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Supplementary information

Autoinducer-fluorophore conjugates enable FRET in LuxR proteins in vitro and in cells

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SUPPLEMENTARY INFORMATION

Autoinducer-Fluorophore Conjugates Enable FRET in LuxR Proteins *in Vitro* and in Cells

Matthew J. Styles,[1] Michelle E. Boursier,[2] Margaret A. McEwan,[3] Emma E. Santa,[1] Margrith E. Mattmann,[4] Betty L. Slinger,[1] and Helen E. Blackwell[1,*]

[1]*Department of Chemistry, University of Wisconsin–Madison, 1101 University Ave., Madison, WI 53706, USA; blackwell@chem.wisc.edu*
[2]*Current address: Amgen, Inc., 1 Amgen Center Dr., Thousand Oaks, CA 91320*
[3]*Current address: Labcorp Drug Development, 3301 Kinsman Blvd., Madison, WI 53704*
[4]*Current address: Truvian, 10300 Campus Point Dr., San Diego, CA 92121*

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Supplementary Table 1: Summary of FRET-probe library spectral characteristics. DNSA (dansyl amide) and 7HC4A (7-hydroxy coumarin 4-acetic acid) are the parent fluorophores and used here as standards.

Probe	$\lambda_{ex,max}$ (nm)[a]	ε ($M^{-1}cm^{-1}$)[b]	$\lambda_{em,max}$ (nm)[c]	ϕ[d]
DNSA	325	4631 ± 34	555	0.37[e]
DA0	330	4589 ± 33	565	0.221 ± 0.003
DA1	330	4522 ± 50	470	0.167 ± 0.003
DA2	330	4422 ± 21	465	0.317 ± 0.006
DA3	325	1888 ± 209	470	0.285 ± 0.005
DA4	330	3228 ± 27	555	0.406 ± 0.007
DA5	330	5256 ± 53	560	0.356 ± 0.006
DA6	330	4666 ± 102	560	0.431 ± 0.006
DA11	310	5688 ± 55	470	0.276 ± 0.004
7HC4A	325	3078 ± 16	455	0.74[f]
CU0	340	2200 ± 20	470	0.298 ± 0.002
CU1	340	2808 ± 18	470	0.397 ± 0.004
CU2	345	9560 ± 81	470	0.312 ± 0.007
CU3	345	9297 ± 92	470	0.264 ± 0.005
CU4	340	6187 ± 60	470	0.284 ± 0.003
CU5	340	4475 ± 20	470	0.317 ± 0.003
CU6	340	8040 ± 423	470	0.277 ± 0.004
CU11	335	7610 ± 178	470	0.473 ± 0.006

[a]The wavelength at which each compound has the maximum absorbance (between 220–420 nm) determined from the absorption spectra in Supplementary Figure 2. Absorbance was measured in 5 nm increments. [b]Extinction coefficient determined from the slopes of the linear regression fits in Supplementary Figure 4. [c]The wavelength at which each compound has the maximum fluorescence intensity (excited at 340 nm) determined from the fluorescence spectra in Supplementary Figure 3. Fluorescence intensity was measured in 5 nm increments. [d]Using the gradients from Supplementary Figure 5 and reference quantum yields, the quantum yield of each compound was determined.[1] [e]This value was used as the reference quantum yield for all dansyl compounds.[2,3] [f]This value was used as the reference quantum yield for all coumarin compounds.[4]

Supplementary Table 2: Summary of model fits of reporter assay dose-response curves for selected FRET-probes. Data were fit using GraphPad Prism software using a four parameter (varied slope) dose-response equation.

Receptor[a]	Ligand	Max Activity (%)[b]	95% CI (%)[c]	EC_{50} (nM)[d]	95% CI (nM)[c]
LasR	OdDHL	103.3	*99.0–107.9*	2.16	*1.68–2.79*
	CU0	93.4	*88.9–98.8*	33 (0.6)[e]	*23.0–49.6*
	CU1	79.1	*69.5–99.0*	13769	*9578–26252*
	DA1	95.6	*86.7–107.6*	5491	*4078–8029*
QscR	OdDHL	95.9	*90.8–101.8*	12.9	*9.64–17.6*
	CU2	77.9	*73.9–82.5*	11705 (1.4)[e]	*10121–13618*
	CU11	85.1	*83.0–87.3*	184 (1.2)[e]	*166–204*
	DA4	106.8	*102.7–111.0*	133 (1.5)[e]	*106–164*
RhlR	BHL	100.4	*94.2–108.9*	3063 (0.6)[e]	*2137–4604*
	CU6	3.2	*2.6–3.7*	----[f]	----
	DA4	13.2	*8.8–17.6*	----	----
	DA6	14.9	*13.1–16.7*	----	----
CviR	HHL	100.1	*98.3–102.1*	111	*103–119*
	CU6	87.7	*83.9–91.5*	14869 (1.7)[e]	*12774–17067*
	CU11	86.9	*81.1–94.6*	1079	*883–1378*
	DA6	98.7	*94.2–103.7*	4234	*3658–4962*
CepR	OHL	101.3	*97.1–105.8*	7.31	*5.64–9.45*
	CU1	25.5	*23.1–24.8*	----	----
	CU11	118.6	*116.5–120.8*	283	*265–303*
	DA1	102.5	*87.9–135.5*	31901	*20919–72429*
SdiAec	OOHL	88.6	*81.8–97.5*	0.66	*0.39–1.13*
	CU1	88.1	*73.9–111.6*	4797	*3089–9284*
	CU2	97.7	*90.7–104.6*	9207	*5999–14244*
	DA1	80.4	*74.9–87.0*	3852	*3155–4757*
	DA2	105.3	*94.3–119.2*	707	*446–1120*
	DA4	92.3	*85.1–100.6*	2603	*1995–3399*
	DA6	84.8	*71.4–114.1*	10629	*6480–24669*
SdiAse	OOHL	94.2	*89.8–98.9*	1.54	*1.09–2.21*
	CU1	72.5	*67.7–78.7*	5350	*4257–7008*
	CU11	109.7	*82.6–136.7*	1902 (0.6)[e]	*997–3416*
	DA1	69.4	*63.2–79.0*	13120	*10210–18406*
	DA4	92.3	*73.5–111.1*	15160	*11392–19989*

[a]See Supplementary Table 1 for reporter strain information. [b]Maximum activation used as the "Top" parameter in the model fit. [c]95% confidence intervals (CI) of the parameter listed in the left adjacent column. [d]Concentration that induces half-maximal activation – the "EC_{50}" parameter in the model fit. [e]If the Hill Slope was significantly different than 1.0 ($p = 0.05$), then the calculated Hill slope is listed next to the EC_{50} in parentheses. [f]Fits did not converge because the dose-response did not plateau by the highest concentration tested; compound solubility limitations prevented testing at higher concentrations. In these cases, the highest concentration tested was used for determining the Max Activity.

Supplementary Table 3: Summary of dose-response curve model fits for cell-based activity reporter, *in vitro* FRET binding, and in-cell FRET binding assays with small molecule modulators of QscR.

Assay[a]	Parameter	OdDHL	dDHL	S3	CL	B7	C10-CPA	R6	Q9	OHHL	C10	D6	S5	DA4
Transcription Reporter Assay	EC_{50} (nM)[b]	15.3	14.7	35.6	16.4	15.4	617	73.8 (0.5)[k]	115 (0.4)[k]	13490	661	2891	86896	472
	SD[c]	1.5	2.1	9.0	3.9	7.2	164	37.9	193	3821	363	1230	266162	53
	Maximum Activation (%)[d]	99	112	88	86	88	90	23	11	74	36	73	47[l]	84
	SD	1	3	3	3	5	5	2	3	6	6	8	80	2
	IC_{50} (nM)[e]	---[m]	---	---	---	---	---	42.1	25.5	158	70.5	185	738	---
	SD	---	---	---	---	---	---		6.1	226	10.5	298	108	---
	Minimum Activation (%)[f]	---	---	---	---	---	---	42	20	65	37	46	6	---
	SD	---	---	---	---	---	---	3	2	8	3	36	4	---
In vitro FRET	IC_{50} (nM)[g]	81.8	29.2	34.9	181	352	4178	42.4	55.8	10423	4046	4864	48529	---
	SD	11.8	3.0	3.8	25	41	699	6.8	6.3	2070	404	370	4875	---
	K_d (nM)[h]	1.15	0.409	0.490	2.53	4.93	58.6	0.594	0.783	146	56.7	68	681	28.4
	SD	0.43	0.149	0.179	0.95	1.82	22.7	0.229	0.288	59	20.6	24	248	10.1
In-cell FRET	IC_{50} (nM)[g]	124	79.3	123	342	826	3420	125	179	17100	5483	10093	96605	313[n]
	SD	4	2.2	7	18	37	194	6	7	1492	367	300	9338	43

[a]See above for assay details; reporter assay data shown in Extended Data Figure 4, *in vitro* FRET assay data shown in Figures 3D–E, and in-cell FRET assay data shown in Figure 4D–E. [b]EC_{50} refers to the concentration needed to activate QscR to 50% relative to the maximum activation in the four parameter (varied slope) activation dose-response fit in GraphPad Prism. [c]SD refers to standard deviation reported for the parameter in GraphPad Prism. [d]Maximum activation refers to the "Top" parameter of the model fit; this is the percent (relative to 10 μM OdDHL) where the data plateaus. [e]IC_{50} refers to the concentration needed to inhibit QscR by 50% relative to the minimum activation in the four parameter (varied slope) inhibition dose-response fit in GraphPad Prism. [f]Minimum activation refers to the "Bottom" parameter of the model fit; this is the percent (relative to DMSO added in the antagonism media – see above for assay details) where the data plateaus. [g]IC_{50} refers to the concentration needed to reduce the Trp FRET by 50% in the four parameter (varied slope) inhibition dose-response fit in GraphPad Prism. [h]K_d refers to the affinity calculated from the IC_{50} using the Cheng-Prusoff equation.[1] [k]If the Hill Slope for the model fit was significantly different than 1.0 ($p = 0.05$), then the calculated Hill slope is listed in parentheses (footnote letters i and j skipped for clarity). [l]The data did not plateau, but the fit still converged; the highest concentration tested had 24% activation with a SD of 14. [m]Fit did not converge because the data failed to plateau. [n]In-cell FRET affinity for **DA4** was determined using a binding assay rather than a displacement assay; therefore, this value is for an EC_{50}, not an IC_{50}, and is most directly comparable to the *in vitro* K_d rather than *in vitro* IC_{50}.

Supplementary Table 4: Summary of binding and displacement model fits for select LuxR-type receptor:FRET-probe pairs.

Binding[a]								
Receptor	LasR	RhlR	CviR	CviR	CviR	CepR	SdiAec	SdiAse
Probe	DA1	DA6	DA6	CU6	CU11	DA1	DA4	DA4
EC_{50} (nM)[b]	11868	----[f]	1641	3609	579	----	643	1897
95% CI[c]	7826–26739	----	1508–1786	2667–4895	516–649	----	515–801	1554–2384
Displacement[d]								
Receptor	LasR	RhlR	CviR	CviR	CviR	CepR	SdiAec	SdiAse
Probe	DA1	DA6	DA6	CU6	CU11	DA1	DA4	DA4
Ligand	OdDHL	S4	HHL	HHL	HHL	OHL	OOHL	OOHL
IC_{50} (nM)[e]	17.2	27493	61677	3596	----	12.8	120	40.6
95% CI[c]	8.8–34.9	16767–51713	54053–71399	2881–4657	----	5.4–29.0	101–142	24.1–67.0

[a]In-cell FRET assay data shown in Extended Data Figure 5. [b]EC_{50} refers to the concentration needed to reach 50% Normalized FRET in the four parameter (varied slope) activation dose-response fit in GraphPad Prism. [c]95% CI refers to 95% confidence interval (CI) for the parameter in GraphPad Prism. [d]In-cell FRET assay data shown in Extended Data Figure 6. [e]IC_{50} refers to the concentration needed to reach 50% Normalize FRET in the four parameter (varied slope) inactivation dose-response fit in GraphPad Prism. [f]Fit did not converge because the data failed to plateau.

Supplementary Table 5: Bacterial strains, plasmids, and primers used in this study.[a]

Strain, plasmid, or primer	Description	Reference
Strains		
JLD271	K-12 *E. coli* Δ*lacX74 sdiA271*::Cam; Cam^R	5
Reporter	JLD271 pSC11 pJN105 (for promoter-receptor pairs) Amp^R,$Gent^R$	
BL21(DE3) pLysS	F^- *ompT hsdSB*(r_b^- m_b^-) *gal dcm* (DE3) pLysS; Cam^R	Invitrogen
Overexpression	BL21(DE3)pLysS pET (for each receptor) ; Amp^R, Cam^R	
Plasmids		
pSC11-plasI	Reporter plasmid for LasR; lasI'-lacZ reporter; Amp^R	6
pSC11-p1897	Reporter plasmid for QscR; PA1897'-lacZ reporter; Amp^R	7
pSC11-prhlI	Reporter plasmid for RhlR; rhlI'-lacZ reporter; Amp^R	8
pSC11-pcepI	Reporter plasmid for CepR; cepI'-lacZ reporter; Amp^R	9
pSC11-pcviI	Reporter plasmid for CviR; cviI'-lacZ reporter; Amp^R	This work
pSC11-pgadW	Reporter plasmid for $SdiA_{ec}$; gadW'-lacZ reporter; Amp^R	10
pSC11-psrgE	Reporter plasmid for $SdiA_{se}$; srgE'-lacZ reporter; Amp^R	11
pJN105-LasR	Arabinose-inducible *lasR* expression vector; pBBRMCS; $Gent^R$	6
pJN105-QscR	Arabinose-inducible *qscR* expression vector; pBBRMCS; $Gent^R$	7
pJN105-RhlR	Arabinose-inducible *rhlR* expression vector; pBBRMCS; $Gent^R$	8
pJN105-CepR	Arabinose-inducible *cepR* expression vector; pBBRMCS; $Gent^R$	9
pJN105-CviR	Arabinose-inducible *cviR* expression vector; pBBRMCS; $Gent^R$	This work
pJN105-$SdiA_{ec}$	Arabinose-inducible $sdiA_{EC}$ expression vector; pBBRMCS; $Gent^R$	10
pJN105-$SdiA_{se}$	Arabinose-inducible $sdiA_{SE}$ expression vector; pBBRMCS; $Gent^R$	11
pET17b-LasR	Full length LasR overexpression vector; Amp^R	12
pET3a-QscR	Full length QscR overexpression vector; Amp^R	13
pET23b-RhlR	Full length RhlR overexpression vector; Amp^R	This work
pET23b-CepR	Full length CepR overexpression vector; Amp^R	This work
pET23b-CviR	Full length CviR overexpression vector; Amp^R	This work
pET23b-$SdiA_{ec}$	Full length $SdiA_{ec}$ overexpression vector; Amp^R	This work
pET23b-$SdiA_{se}$	Full length $SdiA_{se}$ overexpression vector; Amp^R	This work
pET23b-$SdiA_{ehec}H_6$	Full length $SdiA_{ehec}H_6$ overexpression vector; Amp^R	10
Primers		
pcviI-F (pSC11)	cactgtcgacCTGCCGTAGGCAAAGAACTAAATGTAAC	This work
pcviI -R (pSC11)	cactggatccGTGTCTGAACGCCAGCAGGTCGAG	This work
cviR-F (pJN105)	catcgaattcATGGTGATCTCGAAACCCATCAACGC	This work
cviR-R (pJN105)	cattctaga TTATTCGTTCGCTACGGTCGAGGG	This work
CepR-F	aaggcatatgGAACTGCGCTGGCAGGATGCCTA	This work
CepR-R	ttctctcgagTCAGGGTGCTTCGATGAGCCCG	This work
CviR-F	aaggcatatgGTGATCTCGAAACCCATCAACGCAAGAC	This work
CviR-R	ttctctcgagTTATTCGTTCGCTACGGTCGAGGGTG	This work
RhlR-F	catcatatgAGGAATGACGGAGGCTTTTTG	This work
RhlR-R	catctcgagTCAGATGAGACCCAGCGCCG	This work
SdiAec-F	catcatatgCAGGATACGGATTTTTTCAGCTG	This work
SdiAec-R	catctcgagTCAAATTAAGCCAGTAGCGGCC	This work
SdiAse-F	catcatatgCAGGAAAATGATTTCTTCACCTGG	This work
SdiAse-R	catctcgagTATCAGACCTGTCGCCGC	This work

[a]Abbreviations: Cam^R = chloramphenicol resistance; Amp^R = ampicillin resistance; $Gent^R$ = gentamicin resistance.

Supplementary Table 6: Compiled QscR-ligand complex T_m values. OdDHL, C12-HSL, S3, CL, B7, and C10-CPA all have similar DSF profiles as the representative agonist (OdDHL) profile in Extended Data Figure 8. R6 and Q9 are partial agonists and have similar DSF profiles (represented in Extended Data Figure 8 in red by Q9). OHHL, C10, D6, and S5 are non-monotonic partial agonists;[14] they have an upturn in cell-based antagonism reporter assays, see Extended Data Figure 4. These compounds had similar DSF profiles to the representative profile in Extended Data Figure 8 for C10 (in blue).

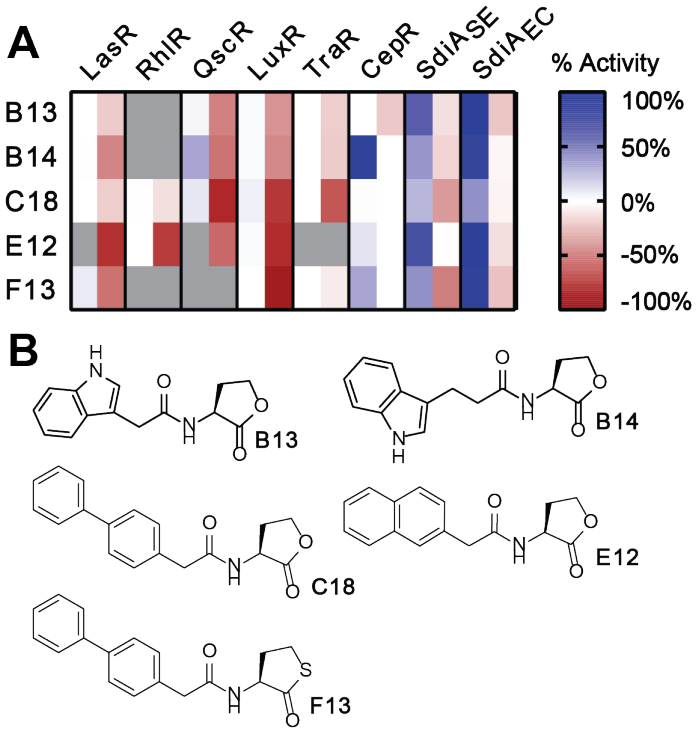
Ligand[a]	**T_m (°C)**[b]	**SD**[c]
OdDHL	57.2	0.9
C12-HSL	47.9	2.7
S3	60.6	0.8
CL	55.7	1.1
B7	53.0	1.0
C10-CPA	47.1	1.5
R6	60.6	1.5
Q9	58.1	1.2
OHHL	44.8	1.4
C10	48.2	0.7
D6	47.1	0.9
S5	42.4	0.9

[a]Numbered ligand names are from our past studies. [b]DSF thermal shift assays performed as described above. T_m values were determined from the minimum of the 1st derivative of the fluorescence profile. Each T_m value is the average of the T_m determined from four independent replicates, each with four technical replicates (total of 16 independent replicates). [c]SD = standard deviation.

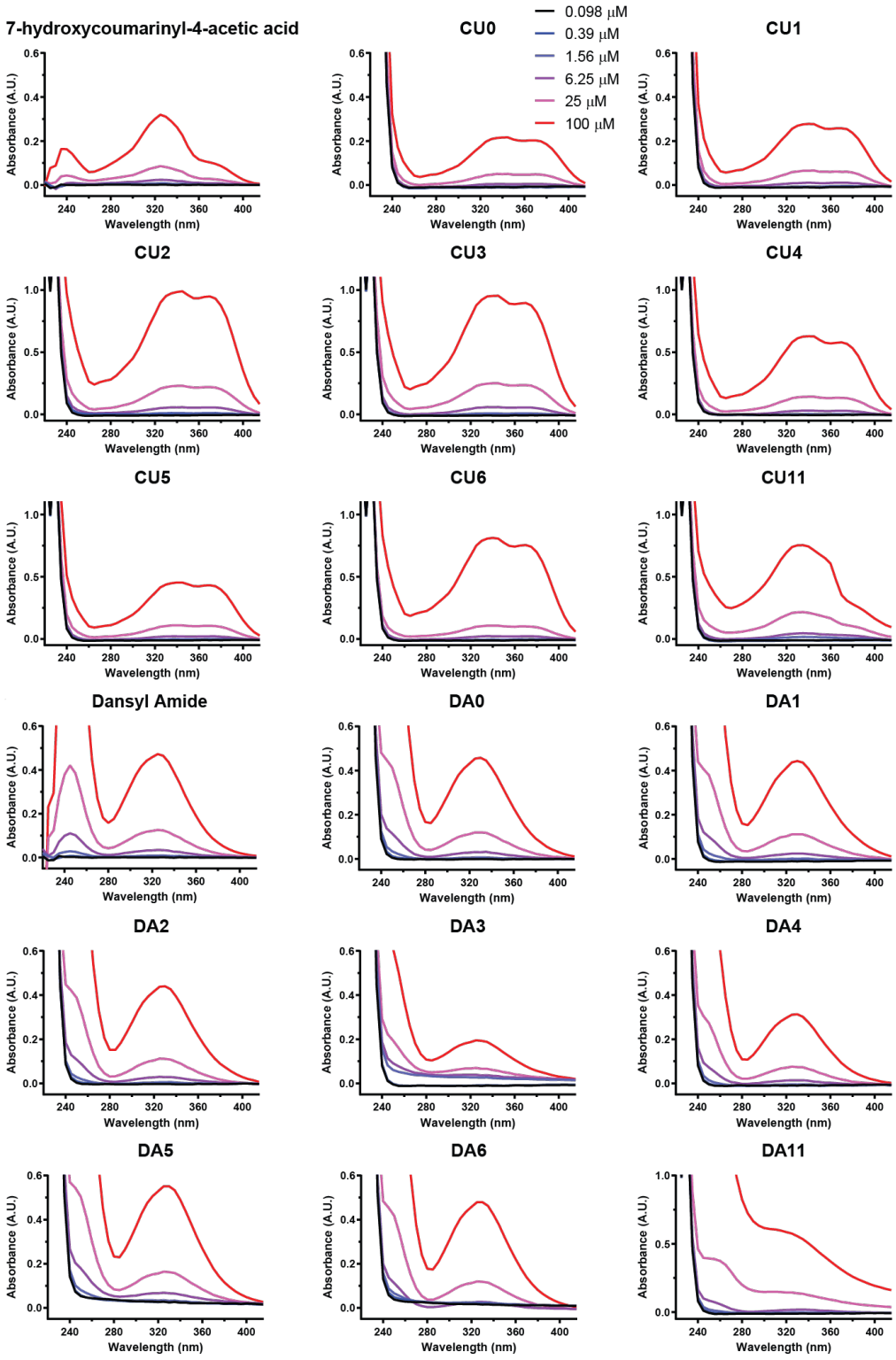
Supplementary Table 7: Excitation and emission wavelengths used for *in vitro* and in-cell FRET assays.

