Regioselective Biocatalytic Self-Sufficient Tishchenko-Type Reaction *via* Formal Intramolecular Hydride Transfer

Erika Tassano,[a] Kemal Merusic,[a] Isa Buljubasic,[a] Olivia Laggner,[a] Tamara Reiter,[a] Andreas Vogel,[b] and Mélanie Hall*[a]

[a] Dr. Erika Tassano, Kemal Merusic, Isa Buljubasic, Olivia Laggner, Tamara Reiter, Dr. Mélanie Hall

Department of Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria

E-mail: melanie.hall@uni-graz.at

[b] Dr. Andreas Vogel

c-LEcta GmbH, Perlickstrasse 5, 04103 Leipzig, Germany

*Corresponding authors: melanie.hall@uni-graz.at

ELECTRONIC SUPPORTING INFORMATION

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General remarks

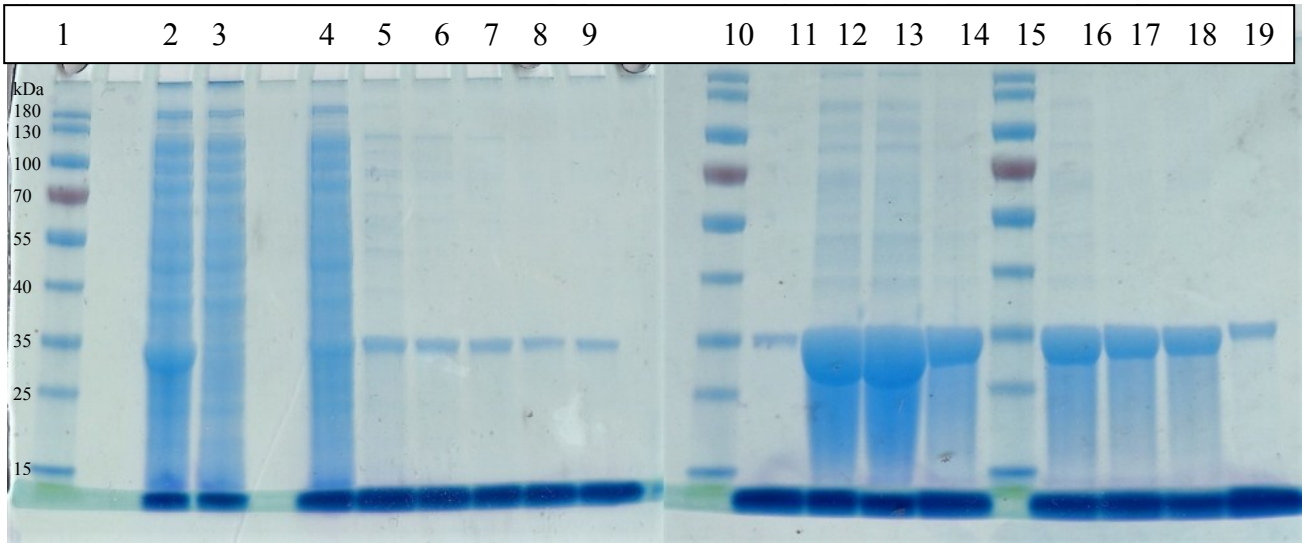
Chemical reagents employed in the synthesis of starting and reference materials were purchased from Alfa Aesar, Sigma-Aldrich/Merck and Fluorochem. TLC analyses were performed on Merck Silica Gel 60 F254 precoated plates and visualized under UV light (λ = 254 nm), stained with Hanessian's stain [dipping into a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (21 g) and $Ce(SO_4)_2\cdot 4H_2O$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming] and with bromocresol green stain (solution of 0.1 g bromocresol green in 500 mL ethanol and 5 mL 0.1 N NaOH). Petroleum ether (40–60 °C) is abbreviated PE. Organic extracts were always dried with anhydrous Na_2SO_4 and filtered before evaporation of the solvent under reduced pressure. Column chromatography was done with the flash methodology[1] by using 220–400 mesh silica. NMR spectra were measured on a Bruker Avance III 300 MHz NMR spectrometer. Chemical shifts are reported in ppm relative to TMS (δ = 0.00 ppm) and the coupling constants (J) in Hertz (Hz). GC-MS analyses were performed on an Agilent 7890A GC system, equipped with an Agilent 5973 mass selective detector and an Agilent HP-5MS column (30 m x 0.25 mm x 0.25 µm).

Enzymes

ADH-A (from *R. ruber*) and HLADH (from *E. caballus*) were cloned and overexpressed as previously reported.[2, 3]

ADH-107, 109, 110, 111, 114, 117, 118, 132, 170, 171, 172, 173, 174, 175 and 182 were obtained from c-LEcta as lyophilized cell-free extracts and are all NADH dependent. The ADH enzyme collection consists of variants obtained by enzyme engineering.[4] The enzymes were expressed in *E. coli* and cell-free extracts were obtained after cell lysis, centrifugation and freeze-drying of the supernatants.

The *N*-terminal *strep* II-tagged Lk-ADH_{mut} (mutations with respect to the wild type from *L. kefir*: L17H; D25T; E29T; H40R; A64V; T71G; A80T; V87L; A94G; V95Y; S96R; N131C; E145L; F147Q; T152A; L153T; N157S; D173L; Y190P; V196M; L199M; E200P; I266V)[5] was cloned in pASK-IBA5plus vector and overexpressed in *E. coli* BL21(DE3). 250 mL LB medium supplemented with ampicillin (100 µg mL^{-1}) and 1 mM $MgCl_2$ were inoculated with 2 mL overnight culture (starter culture, 15 mL LB medium supplemented with ampicillin) and incubated at 37 °C and 120 rpm until an OD_{600} of 0.6 was reached. Then AHTC (anhydrotetracycline, 0.4 µM) was added for induction and the cells left overnight at 20 °C and 120 rpm. The next day the cells were harvested by centrifugation (20 min, 4000 rpm, 4 °C). To obtain the cell-free extract, the cell pellet (4 g from 500 mL culture) was resuspended in buffer W (100 mM TRIS·HCl, 150 mM NaCl, pH = 8.0; 15 mL) and lysed on ice by sonication (time: 2.30 min, pulse ON: 1 s, pulse OFF: 4 s, amplitude: 40%). Afterwards the cell debris were removed by centrifugation (20 min, 15000 rpm, 4 °C) and the cell-free extract was directly applied to Strep-Tactin XT Superflow gravity flow column (5 mL); the purification was performed according to the protocol provided by the supplier (IBA). Fractions containing the purified enzyme (see SDS PAGE below) were collected and concentrated to 2.5 mL; then buffer was exchanged to KPi (50 mM, pH=7.0; supplemented with 2 mM $MgCl_2$); two fractions were collected, frozen in liquid nitrogen and stored at -20 °C.



SDS PAGE: **1**, **10**, **15**: standard (PageRuler^{TM}, 10 to 180 kDa); **2**: supernatant after sonication; **3**: flow-through; **4-9**: washing fraction; **11-19**: elution fractions

Standard biocatalytic procedure

Standard enzymatic Tishchenko-type reaction was run as follows: an aliquot of purified enzyme (1 mg/mL final concentration, unless otherwise stated) was added to a phosphate buffer solution (50 mM, pH 7.0, supplemented with 2 mM $MgCl_2$) containing the substrate (10 mM, from a 200 mM stock solution in CH_3CN, final co-solvent concentration 5 vol%) and the nicotinamide cofactor (0.5 mM). The reaction mixture (500 µL total volume) was incubated at 30 °C and 120 rpm. After 24 h, the product was extracted from the reaction mixture with EtOAc (2 x 250 µL, spiked with 10 mM (*R*)-limonene as internal standard). The combined organic fractions were dried over anhydrous Na_2SO_4 and analyzed by GC. All reactions were run in duplicates.

Acetonitrile was identified as suitable co-solvent to solubilize the substrate during the reaction, while preserving the enzyme activity in most cases. While the actual effect of the co-solvent on the activity of each enzyme was not studied individually, ADHs are usually robust toward the presence of organic solvents at such low concentration (5 vol%). The resulting mixture can be seen as a microemulsion but the state of the solution was not studied in detail, since in most cases the selected condition was found satisfactory with at least several biocatalysts. It cannot be excluded that variation in yields may be related to various degrees of acceptance of the co-solvent.

Supplementary tables

Table S1. Conversion of **1a** to **1b** through ADH-catalyzed reduction-oxidation sequence[a]

Entry	Enzyme[b]	Conv. (%)	$TON_{NAD(P)H}$[c]
1	ADH-107	57	11.4
2	ADH-110	63	12.6
3	ADH-111	74	14.8
4	ADH-114	<1	n.a.
5	ADH-132	59	11.8
6	ADH-170	10	2
7	ADH-172	74	14.8
8	ADH-173	<1	n.a.
9	ADH-174	60	12
10	ADH-175	1	2
11	HLADH	0	n.a.
12	ADH-A	0	n.a.
13	Lk-ADH_{mut}	86	17.2

[a] 10 mM **1a**, 0.5 mM NAD(P)H, 1 mg/mL enzyme, KPi buffer (50 mM, pH 7.0, supplemented with 2 mM $MgCl_2$ in entry 1-10 and 13), 5 vol% CH_3CN, 30 °C, 120 rpm, 16 h. [b] ADH 107, 110,111, 114, 132, 170, 172-175 were obtained from c-LEcta; HLADH and ADH-A were employed as purified enzyme and obtained as reported;[2, 3] Lk-ADH_{mut} was used as purified enzyme and obtained as described herein. [c] $TON_{NAD(P)H}$ calculated based on product formation (1 turnover corresponds to two half-reactions). n.a. not applicable.

Table S2. Substrate and cofactor feeding experiment performed with **1a** and ADH-132.[a]

Entry	**1a** (mM)	NADH (mM)	Reaction time (h)	**1b** (mM)
1	5	0.5	3	2.7
2	10 (5+5)[b]	0.5	6	6.7
3	10 (5+5)[b]	1.0 (0.5+0.5)[b]	6	7.0
4	10	0.5	3	6.1
5	20 (10+10)[b]	0.5	6	10.1
6	20 (10+10)[b]	1.0 (0.5+0.5)[b]	6	14.1

[a] Reaction conditions: 1 mg/mL ADH-132, KPi (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm. [b] Added in two equal aliquots, second aliquot added after 3 h.

Table S3. Conversion of **2a** and **3a** to **2b**-**2c** and **3b**-**3c**, respectively, using a panel of ADHs according to Scheme 2.[a]

Entry	ADH	Conv. (%) to **2b**-**2c**[b]	Ratio **2b**/**2c**[c]	Conv. (%) to **3b**-**3c**[b]	Ratio **3b**/**3c**[d]
1	107	13	23:77	23	62:38
2	109	n.d.	n.a.	42	76:24
3	110	n.d.	n.a.	70	87:13
4	111	n.d.	n.a.	55	79:21
5	114	2	>99:1	56	99:1
6	114[e]	n.t.	n.a.	67	>99:1
7	117	n.d	n.a.	43	77:23
8	118	n.d	n.a.	56	88:12
9	132	n.d.	n.a.	55	83:17
10	170	3	>99:1	24	41:59
11	171	27	70:30	13	54:46
12	172	2	>99:1	67	86:14
13	173	3	>99:1	<2	n.a
14	174	15	20:80	22	63:37
15	175	3	>99:1	<2	n.a.
16	Lk-ADH$_{mut}$	52	90:10	48	73:27
17	Lk-ADH$_{mut}$	81[f]	90:10	69[e]	61:39

[a] Reaction conditions: 10 mM of **2a**/**3a**, 1 mg/mL ADH, 0.5 mM NAD(P)H, KPi buffer (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm, 16 h; entries 1-15: ADHs from c-LEcta available as lyophilized cell lysates. [b] Conversion based on total formation of lactone products. [c] Quantification of **2b** and **2c** using commercial standards. [d] Quantification of **3b** based on calibration curve obtained from synthesized reference material (see the Supporting Information); quantification of **3c** calculated assuming that **3b** and **3c** have similar response factor on GC-FID. [e] After 5 h, additional 10 mM of **3a** and 0.5 mM NADH were added, total reaction time 24 h. [f] 20 mM of **2a**. n.a. not applicable, n.d. not detected, n.t. not tested.

Table S4. Conversion of **4a** to **4b** through ADH-catalyzed reduction-oxidation sequence[a]

Entry	ADH	[**4a**] (mM)	Conv. (%)
1	107	10	2
2	109	10	62
3	110	10	7
4	111	10	40
5	114	10	15
6	117	10	59
7	118	10	43
8	132	10	15
9	170	10	3
10	171	10	15
11	172	10	9
12	173	10	1
13	174	10	2
14	175	10	1
15	Lk-ADH$_{mut}$	5	90
16	Lk-ADH$_{mut}$	10	84
17	Lk-ADH$_{mut}$	15	82
18	Lk-ADH$_{mut}$	20	26
19	Lk-ADH$_{mut}$	50	9

[a] Reaction conditions: 1 mg/mL enzyme, 0.5 mM NAD(P)H, KPi (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm, 16 h.

Table S5. Conversion of **5a-7a** to **5b-7b** and **5c-7b** through ADH-catalyzed reduction-oxidation sequence[a]

Entry	ADH	Conv. (%) to **5b**[b]	Conv. (%) to **6b**/**6c**	Ratio **6b**/**6c**	Conv. (%) to **7b**/**7c**	Ratio **7b**/**7c**
1	Lk-ADH$_{mut}$	5	75	97:3	98	14:86
2	99	n.c.	6	64:36	11	6:94
3	107	n.c.	7	56:44	43	13:87
4	109	1	23	72:28	67	9:91
5	110	8	32	94:6	58	10:90
6	111	1	24	73:27	67	9:91
7	114	n.c.	7	70:30	11	8:92
8	117	1	40	77:24	69	10:90
9	132	32	61	72:28	62	23:77
10	170	n.c.	5	65:35	16	8:92
11	171	n.c.	6	55:45	51	13:87
12	172	1	20	94:6	78	10:90
13	173	n.c.	2	65:35	4	14:86
14	174	n.c.	8	71:29	38	15:85
15	175	n.c.	2	68:32	2	18:82
16	182	n.c.	3	50:50	15	5:95

[a] Reaction conditions: 10 mM substrate, 1 mg/mL ADH, 0.5 mM NAD(P)H, KPi (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm, 24 h. [b] no trace of **5c** was detected. n.c. no conversion.

Table S6. Conversion of **8a-10a** through ADH-catalyzed reduction-oxidation sequence[a]

Entry	ADH	Conv. (%) to **8c**[b]	Conv. (%) to **9c**[b]	Conv. (%) to **10c**
1	Lk-ADH$_{mut}$	4	46	90
2	99	<1	19	14
3	107	<1	3	>99
4	109	1	5	92
5	110	14	15	84
6	111	2	5	90
7	114	4	2	58
8	117	1	6	94
9	118	13	52	n.t.
10	132	57	6	>99
11	170	<1	3	29
12	171	<1	4	93
13	172	5	27	81
14	173	<1	<1	<2
15	174	<1	3	69
16	175	<1	2	4
17	182	<1	2	10

[a] Reaction conditions: 10 mM substrate, 1 mg/mL ADH, 0.5 mM NAD(P)H, KPi (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm, 16 h. [b] no trace of **8b**/**9b** was detected. n.t. not tested.

Table S7. Effect of additional EWG on conversion of **8a**-**10a** (compared to mono-substituted **5a**-**7a**)

Entry	ADH	ΔConv. (%) from **5b** to **8c**	ΔConv. (%) from **6b** to **9c**	ΔConv. (%) from **7c** to **10c**
1	Lk-ADH$_{mut}$	-20	-39	-8
2	99	n.a.	+217	+27
3	107	n.a.	-57	+133
4	109	0	-78	+37
5	110	+75	-53	+45
6	111	+100	-79	+34
7	114	n.a.	-71	+427
8	117	0	-85	+36
9	132	+78	-97	+63
10	170	n.a.	-40	+81
11	171	n.a.	-33	+82
12	172	+400	+35	+4
13	173	n.a.	n.a.	n.a.
14	174	n.a.	-63	+82
15	175	n.a.	0	+100
16	182	n.a.	-33	-33

[a] Reaction conditions: 1 mg/mL ADH, 0.5 mM NAD(P)H, KPi (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm, 16 h. n.a. not applicable.

Supplementary figures

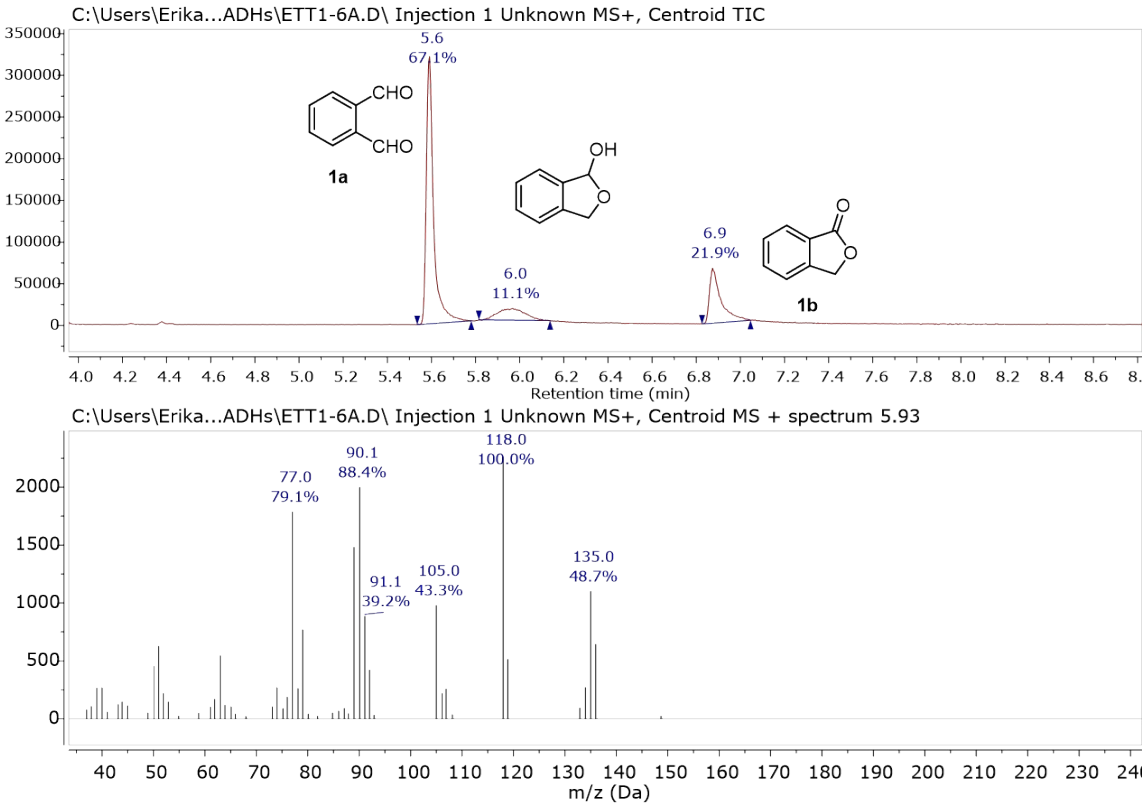


Figure S1. GC-MS analysis of the reaction of **1a** with ADH-170 (corresponding to entry 6 in Table S1) showing accumulation of intermediate lactol. MS fragmentation is of the lactol (peak at 6.0 min) and compatible with the one reported in literature.[6]

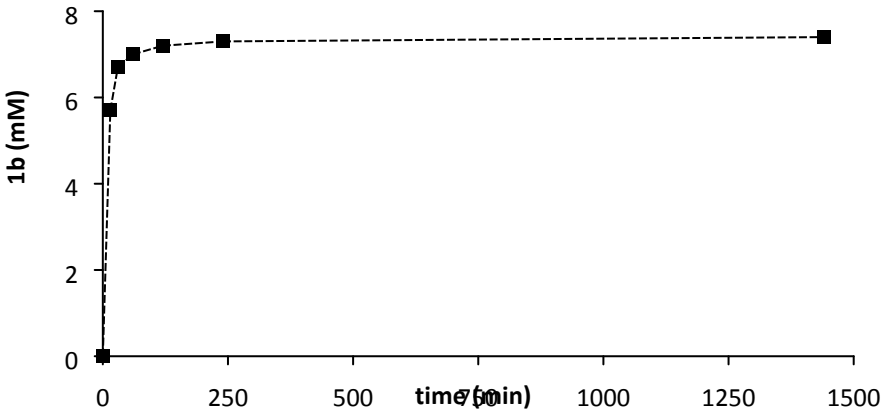
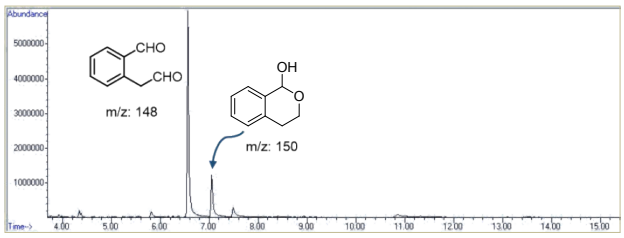


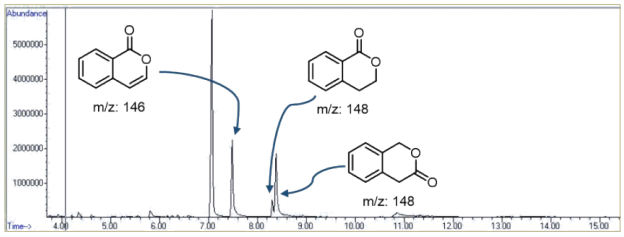
Figure S2. Time study of the conversion of **1a** to **1b** by ADH-172. 10 mM **1a**, 0.5 mM NADH, 1 mg/mL enzyme, KPi buffer (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm.

Trace A



Peak at 7 min: EI MS (70 eV, m/z (%)): 150.0 (M^+, 3), 132.1 (13), 104.1 (100), 103.1 (27), 78.1 (22).

Trace B



Peak at 7.5 min: EI MS (70 eV, m/z (%)): 146.0 (M^+, 61), 118.1 (100), 90.1 (67), 63.0 (30).

Trace C

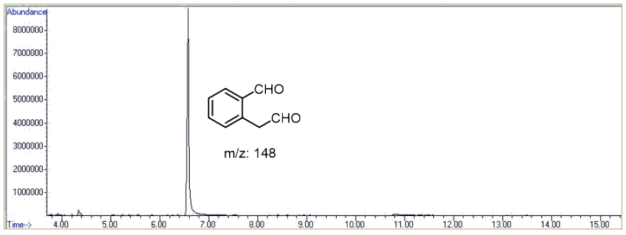


Figure S3. GC-MS traces of biotransformations (A, using ADH-110; B, using ADH-174) and blank reaction (C, no enzyme) using compound **2a** as substrate. 10 mM **2a**, 0.5 mM NADH, 1 mg/mL ADH, KPi buffer (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm, 16 h.

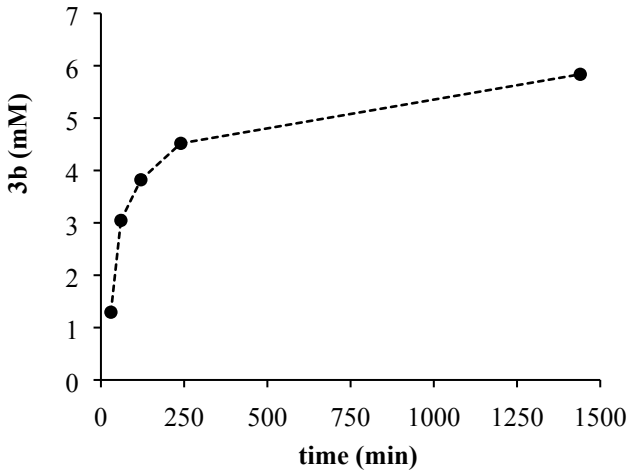


Figure S4. Time study of the conversion of **3a** to **3b** by ADH-114 (**3c** not detected). 10 mM **3a**, 0.5 mM NADH, 1 mg/mL enzyme, KPi buffer (50 mM, pH 7.0, 2 mM $MgCl_2$), 5 vol% CH_3CN, 30 °C, 120 rpm.

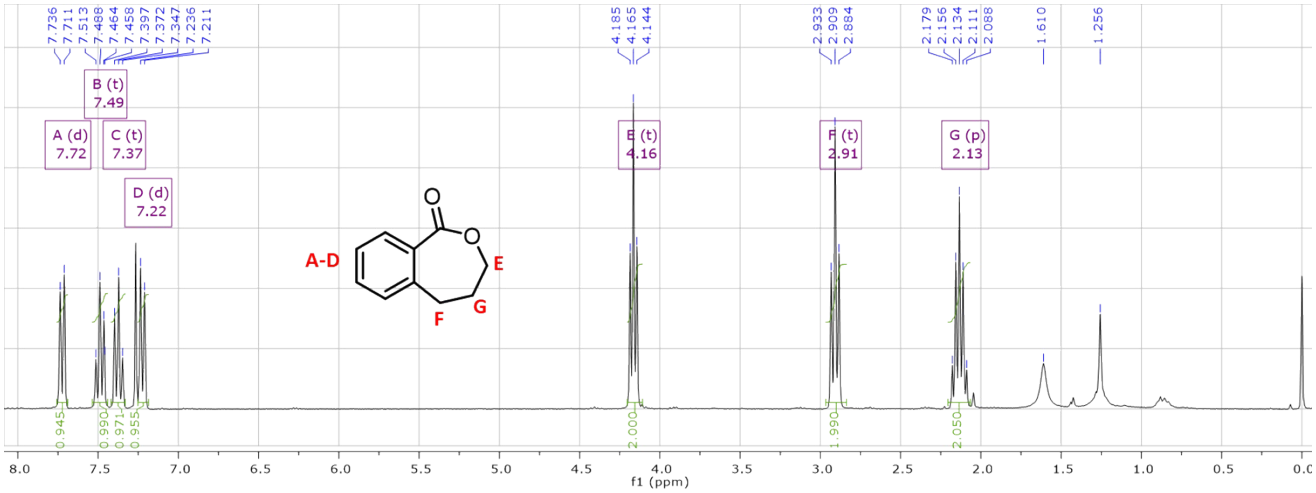
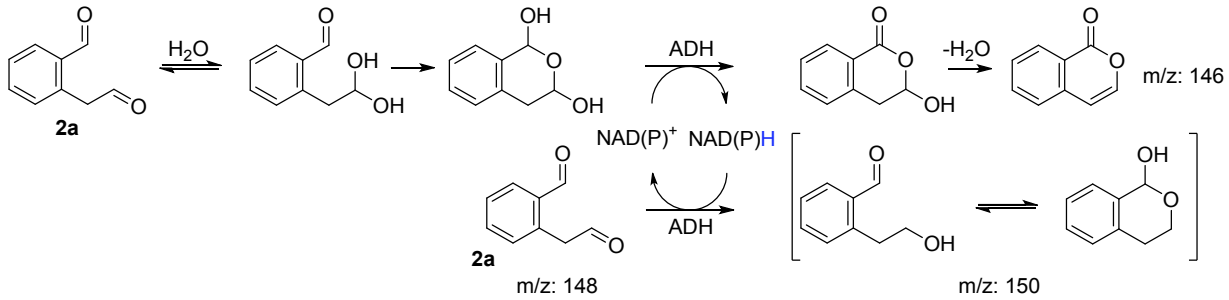
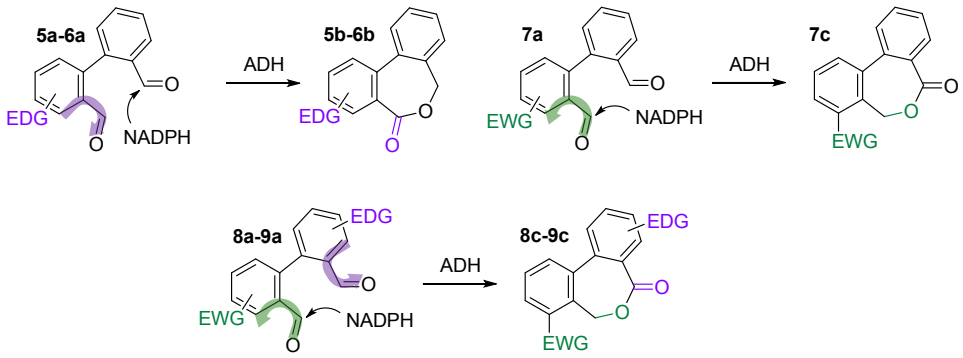


Figure S5. ^{1}H-NMR of lactone **3b** obtained from scale-up biotransformation of **3a** with ADH-114 (200 mg scale, see 'synthetic procedures' section). Only one regioisomer is detected.

