A Comprehensive Study of Molecular Mechanisms on Anti-obesity Effect by the Constituents of Grains of Paradise

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(香辛料 Grains of Paradise 成分の肥満抑制効果と
その分子メカニズムの網羅的解明)

2019

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CONTENTS

OVERVIEW

The cause of death in Japan is largely changing from infections as tuberculosis and pneumonia to lifestyle diseases as cancer, heart disease, and cerebrovascular disease. It's associated with the way a person or group of people lives their life. Among lifestyle disease obesity has especially higher risks that causes several complications. Obesity is generally caused by excessive energy intake more than energy expenditure and the population is increasing year by year. The number of obese people is 710 million in 195 countries at 2015 and it has been two times between 1980 and 2015 (Institute for Health Metrics and Evaluation (IHME), University of Washington, [Global Burden of Diseases Study]) Therefore, development of novel functional materials to ameliorate and/or prevent obesity is necessary. I have studied a spice named Grains of Paradise (GOP) which have long been used in West Africa.

GOP is one of the names given to the dried seeds of the tropical plant *Aframomum melegueta*, which is widely distributed throughout West Africa. The seeds have long been used in folkloric herbal remedies and are known to have, among other properties, antioxidant, antibacterial and antinociceptive activity. However, it is more widely known as a spice and condiment for beer, bread, meats and wines than as a phytomedicine or food. Therefore, the pharmacological action of the plant has not yet been well studied. To discover the novel function of GOP, I subjected GOP extract and the ingredients to animal and measured body weight gain, total cholesterol and triglyceride concentrations in serum and liver of mice. I also tried to isolate compounds in GOP which have high effectiveness on anti-obesity and measured sympathetic nerve activity (SNA) entering brown adipose tissue (BAT) to clarify the anti-obesity mechanism by GOP.

EXPERIMENTS

1 *Extraction, Isolation and Elucidation of Grains of Paradise (GOP) Constituents*

1.1 Introduction

There are large number of anecdotal documents concerning the historical use of spices and herbs for human health benefits.[1] Spices and herbs have been utilized as phytopharmacological medicines, to mask unpleasant tastes and food flavors, and to keep food fresh. Since ancient times, human beings have understood that parts of plants, including the leaves, seeds, and roots, have a pleasant taste, agreeable odors, or medicinal potency; thus, the demand for the plants has increased and they have gradually spread widely throughout various cultures as condiments and herbal medicines.

At present, worldwide interest in the health benefits of spices and herbs is growing. Certain anecdotal information passed down by ancestors has been supported by scientific evidence from research studies of natural product chemistry, biochemistry, and food science. For example, chili pepper extract and the main ingredient capsaicin, which is responsible for the pungency of chili pepper, possess antimicrobial and anti-inflammatory activities[2] and ginger and black pepper extract possess significant antioxidant activity.[3,4] Spices and herbs are rich sources of phytochemicals composed of flavonoids and other phenolic compounds, including carotenoids and sterols.[5–8] Phytochemicals have the potential to exert preventive effects against diseases. Thus, spices not only provide flavor and aroma to food and retard microbial growth, but also provide beneficial effects for human health and prevent certain diseases.[9]

GOP, the dried seed of *Aframomum melegueta*, is the only major spice used by natives of West Africa. GOP was used to flavor spicy wine with cinnamon and ginger during medieval times and as a substitute for pepper in ancient Europe. In the Middle Ages, the seeds, which were initially imported from Africa into Italy, were as popular in Europe as other hot spices, such as cinnamon, cloves, and ginger. At present, GOP is

rarely used as a spice except in veterinary preparations and to flavor traditional liqueurs and vinegars in America. However, it is more widely known as a spice and condiment for beer, bread, meats, and wines than as a phytomedicine or food. Therefore, the pharmacological action of the plant has not yet been well studied. A number of pharmacological studies involving the spice have reported that the pungent principles included in GOP have antifeedant, antiseptic, molluscicidal[10], hepatoprotective[11], and antidiarrheal[12] activities and can induce apoptosis in leukemia (HL-60) cells.[13] In addition to these effective properties, I previously demonstrated that the daily intake of GOP daily decreased body weight gain and hepatic and serum fats in mice.[14] An increase in whole-body energy expenditure and in a decrease visceral fat after ingestion of GOP extract were also reported by Sugita *et al*.[15] To investigate the bioactive ingredients I attempted to isolate and identify the compounds that contributed to obesity prevention.

1.2 Materials and Methods

1.2.1 General Procedures

NMR analysis was performed using a JEOL ECA-600NMR (JEOL, Tokyo, Japan) and a Bruker Biospin AVANCEIII 600 (Bruker corporation, MA, USA) with deuterated solvent signals as internal standards. Matrix-assisted laser desorption ionization TOF-MS (MALDI-TOF-MS) spectra were measured on a Shimadzu AXIMA-Resonance spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with a nitrogen laser (λ = 337 nm). HPLC analysis was performed on a reversed-phase column [Sunniest C18, 5 μm (I.D.) × 250 mm (L)] (ChromaNik Technologies Inc., Osaka, Japan) equipped with an SPD-M20A photodiode array detector (Shimadzu Corporation) to confirm the purity of the isolated compounds. Preparative HPLC, equipped with a 880-PU pump, 875-UV detector, and an Inertsil® ODS-3 column (20

mmφ × 250 mm; GL Sciences, Tokyo, Japan) was conducted to obtain highly purified compounds.

Other commercially available products, including solvents, were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

1.2.2 Plant Material

The dried whole spice of GOP was provided by Share Trade Inc. (Tokyo, Japan).

1.2.3 Extraction and Isolation

Dried GOP powder (approximately 5 kg) was extracted with methanol on a rotary shaker (NR-20, TAITEC, Saitama, Japan) for a whole day at room temperature (20 ± 2 °C). This extraction was repeated three times. The extract was successively extracted with hexane, diethyl ether, ethyl acetate, and methanol, and each solution was concentrated *in vacuo* at 30 °C to obtain each soluble extract. I focused on the hexane soluble extract and performed a separation by silica gel column (75 mm φ × 510 (L) mm) chromatography using a mixture of ethyl acetate-benzene (1:3, v/v) as the eluent and obtained four fractions (HFr.1–4) by monitoring the TLC analysis. The ether soluble extract was also separated by silica gel column (75 mm φ × 525 (L) mm) chromatography using ethyl acetate-benzene (1:3, v/v) as eluents and five fractions were obtained (EFr.1–5) by TLC analysis. Preparative HPLC (JASCO Corporation, Tokyo, Japan) was performed using an Inertsil® ODS-3 column (20 mm φ × 250 mm) with a gradient elution to obtain the isolated and purified compounds. UV spectra were detected with a JASCO 875-UV intelligent UV/VIS Detector (JASCO Corporation) during the preparative HPLC procedure. Compounds **A**–**T** were isolated from HFr.1–4 and EFr.2 and subjected to HPLC analysis for the confirmation of purity following

NMR and MS measurement. The isolated compounds were identified by ^{1}H-, ^{13}C-, and 2D-NMR and MALDI-TOF-MS.

^{1}H- and ^{13}C- NMR spectra were recorded in Methanol-D_4 or Chloroform-D (Wako Pure Chemical Industries, Ltd, Tokyo, Japan) using a JEOL EC600 NMR (JEOL, Tokyo, Japan) or a Bruker Biospin AVANCEIII 600 (Bruker corporation, MA, USA). Coupling constants were expressed in Hz and the chemical shifts were expressed on a δ (ppm) scale. The isolated compounds were mixed with the matrix (2,3-dihydroxybenzoic acid in methanol, 10 mg/mL) and loaded onto a 384-well MALDI sample plate. MALDI-TOF-MS spectra were measured using a Shimadzu AXIMA-Resonance spectrometer (Shimadzu Corporation, Kyoto, Japan). Optical rotations were measured using a JASCO P-2300 system (Easton, MD, USA).

1.2.4 Elucidation of the Compound Structure of GOP

Compound **A**; yellow oil; UV λ_{max} 201, 223, 494, 418, 395 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.87 (3H, m, H-11), 1.26–1.30 (10H, m, H-6–10), 1.57 (2H, m, H-5), 2.22 (2H, m, H-4), 2.56 (2H, t, J = 7.6 Hz, H-2), 2.87 (2H, m, H-1), 3.87 (3H, s, H-1′), 6.68 (1H, m, H-6′), 6.70 (1H, m, H-2′), 6.81 (1H, m, H-5′); ^{13}C-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 150 MHz) δ 15.8 (C-11), 24.3 and 34.4 (C-9 and C-10), 27.3 (C-5) 29.7, 31.6, and 38.4 (C-6, C-7, and C-8), 33.4 (C-1), 40.2 (C-4), 42.5 (C-2), 57.8 (C-12), 112.9 (C-2′), 122.7 (C-6′), 116.2 (C-5′), 134.6 (C-11′), 145.9 (C-4′), 148.3 (C-3′), 195.4 (C-3); MALDI-TOF-MS *m/z* 293.6 $[M+H]^{+}$.

Compound **B**; yellow oil; UV λ_{max} 203, 280, 485, 569, 593 nm; ^{1}H-NMR (Chloroform-D, JEOL EC, 500 MHz) δ 0.87 (3H, m, H-10), 1.24–1.34 (8H, m, H-6–9), 1.56 (2H, m, H-5), 2.24 (1H, t, J = 7.5 Hz, H-2), 2.36 (2H, t, J = 7.5 Hz, H-4), 2.68 (1H, t, J = 7.45 Hz, H-2), 2.83 (2H, m, H-1), 3.85 (3H, s, H-11), 6.65 (1H, br d, J = 8.0 Hz,

H-6′), 6.68 (1H, br s, H-2′), 6.81 (1H, d, *J* = 8.0 Hz, H-5′); ^{13}C-NMR (Chloroform-D, JEOL EC, 125 MHz) δ 14.1 (C-10), 22.7 (C-8), 23.9 (C-5) 29.1–29.3 (C-6 and C-7), 29.6 (C-1), 31.7 (C-9), 43.2 (C-4), 44.7 (C-2), 55.9 (C-11), 111.2 (C-2′), 114.4 (C-5′), 120.8 (C-6′), 133.2 (C-1′), 144.0 (C-4′), 146.5 (C-3′), 210.8 (C-3); MALDI-TOF-MS *m/z* 279.5 $[M+H]^+$.

Compound **C**; yellow oil; $[\alpha]_D^{20}$ −0.46 (*c* 0.29, $CHCl_3$); UV λ_{max} 203, 223, 280, 485, 440 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.86 (3H, t, *J* = 5.7 Hz, H-11), 1.34–1.41 (10H, m, H-6–10), 1.44 (2H, m, H-5), 1.48 (2H, m, H-4), 1.69–1.78 (2H, m, H-2), 2.60–2.73 (2H, m, H-1), 3.62 (1H, m, H-3), 3.87 (3H, s, H-12), 6.69 (1H, m, H-6′), 6.71 (1H, m, H-2′), 6.83 (1H, d, *J* = 7.9 Hz, H-5′); ^{13}C-NMR (Chloroform-D, JEOL EC, 125 MHz) δ 14.2 (C-11), 22.7, 29.4–29.7 (C-6, C-7, C-8, C-9, and C-10), 25.7 (C-5), 31.9 (C-1), 37.7 (C-4), 39.5 (C-2), 56.0 (C-12), 71.5 (C-3), 111.1 (C-2′), 114.3 (C-5′), 121.0 (C-6′), 134.2 (C-1′), 143.8 (C-4′), 146.5 (C-3′); MALDI-TOF-MS *m/z* 294.7 $[M+H]^+$.

Compound **D**; yellow oil; $[\alpha]_D^{20}$ +27.3 (*c* 1.2, $CHCl_3$); UV λ_{max} 201, 227, 280, 422, 443 nm; ^{1}H-NMR (Chloroform-D, JEOL ECA, 500 MHz) δ 0.86 (3H, t, *J* = 6.9 Hz, H-10), 1.27–1.32 (4H, m, H-8 and H-9), 1.36 (2H, m, H-7), 1.45 (2H, m, H-6), 2.50 (2H, m, H-4), 2.70 (2H, t, *J* = 7.4 Hz, H-2), 2.80 (2H, t, *J* = 7.5 Hz, H-1), 3.82 (3H, s, H-11), 4.00 (1H, m, H-5), 6.62 (1H, dd, *J* = 1.8, 8.9 Hz, H-6′), 6.65 (1H, d, *J* = 1.7 Hz, H-2′), 6.78 (1H, d, *J* = 7.5 Hz, H-5′); ^{13}C-NMR (Chloroform-D, JEOL ECA, 125 MHz) δ 14.1 (C-10), 22.7 (C-9), 25.2 (C-7), 29.3 (C-1), 31.8 (C-8), 36.5 (C-6), 45.5 (C-2), 49.4 (C-4), 55.9 (C-11), 67.8 (C-5), 111.2 (C-2′), 114.6 (C-5′), 120.8 (C-6′), 132.7 (C-1′), 144.1 (C-4′), 146.7 (C-3′), 211.6 (C-3); MALDI-TOF-MS *m/z* 294.7 $[M+H]^+$.

Compound **E**; yellowish oil; UV λ_{max} 201, 226, 280, 577 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.88 (3H, t, J = 7.2 Hz, H-10), 1.21–1.31 (6H, m, H-7–9), 1.53 (2H, m, H-6), 1.99 (3H, s, H-13), 2.60 (2H, m, H-4), 2.72 (2H, m, H-2), 2.83 (2H, m, H-1), 3.87 (3H, s, H-11), 5.22 (1H, m, H-5), 6.66 (1H, dd, J = 1.8 and 7.8 Hz, H-6′), 6.69 (1H, d, J = 1.8 Hz, H-2′), 6.82 (1H, d, J = 7.8 Hz, H-5′); ^{13}C-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 150 MHz) δ 15.9 (C-10), 23.0 (C-13), 24.4 (C-9), 26.7 (C-7), 31.2 (C-1), 33.4 (C-8), 36.1 (C-6), 47.1 (C-2), 49.3 (C-4), 57.8 (C-11), 72.3 (C-5), 113.0 (C-2′), 116.2 (C-5′), 122.7 (C-6′), 134.8 (C-1′), 145.8 (C-4′), 148.3 (C-3′), 172.4 (C-12), 209.1 (C-3); MALDI-TOF-MS *m/z* 336.6 $[M+H]^{+}$.

Compound **F**; yellowish oil; $[\alpha]_D^{20}$ +0.67 (c 1.15, $CHCl_3$); UV λ_{max} 201, 226, 280, 413 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.88 (3H, t, J = 7.2 Hz, H-12), 1.26–1.43 (10H, m, H-7–11), 2.41 (2H, dd, J = 4.8 and 16.2 Hz, H-4a), 2.64 (2H, dd, J = 7.8 and 16.2 Hz, H-4b), 2.75 (2H, m, H-2), 2.83 (2H, t, J = 7.8 Hz, H-1), 3.29 (3H, s, H-14), 3.66 (1H, m, H-5), 3.87 (3H, s, H-13), 6.67 (1H, dd, J = 1.8 and 7.8 Hz, H-6′), 6.70 (1H, d, J = 1.8 Hz, H-2′), 6.82 (1H, d, J = 8.4 Hz, H-5′); ^{13}C-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 150 MHz) δ 15.9 (C-12), 24.5, 26.7, 31.6, 32.2 and 33.8 (C-7, C-8, C-9, C-10, and C-11), 31.2 (C-1), 35.7 (C-6), 47.7 (C-2), 49.5 (C-4), 57.8 (C-13), 58.9 (C-14), 78.9 (C-5), 113.0 (C-2′), 116.2 (C-5′), 122.7 (C-6′), 135.0 (C-1′), 145.8 (C-4′), 148.3 (C-3′), 211.0 (C-3); MALDI-TOF-MS *m/z* 336.6 $[M+H]^{+}$.

Compound **G**; yellow oil; UV λ_{max} 201, 226, 280 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.81–1.64 (7H, m, H-8–10), 1.45 (2H, m, H-7), 2.20 (2H, m, H-6), 2.82–2.89 (4H, m, H-1 and H-2), 3.87 (3H, s, H-11), 6.09 (1H, dt, J = 1.8 and 17.4 Hz, H-4), 6.69 (1H, dd, J = 1.8 and 7.7 Hz, H-6′), 6.71 (1H, d, J = 2.4 Hz, H-2′), 6.79–6.84 (2H, m, H-5 and H-5′); ^{13}C-NMR (Chloroform-D, Bruker Biospin

AVANCEIII, 150 MHz) δ 15.9, 24.3, and 33.3 (C-8, C-9, and C-10), 29.7 (C-7) 31.8 (C-1), 34.7 (C-6), 43.9 (C-2), 57.8 (C-11), 113.0 (C-2′), 116.2 (C-5′), 122.7 (C-6′), 132.2 (C-4), 135.2 (C-1′), 145.8 (C-4′), 148.3 (C-3′), 149.8 (C-5), 201.7 (C-3); MALDI-TOF-MS *m/z* 276.5 $[M+H]^+$.

Compound **H**; yellow powder; UV λ_{max} 200, 371, 253, 485, 577 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.91 (3H, t, J = 7.2 Hz, H-10), 1.32–1.35 (4H, m, H-8 and H-9), 1.65 (2H, quint, J = 7.2 Hz, H-7), 2.37 (2H, t, J = 7.2 Hz, H-6), 3.97 (3H, s, H-11), 5.63 (2H, s, H-4), 6.34 (1H, d, J = 15.6 Hz, H-2), 6.92 (1H, d, J = 8.4 Hz, H-5′), 7.02 (1H, d, J = 1.8 Hz, H-2′), 7.08 (1H, dd, J = 1.2 and 7.8 Hz, H-6′), 7.53 (1H, d, J = 15.6 Hz, H-1); ^{13}C-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 150 MHz) δ 15.9 (C-10), 24.4 (C-8), 27.3 (C-9), 33.4 (C-7), 42.0 (C-6), 57.9 (C-11), 102.1 (C-4), 111.4 (C-2′), 116.7 (C-5′), 122.5 (C-2), 124.5 (C-6′), 129.6 (C-1′), 141.8 (C-1), 148.7 (C-4′), 149.6 (C-3′), 180.0 (C-3), 202.1 (C-5); MALDI-TOF-MS *m/z* 291.1 $[M+H]^+$.

Compound **I**; yellowish oil; UV λ_{max} 202, 222, 280, 424, 368 nm; ^{1}H-NMR (CHLOROFORM-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.88 (3H, t, J = 7.2 Hz, H-10), 1.23–1.34 (6H, m, H-7–9), 1.41 (1H, m, H-6a), 1.48 (1H, m, H-6b), 2.41 (1H, dd, J = 4.2 and 16.2 Hz, H-4a), 2.64 (1H, dd, J = 7.8 and 16.2 Hz, H-4b), 2.74 (2H, m, H-2), 2.83 (2H, t, J = 7.8 Hz, H-1), 3.29 (3H, s, H-12), 3.66 (1H, m, H-5), 3.87 (3H, s, H-11), 6.67 (1H, dd, J = 1.8 and 8.4 Hz, H-6′), 6.70 (1H, d, J = 1.8 Hz, H-2′), 6.82 (1H, d, J = 8.4 Hz, H-5′); ^{13}C-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 150 MHz) δ 15.9 (C-10), 24.5 (C-9), 26.7 (C-7), 31.2 (C-1), 33.8 (C-8), 35.7 (C-6), 47.7 (C-2), 49.5 (C-4), 57.8 (C-11), 58.9 (C-12), 78.9 (C-5), 113.0 (C-2′), 116.2 (C-5′), 122.7 (C-6′), 135.0 (C-1′), 145.8 (C-4′), 148.3 (C-3′), 211.0 (C-3); MALDI-TOF-MS *m/z* 308.1 $[M+H]^+$.

Compound **J**; yellow oil; UV λ_{max} 202, 224, 280, 432 nm; ^{1}H-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 600 MHz) δ 0.93 (3H, t, J = 7.2 Hz, H-8), 1.63 (2H, sext, J = 7.2 Hz, H-7), 2.48–2.53 (4H, m, H-2 and H-6), 2.72 (2H, t, J = 7.2 Hz, H-1), 3.88 (3H, s, H-9), 6.11 (1H, dt, J = 1.8 and 15.9 Hz, H-4), 6.67–6.68 (2H, m, H-2′ and H-6′), 6.81–6.86 (2H, m, H-3 and H-5′); ^{13}C-NMR (Chloroform-D, Bruker Biospin AVANCEIII, 150 MHz) δ 15.7 (C-8), 19.6 (C-7), 36.1 (C-1), 36.4 (C-2), 44.0 (C-6), 57.8 (C-9), 112.8 (C-6′), 116.2 (C-5′), 122.8 (C-2′), 132.7 (C-4), 134.6 (C-1′), 145.9 (C-4′), 147.8 (C-3), 148.3 (C-3′), 202.7 (C-5); MALDI-TOF-MS *m/z* 248.4 $[M+H]^{+}$.

Compound **K**; brown oil; UV λ_{max} 201, 223, 494, 418, 395 nm; ^{1}H-NMR (METHANOL-D4, Bruker Biospin AVANCEIII, 600 MHz) δ 2.45–2.49 (2H, m, H-6), 2.62 (2H, t, J = 7.2 Hz, H-7), 2.76–2.79 (2H, m, H-1), 2.82–2.85 (2H, m, H-2), 3.82 (3H, s, H-8), 6.08 (1H, d, J = 16.2 Hz, H-4), 6.49 (1H, dd, J = 1.8, 7.8 Hz, H-6″), 6.59 (1H, dd, J = 1.8, 7.8 Hz, H-6′), 6.62 (1H, d, J = 1.8 Hz, H-2″), 6.66 (1H, d, J = 7.8 Hz, H-5″), 6.68 (1H, d, J = 7.8 Hz, H-5′), 6.75 (1H, d, J = 1.8 Hz, H-2′), 6.87 (1H, dt, J = 7.2, 16.2 Hz, H-5); ^{13}C-NMR (CHLOROFORM-D, Bruker Biospin AVANCEIII, 600 MHz) δ 31.7 (C-1), 35.3 (C-7), 36.1 (C-6), 43.2 (C-2), 56.8 (C-8), 113.6 (C-2′), 116.6 (C-5″), 116.8 (C-5′), 117.0 (C-2″), 121.2 (C-6″), 122.2 (C-6′), 132.1 (C-4), 134.3 (C-1″), 134.5 (C-1′), 145.1 (C-4″), 146.3 (C-4′), 146.7 (C-3″), 149.4 (C-3′), 149.8 (C-5), 203.4 (C-3); MALDI-TOF-MS *m/z* 342.1 $[M+H]^{+}$, 365.2 $[M+Na]^{+}$.

Compound **L**; brown oil; UV λ_{max} 203, 280 nm; ^{1}H-NMR (METHANOL-D4, Bruker Biospin AVANCEIII, 600 MHz) δ 1.50 (4H, m, H-5 and H-6), 2.41 (4H, m, H-4 and H-7), 2.69 (2H, t, J = 6.6 Hz, H-2), 2.75 (2H, t, J = 6.6 Hz, H-1), 3.80 (3H, s, H-8), 6.45 (1H, dd, J = 2.4, 8.4 Hz, H-6″), 6.59 (2H, m, H-6′ and H-2″), 6.65 (1H, d, J = 8.4 Hz, H-5″), 6.68 (1H, d, J = 7.8 Hz, H-5′), 6.74 (1H, d, J = 1.8 Hz, H-2′); ^{13}C-NMR

(METHANOL-D4, Bruker Biospin AVANCEIII, 600 MHz) δ 24.9 and 32.8 (C-5 and C-6), 31.1 (C-1), 36.5 (C-7), 44.2 (C-4), 45.9 (C-2), 56.8 (C-8), 113.6 (C-2′), 116.65 (C-5′), 116.73 (C-5″), 117.0 (C-2″), 121.1 (C-6″), 122.2 (C-6′), 134.6 (C-1′), 135.7 (C-1″), 144.7 (C-4″), 146.2 (C-4′), 146.6 (C-3″), 149.4 (C-3′), 214.1 (C-3); MALDI-TOF-MS *m/z* 344.2 $[M+H]^+$, 367.2 $[M+Na]^+$.

Compound **M**; brownish solid; UV λ_{max} 203, 280, 325 nm; ^{1}H-NMR (METHANOL-D4, JEOL ECA-600, 600 MHz) δ 1.48 (4H, m, H-5 and H-6), 2.39 (4H, m, H-4 and H-7), 2.66 (4H, t, J = 4.8 Hz, H-1 and H-2), 3.76 (3H, s, H-8), 6.26 (1H, d, J = 1.4 Hz, H-6″), 6.28 (1H, d, J = 2.1 Hz, H-2″), 6.43 (1H, dd, J = 2.1, 7.6 Hz, H-6″), 6.55 (1H, d, J = 2.1 Hz, H-2″), 6.62 (1H, d, J = 8.3 Hz, H-5″); ^{13}C-NMR (METHANOL-D4, JEOL ECA-600, 150 MHz) δ 23.0 (C-5), 29.6 (C-1), 30.9 (C-6), 34.6 (C-7), 42.4 (C-4), 44.0 (C-2), 55.2 (C-8), 103.4 (C-2′), 108.4 (C-6′), 114.9 (C-5″), 115.2 (C-2″), 119.3 (C-6″), 132.0 (C-1′ and C-4′), 133.9 (C-1″), 142.8 (C-4″), 144.7 (C-3″), 145.1 (C-5′), 148.3 (C-3′), 212.4 (C-3); MALDI-TOF-MS *m/z* 360.9 $[M+H]^+$.

Compound **O**; yellowish oil; UV λ_{max} 202, 220, 280, 359, 485 nm; ^{1}H-NMR (CHLOROFORM-D, JEOL EC, 600 MHz) δ 0.86 (3H, t, J = 6.84 Hz, H-12), 1.26–1.30 (10H, m, H-7–11), 1.40 (2H, m, H-6), 2.51 (2H, m, H-4), 2.71 (2H, t, J = 7.56 Hz, H-2), 2.82 (2H, t, J = 7.56 Hz, H-1), 3.85 (3H, s, H-13), 4.00 (1H, m, H-5), 6.64 (1H, dd, J = 2.04, 8.22 Hz, H-6′), 6.66 (1H, d, J = 2.04 Hz, H-2′), 6.80 (1H, d, J = 8.22 Hz, H-5′); ^{13}C-NMR (CHLOROFORM-D, JEOL EC, 600 MHz) δ 14.2 (C-12), 22.7 (C-10 or C-11), 25.5 (C-7 or C-8), 29.31 (C-7 or C-8), 29.36 (C-9), 29.6 (C-1), 31.9 (C-10 or C-11), 36.6 (C-6), 45.5 (C-2), 49.4 (C-4), 56.0 (C-13), 67.8 (C-5), 111.1 (C-2′), 114.5

(C-5′), 120.8 (C-6′), 132.7 (C-1′), 144.1 (C-4′), 146.5 (C-3′), 211.5 (C-3); MALDI-TOF-MS *m/z* 322.7 $[M+H]^+$, 345.7 $[M+Na]^+$.

Compound **P**; brown oil; UV λ_{max} (202, 222, 278 nm; ^{1}H-NMR (CHLOROFORM-D, JEOL ECA-600, 600 MHz) δ 1.49–1.60 (4H, m, H-5 and H-6), 2.38 (2H, t, J = 6.9 Hz, H-4), 2.51 (2H, t, J = 7.6 Hz, H-7), 2.67 (2H, t, J = 7.6 Hz, H-2), 2.80 (2H, t, J = 7.6 Hz, H-1), 3.83 (3H, s, H-8), 6.64 (1H, dd, J = 1.3, 8.3 Hz, H-6′), 6.66 (1H, d, J = 2.0 Hz, H-2′), 6.73 (2H, d, J = 8.2 Hz, H-3″ and H-5″), 6.81 (1H, d, J = 8.3 Hz, H-5′), 6.99 (2H, d, J = 8.2 Hz, H-2″ and H-6″); ^{13}C-NMR (CHLOROFORM-D, JEOL ECA-600, 150 MHz) δ 23.4 (C-5),29.6 (C-1), 31.3 (C-6), 34.9 (C-7), 43.0 (C-4), 44.7 (C-2), 55.9 (C-8), 111.2 (C-2′), 114.4 (C-5′), 115.2 (C-3″ or C-5″), 120.8 (C-6′), 129.5 (C-2″ or C-6″), 133.1 (C-1′), 134.3 (C-1″), 143.9 (C-4′), 146.5 (C-3′), 153.8 (C-4″), 210.0 (C-3); MALDI-TOF-MS *m/z* 328.6 $[M+H]^+$, 351.7 $[M+Na]^+$.

Compound **Q**; yellowish oil; UV λ_{max} 202, 220, 280, 359, 485 nm; ^{1}H-NMR (CHLOROFORM-D, JEOL EC, 600 MHz) δ 0.87 (3H, t, J = 6.9 Hz, H-10), 1.40 (2H, m, H-6), 1.21–1.30 (6H, m, H-7–9), 2.51 (1H, m, H-4), 2.73 (2H, t, J = 7.6 Hz, H-2), 2.84 (2H, t, J = 7.6 Hz, H-1), 3.83 (3H, s, H-11), 3.85 (3H, s, H-12), 4.00 (1H, m, H-5), 6.68 (1H, s, H-2′), 6.71 (1H, bd, J = 10.3 Hz, H-6′), 6.77 (1H, d, J = 8.9 Hz, H-5′); ^{13}C-NMR (CHLOROFORM-D, JEOL EC, 600 MHz): δ 14.1 (C-10), 22.7 (C-9), 25.2 (C-7), 29.3 (C-1), 31.8 (C-8), 36.5 (C-6), 45.4 (C-2), 49.4 (C-4), 55.9 (C-12), 56.0 (C-11), 67.7 (C-5), 111.4 (C-5′), 111.7 (C-2′), 120.1 (C-6′), 133.4 (C-1′), 147.5 (C-4′), 149.0 (C-3′), 211.5 (C-3); MALDI-TOF-MS *m/z* 322.7 $[M+H]^+$, 345.7 $[M+Na]^+$.

Compound **R**; brown oil; UV λ_{max} 219, 278, 370, 348, 321nm; ^{1}H-NMR (CHLOROFOLM-D, JEOL ECA-600, 600 MHz) δ 1.26 (2H, m, H-5), 1.47 (2H, m, H-6), 1.52 (2H, t, J = 7.6 Hz, H-4), 1.76 (2H, m, H-2), 1.96 (3H, s, H-9), 2.40 (2H, t, J = 7.6 Hz, H-7), 2.46 (2H, m, H-1), 3.76 (3H, s, H-10), 4.83 (1H, m, H-3), 6.26 (2H, s, H-2′ and H-6′), 6.43 (1H, dd, J = 2.0, 8.2 Hz, H-6″), 6.57 (1H, d, J = 2.0 Hz, H-2″), 6.63 (1H, d, J = 8.2 Hz, H-5″); ^{13}C-NMR (CHLOROFOLM-D, JEOL ECA-600, 150 MHz) δ 19.8 (C-9), 24.3 (C-5), 31.1 (C-6), 31.3 (C-1), 33.6 (C-4), 34.7 (C-7), 35.7 (C-2), 53.3 (C-10), 73.9 (C-3), 103.5 (C-2′ or C-6′), 108.5 (C-2′ or C-6′), 114.9 (C-5″), 115.2 (C-2″), 119.4 (C-6″), 131.9 (C-3′ or C-5′), 132.5 (C-1′), 134.1 (C-1″), 142.8 (C-4″), 144.7 (C-3″), 145.1 (C-3′ or C-5′), 148.2 (C-4′), 171.7 (C-8); MALDI-TOF-MS *m/z* 404.9 $[M+H]^{+}$, 427.9 $[M+Na]^{+}$.

Compound **S**; white solid; UV λ_{max} (205, 220, 279, 322, 391 nm; ^{1}H-NMR (METHANOL-D4, JEOL EC, 600 MHz) δ 6.77 (1H, d, J = 2.0 Hz, C2-H), 7.40 (1H, dd, J = 2.1, 8.2 Hz, C6-H), 7.41 (1H, d, J = 7.6 Hz, C5-H); ^{13}C-NMR (METHANOL-D4, JEOL EC, 150 MHz) δ 114.4 (C-5), 116.4 (C-2), 121.9 (C-1), 122.5 (C-6), 144.7 (C-3), 150.2 (C-4), 169.0 (C-7); MALDI-TOF-MS *m/z* 154.5 $[M+H]^{+}$, 177.5 $[M+Na]^{+}$.

Compound **T**; yellow oil; UV λ_{max} (205, 220, 279, 322, 391 nm; ^{1}H-NMR (METHANOL-D4, JEOL EC, 600 MHz) δ 0.88 (3H, t, J = 6.9 Hz, H-10), 1.22–1.33 (6H, m, H-7–9), 1.41 (2H, m, H-6), 1.49 (2H, quin, J = 4.1 Hz, H-4), 1.69 (2H, m, H-2), 2.60 (2H, m, H-1), 3.76–3.83 (2H, m, H-3 and H-5), 3.79 (3H, s, H-11), 6.60 (1H, dd, J = 2.0, 8.3 Hz, H-6′), 6.67 (1H, d, J = 8.2 Hz, H-5′), 6.74 (1H, d, J = 1.3 Hz, H-2′); ^{13}C-NMR (METHANOL-D4, JEOL EC, 150 MHz) δ 13.2 (C-10), 22.4 (C-9), 25.2 (C-7), 31.3 (C-1), 31.8 (C-8), 37.8 (C-6), 40.1 (C-2), 44.3 (C-4), 55.1 (C-11), 67.4 (C-3), 68.1 (C-5),

111.9 (C-2′), 114.8 (C-5′), 120.5 (C-6′), 140.0 (C-1′), 144.1 (C-4′), 147.5 (C-3′); MALDI-TOF-MS *m/z* 319.8 $[M+Na]^+$.

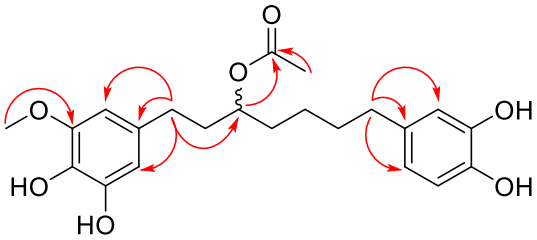
1.3 Results and Discussion

Compounds **C**, **J**, and **R** were identified as novel compounds. According to the NMR data of Compound **C**, the signals at 6.69, 6.71, and 6.83 ppm corresponded to aromatic ring protons related to the ortho, meta, and ortho-meta positions because of their chemical shift, coupling constant, and ^1H-^1H correlation spectroscopy (COSY). The two protons detected at 3.62 ppm as a multiplet and at 3.87 ppm as a singlet indicated the methine and methyl proton, respectively, which were exceedingly shifted to a low magnetic field and the correlations were observed from the hetero-nuclear multiple-bond correlation (HMBC). Moreover, after the consideration of the hetero nuclear multiple quantum coherence (HMQC), COSY, and MS spectra, Compound **C** was determined as a novel vanilloid, 1-(4′-hydroxy-3′-methoxyphenyl)-decan-3-ol.

According to the NMR data of Compound **J**, the signals at 6.11, 6.67–6.68, and 6.81–6.86 ppm corresponded to aromatic ring protons related to the ortho, meta, ortho-meta, and trans-alkene protons owing to their chemical shift, coupling constant, and COSY correlations. The proton detected at 3.88 ppm as a singlet and the carbon detected at 202.7 ppm indicated the methine proton significantly shifted to a lower magnetic field and the carbonyl carbon, respectively. The correlations observed in HMBC are shown in Figure. Moreover, in considering the HMQC, COSY, and MS spectra, Compound **J** was determined as a novel vanilloid, 1-(4′-hydroxy-3′-methoxyphenyl)-3-octen-5-one.

Compound **R** was isolated as a brown oil and exhibited an adduct ion at *m/z* 427.9 $[M+Na]^+$ by MALDI-TOF-MS, corresponding to $C_{22}H_{28}O_7Na$. The ^1H NMR indicated 6 methylene signals at δ_H 1.26 (H-5), 1.47 (H-6), 1.52 (H-4), 1.76 (H-2), 2.40

(H-7), 2.46 (H-1), and 4.83 (H-3), two methyl signals; acetate methyl at δ_H 1.96 (H-9) and methoxyl methyl at δ_H 3.76 (H-10), and 5 aromatic signals at δ_H 6.26 (s, H-2′ and H-6′), 6.43 (H-6″), 6.57 (H-2″), and 6.63 (H-5″). The ^{13}C NMR spectrum desplayed one ester carbon above 170 ppm, 12 aromatic carbons between 110–150 ppm, and other 9 carbons. There were HMBC correlations (Shown in below) from H-1 to the signals δ_C 73.9 (C-3) and aromatic carbons at δ_C 103.5 (C-2′ or C-6′), 108.5 (C-2′ or C-6′), and 132.5 (C-1′), as well as the correlations from H-9 and H-3 to acetate carbonyl at δ_C 171.7 (C-8). Moreover, in considering the HMQC, COSY, and MS spectra, Compound **R** was determined as a novel diarylheptanoid, 7-(3″, 4″-dihydroxylphenyl)-1-(4′, 5′-dihydroxy-3′-methoxyphenyl)-3-acetyl-heptan. However, the compound has not been determined its absolute configuration.

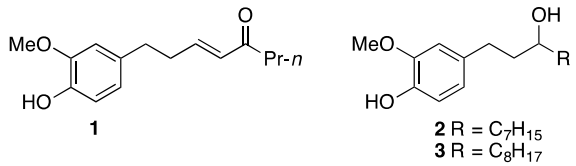


The others were identified as known compounds by the comparison of the NMR data obtained in this study with that of previous reports.[16–19] The other ompounds were identified as 7-paradol (**A**), 6-paradol (**B**), 6-gingerol (**D**), acetyl-6-gingerol (**E**), 1-(4′-hydroxy-3′-methoxyphenyl)-5-methoxy-dodecan-3-one (**F**), 6-shogaol (**G**), 1-dehydrogingerdione (**H**), and 1-(4′-hydroxy-3′-methoxyphenyl)-5-methoxydecan-3-one (**I**), 7-(3″, 4″-dihydrophenyl)-1-(4′-hydroxy-3′-methoxyphenyl)-hept-4-en-3-one (**K**), 7-(3″, 4″-dihydroxylphenyl)-1-(4′-hydroxy-3′-methoxyphenyl)-heptan-3-one (**L**), 7-(3″, 4″-dihydroxylphenyl)-1-(4′,5′-dihydroxy-3′-methoxyphenyl)-heptan-3-one (**M**), 8-gingerol (**O**), 7-(4″-hydroxylphenyl)-1-(4′-hydroxy-3′-methoxyphenyl)-heptan-3-one (**P**), methyl-6-gingerol (**Q**), protocatechuic acid (**S**), 6-gingerdiol (**T**) respectively.

2 *Synthesis of Phenolic Constituents of GOP*

2.1 Introduction

GOP is one of the names given[20] to the dried seeds of the tropical plant *Aframomum melegueta*, which is widely distributed throughout West Africa. The seeds have long been used in folkloric herbal remedies[21] and are known to have, among other properties, antioxidant,[22] antibacterial[23] and antinociceptive[20] activity. In preliminary studies[14] it was found that intake of a methanol extract of GOP has an anti-obesity effect in mice and lowers hepatic and serum fats. A reduction in visceral fat has also been observed in humans, using an ethanol extract.[15] Some of the phenolic compounds present in the seeds[24] were also isolated[25] and found to share structural features with vanilloids that were already known[26] to have anti-obesity properties. Consequently, it was of interest to establish if the vanilloids from *Aframomum melegueta* were responsible for the anti-obesity effect. Most of the compounds could be isolated in adequate quantities and several were found to possess anti-obesity properties,[27] but the isolated amounts of the vanilloids **1**[25] and **2**[25,28] were insufficient for biological evaluation in mice. For this reason I have synthesized both **1** and **2**, as well as the homolog **3**, and the synthetic work is described below. Independently of the initial report of **1**[25] the same compound was isolated from a different plant.[29] Compound **2** has been isolated as a *racemate* by hexane extraction from *Aframomum melegueta*;[30] and by supercritical CO_2 extraction from the rhizomes of ginger (*Zingiber officinale* Roscoe),[31] and has been prepared[32] in *racemic* form by $NaBH_4$ reduction of 6-shogaol. Racemic **2** has been found to promote cholesterol efflux from THP-1-derived macrophages.[33] Our sample of natural **2**,[25,28] as isolated, contained a small impurity[25] and had $[\alpha]_D$ –0.46 (*c* = 0.29 g/100 mL). After I had prepared the *S*-enantiomer I established that the natural material was not racemic but was a 1:1.7 mixture of the *R* and *S* enantiomers.[34]



2.2 Materials and Methods

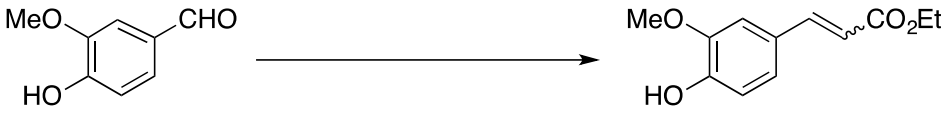
2.2.1 General Procedures

Solvents used for chromatography were distilled before use. Commercial thin layer chromatography plates (silica gel, Merck 60F-254) were used. Silica gel for flash chromatography was Merck type 60 (230−400 mesh). Dry solvents were prepared under an inert atmosphere (N_2) and transferred by syringe or cannula. Unless otherwise indicated, all reactions were done under an inert atmosphere (N_2). The symbols s, d, t, and q used for ^{13}C NMR spectra indicate zero, one, two, or three attached hydrogens, respectively, the assignments being made from APT spectra. Optical rotations were measured at 20 °C. Solutions were evaporated under water pump vacuum, and the residue was then kept under oil pump vacuum. High resolution electrospray mass spectrometric analyses were done with an orthogonal time-of-flight analyzer, and electron ionization mass spectra were measured with a double-focusing sector mass spectrometer. Gradient flash chromatography was done by stepwise small increases in the proportion of the more polar solvent, as described for the individual experiments.

2.2.2 Synthesis of Compound **J**

*Synthesis of (**1**) (Compound **J**)*

*2.2.2.1 Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate and Ethyl (2Z)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate (**1.3**)*[35,36]



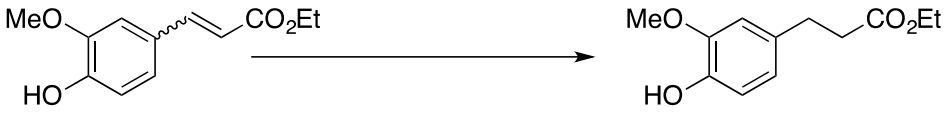
1.1 **1.3**

Dry PhH (150 mL) was added to a flask containing vanillin (8.0 g, 52.6 mmol) and the Wittig reagent **1.2**[37] (19.0 g, 54.6 mmol). The solution was stirred and heated at 80 °C for 4.5 h (oil bath) by which time the reaction was complete (tlc, silica, 1:1 EtOAc-hexane). Evaporation of the solvent and flash chromatography of the residue over silica gel (23 × 5 cm), using 1:1 EtOAc-hexane, gave **1.3** (11.6 g, 99%) as an oil which was a mixture of *Z* and *E* isomers (ca 3:1 *E*:*Z*).

In an earlier run we separated the *Z* and *E* isomers. The *Z* isomer had: FTIR ($CDCl_3$, cast) 3419, 2925, 1515, 1174 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.31 (t, J = 7.0 Hz, 3 H), 3.95 (s, 3 H), 4.21 (q, J = 7.0 Hz, 2 H), 5.83 (d, J = 13.0 Hz, 1 H), 5.83 (d, J = 13.0 Hz, 1 H), 6.81 (d, J = 13.0 Hz, 1 H), 6.90 (d, J = 8.0 Hz, 1 H), 7.13 (dd, J = 1.5, 8.5 Hz, 1 H), 7.79 (d, J = 2.0 Hz, 1 H); exact mass (electrospray) m/z calcd for $C_{12}H_{13}O_4$ $(M–H)^–$ 221.0819, found 221.0816.

The *E* isomer had: FTIR ($CDCl_3$, cast) 3392, 2927, 1514, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.35 (t, J = 7.0 Hz, 3 H), 3.94 (s, 3 H), 4.27 (q, J = 7.0 Hz, 2 H), 5.92 (s, OH), 6.31 (d, J = 15.9 Hz, 1 H), 6.93 (d, J = 8.0 Hz, 1 H), 7.05 (br s, 1 H), 7.09 (br d, J = 8.0 Hz, 1 H), 7.63 (d, J = 15.9 Hz, 1 H); exact mass (electrospray) m/z calcd for $C_{12}H_{13}O_4$ $(M–H)^–$ 221.0819, found 221.0817.

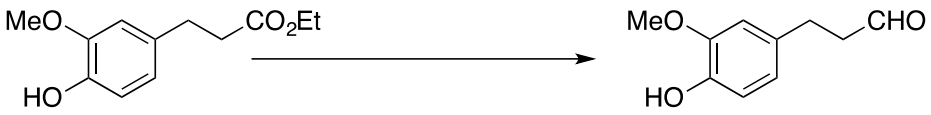
*2.2.2.2 Ethyl 3-(4-hydroxy-3-methoxyphenyl)propanoate (**1.4**)*[35,36]



1.3 **1.4**

10% Pd/C (1.2 g) was added to a solution of **1.3**[35,36] (*Z*,*E* isomer mixture, 11.8 g, 56.0 mmol) in EtOH (120 mL). The flask was flushed with hydrogen (balloon) several times, then kept under a slight pressure of H_2 (balloon), and the mixture was stirred overnight by which time the reaction was over (tlc, silica, 1:4 EtOAc-hexane). The mixture was diluted with EtOH and passed through a short pad of Celite. Evaporation of the filtrate gave **1.4** (10.2 g, 86%) as an oil which was pure enough for the next step. The material had: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.24 (t, J = 7.2 Hz, 3 H), 2.59 (t, J = 7.2 Hz, 2 H), 2.88 (t, J = 7.6 Hz, 2 H), 3.87 (s, 3 H), 4.13 (q, J = 7.2 Hz, 2 H), 5.48 (s, OH), 6.69 (dd, J = 2.0, 8.0 Hz, 1 H), 6.71 (d, J = 2.0 Hz, 1 H), 6.83 (d, J = 8.0 Hz, 1 H).

*2.2.2.3 3-(4-Hydroxy-3-methoxyphenyl)propanal (**1.5**)*[35]

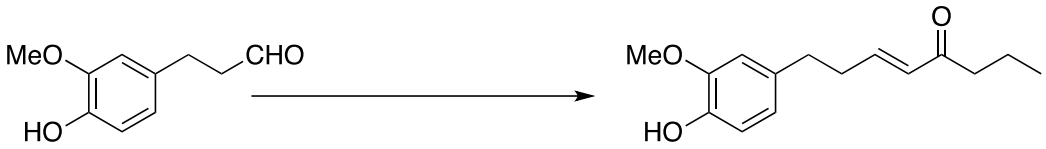


1.4 **1.5**

DIBAL-H (1.1 M in cyclohexane, 30 mL, 33 mmol) was added dropwise by syringe to a stirred and cooled (–78 °C) solution of **1.4** (4.23 g, 18.9 mmol) in dry CH_2Cl_2 (200 mL). After the addition, stirring at –78 °C was continued for 3.5 h, and then the mixture was quenched by addition of MeOH (15 mL). Saturated aqueous

Rochelle salt (400 mL) was added, the cold bath was left in place but not recharged, and stirring was continued overnight. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. Flash chromatography of the residue over silica gel (5 × 23 cm), using first 20% EtOAc-hexane, and then 50% EtOAc-hexane, gave **1.5** (2.5 g, 73%) as an oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.75 (t, J = 7.2 Hz, 2 H), 2.90 (t, J = 7.2 Hz, 2 H), 3.88 (s, 3 H), 5.48 (s, OH), 6.67–6.70 (m, 2 H), 6.84 (d, J = 8.0 Hz, 1 H), 9.82 (s, 1 H); exact mass (EI) m/z calcd for $C_{10}H_{14}O_3$ $(M)^+$ 182.0943; found: 182.0943.

*2.2.2.4 (5E)-8-(4-Hydroxy-3-methoxyphenyl)oct-5-en-4-one (**1**)*



1.5 **1**

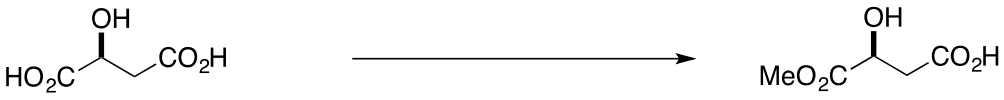
A solution of ylide **1.6**[30,39] (1.3 g, 3.68 mmol) in dry CH_2Cl_2 (24 mL) was added dropwise by syringe to a cooled (ice bath) flask containing **1.5** (552.3 mg, 3.07 mmol) and a magnetic stirring bar. The mixture was stirred and the ice bath was left in place but not recharged. Stirring was continued for 18 h by which time the reaction was over (tlc control, silica, 1:4 EtOAc-hexane). Evaporation of the solvent and flash chromatography of the residue over silica gel (20 × 2 cm), using 1:3 EtOAc-hexane, gave **1** (1.3 g, 77%) as a pale yellow solid which was a single *E* isomer, corresponding spectroscopically (^{1}H and ^{13}C NMR) to the natural product: mp 35–38 °C; FTIR ($CDCl_3$, cast) 3418, 2962, 1516, 1272 cm^{-1}; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 0.92 (t, J = 7.7 Hz, 3 H), 1.62 (sextet, J = 7.7 Hz, 2 H), 2.48–2.52 (m, 4 H), 2.71 (t, J = 7.0 Hz, 2 H), 3.87 (s, 3 H), 5.54 (s, OH), 6.10 (dt, J = 1.4, 15.4 Hz, 1 H), 6.66–6.68 (m, 2 H), 6.81–6.85 (m, 2

H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 13.8 (q), 17.7 (t), 34.2 (t), 34.4 (t), 42.1 (t), 55.9 (q), 110.9 (d), 114.3 (d), 120.9 (d), 130.8 (d), 132.7 (s), 144.0 (s), 145.9 (d), 146.4 (s), 200.7 (s); exact mass (electrospray) *m*/*z* calcd for $C_{15}H_{19}O_3$ $(M–H)^-$ 247.134, found 247.1339.

2.2.3 Synthesis of Compound **C** and the Analogue

*Synthesis of **(3)***

*2.2.3.1 (3S)-3-Hydroxy-4-methoxy-4-oxobutanoic acid (**4.2**)*[40,41]



4.1 **4.2**

The l-(−)-malic acid used in this experiment (99%) had $[\alpha]_D$ –3.22 (*c* = 30.036, MeOH); Lit.[42] $[\alpha]_D$ –2.92 (*c* = 30, MeOH).

