1R and CB2R respectively). The association of the tracer ligand was monitored using TR-FRET in competition with different concentrations of each cold cannabinoid ligand. Data were globally fitted using the model “kinetics of competitive binding” in GraphPad Prism and association rate- k_{on} and dissociation rate- k_{off} were calculated for the different cannabinoid compounds, which are found in [Supplementary Table 4](#) and [Table 5](#).

Supplementary Table 4. Kinetic parameters calculated from the Motulsky and Mahan experimental approach for the cannabinoid compounds tested for CB1R at room temperature (25°C). Data are expressed as mean ± SEM of 4 experiments (except HU-308, N=3) conducted independently.

CB1R (25°C)	k_{on} (M ⁻¹ min ⁻¹)	k_{off} (min ⁻¹)	Residence time (Rt)		pK _d
			(min)	(s)	
Rimonabant	(1.16 ± 0.16) × 10 ⁸	0.36 ± 0.03	2.86 ± 0.23	171 ± 14	8.50 ± 0.03
HU-210	(1.64 ± 0.08) × 10 ⁸	0.38 ± 0.01	2.66 ± 0.03	160 ± 2	8.64 ± 0.02
CP 55,940	(1.02 ± 0.15) × 10 ⁸	2.45 ± 0.19	0.42 ± 0.03	25 ± 2	7.61 ± 0.03
2-AG	(1.20 ± 0.18) × 10 ⁶	4.82 ± 0.79	0.22 ± 0.03	13 ± 2	5.40 ± 0.08
Anandamide	(1.41 ± 0.17) × 10 ⁶	3.84 ± 0.53	0.28 ± 0.04	17 ± 2	5.57 ± 0.01
HU-308	(4.03 ± 0.53) × 10 ⁵	1.73 ± 0.21	0.60 ± 0.08	36 ± 5	5.37 ± 0.01
SR 144528	(1.25 ± 0.16) × 10 ⁷	2.32 ± 0.20	0.44 ± 0.04	26 ± 3	6.72 ± 0.02

Supplementary Table 5. Kinetic parameters calculated from the Motulsky and Mahan experimental approach for the cannabinoid compounds tested for CB2R at room temperature (25°C). Data are expressed as mean ± SEM of 4 experiments conducted independently.

CB2R (25 °C)	k_{on} (M ⁻¹ min ⁻¹)	k_{off} (min ⁻¹)	Residence time (Rt)		pK _d
			(min)	(s)	
Rimonabant	(2.02 ± 0.34) ×10 ⁶	2.02 ± 0.34	0.54 ± 0.08	32 ± 5	6.00 ± 0.04
HU-210	(6.42 ± 0.35) ×10 ⁷	(2.38 ± 0.48) ×10 ⁻²	(4.95 ± 1.28) ×10 ¹	2971 ± 772	9.46 ± 0.12
CP-55940	(1.15 ± 0.10) ×10 ⁸	0.33 ± 0.04	3.19 ± 0.39	191 ± 23	8.55 ± 0.02
2-AG	(6.26 ± 2.28) ×10 ⁶	2.47 ± 0.55	0.48 ± 0.12	29 ± 7	6.36 ± 0.06
Anandamide	(1.44 ± 0.19) ×10 ⁶	1.27 ± 0.20	0.85 ± 0.13	51 ± 8	6.06 ± 0.02
HU-308	(2.46 ± 0.37) ×10 ⁷	0.42 ± 0.05	2.73 ± 0.19	164 ± 12	7.75 ± 0.08
SR-144528	(8.55 ± 0.38) ×10 ⁷	0.16 ± 0.01	6.32 ± 0.45	379 ± 27	8.73 ± 0.05

General synthetic and characterization methods for TR-FRET tracers

Procedure

Unless otherwise noted, all reactions were carried out under nitrogen atmosphere. Compounds 5-10 shown in [Supplementary Figure 6](#) were synthesized according to Gazzi et al. (2022).

Chemicals

All chemicals and solvents were purchased from commercial suppliers and were used without further purification. DMF, DMSO, THF, Et₂O, MeCN, and CH₂Cl₂ were dried using 4 Å molecular sieves or using an LC Technology Solutions solvent purification system (SP-1) under an atmosphere of dry nitrogen. *i*-Pr₂NEt was distilled from KOH under an atmosphere of dry nitrogen.

Chromatography

Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F₂₅₄ TLC glass plates. Purification of reaction products was carried out by flash column chromatography (FCC) using Sigma Aldrich silica 230-400 mesh particle size, 60 Å under 0.3–0.5 bar overpressure or Büchi Pure Chromatography System C-805 Flash with FlashPure silica cartridges.

Nuclear Magnetic Resonance Spectroscopy

NMR spectra were acquired on Bruker AVIII HD 500 MHz and 400 MHz spectrometers operating at the denoted spectrometer frequency given in MHz for the specified nucleus. ¹H NMR spectra are reported with the solvent resonance as the reference (CDCl₃ at 7.26 ppm, CD₃OD at 3.31 ppm, CD₂Cl₂ at 5.32 ppm, (CD₃)₂SO at 2.50 ppm, CD₃CN at 1.94 ppm). Peaks are reported as (s = singlet, bs = broad singlet, d = doublet, bt = broad triplet, t = triplet, q = quartet, m = multiplet or unresolved, coupling constant(s) in Hz, integral). ¹³C NMR spectra were recorded with ¹H-decoupling and are reported in ppm with the solvent resonance as the reference (CDCl₃ at 77.16 ppm, CD₃OD at 49.00 ppm, CD₂Cl₂ at 54.00 ppm, (CD₃)₂SO at 39.52 ppm, CD₃CN at 1.32 ppm). Service measurements were performed by the NMR service team of the Laboratorium für Organische Chemie at ETH Zürich.

High-Resolution Mass Spectrometry

High-resolution mass spectrometric data were obtained at ETH Zürich mass spectrometry service on Bruker Daltonics maXis ESI-QTOF, Thermo Q Exactive EI-Trace 1310 Analyser or a Bruker Daltonics maXis II ESI-QTOF spectrometers and are reported as (*m/z*).

Infrared Spectroscopy

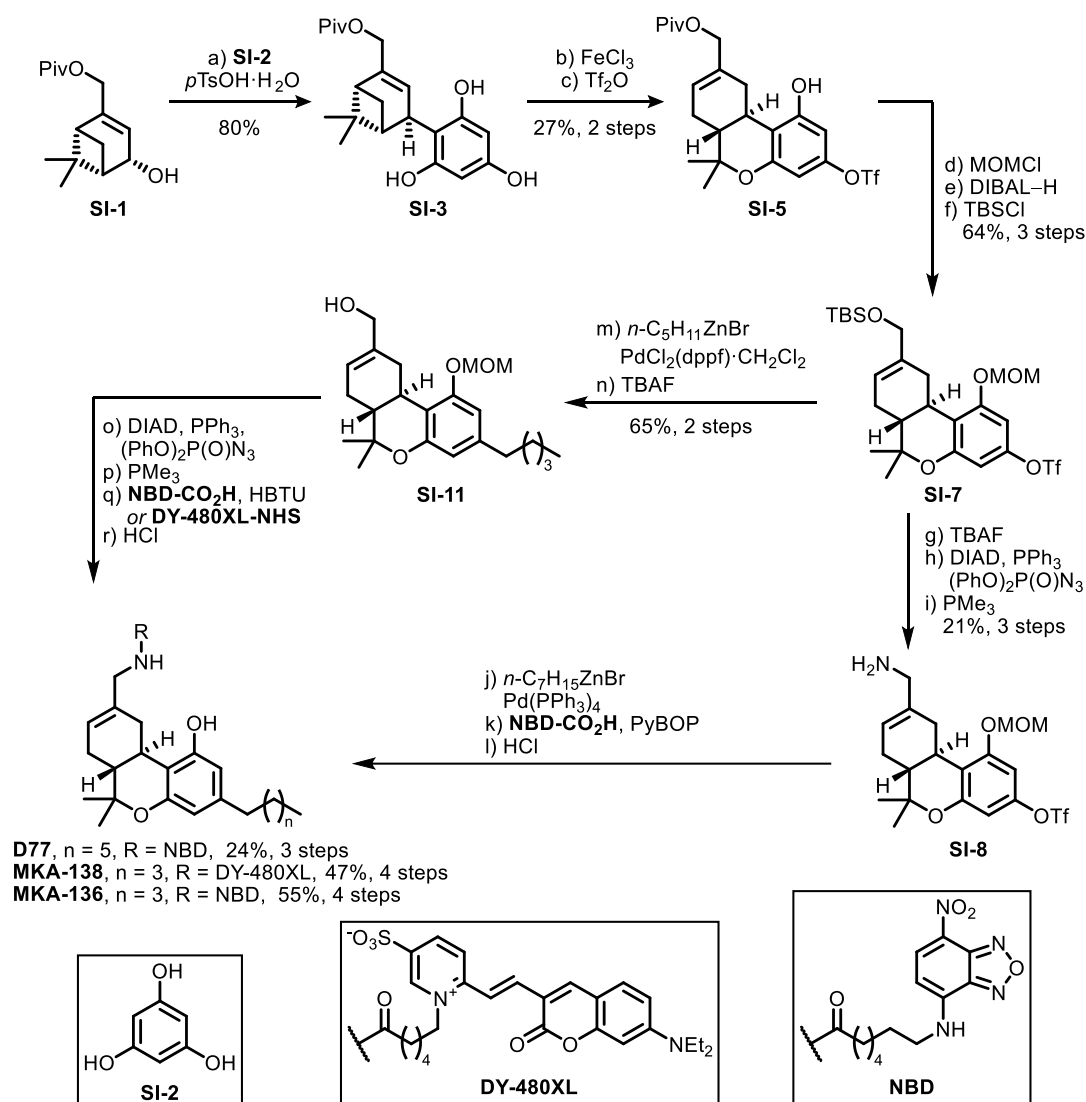
Infrared (IR) spectra were measured neat on a Perkin-Elmer UATR Two FT-IR Spectrometer and the band maxima are reported in wavenumbers (cm⁻¹).

Optical Rotation

Optical rotations ([α]_D^T) were determined using a Jasco P-2000 Polarimeter (10 cm, 1.5 mL cell).

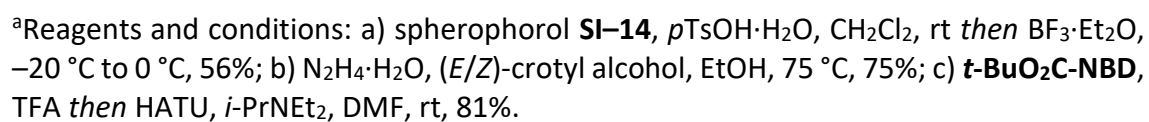
Chemical synthesis of D77 and other fluoroprobes

Our synthesis of D77 commenced with the acid-mediated FRIEDEL–CRAFTS allylation of phloroglucinol (**SI-2**) with (1*R*)-(–)-myrtenol-derived allylic alcohol (**SI-1**) according to a procedure previously reported by our group (Sarott et al. 2021). Upon exposure of **SI-1** and **SI-2** to *p*TsOH·H₂O, adduct **SI-3** was obtained as a single diastereomer in 80% yield (see [Supplementary Scheme S1](#)). Intermediate **SI-5** was accessed by treatment of **SI-3** with FeCl₃ to forge the central benzochromene motif of THC, followed by *para*-selective phenol triflation. Protection of the phenol as MOM ether, reductive cleavage of the pivalate ester, and protection with TBSCl yielded allylic alcohol **SI-7**. To arrive at heterobifunctional linchpin **SI-8**, compound **SI-7** was converted to the corresponding primary amine in a sequence consisting of TBAF-mediated TBS deprotection, azide substitution under MITSUNOBU conditions and STAUDINGER reduction. The aryl triflate and primary amine in **SI-8** served as orthogonal handles for late-stage functionalization with the affinity–controlling alkyl side chain as well as with the fluorophore of choice, respectively. NEGISHI cross-coupling of **SI-8** with *n*-C₇H₁₅ZnBr installed the C₇ alkyl side chain at C(3). The synthesis concluded with introduction of the nitrobenzoxadiazole (NBD) fluorophore by a PyBOP-mediated amide coupling with carboxylic acid **NBD-CO₂H**, followed by MOM ether deprotection to reveal TR-FRET tracer **D77**.



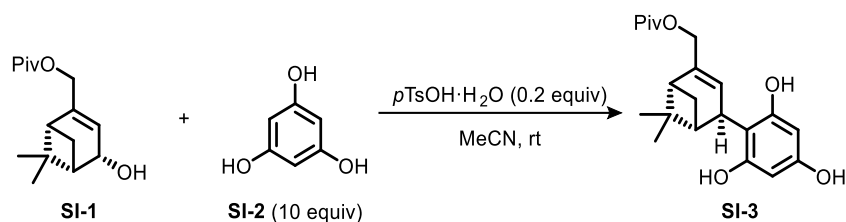
Scheme S1. Synthesis of Δ^8 -THC-derived fluorescent tracers via a heterobifunctional derivative.^a

^aReagents and conditions: a) phloroglucinol **SI-2**, $p\text{TsOH}\cdot\text{H}_2\text{O}$, MeCN, rt, 80%; b) FeCl_3 , CH_2Cl_2 , 4 Å MS, 0 °C to rt; c) Tf_2O , 2,6-lutidine, CH_2Cl_2 , -30 °C to rt, 27% over two steps; d) MOMCl, $i\text{-PrNEt}_2$, CH_2Cl_2 , 0 °C, quant.; e) DIBAL-H, CH_2Cl_2 , 0 °C; f) TBSCl, NEt_3 , DMAP, CH_2Cl_2 , rt, 64% over two steps; g) TBAF, THF, rt, 45%; h) DIAD, PPh_3 , $(\text{PhO})_2\text{P(O)N}_3$, THF, 0 °C to rt; i) PMe_3 , THF, H_2O , rt, 48% over two steps; j) $n\text{-C}_7\text{H}_{15}\text{ZnBr}$, $\text{Pd(PPh}_3)_4$, THF, 70 °C, 39%; k) **NBD-CO₂H**, PyBOP, $i\text{-PrNEt}_2$, CH_2Cl_2 , rt, 69%; l) HCl, MeOH, rt, 89%; m) $n\text{-C}_5\text{H}_{11}\text{ZnBr}$, TBAI, $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$, THF, NMP, 60 °C, quant.; n) TBAF, THF, 0 °C, 65%; o) DIAD, PPh_3 , $(\text{PhO})_2\text{P(O)N}_3$, THF, 0 °C to rt, 75%; p) PMe_3 , THF, H_2O , rt; q) **DY-480XL-NHS**, $i\text{-PrNEt}_2$, DMF, rt or **NBD-CO₂H**, HBTU, $i\text{-PrNEt}_2$, CH_2Cl_2 , rt; r) HCl, MeOH, Et_2O , rt, 63–73% over three steps.



Compound synthesis and characterization

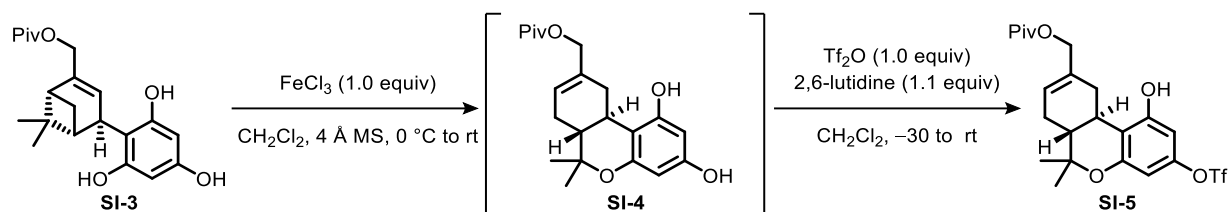
Synthesis of **SI-3**



Allylic alcohol **SI-1** (1.9 g, 7.4 mmol, 1.0 equiv) and phloroglucinol (**SI-2**) (9.4 g, 0.074 mol, 10 equiv) were dissolved in MeCN (300 mL) and $p\text{TsOH}\cdot\text{H}_2\text{O}$ (0.28 g, 1.5 mmol, 0.2 equiv) was added. The resulting solution was stirred at ambient temperature. After 30 min, TLC showed full conversion of limiting allylic alcohol reagent and the reaction was stopped by addition of brine (200 mL). After separation of the organic layer, the aqueous phase was extracted with EtOAc (6 × 75 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The unpurified solid was extracted with hot chloroform (5 × 50 mL, 55 °C), filtered, and the filtrate concentrated under reduced pressure. Purification by flash column chromatography (SiO_2 ; using 10 – 20% EtOAc in hexanes) afforded the product **SI-3** as off-white solid (2.1 g, 80%).

^1H NMR (400 MHz, CD_3CN) δ = 6.71 (br, 3H), 5.93 – 5.86 (m, 1H), 5.82 (s, 2H), 4.68 – 4.40 (m, 2H), 3.90 (t, J = 2.5 Hz, 1H), 2.34 – 2.24 (m, 2H), 2.14 – 2.08 (m, 1H), 1.42 (d, J = 9.1 Hz, 1H), 1.30 (s, 3H), 1.18 (s, 9H), 0.93 (s, 3H). **^{13}C NMR** (101 MHz, CD_3CN) δ = 179.1, 157.7, 157.4, 148.0, 122.3, 107.8, 96.1, 67.3, 48.3, 44.8, 41.40, 39.5, 38.3, 28.3, 27.4, 26.2, 21.1. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3420, 2976, 2936, 2872, 1704, 1628, 1606, 1516, 1465, 1399, 1384, 1367, 1284, 1232, 1162, 1143, 1074, 1030, 1009, 940, 881, 824, 773, 741, 723, 637, 527, 507. **HRMS (ESI)**: m/z = 383.1830 $[\text{M}+\text{Na}]^+$ (calc. for $\text{C}_{21}\text{H}_{28}\text{NaO}_5$ m/z = 383.1834). **$[\alpha]^{23}_{\text{D}}$** = -93.994 ± 0.198 (c = 1.0, MeOH).

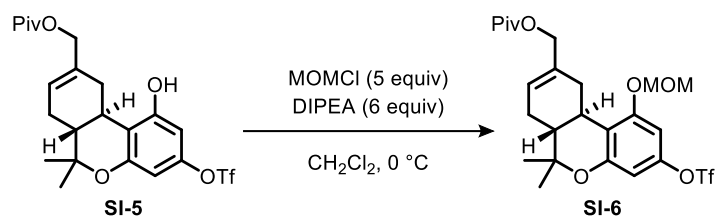
Synthesis of **SI-5**



To a suspension of **SI-3** (1.4 g, 4.0 mmol, 1.0 equiv) and 1.5 g activated 4 Å MS in anhydrous CH_2Cl_2 (16 mL) at 0 °C was added anhydrous FeCl_3 (0.65 g, 4.0 mmol, 1.0 equiv) in three batches. The reaction mixture was then allowed to reach ambient temperature during 90 min, after which full conversion of starting material was observed. The reaction mixture was then filtered through a pad of Celite to remove the molecular sieves. The filtrate was washed with water and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude material by flash column chromatography (SiO_2 ; using 20% EtOAc in hexanes) afforded bisphenol intermediate **SI-4** as light-brown foam (0.91 g, 80% pure, approx. 50% yield). Intermediate **SI-4** was used for subsequent triflation without further purification and reagent equivalents were based on the estimated 50% yield in the cyclization step. Thus, to a solution of **SI-4** in anhydrous CH_2Cl_2 (40 mL) at -30 °C (acetone/dry ice) was added 2,6-lutidine (0.26 mL, 2.2 mmol, 1.1 equiv) and then a solution of triflic anhydride (0.34 mL, 2.0 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (0.5 mL). The resulting orange-yellow solution was allowed to reach ambient temperature in the course of 2 h, after which full conversion was observed. The reaction was stopped by addition of water (20 mL) and after separation of the aqueous layer the organic phase was washed with 1 M HCl, aq. sat. NaHCO_3 and brine (10 mL, each). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude material by flash column chromatography (SiO_2 ; using 3 – 10% EtOAc in hexanes) afforded the product **SI-5** as white foam (0.54 g, 27% over 2 steps).

^1H NMR (400 MHz, CDCl_3) δ = 6.34 (d, J = 2.5 Hz, 1H), 6.28 (d, J = 2.5 Hz, 1H), 5.78 – 5.73 (m, 1H), 4.50 (s, 2H), 3.33 (dd, J = 16.5, 4.3 Hz, 1H), 2.75 – 2.67 (m, 1H), 2.29 – 2.21 (m, 1H), 1.95 – 1.76 (m, 3H), 1.39 (s, 3H), 1.23 (s, 9H), 1.09 (s, 3H). **^{13}C NMR** (101 MHz, CDCl_3) δ = 179.3, 156.3, 156.0, 148.4, 133.5, 123.1, 118.8 (q, J = 320.7 Hz), 113.2, 103.3, 100.8, 77.76, 68.1, 44.5, 39.1, 31.4, 31.3, 27.6, 27.4, 27.4, 18.6. **^{19}F NMR** (376 MHz, CDCl_3) δ = -73.0 (decoupled). **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3374, 2978, 1700, 1598, 1421, 1209, 1180, 1141, 985, 865, 847, 759, 614. **HRMS (ESI)**: m/z = 493.1507 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{22}\text{H}_{28}\text{F}_3\text{O}_7\text{S}$ m/z = 493.1502). **$[\alpha]_D^{23}$** = -145.512 $\pm$ 0.147 (c = 2.0, CHCl_3).

