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Supplementary information

A designed photoenzyme for enantioselective [2+2] cycloadditions

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Supplementary Information

A Designed Photoenzyme for Enantioselective [2+2]-Cycloadditions

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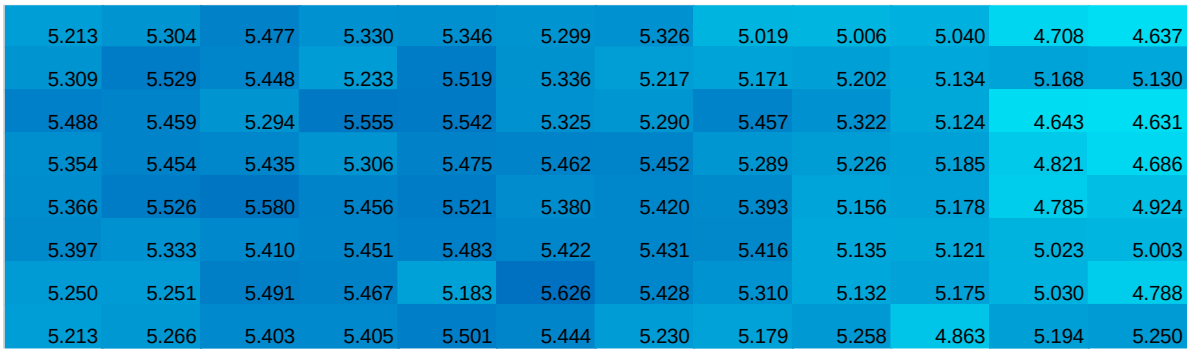
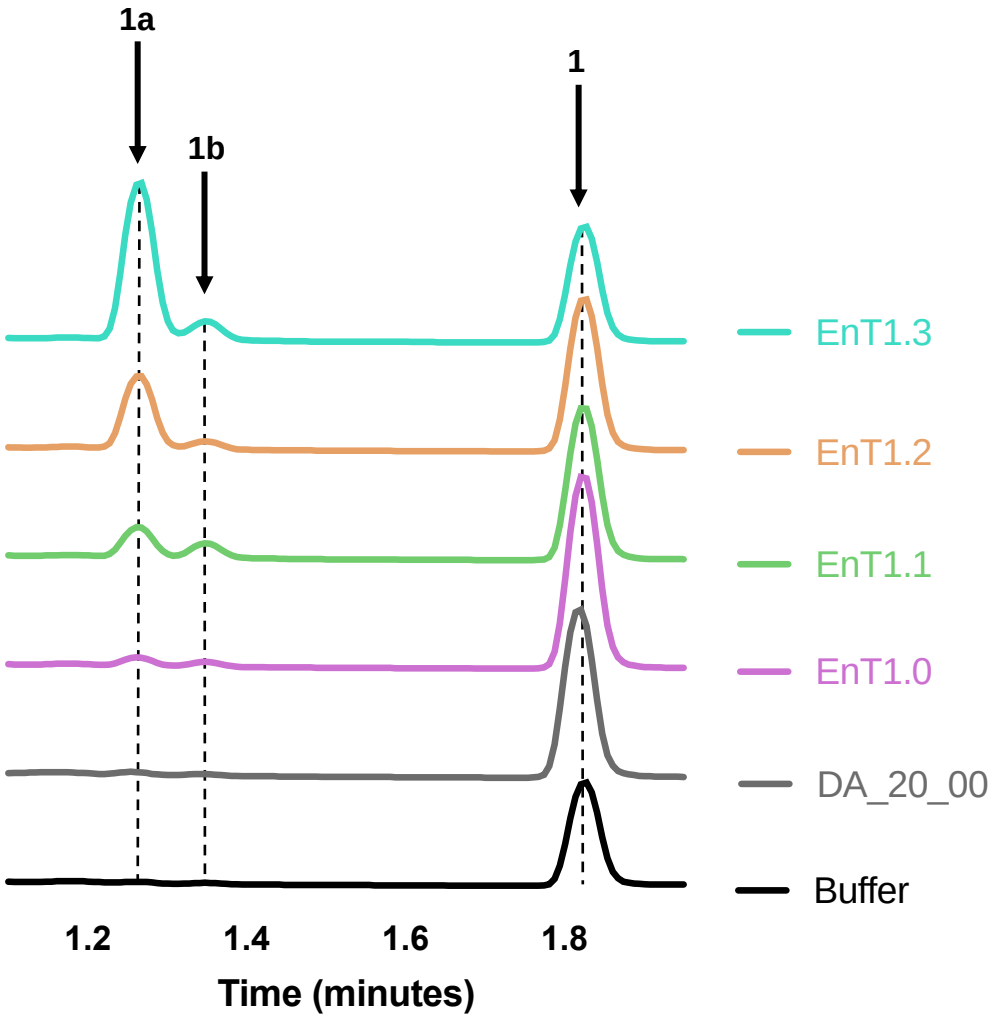
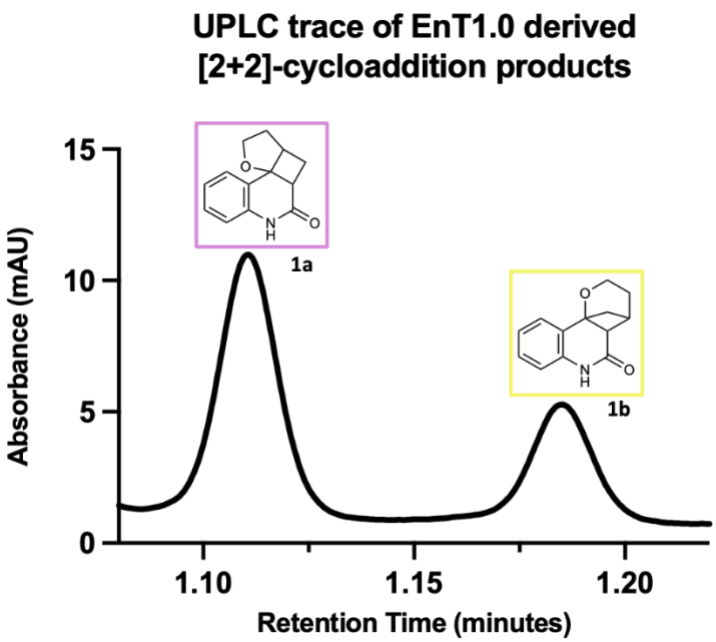


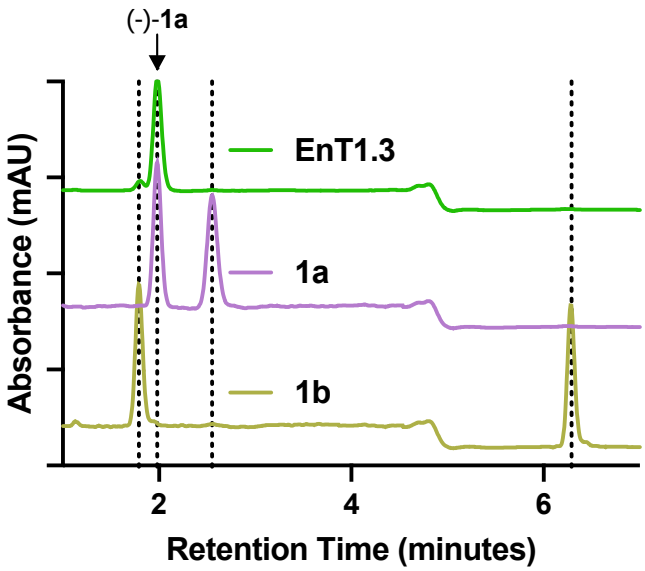
Supplementary Figure 1: Uniformity of light source. Uniformity of light irradiation of a UV curing LED oven chamber (NovaChem) was investigated by monitoring conversions across a 96-well plate. Reaction conditions: 7.5 μM EnT1.0, 400 μM **1**, 30 minutes irradiation (10 seconds on/off pulse) at 365 nm, at 4 °C, in 100 μL PBS (pH = 7.4) with 5% DMSO as a cosolvent. Conversions (**1a** and **1b** combined) are shown as a matrix, correlating to their respective plate positions. The relative coefficient of variance is 4.3%.



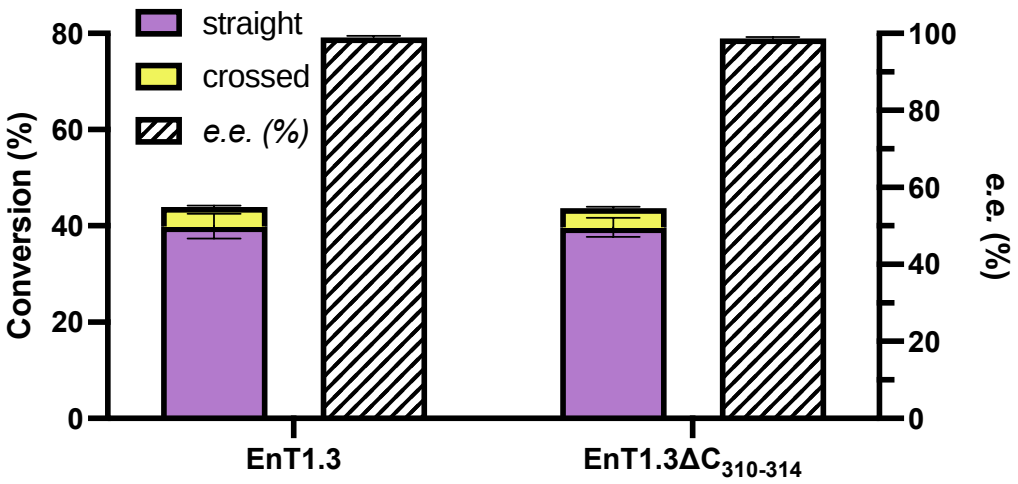
Supplementary Figure 2: UPLC chromatograms of evolution trajectory. UPLC absorbance chromatograms at 260 nm for reactions containing no enzyme, DA_20_00, EnT1.0, EnT1.1, EnT1.2 or EnT1.3. Reaction conditions: 3 μM catalyst, 400 μM **1**, 60 minutes irradiation (10 seconds on/off pulse) at 365 nm, at 4 °C, in 100 μL PBS (pH = 7.4) with 5% DMSO as a cosolvent.



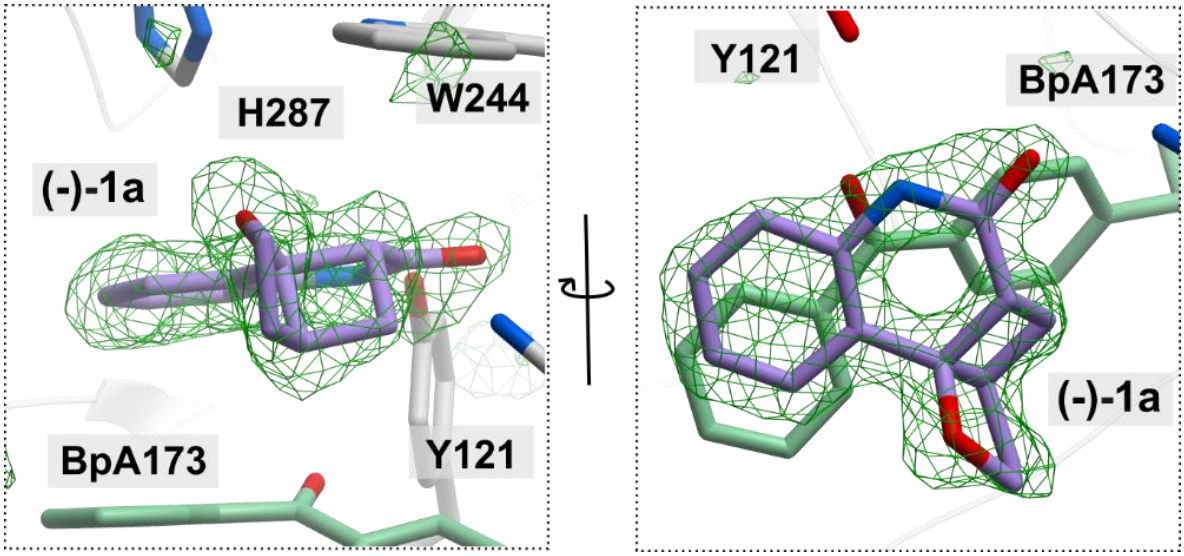
Supplementary Figure 3: UPLC chromatogram showing products derived from reactions with EnT1.0. UPLC chromatogram at 260 nm showing product **1a** and product **1b** (2:1 ratio). Reaction conditions: 7.5 μM EnT1.0, 400 μM **1**, 30 minutes irradiation (10 seconds on/off pulse) at 365 nm, at 4 °C, in 100 μL PBS (pH = 7.4) with 5% DMSO as a cosolvent.



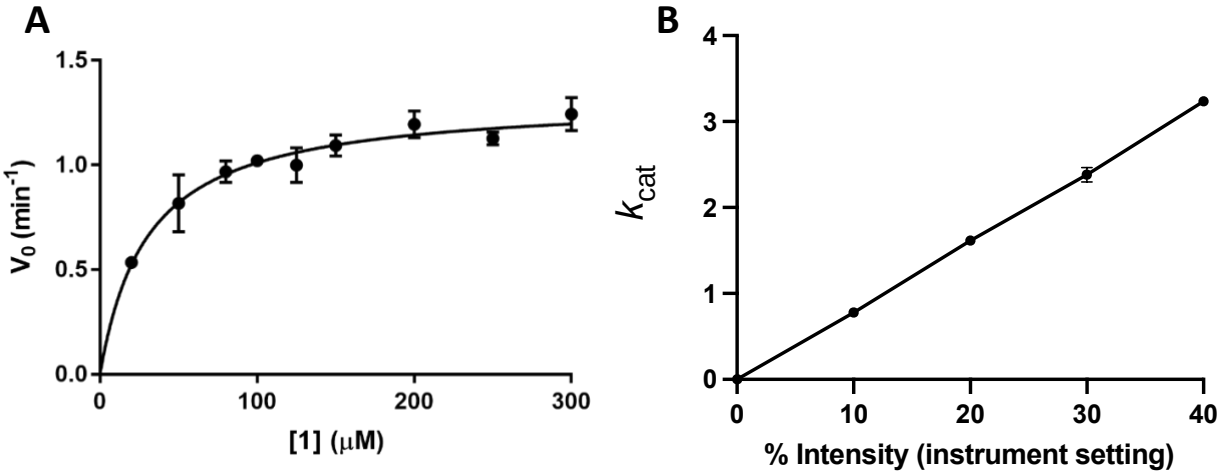
Supplementary Figure 4: SFC chromatograms of racemic product standards and the product of EnT1.3-catalysed reaction using substrate 1. Reaction conditions: 10 µM EnT1.3, 400 µM **1**, 60 minutes irradiation (10 seconds on/off pulse) at 365 nm, at 4 °C, in 100 µL PBS (pH = 7.4) with 5% DMSO as a cosolvent. For SFC analysis, reactions were extracted with 3 volumes of ethyl acetate.



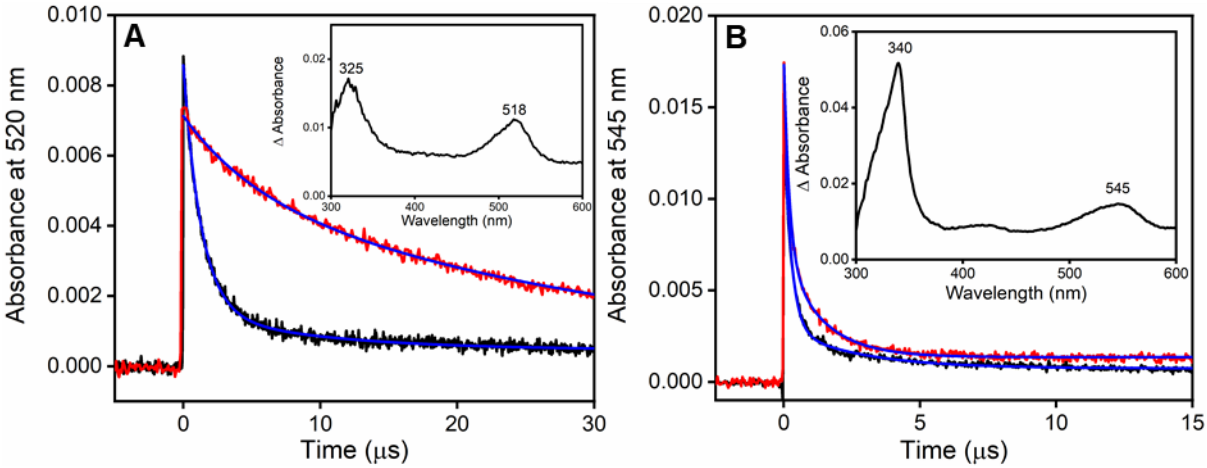
Supplementary Figure 5: A comparison of the activity and selectivity of EnT1.3 and EnT1.3ΔC$_{310-314}$. Reaction conditions: 10 μM catalyst, 400 μM **1**, 15 minutes irradiation (10 seconds on/off pulse) at 365 nm, at 4 °C, PBS (pH = 7.4) with 5% DMSO as a cosolvent. The error bars represent the standard deviation of measurements made in triplicate.



Supplementary Figure 6: **The active site of EnT1.3ΔC$_{310\text{-}314}$ in complex with the product (-)-1a.** The Fo − Fc omit density corresponding to (-)-**1a** is contoured at 3σ (green mesh). The product, BpA173 and active site residues are shown as atom-coloured sticks (with carbons shown in purple, green, and grey, respectively).



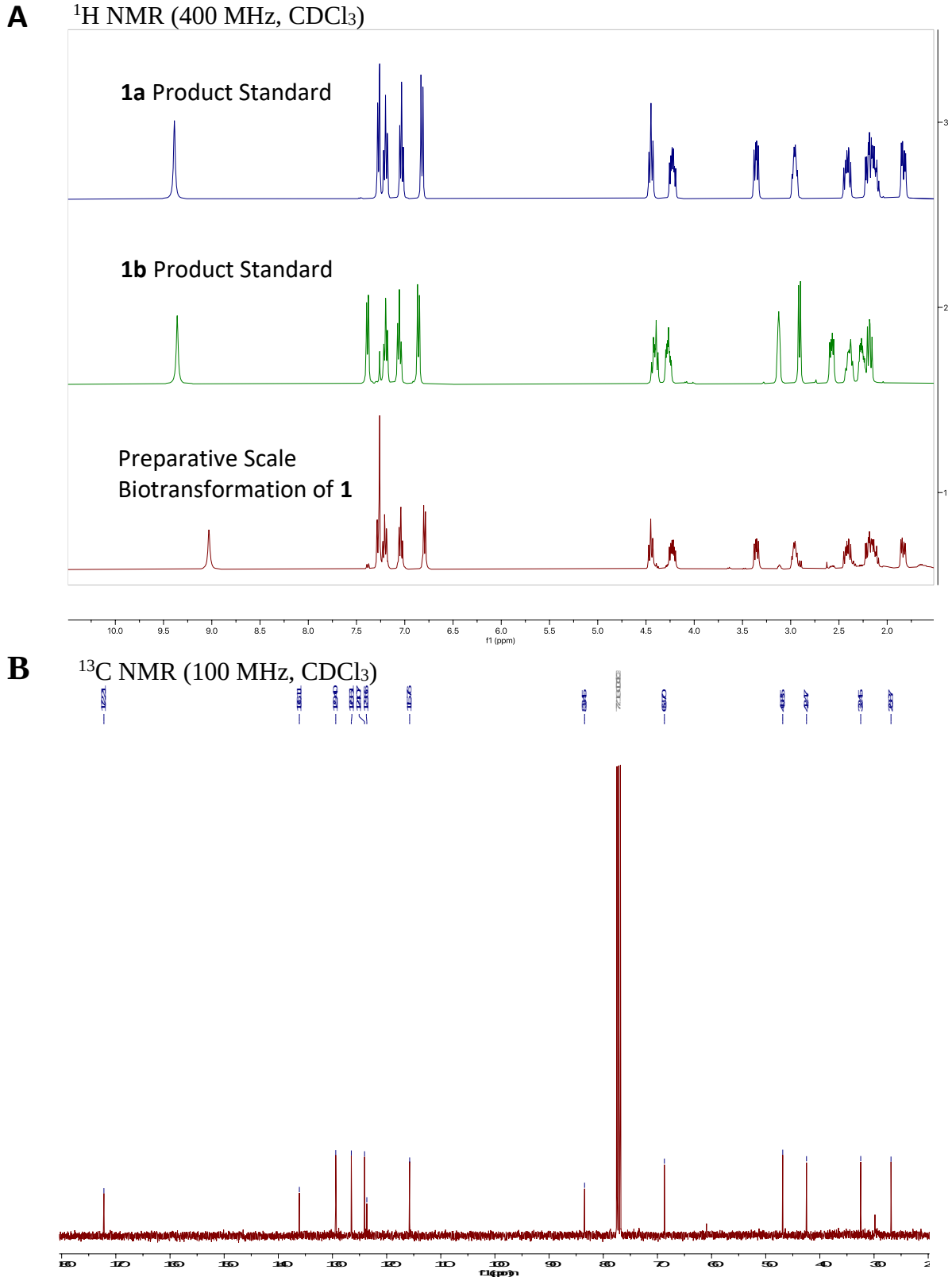
Supplementary Figure 7: Kinetic characterisation of EnT1.3. A) Michaelis-Menten plot for the intramolecular [2+2]-cycloaddition of substrate **1** catalysed by EnT1.3. Kinetic assays were performed at various concentrations of **1**, and 1 μM of EnT1.3 in 250 μL PBS (pH 7.4) with 5% DMSO as a co-solvent in 96-well plate. The plots show the averaged initial rates which were fitted to the Michaelis-Menten equation using Origin software. Data are a mean ± S.D. of measurements made in triplicate. B) The dependence of EnT1.3 v_0 on light intensity. Assays were performed with 400 μM of substrate **1** and 1 μM of EnT1.3 in 500 μL PBS (pH 7.4) with 5% DMSO as a co-solvent in 1 mL glass vials. Intensity was varied from 0%-40% (based on instrument settings) with the reactions irradiated 3 cm directly below the LED array.



C

Sample	Lifetimes (µs)	Relative amplitude
BpA plus O_2	$\tau_1 = 1.31 \pm 0.03$ $\tau_2 = 9.71 \pm 0.99$	$A_1 = 46\,\%$ $A_2 = 54\,\%$
BpA minus O_2	$\tau_1 = 5.16 \pm 0.38$ $\tau_2 = 32.38 \pm 1.15$	$A_1 = 6\,\%$ $A_2 = 94\,\%$
EnT1.3 plus O_2	$\tau_1 = 0.26 \pm 0.04$ $\tau_2 = 2.72 \pm 0.11$	$A_1 = 28\,\%$ $A_2 = 72\,\%$
EnT1.3 minus O_2	$\tau_1 = 0.26 \pm 0.04$ $\tau_2 = 2.18 \pm 0.05$	$A_1 = 33\,\%$ $A_2 = 67\,\%$

Supplementary Figure 8: Photophysical properties of BP and EnT1.3. Kinetic transients showing the decay of the benzophenone triplet state on the µs timescale in the presence (black) and absence (red) of oxygen for A) 50 µM benzophenone with 5% DMSO as a co-solvent and B) 50 µM EnT1.3 in PBS (pH 7.4). Transients were measured at 4°C at the respective wavelength and data shown are the average of 50 traces. Data were fitted to a double exponential equation to obtain lifetimes (blue lines). The insets show the absorbance difference spectra of the triplet under the same conditions using a CCD gate width of 100 ns. C) Kinetic parameters determined from double exponential fits shown in A and B.



Supplementary Figure 9: Preparative 12 mg Scale Biotransformation of Substrate 1. A) ^{1}H NMR spectra (400 MHz, $CDCl_3$) of **1a** and **1b** chemically synthesised product standards, and crude product extracted from the EnT1.3 (10 μM) catalysed intramolecular [2+2]-cycloaddition of substrate **1 (**400 μM) in PBS (pH 7.4) with 5% DMSO as a co-solvent (140 mL reaction volume). B) ^{13}C NMR spectra (400 MHz, $CDCl_3$) of the crude product of the biotransformation.

Supplementary Information, Table 1: Data summary of conversion and selectivity values of EnT1.0. Reaction conditions: 15 μM catalyst, 400 μM **1**, 30 minutes irradiation (10 seconds on/off pulse) at 365 nm, at 4 °C, PBS (pH = 7.4) with 5% DMSO as a cosolvent. Standard deviations are given for measurements in triplicate.

Catalyst	Average Conversion Straight (%)	S.D.	Average Conversion Crossed (%)	S.D.	*e.e.*	S.D.
-	0.33	0.031	0.19	0.079	-	-
Benzophenone	4.5	0.40	3.1	0.29	-	-
DA_20_00	0.55	0.21	0.32	0.044	-	-
EnT1.0	9.0	0.75	4.4	0.19	46	1.53
EnT1.0$^{\Psi}$	0.0	-	0.0	-	-	-

[Ψ] Reaction was performed without exposure to 365 nm light.

Supplementary Information, Table 2: Data summary of conversion values of EnT1.0 and EnT1.3 for Extended Data Figure 4. Reaction conditions: 15 μM catalyst, 400 μM **1**, 10 seconds on/off pulse at 365 nm, 4 °C, PBS (pH 7.4) with 5% DMSO as a cosolvent. At each time point, 100 μL of reaction mix was quenched with 1 volume MeCN and analysed by UPLC. Standard deviations are given for measurements in triplicate.

	EnT1.3		EnT1.0	
Time (minutes)	**Average Conversion (%)**	**S.D.**	**Average Conversion (%)**	**S.D.**
0.0	0.0	-	0.0	-
1.0	4.35	0.07	1.34	0.05
2.0	8.77	0.42	2.38	0.11
3.0	12.76	0.45	3.54	0.17
4.0	17.13	0.55	4.55	0.07
5.0	21.34	0.74	5.49	0.43

Supplementary Information, Table 3: Substrate scope of EnT variants. Reaction conditions for the synthesis of **1a**-**12a** and **1b**, **2b** and **12b**, conversion is displayed as a total of major and minor product where appropriate. All reactions were performed using PBS buffer pH 7.4 and 5% DMSO in triplicate. Standard deviations are given for measurements in triplicate. Reaction conversions are determined based on UPLC calibration curves of authentic standards of starting materials and products. Extinction coefficients and UPLC methods are given in Supplementary Table S8. The *e.e.* values are determined using chiral SFC or HPLC analysis. Chromatograms of substrate standards, product standards, and biotransformations are provided at the end of the Supplementary Information.

Substrate	Product	Variant	Catalyst loading (mol%)	Substrate loading (mM)	Time (hours)	Conversion (%)	S.D.	*e.e.*
1	**1a**, **1b**	EnT1.3	1.5	0.4	2[1]	94	0.70	99
1	**1a, 1b**	EnT1.3	2.5	0.4	1[1]	100	0.07	99
1	**1a, 1b**	EnT1.3 BpA173Ala + BP	2.5	0.4	1[1]	4.2	0.07	12
2	**2a**, **2b**	EnT1.3	10	0.3	4[1]	99	0.29	90
3	**3a**	EnT1.2	7.5	0.2	3[1]	76	0.95	80
4	**4a**	EnT1.3	5	0.5	2[1]	98	0.39	92
5	**5a**	EnT1.3	7.5	0.4	4[2] [†]	95	2.37	82
6	**6a**	EnT1.3	5	0.4	3[2]	100	N.D.	95
7	**7a**	EnT1.3	10	0.4	3[2]	95	0.31	94
8	**8a**	EnT1.2	7.5	0.3	4[2]	98	0.5	26
8	**8a**	EnT1.1 A229S Y37L	7.5	0.3	4[2]	98	0.87	59
9	**9a**	EnT1.3	5	0.4	3[2]	100	N.D.	97
10	**10a**	EnT1.3	7.5	0.5	3[2][†]	89	0.46	87
11	**11a**	EnT1.3	7.5	0.5	3[2][†]	92	0.71	81
12	**12a**	EnT1.2	7.5	0.5	4[2][†]	89	0.9	48
13	**13a**, **13b**	EnT1.3	7.5	1	3[1][†]	69	0.57	36

13	**13a**, **13b**	EnT1.3 Y121F	7.5	1	3[1][†]	94	0.32	78
13	**13a, 13b**	EnT1.3 Y121F S271C	7.5	1	3[1][*]	99	0.05	98
2-Quinolone + methyl vinyl ketone (MVK)	**14**	EnT1.3	5	2 mM quinolone 50 mM MVK	4[2]	71	0.81	97
2-Quinolone + Ethyl vinyl ketone (EVK)	**15**	EnT1.3	5	2 mM quinolone 50 mM EVK	4[2]	73	0.1	97

[1] 365nm irradiation wavelength
[2] 395nm irradiation wavelength
[†] LED intensity was set to 50% to minimise background reactions.
[*] LED intensity was set to 40% to minimise background reactions.

Supplementary Information, Table 4: Protein crystallization conditions.

Variant	Mother liquor composition
EnT1.0	0.2 M Ammonium sulfate, 0.1 M BIS-Tris, pH = 5.5, 25% w/v PEG 3350 JCSG condition H7
EnT1.3	0.2 M Ammonium chloride, 0.1 M HEPES, pH = 7.0, 20% w/v PEG 6000 PACT premier Eco condition C8
EnT1.3$\Delta C_{310\text{-}314}$	0.2 M Magnesium formate dihydrate, 20% w/v PEG 3350 JCSG condition A5

Supplementary Information, Table 5: Data collection and refinement statistics

	EnT1.0	**EnT1.3**	**EnT1.3ΔC:product**
PDB ascension number	7ZP5	7ZP6	7ZP7
Wavelength (Å)	0.9763	0.9762	0.9763
Resolution range	81.57 - 1.54 (1.595 - 1.54) [a]	54.66 - 1.9 (1.968 - 1.9)[a]	74.59 - 1.7 (1.761 - 1.7)[a]
Space group	C 1 2 1	P 2_1 2_1 2_1	P 1 2_1 1
Unit cell dimensions a, b, c, (Å) α, β, γ (°)	173.847, 43.397, 81.7644, 90, 93.9843, 90	50.894, 75.5961, 78.5996 90, 90, 90	50.7517, 73.9581, 74.7216 90, 93.4574, 90
Total reflections	585059 (35012)	329486 (33095)	417082 (35910)
Unique reflections	89549 (8084)	24554 (2405)	60455 (5861)
Multiplicity	6.5 (4.3)	13.4 (13.8)	6.9 (6.1)
Completeness (%)	98.84 (89.62)	99.96 (99.92)	99.64 (97.25)
Mean I/sigma(I)	17.22 (0.67)	8.95 (1.81)	15.85 (2.08)
Wilson B-factor	23.72	18.41	16.81
R-merge	0.05761 (0.8321)	0.2025 (0.873)	0.08916 (0.4921)
R-meas	0.06245 (0.9503)	0.2105 (0.9067)	0.09642 (0.5379)
R-pim	0.02381 (0.448)	0.05688 (0.2423)	0.03641 (0.2143)
$CC_{1/2}$	0.998 (0.801)	0.996 (0.872)	0.997 (0.876)
CC*	1 (0.943)	0.999 (0.965)	0.999 (0.966)
Reflections used in refinement	89457 (8058)	24547 (2403)	60431 (5860)
Reflections used for R-free	4542 (449)	1239 (123)	3069 (264)
R-work	0.1959 (0.3637)	0.1690 (0.2104)	0.1508 (0.1964)
R-free[b]	0.2231 (0.3768)	0.2133 (0.2776)	0.1807 (0.2400)
CC (work)	0.965 (0.844)	0.962 (0.928)	0.974 (0.922)
CC (free)	0.962 (0.840)	0.942 (0.896)	0.961 (0.874)
Number of non-hydrogen atoms	5359	2840	5621
macromolecules	4901	2566	4883
ligands	10	0	68
solvent	448	274	702
Protein residues	617	321	614
RMS (bonds)	0.010	0.005	0.020
RMS (angles)	1.00	0.90	0.87
Ramachandran favoured (%)	96.71	97.15	96.69
Ramachandran allowed (%)	3.29	2.85	3.31
Ramachandran outliers (%)	0.00	0.00	0.00
Rotamer outliers (%)	0.00	0.37	0.58
Clashscore	2.26	4.93	3.71
Average B-factor	31.98	21.37	21.07
macromolecules	31.42	20.56	19.77
ligands	48.84	-	30.61
Solvent	37.75	28.96	29.67
Number of TLS groups	1	1	1

[a]Values in parentheses are for highest resolution shell. [b]R-free was calculated using ~5% of the data separate from the rest

Supplementary Information, Table 6: Cosolvent tolerance of EnT1.3. Reaction conditions: 7.5 μM EnT1.3, 400 μM **1**, 10 seconds on/off pulse at 365 nm, 4 °C, PBS (pH 7.4) with the stated cosolvents. Standard deviations are given for measurements in triplicate.

Co-solvent	Average Conversion Straight (%)	S.D.	Average Conversion Crossed (%)	S.D.	*e.e.*
5% DMSO	33.6	0.5	3.6	0.0	99
10% DMSO	31.1	1.7	3.4	0.2	98
15% DMSO	27.8	0.5	3.1	0.1	98
20% DMSO	24.0	0.3	2.9	0.2	98
25% DMSO	17.8	0.3	2.3	0.0	93
30% DMSO	13.1	0.5	1.8	0.1	90
5% MeCN	28.4	0.8	3.0	0.0	99
10% MeCN	27.8	0.3	3.0	0.1	98
15% MeCN	25.3	0.5	3.0	0.2	94
20% MeCN	16.4	1.8	2.1	0.2	91
25% MeCN	6.3	1.3	1.1	0.1	N/A
30% MeCN	1.4	0.4	0.4	0.1	N/A

Supplementary Information, Table 7: Mass spectrometry data for DA_20_00 and EnT1.0 variants.

Variant	Expected Mass	Observed Mass
DA_20_00	36012.98	36012.6
DA_20_00 G29BpA	36207.2	36206.4
DA_20_00 D30BpA	36149.17	36148.4
DA_20_00 L148BpA	36151.07	36150.8
DA_20_00 Q195BpA	36136.17	36135.8
EnT1.0	36193.19	36193.2
EnT1.0 Y37A	36101.08	36101.3
EnT1.0 I72A	36151.08	36153.05
EnT1.0 M90A	36133.08	36133.2
EnT1.0 P135A	36167.1	36167.0
EnT1.0 I148A	36151.08	36152.94
EnT1.0 Q195A	36136.18	36137.69
EnT1.0 W244A	36078.08	36080.19
EnT1.0 H287A	36127.08	36128.97
EnT1.1 A229S Y37L	36098.85	36098.57
EnT1.2	36136.08	36135.3
EnT1.3	36196.08	36195.8
EnT1.3 Y37F	36180.08	36178.89
EnT1.3 Y121F	36180.08	36179.17
EnT1.3 Q195A	36139.07	36137.99
EnT1.3 S229A	36180.08	36178.99
EnT1.3 W244A	36080.97	36079.32
EnT1.3 H287A	36129.97	36127.63
EnT1.3 Y121F S271C	36195.86	36194.54
EnT1.3 Y121F Y37L	36129.82	36130.18

Supplementary Information, Table 8: Analytical UPLC methods for products 1a-12a and 1b, 2b 12b.