Supplementary schemes



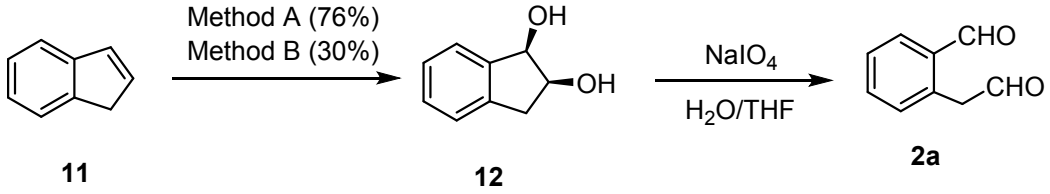
Scheme S1. Possible pathways in the conversion of **2a** leading to depletion of substrate and formation of side-products in a redox-neutral manner, as detected in Figure S3.



Scheme S2. Effect of the electronic properties of the substitution on the regioselectivity of ADH in the conversion of substituted [1,1'-biphenyl]-2,2'-dicarbaldehyde substrates **5a-9a** (EDG: electron-donating group; EWG: electron-withdrawing group).

Synthetic procedures

Synthesis of 2-(2-oxoethyl)benzaldehyde **2a**

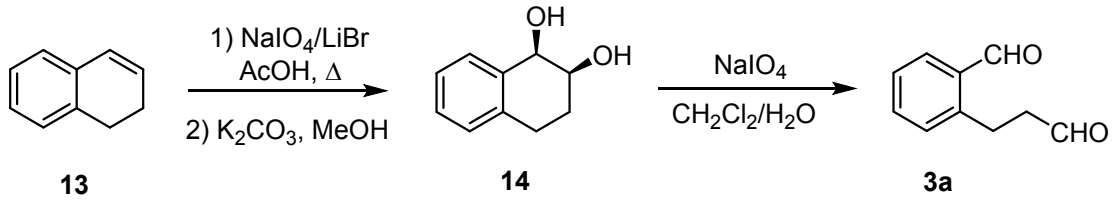


Method A: in a sealed glass vial indene **11** (250 mg, 2.6 mmol) was dissolved in a mixture of THF/H_2O/^{t}BuOH 5:1:1 (total volume: 2.5 mL), then K_2OsO_4 (8 mg, 0.02 mmol) and *N*-methylmorpholine (328 mg, 2.8 mmol) were added, and the mixture was stirred at RT overnight. The reaction was quenched by addition of a 10% Na_2SO_3 solution, stirred 30 minutes and then extracted with EtOAc; the collected organic layers were dried over Na_2SO_4 and solvent was removed under vacuum. The crude was purified by FC (EtOAc), affording the *cis*-diol **12** in 76% yield (247 mg, 1.64 mmol).

Method B: a mixture of indene **11** (350 mg, 3.0 mmol), $NaIO_4$ (193 mg, 0.9 mmol) and LiBr (50 mg, 0.6 mmol) was dissolved in acetic acid (5 mL) and heated at 95 °C overnight. After cooling, the reaction was diluted with water and a 10% $Na_2S_2O_5$ solution and extracted with EtOAc; the collected organic fractions were washed with $NaHCO_3$ sat., brine and the solvent was evaporated. The crude was dissolved in MeOH (20 mL) and K_2CO_3 (620 mg, 4.5 mmol) was added; the mixture was stirred at 40 °C overnight. Then the solvent was removed, the residue was solubilized in NH_4Cl sat. and extracted with EtOAc; the collected organic layers were dried over Na_2SO_4 and solvent was removed under vacuum. The crude was purified by FC (CyHex/EtOAc 4:6), affording the *cis*-diol **12** in 30% yield (135 mg, 0.9 mmol). Spectroscopic data agree with those reported in literature.[7]
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.46 – 7.34 (m, 1H), 7.31 – 7.18 (m, 3H), 4.95 (d, *J* = 5.0 Hz, 1H), 4.52 – 4.37 (m, 1H), 3.09 (dd, *J* = 16.4, 5.7 Hz, 1H), 2.92 (dd, *J* = 16.4, 3.5 Hz, 1H), 2.68 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.1, 140.3, 129.0, 127.3, 125.5, 125.2, 76.1, 73.6, 38.7.

A solution of $NaIO_4$ (240 mg, 1.1 mmol) in H_2O (11 mL) was added to a solution of the diol **12** in THF (4.8 mL) and the mixture was stirred at RT for 60 minutes. The reaction was extracted with CH_2Cl_2 the collected organic fractions were washed with brine, dried over Na_2SO_4 and solvent was removed under vacuum, yielding 2-(2-oxoethyl)benzaldehyde **2a** in 87% yield (122 mg, 0.84 mmol), as an unstable oil. Spectroscopic data agree with those reported in the literature.[8]
^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.05 (s, 1H), 9.80 (s, 1H), 7.85 (dd, *J* = 7.2, 1.8 Hz, 1H), 7.70 – 7.45 (m, 2H), 7.36 – 7.15 (m, 1H), 4.15 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.5, 193.5, 135.8, 134.3, 134.2, 132.9, 128.3, 48.4.

Synthesis of 2-(3-oxopropyl)benzaldehyde **3a**

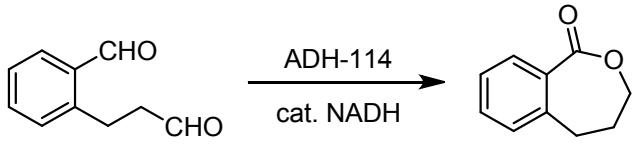


13 **14** **3a**

A mixture of 1,2-dihydronaphtalene **13** (800 mg, 6.1 mmol), $NaIO_4$ (394 mg, 1.84 mmol) and LiBr (106 mg, 1.22 mmol) was dissolved in acetic acid (10 mL) and heated at 95 °C overnight. After cooling, the reaction was diluted with water and a 10% $Na_2S_2O_5$ solution and extracted with EtOAc; the collected organic fractions were washed with $NaHCO_3$ sat., brine and the solvent was evaporated. The crude was dissolved in MeOH (30 mL) and K_2CO_3 (1.26 g , 9.1 mmol) was added; the mixture was stirred at room temperature overnight. Then the solvent was removed, the residue was solubilized in NH_4Cl sat. and extracted with EtOAc; the collected organic layers were dried over Na_2SO_4 and solvent was removed under vacuum. The crude was purified by FC (CyHex/EtOAc 7:3), affording the *cis*-diol **14** in 30% yield (302 mg, 1.8 mmol). Spectroscopic data agree with those reported in literature.[9]
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.47 – 7.40 (m, 1H), 7.25 – 7.20 (m, 2H), 7.16 – 7.10 (m, 1H), 4.70 (s, 1H), 4.09 – 3.96 (m, 1H), 3.04 – 2.91 (m, 1H), 2.86 – 2.70 (m, 1H), 2.37 (s, 1H), 2.12 – 1.76 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.5, 136.3, 130.0, 128.8, 128.3, 126.6, 70.1, 69.7, 27.1, 26.4.

A solution of $NaIO_4$ (472 mg, 0.5 mmol) in H_2O (3.6 mL) was added to a solution of the diol **14** in CH_2Cl_2 (18 mL) and the mixture was stirred at RT for 60 minutes. The two phases were then separated and the organic fraction was dried over Na_2SO_4 and solvent was removed under vacuum, yielding 2-(3-oxopropyl)benzaldehyde in 91% yield (271 mg, 1.67 mmol). Spectroscopic data agree with those reported in the literature.[10]
^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.17 (s, 1H), 9.83 (t, *J* = 1.3 Hz, 1H), 7.81 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.53 (td, *J* = 7.5, 1.5 Hz, 1H), 7.44 (td, *J* = 7.5, 1.4 Hz, 1H), 7.33 (dd, *J* = 7.6, 1.3 Hz, 1H), 3.36 (t, *J* = 7.4, 2H), 2.80 (td, *J* = 7.5, 1.3 Hz, 2H) . ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.4, 193.2, 142.9, 134.0, 133.9, 131.2, 127.2, 45.2, 25.9.

Synthesis of 4,5-dihydrobenzo[*c*]oxepin-1(3*H*)-one **3b**

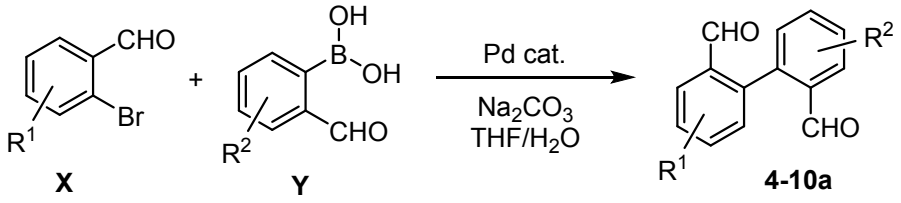


3a **3b**

An aliquot of purified enzyme (30 mg, 1 mg/mL final concentration) was added to a phosphate buffer solution (50 mM, pH 7.0, supplemented with 2 mM $MgCl_2$ and 5 vol% CH_3CN) containing the substrate (100 mg, 10 mM final concentration) and the nicotinamide cofactor (0.5 mM). The reaction mixture was incubated at 30 °C and 120 rpm. After 5 h, additional 10 mM of **3a** and 0.5 mM of NADH were added; total reaction time 24 h. After incubation, the mixture was extracted with EtOAc, and the

crude was purified by FC (CyHex:EtOAc 7:3), yielding 105 mg (0.65 mmol) of lactone **3b** (53% isolated yield from 200 mg of starting material).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.72 (d, J = 7.6 Hz, 1H), 7.49 (t, J = 7.4 Hz, 1H), 7.37 (t, J = 7.5 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 4.16 (t, J = 6.3 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.13 (p, J = 6.8 Hz, 2H). Spectroscopic data agree with literature.[11]

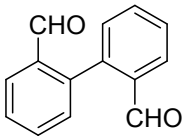
Synthesis of substituted 1,1´-[biphenyl]-2,2´-dicarbaldehydes **4-10a**



4a: R^1= H; R^2 = H
5a: R^1= 3-OMe; R^2 = H
6a: R^1= 5-OMe; R^2 = H
7a: R^1= 3-F; R^2 = H
8a: R^1= 3'-OMe; R^2 = 3-F
9a: R^1= 5'-OMe; R^2 = 3-F
10a: R^1= 3'-F; R^2 = 3-F

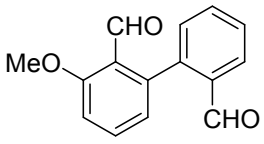
General procedure: a mixture of the bromobenzaldehyde **X**, Pd catalyst, ligand, Na_2CO_3 and the formylphenylboronic acid **Y** were dissolved in THF and H_2O (10 %v/v); the reaction was stirred at 60 °C until completion. After cooling, the reaction was diluted with 0.5 N HCl and extracted with EtOAc; the collected organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum. The crude was purified by FC affording the desired product.

[1,1´-biphenyl]-2,2´-dicarbaldehyde (**4a**)



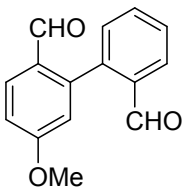
Reaction conditions: 2-bromobenzaldehyde (200 mg, 1.08 mmol), 2-formylphenylboronic acid (211 mg, 1.41 mmol), $Pd(AcO)_2$ (12 mg, 0.05 mmol), PPh_3 (43 mg, 0.16 mmol), Na_2CO_3 (229 mg, 2.16 mmol) in THF (5.40 mL THF) and H_2O (0.54 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 88% (200 mg, 0.95 mmol).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.85 (s, 2H), 8.07 (dd, J = 7.6, 1.6 Hz, 2H), 7.71 – 7.64 (m, 2H), 7.61 (t, J = 7.5 Hz, 2H), 7.36 (dd, J = 7.4, 1.4 Hz, 2H). HRMS: 211.0756 (calc. 211.0753). Spectroscopic data agree with literature.[12]

3-methoxy-[1,1´-biphenyl]-2,2´-dicarbaldehyde (**5a**)



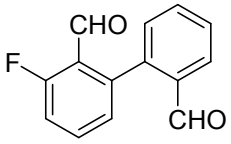
Reaction conditions: 2-bromo-6-methoxybenzaldehyde (200 mg, 0.93 mmol), 2-formylphenylboronic acid (181 mg, 1.21 mmol), $Pd(AcO)_2$ (10 mg, 0.05 mmol), PPh_3 (37 mg, 0.15 mmol), Na_2CO_3 (197 mg, 1.86 mmol) in THF (4.65 mL THF) and H_2O (0.47 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 147 mg (0.61 mmol, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.36 (s, 1H), 9.79 (d, J = 0.7 Hz, 1H), 7.99 (dd, J = 7.7, 1.5 Hz, 1H), 7.66 – 7.46 (m, 3H), 7.22 (dd, J = 7.4, 1.3 Hz, 1H), 7.10 (d, J = 8.5 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 3.99 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 191.6, 190.4, 162.1, 143.8, 141.0, 134.4, 134.1, 133.4, 130.4, 128.1, 128.0, 124.3, 123.8, 111.7, 56.8. HRMS: [M^+] 241.0858 (calc. 241.0859).

5-methoxy-[1,1´-biphenyl]-2,2´-dicarbaldehyde (**6a**)



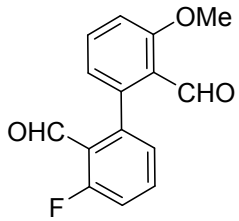
Reaction conditions: 2-bromo-4-methoxybenzaldehyde (200 mg, 0.93 mmol), 2-formylphenylboronic acid (181 mg, 1.21 mmol), $Pd(AcO)_2$ (10 mg, 0.05 mmol), PPh_3 (37 mg, 0.15 mmol), Na_2CO_3 (197 mg, 1.86 mmol) in THF (4.65 mL THF) and H_2O (0.47 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 129 mg (0.54 mmol, 58%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.85 (s, 1H), 9.67 (s, 1H), 8.06 (dd, J = 7.6, 1.6 Hz, 1H), 8.04 (d, J = 8.7 Hz, 1H), 7.67 (td, J = 7.5, 1.6 Hz, 1H), 7.63 – 7.54 (m, 1H), 7.37 (dd, J = 7.4, 1.2 Hz, 1H), 7.12 – 7.06 (m, 1H), 6.81 (d, J = 2.5 Hz, 1H), 3.91 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 191.2, 189.8, 163.5, 143.8, 141.4, 134.7, 133.6, 131.5, 131.2, 129.0, 128.5, 128.3, 116.8, 114.7, 55.9. HRMS: [M^+] 241.0860 (calc. 241.0859). Spectroscopic data agrees with literature.[13]

3-fluoro-[1,1´-biphenyl]-2,2´-dicarbaldehyde (**7a**)



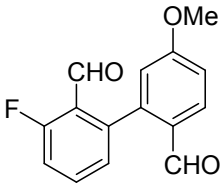
Reaction conditions: 2-bromo-6-fluoro-benzaldehyde (200 mg, 0.98 mmol), 2-formylphenylboronic acid (192 mg, 1.28 mmol), $Pd(AcO)_2$ (11 mg, 0.05 mmol), PPh_3 (39 mg, 0.15 mmol), Na_2CO_3 (208 mg, 2.16 mmol) in THF (4.90 mL THF) and H_2O (0.49 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 161 mg (0.71 mmol, 72%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.17 (s, 1H), 9.82 (s, 1H), 8.06 – 7.92 (m, 1H), 7.76 – 7.49 (m, 3H), 7.37 – 7.18 (m, 2H), 7.09 (d, J = 7.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 191.1, 187.8 (d, J_{CF} = 6.7 Hz), 164.4 (d, J_{CF} = 261.3 Hz), 141.9 (d, J_{CF} = 1.4 Hz), 141.4 (d, J_{CF} = 2.4 Hz), 134.9 (d, J_{CF} = 10.4 Hz), 134.2, 133.6, 130.8, 129.3, 128.8, 127.7 (d, J_{CF} = 3.6 Hz), 123.2 (d, J_{CF} = 7.1 Hz), 116.7 (d, J_{CF} = 21.5 Hz). HRMS: [M^+] 229.0662 (calc. 229.0659).

3-fluoro-3´-methoxy-[1,1´-biphenyl]-2,2´-dicarbaldehyde (**8a**)



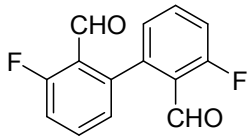
Reaction conditions: 2-bromo-6-methoxybenzaldehyde (225 mg, 1.04 mmol), 3-fluoro-2-formylphenylboronic acid (260 mg, 1.55 mmol), $Pd(dppf)Cl_2$ (20 mg, 0.03 mmol, 0.02 eq.), Na_2CO_3 (254 mg, 2.40 mmol) in THF (6.00 mL THF) and H_2O (0.60 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 244 mg (0.94 mmol, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.38 (d, J = 0.6 Hz, 1H), 10.11 (s, 1H), 7.59 – 7.49 (m, 2H), 7.18 (ddd, J = 10.6, 8.4, 1.1 Hz, 1H), 7.07 (dd, J = 8.5, 0.9 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 3.98 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 190.3, 188.1 (d, J_{CF} = 6.6 Hz), 164.1 (d, J_{CF} = 259.8 Hz), 162.3, 144.5 (d, J_{CF} = 1.6 Hz), 141.1 (d, J_{CF} = 2.4 Hz), 134.7 (d, J_{CF} = 10.4 Hz), 134.6, 126.09 (d, J_{CF} = 3.5 Hz), 123.1, 123.1, 122.6 (d, J_{CF} = 7.3 Hz), 115.8 (d, J_{CF} = 21.5 Hz), 111.6, 56.1. HRMS: 259.0767 (calc. 259.0765).

3-fluoro-5′-methoxy-[1,1′-biphenyl]-2,2′-dicarbaldehyde (**9a**)



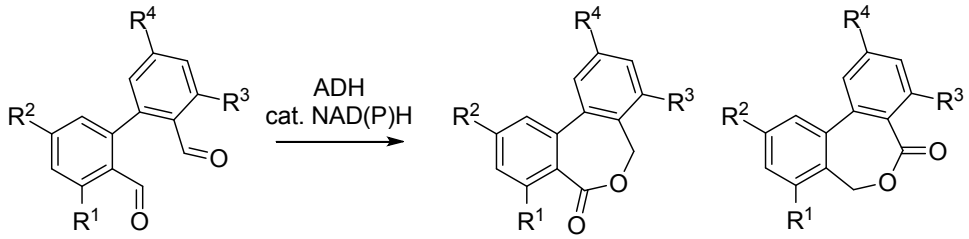
Reaction conditions: 2-bromo-4-methoxybenzaldehyde (200 mg, 0.93 mmol), 3-fluoro-2-formylphenylboronic acid (235 mg, 1.40 mmol), $Pd(dppf)Cl_2$ (16 mg, 0.02 mmol, 0.02 eq.), Na_2CO_3 (229 mg, 2.16 mmol) in THF (5.40 mL THF) and H_2O (0.54 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 197 mg (0.76 mmol, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.14 (s, 1H), 9.66 (d, J = 0.6 Hz, 1H), 7.97 (d, J = 8.7 Hz, 1H), 7.62 (ddd, J = 8.4, 7.6, 5.5 Hz, 1H), 7.32 – 7.21 (m, 1H), 7.14 – 6.94 (m, 2H), 6.72 (d, J = 2.5 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 189.7, 187.7 (d, J_{CF} = 5.9 Hz), 164.0 (d, J_{CF} = 261.6 Hz), 163.6, 143.7 (d, J_{CF} = 2.4 Hz), 142.0 (d, J_{CF} = 1.4 Hz), 134.8 (d, J_{CF} = 10.4 Hz), 132.0, 127.8, 127.3 (d, J_{CF} = 3.6 Hz), 123.2 (d, J_{CF} = 7.1 Hz), 116.8 (d, J_{CF} = 21.4 Hz), 116.2, 114.1, 55.8. HRMS: 259.0770 (calc. 259.0765).

3,3’-difluoro-[1,1′-biphenyl]-2,2′-dicarbaldehyde (**10a**)



Reaction conditions: 2-bromo-6-fluorobenzaldehyde (200 mg, 0.98 mmol), 3-fluoro-2-formylphenylboronic acid (215 mg, 1.28 mmol), $Pd(AcO)_2$ (11 mg, 0.05 mmol), PPh_3 (39 mg, 0.15 mmol), Na_2CO_3 (200 mg, 1.96 mmol) in THF (4.90 mL THF) and H_2O (0.49 mL). The crude was purified by FC (Cyhex/EtOAc 8:2). Yield: 225 mg (0.91 mmol, 93%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.22 (s, 1H), 7.64 – 7.55 (m, 2H), 7.30 – 7.18 (m, 2H), 6.98 (d, J = 7.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 187.6 (d, J_{CF} = 8.4 Hz), 164.9 (d, J_{CF} = 259.4 Hz), 142.0 (d, J_{CF} = 2.1 Hz), 135.1 (d, J_{CF} = 10.4 Hz), 126.4 (d, J_{CF} = 3.6 Hz), 122.6 (d, J_{CF} = 7.3 Hz), 116.4 (d, J_{CF} = 21.7 Hz). HRMS: 247.0565 (calc. 247.0565).

Synthesis of substituted dibenzo[*c,e*]oxepin-5(7*H*)-ones



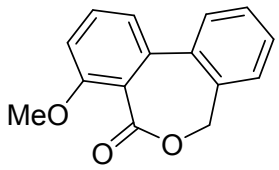
4a-10a **4b-10b** **4c-10c**

	R^1	R^2	R^3	R^4
4a-c	H	H	H	H
5a-c	OMe	H	H	H
6a-c	H	OMe	H	H
7a-c	F	H	H	H
8a-c	F	H	OMe	H
9a-c	F	H	H	OMe
10a-c	F	H	F	H

General procedure: an aliquot of enzyme (final concentration 1 mg/mL) was added to phosphate buffer solution (50 mM, pH 7.0, supplemented with 2 mM $MgCl_2$ and 5 vol% CH_3CN) containing 10 mM substrate and the nicotinamide cofactor (0.5 mM). The reaction mixture was incubated at 30 °C and 120 rpm, 16 h. The reaction mixture was then extracted with EtOAc; the collected organic fractions

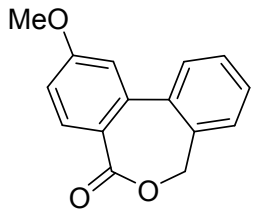
were dried over Na_2SO_4 and the solvent was removed under vacuum. The crude was purified by FC affording the desired product.

4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one (**5b**)



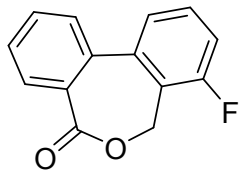
Starting from 48 mg (0.2 mmol) of 5-methoxy-[1,1´-biphenyl]-2,2´-dicarbaldehyde **5a**, using ADH-132 and NADH. The crude was purified by FC (toluene/EtOAc 12:1). Yield: 14 mg (0.058 mmol, 29%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.67 (d, *J* = 7.3 Hz, 1H), 7.72 – 7.64 (m, 1H), 7.59 – 7.45 (m, 2H), 7.45 – 7.36 (m, 2H), 7.14 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.06 (dd, *J* = 8.4, 0.9 Hz, 1H), 5.07 (d, *J* = 12.1 Hz, 1H), 4.90 (d, *J* = 12.1 Hz, 1H), 3.95 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.7, 158.6, 138.9, 138.6, 135.4, 132.3, 130.2, 128.8, 128.7, 128.6, 120.7, 120.4, 111.4, 68.8, 56.5. HRMS: [M^+] 241.0859 (calc. 241.0859).

2-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one (**6b**)



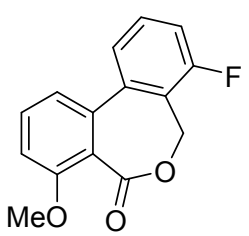
Starting from 24 mg (0.1 mmol) of 3-methoxy-[1,1´-biphenyl]-2,2´-dicarbaldehyde **6a**, using Lk-ADH_{mut} and NADPH. The crude was purified by FC (toluene/EtOAc 10:1). Yield: 16 mg (0.067 mmol, 66%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.98 (d, *J* = 8.6 Hz, 1H), 7.68 – 7.60 (m, 1H), 7.58 – 7.50 (m, 1H), 7.49 – 7.40 (m, 2H), 7.11 – 6.95 (m, 2H), 5.06 (bs, 1H), 4.97 (bs, 1H), 3.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 162.7, 139.4, 139.0, 135.0, 134.4, 130.1, 128.8, 128.6, 123.1, 114.1, 113, 69.1, 55.6. HRMS: [M^+] 241.0855 (calc. 241.0859). Spectroscopic data agree with literature.[14]

8-fluorodibenzo[*c,e*]oxepin-5(*7H*)-one (**7c**)



Starting from 23 mg (0.1 mmol) 3-fluoro-[1,1´-biphenyl]-2,2´-dicarbaldehyde **7a**, using Lk-ADH_{mut} and NADPH. The crude was purified by FC (CyHex/EtOAc 8:2). Yield: 17 mg (0.074 mmol, 74%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.00 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.76 – 7.37 (m, 5H), 7.23 – 7.11 (m, 1H), 5.51 (s, 1H), 4.80 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2, 159.6 (d, J_{CF} = 249.7 Hz), 141.6 (d, J_{CF} = 3.0 Hz), 136.3 (d, J_{CF} = 3.0 Hz), 132.8, 132.2, 131.3 (d, J_{CF} = 9.0 Hz), 130.8, 129.2 , 128.9, 124.3 (d, J_{CF} = 3.5 Hz), 122.6 (d, J_{CF} = 15.8 Hz), 115.5 (d, J_{CF} = 22.3 Hz), 60.6 (d, J_{CF} = 5.9 Hz). HRMS: [M^+] 229.0660 (calc. 229.0659).

