SUPPORTING INFORMATION

From the configurational preference of dihydroceramide desaturase 1 towards Δ⁶-unsaturated substrates to the discovery of a new Des1 inhibitor

Ana Pou,⁰ José Luis Abad,⁰ Yadira F. Ordóñez,⁰ María Garrido,⁰ Josefina Casas,⁰ Gemma Fabriàs*° and Antonio Delgado*°°

⁰. Spanish National Research Council (CSIC), Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Research Unit on Bioactive Molecules (RUBAM), Department of Biomedicinal Chemistry, Jordi Girona 18-26; 08034-Barcelona, Spain

°. University of Barcelona (UB), Faculty of Pharmacy and Food Sciences, Department of Pharmacology, Toxicology and Medicinal Chemistry, Unit of Pharmaceutical Chemistry (Associated Unit to CSIC), Avda. Joan XXIII s/n, 08028-Barcelona, Spain

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General Methods

Dry solvents were obtained by passing through an activated alumina column on a Solvent Purification System (SPS). Methanol was dried over CaH$_2$ and distilled prior to use. Commercially available reagents and solvents were used with no further purification. All reactions were monitored by TLC analysis using ALUGRAM® SIL G/UV$_{254}$ precoated aluminum sheets (Machery-Nagel). UV light was used as the visualizing agent and a 5% (w/v) ethanolic solution of phosphomolybdic acid as the developing agent. Flash column chromatography was carried out with the indicated solvents using flash-grade silica gel (37-70 µm) using the indicated eluting system (in % v/v). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated.

NMR spectra were recorded at room temperature on a Varian Mercury 400 instrument. The chemical shifts (δ) are reported in ppm relative to the solvent signal, and coupling constants (J) are reported in Hertz (Hz). For $^{13}$C NMR spectra recorded in D$_2$O, a 0.5 % (w/v) solution of DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) in D$_2$O was used as external reference (0 ppm). The following abbreviations are used to define the multiplicities in $^1$H NMR spectra: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet, br = broad signal and. High resolution mass spectrometry (HRMS) analyses were carried out on an Acquity UPLC system coupled to a LCT Premier orthogonal accelerated time-of-flight mass spectrometer (Waters) using electrospray ionization (ESI) technique. Optical rotations were measured at room temperature on a Perkin Elmer 341 polarimeter at 25 °C in 1-dm 1-mL cell, using a sodium light lamp (λ = 589 nm). Specific optical rotations values [α]$_D$ are expressed in 10$^{-1}$·deg·cm$^3$·g$^{-1}$, and concentrations (c) are reported in g/100 mL of solvent.

HPLC analyses were performed with an Alliance apparatus coupled to a fluorescence detector using a C18 column (Kromasil, 100 C18, 5µm, 15x0.40 cm, Tracer) precolumn equipped (precolumn ODS, Tracer). Compounds were eluted with 30% H$_2$O and 70% acetonitrile (v/v), flowing at 1 mL/min. The detector was set at an excitation wavelength of 465 nm and measure the emission wavelength at 530 nm. Each sample was run for up to 20 minutes.
1. Synthesis of (E)-Δ⁶ ceramides

Scheme S1. Synthesis of (E)-Δ⁶ ceramides: (a) 3-butenylMgBr, THF; (b) (R)(-)MPA, EDC, DMAP, CH₂Cl₂; (c) n-tridene, Grubbs, 2nd generation, CH₂Cl₂; (d) K₂CO₃, MeOH; (e) AcCl, MeOH; (f) C6NBD acid, HOBt, EDC, CH₂Cl₂; (g) octanoic acid, HOBt, EDC, CH₂Cl₂.

(4S)-tert-butyl 4-(1-hydroxypent-4-en-1-yl)-2,2-dimethyl oxazolidine-3-carboxylate (9)

3-Butenylmagnesium bromide (0.5M solution in THF, 6.15 mL, 3.1 mmol) was added dropwise to a cooled solution of Garner's aldehyde¹ (500 mg, 2.1 mmol) in anhydrous THF (7 mL) at -78 °C. The reaction mixture was stirred at that temperature for 2 h and then allowed to warm to rt. Next, saturated aqueous NH₄Cl was carefully added. The aqueous phase was extracted with Et₂O (3 x 15 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography (hexane/EtOAc 80:20) to give 376 mg (1.31 mmol, 76%) of 9 as an inseparable mixture of diastereomers.

¹H NMR (δ, 400 MHz, CDCl₃, mixture of diastereomers (2S, 3R,S): 5.88-5.76 (m, 1H), 5.03 (d, J= 17.0 Hz, 1H), 4.95 (d, J= 9.5 Hz, 1H), 4.15-3.63 (m, 4H), 2.31-2.26 (m, 1H), 2.22-2.08 (m, 1H), 1.67-1.35 (m, 17H).

¹³C NMR (δ, 101 MHz, CDCl₃, mixture of diastereomers): 155.8, 138.1, 115.2, 99.2, 79.5, 74.0, 70.6, 65.5, 33.7, 30.9, 29.9, 29.0, 28.5.


Analytical data match those reported for this compound in the literature.²

(5S)-tert-butyl 4-((R)-1-((R)-2-methoxy-2-phenylacetoxy)pent-4-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (10)
A solution of (R)-α-methoxy-α-phenylacetic acid (58 mg, 0.35 mmol), EDC (34 mg, 0.18 mmol) and DMAP (22 mg, 0.18 mmol) in dry CH₂Cl₂ (3 mL) was added dropwise to a solution of 9 (50 mg, 0.18 mmol) in CH₂Cl₂ (2 mL) under argon. The reaction mixture was stirred at rt for 7 hours. The organic layer was sequentially washed with 1N HCl, sat NaHCO₃, and water (2 x 1mL each), then dried over MgSO₄ and concentrated under reduced pressure affording crude 10 as an approximate 4:1 mixture of two diastereomers. After careful chromatographic purification with hexane/EtOAc 90:10, major diastereomer (2S,3R)(R)-10 was isolated in a 57% yield. Following the methodology of Riguera,³ the absolute configuration at C3 was assigned as R.

Rᵣ(2S,3R)(R): 0.54; Rᵣ(2S,3S)(R): 0.40

¹H NMR (δ, 400 MHz, CDCl₃. peaks assigned by HSQC): (S,R,R) 7.41-7.37 (m, 2H), 7.31-7.23 (m, 3H), 5.77-5.62 (m, 1H, C6H), 5.21 (br, 1H, C3H), 4.94-4.87 (m, 2H, C7H₂), 4.70 (s, 1H, CαH), 3.93-3.51 (m, 3H, C2H+C1H₂), 3.36 (s, 3H, OMe), 2.07-1.88 (m, 2H, C5H₂), 1.66-1.52 (m, 2H, C4H₂), 1.40 (s, 9H, 2xC9H₃+1xC12H₃)(*), 1.32 (broad, 1H, 1xC12H₃)(*); ¹³C NMR (δ, 101 MHz, CDCl₃): 170.0 (C12), 152.9+151.9 (C10)(*), 137.5+137.0 (C6)(*), 136.1 (Cq arom), 128.7, 128.5, 127.3 (C arom), 115.5+115.0 (C7)(*), 94.2+93.7 (C8)(*), 82.8 (Ca), 80.3 (C11), 73.6+73.1 (C3)(*), 63.5 (C1), 59.2 (C2), 57.3 (OMe), 31.5+31.0 (C4)(*), 29.6 (C5), 28.3 (2xC9 + 1xC12), 26.8+25.9 (1xC12)(*), 24.3+23.0 (1xC12)(*)

(*): signal splitting due to rotameric equilibria; see NMR traces for numbering

HRMS (ESI): calculated for C₂₄H₃₆NO₆[M+H]+ 434.2543; found 434.2540.

(S)-tert-butyl 4-((R,E)-1-((R)-2-methoxy-2-phenylacetoxy)hexadec-4-en-1-yl)-2,2-dimethoxazolidine-3-carboxylate (11)

A two necked round bottom flask fitted with a reflux condenser under argon atmosphere, was charged with 10 (550 mg, 1.26 mmol) and n-tridecene (1.92 mL, 8.11 mmol) in previously degassed CH₂Cl₂ (15 mL). Next, Grubb's 2nd generation catalyst (68 mg, 0.08 mmol) was added portionwise, and the resulting mixture was stirred at reflux temperature for 5 h. The mixture was next allowed to cool down to rt and concentrated in vacuo. Flash chromatography of the crude in hexane/EtOAc 8:2 afforded 11 (393 mg, 53%) as a mixture of two isomers (d.r. (E:Z) = 6:1; major E-isomer: Rᵣ = 0.53)
¹H NMR (δ, 400 MHz, CDCl₃) for major E isomer: 7.43-7.39 (m, 2H), 7.34-7.26 (m, 3H), 5.37-5.23 (m, 4H), 4.72 (s, 1H), 3.95-3.52 (m, 4H), 3.38 (s, 3H), 1.97-1.85 (m, 4H), 1.62-1.50 (m, 2H), 1.42 (s, 9H), 1.39-1.15 (m, 22H), 0.85 (m, 3H).

