Supporting Information for

Comparative Metabolomics Reveals Biogenesis of Ascarosides, a Modular Library of Small Molecule Signals in *C. elegans*

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1. Materials and Methods

1.1. Analytical Instrumentation

NMR spectra were recorded on Varian INOVA 600 (600 MHz for ^{1}H, 151 MHz for ^{13}C), INOVA 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C), or INOVA 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometers. NMR spectra were processed using Varian VNMR, MestreLabs MestReC and Mnova software packages.

Low-resolution HPLC–MS and HPLC–MS/MS was performed using an Agilent 1100 Series HPLC system equipped with a diode array detector and connected to a Quattro II mass spectrometer (Micromass/Waters). High resolution MS/MS was performed using an LTQ Orbitrap Velos Hybrid FT mass spectrometer (Thermo Scientific, Cornell University Life Sciences Core Laboratories Center). High resolution HPLC-MS was performed using a Waters nanoACQUITY UPLC System equipped with a Waters Acquity UPLC HSS C-18 column (2.1 x 100 mm, 1.8 μm particle diameter) connected to a Xevo G2 QTof Mass Spectrometer. MassLynx software was used for MS data acquisition and processing.

Flash column chromatography was performed using a Teledyne ISCO CombiFlash system. HPLC fractionation was performed using the Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5 μm particle diameter) coupled to a Teledyne ISCO Foxy 200 fraction collector.

1.2. *C. elegans* Strains and General Culture Methods

C. elegans variety Bristol, strain N2 (wild type), *acox-1*(*ok2257*), *dhs-28*(*hj8*), *maoc-1*(*hj13*), *maoc-1*(*ok2645*), *daf-22*(*m130*), *daf-22*(*ok693*), F58F9.7(*tm4033*), C48B4.1(*ok2619*), F59F4.1(*ok2119*), and F08A8.3(*tm5192*) were maintained at 20 °C on NGM agar plates, made with Bacto agar (BD Biosciences) and seeded with *E. coli* OP50 grown overnight.

1.3. Preparation of Metabolite Extracts

Wild-type (N2, Bristol) or *acox-1(ok2257)*, *maoc-1(hj13)*, *dhs-28(hj8)*, and *daf-22(ok693)* mutant worms from four 10 cm NGM plates were washed using M9-medium into a 100 mL S-medium pre-culture where they were grown for four days at 22°C on a rotary shaker at 220 rpm. Concentrated *E. coli* OP50 derived from 1 L of bacterial culture was added as food at days 1 and 3. Subsequently, each pre-culture was divided equally into four 500 mL Erlenmeyer flasks containing 100 mL of S-medium on day 4. Two of these cultures, labeled non-starved (NS) were grown for 5 days at 22°C on a rotary shaker and fed with concentrated OP50 derived from 500 mL of bacterial culture every day from day 1 to day 4. The remaining two cultures of each set, labeled starved (S) were fed once with concentrated OP50 derived from 500 mL of bacterial culture on day 1 and grown for an additional 9 days at 22°C on a rotary shaker without food. Subsequently, the cultures were harvested on day 5 for NS and day 10 for S, centrifuged, and the resultant supernatant media and worm pellets were frozen over dry ice-acetone slush and lyophilized separately. The lyophilized materials from the supernatant were extracted with 150 mL of 95% ethanol at room temperature for 16 h. The worm pellets were crushed with ~2 g of granular NaCl using a mortar pestle and extracted with 100 mL of 100% ethanol at room temperature for 16 h. The resulting suspensions were filtered and the filtrate evaporated in vacuum at room temperature, producing media metabolite (the worm "excretome") extracts and worm pellet metabolite extracts.

1.4. Ascaroside Feeding Experiment with *daf-22*(*m130*)

Ascaroside feeding experiments were performed with the *daf-22(m130)* mutant, which is less sensitive to growth defects due to added ascarosides than the *daf-22(ok693)* mutant (Schroeder, unpublished results). HPLC-MS analysis of *daf-22(m130)* showed similar ascaroside profiles as *daf-22(ok693)*, notably a total lack of short chain ascarosides with chain length less than 12 carbons. Non-starved cultures of *daf-22 (m130)* were grown as described before with the addition of 5 μM ascr#3 or a 1:1 mixture of 2.5 μM of each of ascr#10 and oscr#10 per culture on day 1 after pre-culture splitting.

1.5. Sample Preparation

Media or worm pellet metabolite extracts were resuspended in ~15 mL methanol, centrifuged and the supernatant collected. The supernatant were then concentrated in vacuum at room temperature and resuspended in 1 mL methanol, centrifuged, and 30 μL of this extract was directly injected for LC-MS/MS analysis.

1.6. Mass Spectrometric Analysis

HPLC-MS/MS profiling was performed using the Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5 μm particle diameter) connected to the Quattro II mass spectrometer using a 10:1 split. A 0.1% acetic acid – acetonitrile solvent

gradient was used at a flow rate of 3.6 ml/min, starting with an acetonitrile content of 5% for 5 min which was increased to 100% over a period of 40 min. Metabolite extracts were analyzed by HPLC-ESI-MS in negative and positive ion modes using a capillary voltage of 3.5 kV and a cone voltage of -40 V and +20 V respectively. HPLC-MS/MS screening for precursor ions of m/z = 73.0 (negative mode) and neutral loss of 130.0 (positive mode) was performed using argon as collision gas at 2.1 mtorr and 30 eV. Ascaroside fragmentation was further analyzed by high-resolution MS/MS using the LTQ Orbitrap. To confirm elemental composition of new compounds, mutant metabolome samples and fractions were additionally analyzed by high-resolution HPLC-MS using the Xevo G2 QTof.

1.7. Identification and Quantification of Ascarosides

For the identification of ascarosides detected in wild type and mutants (ascr, oscr, bhas, bhos, icas, icos, ibha, ibho and glas; see Table S1-11), HPLC-retention times were plotted versus m/z (or chain length). Components belonging to a homologous series exhibited almost linear elution profiles (Figure S2), indicating that components within a series share the same relative stereochemistry. The structure and stereochemistry of the various series were then identified based on (1) isolation of representative examples and NMR analysis (for example see Figure S3, S4, S9, S12), (2) comparison of representative examples with synthetic standards, (3) molecular formula as established from high-resolution MS, (4) characteristic MS/MS fragmentation (see Figure S1), and (5) HPLC-retention times that matched retention time values extrapolated from those of the synthetic samples. The (*E*)-configuration of α,β-unsaturated ascarosides was established by comparison with ascr#3, (*Z*)-configured ascr#3, and ascr#7, and is also suggested by the stereoselectivity of acyl-CoA-oxidase (ACOX) activity. The (3*R*)-stereochemistry of β-hydroxyascarosides (bhas and bhos series) was deduced from comparison with synthetic standards of bhas#10, bhas#22, and bhos#26 as representative examples, and is also suggested from the sequence homology of MAOC-1 and DHS-28 with (*R*)-selective MFE-2 (Figure S6).

Quantification of ascarosides was performed by integration of LC-MS signals from the corresponding ion-traces. Ascaroside concentrations were calculated using response factors determined for synthetic standards of ascr#1, #3, #5, #7, #9, #10, oscr#9, #10, bhas#22, bhos#26, icas#3, #9, icos#10, and glas#10. For most compounds, mass spectrometer response was roughly linear (less than 10% error) for amounts of 1 pmol to 2 nmol per injection. Response factors for ascarosides that were not synthesized were extrapolated based on data observed for the available standards. Generally, we observed strong differences between the response factors of short-chained members of each series (side-chains less than C7), whereas differences between response factors of longer-chained homologs were small. Since not all short-chained members of all series were synthesized, the systematic errors of the absolute amounts reported for some short-chain ascarosides could be larger than for longer-chained compounds.

In order to roughly account for culture duration and worm biomass, we report ascaroside content of the excretome and worm pellet samples in fmol ascarosides produced per hour of culture time per mg of worm pellet dry weight. All quantitative data reported in the Figures were derived from at least two independent biological repeats.

1.8. Spot Attraction Assays

Attraction assays with hbas#3 were done as previously described.[1,2] For the attraction assays, we harvested 50-60 hermaphrodite worms daily at the early fourth larval stage (L4) and stored them at 20 °C overnight to be used as young adults the following day. hbas#3 was dissolved in water containing 10% ethanol. Aliquots were stored at -20 °C in 20 µL tubes. 10% ethanol in water was used as control.

1.9. Quadrant Assays for Measuring Chemotaxis

Chemotaxis to hbas#3 was assessed on 10 cm four-quadrant petri plates.[3] Each quadrant was separated from adjacent ones by plastic spacers. Pairs of opposite quadrants were filled with nematode growth medium (NGM) agar containing different concentrations of hbas#3. Animals were washed gently in a S-basal buffer and placed in the center of a four-quadrant plate with ascarosides in alternating quadrants, and scored after 15 and 30 min. A chemotaxis index was calculated as (the number of animals on ascaroside quadrants minus the number of animals on buffer quadrants)/(total number of animals).

1.10. Statistical Analyses

We used unpaired student's t-tests with Welch's correction for comparing ascaroside profiles between wild-type and mutant metabolomes and for comparing attraction of hermaphrodites on hbas#3 (*P<0.05, **P< 0.001, ***P<0.0001). For comparing the chemotaxis indices of the different concentrations of hbas#3, we used one-factor ANOVA followed by Dunnett's post-test (*P<0.05, **P< 0.01).

2. Supporting Figures

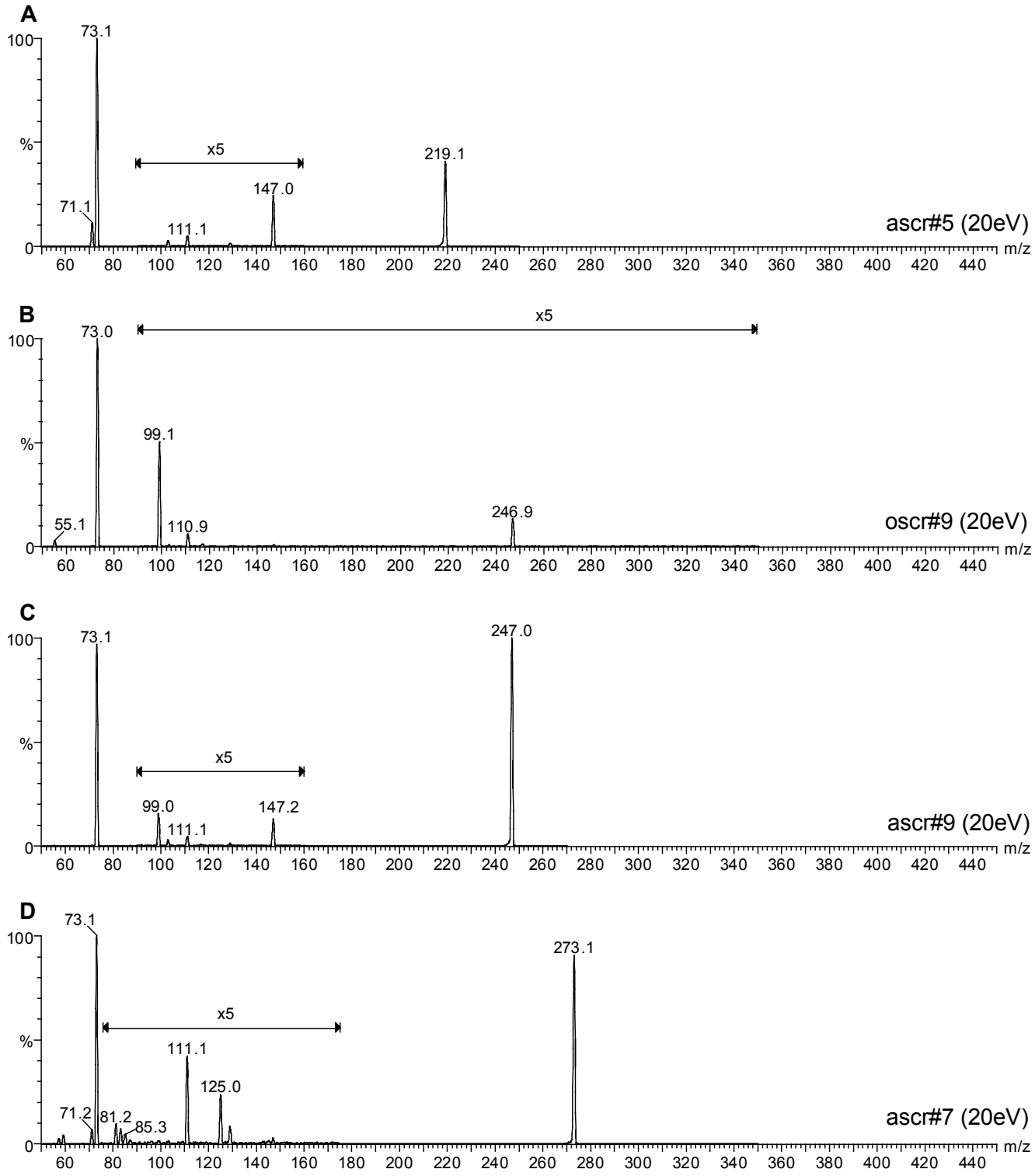


Figure S1. A-D. MS/MS product ion spectra of ascaroside standards. **A.** ascr#5 shows *m/z* 147 [$C_6H_{11}O_4$] for ascarylose and *m/z* 73 for the C_3-sidechain and/or the ascarylose derived $C_3O_2H_5$ fragment of identical molecular composition. **B** and **C.** ascr#9 and oscr#9 show *m/z* 99 [$C_5H_7O_2$] for the C_5-sidechain, *m/z* 147 [$C_6H_{11}O_4$], and *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment. **D.** ascr#7 shows *m/z* 125 [$C_7H_9O_2$] for the C_7-sidechain, *m/z* 111 [$C_6H_7O_2$] and *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment.

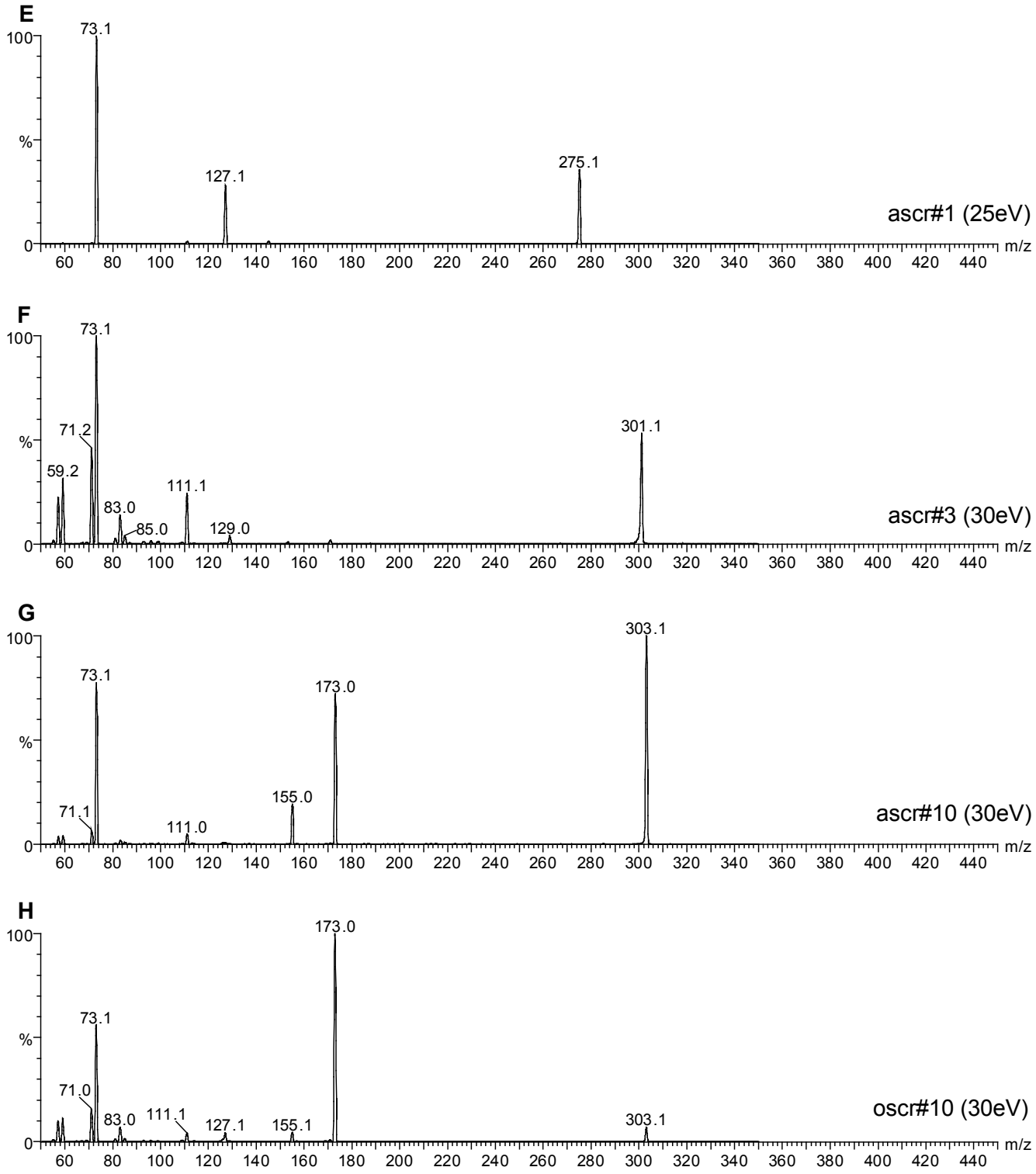


Figure S1. E-H. MS/MS product ion spectra of ascaroside standards. **E.** ascr#1 shows *m/z* 127 [$C_7H_{11}O_2$] for the C_7-sidechain and *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment. **F.** ascr#3 shows *m/z* 111 [$C_6H_7O_2$] and *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment. **G** and **H.** ascr#10 and oscr#10 show *m/z* 173 [$C_9H_{15}O_3$] and 155 [$C_9H_{13}O_2$] for the C_9-sidechain and *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment.

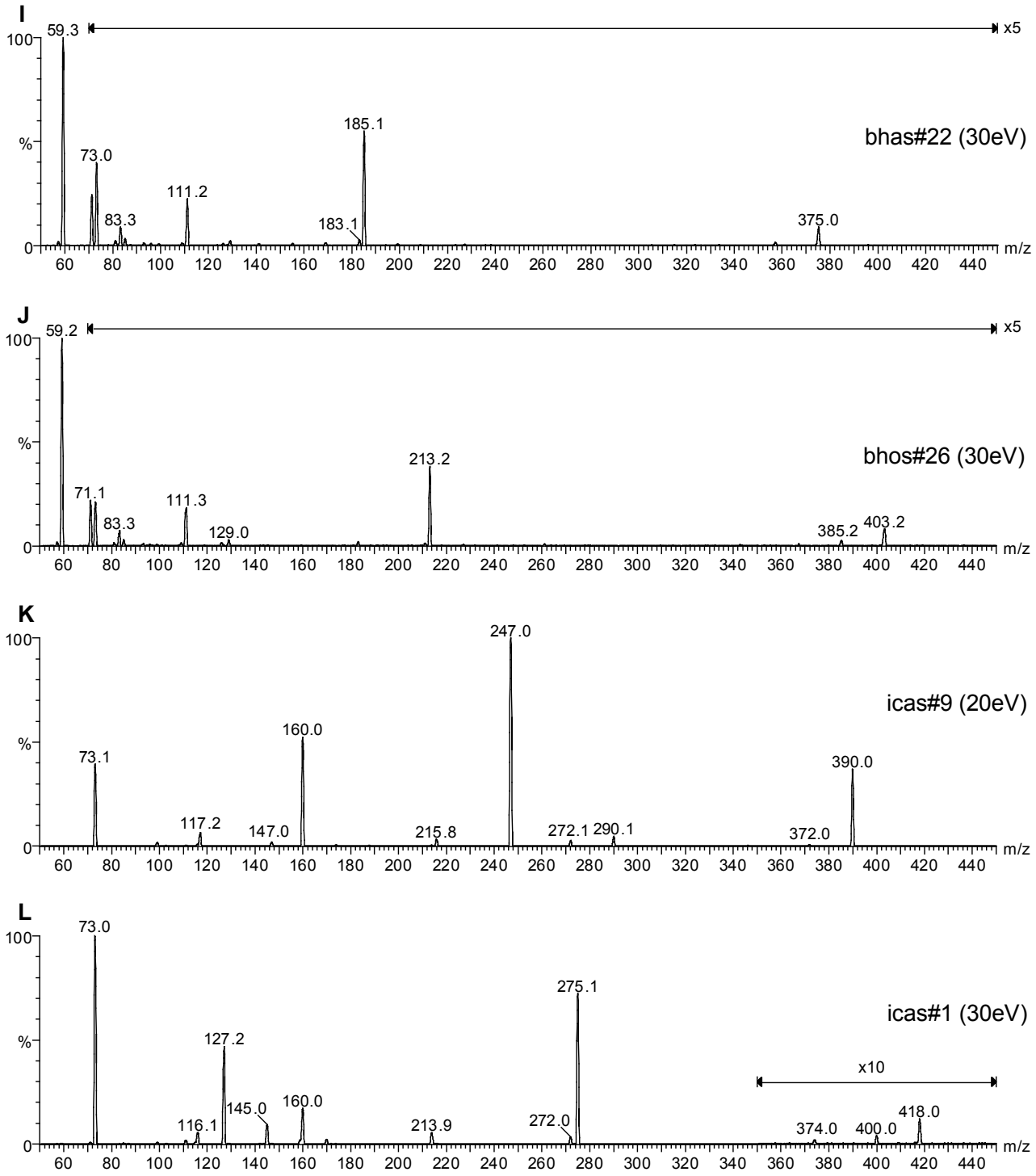


Figure S1. I-L. MS/MS product ion spectra of ascaroside standards. **I** and **J.** β-hydroxy ascarosides such as bhas#22 (C_{13}-sidechain) and bhos#26 (C_{15}-sidechain) show side chain specific fragment ions at *m/z* 185 [$C_{11}H_{21}O_2$] and *m/z* 213 [$C_{13}H_{25}O_2$], respectively, which originate from loss of ascarylose and acetic acid. Furthermore, bhas#22 and bhos#26 show an intensive product ion at *m/z* 59 [$C_2H_3O_2$] for acetate, as well as the diagnostic ion at *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment. **K** and **L**. Indole ascarosides such as icas#9 and icas#1 show side chain specific fragment ions for the corresponding ascarosides at *m/z* 247 [$C_{11}H_{19}O_6$] and *m/z* 275 [$C_{13}H_{23}O_6$], respectively, which originate from loss of the indole carbonyl unit [C_9H_5NO]. Furthermore, icas#9 and icas#1 show *m/z* 160 [$C_9H_6NO_2$] for indole carboxylate ions. Ascaroside product ions of indole ascarosides show additional fragment ions corresponding to the fragmentation pattern of non-indole ascarosides, such as the diagnostic fragment ion at *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment.

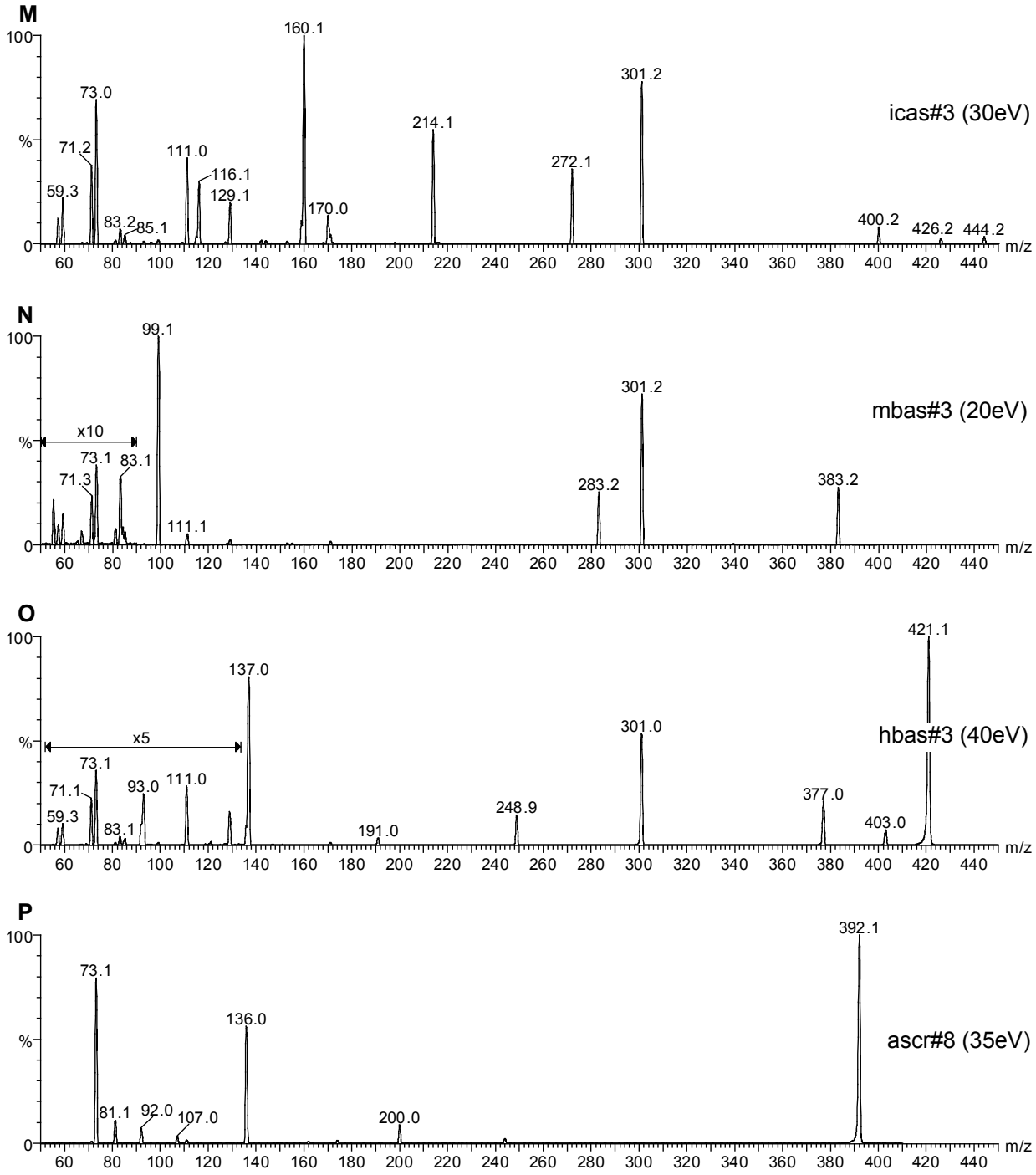


Figure S1. M-P. MS/MS product ion spectra of ascaroside standards. **M.** icas#3 shows *m/z* 301 [$C_{15}H_{25}O_6$] from loss of indole carbonyl unit [C_9H_5NO], along with *m/z* 160 [$C_9H_6NO_2$] for indole carboxylate ions. **N.** mbas#3 shows *m/z* 301 [$C_{15}H_{25}O_6$] and *m/z* 283 [$C_{15}H_{23}O_5$], which originate from loss of tigloyl [C_5H_6O]and tiglate[$C_5H_8O_2$] units, respectively. Furthermore, mbas#3 shows *m/z* 99 [$C_5H_7O_2$] for tiglate ions. The diagnostic fragment ion at *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment is of low intensity. **O.** hbas#3 shows *m/z* 301 [$C_{15}H_{25}O_6$] which originates from loss of a hydroxybenzoyl unit [C_7H_4O]. Furthermore, hbas#3 shows *m/z* 137 [$C_7H_5O_3$] and *m/z* 93 [C_6H_5O] for hydroxybenzoate and phenolate ions. The ascaroside diagnostic fragment ion at *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment is of low intensity. **P.** PABA linked ascr#8 shows characteristic fragment ions at *m/z* 136 [$C_7H_6NO_2$] and *m/z* 92 [C_6H_6N] for PABA and anilid ions, respectively, along with the diagnostic fragment ion at *m/z* 73 for the ascarylose derived $C_3O_2H_5$ fragment.

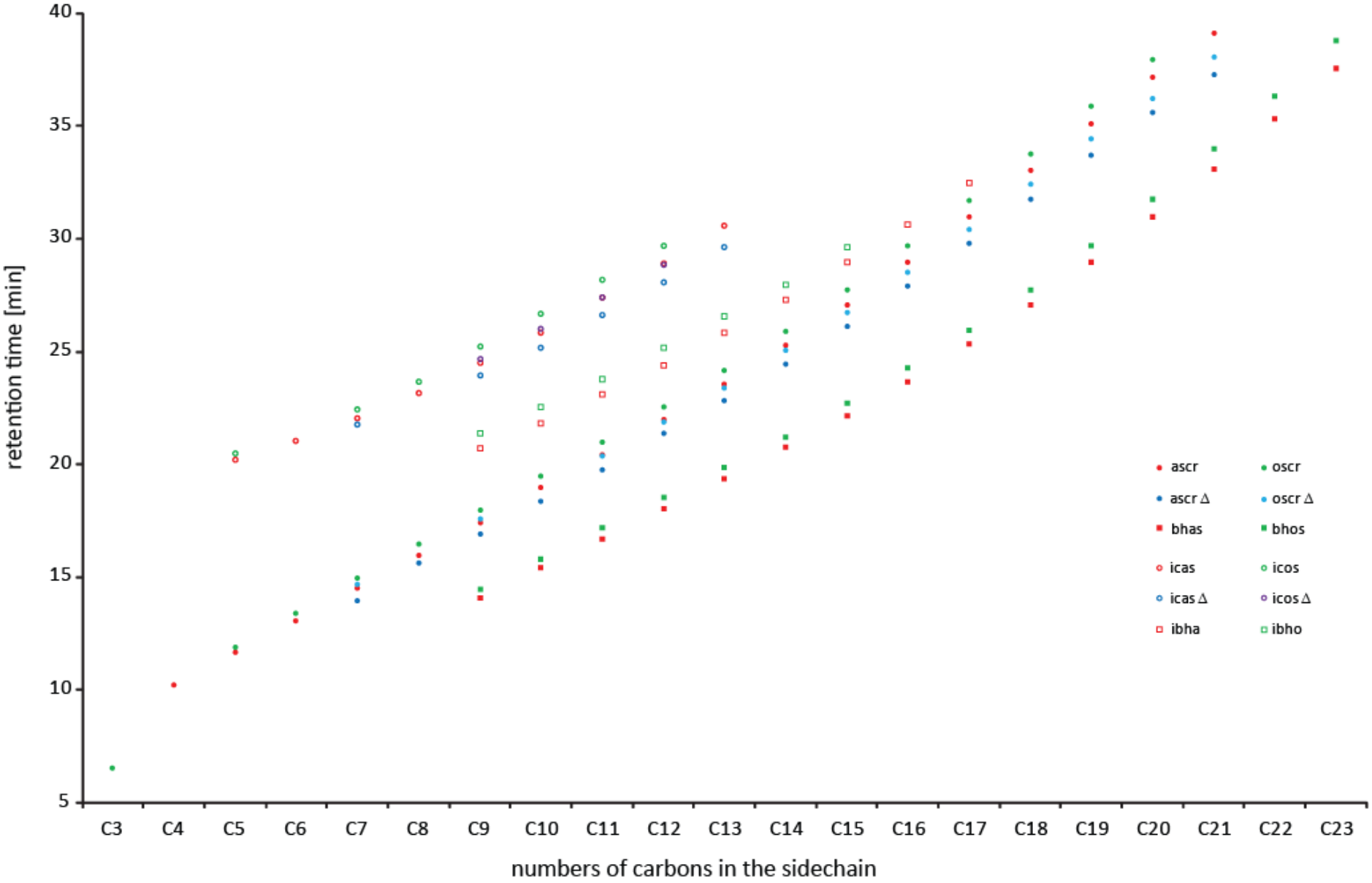


Figure S2. HPLC elution profiles of ascarosides identified in wild-type and mutant excretome extracts of *C. elegans* (Δ indicates components with (*E*)-configured α,β-unsaturated sidechains).

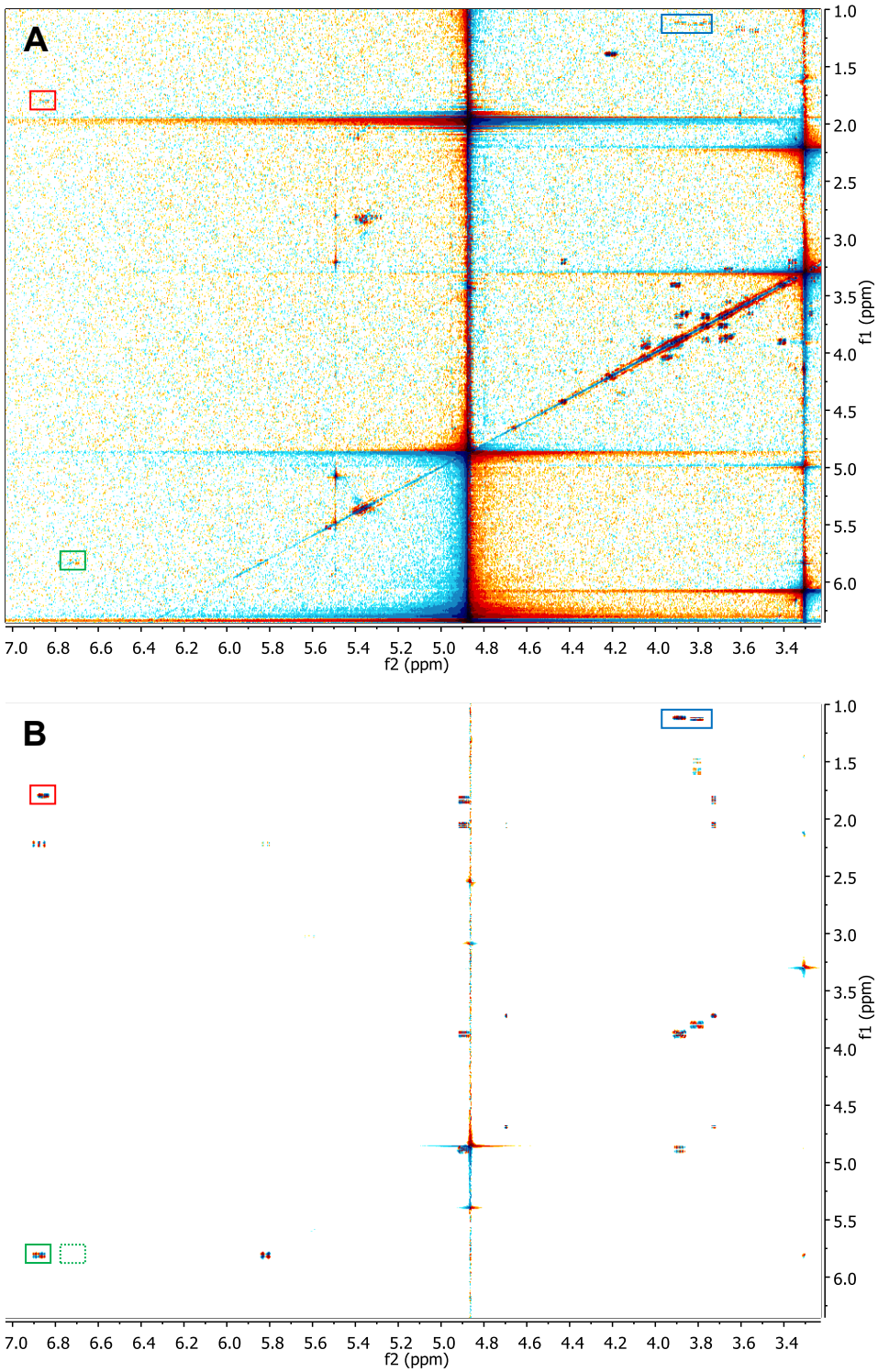


Figure S3. Sections of dqfCOSY spectra (600 MHz, methanol-d_4) of mbas#3-enriched fraction from wild-type *C .elegans* media extracts (A) and synthetic mbas#3 (B) showing characteristic signals for methyl groups of the ascarylose ring and the side chain (blue), the allylic methyl group of the tiglate unit (red), and the pH dependant signal for the side chain double bond (green).

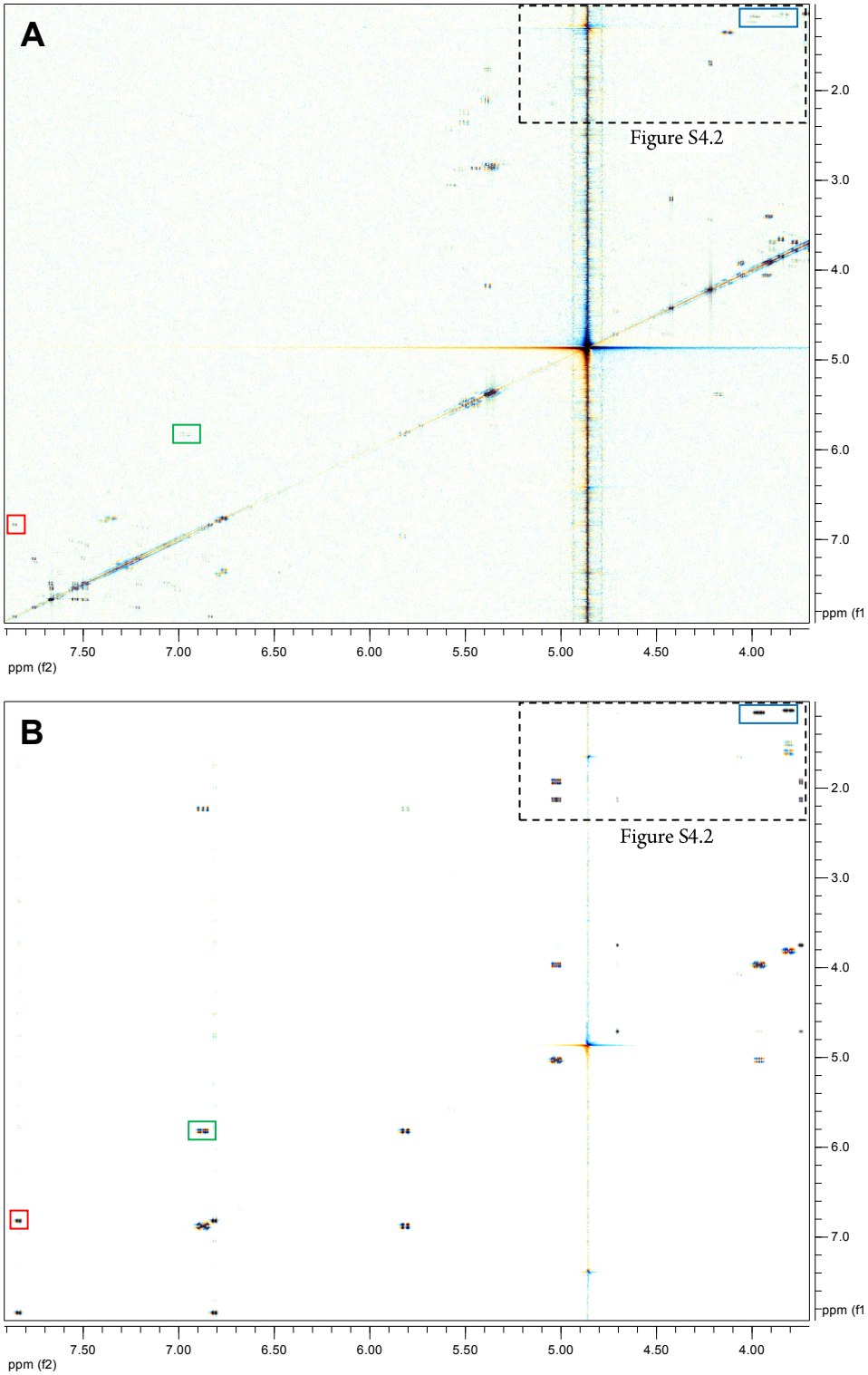


Figure S4.1. Sections of dqfCOSY spectra (600 MHz, methanol-d_4) of hbas#3-enriched fraction from wild-type *C .elegans* media extracts (A) and synthetic hbas#3 (B) showing characteristic signals for methyl groups of the ascarylose ring and the side chain (blue), the *para*-substituted 4-hydroxybenzoyl unit (red), and the side chain double bond (green).

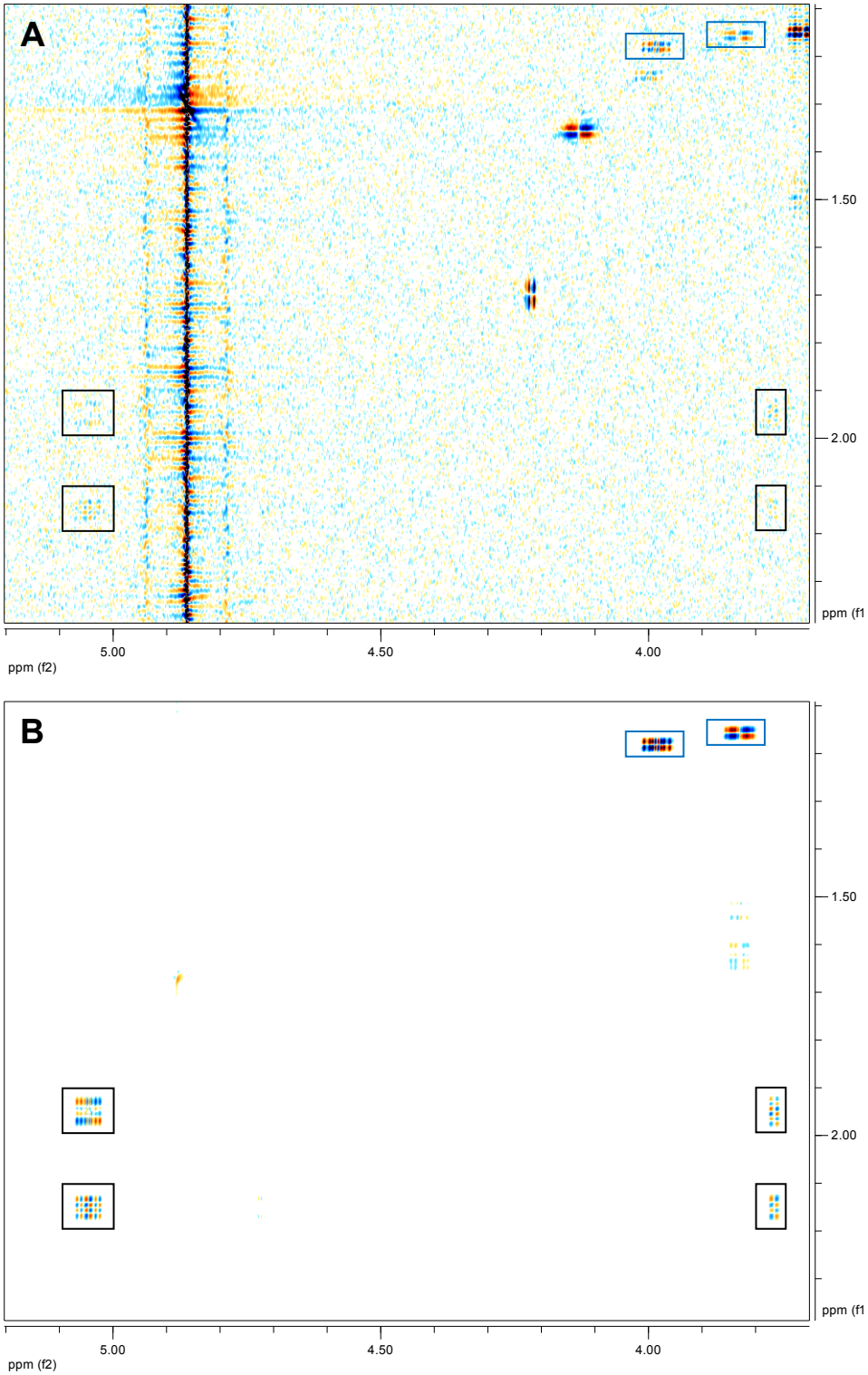


Figure S4.2. Enlarged sections of dqfCOSY spectra (600 MHz, methanol-d_4) of hbas#3-enriched fraction from wild-type *C .elegans* media extracts (A) and synthetic hbas#3 (B) showing characteristic signals for methyl groups of the ascarylose ring (blue), and the ascarylose spin system (black).