Substrate	Flow ($mL.min^{-1}$)	Mobile Phase (% MeCN in MQ H_2O with 0.1% TFA)[1]	Run Time (min)	Extinction Coefficient ($mM^{-1}\ cm^{-1}$)[2]	
				substrate	product(s)
1	1.1	5-40% (1.8 min) 5% (1.81 – 2.2 min)	2.2	747 (**1**)	718 (**1a**) 693 (**1b**)
2	1	20% (2 min) 20-40% (2.1 min) 40% (3 min) 40-20% (3.1 min) 20% (4 min)	4	800 (**2**)	883 (**2a**) 491 (**2b**)
3	1	30%	4	306 (**3**)	628 (**3a**)
4	1	17%	5.5	812 (**4**)	935 (**4a**)
5	1.2	15% (0.3 min) 15-40% (2.7 min) 15% (1min)	4	212 (**5**)	310 (**5a**)
6	1.5	17%	3	804 (**6**)	1241 (**6a**)
7	1.5	20%	3.5	297 (**7**)	929 (**7a**)
8	1	25%	3.5	592 (**8**)	1195 (**8a**)
9	1.8	25%	2.5	522 (**9**)	963 (**9a**)
10	1.5	27%	4	452 (**10**)	671 (**10a**)
11	1	37%	2.5	796 (**11**)	1039 (**11a**)
12	1	30%	3	651 (**12**)	502 (**12a**)
13	1	25% (2 min) 25-50% (2.05 min) 50% (3 min) 50-25% (3.01 min) 25% (4 min)	4	283 (**12**)	496 (**13a**) 488 (**13b**)
Quinolone + MVK	1	15%	1.8	775	873 (**14**)
Quinolone + EVK	1	20%	2	775	918 (**15**)

[1] Column temperature was set to 20 °C
[2] Detector wavelength was set to 260nm

Supplementary Information, Table 9: SFC chiral analytical methods for products 1a-12a and 1b, 2b 12b.

Substrate	Flow ($mL.min^{-1}$)	Mobile Phase (% MeOH in CO_2)	Run Time (min)	Product(s)	Column Temperature (°C)
1	1	25% (4 min) 25-50% (4.01 min) 50% (8 min) 50-25% (8.01 min) 25% (10 min)	10	**1a** **1b**	20
2	0.8	50%	5	**2a** **2b**	20
3	0.8	50%	4	**3a**	20
4	0.8	25%	6	**4a**	30
7	1	30%	5	**7a**	30
9	0.5	30%	6	**9a**	20
10	1	20%	6	**10a**	20
11	1	30%	5	**11a**	30

Supplementary Information, Table 10: Normal phase HPLC chiral analytical methods

Substrate	Flow ($mL.min^{-1}$)	Mobile Phase (% IPA in Hexane)	Run Time (min)	Product(s)	Column Temperature (°C)
5[1]	1	10%	12	**5a**	20
6[2]	1	2%	25	**6a**	20
8[3]	1	6%	18	**8a**	20
12[3]	1	10%	25	**12a**	20
13[2]	1	2%	22	**13a**	20
2-Quinolone[2]	1	10%	6	**14**	20
2-Quinolone[2]	1	10%	6	**15**	20

[1] CHIRALPAK ® OJ-H (0.46 cm x 25 cm) column used.
[2] Diacel 87S82 CHIRALPAK ® IG-3 SFC column (3 mm x 50 mm) column used.
[3] CHIRALPAK ® AD-H (0.46 cm x 25 cm) column used.

Supplementary Information, Table 11: Primers for the alanine scan, rounds 1 & 2.

Flanking Primers	
XHO_R	ATGCATGCCTCGAGCGATCCGA
NDE_F	CATGCATGCATATGGAGATCCCTGTCA
Alanine Scan	
Y37A_F	TATATTGTGGCTCCAGCAGTGGAGGTAAACGGTAAAC
I72A_F	AACGGCTATGGAGGCGCACCAGCCGGGTG
M90A_F	TTATTTGTAGCTGATGCACGTCTGGGCCTTCT
P135A_F	TTATGGATTACGGCCGCAGCGGGCGAGGT
I146A_F	CCTGCCGATTTTACCGCATCGTTACAAGAAAAATTCGGG
Q195A_F	CTGATTGTGGCCGAGGCACCAACTAAAAAGTTATGG
W244A_F	TTGTTAGTTGCCAATGCAGGCTCCTCGCATATC
H287A_F	ATTTTCGTGACGGAGGCAGAAAACAATGCAGTCTGG
Y37A_R	TGGAGCCACAATATAAAAGTC
I72A_R	GCCTCCATAGCCGT
M90A_R	ATCAGCTACAAATAACTGGTTG
P135A_R	GGCCGTAATCCATAAGTT
I146A_R	GGTAAAATCGGCAGGG
Q195A_R	CTCGGCCACAATCAG
W244A_R	ATTGGCAACTAACAAATTGTTG
H287A_R	CTCCGTCACGAAAATGG
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A120x_F	CGTATGCAGGGTTGCNNKTACTGCGCTTTTGATTATGA
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P135x_F	TTATGGATTACGGCCNNKGCGGGCGAGG
I146x_F	CCTGCCGATTTTACCNNKTCGTTACAAGAAAAATTCGGG
L148x_F	GATTTTACCATCTCGNNKCAAGAAAAATTCGGGTCA
Q149x_F	TTTACCATCTCGTTANNKGAAAAATTCGGGTCAATCTATTG
A175x_F	GCATTTCAGTAGCCANNKGGTATTGCCGTGCG
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K225x_F	ATCCCGGGCACTCACNNKGGTGGAGCCGCC
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W244x_F	TTGTTAGTTGCCAATNNKGGCTCCTCGCATATC
E268x_F	ATCCGCTGCCCGTTCNNKAAACCTTCTGCTTTACACTTT
K269x_F	CGCTGCCCGTTCGAANNKCCTTCTGCTTTACACTTTAAGC
S271x_F	CCGTTCGAAAAACCTNNKGCTTTACACTTTAAGCCACAG
H287x_F	ATTTTCGTGACGGAGNNKGAAAACAATGCAGTCTGGA
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M90x_R	ATCAGCTACAAATAACTGGT
A120x_R	GCAACCCTGCATACG

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H287x_R	CTCCGTCACGAAAATGG
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T133x_F	GGAAACTTATGGATTNNKGCCCCAGCGG
A136x_F	TGGATTACGGCCCCANNKGGCGAGGTGGCCCCT
F144x_F	GTGGCCCCTGCCGATNNKACCATCTCGTTAGATGAAAAATT
S147x_F	GCCGATTTTACCATCNNKTTAGATGAAAAATTCGGGTCA
G153x_F	TTAGATGAAAAATTCNNKTCAATCTATTGTTTTACCACCG
Q172x_F	GTGGATACCGCATTTNNKTAGCCAGCTGGTATTGC
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Q195x_F	CTGATTGTGGCCGAGNNKCCAACTAAAAAGTTATGGTCCT
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Y37x_R	TGGAGCCACAATATAAAAGT
I72x_R	GCCTCCATAGCCGT
Y121x_R	GGCGCAACCCTG

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S147x_R	GATGGTAAAATCGGCAGG
G153x_R	GAATTTTTCATCTAACGAGATGGT
Q172x_R	AAATGCGGTATCCACTTG
P174x_R	CTACTGAAATGCGGTATCC
G176x_R	AGCTGGCTACTGAAATGC
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A242x_R	AACTAACAAATTGTTGTCTTCGT
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S271x_R	AGGTTTTTCGAACGGG
A272x_R	AGAAGGTTTTTCGAACGG
Primers used engineering EnT1.3 Y121F towards substrate 12	
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F144x_F	GTGGCCCCTGCCGATNNKACCATCTCGTTAG
I146x_F	GCCCCTGCCGATTTTACCNNKTCGTTAGATGAAAAATTC
L148x_F	CCTGCCGATTTTACCATCTCGNNKGATGAAAAATTCGGGTC
A193x_F	GTCGCCCATATCAACTGATTGTGNNKGAGCAGCGGACTAA
H224x_F	GCACATCCCGGGCACTNNKGAGGGTGGAGCCTC
S229x_F	CTCACGAGGGTGGAGCCNNKGGTATGGATTTCGAC
V34x_R	AATATAAAAGTCCCCATTCTTGTCG
P36x_R	AGCCACAATATAAAAGTCCCCATT
F144x_R	ATCGGCAGGGGCCACC
I146x_R	GGTAAAATCGGCAGGGGC
L148x_R	CGAGATGGTAAAATCGGCAGG
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S229x_R	GGCTCCACCCTCGTGAG

Protein and DNA sequences:
B = 4-benzoylphenylalanine, BpA

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EnT1.1

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EnT1.3 □C

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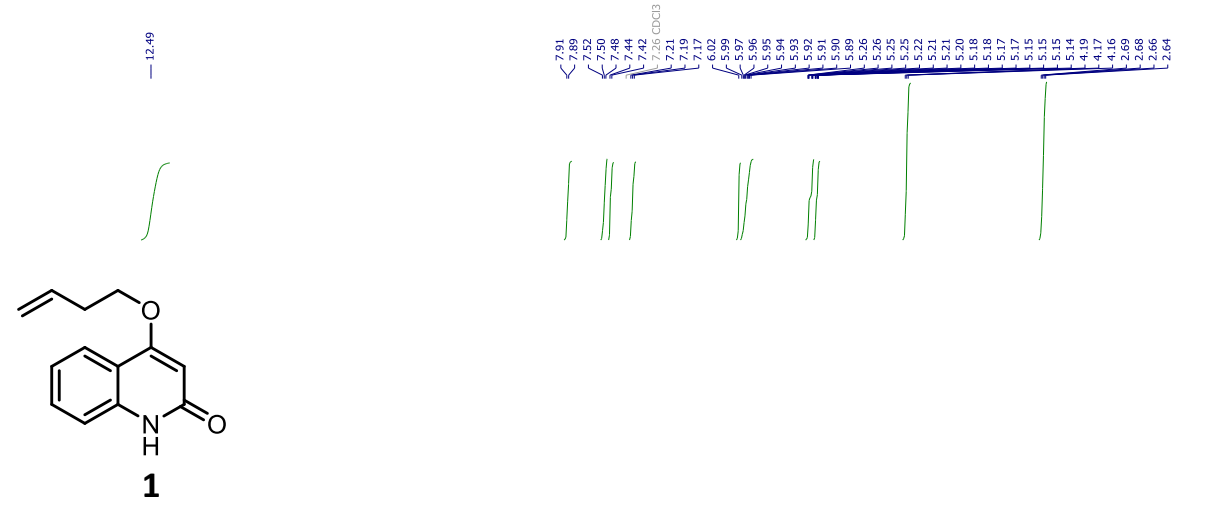
^{1}H and ^{13}C NMR spectra of chemically synthesised substrates and products standards.



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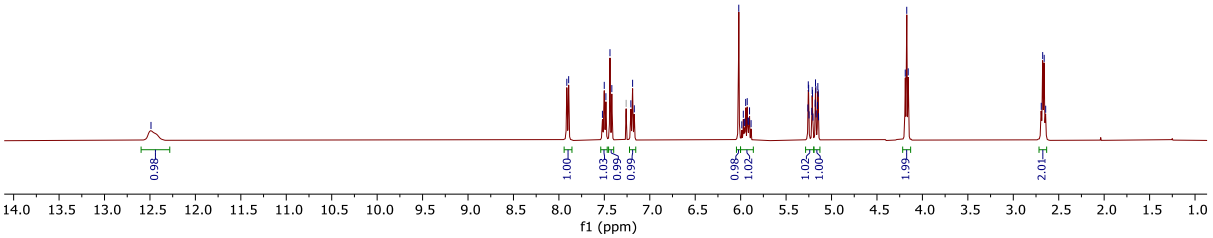

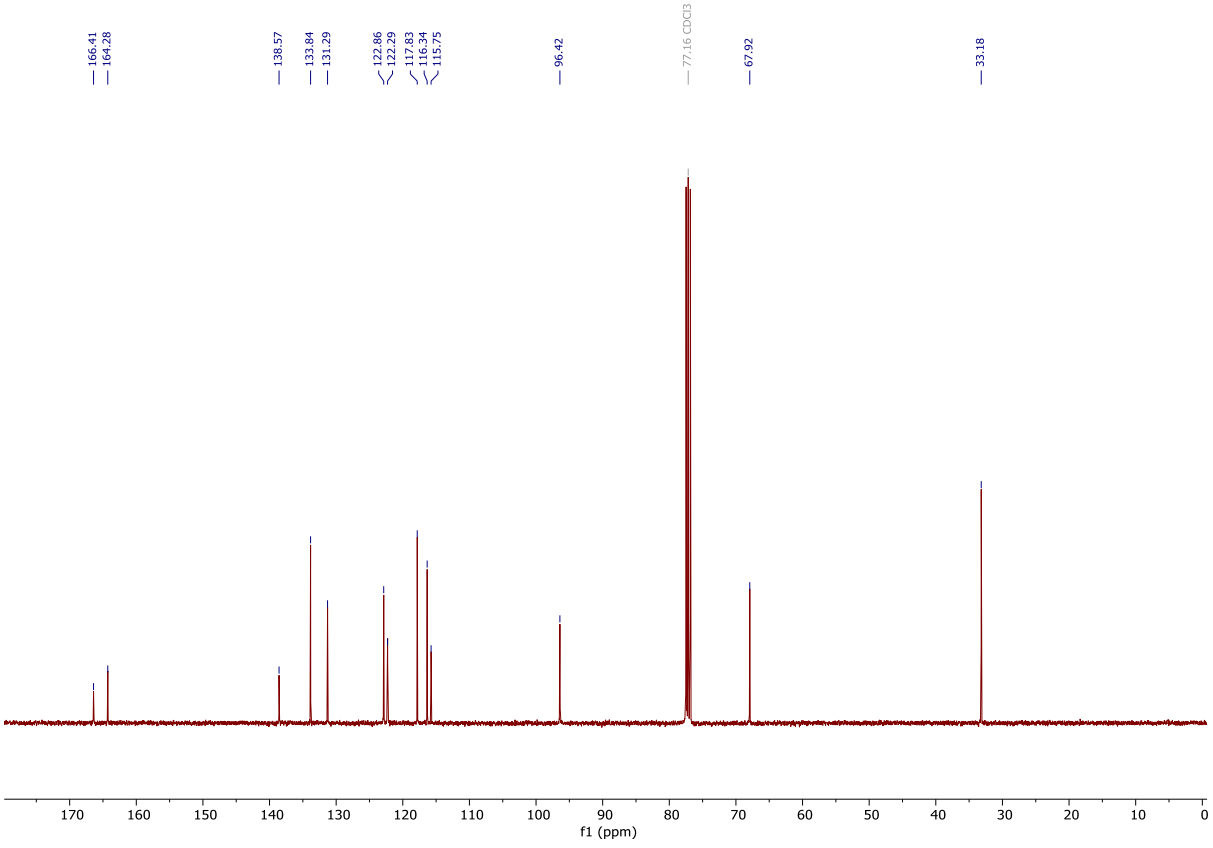

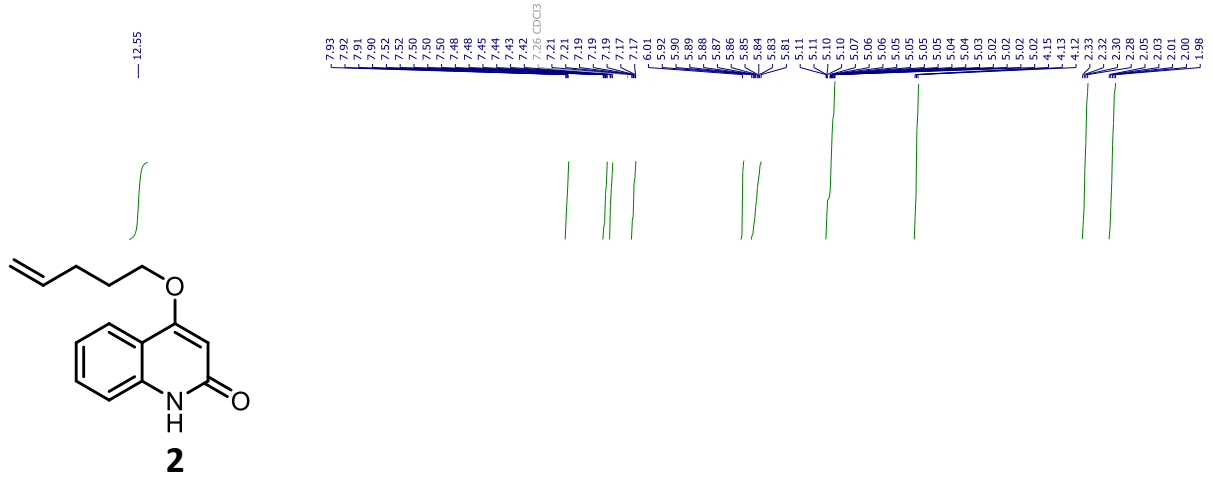
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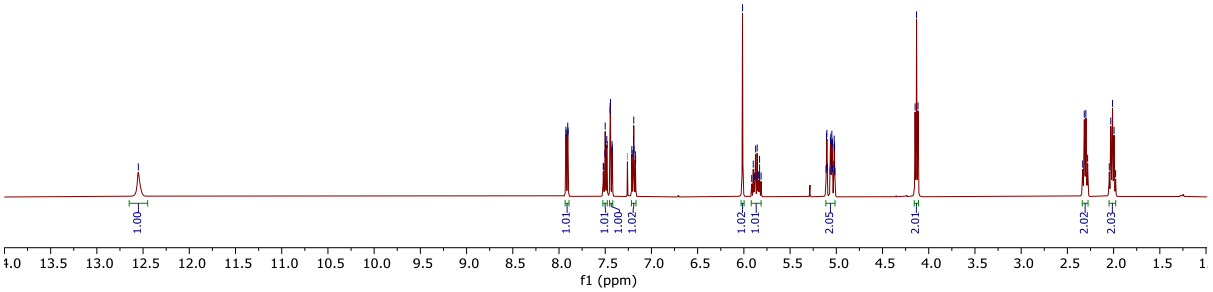
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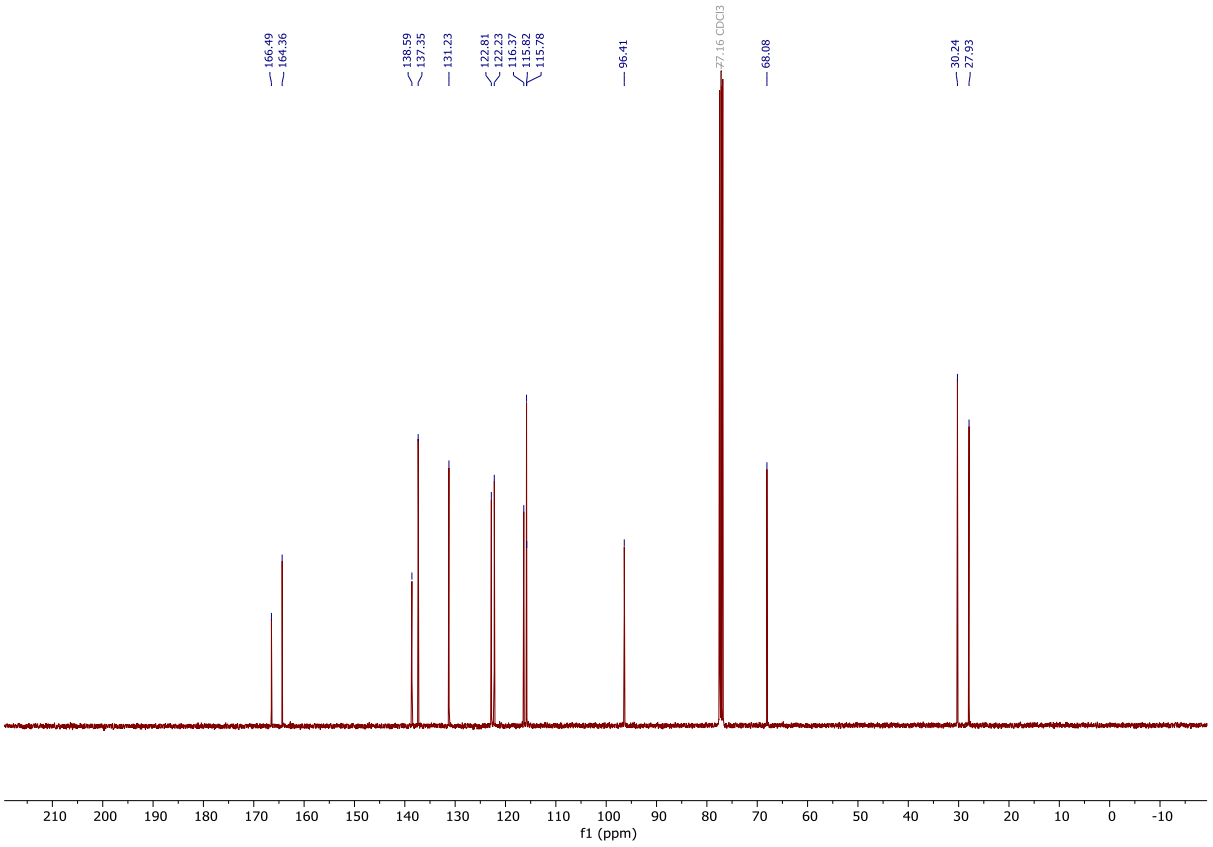
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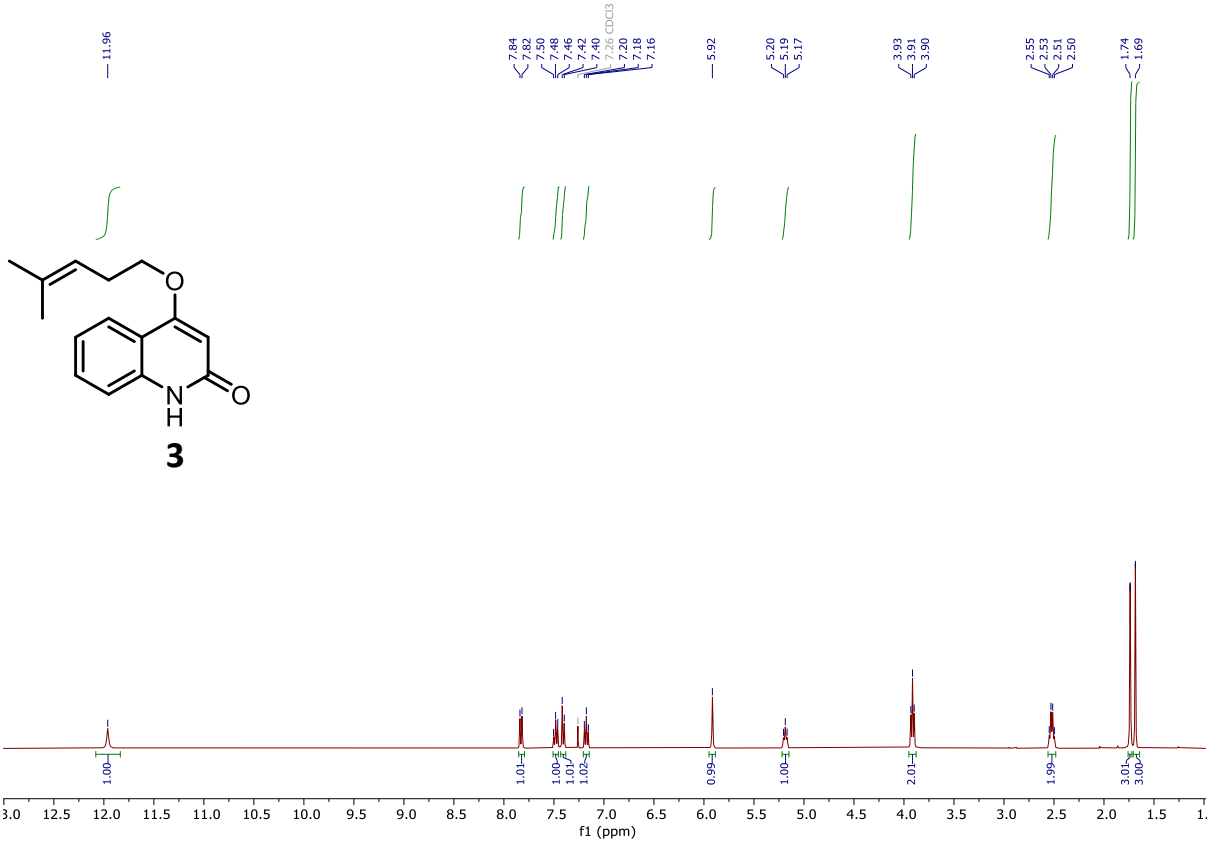
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3

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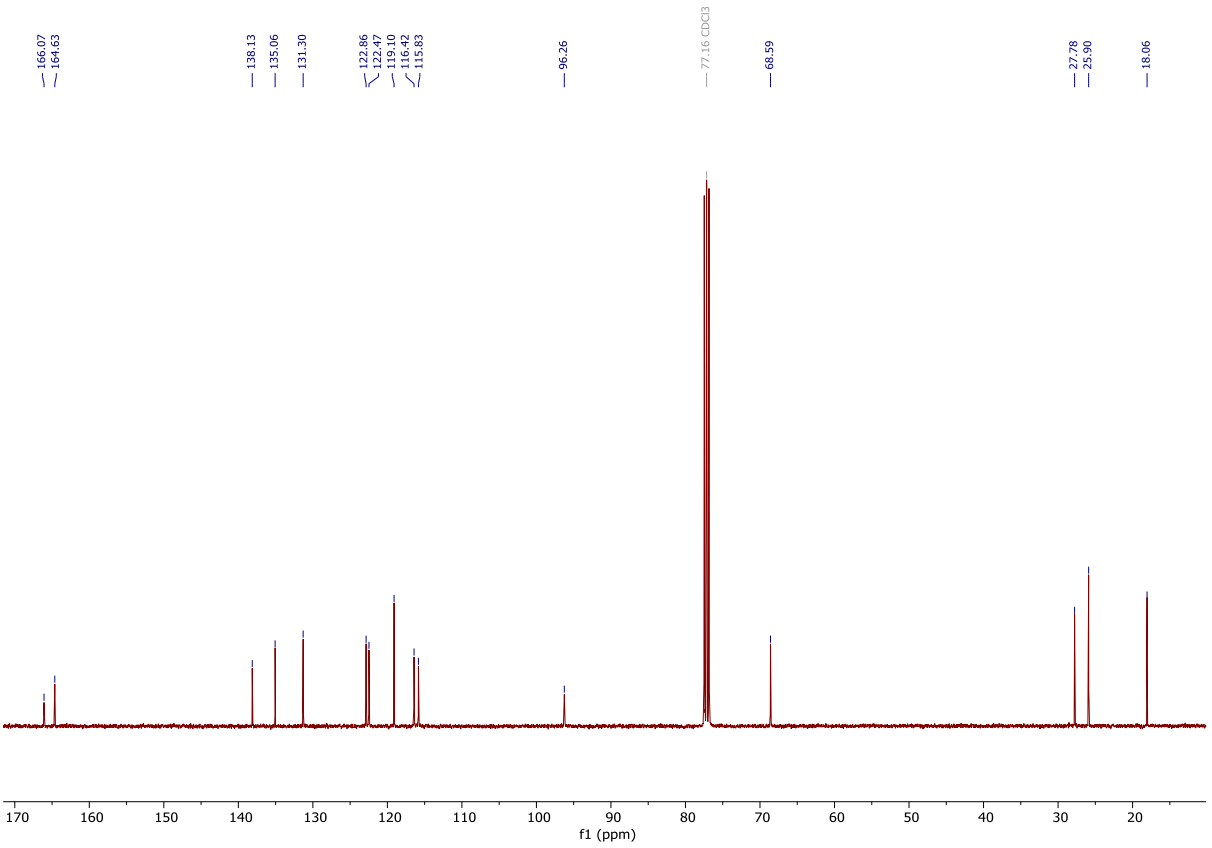
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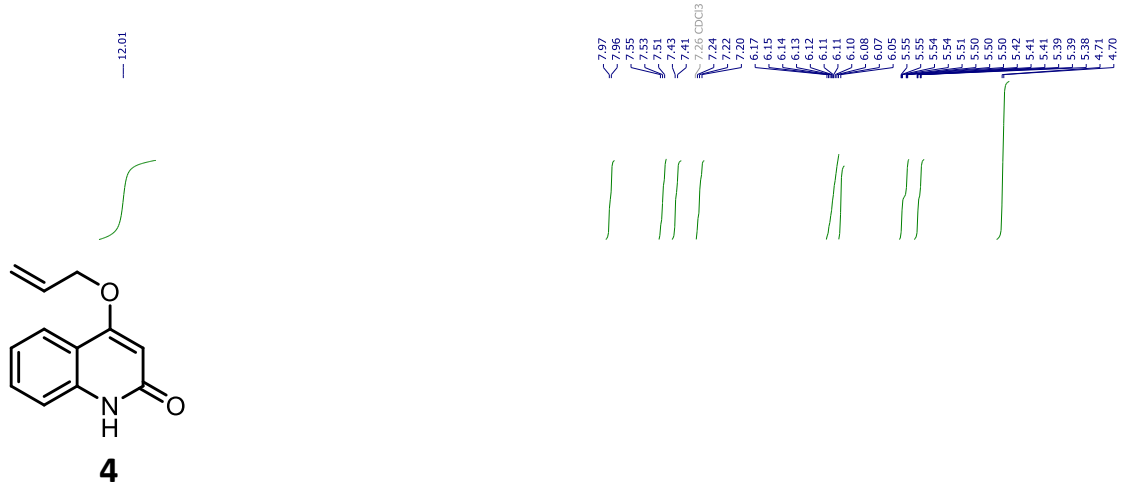
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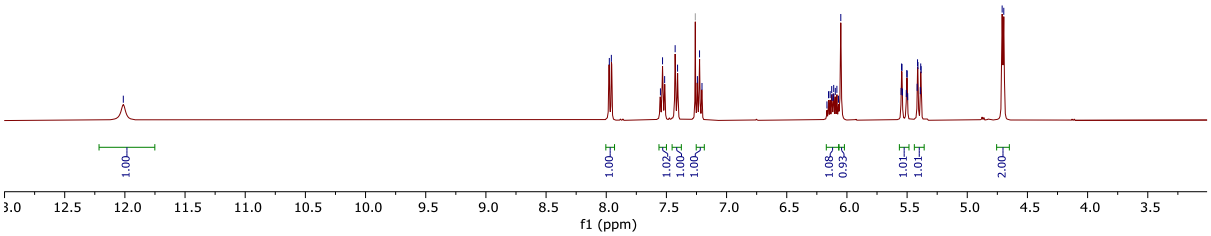
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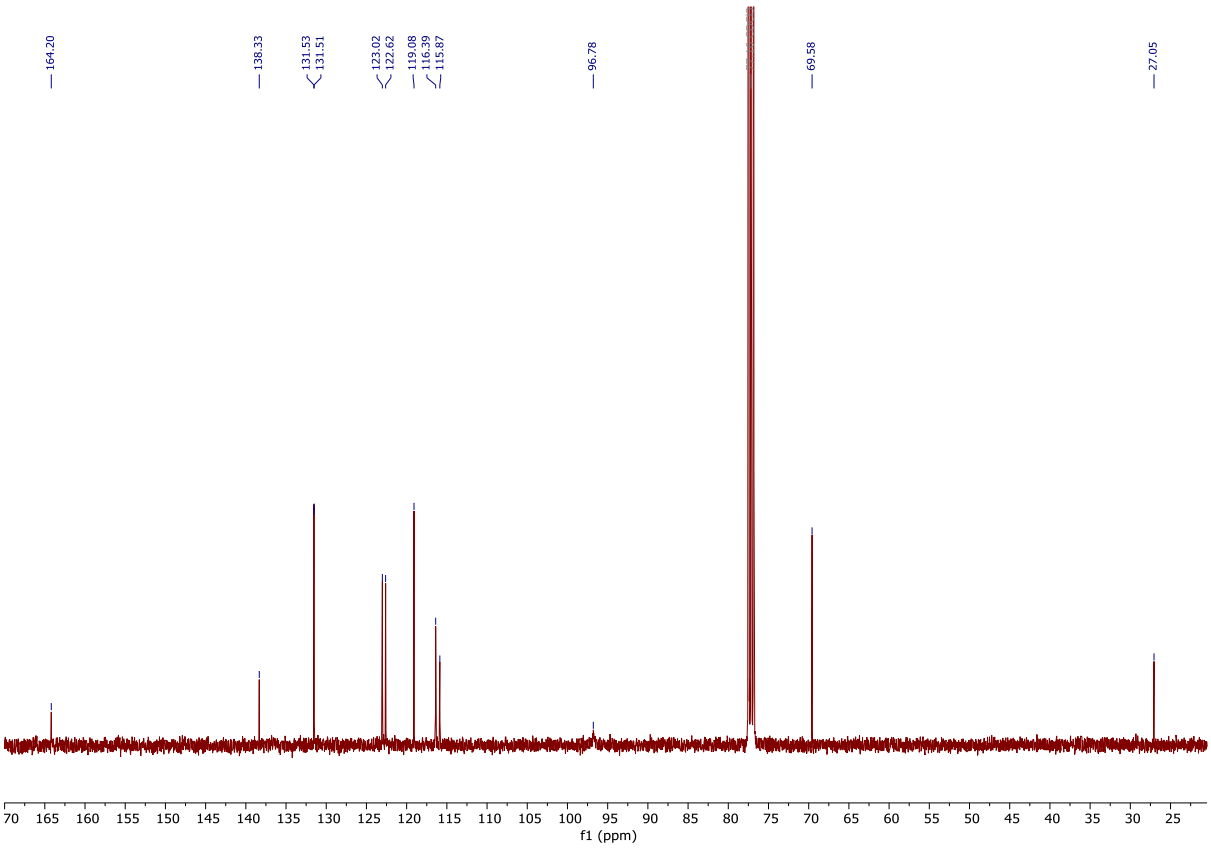
f1 (ppm)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119.08
116.39
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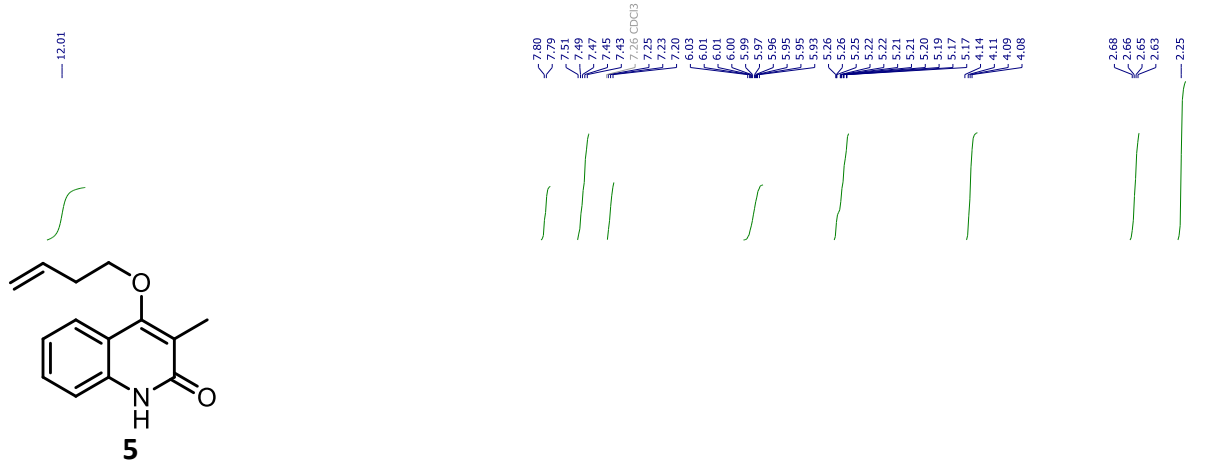
96.78
69.58
27.05
f1 (ppm)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7.80
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7.47
7.45
7.43
7.26 CDCl3
7.25
7.23
7.20
6.03
6.01
6.01
6.00
5.99
5.97
5.96
5.95
5.95
5.93
5.26
5.26
5.25
5.22
5.22
5.21
5.21
5.20
5.19
5.17
5.17
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4.09
4.08
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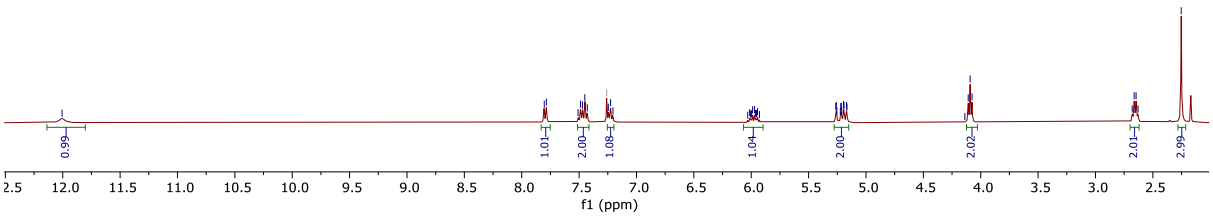
2.66
2.65
2.63
2.25
O
N
H
O