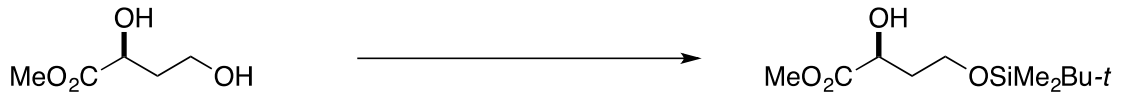
$(CF_3CO)_2O$ (29.3 mL, 207.6 mmol) was added to a stirred sample of l-(–)-malic acid (**4.1**) (11.1 g, 83.0 mmol) and stirring was continued for 90 min (N_2 atmosphere). Residual $(CF_3CO)_2O$ was evaporated under water pump vacuum (protection from moisture). Dry MeOH (35 mL) was added and stirring was continued for 2 h. The MeOH was evaporated and the residue was crystalized from Et_2O to afford **4.2** (12.1 g, 98%): $[\alpha]_D$ –5.57 (*c* = 9.5 g/100 mL); FTIR (MeOH, cast) 3440, 3116, 1732, 1223 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.84 (dd, *J* = 6.5, 16.9 Hz, 1 H), 2.92 (dd, *J* = 4.5, 16.9 Hz, 1 H), 3.81 (s, 3 H), 4.52 (dd, *J* = 4.0, 6.5 Hz, 1 H); ^{13}C NMR (CD_3OD, 175 MHz) δ 39.8 (t), 52.7 (q), 68.6 (d), 173.9 (s), 175.2 (s); exact mass (electrospray) *m*/*z* calcd for $C_5H_8O_5$ $(M–H)^-$ 147.0299, found 147.0300.

*2.2.3.2 Methyl (2S)-2,4-dihydroxybutanoate (**4.3**)*[40]

4.2 **4.3**

$BH_3.SMe_2$ (9.0 mL, 94.9 mmol) was added dropwise by syringe over ca 15 min to a stirred and cooled (0 °C) solution of **4.2** (3.5 g, 23.7 mmol) in THF (20 mL). The ice bath was left in place but not recharged, and stirring was continued overnight. The mixture was quenched by slow addition of MeOH and the solvents were evaporated at *room temperature* under water pump vacuum. The residual oil was diluted with MeOH and the solution was evaporated at room temperature. This procedure was repeated four more times to remove $B(OMe)_3$. The resulting crude diol (**4.3**) was used directly for the next step without purification. The material had: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.88–1.95 (m, 1 H), 2.05–2.11 (m, 2 H), 3.80 (s, 3 H), 4.40 (dd, J = 3.5, 7.5 Hz, 1 H).

*2.2.3.3 Methyl (2S)-4-[(tert-butyldimethylsilyl)oxy]-2-hydroxybutanoate (**4.4**)*[43]



4.3 **4.4**

Crude **4.3** (5.5 g, 41.2 mmol), was dissolved in dry CH_2Cl_2 (25 mL), and Et_3N (6.9 mL, 49.4 mmol) and DMAP (503.2 mg, 4.12 mmol) were then added (N_2 atmosphere). The stirred solution was cooled in an ice bath and solid *t*-$BuMe_2SiCl$ (6.8 g, 45.3 mmol) was added in several small portions by momentarily removing the septum used to close the reaction flask. The ice bath was left in place but not recharged,

and stirring was continued for 30 h. Water (50 mL) was added and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (25 × 4.5 cm), using 15:85 EtOAc-hexane, gave **4.4** (5.6 g, 60% over two steps) as an oil: which contained a small impurity (^{1}H NMR signals at δ 0.13 and 0.16 ppm); $[\alpha]_D$ –5.29 (*c* = 1.369, $CHCl_3$); Lit[33] –37.5 (*c* = 0.5, $CHCl_3$); FTIR ($CHCl_3$, cast) 3494, 2955, 1739, 1101 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.05 (s, 6 H), 0.90 (s, 9 H), 1.83–1.90 (m, 1 H), 2.00–2.06 (m, 1 H), 3.77 (s, 3 H), 3.78–3.82 (m, 2 H), 4.35 (dd, *J* = 3.5, 7.0 Hz, 1 H); ^{13}C NMR (CD_3OD, 175 MHz) δ –5.59 (q), 18.2 (s), 25.8 (q), 36.2 (t), 52.3 (q), 59.8 (t), 68.9 (d), 175.3 (s); exact mass (electrospray) *m*/*z* calcd for $C_{11}H_{24}NaO_4Si$ $(M+Na)^+$ 271.1336, found 271.1333.

*2.2.3.4 Methyl (2S)-2-(benzyloxy)-4-[(tert-butyldimethylsilyl)oxy]butanoate (**4.5**)*[44]

4.4 **4.5**

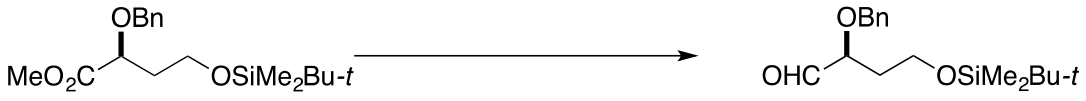
(a) Use of Ag_2O[45]

Freshly-prepared Ag_2O[46] (7.4 g, 32.0 mmol) was tipped into a stirred solution of **4.4** (5.3 g, 21.3 mmol) and BnBr (3.8 mL, 32.0 mmol) in CH_2Cl_2 (60 mL). Stirring was then continued at 35 °C for 15 h with protection from light. The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel (20 × 6 cm), using 7:93 EtOAc-hexane, gave **4.5** (2.2 g, 30%) as an oil.

(b)Use of NaH[47]

Bu_4NI (939.5 mg, 2.54 mmol) was tipped into a stirred mixture of NaH (57–63% dispersion in oil, 1.29 g, 30.5 mmol) in dry DMF (30 mL). Dry DMF (15 mL) was injected into another flask containing **4.4** (6.3 g, 25.4 mmol), followed by BnBr (3.65 mL, 30.5 mmol). The resulting solution was taken up into a syringe and added at a fast dropwise rate to the stirred mixture in the first flask. Stirring was continued for 6 h. The mixture was quenched with ice-cold water and extracted with CH_2Cl_2 (3 × 60 mL). The combined organic extracts were washed with water and brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (26 × 5.5 cm), using 5:95 EtOAc-hexane, gave **4.5** (6.9 g, 79%) as an oil: $[\alpha]_D$ –48.28 (*c* = 1.110, $CHCl_3$); FTIR ($CHCl_3$, cast) 2954, 1753, 1255, 1099 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.04 (s, 6 H), 0.90 (s, 9 H), 1.90–2.04 (m, 2 H), 3.69–3.81 (m, 2 H), 3.75 (s, 3 H), 4.17 (dd, *J* = 4.4, 8.4 Hz, 1 H), 4.43 (d, *J* = 11.2 Hz, 1 H) , 4.71 (d, *J* = 11.2 Hz, 1 H), 7.27–7.37 (m, 5 H); ^{13}C NMR (CD_3OD, 125 MHz) δ –5.4 (q), 18.3 (s), 25.9 (q), 36.1 (t), 51.8 (q), 58.6 (t), 72.6 (t), 75.0 (d), 127.8 (d), 128.0 (d), 128.4 (d), 137.6 (s), 173.5 (s); exact mass (electrospray) *m*/*z* calcd for $C_{18}H_{30}NaO_4Si$ $(M+Na)^+$ 361.1806, found 361.1802.

*2.2.3.5 (2S)-2-(Benzyloxy)-4-[(tert-butyldimethylsilyl)oxy]butanal (**4.6**)*



4.5 **4.6**

DIBAL-H (1 M in hexane, 8.12 mL, 8.12 mmol) was added by syringe at a slow dropwise rate to a stirred and cooled (–78 °C) solution of **4.5** (2.3 g, 6.76 mmol) in dry hexane (10 mL). Stirring at –78 °C was continued for 6 h and the mixture was quenched by dropwise addition of MeOH (5 mL), followed by saturated aqueous

Rochelle salt (40 mL). The cold bath was left in place but not recharged, and stirring was continued overnight. The mixture was extracted with EtOAc (3 × 50 mL) and the combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (22.5 × 4 cm), using 7:93 EtOAc-hexane, gave **4.6** (1.90 g, 91%) as an oil: $[\alpha]_D$ –20.64 (*c* = 1.149, $CHCl_3$); FTIR ($CDCl_3$, cast) 2929, 1732, 1255, 1099 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.05 (s, 6 H), 0.90 (s, 9 H), 1.88–1.98 (m, 2 H), 3.71–3.81 (m, 2 H), 3.97–3.99 (m, 1 H), 4.57 (d, *J* = 12.0 Hz, 1 H), 4.69 (d, *J* = 11.5 Hz, 1 H), 7.30–7.36 (m, 5 H), 9.69 (br s, 1 H); ^{13}C NMR (CD_3OD, 125 MHz) δ –5.47 (q), 18.2 (s), 25.9 (q), 33.9 (t), 58.1 (t), 72.6 (t), 80.8 (d), 127.9 (d), 128.0 (d), 128.5 (d), 137.5 (s), 203.4 (d); exact mass (electrospray) *m*/*z* calcd for $C_{17}H_{28}NaO_3Si$ $(M+Na)^+$ 331.17, found 331.1709.

*2.2.3.6 Diethyl {[4-(benzyloxy)-3-methoxyphenyl]methyl}phosphonate (**3.4**)*[48a]

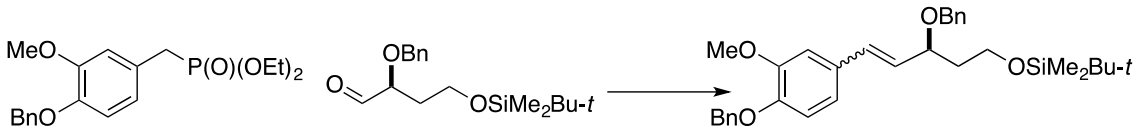
- **3.4**

4-(Benzyloxy)-3-methoxybenzaldehyde[49] was reduced ($NaBH_4$)[50] and the resulting alcohol was converted into the corresponding bromide (PBr_3)[50] to afford 1-(benzyloxy)-4-(bromomethyl)-2-methoxybenzene.

$(EtO)_3P$ (24.4 mL, 142.5 mmol) was added dropwise by syringe to a stirred solution of the bromide (8.8 g, 28.5 mmol) in PhH (50 mL) and the mixture was refluxed (oil bath at 100 °C) for 20 h. The mixture was cooled and evaporated. Flash chromatography of the residue over silica gel (11.5 × 5.5 cm), using 1:4 EtOAc-hexane, gave **3.4** (9.9 g, 95%) as an oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.24 (t, *J* = 7.0 Hz, 3 H),

3.08 (d, *J* = 21.4 Hz, 2 H), 3.88 (s, 3 H), 3.96–4.04 (m, 4 H), 5.13 (s, 2 H), 6.73–6.76 (m, 1 H), 6.82 (d, *J* = 8.0 Hz, 1 H), 6.88 (t, *J* = 2.0 Hz, 1 H), 7.27–7.43 (m, 5 H).

*2.2.3.7 {[(3S,4E)-3-Benzyloxy)-5-[4-benzyloxy)-3-methoxyphenyl]pent-4-en-1-yl]oxy}(tert-butyl)dimethylsilane (E-**5.1**) and {[(3S,4Z)-3-Benzyloxy)-5-[4-benzyloxy)-3-methoxyphenyl]-pent-4-en-1-yl]oxy}(tert-butyl)dimethylsilane (Z-**5.1**)*



3.4 **4.6** **5.1**

$(Me_3Si)_2NLi$ (1 M in THF, 5.37 mL, 5.37 mmol) was added dropwise by syringe to a stirred and cooled (–78 °C) solution of the phosphonate **3.4** (1.95 g, 5.37 mmol) in THF (12 mL) and HMPA (3 mL). Stirring at –78 °C was continued for 1 h and a solution of aldehyde **4.6** (1.4 g, 4.41 mmol) in THF (5 mL) was added dropwise. The cold bath was left in pace but not recharged, and stirring was continued for 23 h. The mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O (3 × 40 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (21.5 × 4.5 cm), using 1:19 EtOAc-hexane, gave *E*-**5.1** (1.6 g, 70%) and *Z*-**5.1** (140.5 mg, 6%) as colorless oils: *Z*-**5.1** had: $[\alpha]_D$ –30.42 (*c* = 1.208, $CHCl_3$); FTIR ($CDCl_3$, cast) 2928, 1513, 1255, 1089 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.05 (d, *J* = 1.6 Hz, 6 H), 0.90 (s, 9 H), 1.83–2.00 (m, 2 H), 3.72–3.77 (m, 1 H), 3.83–3.89 (m, 1 H), 3.88 (s, 3 H), 4.26 (d, *J* = 11.6 Hz, 1 H), 4.55 (d, *J* = 11.6 Hz, 1 H), 4.71 (dt, *J* = 4.0, 12.8 Hz, 1 H), 5.19 (s, 2 H), 5.61 (dd, *J* = 9.6, 12.0 Hz,

1 H), 6.61 (d, J = 11.6 Hz, 1 H), 6.82–6.84 (m, 3 H), 7.22–7.49 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ –5.4 (q), 18.3 (s), 25.9 (q), 38.6 (t), 56.0 (q), 59.4 (t), 70.2 (t), 71.1 (t), 112.9 (d), 113.8 (d), 121.5 (d), 127.2 (d), 127.3 (d), 127.81 (d), 127.83 (d), 128.2 (d), 128.6 (d), 130.2 (s), 131.6 (d), 132.6 (d), 137.2 (s), 138.7 (s) 147.4 (s), 149.3 (s); exact mass (electrospray) m/z calcd for $C_{32}H_{42}NaO_4Si$ $(M+Na)^+$ 541.2745, found 541.2745.

E-**5.1** had: $[\alpha]_D$ –33.95 (c = 1.121, $CHCl_3$); FTIR ($CDCl_3$, cast) 2928, 1512, 1258, 1091 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.04 (s, 6 H), 0.89 (s, 9 H), 1.79 (sextet, J = 6.0 Hz, 1 H), 1.97 (sextet, J = 6.0 Hz, 1 H), 3.68–3.78 (m, 2 H), 3.92 (s, 3 H), 4.11 (dd, J = 8.0, 13.6 Hz, 1 H), 4.40 (d, J = 12.0 Hz, 1 H), 4.62 (d, J = 12.0 Hz, 1 H), 5.17 (s, 2 H), 5.99 (dd, J = 8.0, 16.0 Hz, 1 H), 6.48 (d, J = 16.0 Hz, 1 H), 6.83 (d, J = 8.0 Hz, 1 H), 6.87 (dd, J = 2.0, 8.0 Hz, 1 H), 6.98 (d, J = 1.6 Hz, 1 H), 7.28–7.45 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ –5.3 (q), 18.3 (s), 26.0 (q), 39.2 (t), 56.0 (q), 59.4 (t), 70.2 (t), 71.1 (t), 109.4 (d), 114.0 (d), 119.6 (d), 127.2 (d), 127.4 (d), 127.7 (d), 127.8 (d), 128.3 (d), 128.5 (d), 128.6 (s), 130.3 (d), 132.0 (d), 137.1 (s), 138.8 (s) 148.1 (s), 149.8 (s); exact mass (electrospray) m/z calcd for $C_{32}H_{42}NaO_4Si$ $(M+Na)^+$ 541.2745, found 541.274.

2.2.3.8 Preparation of Z,E-5.1 without separation

$(Me_3Si)_2NLi$ (1 M in THF, 7.26 mL, 7.26 mmol) was added dropwise by syringe to a stirred and cooled (–78 °C) solution of the phosphonate **3.4** (2.65 g, 7.26 mmol) in THF (15 mL) and HMPA (5 mL). Stirring at –78 °C was continued for 1 h and a solution of aldehyde **4.6** (1.9 g, 6.1 mmol) in THF (5 mL) was added dropwise. The cold bath was left in pace but not recharged, and stirring was continued for 18 h. The mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (20 × 4.5 cm), using 7:93 EtOAc-hexane, gave *E*,*Z*-**5.1** (2.3 g, 72%) as a colorless oil.

*2.2.3.9 (3S,4E)-3-(Benzyloxy)-5-[[4-(benzyloxy)-3-methoxyphenyl)pent-4-en-1-ol (**5.2**)*

5.1 **5.2**

Bu_4NF (1 M in THF, 10.6 mL, 10.6 mmol) was added by syringe at a fast dropwise rate to a stirred solution of *E*-**5.1** (1.567 g, 3.02 mmol, containing ca 3% of the *Z* isomer as judged by ^1H NMR) in THF (10 mL). Stirring was continued for 44 h, and the mixture was diluted with water and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (23 × 4 cm), using 1:1 EtOAc-hexane, gave *E*-**5.2** (1.142 g, 93%) and *Z*-**5.2** (78 mg, 6%) as oils. *Z*-**5.2** had: $[\alpha]_D$ –65.10 (*c* = 2.139, $CHCl_3$); FTIR ($CDCl_3$, cast) 3457, 2926, 1513, 1256, 1139 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.85–1.91 (m, 1 H), 1.97–2.04 (m, 1 H), 3.74–3.78 (m, 1 H), 3.82–3.86 (m, 1 H), 3.83 (s, 3 H), 4.23 (d, *J* = 12.0 Hz, 1 H), 4.52 (d, *J* = 11.5 Hz, 1 H), 4.69 (dt, *J* = 4.0, 13.4 Hz, 1 H), 5.18 (s, 2 H), 5.63 (dd, *J* = 9.5, 12.0 Hz, 1 H), 6.65 (d, *J* = 12.0 Hz, 1 H), 6.72 (dd, *J* = 2.0, 8.5 Hz, 1 H), 6.79 (d, *J* = 2.0 Hz, 1 H), 6.85 (d, *J* = 8.5 Hz, 1 H), 7.15–7.45 (m, 10 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 37.5 (t), 56.0 (q), 60.6 (t), 70.2 (t), 71.0 (t), 73.3 (d), 112.6 (d), 113.7 (d), 121.4 (d), 127.2 (d), 127.7 (d), 127.9 (d), 128.0 (d), 128.3 (d), 128.6 (d), 130.0 (s), 131.5 (d), 132.2 (d), 137.1 (s), 138.1 (s) 147.5 (s), 149.4 (s); exact mass (electrospray) *m*/*z* calcd for $C_{26}H_{28}NaO_4$ $(M+Na)^+$ 427.188, found 427.188.

E-**5.2** had: $[\alpha]_D$ –54.53 (*c* = 1.136, $CHCl_3$); FTIR ($CDCl_3$, cast) 3443, 2935, 1512, 1265, 1138 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.85–1.91 (m, 1 H), 1.97–2.04 (m, 1 H), 3.76–3.86 (m, 2 H), 3.94 (s, 3 H), 4.19 (dt, *J* = 4.5, 16.4 Hz, 1 H), 4.43 (d, *J* =

11.5 Hz, 1 H), 4.68 (d, *J* = 12.0 Hz, 1 H), 5.18 (s, 2 H), 6.04 (dd, *J* = 8.5, 15.9 Hz, 1 H), 6.52 (d, *J* = 15.9 Hz, 1 H), 6.86 (d, *J* = 8.5 Hz, 1 H), 6.90 (dd, *J* = 1.5, 8.5 Hz, 1 H), 7.00 (d, *J* = 1.5 Hz, 1 H), 7.29–7.46 (m, 10 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 38.3 (t), 56.1 (q), 60.6 (t), 70.3 (t), 71.1 (t), 79.7 (d), 109.5 (d), 114.0 (d), 119.8 (d), 127.3 (d), 127.6 (d), 127.7 (d), 127.8 (d), 127.9 (d), 128.5 (d), 128.6 (d), 129.9 (s), 132.5 (d), 137.0 (s), 138.3 (s), 148.3 (s), 149.8 (s); exact mass (electrospray) *m*/*z* calcd for $C_{26}H_{28}NaO_4$ $(M+Na)^+$ 427.188, found 427.1878.

*2.2.3.10 (3S,4E)-3-(Benzyloxy)-5-[[4-(benzyloxy)-3-methoxyphenyl)pent-4-enal (E-**5.3**)*

5.2 **5.3**

Dess-Martin reagent (1.30 g, 3.07 mmol) was added in portions to a stirred and cooled (0 °C) mixture of *E*-**5.2** (992.7 mg, 2.45 mmol), $NaHCO_3$ (1.44 g, 17.2 mmol)[51] and CH_2Cl_2 (10 mL). The ice bath was left in place and stirring was continued for 3 h, during which time all the ice melted. The mixture was cooled to 0 °C, quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc (3 × 40 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (23.5 × 4 cm), using 1:2 EtOAc-hexane, gave *E*-**5.3** (943 mg, 95%) as a yellowish oil: $[\alpha]_D$ –51.26 (*c* = 2.233, $CHCl_3$); FTIR ($CDCl_3$, cast) 3031, 2863, 1724, 1512, 1265 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.64 (qd, *J* = 1.6, 4.8, 16.0 Hz, 1 H), 2.85 (qd, *J* = 2.4, 8.0, 16.4 Hz, 1 H), 3.94 (s, 3 H), 4.46 (d, *J* = 12.0 Hz, 1 H), 4.48–4.52 (m, 1 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 5.18 (s, 2 H), 6.03 (dd, *J* = 8.0, 15.6 Hz,

1 H), 6.58 (d, J = 16.0 Hz, 1 H), 6.87 (d, J = 8.4 Hz, 1 H), 6.90 (dd, J = 1.6, 8.4 Hz, 1 H), 6.99 (d, J = 1.6 Hz, 1 H), 7.29–7.47 (m, 10 H), 9.81 (t, J = 2.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 49.6 (t), 56.0 (q), 70.3 (t), 71.0 (t), 75.2 (d), 109.5 (d), 114.0 (d), 119.9 (d), 126.2 (d), 127.2 (d), 127.7 (d), 127.8 (d), 127.9 (d), 128.4 (d), 128.6 (d), 129.5 (s), 133.2 (d), 137.0 (s), 138.0 (s), 148.4 (s), 149.8 (s), 200.7 (d); exact mass (electrospray) m/z calcd for $C_{26}H_{26}NaO_4$ $(M+Na)^+$ 425.1723, found 425.1725.

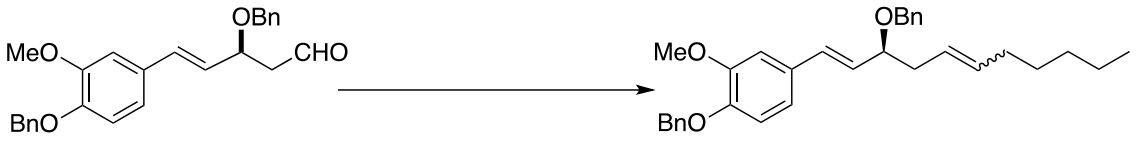
*2.2.3.11 (3S,4E)-3-(Benzyloxy)-5-[[4-(benzyloxy)-3-methoxyphenyl)pent-4-enal and (3S,4Z)-3-(Benzyloxy)-5-[[4-(benzyloxy)-3-methoxyphenyl)pent-4-enal (E,Z-**5.3**)*

***E,Z*-5.2** **E,Z-5.3**

Dess-Martin reagent (1.95 g, 4.59 mmol) was added in portions to a stirred and cooled (0 °C) mixture of *E,Z*-**5.2** (1.55 g, 3.82 mmol), $NaHCO_3$ (2.25 g, 26.8 mmol)[51] and CH_2Cl_2 (10 mL). The ice bath was left in place and stirring was continued for 4 h, during which time all the ice melted. The mixture was cooled to 0 °C, quenched with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc (3 × 40 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (22 × 5 cm), using 1:2 EtOAc-hexane, gave *E,Z*-**5.3** (1.46 g, 94%) as a yellowish oil.

2.2.3.12

*1-(Benzyloxy)-4-[(1E,3S)-3-(benzyloxy)undeca-1,5-dien-1-yl]-2-methoxybenzene (1E-**5.4**) from E-**5.3***



***E*-5.3** **(1*E*)-5.4**

$(Me_3Si)_2NLi$ (1 M in THF, 4.69 mL, 4.69 mmol) was added dropwise by syringe to a stirred and cooled (–78 °C) solution of hexyltriphenylphosphonium bromide[52] (2.00 g, 4.69 mmol) in a mixture of THF (40 mL) and HMPA (5 mL). Stirring at –78 °C was continued for 1 h and then a solution of *E*-**5.3** (926.0 mg, 2.30 mmol) in THF (5 mL) was added dropwise by syringe over ca 5 min. The cold bath was left in place but not recharged, and stirring was continued for 22 h. The mixture was quenched by addition of aqueous phosphate buffer [pH 7.2, prepared[53] by mixing aqueous 1 M Na_2HPO_4 (3.42 volumes) and 1 M NaH_2PO_4 (1.58 volumes)] and extracted with Et_2O (3 × 60 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (21.5 × 4.5 cm), using 7:93 EtOAc-hexane, gave **5.4** (827.1 mg, 76%) as a yellowish oil, which appeared to be a single isomer (^{1}H NMR, ^{13}C NMR) of unestablished C5–C6 geometry: $[\alpha]_D$ – 57.70 (*c* = 1.003, $CHCl_3$); FTIR ($CDCl_3$, cast) 2926, 1265, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, *J* = 7.0 Hz, 3 H), 1.26–1.38 (m, 6 H), 2.05 (dd, *J* = 6.5, 13.9 Hz, 2 H), 2.38–2.44 (m, 1 H), 2.51–2.56 (m, 1 H), 3.92–3.96 (m, 1 H), 3.93 (s, 3 H), 4.45 (d, *J* = 12.0 Hz, 1 H), 4.66 (d, *J* = 12.0 Hz, 1 H), 5.18 (s, 2 H), 5.43–5.52 (m, 2 H), 6.02 (dd, *J* = 8.0, 15.9 Hz, 1 H), 6.48 (d, *J* = 15.4 Hz, 1 H), 6.85 (d, *J* = 8.5 Hz, 1 H), 6.89 (dd, *J* = 2.0, 8.5 Hz, 1 H), 6.99 (d, *J* = 2.0 Hz, 1 H), 7.27–7.46 (m, 10 H); ^{13}C NMR ($CDCl_3$,

175 MHz) δ 14.1 (q), 22.6 (t), 27.5 (t), 29.3 (t), 31.6 (t), 33.9 (t), 56.0 (q), 70.1 (t), 71.1 (t), 80.2 (d), 109.6 (d), 114.1 (d), 119.6 (d), 124.8 (d), 127.2 (d), 127.4 (d), 127.7 (d), 127.9 (d), 128.3 (d), 128.4 (d), 128.6 (d), 130.3 (s), 132.2 (d), 137.1 (s), 138.8 (s), 148.1 (s), 149.8 (s); exact mass (electrospray) *m*/*z* calcd for $C_{32}H_{38}NaO_3$ $(M+Na)^+$ 493.2713, found 493.2716.

*2.2.3.13 1-(Benzyloxy)-4-[(3S)-3-(benzyloxy)undeca-1,5-dien-1-yl]-2-methoxybenzene (1E,1Z-**5.4**) from E,Z-**5.3***

$(Me_3Si)_2NLi$ (1 M in THF, 7.25 mL, 7.25 mmol) was added dropwise by syringe to a stirred and cooled (–78 °C) solution of hexyltriphenylphosphonium bromide[52] (3.1 g, 7.25 mmol) in a mixture of THF (55 mL) and HMPA (7.5 mL). Stirring at –78 °C was continued for 1.5 h and then a solution of *E*,*Z*-**5.3** (1.43 g, 3.55 mmol) in THF (5 mL) was added dropwise by syringe over ca 10 min. The cold bath was left in place but not recharged, and stirring was continued for 15 h. The mixture was quenched by addition of aqueous phosphate buffer [pH 7.2, prepared[53] by mixing aqueous 1 M Na_2HPO_4 (3.42 volumes) and 1 M NaH_2PO_4 (1.58 volumes)] and extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (22 × 4.5 cm), using 5:95 EtOAc-hexane, gave 1*E*-**5.4** as a single isomer and 1*E*,1*Z*-**5.4** (1.5 g in total, 88%) as yellowish oils.

*2.2.3.14 1-(Benzyloxy)-4-[(3S)-3-(benzyloxy)undecyl]-2-methoxybenzene (**5.5**)*

1*E*-5.4 **5.5**

5% Rh/Al_2O_3 (28.4 mg) was added to a solution of 1*E*-**5.4** (single compound of unestablished C5–C6 geometry, 567.8 mg, 1.21 mmol) in EtOH (4 mL) and the diene was hydrogenated at room temperature (H_2-filled balloon) for 3 h. The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel (22.5 × 2 cm), using 1:19 EtOAc-hexane, gave **5.5** (551.3 mg, 96%) as an oil: $[\alpha]_D$ 9.14 (*c* = 1.996, $CHCl_3$); FTIR ($CDCl_3$, cast) 2927, 1513, 1263, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, *J* = 7.0 Hz, 3 H), 1.28–1.42 (m, 12 H), 1.51–1.66 (m, 2 H), 1.77–1.90 (m, 2 H), 2.56–2.62 (m, 1 H), 2.68–2.74 (m, 1 H), 3.42 (quint, *J* = 6.0 Hz, 1 H), 3.87 (s, 3 H), 4.49 (d, *J* = 11.5 Hz, 1 H), 4.55 (d, *J* = 12.0 Hz, 1 H), 5.13 (s, 2 H), 6.65 (dd, *J* = 2.0, 8.0 Hz, 1 H), 6.73 (d, *J* = 2.0 Hz, 1 H), 6.81 (d, *J* = 8.0 Hz, 1 H), 7.27–7.46 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.1 (q), 22.7 (t), 25.3 (t), 29.3 (t), 29.6 (t), 29.8 (t), 31.4 (t), 31.9 (t), 33.8 (t), 35.9 (t), 56.0 (q), 70.8 (t), 71.3 (t), 78.4 (d), 112.4 (d), 114.3 (d), 120.2 (d), 127.3 (d), 127.4 (d), 127.7 (d), 127.8 (d), 128.3 (d), 128.5 (d), 135.9 (s), 137.5 (s), 139.1 (s), 146.3 (s), 149.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_{32}H_{42}NaO_3$ $(M+Na)^+$ 497.3026, found 497.3026.

*2.2.3.15 4-[(3S)-3-Hydroxyundecyl]-2-methoxyphenol [(S)-**3**]*

5.5 (*S*)-**3**

10% Pd/C (7.2 mg) was added to a solution of **5.5** (144.1 mg, 0.30 mmol) in EtOH (3 mL) and the compound was hydrogenated at room temperature (H_2-filled balloon) for 2.5 h. The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel

(17 × 2 cm), using EtOAc, gave (*S*)-**3** (86.2 mg, 96%) as a white solid: mp 53–55 °C; $[\alpha]_D$ 8.35 (*c* = 1.233, $CHCl_3$); FTIR ($CDCl_3$, cast) 3338, 3245, 1516, 1153 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.28–1.51 (m, 14 H), 1.66–1.81 (m, 2 H), 2.57–2.64 (m, 1 H), 2.69–2.76 (m, 1 H), 3.60–3.66 (m, 1 H), 3.86 (s, 3 H), 6.68–6.71 (m, 2 H), 6.83 (d, *J* = 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.1 (q), 22.6 (t), 25.6 (t), 29.2 (t), 29.6 (t), 29.7 (t), 31.8 (t), 31.9 (t), 37.6 (t), 39.4 (t), 55.9 (q), 71.4 (d), 111.0 (d), 114.2 (d), 120.9 (d), 134.1 (s), 143.7 (s), 146.4 (s); exact mass (EI) *m*/*z* calcd for $C_{18}H_{30}O_3$ $(M)^+$ 294.2195, found 294.2191.

Chiral HPLC (CHIRALCEL OD column, 250 × 4.6 mm, 15:85 *i*-PrOH:hexane, 0.5 mL/min, wavelength 230 and 280 nm, 20 °C) showed the compound to have an ee of 90%.

*Preparation of (±)-3 for establishing enantiomeric purity of [(S)-**3**]*

*2.2.3.16 (±)-1-[4-(Benzyloxy)-3-methoxyphenyl]undecan-3-ol [(±)-**3**]*

(a) (3S)-1-[4-(Benzyloxy)-3-methoxyphenyl]undecan-3-ol

(*S*)-3 **-**

K_2CO_3 (218.8 mg, 1.59 mmol) was added to a stirred solution of (*S*)-**3** (155.4 mg, 0.53 mmol) in dry acetone (6 mL) and then BnBr (0.13 mL, 1.06 mmol) was added. The stirred mixture was heated at 60 °C for 10 h. The solvent was evaporated and water (20 mL) was added to the residue. The mixture was extracted with EtOAc (3 × 20 mL) and the combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (21.5 × 2 cm), using 1:4

EtOAc-hexane, gave (3*S*)-1-[4-(benzyloxy)-3-methoxyphenyl]undecan-3-ol (194.4 mg, 95%) as a white solid: mp 53–54 °C; $[\alpha]_D$ 6.78 (*c* = 1.15, $CHCl_3$); FTIR ($CDCl_3$, cast) 3328, 2924, 1514, 1223 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, *J* = 7.0 Hz, 3 H), 1.25–1.49 (m, 14 H), 1.67–1.81 (m, 2 H), 2.58–2.64 (m, 1 H), 2.70–2.76 (m, 1 H), 3.60–3.65 (m, 1 H), 3.88 (s, 3 H), 5.12 (s, 2 H), 6.67 (dd, *J* = 2.0, 8.0 Hz, 1 H), 6.76 (d, *J* = 2.0 Hz, 1 H), 6.81 (d, *J* = 8.5 Hz, 1 H), 7.28–7.45 (m, 5 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.1 (q), 22.6 (t), 25.6 (t), 29.2 (t), 29.6 (t), 29.7 (t), 31.7 (t), 31.9 (t), 37.6 (t), 39.2 (t), 56.0 (q), 71.2 (t), 71.4 (d), 112.4 (d), 114.4 (d), 120.2 (d), 127.3 (d), 127.7 (d), 128.5 (d), 135.6 (s), 137.4 (s), 146.4 (s), 149.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_{25}H_{36}NaO_3$ $(M+Na)^+$ 407.2557, found 407.2552.

(b) 1-[4-(Benzyloxy)-3-methoxyphenyl]undecan-3-one

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$NaHCO_3$ (105.9 mg, 1.26 mmol) and Dess-Martin periodinane (213.9 mg, 0.50 mmol) were added sequentially to a stirred and cooled (0 °C) solution of (3*S*)-1-[4-(benzyloxy)-3-methoxyphenyl]undecan-3-ol (161.5 mg, 0.42 mmol) in dry CH_2Cl_2 (3 mL). Stirring at 0 °C was continued for 3.5 h. The reaction mixture was quenched by addition of saturated aqueous $Na_2S_2O_3$ (4 mL) and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (24 × 2 cm), using 1:4 EtOAc-hexane, gave 1-[4-(benzyloxy)-3-methoxyphenyl]undecan-3-one (125.6 mg 78%) as a white solid: mp 70–72 °C; FTIR ($CDCl_3$, cast) 2920, 1701, 1512, 1136 cm^{-1}; ^1H NMR ($CDCl_3$, 700

MHz) δ 0.88 (t, J = 7.0 Hz, 3 H), 1.24–1.30 (m, 10 H), 1.55 (quint, J = 7.0 Hz, 2 H), 2.37 (t, J = 7.0 Hz, 2 H), 2.69 (t, J = 7.0 Hz, 2 H), 2.83 (t, J = 7.0 Hz, 2 H), 3.87 (s, 3 H), 5.12 (s, 2 H), 6.64 (dd, J = 2.1, 7.7 Hz, 1 H), 6.73 (d, J = 2.1 Hz, 1 H), 6.79 (d, J = 7.7 Hz, 1 H), 7.28–7.43 (m, 5 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.1 (q), 22.6 (t), 23.8 (t), 29.1 (t), 29.2 (t), 29.3 (t), 29.4 (t), 31.8 (t), 43.1 (t), 44.4 (t), 56.0 (q), 71.2 (t), 112.3 (d), 114.3 (d), 120.1 (d), 127.2 (d), 127.7 (d), 128.5 (d), 134.5 (s), 137.4 (s), 146.5 (s), 149.6 (s), 210.5 (s); exact mass (electrospray) m/z calcd for $C_{25}H_{34}NaO_3$ $(M+Na)^+$ 405.24, found 405.2401.

(c) (±)-1-[4-(Benzyloxy)-3-methoxyphenyl]undecan-3-ol

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$NaBH_4$ (10.4 mg, 0.27 mmol) was added in portions to a stirred solution of 1-[4-(benzyloxy)-3-methoxyphenyl]undecan-3-one (105 mg, 0.27 mmol) in dry MeOH (4 mL). Stirring was continued for 2 h, ice water (10 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (20 × 2 cm), using 7:93 EtOAc-hexane, gave (±)-1-[4-(benzyloxy)-3-methoxyphenyl]undecan-3-ol (101.3 mg 96%) as a white solid: mp 59–62 °C; FTIR ($CDCl_3$, cast) 3226, 2918, 1515, 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, J = 7.0 Hz, 3 H), 1.25–1.58 (m, 14 H), 1.67–1.81 (m, 2 H), 2.58–2.64 (m, 1 H), 2.71–2.77 (m, 1 H), 3.60–3.65 (m, 1 H), 3.88 (s, 3 H), 5.13 (s, 2 H), 6.68 (dd, J = 1.5, 8.0 Hz, 1 H), 6.77 (d, J = 1.5 Hz, 1 H), 6.81 (d, J = 8.0 Hz, 1 H), 7.28–7.45 (m,

5 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.1 (q), 22.7 (t), 25.6 (t), 29.3 (t), 29.6 (t), 29.7 (t), 31.7 (t), 31.9 (t), 37.6 (t), 39.2 (t), 56.0 (q), 71.3 (t), 71.4 (d), 112.4 (d), 114.4 (d), 120.2 (d), 127.3 (d), 127.7 (d), 128.5 (d), 135.6 (s), 137.5 (s), 146.4 (s), 149.6 (s); exact mass (electrospray) *m*/*z* calcd for $C_{25}H_{36}NaO_3$ $(M+Na)^+$ 407.2557, found 407.2555.

*(d) (±)-4-[(3-Hydroxyundecyl]-2-methoxyphenol [(±)-**3**]*

- **(±)-3**

10% Pd/C (3.5 mg) was added to a solution of (±)-1-[4-(benzyloxy)-3-methoxyphenyl]undecan-3-ol (70.1 mg, 0.18 mmol) in EtOH (3 mL) and the mixture was stirred under H_2 (balloon) for 1.5 h. The mixture was filtered through a short pad of Celite which was rinsed with EtOAc. Evaporation of the filtrate gave (±)-**3** (52.8 mg, 98%) as a white solid that was pure (^{1}H NMR): 62–63 °C; FTIR ($CDCl_3$, cast) 3390, 2920, 1516, 1154 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J = 7.0 Hz, 3 H), 1.25–1.54 (m, 14 H), 1.66–1.80 (m, 2 H), 2.57–2.63 (m, 1 H), 2.70–2.75 (m, 1 H), 3.60–3.65 (m, 1 H), 3.86 (s, 3 H), 6.69 (dd, J = 2.0, 8.0 Hz, 1 H), 6.71 (d, J = 1.5 Hz, 1 H), 6.83 (d, J = 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.1 (q), 22.7 (t), 25.6 (t), 29.3 (t), 29.6 (t), 29.7 (t), 31.8 (t), 31.9 (t), 37.6 (t), 39.3 (t), 55.8 (q), 71.4 (d), 111.0 (d), 114.3 (d), 120.9 (d), 134.1 (s), 143.7 (s), 146.4 (s); exact mass (electrospray) *m*/*z* calcd for $C_{18}H_{29}O_3$ $(M–H)^–$ 293.2122, found 293.2122.

Synthesis of ***(2)***

2.2.3.17

*1-(Benzyloxy)-4-[(1E,3S)-3-(benzyloxy)deca-1,5-dien-1-yl]-2-methoxybenzene (1E-**6.1**) and 1-(Benzyloxy)-4-[(1E,1Z,3S)-3-(benzyloxy)deca-1,5-dien-1-yl]-2-methoxybenzene (1E,1Z-**6.1**)*

***E*,*Z*-5.3** **E-6.1; 1*E*,1*Z*-6.1**

$(Me_3Si)_2NLi$ (1 M in THF, 14.9 mL, 14.9 mmol) was added dropwise by syringe to a stirred and cooled (–78 °C) solution of pentyltriphenylphosphonium bromide[54] (6.16 g, 14.9 mmol) in a mixture of THF (70 mL) and HMPA (10 mL). Stirring at –78 °C was continued for 1.5 h and then a solution of *Z*,*E*-**5.3** (3.0 g, 7.5 mmol) in THF (5 mL) was added dropwise by syringe over ca 10 min. The cold bath was left in place but not recharged, and stirring was continued for 22 h. The mixture was quenched by addition of aqueous phosphate buffer [pH 7.2, prepared[53] by mixing aqueous 1 M Na_2HPO_4 (3.42 volumes) and 1 M NaH_2PO_4 (1.58 volumes)] and extracted with Et_2O (3 × 80 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (27 × 5.5 cm), using 5:95 EtOAc-hexane, gave 1*Z*,1*E*-**6.1** (1.5 g, 44%) and 1*E*-**6.1** (178.2 mg, 5%), both as yellowish oils. The 1*Z*,1*E* mixture (mainly *E*) had: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, J = 7.0 Hz, 3 H), 1.31–1.37 (m, 4 H), 2.08 (dd, J = 6.5, 13.4 Hz, 2 H), 2.41–2.46 (m, 1 H), 2.53–2.60 (m, 1 H), 3.94–3.98 (m, 1 H), 3.94 (s, 3 H), 4.47 (d, J = 12.0 Hz, 1 H), 4.68 (d, J = 12.5 Hz, 1 H), 5.19 (s, 2 H), 5.45–5.54 (m, 2 H), 6.04 (dd, J = 8.0, 15.4 Hz, 1 H), 6.50 (d, J = 15.9 Hz, 1 H), 6.87 (d, J = 8.5 Hz, 1 H), 6.90 (dd, J = 1.5,

8.5 Hz, 1 H), 7.01 (d, *J* = 1.5 Hz, 1 H), 7.28–7.49 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.0 (q), 22.4 (t), 27.2 (t), 31.8 (t), 33.9 (t), 56.0 (q), 70.1 (t), 71.1 (t), 80.2 (d), 109.5 (d), 114.0 (d), 119.7 (d), 124.9 (d), 127.2 (d), 127.4 (d), 127.7 (d), 127.9 (d), 128.3 (d), 128.4 (d), 128.6 (d), 130.3 (s), 132.1 (d), 132.2 (d), 137.1 (s), 138.8 (s), 148.1 (s), 149.8 (s).

The 1*E* isomer had: $[\alpha]_D$ –39.19 (*c* = 1.043, $CHCl_3$); FTIR ($CDCl_3$, cast) 2928, 1512, 1266, 1138 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, *J* = 7.0 Hz, 3 H), 1.26–1.35 (m, 4 H), 2.05 (dd, *J* = 6.5, 13.4 Hz, 2 H), 2.37–2.43 (m, 1 H), 2.50–2.55 (m, 1 H), 3.91–3.95 (m, 1 H), 3.92 (s, 3 H), 4.44 (d, *J* = 12.0 Hz, 1 H), 4.65 (d, *J* = 12.5 Hz, 1 H), 5.18 (s, 2 H), 5.42–5.51 (m, 2 H), 6.01 (dd, *J* = 8.0, 15.9 Hz, 1 H), 6.47 (d, *J* = 15.9 Hz, 1 H), 6.84 (d, *J* = 8.5 Hz, 1 H), 6.88 (dd, *J* = 2.0, 8.5 Hz, 1 H), 6.98 (d, *J* = 2.0 Hz, 1 H), 7.28–7.45 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 14.0 (q), 22.4 (t), 27.2 (t), 31.7 (t), 33.9 (t), 56.0 (q), 70.1 (t), 71.0 (t), 80.1 (d), 109.5 (d), 114.0 (d), 119.6 (d), 124.8 (d), 127.2 (d), 127.4 (d), 127.6 (d), 127.8 (d), 128.30 (d), 128.34 (d), 128.5 (d), 130.2 (s), 132.1 (d), 132.2 (d), 137.1 (s), 138.8 (s), 148.0 (s), 149.8 (s); exact mass (electrospray) *m*/*z* calcd for $C_{31}H_{36}NaO_3$ $(M+Na)^+$ 479.2557, found 479.2558.

For both fractions the C5–C6 double bond geometry was not determined.

*2.2.3.18 1-(Benzyloxy)-4-[(3S)-3-(benzyloxy)decyl]-2-methoxybenzene (**6.2**)*

1*E*,1*Z*-6.1 **6.2**

5% Rh/Al_2O_3 (32.5 mg) was added to a solution of 1*E*,1*Z*-**6.1** (unestablished C5–C6 geometry, 650.7 mg, 1.43 mmol) in EtOH (10 mL) and the diene was hydrogenated at room temperature (H_2-filled balloon) for 4 h. The mixture was filtered

through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel (26.0 × 4 cm), using 1:19 EtOAc-hexane, gave **6.2** (407.2 mg, 62%) as an oil: $[\alpha]_D$ 6.53 (*c* = 1.041, $CHCl_3$); FTIR ($CDCl_3$, cast) 2928, 1513, 1262, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.92 (t, *J* = 7.0 Hz, 3 H), 1.26–1.53 (m, 10 H), 1.54–1.68 (m, 2 H), 1.79–1.92 (m, 2 H), 2.59–2.65 (m, 1 H), 2.71–2.77 (m, 1 H), 3.44 (quint, *J* = 5.5 Hz, 1 H), 3.88 (s, 3 H), 4.51 (d, *J* = 12.0 Hz, 1 H), 4.57 (d, *J* = 11.5 Hz, 1 H), 5.15 (s, 2 H), 6.67 (dd, *J* = 2.0, 8.0 Hz, 1 H), 6.76 (d, *J* = 2.0 Hz, 1 H), 6.83 (d, *J* = 8.0 Hz, 1 H), 7.28–7.48 (m, 10 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2 (q), 22.7 (t), 25.3 (t), 29.3 (t), 29.9 (t), 31.4 (t), 31.9 (t), 33.8 (t), 36.0 (t), 56.0 (q), 70.9 (t), 71.3 (t), 78.5 (d), 112.5 (d), 114.4 (d), 120.2 (d), 127.3 (d), 127.5 (d), 127.75 (d), 127.79 (d), 128.4 (d), 128.5 (d), 136.0 (s), 137.5 (s), 139.1 (s), 146.3 (s), 149.7 (s); exact mass (electrospray) *m*/*z* calcd for $C_{31}H_{40}NaO_3$ $(M+Na)^+$ 483.287, found 483.2873.

Larger scale experiment

5% Rh/Al_2O_3 (65.0 mg) was added to a solution of 1*E*,1*Z*-**6.1** (unestablished C5–C6 geometry, 1.07 g, 2.35 mmol) in EtOH (10 mL) and the diene was hydrogenated at room temperature (H_2-filled balloon) for 4 h. The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel (25.5 × 4 cm), using 1:19 EtOAc-hexane, gave **6.2** (684 mg, 63%) as an oil.

*2.2.3.19 4-[(3S)-3-Hydroxydecyl]-2-methoxyphenol [(S)-**2**]*

6.2 (***S***)**-2**

10% Pd/C (20.1 mg) was added to a solution of **6.2** (402.5 mg, 0.87 mmol) in EtOH (10 mL) and the compound was hydrogenated at room temperature (H_2-filled balloon) for 2 h. The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel (22.5 × 2 cm), using first 1:4 EtOAc-hexane and then 1:1 EtOAc-hexane, gave (*S*)-**2** (211.4 mg, 86%) as a white solid: mp 48–49 °C; $[\alpha]_D$ 6.31 (*c* = 1.049, $CHCl_3$); FTIR ($CDCl_3$, cast) 3423, 2928, 1515, 1270 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, *J* = 7.0 Hz, 3 H), 1.28–1.34 (m, 10 H), 1.41–1.51 (m, 2 H), 1.67–1.80 (m, 2 H), 1.82 (br s, OH), 2.57–2.63 (m, 1 H), 2.70–2.76 (m, 1 H), 3.60–3.65 (m, 1 H), 3.84 (s, 3 H), 5.81 (s, OH), 6.69 (dd, *J* = 2.0, 8.0 Hz, 1 H), 6.71 (d, *J* = 2.0 Hz, 1 H), 6.83 (d, *J* = 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q), 22.7 (t), 25.7 (t), 29.3 (t), 29.7 (t), 31.78 (t), 31.85 (t), 37.6 (t), 39.4 (t), 55.9 (q), 71.5 (d), 111.2 (d), 114.4 (d), 120.9 (d), 134.2 (s), 143.7 (s), 146.6 (s); exact mass (EI) *m*/*z* calcd for $C_{17}H_{28}NaO_3$ $(M+Na)^+$ 303.1931, found 301.1931.

Larger scale experiment

10% Pd/C (34.2 mg) was added to a solution of **6.2** (684 mg, 1.49 mmol) in EtOH (10 mL) and the compound was hydrogenated at room temperature (H_2-filled balloon) for 17 h. The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse. Evaporation of the filtrate and flash chromatography of the residue over silica gel (25 × 3 cm), using first 1:4 EtOAc-hexane and then 1:1 EtOAc-hexane, gave (*S*)-**2** (354 mg, 85%) as a white solid.

Chiral HPLC (CHIRALCEL OD column, 250 × 4.6 mm, 15:85 *i*-PrOH:hexane, 0.5 mL/min, wavelength 230 and 280 nm, 20 °C) showed the compound to have an ee of 68%.

The material isolated from natural sources had: $[\alpha]_D$ –0.46 (*c* = 0.29, $CHCl_3$). Chiral HPLC (CHIRALCEL OD column, 250 × 4.6 mm, 15:85 *i*-PrOH:hexane, 0.5

mL/min, wavelength 230 and 280 nm, 20 °C) showed the compound to be a 1:1.7 *R*:*S* mixture.

*Preparation of (±)-**2** for establishing enantiomeric purity of [(S)-**2**]*

(a) (3S)-1-[4-(Benzyloxy)-3-methoxyphenyl]decan-3-ol

(*S*)-2 **-**

K_2CO_3 (284.0 mg, 2.06 mmol) was added to a stirred solution of (*S*)-**2** (192.1 mg, 0.69 mmol) in dry acetone (10 mL) and BnBr (0.16 mL, 1.37 mmol) was added. The stirred mixture was then heated at 60 °C for 12 h. The solvent was evaporated, water (20 mL) was added to the residue and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (21 × 2 cm), using 1:4 EtOAc-hexane, gave (3*S*)-1-[4-(benzyloxy)-3-methoxyphenyl]decan-3-ol (247.0 mg, 97%) as a white solid: mp 63–65 °C; $[\alpha]_D$ 5.20 (*c* = 1.085, $CHCl_3$); FTIR ($CDCl_3$, cast) 3334, 2925, 1514, 1260 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, *J* = 7.0 Hz, 3 H), 1.29–1.34 (m, 10 H), 1.43–1.51 (m, 2 H), 1.67–1.81 (m, 2 H), 2.58–2.65 (m, 1 H), 2.71–2.77 (m, 1 H), 3.60–3.65 (m, 1 H), 3.88 (s, 3 H), 5.13 (s, 2 H), 6.68 (dd, *J* = 1.5, 8.0 Hz, 1 H), 6.77 (d, *J* = 1.5 Hz, 1 H), 6.81 (d, *J* = 8.0 Hz, 1 H), 7.28–7.45 (m, 5 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q), 22.7 (t), 25.7 (t), 29.3 (t), 29.7 (t), 31.75 (t), 31.84 (t), 37.7 (t), 39.2 (t), 56.0 (q), 71.3 (t), 71.4 (d), 112.5 (d), 114.4 (d), 120.2 (d), 127.3 (d), 127.7 (d), 128.5 (d), 135.6 (s), 137.5 (s), 146.4 (s), 149.7 (s); exact mass (electrospray) *m*/*z* calcd for $C_{24}H_{34}NaO_3$ $(M+Na)^+$ 393.24, found 393.2395.