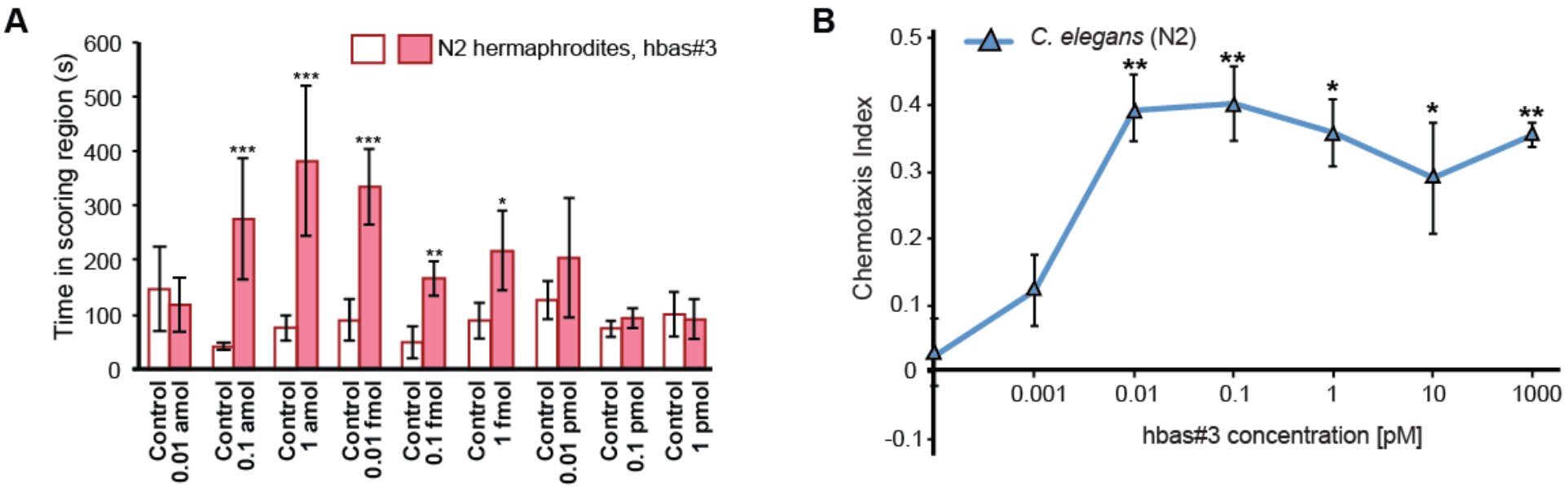


Figure S5 A. Wild-type (N2) hermaphrodites are attracted to hbas#3 in the spot attraction assay in a dose dependent manner. **B.** Dose dependence of hbas#3 attraction for wild-type hermaphrodites in the quadrant chemotaxis assay.

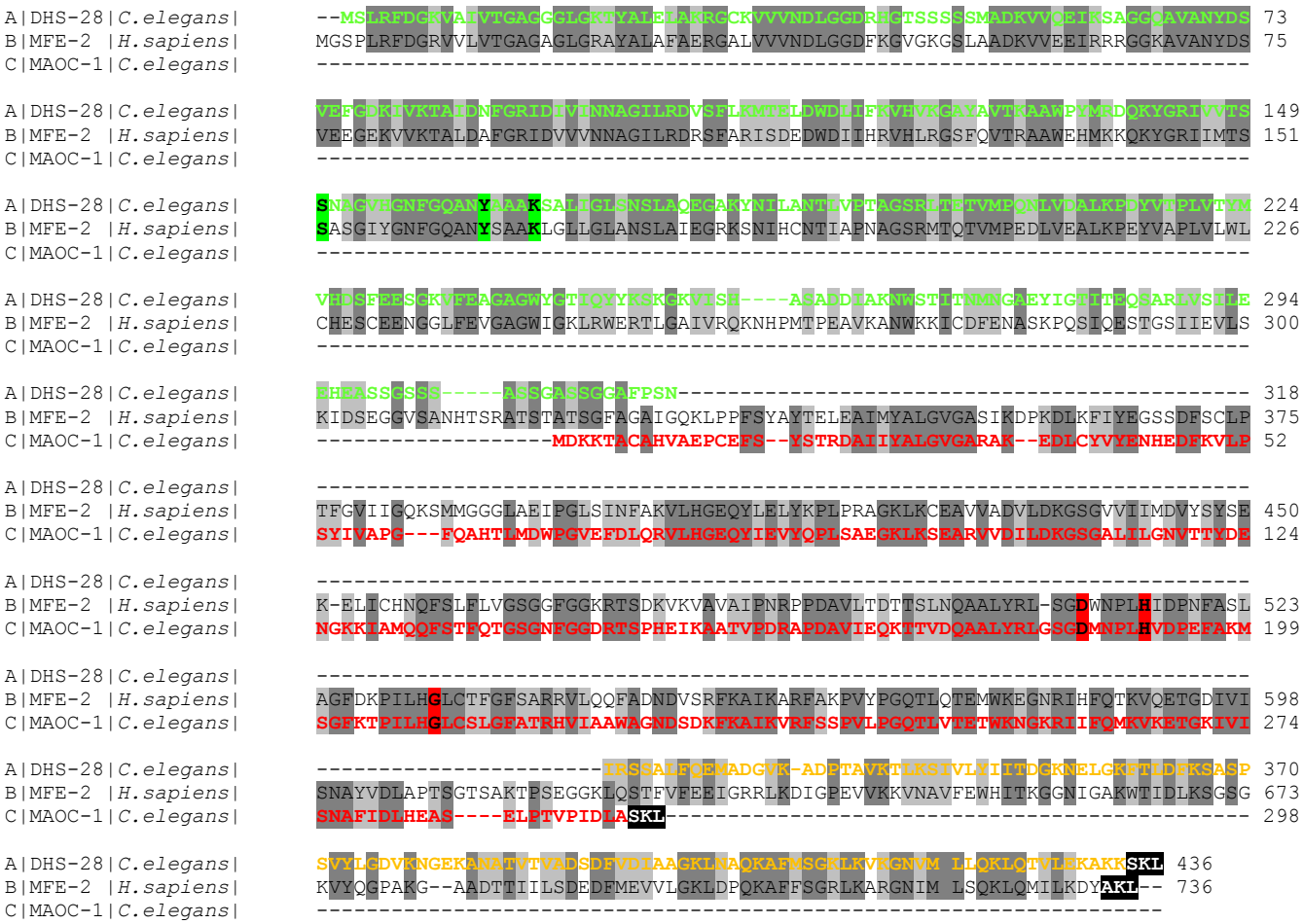


Figure S6. Alignment of human MFE-2 isoform 2 (NP_000405.1) with *C. elegans*' MAOC-1 (NP_495494.1 (red)) and DHS-28 (NP_509146.1; oxidoreductase domain (green), SCP-2 sterol carrier protein domain (yellow)) was performed using ClustalW. Amino acids constituting the catalytic sites are marked with green and red boxes and the peroxisomal targeting signal in black. Identical amino acids are marked in grey and similar amino acids are marked in light grey.

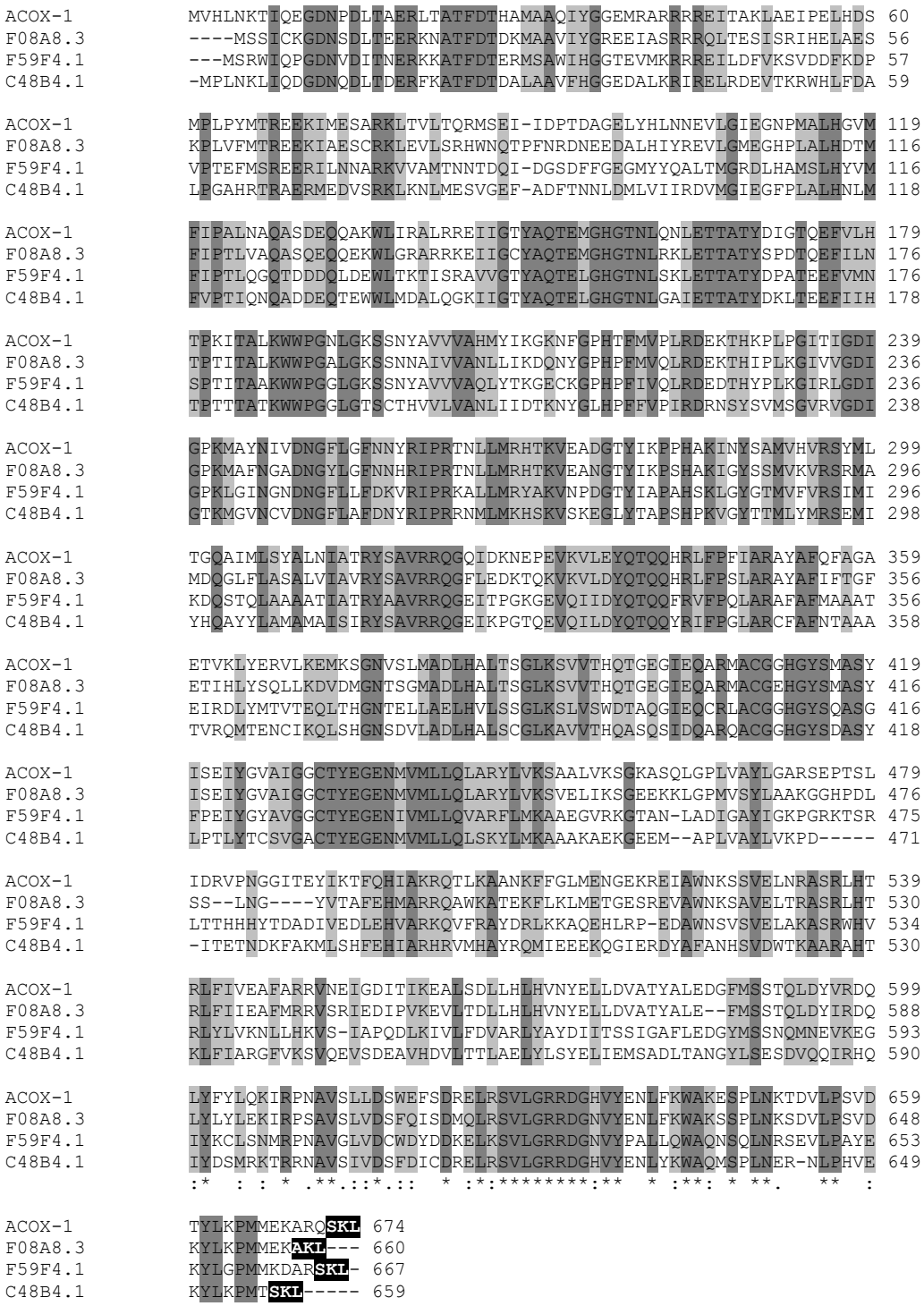


Figure S7. Alignment of *C. elegans'* ACOX-1 isoform a.1 with other peroxisomal acyl-CoA oxidases was performed using ClustalW. Identical amino acids are marked in grey, similar amino acids are marked in light grey, and the peroxisomal targeting signal is marked in black.

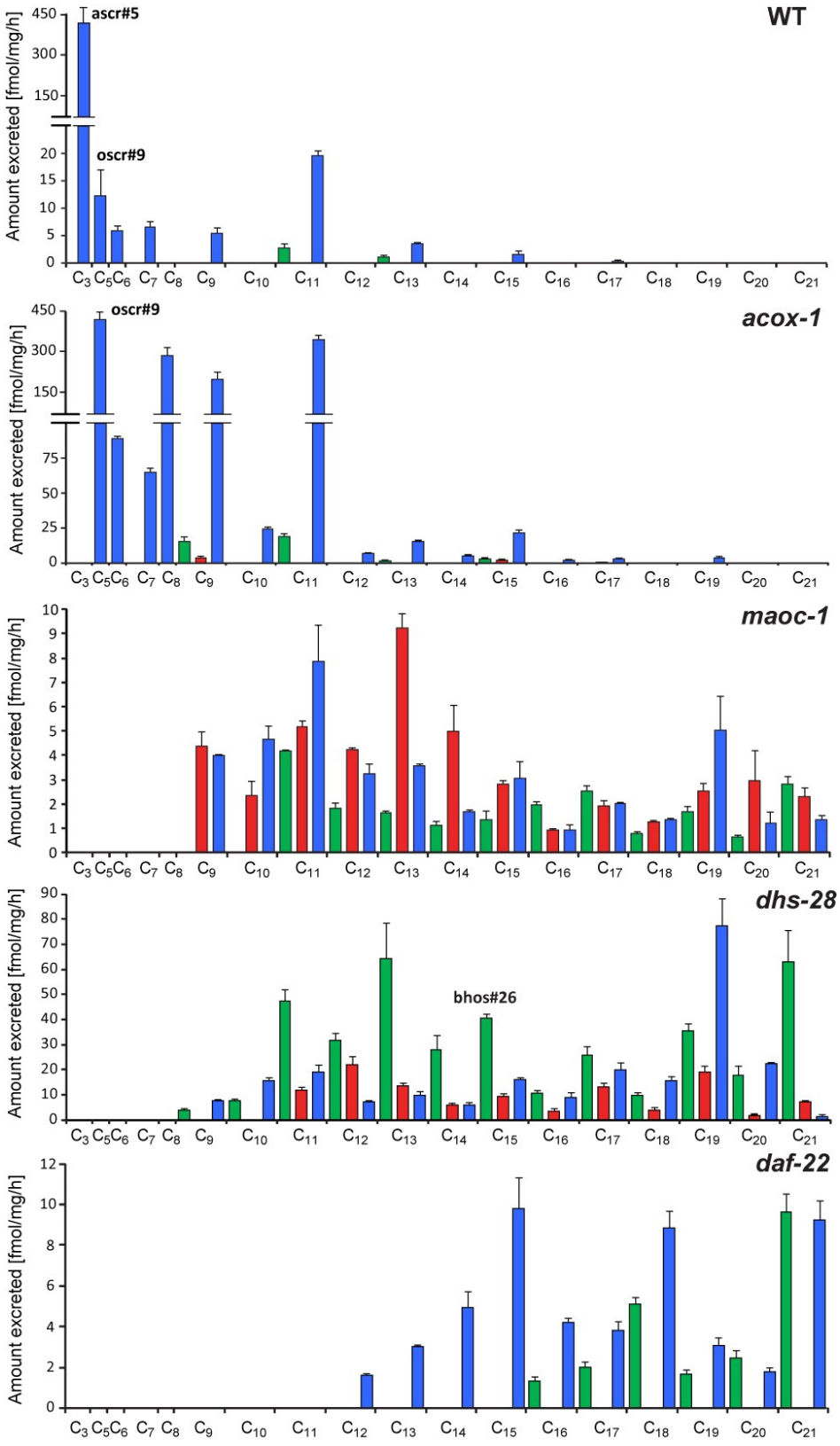


Figure S8. Profiles of (ω)-oxygenated ascaroside in wild-type (N2) and β-oxidation mutants (*acox-1*, *maoc-1*, *dhs-28*, *daf-22*) showing saturated (blue), α,β-unsaturated (red), and β-hydroxylated (green) ascarosides (For (ω-1)-oxygenated ascarosides see Figure 4c).

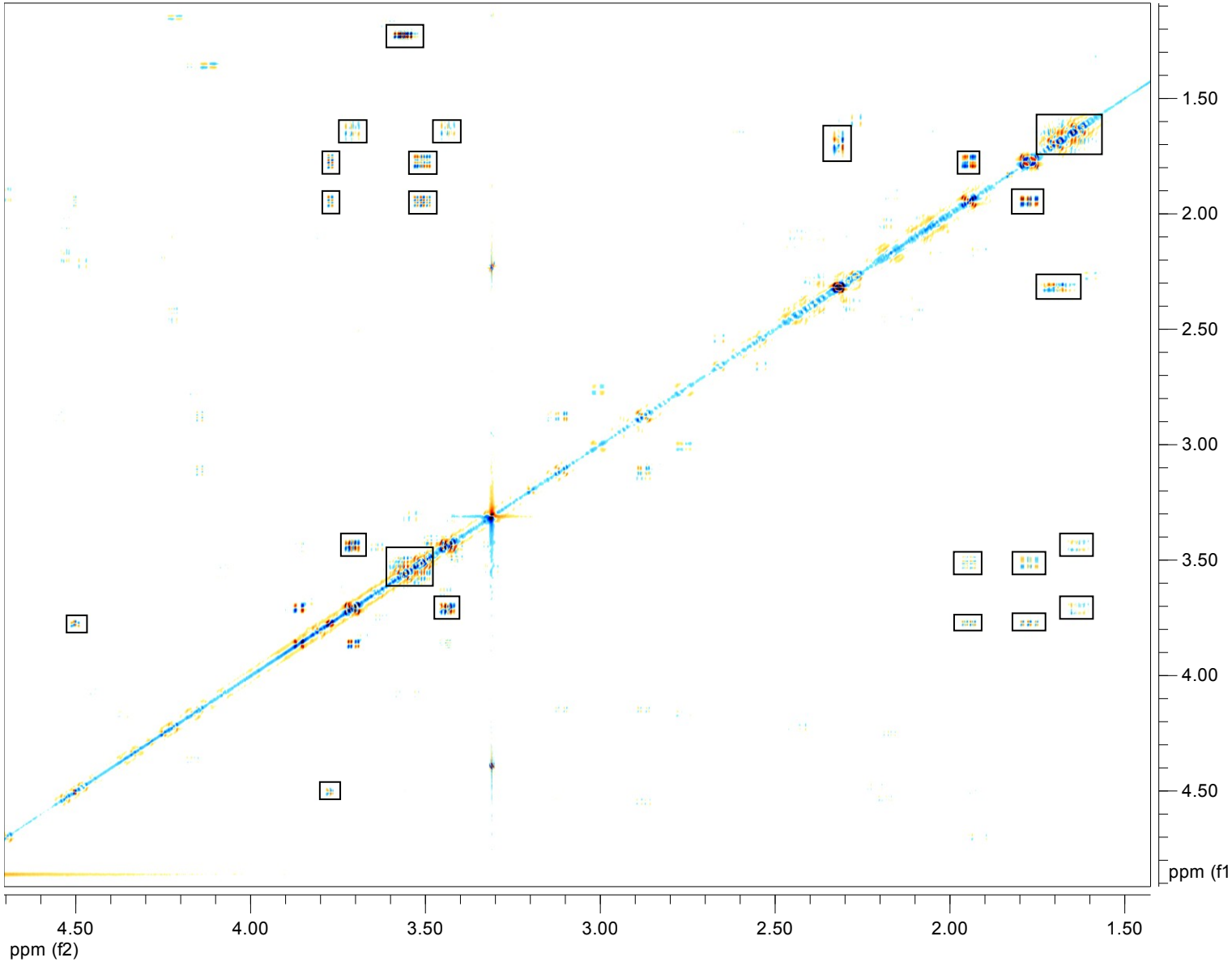


Figure S9. dqfCOSY spectrum (600 MHz, methanol-d_4) of oscr#9–enriched fraction from *acox-1(ok2257)* media extracts.

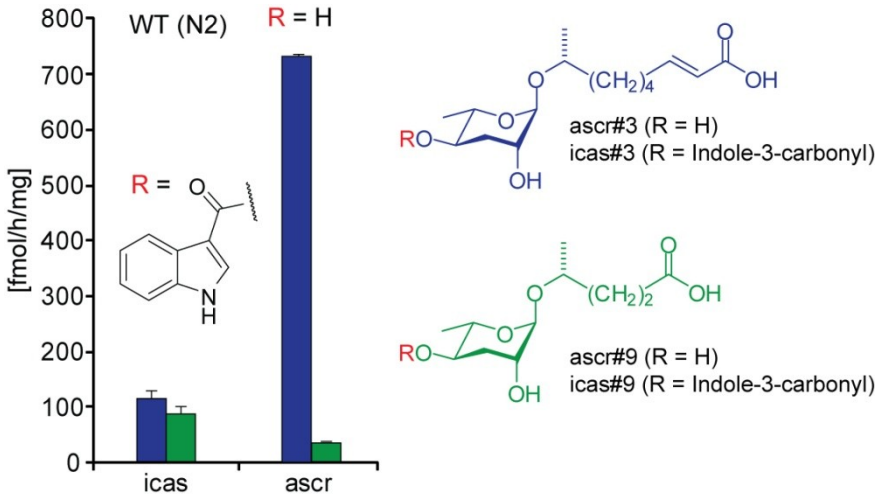


Figure S10. Relative abundance of ascarosides ascr#3 and ascr#9 and their corresponding indole ascarosides icas#3 and icas#9 in wild-type excretome extracts indicates that indole attachment is highly dependent on side chain length.

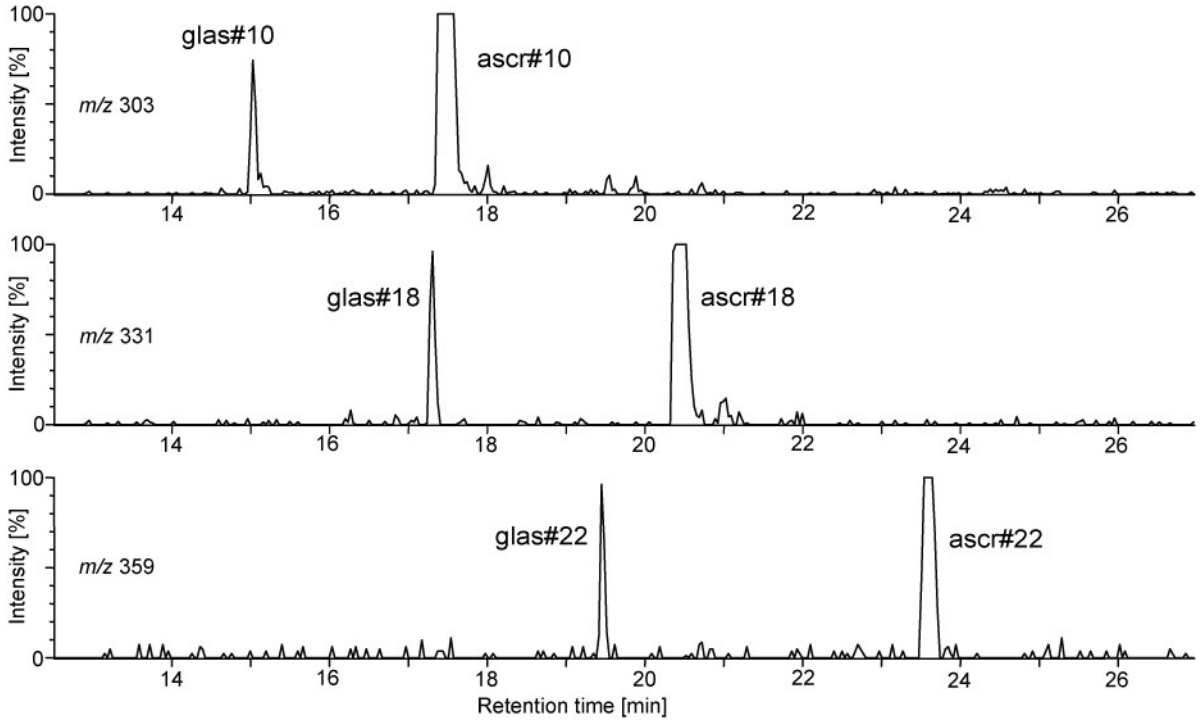


Figure S11. LC-MS/MS chromatograms (precursor ions of m/z = 73) of *acox-1(ok2257)* worm body extracts showing glucosyl esters glas#10, glas#18, and glas#22 and the corresponding non-glycosylated ascarosides ascr#10, ascr#18, and ascr#22.

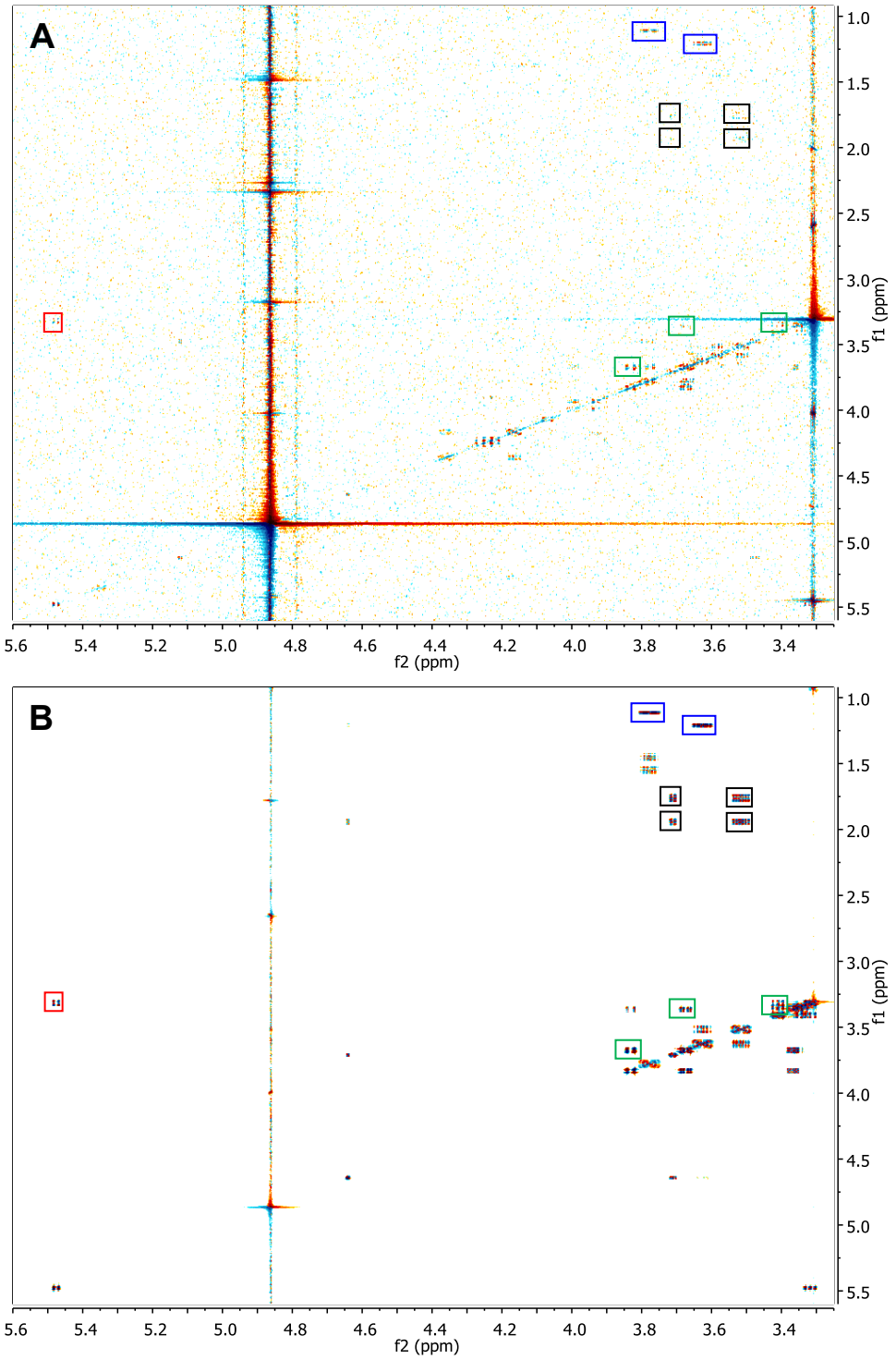


Figure S12. Sections of dqfCOSY spectra (600 MHz, methanol-d_4) of glas#10–enriched fraction from *acox-1(ok2257)* worm pellet extracts (A) and synthetic glas#10 (B), showing characteristic signals for methyl groups of the ascarylose ring and the side chain (blue), the anomeric hydrogen of the glucose unit (red), the glucose spin system (green), and the ascarylose spin system (black).

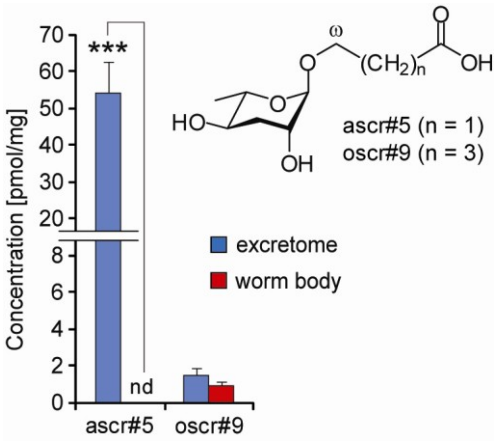


Figure S13. Differential excretion of (ω)-oxygenated ascarosides by wild type *C. elegans* (For (ω-1)-oxygenated ascarosides see Figure 6a).

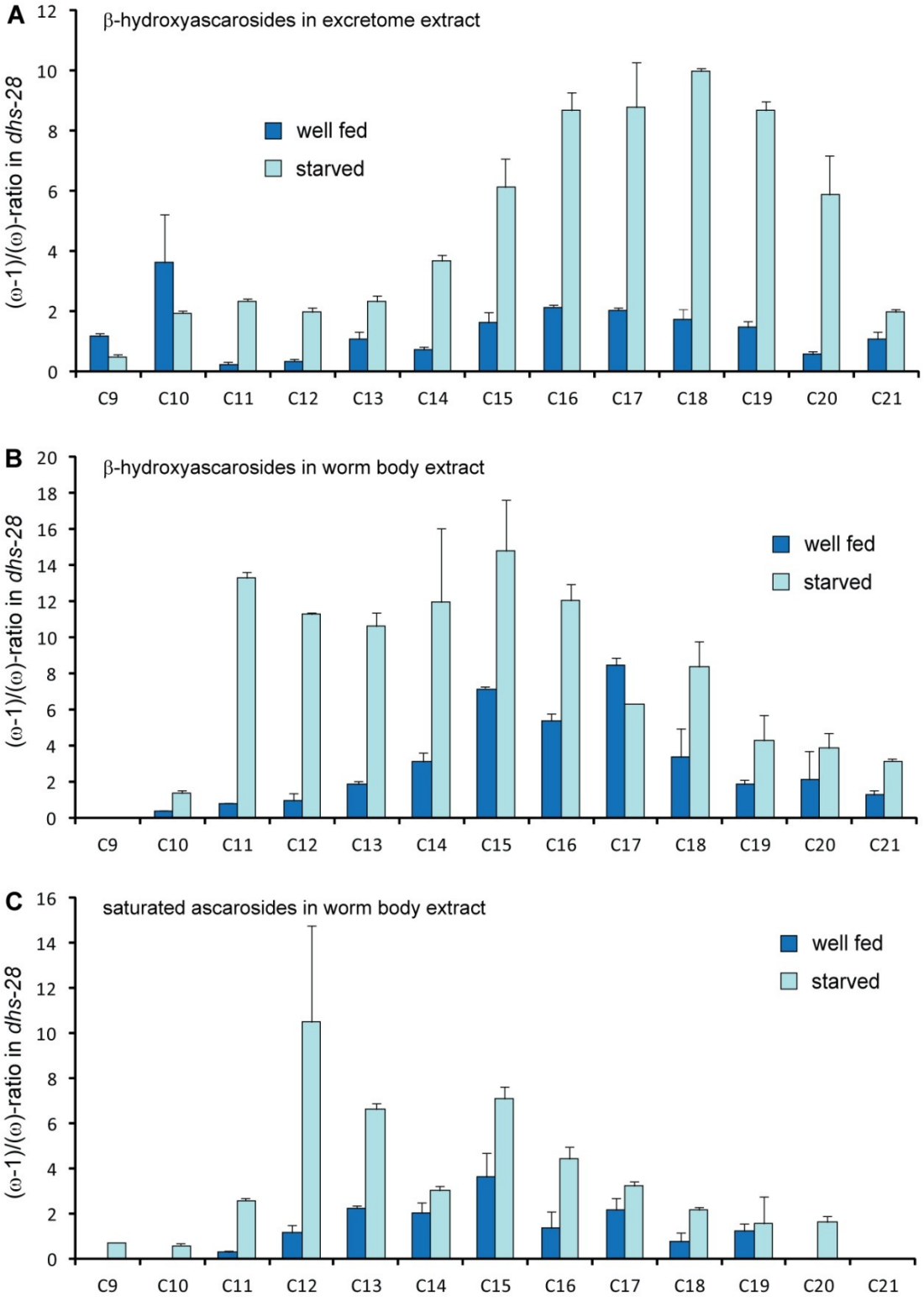


Figure S14. Ratio of (ω-1) to (ω)-linked ascarosides in *dhs-28(hj8)* mutant worms shows strong dependence on nutritional conditions, **A.** β-hydroxy ascarosides in *dhs-28(hj8)* excretome extracts, **B.** β-hydroxy ascarosides in *dhs-28(hj8)* worm body extracts, **C.** saturated ascarosides in *dhs-28(hj8)* worm body extracts (saturated ascarosides in *dhs-28(hj8)* excretome extracts are shown in Figure 6b).

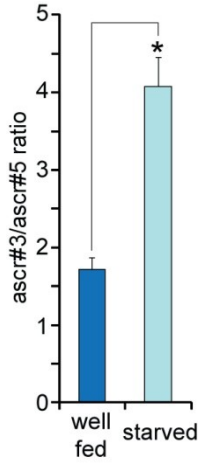


Figure S15. Starvation dependence of ascr#3/ascr#5 ratio in wild type excretome.

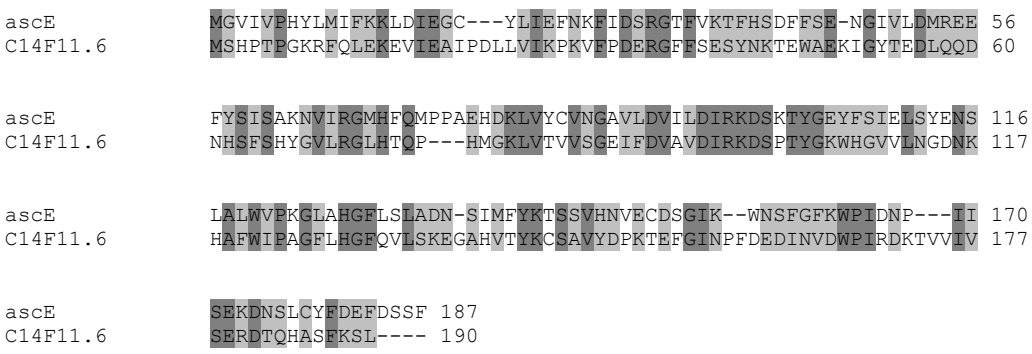
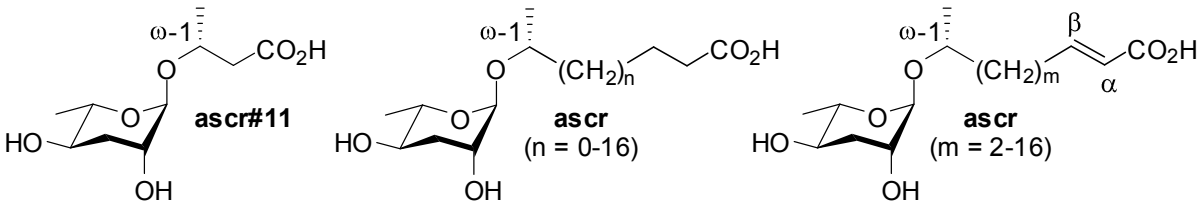


Figure S16. Alignment of *Yersinia pseudotuberculosis* CDP-3, 6-dideoxy-D-glycero-D-glycero-4-hexulose-5-epimerase or *asc*E (AAA88702.1) with *C. elegans* homolog C14F11.6 (CCD64543.1) was performed using ClustalW. Identical amino acids are marked in grey and similar amino acids are marked in light grey.

3. Supporting Tables

Table S1. HPLC-ESI-MS data of (ω-1)-oxygenated ascarosides (ascr).



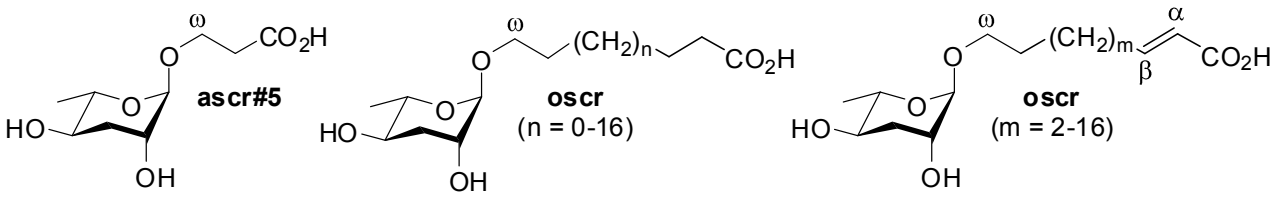
Side chain length (n, m)	SMID[§]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD
C_4	ascr#11*	$C_{10}H_{18}O_6$	234.1103	233.1025	233.1031	10.21 ±0.03
C_5, n = 0	ascr#9*[4]	$C_{11}H_{20}O_6$	248.1260	247.1182	247.1189	11.69 ±0.01
C_6, n = 1	ascr#12	$C_{12}H_{22}O_6$	262.1416	261.1338	261.1343	13.09 ±0.02
ΔC_7, m = 2	ascr#7*[2]	$C_{13}H_{22}O_6$	274.1416	273.1339	273.1337	13.96 ±0.07
C_7, n = 2	ascr#1*[5]	$C_{13}H_{24}O_6$	276.1573	275.1495	275.1497	14.52 ±0.04
ΔC_8, m = 3	ascr#13	$C_{14}H_{24}O_6$	288.1573	287.1495	287.1481	15.61 ±0.18
C_8, n = 3	ascr#14	$C_{14}H_{26}O_6$	290.1729	289.1651	289.1647	15.96 ±0.03
ΔC_9, m = 4	ascr#3*[6]	$C_{15}H_{26}O_6$	302.1729	301.1651	301.1652	16.90 ±0.02
C_9, n = 4	ascr#10*[4]	$C_{15}H_{28}O_6$	304.1886	303.1808	303.1800	17.44 ±0.02
ΔC_{10}, m = 5	ascr#15	$C_{16}H_{28}O_6$	316.1886	315.1808	315.1817	18.34 ±0.02
C_{10}, n = 5	ascr#16	$C_{16}H_{30}O_6$	318.2042	317.1964	317.1959	18.98 ±0.05
ΔC_{11}, m = 6	ascr#17	$C_{17}H_{30}O_6$	330.2042	329.1964	329.1957	19.75 ±0.02
C_{11}, n = 6	ascr#18	$C_{17}H_{32}O_6$	332.2199	331.2121	331.2130	20.43 ±0.02
ΔC_{12}, m = 7	ascr#19[7]	$C_{18}H_{32}O_6$	344.2199	343.2121	343.2120	21.36 ±0.03
C_{12}, n = 7	ascr#20	$C_{18}H_{34}O_6$	346.2355	345.2277	345.2278	21.97 ±0.03
ΔC_{13}, m = 8	ascr#21[7]	$C_{19}H_{34}O_6$	358.2355	357.2277	357.2273	22.83 ±0.02
C_{13}, n = 8	ascr#22	$C_{19}H_{36}O_6$	360.2512	359.2434	359.2437	23.58 ±0.02
ΔC_{14}, m = 9	ascr#23[7]	$C_{20}H_{36}O_6$	372.2512	371.2434	371.2444	24.46 ±0.01
C_{14}, n = 9	ascr#24	$C_{20}H_{38}O_6$	374.2668	373.2590	373.2596	25.29 ±0.03
ΔC_{15}, m = 10	ascr#25[7]	$C_{21}H_{38}O_6$	386.2668	385.2590	385.2598	26.15 ±0.02
C_{15}, n = 10	ascr#26	$C_{21}H_{40}O_6$	388.2825	387.2747	387.2743	27.09 ±0.02
ΔC_{16}, m = 11	ascr#27	$C_{22}H_{40}O_6$	400.2825	399.2747	399.2734	27.89 ±0.03
C_{16}, n = 11	ascr#28	$C_{22}H_{42}O_6$	402.2981	401.2903	401.2901	28.97 ±0.04

ΔC_{17}, m = 12	ascr#29	$C_{23}H_{42}O_6$	414.2981	413.2903	413.2891	29.80 ±0.03
C_{17}, n = 12	ascr#30	$C_{23}H_{44}O_6$	416.3138	415.3060	415.3067	30.96 ±0.03
ΔC_{18}, m = 13	ascr#31	$C_{24}H_{44}O_6$	428.3138	427.3060	427.3075	31.78 ±0.03
C_{18}, n = 13	ascr#32	$C_{24}H_{46}O_6$	430.3294	429.3216	429.3221	33.02 ±0.02
ΔC_{19}, m = 14	ascr#33	$C_{25}H_{46}O_6$	442.3294	441.3216	441.3215	33.74 ±0.03
C_{19}, n = 14	ascr#34	$C_{25}H_{48}O_6$	444.3451	443.3373	443.3374	35.12 ±0.08
ΔC_{20}, m = 15	ascr#35	$C_{26}H_{48}O_6$	456.3451	455.3373	455.3371	35.59 ±0.06
C_{20}, n = 15	ascr#36	$C_{26}H_{50}O_6$	458.3607	457.3529	457.3501	37.14 ±0.07
ΔC_{21}, m = 16	ascr#37	$C_{27}H_{50}O_6$	470.3607	469.3529	469.3519	37.71 ±0.13
C_{21}, n = 16	ascr#38	$C_{27}H_{52}O_6$	472.3764	471.3686	471.3697	39.15 ±0.06

* confirmed using synthetic standards

$ SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S2. HPLC-ESI-MS data of (ω)-oxygenated ascarosides (oscr).



