Supporting Information

Sudemycin K: a synthetic anti-tumor splicing inhibitor variant with improved activity and versatile chemistry

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TABLE OF CONTENTS

Activity assays

Cell culture

HeLa cells were cultured in Glutamax Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum and penicillin/streptomycin antibiotics (500 u ml^{-1} penicillin; 0.5 mg ml^{-1} streptomycin). Cells were kept at 37 °C under 5% CO_2.

Cytotoxicity assays

1000 HeLa cells/well seeded the day before drug treatments. DMSO or drugs diluted in DMSO were added to the medium (1 µl in 100 µl total medium, from 4 or 6 10-fold serial dilutions of 10 mM stock solutions). 68 h after treatment, cell medium was replaced with medium containing 10 µM Alamar Blue/Resazurin[1] (Sigma, R7017), diluted from a 5 mg ml^{-1} stock in PBS. Cells were incubated at 37 °C for 4 h and fluorescence was measured with an Infinite 200 PRO series multiplate reader (TECAN) using 530 nm and 590 nm as fluorescence excitation and emission wavelengths, respectively. IC50 values were calculated by interpolating 50% viability values with GraphPad Prism 6 Software. Average and standard deviation values were calculated from experimental triplicates.

Drug treatment and alternative splicing analyses

HeLa cells seeded in 96 wells plate (10000 cells/well) were treated with serial dilutions of drugs dissolved in DMSO (1 µl of treatment in 100 µl total medium) and incubated for the indicated times. mRNAs were isolated using oligo dT-coated 96 well plates (mRNA catcher PLUS, Life Technologies) and one third of the recovered RNA was reverse-transcribed using Superscript III reverse transcriptase (Invitrogen, Life Technologies). PCR reactions were carried out in 25 µl reactions with 5 µl of cDNA and 38 cycles of amplification. Primers used to detect *MCL1* alternative splicing correspond to sequences AGACCTTACGACGGGTTGG and ACCAGCTCCTACTCCAGCAA in exons 1 and 3, generating amplicons of 401 and 153 bp, for exon 2 inclusion and skipping isoforms, respectively. High throughput capillary electrophoresis measurements for the different splicing isoforms were performed in 96-well format in a Labchip GX Caliper workstation (Caliper, Perkin Elmer) using a HT DNA 5K LabChip chip (Perkin Elmer). Percentages of exon 2 inclusion were normalized to DMSO treatment values and IC50 were calculated by interpolating titration curves with GraphPad Prism 6 Software.

In vitro spliceosome A3' complex formation inhibition assays

This assay was carried out as previously described[2]. Specifically, templates for *in vitro* transcription were PCR-amplified from a plasmid containing AdML sequences in order to generate transcripts containing the last 40 nt of intron 1 and the complete exon 2 (PCR primers: GCTAATACGACTCACTATAGGGtgatgatgtcatacttatc, cccactggaaagaccgcgaaga, with the forward primer including the T7 promoter sequence, indicated with capital letters).

Purified PCR products were *in vitro* transcribed in the presence of 32P-Uridine with T7 RNA Polymerase (Promega) and purified by a Sephadex G-50 column (GE Healthcare).

The obtained RNAs correspond to the full AdML 3'ss but they lack the 5' part of the

intron. The corresponding transcript sequence is the following: ugaugaugucauacuuauccugucccuuuuuuuuccacagCUCGCGGUUGAGGACAAACUCUUCGCG GUCUUUCCAGUGGG (with exonic sequences in capital letters).
Each splicing reaction contained 1 µl of RNA mix (1 fmol of RNA, Creatine Phosphate 200 µM, ATP 10 mM, $MgCl_2$ 27 mM), 3 µl of HeLa nuclear extracts (Cilbiotech), 1µl of drug or DMSO, 2 µl of Polyvynil alcohol 15% prewarmed at 30 °C and Buffer D complemented with fresh 0.1 M KCl and 1 mM DTT up to 9 µl. ATP and Creatine Phosphate were replaced with Buffer D 0.1 M KCl for the –ATP control. The reactions were set up in a 48 wells microplate and incubated at 30 °C for 15'. Subsequently, 5 mg ml^{-1} heparin and 2.2 µl of 6X DNA loading dye were added and the reactions were incubated for 10' at room temperature. The products were subsequently loaded on a 1.5% (w/v) low-melting agarose (Invitrogen) gel in 50 mM Tris base and 50 mM glycine buffer for 90' at 4 °C and run at 75 V. Gels were fixed in 10% (v/v) methanol and 10% (v/v) acetic acid for 10' at room temperature, dried for at least 3 hours at 50 °C and exposed overnight to a PhosphorImager screen. The intensity of the complex A3' band over the signal of the whole well were measured by ImageJ, substracted from the blank (i.e. the signal of an equivalent empty area of the gel) and normalized to the DMSO treatment condition. IC_{50}s were calculated by interpolating titration curves with GraphPad Prism 6 Software.

