

Epimerization of an ascaroside-type glycolipid downstream of the canonical β -oxidation cycle in the nematode *Caenorhabditis nigoni*

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EXPERIMENTAL PROCEDURES

Preparation of exometabolome extracts. Wild-type *Caenorhabditis nigoni* JU1422 (collected in Trivandrum, Kerala, India on 21 Dec 2007, obtained from the *Caenorhabditis elegans* Genetics Center (CGC)) was cultivated at 23 °C on NGM agar seeded with *Escherichia coli* OP50 (uracil auxotroph). Mixed stage nematodes from five 10 cm plates collected in M9 buffer served as inoculums for liquid cultures grown in 100 ml S-medium at 23 °C and 150 rpm. Concentrated *E. coli* OP50 bacteria pellet from an overnight culture in LB medium at 37 °C and 170 rpm was provided as food from day 1 to day 7, after which the cultures were starved for 7 days. After 14 days, nematodes were separated by centrifugation (5 min at 5000 g). The filtered supernatant representing the exometabolome was frozen at -80 °C, lyophilized, and extracted with 3 x 100 ml methanol for 12 h each. The combined extract was filtered, concentrated to dryness at 40 °C under reduced pressure, reconstituted in 1 ml methanol, and aliquots were analyzed by HPLC-HRMS/MS, and HPLC-MS/MS precursor ion scanning.

Liquid Chromatography-Electrospray Ionization-High Resolution Tandem Mass Spectrometry. HPLC-ESI-HRMS/MS analysis of crude nematode exometabolome extracts and *C. nigoni* exometabolome fractions was performed using a Dionex UltiMate 3000 HPLC instrument coupled to a Bruker Maxis ultrahigh resolution (UHR) qTOF mass spectrometer equipped with an electrospray ionization (ESI) unit operated in positive or negative mode. Chromatographic separations were achieved using an Agilent ZORBAX Eclipse XDB-C18 column (250 x 3 mm, 5 µm particle diameter) with a flow rate of 400 µl/min and gradient elution starting at 3% acetonitrile in 0.5% aqueous acetic acid for 5 minutes followed by a linear increase to 100% acetonitrile with 0.5% acetic acid within 35 minutes. Data were analyzed with the Compass DataAnalysis 4.3 software (Bruker).

Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry Precursor Ion Scanning. HPLC-MS/MS precursor ion scanning for the ascaroside-specific fragment ion at m/z 73.0 [$C_3H_5O_3$] was performed using an Agilent 1260 HPLC instrument (Agilent Technologies) coupled to an API5000 Triple Quadrupole mass spectrometer (AB Sciex, Darmstadt) equipped with an electrospray ionization (ESI) unit operated in negative mode. Chromatographic separations were achieved using an Agilent ZORBAX Eclipse XDB-C18 column (50 x 4.6 mm, 1.8 µm particle diameter) (Agilent Technologies) with a flow rate of 1.1 ml/min and gradient elution starting at 5% acetonitrile in 0.05% aqueous formic acid followed by a linear increase to 95% acetonitrile with 0.05% formic acid within 10 minutes. Data were analyzed with the Analyst 1.6 software (AB Sciex).

NMR spectroscopy. NMR spectra were recorded at 400 MHz for 1H and 100 MHz for ^{13}C using a Bruker AVANCE III HD 400 instrument equipped with a 5 mm BBFO probe (MPICE) or Bruker AVANCE II 400 instrument equipped with a 5 mm BBFO+ probe (NPAC, UniNE). Residual solvent signals were used as internal reference with 1H at 3.31 ppm and ^{13}C at 49.05 ppm for CD_3OD or 1H at 7.26 ppm and ^{13}C at 77.16 ppm for $CDCl_3$. Two-dimensional high-resolution double quantum filtered (*dqf*)-COSY spectra were recorded using phase cycling for coherence selection. Spectra were zero-filled prior to Fourier transformation, phased manually, and baseline corrected using the Topspin 3.2 (Bruker) and MNova 9.0 (Mestrelab Research) software.

Isolation of cae-7OH- Δ C9 (11) from *C. nigoni*. The hydroxyacyl caenorhabdoside cae-7OH- Δ C9 (11) was isolated from 1.6 L of *Caenorhabditis nigoni* (JU1422) liquid culture supernatant. The filtered medium was frozen at -80 °C, lyophilized, and the powdered residue extracted with 3 x 100 ml methanol for 12 h each. The filtered extract was concentrated to dryness under reduced pressure and the resulting exometabolome extract was adsorbed onto 2 g of celite and fractionated by solid phase extraction on 5 g RP-C18 cartridges (Chromabond, Macherey-Nagel) using increasing concentrations of methanol in water as eluent (0-100% in 10% steps) to afford 10 fractions of 20 ml each that were concentrated under reduced pressure. The 40% methanol fraction containing the target component according to HPLC-ESI(-)-HR-MS was subsequently submitted to semi-preparative HPLC using an Agilent HP-1100 HPLC instrument equipped with a Grom-Sil 120 ODS-4 HE column (250 x 8 mm, 5 μ m) coupled to a Gilson 206 Abimed fraction collector. A flow rate of 2 ml/min with gradient elution was used starting at 3% acetonitrile in 0.5% aqueous acetic acid for 3 minutes, followed by a linear increase to 100% acetonitrile with 0.5% acetic acid within 30 minutes. Aliquots of 10 μ l were analyzed by HPLC-ESI(-)-HR-MS as described before. The fractions containing the target compound (Figure S2) cae-7OH- Δ C9 (11) along with co-eluting asc-C7 (ascr#1, 9, n = 3) were concentrated to dryness, dissolved in 600 μ l CD₃OD, and analyzed by one- and two-dimensional NMR spectroscopy (Figure S4).

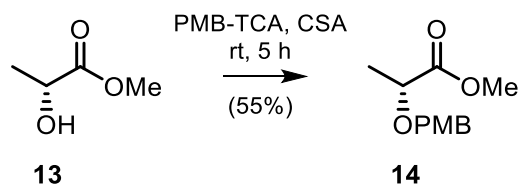
(7R,8R,2E)-threo-8-[(3',6'-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH- Δ C9, 11) isolated from the *C. nigoni* exometabolome (approximately 275 μ g, c = 550 nmol/L), for ¹H and ¹³C NMR data see table 1; HRMS (ESI-TOF) *m/z* (M – H)⁻ calcd for C₁₅H₂₅O₇ 317.1606, found 317.1603.

(6R,2E)-6-[(3',6'-dideoxy- α -L-arabino-hexopyranosyl)oxy]-heptanoic acid (asc-C7, 9, n = 3) isolated from the *C. nigoni* exometabolome (approximately 350 μ g, c = 800 nmol/L), HRMS (ESI-TOF) *m/z* (M – H)⁻ calcd for C₁₃H₂₃O₆ 275.1500, found 275.1502.

Retention assay to evaluate nematode behavioral response. Nematode preference for environments conditioned with known amounts of hydroxyacyl caenorhabdoside (cae-7OH- Δ C9, 11) and hydroxyacyl ascaroside (asc-7OH- Δ C9, 10) was measured using a retention assay. On a 6 cm Petri dish filled with 6 ml peptone-free NGM agar, circular scoring regions with 9 mm diameter were marked. Next, 1 μ l of 10% aqueous methanol (as solvent control) or solutions of cae-7OH- Δ C9 (11) or a 1:1 mixture of cae-7OH- Δ C9 (11) and asc-7OH- Δ C9 (10) in biologically relevant concentration ranging from a 100 pM – 1 μ M in 10% aqueous methanol were placed in the center of the scoring areas and left to dry for 5 minutes. Young adult nematodes from non-starved and non-crowded 6 cm NGM agar plates seeded with *E. coli* OP50 were sorted by sex and transferred to peptone-free NGM agar without food for approximately 1 h before being used for the assay to minimize the amount of concomitant bacteria. Individual worms were placed into the center of the conditioned scoring region and the time required for the nematodes to leave the scoring region was recorded. Nematodes were defined to have left the scoring area when no part of the nematode was still within the circular boundary. A total number of 10 worms per condition were analyzed and experiments were repeated on two separate days. A one-way ANOVA with Dunnett's post-test was performed between subjects using the SPSS statistics software version 26 (IBM) to evaluate the effect of ascarosides on mean times nematode spent in scoring regions. No significant difference between test and control spot was observed.

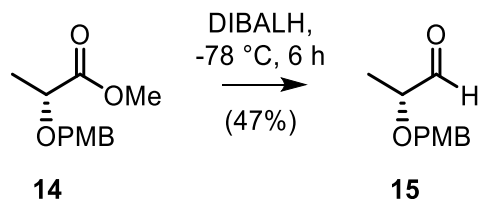
Two-spot *E. coli* preference assay to evaluate nematode behavioral response. Nematode preference for environments conditioned with known amounts of caenorhabdoside (cae-7OH- Δ C9, **11**) was measured using a two-spot preference assay. In a 3 cm Petri dish filled with 3.0 ml peptone-free NGM agar, two 10 μ l spots of washed *E. coli* OP50 bacteria from an overnight culture in LB grown at 37 °C in M9 phosphate buffer (1:2, v/v) were placed in 2 cm distance. Next, 2 μ l of 10% aqueous methanol (as solvent control) or cae-7OH- Δ C9 (**11**) solutions in biologically relevant concentration of 1 μ M, 10 nM, and 100 pM in 10% aqueous methanol were placed in the center of the *E. coli* spots and left to dry for 5 minutes. Young adult nematodes from non-starved and non-crowded 6 cm NGM agar plates seeded with *E. coli* OP50 were sorted by sex and transferred to peptone-free NGM agar without food for approximately 1 h before being used for the assay to minimize the amount of concomitant bacteria. Next, 10 male or female worms were placed in equidistance to the test and control region onto the agar. Distribution of nematodes in the two scoring regions was determined after 1 h and 6 h and the attraction indices determined ($AI = (\text{numbers of worms in compound treated spot} - \text{numbers of worms in the control spot}) / (\text{total number of worms in both spots})$). All experiments were performed in triplicate and repeated on two separate days with comparable results. A one-way ANOVA with Dunett's post-test was performed between subjects using the SPSS statistics software version 26 (IBM) to evaluate the effect of ascarosides on mean times nematode spent in scoring regions. No significant difference between test and control spot was observed.

Synthesis of (*R*)-methyl 2-(4-methoxybenzyloxy)propanoate (**14**).



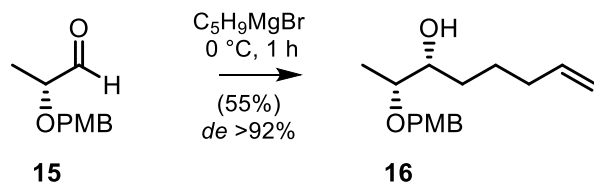
A solution of methyl (*R*)-(+)-lactate (**13**, 1.0 g, 9.6 mmol) in dichloromethane (2 mL) was treated with 4-methoxybenzyl-2,2,2-trichloroacetimidate (4.8 g, 19 mmol) and camphor-10-sulfonic acid (0.5 g, 2.15 mmol). The mixture was stirred for 5 h at room temperature then quenched with methanol and saturated aqueous NaHCO₃ solution. The organic phase was separated, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 10% ethyl acetate in hexane (v/v) as eluent to afford **14** (1.18 g, 5.3 mmol 55% yield). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.29 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.61 (d, *J* = 11.3 Hz, 1H), 4.39 (d, *J* = 11.3 Hz, 1H), 4.05 (q, *J* = 6.9 Hz, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 1.42 (d, *J* = 6.9 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm) 173.9, 159.5, 129.7, 129.7, 113.9, 73.7, 71.8, 55.4, 52.0, 18.8. For NMR spectra see: [1].

Synthesis of (*R*)-2-(4-methoxybenzyloxy)propanal (**15**).



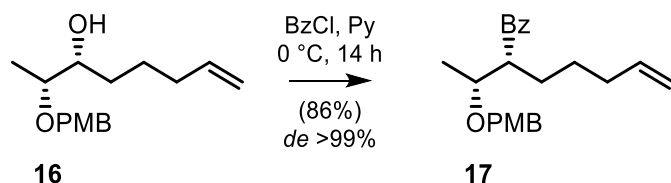
Under N₂ atmosphere a solution of **14** (1.18 g, 5.4 mmol) in dichloromethane (17 mL) at -78 °C was treated dropwise with 1 M diisobutylaluminum hydride (DIBALH) solution in toluene (5.8 mL, 5.8 mmol). After 6 h the reaction was quenched with methanol (0.9 mL) and saturated aqueous potassium sodium tartrate solution (1.8 mL), then stirred for 1 h. The organic phase was extracted with dichloromethane, dried over Na₂SO₄, concentrated under reduced pressure and purified by flash column chromatography on silica gel using 9% ethyl acetate in toluene (v/v) as eluent to afford **15** (494 mg, 47% yield) as a yellowish oil that was directly used for the next step. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 9.63 (d, *J* = 1.8 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.54 (d, *J* = 11.4 Hz, 1H), 3.87 (dq, *J* = 1.8 Hz, *J* = 7.0 Hz, 1H), 3.80 (s, 3H), 1.31 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm) 203.7, 159.7, 129.8, 129.5, 114.1, 79.3, 71.9, 55.4, 15.4. For NMR spectra see: [1].

Synthesis of (2*R*,3*R*)-*threo*-3-hydroxy-2-(4-methoxybenzyloxy)-7-octene (**16**).



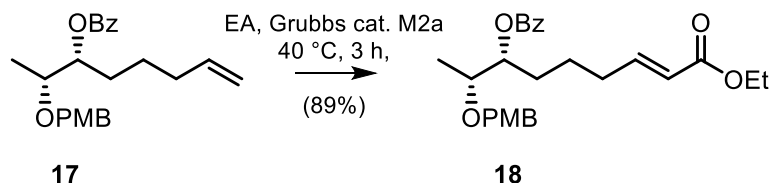
Under N_2 atmosphere at $0\text{ }^\circ\text{C}$, a solution of 4-pentenylmagnesium bromide (5.1 mmol) in diethyl ether (10 ml) (prepared from 5-bromo-1-pentene (767 mg, 5.1 mmol) and magnesium (128 mg, 5.3 mmol)) was treated dropwise with **15** (494 mg, 2.5 mmol) in diethyl ether (1.7 ml). After stirring at $0\text{ }^\circ\text{C}$ for 1 h the reaction was quenched with NH_4Cl solution. The organic phase was extracted with ethyl acetate dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as eluent to afford **16** as a yellowish oil (363 mg, 55% yield) with a diastereomeric excess of $de >92\%$ as determined by ^1H NMR [1]. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 7.26 (d, $J = 8.6\text{ Hz}$, 2H), 6.88 (d, $J = 8.6\text{ Hz}$, 2H), 5.80 (ddt, $J = 16.9$, $J = 10.2\text{ Hz}$, $J = 6.7\text{ Hz}$, 1H), 5.00 (d.br, $J = 17.1\text{ Hz}$, 1H), 4.95 (ddt, $J = 10.2\text{ Hz}$, $J = 2.3\text{ Hz}$, $J = 1.2\text{ Hz}$, 1H), 4.60 (d, $J = 11.2\text{ Hz}$, 1H), 4.36 (d, $J = 11.1\text{ Hz}$, 1H), 3.80 (s, 3H), 3.41 (ddd, $J = 8.0\text{ Hz}$, $J = 6.6\text{ Hz}$, $J = 3.1\text{ Hz}$, 1H), 3.35 (dq, $J = 6.0\text{ Hz}$, $J = 6.3\text{ Hz}$, 1H), 2.15 – 1.99 (m, 2H), 1.67 – 1.54 (m, 1H), 1.54 – 1.36 (m, 4H), 1.17 (d, $J = 6.1\text{ Hz}$, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 159.4, 138.9, 130.6, 129.5, 114.6, 114.0, 78.2, 74.9, 70.8, 55.3, 33.8, 32.4, 24.9, 15.7. For NMR spectra see: [1].

Synthesis of (2*R*,3*R*)-*threo*-3-benzoyloxy-2-(4-methoxybenzyloxy)-7-octene (**17**).



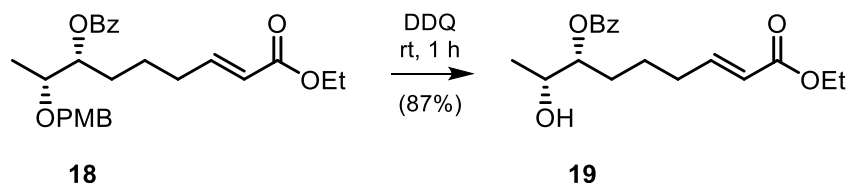
A solution of **16** (363 mg, 1.37 mmol) and dry pyridine (331 μL , 4.11 mmol) in dichloromethane (1 mL) at $0\text{ }^\circ\text{C}$ was treated with a solution of benzoyl chloride (320 μL , 2.74 mmol) in dichloromethane (10 mL). After stirring overnight at room temperature, the mixture was diluted with dichloromethane (10 mL), washed with 1 M HCl (10 mL) and saturated aqueous NaHCO_3 solution (10 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 10% ethyl acetate in hexane (v/v) as eluent to afford **17** (435.5 mg, 86% yield) as a colorless oil with a diastereomeric excess of $de >99\%$ as determined by ^1H NMR [1]. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 8.09 (d, $J = 7.6\text{ Hz}$, 2H), 7.59 (m, 1H), 7.47 (m, 2H), 7.26 (d, $J = 8.5\text{ Hz}$, 2H), 6.86 (d, $J = 8.5\text{ Hz}$, 2H), 5.79 (ddt, $J = 17.0\text{ Hz}$, $J = 10.3\text{ Hz}$, $J = 6.7\text{ Hz}$, 1H), 5.25 (m, 1H), 5.01 (d, $J = 17.1\text{ Hz}$, 1H), 4.96 (d, $J = 10.3\text{ Hz}$, 1H), 4.62 (d, $J = 11.5\text{ Hz}$, 1H), 4.49 (d, $J = 11.5\text{ Hz}$, 1H), 3.81 (s, 3H), 3.74 (dq, $J = 4.9\text{ Hz}$, $J = 6.3\text{ Hz}$, 1H), 2.10 (m, 2H), 1.77 (m, 2H), 1.46 (m, 2H), 1.23 (d, $J = 6.4\text{ Hz}$, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 166.4, 159.3, 138.5, 133.0, 130.7, 129.8, 129.4, 129.0, 128.5, 114.9, 113.9, 76.1, 74.6, 70.9, 55.4, 33.7, 29.0, 25.0, 15.5. For NMR spectra see: [1].

Synthesis of (7*R*,8*R*,2*E*)-threo-ethyl 7-benzoyloxy-8-(4-methoxybenzyloxy)-2-nonenolate (18).



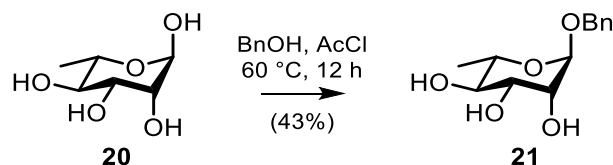
A solution of **17** (435.5 mg, 1.18 mmol) and ethyl acrylate (643 μL , 5.9 mmol) in dichloromethane (35 mL) was treated with Grubbs second generation catalyst M2a (C848) (59 mg, 70 μmol) and stirred at 40 $^\circ\text{C}$ for 3 h. The solution was cooled to room temperature and then concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as eluent to afford **18** (460 mg, 89% yield) as a brownish oil. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 8.07 (d, $J = 7.2$ Hz, 2H), 7.57 (m, 1H), 7.47 (m, 2H), 7.25 (d, $J = 8.6$ Hz, 2H), 6.93 (dt, $J = 15.6$ Hz, $J = 7.0$ Hz, 1H), 6.86 (d, $J = 8.6$ Hz, 2H), 5.82 (d, $J = 15.6$ Hz, 1H), 5.24 (m, 1H), 4.62 (d, $J = 11.5$ Hz, 1H), 4.48 (d, $J = 11.5$ Hz, 1H), 4.19 (q, $J = 7.0$ Hz, 2H), 3.81 (s, 3H), 3.73 (dq, $J = 4.6$ Hz, $J = 6.4$ Hz, 1H), 2.23 (m, 2H), 1.78 (m, 2H), 1.52 (m, 2H), 1.30 (t, $J = 6.9$ Hz, 3H), 1.23 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 166.7, 166.4, 159.3, 148.6, 133.1, 130.7, 130.4, 129.8, 129.5, 128.5, 121.8, 113.9, 75.8, 74.4, 70.9, 60.3, 55.4, 32.1, 29.1, 24.2, 15.4, 14.4. For NMR spectra see: [1].

Synthesis of (7*R*,8*R*,2*E*)-threo-ethyl 7-benzoyloxy-8-hydroxy-2-nonenolate (19).



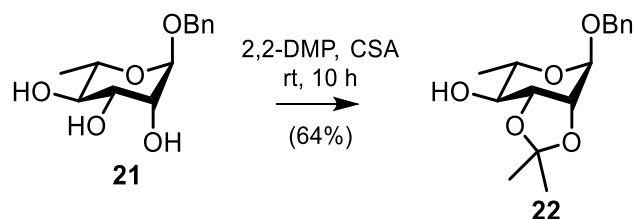
A solution of **18** (480 mg, 1.04 mmol) in dichloromethane (7.5 mL) was treated with water (376 μL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (254 mg, 1.14 mmol). The mixture was stirred for 1 h at room temperature then quenched with water (2 mL). The organic phase was extracted with dichloromethane, dried with Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as eluent to afford **19** (290 mg, 87% yield) as an orange oil. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 8.05 (m, 2H); 7.57 (m, 1H), 7.45 (m, 2H), 6.91 (dt, $J = 15.7$ Hz, $J = 7.0$ Hz, 1H), 5.80 (d, $J = 15.7$ Hz, 1H), 5.05 (dt, $J = 7.8$ Hz, $J = 5.0$ Hz, 1H), 4.16 (q, $J = 7.2$ Hz, 2H), 3.94 (dq, $J = 4.8$ Hz, $J = 6.4$ Hz, 1H), 2.23 (m, 2H), 1.77 (m, 2H), 1.55 (m, 2H), 1.26 (t, $J = 7.0$ Hz, 3H), 1.23 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 166.7, 166.6, 148.4, 133.3, 130.1, 129.8, 128.6, 121.9, 77.9, 69.0, 60.3, 32.0, 30.2, 24.1, 19.6, 14.4. For NMR spectra see: [1].

Synthesis of *O*-benzyl-6-deoxy- α -L-arabino-hexopyranoside (**21**).



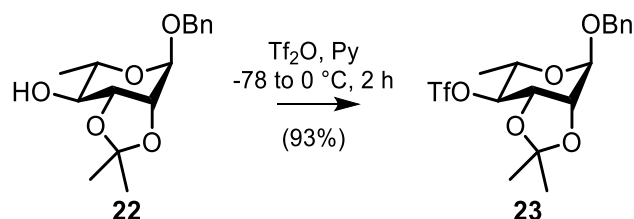
A suspension of L-rhamnose monohydrate (**20**, 5.0 g, 27.4 mmol) in benzyl alcohol (27 mL) was treated with acetyl chloride (8.6 mL, 121.5 mmol). After stirring overnight at 60 °C the reaction was quenched with K₂CO₃ (10.0 g, 72.4 mmol) and stirred for 1 h. The mixture was diluted with ethyl acetate (55 mL) and the precipitating salt removed by filtration and washed with ethyl acetate (2 x 30 mL). The filtrate was concentrated under reduced pressure and the residue fractionated by flash column chromatography on silica gel using a stepwise gradient from 0 to 5% methanol in dichloromethane (v/v) as eluent to afford **21** (3.0 g, 11.8 mmol, 43% yield, α/β mixture: 92/8) as a yellow oil. ¹H NMR (400 MHz, CD₃OD): δ (ppm) 7.34 (m, 5H), 7.29 (m, 1H), 4.76 (d, *J* = 1.8 Hz, 1H), 4.69 (d, *J* = 11.8 Hz, 1H), 4.50 (d, *J* = 11.8 Hz, 1H), 3.82 (dd, *J* = 3.5 Hz, *J* = 1.7 Hz, 1H), 3.68 (dd, *J* = 9.5 Hz, *J* = 3.5 Hz, 1H), 3.62 (m, 1H), 3.39 (t, *J* = 9.5 Hz, 1H), 1.27 (d, *J* = 6.3 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD): δ (ppm): 139.1, 129.4, 129.0, 128.8, 100.9, 74.0, 72.5, 72.3, 70.04, 70.01, 18.0. For comparison see: [2].

Synthesis of *O*-benzyl-6-deoxy-2,3-(*O*-isopropylidene)- α -L-arabino-hexopyranoside (**22**).



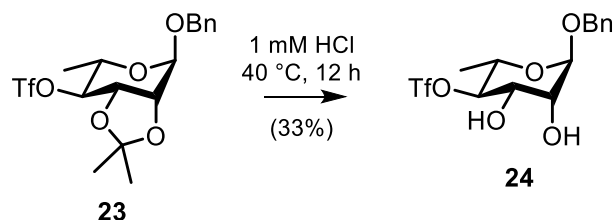
Under N₂ atmosphere a solution of **21** (3.0 g, 11.8 mmol) in dry acetone (50 mL) was treated with 2,2-dimethoxypropane (8 mL, 10.1 mmol) and camphor-10-sulfonic acid (271 mg, 1.18 mmol). After stirring at room temperature for 10 h the reaction was quenched with aqueous 1 M sodium hydroxide solution and concentrated under reduced pressure. The residue was dissolved in dichloromethane and washed with water. The combined organic phases were washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was fractionated by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as eluent to afford **22** (2.2 g, 7.5 mmol, 64% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.34 (m, 4H), 5.05 (s, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.19 (d, *J* = 5.7 Hz, 1H), 4.11 (dd, *J* = 7.2 Hz, *J* = 5.7 Hz, 1H), 3.74 (m, 1H), 3.42 (dd, *J* = 9.2 Hz, *J* = 7.2 Hz, 1H), 1.52 (s, 3H), 1.35 (s, 3H), 1.31 (d, *J* = 6.3 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 137.2, 128.7, 128.4, 128.1, 109.7, 96.5, 78.5, 76.0, 74.7, 69.4, 66.3, 28.1, 26.3, 17.6. For comparison see: [2].

Synthesis of O-benzyl-6-deoxy-2,3-O-(isopropylidene)-4-trifluoromethanesulfonate- α -L-arabino-hexopyranoside (23**).**



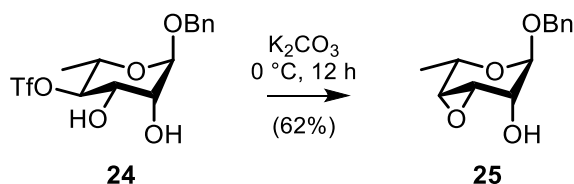
Under N_2 atmosphere a solution of **22** (2.2 g, 7.5 mmol) in dichloromethane (15 mL) at $-78\text{ }^{\circ}\text{C}$ was treated dropwise with anhydrous pyridine (1.83 mL, 22.5 mmol) followed by trifluoromethanesulfonic anhydride (1.88 mL, 11.3 mmol). The mixture was stirred at $-78\text{ }^{\circ}\text{C}$ for 10 min and then at $0\text{ }^{\circ}\text{C}$ for 2 h, after which it was diluted with dichloromethane (15 mL) and washed with cold 1 M HCl (15 mL) and water (15 mL). The organic phase was concentrated under reduced pressure and the residue fractionated by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as eluent to afford **23** (3.0 g, 7.0 mmol, 93% yield) as an orange oil. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 7.35 (m, 5H), 5.09 (s, 1H), 4.70 (d, $J = 11.8\text{ Hz}$, 1H), 4.56 (m, 2H), 4.32 (dd, $J = 7.6\text{ Hz}$, $J = 5.5\text{ Hz}$, 1H), 4.25 (d, $J = 5.4\text{ Hz}$, 1H), 3.95 (dq, $J = 10.0\text{ Hz}$, $J = 6.4\text{ Hz}$, 1H), 1.54 (s, 3H), 1.36 (s, 3H), 1.33 (d, $J = 6.3\text{ Hz}$, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 136.6, 128.8, 128.4, 110.7, 96.1, 89.4, 75.1, 69.8, 63.3, 27.7, 26.4, 17.1. For comparison see: [2].

Synthesis of O-benzyl-6-deoxy-4-trifluoromethanesulfonate- α -L-arabino-hexopyranoside (24**).**



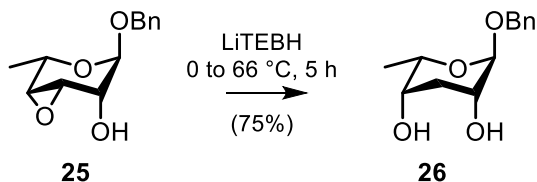
A solution of **23** (3.0 g, 7 mmol) in chloroform (20 mL) was treated with a diluted 1 mM hydrochloric acid solution (0.2 mL). After stirring overnight at $40\text{ }^{\circ}\text{C}$ the mixture was concentrated under reduced pressure and the residue diluted with dichloromethane (20 mL). The solution was washed with water (10 mL), dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was fractionated by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as eluent to afford **24** (900 mg, 2.3 mmol, 33% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 7.34 (m, 5H), 4.89 (s, 1H), 4.69 (m, 2H), 4.54 (d, $J = 11.9\text{ Hz}$, 1H), 4.09 (m, 2H), 3.98 (dq, $J = 9.7\text{ Hz}$, $J = 6.3\text{ Hz}$, 1H), 1.36 (d, $J = 6.3\text{ Hz}$, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 136.7, 128.8, 128.4, 128.2, 98.4, 88.8, 72.1, 69.8, 69.2, 65.4, 17.4. For comparison see: [2].

