Chemistry–A European Journal

Supporting Information

Diversely Functionalised Cytochalasins through Mutasynthesis and Semi-Synthesis

Chongqing Wang,[a] Christopher Lambert,[b, d] Maurice Hauser,[a] Adrian Deuschmann,[a] Carsten Zeilinger,[a] Klemens Rottner,[c, d] Theresia E. B. Stradal,[c] Marc Stadler,[b] Elizabeth J. Skellam,[a] and Russell J. Cox*[a]

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1.0 Fermentationand Metabolite Preparation and Characterisation

1.01 Strains and Culture Conditions

M. grisea NI980 is a *Digitaria smutsii* isolate obtained from our collaborators and was used as the wild-type strain in this study. Strains were cultivated on Oatmeal Agar (OMA) (4% *w/v* oat meal, 0.5% *w/v* sucrose, 2% agar) or Complete Medium (CM) agar at 25°C. For cytochalasan production, strains were cultivated in Soy Sauce Sucrose (SSS) medium (5% *v/v* soy sauce, 5% *w/v* sucrose, in autoclaved tap water) or DPY liquid medium (2% *w/v* dextrin from potato starch, 1% *w/v* polypeptone, 0.5% *w/v*yeast extract, 0.5% *w/v* monopotassium phosphate, 0.05% *w/v* magnesium sulfate, 2.5% *w/v* agar) for 7 days at 25 °C, 110 rpm.

1.02 Fermentation and Extraction Protocols

For extraction, *M. grisea* spores were collected from MG agar plates incubated for 7 days and inoculated into 500mL Erlenmeyer flasks containing 100 mL DPY. The spores were allowed to grow in the liquid culture for 7-8 days on shakers at 110 rpm at 25 °C. The cells were blended in the fermentation broth, filtered to remove the mycelium, and transferred into a separating funnel. An equal volume of EtOAc was added into the separating funnel and the aqueous layer was extracted twice. The organic solvent from the two extractions was combined and dried with $MgSO_4$, filtered and evaporated to dryness. The crude extract was dissolved into 1 mL HPLC grade MeOH.

1.03 Feeding Studies

200 mg of *O*-Methyl-L-tyrosine **3a** (purchased from Sigma-Aldrich) was dissolved into double distilled water (final concentration: 10 mg / mL) and filter sterilized. 200 µl of this solution was fed to the Δ*pyiA* knock-out strain inoculated into 500 mL Erlenmeyer flasks containing 100 mL DPY. The feeding was repeated at 24 hour intervals four times. On the 7th day the cultures were extracted using the method described above.

200 mg of *O*-Propylene-L-tyrosine **3f** was dissolved into double distilled water (final concentration: 10 mg / mL) and filter sterilized. 200 µl of this solution was fed to the Δ*pyiA* knock-out strain inoculated into 500 mL Erlenmeyer flasks containing 100 mL DPY. The feeding was repeated at 24 hour intervals four times. On the 7th day the cultures were extracted using the method described above.

200 mg of *O*-Propyne-L-tyrosine **3g** was dissolved into double distilled water (final concentration: 10 mg / mL) and filter sterilized. 200 µl of this solution was fed to the Δ*pyiA* knock-out strain inoculated into 500 mL Erlenmeyer flasks containing 100 mL DPY. The feeding was repeated at 24 hour intervals four times. On the 7th day the cultures were extracted using the method described above.

200 mg of *para*-fluoro- / chloro- /bromo- /iodo-phenylalanine (**3b-3e,** purchased from Sigma-Aldrich**)** was dissolved into double distilled water (final concentration: 10 mg / mL), stirred at 60 ˚C until dissolved completely and filer sterilized. 200 µl of this solution was fed to the Δ*pyiA* knock-out strain inoculated into 500 mL Erlenmeyer flasks containing 100 mL DPY. The feeding was repeated at 24 hour intervals four times. On the 7th day the cultures were extracted using the method described above.

200 mg of *para*-azido-phenylalanine **3h** (purchased from abcr) was dissolved into double distilled water (final concentration: 10 mg / mL) and filter sterilized. 200 µl of this solution was fed to the Δ*pyiA* knock-out strain inoculated into 500 mL Erlenmeyer flasks containing 100 mL DPY. The feeding was repeated at 24 hour intervals four times. On the 7th day the cultures were extracted using the method described above.

1.04 Analytical LCMS

LCMS data were obtained using a Waters LCMS system comprising of a Waters 2767 autosampler, Waters 2545 pump system and a Phenomenex Kinetex column (2.6 µ, C_{18}, 100 Å, 4.6 × 100 mm) equipped with a Phenomenex Security Guard precolumn (Luna C_5 300 Å) eluted at 1 mL/min. Detection was performed by Waters 2998 diode array detector between 200 and 600 nm; Waters 2424 ELSD and Waters SQD-2 mass detector operating simultaneously in ES^+ and ES^- modes between 100 m/z and 650 m/z. Solvents were A, HPLC-grade H_2O containg 0.05% formic acid; and B, HPLC-grade CH_3CN containing 0.045% formic acid. Gradients were as follows: Method 1 (optimised for non-polar compounds): 0 min, 10% B; 10 min 90% B; 12 min, 90% B; 13 min, 10% B and 15 min, 10% B. Method 2 (optimised for polar compounds): 0 min, 10% B; 10 min 40% B; 12 min, 90% B; 13 min, 10% B and 15 min, 10% B.

1.05 Preparative LCMS

Compounds were purified using a Waters massdirected autopurification system consisting of a Waters 2545 pump and Waters 2767 autosampler. The chromatography column was a Phenomenex Kinetex Axia column (5µ, C_{18}, 100 Å, 21.2 × 250 mm) fitted with a Luna C_5 300 Å Phenomenex Security Guard precolumn. The column was eluted at 20 mL/min at 22 °C. Solvents used were A, H_2O + 0.05% formic acid; and B, CH_3CN + 0.045% formic acid. All solvents were HPLC grade. The column outlet was split (100:1) and the minority flow was supplemented with HPLC-grade MeOH + 0.045% formic acid to 1000 µL/min and diverted for interrogation by diode array (Waters 2998) and evaporative light-scattering (Waters 2424) detectors. The flow was also analysed by mass spectrometry (Waters SQD-2 in ES^+ and ES^- modes). Desired compounds were collected into glass test tubes. Combined fractions were evaporated *in vacuo*, then dissolved directly in HPLC-grade MeOH to make the final concentration 10 mg / mL, 300 µl solution was loaded into the columnfor purification.

1.06 HRMS

HRMS was obtained using a UPLC system (Waters Acquity Ultraperformance, running the same method and column as above) connected to a Q-TOF Premier mass spectrometer.

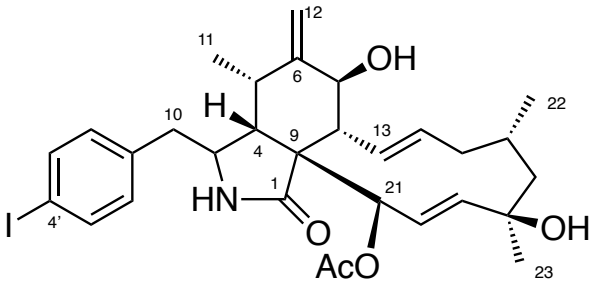
1.07 NMR

A Bruker Avance 500 instrument equipped with a cryo-cooled probe at 500 MHz (^1H)/125 MHz (^{13}C) and 600 MHz (^1H)/150 MHz (^{13}C) were used for all NMR analysis. Standard parameters were used for the collection of 2D spectra (^1H, ^1H-correlation spectroscopy [COSY], heteronuclear single-quantum coherence [HSQC] and HMBC) in the indicated solvents. 1H and 13C spectra are referenced relative to residual protonated solvents. All δ values are quoted in ppm and all *J* values in Hz.

1.08 IR

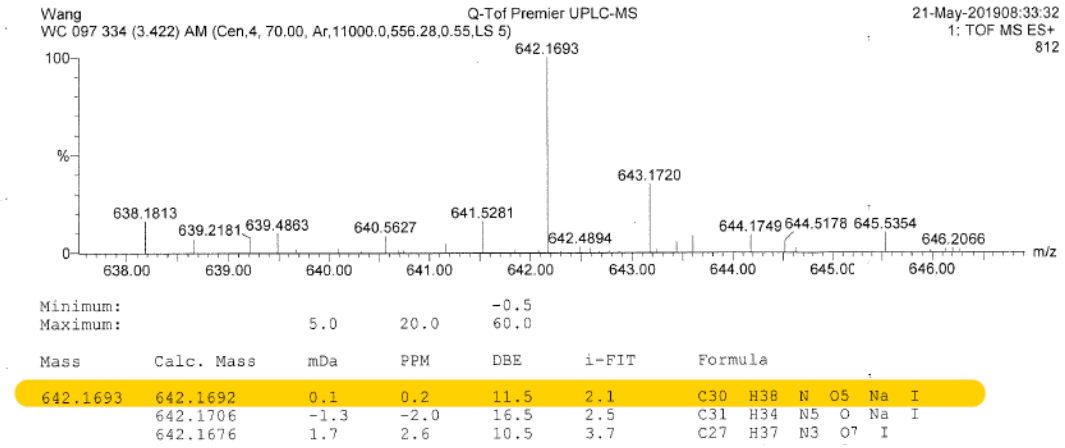
Infrared Spectra were recorded using a Shimadzu IRAffinity-1S fourier transform infrared spectrophotometer and quest atr diamond extended range accessory (black).

1.09 4'-Iodo Cytochalasin H 1e

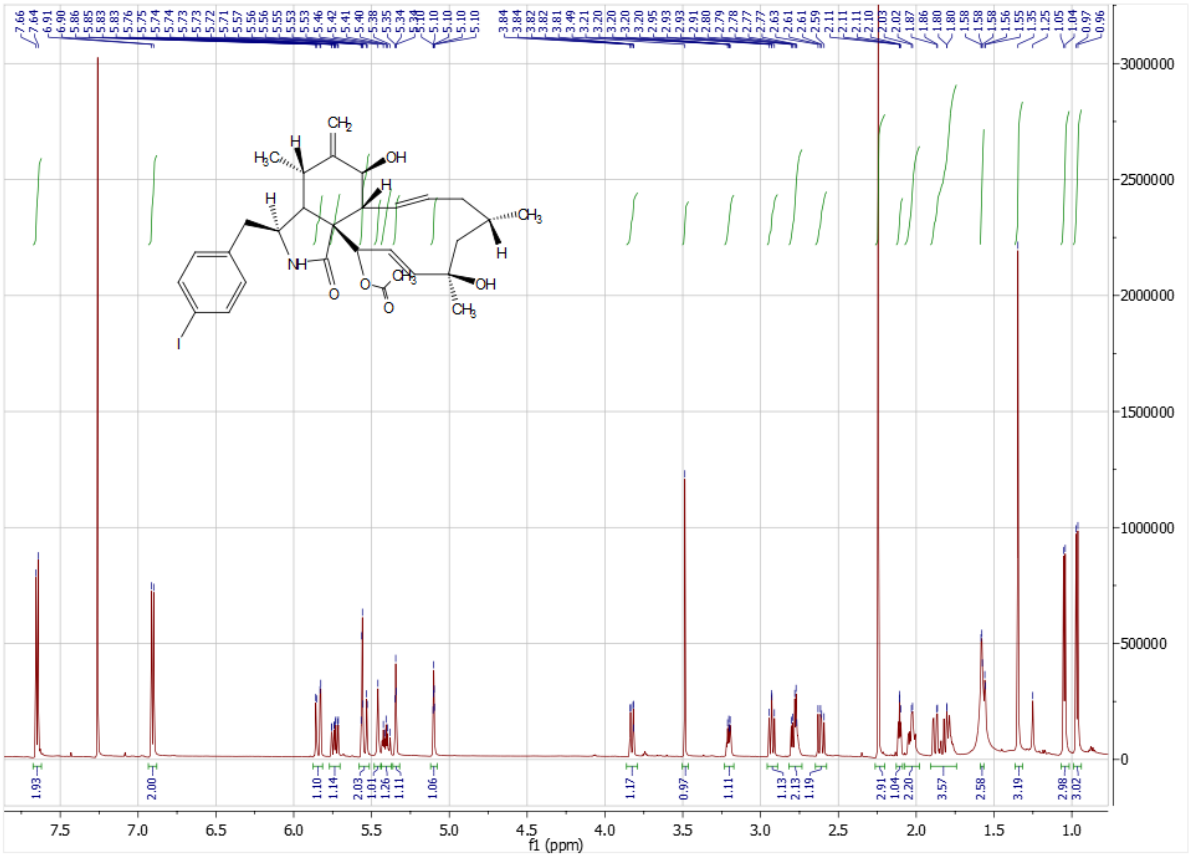


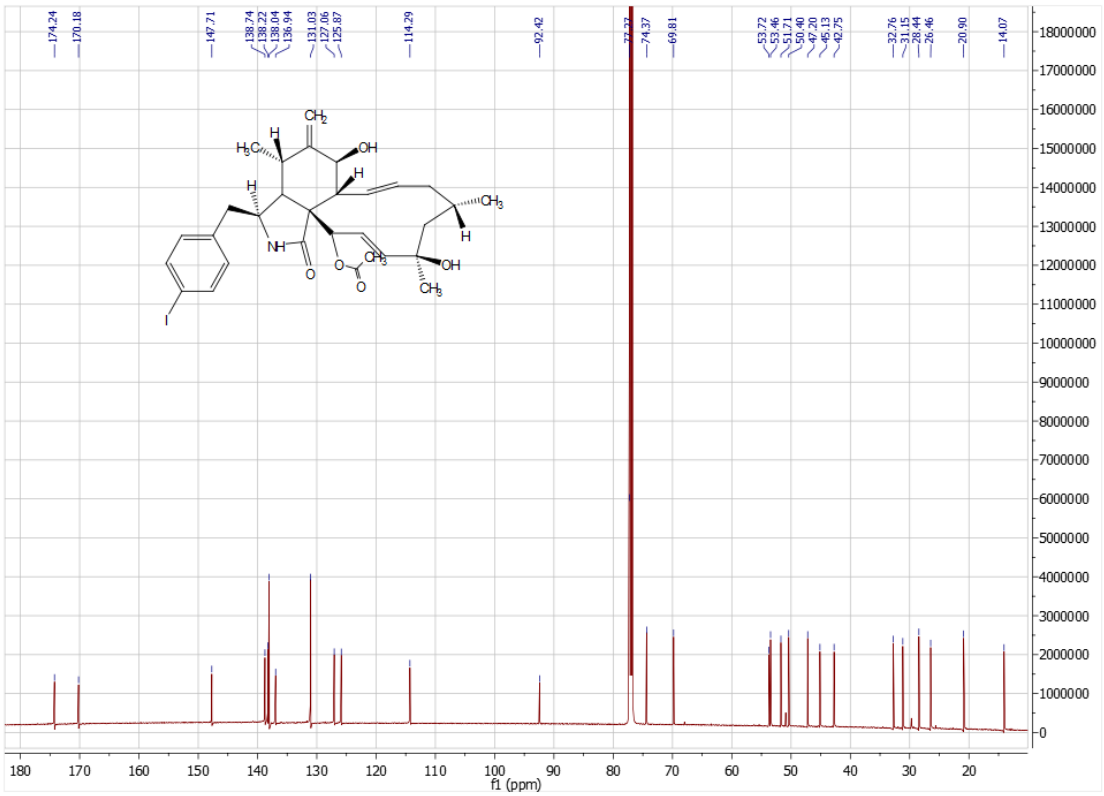
Chemical Formula: $C_{30}H_{38}INO_5$
Exact Mass: 619.1795

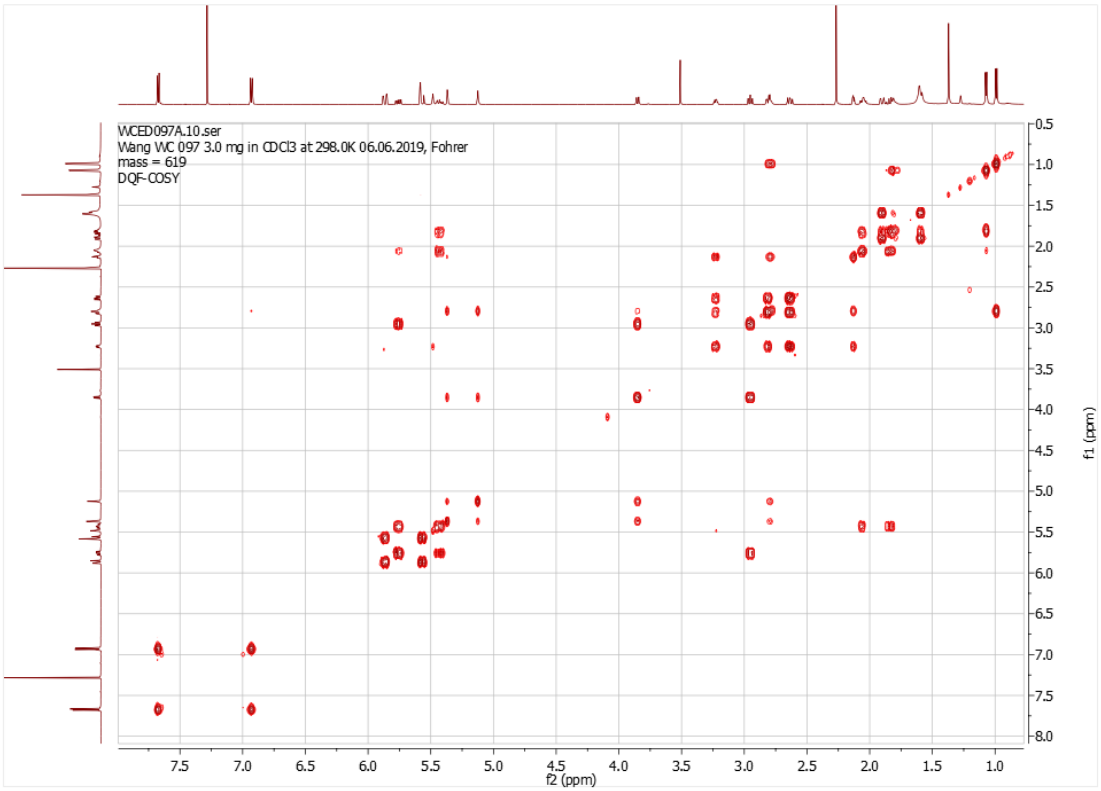
Position	δ_H	M	$J_{H\text{-}H}$/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
2	5.46	brs	-	-	-	-	-
3	3.23	ddd	1.1, 4.8, 9.6	53.5	CH	1', 1, 4, 5	4, 5, 10
4	2.13	dd	3.6, 5.1	50.5	CH	1, 3, 5	3, 5, 10
5	2.80	m	-	32.7	CH	1', 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.84	dd	1.3, 10.8	69.7	CH	5, 6, 8, 12, 13	8, 12
8	2.95	dd	9.8, 10.8	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.7	-	-	-
10a	2.63	dd	9.5, 13.5	45.2	CH_2	1', 3	3, 4, 12
10b	2.82	dd	4.8, 13.5	-	-	-	-
11	0.99	d	6.7	14.0	CH_3	4, 5, 6	5
12a	5.12	brs	-	114.3	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.78	ddd	1.4, 9.6, 15.5	127.1	CH	7, 8, 15	8, 14, 15
14	5.43	ddd	4.8, 10.3, 15.5	138.8	CH	9, 15	13, 15
15a	1.82	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.81	m	-	28.5	CH	13, 14, 17	22
17a	1.60	m	-	53.9	CH_2	18, 19	-
17b	1.89	m	-	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.55	brs	-	138.3	CH	-	20
20	5.88	dd	2.9, 16.4	126.0	CH	18, 19	19
21	5.58	brs	-	77.2	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.5	26.4	CH_3	15, 16, 17	16
23	1.37	s	-	31.1	CH_3	17, 18, 19	-
1'	-	-	-	131.0	-	-	-
2' 6'	6.93	d	8.2	131.1	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.67	d	8.2	138.0	2 x CH	1', 4'	2', 6'
4'	-	-	-	137.3	-	-	-
24	-	-	-	170.2	-	-	-
25	2.27	s	-	20.9	CH_3	21, 24	-

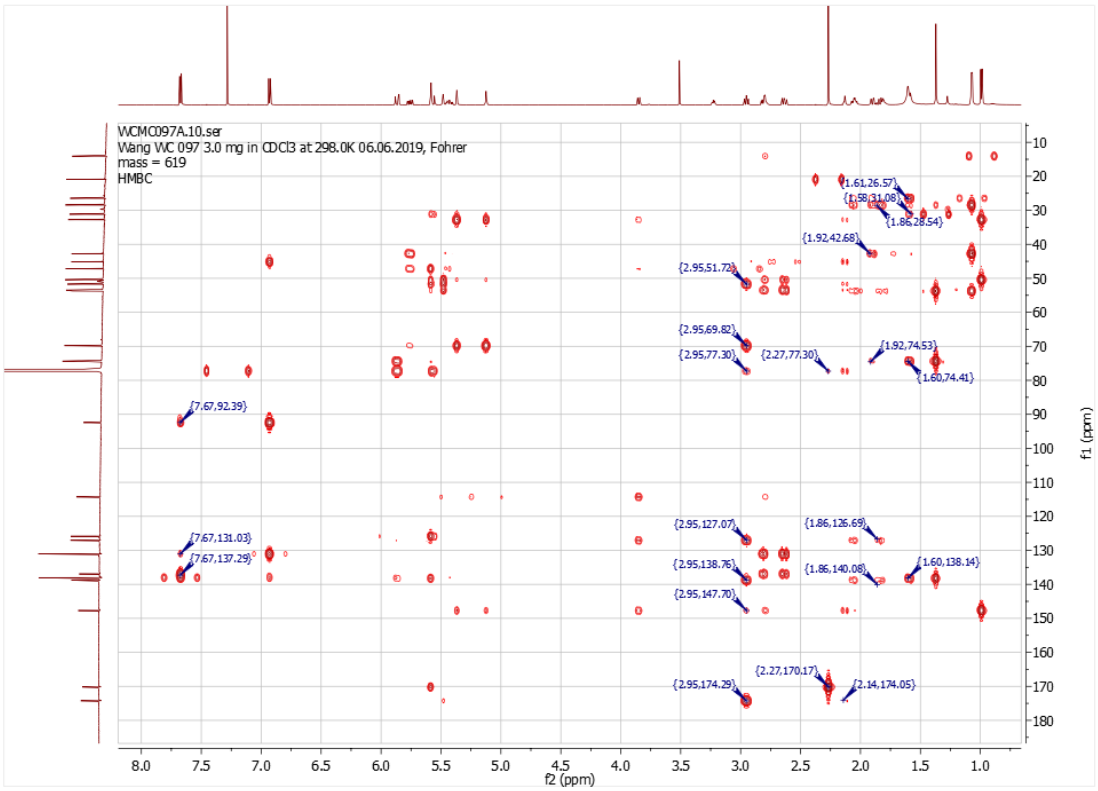


HRMS (*m/z*): calculated for [$C_{30}H_{38}NO_5I$ + Na]: 642.1692, found: 642.1693.




174.24
170.18
147.71
138.74
138.22
138.04
136.94
131.03
127.06
125.87
114.29
92.42
77.37
74.37
69.81
53.72
53.46
51.71
50.40
47.20
46.13
42.75
32.76
31.15
28.44
26.46
20.90
14.07
f1 (ppm)


WCED097A.10.ser
Wang WC 097 3.0 mg in CDCl3 at 298.0K 06.06.2019, Fohrer
mass = 619
DQF-COSY
f2 (ppm)
f1 (ppm)


WCMC097A.10.ser
Wang WC 097 3.0 mg in CDCl3 at 298.0K 06.06.2019, Fohrer
mass = 619
HMBC
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{1.58,31.08}
{1.86,28.54}
{1.92,42.68}
{2.95,51.72}
{2.95,69.82}
{2.95,77.30}
{2.27,77.30}
{1.92,74.53}
{1.60,74.41}
{7.67,92.39}
{7.67,131.03}
{7.67,137.29}
{2.95,127.07}
{1.86,126.69}
{2.95,138.76}
{1.86,140.08}
{1.60,138.14}
{2.95,147.70}
{2.27,170.17}
{2.95,174.29}
{2.14,174.05}
f2 (ppm)
f1 (ppm)


WCMH097A.10.ser
Wang WC 097 3.0 mg in CDCl3 at 298.0K 06.06.2019, Fohrer
mass = 619
13C-HSQC
{0.99,14.00}
{2.27,20.90}
{1.81,28.54}
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{1.37,31.14}
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{1.60,53.92}
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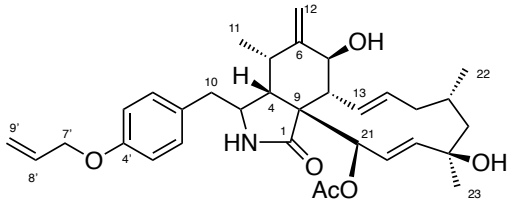
{5.58,77.24}
{5.12,114.35}
{5.37,114.31}
{5.78,127.06}
{6.93,131.06}
{5.88,125.96}
{7.67,137.98}
{5.43,138.79}
{5.58,138.01}
{5.55,138.26}
f2 (ppm)
f1 (ppm)

1.10 4'-(*O*-prop-2-enyl) Cytochalasin H 1f



Chemical Formula: $C_{33}H_{43}NO_6$
Exact Mass: 549.3090

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
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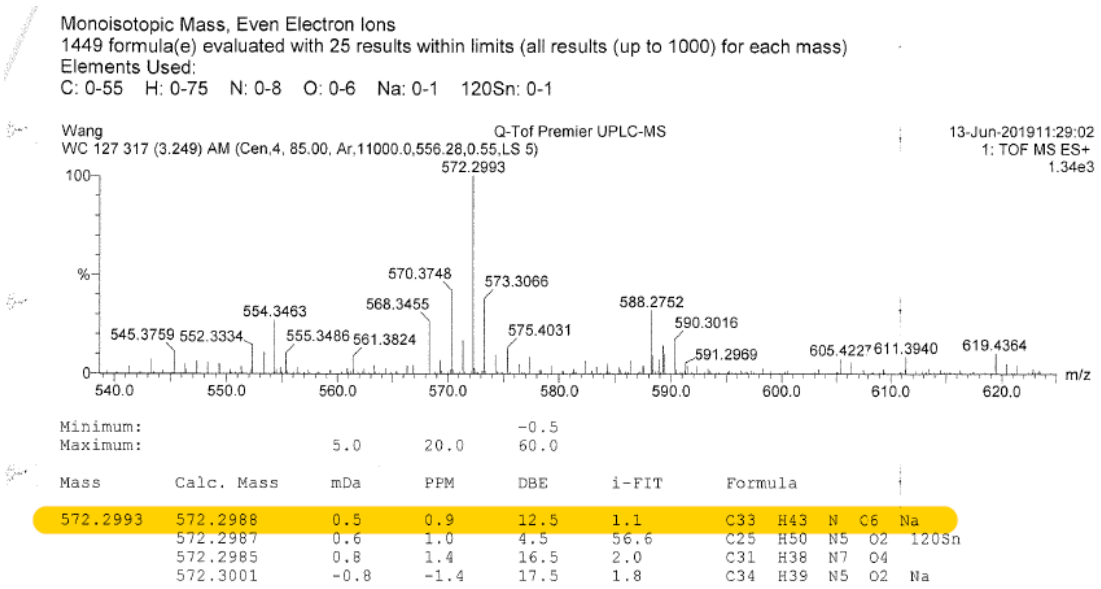
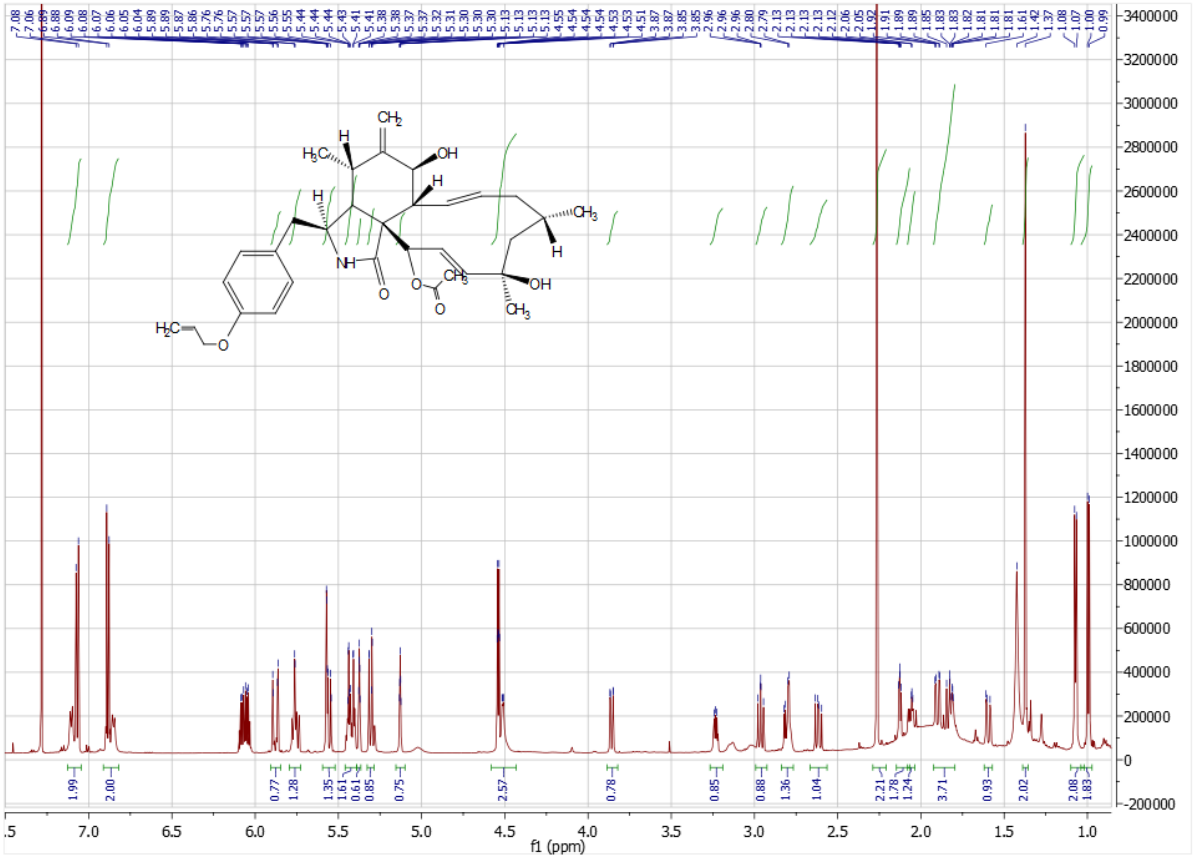
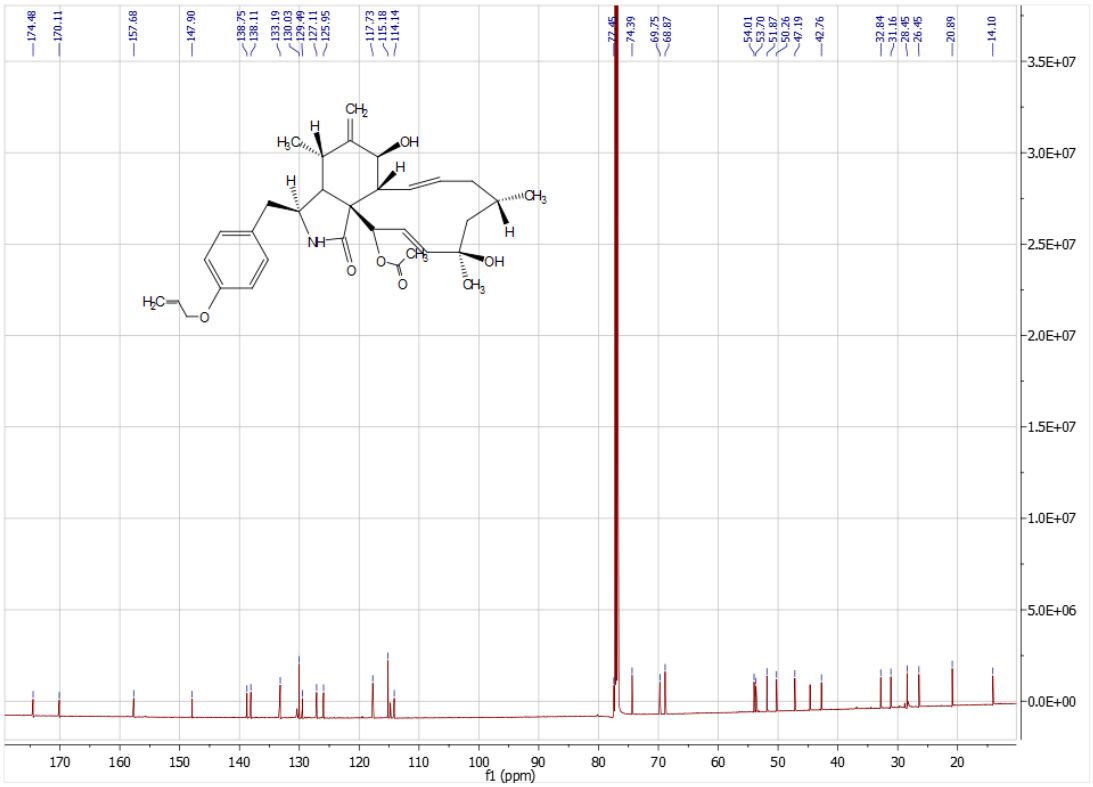
2	5.50	brs	-	-	-	-	-
3	3.23	ddd	1.1, 5.0, 9.6	54.0	CH	1‘, 1, 4, 5	4, 5, 10
4	2.12	dd	3.8, 5.0	50.2	CH	1, 3, 5, 6, 10, 21	3, 5, 10
5	2.79	m	-	33.0	CH	1’, 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.87	dd	1.6, 10.7	69.8	CH	6, 12	8, 12
8	2.96	t	10.7	47.3	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.9	-	-	-
10a	2.62	dd	9.4, 13.6	44.8	CH_2	1’, 3	3, 4, 12
10b	2.81	dd	4.9, 13.6	-	-	-	-
11	0.99	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.13	brs	-	114.0	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.75	ddd	1.4, 9.7, 15.4	127.1	CH	7, 8, 15	8, 14, 15
14	5.42	ddd	4.8, 10.1, 15.4	138.7	CH	9, 15	13, 15
15a	1.84	m	-	42.8	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.81	m	-	28.6	CH	13, 14, 17	22
17a	1.60	dd	3.0, 14.4	53.7	CH_2	18, 19	-
17b	1.89	m	3.0, 14.4	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.57	dd	2.3, 16.2	138.1	CH	-	20
20	5.89	dd	3.0, 16.2	126.0	CH	18, 19	19
21	5.57	dd	2.6, 3.0	77.4	CH	8, 9, 19, 20, 21 23	-
22	1.07	d	6.3	26.6	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	129.5	-	-	-
2’ 6’	7.07	d	8.3	130.0	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	6.88	d	8.3	115.1	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	157.7	-	-	-
24	-	-	-	170.1	-	-	-
25	2.27	s	-	20.9	CH_3	21, 24	-
7’	4.54	t	1.6	68.8	CH_2	4‘, 8‘, 9‘	8‘, 9‘
8’	6.06	dddd	5.3, 10.5, 15.8, 22.6	133.2	CH	7‘	7‘, 9‘
9a’	5.30	dd	1.6, 3.2	117.6	CH_2	7‘, 8‘	7‘, 8‘
9b‘	5.41	m	-	-	-	-	-



HRMS (*m/z*): calculated for [$C_{60}H_{76}N_2O_{10}$ + Na]: 572.2988, found: 572.2993.




174.48
170.11
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138.75
138.11
133.19
130.03
129.49
127.11
125.95
117.73
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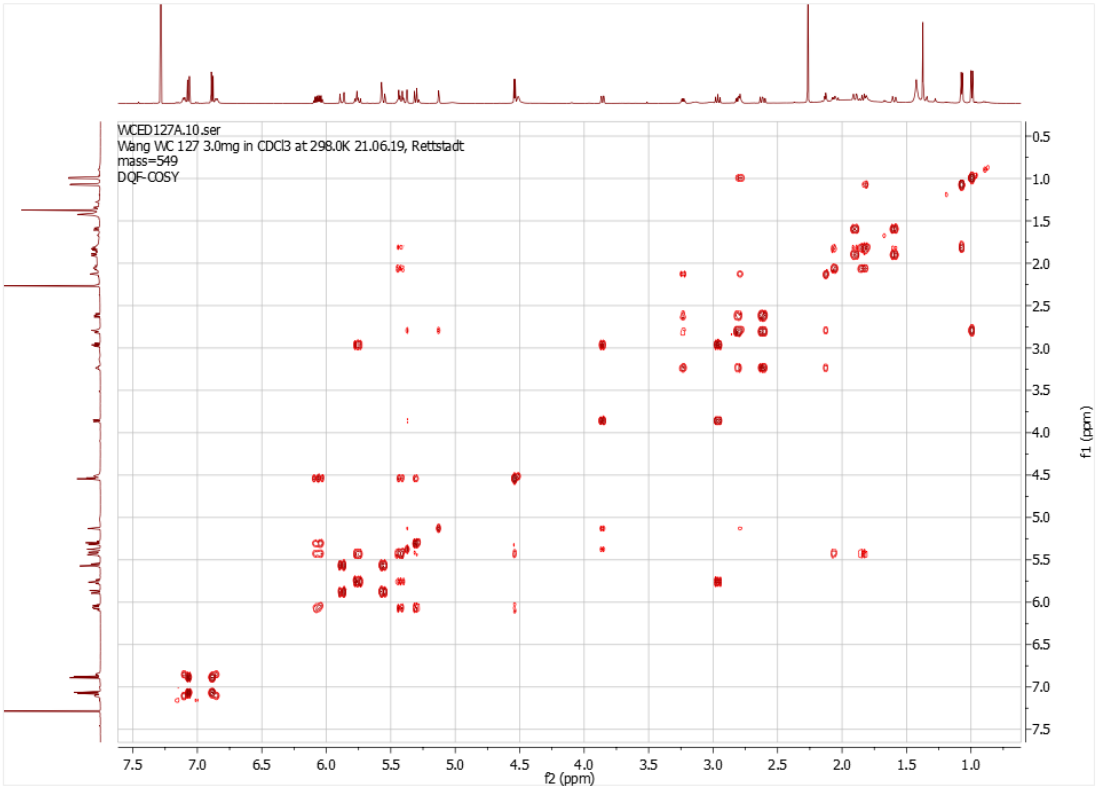
114.14
77.45
74.39
69.75
68.87
54.01
53.70
51.87
50.26
47.19
42.76
32.84
31.16
28.45
26.45
20.89
14.10
3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


WCED127A.10.ser
Wang WC 127 3.0mg in CDCl3 at 298.0K 21.06.19, Rettstadt
mass=549
DQF-COSY
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
f1 (ppm)
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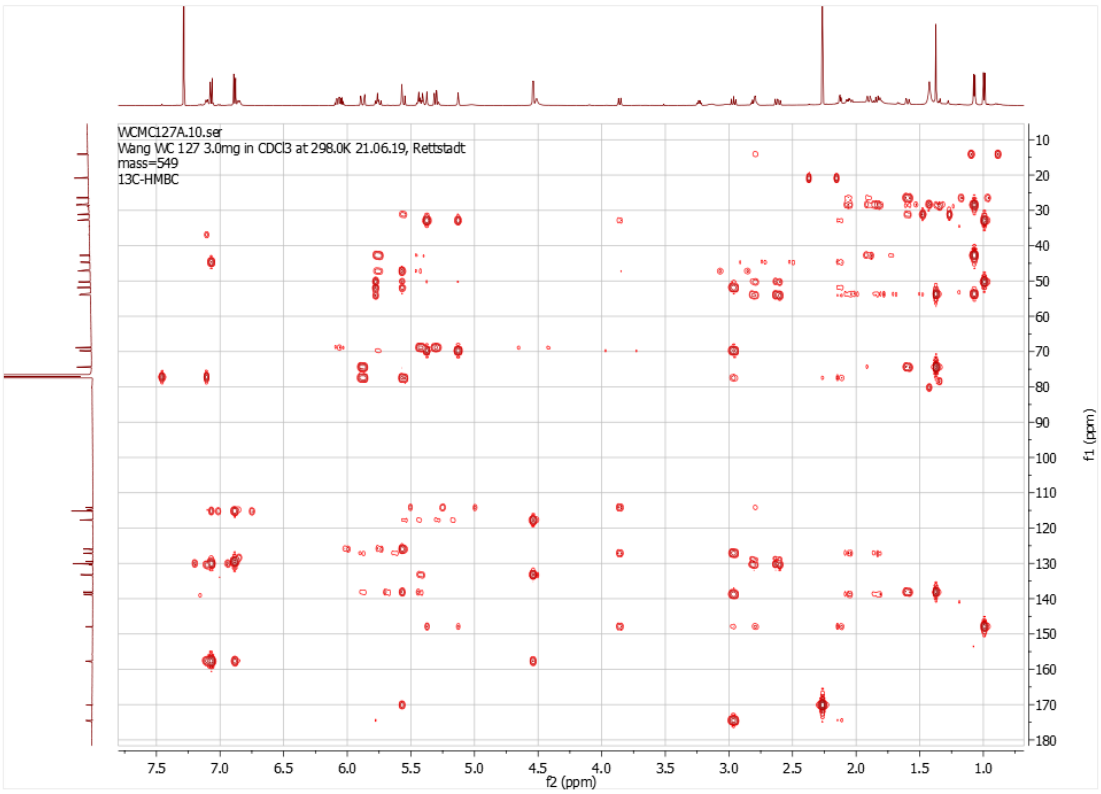
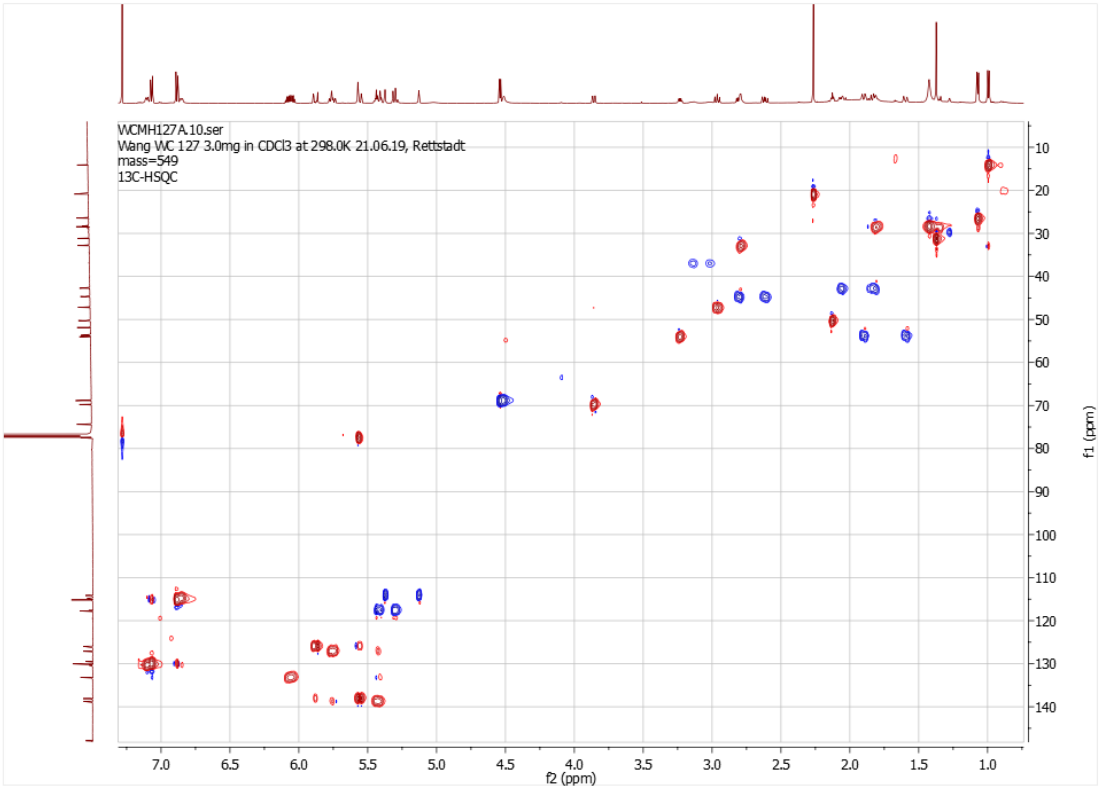
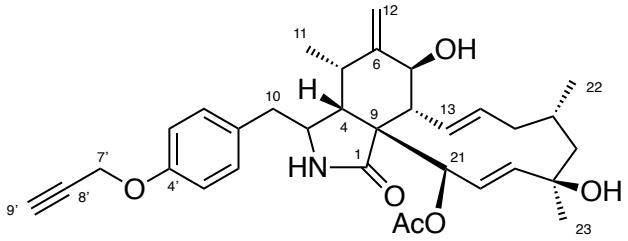
f2 (ppm)


WCMC127A.10.ser
Wang WC 127 3.0mg in CDCl3 at 298.0K 21.06.19, Rettstadt
mass=549
13C-HMBC
f2 (ppm)
f1 (ppm)

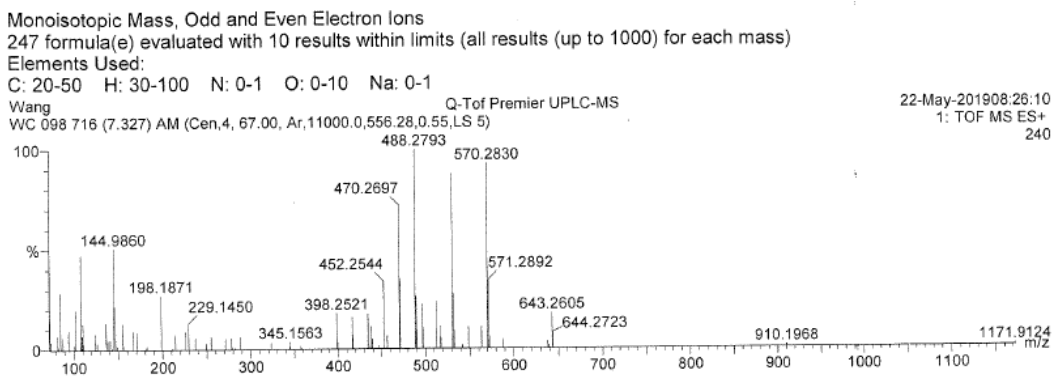

WCMH127A.10.ser
Wang WC 127 3.0mg in CDCl3 at 298.0K 21.06.19, Rettstadt
mass=549
13C-HSQC
f2 (ppm)
f1 (ppm)

1.11 4'-(*O*-propargyl) Cytochalasin H 1g



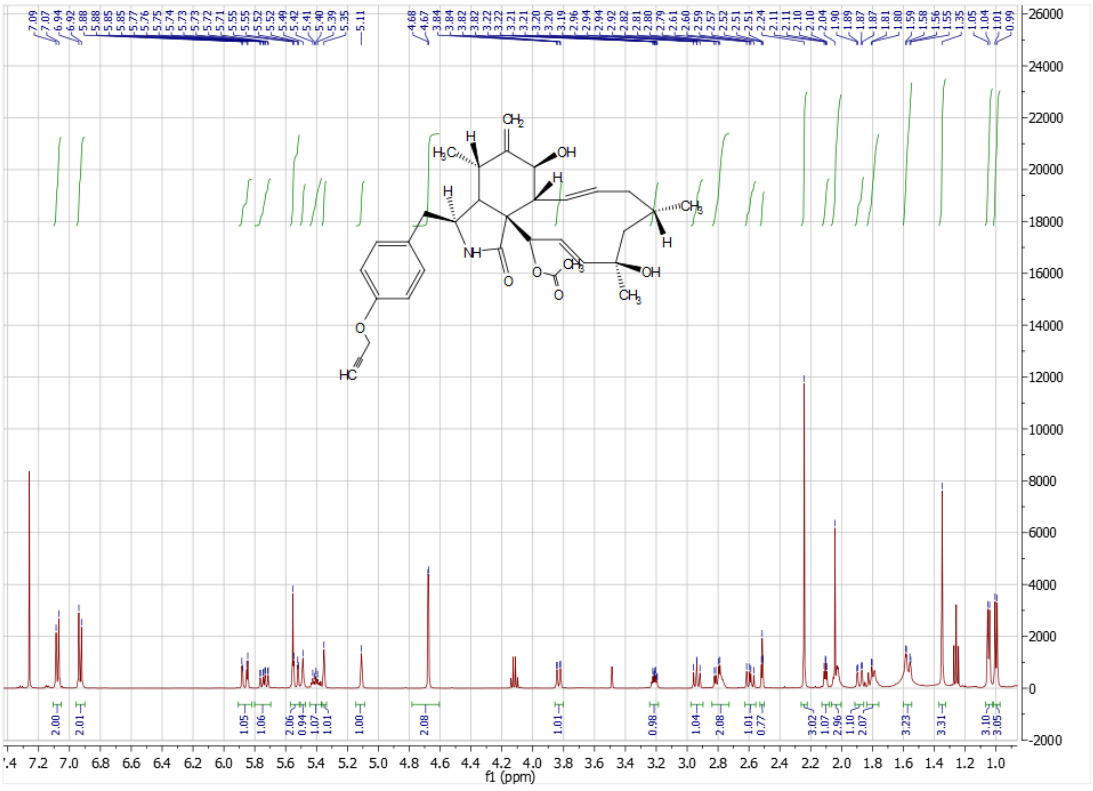
Chemical Formula: $C_{33}H_{41}NO_6$
Exact Mass: 547.2934

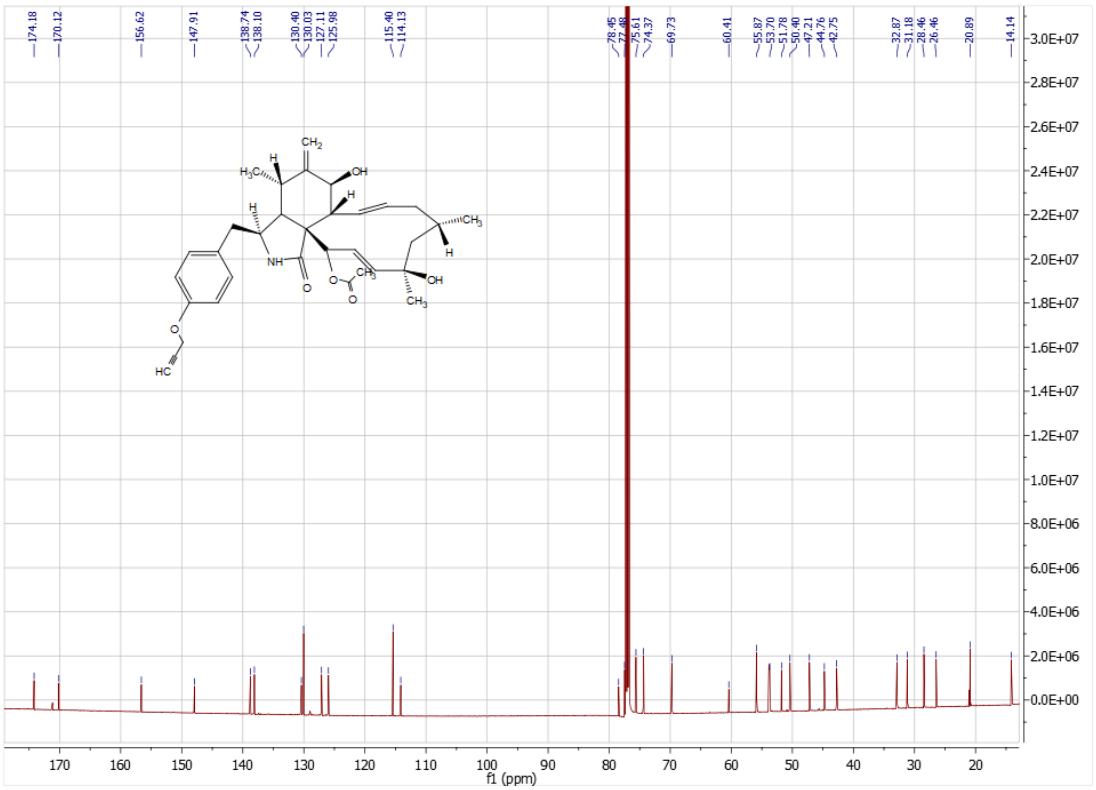
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.2	-	-	-
2	5.50	brs	-	-	-	-	-
3	3.24	ddd	1.1, 5.0, 9.6	53.8	CH	1', 1, 4, 5	4, 5, 10
4	2.13	dd	3.8, 5.0	50.5	CH	1, 3, 5, 6, 10, 21	3, 5, 10
5	2.80	m	-	32.9	CH	1', 3	4, 11, 12
6	-	-	-	147.8	-	-	-
7	3.85	dd	1.6, 10.7	69.6	CH	6, 12	8, 12
8	2.96	t	10.7	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.8	-	-	-
10a	2.61	dd	9.4, 13.6	44.8	CH_2	1', 3	3, 4, 12
10b	2.82	dd	4.9, 13.6	-	-	-	-
11	1.03	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.13	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.76	ddd	1.4, 9.7, 15.4	127.1	CH	7, 8, 15	8, 14, 15
14	5.41	ddd	4.8, 10.1, 15.4	138.8	CH	9, 15	13, 15
15a	1.83	m	-	42.8	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.81	m	-	28.5	CH	13, 14, 17	22
17a	1.60	dd	3.0, 14.4	53.6	CH_2	18, 19	-
17b	1.82	dd	3.0, 14.4	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.54	dd	2.3, 16.2	138.2	CH	-	20
20	5.89	dd	3.0, 16.2	126.0	CH	18, 19	19
21	5.58	dd	2.6, 3.0	77.5	CH	8, 9, 19, 20, 21 23	-
22	1.07	d	6.3	26.4	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1'	-	-	-	130.4	-	-	-
2' 6'	7.09	d	8.3	130.2	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	6.96	d	8.3	115.4	2 x CH	1', 4'	2', 6'
4'	-	-	-	156.7	-	-	-
24	-	-	-	170.2	-	-	-
25	2.26	s	-	20.9	CH_3	21, 24	-
7'	4.70	d	2.4	55.9	CH_2	4', 8', 9'	9'
8'	-	-	-	78.5	-	-	-
9'	2.54	m	-	75.9	CH	7', 8'	7'

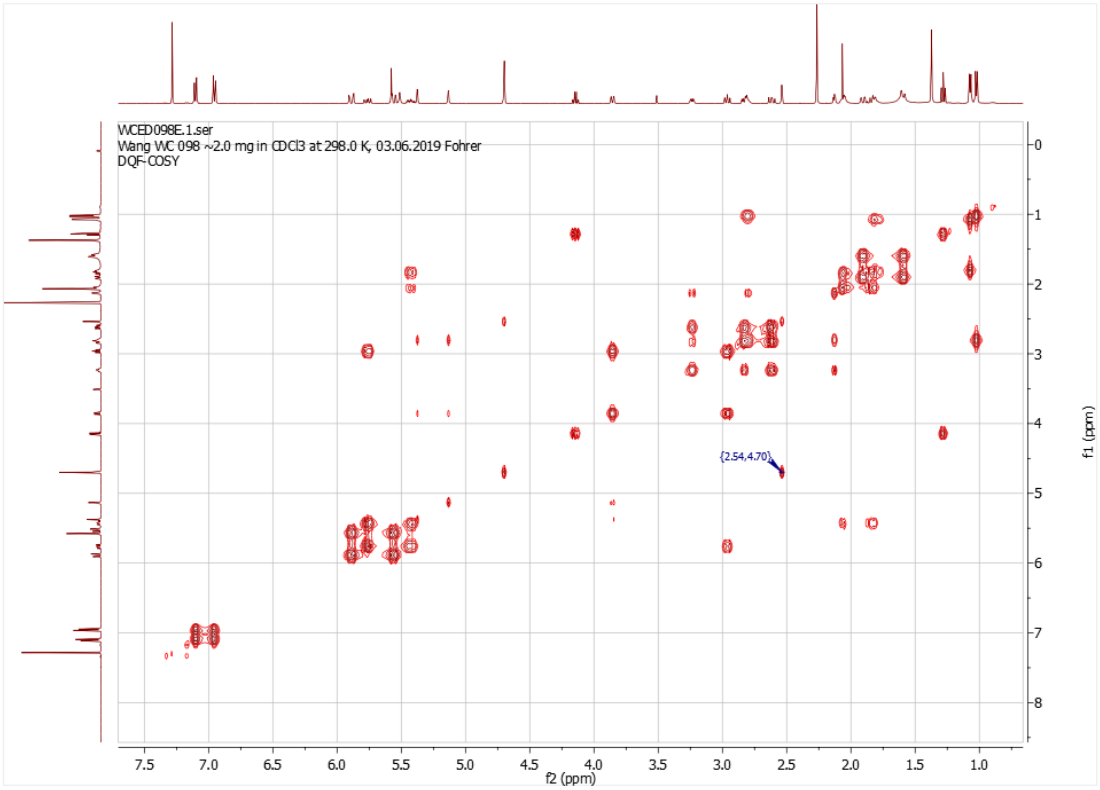


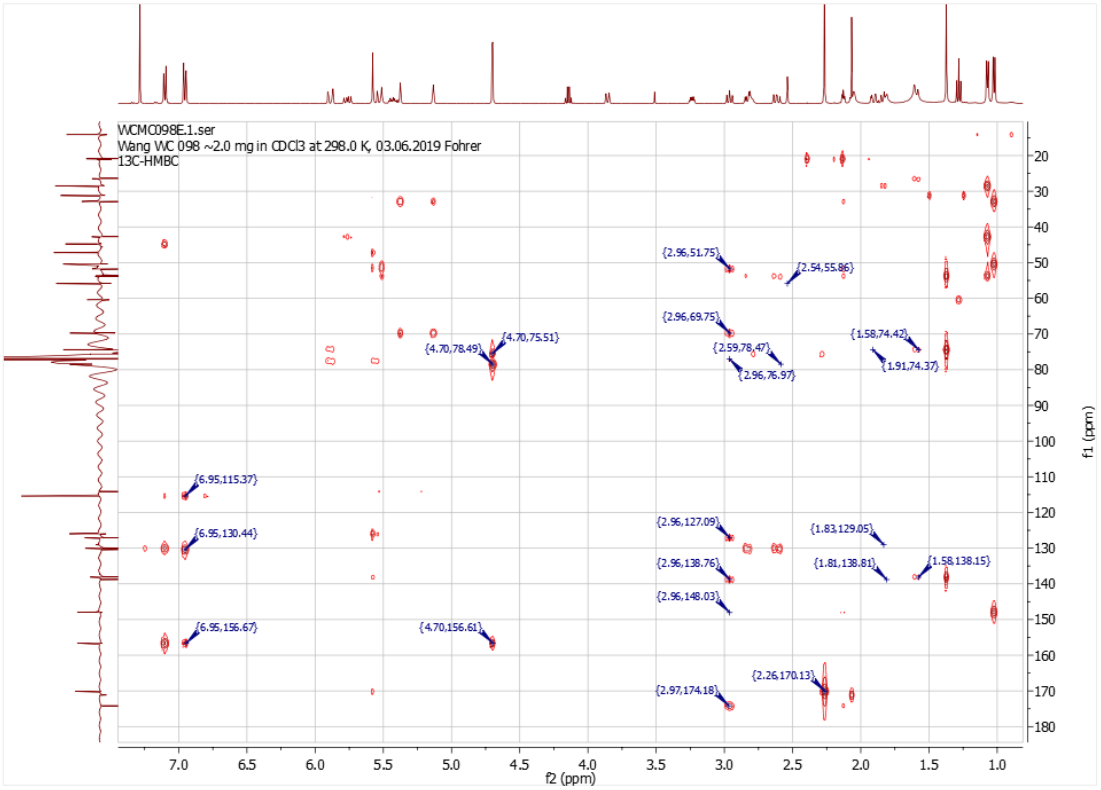
Minimum:		5.0	20.0	-0.5		
Maximum:				60.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
570.2830	570.2829	0.1	0.2	12.0	0.6	C32 H42 O9
	570.2832	-0.2	-0.4	13.5	0.5	C33 H41 N O6 Na
	570.2805	2.5	4.4	9.0	0.4	C30 H43 O9 Na
	570.2856	-2.6	-4.6	16.5	1.2	C35 H40 N O6
	570.2797	3.3	5.8	25.5	4.7	C42 H36 N O
	570.2773	5.7	10.0	22.5	3.2	C40 H37 N O Na
	570.2770	6.0	10.5	21.0	3.0	C39 H38 O4
	570.2899	-6.9	-12.1	22.0	3.7	C41 H39 O Na
	570.2746	8.4	14.7	18.0	1.9	C37 H39 O4 Na
	570.2923	-9.3	-16.3	25.0	5.5	C43 H38 O

HRMS (*m/z*): calculated for [$C_{33}H_{41}NO_6$ + Na]: 570.2832, found: 570.2830.



