

Supporting Information

for

A highly reactive conjugated allene involved in the biosynthesis of volatile oxylipins in the moss *Dicranum scoparium*

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General description

NMR-Spectra were recorded on Bruker Avance 200 MHz or 400 MHz spectrometers for the characterization of synthetic compounds and on a 500 MHz spectrometer for structural elucidation of the novel natural product. Chemical shifts are given in ppm relative to the chemical shift of the solvent signal of chloroform, 7.26 ppm. Accurate mass spectra were measured on a Q-tof micro ESI-TOF (Waters, Manchester, UK) equipped with a lock spray ESI-probe. EI-pos-GC/MS spectra for SPME analysis were recorded on a Perkin Elmer TurboMass mass spectrometer coupled to a Perkin Elmer Auto System XL equipped with a SE 54 column (50 m x 0.25 mm, 0.35 μ m film). For reaction control and characterization of synthesis products a Finnigan Mat Magnum equipped with a DB-5-MS column (30 m x 0.25 mm, 0.25 μ m film) or a GCT (Waters) equipped with a DB-5-MS column (38 m x 0.25 mm, 0.25 μ m film) was used. LC-MS measurements were carried out on an UPLC-MS system equipped with a 2996 PDA detector and a Q-tof micro ESI-TOF (Waters, Manchester, UK). A BEH C18 (2.1 x 50 mm, particle size 1.7 μ m) column was used for LC-MS separation. LC on a semipreparative column was carried out using a LC-8A liquid chromatography system from Shimadzu (Duisburg, Germany) equipped with a SPD-10AV UV-VIS detector and a LiChroCART® 250-10 Purosphere® RP-18 endcapped column (particle size 5 μ m) (Merck, Darmstadt, Germany). Analytical HPLC was done on a Synergi MAX-RP 250-4.6 mm column (particle size 4 μ m) supplied by Phenomenex (Macclesfield, UK). Circular dichroism spectra were recorded on a Jasco P810 instrument. Analytical thin layer chromatography was carried out by using silica gel 60 F₂₅₄ plates. Compounds were detected by UV irradiation or by potassium permanganate staining. Solvents and reagents were purchased from Sigma Aldrich, Deisenhofen, Germany.

(9Z,12Z)-methyl 14-(3-ethyloxiran-2-yl)tetradeca-9,12-dien-6-yneate (2)

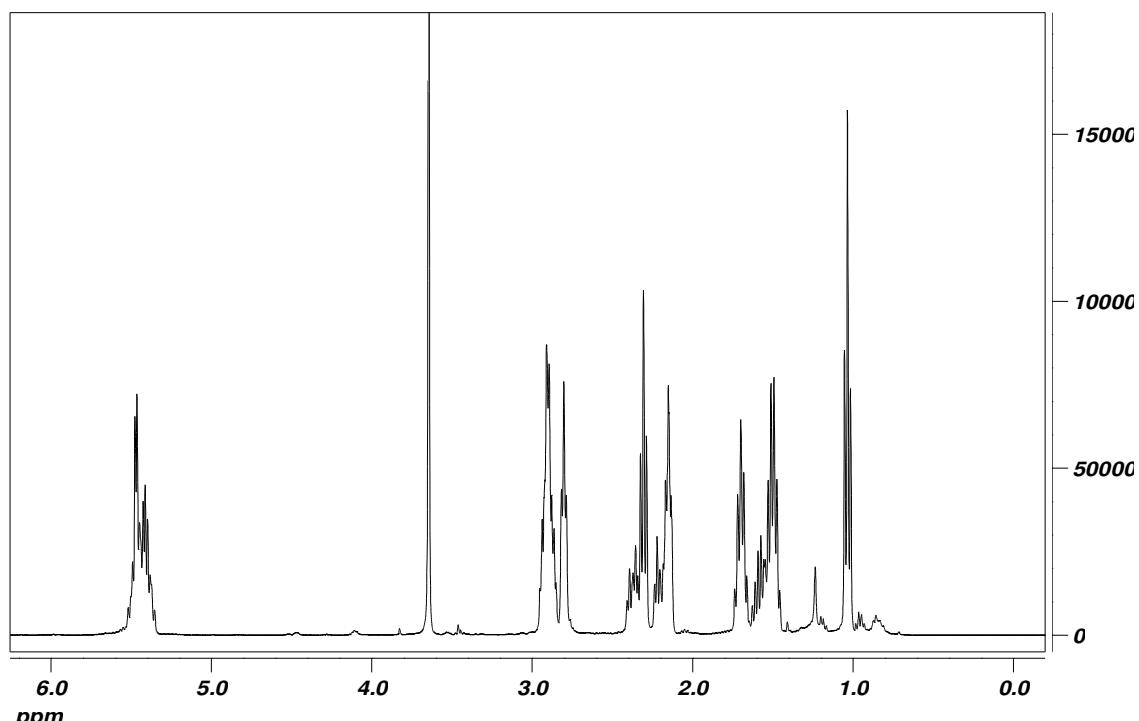
To 50 ml of water/THF (1/2 v/v) and 3.42 mmol NBS, 3.4 mmol of dicranin methyl ester (**1**) (in THF 2 ml) was added at 0°C. The reaction mixture was stirred for 1 h. The progress of the reaction was controlled with TLC. After quantitative conversion, the reaction mixture was allowed to come to room temperature and extracted with dithylether twice and the supernatant was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was taken up in 5 ml methanol and treated with 2 g of K_2CO_3 . Progress of the reaction was monitored by TLC. After 12 h, full conversion was observed. The reaction mixture was acidified with diluted HCl, extracted with diethyl ether and purified by silica gel chromatography. Mobile phase (petrol ether/ diethyl ether) (4/1 v/v).

Yield 73% (754 mg)

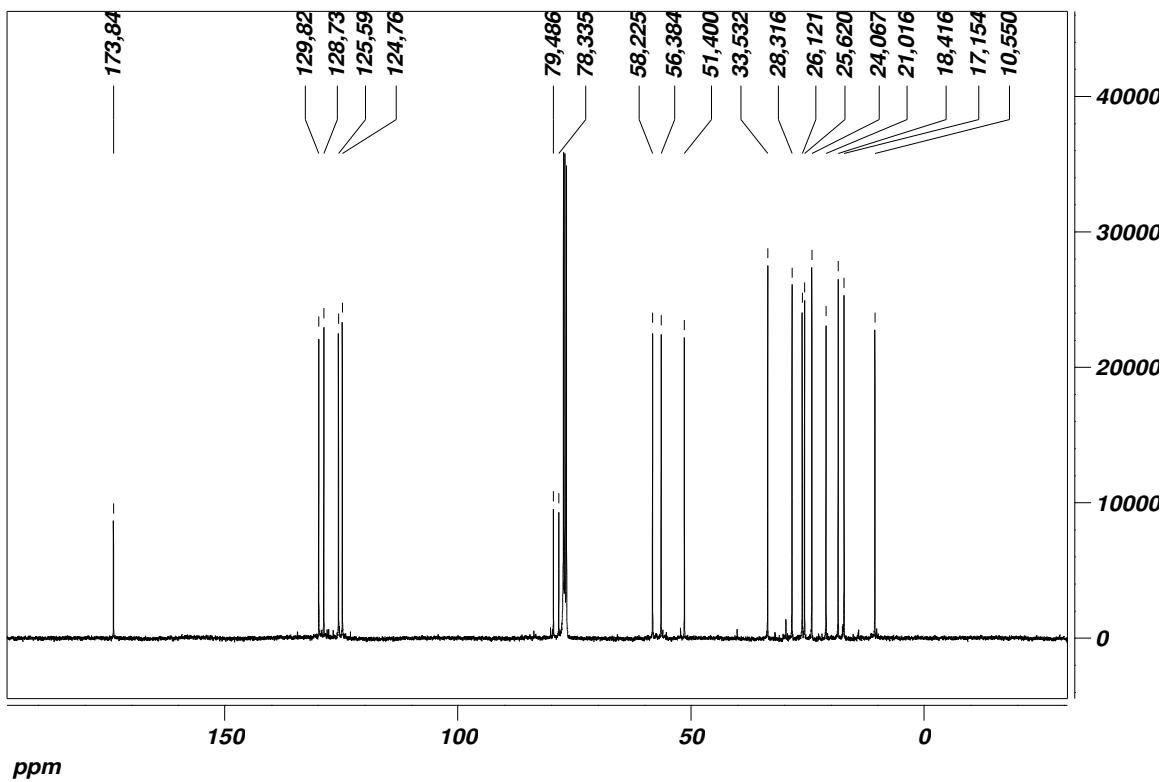
¹H NMR (400 MHz, CDCl₃): δ 5.56-5.32 (m, 4H), 3.65 (s, 3H), 2.98-2.84 (m, 4H), 2.80 (t, *J* = 5.88 Hz, 2H), 2.42-2.33 (m, 1H), 2.31 (t, *J* = 7.45 Hz, 2H), 2.22 (t, *J* = 6.37 Hz, 1H), 2.19-2.10 (m, 2H), 1.75-1.65 (m, 2H), 1.64-1.43 (m, 4H), 1.03 (t, *J* = 7.51 Hz, 3H)