Synthesis of 3,4-anhydro-1-O-benzyl-6-dideoxy- α -L-lyxo-hexopyranoside (**25**).



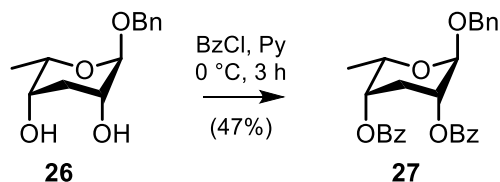
Under N_2 atmosphere a solution of **24** (900 mg, 2.33 mmol) in anhydrous methanol (20 mL) at 0 °C was treated with K_2CO_3 (900 mg, 2.6 mmol). After stirring overnight at room temperature, the mixture was concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 25% ethyl acetate in hexane (v/v) as eluent to afford **25** (345 mg, 1.46 mmol, 62% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 7.34 (m, 4H), 4.70 (d, $J = 11.8$ Hz, 1H), 4.64 (s.br, 1H), 4.51 (d, $J = 11.8$ Hz, 1H), 4.11 (q, $J = 6.5$ Hz, 1H), 3.81 (dd, $J = 11.3$, $J = 5.3$ Hz, 1H), 3.55 (t, $J = 4.8$ Hz, 1H), 3.22 (d, $J = 4.5$ Hz, 1H), 2.51 (d, $J = 11.2$ Hz, 1H), 1.37 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 137.2, 128.7, 128.2, 128.1, 98.5, 69.5, 63.3, 61.4, 54.9, 51.8, 17.5; HRMS (ESI-TOF) m/z ($\text{M} + \text{NH}_4$) $^+$ calcd for $\text{C}_{13}\text{H}_{20}\text{NO}_4$ 254.1387, found 254.1390; m/z ($\text{M} + \text{Na}$) $^+$ calcd for $\text{C}_{13}\text{H}_{16}\text{O}_4\text{Na}$ 259.0941, found 259.0945. For comparison see: [2,3].

Synthesis of O-benzyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**26**).



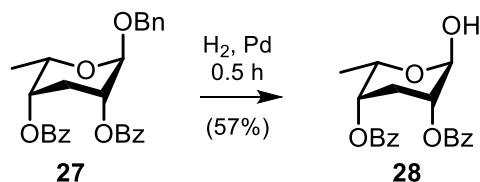
Under N_2 atmosphere, a solution of **25** (345 mg, 1.46 mmol) in dry THF (8 mL) at 0 °C was treated dropwise with 1.7 M lithium triethylborohydride (Super-Hydride[®]) solution in THF (8.3 mL, 14.6 mmol). The reaction was refluxed for 5 h, cooled to room temperature, and quenched with methanol (3 mL). After gas evolution ceased, the solution was poured into ice-cold water (85 mL) and fresh ice was added to the mixture followed by 30% (w/w) aqueous H_2O_2 solution (3.5 mL). The resulting mixture was stirred overnight, concentrated under reduced pressure, and the residue treated with saturated aqueous K_2CO_3 solution (10 mL) and extracted with chloroform. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was fractionated by flash column chromatography on silica gel using 50% ethyl acetate in hexane (v/v) as eluent to afford **26** (262 mg, 1.10 mmol, 75% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 7.35 (m, 4H), 7.29 (m, 1H), 4.81 (s, 1H), 4.74 (d, $J = 11.9$ Hz, 1H), 4.54 (d, $J = 11.8$ Hz, 1H), 3.95 (qd, $J = 6.6$ Hz, $J = 1.3$ Hz, 1H), 3.73 (s.br, 1H), 3.59 (s.br, 1H), 2.03 (m, 2H), 1.23 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 137.7, 128.5, 127.9, 127.8, 99.3, 69.1, 68.1, 66.8, 66.4, 31.4, 17.0. For comparison see: [3].

Synthesis of *O*-benzyl-2,4-di-*O*-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**27**).



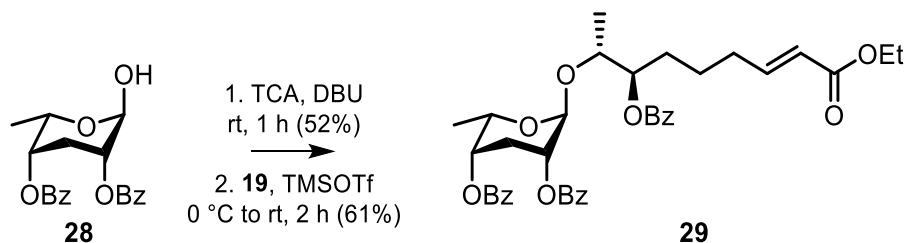
A solution of **26** (154 mg, 646 μmol) in dry pyridine (600 μL) at 0 $^{\circ}\text{C}$ was treated with benzoyl chloride (220 μL , 1.95 mmol). After stirring at 0 $^{\circ}\text{C}$ for 3 h the reaction was quenched with water (1 mL). The organic phase was extracted with hexane (3 x 1 mL), washed with 1.0 M hydrochloric acid (1 mL), dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was fractionated by flash column chromatography on silica gel using dichloromethane as eluent to afford **27** (136 mg, 304 μmol , 47% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 8.10 (m, 2H), 7.90 (m, 2H), 7.53 (m, 1H), 7.43 (m, 1H), 7.40 (m, 4H), 7.32 (m, 3H), 7.15 (m, 2H), 5.08 (s, 2H), 5.08 (s, 1H), 4.79 (d, $J = 11.9$ Hz, 1H), 4.65 (d, $J = 11.9$ Hz, 1H), 4.27 (dq, $J = 1.2$ Hz, $J = 6.6$ Hz, 1H), 2.46 (m, 2H), 1.29 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 166.4, 166.0, 137.4, 133.1, 133.0, 133.3, 130.0, 129.9, 128.7, 128.4, 128.2, 128.1, 97.0, 69.7, 68.3, 67.0, 65.2, 27.9, 17.0; HRMS (ESI-TOF) m/z ($\text{M} + \text{H}$) $^{+}$ calcd for $\text{C}_{27}\text{H}_{27}\text{O}_6$ 447.1802, found 477.1805; m/z ($\text{M} + \text{NH}_4$) $^{+}$ calcd for $\text{C}_{27}\text{H}_{30}\text{NO}_6$ 464.2068, found 464.2072.

Synthesis of 2,4-di-*O*-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranose (**28**).



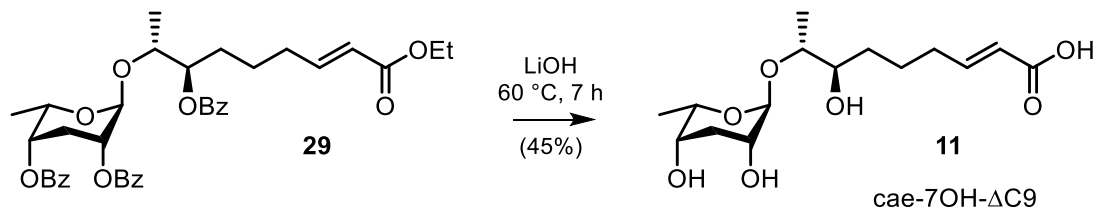
A solution of **27** (134 mg, 300 μmol) in methanol (3 mL) was treated with palladium black (10 mg). After stirring under hydrogen atmosphere (1 atm) for 30 min the mixture was filtered over a thin layer of silica and the catalyst rinsed with ethyl acetate (5 x 3 mL). The filtrate was concentrated under reduced pressure and the residue fractionated by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as eluent to afford **28** (60.8 mg, 171 μmol , 57% yield, α/β mixture: 7:3) as a white solid. ^1H NMR (400 MHz, CDCl_3) for the dominating α -anomer: δ (ppm) 8.09 (m, 2H), 7.99 (ddd, $J = 7.5$ Hz, $J = 3.9$ Hz, $J = 1.9$ Hz, 1H), 7.89 (m, 2H), 7.54 (m, 1H), 7.44 (m, 1H), 7.34 (td, $J = 7.9$ Hz, $J = 1.8$ Hz, 2H), 7.24 (m, 1H), 7.15 (m, 2H), 5.42 (s, 1H), 5.08 (s.br, 1H), 5.03 (s.br, 2H), 4.51 (qd, $J = 6.6$ Hz, $J = 3.4$ Hz, 1H), 2.48 (m, 2H), 1.30 (dd, $J = 6.6$ Hz, $J = 1.9$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 166.4, 166.2, 133.1, 133.1, 130.0, 129.6, 128.5, 128.2, 92.1, 68.3, 67.3, 64.9, 27.3, 17.1; HRMS (ESI-TOF) m/z ($\text{M} + \text{NH}_4$) $^{+}$ calcd for $\text{C}_{20}\text{H}_{24}\text{NO}_6$ 374.1598, found 374.1602; m/z ($\text{M} + \text{Na}$) $^{+}$ calcd for $\text{C}_{20}\text{H}_{20}\text{O}_6\text{Na}$ 379.1152, found 379.1147.

Synthesis of (7*R*,8*R*,2*E*)-threo-ethyl 7-benzoyloxy-8-[(2,4-di-*O*-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-2-nonenolate (29**).**



A solution of **28** (15 mg, 42.1 μmol) in dry dichloromethane (2 ml) was treated with trichloroacetonitrile (9 μl , 80 μmol) followed by 1,8-diazabicyclo-undec-7-ene (0.4 μl , 2.67 μmol). After stirring for 1 h the mixture was concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as eluent to afford the trichloroacetimidate (11 mg, 22.0 μmol , 52% yield) as a yellowish oil that was directly used for the next step. A solution of the trichloroacetimidate in dry dichloromethane (0.2 ml) was treated with **19** (12 mg, 42.2 μmol) in dichloromethane (0.3 ml). The mixture was cooled to 0 $^{\circ}\text{C}$ and treated with trimethylsilyl trifluoromethanesulfonate (0.8 μl). After stirring at room temperature for 2 h the reaction was quenched with saturated aqueous NaHCO_3 solution (0.1 mL) and diluted with dichloromethane. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as eluent to afford **29** (8.8 mg, 13.4 μmol , 61% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3): δ (ppm) 8.07 (m, 4H), 7.85 (m, 2H), 7.60 (m, 1H), 7.53 (m, 1H), 7.48 (m, 2H), 7.42 (m, 1H), 7.32 (m, 2H), 7.12 (m, 2H), 6.94 (dt, $J = 15.6$ Hz, $J = 6.9$ Hz, 1H), 5.83 (dt, $J = 15.6$ Hz, $J = 1.5$ Hz, 1H), 5.28 (dt, $J = 6.3$ Hz, $J = 6.0$ Hz, 1H), 5.10 (s, 1H), 4.93 (dd, $J = 3.8$ Hz, $J = 1.9$ Hz, 1H), 4.77 (s, 1H), 4.18 (q, $J = 7.1$ Hz, 2H), 4.06 (dq, $J = 6.2$ Hz, $J = 6.0$ Hz, 1H), 2.43 – 2.31 (m, 2H), 2.30 – 2.18 (m, 3H), 1.79 (q, $J = 7.4$ Hz, $J = 6.9$ Hz, 3H), 1.67 – 1.55 (m, 3H), 1.27 (d, $J = 6.2$ Hz, 3H), 1.28 (t, $J = 7.1$ Hz, 3H) 1.17 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) 166.7, 166.3, 166.2, 148.3, 133.4, 133.1, 133.0, 130.3, 130.0, 129.9, 129.8, 128.7, 128.4, 128.2, 122.1, 94.2, 75.9, 72.5, 68.2, 67.5, 65.3, 60.4, 32.0, 29.7, 27.7, 24.1, 16.9, 14.9, 14.4; HRMS (ESI-TOF) m/z ($\text{M} + \text{H}$) $^+$ calcd for $\text{C}_{38}\text{H}_{43}\text{O}_{10}$ 659.2851, found 658.2873; m/z ($\text{M} + \text{NH}_4$) $^+$ calcd for $\text{C}_{38}\text{H}_{46}\text{NO}_{10}$ 676.3116, found 676.3137.

Synthesis of (7*R*,8*R*,2*E*)-threo-8-[(3',6'-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (caen#1, **11).**



A solution of **29** (7.7 mg, 11.7 μ mol) in 1,4-dioxane (1 ml) was treated with a solution of lithium hydroxide monohydrate (9.2 mg, 230 μ mol) in water (0.1 ml). After stirring at 60 $^{\circ}$ C for 7 h, the mixture was cooled to room temperature, treated with glacial acetic acid (40 μ l), and concentrated under reduced pressure. The product was purified using semi-preparative Waters Breeze HPLC system (1525 binary HPLC pump with a 2487 dual wavelength absorbance detector) equipped with a reverse-phase XTerra MS C18 OBD column (19 \times 150 mm, 5 μ m) coupled with a Gilson FC 203B fraction collector. Separation was achieved using gradient elution with 15 to 100% (v/v) acetonitrile in water with 0.05% trifluoroacetic acid (v/v) over 60 minutes at a flow rate of 8 ml/min to afford **11** (1.7 mg, 5.3 μ mol, 45% yield) as a colorless oil showing LC-MS and NMR data identical to the natural product isolated from *C. nigoni*. δ (ppm) 6.91 (dt, J = 15.6 Hz, J = 7.0 Hz, 1H, 3'-H), 5.82 (dt, J = 15.6 Hz, J = 1.5 Hz, 1H, 2'-H), 4.78 (s, 1H, 1-H), 3.99 (qd, J = 6.6 Hz, J = 1.0 Hz, 1H, 5-H), 3.75 (dq, J = 4.1 Hz, J = 6.3 Hz, 1H, 8'-H), 3.58 (s.br, 1H, 2-H), 3.57 (s.br, 1H, 4-H), 3.52 (m, 1H, 7'-H), 2.26 (m, 1H, 4'-H), 2.06 (dt, J = 14.3 Hz, J = 3.1 Hz, 1H, 3-H_{ax}), 1.93 (ddt, J = 14.3 Hz, J = 1.0 Hz, J = 3.4 Hz, 1H, 3-H_{eq}), 1.70 (m, 1H, 5'-H), 1.62 – 1.45 (m, 3H, 5'-H, 6'-H), 1.18 (d, J = 6.6 Hz, 3H, 6-H), 1.15 (d, J = 6.3 Hz, 3H, 9'-H); ^{13}C NMR (100 MHz, CD₃OD): δ (ppm) 170.8 (1'-C), 150.0 (3'-C), 123.7 (2'-C), 98.7 (1-C), 75.7 (8'-C), 75.0 (7'-C), 69.1 (4-C), 68.5 (5-C), 68.2 (2-C), 33.02 (4'-C), 32.97 (6'-C), 32.3 (3-C), 25.8 (5'-C), 17.2 (6-C), 14.8 (9'-C); HRMS (ESI-TOF) m/z ($M - H$)⁻ calcd for C₁₅H₂₅O₇ 317.1606, found 317.1601; m/z ($M_2 - H$)⁻ calcd for C₃₀H₅₁O₁₄ 635.3284, found 635.3279.

Supporting Figures

Figure S1: Detection of hydroxyacyl 3,6-dideoxyhexopyranosides in the *Caenorhabditis nigoni* exometabolome using HPLC-MS/MS precursor ion scanning for m/z 73.0 [$C_3H_5O_2$] $^-$.

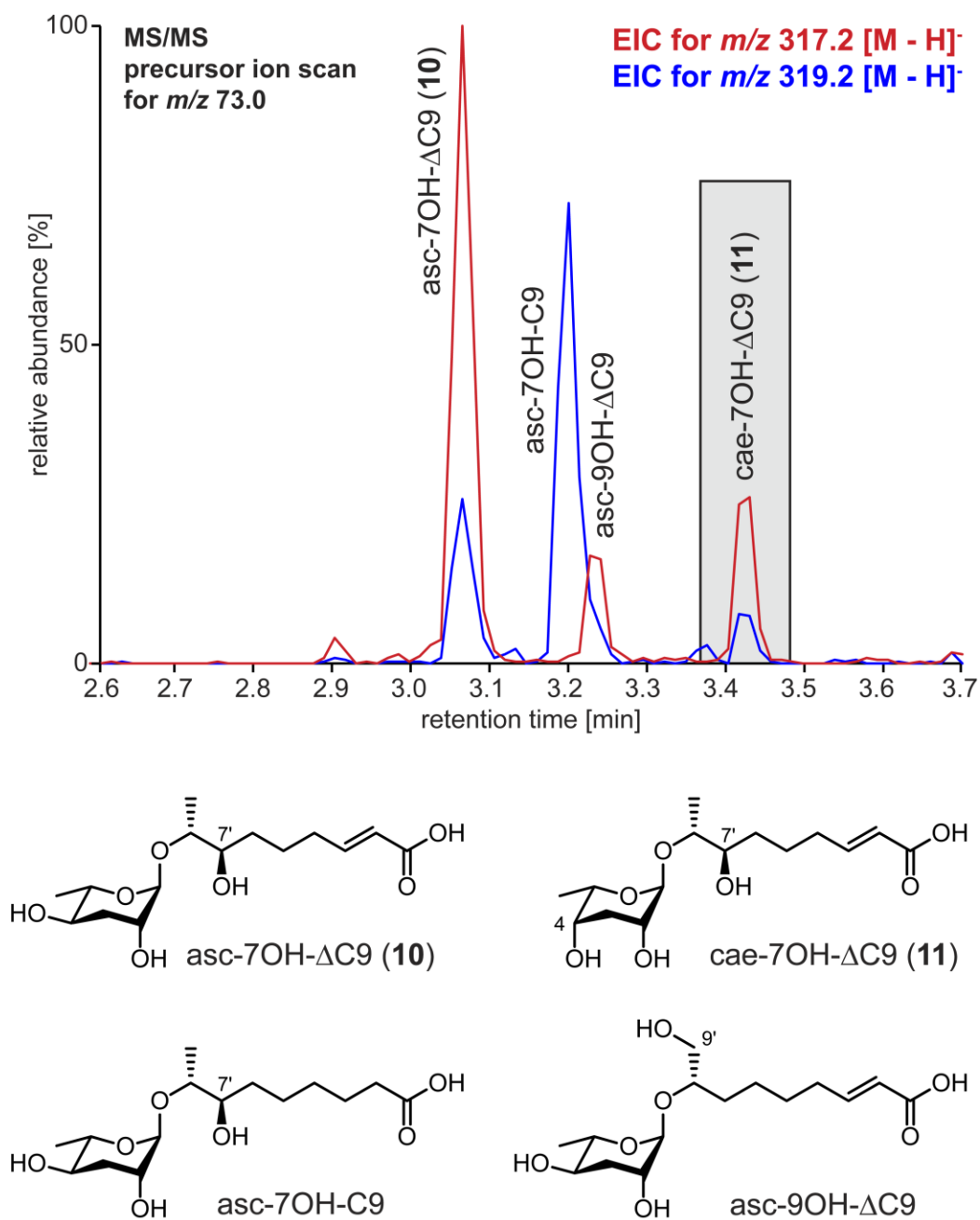


Figure S2: Comparative HPLC-ESI(-)-HR-MS analysis of two consecutive RP-C18 HPLC fractions from *C. nigoni* showing signals for asc-C7 (SMID: ascr#1) with m/z 275.1500 $[M - H]^-$ and cae-7OH- Δ C9 (SMID: caen#1, **11**) with m/z 317.1606 $[M - H]^-$.

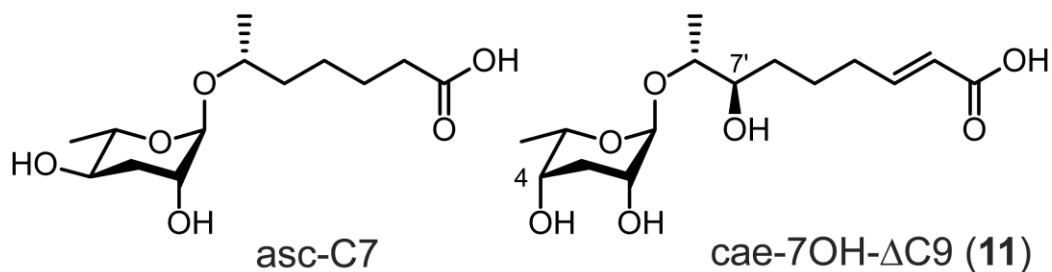
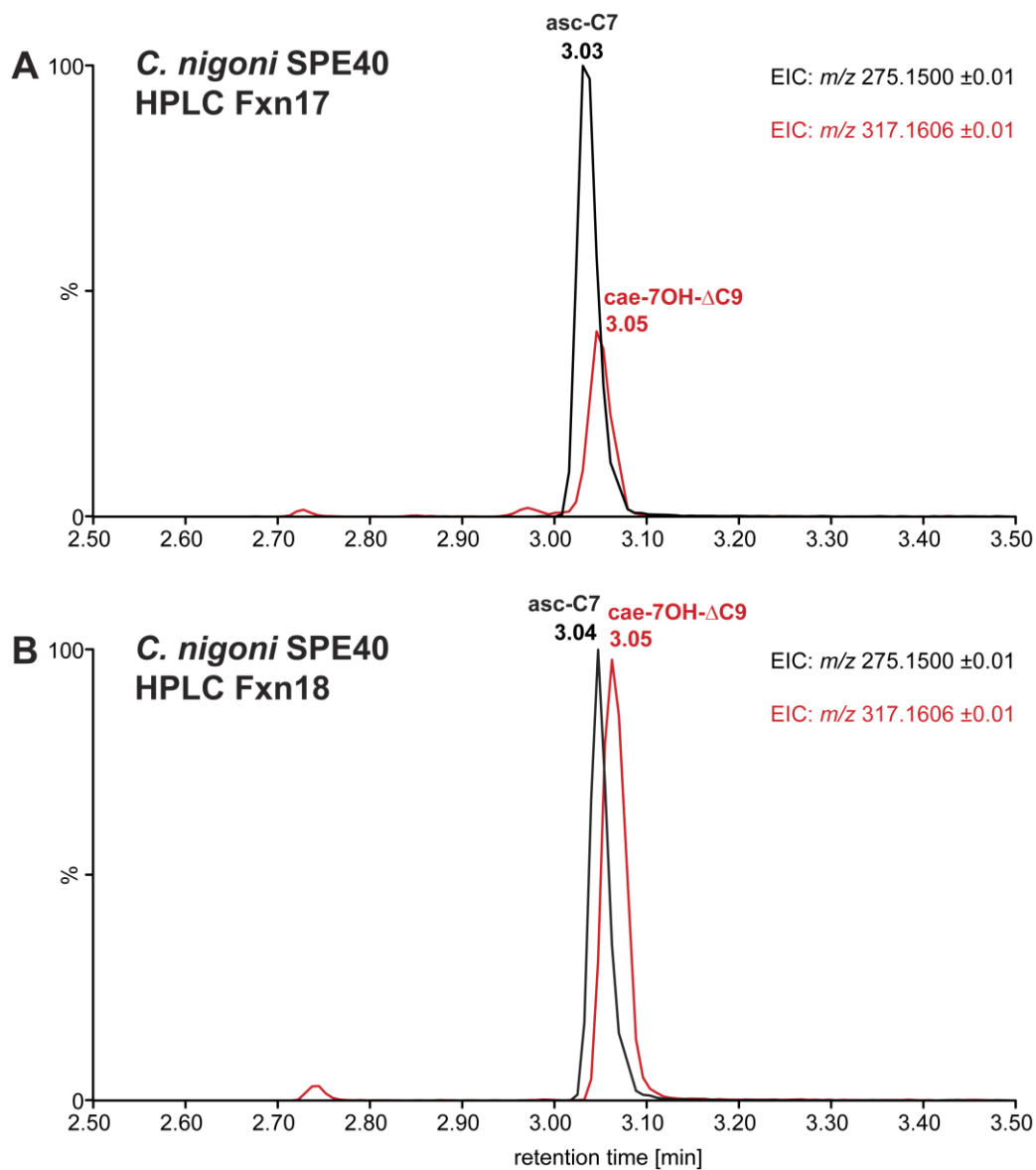


Figure S3: HPLC-ESI(-)-HR-MS/MS spectrum of cae-7OH- Δ C9 (caen#1, **11**) from *C. nigoni*.

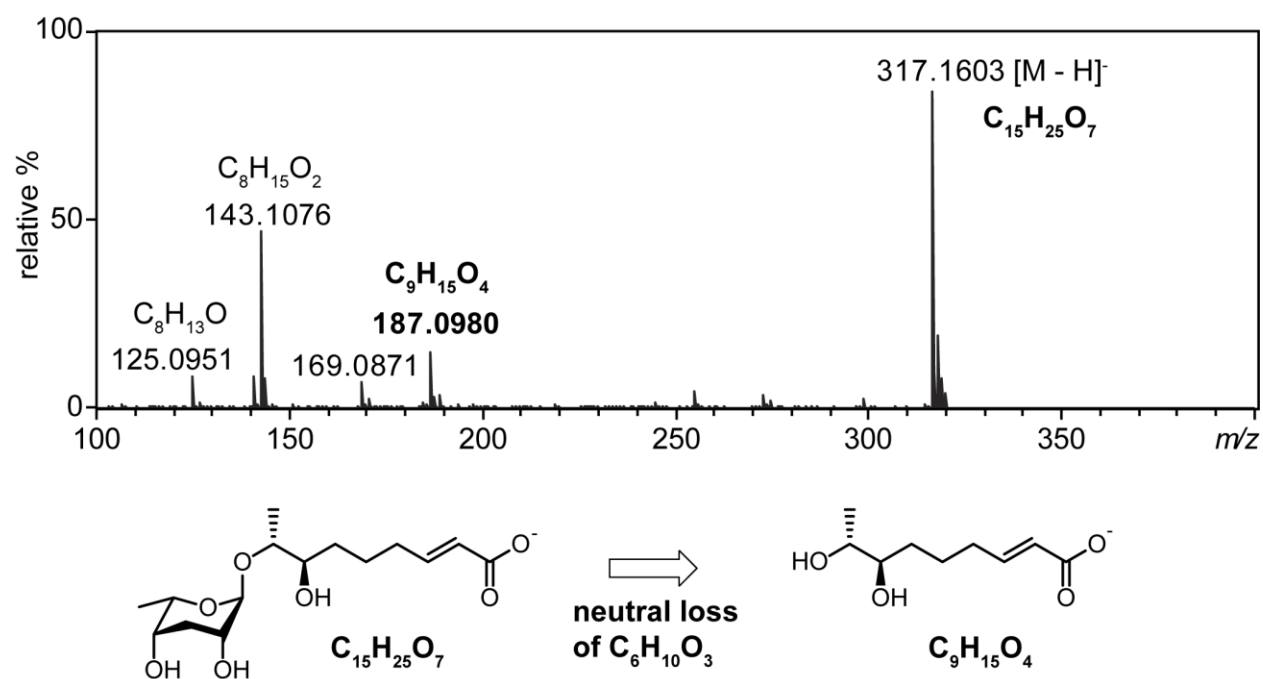


Figure S4a: Comparative analysis of 400 MHz ^1H NMR spectra of two consecutive RP-C18 HPLC fractions from *C. nigoni* highlights signals corresponding to asc-C7 and cae-7OH- ΔC9 (**11**).