¹³C NMR (δ, 101 MHz, CDCl₃) for major E isomer: 170.1, 152.8/151.6 (rotamers), 135.9, 131.2, 130.8, 128.7/128.5 (rotamers), 127.3, 94.3/93.7 (rotamers), 82.9, 80.3, 73.8 (broad due to rotamers), 63.5, 59.2, 57.4, 31.9, 29.6-29.5 (12 C), 28.3, 27.2, 14.1

HRMS (ESI): calculated for C₃₅H₅₈NO₆ [M+H]+ 588.4264; found 588.4273.

(S)-tert-butyl 4-((R,E)-1-hydroxyhexadec-4-en-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (12)

To a solution of 11 (389 mg, 0.66 mmol) in MeOH (10 mL), was added K₂CO₃ (281 mg, 2.03 mmol). The mixture was stirred for 6 h at rt. Next, MeOH was concentrated, and the residue was diluted with EtOAc, washed with water and brine, dried over MgSO₄, filtered, and concentrated in vacuo. Purification of the crude with hexane/EtOAc 8:2 afforded 12 (Rᶠ = 0.45, 75% yield) as a colorless oil.

¹H NMR (δ, 400 MHz, CDCl₃): 5.48-5.33 (m, 2H), 4.12-3.63 (m, 5H), 2.20 (brs, 2H), 2.12-1.99 (m, 2H), 1.98-1.90 (m, 2H), 1.55 (brs, 3H), 1.52-1.38 (s, 12H), 1.33-1.17 (m, 18H), 0.85 (m, 3H).

¹³C NMR (δ, 101 MHz, CDCl₃): 131.4, 129.5, 94.3, 72.3, 64.6, 62.5, 32.6, 31.9, 29.8, 29.78, 29.72, 29.6, 29.5, 29.3, 29.1, 29.0, 28.3, 26.4, 22.6, 14.1.

HRMS (ESI): calculated for C₂₆H₅₀NO₄ [M+H]+ 440.3740; found 440.3752.

N-((2S,3R,E)-1,3-dihydroxyoctadec-6-en-2-yl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanamide (1)

To a solution of 12 (52 mg, 0.12 mmol) in MeOH (3 mL) was added acetyl chloride (0.2 mL) and the mixture was vigorously stirred at rt for 1 h. The reaction mixture was concentrated under reduced pressure and crude amino diol 13 was used in the next step without further purification.

A solution of EDC (36 mg, 0.19 mmol), HOBt (10 mg, 0.14 mmol) and C₆NBD acid (38 mg, 0.13 mmol) in anh CH₂Cl₂ (2 mL) was stirred under argon atmosphere at rt for 10 min, and next added dropwise to a solution of the above amino diol and Et₃N (40 µL, 0.24 mmol) in anh CH₂Cl₂ (2 mL). The reaction mixture was stirred at rt for 4 h under argon. The mixture
was next diluted by addition of CH₂Cl₂ (5 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried over MgSO₄ and filtered. Concentration under reduced pressure afforded a residue, which was purified by flash chromatography with hexane/EtOAc (9:1 to 7:3) followed by CH₂Cl₂/MeOH (100:0 to 97:3) to afford 1 (22 mg, 32% in two steps) as an orange solid; Rₜ = 0.40 (hexane/EtOAc 7:3).

¹H NMR (δ, 400 MHz, CDCl₃): 8.44 (d, J = 8.6 Hz, 1H), 6.88 (brs, 1H), 6.53 (brs, 1H), 6.14 (d, J = 8.5 Hz, 1H), 5.42 (dt, J = 16.5, 6.4 Hz, 2H), 4.01 (m, 1H), 3.87 (m, 1H), 3.77 (m, 2H), 3.49 (m, 1H), 2.29 (t, J = 6.6 Hz, 2H), 2.18 (dt, J = 14.2, 6.9 Hz, 1H), 2.08 (dt, J = 14.2, 6.9 Hz, 1H), 1.94 (q, J = 6.4 Hz, 2H), 1.86-1.69 (m, 4H), 1.65-1.45 (m, 4H), 1.34-1.14 (m, 18H), 0.85 (t, J = 6.7 Hz, 3H).

¹³C NMR (δ, 101 MHz, CDCl₃): 172.9, 144.2, 143.9, 136.5, 132.2, 128.9, 123.4, 98.6, 73.9, 62.4, 53.6, 43.6, 36.0, 34.2, 32.5, 31.8, 29.7, 29.6, 29.5 (2C), 29.3, 29.2, 27.8, 26.2, 24.7, 22.7, 14.1.


HPLC UV-FL: (70:30 ACN:H₂O isocratic) ret time: 16.4 min.

[α]²₀D = - 8 (c = 1.1, MeOH).

**N-((2S,3R,E)-1,3-dihydroxyoctadec-6-en-2-yl)octanamide (2)**

To a solution of 12 (52 mg, 0.12 mmol) in MeOH (3 mL) was added acetyl chloride (0.2 mL) and the mixture was vigorously stirred at rt for 1 h. The reaction mixture was concentrated under reduced pressure and the crude amino diol 13 was used in the next step without further purification.

A solution of EDC (36 mg, 0.19 mmol), HOBt (10 mg, 0.14 mmol) and octanoic acid (55 mg, 0.13 mmol) in anhs CH₂Cl₂ (2 mL) was stirred under argon atmosphere at rt for 10 min, and next added dropwise to a solution of the crude amino diol 13 and Et₃N (40 µL, 0.24 mmol) in anh CH₂Cl₂ (2 mL). The reaction mixture was stirred at rt for 4 h under argon. The mixture was next diluted by addition of CH₂Cl₂ (5 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried over MgSO₄, and filtered. Concentration under reduced pressure afforded a residue, which was purified by flash chromatography with CH₂Cl₂/MeOH (100:0 to 93:7) to afford 2 (30 mg, 60%) as a yellow oil; Rₜ = 0.35, hexane/EtOAc 7:3.
¹H NMR (δ, 400 MHz, CDCl₃): 6.15 (d, J=8.0 Hz, 1H), 5.52-5.34 (m, 2H), 3.98 (t, J=6.0 Hz, 1H), 3.95-3.89 (m, 1H), 3.88-3.75 (m, 2H), 2.66 (brs, 1H), 2.59 (brs, 1H), 2.24 (t, J=8.0 Hz, 2H), 2.10 (q, J=7.0 Hz, 2H), 1.97 (q, J=8.5 Hz, 2H), 1.70-1.49 (m, 4H), 1.41-1.14 (m, 26H), 0.88 (t, J=7.0 Hz, 6H).

¹³C NMR (δ, 101 MHz, CDCl₃): 174.1, 132.0, 129.2, 72.9, 65.6, 53.4, 37.1, 34.2, 32.7, 32.1, 31.9, 29.9, 29.8, 29.7, 29.7, 29.4, 29.2, 29.0, 26.0, 22.9, 22.8, 14.3, 14.2.

HRMS (ESI): calcd. for C₂₆H₅₂NO₃ [M + H]+ 426.3947; found 426.3954.

[α]²⁰D = -1.3 (c = 1.2, CHCl₃).

2. Synthesis of (Z)-Δ⁶ ceramides

![Scheme S2](image)

Scheme S2. Synthesis of (Z)-Δ⁶ ceramides: (a) H₂, Rh cat, MeOH; (b) NaH, THF; (c) TBAF, TFH; (d) IBX, EtOAc; (e) BrPh₂PC₁₂H₂₅, BuLi, HMPA, THF; (f) pTsOH, H₂O-MeOH; (g) NaOH, EtOH; (h) C₆NBD acid, HOBt, EDC, CH₂Cl₂; (i) octanoic acid, HOBt, EDC, CH₂Cl₂

(S)-tert-butyl 4-((R)-4-((tert-butyldimethylsilyl)oxy)-1-hydroxybutyl)-2,2-dimethyloxazolidine-3-carboxylate (15)

Rhodium on alumina (36 mg, 18% weight) was added to a solution of 14 (200 mg, 0.5 mmol) in a freshly degassed MeOH (10 mL). The resulting mixture was vigorously stirred at rt for 3 h under H₂ (1 atm). The mixture was next filtered through a plug of Celite, and the solid was rinsed with MeOH (3 x 10 mL). The combined filtrates were concentrated in vacuo to afford 300 mg of a crude, which was purified by flash chromatography (8/2 hexane/EtOAc, R_f = 0.30) to give 201 mg (99%) of 15 as a yellow oil.
¹H NMR (δ, 400 MHz, CDCl₃): 4.11-3.77 (m, 4H), 3.69 (br, 1H), 3.61 (t, J = 4.9 Hz, 2H), 1.68-1.49 (m, 4H), 1.48-1.34 (m, 15H), 0.84 (s, 9H), 0.02 (s, 6H).

¹³C NMR (δ, 101 MHz, CDCl₃): 153.9, 94.1, 80.7, 64.5, 63.1, 62.3, 28.3, 26.4, 25.8, 18.2, -5.4.

HRMS (ESI): calcd. for C₂₀H₄₂NO₅Si [M + H]⁺ 404.2832; found 404.2835.

[α]²⁰D = -19.8 (c = 1.0, CHCl₃).

