

A New Method for the Rapid Extraction of Natural Products: Efficient Isolation of Shikimic Acid from Star Anise

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Supporting Information

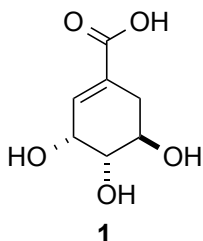
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I. General

Plant material was ground using a Sunbeam spice/coffee bean grinder. Pressurized hot water extraction was undertaken employing a Breville Espresso Machine Model 800ES. Unless otherwise specified, reactions were conducted with magnetic stirring in oven-dried glassware in air. Flash chromatography was carried out using a Reveleris® X2 Flash Chromatography System using silica gel cartridges. All NMR spectra were recorded on a Bruker NMR spectrometer operating at 400 MHz (¹H) and 100 MHz (¹³C).

II. Isolation of Shikimic Acid and Synthesis of Derivatives



Shikimic acid (1)

Dried Chinese star anise (*Illicium verum*) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter (sample compartment) of an espresso machine and extracted using 30% EtOH/H₂O (200 mL of a hot solution) (Figure S1).¹ This was repeated with another sample of star anise (20 g). The

(1) The ethanol content was not increased beyond 30% in order to minimize the flammability of the solvent mixture.

extracts were combined and silica gel (20 g) was added. The ensuing suspension was evaporated to dryness on a rotary evaporator (50 °C water bath temperature). The ensuing brown solid was then loaded onto a sintered glass funnel and washed with CH₂Cl₂ (150 mL) then EtOAc (150 mL) in order to remove anethole and *p*-anisaldehyde (see Appendix I for the ¹H NMR spectrum of the residue obtained from the evaporation of the combined CH₂Cl₂ and EtOAc washings).² These washings were discarded. The resulting solid was then extracted with 10% AcOH/EtOAc (250 mL) and the filtrate was evaporated to dryness (see Appendix II for the ¹H NMR spectrum of this residue). The ensuing solid was then washed with CH₂Cl₂ and then dried under vacuum to provide the title compound as an off-white solid (2.21 g, 5.50 % w / w) (Figure S2).³

¹H NMR (400 MHz, D₂O) δ 6.81 (m, 1H), 4.47 (m, 1H), 4.05 (m, 1H), 3.80 (dd, *J* = 8.2, 4.2 Hz, 1H), 2.77 (ddt, *J* = 18.1, 5.2, 1.4 Hz, 1H), 2.24 (ddt, *J* = 18.2, 6.5, 1.8 Hz, 1H).

¹³C NMR (100 MHz, D₂O) δ 171.1, 136.8, 130.8, 71.5, 66.9, 66.1, 30.9.



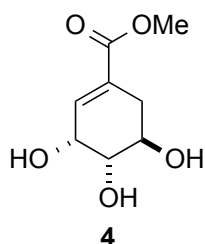
Figure S1: Extraction of star anise using an espresso machine.



Figure S2: Isolated shikimic acid.

(2) It is well-established that anethole and *p*-anisaldehyde are present in star anise. See: Sy, L.-K.; Brown, G. D. *J. Nat. Prod.* **1998**, 61, 987 and references cited therein.

(3) Payne, R.; Edmonds, M. *J. Chem. Educ.* **2005**, 82, 599.

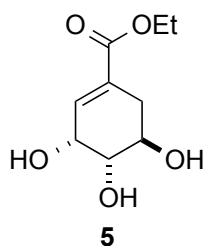


Methyl shikimate (4)

Dried Chinese star anise (*Illicium verum*) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H₂O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and were reduced to 200 mL on a rotary evaporator (to remove EtOH). The ensuing aqueous phase was washed with CH₂Cl₂ (3 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness on a rotary evaporator (50 °C water bath temperature) to yield a red/brown sticky solid. MeOH (100 mL) was added to the ensuing residue and then SOCl₂ (3 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was removed on rotary evaporator. The resulting dark red/black oil was dissolved in 30% MeOH/EtOAc, filtered through a silica plug eluting with 30% MeOH/EtOAc (300 mL) and the solvent removed on rotary evaporator. Automated gradient flash chromatography (silica; MeOH/EtOAc elution) provided the title compound as a light-pink solid (2.03 g, equivalent to 4.7 % yield of shikimic acid w/w).⁴

¹H NMR (400 MHz, D₂O) δ 6.72 (m, 1H), 4.35 (m, 1H), 3.93 (m, 1H), 3.68 (m, 1H), 3.66 (s, 3H), 2.65 (dd, *J* = 18.2, 5.0 Hz, 1H), 2.14 (dd, *J* = 18.3, 6.3 Hz, 1H).

¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.7, 139.8, 127.3, 70.0, 66.8, 65.4, 51.5, 29.6.



Ethyl shikimate (5)

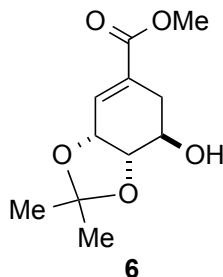
Dried Chinese star anise (*Illicium verum*) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H₂O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and were reduced to 200 mL on a rotary evaporator (to remove EtOH). The ensuing aqueous phase was washed with CH₂Cl₂ (3 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness on a rotary evaporator (50 °C water bath temperature) to yield a red/brown sticky solid. EtOH (100 mL) was added to the ensuing residue and then SOCl₂ (3 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was removed on rotary evaporator. The resulting dark red/black oil was dissolved in 30% MeOH/EtOAc, filtered through a silica plug eluting with 30% MeOH/EtOAc (300 mL) and the solvent removed on rotary evaporator. Automated

(4) Wu, W.; Zou, Y.; Chen, Y.; Li, J.; Lv, Z.; Wei, W.; Huang, T.; Liu, X. *Green Chem.* **2012**, *14*, 363.

gradient flash chromatography (silica; MeOH/EtOAc elution) provided the title compound as a light-brown solid (2.49 g, equivalent to 5.4 % yield of shikimic acid w/w).⁵

¹H NMR (400 MHz, DMSO-d₆) δ 6.62 (m, 1H), 4.82 (m, 2H, OH), 4.61 (d, *J* = 4.3 Hz, 1H, OH), 4.23 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.86 (m, 1H), 3.57 (m, 1H), 2.43 (m, 1H), 2.05 (m, 1H), 1.22 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (100 MHz, DMSO-d₆) δ 166.7, 140.0, 128.1, 70.5, 67.3, 65.9, 60.5, 30.1, 14.6.



Methyl 3,4-*O*-isopropylideneshikimate (6)

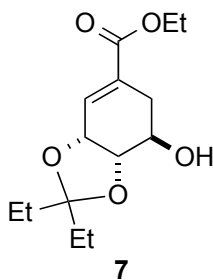
Dried Chinese star anise (*Illicium verum*) pods (15 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H₂O (200 mL of a hot solution). This was repeated with a further 5 samples of star anise (15 g). The extracts were combined and were reduced to 800 mL on a rotary evaporator (to remove EtOH). The ensuing aqueous phase was washed with CH₂Cl₂ (4 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness on a rotary evaporator (50 °C water bath temperature) to yield a red/brown sticky solid. MeOH (300 mL) was added to the ensuing residue and then SOCl₂ (5 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was removed on rotary evaporator. Acetone (100 mL), 2,2-dimethoxypropane (80 mL), and Amberlyst® 15 acidic ion exchange resin (0.8 g) were added to ensuing dark red/black oil. After 3 h at r.t., the mixture was filtered and evaporated to dryness to provide a dark red/black residue which was then dissolved in CH₂Cl₂ (100 mL) and loaded onto a silica plug (4.5 inch diameter, 1.5 inch depth). 7 fractions were collected: (20 % EtOAc/hexanes (5 x 100 mL), EtOAc (1 x 250 mL, 1 x 100 mL). The title compound was present in the final 2 fractions. After the solvent was removed on a rotary evaporator, automated gradient flash chromatography (silica; EtOAc/hexane elution) provided the title compound as a red oil (4.76 g, equivalent to 4.0 % yield of shikimic acid w/w).⁶

¹H NMR (400 MHz, CDCl₃) δ 6.91 (m, 1H), 4.74 (m, 1H), 4.08 (m, 1H), 3.89 (m, 1H), 3.76 (s, 3H), 2.78 (dd, *J* = 17.46, 4.7 Hz, 1H), 2.22 (ddt, *J* = 17.4, 8.4, 1.8 Hz, 1H), 2.20 (br s, 1H, OH), 1.44 (s, 3H), 1.39 (s, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 166.8, 134.2, 130.9, 110.0, 78.2, 72.5, 69.1, 52.4, 29.6, 28.2, 26.0.