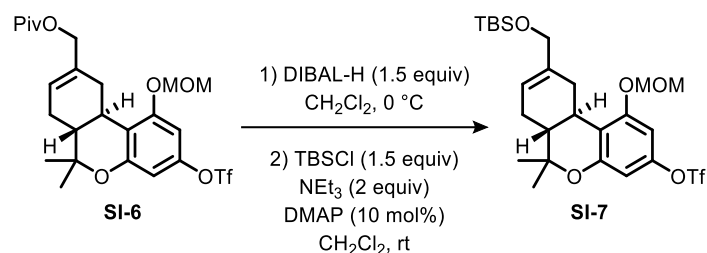
Synthesis of **SI-6**



To a solution of **SI-5** (0.052 g, 0.11 mmol, 1 equiv) in CH_2Cl_2 (1.1 mL) at 0 °C was added DIPEA (0.11 mL, 0.63 mmol, 6 equiv) and MOMCl (0.040 mL, 0.53 mmol, 5 equiv). The resulting solution was stirred at 0 °C for 60 min, after which TLC analysis showed consumption of starting material. The reaction was stopped by addition of aq. sat. NaHCO_3 cooled to ambient temperature and aq. sat. NaHCO_3 was added. The aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure to afford the product **SI-6** as a colorless wax (0.056 g, quantitative).

^1H NMR (500 MHz, CDCl_3) δ = 6.60 (d, J = 2.5 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 5.80 – 5.77 (m, 1H), 5.19 – 5.13 (m, 2H), 4.55 (d, J = 12.5 Hz, 1H), 4.44 (d, J = 12.4 Hz, 1H), 3.47 (s, 3H), 3.29 (dd, J = 17.0, 4.7 Hz, 1H), 2.71 (td, J = 11.0, 4.6 Hz, 1H), 2.29 – 2.19 (m, 1H), 1.95 – 1.76 (m, 2H), 1.40 (s, 3H), 1.22 (s, 9H), 1.10 (s, 3H). **^{13}C NMR** (126 MHz, CDCl_3) δ = 178.4, 157.5, 155.5, 148.8, 133.8, 123.8, 115.1, 104.7, 99.9, 94.7, 77.6, 68.1, 56.5, 44.7, 39.0, 31.8, 31.7, 27.7, 27.4, 27.4, 18.5, (CF_3 too weak). **^{19}F NMR** (471 MHz, CDCl_3) δ = -73.0. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 2977, 1729, 1604, 1423, 1212, 1143, 1055, 992. **HRMS (ESI)**: m/z = 559.1580 $[\text{M}+\text{Na}]^+$ (calc. for $\text{C}_{24}\text{H}_{31}\text{F}_3\text{NaO}_8\text{S}$ m/z = 559.1584).

Synthesis of **SI-7**



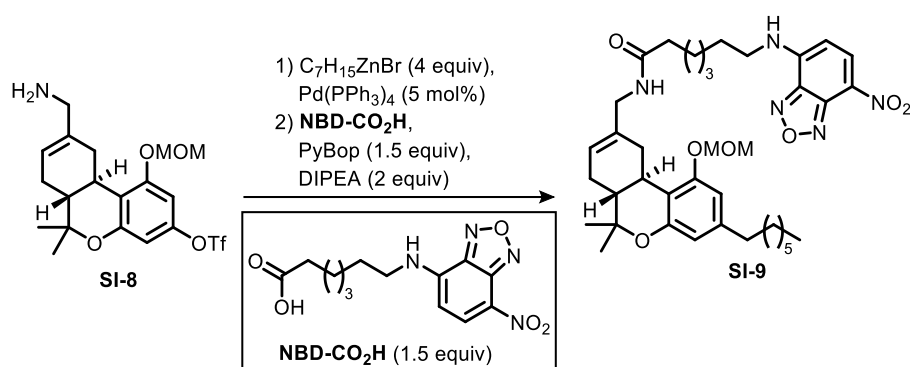
To a solution of **SI-6** (0.056 g, 0.10 mmol, 1.0 equiv) in CH_2Cl_2 (1.1 mL) at 0 °C was added DIBAL-H (1 M in hexanes, 0.16 mL, 0.16 mmol, 1.5 equiv) and the reaction mixture was allowed to reach ambient temperature. After 30 min, TLC (20% EtOAc in hexanes) showed full consumption of starting material. The reaction was stopped by addition of Rochelle's salt solution (1 mL) and the mixture was vigorously stirred until phase separation occurred. The aqueous phase was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting yellowish oil was taken up in CH_2Cl_2 (1.0 mL) and TBSCl (0.024 g, 0.16 mmol, 1.5 equiv), NEt_3 (0.030 mL, 0.21 mmol, 2.0 equiv) as well as DMAP (1.3 mg, 0.010 mmol, 10 mol%) were added. The reaction solution was stirred at ambient temperature for 15 h. Water (1 mL) was added, the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure. Purification by flash column chromatography (SiO_2 ; using 5% Et_2O in hexanes) afforded the product **SI-7** as colorless oil (0.038 g, 64% over two steps).

^1H NMR (400 MHz, CDCl_3) δ = 6.60 (d, J = 2.5 Hz, 1H), 6.44 (d, J = 2.5 Hz, 1H), 5.71 (s, 1H), 5.17 (s, 2H), 4.04 (s, 2H), 3.47 (d, J = 0.8 Hz, 3H), 3.28 (dd, J = 17.0, 4.6 Hz, 1H), 2.69 (td, J = 10.8, 4.6 Hz, 1H), 2.22 (d, J = 13.4 Hz, 1H), 1.94 – 1.69 (m, 3H), 1.39 (s, 3H), 1.09 (s, 3H), 0.92 (s, 9H), 0.08 (d, J = 1.2 Hz, 6H). **^{13}C NMR** (101 MHz, CDCl_3) δ = 157.6, 155.5, 148.7, 138.0, 120.0, 115.5, 104.7, 99.9, 94.7, 77.8, 67.1, 56.4, 45.1, 31.8, 31.4, 27.7, 27.5, 26.1, 18.6, -5.1, -5.1, (CF_3 too weak). **^{19}F NMR** (471 MHz, CDCl_3) δ = -73.0. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 2931, 2857, 1604, 1474, 1424, 1246, 1212, 1143, 1107, 1052, 992, 838. **HRMS (ESI)**: m/z = 589.1864 $[\text{M}+\text{Na}]^+$ (calc. for $\text{C}_{25}\text{H}_{37}\text{F}_3\text{NaO}_7\text{SSi}$ m/z = 589.1874). **$[\alpha]^{25}_{\text{D}}$** = -133.767 ± 0.080 (c = 2.0, CHCl_3)

To a solution of PPh₃ (0.15 g, 0.55 mmol, 2.0 equiv) in THF (1.8 mL) at 0 °C was added DIAD (0.11 mL, 0.55 mmol, 2.0 equiv) and the yellow suspension was stirred at 0 °C for 10 min. A solution of allyl alcohol intermediate (0.12 g, 0.28 mmol, 1.0 equiv) in THF (0.9 mL) was added, then the mixture was removed from the ice bath and (PhO)₂P(O)N₃ (0.12 mL, 0.55 mmol, 2.0 equiv) was added immediately. The resulting mixture was stirred at ambient temperature for 60 min. When TLC (10% EtOAc in hexanes) showed full consumption of starting material, the reaction mixture was concentrated under reduced pressure. Attempted purification by flash column chromatography (SiO₂; using hexanes to 5% Et₂O in hexanes) failed to separate product from DIAD residues, and thus the mixture was taken up in 10:1 THF/water (2.5 mL, separately sparged with N₂). PMe₃ (1 M in THF, 0.55 mL, 0.55 mmol, 2.0 equiv) and the mixture was stirred at ambient temperature overnight. After concentration under reduced pressure, purification by flash column chromatography (SiO₂; using 1 to 15% MeOH in CH₂Cl₂, 1% 7 N NH₃ in MeOH) afforded the product **SI-8** together with POME₃ impurity as yellowish oil (0.060 g, 48% over two steps).

¹H NMR (500 MHz, CDCl₃) δ = 6.57 (d, *J* = 2.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 5.66 (d, *J* = 5.1 Hz, 1H), 5.18 (s, 2H), 3.48 (s, 3H), 3.27 – 3.23 (m, 2H), 3.23 – 3.15 (m, 1H), 2.70 (td, *J* = 11.0, 4.6 Hz, 1H), 2.36 – 2.13 (m, 3H), 1.91 – 1.77 (m, 3H), 1.39 (s, 3H), 1.09 (s, 3H). **¹⁹F NMR** (471 MHz, CDCl₃) δ = -73.0. **¹³C NMR** (126 MHz, CDCl₃) δ = 157.4, 155.5, 148.7, 138.7, 119.4, 118.8 (q, *J* = 231 Hz), 115.3, 104.7, 99.8, 94.8, 77.7, 56.5, 47.6, 45.0, 32.8, 31.9, 27.7, 27.5, 18.5. **IR** (neat, ν_{max}/cm⁻¹) 3370, 2912, 1603, 1476, 1422, 1246, 1212, 1142, 1107, 991. **HRMS (ESI):** *m/z* = 452.1346 [M+H]⁺ (calc. for C₁₉H₂₅F₃NO₆S *m/z* = 452.1349).

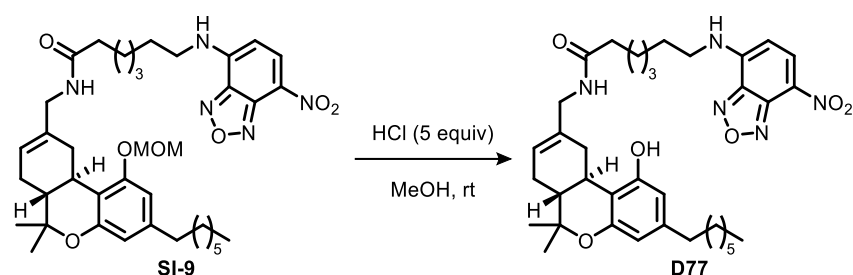
Synthesis of **SI-9**



To a solution of **SI-8** (0.020 g, 0.044 mmol, 1.0 equiv) and $\text{Pd}(\text{PPh}_3)_4$ 2.6 mg, 2.2 μmol , 5 mol) in THF (0.4 mL) was added heptylzincbromide (0.5 M in THF, 0.35 mL, 0.18 mmol, 4 equiv) and the dark solution was stirred at 70 °C overnight. After cooling to ambient temperature, aq. sat. NaHCO_3 was added, the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography (SiO_2 ; using 5% MeOH in CH_2Cl_2 , 1% 7N NH_3 in MeOH) afforded the cross-coupling product in moderate purity (7.0 mg, 0.017 mmol, 39%). This residue was taken up in CH_2Cl_2 (0.2 mL) without further purification, and NBD acid **NBD-CO₂H** (8.4 mg, 0.026 mmol, 1.5 equiv), PyBop (7.0 mg, 0.026 mmol, 1.5 equiv) and DIPEA (6.0 μL , 0.035 mmol, 2.0 equiv) were added. The orange reaction mixture was stirred at ambient temperature for 72 h. Water was added and aqueous layer was extracted with CH_2Cl_2 . The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (SiO_2 ; using 5% MeOH in CH_2Cl_2) afforded the product **SI-9** as an orange solid (6.0 mg, 69%).

¹H NMR (400 MHz, CDCl_3) δ = 8.49 (d, J = 8.6 Hz, 1H), 6.44 (d, J = 1.6 Hz, 1H), 6.34 (d, J = 1.7 Hz, 1H), 6.16 (d, J = 8.6 Hz, 1H), 5.61 (d, J = 5.0 Hz, 1H), 5.47 – 5.39 (m, 1H), 5.18 (d, J = 6.5 Hz, 1H), 5.14 (d, J = 6.5 Hz, 1H), 3.84 (d, J = 5.6 Hz, 2H), 3.48 (s, 3H), 3.47 – 3.44 (m, 2H), 3.25 (dd, J = 15.9, 4.5 Hz, 1H), 2.69 (td, J = 10.9, 4.6 Hz, 1H), 2.50 – 2.43 (m, 2H), 2.20 (q, J = 7.7, 7.2 Hz, 3H), 1.85 – 1.75 (m, 5H), 1.69 (t, J = 7.3 Hz, 2H), 1.56 (m, 2H), 1.51 – 1.43 (m, 4H), 1.37 (s, 3H), 1.31 – 1.25 (m, 8H), 1.09 (s, 3H), 0.90 – 0.84 (m, 3H). **¹³C NMR** (101 MHz, CDCl_3) δ = 172.6, 156.7, 154.5, 144.4, 144.1, 143.2, 136.6, 135.5, 121.5, 112.2, 111.3, 106.4, 98.7, 94.6, 76.5, 56.5, 45.3, 44.0, 36.5, 36.1, 33.2, 32.0, 31.8, 31.2, 29.6, 29.3, 28.8, 28.3, 27.9, 27.7, 26.7, 25.4, 22.8, 18.5, 14.3. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3295, 2927, 2856, 1650, 1619, 1580, 1444, 1299, 1273, 1051. **HRMS (ESI)**: m/z = 714.3831 [$\text{M}+\text{Na}$]⁺ (calc. for $\text{C}_{38}\text{H}_{53}\text{N}_5\text{NaO}_7$ m/z = 714.3837).

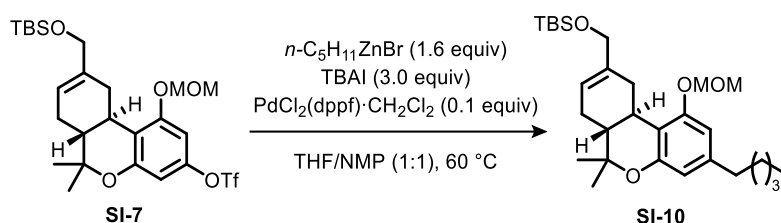
Synthesis of **D77**



To a solution of **SI-9** (6.0 mg, 8.7 μmol , 1.0 equiv) in MeOH (0.1 mL) was added HCl (2 M in THF, 0.022 mL, 0.043 mmol, 5.0 equiv) and the orange solution was stirred at ambient temperature for 2 h. After TLC (10% MeOH in CH_2Cl_2) showed full consumption of starting material, aq. sat. NaHCO_3 was added, the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by pipette chromatography (SiO_2 ; using 1 to 5% MeOH in CH_2Cl_2) afforded the product **D77** as an orange wax (5.0 mg, 89%).

^1H NMR (500 MHz, CDCl_3) δ = 8.47 (d, J = 8.7 Hz, 1H), 6.57 (s, 1H), 6.29 – 6.21 (m, 1H), 6.18 – 6.12 (m, 2H), 5.63 – 5.50 (m, 2H), 3.81 (d, J = 20.8 Hz, 2H), 3.46 (m, 2H), 3.38 – 3.27 (m, 1H), 2.67 (td, J = 10.9, 4.6 Hz, 1H), 2.39 (td, J = 7.4, 3.6 Hz, 2H), 2.24 (t, J = 7.1 Hz, 2H), 2.21 – 2.14 (m, 1H), 1.85 – 1.75 (m, 5H), 1.73 – 1.66 (m, 2H), 1.56 – 1.40 (m, 6H), 1.36 (s, 3H), 1.30 – 1.21 (m, 8H), 1.08 (s, 3H), 0.88 – 0.84 (m, 3H). **^{13}C NMR** (126 MHz, CDCl_3) δ = 173.3, 155.3, 154.9, 144.4, 144.1, 143.1, 136.7, 135.3, 122.0, 110.1, 109.9, 107.9, 98.8, 76.5, 45.5, 45.0, 44.0, 36.6, 35.7, 32.7, 31.9, 31.6, 31.1, 29.5, 29.3, 28.7, 28.2, 27.8, 27.7, 26.6, 25.4, 22.8, 18.6, 14.3. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3320, 2926, 2855, 1622, 1582, 1530, 1444, 1299, 1264. **HRMS (ESI)**: m/z = 648.3747 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{36}\text{H}_{50}\text{N}_5\text{O}_6$ m/z = 648.3756).

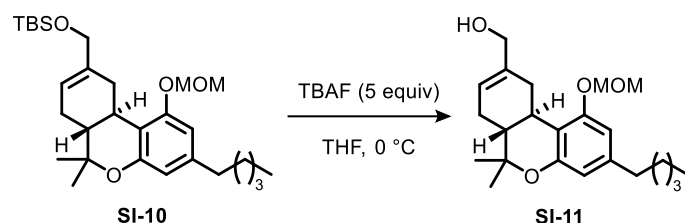
Synthesis of **SI-10**



A mixture of **SI-10** (0.10 g, 0.18 mmol, 1.0 equiv), TBAI (0.13 g, 0.35 mmol, 2.0 equiv), and $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$ (0.014 g, 0.018 mmol, 0.1 equiv) under N_2 was dissolved in 1:1 THF/NMP (0.6 mL). $n\text{-C}_5\text{H}_{11}\text{ZnBr}$ (0.5 M in THF, 0.42 mL, 0.21 mmol, 1.2 equiv) was added at ambient temperature and the resulting brown solution was heated to 60 °C and stirred for 4 h, after which TLC (5% Et_2O in hexanes) showed full consumption of starting material. The reaction mixture was cooled to ambient temperature, and CH_2Cl_2 (1 mL) and aq. sat. NH_4Cl (1 mL) were added. The aqueous phase was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure. Purification by flash column chromatography (SiO_2 ; using 2 to 5% Et_2O in hexanes) afforded the product **SI-10** as a yellow oil (0.086 g, quantitative).

^1H NMR (400 MHz, CDCl_3) δ = 6.48 (d, J = 1.6 Hz, 1H), 6.36 (d, J = 1.6 Hz, 1H), 5.73 – 5.68 (m, 1H), 5.17 (s, 2H), 4.05 (s, 2H), 3.48 (s, 3H), 3.31 (dd, J = 17.1, 4.4 Hz, 1H), 2.70 (td, J = 10.8, 4.6 Hz, 1H), 2.52 – 2.47 (m, 2H), 2.21 (dd, J = 13.2, 4.8 Hz, 1H), 1.91 – 1.74 (m, 3H), 1.64 – 1.54 (m, 2H), 1.38 (s, 3H), 1.34 – 1.30 (m, 2H), 1.29 – 1.24 (m, 2H), 1.10 (s, 3H), 0.92 (s, 9H), 0.91 – 0.86 (m, 3H), 0.08 (s, 3H), 0.08 (s, 3H). **^{13}C NMR** (101 MHz, CDCl_3) δ = 156.9, 154.5, 142.9, 138.3, 119.9, 112.6, 111.3, 106.5, 94.6, 76.5, 67.2, 56.3, 45.5, 36.0, 31.9, 31.9, 31.8, 31.7, 30.9, 27.8, 27.7, 26.1, 22.7, 18.6, 14.2, -5.1, -5.1. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 2954, 2928, 2894, 2856, 1617, 1575, 1403, 1254, 1154, 1112, 1066, 1050, 897. **HRMS (ESI)**: m/z = 489.3395 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{29}\text{H}_{49}\text{O}_4\text{Si}$ m/z = 489.3395). $[\alpha]_{\text{D}}^{25} = -99.240 \pm 0.050$ (c = 2.0, CHCl_3).

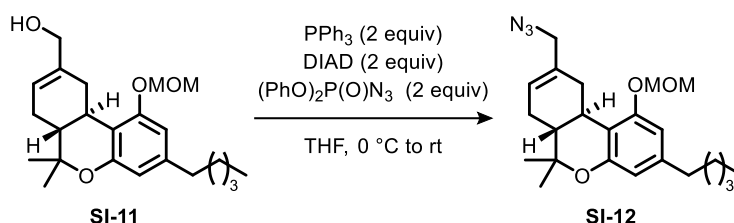
Synthesis of **SI-11**



To a solution of **SI-10** (0.040 g, 0.082 mmol, 1.0 equiv) in THF (0.3 mL) at 0 °C was added TBAF (1 M in THF, 0.41 mL, 0.41 mmol, 5.0 equiv) and the solution was stirred at 0 °C for 1 h, after which TLC (30% EtOAc in hexanes) showed full consumption of starting material. The reaction was stopped by addition of aq. sat. NaHCO_3 and the aqueous phase was extracted with CH_2Cl_2 . The combined organic extracts were washed with brine, dried over Na_2SO_4 , and concentrated under reduced pressure. Purification by flash column chromatography (SiO_2 ; using 20 to 30% EtOAc in hexanes) afforded the product **SI-11** as yellow oil (0.020 g, 65%).

^1H NMR (400 MHz, CDCl_3) δ = 6.44 (d, J = 1.6 Hz, 1H), 6.35 (d, J = 1.6 Hz, 1H), 5.77 – 5.71 (m, 1H), 5.17 (d, J = 1.3 Hz, 2H), 4.08 – 3.97 (m, 2H), 3.49 (s, 3H), 3.38 – 3.29 (m, 1H), 2.70 (td, J = 11.1, 4.8 Hz, 1H), 2.52 – 2.43 (m, 2H), 2.26 – 2.17 (m, 1H), 1.91 – 1.80 (m, 3H), 1.63 – 1.53 (m, 2H), 1.38 (s, 3H), 1.34 – 1.29 (m, 2H), 1.28 – 1.23 (m, 2H), 1.10 (s, 3H), 0.88 (m, 3H). **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3416, 2954, 2928, 2856, 1617, 1574, 1429, 1404, 1182, 1151, 1049, 1010. **HRMS (ESI)**: m/z = 397.2349 $[\text{M}+\text{Na}]^+$ (calc. for $\text{C}_{23}\text{H}_{34}\text{NaO}_4$ m/z = 397.2349).

