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Supplementary Material

Synthesis of novel shikonin derivatives and pharmacological effects of cyclopropylacetylshikonin on melanoma cells

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Content

1. Studies on the enantiomeric purity and associated pharmacological effects of 1

Shikonin and derivatives possess a chiral center in the side chain. In nature, the enantiomeric ratios of shikonin (*R*-isomer) and alkannin (*S*-isomer) varies. Therefore, we determined the optical purity of the isolated shikonins as well as synthesized derivatives by chiral HPLC.

1.1. General

Chiral separations were performed using an Agilent 1100 Series Liquid Chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler and a VWL detector. UV-data were collected at 520 nm. Experiments were carried out at ambient temperature under isocratic conditions with a flow rate of 1.0 ml/min and an injection volume of 15 µl. Data evaluation was performed using a ChemStation for LC 3D Systems Rev. B. 04.03 (Agilent Technologies, Waldbronn, Germany) software. A Chiralcel OD, 250 x 4.6 mm, 3.5 µm from Daicel Chemical Industries (Osaka, Japan) served as CSP with immobilized cellulose tris (3,5-dimethylphenylcarbamate) as chiral selector. Mobile phase consisted of *n*-hexane / 2-propanol = 3 : 1 (v/v).

1.2. Identification of shikonin and alkannin

An authentic sample of previously isolated **1** [8] was hydrolyzed with diluted sodium hydroxide solution [S1]. Chiral HPLC [S2] of the isolated product revealed 70% shikonin and 30% alkannin. The shikonin batch supplied from Chengdu Biopurify Phytochemicals Ltd., Chengdu, People's Republic of China, which was also used for syntheses, and, in addition, an alkannin lot supplied from Chengdu Push Bio-Technology Co. Ltd., Chengdu, People's Republic of China were submitted to the same analysis. Shikonin turned out to consist of 100% *R*-isomer, alkannin was almost a racemate (*R* : *S* = 49 : 51).

References:

S1: Pekin, G.; Ganzera, M.; Senol, S.; Bedir, E.; Korkmaz, K.S.; Stuppner, H. Determination of Naphthazarin Derivatives in Endemic Turkish Alkanna Species by Revered Phase High Performance Liquid Chromatography. *Planta Med* **2007**, *73*, 267-272, DOI: 10.1055/s-2007-967110.

S2: Ikeda, Y.; Ishida, N.; Fukaya, C.; Yokoyama, K.; Tabata, M.; Fukui, H.; Honda, G. Determination of the Ratio between Optical Isomers, Shikonin and Alkannin by High Performance Liquid Chromatography Analysis. *Chem Pharm Bull* **1991**, *39*, 2351-2153.

1.3. Determination of enantiomeric purity of ß,ß-dimethylacrylshikonin samples

To investigate the influence of the chiral center on the cytotoxicity, different hydrolysates of **1** were prepared and analyzed in accordance to the method above (Table S1). These investigations also show that the enantiomeric ratio did not change during synthesis.

Table S1: Enantiomeric ratios of samples of **1**. Racemic *ß,ß*-dimethylacrylshikonin was prepared by acylation of racemic shikonin/alkannin mixture with *ß,ß*-dimethylacrylic acid according to the procedure in the plain text.

Source of 1	Ratio of enantiomers (after hydrolysis)
Isolated from *O. paniculata* [8]	70% *R*-isomer and 30% *S*-isomer
synthesized from 100% shikonin	100% *R*-isomer
synthesized from alkannin	49% *R*-isomer and 51% *S*-isomer

65 *1.4. Influence of enantiomeric purity of **1** on cytotoxicity*

66 Cytotoxicity was investigated using WM164 melanoma cells and pure *R*-isomer, 49% *R*-isomer
67 and two 70:30 mixtures of *R*-isomer (a: mixture of 100% and 49% R-isomer to yield 70/30; b: isolated
68 **1**). Melanoma cells were treated with these compounds up to 10.0 µM and 72 h of incubation (Figure
69 S1). We did not detect significant differences in the cytotoxicity indicating that the chiral center as well
70 as the synthesis process has no influence on the activity.
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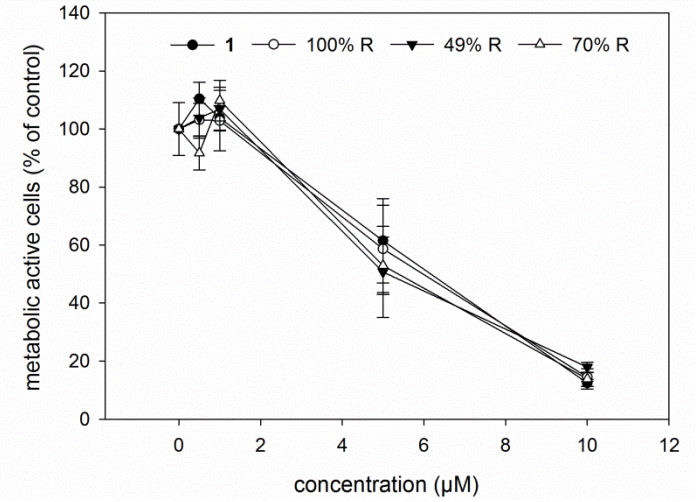

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73 **Figure S1.** Cytotoxicity of the isolated **1** compared to 100% synthesized *R*-isomer of **1** (100% R), 49% *R*-
74 isomer (49% R) and "mixed" 70% *R*-isomer (70% R) as determined by the XTT viability assay and after
75 72 h incubation with different concentration of the compounds (WM164 cells, mean ± sem, $n = 6$).

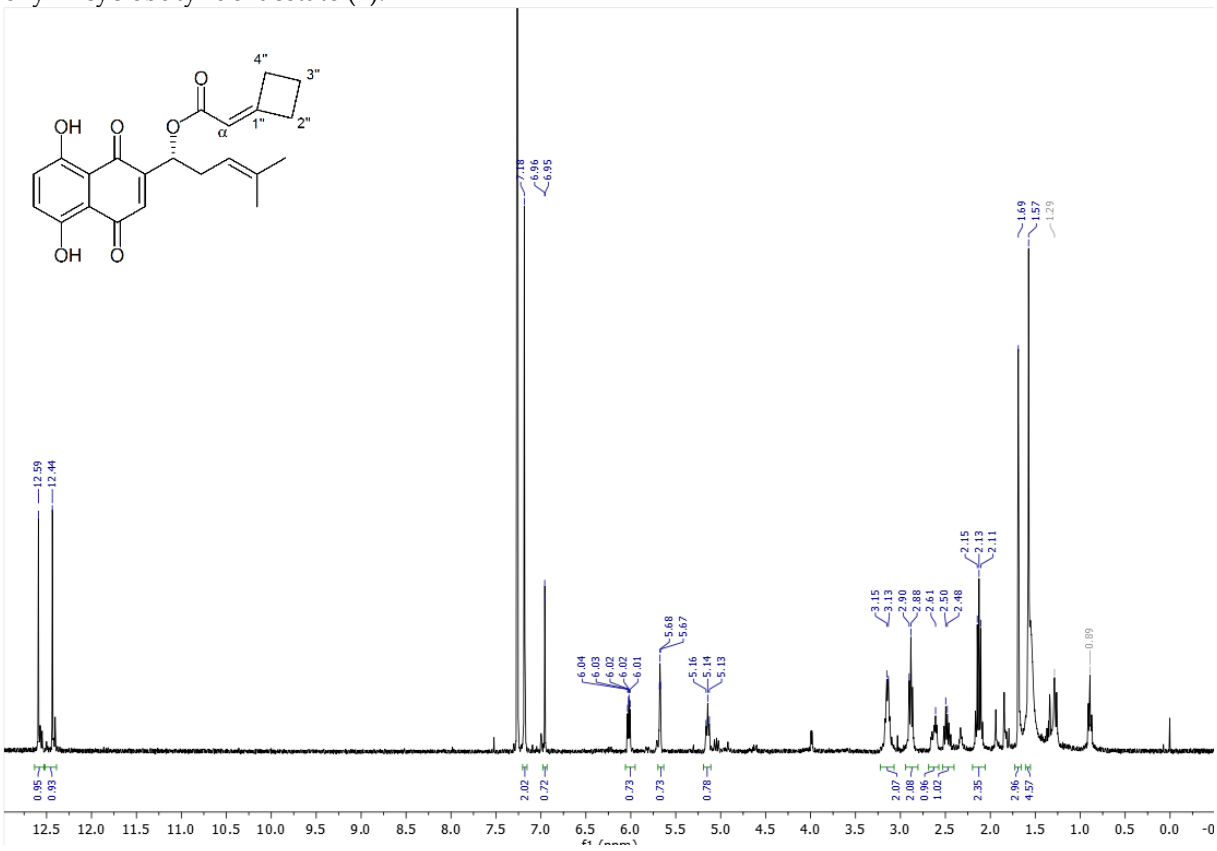
2. NMR-spectra (^{1}H and ^{13}C or HMBC) of the new shikonin derivatives 2 to 10 and 12 to 20

^{1}H and ^{13}C NMR spectra were recorded on Varian 400 MHz UnityINOVA spectrometer (400 and 100 MHz, respectively) using deuterated chloroform ($CDCl_3$) as solvent and TMS as internal standard.

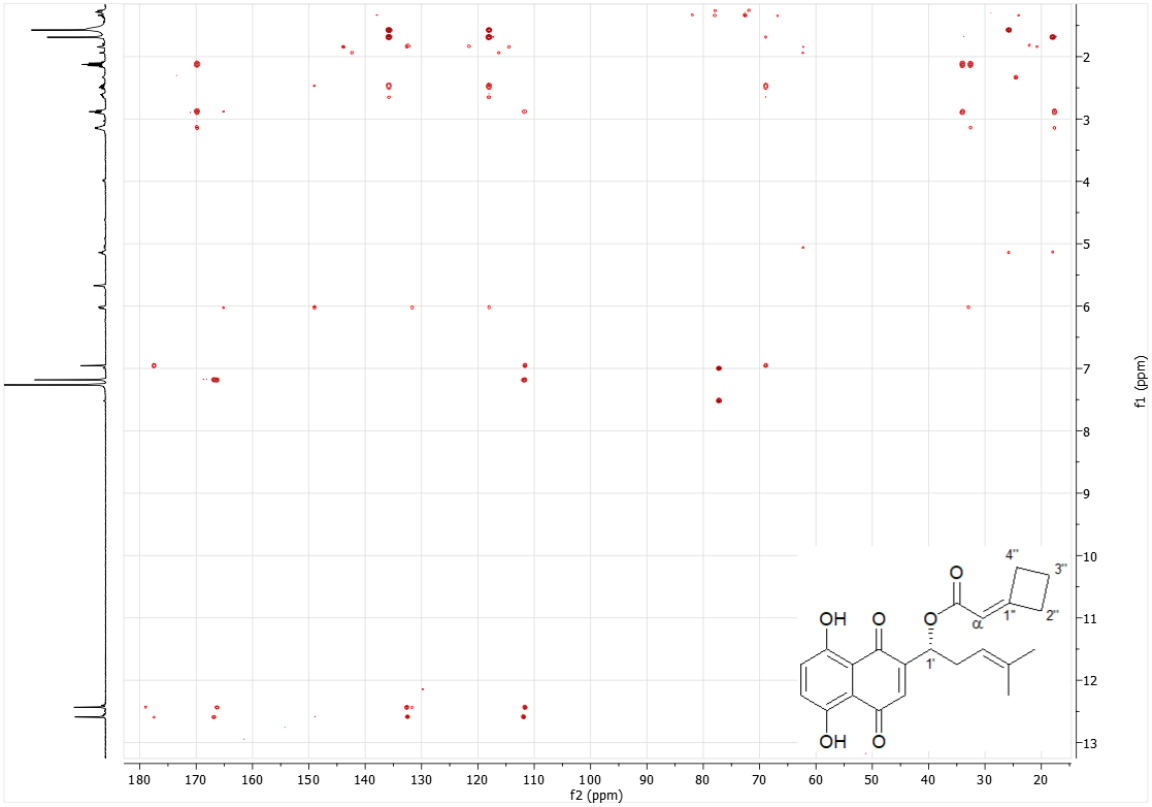
After acylation of shikonin, the side chain was proven by NMR: The ^{1}H NMR signal of H-1′ of the side chain was shifted from 4.92 ppm in shikonin to 6.0 - 6.1 ppm in the esters **2** to **20**, whereas, the signals of the aromatic and the phenolic hydrogen atoms remained almost constant. In the HMBC spectra a cross peak from ester carbonyl carbon to H-1′ of the shikonin moiety proves the esterification at the side chain of shikonin.

NMR-Spectra:

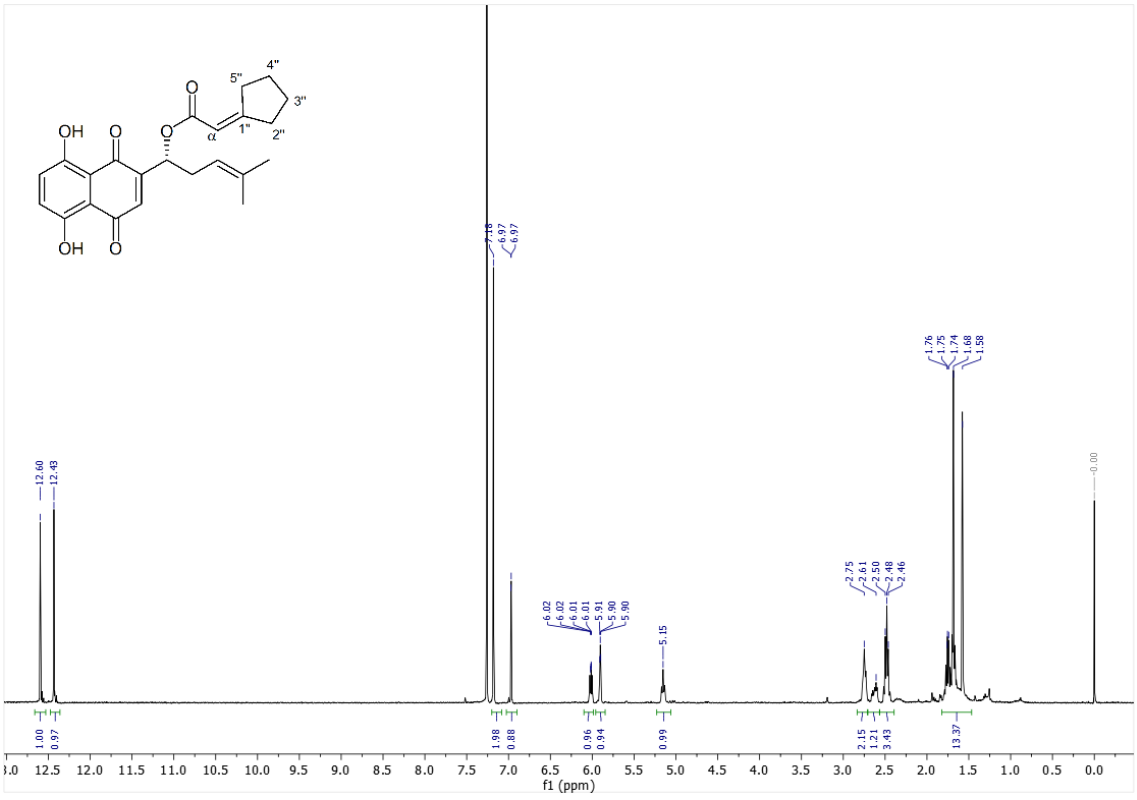
^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclobutylidenacetate (**2**):



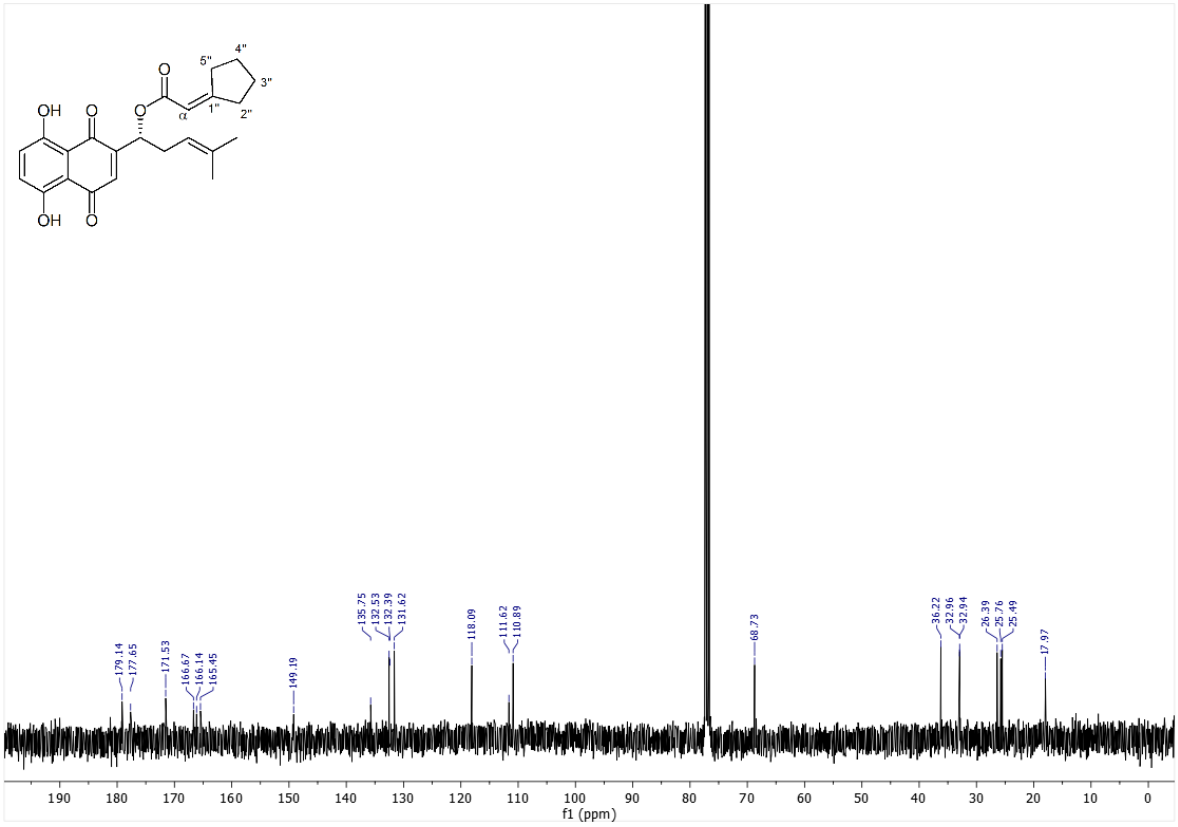
HMBC spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclobutylidenacetate (**2**):



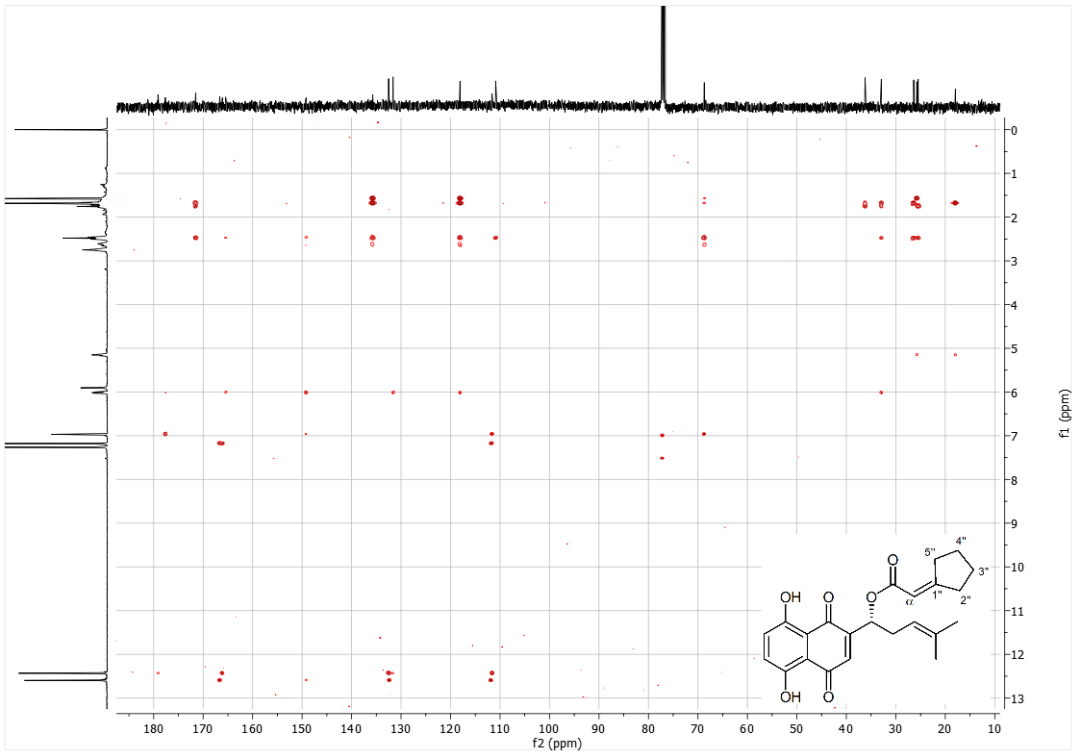
^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclopentylidenacetate (**3**):



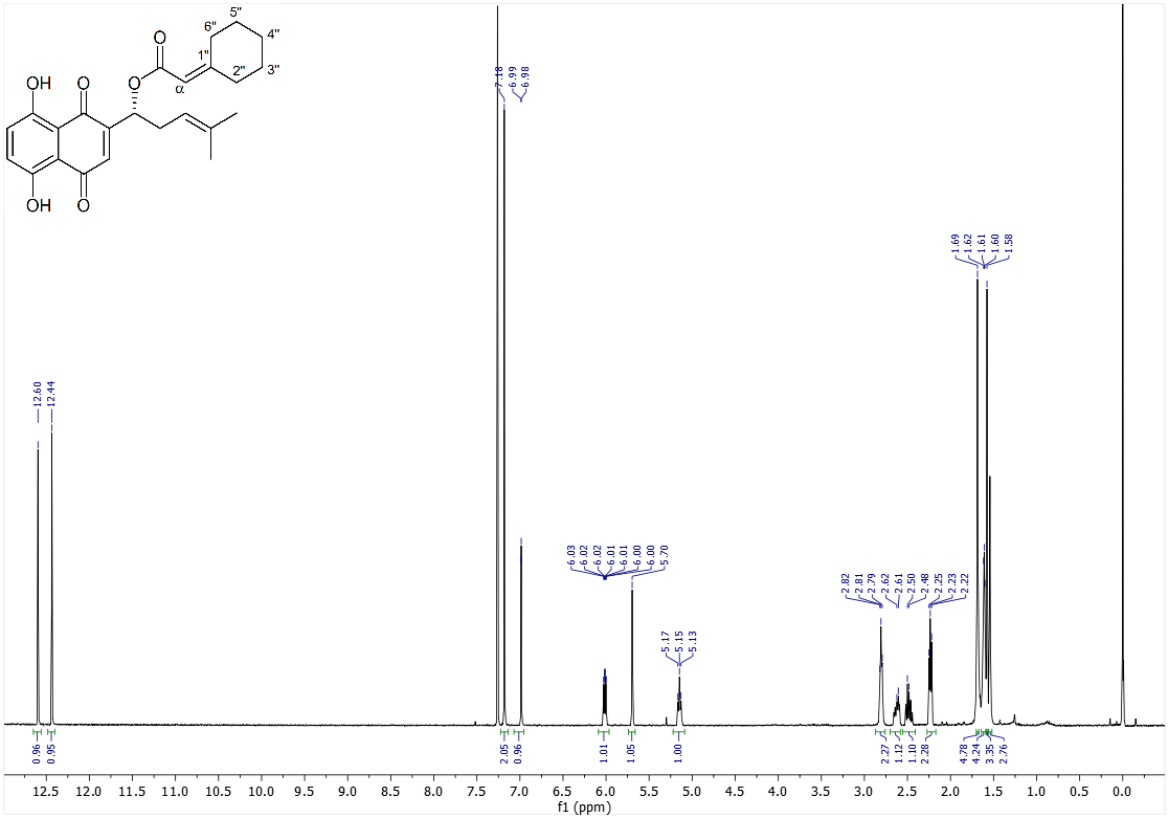
^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclopentylidenacetate (**3**):