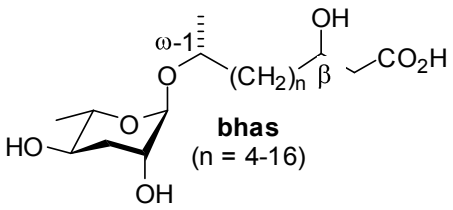
Side chain length (n, m)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD
C_3	ascr#5*[8]	$C_9H_{16}O_6$	220.0947	219.0869	219.0871	6.53 ±0.07
C_5, n = 0	oscr#9*	$C_{11}H_{20}O_6$	248.1260	247.1182	247.1192	11.88 ±0.02
C_6, n = 1	oscr#12	$C_{12}H_{22}O_6$	262.1416	261.1338	261.1345	13.40 ±0.02
ΔC_7, m = 2	oscr#7	$C_{13}H_{22}O_6$	274.1416	273.1339	273.1350	14.67 ±0.02
C_7, n = 2	oscr#1	$C_{13}H_{24}O_6$	276.1573	275.1495	275.1503	14.95 ±0.03
C_8, n = 3	oscr#14	$C_{14}H_{26}O_6$	290.1729	289.1651	289.1672	16.45 ±0.02
ΔC_9, n = 4	oscr#3	$C_{15}H_{26}O_6$	302.1729	301.1651	301.1636	17.58 ±0.03
C_9, n = 4	oscr#10*	$C_{15}H_{28}O_6$	304.1886	303.1808	303.1814	18.00 ±0.02
ΔC_{10}, m = 5	oscr#15	$C_{16}H_{28}O_6$	316.1886	315.1808	315.1816	18.91 ±0.06
C_{10}, n = 5	oscr#16	$C_{16}H_{30}O_6$	318.2042	317.1964	317.1967	19.48 ±0.03
ΔC_{11}, m = 6	oscr#17	$C_{17}H_{30}O_6$	330.2042	329.1964	329.1956	20.39 ±0.01
C_{11}, n = 6	oscr#18	$C_{17}H_{32}O_6$	332.2199	331.2121	331.2124	20.98 ±0.08
ΔC_{12}, m = 7	oscr#19	$C_{18}H_{32}O_6$	344.2199	343.2121	343.2125	21.86 ±0.06
C_{12}, n = 7	oscr#20	$C_{18}H_{34}O_6$	346.2355	345.2277	345.2302	22.54 ±0.03
ΔC_{13}, m = 8	oscr#21	$C_{19}H_{34}O_6$	358.2355	357.2277	357.2271	23.41 ±0.02
C_{13}, n = 8	oscr#22	$C_{19}H_{36}O_6$	360.2512	359.2434	359.2452	24.19 ±0.02
ΔC_{14}, m = 9	oscr#23	$C_{20}H_{36}O_6$	372.2512	371.2434	371.2436	25.04 ±0.03
C_{14}, n = 9	oscr#24	$C_{20}H_{38}O_6$	374.2668	373.2590	373.2589	25.91 ±0.02
ΔC_{15}, m = 10	oscr#25	$C_{21}H_{38}O_6$	386.2668	385.2590	385.2567	26.74 ±0.01
C_{15}, n = 10	oscr#26	$C_{21}H_{40}O_6$	388.2825	387.2747	387.2739	27.73 ±0.02
ΔC_{16}, m = 11	oscr#27	$C_{22}H_{40}O_6$	400.2825	399.2747	399.2728	28.54 ±0.03
C_{16}, n = 11	oscr#28	$C_{22}H_{42}O_6$	402.2981	401.2903	401.2905	29.67 ±0.02
ΔC_{17}, m = 12	oscr#29	$C_{23}H_{42}O_6$	414.2981	413.2903	413.2900	30.42 ±0.02

C_{17}, n = 12	oscr#30	$C_{23}H_{44}O_6$	416.3138	415.3060	415.3080	31.68 ±0.04
ΔC_{18}, m = 13	oscr#31	$C_{24}H_{44}O_6$	428.3138	427.3060	427.3053	32.44 ±0.02
C_{18}, n = 13	oscr#32	$C_{24}H_{46}O_6$	430.3294	429.3216	429.3207	33.78 ±0.02
ΔC_{19}, m = 14	oscr#33	$C_{25}H_{46}O_6$	442.3294	441.3216	441.3218	34.44 ±0.05
C_{19}, n = 14	oscr#34	$C_{25}H_{48}O_6$	444.3451	443.3373	443.3372	35.86 ±0.05
ΔC_{20}, m = 15	oscr#35	$C_{26}H_{48}O_6$	456.3451	455.3373	455.3384	36.23 ±0.05
C_{20}, n = 15	oscr#36	$C_{26}H_{50}O_6$	458.3607	457.3529	457.3545	37.96 ±0.05
ΔC_{21}, m = 16	oscr#37	$C_{27}H_{50}O_6$	470.3607	469.3529	469.3504	38.08 ±0.19
C_{21}, n = 16	oscr#38	$C_{27}H_{52}O_6$	472.3764	471.3686	471.3679	40.19 ±0.13

* confirmed using synthetic standards

$ SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S3. HPLC-ESI-MS data of (ω-1)-oygenated β-hydroxyascarosides (bhas).

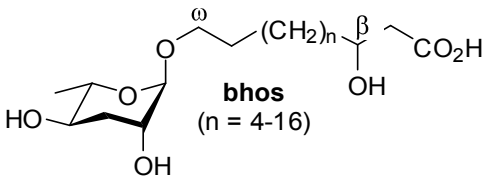


Side chain length (n)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD
C_9, n = 4	bhas#10*	$C_{15}H_{28}O_7$	320.1835	319.1757	319.1764	13.98 ±0.03
C_{10}, n = 5	bhas#16	$C_{16}H_{30}O_7$	334.1992	333.1913	333.1909	15.55 ±0.03
C_{11}, n = 6	bhas#18	$C_{17}H_{32}O_7$	348.2148	347.2070	347.2058	16.72 ±0.02
C_{12}, n = 7	bhas#20	$C_{18}H_{34}O_7$	362.2305	361.2226	361.2235	18.02 ±0.02
C_{13}, n = 8	bhas#22*	$C_{19}H_{36}O_7$	376.2461	375.2383	375.2371	19.39 ±0.02
C_{14}, n = 9	bhas#24[7]	$C_{20}H_{38}O_7$	390.2618	389.2539	389.2537	20.74 ±0.03
C_{15}, n = 10	bhas#26[7]	$C_{21}H_{40}O_7$	404.2774	403.2696	403.2693	22.19 ±0.03
C_{16}, n = 11	bhas#28[7]	$C_{22}H_{42}O_7$	418.2931	417.2852	417.2852	23.66 ±0.03
C_{17}, n = 12	bhas#30[7]	$C_{23}H_{44}O_7$	432.3087	431.3009	431.3009	25.32 ±0.02
C_{18}, n = 13	bhas#32	$C_{24}H_{46}O_7$	446.3244	445.3165	445.3164	27.07 ±0.03
C_{19}, n = 14	bhas#34	$C_{25}H_{48}O_7$	460.3400	459.3322	459.3316	28.97 ±0.02
C_{20}, n = 15	bhas#36	$C_{26}H_{50}O_7$	474.3557	473.3478	473.3471	30.97 ±0.03
C_{21}, n = 16	bhas#38	$C_{27}H_{52}O_7$	488.3713	487.3635	487.3629	33.12 ±0.06

* confirmed using synthetic standards

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S4. HPLC-ESI-MS data of (ω)-oygenated β-hydroxyascarosides (bhos).

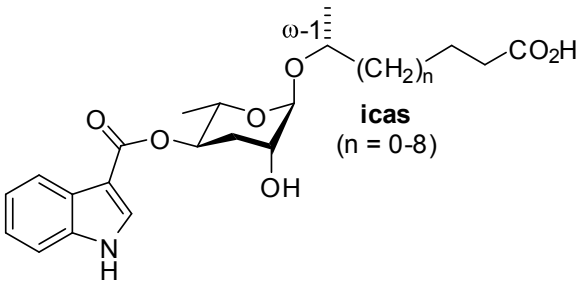


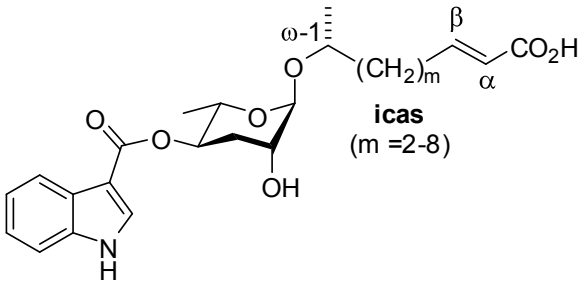
Side chain length (n)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD
C_9, n = 4	bhos#10	$C_{15}H_{28}O_7$	320.1835	319.1757	319.1781	14.44 ±0.01
C_{10}, n = 5	bhos#16	$C_{16}H_{30}O_7$	334.1992	333.1913	333.1902	15.82 ±0.01
C_{11}, n = 6	bhos#18	$C_{17}H_{32}O_7$	348.2148	347.2070	347.2054	17.17 ±0.02
C_{12}, n = 7	bhos#20	$C_{18}H_{34}O_7$	362.2305	361.2226	361.2224	18.51 ±0.01
C_{13}, n = 8	bhos#22	$C_{19}H_{36}O_7$	376.2461	375.2383	375.2370	19.86 ±0.02
C_{14}, n = 9	bhos#24	$C_{20}H_{38}O_7$	390.2618	389.2539	389.2537	21.23 ±0.03
C_{15}, n = 10	bhos#26*	$C_{21}H_{40}O_7$	404.2774	403.2696	403.2691	22.70 ±0.01
C_{16}, n = 11	bhos#28	$C_{22}H_{42}O_7$	418.2931	417.2852	417.2849	24.28 ±0.06
C_{17}, n = 12	bhos#30	$C_{23}H_{44}O_7$	432.3087	431.3009	431.3005	25.95 ±0.07
C_{18}, n = 13	bhos#32	$C_{24}H_{46}O_7$	446.3244	445.3165	445.3164	27.73 ±0.02
C_{19}, n = 14	bhos#34	$C_{25}H_{48}O_7$	460.3400	459.3322	459.3318	29.68 ±0.03
C_{20}, n = 15	bhos#36	$C_{26}H_{50}O_7$	474.3557	473.3478	473.3473	31.76 ±0.03
C_{21}, n = 16	bhos#38	$C_{27}H_{52}O_7$	488.3713	487.3635	487.3640	33.96 ±0.08

* confirmed using synthetic standards

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S5. HPLC-ESI-MS data of (ω-1)-oxygenated indole ascarosides (icas).





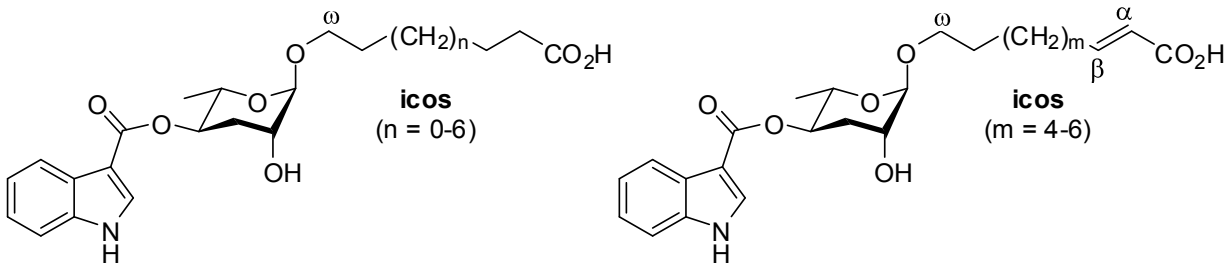
Side chain length (n, m)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]$^-$ calculated	*m/z* [M-H]$^-$ observed	Retention time [min] ±SD	WT (N2)	*acox-1* (*ok2557*)	*maoc-1* (*hj13*)	*dhs-28* (*hj8*)	*daf-22* (*ok693*)
C_5, n = 0	icas#9*[9]	$C_{20}H_{25}NO_7$	391.1631	390.1553	390.1555	20.18 ±0.02	+	+	-	-	-
C_6, n = 1	icas#12	$C_{21}H_{27}NO_7$	405.1788	404.1709	404.1703	21.02 ±0.04	+	+	-	-	-
ΔC_7, m = 2	icas#7*[4]	$C_{22}H_{27}NO_7$	417.1788	416.1709	416.1719	21.74 ±0.03	+	-	-	-	-
C_7, n = 2	icas#1*[4]	$C_{22}H_{29}NO_7$	419.1944	418.1866	418.1864	22.06 ±0.03	+	+	-	-	-
C_8, n = 3	icas#14	$C_{23}H_{31}NO_7$	433.2101	432.2022	432.2013	23.20 ±0.04	+	+	-	-	-
ΔC_9, m = 4	icas#3*[4]	$C_{24}H_{31}NO_7$	445.2101	444.2022	444.2029	23.94 ±0.03	+	-	+	-	-
C_9, n = 4	icas#10[4]	$C_{24}H_{33}NO_7$	447.2257	446.2179	446.2185	24.55 ±0.03	+	+	+	+	-
ΔC_{10}, m = 5	icas#15	$C_{25}H_{33}NO_7$	459.2257	458.2179	458.2198	25.21 ±0.05	-	-	+	+	-
C_{10}, n = 5	icas#16	$C_{25}H_{35}NO_7$	461.2414	460.2335	460.2369	25.89 ±0.04	+	+	+	+	-
ΔC_{11}, m = 6	icas#17	$C_{26}H_{35}NO_7$	473.2414	472.2335	472.2344	26.68 ±0.04	-	-	+	+	-
C_{11}, n = 6	icas#18	$C_{26}H_{37}NO_7$	475.2570	474.2492	474.2494	27.44 ±0.03	+	+	+	+	-
ΔC_{12}, m = 7	icas#19	$C_{27}H_{37}NO_7$	487.2570	486.2492	486.2486	28.20 ±0.06	-	-	+	+	-
C_{12}, n = 7	icas#20	$C_{27}H_{39}NO_7$	489.27265	488.2648	488.2628	28.98 ±0.03	-	+	+	+	-

ΔC_{13}, m = 8	icas#21	$C_{28}H_{39}NO_7$	501.27265	500.2648	500.2640	29.71 ±0.04	-	-	+	+	-
C_{13}, n = 8	icas#22	$C_{28}H_{41}NO_7$	503.28830	502.2805	502.2807	30.66 ±0.03	-	+	+	+	-

* confirmed using synthetic standards

$ SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S6. HPLC-ESI-MS data of (ω)-oxygenated indole ascarosides (icos).

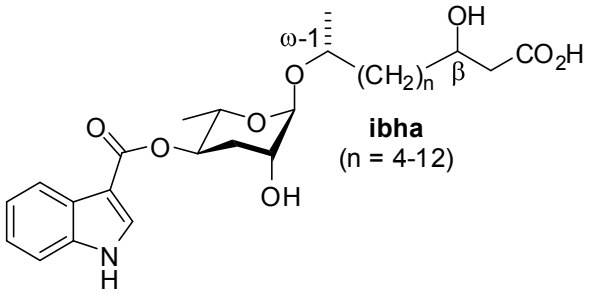


Side chain length (n, m)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD	WT (N2)	*acox-1* (*ok2557*)	*maoc-1* (*hj13*)	*dhs-28* (*hj8*)	*daf-22* (*ok693*)
C_5, n = 0	icos#9	$C_{20}H_{25}NO_7$	391.1631	390.1553	390.1541	20.48 ±0.03	-	+	-	-	-
C_7, n = 2	icos#1	$C_{22}H_{29}NO_7$	419.1944	418.1866	418.1858	22.63 ±0.03	+	+	-	-	-
ΔC_9, m = 4	icos#3	$C_{24}H_{31}NO_7$	445.2101	444.2022	444.2049	24.66 ±0.04	-	-	+	-	-
C_9, n = 4	icos#10*	$C_{24}H_{33}NO_7$	447.2257	446.2179	446.2171	25.29 ±0.03	-	+	+	+	-
ΔC_{10}, m = 5	icos#15	$C_{25}H_{33}NO_7$	459.2257	458.2179	458.2170	26.02 ±0.05	-	-	+	+	-
C_{10}, n = 5	icos#16	$C_{25}H_{35}NO_7$	461.2414	460.2335	460.2350	26.73 ±0.04	-	-	+	+	-
ΔC_{11}, m = 6	icos#17	$C_{26}H_{35}NO_7$	473.2414	472.2335	472.2325	27.45 ±0.03	-	-	+	+	-
C_{11}, n = 6	icos#18	$C_{26}H_{37}NO_7$	475.2570	474.2492	474.2490	28.21 ±0.04	-	+	+	+	-

* confirmed using synthetic standards

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

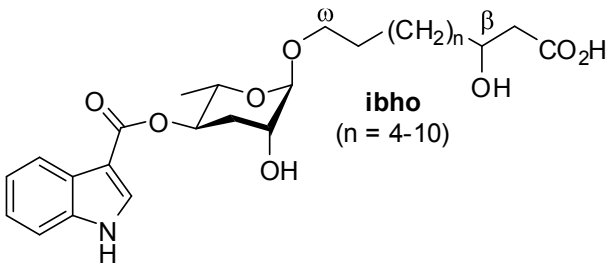
Table S7. HPLC-ESI-MS data of (ω-1)-oxygenated indole β-hydroxy ascarosides (ibha).



Side chain length (n)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD	WT (N2)	*acox-1* (*ok2557*)	*maoc-1* (*hj13*)	*dhs-28* (*hj8*)	*daf-22* (*ok693*)
C_9, n = 4	ibha#10	$C_{24}H_{33}NO_8$	463.2206	462.2128	462.2113	20.72 ±0.03	-	-	-	+	-
C_{10}, n = 5	ibha#16	$C_{25}H_{35}NO_8$	477.2363	476.2284	476.2251	21.81 ±0.04	-	-	-	+	-
C_{11}, n = 6	ibha#18	$C_{26}H_{37}NO_8$	491.2519	490.2441	490.2476	23.08 ±0.04	-	-	+	+	-
C_{12}, n = 7	ibha#20	$C_{27}H_{39}NO_8$	505.2676	504.2597	504.2582	24.38 ±0.03	-	-	+	+	-
C_{13}, n = 8	ibha#22	$C_{28}H_{41}NO_8$	519.2832	518.2754	518.2726	25.86 ±0.03	-	-	+	+	-
C_{14}, n = 9	ibha#24	$C_{29}H_{43}NO_8$	533.2989	532.2910	532.2938	27.30 ±0.04	-	-	-	+	-
C_{15}, n = 10	ibha#26	$C_{30}H_{45}NO_8$	547.3145	546.3067	546.3033	28.96 ±0.04	-	-	-	+	+
C_{16}, n = 11	ibha#28	$C_{31}H_{47}NO_8$	561.3302	560.3223	560.3201	30.63 ±0.05	-	-	-	+	-
C_{17}, n = 12	ibha#30	$C_{32}H_{49}NO_8$	575.3458	574.3380	574.3347	32.46 ±0.01	-	-	-	+	-

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

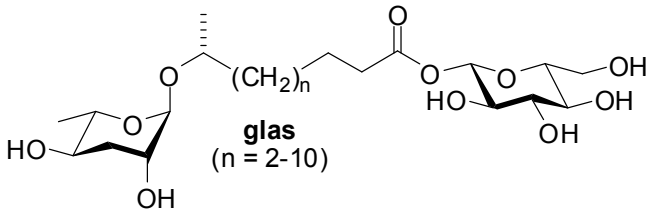
Table S8. HPLC-ESI-MS data of (ω)-oxygenated indole β-hydroxy ascarosides (ibho).

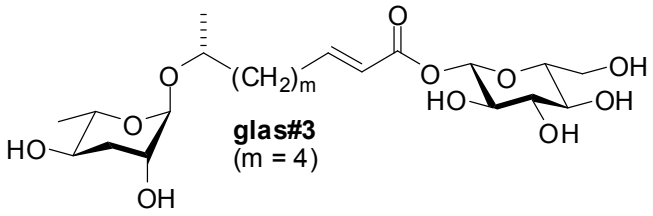


Side chain length (n)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD	WT (N2)	*acox-1* (*ok2557*)	*maoc-1* (*hj13*)	*dhs-28* (*hj8*)	*daf-22* (*ok693*)
C_9, n = 4	ibho#10	$C_{24}H_{33}NO_8$	463.2206	462.2128	462.2114	21.39 ±0.03	-	-	-	+	-
C_{10}, n = 5	ibho#16	$C_{25}H_{35}NO_8$	477.2363	476.2284	476.2252	22.56 ±0.03	-	-	-	+	-
C_{11}, n = 6	ibho#18	$C_{26}H_{37}NO_8$	491.2519	490.2441	490.2438	23.79 ±0.03	-	-	+	+	-
C_{12}, n = 7	ibho#20	$C_{27}H_{39}NO_8$	505.2676	504.2597	504.2580	25.15 ±0.03	-	-	-	+	-
C_{13}, n = 8	ibho#22	$C_{28}H_{41}NO_8$	519.2832	518.2754	518.2719	26.58 ±0.03	-	-	-	+	-
C_{14}, n = 9	ibho#24	$C_{29}H_{43}NO_8$	533.2989	532.2910	532.2886	27.94 ±0.03	-	-	-	+	-
C_{15}, n = 10	ibho#26	$C_{30}H_{45}NO_8$	547.3145	546.3067	546.3094	29.65 ±0.03	-	-	-	+	+

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S9. HPLC-ESI-MS data of glucosyl ascaroside esters (glas).



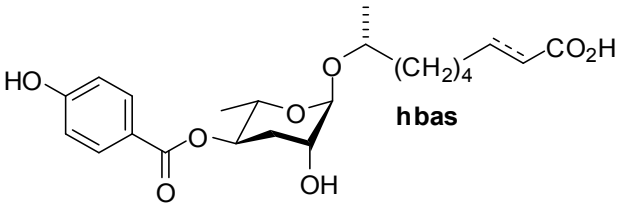


Side chain length (n, m)	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* $[M+Cl]^-$ calculated	*m/z* $[M+Cl]^-$ observed	Retention time [min] ±SD	WT (N2)	*acox-1* (*ok2557*)	*maoc-1* (*hj13*)	*dhs-28* (*hj8*)	*daf-22* (*ok693*)
C_7, n = 2	glas#1	$C_{19}H_{32}O_{11}$	438.2101	473.1795	473.1803	11.99 ±0.04	+	-	-	-	-
ΔC_9, m = 4	glas#3	$C_{21}H_{36}O_{11}$	464.2258	499.1952	499.1932	14.64 ±0.03	+	-	-	-	-
C_9, n = 4	glas#10*	$C_{21}H_{38}O_{11}$	466.2414	501.2108	501.2112	15.05 ±0.03	+	+	-	-	-
C_{10}, n = 5	glas#16	$C_{22}H_{40}O_{11}$	480.2571	515.2265	515.2269	16.19 ±0.05	-	+	-	-	-
C_{11}, n = 6	glas#18	$C_{23}H_{42}O_{11}$	494.2727	529.2421	529.2402	17.34 ±0.04	+	+	-	-	-
C_{12}, n = 7	glas#20	$C_{24}H_{44}O_{11}$	508.2884	543.2578	543.2551	18.41 ±0.04	-	+	-	-	-
C_{13}, n = 8	glas#22	$C_{25}H_{46}O_{11}$	522.3040	557.2734	557.2720	19.49 ±0.05	-	+	-	-	-
C_{14}, n = 9	glas#24	$C_{26}H_{48}O_{11}$	536.3197	571.2891	571.2896	20.59 ±0.04	-	+	-	-	-
C_{15}, n = 10	glas#26	$C_{27}H_{50}O_{11}$	550.3353	585.3047	585.3095	21.77 ±0.04	-	+	-	-	-

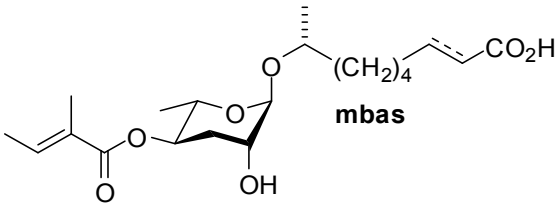
* confirmed using synthetic standards

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

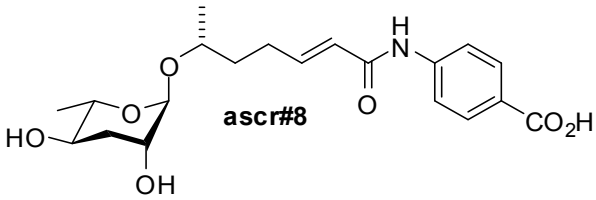
Table S10. HPLC-ESI-MS data of ascr#8, 4-(4-hydroxybenzoyl)- and 4-(2-(*E*)-methyl-2-butenoyl)-ascarosides (hbas and mbas).



hbas



mbas



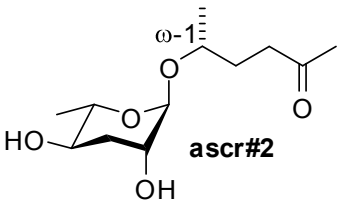
ascr#8

Side chain length	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* [M-H]⁻ calculated	*m/z* [M-H]⁻ observed	Retention time [min] ±SD	WT (N2)	*acox-1* (*ok2557*)	*maoc-1* (*hj13*)	*dhs-28* (*hj8*)	*daf-22* (*ok693*)
ΔC_7	ascr#8*[2]	$C_{20}H_{27}NO_7$	393.1788	392.1709	392.1712	16.77 ±0.04	+	-	-	-	-
ΔC_9	hbas#3*	$C_{22}H_{30}O_8$	422.1941	421.1862	421.1866	22.41 ±0.03	+	-	-	-	-
C_9	hbas#10	$C_{22}H_{32}O_8$	424.2097	423.2019	423.2018	22.94 ±0.04	-	+	-	-	-
ΔC_9	mbas#3*	$C_{20}H_{32}O_7$	384.2148	383.2070	383.2079	25.66 ±0.04	+	-	-	-	-
C_9	mbas#10	$C_{20}H_{34}O_7$	386.2305	385.2226	385.2239	26.38 ±0.04	-	+	-	-	-

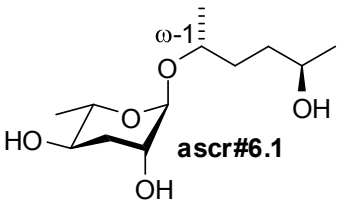
* confirmed using synthetic standards

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

Table S11. HPLC-ESI-MS data of ascr#2 and ascr#6.1.



ascr#2



ascr#6.1

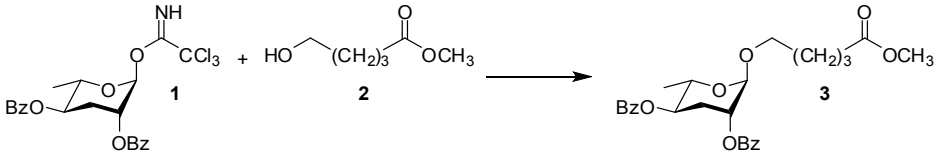
Side chain length	SMID[$]	Molecular formula	Molecular weight [amu]	*m/z* $[M+Na]^+$ calculated	*m/z* $[M+Na]^+$ observed	Retention time [min] ±SD
C_6	ascr#2[*6]	$C_{12}H_{22}O_5$	246.1467	269.1365	269.1371	13.06 ±0.03
C_6	ascr#6[*2]	$C_{12}H_{24}O_5$	248.1624	271.1521	271.1532	12.72 ±0.04

* confirmed using synthetic standards

[$] SMID: Small Molecule IDentifier for small molecules identified from *C. elegans* and other nematodes. The SMID database (www.smid-db.org) is an electronic resource maintained by Frank C. Schroeder and Lukas Mueller at the Boyce Thompson Institute in collaboration with Wormbase (www.wormbase.org). The purpose of this database is to introduce searchable, gene-style identifiers, "SMIDs", for all small molecules newly identified from *C. elegans* and other nematodes.

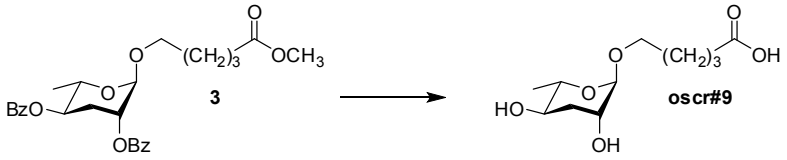
4. Syntheses and Spectroscopic Data

4.1. Methyl 5-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)pentanoate (3)



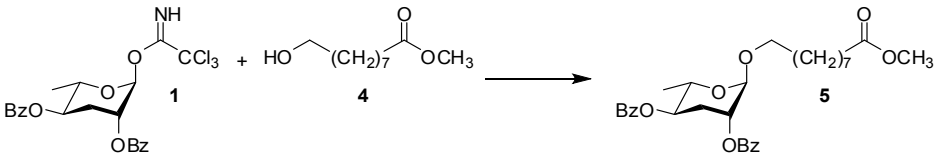
A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide)[6] (**1**, 132 mg, 263 µmol) and methyl 5-hydroxypentanoate[10] (**2**, 125 mg, 950 µmol) in dry dichloromethane (3 ml) at 0 °C was treated with trimethylsilyloxy triflate (5µl). After 3 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica using a gradient of 20 – 40% ethyl acetate in hexanes afforded **3** (66.8 mg, 142 µmol, 47%) as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 1.28 (d, *J* = 6.2 Hz, 3H), 1.67-1.80 (m, 4H), 2.23 (m, 1H), 2.40 (t, *J* = 6.9 Hz, 2H), 2.48 (m, 1H), 3.58 (m, 1H), 3.64 (s, 3H), 3.83 (m, 1H), 4.13 (dq, *J* = 9.8 Hz, *J* = 6.0 Hz, 1H), 4.87 (s.br, 1H), 5.15 (ddd, *J* = 11.0 Hz, *J* = 10.4 Hz, *J* = 4.5 Hz, 1H), 5.18 (s.br, 1H), 7.50-7.60 (m, 4H), 7.62-7.72 (m, 2H), 8.05 (d, *J* = 7.5 Hz, 2H), 8.11 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 18.3, 22.5, 29.6, 30.4, 34.0, 51.5, 67.5, 67.9, 71.4, 71.5, 97.0, 129.4, 129.5, 130.3, 130.4, 131.0, 131.0, 134.1, 134.2, 165.9, 166.0, 174.0.

4.2. 5-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)pentanoic acid (oscr#9)



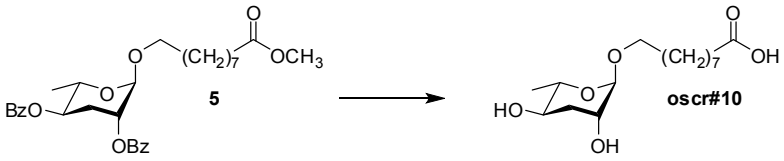
A solution of **3** (66.8 mg, 142 µmol) in dry tetrahydrofuran (0.5 ml) was added to a mixture of LiOH (28 mg, 1.4 mmol) and water (0.6 ml) in 1,4-dioxane (4 ml). After stirring at 66 °C for 2 h the solution was acidified with glacial acetic acid and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 30% methanol in dichloromethane containing 1% acetic acid afforded oscr#9(26 mg, 105 µmol, 74%) as a colorless oil. ^{1}H NMR (400 MHz, methanol-d_4): δ (ppm) 1.22 (d, *J* = 6.0 Hz, 3H), 1.58-1.72 (m, 4H), 1.77 (ddd, *J* = 13.1 Hz, *J* = 11.1 Hz, *J* = 3.2 Hz, 1H), 1.95 (ddt, *J* = 13.1 Hz, *J* = 3.7 Hz, *J* = 0.9 Hz, 1H), 2.33 (t, *J* = 7.2 Hz, 2H), 3.43 (dt, *J* = 9.6 Hz, *J* = 6.0 Hz, 1H) 3.47 – 3.59 (m, 2H), 3.71 (dt, *J* = 9.8 Hz, *J* = 6.2 Hz, 1H), 3.77 (m, 1H), 4.50 (s, 1H); ^{13}C NMR (100 MHz, methanol-d_4): δ (ppm) 18.1, 23.0, 30.1, 34.7, 36.0, 67.9, 68.3, 69.4, 70.9, 100.4, 177.5.

4.3. Methyl 9-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoate (5)



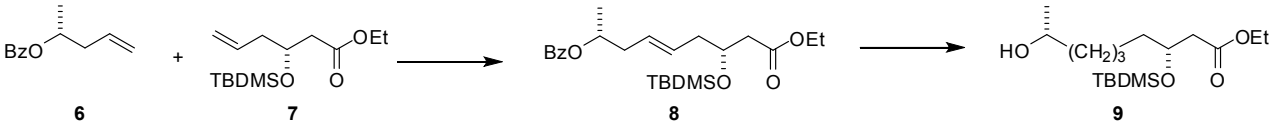
A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide)[6] (**1**, 132 mg, 263 µmol) and methyl 9-hydroxynonanoate[11] (**4**, 112.8 mg, 600 µmol) in dry dichloromethane (3 ml) at 0 °C was treated with trimethylsilyloxy triflate (5 µl). After 3 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4 and concentrated in vacuum. Flash column chromatography on silica using a gradient of 20 – 40% ethyl acetate in hexanes afforded **5** (99.3 mg, 190 µmol, 72%) as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 1.28 (d, *J* = 6.2 Hz, 3H), 1.30 – 1.40 (m, 6H), 1.40 – 1.49 (m, 2H), 1.56 – 1.72 (m, 2H), 2.22 (ddd, *J* = 13.6 Hz, *J* = 11.5 Hz, *J* = 3.2 Hz, 1H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.46 (m, 1H), 3.55 (dt, *J* = 9.8 Hz, *J* = 6.5 Hz, 1H), 3.60 (s, 3H), 3.81 (dt, *J* = 9.6 Hz, *J* = 6.6 Hz, 1H), 4.13 (dq, *J* = 9.7 Hz, *J* = 6.2 Hz, 1H), 4.86 (s.br, 1H), 5.15 (ddd, *J* = 11.4 Hz, *J* = 9.8 Hz, *J* = 4.6 Hz, 1H), 5.18 (m, 1H), 7.50 – 7.60 (m, 4H), 7.63 – 7.71 (m, 2H), 8.04 (m, 2H), 8.11 (m, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 18.3, 25.6, 26.8, 29.7, 29.9, 30.0, 30.2, 30.4, 34.4, 51.4, 67.4, 68.2, 71.4, 71.5, 97.0, 129.4, 129.5, 130.2, 130.3, 130.9, 131.0, 134.1, 134.2, 165.9, 165.9, 174.3.

4.4. 9-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoic acid (oscr#10)



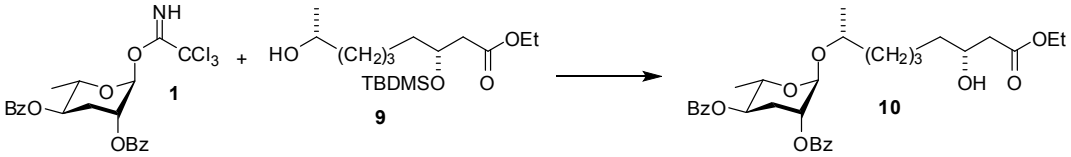
A solution of **10** (99.3 mg, 190 µmol) in tetrahydrofuran (500 µl) was added to a mixture of LiOH (38 mg, 1.9 mmol) and water (800 µl) in 5 ml 1,4-dioxane (5 ml). After stirring at 66 °C for 3 h the solution was acidified with acetic acid and concentrated in vacuum. Flash column chromatography on silica gel using a gradient of 5 – 30% methanol in dichloromethane containing 1% glacial acetic acid afforded oscr#10 (49 mg, 161 µmol, 85%) as a colorless oil. ^{1}H NMR (400 MHz, methanol-d_4): δ (ppm) 1.22 (d, *J* = 6.1 Hz, 3H), 1.32-1.43 (m, 8H), 1.56-1.63 (m, 4H), 1.77 (ddd, *J* = 13.1 Hz, *J* = 11.1 Hz, *J* = 3.2 Hz, 1H), 1.96 (ddt, *J* = 13.1 Hz, *J* = 3.7 Hz, *J* = 0.9 Hz, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 3.41 (dt, *J* = 9.6 Hz, *J* = 6.2 Hz, 1H) 3.49 – 3.59 (m, 2H), 3.68 (dt, *J* = 9.8 Hz, *J* = 5.5 Hz, 1H), 3.76 (m, 1H), 4.49 (s, 1H); ^{13}C NMR (100 MHz, methanol-d_4): δ (ppm) 17.3, 25.2, 26.4, 28.0, 29.3, 29.5, 29.6, 30.5, 34.1, 61.1, 67.4, 68.5, 69.9, 99.4, 176.8.

4.5. Ethyl (8*R*)-hydroxy-(3*R*)-*tert*-butyldimethylsilyloxynonanoate (9)



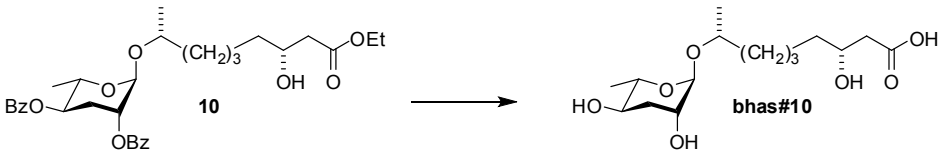
A solution of **6**[12] (366 mg, 1.91 mmol) and **7**[13] (104 mg, 380 µmol) in dry dichloromethane (10 ml) was treated with 1,4-benzoquinone (4 mg, 38 µmol) in dichloromethane (0.5 ml) and stirred for 10 min. A solution of Grubbs 2nd generation catalyst (16 mg, 19 µmol) in dichloromethane (0.5 ml) was added and the mixture stirred at 40 °C. After 20 h the mixture was filtered over a small layer of silica and concentrated in vacuum. Flash column chromatography on silica using a gradient of 0 – 20% ethyl acetate in hexane afforded a mixture of the desired product **8** and the homodimer of **6** which was not purified further. Instead the crude mixture (160 mg) was dissolved in ethanol (2 ml), treated with Pd/C (15 mg, 10%, *w/w*) and hydrogenated for 40 h. The mixture was filtered, concentrated in vacuum and purified by flash column chromatography on silica gel using a gradient of 10 – 30% ethyl acetate in hexane to afford **9** (48 mg, 144 µmol, 38% over two steps) as a colorless oil. ^{1}H NMR (500 MHz, chloroform-d_1): δ (ppm) 0.03 (s, 3H), 0.06 (s, 3H), 0.86 (s, 9H), 1.19 (d, *J* = 6.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.29-1.47 (m, 6H), 1.47-1.53 (m, 2H), 2.40 (dd, *J* = 14.6 Hz, *J* = 5.7 Hz, 1H), 2.44 (dd, *J* = 14.6 Hz, *J* = 7.0 Hz, 1H), 3.75-3.83 (m, 1H), 4.09-4.15 (m, 3H).

4.6. Ethyl (8*R*)-(3'*R*,5'*R*-Dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoate (10)



A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide)[6] (**1**, 62 mg, 120 µmol) in dry dichloromethane (2 ml) at -10 °C was treated with **9** (47 mg, 141 µmol) and trimethylsilyloxy triflate (10 µl). After 3.5 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4 and concentrated in vacuum. Flash column chromatography on silica using a gradient of 10 – 40% ethyl acetate in hexane afforded **10** (4.0 mg, 7.2 µmol, 6%) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-d_1): δ (ppm) 1.19 (d, *J* = 6.1 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.28 (d, *J* = 6.4 Hz, 3H), 1.33–1.72 (m, 8H), 2.20 (ddd, *J* = 14.3 Hz, *J* = 11.6 Hz, *J* = 3.2 Hz, 1H), 2.42 (dd, *J* = 16.5 Hz, *J* = 9.0 Hz, 1H), 2.38-2.45 (m, 1H), 2.52 (dd, *J* = 16.5 Hz, *J* = 3.0 Hz, 1H), 3.00 (d, *J* = 3.9 Hz, 1H), 3.80-3.89 (m, 1H), 3.98-4.07 (m, 1H), 4.11 (dq, *J* = 9.7 Hz, *J* = 6.1 Hz, 1H), 4.17 (q, *J* = 7.2 Hz, 2H), 4.95 (s.br, 1H), 5.12-5.22 (m, 2H), 7.43-7.50 (m, 4H), 7.55-7.62 (m, 2H), 8.05 (d, *J* = 7.5 Hz, 2H), 8.11 (d, *J* = 7.5 Hz, 2H).

4.7. (8*R*)-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoic acid (bhas#10)

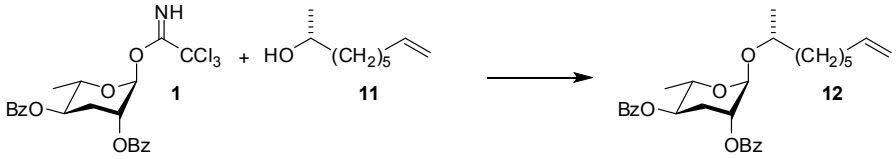


A solution of **10** (4 mg, 7.2 µmol) in tetrahydrofuran (150 µl) was treated with LiOH (7 mg, 290 µl) in water (100 µl) and 1,4-dioxane (250 ml) at 67 °C for 5 h. The reaction mixture was acidified with acetic acid (100 µl), concentrated in vacuum, treated with methanol (2 ml) and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 25% methanol in dichloromethane with 0.5% glacial acetic acid afforded bhas#10 (1.5 mg, 4.7 µmol; 65%) as a colorless oil.

NMR Spectroscopic Data of bhas#10. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for bhas#10 in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

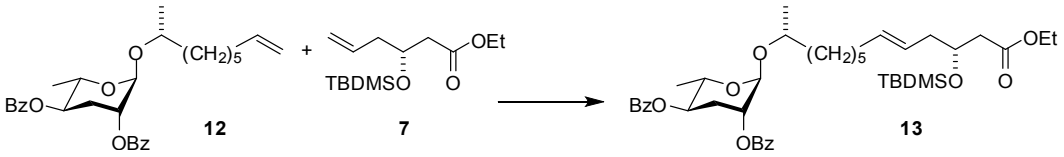
Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	178.5			
2	44.8	2.23-2.33 2.34-2.44		
3	70.0	3.89-3.98		
4	38.0	1.44-1.52		
5-6	26.8, 27.0	1.33-1.52		
7	38.4	1.44-1.63		C6, C8,
8	72.5	3.78		C1′, C-7, C-9
9	19.3	1.12	$J_{8,9}$ = 6.2	C-8, C-7
1′	97.5	4.64		C-3′, C-5′, C-8
2′	70.0	3.71		
3′	35.9	1.77 (*ax*)	$J_{3'ax,3'eq}$ = 13.2, $J_{3'ax,4'}$ = 11.5, $J_{2',3'ax}$ = 3.1	C-4′, C-5′
		1.95 (*eq*)	$J_{2',3'eq}$ = 3.2, $J_{3'eq,4'}$ = 4.7	C-1′, C-2′, C-4′, C-5′
4′	68.4	3.51	$J_{4',5'}$ = 9.4	C-3′, C-5′, C-6′
5′	71.2	3.63		C-1′, C-3′, C-4′, C-6′
6′	18.1	1.22	$J_{5',6'}$ = 6.2	C4′, C-5′

4.8. (8*R*)-(3'*R*,5'*R*-Dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-1-ene (12)



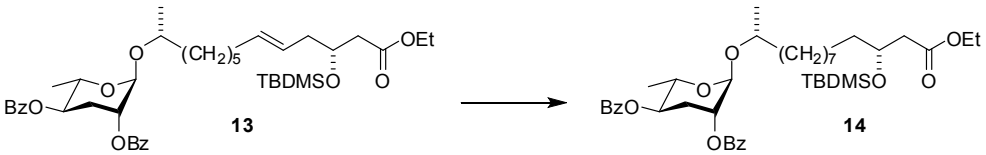
A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide)[6] (**1**, 132 mg, 263 µmol) in dry dichloromethane (3 ml) at 0 °C was treated with (8*R*)-hydroxynon-1-ene[14] (**11**, 85.2 mg, 600 µmol) and trimethylsilyloxy triflate (5 µl). After 3 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4 and concentrated in vacuum. Flash column chromatography on silica using a gradient of 10 – 30% ethyl acetate in hexanes afforded **12** (71.0 mg, 148 µmol, 56%) as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 1.20 (d, *J* = 6.1 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 3H), 1.33–1.72 (m, 8H), 2.09 (m, 2H), 2.23 (ddd, *J* = 13.5 Hz, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H), 2.47 (m, 1H), 3.91 (m, 1H), 4.20 (dq, *J* = 9.6 Hz, *J* = 6.1 Hz, 1H), 4.93 (ddt, *J* = 10.2 Hz, *J* = 2.2 Hz, *J* = 1.3 Hz, 1H), 5.01 (s.br, 1H), 5.02 (ddt, *J* = 17.1, Hz, *J* = 2.2 Hz, *J* = 1.6 Hz, 1H), 5.13 (m, 1H), 5.16 (ddd, *J* = 11.3 Hz, *J* = 9.8 Hz, *J* = 4.6 Hz, 1H), 5.84 (ddt, *J* = 17.1 Hz, *J* = 10.3 Hz, *J* = 6.8 Hz, 1H), 7.50-7.60 (m, 4H), 7.63-7.71 (m, 2H), 8.04 (m, 2H), 8.12 (m, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 18.3, 19.5, 26.3, 29.7, 29.7, 30.4, 34.4, 37.8, 67.7, 71.5, 72.1, 72.9, 94.4, 114.8, 129.4, 129.5, 130.2, 130.4, 131.0, 131.0, 134.1, 134.2, 139.8, 165.9, 166.0.