Measurement Type	Fluorophore	Excitation wavelength	Emission wavelength
FRET	Coumarin	280 nm	465 nm
	Dansyl	280 nm	535 (540)[a] nm
Direct Excitation of FRET-probe	Coumarin	335 (340)[a] nm	465 nm
	Dansyl	335 (340)[a] nm	535 (540)[a] nm

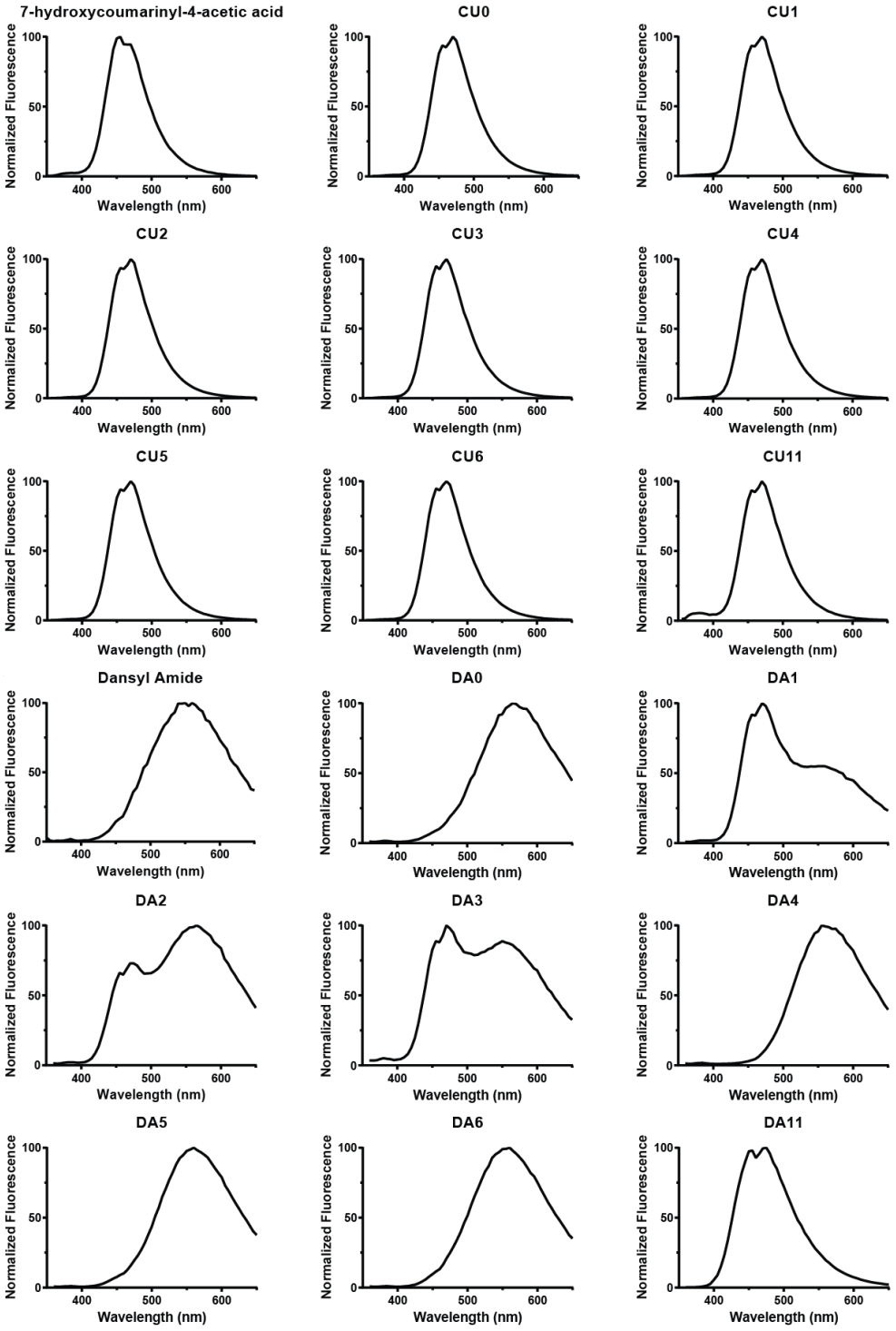
[a]Values in parentheses were used when excitation was performed on the Tecan Infinity M100 Pro plate reader, which has a monochromator; bandwidths of 5 nm were used. For measurements from the Envision plate reader (values w/o parentheses), bandwidths varied by the filter used: 280 nm,10 nm; 335 nm, 40 nm; 465 nm, 25 nm; and 535 nm, 25 nm.



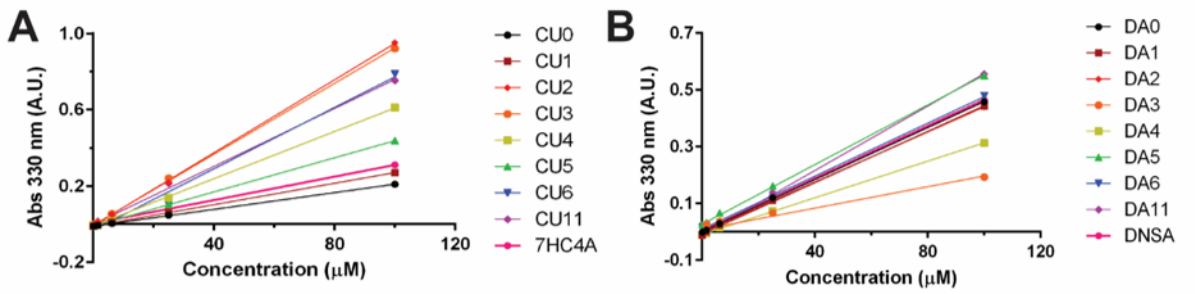
Supplementary Figure 1: Prior screening results for AHLs with bicyclic acyl tail substituents. A) Heatmap representation of the activity of a set of bicyclic-tailed AHL analogues in LuxR-type receptors from prior studies as measured using transcriptional reporter assays (see main text for key citations). Each column has a subcolumn for the results of an agonism assay (gradient from 100% activation in blue to 0% in white) and a subcolumn for the results of an antagonism assay (gradient from 100% inhibition in red to 0% in white). Grey indicates that the compound was not screened. **B)** Structures of the compounds listed in part **A**. The methods used for screening prior Blackwell lab compound libraries were summarized previously.[3]



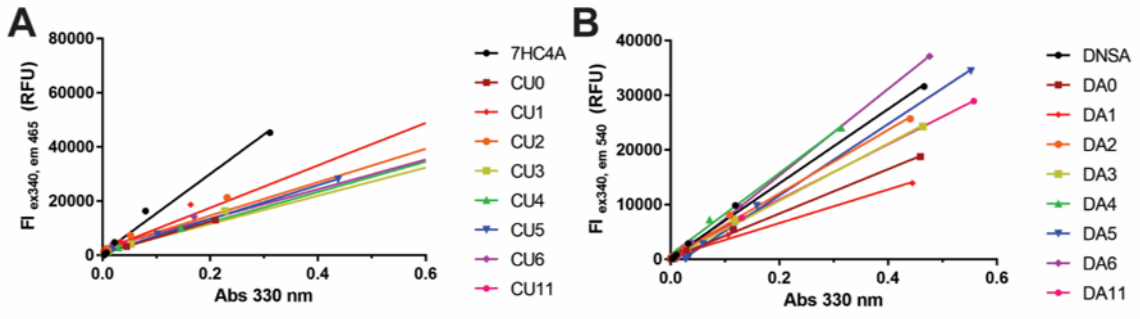
Supplementary Figure 2: FRET-probe library absorbance spectra. Each compound (listed above each plot) was serial diluted to different concentrations (0.39–100 μM) in low salt TEDG buffer with DMSO held at 1% (v/v). Absorbance was measured in 5 nm steps from 220 nm to 420 nm. Samples were measured in 1 cm pathlength quartz crystal sample holders. Each plot is of a single replicate.



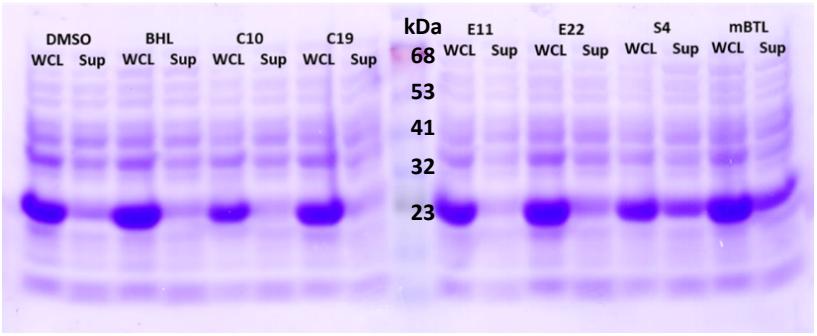
Supplementary Figure 3: FRET-probe library emission spectra. Fluorescence emission (with excitation at 340 nm) of each indicated compound (listed above each plot) at 25 μM in low salt TEDG buffer with DMSO (1% (v/v)) was measured from 350 nm to 650 nm in 5 nm increments. Fluorescence intensities were normalized to the maximum fluorescence in this range. Plots are the average of duplicate measurements obtained on the Tecan instrument.



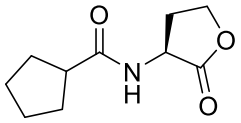
Supplementary Figure 4: Determination of FRET-probe extinction coefficients. Linear regression analysis of the absorbance of each FRET-probe at various concentrations in low salt TEDG buffer with DMSO (1% (v/v)) was used to determine the extinction coefficient (see Supplementary Table 2 for values) of each probe at 330 nm using Beer's Law. **A)** CU compounds and **B)** DA compounds. Each point represents a single replicate.



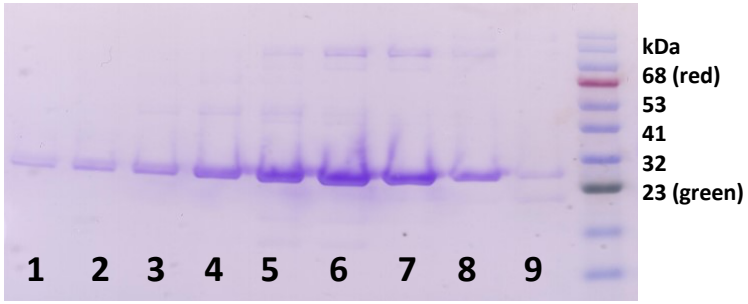
Supplementary Figure 5: Determination of FRET-probe quantum yields. Each compound was serial diluted to different concentrations (10 nM – 10 μM) in low salt TEDG buffer with DMSO (1% (v/v)). Extinction coefficients determined from the plots in Supplementary Figure 4 were used to calculate absorbances for each compound at each concentration. **A)** CU compounds and **B)** DA compounds. Each data point is the average of four technical replicates. Linear regression analysis of the absorbance of each FRET-probe versus the fluorescence intensity (excitation 340 nm, emission **A)** 465 nm or **B)** 540 nm) was used to determine the gradient for each compound, which was then used to calculate the quantum yield of each compound (see Supplementary Table 2 for values).[1]



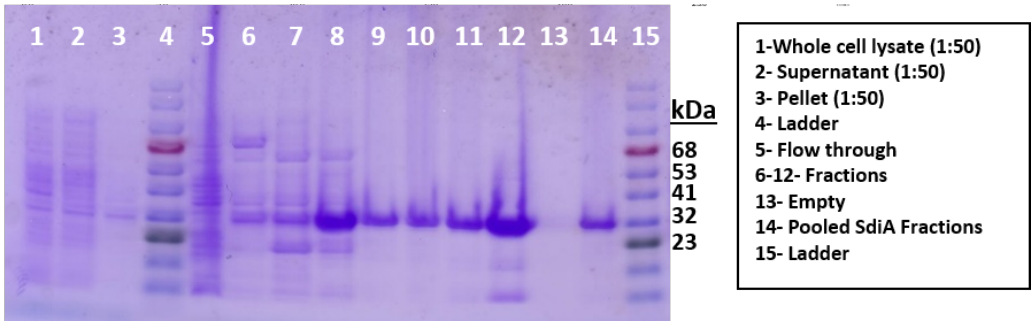
S4



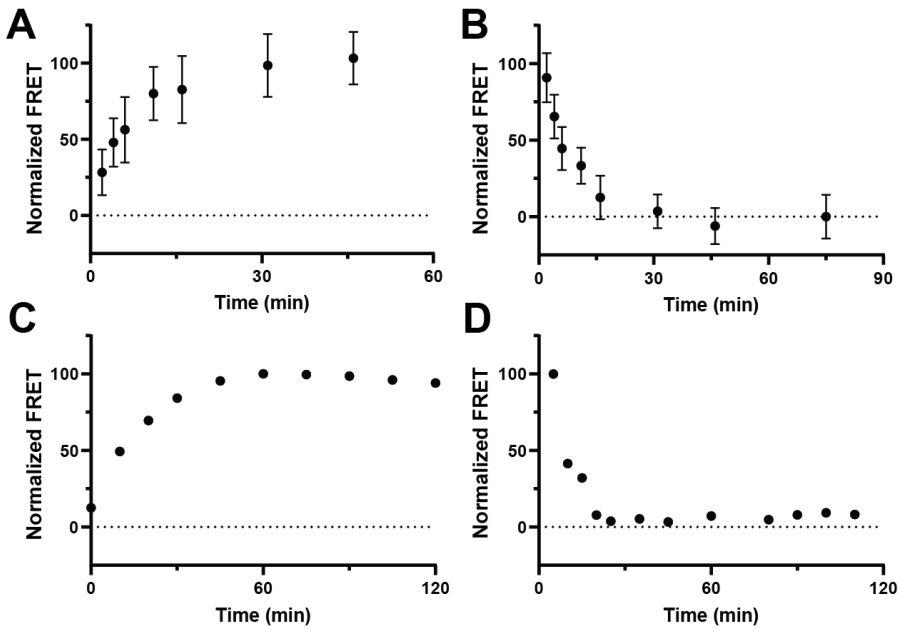
Supplementary Figure 6: Selection of a ligand for soluble accumulation of RhlR upon overexpression. RhlR was overexpressed in *E. coli* from a T7 promoter as described above for the in-cell FRET assay with either DMSO (no compound) or 250 μM of several compounds that potently activate or inhibit RhlR activity in cell-based reporter assays, including BHL (the native AHL signal of RhlR) and S4 (a strong synthetic agonist of RhlR).[15] After pelleting the cells, they were resuspended in low-salt TEDG media containing DMSO or the ligand at the concentration used during the overexpression. The cells were gently lysed via sonication to produce the whole cell lysate (WCL) samples. The lysate was centrifuged to pellet insoluble material (30,000xg for 20 min at 4 °C); the supernatant was used as the "Sup" samples. SDS PAGE was performed on these samples (Sup and WCL for each), followed by Coomassie staining. The same volume of Sup and WCL were added to each well. The structure of S4 is shown to the right of the SDS PAGE gel image. Solubilization of RhlR using S4 has been repeated in an independent protein overexpression experiment.



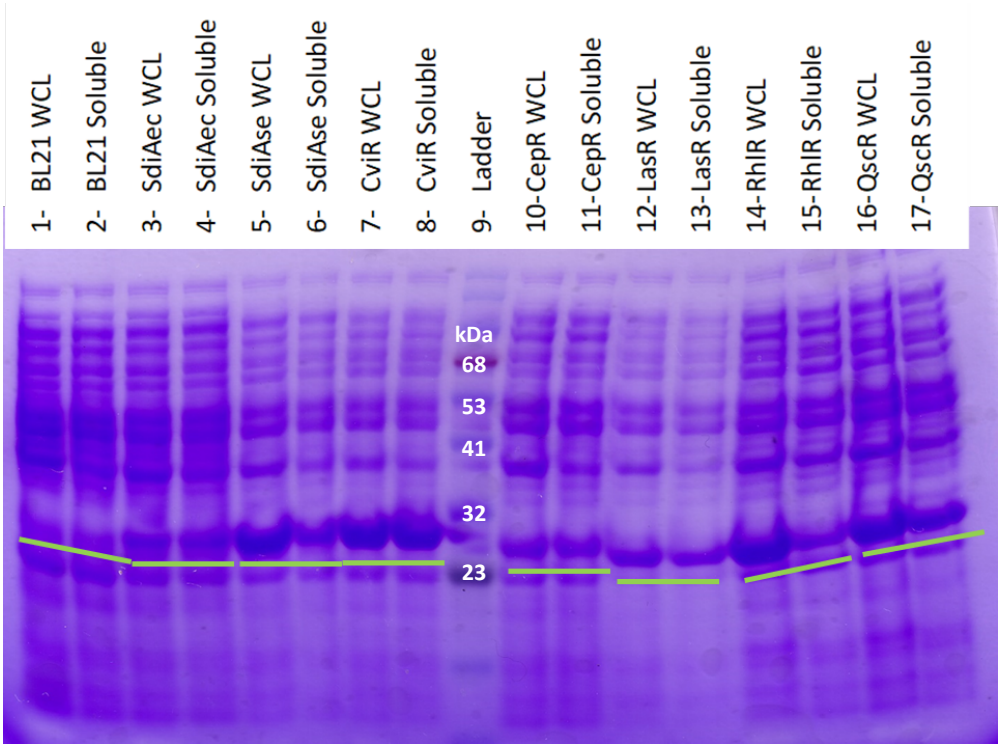
Supplementary Figure 7: SDS PAGE gel for QscR and purity data for fractions 24–31, in lanes 1–9, respectively. The estimated purity is listed next to each fraction. Fractions 28 and 29 (lanes 5 and 6) were used for *in vitro* FRET and DSF assays.



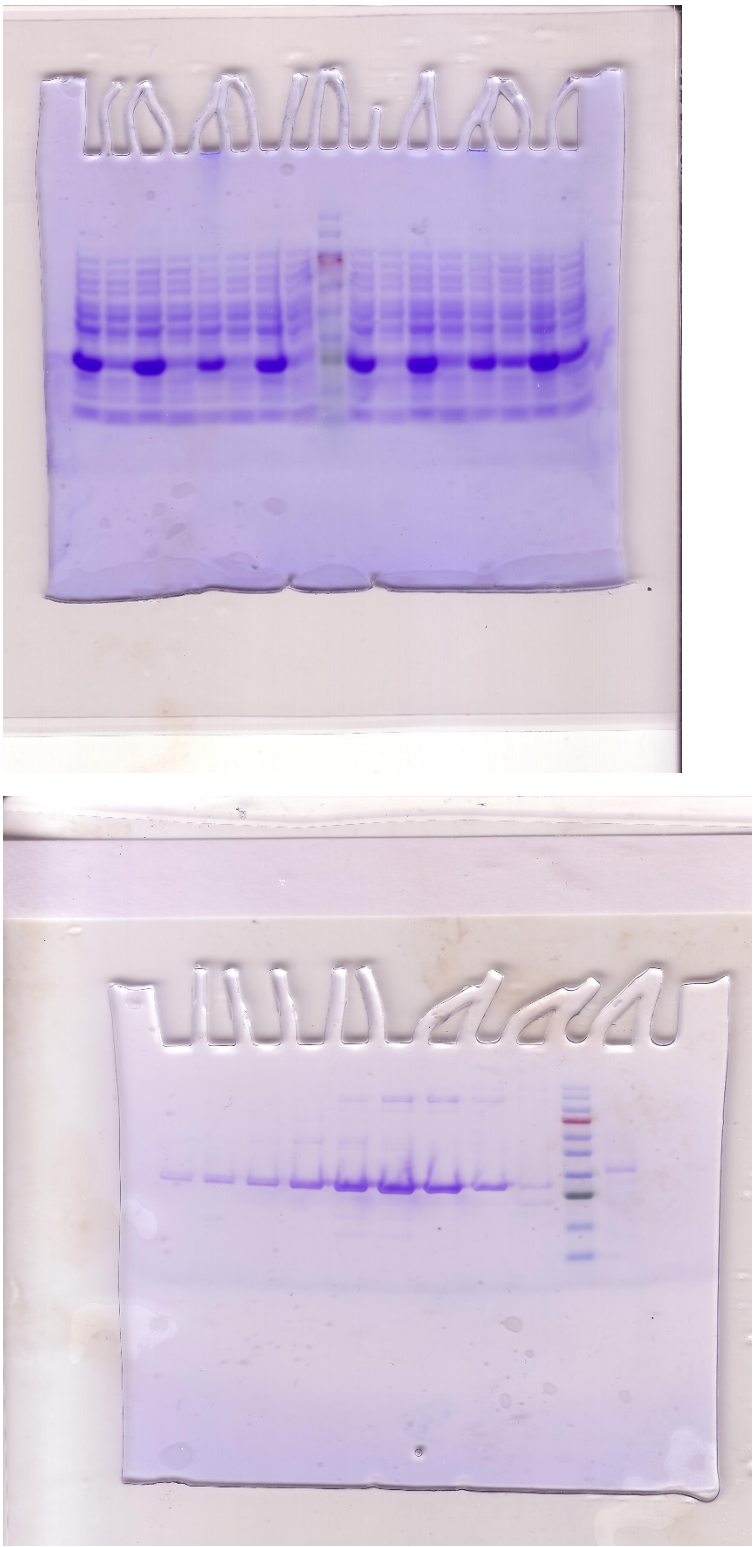
Supplementary Figure 8: SDS PAGE gel for $SdiA_{ec}H_6$ and purity data for specific fractions. The legend at the right relates sample content to each gel lane. Dilute (1:50 dilution in buffer) whole cell lysate, supernatant with soluble protein, and pelleted insoluble protein samples were run in lanes 1–3, respectively, showing that expressed $SdiA_{ec}H_6$ was in the soluble fraction (and the pellet). Nickel affinity column fractions containing $SdiA_{ec}H_6$ from the soluble fraction of the lysate are shown in lanes 7–12; these fractions were pooled (lane 14 indicating >95% pure $SdiA_{ec}H_6$), concentrated, and used as the $SdiA_{ec}H_6$ stock solution for the data shown in Figure 6C.