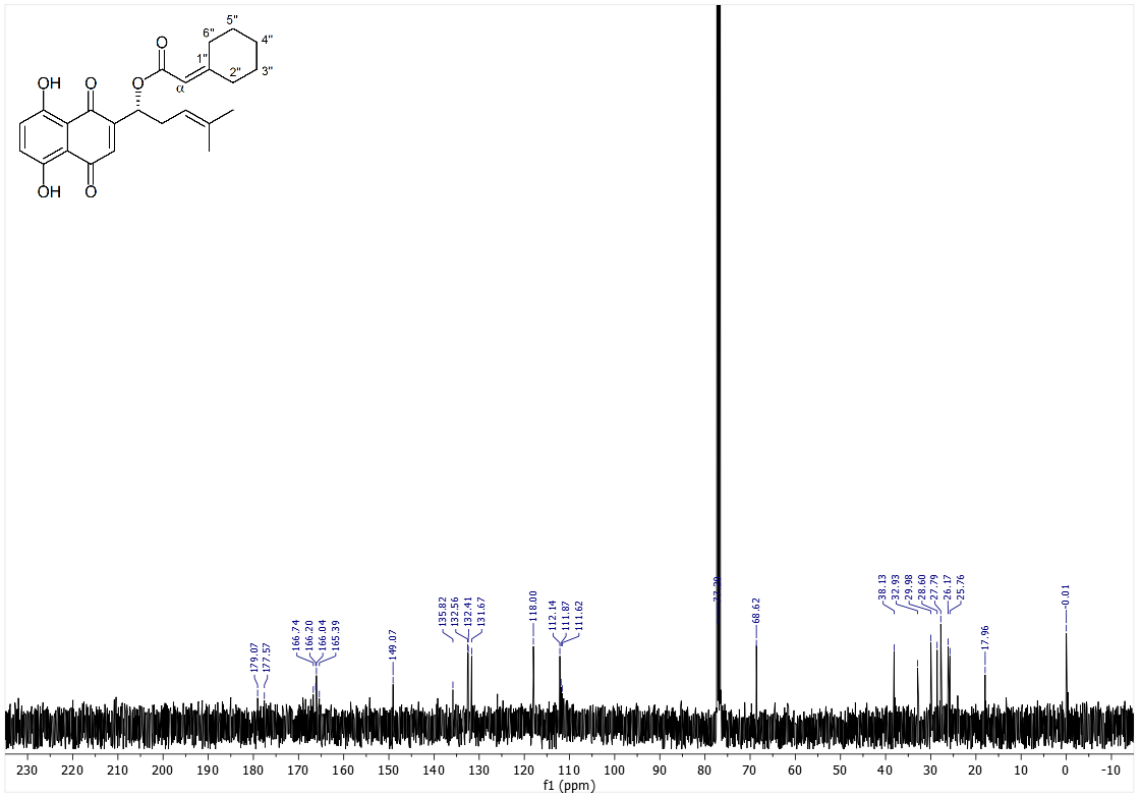
HMBC spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclopentylidenacetate (**3**):



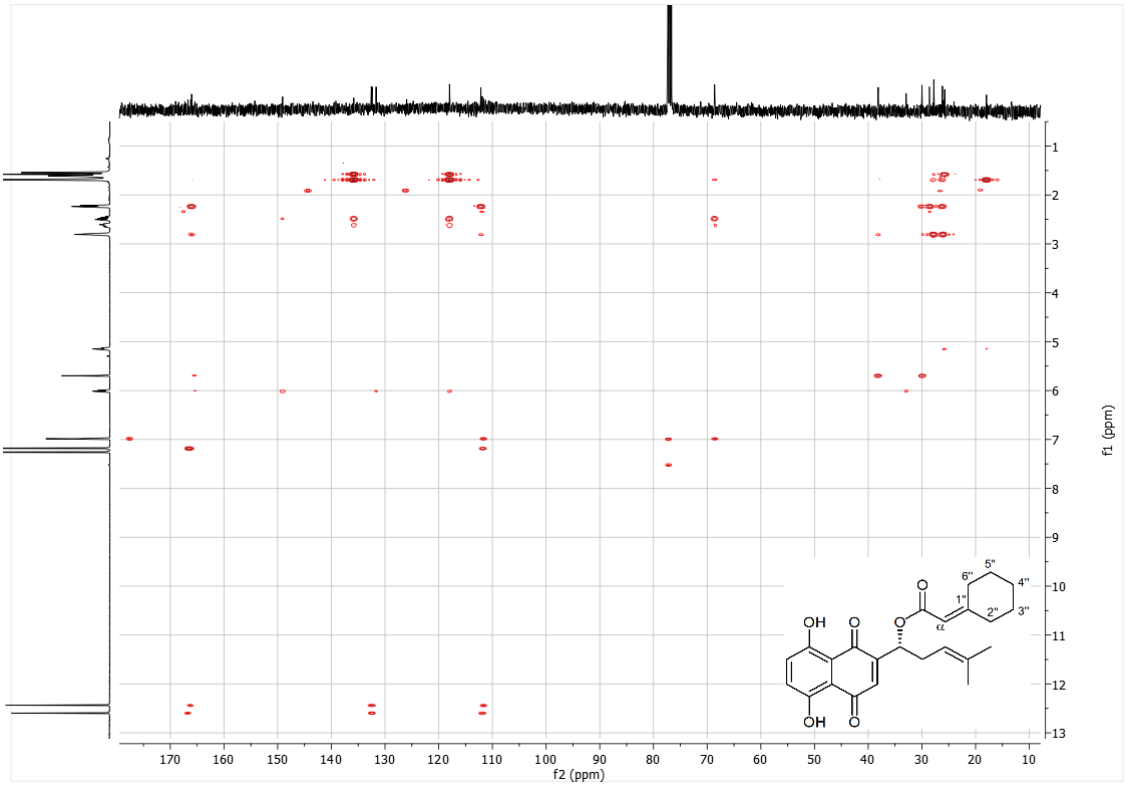
[1]H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclohexylidenacetate (**4**):



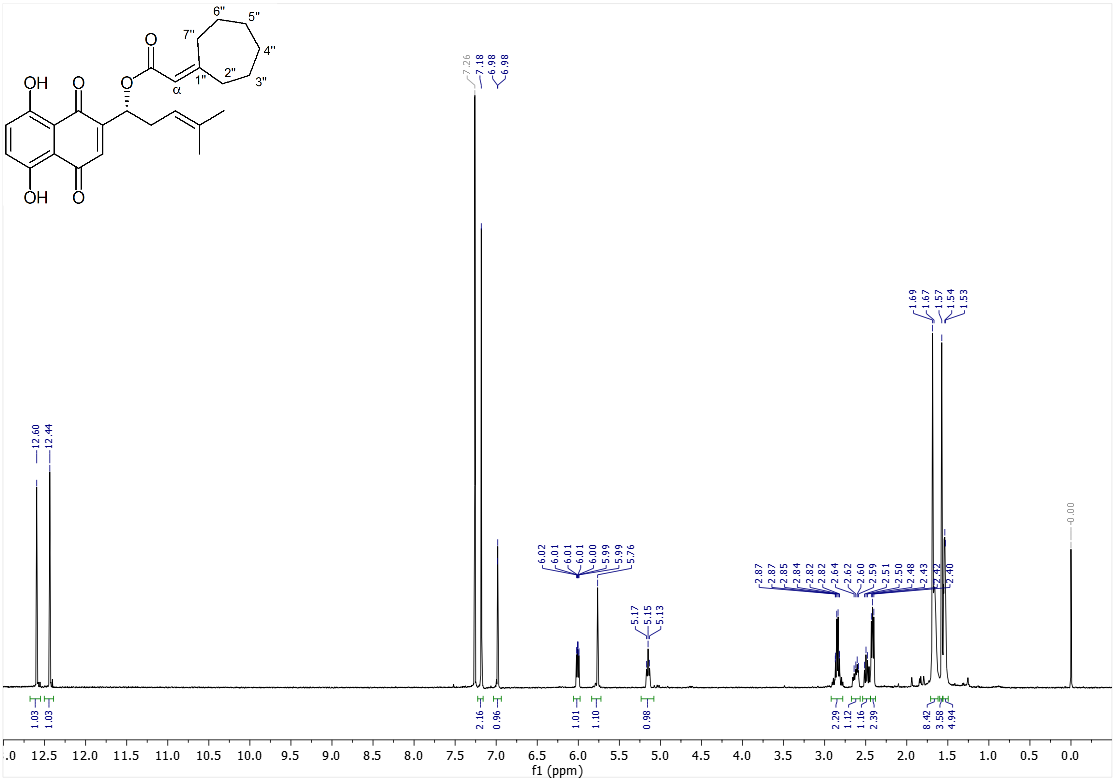
[13]C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclohexylidenacetate (**4**):



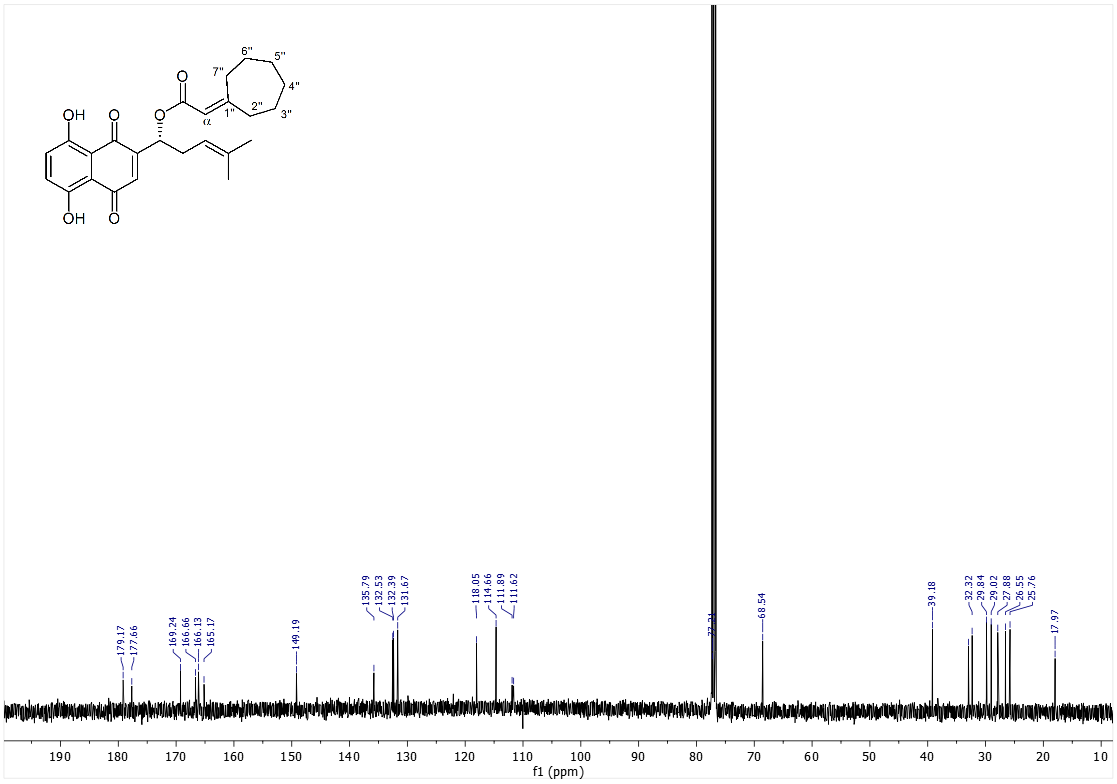
HMBC spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cyclohexylidenacetate (**4**):



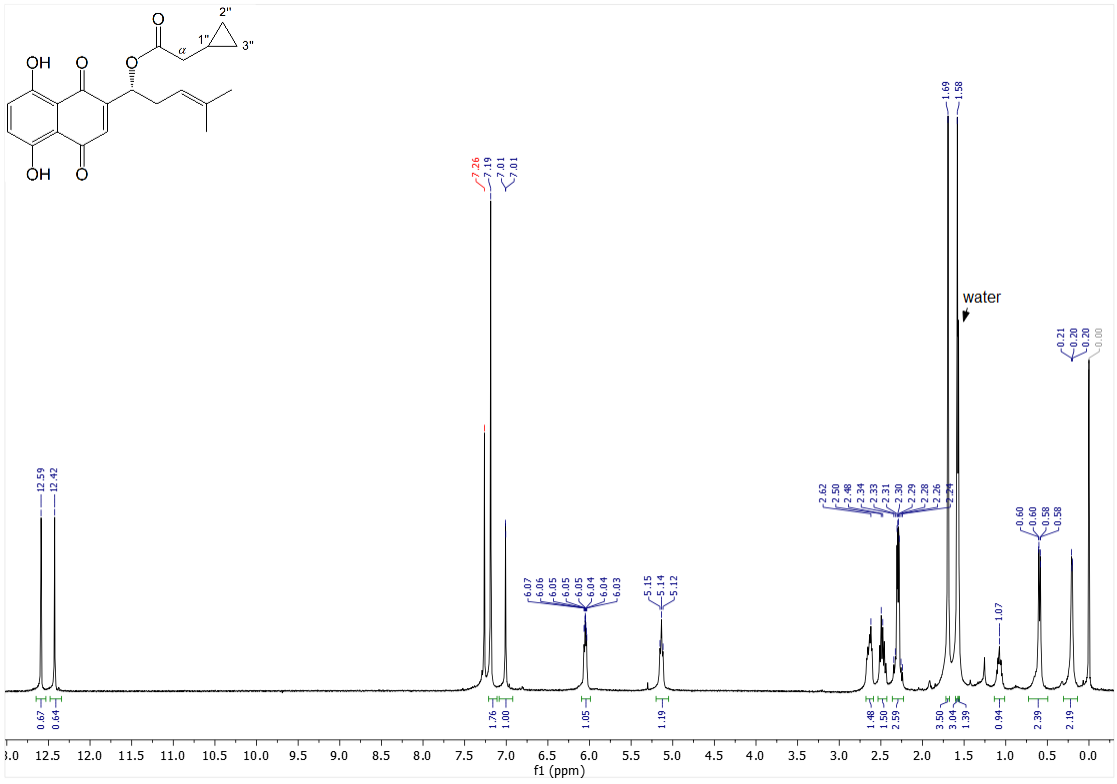
^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cycloheptylidenacetate (**5**):



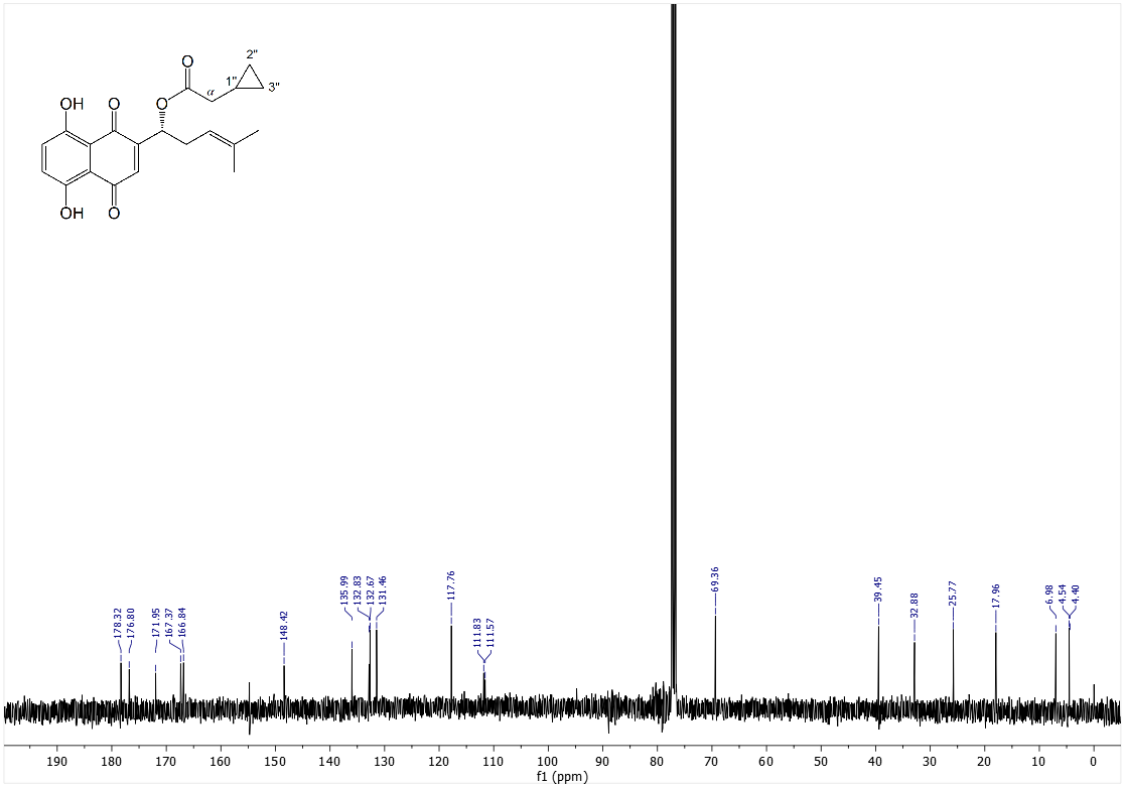
^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl 2-cycloheptylidenacetate (**5**):



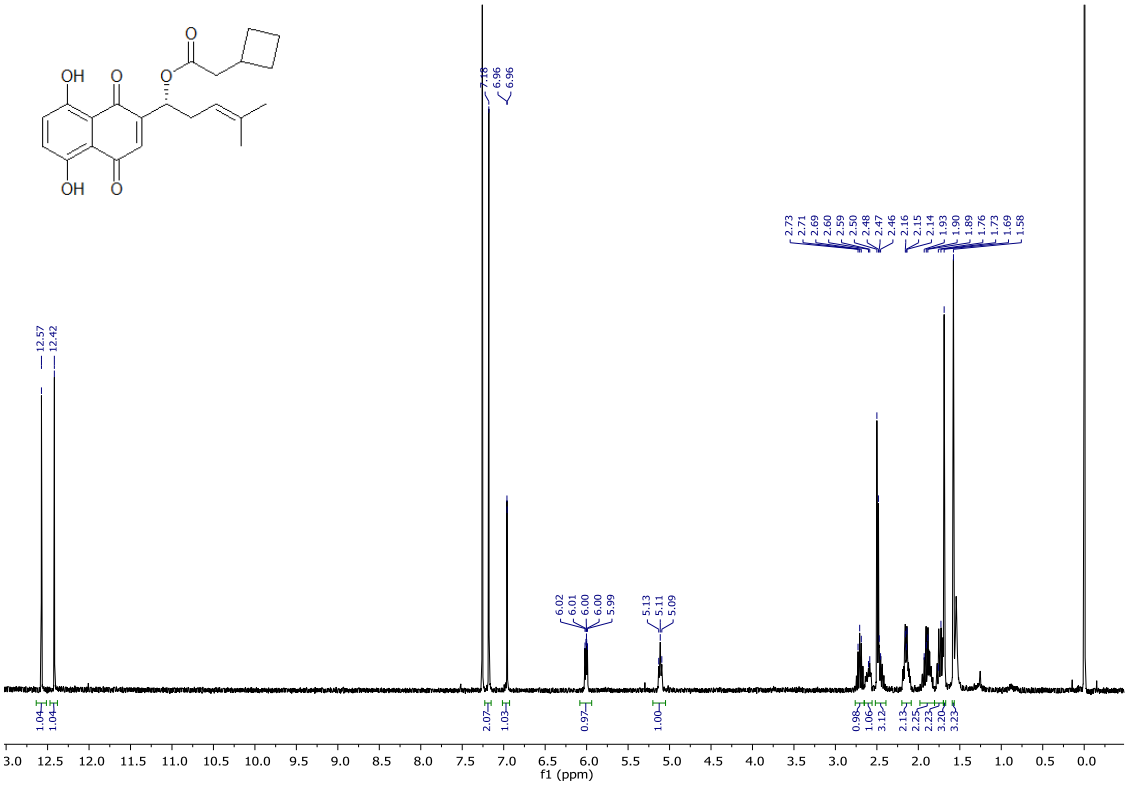
^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl cyclopropylacetate (**6**):



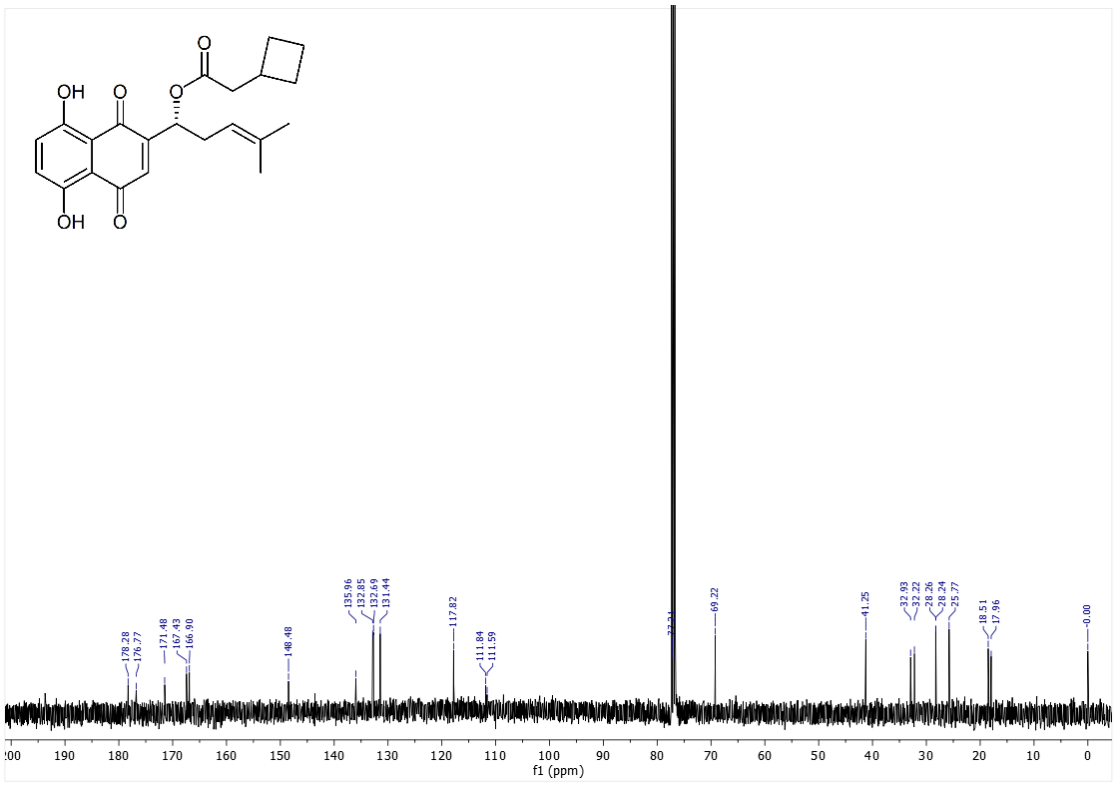
^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl cyclopropylacetate (**6**):



^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl cyclobutylacetate (**7**):



127 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
128 enyl cyclobutylacetate (**7**):



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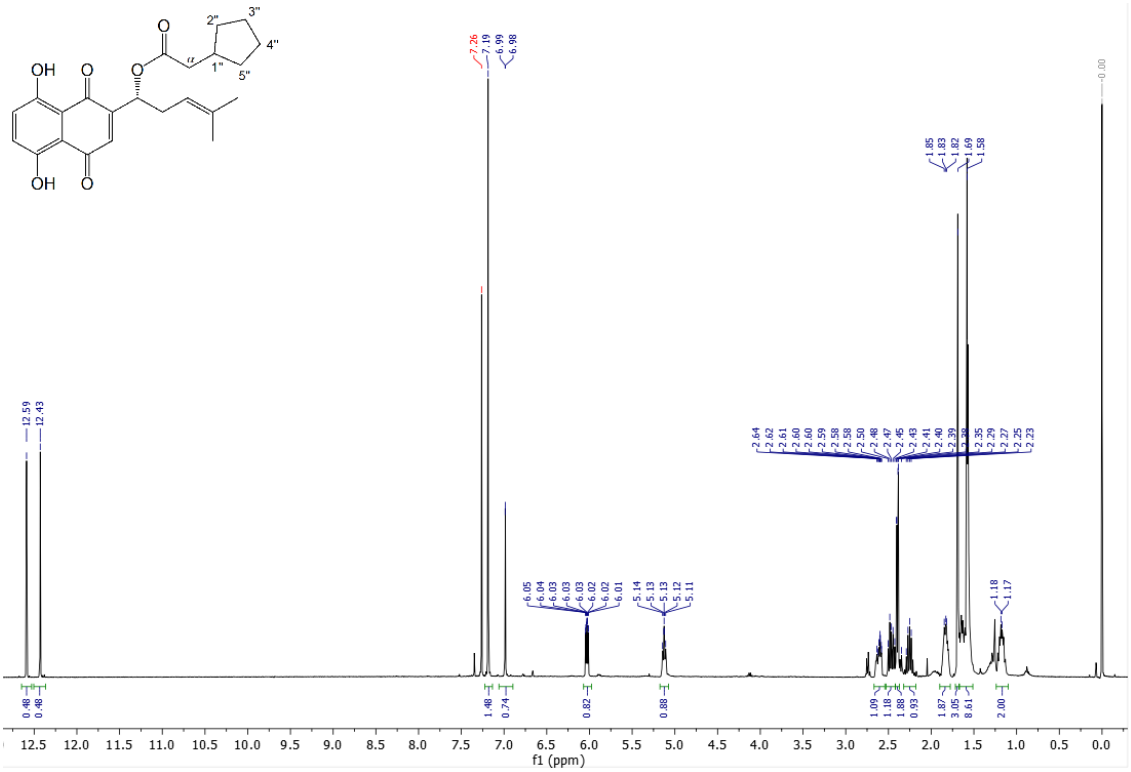
130 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
131 enyl cyclopentylacetate (**8**):