¹³C NMR (101 MHz, *CDCl*₃): δ 173.8, 129.8, 128.7, 125.5, 124.7, 79.4, 78.3, 58.2, 56.3, 51.4, 33.5, 28.3, 26.1, 25.6, 24.1, 21.0, 18.4, 17.1, 10.6

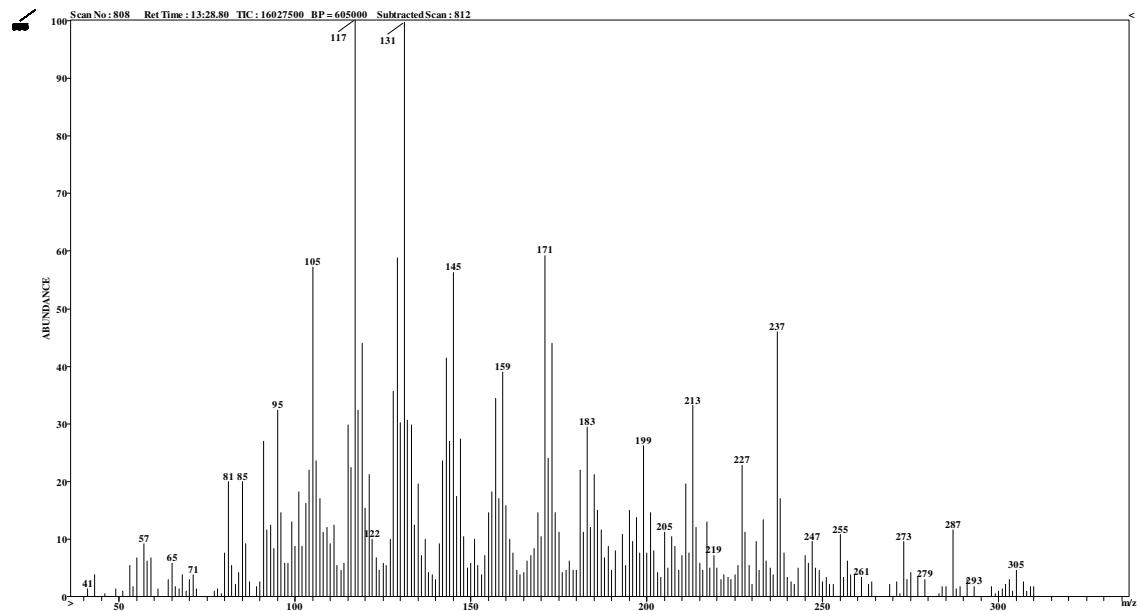
El-pos-HRMS: m/z calcd. for $C_{19}H_{28}O_3$: 304.2038; found: 304.2046



¹H-NMR spectrum of (9Z,12Z)-methyl 14-(3-ethyloxiran-2-yl)tetradeca-9,12-dien-6-ynoate (2)



^{13}C -NMR spectrum of (9Z,12Z)-methyl 14-(3-ethyloxiran-2-yl)tetradeca-9,12-dien-6-yneate (**2**)



EI-pos MS spectrum of (9Z,12Z)-methyl 14-(3-ethyloxiran-2-yl)tetradeca-9,12-dien-6-yneate (**2**)

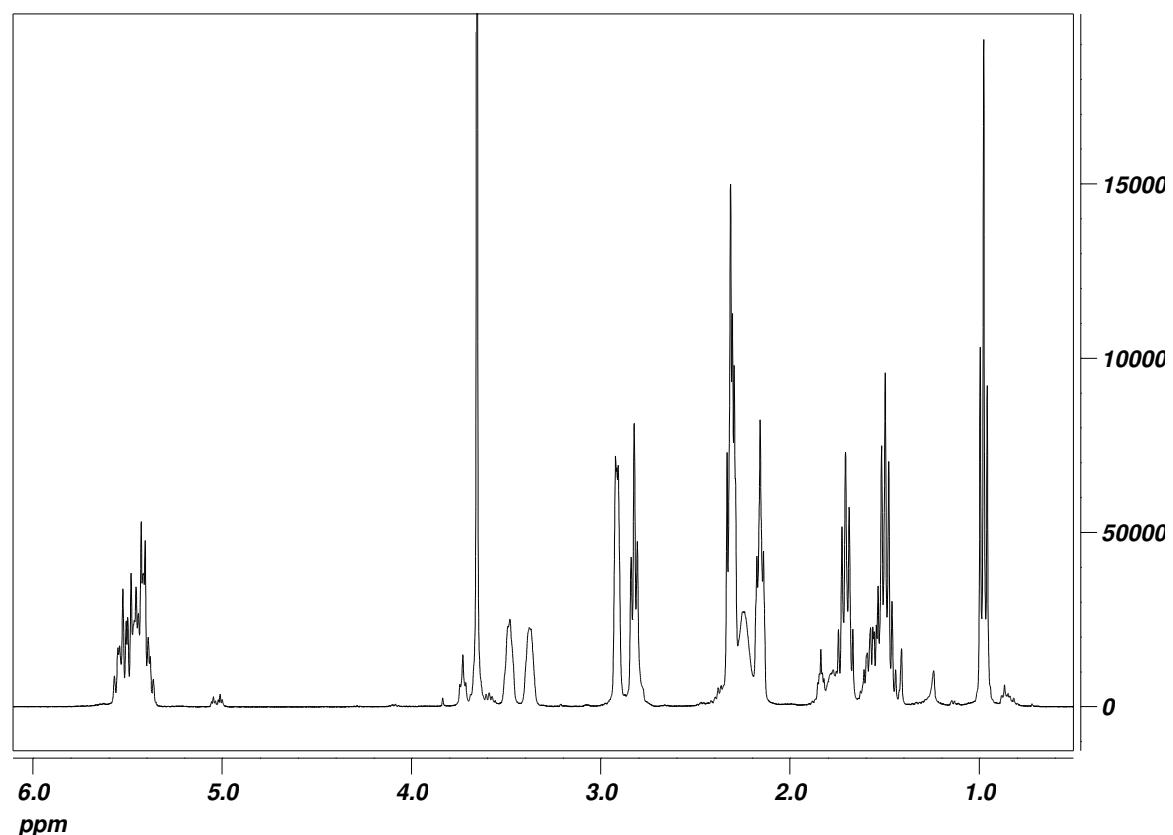
(9Z,12Z)-methyl 15,16-dihydroxyoctadeca-9,12-dien-6-ynoate (3)

Synthesis was prepared following a published procedure developed for the transformation of arachidonic acid.¹