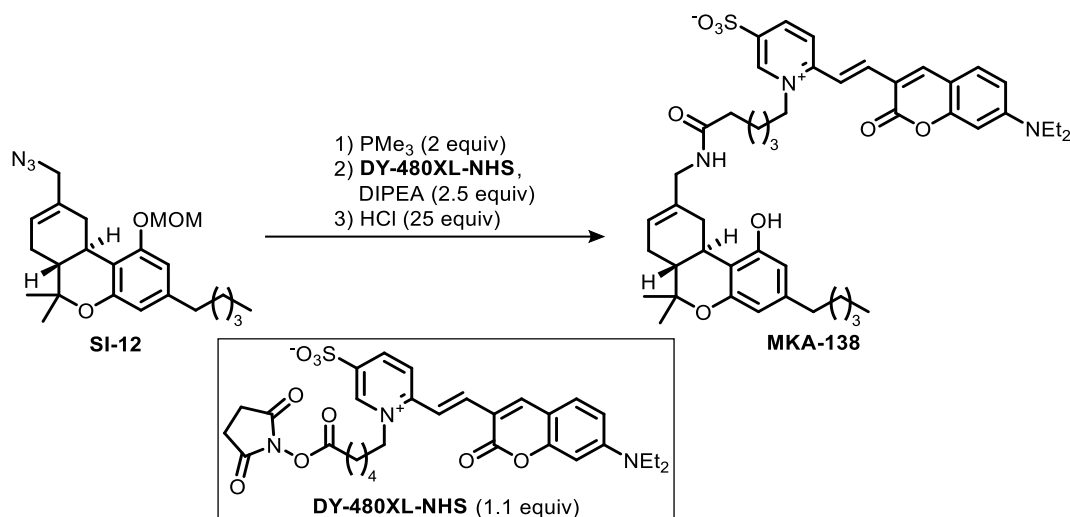
Synthesis of **SI-12**



To a solution of PPh_3 (0.028 g, 0.107 mmol, 2.0 equiv) in THF (0.35 mL) at 0 °C was added DIAD (0.021 mL, 0.107 mmol, 2.0 equiv) and the yellow suspension was stirred at 0 °C for 10 min. A solution of **SI-11** (0.020 g, 0.053 mmol) in THF (0.18 mL) was added, then the mixture was removed from the ice bath and $(\text{PhO})_2\text{P(O)N}_3$ (0.023 mL, 0.107 mmol, 2.0 equiv) was added immediately. The resulting mixture was stirred at ambient temperature for 60 min. When TLC (10% EtOAc in hexanes) showed full consumption of starting material, the reaction mixture was concentrated under reduced pressure. Purification by flash column chromatography (SiO_2 ; using hexanes to 5% Et_2O in hexanes) afforded the product **SI-12** as a yellow oil (0.016 g, 75%).

^1H NMR (400 MHz, CDCl_3) δ = 6.46 (d, J = 1.6 Hz, 1H), 6.36 (d, J = 1.6 Hz, 1H), 5.80 – 5.74 (m, 1H), 5.21 – 5.15 (m, 2H), 3.78 – 3.63 (m, 2H), 3.50 (s, 3H), 3.43 – 3.32 (m, 1H), 2.73 (td, J = 10.9, 4.6 Hz, 1H), 2.53 – 2.45 (m, 2H), 2.32 – 2.21 (m, 1H), 1.98 – 1.78 (m, 3H), 1.63 – 1.53 (m, 2H), 1.39 (s, 3H), 1.35 – 1.27 (m, 4H), 1.11 (s, 3H), 0.92 – 0.86 (m, 3H). **^{13}C NMR** (101 MHz, CDCl_3) δ = 156.7, 154.4, 143.2, 133.6, 125.1, 112.0, 111.3, 106.4, 94.6, 76.4, 57.4, 56.4, 45.0, 36.0, 33.1, 31.8, 31.8, 30.9, 27.9, 27.7, 22.7, 18.5, 14.2. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 2956, 2929, 2857, 2096, 1617, 1575, 1237, 1151. **HRMS (ESI)**: m/z = 422.2413 $[\text{M}+\text{Na}]^+$ (calc. for $\text{C}_{23}\text{H}_{33}\text{NaN}_3\text{O}_3$ m/z = 422.2414).

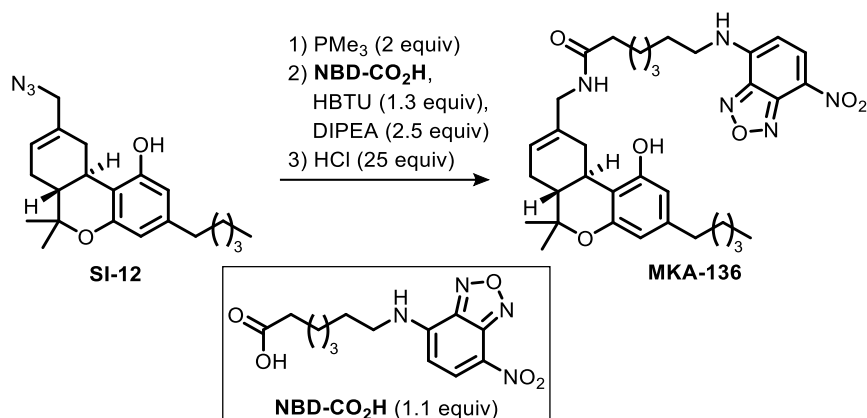
Synthesis of **MKA-138**



To a solution of **SI-12** (3.5 mg, 8.8 μmol , 1.0 equiv.) in 10:1 THF/water (0.1 mL) was added PMe_3 (1 M in THF, 0.018 mL, 0.018 mmol, 2.0 equiv.) and the reaction was stirred at ambient temperature for 4 h, after which TLC (10% EtOAc in hexanes) showed full consumption of starting material. The reaction mixture was concentrated under reduced pressure, and the residue was taken up in CH_2Cl_2 (0.1 mL). **DY-480XL-NHS** (6.0 mg, 9.7 μmol , 1.1 equiv.) and DIPEA (8.8 μL , 0.022 mmol, 2.5 equiv.) were added and the reaction was stirred overnight at ambient temperature. The mixture was concentrated under reduced pressure, the residue was dissolved in MeOH (0.1 mL) and HCl (2 M in Et_2O , 0.10 mL, 0.20 mmol, 25 equiv) was added. The mixture was stirred at ambient temperature for 2 h, then the reaction was concentrated *in vacuo* and directly purified by preparative TLC (SiO_2 ; using 5% MeOH in DCM) afforded the product **MKA-138** as a red solid (4.6 mg, 63% over three steps).

^1H NMR (500 MHz, CD_2Cl_2) δ 9.14 (s, 1H), 8.59 (d, $J = 8.5$ Hz, 1H), 8.06 (d, $J = 8.9$ Hz, 1H), 8.02 (d, $J = 15.4$ Hz, 1H), 7.85 (s, 1H), 7.45 – 7.35 (m, 2H), 6.70 – 6.66 (m, 1H), 6.63 (bs, 1H), 6.54 – 6.45 (m, 1H), 6.22 (d, $J = 1.7$ Hz, 1H), 6.07 (d, $J = 1.6$ Hz, 1H), 5.62 (bs, 1H), 4.67 – 4.53 (m, 2H), 3.82 – 3.68 (m, 2H), 3.51 – 3.35 (m, 5H), 2.58 (td, $J = 10.7, 4.2$ Hz, 1H), 2.37 – 1.96 (m, 10H), 1.85 – 1.39 (m, 6H), 1.37 – 1.14 (m, 13H), 0.99 (s, 3H), 0.82 (t, $J = 7.0$ Hz, 3H). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ 173.4, 160.9, 157.5, 156.9, 155.1, 153.5, 148.1, 143.1, 136.0, 131.5, 124.8, 122.6, 116.1, 113.9, 110.9, 110.8, 109.5, 109.2, 108.5, 97.2, 76.6, 59.4, 46.0, 45.8, 45.6, 36.2, 36.1, 32.7, 32.2, 32.1, 31.4, 29.4, 28.2, 27.9, 25.9, 25.1, 23.1, 18.7, 14.4, 12.8. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3331, 2927, 2856, 1713, 1618, 1579, 1504, 1042.

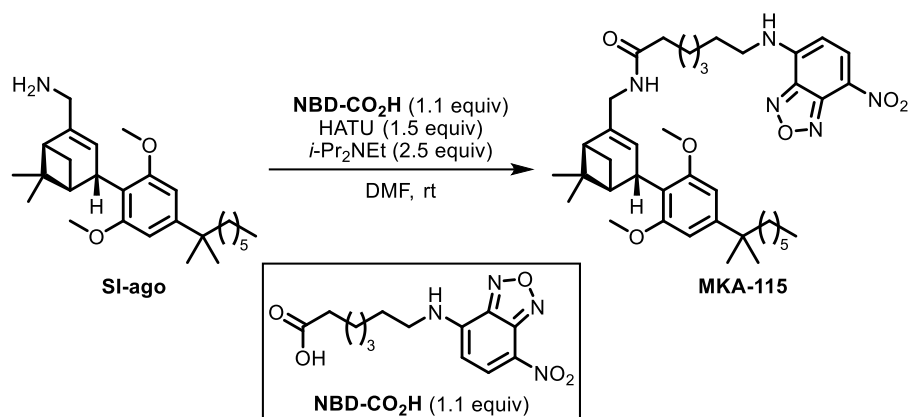
Synthesis of **MKA-136**



To a solution of **SI-12** (3.5 mg, 8.8 μmol , 1.0 equiv) in 10:1 THF/water (0.1 mL) was added PMe_3 (1 M in THF, 0.018 mL, 0.018 mmol, 2.0 equiv.) and the reaction was stirred at ambient temperature for 4 h, after which TLC (10% EtOAc in hexanes) showed full consumption of starting material. The reaction mixture was concentrated under reduced pressure, and the residue was taken up in CH_2Cl_2 (0.1 mL). Acid **NBD-CO₂H** (3.1 mg, 9.1 μmol , 1.1 equiv.), HBTU (5.4 mg, 0.010 mmol, 1.3 equiv.), DIPEA (3.6 μL , 0.021 mmol, 2.5 equiv.) were added and after stirring for 1 h full conversion was observed by TLC (5% MeOH in DCM, 1% 7 N NH_3 in MeOH). Aq. sat. NaHCO_3 was added, and the aqueous layer was extracted with CH_2Cl_2 . The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Finally, the residue was dissolved in MeOH (0.1 mL) and HCl (2 M in Et_2O , 0.10 mL, 0.20 mmol, 25 equiv) was added. The mixture was stirred at ambient temperature for 2 h, then the reaction was stopped by addition of aq. sat. NaHCO_3 . The aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (SiO_2 ; using 60% EtOAc in hexanes) afforded the product **MKA-136** as an orange solid (4.0 mg, 73% over three steps).

¹H NMR (500 MHz, CD_2Cl_2) δ = 8.47 (dd, J = 8.7, 0.5 Hz, 1H), 6.61 (br, 1H), 6.26 – 6.13 (m, 3H), 6.02 (br, 1H), 5.69 – 5.56 (m, 2H), 3.87 – 3.72 (m, 2H), 3.53 – 3.41 (m, 2H), 3.31 (dd, J = 16.0, 4.6 Hz, 1H), 2.66 (td, J = 11.1, 4.8 Hz, 1H), 2.39 (td, J = 7.4, 1.9 Hz, 2H), 2.27 – 2.13 (m, 3H), 1.85 – 1.63 (m, 5H), 1.59 – 1.24 (m, 12H), 1.33 (s, 3H), 1.06 (s, 3H), 0.87 (t, J = 7.0 Hz, 3H). **¹³C NMR** (126 MHz, CD_2Cl_2) δ = 173.6, 156.0, 155.3, 145.0, 143.4, 137.3, 135.9, 121.8, 110.6, 110.1, 108.2, 76.8, 45.6, 44.5, 37.0, 36.0, 33.2, 32.1, 32.0, 31.4, 29.2, 28.7, 28.2, 27.9, 27.1, 25.9, 23.1, 18.7, 14.4. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$) 3314, 2926, 2855, 1621, 1582, 1530, 1443, 1426, 1351, 1298, 1264, 1185. **HRMS (ESI)**: m/z = 620.3440 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_{34}\text{H}_{46}\text{N}_5\text{O}_6$ m/z = 620.3443). $[\alpha]^{25}_{\text{D}} = -86.767 \pm 0.577$ (c = 0.20, CHCl_3).

Synthesis of **MKA-115**

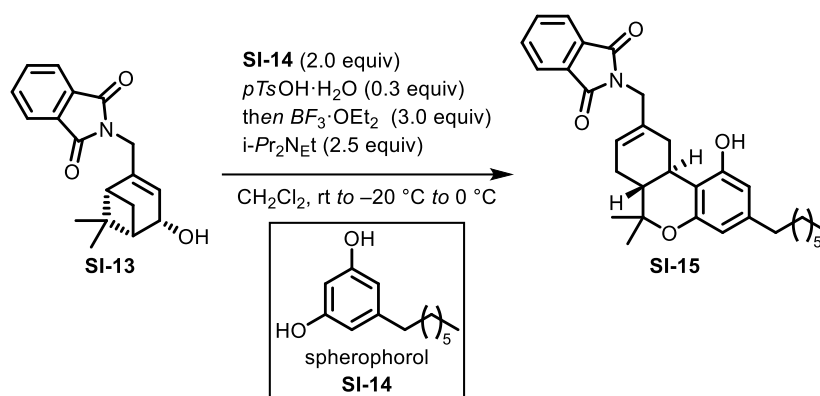


Synthesis of **SI-ago** can be found in Westphal et al. (2020).

To a solution of **NBD-CO₂H** (4.2 mg, 13.5 μ mol, 1.1 equiv) in DMF (0.1 mL) was added HATU (7.0 mg, 18.4 μ mol, 1.5 equiv) and *i*-Pr₂NEt (5 μ L, 30.8 μ mol, 2.5 equiv) and the reaction mixture was stirred at 0 °C for 5 min before being added to a mixture of **SI-ago** (5.1 mg, 12.3 μ mol, 1.0 equiv) in DMF (50 μ L). The reaction mixture was stirred at rt for 1 h and concentrated *in vacuo*. The crude product was purified by preparative TLC (SiO₂, 50% EtOAc in hexanes) to afford the title product **MKA-116** as an orange solid (7.6 mg, 88%).

¹H NMR (400 MHz, CD₂Cl₂) δ 8.47 (d, *J* = 8.7 Hz, 1H), 6.49 (s, 2H), 6.19 (d, *J* = 8.7 Hz, 1H), 5.59 (dt, *J* = 2.9, 1.5 Hz, 1H), 5.39 (t, *J* = 5.7 Hz, 1H), 3.98 – 3.93 (m, 1H), 3.90 – 3.76 (m, 2H), 3.72 (s, 6H), 3.53 – 3.44 (m, 2H), 2.23 – 2.12 (m, 3H), 2.08 (td, *J* = 5.7, 1.4 Hz, 1H), 2.04 – 1.97 (m, 1H), 1.87 – 1.74 (m, 2H), 1.71 – 1.62 (m, 3H), 1.61 – 1.39 (m, 6H), 1.32 – 1.20 (m, 8H), 1.27 (s, 3H), 1.25 (s, 6H), 1.15 – 1.03 (m, 2H), 0.95 (s, 3H), 0.85 (t, *J* = 6.7 Hz, 2H). **¹³C NMR** (101 MHz, CD₂Cl₂) δ 172.29, 158.49, 149.71, 144.53, 144.16, 138.68, 136.77, 123.48, 117.41, 102.81, 55.74, 47.47, 44.46, 44.37, 44.25, 43.94, 40.72, 38.00, 37.52, 36.52, 31.85, 30.09, 29.76, 28.81, 28.67, 28.21, 27.60, 26.55, 26.08, 25.43, 24.74, 22.73, 20.79, 13.90. **IR** (neat, ν_{max} /cm⁻¹) 3312, 2926, 2856, 1649, 1621, 1580, 1530, 1502, 1448, 1410, 1350, 1299, 1272, 1239, 1186, 1162, 1123, 1040. **HRMS (ESI)**: *m/z* = 704.4374 [M+H]⁺ (calc. for C₄₀H₅₈N₅O₆ *m/z* = 704.4382). **[α]_D²⁵** = +38.271 $\pm$ 0.471 (*c* = 0.31, CH₂Cl₂).

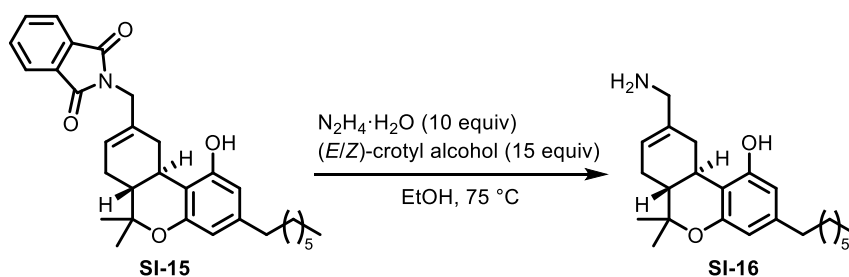
Synthesis of **SI-15**



Verbenol **SI-13** (149 mg, 500 μ mol, 1.0 equiv) was added to a solution of spherophorol **SI-14** (208 mg, 1.00 mmol, 2.0 equiv) and TsOH·H₂O (28.5 mg, 150 μ mol, 0.3 equiv) in CH₂Cl₂ (10 mL), and the mixture was stirred for 1 h at rt. The reaction mixture was cooled to -20 °C. BF₃·OEt₂ (190 μ mol, 1.54 mmol, 3.0 equiv) was added dropwise, and the resulting pale green solution was allowed to warm to 0 °C over the course of 2 h while stirring. The reaction was quenched by addition of brine (25 mL). The phases were separated, and the aqueous layer was extracted with Et₂O (4 $\times$ 20 mL). Combined organic extracts were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO₂; 5 – 15% Acetone in hexanes) afforded the product **SI-15** as an off-white foam (136 mg, 56%).

¹H NMR (400 MHz, CDCl₃) δ 7.91 – 7.82 (m, 2H), 7.77 – 7.67 (m, 2H), 6.24 (s, 2H), 5.98 – 5.92 (m, 1H), 4.44 (ddd, *J* = 15.5, 3.0, 1.9 Hz, 1H), 4.27 – 4.17 (m, 1H), 4.04 – 3.97 (m, 1H), 2.44 – 2.36 (m, 2H), 2.34 – 2.19 (m, 3H), 1.57 – 1.48 (m, 2H), 1.47 (d, *J* = 9.1 Hz, 1H), 1.29 (s, 3H), 1.29 – 1.21 (m, 8H), 0.97 (s, 3H), 0.86 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 168.5, 155.4, 148.0, 143.3, 134.3, 132.0, 123.7, 120.6, 111.9, 108.7, 47.4, 44.3, 43.2, 41.2, 37.8, 35.6, 31.9, 31.0, 29.4, 29.3, 28.1, 26.0, 22.8, 20.7, 14.2. IR (neat, ν_{max} /cm⁻¹): 3449, 2925, 2855, 1770, 1708, 1624, 1578, 1467, 1430, 1392, 1341, 1282, 1111, 1019, 947, 756, 728. HRMS (ESI): *m/z* = 488.279 [M+H]⁺ (calc. for C₃₁H₃₈NO₄ *m/z* = 488.2795). [α]_D²⁵ = -98.221 $\pm$ 0.098 (*c* = 1.0, CHCl₃).

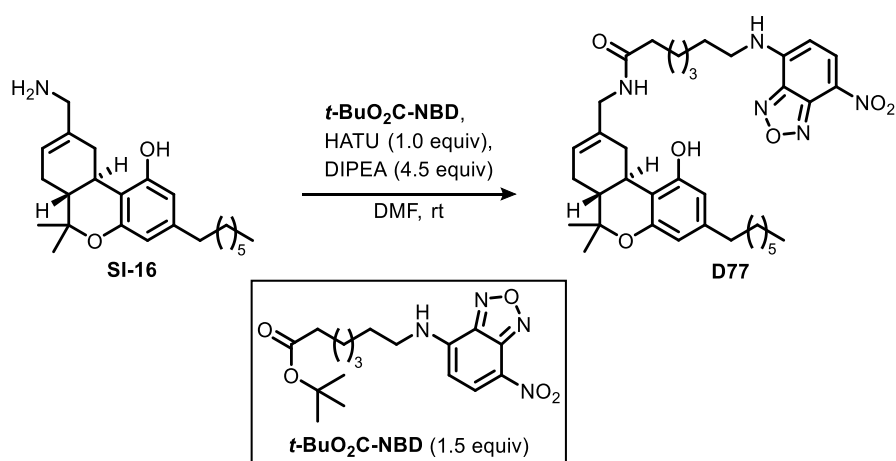
Synthesis of **SI-16**



To a solution of **SI-15** (150 mg, 307 μmol , 1.0 equiv) in EtOH (6 mL) were added $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ (150 μL , 3.08 mmol, 10 equiv) and $(E/Z)\text{-crotyl alcohol}$ (400 μL , 4.69 mmol, 15 equiv), and the resulting mixture was stirred at 75 $^\circ\text{C}$ for 2 h. The mixture was cooled to rt, filtered through a pad of Celite[®] and washed with EtOH (3 $\times$ 30 mL). Purification by flash column chromatography (SiO_2 ; 1% 6M NH_3 in MeOH, 9% MeOH in CH_2Cl_2) afforded the product **SI-16** as a pink oil (82.7 mg, 75%).

^1H NMR (500 MHz, CD_3OD) δ 6.17 (d, $J = 1.6$ Hz, 1H), 6.09 (d, $J = 1.6$ Hz, 1H), 5.71 – 5.67 (m, 1H), 3.41 (ddd, $J = 17.3, 4.8, 2.0$ Hz, 1H), 3.25 – 3.23 (m, 2H), 2.69 – 2.62 (m, 1H), 2.43 – 2.38 (m, 2H), 2.29 – 2.20 (m, 1H), 1.95 – 1.85 (m, 1H), 1.83 – 1.76 (m, 1H), 1.75 (td, $J = 11.7, 4.5$ Hz, 1H), 1.59 – 1.51 (m, 2H), 1.35 (s, 3H), 1.34 – 1.21 (m, 8H), 1.08 (s, 3H), 0.90 (t, $J = 7.0$ Hz, 3H). **^{13}C NMR** (126 MHz, CD_3OD) δ 157.8, 155.8, 143.6, 138.2, 122.2, 111.4, 109.8, 108.5, 77.2, 47.7, 46.8, 36.6, 34.0, 33.1, 33.0, 32.3, 30.3, 30.3, 28.8, 28.0, 23.7, 18.6, 14.4. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$): 2924, 2853, 1619, 1574, 1424, 1382, 1264, 1184, 1109, 1084, 1049, 839. **HRMS (ESI)**: $m/z = 358.2736$ [$\text{M} + \text{H}$]⁺ (calc. for $\text{C}_{23}\text{H}_{36}\text{NO}_2$ $m/z = 358.2741$). $[\alpha]_{\text{D}}^{25} = -143.774 \pm 0.377$ ($c = 1.0$, CHCl_3).