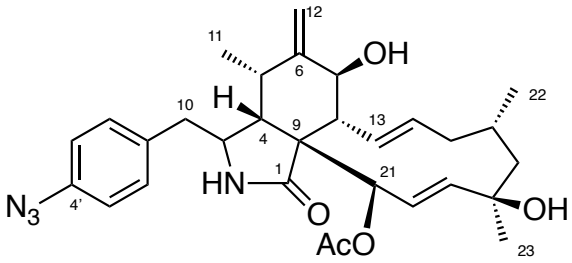

174.18
170.12
156.62
147.91
138.74
138.10
130.40
130.03
127.11
125.98
115.40
114.13
78.45
77.48
75.61
74.37
69.73
60.41
55.87
53.70
51.78
50.40
47.21
44.76
42.75
32.87
31.18
28.46
26.46
20.89
14.14
f1 (ppm)


WCED098E.1.ser
Wang WC 098 ~2.0 mg in CDCl3 at 298.0 K, 03.06.2019 Fohrer
DQF-COSY
(2.54,4.70)
f2 (ppm)
f1 (ppm)


WCMC098E.1.ser
Wang WC 098 ~2.0 mg in CDCl3 at 298.0 K, 03.06.2019 Fohrer
13C-HMBC
f2 (ppm)
f1 (ppm)

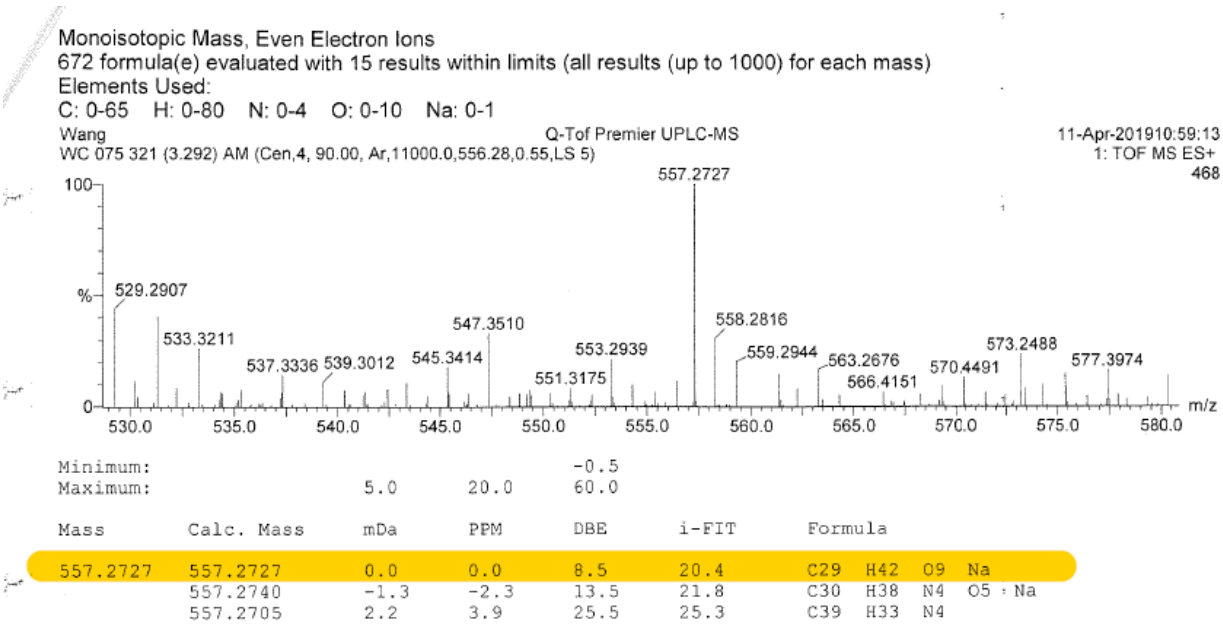

WCMH098E.1.ser
Wang WC 098 ~2.0 mg in CDCl3 at 298.0 K, 03.06.2019 Fohrer
13C-HSQC
f2 (ppm)
f1 (ppm)

1.12 4'-Azido Cytochalasin H 1h



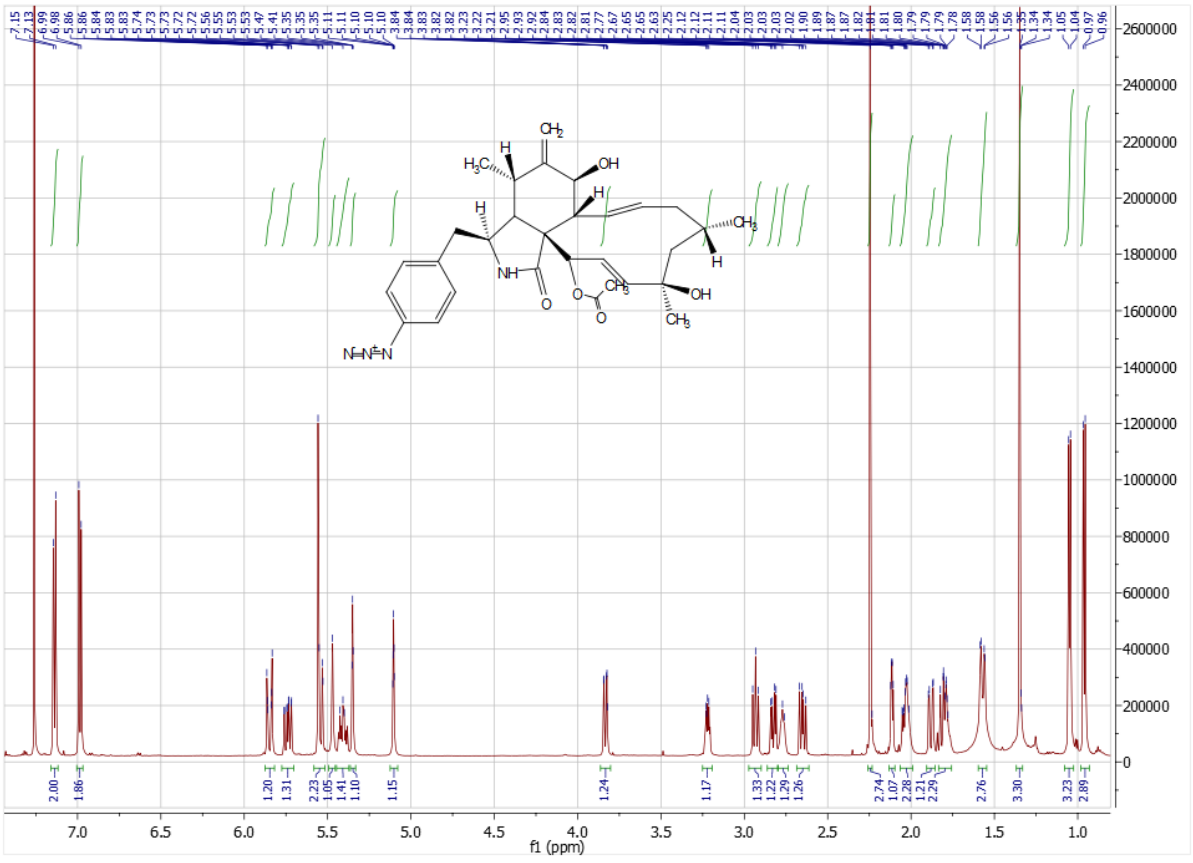
Chemical Formula: $C_{30}H_{38}N_4O_5$
Exact Mass: 534.2842

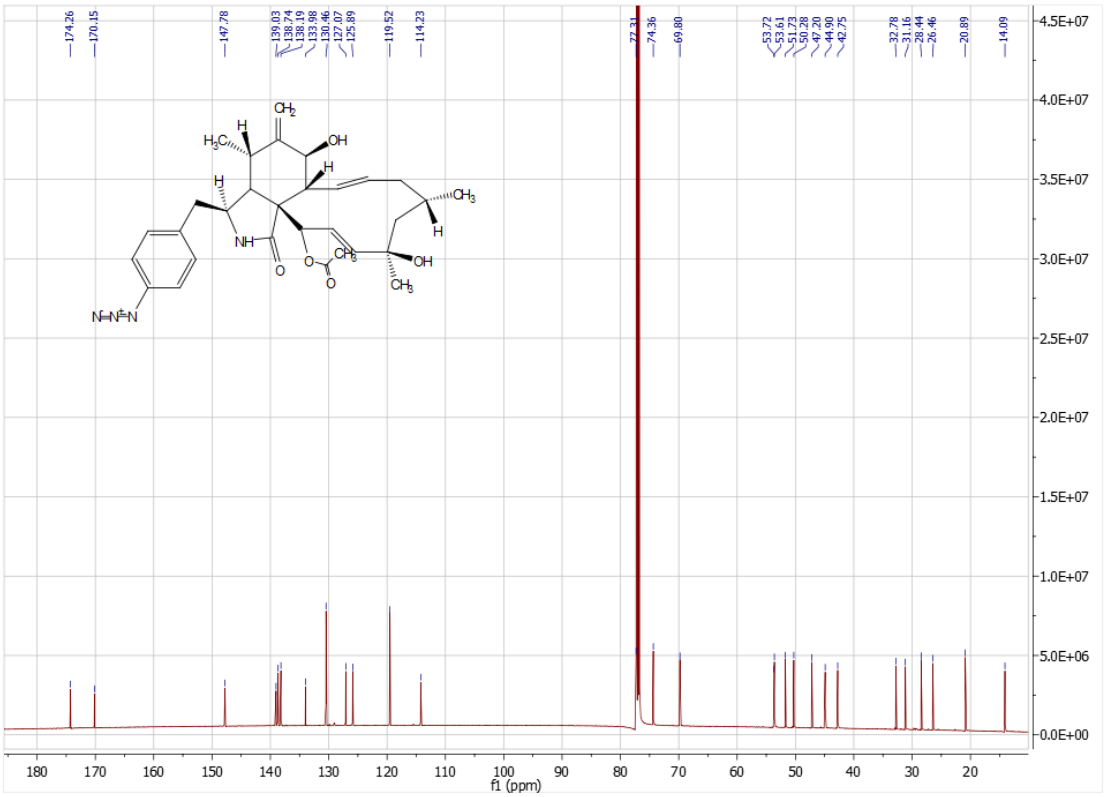
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.2	-	-	-
2	5.47	brs	-	-	-	-	-
3	3.22	ddd	1.1, 5.0, 9.6	53.7	CH	1', 1, 4, 5	4, 5, 10
4	2.11	dd	3.8, 5.0	50.3	CH	1, 3, 5, 6, 10, 21	3, 5, 10
5	2.77	m	-	32.8	CH	1', 3	4, 11, 12
6	-	-	-	147.7	-	-	-
7	3.84	dd	1.4, 10.2	69.8	CH	5, 6, 8, 12, 13	8, 12
8	2.93	t	10.2	47.3	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.6	-	-	-
10a	2.65	dd	9.3, 13.6	44.9	CH_2	1', 3	3, 4, 12
10b	2.84	dd	4.9, 13.6	-	-	-	-
11	0.96	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.10	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.35	brs	-	-	-	-	-
13	5.74	ddd	1.4, 9.7, 15.5	127.1	CH	7, 8, 15	8, 14, 15
14	5.42	ddd	4.8, 10.2, 15.5	138.7	CH	9, 15	13, 15
15a	1.82	m	-	42.8	CH_2	13, 14, 16, 17	13, 14,
15b	2.04	m	-	-	-	-	-
16	1.79	m	-	28.6	CH	13, 14, 17	22
17a	1.58	dd	3.1, 14.3	53.9	CH_2	18, 19	-
17b	1.87	dd	3.2, 14.3	-	-	-	-
18	-	-	-	74.3	-	-	-
19	5.56	dd	3.8, 15.2	138.1	CH	-	20
20	5.83	m	-	125.8	CH	9, 18, 19	19
21	5.55	brs	-	77.3	CH	19, 20, 24	-
22	1.05	d	6.4	26.6	CH_3	15, 16, 17	16
23	1.35	s	-	31.3	CH_3	17, 18, 19	-
1'	-	-	-	133.8	-	-	-
2' 6'	7.14	d	8.4	130.4	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	6.99	d	8.4	119.1	2 x CH	1', 4'	2', 6'
4'	-	-	-	138.9	-	-	-
24	-	-	-	170.2	-	-	-
25	2.25	s	-	21.0	CH_3	21, 24	-

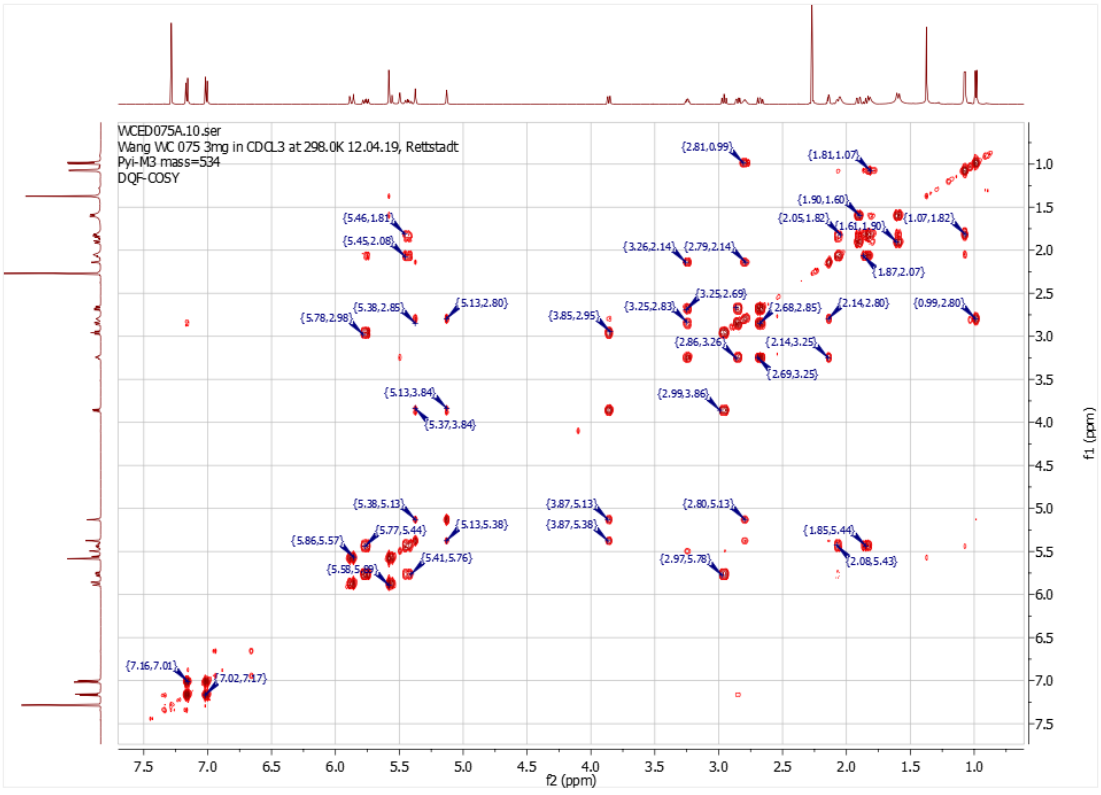


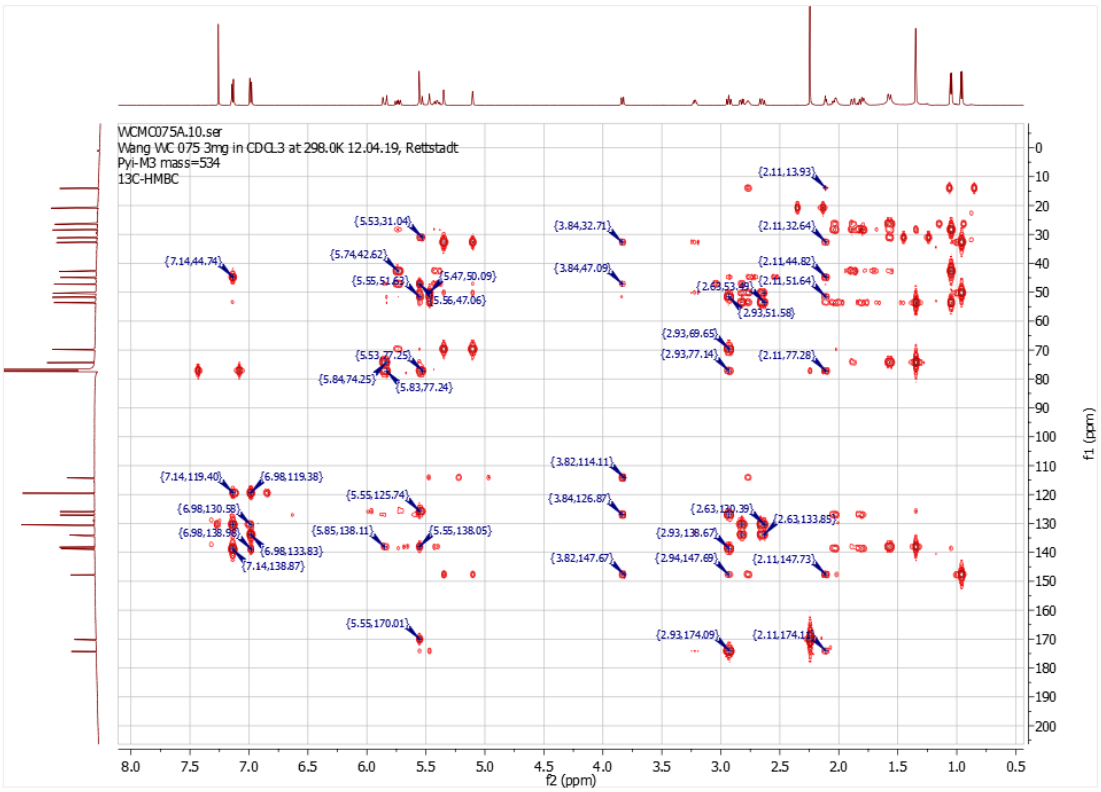
HRMS (*m/z*): calculated for [$C_{30}H_{38}N_4O_5$ + Na]: 557.2727, found: 557.2727.

IR (powder, cm^{-1}): 3388, 2920, 2112, 1685, 1506, 1369, 1280, 1226, 1045, 1014, 962, 534.




174.26
170.15
147.78
139.03
138.74
138.19
133.98
130.46
127.07
125.89
119.52
114.23
77.31
74.36
69.80
53.72
53.61
53.23
50.28
47.20
44.90
42.75
32.78
31.16
28.44
26.46
20.89
14.09
4.5E+07
4.0E+07
3.5E+07
3.0E+07
2.5E+07
2.0E+07
1.5E+07
1.0E+07
5.0E+06
0.0E+00
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

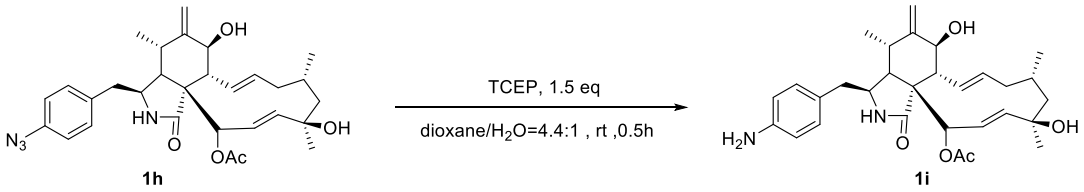

WCED075A.10.ser
Wang WC 075 3mg in CDCL3 at 298.0K 12.04.19, Rettstadt
Pyi-M3 mass=534
DQF-COSY
f1 (ppm)
f2 (ppm)


WCMC075A.10.ser
Wang WC 075 3mg in CDCL3 at 298.0K 12.04.19, Rettstadt
Pyi-M3 mass=534
13C-HMBC
f2 (ppm)
f1 (ppm)

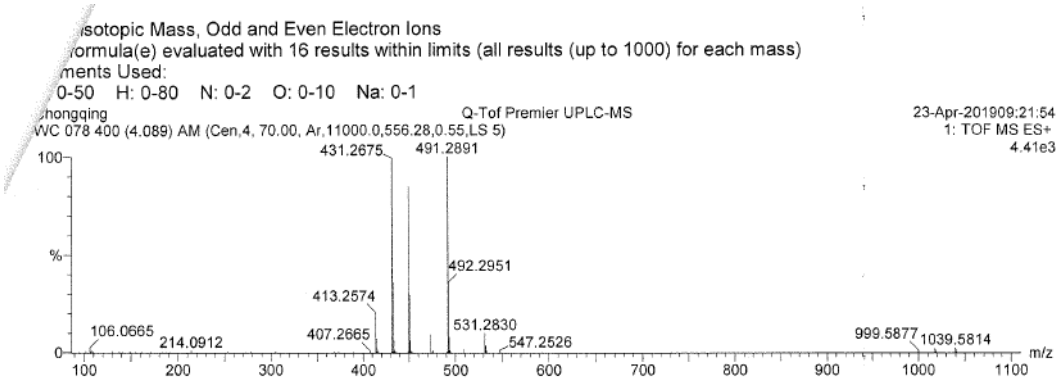

WCMH075A.10.ser
Wang WC 075 3mg in CDCL3 at 298.0K 12.04.19, Rettstadt
Pyi-N3 mass=534
13C-HSQC
f2 (ppm)
f1 (ppm)

2.0 Synthetic Procedures and Characterisation

2.01 4'-Amino Cytochalasin H 1i

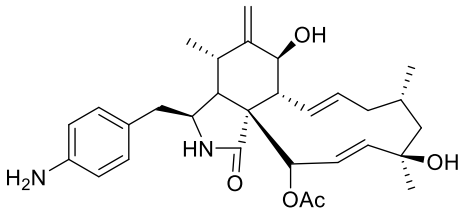


To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) in 2.0 mL dioxane-water mixture (4.4:1, *v/v*) was added TCEP (7.0mg, 0.028 mmol, 1.5 eq). The mixture was stirred at room temperature for 1 hour and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 6.7 mg of desired product (70.2%, 0.013 mmol) as a white solid.



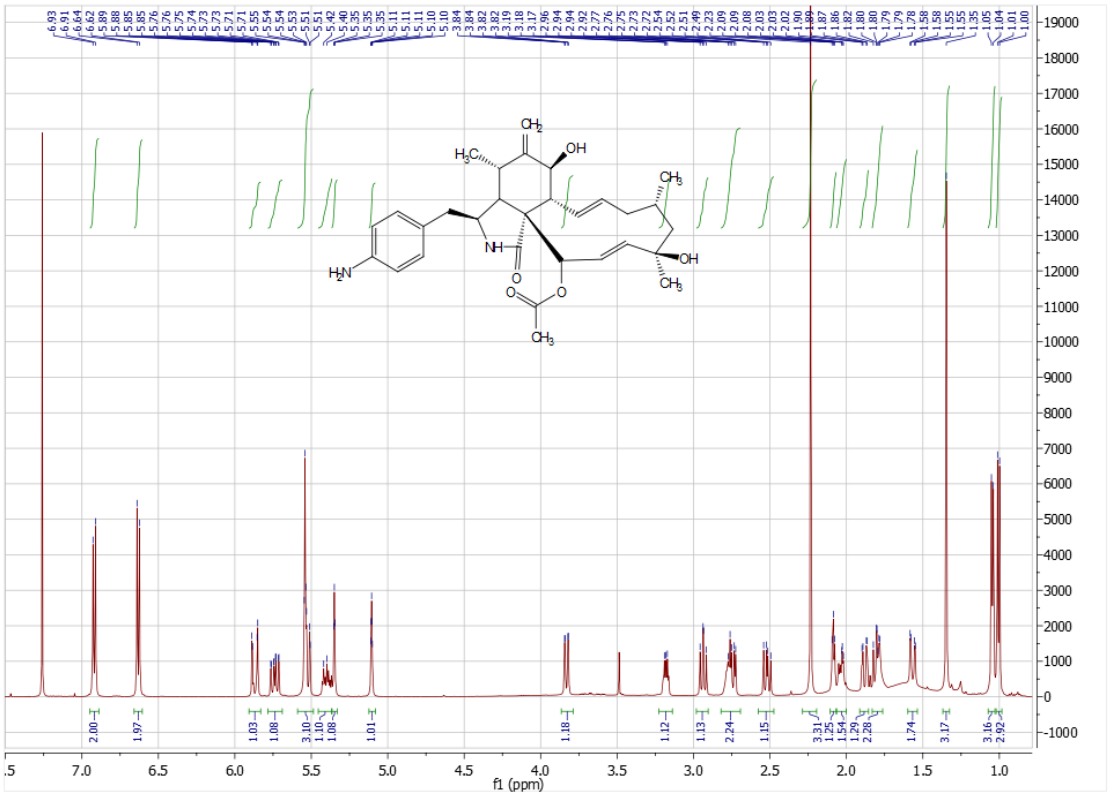
Minimum:				-0.5		
Maximum:		5.0	20.0	60.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
531.2830	531.2832	-0.2	-0.4	10.0	0.7	C29 H41 N O8
	531.2835	-0.5	-0.9	11.5	0.9	C30 H40 N2 O5 Na
	531.2808	2.2	4.1	7.0	1.7	C27 H42 N O8 Na
	531.2859	-2.9	-5.5	14.5	0.8	C32 H39 N2 O5
	531.2800	3.0	5.6	23.5	4.5	C39 H35 N2

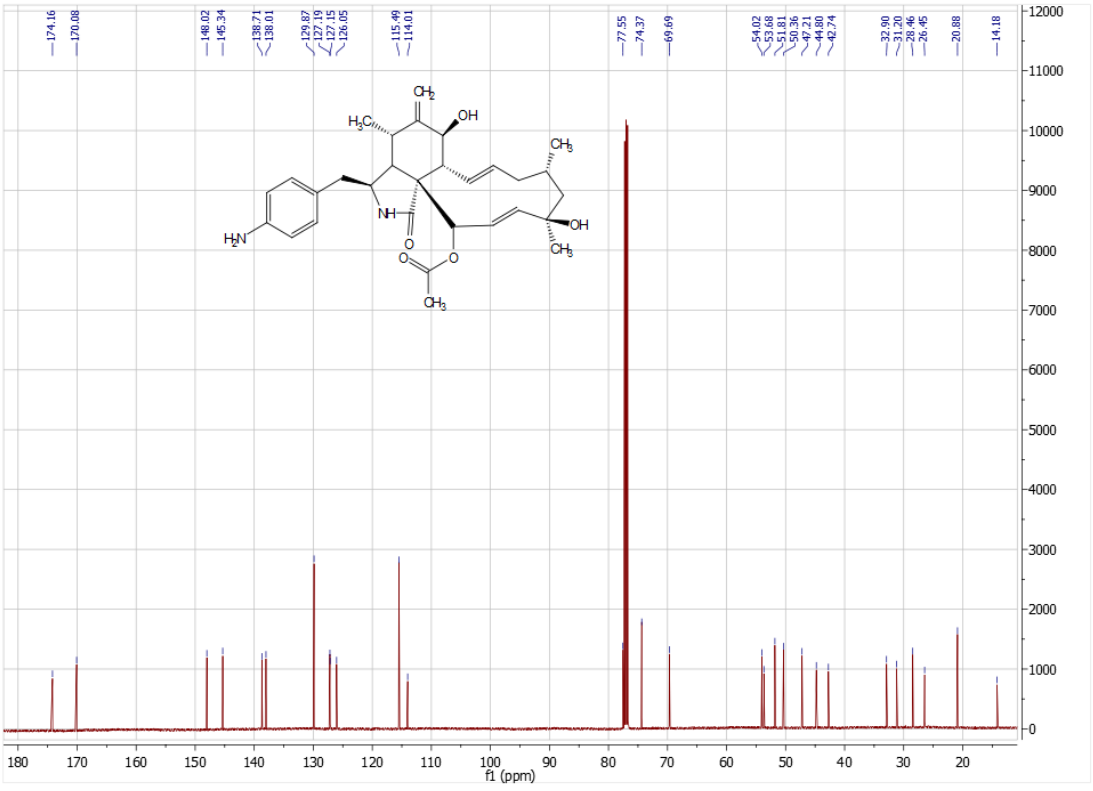
HRMS (*m/z*): calculated for [$C_{60}H_{76}N_2O_{10}$ + Na]: 531.2835, found: 531.2830.

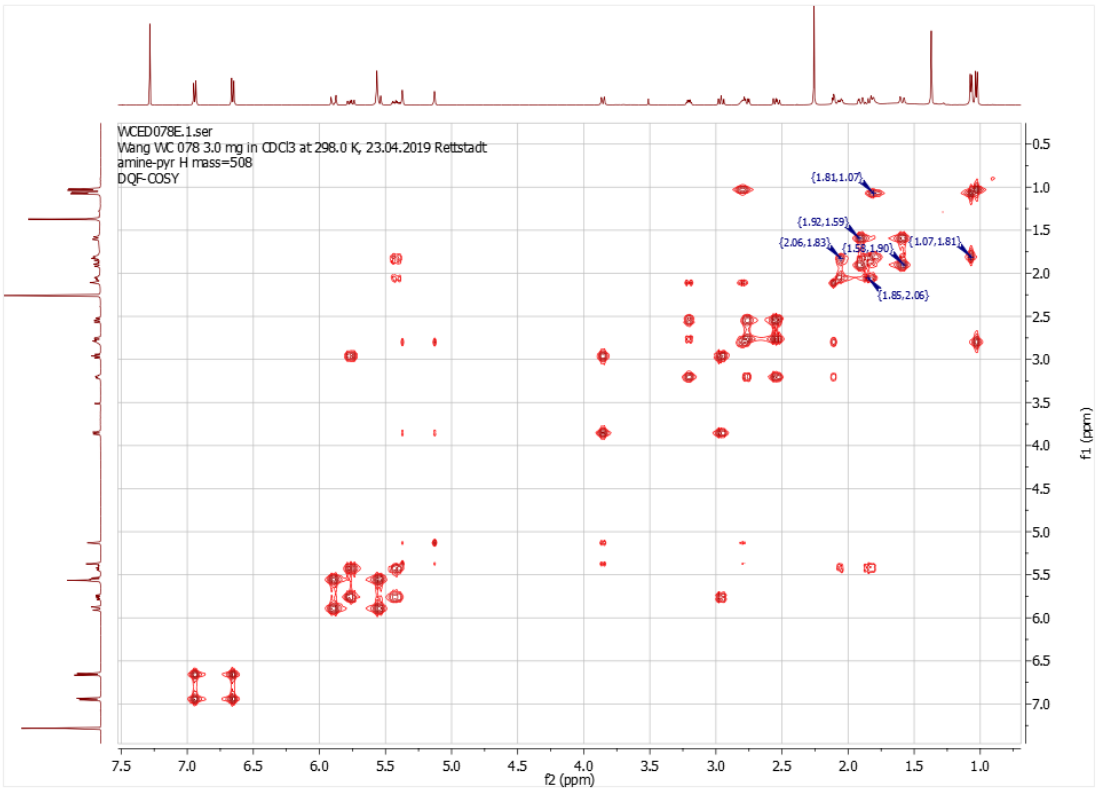


1i

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.1	-	-	-
2	5.56	brs	-	-	-	-	-
3	3.20	ddd	1.1, 4.7, 9.4	53.9	CH	1‘, 1, 4, 5	4, 5, 10
4	2.11	dd	4.4, 4.7	50.2	CH	1, 3, 5, 6, 10, 21	3, 5, 10
5	2.80	m	-	32.9	CH	1’, 3	4, 11, 12
6	-	-	-	148.1	-	-	-
7	3.86	dd	1.4, 10.8	69.6	CH	6, 12	8, 12
8	2.96	dd	9.8, 10.8	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.8	-	-	-
10a	2.56	dd	9.8, 13.7	44.8	CH_2	1’, 3	3, 4, 12
10b	2.75	dd	4.6, 13.7	-	-	-	-
11	1.02	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.13	brs	-	113.9	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.76	ddd	1.3, 9.6, 15.4	127.1	CH	7, 8, 15	8, 14, 15
14	5.40	ddd	4.9, 10.2, 15.4	138.7	CH	9, 15	13, 15
15a	1.82	m	-	42.6	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.81	m	-	28.4	CH	13, 14, 17	22
17a	1.58	dd	2.9, 14.3	53.8	CH_2	18, 19	-
17b	1.89	m	3.1, 14.3	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.53	dd	2.3, 17.2	138.0	CH	-	20
20	5.89	dd	2.1, 17.2	126.0	CH	18, 19	19
21	5.58	m	-	77.2	CH	8, 9, 19, 20, 21 23	-
22	1.06	d	6.3	26.5	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	130.0	-	-	-
2’ 6’	6.95	d	8.3	129.8	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	6.65	d	8.3	115.3	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	145.3	-	-	-
24	-	-	-	170.1	-	-	-
25	2.25	s	-	20.9	CH_3	21, 24	-


f1 (ppm)

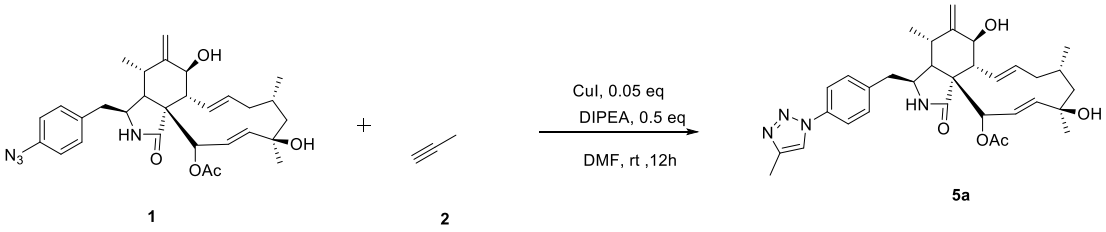

f1 (ppm)


WCED078E.1.ser
Wang WC 078 3.0 mg in CDCl3 at 298.0 K, 23.04.2019 Rettstadt
amine-pyr H mass=508
DQF-COSY
{1.81,1.07}
{1.92,1.59}
{2.06,1.83}
{1.58,1.90}
{1.07,1.81}
{1.85,2.06}
f2 (ppm)
f1 (ppm)

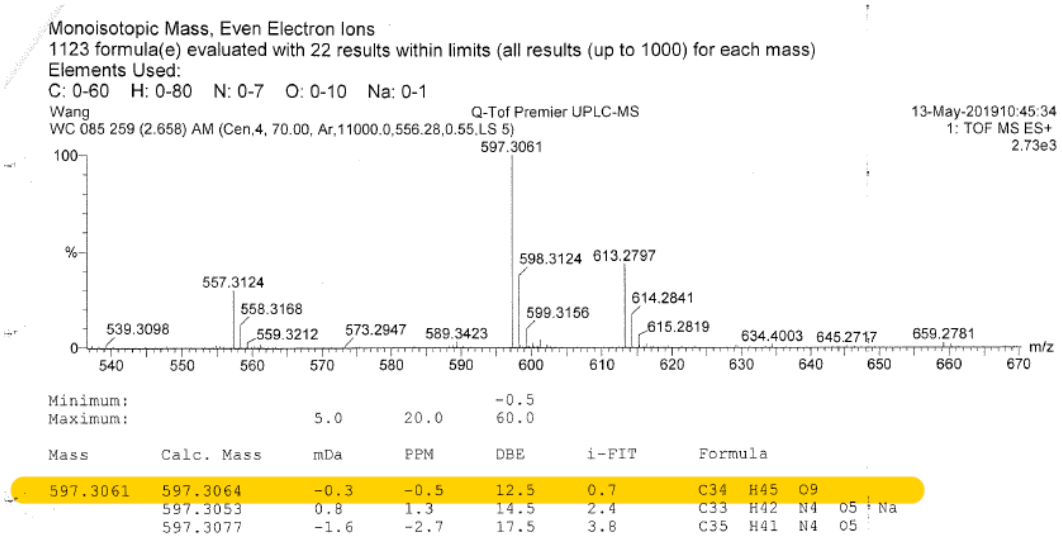

WCMH078E.1.ser
Wang WC 078 3.0 mg in CDCl3 at 298.0 K, 23.04.2019 Rettstadt
amine-pyr H mass=508
13C-HSQC
{1.02,14.09}
{2.25,20.93}
{1.06,26.46}
{1.81,28.44}
{2.80,32.85}
{1.37,31.19}
{2.75,44.87}
{2.06,42.72}
{2.96,47.18}
{2.11,50.24}
{1.82,42.61}
{3.20,53.90}
{1.89,53.83}
{1.58,53.73}
{3.86,69.57}
{5.58,77.15}
{6.65,115.29}
{5.37,113.95}
{5.13,113.85}
{5.89,125.96}
{5.42,127.09}
{6.95,129.79}
{5.76,127.08}
{5.40,138.65}
{5.53,138.01}
f2 (ppm)
f1 (ppm)


WCMH078E.1.ser
Wang WC 078 3.0 mg in CDCl3 at 298.0 K, 23.04.2019 Rettstadt
amine-pyr H mass=508
13C-HSQC
{1.02,14.09}
{2.25,20.93}
{1.81,28.44}
{1.06,26.46}
{2.80,32.85}
{1.37,31.19}
{2.06,42.72}
{2.75,44.87}
{2.96,47.18}
{1.82,42.61}
{2.11,50.24}
{3.20,53.90}
{1.89,53.83}
{1.58,53.73}
{3.86,69.57}
{5.58,77.15}
{6.65,115.29}
{5.37,113.95}
{5.13,113.85}
{5.89,125.96}
{5.42,127.09}
{6.95,129.79}
{5.76,127.08}
{5.40,138.65}
{5.53,138.01}
f2 (ppm)
f1 (ppm)

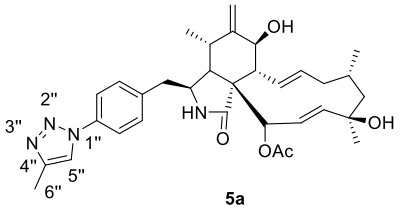
2.02 4'-(1-(4-methyltriazolyl)) Cytochalasin H 5a



To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and propyne (2.0 mg, 0.038 mmol, 2.0 eq, purchased from Acros) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 8.2 mg of desired product **5a** (76.4%, 0.014 mmol) as a white solid.



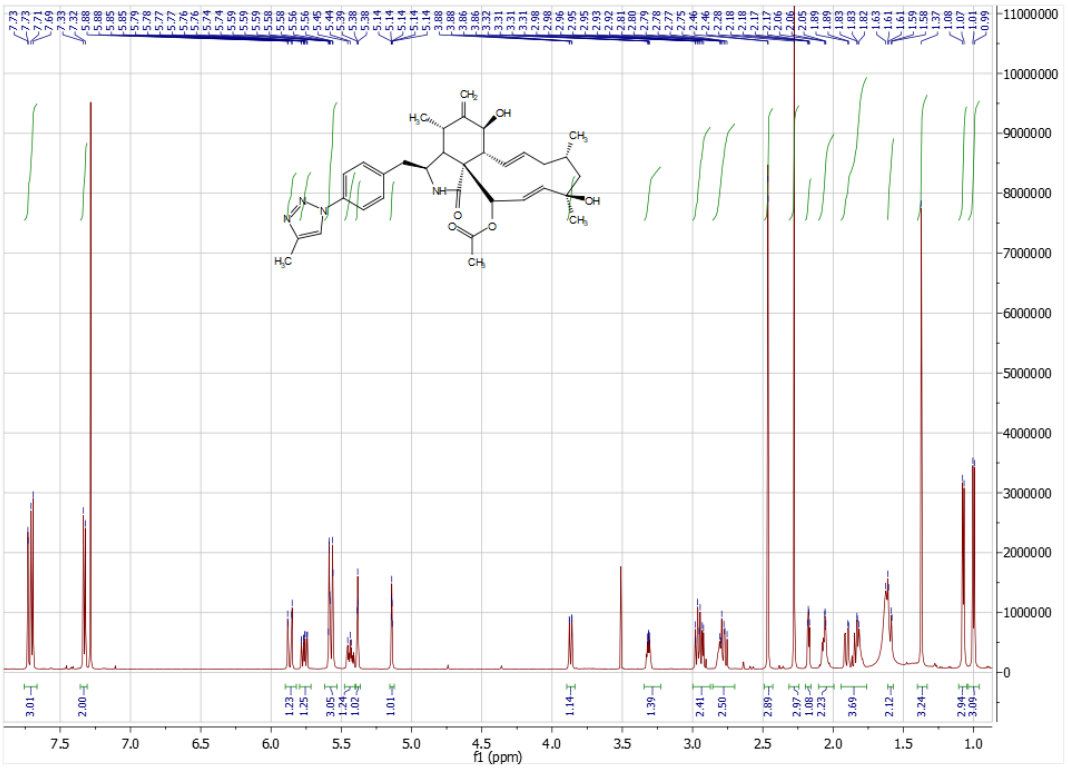
HRMS (*m/z*): calculated for [$C_{60}H_{76}N_2O_{10}$ + Na]: 597.3053, found: 597.3061.

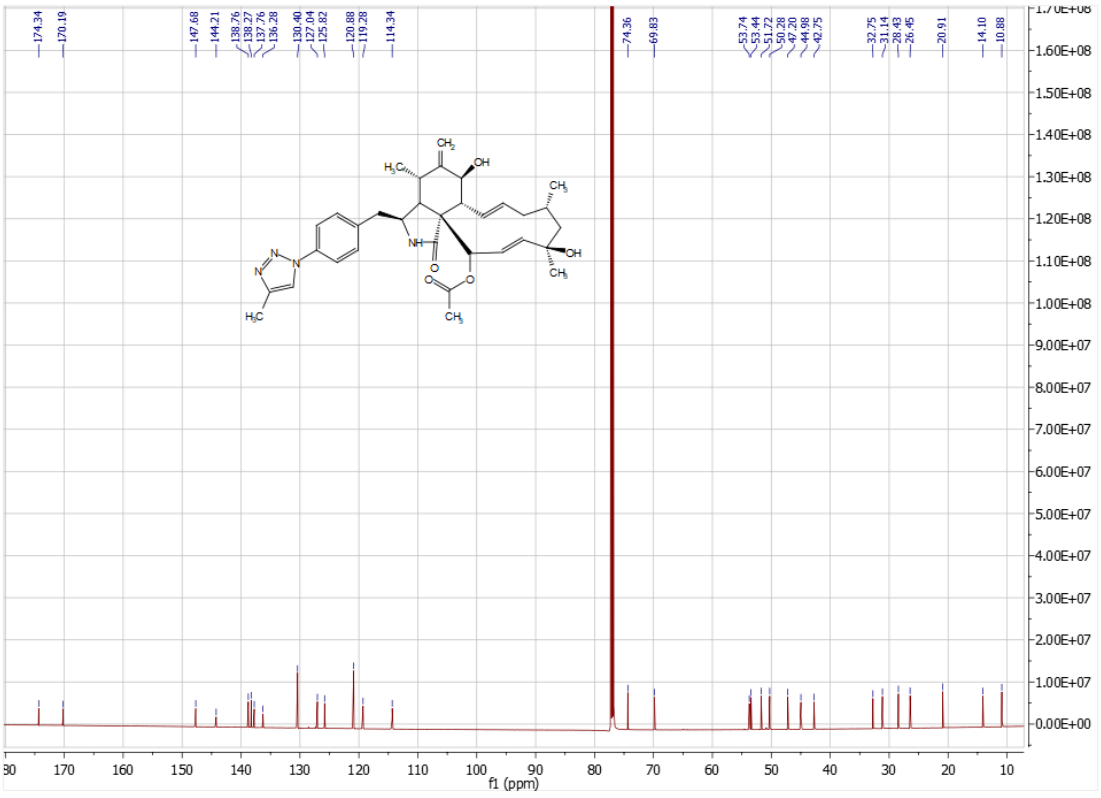


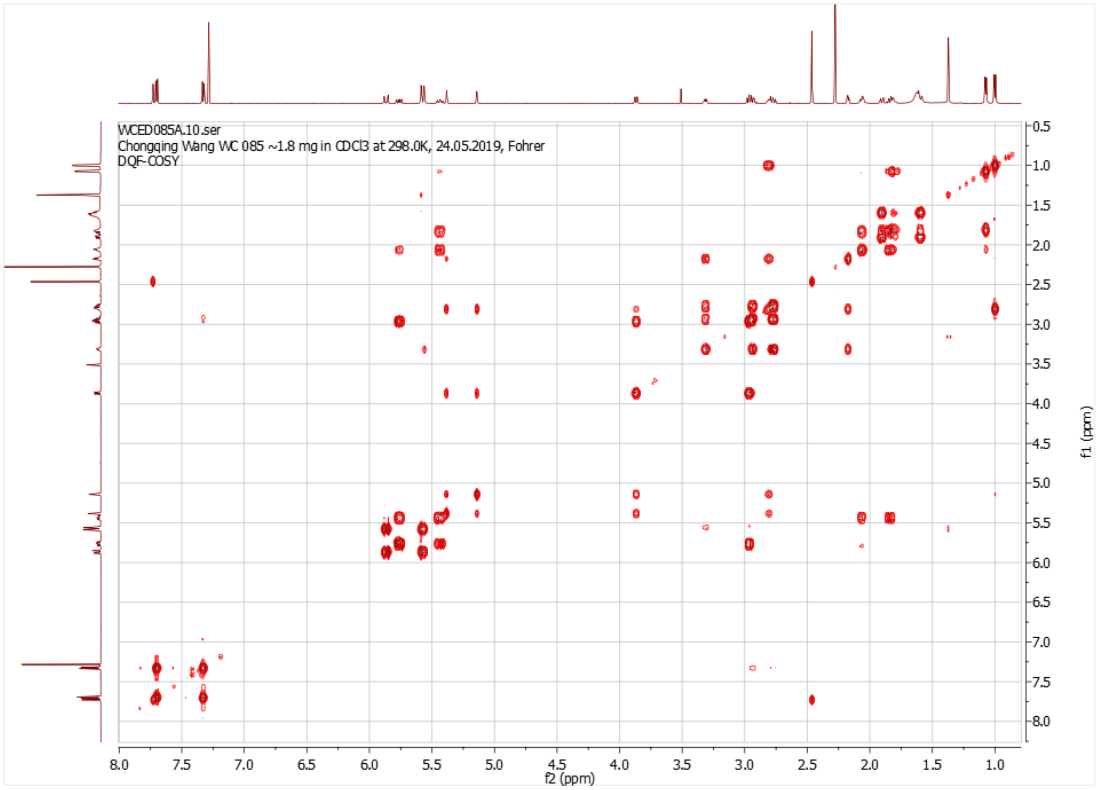
5a

Chemical Formula: $C_{33}H_{42}N_4O_5$
Exact Mass: 574.3155

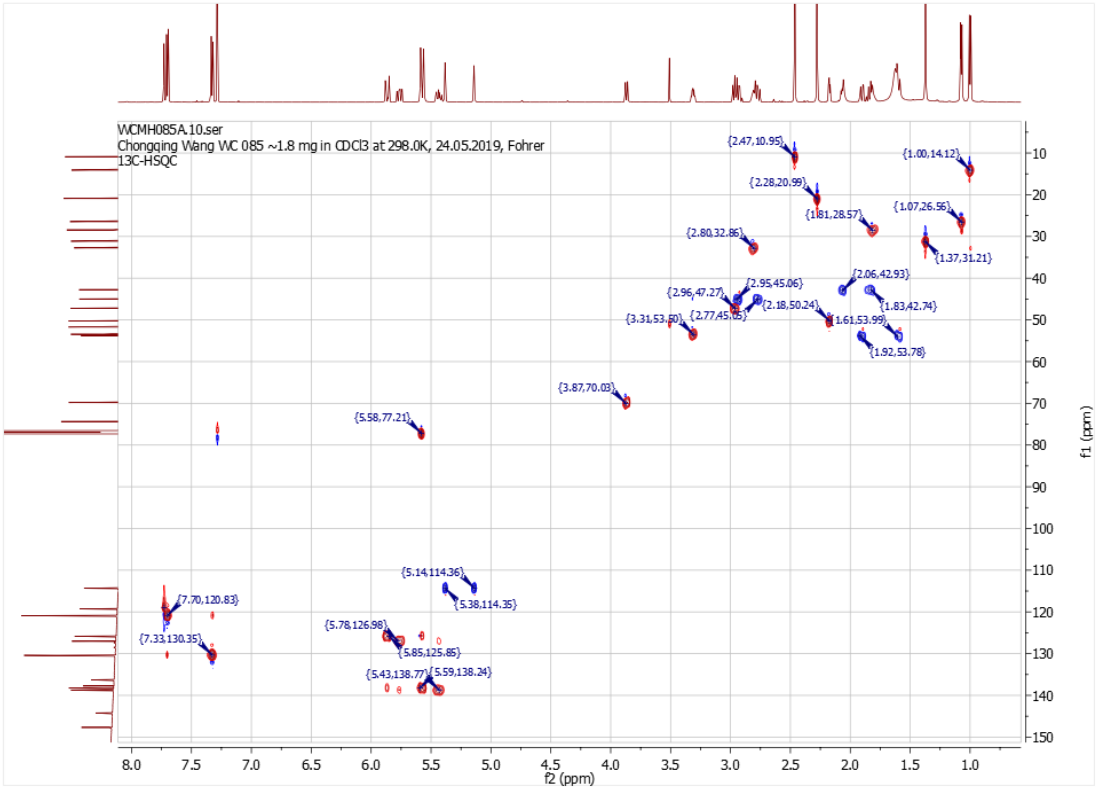
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
2	5.56	brs	-	-	-	-	-
3	3.31	m	-	53.5	CH	1', 1, 4, 5	4, 5, 10
4	2.18	dd	3.8, 5.0	50.2	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.80	m	-	32.7	CH	1', 3	4, 11, 12
6	-	-	-	147.7	-	-	-
7	3.87	dd	1.3, 10.8	70.0	CH	6, 12	8, 12
8	2.96	m	-	47.3	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.7	-	-	-
10a	2.77	m	-	45.1	CH_2	1', 3	3, 4, 12
10b	2.95	m	-	-	-	-	-
11	1.00	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.4	CH_2	5, 6, 7	5, 7, 11
12b	5.38	brs	-	-	-	-	-
13	5.78	ddd	1.4, 9.7, 15.4	127.0	CH	7, 8, 15	8, 14, 15
14	5.43	ddd	4.8, 10.1, 15.4	138.8	CH	9, 15	13, 15
15a	1.83	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.81	m	-	28.6	CH	13, 14, 17	22
17a	1.61	m	-	53.8	CH_2	18, 19	-
17b	1.92	dd	3.0, 14.4	-	-	-	-
18	-	-	-	74.3	-	-	-
19	5.59	brs	-	138.2	CH	-	20
20	5.85	dd	3.0, 16.2	125.9	CH	18, 19	19
21	5.58	brs	-	77.2	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.5	26.6	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1'	-	-	-	137.8	-	-	-
2' 6'	7.33	d	8.5	130.4	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.70	d	8.5	120.8	2 x CH	1', 4'	2', 6'
4'	-	-	-	120.9	-	-	-
24	-	-	-	170.2	-	-	-
25	2.28	s	-	21.0	CH_3	24	-
4''	-	-	-	144.2	-	-	-
5''	7.73	s	-	118.9	CH	4''	-
6''	2.47	s	-	11.0	CH_3	4'', 5''	-


f1 (ppm)

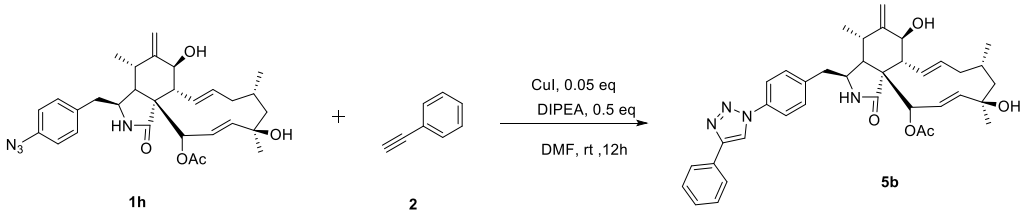

f1 (ppm)


WCED085A.10.ser
Chongqing Wang WC 085 ~1.8 mg in CDCl3 at 298.0K, 24.05.2019, Fohrer
DQF-COSY
f2 (ppm)
f1 (ppm)

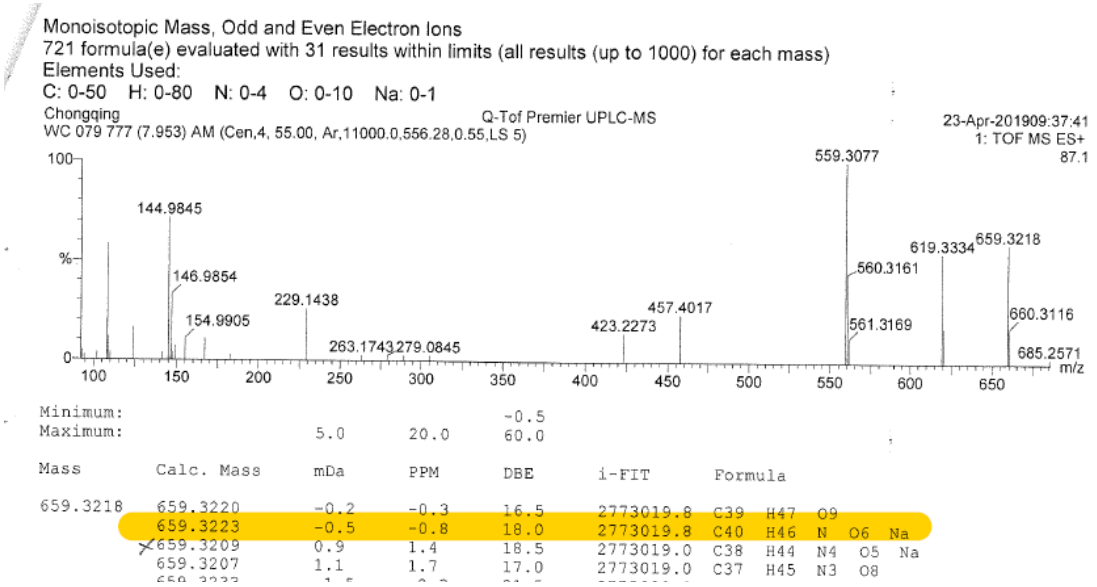

WCMC085A.10.ser
Chongqing Wang WC 085 ~1.8 mg in CDCl3 at 298.0K, 24.05.2019, Fohrer
HMBC
{3.34,32.73}
{5.47,42.63}
{5.59,47.09}
{5.38,50.32}
{5.55,53.49}
{2.96,51.71}
{2.79,53.35}
{3.34,50.12}
{2.79,50.29}
{2.97,69.83}
{2.96,77.24}
{5.86,77.28}
{5.86,77.20}
{5.59,77.23}
{7.70,120.89}
{7.70,130.41}
{7.70,137.77}
{7.73,144.22}
{5.58,124.01}
{5.58,138.27}
{5.88,138.26}
{3.86,127.08}
{2.94,125.20}
{2.94,130.38}
{2.79,130.53}
{2.94,138.74}
{2.76,137.87}
{3.31,137.04}
{3.86,147.72}
{5.59,170.19}
{2.28,170.22}
{2.96,174.38}
{3.29,174.43}
{3.34,174.26}
f2 (ppm)
f1 (ppm)


WCMH085A.10.ser
Chongqing Wang WC 085 ~1.8 mg in CDCl3 at 298.0K, 24.05.2019, Fohrer
13C-HSQC
{2.47,10.95}
{1.00,14.12}
{2.28,20.99}
{1.81,28.57}
{1.07,26.56}
{2.80,32.86}
{1.37,31.21}
{2.06,42.93}
{2.96,47.27}
{2.95,45.06}
{2.18,50.24}
{1.83,42.74}
{3.31,53.50}
{2.77,45.05}
{1.61,53.99}
{1.92,53.78}
{3.87,70.03}
{5.58,77.21}
{5.14,114.36}
{5.38,114.35}
{7.70,120.83}
{5.78,126.98}
{7.33,130.35}
{5.85,125.85}
{5.43,138.77}
{5.59,138.24}
f2 (ppm)
f1 (ppm)

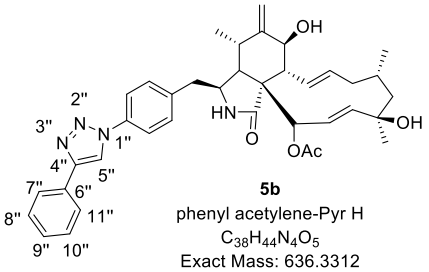
2.03 4'-(1-(4-phenyltriazolyl)) Cytochalasin H 5b



To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and phenylacetylene (4 mg, 0.038 mmol, 2.0 eq, purchased from Acros) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 7.4 mg of desired product (61.8%, 0.011 mmol) as a white solid.

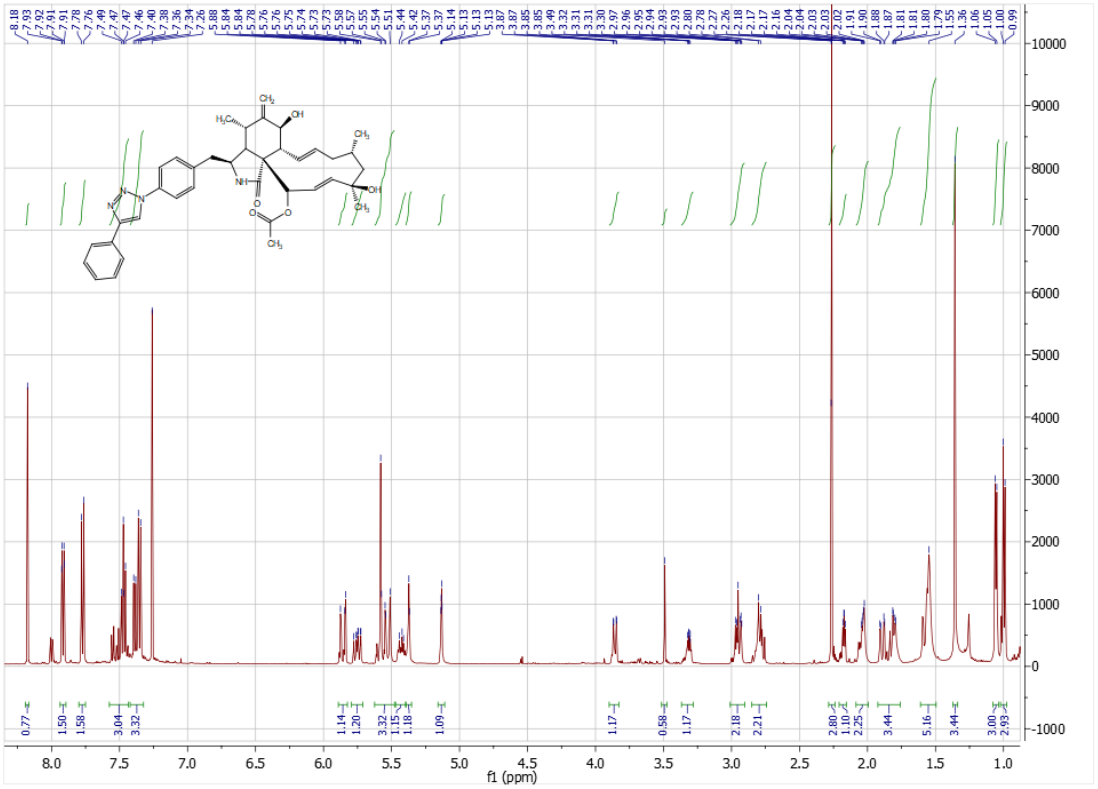


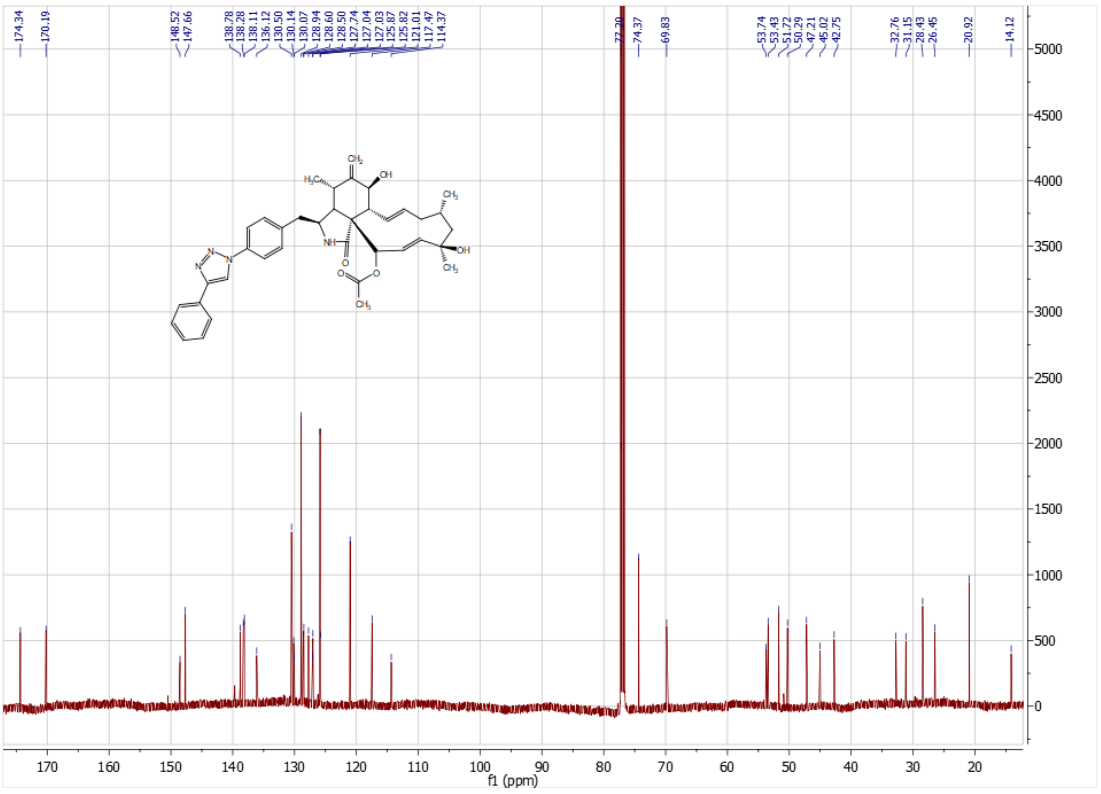
HRMS (*m/z*): calculated for [$C_{40}H_{46}N_1O_6$ + Na]: 659.3223, found: 659.3218.



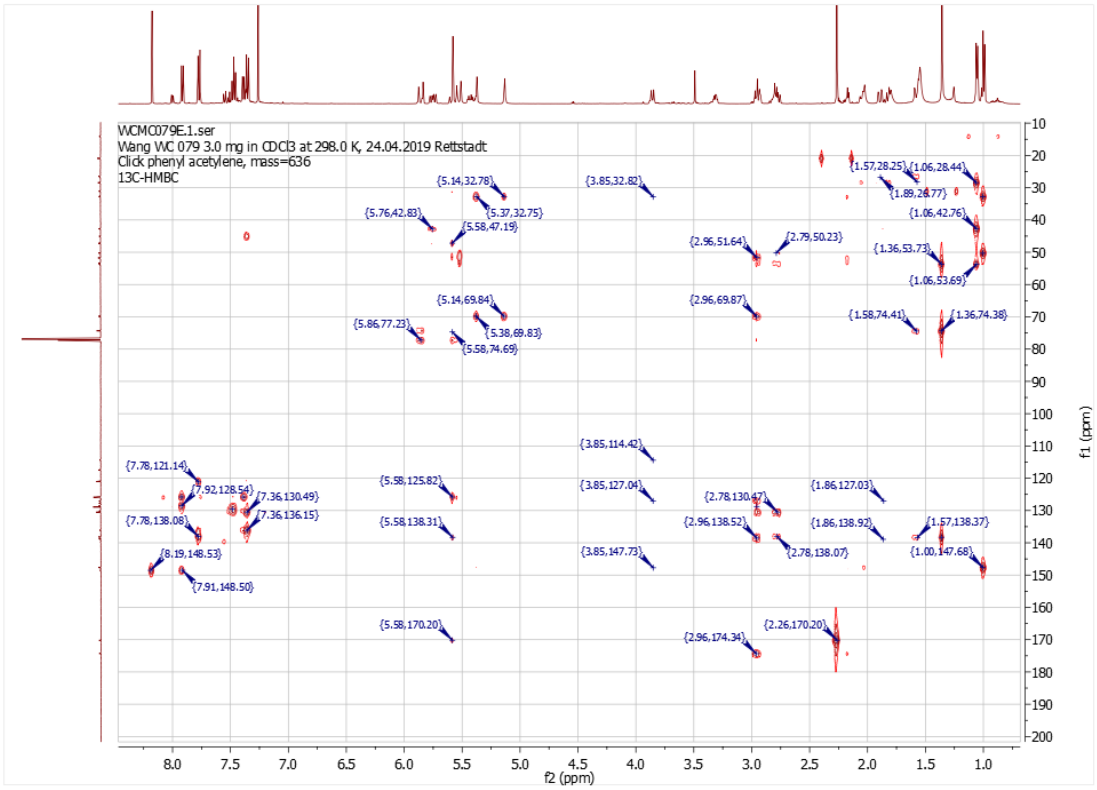
5b
phenyl acetylene-Pyr H
$C_{38}H_{44}N_4O_5$
Exact Mass: 636.3312

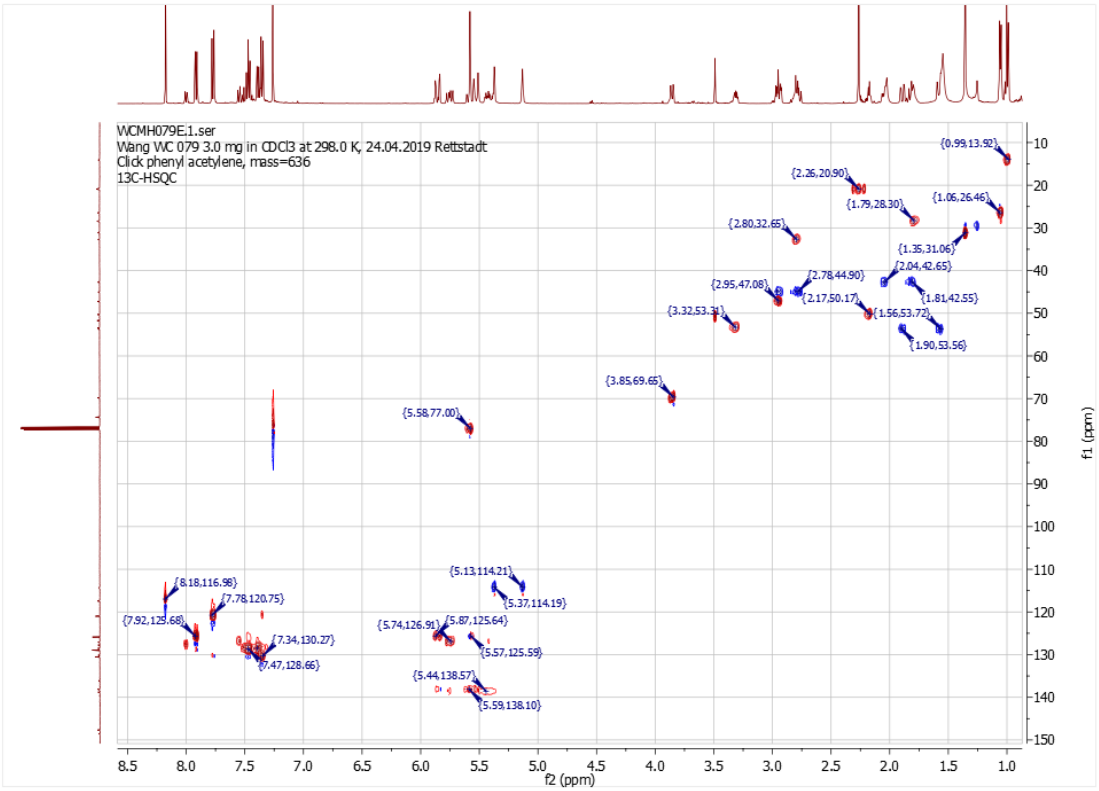
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.2	-	-	-
2	5.56	brs	-	-	-	-	-
3	3.32	m	-	53.3	CH	1', 1, 4, 5	4, 5, 10
4	2.17	dd	3.8, 5.0	50.2	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.80	m	-	32.7	CH	1', 3	4, 11, 12
6	-	-	-	147.7	-	-	-
7	3.85	dd	1.6, 10.7	69.7	CH	6, 12	8, 12
8	2.95	m	-	47.1	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.6	-	-	-
10a	2.65	m	-	44.9	CH_2	1', 3	3, 4, 12
10b	2.81	m	-	-	-	-	-
11	0.99	d	6.7	13.9	CH_3	4, 5, 6	5
12a	5.13	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.74	ddd	1.4, 9.7, 15.4	126.9	CH	7, 8, 15	8, 14, 15
14	5.44	ddd	4.8, 10.1, 15.4	138.6	CH	9, 15	13, 15
15a	1.82	m	-	42.8	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.79	m	-	28.3	CH	13, 14, 17	22
17a	1.58	dd	2.9, 14.4	53.9	CH_2	18, 19	-
17b	1.89	m	-	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.58	brs	2.3, 16.2	138.1	CH	-	20
20	5.87	dd	3.0, 16.2	125.6	CH	18, 19	19
21	5.59	brs	-	77.0	CH	8, 9, 19, 20, 23	-
22	1.06	d	6.5	26.5	CH_3	15, 16, 17	16
23	1.35	s	-	31.1	CH_3	17, 18, 19	-
1'	-	-	-	136.3	-	-	-
2' 6'	7.34	d	8.5	130.3	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.78	d	8.5	120.8	2 x CH	1', 4'	2', 6'
4'	-	-	-	138.1	-	-	-
24	-	-	-	170.2	-	-	-
25	2.26	s	-	20.9	CH_3	24	-
4''	-	-	-	148.5	-	-	-
5''	8.18	s	-	117.0	CH	4''	-
6''	-	-	-	129.6	-	-	-
7'' 11''	7.92	dd	1.4, 8.4	125.7	2 x CH	4'', 9''	8'', 10''
9''	7.40	dd	8.4, 18.6	128.5	CH	7'', 11''	8'', 10''
8'' 10''	7.47	dd	7.4, 8.4	128.7	2 x CH	6''	7'', 11''


f1 (ppm)

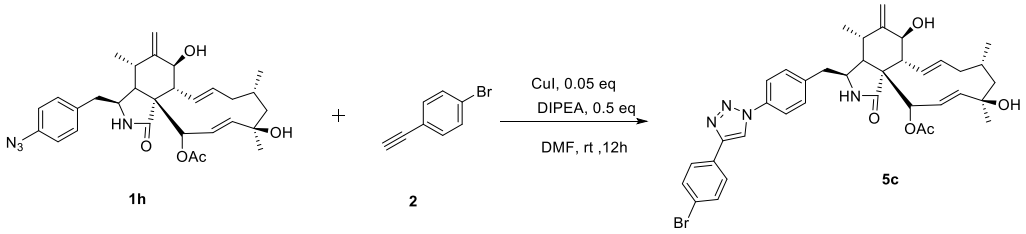

f1 (ppm)


WCED079E.1.ser
Wang WC 079 3.0 mg in CDCl3 at 298.0 K, 24.04.2019 Rettstadt
Click phenyl acetylene, mass=636
DQF-COSY
f2 (ppm)
f1 (ppm)

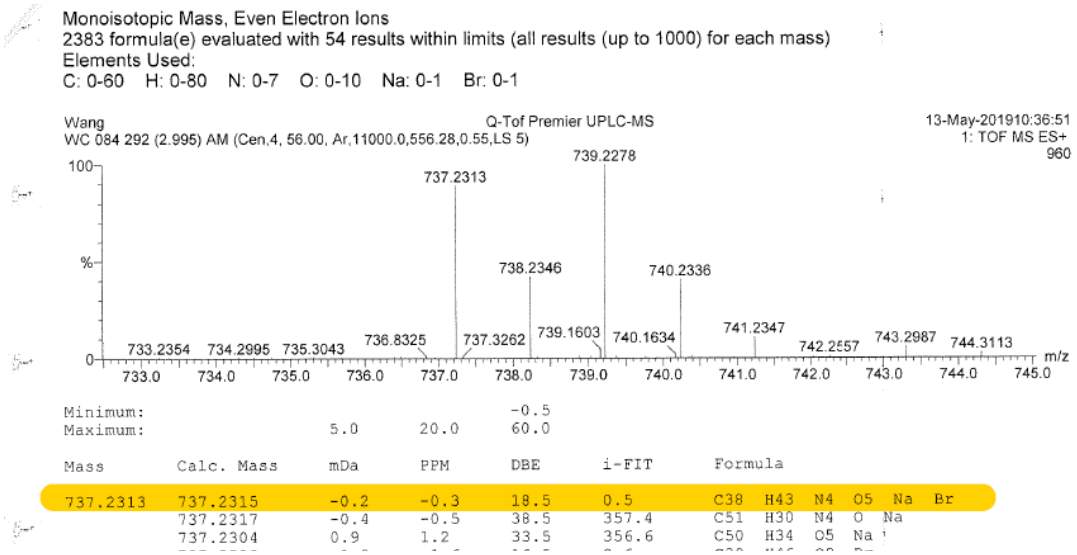

WCMC079E.1.ser
Wang WC 079 3.0 mg in CDCl3 at 298.0 K, 24.04.2019 Rettstadt
Click phenyl acetylene, mass=636
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH079E.1.ser
Wang WC 079 3.0 mg in CDCl3 at 298.0 K, 24.04.2019 Rettstadt
Click phenyl acetylene, mass=636
13C-HSQC
{0.99,13.92}
{2.26,20.90}
{1.79,28.30}
{1.06,26.46}
{2.80,32.65}
{1.35,31.06}
{2.04,42.65}
{2.78,44.90}
{2.95,47.08}
{2.17,50.17}
{1.81,42.55}
{3.32,53.31}
{1.56,53.72}
{1.90,53.56}
{3.85,69.65}
{5.58,77.00}
{8.18,116.98}
{7.78,120.75}
{7.92,129.68}
{7.34,130.27}
{7.47,128.66}
{5.13,114.21}
{5.37,114.19}
{5.74,126.91}
{5.87,125.64}
{5.57,125.59}
{5.44,138.57}
{5.59,138.10}
f2 (ppm)
f1 (ppm)

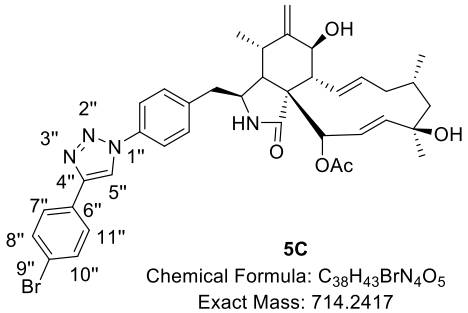
2.04 4'-(1-(4-*p*-bromophenyltriazolyl)) Cytochalasin H 5c



To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and *p*-bromophenylacetylene (6.8 mg, 0.038 mmol, 2.0 eq, purchased from Acros) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at 80 °C for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 8.1 mg of desired product (60.6%, 0.011 mmol) as a white solid.