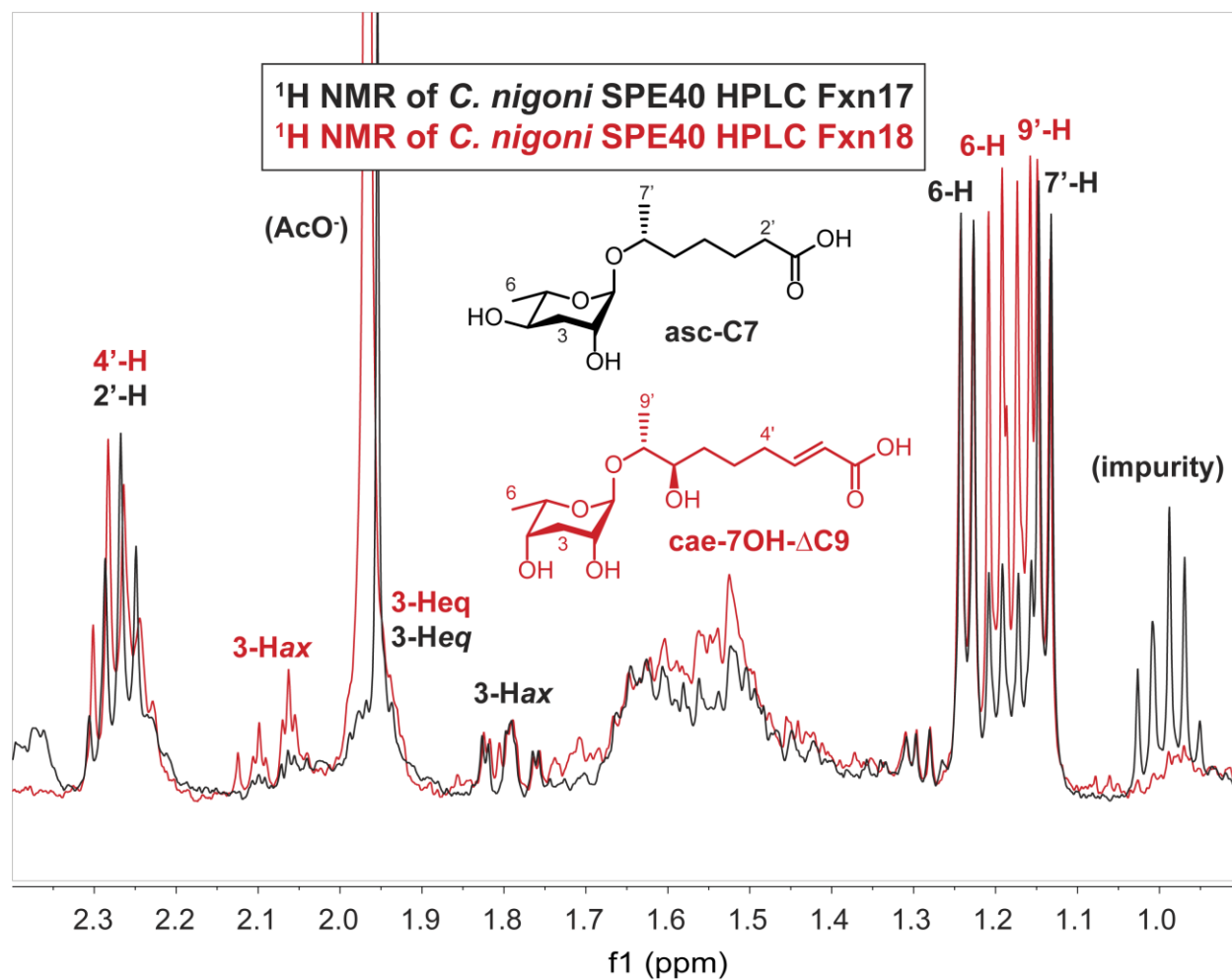


Figure S4b: Comparative analysis of 400 MHz ^1H NMR spectra of two consecutive RP-C18 HPLC fractions from *C. nigoni* highlights signals corresponding to asc-C7 and cae-7OH- ΔC9 (11).

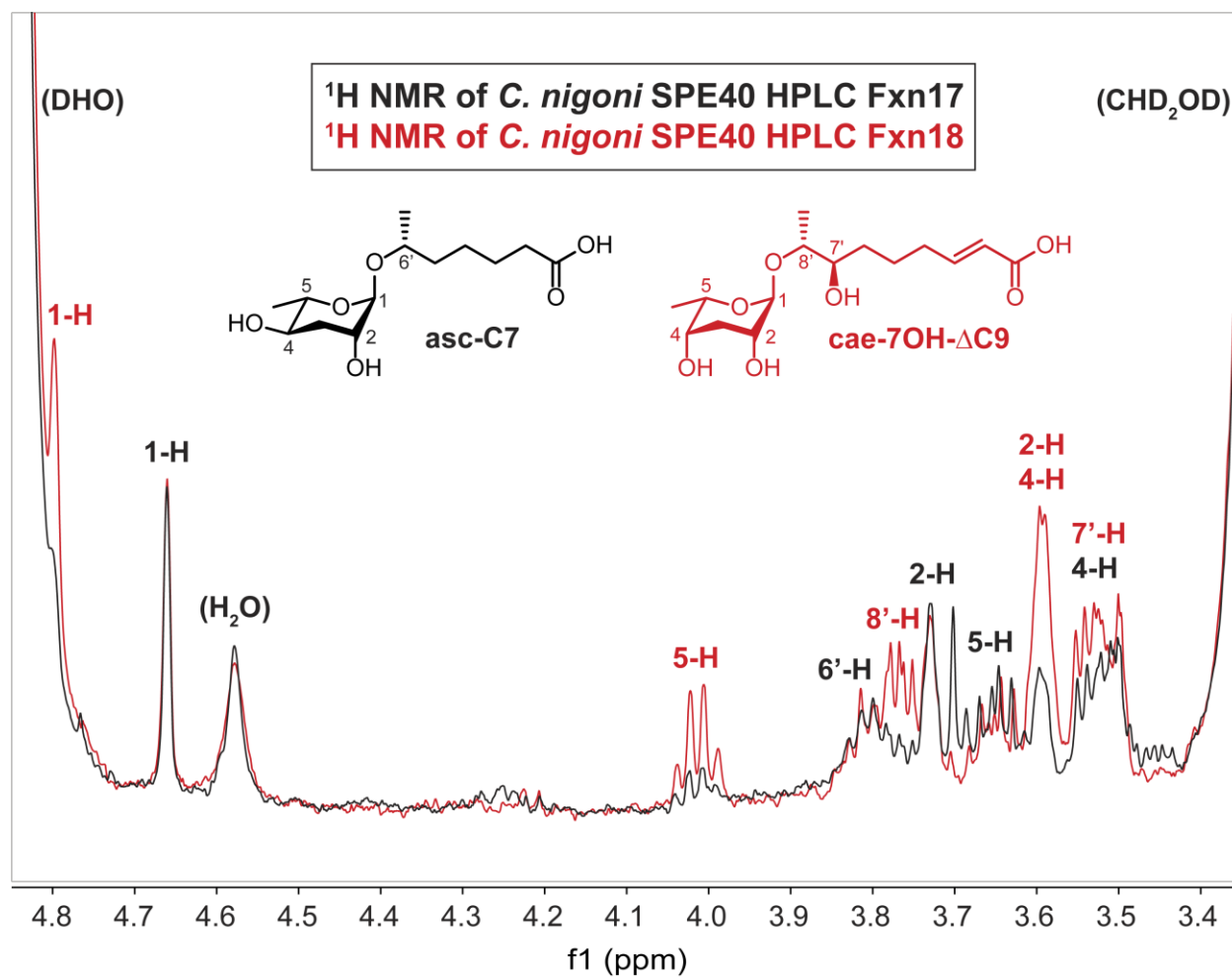


Figure S5: Comparative analysis of 400 MHz *dqf*-COSY spectra of (A) natural cae-7OH- Δ C9 (**11**) isolated from *C. nigoni*; (B) synthetic *threo*-asc-7OH- Δ C9 (**10**) identified in *C. nigoni* [1], and (C) synthetic *erythro*-asc-7OH- Δ C9 [1] (in CD₃OD) indicates the *threo*-configuration of natural **11**.

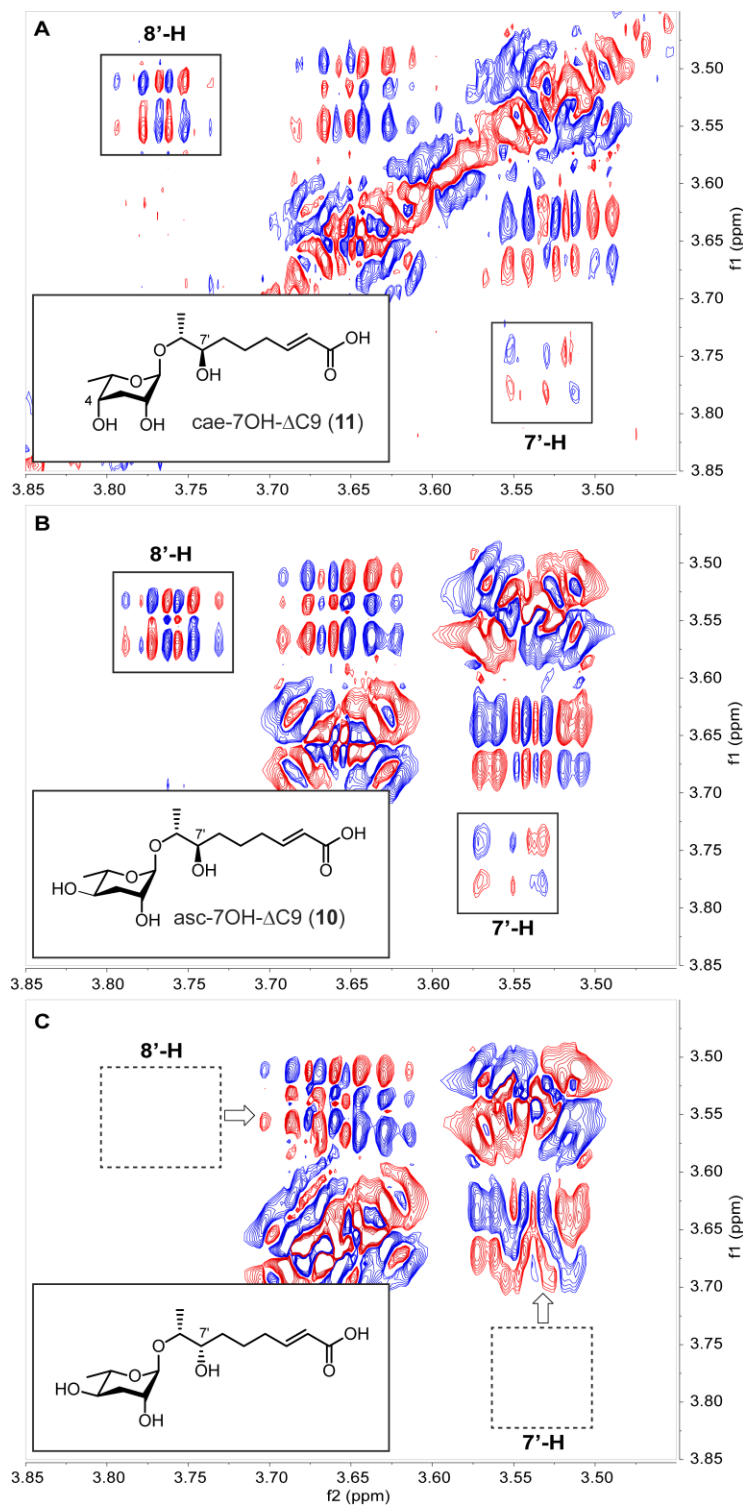


Figure S6a: Section of the 400 MHz *dqf*-COSY spectrum of a mixture of cae-7OH- Δ C9 (**11**) and asc-C7 isolated from *C. nigoni* (in CD₃OD).

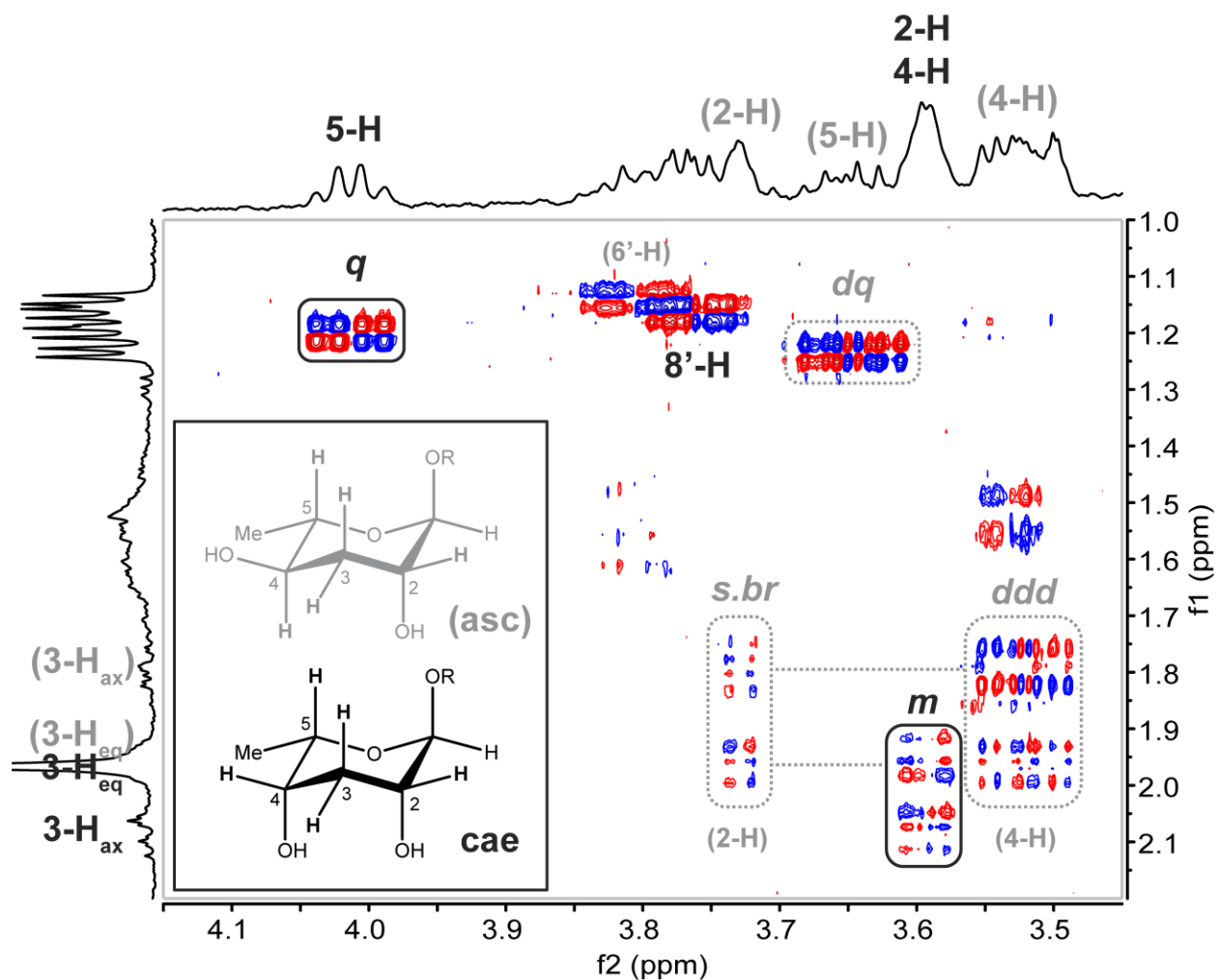


Figure S6b: Section of the 400 MHz *dqf*-COSY spectrum of a mixture of cae-7OH- Δ C9 (**11**) and asc-C7 isolated from *C. nigoni* (in CD₃OD).

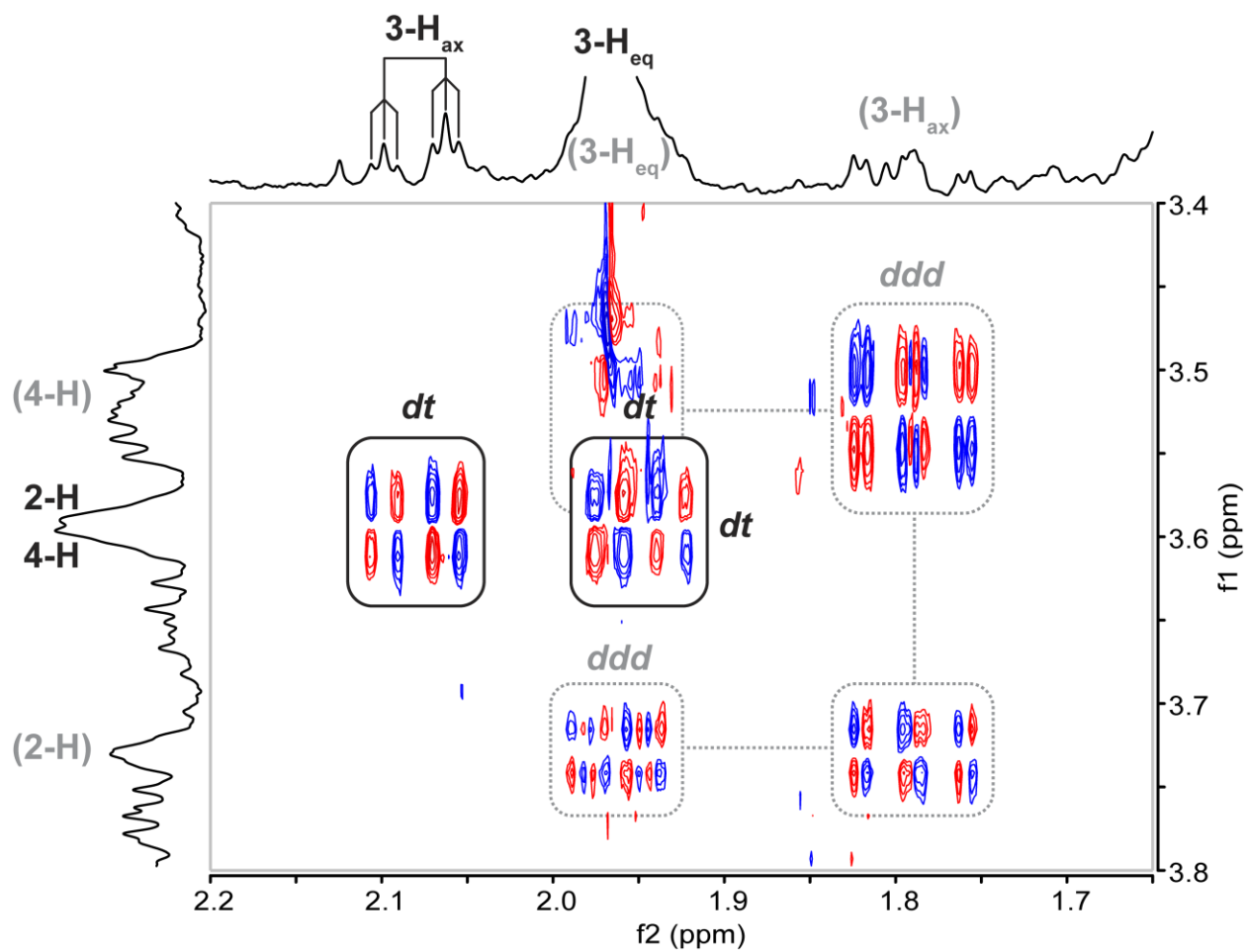
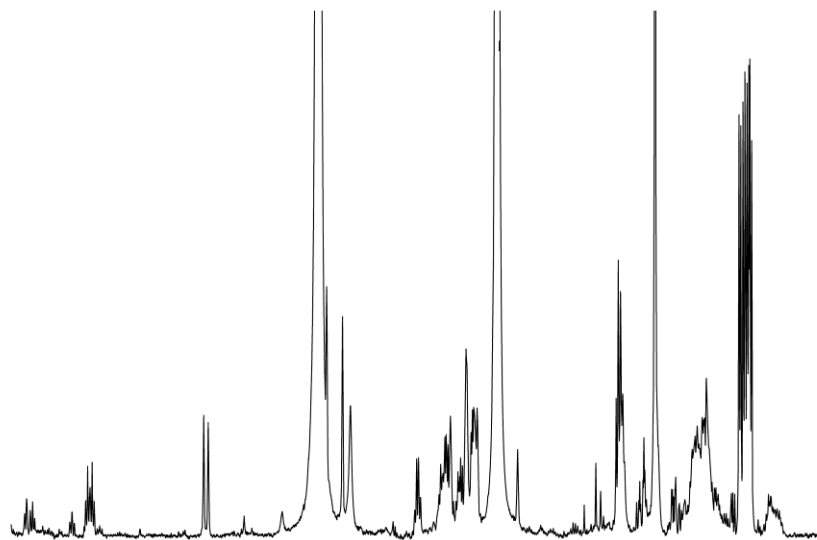


Figure S7a: Comparative analysis of 400 MHz ^1H NMR spectra of natural products isolated from *C. nigoni* and synthetic cae-7OH- ΔC9 (**11**) (in CD_3OD).

A) isolated from *C. nigoni*



B) synthetic cae-7OH- ΔC9 (**11**)

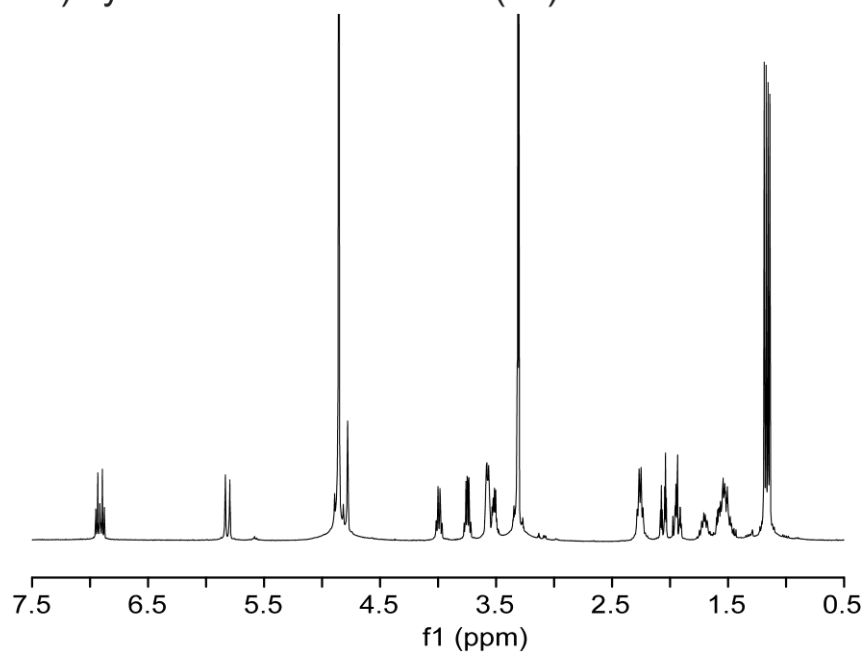


Figure S7b: Comparative analysis of sections of the 400 MHz ^1H NMR spectra of natural products isolated from *C. nigoni* and synthetic cae-7OH- ΔC9 (**11**) and synthetic asc-C7 (**9**, $n = 3$) (in CD_3OD).

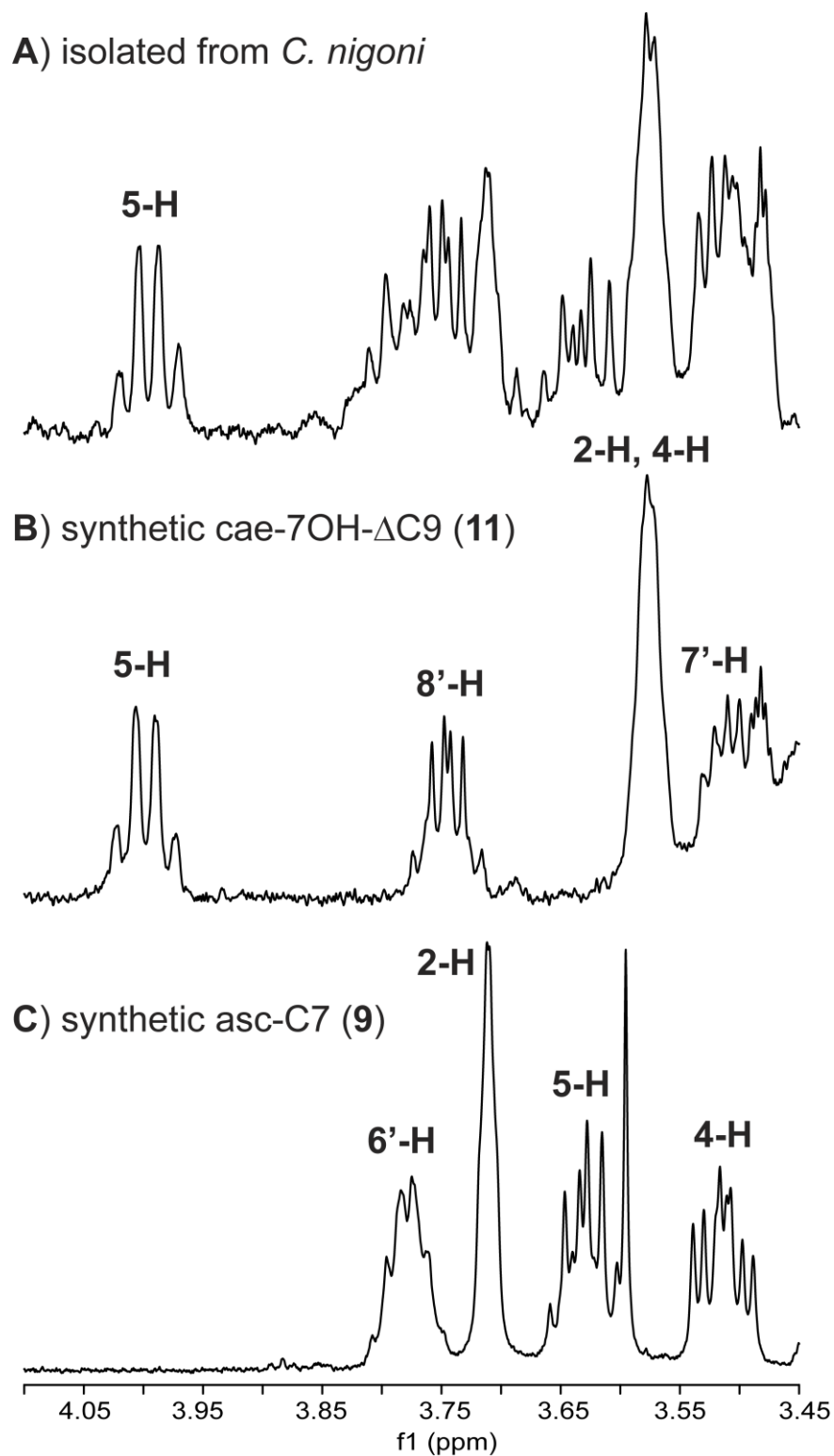


Figure S7c: Comparative analysis of sections of the 400 MHz ^1H NMR spectra of natural products isolated from *C. nigoni* and synthetic cae-7OH- ΔC9 (**11**) and synthetic asc-C7 (**9**, $n = 3$) (in CD_3OD).

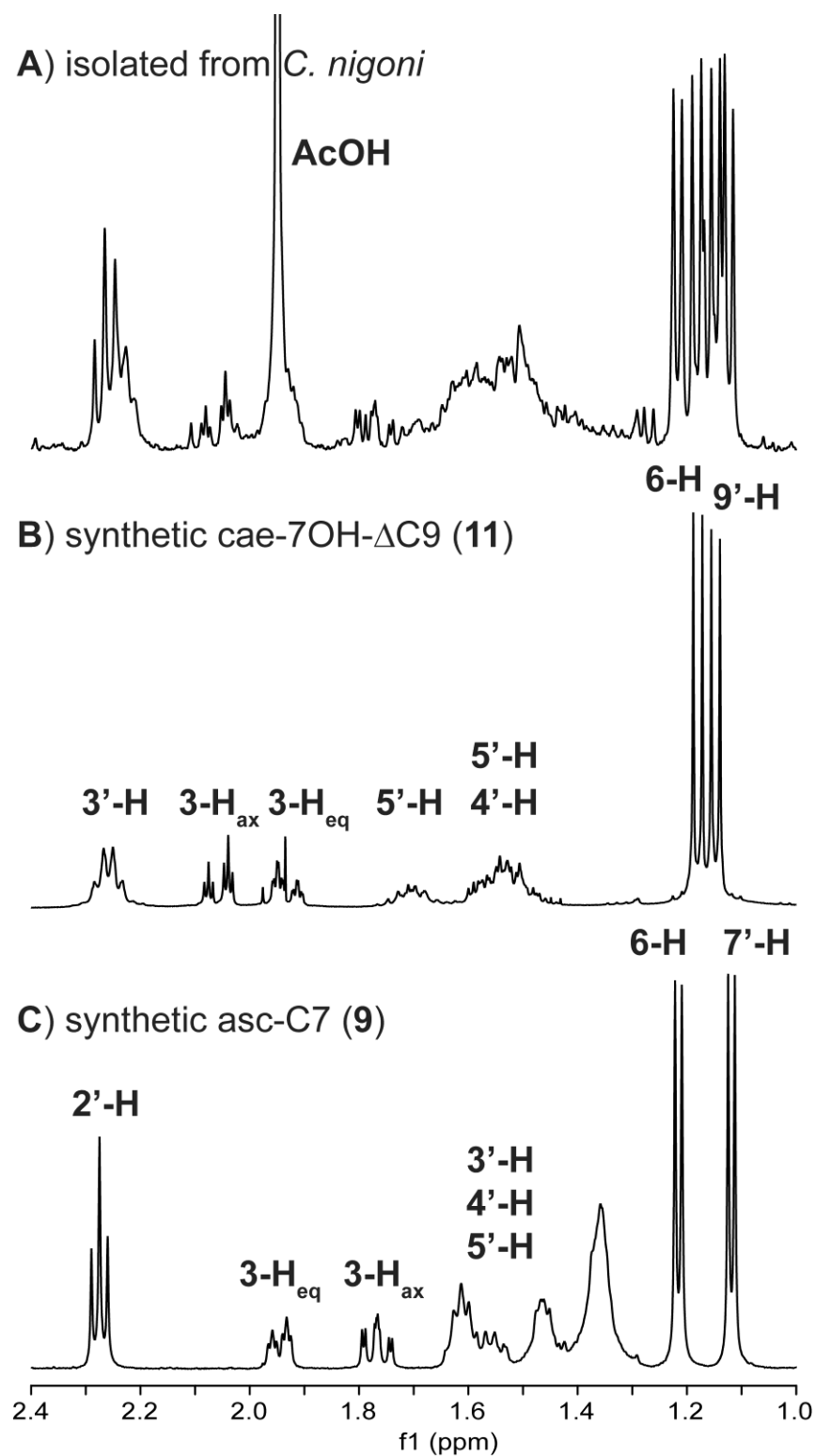


Figure S8: Comparative HPLC-HR-MS/MS analysis of **(A)** synthetic asc-7OH- Δ C9 (**10**) and cae-7OH- Δ C9 (**11**) and **(B)** the natural products isolated from *C. nigoni*.