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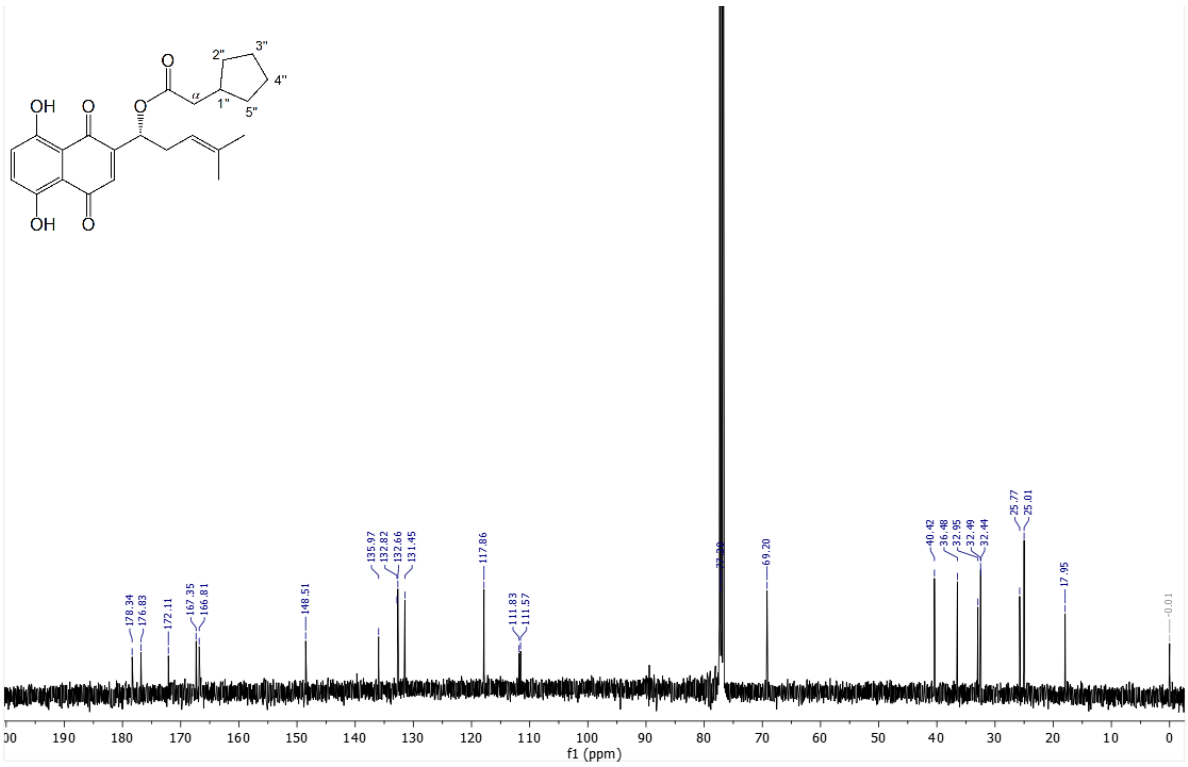
133 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
134 enyl cyclopentylacetate (**8**):



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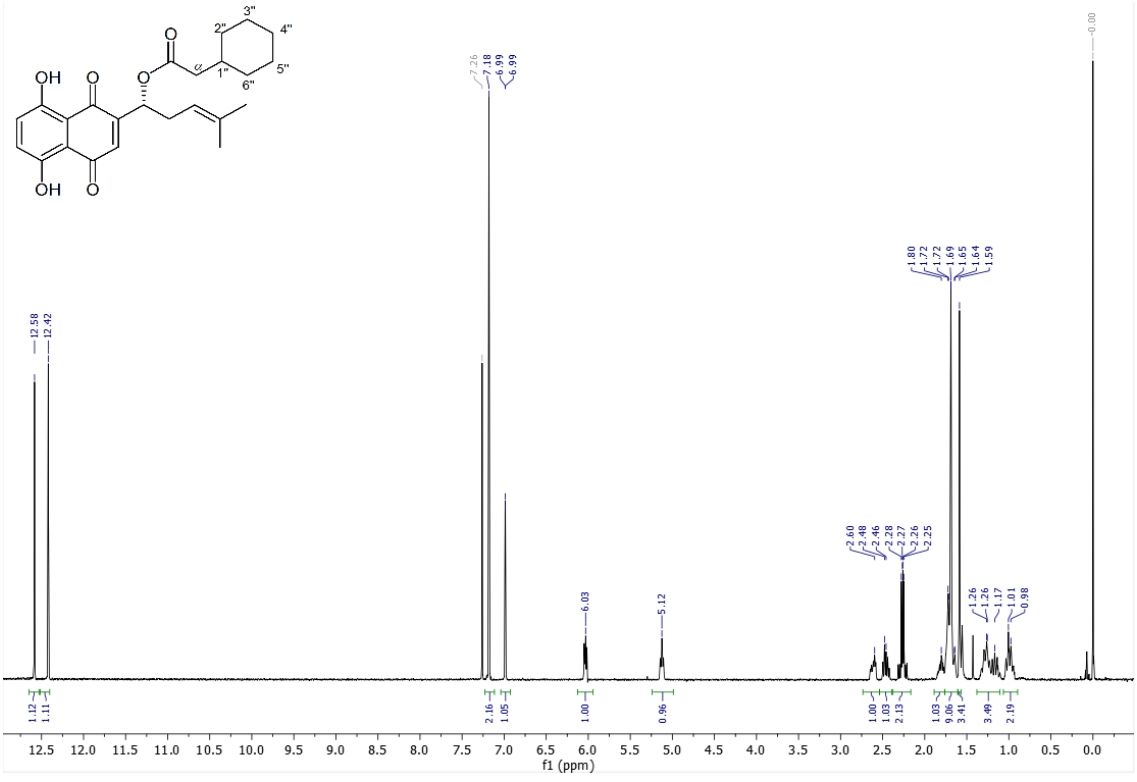
136 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
137 enyl cyclohexylacetate (**9**):



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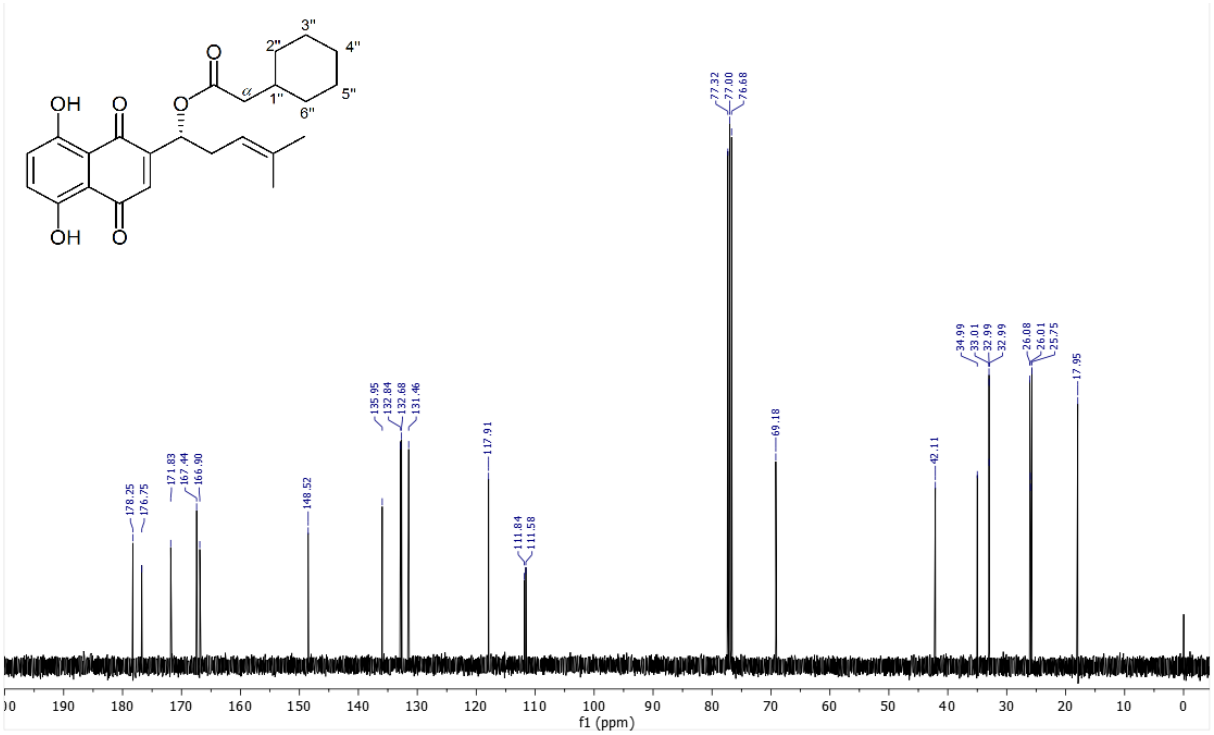
139 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
140 enyl cyclohexylacetate (**9**):



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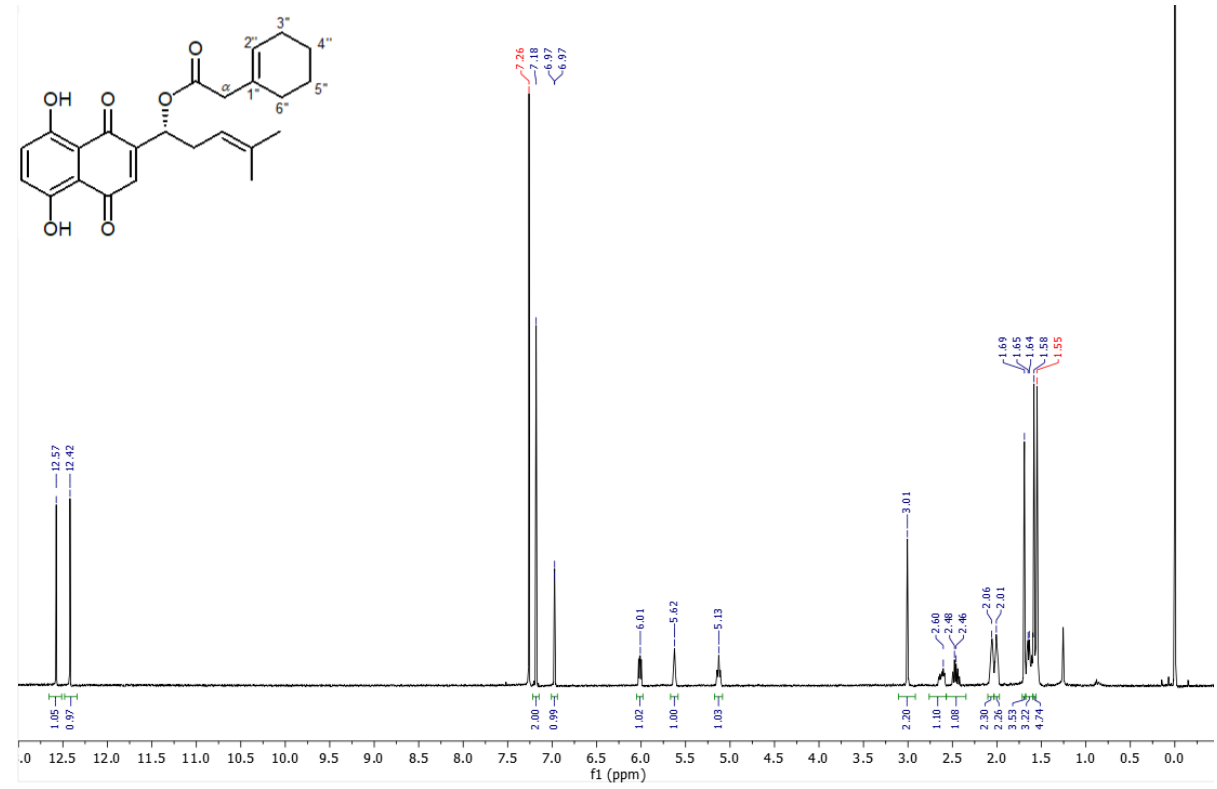
142 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
143 enyl 1-cyclohexen-1-ylacetate (**10**):



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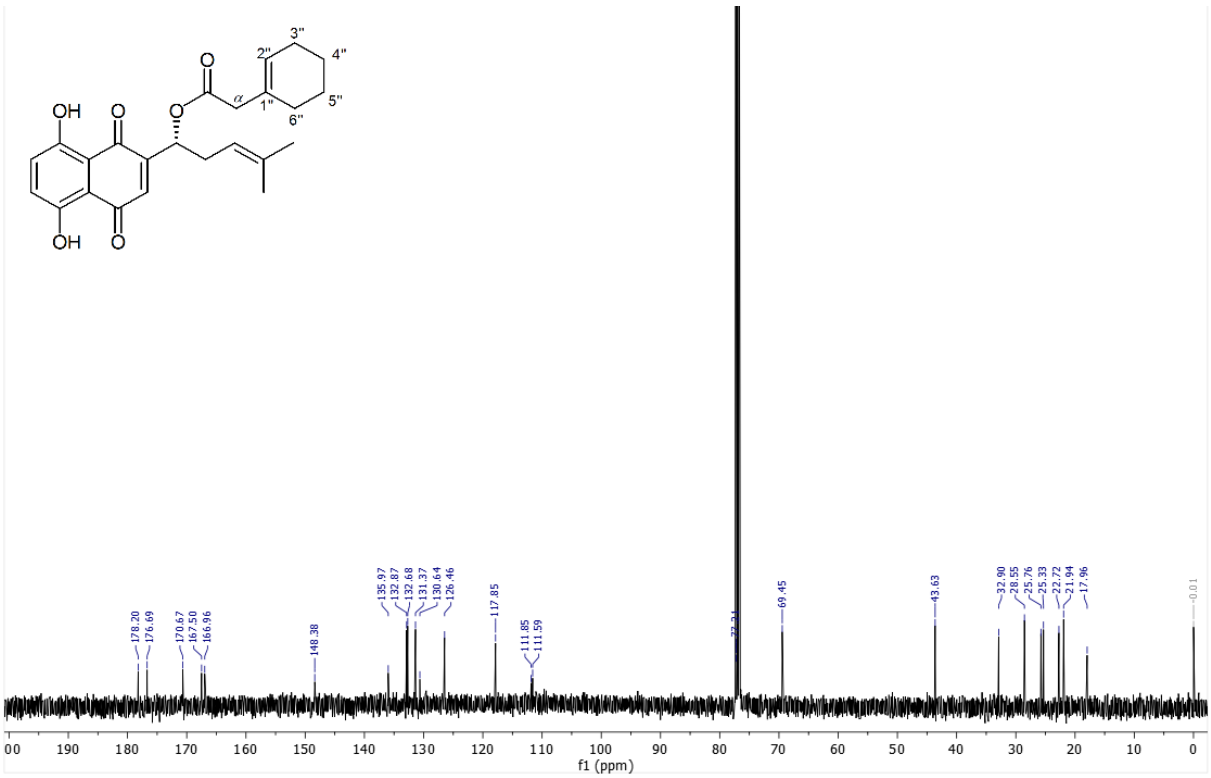
145 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
146 enyl 1-cyclohexen-1-ylacetate (**10**):



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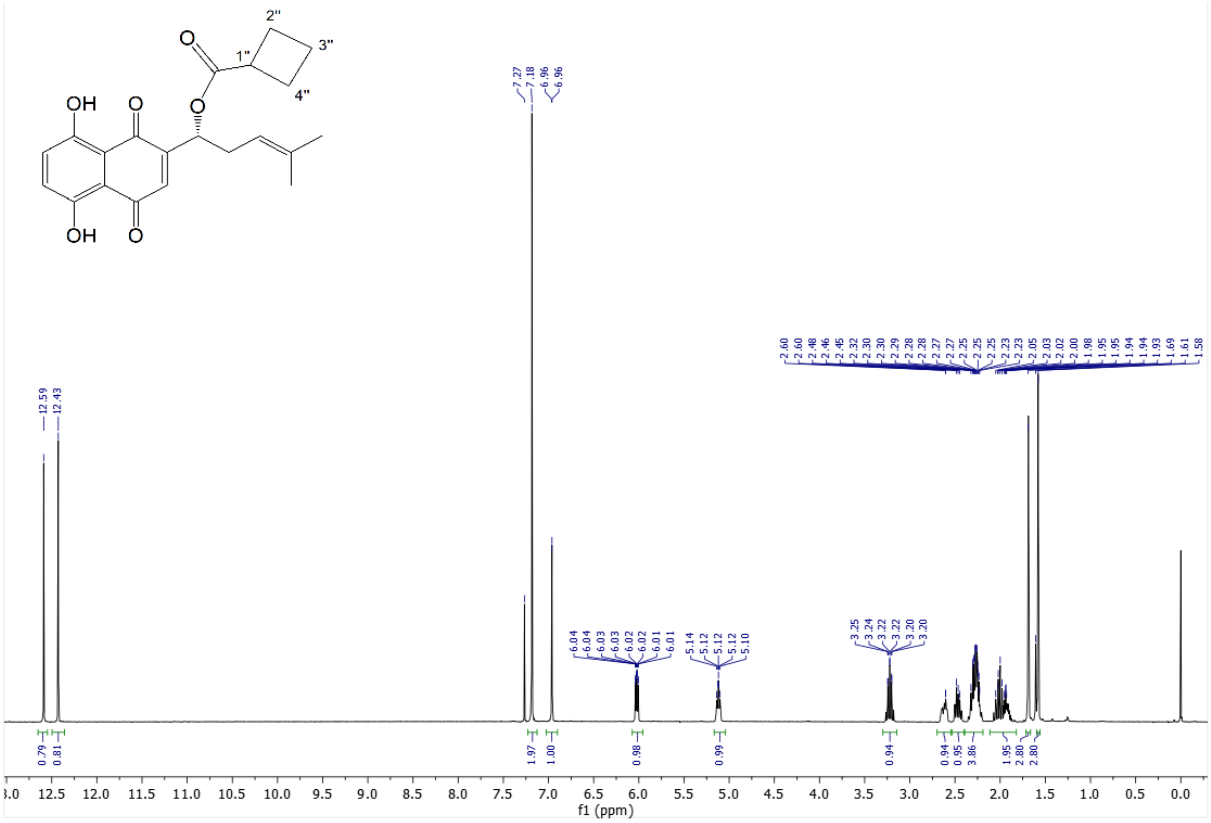
148 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
149 enyl cyclobutanecarboxylate (**12**):



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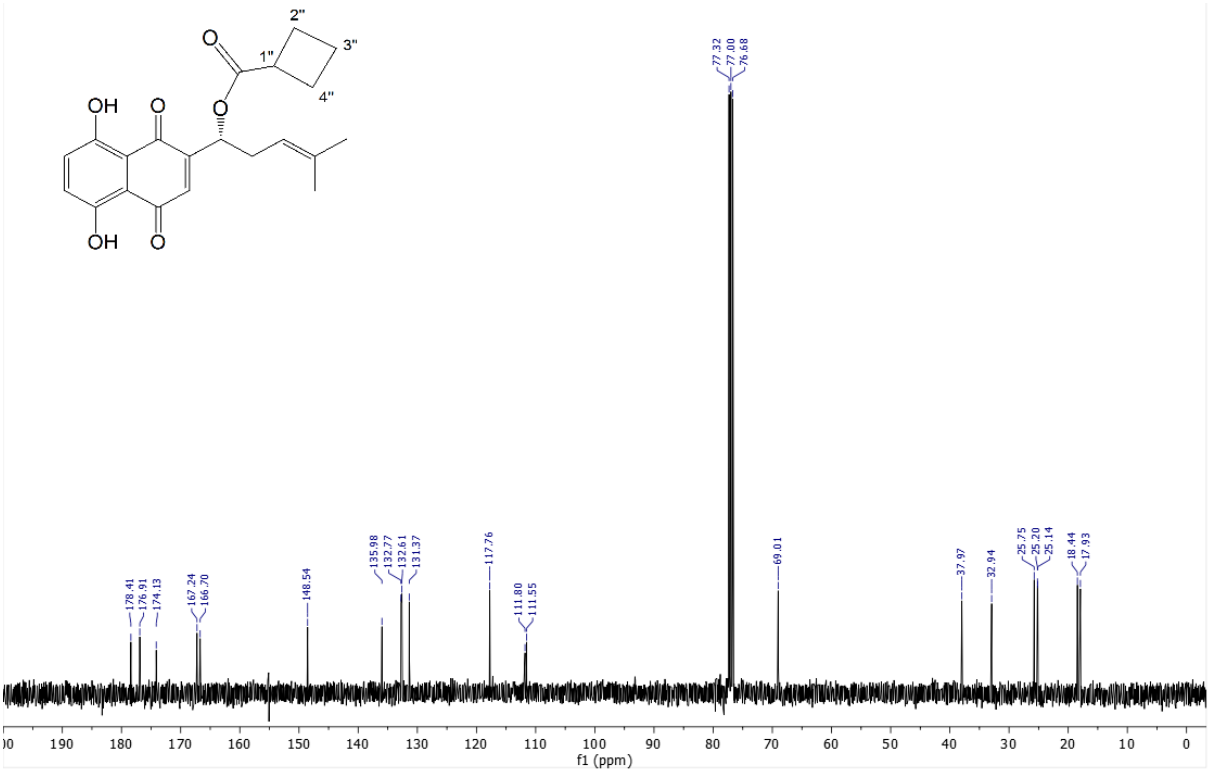
151 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
152 enyl cyclobutanecarboxylate (**12**):



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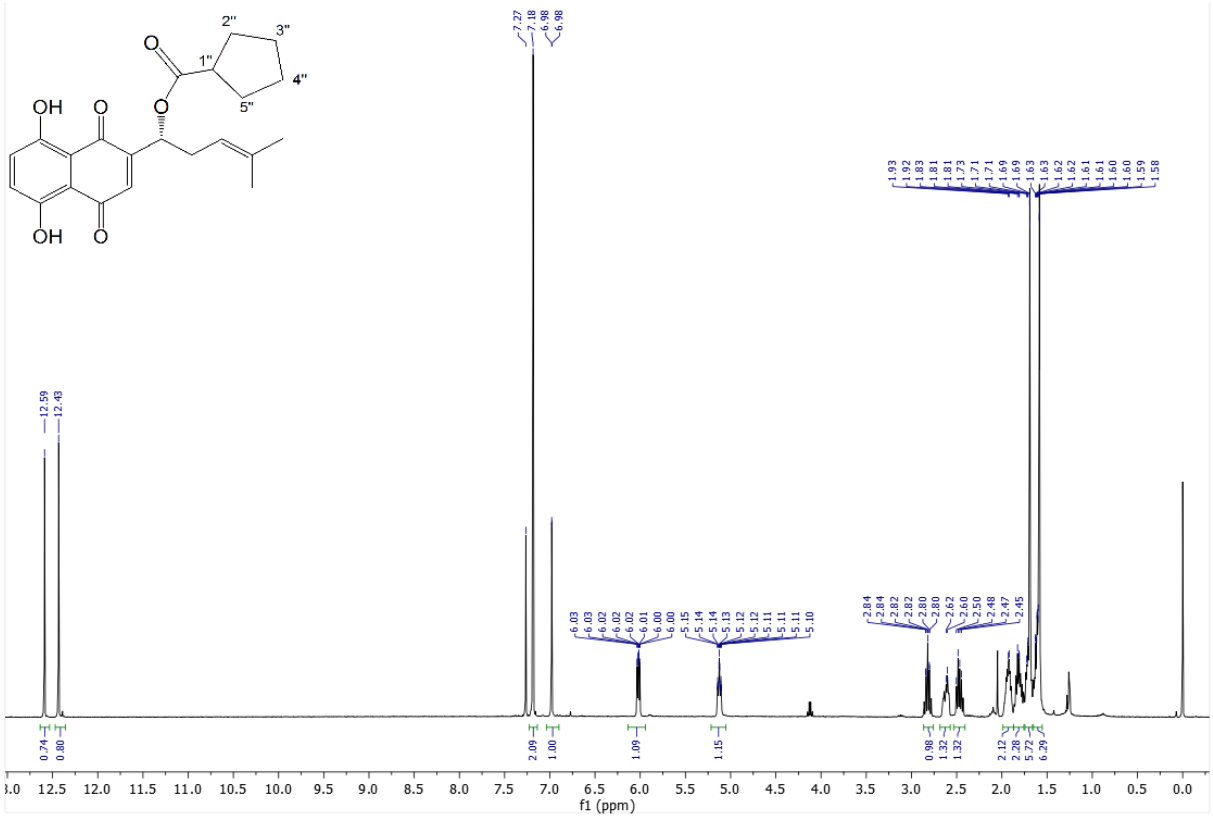
154 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
155 enyl cyclopentanecarboxylate (**13**):



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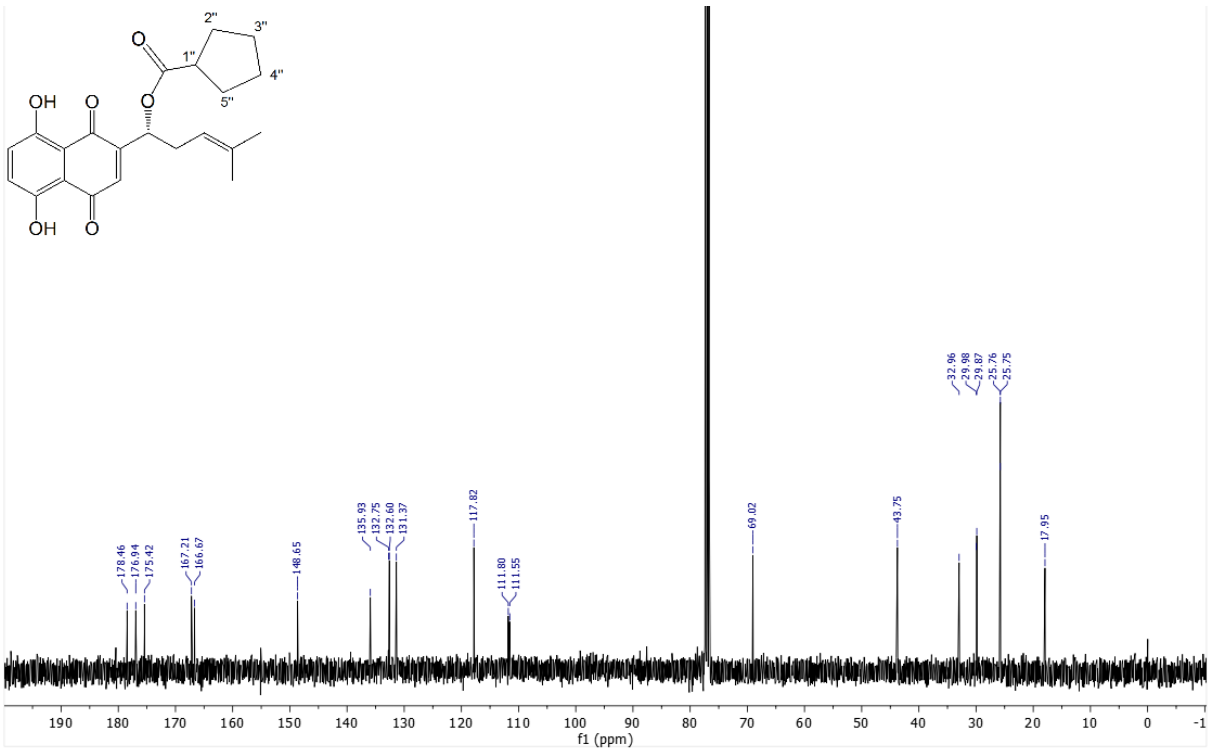
157 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
158 enyl cyclopentanecarboxylate (**13**):



