Synthetic Access to Non-Canonical Strigolactones – Syntheses of Carlactonic Acid and Methyl Carlactonoate

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Supporting Information

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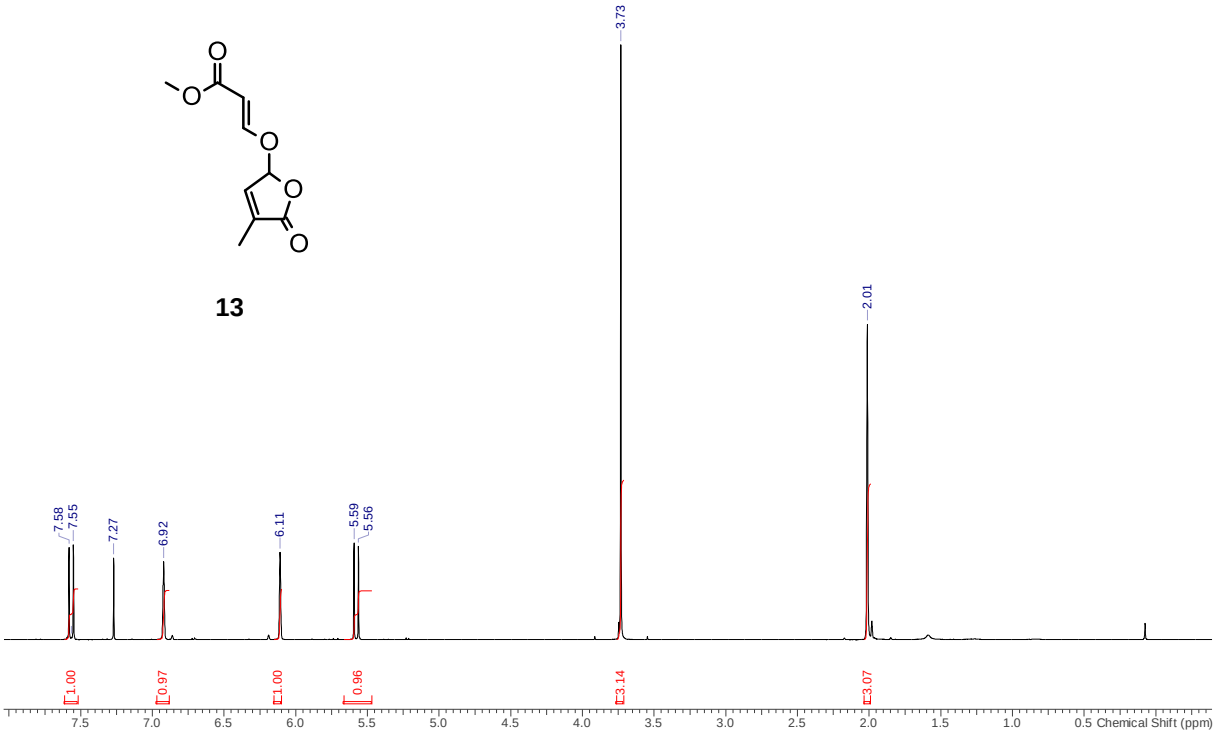
1. ^{1}H- and ^{13}C-NMR spectra



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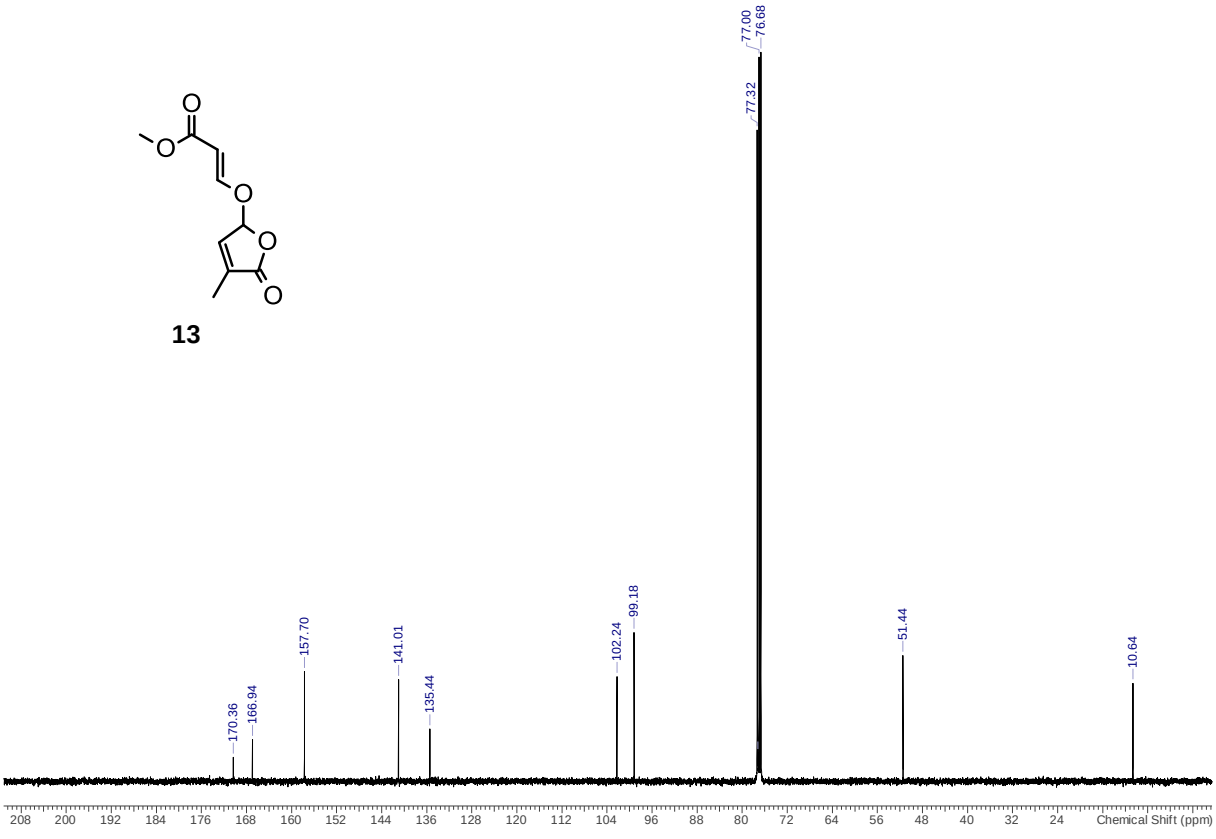

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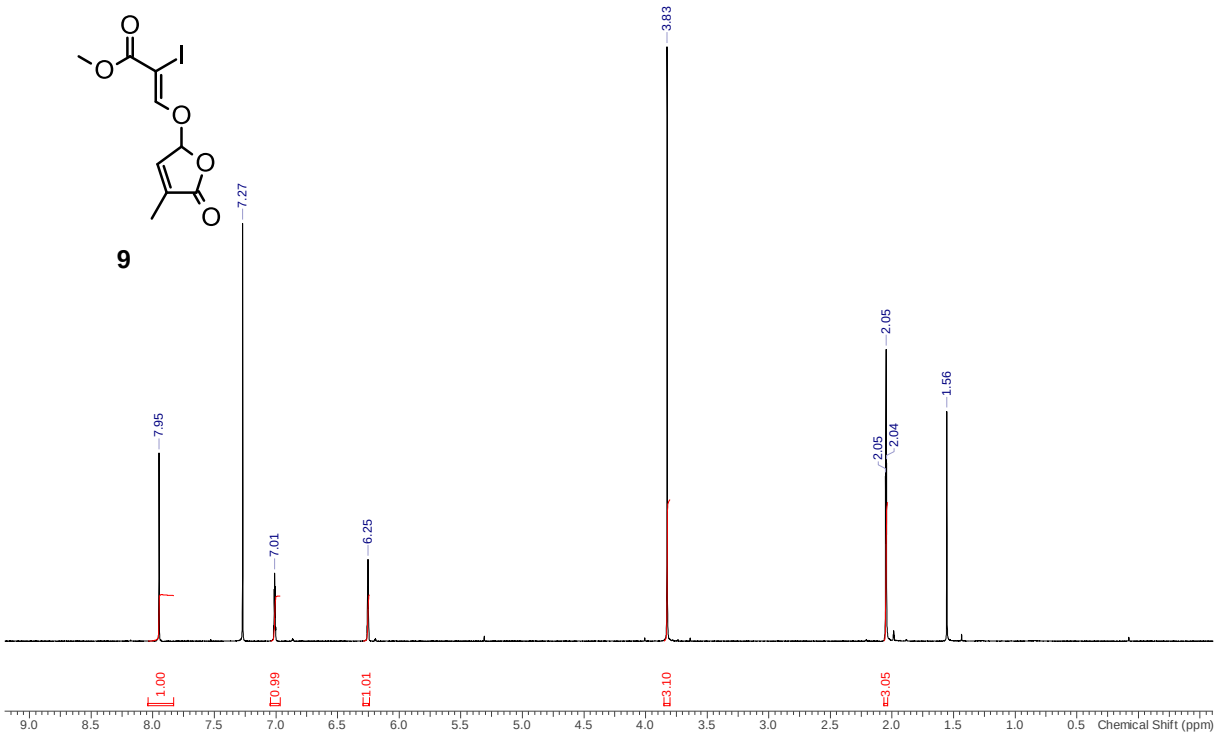
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2.05
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7.95
2.05
2.04
7.01
6.25
1.00
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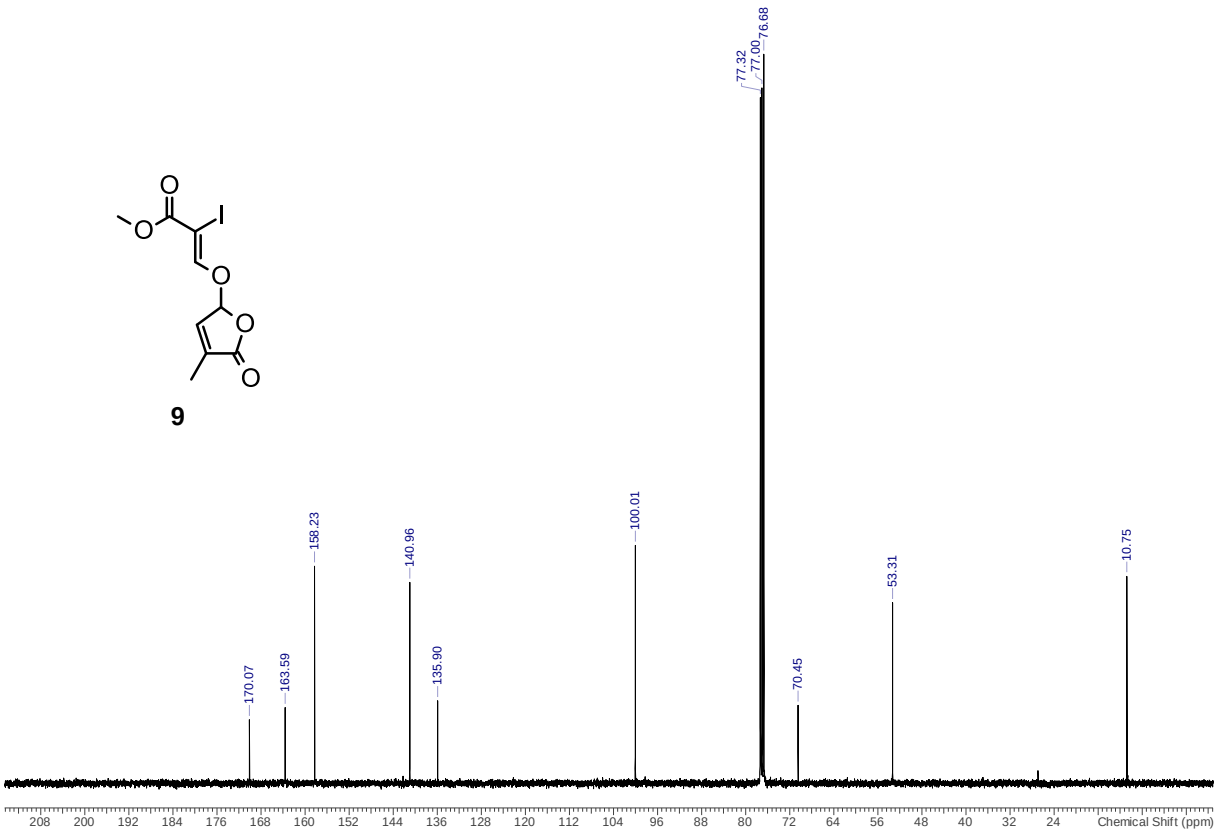
1.01
3.10
3.05
Chemical Shift (ppm)


9
77.32
77.00
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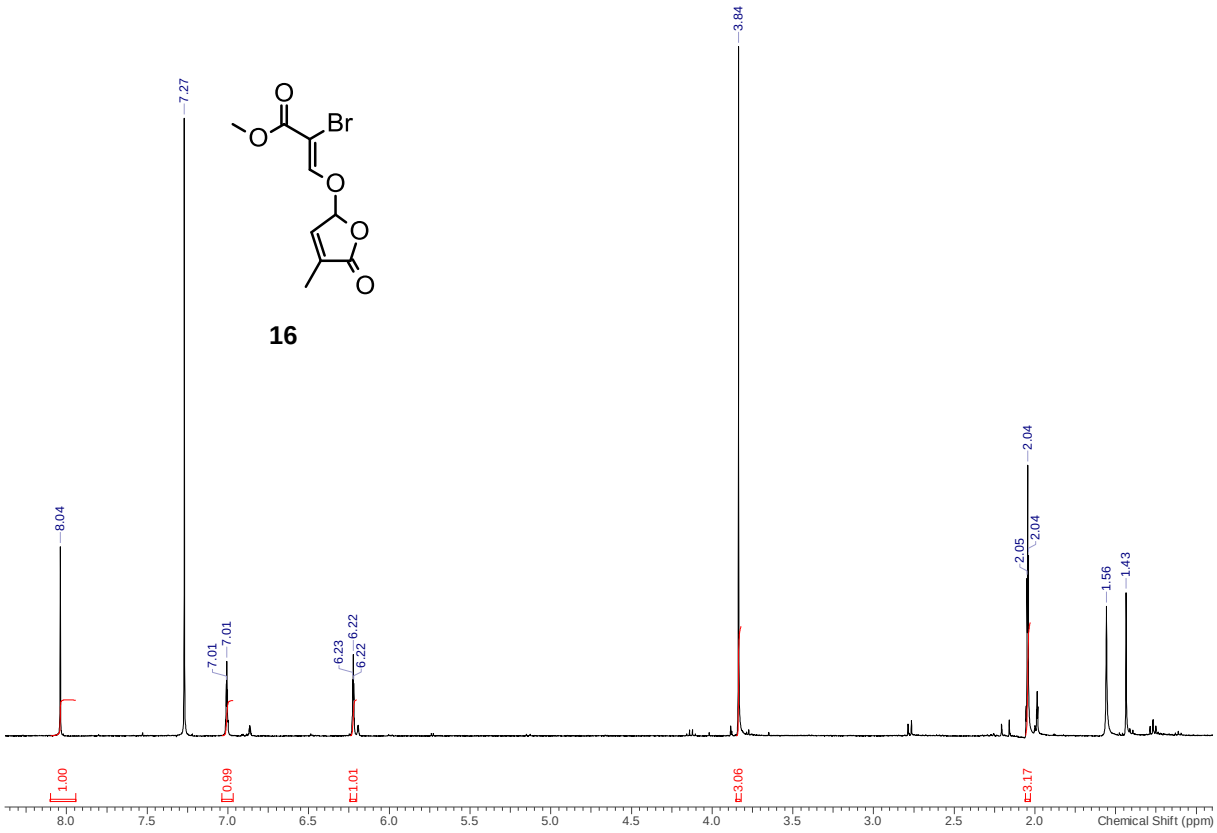
10.75
170.07
163.59
135.90
70.45
Chemical Shift (ppm)