(5) Kalashnikov, A. I.; Sysolyatin, S. V.; Sakovich, G. V.; Sonina, E. G.; Shchurova, I. A. *Russ. Chem. Bull.* **2014**, 62, 163.

(6) Tavassoli, A.; Duffy, J. E. S.; Young, D. W. *Org. Biomol. Chem.* **2006**, 4, 569.

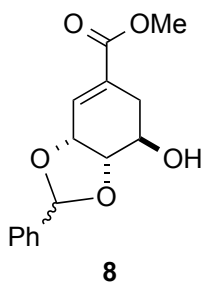


Ethyl 3,4-O-(3-pentylidene)shikimate (7)

Dried Chinese star anise (*Illicium verum*) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H₂O (200 mL of a hot solution). This was repeated with a further 9 samples of star anise (20 g). The extracts were combined and were reduced to 800 mL on a rotary evaporator (to remove EtOH). The ensuing aqueous phase was washed with CH₂Cl₂ (4 x 100 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness on a rotary evaporator (50 °C water bath temperature) to yield a red /brown sticky solid. EtOH (300 mL) was added to the ensuing residue and then SOCl₂ (10 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was removed on rotary evaporator. 3-Pentanone (200 mL), and Amberlyst® 15 acidic ion exchange resin (1.6 g) were added to ensuing dark red /black oil. After 2.5 h at r.t., the mixture was evaporated to dryness to provide a dark red /black residue which was then dissolved in CH₂Cl₂ (100 mL) and loaded onto a silica plug (4.5 inch diameter, 2 inch depth). 7 fractions were collected: (30% EtOAc/hexanes (2 x 150 mL), 50% EtOAc/hexanes (2 x 200 mL), EtOAc (1 x 100 mL). Evaporation of fractions 2–4 provided the title compound as a yellow oil (13.15 g, equivalent to 4.2 % yield of shikimic acid w/w).⁷

¹H NMR (400 MHz, CDCl₃) δ 6.93 (m, 1H), 4.75 (m, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.11 (m, 1H), 3.91 (m, 1H), 2.77 (dd, *J* = 17.3, 4.6 Hz, 1H), 2.25 (ddt, *J* = 17.3, 8.2, 1.9 Hz, 1H), 1.67 (q, *J* = 7.5 Hz, 4H), 1.65 (q, *J* = 7.5 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.5 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ 166.4, 134.3, 130.6, 113.9, 78.1, 72.5, 69.2, 61.3, 29.9, 29.5, 29.3, 14.4, 8.7, 8.1.



Methyl 3,4-O-benzylideneshikimate (8)

Dried Chinese star anise (*Illicium verum*) pods (20 g) were finely ground using a coffee bean grinder. This material was mixed with sand (2 g), placed into the portafilter of an espresso machine and extracted using 30% EtOH/H₂O (200 mL of a hot solution). This was repeated with another sample of star anise (20 g). The extracts were combined and were reduced to 200 mL on a rotary evaporator (to remove EtOH). The ensuing aqueous phase was washed with CH₂Cl₂ (3 x 80 mL) and the organic phases were discarded. The aqueous layer was then evaporated to dryness on a rotary evaporator (50 °C water bath temperature) to yield a

(7) Carr, R.; Ciccone, F.; Gabel, R.; Guinn, M.; Johnston, D.; Mastriona, J.; Vandermeer, T.; Groaning, M. *Green Chem.* **2008**, *10*, 743.

red/brown sticky solid. MeOH (100 mL) was added to the ensuing residue and then SOCl₂ (3 mL) was added dropwise. The mixture was heated at reflux for 1.5 h, cooled and then the solvent was removed on rotary evaporator. THF (100 mL), benzaldehyde (3 mL), and Amberlyst® 15 acidic ion exchange resin (1.6 g) were added to ensuing black oil. After 2 h at r.t., the mixture was evaporated to dryness to provide a black residue which was then loaded onto a silica plug (4.5 inch diameter, 1.5 inch depth). 4 fractions were collected: 50% EtOAc/hexanes (3 x 150 mL), 70 % EtOAc/hexanes (1 x 150 mL). The title compound was present in fractions 2–4. After the solvent was removed on a rotary evaporator, automated gradient flash chromatography (silica; EtOAc/hexane elution) provided the title compound as a mixture of diastereoisomers as a yellow oil (2.34 g, equivalent to 3.5 % yield of shikimic acid w/w).⁸ These diastereoisomers were partially separable by chromatography.

(*R*)-Methyl 3,4-*O*-benzylideneshikimate

¹H NMR (400 MHz, CDCl₃) δ 7.43 (m, 2H), 7.37 (m, 3H), 7.00 (m, 1H), 5.94 (s, 1H), 4.80 (m, 1H), 4.18 (m, 1H), 3.93 (m, 1H), 3.78 (s, 3H), 2.83 (dd, *J* = 17.4, 4.8 Hz, 1H), 2.27 (ddt, *J* = 17.5, 9.0, 1.9 Hz, 1H).

¹³C NMR (100 MHz, CDCl₃) δ 166.6, 136.6, 133.0, 131.7, 129.9, 128.7, 127.0, 104.6, 78.8, 74.2, 69.3, 52.4, 29.7.

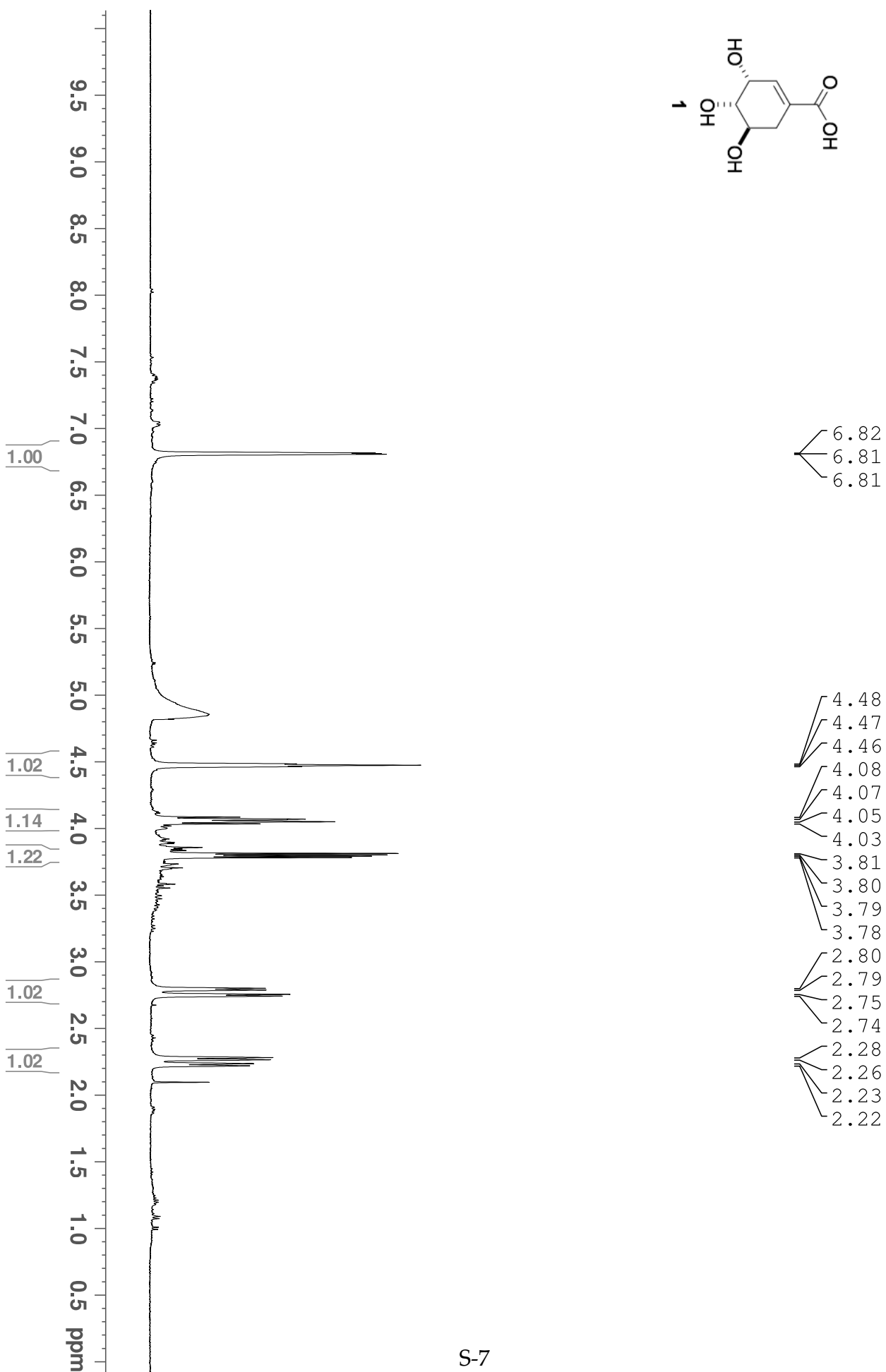
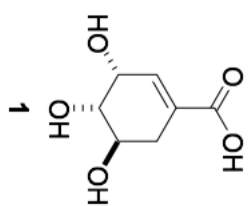
(*S*)-Methyl 3,4-*O*-benzylideneshikimate

¹H NMR (400 MHz, CDCl₃) δ 7.45 (m, 2H), 7.38 (m, 3H), 6.93 (m, 1H), 5.97 (s, 1H), 4.87 (m, 1H), 4.34 (m, 1H), 4.14 (m, 1H), 3.79 (s, 3H), 2.84 (dd, *J* = 17.3, 4.5 Hz, 1H), 2.34 (ddt, *J* = 17.5, 7.2, 1.8 Hz, 1H).