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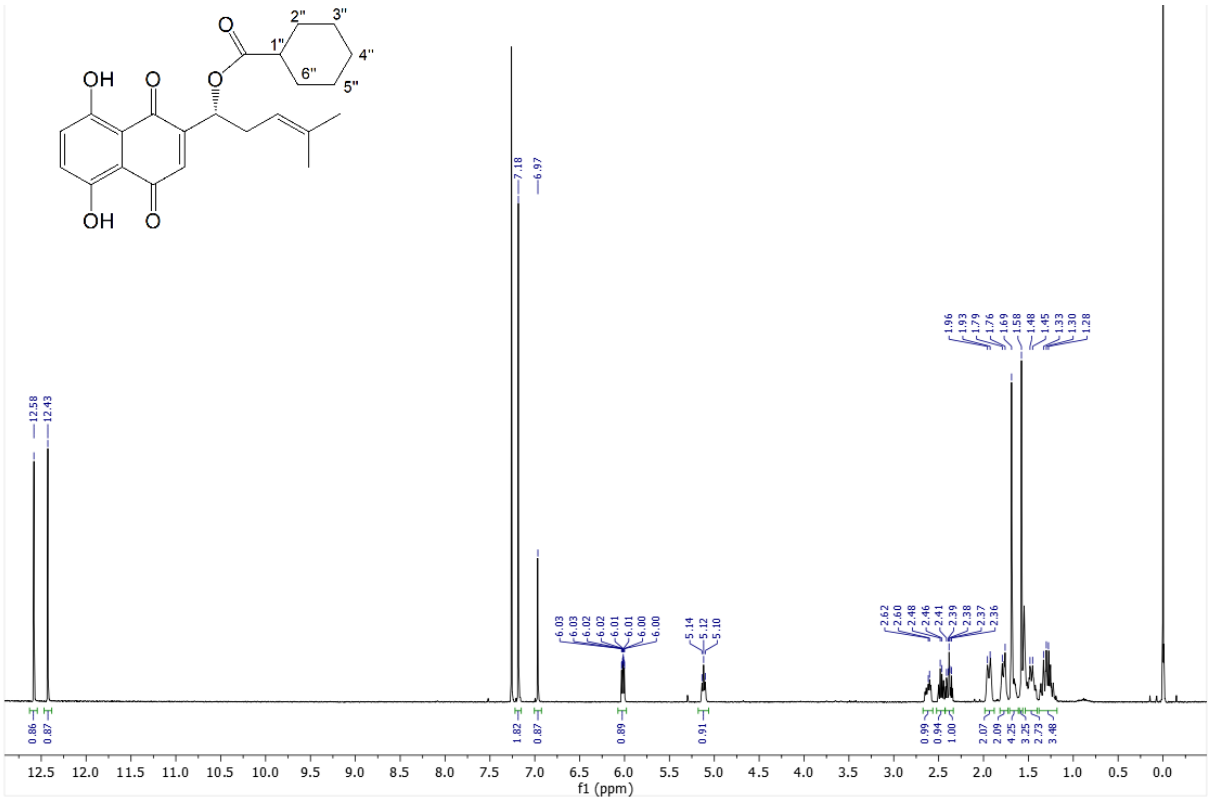
160 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
161 enyl cyclohexanecarboxylate (**14**):



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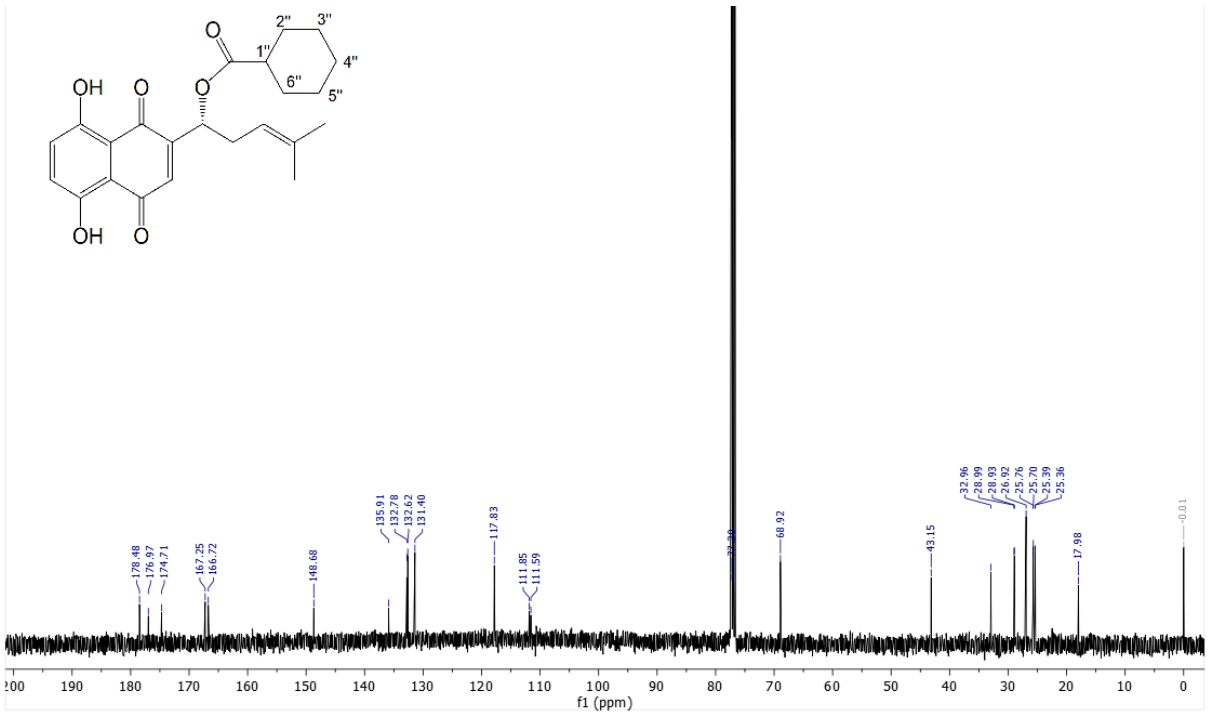
163 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-

164 enyl cyclohexanecarboxylate (**14**):



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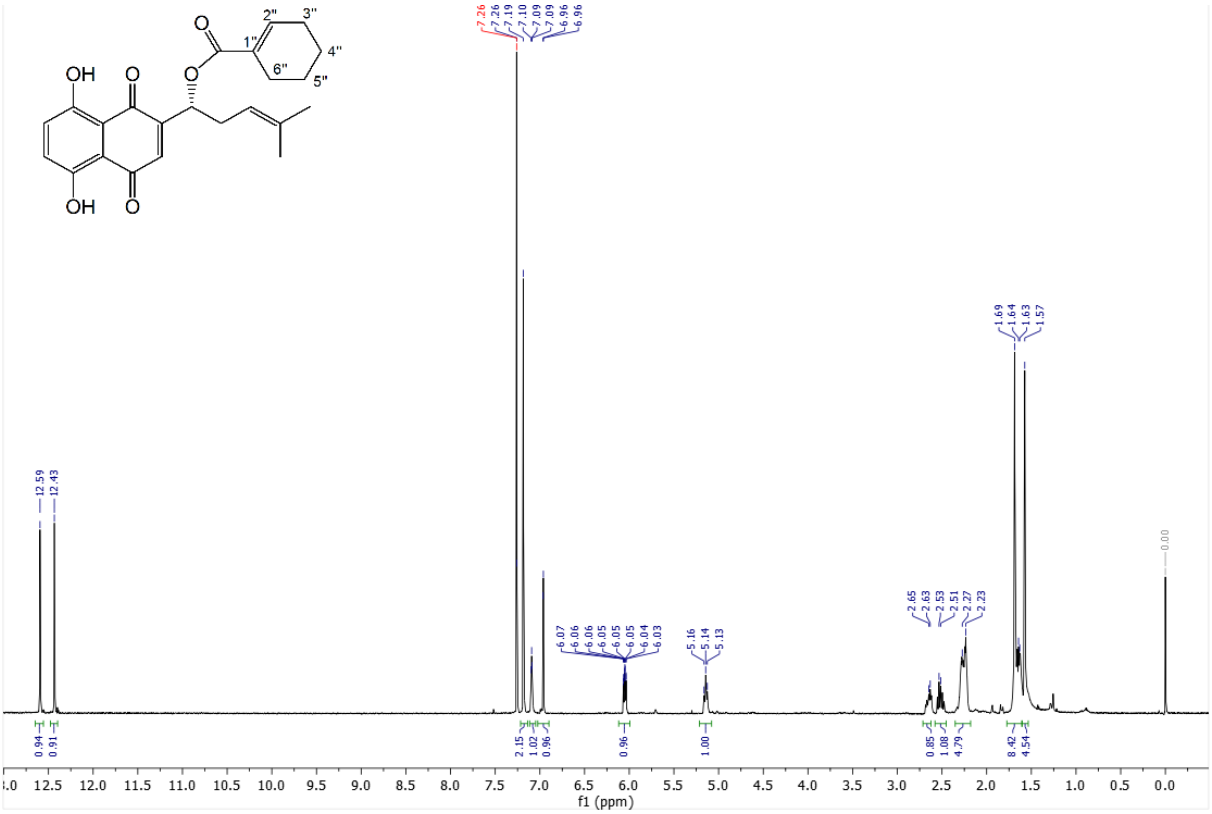
166 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-

167 enyl cyclohex-1-enecarboxylate (**15**):



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169 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
170 enyl cyclohex-1-enecarboxylate (**15**):
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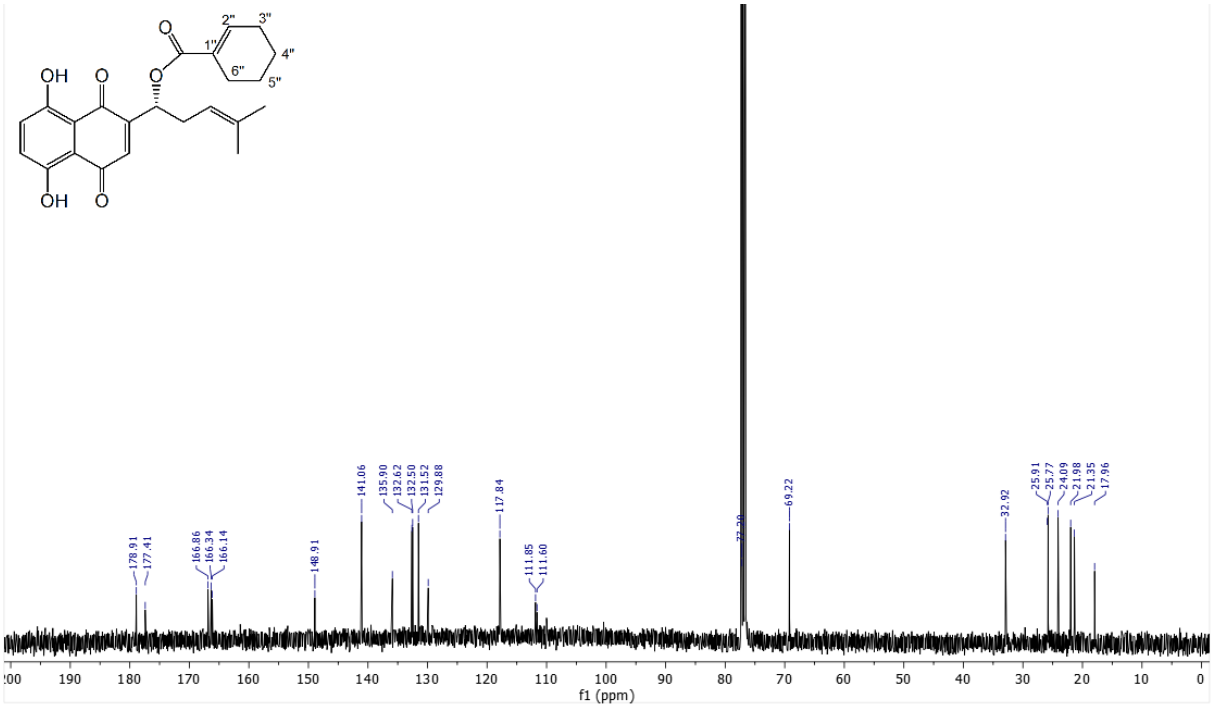

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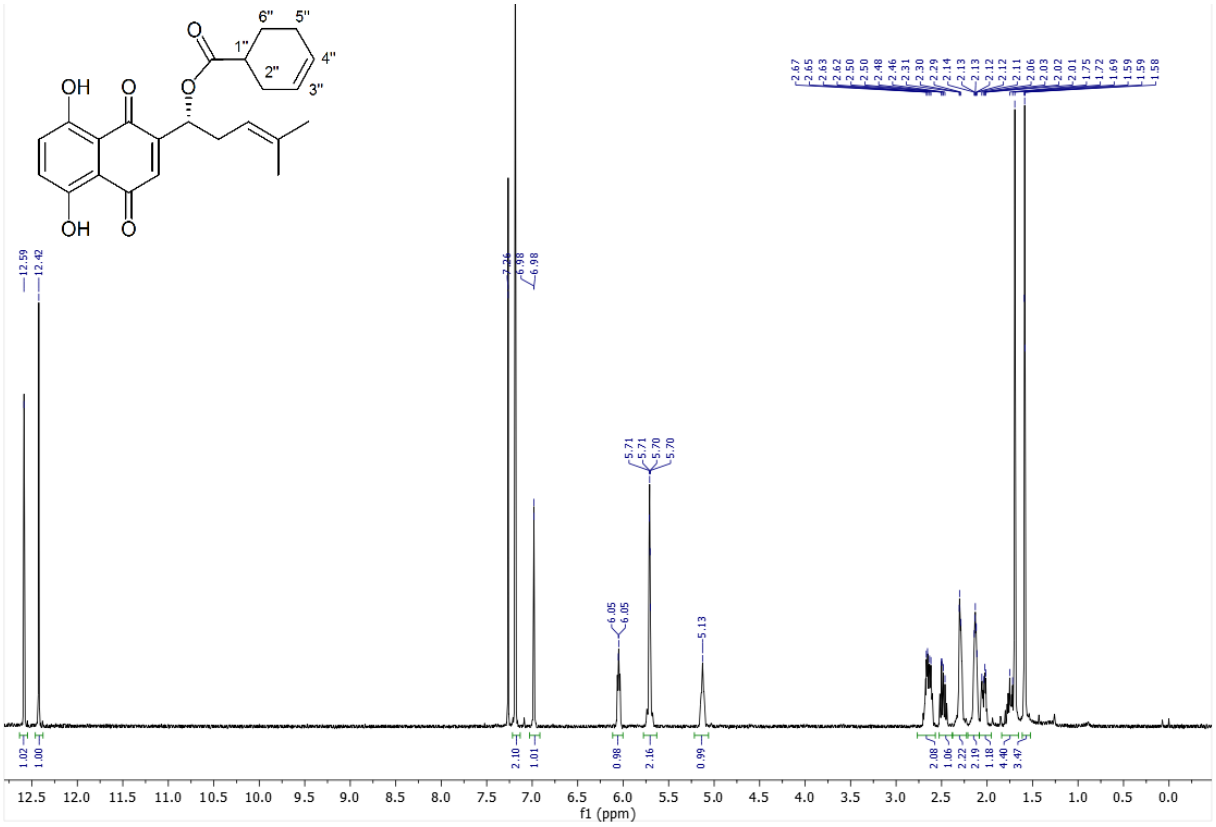
173 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
174 enyl cyclohex-3-enecarboxylate (**16**):



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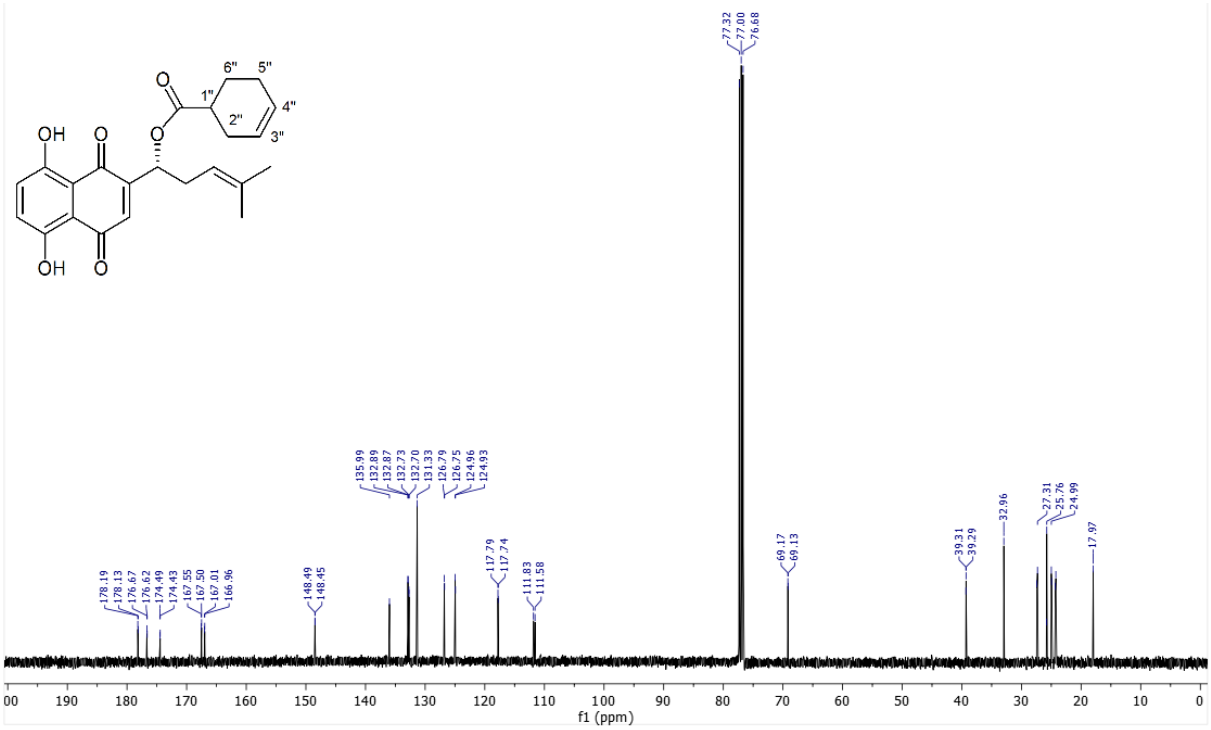
176 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
177 enyl cyclohex-3-enecarboxylate (**16**):



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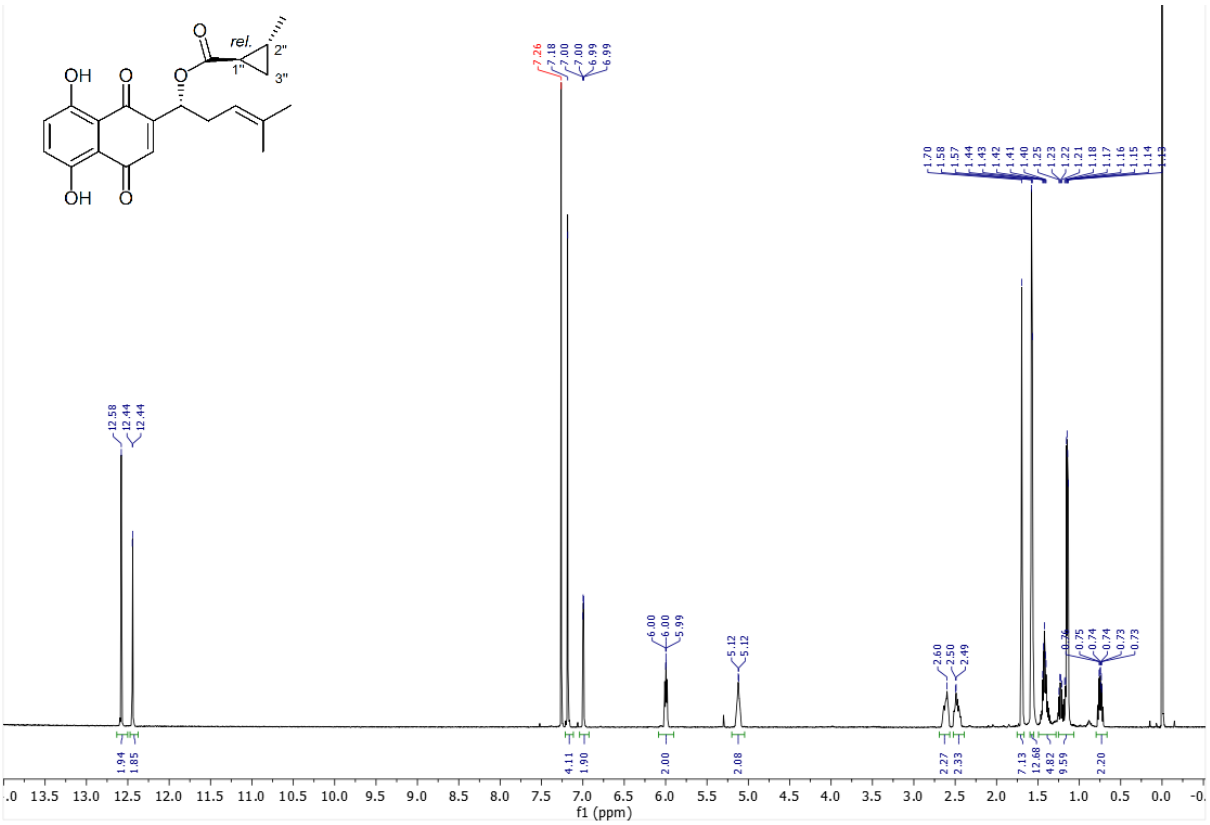
179 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
180 enyl trans 2-methylcyclopropanecarboxylate (**17**):



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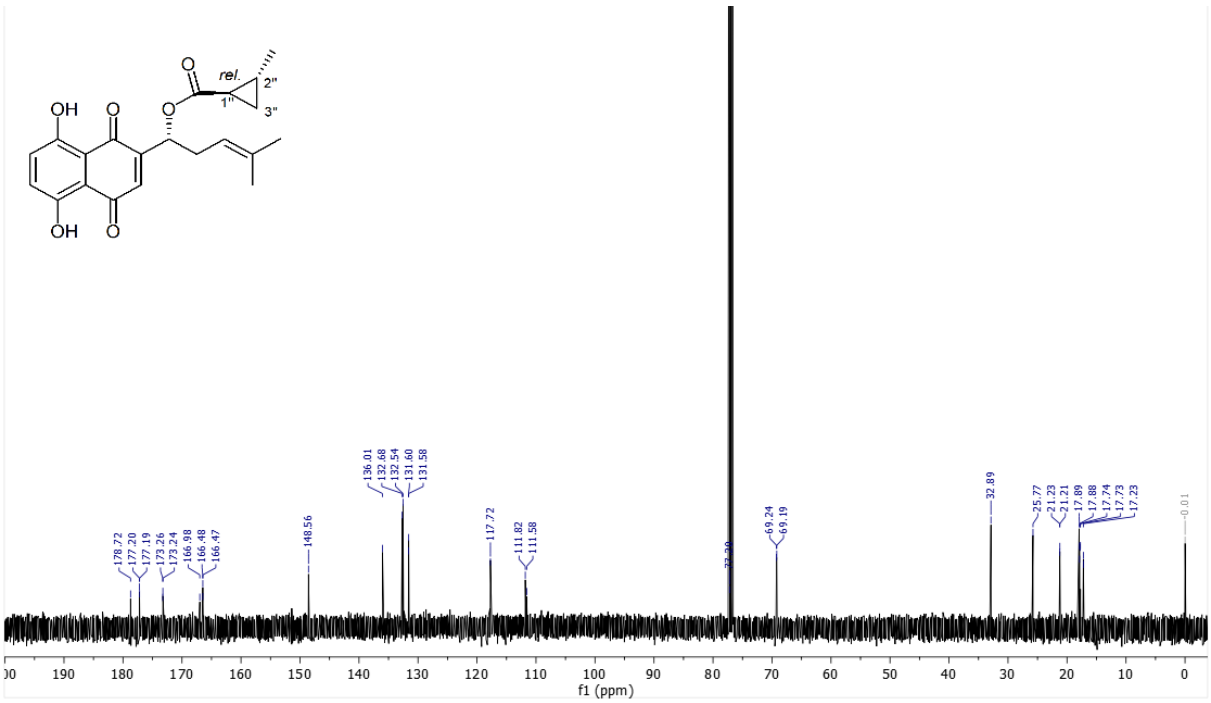
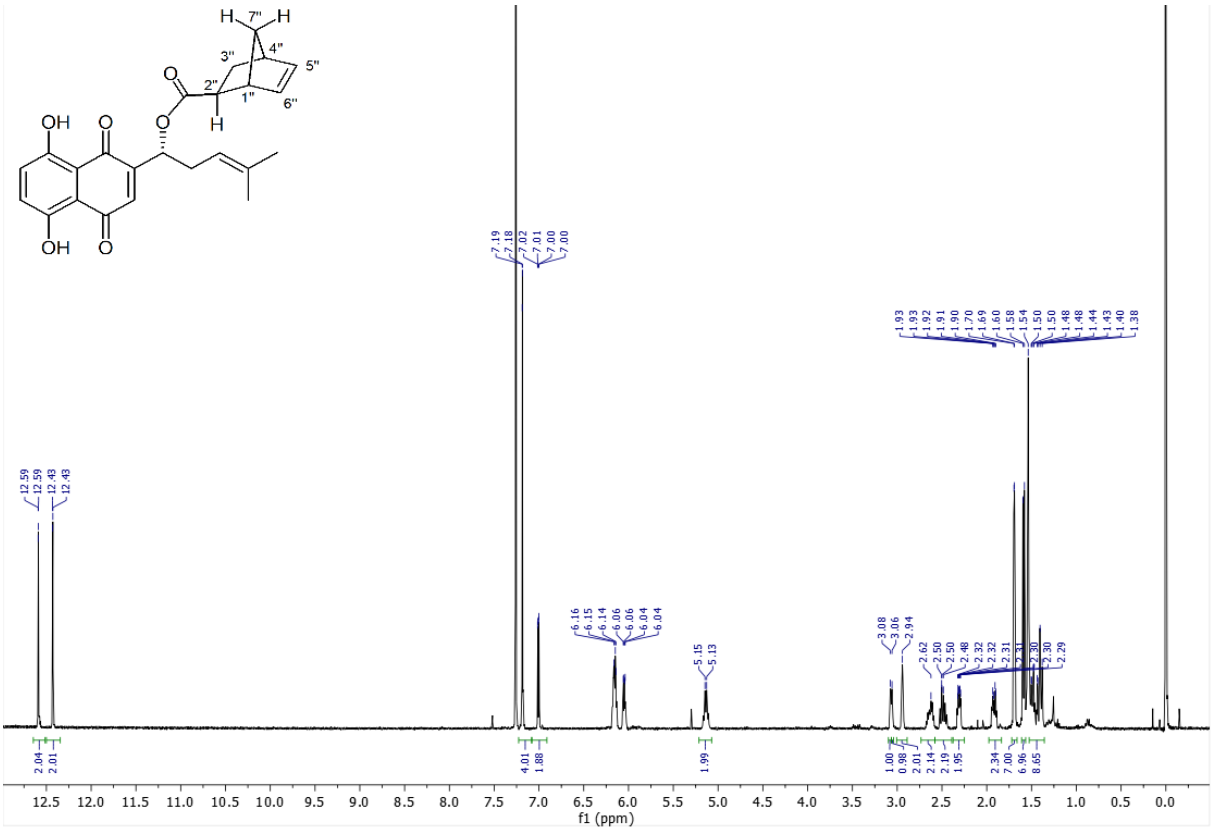
182 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
183 enyl cyclohex-3-enecarboxylate (**17**):