(1R,7aS)-1-(3-((tert-butyldimethylsilyl)oxy)propyl)-5,5-dimethylidihydro-1H-oxazolo[3,4-c]oxazol-3(5H)-one (16)

To a solution of RBM8-101 (370 mg, 0.9 mmol) in anhydrous THF (15 mL) was added to a suspension of NaH (370 mg, 9.2 mmol) in anhydrous THF (5 mL) at rt. The reaction mixture was vigorously stirred for 16 hours at 50 °C under argon. The reaction was next quenched by dropwise addition of aqueous sat. NaHCO₃ at 0 °C until H₂ evolution was not observed. The aqueous phase was next extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo to give a crude, which was purified by flash chromatography (80:20 hexane/EtOAc, Rf = 0.45,) to afford 258 mg (85%) of 16.

¹H NMR (δ, 400 MHz, CDCl₃): 4.61 (td, J = 8.3, 4.9 Hz, 1H), 4.29 (td, J = 8.5, 6.3 Hz, 1H), 3.90 (dd, J = 8.5, 6.2 Hz, 1H), 3.69 (t, 2H), 3.67-3.49 (m, 1H), 1.69 (s, 3H), 1.78-1.58 (m, 3H), 1.58-1.46 (m, 1H), 1.41 (s, 3H), 0.85 (s, 9H), 0.03 (s, 6H).

¹³C NMR (δ, 101 MHz, CDCl₃): 156.8, 94.7, 74.4, 63.5, 62.1, 61.2, 28.7, 28.2, 27.4, 25.9, 23.4, 18.2, -5.4.

HRMS (ESI): calcd. for C₁₆H₃₂NO₄Si [M + H]⁺ 330.2101; found 330.2117.

[α]²⁰D = -29.8 (c = 1.1, CHCl₃).

(1R,7aS)-1-(3-hydroxypropyl)-5,5-dimethylidihydro-1H-oxazolo[3,4-c]oxazol-3(5H)-one (17)

A solution of TBAF (0.61 mL, 0.61 mmol, 1M in THF) is added dropwise to a solution of 16 (200 mg, 0.61 mmol) in THF (5 mL) at 0°C. The reaction mixture was stirred for 1 h at rt. An excess of aqueous NH₄Cl was added and the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo
to afford 151 mg (86% yield) of 17, which was used in the next step without further purification.

\[ ^{1}H\text{ NMR (}\delta, 400\text{ MHz, CDCl}_{3}\text{): 4.65 (qd, }J = 8.8, 8.2, 4.8\text{ Hz, 1H), 4.34 (td, }J = 8.5, 6.2\text{ Hz, 1H), 3.93 (dd, }J = 8.5, 6.2\text{ Hz, 1H), 3.82-3.62 (m, 3H), 1.82-1.73 (m, 2H), 1.72 (s, 3H), 1.71-1.58 (m, 2H), 1.44 (s, 3H). } \]

\[ ^{13}C\text{ NMR (}\delta, 101\text{ MHz, CDCl}_{3}\text{): 156.8, 94.8, 74.5, 63.6, 61.8, 61.2, 28.8, 28.1, 27.3, 23.4. } \]

HRMS (ESI): calcd. for C_{10}H_{17}NNaO_{4}[M + Na]^+ 238.1055; found 238.1048.

\[ [\alpha]^{20}_{D} = -25.7 (c = 1.09, \text{ CHCl}_{3}). \]

3-((1R,7aS)-5,5-dimethyl-3-oxotetrahydro-1H-oxazolo[3,4-c]oxazol-1-yl)propanal (18)

To a solution of 17 (100 mg, 0.46 mmol) in EtOAc (20 mL) was added 2-iodoxybenzoic acid (195 mg, 0.70 mmol) at rt under argon. The reaction was warmed to 85 °C and stirred at this temperature for 16 h. The reaction mixture was then cooled down to rt and left in an ice-bath for additional 2 h. The suspension was filtered through a medium porosity sintered-glass funnel, the solids were thoroughly rinsed with EtOAc, and the filtrates were concentrated \textit{in vacuo}. Purification of the crude with CH_{2}Cl_{2}/MeOH (100% to 95%) gave 85 mg (87%) of aldehyde 18; \( R_f = 0.30 \) in hexane/EtOAc 1:1

\[ ^{1}H\text{ NMR (}\delta, 400\text{ MHz, CDCl}_{3}\text{): 9.71 (s, 1H), 4.53 (ddd, }J = 10.1, 8.1, 3.9\text{ Hz, 1H), 4.36 (td, }J = 8.3, 6.3\text{, 1H), 3.87 (dd, }J = 8.7, 6.3\text{, 1H), 3.68 (t, }J = 8.6\text{, 1H), 2.77-2.52 (m, 2H), 1.90-1.73 (m, 2H), 1.61 (s, 3H, 1.34 (s, 3H). } \]

\[ ^{13}C\text{ NMR (}\delta, 101\text{ MHz, CDCl}_{3}\text{): 220.2, 156.3, 94.8, 73.6, 63.4, 61.1, 39.9, 27.8, 23.4, 23.1. } \]

HRMS (ESI): calcd. for C_{10}H_{16}NO_{4}[M + H]^+ 214.1079; found 214.1059.

\[ [\alpha]^{20}_{D} = -12.02 (c = 1.0, \text{ CHCl}_{3}). \]

(1R,7aS)-5,5-dimethyl-1-((Z)-pentadec-3-en-1-yl)dihydro-1H-oxazolo[3,4-c]oxazol-3(5H)-one (19)

A solution of dodecyltriphenylphosphonium bromide (300 mg, 0.58 mmol) in anhydrous THF (10 mL) and HMPA (0.75 mL) was cooled down to -78°C, followed by dropwise addition of \( n\text{BuLi (0.24 mL, 0.61 mmol, 2.5 M in hexane) over 30 min under argon. The resulting mixture was allowed to warm to 0°C, and next stirred for additional 30 min. After cooling down to -78°C, a solution of aldehyde 18 (78 mg, 0.36 mmol) in anh THF (5 mL) was added dropwise. After vigorous stirring at -78°C for 15 min, the reaction was allowed to warm to rt
and kept at this temperature for 2 h under stirring. The reaction mixture was next quenched by addition of aqueous sat. NH₄Cl (10 mL), and stirred for 30 min. The aqueous phase was extracted with EtOAc (3 x 10 mL), and the combined organic layers were dried over MgSO₄ and filtered. Concentration under reduced pressure afforded a crude, which was purified by flash chromatography (10:0 to 8:2 hexane/EtOAc gradient) to afford 83 mg (0.23 mmol, 64%) of 19 as a colorless oil. R₇ = 0.25 in hexane/EtOAc 9:1

¹H NMR (δ, 400 MHz, CDCl₃): 5.53-5.35 (m, 1H), 5.30 (dt, J= 10.8, 7.3 Hz, 1H), 4.69-4.52 (m, 1H), 4.30 (td, J= 8.5, 6.2 Hz, 1H), 3.91 (dd, J= 8.5, 6.2 Hz, 1H), 3.71 (t, J= 8.8 Hz, 1H), 2.28-2.10 (m, 2H), 2.02 (q, J= 6.7 Hz, 2H), 1.85-1.75 (m, 1H), 1.72 (s, 3H), 1.58-1.48 (m, 1H), 1.44 (s, 3H), 1.35-1.21 (m, 18 H), 0.94-0.81 (m, 3H).

¹³C NMR (δ, 101 MHz, CDCl₃): 156.8, 132.7 127.5, 94.8, 73.9, 63.6, 61.2, 31.9, 30.7, 29.7, 29.6, 29.5, 29.3, 29.2, 28.1, 27.2, 23.4, 22.7, 14.1.

HRMS (ESI): calcd. For C₂₂H₄₀NO₃ [M+H]+ 366.3008; found 366.3013.

[α]²⁰ₒ = -17.6 (c = 1.05, CHCl₃).

(4S,5R)-4-(hydroxymethyl)-5-((Z)-pentadec-3-en-1-yl)oxazolidin-2-one (33)

Solid p-TsOH (7 mg, 0.03 mmol) was added portionwise to a solution of 19 (80 mg, 0.22 mmol) in MeOH (5 mL). After vigorous stirring at rt for 3 h, Et₃N was added dropwise and the reaction mixture was concentrated in vacuo. Purification of the crude (95:5 to 80:20 CH₂Cl₂:MeOH gradient) gave 60 mg (0.18 mmol, 84%) of 33 as a white solid. R₇ = 0.25 in hexane/EtOAc 1:1.

¹H NMR (δ, 400 MHz, CDCl₃): 6.76 (s, 1H), 5.51-5.39 (m, 1H), 5.37-5.24 (m, 1H), 4.64 (ddd, J= 10.1, 7.7, 3.7 Hz, 1H), 3.95-3.84 (m, 1H), 3.80 (td, J= 7.5, 7.0, 3.6 Hz, 1H), 3.73-3.62 (m, 2H), 2.30-2.09 (m, 2H), 2.01 (qd, J= 7.1, 1.5 Hz, 2H), 1.96-1.81 (m, 1H), 1.61 (ddddd, J= 14.0, 8.7, 7.3, 3.8 Hz, 1H), 1.41-1.09 (m, 16 H), 0.94-0.80 (m, 3H).

¹³C NMR (δ, 101 MHz, CDCl₃): 160.7, 131.9, 127.3, 79.0, 61.0, 56.8, 31.9, 29.7, 29.64, 29.62, 29.56, 29.32, 20.30, 28.9, 27.2, 23.7, 22.7, 14.1.