5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2.02
2.01
2.99
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11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
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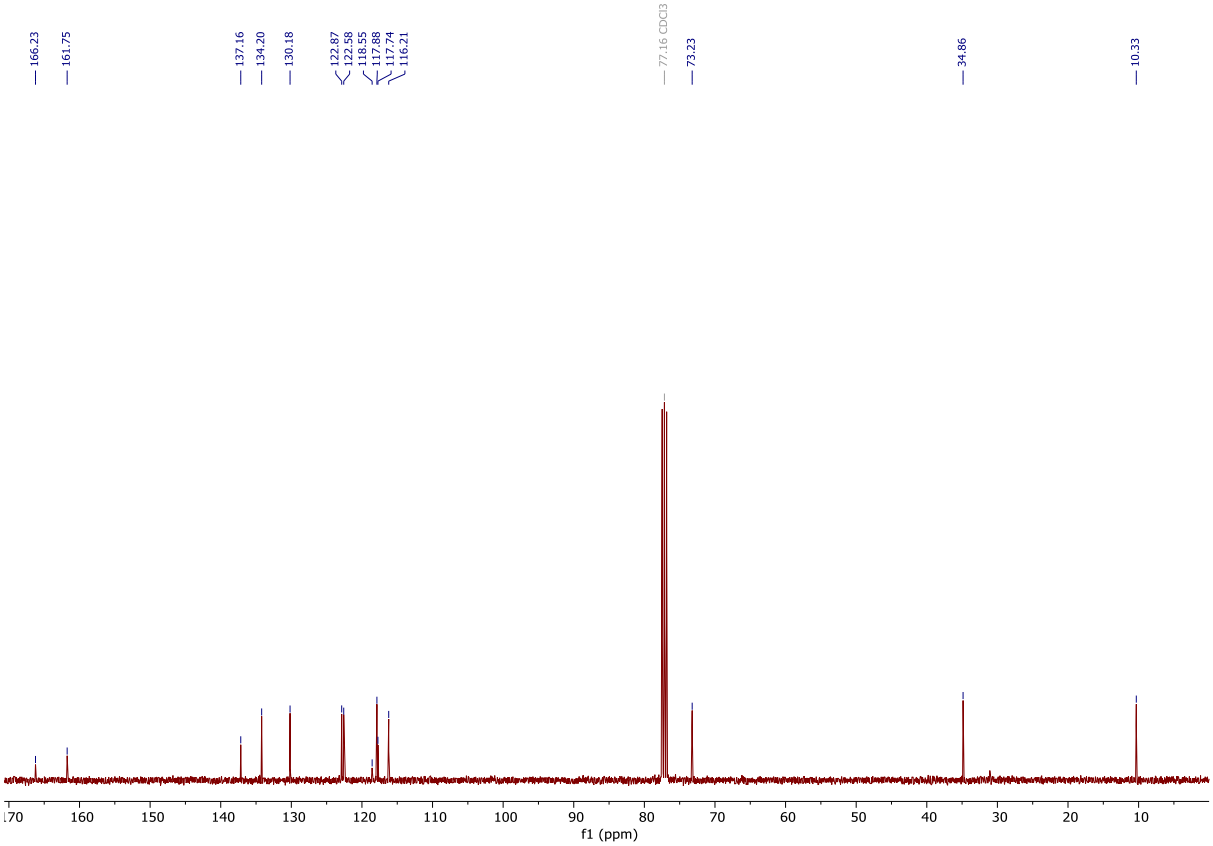
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)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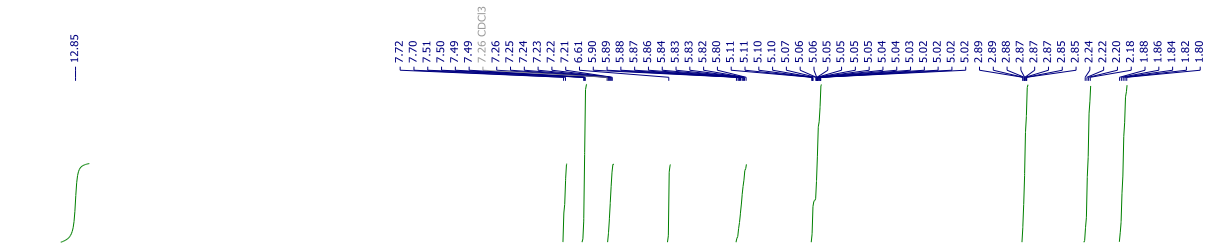
118.55
117.88
117.74
116.21
77.16 CDCl3
73.23
34.86
10.33
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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7.25
7.24
7.23
7.22
7.21
6.61
5.90
5.89
5.88
5.87
5.86
5.84
5.83
5.83
5.82
5.80
5.11
5.11
5.10
5.10
5.07
5.06
5.06
5.05
5.05
5.05
5.05
5.04
5.04
5.03
5.02
5.02
5.02
5.02
2.89
2.89
2.88
2.87
2.87
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2.18
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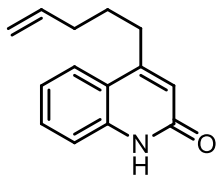
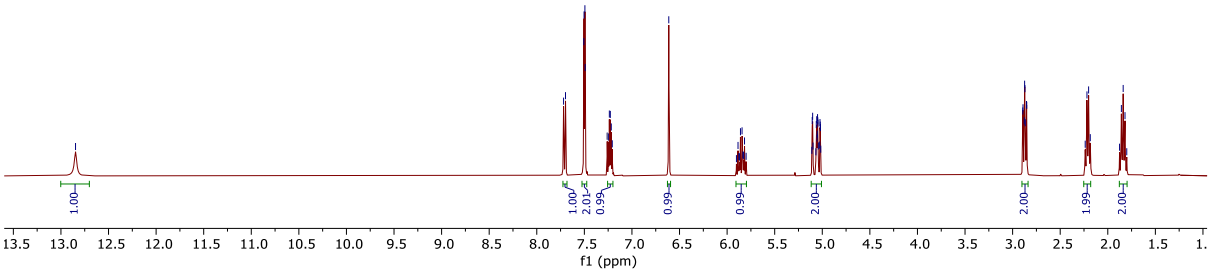
1.86
1.84
1.82
1.80


N
H
O

6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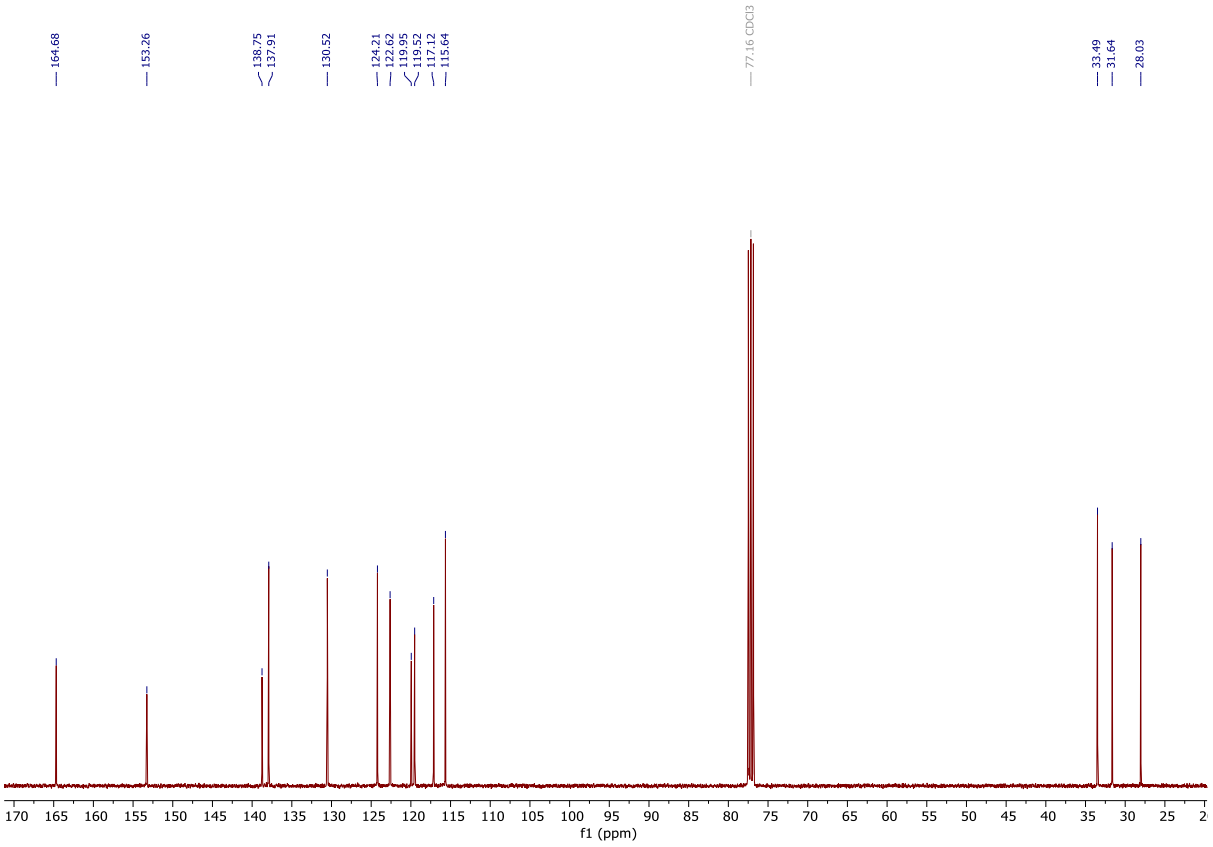
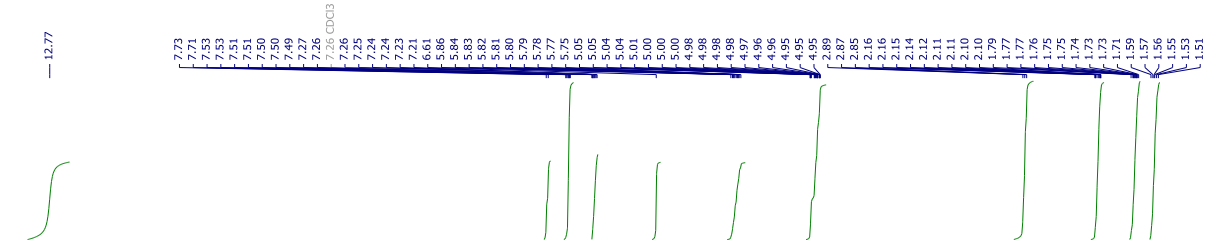
0.99
0.99
0.99
2.00
2.00
1.99
2.00
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)


164.68
153.26
138.75
137.91
130.52
124.21
122.62
119.95
119.52
117.12
115.64
77.16 CDCl3
33.49
31.64
28.03
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 2
f1 (ppm)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7.71
7.53
7.53
7.51
7.51
7.50
7.50
7.49
7.27
7.26
CDCl3
7.26
7.25
7.24
7.24
7.23
7.21
6.61
5.86
5.84
5.83
5.82
5.81
5.80
5.79
5.78
5.77
5.75
5.05
5.05
5.04
5.04
5.01
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5.00
5.00
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2.16
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2.14
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2.11
2.11
2.10
2.10
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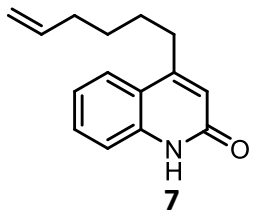
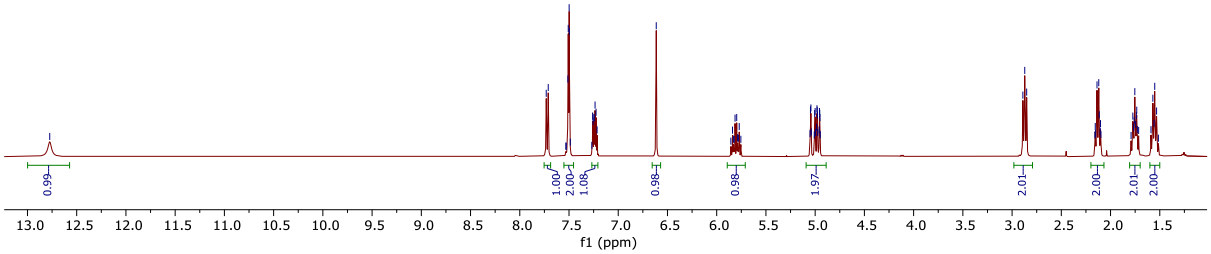
1.77
1.76
1.75
1.75
1.74
1.73
1.73
1.71
1.59
1.57
1.56
1.55
1.53
1.51


N
H
O

7


0.99
1.00
2.00
1.08
0.98
0.98
1.97
2.01
2.00
2.01
2.00
13.0
12.5
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11.5
11.0
10.5
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9.5
9.0
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7.5
7.0
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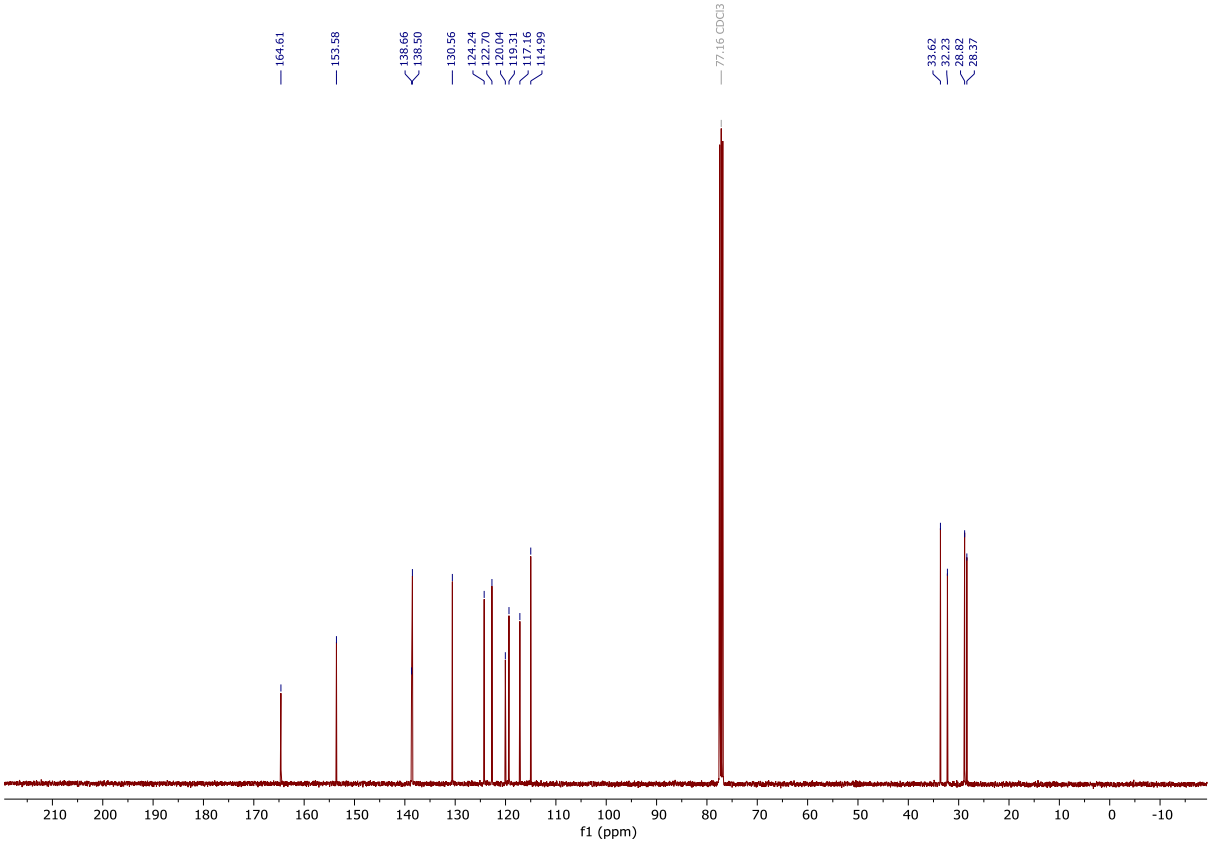
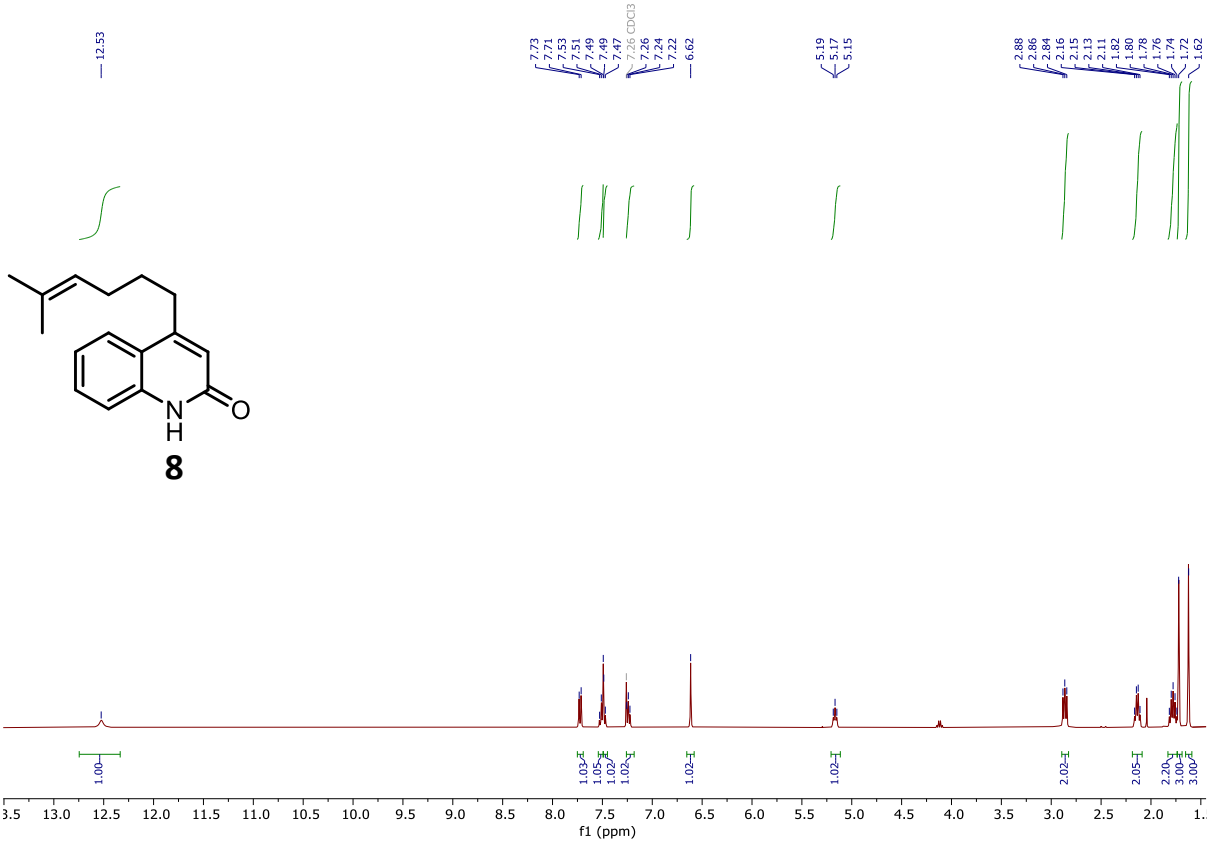
3.0
2.5
2.0
1.5
f1 (ppm)


164.61
153.58
138.66
138.50
130.56
124.24
122.70
120.04
119.31
117.16
114.99
77.16
CDCl3
33.62
32.23
28.82
28.37
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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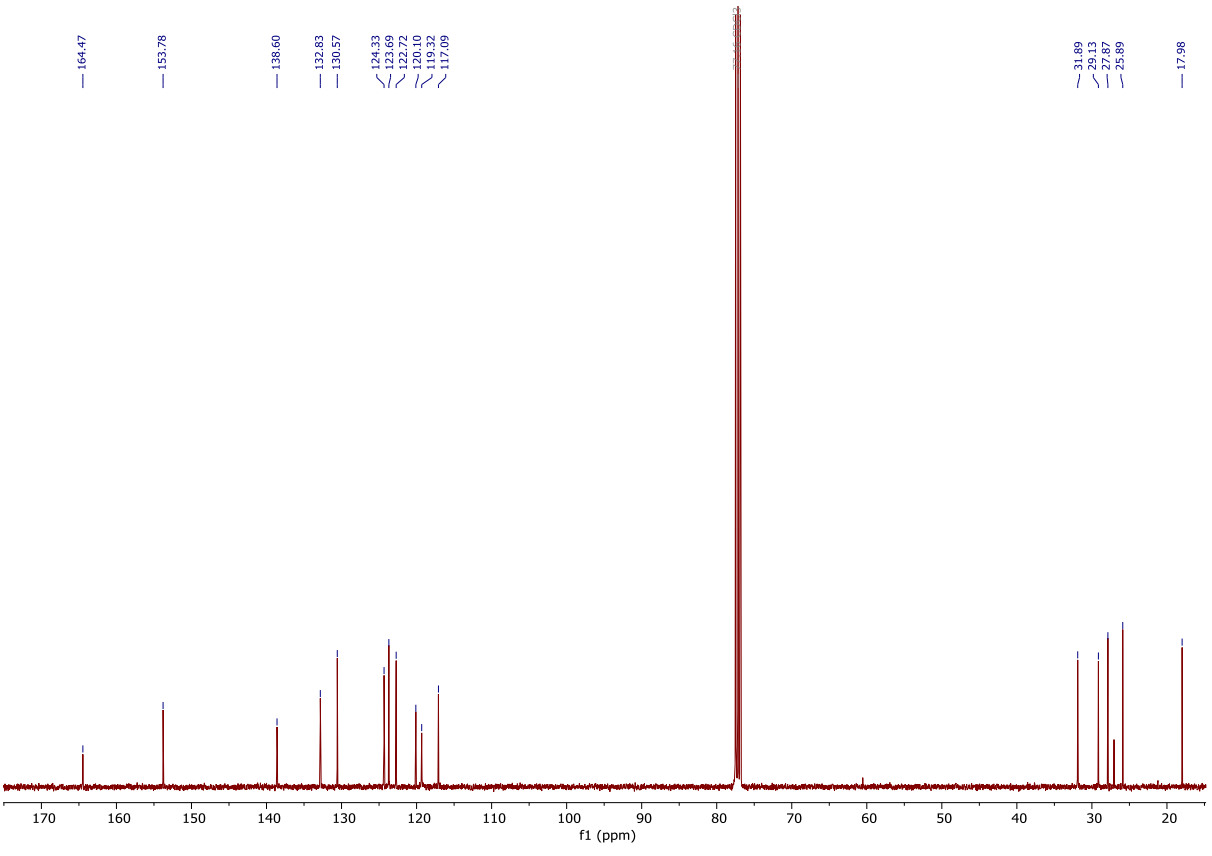
7.73
7.71
7.53
7.51
7.49
7.49
7.47
7.26 CDCl3
7.26
7.24
7.22
6.62
5.19
5.17
5.15
2.88
2.86
2.84
2.16
2.15
2.13
2.11
1.82
1.80
1.78
1.76
1.74
1.72
1.62
N
H
O
1.00
1.03
1.05
1.02
1.02
1.02
2.02
2.05
2.20
3.00
3.00
f1 (ppm)

8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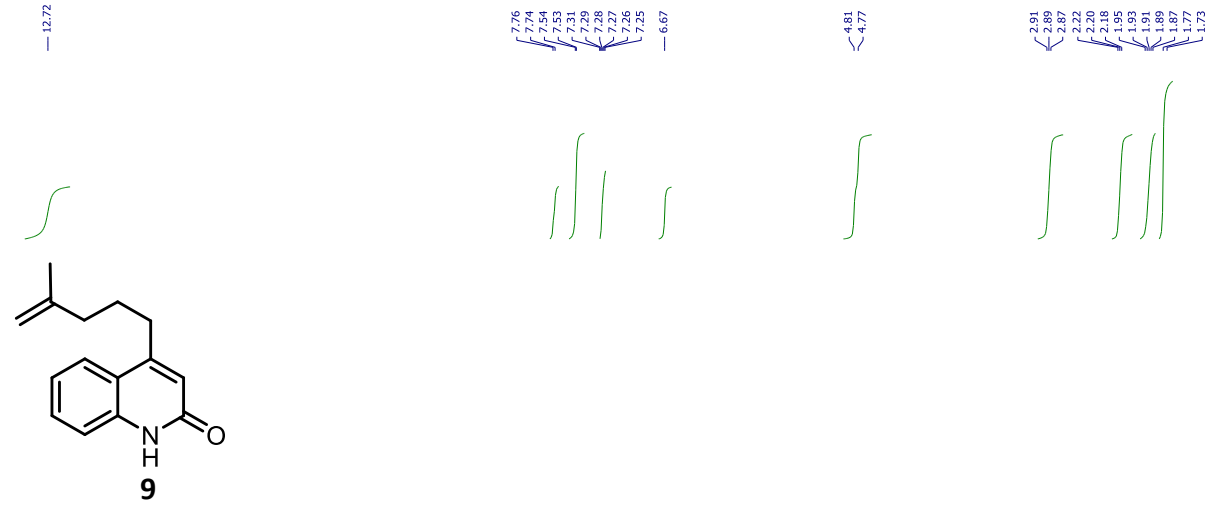
130.57
124.33
123.69
122.72
120.10
119.32
117.09
77.46 CDCl3
31.89
29.13
27.87
25.89
17.98
f1 (ppm)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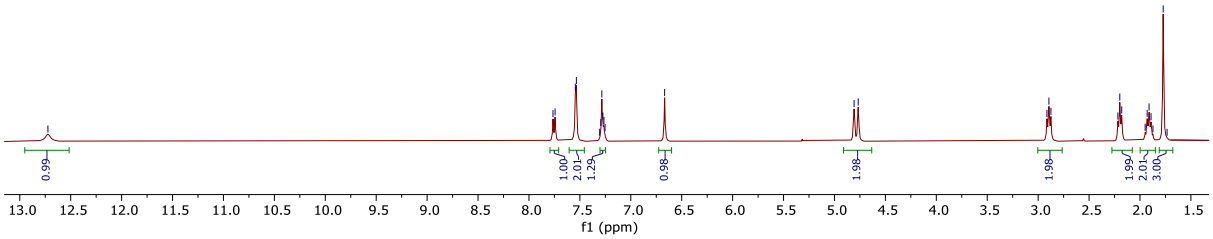
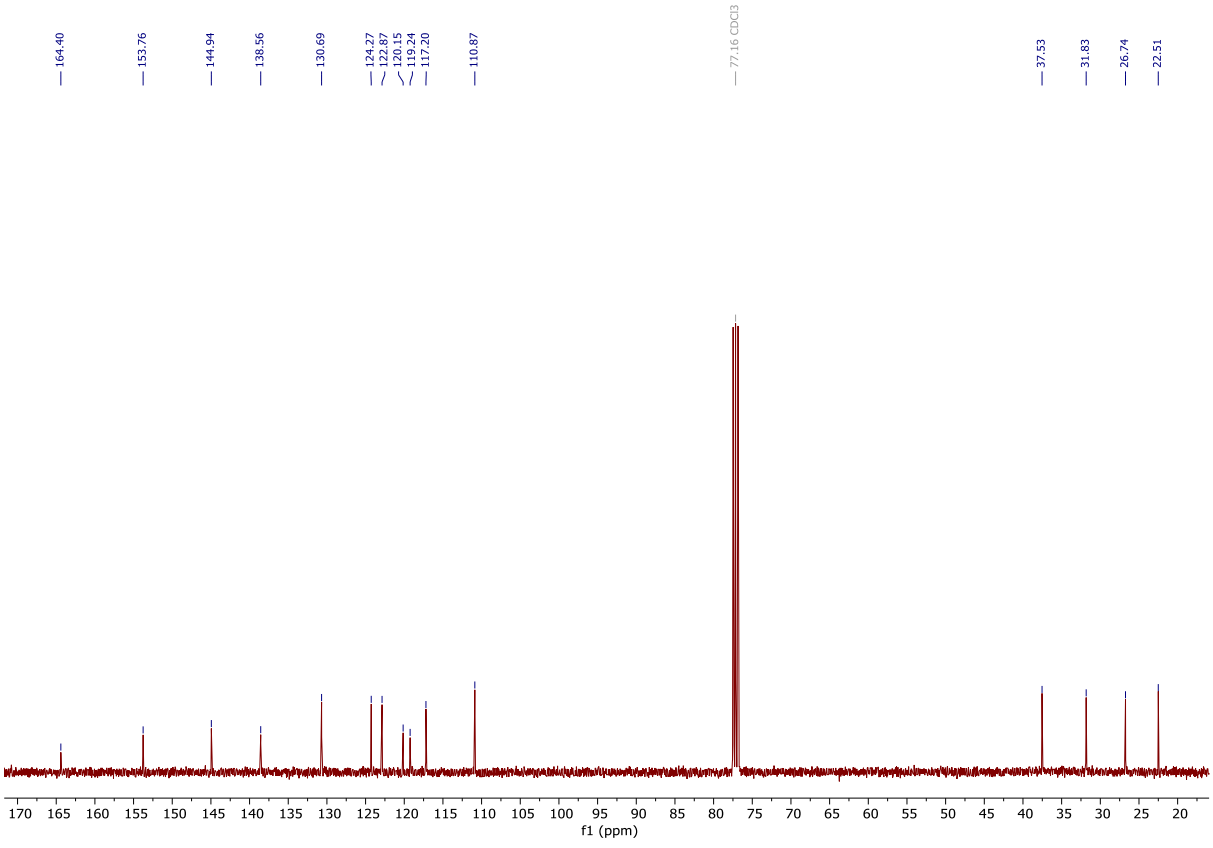
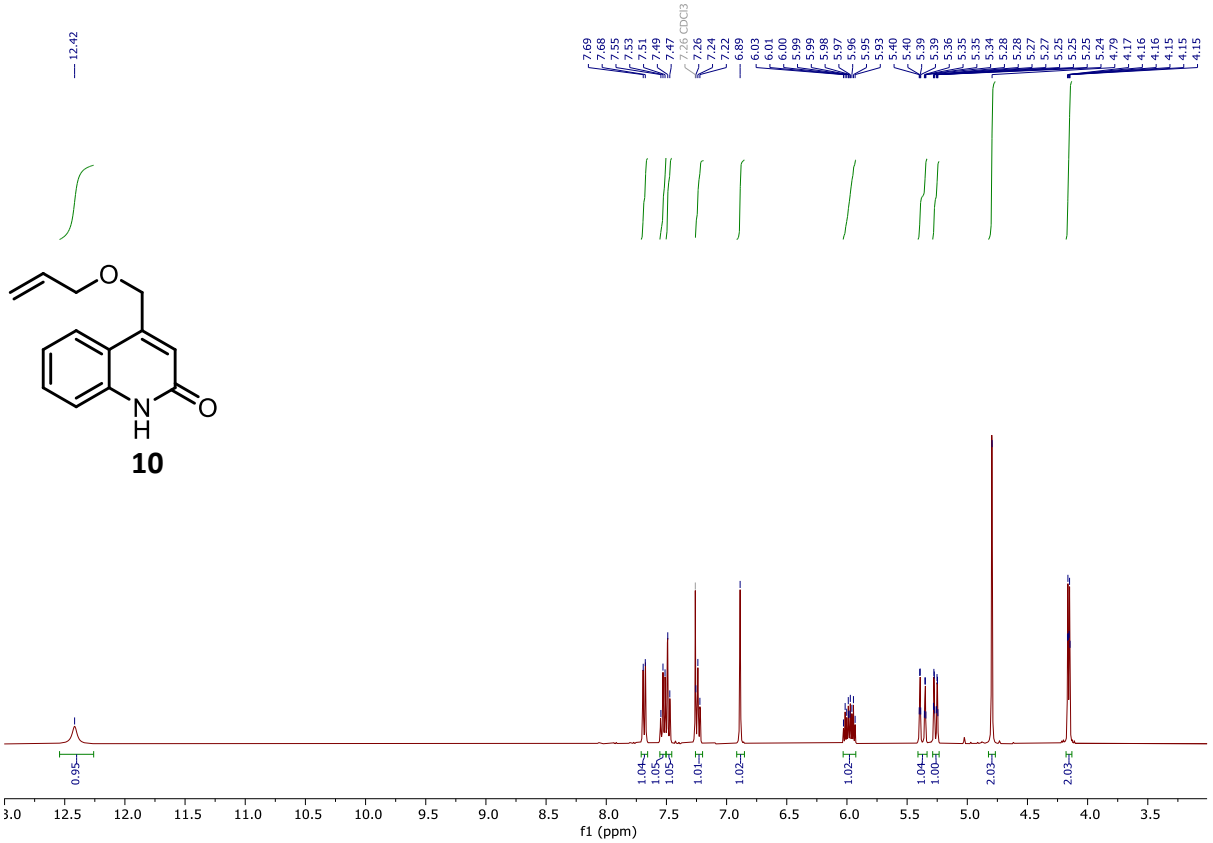
7.53
7.31
7.29
7.28
7.27
7.26
7.25
6.67
4.81
4.77
2.91
2.89
2.87
2.22
2.20
2.18
1.95
1.93
1.91
1.89
1.87
1.77
1.73
N
H
O

9


0.99
1.00
2.01
1.25
0.98
1.98
1.98
1.99
2.01
3.00
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)


164.40
153.76
144.94
138.56
130.69
124.27
122.87
120.15
119.24
117.20
110.87
77.16 CDCl3
37.53
31.83
26.74
22.51
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20
f1 (ppm)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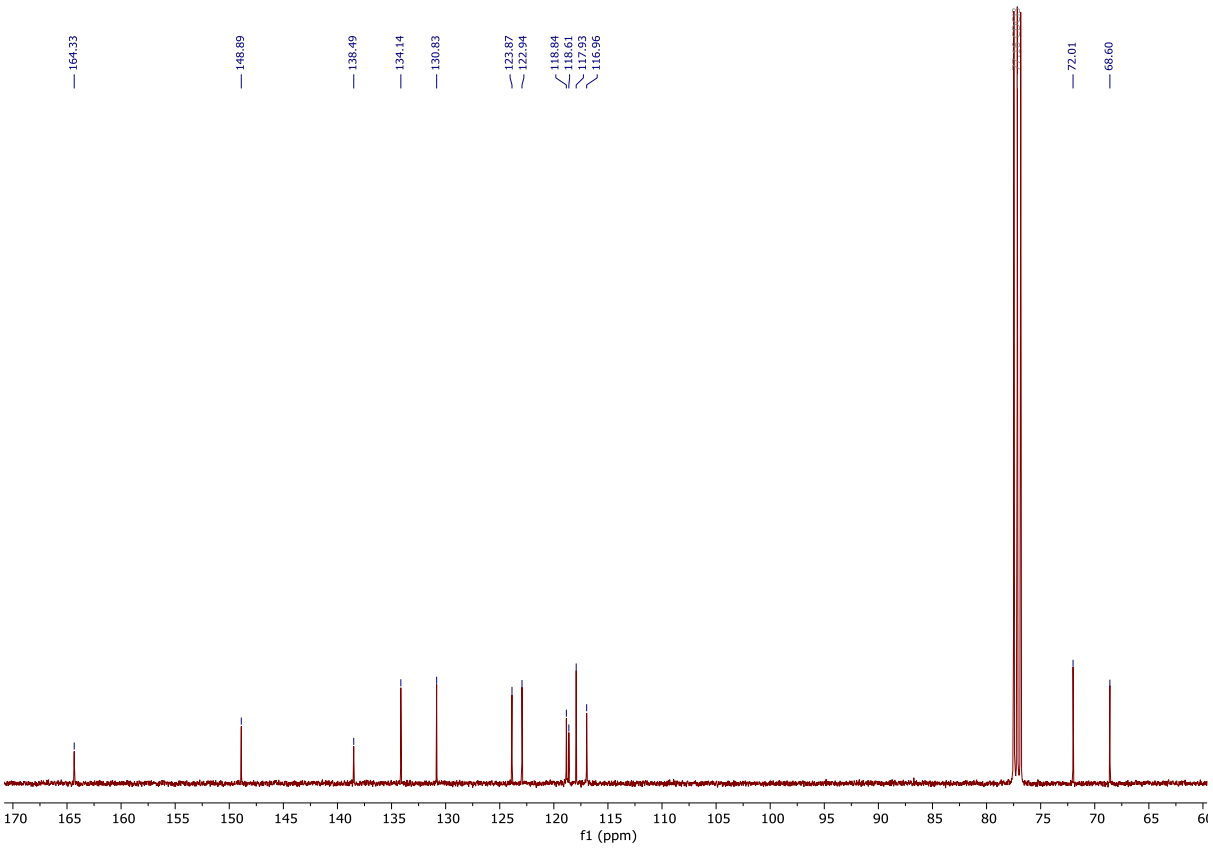
7.68
7.55
7.53
7.51
7.49
7.47
7.26 CDCl3
7.26
7.24
7.22
6.89
6.03
6.01
6.00
5.99
5.99
5.98
5.97
5.96
5.95
5.93
5.40
5.40
5.39
5.39
5.36
5.35
5.35
5.34
5.28
5.28
5.27
5.27
5.25
5.25
5.25
5.24
4.19
4.17
4.16
4.16
4.15
4.15
4.15
O
N
H
O
0.95
1.04
1.05
1.05
1.01
1.02
1.02
1.04
1.00
2.03
2.03
f1 (ppm)