HRMS (*m/z*): calculated for [$C_{38}H_{43}N_4O_5Br$ + Na]: 737.2315, found: 737.2313.

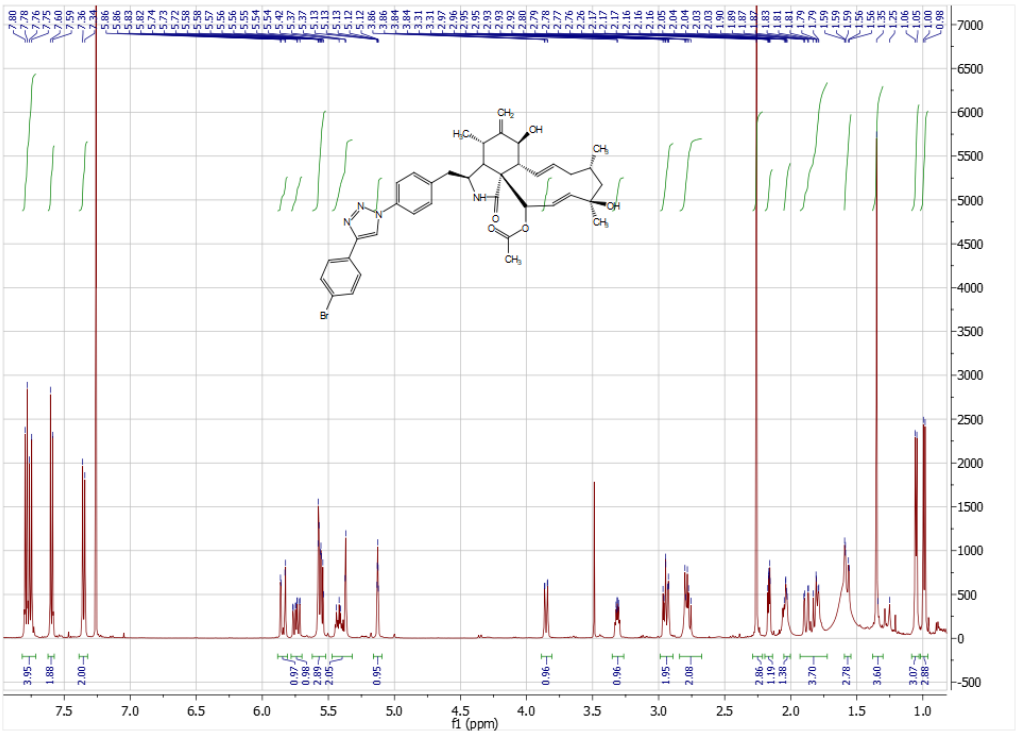


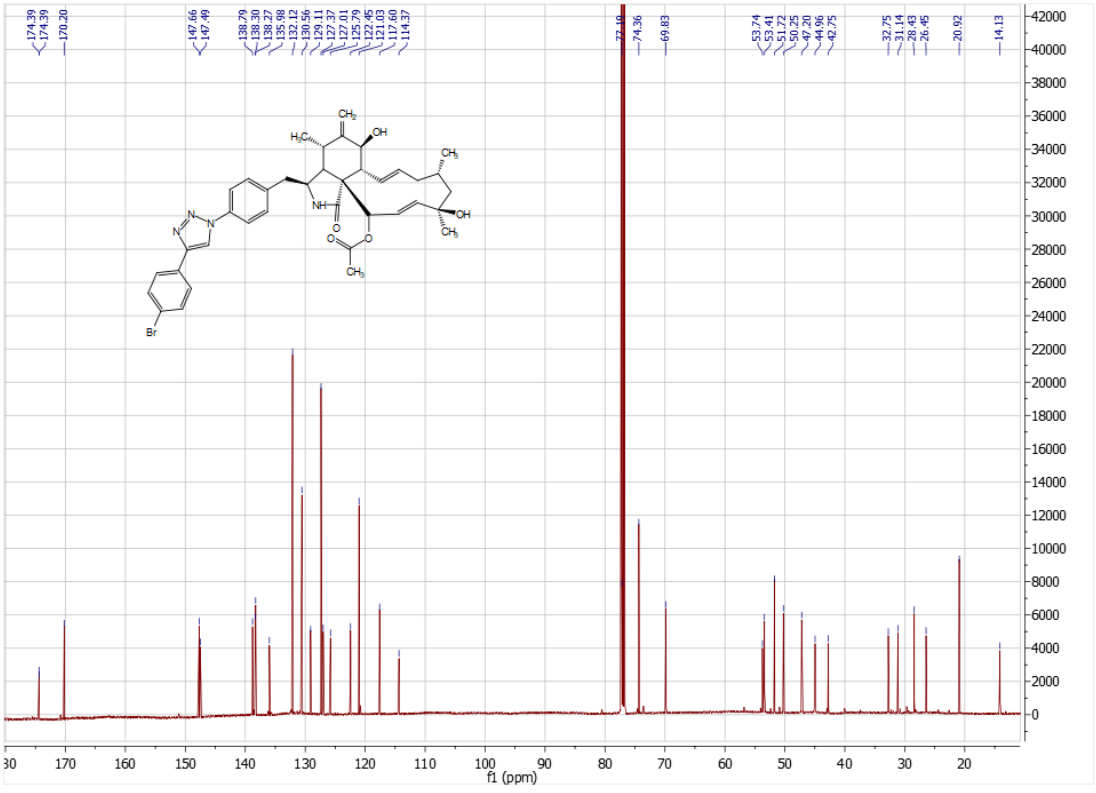
5C

Chemical Formula: $C_{38}H_{43}BrN_4O_5$

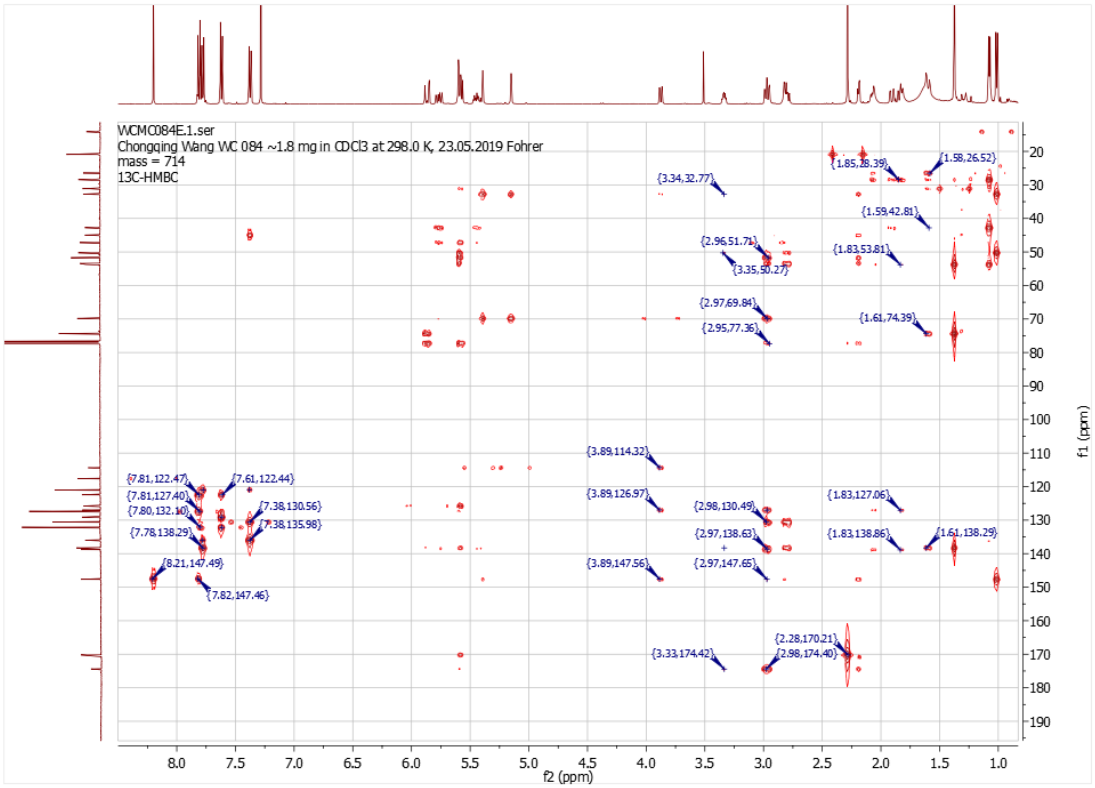
Exact Mass: 714.2417

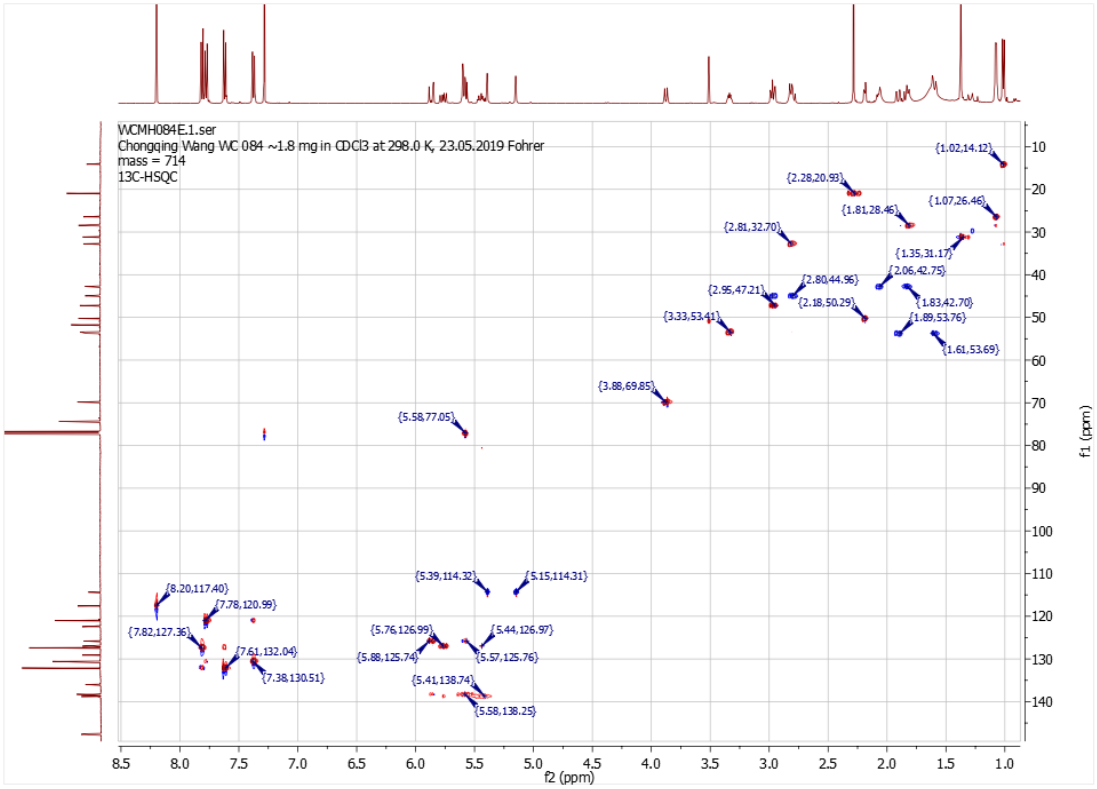
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.4	-	-	-
2	5.56	brs	-	-	-	-	-
3	3.33	m	-	53.4	CH	1‘, 1, 4, 5	4, 5, 10
4	2.18	dd	3.8, 5.0	50.3	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.81	m	-	32.7	CH	1’, 3	4, 11, 12
6	-	-	-	147.6	-	-	-
7	3.88	dd	1.6, 10.7	69.9	CH	6, 12	8, 12
8	2.95	m	-	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.7	-	-	-
10a	2.80	m	-	44.9	CH_2	1’, 3	3, 4, 12
10b	2.97	m	-	-	-	-	-
11	1.02	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.15	brs	-	114.3	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.76	ddd	1.4, 9.7, 15.4	127.0	CH	7, 8, 15	8, 14, 15
14	5.41	ddd	4.8, 10.1, 15.4	138.7	CH	9, 15	13, 15
15a	1.83	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.81	m	-	28.5	CH	13, 14, 17	22
17a	1.61	dd	2.9, 14.4	53.7	CH_2	18, 19	-
17b	1.89	dd	3.0, 14.4	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.58	brs	-	138.3	CH	-	20
20	5.88	dd	3.0, 16.2	125.7	CH	18, 19	19
21	5.58	brs	-	77.1	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.5	26.5	CH_3	15, 16, 17	16
23	1.35	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	136.1	-	-	-
2’ 6’	7.38	d	8.5	130.5	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.78	d	8.5	121.0	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	120.9	-	-	-
24	-	-	-	170.2	-	-	-
25	2.28	s	-	20.9	CH_3	24	-
4‘‘	-	-	-	147.5	-	-	-
5‘‘	8.20	s	-	117.4	CH	4‘‘	-
6‘‘	-	-	-	129.1	-	-	-
7‘‘ 11‘‘	7.82	dd	1.4, 8.4	127.4	2 x CH	4‘‘, 9‘‘	8‘‘, 10‘‘
8‘‘ 10‘‘	7.61	dd	7.4, 8.4	132.0	2 x CH	6‘‘	7‘‘, 11‘‘


f1 (ppm)

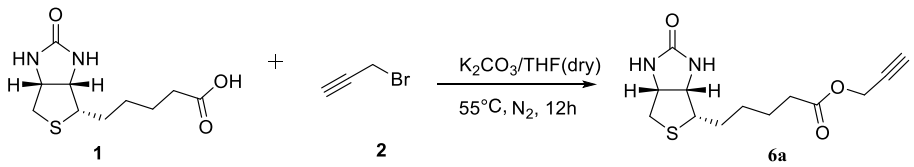

f1 (ppm)


WCED084E.1.ser
Chongqing Wang WC 084 ~1.8 mg in CDCl3 at 298.0 K, 23.05.2019 Fohrer
mass = 714
DQF-COSY
f2 (ppm)
f1 (ppm)


WCMC084E.1.ser
Chongqing Wang WC 084 ~1.8 mg in CDCl3 at 298.0 K, 23.05.2019 Fohrer
mass = 714
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH084E.1.ser
Chongqing Wang WC 084 ~1.8 mg in CDCl3 at 298.0 K, 23.05.2019 Fohrer
mass = 714
13C-HSQC
{1.02,14.12}
{2.28,20.93}
{1.81,28.46}
{1.07,26.46}
{2.81,32.70}
{1.35,31.17}
{2.06,42.75}
{2.80,44.96}
{2.95,47.21}
{2.18,50.29}
{1.83,42.70}
{3.33,53.41}
{1.89,53.76}
{1.61,53.69}
{3.88,69.85}
{5.58,77.05}
{5.39,114.32}
{5.15,114.31}
{8.20,117.40}
{7.78,120.99}
{7.82,127.36}
{5.76,126.99}
{5.44,126.97}
{5.88,125.74}
{5.57,125.76}
{7.61,132.04}
{7.38,130.51}
{5.41,138.74}
{5.58,138.25}
f2 (ppm)
f1 (ppm)

2.05 Biotin propargyl ester 6a



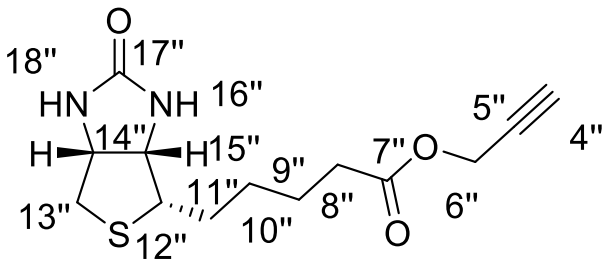
1 2 6a

To a stirred solution of **biotin** (100.0 mg, 0.41 mmol, 1.0 eq, purchased from Sigma-Aldrich) and propargyl bromide (0.1mL, 0.82 mmol, 2.0 eq, purchased from Acros) in dry THF (4 mL) was added K_2CO_3 (50.0 mg, 0.49 mmol, 1.2 eq). The mixture was stirred at 55 °C for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4 mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 69.0 mg of desired product (59.7%, 0.230 mmol) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ (Hz) = 4.70 (d, J = 2.5 Hz, 2H), 4.56 (m, 1H), 4.37 (m, 2H), 3.20 (m, 1H), 2.95 (dd, J = 5.0 and 12.9 Hz, 1H), 2.77 (d, J = 12.9 Hz, 1H), 2.51 (t, J = 2.5 Hz, 2H), 2.41 (t, J = 7.1 Hz, 2H), 1.73 (m, 2H), 1.50 (m, 2H), 1.27 (m, 2H).

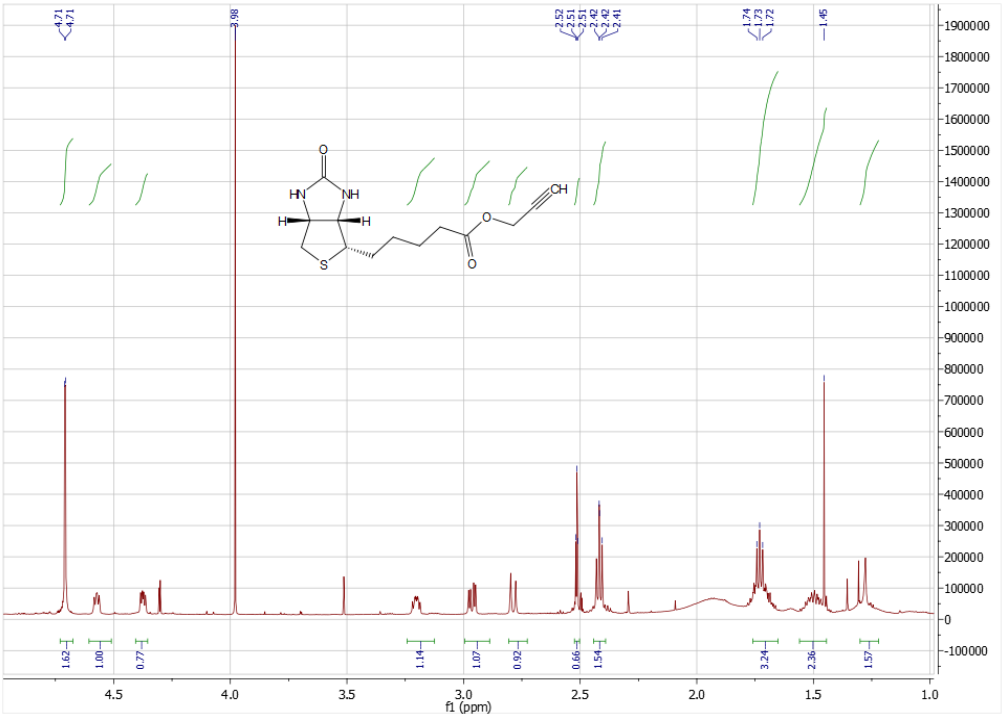
^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.6, 163.1, 79.5, 75.5, 62.0, 60.3, 55.2, 51.9, 40.6, 33.6, 29.8, 28.3, 24.6.

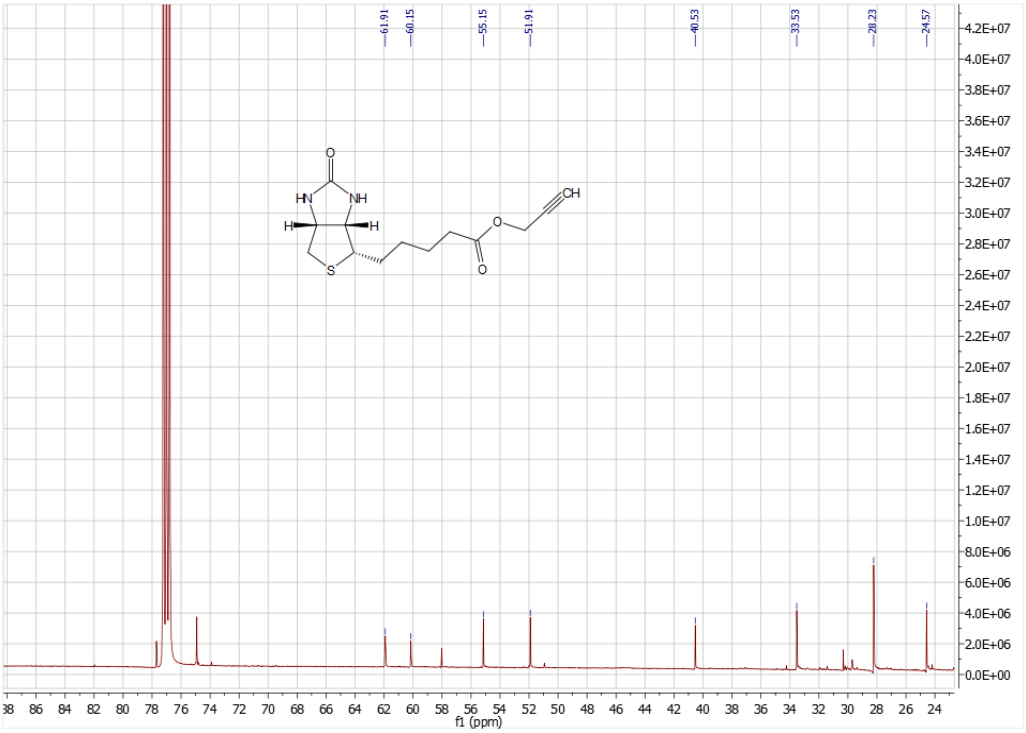
HRMS (*m/z*): calculated for [$C_{13}H_{18}N_2O_3S$ + Na]: 305.0936, found: 305.0936.

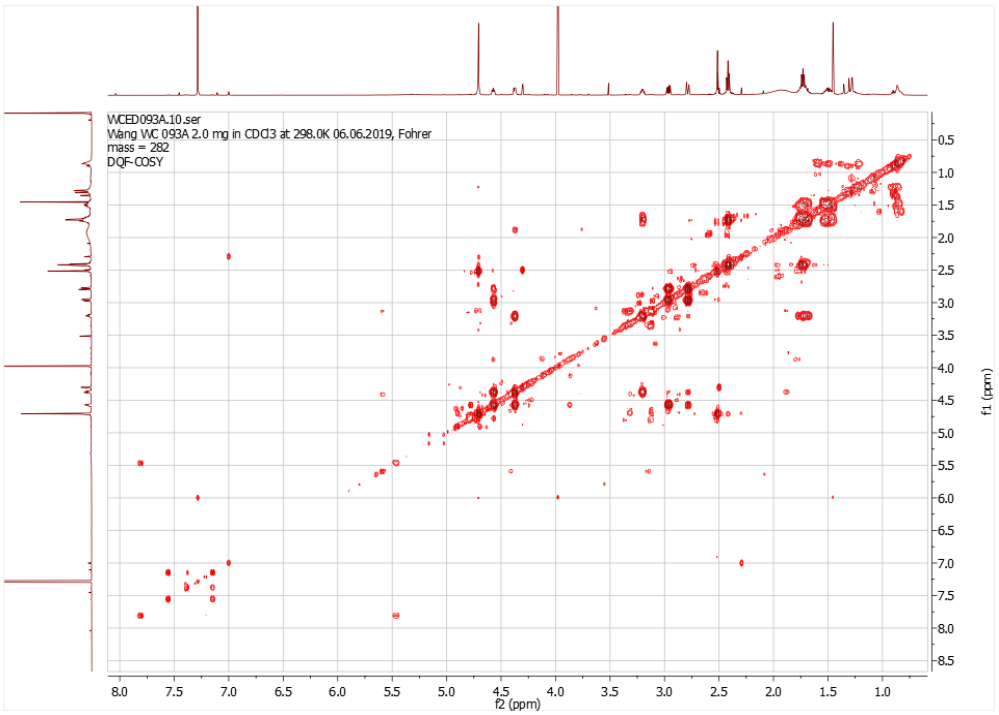


6a

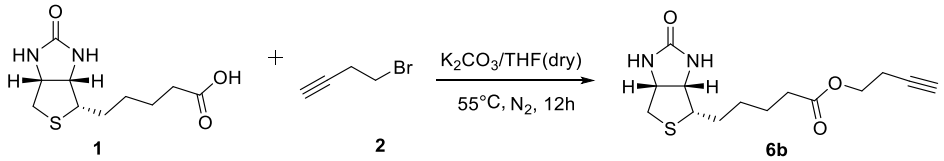
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
4''	2.51	t	2.5	75.5	CH	5'', 6''	5''
5''	-	-	-	79.5	-	-	6''
6''	4.70	d	2.5	51.9	CH_2	4'', 6''	5''
7''	-	-	-	172.6	-	-	-
8''	2.41	t	7.1	33.6	CH_2	7'', 9''	9''
9a''	1.50	m	-	28.3	CH_2	8'', 10''	8''
9b''	1.73	m	-	-	-	-	-
10''	1.27	m	-	29.8	CH_2	-	-
11''	1.73	m	-	24.6	CH_2	9'', 12''	12''
12''	3.20	m	-	55.2	CH	9''	11'', 15''
13a''	2.77	d	12.9	40.6	CH_2	12'', 14''	14''
13b''	2.95	dd	5.0, 12.9	-	-	-	-
14''	4.56	m	-	60.3	CH	12'', 13'', 17''	13'', 15''
15''	4.37	m	-	62.0	CH	12'', 13'', 17''	12'', 14''
17''	-	-	-	163.1	-	-	-




61.91
60.15
55.15
51.91
40.53
33.53
28.23
24.57
HN
NH
H
H
S
O
O
O
CH
f1 (ppm)


WCED093A.10.ser
Wang WC 093A 2.0 mg in CDCl3 at 298.0K 06.06.2019, Fohrer
mass = 282
DQF-COSY
f2 (ppm)
f1 (ppm)

2.06 Biotin but-3-ynyl ester 6b

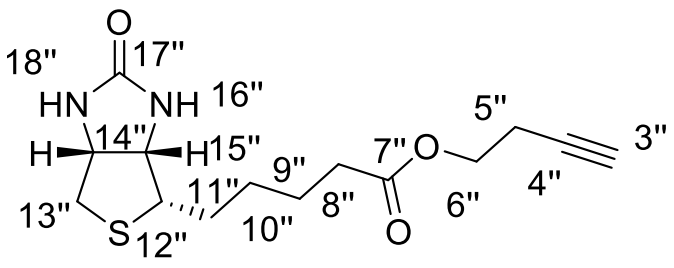


To a stirred solution of **biotin** (100.0 mg, 0.41 mmol, 1.0 eq, purchased from Sigma-Aldrich) and 1-bromo-but-3-yne (80.0 mg, 0.61 mmol, 1.5 eq, purchased from Sigma-Aldrich) in dry THF (4 mL) was added K_2CO_3 (50.0 mg, 0.49 mmol, 1.2 eq). The mixture was stirred at 55 °C for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4 mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 100.2 mg of desired product (82.6%, 0.313 mmol) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ (Hz) = 4.54 (m, 1H), 4.35 (m, 1H), 4.21 (t, J = 6.8 Hz 2H), 3.19 (m, 1H), 2.96 (dd, J = 5.1 and 12.8 Hz, 1H), 2.75 (dd, J = 1.0 and 12.8 Hz, 1H), 2.55 (ddd, J = 2.7, 6.8 and 13.5 Hz 2H), 2.39 (t, J = 7.4 Hz, 2H), 1.73 (m, 2H), 1.71 (m, 1H), 1.46 (m, 1H), 1.28 (m, 2H).

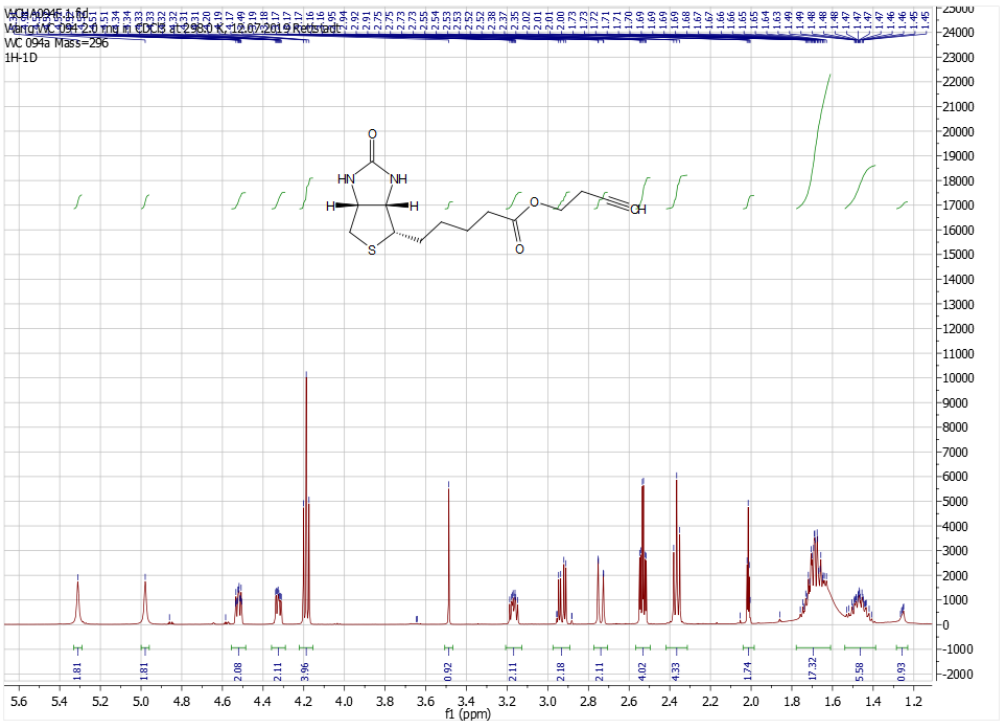
^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.3, 163.1, 80.1, 69.9, 62.0, 61.9, 60.1, 55.2, 40.6, 33.7, 29.7, 28.1, 24.7.

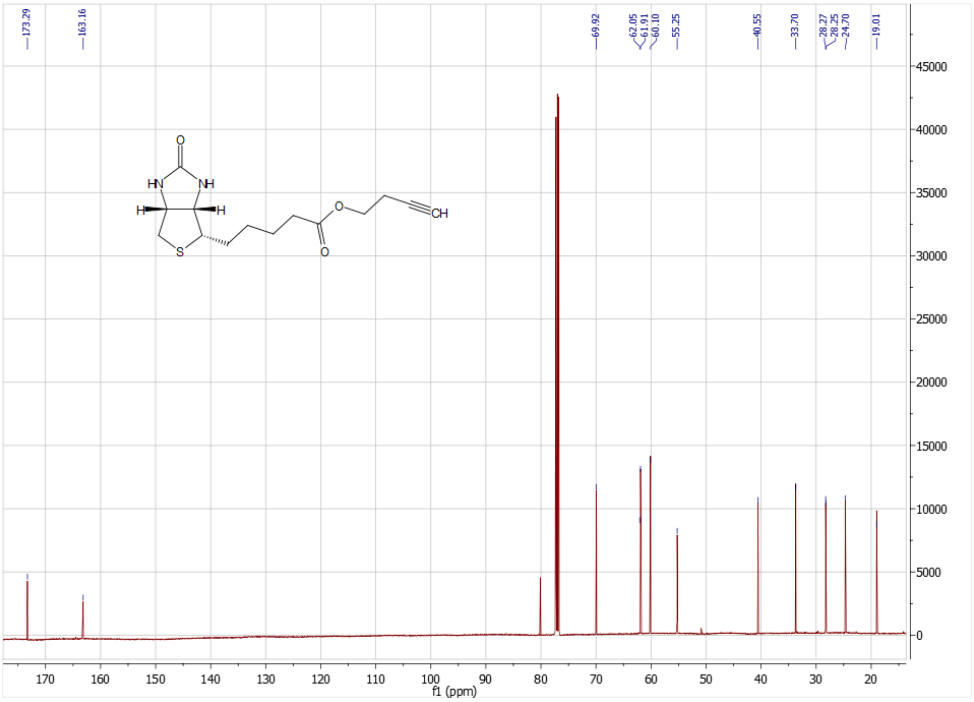
HRMS (*m/z*): calculated for [$C_{14}H_{20}N_2O_3S$ + Na]: 319.1092, found: 319.1087.

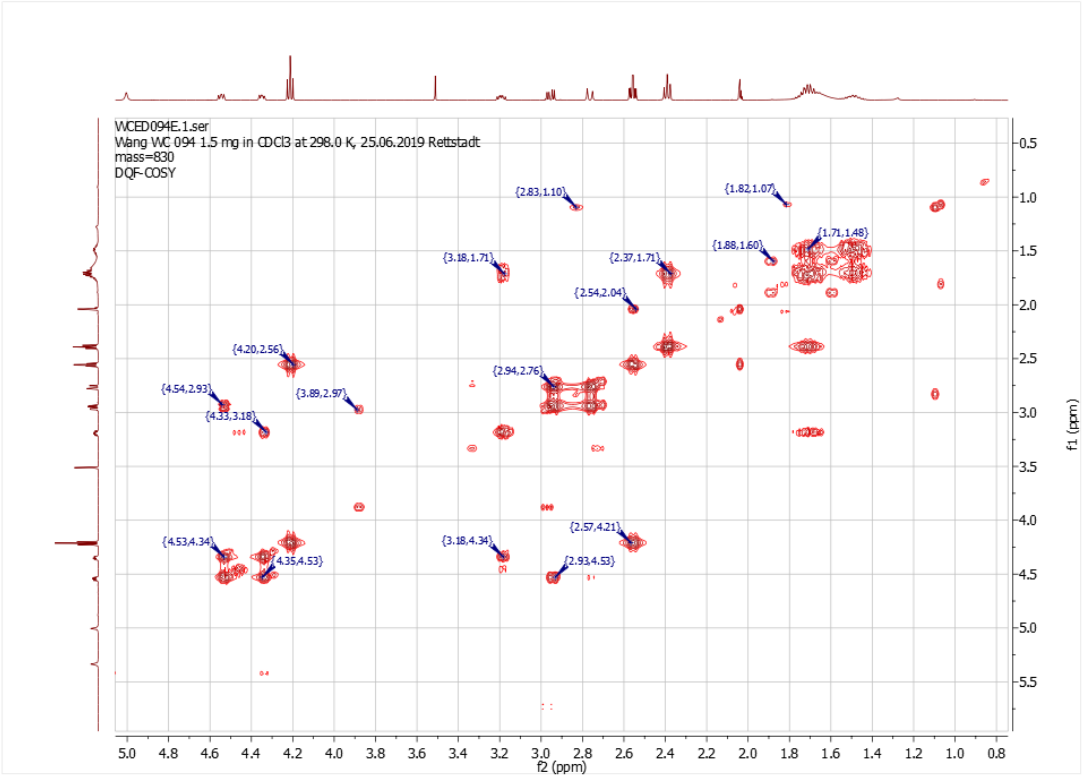


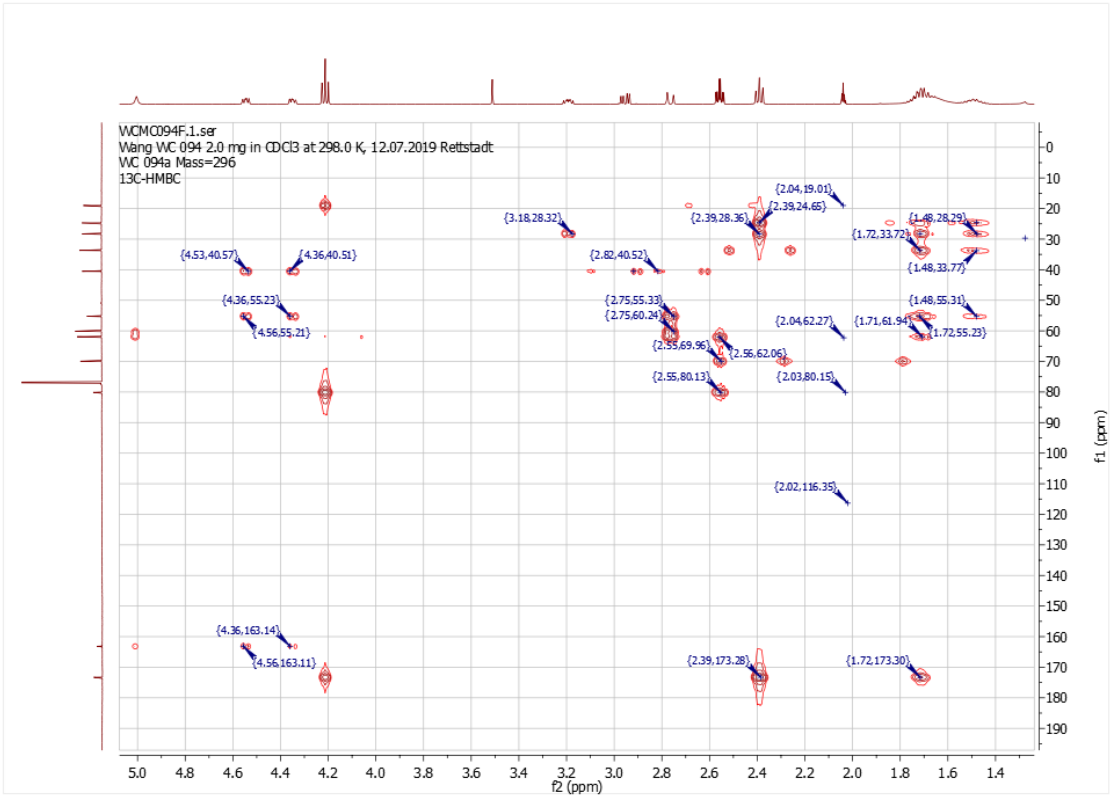
6b

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
3''	2.04	dd	2.6, 5.3	69.9	CH	4'', 5'', 6''	5''
4''	-	-	-	80.1	-	-	-
5''	2.55	ddd	2.7, 6.8, 13.5	19.0	CH_2	3'', 4'', 6''	3'', 6''
6''	4.21	t	6.8	62.0	CH_2	-	5''
7''	-	-	-	173.3	-	-	-
8''	2.39	t	7.4	33.7	CH_2	7'', 9'', 11''	9''
9a''	1.46	m	-	28.1	CH_2	8'', 12''	8''
9b''	1.71	-	-	-	-	-	-
10''	1.28	m	-	29.7	CH_2	-	
11''	1.73	m	-	24.7	CH_2	9'', 12''	12''
12''	3.19	m	-	55.2	CH	9''	11'', 15''
13a''	2.75	dd	1.0, 12.8	40.6	CH_2	12'', 14''	14''
13b''	2.96	dd	5.1, 12.8	-	-	-	-
14''	4.54	m	-	60.1	CH	12'', 13'', 17''	13'', 15''
15''	4.35	m	-	61.9	CH	12'', 13'', 17''	12'', 14''
17''	-	-	-	163.1	-	-	-



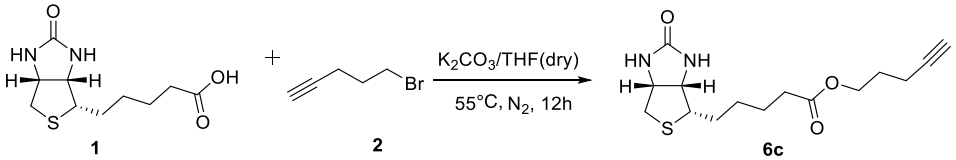

173.29
163.16
69.92
62.05
61.91
60.10
55.25
40.55
33.70
28.27
28.25
24.70
19.01
O
HN
NH
H
H
S
O
O
CH
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)


WCED094E.1.ser
Wang WC 094 1.5 mg in CDCl3 at 298.0 K, 25.06.2019 Rettstadt
mass=830
DQF-COSY
{2.83,1.10}
{1.82,1.07}
{1.71,1.48}
{1.88,1.60}
{3.18,1.71}
{2.37,1.71}
{2.54,2.04}
{4.20,2.56}
{2.94,2.76}
{4.54,2.93}
{3.89,2.97}
{4.33,3.18}
{4.53,4.34}
{4.35,4.53}
{3.18,4.34}
{2.57,4.21}
{2.93,4.53}
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
f1 (ppm)
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8
f2 (ppm)


WCMC094F.1.ser
Wang WC 094 2.0 mg in CDCl3 at 298.0 K, 12.07.2019 Rettstadt
WC 094a Mass=296
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH094F.1.ser
Wang WC 094 2.0 mg in CDCl3 at 298.0 K, 12.07.2019 Rettstadt
WC 094a Mass=296
13C-HSQC
f2 (ppm)
f1 (ppm)

2.07 Biotin pent-4-ynyl ester 6c

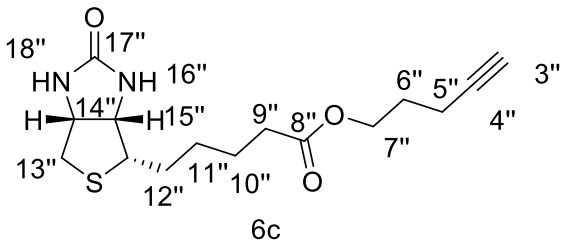


To a stirred solution of **biotin** (100.0 mg, 0.41 mmol, 1.0 eq, purchased from Sigma-Aldrich) and 1-bromopent-4-yne (90.0 mg, 0.61 mmol, 1.5 eq, purchased from Alfa Aesar) in dry THF (4 mL) was added K_2CO_3 (50.0 mg, 0.49 mmol, 1.2 eq). The mixture was stirred at 55 °C for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4 mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 96.0 mg of desired product (75.6%, 0.288 mmol) as a white solid.

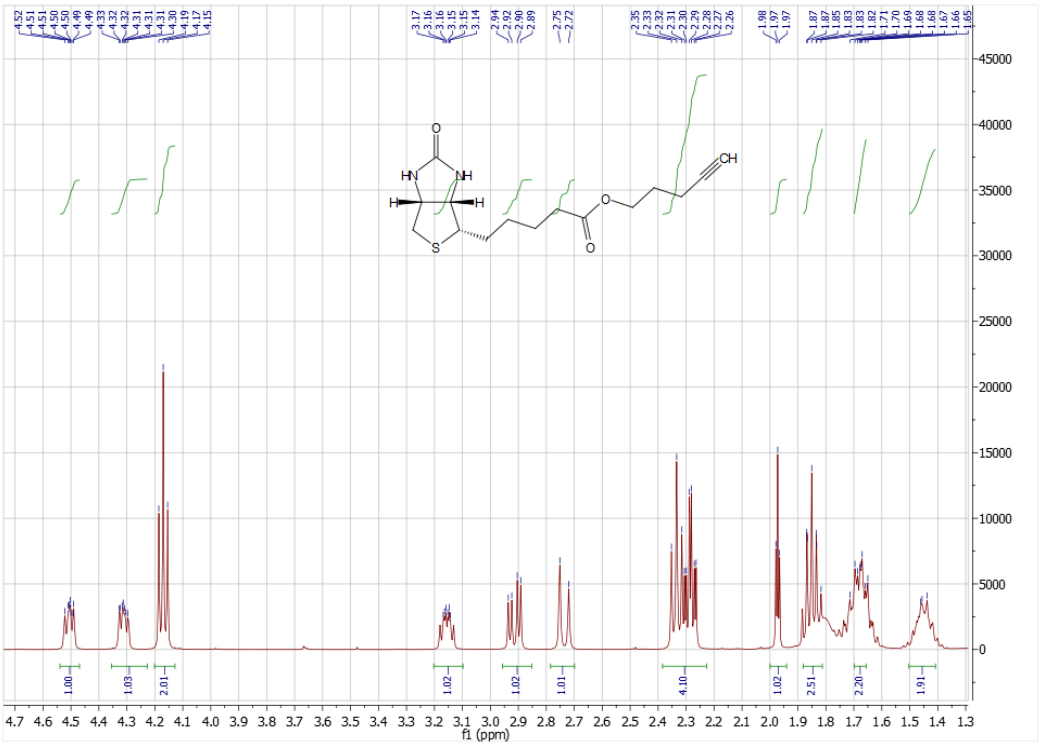
^{1}H NMR (500 MHz, $CDCl_3$): δ (Hz) = 4.53 (m, 1H), 4.33 (m, 1H), 4.19 (t, J = 6.3 Hz 2H), 2.92 (dd, J = 5.0 and 12.8 Hz, 1H), 2.78 (d, J = 12.8 Hz, 1H), 2.29 (m, 2H), 1.99 (t, J = 2.6 Hz, 1H), 1.87 (m, 2H), 1.73 (m, 2H), 1.71 (m, 1H), 1.48 (m, 1H), 1.28 (m, 2H).

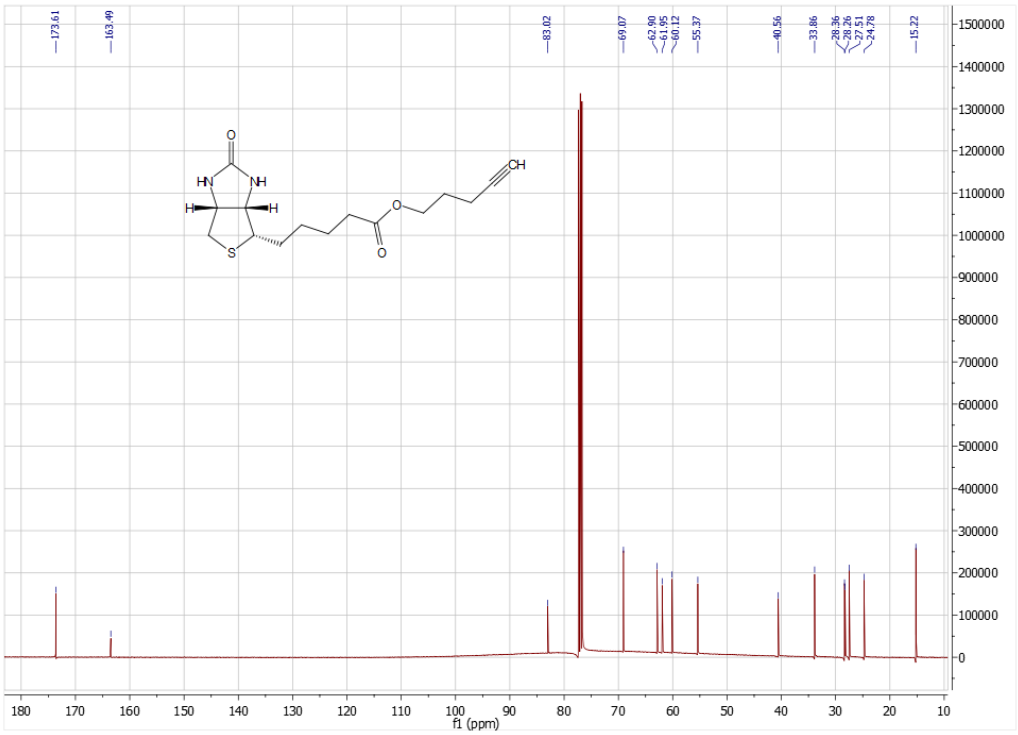
^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.6, 163.6, 83.1, 69.5, 62.9, 62.0, 60.1, 40.3, 29.7, 28.6, 27.6, 24.8, 24.7, 15.4.

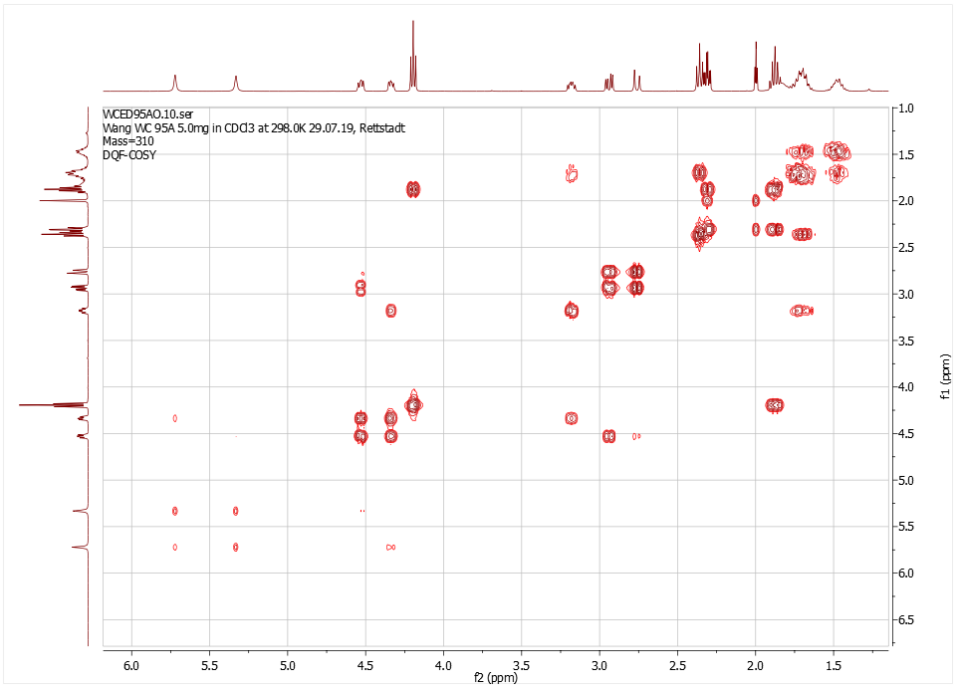
HRMS (*m/z*): calculated for [$C_{15}H_{22}N_2O_3S$ + Na]: 333.1249, found: 333.1246.

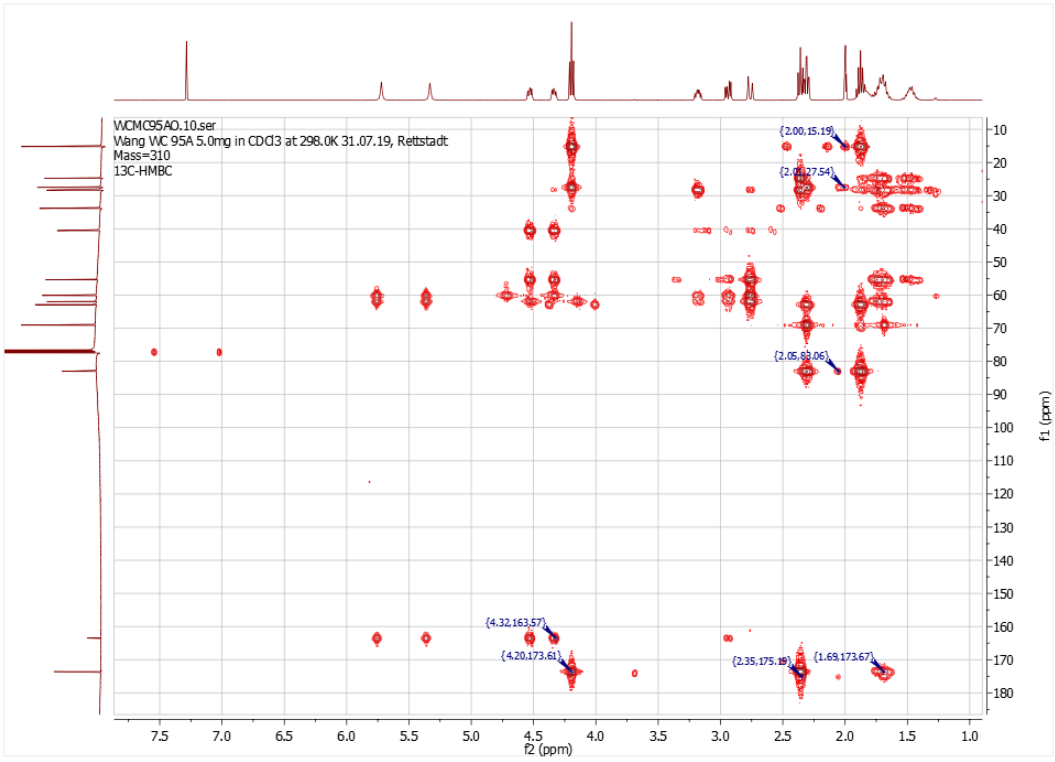


Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
3''	1.99	t	2.6	69.5	CH	4'', 5'', 6''	5''
4''	-	-	-	83.1	-	-	-
5''	2.29	m	-	15.4	CH_2	3'', 4'', 6''	3'', 6''
6''	1.87	m	-	27.6	CH_2	-	5''
7''	4.19	t	6.3	62.9	-	-	-
8''	-	-	-	173.6	CH_2	7'', 9'', 11''	9''
9a''	1.48	m	-	28.6	CH_2	8'', 12''	8''
9b''	1.73	m	-	-	-	-	-
10''	1.28	m	-	29.7	CH_2	-	-
11''	1.73	m	-	24.7	CH_2	9'', 12''	12''
12''	1.71	m	-	24.8	CH	9''	11'', 15''
13a''	2.78	d	12.8	40.3	CH_2	12'', 14''	14''
13b''	2.92	dd	5.0, 12.8	-	-	-	-
14''	4.53	m	-	60.1	CH	12'', 13'', 17''	13'', 15''
15''	4.33	m	-	62.0	CH	12'', 13'', 17''	12'', 14''
17''	-	-	-	163.6	-	-	-


f1 (ppm)

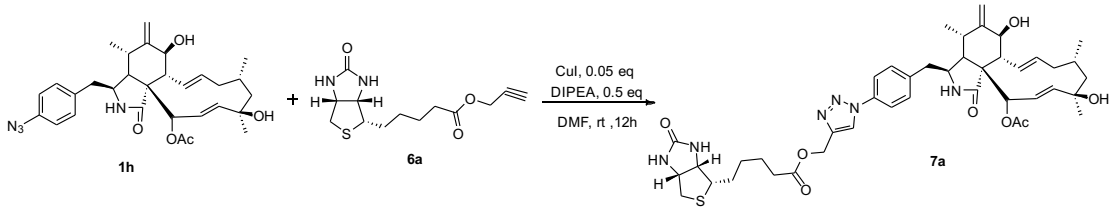

f1 (ppm)


WCED95AO.10.ser
Wang WC 95A 5.0mg in CDCl3 at 298.0K 29.07.19, Rettstadt
Mass=310
DQF-COSY
f2 (ppm)
f1 (ppm)

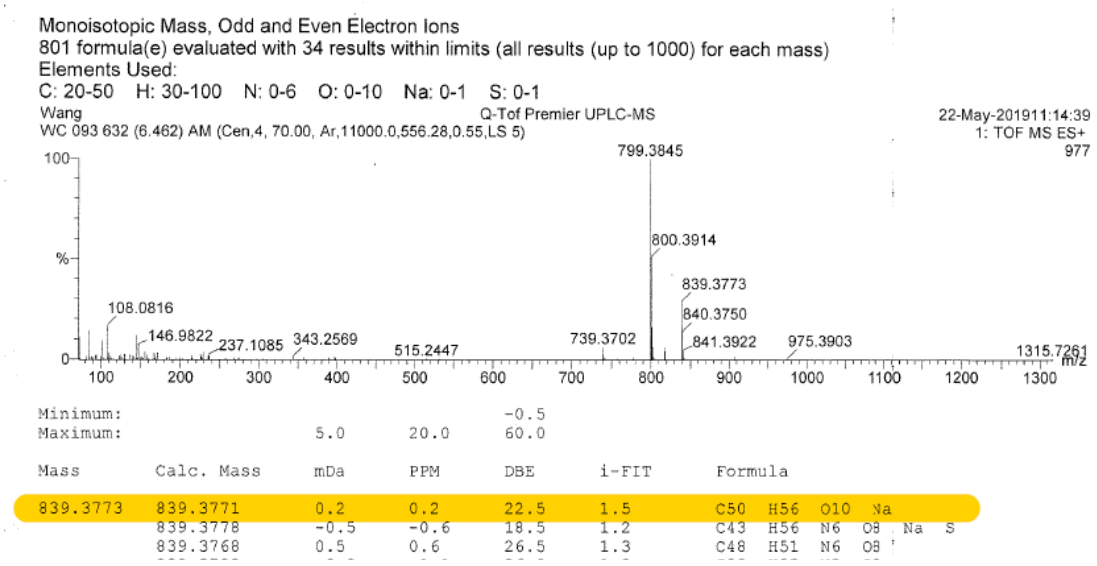

WCMC95AO.10.ser
Wang WC 95A 5.0mg in CDCl3 at 298.0K 31.07.19, Rettstadt
Mass=310
13C-HMBC
{2.00,15.19}
{2.00,27.54}
{2.05,83.06}
{4.32,163.57}
{4.20,173.61}
{2.35,175.19}
{1.69,173.67}
f2 (ppm)
f1 (ppm)


WCMH95AO.10.ser
Wang WC 95A 5.0mg in CDCl3 at 298.0K 29.07.19, Rettstadt
Mass=310
13C-HSQC
{2.29,15.37}
{1.71,24.80}
{1.48,28.57}
{2.36,34.06}
{2.92,40.45}
{2.78,40.34}
{3.18,55.64}
{4.53,60.11}
{1.99,69.47}
f2 (ppm)
f1 (ppm)

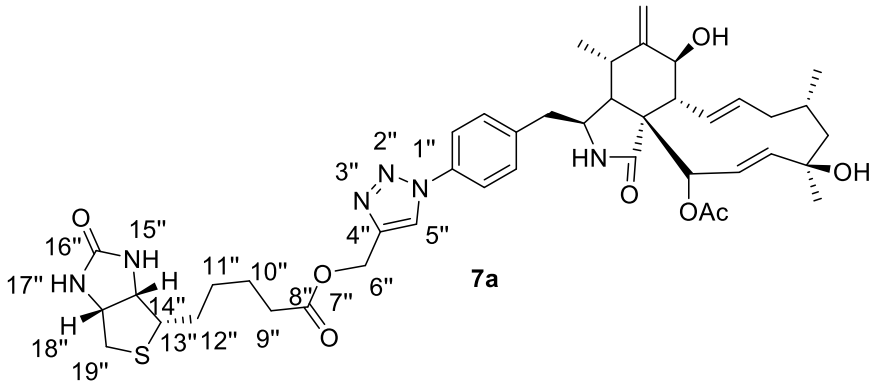
2.08 4'-Biotin-triazole-linked Cytochalasin H 7a



To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and **6a** (10.7 mg, 0.038 mmol, 2.0 eq) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 8.6 mg of desired product (56.4%, 0.010 mmol) as a light yellow solid.



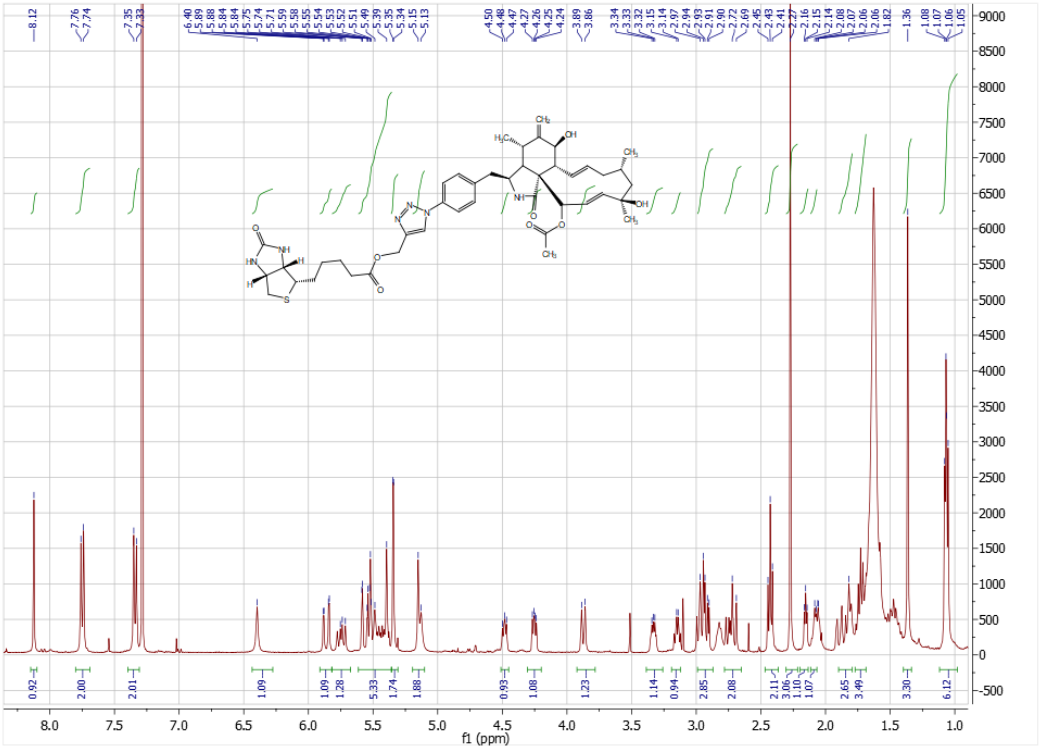
HRMS (*m/z*): calculated for [$C_{43}H_{56}N_6O_8S$ + Na]: 839.3778, found: 839.3773.