4.9. Ethyl (12*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxytridec-5-enoate (13)



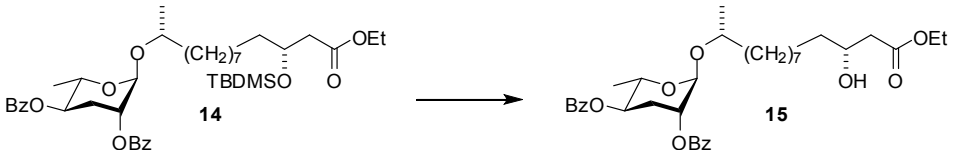
A solution of **12** (38 mg, 80 µmol) and **7**[13] (65 mg, 240 µmol) in dry dichloromethane (2 ml) was treated with 1,4-benzoquinone (1 mg, 8 µmol) in dichloromethane (0.5 ml) and stirred for 10 min. A solution of Grubbs 2nd generation catalyst (3 mg, 4 µmol) in dichloromethane (0.5 ml) was added and the mixture stirred at 40 °C. After 20 h the mixture was filtered over a small layer of silica and concentrated in vacuum. Flash column chromatography on silica using a 5:1 mixture of hexanes and ethyl acetate afforded **13** (17.0 mg, 23 µmol, 29%) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-d_1): δ (ppm) 0.03 (s, 3H), 0.06 (s, 3H), 0.86 (s, 9H), 1.19 (d, J = 6.2 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.28 (d, *J* = 6.3 Hz, 3H), 1.32-1.44 (m, 4H), 1.44-1.52 (m, 2H), 1.61-1.68 (m, 2H), 1.99-2.06 (m, 2H), 2.17-2.22 (m, 3H), 2.38-2.43 (m, 3H), 3.84 (m, 1H), 4.06-4.18 (m, 4H), 4.95 (s, 1H), 5.14 (s.br, 1H), 5.18 (dt, *J* = 4.2 Hz, *J* = 10.6 Hz, 1H), 5.39 (dt, *J* = 15.2 Hz, *J* = 6.8 Hz, 1H), 5.47 (dt, *J* = 15.2 Hz, *J* = 6.3 Hz, 1H), 7.43-7.49 (m, 4H), 7.56-7.61 (m, 2H), 8.04 (d, *J* = 7.4 Hz, 2H), 8.11 (d, *J* = 7.2 Hz, 2H).

4.10. Ethyl (12*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxytridecanoate (14)



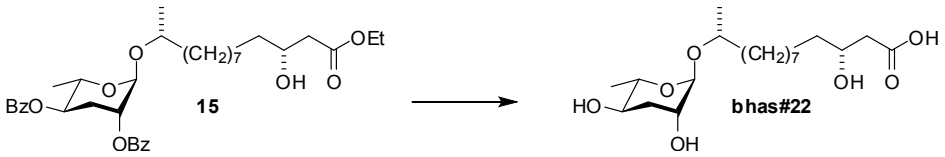
A solution of **13** (14.2 mg, 18.9 µmol) in methanol (1.5 ml) was treated with Pd/C and hydrogenated for 24 h. The mixture was filtered and concentrated in vacuum to afford **14** (12.8 mg, 17.0 µmol, 90%) as a colorless oil. ^{1}H NMR (600 MHz, chloroform-d_1): δ (ppm) 0.03 (s, 3H), 0.05 (s, 3H), 0.86 (s, 9H), 1.19 (d, *J* = 6.1 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.28 (d, *J* = 6.3 Hz, 3H), 1.29-1.40 (m, 10H), 1.42-1.53 (m, 4H), 1.58-1.68 (m, 2H), 2.21 (ddd, *J* = 14.0 Hz, *J* = 11.7 Hz, *J* = 2.8 Hz, 1H), 2.37-2.45 (m, 3H), 3.84 (m, 1H), 4.08-4.15 (m, 4H), 4.95 (s, 1H), 5.14 (s.br, 1H), 5.18 (dt, *J* = 4.2 Hz, *J* = 10.6 Hz, 1H), 7.43-7.49 (m, 4H), 7.56-7.61 (m, 2H), 8.04 (d, *J* = 7.4 Hz, 2H), 8.11 (d, *J* = 7.2 Hz, 2H).

4.11. Ethyl (12*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoate (15)



A solution of **14** (19.5 mg, 26.8 µmol) in acetonitrile (1 ml) was treated with 40% aqueous hydrofluoric acid (10 µl) in acetonitrile (100 µl). After stirring for 1 h, the solution was treated with $NaHCO_3$ (100 mg) for 15 min, dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 80% ethyl acetate in hexanes afforded **15** (12.0 mg, 19.6 µmol; 73%) as a colorless oil. ^{1}H NMR (501 MHz, chloroform-d_1): δ (ppm) 1.19 (d, *J* = 6.1 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.28 (d, *J* = 6.4 Hz, 3H), 1.30-1.55 (m, 14H), 1.60-1.68 (m, 2H), 2.21 (ddd, *J* = 13.5 Hz, *J* = 11.6 Hz, *J* = 3.1 Hz, 1H), 2.38 (dd, *J* = 16.4 Hz, *J* = 9.2 Hz, 1H), 2.41 (m, 1H), 2.49 (dd, *J* = 16.3 Hz, *J* = 3.1 Hz, 1H), 3.84 (m, 1H), 3.99 (m, 1H), 4.12 (dq, *J* = 9.8 Hz, *J* = 6.2 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.95 (s, 1H), 5.15 (s.br, 1H), 5.18 (ddd, *J* = 11.2 Hz, *J* = 9.9 Hz, *J* = 4.5 Hz, 1H), 7.43 – 7.49 (m, 4H), 7.56 – 7.61 (m, 2H), 8.04 (m, 2H), 8.11 (m, 2H).

4.12. (12*R*)-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoic acid (bhas#22)

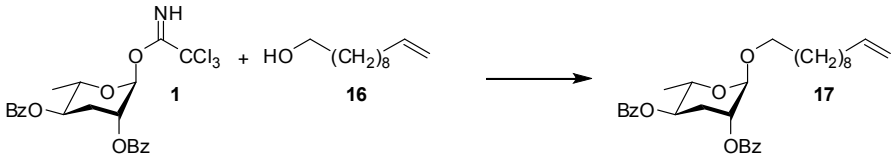


A solution of **15** (12 mg, 19.6 µmol) in tetrahydrofuran (1 ml) was treated with LiOH (15 mg) in water (200 µl) and 1,4-dioxane (2 ml) at 67 °C for 3 h. The reaction mixture was acidified with acetic acid (100 µl) , concentrated in vacuum, treated with methanol (2 ml) and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 30% methanol in dichloromethane with 0.2% acetic acid afforded bhas#22 (7.3 mg, 19.4 µmol; 99 %) as a colorless oil.

NMR Spectroscopic Data of bhas#22. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for bhas#22 in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

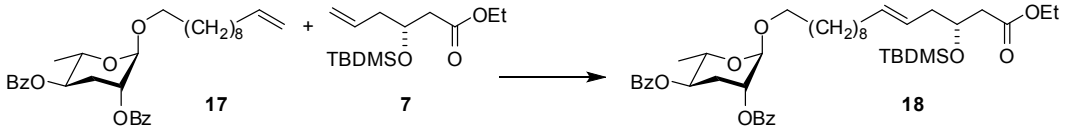
Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	176.4			
2	43.4	2.34, 2.42	$J_{2,2}$ = 15.2, $J_{2,3}$ = 8.1	C-1, C-3, C-4
3	69.2	3.95		C-4, C-5
4	37.8	1.45		
5-10	26.4 - 30.4	1.26-1.39		
11	38.0	1.40-1.50		C9, C10, C12, C13
12	72.2	3.78		C1′, C-9, C-10, C11
13	18.9	1.12	$J_{12,13}$ = 6.2	C-11, C-12
1′	97.2	4.64		C-2′, C-3′, C-5′, C-12
2′	69.6	3.71		C-3′, C-4′
3′	35.5	1.77 (*ax*)	$J_{3'ax,3'eq}$ = 13.1, $J_{3'ax,4'}$ = 11.4, $J_{2',3'ax}$ = 3.0	C-2′, C-4′, C-5′
		1.95 (*eq*)	$J_{2',3'eq}$ = 3.2, $J_{3'eq,4'}$ = 4.7	C-1′, C-2′, C-4′, C-5′
4′	68.0	3.52	$J_{4',5'}$ = 9.6	C-1′, C-3′, C-6′
5′	70.9	3.63	$J_{5',6'}$ = 6.3	C-1′, C-3′, C-4′, C-6′
6′	17.8	1.21		C4′, C-5′

4.13. 11-(3'*R*,5'*R*-Dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)undec-1-ene (17)



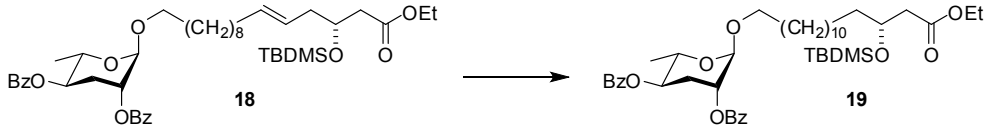
A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide)[6] (**1**, 132 mg, 263 µmol) in dry dichloromethane (3 ml) at 0 °C was treated with 11-hydroxyundec-1-ene (**16**, 102 mg, 600 µmol) and trimethylsilyloxy triflate (5 µl). After 3 h the solution was washed with saturated aqueous $NaHCO_3$ solution (0.5 ml), dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica using gradient of 10 – 30% (*v*/*v*) ethyl acetate in hexanes afforded **17** (92.3 mg, 182 µmol, 69%) as a colorless oil. ^{1}H NMR (400 MHz, acetone-d_6): δ (ppm) 1.28 (d, *J* = 6.2 Hz, 3H), 1.30-1.49 (m, 11H), 1.63-1.72 (m, 2H), 2.03 (m, 2H), 2.22 (ddd, *J* = 13.5 Hz, *J* = 11.3 Hz, *J* = 3.2 Hz, 1H), 2.46 (m, 1H), 3.55 (dt, *J* = 9.7 Hz, *J* = 6.5 Hz, 1H), 3.80 (dt, *J* =9.8 Hz, *J* = 6.7 Hz, 1H), 4.13 (dq, *J* = 9.8 Hz, *J* = 6.3 Hz, 1H), 4.86 (s.br, 1H), 4.90 (ddt, *J* = 10.2 Hz, *J* = 2.2 Hz, *J* = 1.3 Hz, 1H), 5.98 (ddt, *J* = 17.1, Hz, *J* = 2.2 Hz, *J* = 1.6 Hz, 1H), 5.15 (ddd, *J* = 11.3 Hz, *J* = 9.8 Hz, *J* = 4.6 Hz, 1H), 5.18 (m, 1H), 5.80 (ddt, *J* = 17.1 Hz, *J* = 10.3 Hz, *J* = 6.8 Hz, 1H), 7.49-7.59 (m, 4H), 7.62-7.71 (m, 2H), 8.04 (m, 2H), 8.11 (m, 2H); ^{13}C NMR (100 MHz, acetone-d_6): δ (ppm) 18.28, 26.88, 29.67, 29.83, 30.10, 30.16, 30.28, 34.46, 67.43, 68.25, 71.43, 71.53, 97.00, 114.66, 129.42, 129.48, 130.23, 130.35, 130.95, 130.96, 134.08, 134.19, 139.78, 165.89, 165.91.

4.14. Ethyl 15-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxypentadec-5-enoate (18)



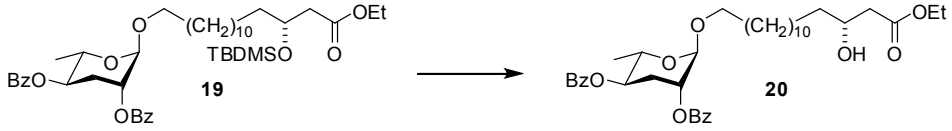
A solution of **17** (30.8 mg, 60 µmol) and **7**[13] (50 mg, 180 µmol) in dry dichloromethane (2 ml) was treated with 1,4-benzoquinone (1 mg, 8 µmol) in dichloromethane (0.5 ml) and stirred for 10 min. A solution of Grubbs 2nd generation catalyst (3 mg, 4 µmol) in dichloromethane (0.5 ml) was added and the mixture stirred at 40 °C. After 20 h the mixture was filtered over a small layer of silica and concentrated in vacuum. Flash column chromatography on silica using a 5:1 mixture of hexanes and ethyl acetate afforded **18** (14.2 mg, 18.9 µmol, 31%) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-d_1): δ (ppm) 0.03 (s, 3H), 0.06 (s, 3H), 0.86 (s, 9H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.27-1.43 (m, 12H), 1.61-1.68 (m, 2H), 1.95-2.02 (m, 2H), 2.16-2.25 (m, 2H), 2.35-2.46 (m, 3H), 3.50 (dt, *J* = 9.6 Hz, *J* = 6.5 Hz, 1H), 3.76 (dt, *J* = 9.6 Hz, *J* = 6.8 Hz, 1H), 4.04-4.18 (m, 4H), 4.82 (s, 1H), 5.18 (ddd, *J* = 11.2 Hz, *J* = 9.9 Hz, *J* = 4.7 Hz, 1H), 5.21 (s.br, 1H.), 5.37 (dt, *J* = 15.2 Hz, *J* = 7.0 Hz, 1H), 5.45 (dt, *J* = 15.3 Hz, *J* = 6.9 Hz, 1H), 7.43-7.50 (m, 4H), 7.56-7.61 (m, 2H), 8.04 (m, 2H), 8.11 (m, 2H).

4.15. Ethyl 15-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxypentadecanoate (19)



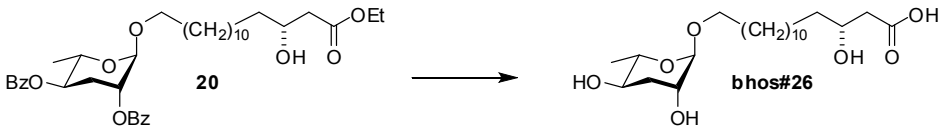
A solution of **18** (14.2 mg, 18.9 µmol) in methanol (1.5 ml) was treated with Pd/C (10 mg, 10%, *w/w*) and hydrogenated for 24 h. The mixture was filtered and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 80% ethyl acetate in hexanes afforded **19** (12.8 mg, 17.0 µmol, 90%) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-d_1): δ (ppm) 0.03 (s, 3H), 0.06 (s, 3H), 0.86 (s, 9H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.25-1.36 (m, 14H), 1.36-1.42 (m, 2H), 1.45-1.51 (m, 2H), 1.61-1.68 (m, 2H), 2.21 (ddd, *J* = 14.3 Hz, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H), 2.35-2.46 (m, 3H), 3.50 (dt, *J* = 9.6 Hz, *J* = 6.5 Hz, 1H), 3.76 (dt, *J* =9.6 Hz, *J* = 6.8 Hz, 1H), 4.04-4.18 (m, 4H), 4.82 (s, 1H), 5.18 (ddd, *J* = 11.2 Hz, *J* = 9.9 Hz, *J* = 4.7 Hz, 1H), 5.20 (s.br, 1H.), 7.43-7.50 (m, 4H), 7.56-7.61 (m, 2H), 8.04 (m, 2H), 8.11 (m, 2H).

4.16. Ethyl 15-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoate (20)



A solution of **19** (9.2 mg, 12.2 µmol) in acetonitrile (2 ml) was treated with 40% hydrofluoric acid (20 µl). After stirring for 1 h the solution was treated with $NaHCO_3$ (100 mg) for 15 min, dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 80% ethyl acetate in hexanes afforded **20** (4.4 mg, 6.9 µmol; 57%).^{1}H NMR (400 MHz, chloroform-d_1): δ (ppm) 1.27 (t, *J* = 7.2 Hz, 3H), 1.30 (d, *J* = 6.2 Hz, 3H), 1.25-1.36 (m, 16H), 1.36-1.42 (m, 2H), 1.45-1.51 (m, 2H), 1.61-1.68 (m, 2H), 2.21 (ddd, *J* = 14.3 Hz, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H), 2.39 (dd, *J* = 16.4 Hz, *J* = 9.0 Hz, 1H), 2.41 (m, 1H), 2.50 (dd, *J* = 16.4 Hz, *J* = 3.0 Hz, 1H), 3.50 (dt, *J* = 9.6 Hz, *J* = 6.5 Hz, 1H), 3.76 (dt, *J* =9.6 Hz, *J* = 6.8 Hz, 1H), 3.99 (m, 1H), 4.07 (dq, *J* = 10.0 Hz, *J* = 6.2 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.82 (s, 1H), 5.18 (ddd, *J* = 11.2 Hz, *J* = 9.9 Hz, *J* = 4.7 Hz, 1H), 5.20 (s.br, 1H), 7.43-7.50 (m, 4H), 7.56-7.61 (m, 2H), 8.04 (m, 2H), 8.11 (m, 2H).

4.17. 15-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoic acid (bhos#26)

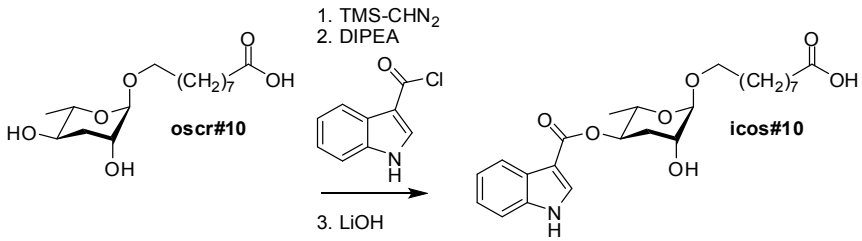


A solution of **20** (4.4 mg, 6.9 μmol) in tetrahydrofuran (1 ml) was treated with a solution of LiOH (5 mg) in water (200 μl) and 1,4-dioxane (2 ml) and stirred at 67 °C. After 3 h the solution was acidified with glacial acetic acid (50 μl) and concentrated in vacuum. Flash column chromatography on silica using a using a gradient of 5 – 50% methanol in dichloromethane with 0.2% acetic acid afforded ω-oxygenated β-hydroxyascaroside#26 (**bhos#26**; 2.3 mg, 5.7 μmol; 83%).

NMR Spectroscopic Data of bhos#26. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for **bhos#26** in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	180.0			
2	45.0	2.23, 2.33	$J_{2,2}$ = 16.0, $J_{2,3}$ = 7.0	C-1, C-3, C-4
3	70.0	3.88		C-4, C-5
4	37.7	1.44, 1.59		
5-12	30.40-30.50	1.30-1.59		
13	27.2	1.37		
14	30.5	1.57		
15	68.0	3.41, 3.68	$J_{15,15}$ = 9.6 Hz, $J_{15,14}$ = 6.3	C-1′, C-14,
1′	100.0	4.49		C-2′, C-3′, C-5′, C-15
2′	69.1	3.76		C-3′, C-4′
3′	35.6	1.77 (*ax*)	$J_{3'ax,3'eq}$ = 13.1, $J_{3'ax,4'}$ = 11.3, $J_{2',3'ax}$ = 3.1	C-2′, C-4′, C-5′
		1.95 (*eq*)	$J_{2',3'eq}$ = 3.2, $J_{3'eq,4'}$ = 4.7	C-2′, C-4′, C-5′
4′	68.0	3.51	$J_{4',5'}$ = 9.6	C-5′, C-6′
5′	70.5	3.56	$J_{5',6'}$ = 6.1	C-3′, C-4′, C-6′
6′	17.8	1.23		C4′, C-5′

4.18. 9-(5'*R*-((*1H*)-Indole-3-carbonyloxy)-3'*R*-hydroxy-6'*S*-methyl-tetrahydro-(2*H*)-pyran-2'-yloxy)nonanoic acid (icos#10)

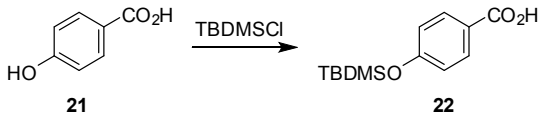


A solution of oscr#10 (12 mg, 39.5 µmol) in a mixture of methanol (1 ml) and toluene (1 ml) was treated with 2.0 M (trimethylsilyl)diazomethane in diethyl ether (23 µl, 46 µmol). After stirring for 30 min excess reagent was quenched by addition of acetic acid (20 µl) and the solution concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 10% methanol in dichloromethane afforded the methyl ester (11.3 mg, 35.5 µmol, 90%) as a colorless solid. A solution of the methyl ester in dichloromethane (1 ml) at -20 °C was treated with DIPEA (175 µl, 1 mmol) and indolecarboxylic acid chloride (freshly prepared by treatment of indole-3-carboxylic acid (68 mg, 420 µmol) in dichloromethane (2 ml) at 0 °C with dimethylformamide (10 µl) and $SOCl_2$ (72 µl, 840 µmol). After stirring for 20 min at RT the solution was concentrated in vacuum) in dichloromethane (2 ml) was added drop wise. The solution was allowed to come to -7 °C, quenched with saturated aqueous $NaHCO_3$ solution (2 ml), and the aqueous phase extracted with dichloromethane (3 x 2 ml). The combined organic phases were dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 10% methanol in dichloromethane afforded a isomeric mixture indole carboxylate esters (8.4 mg, 18.2 µmol, 51%) which was dissolved in tetrahydrofuran (1 ml) and treated with a solution of LiOH (2.8 mg, 116 µmol) in water (0.5 ml) and 1,4-dioxane (2 ml) at 67 °C. After stirring for 2 h the solution was acidified with acetic acid (30 µl) and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 20% methanol in dichloromethane containing 0.2% acetic acid afforded a isomeric mixture of icos#10 isomers. HPLC afforded pure samples of icos#10 (0.6 mg, 1.3 µmol, 7%) and its isomer (0.3 mg, 0.67 µmol, 3.7%).

NMR Spectroscopic Data of icos#10. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for **icos#10** in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

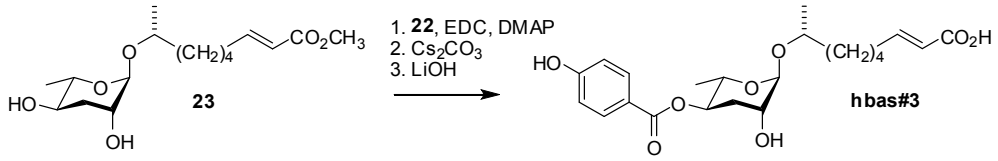
Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	176.4			
2	35.5	2.26	$J_{2,3}$ = 7.5	C-1, C-3, C-4
3	26.1	1.62		
4-5	30.1	1.38		
6	21.0	1.38		
7	27.1	1.43		
8	30.4	1.65		
9	68.2	3.49, 3.77	$J_{9,9}$ = 9.7, $J_{9,8}$ = 6.5	C-1′
1′	100.2	4.60		C-3′, C-9
2′	68.8	3.85		
3′	33.2	2.02 (*ax*)	$J_{3'ax,3'eq}$ = 13.1, $J_{3'ax,4'}$ = 11.3, $J_{2',3'ax}$ = 3.0	C-4′, C-5′
		2.21 (*eq*)	$J_{3'ax,3'eq}$ = 12.9, $J_{3'eq,4'}$ = 4.0	C-1′, C-4′, C-5′
4′	70.3	5.13	$J_{4',5'}$ = 9.8	C-5′, C-6′, C3″
5′	68.2	4.00	$J_{5',6'}$ = 6.3	
6′	17.8	1.25		C4′, C-5′
2″	133.1	7.96		C-3″, C3a″, C7a″
3″	108.1			
3″-CO	166.0			
3a″	137.9			
4″	121.5	8.02		C5″, C3a″
5″	123.4	7.20		C4″, C3a″
6″	122.3	7.20		C7″, C7a″
7″	112.7	7.45		C6″, C7a″
7a″	127.0			

4.19. 4-*tert*-Butyldimethylsilyloxybenzoic acid (22)



A solution of 4-hydroxybenzoic acid (**21**, 1.52 g, 10 mmol) in dimethylformamide (7 ml) was treated with DIPEA (5.2 ml, 30 mmol) and *tert*-butyldimethylsilyl chloride (3.7 g, 24.5 mmol). After 12 h the mixture was brought to pH 4 by addition of 1 M H_3PO_4, extracted twice with hexanes (15 ml), and the organic phase washed twice with water (15 ml), dried over Na_2SO_4, and concentrated in vacuum. The residue (3.7 g) was dissolved in THF (10 ml) and treated with water (7 ml) and glacial acetic acid (21 ml). After stirring for 90 min the mixture was added to ice water, extracted twice with a 1:1 mixture (*v/v*) of diethyl ether and hexanes (30 ml), and the organic phase washed with water (30 ml), dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 20% methanol in dichloromethane containing 0.2 % acetic acid afforded 4-*tert*-butyldimethylsilyloxybenzoic acid (**22**, 1.42 g, 5.3 mol, 53%) as a white solid. ^{1}H NMR (400 MHz, chloroform-d_1): δ (ppm) 0.24 (s, 6H), 0.99 (s, 9H), 6.89 (d, *J* = 8.8 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (100 MHz, chloroform-d_1): δ (ppm) -4.22, 18.40, 25.73, 120.08, 122.39, 132.46, 132.46, 161.01, 172.23.

4.20. (8*R*)-(3'*R*-Hydroxy-5'*R*-(4-hydroxybenzoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (hbas#3)

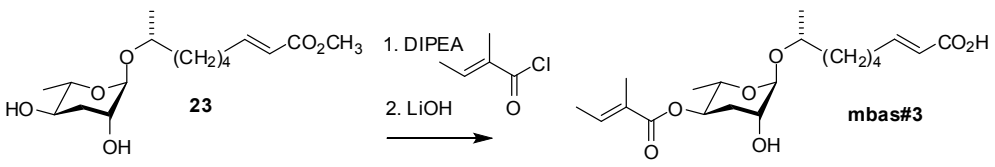


A solution of ascaroside#3 methyl ester[4] (**23**, 5.7 mg, 18 µmol) in dry dichloromethane (500 µl) was treated with 4-*tert*-butyldimethylsilyloxybenzoic acid (**22**, 11.0 mg, 41 µmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 9.0 mg, 47 µmol), and 4-dimethylaminopyridine (DMAP, 6.2 mg, 51 µmol). After stirring for 48 h the solution was concentrated in vacuum, treated with H_2O (500 µL), and the products were extracted with dichloromethane (3 x 1 ml), dried over Na_2SO_4 and concentrated in vacuum. Flash column chromatography on silica using a gradient of 0–30% methanol in dichloromethane afforded an isomeric mixture of 4-*tert*-butyldimethylsilyloxybenzoyl-ascaroside#3 methyl esters (7.3 mg, 12.9 µmol; 72%). For deprotection of the aromatic *tert*-butyldimethylsilyloxy group[15] a mixture of (4-*tert*-butyldimethylsilyloxybenzoyl)-ascaroside#3 methyl esters (6.0 mg, 10.7 µmol) in dimethylformamide (360 µl) was treated with Cs_2CO_3 (1.9 mg, 5.4 µmol) in H_2O (36 µl) and stirred for 3.5 h. The products were extracted with dichloromethane (2 x 1 ml), dried over Na_2SO_4, and concentrated in vacuum. Flash column chromatography on silica gel using a gradient of 0 – 30% methanol in dichloromethane afforded a isomeric mixture of 4-hydroxybenzoyl-ascaroside#3 methyl esters (4.5 mg, 10.0 µmol; 93%). For cleavage of the methyl ester group a mixture of 4-hydroxybenzoyl-ascaroside#3 methyl esters (4.5 mg, 10.0 µmol) in THF (100 µl) was treated with LiOH (2.3 mg) in H_2O (30 µl) and 1,4-dioxane (500 µl) at 67 °C. After 2 h the reaction was quenched by addition of glacial acetic acid (50 µl) and the solution concentrated in vacuum. Flash column chromatography on silica using a gradient of 5 – 20% methanol in dichloromethane with 0.2% acetic acid afforded an isomeric mixture of hbas#3 isomers (1.2 mg, 2.8 µmol, 28%). HPLC afforded pure samples of hbas#3 (0.6 mg, 1.4 µmol; 14%) and its isomer (0.6 mg, 1.4 µmol; 14%).

NMR Spectroscopic Data of hbas#3. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for **hbas#3** in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	171.7			
2	124.4	5.83	$J_{2,3}$ = 15.6	C-4
3	149.0	6.90	$J_{3,4}$ = 7.0	C-1, C-4, C-5
4	33.1	2.26		C-2, C-3, C5, C-6
5, 6	29.2, 26.5	1.40 – 1.54		C-4, C-6
7	38.1	1.53, 1.62		C-5, C-6, C-8
8	72.7	3.83		C-6, C-7, C-9
9	19.4	1.16	$J_{8,9}$ = 6.1	C-7, C-8
1′	97.5	4.73		C-3′, C-5′, C-8
2′	69.5	3.77		C-4′
3′	33.1	1.95 (*ax*)	$J_{3'ax,3'eq}$ = 12.9, $J_{3'ax,4'}$ = 11.2, $J_{2',3'ax}$ = 2.9	C-4′, C-5′
		2.15 (*eq*)	$J_{2',3'eq}$ = 3.2, $J_{3'eq,4'}$ = 4.7	C-2′, C-4′, C-5′
4′	71.5	5.05	$J_{4',5'}$ = 9.6	C-5′, C-6′, C-7″
5′	68.4	3.98	$J_{5',6'}$ = 6.3	C-4′, C-6′
6′	18.1	1.18		C4′, C-5′
7″-COO	167.2			
1″	122.1			
2″,6″	132.8	7.85	J = 8.9	C-1″,C-3″,5″,C-4″,C-7″
3″,5″	116.2	6.83		C-2″,6″, C-4″
4″	163.6			

4.21. (8*R*)-(3'*R*-Hydroxy-5'*R*-(*E*)-(2-methylbut-2-enoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (mbas#3)



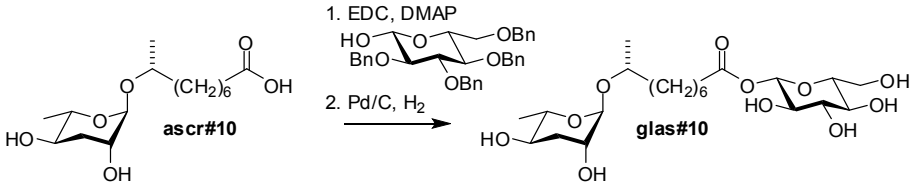
A solution of ascaroside#3 methyl ester[4] (**23**, 10 mg, 31.4 µmol) in dry dichloromethane (1 ml) at 0 °C was treated with DIPEA (110 µl, 630 µmol) and (*E*)-2-methylbut-2-enoic acid chloride (35 µl, 316 µmol) in dichloromethane (0.5 ml) was added drop wise. After stirring at 0 °C

for 1 h the solution was allowed to return to RT and treated with saturated aqueous $NaHCO_3$ solution (0.5 ml). The product was extracted with ethyl acetate, dried over Na_2SO_4 and concentrated in vacuum. Flash chromatography on silica using a gradient of 5 – 25% ethyl acetate in hexanes afforded the di-tiglate ester (5.2 mg, 10.8 µmol, 34%) as a yellowish solid. The product (4.5 mg, 9.4 µmol) was dissolved in tetrahydrofuran (1 ml) and treated with LiOH (0.5 mg, 22 µmol) in water (100 µl) and 1,4-dioxane (2 ml). After stirring at 67 °C for 3 h the reaction was quenched by addition of glacial acetic acid (100 µl). The solution was concentrated in vacuum, the residue dissolved in methanol and concentrated in vacuum. Flash column chromatography on silica gel using a gradient of 0 – 20% methanol in dichloromethane containing 0.2% acetic acid afforded a mixture of mbas#3 isomers (2.1 mg, 5.45 µmol). HPLC provided a pure sample of mbas#3 (1.2 mg, 3.1 µmol; 33% yield) identical to the natural product from *C. elegans*.

NMR Spectroscopic Data of mbas#3. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for **mbas#3** in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	171.7			
2	124.4	5.82	$J_{2,3}$ = 15.6	
3	149.0	6.88	$J_{3,4}$ = 7.0	
4	33.0	2.23		C-5, C-6
5, 6	29.3, 26.4	1.42 – 1.55		
7	37.9	1.60 1.52		
8	72.4	3.81		
9	18.9	1.14	$J_{8,9}$ = 6.1	C-7, C-8
1′	97.4	4.70		C-3′, C-5′, C-8′
2′	69.3	3.73		
3′	32.8	1.85 (*ax*)	$J_{3'ax,3'eq}$ = 12.9, $J_{3'ax,4'}$ = 11.2, $J_{2',3'ax}$ = 2.9	
		2.07 (*eq*)	$J_{2',3'eq}$ = 3.2, $J_{3'eq,4'}$ = 4.7	
4′	71.4	4.90	$J_{4',5'}$ = 9.6	
5′	68.2	3.89	$J_{5',6'}$ = 6.1	
6′	17.8	1.13		C-4′, C-5′
1″-COO	168.5			
2″	129.2			
3″	138.8	6.85	$J_{3'',4''}$ = 7.0	
4″	14.2	1.81		C-2″, C-3″
5″	11.8	1.82		C-1″, C-2″, C-3″

4.22. 2-(8*R*)-(3'*R*,5'*R*-Dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoyl-3,4,5-trihydroxy-6-hydroxymethyl-(2*H*)-tetrahydropyran (glas#10)



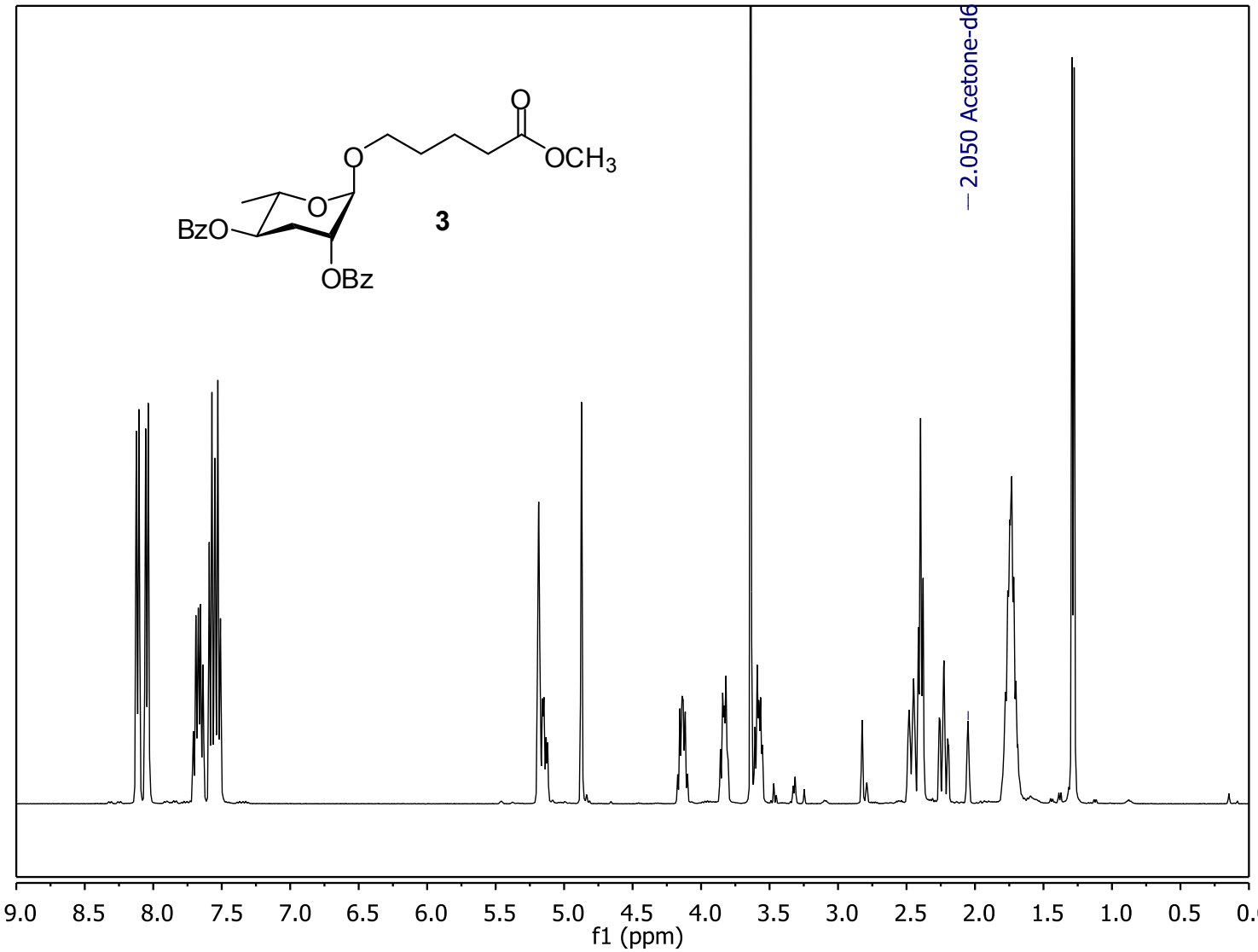
A solution of ascr#10 (3 mg, 9.9 μmol) in dry dimethylformamide (2 ml) was treated with 2,3,4,6-tetra-*O*-benzyl-D-glucose (11 mg, 20 μmol), DMAP (12.2 mg, 100 μmol), and EDC (19.2 mg, 100 μmol). After stirring at RT for 18 h the solution was concentrated in vacuum. The residue was treated with aqueous acetic acid (200 μl), concentrated, and purified by flash column chromatography on silica using a gradient of 5 – 50% methanol in dichloromethane. The product was dissolved in ethanol (1 ml), treated with Pd/C (10 mg, 10% Pd (*w/w*)), and hydrogenated for 24 h. The mixture was filtered and concentrated in vacuum. HPLC provided pure samples of β-D-glucosyl-ascaroside#10 (1.5 mg, 3.22 μmol, 33%) and α-D-glucosyl-ascaroside#10 (1.2 mg, 2.58 μmol, 26%).

NMR Spectroscopic Data of glas#10. ^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for **glas#10** in methanol-d_4. Chemical shifts were referenced to ($CD_2\underline{H}OD$) = 3.31 ppm and ($\underline{C}D_2HOD$) = 49.05 ppm.