HRMS (ESI): calcd. For C₁₉H₃₆NO₃ [M+H]+ 326.2695; found 326.2704.

[α]²⁰ₒ = -11.3 (c = 0.99, CHCl₃).

(2S,3R,Z)-2-aminoctadec-6-ene-1,3-diol (20)

To a solution of 33 (50 mg, 0.15 mmol) in EtOH (5 mL) was added NaOH 2N (5 mL) and the mixture was vigorous stirred at reflux temperature for 2 h. The reaction mixture was cooled to
rt, concentrated under reduced pressure, and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo to give a crude, which was purified by flash chromatography (100:0 to 90:10 CH₂Cl₂/EtOAc), affording 32 mg (70%) of 20 as a white waxy solid. Rᵣ = 0.20 in hexane/EtOAc 1.1.

¹H NMR (δ, 400 MHz, CD₃OD): 5.42 (td, J = 7.9, 6.9, 3.9 Hz, 2H), 3.76 (dd, J = 10.9, 4.1 Hz, 1H), 3.63-3.47 (m, 2H), 2.85-2.70 (m, 1H), 2.29 (ddt, J = 19.3, 10.1, 4.4 Hz, 1H), 2.22-2.05 (m, 3H), 1.61 (tdd, J = 16.6, 8.4, 5.0 Hz, 1H), 1.50 (ddt, J = 19.3, 10.1, 4.4 Hz, 1H), 1.45-1.24 (m, 18 H), 1.01-0.85 (m, 3H).


HRMS (ESI): calcd. For C₁₈H₃₈NO₂ [M+H]⁺ 300.2903; found 300.2903.

[α]²⁰D = -0.7 (c = 1.0, CHCl₃).

N-((2S,3R,Z)-1,3-dihydroxyoctadec-6-en-2-yl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanamide (3)

A solution of EDC (36 mg, 0.19 mmol), HOBt (28 mg, 0.21 mmol) and C₆NBD acid (82 mg, 0.28 mmol) in anhydrous CH₂Cl₂ (7 mL) was stirred under argon at rt for 10 min, and next added dropwise to a solution of 20 (53 mg, 0.17 mmol) in anh CH₂Cl₂ (8 mL). The reaction mixture was stirred at rt for 5 h under argon. The mixture was diluted by addition of CH₂Cl₂ (10 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried over MgSO₄ and filtered. Concentration under reduced pressure afforded a crude, which was purified by flash chromatography in hexane/EtOAc (9:1 to 7:3), followed by CH₂Cl₂/MeOH (100:0 to 97:3) to afford 3 (80%) as an orange waxy solid. Rᵣ = 0.35 in hexane/EtOAc 1:1.

¹H NMR (δ, 400 MHz, CDCl₃): 8.49 (d, J = 8.6 Hz, 1H, o-NO₂), 6.64 (s, 1H), 6.41 (d, J = 7.7 Hz, 1H), 6.17 (d, J = 8.7 Hz, 1H, m-NO₂), 5.40 (tdd, J = 18.0, 10.9, 9.7 Hz, 2H), 4.05 (dd, J = 11.3, 3.4 Hz, 1H, C1H), 3.94-3.85 (m, 1H, C2H), 3.85-3.80 (m, 1H, C3H), 3.78 (dd, J = 11.3, 3.0 Hz, 1H, C1H'), 3.52 (dt, J = 10.6, 5.4 Hz, 2H, C5'H2), 2.32 (q, J = 7.6 Hz, 2H, C1'H), 2.18 (ddt, J = 20.8, 14.2, 7.4 Hz, 2H), 2.03 (q, J = 6.8 Hz, 2H), 1.91-1.71 (m, 4H), 1.71-1.46 (m, 4H), 1.25 (m, 18H), 0.88 (t, J = 6.9 Hz, 3H).
\(^{13}\)C NMR (\(\delta\), 101 MHz, CDCl\(_3\)): 172.8, 144.2, 143.9, 136.4 (C o-NO\(_2\)), 131.6 (C=), 128.3 (C=), 98.5 (C m-NO\(_2\)), 74.2, 62.4, 53.6, 43.4, 36.0, 34.4, 31.9, 29.7, 29.6, 29.5, 29.3, 27.9, 27.3, 26.1, 24.6, 23.8, 22.7, 14.1.

HSQC (see figure in NMR spectra for atom numbering)

HRMS (ESI): calcd. For C\(_{30}\)H\(_{50}\)N\(_5\)O\(_6\) [M+H]\(^+\) 576.3761; found 576.3779.

HPLC UV-FL: (70:30 ACN:H\(_2\)O) rt: 17.3 min.

\([\alpha]\)\(^{20}\)\(_D\) = -13 (c = 0.8, MeOH).

\(N\)-((2S,3R,Z)-1,3-dihydroxyoctadec-6-en-2-yl)octanamide (4)

A solution of EDC (42 mg, 0.22 mmol), HOBt (28 mg, 0.20 mmol) and octanoic acid (38 mg, 0.28 mmol) in anh CH\(_2\)Cl\(_2\) (7 mL) was stirred under argon at rt for 10 min, and next added dropwise to a solution of 20 (51 mg, 0.17 mmol) in anh CH\(_2\)Cl\(_2\) (8 mL). The reaction mixture was stirred at rt for 3 h under argon. The mixture was next diluted by addition of CH\(_2\)Cl\(_2\) (10 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried over MgSO\(_4\), and filtered. Concentration under reduced pressure afforded a crude, which was purified by flash chromatography in CH\(_2\)Cl\(_2\)/MeOH (100:0 to 95:5) to afford 4 (87\%) as a waxy white solid. \(R_\text{f}\) = 0.25 in hexane/EtOAc 1:1.

\(^1\)H NMR (\(\delta\), 400 MHz, CDCl\(_3\)): 6.38 (br, 1H), 5.51-5.31 (m, 2H), 3.99 (dd, \(J\) = 11.3, 3.3 Hz, 1H), 3.92-3.67 (m, 3H), 2.25-2.19 (m, 2H), 2.03 (q, \(J\) = 13.5, 6.5 Hz, 2H), 1.61 (td, \(J\) = 13.0, 6.4, 4H), 1.36-1.21 (m, 28H), 0.87 (t, \(J\) = 6.8, 6H).

\(^{13}\)C NMR (\(\delta\), 101 MHz, CDCl\(_3\)): 173.6, 131.4, 128.4, 73.9, 62.5, 53.9, 36.8, 34.3, 31.9, 31.6, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 28.9, 27.3, 25.7, 23.8, 22.6, 22.6, 14.1, 14.02.

HRMS (ESI): calcd. For C\(_{26}\)H\(_{52}\)NO\(_3\) [M+H]\(^+\) 426.3947; found 426.3962.

\([\alpha]\)\(^{20}\)\(_D\) = -1.02 (c = 1.3, CHCl\(_3\)).
3. Synthesis of (E,E)-Δ⁴,6 ceramides

Scheme S3. Synthesis of (E,E)-Δ⁴,6 ceramides: (a) (E)-1-iodotridec-1-ene, Pd(PPh₃)₄, CuI, THF; (b) RedAl, THF, 0°C to rt; (c) NaH, THF; (d) p-TsOH, MeOH; (e) NaOH/EtOH (1:1); (f) C₆NBD acid, HOBt, EDC, Et₃N, CH₂Cl₂; (g) octanoic acid, HOBt, EDC, Et₃N, CH₂Cl₂

(S)-tert-butyl 4-((R,E)-1-hydroxyhexadec-4-en-2-yn-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (22)

To a solution of Pd(PPh₃)₄ (31 mg, 0.03 mmol) and CuI (5 mg, 0.03 mmol) in piperidine (3 mL) was added a solution of 21 (42 mg, 0.27 mmol) in THF (5 mL) and a solution of (E)-1-iodotridec-1-ene (100 mg, 0.32 mmol) in piperidine (3 mL). The reaction mixture was stirred at rt for 2 h, and then quenched by adding saturated solution of NH₄Cl (5 mL) at 0 °C. The aqueous layer was extracted with Et₂O (4 x 5 mL). The resulting organic layer was then dried over MgSO₄ and concentrated to give a crude that was purified by flash chromatography (hexane/EtOAc 7:3, Rf = 0.35) to give 22 (82 mg, 70%) as an orange oil.

¹H NMR (δ, 400 MHz, CDCl₃): 0.86 (m, 3H), 1.19-1.30 (m, 18H), 1.30-1.39 (m, 3H), 1.48 (s, 6H), 1.49 (s, 3H), 1.54-1.61 (m, 3H), 2.02-2.10 (m, 2H), 3.80-3.90 (m, 1H), 4.02-4.12 (m, 1H), 4.12-4.21 (m, 1H), 4.53-4.61 (brs, 1H), 5.44, (d, J = 15.9 Hz, 1H), 6.05-6.16 (m, 1H).

¹³C NMR (δ, 101 MHz, CDCl₃): 145.4, 108.8, 108.5, 95.0, 85.4, 81.3, 65.2, 64.9, 62.9, 33.0, 31.9, 29.60, 29.58, 29.54, 29.45, 29.32, 29.30, 29.0, 28.7, 25.6, 25.4, 22.6, 14.1.