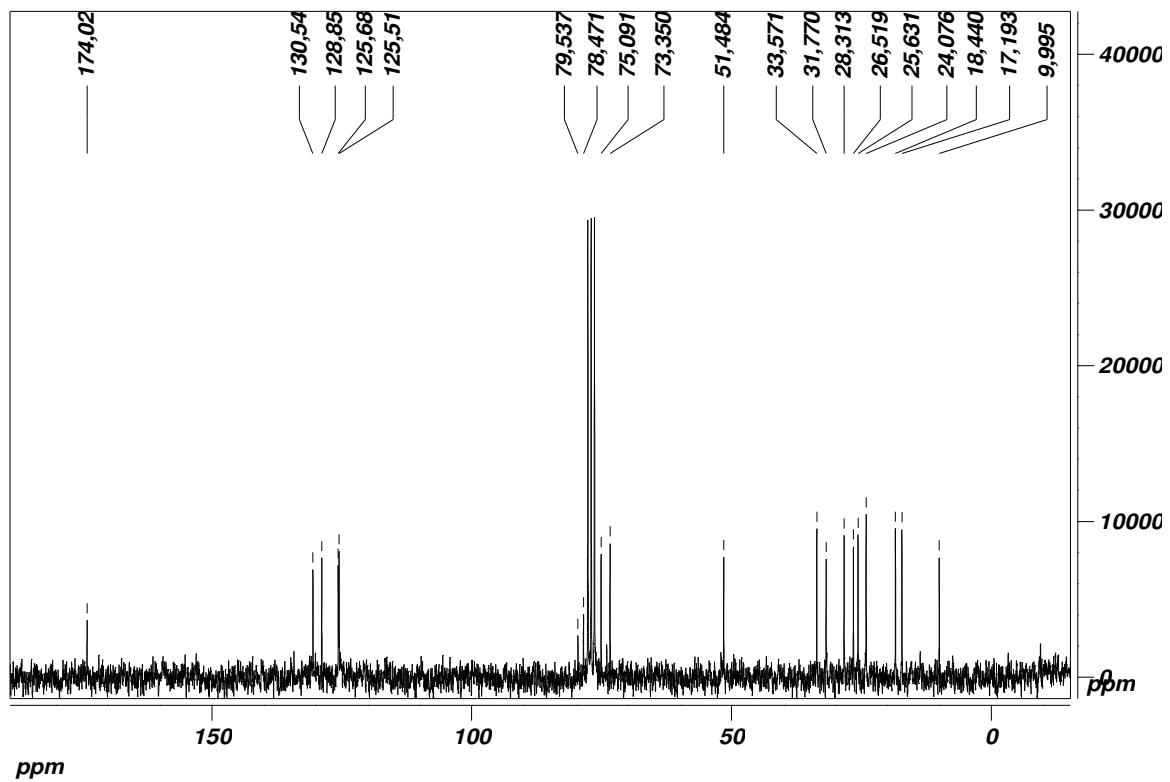
Yield: 52%

¹H NMR (400 MHz, *CDCl*₃): δ 5.67-5.31 (m, 4H), 3.65 (s, 3H), 3.52-3.44 (m, 1H), 3.41-3.34 (m, 1H), 2.94-2.87 (m, 2H), 2.82 (t, *J* = 6.51 Hz, 2H), 2.35-2.27 (m, 4H), 2.19-2.13 (m, 2H), 1.76-1.64 (m, 2H), 1.63-1.39 (m, 4H), 0.98 (t, *J* = 7.43 Hz, 3H)

¹³C NMR (101 MHz, *CDCl*₃): δ 174.0, 130.5, 128.8, 125.7, 125.5, 79.5, 78.5, 75.1, 73.3, 51.5, 33.6, 31.8, 28.3, 26.5, 25.6, 24.1, 18.4, 17.2, 9.9



¹H-NMR spectrum of (9Z,12Z)-methyl 15,16-dihydroxyoctadeca-9,12-dien-6-ynoate (3)



¹³C-NMR spectrum of (9Z,12Z)-methyl 15,16-dihydroxyoctadeca-9,12-dien-6-yneate (**3**)

(9Z,12Z)-methyl 15-oxopentadeca-9,12-dien-6-ynoate (4)

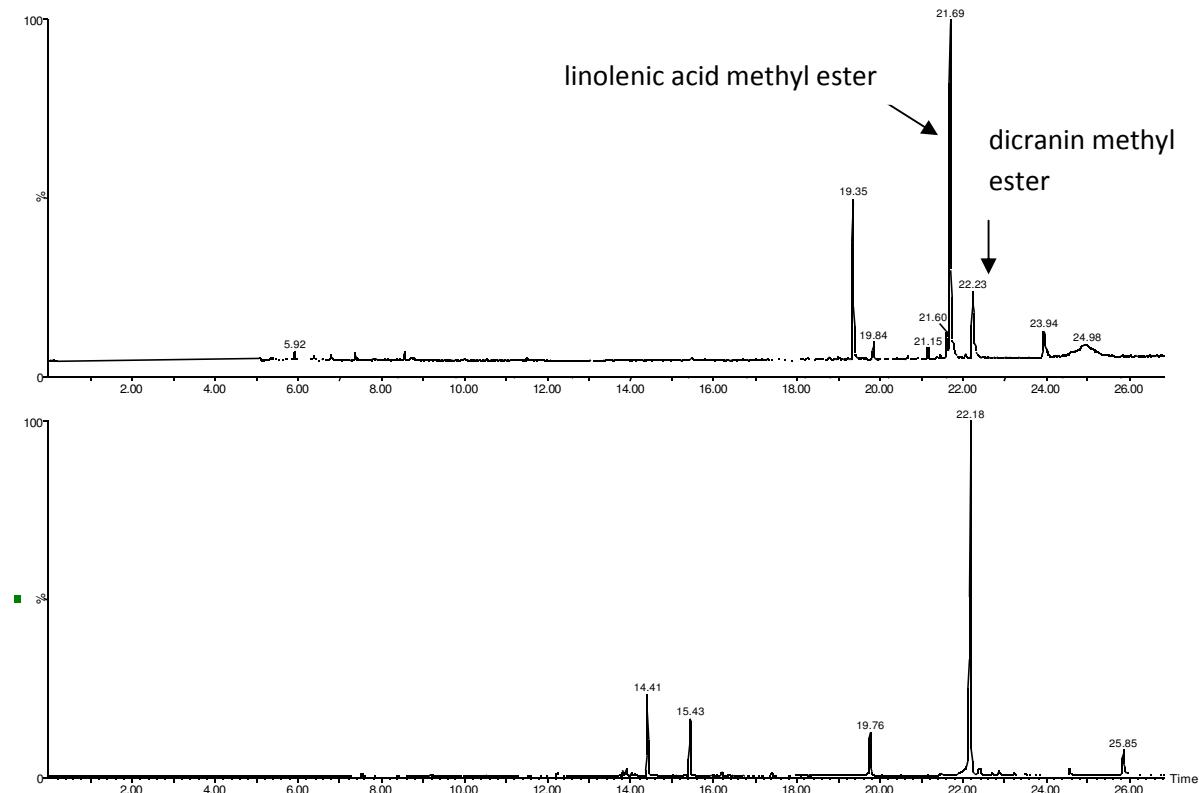
Synthesis was performed following a published procedure developed for the transformation of arachidonic acid.¹ The product was used for the Wittig reaction without further purification.

[16,17,17,18,18,18-²H₆]dicranin methyl ester (6)

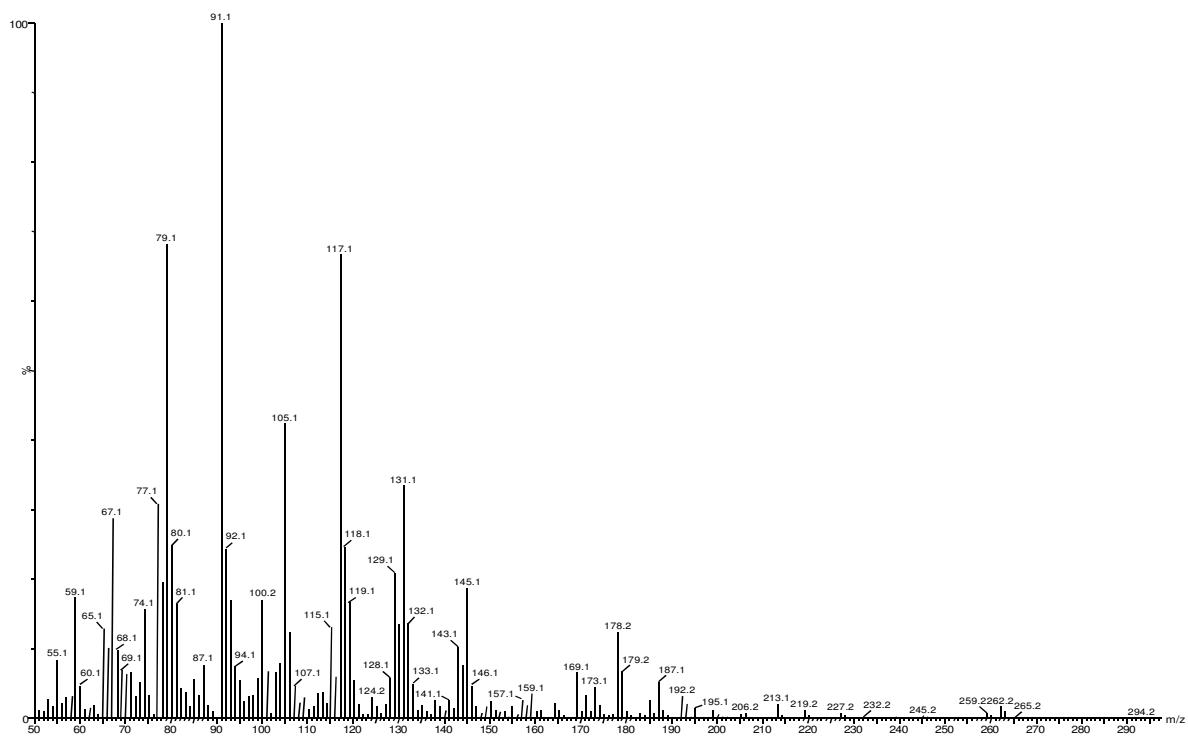
Synthesis was prepared following a published procedure developed for the transformation of arachidonic acid.¹

Yield: 8% (8 mg) over two steps.