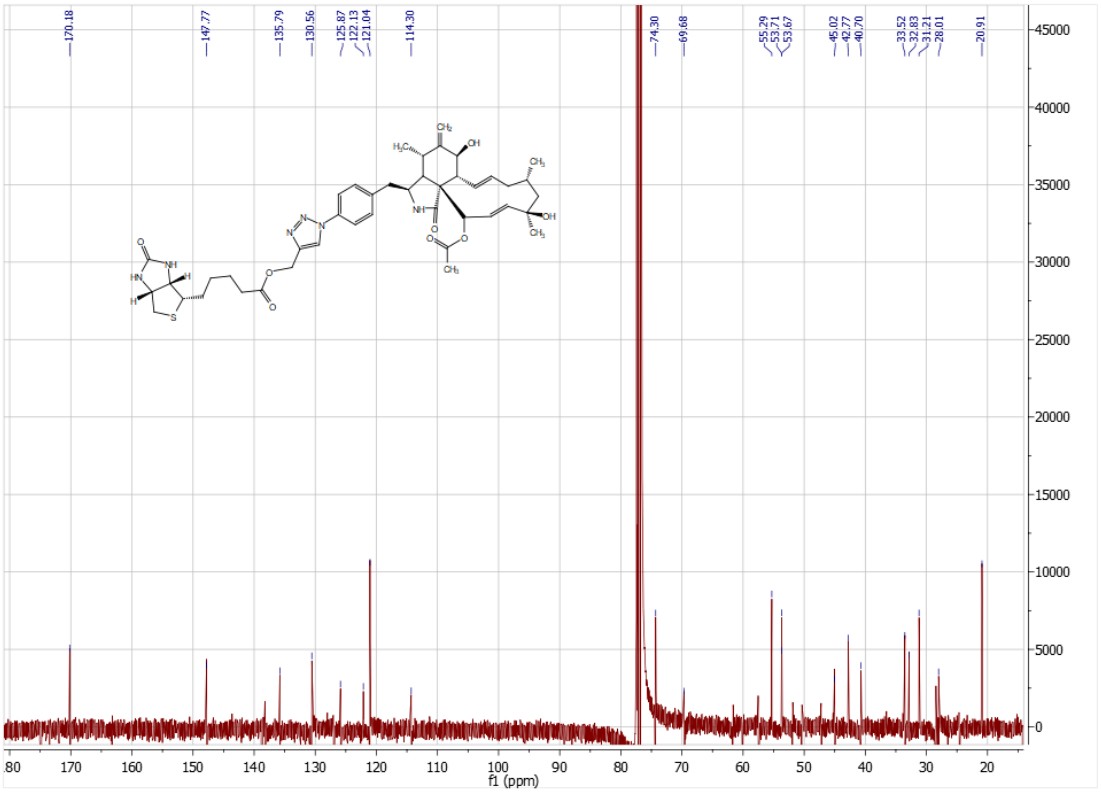


7a

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.8	-	-	-
3	3.33	m	-	53.7	CH	1‘,4, 5	4, 5, 10
4	2.15	t	4.3	50.5	CH	3, 5	3, 5, 10
5	2.82	m	-	32.9	CH	1’, 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.86	d	10.8	69.6	CH	5, 6, 8, 12, 13	8, 12
8	2.97	m	-	47.2	CH	1, 7, 9, 13, 21	7, 13
9	-	-	-	51.9	-	-	-
10a	2.74	m	-	45.1	CH_2	1’, 3	3, 4, 12
10b	2.94	dd	4.8, 13.5	-	-	-	-
11	1.07	d	6.7	14.4	CH_3	4, 5, 6	5
12a	5.15	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.74	ddd	1.4, 9.6, 15.5	127.0	CH	7, 8, 15	8, 14, 15
14	5.42	ddd	4.8, 10.3, 15.5	138.8	CH	9, 15	13, 15
15a	1.82	m	-	42.8	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.82	m	-	28.5	CH	13, 14, 17	22
17a	1.61	m	-	53.8	CH_2	18, 19	-
17b	1.91	m	-	-	-	-	-
18	-	-	-	74.3	-	-	-
19	5.58	brs	-	138.2	CH	-	20
20	5.88	dd	2.7, 16.5	125.9	CH	18, 19	19
21	5.52	brs	-	77.4	CH	8, 9, 19, 20, 23	-
22	1.06	d	6.5	26.5	CH_3	15, 16, 17	16
23	1.35	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	130.6	-	-	-
2’ 6’	7.33	d	8.2	130.6	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.75	d	8.2	120.9	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	138.2	-	-	-
24	-	-	-	170.2	-	-	-
25	2.27	s	-	21.0	CH_3	21, 24	-
4‘‘	-	-	-	145.1	-	-	-
5‘‘	8.16	s	-	119.6	CH	4‘‘	-
6‘‘	5.34	s	-	25.5	CH_2	4‘‘, 5‘‘, 7‘‘	7‘‘
8‘‘	-	-	-	173.5	-	-	-
9‘‘	2.43	t	6.9	33.6	CH_2	8‘‘, 10‘‘	10‘‘, 12‘‘
10‘‘	1.67	m	-	28.3	CH_2	8‘‘, 9‘‘, 11‘‘, 12‘‘, 13‘‘	9‘‘, 11‘‘

11‘‘	1.48	m	-	28.1	CH_2	9‘‘, 10‘‘,12‘‘, 13‘‘	10‘‘
12‘‘	1.73	m	-	24.6	CH_2	11‘‘, 13‘‘, 14‘‘,	9‘‘
13‘‘	3.15	m	-	55.3	CH	11‘‘, 14‘‘	14‘‘
14‘‘	4.25	dd	4.4, 7.8	61.6	CH	16‘‘	13‘‘, 18‘‘
16‘‘	-	-	-	163.3	-	-	-
18‘‘	4.48	m	-	60.1	CH	13‘‘, 16‘‘	14‘‘, 19‘‘
19a‘‘	2.69	m	-	40.6	CH_2	13‘‘, 18‘‘	18‘‘
19b‘‘	2.94	m	-	-	-	-	-




170.18
147.77
135.79
130.56
125.87
122.13
121.04
114.30
74.30
69.68
55.29
53.71
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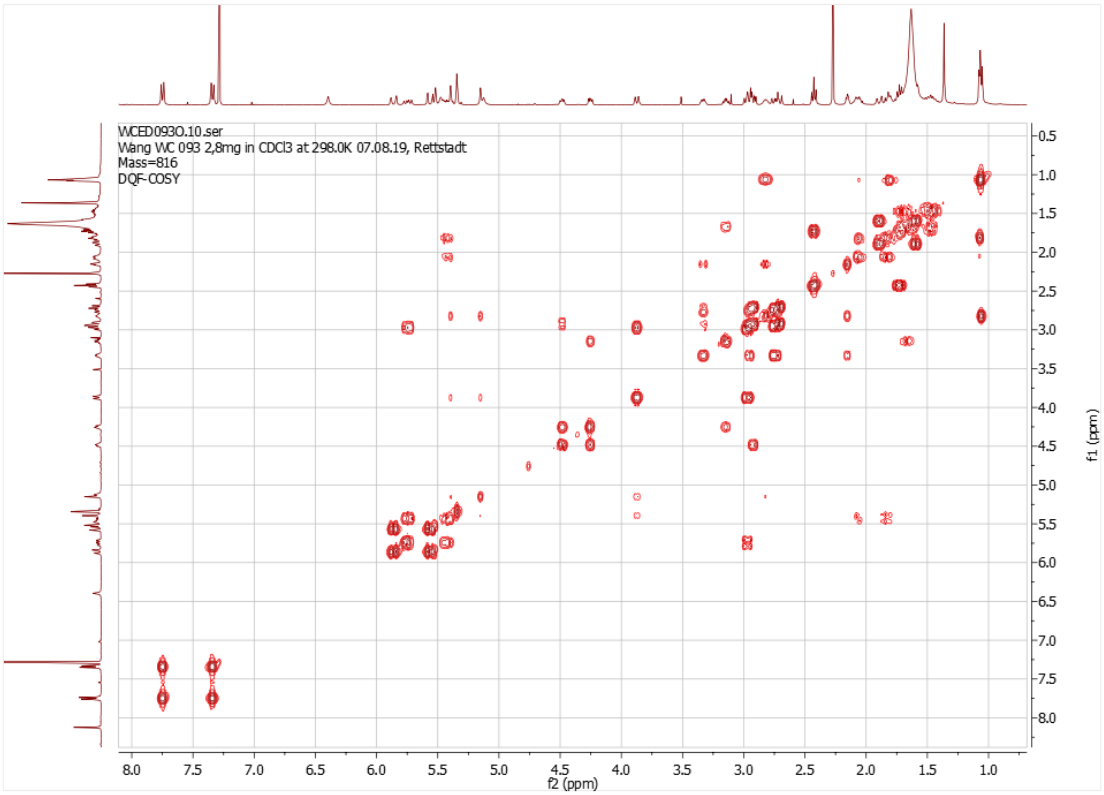
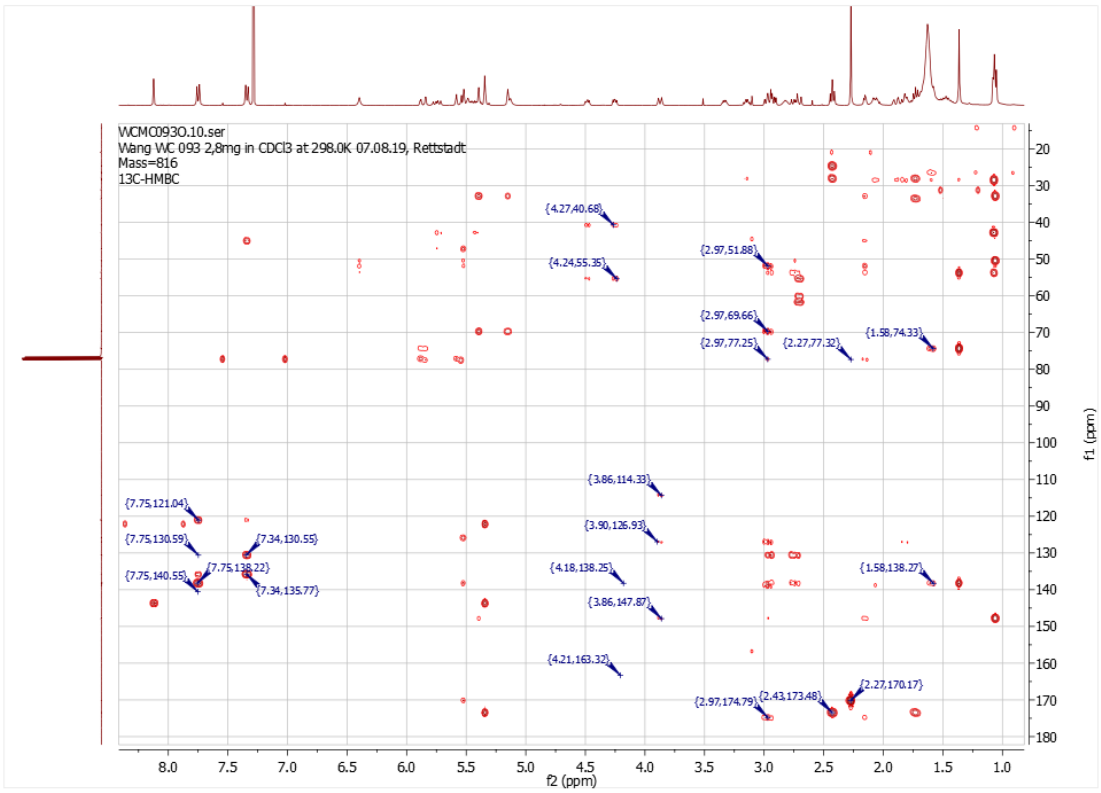
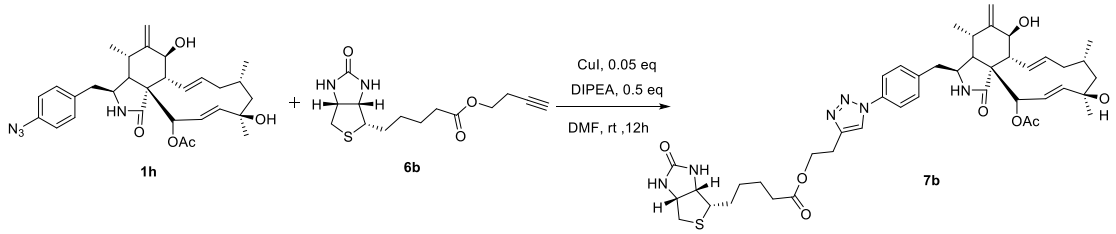
40.70
33.52
32.83
31.21
28.01
20.91
f1 (ppm)


WCED093O.10.ser
Wang WC 093 2,8mg in CDCl3 at 298.0K 07.08.19, Rettstadt
Mass=816
DQF-COSY
f2 (ppm)
f1 (ppm)

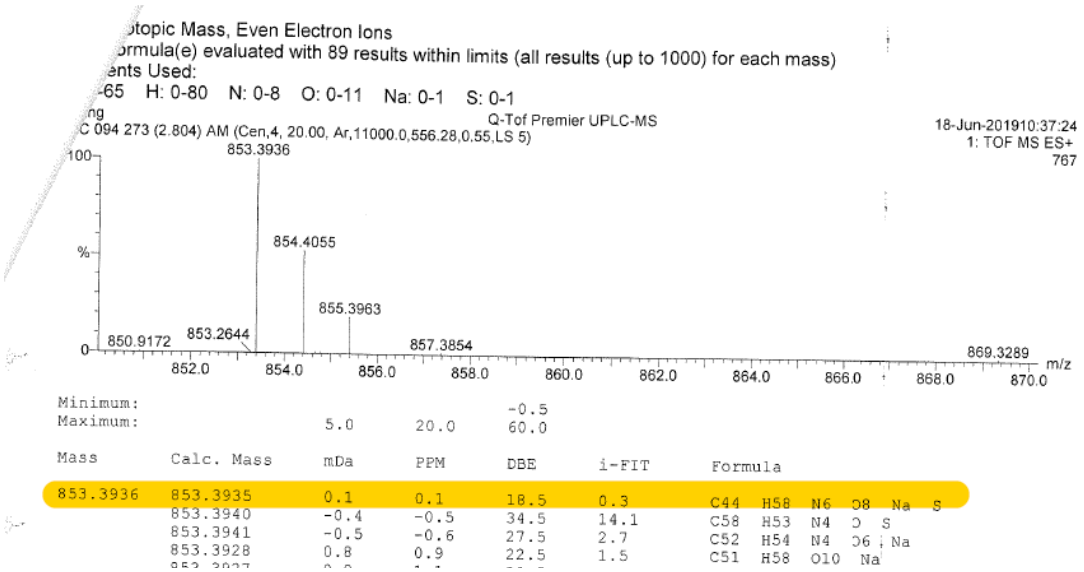

WCMC093O.10.ser
Wang WC 093 2,8mg in CDCl3 at 298.0K 07.08.19, Rettstadt
Mass=816
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH093O.10.ser
Wang WC 093 2,8mg in CDCl3 at 298.0K 07.08.19, Rettstadt
Mass=816
13C-HSQC
f2 (ppm)
f1 (ppm)

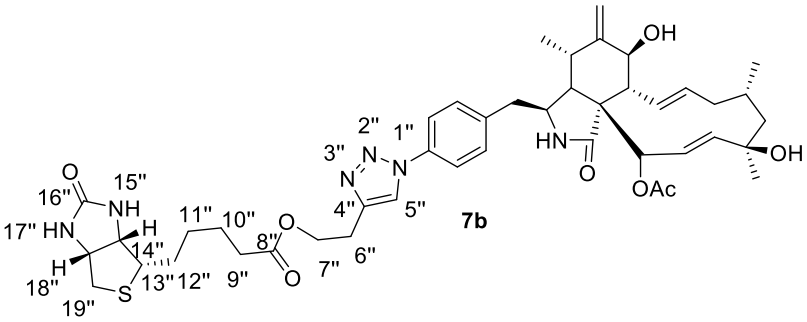
2.09 4'-Biotin-triazole-linked Cytochalasin H 7b



To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and **6b** (11.2 mg, 0.038 mmol, 2.0 eq) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 9.6 mg of desired product (61.9%, 0.011 mmol) as a white solid.



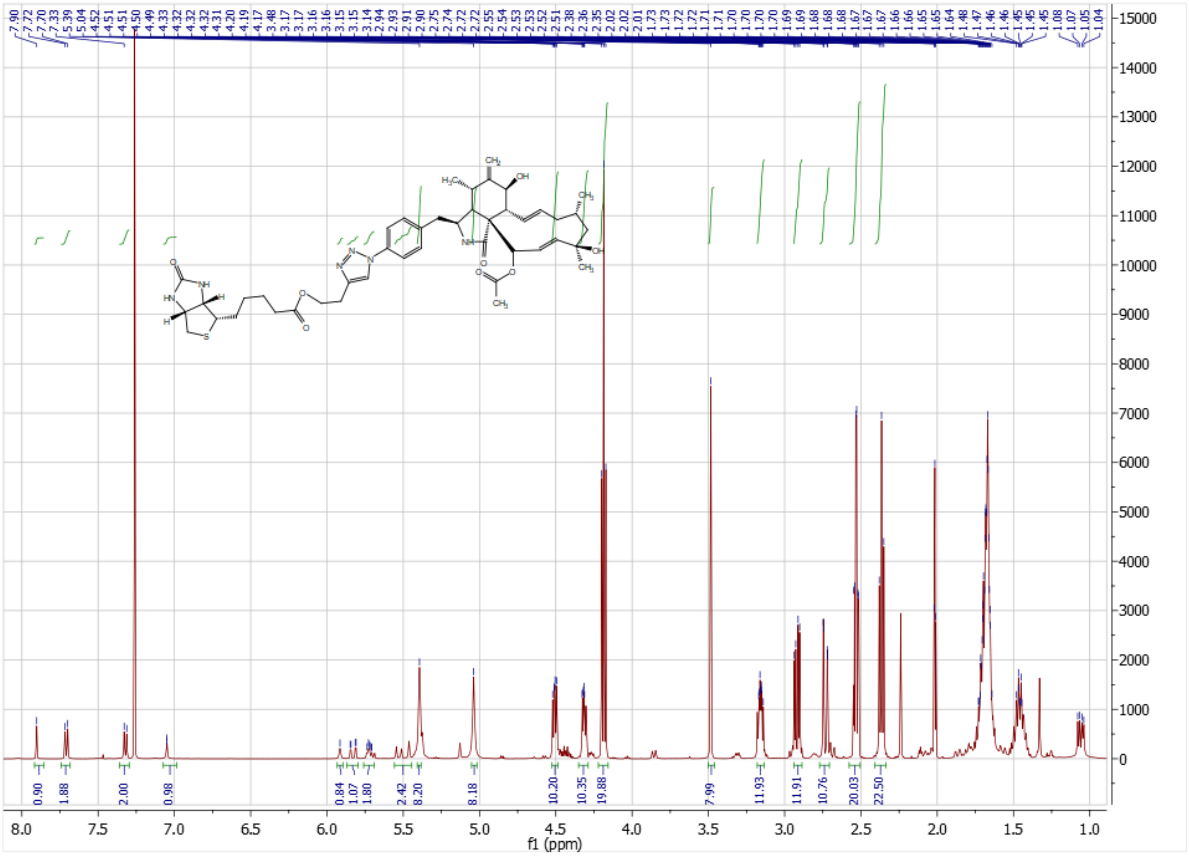
HRMS (*m/z*): calculated for [$C_{44}H_{58}N_6O_8S$ + Na]: 853.3935, found: 853.3936.

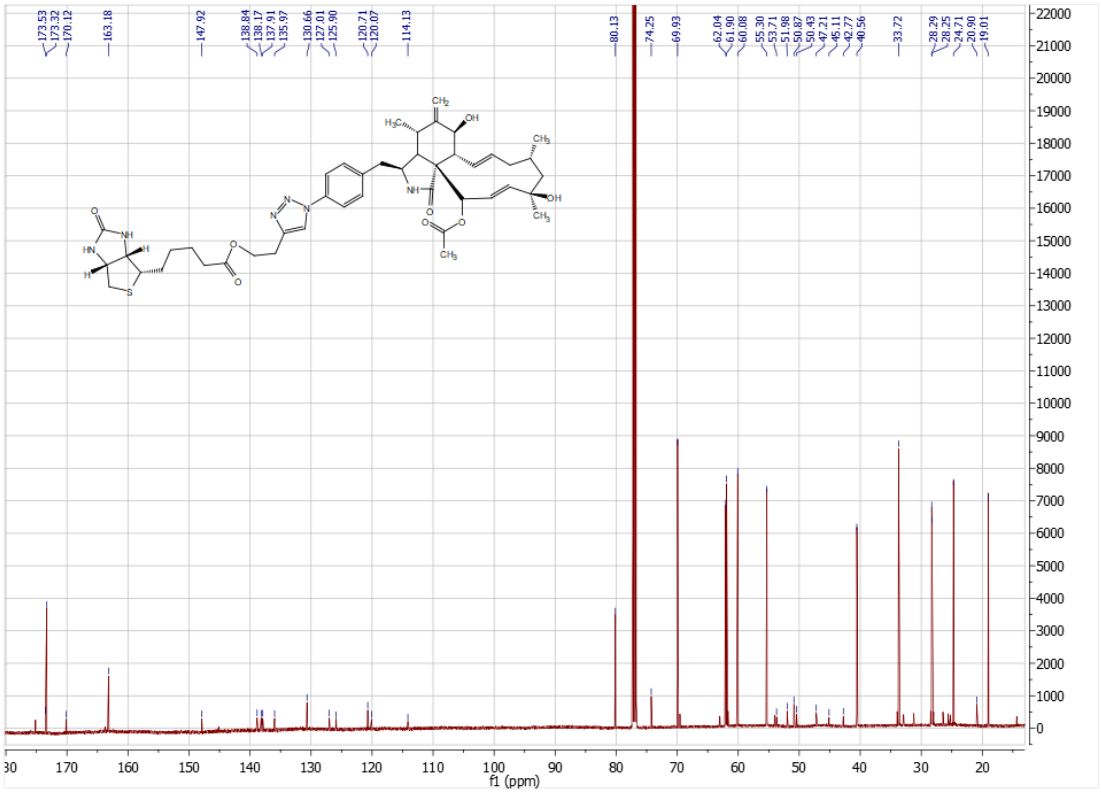


7b

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	175.2	-	-	-
3	3.33	m	-	53.9	CH	1‘,4, 5	4, 5, 10
4	2.13	m	-	50.4	CH	3, 5	3, 5, 10
5	2.83	m	-	32.9	CH	1’, 3	4, 11, 12
6	-	-	-	147.7	-	-	-
7	3.87	dd	1.7, 11.0	69.5	CH	5, 6, 8, 12, 13	8, 12
8	2.97	dd	5.0, 11.0	47.1	CH	1, 7, 9, 13, 21	7, 13
9	-	-	-	52.4	-	-	-
10a	2.73	dd	1.1, 12.9	45.1	CH_2	1’, 3	3, 4, 12
10b	2.93	dd	5.0, 12.9	-	-	-	-
11	1.10	d	6.7	14.3	CH_3	4, 5, 6	5
12a	5.15	brs	-	114.1	CH_2	5, 6, 7	5, 7, 11
12b	5.40	brs	-	-	-	-	-
13	5.74	m	-	126.8	CH	7, 8, 15	8, 14, 15
14	5.44	m	-	138.8	CH	9, 15	13, 15
15a	1.80	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.80	m	-	28.6	CH	13, 14, 17	22
17a	1.61	m	-	53.6	CH_2	18, 19	-
17b	1.88	m	-	-	-	-	-
18	-	-	-	74.2	-	-	-
19	5.55	brs	-	138.2	CH	-	20
20	5.87	dd	2.9, 16.4	125.7	CH	18, 19	19
21	5.49	brs	-	77.3	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.5	26.5	CH_3	15, 16, 17	16
23	1.35	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	130.7	-	-	-
2’ 6’	7.34	d	8.2	130.6	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.74	d	8.2	120.6	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	137.9	-	-	-
24	-	-	-	170.1	-	-	-
25	2.26	s	-	20.9	CH_3	21, 24	-
4‘‘	-	-	-	145.1	-	-	-
5‘‘	7.93	s	-	119.6	CH	4‘‘	-
6‘‘	3.18	m	-	25.6	CH_2	4‘‘, 5‘‘, 7‘‘	7‘‘
7‘‘	4.21	m	-	61.7	CH_2	6‘‘, 8‘‘	6‘‘
8‘‘	-	-	-	173.4	-	-	-
9‘‘	2.39	m	-	33.7	CH_2	8‘‘, 10‘‘	10‘‘, 12‘‘
10‘‘	1.71	m	-	28.2	CH_2	8‘‘, 9‘‘, 11‘‘, 12‘‘, 13‘‘	9‘‘, 11‘‘
11‘‘	1.46	m	-	28.1	CH_2	9‘‘, 10‘‘,12‘‘, 13‘‘	10‘‘
12‘‘	1.69	m	-	24.6	CH_2	11‘‘, 13‘‘, 14‘‘,	9‘‘

13‘‘	3.16	m	-	55.2	CH	11‘‘, 14‘‘	14‘‘
14‘‘	4.34	m	-	61.8	CH	16‘‘	13‘‘, 18‘‘
16‘‘	-	-	-	163.2	-	-	-
18‘‘	4.53	m	-	60.0	CH	13‘‘, 16‘‘	14‘‘, 19‘‘
19a‘‘	2.72	m	-	40.5	CH_2	13‘‘, 18‘‘	18‘‘
19b‘‘	2.95	m	-	-	-	-	-




173.53
173.32
170.12
163.18
147.92
138.84
138.17
137.91
135.97
130.66
127.01
125.90
120.71
120.07
114.13
80.13
74.25
69.93
62.04
61.90
60.08
55.30
53.71
51.98
50.87
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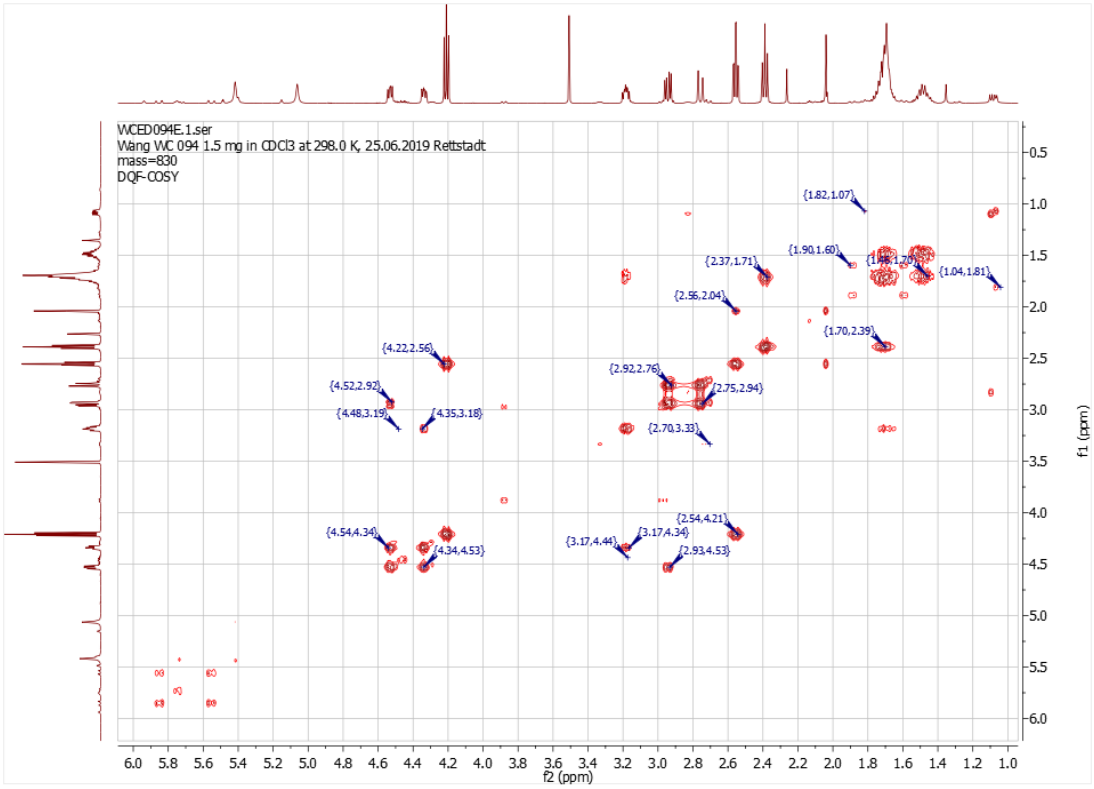
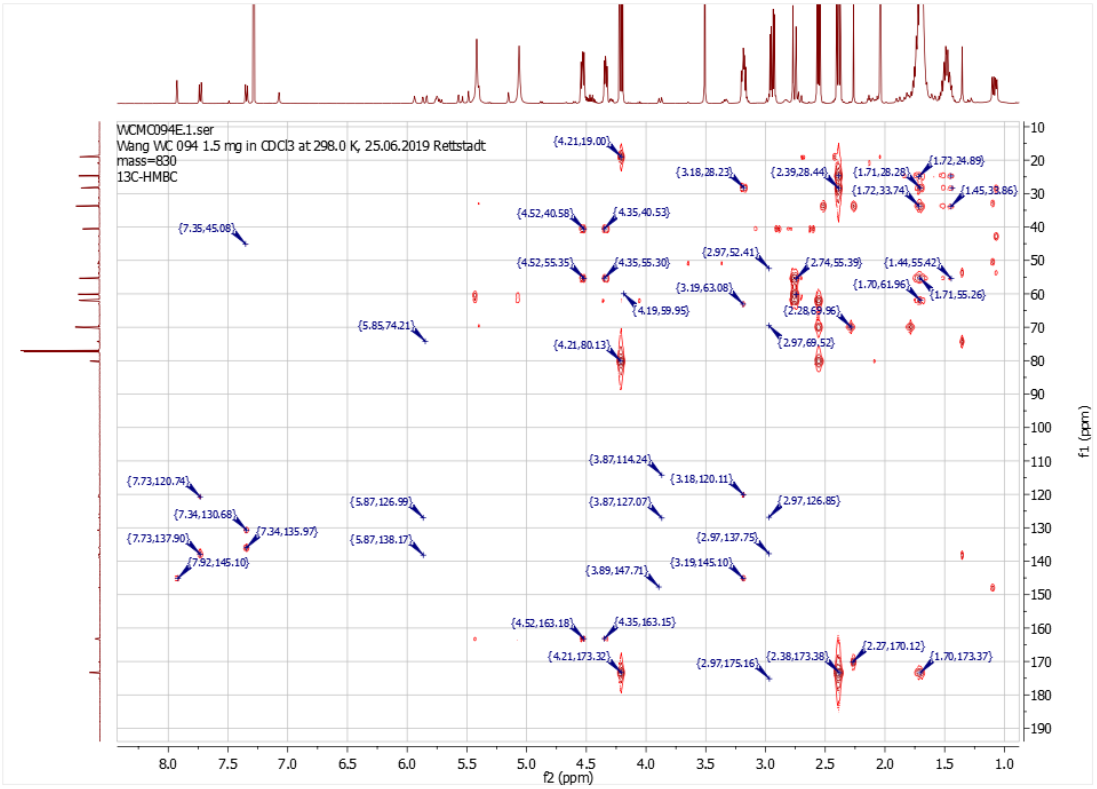
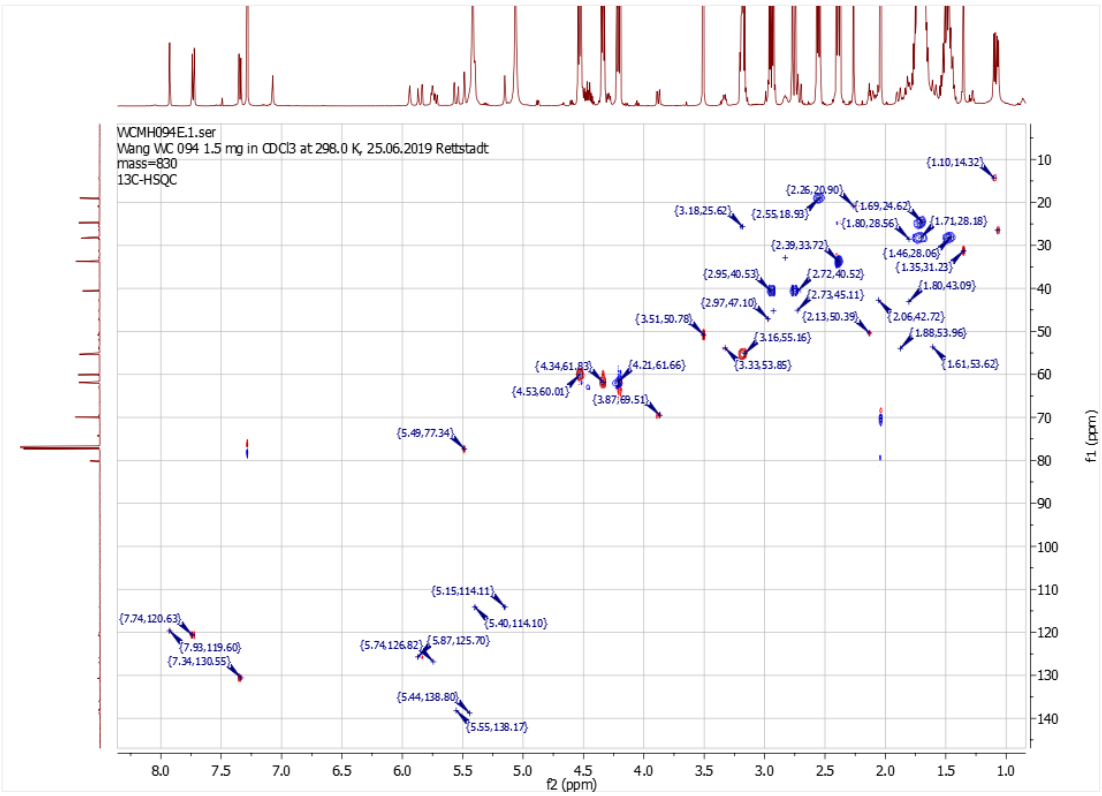
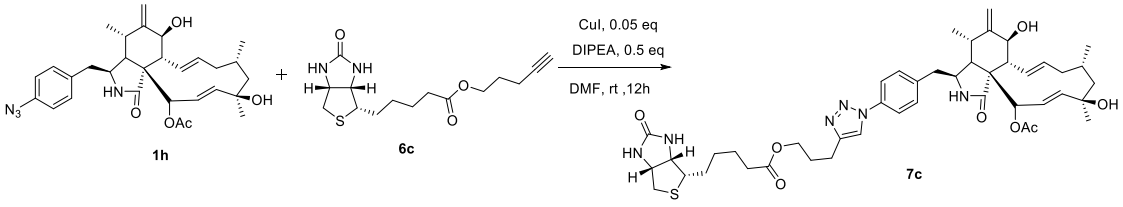
28.29
28.25
24.71
20.90
19.01
f1 (ppm)


WCED094E.1.ser
Wang WC 094 1.5 mg in CDCl3 at 298.0 K, 25.06.2019 Rettstadt
mass=830
DQF-COSY
{1.82,1.07}
{1.90,1.60}
{1.04,1.81}
{2.37,1.71}
{2.56,2.04}
{1.70,2.39}
{4.22,2.56}
{2.92,2.76}
{4.52,2.92}
{2.75,2.94}
{4.48,3.19}
{4.35,3.18}
{2.70,3.33}
{4.54,4.34}
{2.54,4.21}
{3.17,4.44}
{3.17,4.34}
{4.34,4.53}
{2.93,4.53}
f2 (ppm)
f1 (ppm)

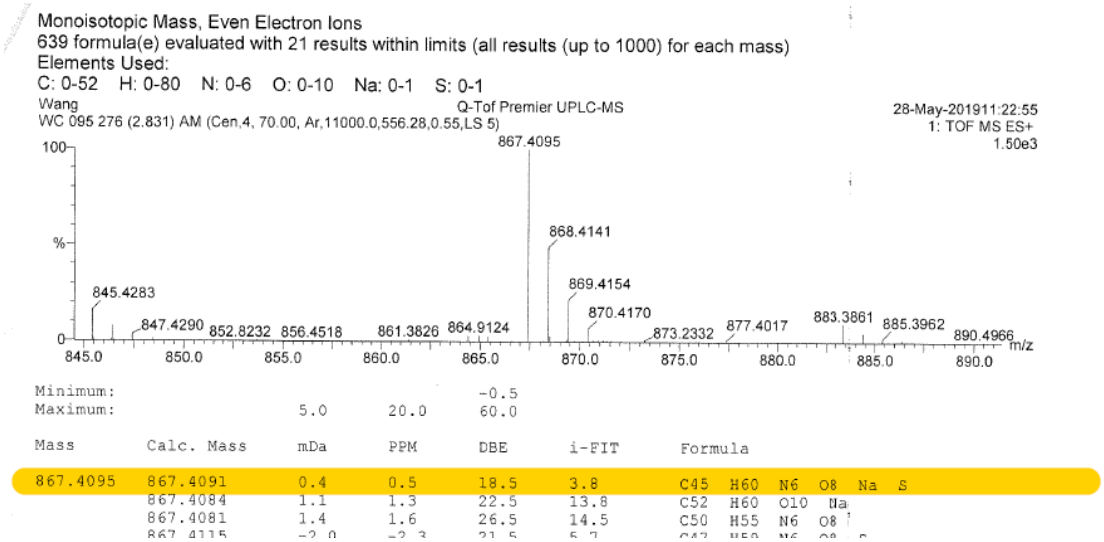

WCMC094E.1.ser
Wang WC 094 1.5 mg in CDCl3 at 298.0 K, 25.06.2019 Rettstadt
mass=830
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH094E.1.ser
Wang WC 094 1.5 mg in CDCl3 at 298.0 K, 25.06.2019 Rettstadt
mass=830
13C-HSQC
f2 (ppm)
f1 (ppm)

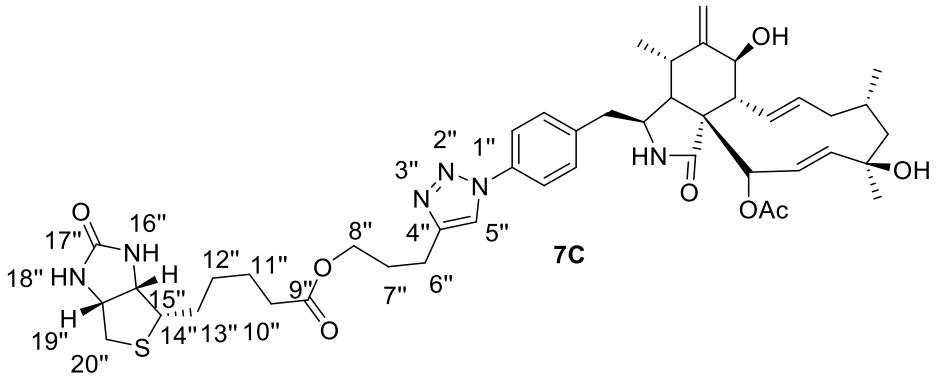
2.10 4'-Biotin-triazole-linked Cytochalasin H 7c



To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and **6c** (11.7 mg, 0.038 mmol, 2.0 eq) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 10.1 mg of desired product (63.8%, 0.011 mmol) as a white yellow solid.



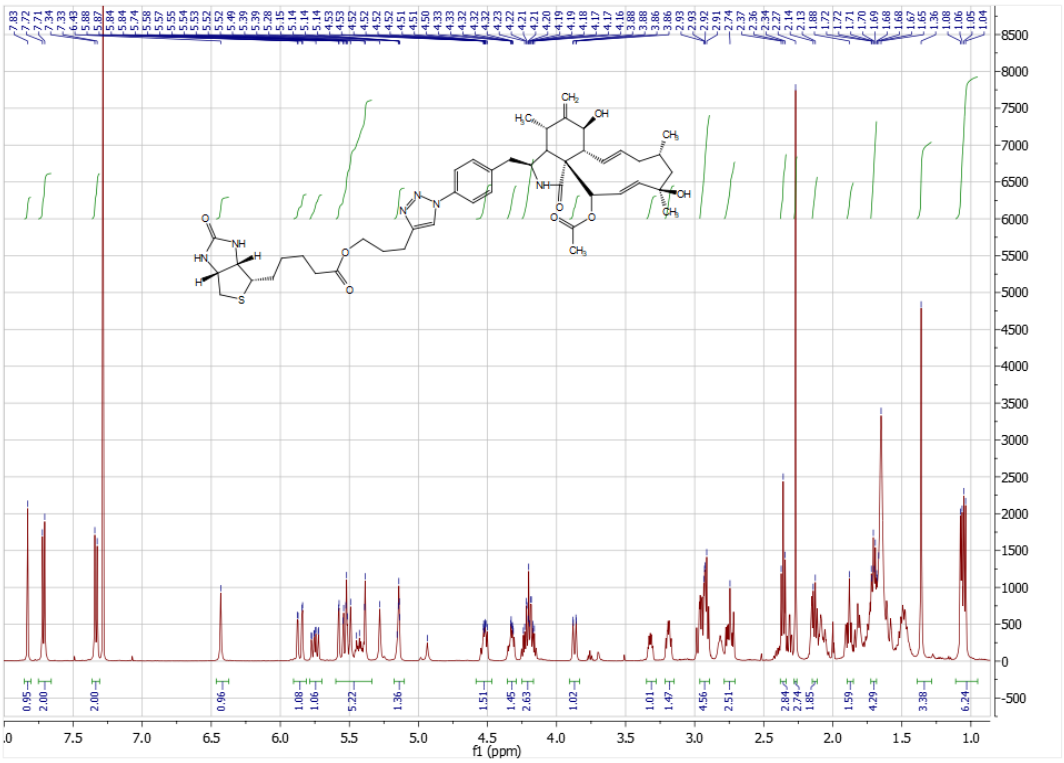
HRMS (*m/z*): calculated for [$C_{45}H_{60}N_6O_8S$ + Na]: 867.4091, found: 867.4095.

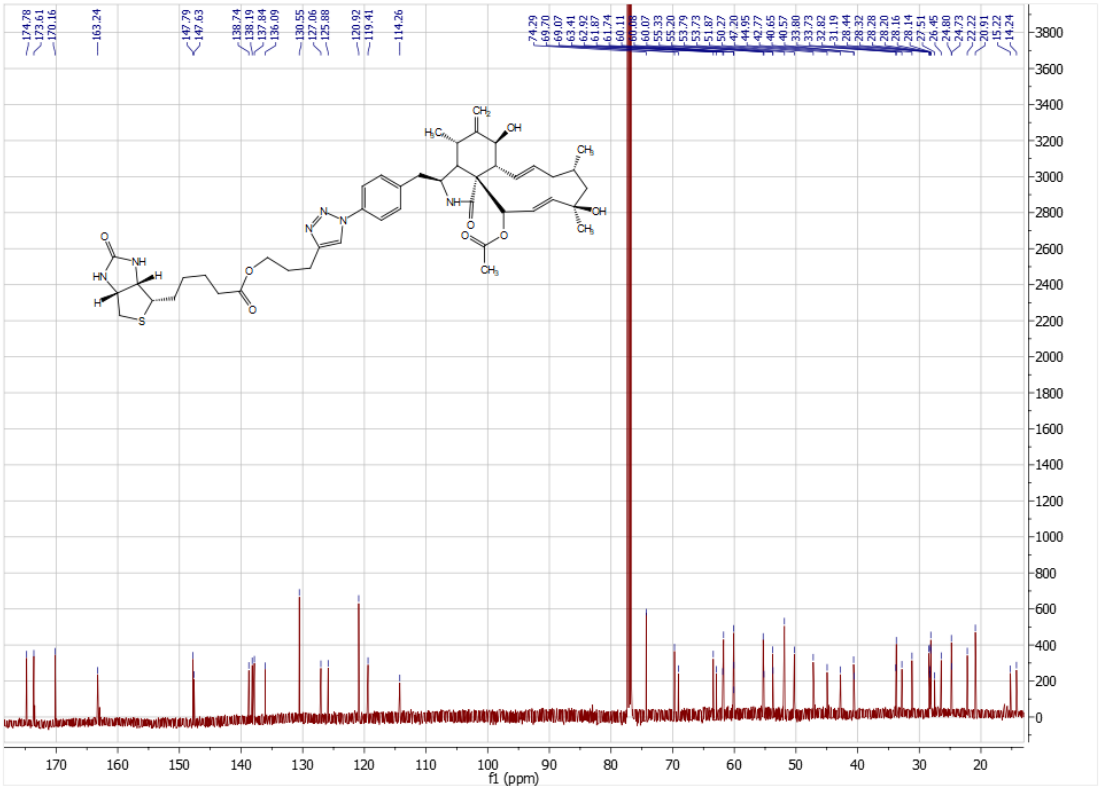


7C

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	175.2	-	-	-
3	3.33	ddd	0.9, 4.8, 9.6	53.7	CH	1‘,4, 5	4, 5, 10
4	2.15	m	-	50.2	CH	3, 5	3, 5, 10
5	2.81	m	-	32.8	CH	1’, 3	4, 11, 12
6	-	-	-	147.7	-	-	-
7	3.88	dd	1.5, 10.8	69.7	CH	5, 6, 8, 12, 13	8, 12
8	2.94	m	-	47.2	CH	1, 7, 9, 13, 21	7, 13
9	-	-	-	52.4	-	-	-
10a	2.75	m	-	45.0	CH_2	1’, 3	3, 4, 12
10b	2.93	m	-	-	-	-	-
11	1.04	d	6.6	14.2	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.74	ddd	1.4, 9.6, 15.5	127.1	CH	7, 8, 15	8, 14, 15
14	5.45	ddd	4.8, 10.3, 15.5	138.7	CH	9, 15	13, 15
15a	1.82	m	-	42.6	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.80	m	-	28.4	CH	13, 14, 17	22
17a	1.61	m	-	53.7	CH_2	18, 19	-
17b	1.91	m	-	-	-	-	-
18	-	-	-	74.2	-	-	-
19	5.54	brs	-	138.2	CH	-	20
20	5.84	dd	2.7, 16.5	125.8	CH	18, 19	19
21	5.52	brs	-	77.2	CH	8, 9, 19, 20, 23	-
22	1.06	d	6.5	26.2	CH_3	15, 16, 17	16
23	1.36	s	-	31.3	CH_3	17, 18, 19	-
1‘	-	-	-	130.7	-	-	-
2’ 6’	7.33	d	8.2	130.4	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.72	d	8.2	120.8	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	137.9	-	-	-
24	-	-	-	170.1	-	-	-
25	2.27	s	-	21.0	CH_3	21, 24	-
4‘’	-	-	-	147.6	-	-	-
5‘’	7.83	s	-	119.1	CH	4‘’	-
6‘’	2.13	m	-	28.1	CH_2	4‘’, 5‘’, 7‘’	7‘’
7‘’	1.89	m	-	27.6	CH_2	6‘’, 8‘’	6‘’
8‘’	4.18	m	-	63.1	CH_2	-	-
9‘’	-	-	-	173.6	-	-	-
10‘’	2.37	m	-	33.8	CH_2	8‘’, 10‘’	9‘’, 11‘’
11‘’	1.70	m	-	28.2	CH_2	8‘’, 9‘’,	10‘’

12‘‘	1.48	m	-	28.2	CH_2	11‘‘,12‘‘, 13‘‘ 9‘‘, 10‘‘, 12‘‘, 13‘‘	9‘‘
13‘‘	1.69	m	-	24.7	CH	11‘‘, 13‘‘, 14‘‘	14‘‘
14‘‘	3.19	m	-	55.2	CH	11‘‘	13‘‘, 15‘‘, 18‘‘
15‘‘	4.33	m	-	61.7	CH	14‘‘, 17‘‘, 20‘‘	14‘‘, 19‘‘
17‘‘	-	-	-	163.3	-	-	-
19‘‘	4.52	m	-	60.0	CH	13‘‘, 16‘‘	15‘‘, 20‘‘
20a‘‘	2.74	m	-	40.6	CH_2	13‘‘, 18‘‘	18‘‘
20b‘‘	2.93	m	-	-	-	-	-




174.78
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147.63
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138.19
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130.55
127.26
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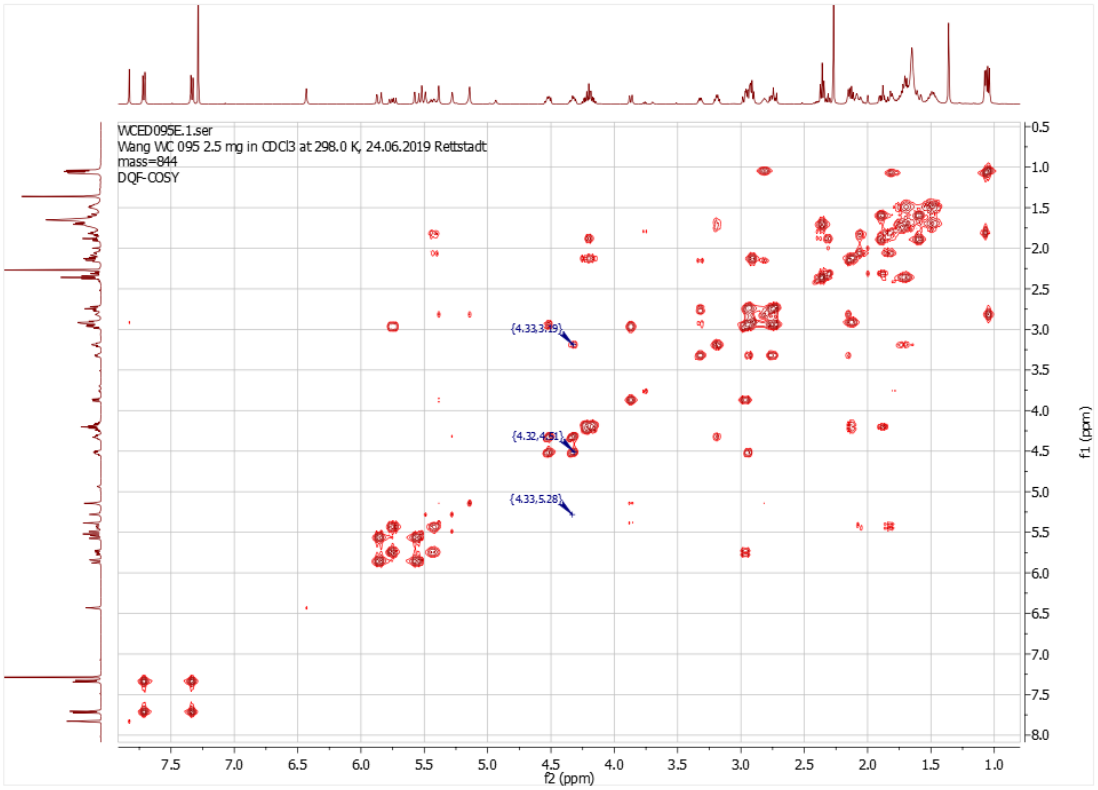
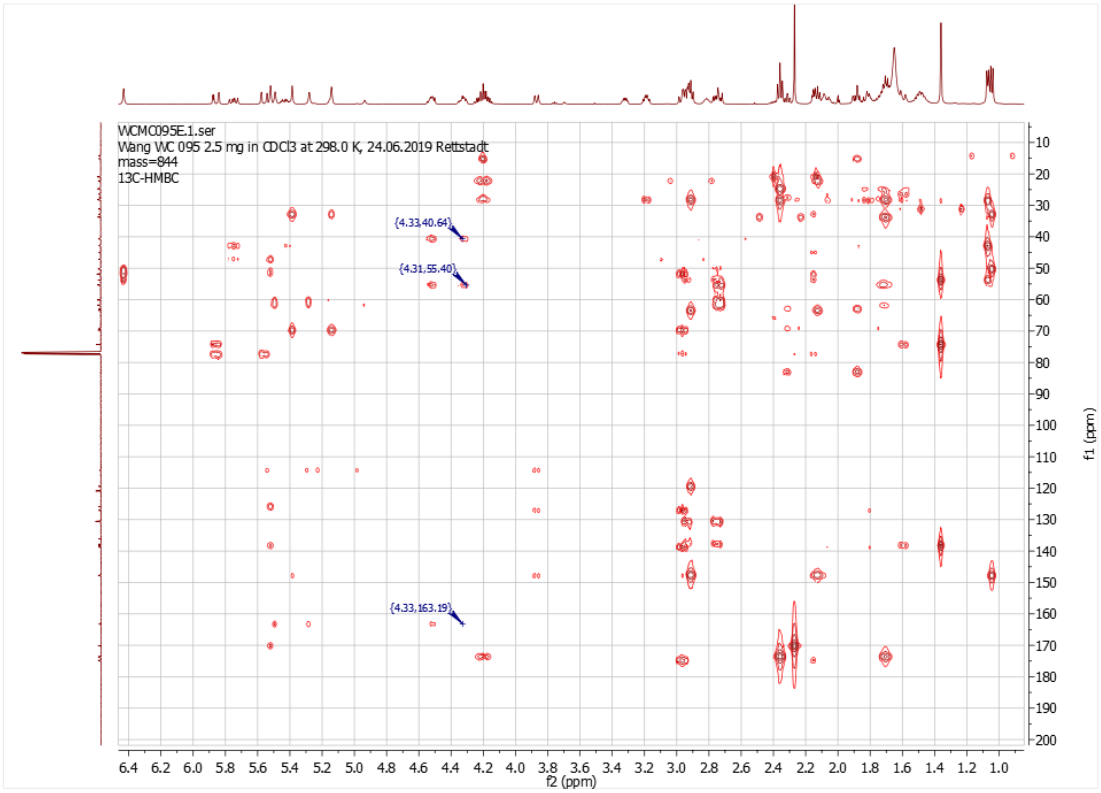
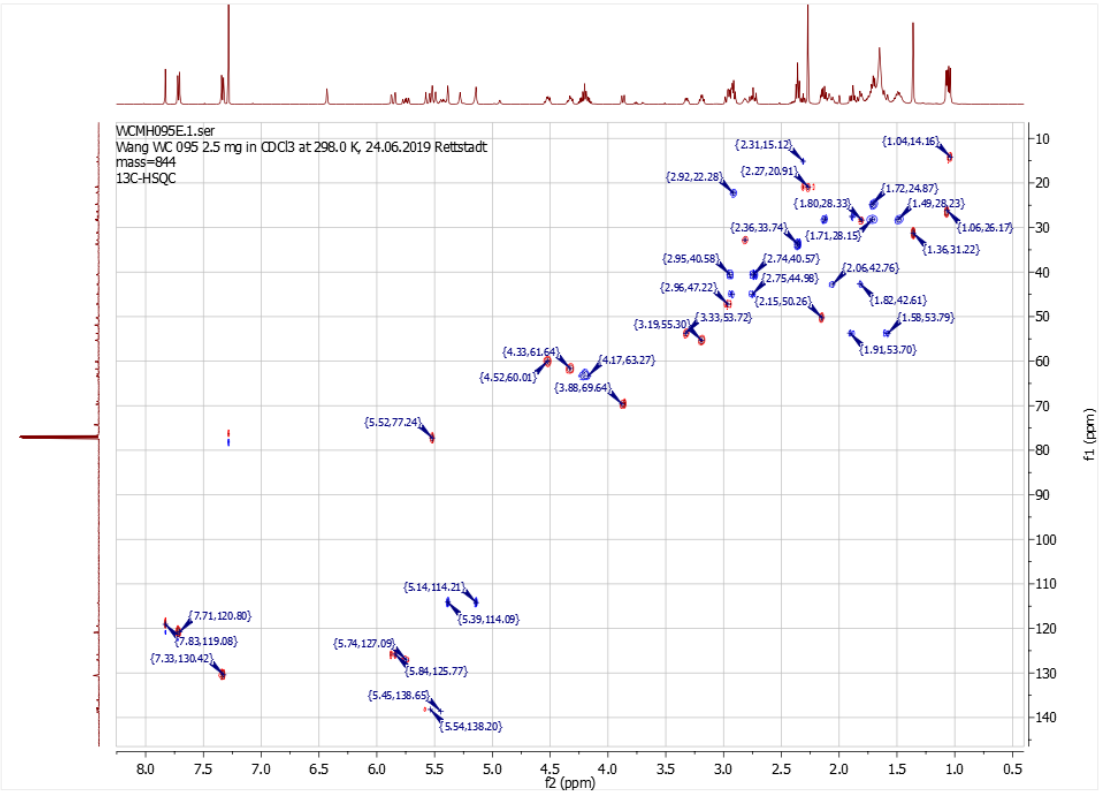
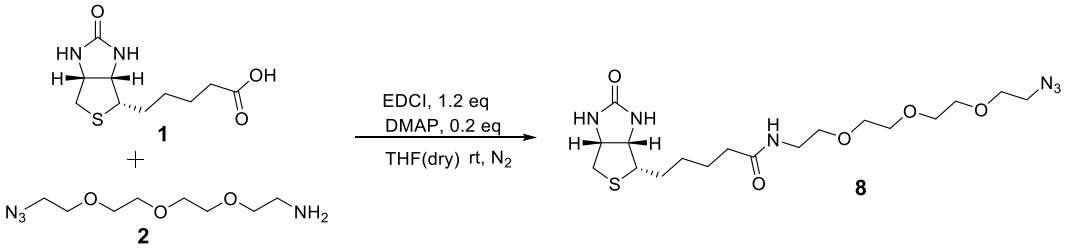
114.26
74.29
69.70
69.07
63.41
62.92
61.87
61.74
61.11
60.16
60.07
55.33
55.20
53.79
53.73
51.87
50.27
44.20
44.05
42.77
40.65
40.57
33.80
33.73
32.82
31.19
28.44
28.32
28.28
28.20
28.16
28.14
27.51
26.45
24.80
24.73
23.22
20.91
15.22
14.24
f1 (ppm)


WCED095E.1.ser
Wang WC 095 2.5 mg in CDCl3 at 298.0 K, 24.06.2019 Rettstadt
mass=844
DQF-COSY
{4.33,3.49}
{4.32,4.51}
{4.33,5.28}
f2 (ppm)
f1 (ppm)

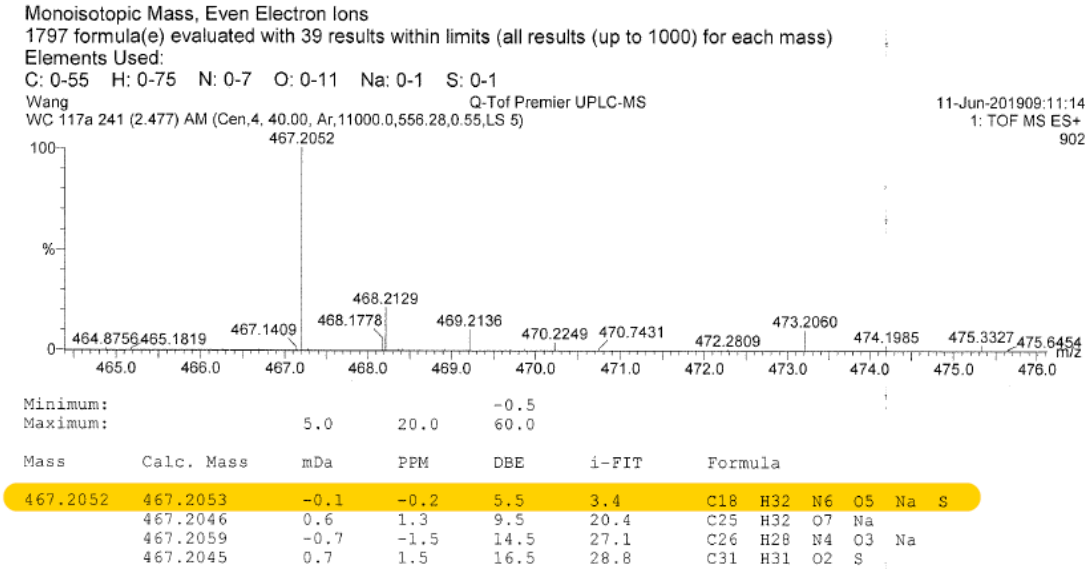

WCMC095E.1.ser
Wang WC 095 2.5 mg in CDCl3 at 298.0 K, 24.06.2019 Rettstadt
mass=844
13C-HMBC
{4.33,40.64}
{4.31,55.40}
{4.33,163.19}
f2 (ppm)
f1 (ppm)


WCMH095E.1.ser
Wang WC 095 2.5 mg in CDCl3 at 298.0 K, 24.06.2019 Rettstadt
mass=844
13C-HSQC
{2.31,15.12}
{1.04,14.16}
{2.92,22.28}
{2.27,20.91}
{1.72,24.87}
{1.80,28.33}
{1.49,28.23}
{2.36,33.74}
{1.71,28.15}
{1.06,26.17}
{1.36,31.22}
{2.95,40.58}
{2.74,40.57}
{2.06,42.76}
{2.96,47.22}
{2.75,44.98}
{2.15,50.26}
{1.82,42.61}
{3.19,55.30}
{3.33,53.72}
{1.58,53.79}
{1.91,53.70}
{4.33,61.64}
{4.17,63.27}
{4.52,60.01}
{3.88,69.64}
{5.52,77.24}
{5.14,114.21}
{5.39,114.09}
{7.71,120.80}
{7.83,119.08}
{7.33,130.42}
{5.74,127.09}
{5.84,125.77}
{5.45,138.65}
{5.54,138.20}
f2 (ppm)
f1 (ppm)

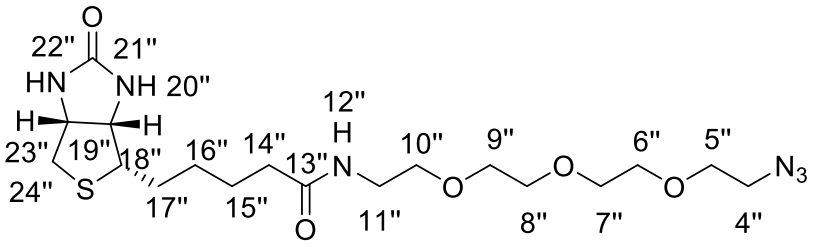
2.11 Biotin azide linker 8



To a stirred solution of **biotin** (100.0 mg, 0.41 mmol, 1.0 eq, purchased from Sigma-Aldrich) and **2** (98.0 mg, 0.45 mmol, 1.1 eq, purchased from Acros) in dry THF (4 mL) was added EDCI (94.0 mg, 0.49 mmol, 1.2 eq) and DMAP (10.0 mg, 0.082 mmol, 0.2 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 111.5 mg of desired product (61.3%, 0.239 mmol) as a white solid.

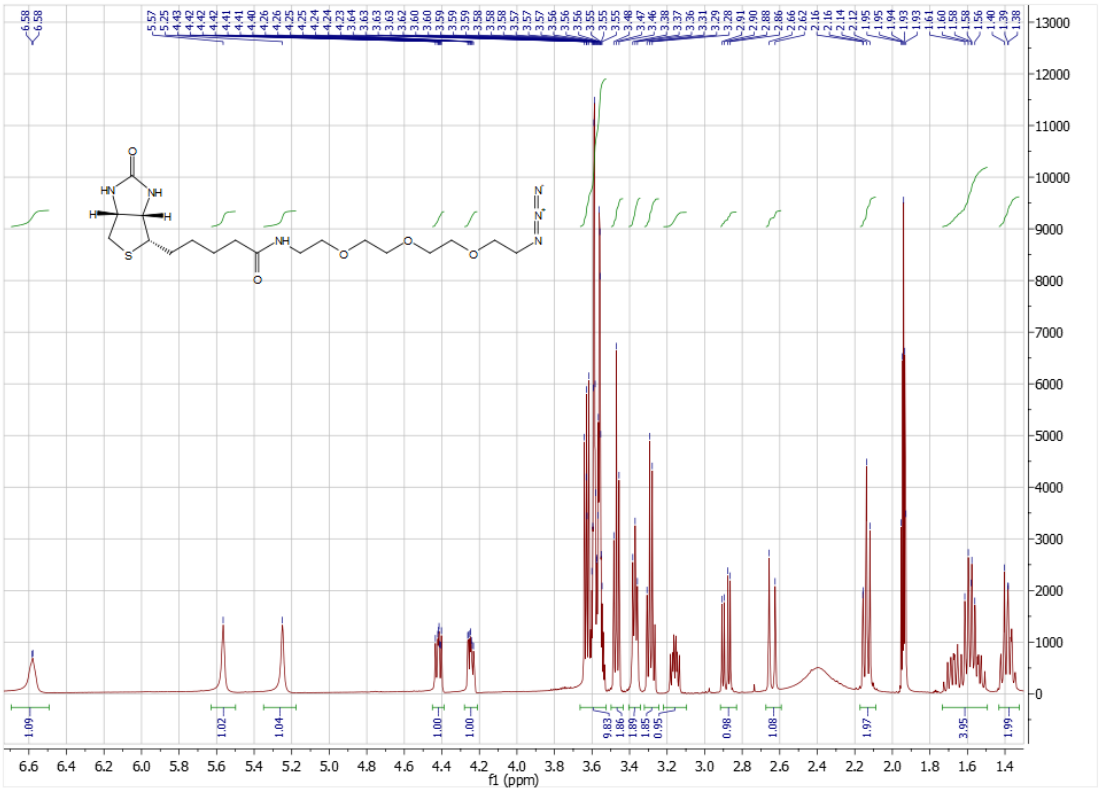


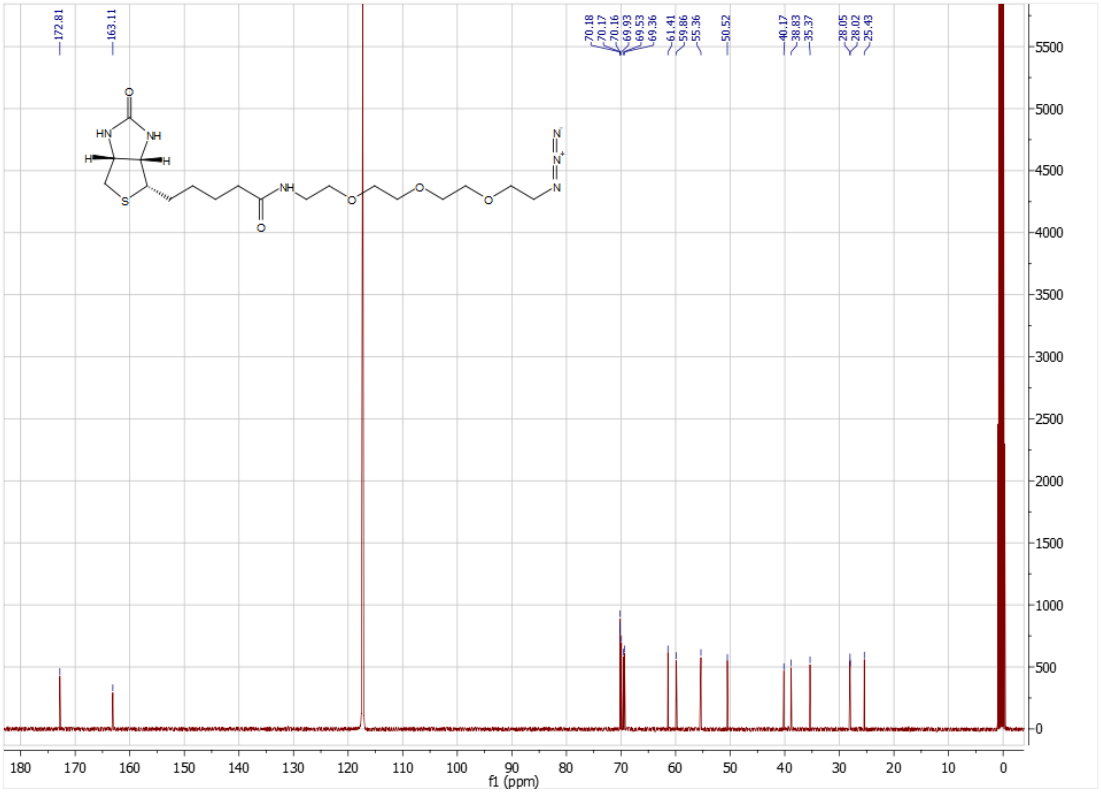
HRMS (*m/z*): calculated for [$C_{18}H_{32}N_6O_5S$ + Na]: 467.2053, found: 467.2052.