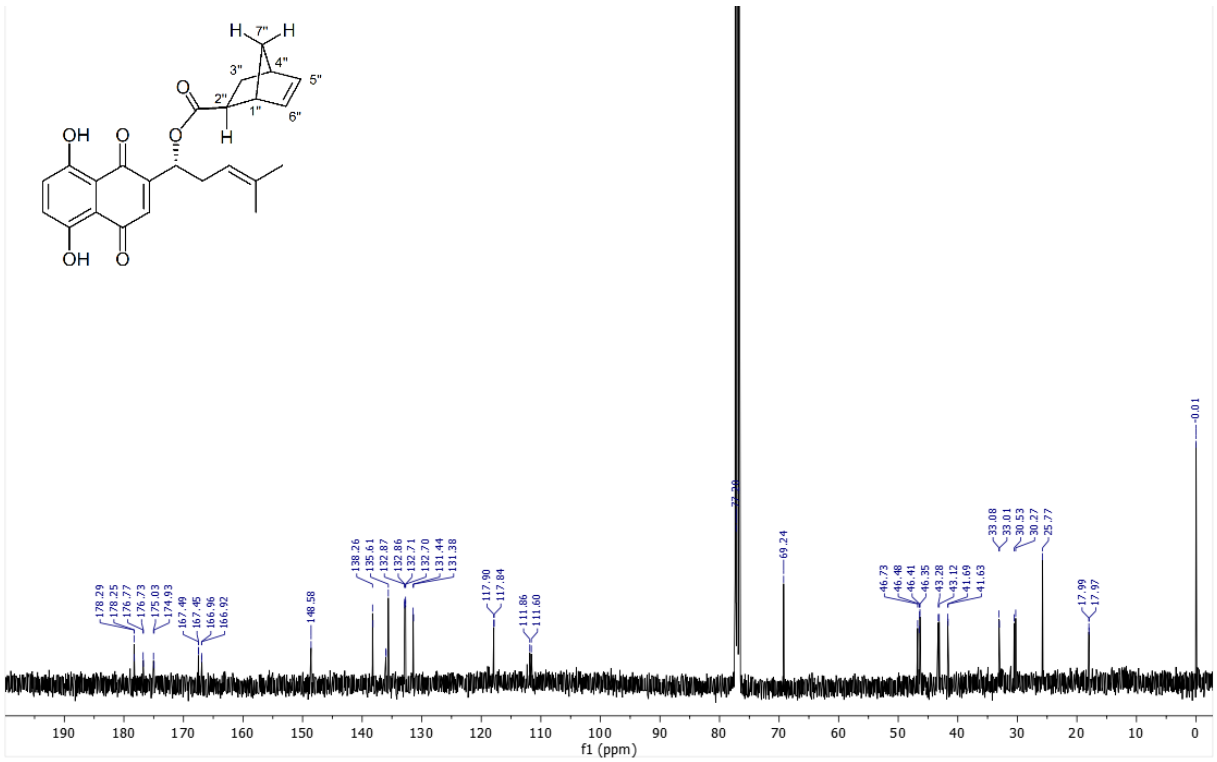
184

185 ^{1}H-NMR spectrum of Exo-(*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-3-yl)-4-methylpent-
186 3-enyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (**18**):

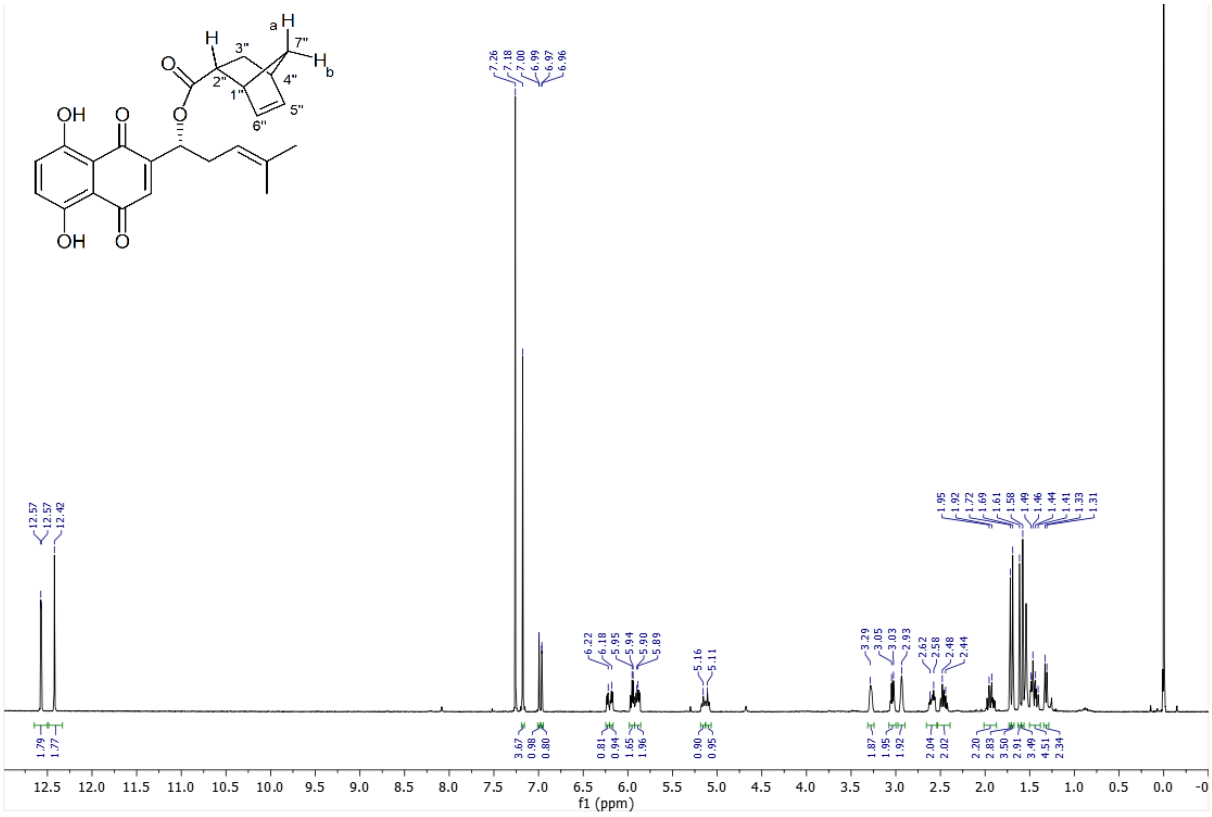


187

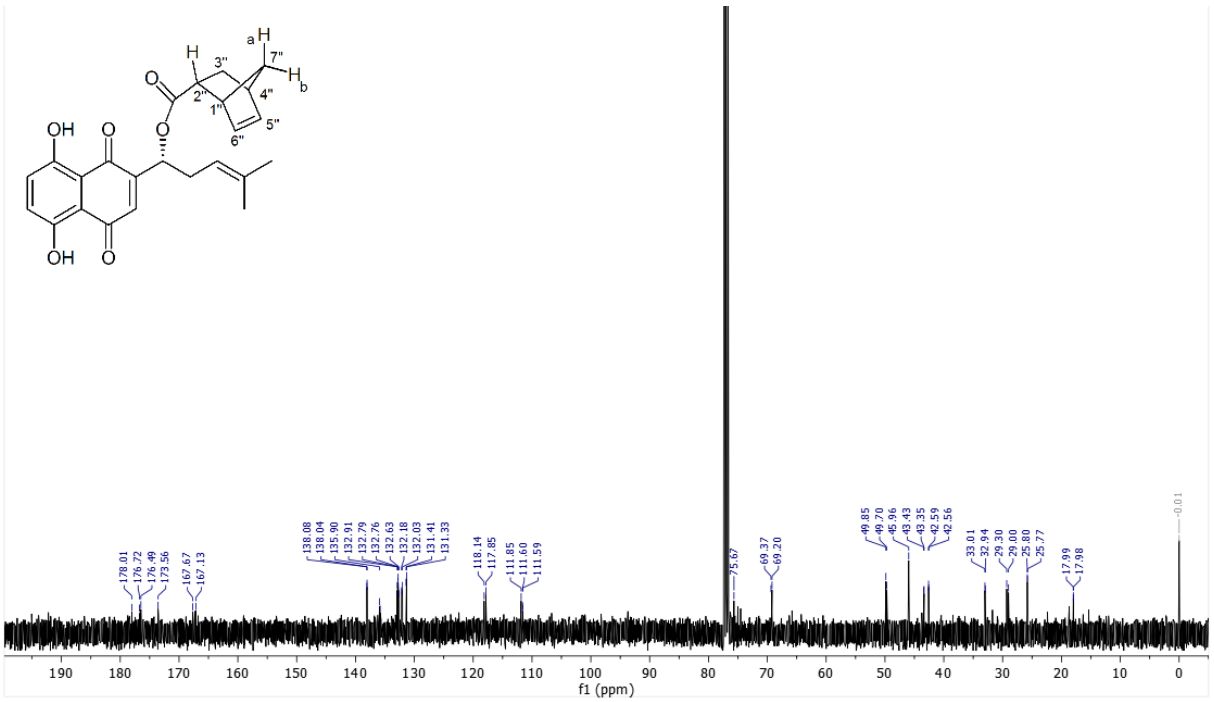
188 ^{13}C-NMR spectrum of Exo-(*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-
189 3-enyl cyclohex-3-enecarboxylate (**18**):



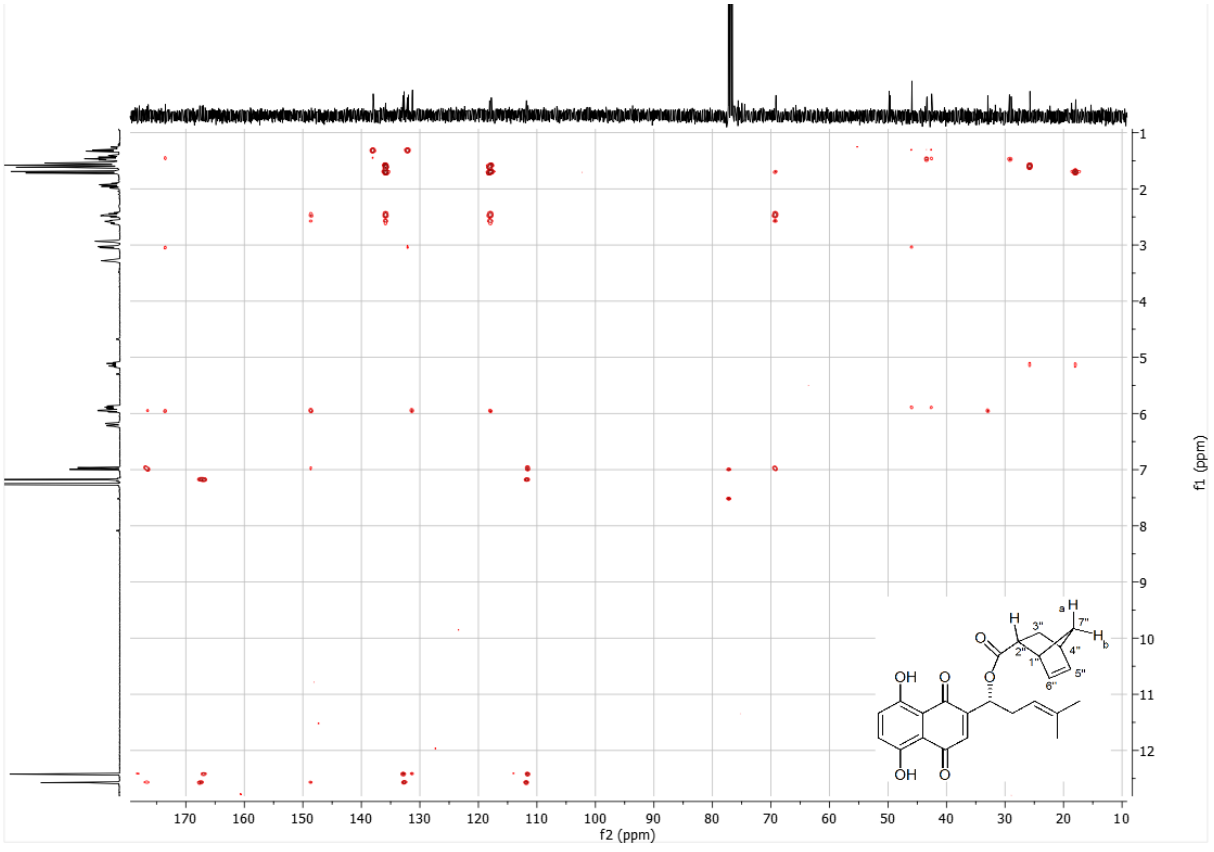
191 ^{1}H-NMR spectrum of Endo-(*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-3-yl)-4-
192 methylpent-3-enyl bicyclo[2.2.1]hept-5-ene-2-carboxylate (**19**):



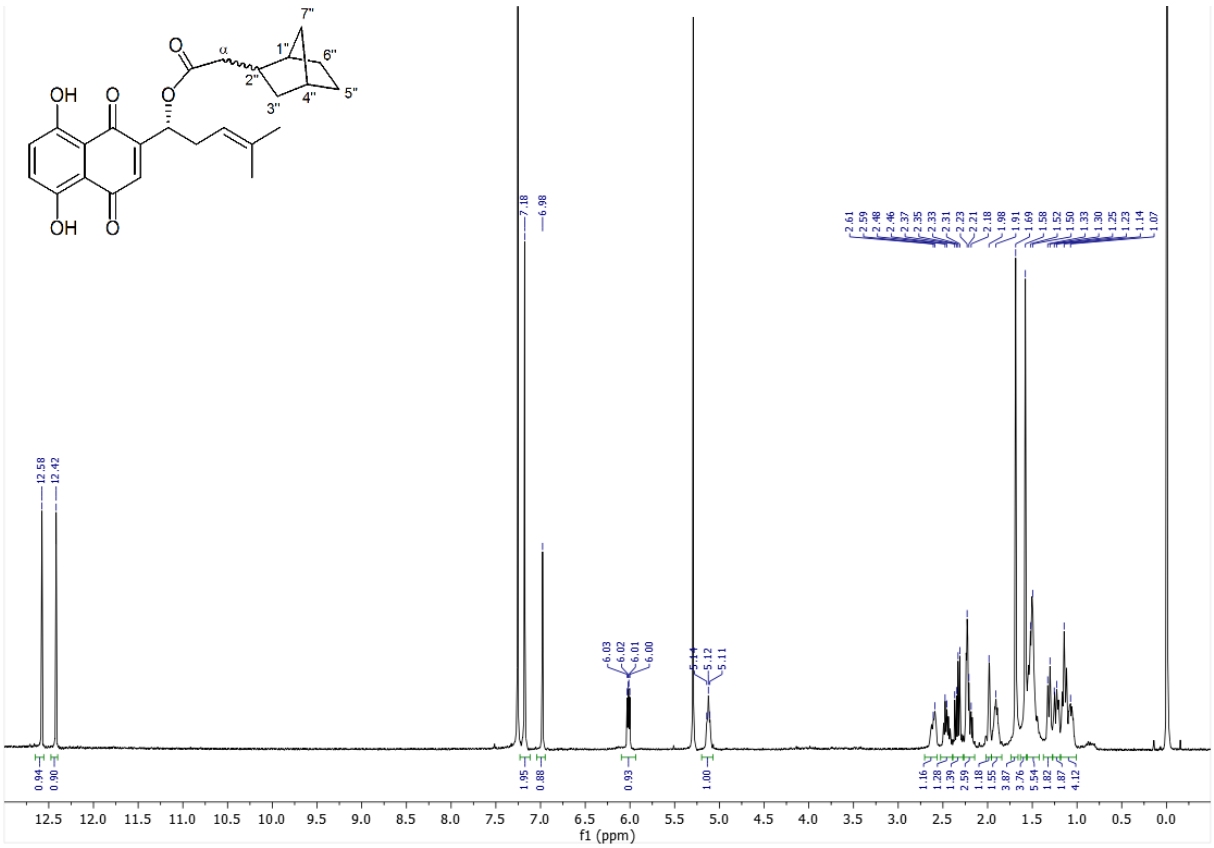
^{13}C-NMR spectrum of Endo-(*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl cyclohex-3-enecarboxylate (**19**):



HMBC spectrum of Endo-(*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl cyclohex-3-enecarboxylate (**19**):

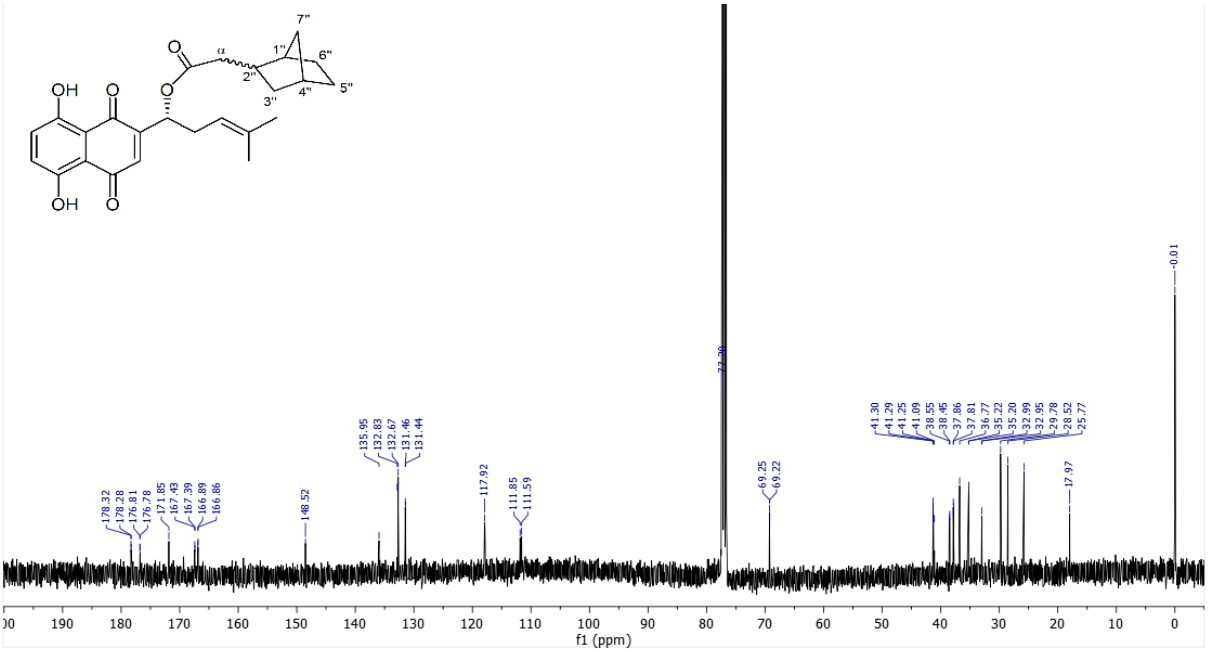


200 ^{1}H-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
201 enyl bicyclo[2.2.1]heptane-2-ylacetate (**20**):



202

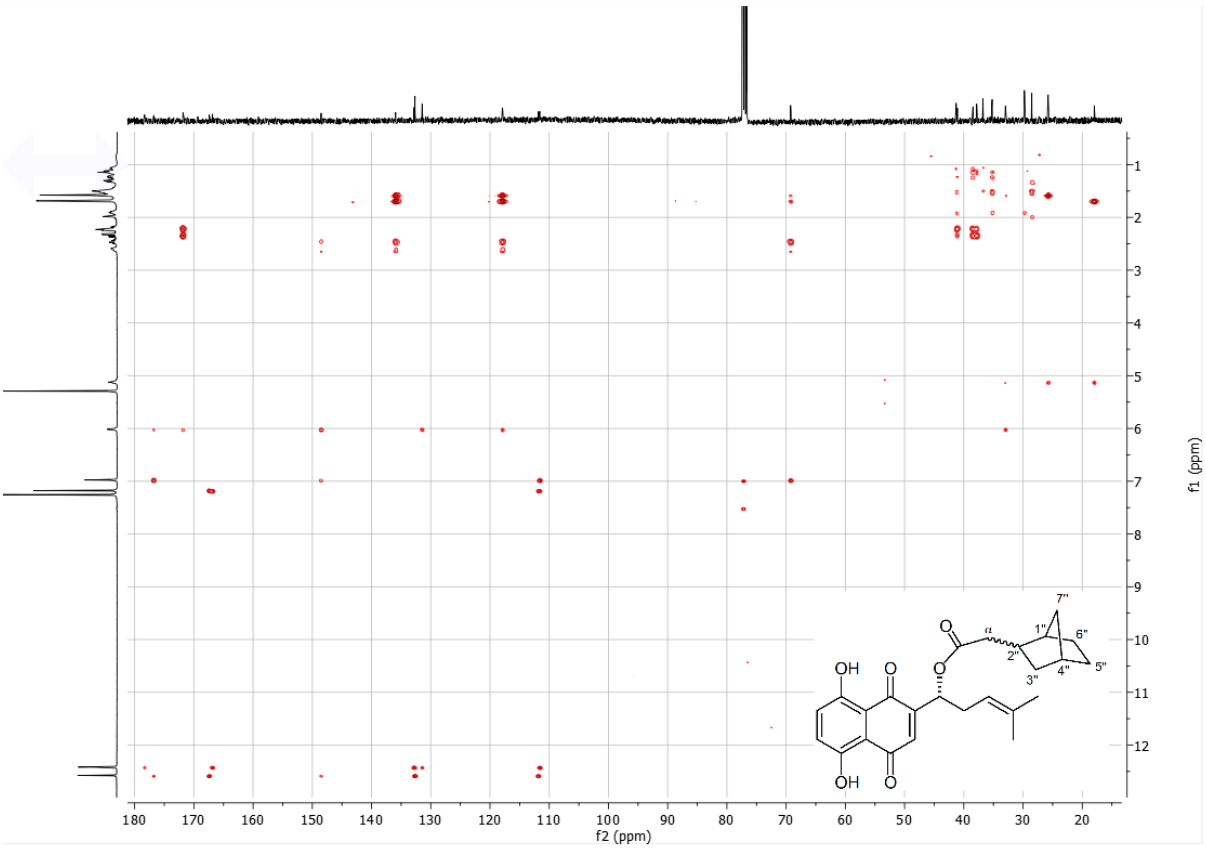
203 ^{13}C-NMR spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-
204 enyl bicyclo[2.2.1]heptane-2-ylacetate (**20**):



205

206

207 HMBC spectrum of (*R*)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl
208 bicyclo[2.2.1]heptane-2-acetate (**20**):



209
210

3. Syntheses of cycloalkylideneacids 2a to 5a and cyclobutylacetic acid (7a)

*Cyclobutylideneacetic acid (**2a**)*

At 0 °C triethylphosphonoacetate (10.2 mL, 11.5 g, 51.4 mmol) was added to a suspension NaH (60% in mineral oil; 2.05 g, 66.8 mmol) in abs. Et_2O (120 mL). After stirring for 5 min, a solution of cyclobutanone (3.74 mL, 3.50 g, 50 mmol) in abs. Et_2O (10 mL) was added. After 4 h at room temperature, water (100 mL) was added. The organic layer was separated and the aqueous layer was extracted with Et_2O (3 x 50 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated resulting in 6.3 g raw ester (contained ca. 20 % ethyl cyclopentenylacetate) which was used for the next reaction without further purification.