10


164.33
148.89
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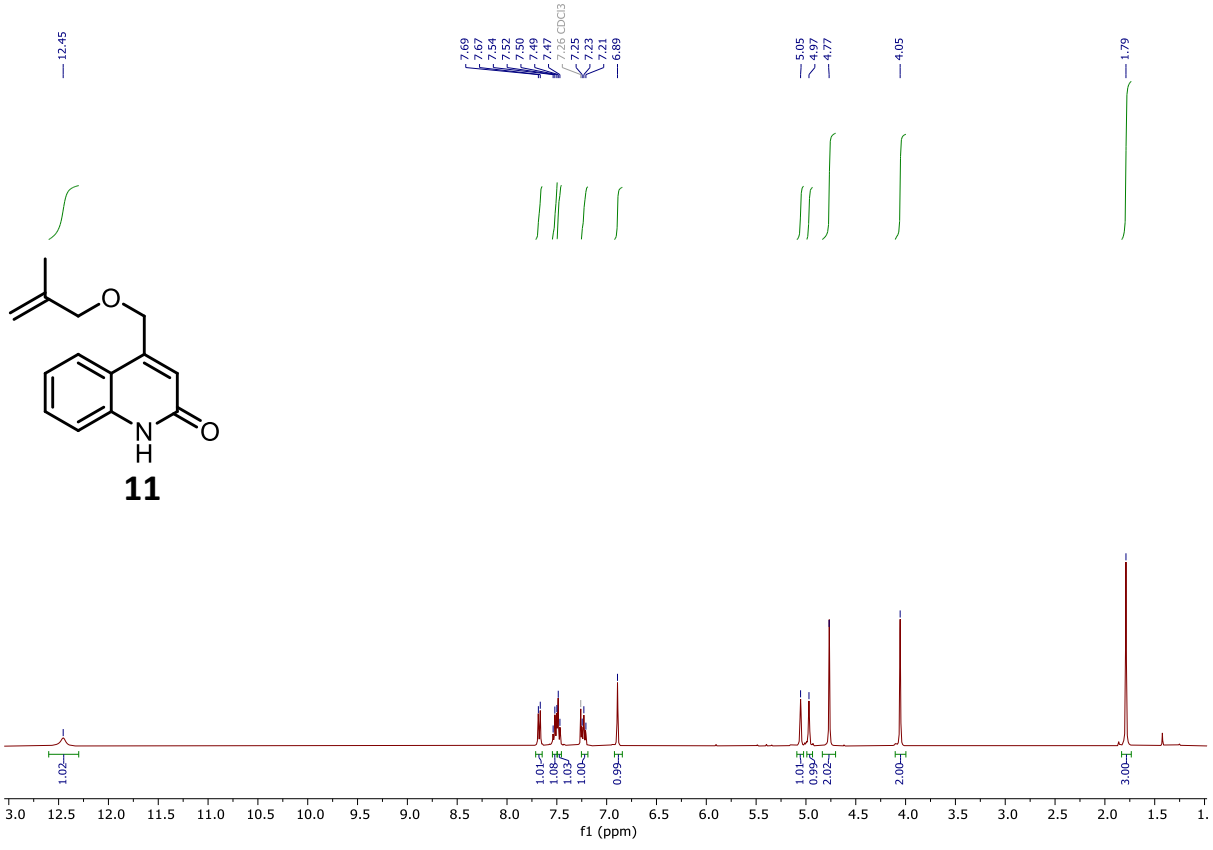
130.83
123.87
122.94
118.84
118.61
117.93
116.96
72.01
68.60
f1 (ppm)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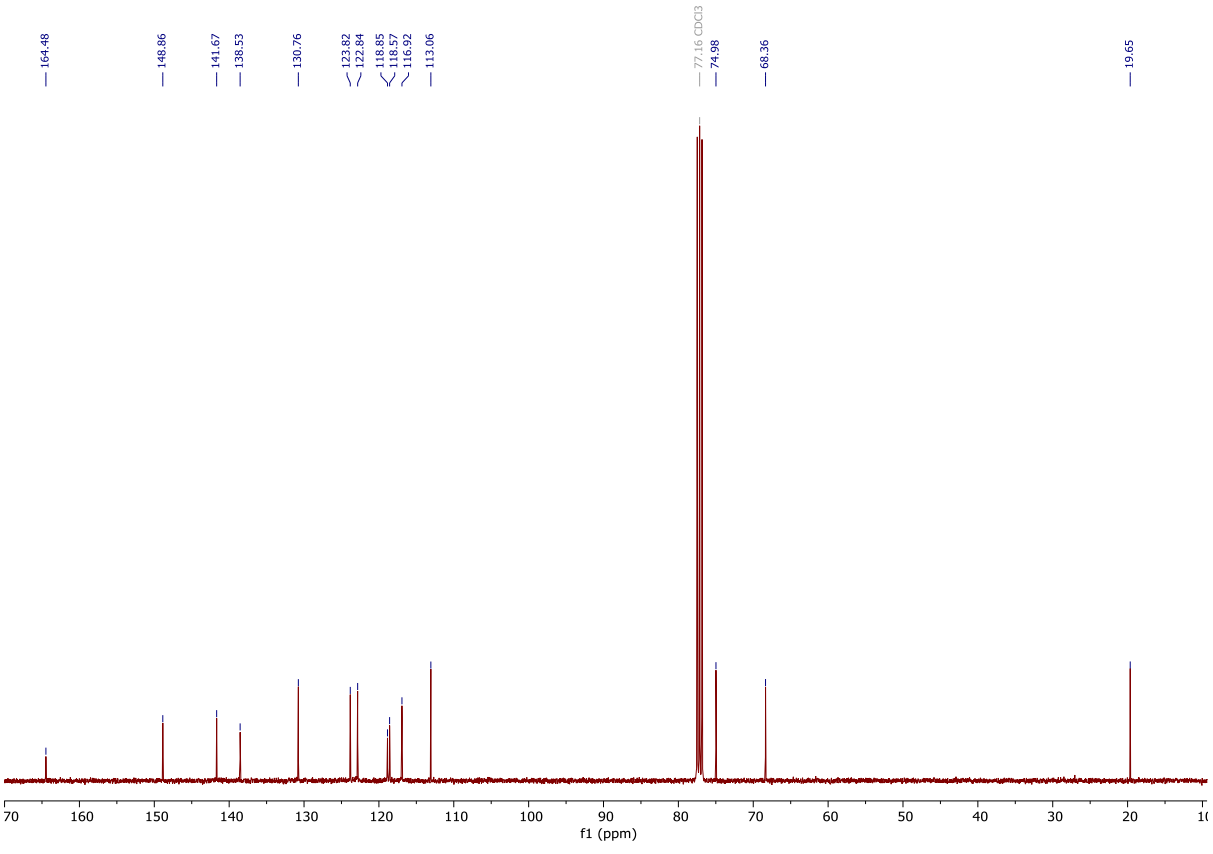
7.67
7.54
7.52
7.50
7.49
7.47
7.26 CDCl3
7.25
7.23
7.21
6.89
5.05
4.97
4.77
4.05
1.79
O
N
H
O
1.02
1.01
1.08
1.03
1.00
0.99
1.01
0.99
2.02
2.00
3.00
f1 (ppm)

11


164.48
148.86
141.67
138.53
130.76
123.82
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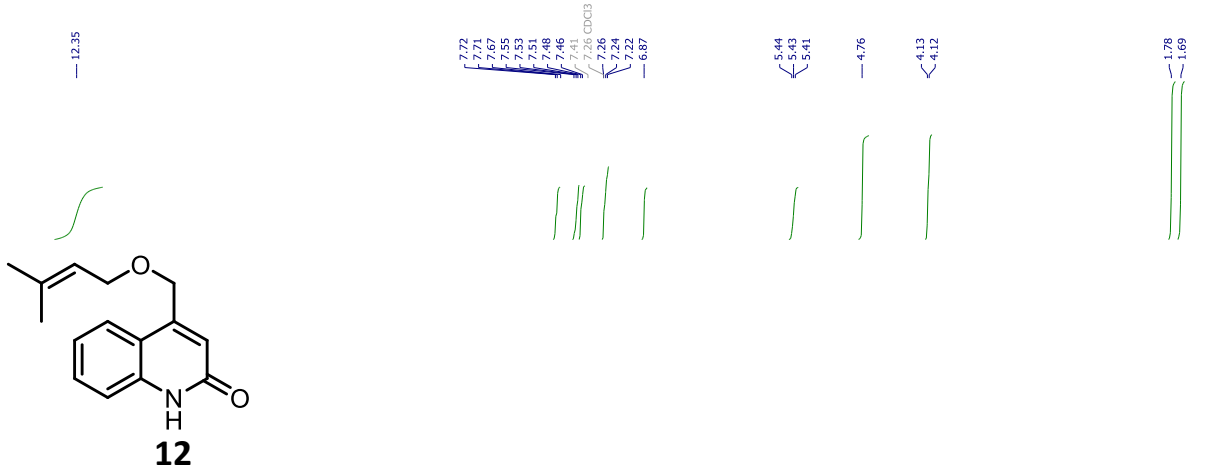
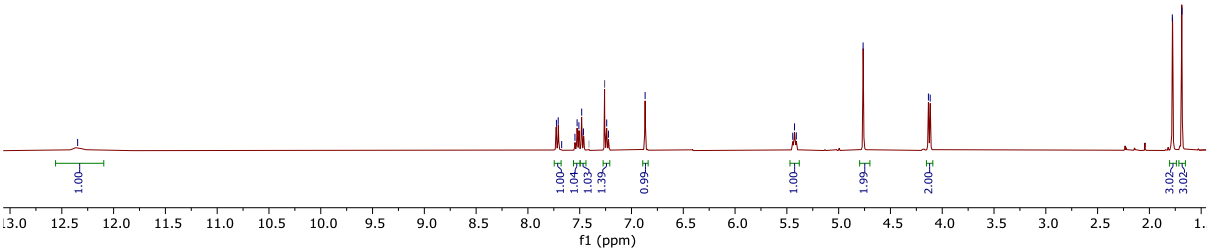
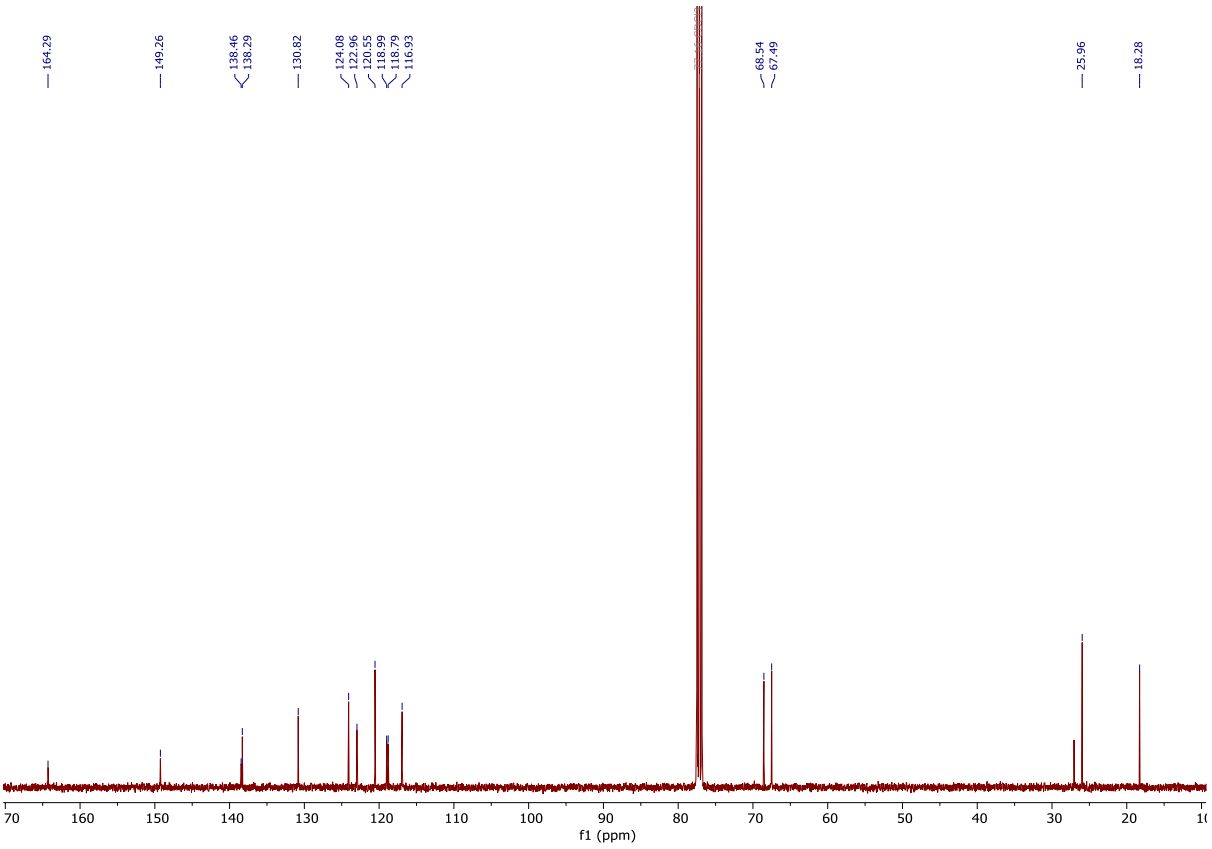
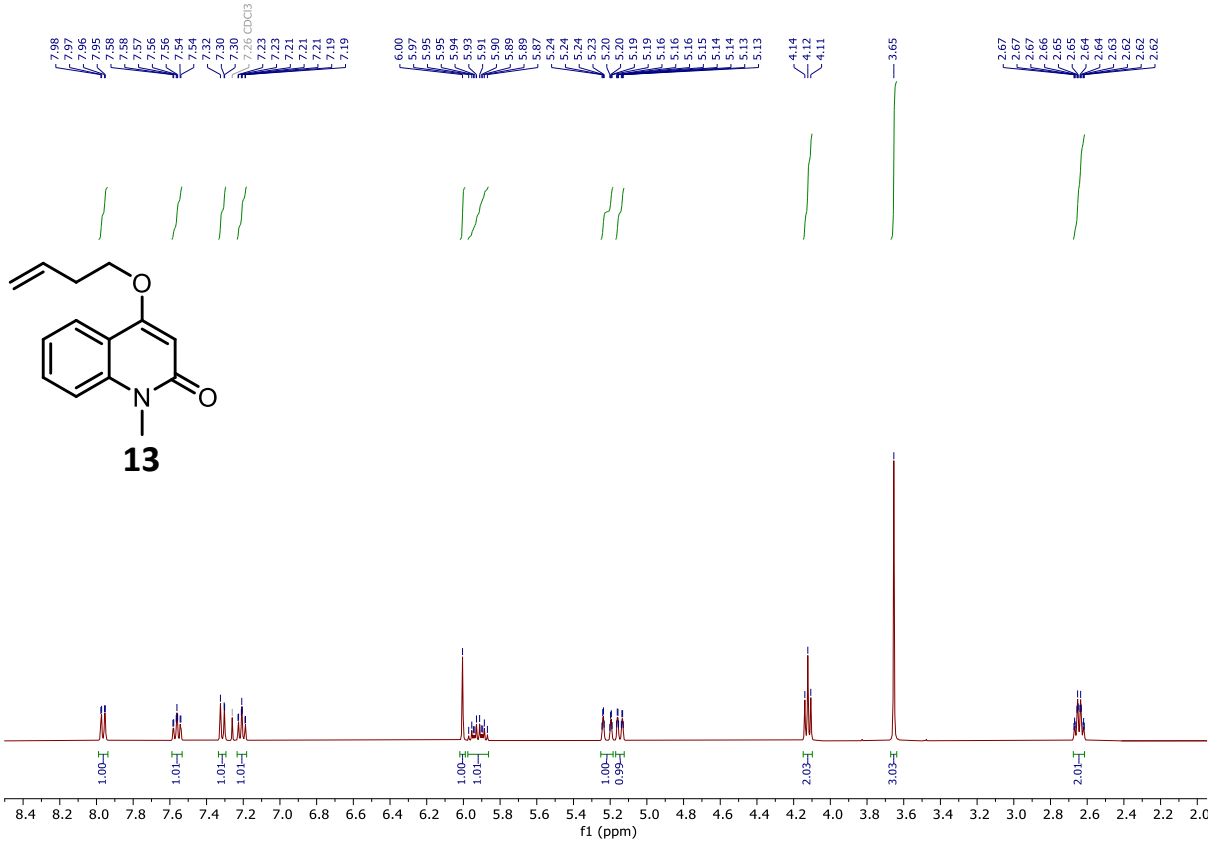
118.85
118.57
116.92
113.06
77.16 CDCl3
74.98
68.36
19.65
f1 (ppm)

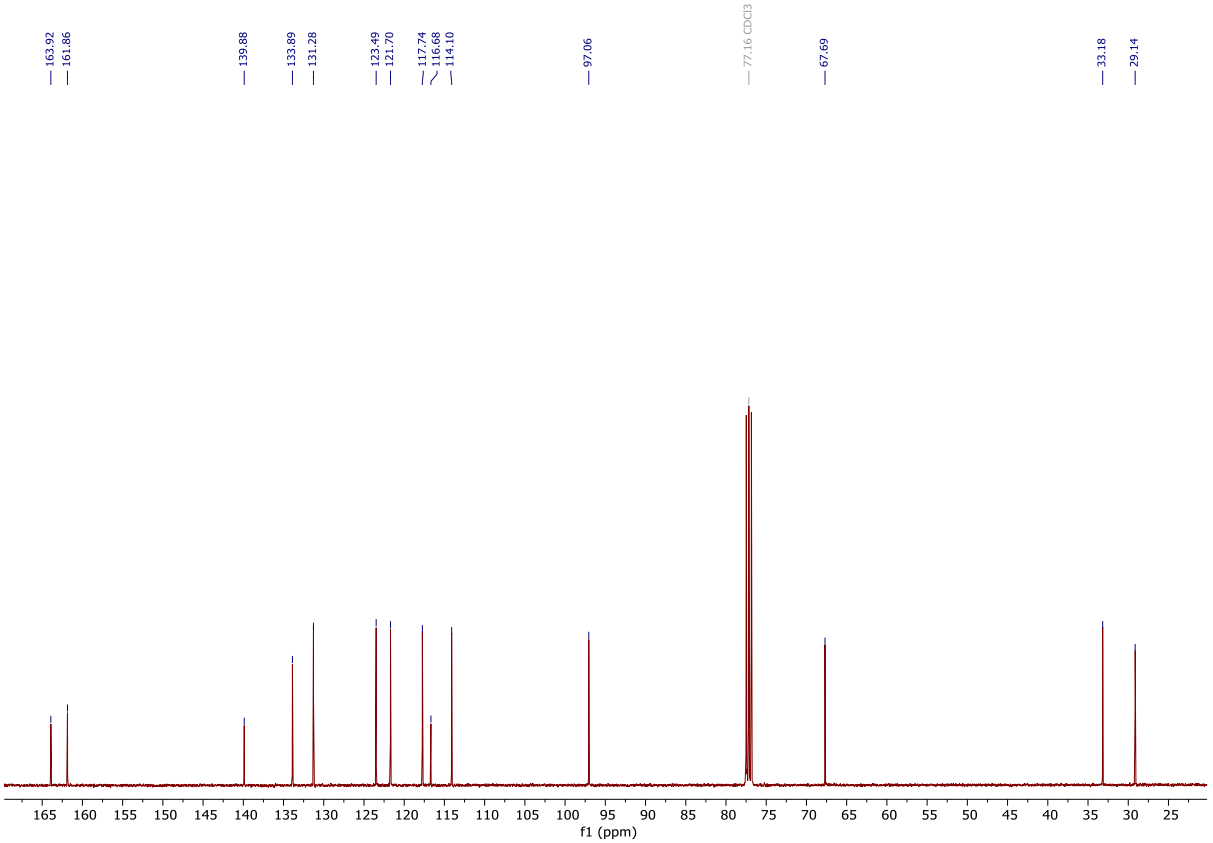

12.35
7.72
7.71
7.67
7.55
7.53
7.51
7.48
7.46
7.41
7.26 CDCl3
7.26
7.24
7.22
6.87
5.44
5.43
5.41
4.76
4.13
4.12
1.78
1.69
O
N
H
O

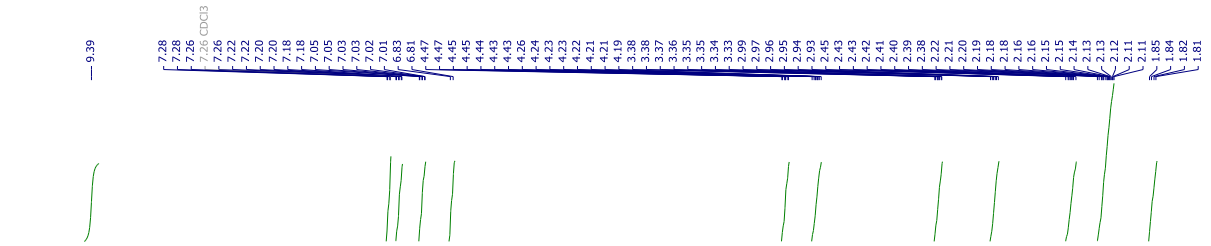
12

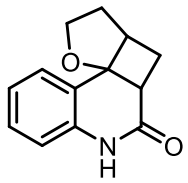

1.00
1.00
1.04
1.03
1.39
0.99
1.00
1.99
2.00
3.02
3.02
f1 (ppm)


164.29
149.26
138.46
138.29
130.82
124.08
122.96
120.55
118.99
118.79
116.93
68.54
67.49
25.96
18.28
f1 (ppm)

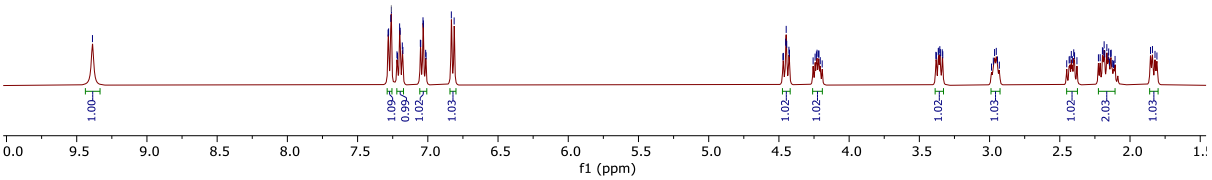

7.98 7.97 7.96 7.95 7.58 7.58 7.57 7.56 7.56 7.54 7.54 7.32 7.30 7.30 7.26 CDCl3 7.23 7.23 7.21 7.21 7.21 7.19 7.19
6.00 5.97 5.95 5.95 5.94 5.93 5.91 5.90 5.89 5.89 5.87 5.24 5.24 5.23 5.20 5.20 5.19 5.19 5.16 5.16 5.16 5.15 5.14 5.14 5.13 5.13
4.14 4.12 4.11
3.65
2.67 2.67 2.67 2.66 2.65 2.65 2.64 2.64 2.63 2.62 2.62 2.62
O
N
O
13
1.00 1.01 1.01 1.01 1.00 1.01 1.00 0.99 2.03 3.03 2.01
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 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
f1 (ppm)

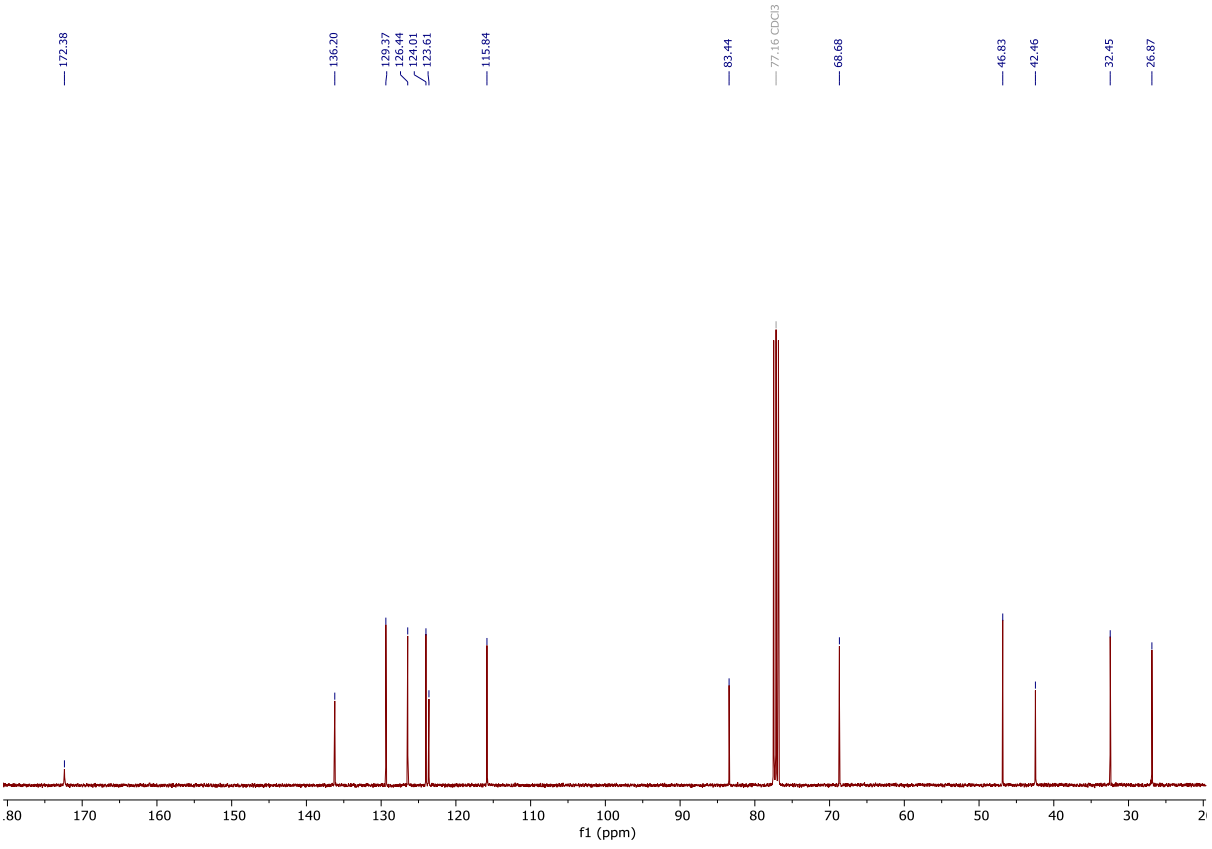

163.92 161.86 139.88 133.89 131.28 123.49 121.70 117.74 116.68 114.10 97.06 77.16 CDCl3 67.69 33.18 29.14
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)

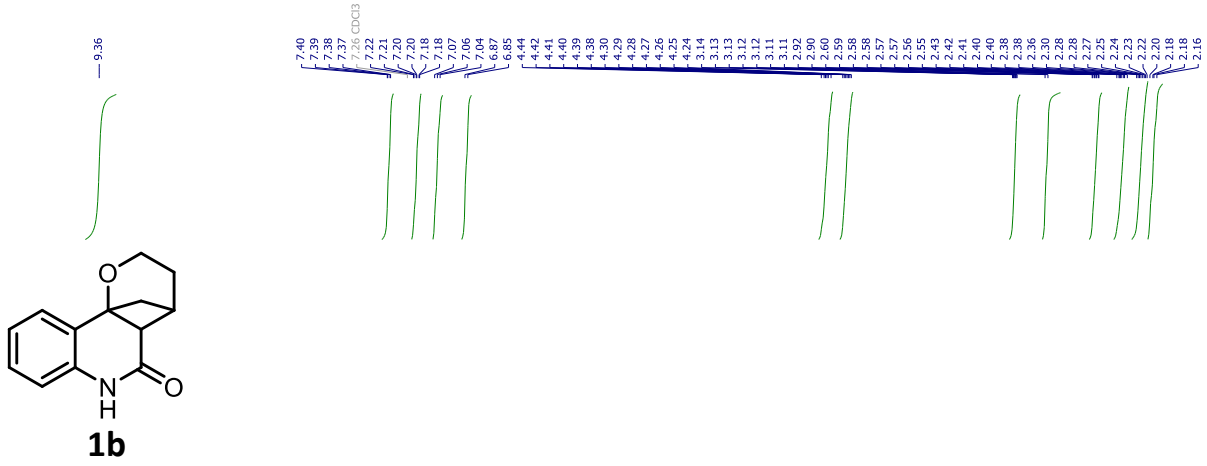

9.39
7.28
7.26
CDCl3
6.83
6.81
4.47
4.45
4.44
4.43
4.26
4.24
4.23
4.22
4.21
4.19
3.38
3.37
3.36
3.35
3.34
3.33
2.99
2.97
2.96
2.95
2.94
2.93
2.45
2.43
2.42
2.41
2.40
2.39
2.38
2.22
2.21
2.20
2.19
2.18
2.16
2.15
2.14
2.13
2.12
2.11
1.85
1.84
1.82
1.81


O
N
H
O

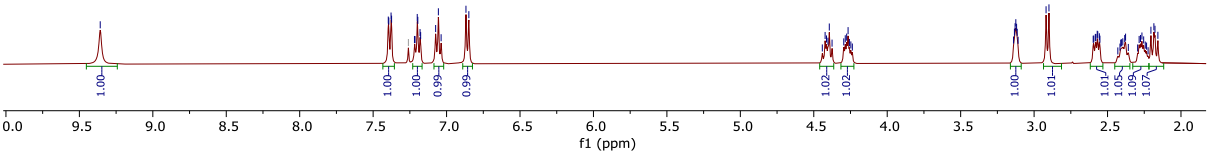
1a

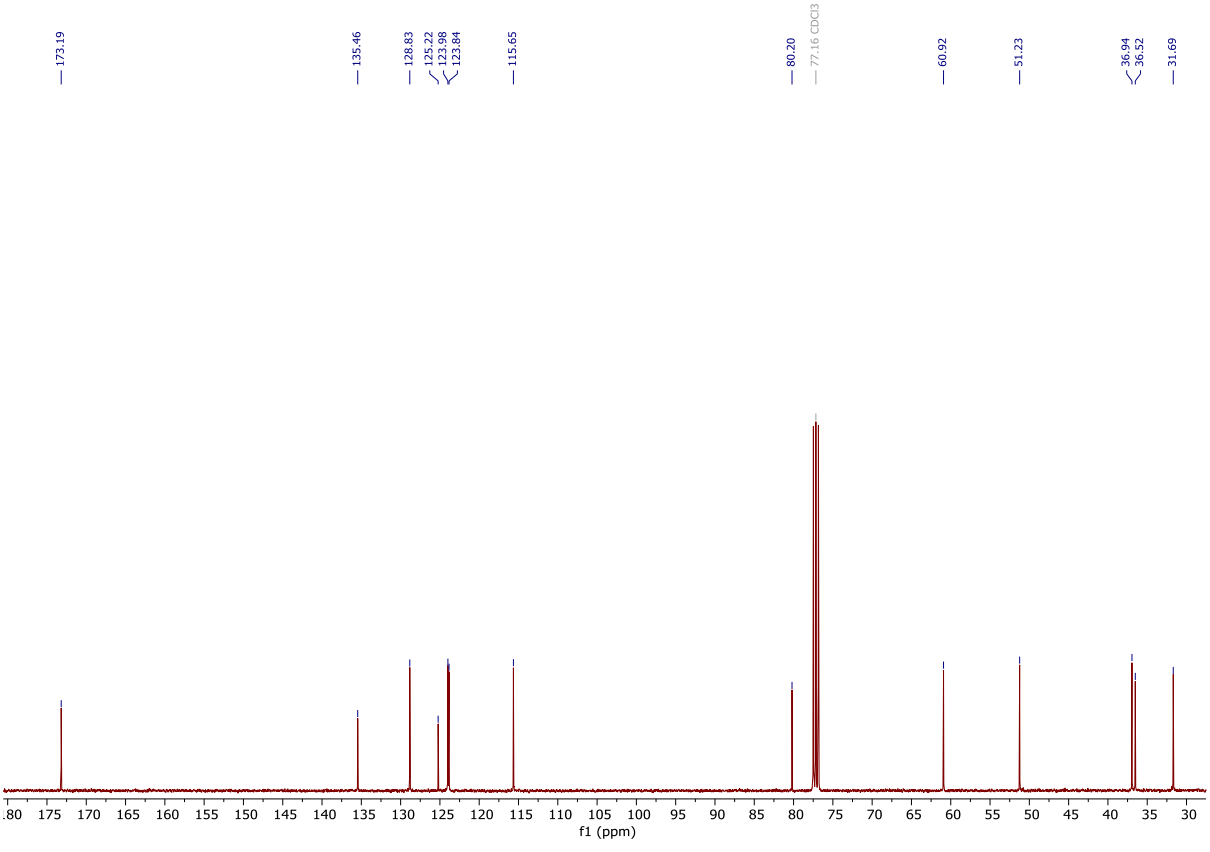

1.00
1.09
0.99
1.02
1.03
1.02
1.02
1.02
1.03
1.02
2.03
1.03
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

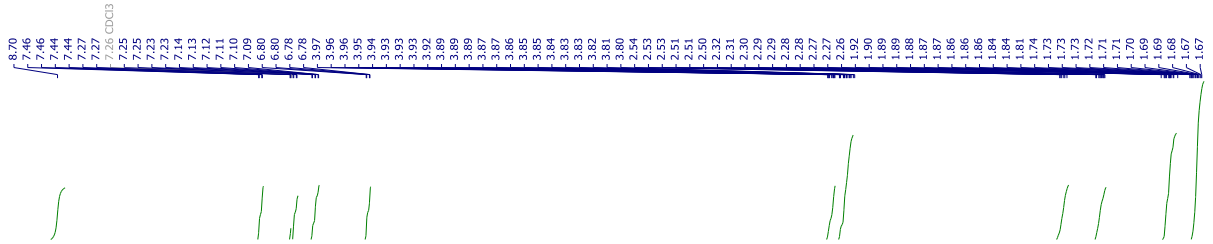

172.38
136.20
129.37
126.44
124.01
123.61
115.84
83.44
77.16 CDCl3
68.68
46.83
42.46
32.45
26.87
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

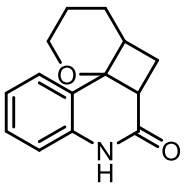

9.36
7.40
7.39
7.38
7.37
7.26 CDCl3
7.22
7.21
7.20
7.20
7.18
7.18
7.07
7.06
7.04
6.87
6.85
4.44
4.42
4.41
4.40
4.39
4.38
4.30
4.29
4.28
4.27
4.26
4.25
4.24
3.14
3.13
3.13
3.12
3.12
3.11
3.11
2.92
2.90
2.60
2.59
2.58
2.58
2.57
2.57
2.56
2.55
2.43
2.42
2.41
2.40
2.40
2.38
2.38
2.36
2.30
2.28
2.28
2.27
2.25
2.24
2.23
2.22
2.20
2.18
2.16
O
N
H
O