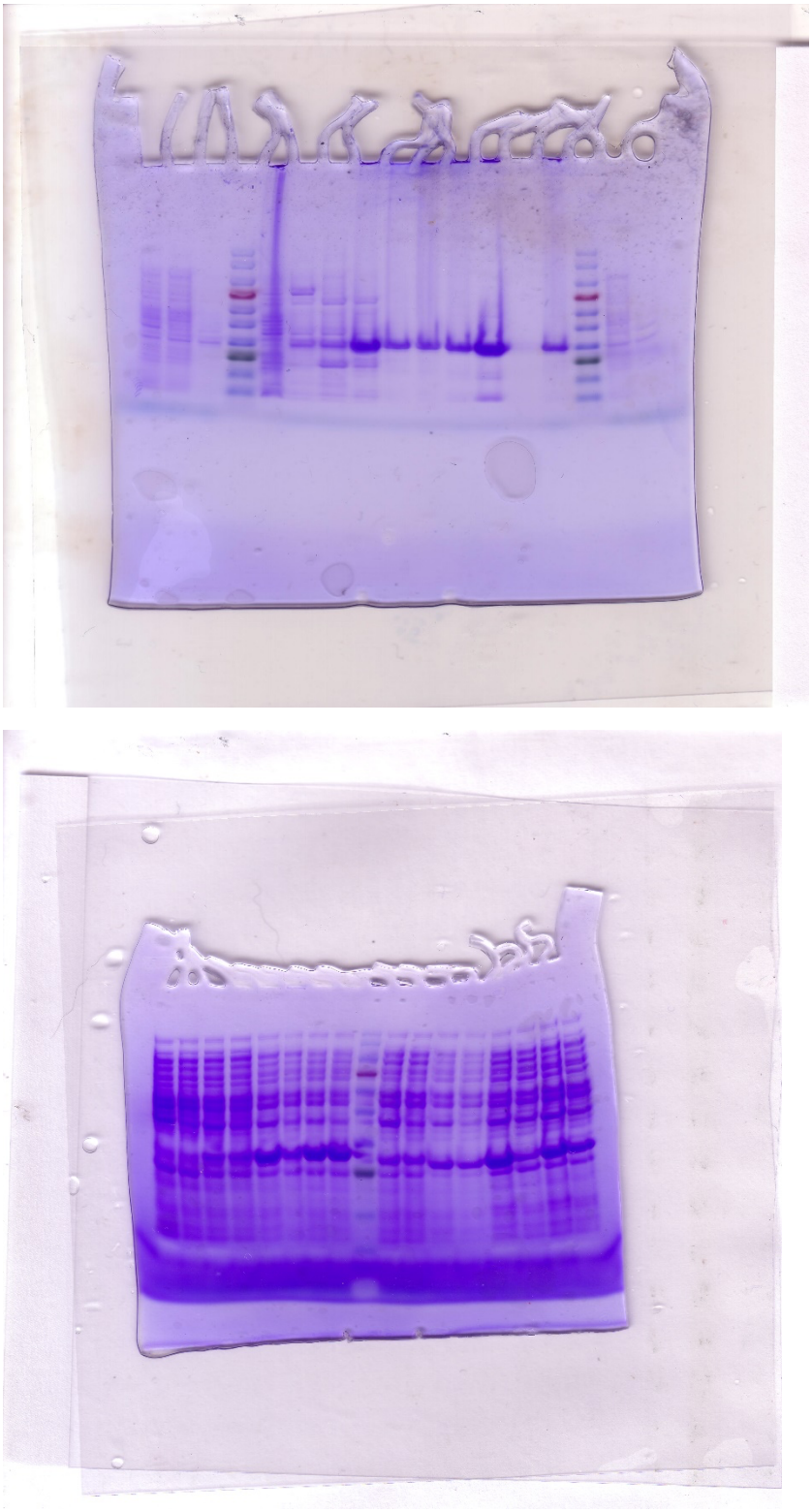
Supplementary Figure 9: Time course plots for DA4-QscR binding and displacement. A) *In vitro* FRET kinetic monitoring of 10 nM QscR mixed with 2 μM DA4. **B)** *In vitro* FRET kinetic monitoring of 10 μM OdDHL displacing DA4 from QscR. A 10 nM solution of QscR was mixed with 2 μM DA4 and allowed to equilibrate for 1 hr at room temperature prior to mixing with OdDHL. **C)** In-cell FRET kinetic monitoring of QscR overexpressed in cells and incubated with 2 μM DA4. **D)** In-cell FRET kinetic monitoring of 10 μM OdDHL displacing DA4 from QscR. Overexpressed QscR cells were mixed with 2 μM DA4 and allowed to equilibrate for 1 hr at room temperature prior to mixing with OdDHL. Time (on x-axis) refers to minutes after all the components were mixed. Samples were incubated at 30 °C in the plate reader and shaken briefly (10 sec) prior to each FRET and fluorescence intensity measurements (see above for assay protocol details). Each data point is the average of eight technical replicates, and error bars represent SD.



Supplementary Figure 10: Accumulation of soluble LuxR-type receptors upon overexpression. Receptors were overexpressed from T7 promoters in *E. coli* BL21(DE3) pLysS strains with pET vectors (see Supplementary Table 1) as described above in the protocol for the in-cell FRET assay. After pelleting the cells, they were resuspended in low-salt TEDG media with added AHL ligand (at the concentration used during the overexpression): 25 μM of OdDHL for LasR; 25 μM of OHHL for QscR; 25 μM of HHL for CviR; 25 μM of OHL for CepR; 250 μM of S4 for RhlR; 10 μM OOHL for $SdiA_{se}$; and no AHL for $SdiA_{ec}$. These cells were gently lysed via sonication to produce the whole cell lysate (WCL) samples. The lysate was centrifuged to pellet insoluble material (30,000xg for 20 min at 4 °C); the supernatant was used as the soluble sample. SDS PAGE was run with the samples listed above for each lane, followed by Coomassie staining. The same volume of WCL and soluble fraction were added to each well. A green line is shown for each WCL/Soluble pair to highlight where the receptor is located on the gel (directly above the green line). Solubility under these expression conditions was replicated once.



Supplementary Figure 11: Unprocessed gel images for Supplementary Figures 6 and 7. Image for Supplementary Figure 6 shown at top, and image for Supplementary Figure 7 shown at bottom.

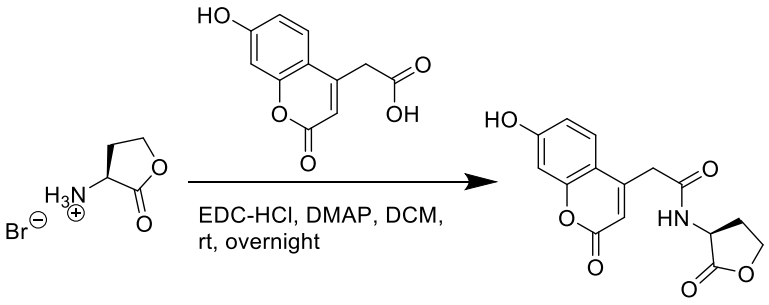


Supplementary Figure 12: Unprocessed gel images for Supplementary Figures 8 and 10. Image for Supplementary Figure 8 shown at top, and image for Supplementary Figure 10 shown at bottom.

Supplementary Note 1 — Synthetic details, compound characterization data, and spectra

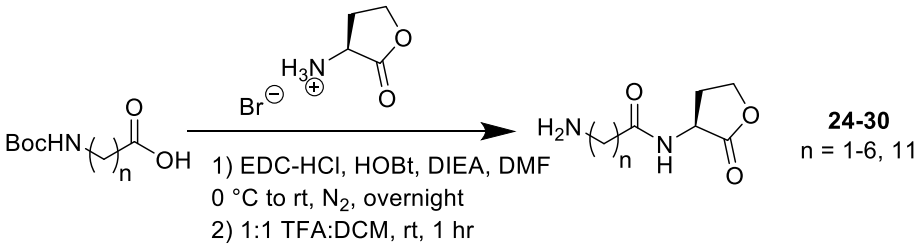
Chemical shifts in NMR data are reported in parts per million relative to residual solvent peaks as a reference. Coupling constants are reported in hertz (Hz). ^{1}H-NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, ddd = doublet of doublets of doublets, dddd = doublet of doublets of doublets of doublets, td = triplet of doublets, dtd = doublet of triplets of doublets, m = multiplet), coupling constant (Hz), and integration. NMR spectral data for final probe products are provided at the end of this section.

*Synthesis of **9** (CU-0)*



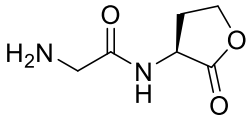
To a solution of 7-hydroxycoumarinyl-4-acetic acid (0.3 mmol, 1 eq.), EDC-HCl (0.36 mmol, 1.2 eq.), and hydroxybenzotriazole (HOBT, 0.36 mmol, 1.2 eq.) in DMF (5 mL), *N,N*-diisopropylethylamine (DIEA, 0.75 mmol, 2.5 eq.) and HBr L-homoserine lactone (0.3 mmol, 1 eq) was added at rt. The solution stirred for 24 hours at rt. The mixture was diluted with distilled water, extracted with dichloromethane, washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield the product CU-0 (white solid, 38 mg, 43% yield). The identity and purity of **9** (CU-0) was confirmed using mass spectrometry and ^{1}H- and ^{13}C-NMR. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.74 (s, 1H), 8.96 (d, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 6.81 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.76 (d, *J* = 2.4 Hz, 1H), 6.20 (s, 1H), 4.55 (ddd, *J* = 10.9, 9.1, 7.8 Hz, 1H), 4.34 (td, *J* = 8.8, 1.8 Hz, 1H), 4.19 (ddd, *J* = 10.5, 8.7, 6.5 Hz, 1H), 3.72 (s, 2H), 2.39 (m, 1H), 2.16 (dtd, *J* = 12.1, 10.6, 8.9 Hz, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.51, 168.45, 161.74, 160.69, 155.46, 151.25, 127.00, 113.40, 112.06, 111.80, 102.77, 65.80, 48.70, 38.93, 28.48. ESI-EMM: $[M+H]^+$ calculated 415.212; measured 415.211.

*Synthesis of **24**–**30** (HSL-n) compounds (CU-n intermediates; n = 1-6, 11)*



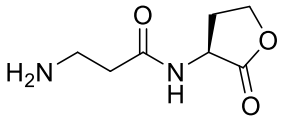
To a solution of Boc-protected amino acid (1.0 mmol, 1.0 eq.), HBr L-homoserine lactone (1.0 mmol, 1.0 eq.), EDC-HCl (1.2 mmol, 1.2 eq.), and hydroxybenzotriazole (HOBT, 1.2 mmol, 1.2

eq.) in DMF (10 mL) under N_2, *N,N*-diisopropylethylamine (DIEA, 2.5 mmol, 2.5 mmol, 2.5 eq.) was added at room temperature (rt). The solution was placed in an ice bath and stirred for 24 hours under N_2 while warming to rt. The mixture was diluted with distilled water, extracted with dichloromethane, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to yield the Boc-protected HSL. This compound was deprotected by stirring for 1 hour at rt in a 1:1 solution of dichloromethane and trifluoroacetic acid. The final HSL product was concentrated under reduced pressure, and its purity and identity were characterized via ^{1}H-NMR before proceeding to the next synthetic step.



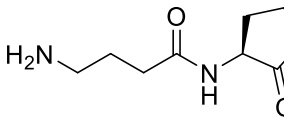
24

24 (HSL-1) was prepared according to the general procedure to afford the product (yellow oil, 121.0 mg, 77% yield). ^{1}H-NMR (500 MHz, D_2O) δ 4.80 (m, 1H), 4.59 (td, *J* = 9.2, 1.5 Hz, 1H), 4.43 (ddd, *J* = 10.9, 9.5, 6.5 Hz, 1H), 3.91 (s, 2H), 2.68 (dddd, *J* = 12.6, 9.3, 6.4, 1.5 Hz, 1H), 2.34 (dtd, *J* = 12.8, 11.1, 9.4 Hz, 1H).



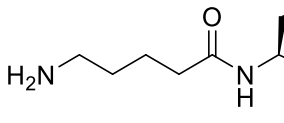
25

25 (HSL-2) was prepared according to the general procedure to afford the product (yellow oil, 168.3 mg, 98% yield). ^{1}H-NMR (400 MHz, D_2O) δ 4.72 (dd, *J* = 11.2, 9.3 Hz, 1H), 4.56 (td, *J* = 9.1, 1.8 Hz, 1H), 4.40 (ddd, *J* = 10.8, 9.2, 6.5 Hz, 1H), 3.29 (t, *J* = 6.5 Hz, 2H), 2.75 (t, *J* = 6.5 Hz, 2H), 2.63 (dddd, *J* = 12.6, 9.3, 6.6, 1.8 Hz, 1H), 2.36 (dtd, *J* = 12.6, 11.0, 9.2 Hz, 1H).



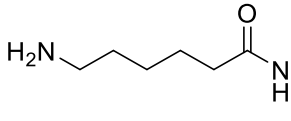
26

26 (HSL-3) was prepared according to the general procedure to afford the product (pink oil, 162.0 mg, 87% yield). ^{1}H-NMR (500 MHz, D_2O) δ 4.69 (dd, *J* = 11.0, 9.3 Hz, 1H), 4.56 (td, *J* = 9.3, 1.9 Hz, 1H), 4.43 (ddd, *J* = 10.5, 9.1, 6.6 Hz, 1H), 3.07 (t, *J* = 7.7 Hz, 2H), 2.65 (dddd, *J* = 12.6, 9.0, 6.7, 1.9 Hz, 1H), 2.48 (t, *J* = 7.3 Hz, 2H), 2.38 (dtd, *J* = 12.6, 10.8, 9.2 Hz, 1H), 2.00 (p, *J* = 7.6 Hz, 2H).



27

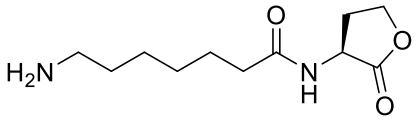
27 (HSL-4) was prepared according to the general procedure to afford the product (brown oil, 268 mg, 89% yield). ^{1}H-NMR (500 MHz, D_2O) δ 4.66 (dd, *J* = 10.8, 9.3 Hz, 1H), 4.57 (td, *J* = 9.3, 2.0 Hz, 1H), 4.41 (ddd, *J* = 10.4, 9.1, 6.7 Hz, 1H), 3.03 (t, *J* = 6.7 Hz, 2H), 2.63 (dddd, *J* = 12.5, 9.1, 6.7, 1.9 Hz, 1H), 2.42-2.32 (m, 3H), 1.71 (p, *J* = 3.6, 4H).



28

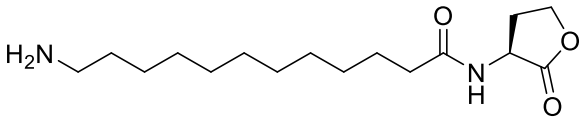
28 (HSL-5) was prepared according to the general procedure to afford the product (yellow oil, 199.3 mg, 93% yield). ^{1}H-NMR (500 MHz, D_2O) δ 4.63 (t, *J* = 10.1 Hz, 1H), 4.54 (td, *J* = 9.3, 1.7

Hz, 1H), 4.38 (ddd, *J* = 10.6, 9.4, 6.7 Hz, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.60 (dddd, *J* = 12.4, 8.7, 6.5, 1.6 Hz, 1H), 2.38-2.28 (m, 3H), 1.67 (p, *J* = 7.5 Hz, 2H), 1.64 (p, *J* = 7.5 Hz, 2H), 1.39 (p, *J* = 7.5 Hz, 2H).



29

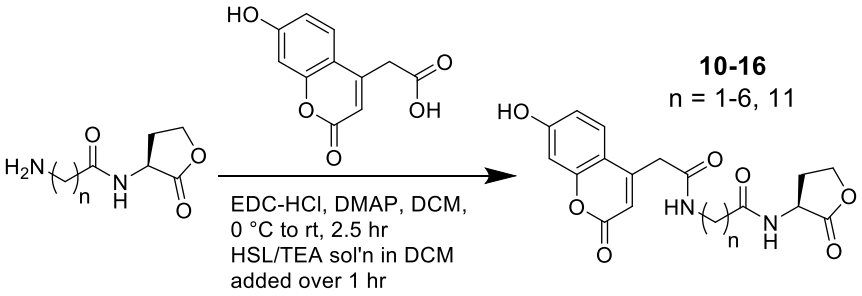
29 (HSL-6) was prepared according to the general procedure to afford the product (yellow oil, 221.4 mg, 97% yield). ^{1}H-NMR (500 MHz, D_2O) δ 4.62 (dd, *J* = 10.9, 9.4 Hz, 1H), 4.53 (td, *J* = 9.2, 1.8 Hz, 1H), 4.38 (ddd, *J* = 10.5, 9.3, 6.7 Hz, 1H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.60 (dddd, *J* = 12.5, 8.6, 6.6, 1.8 Hz, 1H), 2.37-2.27 (m, 3H), 1.65 (p, *J* = 7.2 Hz, 2H), 1.61 (p, *J* = 7.2 Hz, 2H), 1.42-1.30 (m, 4H).



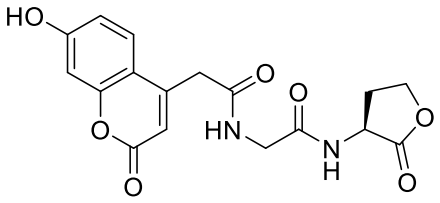
30

30 (HSL-11) was prepared according to the general procedure to afford the product (yellow oil, 244.7 mg, 82% yield). ^{1}H-NMR (500 MHz, D_2O) δ 4.61 (dd, *J* = 11.3, 9.4 Hz, 1H), 4.53 (td, *J* = 9.2, 1.9 Hz, 1H), 4.37 (ddd, *J* = 10.9, 9.6, 6.6 Hz, 1H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.60 (dddd, *J* = 12.7, 8.8, 6.8, 1.9 Hz, 1H), 2.36-2.24 (m, 3H), 1.63 (p, *J* = 7.3 Hz, 2H), 1.58 (p, *J* = 7.2 Hz, 2H), 1.38-1.24 (m, 14H).

*Synthesis of **10**–**16** (CU-n compounds (n = 1-6, 11))*

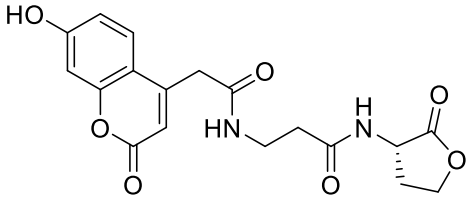


To a solution of 7-hydroxycoumarinyl-4-acetic acid (0.21 mmol, 0.95 eq.), EDC-HCl (0.26 mmol, 1.2 eq.), and 4-dimethylaminopyridine (DMAP, 0.06 mmol, 0.25 eq.) in dichloromethane (6 mL), a solution of the precursor HSL (0.22 mmol, 1.0 eq.) and triethylamine (TEA, 0.22 mmol, 1.0 eq) in dichloromethane (3 mL) was added dropwise over 90 min at 0 °C under N_2. The solution stirred for 2 hours under N_2 at 0 °C. The reaction mixture was concentrated under reduced pressure to yield the crude product. All desired products were purified to homogeneity via flash silica gel column chromatography with various eluent mixtures. The identity and purity of each product was confirmed using mass spectrometry and ^{1}H- and ^{13}C-NMR.



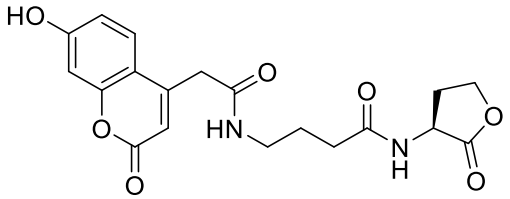
10

10 (CU-1) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (white solid, 2.89 mg, 5.2% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.54 (t, *J* = 5.9 Hz, 1H), 8.44 (d, *J* = 8.0 Hz, 1 H), 7.63 (d, *J* = 8.7 Hz, 1H), 6.77 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.71 (d, *J* = 2.41 Hz, 1H), 6.21 (s, 1H), 4.61 (ddd, *J* = 11.0, 8.6, 8.0 Hz, 1H), 4.35 (td, *J* = 8.8, 1.8 Hz, 1H), 4.22 (ddd, *J* =10.6, 8.7, 6.4 Hz, 1H), 3.76 (d, *J* = 5.7 Hz, 2H), 3.72 (s, 2H), 2.39 (dddd, *J* = 12.1, 8.7, 6.4, 1.8 Hz, 1H), 2.14 (dtd, *J* = 12.0, 10.7, 8.9 Hz, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.11, 168.75, 168.11, 160.22, 154.97, 151.05, 126.80, 112.88, 102.20, 65.25, 47.85, 41.87, 38.45, 28.27. ESI-EMM: $[M+H]^+$ calculated 361.1030; measured 361.1025.



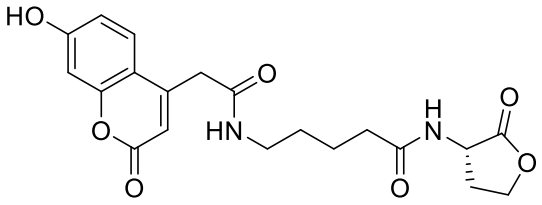
11

11 (CU-2) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (white solid, 15.39 mg, 28% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.54 (broad s, 1H), δ 8.41 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 6.79 (dd, J = 8.7, 2.4 Hz, 1H), 6.71 (d, J = 2.4 Hz, 1H), 6.15 (s, 1H) 4.57 – 4.50 (m, 1H), 4.34 (ddd, J = 8.7, 6.9, 5.0 Hz, 1H), 4.21 (ddd, J = 10.5, 8.7, 6.5 Hz, 1H), 3.62 (d, J = 5.0 Hz, 2H), 3.43 (m, 1H), 3.31 (s, 2H), 3.26 (m, 1H), 2.31(m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.76, 173.43, 171.03, 168.12, 160.71, 155.47, 151.69, 127.20, 113.40, 112.16, 102.73, 65.74, 57.49, 52.23, 49.65, 48.32, 28.74. ESI-EMM: $[M+H]^+$ calculated 375.1187; measured 375.1183.



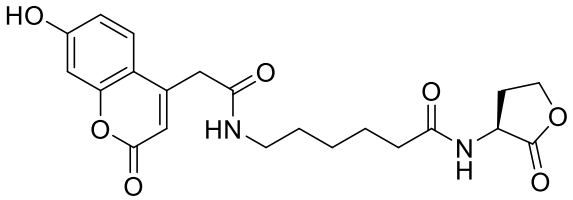
12

12 (CU-3) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (light pink solid, 9.41 mg, 13% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.54 (broad s, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.20 (t, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 6.79 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.71 (d, *J* = 2.3 Hz, 1H), 6.15 (s, 1H), 4.53 (ddd, *J* = 10.9, 9.2, 8.5 Hz, 1H), 4.34 (td, *J* = 8.7, 1.9 Hz, 1H), 4.20 (ddd, *J* = 10.4, 8.7, 6.5 Hz, 1H), 3.63 (s, 2H), 3.06 (q, *J* = 6.6, 2 H), 2.38 (ddd, J = 12.1, 8.7, 6.5, 1.9 Hz, 1H), 2.18-2.08 (m, 3H), 1.64 (p, *J* = 7.3 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.34, 171.77, 167.51, 160.22, 154.98, 151.19, 126.65, 112.88, 111.67, 111.44, 102.77, 65.21, 59.72, 47.83, 38.80, 38.40, 32.46, 28.21, 25.01. ESI-EMM: $[M+H]^+$ calculated 389.1343; measured 389.1336.



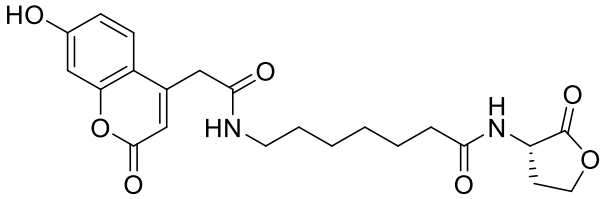
13

13 (CU-4) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (white solid, 14.94 mg, 26% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.20 (t, J = 5.6 Hz, 1H), 8.13 (d, J = 7.4 Hz, 1H), 7.60 (d, J = 8.7 Hz, 1H), 6.79 (d, J = 8.7 Hz, 1H), 6.71 (d, J = 2.3 Hz, 1H), 6.14 (s, 1H), 4.53 (dt, J = 11.0, 8.7 Hz, 1H), 4.35 – 4.29 (m, 1H), 4.21 (ddd, J = 10.5, 8.8, 6.5 Hz, 1H), 3.62 (d, J = 12.3 Hz, 2H), 3.53 – 3.36 (m, 2H), 3.06 (td, J = 7.3, 3.7 Hz, 2H), 2.43 – 2.34 (m, 1H), 2.12 (dd, J = 8.1, 6.3 Hz, 2H), 1.50 (h, J = 7.0 Hz, 2H), 1.40 (td, J = 8.0, 4.2 Hz, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.84, 173.51, 172.79, 167.93, 160.75, 158.15, 155.51, 151.81, 127.12, 118.97, 116.58, 113.47, 102.79, 65.70, 57.53, 52.16, 49.59, 48.29, 34.48, 28.94, 23.11. ESI-EMM: $[M+H]^+$ calculated 403.1500; measured 403.1494.