A solution of LiOH · H_2O (4.20 g, 100 mmol) in water (25 mL) was added to a solution of raw ethyl cyclobutylidenacetat (1.40 g, 10 mmol) in THF (25 mL). The mixture was stirred for 18 h at room temperature. TLC showed very little conversion. THF, water and MeOH (10 mL each), were added and the mixture was stirred for further three days. No starting material was visible in TLC. The mixture was concentrated under reduced pressure to about 30 mL, washed with ether (2 x 10 mL), acidified with conc. HCl (ca. 8 mL) and extracted with Et_2O (3 x 10 mL). This extract was dried over anhydrous Na_2SO_4 and evaporated. 580 mg of the raw product (0.99 g) were purified by CC (silica, cyclohexane / EtOAc = 3:1 to 2:1) resulting in 352 mg cyclobutylideneacetic acid (**2a**) (54%).

^{1}H-NMR ($CDCl_3$): δ 2.11 (quint, *J* = 8.0 Hz, 2H, $CH_2CH_2CH_2$), 2.86 (tm, *J* = 7.9 Hz, 2H, trans CH_2-C=), 3.14 (tm, *J* = 8.1 Hz, 2H, cis CH_2-C=), 5.59 (quint, *J* = 2.3 Hz, 1H, =CH), 11.05 (s, br, 1H, COOH); ^{13}C-NMR ($CDCl_3$): δ 17.5 ($CH_2CH_2CH_2$), 32.5, 34.1 (2 x CH_2-C=), 112.0 (=CH), 171.4, 172.3 (C=CH-CO).

*Cyclopentylideneacetic acid (**3a**) and 2-cyclopentenyl acetic acid (**3b**)*

At 0 °C triethylphosphonoacetate (10.2 mL, 11.5 g, 51.4 mmol) was added to a suspension NaH (60% in mineral oil; 2.05 g, 66.8 mmol) in abs. Et_2O (120 mL). After stirring for 5 min, a solution of cyclopentanone (4.43 mL, 4.21 g, 50 mmol) in abs. Et_2O (10 mL) was added. After 4 h (stirrer stuck after ca. 1 h) at room temperature, water (100 mL) was added. The organic layer was separated and the aqueous layer was extracted with Et_2O (3 x 50 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and evaporated resulting in 8.1 g raw ester (ethyl cyclopentylideneacetate with ca. 20 % ethyl 2-cyclopentenylacetate) which was used for the next reaction without further purification.

0.22 g (5.2 mmol) LiOH · H_2O in 20 mL water was added to a solution of 0.77 g (5 mmol) raw ethyl cyclopentylidenacetate in THF (25 mL) and MeOH (10 mL). After stirring for 2 h at 50 °C (bath temperature), the mixture was concentrated under reduced pressure to approx. 25 mL, washed with ether (3 x 10 mL), acidified with conc. HCl (ca. 0.4 mL) and extracted with ether (3 x 10 mL). The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue consisted in 500 mg of a mixture of **3a** and **3b** (1 : 3; 79 %).

The raw product was submitted to CC (silica, cyclohexane / EtOAc = 3 : 1 to 2 : 1) resulting in 66 mg **3a** (10 %), **3b** 100 mg (16 %) and 66 mg of an isomeric mixture (10 %).

Cyclopentylideneacetic acid (**3a**): ^{1}H-NMR ($CDCl_3$): δ 1.63-1.80 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.47 (tm, *J* = 7.1 Hz, 2H, trans CH2-C=), 2.78 (tm, *J* = 7.1 Hz, 2H, cis CH2-C=), 5.83 (s, 1H, =CH), 11.63 (s, br, 1H, COOH); ^{13}C-NMR ($CDCl_3$): δ 25.4, 26.3 ($CH_2CH_2CH_2CH_2$), 33.0 (cis CH2-C=), 36.3 (trans CH_2-C=), 111.1 (=CH), 172.6, 173.0 (C=CH-COO).

2-Cyclopentenylacetic acid (**3b**): ^{1}H-NMR ($CDCl_3$): δ 1.91 (quint, *J* = 7.5 Hz, 2H, H-4), 2.35 (t, *J* = 7.5 Hz, 4H, H-3 and H-5), 3.17 (s, 2H, CH_2-α), 5.59 (sm, 1H, =CH), 11.25 (s, vbr, 1H, COOH); ^{13}C-NMR (CDCl3): δ 23.4, (C-4), 32.5 (C-α), 35.0, 36.7 (C-3 and C-5), 128.9 (C-2), 135.8 (C-1), 178.2 (COO).

*Cyclohexylideneacetic acid (**4a**)*

Diethylphosphit (1.29 mL, 1.38 mg, 10 mmol) was added to a suspension of NaH (60 % in mineral oil; 1.20 g, 30 mmol) in abs. glyme (32 mL). After stirring for 10 min, a solution of chloroacetic acid (945 mg, 10 mmol) in abs. glyme (10 mL) was added and stirred for 40 min till gas evolution had ceased. Cyclohexanone (1.03 mL, 980 mg, 10 mmol) was added. After stirring for further 90 min and addition of EtOH (1.7 mL), the mixture was poured into water (160 mL). Washing with Et_2O (4 x 50 mL), acidification with conc. HCl (ca. 3 mL), extraction with Et_2O (4 x 50 mL), drying over anhydrous Na_2SO_4 and evaporation resulted in 707 mg raw product. 668 mg of this material was submitted to CC (silica, cyclohexane / EtOAc = 3:1) resulting in 261 mg cyclohexylideneacetic acid (**4a**) (20 %).

^{1}H-NMR ($CDCl_3$): δ 1.56-1.71 (m, 6H, 3 x CH_2), 2.22 (t, *J* = 6.0 Hz, 2H, trans CH_2-C=), 2.83 (t, *J* = 5.8 Hz, 2H, cis CH2-C=), 5.70 (quint, *J* = 1.2 Hz, 1H, =CH), 11.15 (s, br, 1H, COOH); ^{13}C-NMR ($CDCl_3$): δ 26.1, 27.8, 28.6 (3 x CH_2), 30.1 (cis CH_2-C=), 38.3 (trans CH_2-C=), 115.2 (=CH), 167.5 (C=CH-CO), 172.6 (CO).

*Cycloheptylideneacetic acid (**5a**)*

Diethylphosphit (0.78 mL, 872 mg, 6.31 mmol) was added to a suspension of NaH (60 % in mineral oil; 1.10 g, 27.5 mmol) in abs. glyme (20 mL). After stirring for 10 min, a solution of chloroacetic acid (570 mg, 6.03 mmol) in abs. glyme (7 mL) was added and stirred for 30 min till gas evolution had ceased. Cycloheptanone (0.71 mL, 677 mg, 6.03 mmol) was added. After stirring for further 3 h and addition of EtOH (1 mL), the mixture was poured into water (100 mL). Washing with Et_2O (2 x 50 mL), acidification with conc. HCl to pH=4, extraction with Et_2O (4 x 30 mL), drying over anhydrous Na_2SO_4 and evaporation resulted in 360 mg raw product. 300 mg of this material was submitted to CC (silica, cyclohexane / EtOAc = 3:1 to 2:1) resulting in 85 mg cycloheptylideneacetic acid (**5a**) (11 %).

^{1}H-NMR ($CDCl_3$): δ 1.50-1.58 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.62-1.72 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.40 (ddd, *J* = 6.0, ~4.5, 0.6 Hz, 2H, trans CH2-C=), 2.88 (ddd, *J* = 6.2, ~5, 1.2 Hz, 2H, cis CH2-C=), 5.63 (s, 1H, =CH), 11.7 (s, br, 1H, COOH); ^{13}C-NMR ($CDCl_3$): δ 26.4, 27.9, 28.9, 29.7 (4 x CH2), 32.3(cis CH_2-C=), 39.2 (trans CH_2-C=), 115.2 (=CH), 169.9 (C=CH-CO), 172.0 (CO).

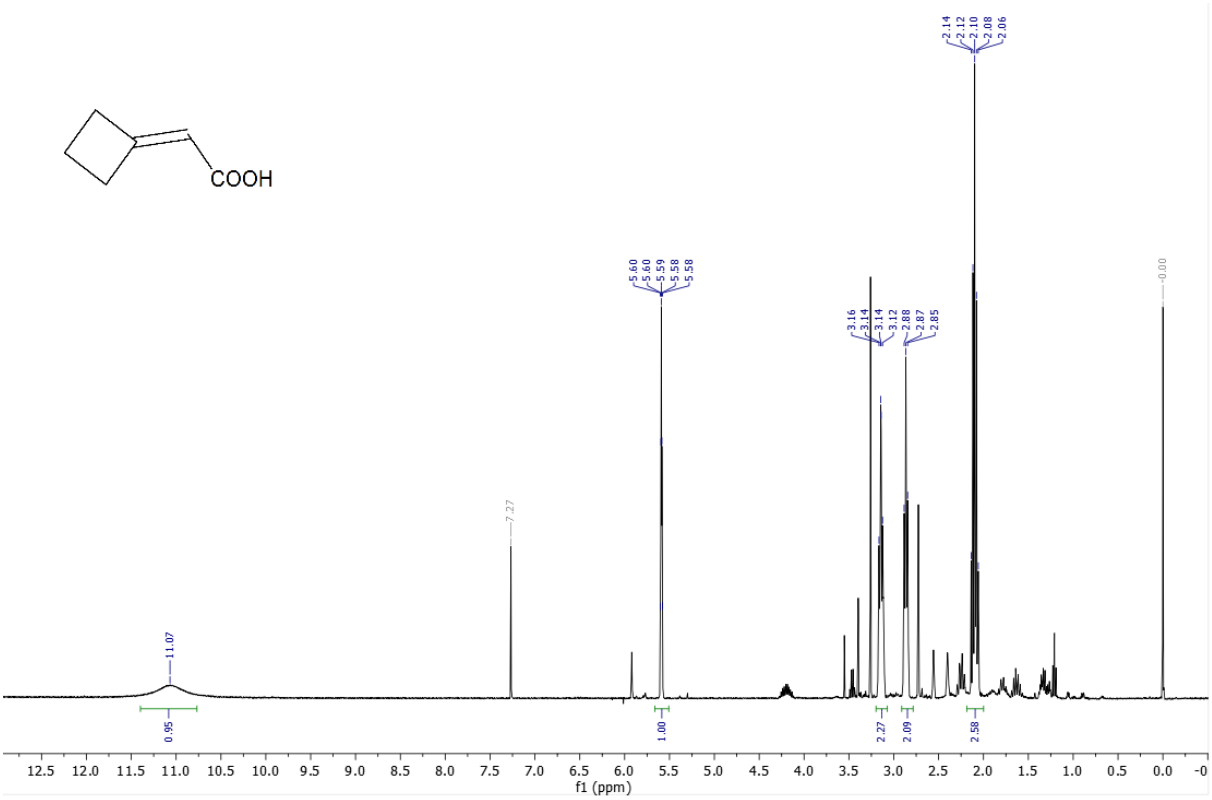
*Cyclobutylacetic acid (**7a**)*

Blank magnesium chips (370 mg, 15.2 mmol) were covered with abs. THF (ca. 0.5 mL). Two drops (bromomethyl)cyclobutane (from 2.09 g, 14.0 mmol) were added. As the reaction started, the rest of the bromide was solved in abs. THF (4 mL) and the solution was added dropwise with stirring. After addition, the mixture was allowed to stand for 18 h at room temperature. The mixture was diluted abs. THF (10 mL), warmed near to reflux und slowly poured on crushed dry ice (100 mL). After warming up to about 0 °C and addition of EtOAc (10 mL), the mixture was washed with 2 M HCl (10 mL) and saturated with NaCl. The organic layer was separated and the aqueous layer was extracted with EtOAc (10 mL), the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure resulting in 1.02 g cyclobutylacetic acid (**7a**) (64 %).

^{1}H-NMR ($CDCl_3$): δ 1.66-1.77 (m, 2H, trans, $CH_2CH_2CH_2$), 1.79-1.95 (m, 2H, $CH_2CH_2CH_2$), 2.10-2.19 (m, 2H, cis, $CH_2CH_2CH_2$), 2.44 (d, *J* = 7.5 Hz, 2H, CH_2CO), 2.68 (sept., *J* = 7.8 Hz, 1H, CH), 11.08 (s, br, 1H, COOH); ^{13}C-NMR ($CDCl_3$): δ 18.4 ($CH_2CH_2CH_2$), 28.1 ($CH_2CH_2CH_2$), 31.9 (CH), 40.9 (C-α), 179.5 (COO).

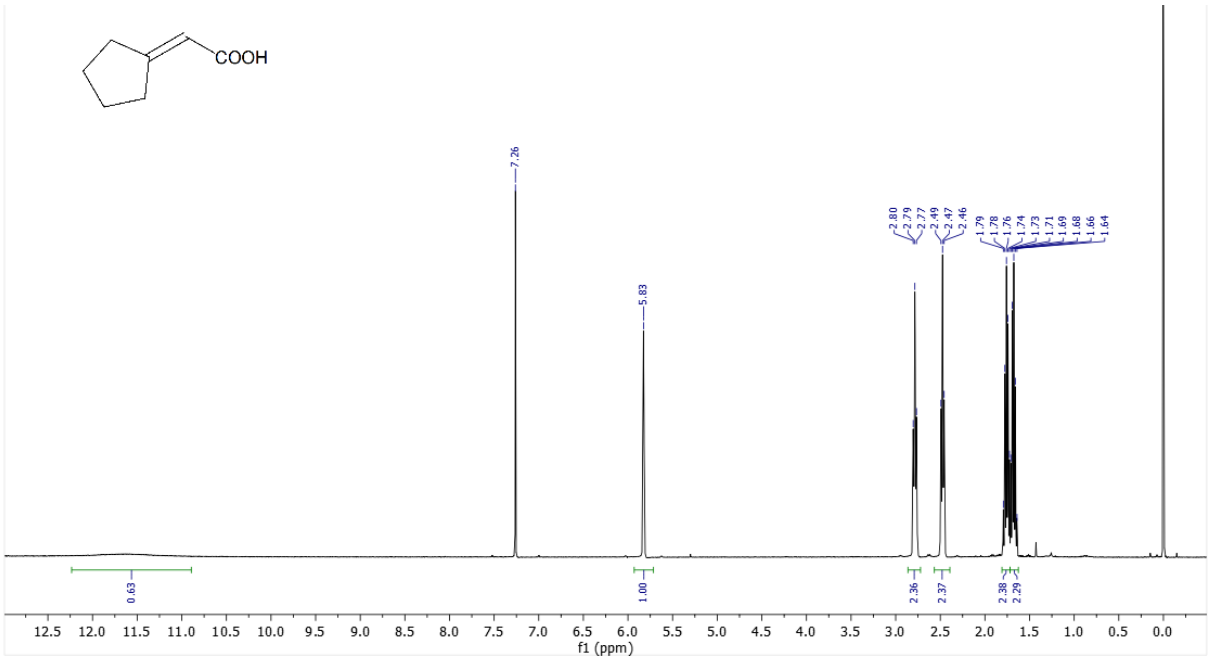
295 **4. ^{1}H NMR-spectra of synthesized acids** (400 MHz; $CDCl_3$) TMS as internal standard

296 ^{1}H-NMR spectrum of cyclobutylideneacetic acid (**2a**):



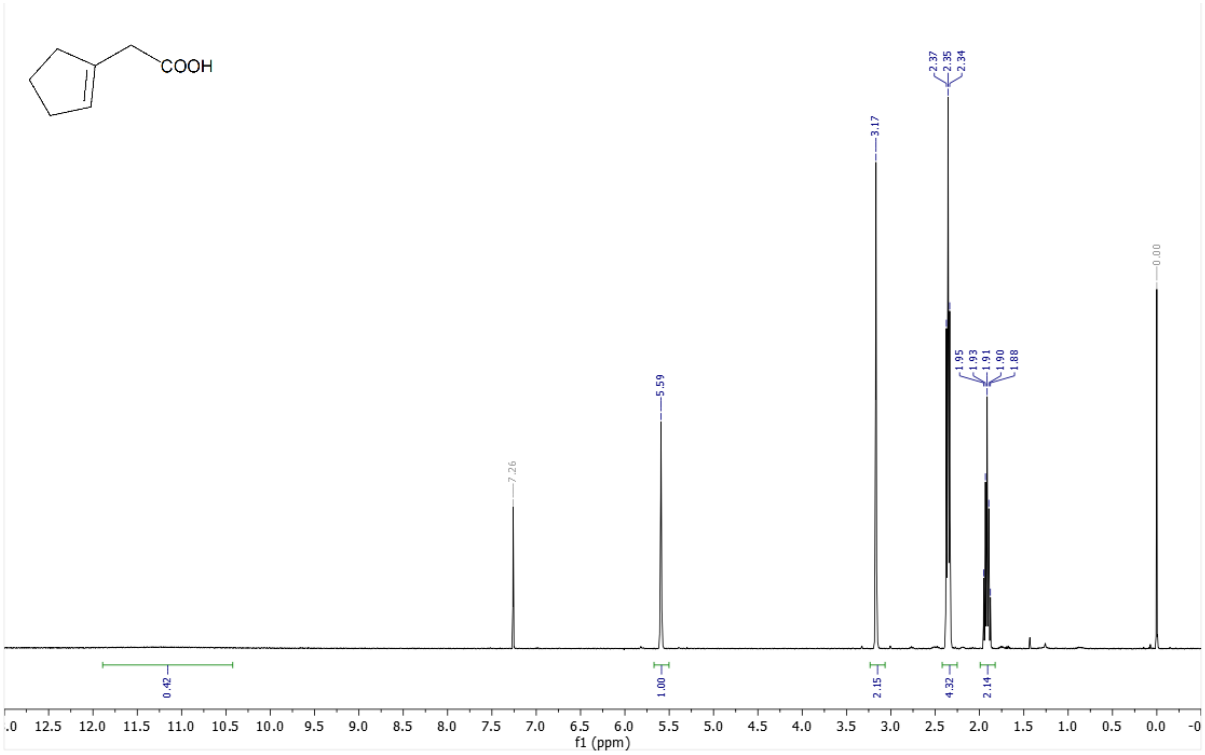
297

298 ^{1}H-NMR spectrum of cyclopentylideneacetic acid (**3a**):



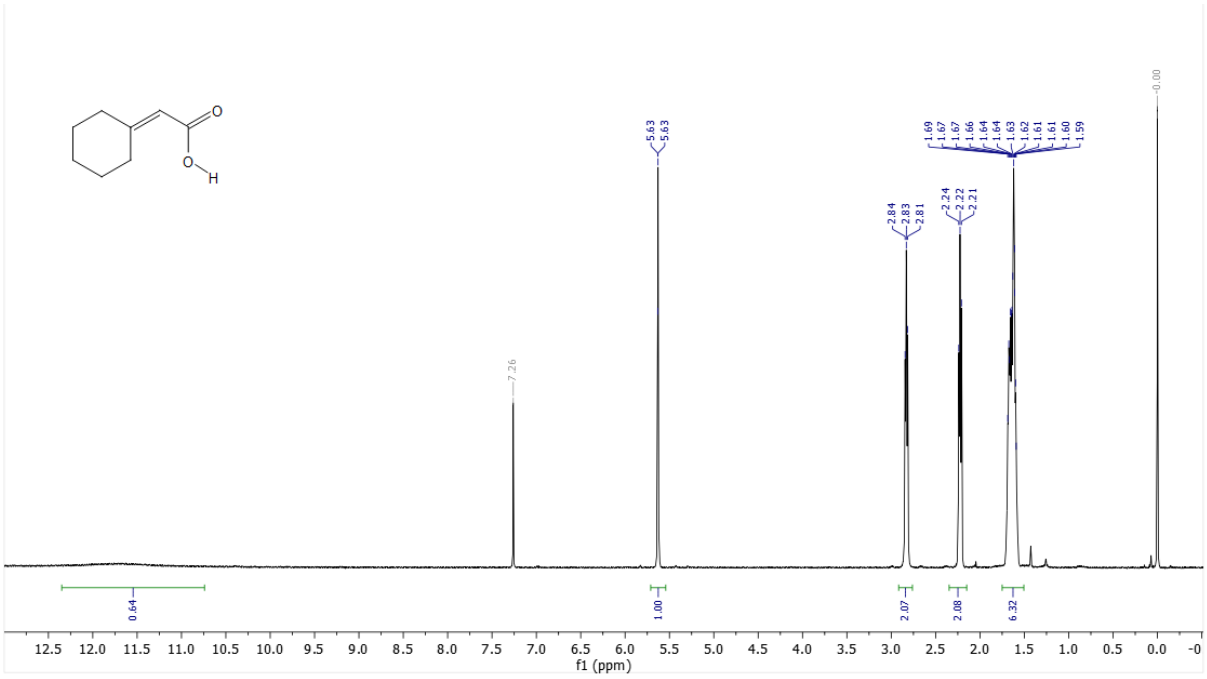
299

300 ^{1}H-NMR spectrum of 2-cyclopentenylacetic acid (**3b**):



301

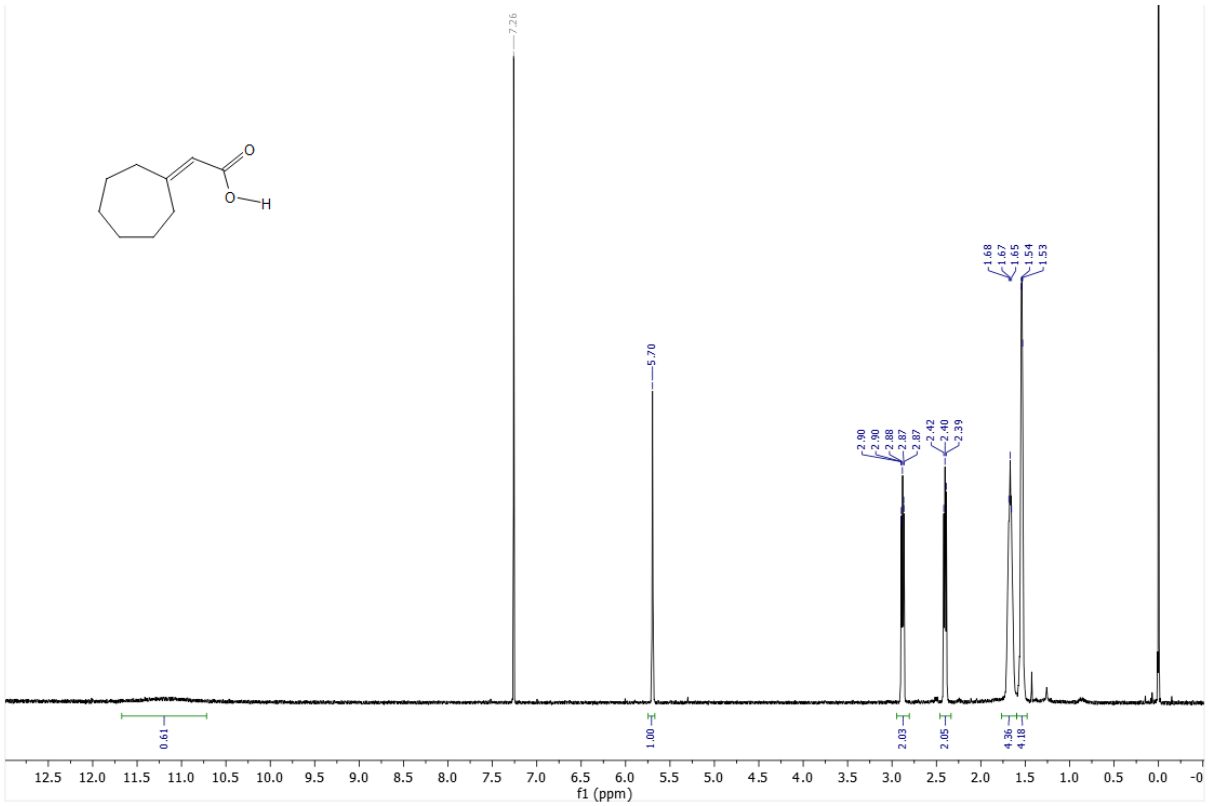
302 ^{1}H-NMR spectrum of cyclohexylideneacetic acid (**4a**):