O
Br
O
O
O
O
16
8.04
7.27
7.01
7.01
6.23
6.22
3.84
2.04
2.05
2.04
1.56
1.43
1.00
0.99
1.01
3.06
3.17
8.0
7.5
7.0
6.5
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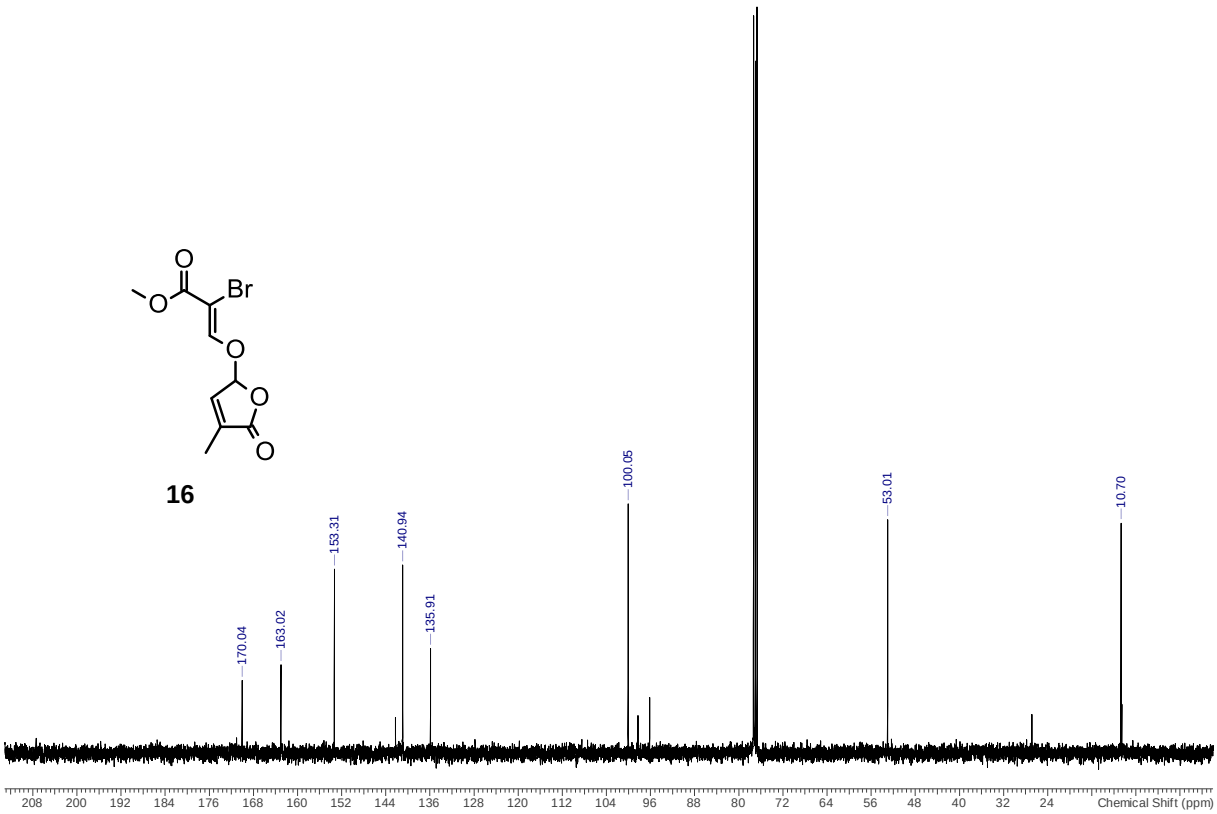
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)


O
Br
O
O
O
O
16
170.04
163.02
153.31
140.94
135.91
100.05
53.01
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208
200
192
184
176
168
160
152
144
136
128
120
112
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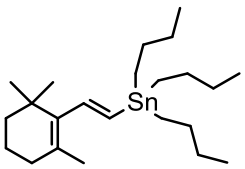
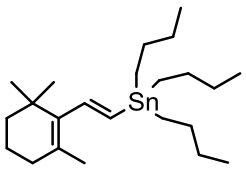
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)


Sn

10

1.00
0.89
0.91
0.87
1.67
1.67
1.54
1.52
1.33
1.31
1.43
1.30
1.28
1.07
1.56
1.62
1.71
1.99
1.98
1.96
0.86
0.85
0.85
0.83
0.18
0.16
6.39
6.38
6.34
6.33
5.89
5.84
5.32
5.32
5.32
1.00
0.97
2.11
3.05
2.28
6.50
2.24
8.18
6.02
17.48
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
0.5
Chemical Shift (ppm)


Sn

10

54.00
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53.72
54.54
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27.84
14.08
10.07
40.01
34.18
33.13
21.85
19.89
27.49
146.52
141.91
132.94
127.95
208
200
192
184
176
168
160
152
144
136
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120
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104
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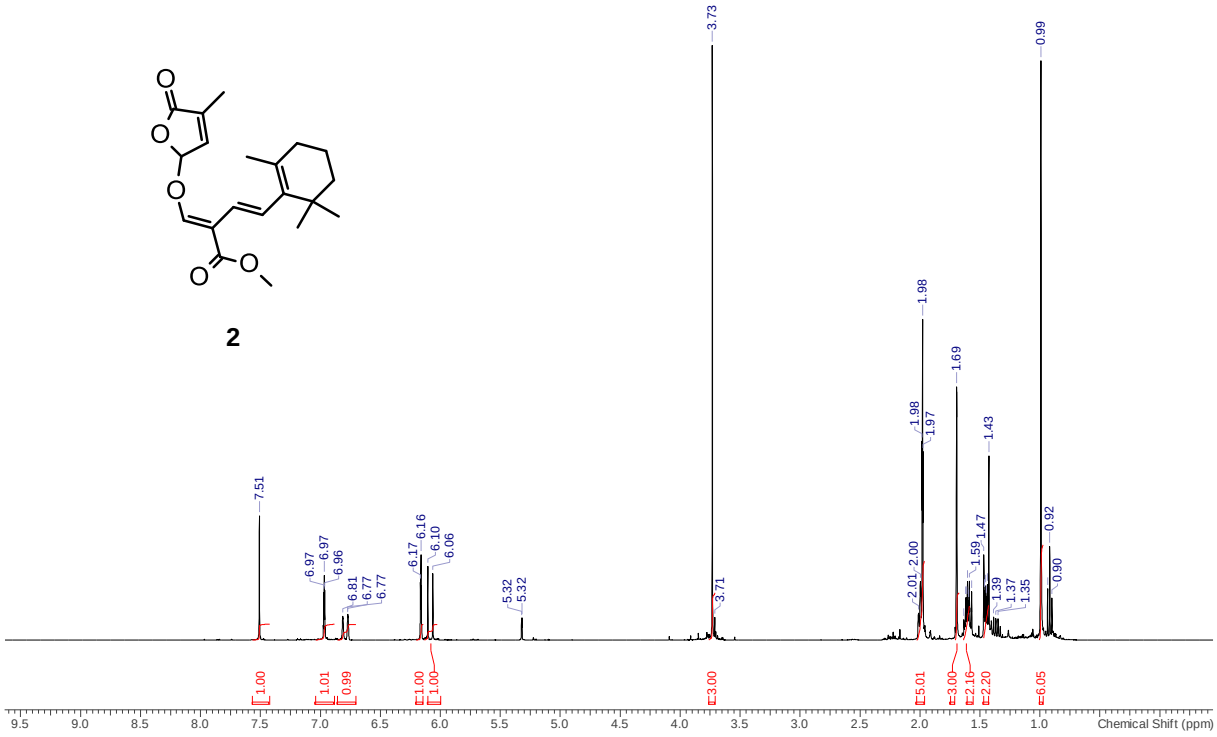
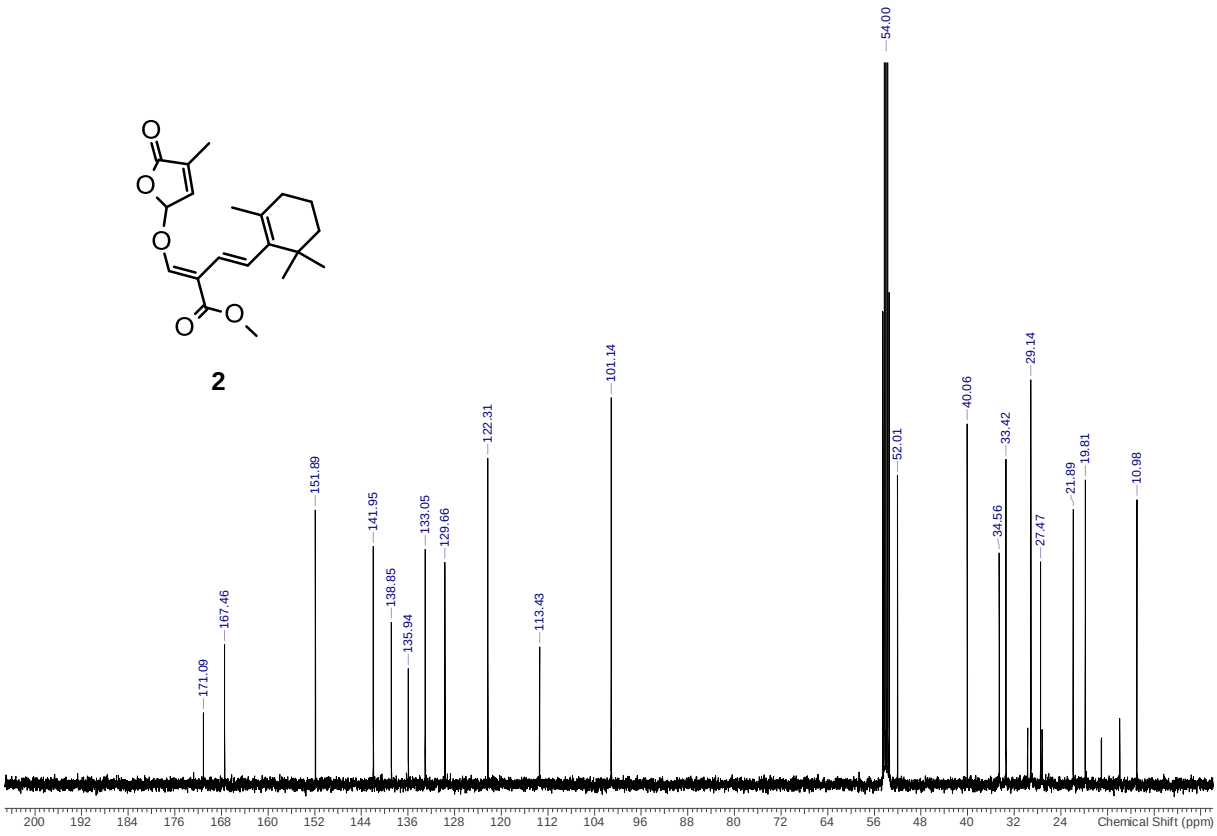
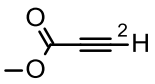
88
80
72
64
56
48
40
32
24
16
Chemical Shift (ppm)


2
7.51
6.97
6.97
6.96
6.81
6.77
6.77
6.17
6.16
6.10
6.06
5.32
5.32
3.73
3.71
2.01
2.00
1.98
1.97
1.69
1.59
1.47
1.43
1.39
1.37
1.35
0.99
0.92
0.90
1.00
1.01
0.99
1.00
1.00
3.00
5.01
3.00
2.16
2.20
6.05
Chemical Shift (ppm)

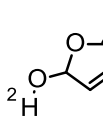

2
171.09
167.46
151.89
141.95
138.85
135.94
133.05
129.66
122.31
113.43
101.14
54.00
52.01
40.06
34.56
33.42
29.14
27.47
21.89
19.81
10.98
Chemical Shift (ppm)



19

97.5% deuteration

(0.55 eq DCM present)



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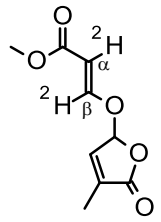
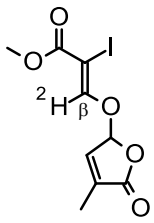
50% deuteration