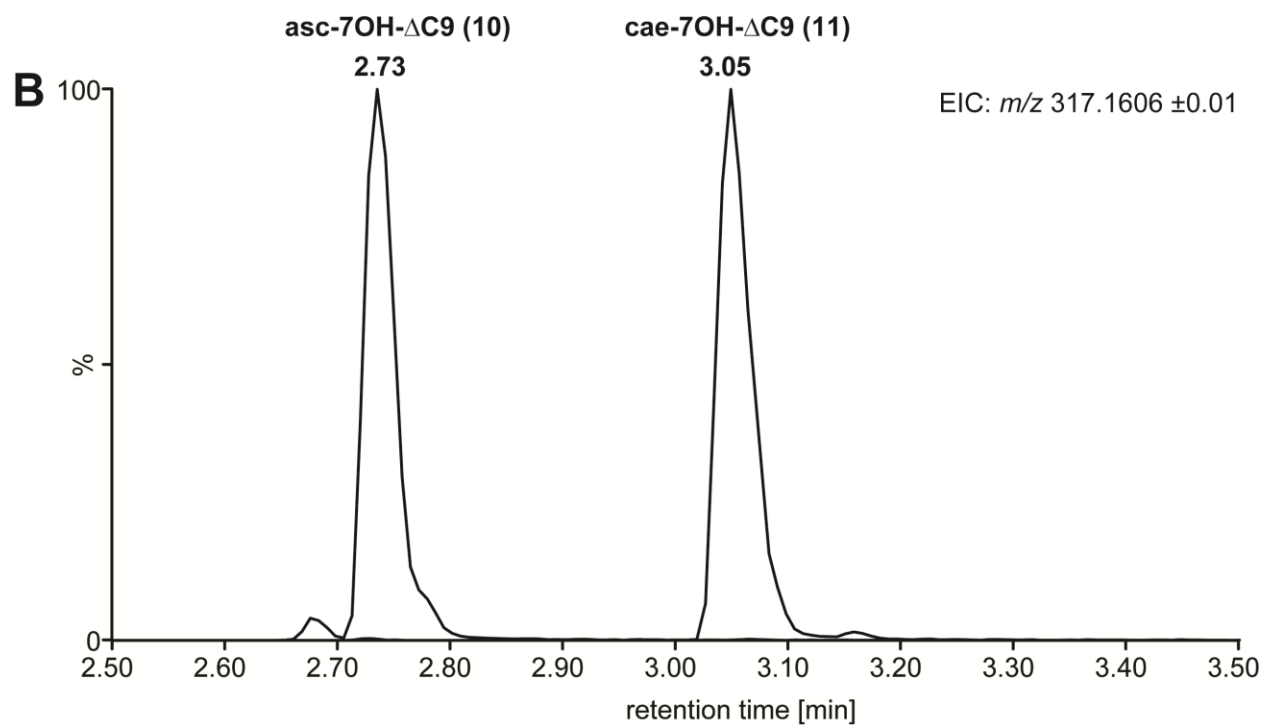
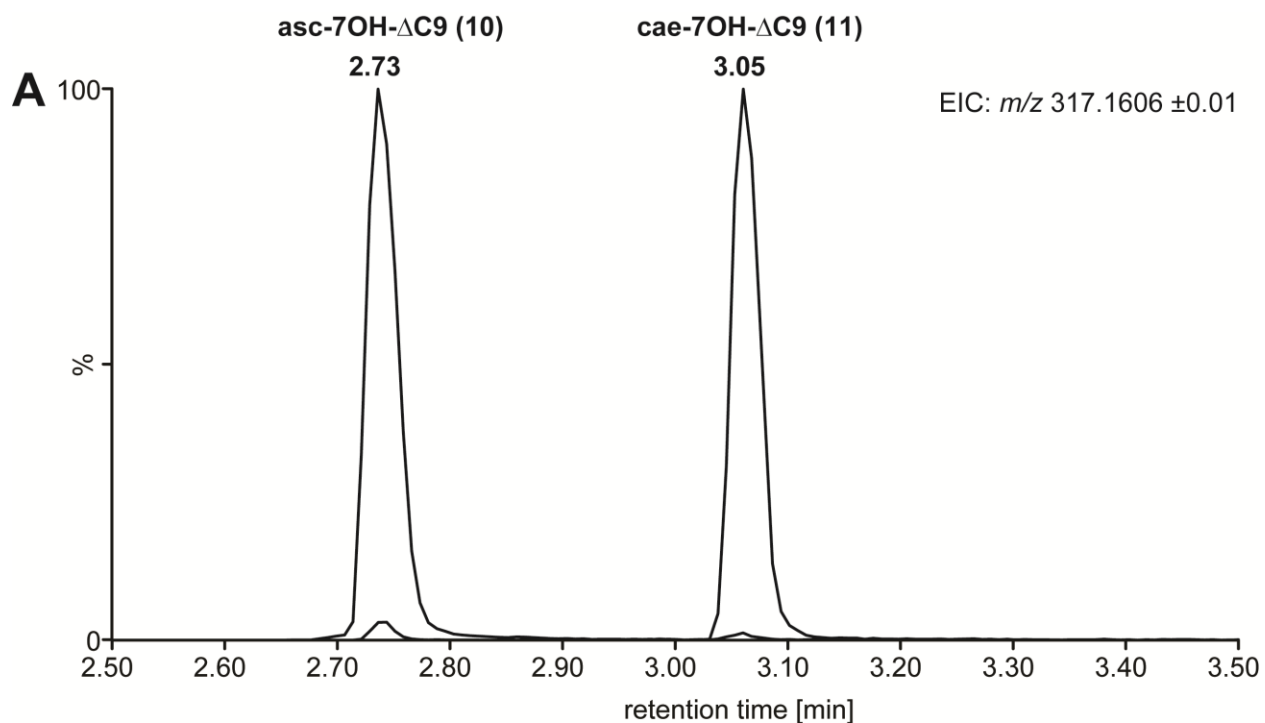
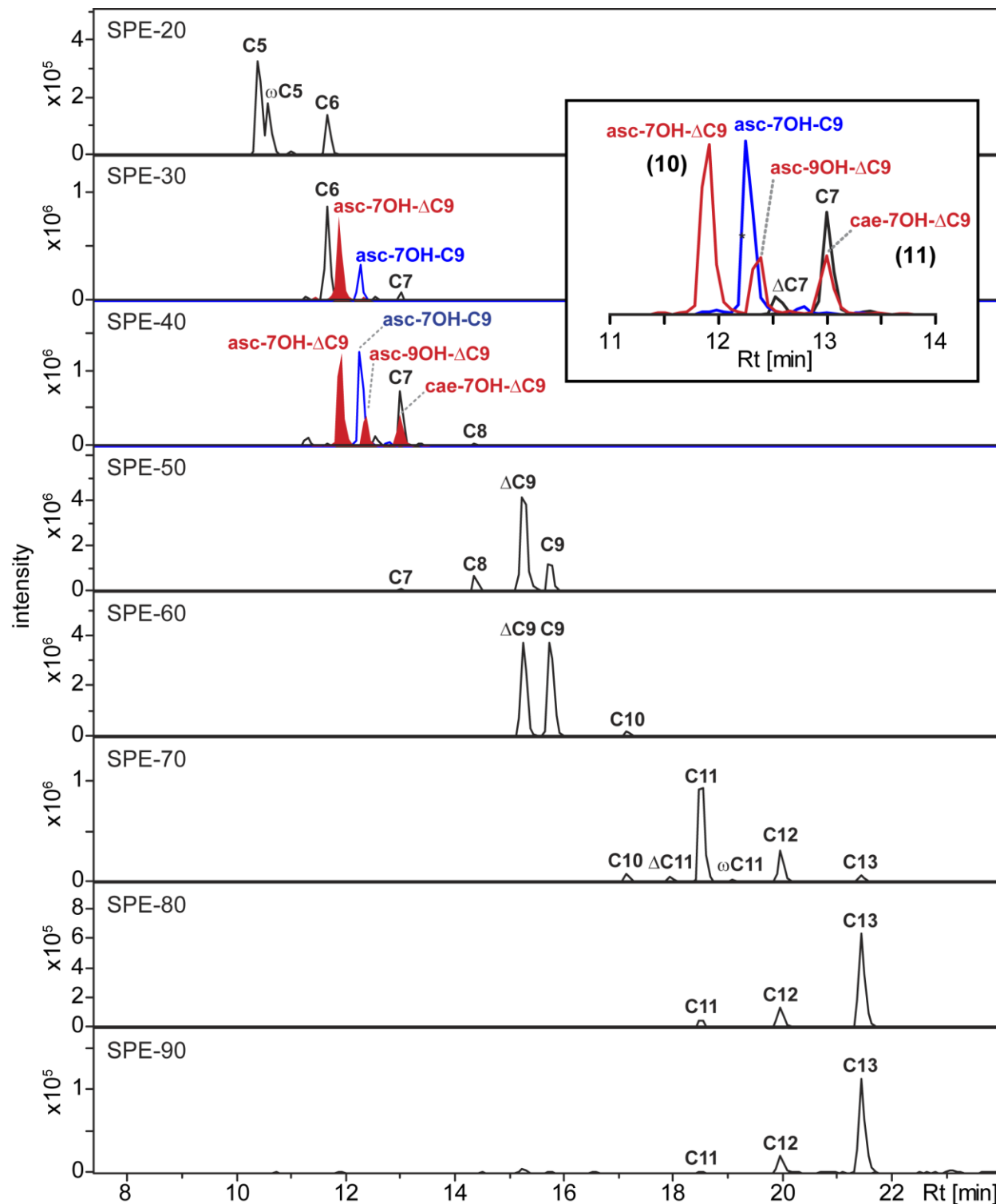


Figure S9: HPLC-ESI(-)-MS analysis of simple ascarosides in *C. nigoni* JU1422 SPE fractions shows the pair of hydroxyacyl ascaroside asc-7OH- Δ C9 (**10**) and hydroxyacyl caenorhabdoside cae-7OH- Δ C9 (**11**) but no other 4-epimeric ascaroside-type component, indicating that 4-epimerization is tightly controlled and specific for the *threo*-7-hydroxynonenoic acid aglycone.



Supplementary NMR Spectra

Figure	Page
S10 ^1H NMR of natural asc-C7 (9) and cae-7OH- Δ C9 (11) from <i>C. nigoni</i>	S29
S11 <i>dqf</i> -COSY of natural asc-C7 (9) and cae-7OH- Δ C9 (11) from <i>C. nigoni</i>	S30
S12 HSQC of natural asc-C7 (9) and cae-7OH- Δ C9 (11) from <i>C. nigoni</i>	S31
S13 ^1H NMR of O-benzyl-6-deoxy- α -L- <i>arabino</i> -hexopyranoside (21)	S32
S14 ^{13}C NMR of O-benzyl-6-deoxy- α -L- <i>arabino</i> -hexopyranoside (21)	S33
S15 ^1H NMR of O-benzyl-6-deoxy-2,3-(O-isopropylidene)- α -L- <i>arabino</i> -hexopyranoside (22)	S34
S16 ^{13}C NMR of O-benzyl-6-deoxy-2,3-(O-isopropylidene)- α -L- <i>arabino</i> -hexopyranoside (22)	S35
S17 ^1H NMR of O-benzyl-6-deoxy-2,3-O-(isopropylidene)-4-trifluoromethanesulfonate- α -L- <i>arabino</i> -hexopyranoside (23)	S36
S18 ^{13}C NMR of O-benzyl-6-deoxy-2,3-O-(isopropylidene)-4-trifluoromethanesulfonate- α -L- <i>arabino</i> -hexopyranoside (23)	S37
S19 ^1H NMR of O-benzyl-6-deoxy-4-trifluoromethanesulfonate- α -L- <i>arabino</i> -hexopyranoside (24)	S38
S20 ^{13}C NMR of O-benzyl-6-deoxy-4-trifluoromethanesulfonate- α -L- <i>arabino</i> -hexopyranoside (24)	S39
S21 ^1H NMR of O-benzyl-3,4-anhydro-6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (25)	S40
S22 ^{13}C NMR of O-benzyl-3,4-anhydro-6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (25)	S41
S23 ^1H NMR of O-benzyl-3,6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (26)	S42
S24 ^{13}C NMR of O-benzyl-3,6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (26)	S43
S25 ^1H NMR of O-benzyl-2,4-di-O-benzoyl-3,6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (27)	S44
S26 ^{13}C NMR of O-benzyl-2,4-di-O-benzoyl-3,6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (27)	S45
S27 HSQC of O-benzyl-2,4-di-O-benzoyl-3,6-dideoxy- α -L- <i>lyxo</i> -hexopyranoside (27)	S46
S28 ^1H NMR of 2,4-di-O-benzoyl-3,6-dideoxy- α -L- <i>lyxo</i> -hexopyranose (28)	S47

S29	¹³ C NMR of 2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranose (28)	S48
S30	<i>dqf</i> -COSY of 2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranose (28)	S49
S31	HSQC of 2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranose (28)	S50
S32	¹ H NMR of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -ethyl 7-benzoyloxy-8-[(2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-2-nonenoate (29)	S51
S33	¹³ C NMR of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -ethyl 7-benzoyloxy-8-[(2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-2-nonenoate (29)	S52
S34	<i>dqf</i> -COSY of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -ethyl 7-benzoyloxy-8-[(2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-2-nonenoate (29)	S53
S35	HSQC of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -ethyl 7-benzoyloxy-8-[(2,4-di-O-benzoyl-3,6-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-2-nonenoate (29)	S54
S36	¹ H NMR of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S55
S37	¹³ C NMR of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S56
S38a	<i>dqf</i> -COSY of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S57
S38b	Section of the <i>dqf</i> -COSY of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S58
S39a	HSQC of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S59
S39b	Section of the HSQC of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S60
S40	HMBC of (7 <i>R</i> ,8 <i>R</i> ,2 <i>E</i>)- <i>threo</i> -8-[(3',6'-dideoxy-α-L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (cae-7OH-ΔC9, 11)	S61

Figure S10: ^1H NMR (400 MHz, CD_3OD) of natural cae-7O- Δ C9 (**11**) (and asc-C7 (**9**)) from *C. nigoni*.

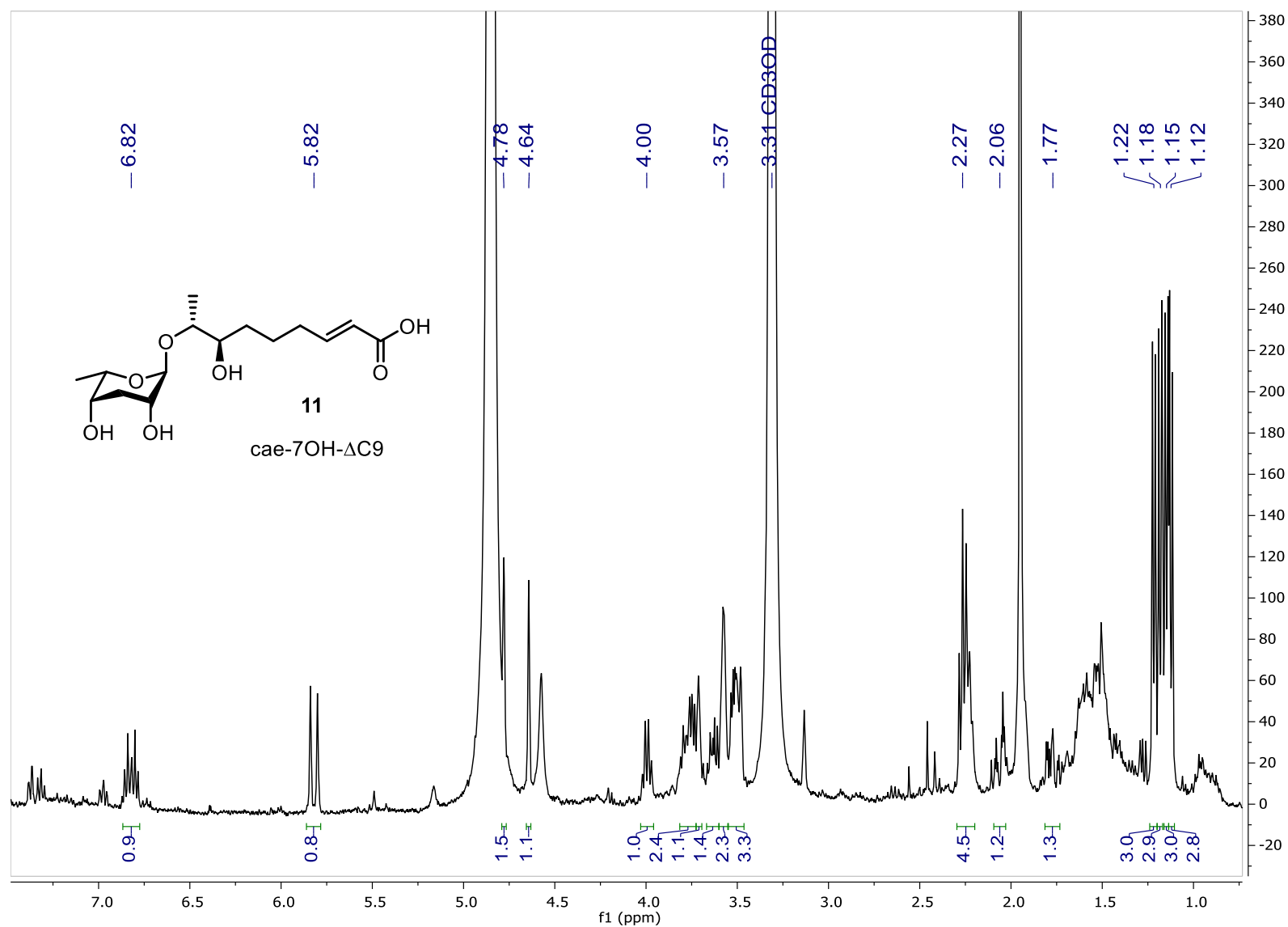


Figure S11: *dqf*-COSY (400 MHz, CD₃OD) of natural cae-7O-ΔC9 (**11**) (and asc-C7 (**9**)) from *C. nigrum*.

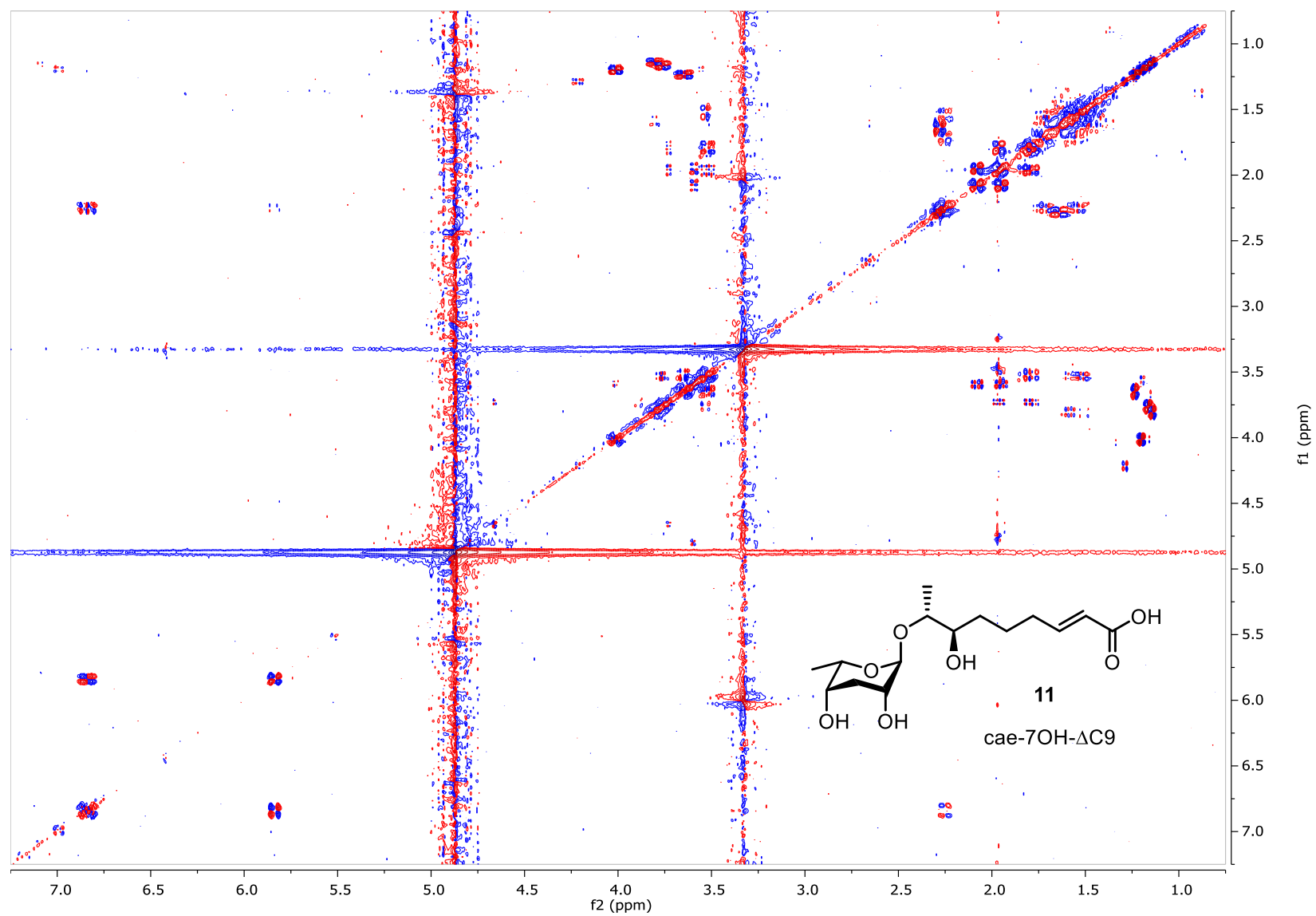


Figure S12: HSQC (400 MHz, CD₃OD) of natural cae-7O- Δ C9 (**11**) (and asc-C7 (**9**)) from *C. nigoni*.

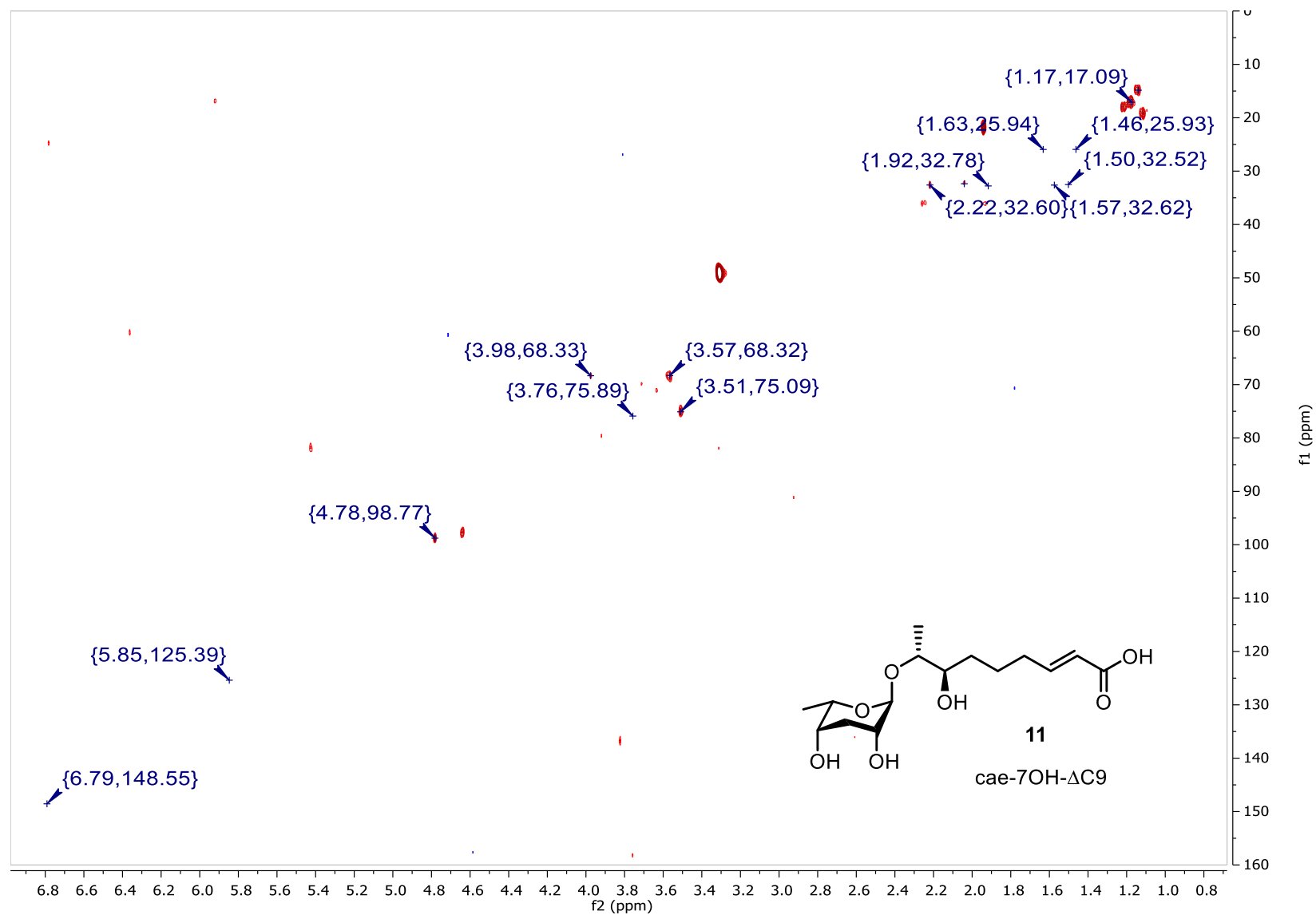


Figure S13: ^1H NMR (400 MHz, CD_3OD) of *O*-benzyl-6-deoxy- α -L-*arabino*-hexopyranoside (**21**)

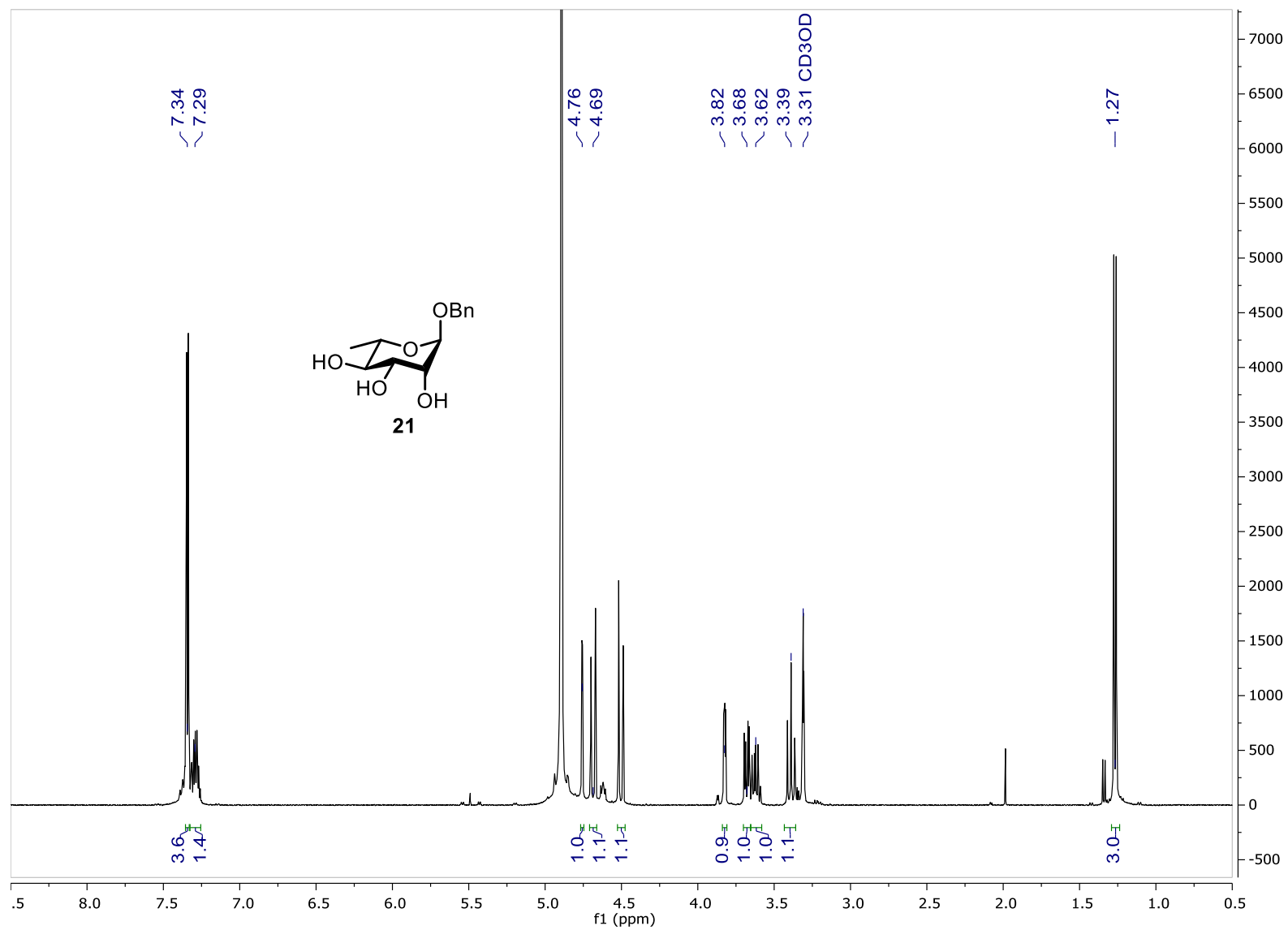


Figure S14: ^{13}C NMR (100 MHz, CD_3OD) of *O*-benzyl-6-deoxy- α -L-*arabino*-hexopyranoside (**21**)