303

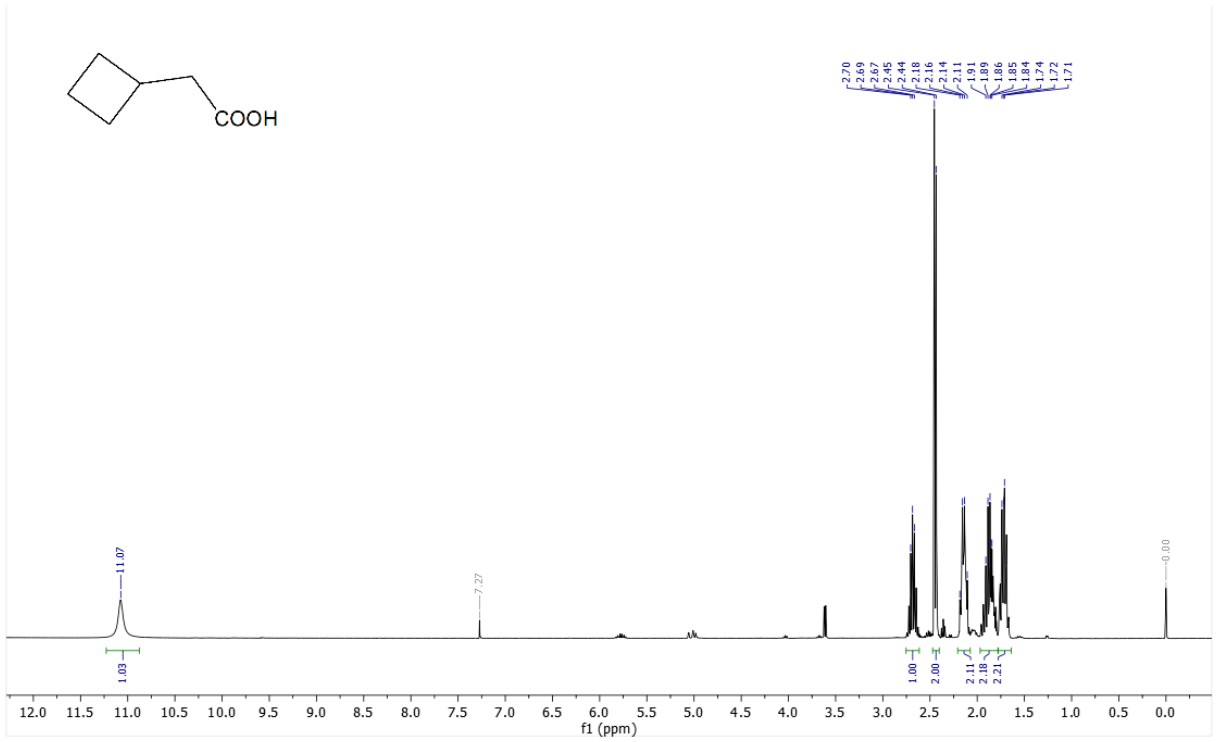
304

305 ^{1}H-NMR spectrum of cycloheptylideneacetic acid (**5a**):



306

307 ^{1}H-NMR spectrum of cyclobutylacetic acid (**7a**):



308

309

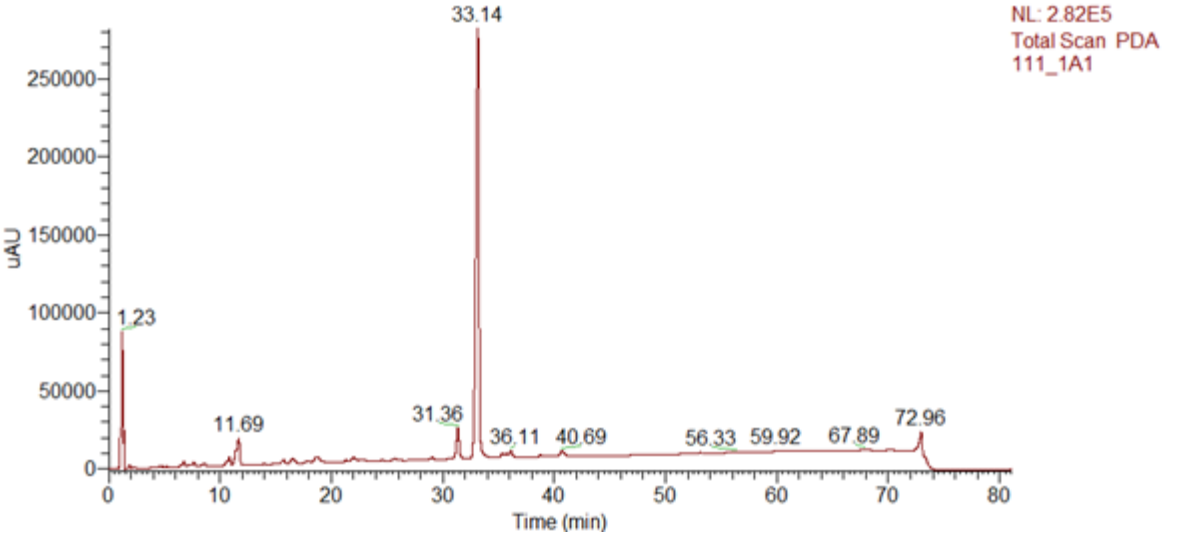
310 **5. HPLC runs of tested compounds**

311 HPLC analyses were carried out on a Dionex Ultimate 3000 UHPLC. Stationary phase: Kinetex
312 C18 column (2.6 μm, 100 × 2.10mm) (Phenomenex Inc., Torrance, CA, USA). Mobile phase: Water (A)
313 and acetonitrile (B); gradient: 0-45 min: 55-100 % B; flow rate: 0.2 mL/min; column temperature: 30 °C;
314 wavelength: 500 nm.

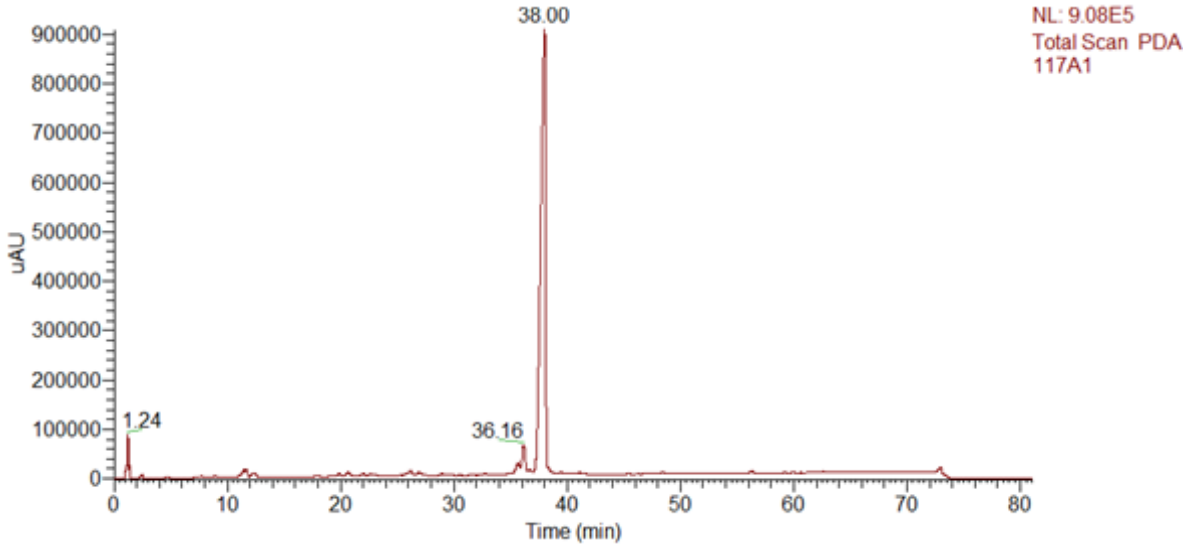
315

cpd

2



3



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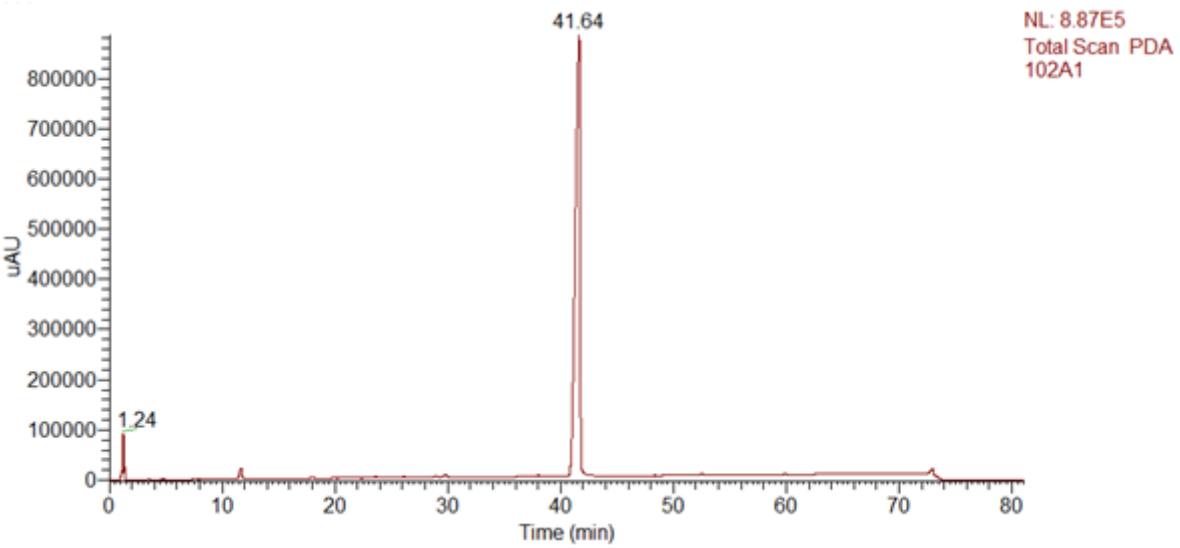
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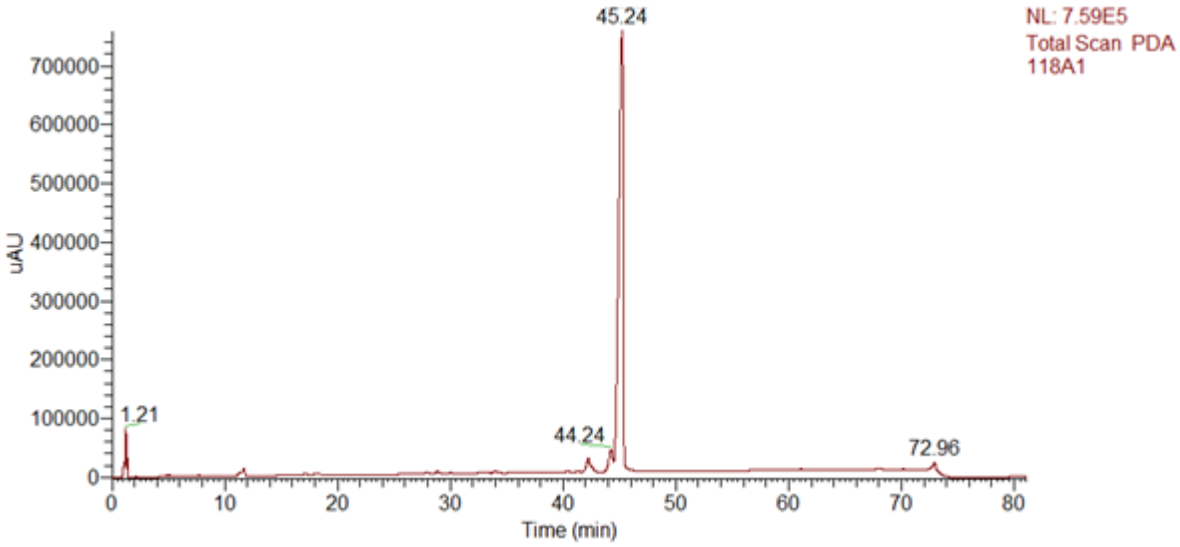
Total Scan PDA
102A1
1.24
uAU
Time (min)

5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NL: 7.59E5
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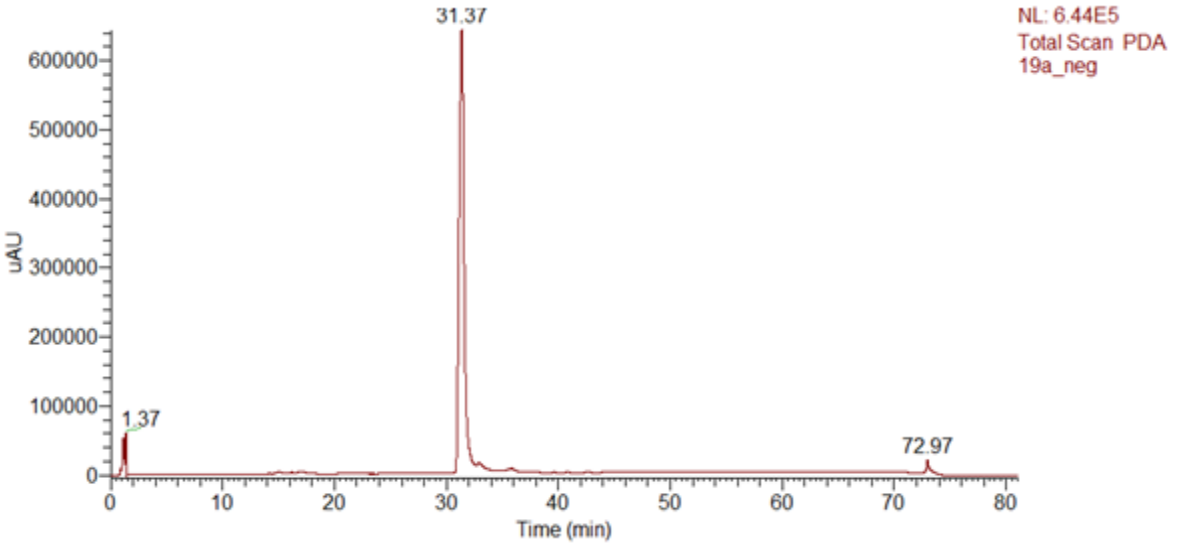
118A1
1.21
44.24
72.96
uAU
Time (min)

6


31.37
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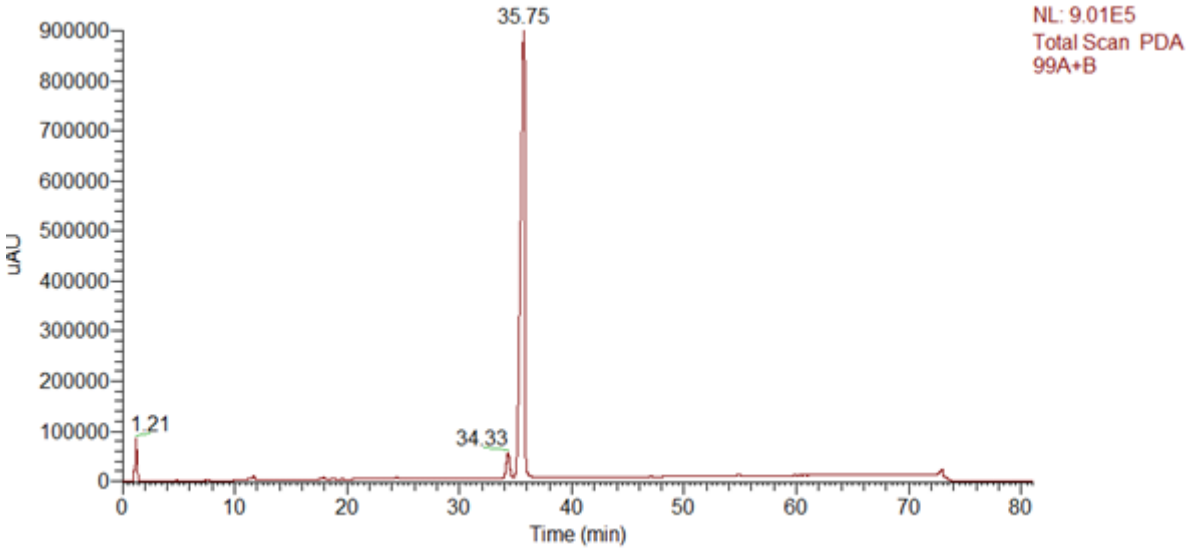
19a_neg
1.37
72.97
uAU
Time (min)

7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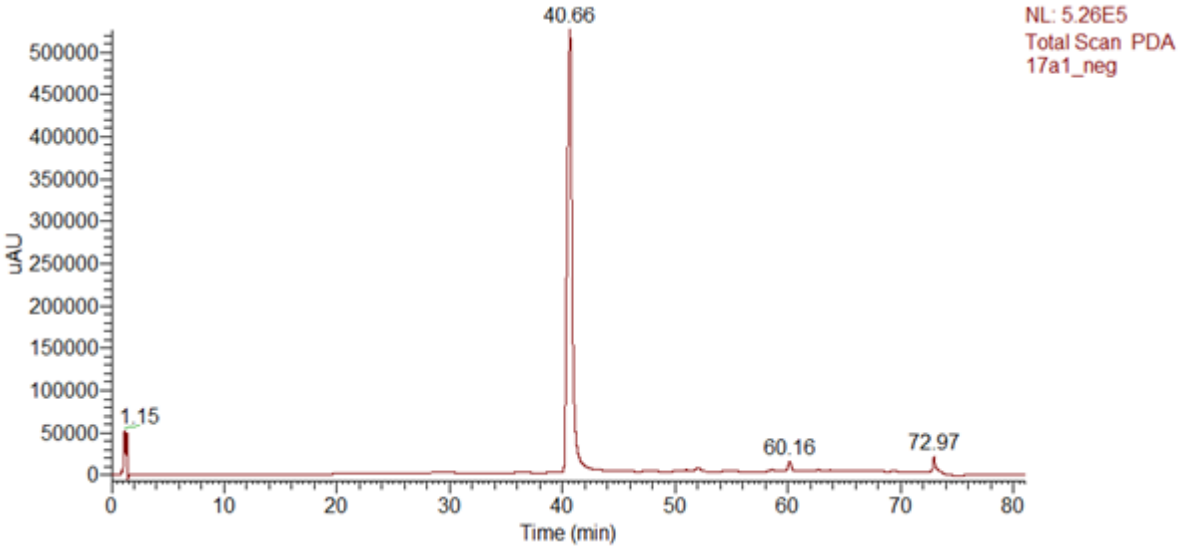
1.21
34.33
uAU
Time (min)

8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17a1_neg
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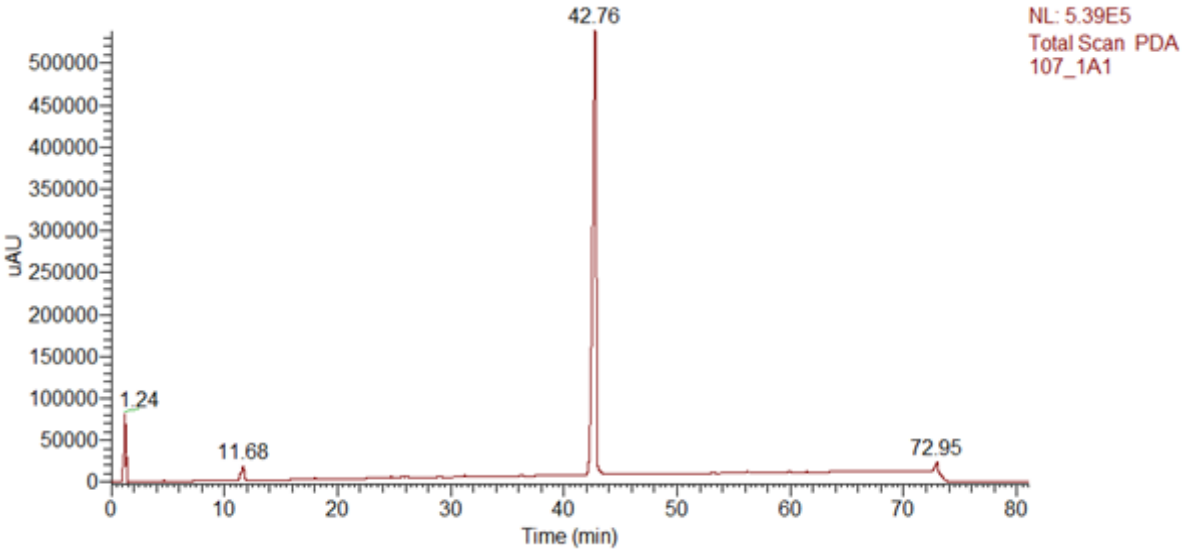
1.15
60.16
72.97
uAU
Time (min)

9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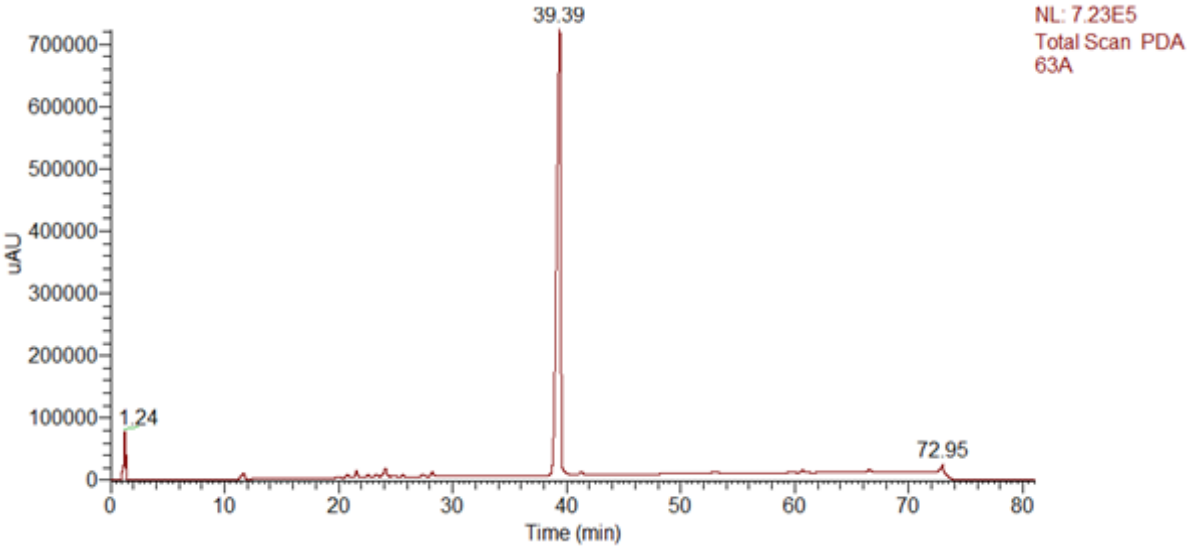
11.68
72.95
uAU
Time (min)

10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63A
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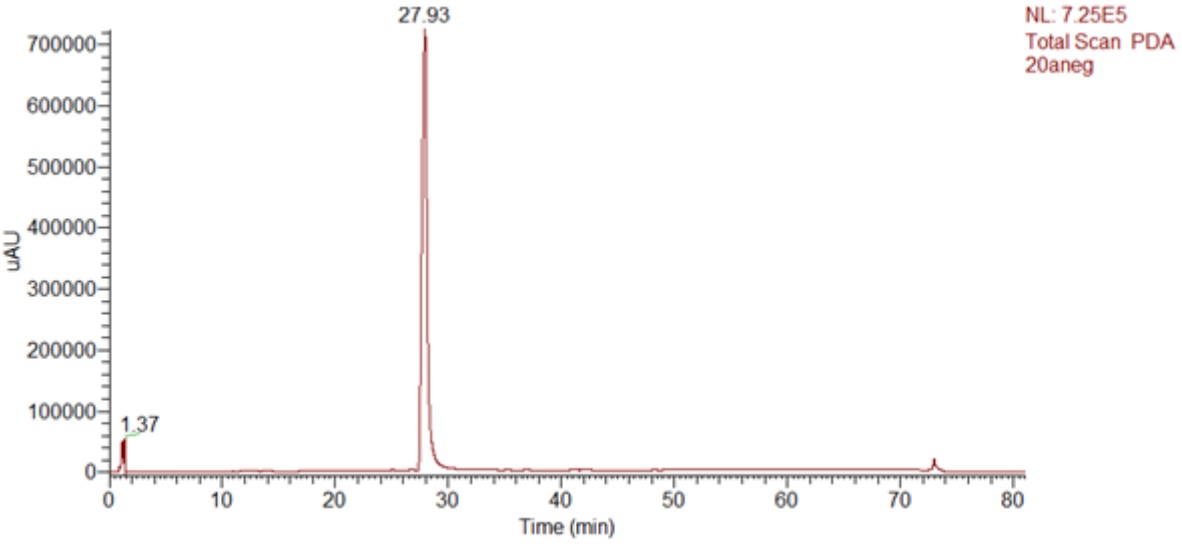
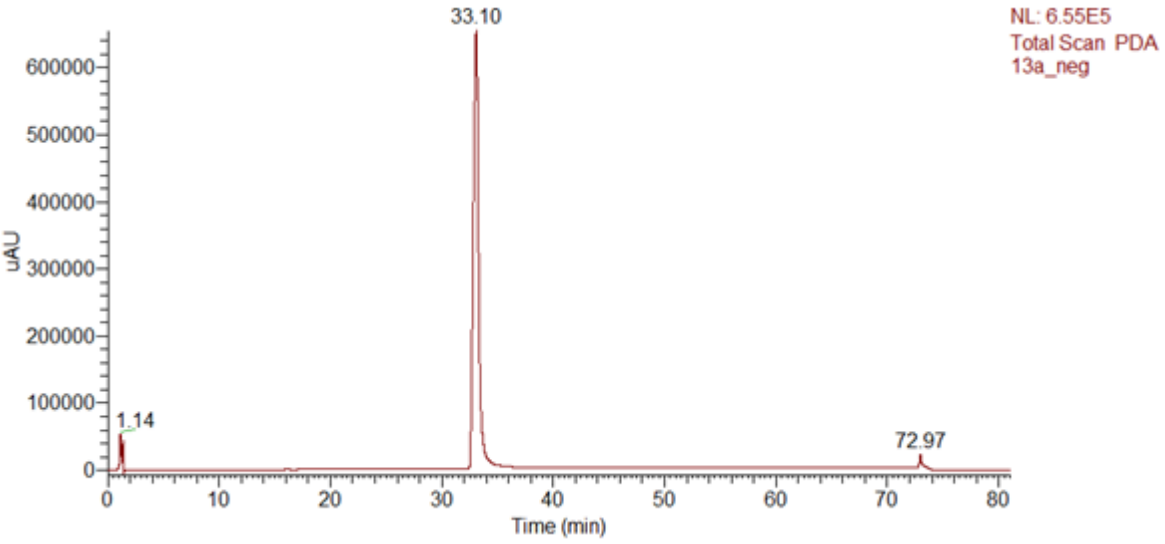
72.95
uAU
Time (min)