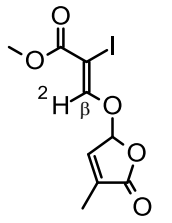
21

92% β-deuteration
78% α-deuteration



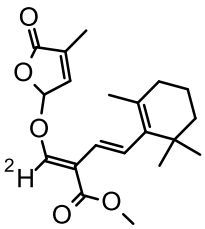
22

91% deuteration



22

91% deuteration



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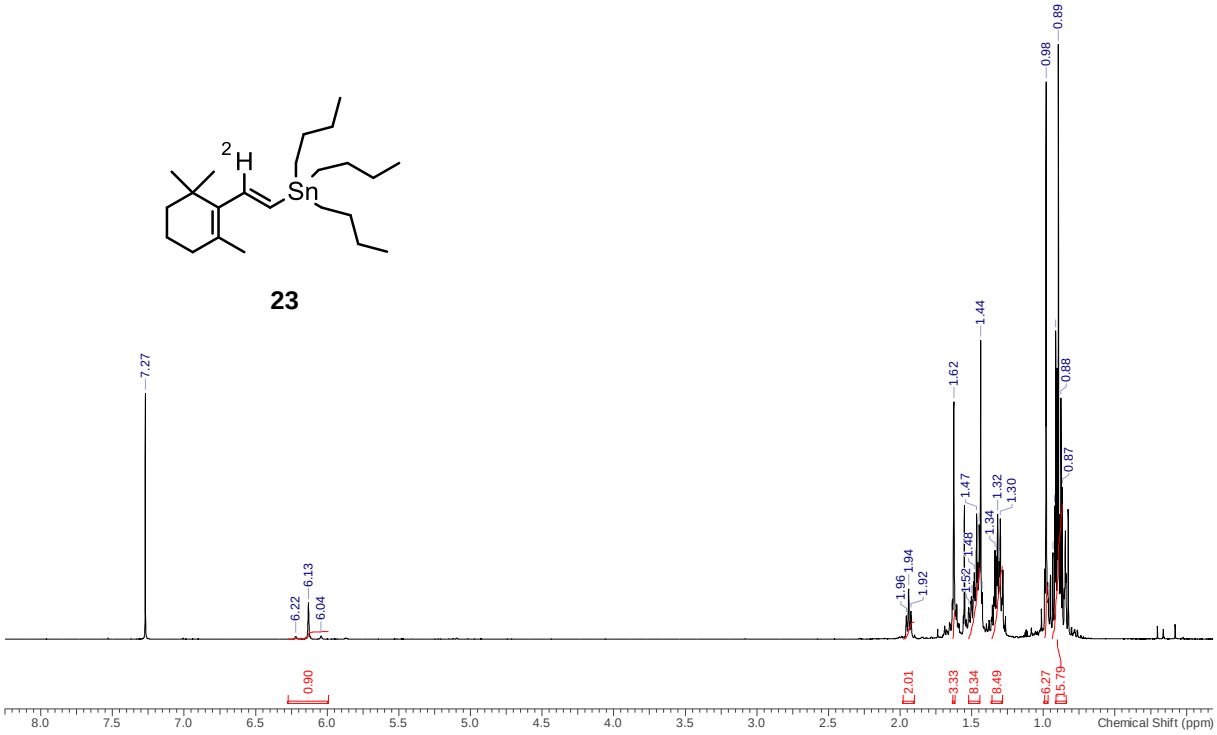
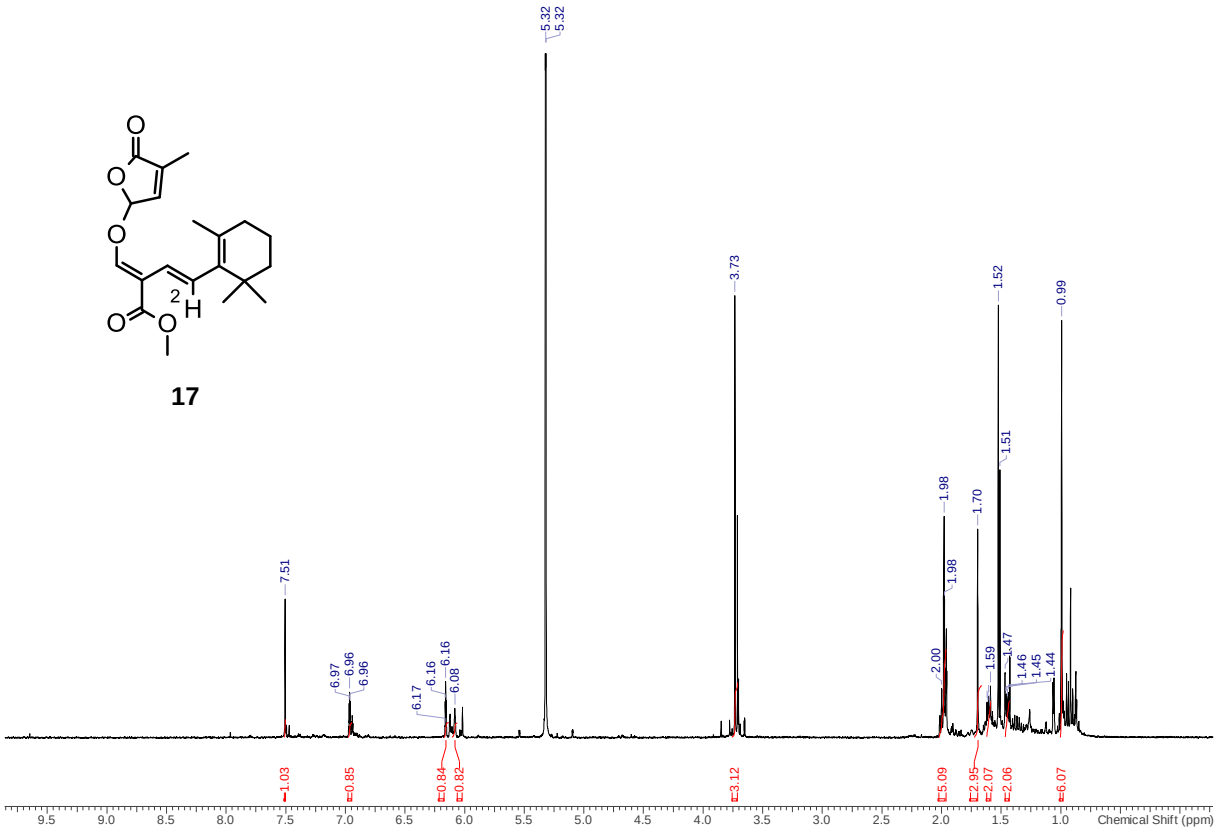
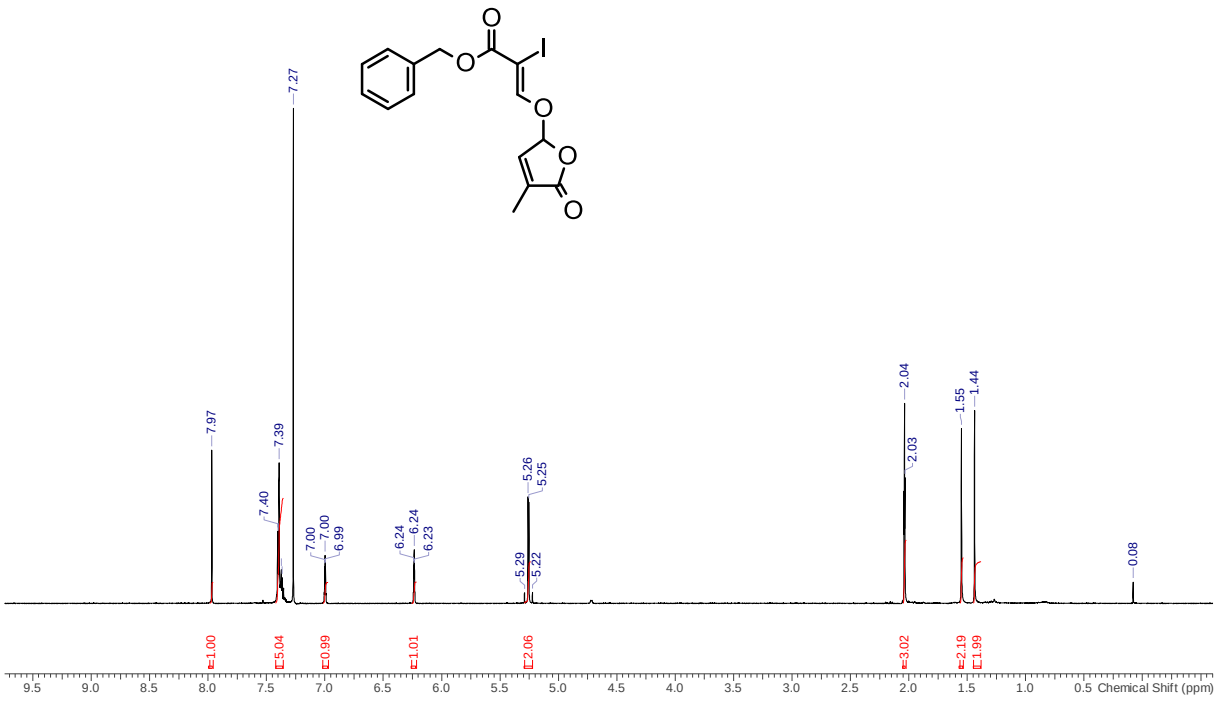
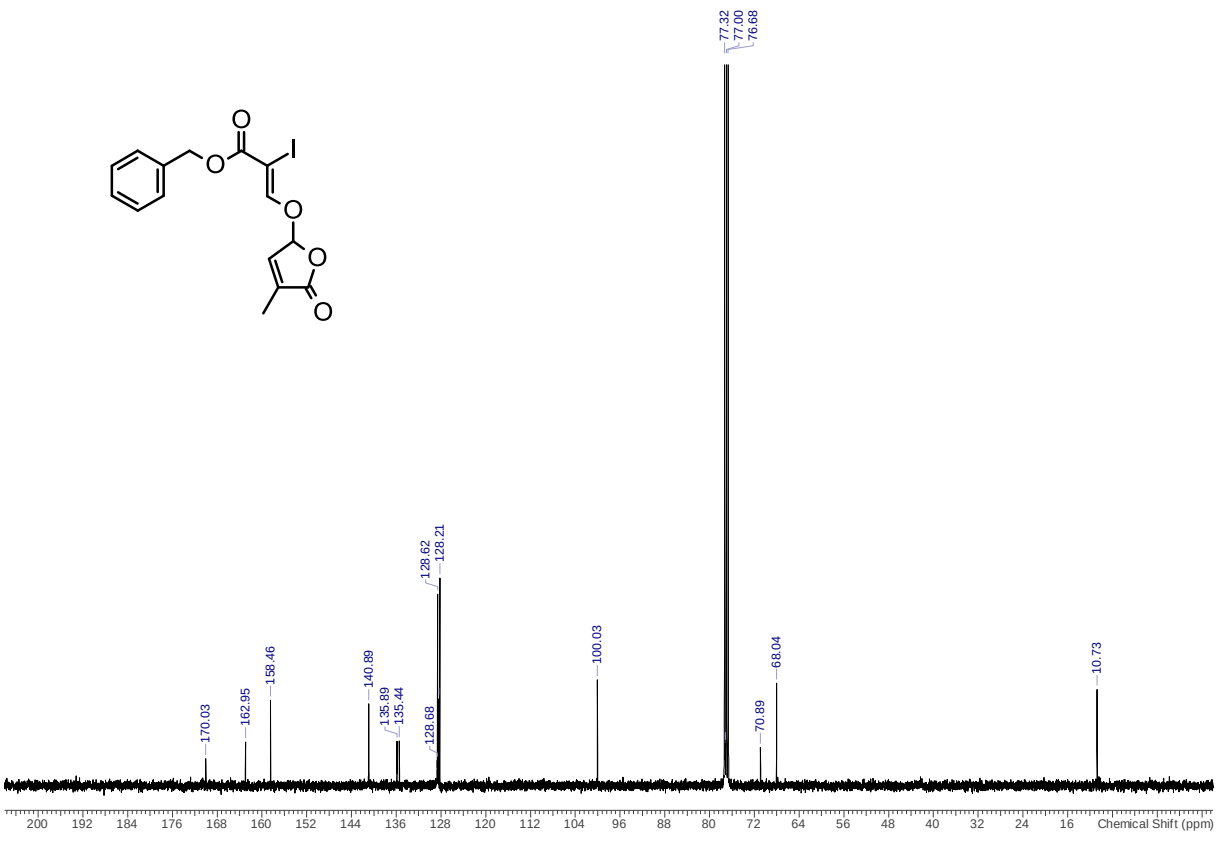

2
H
Sn
7.27
6.22
6.13
6.04
1.96
1.94
1.92
1.62
1.52
1.48
1.47
1.44
1.34
1.32
1.30
0.98
0.89
0.88
0.87
0.90
2.01
3.33
8.34
8.49
6.27
15.79
Chemical Shift (ppm)

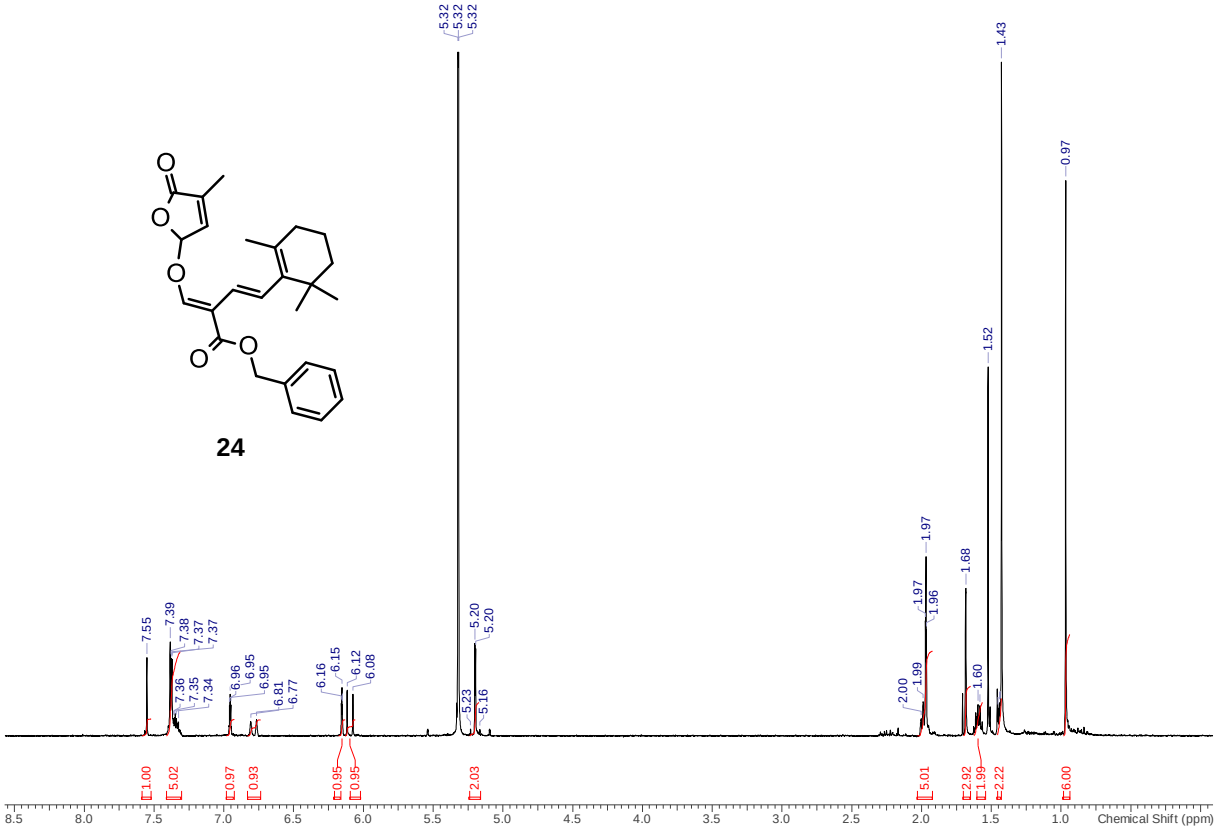
23

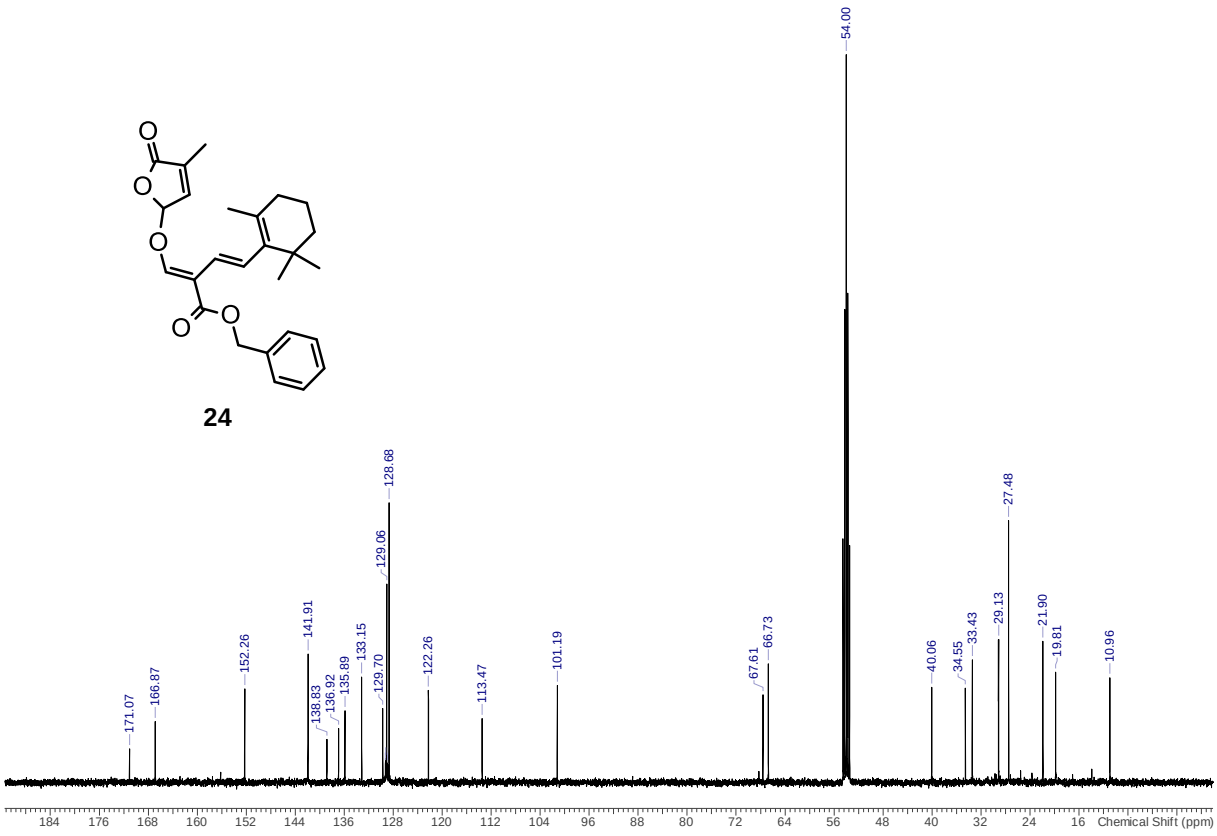

O
O
O
O
O
2
H
5.32
5.32
7.51
6.97
6.96
6.96
6.17
6.16
6.16
6.08
3.73
2.00
1.98
1.98
1.70
1.59
1.52
1.51
1.47
1.46
1.45
1.44
0.99
1.03
0.85
0.84
0.82
3.12
5.09
2.95
2.07
2.06
6.07
Chemical Shift (ppm)