EI-pos-HRMS: *m/z* calcd. for C₁₉H₂₂D₆O₂: 294.2466; found: 294.2461



GC-MS profile of fatty acid methyl esters of *D. scoparium* (upper panel RT 22.23 min) and purified [16,17,17,18,18,18-²H₆]dicranin methyl ester (lower panel RT 22.18 min)

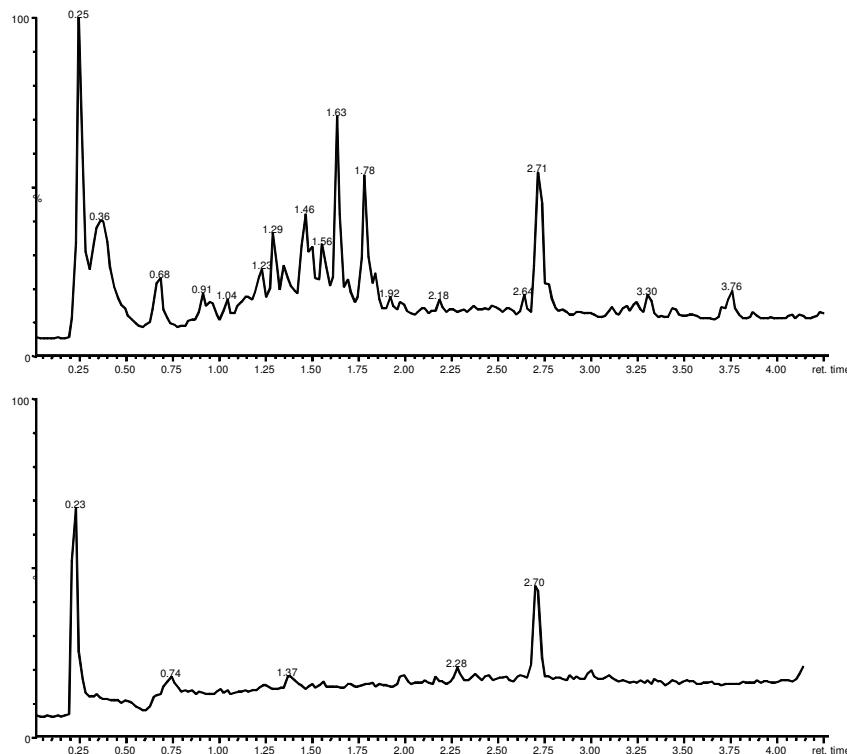


EI-pos-MS spectrum of [16,17,17,18,18,18-²H₆]dicranin methyl ester (**6**)

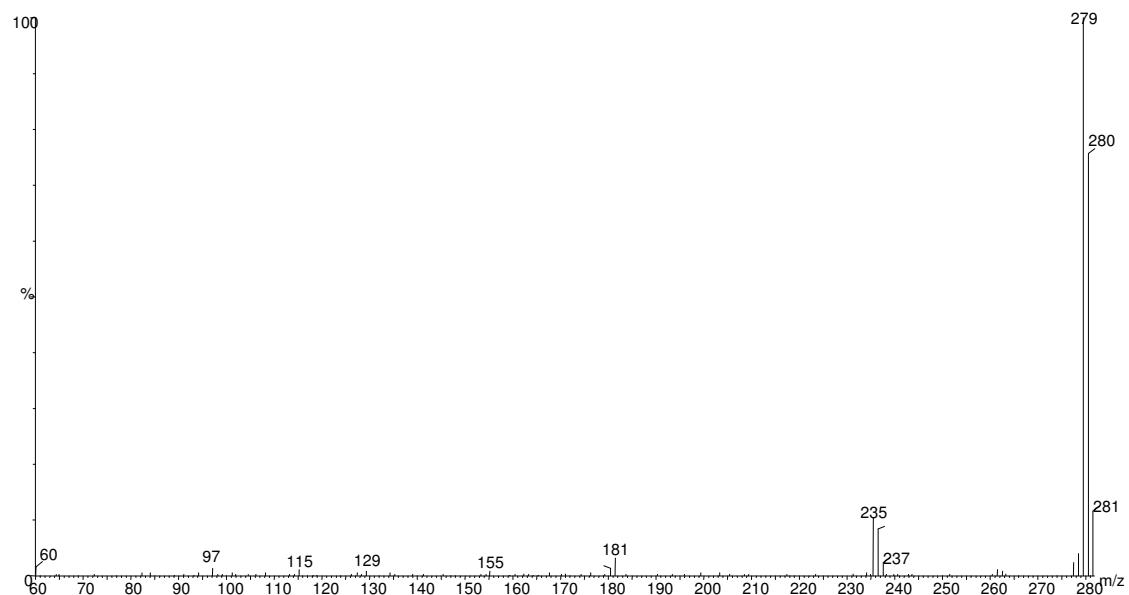
[16,17,17,18,18,18- 2 H₆]dicranin (7a)

Synthesis was performed according to a published saponification procedure.²

Yield: 69% (5 mg)



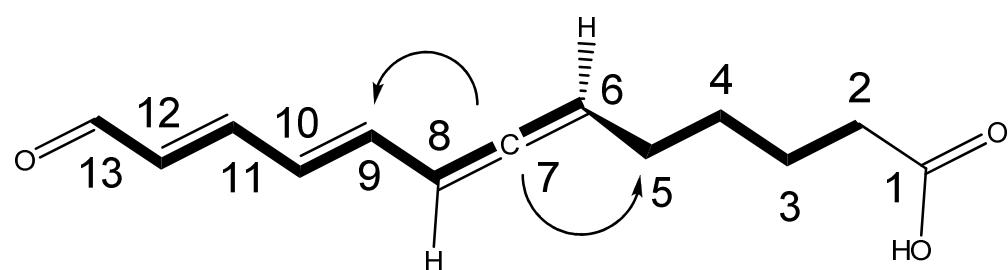
LC-ESI (neg) Chromatogram of extracted dicranin from *D. scoparium* (upper panel RT 2.71 min) and saponified [16,17,17,18,18,18- 2 H₆]dicranin (lower panel RT 2.70 min)



LC-ESI-MSMS (neg) spectrum of [16,17,17,18,18,18- 2 H₆]dicranin (7a)

(S,9E,11E)-13-oxotrideca-6,7,9,11-tetraenoic acid (8)

6.7 kg of freshly collected *D. scoparium* was used to obtain about 2 kg of phyllodes with minor remaining stem parts by cutting of the green parts with a pair of scissors. The biomass was frozen in liquid nitrogen, crushed in the cold and warmed up to room temperature. After 30 min at room temperature, the moss tissue was transferred into brown glass bottles and extracted twice with 5 l ethyl acetate under an inert atmosphere of argon. The liquid phase was filtered off quickly. The solvent was removed at 0°C under reduced pressure and the residue was then immediately taken up in a mixture of 200 ml of acetonitrile/H₂O (1/1 v/v). The precipitated chlorophyll-rich fraction was removed by centrifugation at 6000 rpm and discarded. The supernatant was then concentrated under reduced pressure and the residue (ca. 8 g) was pre-purified by flash-column chromatography on silica gel (solvent system: chloroform / isopropanol / acetone 80/20/15 (v/v/v)). The oxylipin-rich fraction (verified by UPLC-MS) was then evaporated and portions of the residue (5 g) were separated by semipreparative HPLC (solvent A: water acidified with 0.1% formic acid (v/v).; solvent B: acetonitrile. Gradient: 0 min at 25 % B; 18 min 60 % B; 22 min to 80 % B; 24 min to 100 % B; 29 min 100 % B; 32 min 25 % B). All fractions of the separation steps were immediately transferred into brown glass vials and stored in the freezer. The semipreparative HPLC fraction containing the allenic oxylipin was further purified by analytical HPLC on a Syngi MAX-RP column (Solvent A: water acidified with 0.1% formic acid (v/v) and 1% acetonitrile (v/v); solvent B: acetonitrile. Gradient: 0 min at 10 % B; 10 min 60 % B; 15 min to 100 % B; 18 min at 100 % B; 21 min 10 % B). Pure fractions were concentrated in the cold, taken up in chloroform and immediately submitted to NMR analysis. In total 7 mg of (S,9E,11E)-13-oxotrideca-6,7,9,11-tetraenoic acid were obtained.