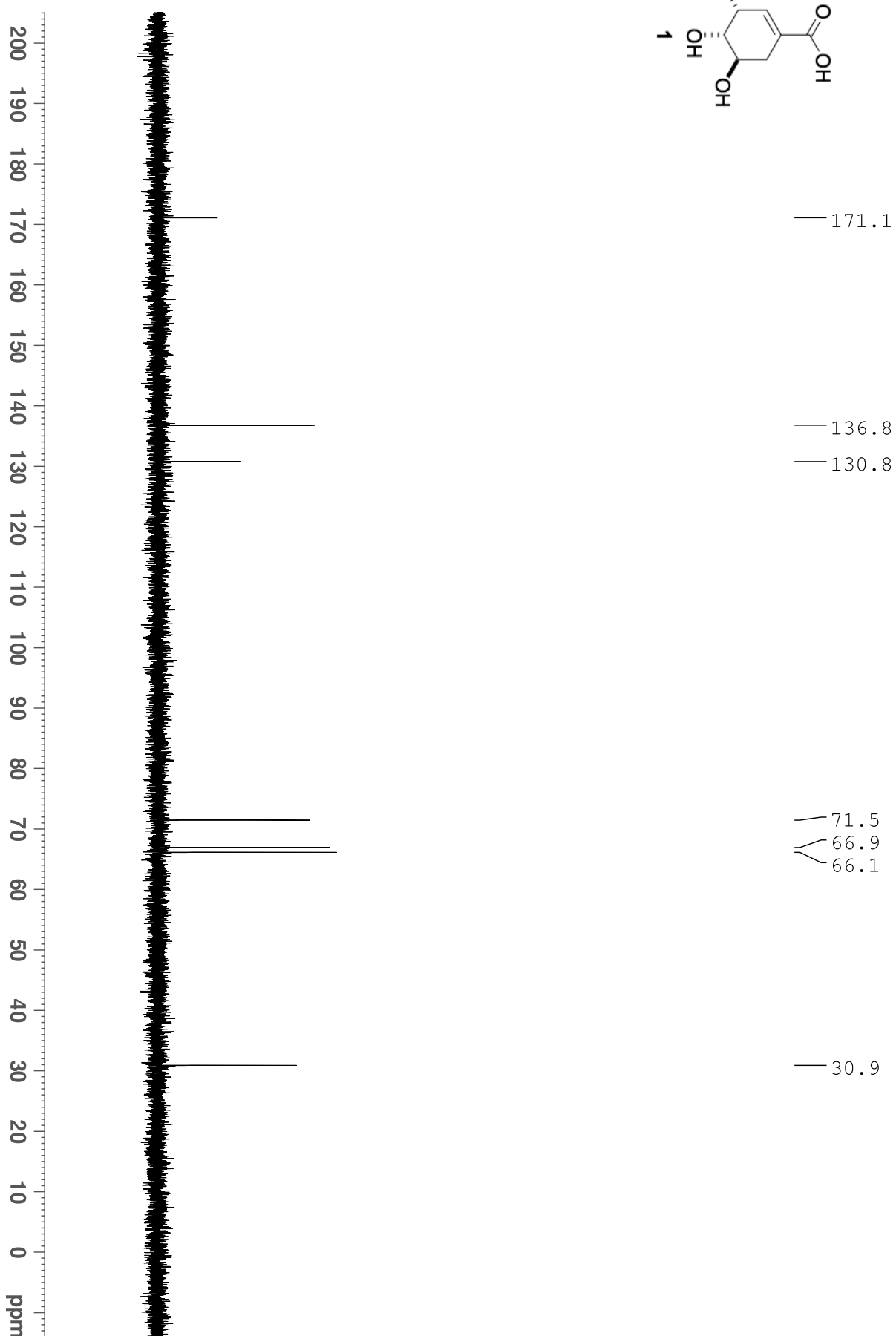
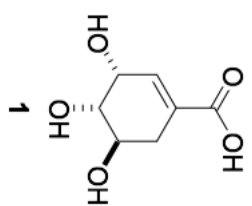
¹³C NMR (100 MHz, CDCl₃) δ 166.7, 137.9, 133.7, 131.3, 129.5, 128.6, 126.5, 102.9, 78.1, 72.7, 67.3, 52.4, 29.1.

(8) Adams, H.; Bailey, N. A.; Brettell, R.; Cross, R.; Frederickson, M.; Haslam, E.; MacBeath, F. S.; Davies, G. M. *Tetrahedron* **1996**, 52, 8565.