Synthesis of **D77**

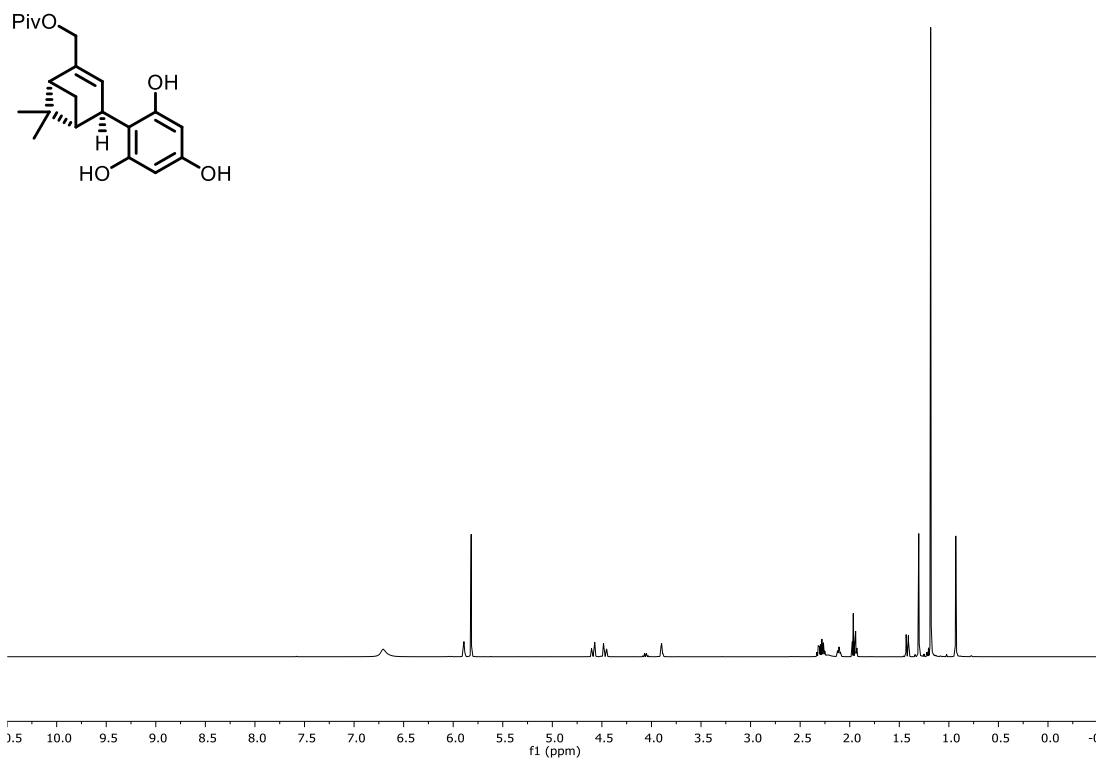


t-BuO₂C-NBD (56.3 mg, 155 μmol , 1.5 equiv) was dissolved in TFA (1.0 mL) and the solution was stirred for 2 h at rt. The mixture was concentrated *in vacuo* and used directly in the next step without further purification. To the solution of this crude, amine **SI-16** (36.5 mg, 102 μmol , 1.0 equiv) and DIPEA (80 μL , 459 μmol , 4.5 equiv) in DMF (0.30 mL), was added HATU (39.0 mg, 103 μmol , 1.0 equiv). The reaction mixture was stirred at rt for 1 h and subsequently quenched with sat. aq. NH_4Cl (20 mL). The mixture was diluted with CH_2Cl_2 (10 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (3 $\times$ 15 mL). Combined organic extracts were dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2 ; 1 – 3%, MeOH in CH_2Cl_2) afforded the product **D77** as a red wax (53.8 mg, 81%).

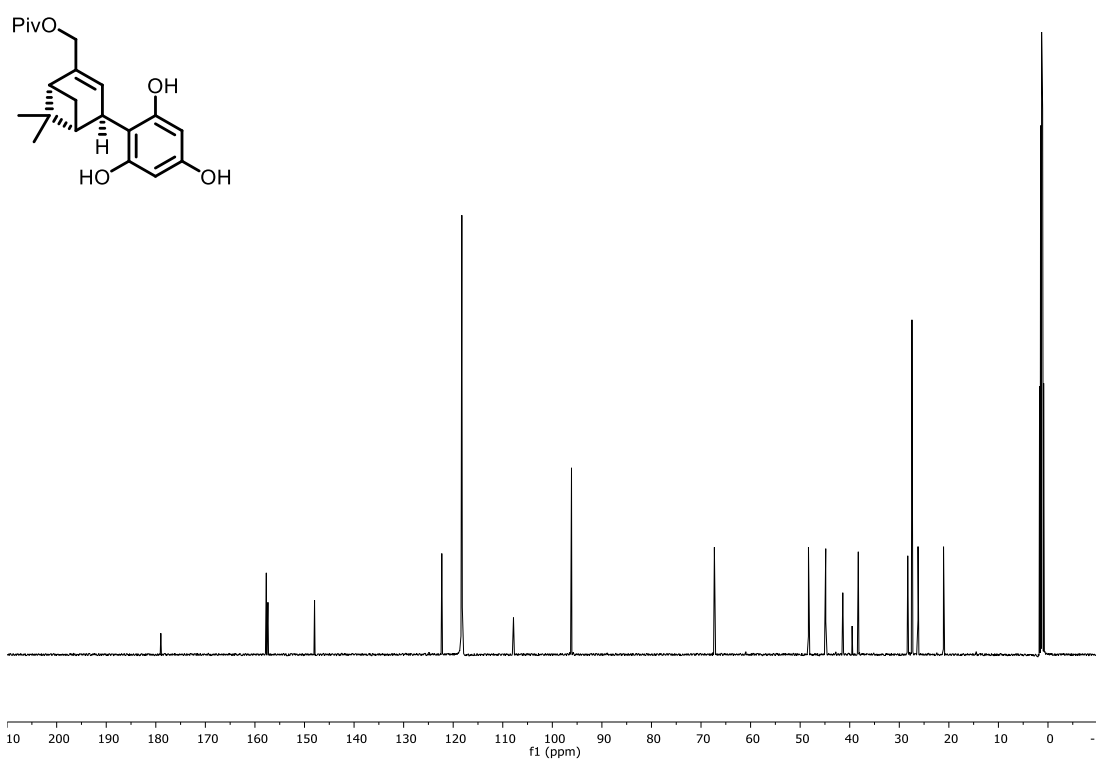
¹H NMR (500 MHz, CDCl_3) δ 8.43 (d, J = 8.7 Hz, 1H), 7.11 (m, 1H), 6.96 (m, 1H), 6.19 (s, 2H), 6.13 (d, J = 8.7 Hz, 1H), 5.86 (t, J = 5.9 Hz, 1H), 5.56 (d, J = 4.9 Hz, 1H), 3.80 (d, J = 5.8 Hz, 2H), 3.53 – 3.40 (m, 2H), 3.34 (dd, J = 15.3, 3.6 Hz, 1H), 2.63 (td, J = 10.9, 4.7 Hz, 1H), 2.35 (td, J = 7.5, 3.6 Hz, 2H), 2.27 (t, J = 7.2 Hz, 2H), 2.18 – 2.08 (m, 1H), 1.85 – 1.64 (m, 5H), 1.54 – 1.43 (m, 6H), 1.32 (s, 3H), 1.30 – 1.16 (m, 10H), 1.04 (s, 3H), 0.84 (t, J = 6.9 Hz, 3H). **¹³C NMR** (126 MHz, CDCl_3) δ 173.8, 155.8, 154.8, 144.5, 144.4, 144.1, 142.9, 136.9, 135.2, 123.4, 121.7, 110.1, 109.5, 108.0, 98.8, 76.4, 45.5, 45.1, 44.1, 36.6, 35.7, 32.7, 31.9, 31.6, 31.1, 29.5, 29.3, 28.8, 28.2, 27.8, 27.6, 26.6, 25.5, 22.8, 18.5, 14.2. **IR** (neat, $\nu_{\text{max}}/\text{cm}^{-1}$): 3317, 2924, 2853, 1620, 1580, 1529, 1442, 1350, 1296, 1262, 1184. **HRMS (ESI)**: m/z = 670.3576 [$\text{M}+\text{Na}$]⁺ (calc. for $\text{C}_{36}\text{H}_{49}\text{N}_5\text{NaO}_6$ m/z = 670.3575). $[\alpha]_{\text{D}}^{25} = -96.006 \pm 0.321$ (c = 1.0, CHCl_3).

NMR spectra

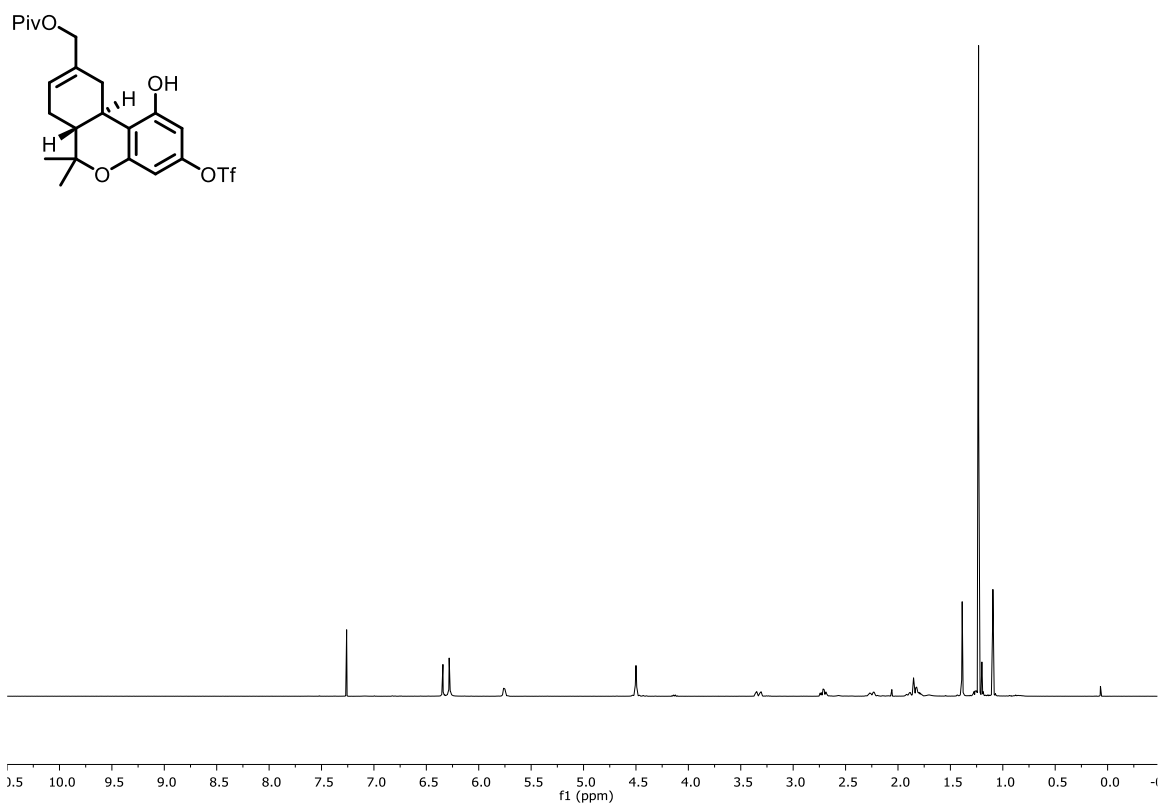
^1H NMR (400 MHz, CD_3CN) of **SI-3**



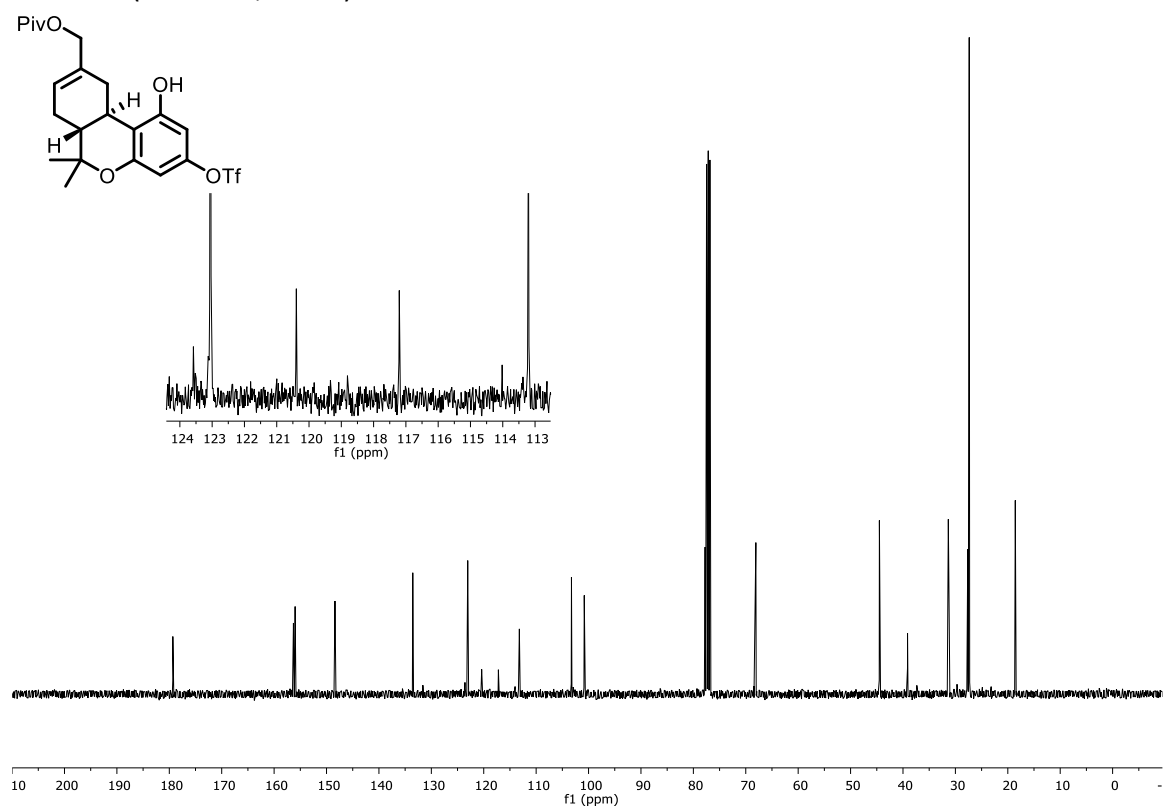
^{13}C NMR (101 MHz, CD_3CN) of **SI-3**



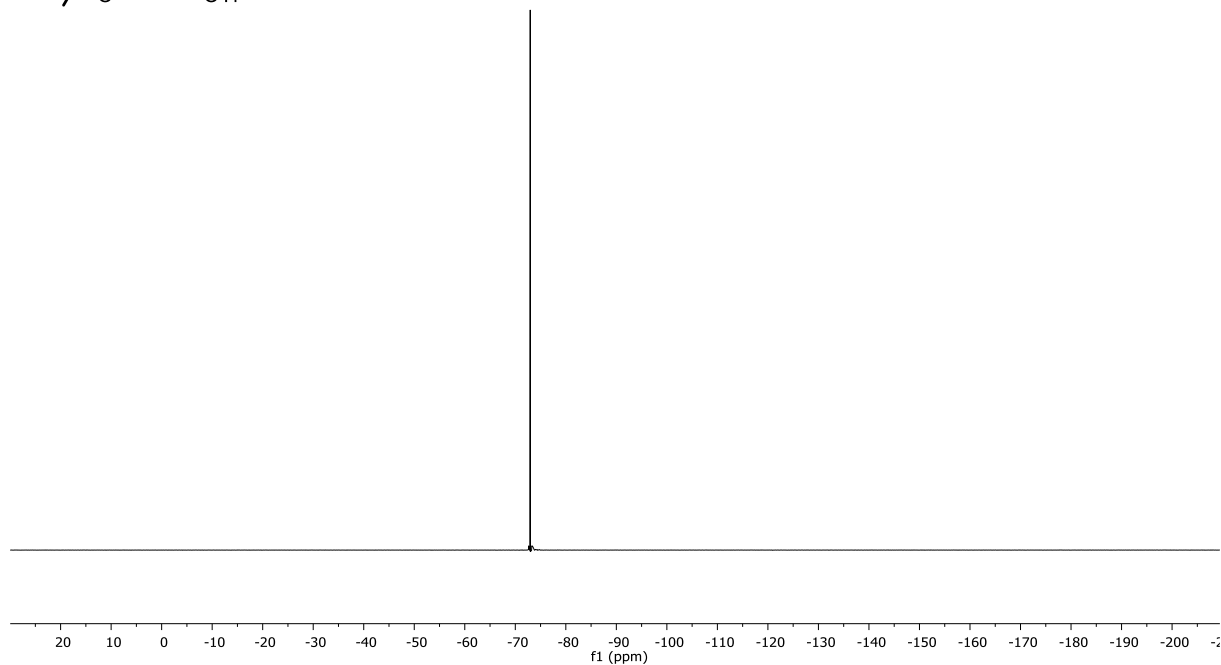
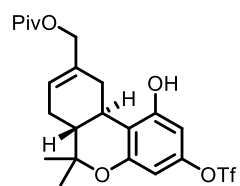
¹H NMR (400 MHz, CDCl₃) of **SI-5**



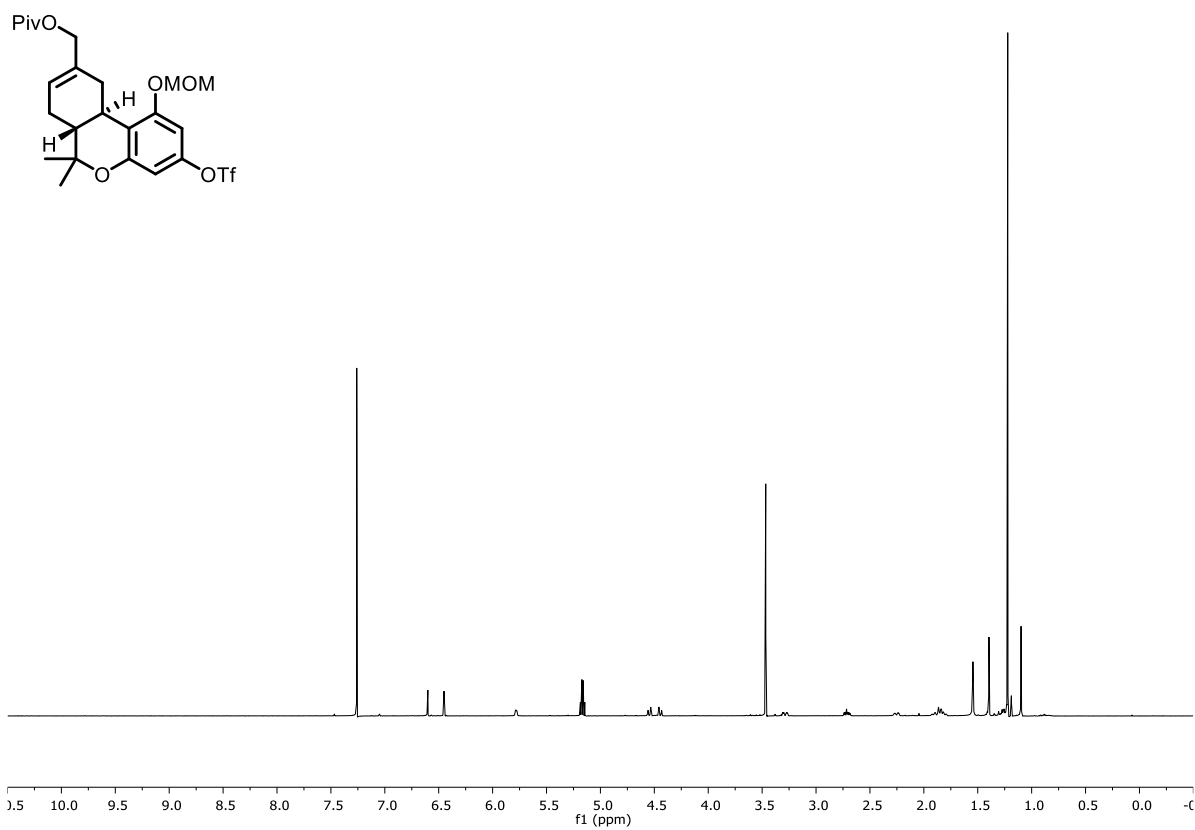
¹³C NMR (101 MHz, CDCl₃) of **SI-3**



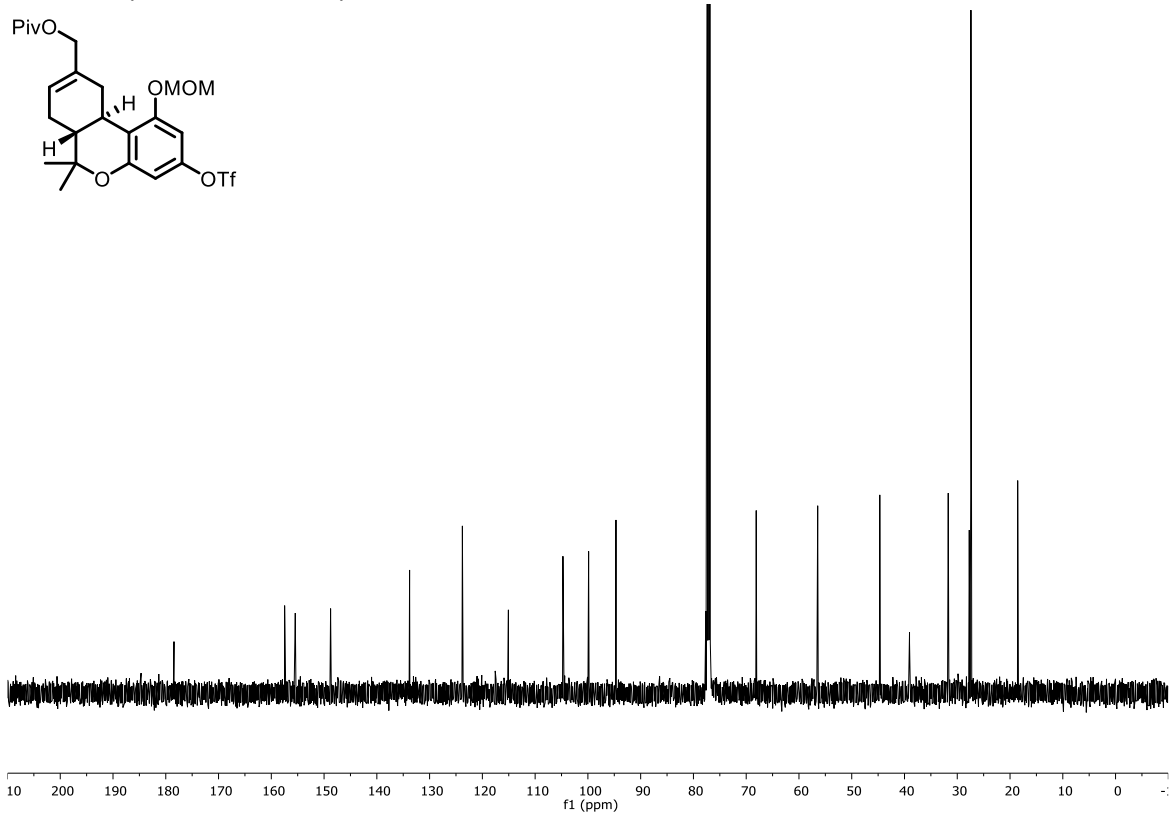
^{19}F NMR (376 MHz, CDCl_3) of **SI-5**