Curly arrow indicate HMBC correlations

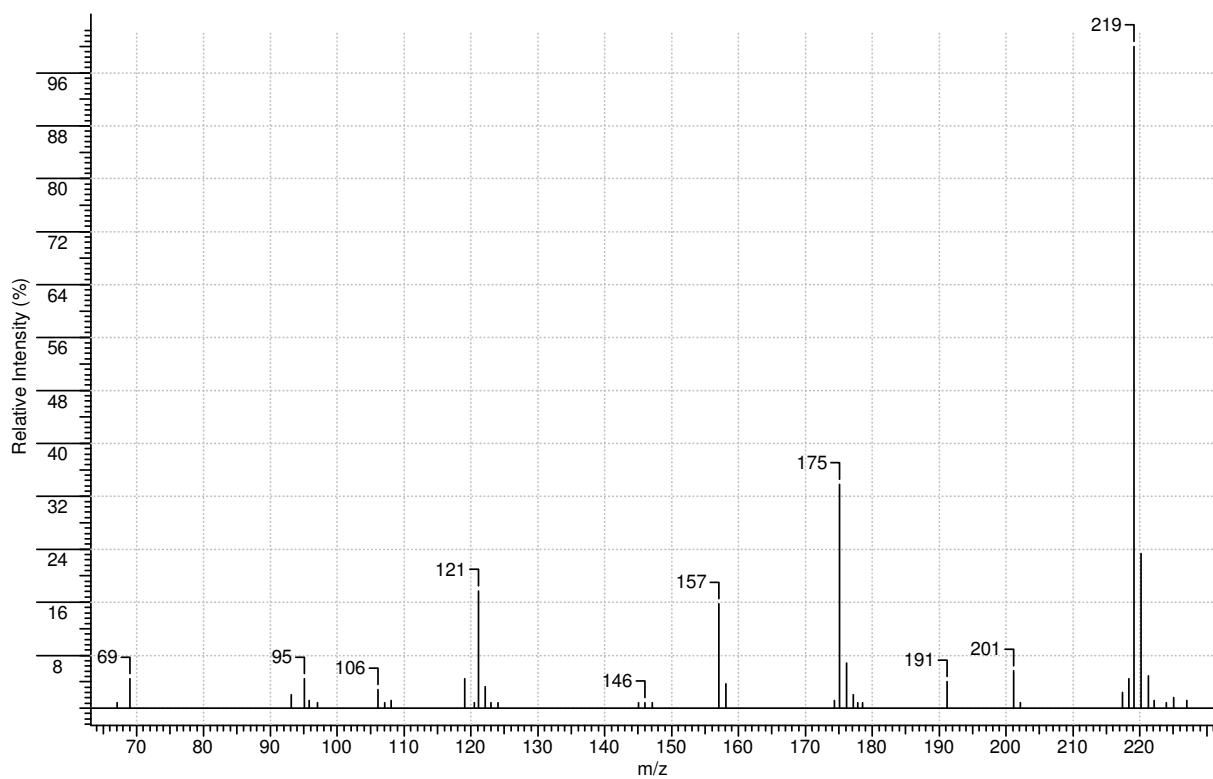
— indicate ¹H, ¹H-COSY correlations

¹H NMR (500 MHz, CDCl₃) δ 9.55 (d, *J* = 8.0 Hz; H13), 7.12 (dd, *J* = 15.2, 11.0 Hz; H11), 6.41 (dd, *J* = 15.0, 11.1 Hz; H10), 6.54 (dd, *J* = 15.0, 10.7 Hz; H9), 6.13 (dd, *J* = 15.2, 8.0 Hz; H12), 5.95 (m, H8), 5.45 (m; H6), 2.11 (m; H5), 1.55-1.47 (m; H4), 1.70 (m; H3), 2.38 (m; H2)

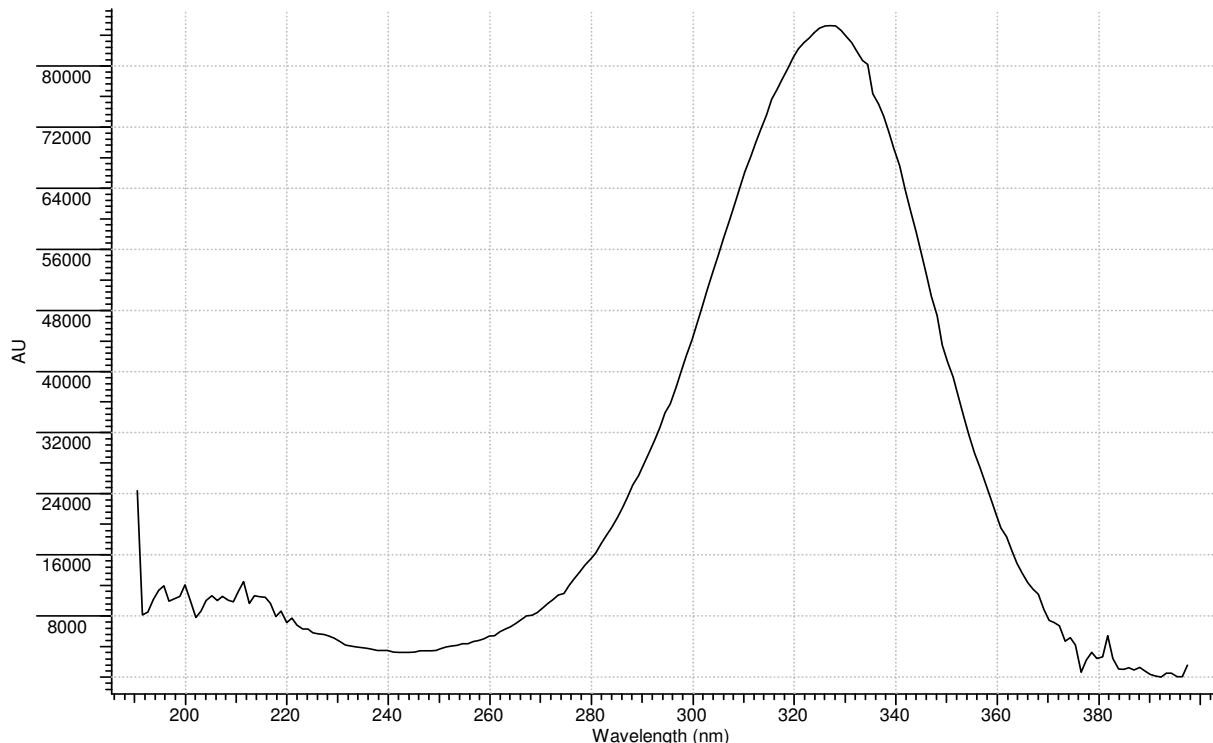
¹³C NMR (126 MHz, CDCl₃ [indirect from HSQC and HMBC]) δ 210.5 (C7); 193.9 (C13); 177.7 (C1); 151.7 (C11); 140.1 (C10); 131.1 (C12); 125.7 (C9); 94.5 (C8); 92.9 (C6); 20.9 (C5); 27.8 (C4); 23.8 (C3); 33.5 (C2)