1b

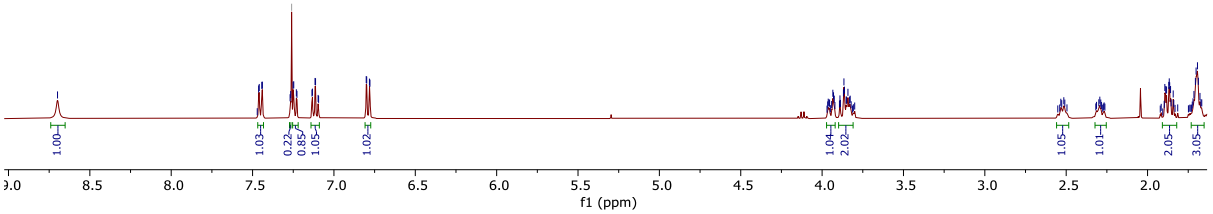

1.00
1.00
1.00
0.99
0.99
1.02
1.02
1.00
1.01
1.01
1.05
1.09
1.07
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

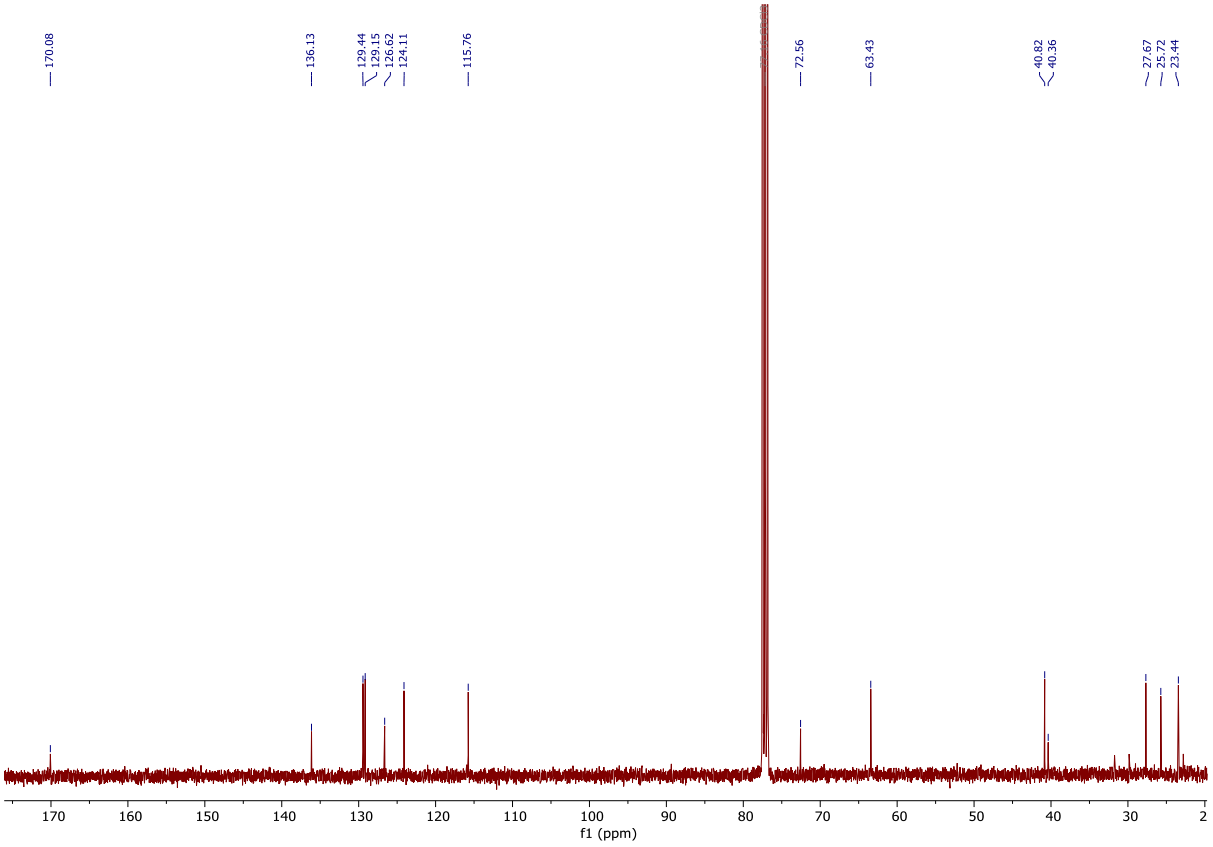

173.19
135.46
128.83
125.22
123.98
123.84
115.65
80.20
77.16 CDCl3
60.92
51.23
36.94
36.52
31.69
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
f1 (ppm)

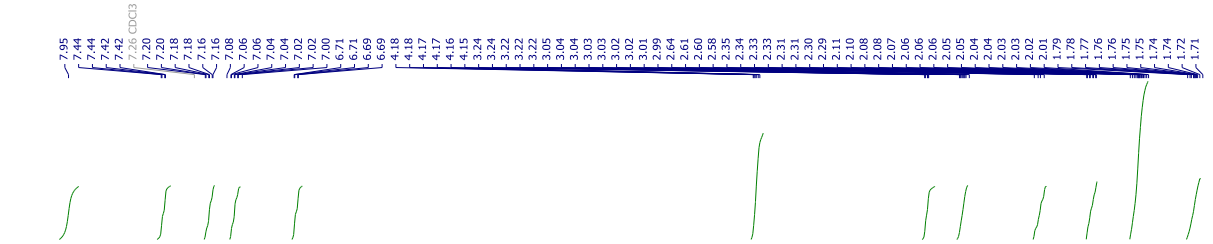


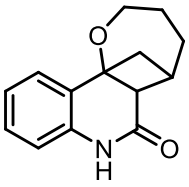


2a

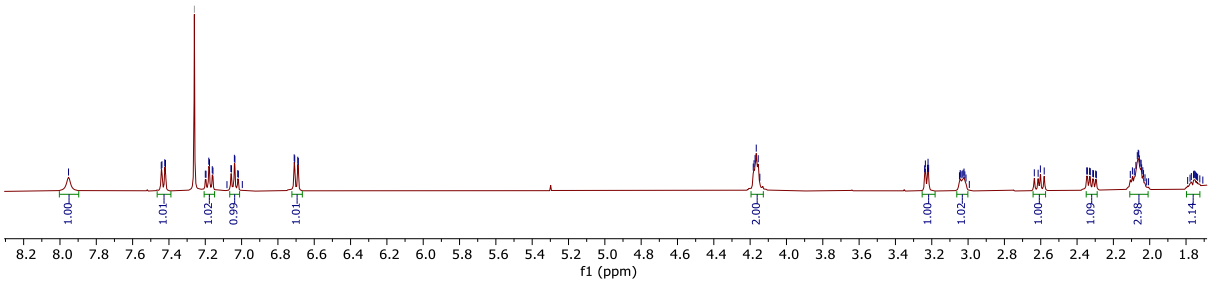

1.00
1.03
0.22
0.85
1.05
1.02
1.04
2.02
1.05
1.01
2.05
3.05
f1 (ppm)

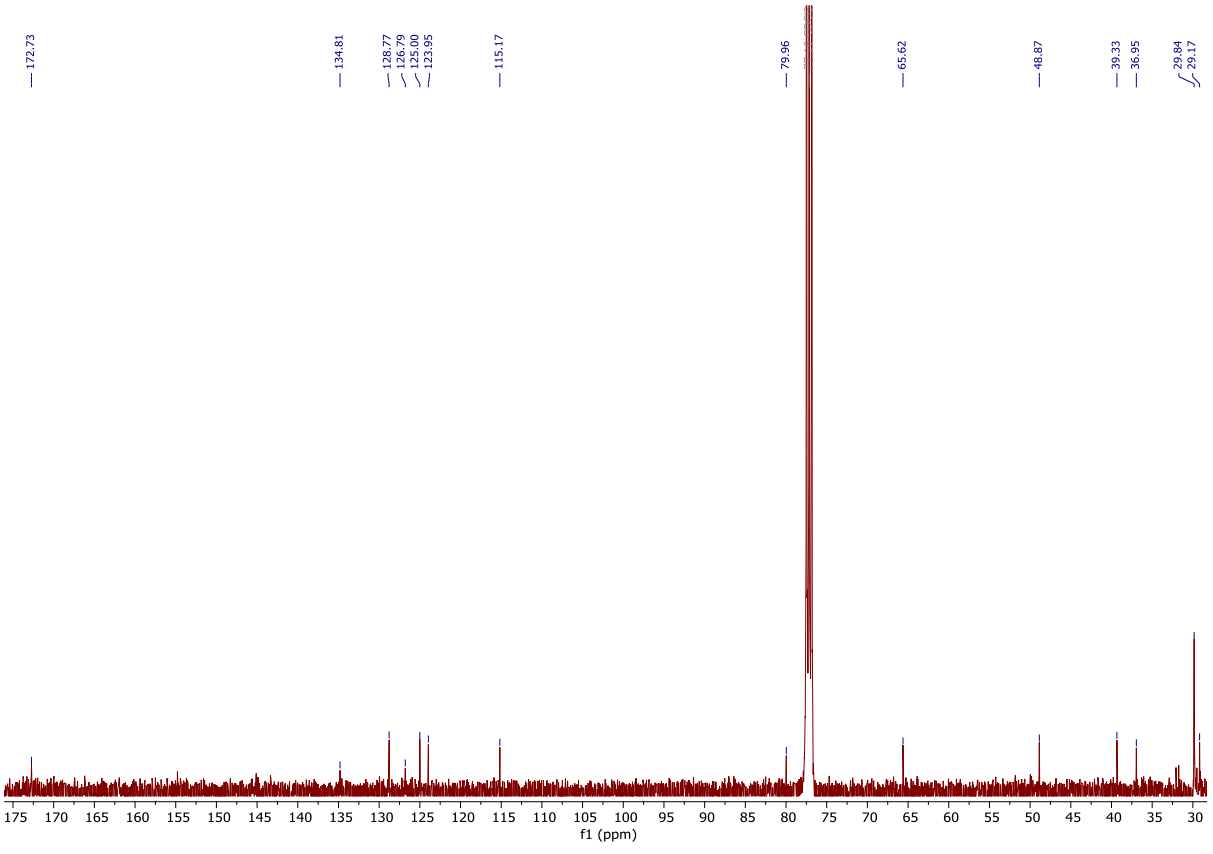

170.08
136.13
129.44
129.15
126.62
124.11
115.76
72.56
63.43
40.82
40.36
27.67
25.72
23.44
f1 (ppm)


7.95
7.44
7.44
7.43
7.42
7.42
7.26
CDCl3
7.20
7.18
7.18
7.16
7.16
7.08
7.06
7.06
7.04
7.04
7.02
7.02
7.00
6.71
6.71
6.69
6.69
4.18
4.18
4.17
4.17
4.16
4.15
3.24
3.24
3.22
3.22
3.22
3.05
3.04
3.04
3.03
3.03
3.02
3.02
3.01
2.99
2.64
2.61
2.60
2.58
2.35
2.34
2.33
2.33
2.31
2.31
2.30
2.29
2.11
2.10
2.08
2.08
2.07
2.06
2.06
2.06
2.05
2.05
2.04
2.04
2.03
2.03
2.02
2.01
2.01
1.79
1.78
1.77
1.76
1.76
1.75
1.75
1.74
1.74
1.72
1.71


O
N
H
O

2b

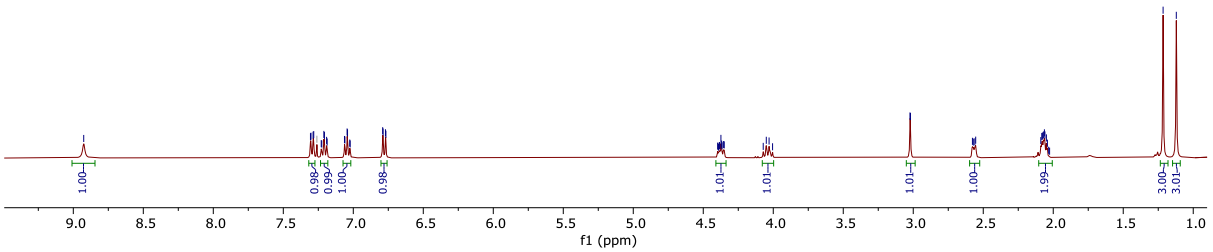

1.00
1.01
1.02
0.99
1.01
2.00
1.00
1.02
1.00
1.09
2.98
1.14
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)

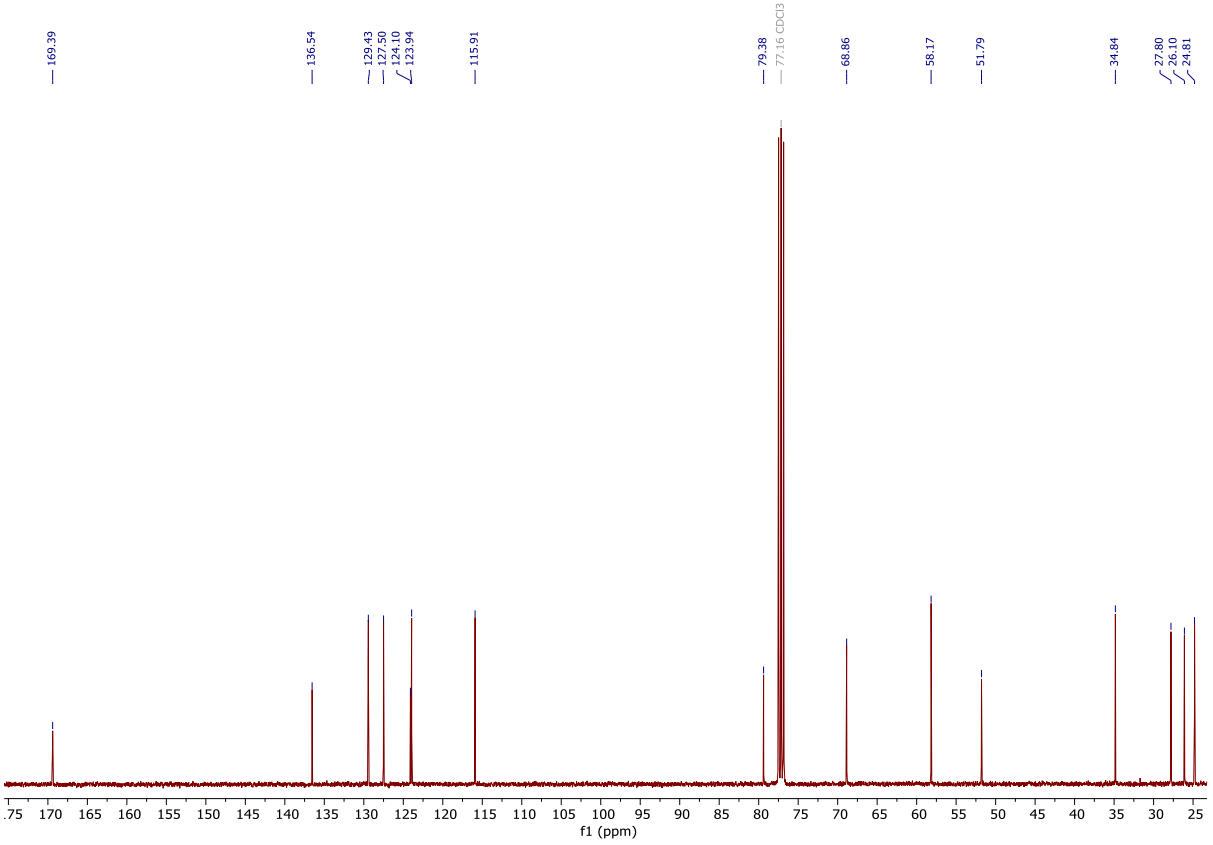

172.73
134.81
128.77
126.79
125.00
123.95
115.17
79.96
65.62
48.87
39.33
36.95
29.84
29.17
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (ppm)

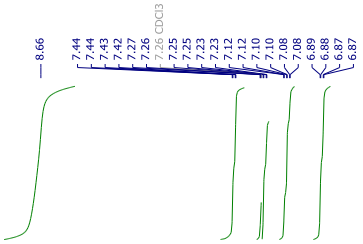

8.93
7.31
7.30
7.29
7.28
7.26 CDCl3
7.23
7.23
7.21
7.21
7.19
7.19
7.06
7.06
7.04
7.04
7.03
7.02
6.79
6.79
6.77
6.77
4.40
4.39
4.39
4.38
4.38
4.37
4.37
4.37
4.36
4.35
4.07
4.05
4.03
4.00
3.02
3.02
2.58
2.57
2.56
2.55
2.09
2.08
2.08
2.07
2.06
2.06
2.05
2.04
2.03
2.03
1.22
1.12

O
N
H
O

3a


1.00
0.98
0.99
1.00
0.98
1.01
1.01
1.01
1.00
1.99
3.00
3.01
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


169.39
136.54
129.43
127.50
124.10
123.94
115.91
79.38
77.16 CDCl3
68.86
58.17
51.79
34.84
27.80
26.10
24.81
.75
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
f1 (ppm)


8.66
7.44
7.44
7.43
7.42
7.27
7.26
7.26 CDCl3
7.25
7.25
7.23
7.23
7.12
7.12
7.10
7.10
7.08
7.08
6.89
6.88
6.87
6.87
4.16
4.14
4.12
4.11
3.46
3.46
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2.66
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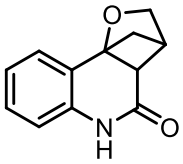
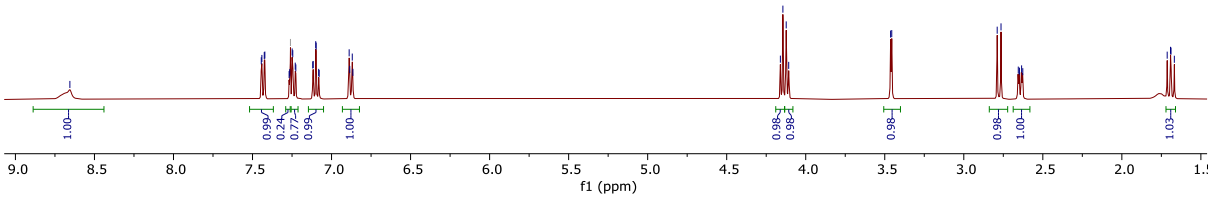
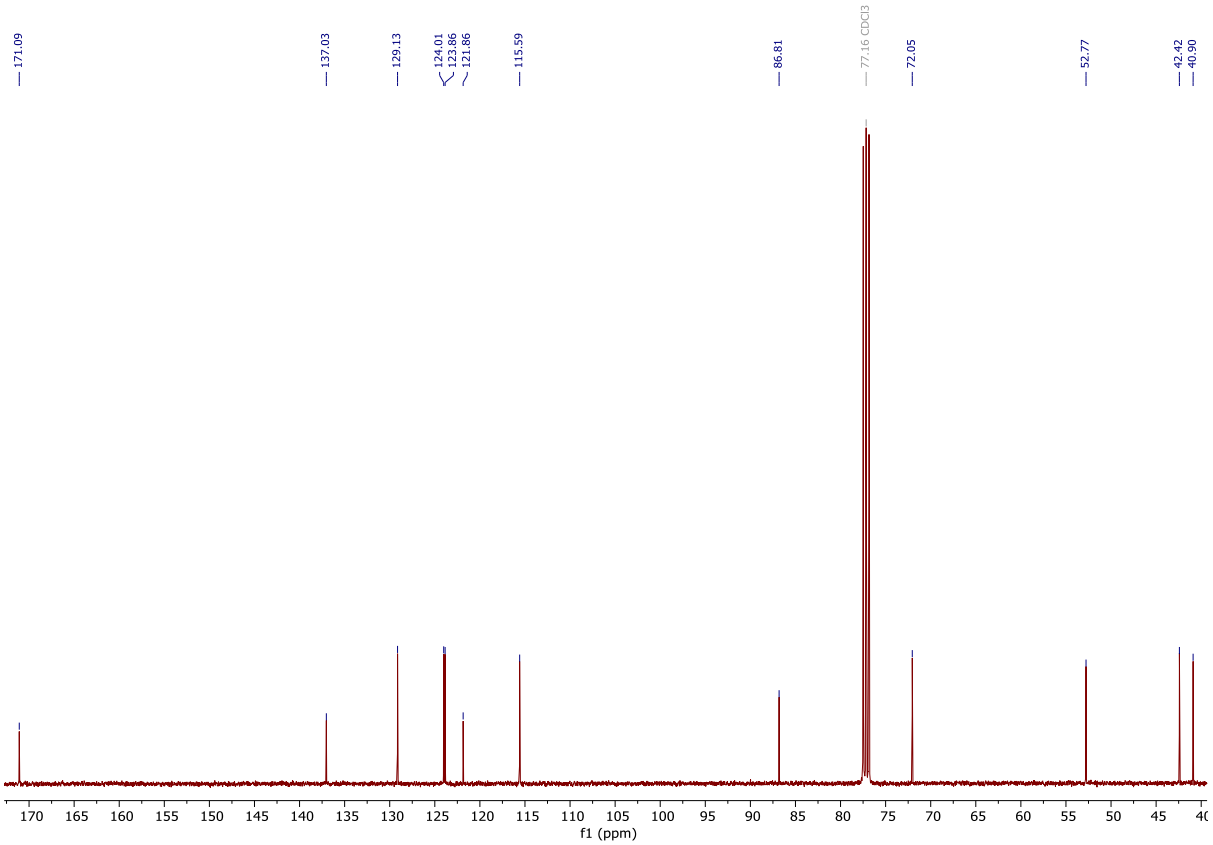
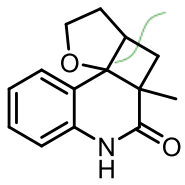
2.63
2.63
1.71
1.69
1.69
1.69
1.67


O
N
H
O

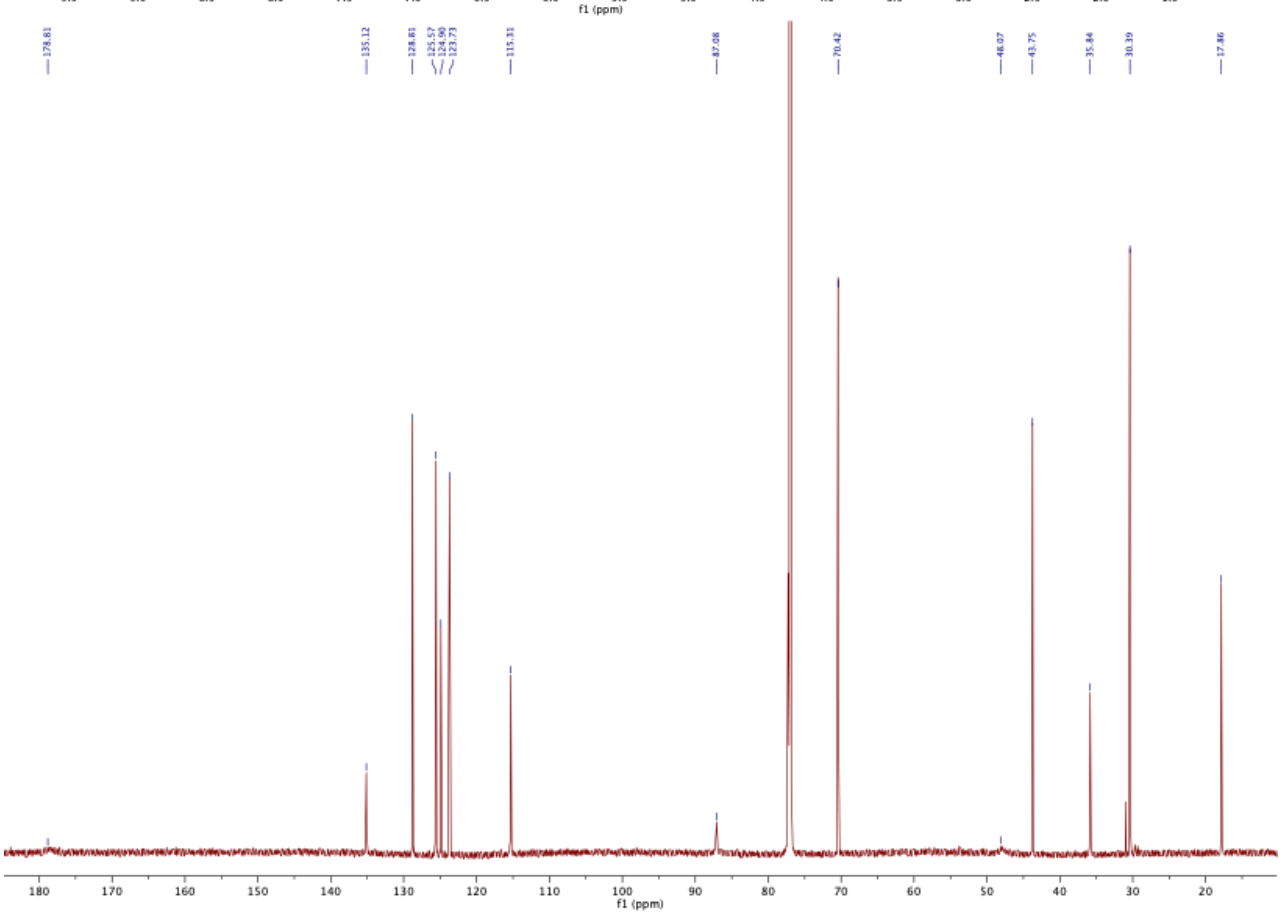
4a


1.00
0.99
0.24
0.77
0.99
1.00
0.98
0.98
0.98
0.98
1.00
1.03
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

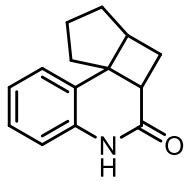

171.09
137.03
129.13
124.01
123.86
121.86
115.59
86.81
77.16 CDCl3
72.05
52.77
42.42
40.90
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
f1 (ppm)



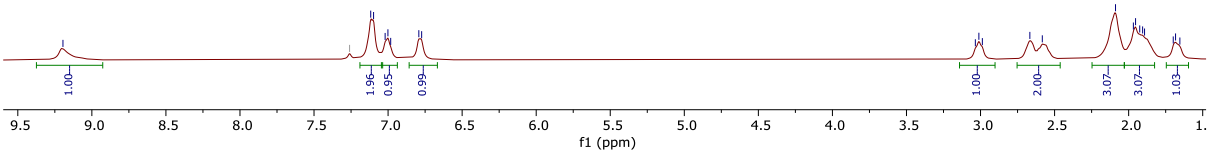
5a



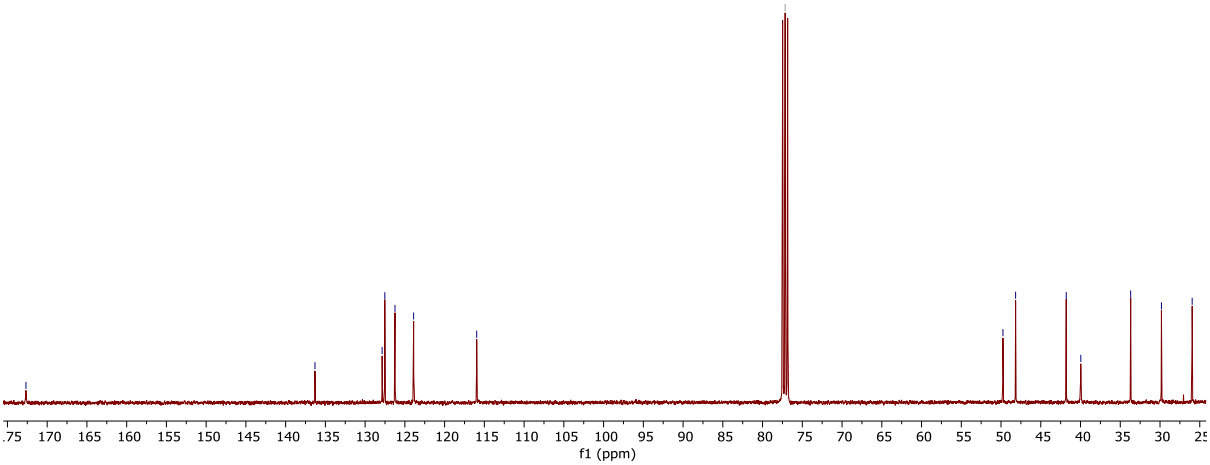

9.20
7.26 CDCl3
7.12
7.10
7.02
7.00
6.98
6.79
6.77
3.03
3.01
2.99
2.67
2.58
2.09
1.97
1.95
1.93
1.91
1.89
1.70
1.68
1.65

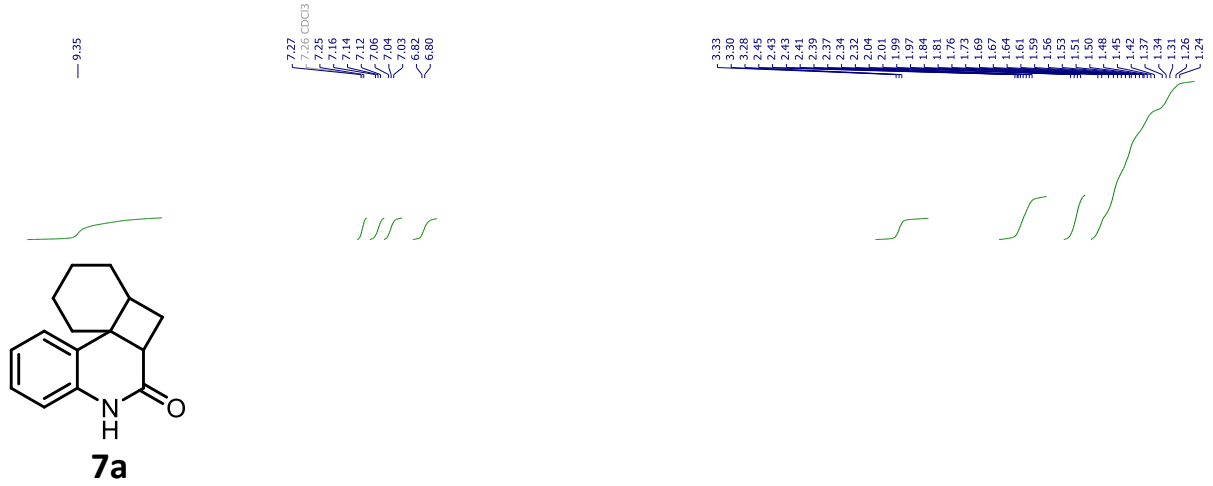

N
H
O

6a

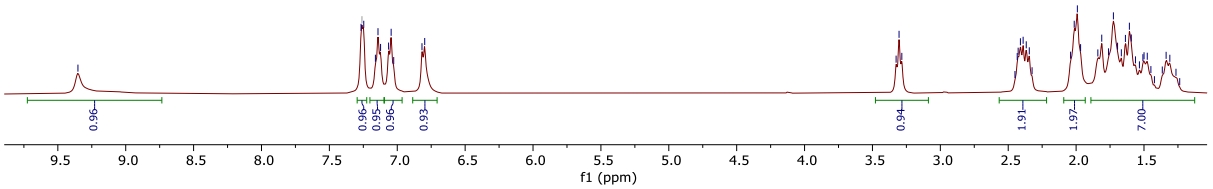

1.00
1.96
0.95
0.99
1.00
2.00
3.07
3.07
1.03
f1 (ppm)

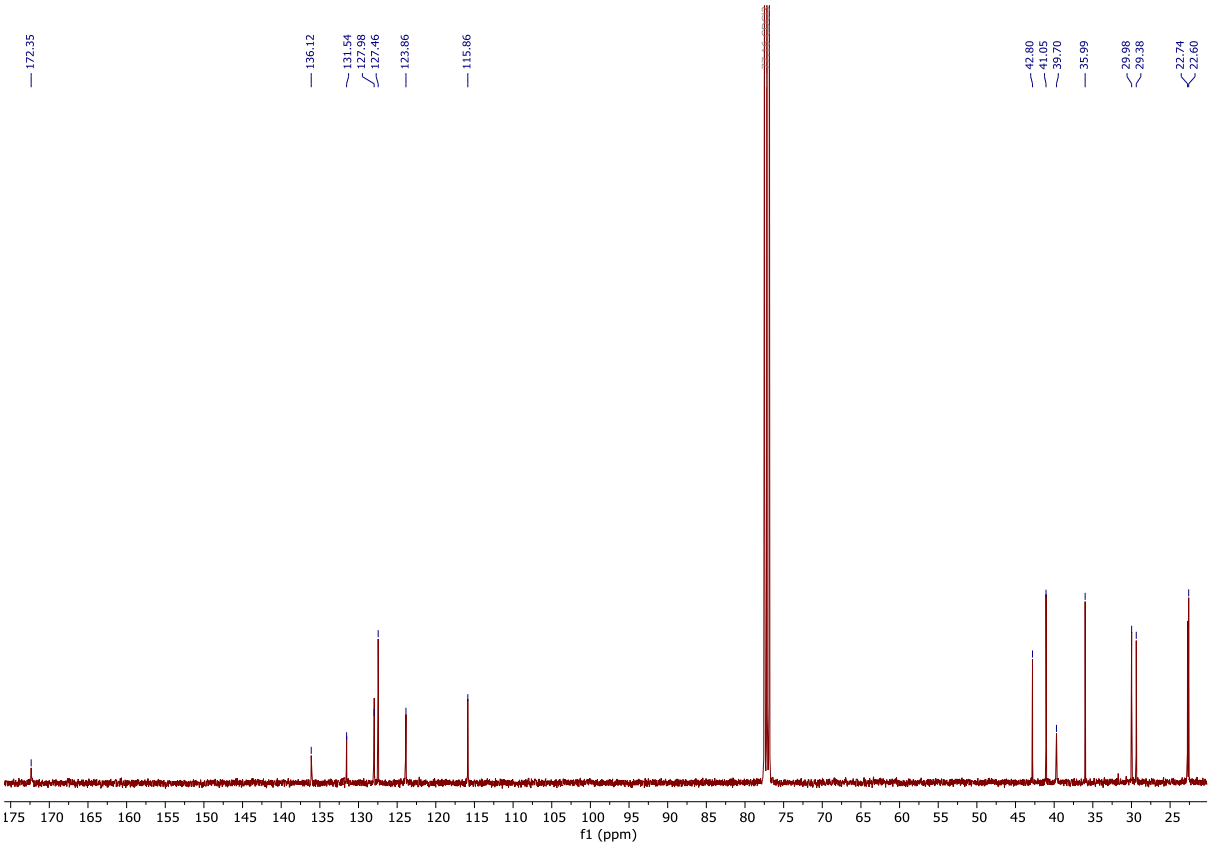

172.67
136.31
127.86
127.53
126.24
123.90
115.97
77.16 CDCl3
49.76
48.17
41.80
39.96
33.69
29.82
25.96