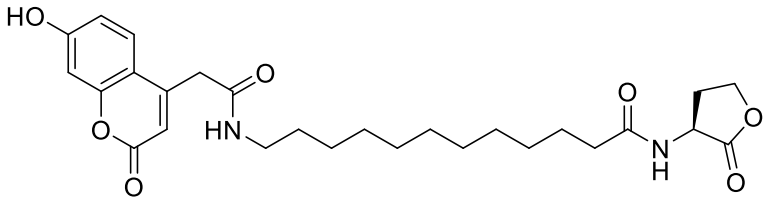
14

14 (CU-5) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (white solid, 22.04 mg, 37% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.60 (broad s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 8.18 (t, *J* = 5.6 Hz, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 6.78 (dd, *J* = 8.7, 1.2 Hz, 1H), 6.71 (d, *J* = 1.4 Hz, 1H), 6.14 (s, 1H), 4.52 (ddd, *J* = 10.8, 9.2, 8.6 Hz, 1H), 4.34 (td, *J* = 8.9, 1.9 Hz, 1H), 4.20 (ddd, *J* = 10.5, 8.7, 6.5 Hz, 1H), 3.62 (s, 2H), 3.04 (q, *J* = 6.6 Hz, 2H), 2.38 (m, 1H), 2.13 (m, 1H), 2.09 (t, *J* = 7.4 Hz, 2H), 1.49 (p, *J* = 7.5 Hz, 2H), 1.40 (p, *J* = 7.5 Hz, 2H), 1.28-1.20 (m, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.86, 172.62, 167.94, 160.85, 158.53, 158.28, 155.61, 151.87, 127.03, 118.90, 116.52, 102.88, 65.69, 48.28, 39.33, 39.13, 35.43, 29.14, 28.68, 26.37, 25.23. ESI-EMM: $[M+H]^+$ calculated 417.1656; measured 417.1652.



15

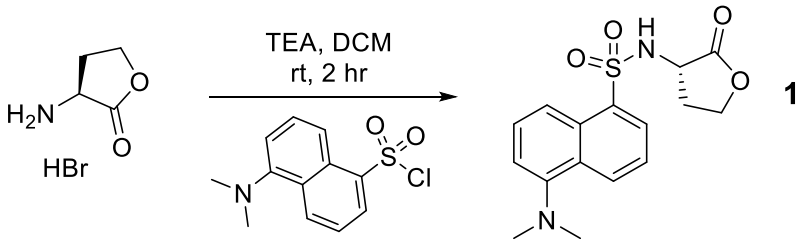
15 (CU-6) was prepared according to the general procedure and purified by flash column chromatography (10% MeOH in DCM) to afford the product (white solid, 4.65 mg, 7% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 8.15 (t, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 6.79 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.72 (d, *J* = 2.3 Hz, 1H), 6.15 (s, 1H), 4.52 (ddd, *J* = 11.0, 9.6, 8.3 Hz, 1H), 4.34 (td, *J* = 8.9, 1.9 Hz, 1H), 4.20 (ddd, *J* = 10.5, 8.7, 6.6 Hz, 1H), 3.60 (s, 2H), 3.05 (q, *J* = 6.4 Hz, 2H), 2.38 (m, 1H), 2.13 (m, 1H), 2.10 (t, *J* = 7.4 Hz, 2H), 1.52-1.44 (m, 2H), 1.42-1.35 (m, 2H), 1.28-1.20 (m, 4H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.86, 172.67, 167.87, 161.66, 160.72, 155.48, 151.80, 127.14, 113.31, 112.13, 111.96, 102.76, 65.69, 48.27, 39.36, 39.20, 35.47, 29.29, 28.72, 28.65, 26.58, 25.49. ESI-EMM: $[M+H]^+$ calculated 431.1813; measured 431.1807.



16

16 (CU-11) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (white solid, 16.01 mg, 21% yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.5 (s, 1H), 8.30 (d, *J* = 8.1 Hz, 1H), 8.15 (t, *J* = 5.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 6.78 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.72 (d, *J* = 2.4 Hz, 1H), 6.16 (s, 1H), 4.52 (ddd, *J* = 10.9, 9.3, 8.5 Hz, 1H), 4.34 (td, *J* = 8.8, 1.9 Hz, 1H), 4.19 (ddd, *J* = 10.4, 8.7, 6.4 Hz, 1H), 3.62 (s, 2H), 3.05 (q, *J* = 6.4 Hz, 2H), 2.37 (m, 1H), 2.12 (m, 1H), 2.09 (t, *J* = 7.3 Hz, 2H), 1.53-1.44 (m, 2H), 1.42-1.33 (m, 2H), 1.25-1.20 (m, 14H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 175.35, 172.21, 167.35, 161.14, 160.21, 154.98, 151.29, 126.65, 112.77, 111.64, 111.44, 102.25, 65.17, 47.76, 38.68, 35.04, 28.97, 28.91, 28.89, 28.73, 28.68, 28.48, 28.23, 26.30, 25.06. ESI-EMM: $[M+H]^+$ calculated 501.2595; measured 501.2590.

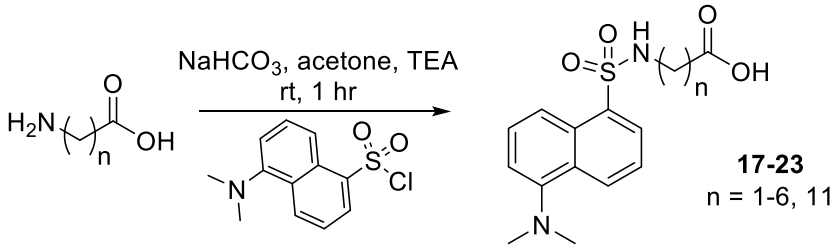
*Synthesis of **1** (DA-0)*



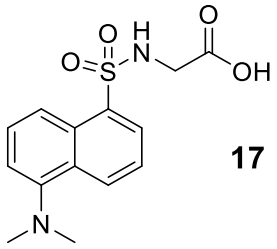
1

Dansyl chloride (0.285 mmol, 0.95 eq.), HBr L-homoserine lactone (0.3 mmol, 1.0 eq.), and TEA (0.75 mmol, 2.5 eq) were dissolved in dichloromethane (5 mL) at rt. The solution was stirred overnight at rt. The mixture was diluted with distilled water, extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure to yield the product **1** (DA-0) (white solid, 68 mg, 71% yield). The identity and purity of DA-0 was confirmed using mass spectrometry and ^{1}H- and ^{13}C-NMR. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.57 (d, *J* = 8.5 Hz, 1H), 8.28-8.22 (m, 2H), 7.6 (dd, *J* = 8.6, 7.6 Hz, 1H), 7.52 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 5.35 (d, *J* = 3.0 Hz, 1H), 4.34 (t, *J* = 9.1 Hz, 1H), 4.10 (11.7, 9.4, 5.6 Hz, 1H), 3.85 (ddd, *J* = 11.4, 8.2, 3.0 Hz, 1H), 2.88 (s, 6H), 2.59 (ddd, *J* = 13.3, 8.2, 5.4 Hz, 1H), 2.13 (qd, *J* = 12.2, 8.8 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 174.09, 152.30, 133.76, 131.49, 130.22, 129.99, 129.77, 129.21, 123.24, 118.72, 115.85, 66.28, 52.12, 45.65, 31.47. ESI-EMM: $[M+H]^+$ calculated 335.1060; measured 335.1053.

*Synthesis of **17**–**23** (DA-n-i compounds (DA-n intermediates; n = 1-6,11))*

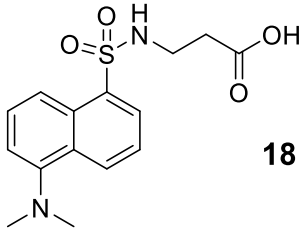


A solution of dansyl chloride (0.74 mmol, 1.0 eq.) in TEA (0.75 mL) and acetone (3 mL) was added to a solution of amino acid (0.93 mmol, 1.25 eq.) in saturated sodium bicarbonate. The mixture stirred for 1 hour at rt. The mixture was then acidified using hydrochloric acid (2M) until it reached a pH of 3 (as monitored by pH paper). The mixture was diluted with water (5 mL) and extracted with ethyl acetate (10 mL). The organic layer was then washed with brine (5 mL), dried over magnesium sulfate, and concentrated under reduced pressure to yield the crude product. All desired products were purified to homogeneity via flash column chromatography with dichloromethane and methanol as eluent. The purity and identity of each intermediate was characterized via ^{1}H-NMR before proceeding to the next synthetic step.



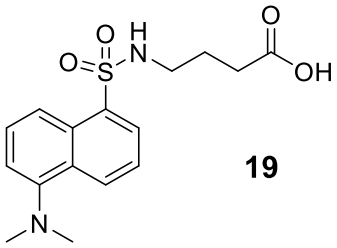
17

17 (DA-1-i) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (yellow solid, 117.9 mg, 51% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.91 (broad s, 1H), 8.50 (d, *J* = 8.6 Hz, 1H), 8.28 (d, *J* = 8.7 Hz, 1H), 8.21 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.54 (dd, *J* = 8.7, 7.6 Hz, 1H), 7.48 (dd, *J* = 8.7, 7.4 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 1H), 5.39 (broad t, *J* = 4.7 Hz, 1H), 3.70 (d, *J* = 4.4 Hz, 2H), 2.86 (s, 6H).



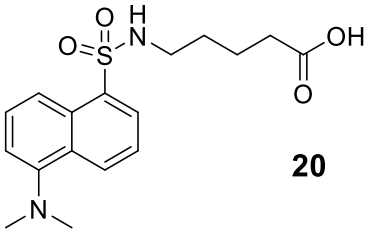
18

18 (DA-2-i) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (yellow solid, 57.0 mg, 35% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 8.7 Hz, 1H), 8.23 (d, *J* = 8.7 Hz, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 7.45 (q, *J* = 7.6 Hz, 2H), 7.10 (d, *J* = 7.6 Hz, 1H), 6.01 (broad s, 1H), 3.10 (broad t, *J* = 5.8 Hz, 2H), 2.82 (s, 6H), 2.44 (broad t, *J* = 6.2 Hz, 2H).



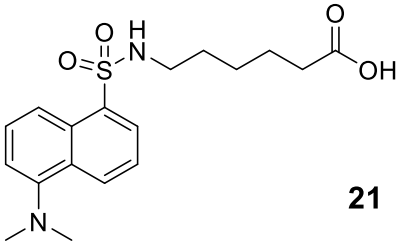
19

19 (DA-3-i) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (yellow solid, 23.3 mg, 7% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 8.5 Hz, 1H), 8.23 (d, *J* = 8.7 Hz, 1H), 8.17 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.48 (dd, *J* = 8.6, 7.5 Hz, 1H), 7.45 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 5.45 (broad s, 1H), 2.89 (broad t, *J* = 6.7 Hz, 2H), 2.83 (s, 6H), 2.30-2.20 (m, 2H), 1.69 (p, *J* = 6.8 Hz, 2H).



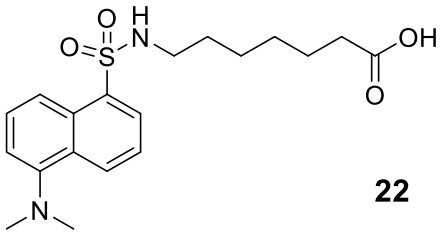
20

20 (DA-4-i) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (green solid, 281.2 mg, 84% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.8 Hz, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.22 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.51 (m, 2H), 7.16 (d, *J* = 7.6 Hz, 1H), 5.42 (t, *J* = 6.0 Hz, 1H), 2.88 (m, 2H), 2.86 (s, 6H), 2.18 (t, *J* = 7.2 Hz, 2H), 1.46 (m, 4H).



21

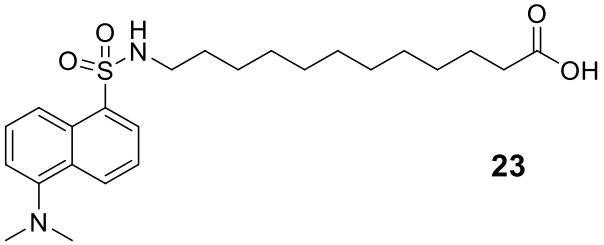
21 (DA-5-i) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (yellow solid, 40.4 mg, 15% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 8.5 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.21 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.51 (dd, *J* = 8.7, 7.5 Hz, 1H), 7.49 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 4.90 (broad s, 1H), 2.89-2.83 (m, 2H), 2.87 (s, 6H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.42 (p, *J* = 7.6 Hz, 2H), 1.36 (p, *J* = 7.2 Hz, 2H), 1.24-1.13 (m, 2H).



22

22 (DA-6-i) was prepared according to the general procedure and purified by flash column chromatography (10% MeOH in DCM) to afford the product (yellow solid, 53.7 mg, 94% yield).

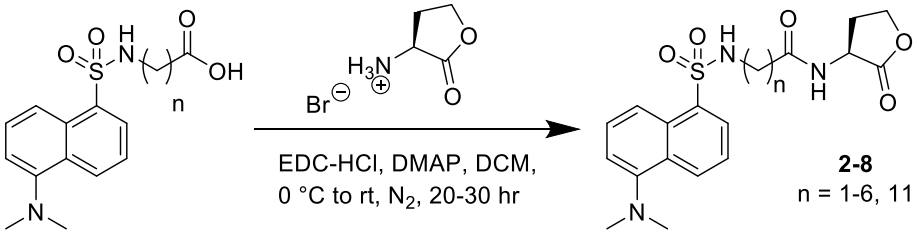
^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* = 8.6 Hz, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 8.22 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.53 (dd, *J* = 8.9, 7.8 Hz, 1H), 7.50 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 4.80 (broad t, *J* = 6.2 Hz, 1H), 2.88-2.83 (m, 2H), 2.86 (s, 6H), 2.20 (t, *J* = 7.5 Hz, 2H), 1.44 (p *J* = 7.3 Hz, 2H), 1.34 (p, *J* = 7.1 Hz, 2H), 1.16-1.10 (m, 4H).



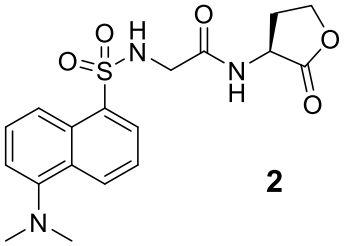
23

23 (DA-11-i) was prepared according to the general procedure and purified by flash column chromatography (10% MeOH in DCM) to afford the product (yellow solid, 27.7 mg, 31% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.5 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.22 (dd, *J* = 7.3, 1.2 Hz, 1H), 7.54 (dd, *J* = 8.7, 7.6 Hz, 1H), 7.50 (dd, *J* = 8.6, 7.4 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 4.81 (broad t, *J* = 6.0 Hz, 1H), 2.89-2.83 (m, 2H), 2.87 (s, 6H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.60 (p, *J* = 7.4 Hz, 2H), 1.37-1.02 (m, 14H), 0.88-0.80 (m, 2H).

*Synthesis of **2**–**8** (DA-n compounds (n = 1-6, 11))*



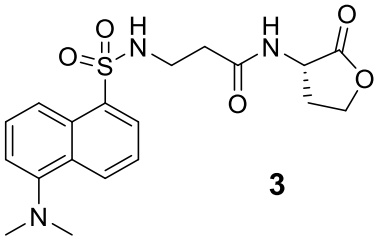
To a solution of the precursor DA-n-i (0.16 mmol, 1.0 eq.), L-homoserine lactone (0.16 mmol, 1.0 eq.), and DMAP (0.04 mmol, 0.25 eq.) in dichloromethane (4 mL), a solution of EDC-HCl (0.20 mmol, 1.2 eq.) in dichloromethane (2 mL) was added via syringe at 0 °C under N_2. The solution stirred for 24 hours under N_2 while warming to rt. The reaction mixture was concentrated under reduced pressure to yield the crude product. All desired products were purified to homogeneity via flash column chromatography with various eluent mixtures. The identity and purity of each product was confirmed using mass spectrometry and ^{1}H- and ^{13}C-NMR.



2

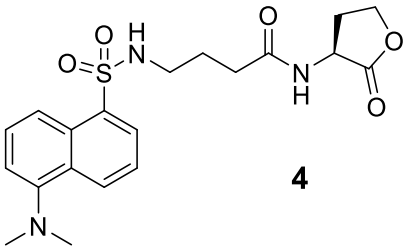
2 (DA-1) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (yellow solid, 7.31 mg, 11% yield). ^{1}H-NMR

(500 MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.5 Hz, 1H), 8.23 (t, *J* = 8.5 Hz, 2H), 7.58 (dd, *J* = 8.7, 7.6 Hz, 1H), 7.51 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 7.5 Hz, 1H), 5.82 (broad t, *J* = 6.5 Hz, 1H), 4.58 (ddd, *J* = 11.6, 8.8, 7.5 Hz, 1H), 4.38 (td, *J* = 9.0, 1.4 Hz, 1H), 4.21 (ddd, *J* = 11.1, 9.3, 6.1 Hz, 1H), 3.62 (dd, *J* = 17.4, 6.6 Hz, 1H), 3.56 (dd, *J* = 17.4, 6.5 Hz, 1H), 2.87 (s, 6H), 2.55 (dddd, *J* = 12.5, 8.8, 6.0, 1.3 Hz, 1H), 1.97 (qd, *J* = 11.8, 9.0 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.10, 168.95, 152.50, 133.38, 131.44, 130.49, 130.15, 129.60, 129.33, 123.44, 118.28, 115.70, 66.07, 48.91, 46.07, 45.61, 29.60, 15.49. ESI-EMM: $[M+H]^+$ calculated 392.1275; measured 392.1266.



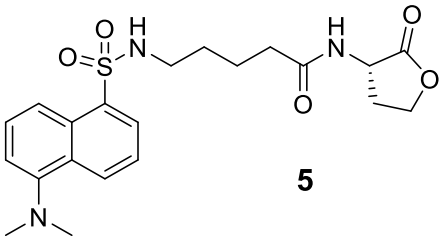
3

3 (DA-2) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (yellow solid, 9.64 mg, 15% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 8.6 Hz, 1H), 8.24 (d, *J* = 6.2 Hz, 1H), 8.22 (dd, *J* = 6, 1.2 Hz, 1H), 7.56 (dd, *J* = 8.6, 7.5 Hz, 1H), 7.51 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.17 (d, *J* = 7.2 Hz, 1H), 5.96 (broad d, *J* = 6.2 Hz, 1H), 5.59 (broad t, *J* = 6.5 Hz, 1H), 4.47-4.38 (m, 2H), 4.24 (ddd, *J* = 11.3, 9.3, 6.0 Hz, 1H), 3.19 (q, *J* = 6.0 Hz, 2H), 2.87 (s, 6H), 2.71 (dddd, *J* = 12.6, 8.7, 6.0, 1.4 Hz, 1H), 2.41-2.36 (m, 2H), 2.08 (qd, *J* = 11.7, 8.9 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.43, 171.43, 162.77, 152.23, 134.88, 130.81, 130.12, 129.76, 129.69, 128.72, 123.40, 118.97, 115.49, 66.28, 49.47, 39.60, 36.71, 35.78, 29.95. ESI-EMM: $[M+H]^+$ calculated 406.1431; measured 406.1428.



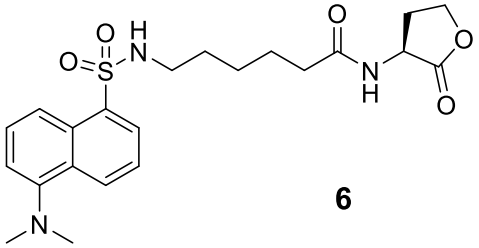
4

4 (DA-3) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (yellow solid, 1.59 mg, 6% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 8.5 Hz, 1H), 8.23 (d, *J* = 8.6 Hz, 1H), 8.19 (dd, J = 7.3, 1.3 Hz, 1H), 7.53 (dd, J = 8.6, 7.5 Hz, 1H), 7.49 (dd, J = 8.5, 7.2 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.30-6.22 (m, 1H), 5.24-5.17 (m, 1H), 4.50 (ddd, J = 11.6, 8.7, 6.5 Hz, 1H), 4.44 (td, J = 9.1, 1.4 Hz, 1H), 2.96-2.87 (m, 2H), 2.86 (s, 6H), 2.72 (dddd, J = 13.5, 8.2, 5.8, 1.2 Hz, 1H), 2.33-2.22 (m, 3H), 1.83-1.70 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.28, 173.27, 152.30, 134.74, 130.67, 130.11, 129.92, 129.75, 128.63, 123.45, 118.79, 115.42, 66.16, 49.38, 45.59, 42.61, 32.93, 30.14, 25.23. ESI-EMM: $[M+H]^+$ calculated 420.1588; measured 420.1582.



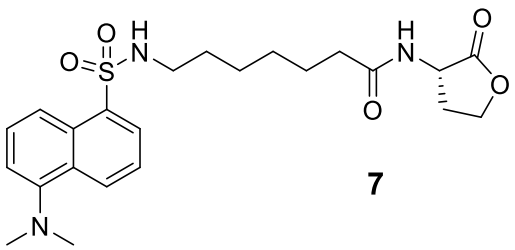
5

5 (DA-4) was prepared according to the general procedure and purified by flash column chromatography (5% MeOH in DCM) to afford the product (yellow solid, 86.6 mg, 87% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 8.5 Hz, 1H), 8.28 (d, *J* = 8.6 Hz, 1H), 8.19 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.52 (dd, *J* = 8.8, 7.8 Hz, 1H), 7.49 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 6.58 (broad d, *J* = 6.9 Hz, 1H), 5.52 (broad t, *J* = 6.2 Hz, 1H), 4.57 (ddd, *J* = 11.4, 8.9, 6.9 Hz, 1H), 4.41 (td, 9.1, 1.5 Hz, 1H), 4.23 (ddd, *J* = 11.0, 9.3, 6.1 Hz, 1H), 2.89-2.84 (m, 2H), 2.86 (s, 6H), 2.64 (dddd, *J* = 12.5, 9.0, 6.1, 1.5 Hz, 1H), 2.22-2.11 (m, 3H), 1.61 (p, *J* = 7.4 Hz, 2H), 1.45 (p, *J* = 7.0 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 176.04, 173.66, 152.11, 134.97, 130.55, 130.07, 129.79, 129.62, 128.58, 123.41, 119.05, 115.43, 66.32, 53.63, 49.22, 25.62, 42.88, 35.34, 29.87, 28.91, 22.42. ESI-EMM: $[M+H]^+$ calculated 433.52; measured 433.50.



6

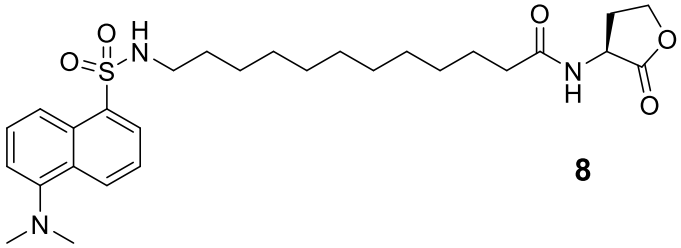
6 (DA-5) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (yellow solid, 13.41 mg, 27% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.6 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.21 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.55 (dd, *J* = 8.6, 7.5 Hz, 1H), 7.51 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.17 (d, *J* = 7.5 Hz, 1H), 6.07 (broad d, *J* = 6.0 Hz, 1H), 4.90 (broad t, *J* = 6.3 Hz, 1H), 4.53 (ddd, *J* = 11.6, 8.7, 6.1 Hz, 1H), 4.47 (td, *J* = 9.1, 1.4 Hz, 1H), 4.27 (ddd, *J* = 11.1, 9.3, 6.1 Hz, 1H), 2.91-2.85 (m, 2H), 2.87 (s, 6H), 2.77 (dddd, *J* = 12.5, 8.7, 6.0, 1.4 Hz, 1H), 2.18 (dtd, *J* = 12.5, 11.3, 8.9 Hz, 1H), 2.11 (t, *J* = 7.2 Hz, 2H), 1.5 (p, *J* = 7.2 Hz, 2H), 1.42 (p, *J* = 7.1 Hz, 2H), 1.29-1.22 (m, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.61, 173.31, 134.75, 130.42, 129.89, 129.61, 128.41, 123.24, 118.74, 115.20, 66.14, 49.28, 45.44, 42.87, 35.41, 30.15, 29.00, 25.24, 24.34. ESI-EMM: $[M+H]^+$ calculated 448.1901; measured 448.1898.