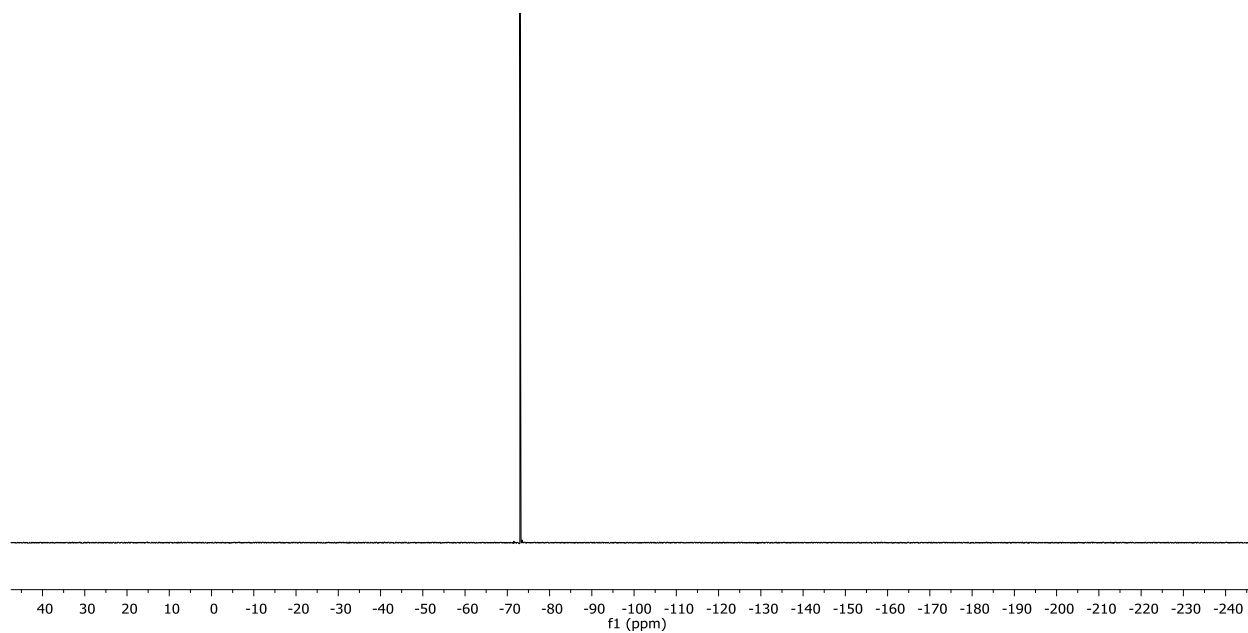
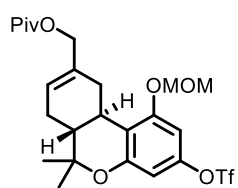
^1H NMR (500 MHz, CDCl_3) of **SI-6**



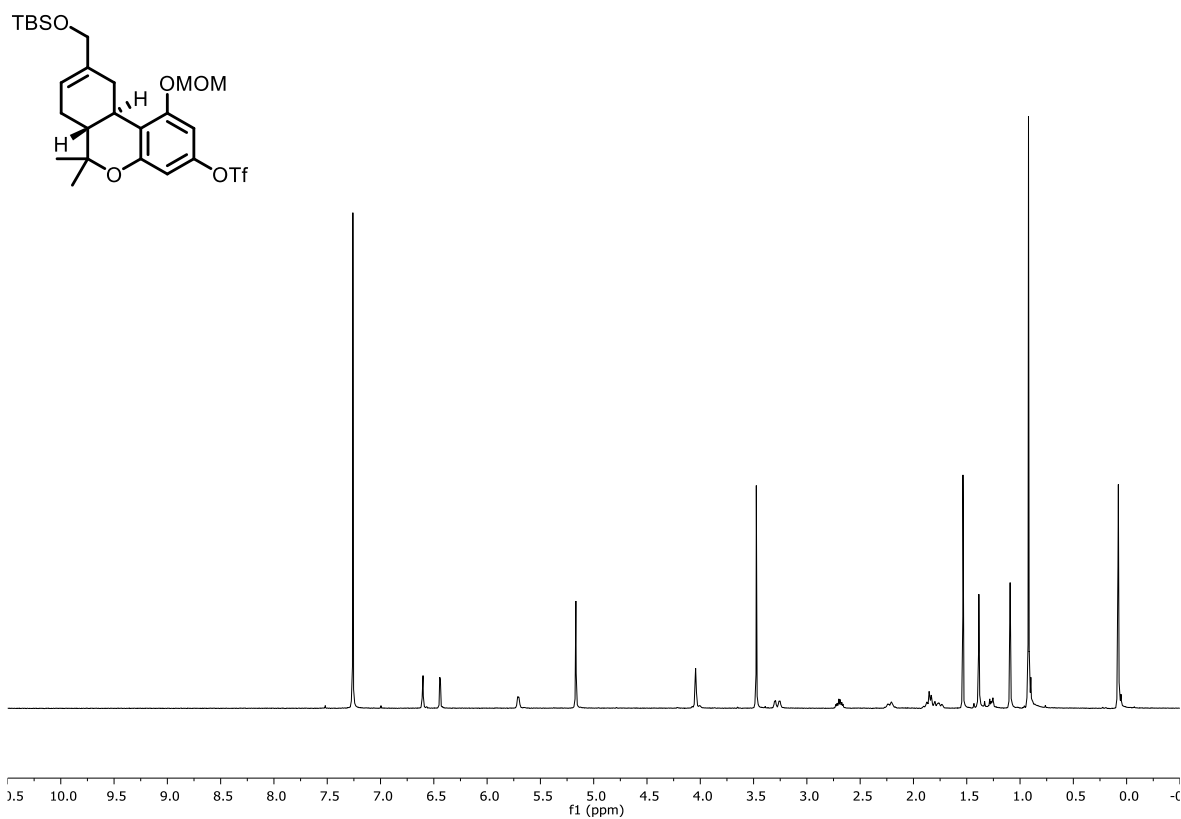
^{13}C NMR (126 MHz, CDCl_3) of **SI-6**



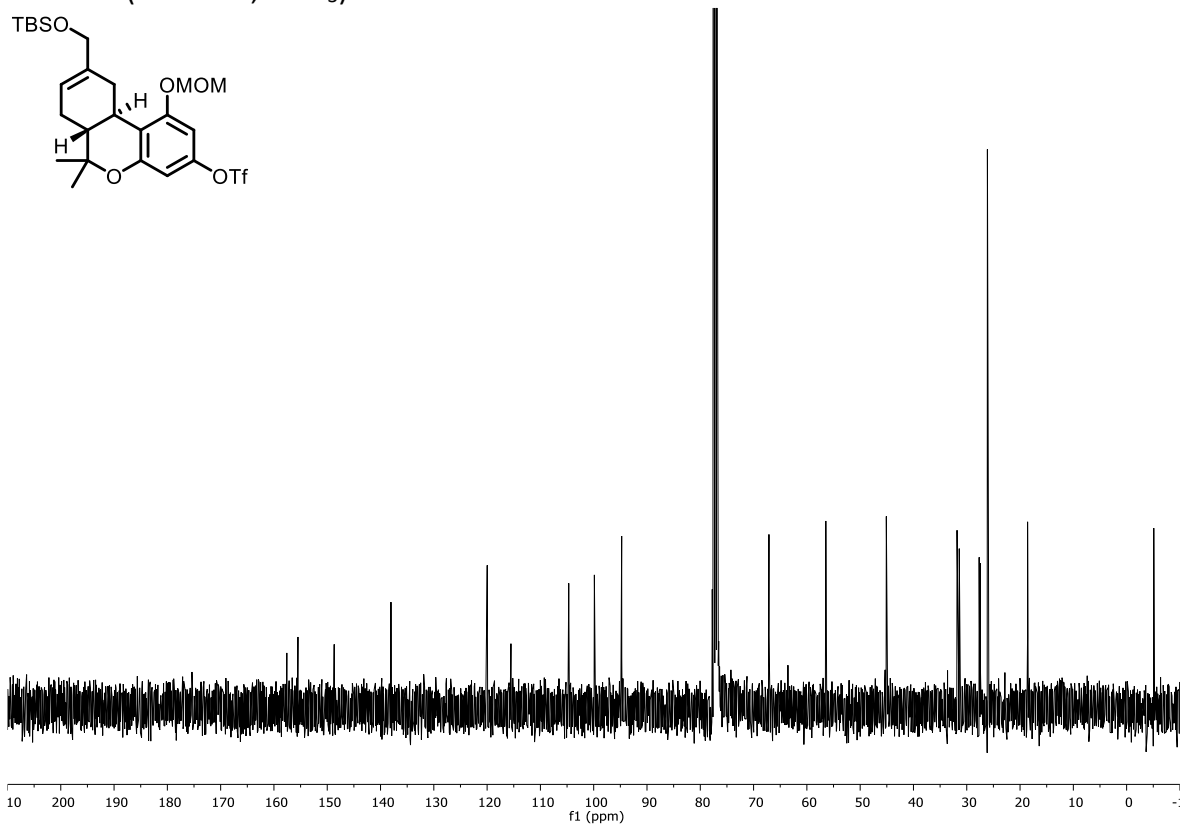
^{19}F NMR (471 MHz, CDCl_3) of **SI-6**



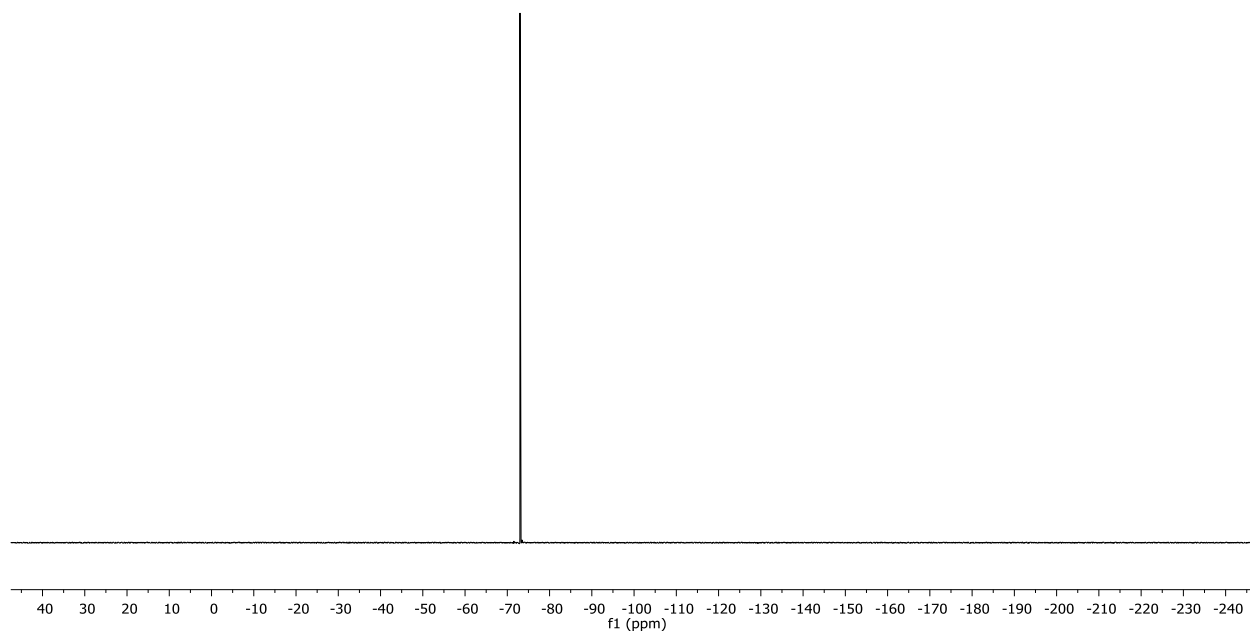
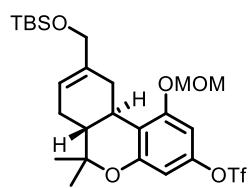
¹H NMR (400 MHz, CDCl₃) of **SI-7**



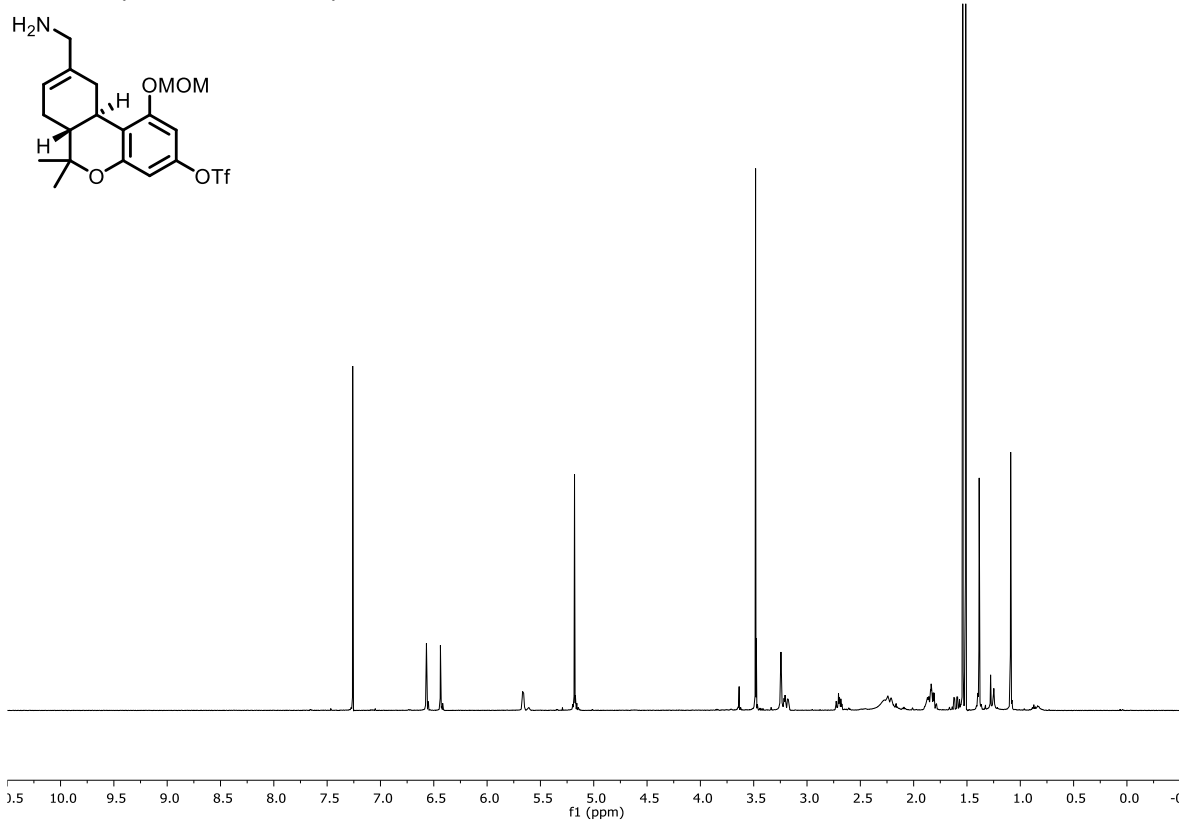
¹³C NMR (101 MHz, CDCl₃) of **SI-7**



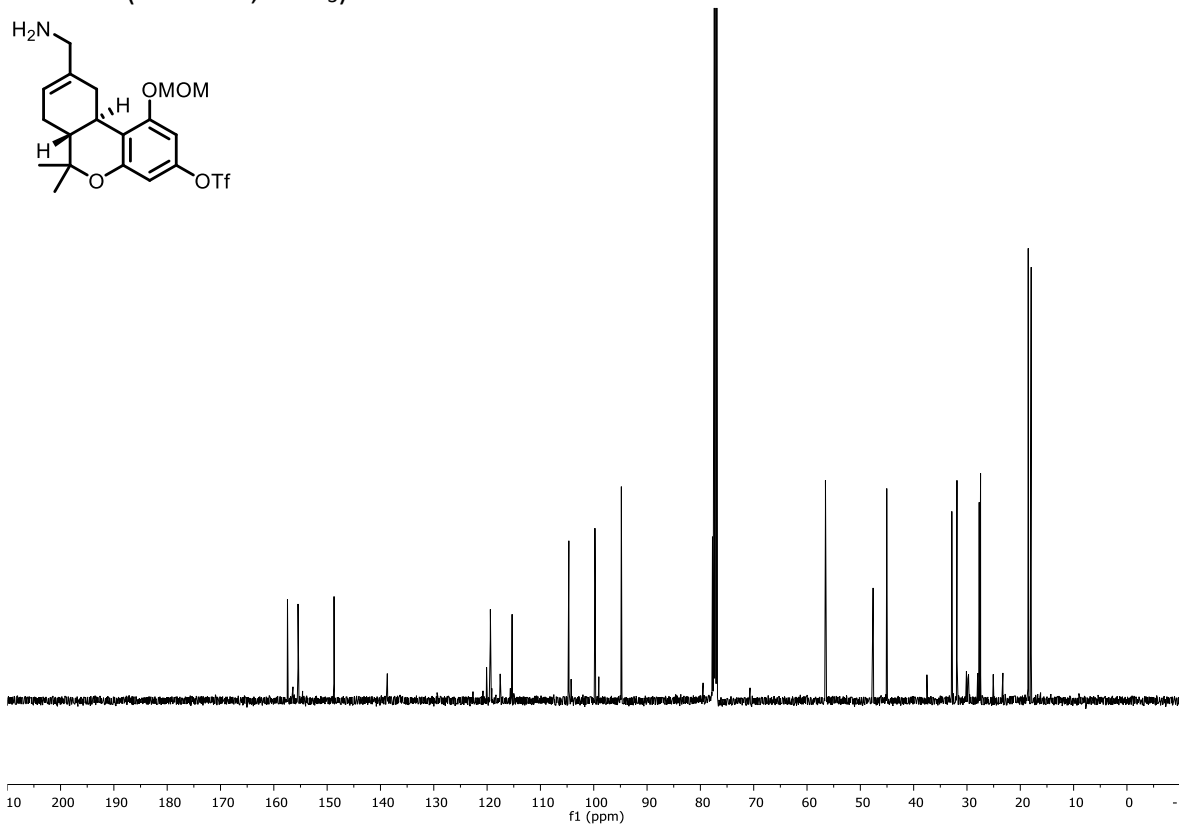
^{19}F NMR (376 MHz, CDCl_3) of **SI-7**