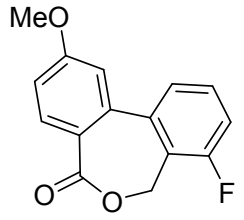
8-fluoro-4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one (**8c**)



Starting from 77 mg (0.30 mmol) of 3-fluoro-3'-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **8a**, enzyme: ADH132 (C_{fin}: 2 mg/mL), NADH. The crude was purified by FC (toluene/EtOAc 12:1). Yield: 22 mg (0.85 mmol, 28%).

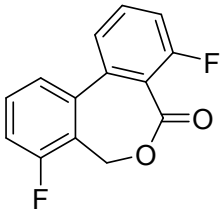
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.56 (t, J = 8.1 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.18 – 7.05 (m, 3H), 5.43 (d, J = 12.5 Hz, 1H), 4.79 (d, J = 12.5 Hz, 1H), 3.95 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.6, 159.7 (d, J_{CF} = 249.5 Hz), 158.6, 141.3 (d, J_{CF} = 3.0 Hz), 137.5 (d, J_{CF} = 2.7 Hz), 132.5, 131.2 (d, J_{CF} = 9.0 Hz), 124.2 (d, J = 3.4 Hz), 123.0 (d, J_{CF} = 16.0 Hz), 120.7, 120.2, 115.5 (d, J_{CF} = 22.3 Hz), 111.9, 60.1 (d, J_{CF} = 5.7 Hz), 56.5. HRMS: [M^+] 259.0764 (calc. 259.0764).

8-fluoro-2-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one (**9c**)



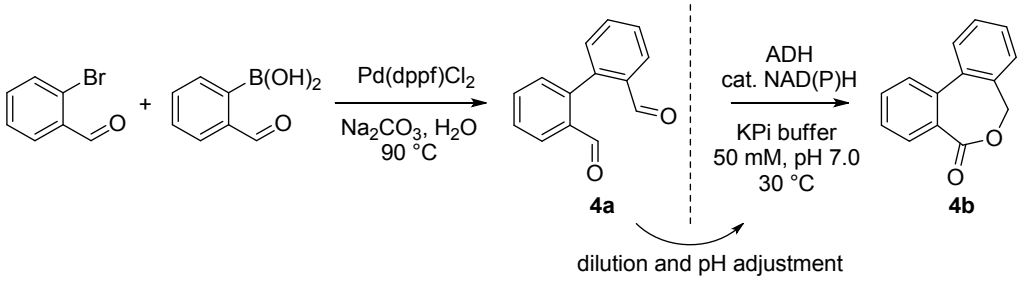
Starting from 52 mg (0.2 mmol) of 3-fluoro-5'-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **9a** using Lk-ADH_{mut} and NADPH. The crude was purified by FC (toluene/EtOAc 13:1). Yield: 30 mg (0.12 mmol; 58 %).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.99 (d, J = 8.8 Hz, 1H), 7.58 – 7.37 (m, 2H), 7.18 (t, J = 8.4 Hz, 1H), 7.07 (s, 2H), 5.46 (s, 1H), 4.83 (s, 1H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 162.8, 159.6 (d, J_{CF} = 249.6 Hz), 141.5 (d, J_{CF} = 3.1 Hz), 138.4 (d, J_{CF} = 3.0 Hz), 134.7, 131.2 (d, J_{CF} = 8.9 Hz), 124.3 (d, J_{CF} = 3.4 Hz), 123.1, 122.7 (d, J_{CF} = 15.9 Hz), 115.7 (d, J_{CF} = 22.2 Hz), 114.6, 114.1, 60.5 (d, J_{CF} = 5.7 Hz), 55.8. HRMS: [M^+] 259.0766 (calc. 259.0764).

4,8-difluorodibenzo[*c,e*]oxepin-5(*7H*)-one (**10c**)



Starting from 100 mg (0.41 mmol) of 3,3'-difluoro-[1,1´-biphenyl]-2,2´-dicarbaldehyde **10a**, using Lk-ADH_{mut} and NADPH. The crude was purified by FC (CyHex/EtOAc 8:2). Yield: 57 mg (0.23 mmol, 57%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.63 (td, J = 8.1, 5.4 Hz, 1H), 7.56 – 7.42 (m, 2H), 7.39 (d, J = 7.8 Hz, 1H), 7.35 – 7.23 (m, 1H), 7.27 – 7.14 (m, 1H), 5.50 (d, J = 12.6 Hz, 1H), 4.82 (d, J = 12.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.3 (d, J_{CF} = 258.0 Hz), 159.7 (d, J_{CF} = 250.1 Hz), 140.4 (d, J_{CF} = 2.6 Hz), 138.0 (d, J_{CF} = 2.8 Hz), 133.3 (d, J_{CF} = 9.6 Hz), 131.6 (d, J_{CF} = 8.9 Hz), 124.4 (d, J_{CF} = 6.1 Hz), 124.3 (d, J_{CF} = 6.1 Hz), 122.7 (d, J_{CF} = 16.1 Hz), 119.5 (d, J_{CF} = 12.9 Hz), 116.9 (d, J_{CF} = 22.2 Hz), 116.1 (d, J_{CF} = 22.3 Hz), 60.4 (d, J_{CF} = 5.8 Hz). HRMS: [M^+] 247.0564 (calc. 247.0565).

One-pot synthesis of dibenzo[*c,e*]oxepin-5(7*H*)-one (**4b**)



2-Bromobenzaldehyde (50 mg, 0.27 mmol), $Pd(dppf)Cl_2$ (4 mg, 0.05mmol, 0.02 eq.), Na_2CO_3 (85 mg, 0.81 mmol, 3 eq.) and 2-formylphenylboronic acid (45 mg, 0.30 mmol, 1.1 eq.) were dissolved in degassed H_2O; the reaction was stirred at 90 °C until completion (checked by GC-MS). After cooling, the reaction was diluted with phosphate buffer (50 mM, pH 7.0, 2 mM $MgCl_2$) to a final volume of 18 mL and supplemented with 5% vol. CH_3CN as cosolvent; the enzyme (Lk-ADH_{mut}, final concentration: 1 mg/mL) and the cofactor (NADPH, final concentration 0.5 mM) were added and the reaction was incubated at 30 °C, 120 rpm for 18 hours. The biotransformation was extracted with EtOAc; the collected organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum. The crude was purified by FC (Cyhex/EtOAc 8:2) affording 27 mg of the desired product (0.13 mmol, 48% isolated yield).

^1H NMR (300 MHz, $CDCl_3$) δ = 8.01 (d, J = 8.2 Hz, 1H), 7.74 – 7.61 (m, 3H), 7.60 – 7.52 (m, 2H), 7.51 – 7.42 (m, 2H), 5.05 (d, J = 15.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4, 139.1, 137.4, 134.9, 132.7, 132.1, 130.8, 130.3, 128.8, 128.8, 128.7, 128.7, 128.6, 69.3. Spectroscopic data agree with literature.[15]

GC analyses

GC analyses were carried out on an Agilent 7890A, with FID detector, equipped with an Agilent HP5 column (30 m x 0.25 mm x 0.25 µm; method **1**) or an Agilent DB-1701 column (30 m x 0.25 mm x 0.25 µm; method **B** and **C**), using H_2 as carrier gas; injector temperature 250 °C, detector temperature 250 °C.

Method **A**: flow 1.4 mL/min, injection volume 5 µL, split ratio 50:1; temperature program: 100 °C, hold 1 min; 10 °C/min to 150 °C, hold 0 min, 20 °C/min to 300 °C, hold 1 min.

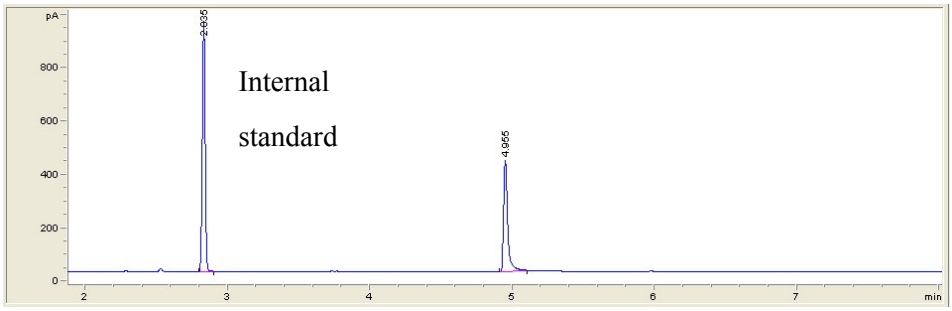
Method **B**: flow 1.0 mL/min, injection volume 5 µL, split ratio 20:1; temperature program: 100 °C, hold 5 min; 10 °C/min to 230 °C, hold 0 min, 20 °C/min to 280 °C, hold 2 min.

Method **C**: flow 1.0 mL/min, injection volume 5 µL, split ratio 20:1; temperature program: 100 °C, hold 1 min; 10 °C/min to 280 °C, hold 5 min.

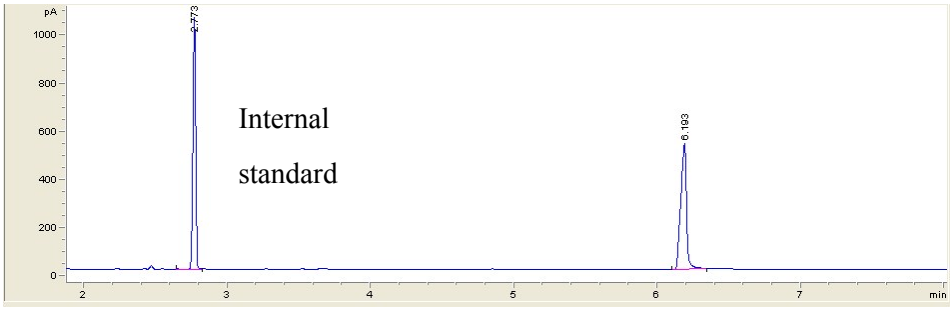
Compounds **1a-b**

Retention times (method **A**): 4.9 min **1a**; 6.2 min **1b**.

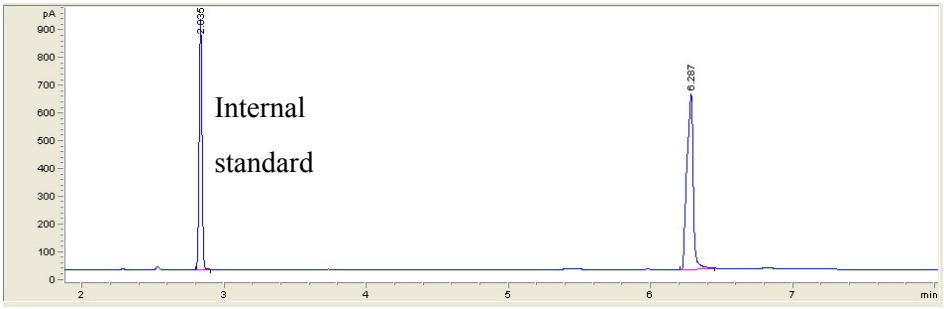
o-phthalaldehyde **1a** (purchased):



Phthalide **1b** (purchased):



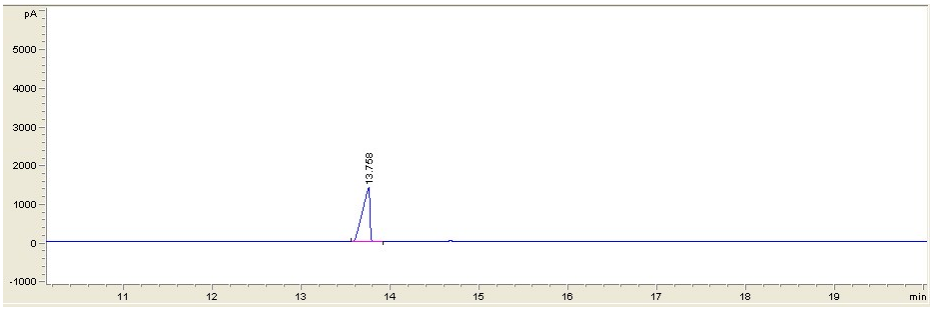
Biotransformation:



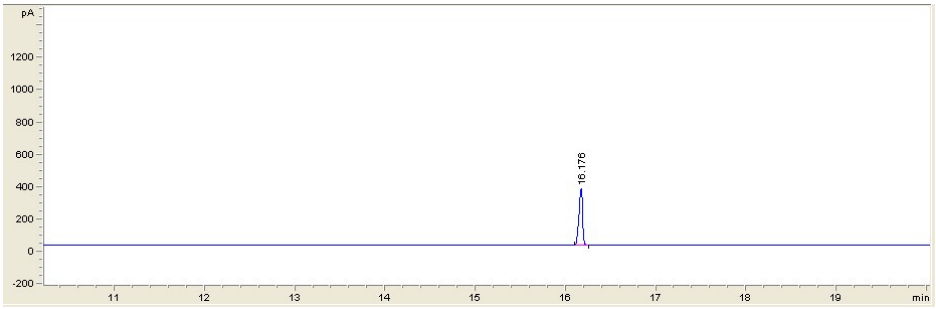
Compounds **2a-c**

Retention times (method **B**): 13.7 min **2a**; 16.2 min **2b**; 16.5 min **2c**.

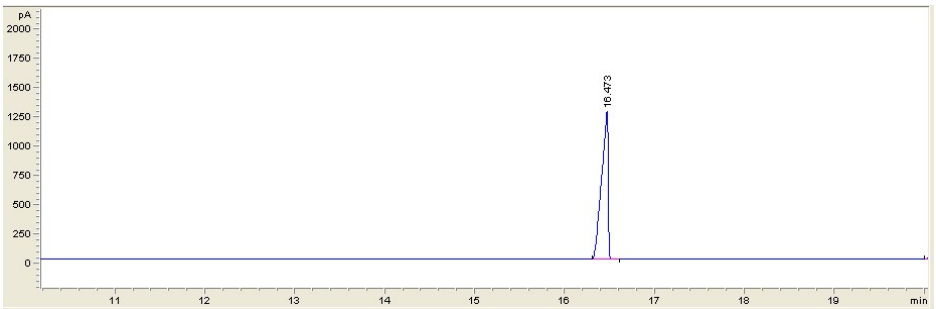
2-(2-oxoethyl)benzaldehyde **2a**:



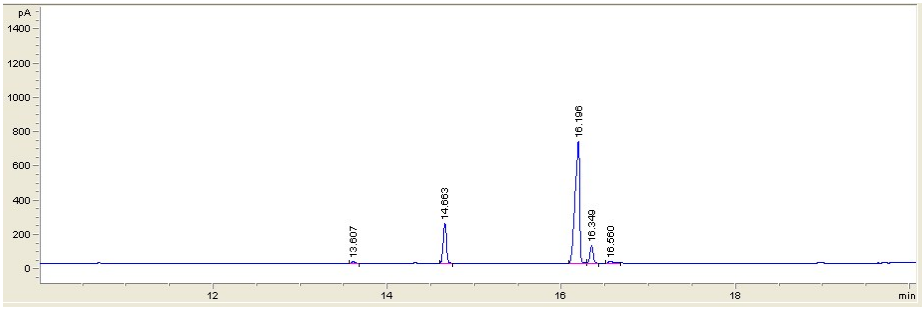
Isochroman-1-one **2b** (purchased):



Isochroman-3-one **2c** (purchased):



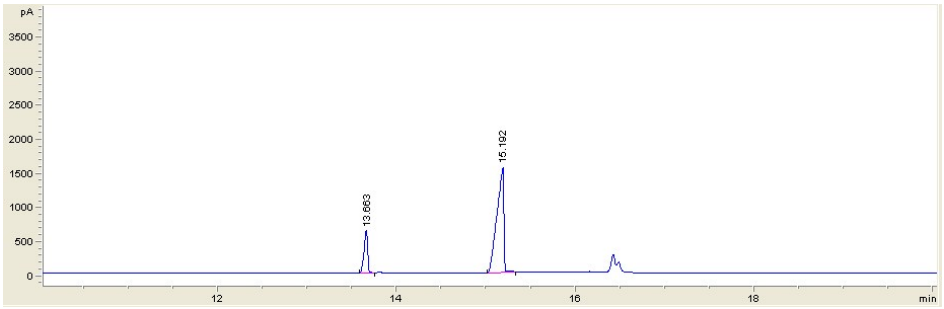
Biotransformation:



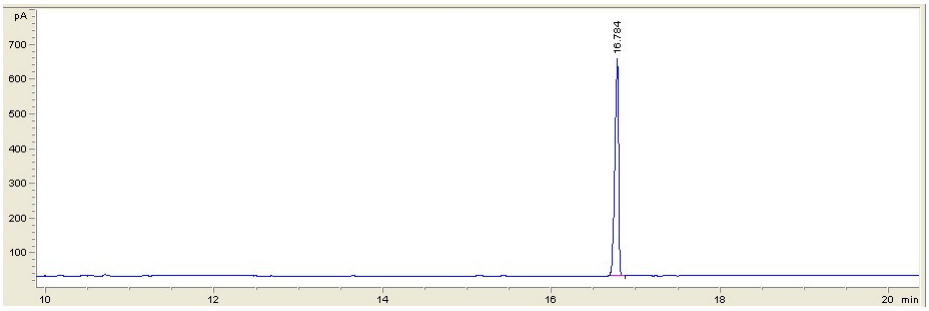
Compounds **3a-c**

Retention times (method **B**): 15.1 min **3a**; 16.8 min **3b**; 18.4 min **3c**.

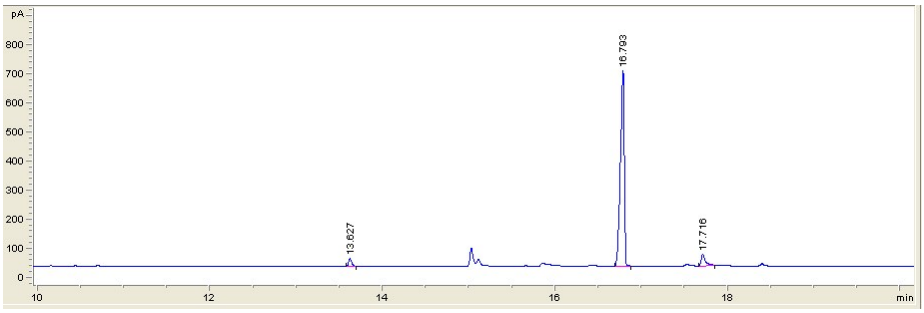
2-(3-oxopropyl)benzaldehyde **3a**:



4,5-dihydrobenzo[*c*]oxepin-1(3*H*)-one **3b**:



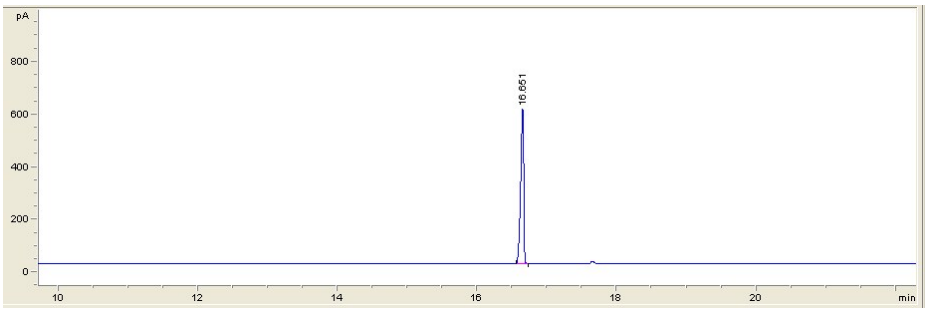
Biotransformation:



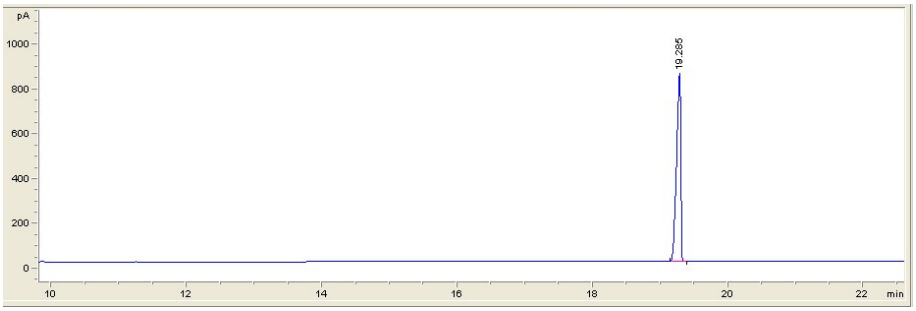
Compounds **4a-b**

Retention times (method **C**): 16.6 min **4a**; 19.3 min **4b**.

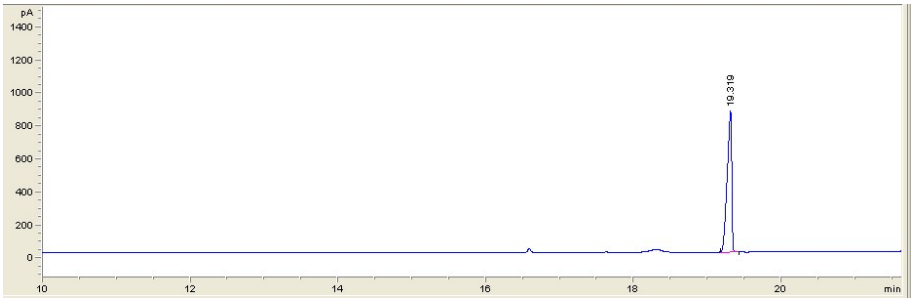
[1,1'-biphenyl]-2,2'-dicarbaldehyde **4a**:



dibenzo[*c,e*]oxepin-5(7*H*)-one **4b** (purchased):



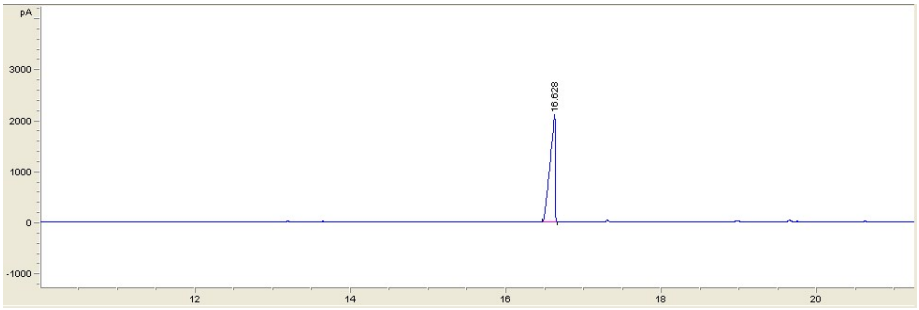
Biotransformation:



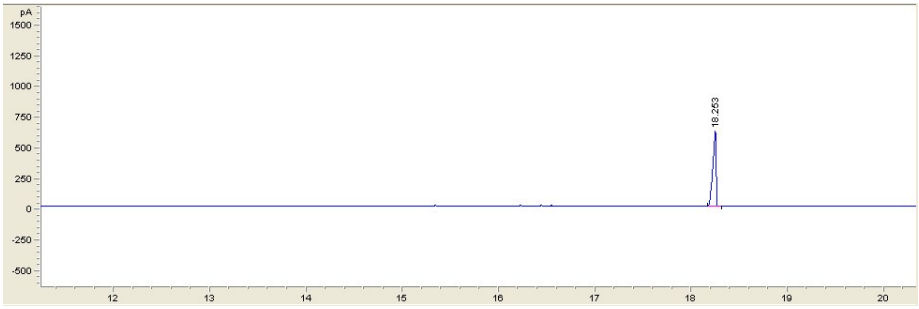
Compounds **5a-c**

Retention times (method **C**): 16.7 min **5a**; 18.2 min **5b**.

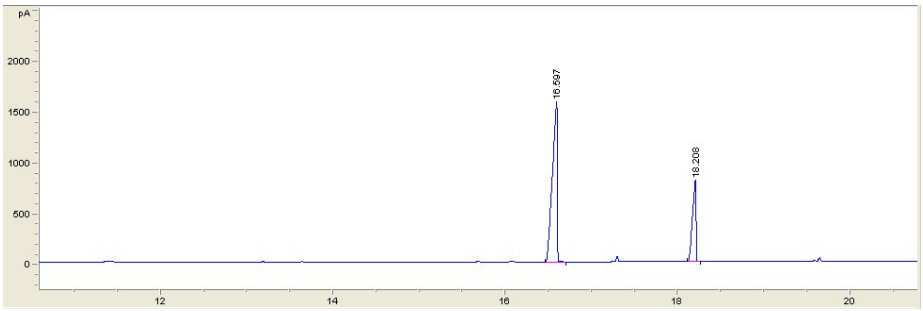
3-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **5a**:



4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **5b**:



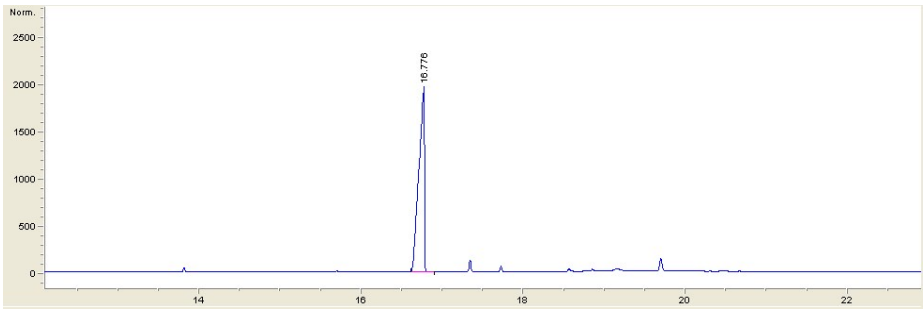
Biotransformation:



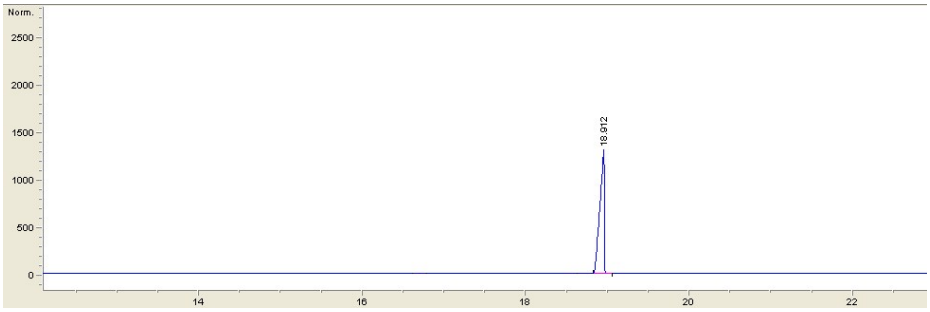
Compounds **6a-b**

Retention times (method **C**): 16.7 min **6a**; 18.6 min **6c**, 18.9 min **6b**.