11


27.93
NL: 7.25E5
Total Scan PDA
20aneg
1.37
uAU
Time (min)

12


33.10
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13a_neg
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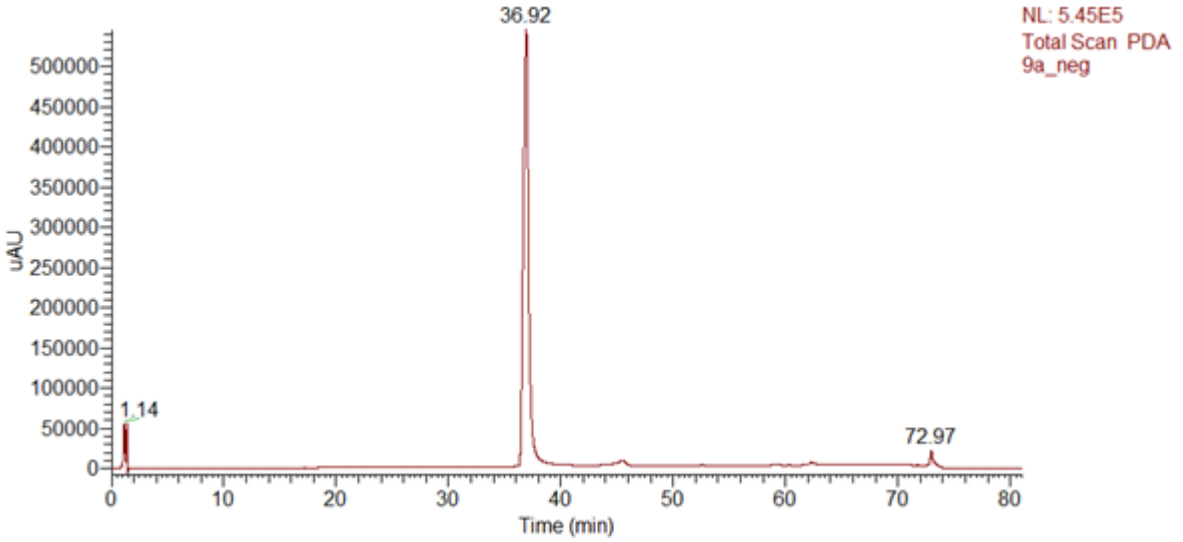
72.97
uAU
Time (min)

13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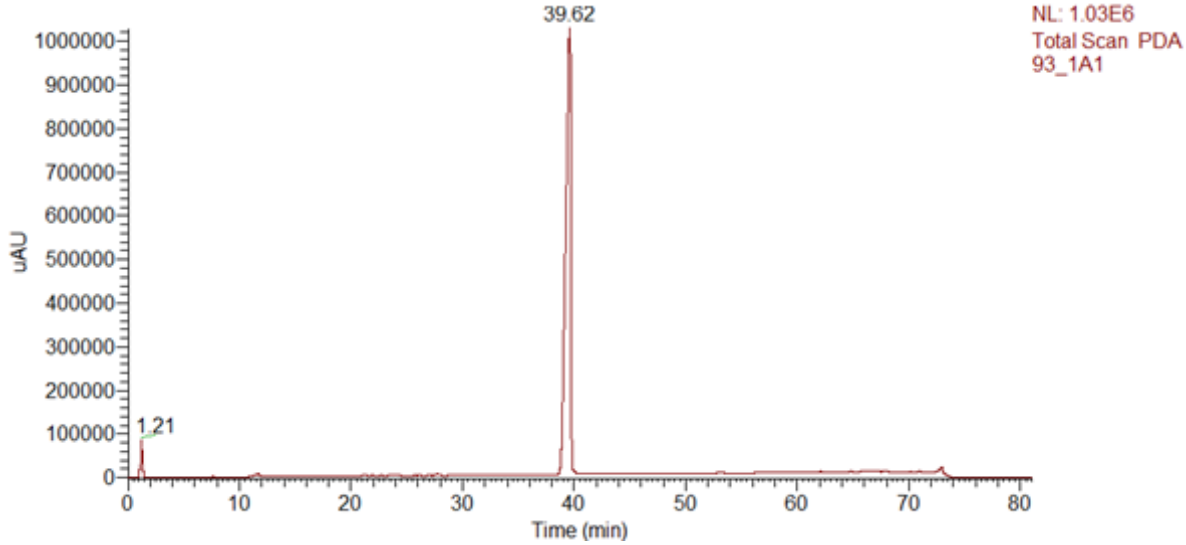
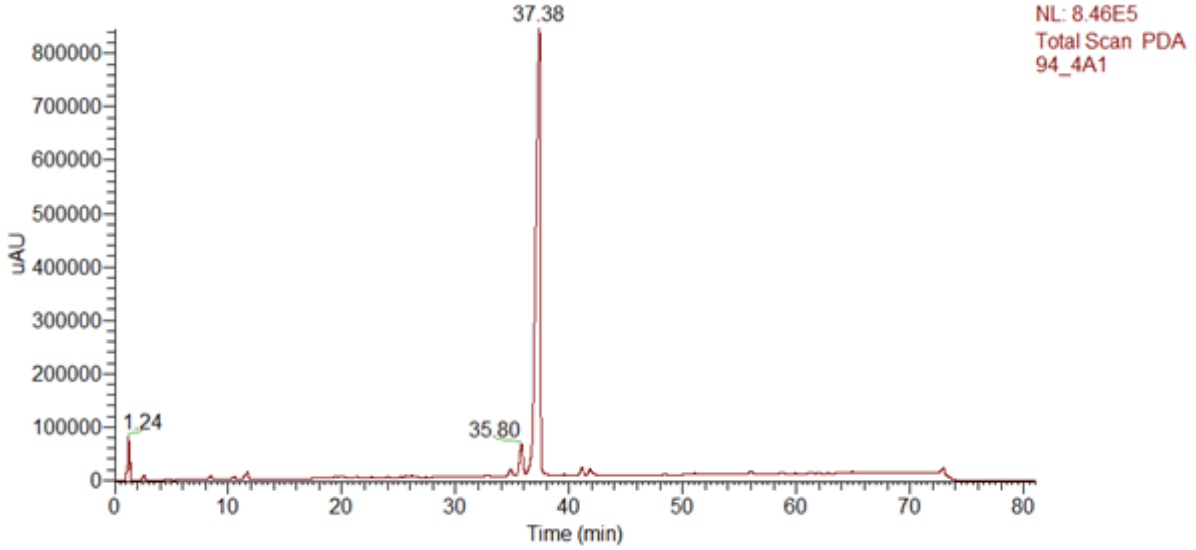
72.97
uAU
Time (min)

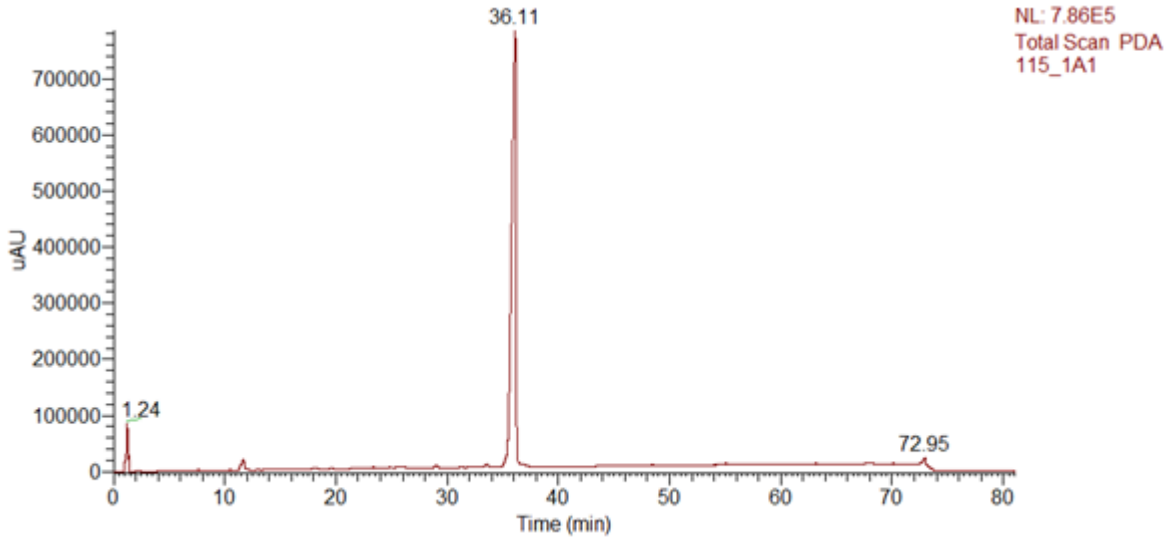
14


39.62
NL: 1.03E6
Total Scan PDA
93_1A1
1.21
uAU
Time (min)

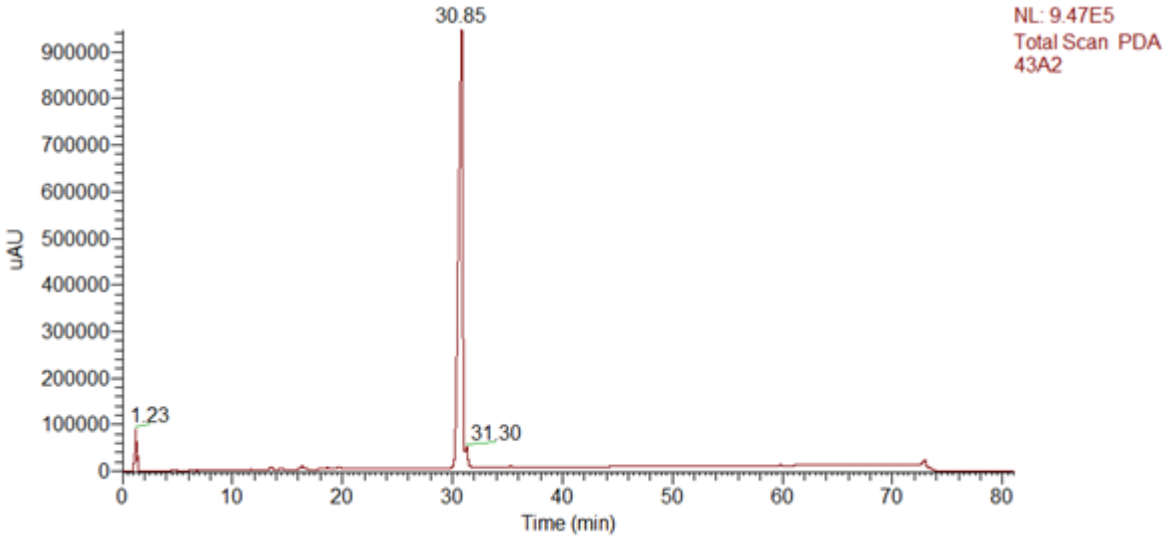
15


37.38
NL: 8.46E5
Total Scan PDA
94_4A1
1.24
35.80
uAU
Time (min)

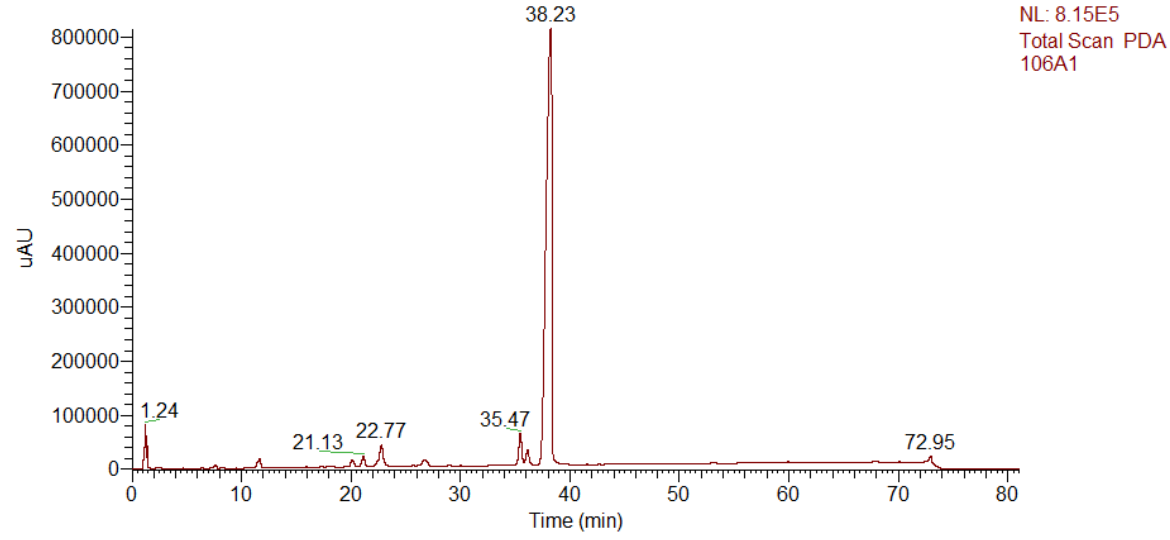
16


NL: 7.86E5
Total Scan PDA
115_1A1
36.11
1.24
72.95
uAU
Time (min)

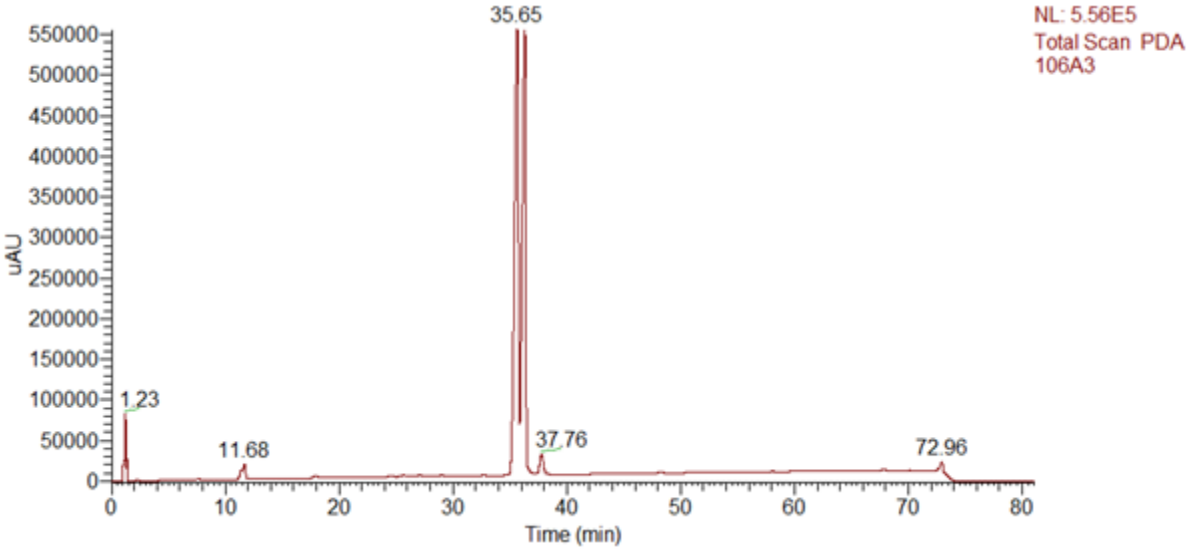
17


NL: 9.47E5
Total Scan PDA
43A2
30.85
1.23
31.30
uAU
Time (min)

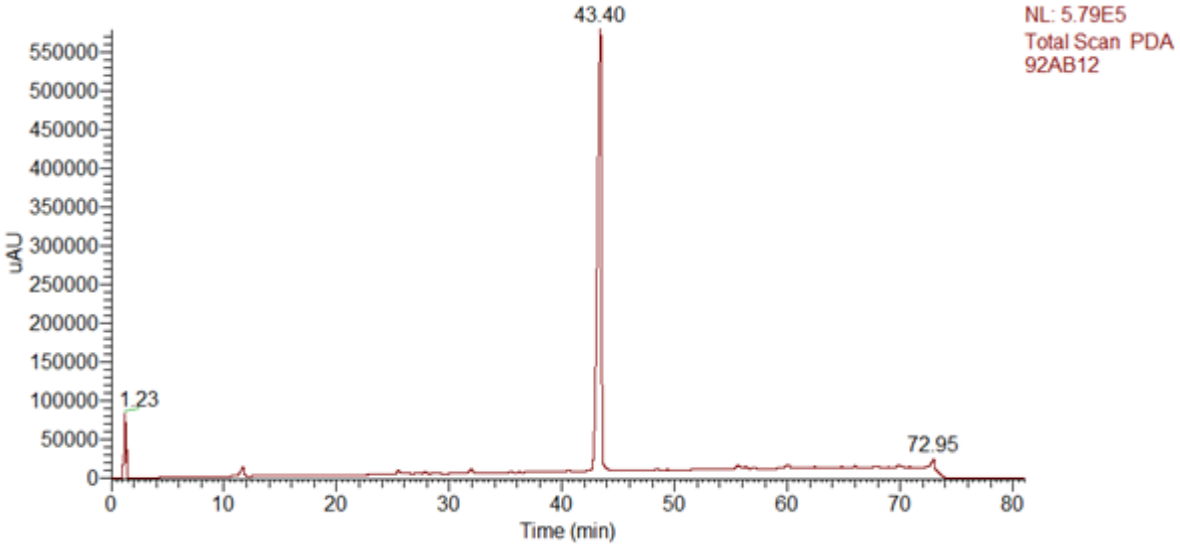
18


NL: 8.15E5
Total Scan PDA
106A1
38.23
1.24
21.13
22.77
35.47
72.95
uAU
Time (min)

19


35.65
NL: 5.56E5
Total Scan PDA
106A3
1.23
11.68
37.76
72.96
uAU
Time (min)

20


43.40
NL: 5.79E5
Total Scan PDA
92AB12
1.23
72.95
uAU
Time (min)

316

317

6. Results of the XTT assay of compounds 1-20

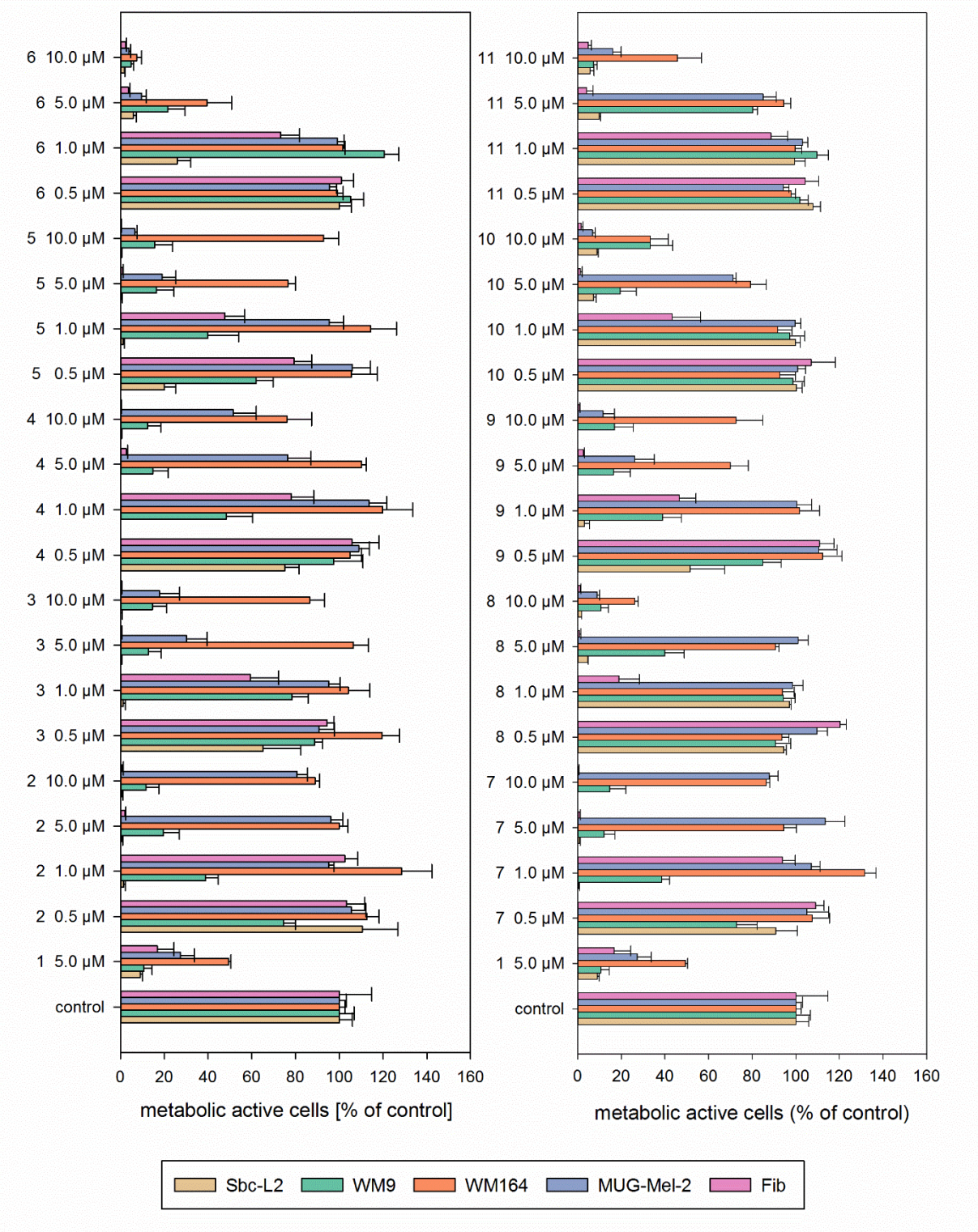


Figure S2. Results of the XTT assay of different concentrations of compounds **2**-**11** towards melanoma cells from non-metastatic (SBcl2) and metastatic lesions (WM9, WM164, MUG-Mel2) and skin fibroblasts (Fib) after 72 h of treatment (mean ± SEM, n = 4) and in comparison to 5.0 µM of **1**.

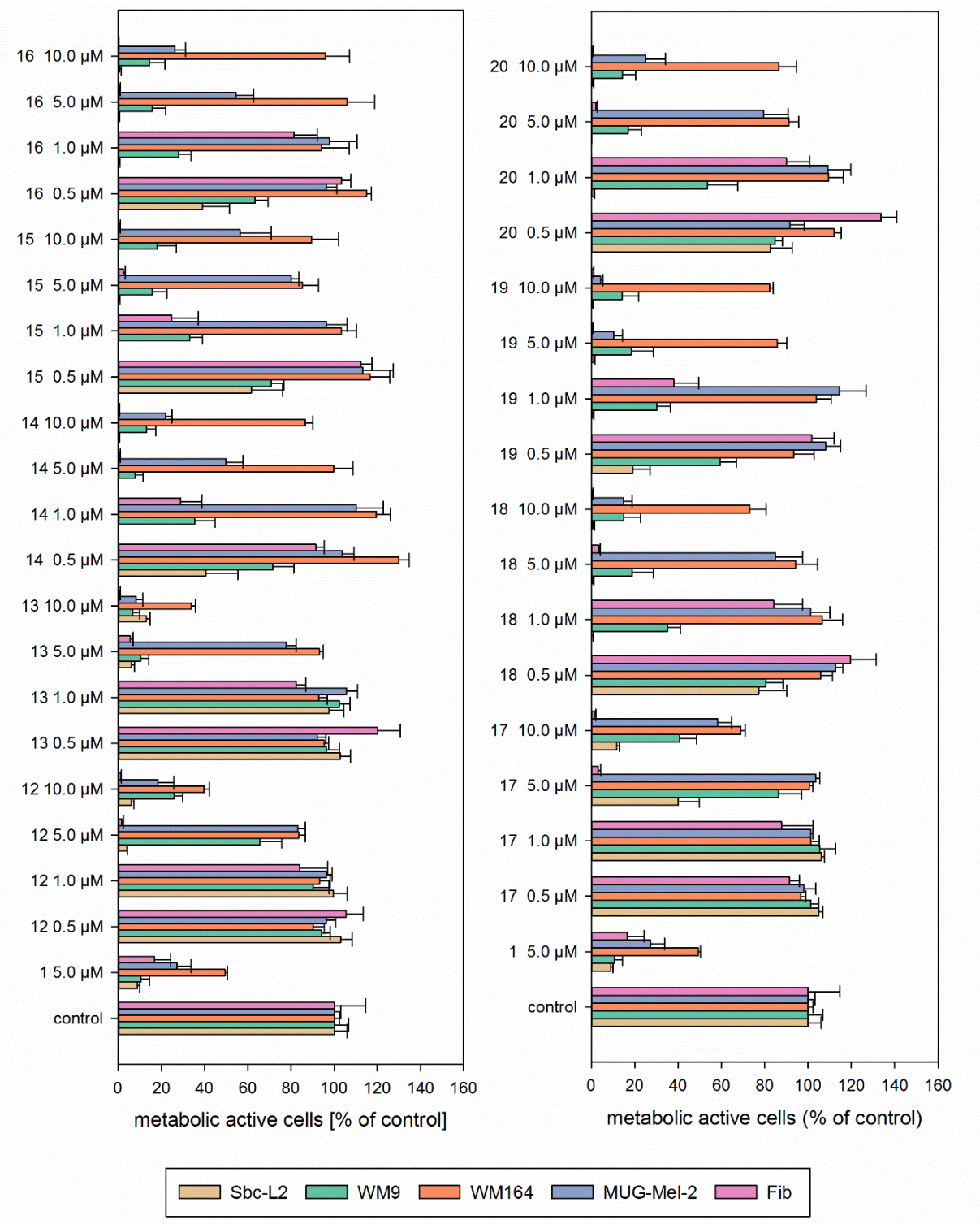


Figure S3. Results of the XTT assay of different concentrations of compounds **12**-**20** towards melanoma cells from non-metastatic (SBcl2) and metastatic lesions (WM9, WM164, MUG-Mel2) and skin fibroblasts (Fib) after 72 h of treatment (mean ± SEM, n = 4) and in comparison to 5.0 µM of **1**.