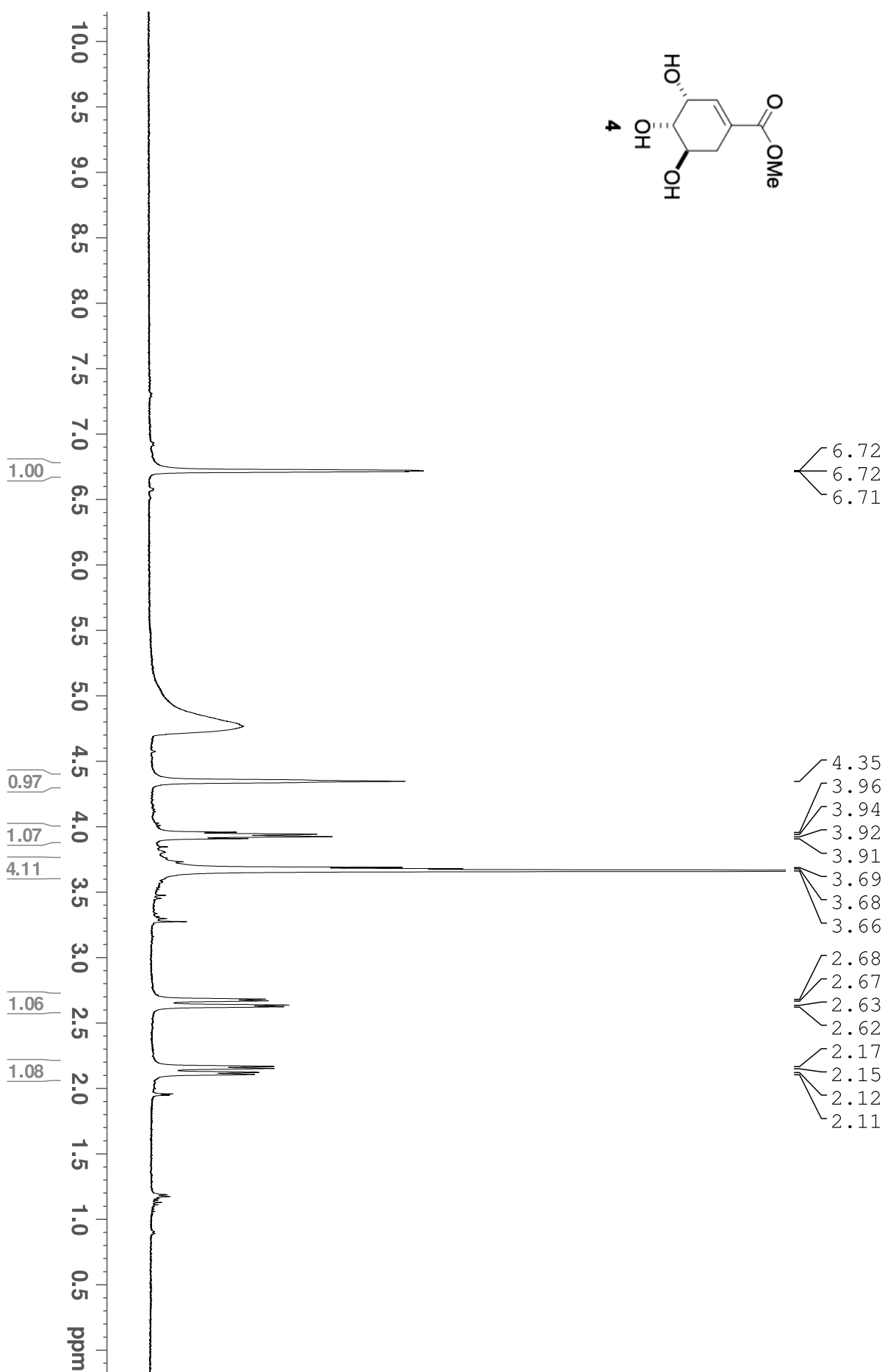
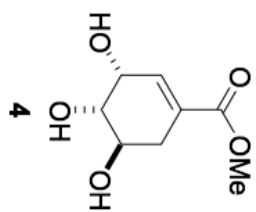
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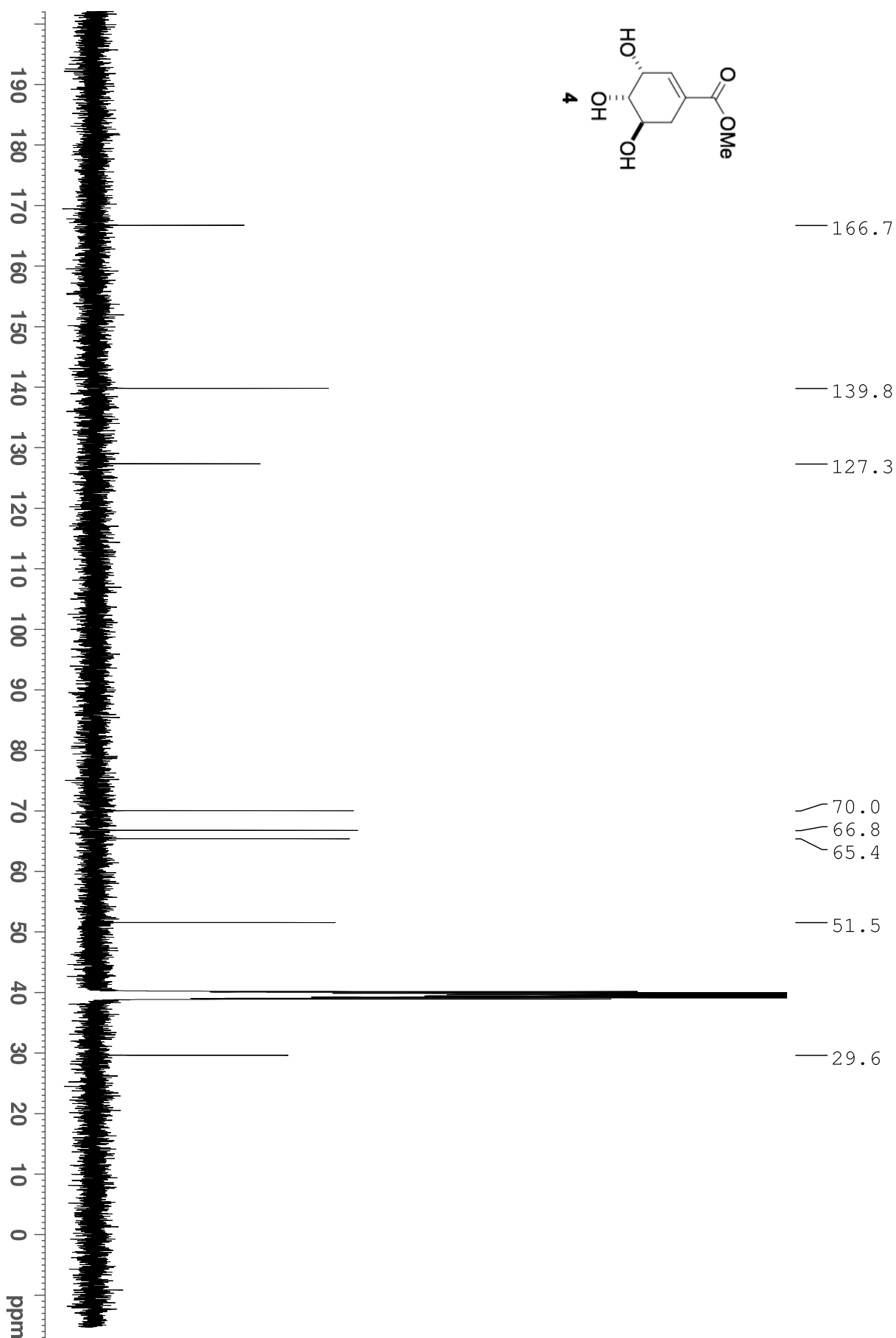
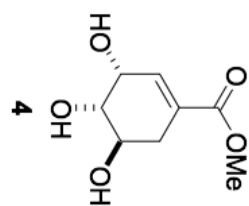
Shikimic acid D2O 13C

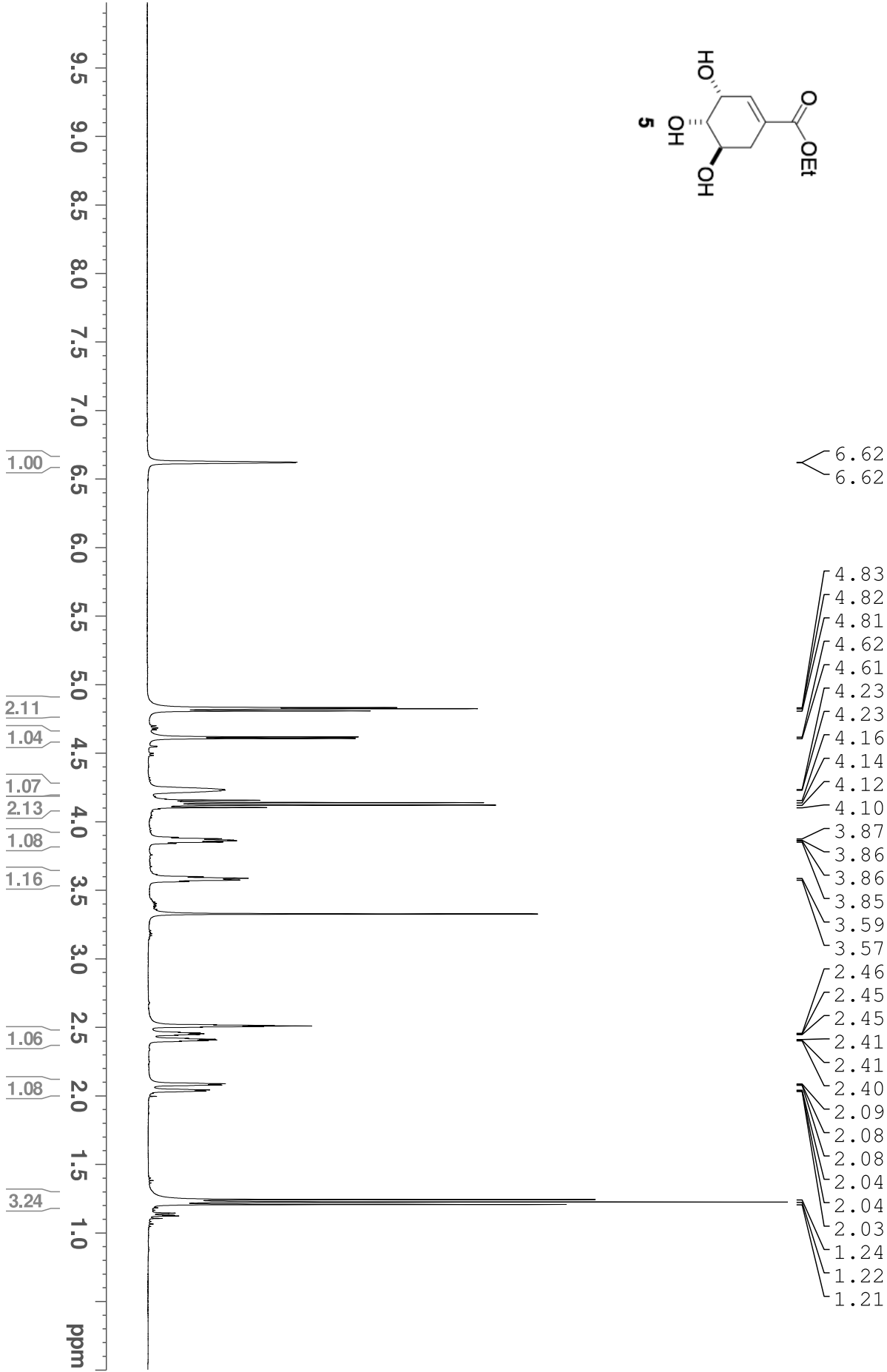
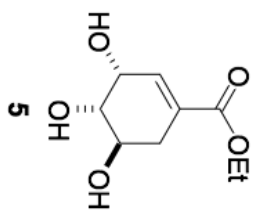