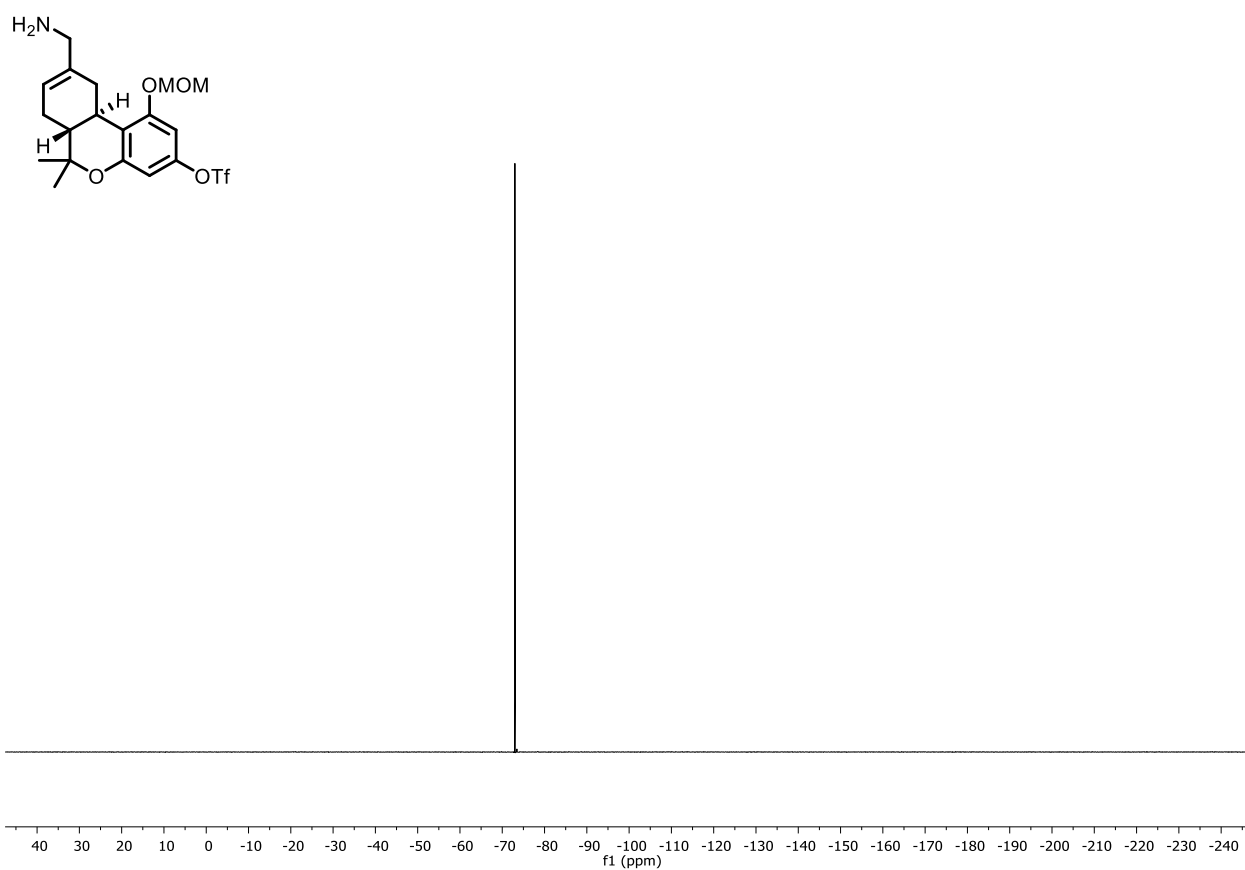
¹H NMR (500 MHz, CDCl₃) of **SI-8**



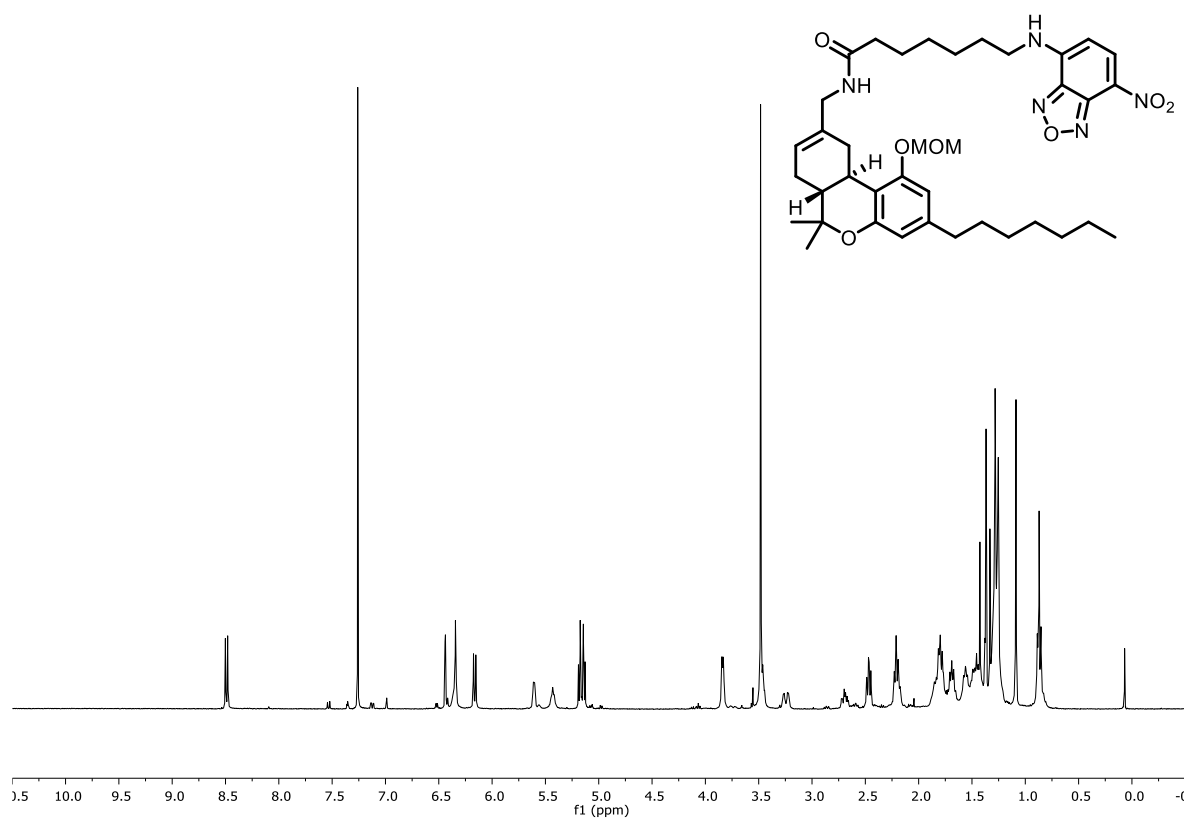
¹³C NMR (126 MHz, CDCl₃) of **SI-8**



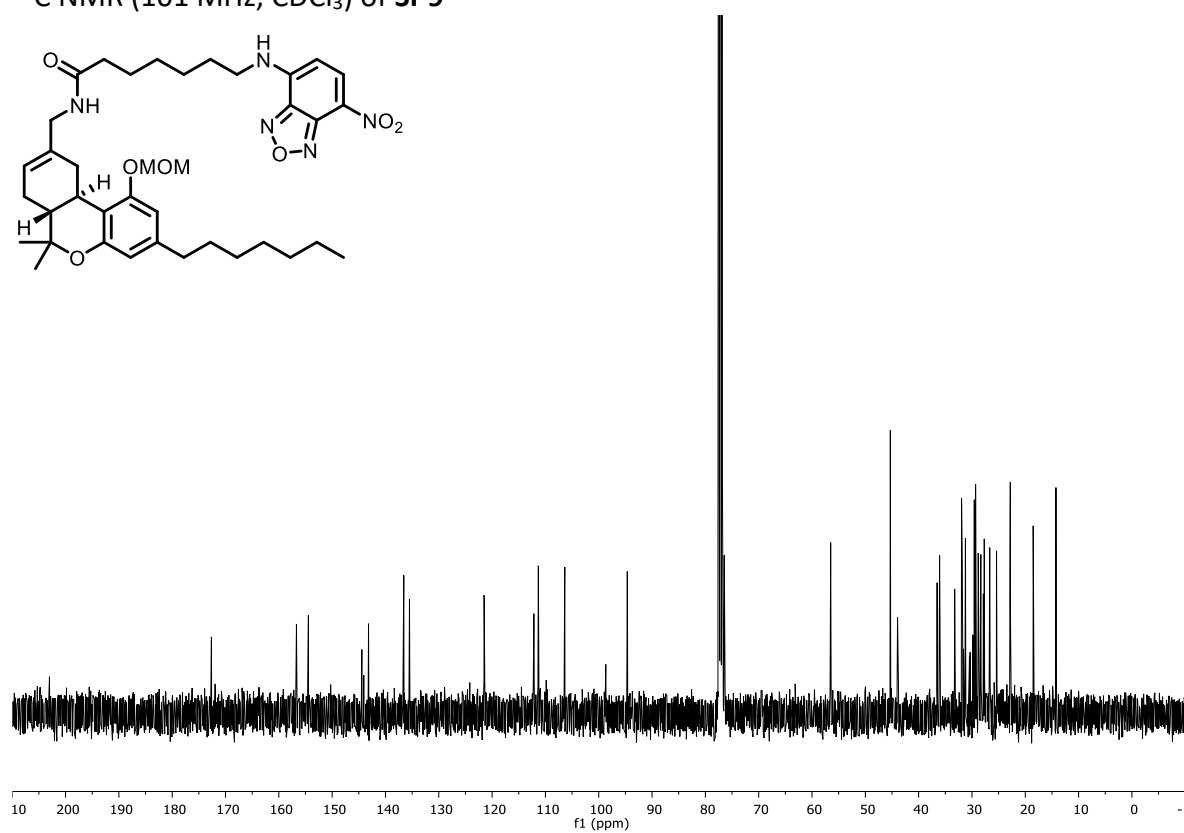
^{19}F NMR (471 MHz, CDCl_3) of **SI-8**



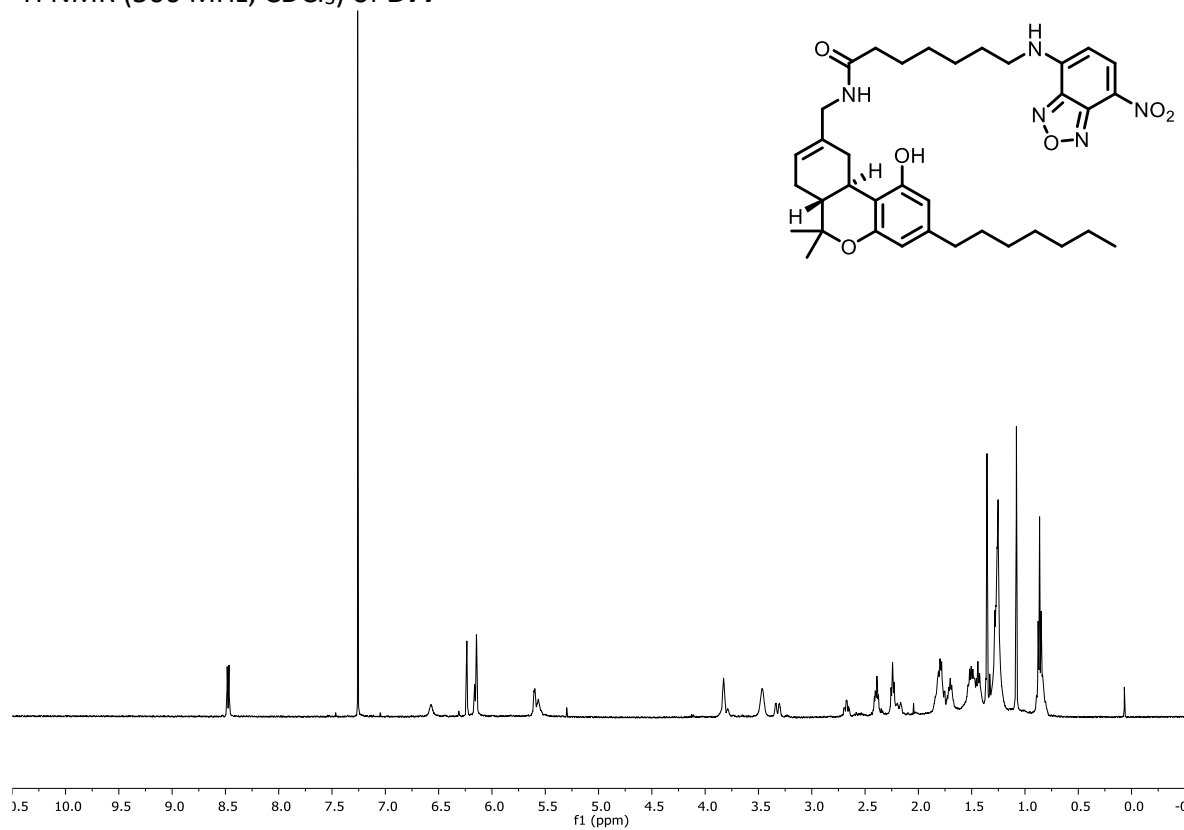
^1H NMR (400 MHz, CDCl_3) of **SI-9**



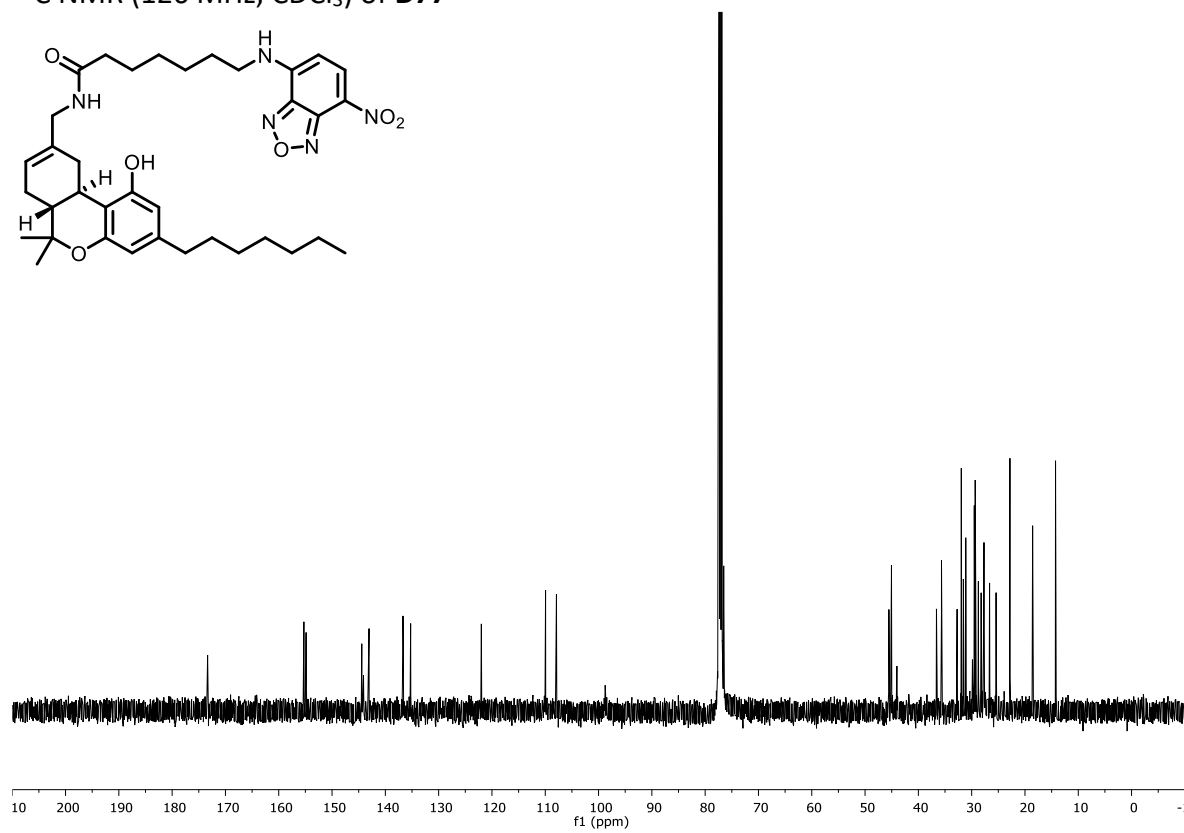
^{13}C NMR (101 MHz, CDCl_3) of **SI-9**