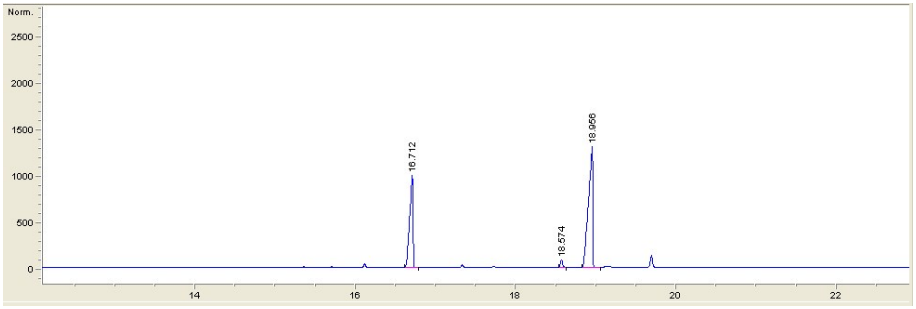
5-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **6a**:



2-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **6b**: 18.9 min



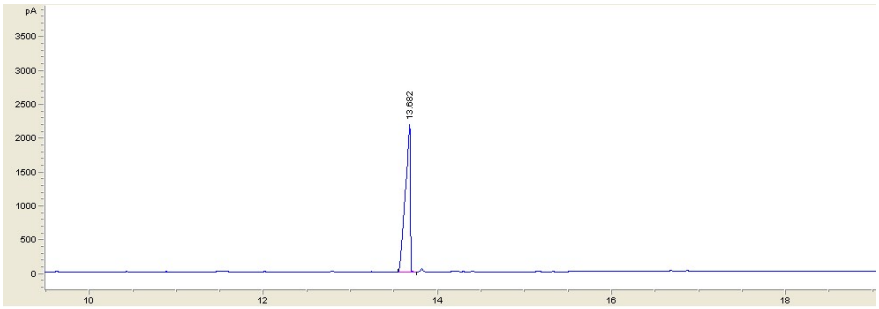
Biotransformation:



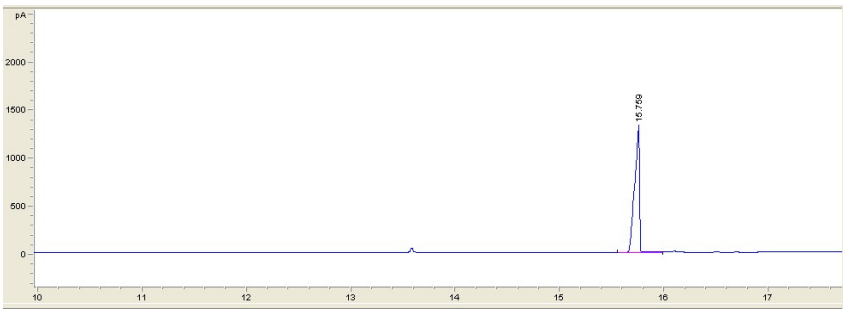
Compounds **7a-c**

Retention times (method **C**): 13.7 min **7a**; 15.8 min **7c**; 16.7 min **7b**.

3-fluoro-[1,1'-biphenyl]-2,2'-dicarbaldehyde **7a**:



8-fluorodibenzo[*c,e*]oxepin-5(7*H*)-one **7c**:



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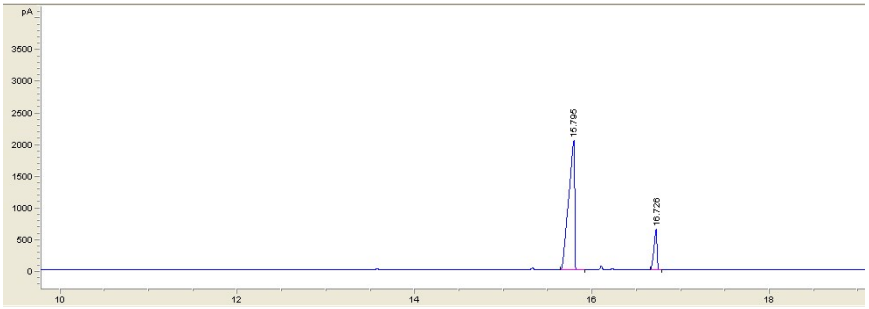
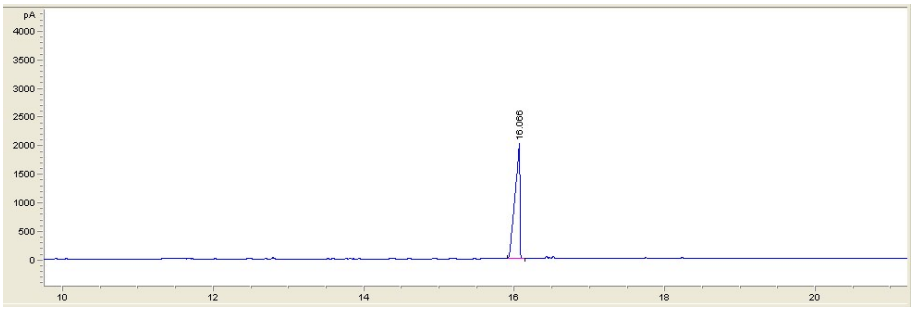


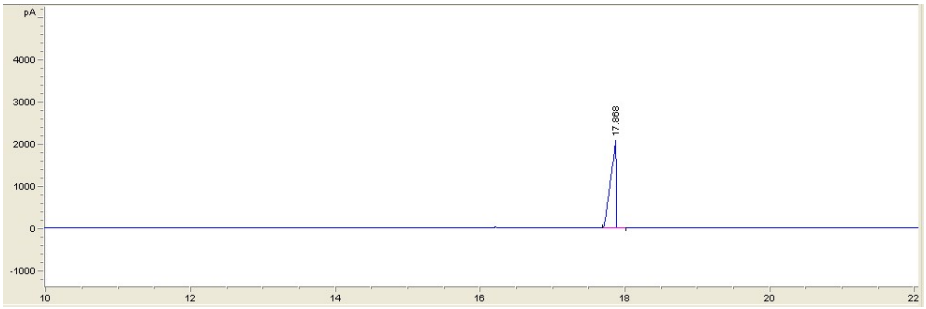
Compounds **8a-c**

Retention times (method **C**): 16.0 min **8a**; 17.8 min **8c**.

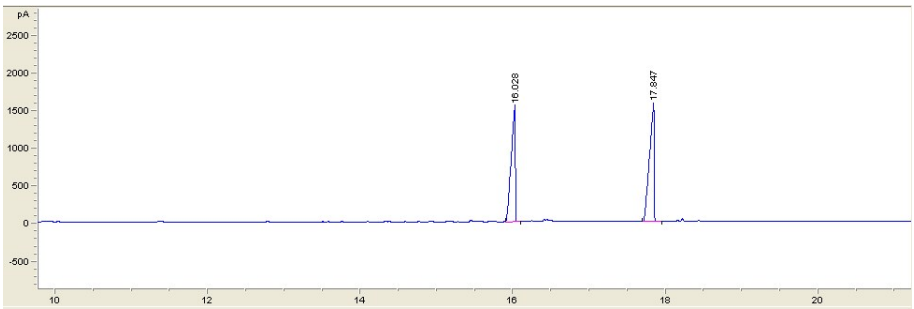
3-fluoro-3'-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **8a**:



8-fluoro-4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **8c**:



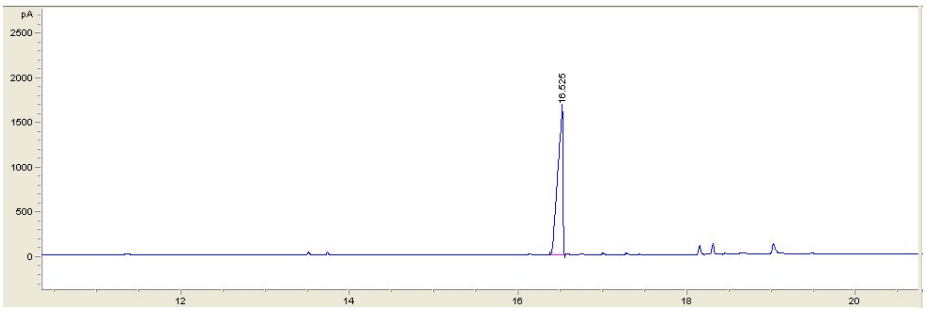
Biotransformation:



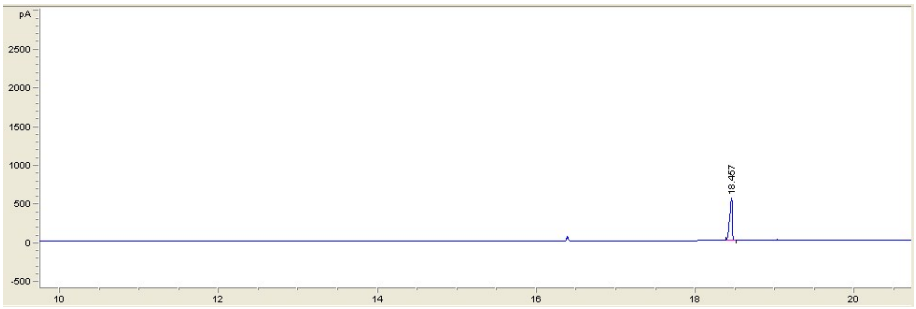
Compounds **9a-c**

Retention times (method **C**): 16.5 min **9a**; 18.5 min **9c**.

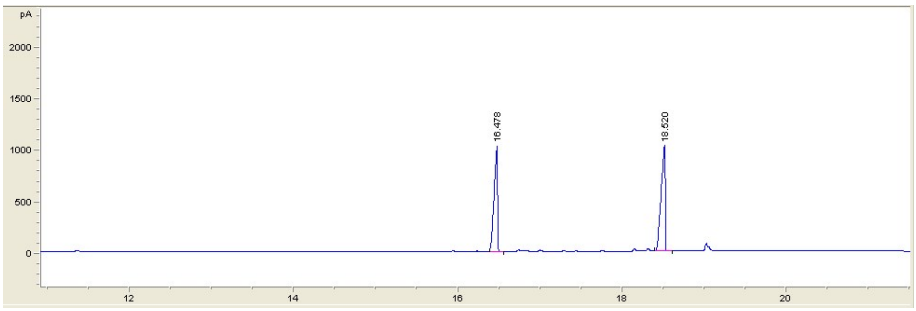
3-fluoro-5'-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **9a**:



8-fluoro-2-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **9c**:



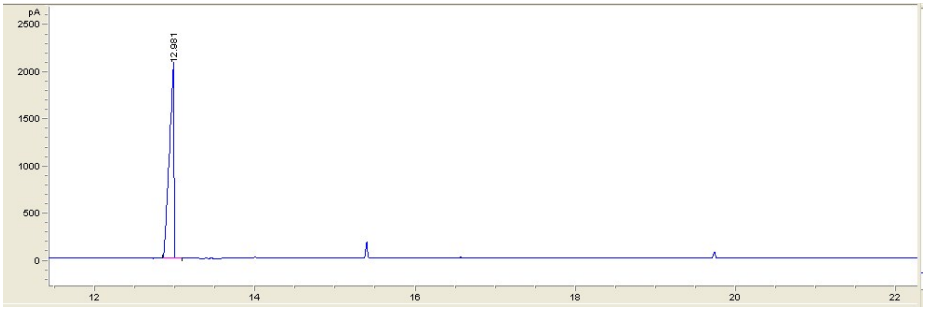
Biotransformation:



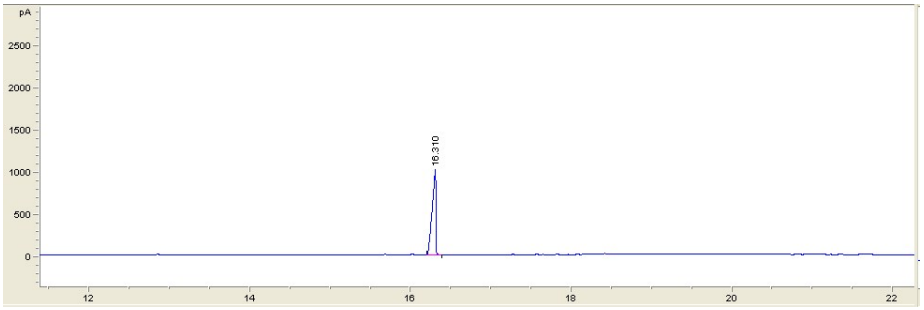
Compounds **10a-c**

Retention times (method **C**): 12.9 min **10a**; 16.3 min **10c**.

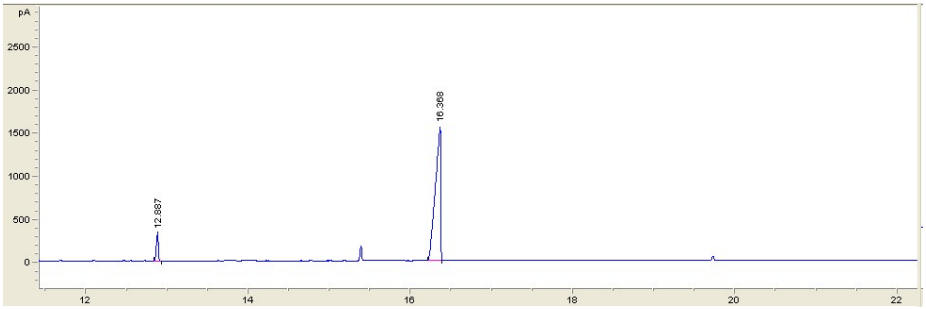
3,3‘-difluoro-[1,1'-biphenyl]-2,2'-dicarbaldehyde **10a**:



4,8-difluorodibenzo[*c,e*]oxepin-5(7*H*)-one **10c**:

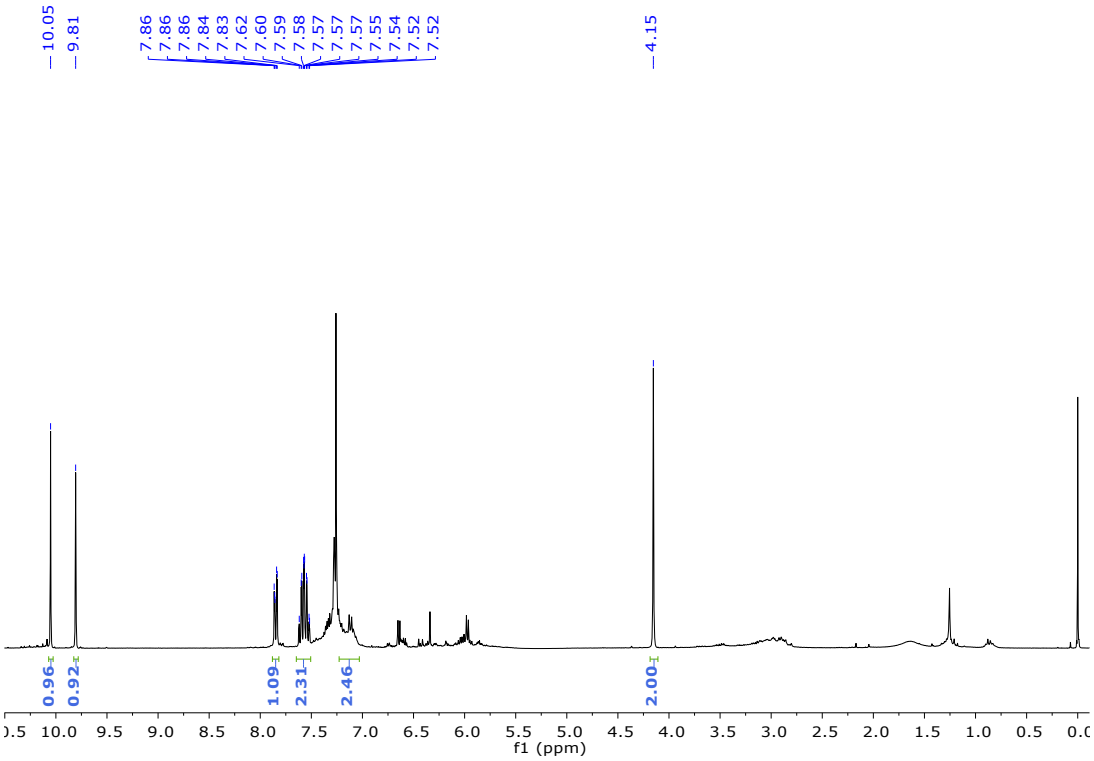


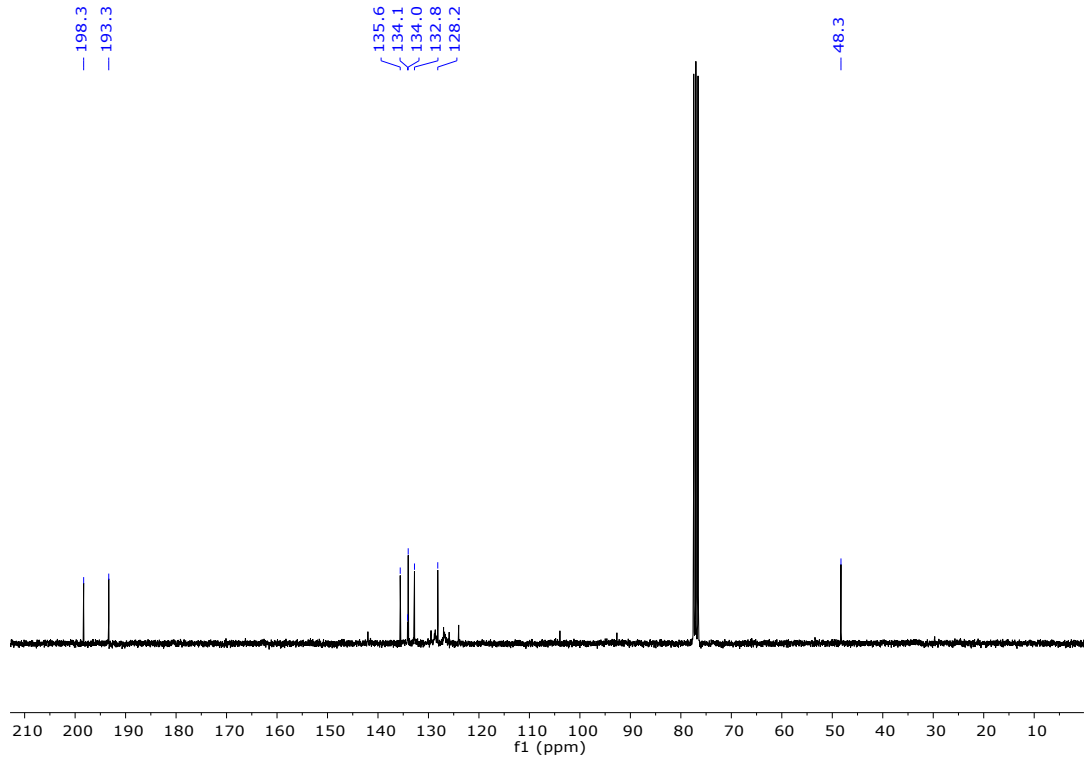
Biotransformation:



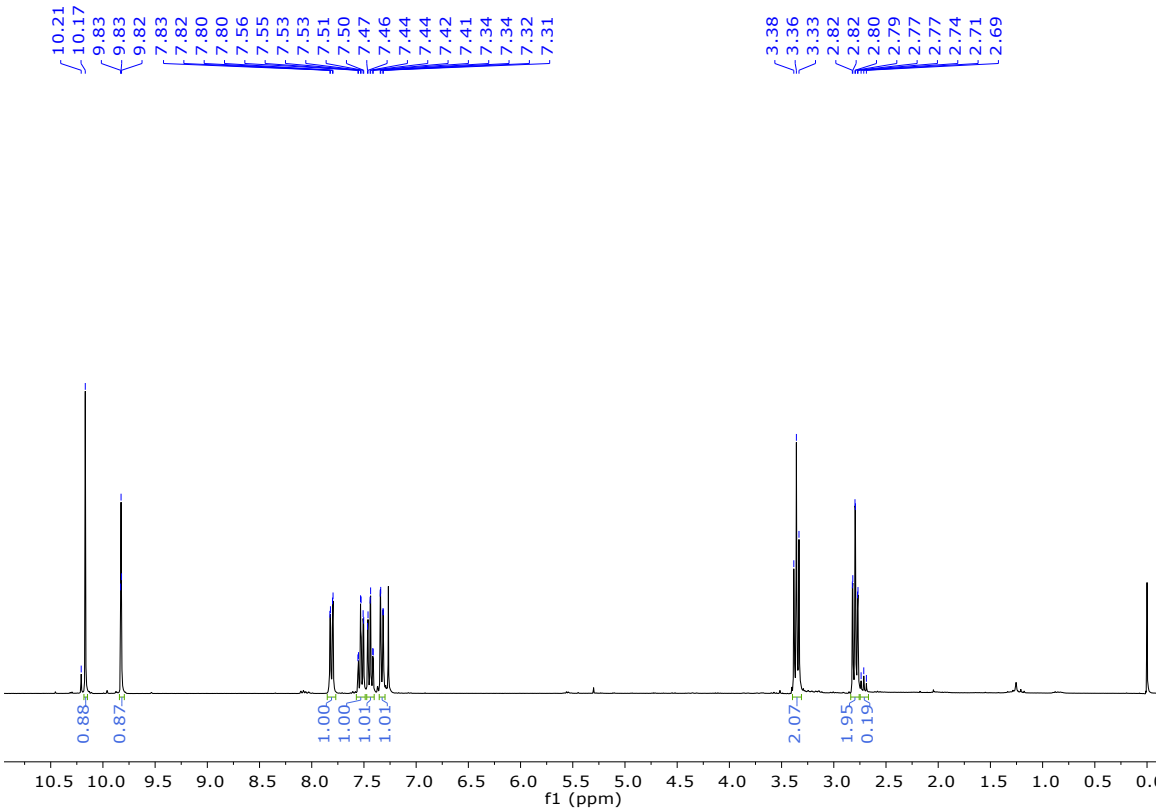
NMR spectra

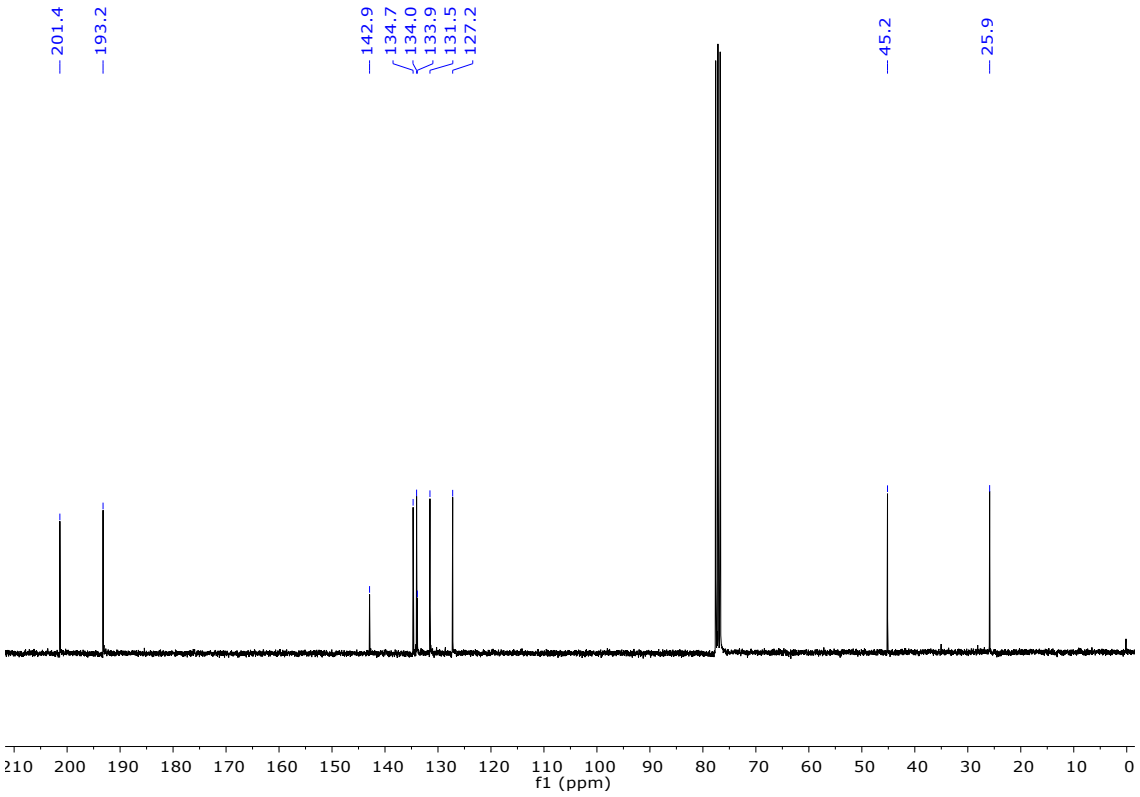
2-(2-oxoethyl)benzaldehyde **2a**



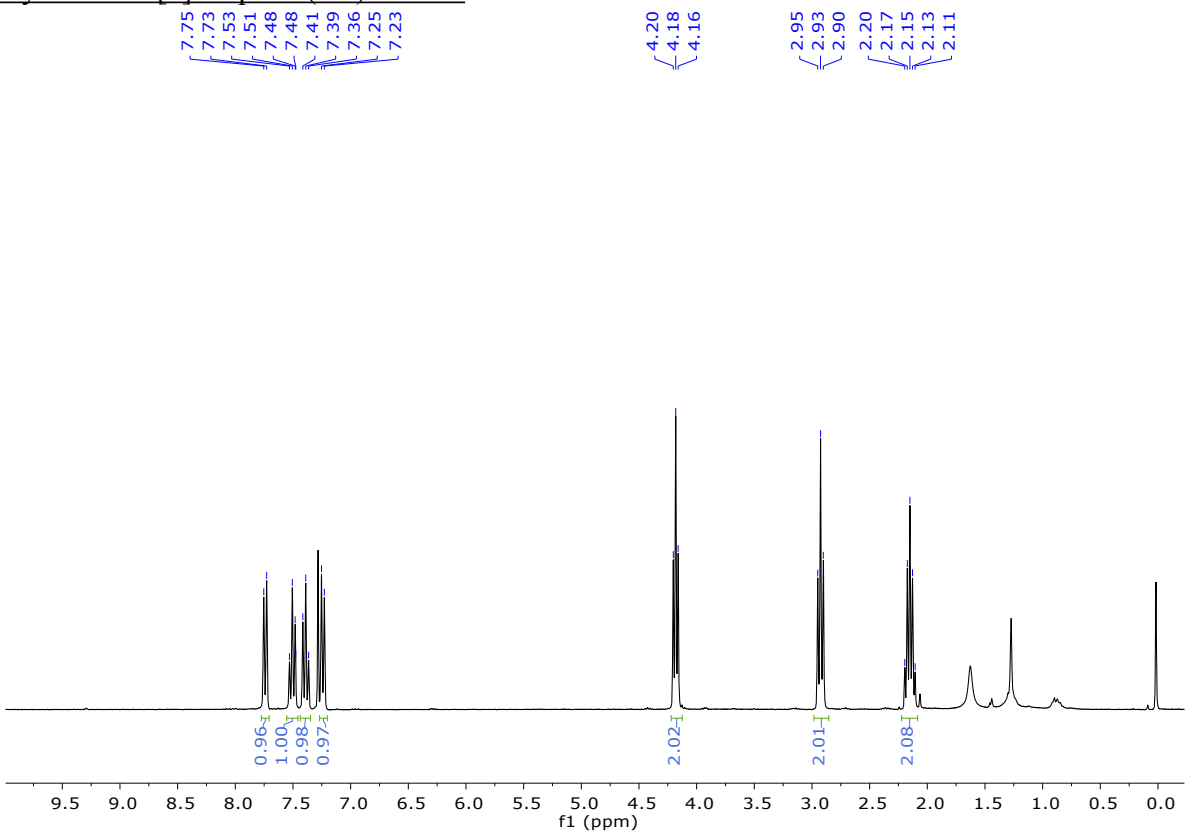


2-(3-oxopropyl)benzaldehyde **3a**

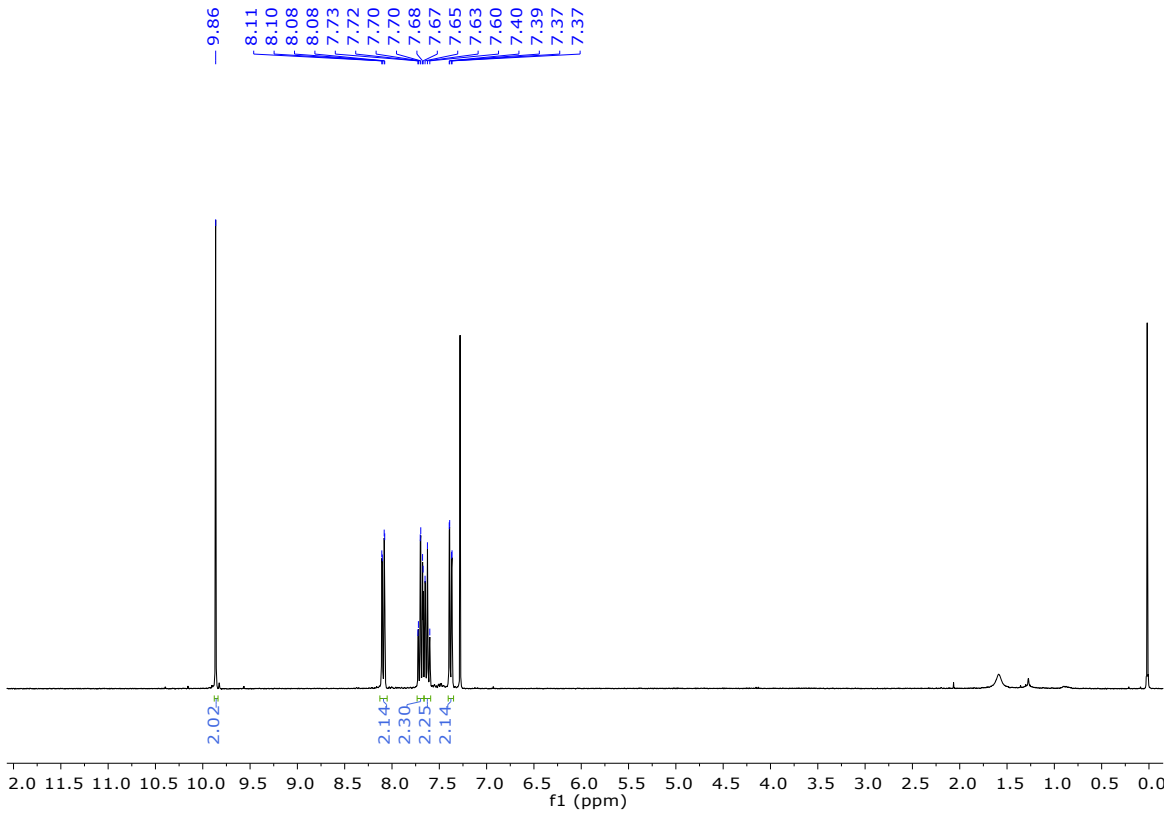




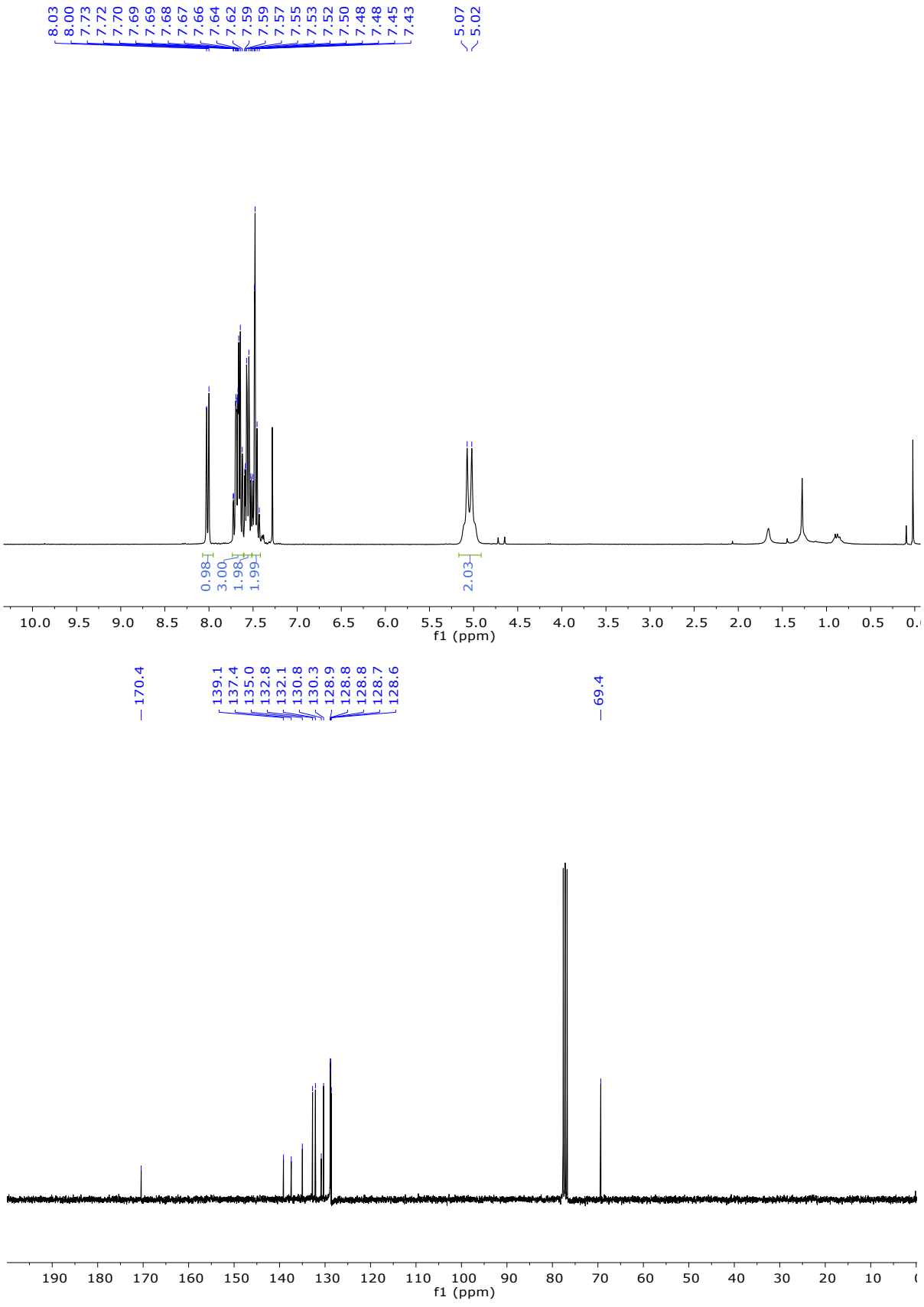
4,5-dihydrobenzo[*c*]oxepin-1(3*H*)-one **3b**



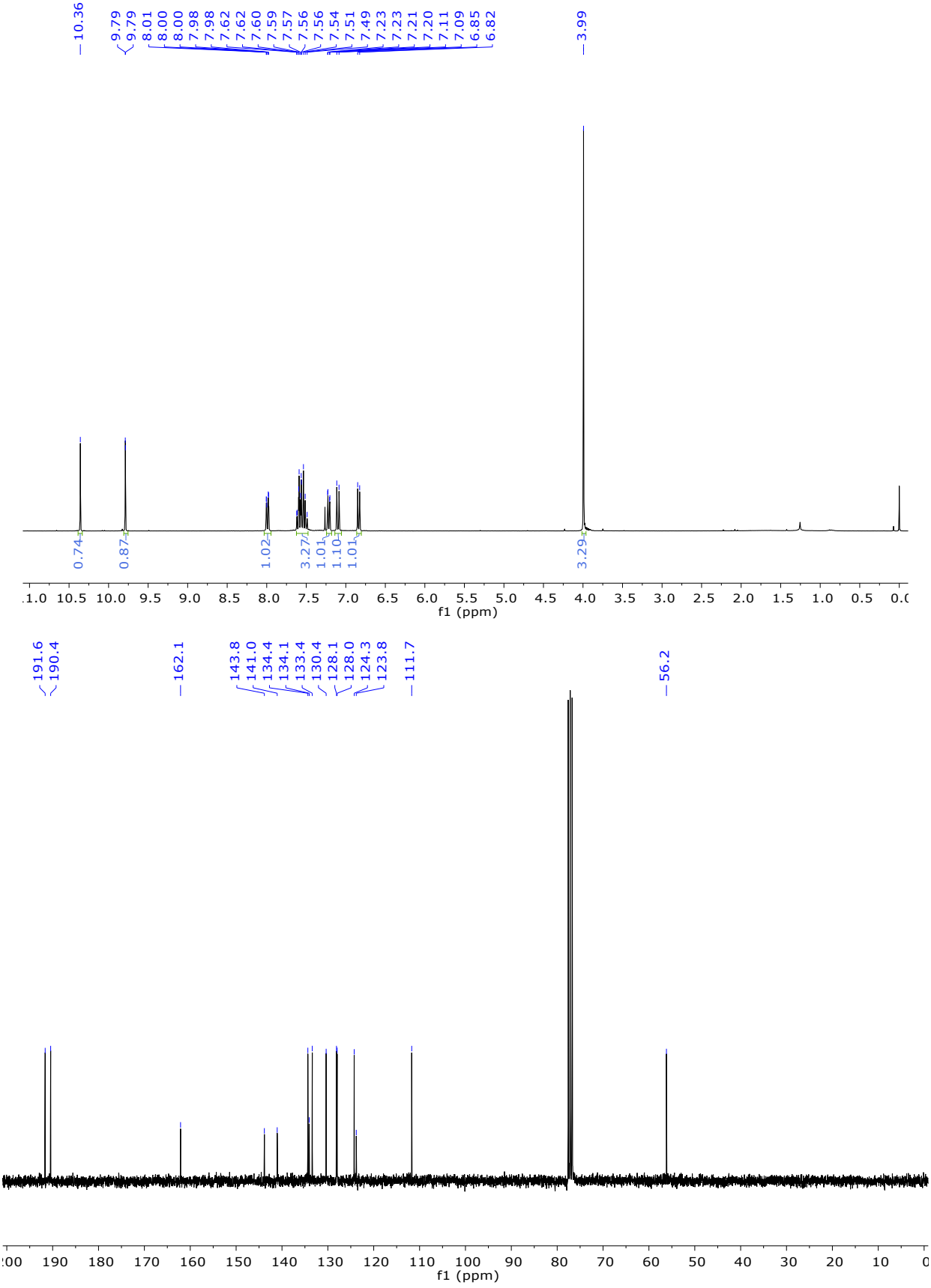
[1,1'-biphenyl]-2,2'-dicarbaldehyde **4a**



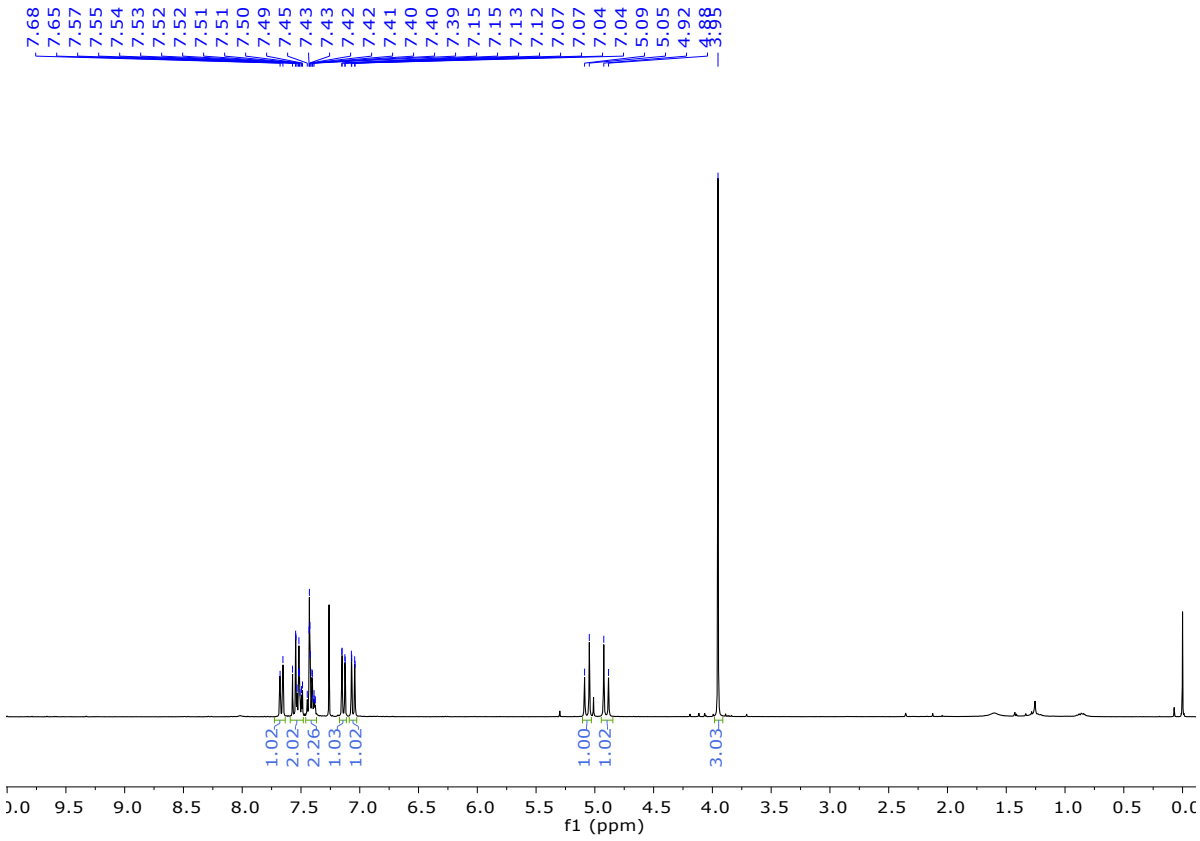
dibenzo[*c,e*]oxepin-5(7*H*)-one **4b**

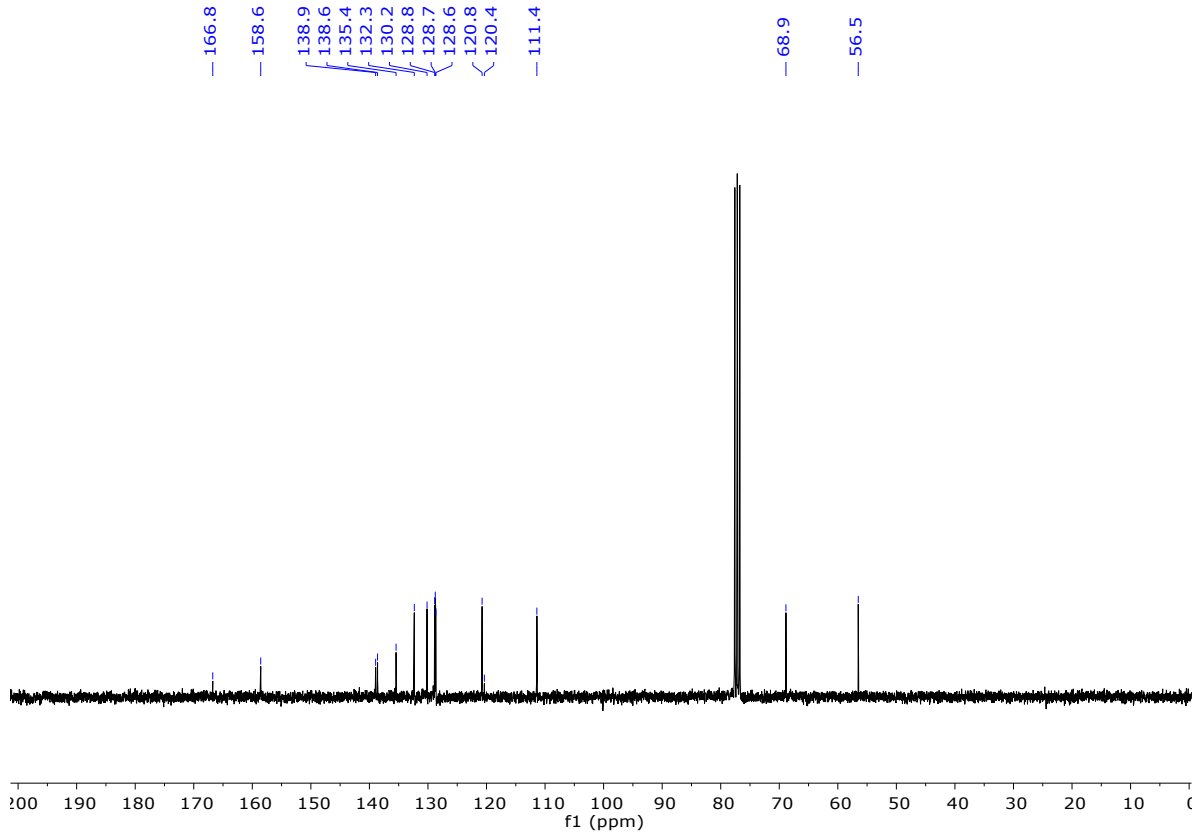


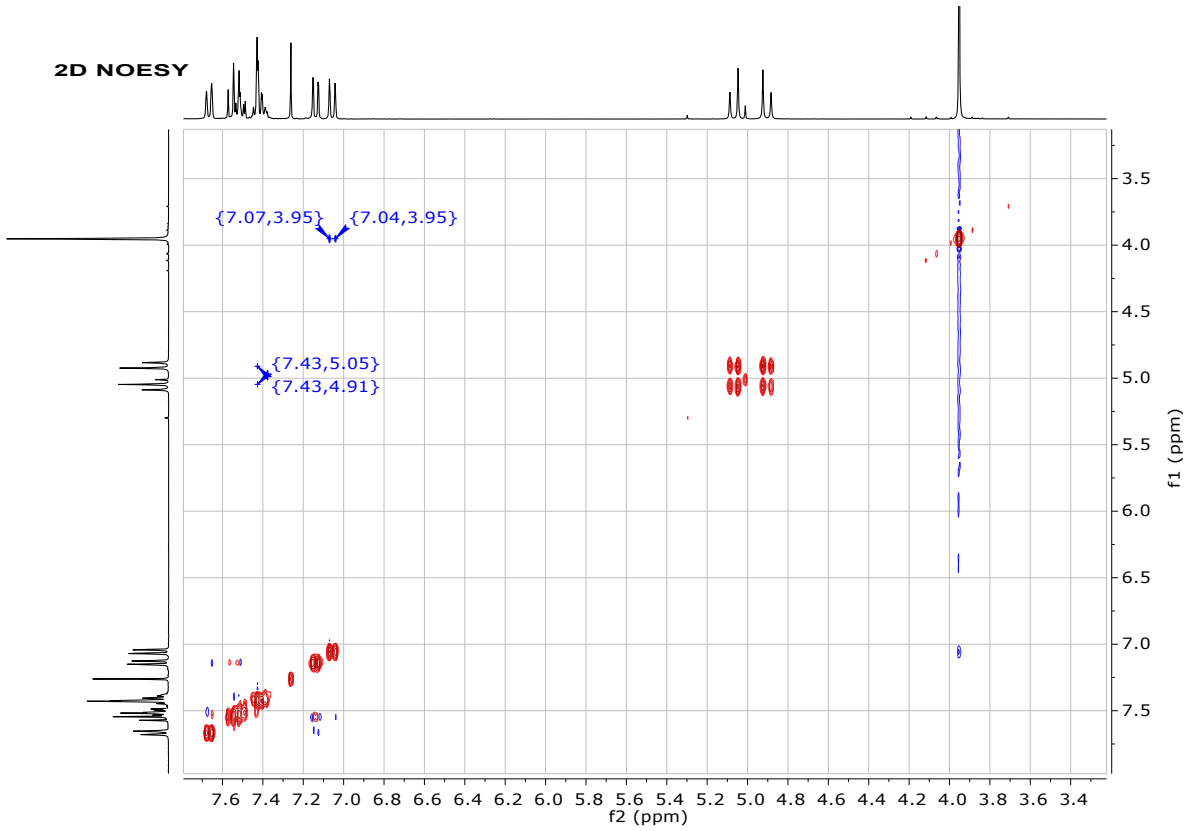
3-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **5a**