(b) 1-[4-(Benzyloxy)-3-methoxyphenyl]decan-3-one[55]

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$NaHCO_3$ (153.2 mg, 1.82 mmol) and Dess-Martin periodinane (309.5 mg, 0.73 mmol) were added sequentially to a stirred and cooled (0 °C) solution of (3*S*)-1-[4-(benzyloxy)-3-methoxyphenyl]decan-3-ol (225.0 mg, 0.61 mmol) in dry CH_2Cl_2 (4 mL). Stirring at 0 °C was continued for 14 h. The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ (4 mL) and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (22.5 × 2 cm), using 1:5 EtOAc-hexane, gave 1-[4-(benzyloxy)-3-methoxyphenyl]decan-3-one (203.1 mg, 90%) as a white solid: mp 53–55 °C; FTIR ($CDCl_3$, cast) 2928, 1712, 1514, 1262 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, J = 7.5 Hz, 3 H), 1.24–1.32 (m, 8 H), 1.53–1.59 (m, 2 H), 2.37 (t, J = 7.5 Hz, 2 H), 2.69 (t, J = 7.5 Hz, 2 H), 2.83 (t, J = 7.5 Hz, 2 H), 3.87 (s, 3 H), 5.12 (s, 2 H), 6.65 (dd, J = 1.5, 8.5 Hz, 1 H), 6.74 (d, J = 1.5 Hz, 1 H), 6.80 (d, J = 8.5 Hz, 1 H), 7.27–7.44 (m, 5 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q), 22.6 (t), 23.8 (t), 29.1 (t), 29.2 (t), 29.5 (t), 31.7 (t), 43.1 (t), 44.4 (t), 56.0 (q), 71.2 (t), 112.4 (d), 114.4 (d), 120.1 (d), 127.3 (d), 127.8 (d), 128.5 (d), 134.6 (s), 137.4 (s), 146.6 (s), 149.7 (s), 210.4 (s); exact mass (electrospray) m/z calcd for $C_{24}H_{32}NaO_3$ $(M+Na)^+$ 391.2244, found 391.2240.

(c) (±)-1-[4-(Benzyloxy)-3-methoxyphenyl]decan-3-ol

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$NaBH_4$ (20.2 mg, 0.53 mmol) was added in portions to a stirred solution of 1-[4-(benzyloxy)-3-methoxyphenyl]decan-3-one (196.3 mg, 0.53 mmol) in dry MeOH (5 mL). Stirring was continued for 3 h and then ice water (20 mL) was added. The mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated to give (±)-1-[4-(benzyloxy)-3-methoxyphenyl]decan-3-ol (193.7 mg 97%) as a white solid: mp 65–66 °C; FTIR ($CDCl_3$, cast) 3230, 2920, 1515, 1256 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, J = 7.0 Hz, 3 H), 1.26–1.34 (m, 10 H), 1.42–1.52 (m, 2 H), 1.67–1.81 (m, 2 H), 2.59–2.65 (m, 1 H), 2.71–2.77 (m, 1 H), 3.60–3.65 (m, 1 H), 3.88 (s, 3 H), 5.13 (s, 2 H), 6.68 (dd, J = 2.0, 8.0 Hz, 1 H), 6.77 (d, J = 2.0 Hz, 1 H), 6.81 (d, J = 8.0 Hz, 1 H), 7.28–7.45 (m, 5 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q), 22.7 (t), 25.7 (t), 29.3 (t), 29.7 (t), 31.76 (t), 31.84 (t), 37.7 (t), 39.2 (t), 56.0 (q), 71.3 (t), 71.4 (d), 112.5 (d), 114.4 (d), 120.2 (d), 127.3 (d), 127.7 (d), 128.5 (d), 135.6 (s), 137.5 (s), 146.4 (s), 149.7 (s); exact mass (electrospray) m/z calcd for $C_{24}H_{34}NaO_3$ $(M+Na)^+$ 393.2400, found 393.2401.

*(d) (±)-4-[(3-Hydroxydecyl]-2-methoxyphenol [(±)-**2**]*[32]

- **(±)-2**

10% Pd/C (9.4 mg) was added to a solution of (±)-1-[4-(benzyloxy)-3-methoxyphenyl]decan-3-ol (190.0 mg, 0.51 mmol) in EtOH (6 mL) and the mixture was stirred under H_2 (balloon) for 1.5 h. The mixture was filtered through a short pad of Celite which was rinsed with EtOAc. Evaporation of the solvent gave (±)-**2** (143.5 mg, 99%) as a white solid that was pure (^{1}H NMR): 59–60 °C; FTIR ($CDCl_3$, cast) 3400, 2921, 1517, 1154 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (t, J = 7.0 Hz, 3 H), 1.25–1.34 (m, 10 H), 1.41–1.52 (m, 2 H), 1.54 (br s, OH), 1.67–1.80 (m, 2 H), 2.57–2.63 (m, 1 H), 2.70–2.76 (m, 1 H), 3.60–3.65 (m, 1 H), 3.86 (s, 3 H), 6.69–6.71 (m, 2 H), 6.83 (d, J = 8.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.1 (q), 22.7 (t), 25.6 (t), 29.3 (t), 29.7 (t), 31.79 (t), 31.83 (t), 37.6 (t), 39.4 (t), 55.9 (q), 71.5 (d), 111.1 (d), 114.3 (d), 120.9 (d), 134.2 (s), 143.7 (s), 146.5 (s); exact mass (electrospray) m/z calcd for $C_{17}H_{27}O_3$ $(M–H)^–$ 279.1966, found 279.1966.

2.2.4 Synthesis of Compound **R**

*Synthesis of (4) (Compound **R**)*

2.2.4.1 Methyl

*(2S)-4-[(tert-butyldimethylsilyl)oxy]-2-[(4-methoxyphenyl)methoxy]butan-oate (**7.1**)*

4.4 **7.1**

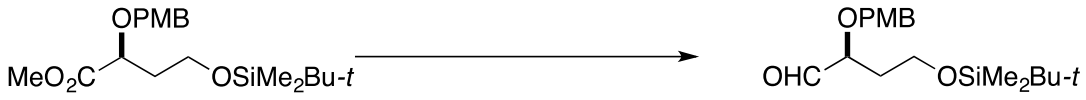
Freshly made Ag_2O (170 mg, 0.73 mmol) was added to a solution of freshly made PmbBr (118 mg, 0.59 mmol) and **4.4** (121.5 mg, 0.49 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred at 35 °C for 12 h (protection from light, N_2 atmosphere). The mixture was filtered through a pad of Celite, using CH_2Cl_2 as a rinse and the filtrate was evaporated to give the crude product. By tlc (silica, 1:5 EtOAc-hexane) the mixture was cleaner than when NaH as used.

4.4 (146.8 mg, 0.59 mmol) and freshly made PmbBr (142.6 mg, 0.71 mmol) in dry DMF (1.5 mL) was added to a stirred suspension of NaH (57-63% w/w in mineral oil, 17.0 mg, 0.71 mmol) and Bu_4NI (21.8 mg, 0.06 mmol) in DMF (3 mL) (N_2 atmosphere). Stirring was continued for 11 h, ice water was added and the mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed several times with water and then with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (21.5 × 2 cm), using 5:95 (EtOAc-hexane), gave **7.1** (60.3 mg, 28.5%) as an oil.

A solution of **4.4** (232.2 mg, 0.93 mmol) and freshly prepared PBMTCA (394.0 mg, 1.40 mmol) (1.5 eq) in toluene (10 mL) was treated with La(OTf)3 (27.4 mg, 0.05

mmol) (0.05 eq) at room temperature. After completion of the reaction in 10 min., the reaction mixture was evaporated and purified by column chromatography on silica gel (19.5 × 2.5 cm) using 5:95 (EtOAc-hexane) to afford **7.1** (94.6 mg, 27.5%) as an oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0,04 (s, 6 H), 0.88 (s, 9 H), 1.86–2.00 (m, 2 H), 3.67–3.76 (m, 2 H), 3.74 (s, 3 H), 3.80 (s, 3 H), 4.14 (q, J = 4.2 Hz, 1 H), 4.36 (d, J = 10.8 Hz, 1 H), 4.62 (d, J = 12.0 Hz, 1 H), 6.86–6.88 (m, 2 H), 7.27–7.29 (m, 2 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ −5.3, 18.3, 26.0, 36.2, 51.9, 55.3, 58.7, 72.3, 74.7, 113.9, 129.5, 129.8, 159.4, 173.7.

*2.2.4.2 (2S)-2-Benzyloxy-4-[(tert-butyldimethylsilyl)oxy]butanal (**7.2**)*



7.1 **7.2**

DIBAL-H (1 M in hexane, 0.24 mL, 0.24 mmol) (1.2 eq) was added by syringe at a slow dropwise rate to a stirred and cooled (–78 °C) solution of **7.1** (73.6 mg, 0.20 mmol) in dry hexane (5 mL). Stirring at –78 °C was continued for 2.5 h and the mixture was quenched by dropwise addition of MeOH (5 mL), followed by saturated aqueous Rochelle salt (40 mL). The mixture was extracted with EtOAc (3 × 20 mL) and the combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (21.0 × 2.5 cm), using 5:95 EtOAc-hexane, gave **7.2** (50 mg, 74%) as an oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0,04 (s, 6 H), 0.88 (s, 9 H), 1.83–1.95 (m, 2 H), 3.70–3.78 (m, 2 H), 3.81 (s, 3 H), 3.94–3.97 (m, 1 H), 4.50 (d, J = 11.4 Hz, 1 H), 4.61 (d, J = 11.4 Hz, 1 H), 6.87–6.90 (m, 2 H),

7.27–7.29 (m, 2 H), 9.65 (d, J = 2.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ −5.3, 18.4, 26.0, 34.0, 55.4, 58.2, 72.4, 80.5, 114.1, 129.7, 129.8, 159.6, 203.9.

*2.2.4.3 4-Hydroxy-3-iodo-5-methoxybenzaldehyde (**7.3**)*

1.1 **7.3**

Vanillin (2.02 g, 13.3 mmol) as added to a vigorously stirred solution of $NaHCO_3$ (1.34 g, 16.0 mmol) and KI (3.31 g, 19.9 mmol) in water (50 mL). I_2 (3.37 g, 13.3 mmol) was added in four portions over 30 min. Stirring was continued for 36 h and the mixture was filtered (filter paper in Buchner funnel). The solid was washed in the filter funnel with 20%w/v aqueous $Na_2S_2O_3$ and then with water, and dried under oil pump vacuum to give the **7.3** (3.50 g, 94.6 %) as a pale brown solid: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.97 (s, 3 H), 6.68 (s, OH), 7.38 (d, J = 1.8 Hz, 1 H), 7.82 (d, J = 1.8 Hz, 1 H), 9.77 (s, 1 H).

*2.2.4.4 3,4-Dihydroxy-5-methoxybenzaldehyde (**7.4**)*

7.3 **7.4**

20% aqueous NaOH (75 mL) was poured into a flask containing **7.3** (3.5 g, 12.6 mmol) and $CuSO_4.5H_2O$ (627.3 mg, 2.51 mmol) and the mixture was stirred and refluxed for 17 h. The mixture was cooled to below 10 °C and acidified with 6 N hydrochloric acid to pH 2 (pH paper). The mixture was filtered and the filtrate was extracted with EtOAc (3 × 150 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. The dark grey residue was crystallized from PhMe to afford **7.4** (1.94 g, 92%) as a solid: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.91 (s, 3 H), 7.04 (d, J = 1.8 Hz, 1 H), 7.09 (d, J = 1.2 Hz, 1 H), 9.68 (s, 1 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 56.7, 105.8, 112.5, 129.4, 142.5, 147.0, 149.9, 193.0.

*2.2.4.5 3,4-Bis(benzyloxy)-5-methoxybenzaldehyde (**7.5**)*

7.4 **7.5**

BnBr (0.21 mL, 1.74 mmol) was added to a stirred mixture of **7.4** (4.77 g, 28.3 mmol) and K_2CO_3 (9.4 g, 68.0 mmol) (2.4 eq) in dry acetone (100 mL). The mixture was refluxed for 8 h, cooled, filtered inorganic salt, and washed with acetone. The filtrate was evaporated and flash chromatography of the residue over silica gel (14.0 × 6.5 cm), using 1:4 EtOAc-hexane, gave **7.5** (6.41 g, 64.9%) as an oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.89 (s, 3 H), 5.14 (s, 2 H), 5.18 (s, 2 H), 7.14 (d, J = 1.2 Hz, 1 H), 7.19 (d, J = 1.8 Hz, 1 H), 7.29–7.46 (m, 10 H), 9.83 (s, 1 H).

*2.2.4.6 [3,4-Bis(benzyloxy)-5-methoxyphenyl]methanol (**7.6**)*

7.5 **7.6**

$NaBH_4$ (1.34 g, 35.4 mmol) (1.2 eq) was added portionwise to a stirred and cooled (0 °C) solution of **7.5** (10.3 g, 29.5 mmol) in dry MeOH (60 mL). The cold bath was left in place but not recharged and stirring was continued for 2 h. The MeOH was evaporated and ice-cold water was added to the residue. The mixture was extracted with EtOAc (3 × 60 mL) and the combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (14.0 × 6.5 cm), using 1:3, 1:2 EtOAc-hexane, gave **7.6** (9.5 g, 91.9%) as an oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.83 (s, 3 H), 4.56 (s, 2 H), 5.04 (s, 2 H), 5.08 (s, 2 H), 6.60 (d, J = 1.2 Hz, 1 H), 6.64 (d, J = 1.8 Hz, 1 H), 7.28–7.49 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 56.2, 65.4, 71.2, 75.2, 104.4, 105.9, 127.5, 127.9, 128.0, 128.3, 128.60, 128.65, 136.9, 137.0, 137.2, 137.9, 152.8, 153.9.

*2.2.4.7 1,2-Bis(benzyloxy)-5-(bromomethyl)-3-methoxybenzene (**7.7**)*

7.6 **7.7**

A solution of PBr_3 (34.8 μL, 0.37 mmol) (1.3 eq) in dry Et_2O (1 mL) was added slowly by syringe to a stirred solution of **7.6** (98.9 mg, 0.28 mmol) in dry Et_2O (5 mL). Stirring was continued for 16 h and the reaction mixture was poured into cold water and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with water and brine, dried (Na_2SO_4) and evaporated to give the **7.7** (109.9 mg, 94.2%) as an oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.85 (s, 3 H), 4.44 (s, 2 H), 5.02 (s, 2 H), 5.10 (s, 2 H), 6.63 (d, J = 2.4 Hz, 1 H), 6.68 (d, J = 1.8 Hz, 1 H), 7.28–7.45 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 34.4, 56.4, 71.4, 75.3, 106.8, 108.5, 127.6, 128.0, 128.1, 128.3, 128.6, 128.7, 133.3, 137.0, 137.9, 138.1, 152.8, 153.9.

*2.2.4.8 Diethyl {[3,4-bis(benzyloxy)-5-methoxyphenyl]methyl}phosphonate (**7.8**)*

7.7 **7.8**

$(EtO)_3P$ (6.84 mL, 39.9 mmol) (5 eq) was added to a stirred solution of **7.7** (3.3 g, 7.97 mmol) in dry PhH (150 mL) and the solution was refluxed for 40 h and then evaporated. Flash chromatography of the residue over silica gel (15.5 × 6.5 cm), using EtOAc, gave **7.8** (3.7 g, 99.5%) as an oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.21 (t, J = 7.2 Hz, 6 H), 3.03 (s, 1 H), 3.07 (s, 1 H), 3.81 (s, 3 H), 3.90–4.01 (m, 4 H), 5.01 (s, 2 H), 5.08 (s, 2 H), 6.53 (d, J = 1.8 Hz, 1 H), 6.57 (d, J = 1.8 Hz, 1 H), 7.24−7.44 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 16.5, 16.6, 34.5, 56.3, 62.25, 62.30, 71.2, 75.1, 107.5, 109.1, 127.5, 127.88, 127.94, 128.2, 128.58, 128.62, 136.70, 136.73, 137.2, 138.0, 152.6, 153.7.

*2.2.4.9 4-(2-Chloroethyl)benzene-1,2-diol (**8.2**)*

8.1 **8.2**

Et_3SiH (8.64 mL, 54.1 mmol) (1 eq) was added dropwise to a stirred suspension of **8.1** (10.1 g, 54.1 mmol) in CF_3CO_2H (60 mL). Stirring was continued for 10 h and then more Et_3SiH (8.64 mL, 54.1 mmol) (1 eq) was added (the reaction was not complete as judged by tlc) and stirring was continued for an additional 24 h. The mixture was poured into ice water (200 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to afford a white solid that was washed with petroleum ether (bp 35–60 °C) and dried under oil pump vacuum to give **8.2** (6.7 g, 71%) as a white solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.96 (t, J = 7.5 Hz, 2 H), 3.66 (t, J = 7.5 Hz, 2 H), 4.99 (s, OH), 5.09 (s, OH), 6.66 (dd, J = 2.0, 8.0 Hz, 1 H), 6.75 (d, J = 2.0 Hz, 1 H), 6.81 (d, J = 8.0 Hz, 1 H).

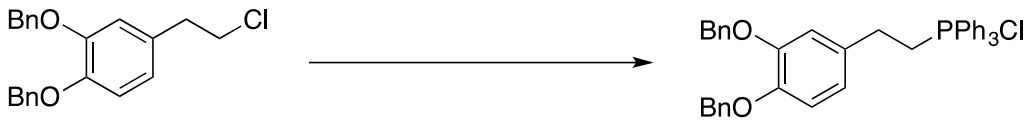
*2.2.4.10 1,2-Bis(benzyloxy)-4-(2-chloroethyl)benzene (**8.3**)*

8.2 **8.3**

BnCl (13.4 mL, 116.2 mmol) was added to a stirred mixture of **8.2** (6.69 g, 38.7 mmol), K_2CO_3 (13.4 g, 96.8 mmol) and NaI (2.9 g, 19.4 mmol) in dry acetone (60

mL). The stirred mixture was refluxed for 48 h, cooled and diluted with water (150 mL). The mixture was extracted with Et_2O (3 × 100 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (27 × 5.5 cm), using 5:95 EtOAc-hexane, gave the **8.3** (10.8 g, 79%) as an oil, which was pure as judged by H NMR. The monobenzyl phenol (1.7 g, 16.9%) was also isolated; evidently, I need to add more benzyl chloride or, better, recycle the monobenzyl compound: ^{1}H NMR ($CDCl_3$, 700 MHz) δ 2.95 (t, J = 7.7 Hz, 2 H), 3.63 (t, J = 7.7 Hz, 2 H), 5.14 (s, 2 H), 5.15 (s, 2 H), 6.73 (dd, J = 2.1, 8.4 Hz, 1 H), 6.81 (d, J = 1.4 Hz, 1 H), 6.89 (d, J = 7.7 Hz, 1 H), 7.30–7.45 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 38.7 (t), 45.1 (t), 71.41 (t), 71.47 (t), 115.2 (d), 116.1 (d), 121.8 (d), 127.3 (d), 127.4 (d), 127.77 (d), 127.81 (d), 131.5 (s), 137.3 (s), 137.4 (s), 148.1 (s), 148.9 (s).

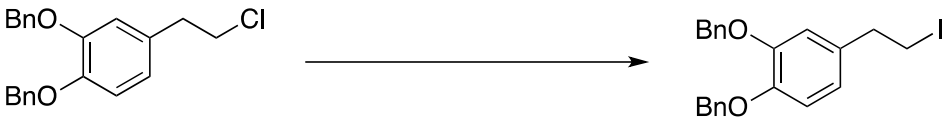
*2.2.4.11 (3,4-bis(benzyloxy)phenethyl)chlorotriphenyl- λ^5-phosphane (**8.4**)*



8.3 **8.4**

This reaction does not work, so I made the iodide.

*2.2.4.12 1,2-Bis(benzyloxy)-4-(2-iodoethyl)benzene (**8.5**).*



8.3 **8.5**

NaI (13.8 g, 91.9 mmol) was added to a solution of **8.3** (10.8 g, 30.6 mmol) in butan-2-one (150 mL) and the mixture was refluxed for 66 h. The solvent was evaporated and CH_2Cl_2 100 mL) was added to the residue. The resulting suspension was filtered and the filtrate was dried (Na_2SO_4) and evaporated. Flash chromatography of the residue over silica gel (27 × 5.5 cm), using 1:6 EtOAc-hexane, gave the **8.5** (12.6 g, 92.9%) as a white solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.07 (t, J = 8.0 Hz, 2 H), 3.27 (t, J = 8.0 Hz, 2 H), 5.15 (s, 2 H), 5.16 (s, 2 H), 6.72 (dd, J = 2.0, 8.0 Hz, 1 H), 6.79 (d, J = 2.5 Hz, 1 H), 6.89 (d, J = 8.0 Hz, 1 H), 7.30–7.47 (m, 10 H); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 5.9 (t), 39.9 (t), 71.4 (t), 71.5 (t), 115.3 (d), 115.6 (d), 121.3 (d), 127.3 (d), 127.4 (d), 127.78 (d), 127.82 (d), 128.47 (d), 128.49 (d), 134.1 (s), 137.3 (s), 137.4 (s), 148.0 (s), 149.0 (s).

*2.2.4.13 (3,4-bis(benzyloxy)phenethyl)iodotriphenyl- λ^5-phosphane(**8.6**).*

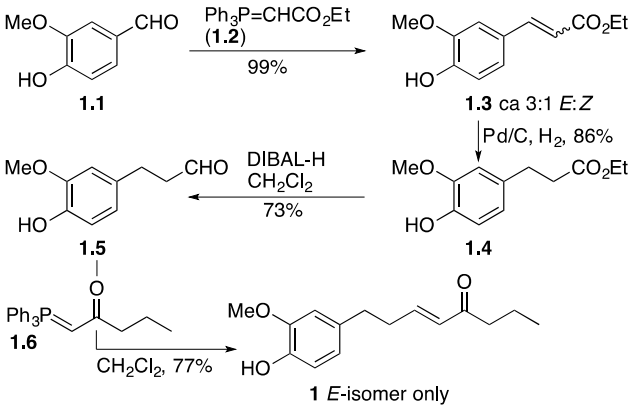
8.5 **8.6**

Ph_3P (11.2 g, 42.7 mmol) was added to a stirred solution of **8.5** (12.6 g, 28.4 mmol) in PhMe (150 mL) and the mixture was refluxed for 72 h (N_2 atmosphere), cooled and evaporated. The residue was washed several times with Et_2O and dried under oil pump vacuum to give the **8.6** (20.4 g, 101%) as a white solid, which evidently contained some (1.3%) Ph_3P: ^{1}H NMR ($CDCl_3$, 700 MHz) δ 2.95 (m, 2 H), 4.06 (m, 2 H), 5.08 (s, 2 H), 5.26 (s, 2 H), 6.56 (dd, J = 2.1, 8.4 Hz, 1 H), 6.70 (d, J = 8.4 Hz, 1 H), 7.16–7.79 (m, 26 H).

2.3 Results and Discussion

2.3.1 Synthesis of compound 1

Compound **1** was synthesized by the method summarized in Scheme 1. Vanillin (**1.1**) underwent efficient Wittig reaction with ethyl 2-(triphenyl-λ^5-phosphanylidene)acetate (**1.2**) to form a 3:1 mixture of *E* and *Z* esters **1.3**.[35] Hydrogenation afforded the saturated ester **1.4** and DIBAL-H reduction then gave aldehyde **1.5**.[35] This underwent Wittig reaction with the readily available keto ylide **1.6**,[36] and the resulting enone **1** could be isolated in good yield as the desired *E* isomer which was spectroscopically identical with the compound extracted[25] from GOP. Unlike the natural material which was an oil, the synthetic enone was obtained as a crystalline solid, mp 35–38 °C.



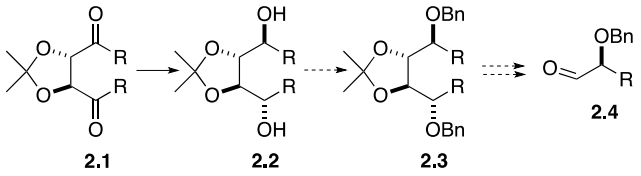
Scheme 1. Synthesis of compound 1

2.3.2 Synthesis of compound 3

I first developed a route to the unnatural homolog **3** before making the natural material **2**.

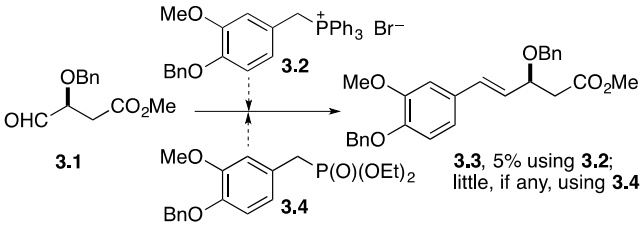
As the absolute configuration and optical purity of compound **2** was unknown at the time, I made the arbitrary decision to use tartaric acid for our work, along the lines shown in Scheme 2. This plan was based on a report[56] that the diketone **2.1** (R =

n-C_5H_{11}) derived from d-(–)-tartaric acid could be reduced stereoselectively with K-Selectride to the diol **2.2** (R = *n*-C_5H_{11}). However, when I attempted to follow an analogous sequence to make **2.4** (R = *n*-C_8H_{17}), using *n*-octylmagnesium bromide instead of the reported pentyl reagent, I obtained low yields (ca 43%) of the desired diketone **2.1** (R = C_8H_{17}). This outcome prompted us to change to a route based on l-(–)-malic acid. The enantiomer is, of course, available, although it is more expensive, and there would also be an opportunity to invert the stereochemistry of the hydroxyl-bearing carbon at a suitable stage.

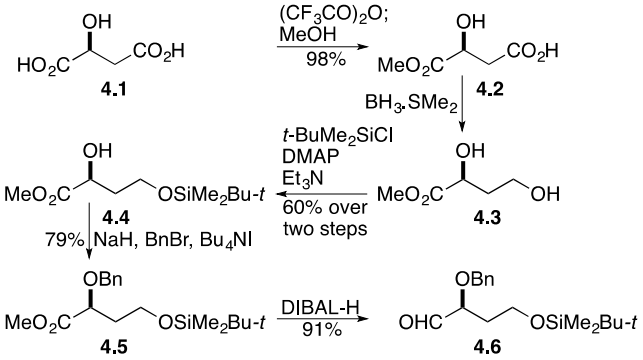


Scheme 2. Initial approach based on tartaric acid

Initially, I converted malic acid into its *O*-benzyl dimethyl ester and reduced that selectively (Scheme 3) to methyl (2*S*)-4-oxo-3-(phenylmethoxy)butanoate (**3.1**),[57] but in our hands (working at –78 °C instead of the reported[57a] temperature of –90 °C) the yield in the reduction step (DIBAL-H) was low (47%). In addition, attempts to form the olefin **3.3** either by Wittig reaction with the phosphonium salt **3.2**[58] or by the Horner-Wadsworth-Emmons process, using phosphonate **3.4**,[48] were unsatisfactory (Scheme 3). Accordingly, we modified the route to one that involves conversion of malic acid into aldehyde **4.6** (Scheme 4), followed by olefination with a benzylic phosphonate.

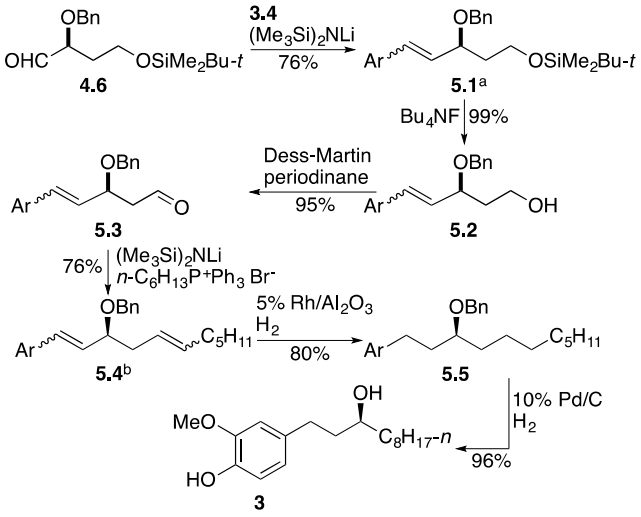


Scheme 3. Initial approach based on malic acid



Scheme 4. Synthesis of aldehyde 4.6

The aldehyde **4.6** was made by regioselective esterification of l-malic acid (**4.1**→**4.2**), following a literature procedure.[40] The remaining carboxyl group was then easily reduced[40] with $BH_3.SMe_2$ and the resulting primary hydroxyl was protected by silylation (**4.2**→**4.3**→**4.4**). The next step, *O*-benzylation of the secondary hydroxyl in **4.4** was tried by two methods. Use of freshly-prepared Ag_2O[46] and BnBr in CH_2Cl_2[45] gave the desired product in 30% yield, but the efficiency of the benzylation was improved (79% yield) by using NaH and BnBr in DMF in the presence[47] of Bu_4NI. Finally, DIBAL-H reduction afforded the aldehyde **4.6** needed as one of the components for the intended olefination.



[a]Ar = (4-benzyloxy-3-methoxy)phenyl. [b]A single isomer was obtained when starting from *E*-**5.3**.

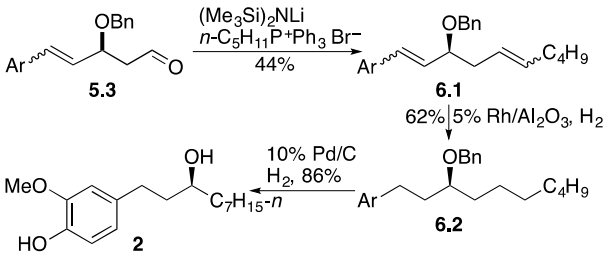
Scheme 5. Olefination of aldehyde 4.6 [Ar = (4-benzyloxy-3-methoxy)phenyl]

Deprotonation of phosphonate **3.4** with $(Me_3Si)_2NLi$ in a 4:1 (v/v) mixture of THF and HMPA,[59] followed by addition of aldehyde **4.6** gave the olefins **5.1**. In the first experiment the *E* and *Z* isomers were isolated in yields of 70% and 6%, respectively, but in subsequent work the isomer mixture was used. Use of HMPA in the olefination was essential; without it very low yields were obtained. Desilylation proceeded without incident, but oxidation of the resulting alcohols (**5.2**) with PCC gave a very low yield. Fortunately, the Dess-Martin periodinane was extremely effective in generating the desired aldehyde **5.3** (95% yield), and a Wittig reaction then took the route as far as **5.4**, which was obtained as a mixture of isomers. I had originally intended to subject **5.4** to hydrogenation of the double bonds over Pd/C and then in situ hydrogenolysis of the benzyloxy groups but, in the event, significant hydrogenolysis of the allylic C—O bond occurred. Therefore **5.4** was first reduced over 5% Rh-Al_2O_3 to saturate the double bonds (monitored by ^{1}H NMR and tlc), and then the benzyl groups were removed by hydrogenolysis (Pd/C) so as to obtain the target alcohol (**5.4**→**5.5**→**3**).

In order to establish the optical purity of **3** I needed a racemic sample. Several attempts to selectively oxidize the aliphatic hydroxyl of **3** were unsuccessful,[60] but the phenolic hydroxyl was easily benzylated (BnBr, K_2CO_3, 96%) and the remaining secondary hydroxyl could be oxidized with the Dess-Martin reagent. Then reduction ($NaBH_4$) and hydrogenolysis provided a reference sample of racemic **3**. Chiral HPLC analysis served to establish that the ee of (*S*)-**3** was 90%.

2.3.3 Synthesis of compound 2

With a practical route to (*S*)-**3** I next diverted the advanced intermediate **5.3** to the natural product **2**. To this end, **5.3** was subjected to Wittig reaction with the ylide generated from pentyltriphenylphosphonium bromide (Scheme 6). The resulting dienes were first hydrogenated over Rh-Al_2O_3 and then subjected to hydrogenolysis over Pd/C to afford (*S*)-**2** as a crystalline solid (**6.1**→**6.2**→**2**). To make a racemic sample, the phenolic hydroxyl of (*S*)-**2** was benzylated, following our earlier procedure, and the secondary alcohol was oxidized to a ketone, which was then reduced ($NaBH_4$) and subjected to hydrogenolysis to liberate the phenol. With racemic and optically active samples in hand, chiral HPLC showed that synthetic (*S*)-**2** had an ee of 68% and, surprisingly, that the natural sample was a 1:1.7 mixture of *R* and *S* isomers. I did not establish why the optical purity of (*S*)-**2** is lower than that of the homolog (*S*)-**3**. The negative value of the specific rotation of natural phenolic alcohol **2** must be due to a minor impurity, as the predominance of the *S*-isomer should result in a positive value.



Scheme 6. Conversion of 5.3 to 6.3

3 *Evaluation of Anti-obesity Effect by GOP Extract and the Constituents*

3.1 Introduction

Obesity is defined as an abnormal or excessive fat accumulation and is recognized as a major risk factor for diabetes, cardiovascular disease, and cancer by the World Health Organization (WHO). The number of obese people more than doubled between 1980 and 2014. Reducing lipid accumulation causing body weight loss is crucial to management of obesity. The development of effective treatments for overweight and obese patients has become very desirable in recent years.

Adipose tissue is a major metabolic organ, and roughly divided into two types: one is white adipose tissue (WAT) that stores energy in the form of triglyceride; the other is brown adipose tissue (BAT). BAT is involved in the dissipation and expenditure of energy as heat. This process of thermogenesis is induced by high-fat diets and cold exposure.[1,2] Therefore, adipocytes play a key role in energy homeostasis. Activating BAT by stimulating sympathetic nerve activity (SNA) is one of the principal strategies to enhance energy expenditure and lipolysis.[63–65] This is an effective and practical approach to treating obesity-related diseases.

Aframomum melegueta is a herbaceous plant, widely distributed throughout Nigeria, Ghana, Guinea, and other countries in West Africa.[66] Its seeds are called Grains of Paradise (GOP), Guinea pepper, alligator pepper, or melegueta pepper. It has been traditionally used as a spice for flavouring food and as a remedy for digestive and intestinal health, dysentery, migraine, and fever.[67] Recently, a number of studies have reported that GOP extract has a range of activities such as antibacterial, repellent,[68,69] antioxidant, anti-inflammatory,[22,70] and hypoglycemic[71] effects. Moreover, GOP contains many non-volatile pungent compounds such as 6-paradol, 6-gingerol, and 6-shogaol.[11] These compounds possess a vanillyl moiety, and are structurally similar to capsaicin and capsiate, which are found in chili pepper.[72] It is well known that capsinoids, including capsaicin and capsiate, exert an anti-obesity effect by stimulating

SNA and hence BAT thermogenesis.[26a] Similarly, it might be expected that GOP extract containing vanilloids would activate BAT SNA and BAT thermogenesis. In the present study, GOP extract was administered during an animal breeding test to investigate its efficacy in obesity prevention.

3.2 Materials and Methods

3.2.1 General Procedures

Tween 80 was purchased from MP Biomedicals, LLC (Santa Ana, CA, USA). Saline was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Other commercially available products, including urethane for anesthesia, were purchased from Wako Chemicals (Osaka, Japan).

3.2.2 Animal Breeding

GOP was provided by Share Trade Inc (Tokyo, Japan). Dried GOP seed powder (about 5 kg) underwent methanol extraction all night at room temperature (20 ± 2 °C) and the extract was obtained in 5.6% yield based on the powder. The extract was dissolved in 10% Tween 80 saline solution containing 10% ethanol, and was used for oral administration during neural recording.[73,74]

Five-week-old male mice (24.8 ± 0.9 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan) and placed in a breeding environment (25 ± 1 °C, 12 h light-dark cycle). The mice were fed a normal diet (ND) for a period of one week. Both feed and water were available *ad libitum*. After preliminary breeding, they were divided into five groups. Three of the groups were fed a high-fat diet (HFD), composed of ND, 20% lard, 1% cholesterol powder, and 0.25% sodium cholate, containing either 2% GOP seed powder, 0.3% GOP extract, or 1% GOP extract.[75] Two control groups were fed either an

ND or HFD only. At 11 weeks of age, the mice were dissected to obtain samples of serum, the liver, and fat.

All experimental procedures were approved by the Gifu University Animal Care and Use Committee.

3.2.3 Lipid Analysis of Serum and Liver

3.2.3.1 Measurement of Serum Total Cholesterol (TC) Concentration

Blood samples were stored at room temperature (20 ± 2 °C) for 1 h, and then centrifuged at 3500 rpm for 15 min at room temperature (20 ± 2 °C). The TC analysis was performed using TC E-test kits according to the manufacture's protocol (Wako Pure Chemical Industries, Ltd.).

3.2.3.2 Measurement of Serum High-Density Lipoprotein Cholesterol (HDL-C) Concentraion

The HDL-C analysis was performed using HDL-C E-test kits according to the manufacturer's protocol (Wako Pure Chemical Industries, Ltd.).

3.2.3.3 Measurement of Serum Triglyceride (TG) Concentration

The TG analysis was performed using TG E-test kits according to the manufacturer's protocol (Wako Pure Chemical Industries, Ltd.).

3.2.3.4 Hepatic Lipid Extraction

Liver tissue (40 mg) was homogenized with 0.1 methyl acetate, methanol, and chloroform (4 : 10 : 5) at 4000 rpm for 1 min, and the homogenates were centrifuged at 5800 rpm for 10 min.

3.2.3.5 Measurement of Hepatic TC Concentration

The TC analysis was performed using TC E-test kits according to the manufacturer's protocol (Wako Pure Chemical Industries, Ltd.).

3.2.3.6 Measurement of Hepatic TG Concentration

The TG analysis was performed using TG E-test kits according to the manufacturer's protocol (Wako Pure Chemical Industries, Ltd.).

3.2.4 Statistical Analysis

All data were expressed as means ± SE values. Statistical significance of differences was evaluated using the Student's t-test. The difference was considered to be significant if $p < 0.05$.

3.3 Results and Discussion

To investigate the anti-obesity effect of GOP and GOP extract, the mice were divided into five groups after preliminary breeding. Mice willingly ate the feed provided in five meals, including those in the group receiving 2% GOP. Despite this, the 2% GOP group exhibited significantly lower body weight gain over the five weeks of feeding compared with the HFD group ($p < 0.01$) (Fig. 1). The body weight gain of the groups fed GOP extract with the HFD notably decreased, to a similar level as those of the mice in the ND control group (0.3% GOP extract group: $p < 0.001$, 1% GOP extract group: $p < 0.001$). Epididymal fat and mesenteric fat weights significantly decreased in the groups receiving GOP in HFD when compared with those in the ND control group (0.3% GOP extract group: $p < 0.001$ and < 0.01, respectively; 1% GOP extract group: $p < 0.001$ and < 0.01, respectively; 2% GOP group: $p < 0.01$ and < 0.001, respectively).

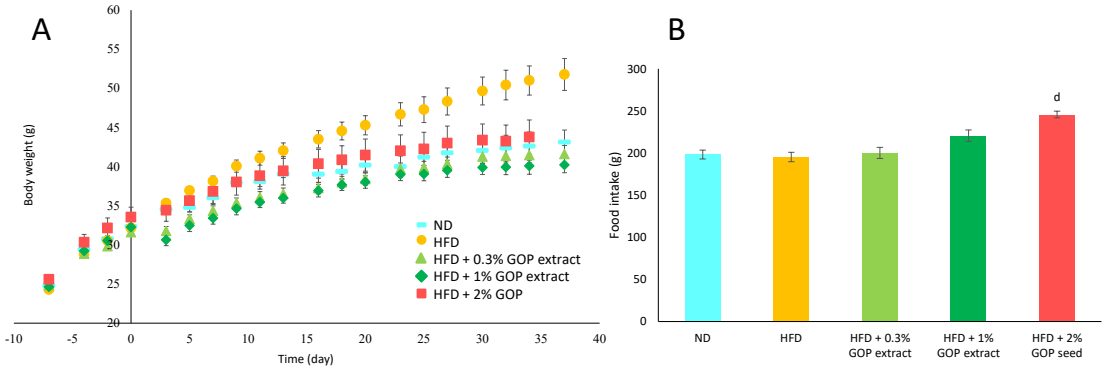


Fig. 1. Body weight over time in mice (n = 9) fed a normal diet (ND), high-fat diet (HFD), HFD including 0.3% GOP extract, HFD including 1% GOP extract, and HFD including 2% GOP (a) and the total food intake per mouse (b). d: $p < 0.01$ compared with HFD values

Lipid accumulation was further investigated to explore the anti-obesity effect of GOP on mice. HFD intake significantly increased the total cholesterol (TC) and triglyceride (TG) concentration in the liver. GOP intake had no significant impact on liver weight (Fig. 2). However, the GOP and GOP extract groups had significantly decreased levels of hepatic TC and TG. This effect was most marked in the 1% GOP extract group, whose TC and TG levels (190 and 260 mg/dL, respectively) were far lower than those of the HFD group (257 and 388 mg/dL, respectively). In contrast, there was no significant difference in serum levels of either TC or high-density lipoprotein-cholesterol (HDL-C) among any of the groups on the HFD. Serum TG concentrations significantly increased in the GOP intake group (0.3% GOP extract group: $p < 0.05$; 1% GOP extract group: $p < 0.01$) (Fig. 3).

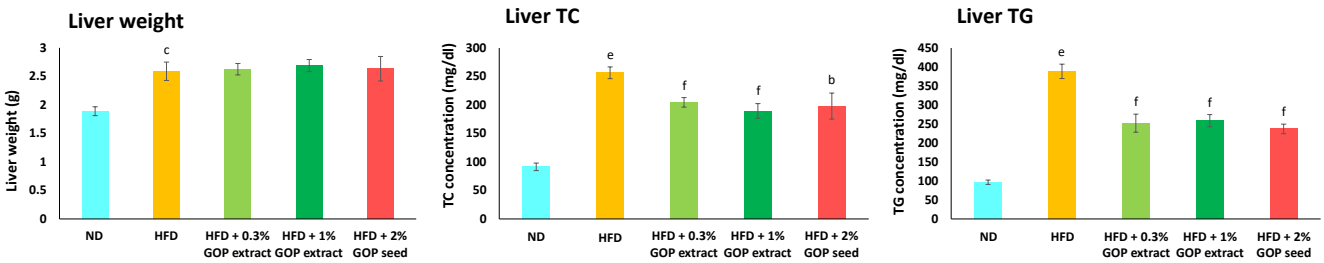


Fig. 2. Liver weight, total cholesterol (TC), and triglyceride (TG) concentrations in mice (n = 9) fed a normal diet (ND), high-fat diet (HFD), HFD including 0.3% GOP extract, HFD including 1% GOP extract, and HFD including 2% GOP. (c, e): $p < 0.01$, 0.001 compared with ND values respectively. (b, f): $p < 0.05$, 0.001 compared with HFD values, respectively.

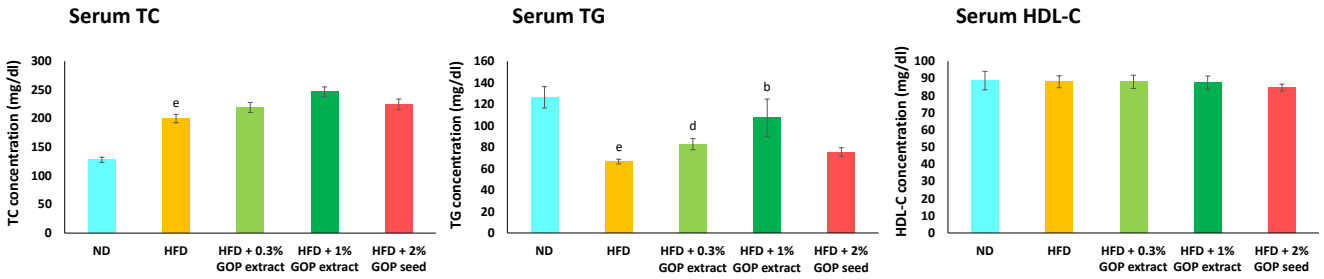


Fig. 3. Total cholesterol (TC), triglyceride (TG), and high density lipoprotein-cholesterol (HDL-C) concentrations in serum of mice (n = 9) fed a normal diet (ND), high-fat diet (HFD), HFD including 0.3% GOP extract, HFD including 1% GOP extract, and HFD including 2% GOP. (e): $p < 0.001$ compared with ND values. (b, d): $p < 0.05$, 0.01 compared with HFD values, respectively.

The animal breeding experiment in this study was performed to investigate the anti-obesity effects of GOP and GOP extract. The results are shown in Fig. 1–3. The feeding of HFD containing 1% GOP extract for five weeks greatly suppressed body weight gain and fat accumulation in mice. This suggested that GOP has the potential to inhibit lipid accumulation. GOP intake significantly decreased TC and TG concentrations in liver tissue, and prevented the pale discoloration of the liver, which was observed in the HFD group; however, it had no significant impact on liver weight.

The serum TC and HDL-C concentrations in the GOP intake groups were unchanged compared with those in the HFD group mice. Serum TG concentrations in the GOP intake groups significantly increased. These results suggested that hepatic lipid metabolism was improved by GOP intake. Consequently, GOP intake potently decreases fat accumulation in HFD mice. De Creamer et al. (1994)[76] reported that hepatomegaly was observed in mice fed a diet rich in fish oil, with no significant changes in body, heart, or kidney weights. They concluded that this could be caused by induction of a liver peroxisome, which regulates β-oxidation and biosynthesis of bile acid. This metabolic change occurs via peroxisome proliferator-activated receptors (PPAR), which are involved in adipocyte differentiation.[77–80] One of the components of GOP, 6-shogaol, has been proven to activate PPAR.[81] Hence, the unchanged liver weight of mice in the GOP groups compared with those of mice in the HFD group observed in this present study might be due to increased PPAR-stimulated hepatic peroxisome induction.

4 *Elucidation of the Anti-obesity Effect of GOP Components*

4.1 Introduction

One anti-obesity mechanism is the activation of the sympathetic nervous system in BAT. BAT is distributed throughout the interscapular region and the perirenal area in mice, the paravertebral, supraclavicular, and suprarenal areas in adult humans,[61] and plays a prominent role in non-shivering thermogenesis to produce heat and maintain body temperature.[63] Hence, an increase in the SNA that leads to BAT stimulation, which contributes to the promotion of energy expenditure and the decomposition of fats, is considered one of the most effective ways to improve obesity. In this context, I applied GOP extract and 6-gingerol isolated from GOP extract prepared at various concentrations to rats to elucidate the mechanism of the anti-obesity effect by using electrophysiological techniques. Furthermore, electrical activity in BAT interscapular

nerves was recorded using an electrophysiological method to investigate the anti-obesity mechanism of GOP

4.2 Materials and Methods

4.2.1 General Procedures

Tween 80 (MP Biomedicals, LLC, Santa Ana, CA, USA) and saline (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) were used for preparing the sample solution for oral administration. A Bioelectric Amplifier ER-1 (Bio Research Center Co., Ltd, Nag) was used for amplifying and filtring sympathetic efferent nerve impulses. A PowerLab (AD Instruments Japan Inc., Nagoya, Japan) was used for converting the amplified signals, which were then recorded on a computer using Chart 5 software (AD Instruments Japan Inc.).

4.2.2 Animal

Male Wistar rats aged 11–13 week were purchased from Japan SLC Inc. (Hamamatsu, Japan) and placed in a breeding environment (25 ± 1 °C, 12 h light-dark cycle). They accessed to water and food *ad libitum*.

4.2.3 Neural Activity Measurement

The GOP extract and 6-gingerol were first dissolved in Tween 80 (MP Biomedicals, LLC, Santa Ana, CA, USA) in warm water and then diluted in ethanol and saline (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) solution containing 10% ethanol.[73,74] The 10% Tween 80/saline reagent solution was orally administered using a gastric tube at concentrations of 10 and 30 mg/kg body weight for GOP extract, 5 and 10 mg/kg body weight for 6-gingerol, and 5 mg/kg body weight for capsaicin. The interscapular nerves activity innervating BAT was recorded as previously described.[14]

In short, the rats were anesthetized by intraperitoneal injection with urethane solution (1 g urethane/1 kg body weight) and placed in the prone position and a small incision was made between the scapula. The sympathetic nerves entering BAT were separated from the muscle and cut on the peripheral side. Four efferent nerves were identified and one of the branches was isolated and separated from the connective tissues. The isolated nerve was placed on a pair of silver/silver electrodes (0.3 mm, AG 401325, Nilaco Corp., Tokyo, Japan) and placed in liquid paraffin to protect the nerve against dryness and external noises. Sympathetic efferent discharges detected with electrodes were amplified and filtered by a Bioelectric Amplifier ER-1 (Bio Research Center Co., Ltd, Nagoya, Japan). The amplified impulses were converted to digital signals using a PowerLab system (AD Instruments Japan Inc., Nagoya, Japan) and recorded on a computer using Chart 5 software (AD Instruments Japan Inc.). Spikes above a threshold voltage set just above background levels were counted by the spike histogram (AD Instruments Japan Inc.). The baseline activity was recorded for at least 30 min, after which GOP extract (10 and 30 mg/kg body weight) or 6-gingerol (5 and 10 mg/kg body weight) was oral administered *via* a gastric tube. The BAT SNA was continuously monitored every 5 min.

All experimental procedures were approved by the Gifu University Animal Care and Use Committee.

4.3 Results and Discussion

To clarify the anti-obesity effect of GOP extract in mice, BAT SNA was determined using an electrophysiological method. An initial intragastric infusion of GOP extract (5 mg/kg body weight) immediately decreased BAT SNA by around 10%, and this decrease lasted for at least 1 h. After BAT SNA recovery, a second intragastric

infusion of GOP extract at same concentration produced the same effect on BAT SNA. This procedure was performed on three rats and produced similar results for each rats.

The electrical activity of the sympathetic nerves entering BAT is key to the function of thermogenesis in rodents and humans. I also investigated the effect of GOP extract with different concentrations, 6-gingerol, and capsaicin on SNA using an electrophysiological method to illustrate their role in the stimulation of sympathetic nerve innervating BAT. A single administration of GOP extract (10 and 30 mg/kg body weight) resulted in an immediately decrease in BAT SNA and the decrease in SNA lasted for 70 min (Fig. 4C and D), which was similar to those previously reported using a concentration of 5 mg/kg body weight in this study.[14] Similarly, the efferent discharge of the sympathetic nerve innervating the BAT decreased as a consequence of the intragastric administration of 6-gingerol (5 and 10 mg/kg body weight) and the decrease in SNA lasted for at least 50 min (Fig. 4E and F). Similar results of decreased BAT SNA after GOP extract and 6-gingerol injection were observed in at least three rats. The 10% Tween 80 solution also decrease BAT SNA and the decrease in SNA lasted less than 20 min. The nerve discharges were transiently declined after the oral injection of 10% Tween 80 solution, thereafter, the discharge was recovered around within 20 minutes (Fig. 4A). In this experiment, Tween 80 solution was used as a dissolved solvent of samples, so the all experiment should consider the decreasing BAT SNA for about 20 minutes. In case of the administration of capsaicin (5 mg/kg body weight), the BAT SNA was increased drastically just after oral injection (Fig. 4B).