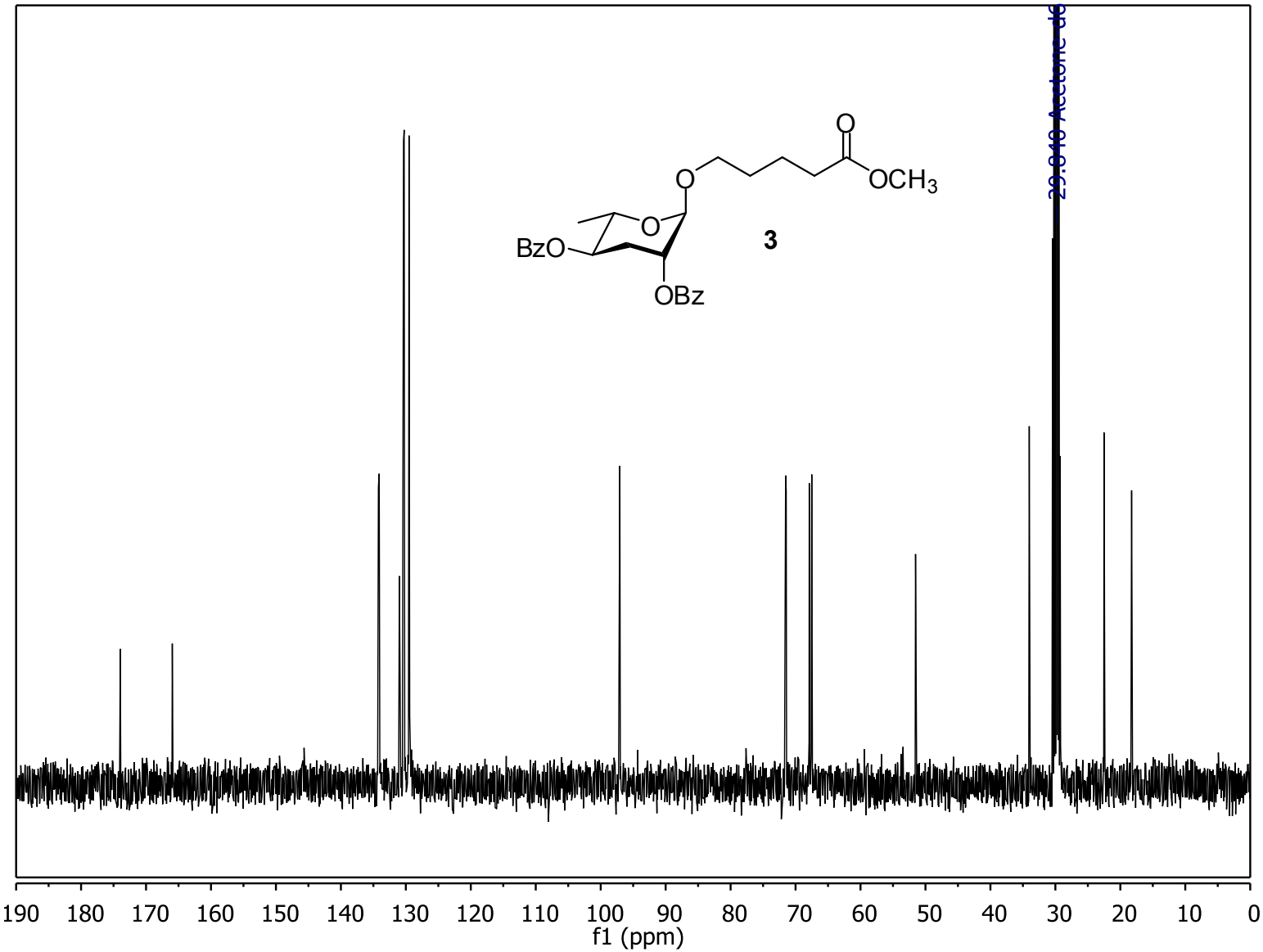
Position	δ ^{13}C [ppm]	δ ^{1}H [ppm]	^{1}H-^{1}H-coupling constants [Hz]	Relevant HMBC correlations
1	173.8			
2	34.7	2.41	$J_{2,2}$ = 15.4, $J_{2,3}$ = 7.8	C-1
3	25.5	1.64		C-1, C-2
4		1.37		
5, 6	29.8, 30.1	1.30-1.50		
7	26.6	1.45 – 1.55		
8	72.3	3.78		
9	19.2	1.12	$J_{8,9}$ = 6.1	C-8, C-7
1′	97.4	4.60		C-9, C-3′, C-5′
2′	69.8	3.71		
3′	35.8	1.77 (*ax*)	$J_{3'ax,3'eq}$ = 13.0, $J_{3'ax,4'}$ = 11.4, $J_{2',3'ax}$ = 2.9	
		1.95 (*eq*)	$J_{2',3'eq}$ = 3.2, $J_{3'eq,4'}$ = 4.7	
4′	68.2	3.52	$J_{4',5'}$ = 9.5	
5′	71.0	3.63	$J_{5',6'}$ = 6.3, $J_{5',4'}$ = 9.3	
6′	18.0	1.22		C-5′, C-4′
1″	95.4	5.47	$J_{1'',2''}$ = 8.1	C-1
2″	73.8	3.32	$J_{2'',3''}$ = 9.1	
3″	77.8	3.41	$J_{3'',4''}$ = 9.7	
4″	70.9	3.35	$J_{4'',5''}$ = 9.7	
5″	78.7	3.36		
6″	62.2	3.67	$J_{6'',6''}$ = 12.0, $J_{6'',5''}$ = 4.8	
		3.83	$J_{6'',6''}$ = 12.0, $J_{6'',5''}$ = 1.9	

5. NMR Spectra of Synthetic Compounds

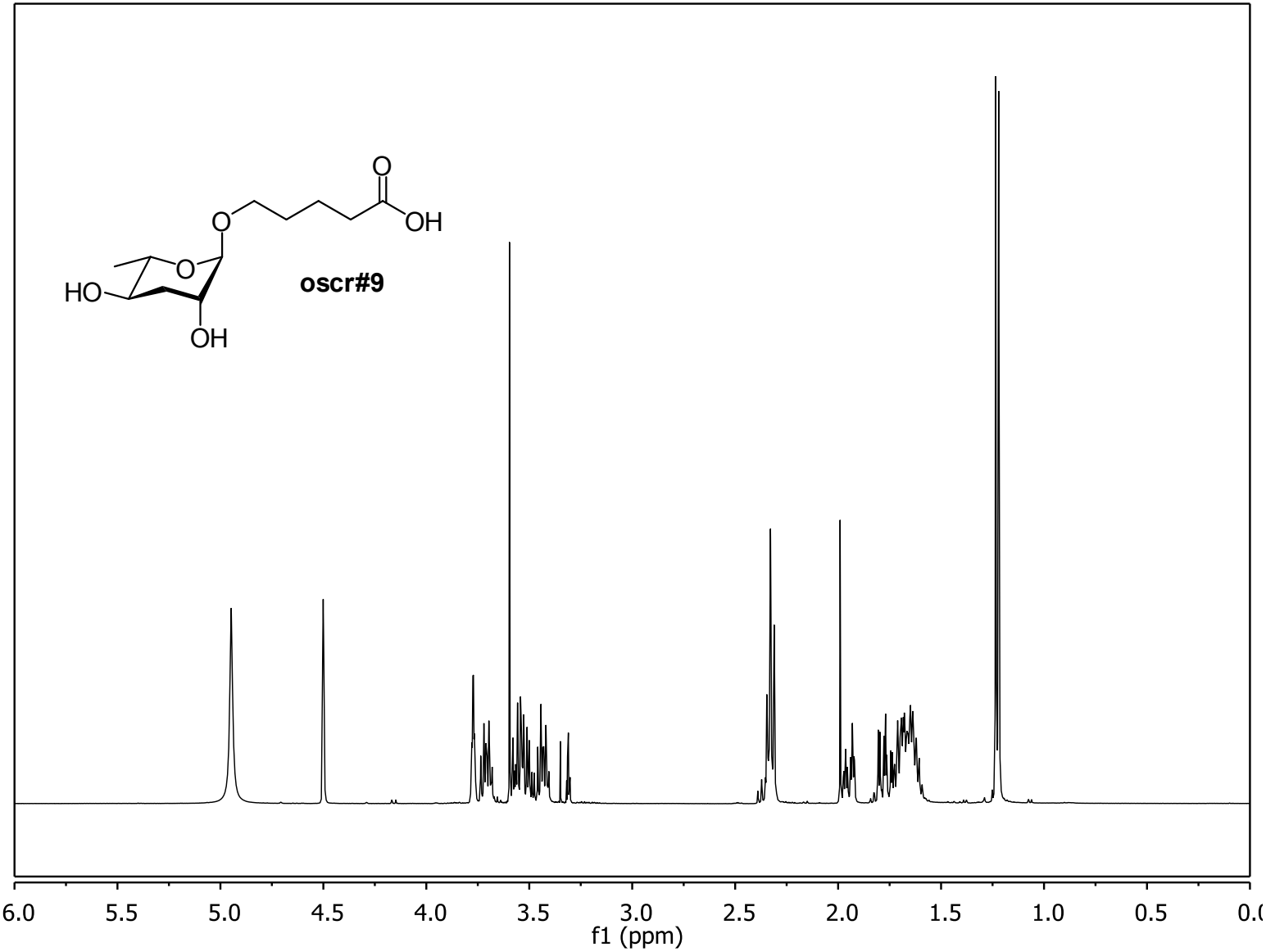
5.1A. ^{1}H NMR Spectrum (400 MHz, acetone-d_6) of Methyl 5-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)pentanoate (**3**)



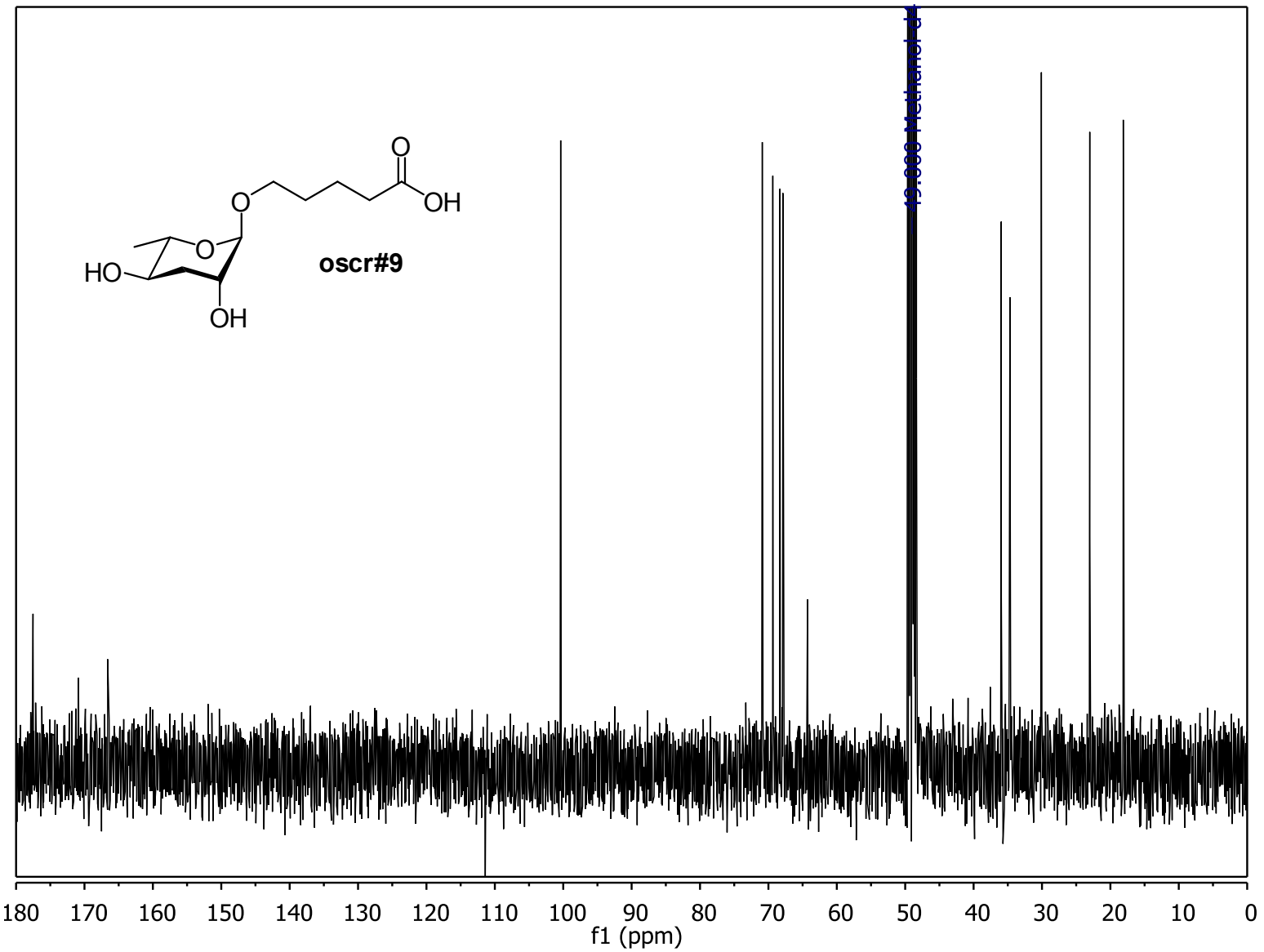
5.1B. ^{13}C NMR Spectrum (100 MHz, acetone-d_6) of Methyl 5-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)pentanoate (**3**)



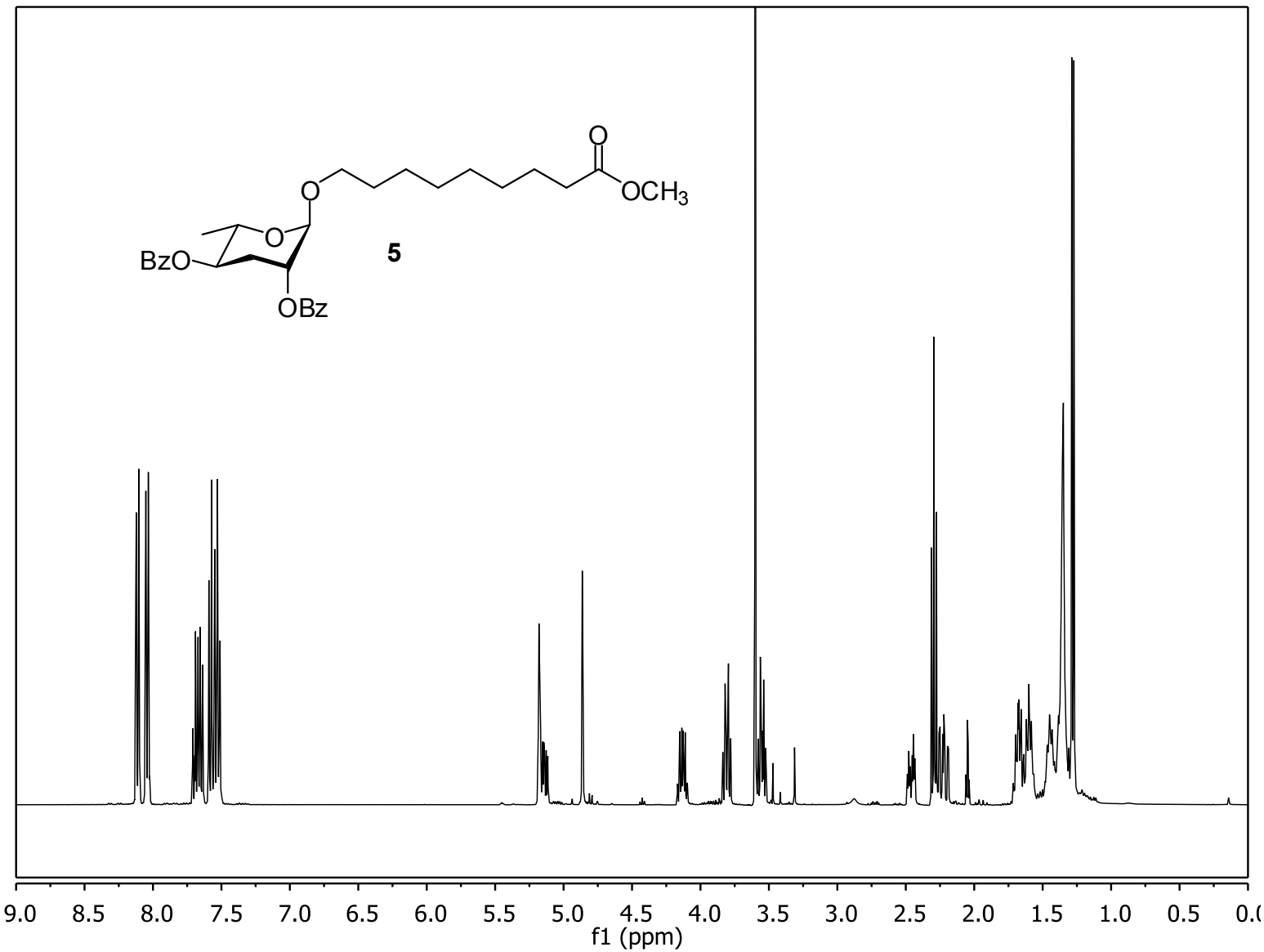
5.2A ^{1}H NMR Spectrum (400 MHz, methanol-d_4) of 5-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)pentanoic acid (**oscr#9**)



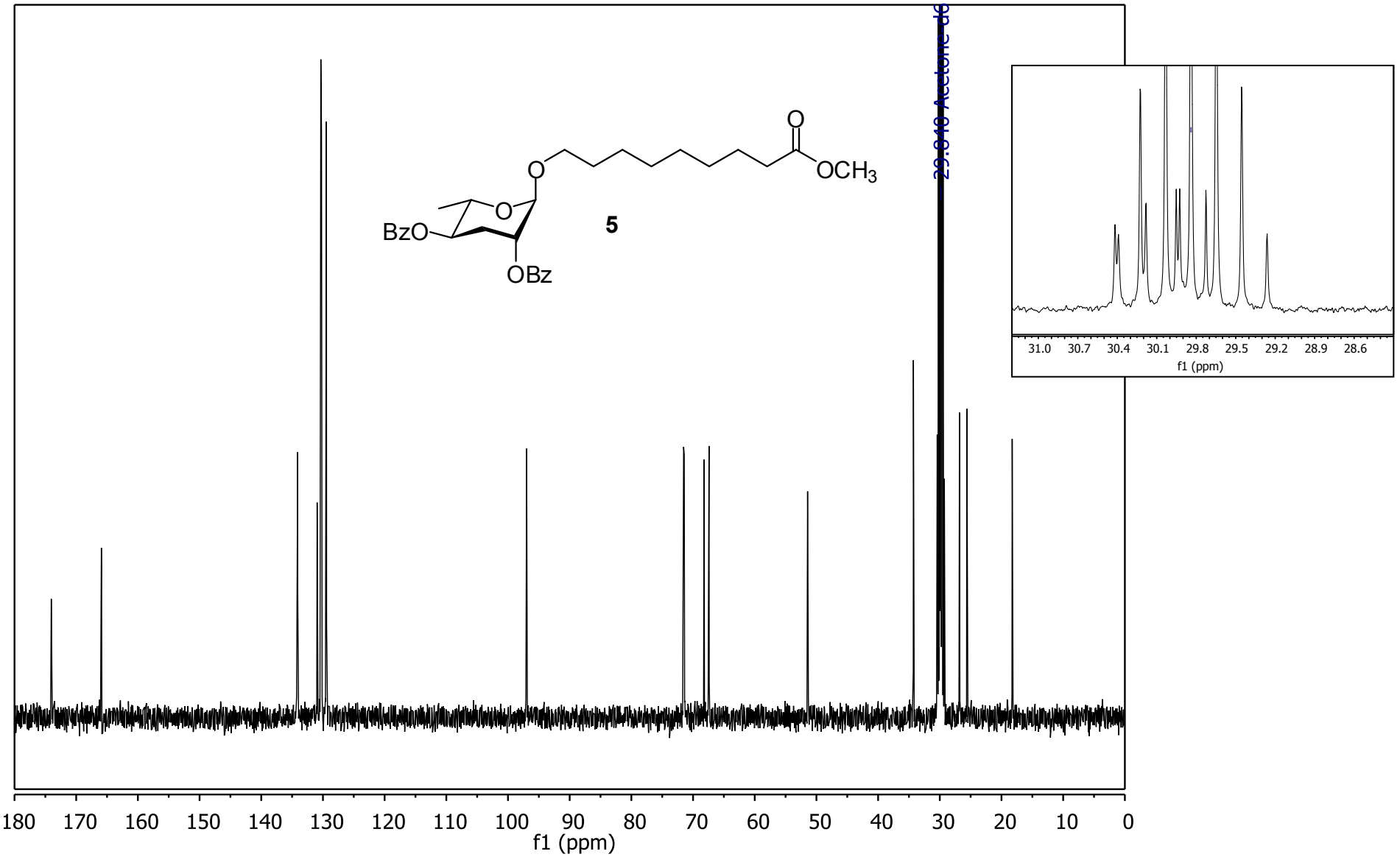
5.2B. ^{13}C NMR Spectrum (100 MHz, methanol-d_4) of 5-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)pentanoic acid (**oscr#9**)



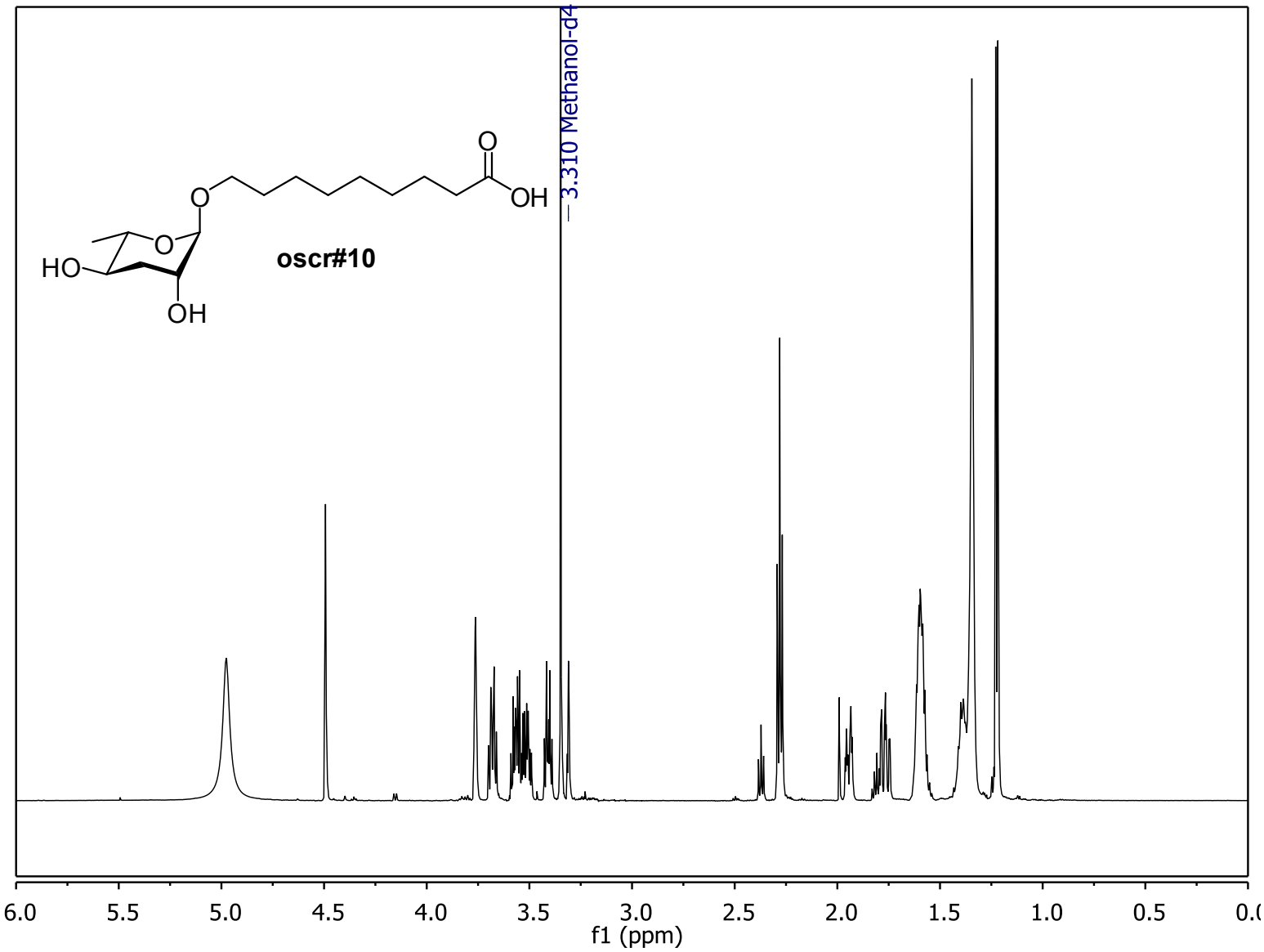
5.3A. ^{1}H NMR Spectrum (400 MHz, acetone-d_6) of Methyl 9-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoate (**5**)



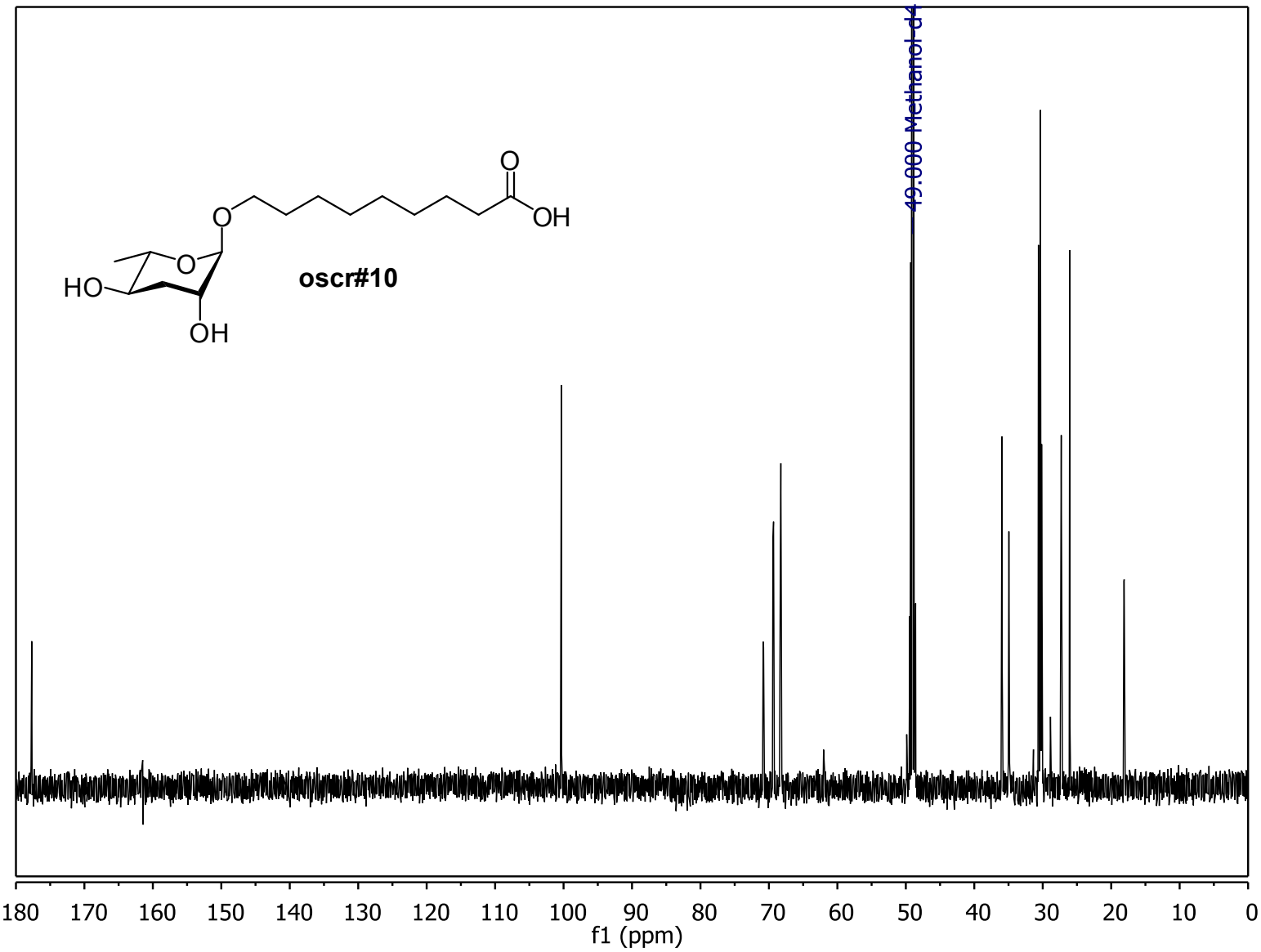
5.3B. ^{13}C NMR Spectrum (100 MHz, acetone-d_6) of Methyl 9-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoate (**5**)



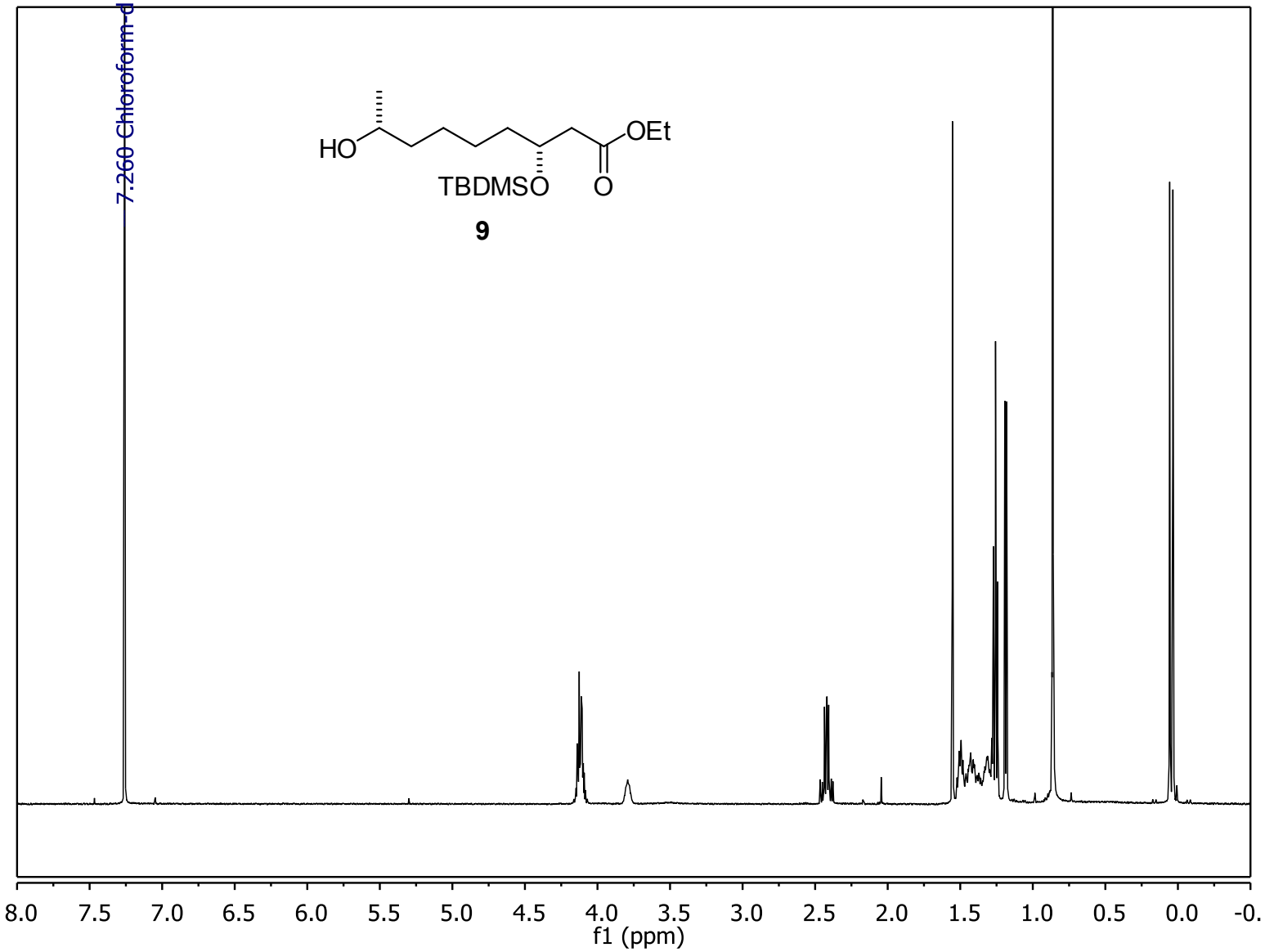
5.4A. ^{1}H NMR Spectrum (600 MHz, methanol-d_4) of 9-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoic acid (**oscr#10**)



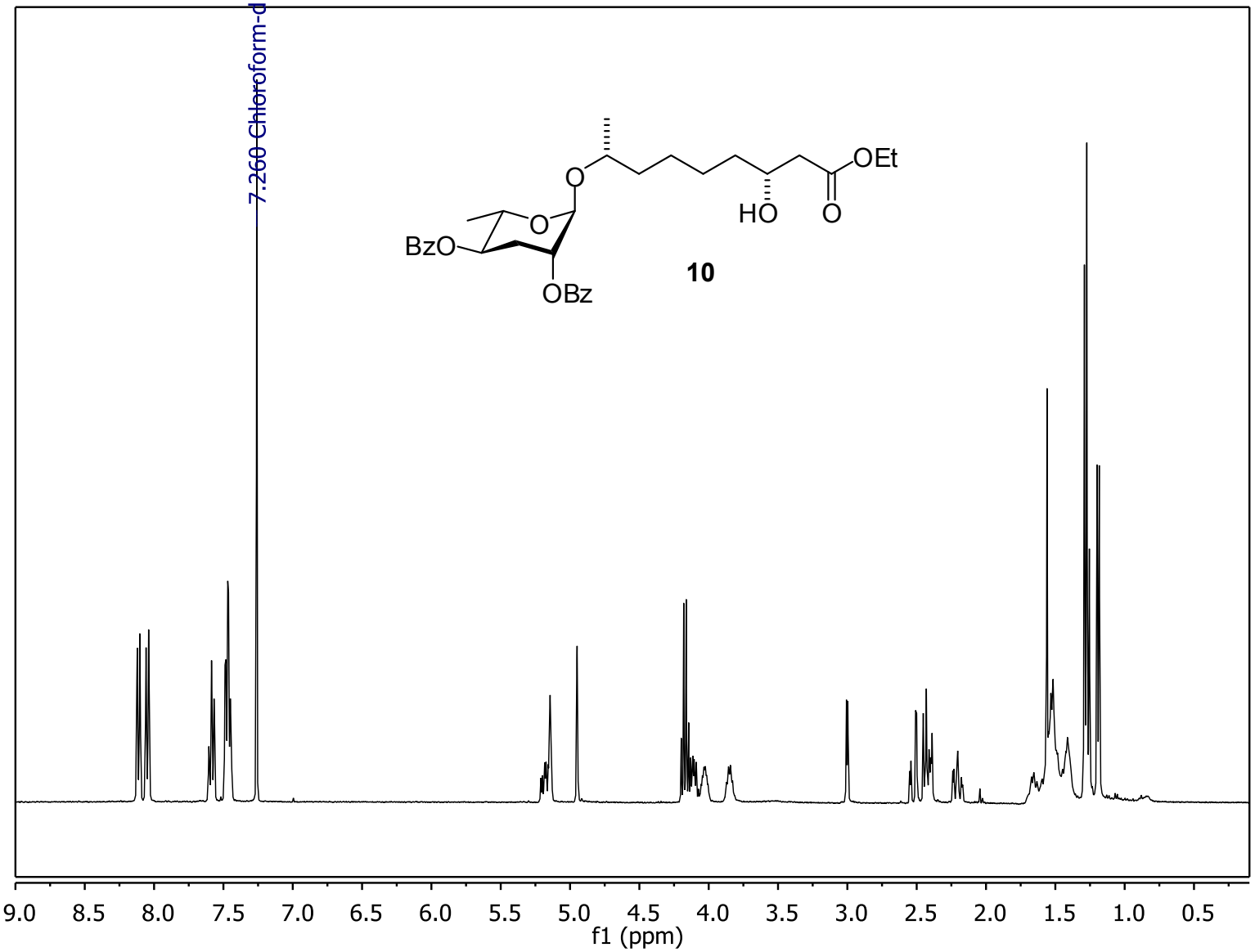
5.4B. ^{13}C NMR Spectrum (100 MHz, methanol-d_4) of 9-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoic acid (**oscr#10**)



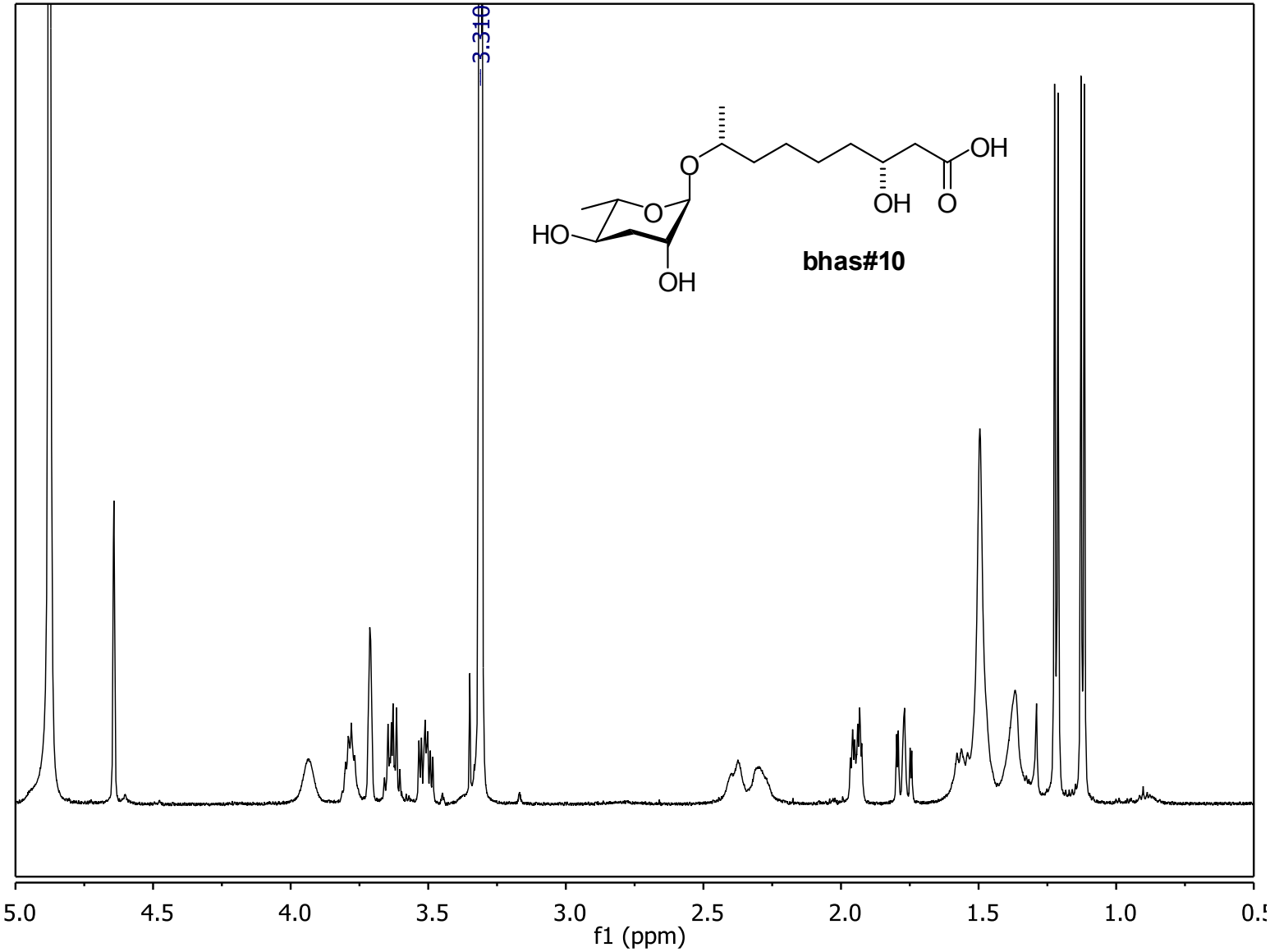
5.5. ^{1}H NMR Spectrum (500 MHz, chloroform-d_1) of Ethyl (8*R*)-hydroxy-(3*R*)-*tert*-butyldimethylsilyloxynonanoate (**9**)



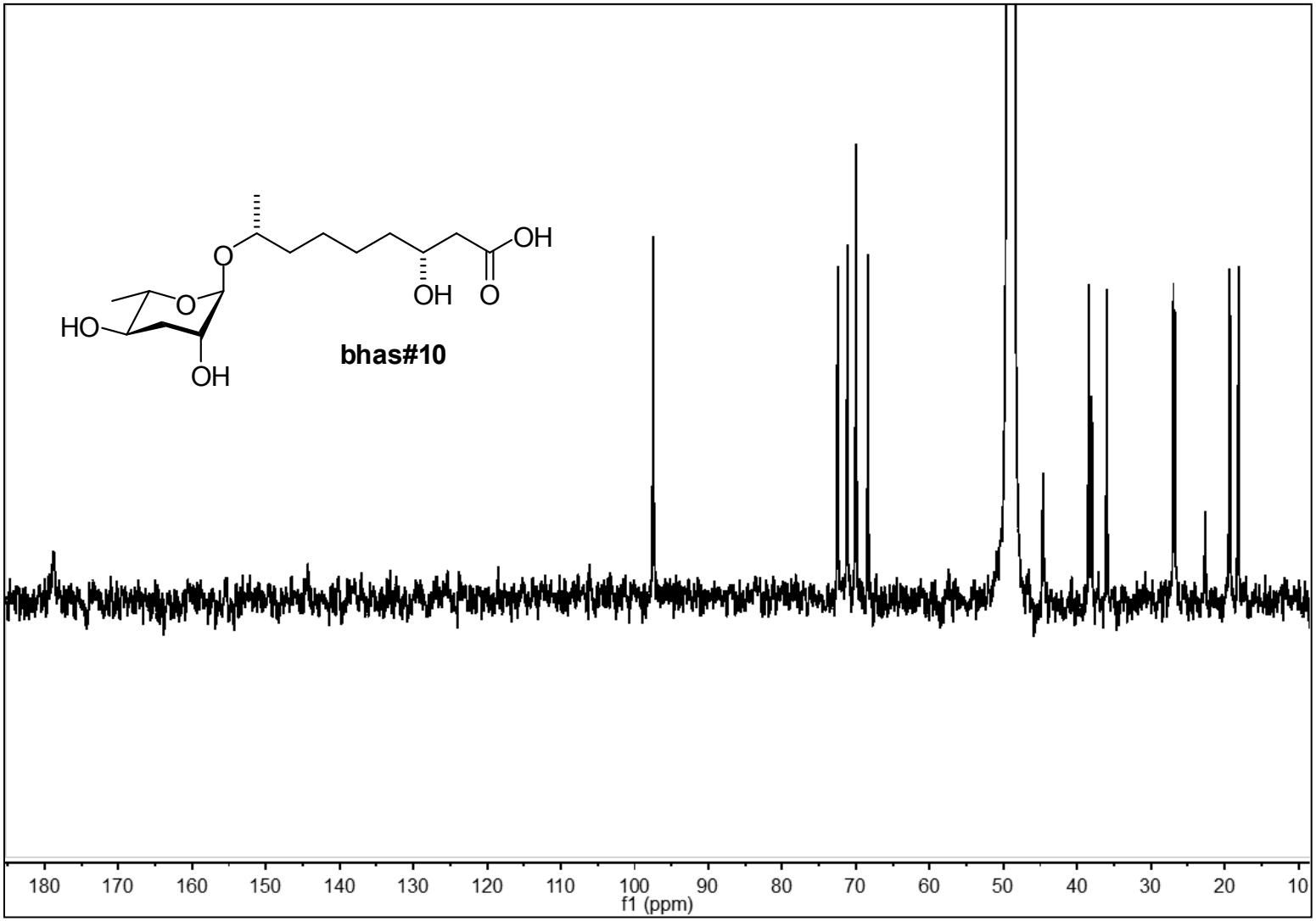
5.6. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of Ethyl (8*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoate (**10**)



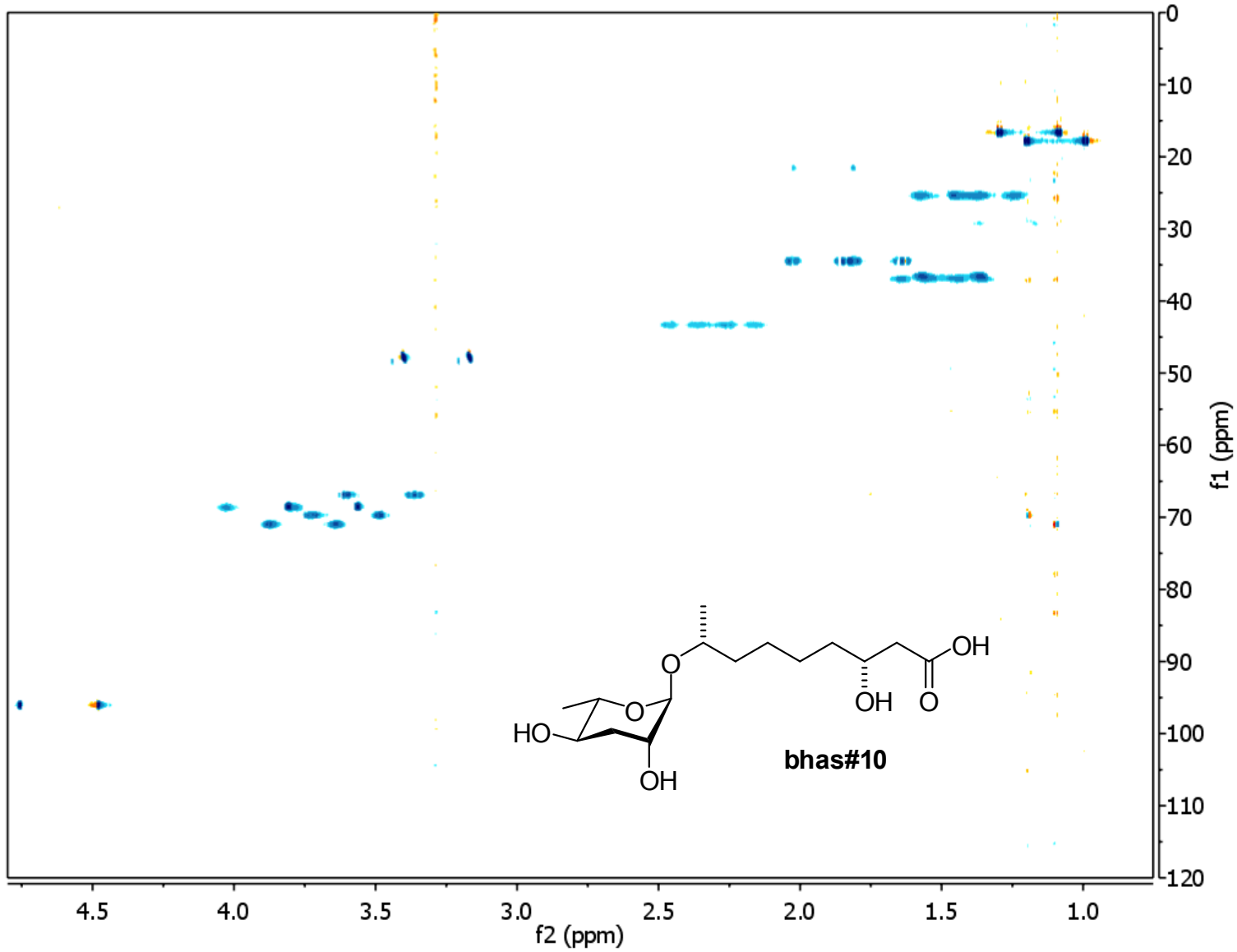
5.7A. ^{1}H NMR Spectrum (500 MHz, methanol-d_4) of (8*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoic acid (**bhas#10**)