Methyl shikimate D2O

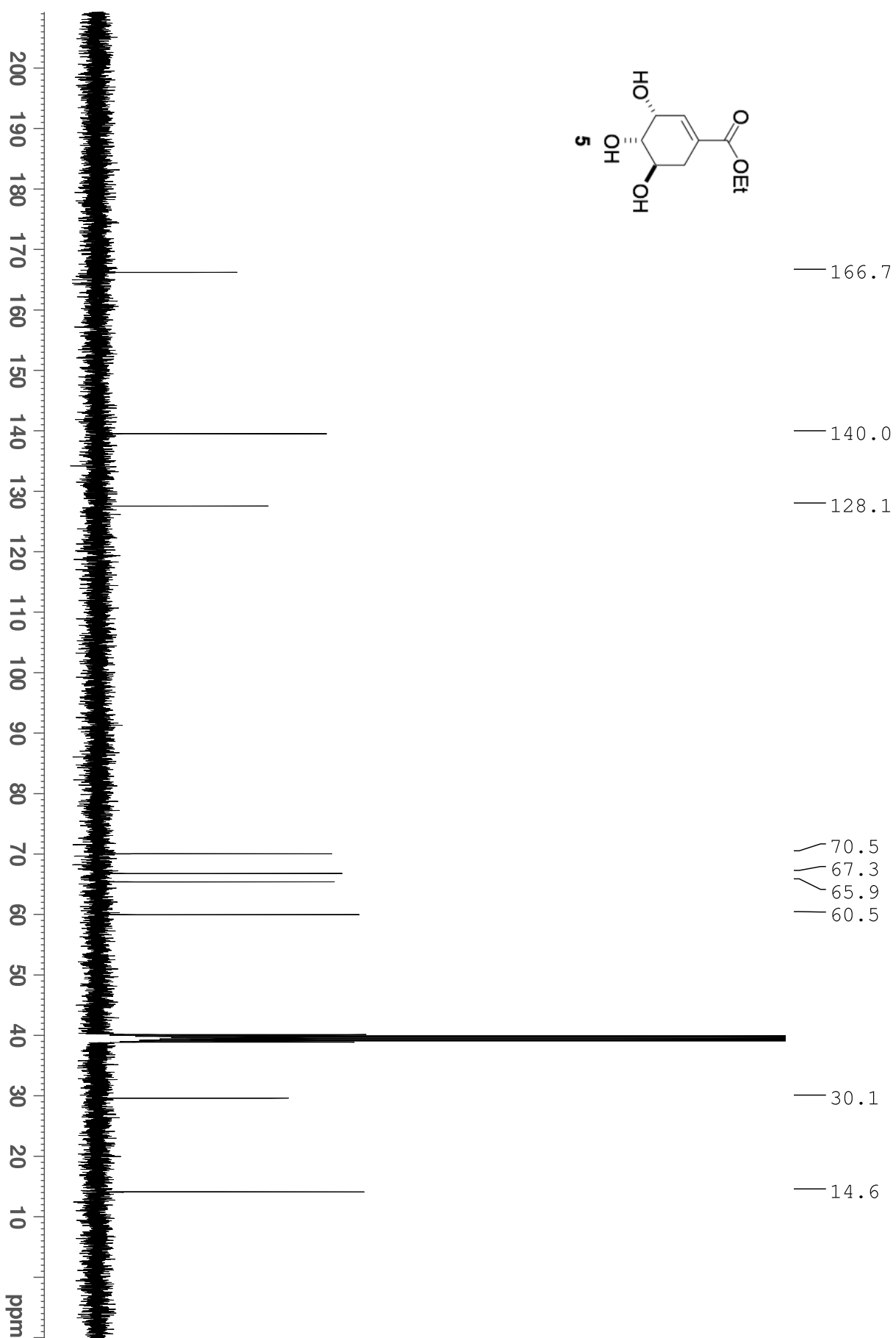
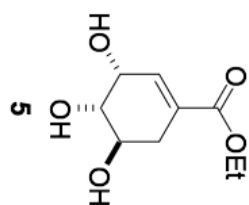


Methyl shikimate DMSO d6 13C

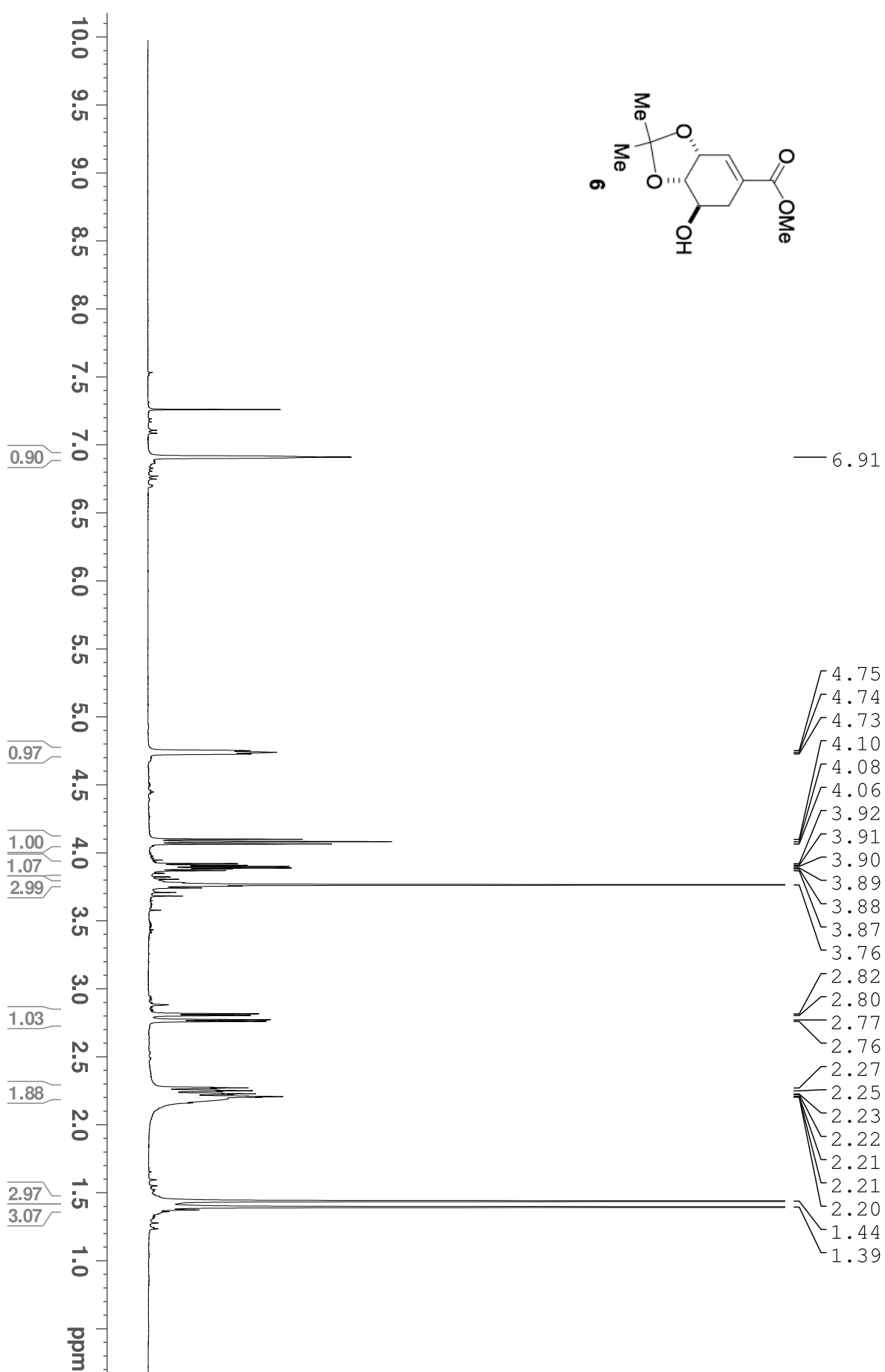
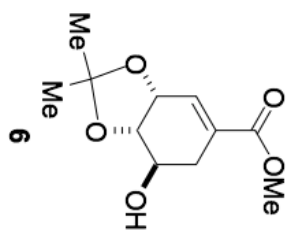