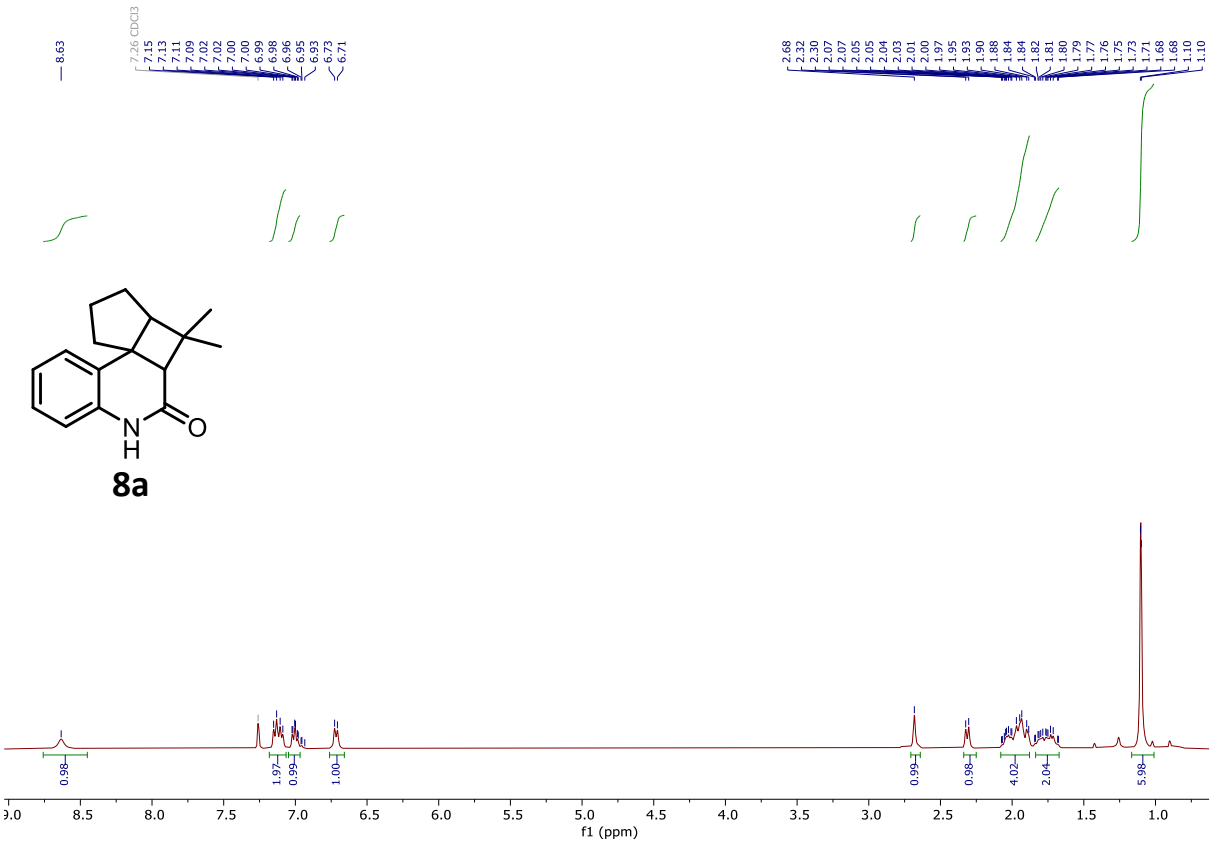

f1 (ppm)

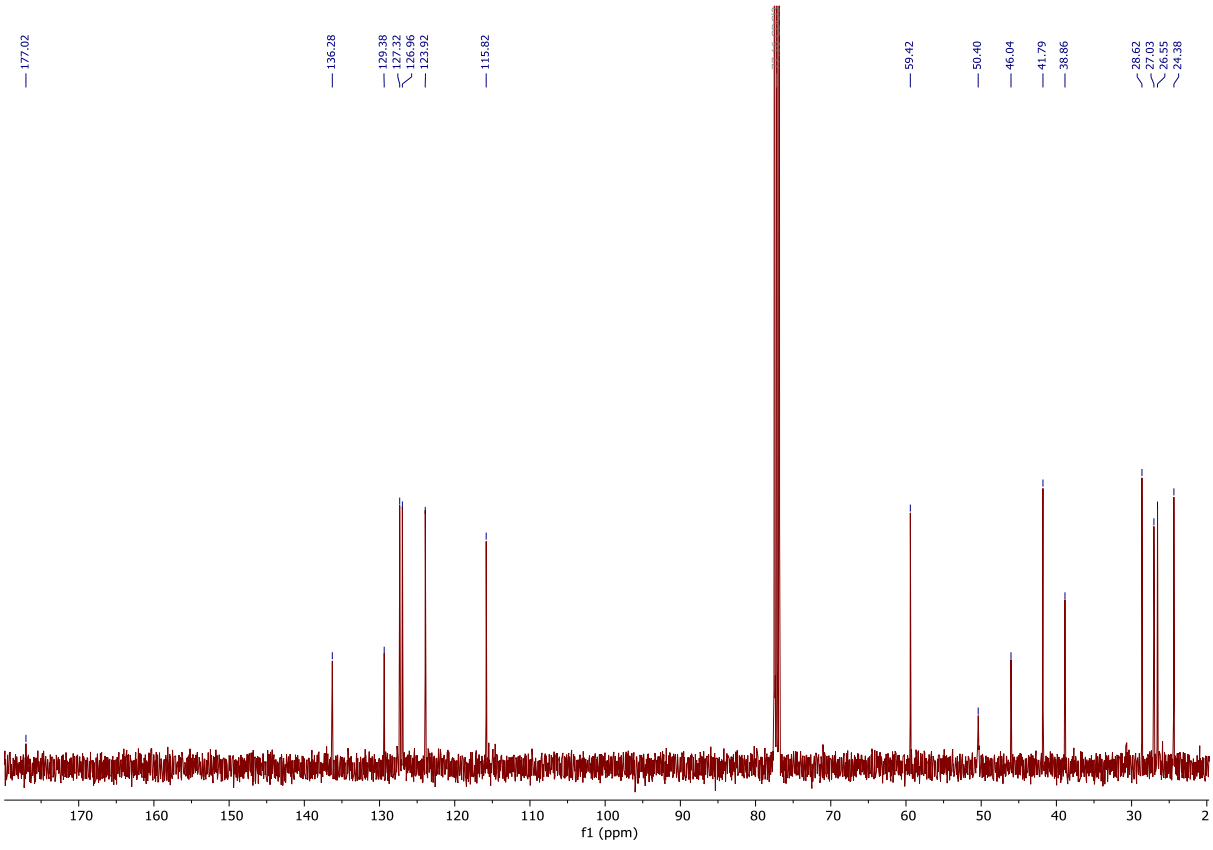

9.35
7.27
7.26 CDCl3
7.25
7.16
7.14
7.12
7.06
7.04
7.03
6.82
6.80
3.33
3.30
3.28
2.45
2.43
2.43
2.41
2.39
2.37
2.34
2.32
2.04
2.01
1.99
1.97
1.84
1.81
1.76
1.73
1.69
1.67
1.64
1.61
1.59
1.56
1.53
1.51
1.50
1.48
1.45
1.42
1.37
1.34
1.31
1.26
1.24
N
H
O

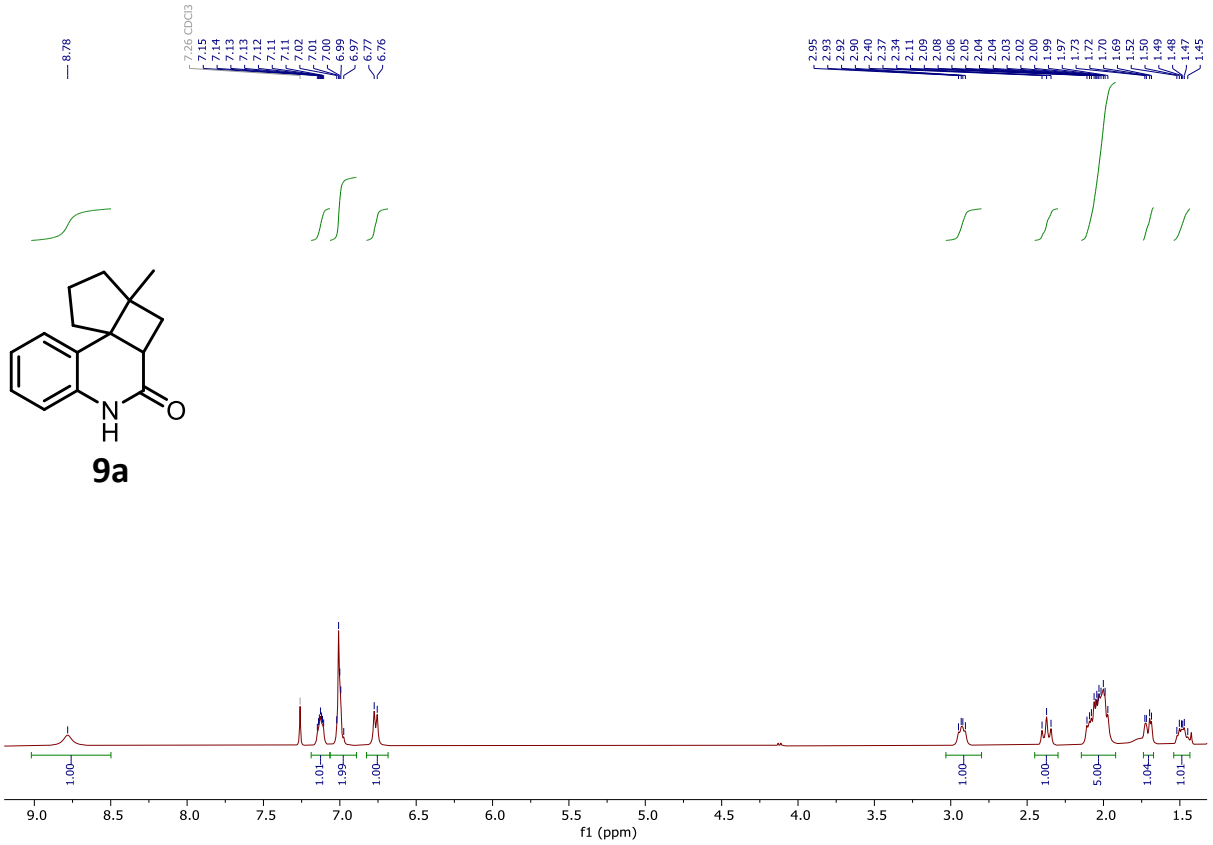
7a


0.96
0.96
0.95
0.96
0.93
0.94
1.91
1.97
7.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)

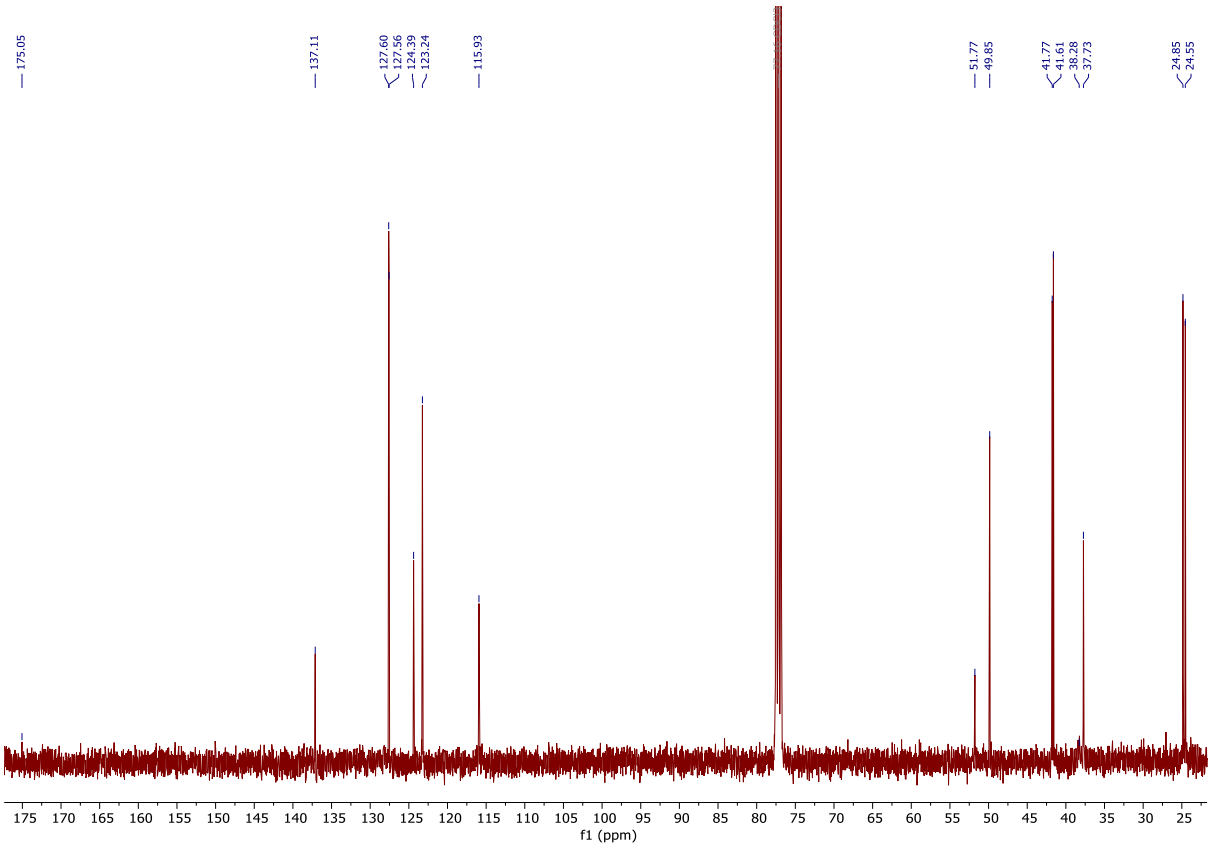

172.35
136.12
131.54
127.98
127.46
123.86
115.86
42.80
41.05
39.70
35.99
29.98
29.38
22.74
22.60
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)


8.63
7.26 CDCl3
7.15
7.13
7.11
7.09
7.02
7.02
7.00
7.00
6.99
6.98
6.96
6.95
6.93
6.73
6.71
2.68
2.32
2.30
2.07
2.07
2.05
2.05
2.04
2.03
2.01
2.00
1.97
1.95
1.93
1.90
1.88
1.84
1.84
1.82
1.81
1.80
1.79
1.77
1.76
1.75
1.73
1.71
1.68
1.68
1.10
1.10
N
H
O
8a
0.98
1.97
0.99
1.00
0.99
0.98
4.02
2.04
5.98
f1 (ppm)

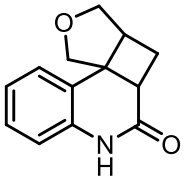

177.02
136.28
129.38
127.32
126.96
123.92
115.82
59.42
50.40
46.04
41.79
38.86
28.62
27.03
26.55
24.38
f1 (ppm)


8.78
7.26 CDCl3
7.15
7.14
7.13
7.13
7.12
7.11
7.11
7.02
7.01
7.00
6.99
6.97
6.77
6.76
2.95
2.93
2.92
2.90
2.40
2.37
2.34
2.11
2.09
2.08
2.06
2.05
2.04
2.04
2.03
2.02
2.00
1.99
1.97
1.73
1.72
1.70
1.69
1.52
1.50
1.49
1.48
1.47
1.45
N
H
O
1.00
1.01
1.99
1.00
1.00
1.00
5.00
1.04
1.01
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

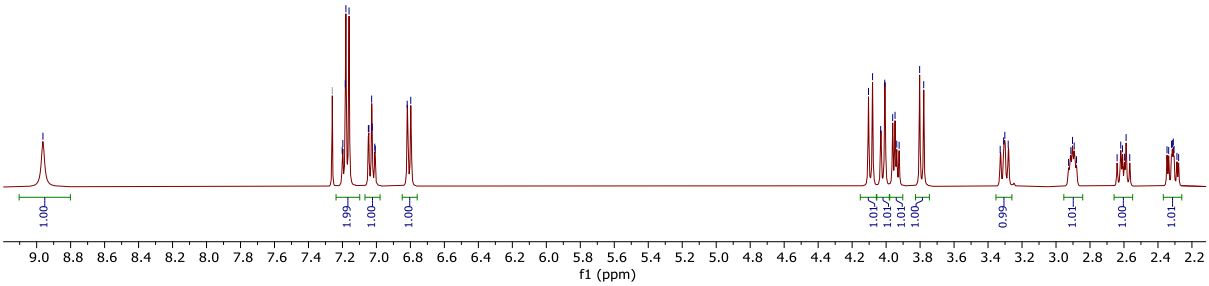
9a

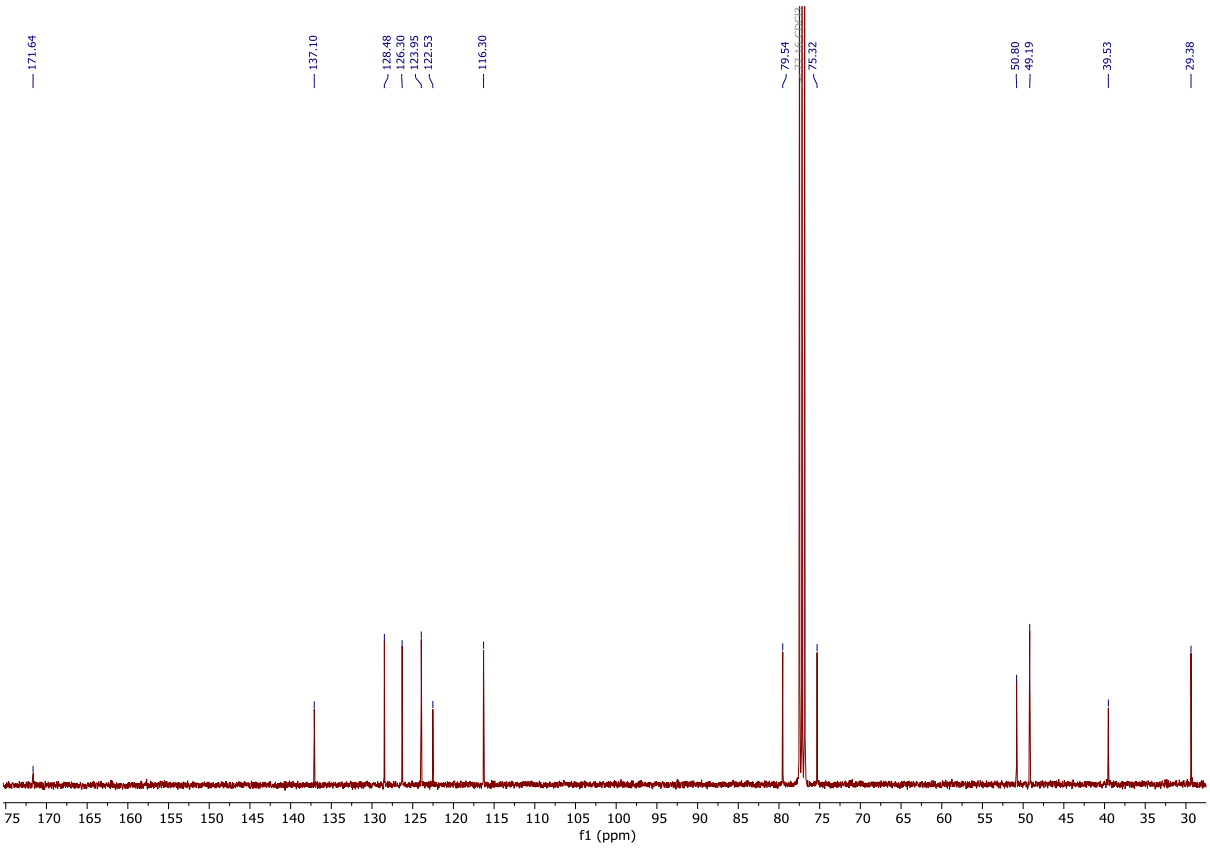

175.05
137.11
127.60
127.56
124.39
123.24
115.93
51.77
49.85
41.77
41.61
38.28
37.73
24.85
24.55
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
f1 (ppm)

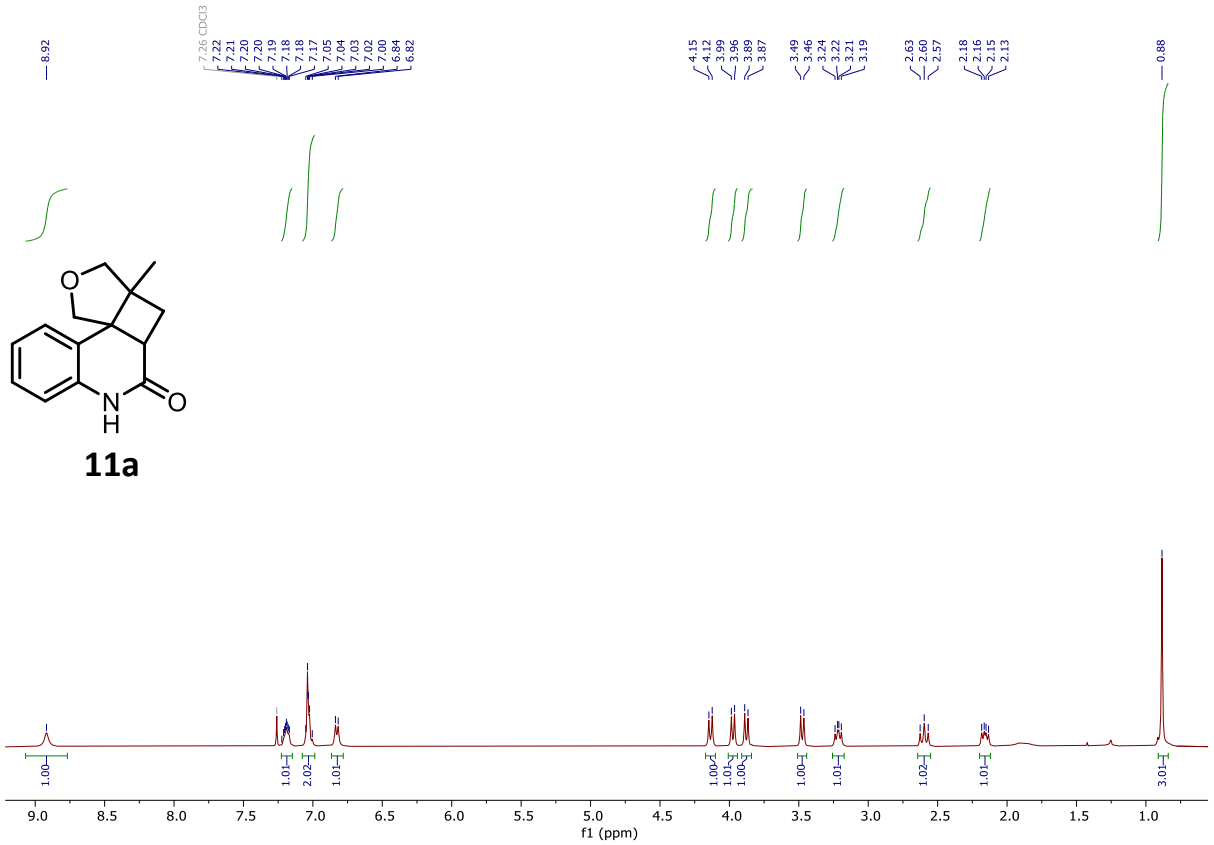

8.96
7.26 CDCl3
7.20
7.20
7.18
7.18
7.16
7.05
7.04
7.03
7.02
7.01
7.01
6.82
6.80
4.10
4.08
4.03
4.01
3.96
3.95
3.94
3.92
3.80
3.78
3.33
3.31
3.30
3.28
2.93
2.91
2.90
2.89
2.88
2.64
2.62
2.61
2.60
2.59
2.57
2.35
2.34
2.32
2.31
2.31
2.31
2.29
2.28

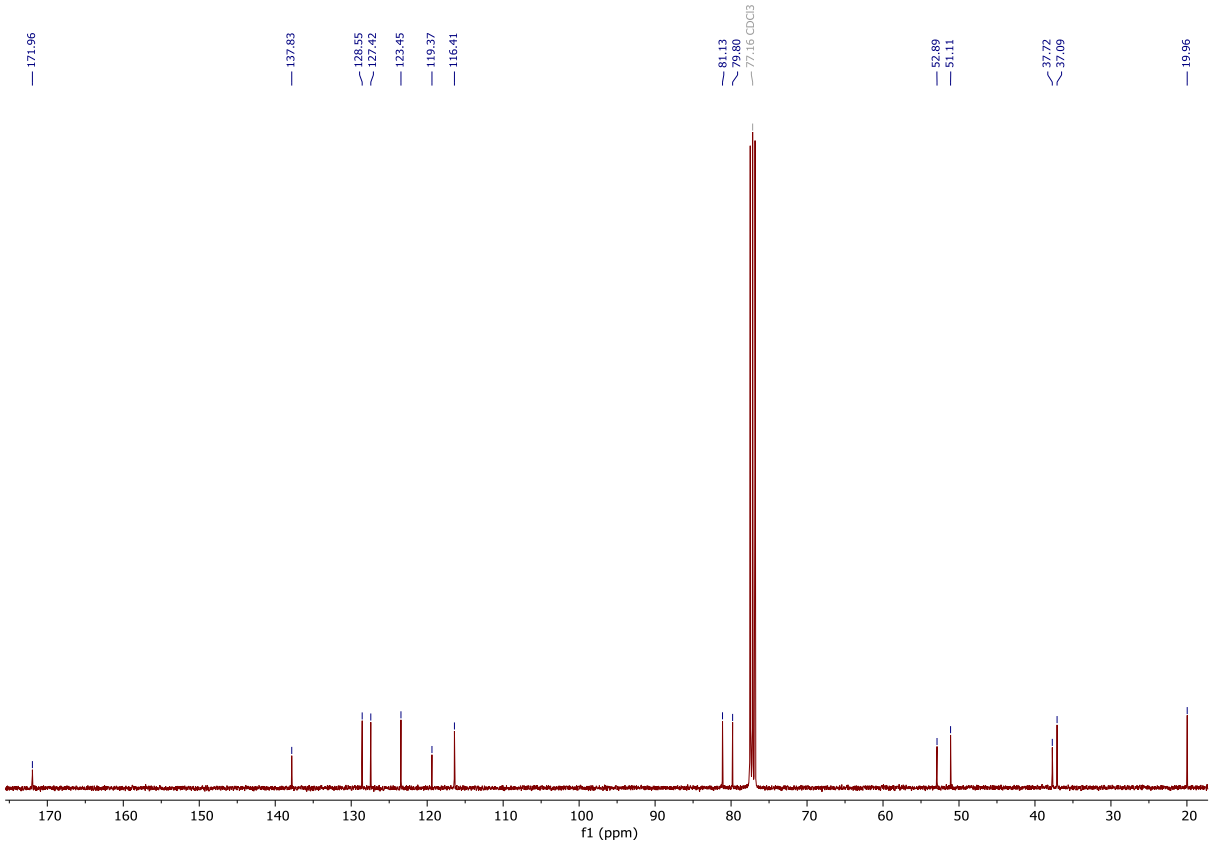

O
N
H
O

10a


1.00
1.99
1.00
1.00
1.01
1.01
1.01
1.00
0.99
1.01
1.00
1.01
9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 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
f1 (ppm)


171.64
137.10
128.48
126.30
123.95
122.53
116.30
79.54
77.16 CDCl3
75.32
50.80
49.19
39.53
29.38
75 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (ppm)


8.92
7.26 CDCl3
7.22
7.21
7.20
7.20
7.19
7.18
7.18
7.17
7.05
7.04
7.03
7.02
7.00
6.84
6.82
4.15
4.12
3.99
3.96
3.89
3.87
3.49
3.46
3.24
3.22
3.21
3.19
2.63
2.60
2.57
2.18
2.16
2.15
2.13
0.88
O
N
H
O
11a
1.00
1.01
2.02
1.01
1.00
1.01
1.00
1.00
1.01
1.02
1.01
3.01
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


171.96
137.83
128.55
127.42
123.45
119.37
116.41
81.13
79.80
77.16 CDCl3
52.89
51.11
37.72
37.09
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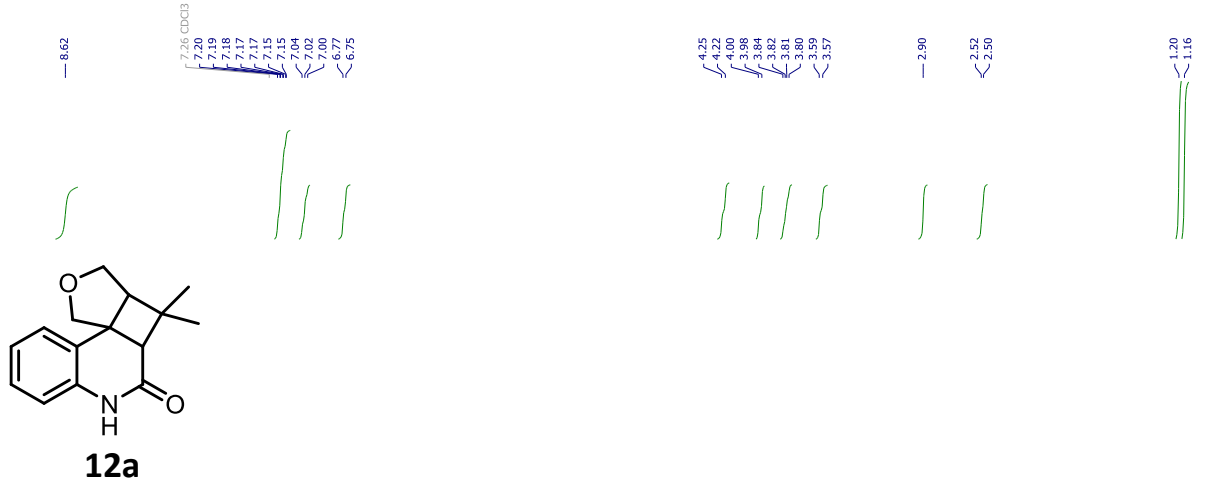
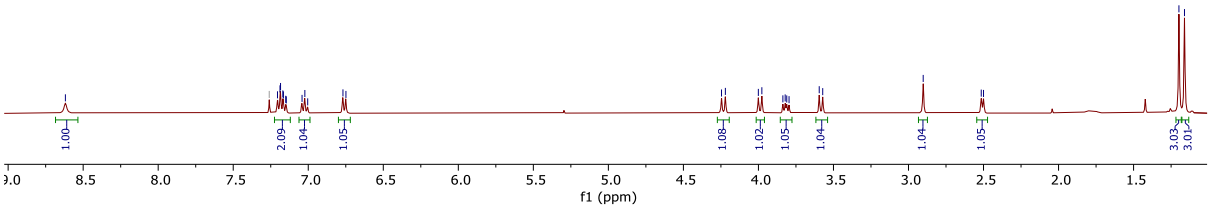
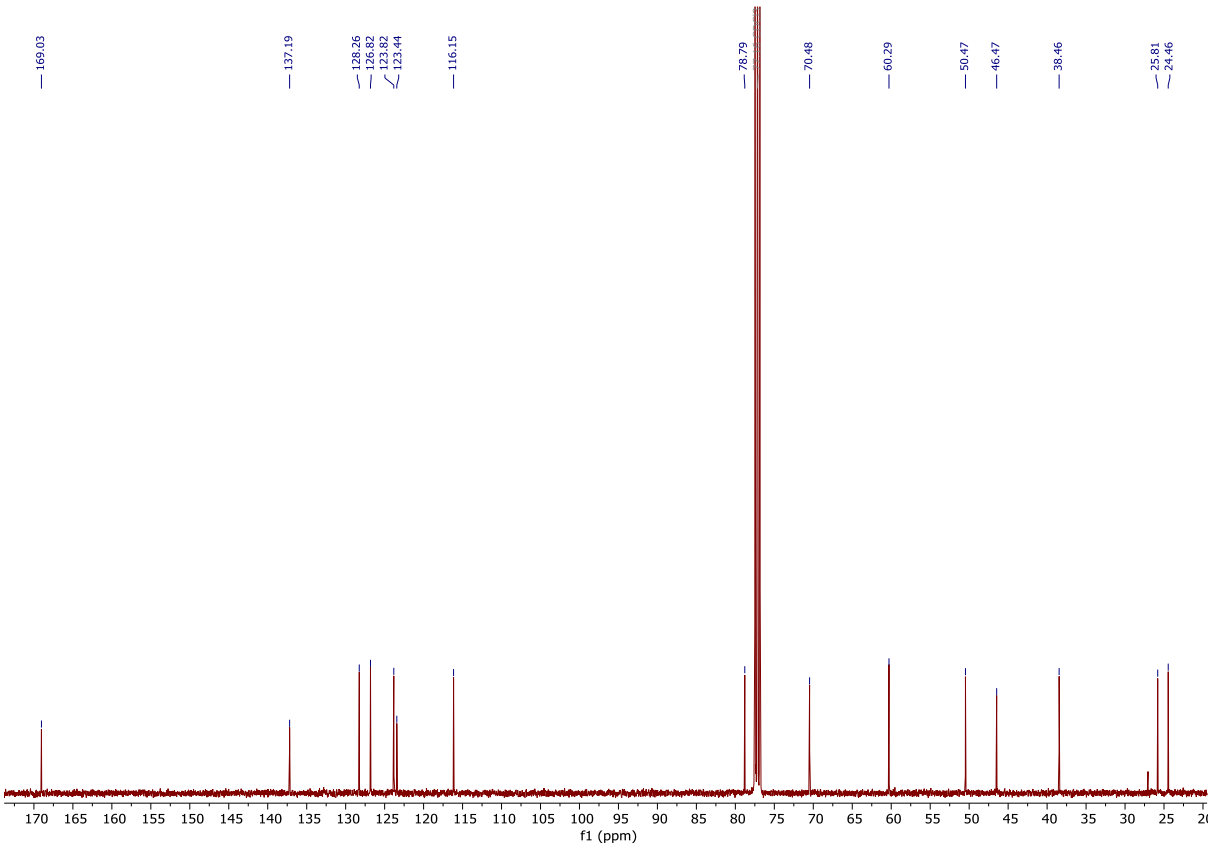
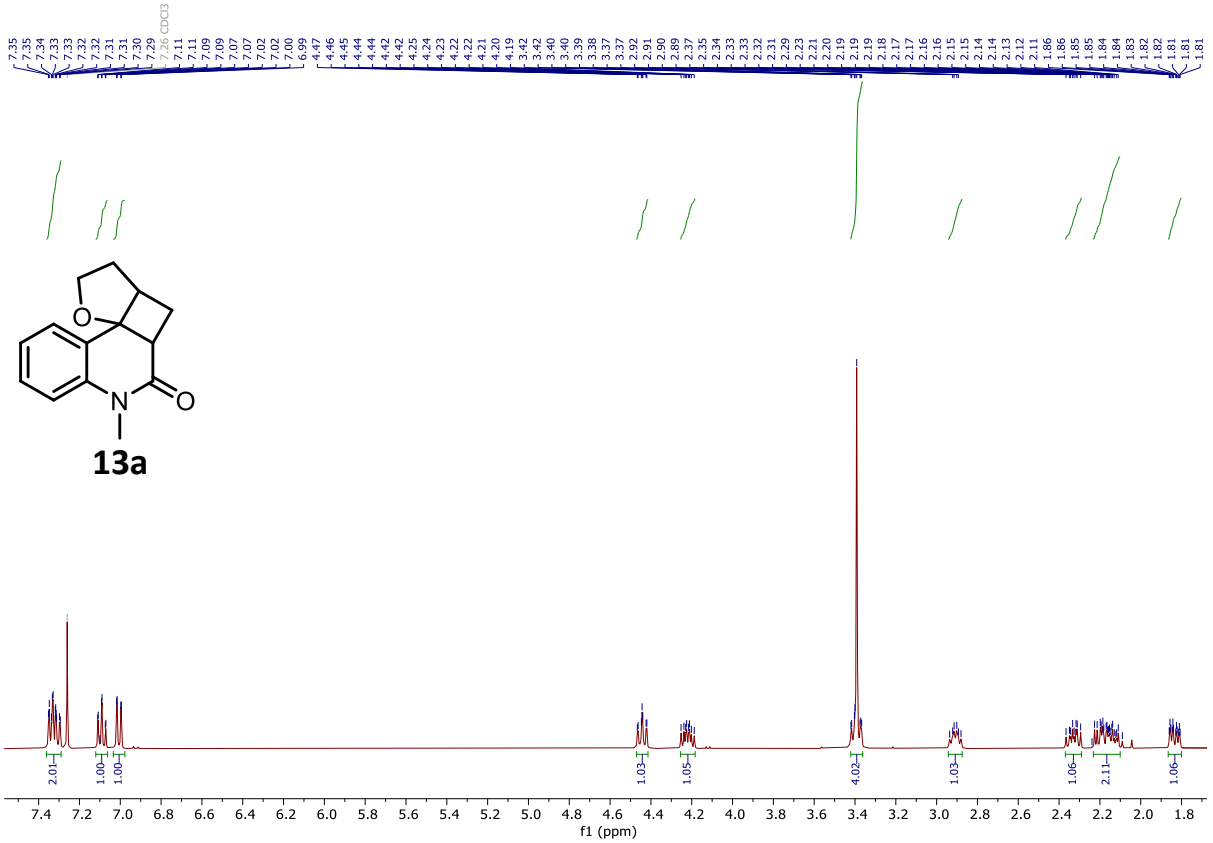
80
70
60
50
40
30
20
f1 (ppm)