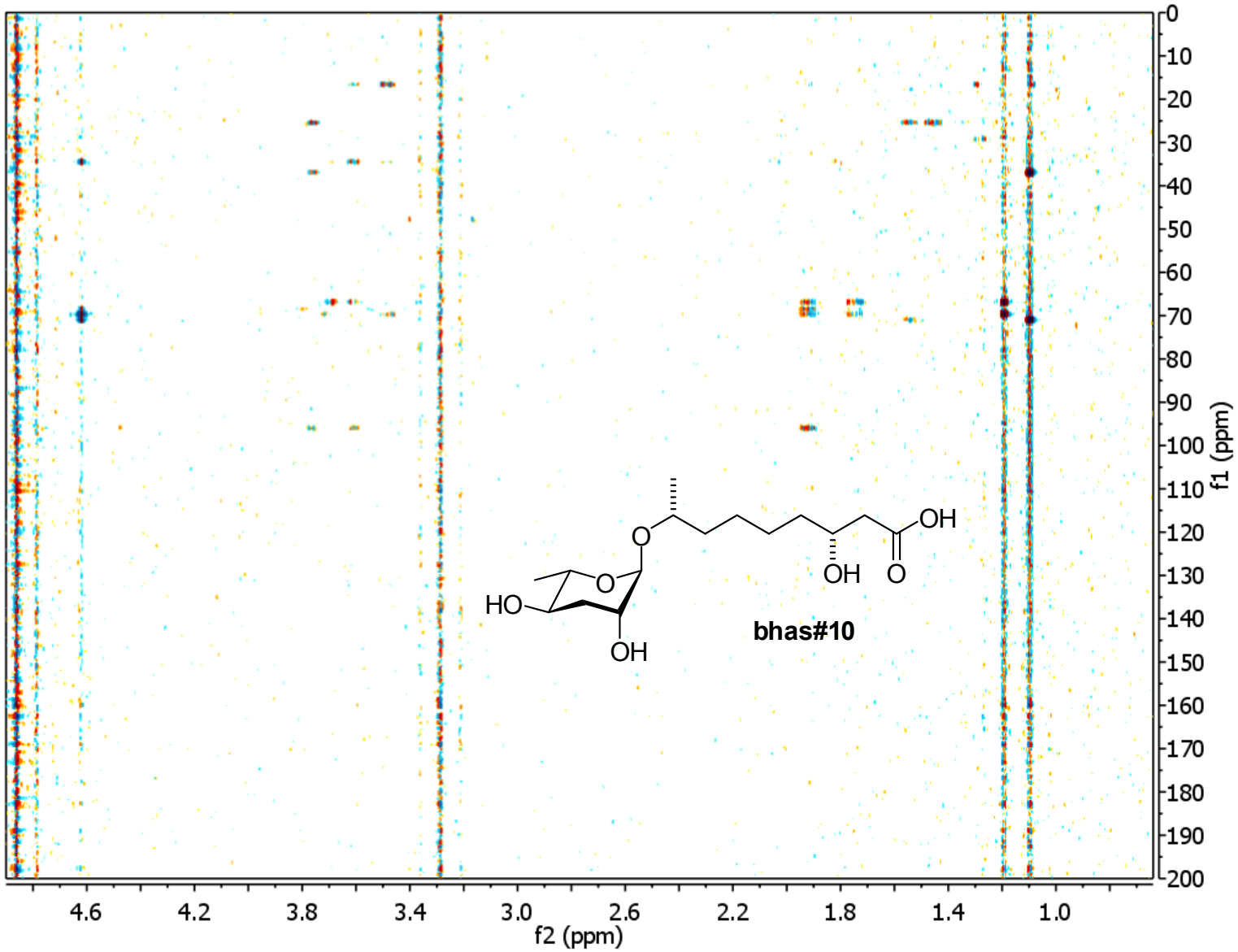
5.7B. ^{13}C NMR Spectrum (125 MHz, methanol-d_4) of (8*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoic acid (**bhas#10**)



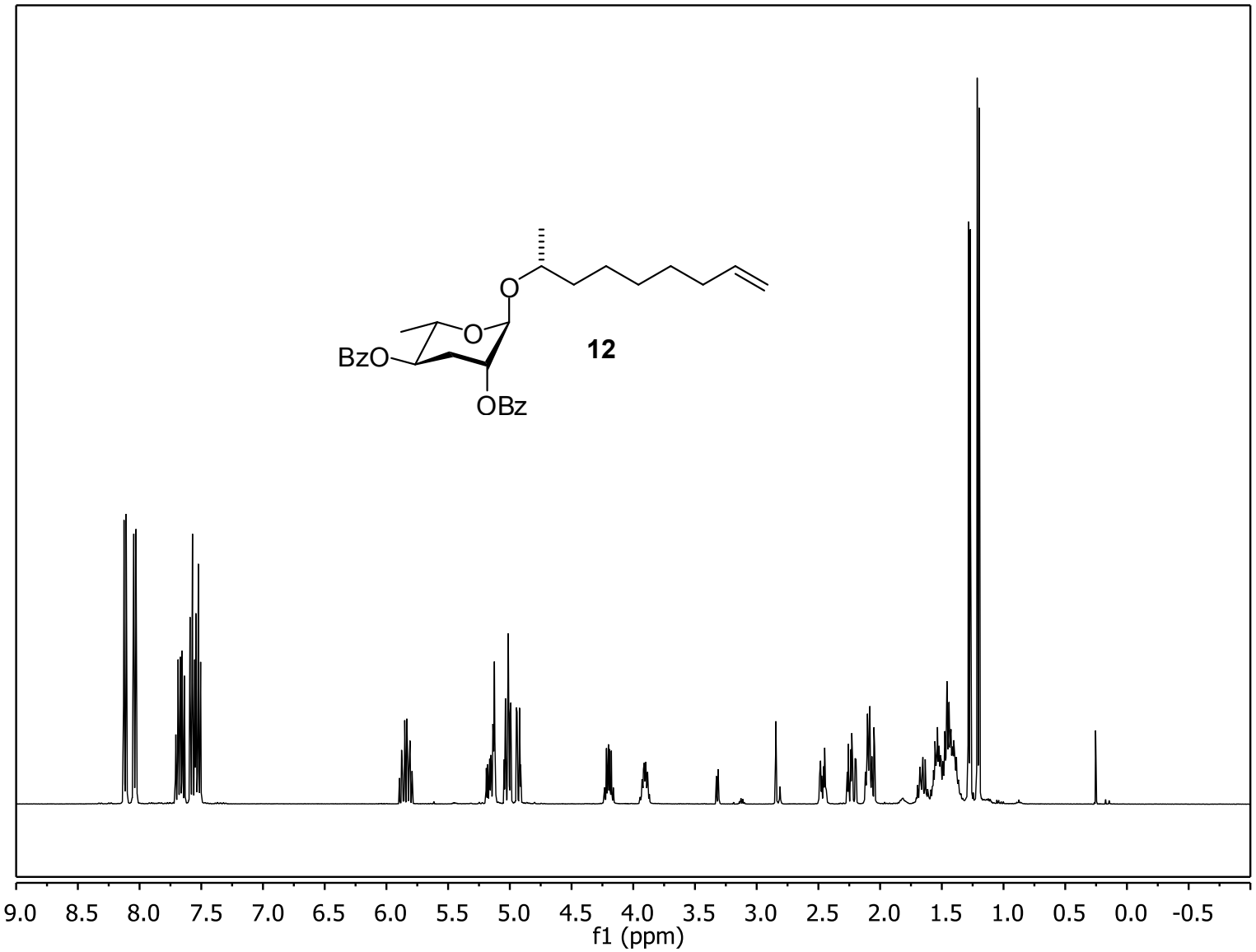
5.7C. HSQC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of (8*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoic acid (**bhas#10**)



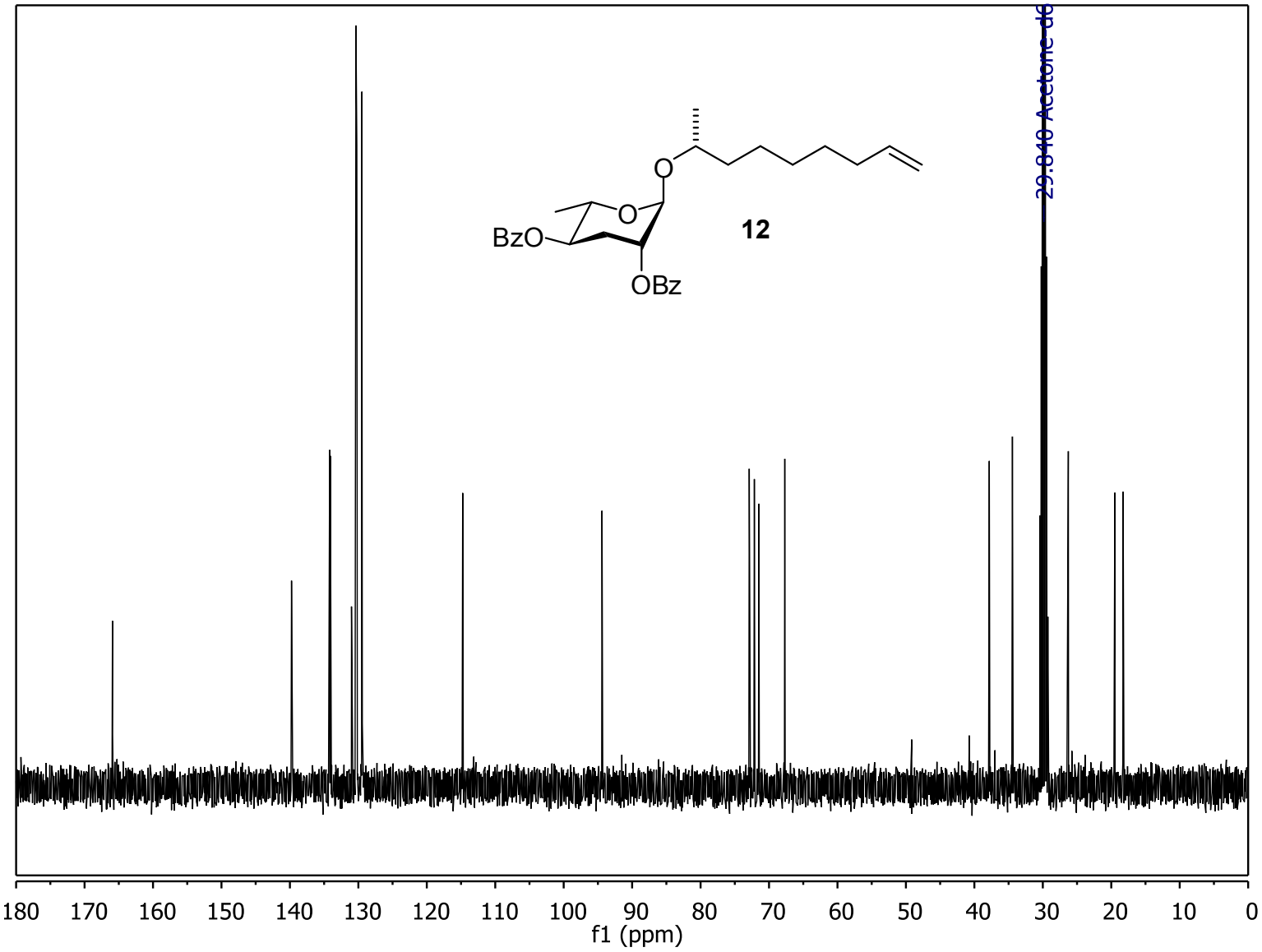
5.7D. HMBC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of (8*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxynonanoic acid (**bhas#10**)



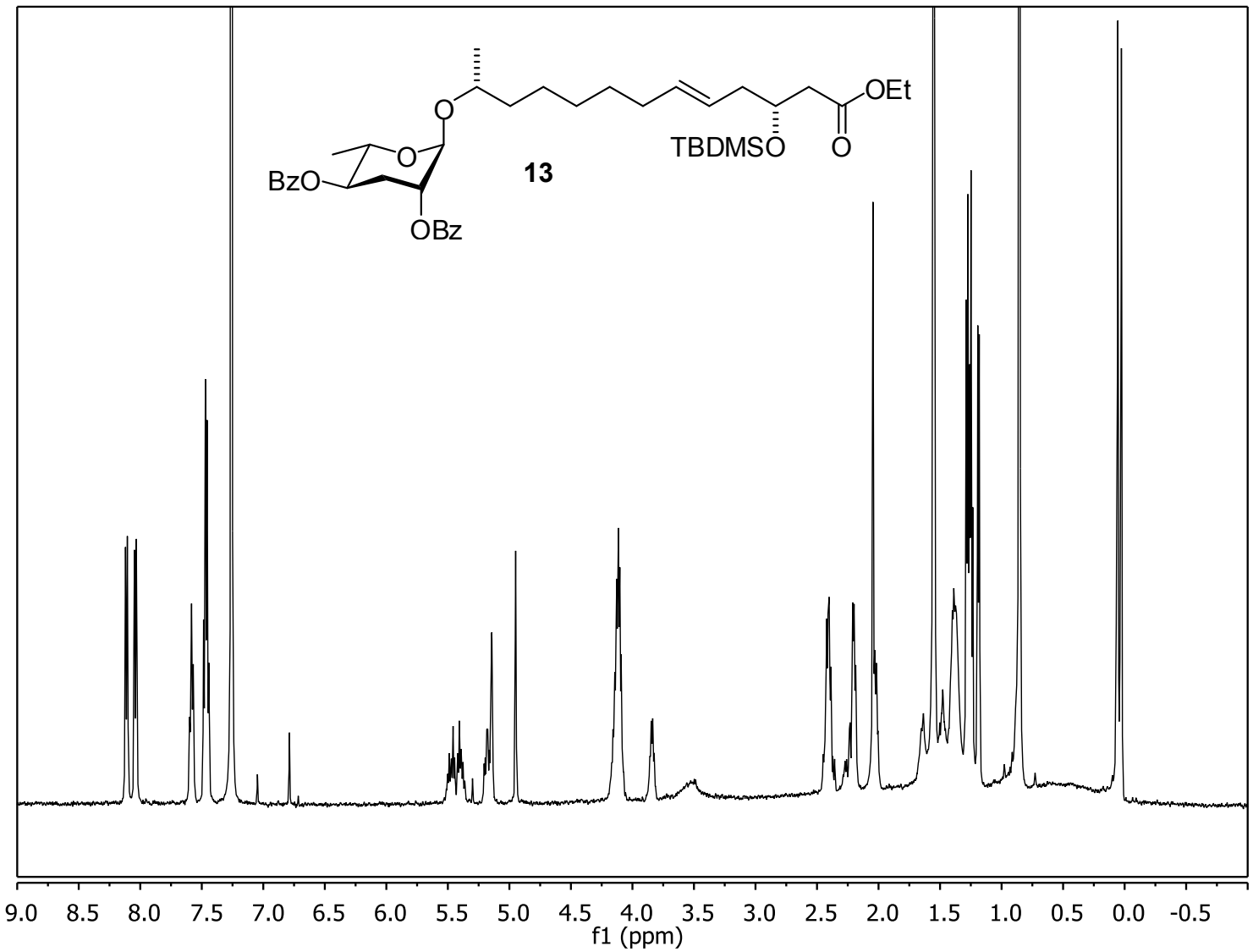
5.8A. ^{1}H NMR Spectrum (400 MHz, acetone-d_6) of (8*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-1-ene (**12**)



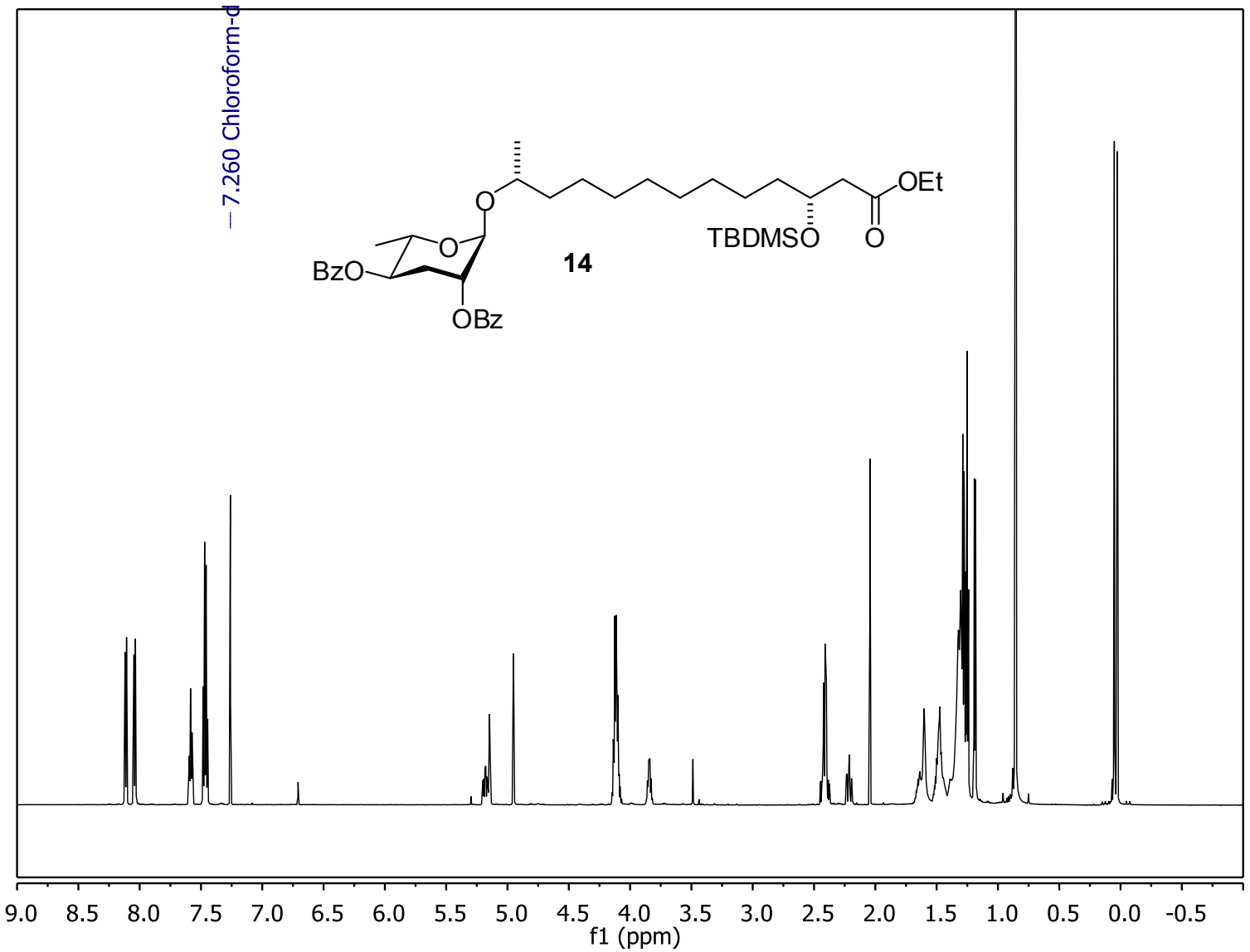
5.8B. ^{13}C NMR Spectrum (100 MHz, acetone-d_6) of (8*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-1-ene (**12**)



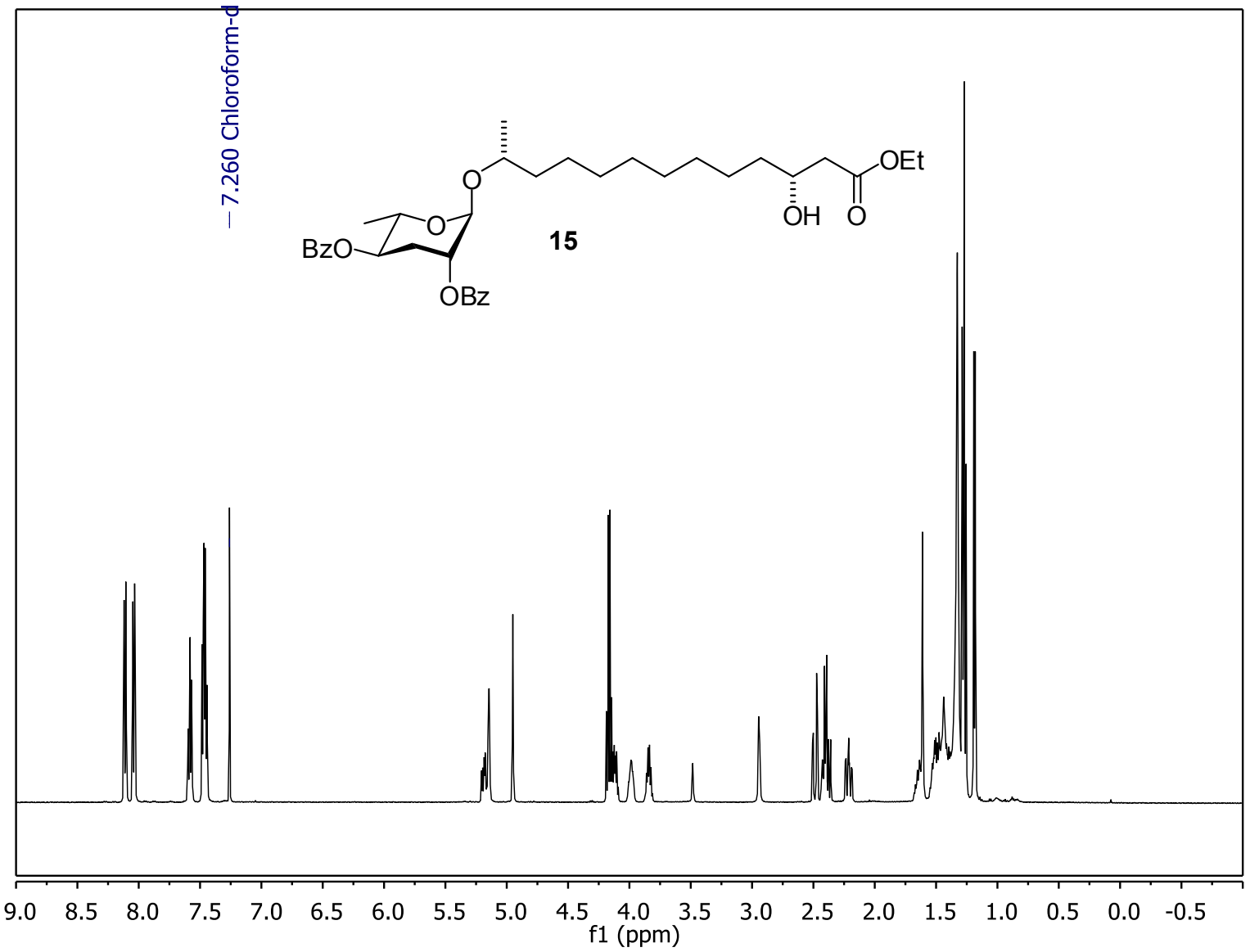
5.9. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of (12*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxytridec-5-enoic acid ethyl ester (**13**)



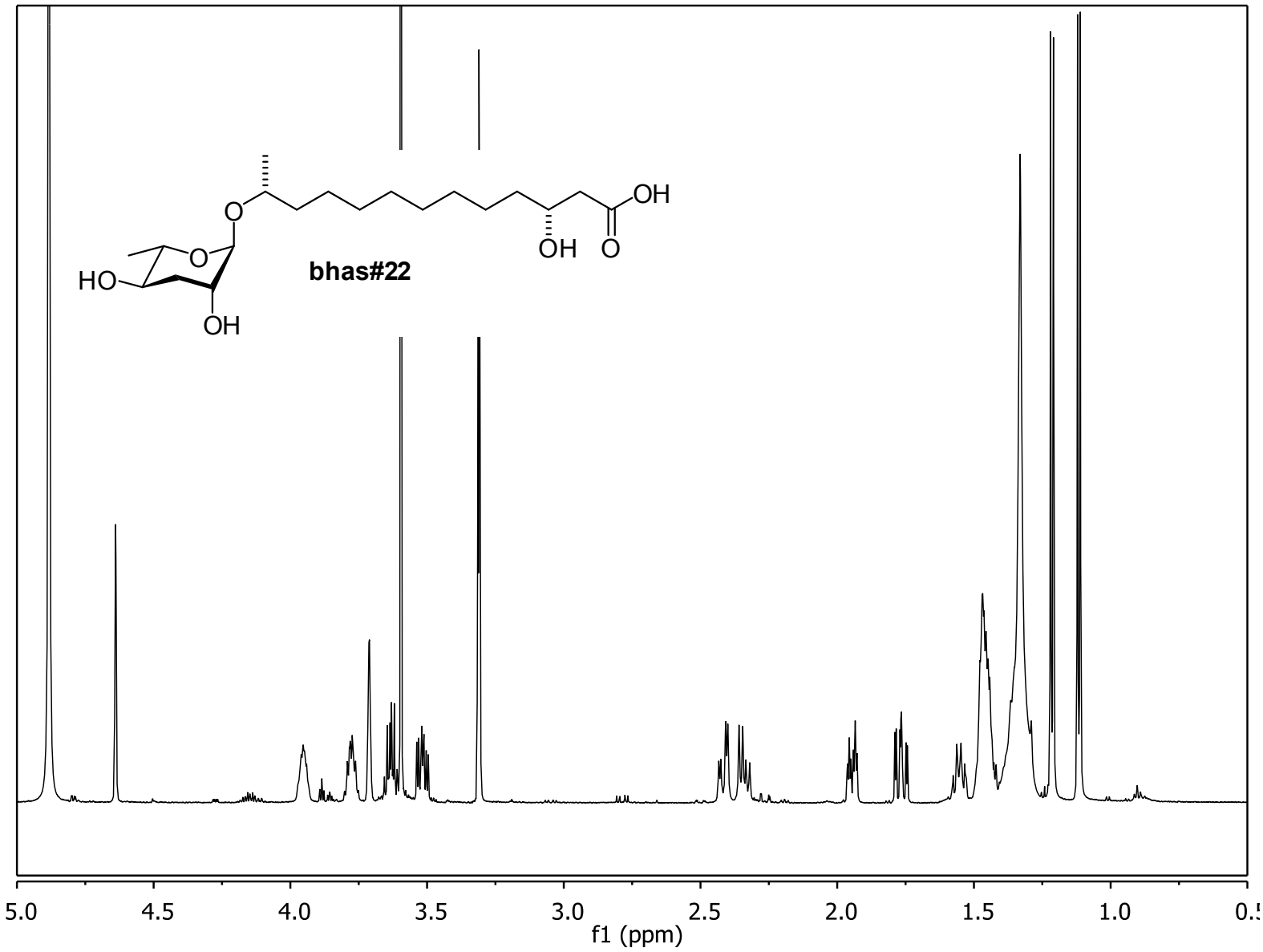
5.10. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of (12*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxytridecanoic acid ethyl ester (**14**)



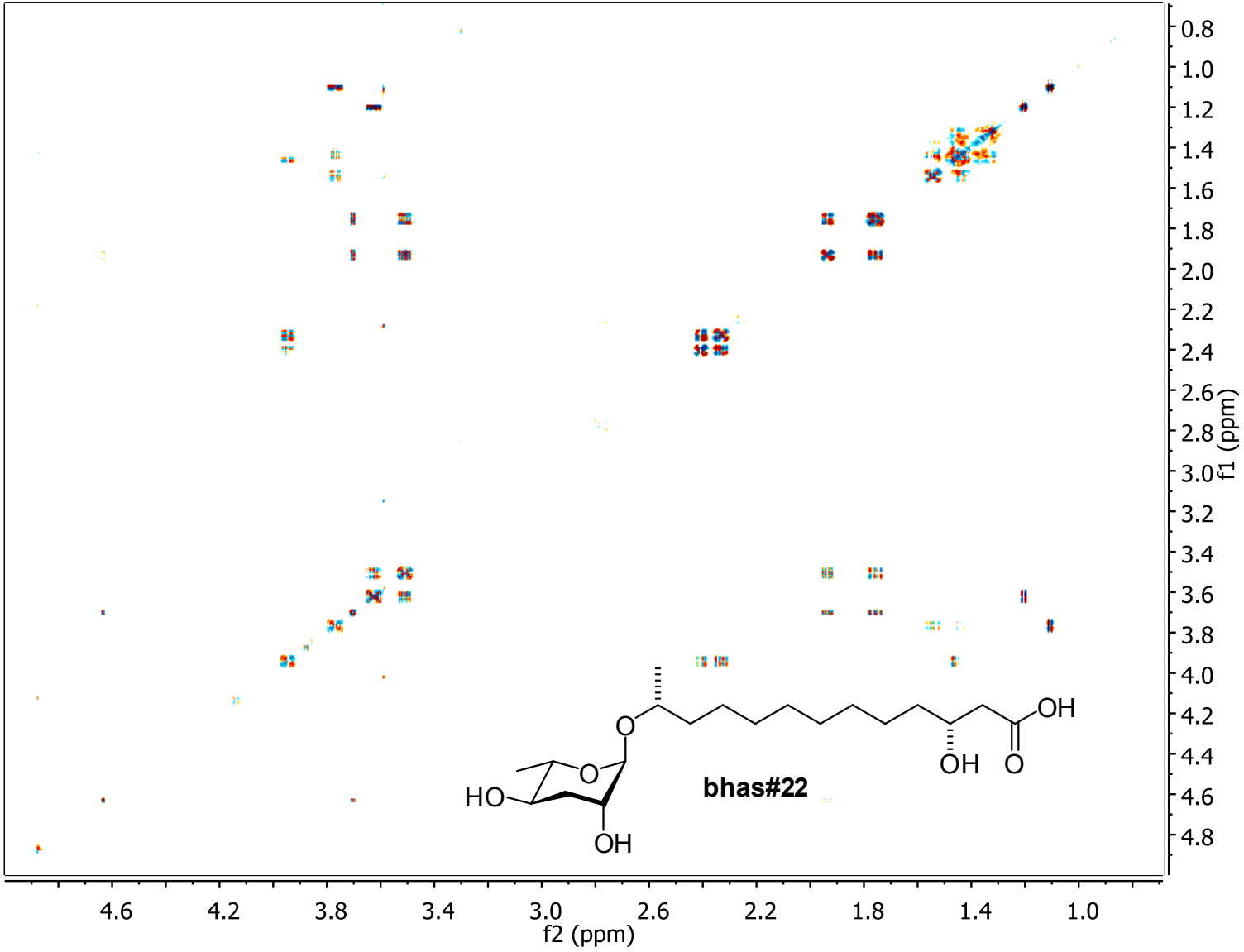
5.11. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of (12*R*)-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoic acid ethyl ester (**15**)



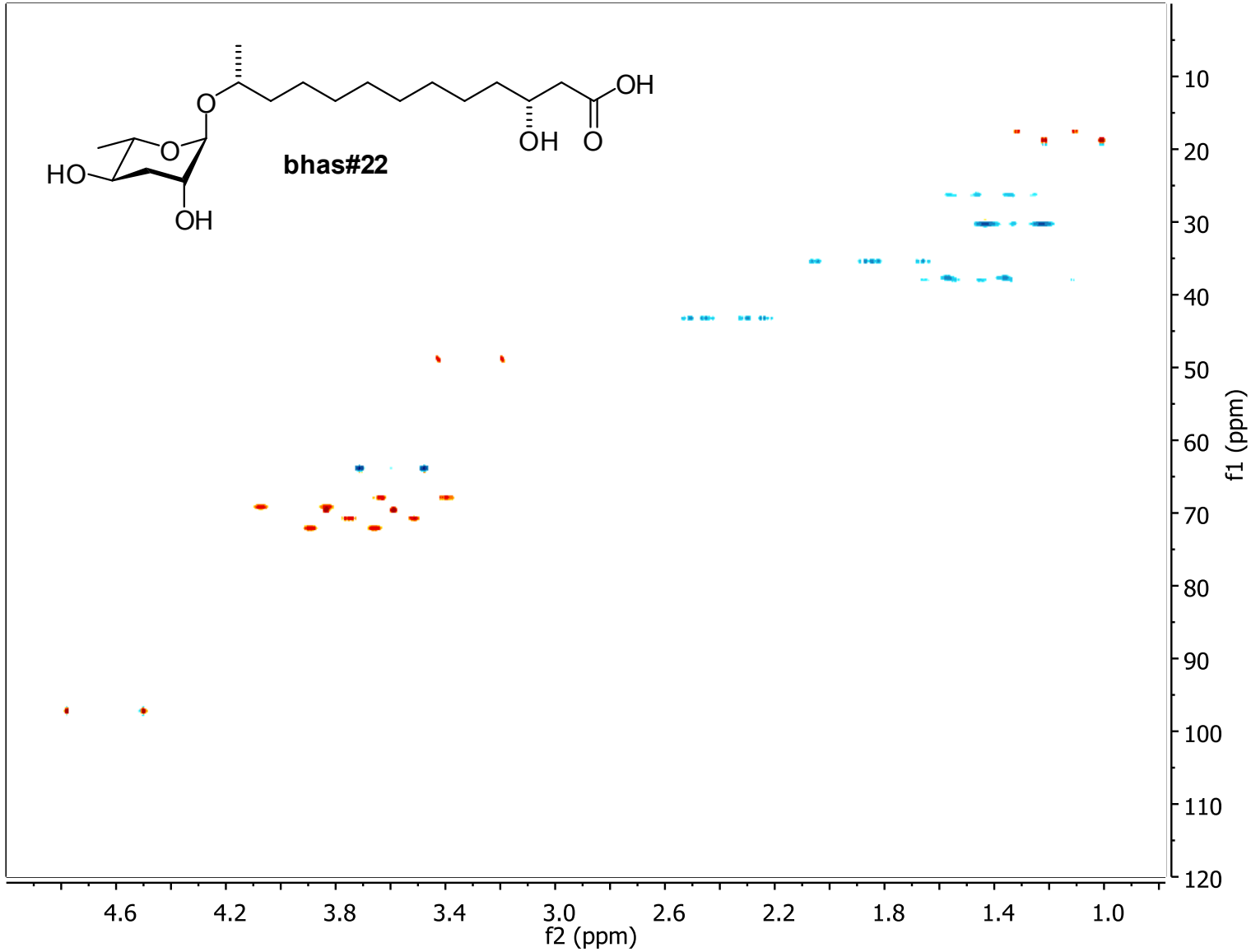
5.12A. ^{1}H NMR Spectrum (600 MHz, methanol-d_4) of (12*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoic acid (**bhas#22**)



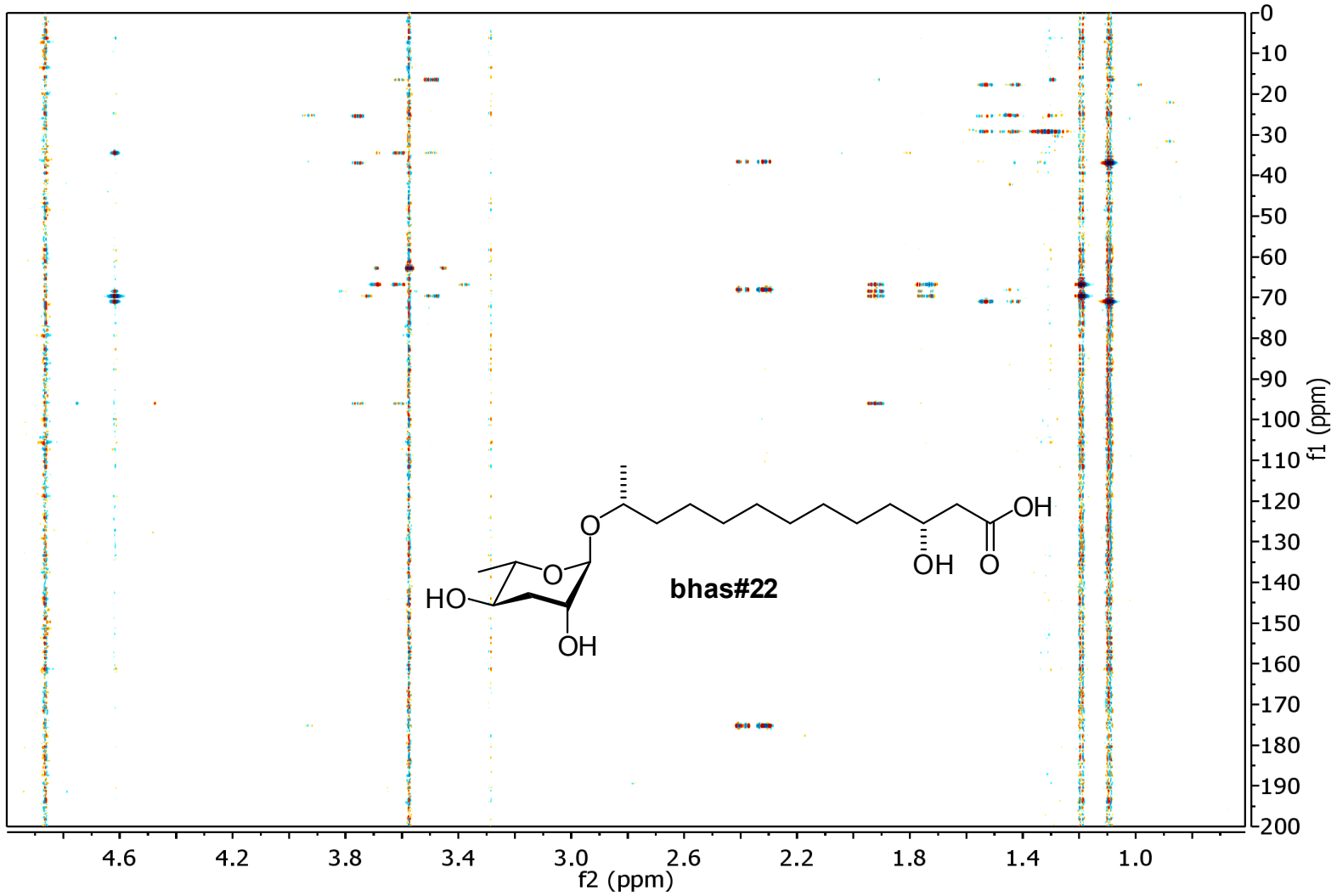
5.12B. dqfCOSY Spectrum (600 MHz, methanol-d_4) of (12*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoic acid (**bhas#22**)



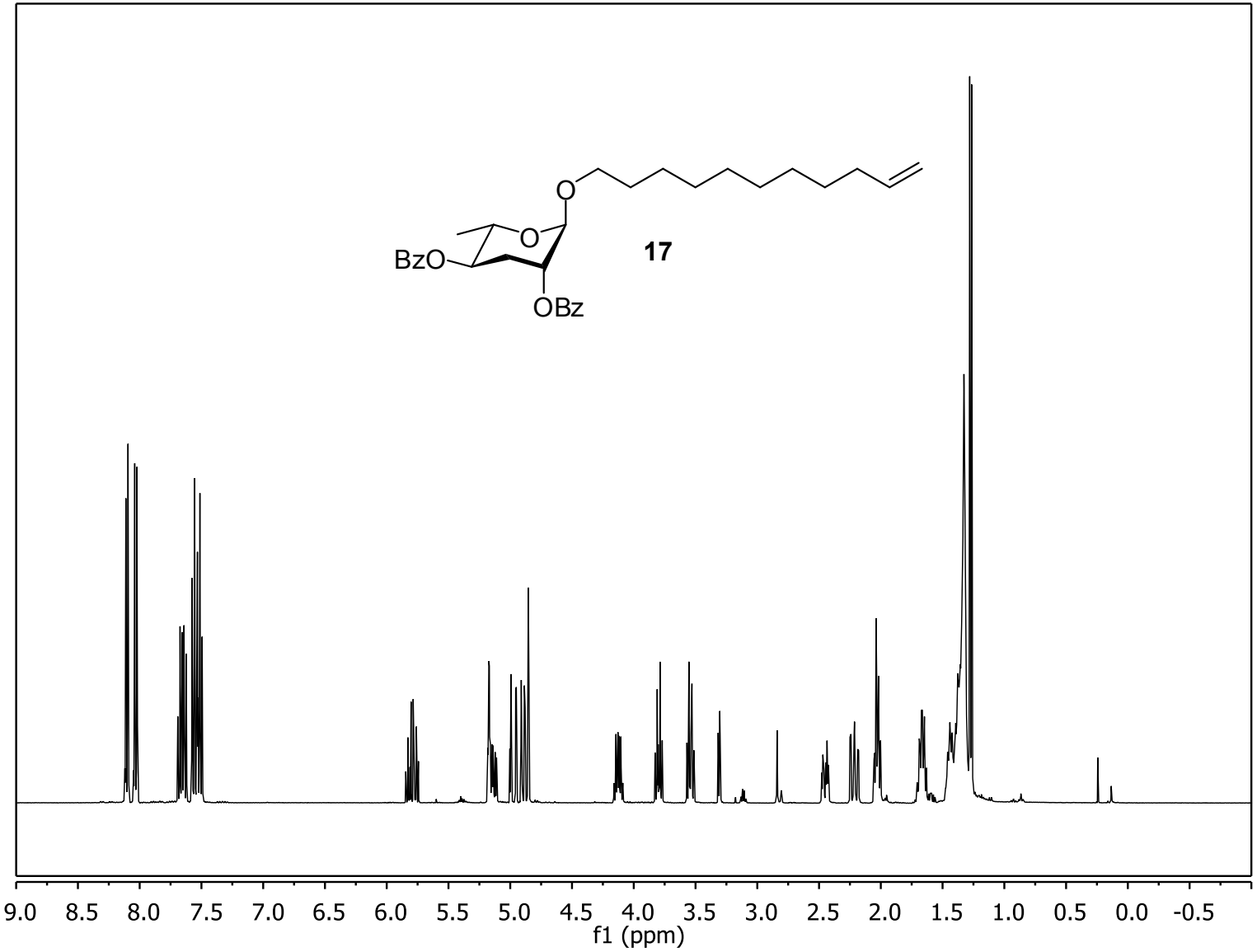
5.12C. HSQC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of (12*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoic acid (**bhas#22**)



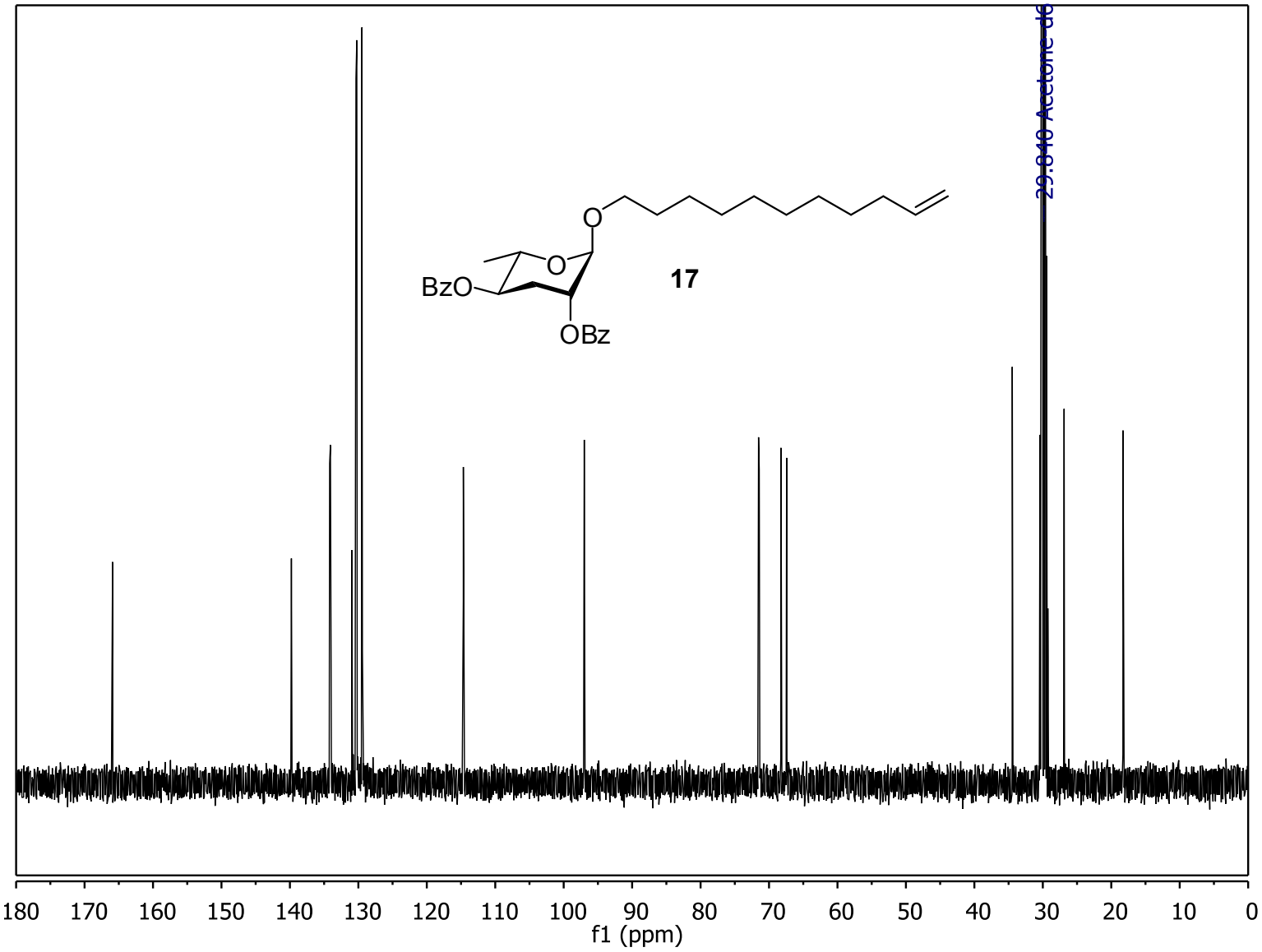
5.12D. HMBC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of (12*R*)-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxytridecanoic acid (**bhas#22**)