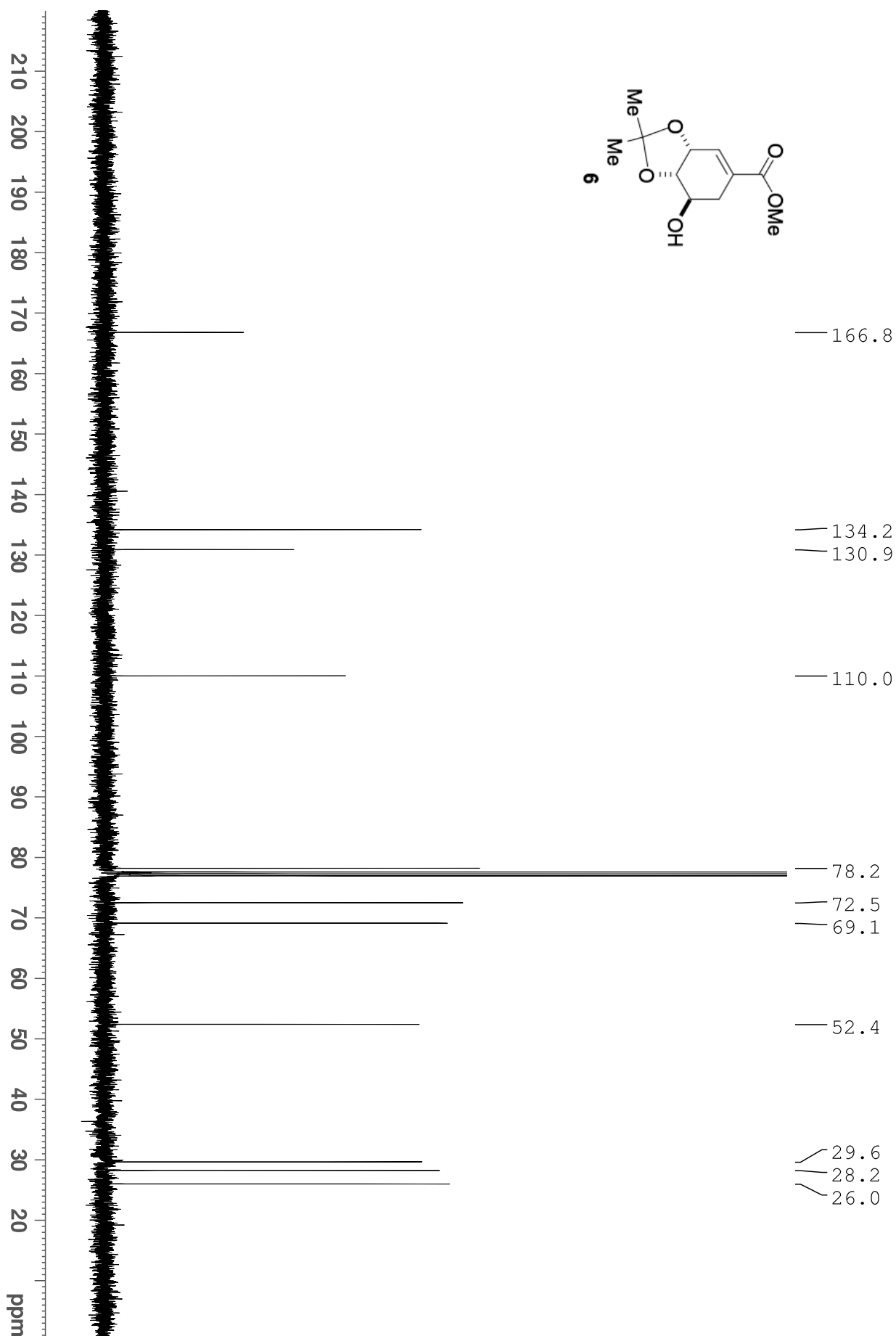
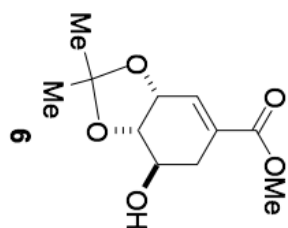
Ethyl shikimate DMSO d6 13C



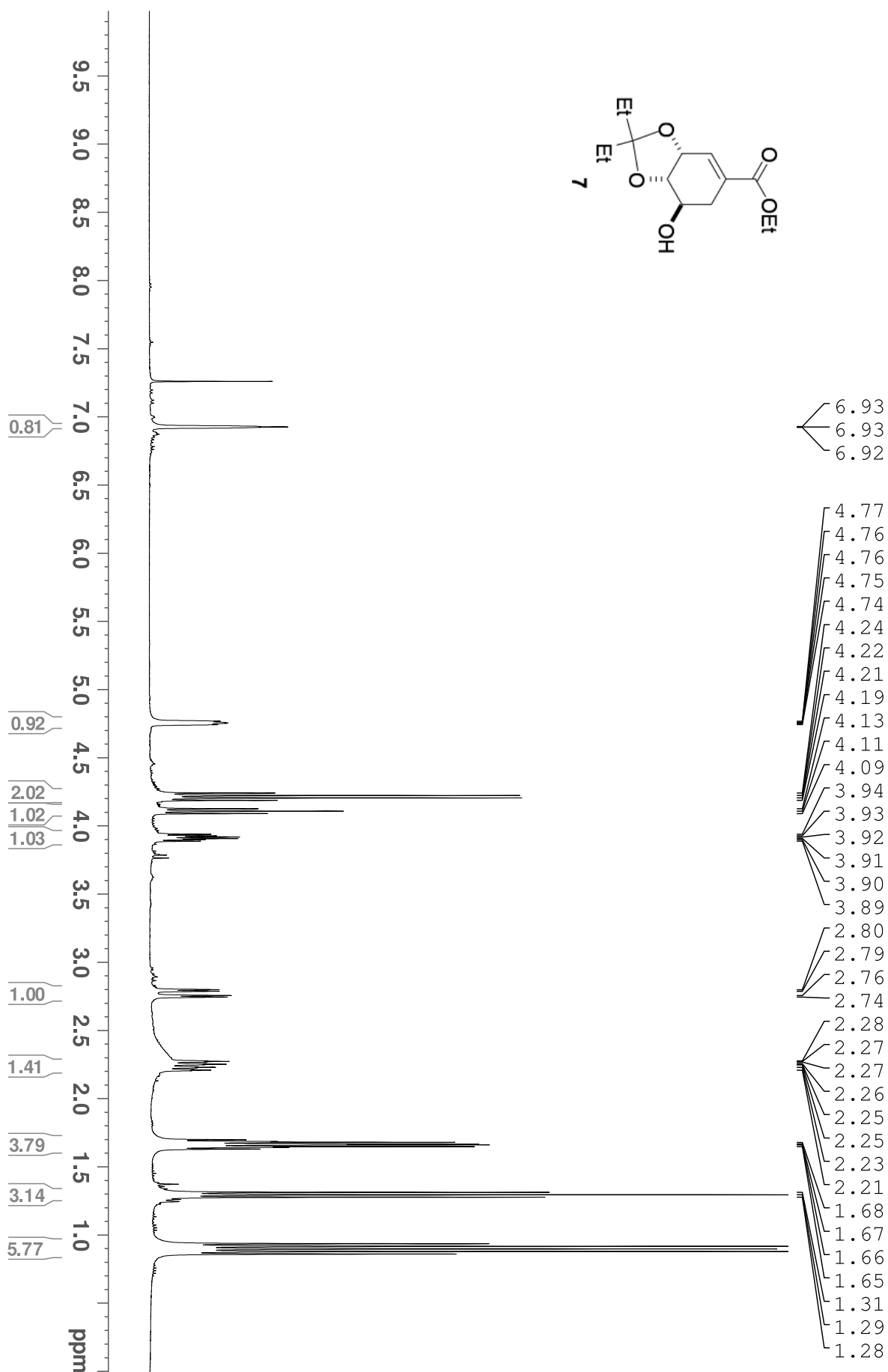
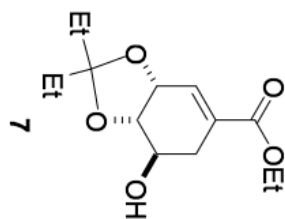
Methyl shikimate, propylidene CDCl₃