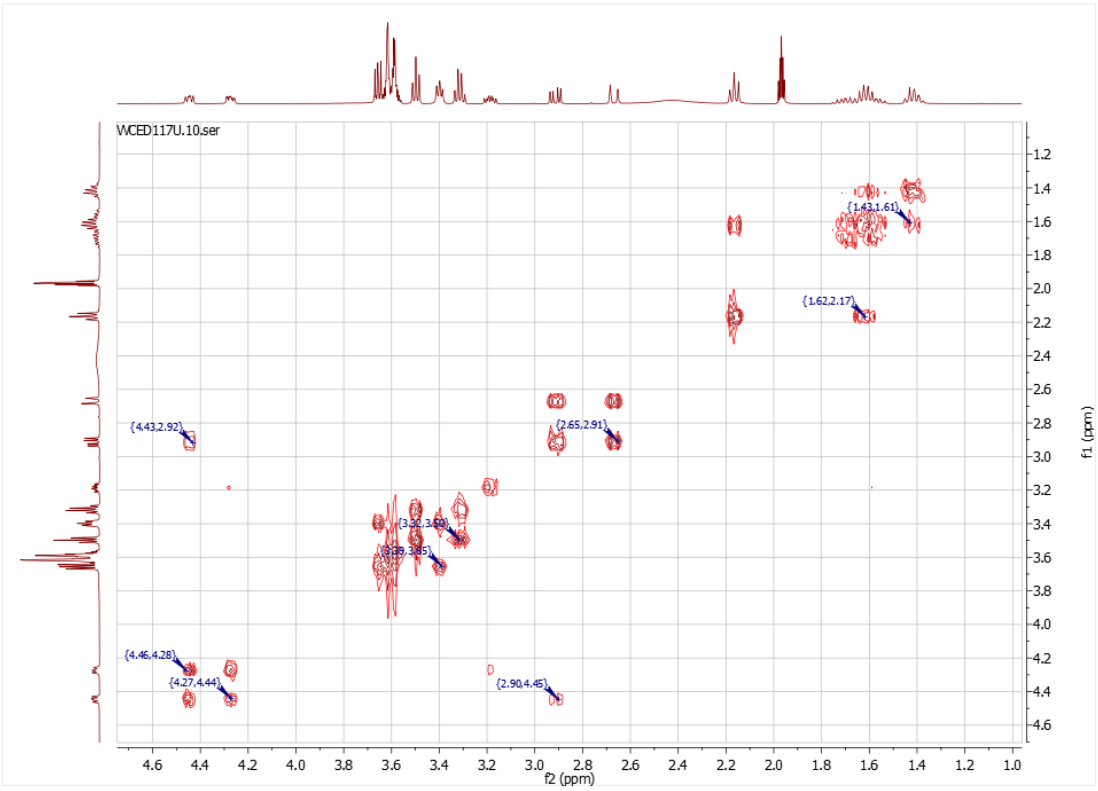


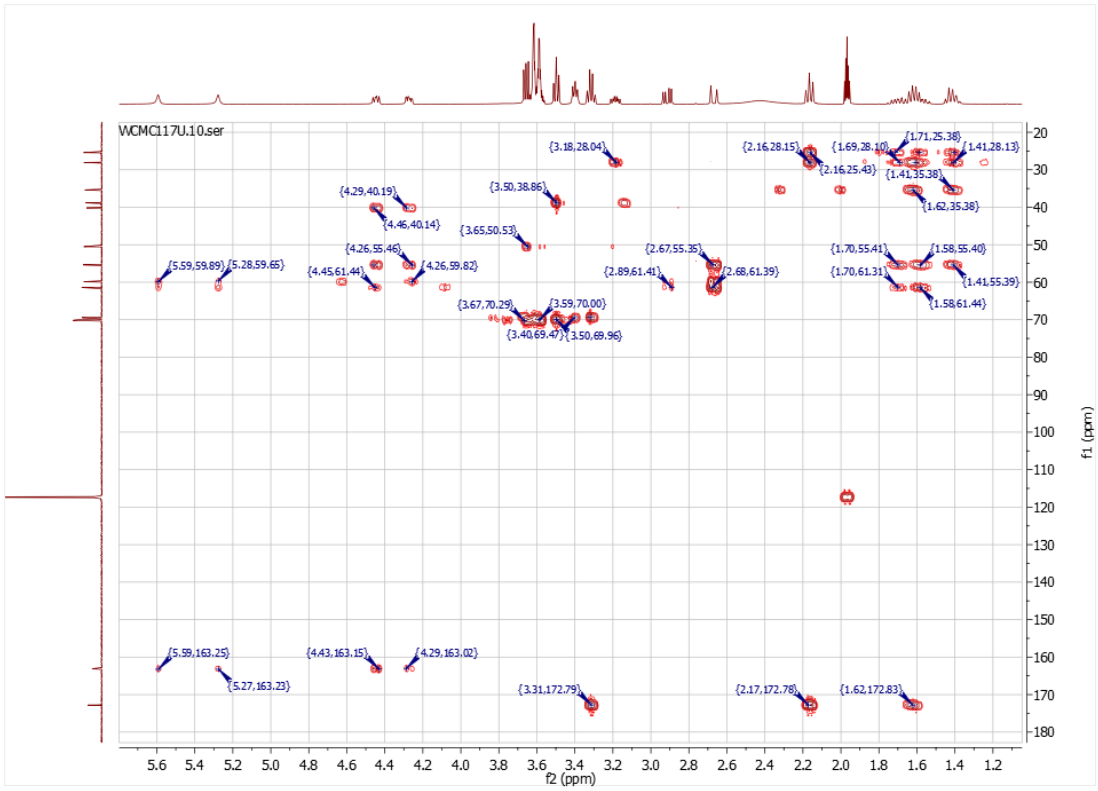
8

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
4''	3.40	t	5.6	50.5	CH_2	5''	5''
5''	3.65	m	-	69.6	CH_2	4'', 6''	4''
6''9''	3.59	m	-	69.8	CH_2	7'', 8''	-
7'' 8''	3.62	m	-	70.2	CH_2	6'', 9''	-
10''	3.50	m	-	69.3	CH_2	9'', 11''	11''
11''	3.32	dd	5.6, 11.2	38.3	CH_2	10'', 13''	10''
12''	6.58	s	-	-	-	-	-
13''	-		-	172.8	-	-	-
14''	2.15	dd	1.0, 7.9	35.4	CH_2	13'', 15'', 16''	15''
15''	1.62	m	-	25.3	CH_2	13'', 14'', 16''	14'', 16''
16''	1.39	m	-	28.1	CH_2	14'', 15'', 17'', 18''	15''
17''	1.57	m	-	27.9	CH_2	18'', 19''	-
18''	3.18	-	-	55.4	CH	17''	-
19''	4.27	m	-	61.4	CH_2	18'', 21'', 23'', 24''	23''
20''	5.28	s	-	-	-	-	-
21''	-			163.2	-	-	-
22''	5.59	s	-	-	-	-	-
23''	4.46	m	-	59.9	CH	18'', 19'', 21'', 23''	19'', 24''
24a''	2.68	d	12.7	40.1	CH_2	18'', 19''	23''
24b''	2.90	dd	5.0, 12.7	-	-	-	-


f1 (ppm)


f1 (ppm)


WCED117U.10.ser
{1.43,1.61}
{1.62,2.17}
{4.43,2.92}
{2.65,2.91}
{4.46,4.28}
{4.27,4.44}
{2.90,4.45}
f2 (ppm)
f1 (ppm)


WCMC117U.10.ser
{3.18,28.04}
{2.16,28.15}
{1.69,28.10}
{1.71,25.38}
{1.41,28.13}
{2.16,25.43}
{1.41,35.38}
{1.62,35.38}
{4.29,40.19}
{3.50,38.86}
{4.46,40.14}
{3.65,50.53}
{4.26,55.46}
{2.67,55.35}
{1.70,55.41}
{1.58,55.40}
{5.59,59.89}
{5.28,59.65}
{4.45,61.44}
{4.26,59.82}
{2.89,61.41}
{2.68,61.39}
{1.70,61.31}
{1.41,55.39}
{3.67,70.29}
{3.59,70.00}
{1.58,61.44}
{3.40,69.47}
{3.50,69.96}
{5.59,163.25}
{4.43,163.15}
{4.29,163.02}
{5.27,163.23}
{3.31,172.79}
{2.17,172.78}
{1.62,172.83}
f2 (ppm)
f1 (ppm)


WCMH117U.10.ser
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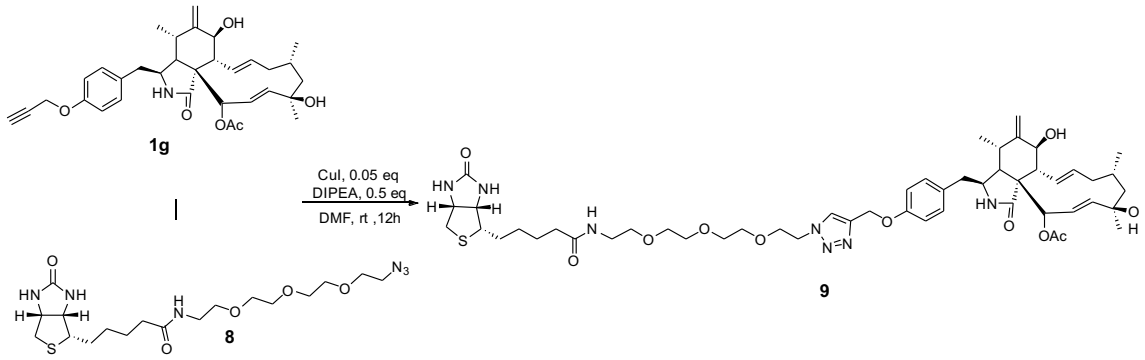
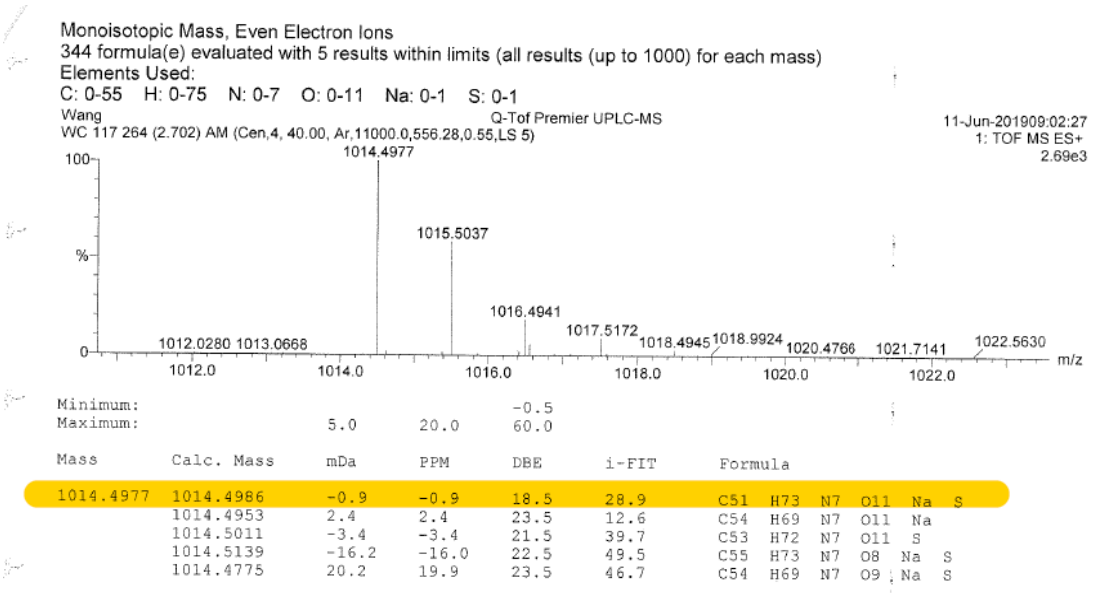
{1.71,27.98}
{1.63,27.99}
{2.15,35.37}
{3.32,38.83}
{2.93,40.12}
{2.68,40.17}
{2.65,40.16}
{2.90,40.18}
{3.40,50.51}
{3.18,55.37}
{4.46,59.85}
{4.27,61.39}
{3.62,70.24}
{3.40,69.56}
f2 (ppm)
f1 (ppm)

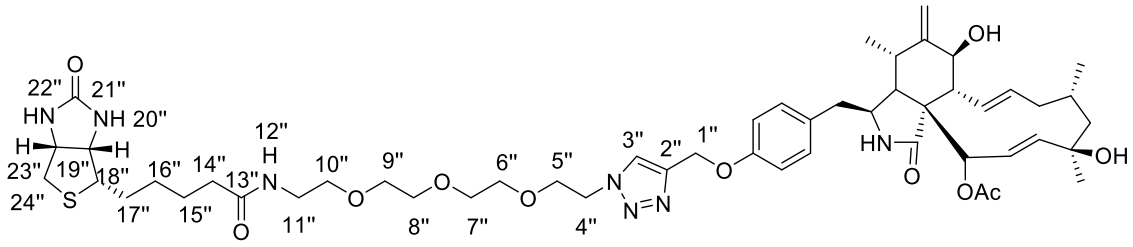
2.12 4'-*O*-biotin-linked Cytochalasin H 9



To a stirred solution of **1g** (13.0 mg, 0.024 mmol, 1.1 eq) and **8** (10.0 mg, 0.022 mmol, 1.0 eq) in DMF (2 mL) was added CuI (0.23 mg, 1.2 µmol, 0.05 eq) and DIPEA (1.5 mg, 12 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 14.3 mg of desired product (64.3%, 0.014 mmol) as a white solid.



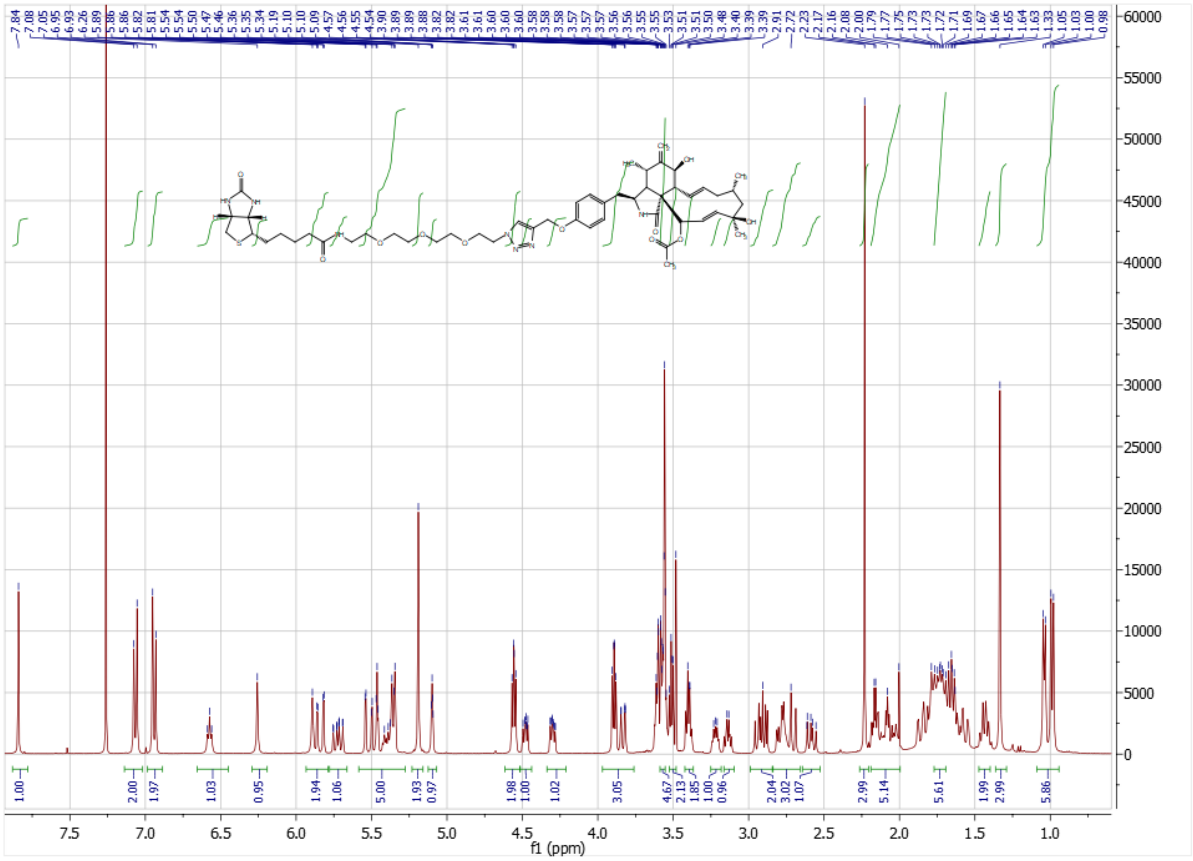
HRMS (*m/z*): calculated for [$C_{51}H_{73}N_7O_{11}S$ + Na]: 1014.4986, found: 1014.4977.

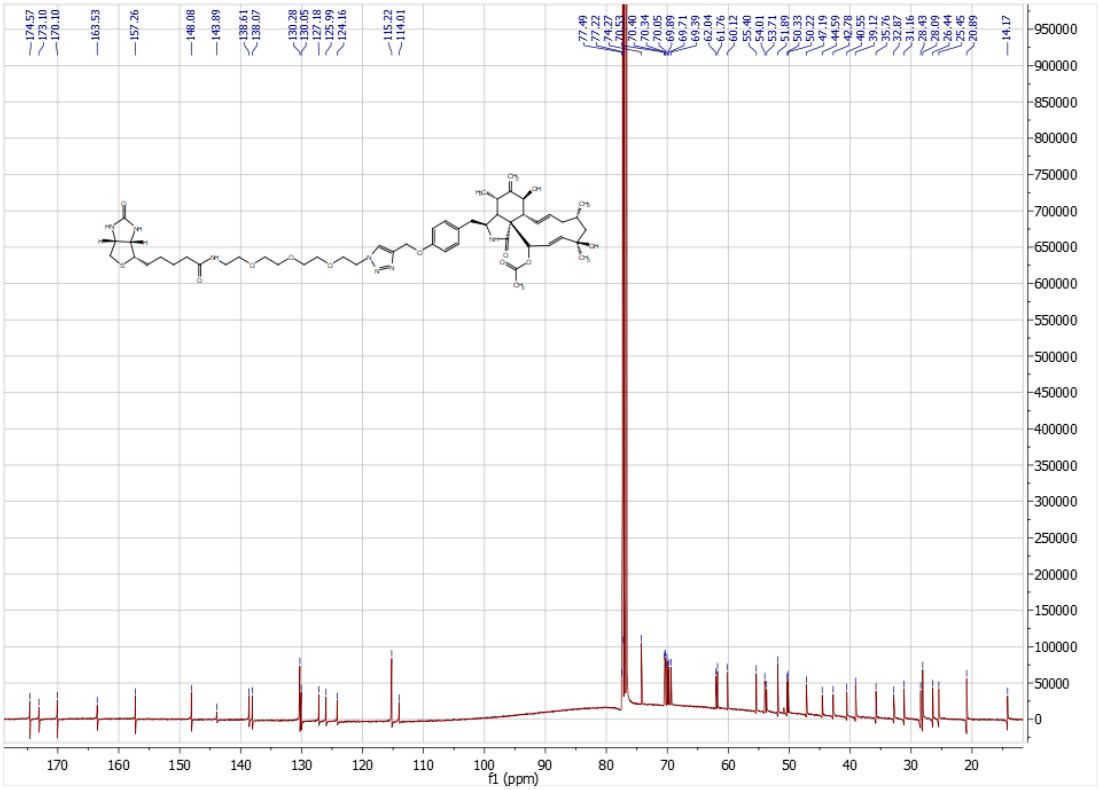


9

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.6	-	-	-
3	3.24	m	-	54.0	CH	1', 1, 4, 5	4, 5, 10
4	2.10	m	-	50.2	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.79	m	-	33.0	CH	1', 3	4, 11, 12
6	-	-	-	150.0	-	-	-
7	3.84	dd	1.3, 10.8	69.8	CH	6, 12	8, 12
8	2.96	m	-	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.9	-	-	-
10a	2.61	m	-	44.5	CH_2	1', 3	3, 4, 12
10b	2.80	m	-	-	-	-	-
11	1.00	d	6.7	14.3	CH_3	4, 5, 6	5
12a	5.12	brs	-	114.0	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.74	ddd	1.3, 9.7, 15.3	127.2	CH	7, 8, 15	8, 14, 15
14	5.41	ddd	5.1, 10.4, 15.3	138.5	CH	9, 15	13, 15
15a	1.81	m	-	43.0	CH_2	13, 14, 16, 17	13, 14,
15b	2.07	m	-	-	-	-	-
16	1.82	m	-	28.6	CH	13, 14, 17	22
17a	1.61	m	-	53.9	CH_2	18, 19	-
17b	1.90	m	-	-	-	-	-
18	-	-	-	74.3	-	-	-
19	5.56	brs	-	138.2	CH	-	20
20	5.88	dd	2.6, 16.6	126.0	CH	18, 19	19
21	5.49	brs	-	77.6	CH	8, 9, 19, 20, 23	-
22	1.06	d	6.4	26.7	CH_3	15, 16, 17	16
23	1.38	s	-	31.2	CH_3	17, 18, 19	-
1'	-	-	-	130.1	-	-	-
2' 6'	7.10	d	8.5	130.3	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	6.97	d	8.5	115.2	2 x CH	1', 4'	2', 6'
4'	-	-	-	157.3	-	-	-
24	-	-	-	170.1	-	-	-
25	2.26	s	-	20.9	CH_3	24	-
1''	5.21	s	-	62.1	-	2'', 3'', 4'	3''
2''	-	-	-	143.9	-	-	-
3''	7.86	s	-	124.0	CH	2''	1'', 4''
4''	4.58	dd	4.5, 5.6	50.3	CH_2	5''	3'', 5''
5''	3.91	dd	4.5, 5.5	69.4	CH_2	4'', 6''	4''
6''9''	3.58	m	-	70.2	CH_2	7'', 8''	-
7'' 8''	3.62	m	-	70.4	CH_2	6'', 9''	-
10''	3.54	m	-	69.9	CH_2	9'', 11''	11''
11''	3.42	dd	5.3, 10.5	39.2	CH_2	10'', 13''	10''
12''	6.56	t	5.6	-	-	-	-

13‘‘	-	-	-	173.1	-	-	-
14‘‘	2.19	m	-	35.8	CH_2	13’’, 15’’, 16’’	15’’
15‘‘	1.68	m		25.7	CH_2	13‘‘, 14‘‘, 16‘‘	14‘‘, 16‘‘
16‘‘	1.47	m	-	28.4	CH_2	14’’, 15’’, 17’’, 18’’	15’’
17‘‘	1.69	m	-	28.2	CH_2	18‘‘, 19‘‘	-
18‘‘	3.16	m	-	55.4	CH	17’’	-
19‘‘	4.32	m	-	61.8	CH	18‘‘, 21‘‘, 23‘‘, 24‘‘	23‘‘
21‘‘	-	-	-	163.6	-	-	-
22‘‘	-	-	-	-	-	-	-
23‘‘	4.50	m	-	60.1	CH	18‘‘, 19‘‘, 21‘‘, 23‘‘	19‘‘, 24‘‘
24a‘‘	2.74	m	-	40.5	CH_2	18‘‘, 19‘‘	23‘‘
24b‘‘	2.91	m	-	-	-	-	-




174.57
173.10
170.10
163.53
157.26
148.08
143.89
138.61
138.07
130.28
130.05
127.18
125.99
124.16
115.22
114.01
77.49
77.22
76.97
74.23
70.40
70.34
70.05
69.89
69.71
69.39
62.04
61.76
60.12
55.60
54.41
53.71
51.89
50.33
50.22
47.19
44.59
42.78
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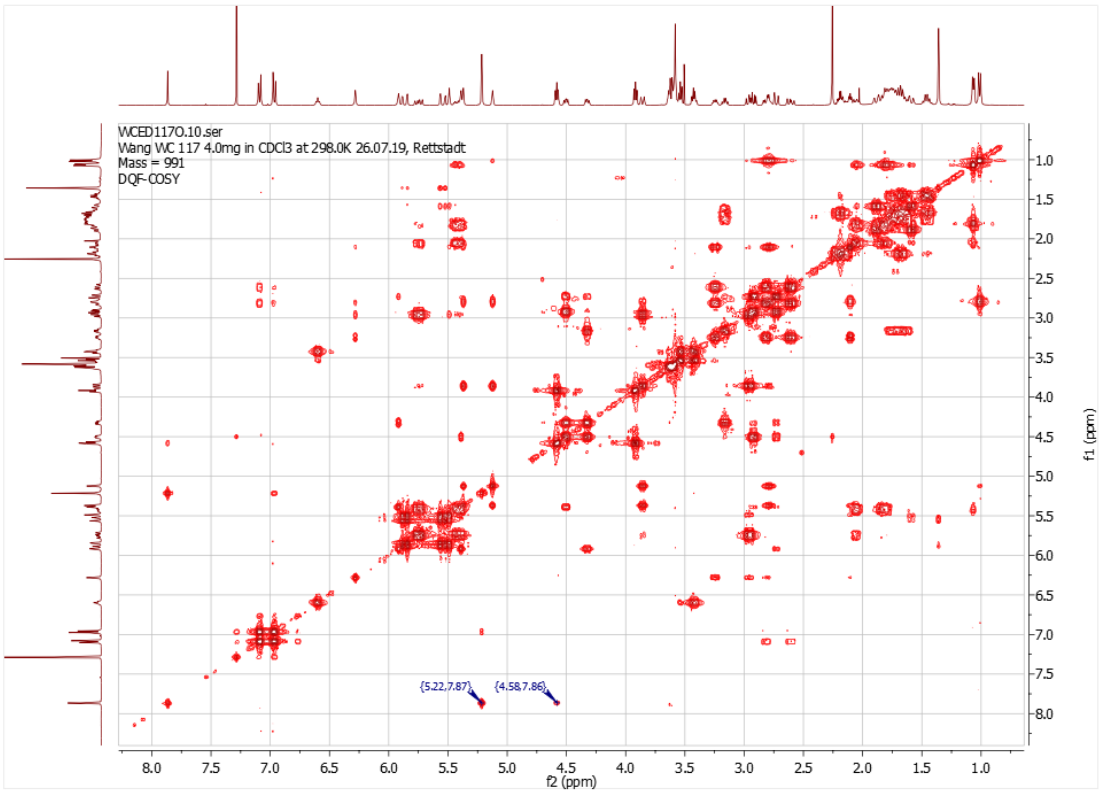
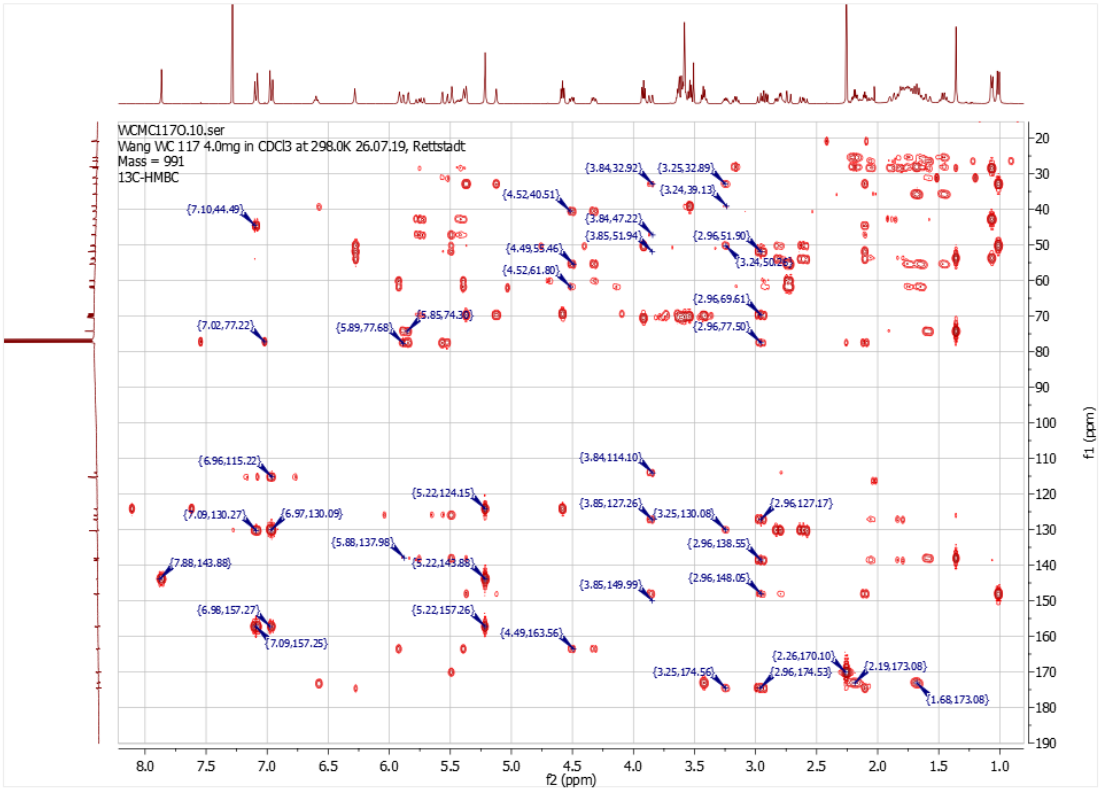
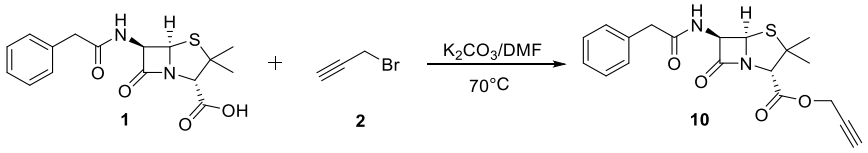
32.87
31.16
28.43
28.09
26.44
25.45
20.89
14.17
f1 (ppm)


WCED117O.10.ser
Wang WC 117 4.0mg in CDCl3 at 298.0K 26.07.19, Rettstadt
Mass = 991
DQF-COSY
{5.22,7.87}
{4.58,7.86}
f2 (ppm)
f1 (ppm)


WCMC117O.10.ser
Wang WC 117 4.0mg in CDCl3 at 298.0K 26.07.19, Rettstadt
Mass = 991
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH117O.10.ser
Wang WC 117 4.0mg in CDCl3 at 298.0K 26.07.19, Rettstadt
Mass = 991
13C-HSQC
f2 (ppm)
f1 (ppm)

2.13 *O*-Propargyl Penicillin G 10



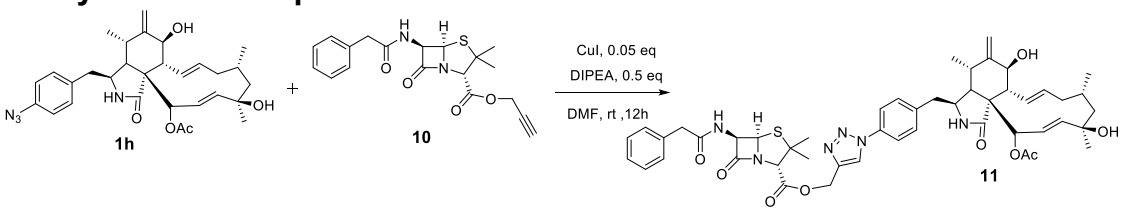
To a stirred solution of Penicillin G **1** (100.0 mg, 0.30 mmol, 1.0 eq, purchased from TCI) and **2** (0.1mL, 0.60 mmol, 2.0 eq, purchased from Acros) in dry DMF (4 mL) was added K_2CO_3 (50.0 mg, 0.49 mmol, 1.2 eq, purchased from Acros). The mixture was stirred at 70 °C for 12 hours under nitrogen and the solvent was removed in vacuo. The residue was dissolved in EtOAc (4 mL) and washed with water (3 mL x 2). The organic phase was dried over MgSO4, filtered and evaporated to dryness in vacuo. The residue was dissolved in MeOH and purified by LCMS to afford 77.3 mg of desired product (69.4%, 0.196 mmol) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ (Hz) = 7.38-7.29 (m, 5H), 5.67 (dd, J = 4.2 and 9.0 Hz, 1H), 5.53 (d, J = 4.2 Hz, 1H), 4.82 (d, J = 2.5 Hz, 1H), 4.71 (d, J = 2.5 Hz, 1H), 4.42 (s, 1H), 3.65 (s, 2H), 2.53 (t, J = 2.5 Hz, 1H), 1.50 (s, 3H), 1.48 (s, 3H).

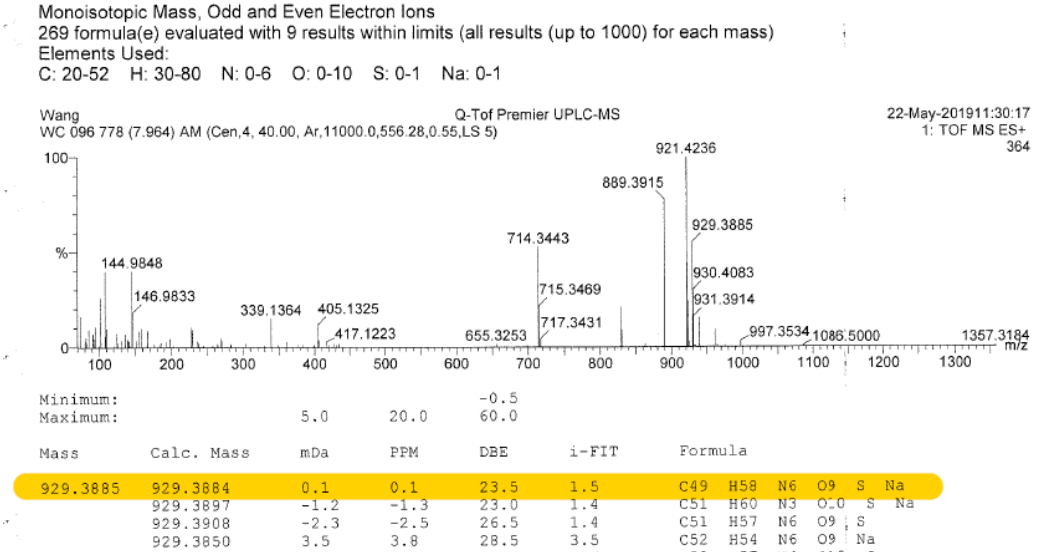
^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.5, 170.5, 166.8, 133.8, 129.4, 129.0, 127.6, 76.4, 76.2, 70.0, 67.9, 64.7, 58.7, 52.7, 43.6, 26.8.

HRMS (*m/z*): calculated for [$C_{19}H_{20}N_2O_4S$ + Na]: 395.1041, found: 395.1038.

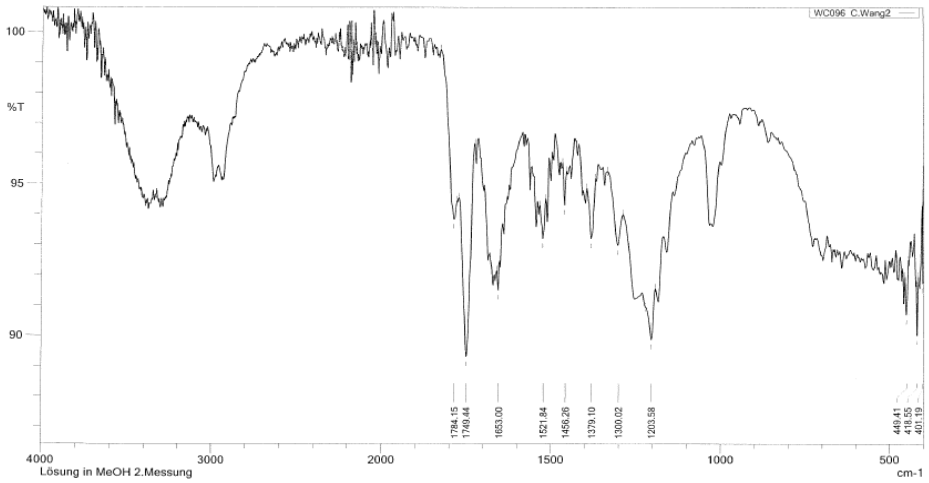
2.14 Cytochalasin H penicillin G adduct 11



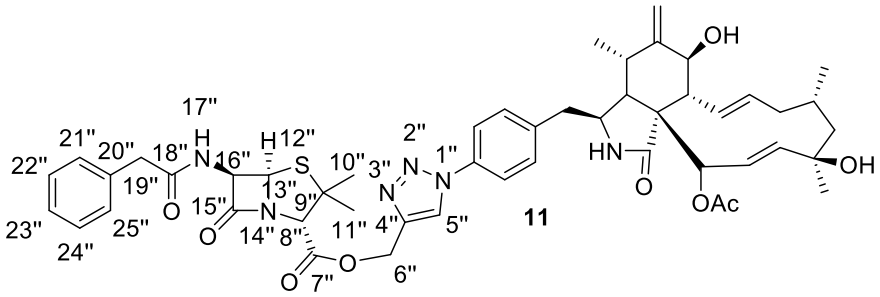
To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and **10** (14.1 mg, 0.038 mmol, 2.0 eq) in DMF (4 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4 mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 15.0 mg of desired product (88.6%, 0.015 mmol) as a white yellow solid.



HRMS (*m/z*): calculated for [$C_{49}H_{58}N_6O_9S$ + Na]: 1007.5398, found: 1007.5402.



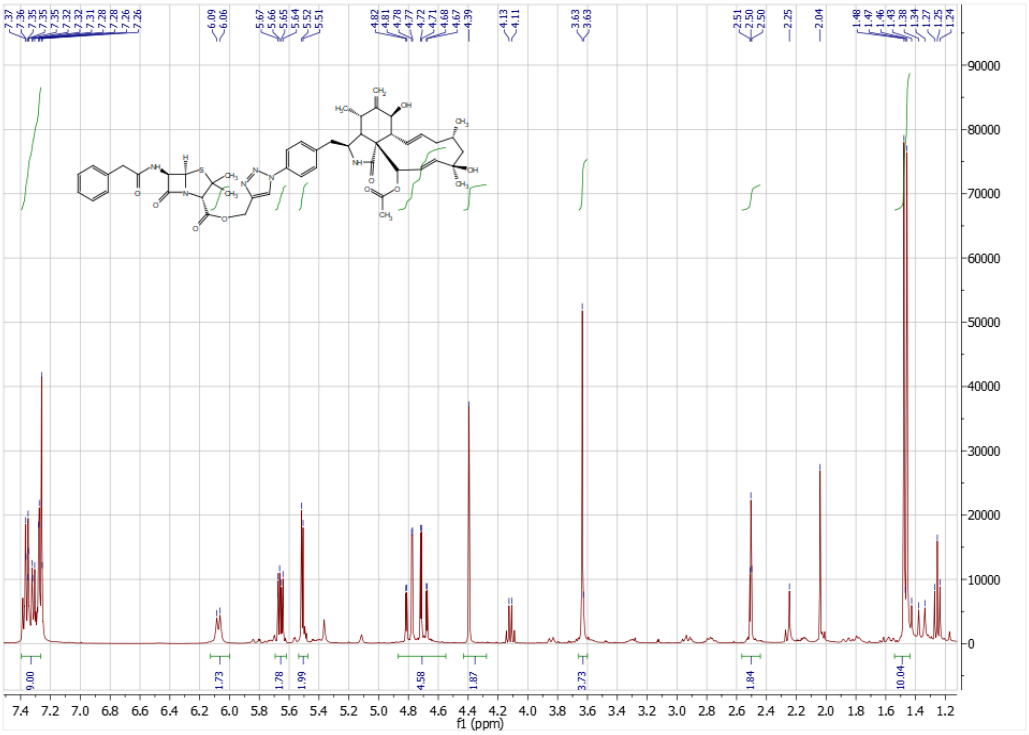
IR (powder, cm^{-1}): 1784, 1749, 1653, 1521, 1456, 1379, 1300, 1203, 449, 418.

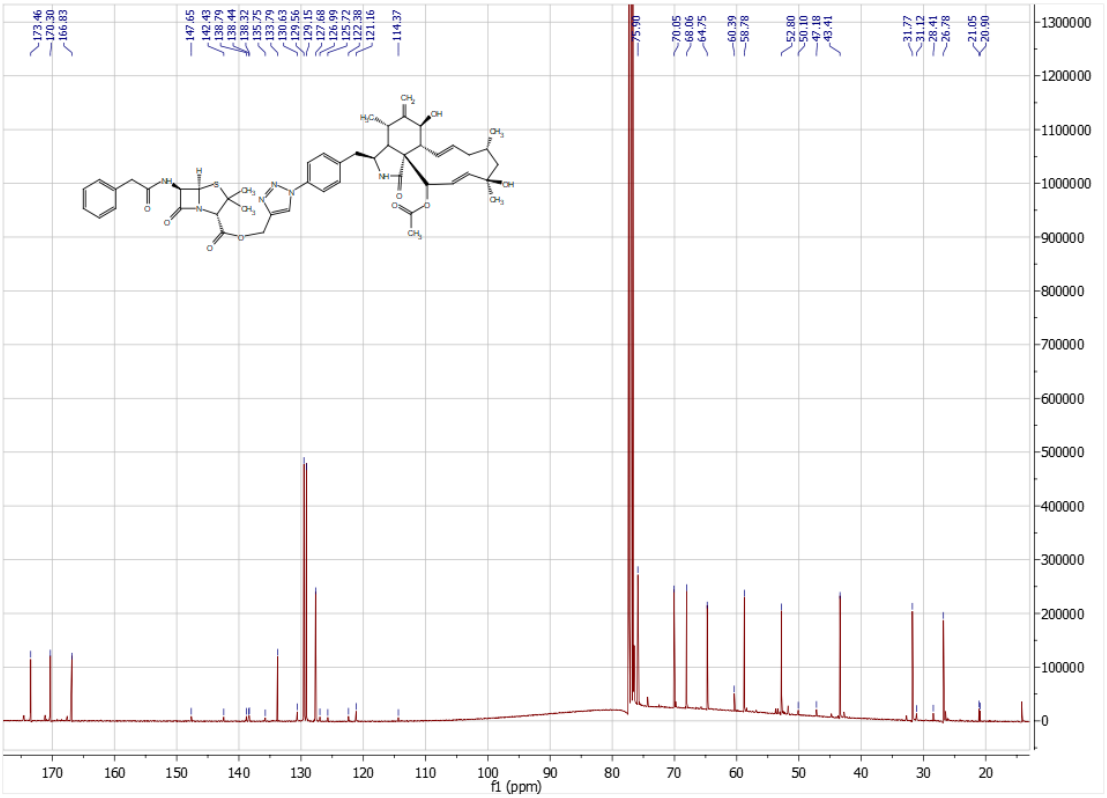


11

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.6	-	-	-
3	3.33	m	-	53.2	CH	1‘, 1, 4, 5	4, 5, 10
4	2.17	m	-	49.9	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.85	m	-	32.6	CH	1’, 3	4, 11, 12
6	-	-	-	147.5	-	-	-
7	3.88	d	10.6	69.7	CH	6, 12	8, 12
8	2.96	m	-	47.0	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.9	-	-	-
10a	2.74	m	-	44.7	CH_2	1’, 3	3, 4, 12
10b	2.90	m	-	-	-	-	-
11	0.98	d	6.6	13.8	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.3	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.75	m	-	127.1	CH	7, 8, 15	8, 14, 15
14	5.43	m	-	138.9	CH	9, 15	13, 15
15a	1.82	m	-	42.6	CH_2	13, 14, 16, 17	13, 14,
15b	2.08	m	-	-	-	-	-
16	1.82	m	-	28.2	CH	13, 14, 17	22
17a	1.61	m	-	53.5	CH_2	18, 19	-
17b	1.91	m	-	-	-	-	-
18	-	-	-	74.1	-	-	-
19	5.55	brs	-	138.4	CH	-	20
20	5.86	dd	2.6, 16.5	125.8	CH	18, 19	19
21	5.52	brs	-	77.1	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.4	26.3	CH_3	15, 16, 17	16
23	1.38	s	-	31.4	CH_3	17, 18, 19	-
1‘	-	-	-	129.5	-	-	-
2’ 6’	7.34	d	8.5	127.8	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.72	d	8.5	121.1	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	135.7	-	-	-
24	-	-	-	170.2	-	-	-
25	2.26	s	-	21.1	CH_3	24	-
4‘‘	-	-	-	142.4	-	-	-
5‘‘	8.07	s	-	122.2	CH	4‘‘	-
6‘‘	5.39	s	-	58.1	CH_2	4‘‘, 5‘‘,7‘‘	-
7‘‘	-	-	-	173.4	-	-	-
8‘‘	4.42	s	-	70.1	CH	7‘‘, 9‘‘, 10‘‘, 11‘‘, 13‘‘,	-
9‘‘	-	-	-	64.7	-	-	-
10‘‘11‘‘	1.50	s	-	26.9	2 x CH_3	8‘‘, 9‘‘	-
13‘‘	5.54	d	4.2	67.9	CH	15‘‘, 16‘‘	16‘‘
15‘‘	-	-	-	173.5	-	-	-
16‘‘	5.69	dd	4.2, 9.1	58.5	CH	13‘‘, 15‘‘, 18‘‘	13‘‘
18‘‘	-	-	-	170.3	-	-	-

19‘‘	3.66	s	-	43.4	CH_2	18‘‘, 20‘‘, 21‘‘, 25‘‘	-
20‘‘	-	-	-	133.8	-	-	-
21‘‘25‘‘	7.30	m	-	129.6	2 x CH	22‘‘ 24‘‘	22‘‘ 24‘‘
22‘‘ 24‘‘	7.39	m	-	129.2	2 x CH	21‘‘25‘‘	21‘‘25‘‘
23‘‘	7.28	m	-	127.6	CH	21‘‘, 22‘‘, 24‘‘, 25‘‘	22‘‘, 24‘‘




173.46
170.30
166.83
147.65
142.43
138.79
138.44
138.32
135.75
133.79
130.63
129.56
129.15
127.68
126.99
125.72
122.38
121.16
114.37
75.90
70.05
68.06
64.75
60.39
58.78
52.80
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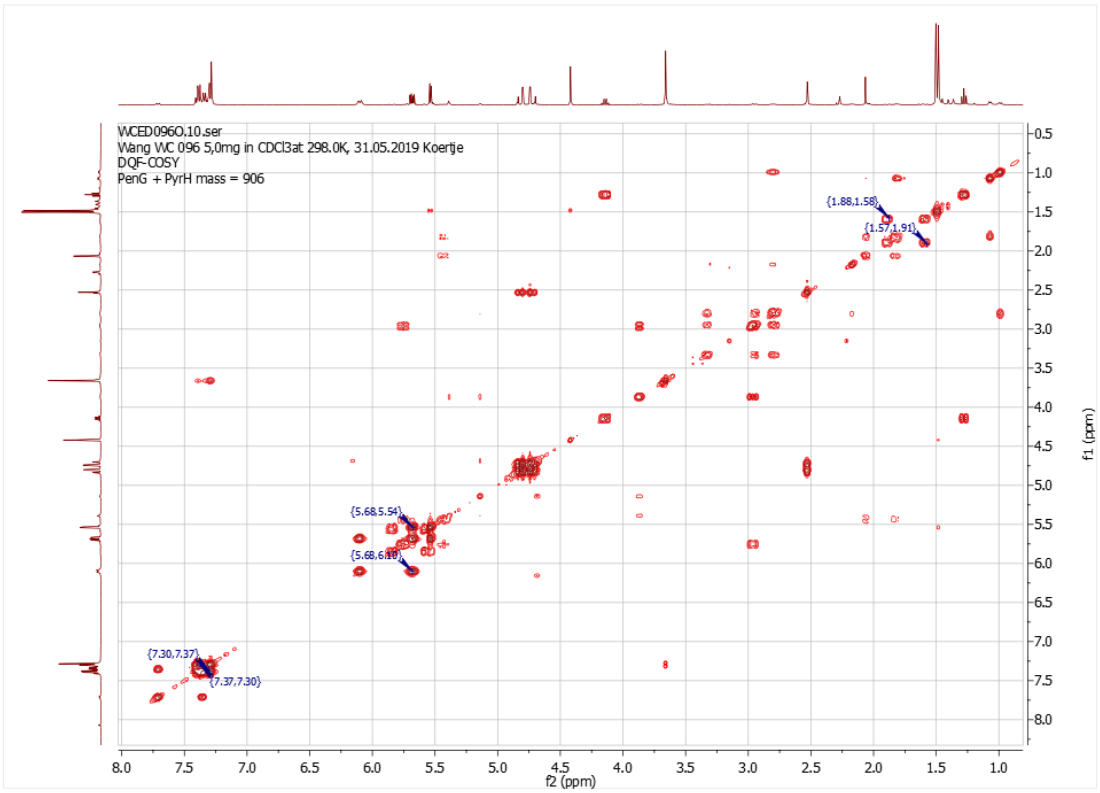
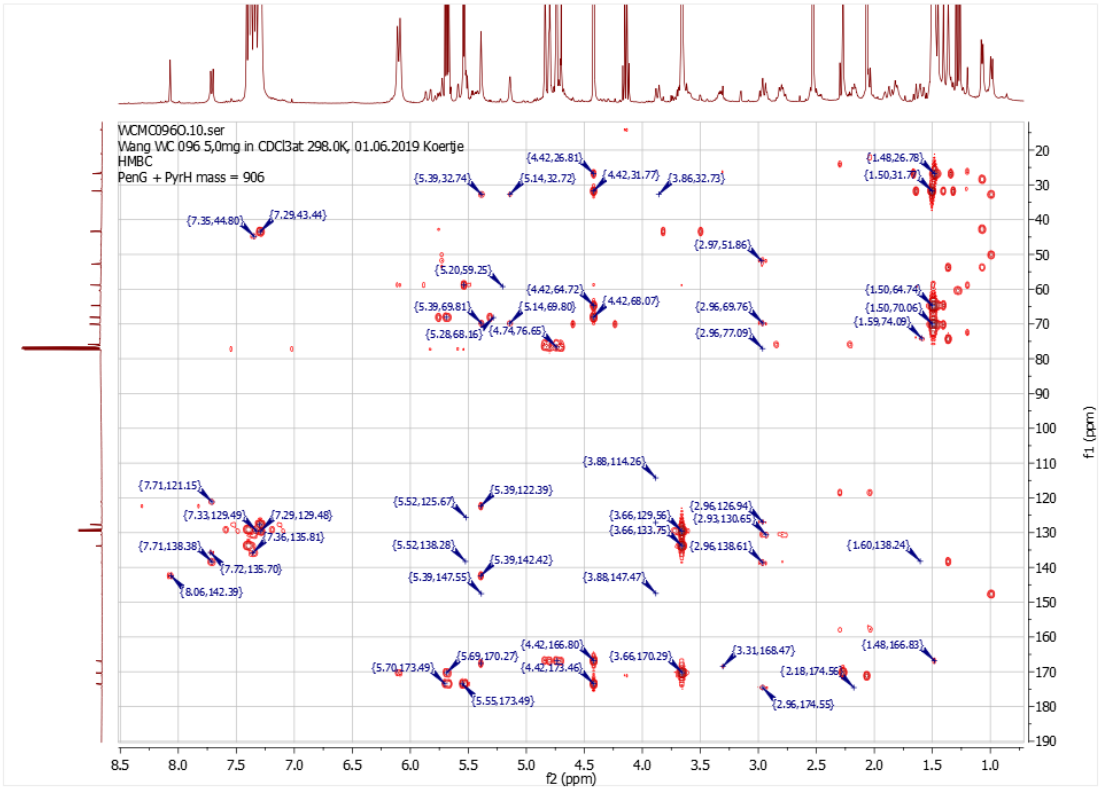
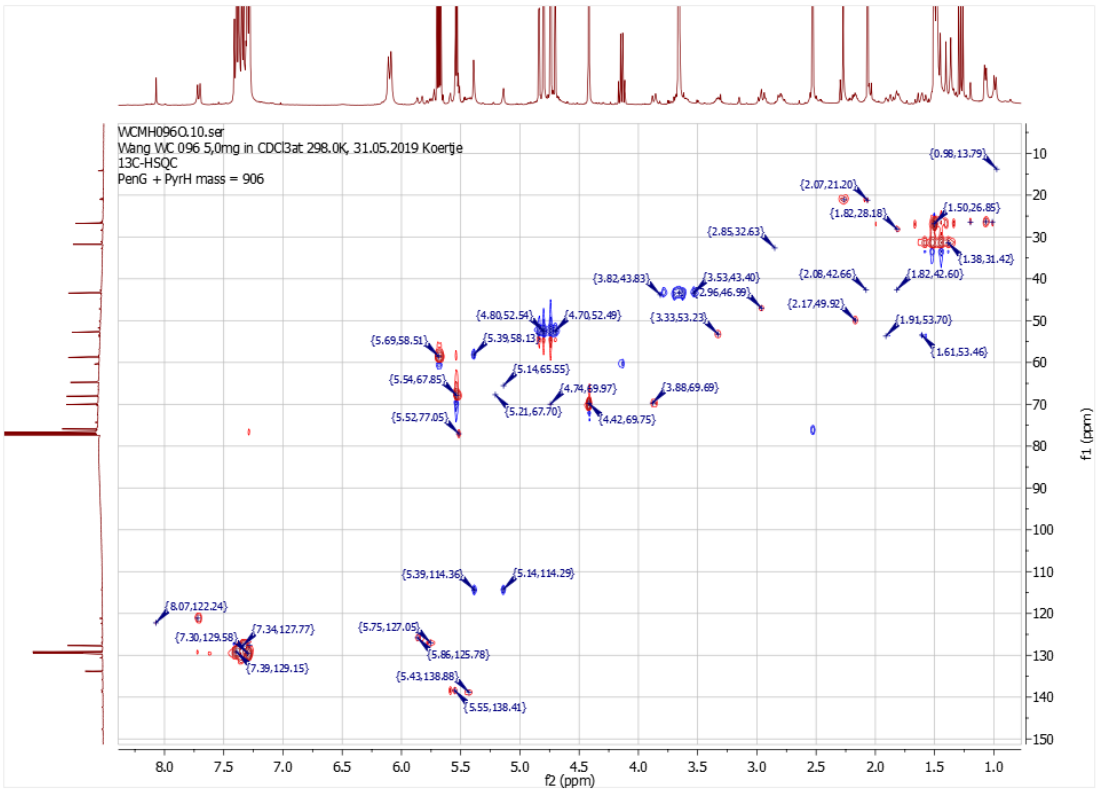
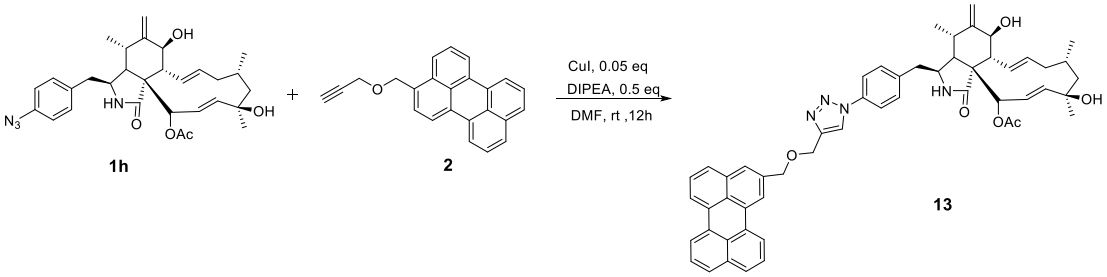
31.77
31.12
28.41
26.78
21.05
20.90
f1 (ppm)


WCED096O,10.ser
Wang WC 096 5,0mg in CDCl3at 298.0K, 31.05.2019 Koertje
DQF-COSY
PenG + PyrH mass = 906
{1.88,1.58}
{1.57,1.91}
{5.68,5.54}
{5.68,6.18}
{7.30,7.37}
{7.37,7.30}
f2 (ppm)
f1 (ppm)

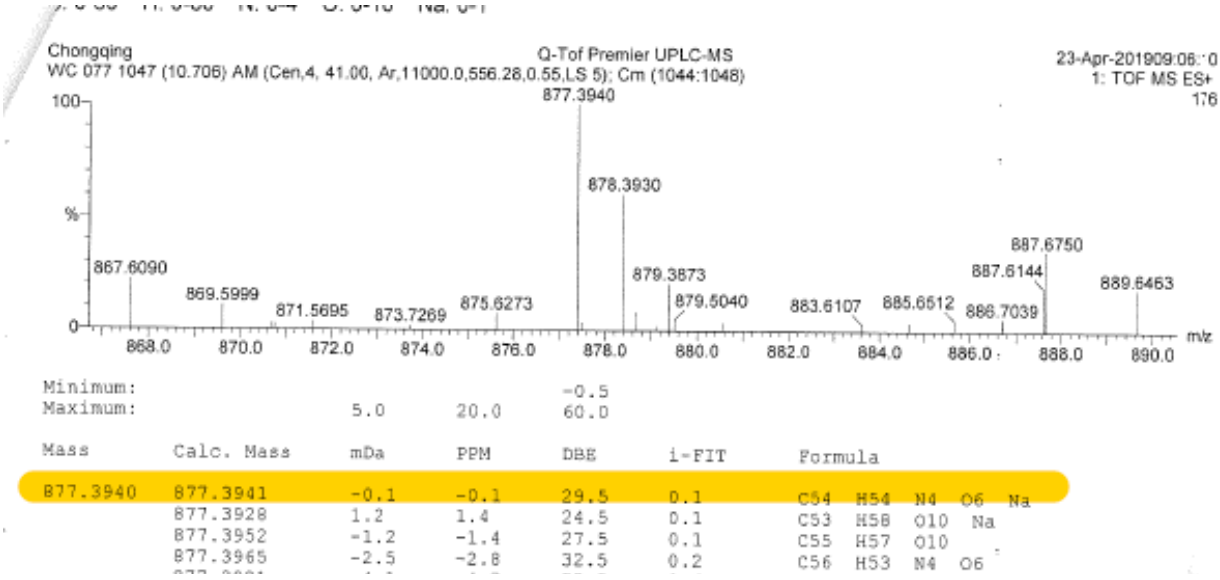

WCMC096O.10.ser
Wang WC 096 5,0mg in CDCl3at 298.0K, 01.06.2019 Koertje
HMBC
PenG + PyrH mass = 906
f2 (ppm)
f1 (ppm)


WCMH096O.10.ser
Wang WC 096 5,0mg in CDCl3at 298.0K, 31.05.2019 Koertje
13C-HSQC
PenG + PyrH mass = 906
f2 (ppm)
f1 (ppm)

2.15 4'-Perylene linked Cytochalasin H 13

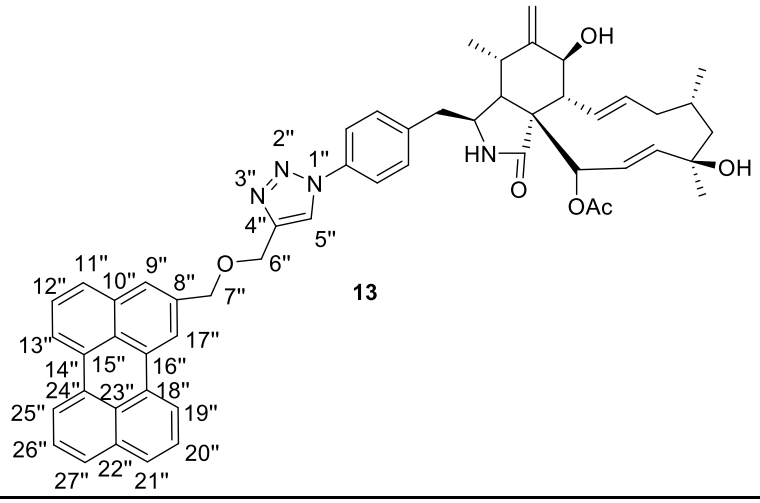


To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and perylene-linked acetylene **2** (12.2 mg, 0.038 mmol, 2.0 eq) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 8.0 mg of desired product (72.5%, 0.009 mmol) as a yellow solid.



HRMS (m/z): calculated for [$C_{54}H_{54}N_4O_6$ + Na]: 877.3941, found: 877.3940.

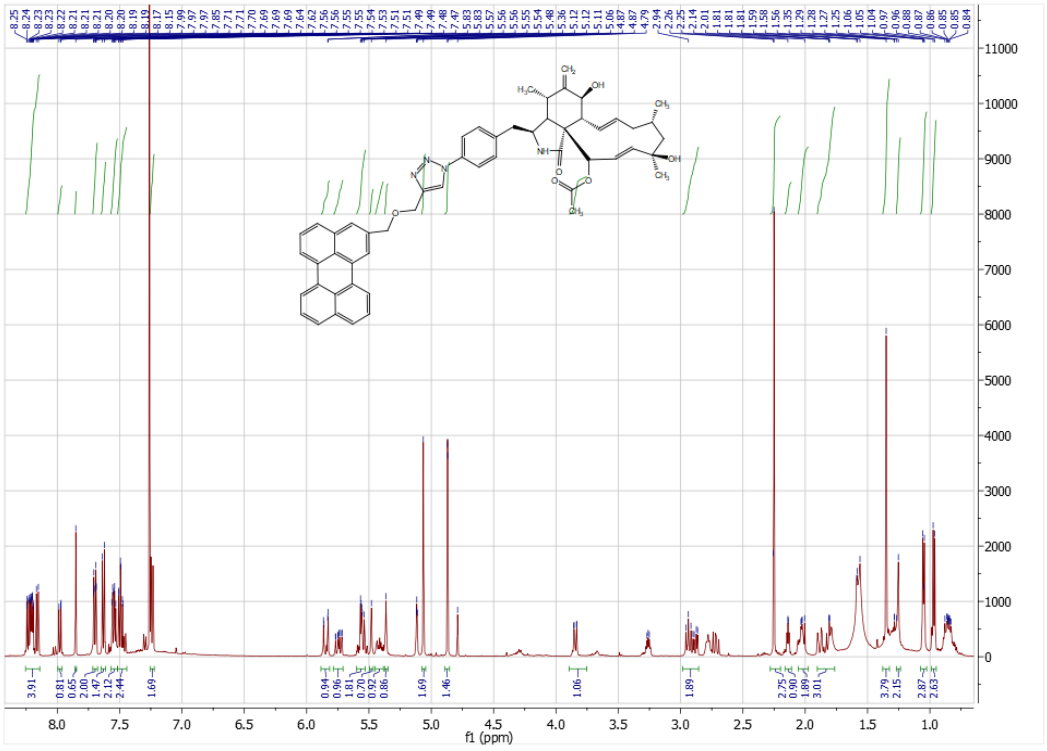
IR (powder, cm^{-1}): 2922, 1735, 1693, 1519, 1369, 1228, 1043, 1014, 962, 812, 767, 542.