17


7.97
7.39
7.27
7.40
7.00
7.00
6.99
6.24
6.24
6.23
5.29
5.26
5.25
5.22
2.04
2.03
1.55
1.44
0.08
1.00
5.04
0.99
1.01
2.06
3.02
2.19
1.99
Chemical Shift (ppm)


77.32
77.00
76.68
128.62
128.21
170.03
162.95
158.46
140.89
135.89
135.44
128.68
100.03
70.89
68.04
10.73
Chemical Shift (ppm)


24
5.32
5.32
5.32
1.43
0.97
1.52
1.97
1.68
1.97
1.96
7.55
7.39
7.38
7.37
7.37
7.36
7.35
7.34
6.96
6.95
6.95
6.81
6.77
6.16
6.15
6.12
6.08
5.20
5.20
5.23
5.16
2.00
1.99
1.60
1.00
5.02
0.97
0.93
0.95
0.95
2.03
5.01
2.92
1.99
2.22
6.00
Chemical Shift (ppm)


24
54.00
128.68
129.06
27.48
141.91
133.15
129.70
152.26
122.26
113.47
101.19
67.61
66.73
40.06
34.55
33.43
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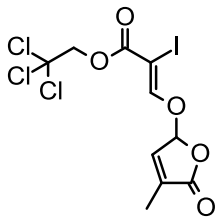
19.81
10.96
171.07
166.87
138.83
136.92
135.89
Chemical Shift (ppm)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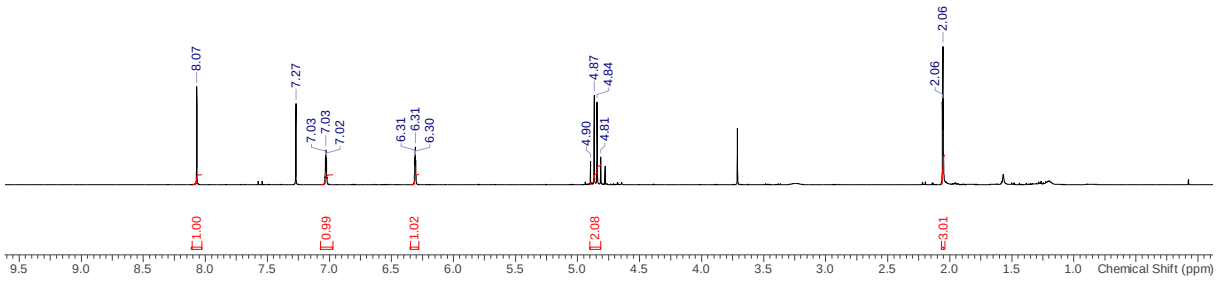
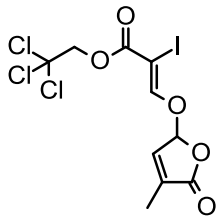
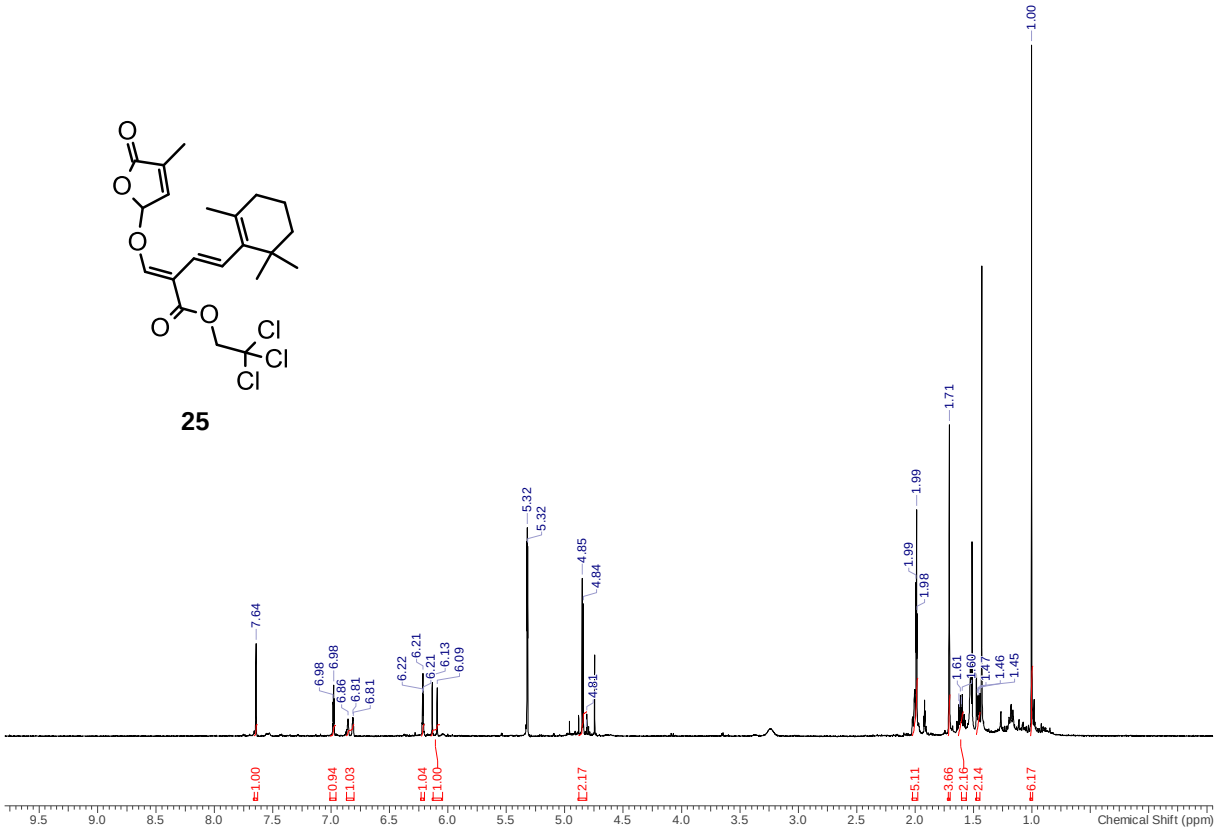
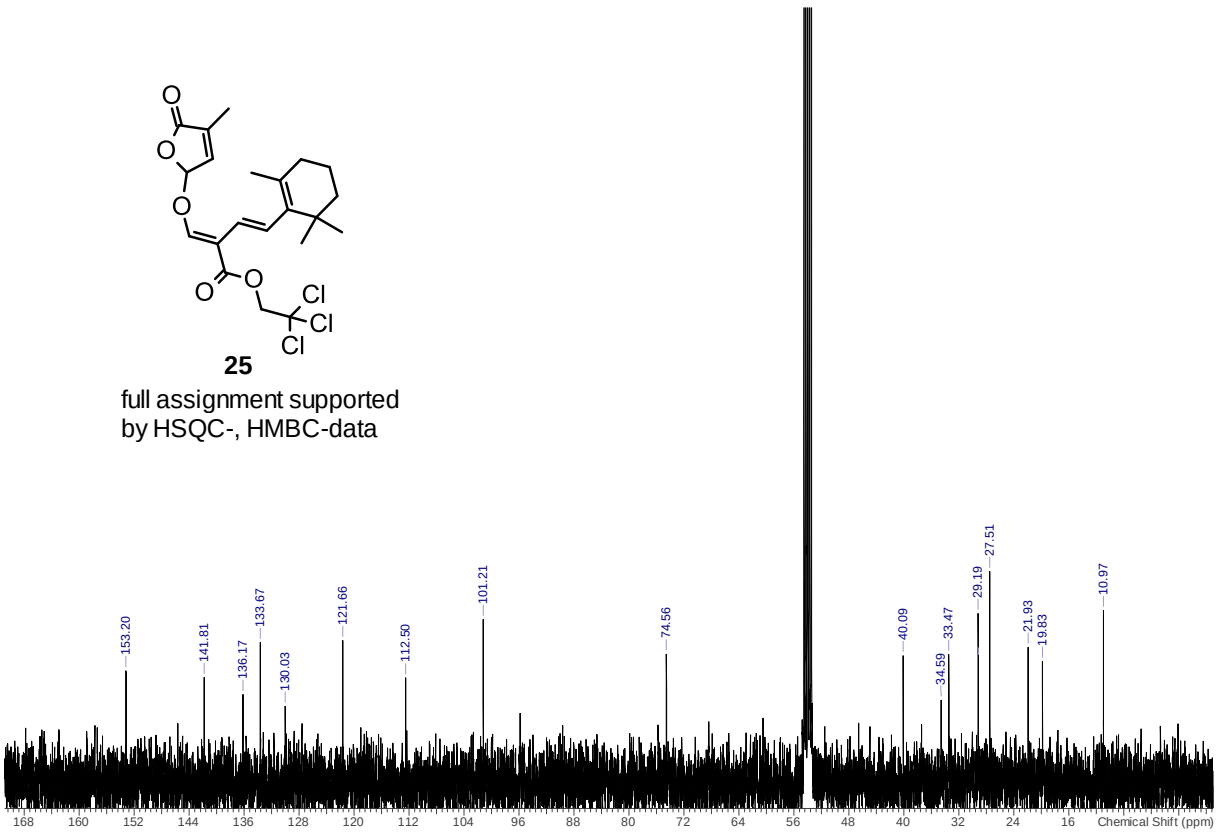
Cl
Cl
O
O
I
O
O
O


8.07
7.27
7.03
7.03
7.02
6.31
6.31
6.30
4.90
4.87
4.84
4.81
2.06
2.06
1.00
0.99
1.02
2.08
3.01
Chemical Shift (ppm)

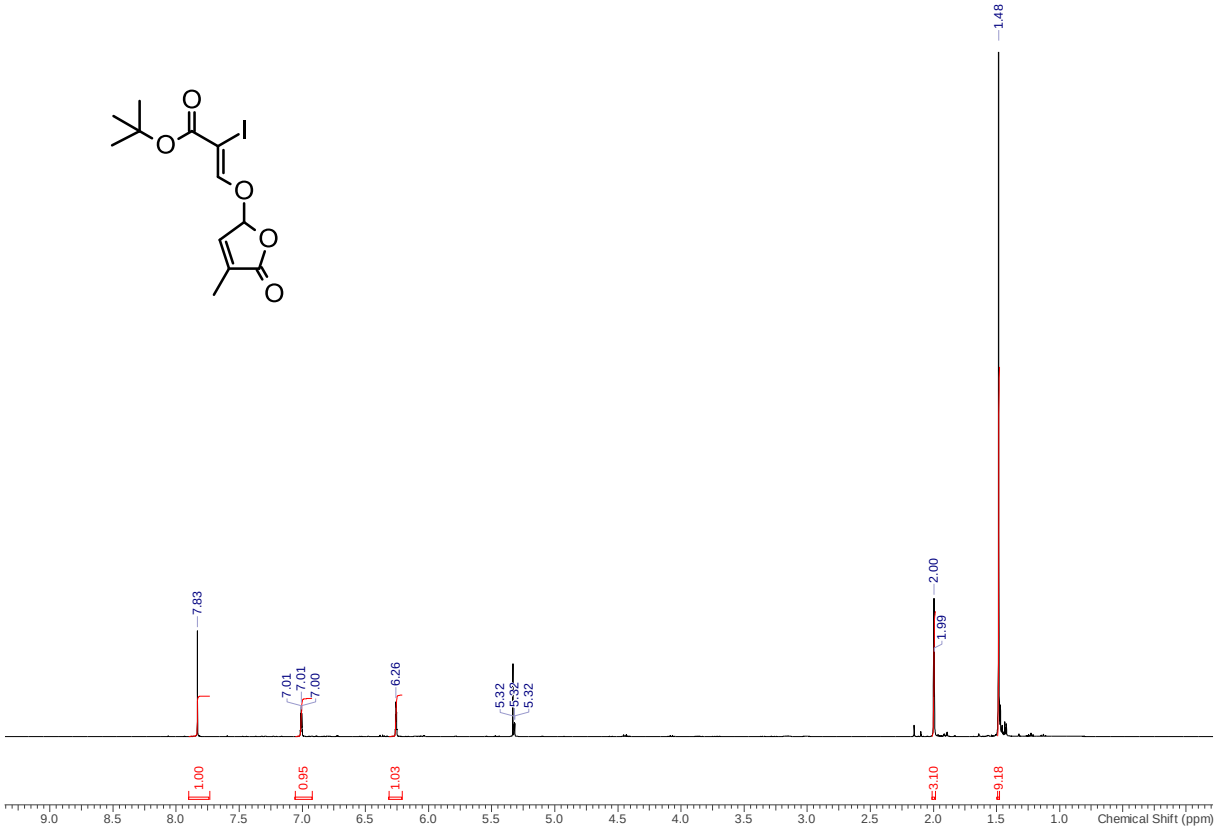

Cl
Cl
Cl
O
O
I
O
O
O

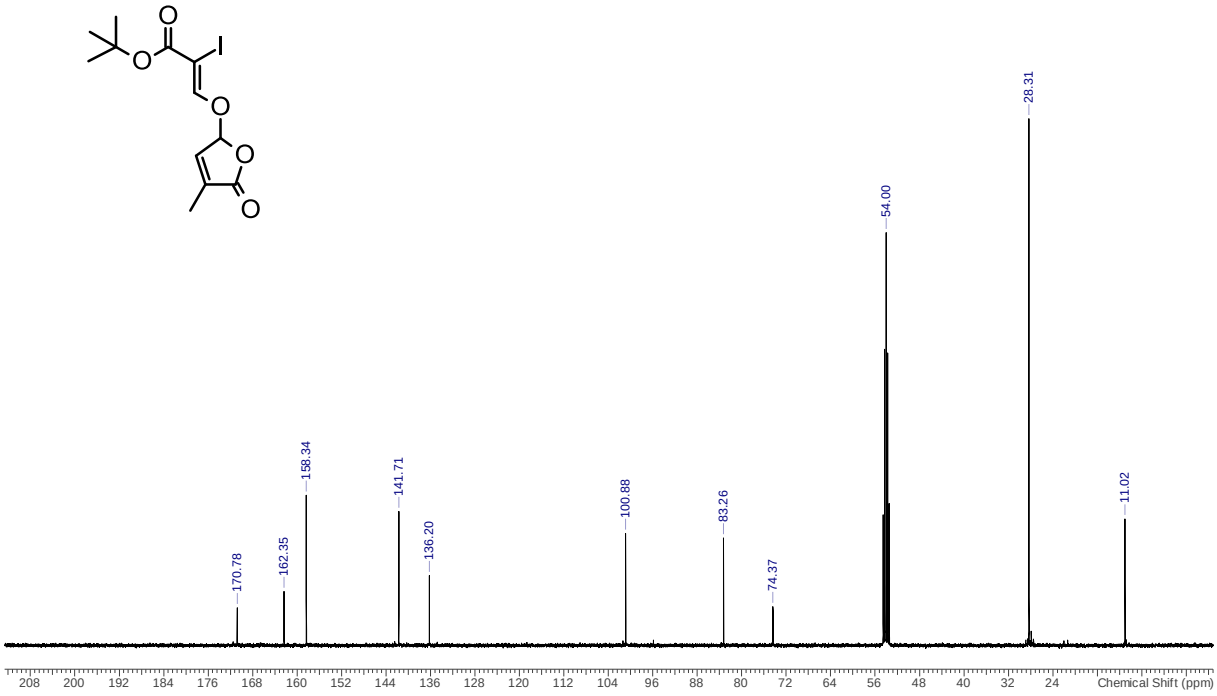
77.32
77.00
76.68
169.90
161.61
159.68
140.78
136.10
100.12
94.66
75.28
68.86
67.09
10.75
Chemical Shift (ppm)