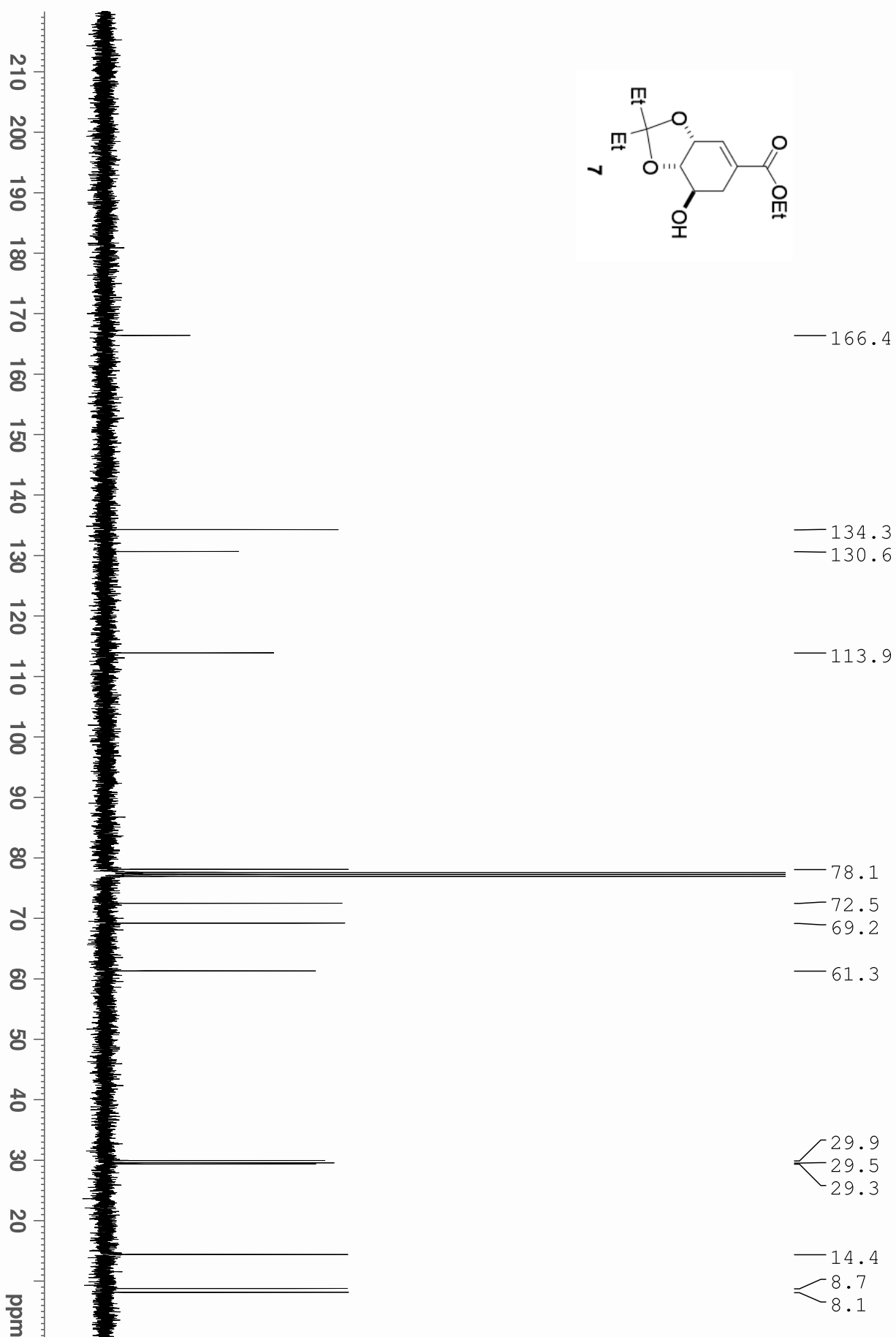
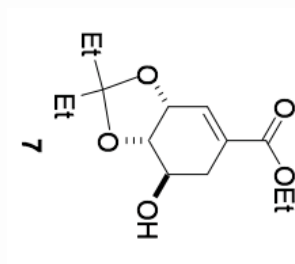
Methyl shikimate, propylidene CDCl₃ 13C



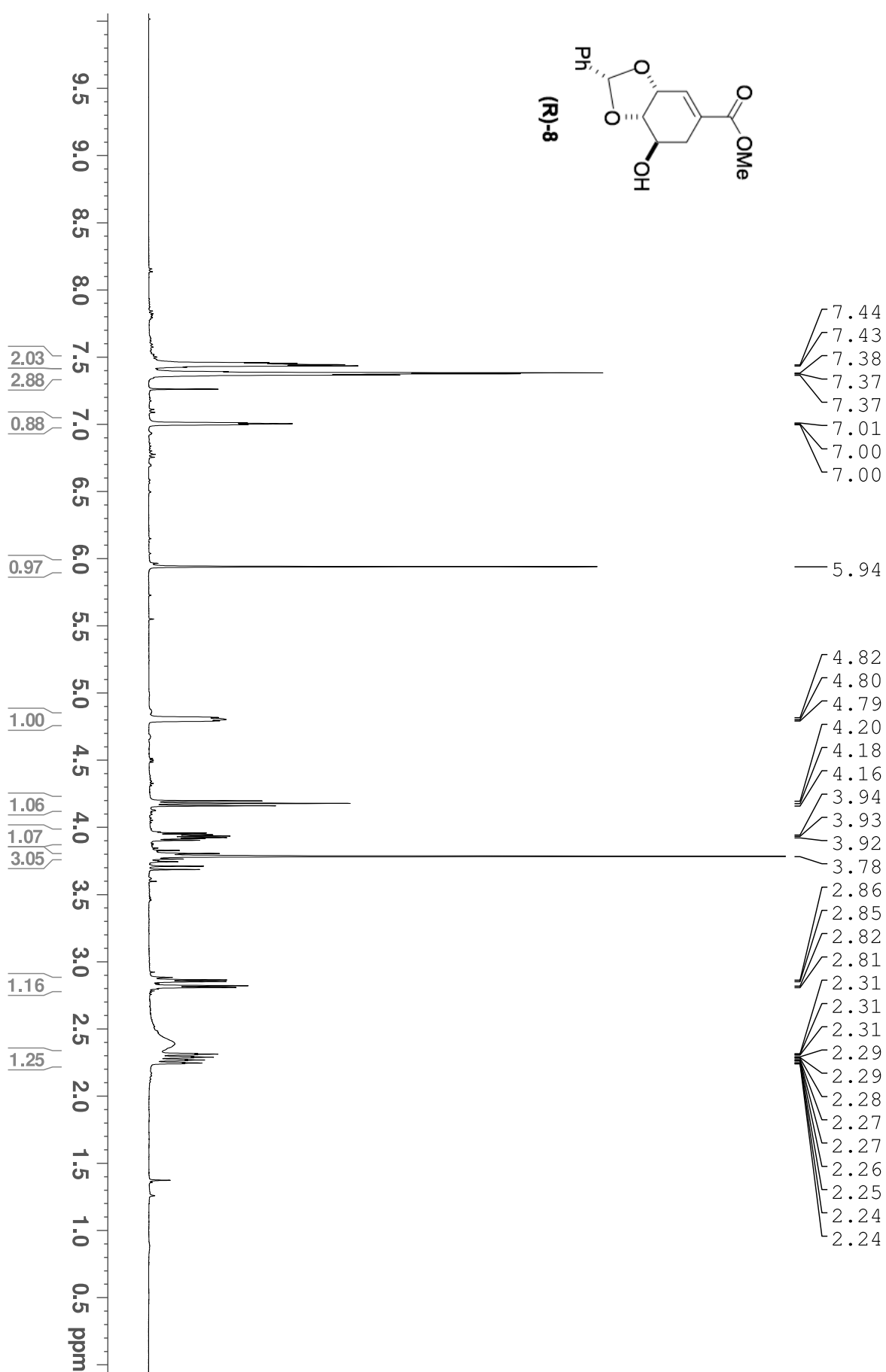
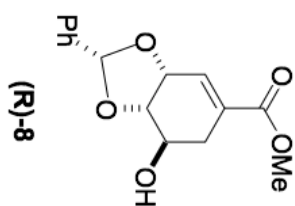
Ethyl shikimate, pentylidene CDCl₃