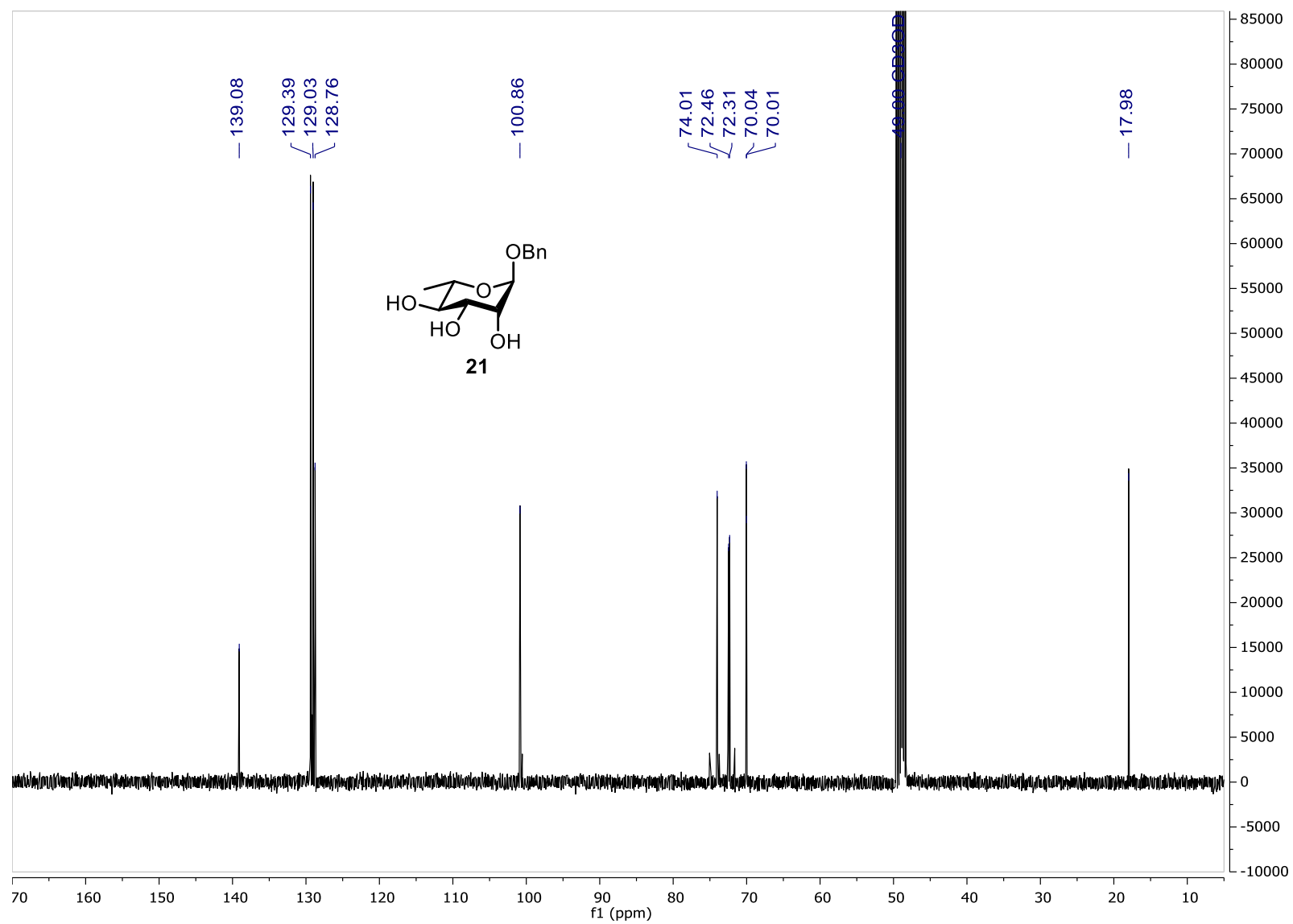


Figure S15: ^1H NMR (400 MHz, CDCl_3) of O-benzyl-6-deoxy-2,3-(O-isopropylidene)- α -L-*arabino*-hexopyranoside (**22**)

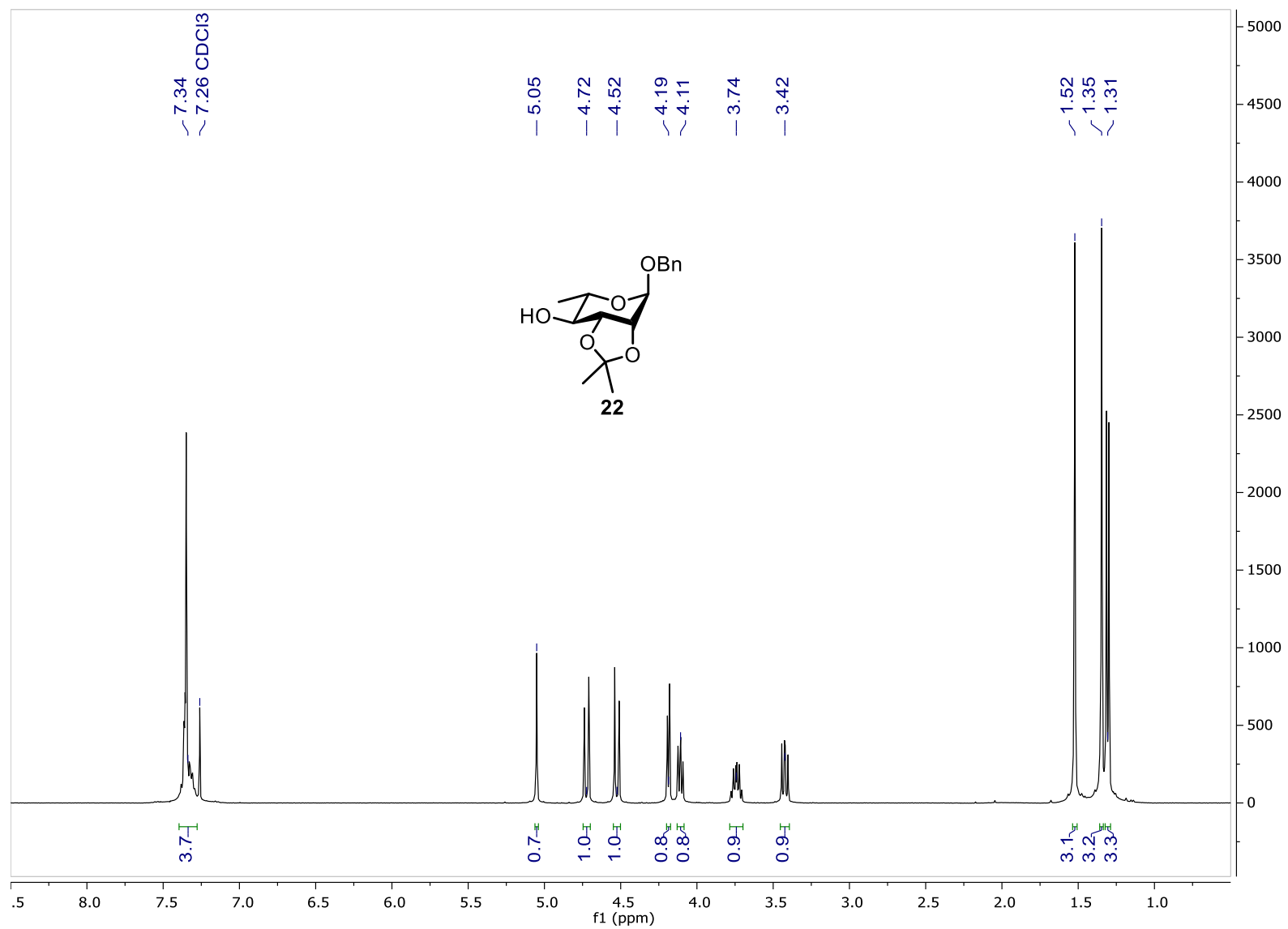


Figure S16: ^{13}C NMR (100 MHz, CDCl_3) of O-benzyl-6-deoxy-2,3-(O-isopropylidene)- α -L-arabino-hexopyranoside (**22**)

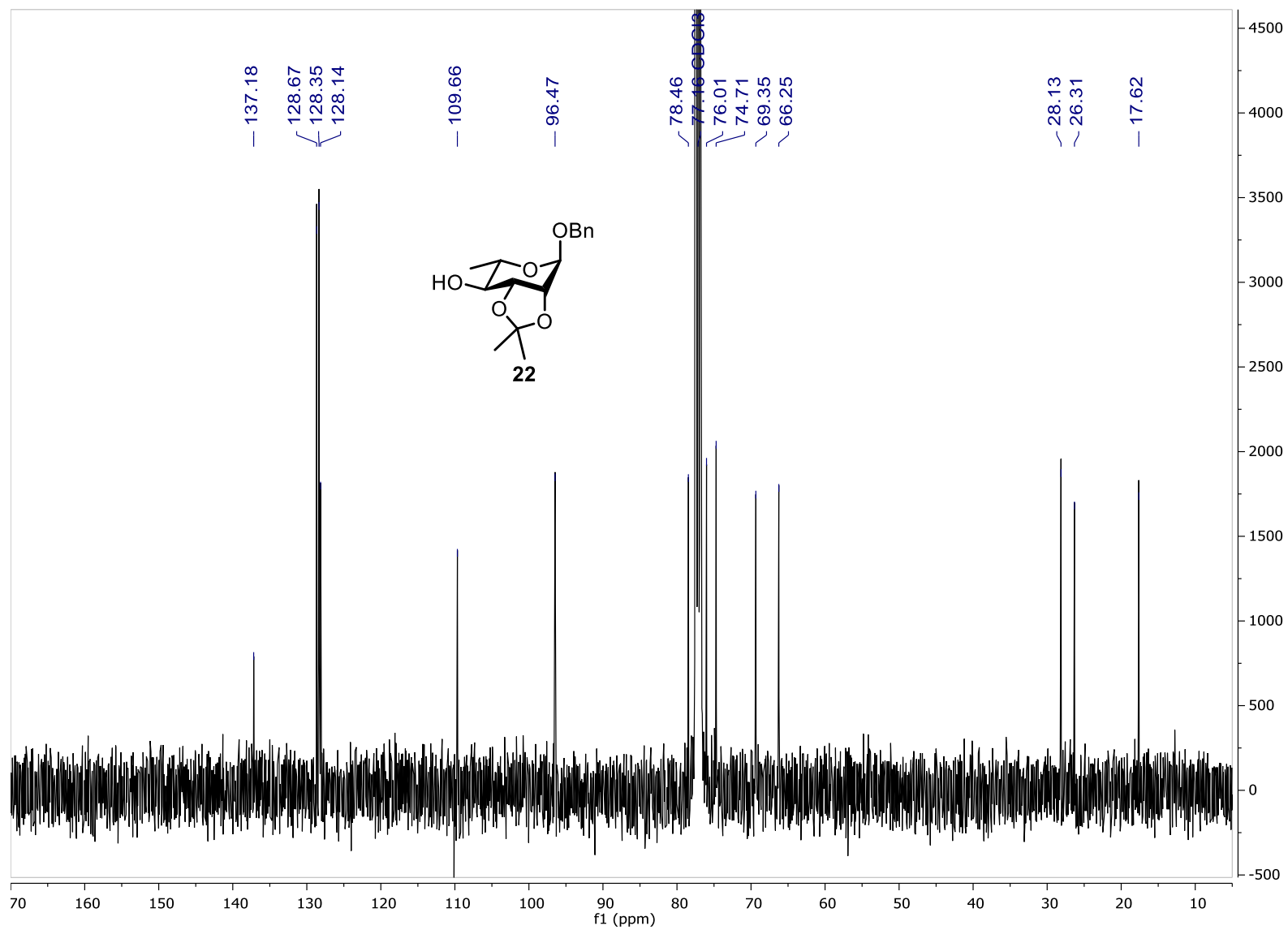


Figure S17: ^1H NMR (400 MHz, CDCl_3) of O-benzyl-6-deoxy-2,3-O-(isopropylidene)-4-trifluoromethanesulfonate- α -L-arabino-hexopyranoside (**23**)

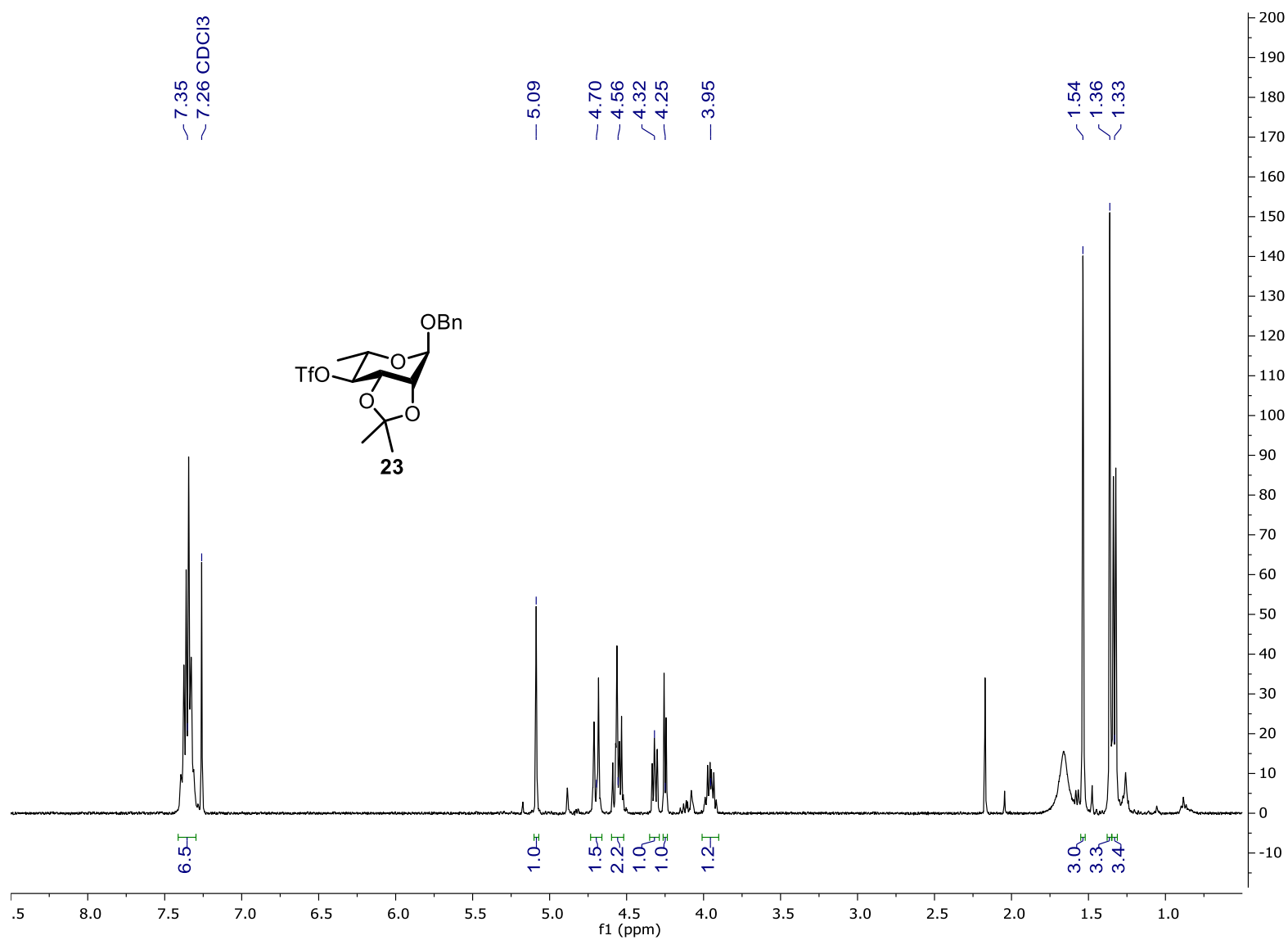


Figure S18: ^{13}C NMR (100 MHz, CDCl_3) of O-benzyl-6-deoxy-2,3-O-(isopropylidene)-4-trifluoromethanesulfonate- α -L-arabino-hexopyranoside (**23**)

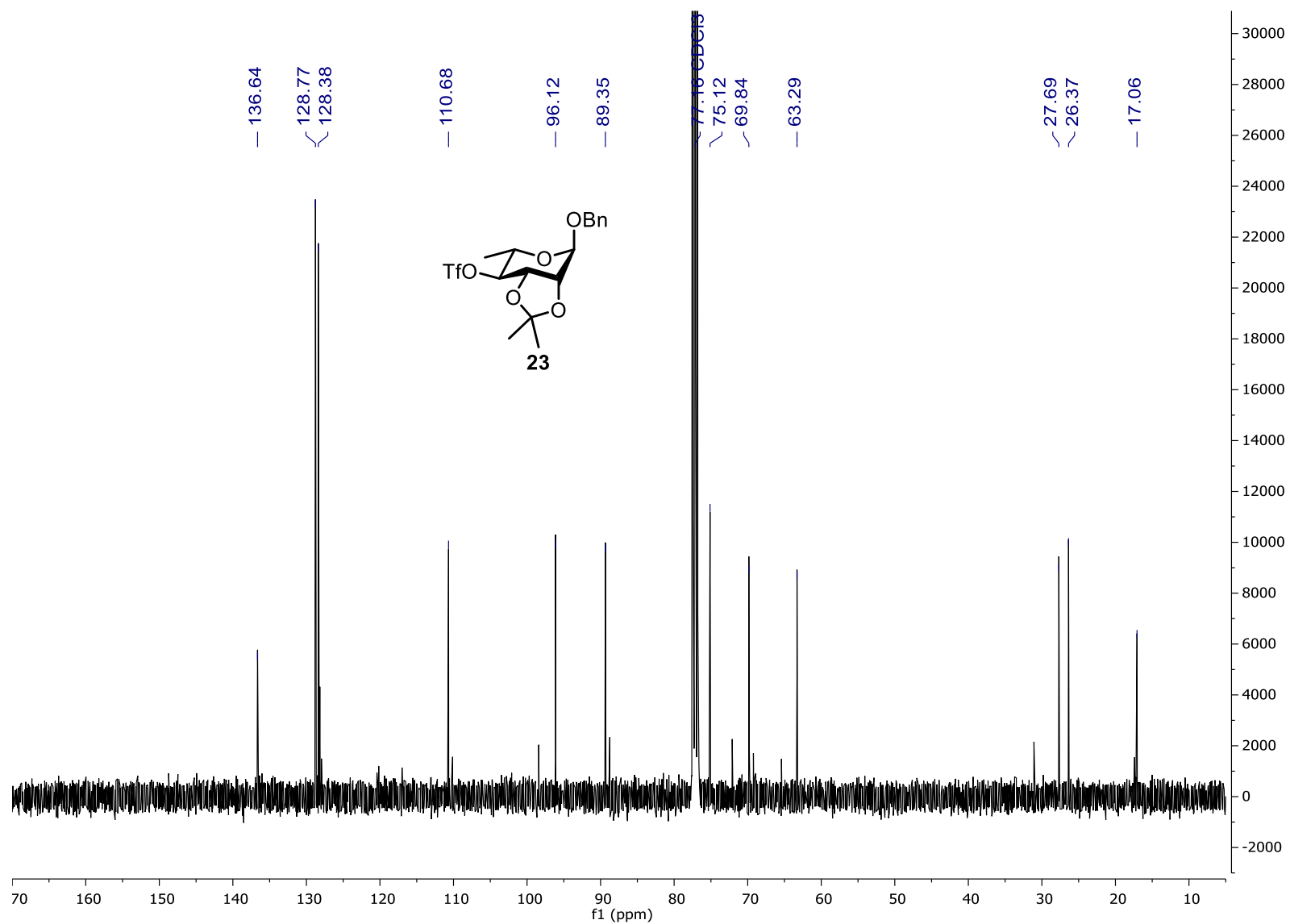


Figure S19: ^1H NMR (400 MHz, CDCl_3) of O-benzyl-6-deoxy-4-trifluoromethanesulfonate- α -L-*arabino*-hexopyranoside (**24**)

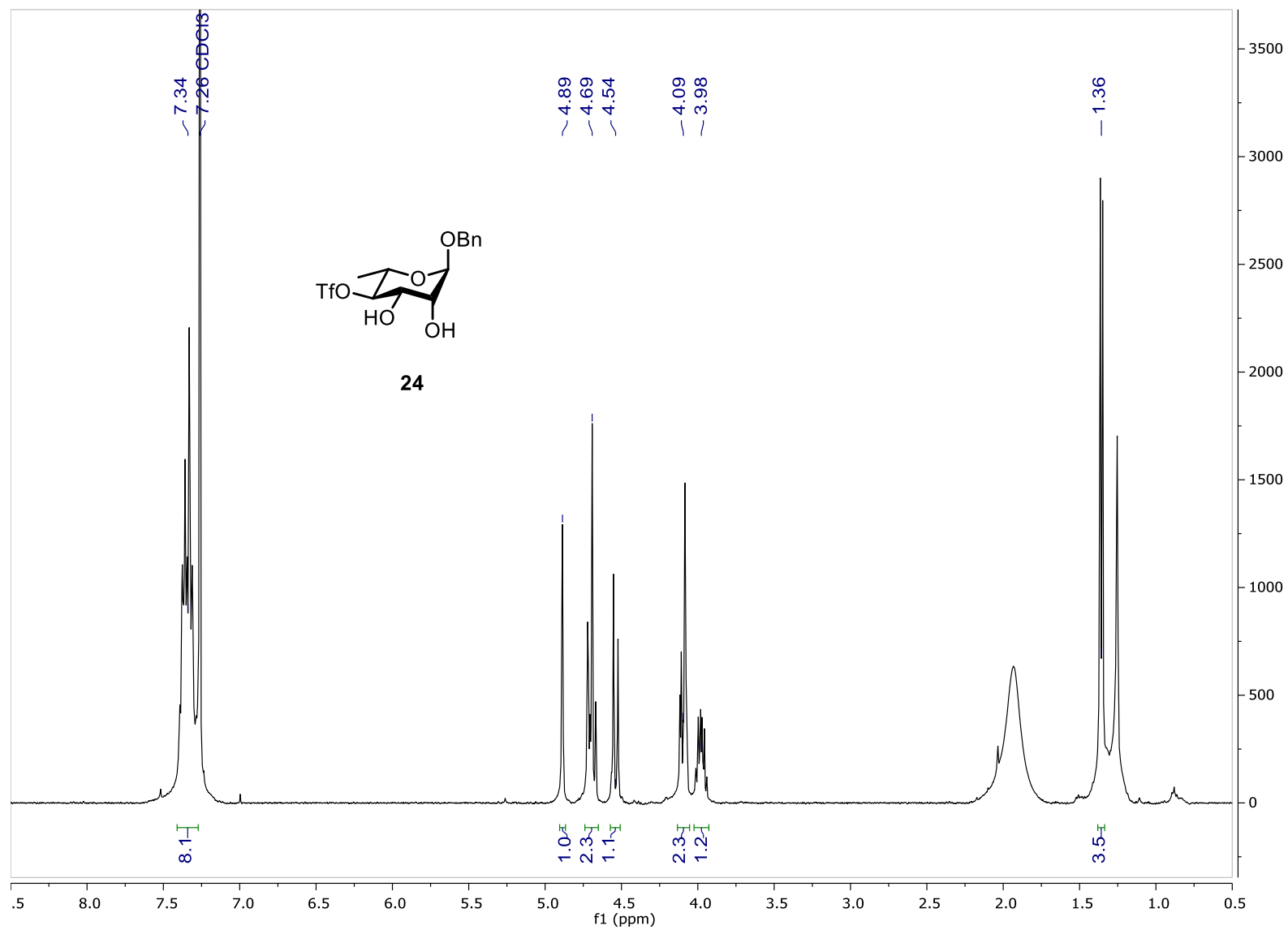


Figure S20: ^{13}C NMR (100 MHz, CDCl_3) of O-benzyl-6-deoxy-4-trifluoromethanesulfonate- α -L-arabino-hexopyranoside (**24**)

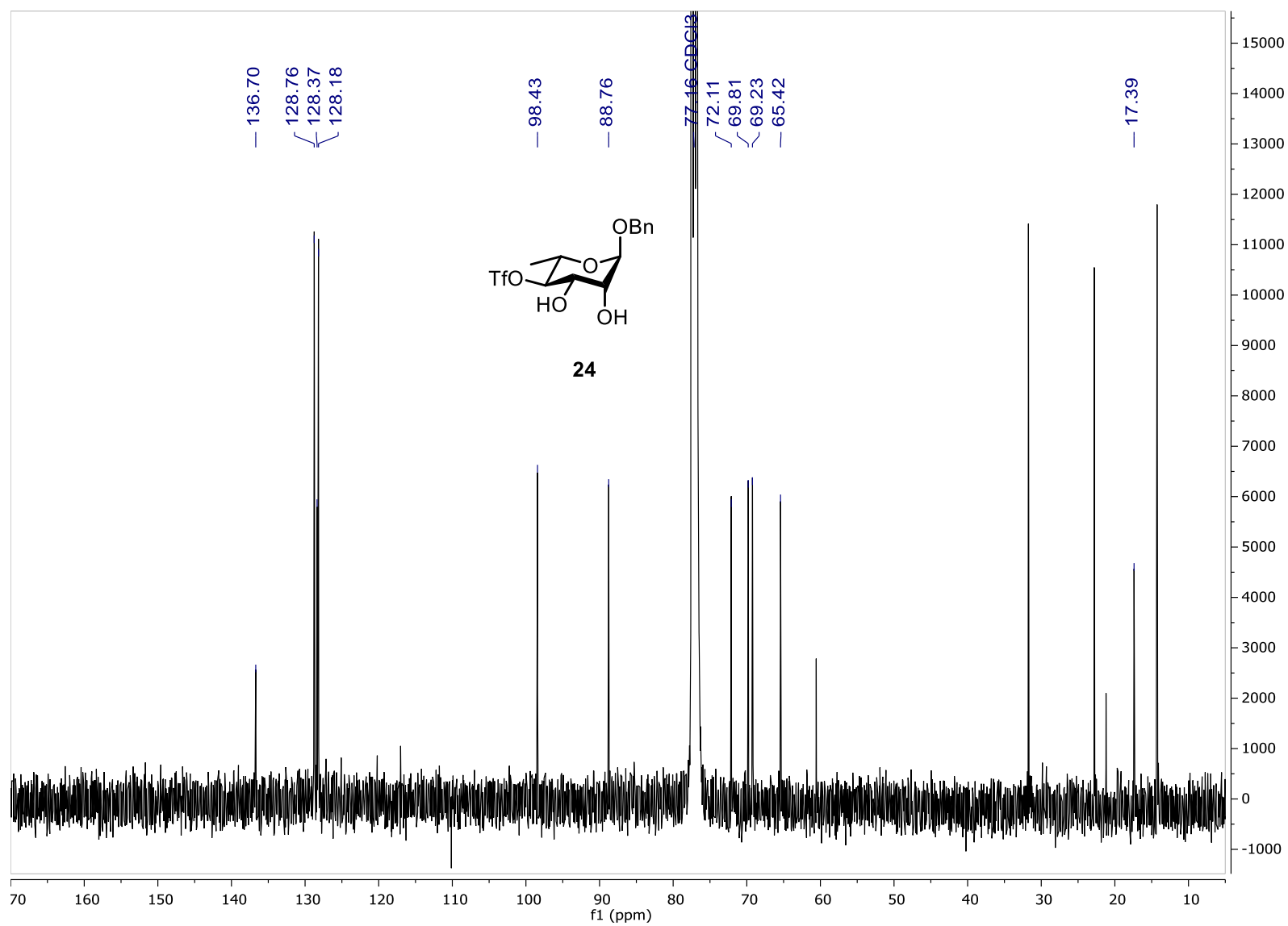


Figure S21: ^1H NMR (400 MHz, CDCl_3) of O-benzyl-3,4-anhydro-6-dideoxy- α -L-lyxo-hexopyranoside (**25**)