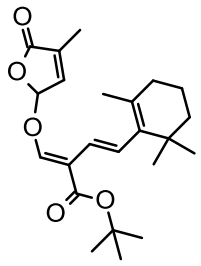




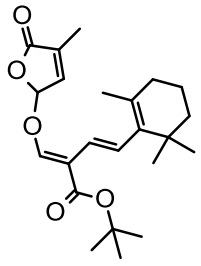
full assignment supported
by HSQC-, HMBC-data


1.48
7.83
7.01
7.01
7.00
6.26
5.32
5.32
5.32
2.00
1.99
1.00
0.95
1.03
3.10
9.18
Chemical Shift (ppm)

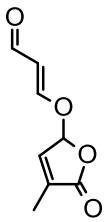

28.31
54.00
158.34
141.71
170.78
162.35
136.20
100.88
83.26
74.37
11.02
Chemical Shift (ppm)



26
+ 0.5 eq dibenzylidene-acetone (dba) present



26
+ 0.5 eq dibenzylidene-acetone (dba) present



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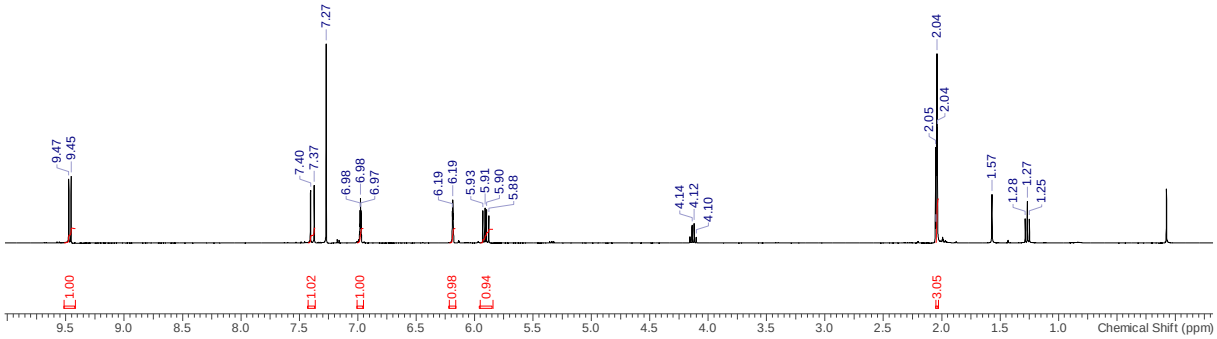
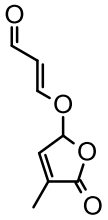

9.47
9.45
7.40
7.37
7.27
6.98
6.98
6.97
6.19
6.19
5.93
5.91
5.90
5.88
4.14
4.12
4.10
2.05
2.04
2.04
1.57
1.28
1.27
1.25
1.00
1.02
1.00
0.98
0.94
3.05
Chemical Shift (ppm)



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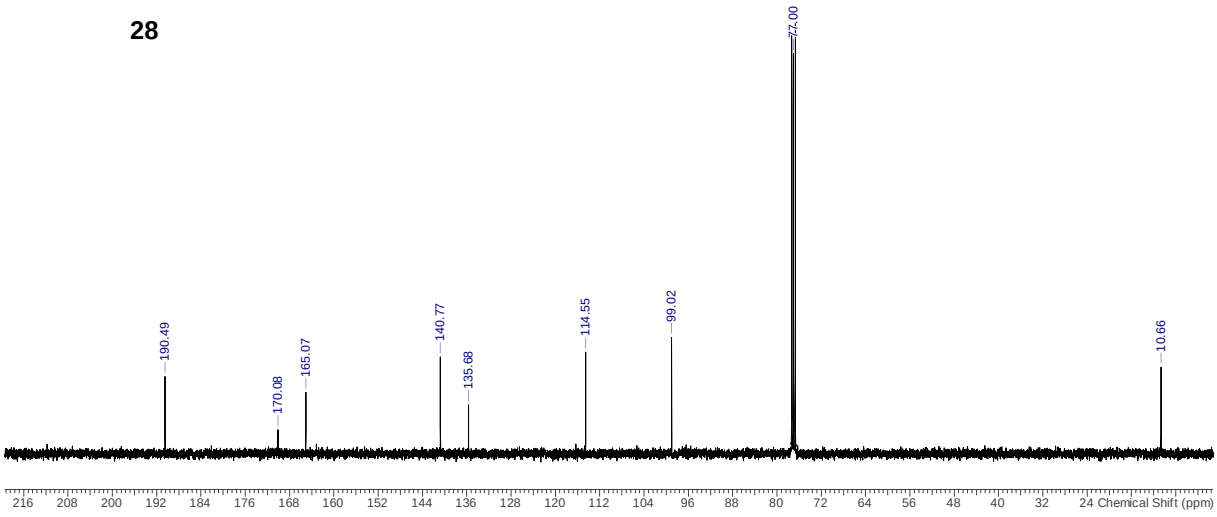
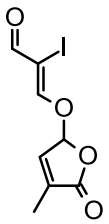

190.49
170.08
165.07
140.77
135.68
114.55
99.02
77.00
10.66
Chemical Shift (ppm)



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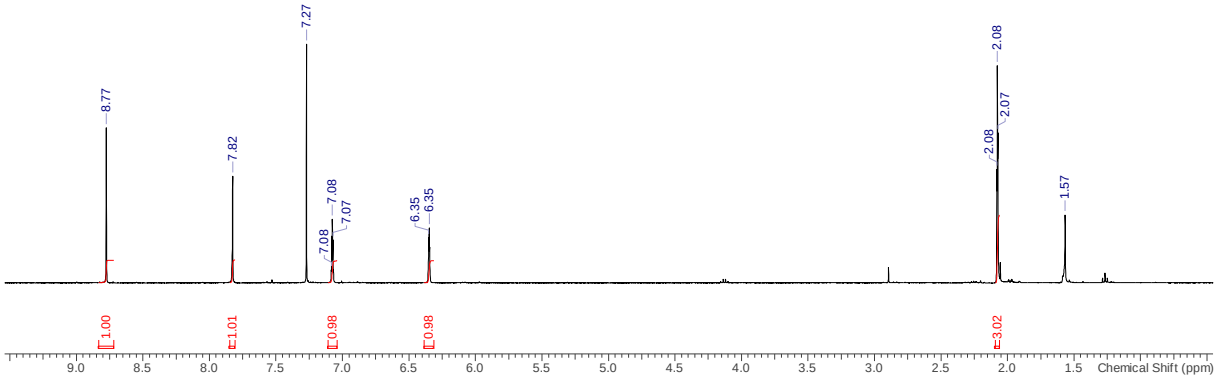
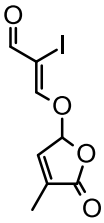
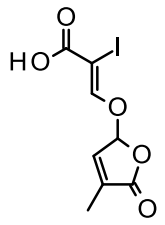

8.77
7.82
7.27
7.08
7.08
7.07
6.35
6.35
2.08
2.07
2.08
1.57
1.00
1.01
0.98
0.98
3.02
Chemical Shift (ppm)

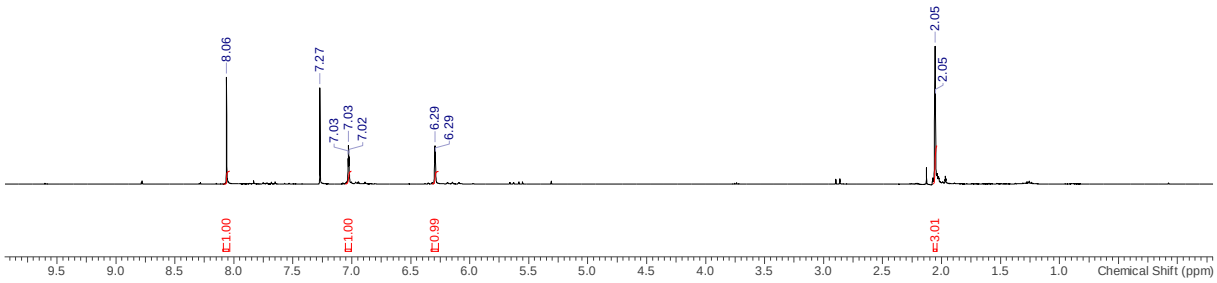


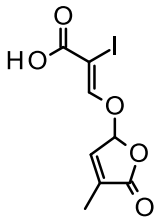
29

77.00
185.21
169.72
165.32
140.69
136.27
100.07
87.76
67.83
10.81
Chemical Shift (ppm)



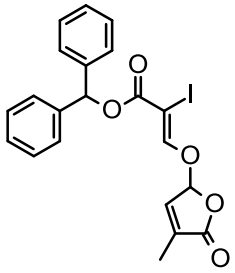
31


8.06
7.27
7.03
7.03
7.02
6.29
6.29
2.05
2.05
1.00
1.00
0.99
3.01
Chemical Shift (ppm)

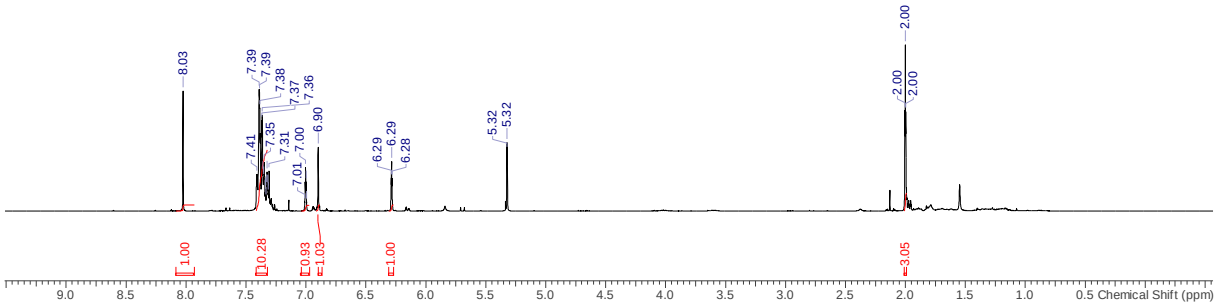


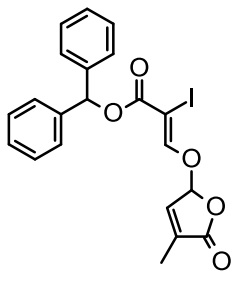
31

77.00
169.97
168.45
160.30
140.81
136.06
100.13
69.67
10.77
Chemical Shift (ppm)



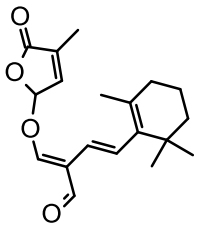
32


8.03
7.39
7.39
7.38
7.37
7.36
7.41
7.35
7.31
7.00
7.01
6.90
6.29
6.29
6.28
5.32
5.32
2.00
2.00
2.00
1.00
0.28
0.93
1.03
1.00
3.05
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
0.5 Chemical Shift (ppm)



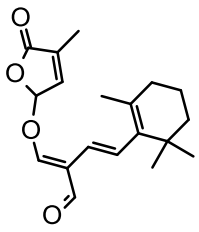
32

54.00
129.16
127.46
127.40
159.49
170.66
162.69
141.57
140.59
136.38
126.93
100.97
79.58
70.71
11.04
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)