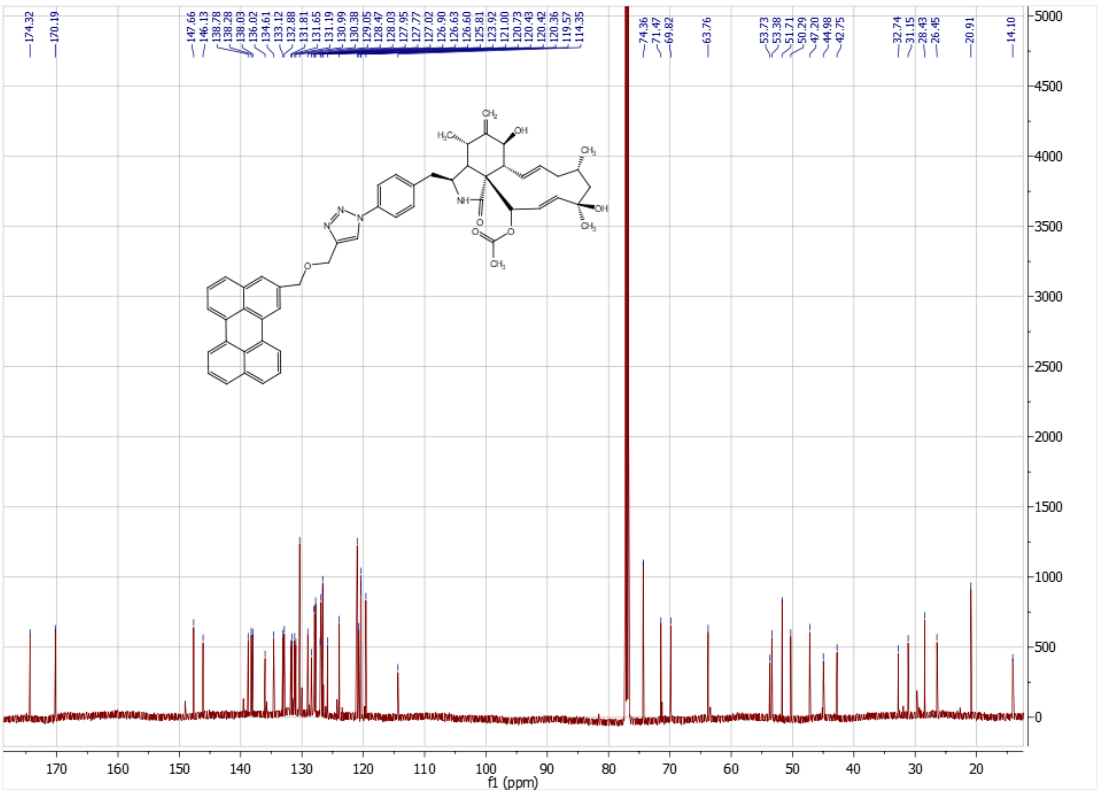


13

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
3	3.29	m	-	52.9	CH	1‘, 1, 4, 5	4, 5, 10
4	2.16	dd	3.7, 5.0	49.9	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.81	m	-	32.4	CH	1’, 3	4, 11, 12
6	-	-	-	147.7	-	-	-
7	3.88	dd	1.3, 10.8	69.4	CH	6, 12	8, 12
8	2.98	m	-	46.8	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.6	-	-	-
10a	2.74	m	-	44.7	CH_2	1’, 3	3, 4, 12
10b	2.90	m	-	-	-	-	-
11	0.99	d	6.6	13.7	CH_3	4, 5, 6	5
12a	5.14	brs	-	113.8	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.76	ddd	1.4, 9.7, 15.4	126.7	CH	7, 8, 15	8, 14, 15
14	5.42	ddd	4.8, 10.1, 15.4	138.4	CH	9, 15	13, 15
15a	1.83	m	-	42.3	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.82	m	-	28.1	CH	13, 14, 17	22
17a	1.61	m	-	53.3	CH_2	18, 19	-
17b	1.90	m	-	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.56	brs	-	137.9	CH	-	20
20	5.85	dd	2.2, 17.0	125.4	CH	18, 19	19
21	5.58	brs	-	76.8	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.4	26.1	CH_3	15, 16, 17	16
23	1.38	s	-	30.8	CH_3	17, 18, 19	-
1‘	-	-	-	138.0	-	-	-
2’ 6’	7.27	d	8.5	130.3	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.65	d	8.5	120.6	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	136.0	-	-	-
24	-	-	-	170.2	-	-	-
25	2.28	s	-	20.6	CH_3	24	-
4‘‘	-	-	-	146.1	-	-	-
5‘‘	7.87	s	-	120.4	CH	4‘‘	-
6‘‘	4.90	s	-	63.4	CH_2	4’’, 5’’,7’’	-
7‘‘	5.09	s	-	71.1	CH_2	6‘‘, 8‘‘, 17‘‘	-

8‘‘	-	-	-	133.0	-	-	-
9‘‘	8.18	s	-	119.2	CH	8‘‘, 10‘‘	-
10‘‘	-	-	-	129.1	-	-	-
11‘‘	8.00	dd	0.9, 8.3	123.2	CH	10‘‘, 13‘‘, 15‘‘	12‘‘, 13‘‘
12‘‘20‘‘26‘‘	7.56	m	-	127.3	3 x CH	13‘‘, 19‘‘, 25‘‘, 16‘‘, 22‘‘	11‘‘, 13‘‘
13‘‘19‘‘25‘‘	8.23	dd	1.0, 7.6	120.0	3 x CH	14‘‘, 15‘‘, 23‘‘	11‘‘, 12‘‘
14‘‘	-	-	-	128.3	-	-	-
15‘‘	-	-	-	132.8	-	-	-
16‘‘	-	-	-	131.1	-	-	-
17‘‘	7.72	d	8.2	127.6	CH	8‘‘, 9‘‘, 16‘‘, 18‘‘	21‘‘
18‘‘	-	-	-	128.5	-	-	-
21‘‘27‘‘	7.52	dd	1.6, 7.8	126.2	2 x CH	19‘‘, 22‘‘	17‘‘
22‘‘	-	-	-	134.7	-	-	-
23‘‘	-	-	-	135.1	-	-	-
24‘‘	-	-	-	132.0	-	-	-




174.32
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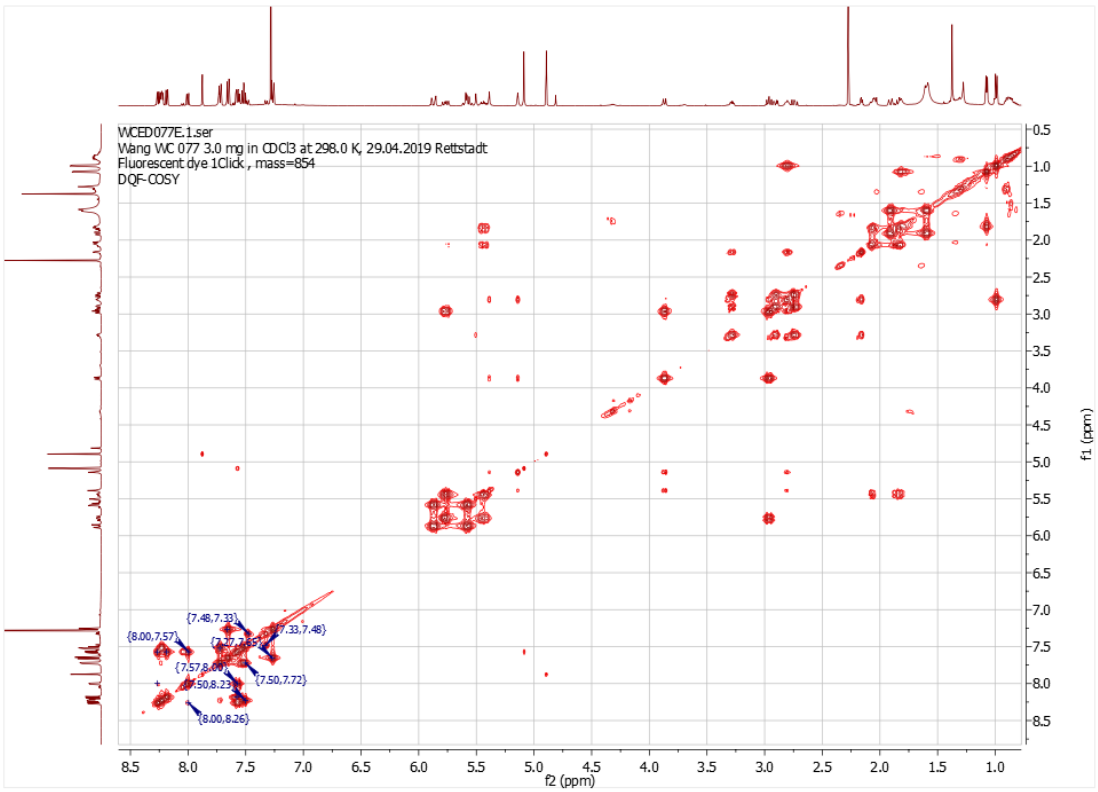
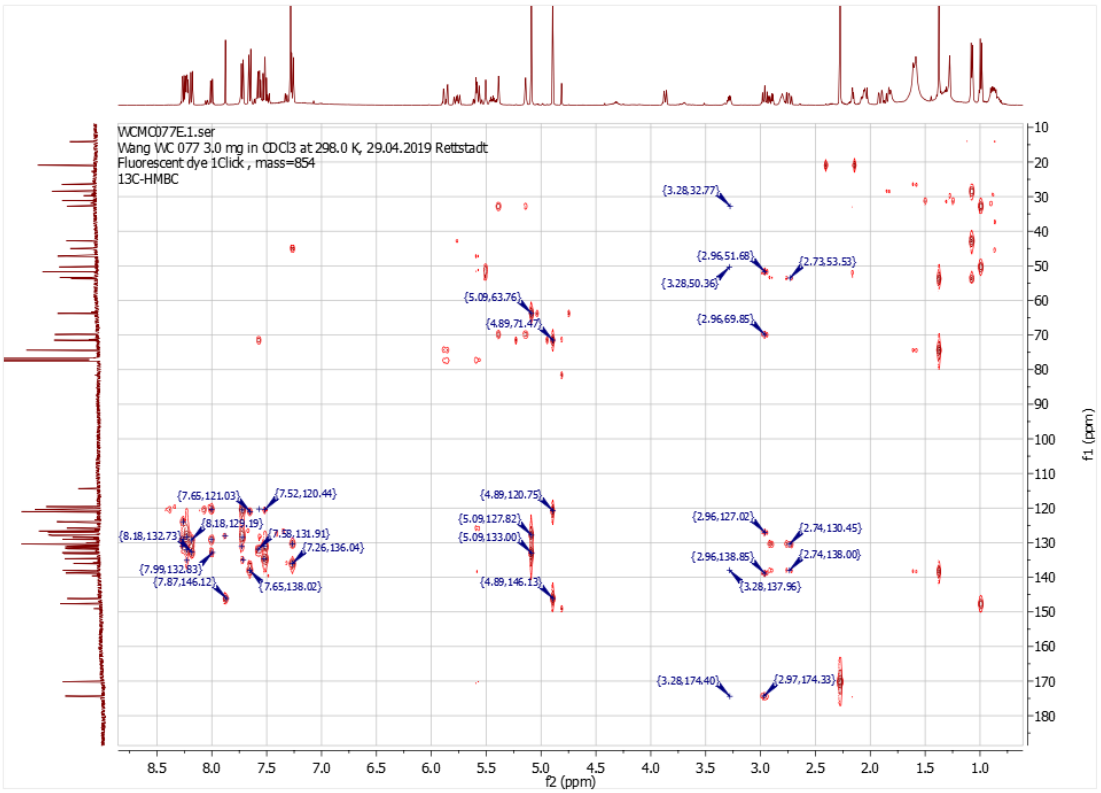
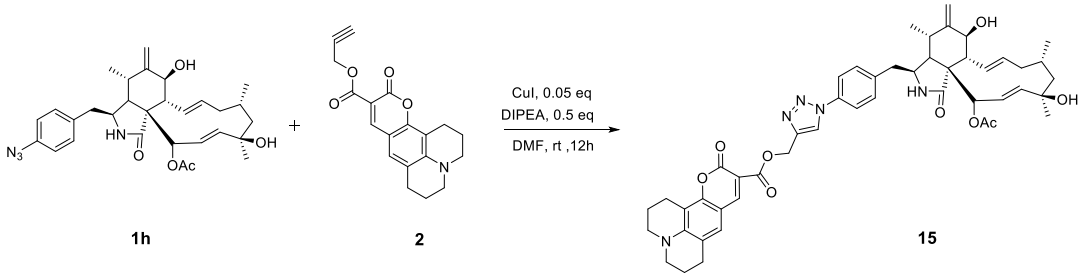
146.13
138.78
138.28
136.69
136.02
134.61
133.12
132.88
131.81
131.65
131.19
130.99
130.38
130.15
128.47
128.03
127.95
127.77
127.02
126.90
126.63
126.60
125.81
123.92
121.00
120.73
120.43
120.42
120.36
119.57
114.35
74.36
71.47
69.82
63.76
53.73
53.38
51.71
50.29
47.20
44.88
42.75
32.74
31.15
28.43
26.45
20.91
14.10
f1 (ppm)


WCED077E.1.ser
Wang WC 077 3.0 mg in CDCl3 at 298.0 K, 29.04.2019 Rettstadt
Fluorescent dye 1Click , mass=854
DQF-COSY
{7.48,7.33}
{8.00,7.57}
{7.33,7.48}
{7.57,8.00}
{7.50,8.23}
{7.50,7.72}
{8.00,8.26}
f2 (ppm)
f1 (ppm)


WCMC077E.1.ser
Wang WC 077 3.0 mg in CDCl3 at 298.0 K, 29.04.2019 Rettstadt
Fluorescent dye 1Click , mass=854
13C-HMBC
f2 (ppm)
f1 (ppm)

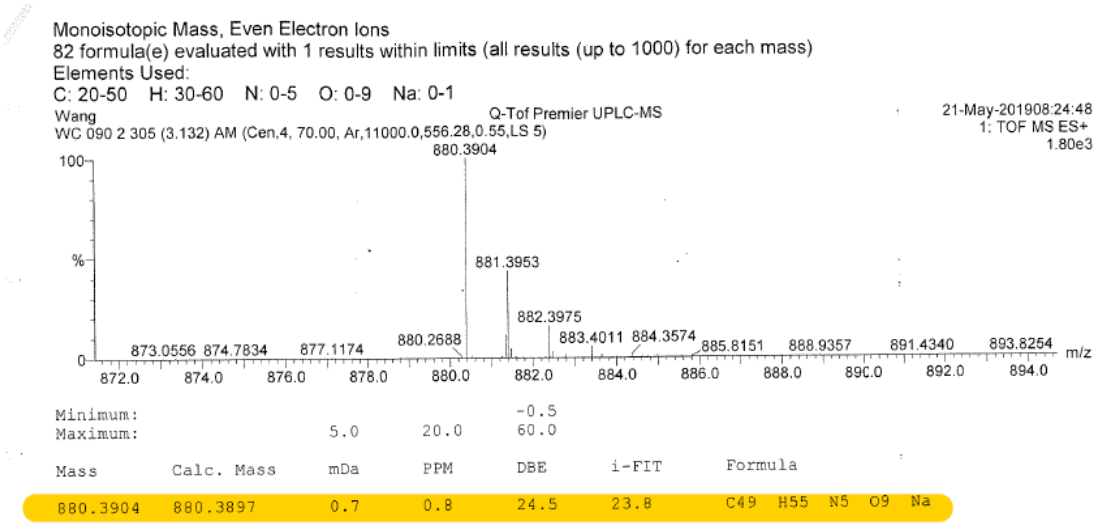

WCMH077E.1.ser
Wang WC 077 3.0 mg in CDCl3 at 298.0 K, 29.04.2019 Rettstadt
Fluorescent dye 1Click , mass=854
13C-HSQC
f2 (ppm)
f1 (ppm)

2.16 4-Coumarin dye-linked Cytoclasin H 15



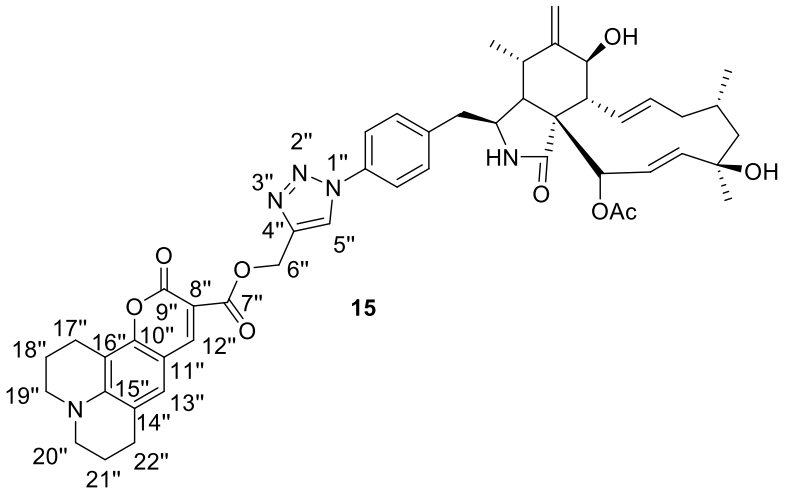
1h **2** **15**

To a stirred solution of **1h** (5 mg, 0.01 mmol, 1.0 eq) and acetylene-linked coumarin **2** (3.0 mg, 0.01 mmol, 1.0 eq) in DMF (2 mL) was added CuI (0.1 mg, 0.5 µmol, 0.05 eq) and DIPEA (0.6 mg, 4.8 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 6.3 mg of desired product (78.2%, 0.007 mmol) as a deep yellow solid.



HRMS (*m/z*): calculated for [$C_{49}H_{55}N_5O_9$ + Na]: 880.3897, found: 880.3904.

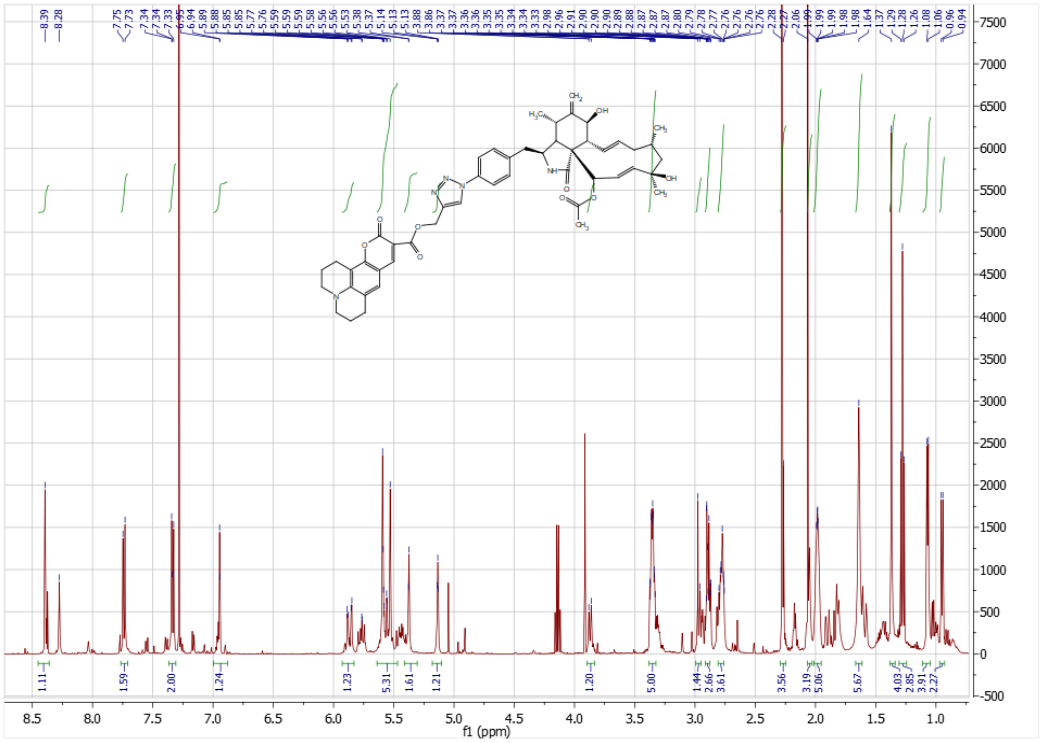
IR (powder, cm^{-1}): 3419, 2924, 1735, 1685, 1618, 1585, 1560, 1517, 1442, 1369, 1309, 1238, 1197, 1172, 1111, 1045, 962, 792, 439.

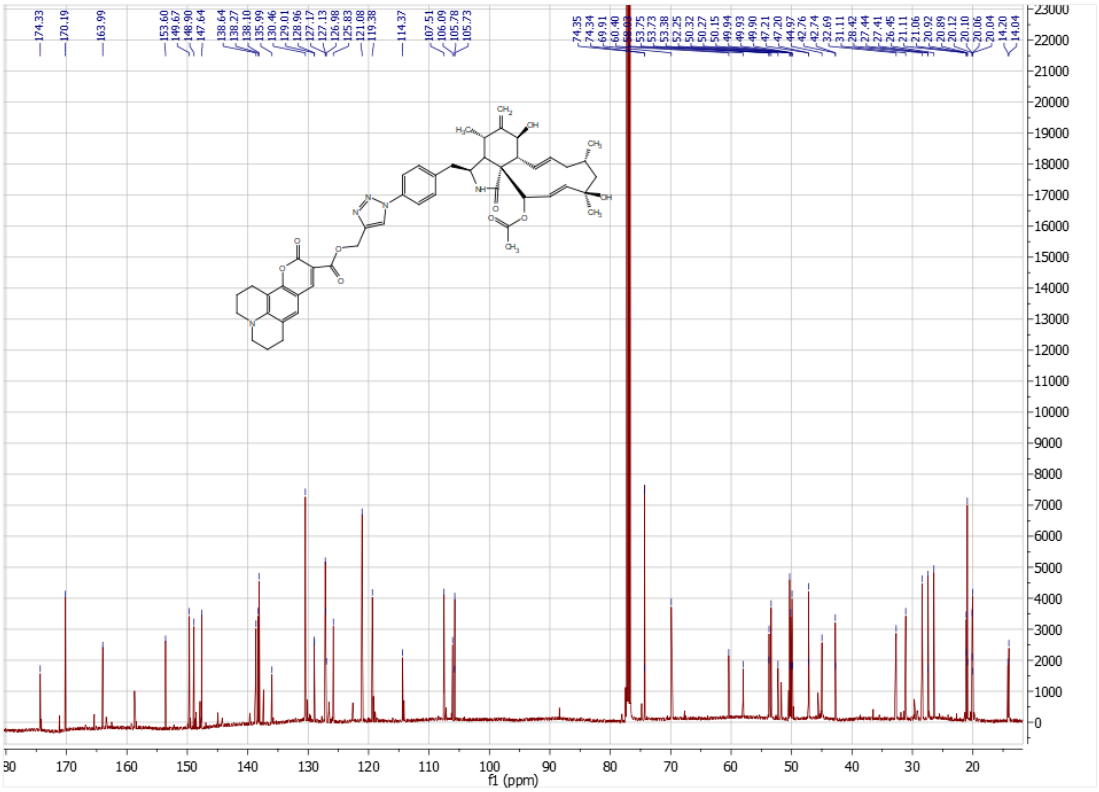


15

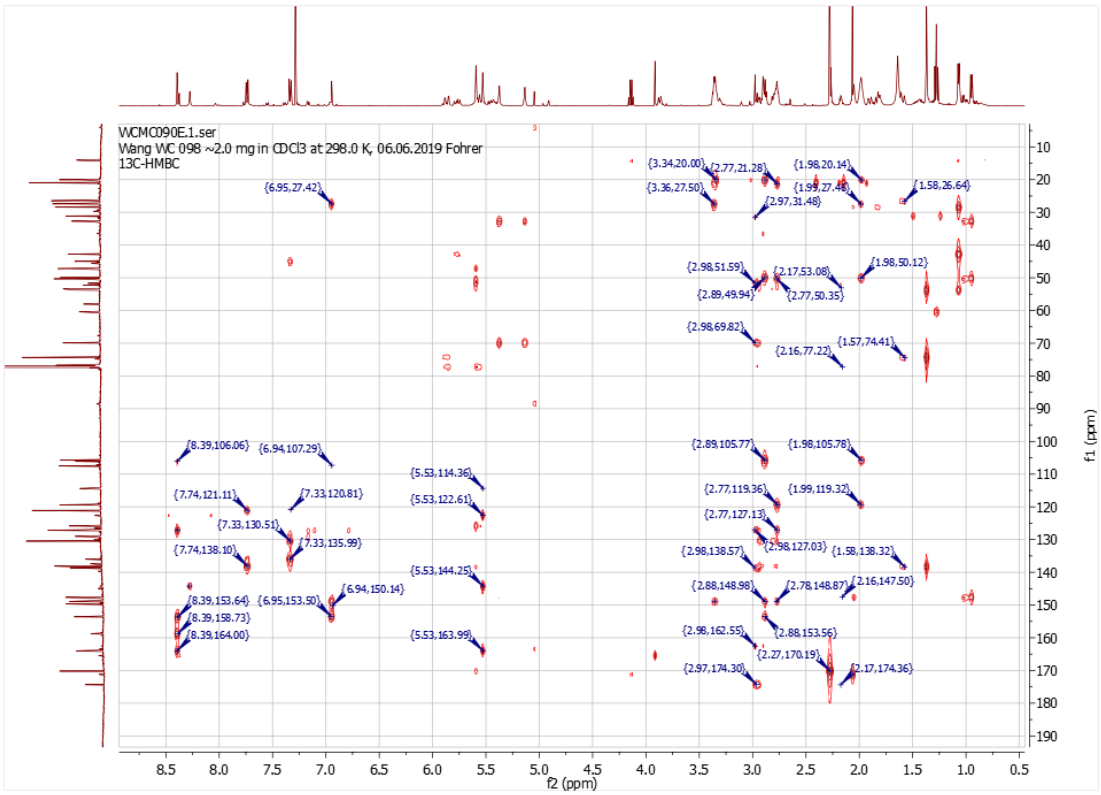
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
3	3.31	m	-	53.3	CH	1‘, 1, 4, 5	4, 5, 10
4	2.17	dd	3.7, 5.0	50.1	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.79	m	-	32.7	CH	1’, 3	4, 11, 12
6	-	-	-	148.9	-	-	-
7	3.86	dd	1.3, 10.8	69.8	CH	6, 12	8, 12
8	2.96	m	-	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.6	-	-	-
10a	2.77	m	-	45.0	CH_2	1’, 3	3, 4, 12
10b	2.90	m	-	-	-	-	-
11	0.94	d	6.6	14.0	CH_3	4, 5, 6	5
12a	5.13	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.76	ddd	1.4, 9.7, 15.4	127.1	CH	7, 8, 15	8, 14, 15
14	5.41	ddd	4.8, 10.1, 15.4	138.6	CH	9, 15	13, 15
15a	1.82	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.81	m	-	28.4	CH	13, 14, 17	22
17a	1.58	m	-	53.8	CH_2	18, 19	-
17b	1.88	m	-	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.59	brs	-	138.2	CH	-	20
20	5.85	dd	2.2, 17.0	125.8	CH	18, 19	19
21	5.56	brs	-	77.2	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.4	26.5	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	138.0	-	-	-
2’ 6’	7.33	d	8.5	130.3	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.73	d	8.5	121.0	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	136.0	-	-	-
24	-	-	-	170.2	-	-	-
25	2.27	s	-	20.9	CH_3	24	-
4‘‘	-	-	-	144.2	-	-	-
5‘‘	8.27	s	-	122.1	CH	4‘‘	-
6‘‘	5.53	s	-	58.0	CH_2	4’’, 5’’,7’’	-
7‘‘	-	-	-	164.0	-	-	-

8‘‘	-	-	-	106.1	-	-	-
9‘‘	-	-	-	158.7	-	-	-
10‘‘	-	-	-	153.6	-	-	-
11‘‘	-	-	-	107.3	-	-	
12‘‘	8.38	m	-	149.6	CH	7‘‘, 8‘‘, 9‘‘, 10‘‘, 13‘‘,	-
13‘‘	6.94	s	-	127.0	CH	10‘‘, 11‘‘, 12‘‘, 22‘‘	-
14‘‘	-	-	-	119.4	-	-	-
15‘‘	-	-	-	149.0	-	-	-
16‘‘	-	-	-	105.8	-	-	-
17‘‘	2.89	d	8.2	20.0	CH_2	10‘‘, 15‘‘, 16‘‘, 18‘‘, 19‘‘	18‘‘
18‘‘21‘‘	1.98	m	-	20.4	2 x CH_2	17’’, 19’’, 16’’, 22’’	17’’, 20’’, 22’’
19‘‘20‘‘	3.35	m	-	49.8	2 x CH_2	15‘‘, 18‘‘, 22‘‘	21‘‘
22‘‘	2.77	m	-	27.4	CH_2	13’’, 14’’, 15’’, 19’’, 21’’,	21’’



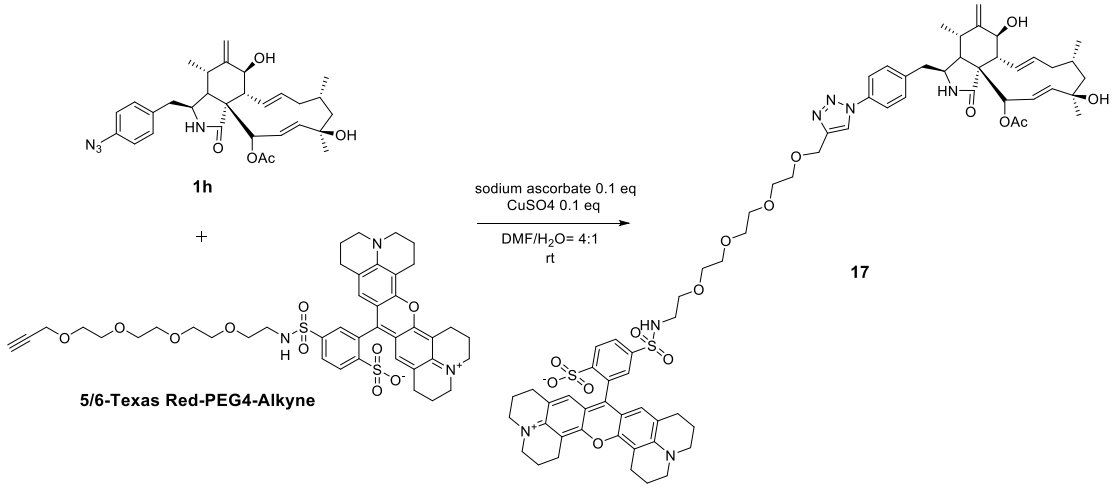

f1 (ppm)


WCED090E.1.ser
Wang WC 098 ~2.0 mg in CDCl3 at 298.0 K, 06.06.2019 Fohrer
DQF-COSY
f2 (ppm)
f1 (ppm)

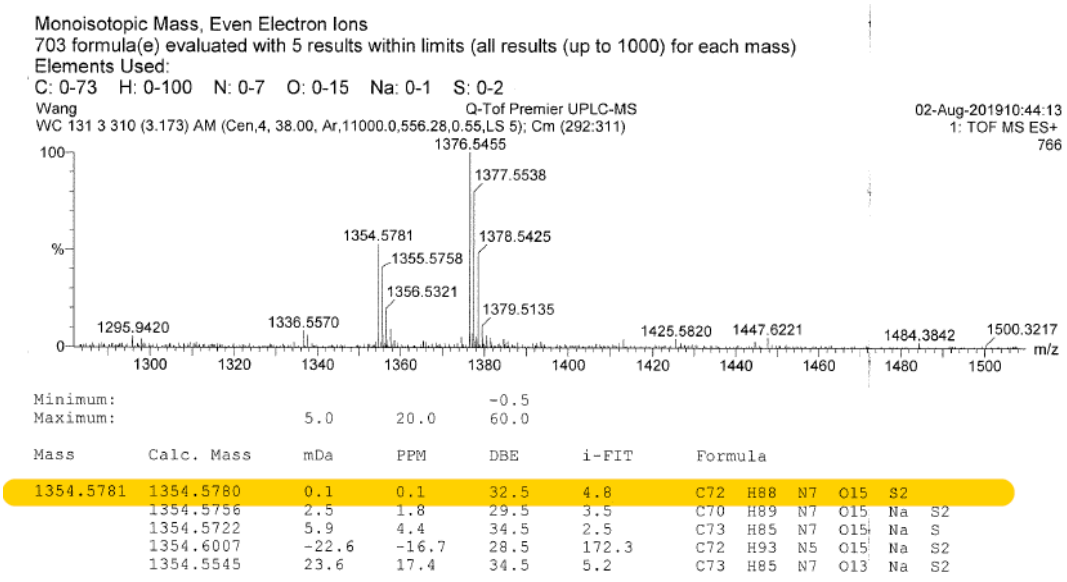

WCMC090E.1.ser
Wang WC 098 ~2.0 mg in CDCl3 at 298.0 K, 06.06.2019 Fohrer
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH090E.1.ser
Wang WC 098 ~2.0 mg in CDCl3 at 298.0 K, 06.06.2019 Fohrer
13C-HSQC
f2 (ppm)
f1 (ppm)

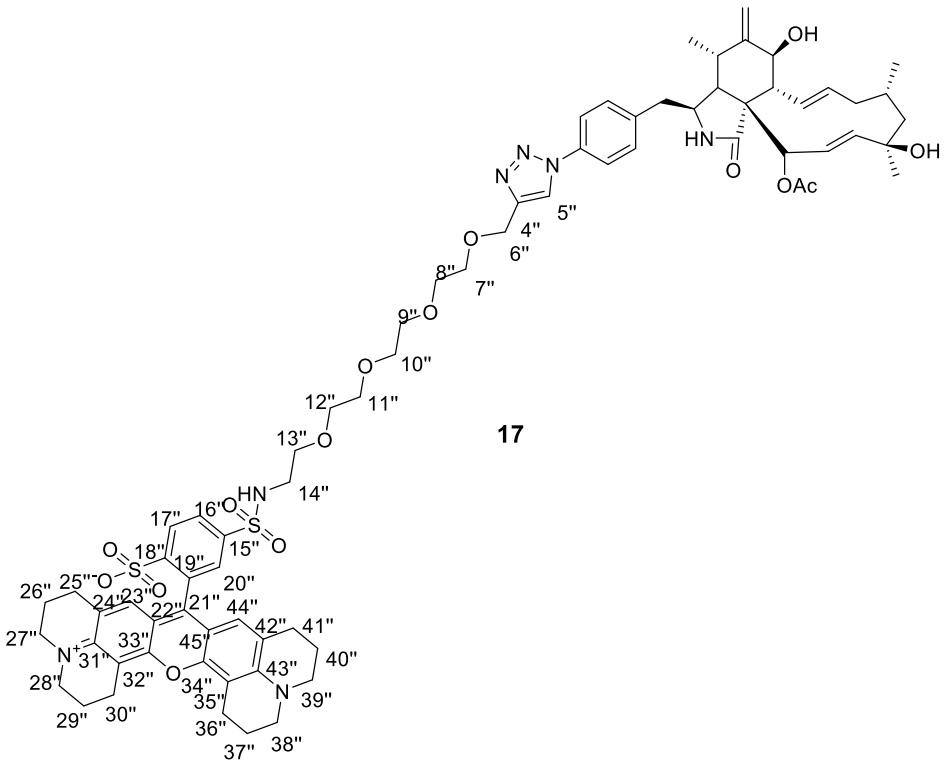
2.17 Dye-linked cytochalasin 17



To a stirred solution of **1h** (5.6 mg, 0.02 mmol, 2.0 eq) and **5/6-Texas Red-PEG$_4$-Alkyne** (4.0 mg, 0.005 mmol, 1.0 eq, purchased from Jena Bioscience) in 1.25 mL DMF-water mixture (4:1, *v/v*) was added sodium ascorbate (0.1 mg, 0.5 µmol, 0.1 eq) and copper sulfate (0.06 mg, 0.5 µmol, 0.1 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 2.6 mg of desired product (38.6%, 0.002 mmol) as a purple solid.



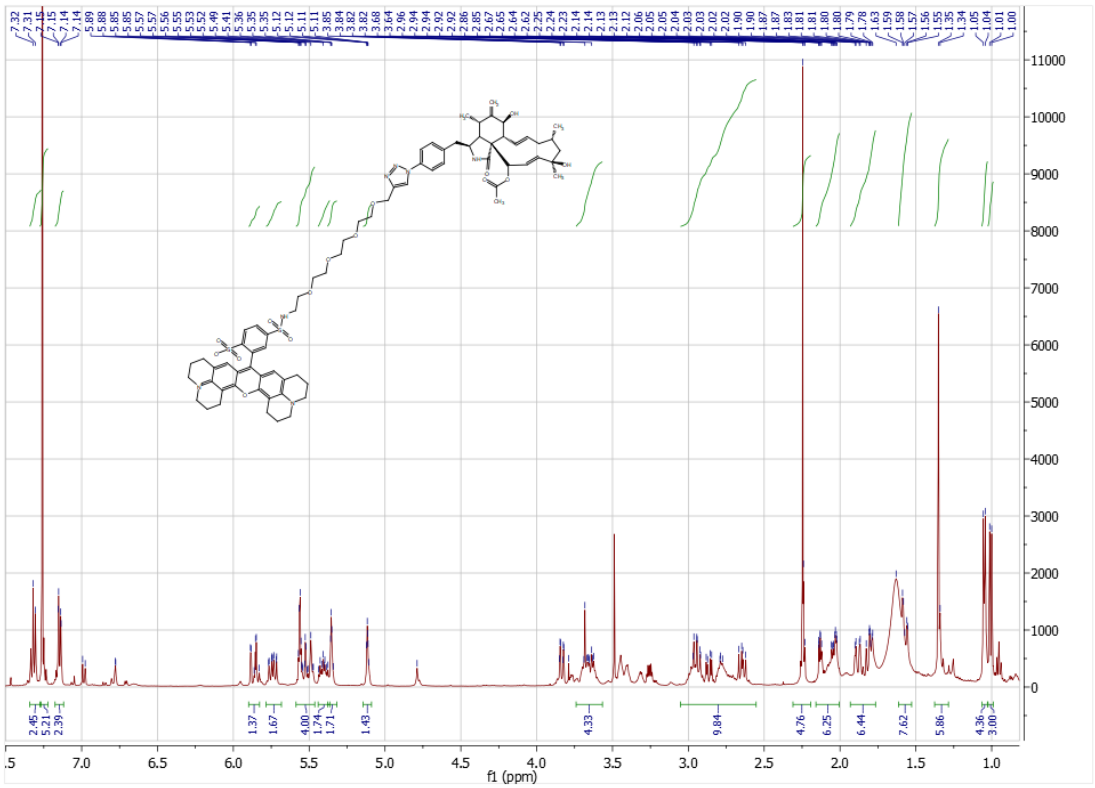
HRMS (*m/z*): calculated for [$C_{70}H_{89}N_7O_{15}S_2$ + H]: 1354.5756, found: 1354.5781.

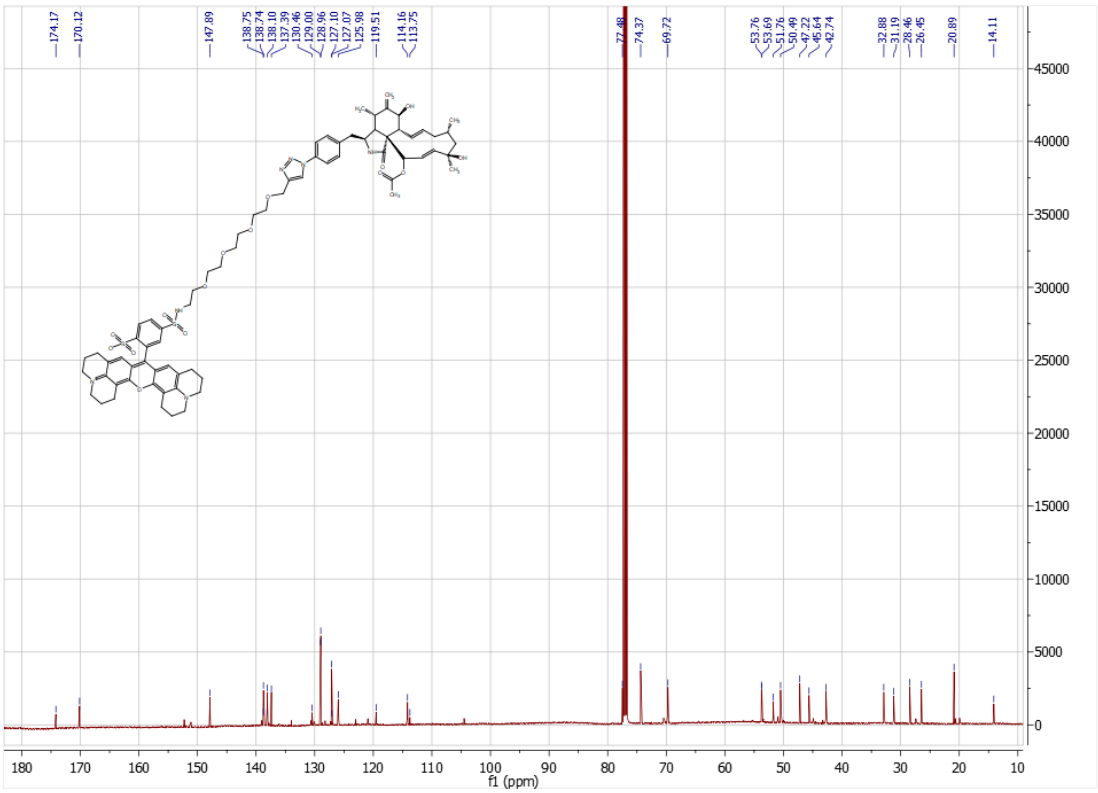


17

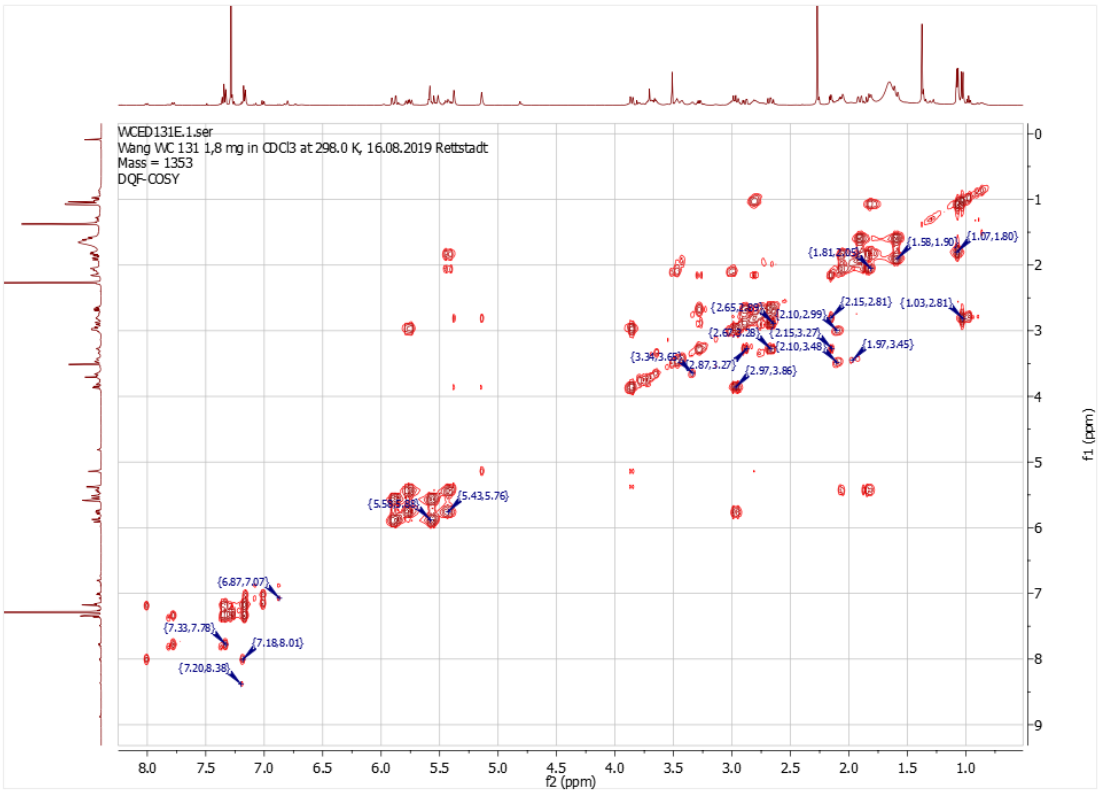
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.2	-	-	-
3	3.27	m	-	53.6	CH	1', 1, 4, 5	4, 5, 10
4	2.15	dd	3.7, 4.7	50.4	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.80	m	-	32.9	CH	1', 3	4, 11, 12
6	-	-	-	148.0	-	-	-
7	3.85	dd	1.4, 10.8	69.7	CH	6, 12	8, 12
8	2.97	m	-	47.2	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.7	-	-	-
10a	2.77	m	-	45.0	CH_2	1', 3	3, 4, 12
10b	2.90	m	-	-	-	-	-
11	1.04	d	6.7	14.2	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.38	brs	-	-	-	-	-
13	5.76	ddd	1.4, 9.7, 15.4	127.1	CH	7, 8, 15	8, 14, 15
14	5.45	m	-	138.7	CH	9, 15	13, 15
15a	1.83	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.81	m	-	28.5	CH	13, 14, 17	22
17a	1.58	m	-	53.8	CH_2	18, 19	-
17b	1.89	m	-	-	-	-	-
18	-	-	-	74.3	-	-	-
19	5.59	brs	-	138.1	CH	-	20
20	5.91	dd	2.8, 16.5	125.8	CH	18, 19	19
21	5.59	brs	-	77.4	CH	8, 9, 19, 20, 23	-
22	1.08	d	6.5	26.5	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1'	-	-	-	137.7	-	-	-
2' 6'	7.36	d	8.1	128.8	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.79	d	8.1	120.8	2 x CH	1', 4'	2', 6'
4'	-	-	-	137.5	-	-	-

24	-	-	-	170.1	-	-	-
25	2.26	s	-	20.9	CH_3	24	-
4''	-	-	-	145.9	-	-	-
5''	8.36	s	-	121.2	CH	4''	-
6''	4.81	s	-	64.3	CH_2	4'', 5'', 7''	-
7'', 8'', 9'', 10'', 11'', 12''	3.68	m	-	70.2	6 x CH_2	7'',8'',9'', 10'',11'',12''	-
13''	3.79	m	-	69.7	CH_2	-	14''
14''	3.34	m	-	43.3	CH_2	-	13''
15''	-	-	-	145.7	-	-	-
16'', 17''	8.01	d	7.9	126.8	2 x CH	18'', 19''	-
18''	-	-	-	127.4	-	-	-
19''	-	-	-	131.5	-	-	-
20''	8.36	s	-	121.2	CH	21'', 22''	-
21''	-	-	-	134.4	-	-	-
22''	-	-	-	126.5	-	-	-
23''	7.27	s	-	127.1	CH	24'', 31''	-
24''	-	-	-	129.1	-	-	-
25'', 30''	2.73	m	-	27.4	2 x CH_2	23'', 24''	-
26'', 29''	1.28	m	-	29.6	2 x CH_2	25'', 27''	-
27'', 28''	3.85	m	-	47.2	2 x CH_2	26''	-
31''	-	-	-	137.5	-	-	-
32''	-	-	-	73.6	-	-	-
33''	-	-	-	150.0	-	-	-
34''	-	-	-	129.0	-	-	-
35''	-	-	-	151.3	-	-	-
36'', 41''	2.66	m	-	27.4	2 x CH_2	34'', 38''	38''
37'', 40''	1.97	m	-	20.6	2 x CH_2	36''	38''
38'', 25'', 27'', 39''	3.47	m	-	50.5	2 x CH_2	37'', 43''	36'', 37''
42''	-	-	-	113.7	-	-	-
43''	-	-	-	152.7	-	-	-
44''	6.83	s	-	128.1	CH	41'', 42'', 43'', 45''	-
45''	-	-	-	104.6	-	-	-


f1 (ppm)

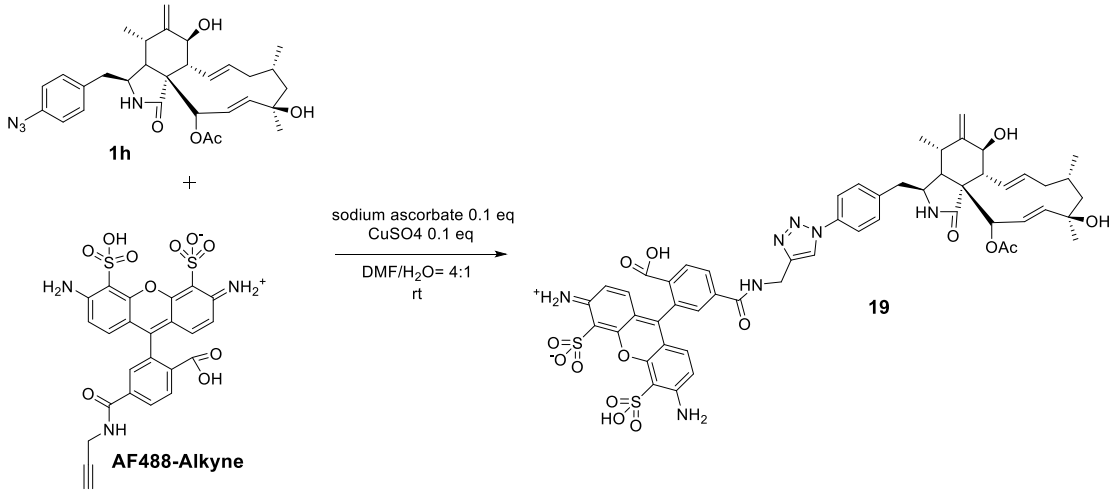

f1 (ppm)


WCMC131E.1.ser
Wang WC 131 1,8 mg in CDCl3 at 298.0 K, 16.08.2019 Rettstadt
Mass = 1353
13C-HMBC
f2 (ppm)
f1 (ppm)

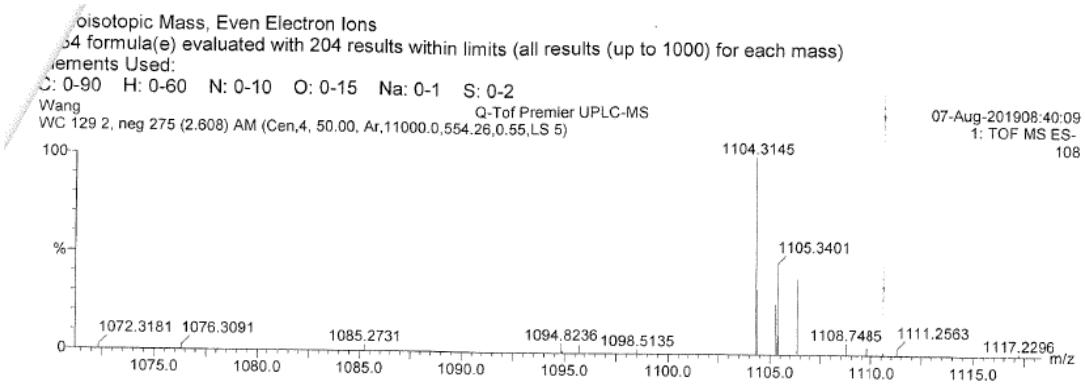

WCED131E.1.ser
Wang WC 131 1,8 mg in CDCl3 at 298.0 K, 16.08.2019 Rettstadt
Mass = 1353
DQF-COSY
f2 (ppm)
f1 (ppm)


WCMH131E.1.ser
Wang WC 131 1,8 mg in CDCl3 at 298.0 K, 16.08.2019 Rettstadt
Mass = 1353
13C-HSQC
{1.04,14.15}
{2.99,19.91}
{2.26,20.92}
{1.97,20.61}
{2.73,27.43}
{1.81,28.50}
{1.08,26.53}
{1.28,29.61}
{2.80,32.85}
{2.66,27.35}
{1.37,31.22}
{3.34,43.26}
{2.05,42.74}
{1.83,42.64}
{3.85,47.23}
{2.97,47.17}
{2.15,50.39}
{3.27,53.63}
{1.58,53.76}
{3.47,50.46}
{1.89,53.76}
{4.81,64.29}
{4.11,63.45}
{3.68,70.24}
{3.85,69.70}
{3.79,69.68}
{5.59,77.39}
{5.38,114.17}
{5.14,114.04}
{8.36,121.23}
{7.79,120.81}
{7.00,119.43}
{7.14,128.85}
{6.83,128.09}
{5.76,127.12}
{8.01,126.81}
{5.91,125.79}
{8.39,130.24}
{7.36,128.82}
{5.45,138.65}
{5.59,138.06}
f2 (ppm)
f1 (ppm)

2.18 Dye-Linked Cytochalasin H 19

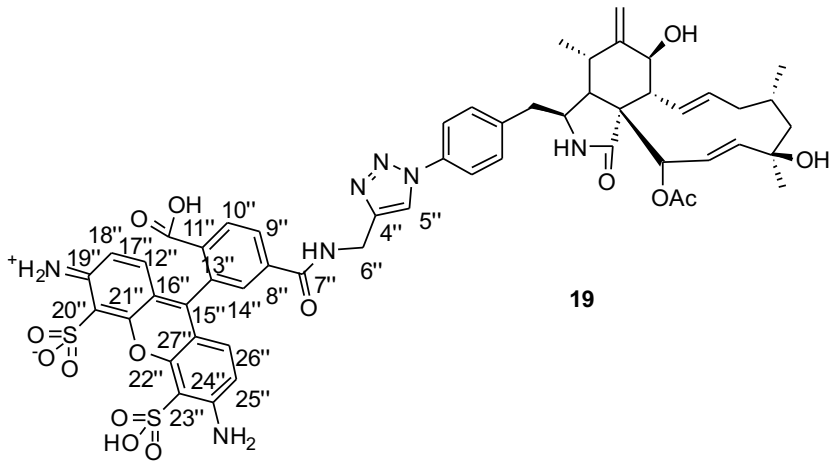


To a stirred solution of **1h** (5.6 mg, 0.02 mmol, 2.0 eq) and **AF488-Alkyne** (3.0 mg, 0.01 mmol, 1.0 eq, purchase from Jena Bioscience) in 4.25 mL DMF-water mixture (4:1, *v/v*) was added sodium ascorbate (0.2 mg, 1 µmol, 0.1 eq) and copper sulfate (0.12 mg, 1 µmol, 0.1 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 1.7 mg of desired product (28.5%, 0.001 mmol) as a red solid.



Minimum:				-0.5		
Maximum:		5.0	20.0	60.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1104.3145	1104.3146	-0.1	-0.1	54.5	8.5	C71 H42 N7 O7
	1104.3148	-0.3	-0.3	57.5	9.0	C79 H46 N O4 S
	1104.3142	0.3	0.3	41.5	6.3	C65 H51 N3 O11 Na S
	1104.3141	0.4	0.4	48.5	7.0	C71 H50 N3 O6 S2
	1104.3149	-0.4	-0.4	50.5	8.4	C73 H47 N O9 Na
	1104.3149	-0.4	-0.4	37.5	4.7	C58 H51 N9 O9 Na S2
	1104.3140	0.5	0.5	38.5	6.7	C57 H47 N9 O14 Na
	1104.3139	0.6	0.5	45.5	6.3	C63 H46 N9 O9 S
	1104.3137	0.8	0.7	59.5	9.2	C78 H43 N5 Na S
	1104.3136	0.9	0.8	32.5	4.2	C57 H55 N5 O13 Na S2
	1104.3154	-0.9	-0.8	53.5	7.6	C72 H46 N7 O2 S2

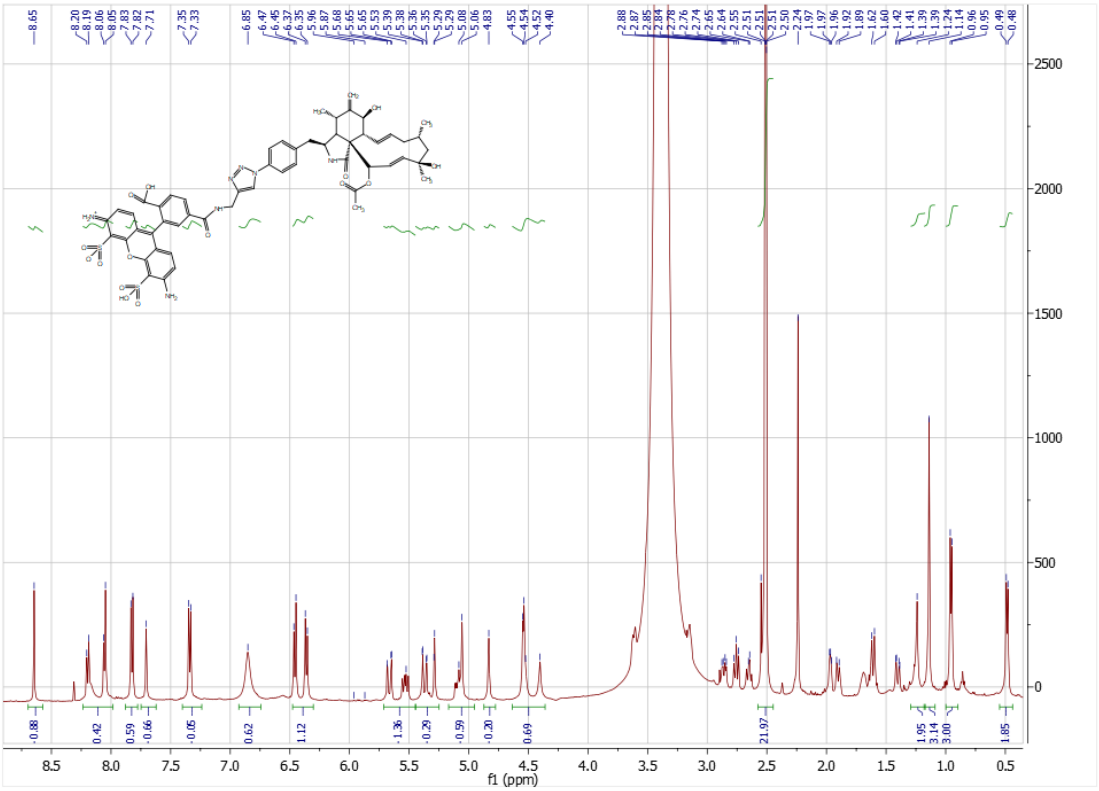
HRMS (*m/z*): calculated for [$C_{57}H_{47}N_9O_9S_2$ + Na]: 1104.3140, found: 1104.3145.

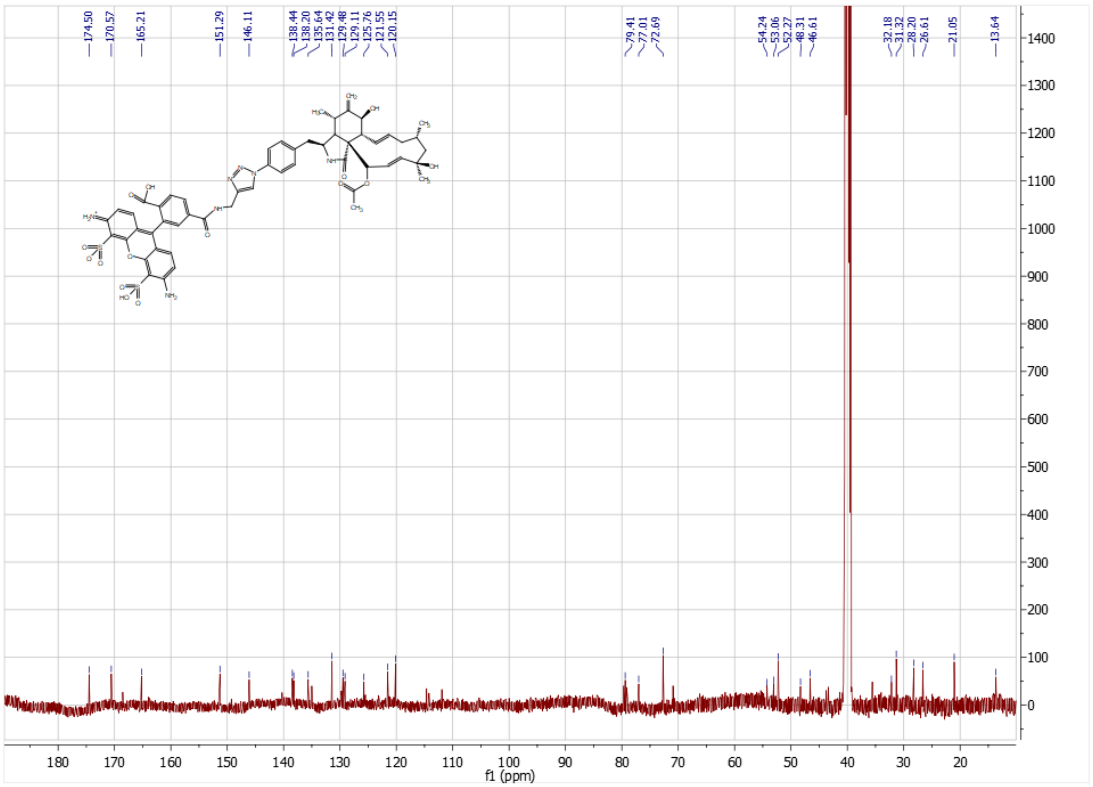


19

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.6	-	-	-
3	3.15	m	-	53.0	CH	1	10
4	1.97	dd	3.7, 5.0	48.3	CH	3, 5	5
5	2.51	m	-	32.2	CH	1', 3	4
6	-	-	-	146.1	-	-	-
7	3.63	dd	1.3, 10.8	71.0	CH	-	8
8	2.74	m	-	46.6	CH	-	7
9	-	-	-	52.4	-	-	-
10a	2.66	m	-	43.8	CH_2	1', 3	3
10b	2.85	m	-	-	-	-	-
11	0.48	d	6.6	13.7	CH_3	4, 5, 6	5
12a	4.84	brs	-	111.8	CH_2	5, 6, 7	7
12b	5.06	brs	-	-	-	-	-
13	5.54	ddd	1.4, 9.7, 15.4	129.2	CH	7, 8, 15	8
14	5.09	ddd	4.8, 10.1, 15.4	134.9	CH	9, 15	13, 15
15a	1.62	m	-	43.5	CH_2	114, 16,	13, 14,
15b	1.90	m	-	-	-	-	-
16	1.69	m	-	28.2	CH	13, 14, 17	22
17a	1.39	m	-	54.3	CH_2	18, 19	-
17b	1.60	m	-	-	-	-	-
18	-	-	-	72.7	-	-	-
19	5.39	brs	-	138.4	CH	-	20
20	5.68	dd	2.2, 17.0	125.8	CH	18, 19	19
21	5.29	brs	-	77.0	CH	19, 20	-
22	0.95	d	6.4	26.7	CH_3	15, 16, 17	16
23	1.14	s	-	31.4	CH_3	17, 18, 19	-
1'	-	-	-	138.3	-	-	-
2' 6'	7.35	d	8.5	131.3	2 x CH	3', 4', 5'	3', 5'
3' 5'	7.84	d	8.5	120.0	2 x CH	1', 4'	2', 6'
4'	-	-	-	135.6	-	-	-
24	-	-	-	170.6	-	-	-
25	2.24	s	-	21.1	CH_3	24	-
4''	-	-	-	146.0	-	-	-
5''	8.65	s	-	121.0	CH	4''	-
6''	4.54	s	-	35.6	CH_2	4'', 5'',7''	-
7''	-	-	-	168.7	-	-	-
8''	-	-	-	140.4	-	-	-
9''	8.05	d	7.7	125.3	CH	8''	10''

10‘‘	8.19	d	7.7	129.6	CH	9’’, 11’’, 12’’	9’’
11‘‘	-	-	-	130.3	-	-	-
12‘‘	-	-	-	165.5	-	-	-
13‘‘	-	-	-	138.3	-	-	-
14‘‘	7.71	s	-	123.1	CH	12’’, 15’’	-
15‘‘	-	-	-	129.3	-	-	-
16‘‘	-	-	-	130.0	-	-	-
17‘‘	5.68	dd	8.8, 15.5	138.3	CH	-	-
18‘‘	6.47	d	8.8	114.1	CH	19‘‘, 20‘‘	-
19‘‘, 24‘‘	-	-	-	114.7	2 x CH	-	-
20‘‘	-	-	-	105.2	-	-	-
21‘‘	-	-	-	151.2	-	-	-
22‘‘	-	-	-	149.4	-	-	-
23‘‘	-	-	-	114.6	-	-	-
25‘‘	6.36	d	8.8	129.5	CH	22‘‘	-
26‘‘	5.06	m	-	134.9	2 x CH	-	-
27‘‘	-	-	-	105.5	-	-	-




174.50
170.57
165.21
151.29
146.11
138.44
138.20
135.64
131.42
129.48
129.11
125.76
121.55
120.15
79.41
77.01
72.69
54.24
53.06
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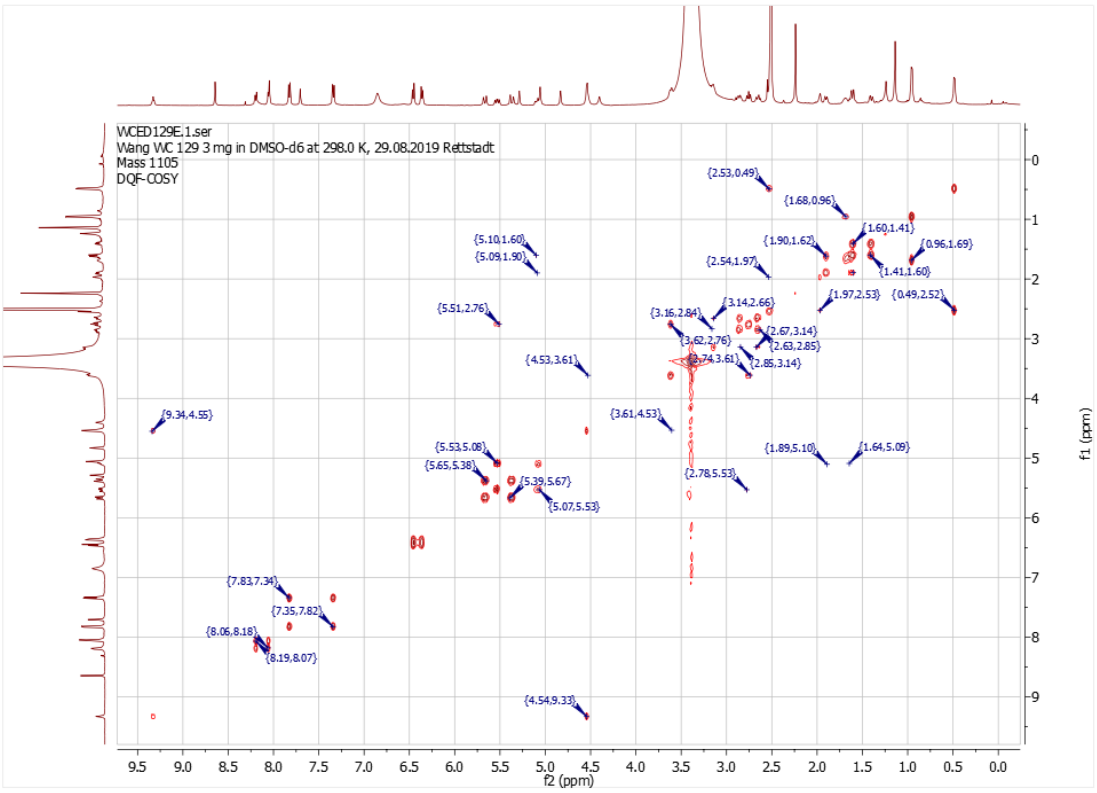
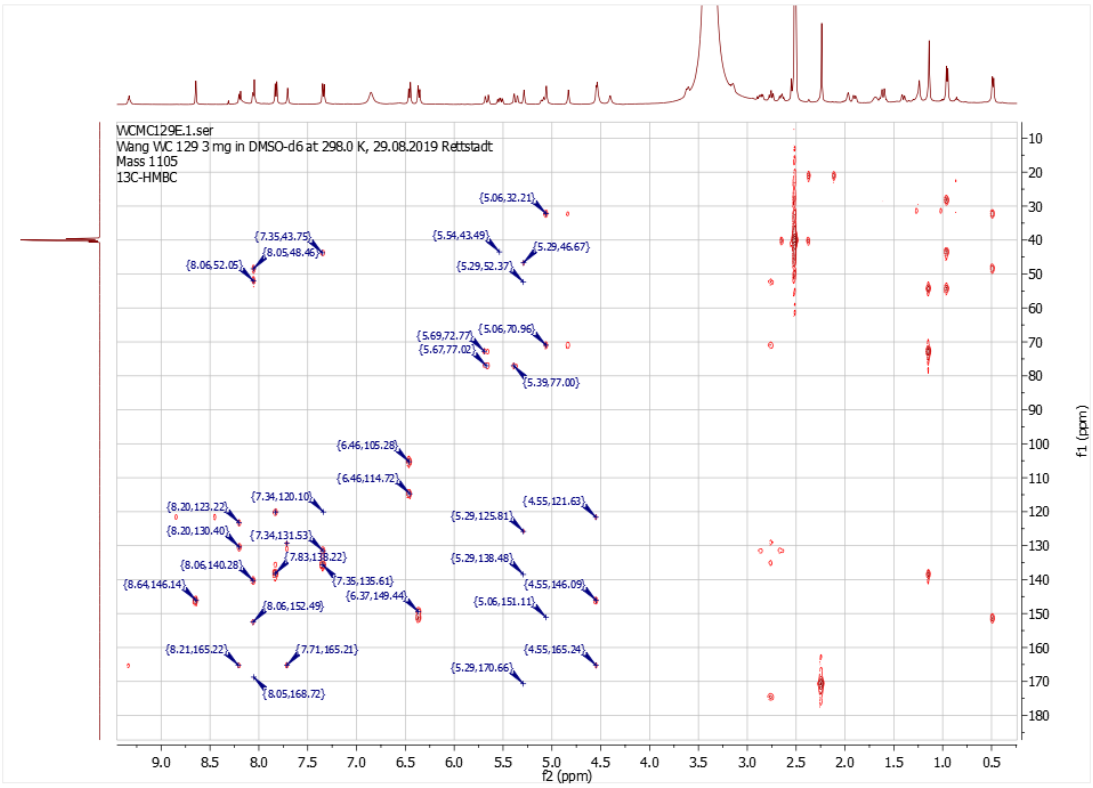
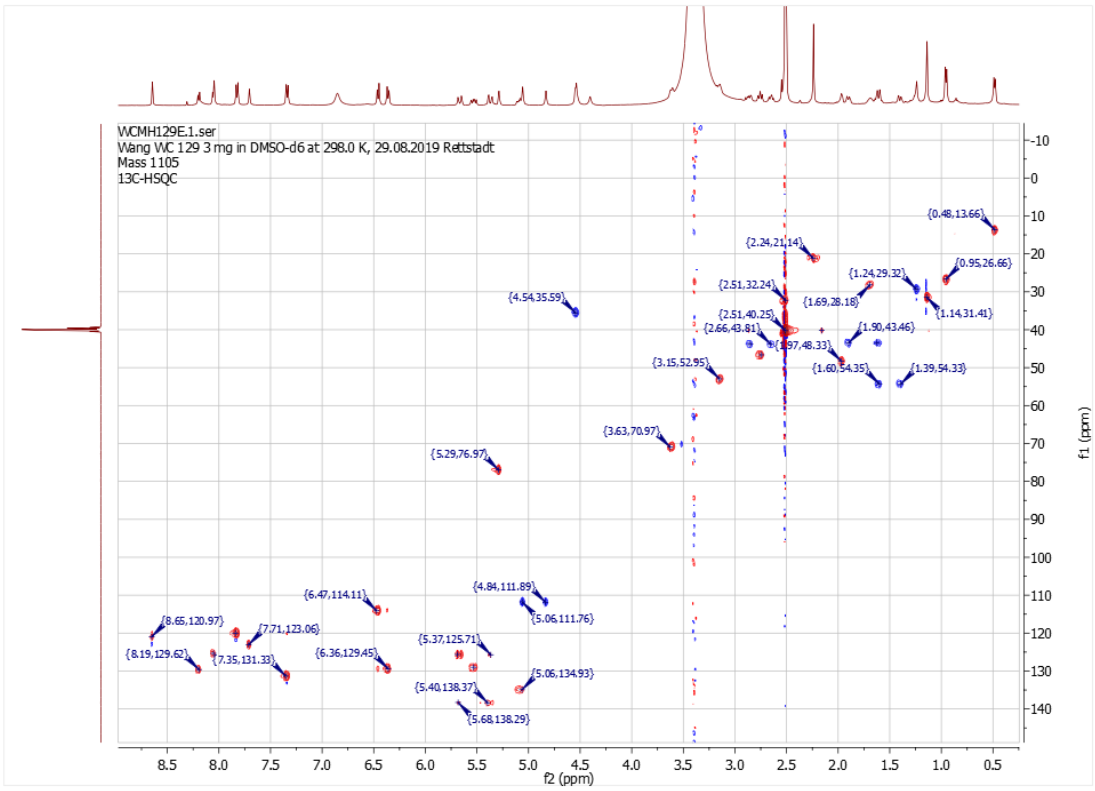
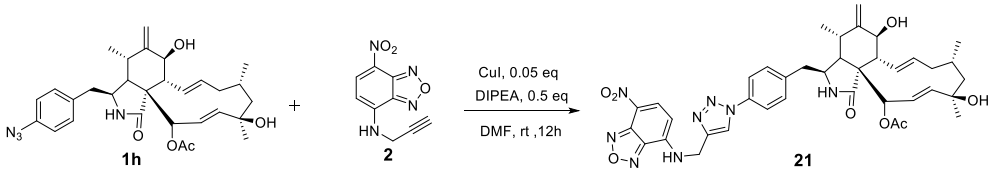
48.31
46.61
32.18
31.32
28.20
26.61
21.05
13.64
f1 (ppm)


WCED129E.1.ser
Wang WC 129 3 mg in DMSO-d6 at 298.0 K, 29.08.2019 Rettstadt
Mass 1105
DQF-COSY
{2.53,0.49}
{1.68,0.96}
{1.60,1.41}
{0.96,1.69}
{1.41,1.60}
{5.10,1.60}
{5.09,1.90}
{1.90,1.62}
{2.54,1.97}
{1.97,2.53}
{0.49,2.52}
{5.51,2.76}
{3.14,2.66}
{3.16,2.84}
{2.67,3.14}
{3.62,2.76}
{2.63,2.85}
{3.74,3.61}
{2.85,3.14}
{4.53,3.61}
{3.61,4.53}
{9.34,4.55}
{5.53,5.08}
{5.65,5.38}
{5.39,5.67}
{5.07,5.53}
{1.89,5.10}
{1.64,5.09}
{2.78,5.53}
{7.83,7.34}
{7.35,7.82}
{8.06,8.18}
{8.19,8.07}
{4.54,9.33}
f2 (ppm)
f1 (ppm)


WCMC129E.1.ser
Wang WC 129 3 mg in DMSO-d6 at 298.0 K, 29.08.2019 Rettstadt
Mass 1105
13C-HMBC
f2 (ppm)
f1 (ppm)

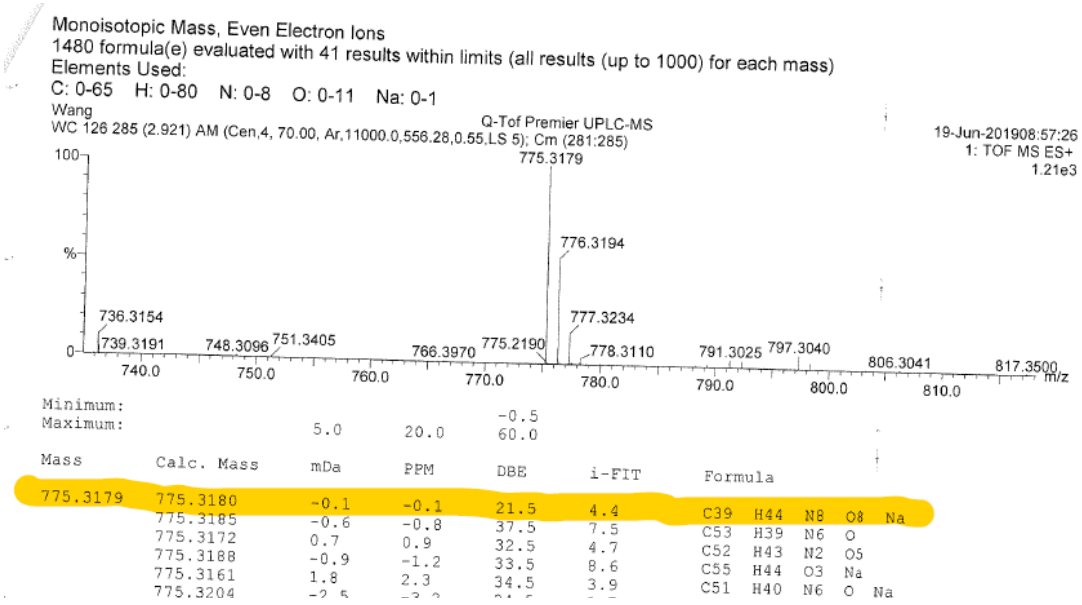

WCMH129E.1.ser
Wang WC 129 3 mg in DMSO-d6 at 298.0 K, 29.08.2019 Rettstadt
Mass 1105
13C-HSQC
f2 (ppm)
f1 (ppm)

2.19 4'-Dye-Linked Cytochalasin H 21

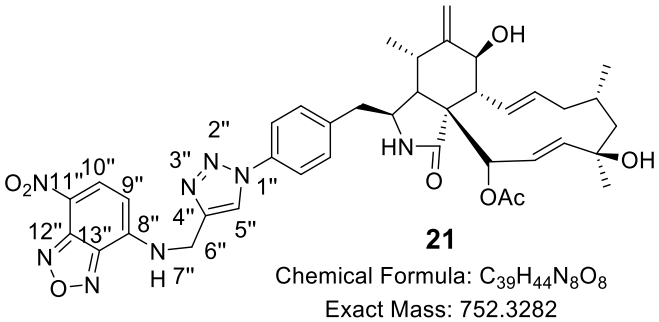


1h 2 21

To a stirred solution of **1h** (10 mg, 0.019 mmol, 1.0 eq) and 7-nitro-4-(prop-2-ynylamino) benzofuran **2** (4.5 mg, 0.021 mmol, 1.1 eq) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 8.6 mg of desired product (61.1%, 0.011 mmol) as a brown solid.