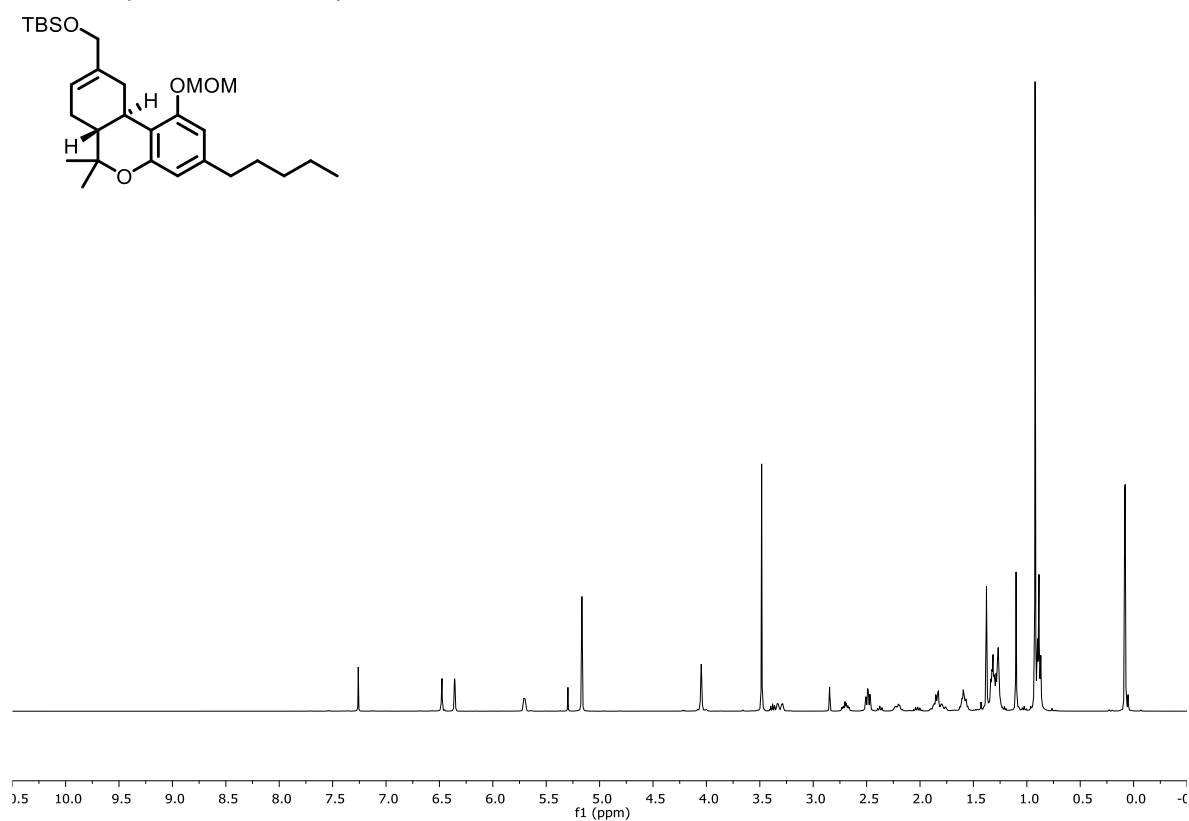
¹H NMR (500 MHz, CDCl₃) of **D77**



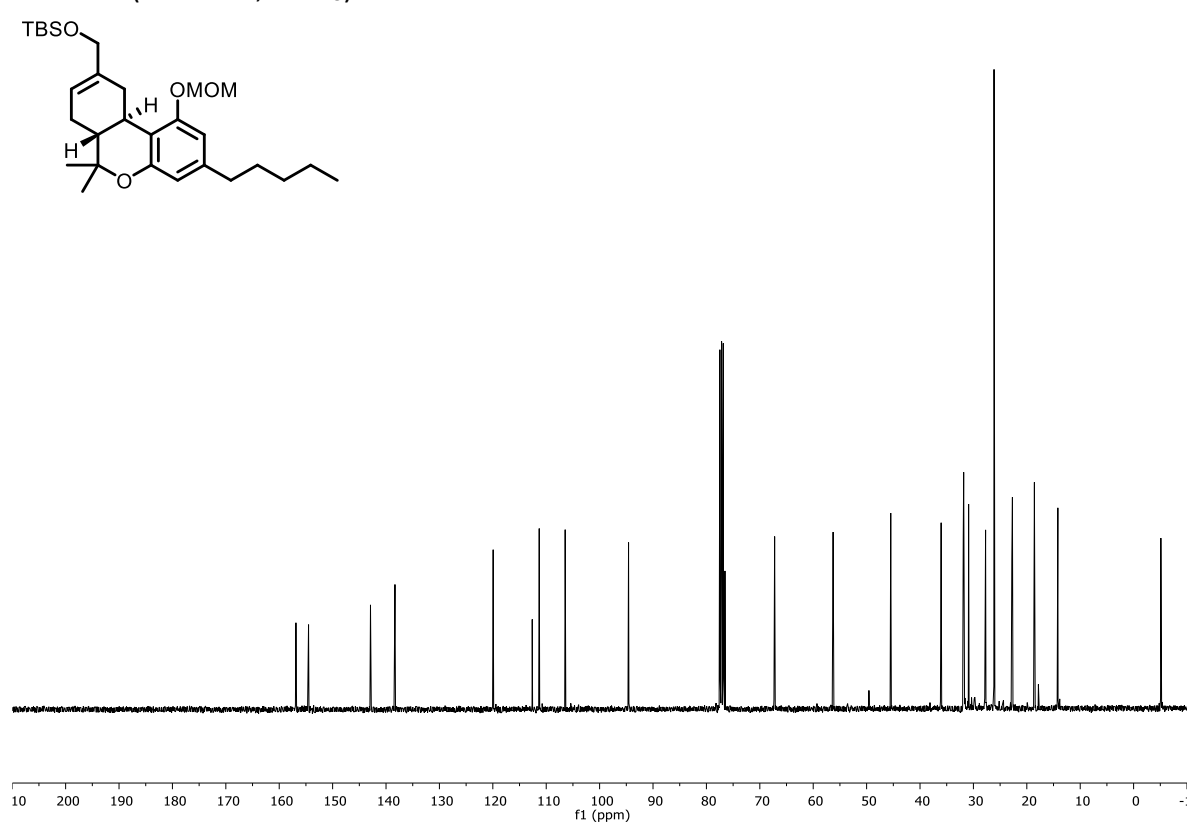
¹³C NMR (126 MHz, CDCl₃) of **D77**

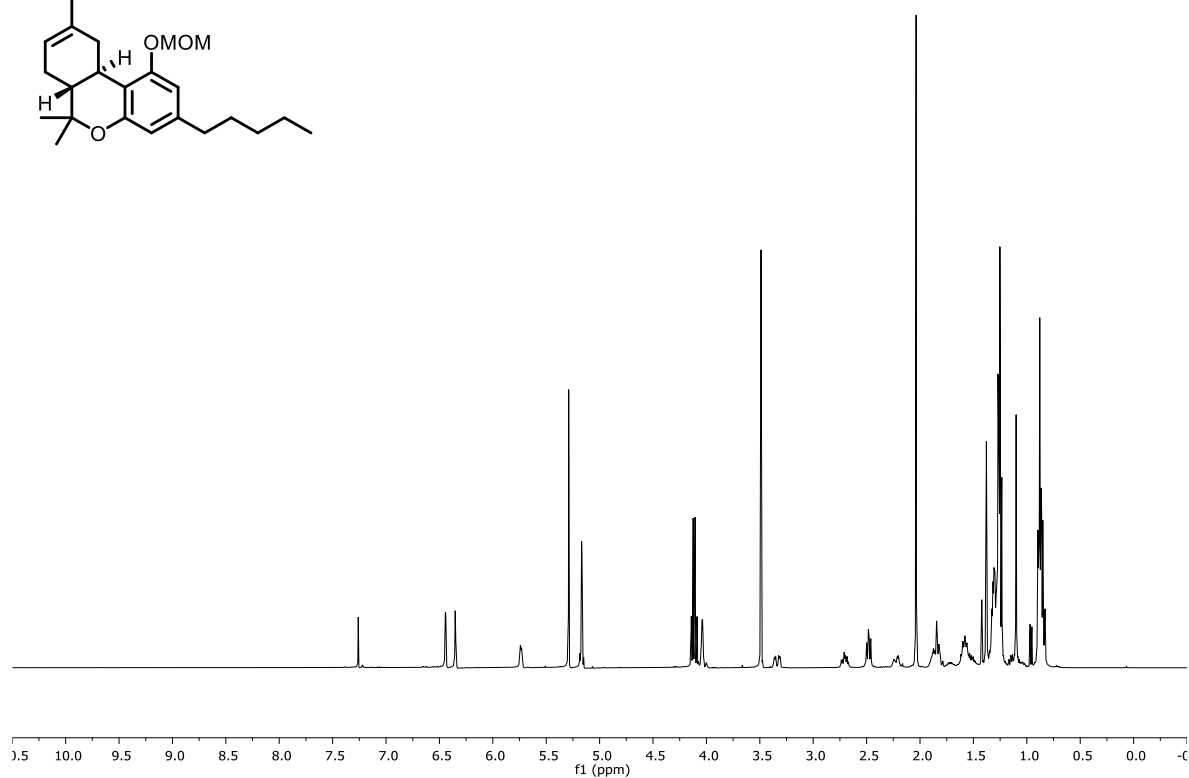
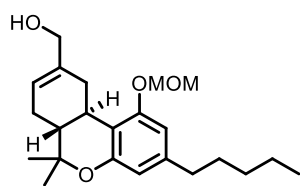


¹H NMR (400 MHz, CDCl₃) of **SI-10**

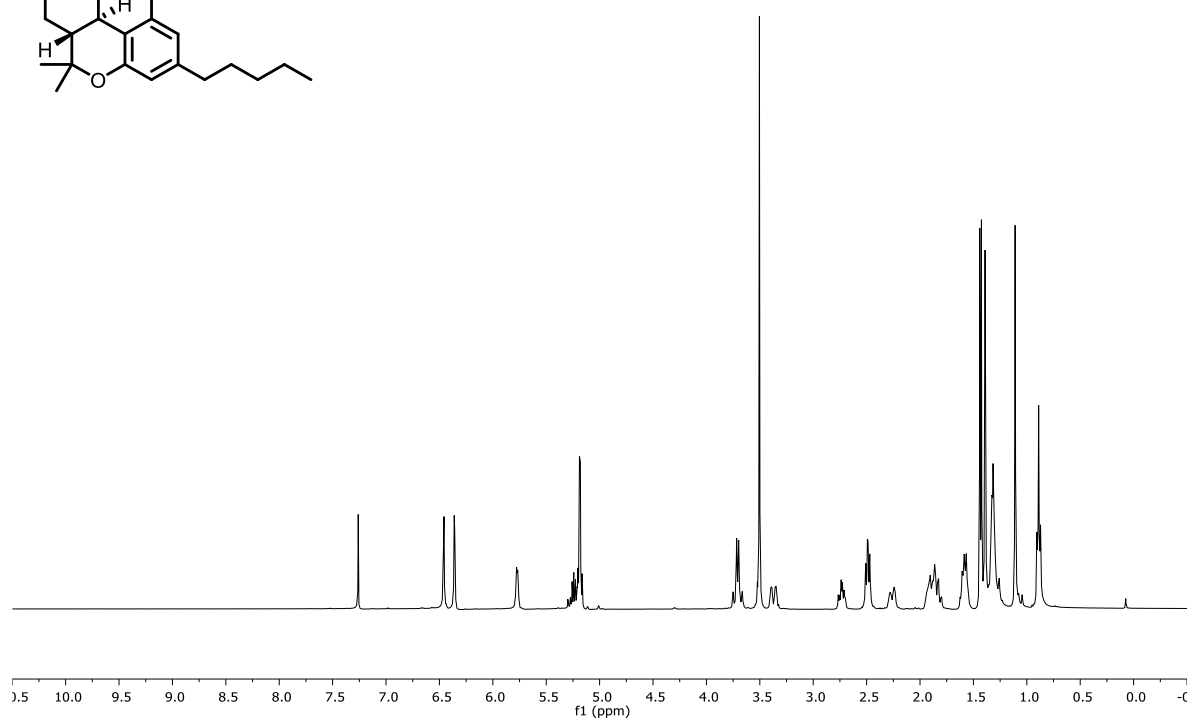
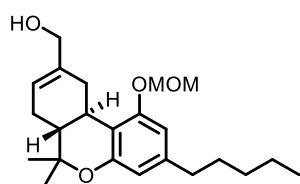