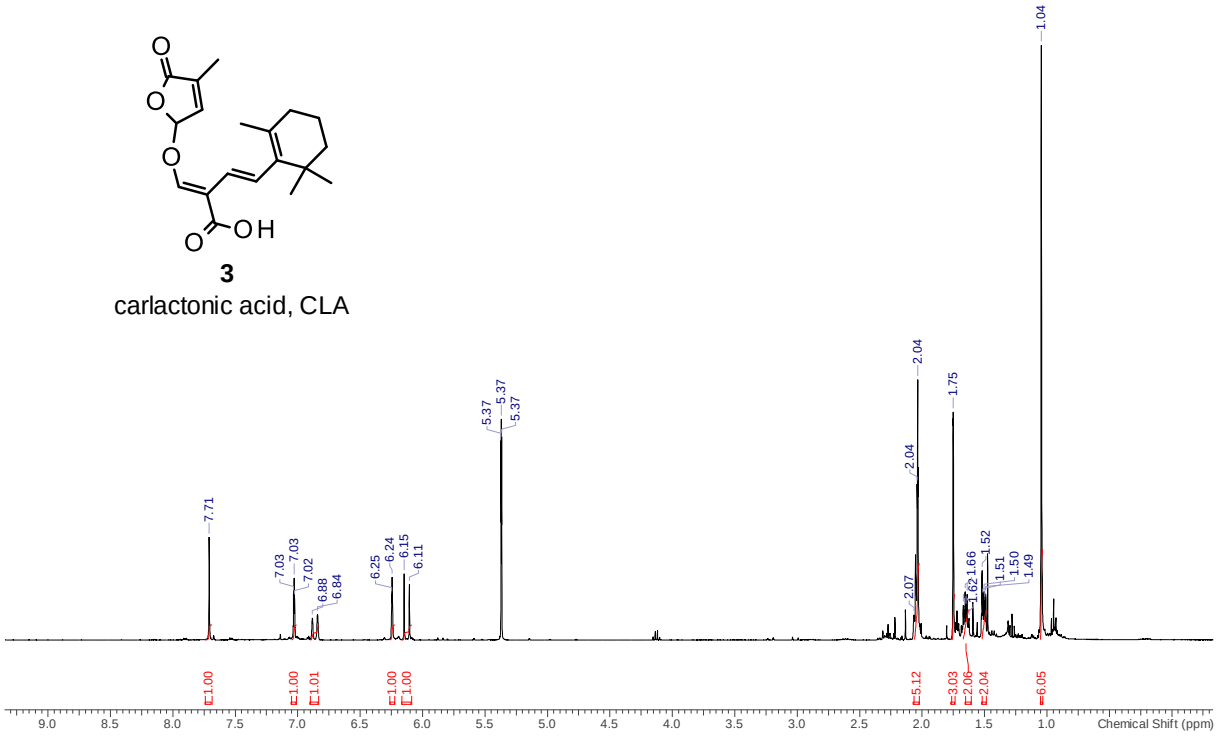
30

19-oxo-CL



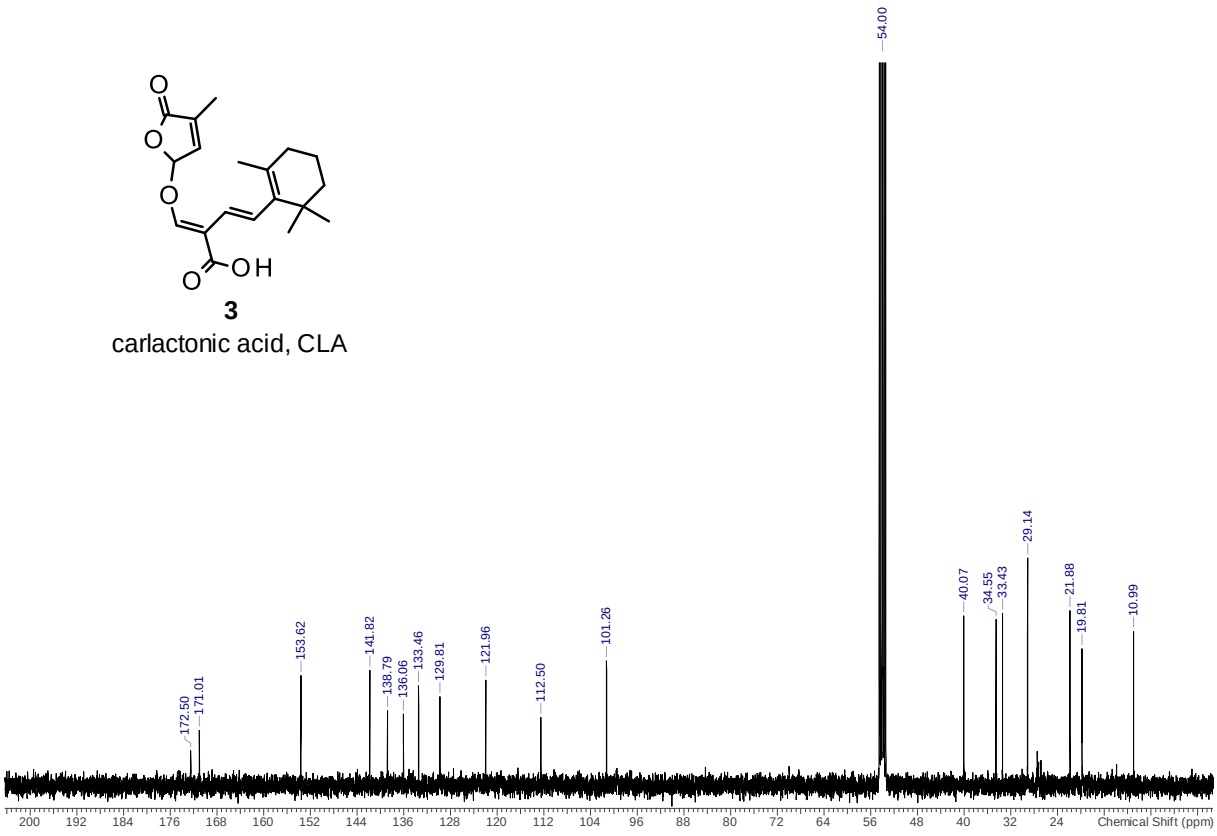
30

19-oxo-CL



3

carlactonic acid, CLA



3

carlactonic acid, CLA

2. HPLC spectra

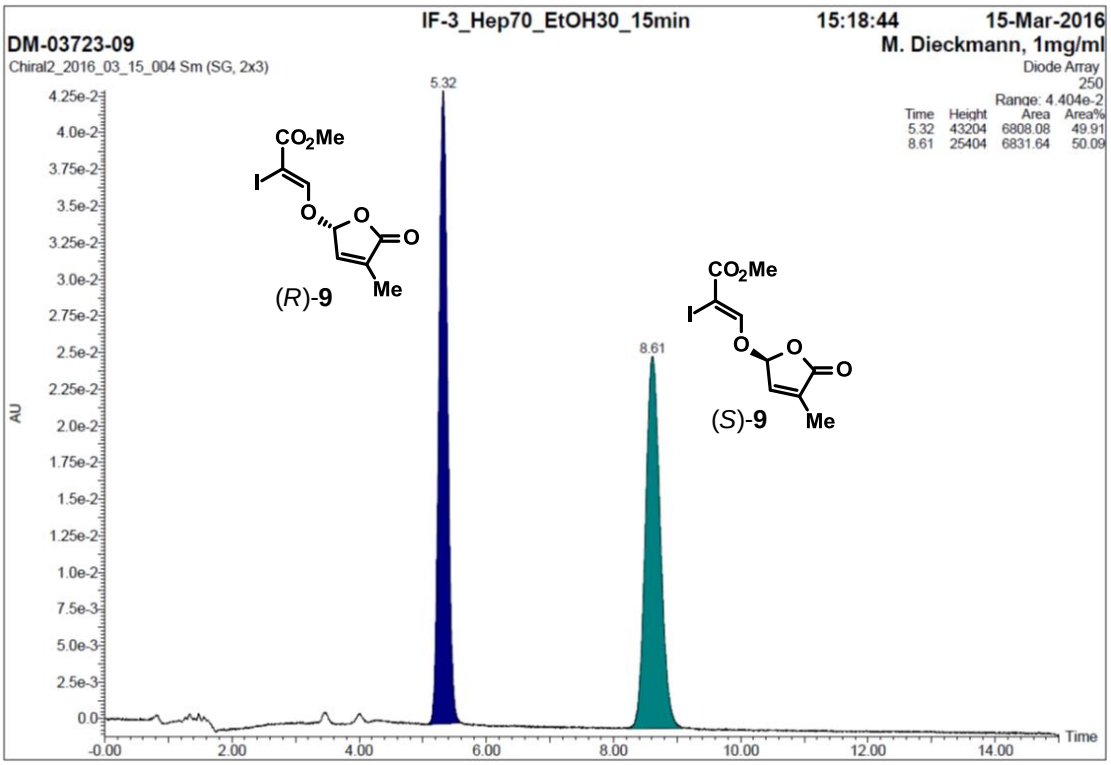


Figure S1: HPLC-separation of both enantiomers: *R*-stereoisomer: R_t = 5.32 min; $[\alpha]_D^{20}$ = +69.1° (c = 1.28, $CHCl_3$, 20 °C), stereochemical assignment: CD-analysis and *Orobanche cumana* germination assay; *S*-stereoisomer: R_t = 8.61 min; $[\alpha]_D^{20}$ = –74.5° (c = 1.07, $CHCl_3$, 20 °C), stereochemical assignment: CD-analysis and *Orobanche cumana* germination assay.

Synthesized (*R*)- and (*S*)-methyl carlactonoate:

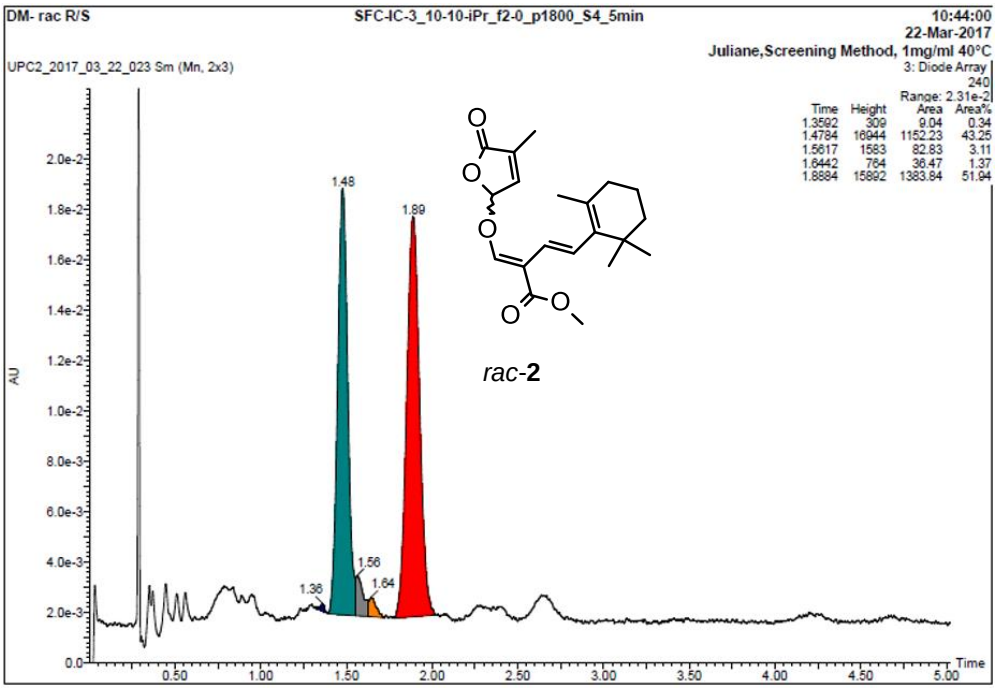


Figure S2: racemic methyl carlactonoate (*rac*-Me-CLA, *rac*-**2**).

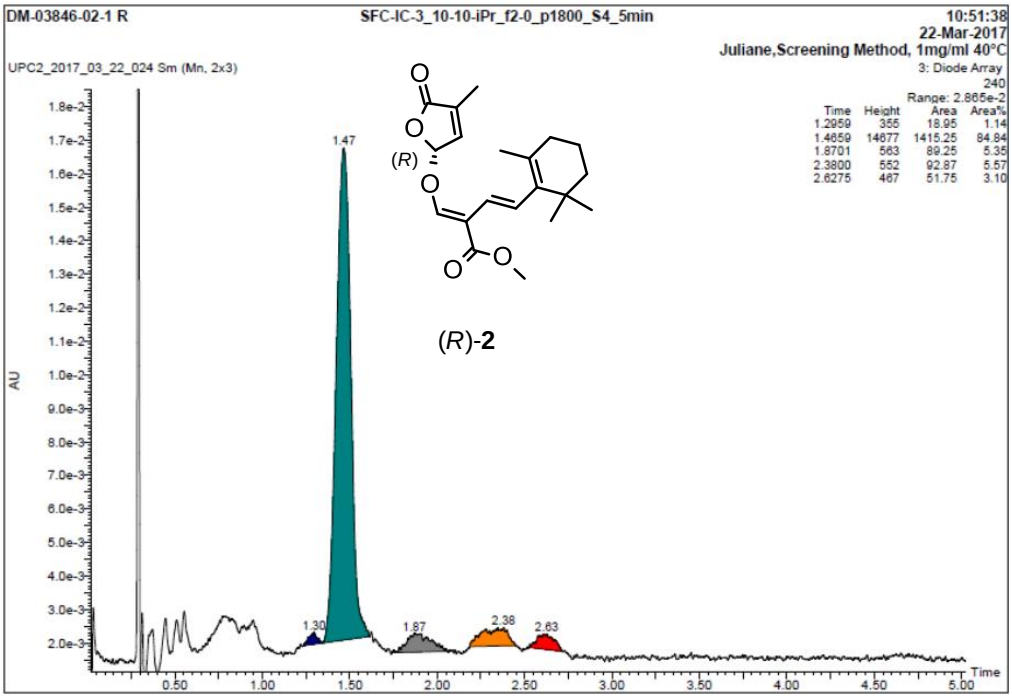


Figure S3: Synthesized (*R*)-methyl carlactonoate ((*R*)-Me-CLA, (*R*)-**2**); $[\alpha]_D^{20}$ ((*R*)-Me-CLA) = +9.9° (c = 0.53, CH_2Cl_2, 20 °C).

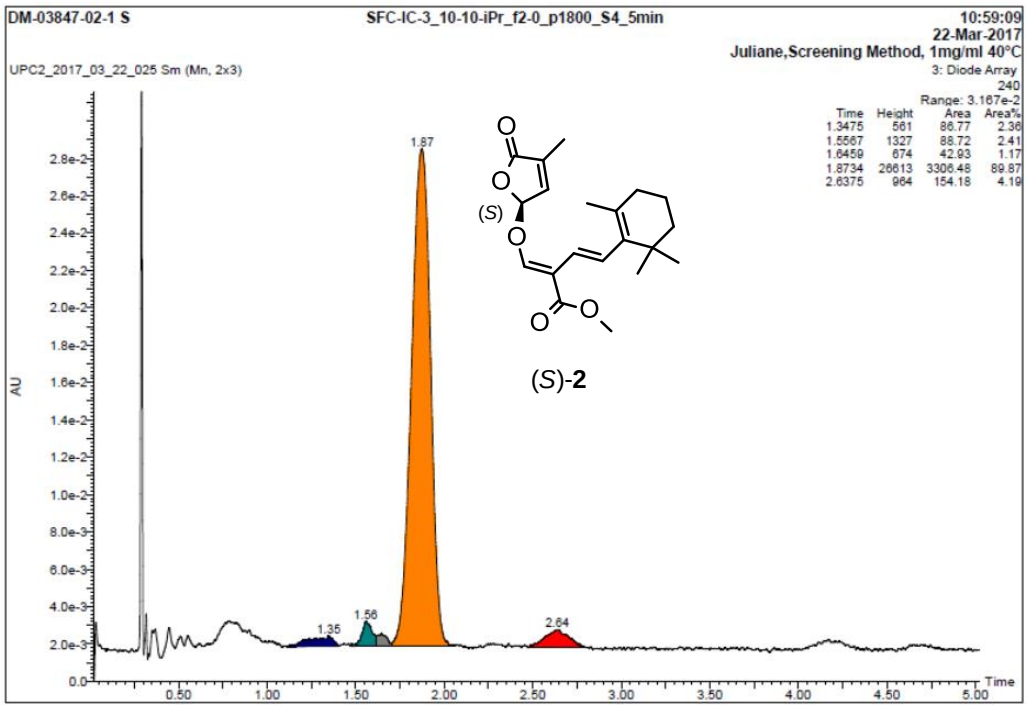


Figure S4: Synthesized (*S*)-methyl carlactonoate ((*S*)-Me-CLA, (*S*)-**2**); $[\alpha]_D^{20}$ ((*S*)-Me-CLA) = –30.4° (c = 0.57, CH_2Cl_2, 20 °C).