ESI-neg-HRMS: *m/z* calcd. for [M-H]⁻ C₁₃H₁₅O₃: 219.1021; found: 219.1019

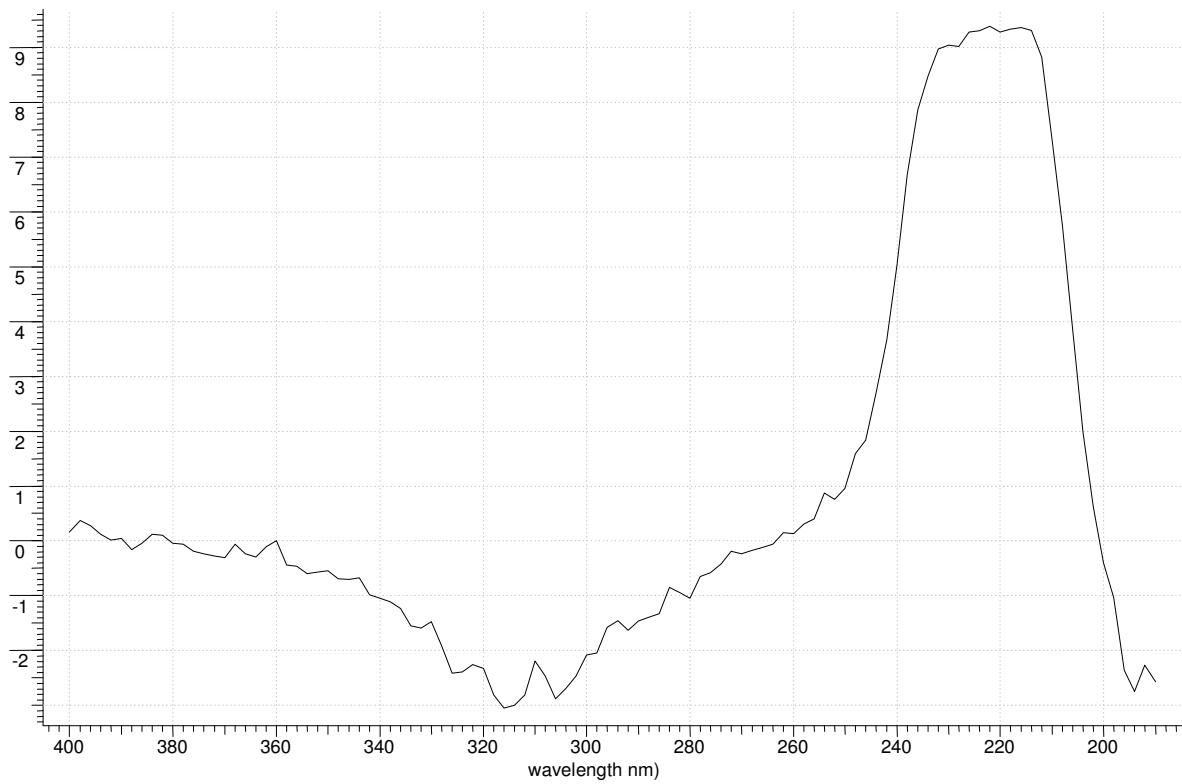
The optical rotation is [α]²⁰_D 65.2° [c=2 mg/ml, acetonitrile]



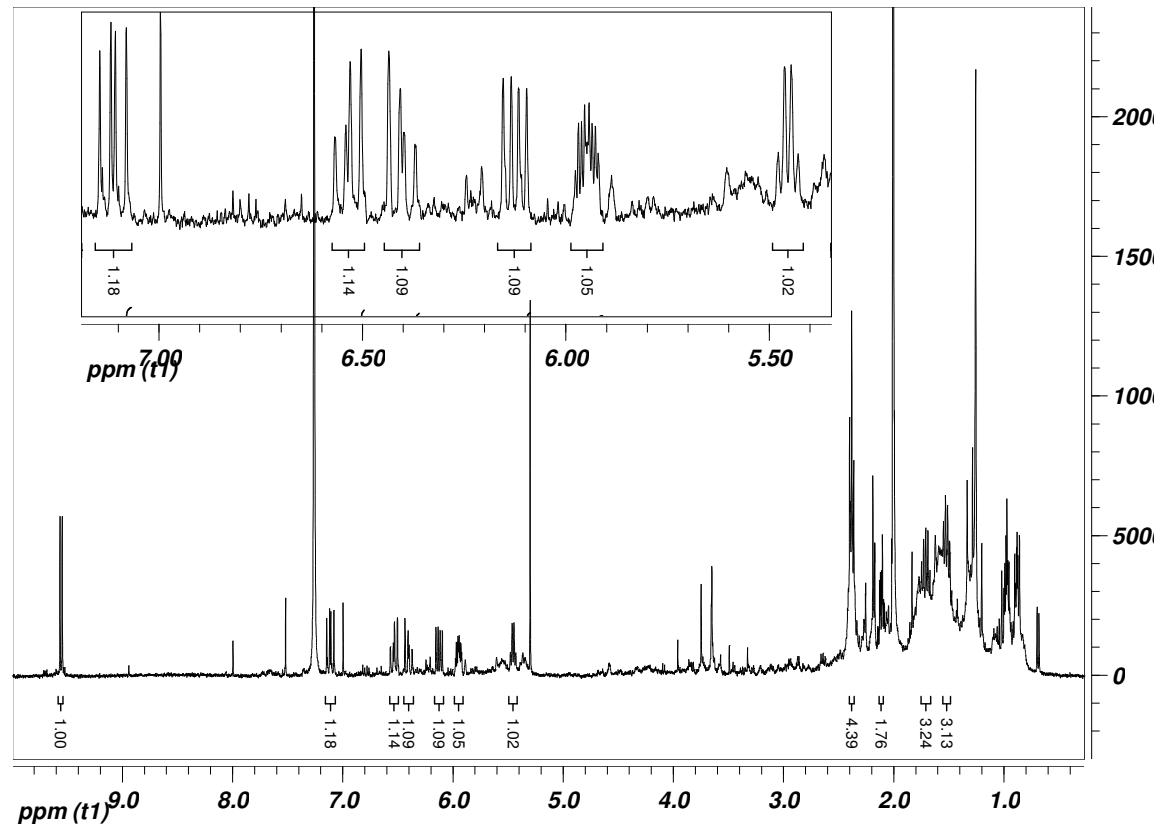
LC-ESI-mass spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**)



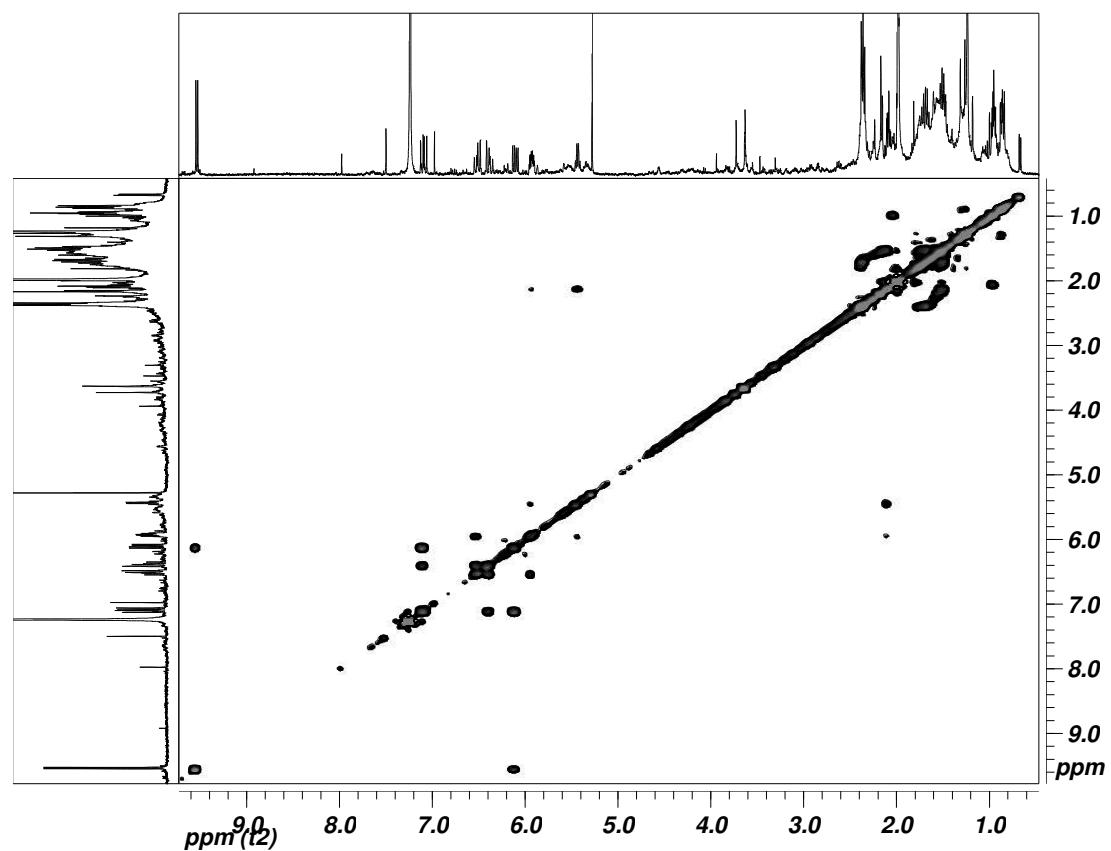
UV-DAD-spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**) in water / acetonitrile / formic acid 53/47/0.1.