HRMS (ESI): calcd. for C₂₆H₄₆NO₄ [M + H]⁺ 436.3427; found 436.3427.
(S)-tert-butyl 4-((R,2E,4E)-1-hydroxyhexadeca-2,4-dien-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (23)

To a solution of 22 (370 mg, 0.85 mmol) in THF (7 mL) previously cooled to 0 °C, was added dropwise a solution of RedAl (wt. 65% in toluene, 2.6 mL, 8.5 mmol). The mixture was stirred at rt for 4 h. Next, the mixture was cooled to 0 °C and quenched by addition of MeOH (10 mL). After dilution with Et₂O (15 mL), 30 mL of a saturated solution of Rochelle salt (Na-K tartrate) was added. The aqueous layer was extracted with Et₂O. The organic layers were washed with a sat Rochelle's salt and dried over MgSO₄. Purification by flash chromatography (hexane/EtOAc 8:2, Rf = 0.30) yielded 85% of 23 as a colorless oil.

¹H NMR (δ, 400 MHz, CDCl₃): 0.86 (t, J= 6.8 Hz, 3H), 1.20-1.31 (m, 18H), 1.43-1.50 (m, 15H), 2.00-2.08 (m, 2H), 3.80-3.88 (m, 1H), 3.95-4.04 (m, 1H), 4.13 (s, 1H), 4.28 (s, 1H), 5.52 (dd, J= 15.6, 6.1 Hz, 1H), 5.60-5.73 (m, 1H), 6.00 (dd, J= 15.1, 10.5, Hz, 1H), 6.19-6.28 (m, 1H).

¹³C NMR (δ, 101 MHz, CDCl₃):  135.4, 131.9, 129.4, 128.8, 94.4, 80.9, 73.8, 64.7, 62.3, 32.6, 31.9, 29.7, 29.60, 20.58, 29.56, 29.47, 29.3, 29.21, 29.19, 29.14, 28.3, 28.2, 26.3, 22.6, 14.1.

HRMS (ESI): calcd. for C₂₆H₄₇NNaO₄ [M + Na]⁺ 460.3403; found 460.3396.

(1S,7aS)-5,5-dimethyl-1-((1E,3E)-pentadeca-1,3-dien-1-yl)dihydro-1H-oxazolo[3,4-c]oxazol-3(5H)-one (24)

A solution of 23 (295 mg, 0.67 mmol) in anh THF (5 mL) was added to a suspension of NaH (270 mg, 6.74 mmol) in anh THF (5 mL) at 0 °C. The reaction mixture was vigorously stirred at rt overnight under argon. The reaction mixture was next quenched by dropwise addition of aqueous sat. NaHCO₃ at 0 °C, until H₂ evolution ceased. The aqueous phase was next extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo to give a residue, which was purified by flash chromatography (80:20 hexane/EtOAc, 7:3, Rf = 0.30) to afford 167 mg (0.46 mmol, 67%) of pure trans/trans 24 as a colorless oil.

¹H NMR (δ, 400 MHz, CDCl₃): 0.83-0.88 (m, 3H), 1.18-1.33 (m, 18H), 1.43 (s, 3H), 1.71 (s, 3H), 2.1 (q, J= 7.5 Hz, 2H), 3.67, (t, J= 8.6 Hz, 1H), 3.83 (dd, J= 8.8, 6.3 Hz, 1H), 4.41 (dt, J= 8.3, 6.3 Hz, 1H), 5.01 (t, J= 8.0 Hz, 1H), 5.41 (dd, J= 16.0, 8.0Hz, 1H), 5.79 (m, 1H), 6.00 (dd, J= 16.0, 12.0Hz, 1H), 6.31 (dd, J= 16.0, 12.0Hz, 1H).
¹³C NMR (δ, 101 MHz, CDCl₃): 159.8, 138.9, 135.7, 128.2, 121.6, 95.1, 75.2, 64.3, 61.7, 32.6, 31.9, 29.61, 29.58, 29.53, 29.42, 29.29, 29.16, 28.9, 27.8, 23.4, 22.6, 14.1.

HRMS (ESI): calcd. for C₂₂H₃₈NO₃ [M + H]⁺ 364.2852; found 364.2852.

(4S,5R)-4-(hydroxymethyl)-5-((1E,3E)-pentadeca-1,3-dien-1-yl)oxazolidin-2-one (25)

Solid p-TsOH (8 mg, 0.04 mmol) was added portionwise to a solution of 24 (160 mg, 0.44 mmol) in MeOH (10 mL). After vigorous stirring at rt for 4 h, the reaction mixture was quenched with sat. aqueous NaHCO₃ and concentrated in vacuo. Purification of the crude (95:5 CH₂Cl₂:MeOH) gave 100 mg (0.30 mmol, 82%) of 25 as a white solid. Rₜ = 0.25, hexane/EtOAc 1:1.

¹H NMR (δ, 400 MHz, CD₂OD): 0.85-0.90 (m, 3H), 1.21-1.34 (m, 16H), 1.35-1.45 (m, 2H), 2.05-2.13 (m, 2H), 3.45-3.58 (m, 2H), 3.81-3.87 (m, 1H), 5.13 (td, J= 8.1, 1.0 Hz, 1H), 5.68-5.85 (m, 2H), 6.06-6.14 (m, 1H), 6.35 (dd, J= 15.2, 10.4 Hz, 1H).

¹³C NMR (δ, 101 MHz, CD₂OD): 137.3, 135.8, 128.7, 122.5, 79.9, 60.7, 57.3, 32.2, 31.6, 29.32, 29.29, 29.27, 29.14, 29.02, 28.86, 28.81, 22.3, 13.0.

HRMS (ESI): calcd. for C₁₉H₃₄NO₃ [M + H]⁺ 324.2539; found 324.2549. m.p. 115 °C.

(2S,3R,4E,6E)-2-aminooctadeca-4,6-diene-1,3-diol (26)

To a solution of 25 (80 mg, 0.25 mmol) in EtOH (5 mL) was added 5 mL of 2N NaOH and the mixture was stirred under reflux for 4 h. Next, the reaction mixture was cooled down to rt and EtOH was concentrated under reduced pressure. The aqueous residue was extracted with CH₂Cl₂ (3 x 10 mL) and the combined organic phases were dried and evaporated to dryness. The resulting compound 26 (60 mg, 0.20 mmol, 82 %) was used in the next step without further purification.

¹H NMR (δ, 400 MHz, CDCl₃): 0.82-0.88 (m, 3H), 1.18-1.41 (m, 18H), 2.05 (q, J= 7.2 Hz, 2H), 2.41 (brs, 2H), 2.84 (s, 1H), 3.58-3.63 (m, 2H), 4.09 (t, J= 6.3 Hz, 1H), 5.54 (dd, J= 15.3, 7.0 Hz, 1H), 5.70 (dt, J= 14.7, 7.0 Hz, 1H), 6.01 (dd, J= 15.1, 10.4 Hz, 1H), 6.24 (dd, 15.3, 10.5 Hz, 1H).

¹³C NMR (δ, 101 MHz, CDCl₃): 136.4, 133.0, 129.6, 129.1, 75.1, 64.0, 56.2, 32.6, 31.9, 29.7, 29.61, 29.58, 29.48, 29.3, 29.2, 29.1, 22.7, 14.1.

HRMS (ESI): calcd. for C₁₈H₃₆NO₂ [M + H]⁺ 298.2746; found 298.2740.
N-((2S,3R,4E,6E)-1,3-dihydroxyoctadeca-4,6-dien-2-yl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanamide (5)

A solution of EDC (52 mg, 0.27 mmol), HOBt (27 mg, 0.20 mmol) and C₆NBD acid (53 mg, 0.18 mmol) in anh CH₂Cl₂ (7 mL) was stirred under argon atmosphere at rt for 10 min and next added dropwise to a solution of the 26 (50 mg, 0.17 mmol) in anh CH₂Cl₂ (8 mL). The reaction mixture was stirred at rt for 2.5 h under argon. The mixture was diluted by addition of CH₂Cl₂ (10 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried over MgSO₄ and filtered. Concentration under reduced pressure afforded a residue, which was purified by flash chromatography in hexane/EtOAc (9:1 to 7:3) followed by CH₂Cl₂/MeOH (100:0 to 97:3) to afford 5 (64 mg, 66%) as an orange waxy solid. R_f = 0.30 in hexane/EtOAc 1:1.

¹H NMR (δ, 400 MHz, CDCl₃): 0.82-0.88 (m, 3H), 1.17-1.41 (m, 18H), 1.45-1.56 (m, 2H), 1.68-1.77 (m, 2H), 1.78-1.87 (m, 2H), 2.05 (q, J = 7.2 Hz, 2H), 2.29 (t, J = 7.0 Hz, 2H), 3.46-3.55 (m, 2H), 3.66-3.73 (m, 1H), 3.94-4.00 (m, 2H), 4.38-4.42 (m, 1H), 5.60 (dd, J = 15.3, 6.2 Hz, 1H), 5.72 (dt, J = 14.6, 7.0 Hz, 1H), 6.01 (dd, J = 15.2, 10.4 Hz, 1H), 6.14 (d, J = 8.7, 1H), 6.28 (dd, J = 15.3, 10.4 Hz, 1H), 6.40 (d, J = 7.5 Hz, 1H), 6.86 (brs, 1H), 8.45 (d, J = 8.6 Hz, 1H).