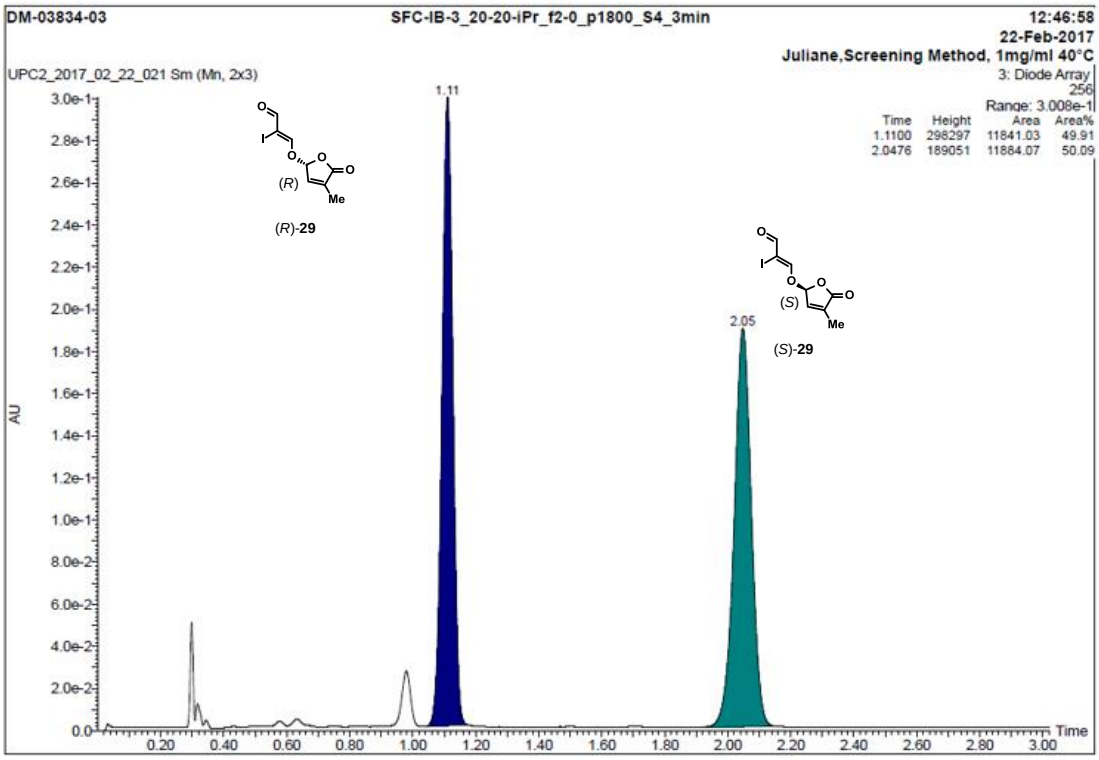


Figure S5: Separation of enantiomers via chiral HPLC: SFC Daicel CHIRALPAK ® IB, CO_2 /*i*PrOH; *R*-stereoisomer, (*R*)-**29**: R_t = 1.11 min; $[\alpha]_D^{20}$ = +119.1° (c = 1.00, $CHCl_3$, 20 °C), stereochemical assignment: CD-analysis; *S*-stereoisomer, (*S*)-**29**: R_t = 2.05 min; $[\alpha]_D^{20}$ = –124.3° (c = 1.07, $CHCl_3$, 20 °C), stereochemical assignment: CD-analysis.

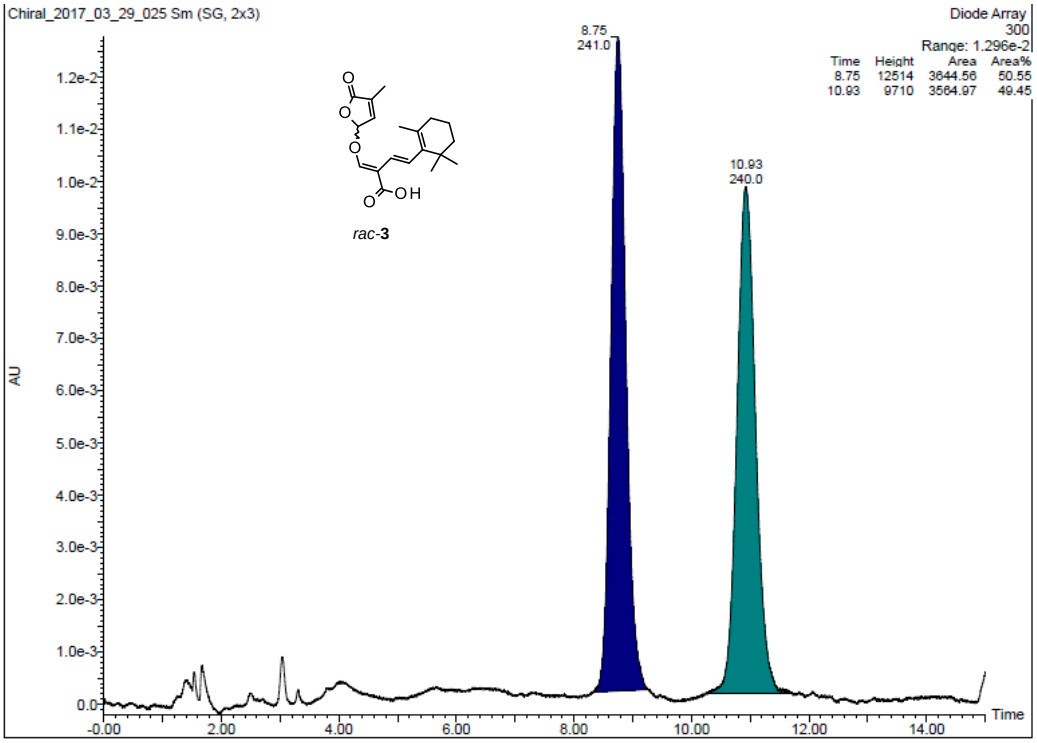


Figure S6: Racemic carlactonic acid (*rac*-CLA, *rac*-**3**).

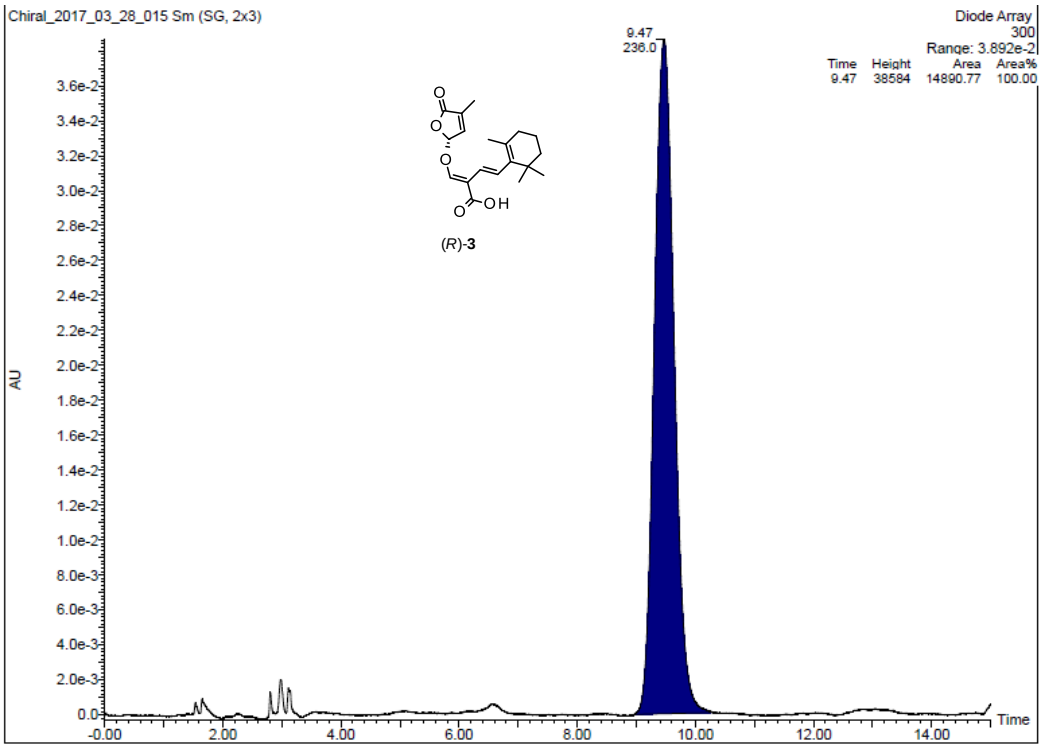


Figure S7: Synthesized (*R*)-carlactonic acid ((*R*)-CLA, (*R*)-**3**); $[\alpha]_D^{20}$ ((*R*)-CLA) = +63.4° (c = 0.30, CH_2Cl_2, 20 °C).

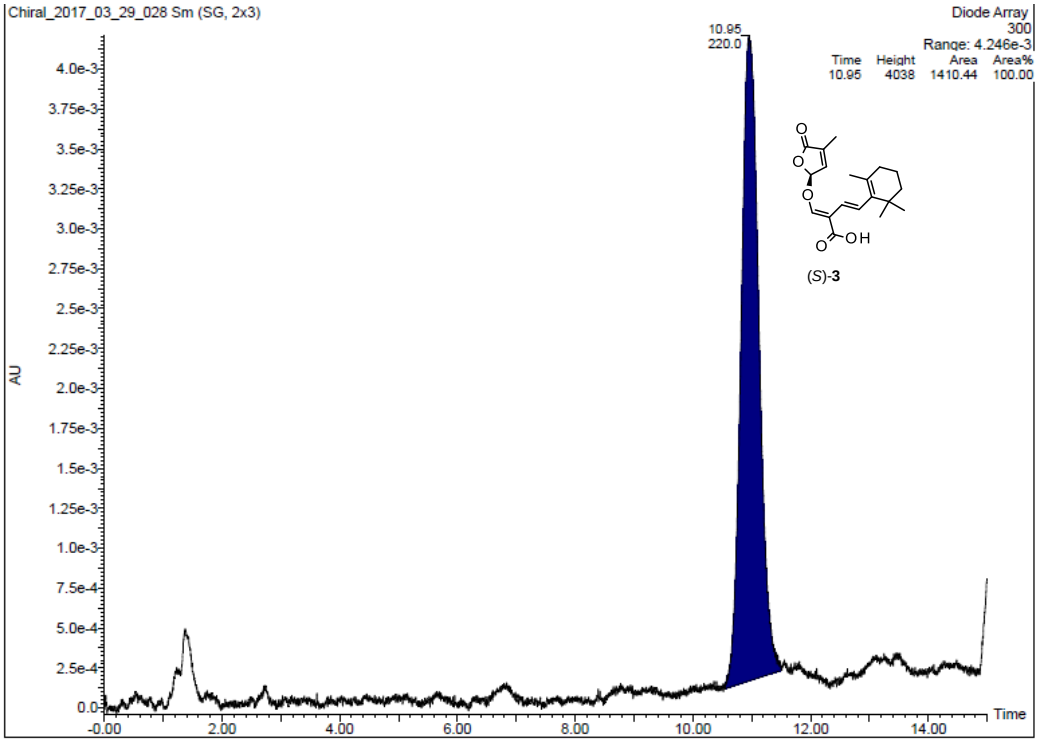


Figure S8: Synthesized (*S*)-carlactonic acid ((*S*)-CLA, (*S*)-**3**); $[\alpha]_D^{20}$ ((*S*)-CLA) = –62.9° (c = 0.69, CH_2Cl_2, 20 °C).

3. Stereochemical assignment: CD-spectra and germination assay on *Orobanche cumana*

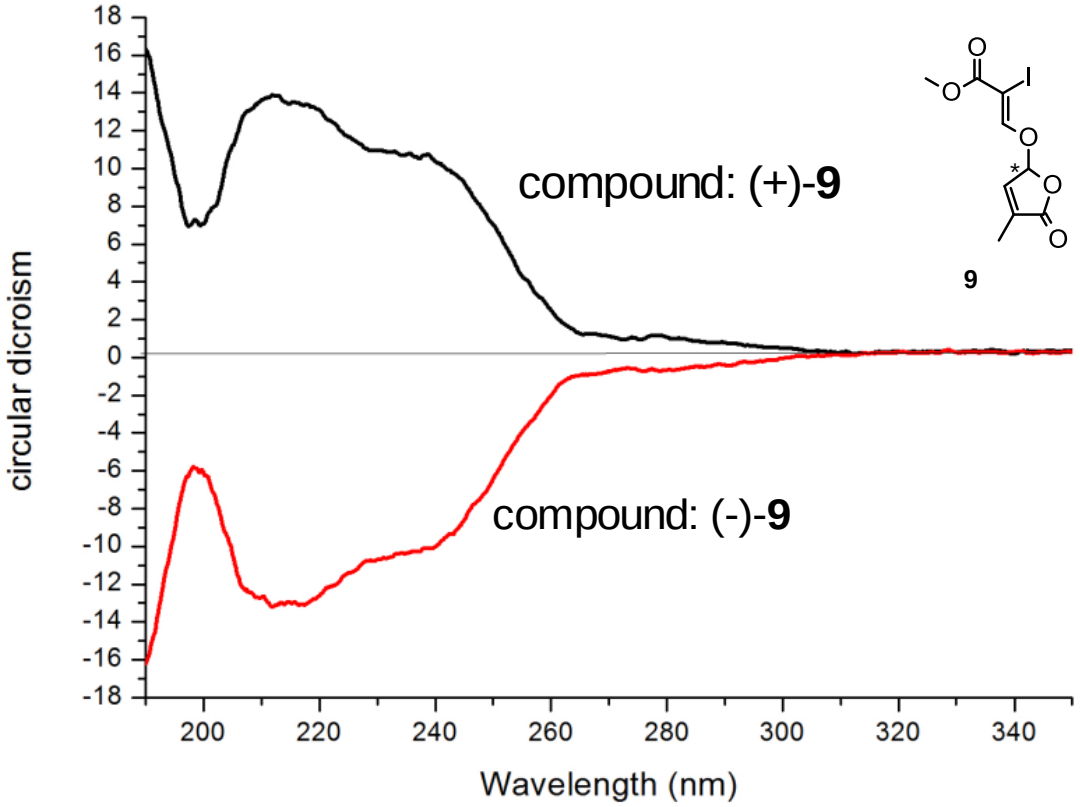


Figure S9: CD-spectra recorded for (+)-**9** (black curve, (+)-compound, peak: 5.32 min) and (–)-**9** (red curve, (–)-compound, peak: 8.61 min), c = 0.15 mg/mL, solvent: acetonitrile.