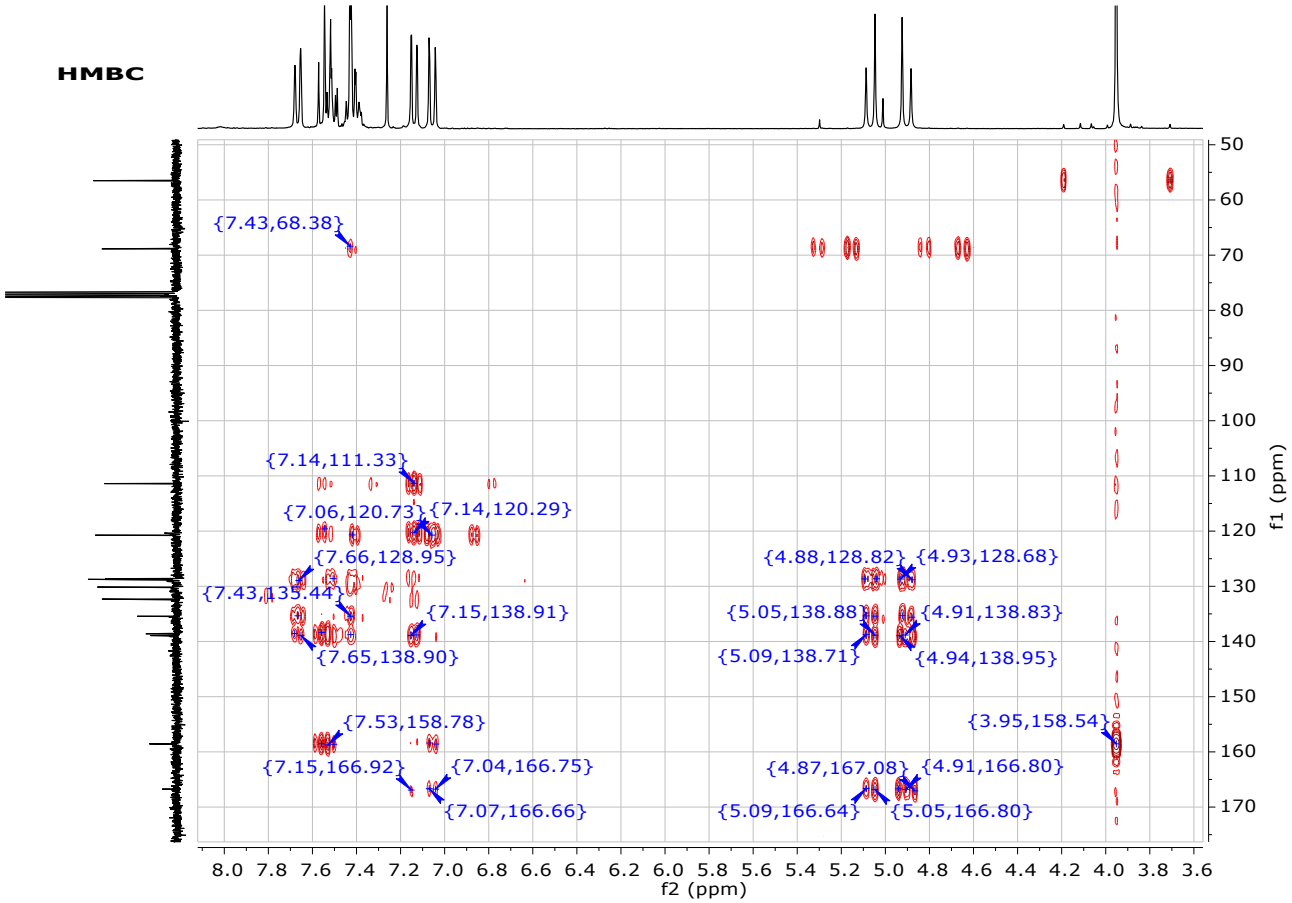


4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **5b**

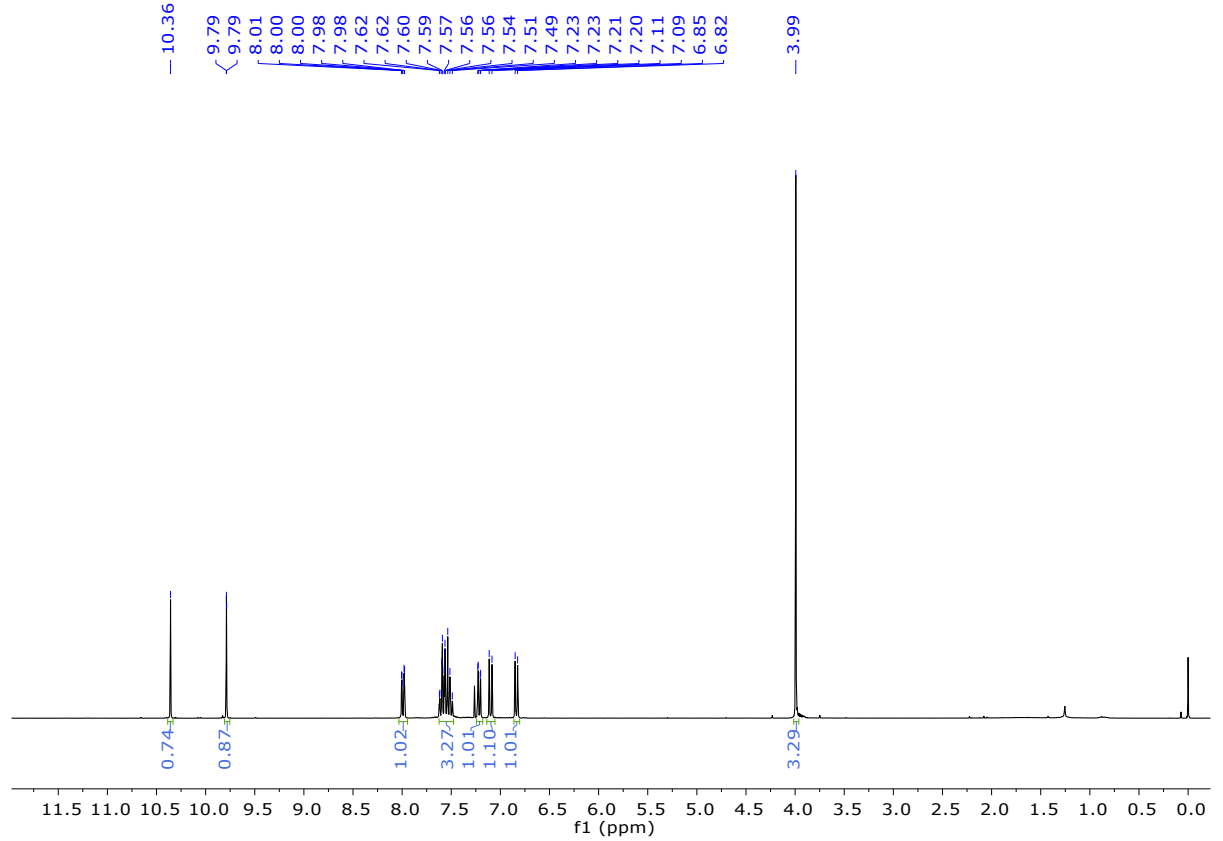


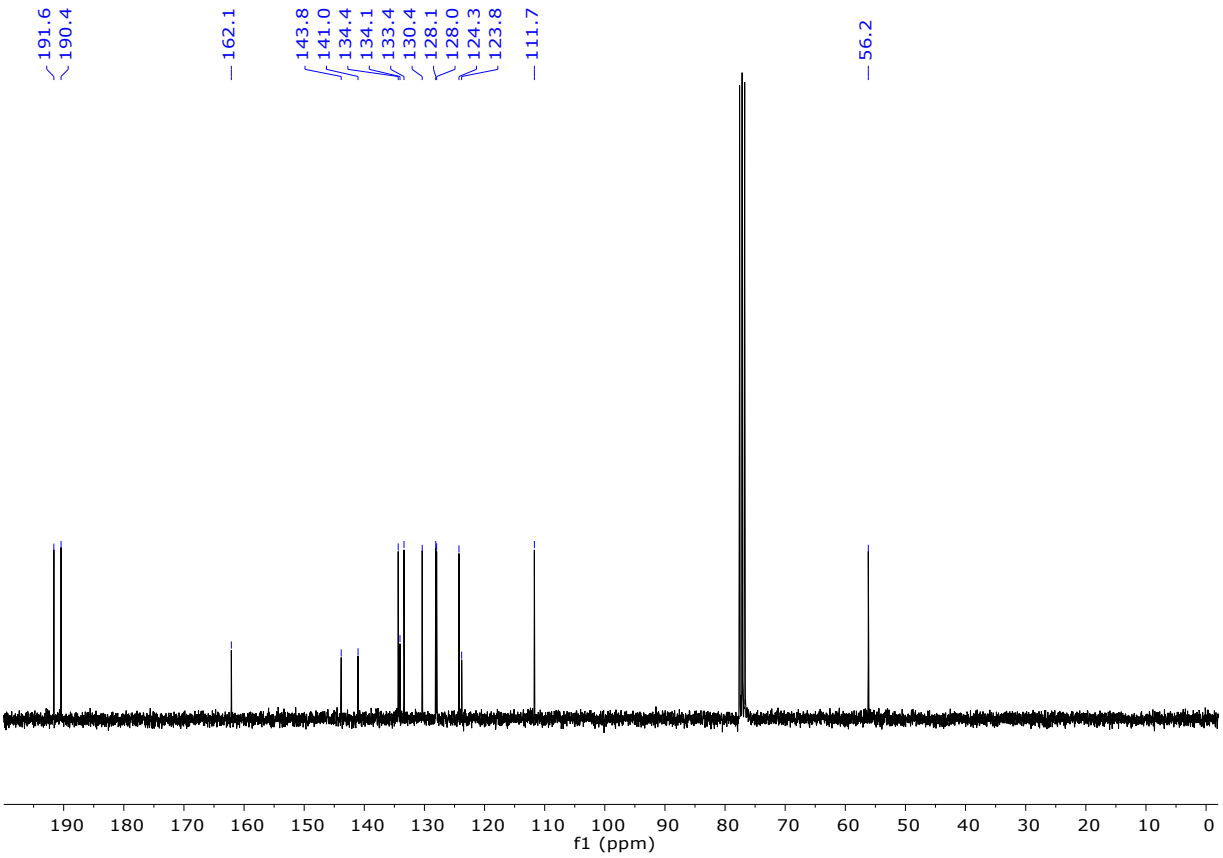



2D NOESY
{7.07,3.95}
{7.04,3.95}
{7.43,5.05}
{7.43,4.91}
f1 (ppm)
f2 (ppm)

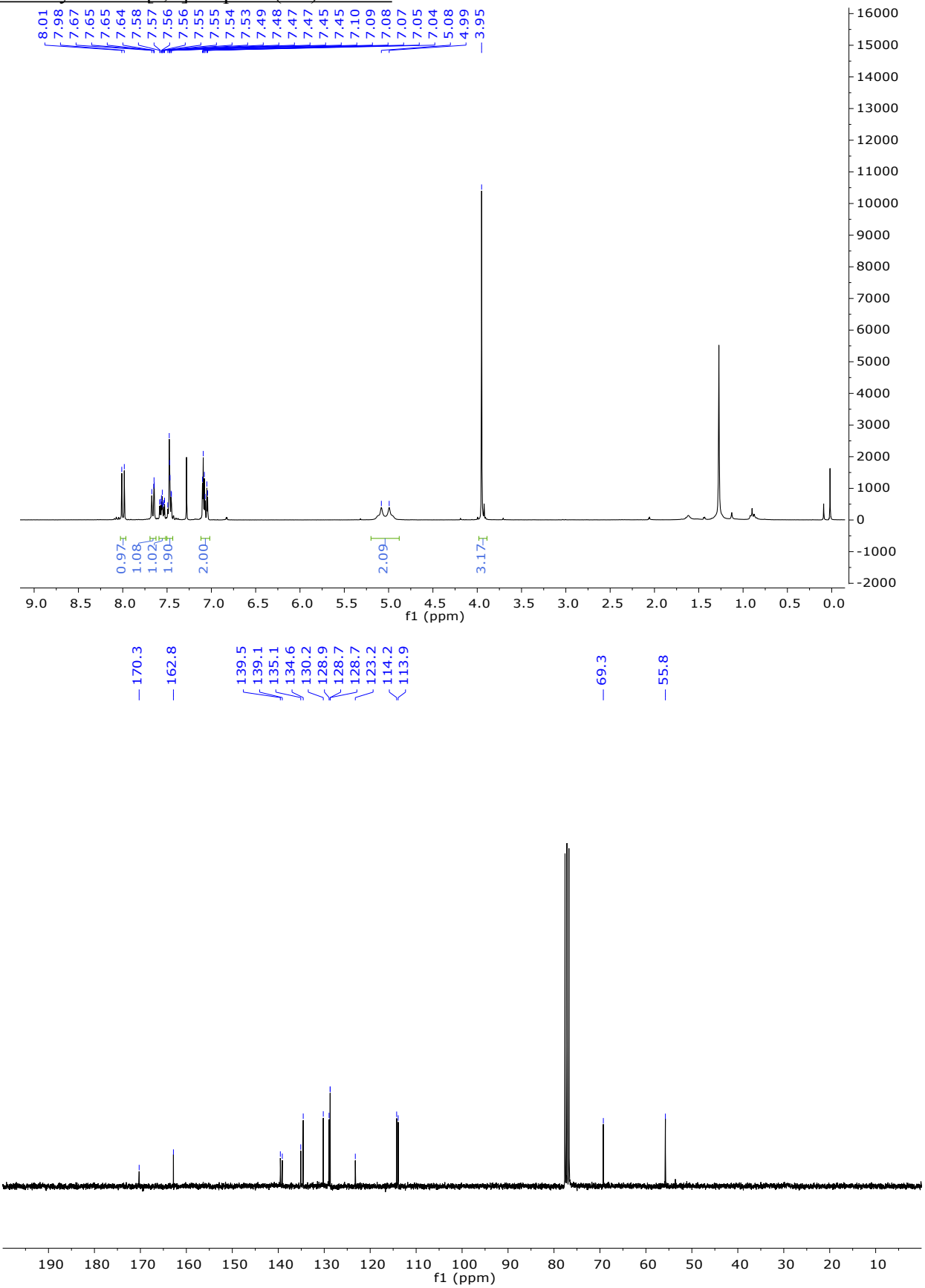

HMBC
{7.43,68.38}
{7.14,111.33}
{7.06,120.73}
{7.14,120.29}
{7.66,128.95}
{7.43,135.44}
{7.15,138.91}
{7.65,138.90}
{7.53,158.78}
{7.15,166.92}
{7.04,166.75}
{7.07,166.66}
{4.88,128.82}
{4.93,128.68}
{5.05,138.88}
{4.91,138.83}
{5.09,138.71}
{4.94,138.95}
{3.95,158.54}
{4.87,167.08}
{4.91,166.80}
{5.09,166.64}
{5.05,166.80}
f1 (ppm)
f2 (ppm)

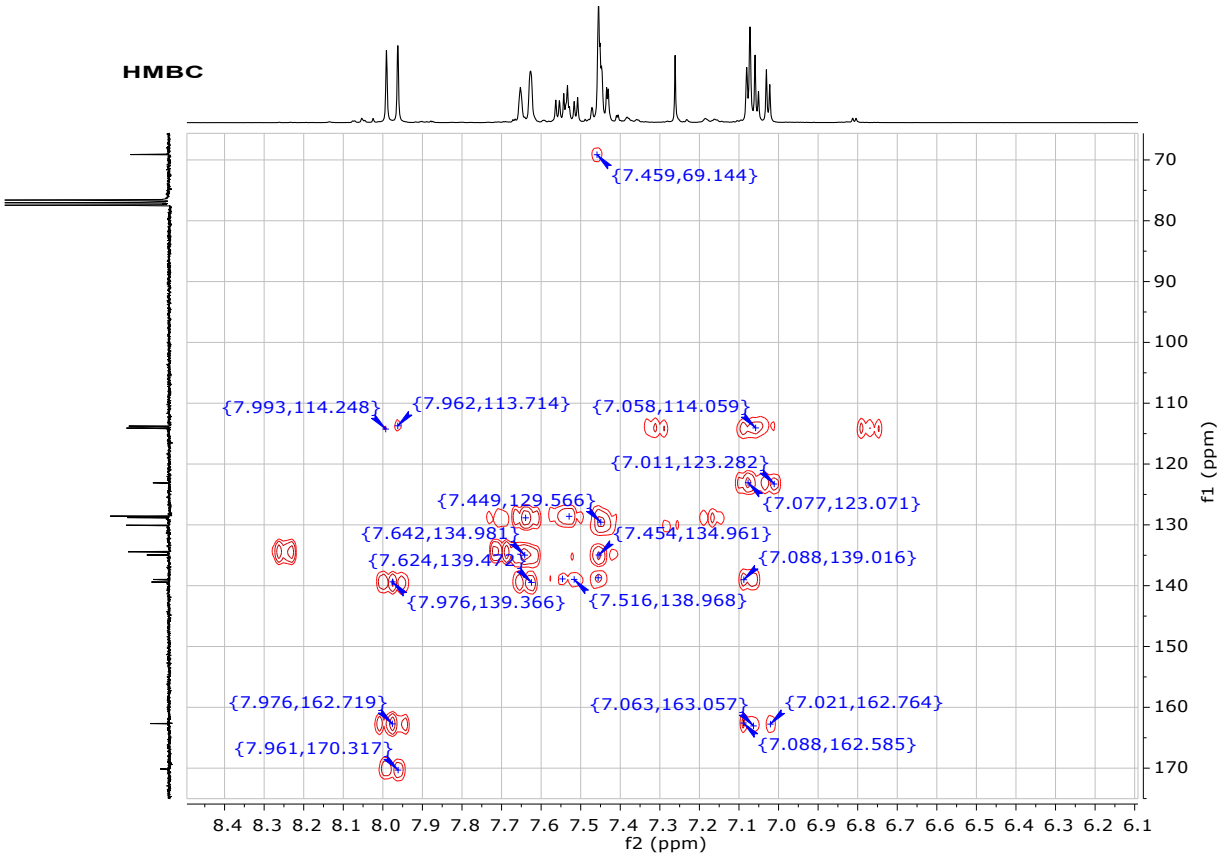
5-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **6a**

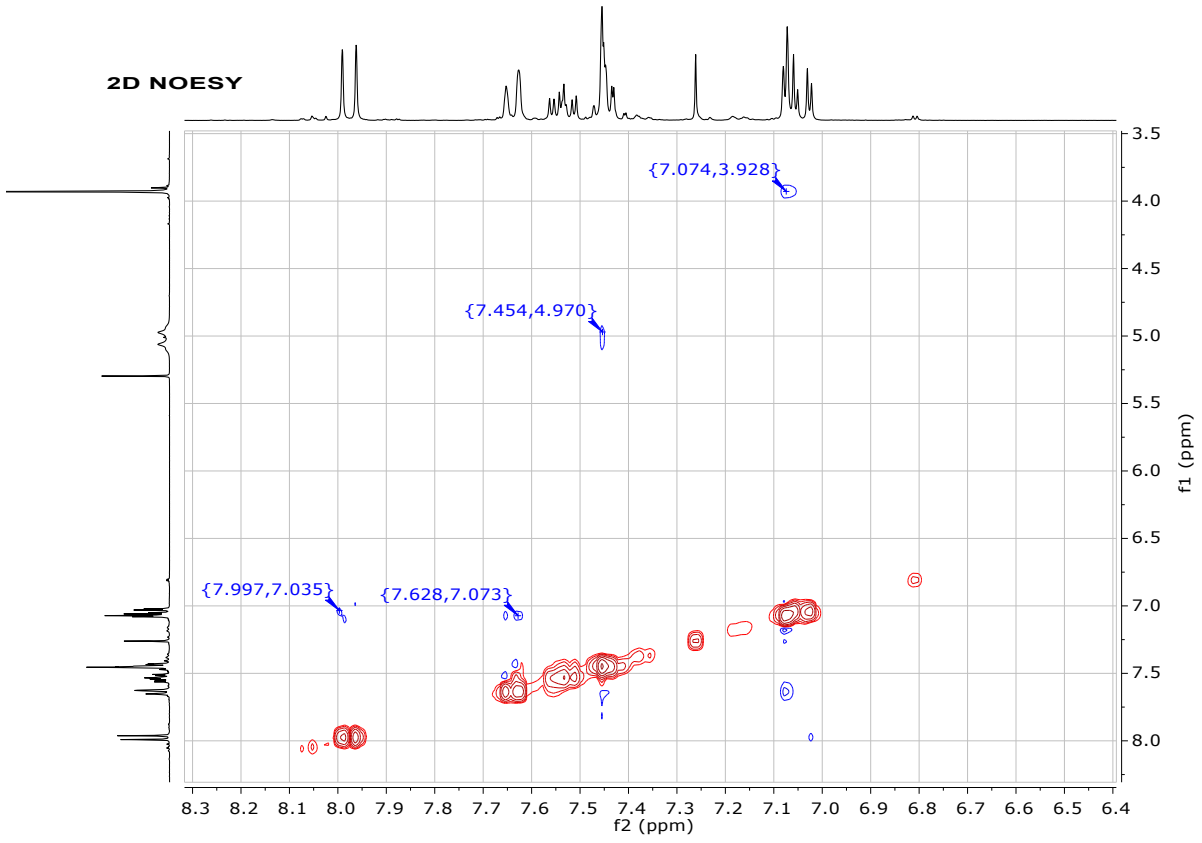




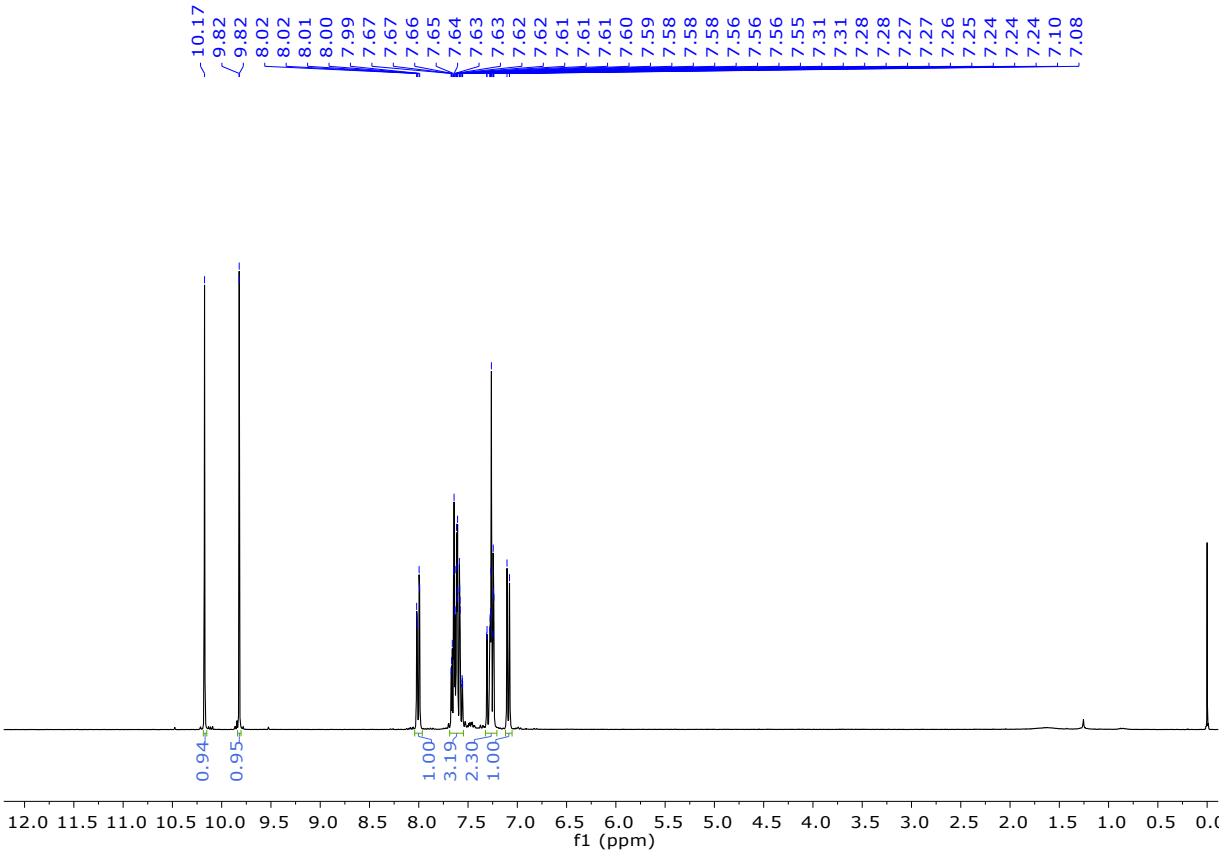
4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **6b**

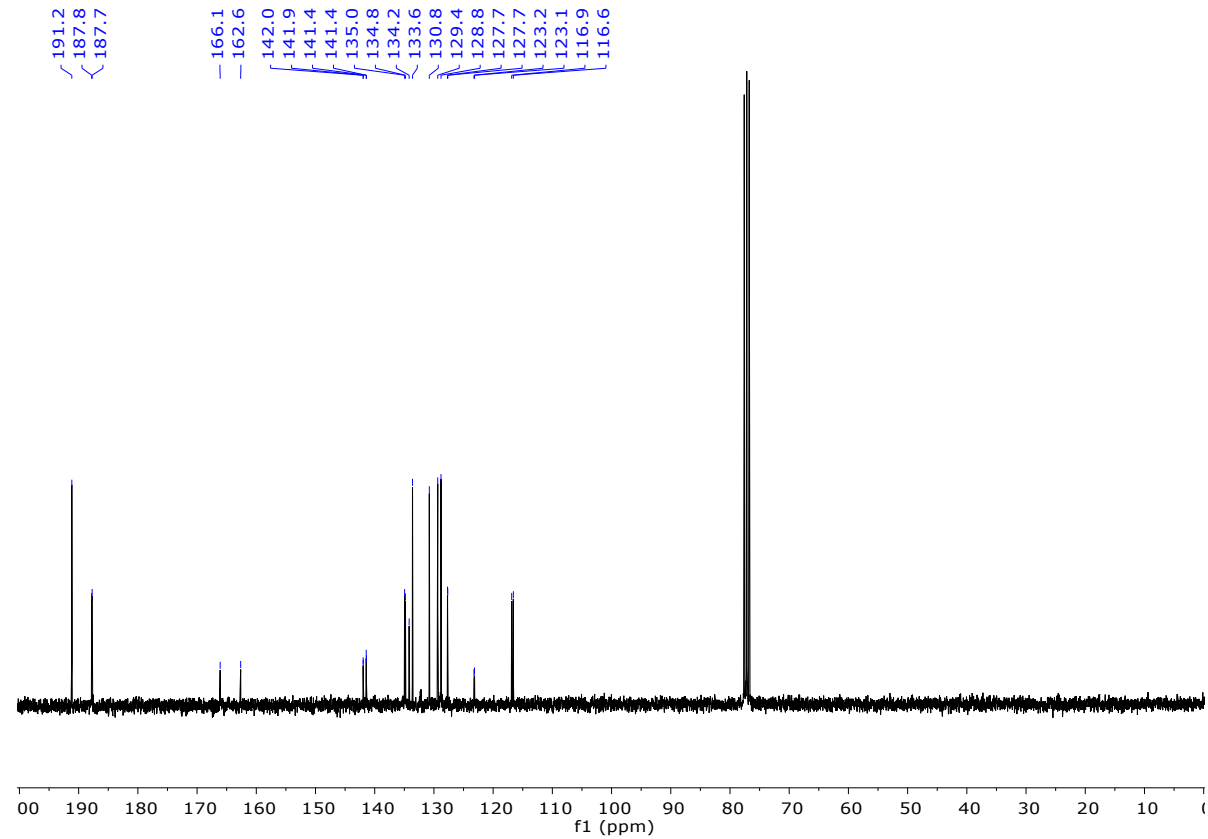



HMBC
{7.459,69.144}
{7.993,114.248}
{7.962,113.714}
{7.058,114.059}
{7.011,123.282}
{7.077,123.071}
{7.449,129.566}
{7.642,134.981}
{7.454,134.961}
{7.624,139.472}
{7.088,139.016}
{7.976,139.366}
{7.516,138.968}
{7.976,162.719}
{7.063,163.057}
{7.021,162.764}
{7.961,170.317}
{7.088,162.585}
f1 (ppm)
f2 (ppm)

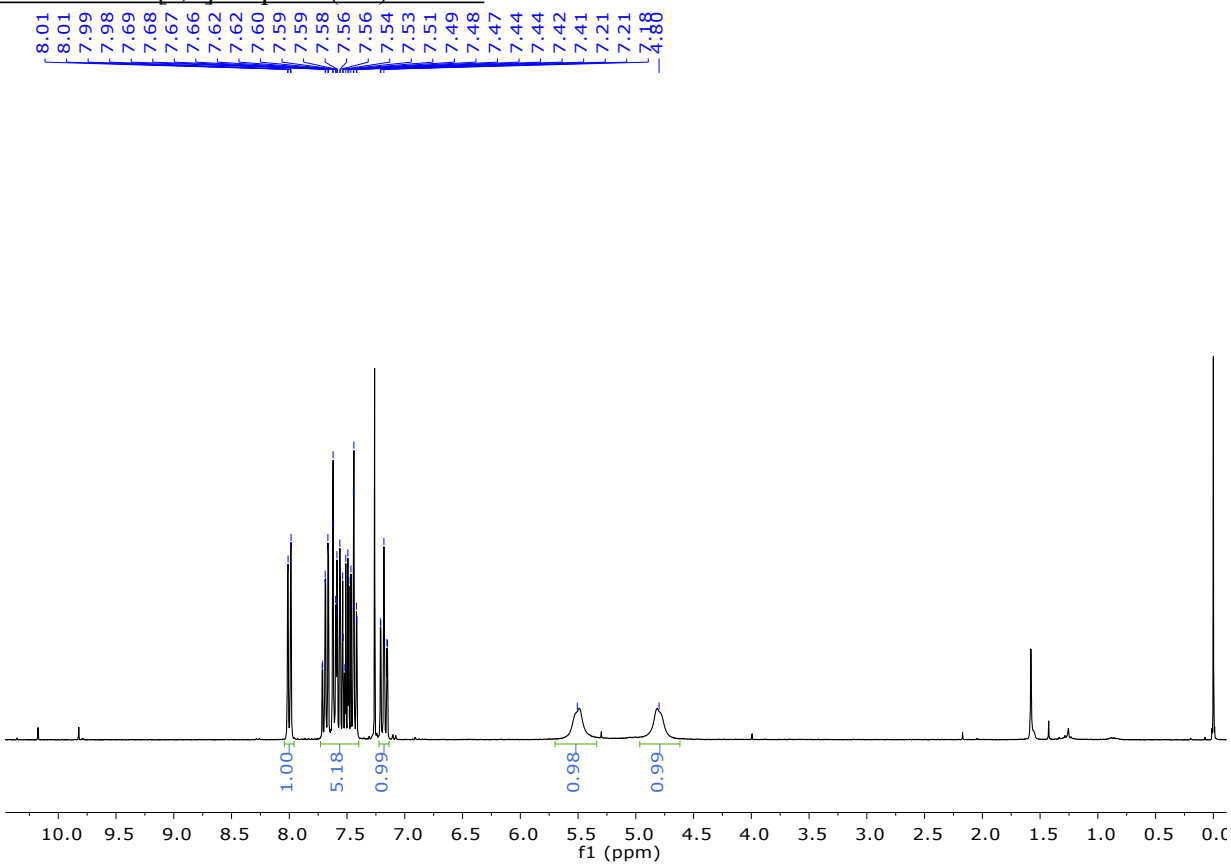

2D NOESY
{7.074,3.928}
{7.454,4.970}
{7.997,7.035}
{7.628,7.073}
f1 (ppm)
f2 (ppm)