HRMS (*m/z*): calculated for [$C_{39}H_{44}N_8O_8$ + Na]: 775.3180, found: 775.3179.

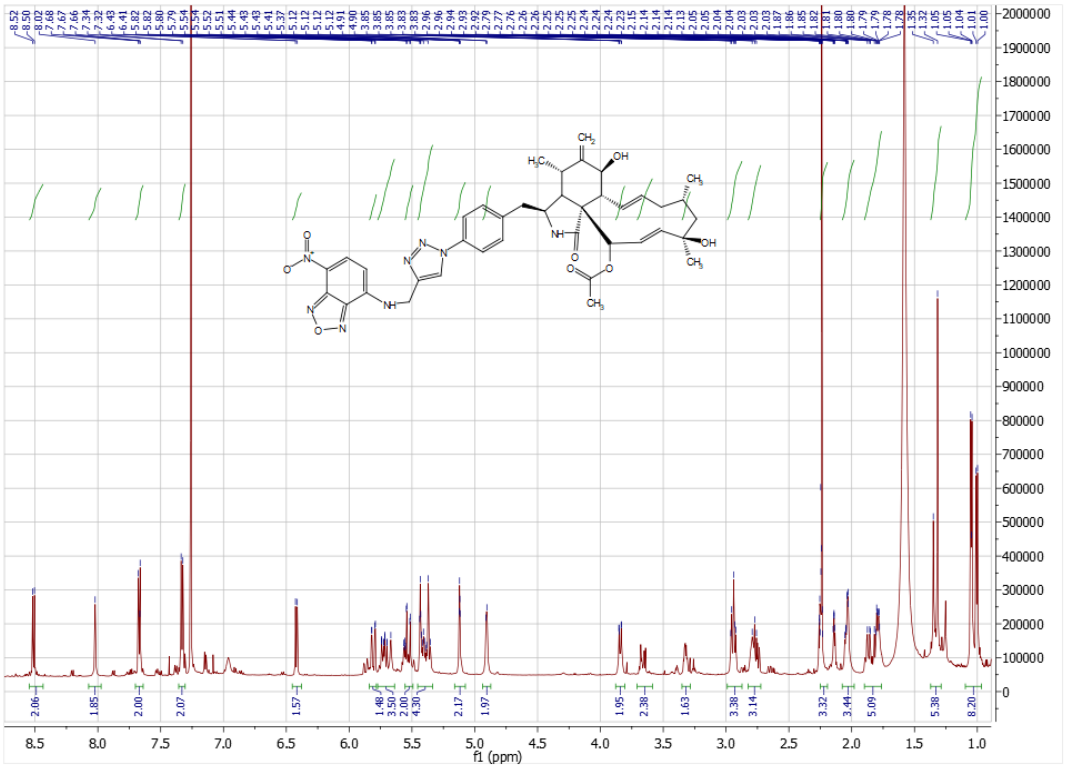


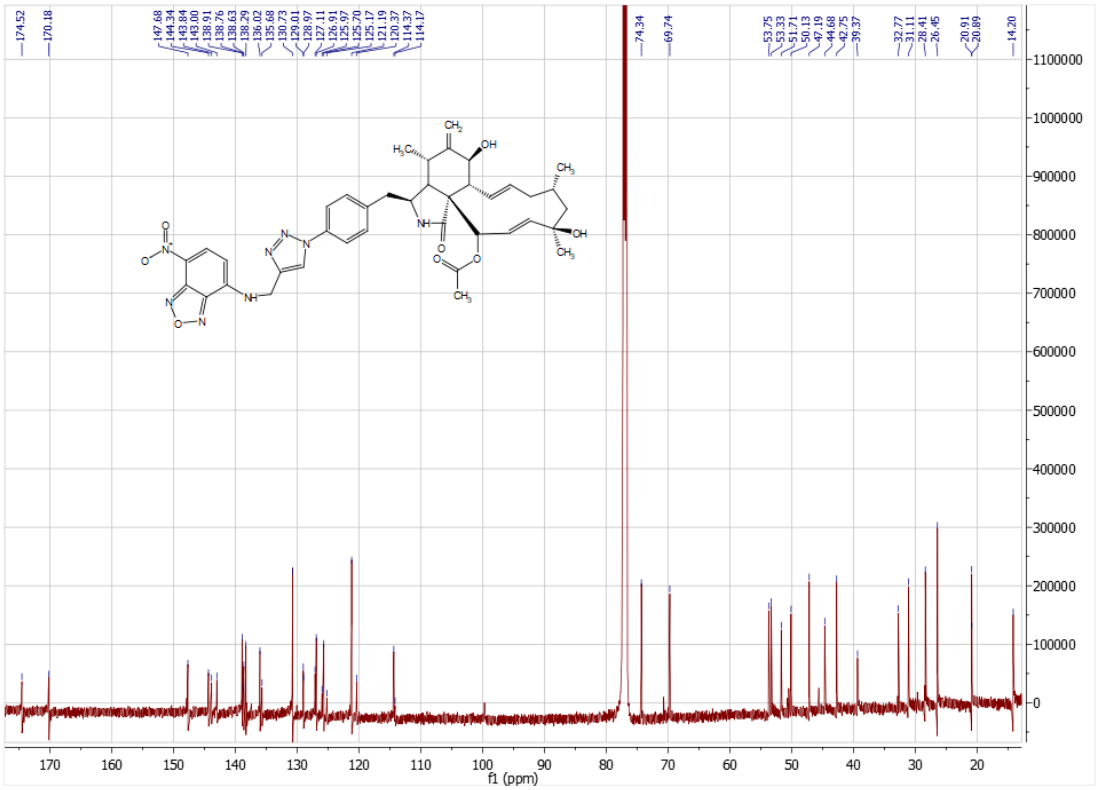
21

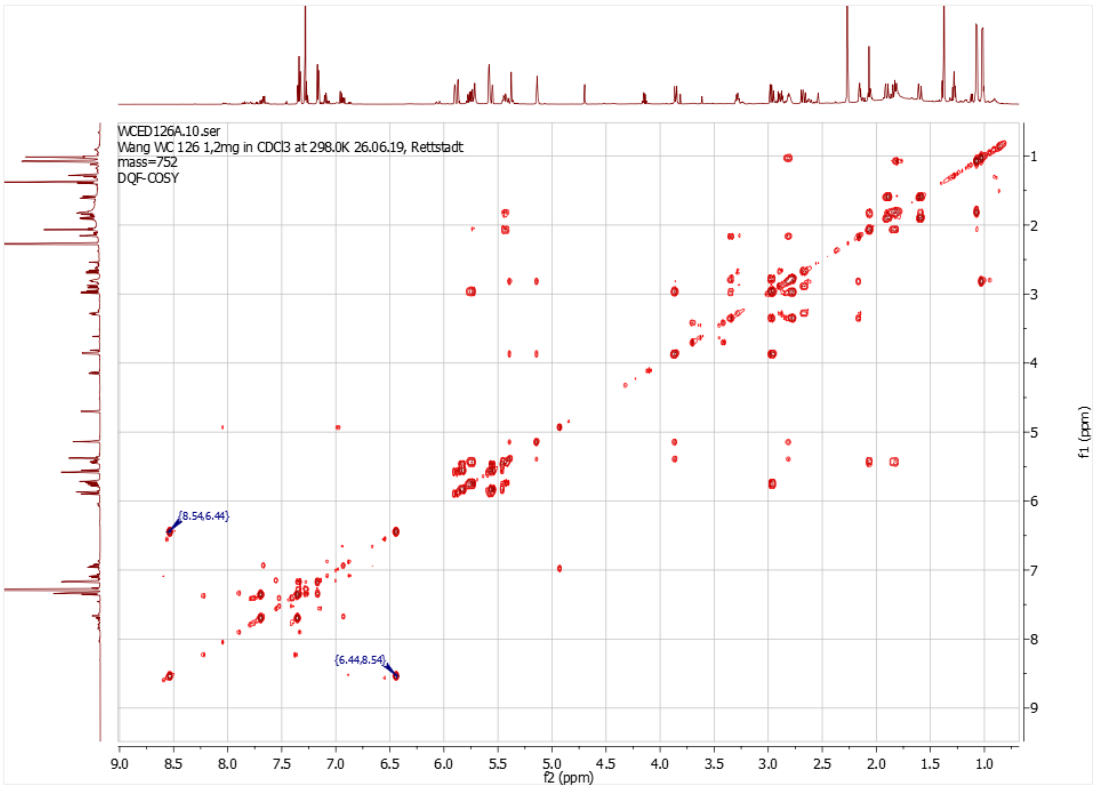
Chemical Formula: $C_{39}H_{44}N_8O_8$

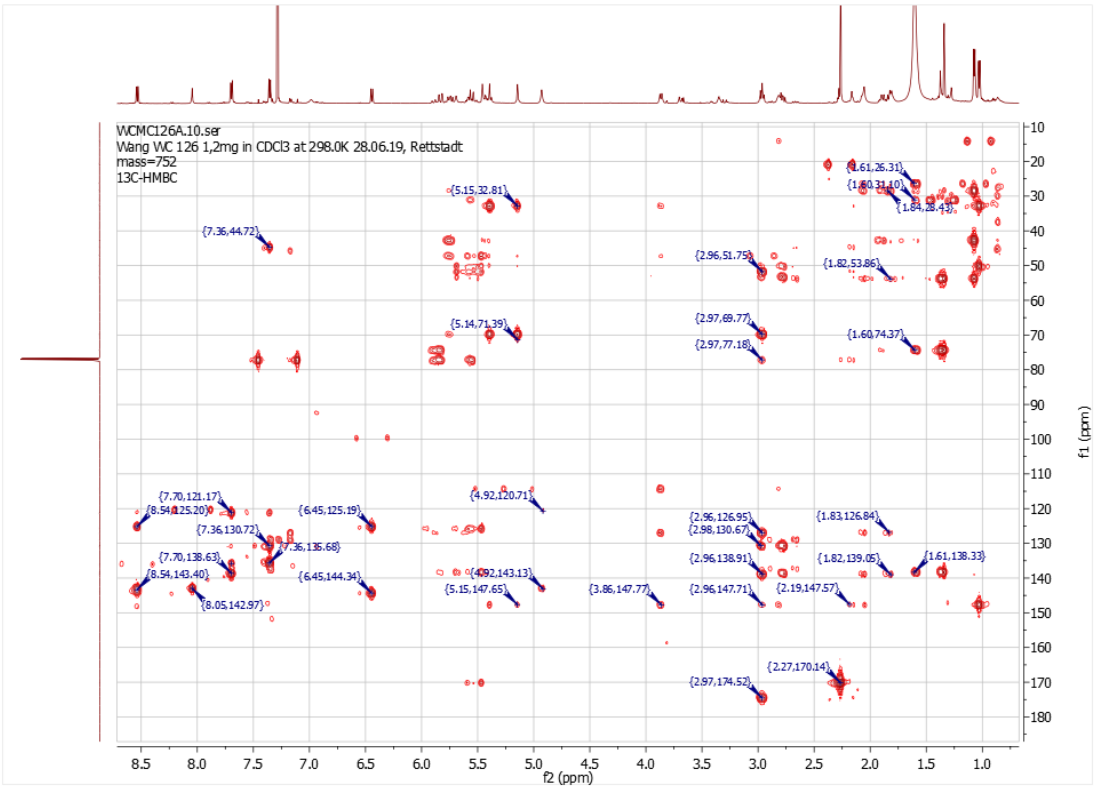
Exact Mass: 752.3282

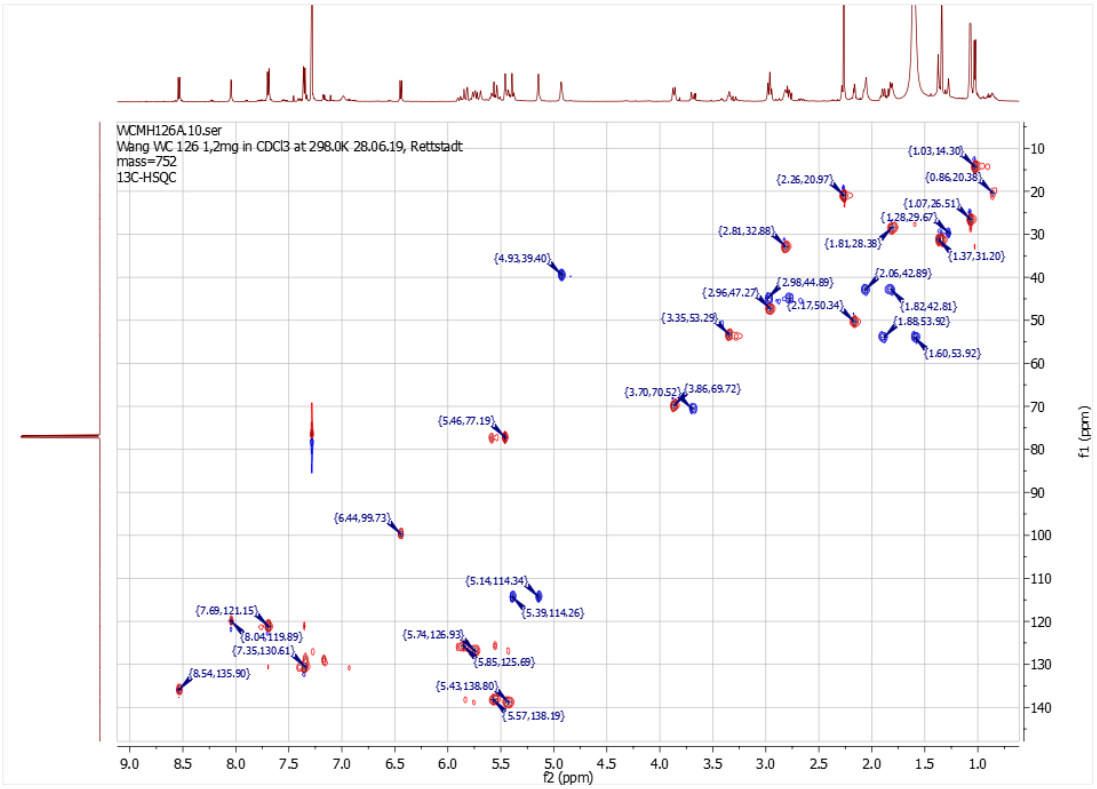
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.5	-	-	-
2	5.56	brs	-	-	-	-	-
3	3.35	m	-	53.3	CH	-	4, 5, 10
4	2.17	dd	3.8, 5.0	50.3	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.81	m	-	32.9	CH	1', 3	4, 11, 12
6	-	-	-	147.8	-	-	-
7	3.86	dd	1.6, 10.7	69.7	CH	6, 12	8, 12
8	2.96	m	-	47.3	CH	1, 7, 9, 13, 20, 21	7, 13
9	-	-	-	51.8	-	-	-
10a	2.78	m	-	44.9	CH_2	1', 3	3, 4, 12
10b	2.98	m	-	-	-	-	-
11	1.03	d	6.7	14.3	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.3	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.74	m	-	126.9	CH	7, 8, 15	8, 14, 15
14	5.43	m	-	138.8	CH	9, 15	13, 15
15a	1.82	m	-	42.8	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.81	m	-	28.4	CH	13, 14, 17	22
17a	1.60	m	-	53.9	CH_2	18, 19	-
17b	1.88	m	-	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.57	brs	-	138.2	CH	-	20
20	5.85	dd	3.0, 16.2	125.7	CH	18, 19	19
21	5.46	brs	-	77.2	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.5	26.5	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1'	-	-	-	130.7	-	-	-
2' 6'	7.35	d	8.5	130.6	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.69	d	8.5	121.2	2 x CH	1', 4'	2', 6'
4'	-	-	-	135.6	-	-	-
24	-	-	-	170.1	-	-	-
25	2.26	s	-	21.0	CH_3	24	-
4''	-	-	-	144.2	-	-	-
5''	8.04	s	-	119.9	CH	4''	-
6''	4.93	s	-	39.4	CH_2	-	-
8''	-	-	-	143.1	-	-	-
9''	6.44	d	8.6	99.7	CH	11'', 12''	10''
10''	8.54	d	8.5	135.9	CH	11'', 13''	9''
11''	-	-	-	125.2	-	-	-
12''	-	-	-	144.3	-	-	-
13''	-	-	-	143.4	-	-	-


f1 (ppm)

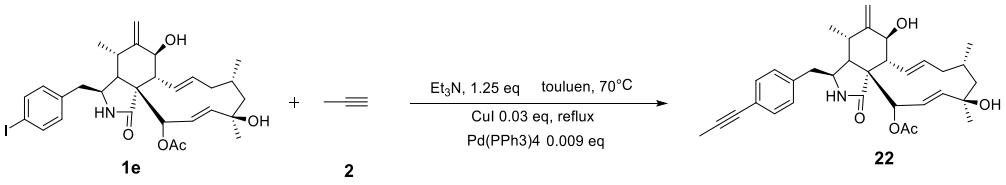

f1 (ppm)


WCED126A.10.ser
Wang WC 126 1,2mg in CDCl3 at 298.0K 26.06.19, Rettstadt
mass=752
DQF-COSY
{8.54,6.44}
{6.44,8.54}
f2 (ppm)
f1 (ppm)

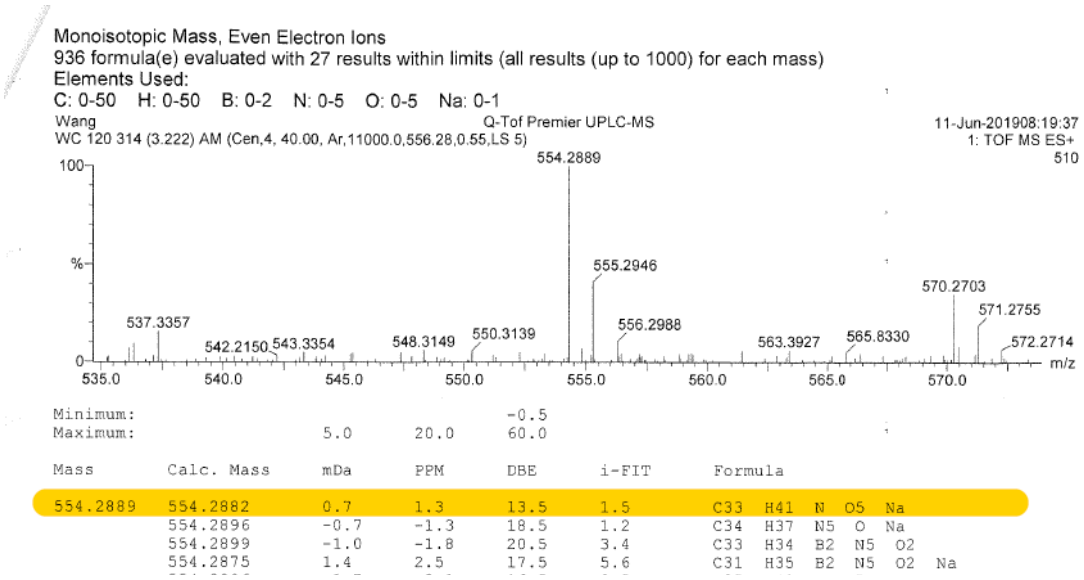

WCMC126A.10.ser
Wang WC 126 1,2mg in CDCl3 at 298.0K 28.06.19, Rettstadt
mass=752
13C-HMBC
{5.15,32.81}
{7.36,44.72}
{5.14,71.39}
{7.70,121.17}
{8.54,125.20}
{6.45,125.19}
{7.36,130.72}
{7.36,135.68}
{7.70,138.63}
{8.54,143.40}
{6.45,144.34}
{8.05,142.97}
{4.92,120.71}
{4.092,143.13}
{5.15,147.65}
{3.86,147.77}
{2.61,26.31}
{1.80,31.10}
{1.64,28.41}
{2.96,51.75}
{1.82,53.86}
{2.97,69.77}
{2.97,77.18}
{1.60,74.37}
{2.96,126.95}
{2.98,130.67}
{1.83,126.84}
{2.96,138.91}
{1.82,139.05}
{1.61,138.33}
{2.96,147.71}
{2.19,147.57}
{2.27,170.14}
{2.97,174.52}
f2 (ppm)
f1 (ppm)


WCMH126A.10.ser
Wang WC 126 1,2mg in CDCl3 at 298.0K 28.06.19, Rettstadt
mass=752
13C-HSQC
{1.03,14.30}
{0.86,20.38}
{2.26,20.97}
{1.07,26.51}
{1.28,29.67}
{2.81,32.88}
{1.81,28.38}
{1.37,31.20}
{4.93,39.40}
{2.06,42.89}
{2.98,44.89}
{2.96,47.27}
{2.17,50.34}
{1.82,42.81}
{3.35,53.29}
{1.88,53.92}
{1.60,53.92}
{3.70,70.52}
{3.86,69.72}
{5.46,77.19}
{6.44,99.73}
{5.14,114.34}
{5.39,114.26}
{7.69,121.15}
{8.04,119.89}
{7.35,130.61}
{5.74,126.93}
{5.85,125.69}
{8.54,135.90}
{5.43,138.80}
{5.57,138.19}
f2 (ppm)
f1 (ppm)

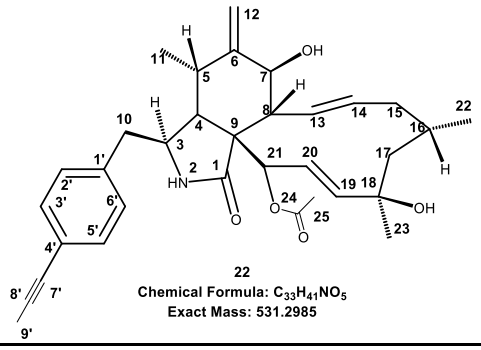
2.20 4'-Prop-1-ynyl Cytochalsin H 22



To a stirred solution of **1e** (10 mg, 0.019 mmol, 1.0 eq) and **2** (2.0 mg, 0.038 mmol, 2.0 eq, purchased from Signma-Aldrich) in toluene (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq), Et_3N (3.0 mg, 0.03 mmol, 1.5 eq) and $Pd(PPh_3)_4$ (0.2 mg, 0.1 µmol, 0.009 eq). The mixture was stirred at 80 °C for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 6.7 mg of desired product (78.2%, 0.012 mmol) as a yellow solid.

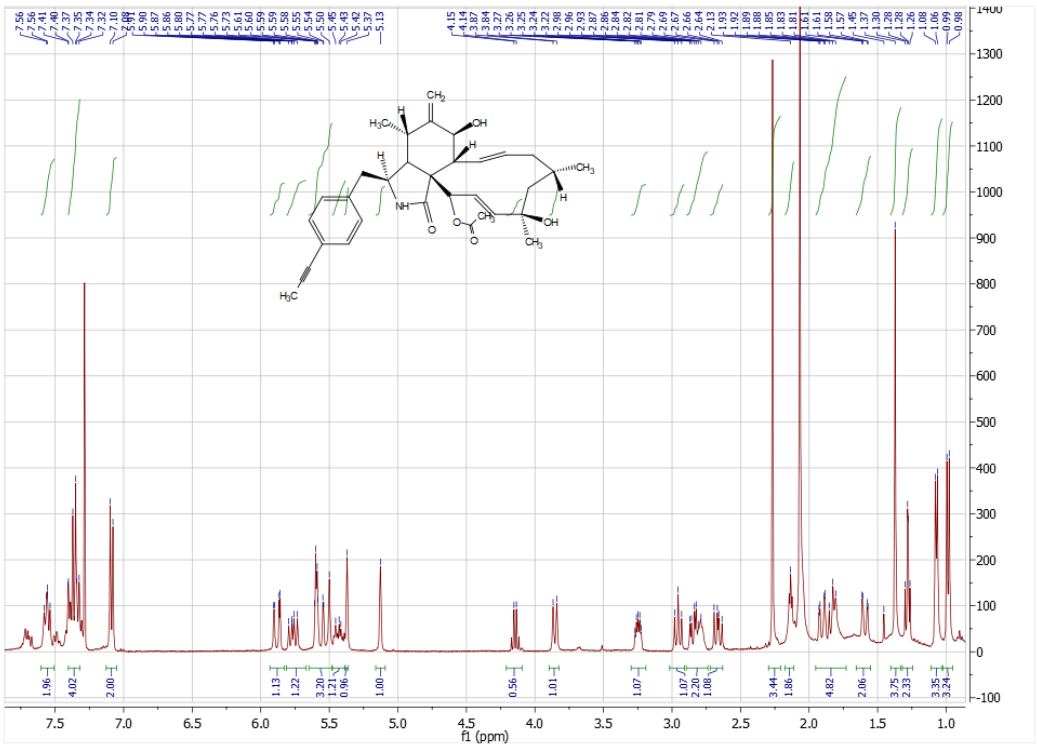


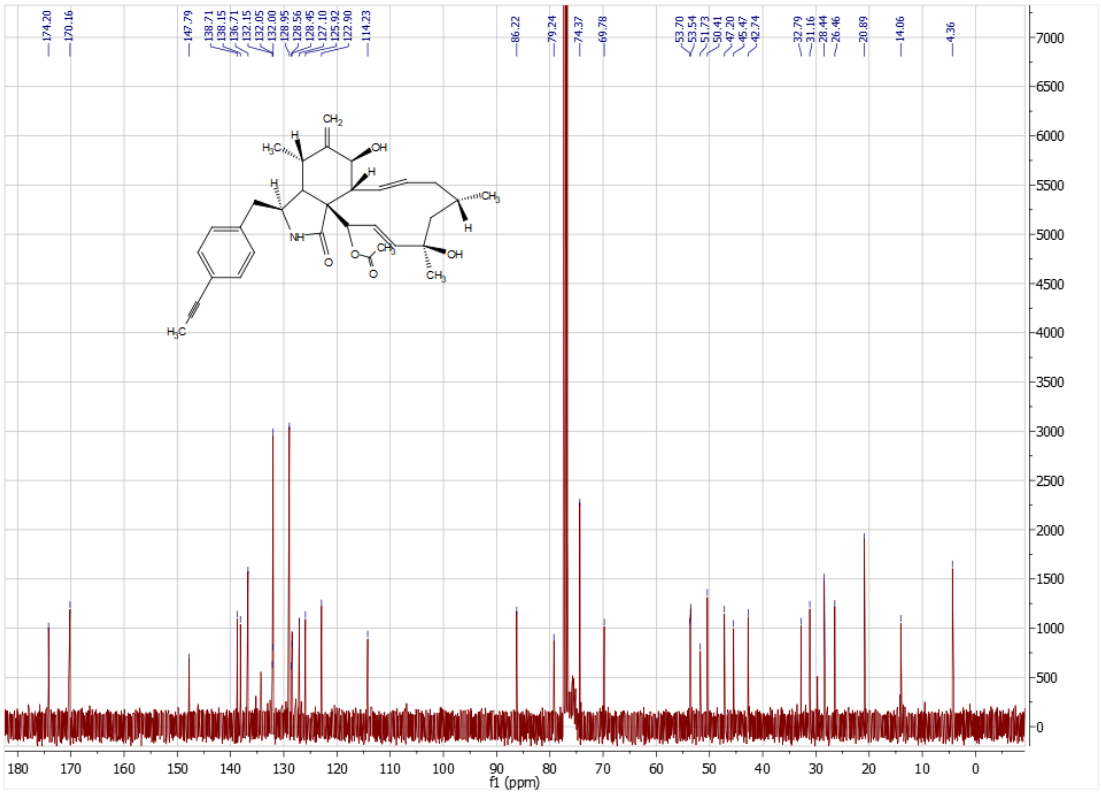
HRMS (*m/z*): calculated for [$C_{33}H_{41}NO_5$ + Na]: 554.2882, found: 554.2889.

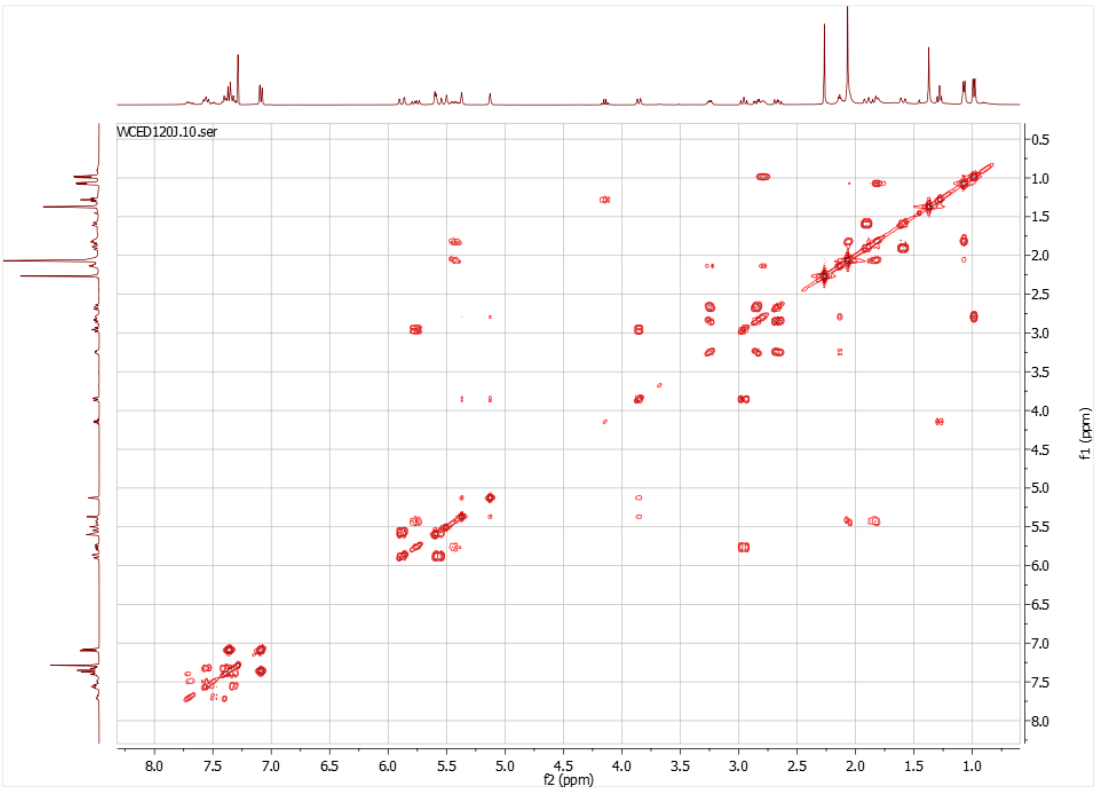


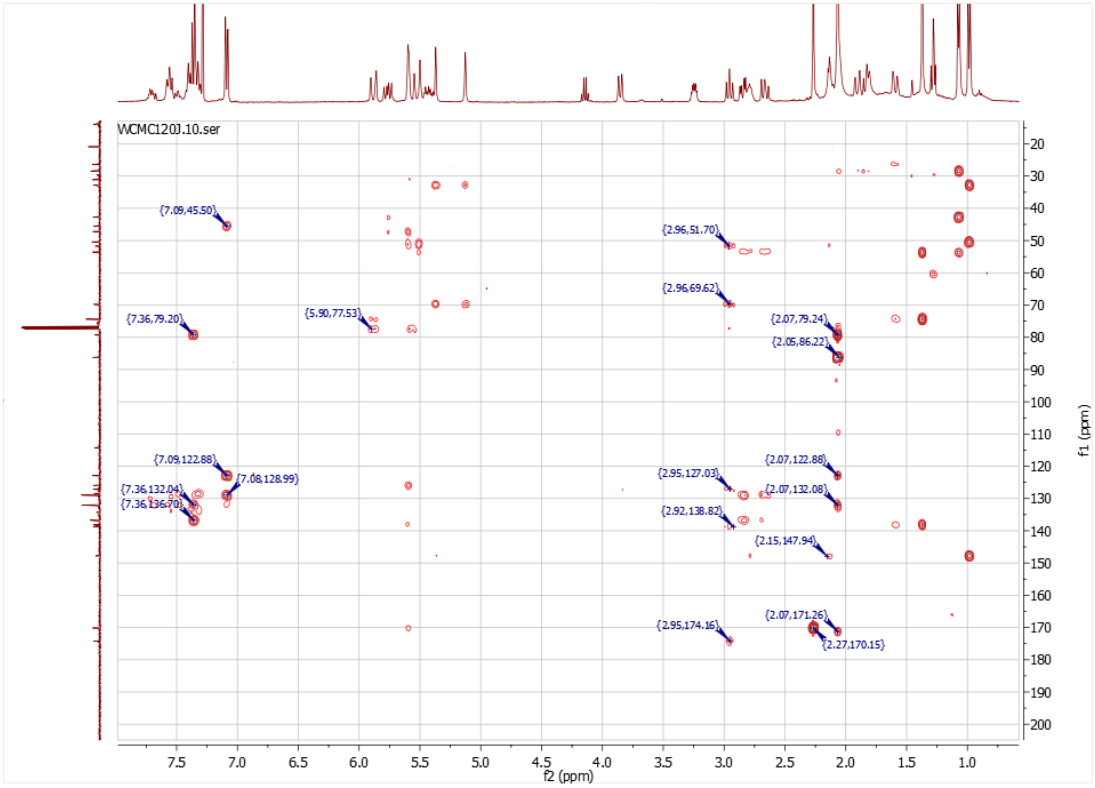
22
Chemical Formula: $C_{33}H_{41}NO_5$
Exact Mass: 531.2985

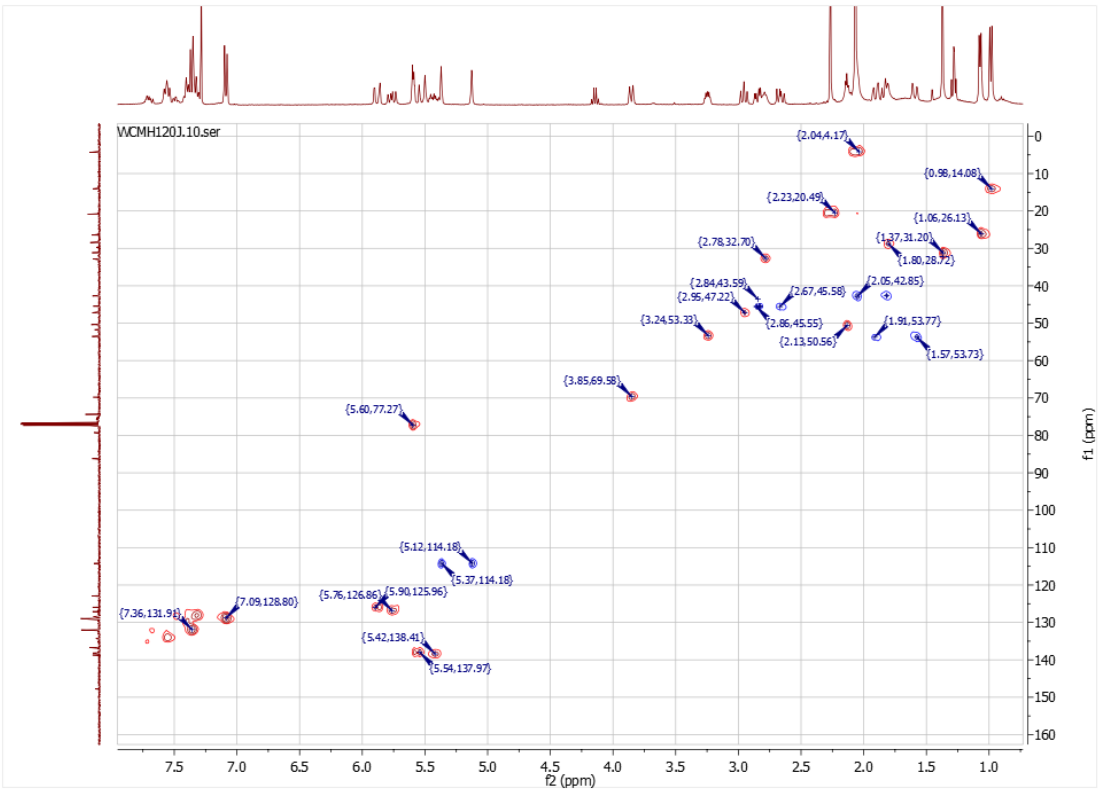
Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.2	-	-	-
3	3.24	ddd	1.1, 4.4, 9.1	53.3	CH	1‘,4, 5	4, 5, 10
4	2.13	t	4.4	50.6	CH	3, 5	3, 5, 10
5	2.78	m	-	32.7	CH	1’, 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.85	d	10.2	69.6	CH	5, 6, 8, 12, 13	8, 12
8	2.95	t	10.2	47.2	CH	1, 7, 9, 13, 21	7, 13
9	-	-	-	51.7	-	-	-
10a	2.67	dd	9.5, 13.5	45.6	CH_2	1’, 3	3, 4, 12
10b	2.82	dd	4.8, 13.5	-	-	-	-
11	0.98	d	6.7	14.1	CH_3	4, 5, 6	5
12a	5.12	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.37	brs	-	-	-	-	-
13	5.76	ddd	1.4, 9.6, 15.5	126.9	CH	7, 8, 15	8, 14, 15
14	5.42	ddd	4.8, 10.3, 15.5	138.4	CH	9, 15	13, 15
15a	1.82	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.80	m	-	28.7	CH	13, 14, 17	22
17a	1.57	m	-	53.7	CH_2	18, 19	-
17b	1.91	m	-	-	-	-	-
18	-	-	-	74.4	-	-	-
19	5.54	brs	-	138.0	CH	-	20
20	5.90	dd	2.9, 16.4	126.0	CH	18, 19	19
21	5.60	brs	-	77.5	CH	8, 9, 19, 20, 23	-
22	1.06	d	6.5	26.1	CH_3	15, 16, 17	16
23	1.37	s	-	31.2	CH_3	17, 18, 19	-
1‘	-	-	-	132.0	-	-	-
2’ 6’	7.09	d	8.2	128.8	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.36	d	8.2	131.9	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	122.9	-	-	-
24	-	-	-	170.2	-	-	-
25	2.27	s	-	20.9	CH_3	21, 24	-
7’	-	-	-	86.2	-	-	-
8’	-	-	-	79.2	-	-	-
9’	2.04	s	-	4.2	CH_3	3‘, 4‘, 5‘, 7‘, 8‘	-


f1 (ppm)

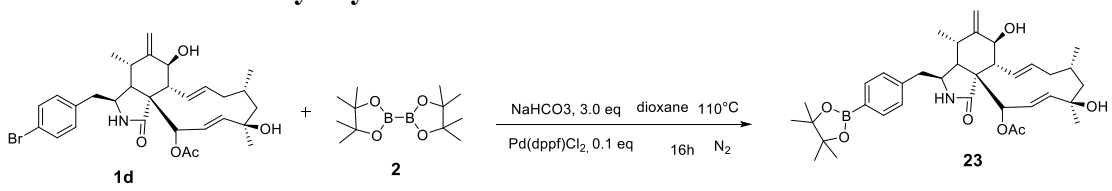

f1 (ppm)


WCED120J.10.ser
f2 (ppm)
f1 (ppm)


WCMC120J.10.ser
{7.09,45.50}
{7.36,79.20}
{5.90,77.53}
{2.96,51.70}
{2.96,69.62}
{2.07,79.24}
{2.05,86.22}
{7.09,122.88}
{7.08,128.99}
{7.36,132.04}
{7.36,136.70}
{2.95,127.03}
{2.07,122.88}
{2.07,132.08}
{2.92,138.82}
{2.15,147.94}
{2.95,174.16}
{2.07,171.26}
{2.27,170.15}
f2 (ppm)
f1 (ppm)

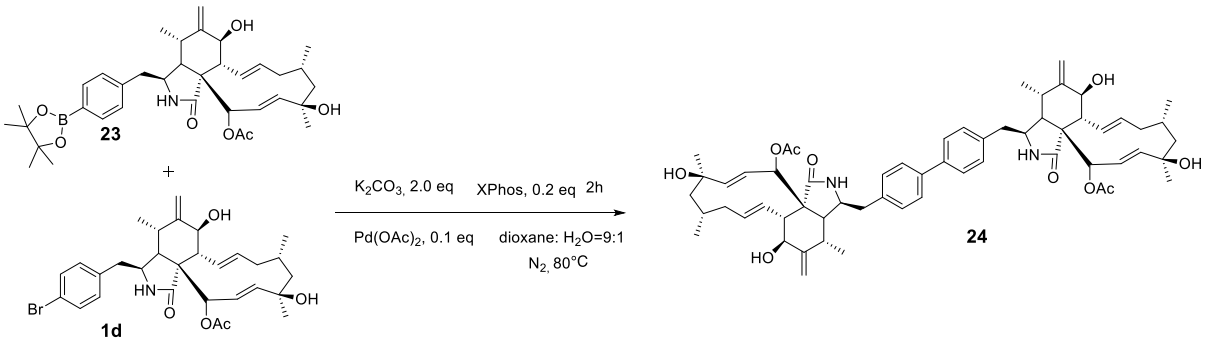

WCMH120J.10.ser
{2.04,4.17}
{0.98,14.08}
{2.23,20.49}
{1.06,26.13}
{2.78,32.70}
{1.37,31.20}
{1.80,28.72}
{2.84,43.59}
{2.95,47.22}
{2.67,45.58}
{2.05,42.85}
{3.24,53.33}
{2.86,45.55}
{2.13,50.56}
{1.91,53.77}
{1.57,53.73}
{3.85,69.58}
{5.60,77.27}
{5.12,114.18}
{5.37,114.18}
{5.76,126.86}
{5.90,125.96}
{7.36,131.91}
{7.09,128.80}
{5.42,138.41}
{5.54,137.97}
f2 (ppm)
f1 (ppm)

2.21 4'-Pinacolborolanyl Cytochalasin H 23



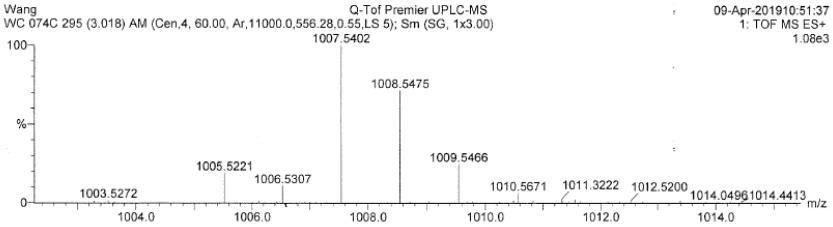
To a stirred solution of **1d** (20 mg, 0.035 mmol, 1.0 eq) and Bis(pinacolato)diboron **2** (17.8 mg, 0.07 mmol, 2.0 eq, purchased from Sigma-Aldrich) in dioxane (3 mL) was added $Pd(dppf)Cl_2$ (2.6 mg, 0.0035 mmol, 0.1 eq), $NaHCO_3$ (10.2 mg, 0.11 mmol, 3.0 eq). The mixture was stirred at 110 °C for 8 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 12.6 mg of desired product (58.3%, 0.020 mmol) as a white solid.

2.22 4'-4' Cytochalasin H dimer 24



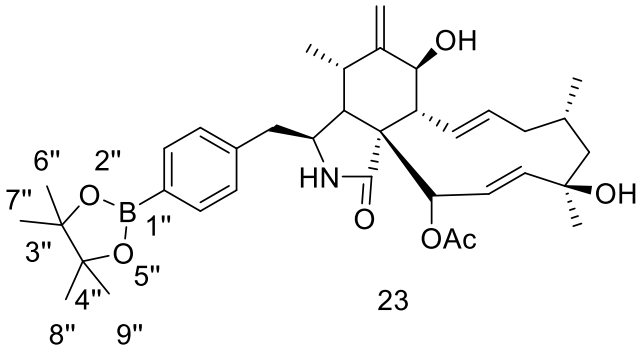
To a stirred solution of **1d** (7.6 mg, 0.012 mmol, 1.5 eq) and **23** (7 mg, 8 µmol, 1.0 eq) in dioxane (4 mL) was added $Pd(OAc)_2$ (0.12 mg, 0.8 µmol, 0.1 eq) and XPhos (0.76 mg, 1.6 µmol, 0.2 eq) and K_2CO_3 (2.2 mg, 16 µmol, 2.0 eq). The mixture was stirred at 80 °C for 2 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (4 mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 5.1 mg of desired product (42.6%, 0.005 mmol) as a white solid.

Monoisotopic Mass, Even Electron Ions
270 formula(e) evaluated with 17 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-65 H: 0-80 B: 0-2 N: 0-2 O: 0-10 Na: 0-1



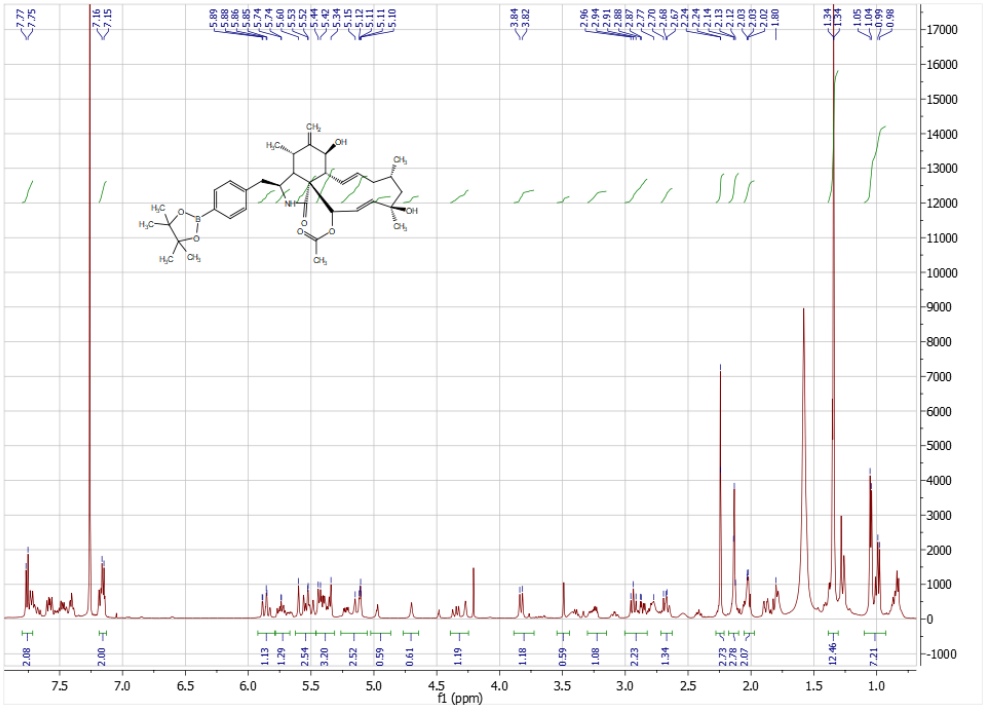
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
	Minimum:			-0.5		
	Maximum:	5.0	20.0	60.0		
1007.5402	1007.5398	0.4	0.4	23.5	0.7	C60 H76 N2 O10 Na
	1007.5417	-1.5	-1.5	26.5	5.5	C62 H74 B2 O9 Na
	1007.5382	2.0	2.0	30.5	1.0	C64 H72 B N2 O8
	1007.5422	-2.0	-2.0	26.5	1.2	C62 H75 N2 O10
	1007.5438	-3.6	-3.6	27.5	2.6	C65 H76 O8 Na
	1007.5441	-3.9	-3.9	29.5	3.6	C64 H73 B2 O9
	1007.5358	4.4	4.4	27.5	1.6	C62 H73 B N2 O8 Na
	1007.5318	8.4	8.3	31.5	3.8	C64 H70 B2 N2 O6 Na
	1007.5529	-12.7	-12.6	26.5	7.1	C61 H74 B2 N2 O8 Na
	1007.5269	13.3	13.2	30.5	2.2	C65 H72 B O9
	1007.5550	-14.8	-14.7	27.5	3.0	C64 H76 N2 O7 Na
	1007.5553	-15.1	-15.0	29.5	5.3	C63 H73 B2 N2 O8
	1007.5245	15.7	15.6	27.5	2.8	C63 H73 B O9 Na
	1007.5569	-16.7	-16.6	22.5	4.9	C59 H77 B N2 O10 Na
	1007.5593	-19.1	-19.0	25.5	3.8	C61 H76 B N2 O10
	1007.5210	19.2	19.1	31.5	5.6	C65 H71 N2 O8
	1007.5205	19.7	19.6	31.5	5.2	C65 H70 B2 O7 Na

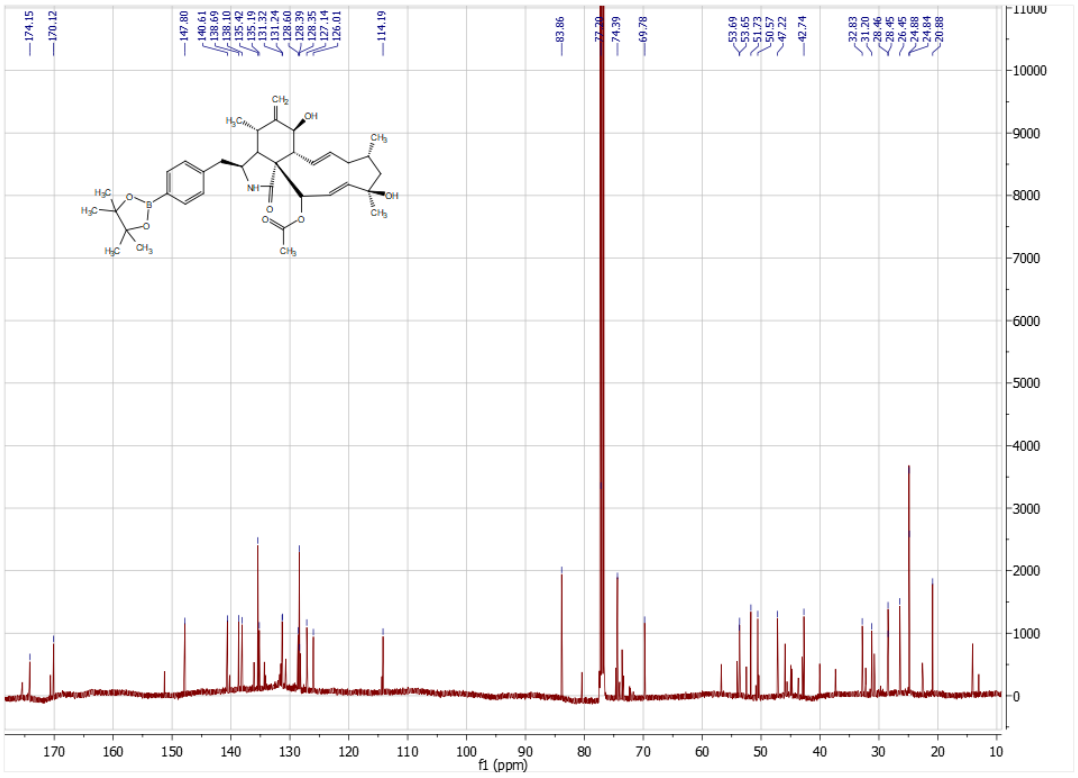
HRMS (*m/z*): calculated for [$C_{60}H_{76}N_2O_{10}$ + Na]: 1007.5398, found: 1007.5402.

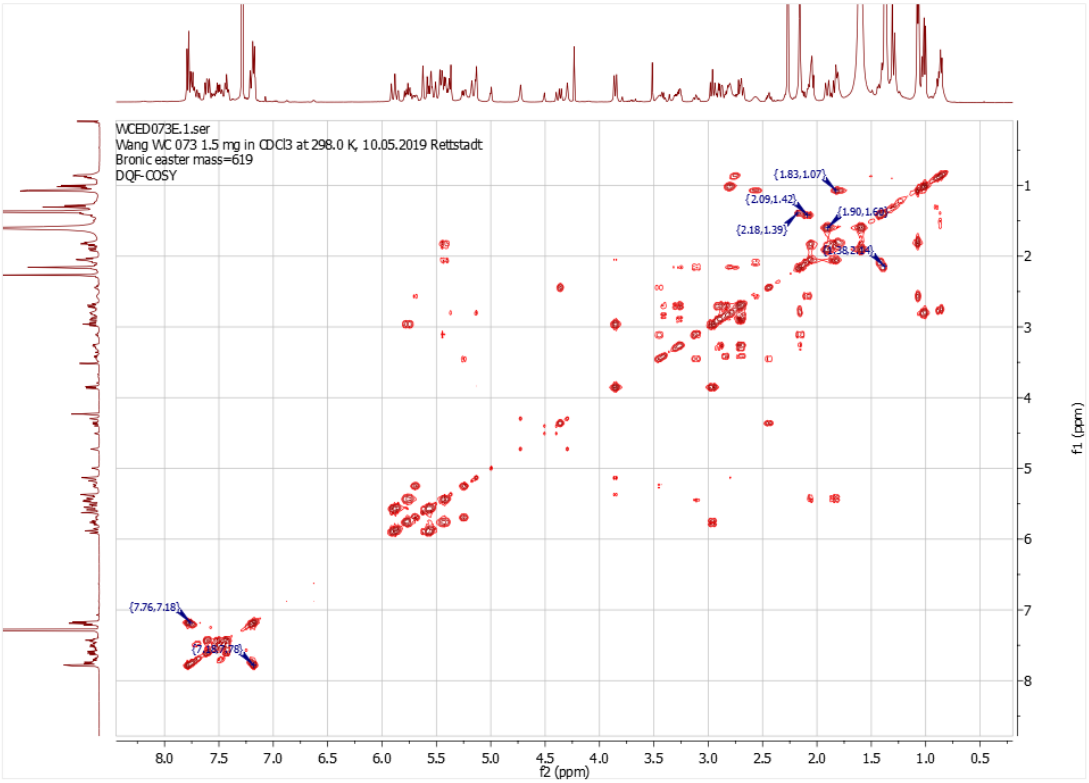


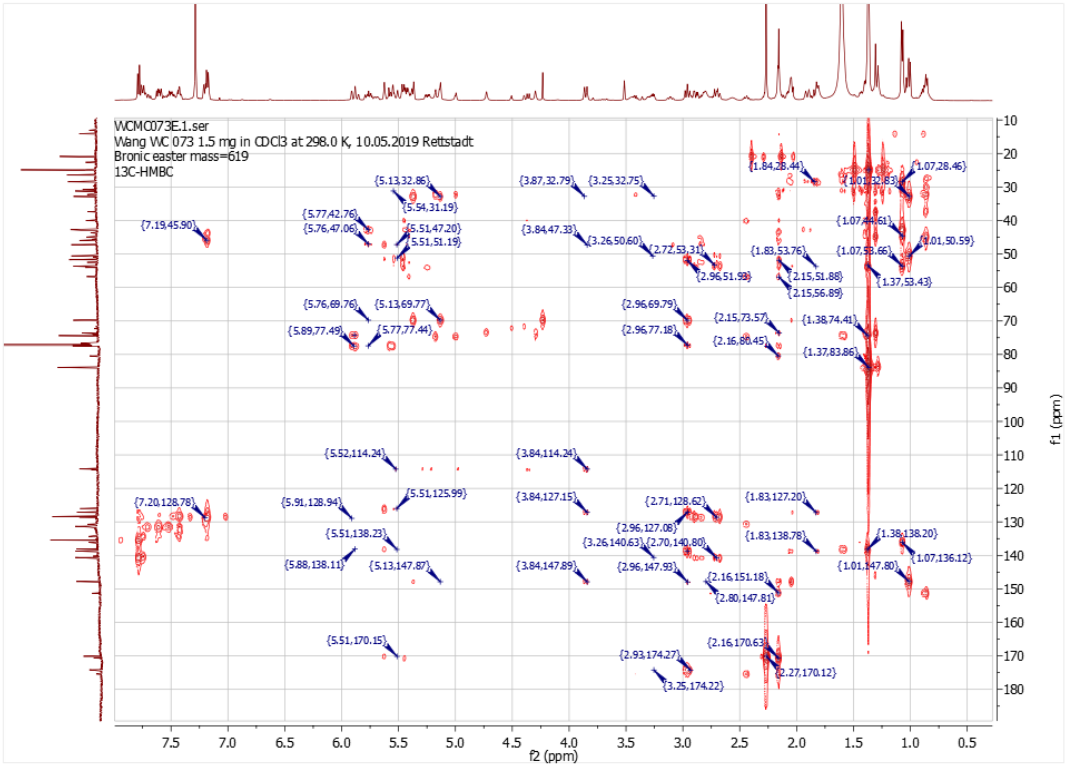
23

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
2	-	-	-	-	-	-	-
3	3.27	m	-	53.4	CH	1', 1, 4, 5	4, 5, 10
4	2.16	m	-	50.2	CH	3, 5	3, 5, 10
5	2.80	m	-	32.5	CH	1', 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.86	d	10.9	69.5	CH	5, 6, 8, 12, 13	8, 12
8	2.96	m	-	47.0	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.9	-	-	-
10a	2.70	m	-	45.5	CH_2	1', 3	3, 4, 12
10b	2.90	m	-	-	-	-	-
11	1.00	d	6.7	13.8	CH_3	4, 5, 6	5
12a	5.13	brs	-	114.0	CH_2	5, 6, 7	5, 7, 11
12b	5.38	brs	-	-	-	-	-
13	5.76	m	-	126.9	CH	7, 8, 15	8, 14, 15
14	5.45	m	-	138.4	CH	9, 15	13, 15
15a	1.82	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.83	m	-	28.3	CH	13, 14, 17	22
17a	1.56	brs	-	53.7	CH_2	18, 19	-
17b	1.90	brs	-	-	-	-	-
18	-	-	-	74.3	-	-	-
19	5.54	brs	-	137.9	CH	-	20
20	5.88	dd	2.6, 16.5	125.7	CH	18, 19	19
21	5.51	brs	-	77.2	CH	8, 9, 19, 20, 23	-
22	1.08	d	6.3	26.3	CH_3	15, 16, 17	16
23	1.38	s	-	31.0	CH_3	17, 18, 19	-
1'	-	-	-	128.6	-	-	-
2' 6'	7.19	d	7.9	128.1	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.78	d	7.9	134.9	2 x CH	1', 4'	2', 6'
4'	-	-	-	128.9	-	-	-
24	-	-	-	170.1	-	-	-
25	2.27	s	-	20.8	CH_3	24	-
3''4''	-	-	-	83.9	-	-	-
6''7''8''9''	1.37	brs	-	24.7	4x CH_3	3'', 4''	-

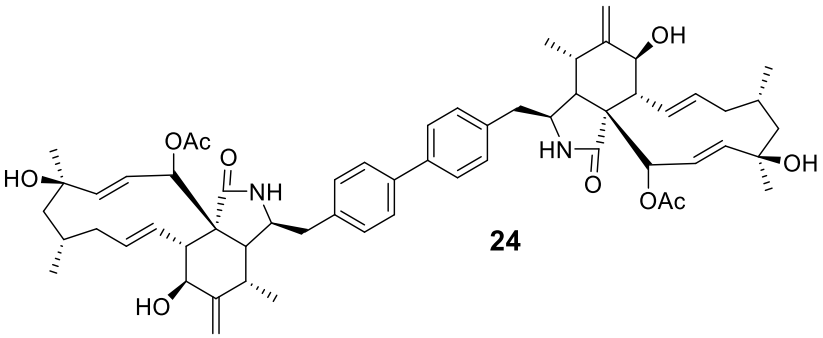

f1 (ppm)


f1 (ppm)


WCED073E.1.ser
Wang WC 073 1.5 mg in CDCl3 at 298.0 K, 10.05.2019 Rettstadt
Bronic easter mass=619
DQF-COSY
f2 (ppm)
f1 (ppm)


WCMC073E.1.ser
Wang WC 073 1.5 mg in CDCl3 at 298.0 K, 10.05.2019 Rettstadt
Bronic easter mass=619
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH073E.1.ser
Wang WC 073 1.5 mg in CDCl3 at 298.0 K, 10.05.2019 Rettstadt
Bronic easter mass=619
13C-HSQC
f2 (ppm)
f1 (ppm)