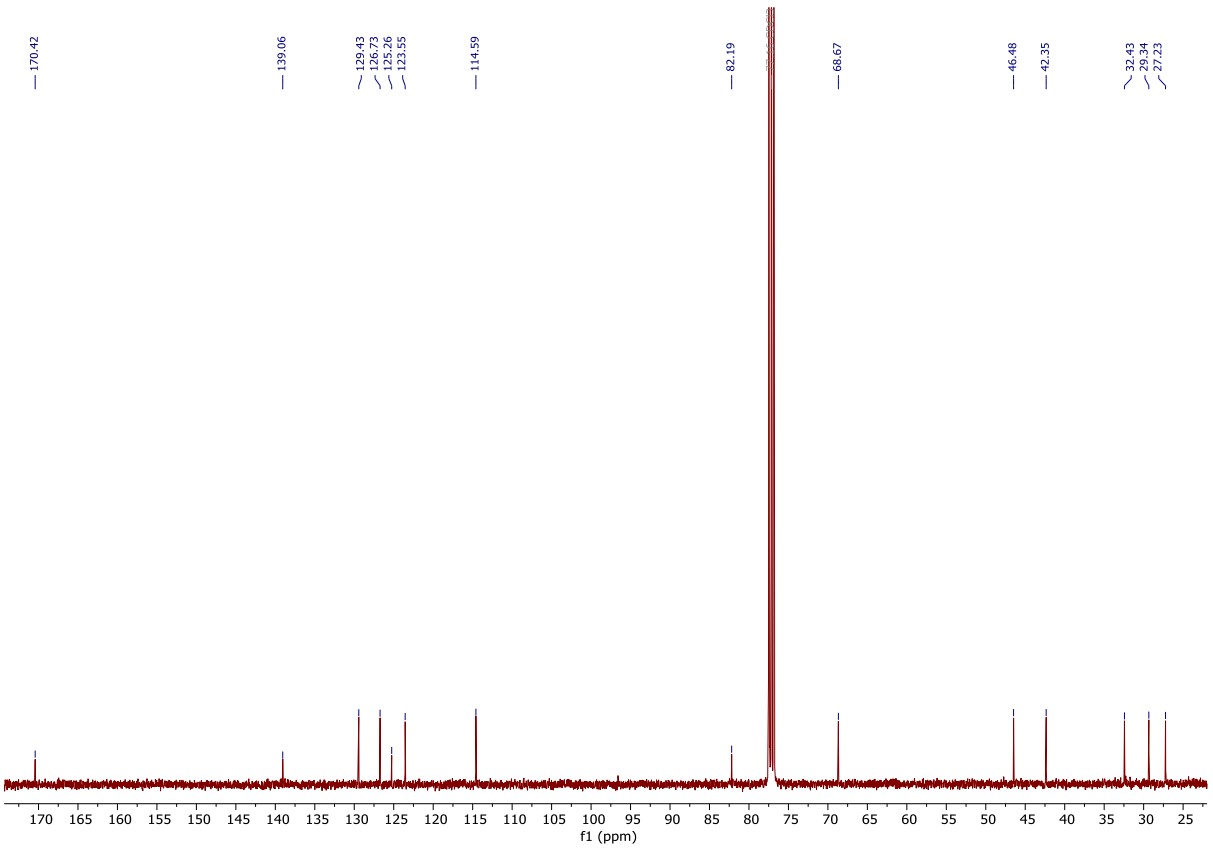

8.62
7.26 CDCl3
7.20
7.19
7.18
7.17
7.17
7.15
7.15
7.04
7.02
7.00
6.77
6.75
4.25
4.22
4.00
3.98
3.84
3.82
3.81
3.80
3.59
3.57
2.90
2.52
2.50
1.20
1.16
O
N
H
O

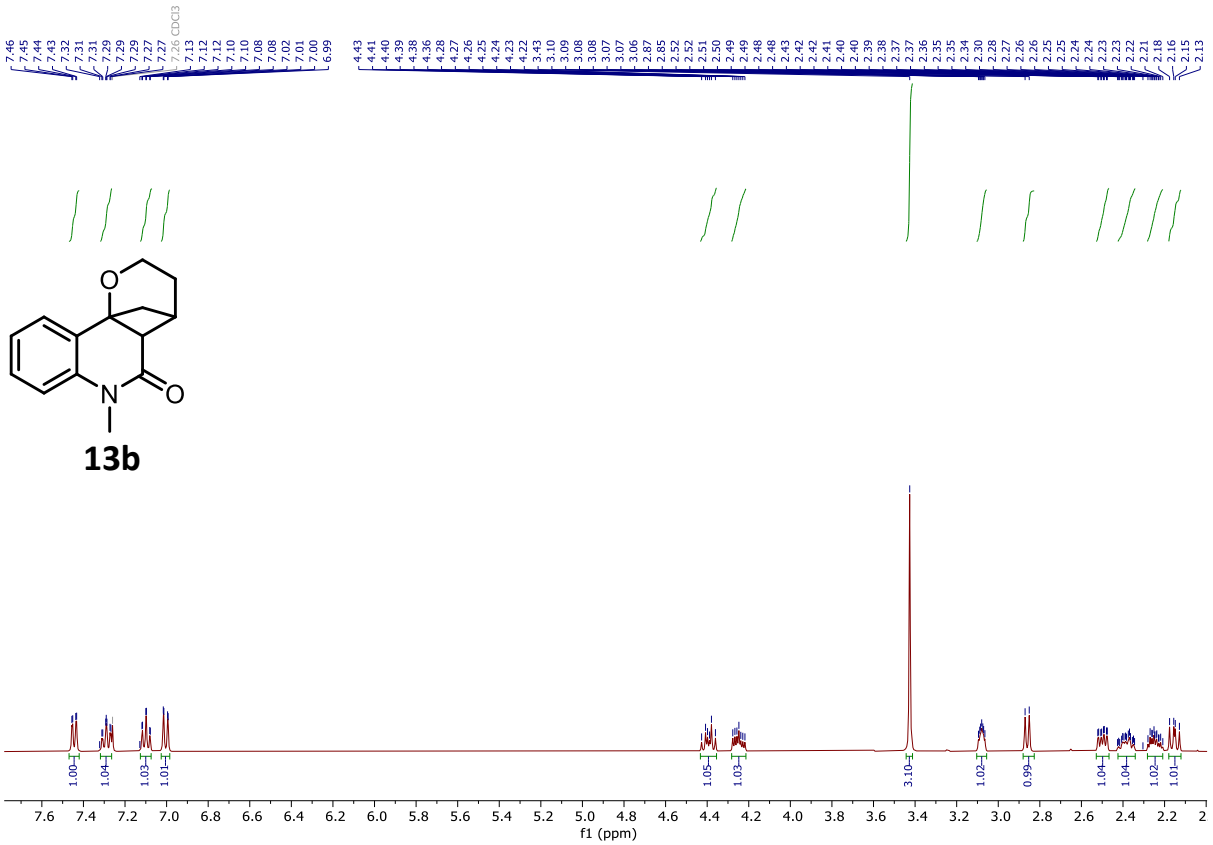
12a

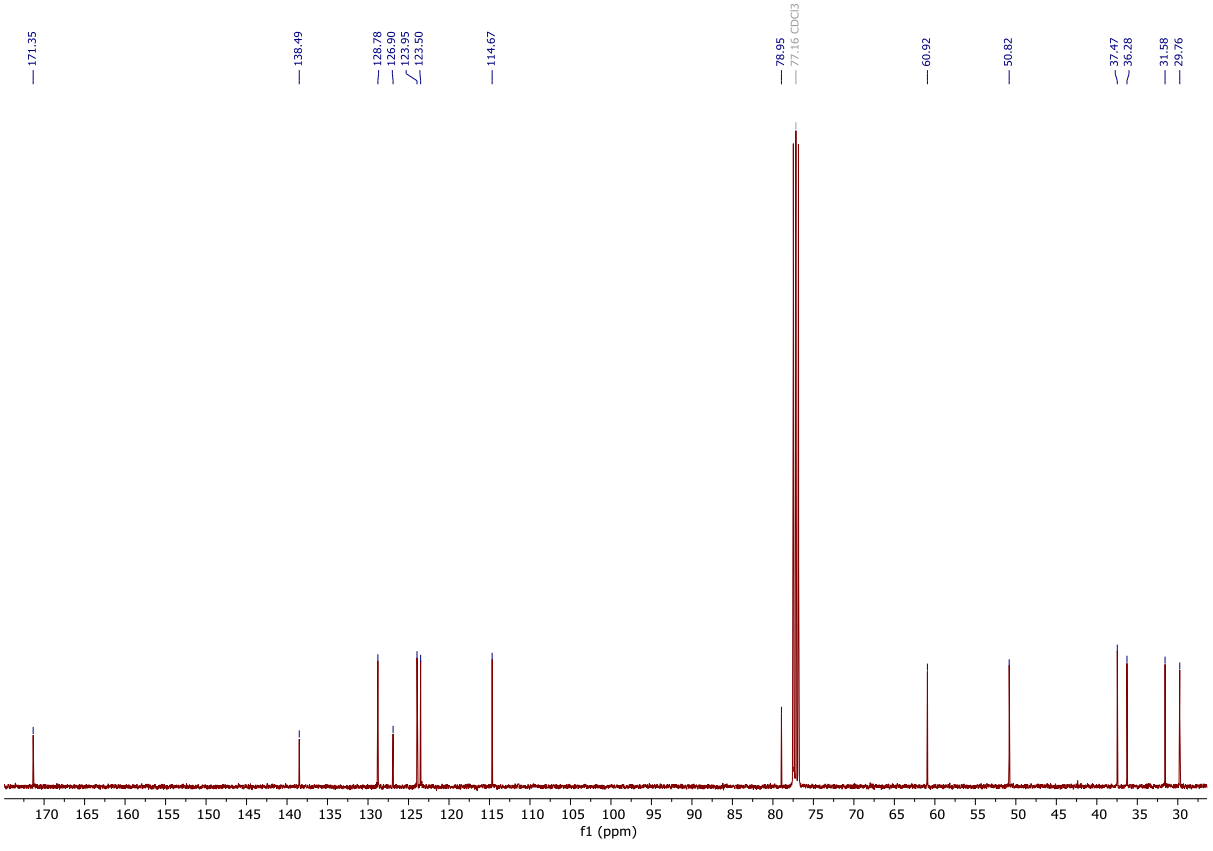

1.00
2.09
1.04
1.05
1.08
1.02
1.05
1.04
1.04
1.05
3.03
3.01
f1 (ppm)

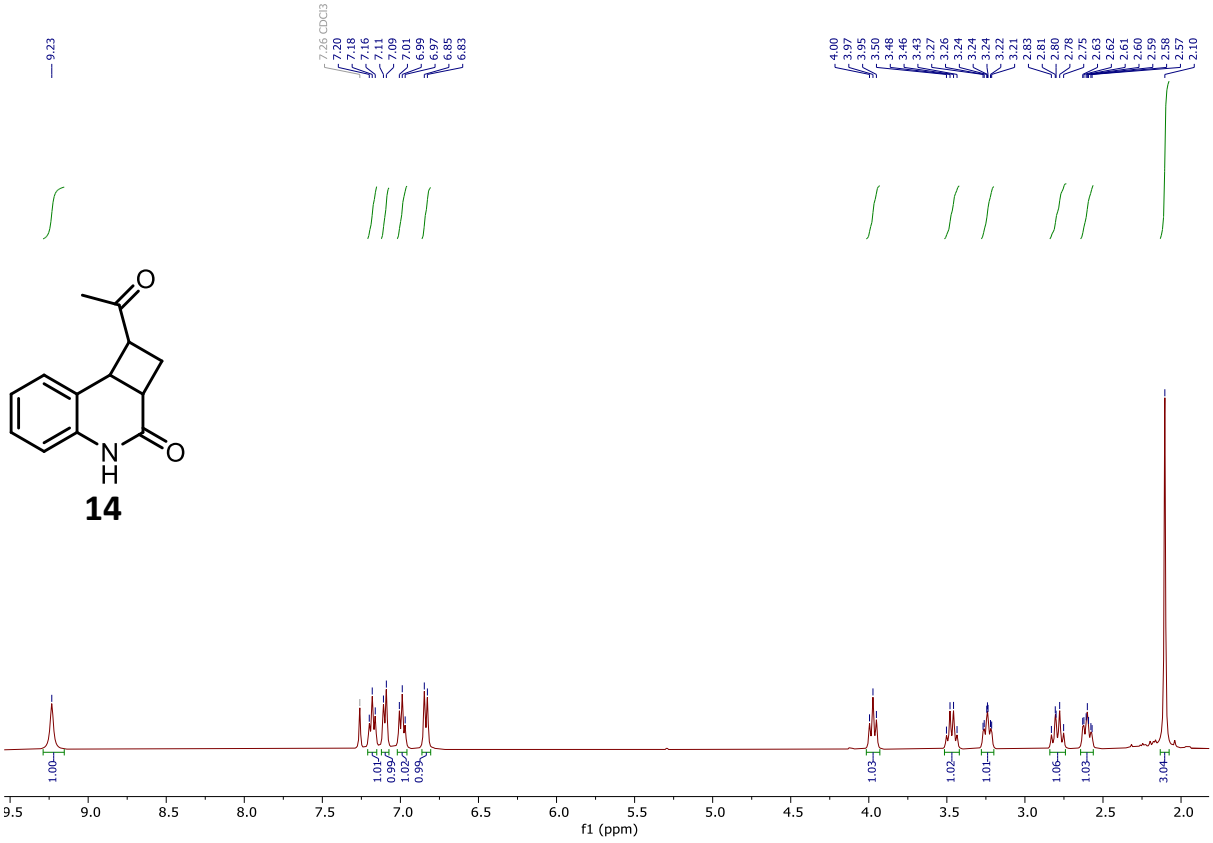

169.03
137.19
128.26
126.82
123.82
123.44
116.15
78.79
70.48
60.29
50.47
46.47
38.46
25.81
24.46
f1 (ppm)

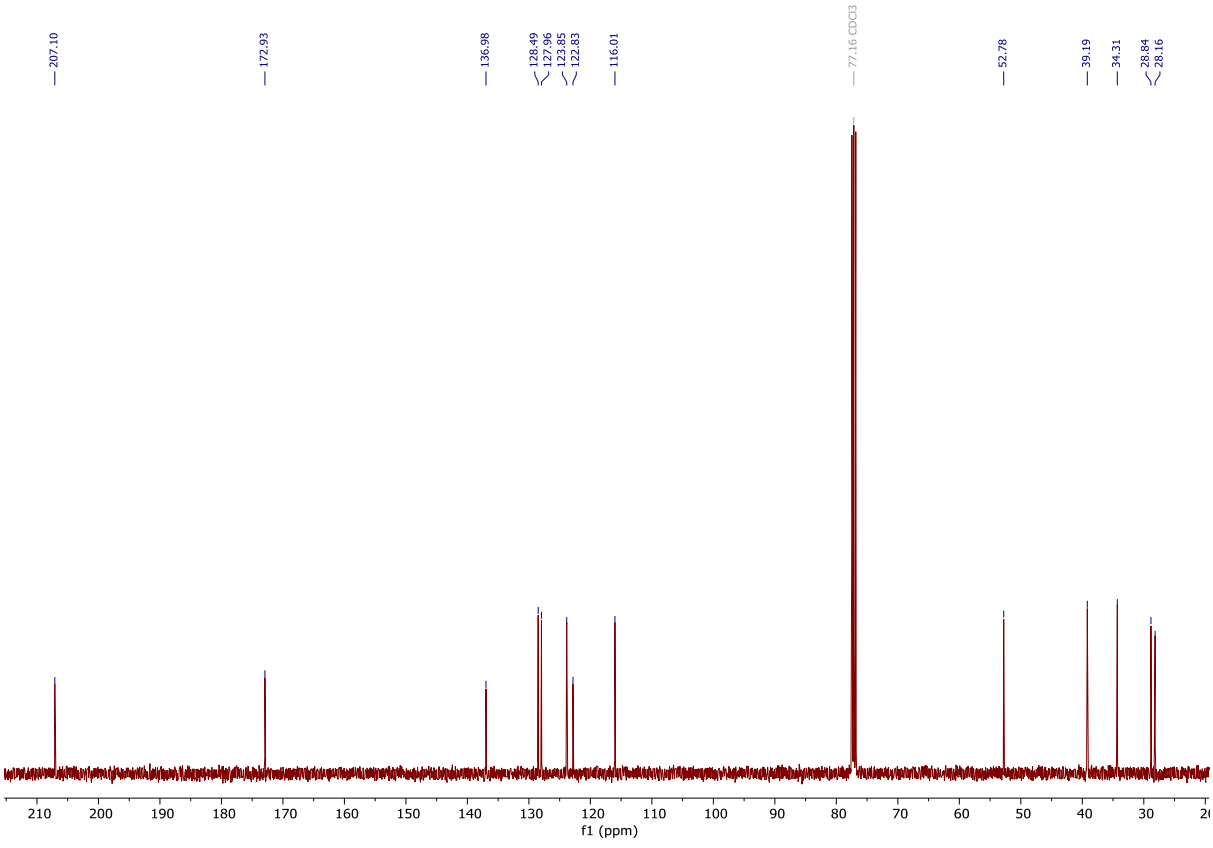

7.35 7.35 7.34 7.33 7.33 7.32 7.32 7.31 7.31 7.30 7.29 7.26 CDCl3 7.11 7.11 7.09 7.09 7.07 7.07 7.02 7.02 7.00 6.99
4.47 4.46 4.45 4.44 4.44 4.42 4.42 4.25 4.24 4.23 4.22 4.22 4.21 4.20 4.19 3.42 3.42 3.40 3.40 3.39 3.38 3.37 3.37 2.92 2.91 2.90 2.89 2.37 2.35 2.34 2.33 2.32 2.31 2.29 2.23 2.21 2.20 2.19 2.19 2.18 2.17 2.17 2.16 2.16 2.15 2.15 2.14 2.14 2.13 2.12 2.11 1.86 1.86 1.85 1.85 1.84 1.84 1.83 1.82 1.82 1.81 1.81 1.81
O
N
O
13a
2.01 1.00 1.00 1.03 1.05 4.02 1.03 1.06 2.11 1.06
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 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
f1 (ppm)

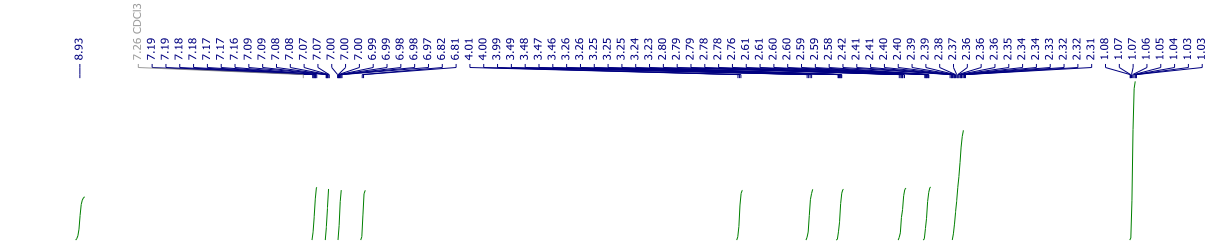

170.42 139.06 129.43 126.73 125.26 123.55 114.59 82.19 68.67 46.48 42.35 32.43 29.34 27.23
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)

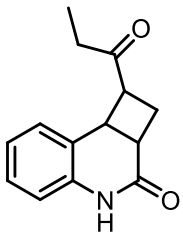

7.46 7.45 7.44 7.43 7.32 7.31 7.31 7.29 7.29 7.29 7.27 7.27 7.26 CDCl3 7.13 7.12 7.12 7.10 7.10 7.08 7.08 7.02 7.01 7.00 6.99
4.43 4.41 4.40 4.39 4.38 4.36 4.28 4.27 4.26 4.25 4.24 4.23 4.22 3.43 3.10 3.09 3.08 3.08 3.07 3.07 3.06 2.87 2.85 2.52 2.52 2.51 2.50 2.49 2.49 2.48 2.48 2.43 2.42 2.42 2.41 2.40 2.40 2.39 2.38 2.37 2.37 2.36 2.35 2.35 2.34 2.30 2.28 2.27 2.26 2.26 2.25 2.25 2.24 2.24 2.23 2.23 2.22 2.21 2.18 2.16 2.15 2.13
O
N
O
13b
1.00 1.04 1.03 1.01 1.05 1.03 3.10 1.02 0.99 1.04 1.04 1.02 1.01
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 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
f1 (ppm)


171.35 138.49 128.78 126.90 123.95 123.50 114.67 78.95 77.16 CDCl3 60.92 50.82 37.47 36.28 31.58 29.76
170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (ppm)

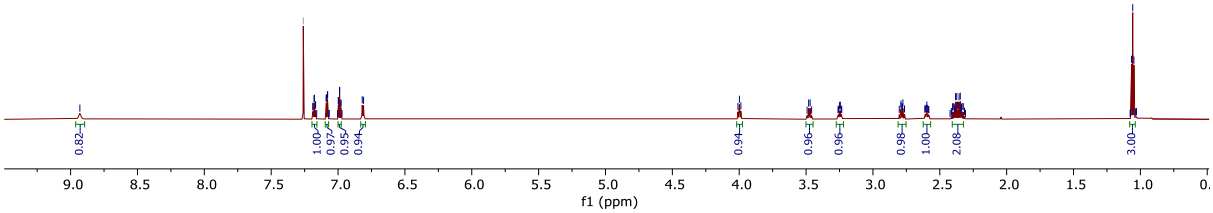

9.23
7.26 CDCl3
7.20
7.18
7.16
7.11
7.09
7.01
6.99
6.97
6.85
6.83
4.00
3.97
3.95
3.50
3.48
3.46
3.43
3.27
3.26
3.24
3.24
3.24
3.22
3.21
2.83
2.81
2.80
2.78
2.75
2.63
2.62
2.61
2.60
2.59
2.58
2.57
2.10
O
N
H
O
14
1.00
1.01
0.99
1.02
0.99
1.03
1.02
1.01
1.06
1.03
3.04
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f1 (ppm)

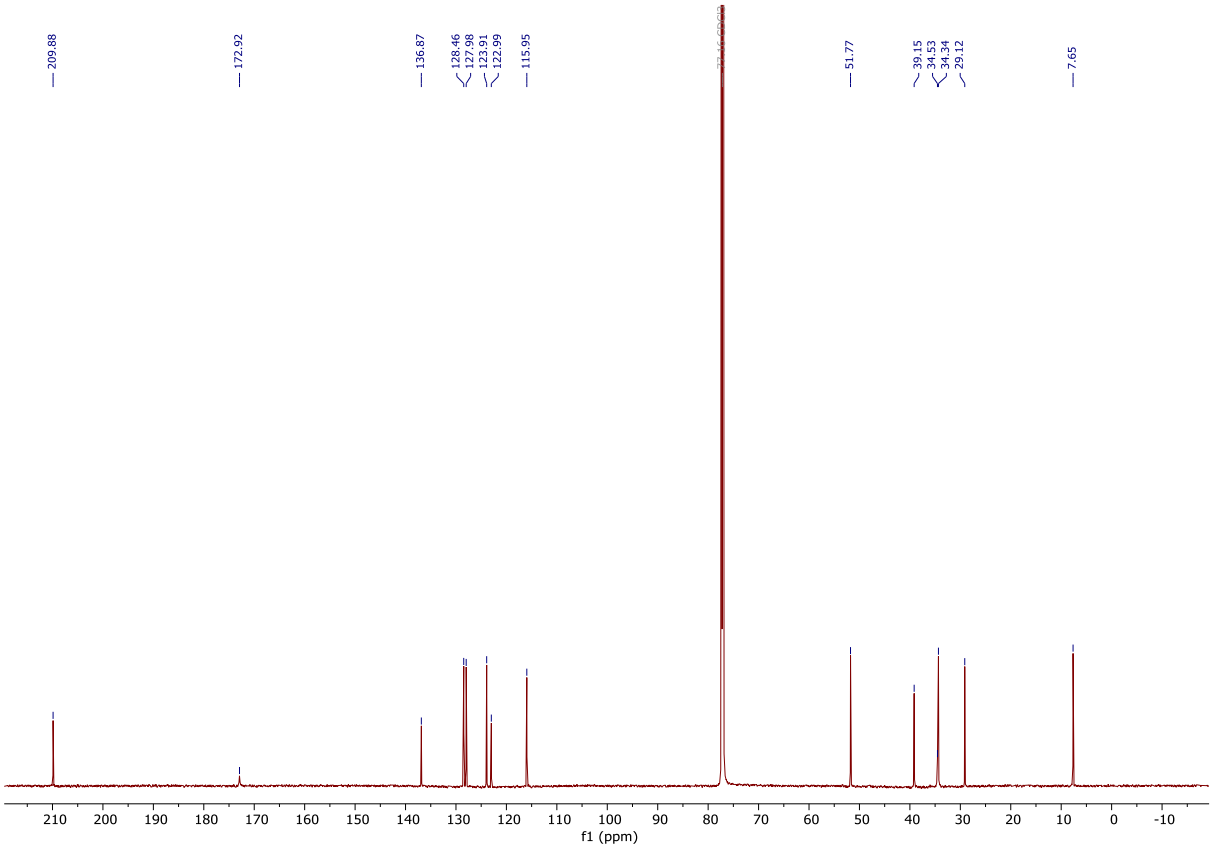

207.10
172.93
136.98
128.49
127.96
123.85
122.83
116.01
77.16 CDCl3
52.78
39.19
34.31
28.84
28.16
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)



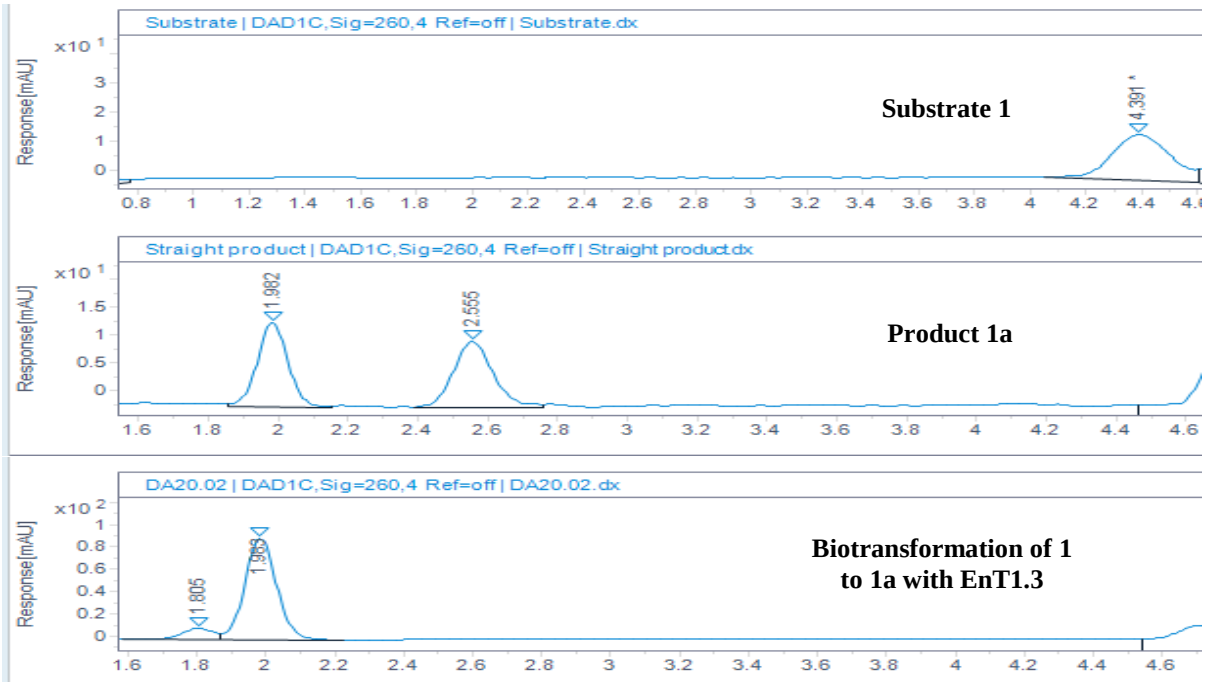


15


0.82
1.00
0.97
0.95
0.94
0.94
0.96
0.96
0.98
1.00
2.08
3.00
f1 (ppm)

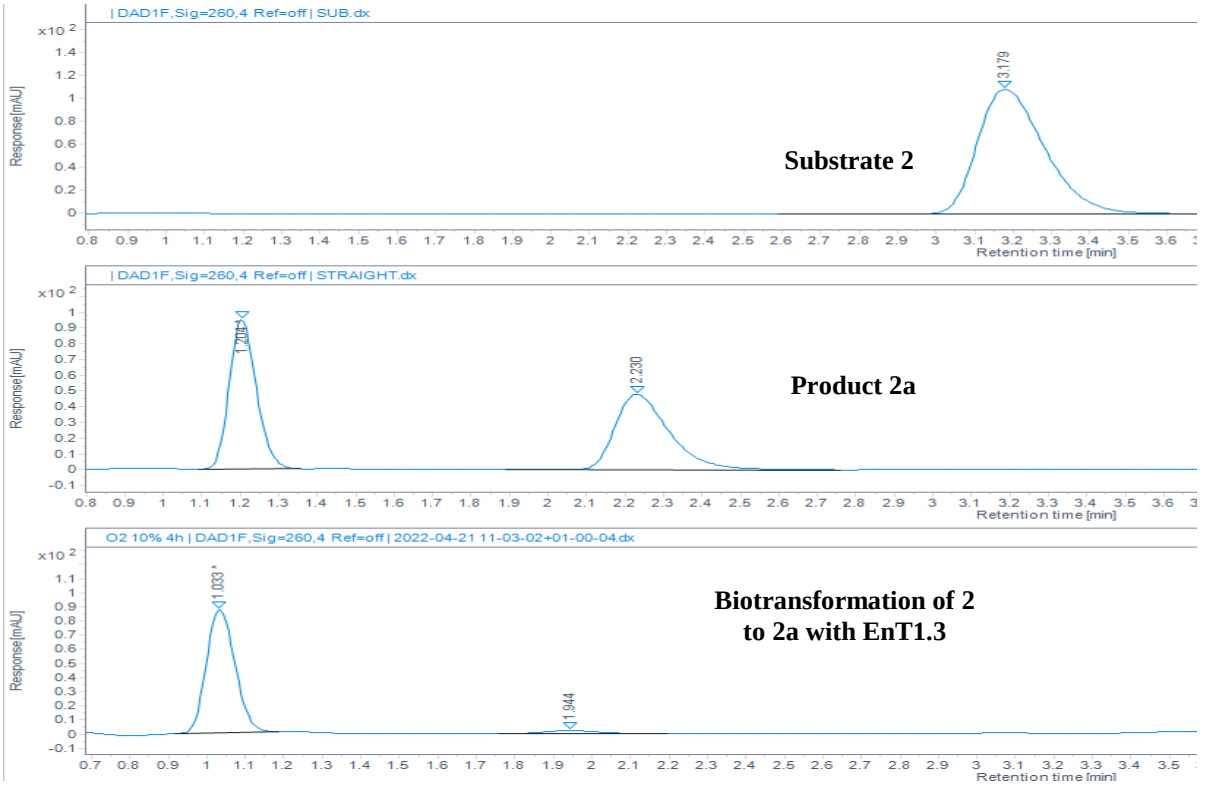

209.88
172.92
136.87
128.46
127.98
123.91
122.99
115.95
77.16
51.77
39.15
34.53
34.34
29.12
7.65
f1 (ppm)

Substrate 1



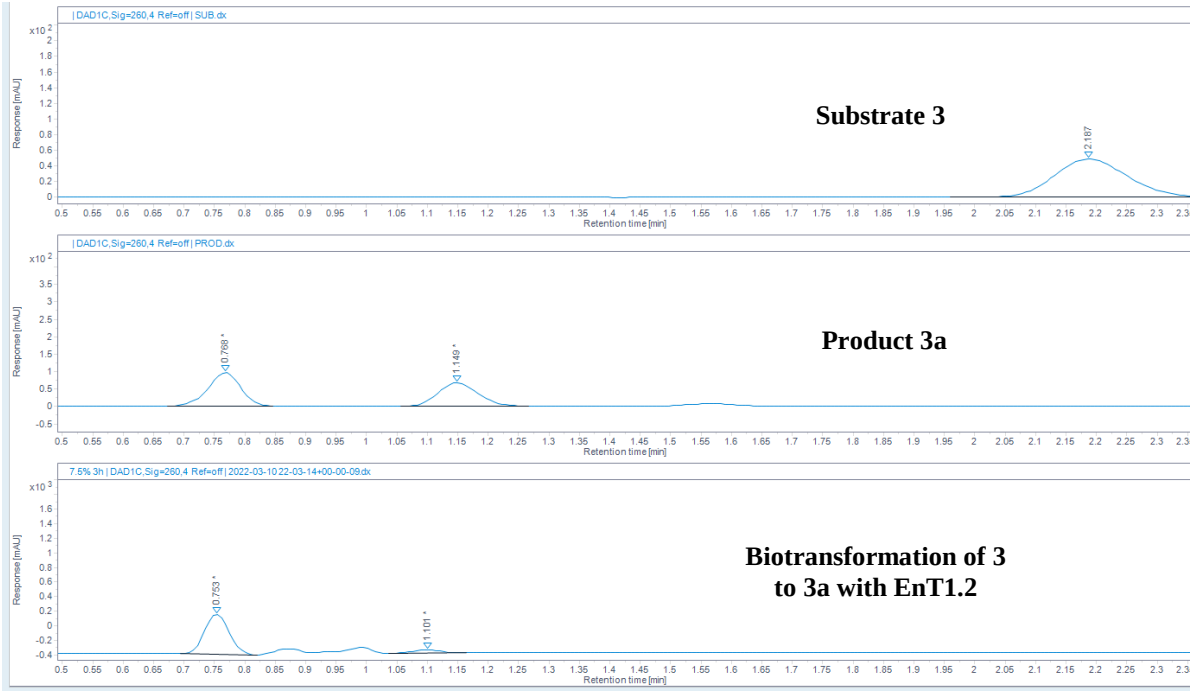
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=75:25)= **1**= 4.4 min; (-)-**1a**= 1.9 min; (+)-**1a**=2.5 min. (Note: the peak at 1.8 min is one enantiomer of product **1b**).

Substrate 2



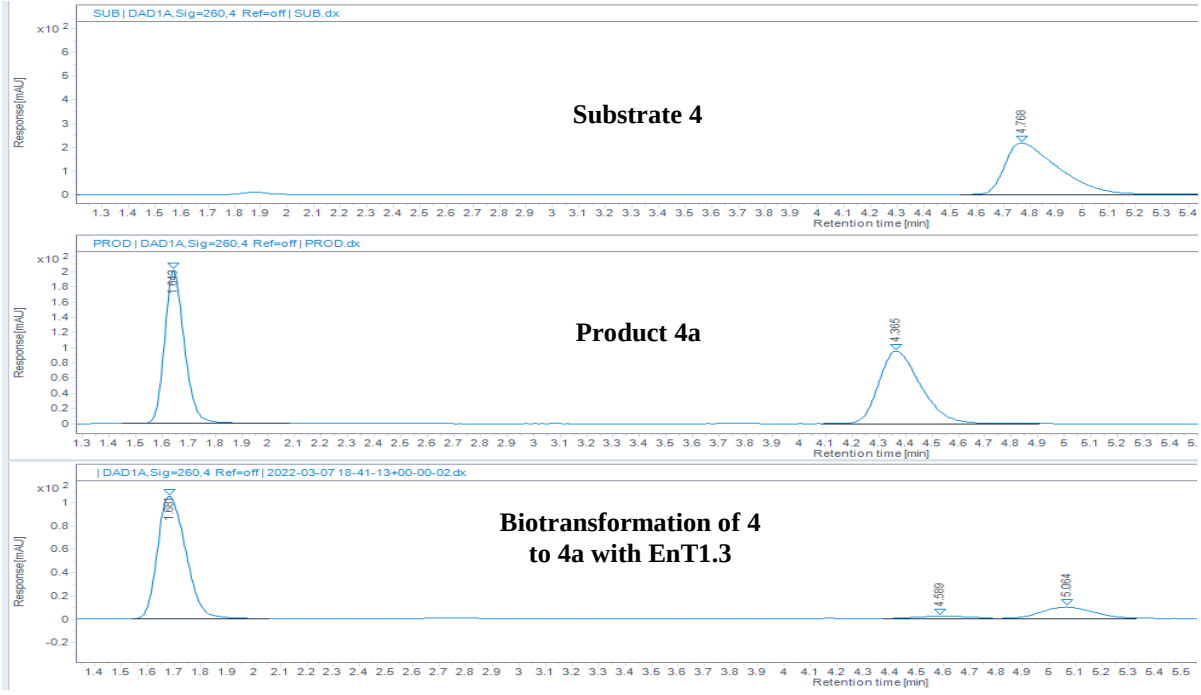
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=50:50)= **2**= 3.1 min; (-)-**2a**= 1.0 min; (+)-**2a**=1.9 min.