¹³C NMR (δ, 101 MHz, CDCl₃): 173.3, 144.23, 143.9, 136.9, 136.5, 132.8, 128.8, 128.7, 74.4, 62.3, 54.3, 43.6, 46.0, 32.7, 31.9, 29.7, 29.60, 29.57, 29.47, 29.31, 29.22, 29.13, 27.8, 26.1, 24.7, 22.7, 14.1.


HPLC-FD (excitation at 465 nm and emission at 530 nm): rt: 13.2 min.

N-((2S,3R,4E,6E)-1,3-dihydroxyoctadeca-4,6-dien-2-yl)octanamide (6)

A solution of EDC (20 mg, 0.14 mmol), HOBt (12 mg, 0.11 mmol) and octanoic acid (15 mg, 0.11 mmol) in anhy CH₂Cl₂ (4 mL) was stirred under argon a at rt for 10 min, and next added dropwise to a solution of 26 (22 mg, 0.11mmol) in anh CH₂Cl₂ (3 mL). The reaction mixture was stirred at rt for 2 h under argon. The mixture was next diluted by addition of CH₂Cl₂ (5 mL) and washed successively with water (5 mL) and brine (5 mL). The organic layer was dried over MgSO₄ and filtered. Concentration under reduced pressure afforded a residue, which was purified by flash chromatography with CH₂Cl₂/MeOH (100:0 to 95:5) to afford 6 (81%) as a white waxy solid. R_f = 0.30 in hexanes/EtOAc 1:1.
\(^1\)H NMR (\(\delta, 400\) MHz, CDCl\(_3\)): 0.88 (t, \(J=7.0\) Hz, 10H), 1.20-4.42 (m, 45.5H), 1.60-1.68 (m, 3.8H), 2.08 (q, \(J=7.0\) Hz, 1.3H), 2.18 (q, \(J=7.5\) Hz, 2H), 2.21-2.27 (m, 2.8H), 3.68-3.74 (m, 1.5H), 3.91-4.01 (m, 2.6H), 4.41 (t, \(J=4.5\) Hz, 0.5H), 4.46 (t, \(J=4.5\) Hz, 0.7H), 5.50 (dd, \(J=18.0, 7.5, 0.9\)H), 5.61 (dd, \(J=15.5, 6.5, 0.7\)H), 5.67-5.78 (m, 1.5H), 5.95-6.08 (m, 1.6H), 6.21-6.34 (m, 2H), 6.62 (dd, \(J=15.0, 11.0\) Hz, 0.8H).

HRMS calculated for C\(_{26}\)H\(_{49}\)NNaO\(_3\): 446.3610 \([M+Na]^+\). Found: 446.3603.

### 4. Synthesis of (E,Z)-\(\Delta^{4,6}\) ceramides

![Scheme S4](image)

Scheme S4. Synthesis of (E,Z)-\(\Delta^{4,6}\) ceramides: (a) (Z)-1-iodotridec-1-ene, Pd(PPh\(_3\))\(_4\), CuI, THF; (b) RedAl, THF, 0°C to rt; (c) NaH, THF; (d) \(p\)-TsOH, MeOH; (e) NaOH/EtOH (1:1); (f) C\(_6\)NBD acid, HO\(_2\)t, EDC, Et\(_3\)N, CH\(_2\)Cl\(_2\); (g) octanoic acid, HO\(_2\)t, EDC, Et\(_3\)N, CH\(_2\)Cl\(_2\)

(S)-tert-butyl 4-((R,Z)-1-hydroxyhexadec-4-en-2-yn-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (28)

To a solution of Pd(PPh\(_3\))\(_4\) (570 mg, 0.5 mmol) and CuI (93 mg, 0.5 mmol) in piperidine (30 mL) was added a solution of 21\(^5\) (1.25 g, 4.9 mmol) in THF (50 mL) and a solution of (Z)-1-iodotridec-1-ene\(^7,8\) (1.48 g, 4.9 mmol) in piperidine (20 mL). The reaction mixture was stirred at rt for 90 min and then quenched by adding a saturated solution of NH\(_4\)Cl (50 mL) at 0 °C. The aqueous layer was extracted with Et\(_2\)O (3 x 20 mL). The resulting organic layer was then dried over MgSO\(_4\) and concentrated to give a crude, which was purified by flash chromatography (hexane/ethyl acetate 90/10, \(R_f = 0.45\)) to give compound 28 (1.5 g, 72%) as a colorless oil.
**1H NMR (400 MHz, CDCl₃)** δ 5.87 (d, J = 9.1 Hz, 1H), 5.42 (d, J = 10.8 Hz, 1H), 4.86 – 4.58 (m, 1H), 4.36 – 3.79 (m, 3H), 2.25 (q, J = 7.2 Hz, 2H), 1.73 – 1.40 (m, 15H), 1.23 (s, 18H), 0.85 (t, J = 6.8 Hz, 3H).

**13C NMR (100 MHz, CDCl₃)** δ 144.7, 108.2, 95.0, 91.1, 81.3, 82.8, 65.1, 64.7, 62.7, 31.9, 30.3, 29.62, 29.60, 29.56, 29.49, 29.3, 29.2, 28.8, 28.3, 25.8, 25.3, 22.6, 14.1.

HRMS (ESI): calcd. For C₂₆H₄₆NO₄[M+H]⁺ 436.3403; found 436.3422.

[α]²⁰D = -54 (c = 1.08, CHCl₃).

**{(S)-tert-butyl 4-((R,2E,4Z)-1-hydroxyhexadeca-2,4-dien-1-yl)-2,2-dimethyloxazolidine-3-carboxylate (29)}**

A solution of 28 (540 mg, 1.2 mmol) in ether (1 mL) was added dropwise to a solution of RedAl (3.8 mL, aprox 60% in toluene, around 12.0 mmol) in ether (2 mL) at 0°C. After stirring at rt for 2 h, the reaction was quenched with a saturated solution of Rochelle salt, and the aqueous layer was extracted with ether. The combined ethereal extracts were washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure. Purification by flash chromatography (hexane/ethyl acetate 80:20, Rₚ = 0.35) afforded 515 mg (95 %) of 29.

**1H NMR (δ, 400 MHz, CDCl₃):** δ 6.69-6.42 (m, 1H), 5.95 (t, J = 11.0 Hz, 1H), 5.64 (broad, 1H), 5.41 (broad, 1H), 4.49-3.70 (m, 5H), 2.13 (q, J = 6.9 Hz, 2H), 1.66-1.37 (broad), 1.44 (s, 9H), 1.22 (s, 6H), 0.85 (t, J = 6.8 Hz, 3H).

**13C NMR (δ, 101 MHz, CDCl₃):** δ 132.8, 131.2, 127.7, 126.8, 94.4, 81.1, 73.9, 64.9, 62.4, 31.9, 29.62, 29.60, 29.57, 29.51, 29.31, 29.26, 28.3, 27.8, 26.2, 24.5, 22.7, 14.1.

HRMS (ESI): calcd. For C₂₆H₄₇NO₄Na[M+Na]⁺ 460.3403; found 460.3386.

[α]²⁰D = -12 (c = 1.0, CHCl₃).

**{(1R,7aS)-5,5-dimethyl-1-((1E,3Z)-pentadeca-1,3-dien-1-yl)dihydro-1H-oxazolo[3,4-c]oxazol-3(5H)-one (30)}**

A solution of 29 (300 mg, 0.68 mmol) in anh THF (10 mL) was added dropwise to a suspension of NaH (46 mg, 1.14 mmol) in anh THF (10 mL) at rt. The reaction mixture was vigorously stirred for 16 hour at 50 °C under argon. The reaction was next quenched by dropwise addition of aqueous sat. NaHCO₃ at 0 °C, until H₂ evolution ceased. The aqueous phase was next extracted with EtOAc (3 x 10 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated in vacuo to give a crude, which was purified by flash...
chromatography (100:0 to 80:20 hexane/EtOAc), affording 200 mg (80 %) of 30. R$_f$ = 0.35 in hexane/EtOAc 8:2.

$^1$H NMR (δ, 400 MHz, CDCl$_3$): δ 6.67 (dd, $J = 15.1, 11.1$ Hz, 1H), 5.98 (t, $J = 11.0$ Hz, 1H), 5.69 – 5.42 (m, 2H), 5.13 (t, $J = 7.6$ Hz, 1H), 4.40 (td, $J = 8.3, 6.4$ Hz, 1H), 3.96 – 3.80 (m, 1H), 3.69 (t, $J = 8.6$ Hz, 1H), 2.27 – 2.07 (m, 2H), 1.72 (s, 3H), 1.45 (s, 3H), 1.37 – 1.10 (m, 18H), 0.86 (t, $J = 11.0$ Hz, 3H).

$^{13}$C NMR (δ, 101 MHz, CDCl$_3$): δ 156.7, 136.1, 130.5, 126.4, 123.8, 95.1, 75.0, 64.3, 61.7, 31.9, 29.7, 29.62, 29.60, 29.57, 29.47, 29.45, 29.3, 29.2, 27.9, 23.4, 22.7, 14.1.

HRMS (ESI): calcd. For C$_{22}$H$_{38}$NO$_3$ [M+H]$^+$ 364.2852; found 364.2842.

[α]$^2$₀D = -17 (c = 0.975, CHCl$_3$).