7

7 (DA-6) was prepared according to the general procedure and purified by flash column chromatography (100% EtOAc) to afford the product (yellow solid, 8.60 mg, 35% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.6 Hz, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.21 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.55 (dd, *J* = 8.8, 7.7 Hz, 1H), 7.51 (dd, *J* = 8.7, 7.3 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 6.02 (broad d, *J* = 5.8 Hz, 1H), 4.75 (broad t, *J* = 6.1 Hz, 1H), 4.54 (ddd, *J* = 11.5, 8.7, 6.1 Hz, 1H),

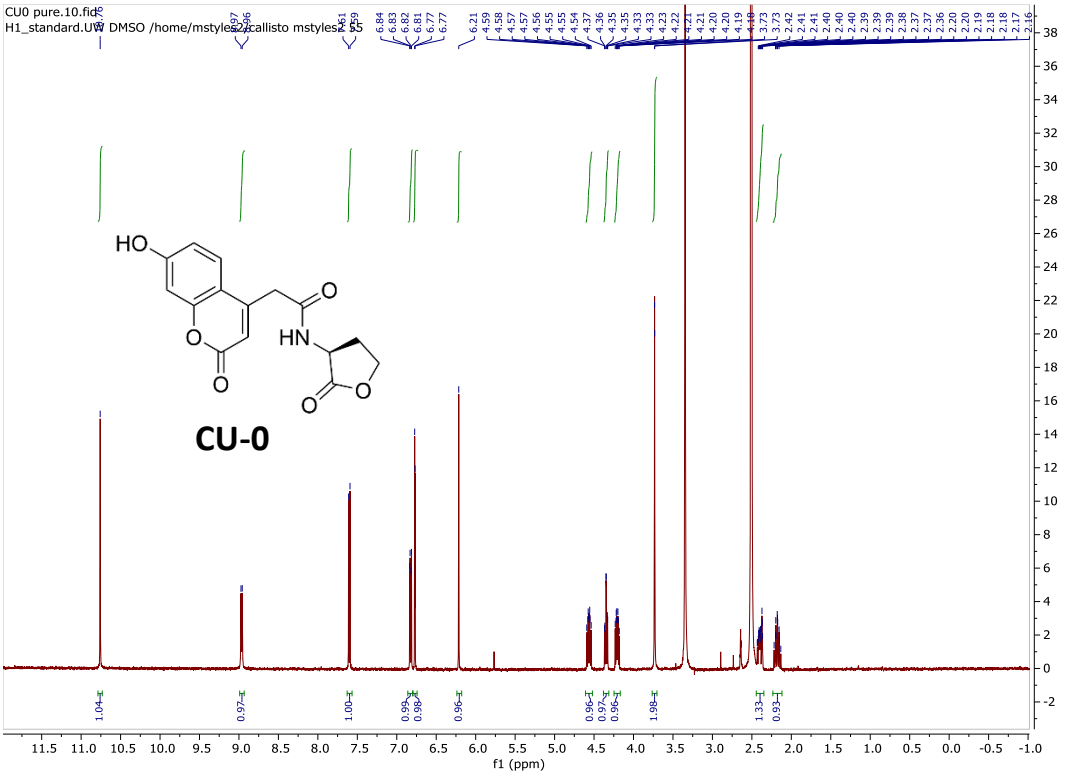
4.45 (td, *J* = 9.0, 1.3 Hz, 1H), 4.26 (ddd, *J* = 11.2, 9.3, 6.0 Hz, 1H), 2.91-2.85 (m, 2H), 2.87 (s, 6H), 2.79 (dddd, *J* = 12.5, 8.8, 5.9, 1.3 Hz, 1H), 2.19-2.07 (m, 3H), 1.5 (p, *J* = 7.0 Hz, 2H), 1.39 (p, *J* = 6.8 Hz, 2H), 1.27-1.17 (m, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.82, 173.73, 152.28, 130.59, 129.86, 129.78, 128.60, 123.45, 118.94, 115.41, 66.34, 49.46, 45.65, 43.23, 35.91, 30.57, 29.33, 28.32, 25.83, 25.07. ESI-EMM: $[M+H]^+$ calculated 462.2057; measured 462.2054.

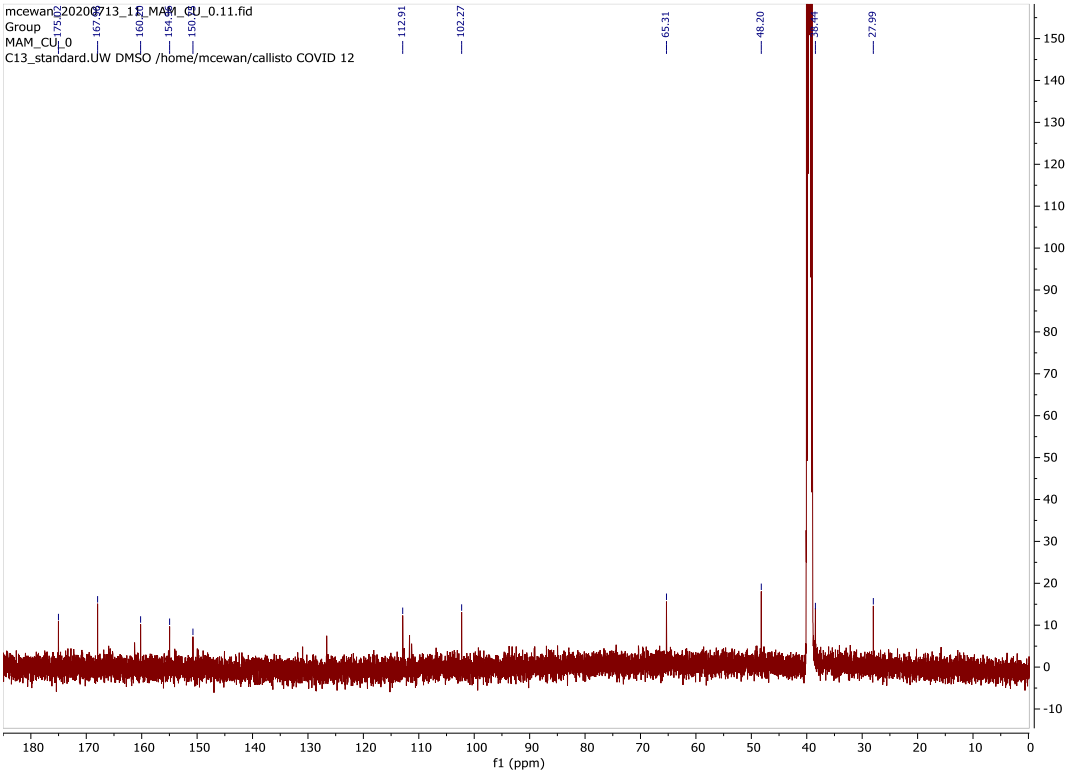


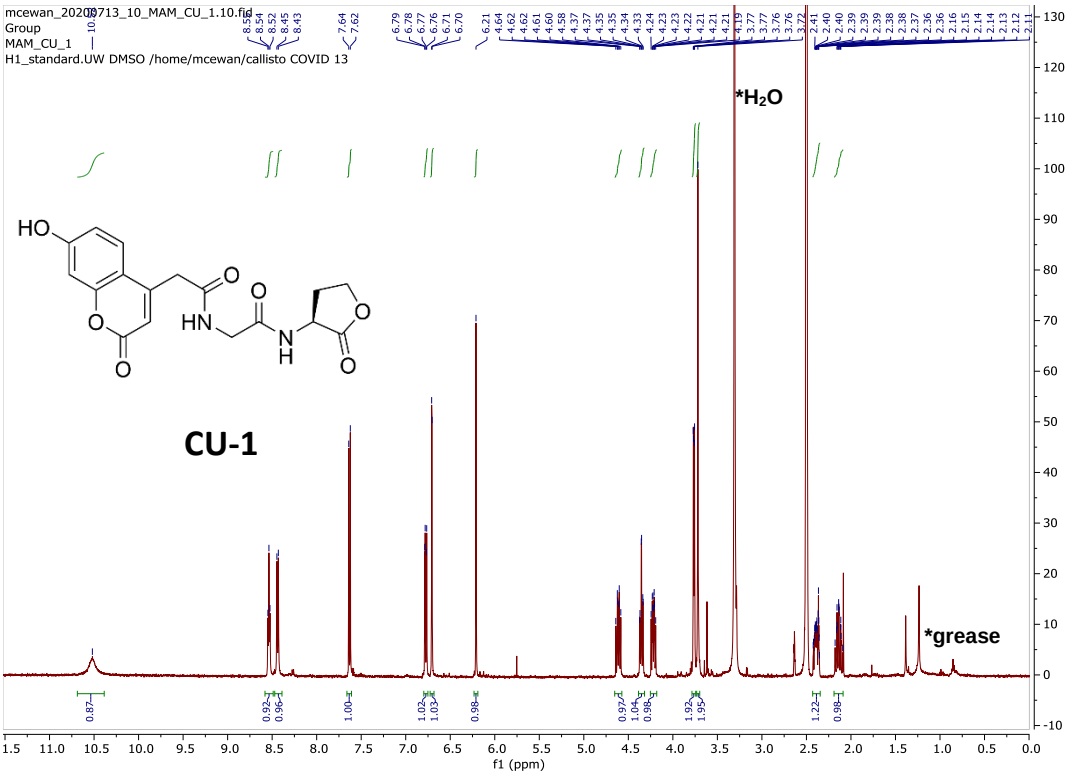
8

8 (DA-11) was prepared according to the general procedure and purified by flash column chromatography (20% MeOH in DCM) to afford the product (yellow solid, 9.05 mg, 38% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 8.5 Hz, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 8.22 (dd, *J* = 7.3, 1.3 Hz, 1H), 7.54 (dd, *J* = 8.8, 7.7 Hz, 1H), 7.50 (dd, *J* = 8.7, 7.4 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 5.98 (broad s, 1H), 4.60 (broad t, *J* = 5.3 Hz, 1H), 4.53 (ddd, *J* = 11.6, 8.6, 5.8 Hz, 1H), 4.44 (td, *J* = 9.1, 1.2 Hz, 1H), 4.26 (ddd, *J* = 11.3, 9.3, 5.8 Hz, 1H), 2.88-2.80 (m, 3H), 2.87 (s, 6H), 2.22 (td, *J* = 7.4, 1.4 Hz, 2H), 2.10 (dtd, *J* = 12.8, 11.7, 8.8 Hz, 1H), 1.62 (p, J = 7.5 Hz, 2H), 1.34 (p, J = 7.1 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 175.54, 173.76, 152. 06, 130.39, 129.67, 128.36, 123.21, 118.68, 115.16, 66.13, 49.29, 45.43, 43.33, 36.19, 36.08, 31.60, 30.68, 29.48, 29.13, 29.07, 29.05, 28.78,26.27, 25.36, 22.67, 18.77, 11.45. ESI-EMM: $[M+H]^+$ calculated 532.2840; measured 532.2842.

^{1}H- and ^{13}C-NMR spectra for the FRET-probe library (^{1}H-NMR only for intermediates)