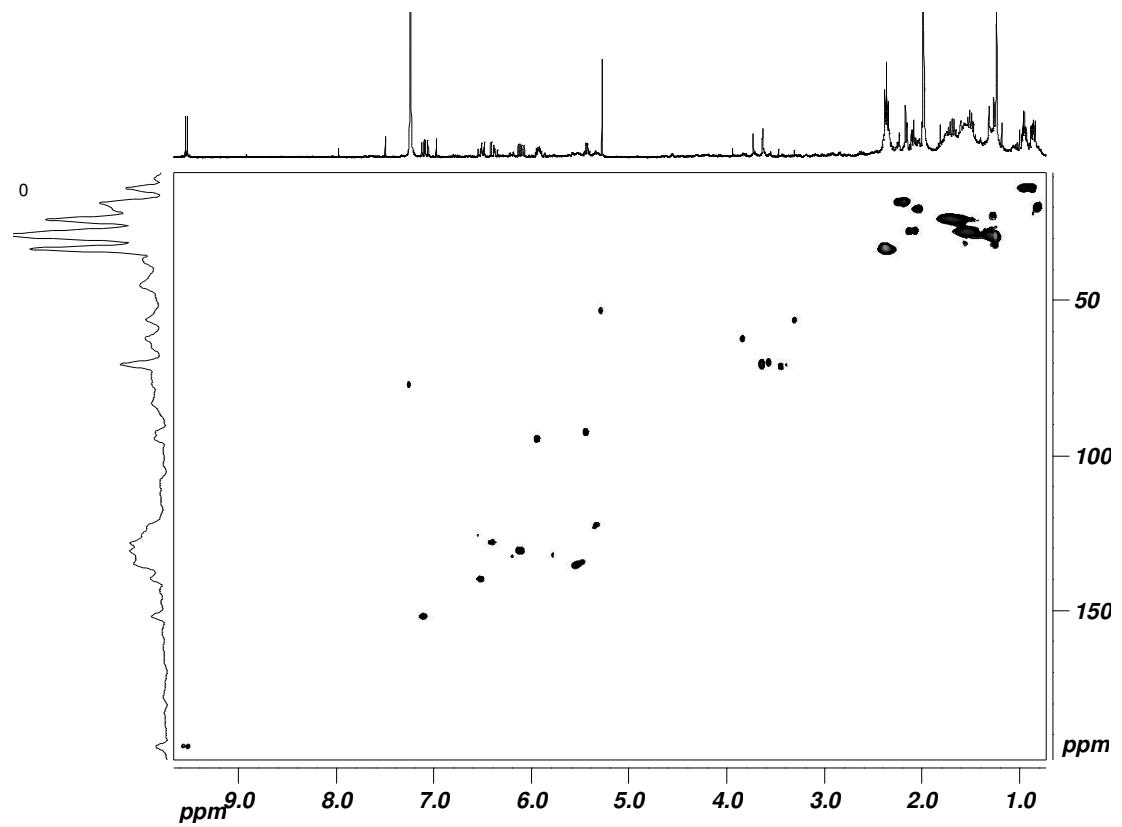
CD-spectrum of (S,9E,11E)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**)



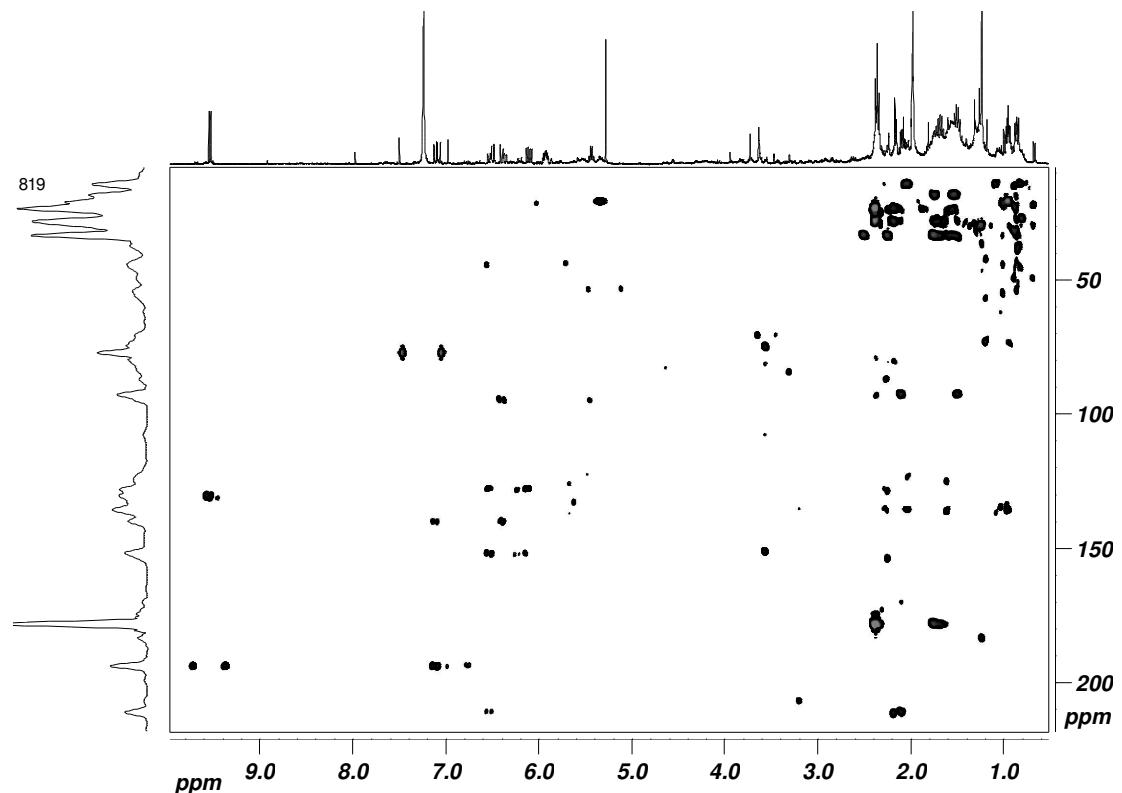
^1H -NMR spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**)



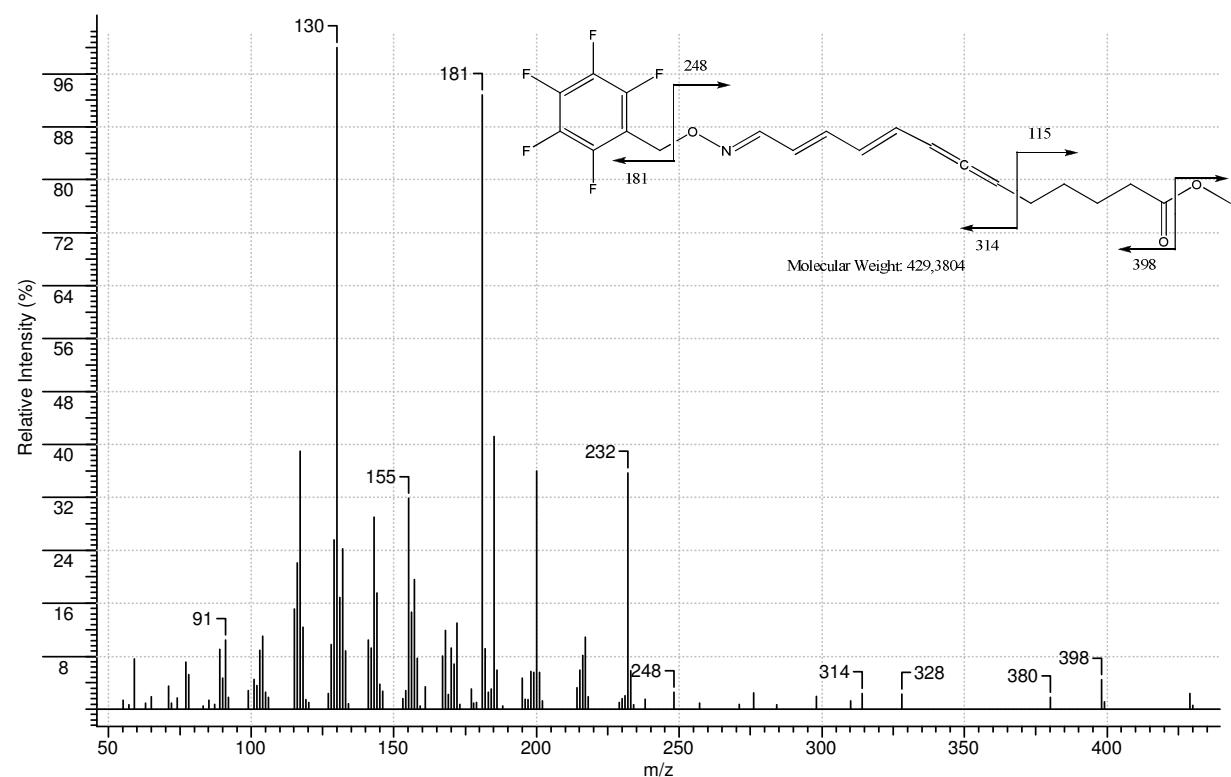
^1H , ^1H COSY spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**)