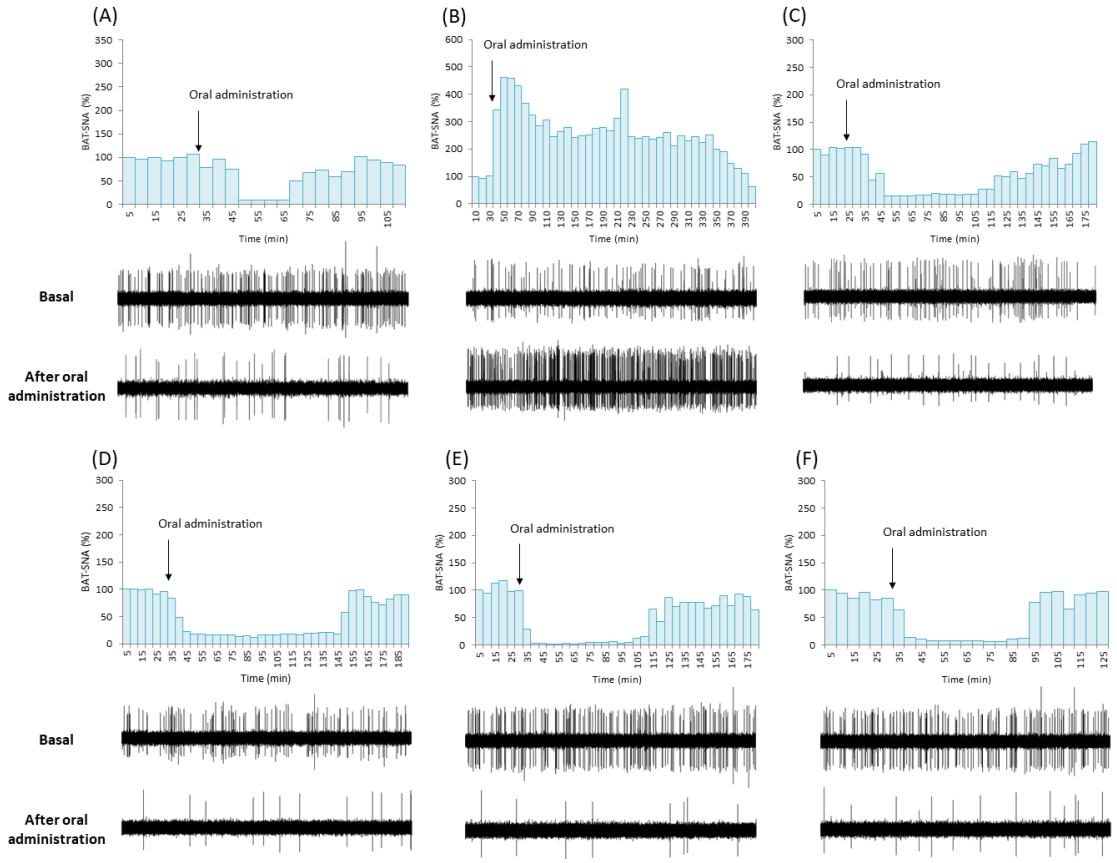


Fig. 4. Effects of (a) 10% Tween 80 solution, (b) capsaicin at 5 mg kg^{-1} body weight, GP extract at (c) 10 and (d) 30 mg kg^{-1} body weight and 6-gingerol at (e) 5 and (f) 10 mg kg^{-1} body weight on SNA in BAT. After recording baseline activity for 30 min, all samples were injected using a gastric tube. Basal nerve activity and nerve activity after oral administration were picked up during 20 s at 20 and 60 min from the start respectively. Similar results were observed from three independent experiments.

The hypothalamus nervous system, especially the ventromedial hypothalamic nucleus (VMH)-sympathetic nervous system and the lateral hypothalamus-parasympathetic nervous system, controls over various metabolic processes, such as ATP, proteins, fatty acid biosynthesis, glycolysis, and the glyconeogenesis system. To regulate the effect on the glucose metabolism of VMH-sympathetic nervous system, electrical stimulus enhanced the selective glucose

uptake in BAT, heart, and skeletal muscles, but not in white adipose tissue and the diaphragm.[82–84] Thus, sympathetic nerves innervating organs and tissues are independent of each other.

In the previous studies, 95% ethanol GOP extract has increased the intercostal sympathetic nerve activity of rat by an intragastric injection,[75] and capsaicin which is a main ingredient of red pepper has also increased interscapular sympathetic nerve activity of rat with decreasing body weight and fat accumulation by oral administration.[85] While on the other hand, GOP methanol extract decreased interscapular sympathetic nerve activity with inhibiting body weight gain and fat accumulation and improving lipid metabolism in our previous study.[14] The main ingredient 6-gingerol which has very similar structure with capsaicin also decreased interscapular BAT SNA in this study. These differences are thought to be due to the experimental method, types of sympathetic nerve or administration method. Otherwise other minor ingredients in GOP extract might affect to decrease sympathetic nerve activity or GOP extract might contribute to preventing fat absorption, lipid biosynthesis, and differentiation of adipocytes.

With regard to fat metabolism, the main ingredient 6-shogaol would be a key compound against PPARγ. 6-Shogaol acts as an agonist of PPARγ, which plays an important role in adipocyte differentiation. In contrast, it has been demonstrated that 6-gingerol does not function as a PPARγ agonist.[80] In addition, PPARγ, which is a transcription factor associated with the terminal differentiation of adipocytes, is mainly expressed in adipose tissues and the expression is increased by the activation of the sympathetic nerve in conjunction with UCP-1 expression, which is related to non-shivering thermogenesis in BAT.[86] In other words, the increase in PPARγ expression through the sympathetic nervous system was closely related to the anti-obesity effects. The results obtained in the present study suggested that 6-gingerol

has a small contribution to PPARγ activation caused by the stimulation of the sympathetic nerve.

Adipose tissues are classified as either WAT or BAT, which have different functions and morphology.[81] BAT is found in infants, young children, and rodents. Recently, BAT has been observed around the scapula, axilla, and vertebral column of adult humans using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) and positron-emission tomography (PET).[63,99,88] Thermogenesis in BAT is mediated by sympathetic stimulation of BAT mitochondrial uncoupling protein 1 (UCP1). I hypothesized that the anti-obesity activity of GOP intake in this mouse breeding test was due to this mechanism, and BAT SNA was involved in the effect of GOP extract.

Capsaicin, which is the major ingredient in chili pepper, has the potential to increase energy expenditure and decrease body fat by activating BAT in humans and rodents.[89–91] This results in stimulation of SNA by transient receptor potential vanilloid 1 (TRPV1), which is expressed in the gastrointestinal tract and sensory nervous system and specifically combines with capsaicin at the vanillyl moiety.[28,92] The results of the present study were consistent with data obtained from experiments with capsaicin and capsiate, which is also found in chili peppers. Surprisingly however, GOP extract exhibits a different mode of action from that of capsaicin and capsiate; GOP extract decreases SNA, rather than increasing it as seen with capsaicin and capsiate.

Capsaicin and capsiate bear structural similarities to the non-volatile vanilloids found in GOP.[93–95] It has also been reported that 6-paradol and 6-shogaol in GOP extract were found to activate TRPV1 through non-covalent bonding.[96] The HPLC analysis showed that GOP extract contains many vanilloid compounds possessing hydrocarbon side chains with a double bond and/or carbonyl group (data not shown). These substructures of vanilloids may be responsible for activating BAT SNA. This might suggest that the vanilloids in GOP contribute to the anti-obesity effect by stimulating

BAT SNA. However, contrary to expectations, BAT SNA was decreased by oral administration of GOP extract.

Crocin, ezetimibe, catechins, and caffeine inhibit intestinal absorption of cholesterol and fat by inactivating pancreatic lipase.[97–99] The aqueous extract of *Zingiber officinale* Roscoe also prevents intestinal lipid absorption through the actions of vanilloid compounds such as 6-gingerol and 6-shogaol.[100] These reports suggest that GOP may inhibit body weight gain in mice by suppressing lipid absorption rather than by activating BAT SNA.

Ⅲ CONCLUSIONS

In conclusion, I isolated and determined the structures of the ingredients in GOP extract. Among the isolated compounds, I identified the novel vanilloids compound **C** and **J**, 1-(4′-hydroxy-3′-methoxyphenyl)-decan-3-ol and 1-(4′-hydroxy-3′-methoxyphenyl)-3-octen-5-one, respectively, and demonstrated the first isolation of compounds **E**, **F**, **H**, and **I** from GOP. The two naturally occurring vanilloids **1** and **2**, isolated from the seeds of *Aframomum melegueta*, were synthesized by routes that provide adequate quantities for biological testing and that should allow variation in alkyl chain length with consequent alteration in the lipophilicity of the final vanilloid. Unlike other reports on the isolation of **2**, the natural material was not racemic but was a 1:1.7 mixture of *R* and *S* isomers, and the present synthetic route should allow biological evaluation of the individual enantiomers since both enantiomers of malic acid are available. Furthermore, the present study demonstrated that GOP and its extract exert an anti-obesity effect in HFD-fed mice. Prevention of body weight gain and fat accumulation occured through improved hepatic lipid metabolism. In addition, the oral administration of GOP extract and 6-gingerol, which are the principle pungent compounds in GOP, clearly decreased interscapular BAT SNA. This phenomenon suggested that GOP extract and 6-gingerol operate to prevent fat accumulation through different mechanisms to capsaicin and capsiate, which activate the sympathetic nervous system following BAT thermogenesis.

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V ACKNOWLEDGEMENTS

I would first like to appreciate my supervisor Professor Tohru Mitsunaga, the United Graduate School of Agricultural Science, Gifu University, for great coutinuous support, knowledge and motivation in all my PhD period. Besides, I thank to my co-supervisor, Professor Shingo Kawai, Faculty of Agriculture, Shizuoka University and Associate professor Masaya Shimada, Fuculty of Applied Biological Sciences, Gifu University for their encouragements, comments, and discussions.

I am also greatly thankful to Professor Derrick L J Clive, Faculty of Chemistry, University of Alberta, for giving me a valuable chance to learn new experimental technique and knowledge. He tought me a lot of things better to know and supported and made my thesis more interesting.

My grateful thanks are also extended to all colleague in Natural Product Chemistry Lab and staff in the United Graduate School of Agricultural Science, Gifu University for their friendship, kindness, and encouragements during my research

Ⅵ　APPENDIX. SUPPLEMENTARY DATA

SUPPORTING INFORMATION

A Comprehensive Study of Molecular Mechanisms on

Anti-obesity Effect by the Constituents of Grains of Paradise

(香辛料 Grains of Paradise 成分の肥満抑制効果とその分子メカニズムの網羅的解明)

By Hiroyuki Hattori

2019

The United Graduate School of Agricultural Science, Gifu University

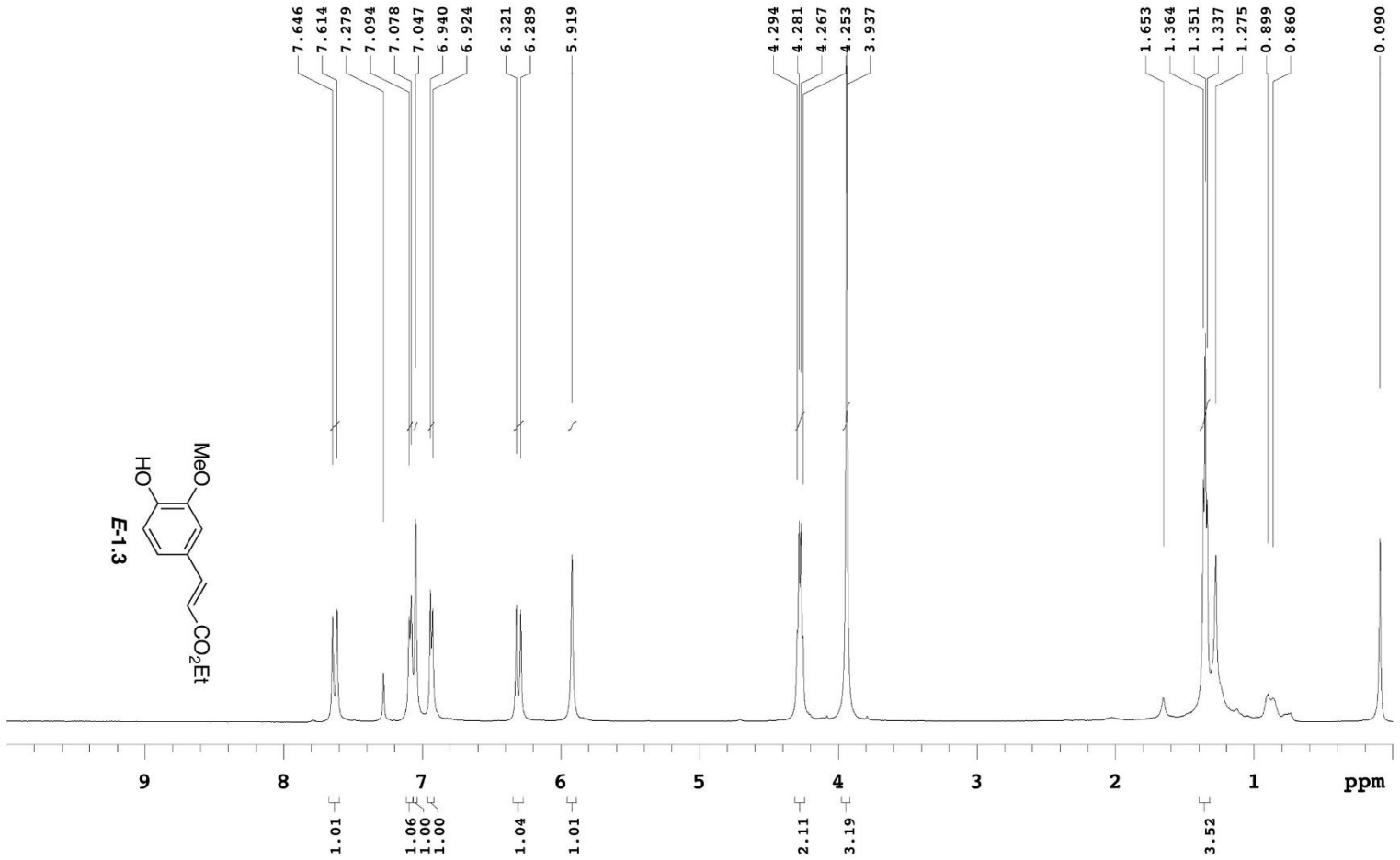
Science of Biological Resources

Agilent Technologies

Department of Chemistry, University of Alberta

Recorded on: **ibd5, Apr 3 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.04.03.i5_wittig_reaction_2_H1_1D

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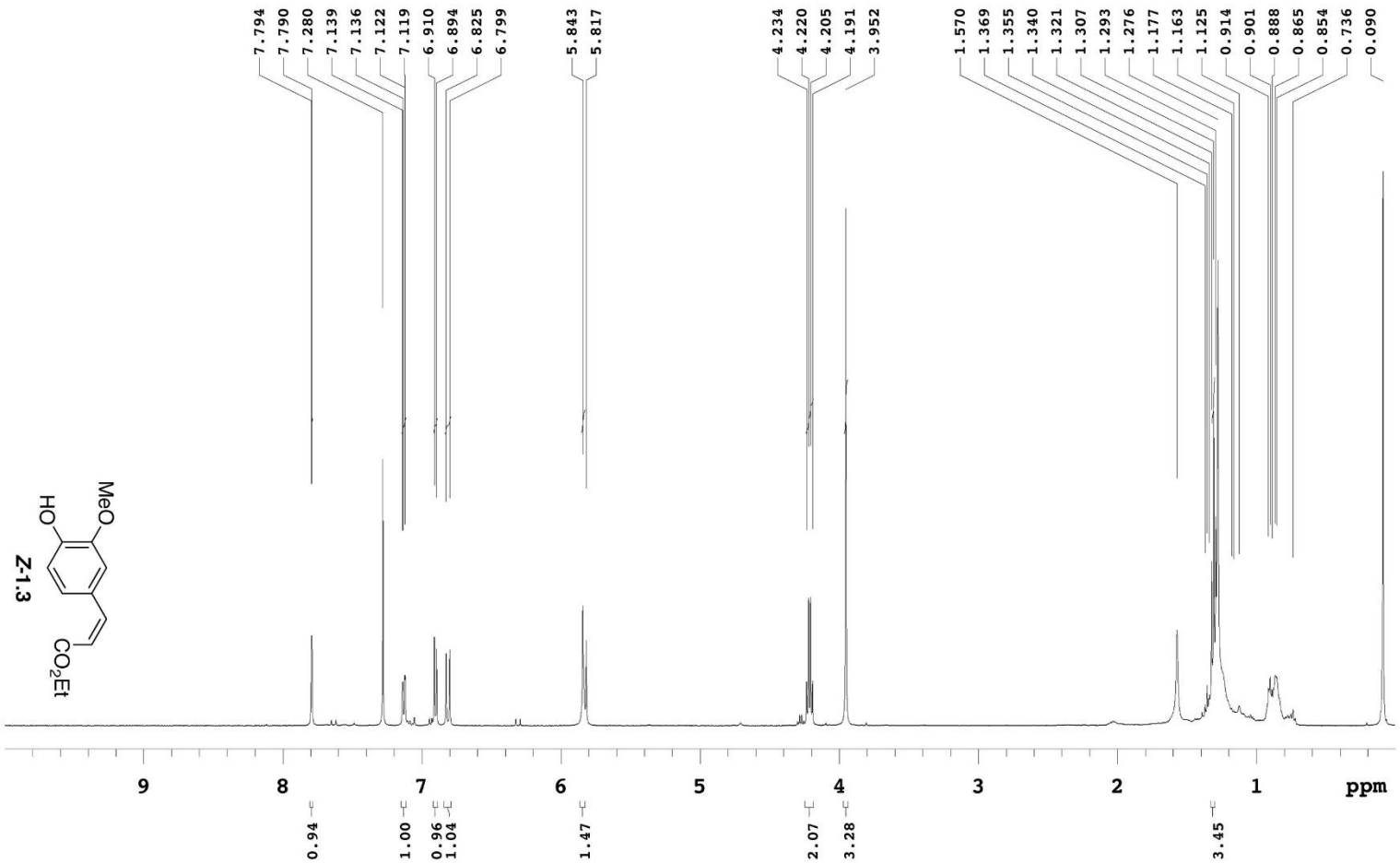
Department of Chemistry, University of Alberta

Recorded on: **ibd5, Apr 3 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.74**	Completed Scans **16**

498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.04.03.i5_wittig_reaction_1_H1_1D

Agilent Technologies

Department of Chemistry, University of Alberta

Recorded on: **mr400, Apr 17 2018**	Sweep Width(Hz): **4807.69**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.07**	Hz per mm(Hz/mm): **16.66**	Completed Scans **16**

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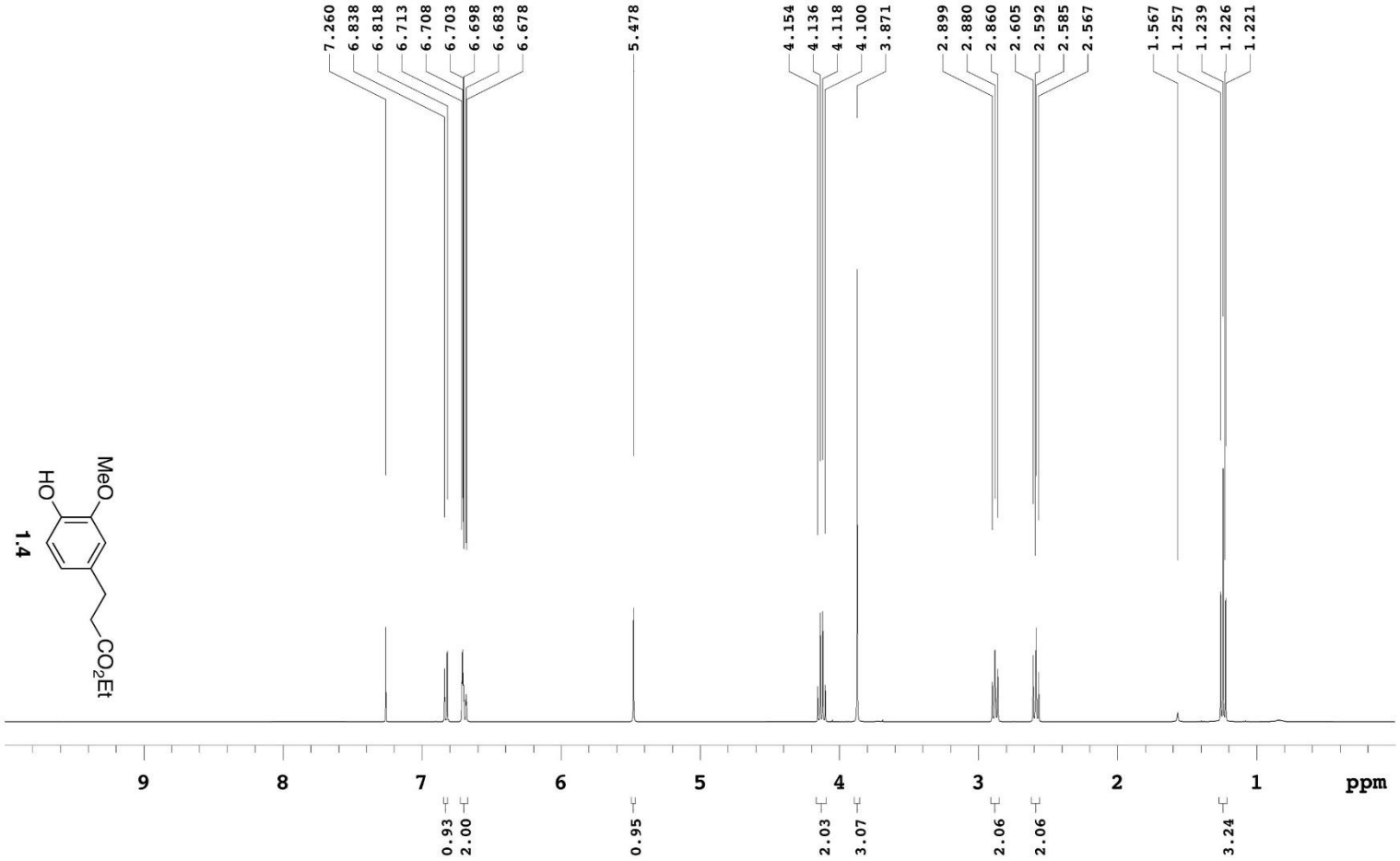
temp 25.9 C -> actual temp = 27.0 C, onenmr probe



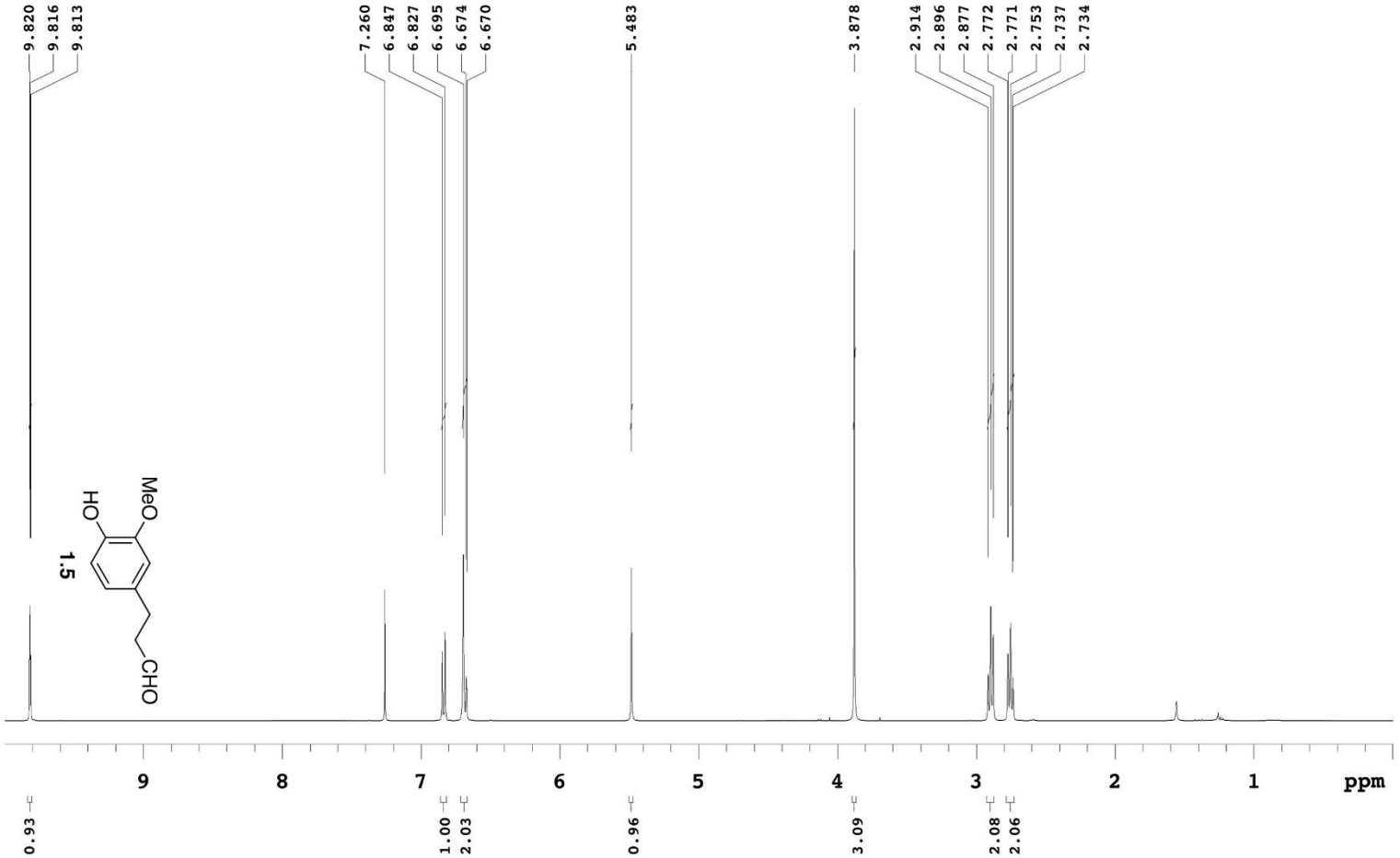
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.04.17.mr4_HH-1-11_H1_1D

Agilent Technologies

Department of Chemistry, University of Alberta

Recorded on: **mr400, Apr 12 2018**	Sweep Width(Hz): **4807.69**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.07**	Hz per mm(Hz/mm): **16.67**	Completed Scans **16**

399.978 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 25.9 C -> actual temp = 27.0 C, onenmr probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.04.12.mr4_HH-1-7B_H1_1D

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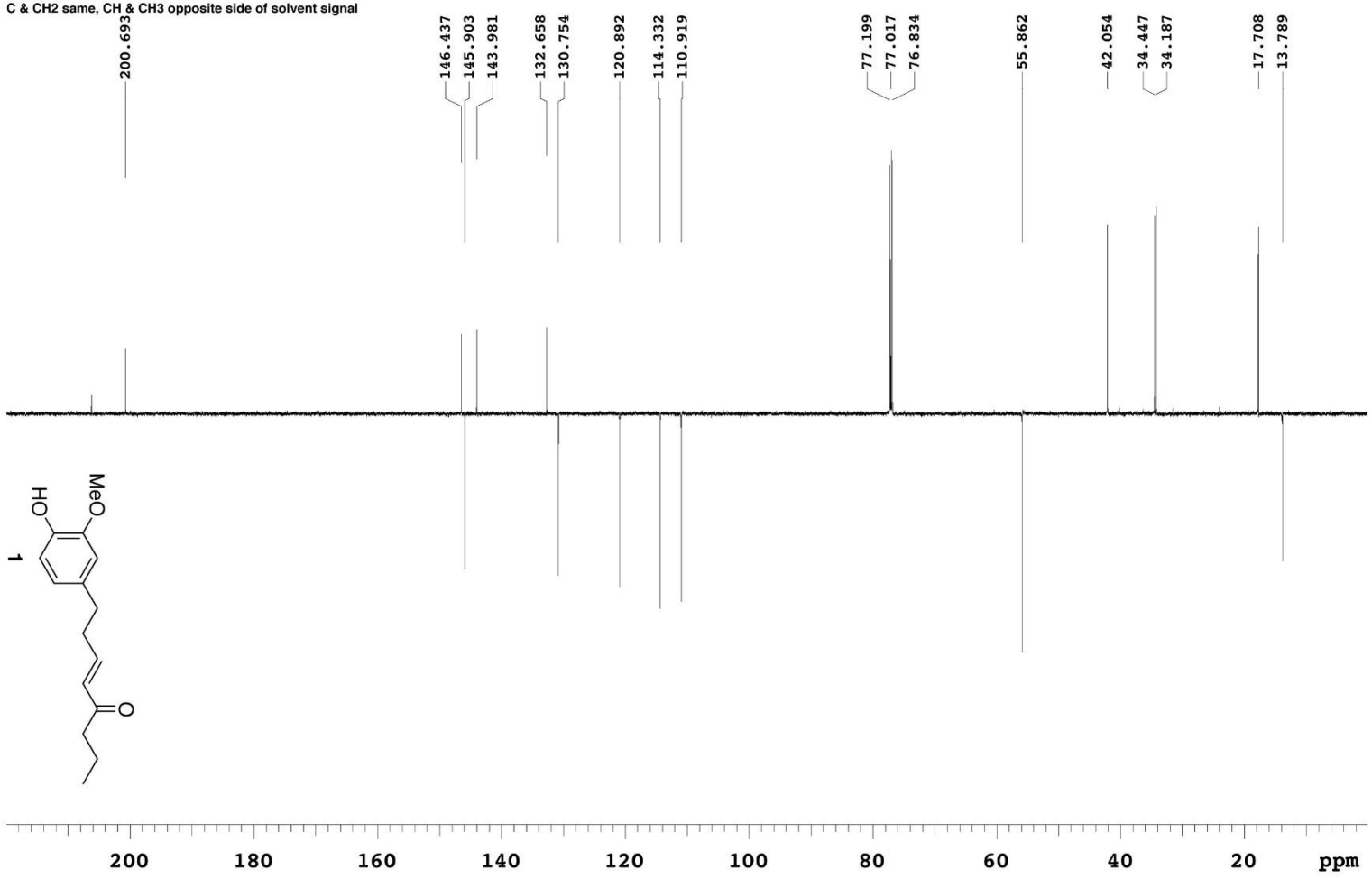
Recorded on: **v700, Dec 20 2018**	Sweep Width(Hz): **48076.9**	Acquisiton Time(s): **1**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.37**	Hz per mm(Hz/mm): **161.17**	Completed Scans **92**

Hiroyuki, Compound_J
175.975 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
C & CH2 same, CH & CH3 opposite side of solvent signal



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.20.v7_Compound_J_loc11_04.33_C13_APT_ad

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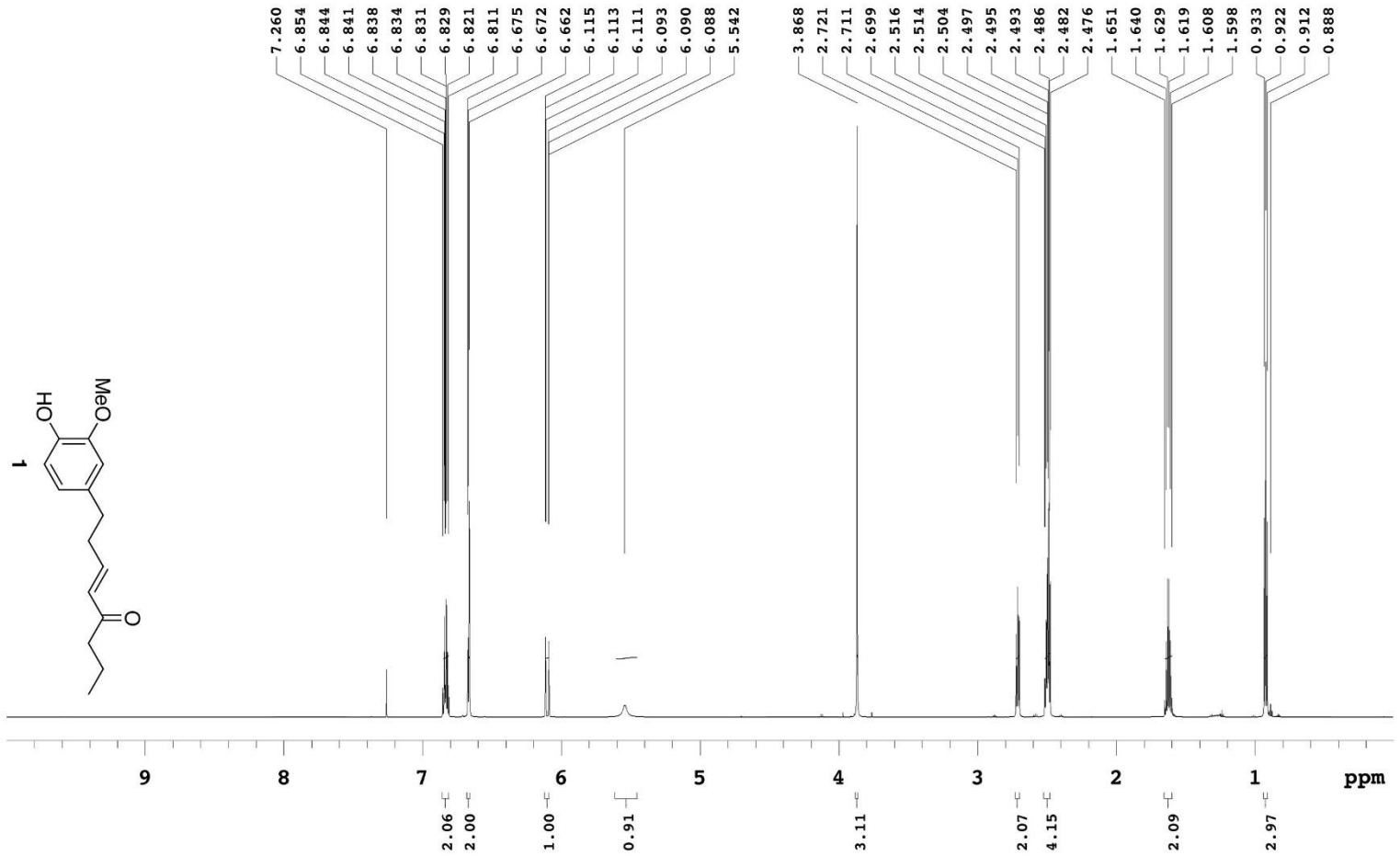
Recorded on: **v700, Dec 20 2018**	Sweep Width(Hz): **8389.26**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **PRESAT**	Digital Res.(Hz/pt): **0.13**	Hz per mm(Hz/mm): **29.14**	Completed Scans **8**

Hiroyuki, Compound_J
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.20.v7_Compound_J_loc11_04.32_H1_1D

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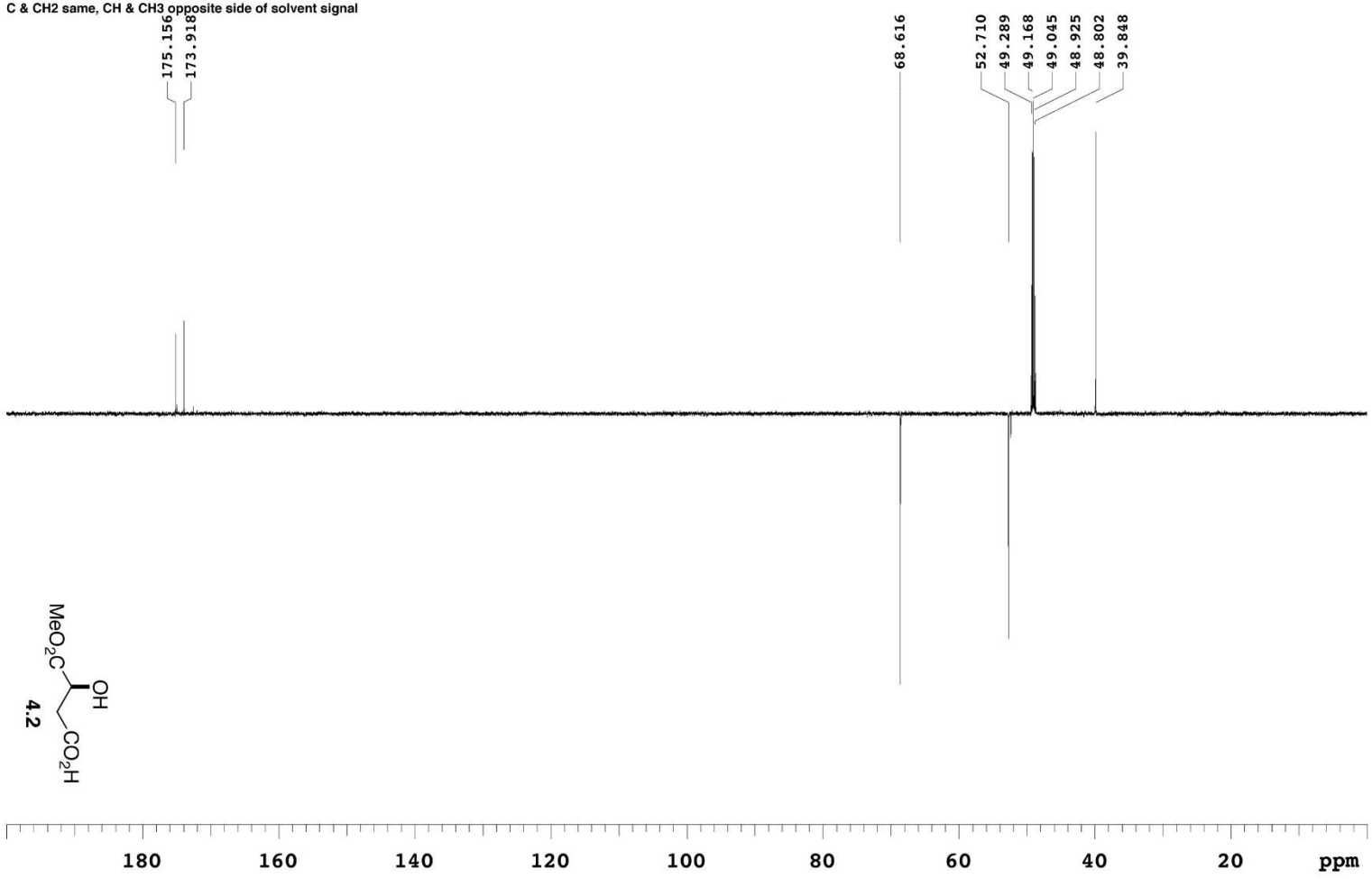
Recorded on: **v700, Nov 30 2018**	Sweep Width(Hz): **48076.9**	Acquisiton Time(s): **1**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.37**	Hz per mm(Hz/mm): **146.68**	Completed Scans **8**

Hiroyuki, HH-2-173
175.975 MHz C13 APT_ad in cd3od (ref. to CD3OD @ 49.0 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
C & CH2 same, CH & CH3 opposite side of solvent signal



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.30.v7_HH-2-173_loc9_15.32_C13_APT_ad

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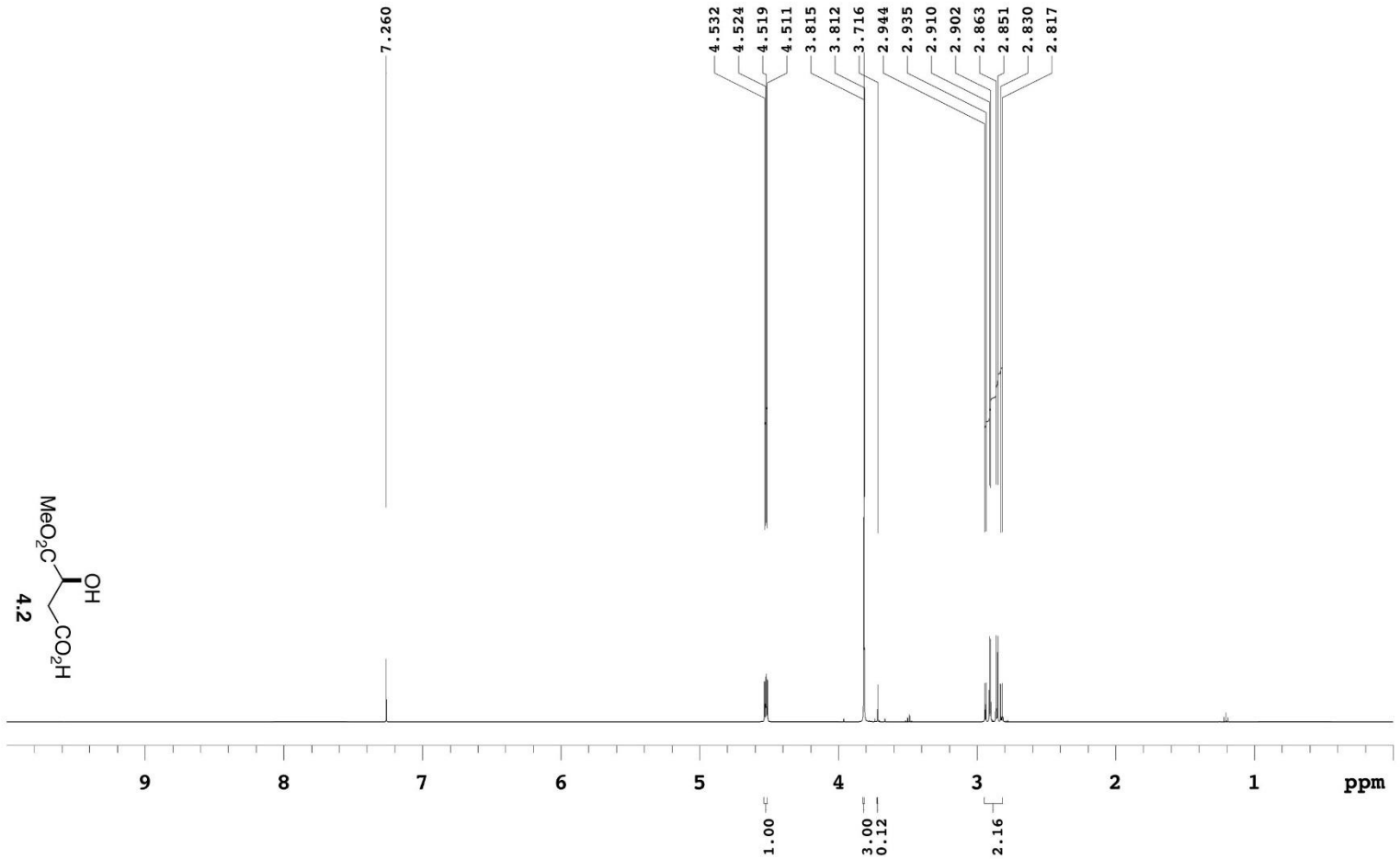
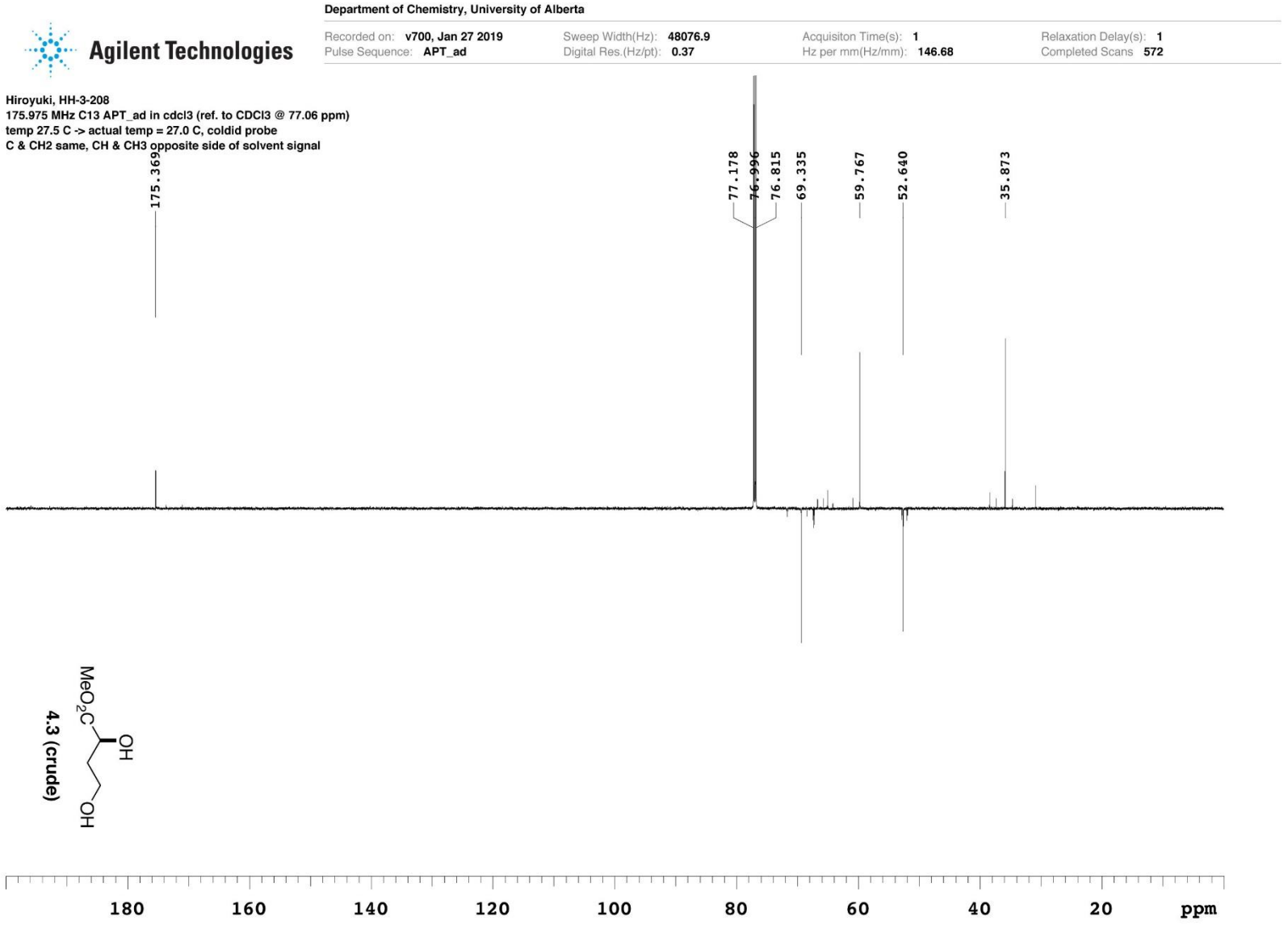
Recorded on: **ibd5, Nov 21 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-2-167 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.11.21.i5_HH-2-167_H1_1D



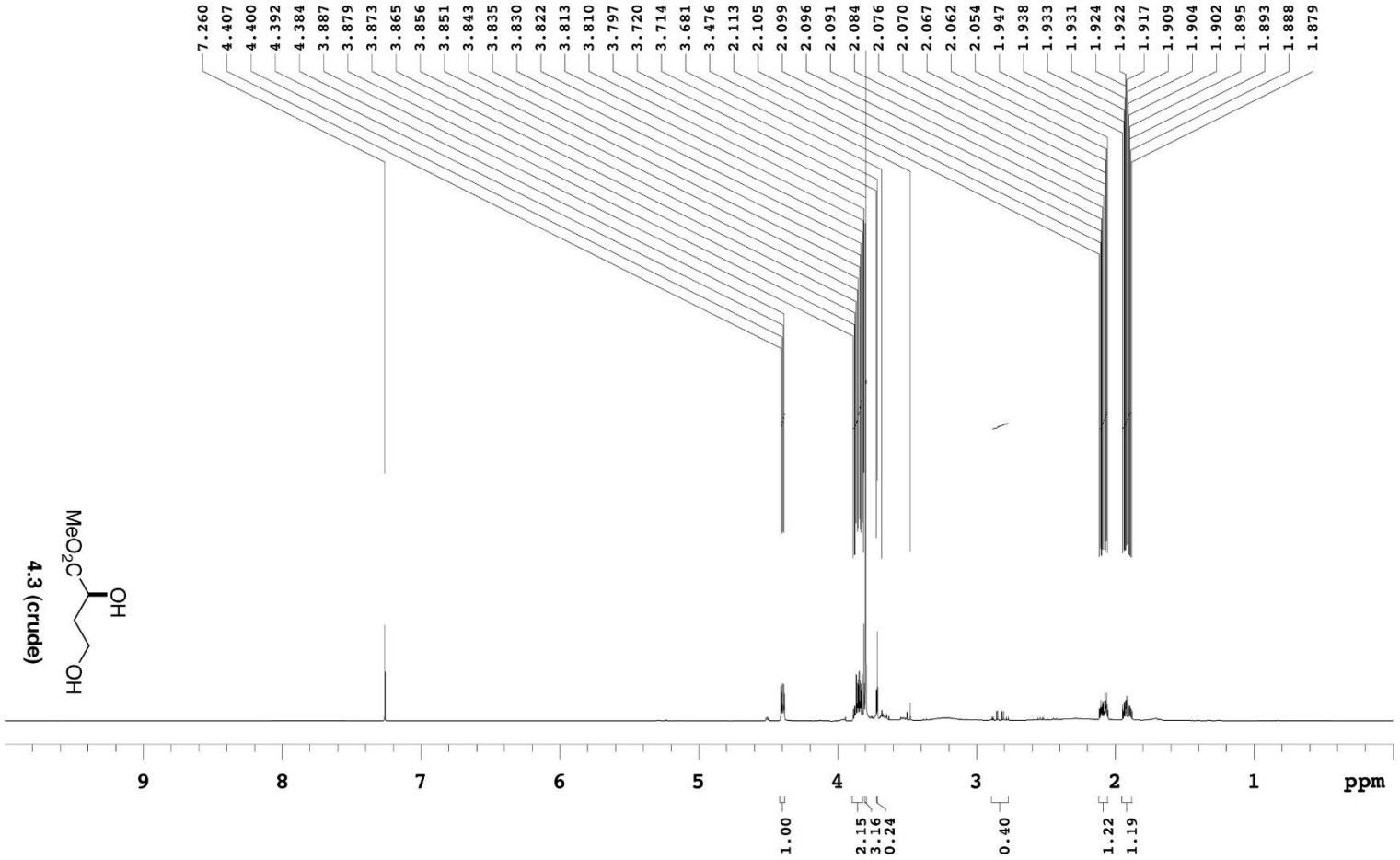
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2019.01/2019.01.27.v7_HH-3-208_loc24_21.17_C13_APT_ad