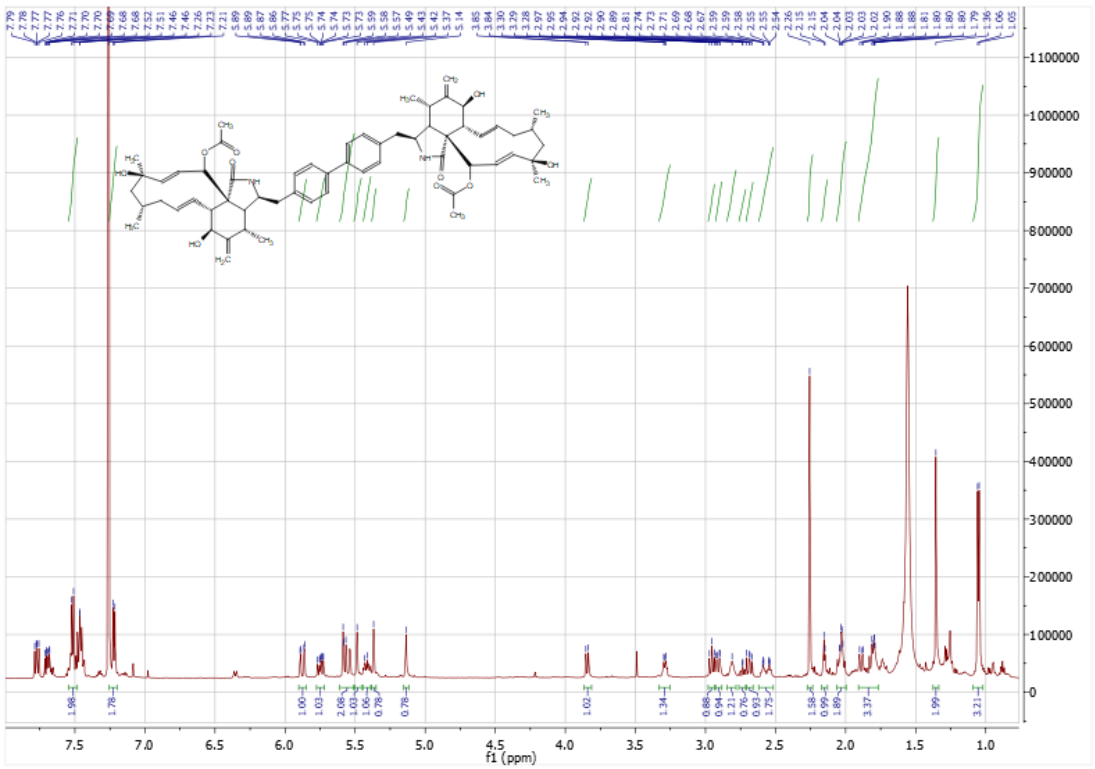


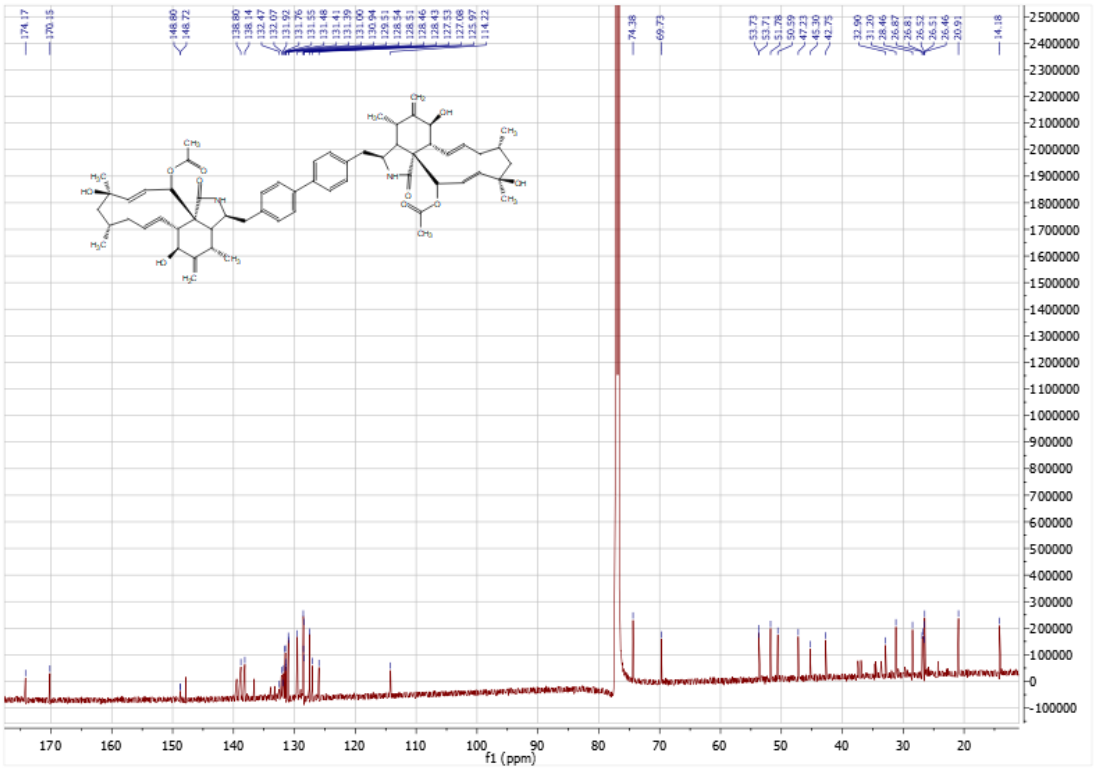
24

Chemical Formula: $C_{60}H_{76}N_2O_{10}$
Exact Mass: 984.5500

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
2	5.48	brs	-	-	-	-	3
3	3.29	ddd	0.9, 9.6, 4.6	53.4	CH	-	2
4	2.16	dd	4.6, 8.9	50.3	CH	9, 21	5
5	2.81	m	-	32.5	CH	-	4, 11, 12
6	-	-	-	148.1	-	-	-
7	3.83	d	10.8	69.3	CH	6, 12, 13	8, 12
8	2.95	dd	10.2, 10.8	46.9	CH	1, 3, 7, 13, 14	7, 13
9	-	-	-	51.7	-	-	-
10a	2.68	dd	13.5, 9.9	44.9	CH_2	1', 2', 6', 3	
10b	2.92	dd	13.5, 4.4	-	-	-	-
11	1.05	d	6.5	13.9	CH_3	4, 5, 6	5
12a	5.13	brs	-	113.8	CH_2	5, 6, 7	5, 7
12b	5.37	brs	-	-	-	-	
13	5.75	ddd	9.7, 15.5, 1.3	126.7	CH	8, 9	8, 14
14	5.41	ddd	15.5, 4.7, 10.0	138.4	CH	-	13, 15
15a	1.80	m	-	42.5	CH_2	13, 14, 16	14, 17
15b	2.04	m	-	-	-	-	-
16	1.79	m	-	28.2	CH	-	17
17a	1.56	m	-	53.5	CH_2	18, 19	15, 16
17b	1.88	dd	3.0, 14.4	-	-	-	-
18	-	-	-	74.6	-	-	-
19	5.57	dd	16.6, 2.4	137.8	CH	8, 9, 20	20
20	5.89	dd	16.6, 2.7	125.6	CH	18, 21	21
21	5.58	dd	2.5, 2.7	77.1	CH	19, 20	20
22	1.05	d	6.5	26.2	CH_3	15, 16, 17	15
23	1.37	s	-	30.8	CH_3	16, 17, 18, 19	-
1'	-	-	-	129.7	-	-	-
2' 6'	7.22	d	8.1	129.1	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.51	d	8.1	127.1	2 x CH	2', 6', 4'	2', 6'
4'	-	-	-	139.6	-	-	-
24	-	-	-	170.3	-	-	-
25	2.26	s	-	20.6	CH_3	24	-

NMR data of **24**recorded at 500 MHz in $CDCl_3$.


f1 (ppm)

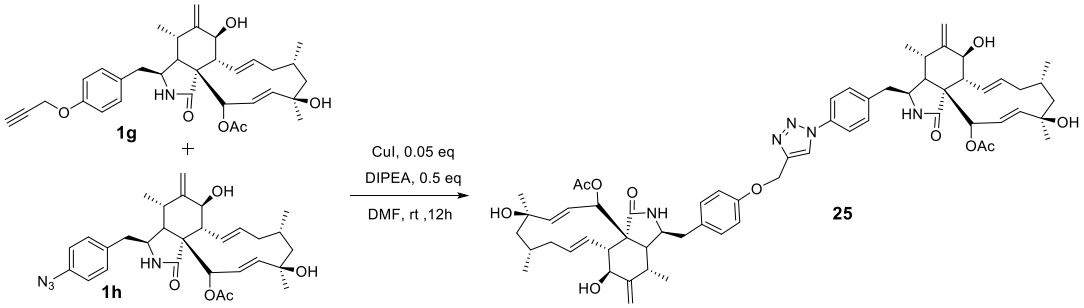

f1 (ppm)


WCED074A10.ser
Wang WC074 1.2mg in CDCl3 at 298.0 K 09.04.19, Rettstadt
DQF-COSY
f2 (ppm)
f1 (ppm)

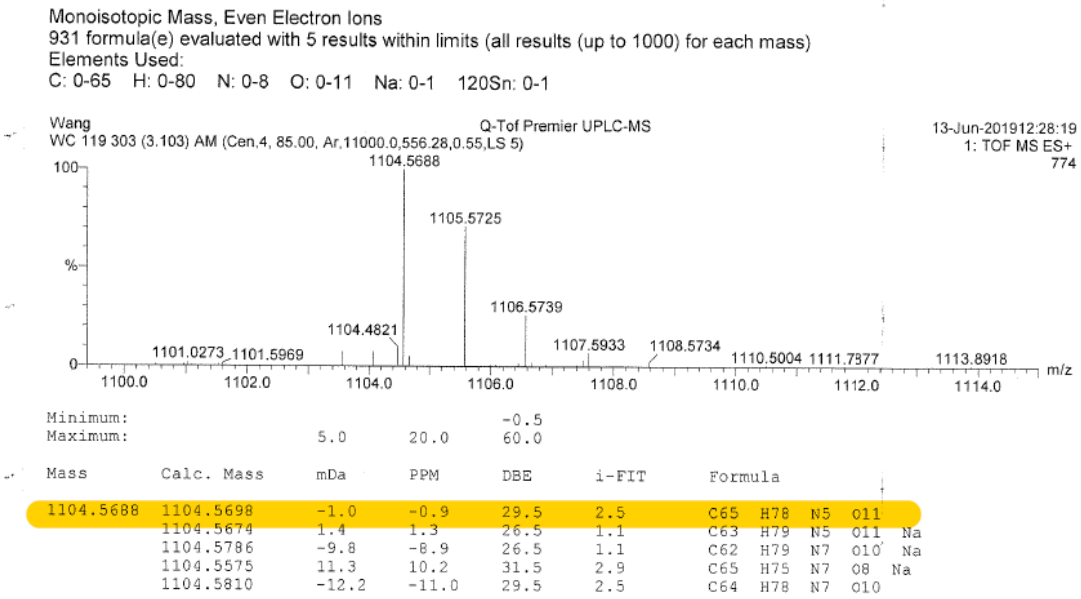

WCMC074A10.ser
Wang WC074 1.2mg in CDCl3 at 298.0 K 09.04.19, Rettstadt
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH074A10.ser
Wang WC074 1.2mg in CDCl3 at 298.0K 09.04.19, Rettstadt
13C-HSQC
f2 (ppm)
f1 (ppm)

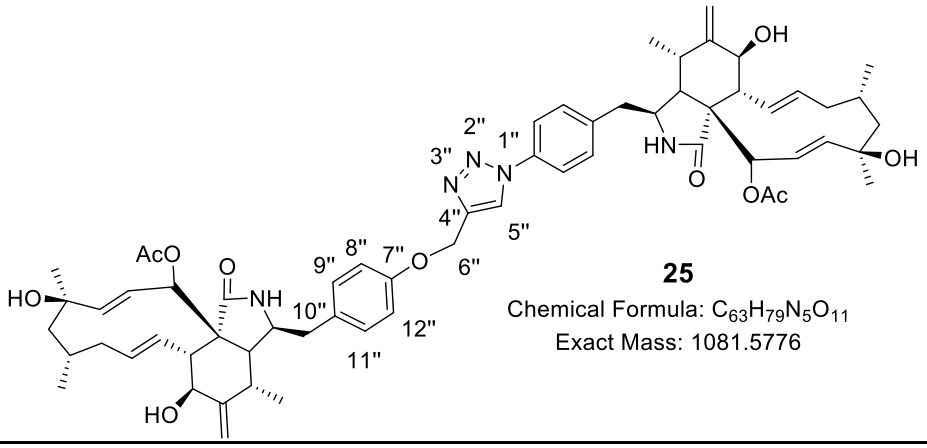
2.23 Cytochalasin H Dimer 25



To a stirred solution of **1h** (11.0 mg, 0.021 mmol, 1.1 eq) and **1g** (10.0 mg, 0.019 mmol, 1.0 eq) in DMF (2 mL) was added CuI (0.2 mg, 0.95 µmol, 0.05 eq) and DIPEA (1.2 mg, 9.5 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2mL) and washed with water (2 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 16.5 mg of desired product (81.5%, 0.015 mmol) as a white solid.



HRMS (*m/z*): calculated for [$C_{60}H_{76}N_2O_{10}$ + Na]: 1104.5674, found: 1104.5688.

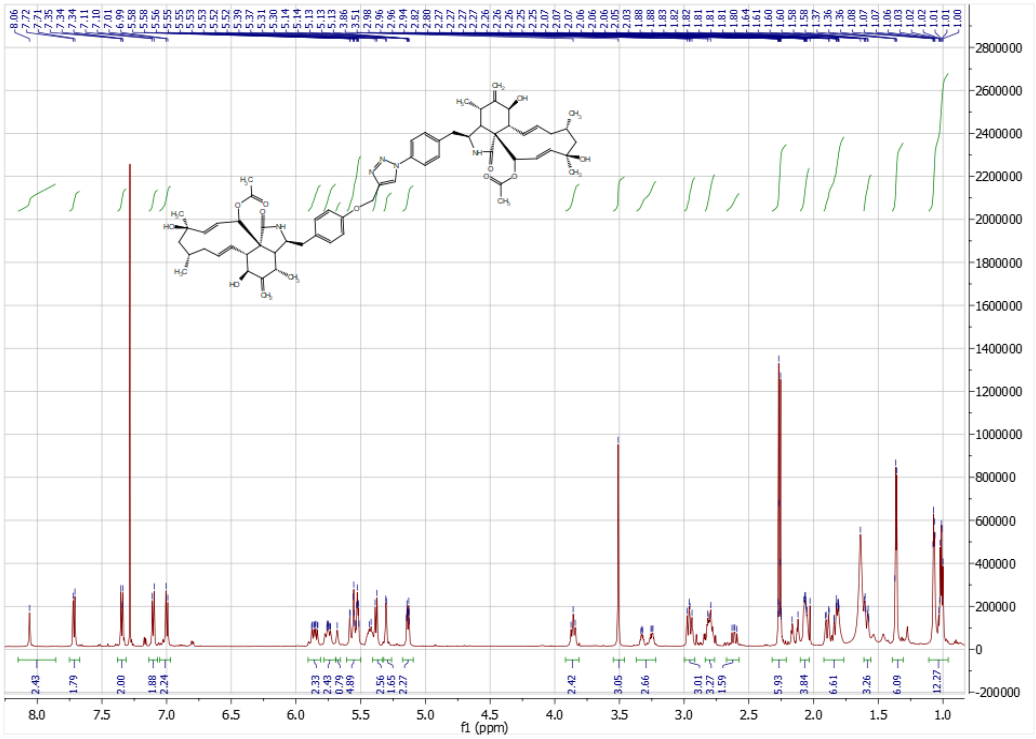


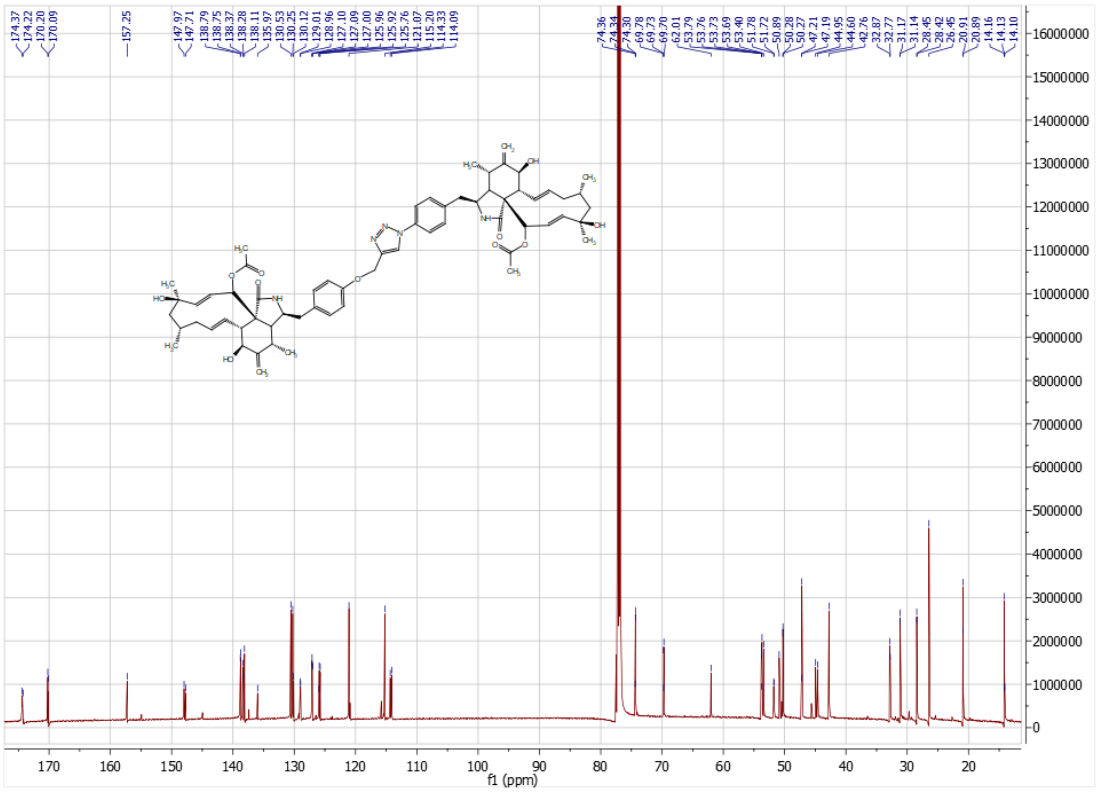
25

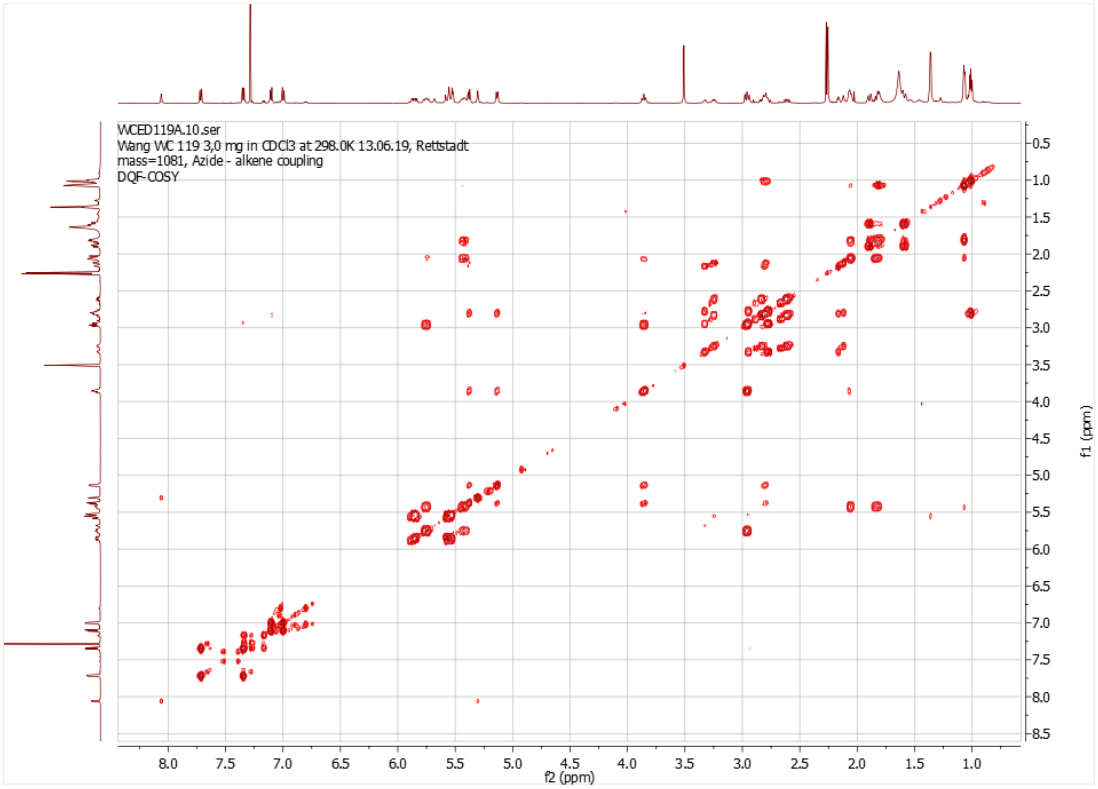
Chemical Formula: $C_{63}H_{79}N_5O_{11}$

Exact Mass: 1081.5776

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
3	3.25	m	-	53.8	CH	1‘, 1, 4, 5	4, 5, 10
4	2.16	dd	3.9, 5.1	50.4	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.80	m	-	32.8	CH	1’, 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.86	dd	2.2, 11.8	69.6	CH	6, 12	8, 12
8	2.96	m	-	47.3	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.8	-	-	-
10a	2.79	m	-	44.9	CH_2	1’, 3	3, 4, 12
10b	2.96	m	-	-	-	-	-
11	1.01	d	6.6	14.2	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.38	brs	-	-	-	-	-
13	5.75	m	-	127.9	CH	7, 8, 15	8, 14, 15
14	5.42	m	-	138.8	CH	9, 15	13, 15
15a	1.82	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.05	m	-	-	-	-	-
16	1.81	m	-	28.6	CH	13, 14, 17	22
17a	1.58	m	-	53.7	CH_2	18, 19	-
17b	1.91	m	-	-	-	-	-
18	-	-	-	74.7	-	-	-
19	5.56	brs	-	138.2	CH	-	20
20	5.86	m	-	125.8	CH	18, 19	19
21	5.52	brs	-	77.5	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.4	26.5	CH_3	15, 16, 17	16
23	1.36	s	-	31.3	CH_3	17, 18, 19	-
1‘	-	-	-	138.3	-	-	-
2’ 6’	7.34	d	8.5	130.4	2 x CH	3’, 4’, 5’, 10	3’, 5’
3’ 5’	7.71	d	8.5	121.0	2 x CH	1’, 4’	2’, 6’
4’	-	-	-	136.0	-	-	-
24	-	-	-	170.2	-	-	-
25	2.26	s	-	21.0	CH_3	24	-
4‘‘	-	-	-	145.0	-	-	-
5‘‘	8.08	s	-	120.5	CH	4‘‘	-
6‘‘	5.30	s	-	62.0	CH_2	4’’, 5’’,7’’	-
7‘‘	-	-	-	157.3	CH_2	6‘‘, 8‘‘, 17‘‘	-
8‘‘12‘‘	7.00	d	8.6	115.2	2 x CH	7’’, 9’’, 11’’	9’’, 11’’
9‘‘ 11‘‘	7.10	d	8.6	130.2	2 x CH	8‘‘, 10‘‘	8’’, 12’’
10‘‘	-	-	-	138.3	-	-	-

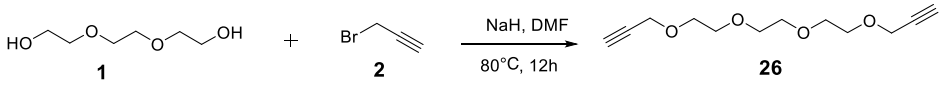

f1 (ppm)


f1 (ppm)


WCED119A.10.ser
Wang WC 119 3,0 mg in CDCl3 at 298.0K 13.06.19, Rettstadt
mass=1081, Azide - alkene coupling
DQF-COSY
f2 (ppm)
f1 (ppm)

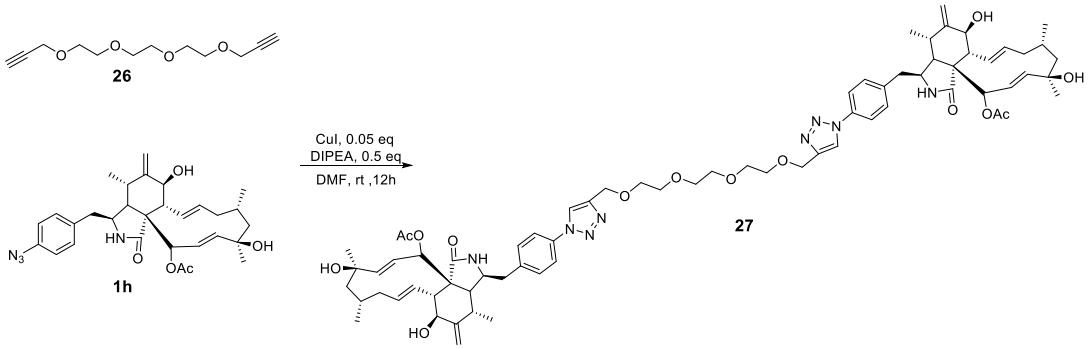

WCMC119A.10.ser
Wang WC 119 3,0 mg in CDCl3 at 298.0K 13.06.19, Rettstadt
mass=1081, Azide - alkene coupling
13C-HMBC
f2 (ppm)
f1 (ppm)

2.24 Bis acetylene linker 26

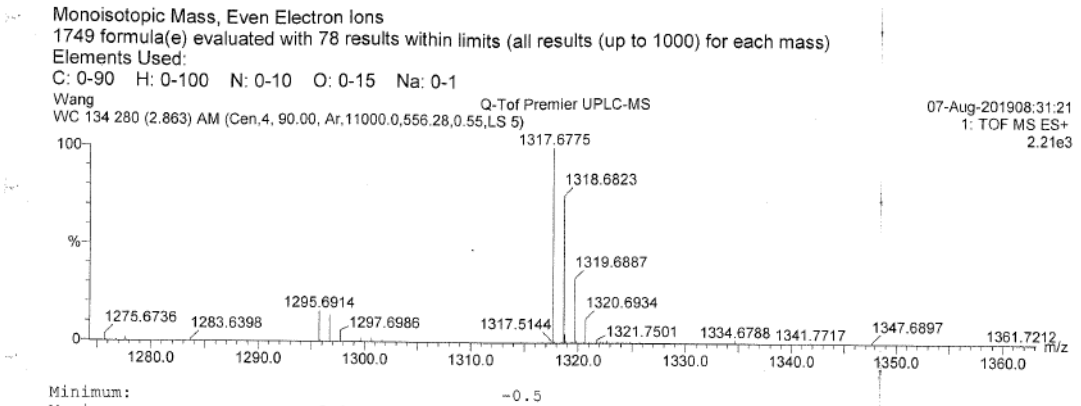


To a stirred solution of **PEG** (100 mg, 0.67 mmol, 1.0 eq, purchased from Sigma-Aldrich) in dry DMF (3 mL) was added sodium hydride (32.2 mg, 1.34 mmol, 2.0 eq). The mixture was stirred at room temperature for 1 hour under nitrogen after which propargyl bromide (0.6 mL, 6.7 mmol, 10.0 eq, purchased from Acros) was added. The reaction solution was stirred at 80 °C for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was diluted with water (10 mL) and then neutralized with 0.1 M HCl (15 mL). The resulting mixture was extracted with EtOAc (15 mL × 2) and the exract was washed with brine (20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The crude light yellow oil (100 mg) was directly used in the next step without further purification.

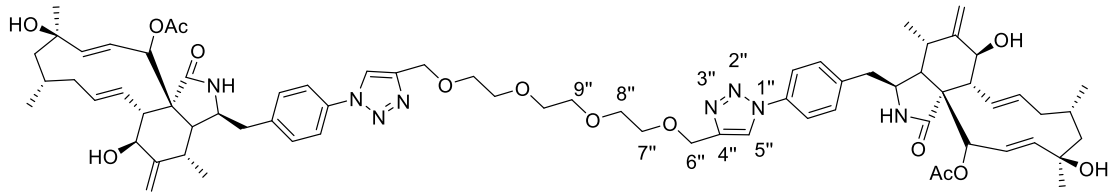
2.25 Dimeric Cytochalasin H 27



To a stirred solution of **1h** (20 mg, 0.038 mmol, 1.0 eq) and **26** (25.8 mg, 0.114 mmol, 3. 0 eq) in DMF (4 mL) was added CuI (0.4 mg, 1.90 µmol, 0.05 eq) and DIPEA (2.4 mg, 19.0 µmol, 0.5 eq). The mixture was stirred at room temperature for 12 hours under nitrogen and the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (2 mL) and washed with water (3 mL x 2). The organic phase was dried over $MgSO_4$, filtered and evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and purified by LCMS to afford 15.4 mg of desired product (31.7%, 0.012 mmol) as a brown solid.



HRMS (*m/z*): calculated for [$C_{872}H_{94}N_8O_{14}$ + Na]: 1317.6787, found: 1317.6775.

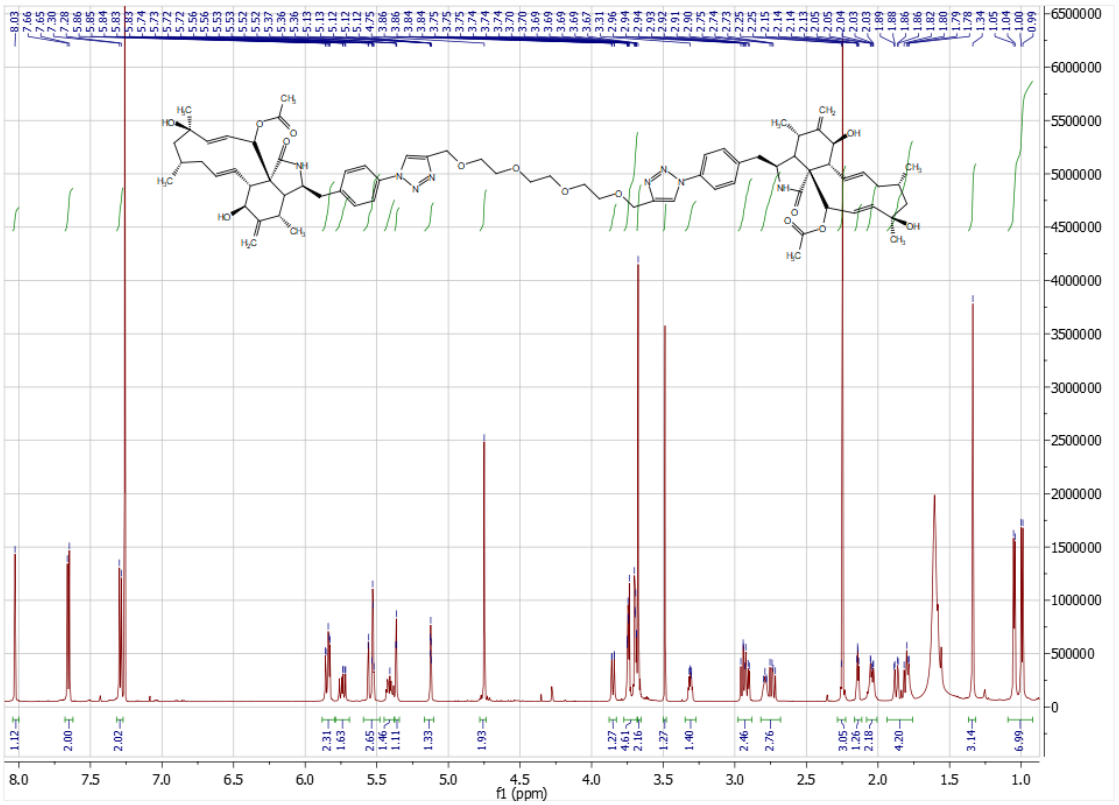


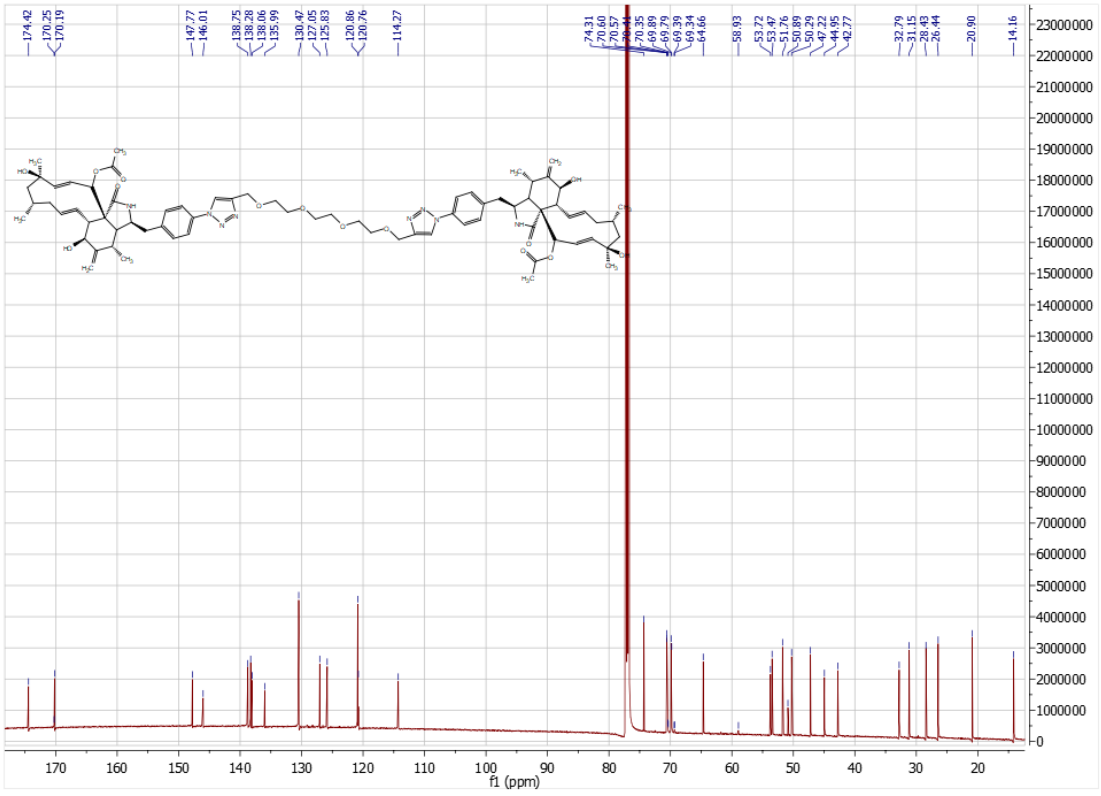
27

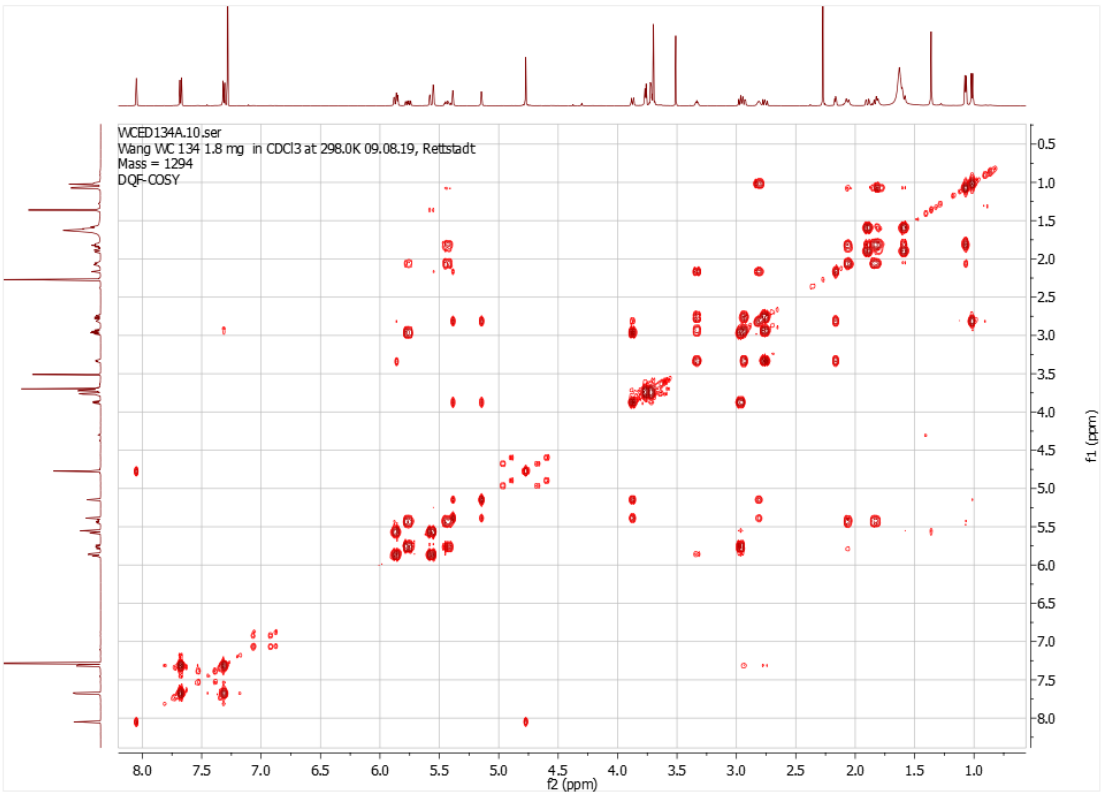
Chemical Formula: $C_{72}H_{94}N_8O_{14}$

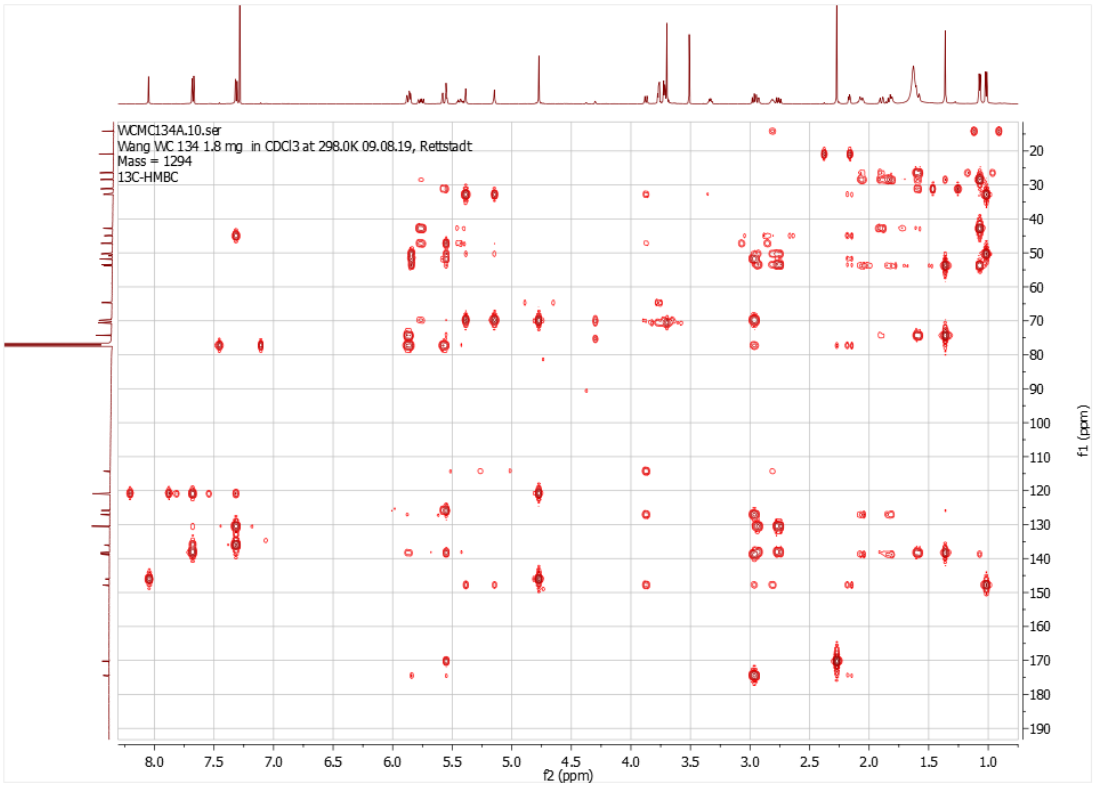
Exact Mass: 1294.6889

Position	δ_H	M	J_{H-H}/Hz	δ_C	HSQC	HMBC H to C	H-H COSY
1	-	-	-	174.3	-	-	-
3	3.33	m	-	53.5	CH	1', 1, 4, 5	4, 5, 10
4	2.16	dd	3.8, 5.1	50.3	CH	1, 3, 5, 6, 10	3, 5, 10
5	2.81	m	-	32.8	CH	1', 3	4, 11, 12
6	-	-	-	147.9	-	-	-
7	3.88	dd	1.3, 10.8	69.9	CH	6, 12	8, 12
8	2.96	m	-	47.3	CH	7, 9, 13, 20, 21	7, 13
9	-	-	-	51.8	-	-	-
10a	2.76	m	-	45.1	CH_2	1', 3	3, 4, 12
10b	2.93	m	-	-	-	-	-
11	1.02	d	6.7	14.2	CH_3	4, 5, 6	5
12a	5.14	brs	-	114.2	CH_2	5, 6, 7	5, 7, 11
12b	5.39	brs	-	-	-	-	-
13	5.75	ddd	1.4, 9.7, 15.4	126.9	CH	7, 8, 15	8, 14, 15
14	5.42	ddd	4.8, 10.1, 15.4	138.8	CH	9, 15	13, 15
15a	1.82	m	-	42.7	CH_2	13, 14, 16, 17	13, 14,
15b	2.06	m	-	-	-	-	-
16	1.81	m	-	28.6	CH	13, 14, 17	22
17a	1.60	m	-	53.6	CH_2	18, 19	-
17b	1.88	m	-	-	-	-	-
18	-	-	-	74.7	-	-	-
19	5.56	brs	-	138.2	CH	-	20
20	5.88	dd	2.2, 17.0	125.7	CH	18, 19	19
21	5.55	brs	-	77.3	CH	8, 9, 19, 20, 23	-
22	1.07	d	6.3	26.5	CH_3	15, 16, 17	16
23	1.36	s	-	31.3	CH_3	17, 18, 19	-
1'	-	-	-	138.3	-	-	-
2' 6'	7.31	d	8.5	130.4	2 x CH	3', 4', 5', 10	3', 5'
3' 5'	7.68	d	8.5	120.8	2 x CH	1', 4'	2', 6'
4'	-	-	-	136.0	-	-	-
24	-	-	-	170.2	-	-	-
25	2.27	s	-	21.0	CH_3	24	-
4''	-	-	-	145.0	-	-	-
5''	8.04	s	-	120.4	CH	4''	-
6''	4.77	s	-	64.7	CH_2	4'', 5'',7''	-
7''	3.76	m	-	69.9	CH_2	6'', 8'', 17''	-
8''9''	3.70	m	-	70.6	CH_2	8'', 9''	-


f1 (ppm)


f1 (ppm)


WCED134A.10.ser
Wang WC 134 1.8 mg in CDCl3 at 298.0K 09.08.19, Rettstadt
Mass = 1294
DQF-COSY
f2 (ppm)
f1 (ppm)


WCMC134A.10.ser
Wang WC 134 1.8 mg in CDCl3 at 298.0K 09.08.19, Rettstadt
Mass = 1294
13C-HMBC
f2 (ppm)
f1 (ppm)


WCMH134A.10.ser
Wang WC 134 1.8 mg in CDCl3 at 298.0K 09.08.19, Rettstadt
Mass = 1294
13C-HSQC
{1.02,14.20}
{2.27,20.98}
{1.81,28.57}
{1.07,26.53}
{2.81,32.83}
{1.36,31.25}
{2.06,42.88}
{2.93,45.11}
{2.96,47.27}
{1.82,42.71}
{3.51,50.92}
{2.16,50.30}
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{1.88,53.94}
{1.60,53.64}
{4.77,64.69}
{3.70,70.57}
{3.88,69.93}
{5.55,77.29}
{5.14,114.23}
{5.39,114.18}
{7.68,120.82}
{5.75,126.93}
{5.88,125.74}
{7.31,130.42}
{5.44,138.71}
{5.58,138.25}
f2 (ppm)
f1 (ppm)

3.0 *In vivo* Procedures

3.01 Growth Inhibition of Fungal Strains

Pyrichalasin H **1a** and compounds **1e - 27** (Table 3.1) were subjected to a serial dilution assay in 96-well plate format, with selected microorganisms grown for 24 (*S. pombe*. *P. anomala*. *M. hiemalis*. *R. glutinis*) and 48 hours (*C. albicans*), as described by Mulwa et al. 2018.[1] Inhibitory concentrations are given in µM. Observed alleviated, but incomplete inhibition of growth is marked in brackets.

Table 3.1: Growth Inhibitory concentrations of pyrichalasin H **1a** and derivatives in a serial dilution assay *versus* selectedfungi. Inhibiting concentrations are given in µM (µmol/L). n.i. = no inhibition. Lowest value = red; highest value = green.

Compound	*Schizosaccharomyces pombe*	*Pichia anomala*	*Mucor hiemalis*	*Candida albicans*	*Rhodotorula glutinis*
1a	31.722	n.i.	(31.722)	n.i.	63.635
1e	3.392	n.i.	n.i.	n.i.	6.783
1f	60.622	n.i.	n.i.	n.i.	121.971
1g	30.331	n.i.	n.i.	n.i.	60.845
1h	7.861	n.i.	62.326	n.i.	31.067
1i	n.i.	n.i.	n.i.	n.i.	n.i.
5a	n.i.	n.i.	n.i.	n.i.	n.i.
5b	n.i.	n.i.	n.i.	n.i.	n.i.
5c	n.i.	n.i.	n.i.	n.i.	93.806
7a	n.i.	n.i.	n.i.	n.i.	n.i.
7b	n.i.	n.i.	n.i.	n.i.	n.i.
7c	n.i.	n.i.	n.i.	n.i.	n.i.
9	n.i.	n.i.	n.i.	n.i.	n.i.
11	n.i.	n.i.	n.i.	n.i.	n.i.
13	n.i.	n.i.	n.i.	n.i.	n.i.
15	78.143	n.i.	19.361	4.899	78.143
17	12.264	n.i.	n.i.	n.i.	49.499
19	n.i.	n.i.	n.i.	n.i.	n.i.
21	n.i.	n.i.	n.i.	n.i.	n.i.
22	126.106	n.i.	n.i.	n.i.	n.i.
23	108.175	n.i.	n.i.	n.i.	n.i.
24	n.i.	n.i.	n.i.	n.i.	n.i.
25	n.i.	n.i.	n.i.	n.i.	n.i.
27	n.i.	n.i.	n.i.	n.i.	n.i.

3.02 Cytotoxicity Assays

Cytotoxicity was assessed by subjection of pyrichalasin H **1a** and synthetic derivatives **1e - 27 (Table 3.2)** to a standardized metabolic activity test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) on mouse fibroblast cells (L929) and different cancer cell lines (cervix cancer, KB3.1; prostate cancer, PC3; lung cancer, A549; breast cancer, MCF-7; epidemoid carcinoma, A431; ovarian cancer, SKOV-3), as available. Concentrations are given for 50% growth inhibition observed with the no-compound control as reference[1] (IC_{50} in µM).

Table 3.2: Concentrations (µM) of previously described compounds found to have metabolism-inhibitory effects according to the MTT assay half-maximal to complete inhibition (IC50). Epothilon B served as the inhibited-growth negative control.
* = no visible effect; ** = small proliferation inhibition; n.d. = not determined. Lowest value = red; highest value = green.

Compound	L929	KB3.1	PC3	A549	MCF-7	A431	SKOV-3
1a	0.21	0.036	0.153	0.067	0.055	0.044	0.034
1e	0.128	0.019	1.292	0.032	0.015	0.016	0.021
1f	0.419	0.273	0.255	0.273	0.455	0.291	0.419
1g	0.329	0.08	0.183	0.256	0.164	0.183	0.238
1h	0.082	0.036	0.071	n.d.	0.026	n.d.	n.d.
1i	2.3601	0.393	60.005	n.d.	0.452	n.d.	n.d.
5a	10.621	1.445	2.089	4.353	1.254	1.915	2.089
5b	0.503	0.22	6.286	n.d.	0.22	n.d.	n.d.
5c	0.364	0.518	1.82	2.38	1.68	0.504	1.386
7a	**	19.599	n.d.	n.d.	30.623	n.d.	n.d.
7b	**	40.944	n.d.	n.d.	n.d.	n.d.	n.d.
7c	**	14.211	n.d.	n.d.	n.d.	n.d.	n.d.
9	*	**	n.d.	n.d.	n.d.	n.d.	n.d.
11	25.375	6.84	n.d.	n.d.	17.652	n.d.	n.d.
13	43.305	4.565	**	n.d.	3.745	n.d.	n.d.
15	2.799	0.606	1.749	2.333	1.061	1.633	0.805
17	0.096	0.021	0.096	0.051	0.055	0.035	0.03
19	27.14	21.713	n.d.	n.d.	n.d.	n.d.	n.d.
21	1.861	0.518	0.425	0.518	0.332	0.412	0.385
22	5.27	2.635	3.388	3.388	2.447	5.458	6.023
23	1.937	0.291	0.549	0.581	0.371	0.387	0.452
24	8.227	1.117	6.704	n.d.	0.828	n.d.	n.d.
25	1.664	0.12	0.647	0.629	0.592	0.629	1.109
27	**	6.565	n.d.	n.d.	n.d.	n.d.	n.d.

3.03 *In vivo* Effects on the Actin Cytoskeleton

Effects on the actin cytoskeleton were characterized in further detail using osteosarcoma cells (U2OS), following the procedure described by Kretz et al. 2019.[2] All compounds affected the actin cytoskeleton, confirming retained activities as functionalized compounds even upon heavy modification (Figure 3.1A, Figure 3.1B).

Fluorescently-labelled cytochalasins were further investigated concerning their potential use in staining the actin cytoskeleton. Here, U2OS cells were grown on fibronectin-coated cover slips overnight similar to the methodology employed before. Untreated, well-spread cells were fixed with 500µl of warm 4% paraformaldehyde (PFA) in PBS solution for 20 minutes and washed with 500µl PBS. After cell permeabilisation with 500µl 0.1% Triton X-100 in PBS and additional washing steps with 500µl PBS (3x), coverslips were treated with labelled cytochalasin derivatives (100µg/ml) and fluorescently-labelled phalloidins (ALEXA 488, green fluorescence; ATTO 594, red fluorescence), in each case complementary in colour, by incubation in a wet chamber on parafilm. Samples were incubated for 1h in the dark, followed by procedures for nuclear staining and image recording, as stated above.

Initial staining attempts using **21** as a fluorophore did not show any interpretable fluorescence, thus it was excluded from future experiments (data not shown). Out of the four fluorescently-coupled derivatives, both **19** and **17**displayed a staining pattern at least partially overlapping with actin filament-containing stress fibre-like structures, as confirmed by counterstaining with phalloidin (Fig. 3.2). However, in follow-up experiments, stainings appeared more specific with **17.** Moreover, signal intensities with **17** were further improved upon inclusion of 0.25% glutaraldehyde (GA, Fig. 3.3) as fixative as compared to using 4% para-formaldehyde (PFA, Fig. 3.2) alone, assumed to counteract partial filament loss seen with weaker fixatives such as PFA.[3] Notably, unspecific staining patterns obtained with **13** and **15** were not improved further upon addition of 0.25% GA.

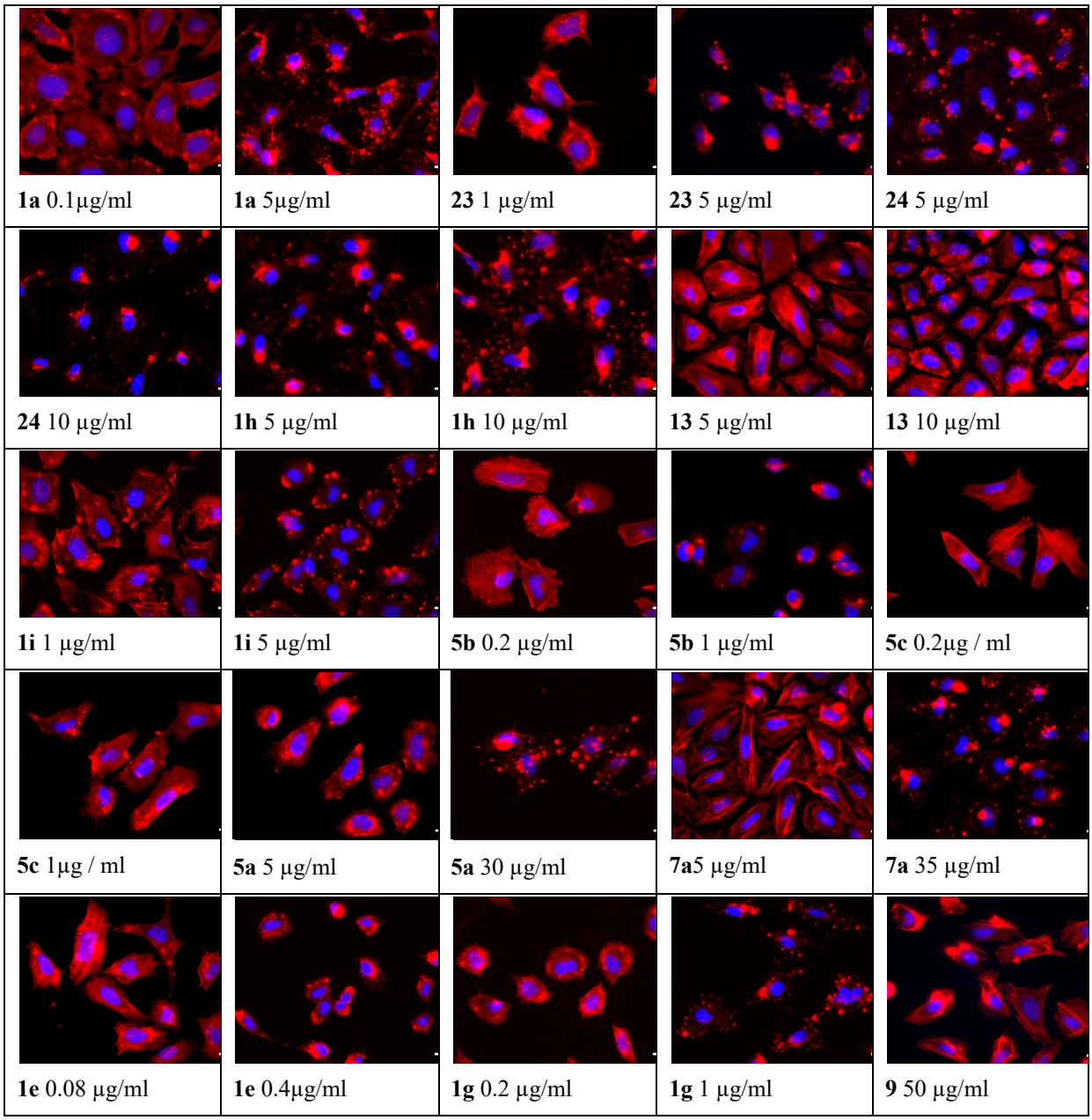

Figure 3.1A: Summary of fluorescence microscopy images showing the actin cytoskeleton (pseudocoloured in red) stained by fluorescently-coupled phalloidin (atto 594) and nuclei stained by DAPI (blue). DMSO served as a vehicle control. U2OS cells were treated for 1 hour with respective compound and concentration, and directly fixed with 4% PFA. Images show the collapse of cytoskeletal actin to different degrees of severity. See Figure 3.1B for the effect of cytochalasin B (CytB).

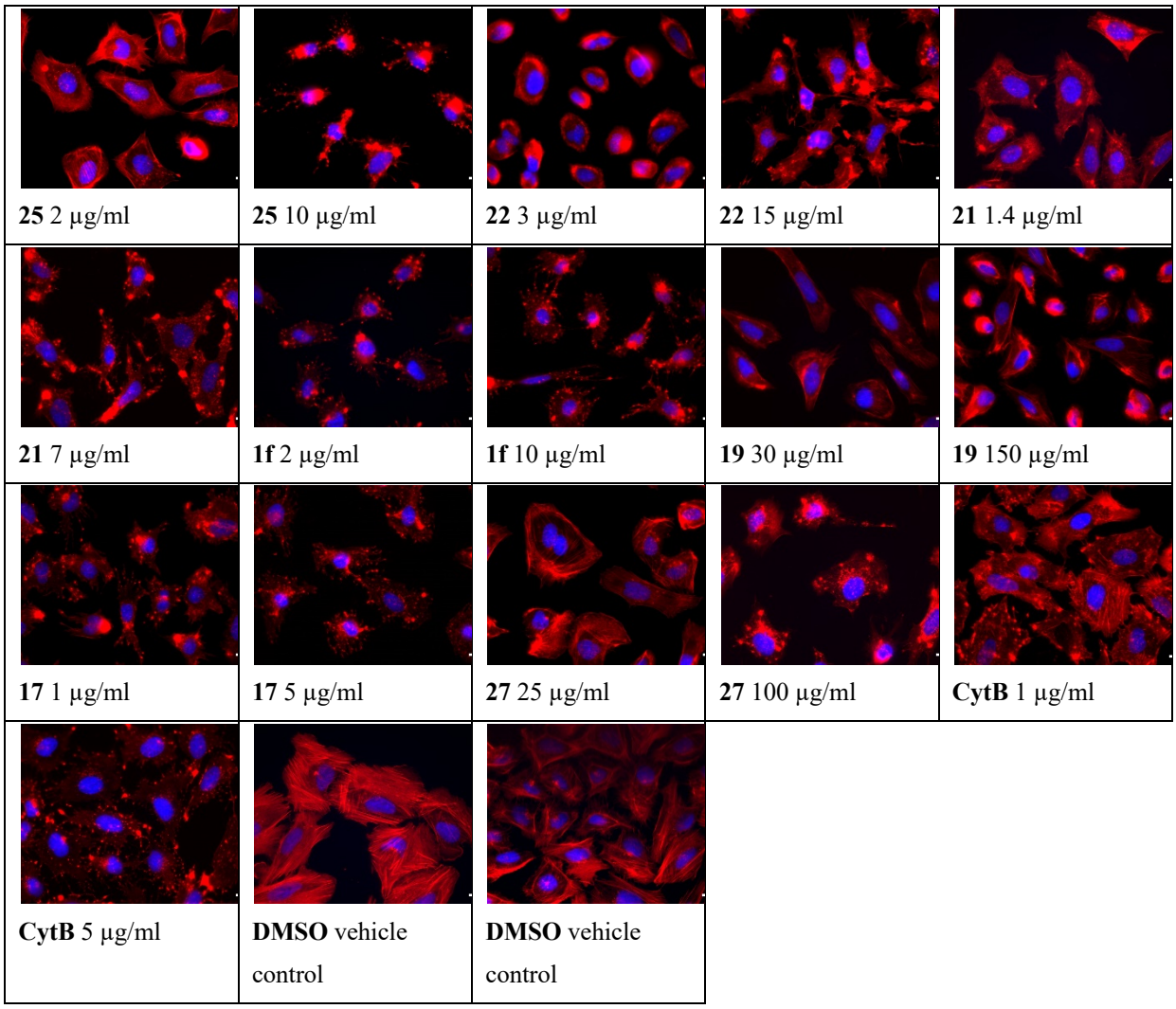

Figure 3.1B: Summary of fluorescence microscopy images showing the actin cytoskeleton (pseudocoloured in red) stained by fluorescently-coupled phalloidin (atto 594) and nuclei stained by DAPI (blue). DMSO served as a vehicle control. U2OS cells were treated for 1 hour with respective compound and concentration, and directly fixed with 4% PFA. Images show the collapse of cytoskeletal actin to different degrees of severity. The effect of cytochalasin B (CytB) is shown in comparison.

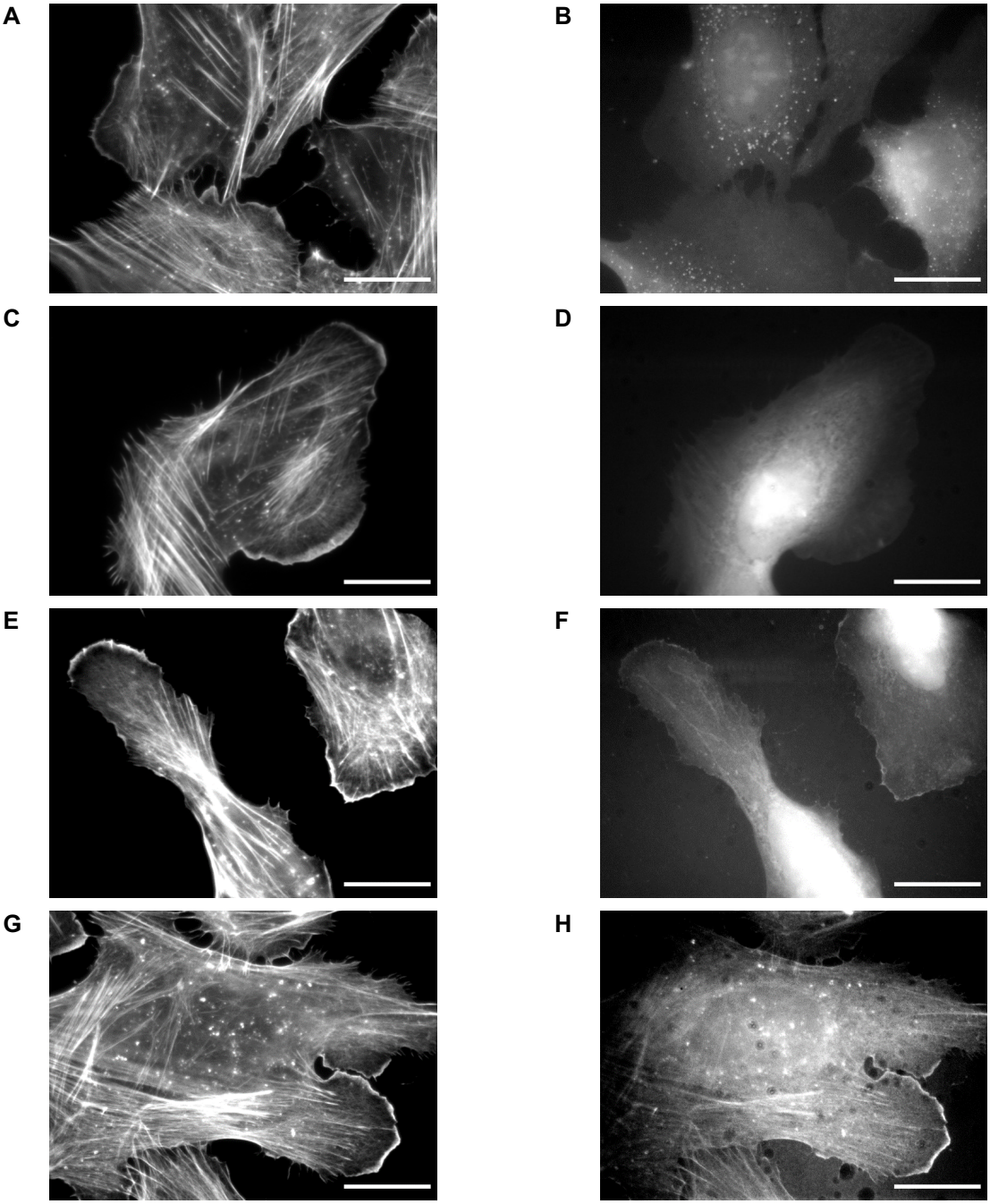

Figure 3.2: Fluorescence microscopy images of untreated U2OS cells simultaneously stained with fluorescently-coupled phalloidin (ALEXA 488, ATTO 594) and differentially-derivatised cytochalasin variants upon fixation with 4% PFA. **A**, **C**, **E**, **G**: Actin cytoskeleton stained with phalloidin. Images **B**, **D**: Signals obtained with **13** and **15**, respectively, and lacking any obvious specificity for the actin cytoskeleton. Image **F**: **19** displaying mostly cytosolic signal, and very weak association with actin cytoskeletal structures, such as lamellipodia-like structures at the cell periphery. Image **H**: More prominent association of **17** with the actin cytoskeleton. Note that prominently stained structures at least partly overlap with the phalloidin staining in image **G**. Scale = 20µm.

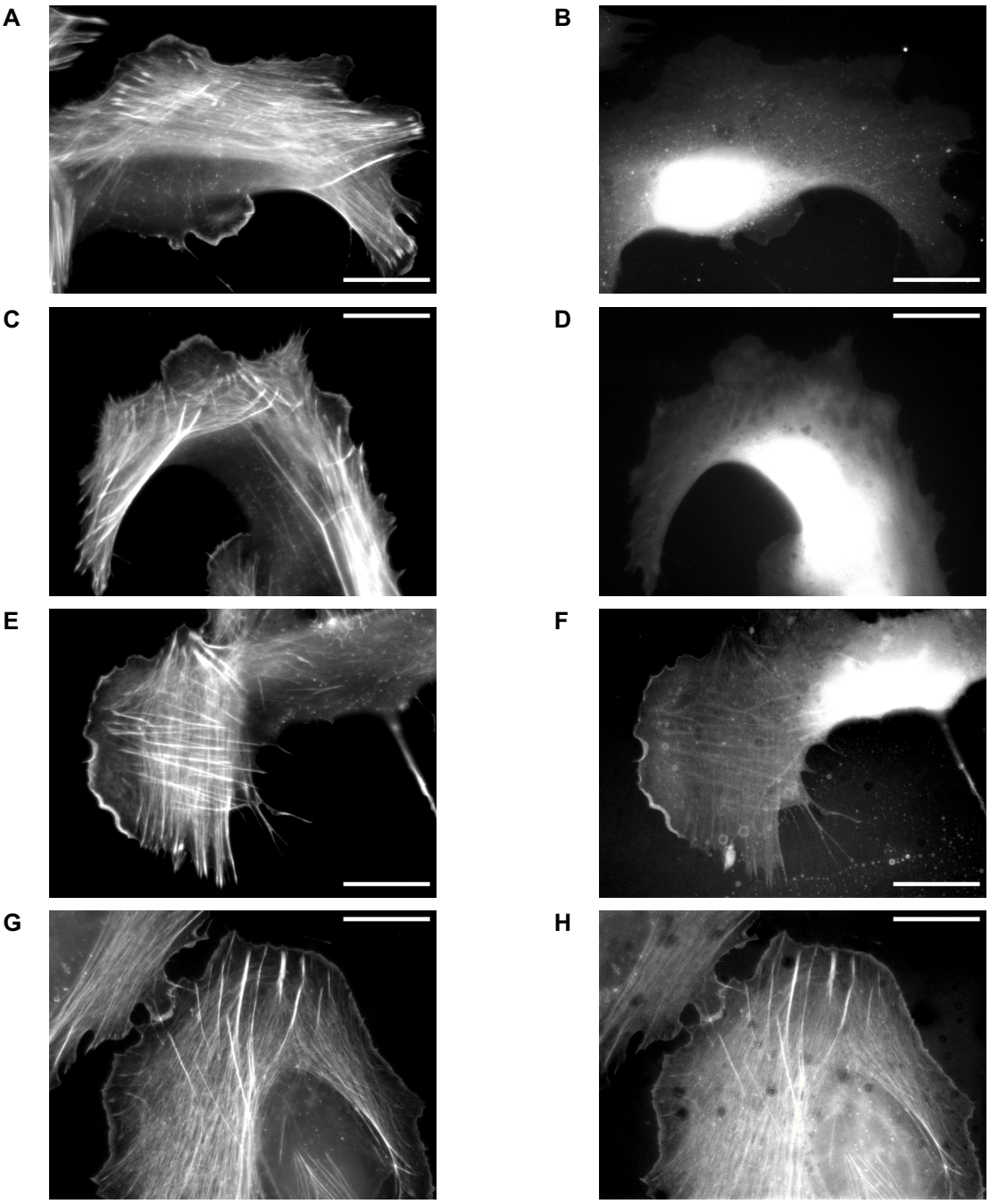

Figure 3.3: Fluorescence microscopy of untreated U2OS cells simultaneously stained with fluorescently-coupled phalloidin (ALEXA 488, ATTO 594) and cytochalasin derivatives upon fixation with 4% para-formaldehyde (PFA) and 0.25% glutaraldehyde (GA). **A**, **C**, **E**, **G**: Actin cytoskeleton as stained with phalloidin. Images **B**, **D**: lack of association with the actin cytoskeleton, as already observed for **13** and **15** upon fixation with PFA alone (Figure 3.2). Image **F**: Clearly discernible staining of actin cytoskeletal structures with **19**, which appears enhanced upon PFA/GA-fixation as compared to PFA alone (see Figure 3.2), and **H**: **17** displaying quite robust labelling of actin filament networks, reasonably well overlapping in these conditions with the phalloidin staining in G. Scale bars = 20µm.

4.0 References

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