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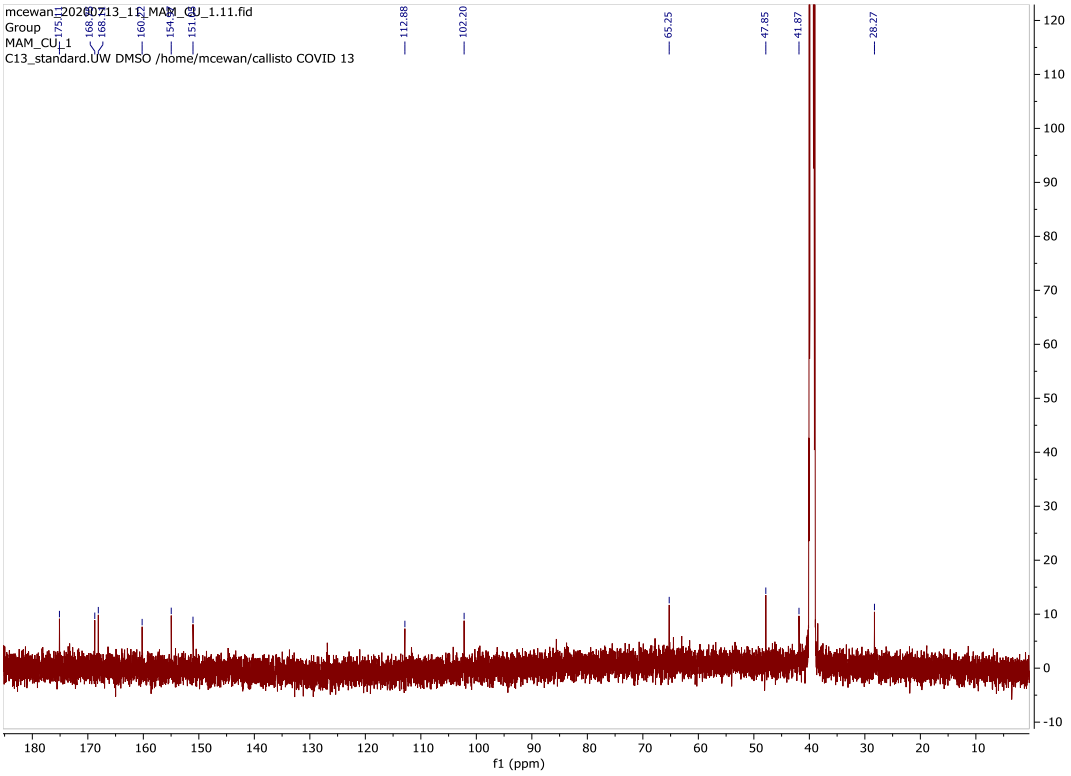
CU-1
*grease
f1 (ppm)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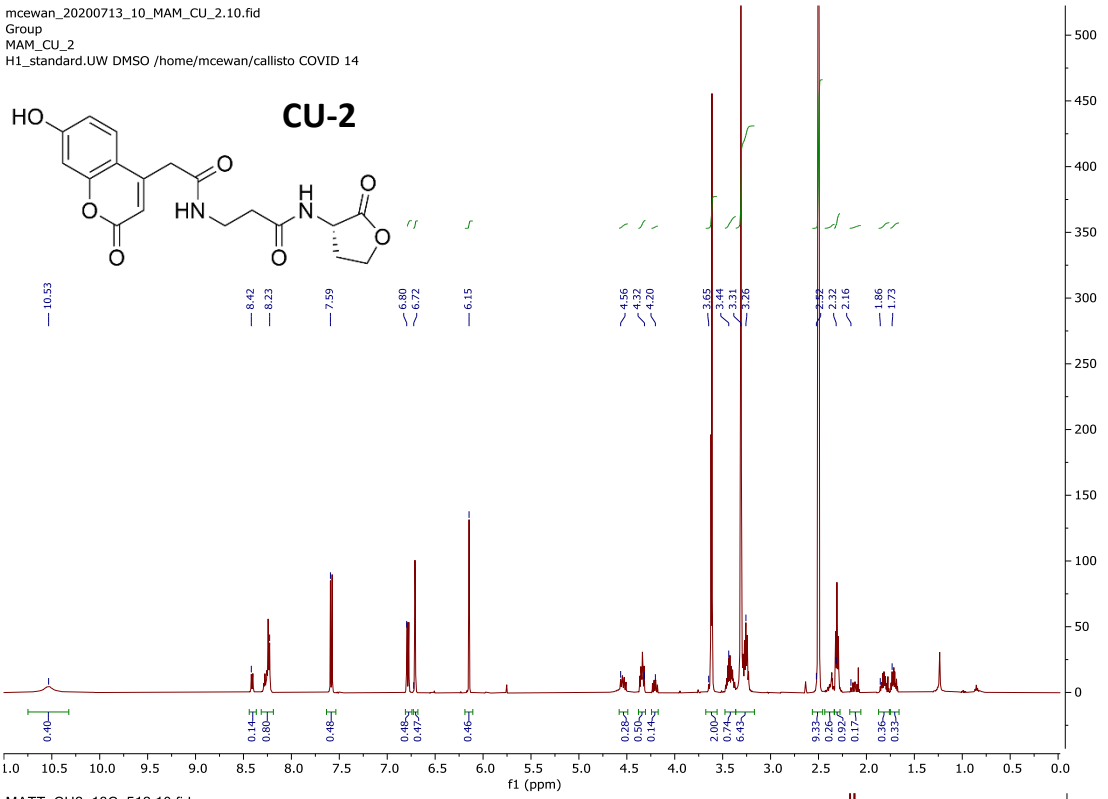
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O
O
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8.23
7.59
6.80
6.72
6.15
4.56
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4.20
3.65
3.44
3.31
3.26
2.52
2.32
2.16
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1.73
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0.47
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0.33
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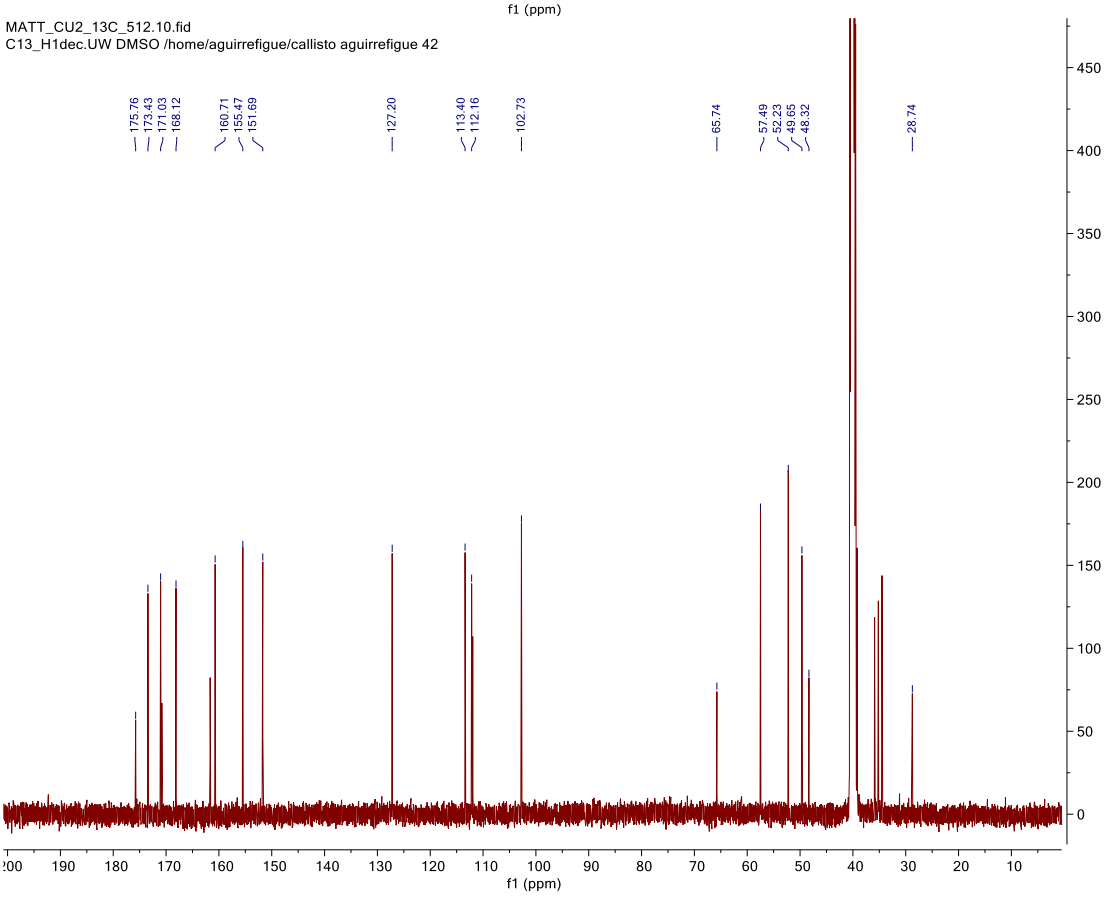
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168.12
160.71
155.47
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112.16
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65.74
57.49
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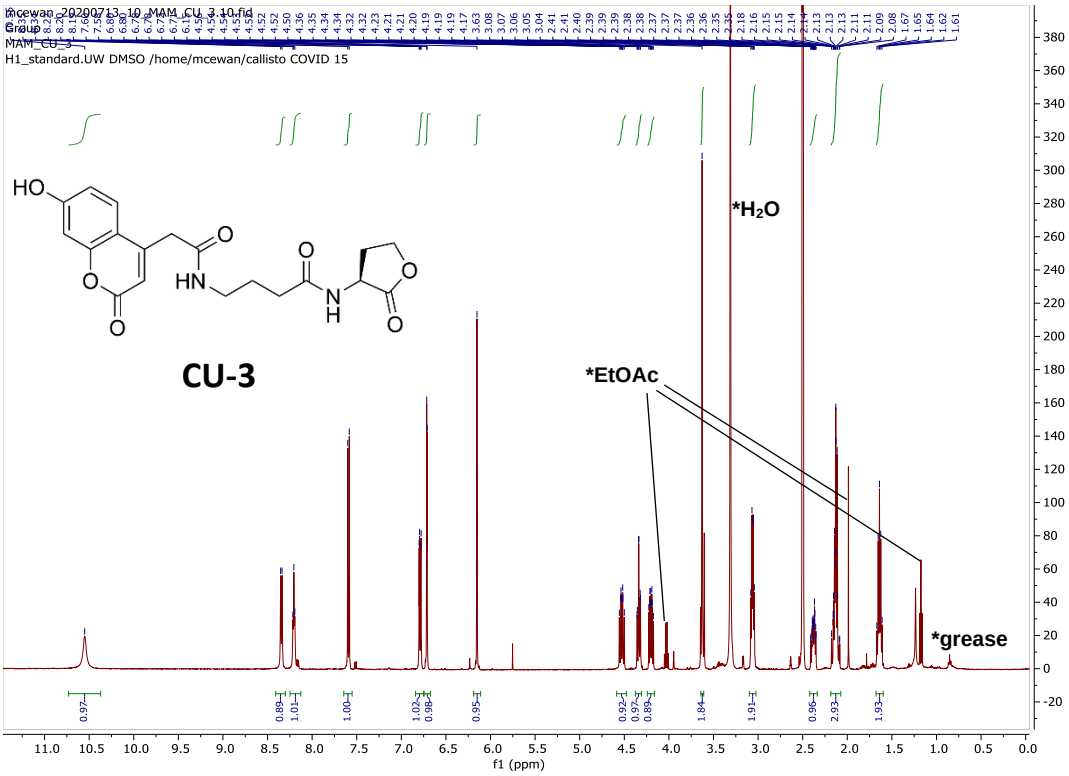
48.32
28.74
f1 (ppm)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CU-3
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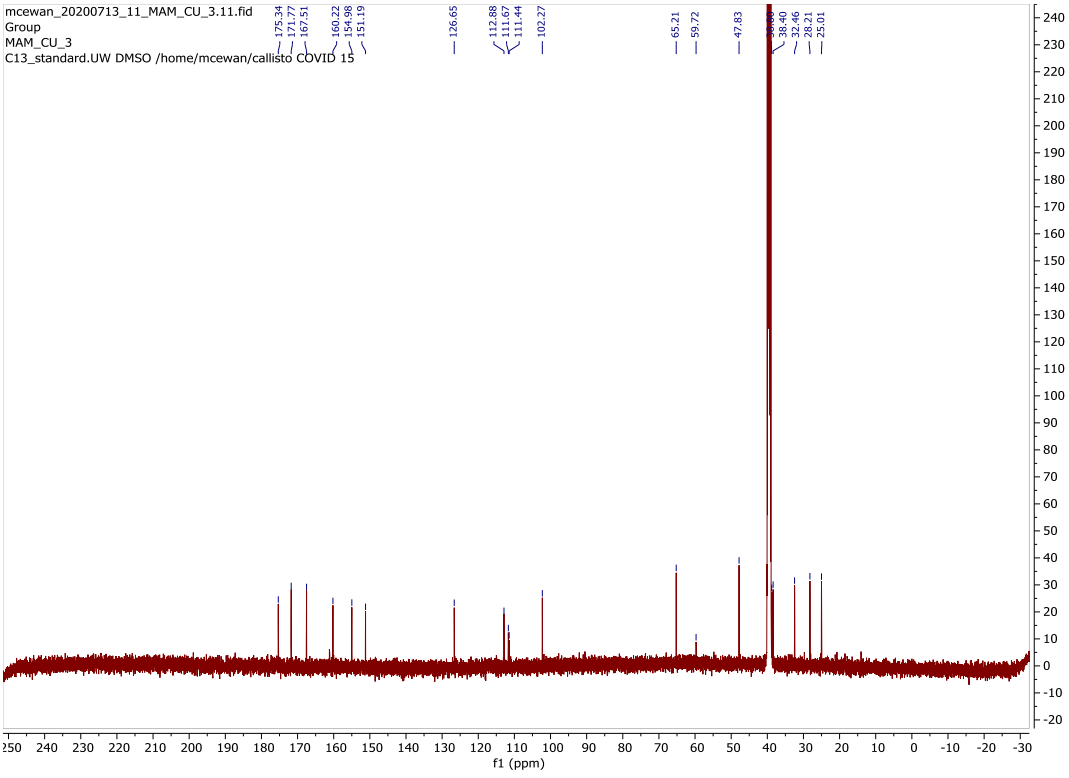
*EtOAc
*grease
f1 (ppm)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126.65
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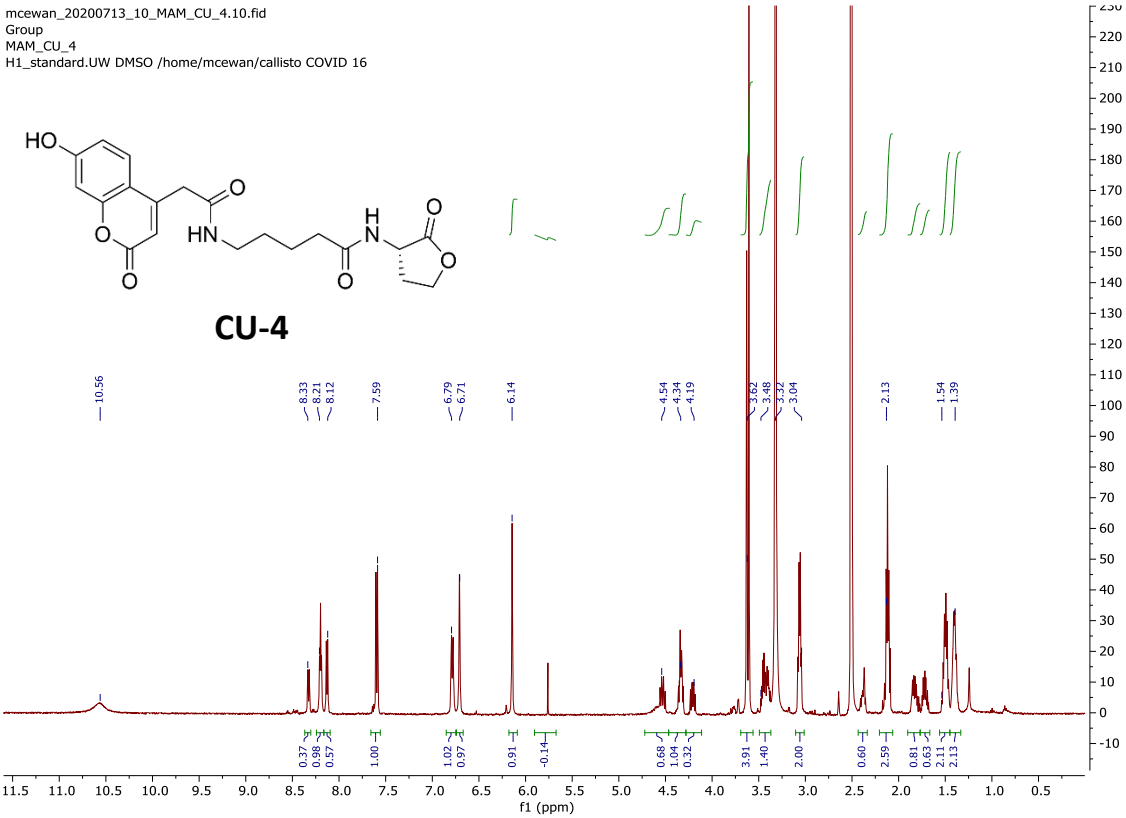
f1 (ppm)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6.79
6.71
6.14
4.54
4.34
4.19
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3.48
3.32
3.04
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1.54
1.39
0.37
0.98
0.57
1.00
1.02
0.97
0.91
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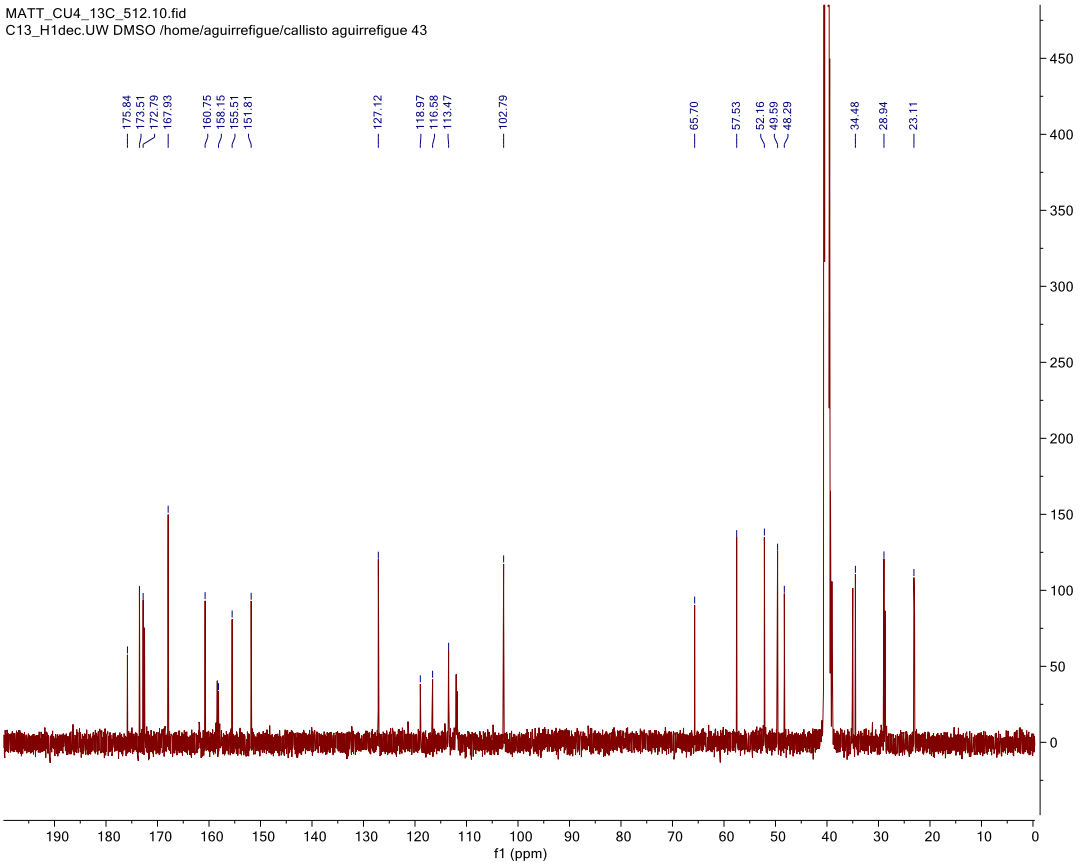
2.13