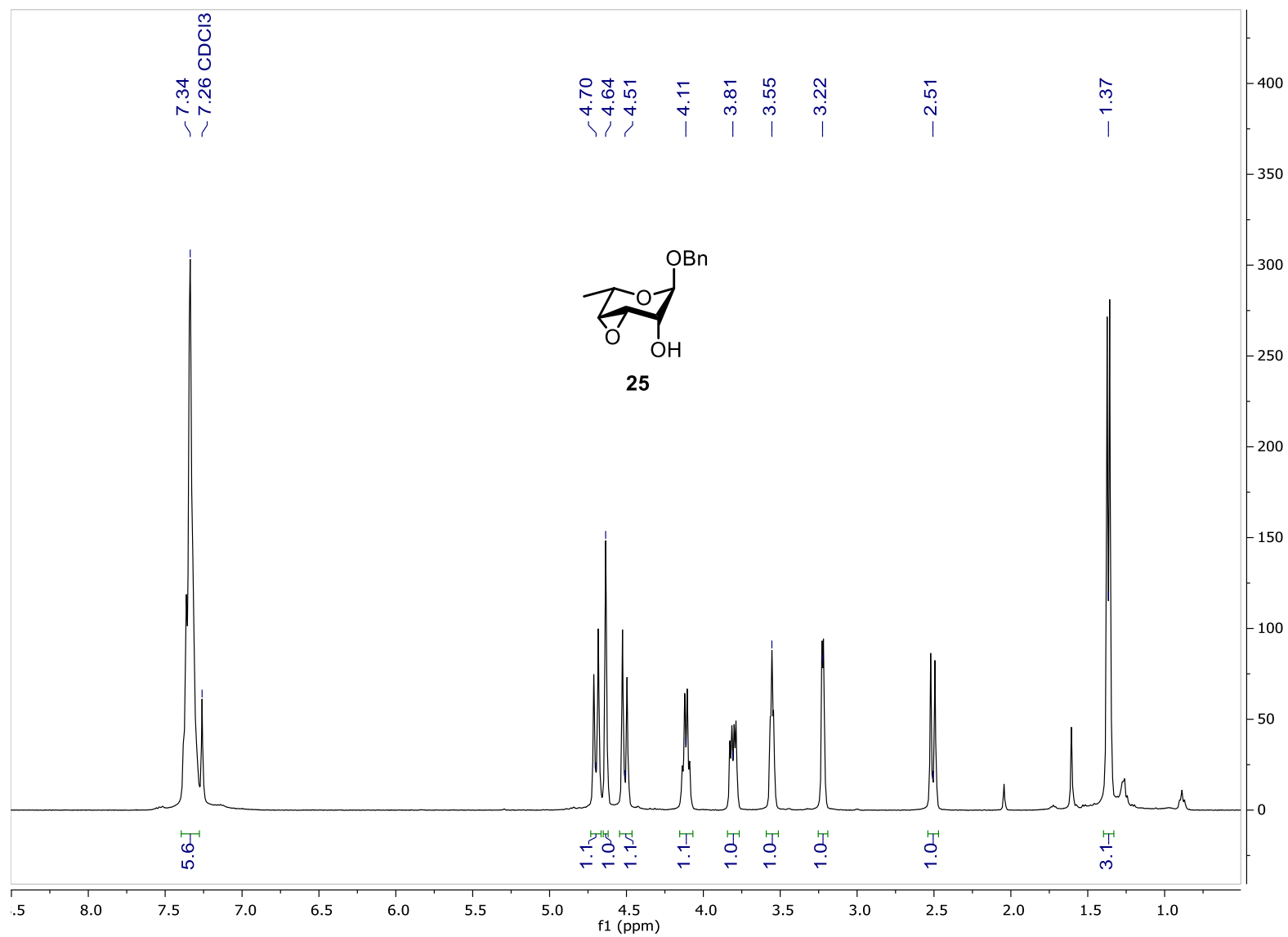


Figure S22: ^{13}C NMR (100 MHz, CDCl_3) of O-benzyl-3,4-anhydro-6-dideoxy- α -L-lyxo-hexopyranoside (**25**)

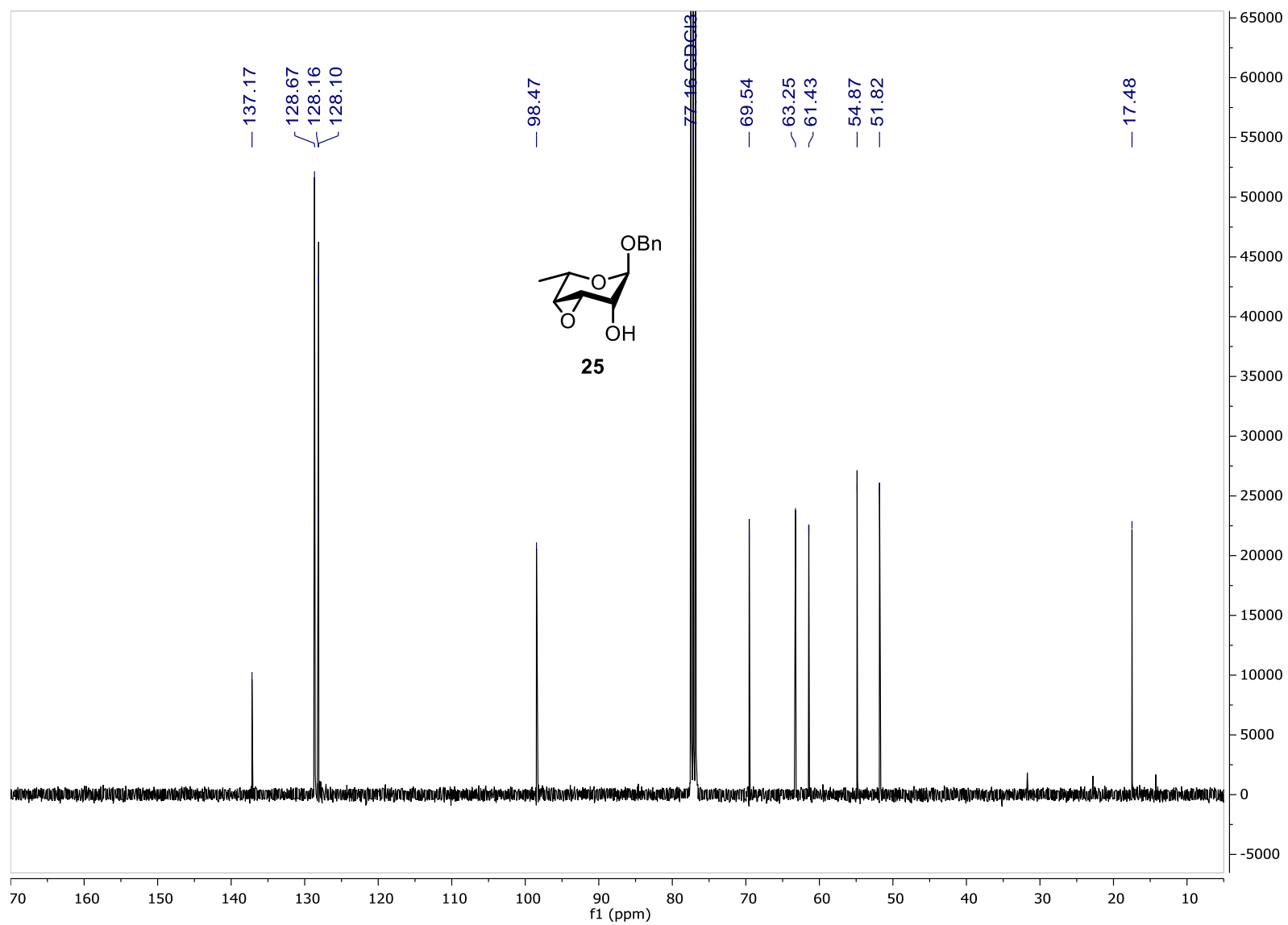


Figure S23: ^1H NMR (400 MHz, CDCl_3) of O-benzyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**26**)

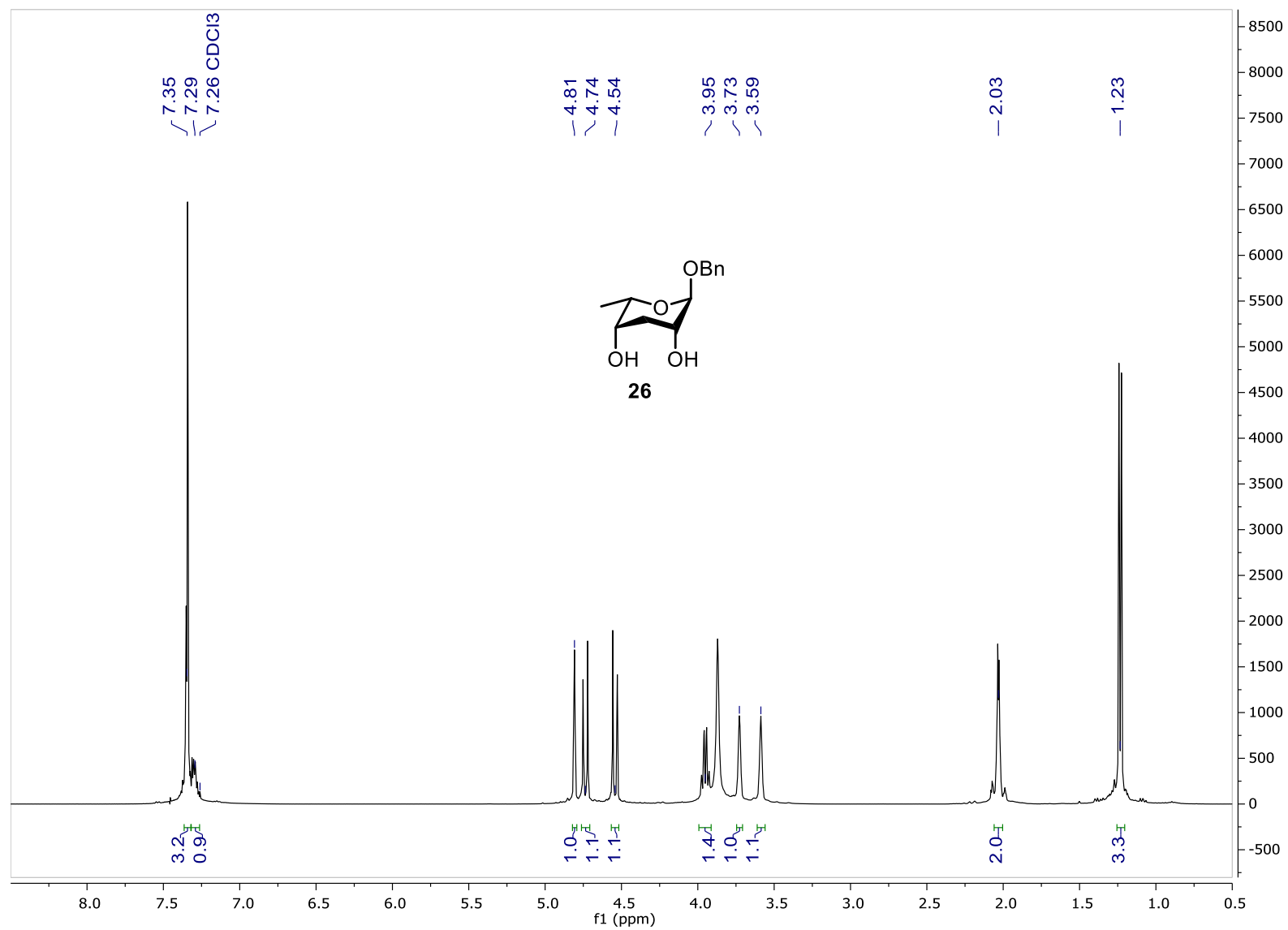


Figure S24: ^{13}C NMR (100 MHz, CDCl_3) of O-benzyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**26**)

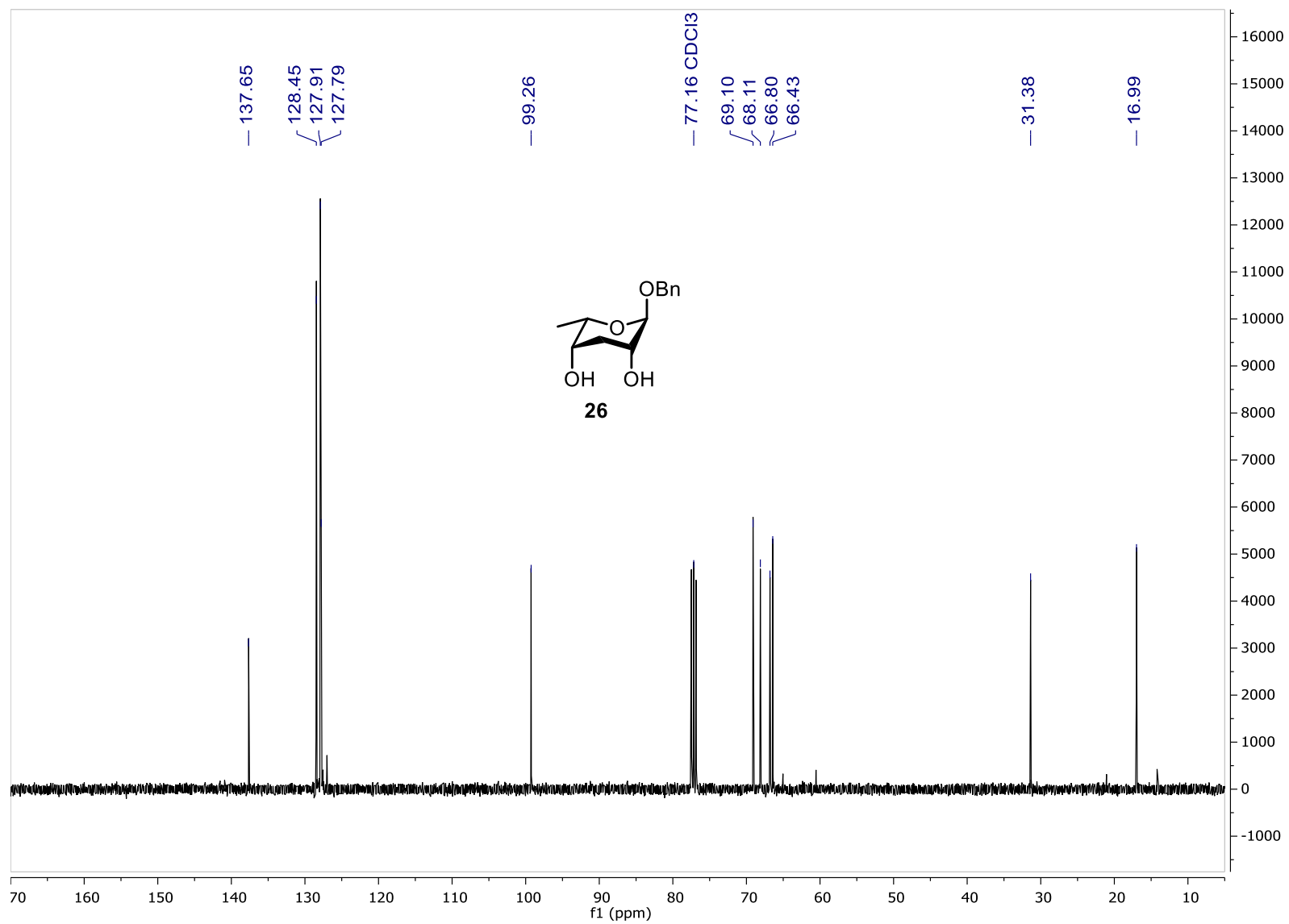


Figure S25: ^1H NMR (400 MHz, CDCl_3) of O-benzyl-2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**27**)

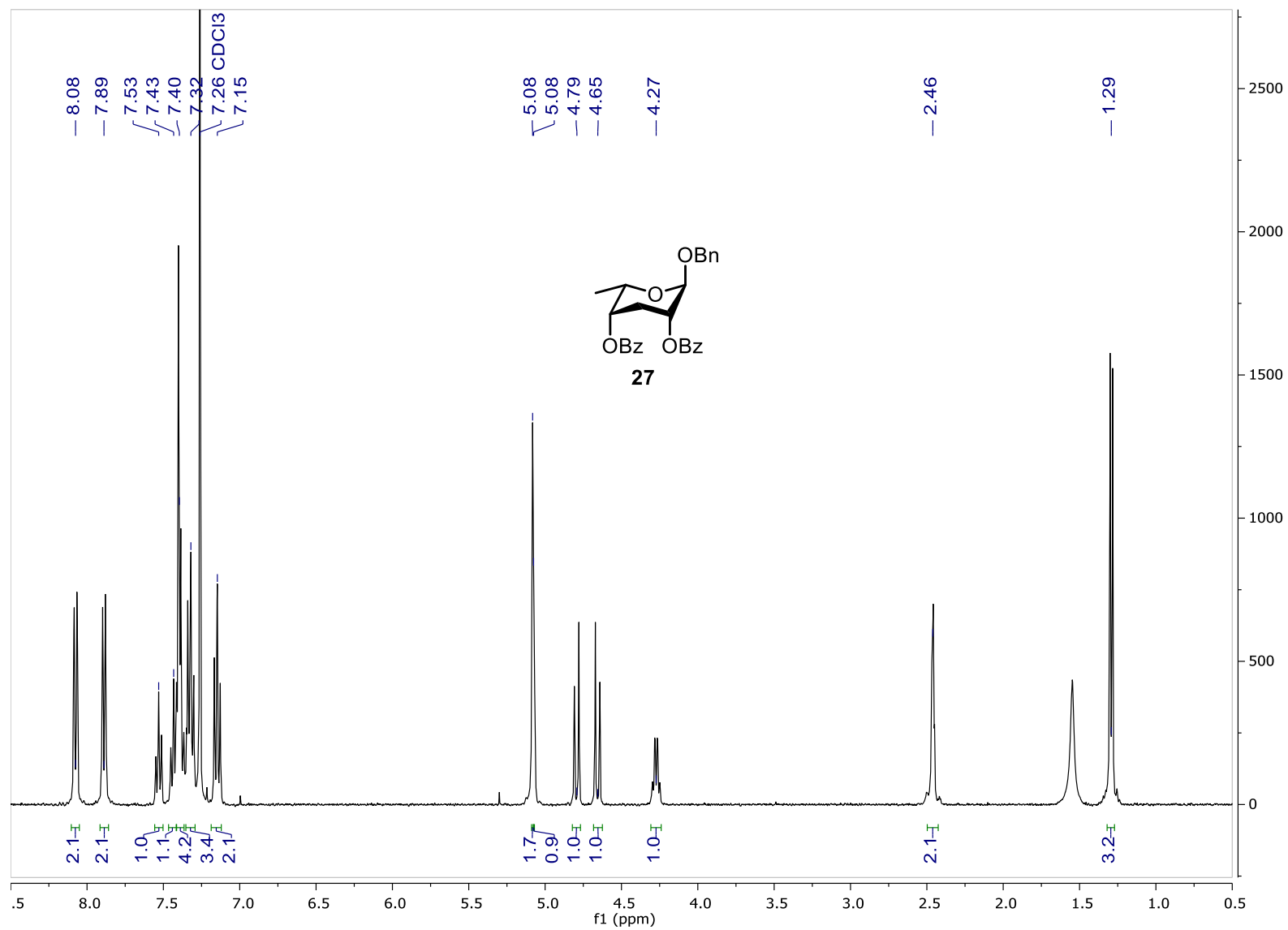


Figure S26: ^{13}C NMR (100 MHz, CDCl_3) of O-benzyl-2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**27**)

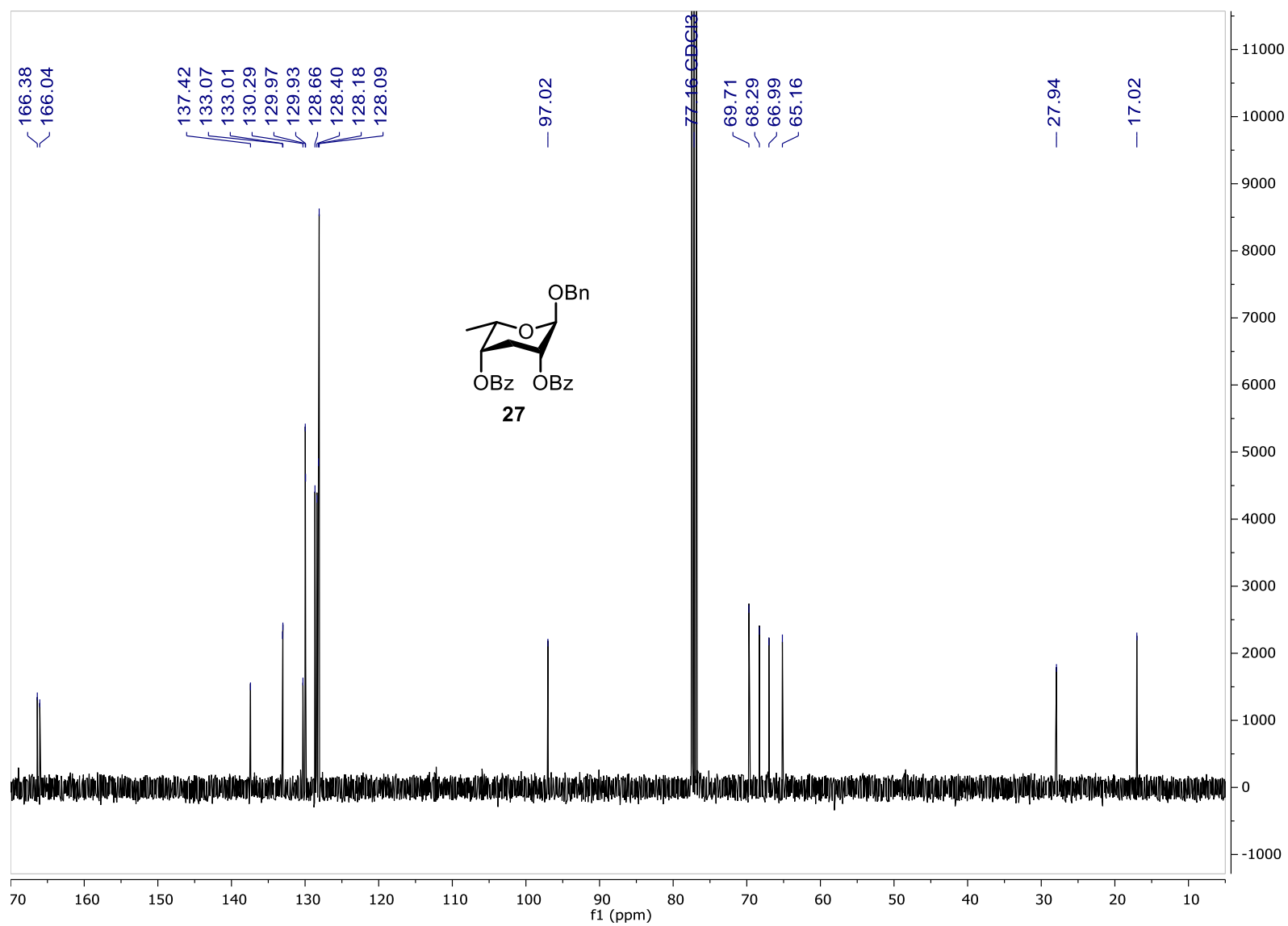


Figure S27: HSQC (400 MHz, CDCl₃) of O-benzyl-2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranoside (**27**)

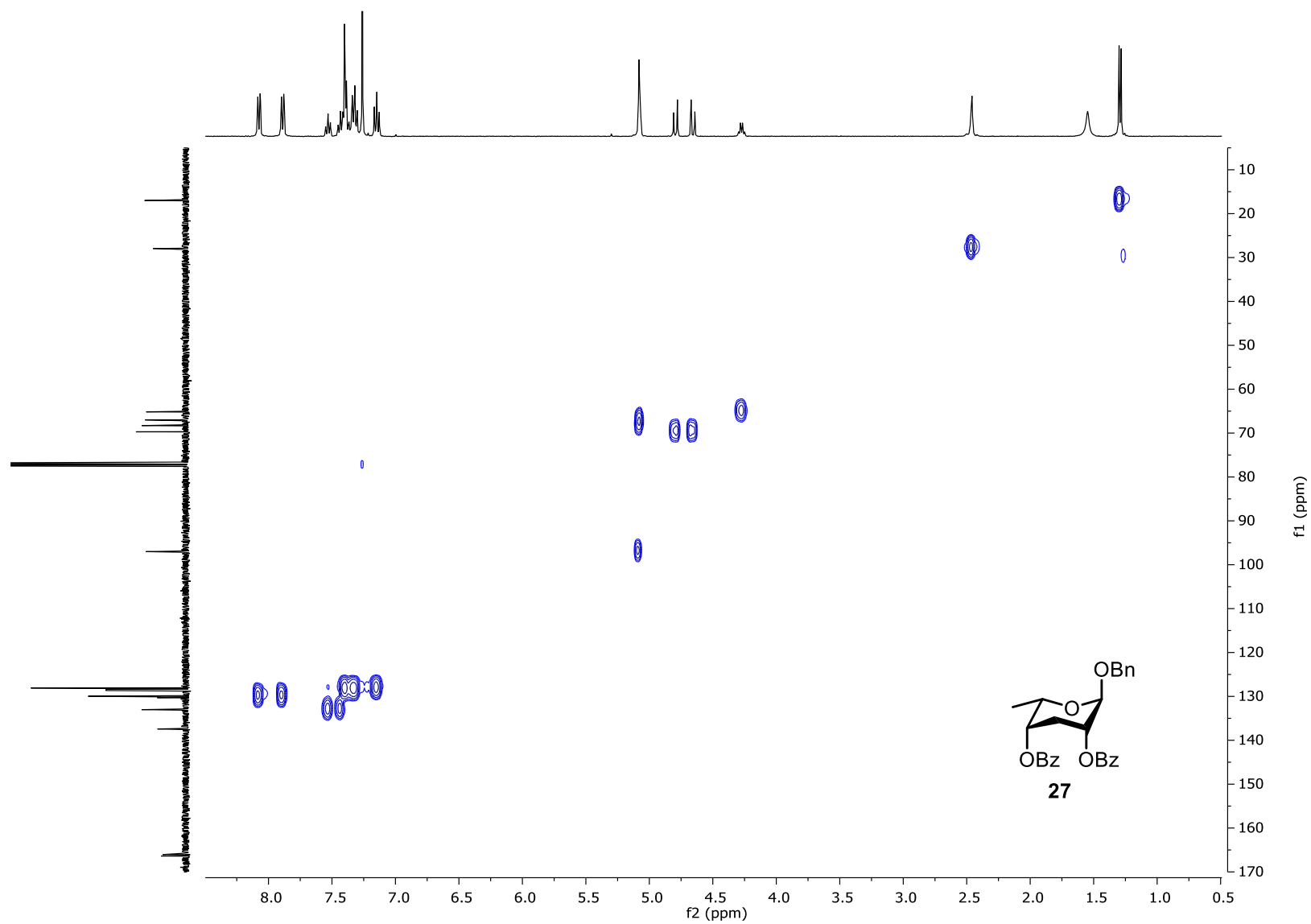


Figure S28: ^1H NMR (400 MHz, CDCl_3) of 2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranose (**28**)