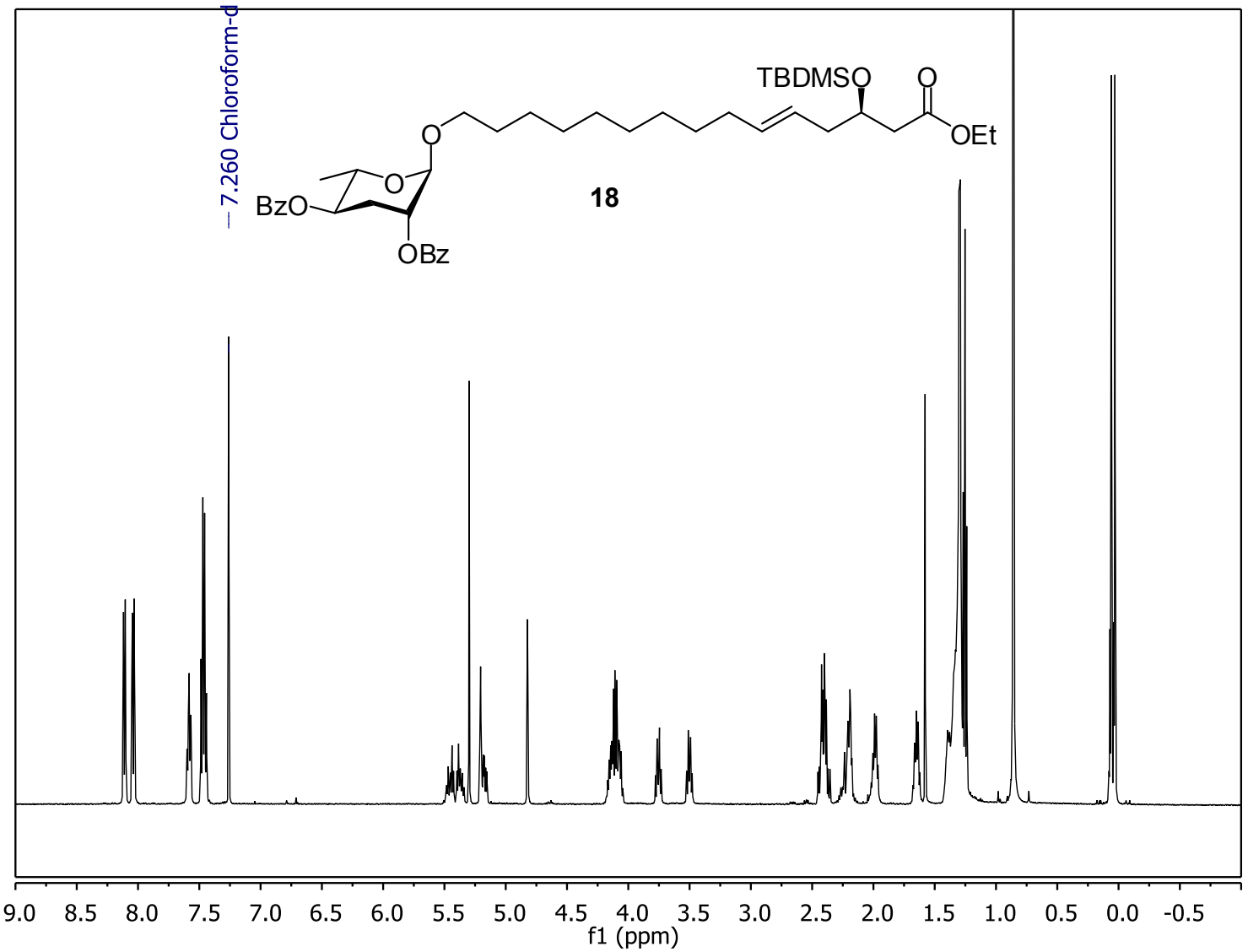
5.13A. ^{1}H NMR Spectrum (400 MHz, acetone-d_6) of 11-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)undec-1-ene (**17**)



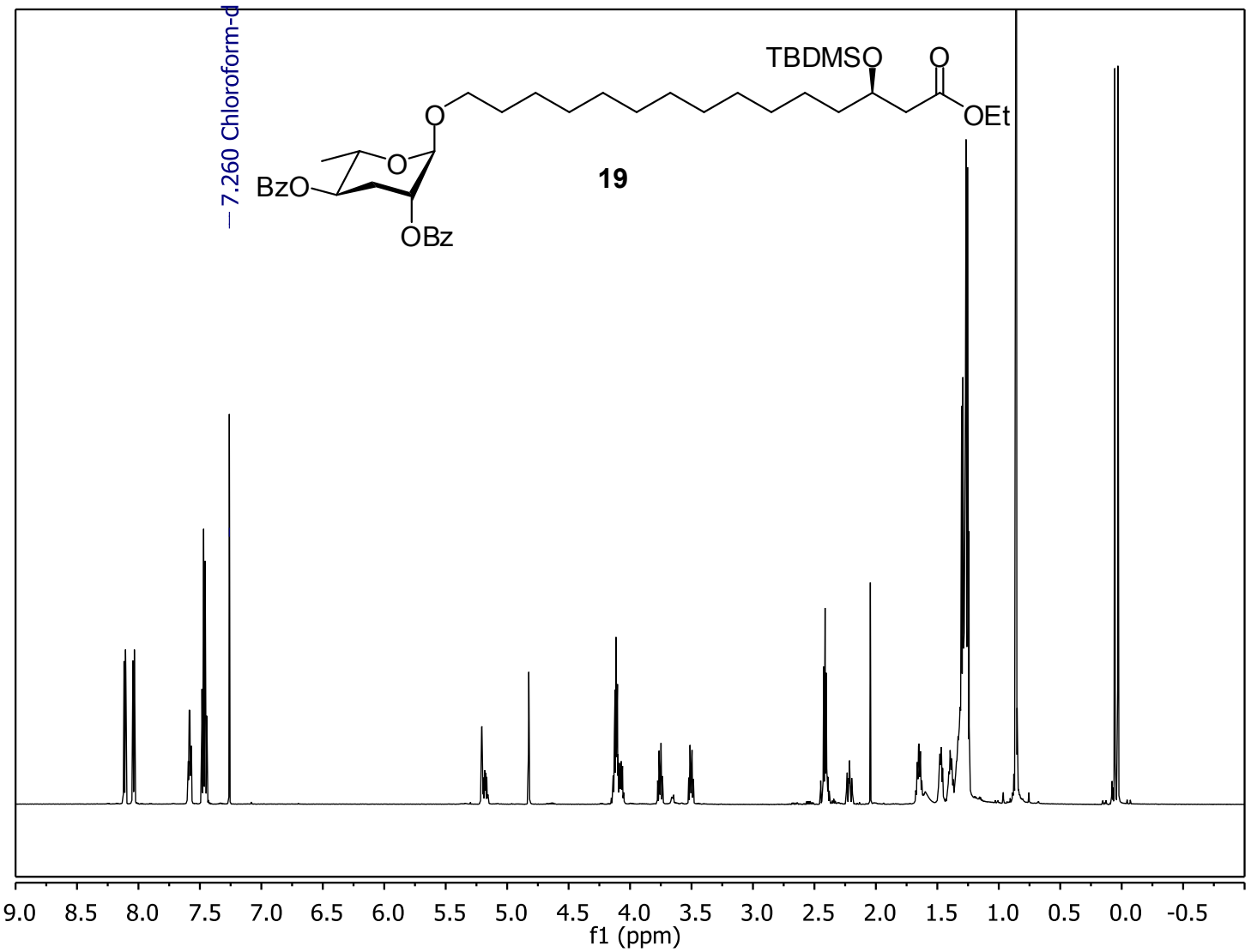
5.13B. ^{13}C NMR Spectrum (100 MHz, acetone-d_6) of 11-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)undec-1-ene (**17**)



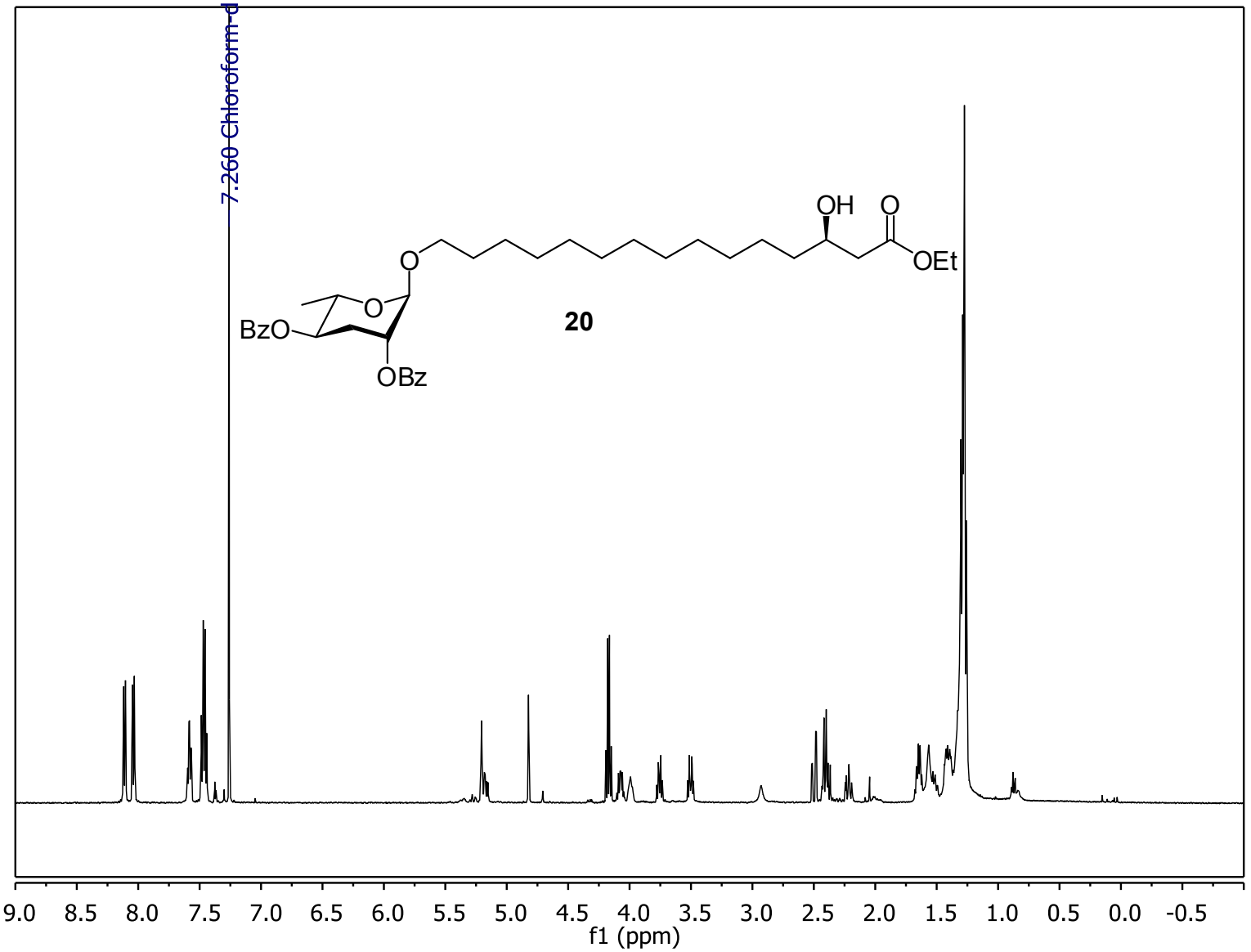
5.14. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of 15-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxypentadec-5-enoic acid ethyl ester (**18**)



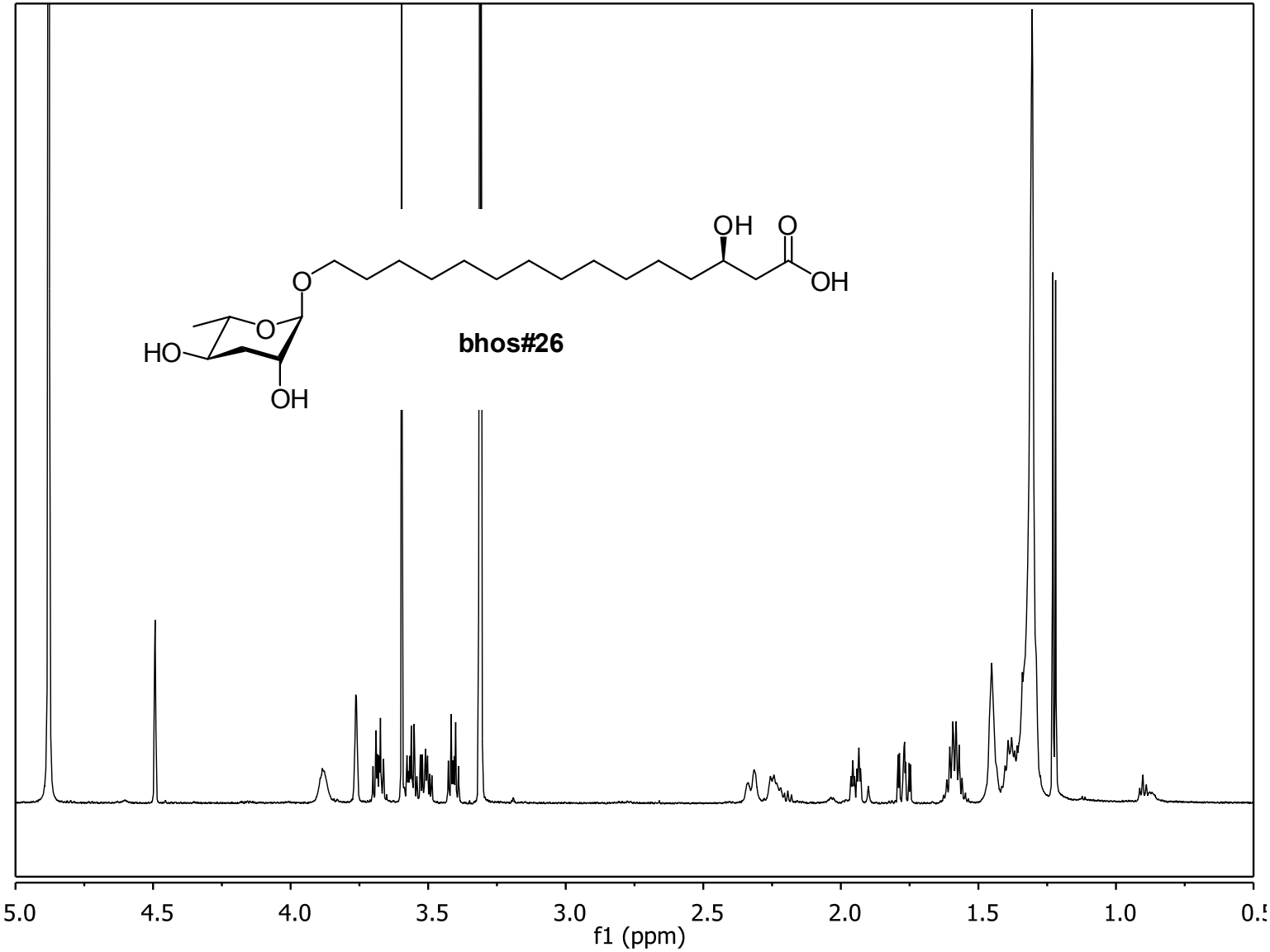
5.15. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of 15-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-*tert*-butyldimethylsilyloxypentadecanoic acid ethyl ester (**19**)



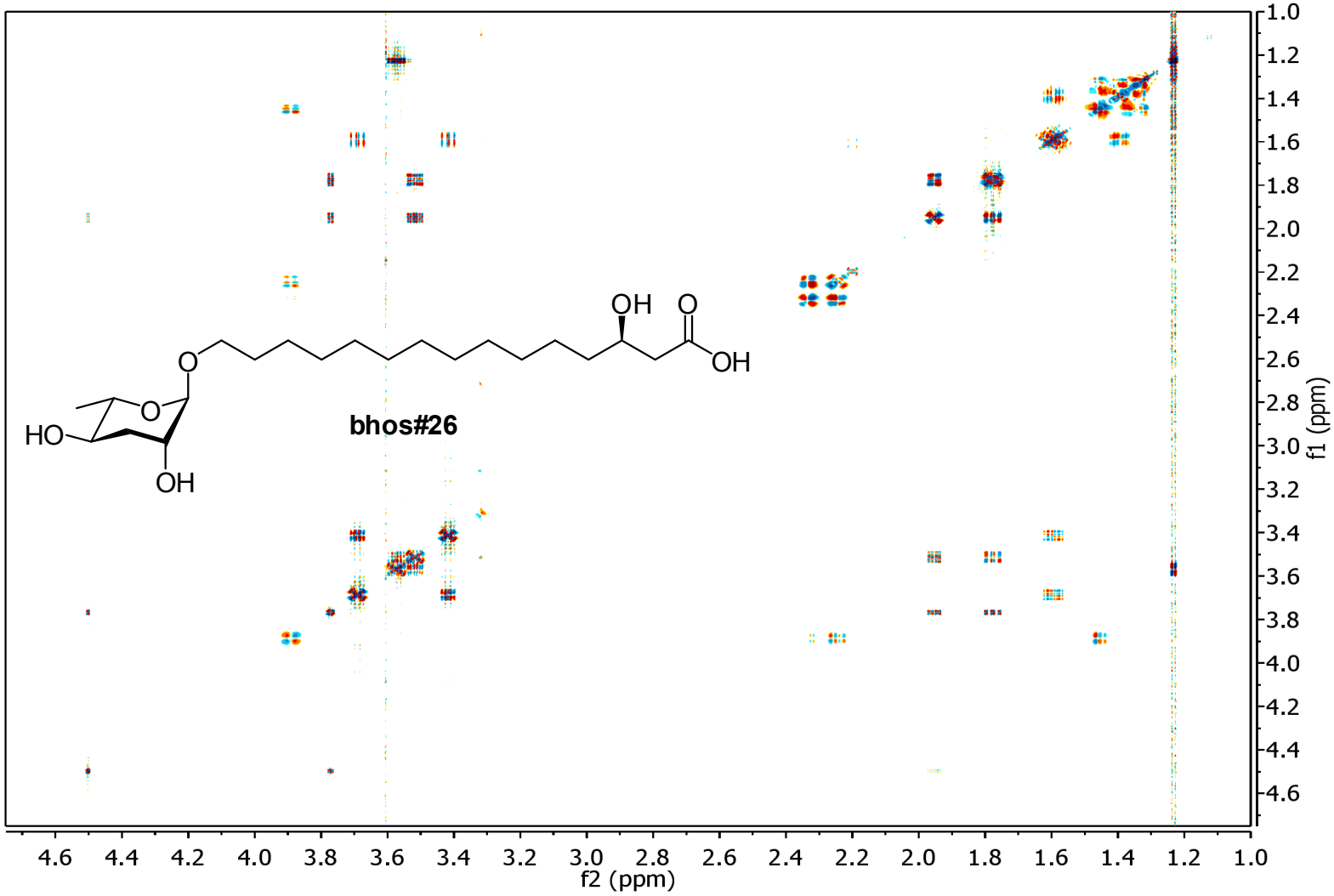
5.16. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of 15-(3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoic acid ethyl ester (**20**)



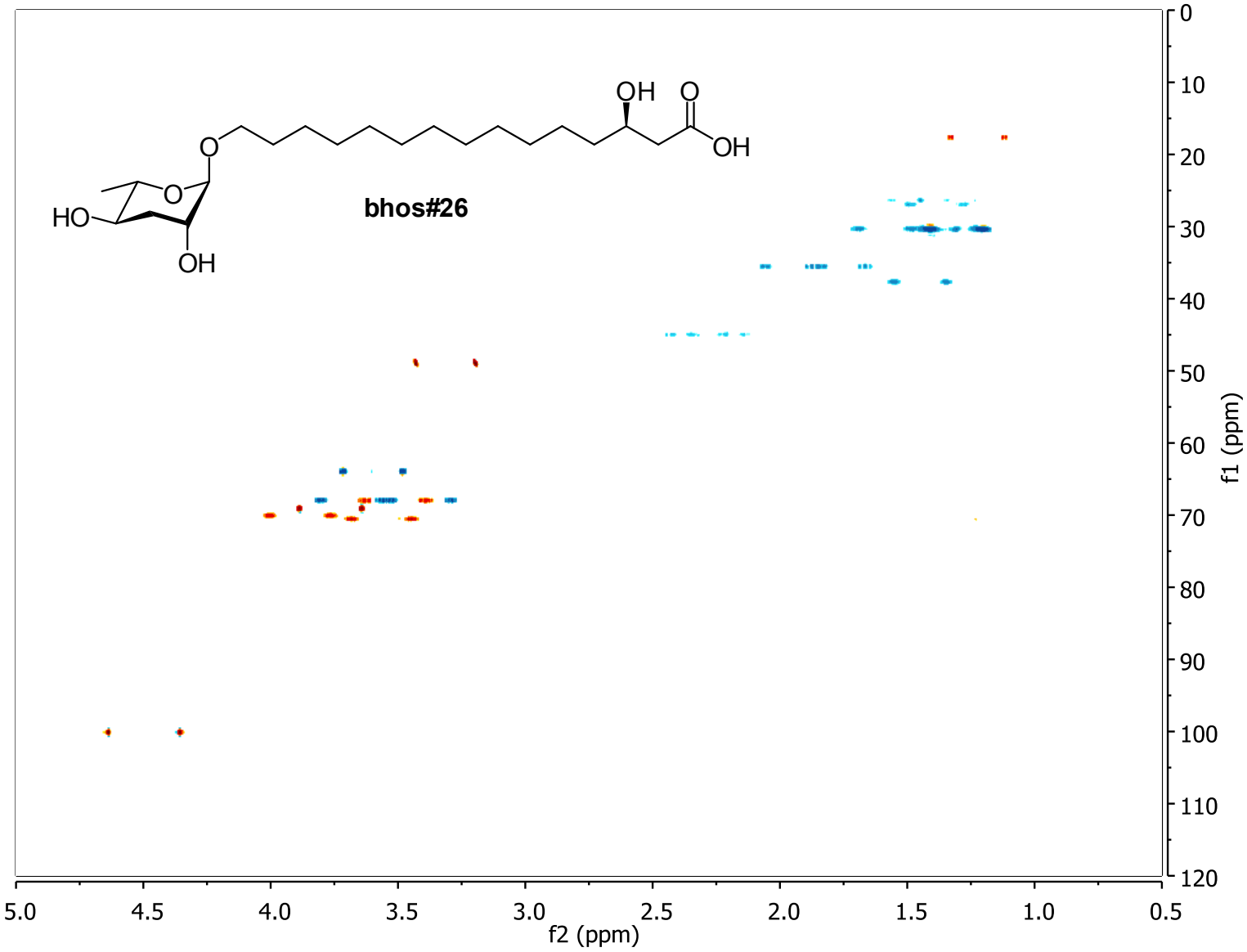
5.17A. ^{1}H NMR Spectrum (400 MHz, methanol-d_4) of 15-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoic acid (**bhos#26**)



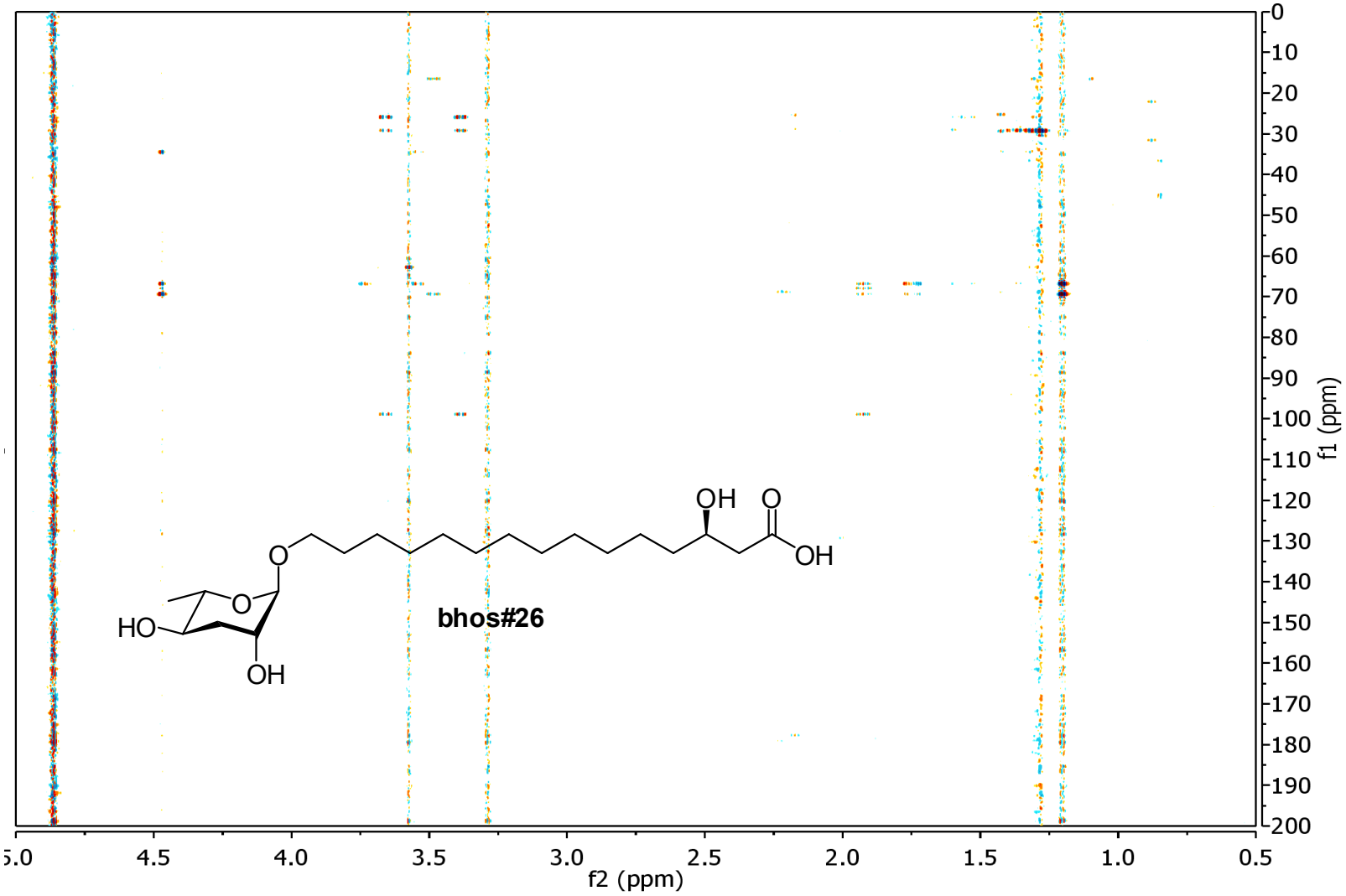
5.17B. dqfCOSY Spectrum (600 MHz, methanol-d_4) of 15-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoic acid (**bhos#26**)



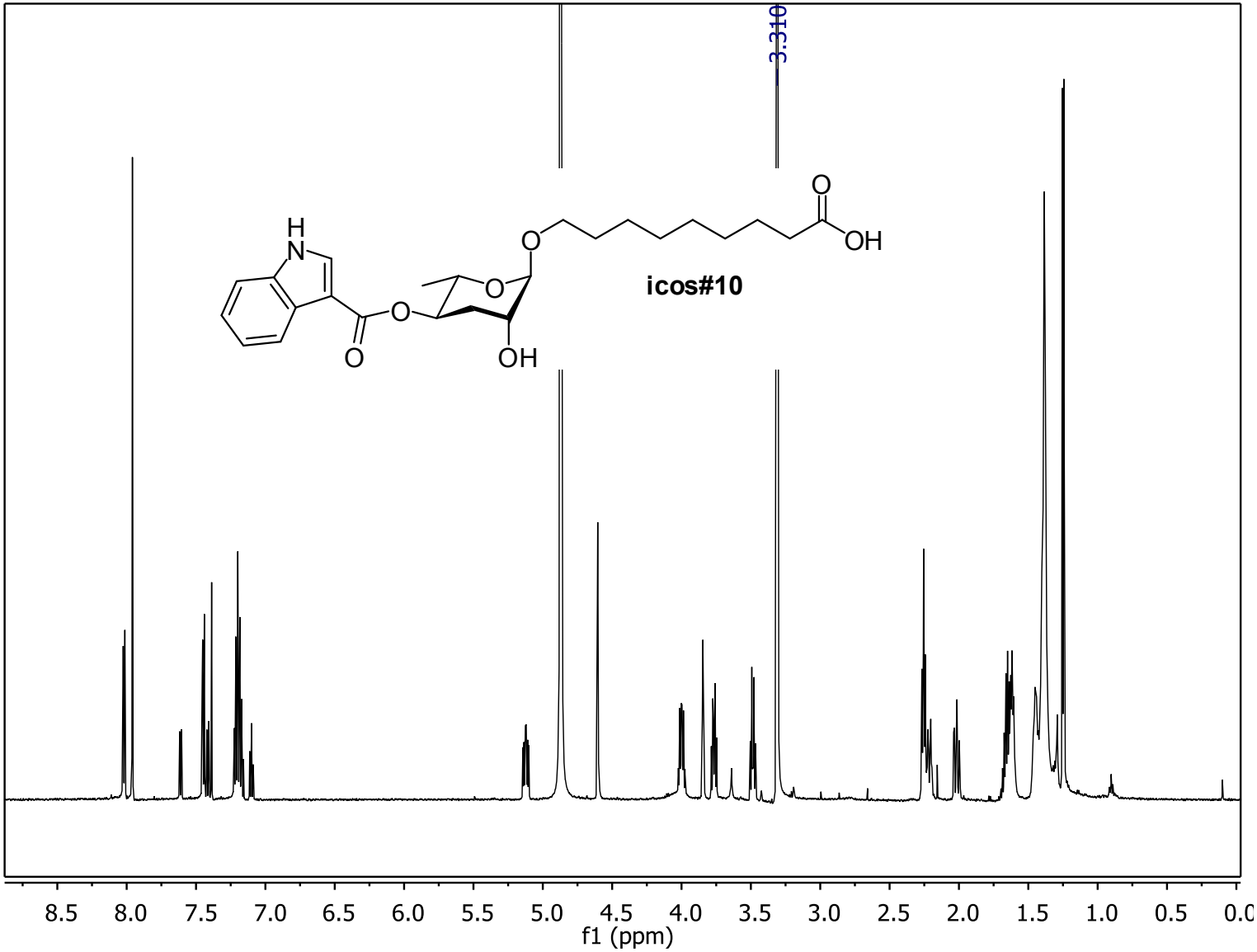
5.17C. HSQC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of 15-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoic acid (**bhos#26**)



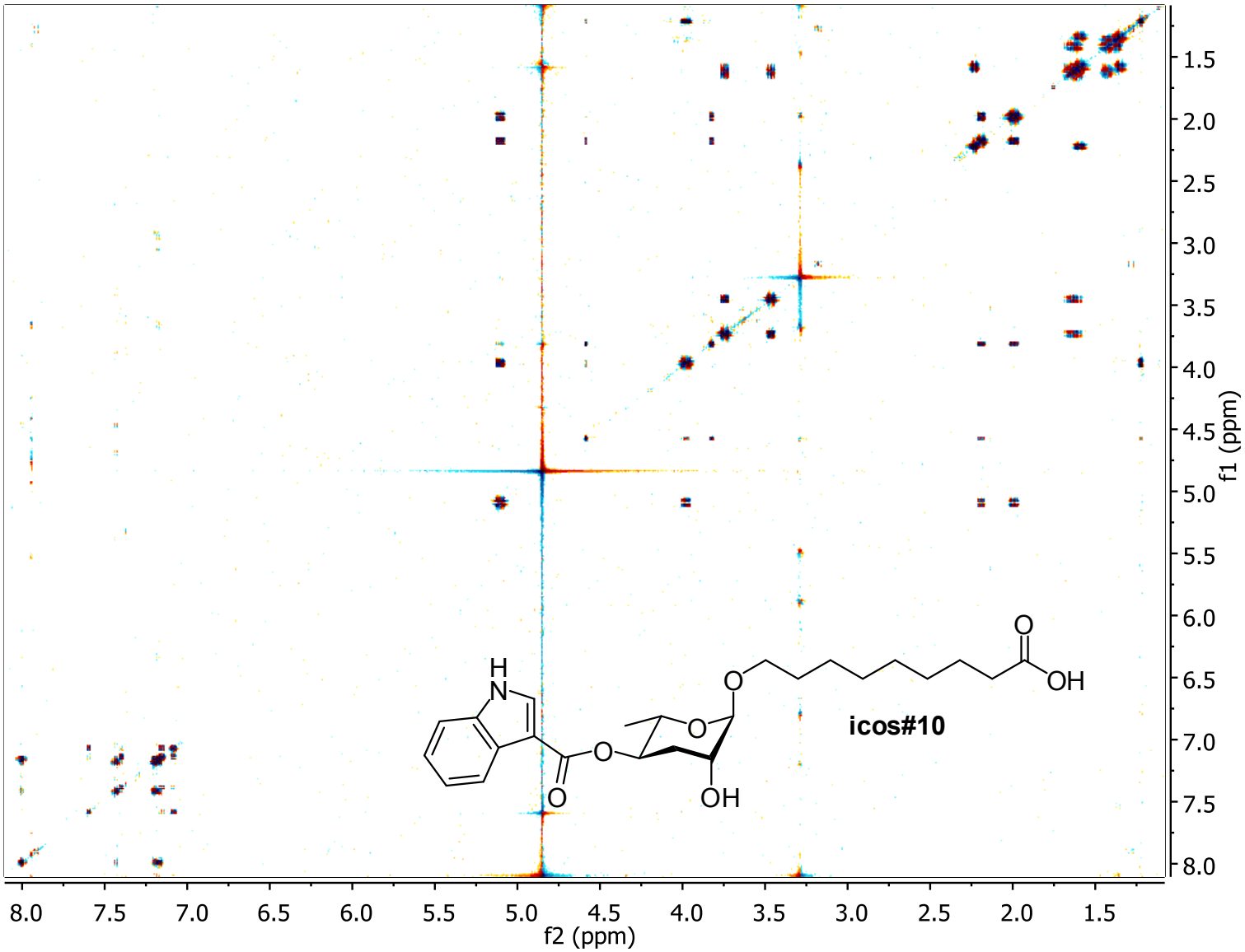
5.17D. HMBC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of 15-(3'*R*,5'*R*-dihydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)-(3*R*)-hydroxypentadecanoic acid (**bhos#26**)



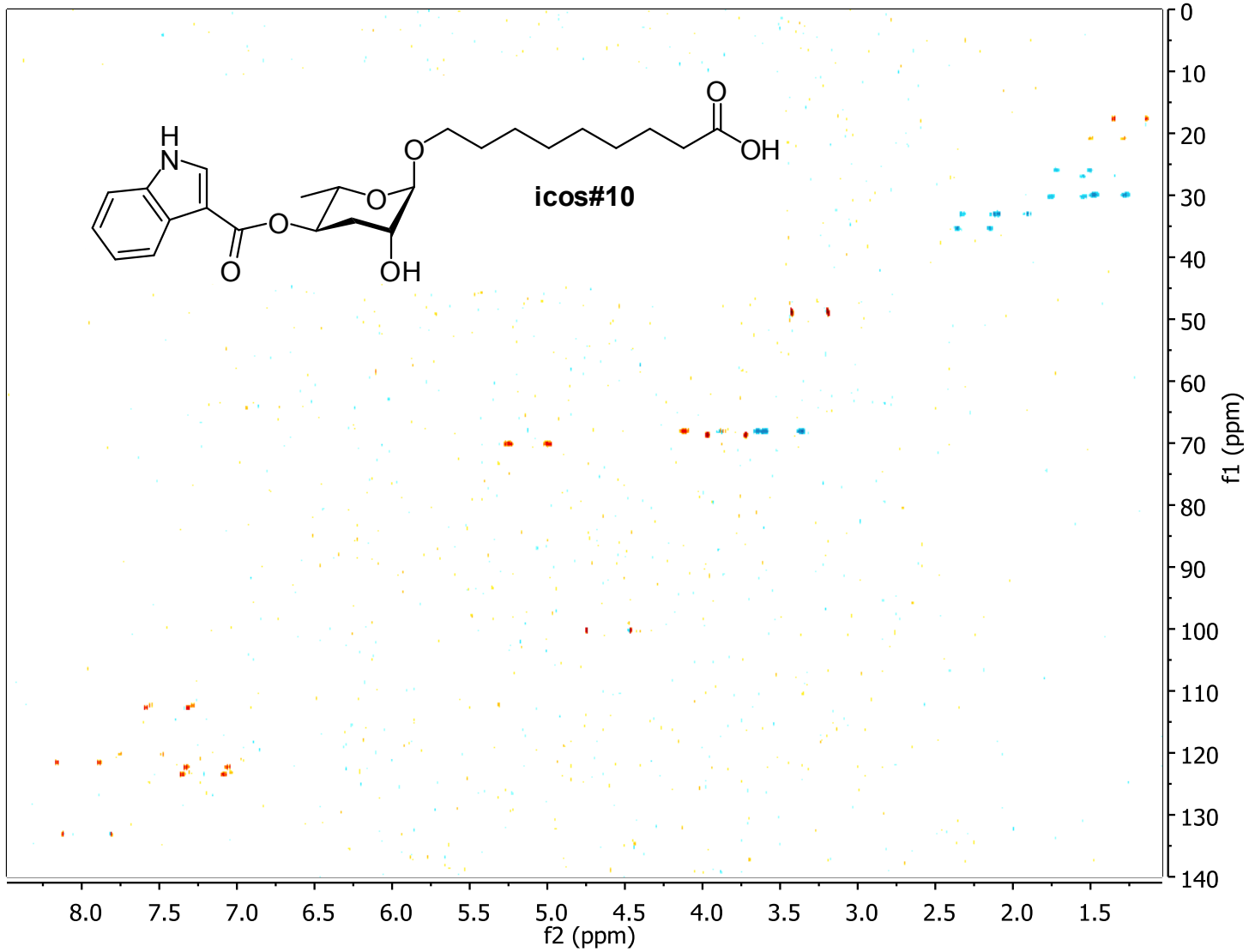
5.18A. ^{1}H NMR Spectrum (600 MHz, methanol-d_4) of 9-(5'*R*-(1*H*-indole-3-carbonyloxy)-3'*R*-hydroxy-6'*S*-methyl-tetrahydro-(2*H*)-pyran-2'-yloxy)nonanoic acid (**icos#10**)



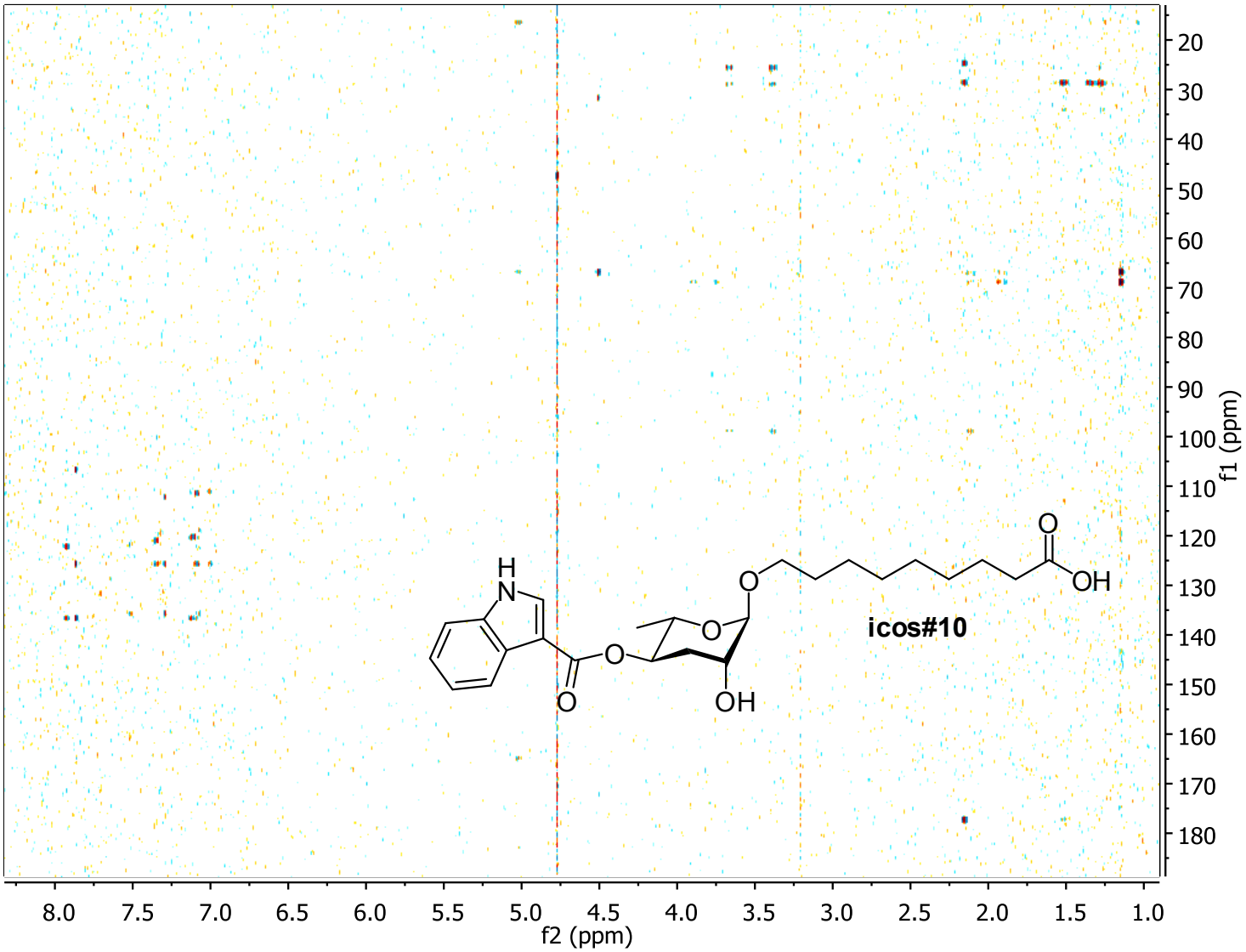
5.18B. dqfCOSY Spectrum (600 MHz, methanol-d_4) of 9-(5'*R*-(1*H*-indole-3-carbonyloxy)-3'*R*-hydroxy-6'*S*-methyl-tetrahydro-(2*H*)-pyran-2'-yloxy)nonanoic acid (**icos#10**)