f1 (ppm)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151.81
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52.16
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34.48
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23.11
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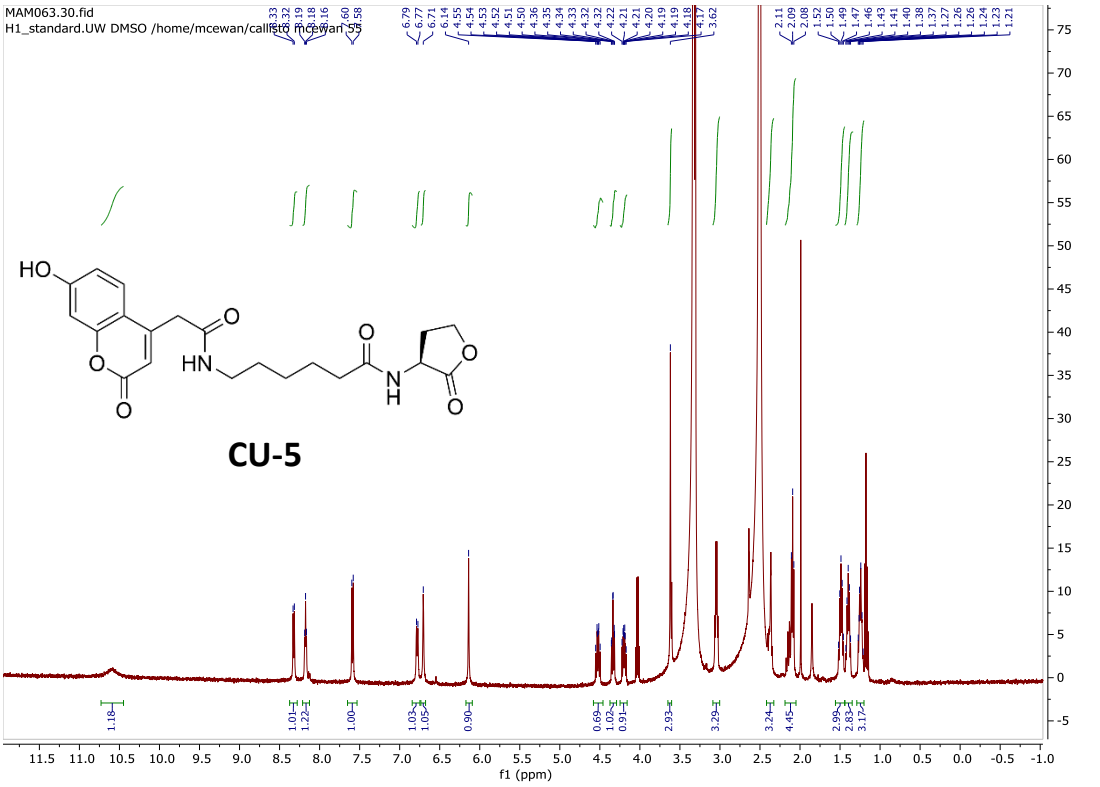
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H
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O
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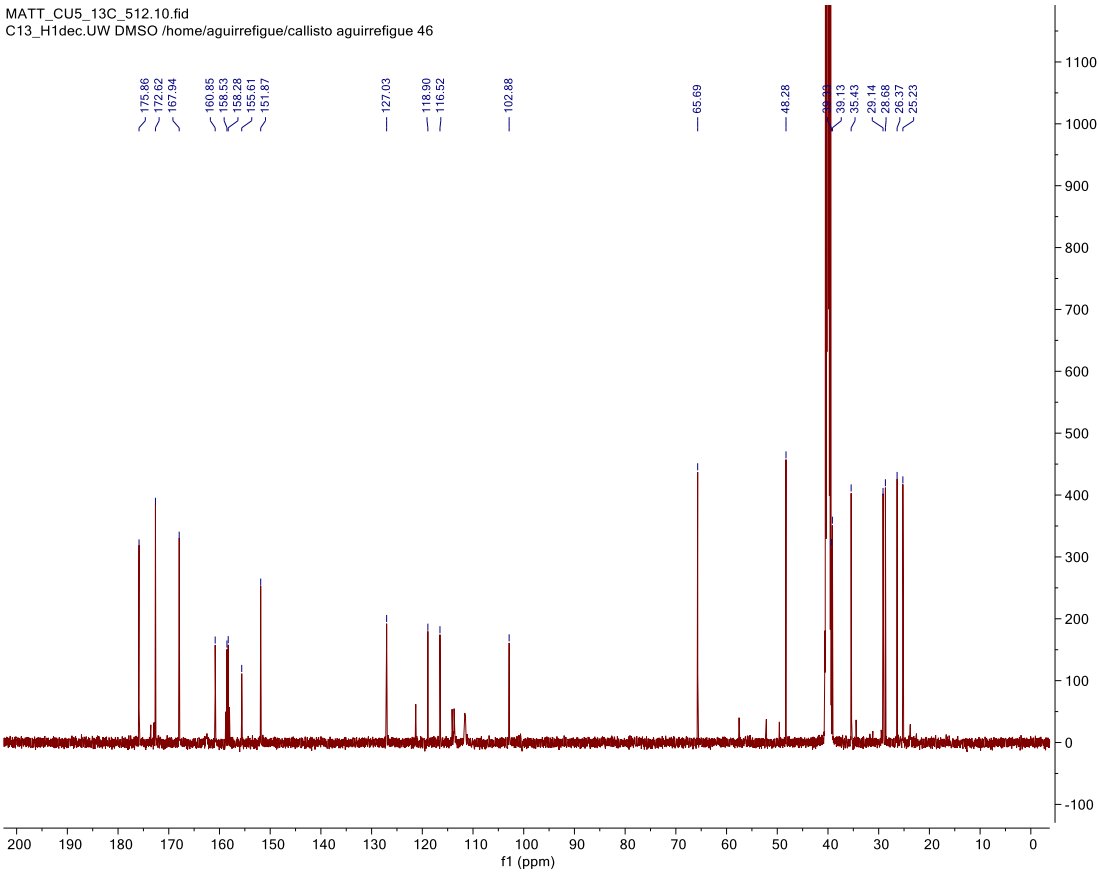
CU-5
f1 (ppm)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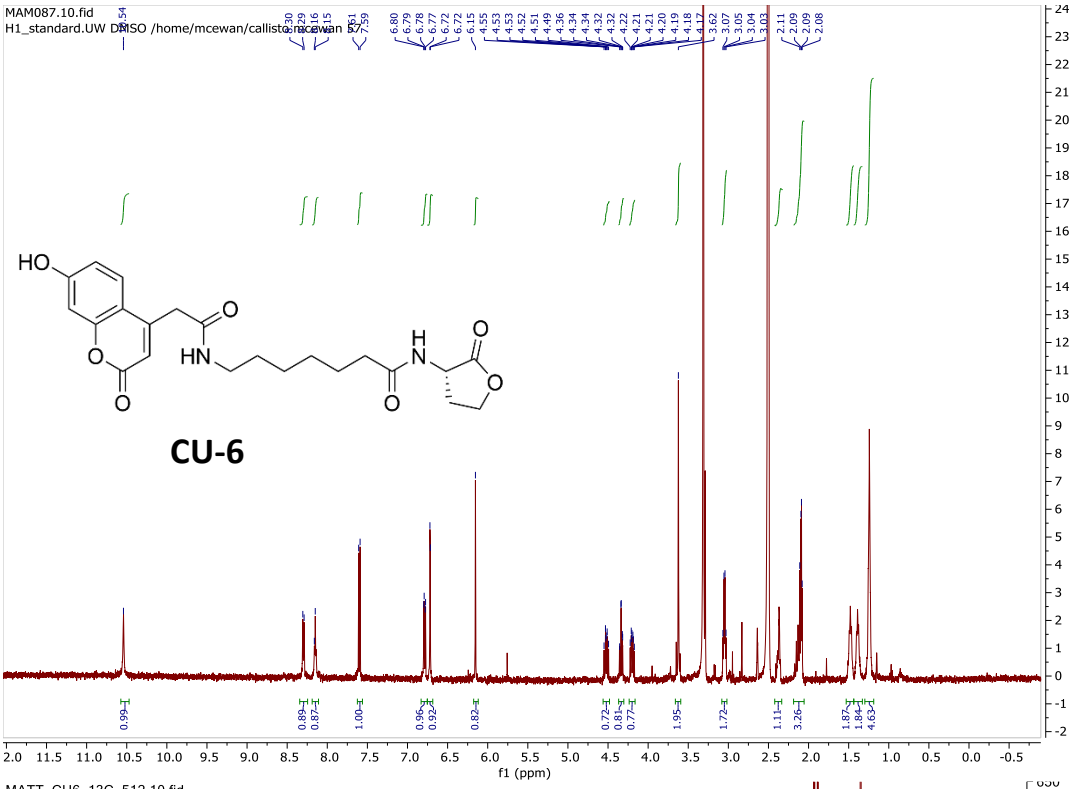
25.23
f1 (ppm)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HN
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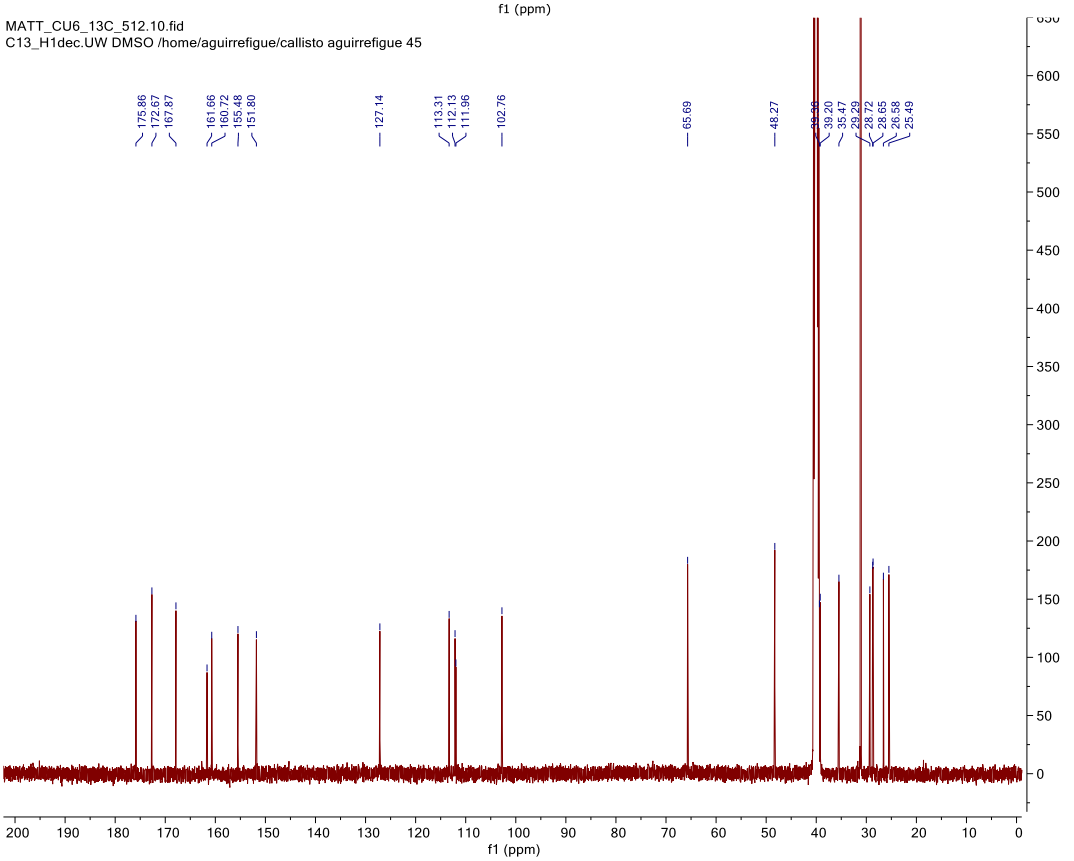
H
N
O
O
O
CU-6
f1 (ppm)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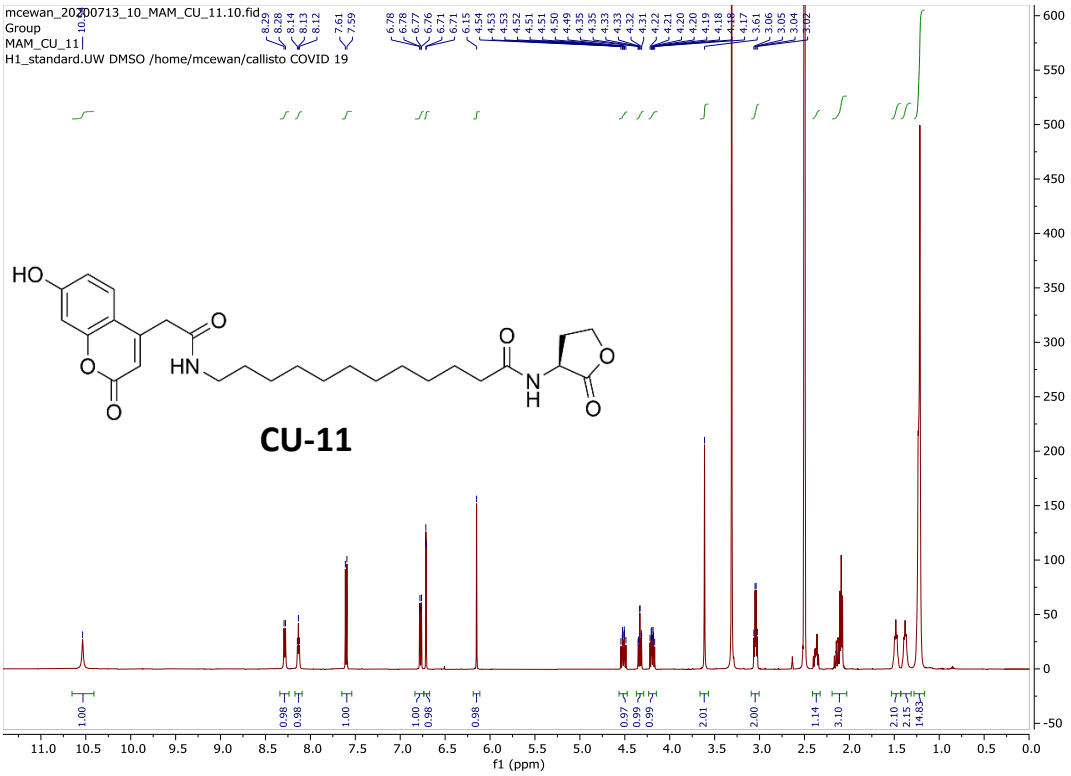
f1 (ppm)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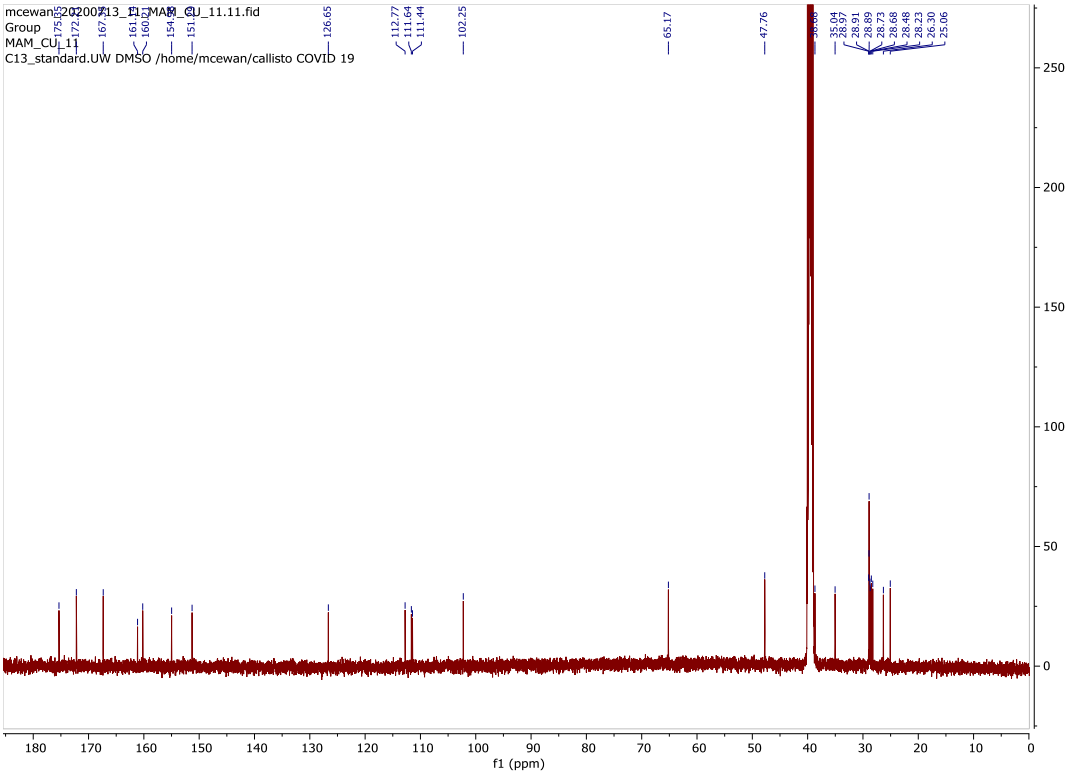
H
O
O
O
O
CU-11
f1 (ppm)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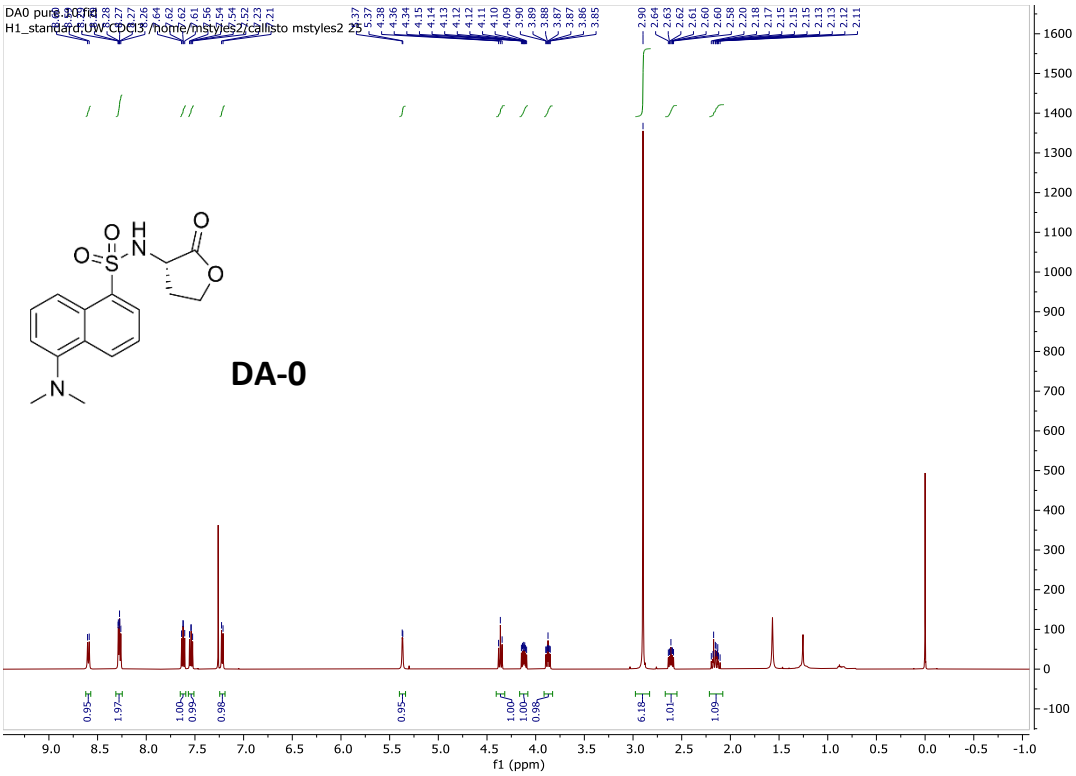
f1 (ppm)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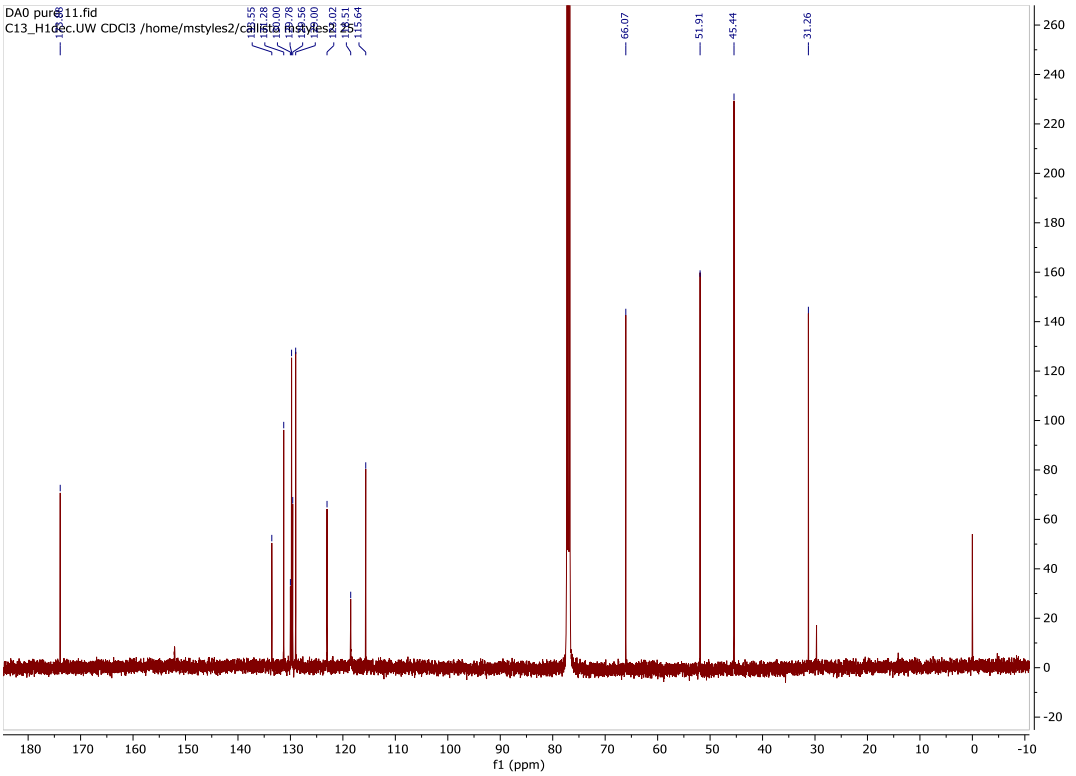
DA-0
f1 (ppm)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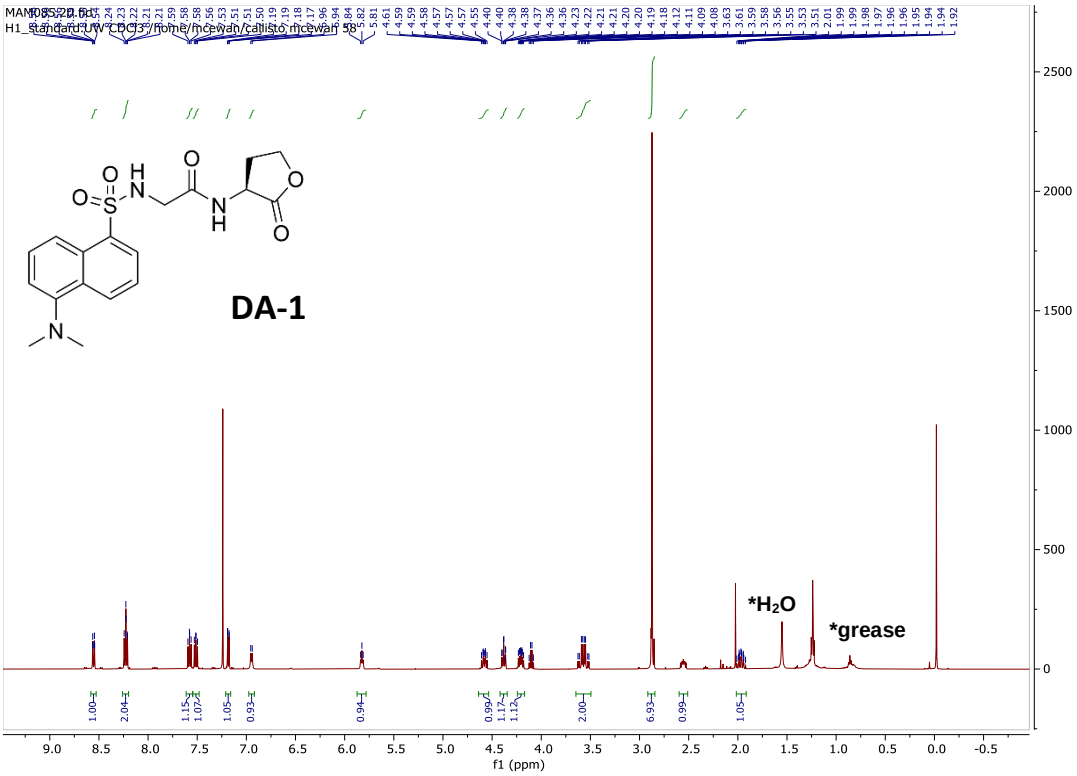
f1 (ppm)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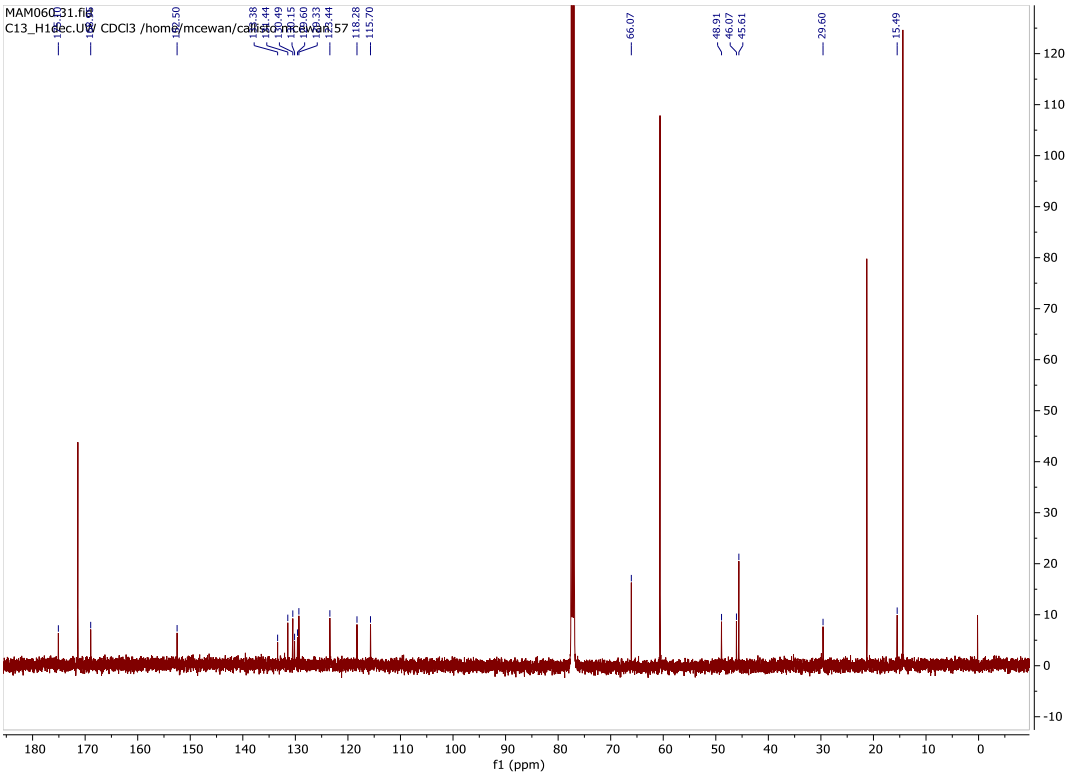
*grease
f1 (ppm)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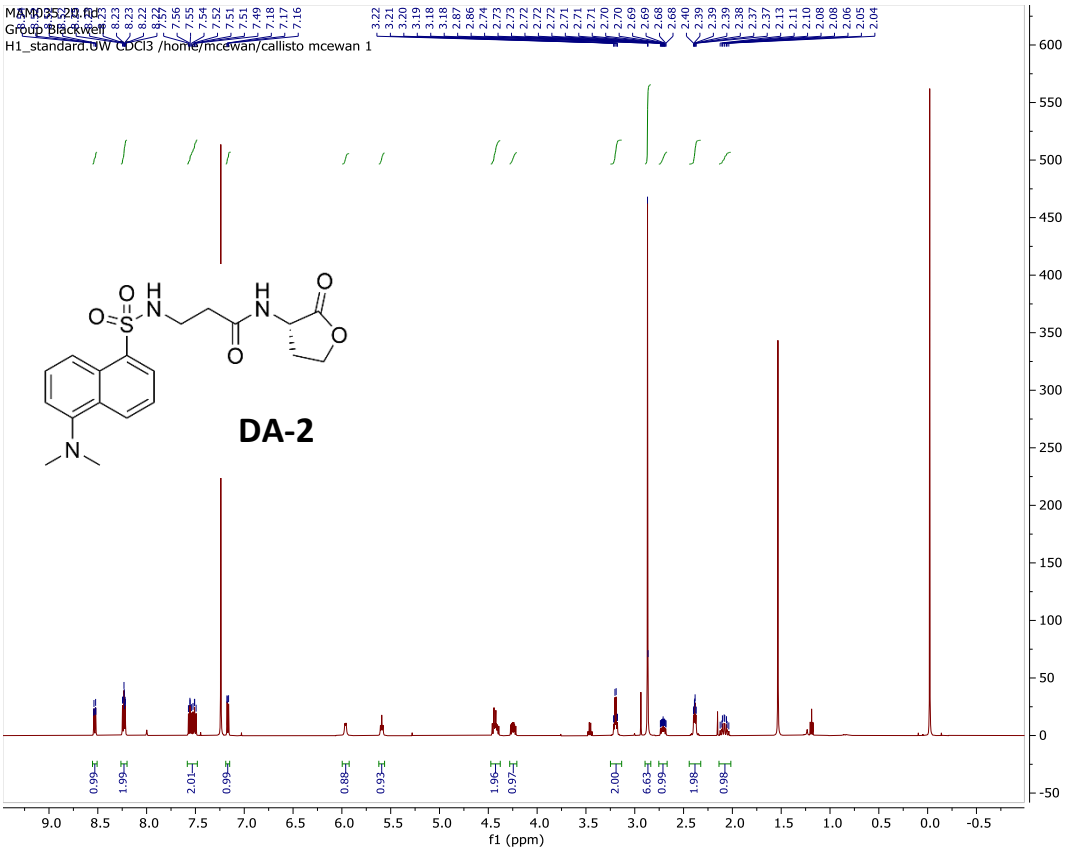
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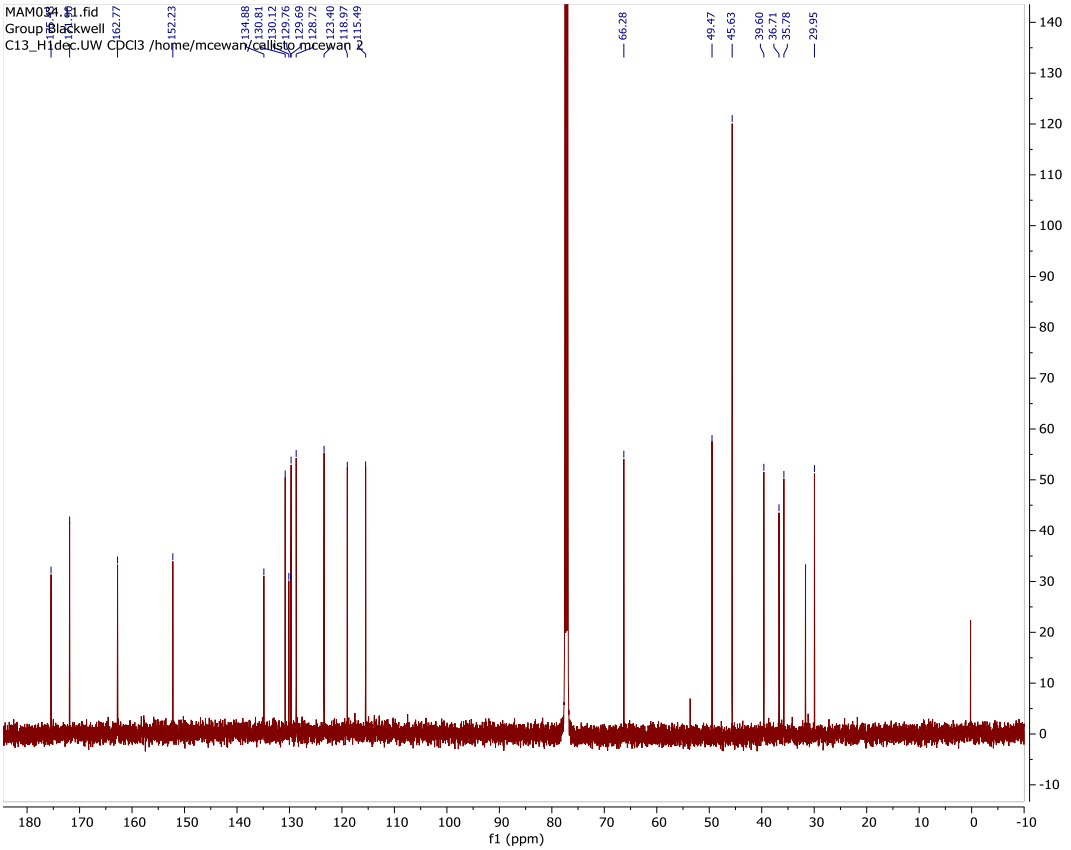
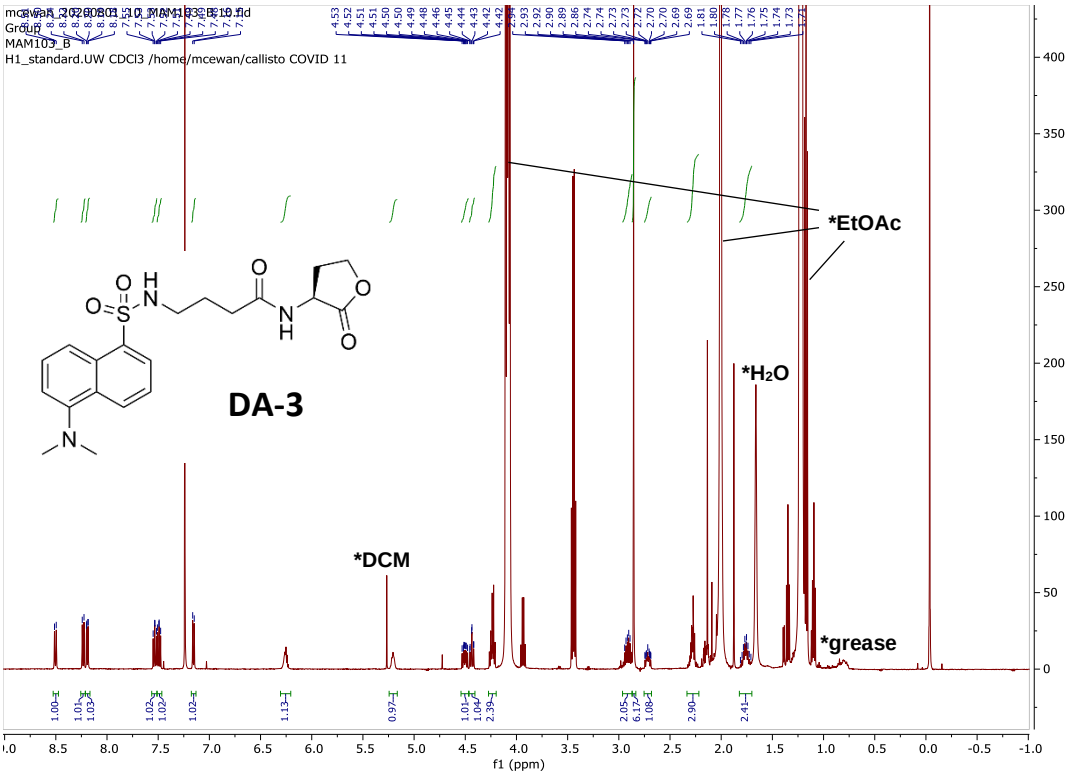
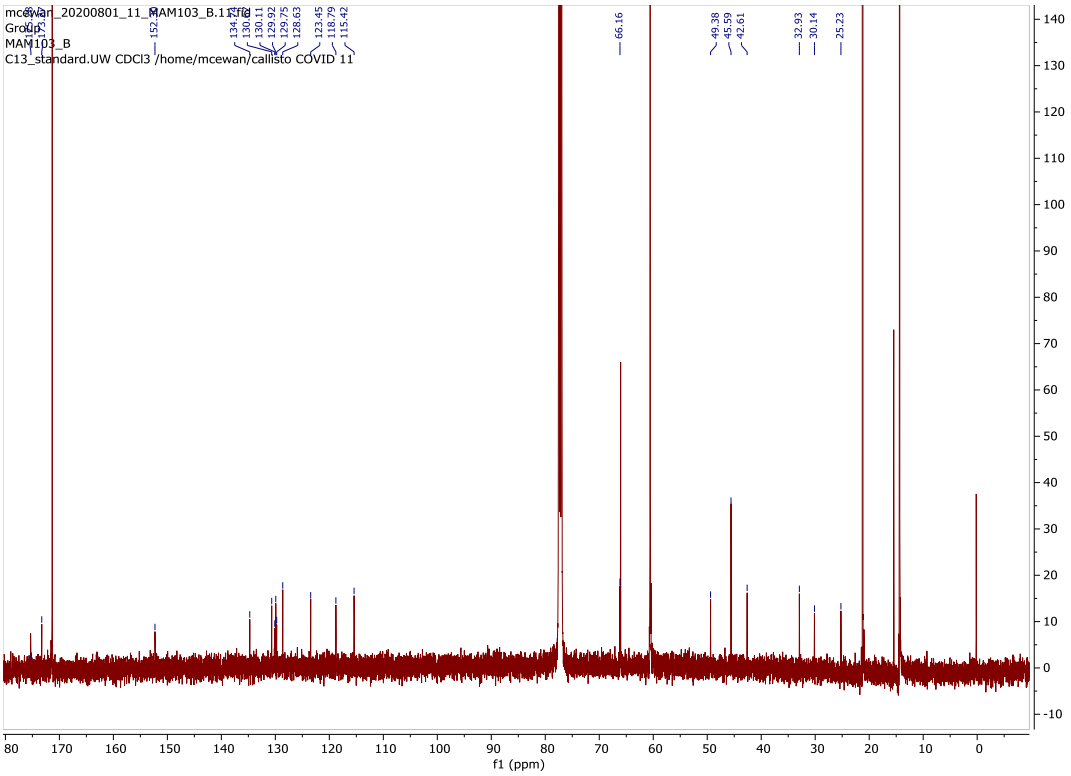
DA-2
f1 (ppm)