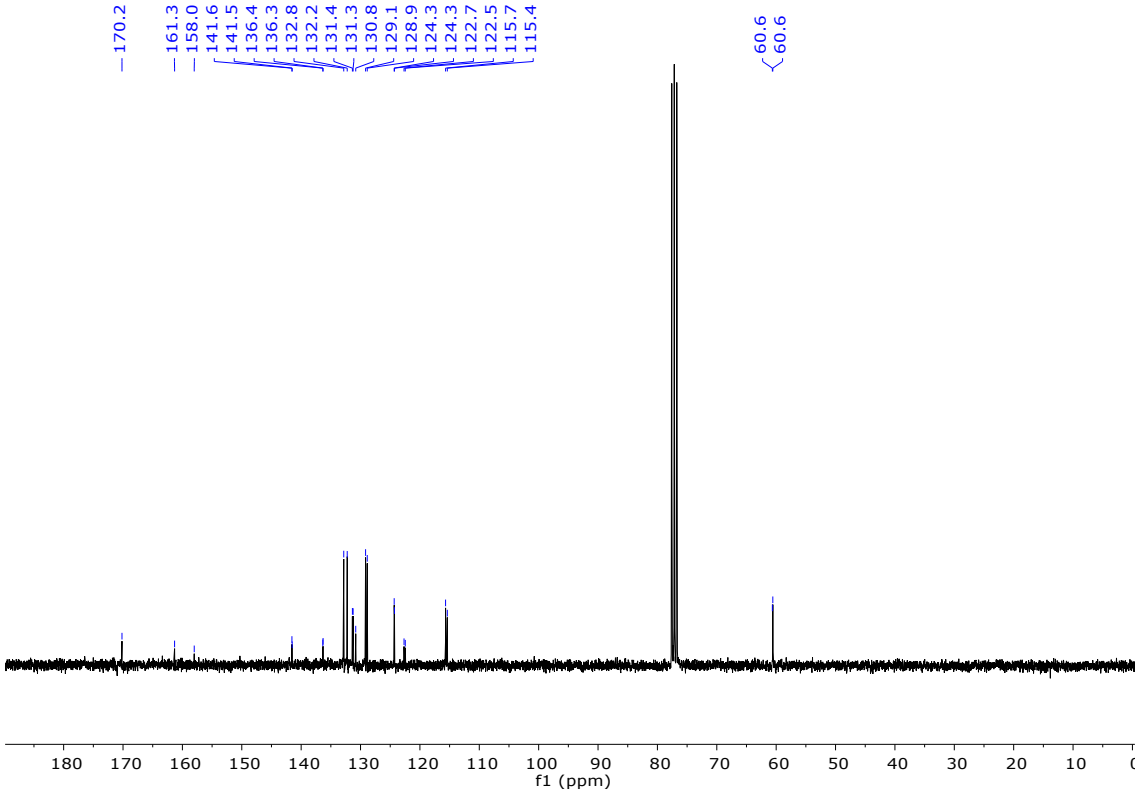
3-fluoro-[1,1'-biphenyl]-2,2'-dicarbaldehyde **7a**



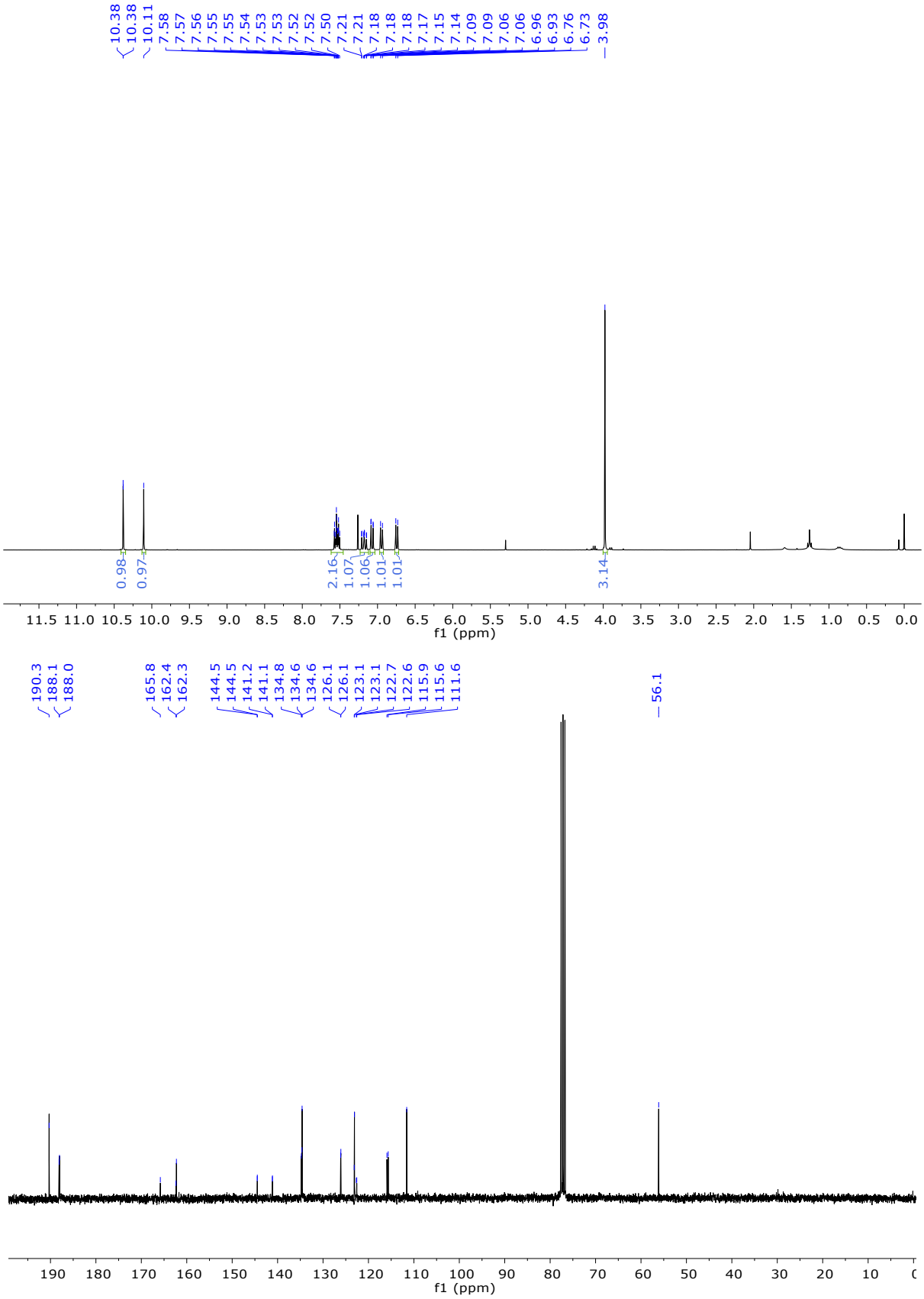


8-fluorodibenzo[*c,e*]oxepin-5(7*H*)-one **7c**

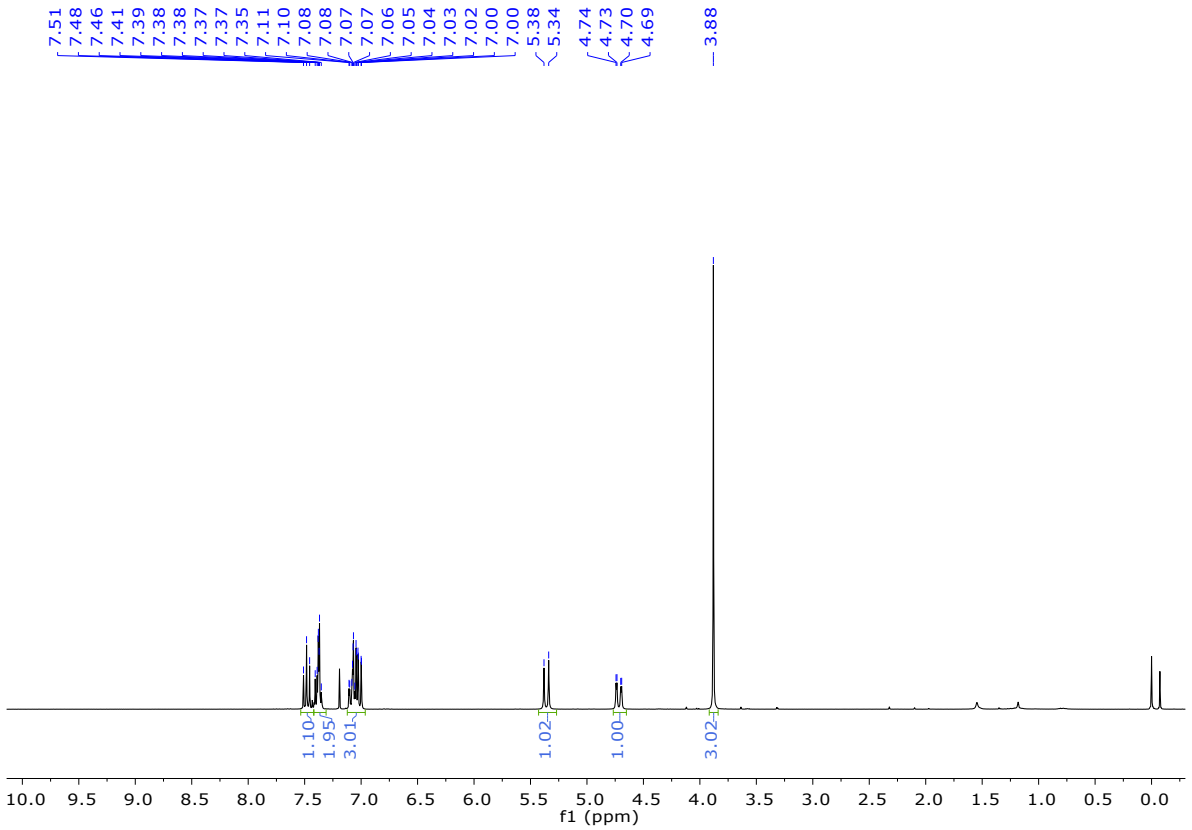


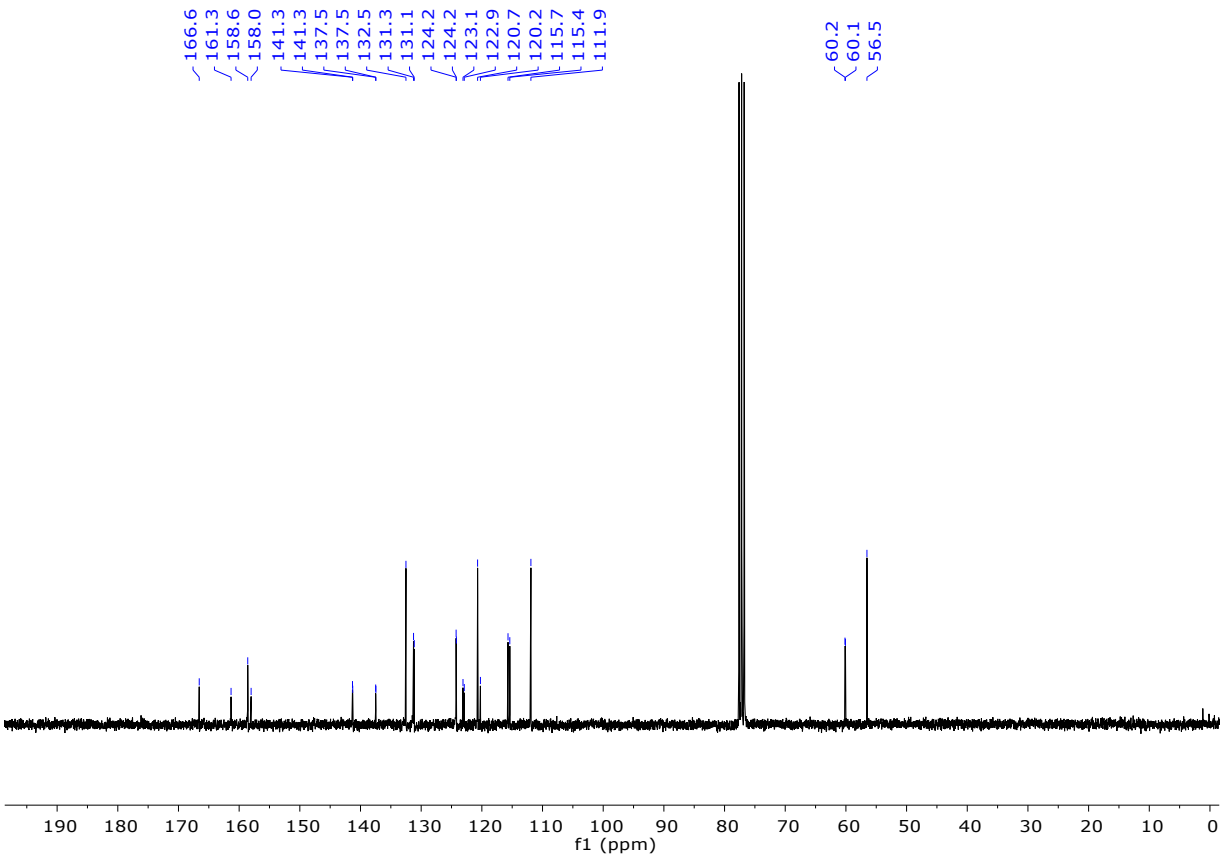


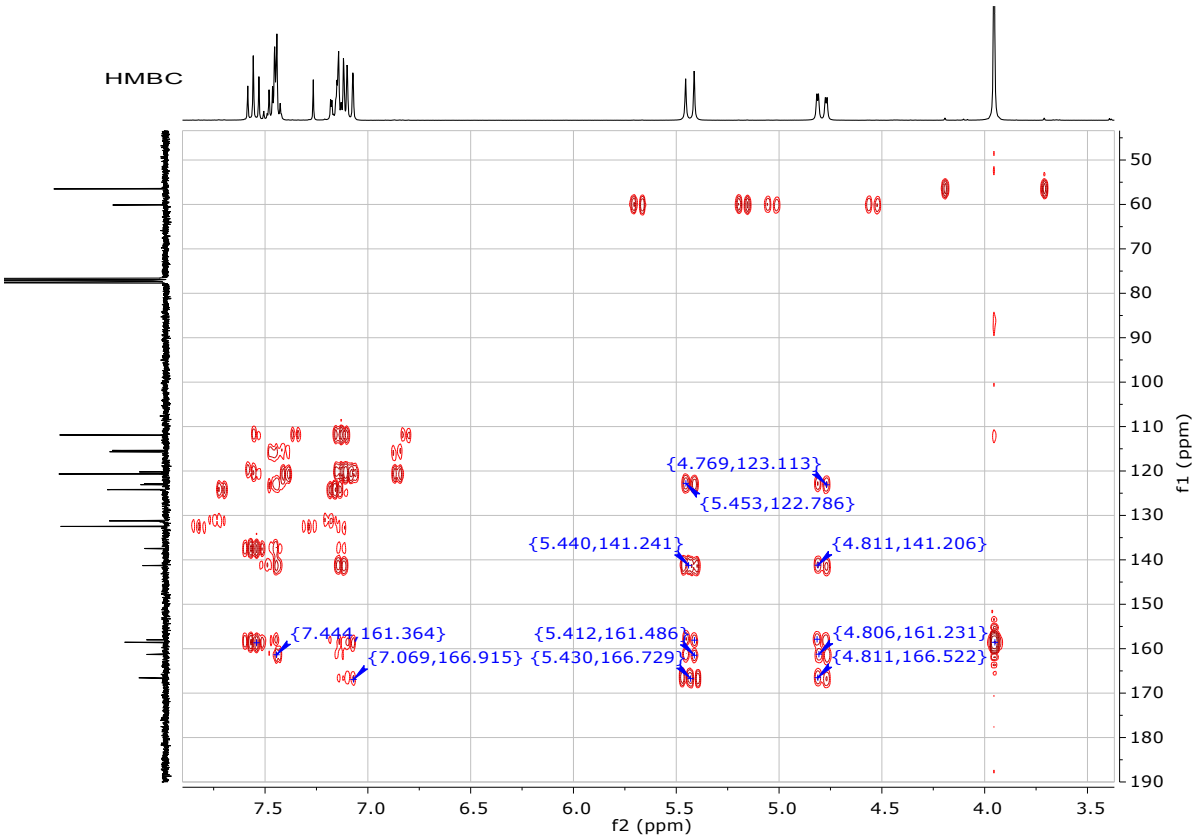
3-fluoro-3'-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **8a**

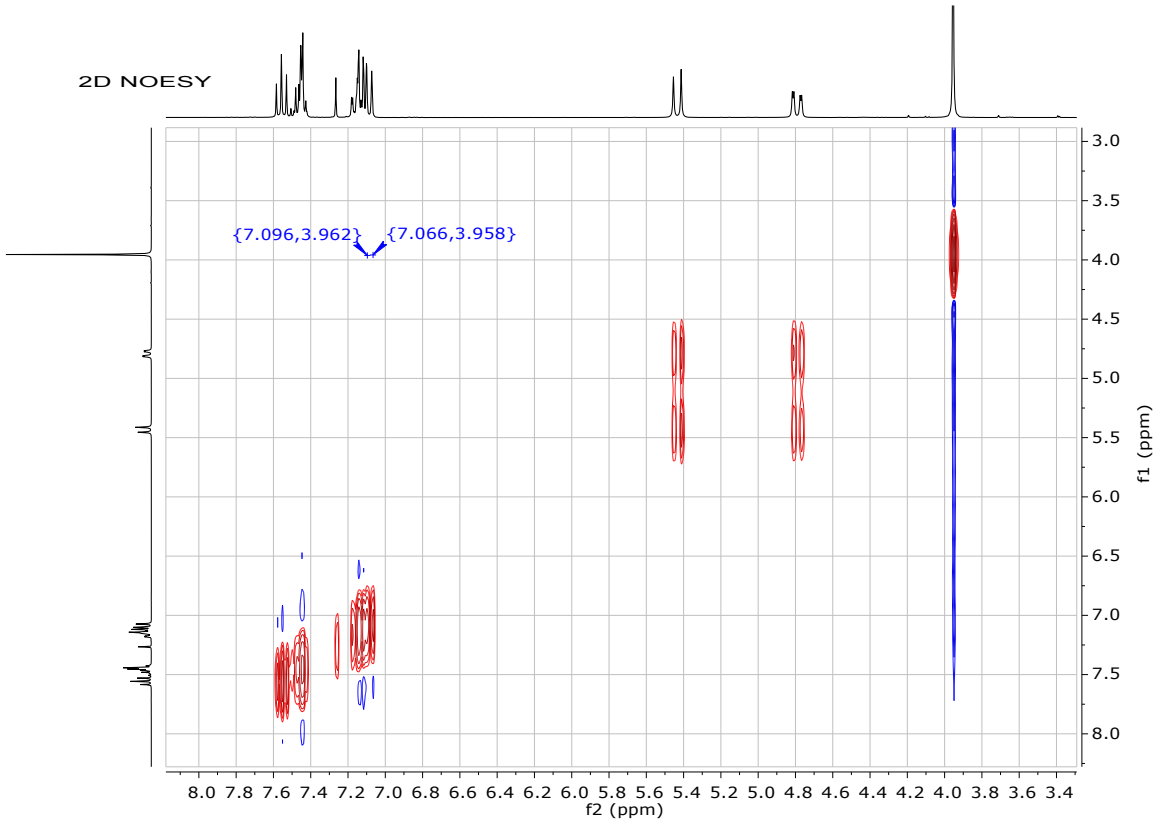


8-fluoro-4-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **8c**

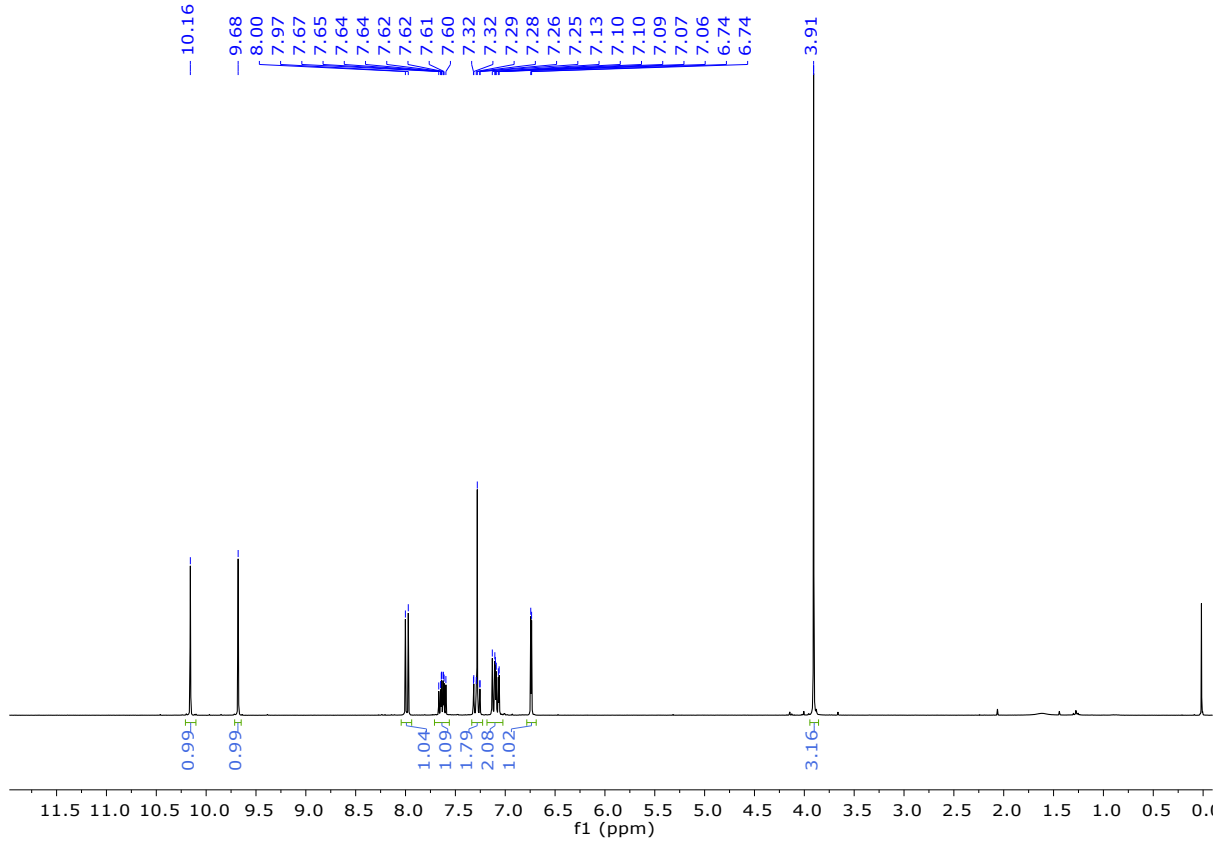


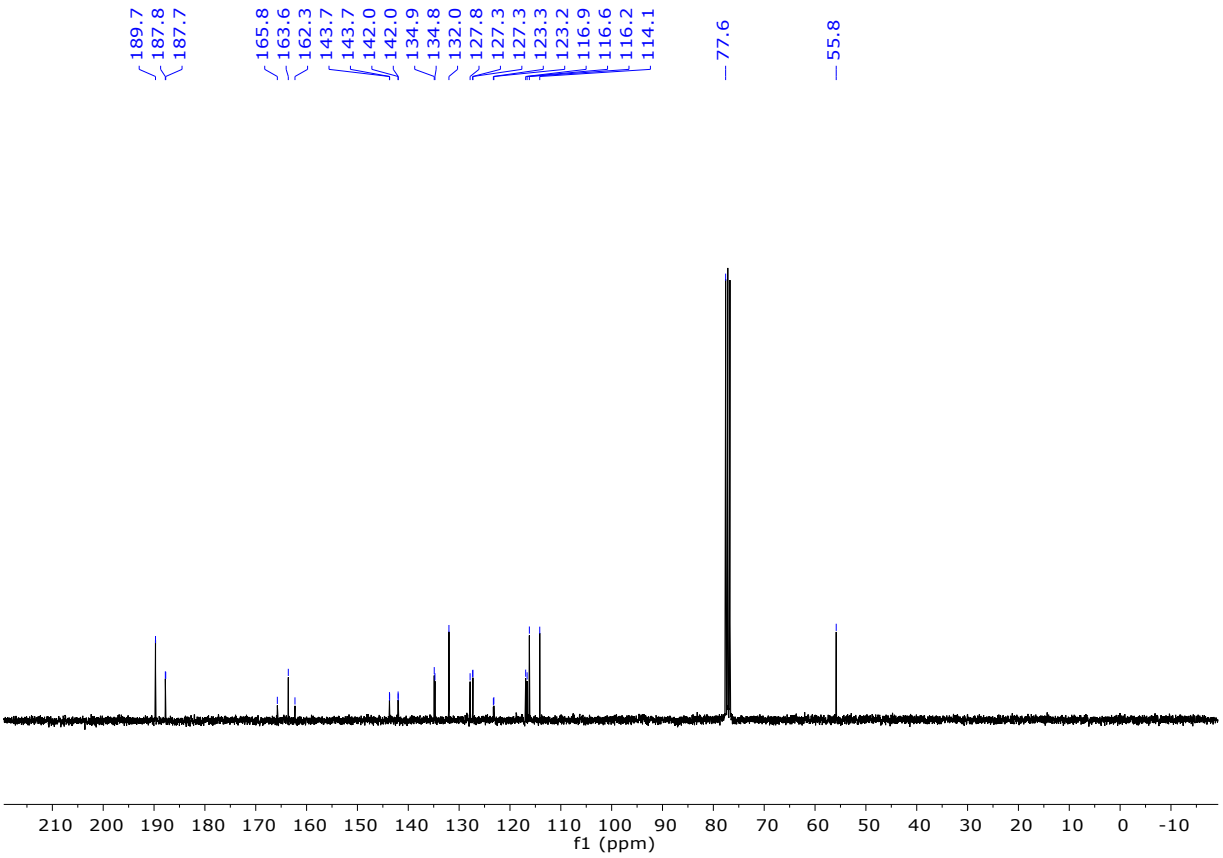



HMBC
{4.769,123.113}
{5.453,122.786}
{5.440,141.241}
{4.811,141.206}
{7.444,161.364}
{7.069,166.915}
{5.412,161.486}
{5.430,166.729}
{4.806,161.231}
{4.811,166.522}
f2 (ppm)
f1 (ppm)