Agilent Technologies

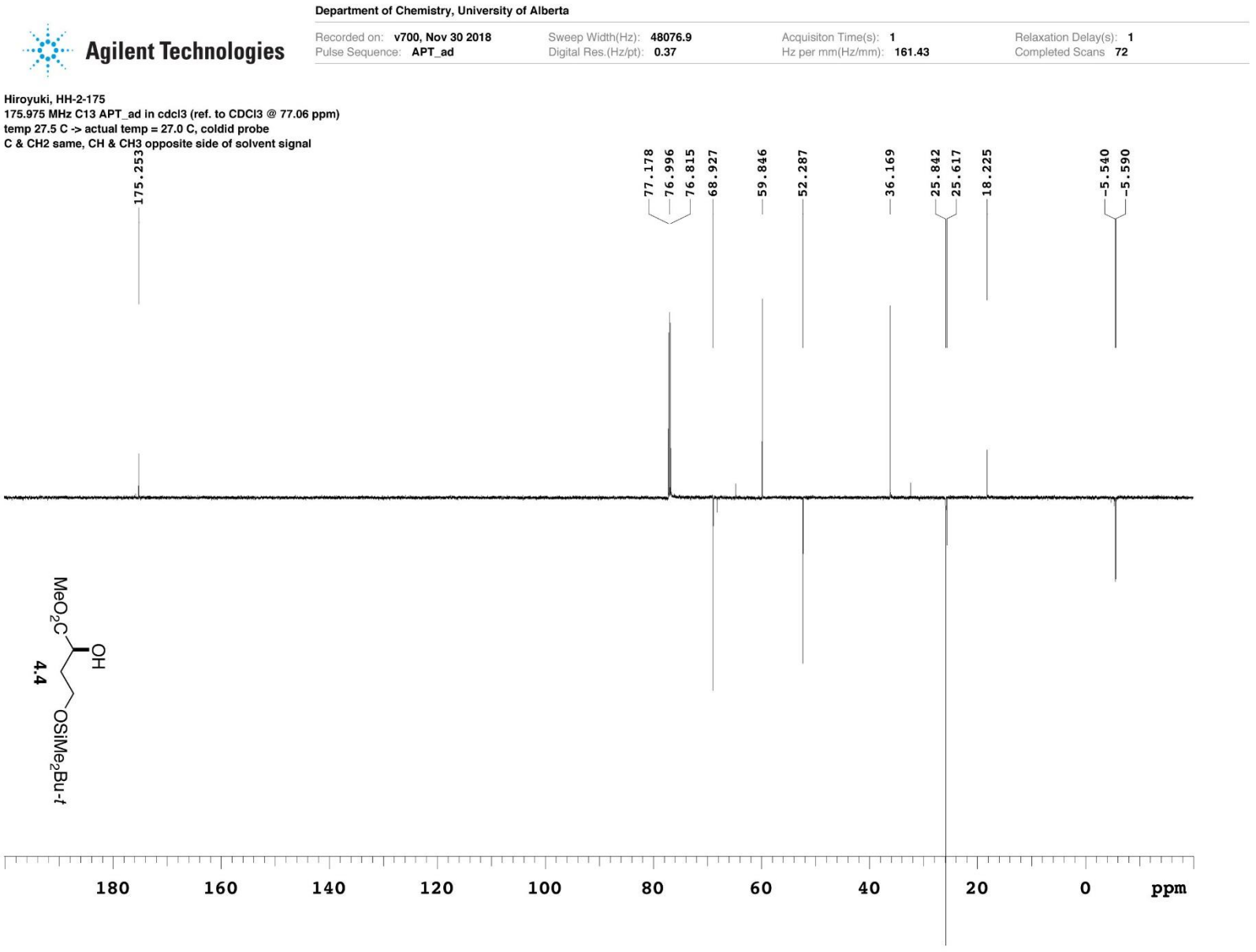
Department of Chemistry, University of Alberta

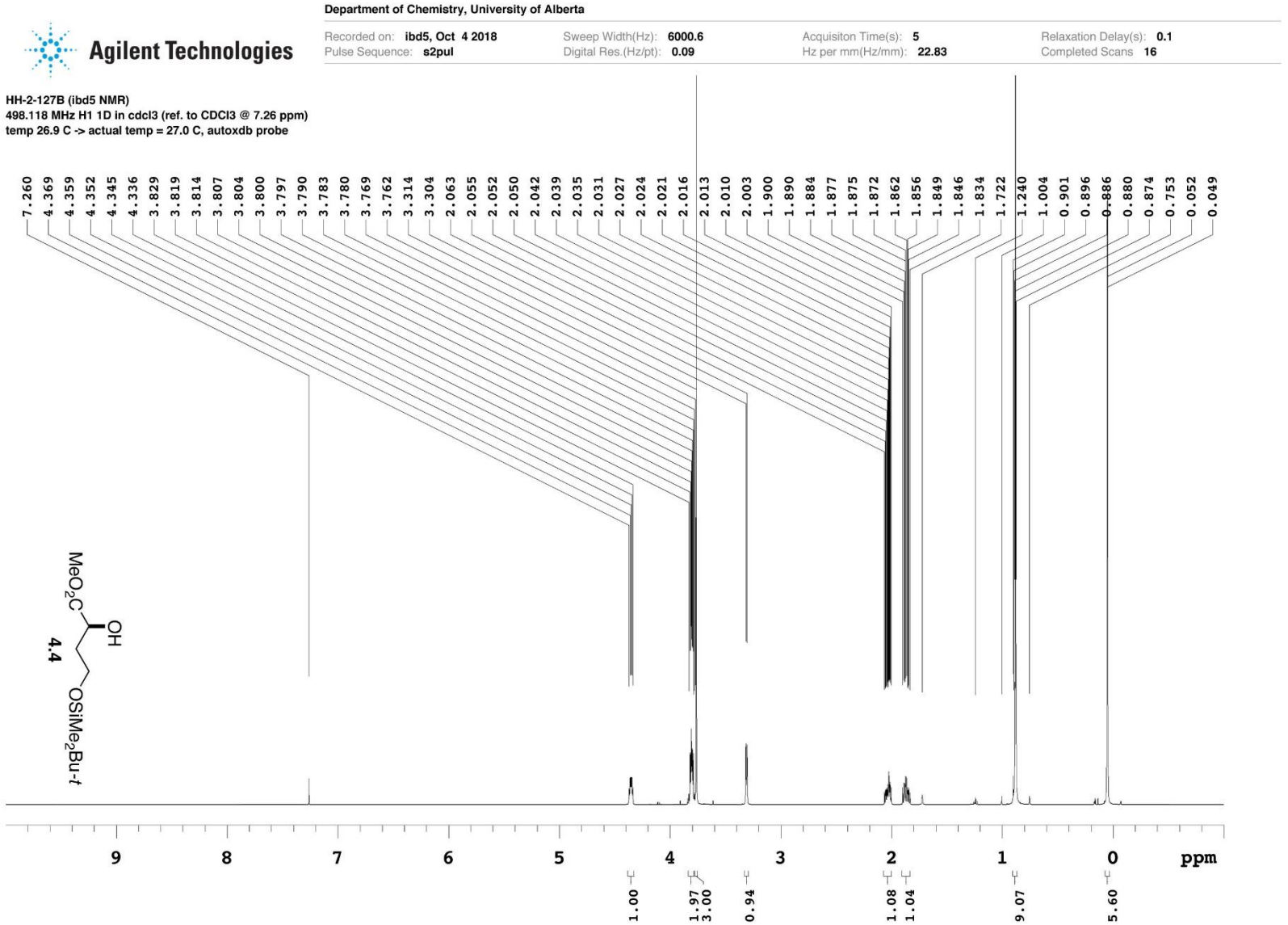
Recorded on: **ibd5, Oct 3 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-2-126 crude (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.10.03.i5_HH-2-126_crude_H1_1D


Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: v700, Nov 30 2018
Pulse Sequence: APT_ad
Sweep Width(Hz): 48076.9
Digital Res.(Hz/pt): 0.37
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 161.43
Relaxation Delay(s): 1
Completed Scans 72
Hiroyuki, HH-2-175
175.975 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
C & CH2 same, CH & CH3 opposite side of solvent signal
175.253
77.178
76.996
76.815
68.927
59.846
52.287
36.169
25.842
25.617
18.225
-5.540
-5.590
MeO2C
OH
4.4
OSiMe2Bu-t
180
160
140
120
100
80
60
40
20
0
ppm
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.30.v7_HH-2-175_loc10_15.37_C13_APT_ad



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.10.04.i5_HH-2-127B_H1_1D

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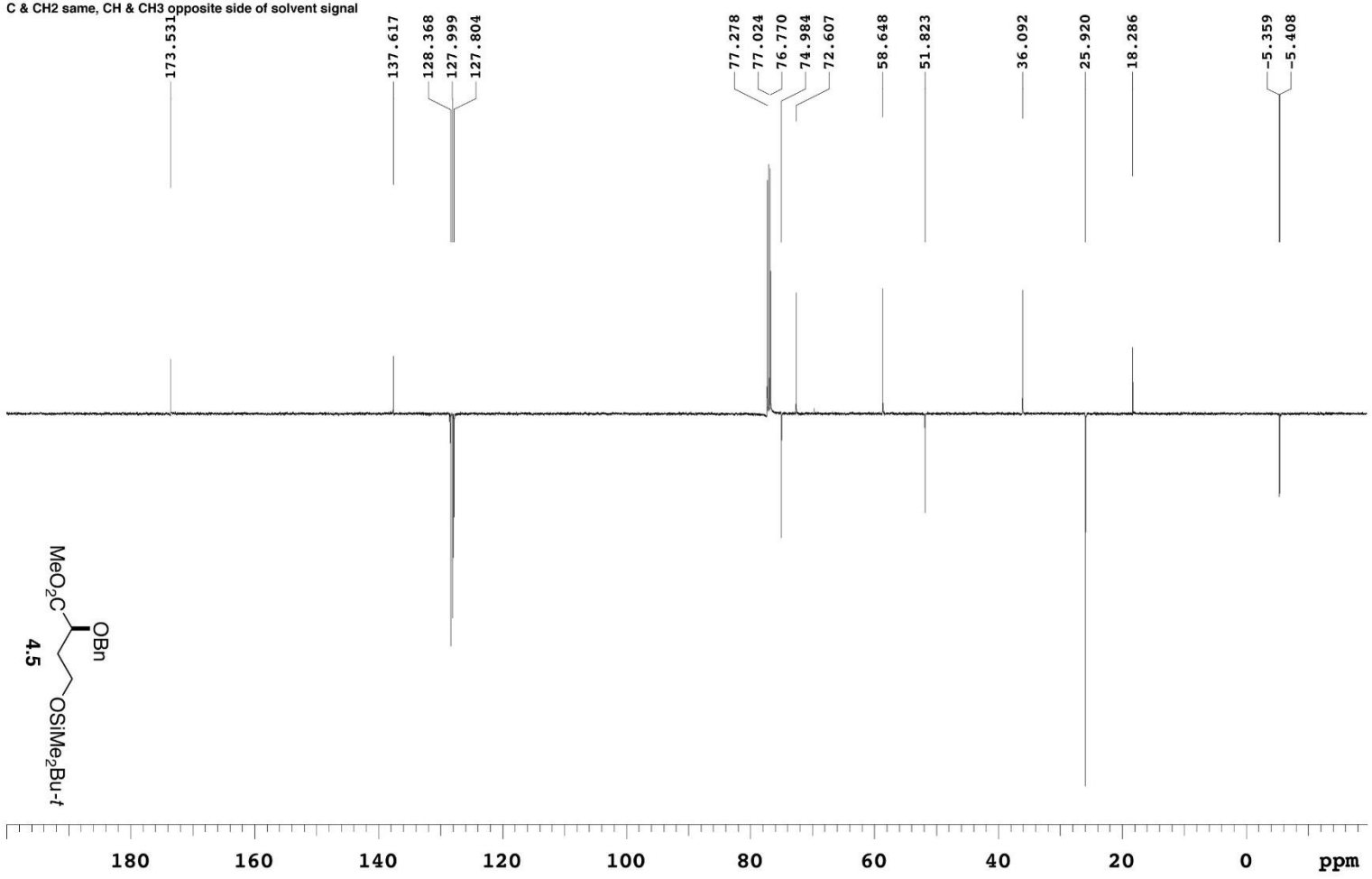
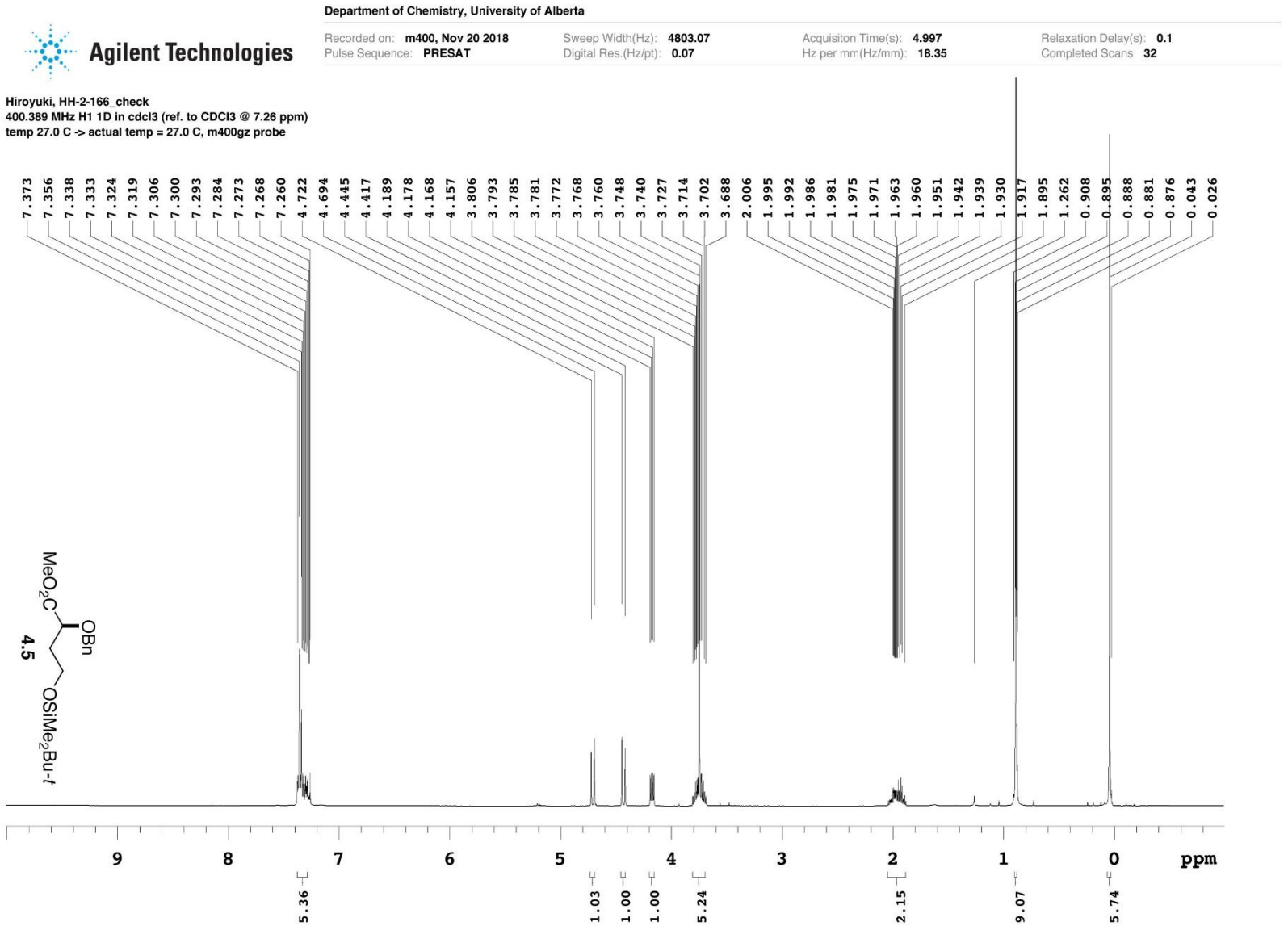
Recorded on: u500, Oct 9 2018	Sweep Width(Hz): 33783.8	Acquisiton Time(s): 1	Relaxation Delay(s): 1
Pulse Sequence: APT_ad	Digital Res.(Hz/pt): 0.26	Hz per mm(Hz/mm): 115	Completed Scans 84

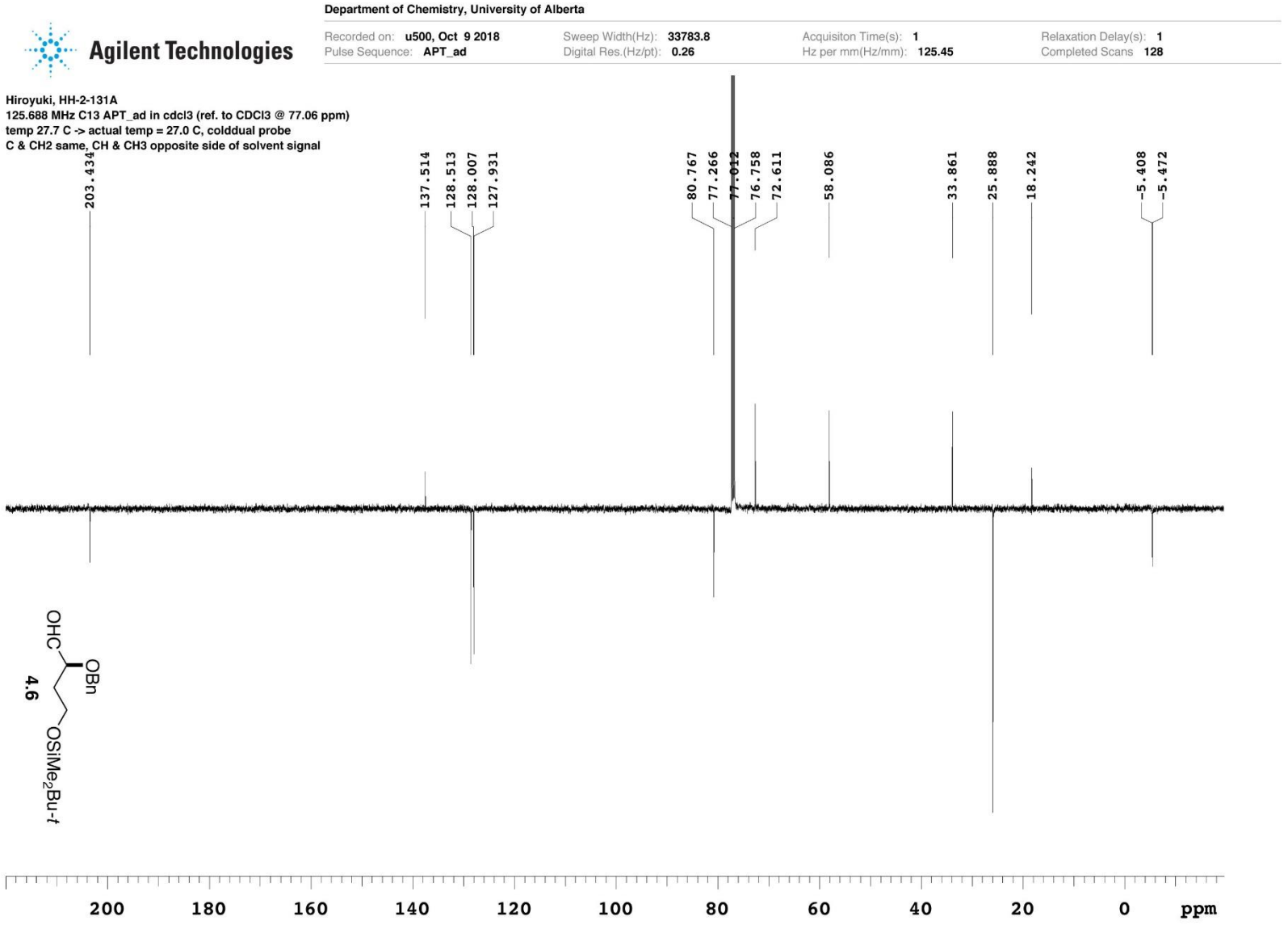
Hiroyuki, HH-2-129C
125.688 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
C & CH2 same, CH & CH3 opposite side of solvent signal



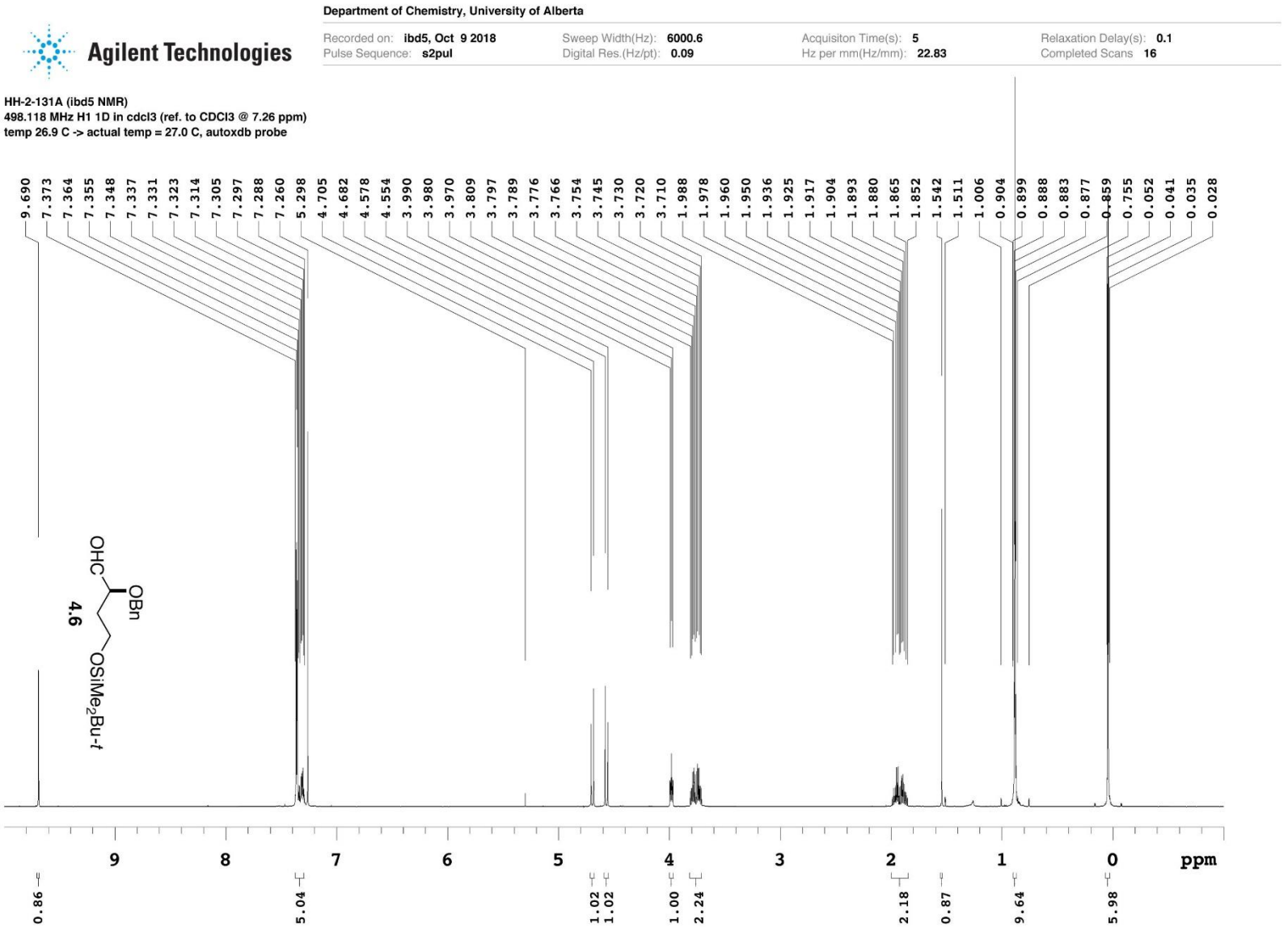
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.10/2018.10.09.u5_HH-2-129C_loc1_17.48_C13_APT_ad



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.20.m4_HH-2-166_check_loc9_13.56_H1_1D



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.10/2018.10.09.u5_HH-2-131A_loc1_14.36_C13_APT_ad



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.10.09.i5_HH-2-131A_H1_1D

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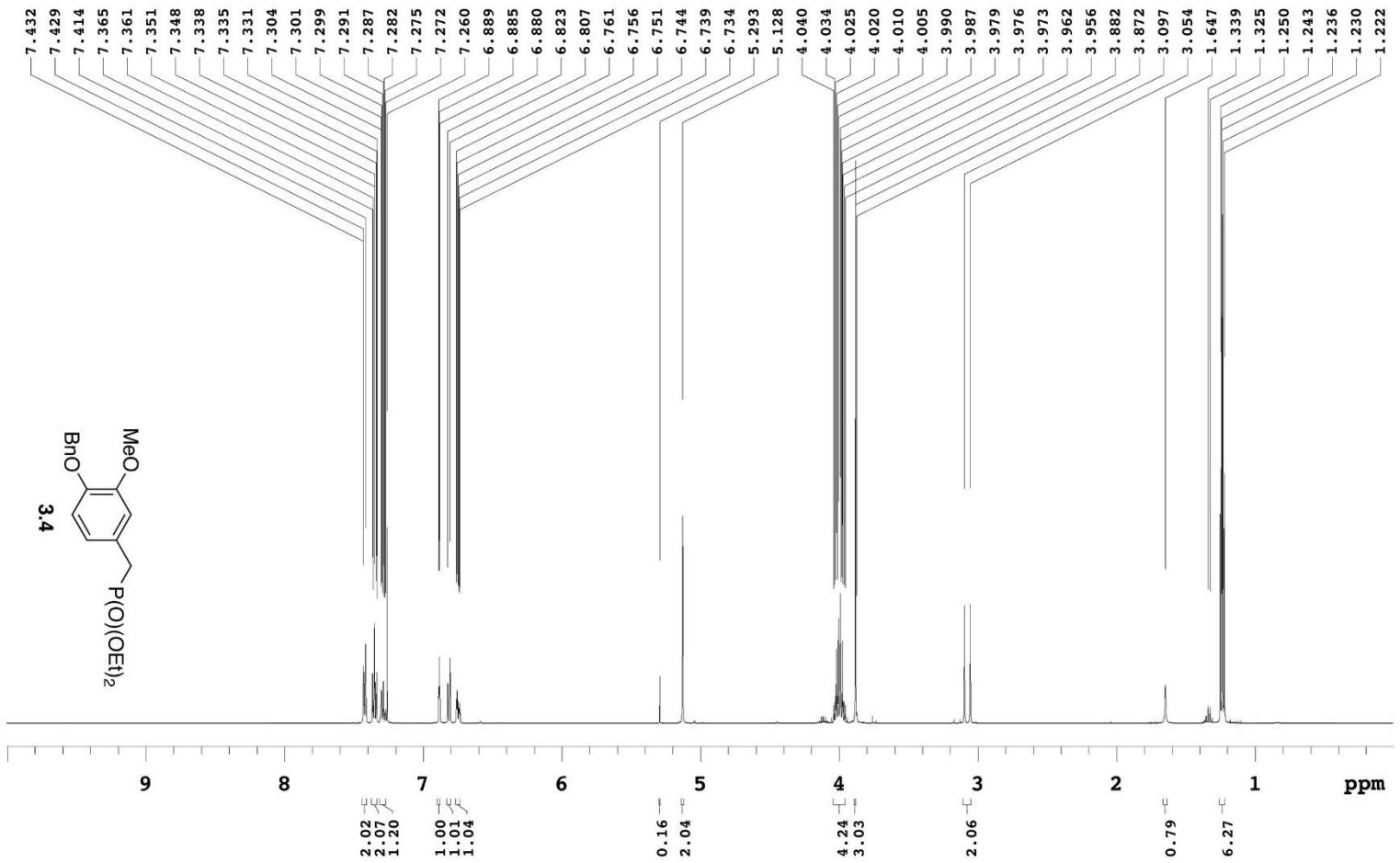
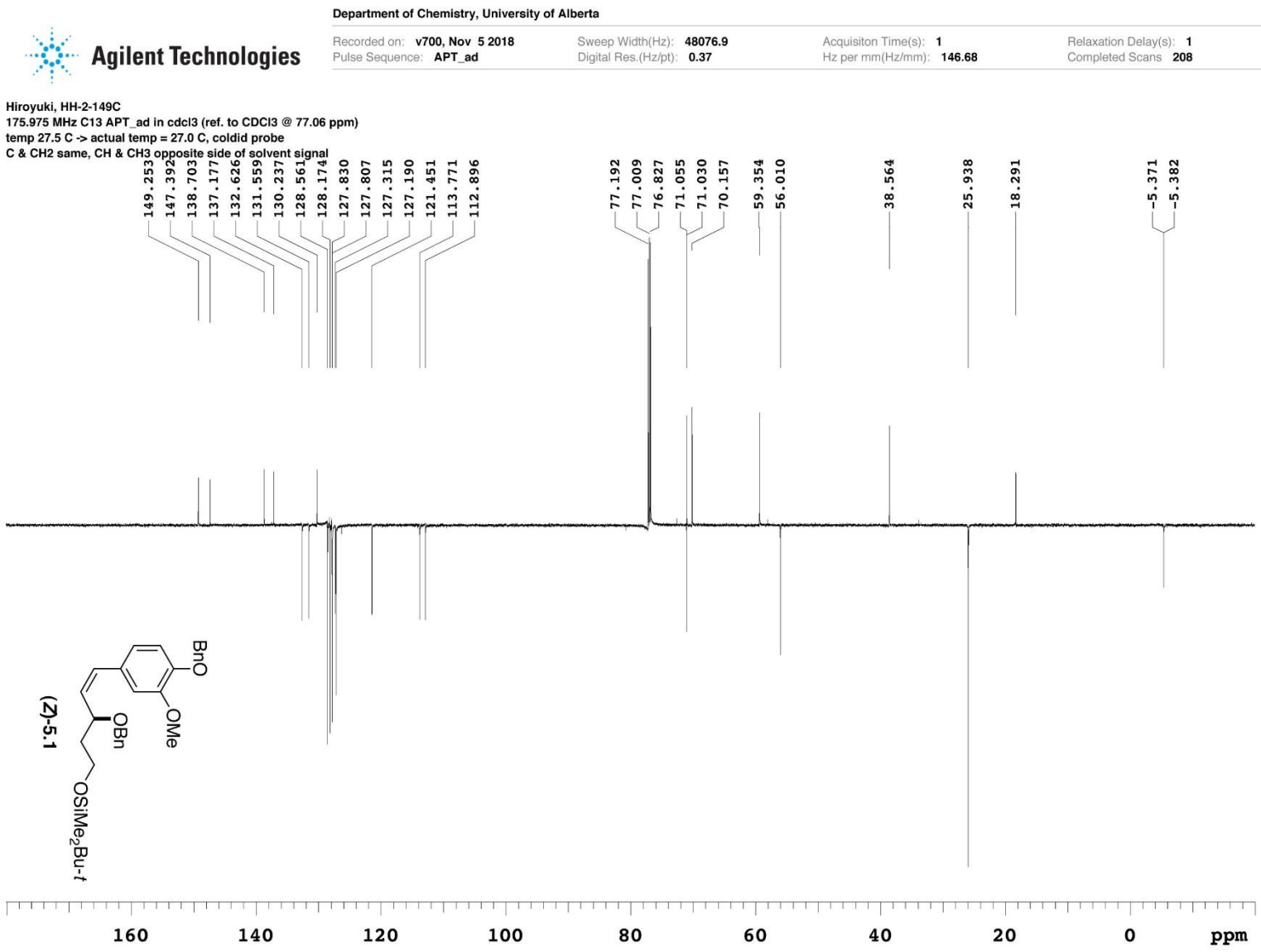
Recorded on: **ibd5, Oct 12 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

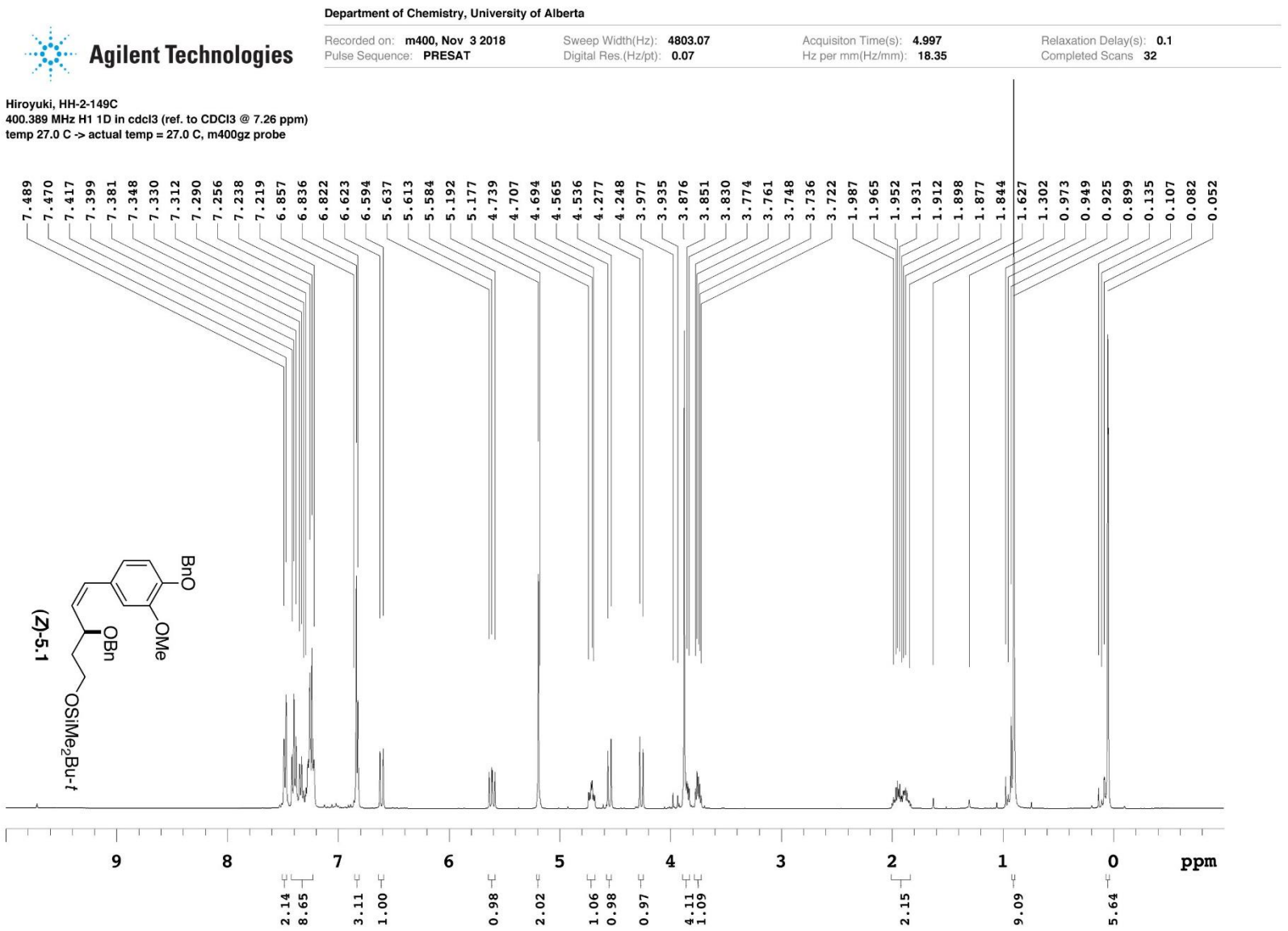
HH-2-117_1 check (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe

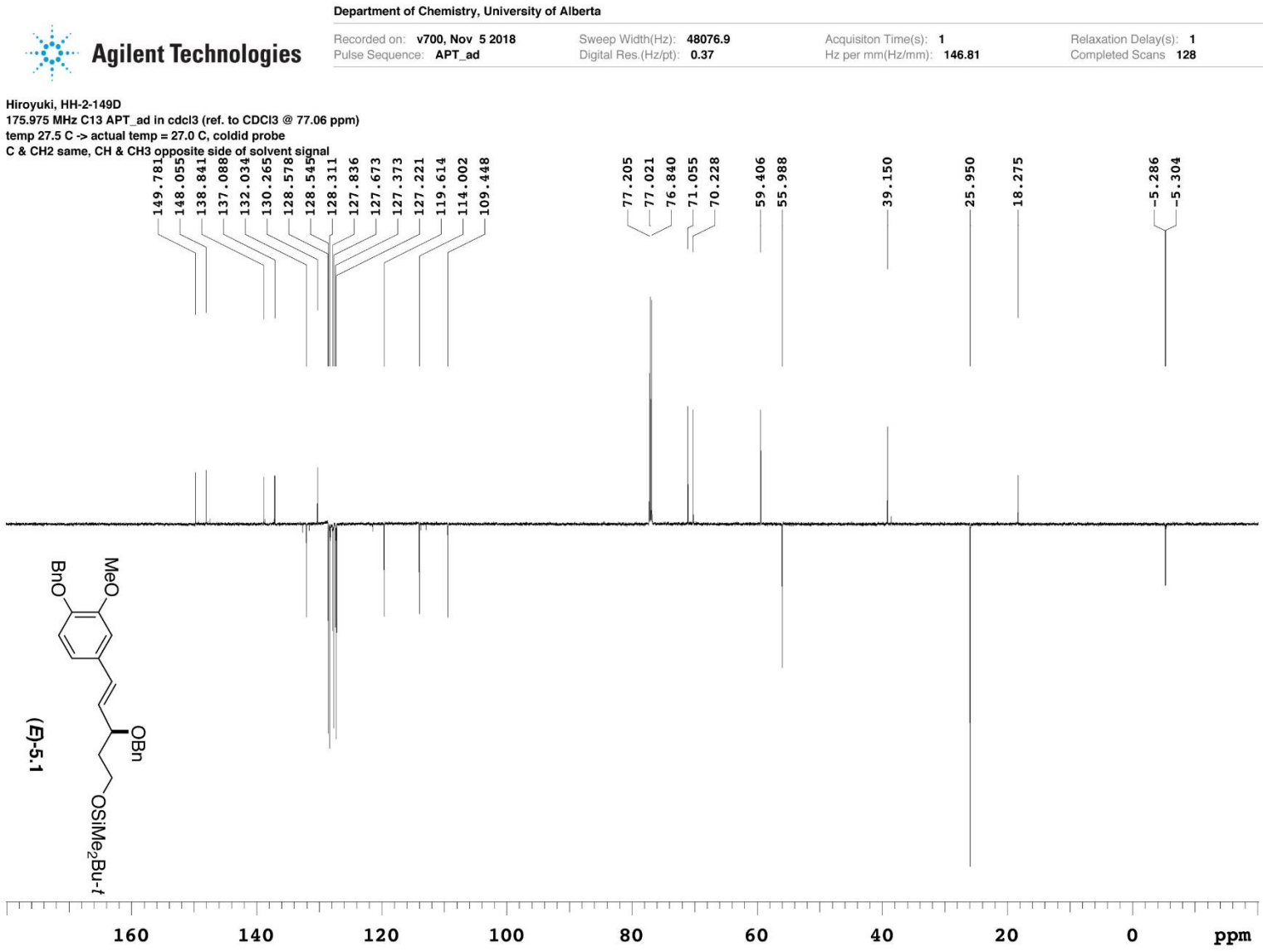


File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.10.12.i5_HH-2-117_1_check_H1_1D

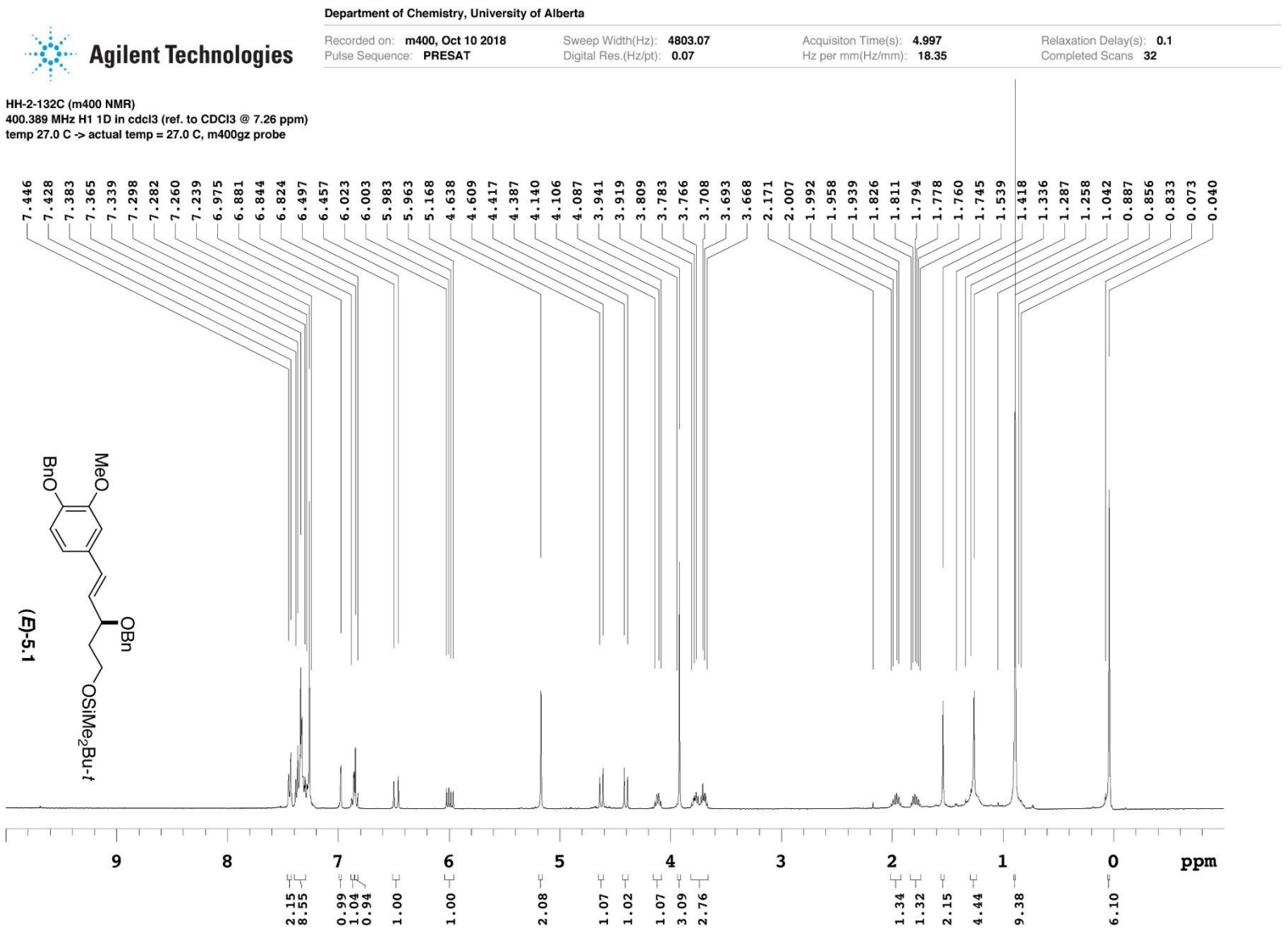


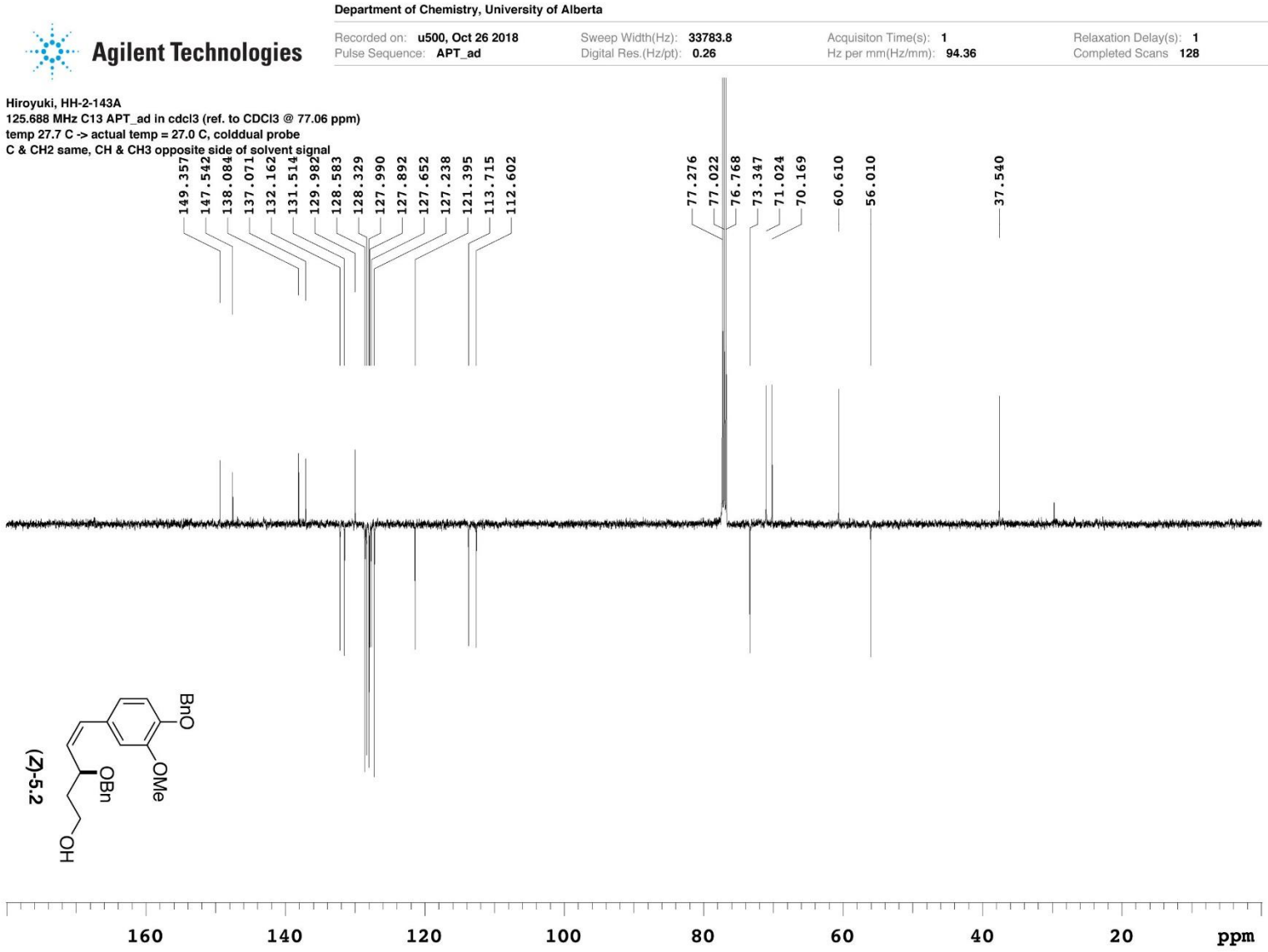
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.05.v7_HH-2-149C_loc40_12.12_C13_APT_ad


Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: m400, Nov 3 2018
Pulse Sequence: PRESAT
Sweep Width(Hz): 4803.07
Digital Res.(Hz/pt): 0.07
Acquisiton Time(s): 4.997
Hz per mm(Hz/mm): 18.35
Relaxation Delay(s): 0.1
Completed Scans 32
Hiroyuki, HH-2-149C
400.389 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.0 C -> actual temp = 27.0 C, m400gz probe
7.489 7.470 7.417 7.399 7.381 7.348 7.330 7.312 7.290 7.256 7.238 7.219 6.857 6.836 6.822 6.623 6.594 5.637 5.613 5.584 5.192 5.177 4.739 4.707 4.694 4.565 4.536 4.277 4.248 3.977 3.935 3.876 3.851 3.830 3.774 3.761 3.748 3.736 3.722 1.987 1.965 1.952 1.931 1.912 1.898 1.877 1.844 1.627 1.302 0.973 0.949 0.925 0.899 0.135 0.107 0.082 0.052
BnO
OMe
OBn
OSiMe2Bu-t
(Z)-5.1
9 8 7 6 5 4 3 2 1 0 ppm
2.14 8.65 3.11 1.00 0.98 2.02 1.06 0.98 0.97 4.11 1.09 2.15 9.09 5.64
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.03.m4_HH-2-149C_loc3_12.50_H1_1D

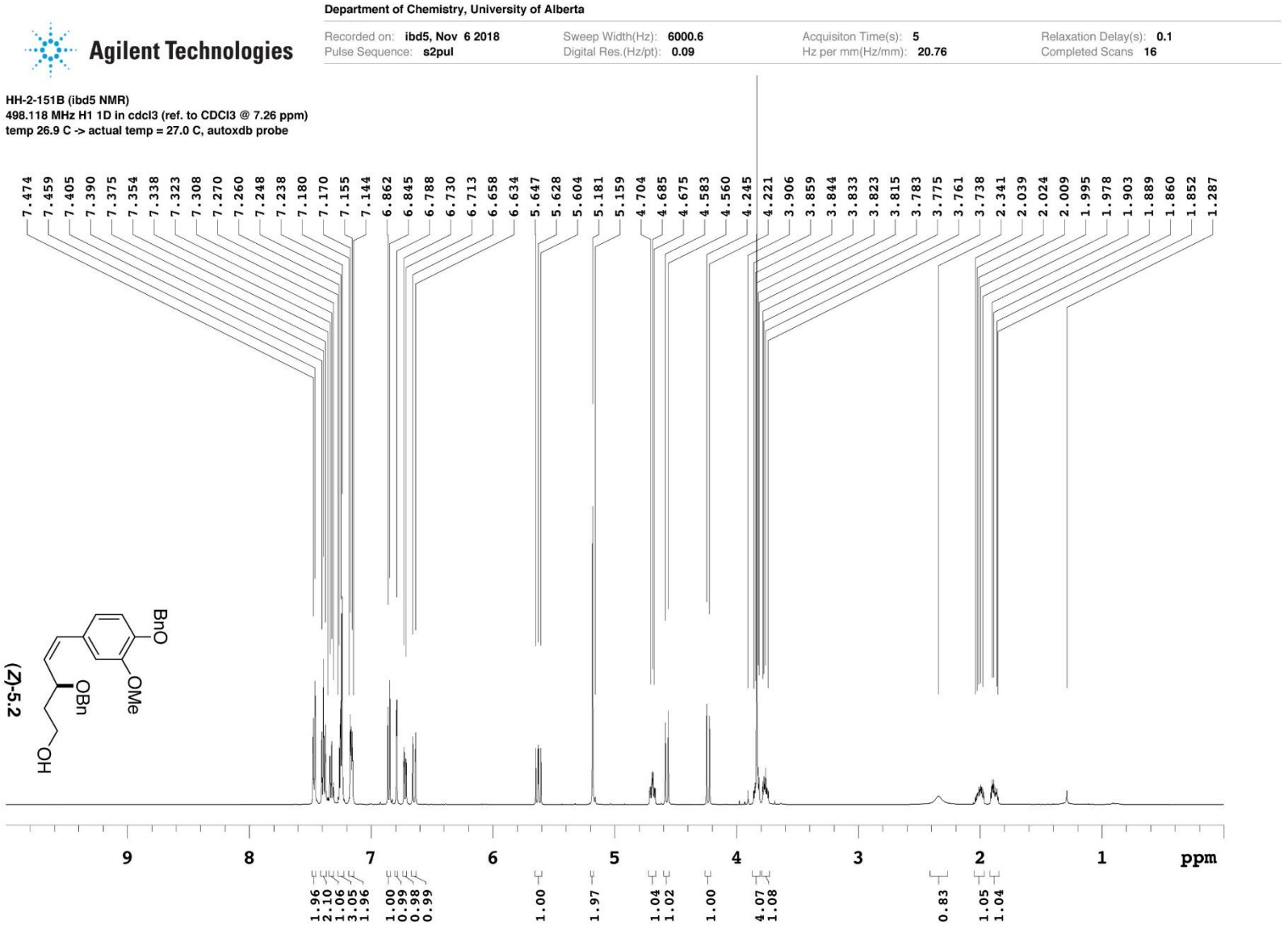


File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.05.v7_HH-2-149D_loc41_12.23_C13_APT_ad


Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: m400, Oct 10 2018
Pulse Sequence: PRESAT
Sweep Width(Hz): 4803.07
Digital Res.(Hz/pt): 0.07
Acquisiton Time(s): 4.997
Hz per mm(Hz/mm): 18.35
Relaxation Delay(s): 0.1
Completed Scans 32
HH-2-132C (m400 NMR)
400.389 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.0 C -> actual temp = 27.0 C, m400gz probe
7.446 7.428 7.383 7.365 7.339 7.298 7.282 7.260 7.239 6.975 6.881 6.844 6.824 6.497 6.457 6.023 6.003 5.983 5.963 5.168 4.638 4.609 4.417 4.387 4.140 4.106 4.087 3.941 3.919 3.809 3.783 3.766 3.708 3.693 3.668 2.171 2.007 1.992 1.958 1.939 1.826 1.811 1.794 1.778 1.760 1.745 1.539 1.418 1.336 1.287 1.258 1.042 0.887 0.856 0.833 0.073 0.040
BnO
MeO
OBn
OSiMe2Bu-t
(E)-5.1
9 8 7 6 5 4 3 2 1 0 ppm
2.15 8.55 0.99 1.04 0.94 1.00 1.00 2.08 1.07 1.02 1.07 3.09 2.76 1.34 1.32 2.15 4.44 9.38 6.10
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.10/2018.10.10.m4_HH-2-132C_loc5_22.14_H1_1D



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.10/2018.10.26.u5_HH-2-143A_loc3_03.39_C13_APT_ad



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.11.06.i5_HH-2-151B_H1_1D

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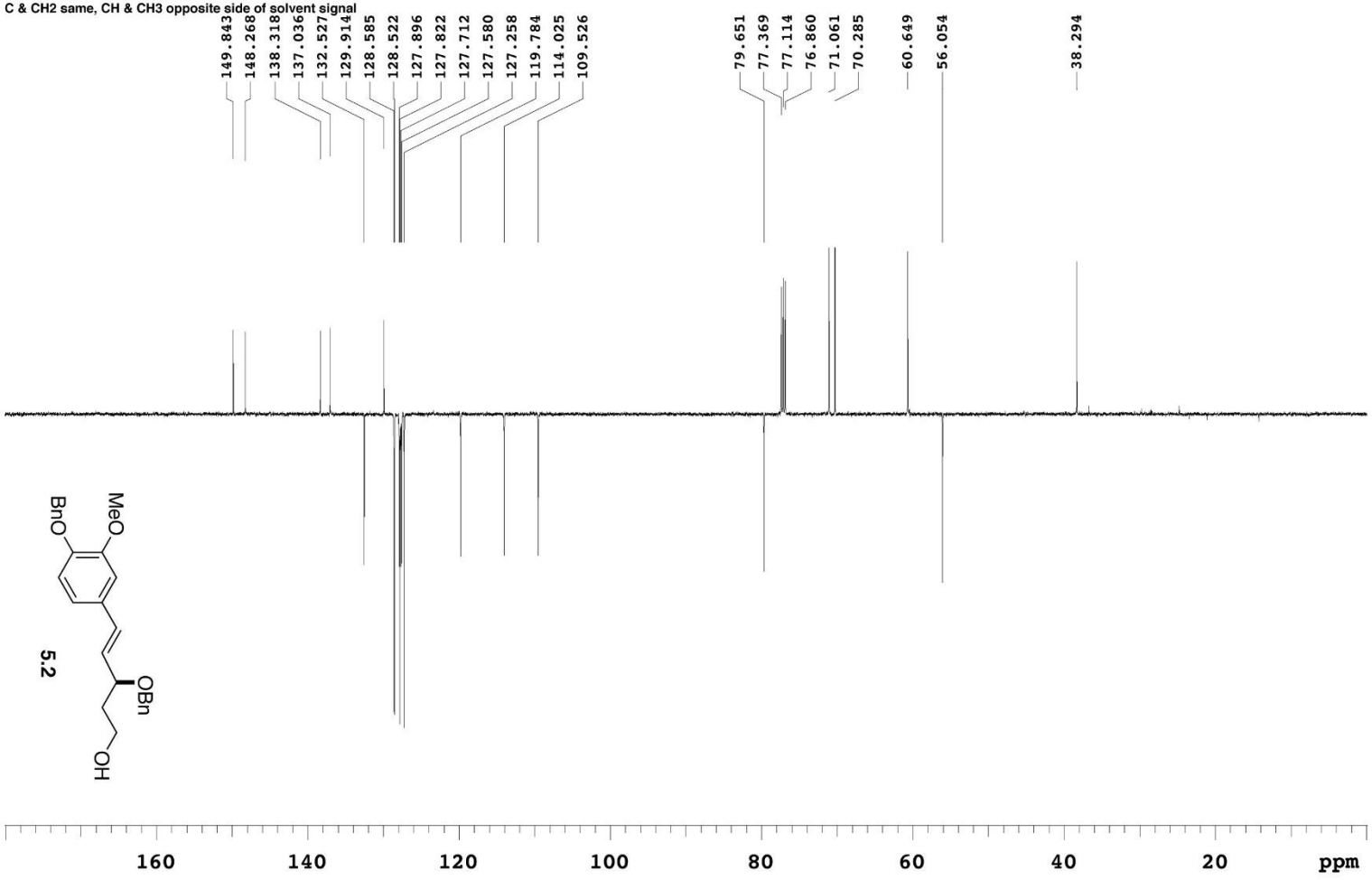
Recorded on: **u500, Oct 26 2018**	Sweep Width(Hz): **33783.8**	Acquisiton Time(s): **1**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.26**	Hz per mm(Hz/mm): **94.36**	Completed Scans **20**

Hiroyuki, HH-2-143B
125.688 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
C & CH2 same, CH & CH3 opposite side of solvent signal



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.10/2018.10.26.u5_HH-2-143B_loc2_03.34_C13_APT_ad

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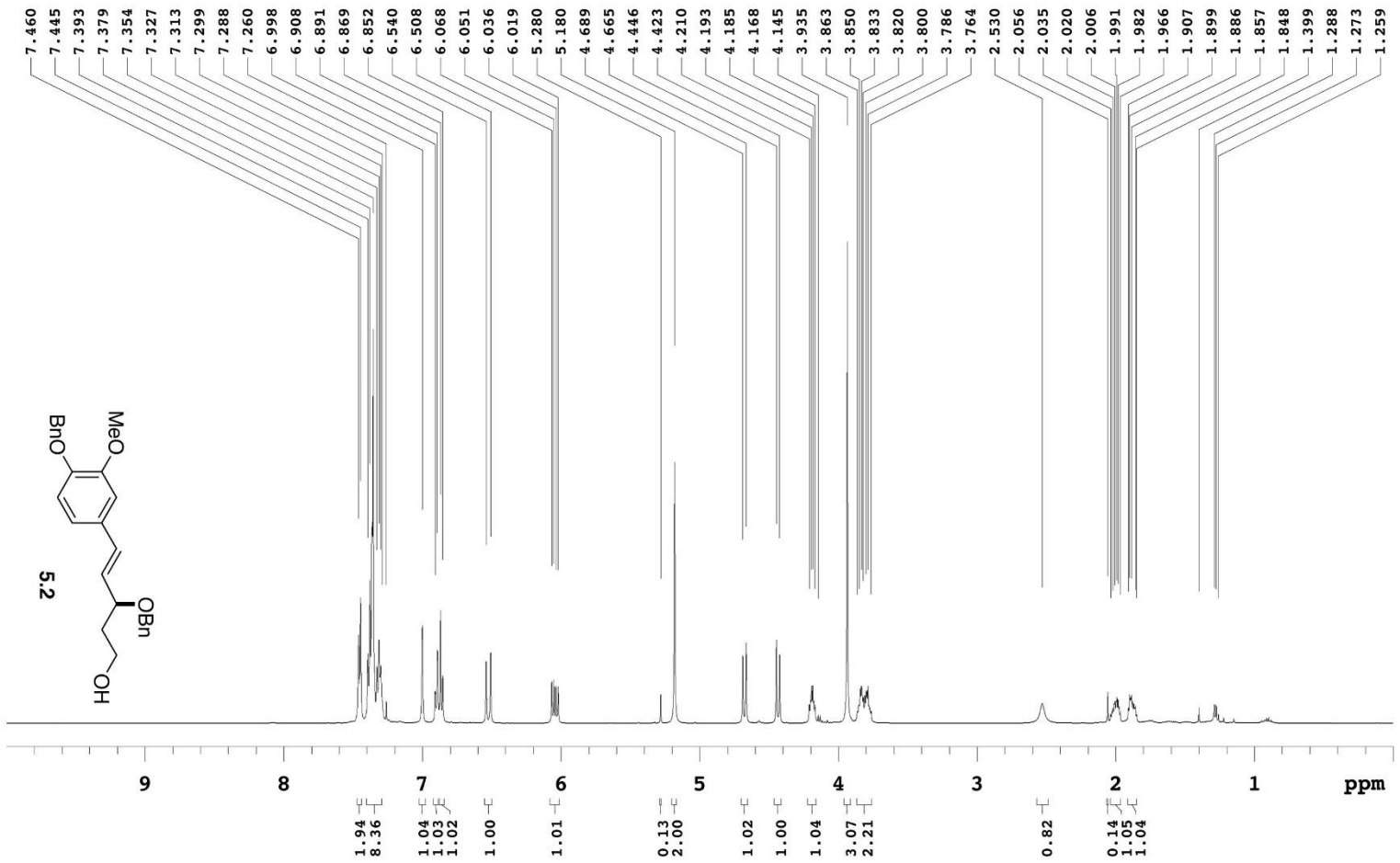
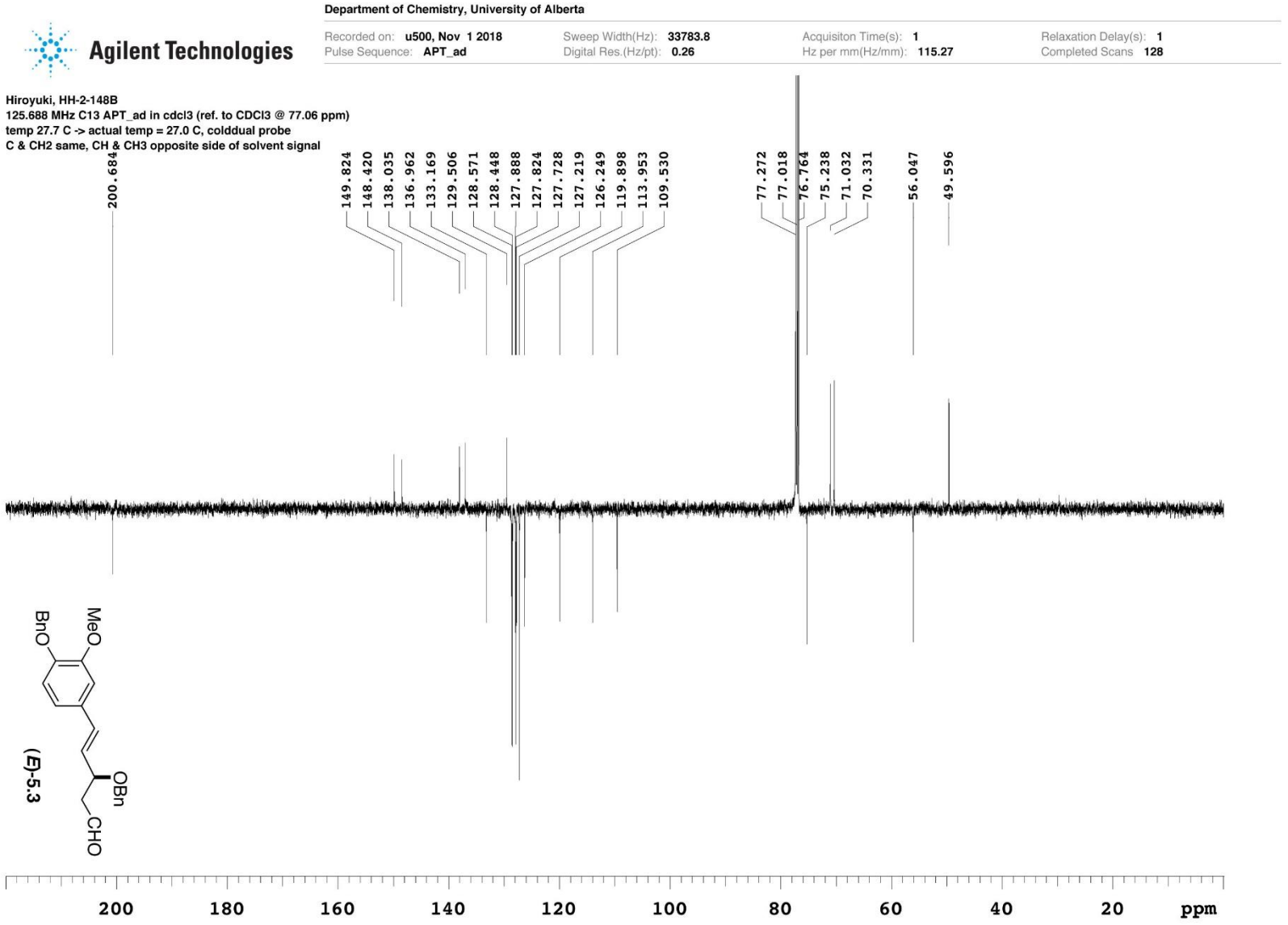
Recorded on: **ibd5, Oct 25 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-2-143B (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.10.25.i5_HH-2-143B_H1_1D



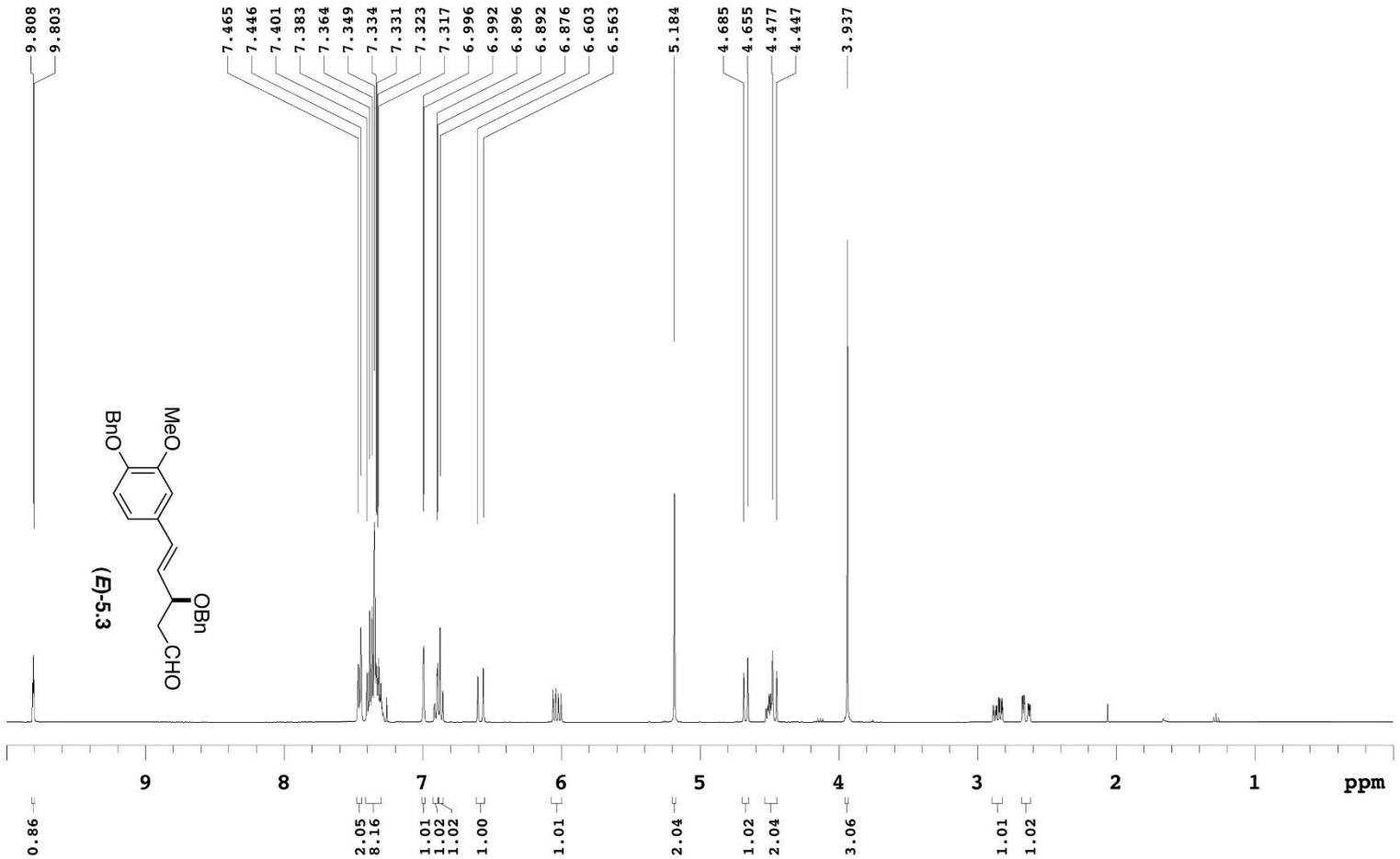
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.01.u5_HH-2-148B_loc1_06.23_C13_APT_ad

Department of Chemistry, University of Alberta

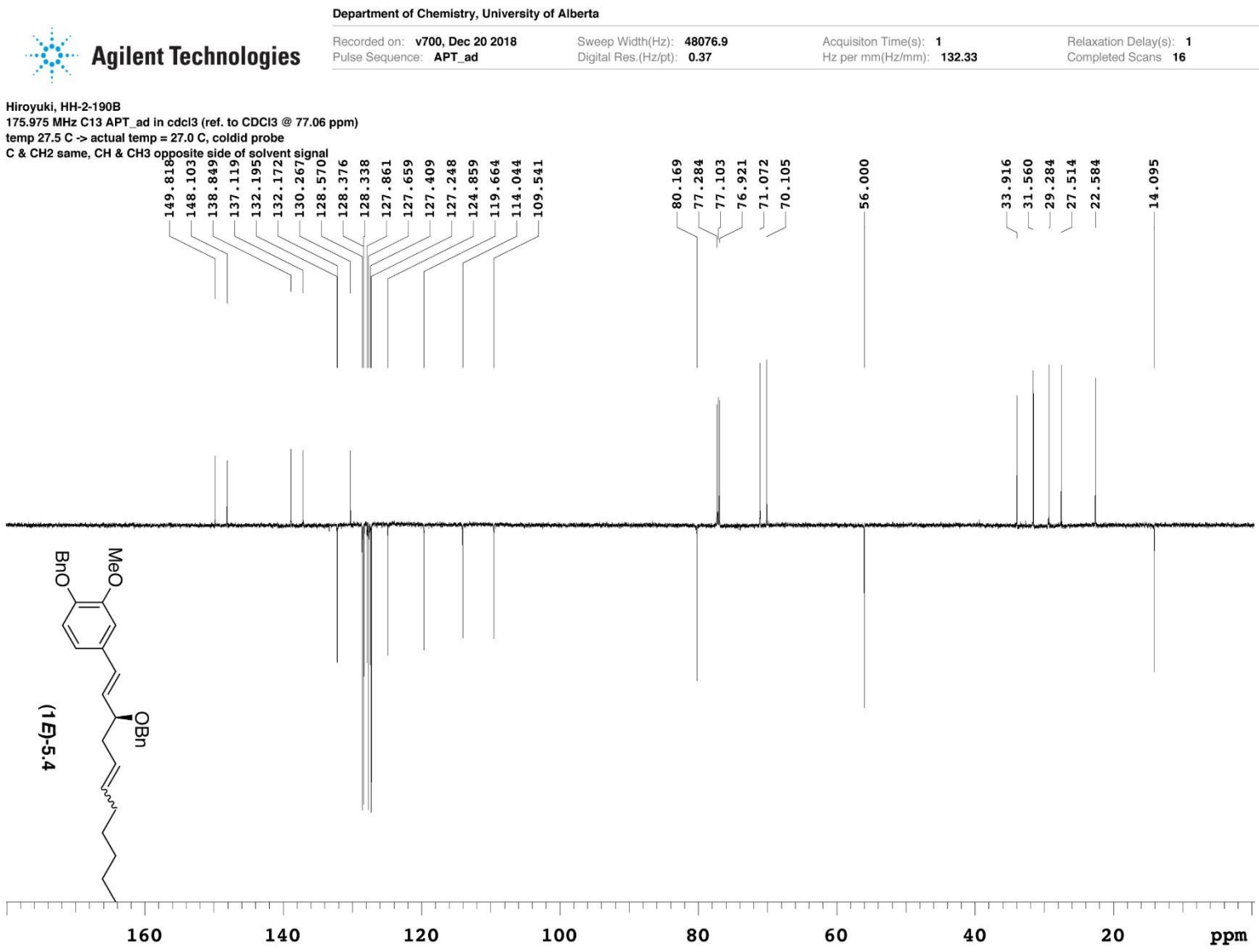
Agilent Technologies

Recorded on: **m400, Nov 15 2018**	Sweep Width(Hz): **4803.07**	Acquisiton Time(s): **4.997**	Relaxation Delay(s): **0.1**
Pulse Sequence: **PRESAT**	Digital Res.(Hz/pt): **0.07**	Hz per mm(Hz/mm): **16.69**	Completed Scans **32**

Hiroyuki, HH-2-157
400.389 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.0 C -> actual temp = 27.0 C, m400gz probe



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.15.m4_HH-2-157_ioc5_15.00_H1_1D



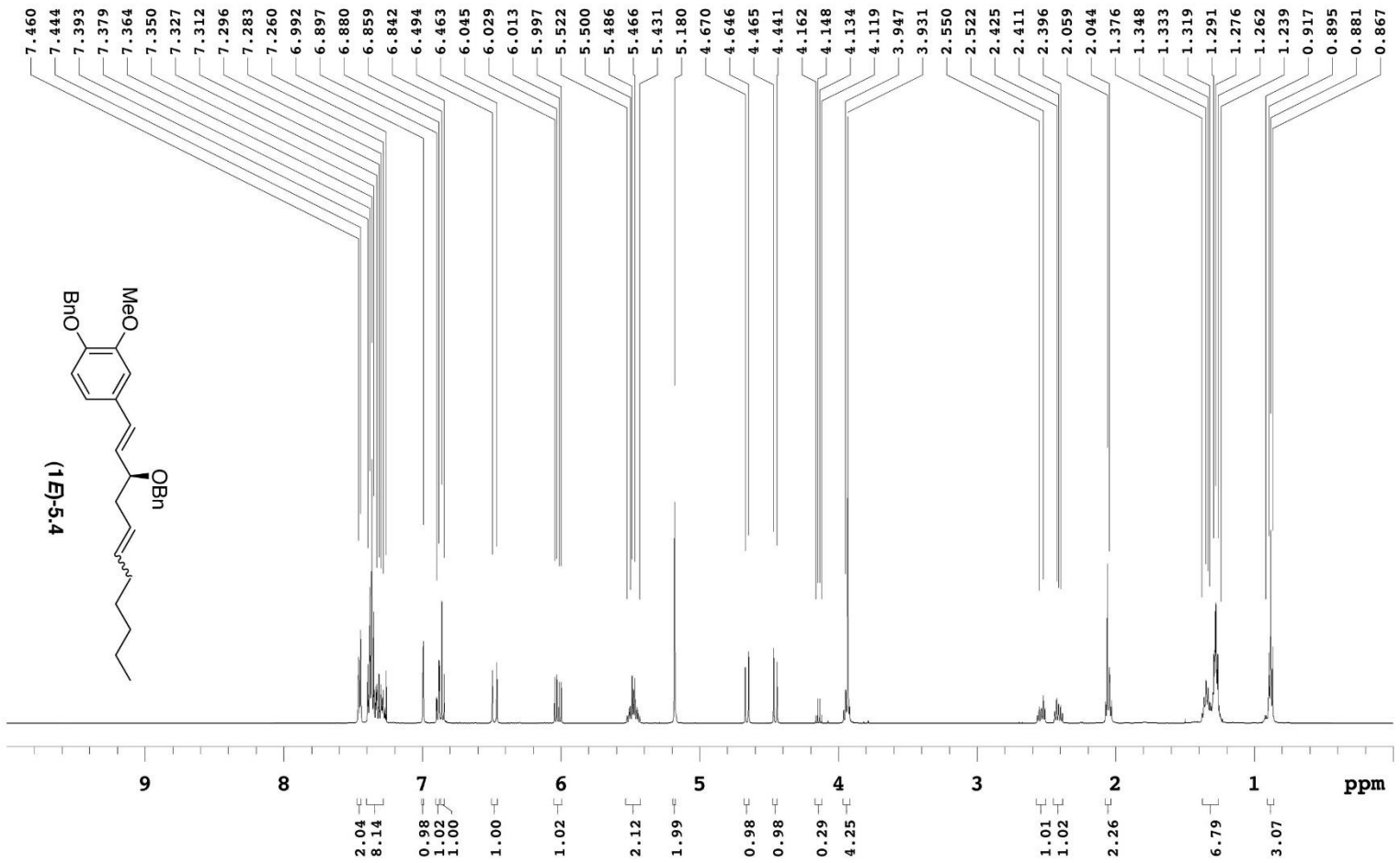
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.20.v7_HH-2-190B_loc12_11.58_C13_APT_ad

Agilent Technologies

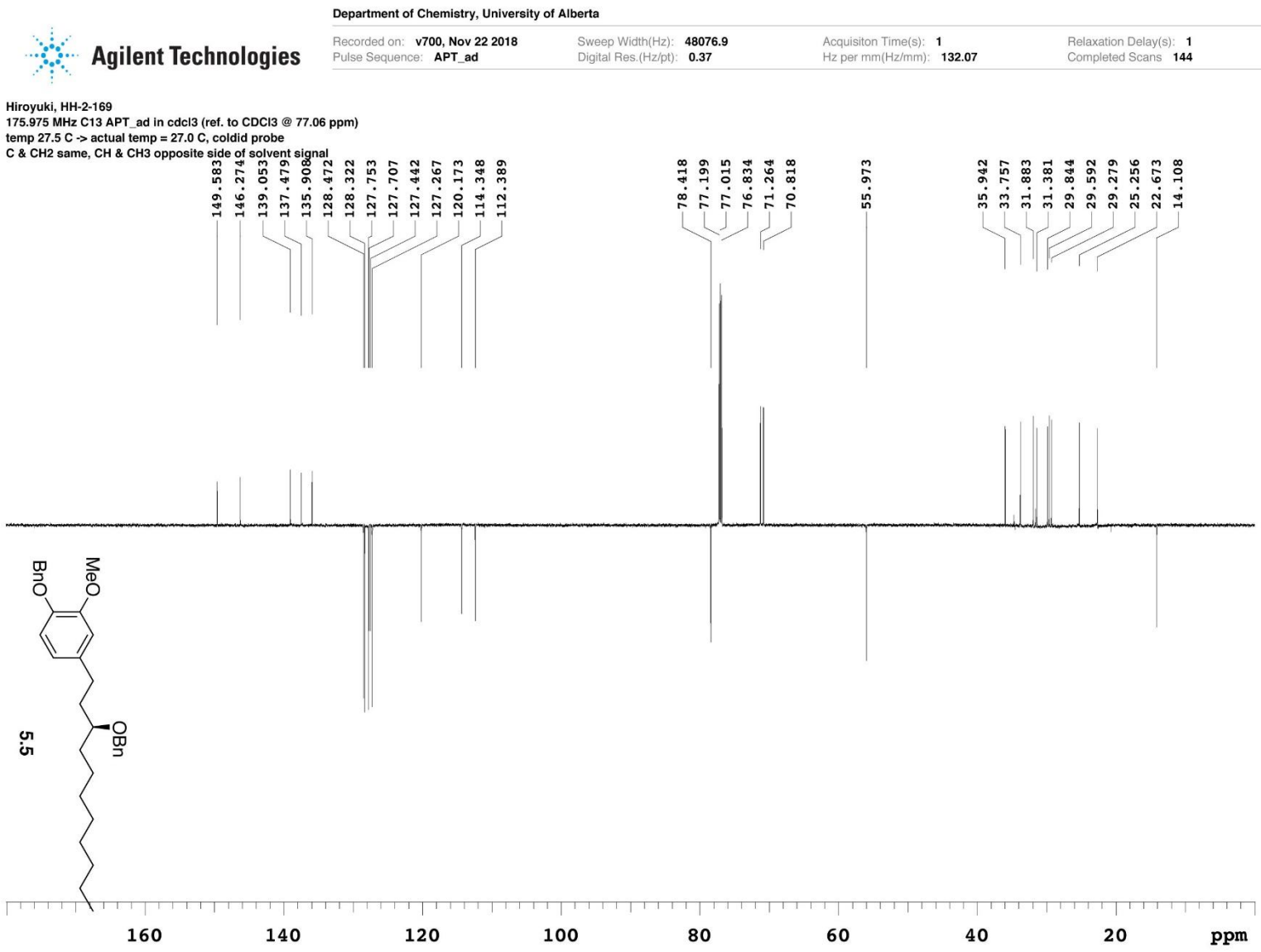
Department of Chemistry, University of Alberta

Recorded on: **ibd5, Nov 21 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-2-165B (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.11.21.i5_HH-2-165B_H1_1D



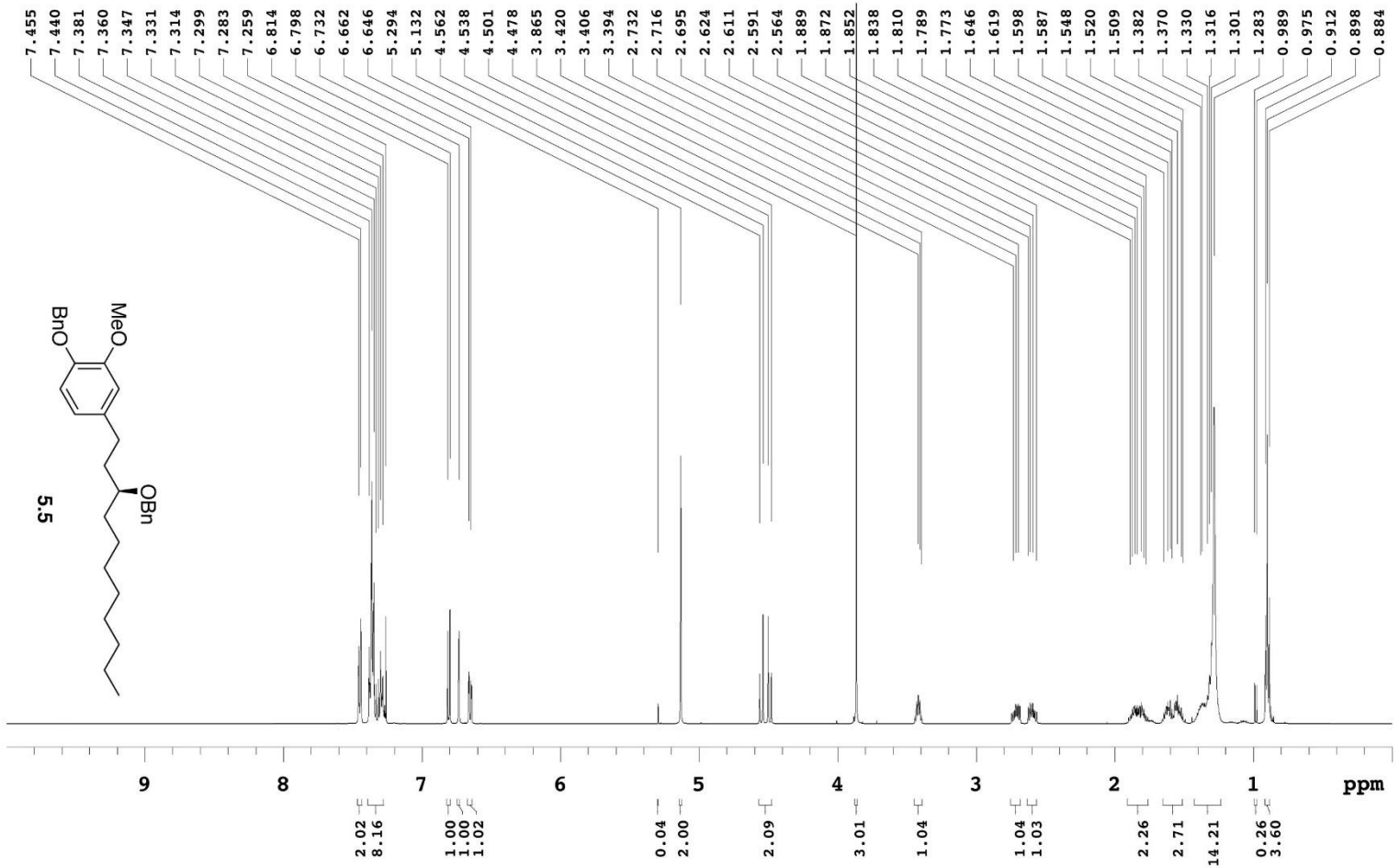
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.22.v7_HH-2-169_loc18_18.05_C13_APT_ad

Department of Chemistry, University of Alberta

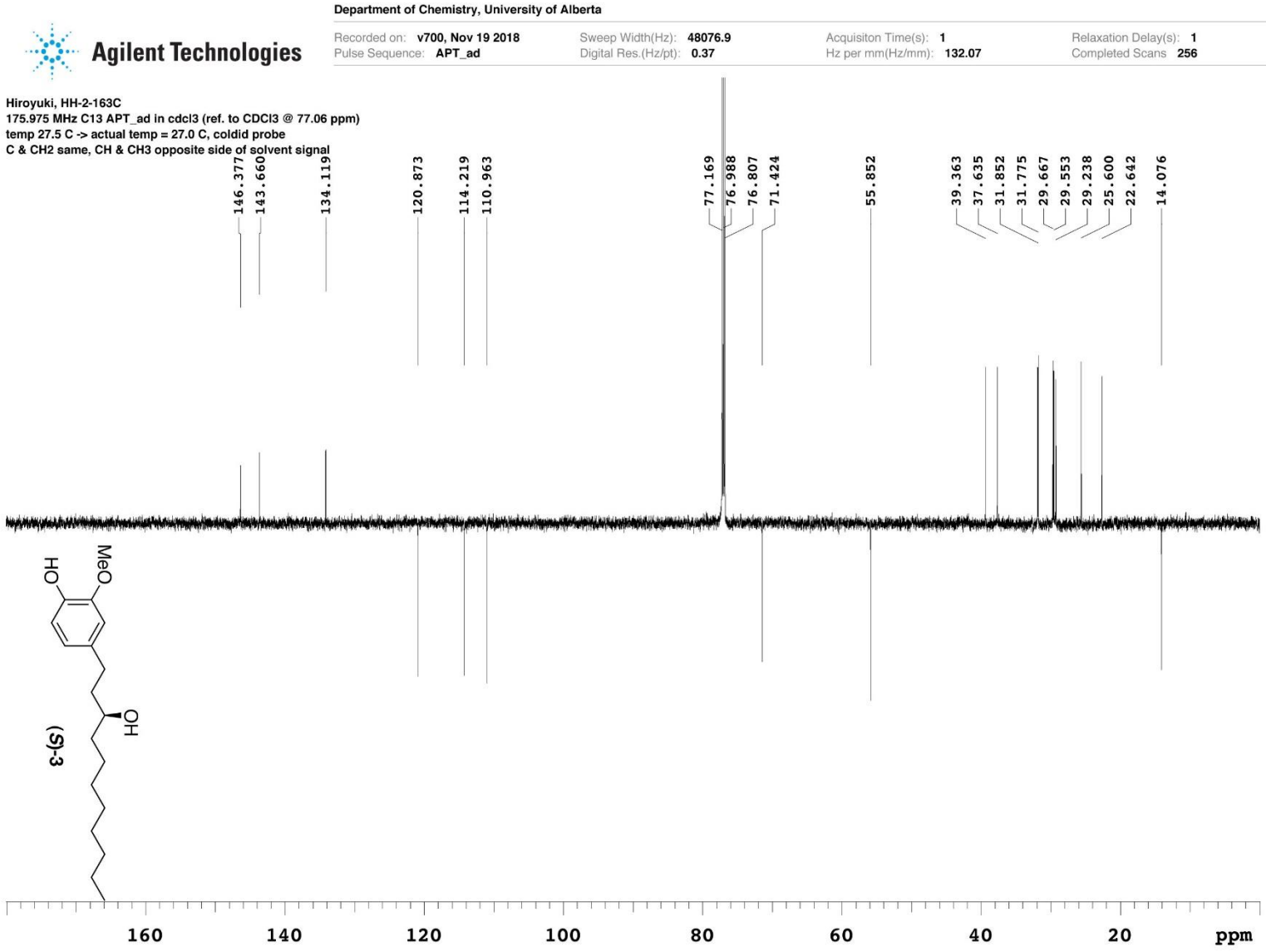
Agilent Technologies

Recorded on: **ibd5, Nov 22 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-2-169 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.11.22.i5_HH-2-169_H1_1D



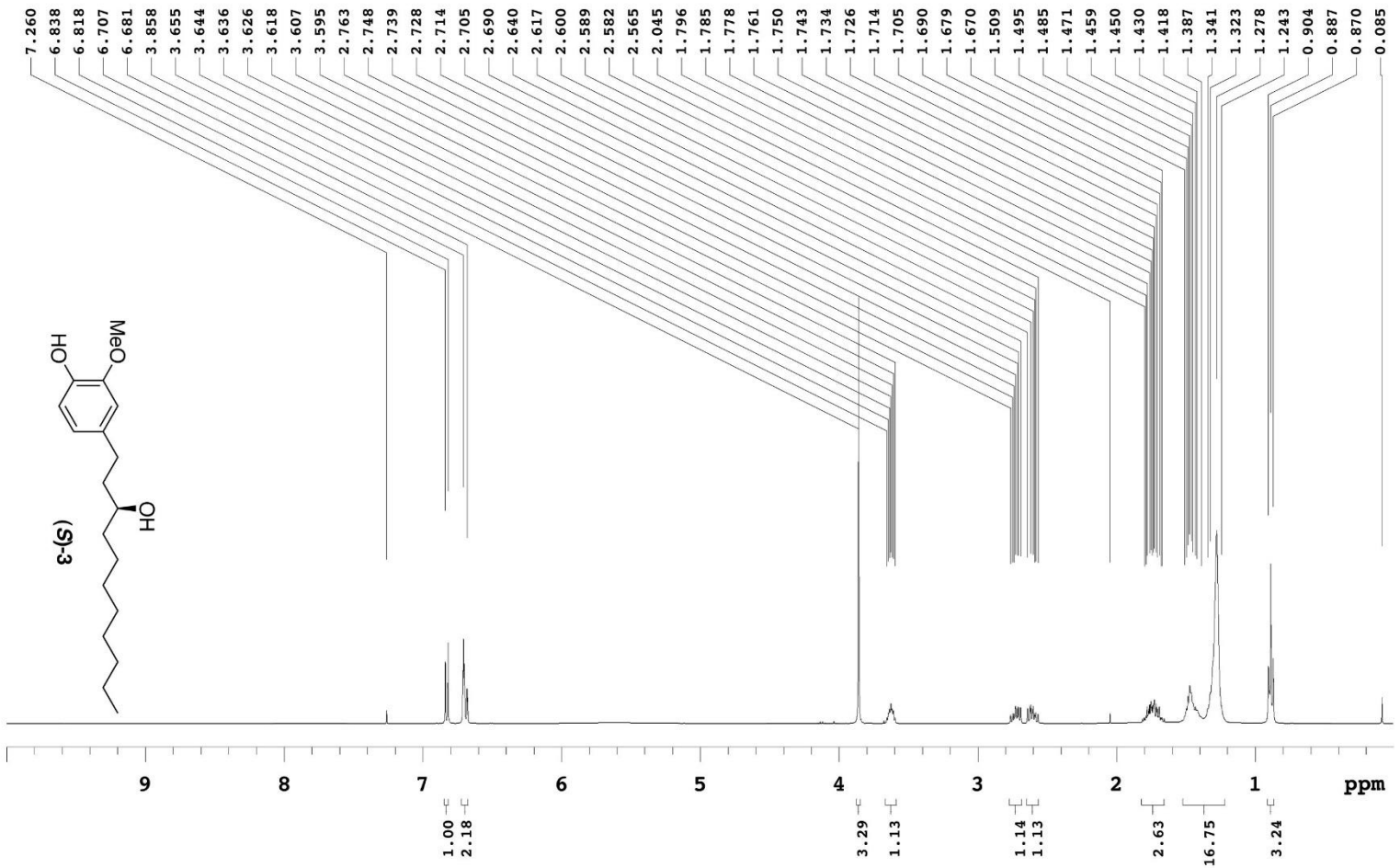
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.11/2018.11.19.v7_HH-2-163C_loc11_15.28_C13_APT_ad

Department of Chemistry, University of Alberta

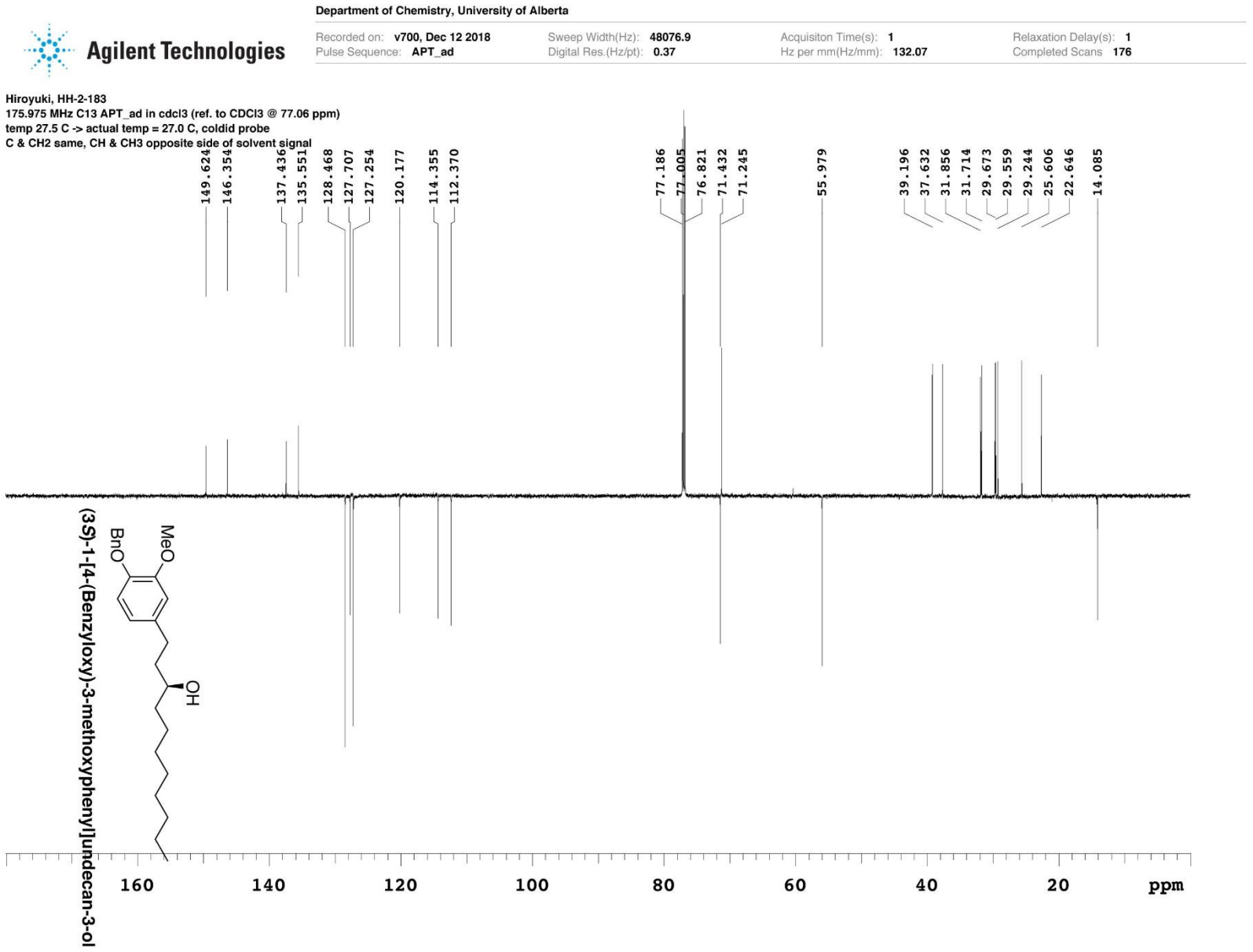
Agilent Technologies

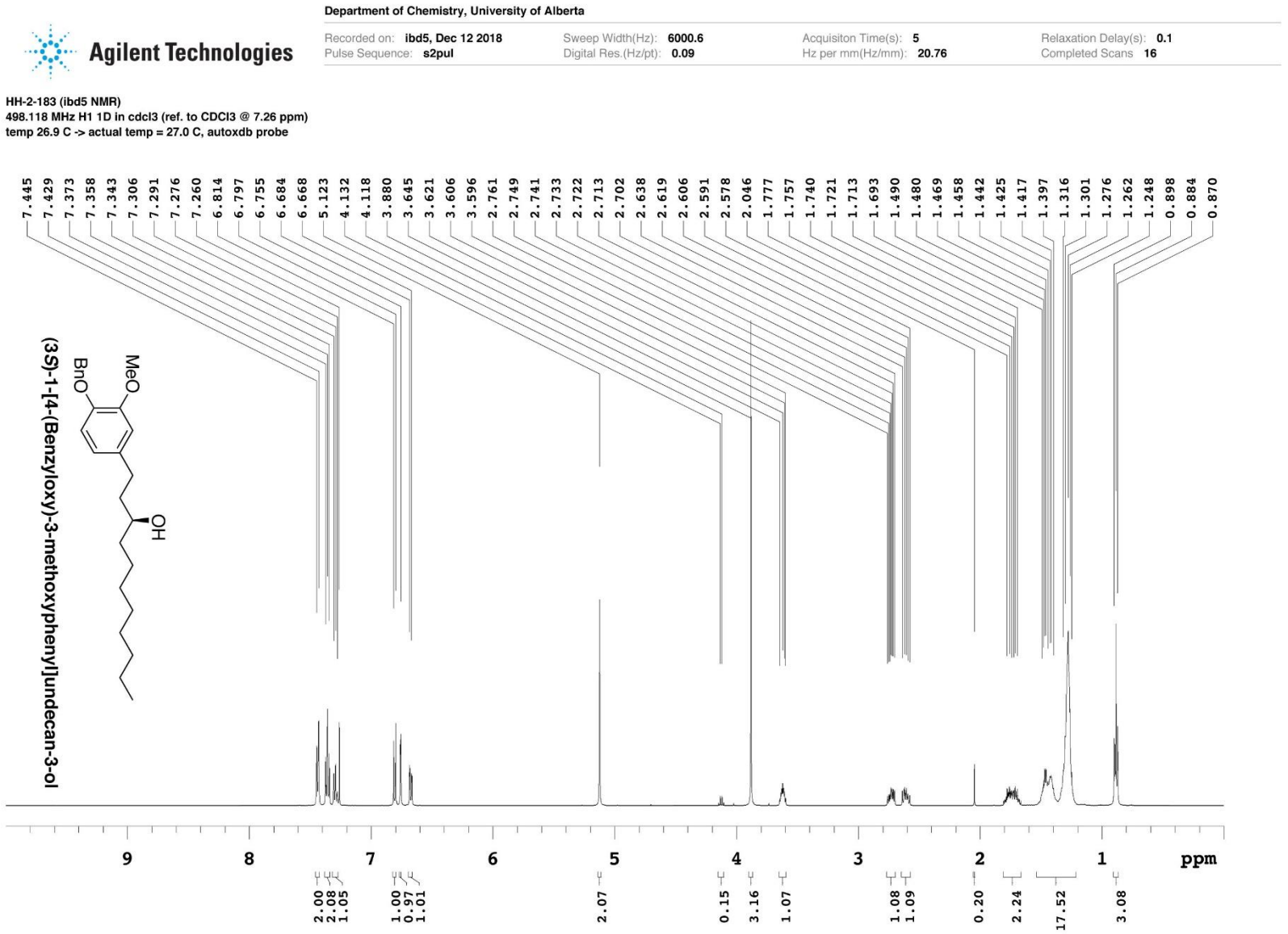
Recorded on: **mr400, Nov 23 2018**	Sweep Width(Hz): **4807.69**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.07**	Hz per mm(Hz/mm): **16.66**	Completed Scans **16**

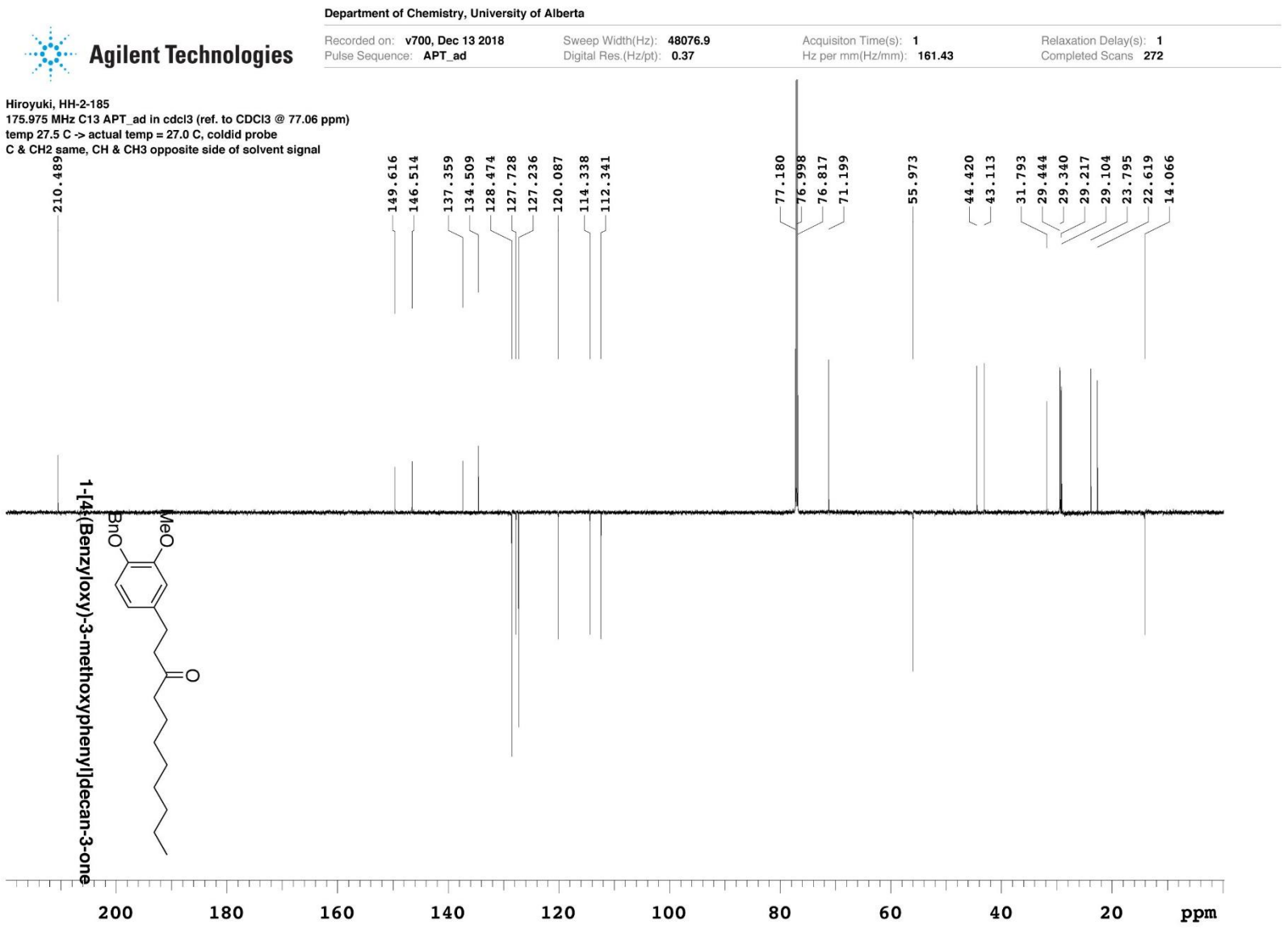
HH-2-170 (mr400 NMR)
399.978 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 25.9 C -> actual temp = 27.0 C, onenmr probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.11.23.mr4_HH-2-170_H1_1D


Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: v700, Dec 12 2018
Pulse Sequence: APT_ad
Sweep Width(Hz): 48076.9
Digital Res.(Hz/pt): 0.37
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 132.07
Relaxation Delay(s): 1
Completed Scans 176
Hiroyuki, HH-2-183
175.975 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
C & CH2 same, CH & CH3 opposite side of solvent signal
149.624
146.354
137.436
135.551
128.468
127.707
127.254
120.177
114.355
112.370
77.186
77.005
76.821
71.432
71.245
55.979
39.196
37.632
31.856
31.714
29.673
29.559
29.244
25.606
22.646
14.085
(3S)-1-[4-(Benzyloxy)-3-methoxyphenyl]undecan-3-ol
BnO
MeO
OH
160
140
120
100
80
60
40
20
ppm
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.12.v7_HH-2-183_loc7_16.32_C13_APT_ad


Agilent Technologies
Department of Chemistry, University of Alberta
Recorded on: ibd5, Dec 12 2018
Pulse Sequence: s2pul
Sweep Width(Hz): 6000.6
Digital Res.(Hz/pt): 0.09
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 20.76
Relaxation Delay(s): 0.1
Completed Scans 16
HH-2-183 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
7.445 7.429 7.373 7.358 7.343 7.306 7.291 7.276 7.260 6.814 6.797 6.755 6.684 6.668 5.123 4.132 4.118 3.880 3.645 3.621 3.606 3.596 2.761 2.749 2.741 2.733 2.722 2.713 2.702 2.638 2.619 2.606 2.591 2.578 2.046 1.777 1.757 1.740 1.721 1.713 1.693 1.490 1.480 1.469 1.458 1.442 1.425 1.417 1.397 1.316 1.301 1.276 1.262 1.248 0.898 0.884 0.870
BnO
MeO
OH
(3S)-1-[4-(Benzyloxy)-3-methoxyphenyl]undecan-3-ol
9 8 7 6 5 4 3 2 1 ppm
2.00 2.08 1.05 1.00 0.97 1.01 2.07 0.15 3.16 1.07 1.08 1.09 0.20 2.24 17.52 3.08
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.12.12.i5_HH-2-183_H1_1D



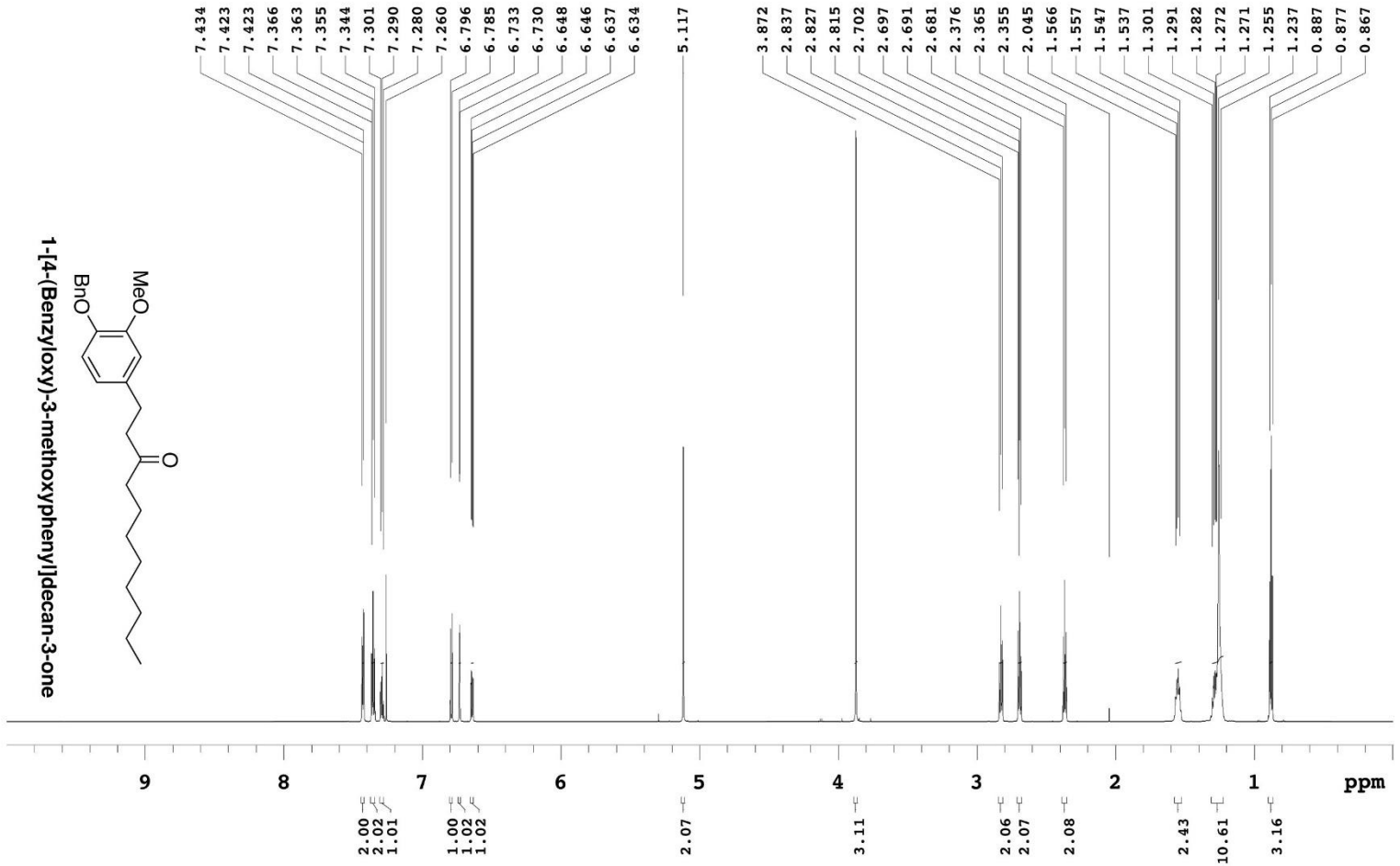
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.13.v7_HH-2-185_loc7_13.26_C13_APT_ad

Department of Chemistry, University of Alberta

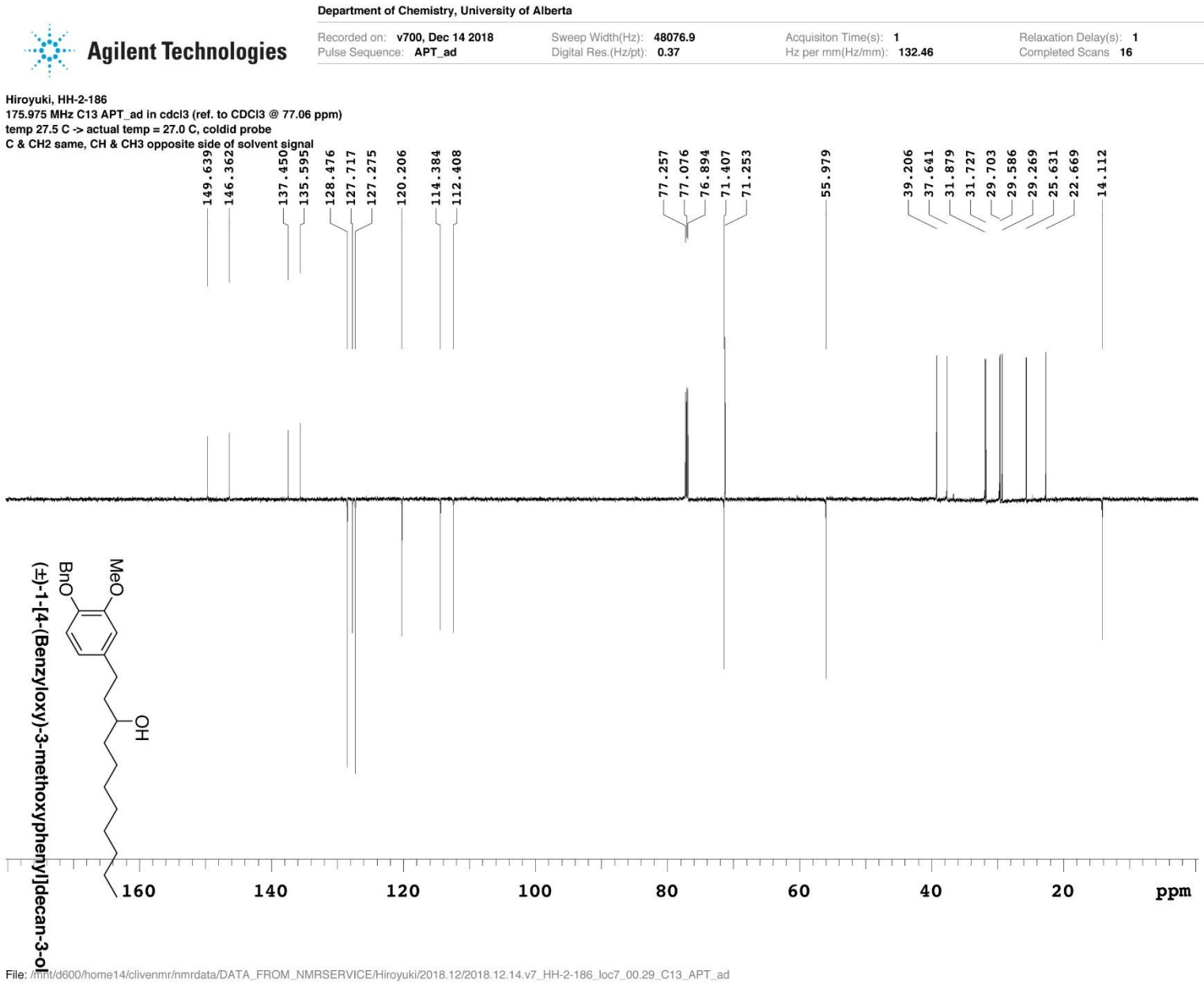
Agilent Technologies

Recorded on: **v700, Dec 13 2018**	Sweep Width(Hz): **8389.26**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **PRESAT**	Digital Res.(Hz/pt): **0.13**	Hz per mm(Hz/mm): **29.17**	Completed Scans **8**

HH-2-185 (v700 NMR)
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.13.v7_HH-2-185_loc7_13.25_H

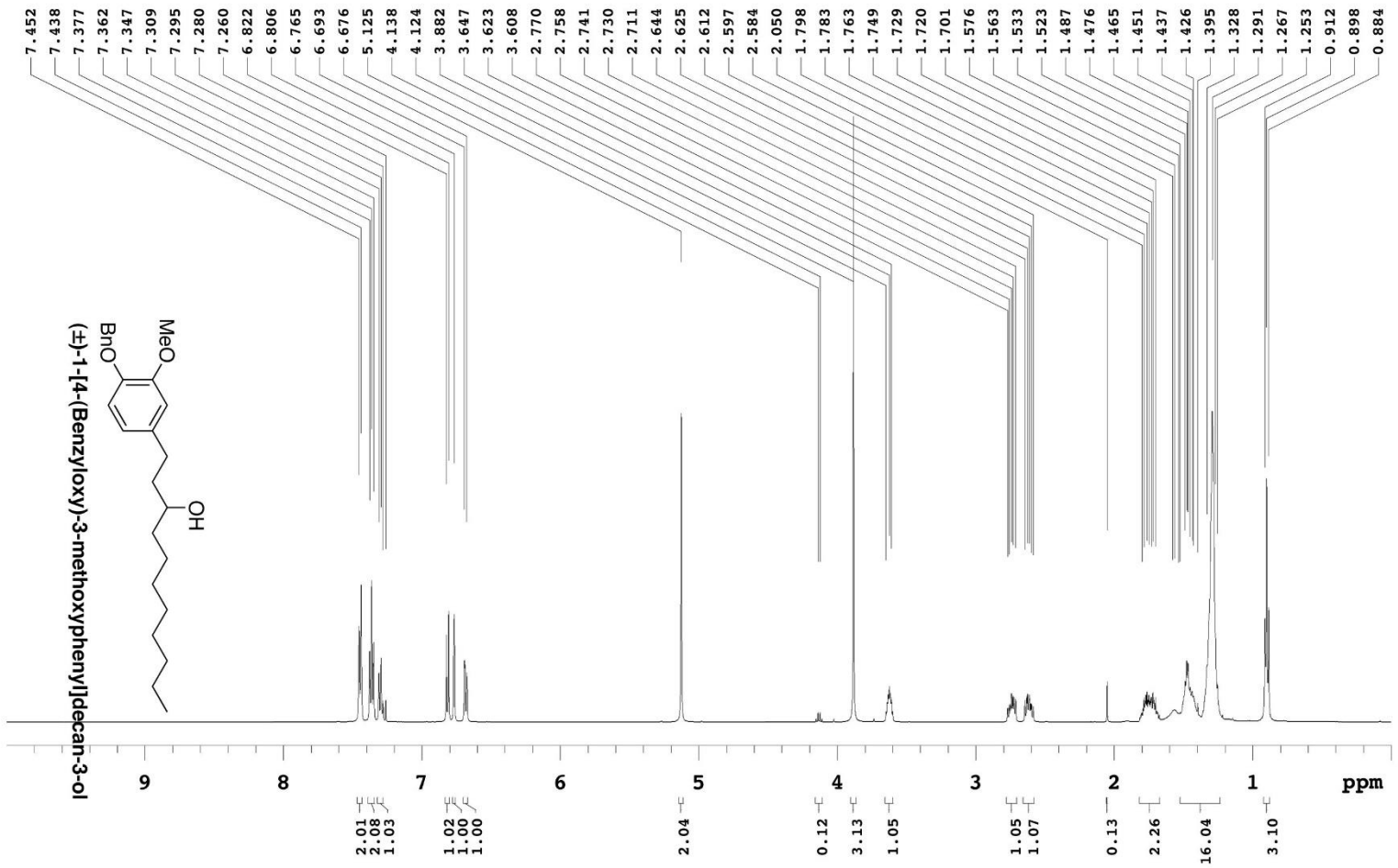

Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: v700, Dec 14 2018
Pulse Sequence: APT_ad
Sweep Width(Hz): 48076.9
Digital Res.(Hz/pt): 0.37
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 132.46
Relaxation Delay(s): 1
Completed Scans 16
Hiroyuki, HH-2-186
175.975 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
C & CH2 same, CH & CH3 opposite side of solvent signal
149.639
146.362
137.450
135.595
128.476
127.717
127.275
120.206
114.384
112.408
77.257
77.076
76.894
71.407
71.253
55.979
39.206
37.641
31.879
31.727
29.703
29.586
29.269
25.631
22.669
14.112
BnO
MeO
OH
(±)-1-[4-(Benzyloxy)-3-methoxyphenyl]decan-3-ol
160
140
120
100
80
60
40
20
ppm
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.14.v7_HH-2-186_loc7_00.29_C13_APT_ad

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Dec 14 2018**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-2-186 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



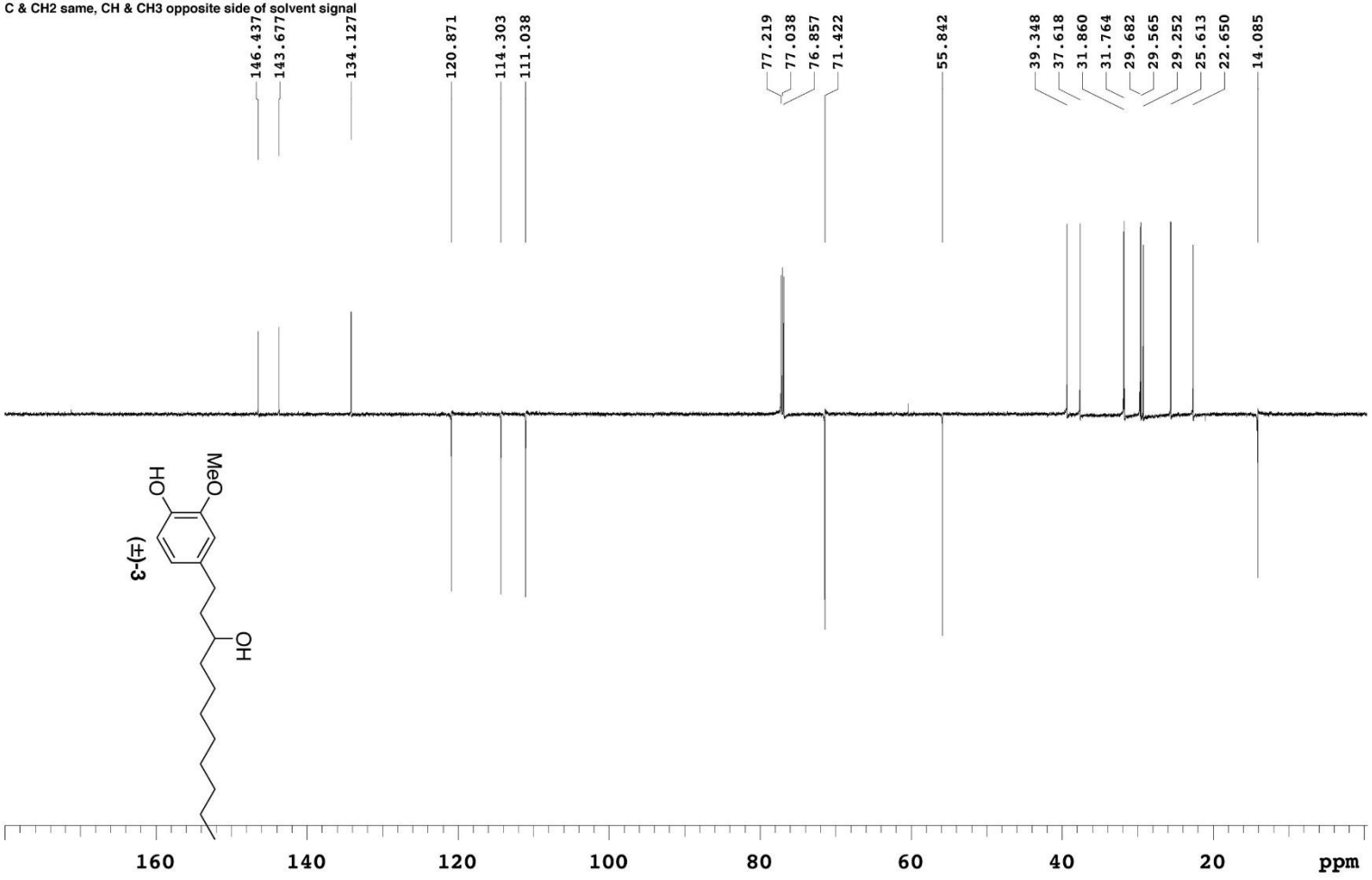
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.12.14.i5_HH-2-186_H1_1D

Department of Chemistry, University of Alberta

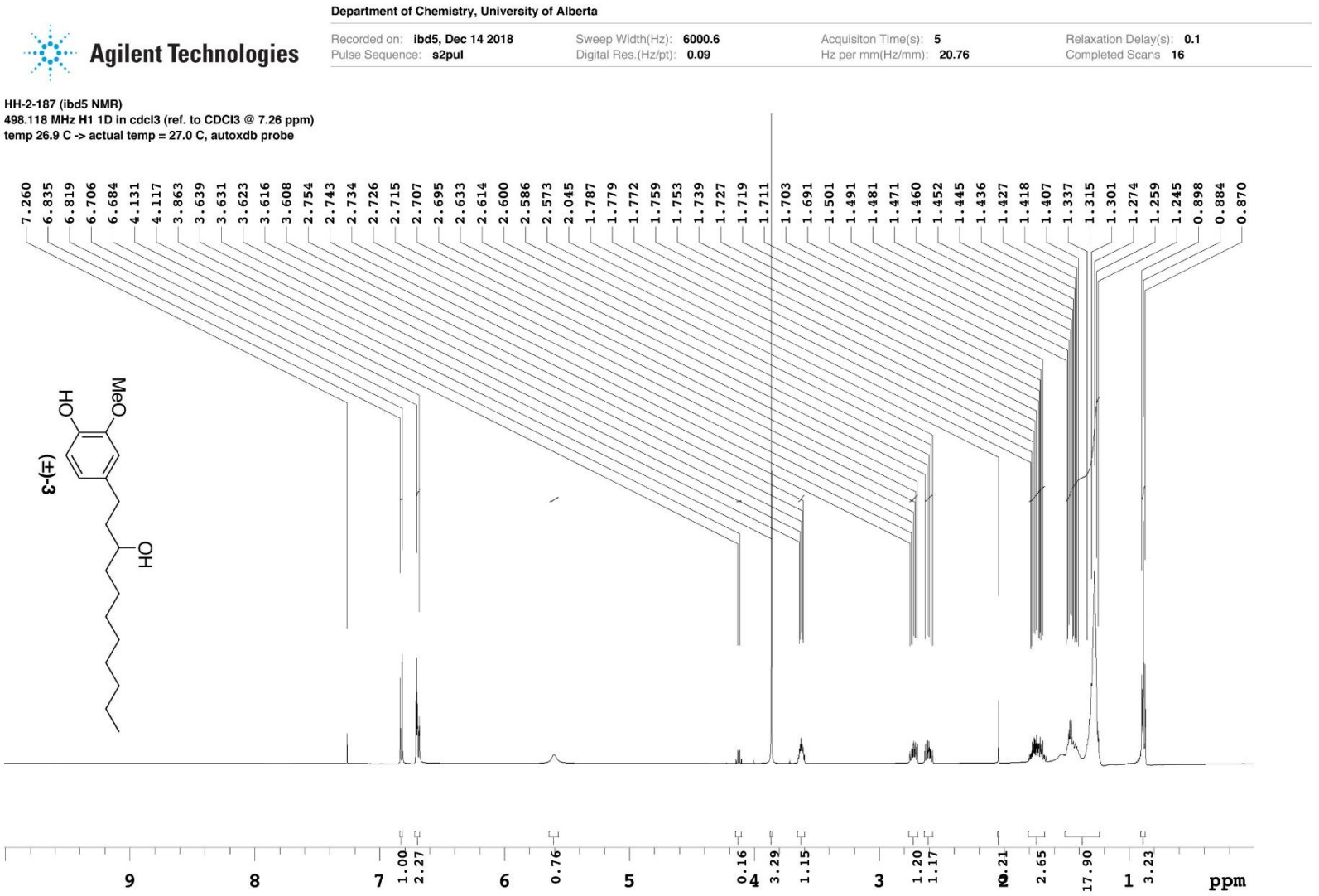
Agilent Technologies

Recorded on: v700, Dec 14 2018	Sweep Width(Hz): 48076.9	Acquisiton Time(s): 1	Relaxation Delay(s): 1
Pulse Sequence: APT_ad	Digital Res.(Hz/pt): 0.37	Hz per mm(Hz/mm): 132.28	Completed Scans 32

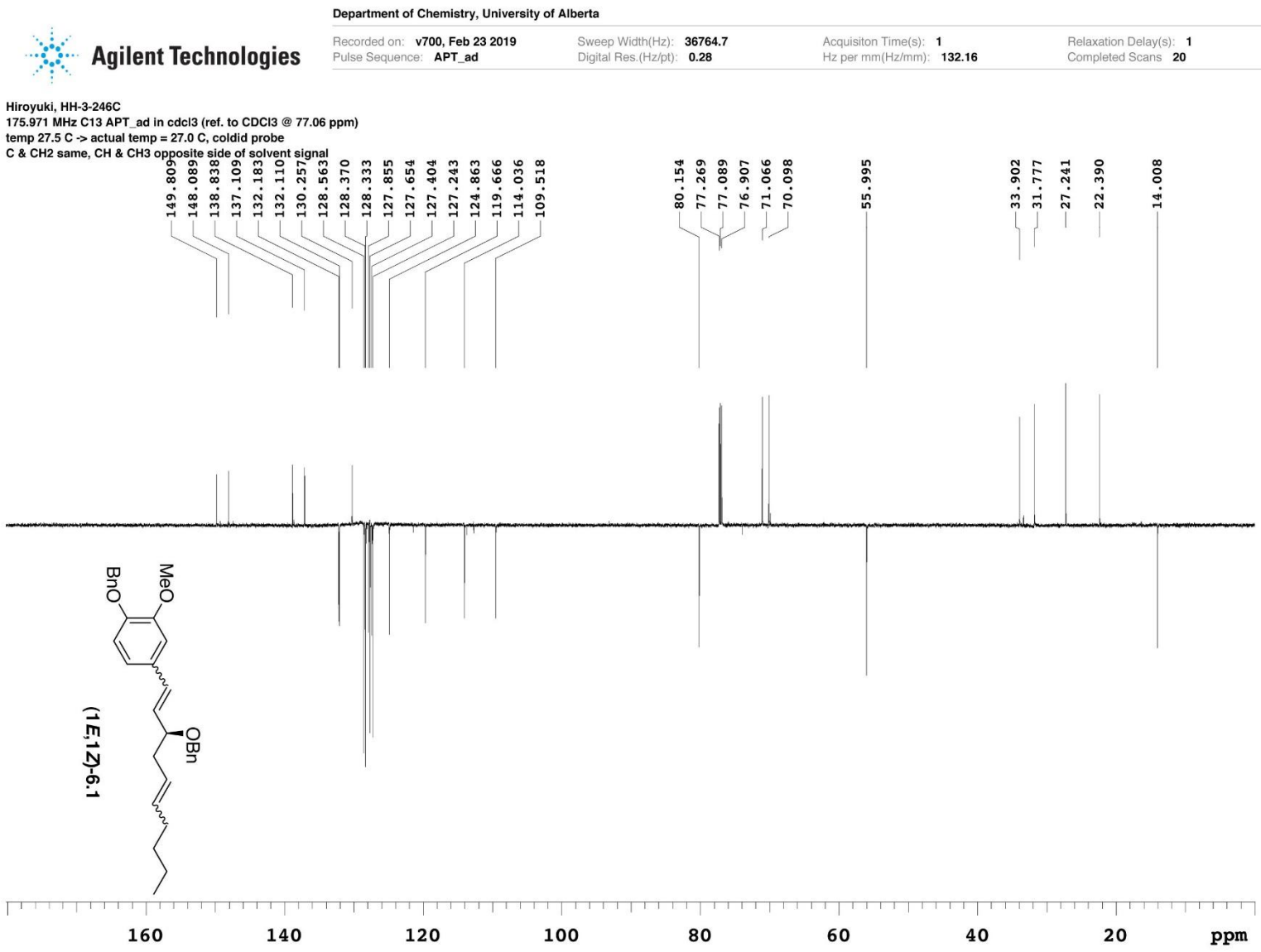
Hiroyuki, HH-2-187
175.975 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
C & CH2 same, CH & CH3 opposite side of solvent signal



File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2018.12/2018.12.14.v7_HH-2-187_loc11_15.42_C13_APT_ad



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2018.12.14.i5_HH-2-187_H1_1D



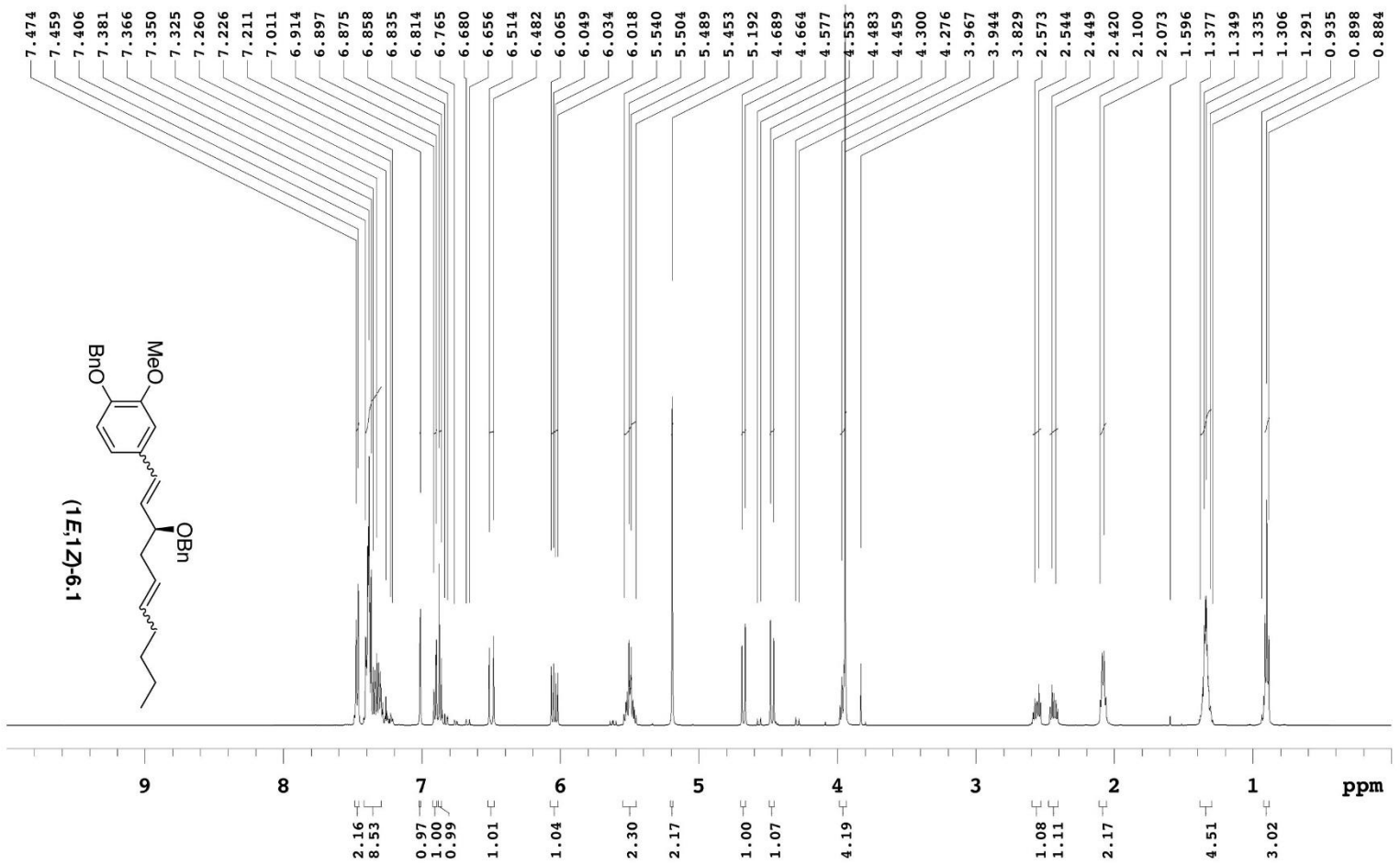
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2019.02/2019.02.23.v7_HH-3-246C_loc17_20.10_C13_APT_ad

Agilent Technologies

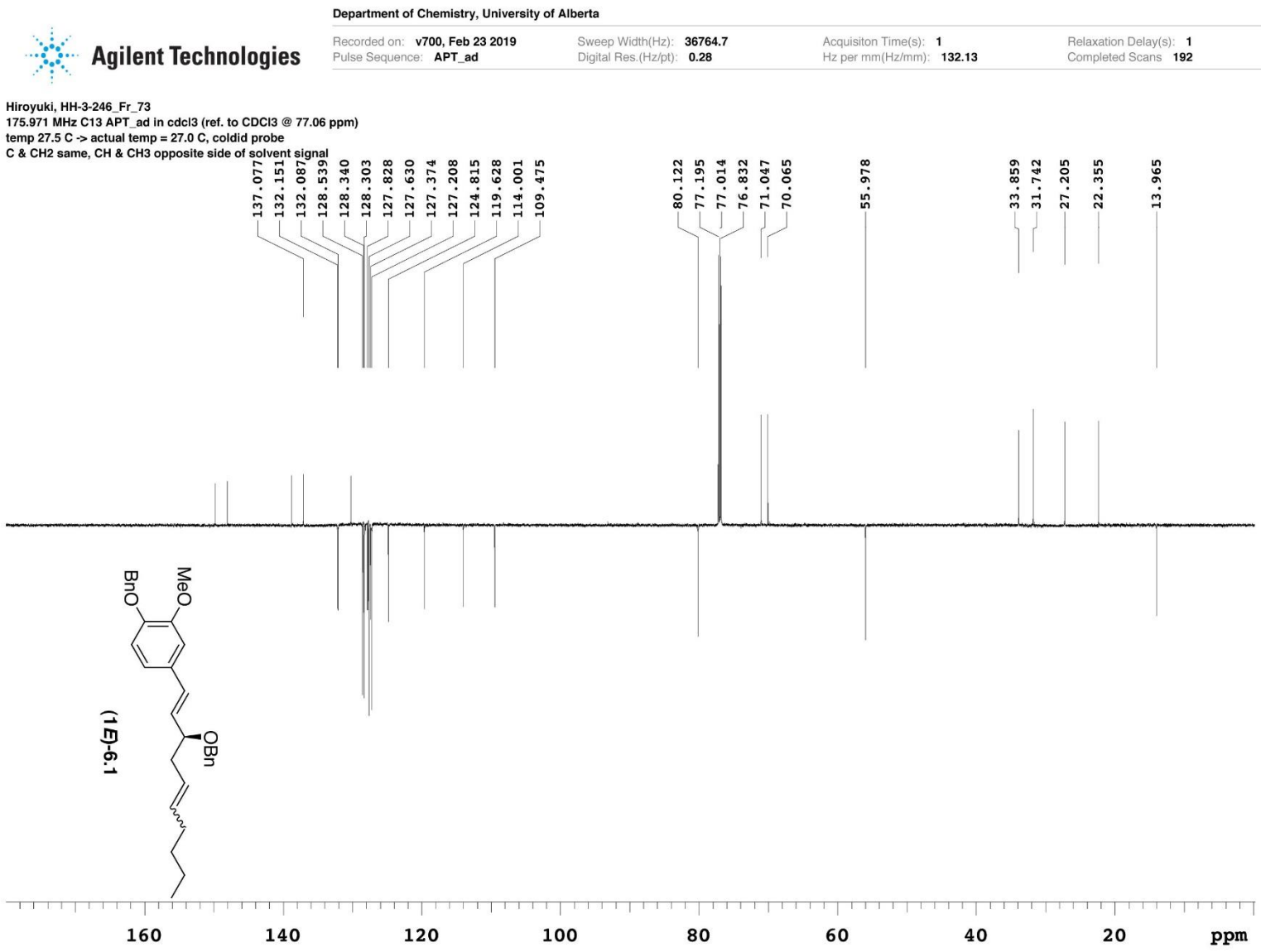
Department of Chemistry, University of Alberta

Recorded on: **ibd5, Feb 23 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-246C (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.23.i5_HH-3-246C_H1_1D



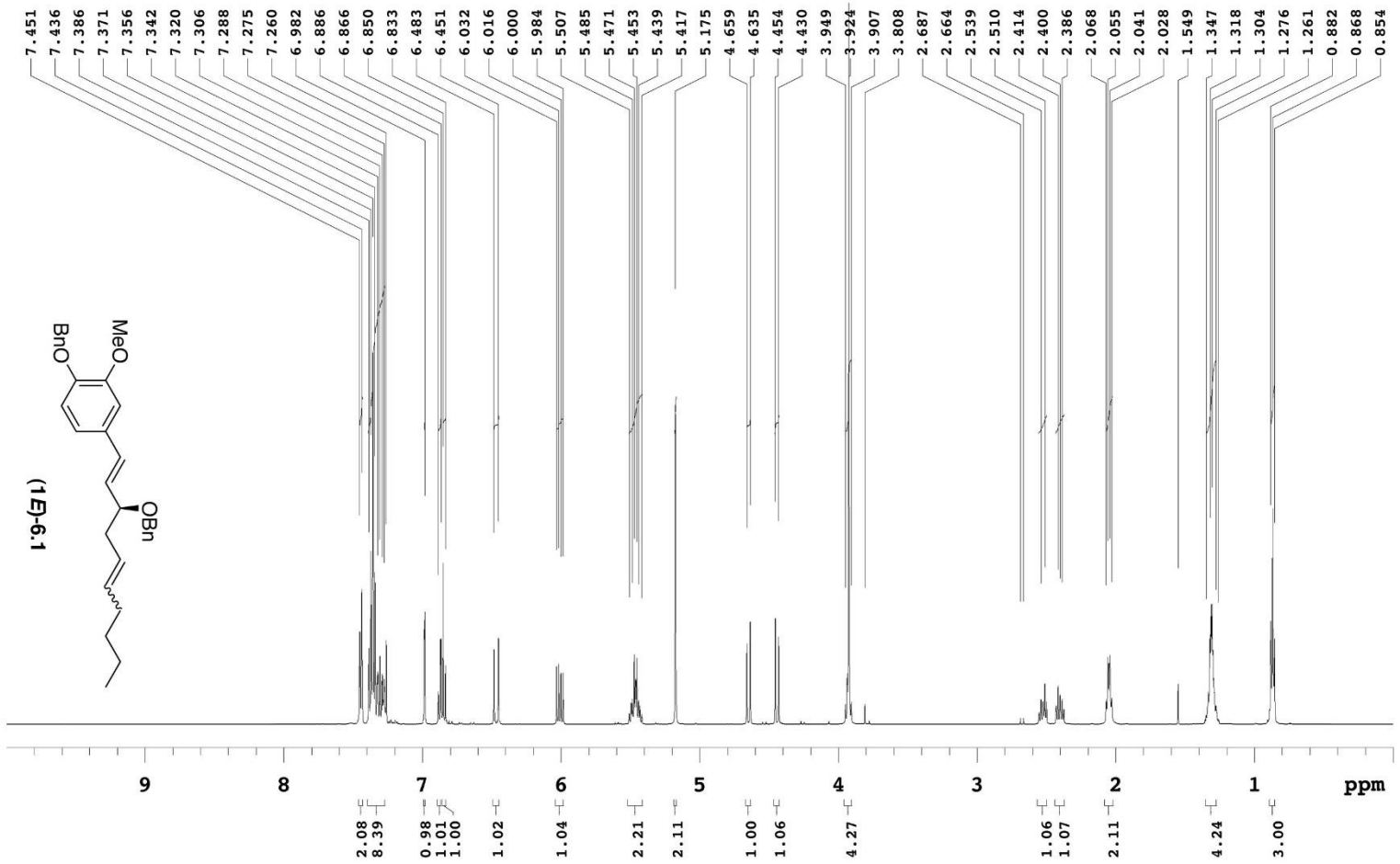
File: /mnt/d600/home14/clivenmr/nmrdata/DATA_FROM_NMRSERVICE/Hiroyuki/2019.02/2019.02.23.v7_HH-3-246_Fr_73_loc18_20.16_C13_APT_ad

Department of Chemistry, University of Alberta

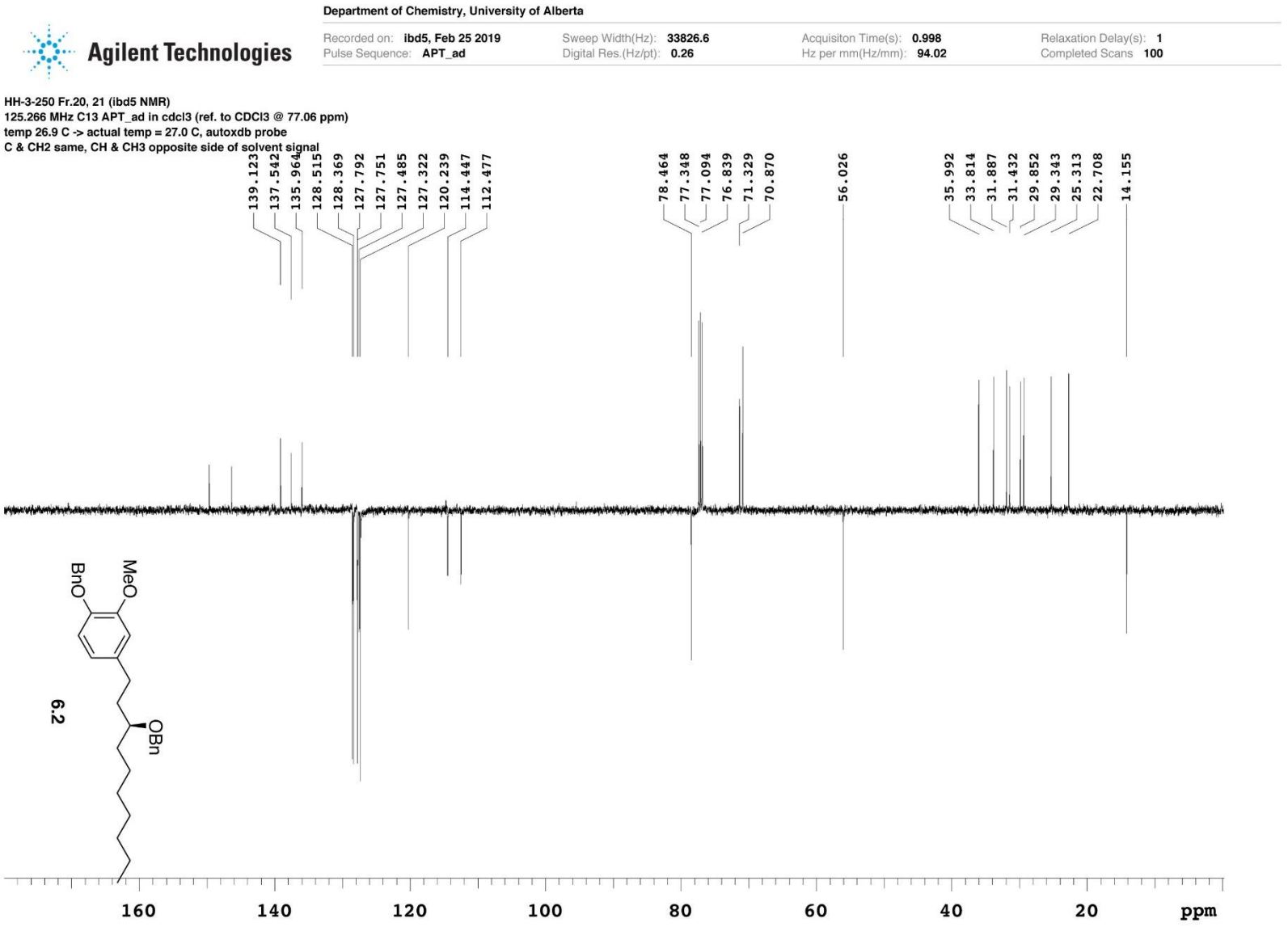
Agilent Technologies

Recorded on: **ibd5, Feb 23 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-246_Fr.73
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.23.i5_HH-3-246_Fr.73_H1_1D

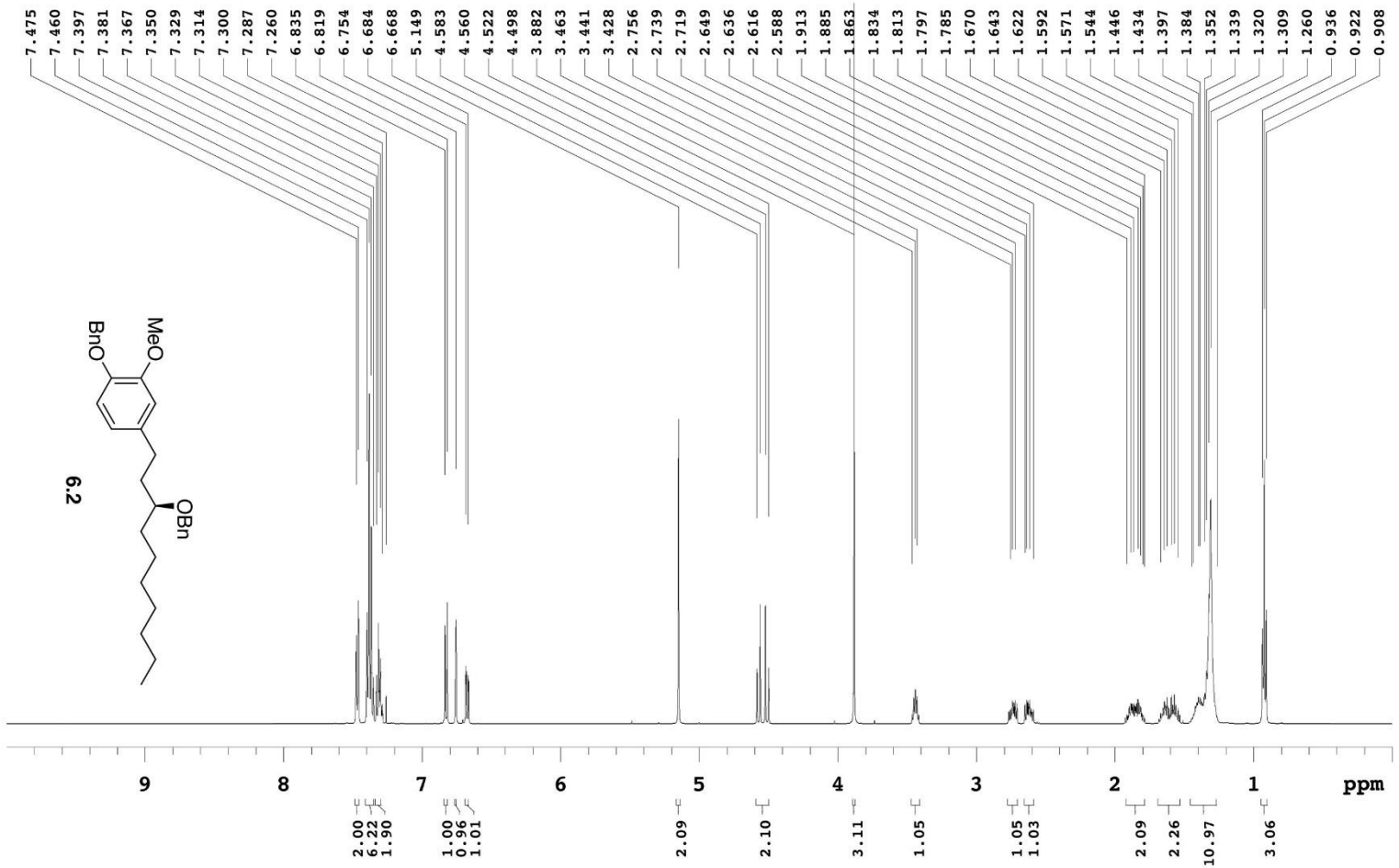

Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: ibd5, Feb 25 2019
Pulse Sequence: APT_ad
Sweep Width(Hz): 33826.6
Digital Res.(Hz/pt): 0.26
Acquisiton Time(s): 0.998
Hz per mm(Hz/mm): 94.02
Relaxation Delay(s): 1
Completed Scans 100
HH-3-250 Fr.20, 21 (ibd5 NMR)
125.266 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal
139.123
137.542
135.964
128.515
128.369
127.792
127.751
127.485
127.322
120.239
114.447
112.477
78.464
77.348
77.094
76.839
71.329
70.870
56.026
35.992
33.814
31.887
31.432
29.852
29.343
25.313
22.708
14.155
BnO
MeO
OBn
6.2
160
140
120
100
80
60
40
20
ppm
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.25.i5_HH-3-250_Fr.20__21_C13_APT_ad

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Feb 25 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-250 Fr20, 21 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.25.i5_HH-3-250_Fr.20__21_H1_1D

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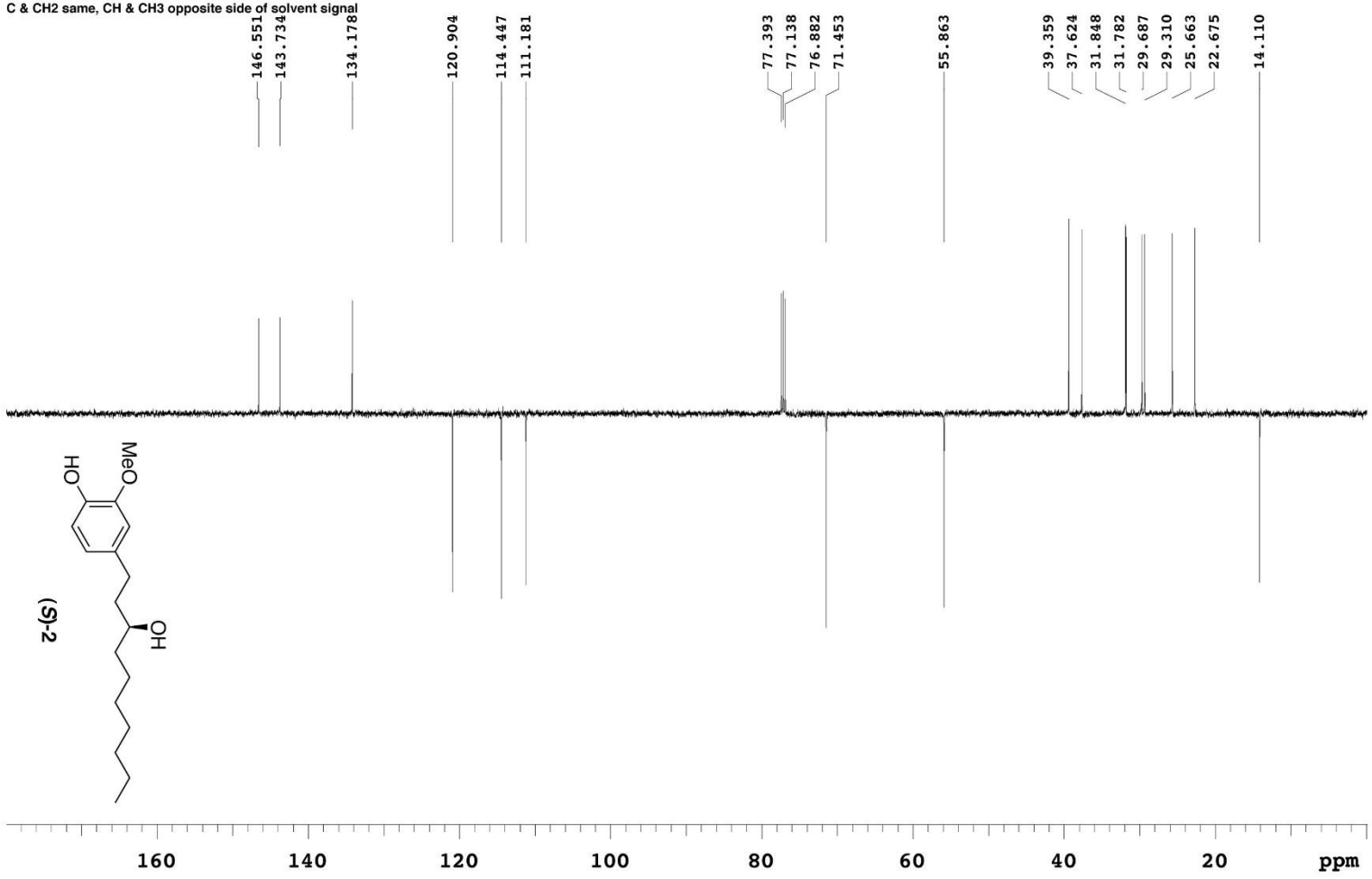
Agilent Technologies

Recorded on: **ibd5, Feb 26 2019**	Sweep Width(Hz): **33826.6**	Acquisiton Time(s): **0.998**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.26**	Hz per mm(Hz/mm): **93.96**	Completed Scans **32**

HH-3-252 (ibd5 NMR)
125.266 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal