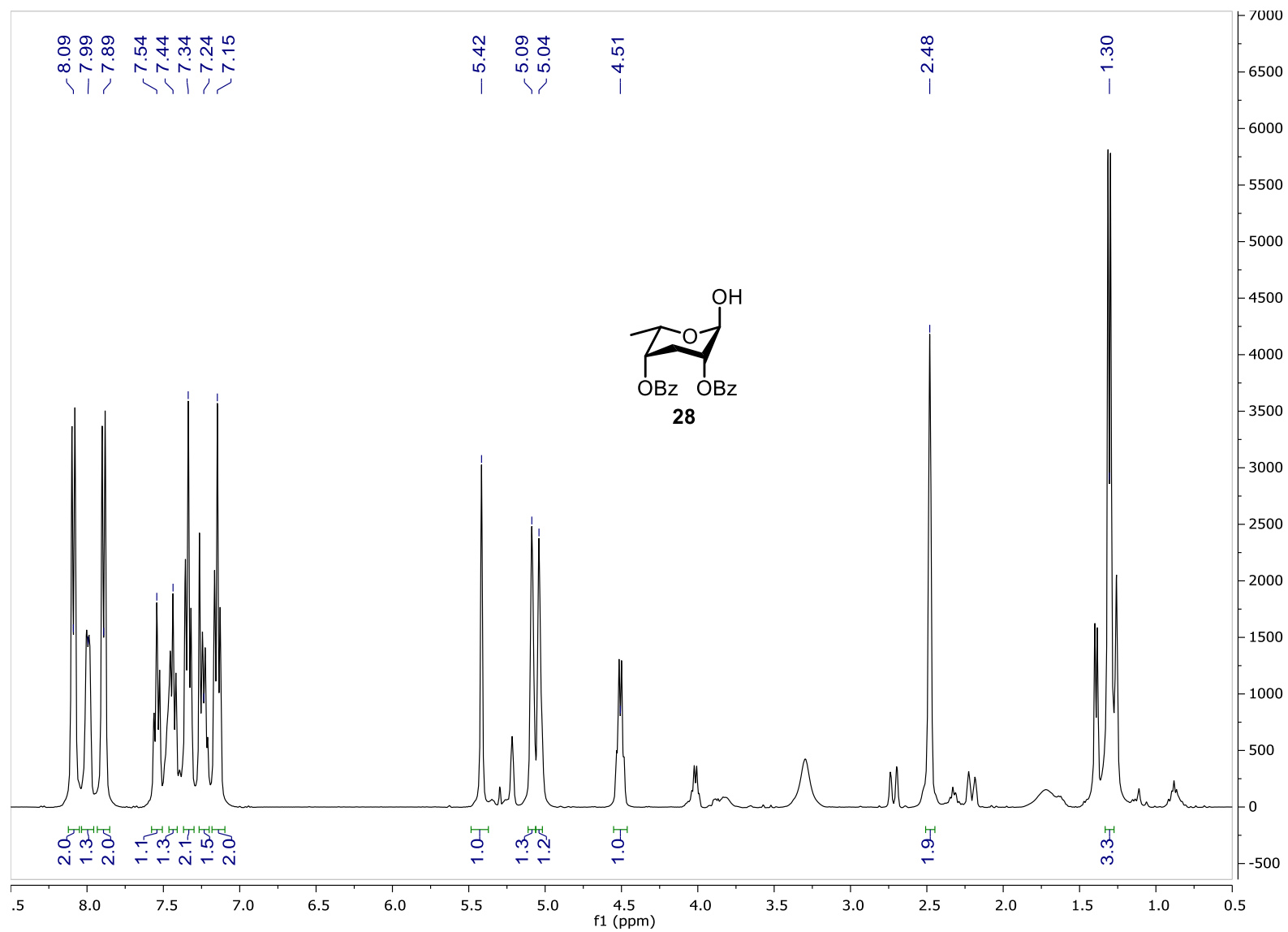


Figure S29: ^{13}C NMR (100 MHz, CDCl_3) of 2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranose (**28**)

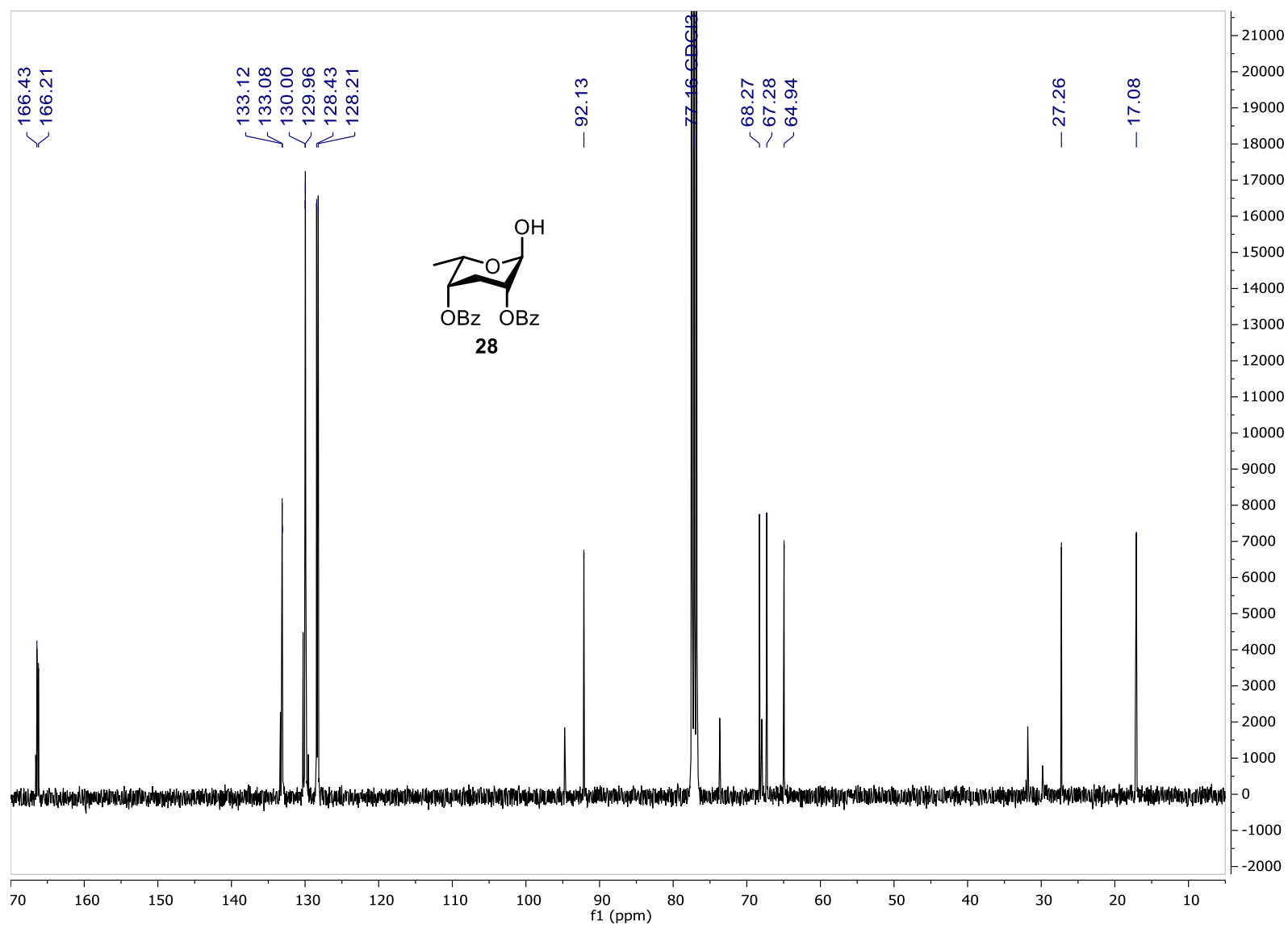


Figure S30: *dqf*-COSY (400 MHz, CDCl₃) of 2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranose (**28**)

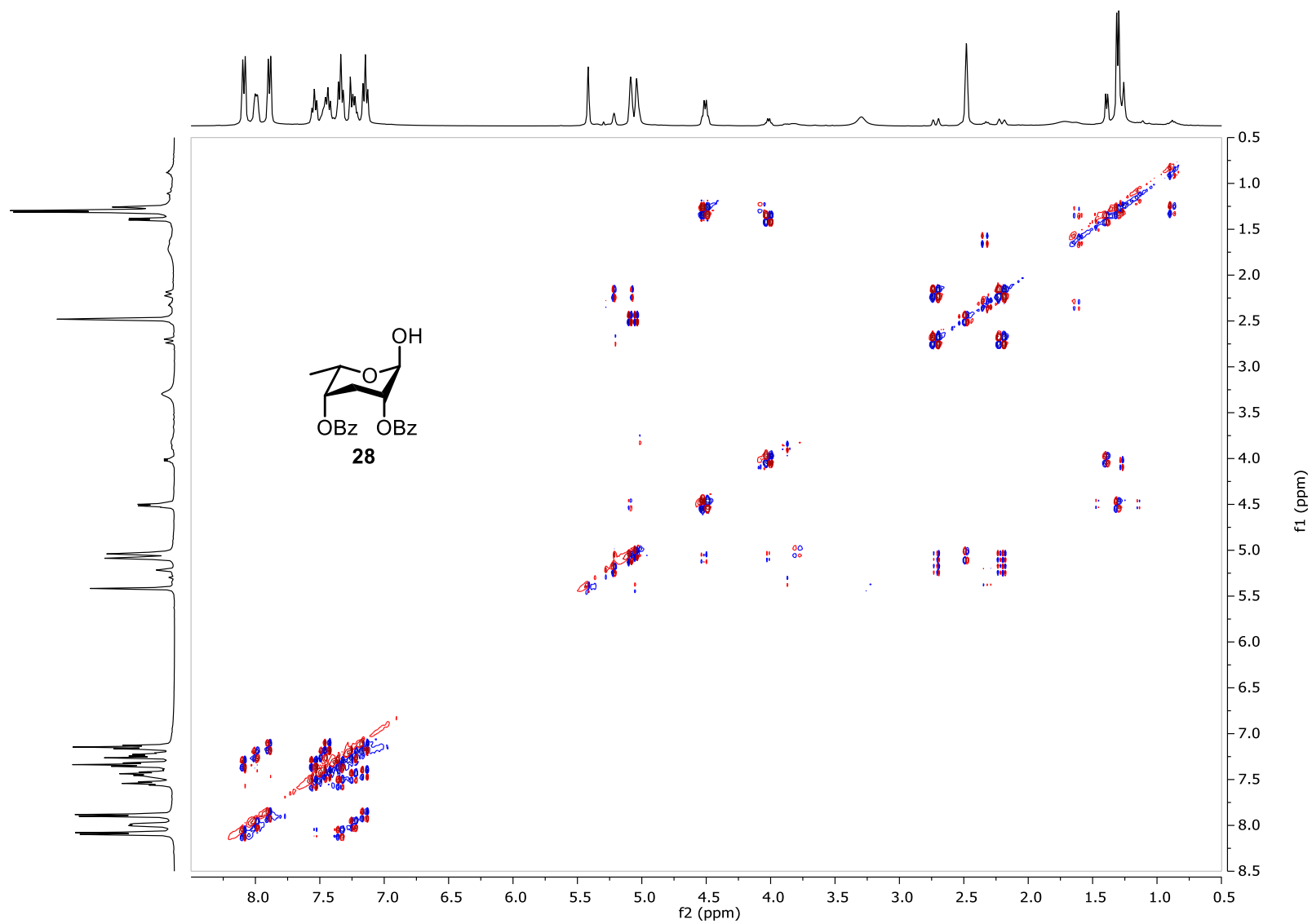


Figure S31: HSQC (400 MHz, CDCl₃) of 2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranose (**28**)

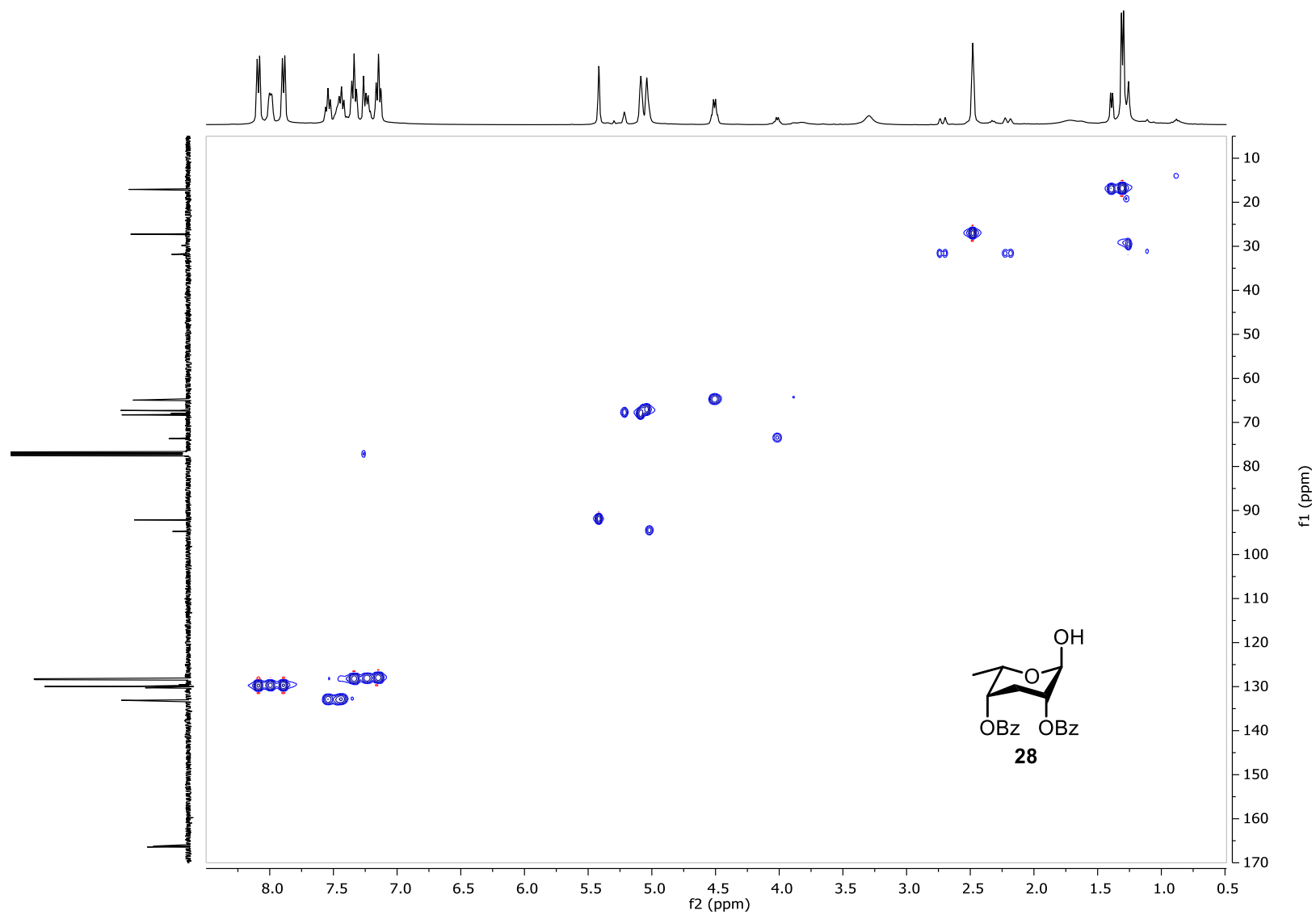


Figure S32: ^1H NMR (400 MHz, CDCl_3) of (7*R*,8*R*,2*E*)-*threo*-ethyl 7-benzoyloxy-8-[(2,4-di-*O*-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-2-nonenoate (**29**)

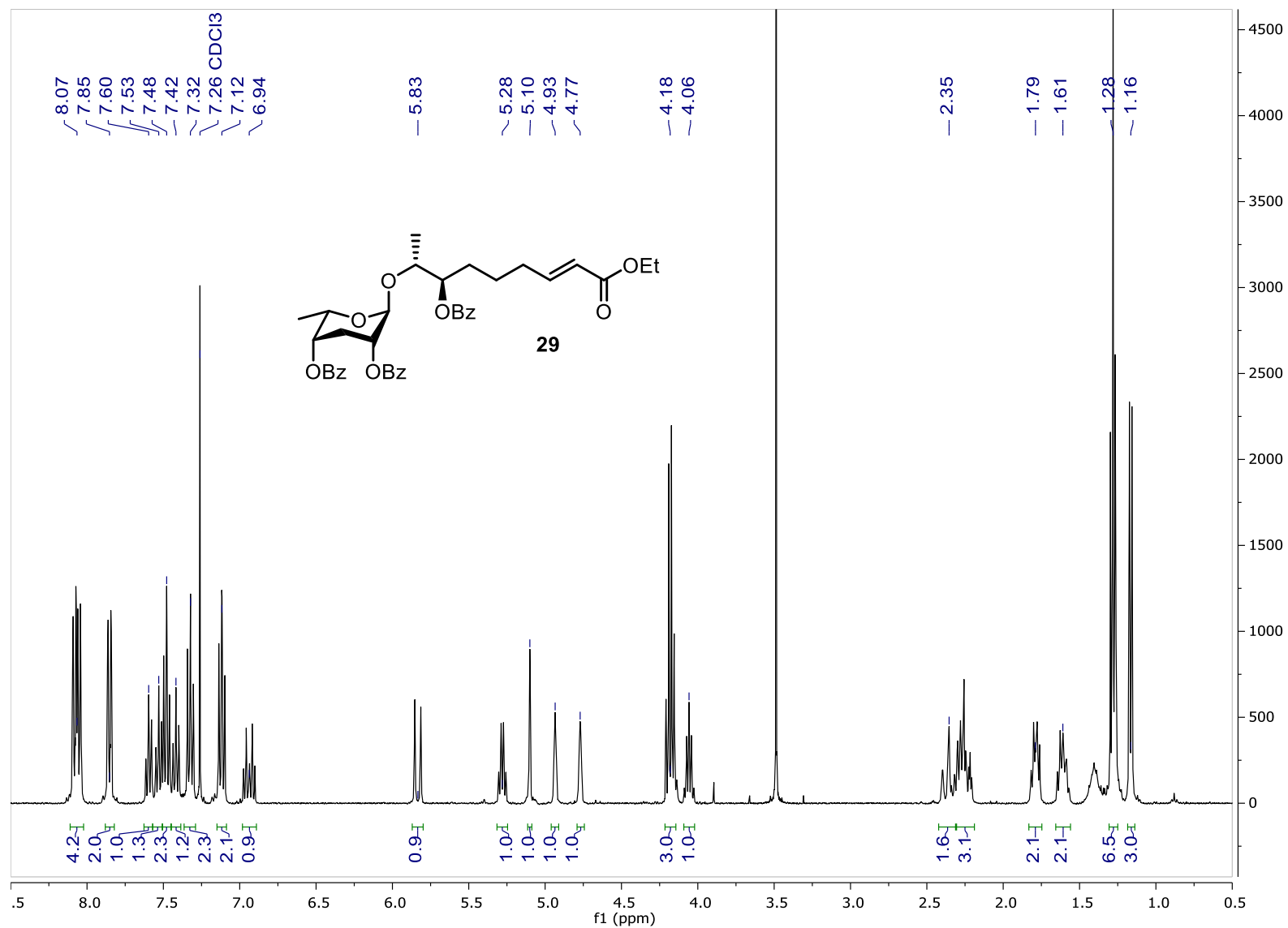


Figure S33: ^{13}C NMR (100 MHz, CDCl_3) of (7*R*,8*R*,2*E*)-*threo*-ethyl 7-benzoyloxy-8-[(2,4-di-*O*-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-2-nonenolate (**29**)

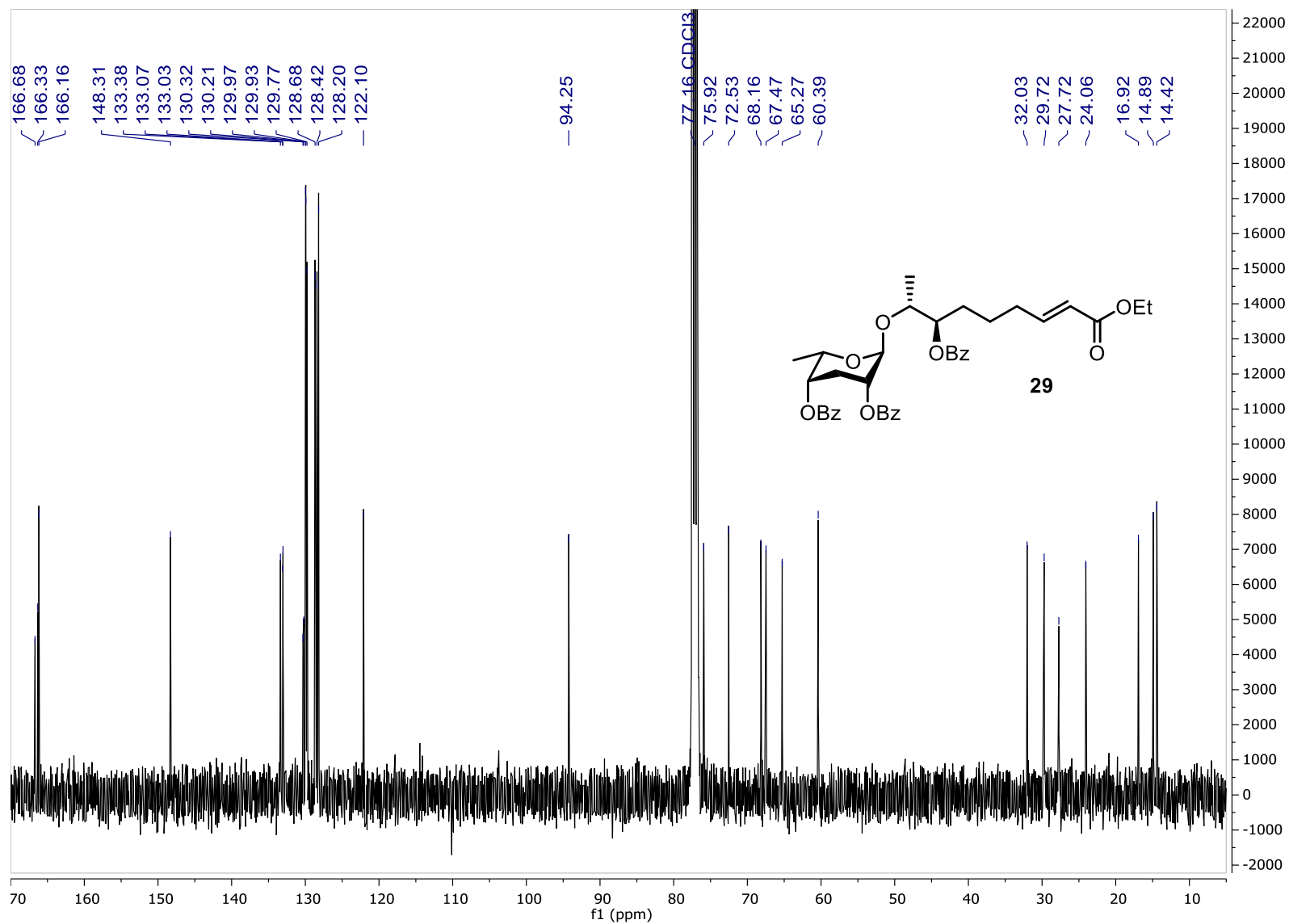


Figure S34: *dqf*-COSY (400 MHz, CDCl₃) of (7*R*,8*R*,2*E*)-*threo*-ethyl 7-benzoyloxy-8-[(2,4-di-*O*-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-2-nonenoate (**29**)

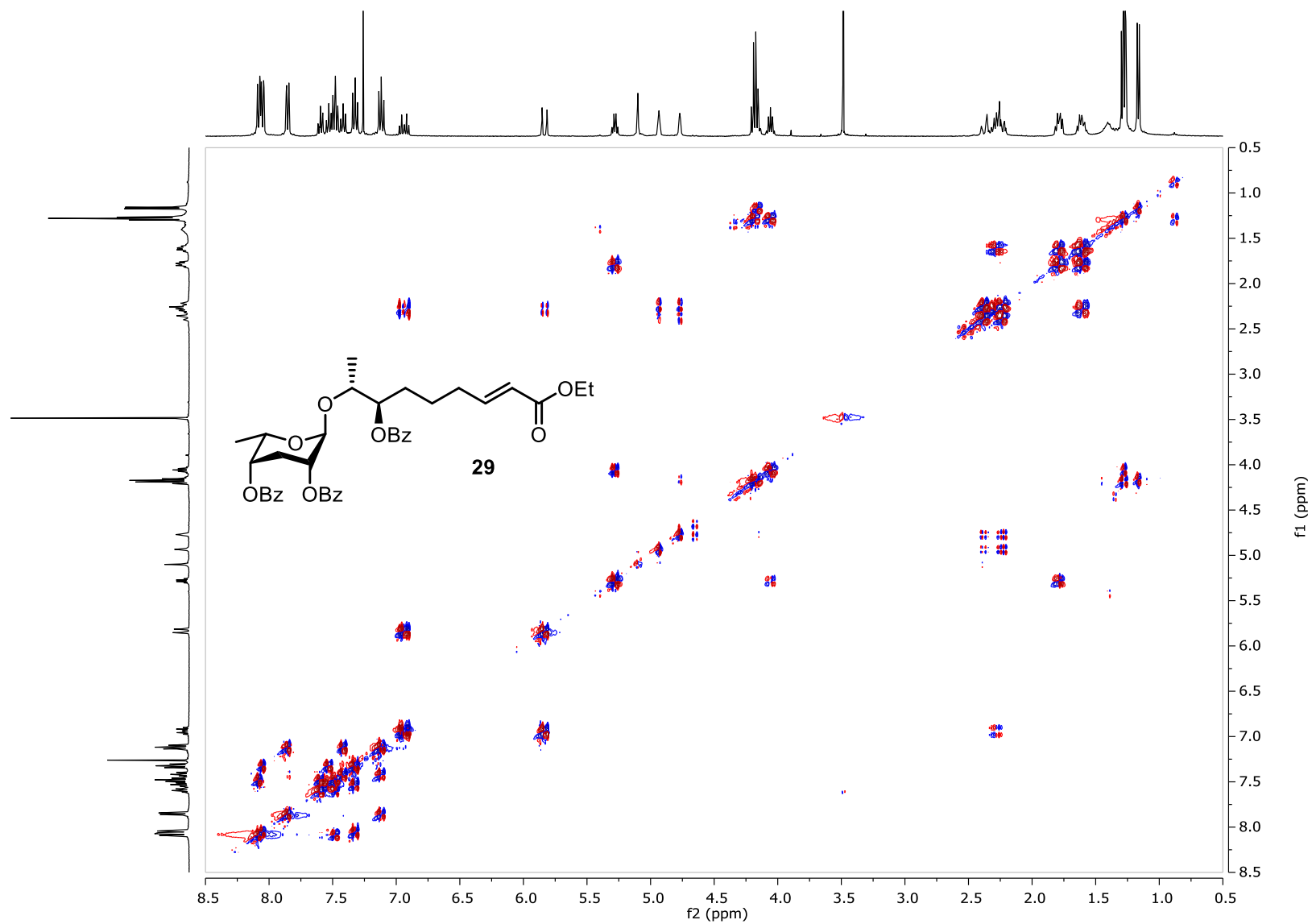


Figure S35: HSQC (400 MHz, CDCl₃) of (7*R*,8*R*,2*E*)-*threo*-ethyl 7-benzoyloxy-8-[(2,4-di-O-benzoyl-3,6-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-2-nonenoate (**29**)

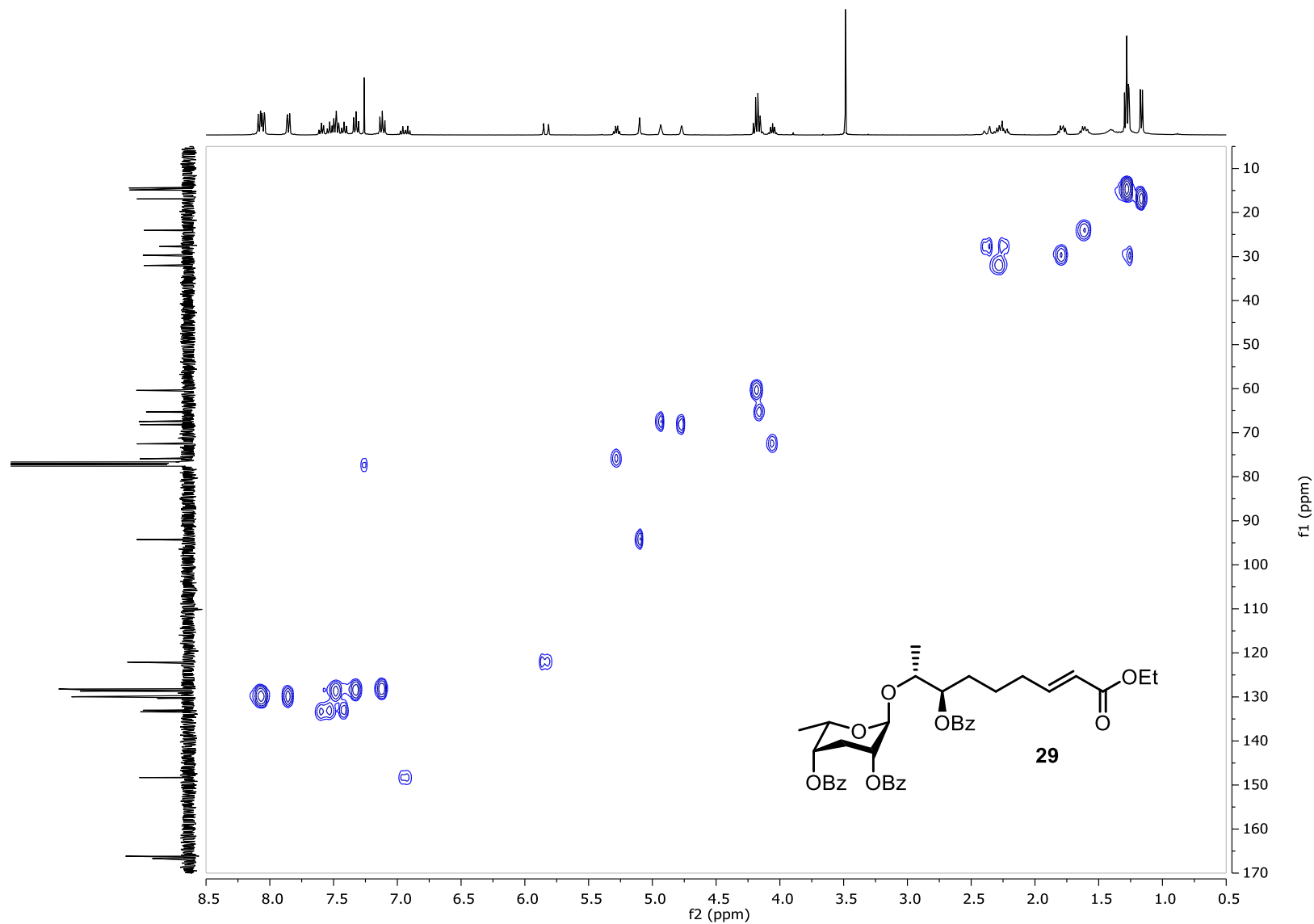


Figure S36: ^1H NMR (400 MHz, CD_3OD) of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-*lyxo*-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**)