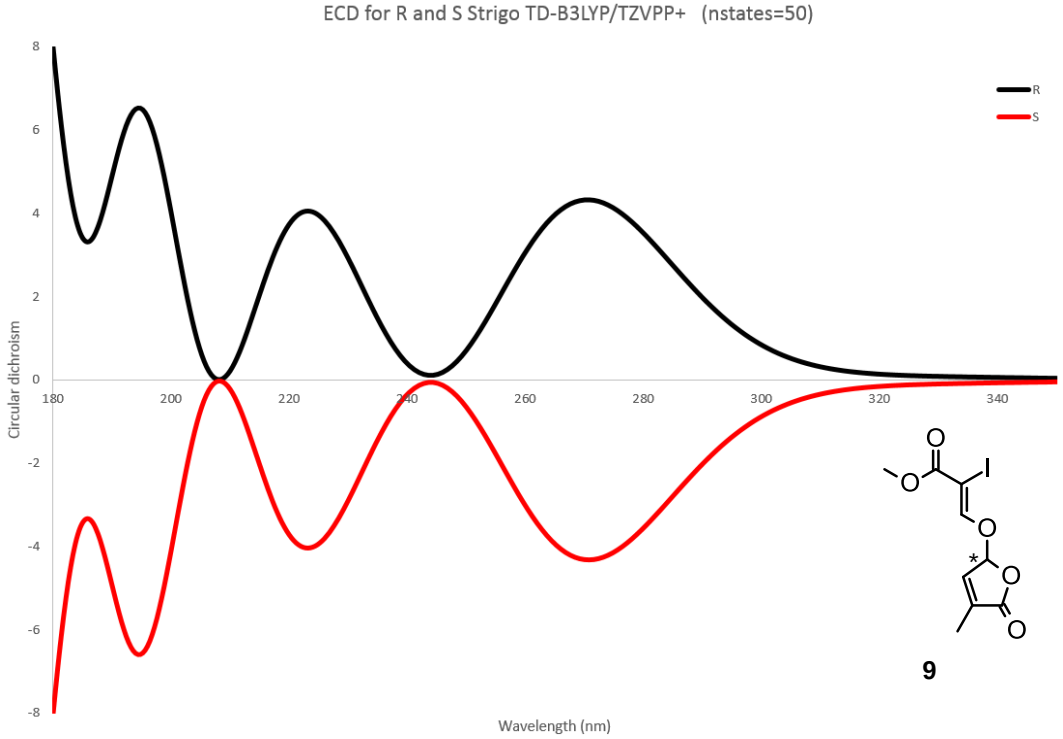


Figure S10: calculated CD-spectra corresponding to (*R*)- (black) and (*S*)-configuration (red) for compound **9**.

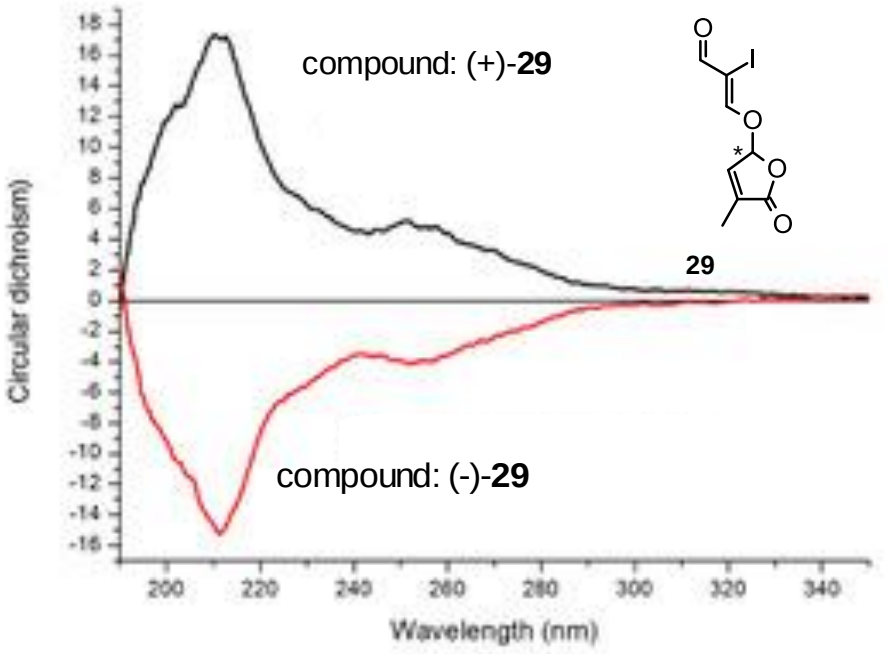


Figure S11: CD-spectra recorded for (+)-**29** (black curve, (+)-compound, peak: 1.11 min) and (–)-**9** (red curve, (–)-compound, peak: 2.05 min), c = 0.10 mg/mL, solvent: acetonitrile.

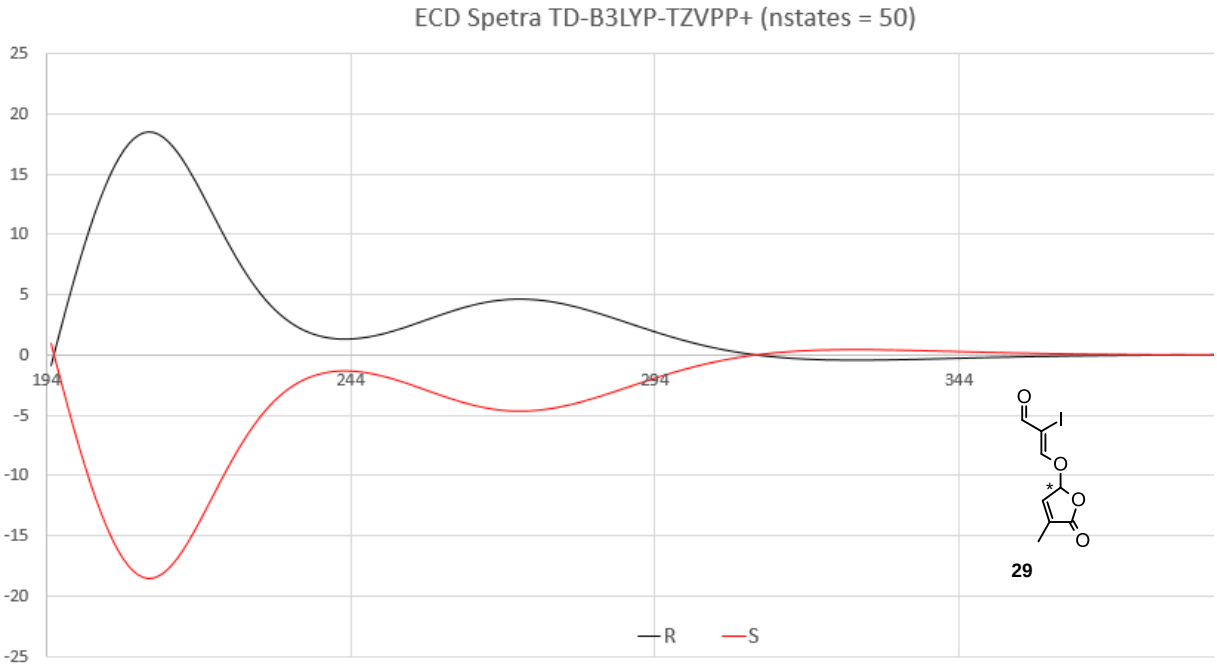


Figure 12: calculated CD-spectra corresponding to (*R*)- (black) and (*S*)-configuration (red) for compound **29**.

2016-039			Promotion of O. cumana germination;	
Treatment no.	Info	AI	Rate µM	% germination (mean of 5 reps) (human estimation A)
1.1	test	(+)-compound	$3x10^{-2}$	96
1.2			$3x10^{-4}$	26
1.3			$3x10^{-6}$	11
2.1	test	(-)-compound	$3x10^{-2}$	25
2.2			$3x10^{-4}$	28
2.3			$3x10^{-6}$	3
3		Water		3
4		Water & acetone		3

Figure S13: Germination assay on *Orobanche Cumana* seeds for (+)-**9** ((+)-compound, peak: 5.32 min) and (–)-**9** ((–)-compound, peak: 8.61 min).

Protocol:

Orobanche cumana germination assay: Seeds of Orobanche cumana were collected from a sunflower field in Manzanilla (Seville, Spain), cleaned by sucrose floatation technique, and disinfected 2 min in 1% sodium hypochlorite solution and 0.025% (v/v) Tween 20. Seeds were decanted onto two layers of cheesecloth, rinsed with sterile deionised water and resuspended in sterile deionised water. Two ml seed suspension containing approximately 150–400 seeds were spread evenly on two layers of sterile wet glass fiber filter paper disc in Petri dishes (ø 9 mm). Seeds were incubated 10 days at 20 °C in the dark for seed conditioning. The upper disc with seeds was briefly dried, transferred to a petri dish lined with a dry GFFP disc, and wetted with 6 ml of the appropriate test solution. Compounds were tested at concentrations of 0.001, 0.01, and 0.1 mg. The strigolactone analogue GR24 was included as positive control and 0.01% DMSO as negative control. All treatments were tested in five replicates. Seeds were re-incubated at 20 °C in the dark and examined for germination 10 days later. The radicles of germinated seeds were stained for 5 min with blue ink (MIGROS, Switzerland) in 5% acetic acid. Seeds were photographed and germination of 100 seeds per replicate was evaluated on digital images. Seeds were considered germinated when the radicle protruded from the seed coat.

4. X-ray data

Structure of *rac*-**9**

A single crystal grown from cyclohexane and ethyl acetate was selected for single crystal X-ray data analysis. The crystal sample mounted had dimensions of 0.15 mm x 0.05 mm x 0.05 mm and was a small piece broken from a very long colourless needle. Data collection was performed on a Rigaku Supernova diffractometer at 117 K. The unit cell was determined to be monoclinic (space group P21/n). Crystallographic data is summarized in Table S1. Coordinates and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1573373).

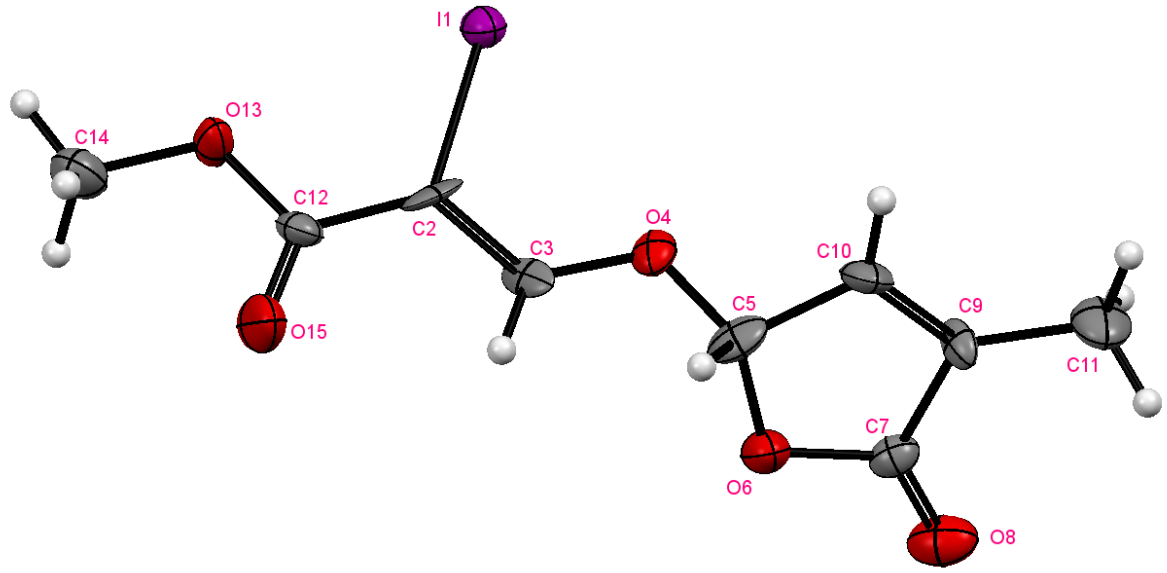


Figure S14: ORTEP representation of *rac*-**9** with thermal ellipsoids shown at the 50% probability level. Hydrogen atoms of arbitrary size.

Table S1. Crystal data and structure refinement for compound *rac*-9	
formula	$C_9 H_9 I_1 O_5$
M_r	324.07
crystal system	monoclinic
space group	*P*21/n
Temperature (K)	117
a, *b*, *c* (Å)	4.2649(18), 27.927(7), 9.166(2)
β (˚)	100.79(3)
V (Å^3)	1072.4(3)

Z	4
radiation type (wavelength)	Cu $K\alpha$ (1.54184 Å)
μ (mm^{-1})	23.51
crystal size (mm)	0.15 x 0.05 x 0.05
diffractometer	Rigaku Oxford Diffraction SuperNova
absorption correction	multi-scan
measured reflections	2309
independent reflections	1426
R_{int}	0.050
Θ_{max} (°)	58.9
$(\sin \Theta/\lambda)_{max}$ ($Å^{-1}$)	0.555
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.074, 0.146, 1.10

Structure of *rac*-**29**

A single crystal grown from cyclohexane, ethyl acetate and dichloromethane was selected for single crystal X-ray data analysis. The crystal sample mounted was a pale yellow needle with dimensions of 0.25 mm x 0.07 mm x 0.01 mm. Data collection was performed on a Rigaku Supernova diffractometer at 117 K. The unit cell was determined to be orthorhombic (space group Fdd2). Crystallographic data is summarized in Table S2. Coordinates and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1573432).

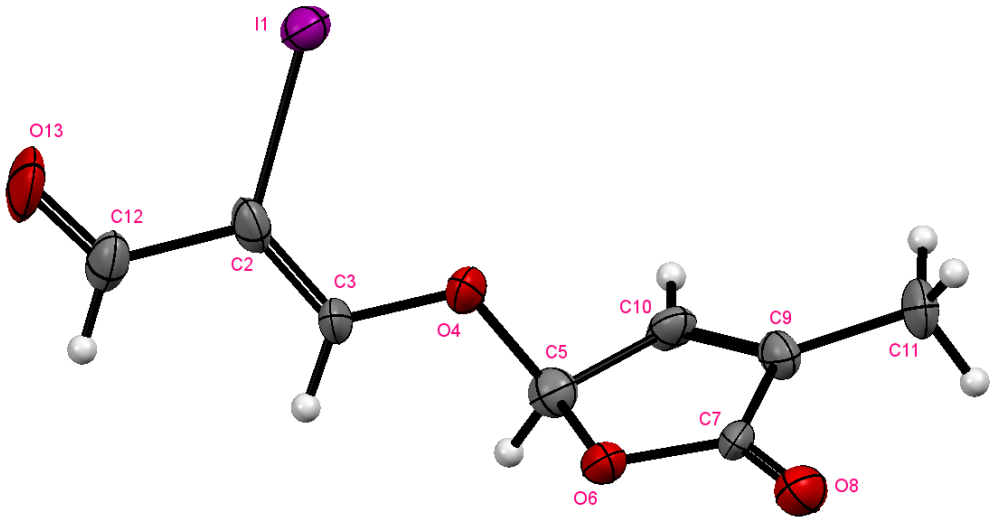


Figure S15: ORTEP representation of *rac*-**29** with thermal ellipsoids shown at the 50% probability level. Hydrogen atoms of arbitrary size.

Table S2. Crystal data and structure refinement for compound *rac*-29	
formula	$C_8 H_7 I_1 O_4$
M_r	294.05
crystal system	orthorhombic
space group	Fdd2
Temperature (K)	117
a, *b*, *c* (Å)	33.7382(8), 26.6877(8), 4.17233(11)
α , β, γ (˚)	90, 90, 90
V (Å^3)	3756.74(10)
Z	16
radiation type (wavelength)	Cu *Kα* (1.54184 Å)
μ (mm^{-1})	26.68
crystal size (mm)	0.25 x 0.07 x 0.01
diffractometer	Rigaku Oxford Diffraction SuperNova
absorption correction	multi-scan
measured reflections	4172
independent reflections	1366
R_{int}	0.045
Θ_{max} (˚)	61.3
$(\sin \Theta/\lambda)_{max}$ (Å^{-1})	0.569
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.068, 0.161, 0.94