HSQC spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**)



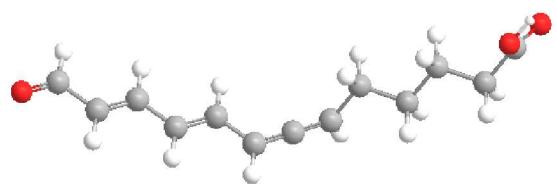
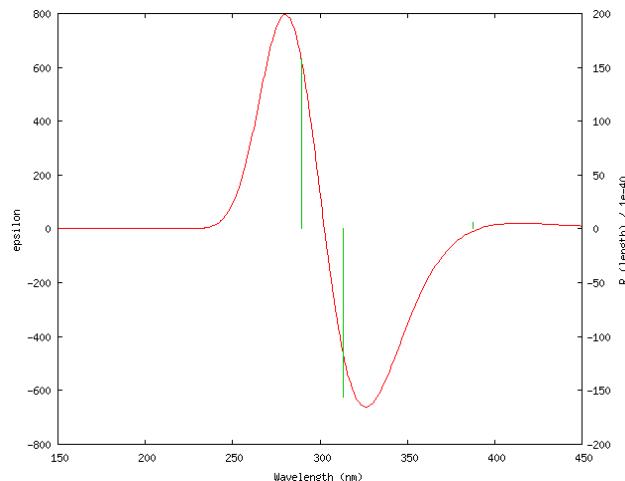
HMBC spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**)



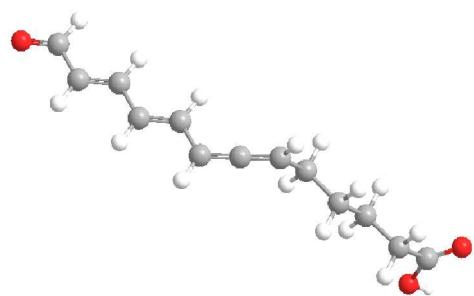
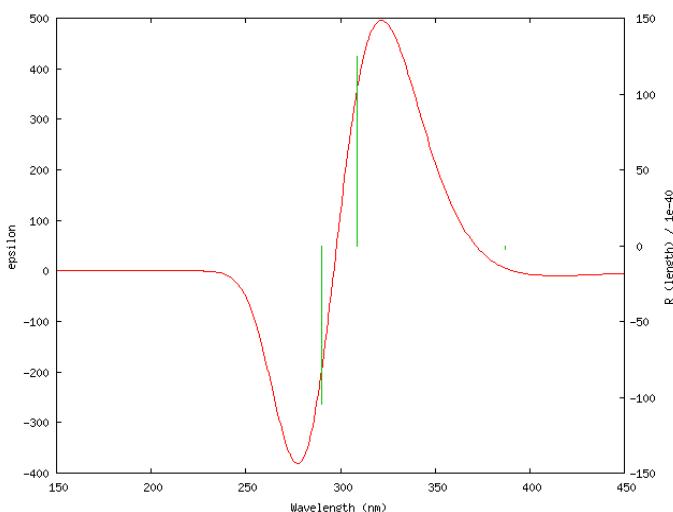
GC-MS spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid (**8**) after derivatisation with 1) TMSDM (trimethylsilyl diazomethane) and 2) PFBHA (pentafluorhydroxylamine hydrochloride)

CD-Spectra calculations for the stereoisomers of (*9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid

All calculations were performed according to³.



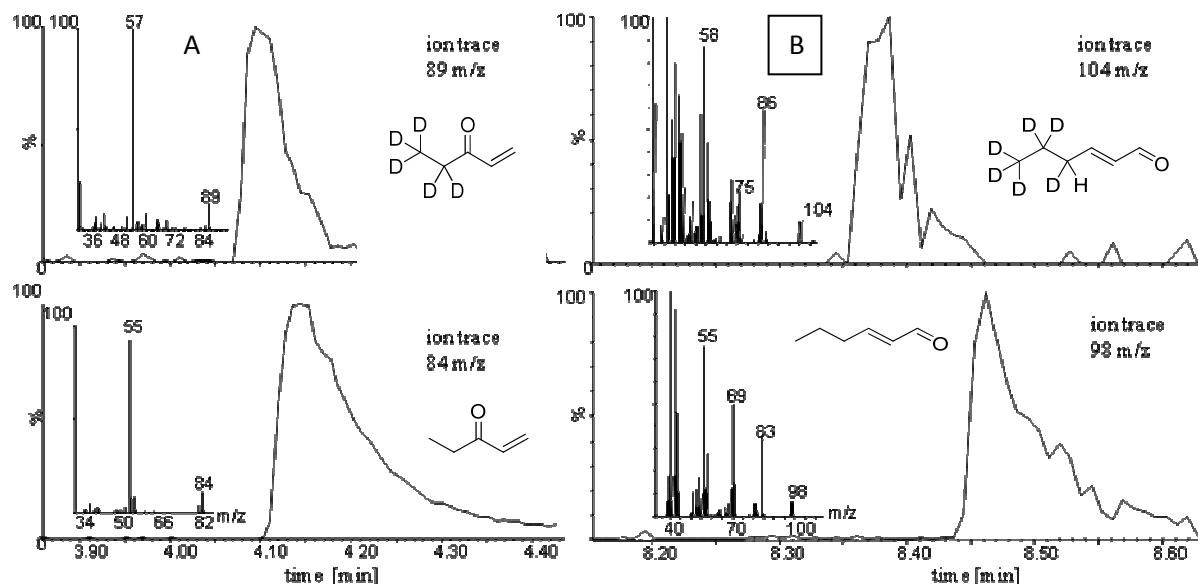
Calculated CD-spectrum of (*S,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid



Calculated CD-spectrum of (*R,9E,11E*)-13-oxotrideca-6,7,9,11-tetraenoic acid

Incorporation experiments with [16,17,17,18,18,18-²H₆]dicranin (7a)

Incorporation experiments were performed according to ⁴.



GC/MS profiles of SPM extracts after application of [16,17,17,18,18,18-²H₆]dicranin (7a) to mechanically wounded *D. scoparium*. The inserts show the background subtracted EI-mass spectra. Comparison of retention time and mass spectra of A) [²H₅]pentenone and unlabelled pentenone and B) [²H₆]hexenal and hexenal are shown

References:

¹ Hombeck, M.; Boland, W., Biosynthesis of the algal pheromone fucoserratene by the freshwater diatom *Asterionella formosa* (Bacillariophyceae). *Tetrahedron* **1998**, 54, (37), 11033-11042.

² Pohnert, G.; Adolph, S.; Wichard, T., Short synthesis of labeled and unlabeled 6Z,9Z,12Z,15-hexadecatetraenoic acid as metabolic probes for biosynthetic studies on diatoms. *Chemistry and Physics of Lipids* **2004**, 131, (2), 159-166.

³ Rempt, M.; Pohnert, G., Novel acetylenic oxylipins from the moss *Dicranum scoparium* with antifeeding activity against herbivorous slugs. *Angewandte Chemie-International Edition* **2010**, 49, (28), 4755-4758.

⁴ Croisier, E.; Rempt, M.; Pohnert, G., Survey of volatile oxylipins and their biosynthetic precursors in bryophytes. *Phytochemistry* **2010**, 71, (5-6), 574-580.