MAM034-C1.fid
Group Blackwell
C13_H1dec.UW CDCl3 /home/mcewan/callisto mcewan
f1 (ppm)


mcewan_20200801_10_MAM103_B.10.fid
Group1
MAM103_B
H1_standard.UW CDCl3 /home/mcewan/callisto COVID 11
*EtOAc
*H2O
DA-3
*DCM
*grease
f1 (ppm)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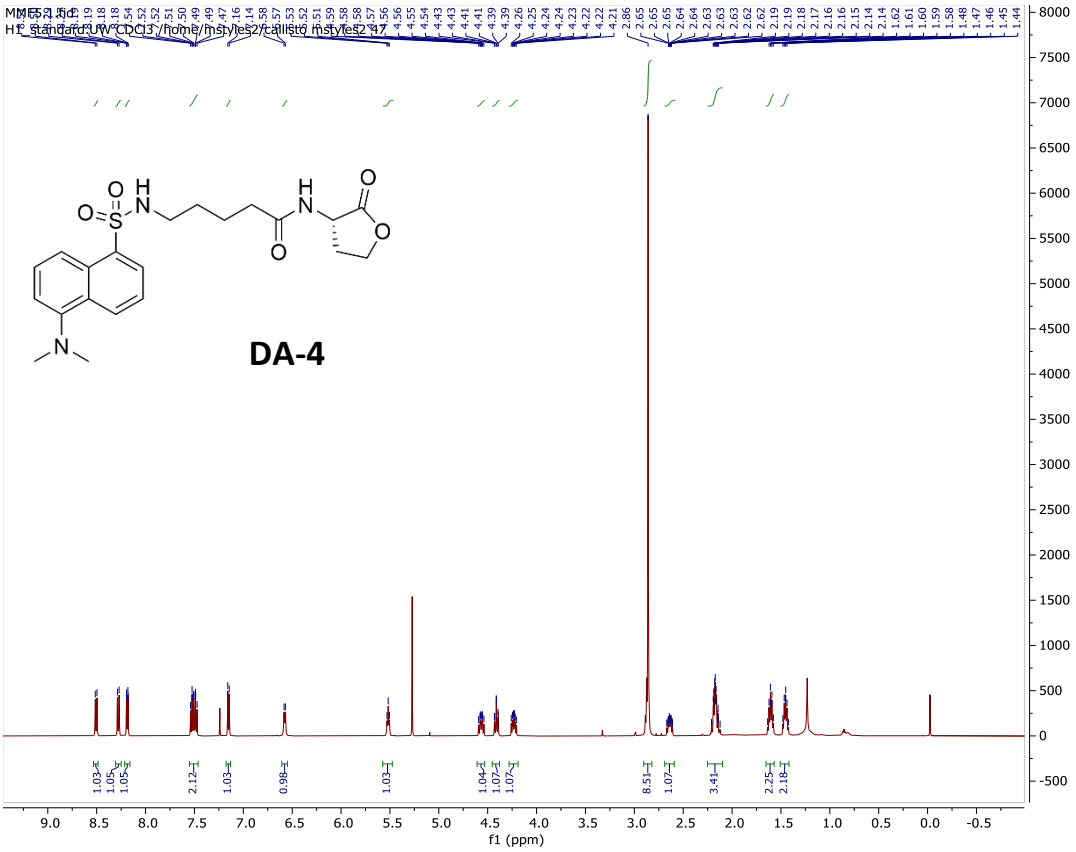
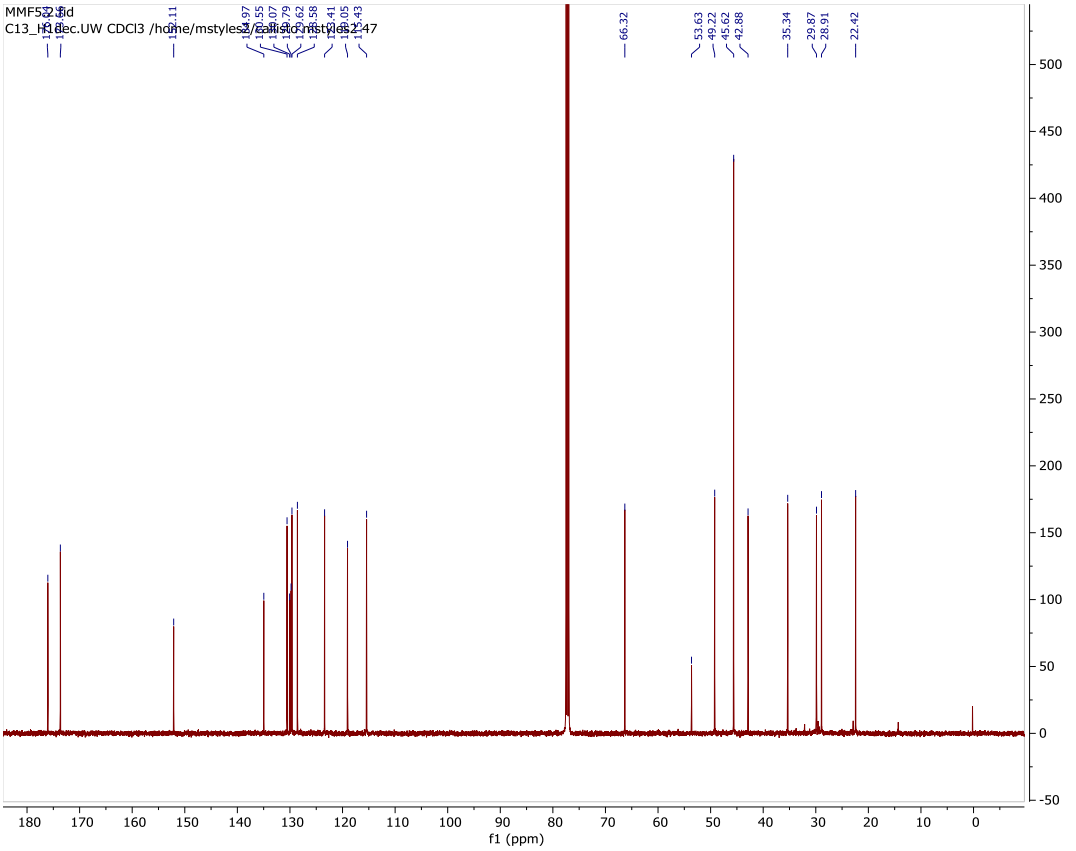
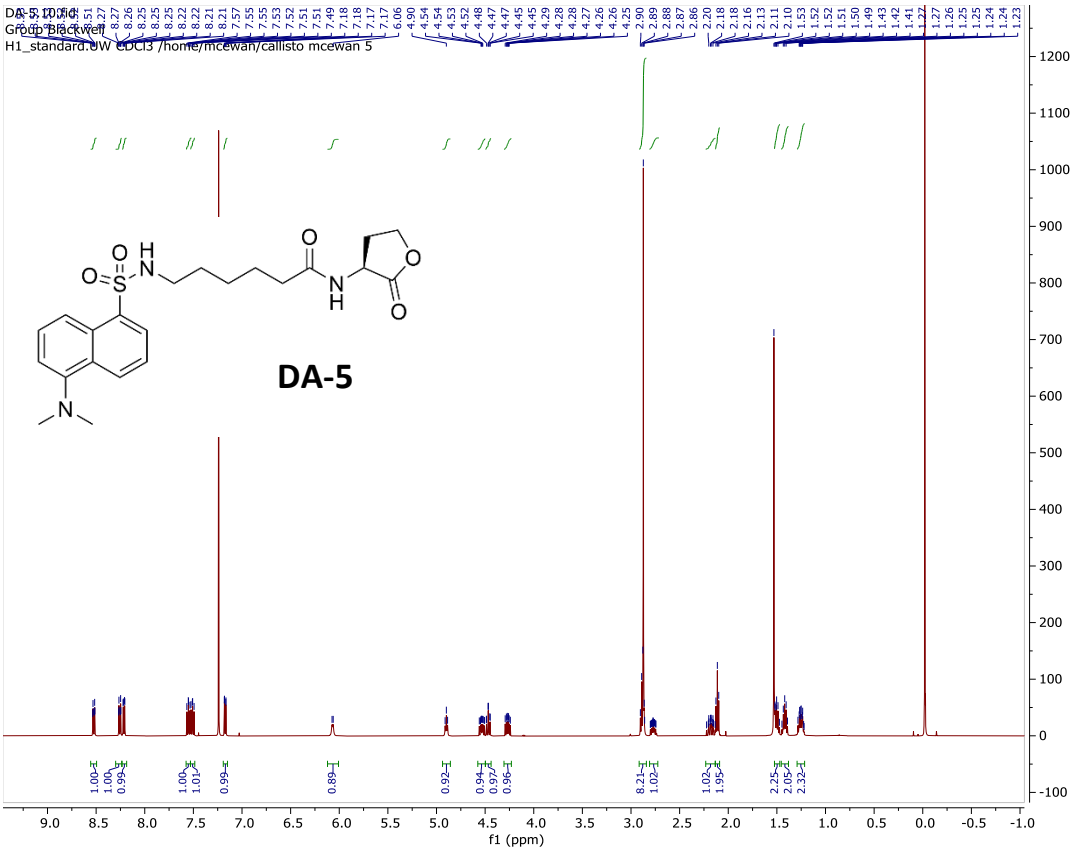
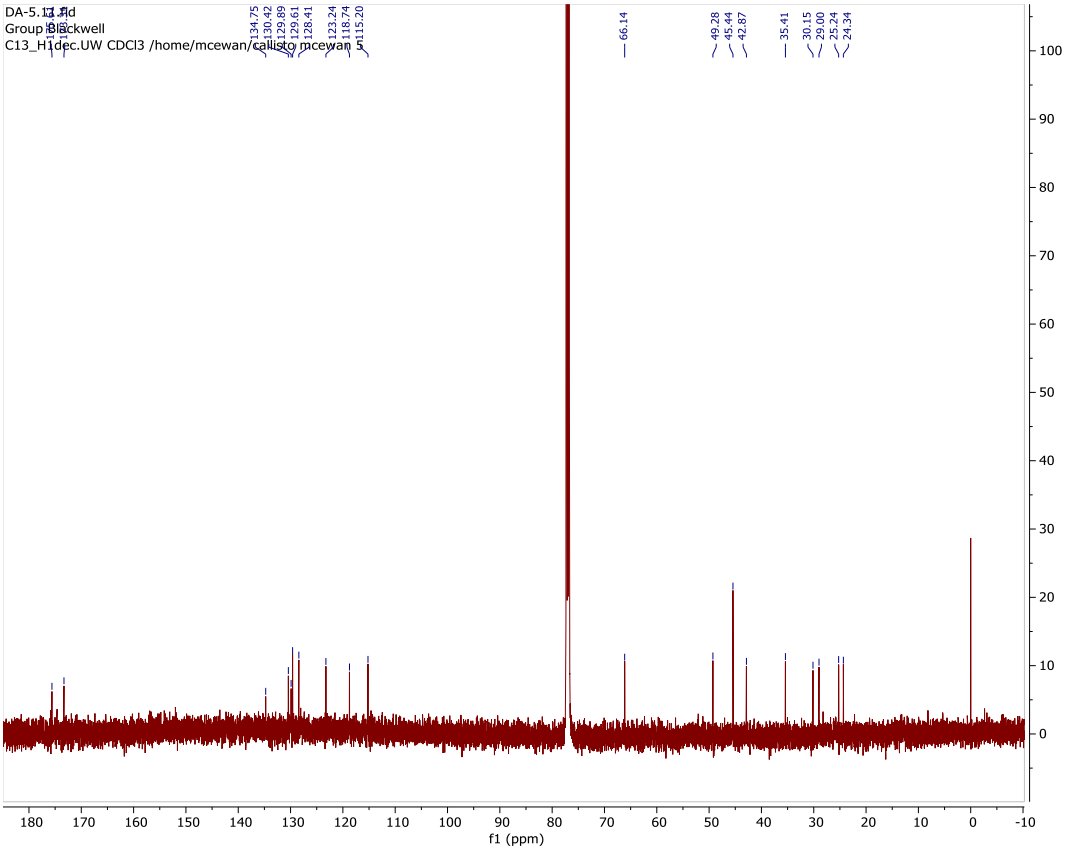
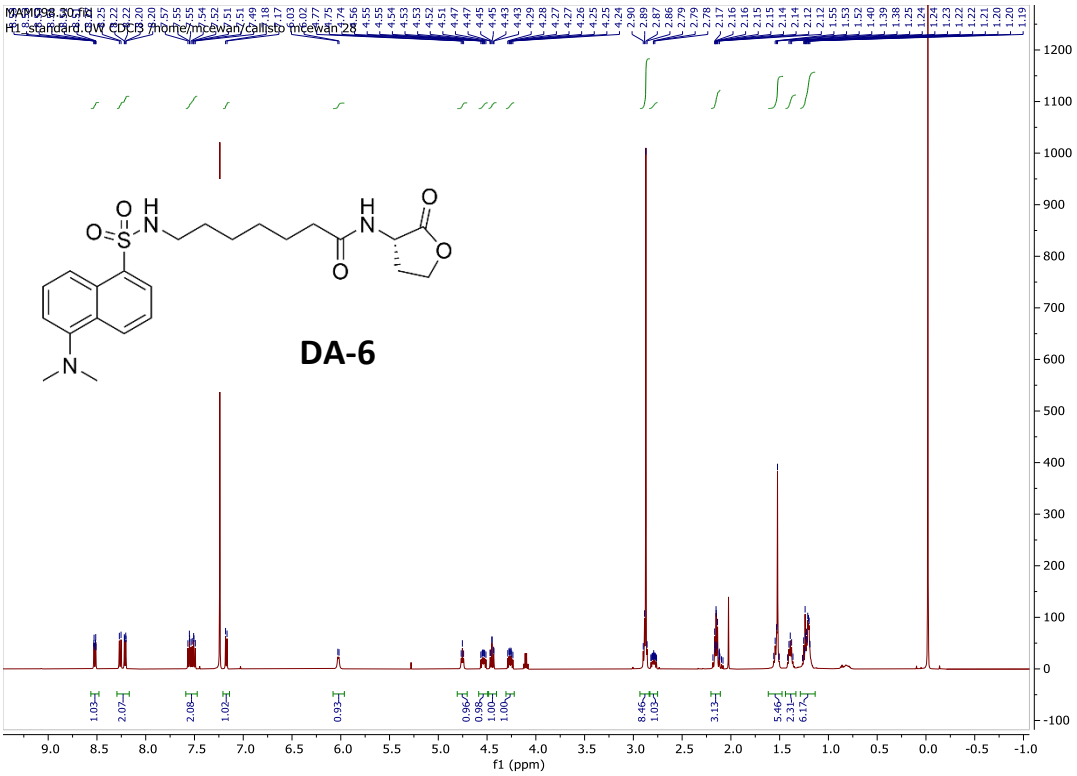
Group1
MAM103_B
C13_standard.UW CDCl3 /home/mcewan/callisto COVID 11
f1 (ppm)

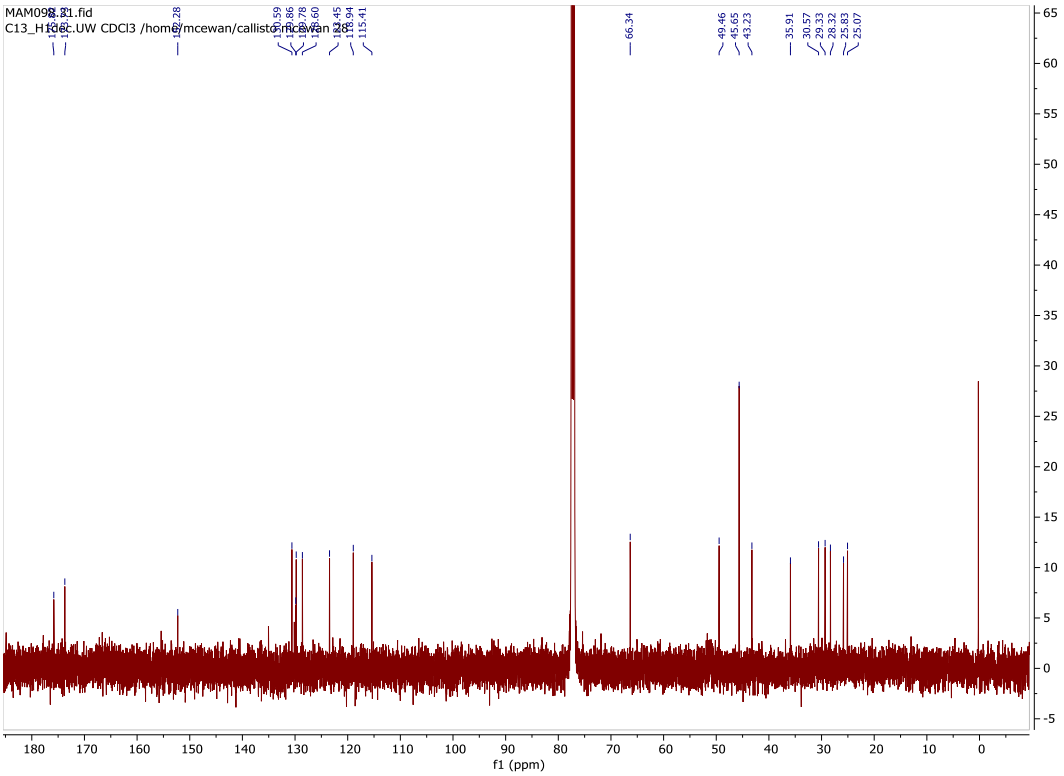

DA-4
f1 (ppm)

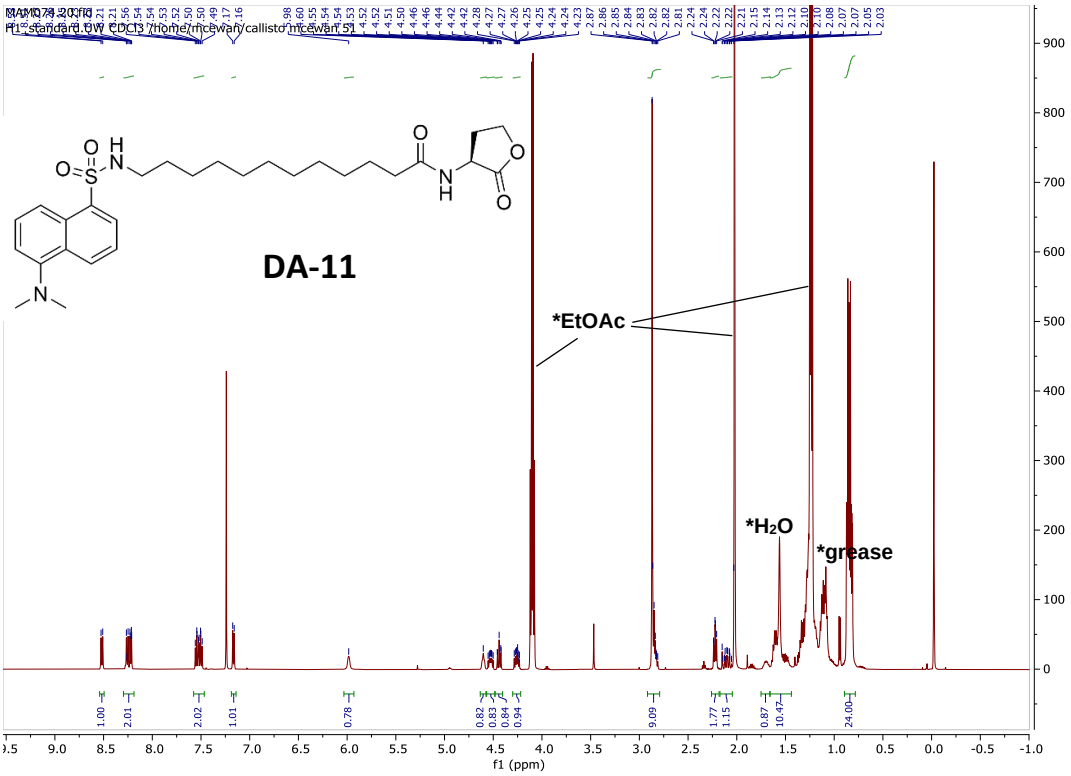

f1 (ppm)

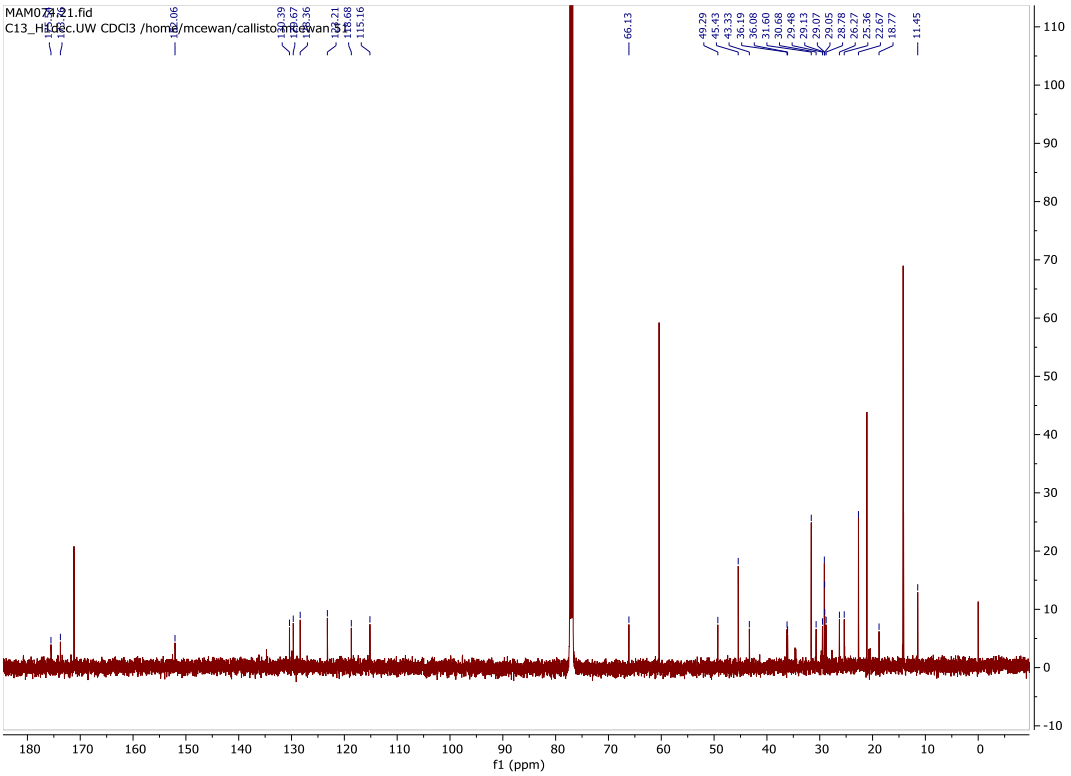

DA-5.10.fid
Group Blackwell
H1_standard.UW CDCl3 /home/mcewan/callisto mcewan 5
DA-5


Group Blackwell
C13_H1dec.UW CDCl3 /home/mcewan/callisto mcewan
f1 (ppm)


DA-6
f1 (ppm)


f1 (ppm)


DA-11
*EtOAc
*H₂O
*grease
f1 (ppm)


f1 (ppm)

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