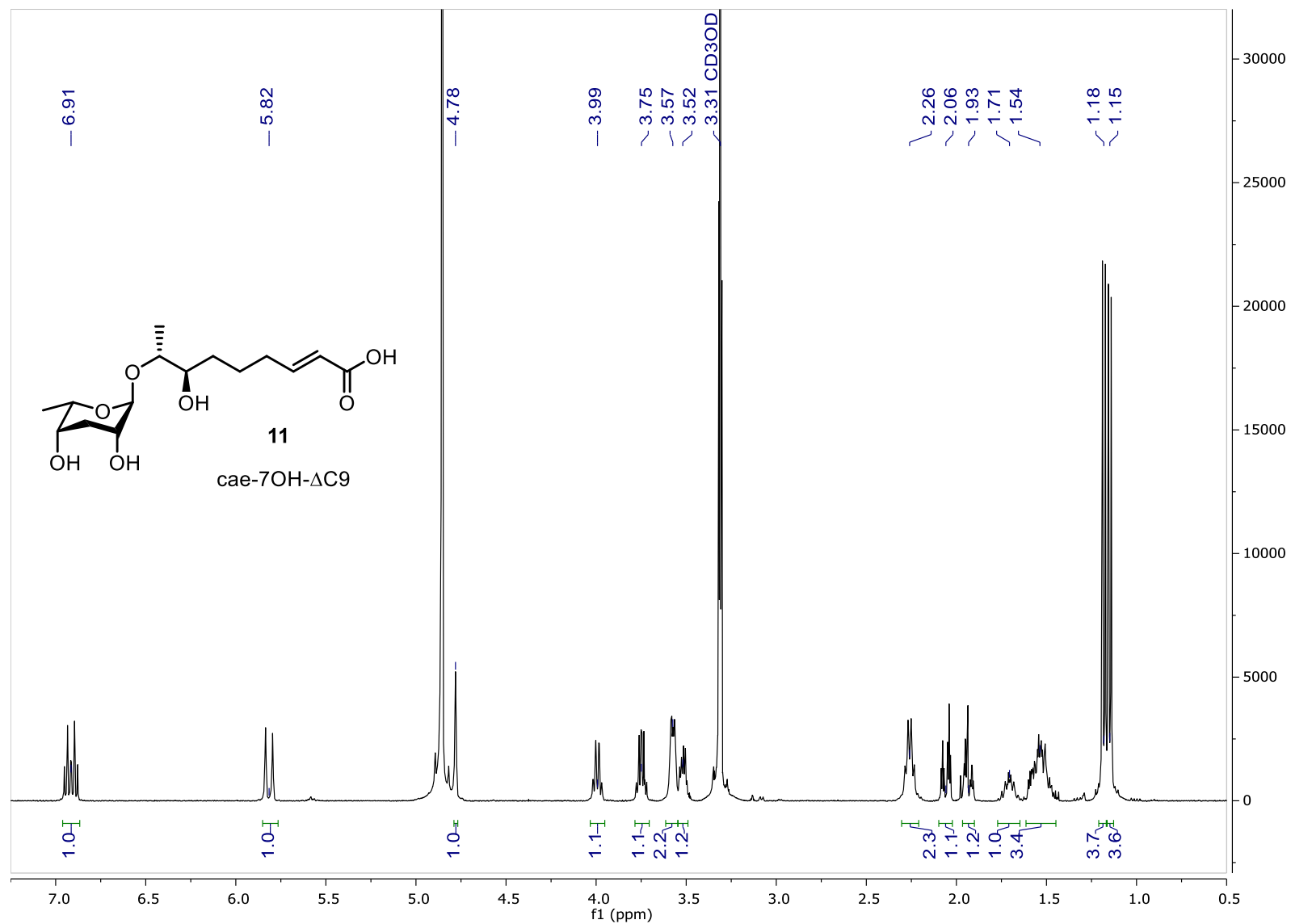


Figure S37: ^{13}C NMR (100 MHz, CD_3OD) of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-*lyxo*-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**)

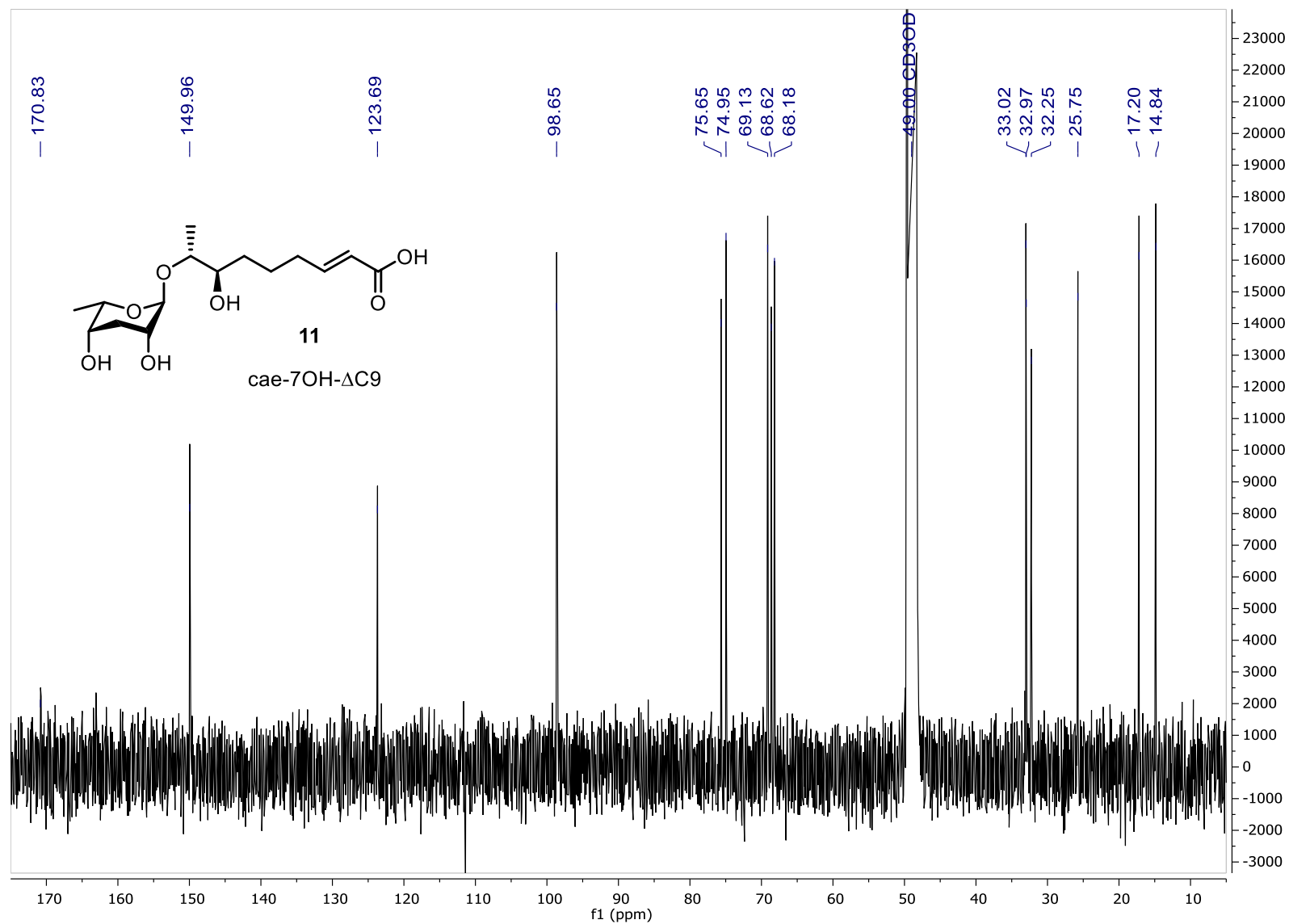


Figure S38a: *dqf*-COSY (400 MHz, CD₃OD) of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-*lyxo*-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**)

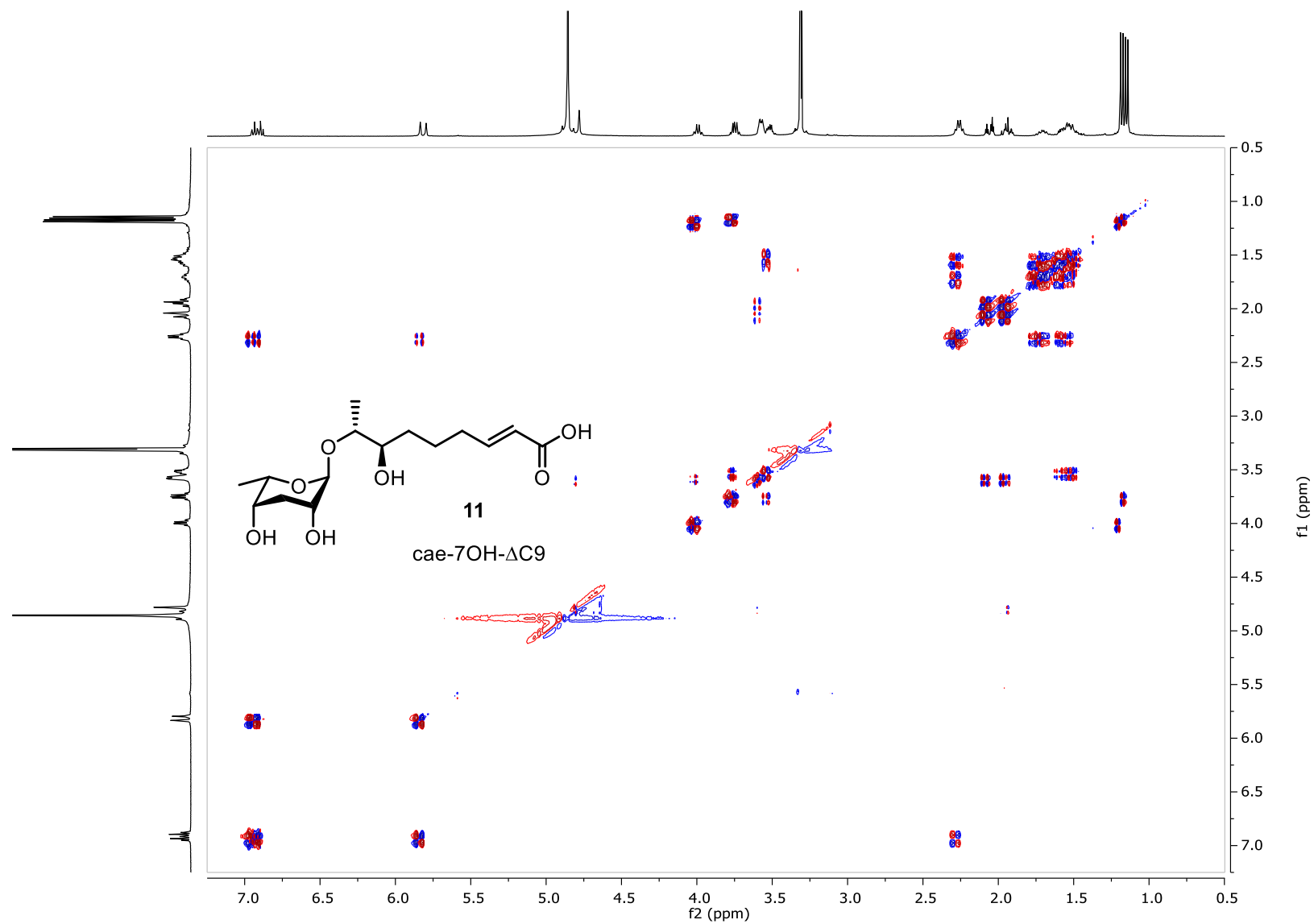


Figure S38b: Section of the *dqf*-COSY (400 MHz, CD₃OD) of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-*lyxo*-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**) showing ⁴*J*-W-coupling of the anomeric 1-H and the equatorial methylene proton 3-H.

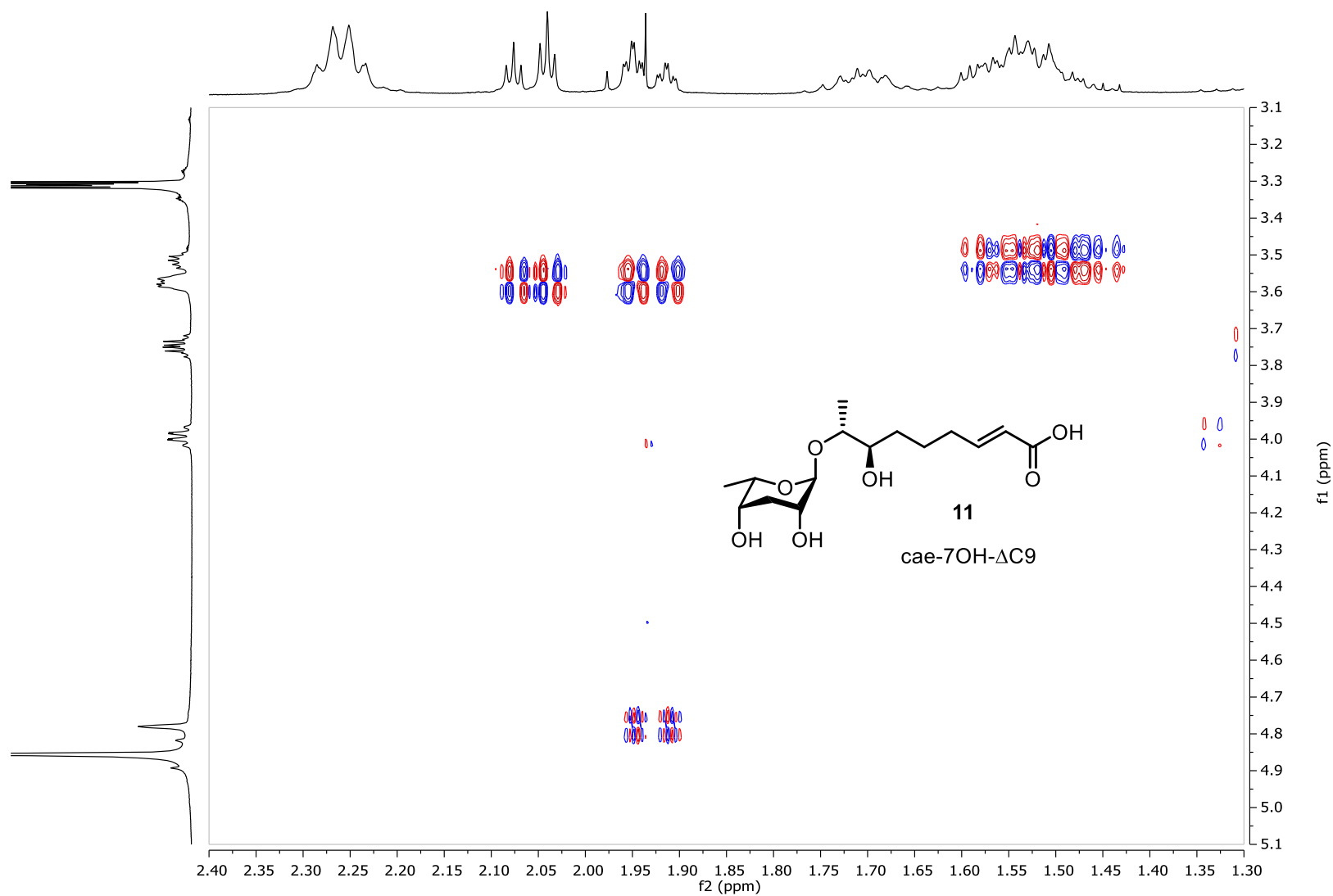


Figure S39a: HSQC (400 MHz, CD₃OD) of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-*lyxo*-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**)

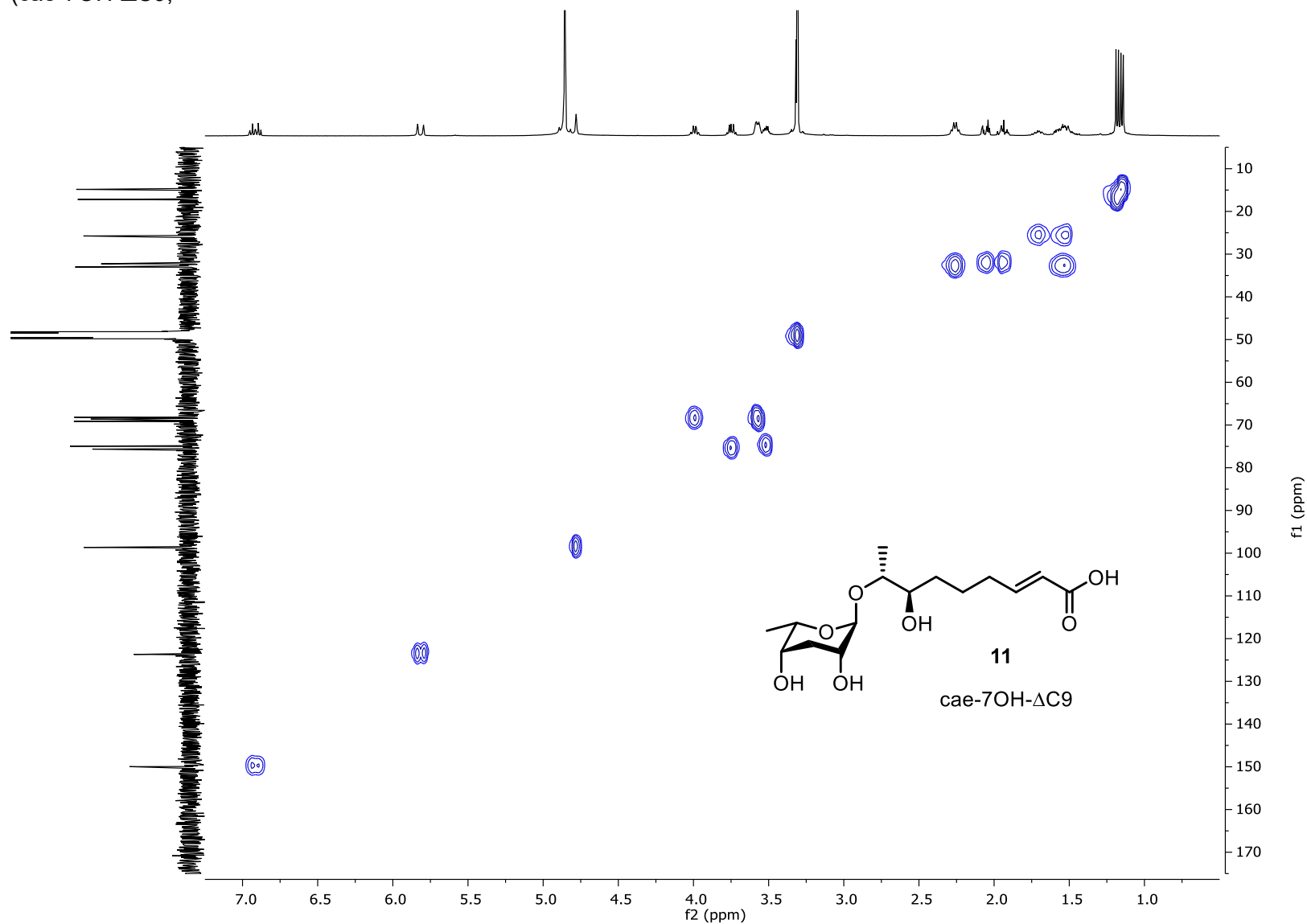


Figure S39b: Section of the HSQC (400 MHz, CD₃OD) spectrum of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-lyxo-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**) showing assignment of ¹H and ¹³C NMR signals of 2-C, 4-C, and 5-C.

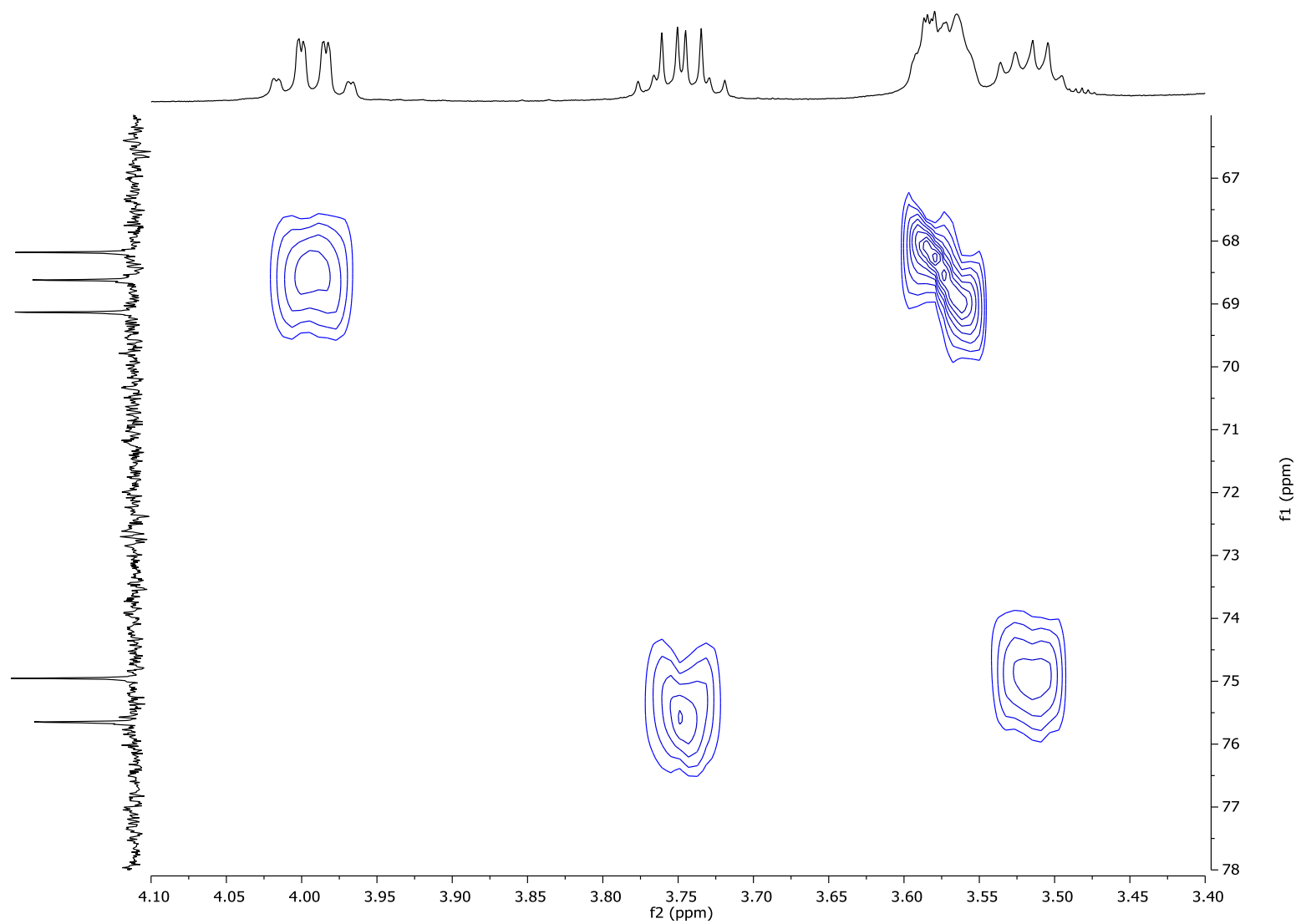
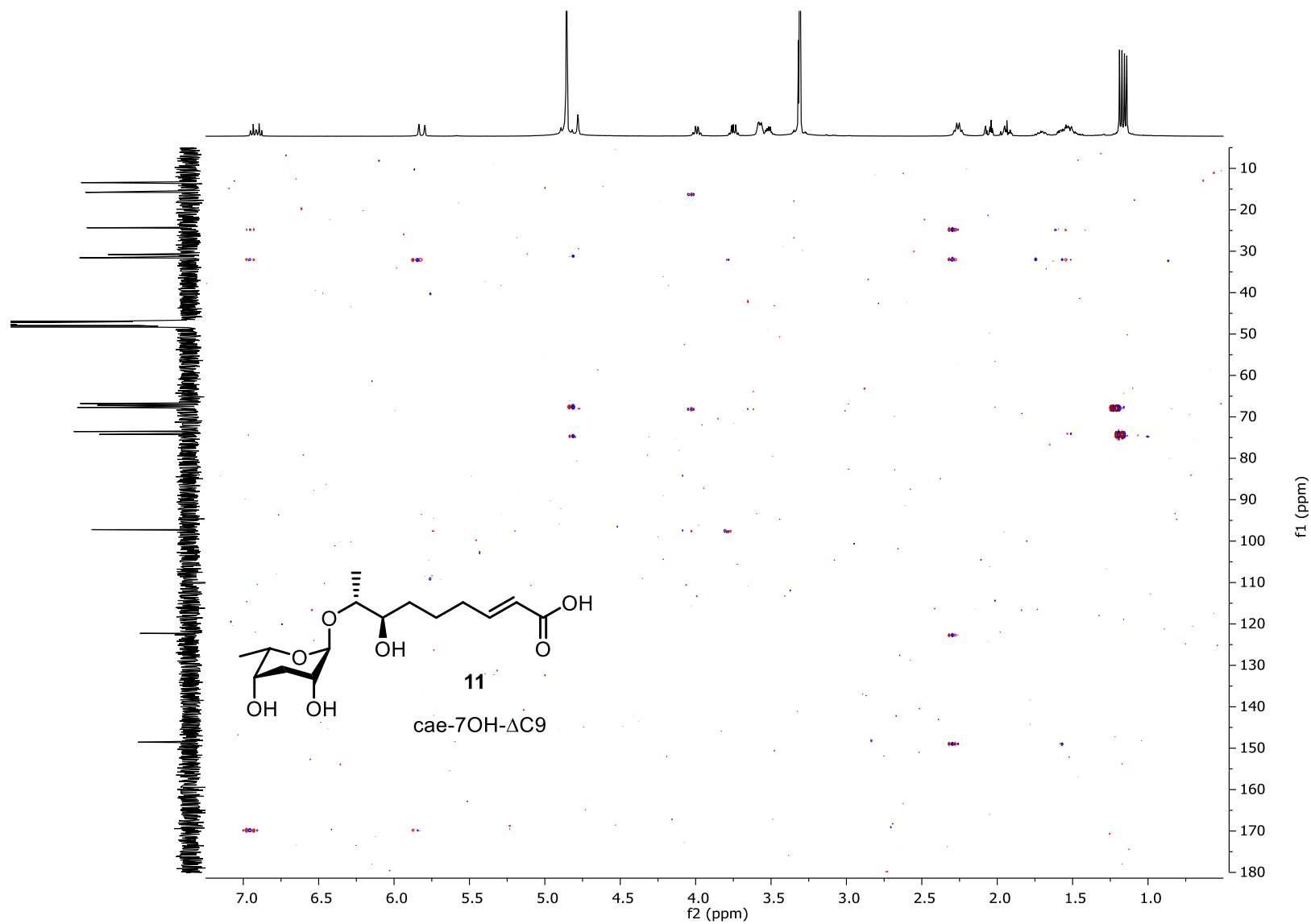


Figure S40: HMBC (400 MHz, CD₃OD) of (7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy- α -L-*lyxo*-hexopyranosyl)oxy]-7-hydroxy-2-nonenic acid (cae-7OH- Δ C9, **11**)



References

- (1) Dong, C.; Reilly, D. K.; Bergame, C.; Dolke, F.; Srinivasan, J.; von Reuss, S. H. *J. Org. Chem.* **2018**, 83, 7109-7120.
- (2) Zunk, M.; Kiefel, M. J. *Tetrahedron Lett.* **2011**, 52, 1296-1299.
- (3) Cuccarese, M. F.; Wang, H.-Y. L.; O'Doherty, G. A. *Eur. J. Org. Chem.* **2013**, 2013, 3067-3075.