(4S,5R)-4-(Hydroxymethyl)-5-((1E,3Z)-pentadeca-1,3-dien-1-yl)oxazolidin-2-one (31)

Solid p-TsOH (3 mg, 0.01 mmol) was added to a solution of 30 (50 mg, 0.13 mmol) in MeOH (3 mL). After vigorous stirring at rt for 1 h, Et$_3$N was added dropwise and the reaction mixture was concentrated in vacuo. Purification of the crude (100:0 to 97:03 CH$_2$Cl$_2$:MeOH gradient) gave 36 mg (85%) of 31 as a white waxy solid. R$_f$ = 0.45 in hexane/EtOAc 1:1.

$^1$H NMR (δ, 400 MHz, CDCl$_3$): δ 6.65 (dd, $J = 15.2, 11.1$ Hz, 1H), 6.58 (s, 1H), 5.99 (t, $J = 11.1$ Hz, 1H), 5.73 (dd, $J = 15.2, 8.3$ Hz, 1H), 5.62 – 5.50 (m, 1H), 5.16 (t, $J = 8.2$ Hz, 1H), 3.88 (ddd, $J = 8.4, 6.0, 3.9$ Hz, 1H), 3.70 – 3.54 (m, 2H), 3.40 (brs, 1H), 2.16 (q, $J = 6.9$ Hz, 2H), 1.34 (dd, $J = 19.7, 5.5$ Hz, 18H), 0.86 (t, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (δ, 101 MHz, CDCl$_3$): δ 160.2, 135.9, 131.7, 126.6, 123.7, 80.1, 61.7, 57.5, 31.9, 29.63, 29.60, 29.57, 29.49, 29.32, 29.26, 27.9, 22.7, 14.1.

HRMS (ESI): calcd. For C$_{19}$H$_{34}$NO$_3$ [M+H]$^+$ 324.2539; found 324.2531.

[α]$^2$₀D = -23 (c = 1.0, CHCl$_3$).

(2S,3R,4E,6Z)-2-Aminooctadeca-4,6-diene-1,3-diol (32)

A solution of 2N NaOH (5 mL) was added dropwise to a solution of 31 (50 mg, 0.18 mmol) in EtOH (5 mL). After vigorously stirring at reflux temperature for 2 h, the reaction mixture was cooled to rt, concentrated under reduced pressure to eliminate EtOH, and extracted with CH$_2$Cl$_2$ (3 x 10 mL). The combined organic extracts were dried over MgSO$_4$, filtered, and concentrated in vacuo to give a crude, which was purified by flash chromatography (100:0 to
90:10 CH₂Cl₂/EtOAc) to afford 45 mg (98%) of 32 as a white waxy solid. R₇ = 0.40 in hexane/EtOAc 1:1.

¹H NMR (δ, 400 MHz, CD₃OD): δ 6.63 (dd, J = 15.2, 11.1 Hz, 1H), 6.05 (t, J = 11.1 Hz, 1H), 5.72 (dd, J = 15.2, 6.9 Hz, 1H), 5.47 (dt, J = 10.7, 7.7 Hz, 1H), 4.12 (t, J = 6.5 Hz, 1H), 3.68 (dd, J = 11.0, 4.5 Hz, 1H), 3.52 (dd, J = 11.0, 7.3 Hz, 1H), 2.81 (q, J = 6.3, 1H), 2.27-2.19 (m, 2H), 1.46-1.26 (m, 18H), 0.99-0.72 (m, 3H).


HRMS (ESI): calcd. For C₁₈H₃₆NO₂ [M+H]⁺ 298.2746; found 298.2729. 

[α]²⁰D = +4 (c = 1.0, CHCl₃).

N-((2S,3R,4E,6Z)-1,3-Dihydroxyoctadeca-4,6-dien-2-yl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexanamide (7)

A solution of EDC (38 mg, 0.18 mmol), HOBt (29 mg, 0.21 mmol) and C₆NBD acid (88 mg, 0.30 mmol) in anh CH₂Cl₂ (7 mL) was stirred under argon at rt for 10 min, and next added dropwise to a solution of 32 (53 mg, 0.18 mmol) in anh CH₂Cl₂ (5 mL). The reaction mixture was stirred at rt for 5 h under argon. The mixture was next diluted by addition of CH₂Cl₂ (10 mL) and washed successively with water (10 mL) and brine (10 mL). The organic layer was dried over MgSO₄, and filtered. Concentration under reduced pressure afforded a residue, which was purified by flash chromatography with CH₂Cl₂/MeOH (100:0 to 95:5) to afford 7 (80%) as a white waxy solid. R₇ = 0.35 in hexane/EtOAc 1:1

¹H NMR (δ, 400 MHz, CDCl₃): 8.44 (d, J = 8.7 Hz, 1H), 6.87 (s, 1H), 6.60 (dd, J = 15.2, 11.1 Hz, 1H), 6.44 (d, J = 7.8 Hz, 1H), 6.14 (d, J = 8.7 Hz, 1H), 5.95 (t, J = 11.0 Hz, 1H), 5.69 (dd, J = 15.2, 6.2 Hz, 1H), 5.47 (dt, J = 10.7, 7.6 Hz, 1H), 4.44 (s, 1H), 4.10-3.86 (m, 2H), 3.78-3.65 (m, 1H), 3.50 (d, J = 5.7 Hz, 2H), 2.29 (d, J = 7.0 Hz, 2H), 2.18-2.09 (m, 2H), 1.86-1.67 (m, 4H), 1.28-1.11 (m, 20H), 0.85 (t, J = 6.8 Hz, 3H).

¹³C NMR (δ, 101 MHz, CDCl₃): 173.4, 144.2, 136.6, 134.2, 131.0, 127.7, 127.1, 74.5, 62.2, 54.3, 43.6, 36.0, 31.9, 29.64, 29.60, 29.59, 29.52, 29.31, 29.29, 27.9, 27.8, 26.2, 24.7, 22.7, 14.1.

HRMS (ESI): calcd. For C₃₀H₄₇N₅NaO₆[M+Na]⁺ 596.3424; found 596.3415.

[α]²⁰D = -11 (c = 0.45, MeOH).
**N-((2S,3R,4E,6Z)-1,3-Dihydroxyoctadeca-4,6-dien-2-yl)octanamide (8)**

A solution of EDC (13 mg, 0.09 mmol), HOBt (8 mg, 0.07 mmol) and octanoic acid (10 mg, 0.09 mmol) in anhy CH₂Cl₂ (3 mL) was stirred under argon at rt for 10 min, and next added dropwise to a solution of 32 (15 mg, 0.07 mmol) in anh CH₂Cl₂ (3 mL). The reaction mixture was stirred at rt for 2 h under argon. The mixture was next diluted by addition of CH₂Cl₂ (5 mL) and washed successively with water (5 mL) and brine (5 mL). The organic layer was dried over MgSO₄ and filtered. Concentration under reduced pressure afforded a residue, which was purified by flash chromatography with CH₂Cl₂/MeOH (100:0 to 95:5) to afford 8 (87%) as a white waxy solid. Rᵣ = 0.30 in hexane/EtOAc 1:1.

¹H NMR (δ, 400 MHz, CDCl₃): 6.60 (dd, J = 15.2, 11.1 Hz, 1H), 6.26 (d, J = 7.4 Hz, 1H), 5.97 (t, J = 11.0 Hz, 1H), 5.68 (dd, J = 15.2, 6.2 Hz, 1H), 5.54-5.40 (m, 1H), 4.49-4.35 (m, 1H), 4.08-3.83 (m, 2H), 3.76-3.64 (m, 1H), 2.38-1.99 (m, 4H), 1.69-1.52 (m, 2H), 1.43-1.16 (m, 24H), 1.06-0.64 (m, 6H).

¹³C NMR (δ, 101 MHz, CDCl₃): 173.9, 134.1, 131.2, 127.6, 127.2, 74.6, 62.4, 54.4, 36.8, 31.9, 31.6, 29.64, 29.61, 29.59, 29.52, 29.32, 29.29, 29.19, 28.98, 27.9, 25.7, 22.7, 22.6, 14.1, 14.0.

HRMS (ESI): calcd. For C₂₆H₄₉NNaO₃ [M+Na]+ 446.3610; found 446.3605. 

[α]ᵢ⁰D = -22 (c = 0.99, MeOH).
Experimental procedures: Biochemistry

Cell culture

The human gastric cancer cell line HGC 27 was cultured at 37°C in 5% CO 2 in minimum essential medium supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and 100 ng/ml each of penicillin and streptomycin. Cells were routinely grown at a 60% maximum confluence. Human glioblastoma cell lines T98 and U87 were cultured at 37°C in 5% CO 2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 100 μg/ml each of penicillin and streptomycin. All cell lines were obtained from American Type Culture Collection.

Cell viability

In all cell lines, cell viability was measured in triplicate samples by the MTT assay. Cells were seeded in 96 well plates at a density of 1x10^5 cells/ml and then subjected to various treatments for 24 h. At the end of the treatments MTT was added to each well and incubated for 3 h. The supernatant was aspirated, and the formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm.

Des1 activity assay

Des1 activity was determined in HGC27 cell lysates using the fluorescent derivatives 1, 3, or dhCerC6NBD9 (positive control of enzyme activity). dhCerC6NBD was used as substrate in the inhibition studies by 2 and 4.