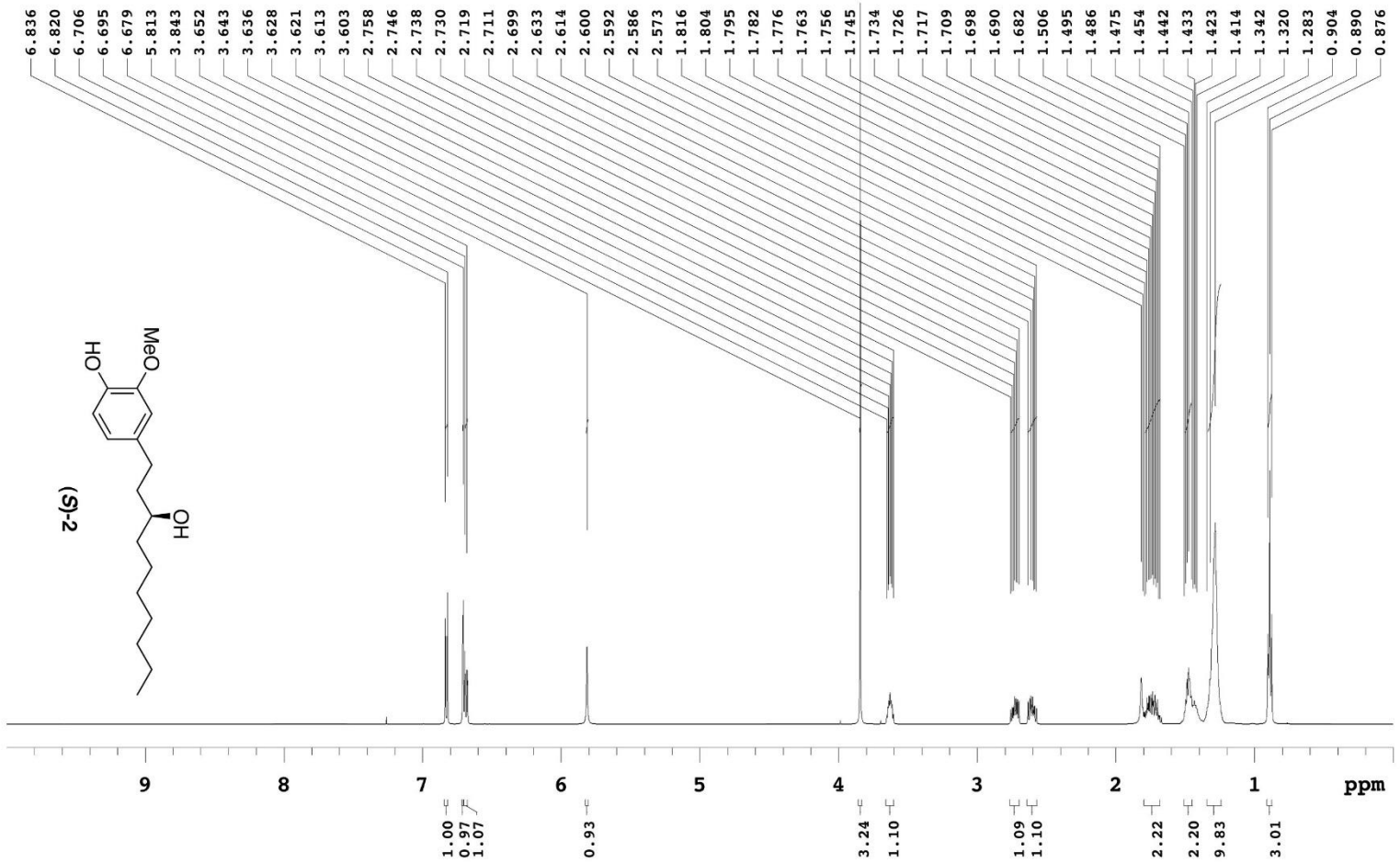
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.26.i5_HH-3-252_C13_APT_ad

Department of Chemistry, University of Alberta

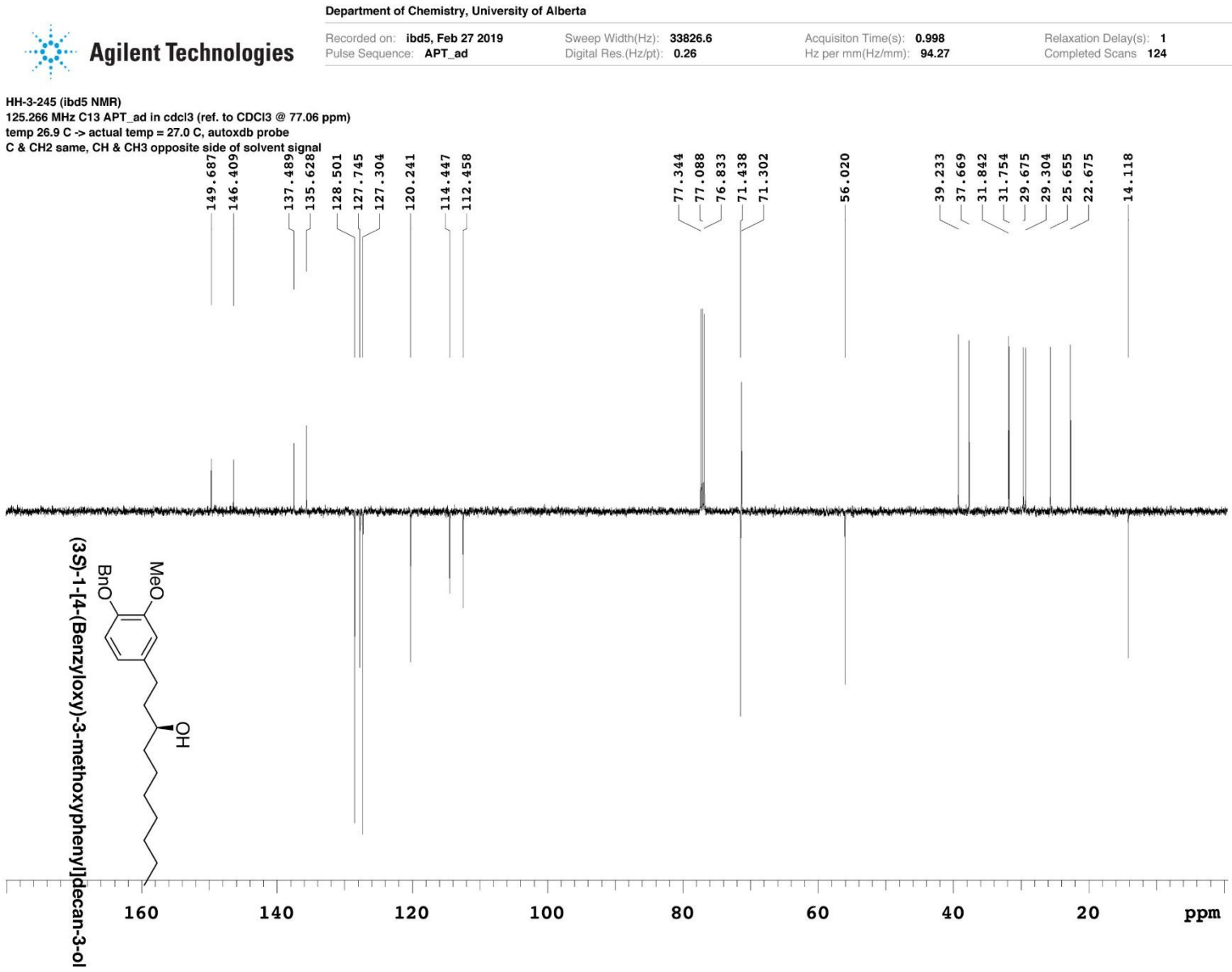
Agilent Technologies

Recorded on: **ibd5, Feb 26 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-252 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.26.i5_HH-3-252_H1_1D

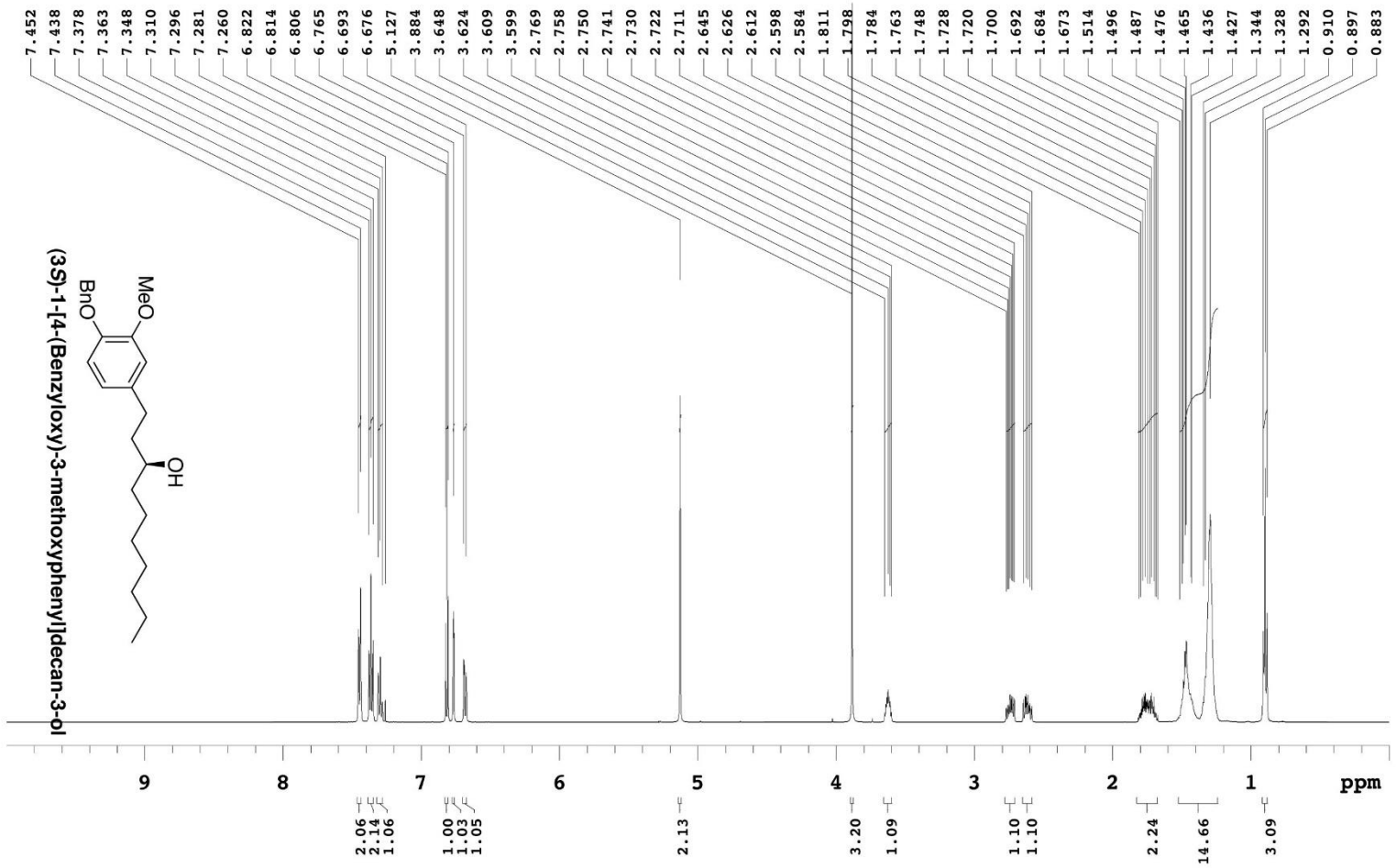

Department of Chemistry, University of Alberta
Agilent Technologies
Recorded on: ibd5, Feb 27 2019
Pulse Sequence: APT_ad
Sweep Width(Hz): 33826.6
Digital Res.(Hz/pt): 0.26
Acquisiton Time(s): 0.998
Hz per mm(Hz/mm): 94.27
Relaxation Delay(s): 1
Completed Scans 124
HH-3-245 (ibd5 NMR)
125.266 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal
149.687
146.409
137.489
135.628
128.501
127.745
127.304
120.241
114.447
112.458
77.344
77.088
76.833
71.438
71.302
56.020
39.233
37.669
31.842
31.754
29.675
29.304
25.655
22.675
14.118
BnO
MeO
OH
(3S)-1-[4-(Benzyloxy)-3-methoxyphenyl]decan-3-ol
160
140
120
100
80
60
40
20
ppm
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.27.i5_HH-3-254_C13_APT_ad

Agilent Technologies

Department of Chemistry, University of Alberta

Recorded on: **ibd5, Feb 27 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-254 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



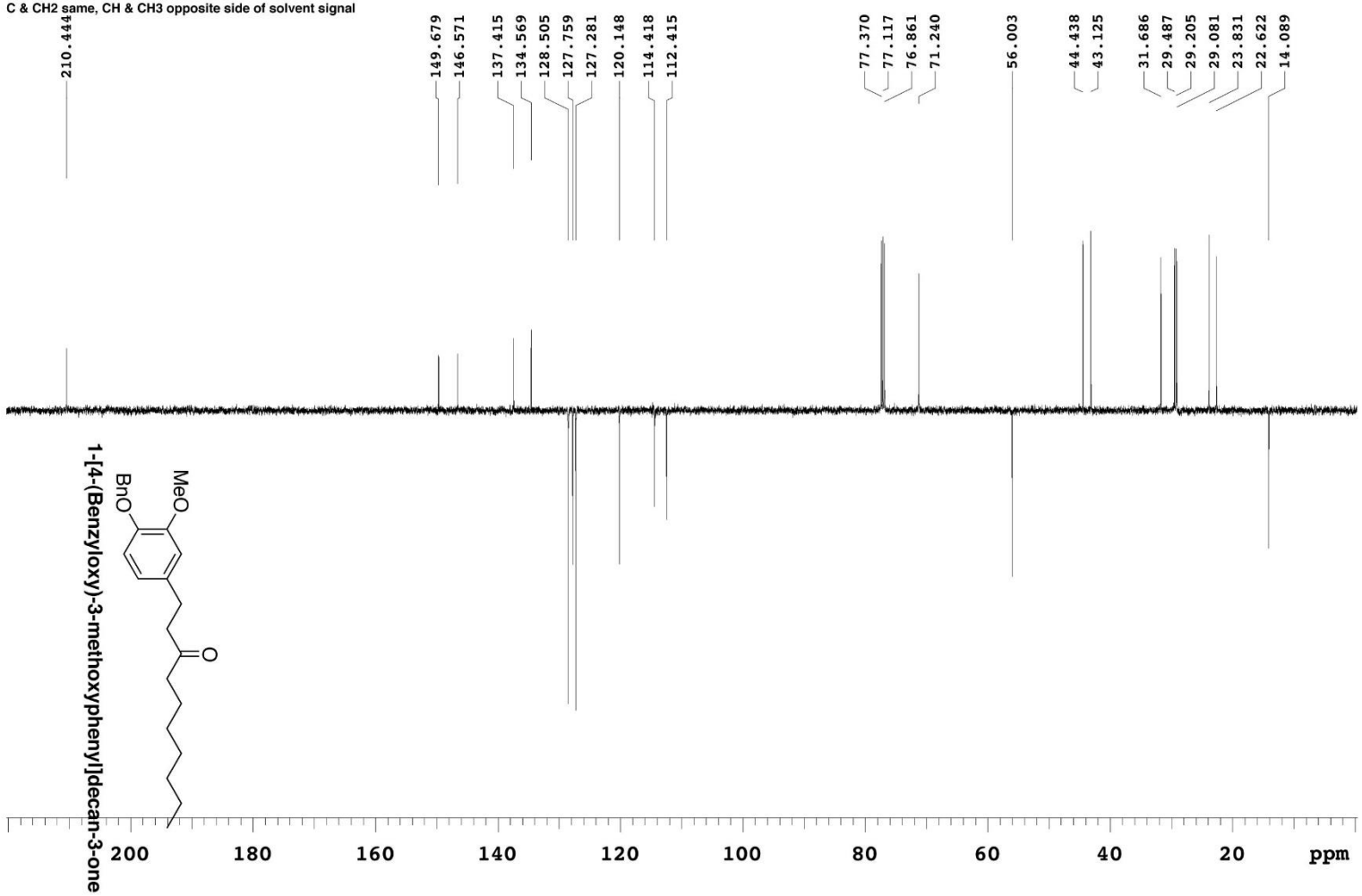
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.27.i5_HH-3-254_H1_1D

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Feb 28 2019**	Sweep Width(Hz): **33826.6**	Acquisiton Time(s): **0.998**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.26**	Hz per mm(Hz/mm): **115.15**	Completed Scans **64**

HH-3-256 (ibd5 NMR)
125.266 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal



1-[4-(Benzyloxy)-3-methoxyphenyl]decan-3-one

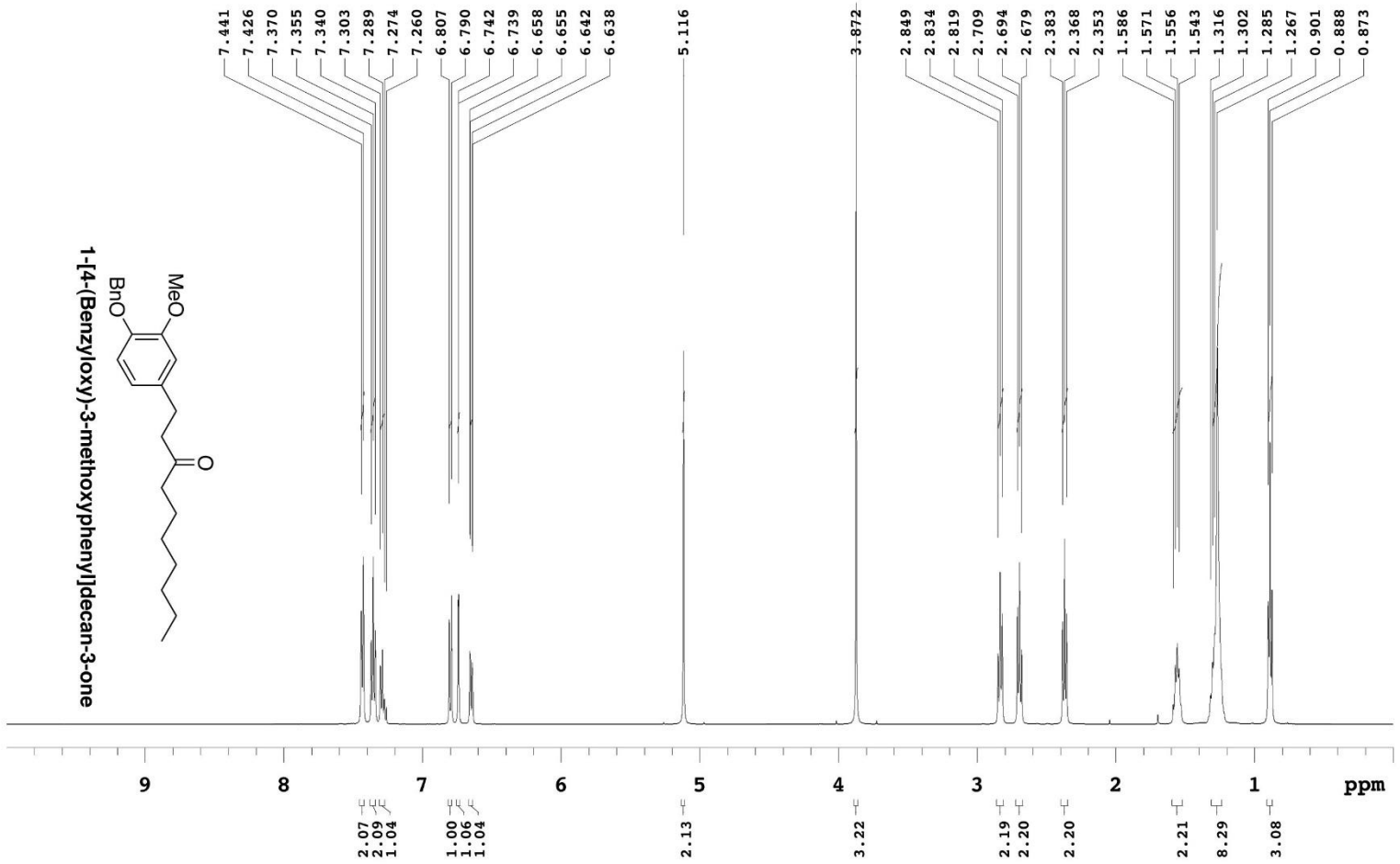
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.28.i5_HH-3-256_C13_APT_ad

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Feb 28 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-256 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



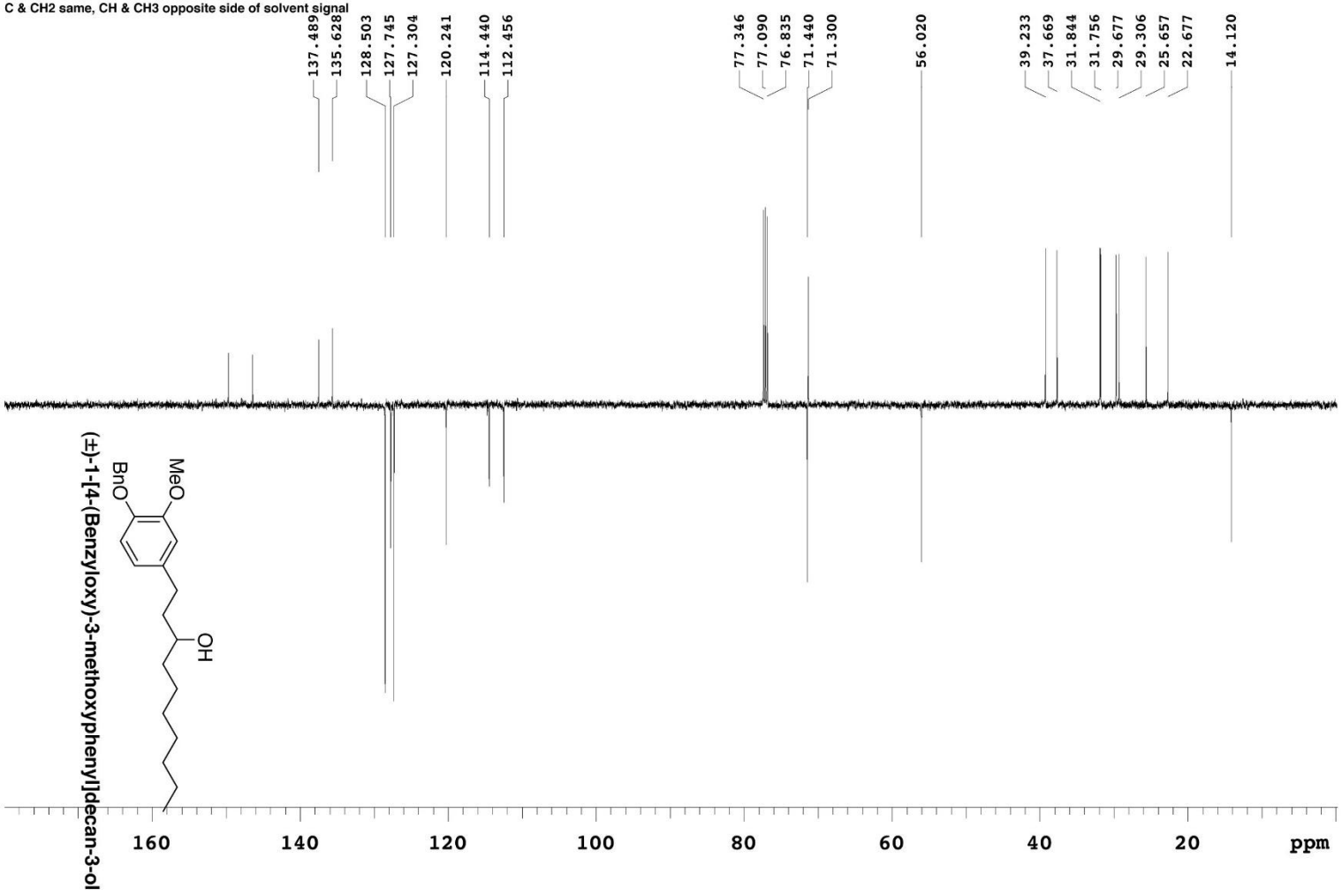
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.28.i5_HH-3-256_H1_1D

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Feb 28 2019**	Sweep Width(Hz): **33826.6**	Acquisiton Time(s): **0.998**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.26**	Hz per mm(Hz/mm): **94.02**	Completed Scans **96**

HH-3-257 (ibd5 NMR)
125.266 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal



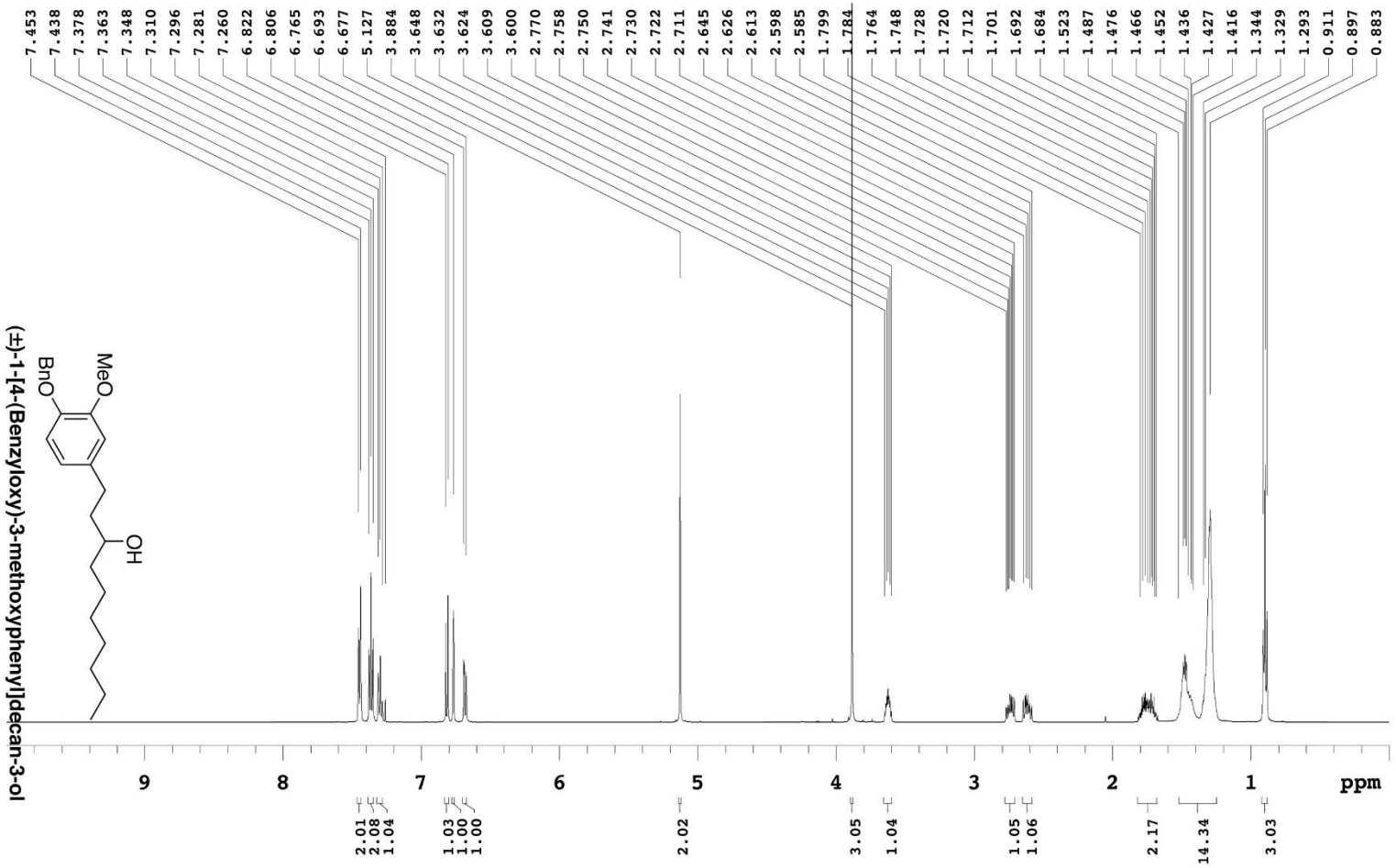
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.28.i5_HH-3-257_C13_APT_ad

Agilent Technologies

Department of Chemistry, University of Alberta

Recorded on: **ibd5, Feb 28 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.76**	Completed Scans **16**

HH-3-257 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



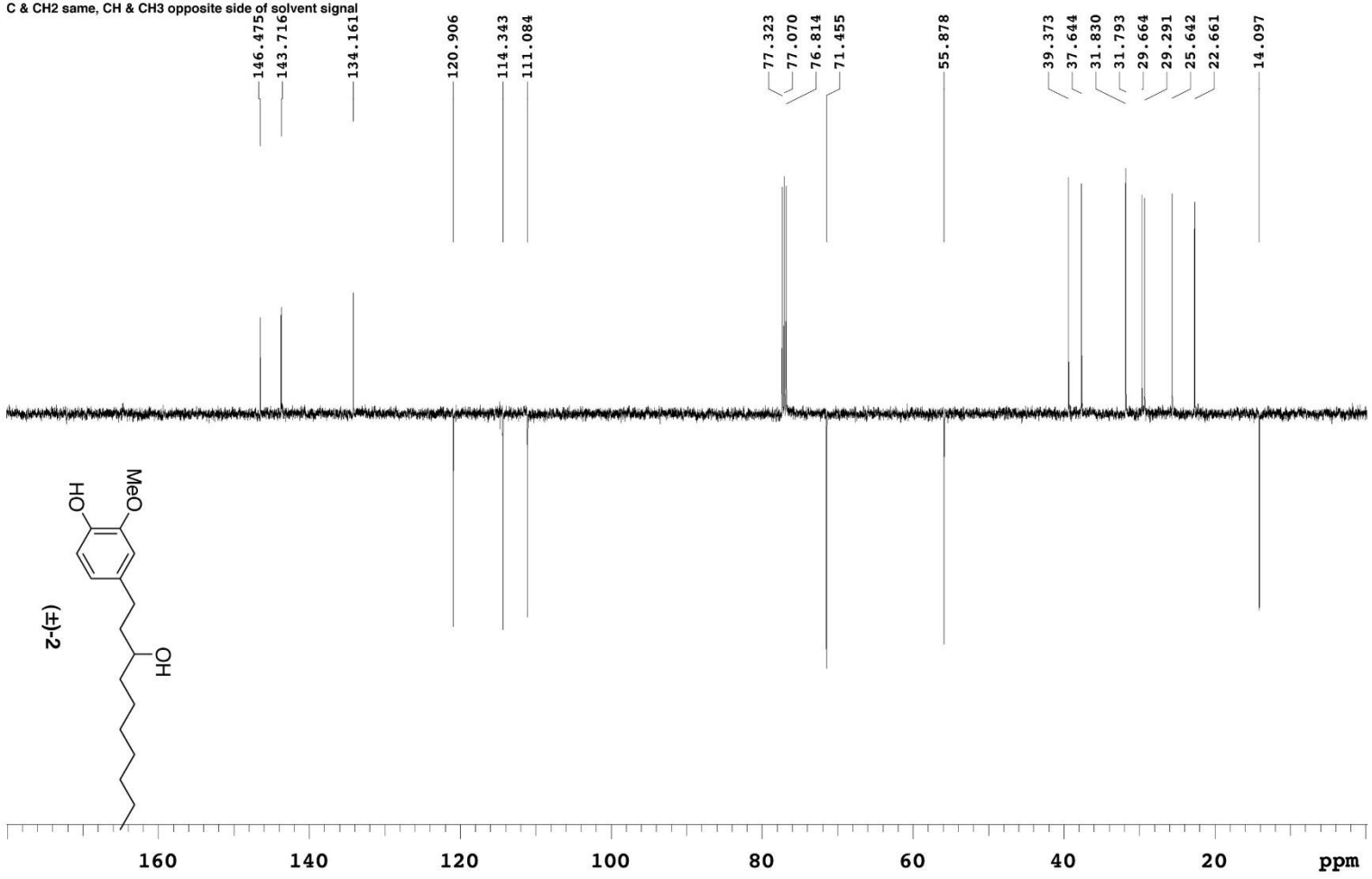
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.02.28.i5_HH-3-257_H1_1D

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Mar 1 2019**	Sweep Width(Hz): **33826.6**	Acquisiton Time(s): **0.998**	Relaxation Delay(s): **1**
Pulse Sequence: **APT_ad**	Digital Res.(Hz/pt): **0.26**	Hz per mm(Hz/mm): **94.11**	Completed Scans **80**

HH-3-258 (ibd5 NMR)
125.266 MHz C13 APT_ad in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe
C & CH2 same, CH & CH3 opposite side of solvent signal



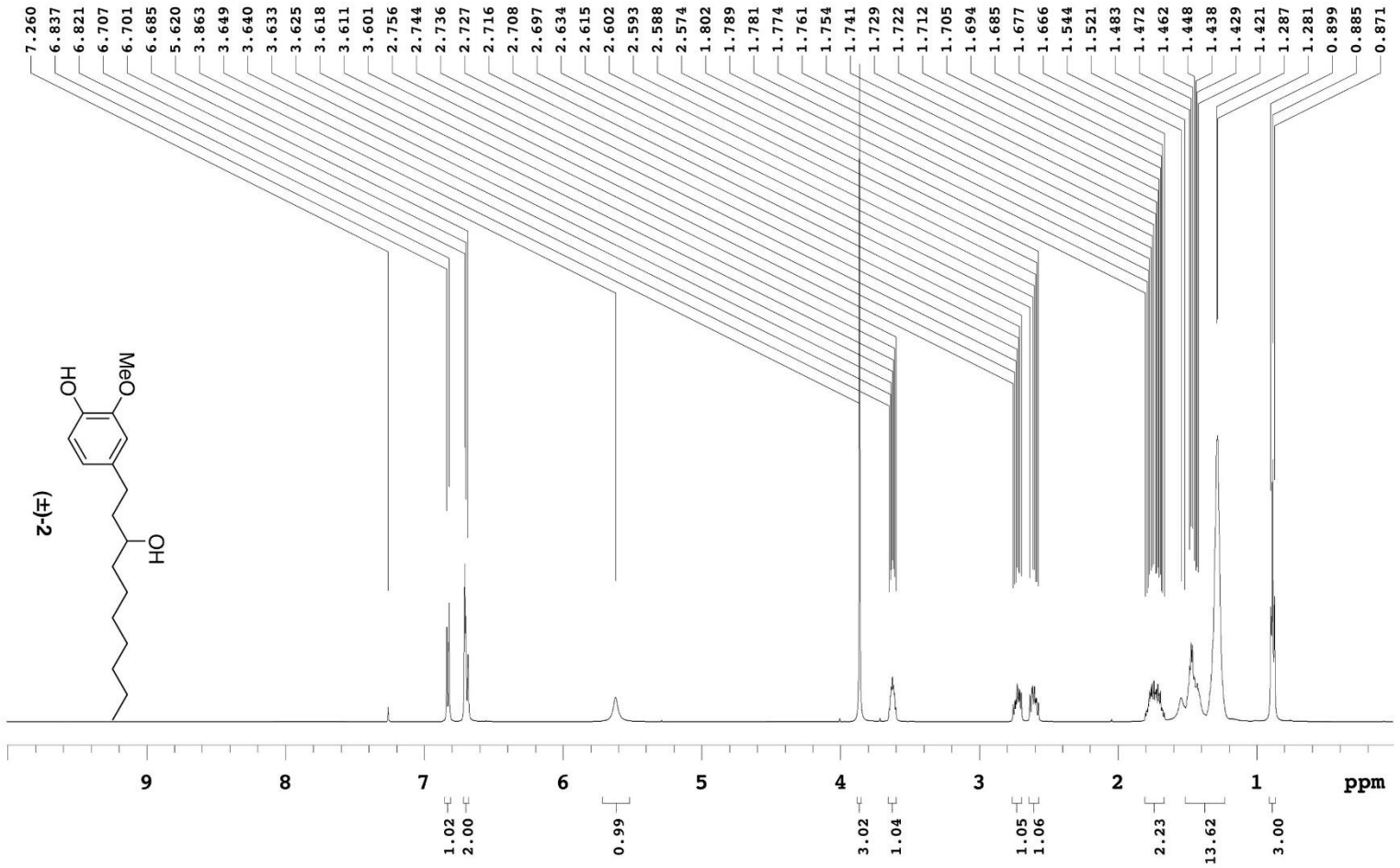
File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.03.01.i5_HH-3-258_C13_APT_ad

Department of Chemistry, University of Alberta

Agilent Technologies

Recorded on: **ibd5, Mar 1 2019**	Sweep Width(Hz): **6000.6**	Acquisiton Time(s): **5**	Relaxation Delay(s): **0.1**
Pulse Sequence: **s2pul**	Digital Res.(Hz/pt): **0.09**	Hz per mm(Hz/mm): **20.74**	Completed Scans **16**

HH-3-258 (ibd5 NMR)
498.118 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.9 C -> actual temp = 27.0 C, autoxdb probe



File: /mnt/d600/home14/clivenmr/nmrdata/hattori/2019.03.01.i5_HH-3-258_H1_1D

HPLC of natural compound 2

Data File C:\HPCHEM\1\DATA\ED\19030505.D Sample Name: CL NP

OD column, IPA:Hex=15:85 0.5 mL/min 20C

```
==================================================================
Injection Date  : 3/5/2019 3:15:36 PM          Seq. Line :   5
Sample Name     : CL NP                         Location : Vial 28
Acq. Operator   : Ed                               Inj :   1
Acq. Instrument : Instrument 1                 Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 0.1 µl
Acq. Method     : C:\HPCHEM\1\METHODS\EDH85.M
Last changed    : 3/5/2019 11:26:00 AM by Ed
Analysis Method : C:\HPCHEM\1\METHODS\MEH98.M
Last changed    : 3/5/2019 2:19:28 PM by Ed
                  (modified after loading)
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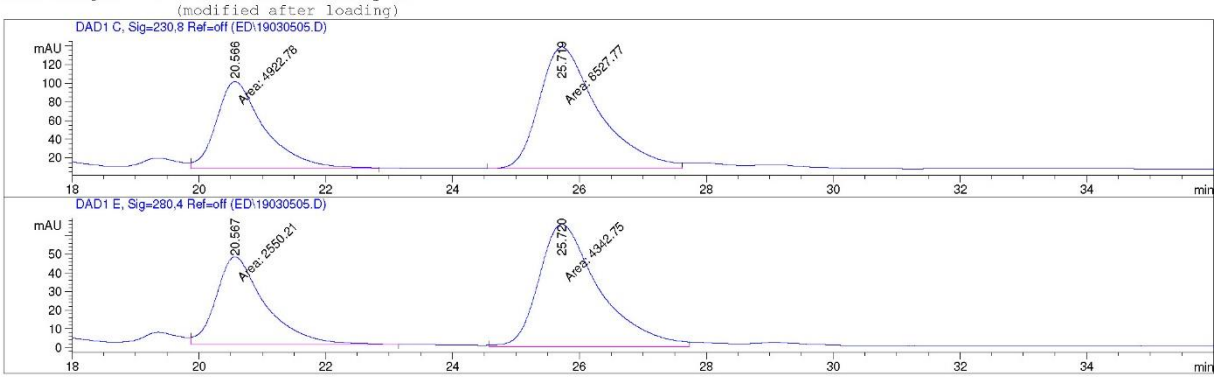



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=230,8 Ref=off
```

Instrument 1 3/6/2019 8:35:40 AM Ed Page 1 of 2

Data File C:\HPCHEM\1\DATA\ED\19030505.D Sample Name: CL NP

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.566	MM	0.8785	4922.77539	93.39045	36.5991
2	25.719	MF	1.0883	8527.76562	130.59981	63.4009
Totals :				1.34505e4	223.99026	

Signal 2: DAD1 E, Sig=280,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.567	MM	0.8990	2550.20557	47.28008	36.9973
2	25.720	MM	1.1036	4342.74561	65.58202	63.0027
Totals :				6892.95117	112.86211	

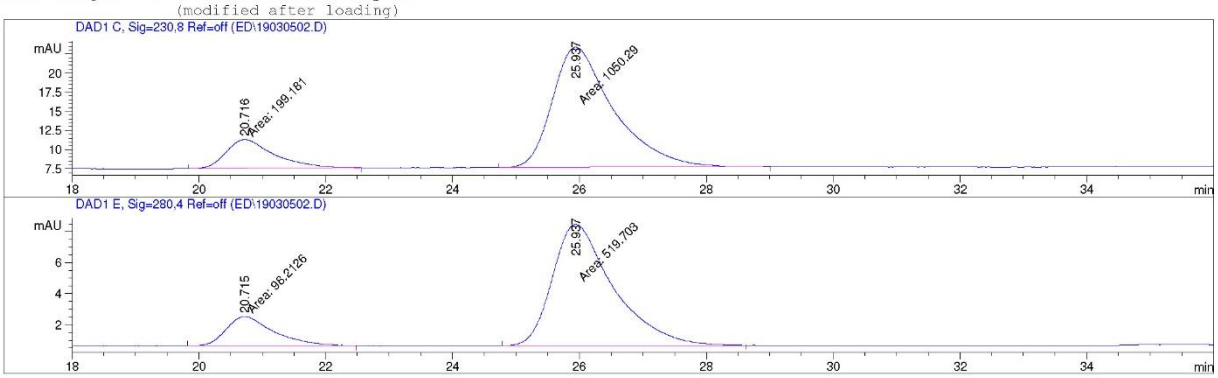
*** End of Report ***

HPLC of synthetic compound 2

Data File C:\HPCHEM\1\DATA\ED\19030502.D Sample Name: HH-3-252

OD column, IPA:Hex=15:85 0.5 mL/min 20C

```
======================================================================
Injection Date  : 3/5/2019 12:27:09 PM          Seq. Line :   2
Sample Name     : HH-3-252                       Location : Vial 29
Acq. Operator   : Ed                                Inj :   1
Acq. Instrument : Instrument 1                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 0.3 µl
Acq. Method     : C:\HPCHEM\1\METHODS\EDH85.M
Last changed    : 3/5/2019 11:26:00 AM by Ed
Analysis Method : C:\HPCHEM\1\METHODS\MEH98.M
Last changed    : 3/5/2019 2:19:28 PM by Ed
                  (modified after loading)
```




```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=230,8 Ref=off
```

Instrument 1 3/5/2019 2:21:40 PM Ed Page 1 of 2

Data File C:\HPCHEM\1\DATA\ED\19030502.D Sample Name: HH-3-252

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.716	MM	0.8784	199.18086	3.77929	15.9413
2	25.937	MM	1.1152	1050.28552	15.69707	84.0587
Totals :				1249.46638	19.47636	

Signal 2: DAD1 E, Sig=280,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.715	MM	0.8710	98.21260	1.87927	15.8942
2	25.937	MM	1.1092	519.70337	7.80871	84.1058
Totals :				617.91597	9.68798	

*** End of Report ***

HPLC of racemic compound 2

Data File C:\HPCHEM\1\DATA\ED\19030503.D Sample Name: HH-3-258

OD column, IPA:Hex=15:85 0.5 mL/min 20C

```
==================================================================
Injection Date  : 3/5/2019 1:23:17 PM          Seq. Line :   3
Sample Name     : HH-3-258                      Location : Vial 30
Acq. Operator   : Ed                               Inj :   1
Acq. Instrument : Instrument 1                 Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 0.3 µl
Acq. Method     : C:\HPCHEM\1\METHODS\EDH85.M
Last changed    : 3/5/2019 11:26:00 AM by Ed
Analysis Method : C:\HPCHEM\1\METHODS\MEH98.M
Last changed    : 3/5/2019 2:19:28 PM by Ed
                  (modified after loading)
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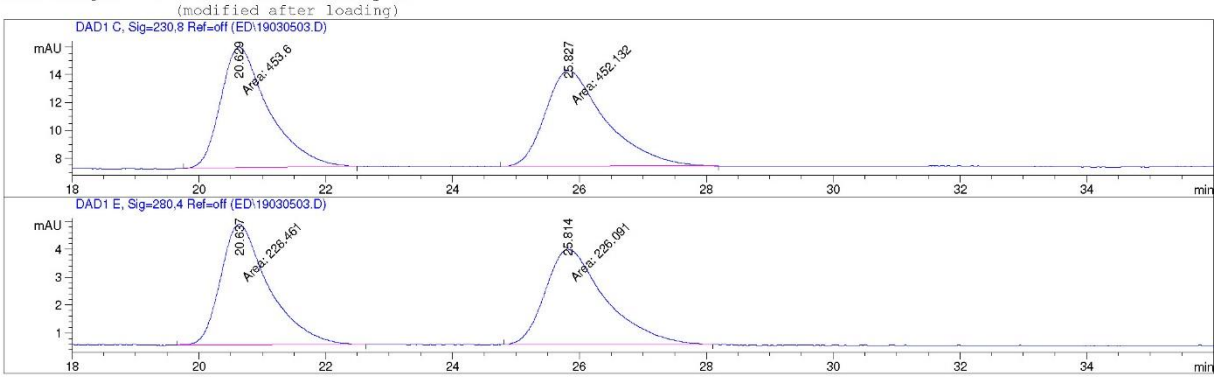



```
==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=230,8 Ref=off
```

Instrument 1 3/5/2019 2:20:15 PM Ed Page 1 of 2

Data File C:\HPCHEM\1\DATA\ED\19030503.D Sample Name: HH-3-258

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.629	MM	0.8748	453.60031	8.64238	50.0810
2	25.827	MM	1.0985	452.13226	6.86014	49.9190
Totals :				905.73257	15.50252	

Signal 2: DAD1 E, Sig=280,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.637	MM	0.8789	228.46127	4.33252	50.2608
2	25.814	MM	1.0990	226.09076	3.42874	49.7392
Totals :				454.55203	7.76126	

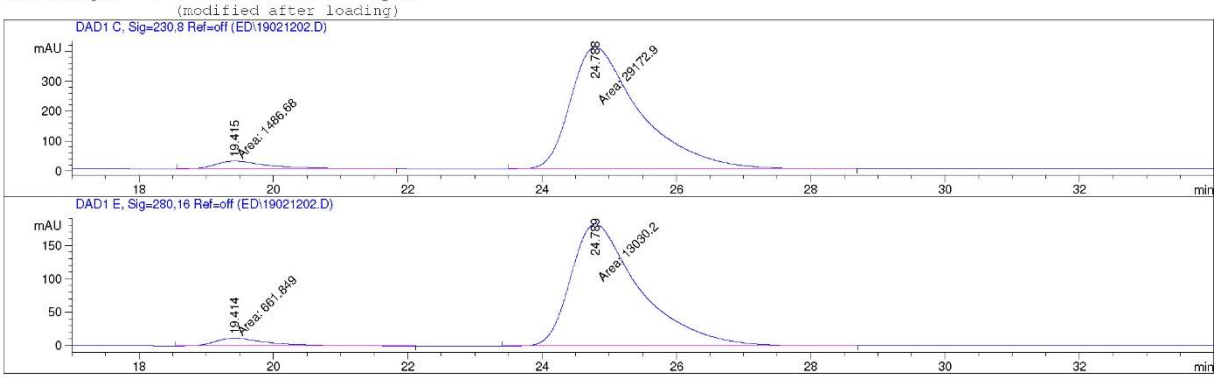
*** End of Report ***

HPLC of Synthetic compound 3

Data File C:\HPCHEM\1\DATA\ED\19021202.D Sample Name: CL S-isomer

OD column, IPA:Hex=15:85 0.5 mL/min 20C
Chiral from S-isomer

```
==================================================================
Injection Date  : 2/12/2019 9:35:39 AM          Seq. Line :   2
Sample Name     : CL S-isomer                    Location : Vial 28
Acq. Operator   : Ed                                Inj :   1
Acq. Instrument : Instrument 1                  Inj Volume : 1 µl
Acq. Method     : C:\HPCHEM\1\METHODS\EDH85.M
Last changed    : 12/4/2018 10:37:52 AM by Carl
Analysis Method : C:\HPCHEM\1\METHODS\MEH98.M
Last changed    : 2/12/2019 1:03:17 PM by ed
                  (modified after loading)
```

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==================================================================
                        Area Percent Report
==================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=230,8 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  19.415 MM    0.9454 1486.67908   26.20981   4.8490
   2  24.788 MM    1.1950 2.91729e4   406.86475  95.1510
```

Instrument 1 2/12/2019 1:04:37 PM ed Page 1 of 2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				3.06596e4	433.07456	

Signal 2: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.414	MM	0.9426	661.84857	11.70214	4.8338
2	24.789	MM	1.1909	1.30302e4	182.36313	95.1662
Totals :				1.36921e4	194.06526	

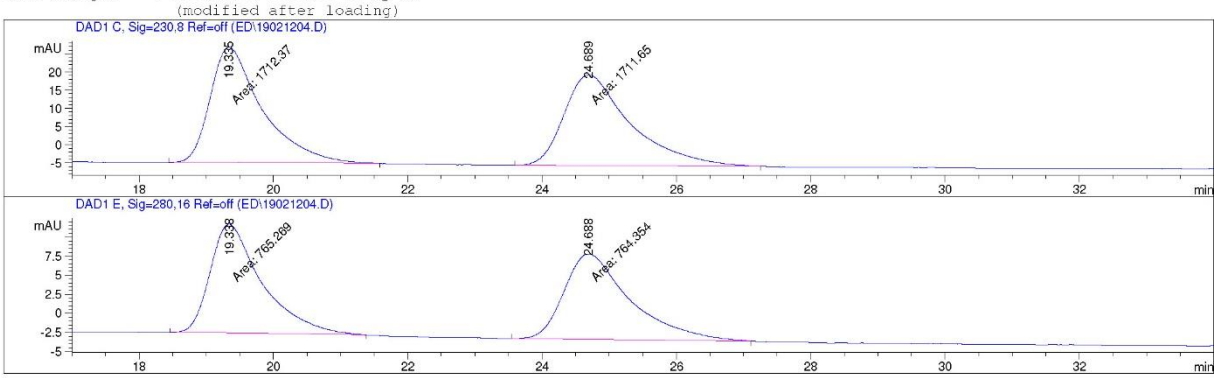
*** End of Report ***

HPLC of racemic compound 3

Data File C:\HPCHEM\1\DATA\ED\19021204.D Sample Name: CL Racemate

OD column, IPA:Hex=15:85 0.5 mL/min 20C
Racemate

```
======================================================================
Injection Date  : 2/12/2019 12:27:31 PM          Seq. Line :   4
Sample Name     : CL Racemate                     Location : Vial 30
Acq. Operator   : Ed                                 Inj :   1
Acq. Instrument : Instrument 1                  Inj Volume : 1 µl
Acq. Method     : C:\HPCHEM\1\METHODS\EDH85.M
Last changed    : 12/4/2018 10:37:52 AM by Carl
Analysis Method : C:\HPCHEM\1\METHODS\MEH98.M
Last changed    : 2/12/2019 1:03:17 PM by ed
                  (modified after loading)
```

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======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 C, Sig=230,8 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]      [min]    [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  19.335 MM    0.8978 1712.36523   31.78810  50.0105
   2  24.689 MM    1.1423 1711.64526   24.97433  49.9895
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				3424.01050	56.76243	

Signal 2: DAD1 E, Sig=280,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.338	MM	0.8942	765.26886	14.26325	50.0299
2	24.688	MM	1.1380	764.35364	11.19423	49.9701
Totals :				1529.62250	25.45748	

*** End of Report ***