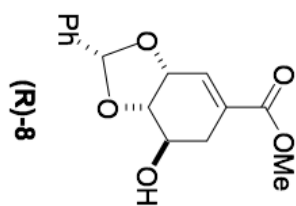
Ethyl shikimate, pentylidene CDCl₃ 13C



Methyl shikimate, benzylidene CDCl₃
(R)-isomer



Methyl shikimate, benzylidene CDCl₃
(R)-isomer



— 166.6

136.6
133.0
131.7
129.9
128.7
127.0

— 104.6

— 78.8

— 74.2

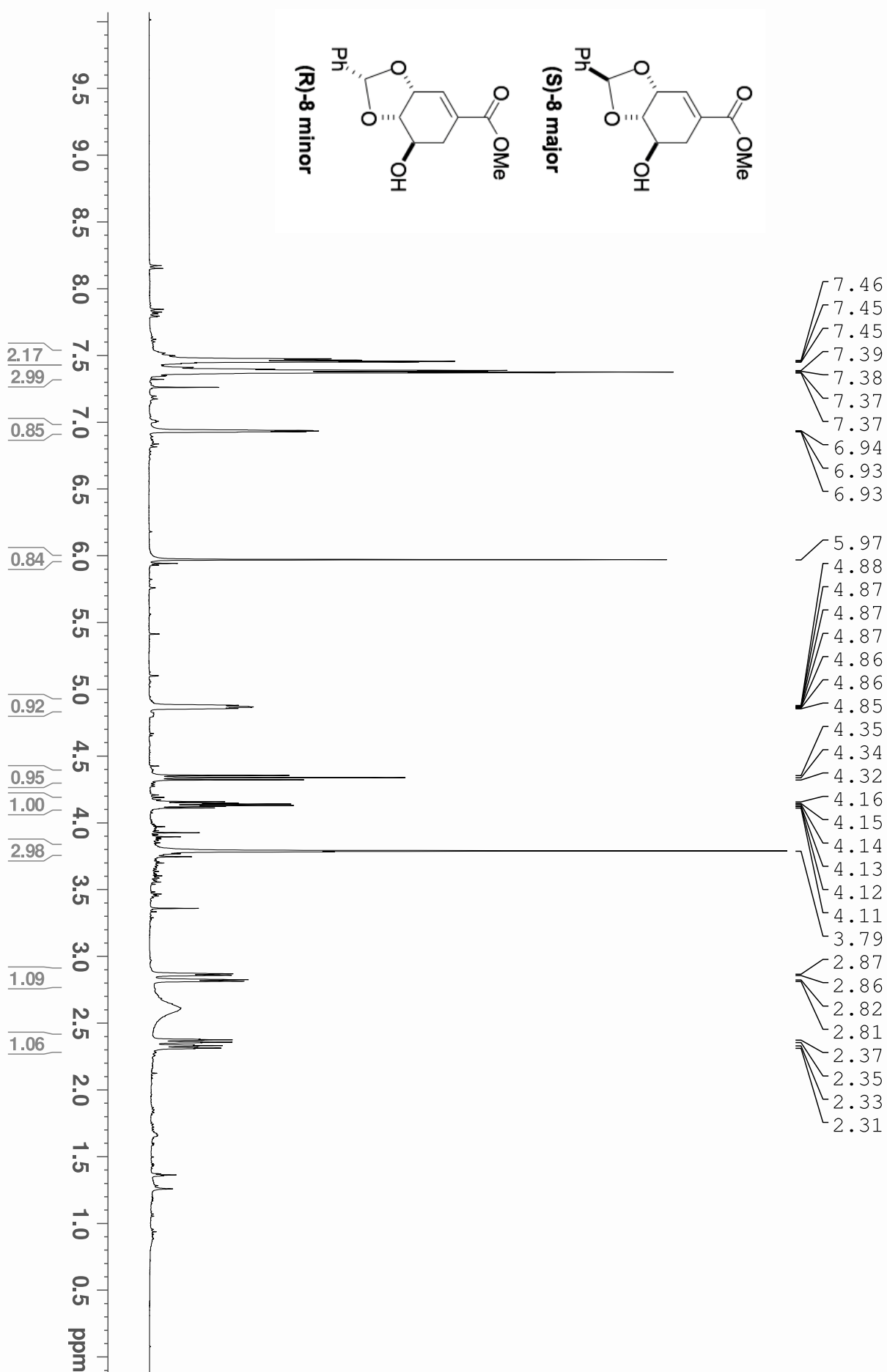
— 69.3

— 52.4

— 29.7



Methyl shikimate, benzylidene CDCl₃
(S)-isomer



Methyl shikimate, benzylidene CDCl₃ 13C
(S)-isomer

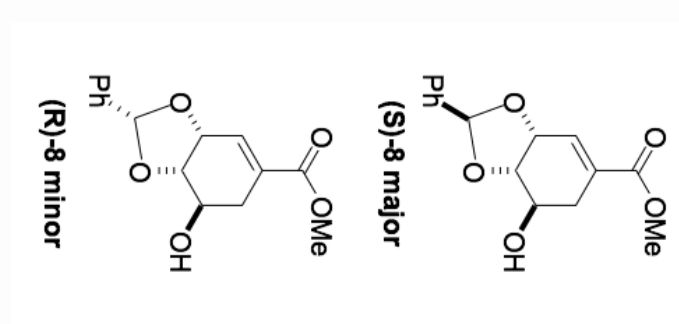
— 166.70
/ 137.94
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/ 131.33
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/ 128.61
/ 126.47

— 102.85

— 78.11
— 72.65
— 67.31

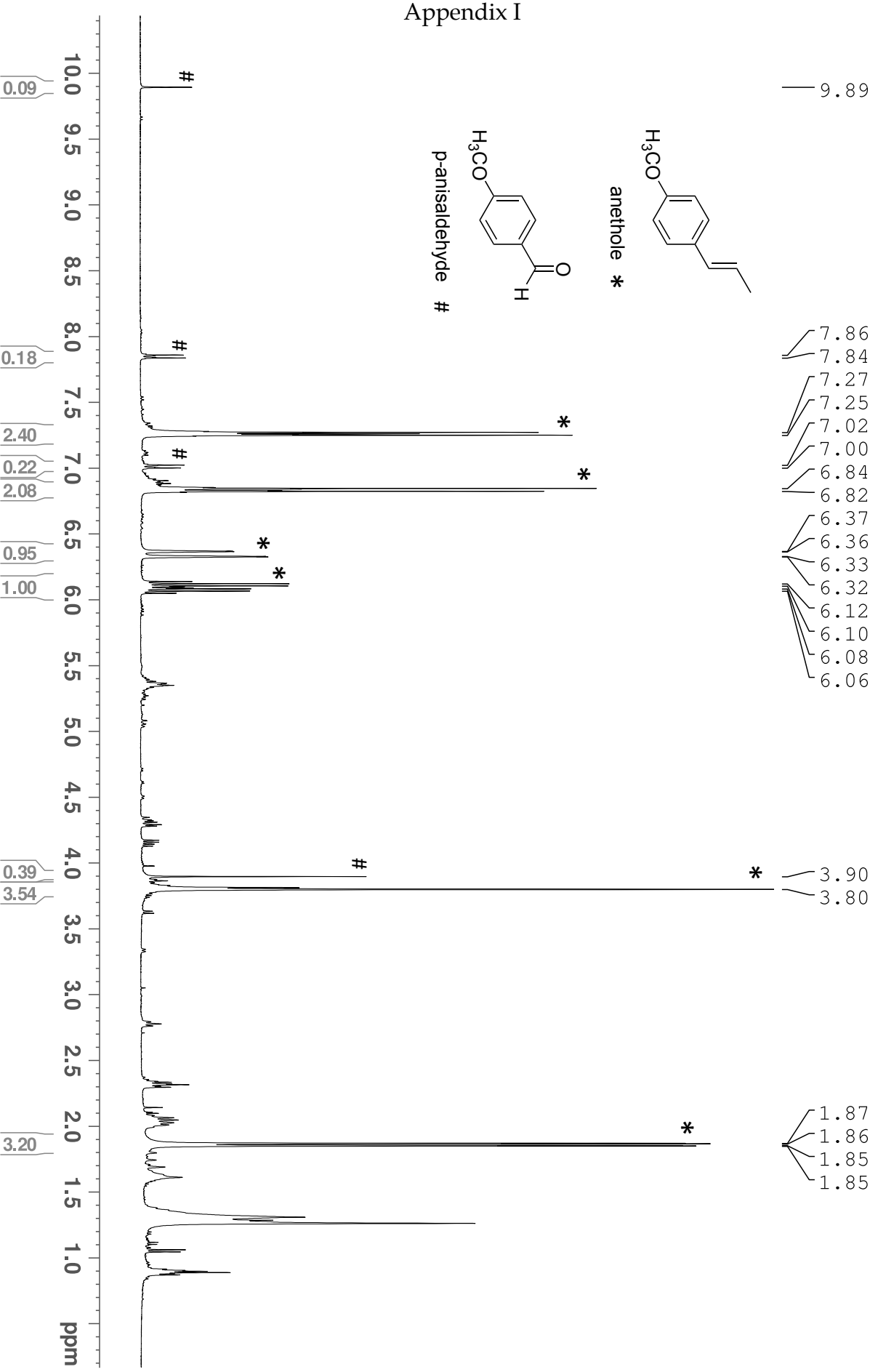
— 52.37

— 29.13



200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Star anise extract combined CH₂Cl₂, EtOAc washings CDCl₃



shikimic acid prior to final wash D2O