To prepare the cell lysate, a suspension of 10^6 cells/ml per sample was centrifuged (190 g/3 min), the pellets were washed twice with PBS and resuspended in 0.4 ml of 0.2 M phosphate buffer pH 7.4 (PB). Then 100 μL of PB were added to each pellet and sonicated at 75 Watts (Branson SFX150 sonifier) for 5 seconds. A 3.5 % (v/v) solution of the required amount of stock substrate solution (1 mM in EtOH) in a BSA solution (3.3 mg/ml in PB) was prepared to have the needed substrate concentrations (35 μM for a 10 μM final concentration in standard assays). In inhibition studies, the required amount of test compound was added at this point. To each tube containing the lysate from 10^6 cells was added: 85 μL of the BSA-substrate-inhibitor/vehicle mix (final substrate concentrations in standard assays was 10 μM), 30 μL of NADH solution (20 mg/ml in PB) and 85 μL of PB to have a final volume of 300 μL. Unless indicated otherwise, the reaction mixture was incubated at 37°C for 4 h. To stop the reaction, 700 μL/sample of methanol was added to each tube and the reaction mixture was stirred by vortex and kept at 4°C overnight. The mixture was centrifuged (9.300 g/3 min), the
clear supernatants were transferred to HPLC vials and 20 μL were injected. HPLC analyses were performed with an Alliance apparatus coupled to a fluorescence detector using a C18 column (Kromasil 100 C18, 5 μm, 15 x 0.40 cm, Tracer) precolumn equipped ( precolumn ODS, Tracer). Compounds were eluted with 25% H₂O and 75% acetonitrile, both with a 0.1% trifluoroacetic acid, flowing at 1 mL/min. The detector was set at an excitation wavelength of 465 nm and an emission wavelength at 530 nm. Each sample was run for up to 22 minutes.

Specific assays conditions were: To determine the kinetic parameters of 2 as Des1 substrate (Fig. 2A), concentrations of 2 were 20, 15, 10, 5, 2.5, 1.25, 0.675, 0.327 and 0 μM. To determine the CC₅₀ values of compounds 2 and GT11 (Fig. 4A), compounds concentrations were 7.5, 5.0, 2.5, 2.0, 1.0, 0.5, 0.25, 0.1, 0.01 and 0 μM (2) and 2.5, 1.0, 0.1, 0.05, 0.02 and 0 μM (GT11). Reaction time was 4 h. To determine reversibility of Des1 inhibition by 2 (Fig. 4B), substrate concentration (dhCerC6NBD) was 10 μM, test compound concentration was 150 nM and the reaction times were 1, 2.5, 4 and 6 h. For Ki determination (Fig. 4C, D), substrate (dhCerC6NBD) concentrations were 20, 15, 10 and 5 μM and inhibitor concentrations were 200, 100 and 0 nM. Reaction time was 4 h.

**Lipid analyses**

Cells were seeded at 1x10⁵ cells into 6 well plates (1 ml/well) and were allowed to adhere for 24 h. Medium was replaced with fresh medium containing the test compounds at the specified concentrations or EtOH as control. The medium was removed after 2 and 24 h, and cells were washed with PBS and harvested by trypsinization. Sphingolipid extracts, fortified with internal standards (N-dodecanoylsphingosine, N-dodecanoylglucosylsphingosine, N-dodecanoylsphingosylphosphorylcoline, C17-sphinganine and C17-sphinganine 1-phosphate, 0.2 nmol each) were prepared and analysed as reported by UPLC-TOF MS.
Figure S1. Full HPLC-FD chromatograms showing the $\Delta^4$ desaturation of dhCerC6NBD (positive control of Des activity) and $\Delta^6$ monoenoic ceramides 1 and 3 in HGC27 cell lysates. The experiment was carried out as detailed in the experimental section. Analytical conditions were: 15 cm reversed-phase C18 column, flow rate 1 mL/min, injection volume 20 µL, mobile phase 25% H$_2$O-75% acetonitrile, both with a 0.1% trifluoroacetic acid. Fluorescence detector, excitation at 465 nm and emission at 530 nm. AU: arbitrary units.
Figure S2 A. Residuals plot of the direct Michaelis-Menten plot given in Fig. 2D. B. Lineweaver-Burk plot for Δ^4 desaturation of 3. Linear regression with GraphPad Prism 6 afforded $K_{m(app)}$ and $V_{max(app)}$ values of 7.6 ($\pm 1.0$) µM and 23.03 ($\pm 1.5$) pmols/h/mg protein, respectively ($y = 0.3317x + 0.04341$). C. Direct linear plot^{10} of the velocity values obtained at the different substrate concentrations. For 8 substrate concentrations, there are 28 different intersections ($8^*(8-1)/2$). The median of all intersections affords $K_{m(app)}$=8.62 µM and $V_{max(app)}$=27.18 pmol/h/mg.
Figure S3. Effect of compounds on cell viability. U87 and T98 cells were exposed to the compounds for 24 h and cell viability was determined by the MTT test. Curve fitting with the sigmoidal dose-response (variable slope) equation afforded the \( \text{CC}_{50} \) values (\( \mu \text{M} \)) (95% confidence intervals) depicted in the table. Data were obtained from 3 experiments with triplicates.
**Figure S4.** Amounts of total dihydroceramides and ceramides in T98 and U87 cells incubated with vehicle (EtOH) or 10 µM of compounds 2 and 4 for 24 h. Lipids were analyzed by UPLC-TOF MS analysis. Results are means ± SD of two independent experiments with triplicates. Asterisks indicate statistically significant difference of means (P < 0.05, unpaired, two tailed t test). Note that levels of ceramides in treatments with 2 decreased over controls, in agreement with Des1 inhibition.
Figure S5. Evaluation of the reversible inhibitory activity of 2. Cell lysates (4x10^6 cells/mL per sample) were prepared as detailed in the experimental section and were preincubated with 2 at 150 nM for 30 min. Then the solution was diluted 4-fold with substrate (10 μM final concentration) and NADH (0.6 mg) solution and the reaction was allowed to proceed for 4 h. Results obtained from 3 independent experiments with duplicates are shown as the mean ± SD. Asterisk indicates statistical significance at P<0.00001 (unpaired, two-tailed t test).
Table S1. MS-Based assignments of metabolites of 2 and 4 present in cell lipid extracts.

<table>
<thead>
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<th>Compound</th>
<th>Exp. Mass&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Calc. Mass&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Error (ppm)</th>
<th>Formula</th>
<th>RT (min)&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>2</td>
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<td>426.3947</td>
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<td>424.3791</td>
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<td>3.83&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>424.3791</td>
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<sup>a</sup>in ESI-positive mode. <sup>b</sup>RT, retention time. <sup>c</sup>identical to synthetic standards.

References

The image contains a chemical structure labeled as compound 10, along with a NMR spectrum graph. The structure includes atoms numbered 1 to 12, with specific groups such as Ph (phenyl) and OMe (methoxy). The spectrum graph shows peaks at various chemical shifts (f1) in ppm (parts per million).
O

N

O

O

P

h

O

M

e

H

O

B

o

1

Boc 11
14

\[ \text{N} \text{Boc} \text{O} \text{H} \text{OTBS} \]

f1 (ppm)

-2000 -1800 -1600 -1400 -1200 -1000 -800 -600 -400 -200 0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800 3000 3200 3400 3600 3800
18.23
23.37
25.86
27.37
28.14
28.69
61.19
62.08
63.54
74.44
94.69
156.80

O
N
O
O
T
B
S
16
17

\[ \text{f1 (ppm)} \]

\[
\begin{align*}
23.38 & \quad 27.34 & \quad 28.08 & \quad 28.73 & \quad 61.23 & \quad 61.82 & \quad 63.56 & \quad 74.50 & \quad 94.77 & \quad 156.75
\end{align*}
\]
23

\[ \text{O} \]
\[ \text{N} \]
\[ \text{O} \]

\[ \text{OH} \]

\[ \text{O} \]

\[ \text{O} \]

23

\[ \text{OH} \]

\[ \text{O} \]

\[ \text{O} \]

23

\[ \text{O} \]

\[ \text{N} \]

\[ \text{O} \]

23

\[ \text{OH} \]

\[ \text{O} \]

23

\[ \text{O} \]

\[ \text{N} \]

\[ \text{O} \]

23
\[
\text{HO-}\text{NH}_2
\]

\[
\overset{\text{32}}{\text{C=C=CHCH=CHCH=CHCH=CHCH=CHCH=CH}}\text{CH}_2\text{CH}_2\text{OH}
\]

\[
f_1 (\text{ppm})
\]

\[
\begin{align*}
28.9 & \quad 29.0 & \quad 29.1 & \quad 29.2 & \quad 29.3 & \quad 29.4 & \quad 29.5 \\
28.97 & \quad 29.08 & \quad 29.26 & \quad 29.36 & \quad 29.39 & \quad 29.41 & \quad 28.97
\end{align*}
\]

\[
\begin{align*}
73.42 & \quad 62.95 & \quad 56.72 & \quad 31.47 & \quad 27.31 & \quad 23.33 & \quad 13.08
\end{align*}
\]

\[
\begin{align*}
13.08 & \quad 22.33 & \quad 27.31 & \quad 31.67 & \quad 56.72 & \quad 62.95 & \quad 73.42
\end{align*}
\]