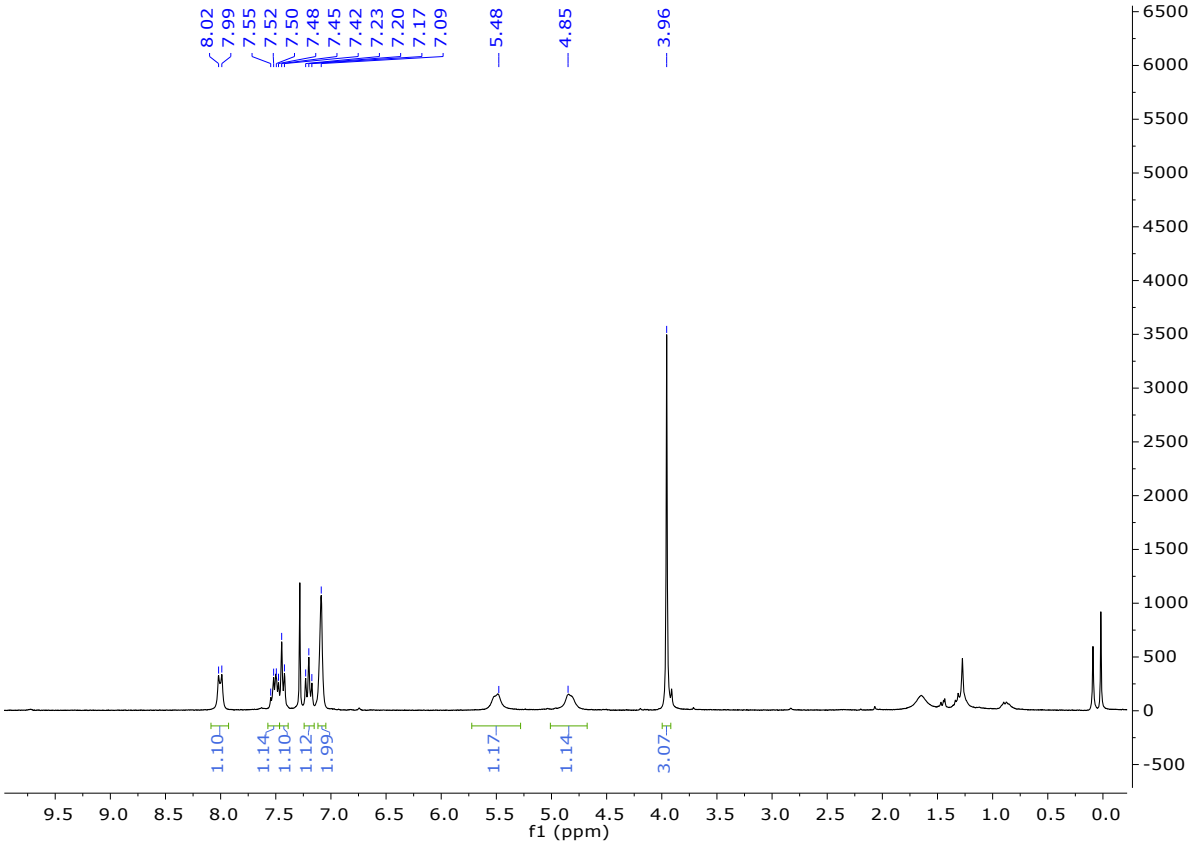

2D NOESY
{7.096,3.962}
{7.066,3.958}
f2 (ppm)
f1 (ppm)

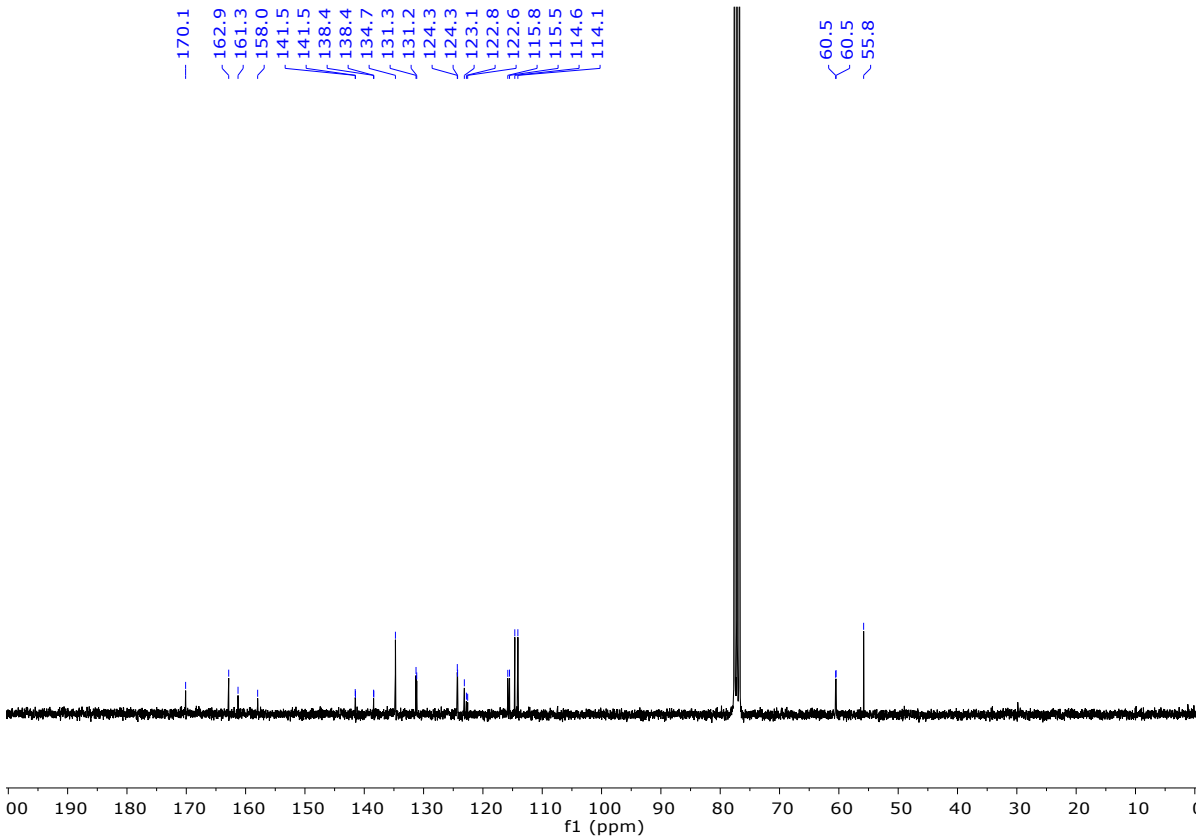
3-fluoro-5'-methoxy-[1,1'-biphenyl]-2,2'-dicarbaldehyde **9a**



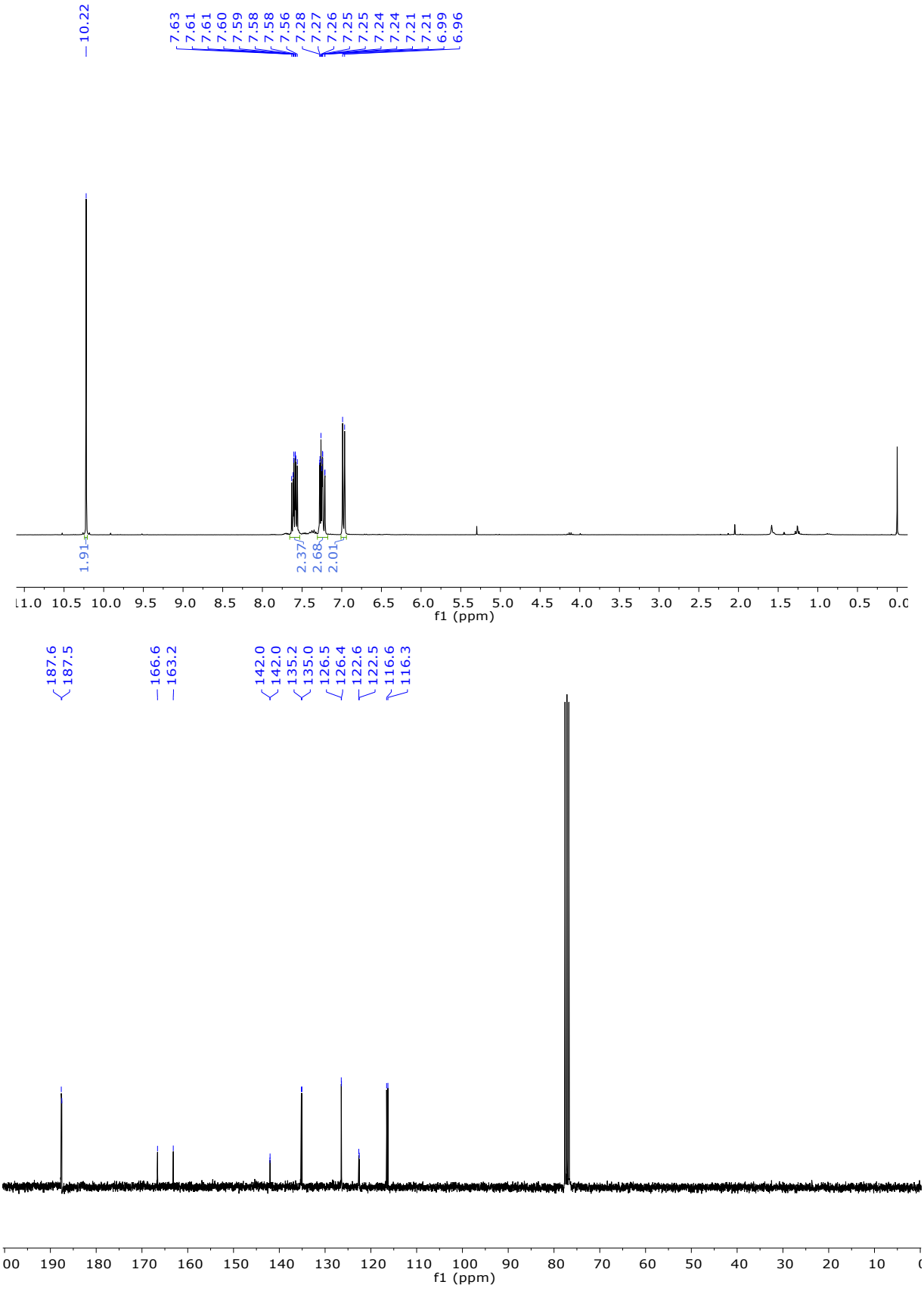


8-fluoro-2-methoxydibenzo[*c,e*]oxepin-5(7*H*)-one **9c**

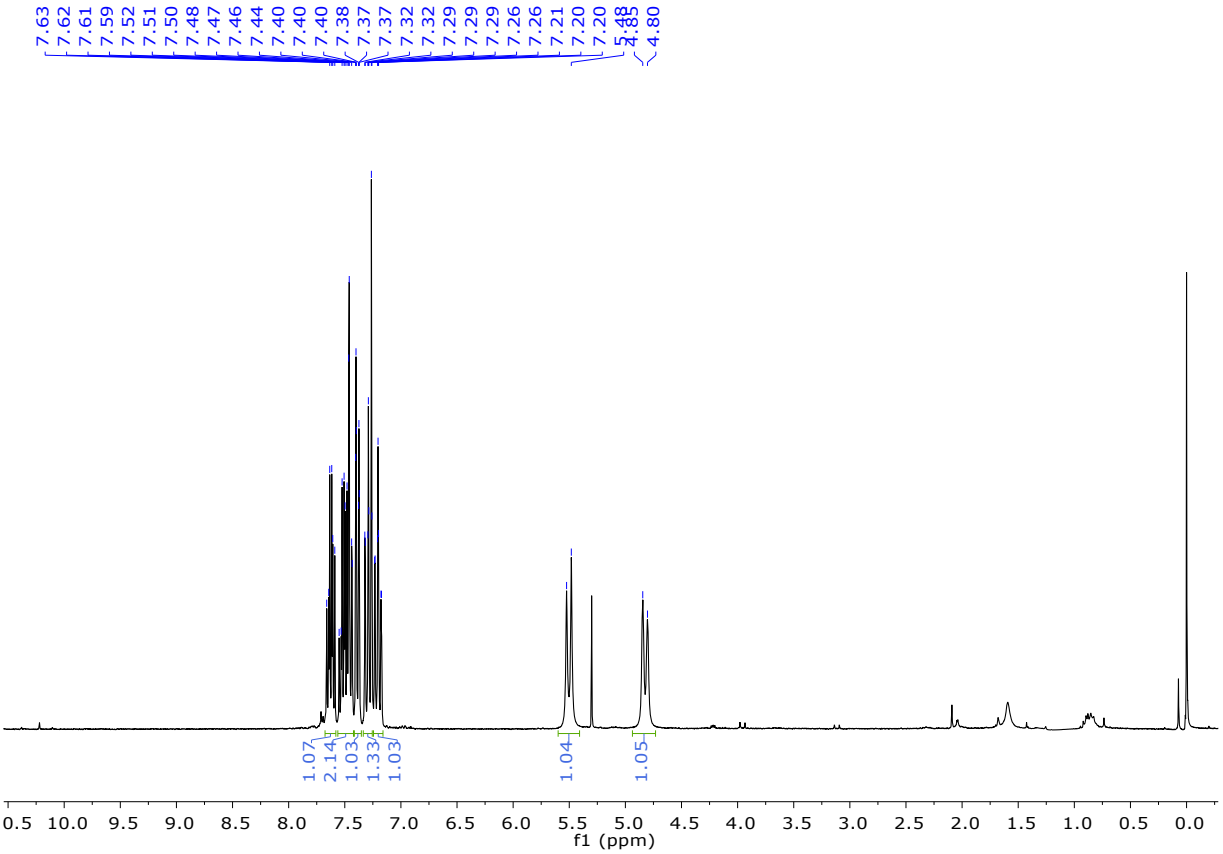


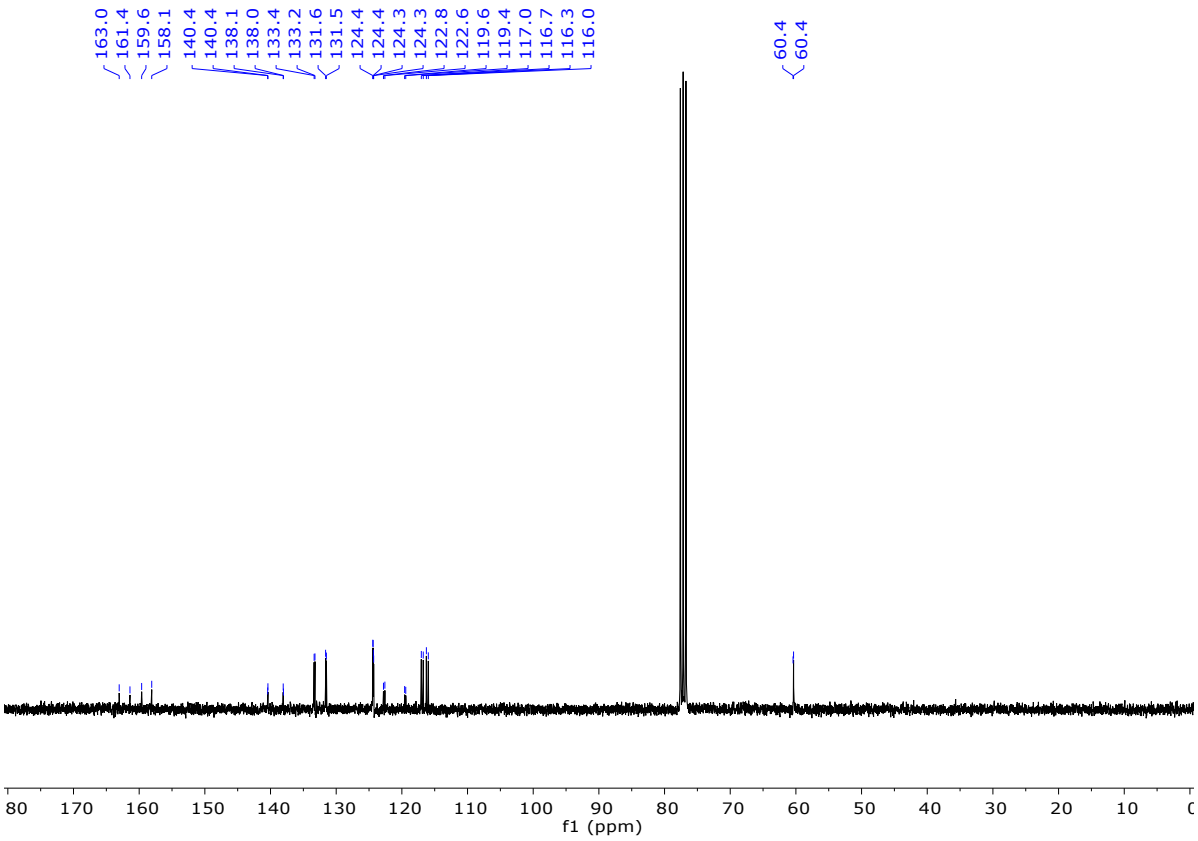


3,3‘-difluoro-[1,1'-biphenyl]-2,2'-dicarbaldehyde **10a**

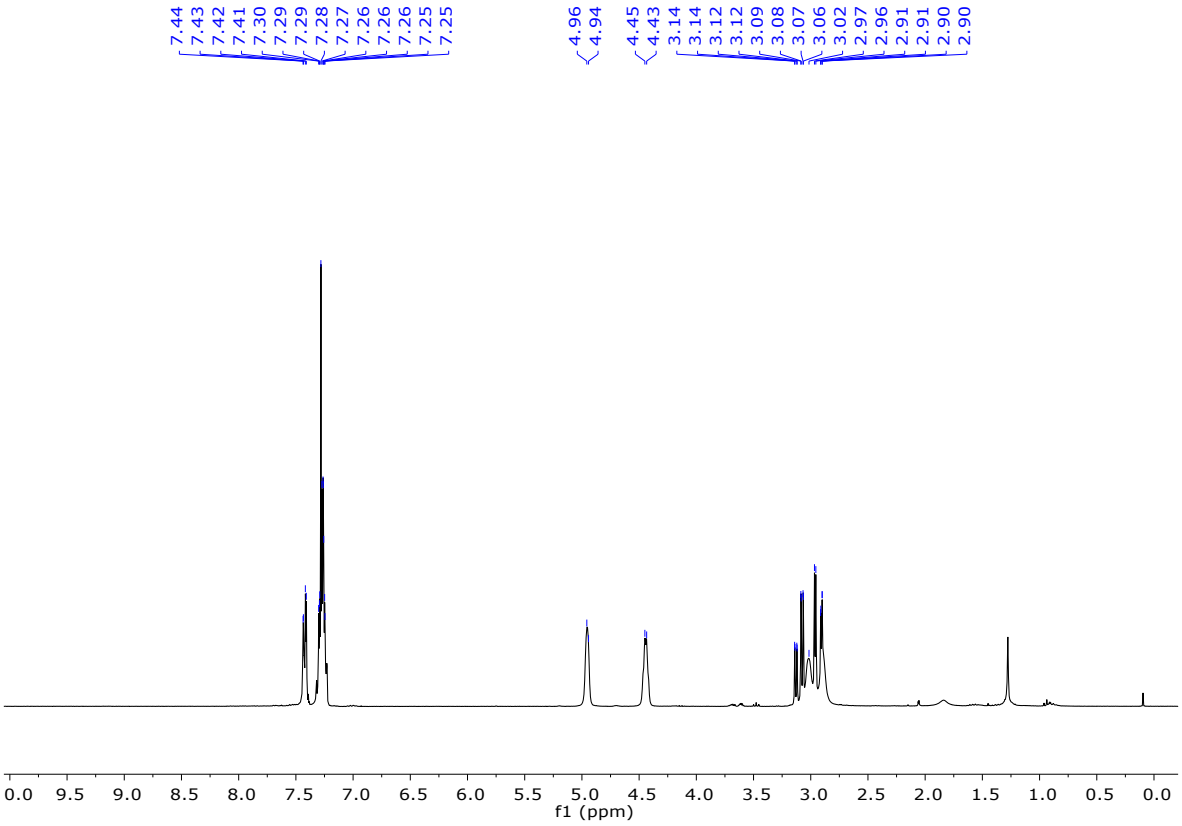


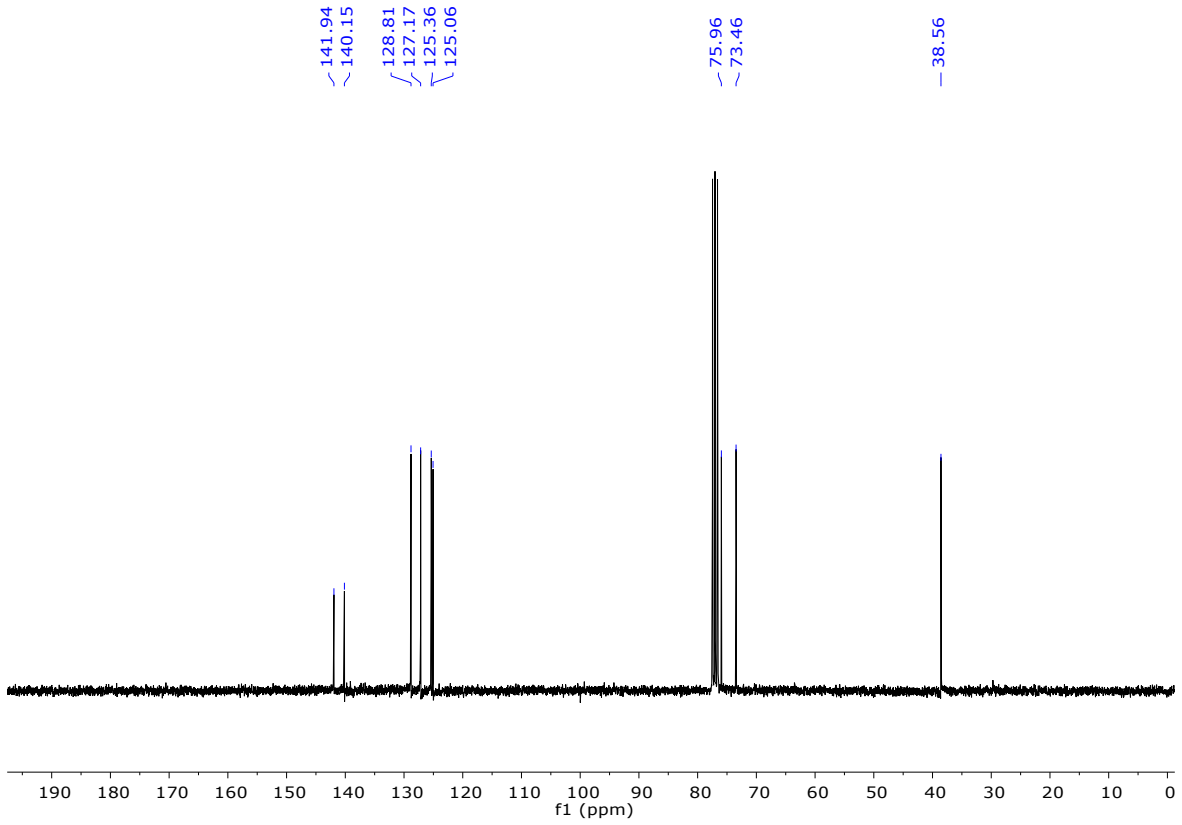
4,8-difluorodibenzo[*c,e*]oxepin-5(7*H*)-one **10c**



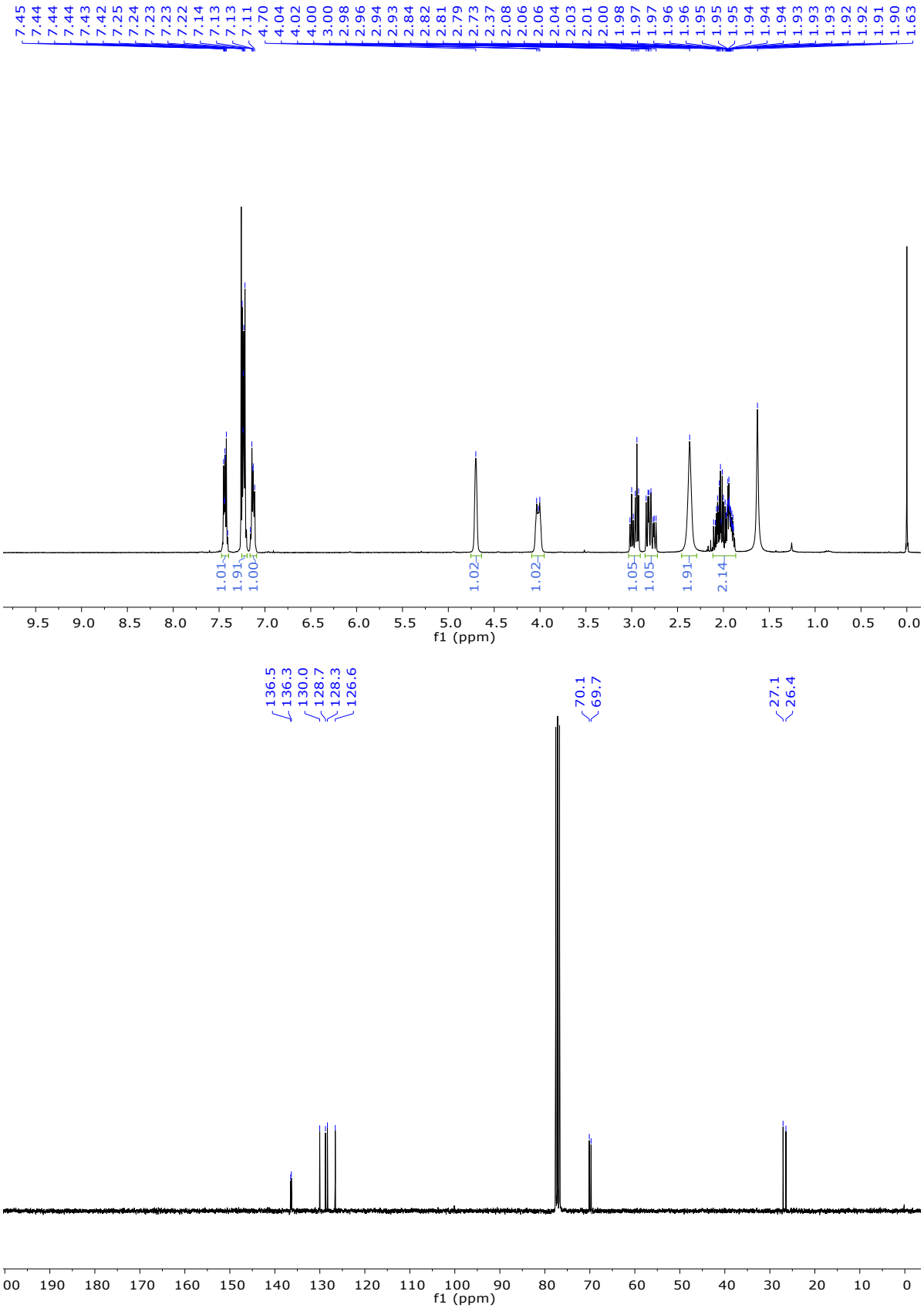


cis-2,3-dihydro-1*H*-indene-1,2-diol **12**





cis-1,2,3,4-tetrahydronaphthalene-1,2-diol **14**



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