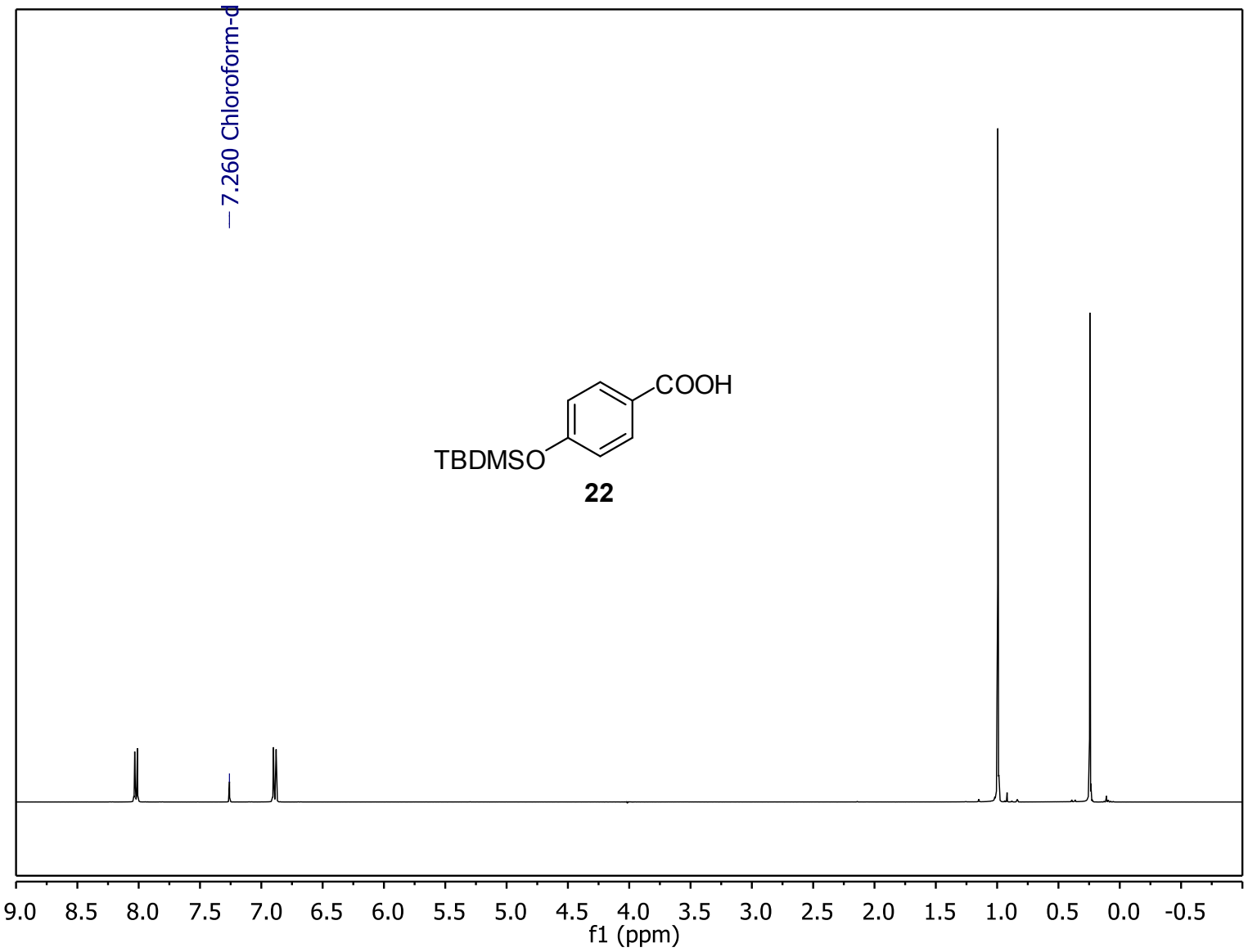
5.18C. HSQC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of 9-(5'*R*-(1*H*-indole-3-carbonyloxy)-3'*R*-hydroxy-6'*S*-methyl-tetrahydro-(2*H*)-pyran-2'-yloxy)nonanoic acid (**icos#10**)



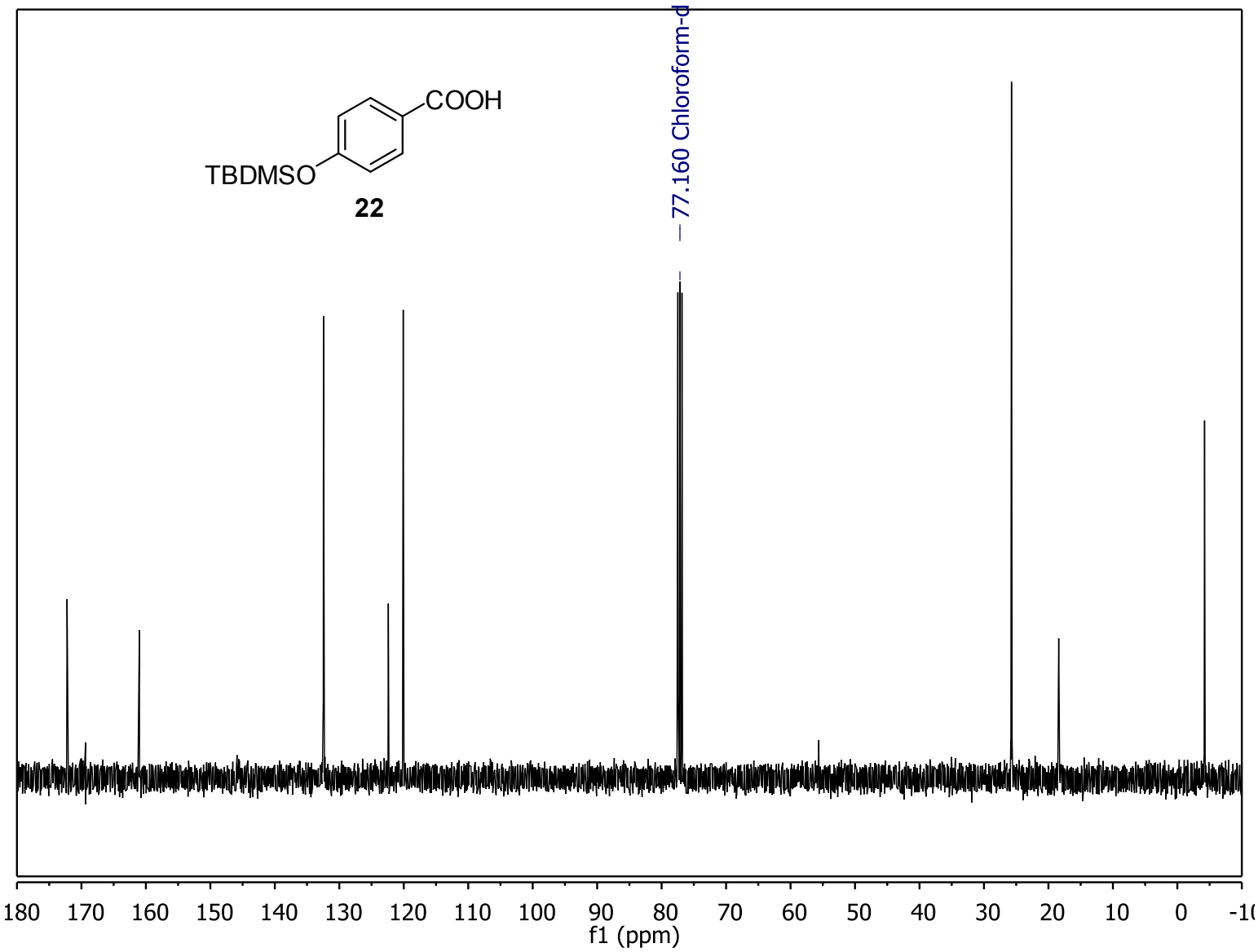
5.18D. HMBC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of 9-(5'*R*-(*1H*-indole-3-carbonyloxy)-3'*R*-hydroxy-6'*S*-methyl-tetrahydro-(*2H*)-pyran-2'-yloxy)nonanoic acid (**icos#10**)



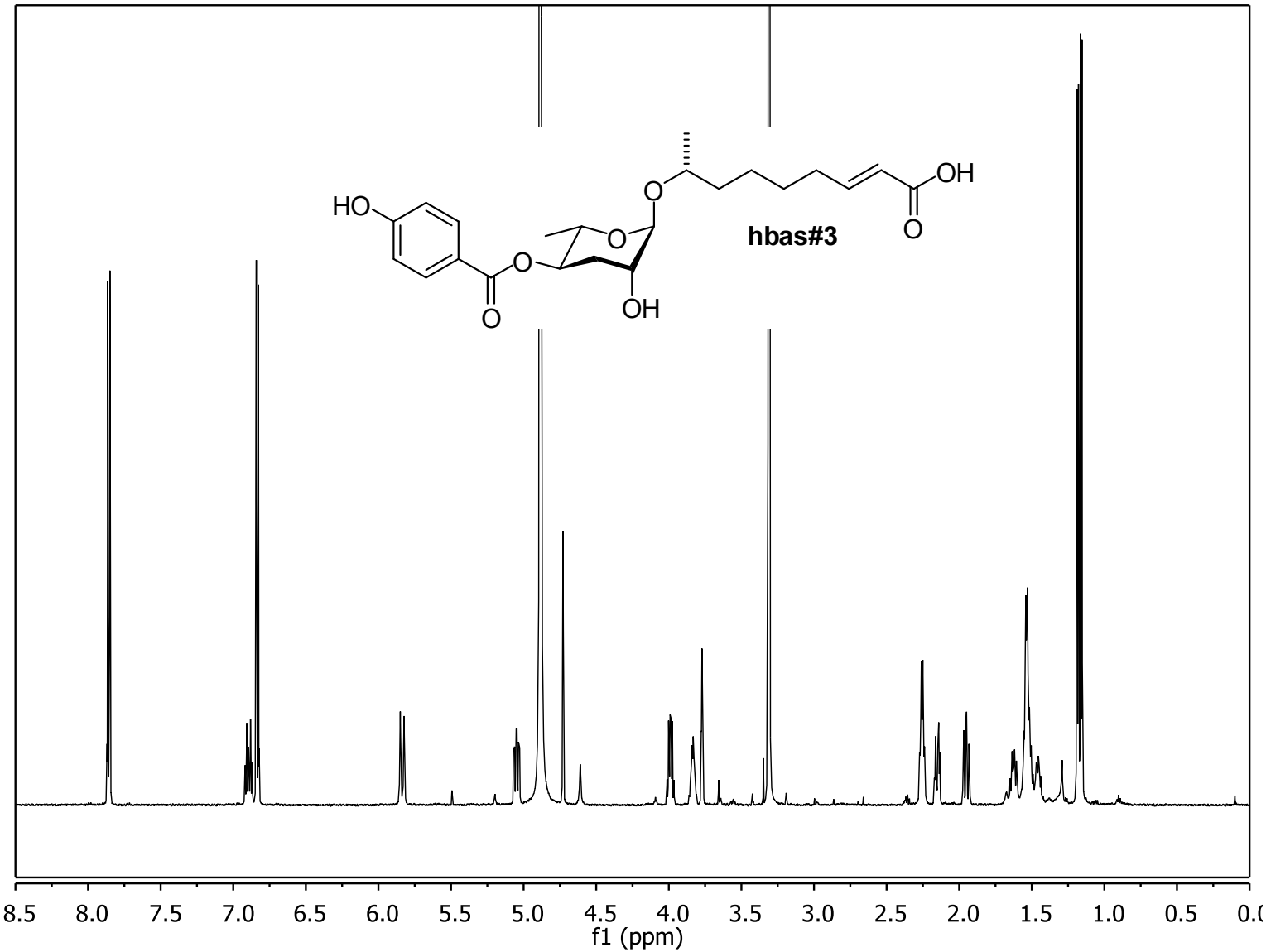
5.19A. ^{1}H NMR Spectrum (400 MHz, chloroform-d_1) of 4-*tert*-butyldimethylsilyloxybenzoic acid (**22**)



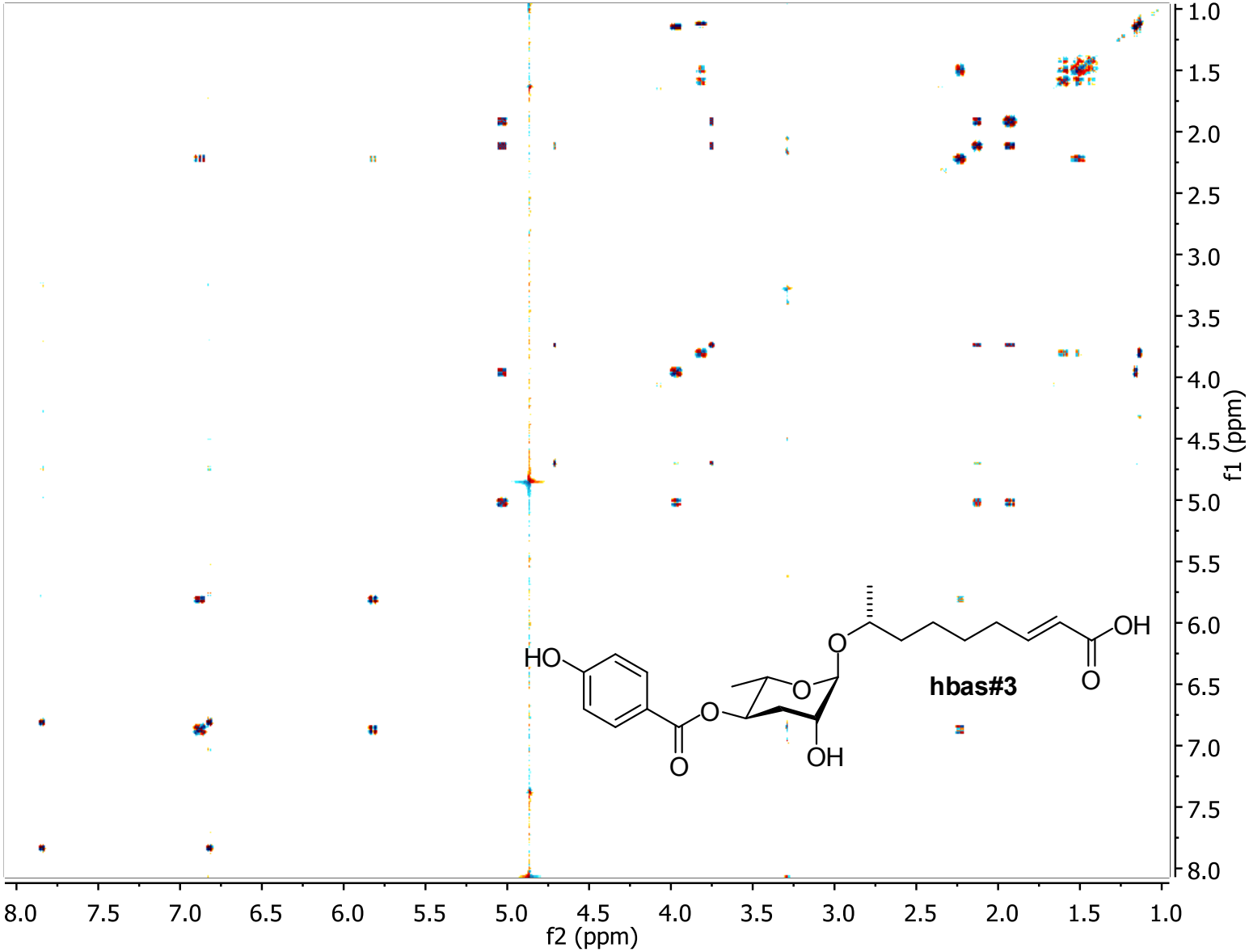
5.19B.^{13}C NMR Spectrum (100 MHz, chloroform-d_1) of 4-*tert*-butyldimethylsilyloxybenzoic acid (**22**)



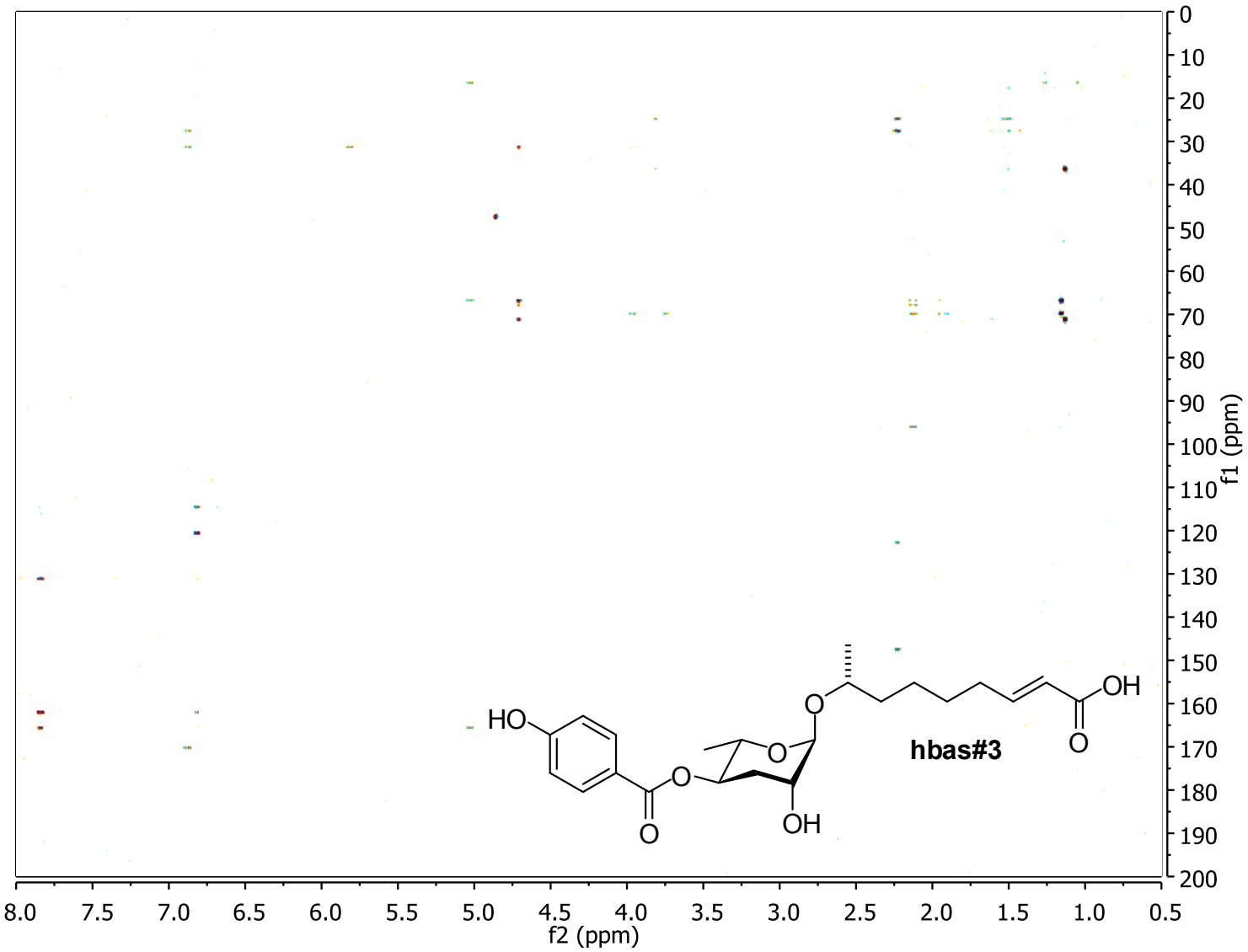
5.20A. ^{1}H NMR Spectrum (600 MHz, methanol-d_4) of (8*R*)-(3'*R*-hydroxy-5'*R*-(4-hydroxybenzoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (**hbas#3**)



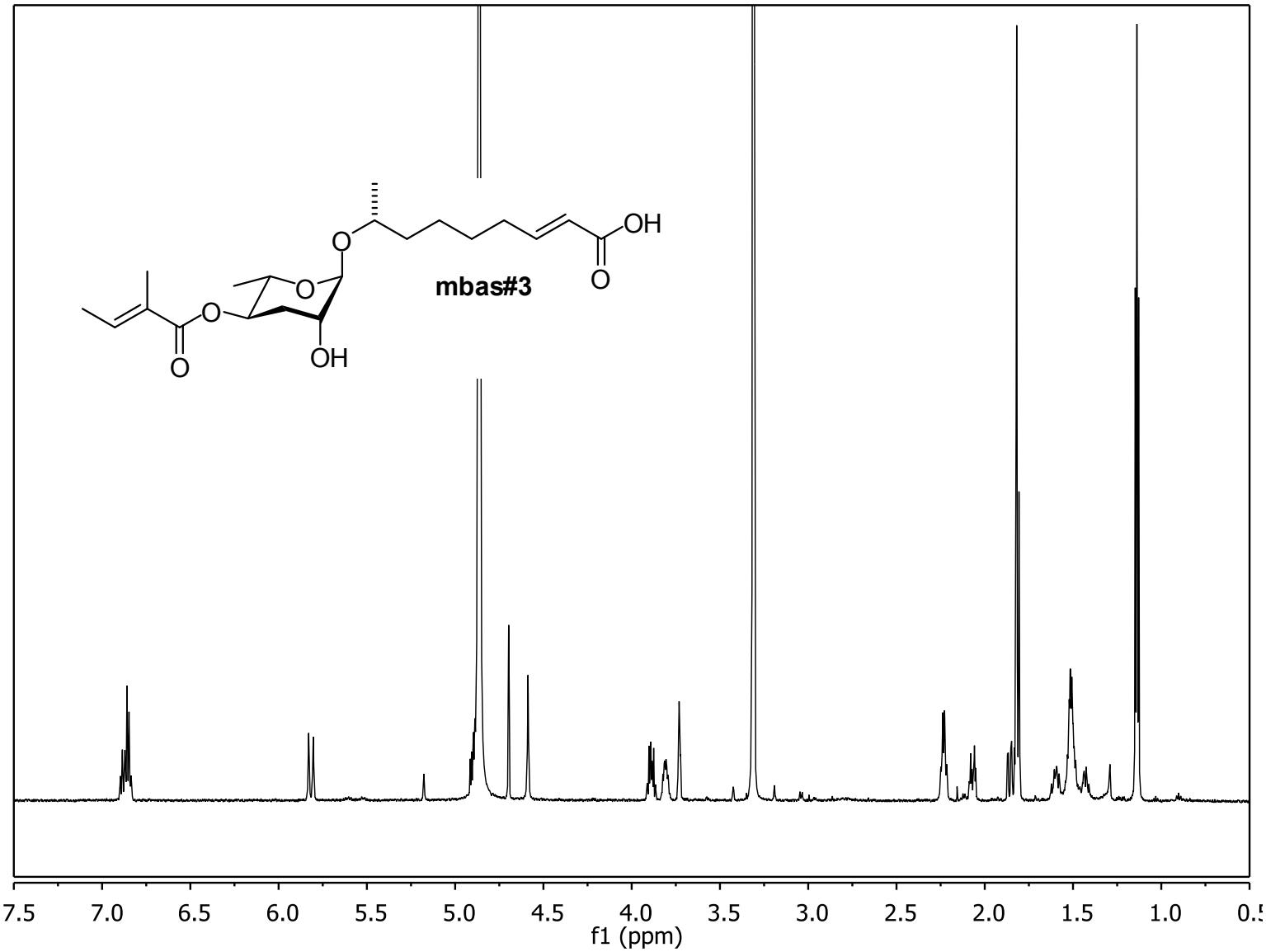
5.20B. dqfCOSY Spectrum (600 MHz, methanol-d_4) of (8*R*)-(3'*R*-hydroxy-5'*R*-(4-hydroxybenzoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (**hbas#3**)



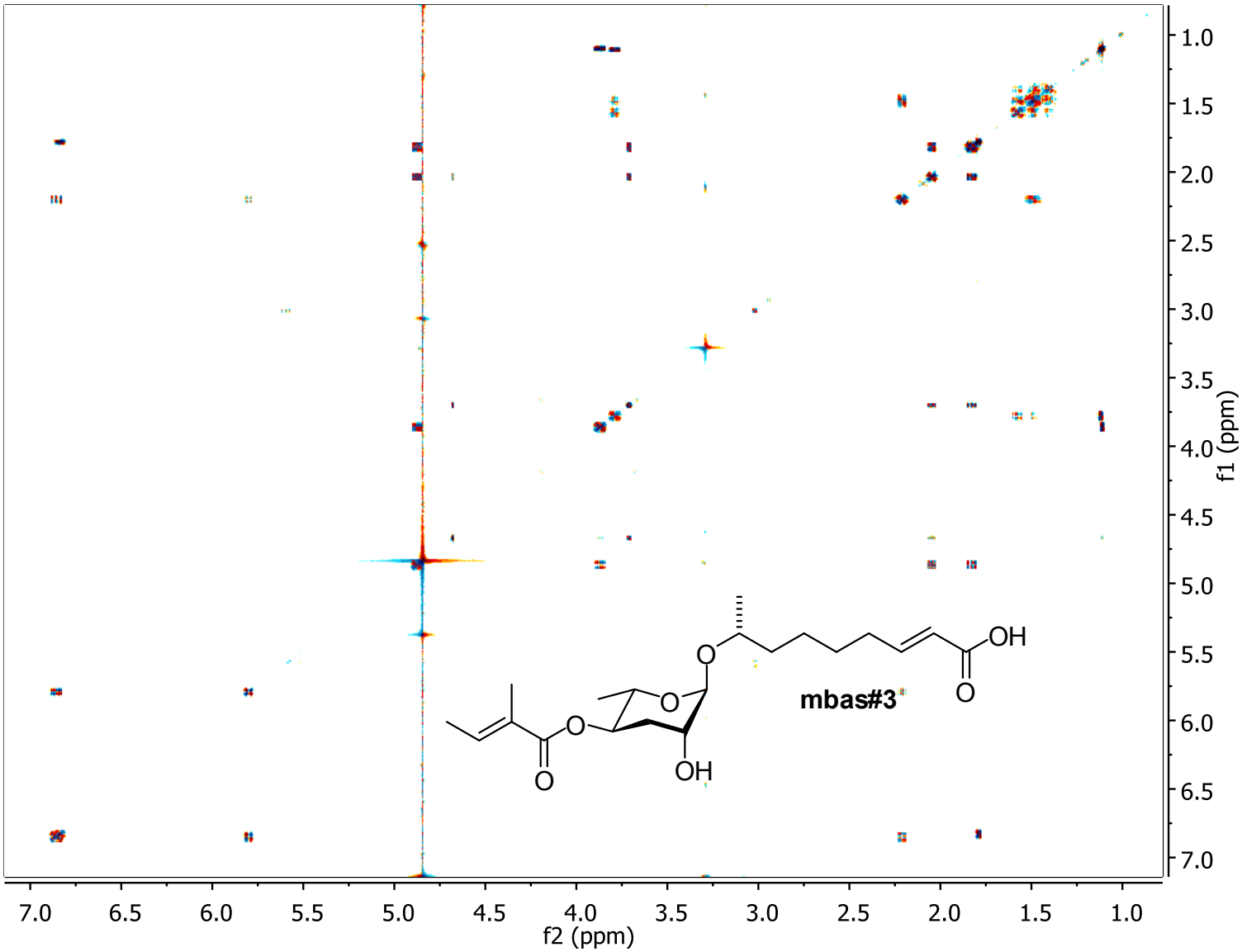
5.20C. HMBC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of (8*R*)-(3'*R*-hydroxy-5'*R*-(4-hydroxybenzoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (**hbas#3**)



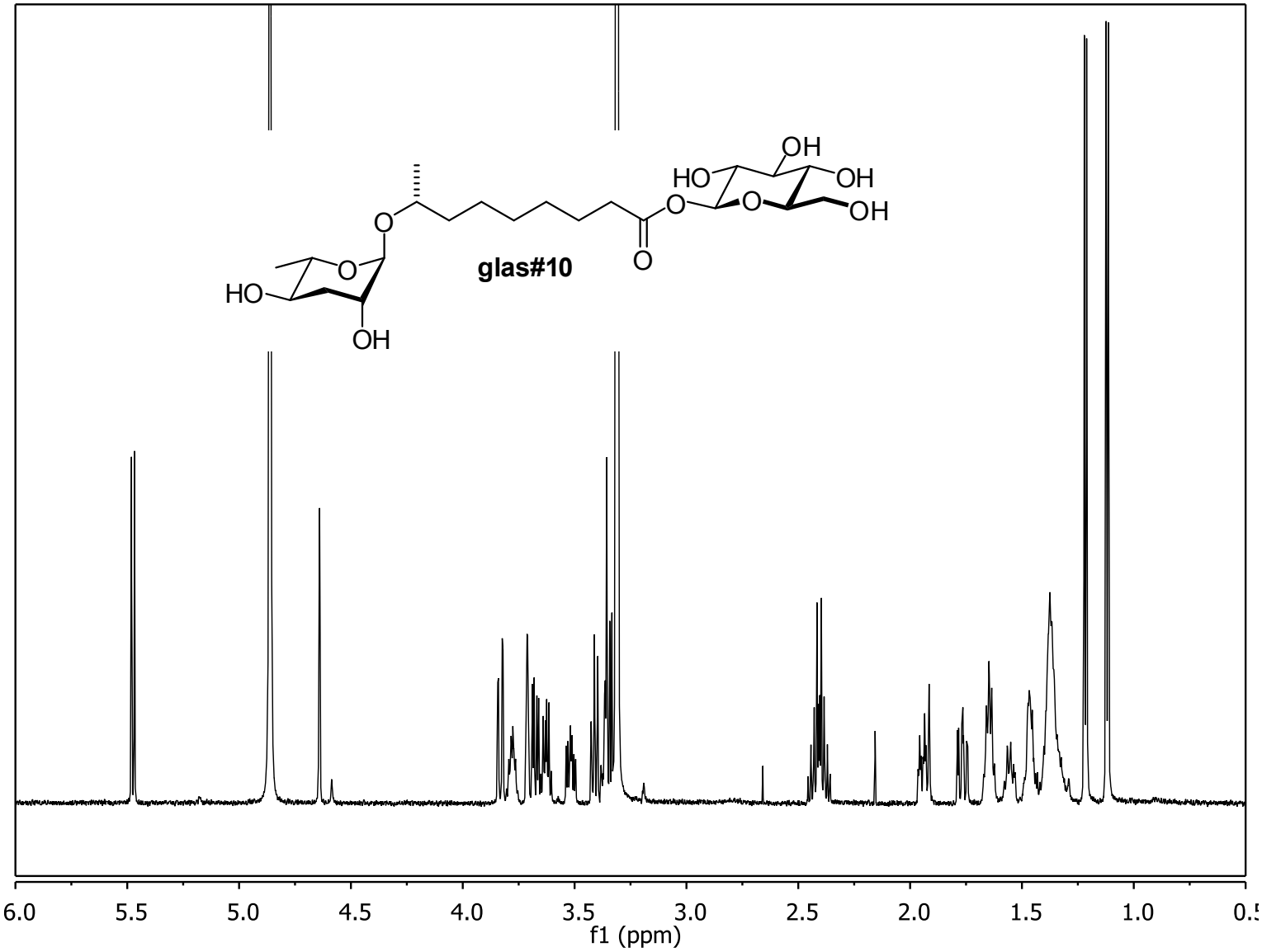
5.21A. ^{1}H NMR Spectrum (600 MHz, methanol-d_4) of (8*R*)-(3'*R*-hydroxy-5'*R*-(*E*)-(2-methylbut-2-enoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (**mbas#3**)



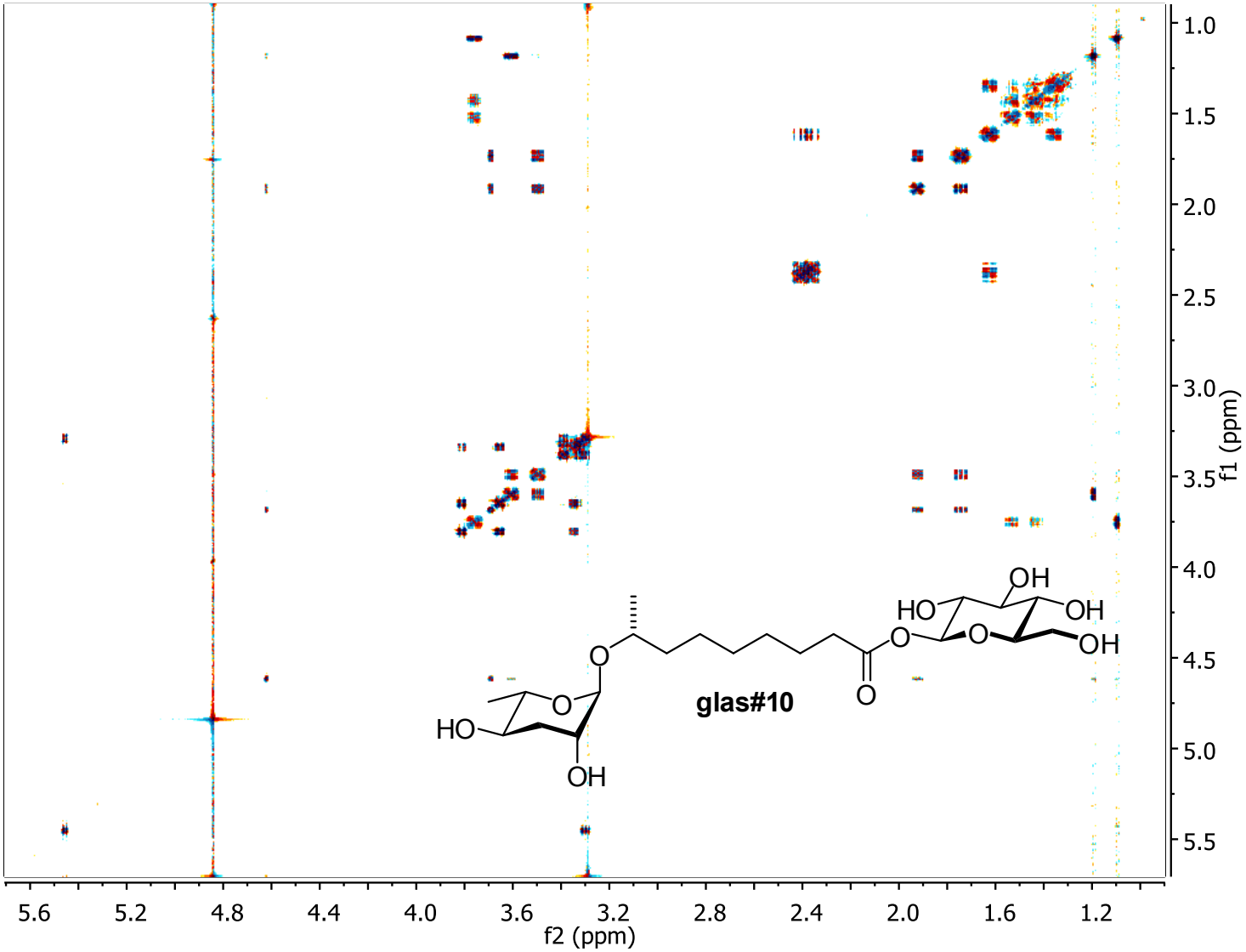
5.21B. dqfCOSY Spectrum (600 MHz, methanol-d_4) of (8*R*)-(3'*R*-hydroxy-5'*R*-(*E*)-(2-methylbut-2-enoyloxy)-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)non-(2*E*)-enoic acid (**mbas#3**)



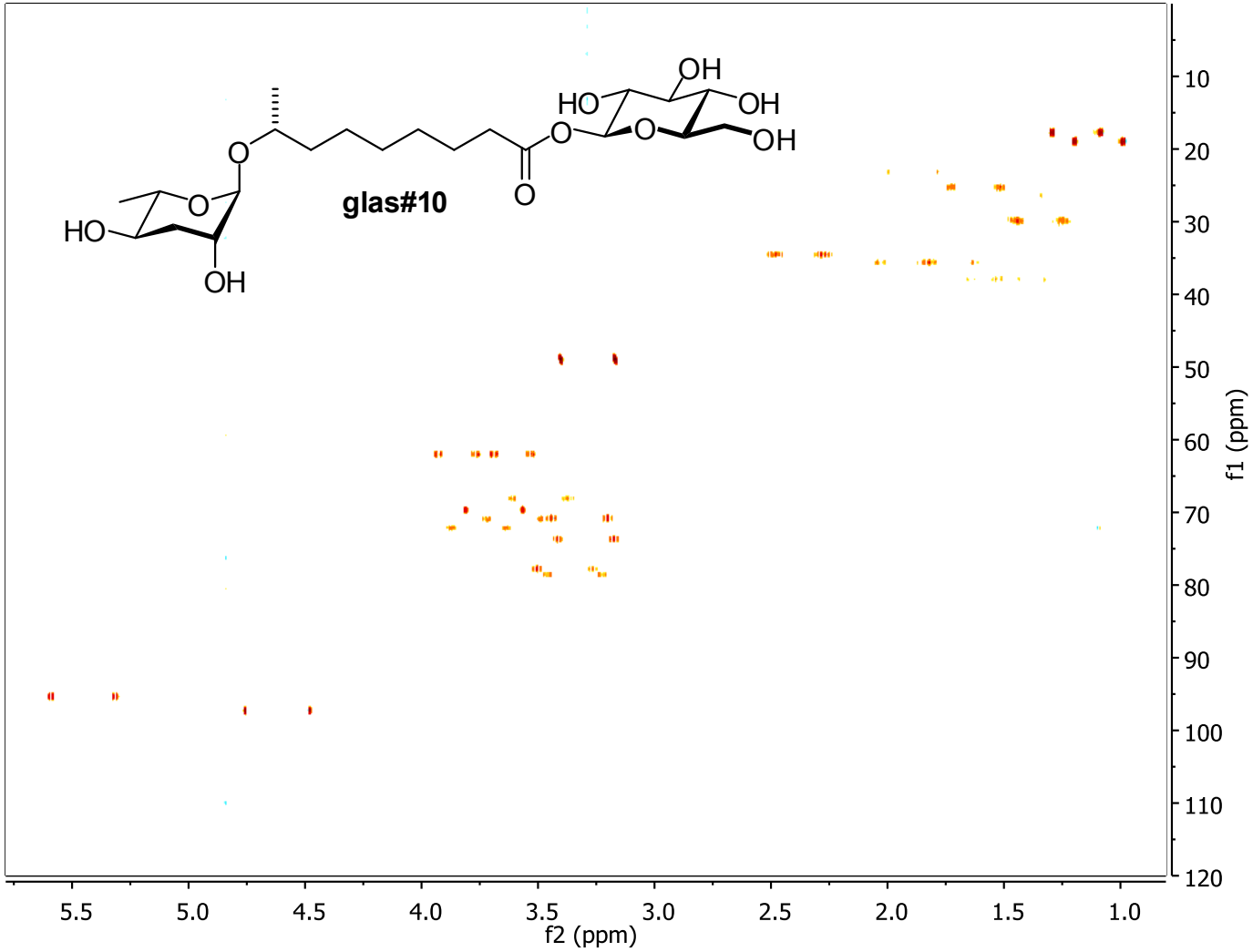
5.22A. ^{1}H NMR Spectrum (600 MHz, methanol-d_4) of 2-(8*R*)-(3'*R*,5'*R*-di-hydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoyl-3,4,5-trihydroxy-6-hydroxymethyl-(2*H*)-tetrahydropyran (**glas#10**)



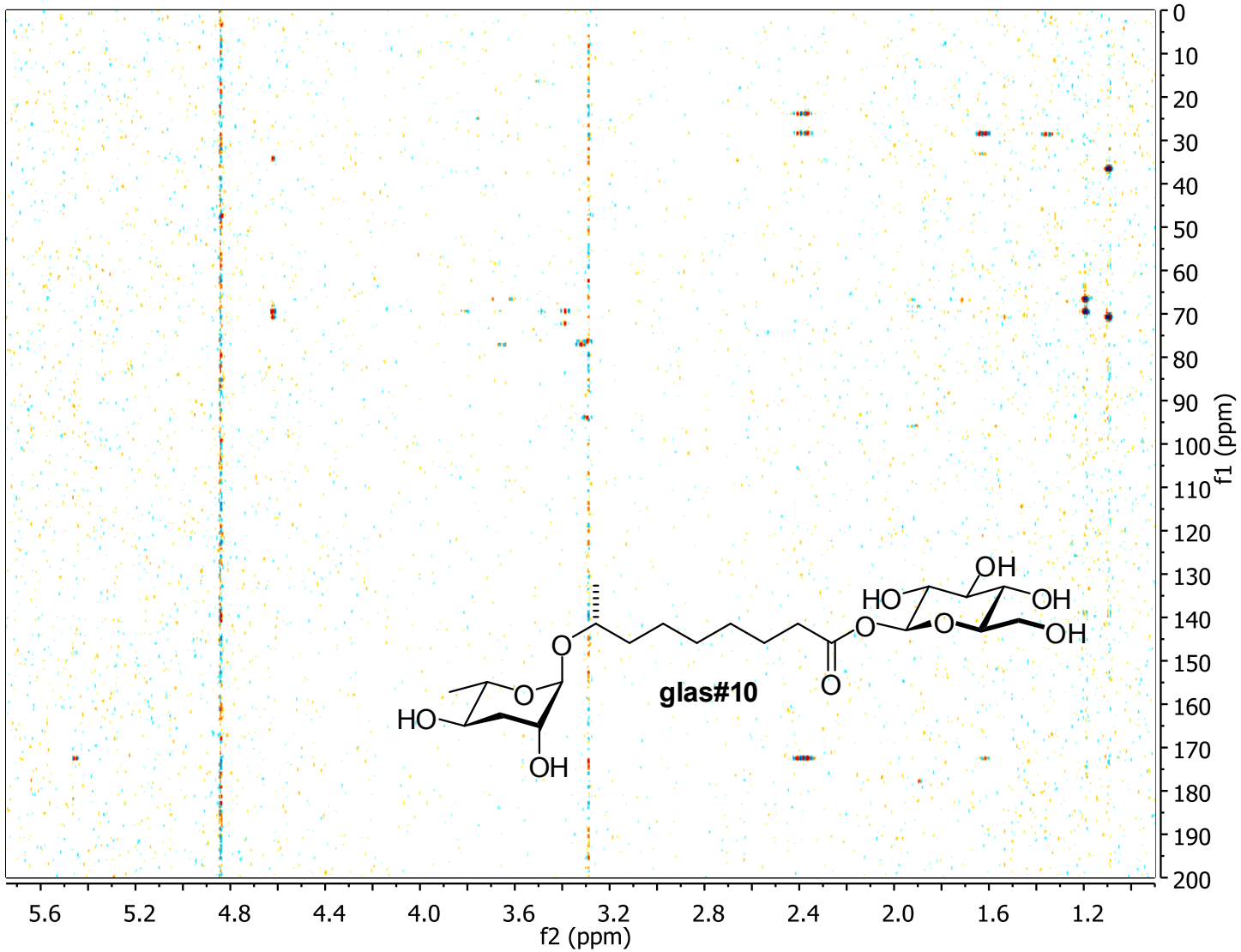
5.22B. dqfCOSY Spectrum (600 MHz, methanol-d_4) of 2-(8*R*)-(3'*R*,5'*R*-di-hydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoyl-3,4,5-trihydroxy-6-hydroxymethyl-(2*H*)- tetrahydropyran (**glas#10**)



5.22C. HMQC Spectrum (600 MHz for ^{1}H, 151 MHz for ^{13}C, methanol-d_4) of 2-(8*R*)-(3'*R*,5'*R*-di-hydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoyl-3,4,5-trihydroxy-6-hydroxymethyl-(2*H*)-tetrahydropyran (**glas#10**)



5.22D. HMBC Spectrum (600 MHz, methanol-d_4) of 2-(8*R*)-(3'*R*,5'*R*-di-hydroxy-6'*S*-methyl-(2*H*)-tetrahydropyran-2'-yloxy)nonanoyl-3,4,5-trihydroxy-6-hydroxymethyl-(2*H*)-tetrahydropyran (**glas#10**)



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