Substrate 3



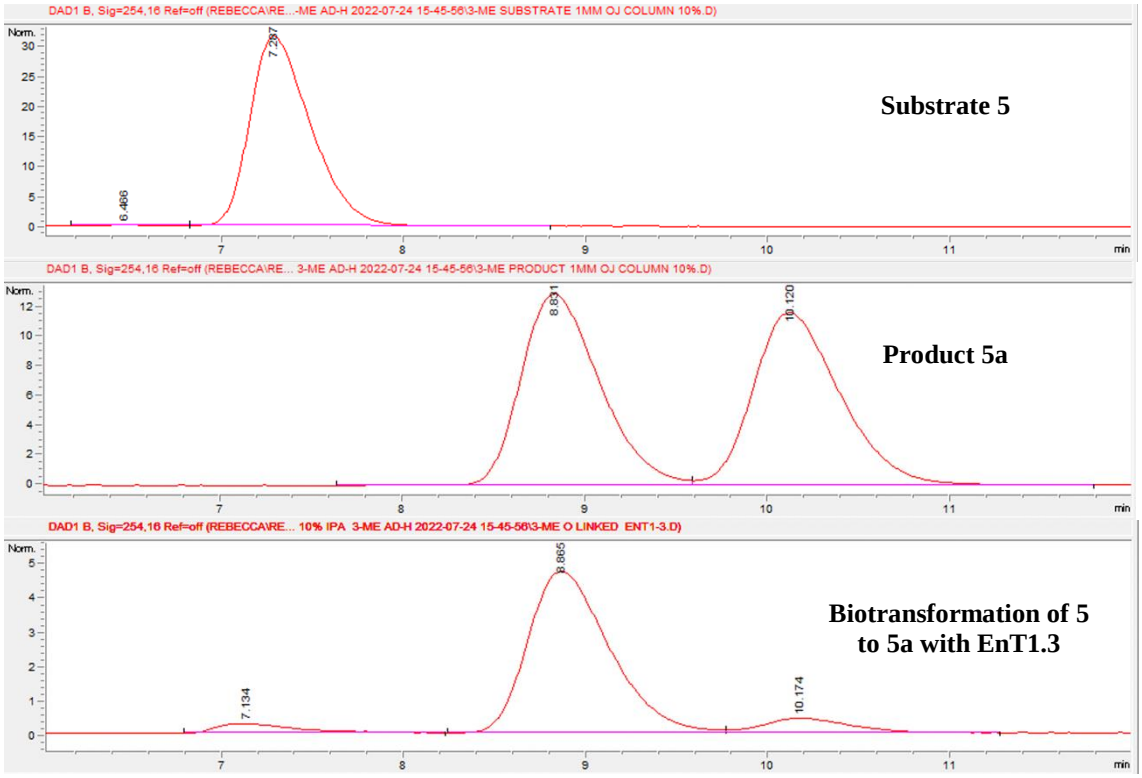
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=50:50)= **3**= 2.1 min; (-)-**3a**= 0.7 min; (+)-**3a**=1.1 min. With this substrate there are minor additional impurity peaks observed between 0.8-1 min that arise from a non-enzymatic background reaction.

Substrate 4



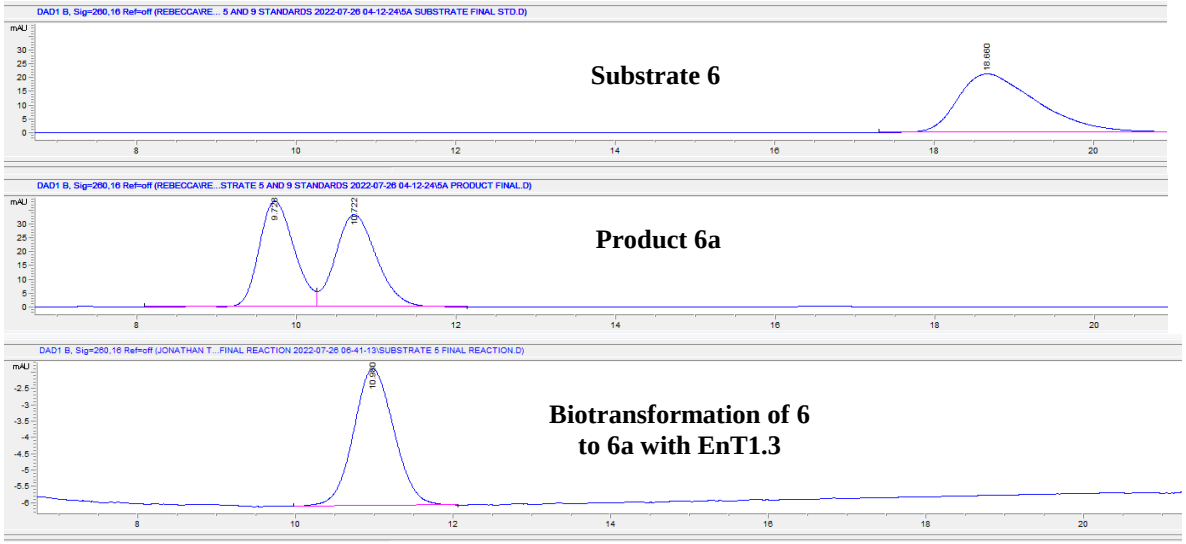
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=75:25)= **4**= 4.7 min; (-)-**4a**= 1.6 min; (+)-**4a**=4.5 min.

Substrate 5



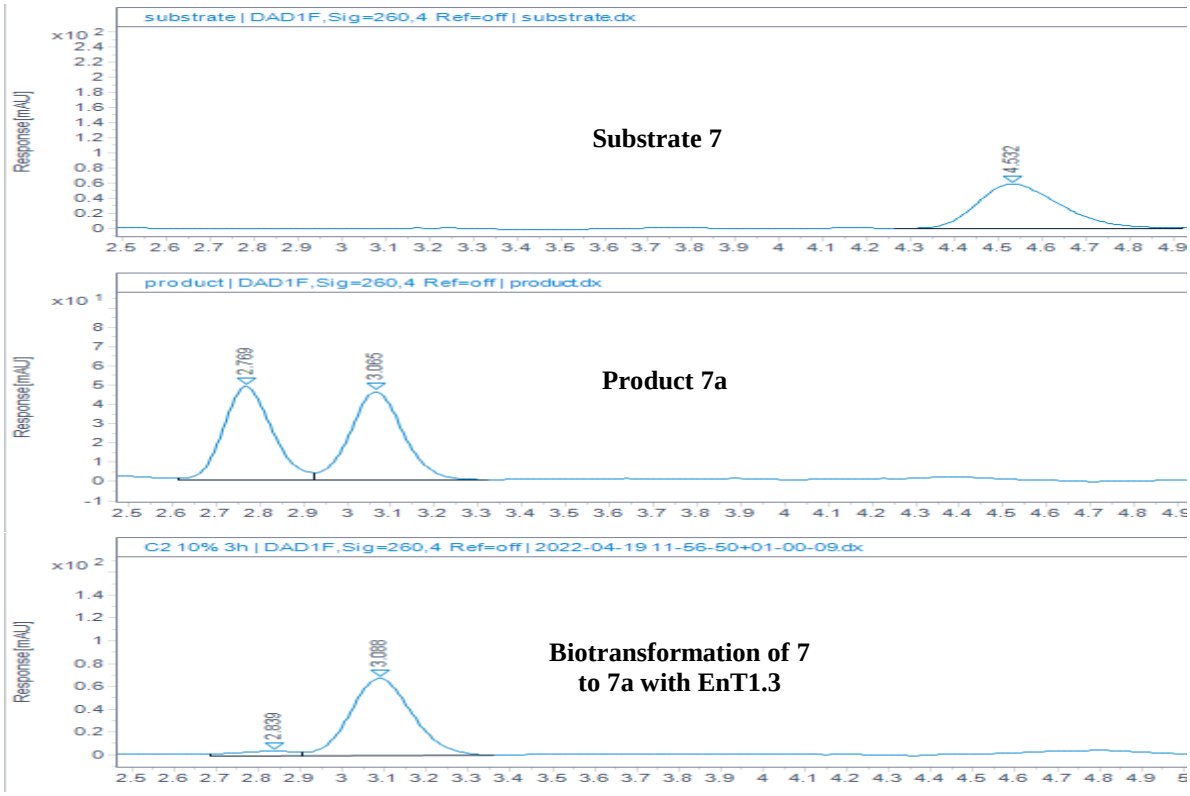
HPLC (AD-H, 0.46cm x 25cm, n-hexane/iso-propanol=90:10)= **5**= 7.1 min; (-)-**5a**= 8.9 min; (+)-**5a**=10.2 min.

Substrate 6



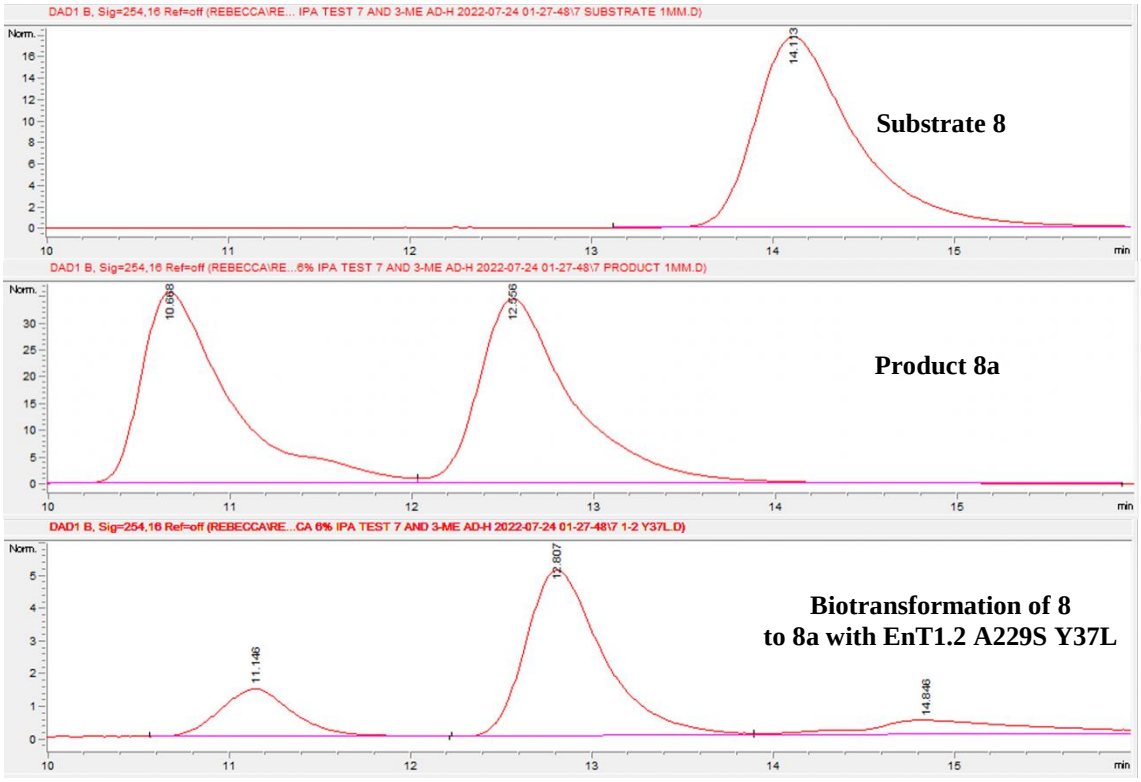
HPLC (IG-3 SFC column, 3 mm x 50 mm, n-hexane/iso-propanol=98:2)= **6**= 18.6 min; (-)-**6a**= 10.7 min; (+)-**6a**= 9.7 min.

Substrate 7



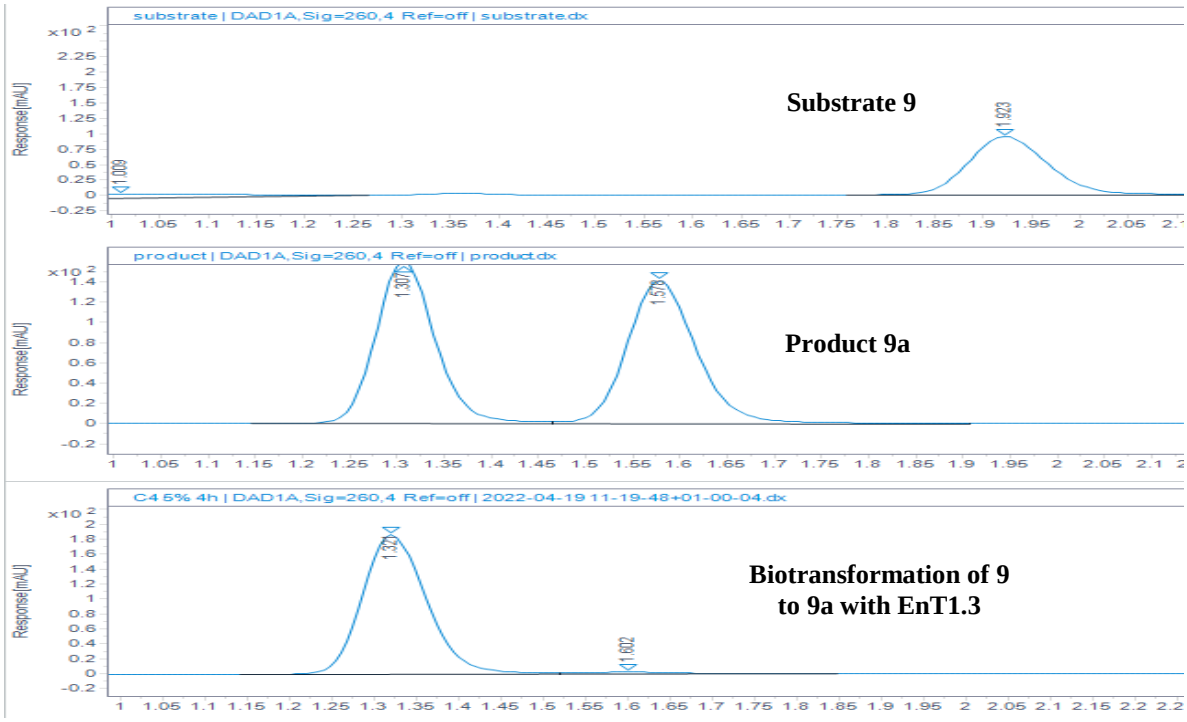
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=80:20)= **7**= 4.5 min; (-)-**7a**= 3.0 min; (+)-**7a**=2.8 min.

Substrate 8



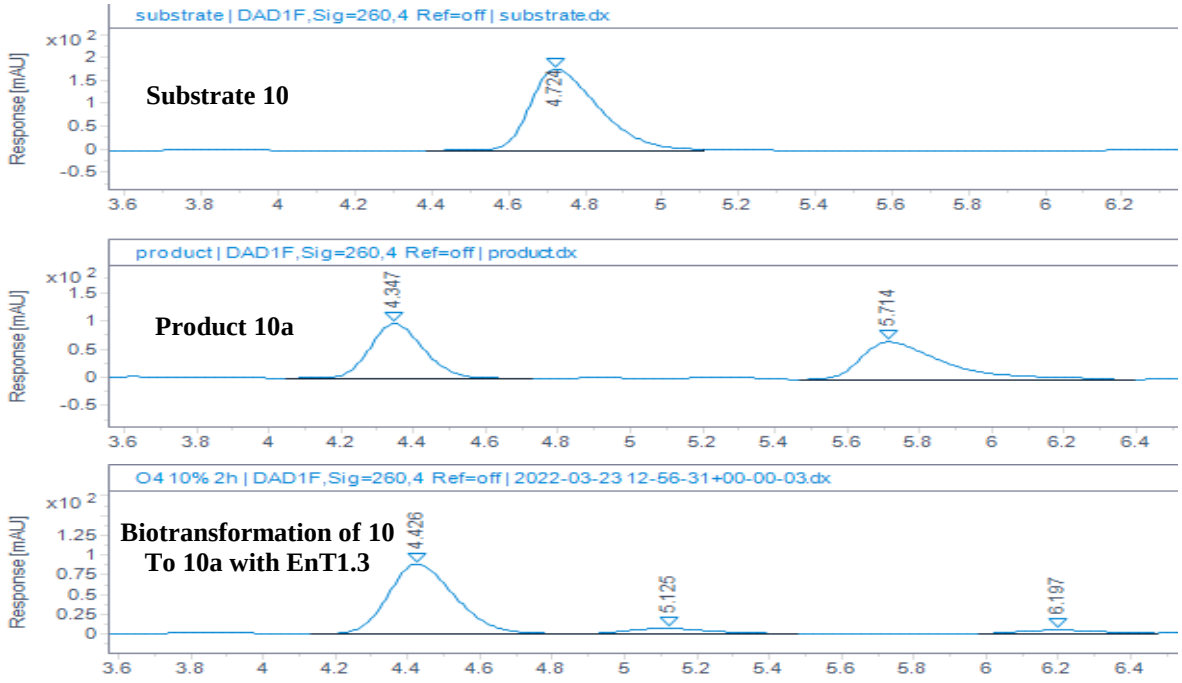
HPLC (AD-H, 0.46cm x 25cm, n-hexane/iso-propanol=94:6)= **7**= 14.8 min; (-)-**7**= 12.8 min; (+)-**7**=11.1 min.

Substrate 9



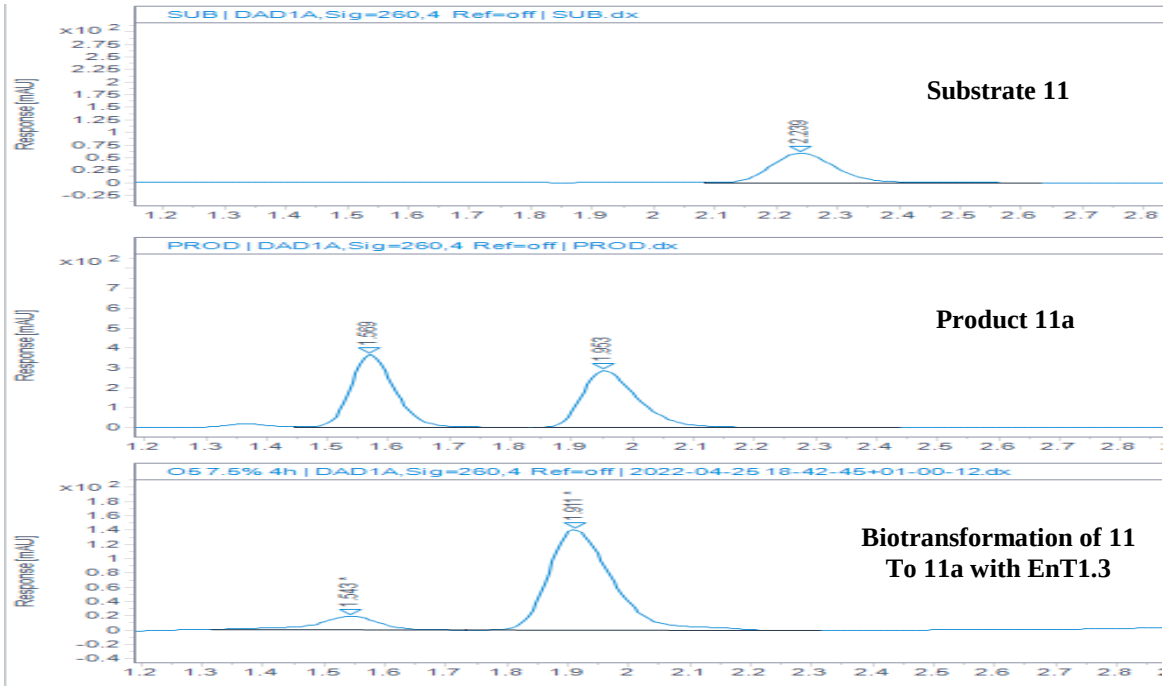
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=70:30)= **9**= 1.9 min; (-)-**9a**= 1.3 min; (+)-**9a**=1.6 min.

Substrate 10



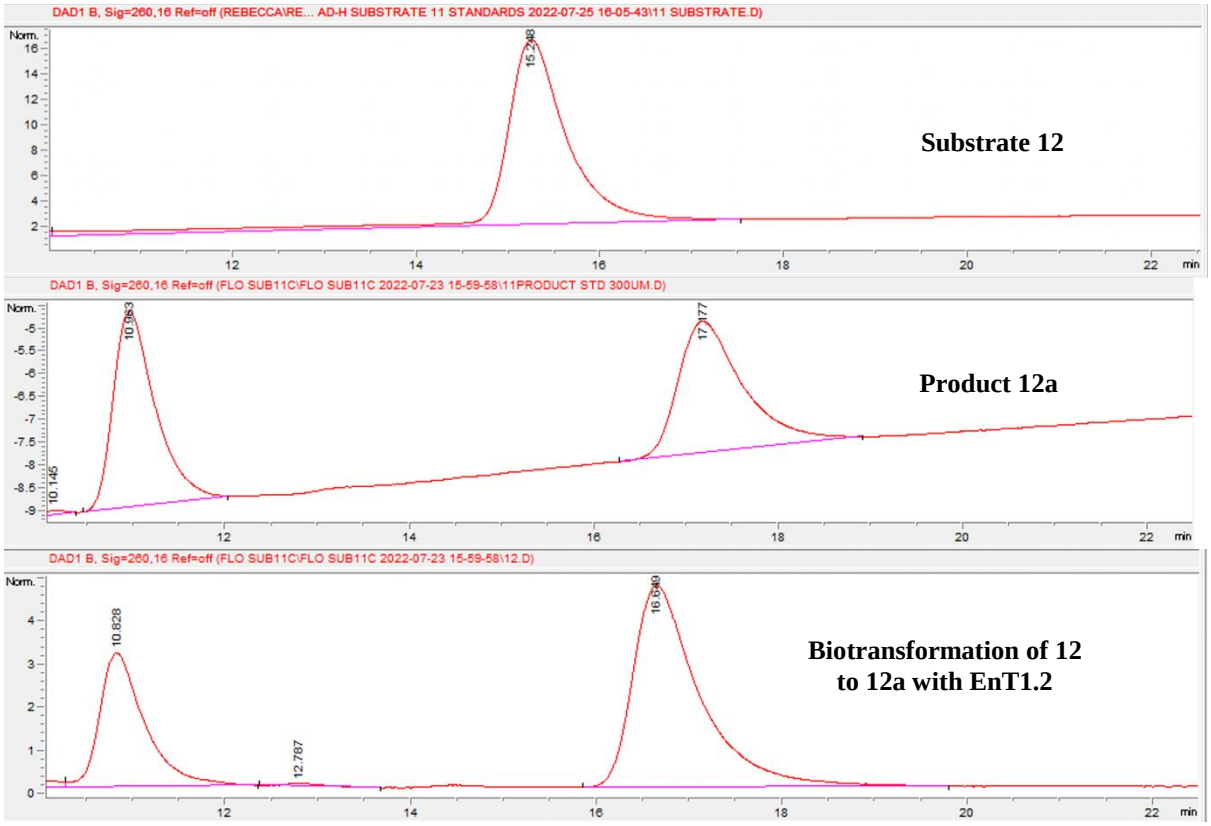
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=85:15)= **10**= 4.7 min; (-)-**10a**= 4.4 min; (+)-**10a**=5.7 min.

Substrate 11



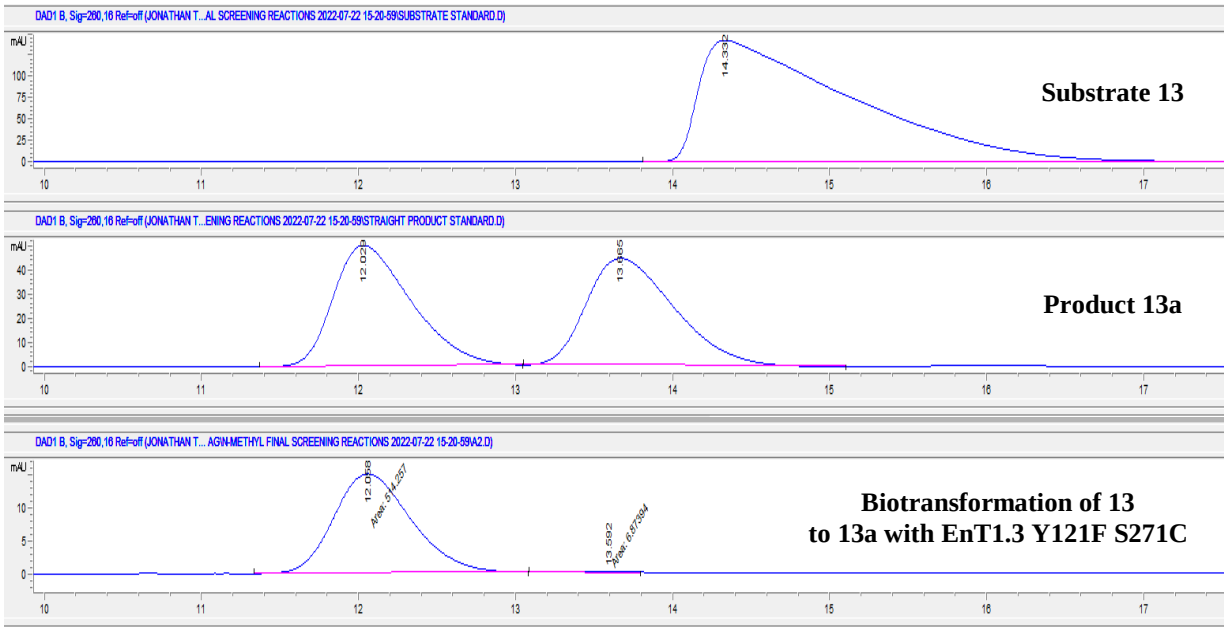
HPLC (IG-3 SFC column, 3 mm x 50 mm, CO_2/methanol=70:30)= **11**= 2.2 min; (-)-**11a**= 1.9 min; (+)-**11a**=1.5 min.

Substrate 12



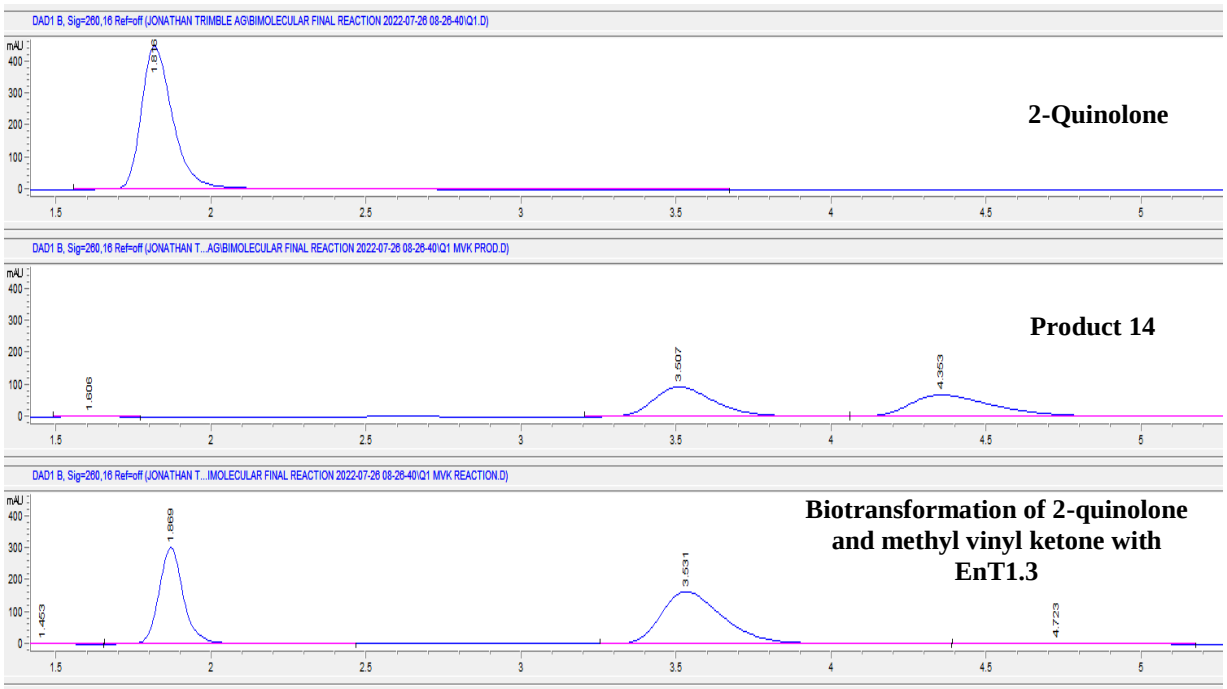
HPLC (AD-H, 0.46cm x 25cm, n-hexane/iso-propanol=90:10)= **12**= 15.2 min; (-)-**12**= 17 min; (+)-**12**=10.9 min.

Substrate 13



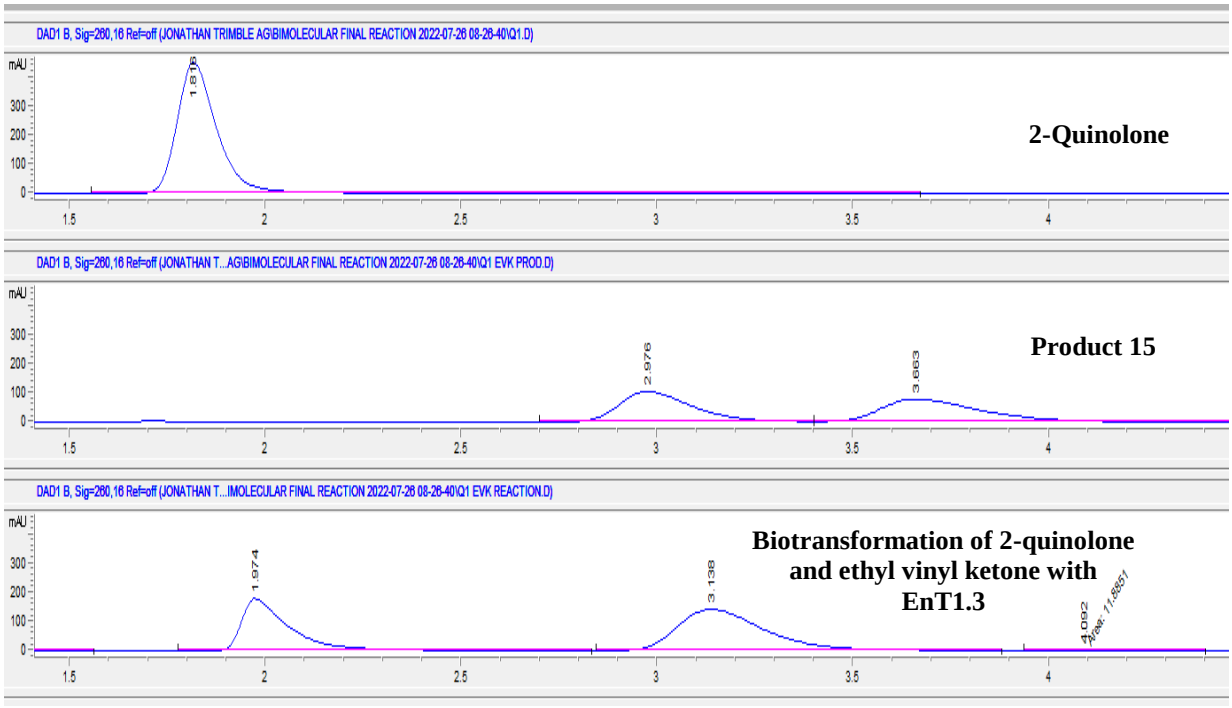
HPLC (IG-3 SFC column, 3 mm x 50 mm, n-hexane/iso-propanol=98:2)= **13**= 14.3 min; (-)-**13a**= 12.0 min; (+)-**13a**= 13.6 min.

Substrate 14



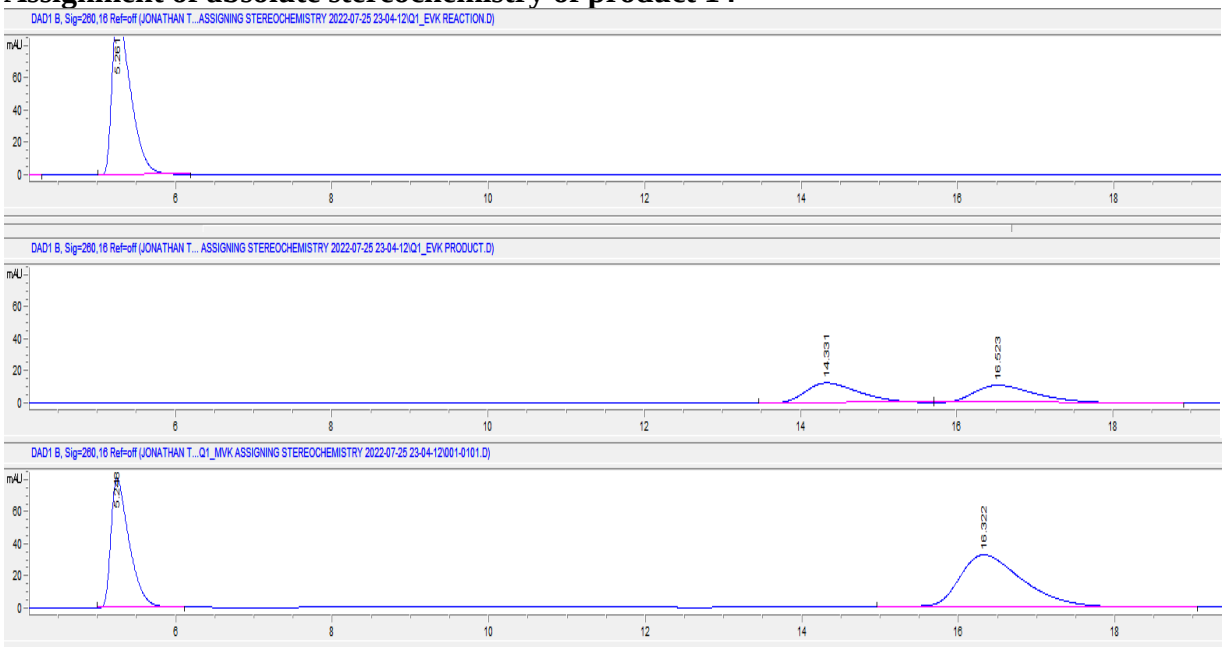
HPLC (IG-3 SFC column, 3 mm x 50 mm, n-hexane/iso-propanol=90:10)= **2-quinolone**= 1.8 min; (-)-**14**= 3.5 min; (+)-**14**= 4.4 min.

Substrate 15



HPLC (IG-3 SFC column, 3 mm x 50 mm, n-hexane/iso-propanol=90:10)= **2-quinolone**= 1.9 min; (-)-**15**= 3.1 min; (+)-**15**= 4.0 min.

Assignment of absolute stereochemistry of product 14



HPLC (OJ-H, 0.46cm x 25cm, n-hexane/iso-propanol=80:20)= **2-quinolone**= 5.2 min; (-)-**14**= 16.3 min; (+)-**14**=14.3 min. This HPLC method is identical to that described in reference 37 in order to assign the absolute stereochemistry of product **14** based on comparison of retention times.