¹³C NMR (101 MHz, CDCl₃) of **SI-10**

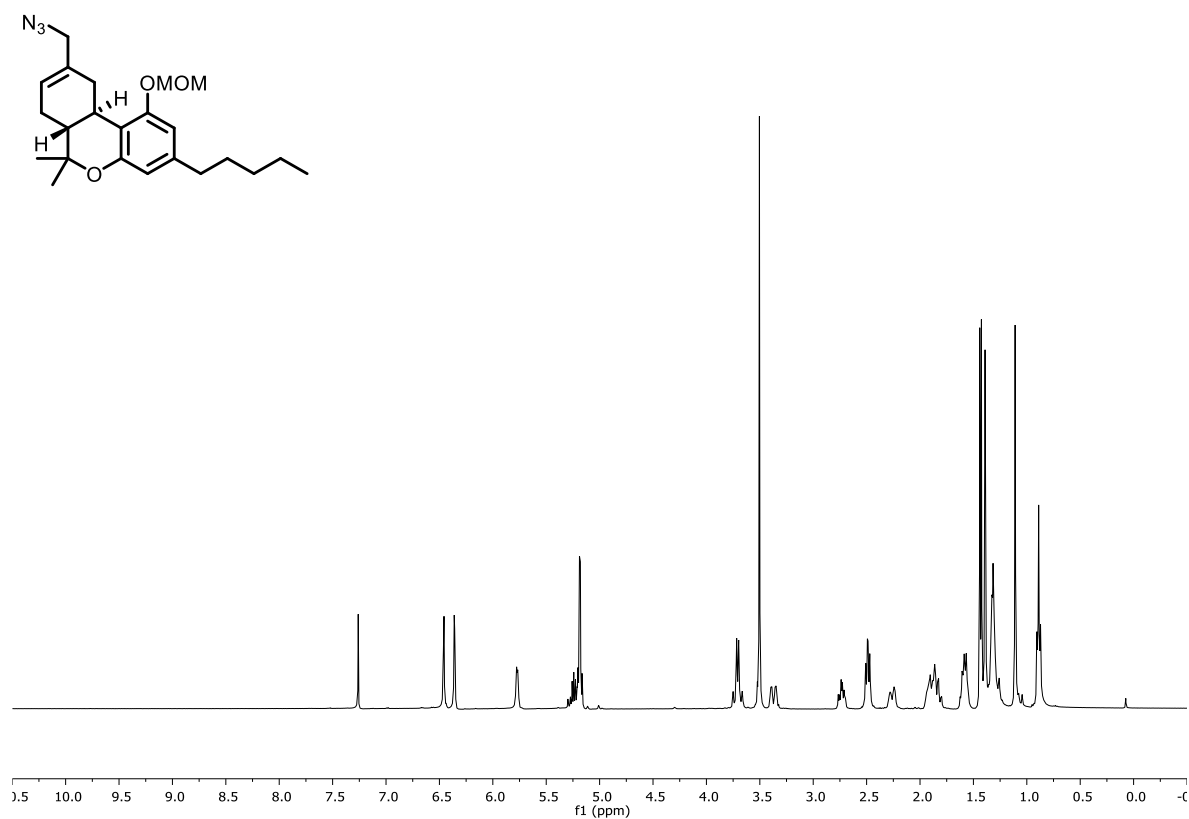


¹H NMR (400 MHz, CDCl₃) of **SI-11**

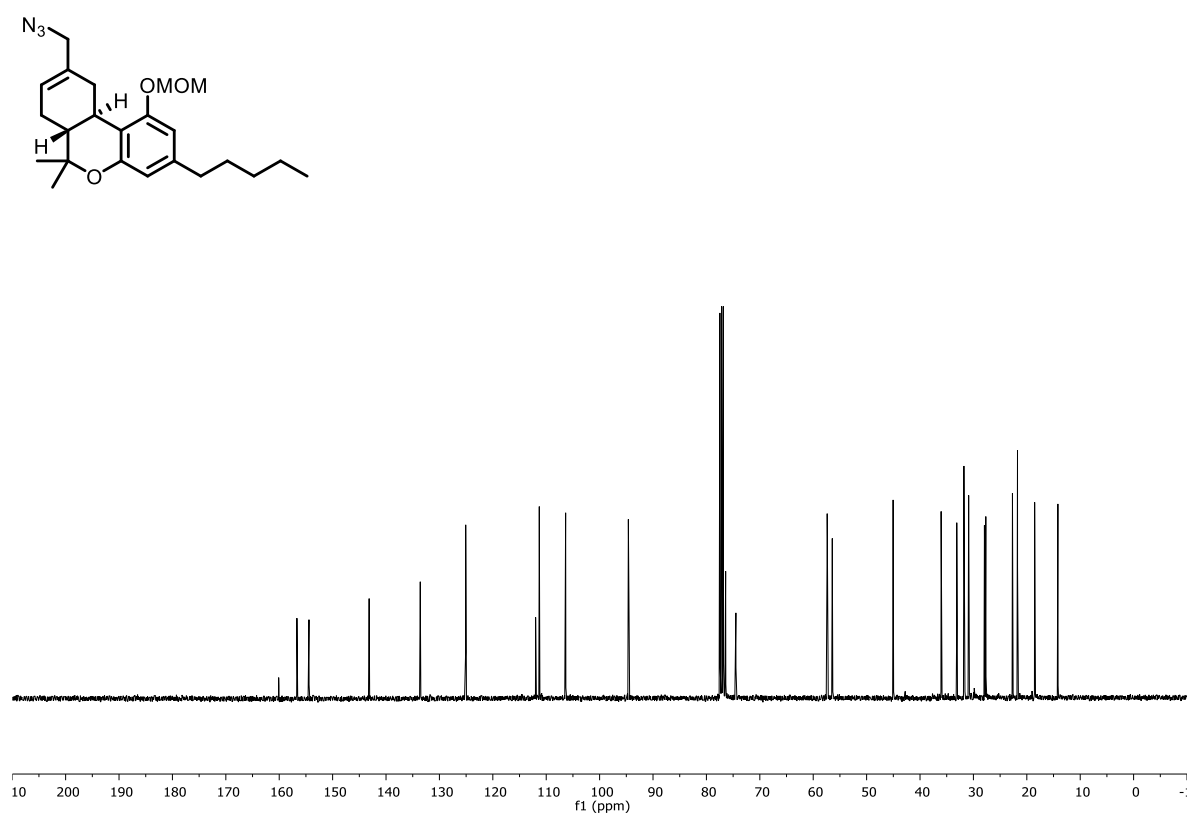
¹³C NMR (101 MHz, CDCl₃) of **SI-11**



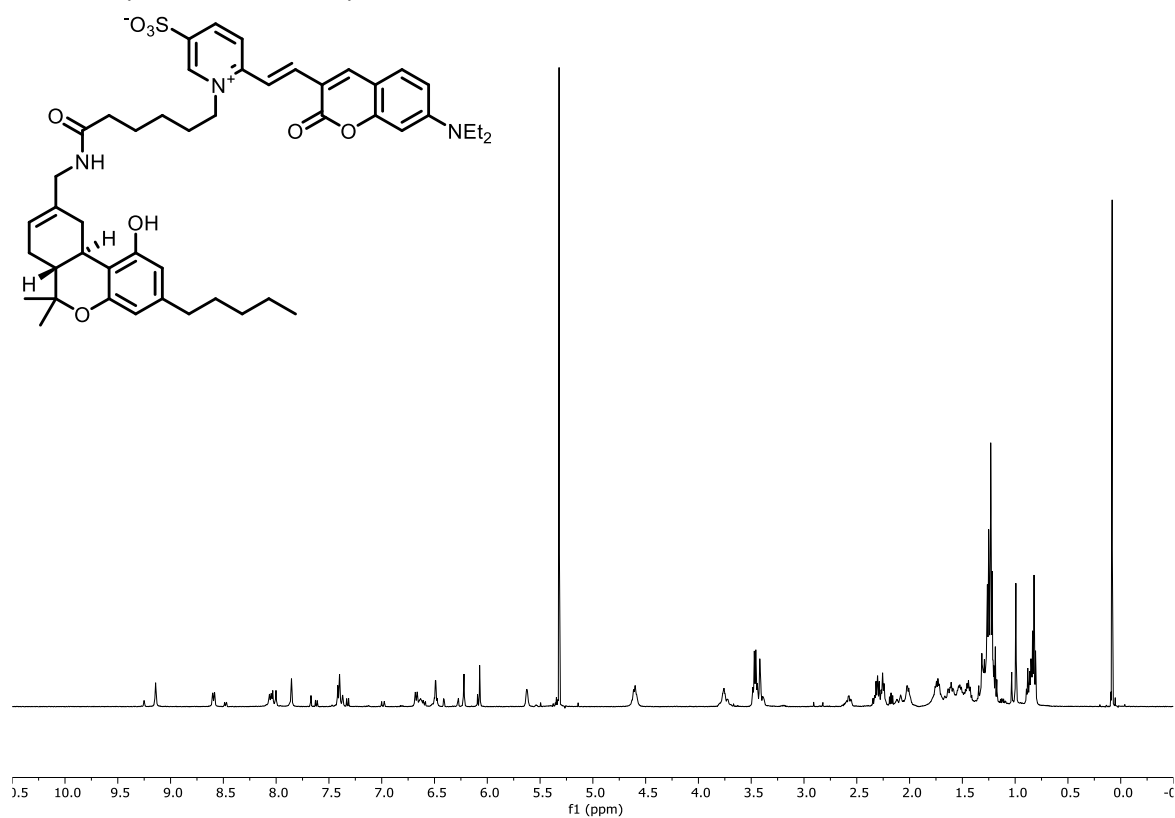
¹H NMR (400 MHz, CDCl₃) of **SI-12**



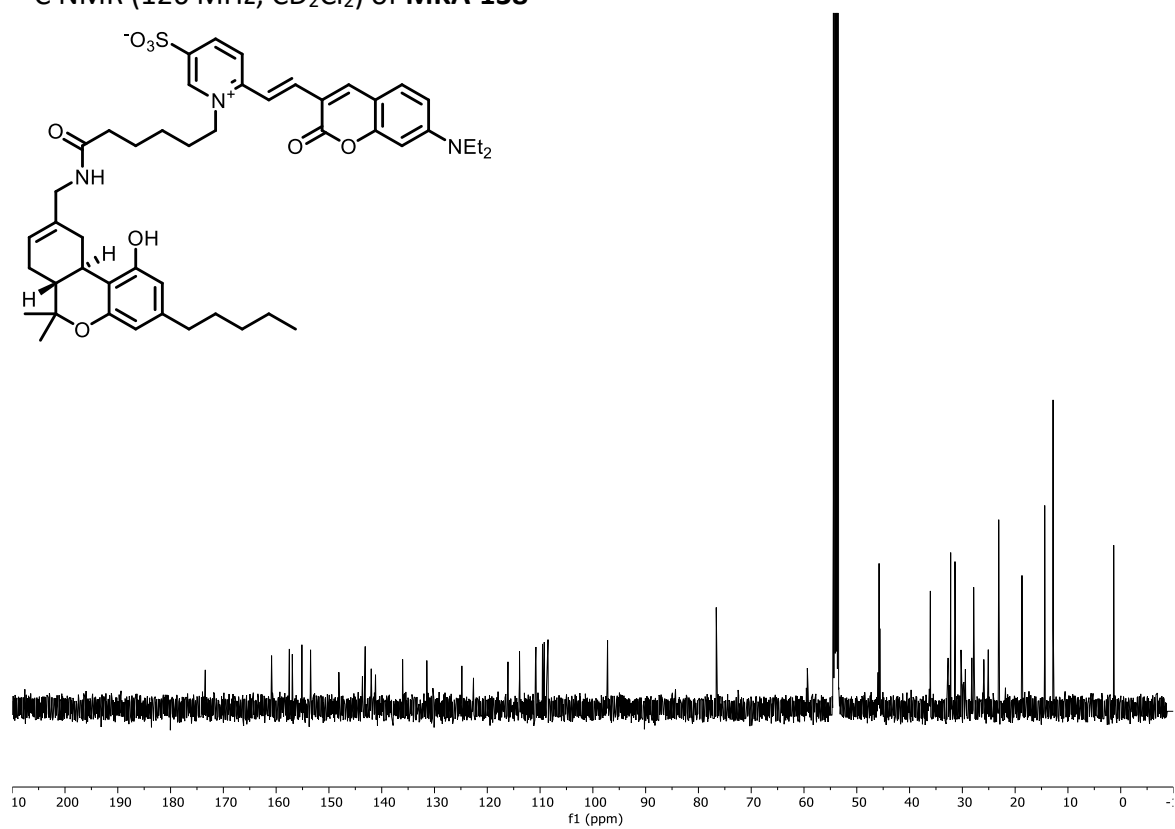
¹³C NMR (101 MHz, CDCl₃) of **SI-12**



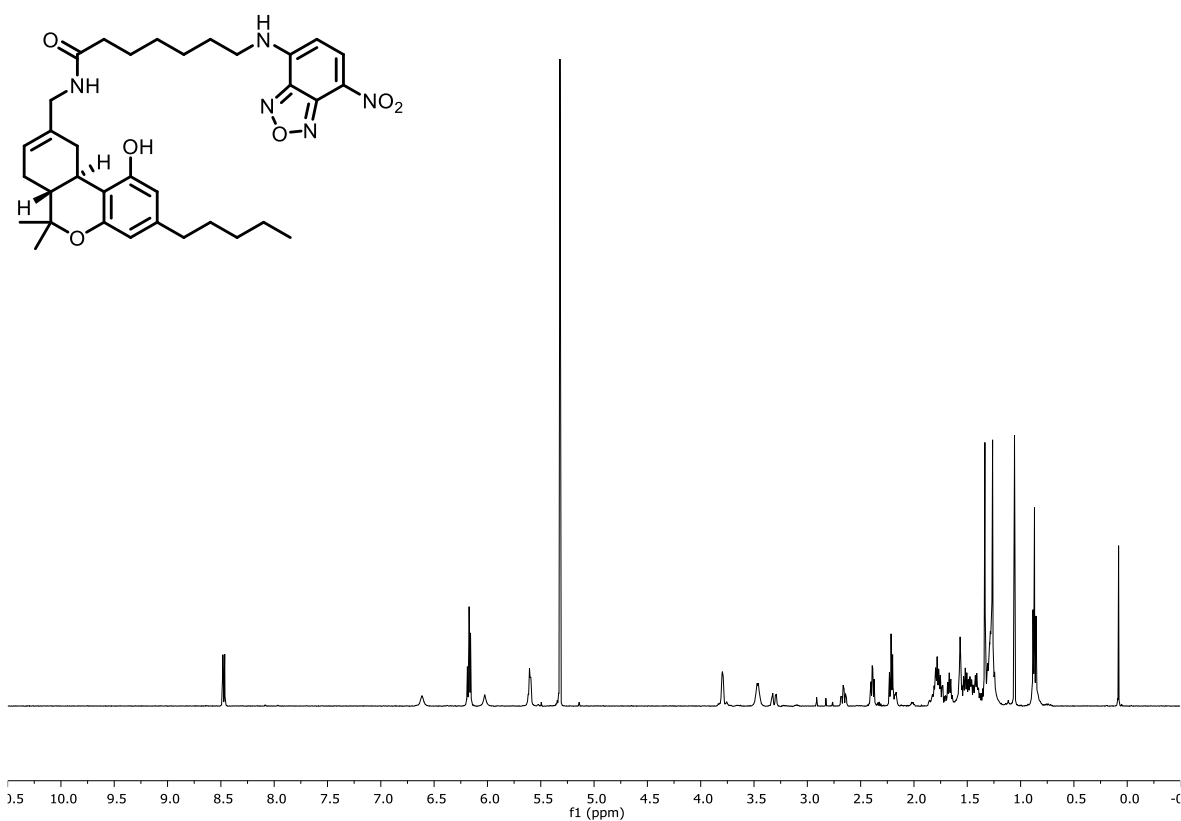
¹H NMR (500 MHz, CD₂Cl₂) of **MKA-138**



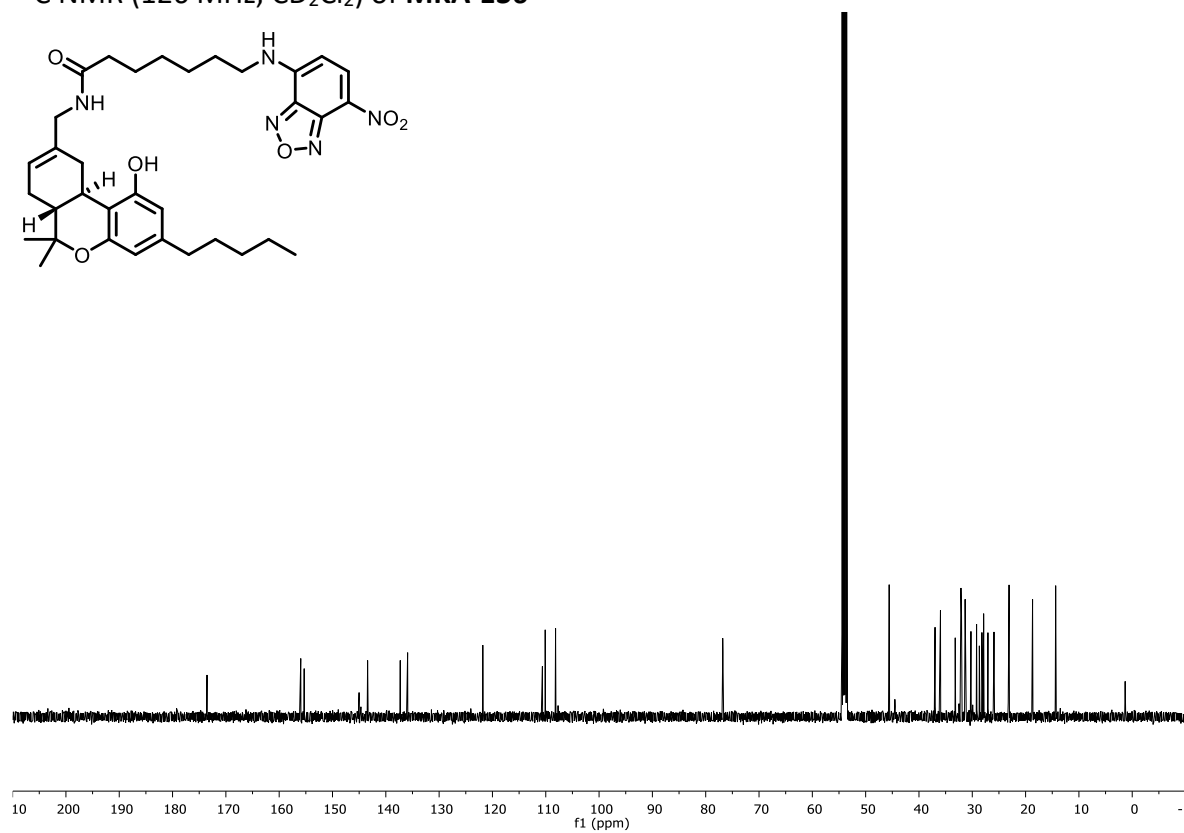
¹³C NMR (126 MHz, CD₂Cl₂) of **MKA-138**



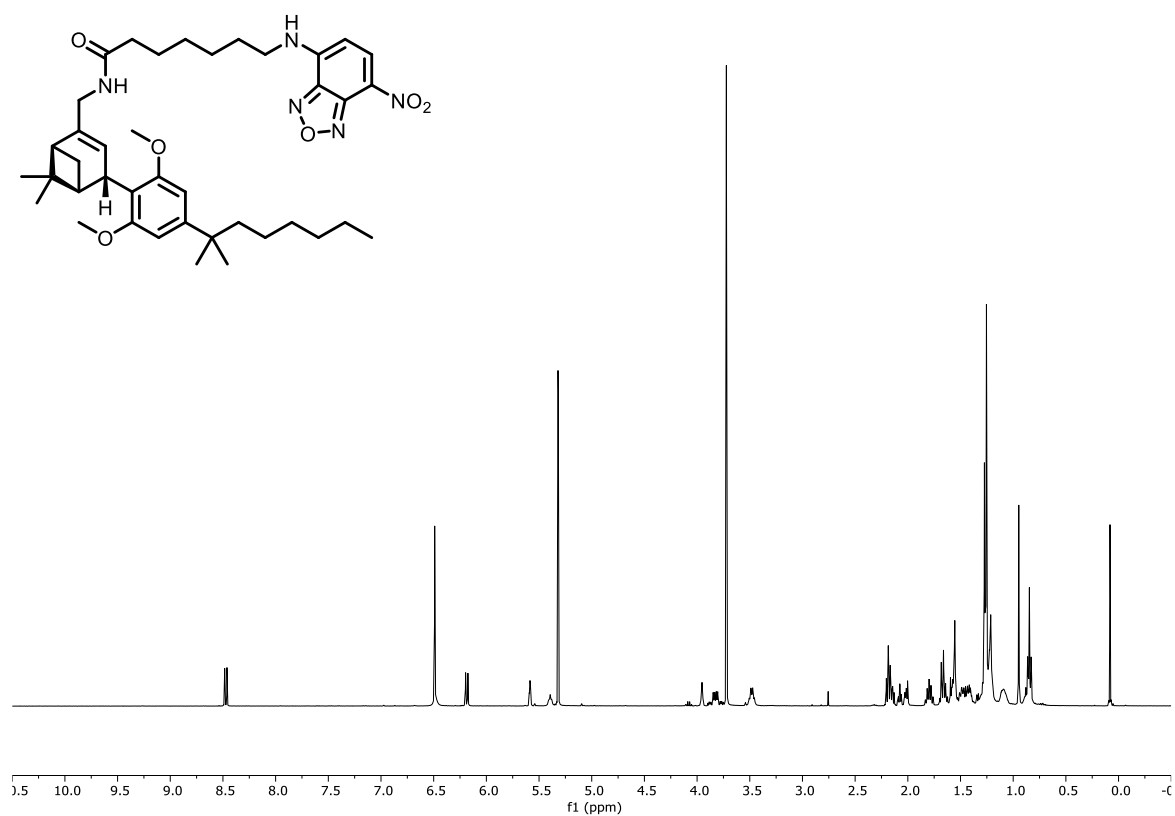
¹H NMR (500 MHz, CD₂Cl₂) of **MKA-136**



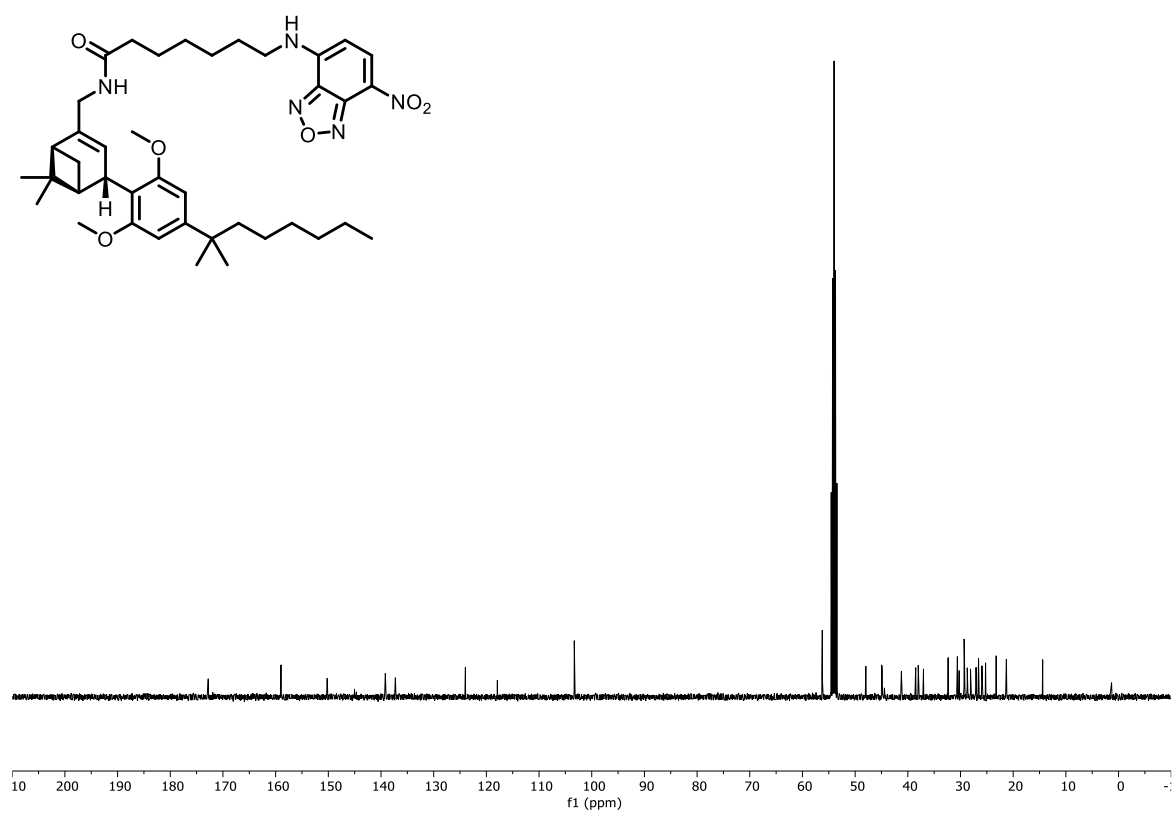
¹³C NMR (126 MHz, CD₂Cl₂) of **MKA-136**



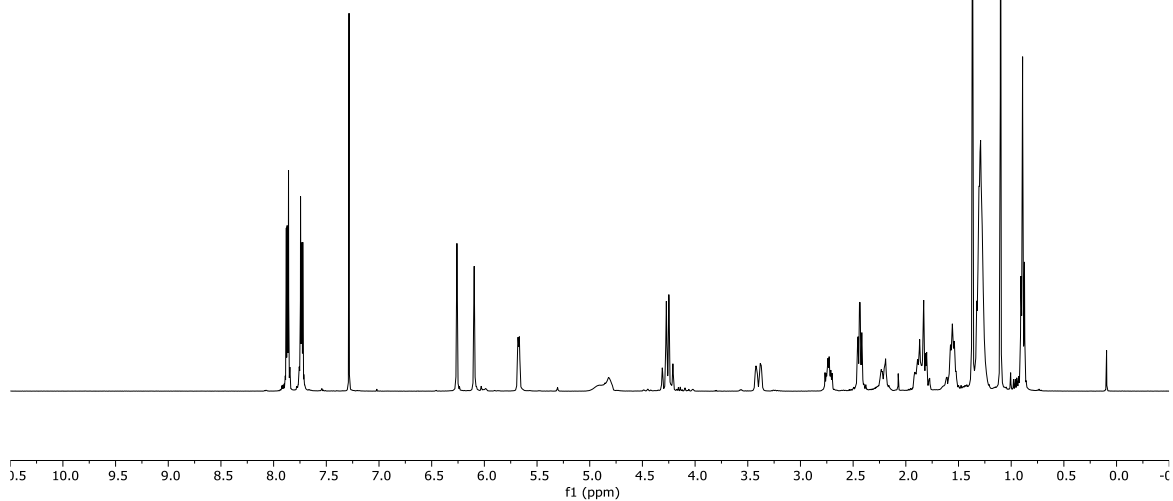
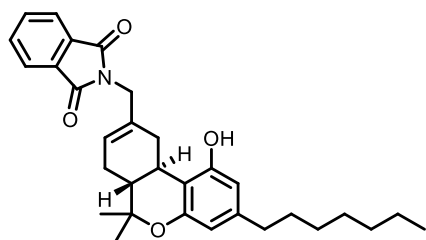
¹H NMR (400 MHz, CD₂Cl₂) of **MKA-115**



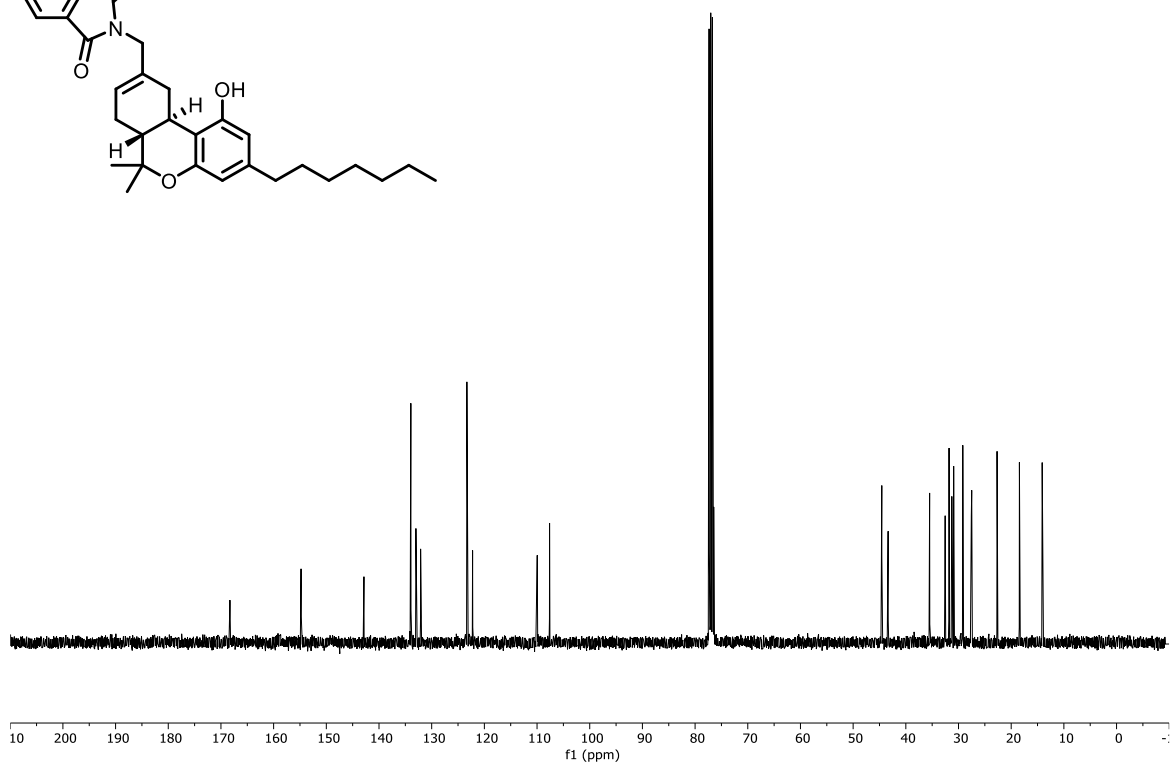
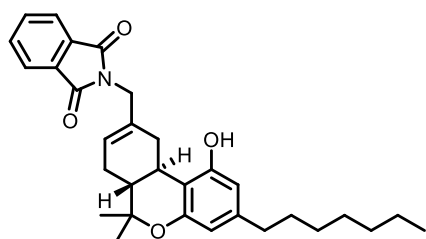
¹³C NMR (101 MHz, CD₂Cl₂) of **MKA-115**



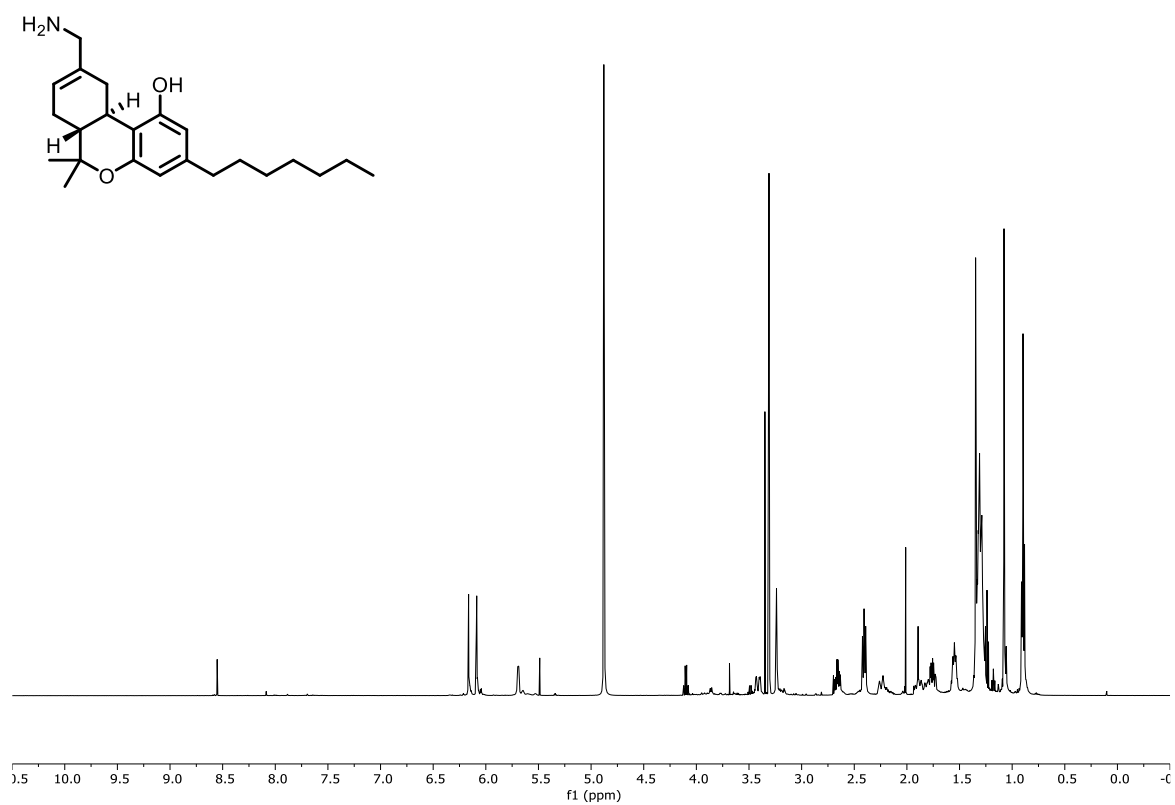
^1H NMR (400 MHz, CDCl_3) of **SI-15**



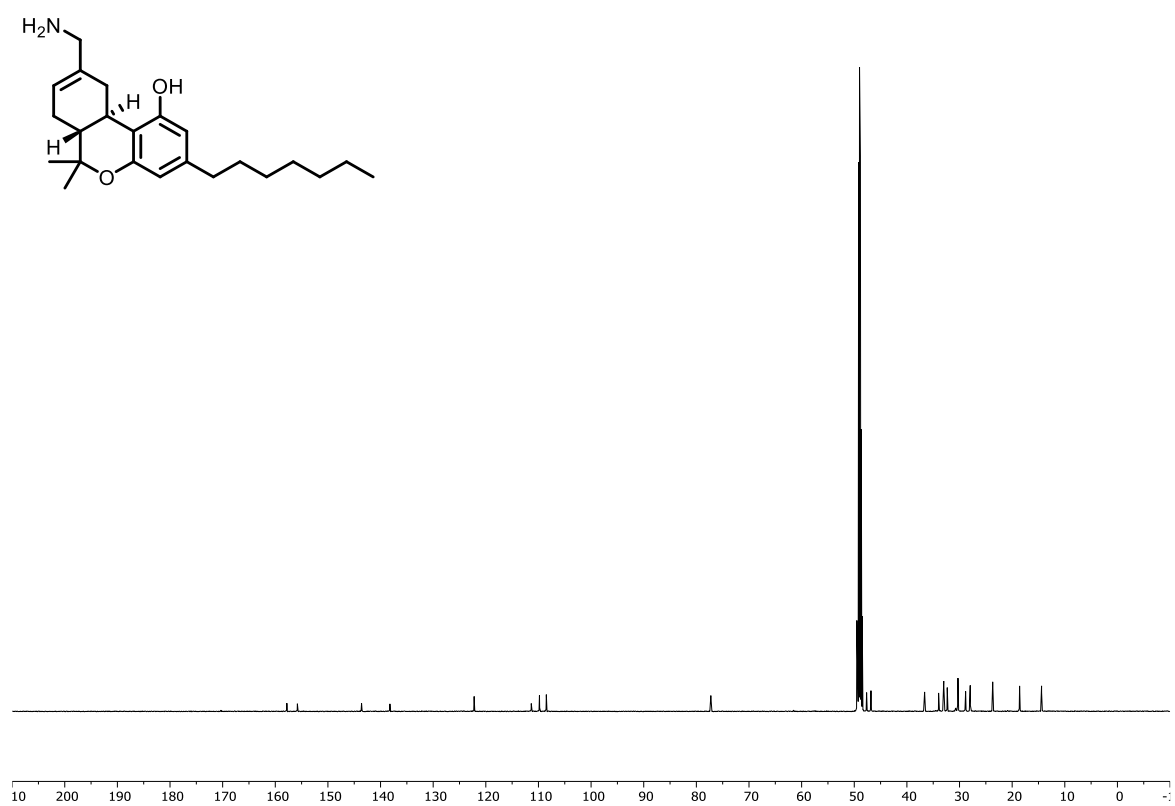
^{13}C NMR (101 MHz, CDCl_3) of **SI-15**



¹H NMR (500 MHz, CD₃OD) of **SI-16**



¹³C NMR (126 MHz, CD₃OD) of **SI-16**



Supplementary references

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