In vitro full spliceosome assembly assay

This assay was carried out as the previous one, but with RNAs containing both AdML exons 1 and 2 and the full intron. the corresponding transcript sequence is: (AAUACACGGAAUUCGAGCUCGCCCACUCUUGGAUCGGAAA-
CCCGUCGGCCUCCGAACGguaagagccuagcauguagaacugguuaccugcagcccaagcuugcugcacguc uagggcgcaguaguccaggguuuccuugaugaugucauacuuauccugucccuuuuuuuuccacagCUCGCGG UUGAGGACAAACUCUUCGCGGUCUUUCCAGUGGG). Templates for T7 *in vitro* transcription were PCR-amplified as specified previously (with the same reverse primer and a T7 forward primer GCTAATACGACTCACTATAGGGaatacacggaattcgagctcg).
Reactions were set up as before, but the final concentration of DMSO was kept lower than 2% to avoid DMSO-related inhibitory effects[3]. Splicing mixes were incubated at 30 °C for 30'. 5 mg ml^{-1} were added to the reaction and after a 10' incubation at room temperature, 3 µl of 50% (v/v) glycerol were added. 2% (w/v) low-melting agarose gels were run in 20 mM Tris base and 20 mM glycine buffer for 3 h 50' at 75 V, at 4 °C, as previously reported[3]. Gels were dried for 2 h at 50 °C and exposed overnight to a PhosphorImager screen.

Pulsed treatments and stability experiments in culture medium

For pulsed drug treatments, cytotoxicity and *MCL1* alternative splicing assays were carried out as previously described, but culture medium was changed 30' post treatment.
For drug stability experiments, complete culture medium containing 10% (v/v) fetal

bovine serum (Gibco, Life Technologies) containing 1 µM drug or the equivalent volume of DMSO was incubated at 37 °C for the indicated times and subsequently added to plated cells. *MCL1* alternative splicing assays were carried out as described above.

Comparative analysis of Sudemycins solubility

The solubilities of Sudemycin K, D1 and D6 were compared by performing serial dilutions of the drugs (initially dissolved in DMSO at a concentration of 10 mM) in a solution of PBS and isopropanol (1:1 v/v). The dilutions were incubated at room temperature for 24 h, centrifuged in a table-top centrifuge (Eppendorf) at maximum speed for 15 minutes and optical density of the supernatants was measured at the peak of spectral absorbance (236 nM). Tests were performed in triplicate. The results showed that Sudemycins K and D6 displayed equivalent solubilities (estimated at > 10 µM by previous work[4]). Consistent with previous results[4], the solubility of Sudemycin D1 was found to be at least 2-fold lower.

Chemical Synthesis of Sudemycin derivatives.

General procedures

Tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were dried using a PureSolv solvent purification system. All other solvents and reagents were used as purchased, without further purification, unless otherwise indicated. Flash column chromatography was performed on silica gel (60A 35-70 µm) as stationary phase. Analytical and/or preparative HPLC were performed on a Waters instrument. The purification of chiral compounds was carried out using a Phenomenex Lux 5u Cellulose-2 column. Analytical TLC was performed on pre-coated silica gel 60 F254 plates (0.2 mm thick, 20x20 cm) and visualized under UV light (254 and 360 nm), with anisaldehyde in conc. H_2SO_4, with phosphomolybdic acid in ethanol or in ninhydrin in ethanol. Polarimetry studies were performed on a Perkin-Elmer 241 or JascoP-2000 polarimeter equipped with a Na-lamp. IR spectra were recorded on a Thermo Nicolet FT-IR Nexus spectrometer. NMR spectra were recorded on a Varian Mercury 400MHz. Chemical shifts are reported in ppm referenced to the appropriate residual solvent peaks ($CDCl_3$ or CD_3OD) and coupling constants are reported in Hz. Multiplicity of the carbons was assigned with gHSQC experiments. Standard abbreviations for off-resonance decoupling were employed: s = singlet, d = doublet, t = triplet, q = quadruplet. The same abbreviations were also used for the multiplicity of signals in ^{1}H-NMR and also, bs = broad singlet, bd =broad doublet, m = multiplet. High Resolution Mass Spectroscopy (HRMS) was performed an Agilent LC/MSD-TOF 2006 system using the ESI-MS technique.

General procedure 1. Staudinger reduction of azide to amine and coupling with acid. Synthesis of compounds S3, S5, 17, Sudemycin K and 3:

The dry azide (1 eq) was dissolved in dry benzene and treated with Ph_3P (2 eq) at rt. The reaction solution was degassed with nitrogen and heated at 55 ˚C for 2 h, the transformation of starting material was controlled by TLC. Water (10 eq) was added and the reaction mixture heated again at 55 ˚C for another 2 h. The reaction mixture was cooled to rt and diluted with a mixture of CH_2Cl_2 and diethyl ether (8:2). This mixture was shaken vigorously, dried over $MgSO_4$, filtered, and concentrated. The resulting amine intermediate was used in the next step without further purification.
A stirred solution of crude amine (1 eq) in dry MeCN (2 ml) was treated with *i*-Pr_2EtN (5 eq) and a solution of acid (1.7 eq) in MeCN (0.5 ml). This mixture was cooled in an ice-bath and HBTU (1.45 eq) was added portion wise to the reaction solution. The reaction suspension warmed to rt and stirred 2 h and diluted with EtOAc. After successive washing with saturated aqueous $NaHCO_3$, saturated aqueous NH_4Cl, saturated $NaHCO_3$ solutions and brine, the organic layer was dried over $MgSO_4$ and evaporated. The residue was purified on silica gel column chromatography with hexane-EtOAc to give corresponding amide.

General procedure 2. Julia-Kocienski Olefination. Synthesis of compounds 12, 14 and 21:

A mixture of sulfone **11**[4] (1.3 eq) and aldehyde (1 eq) was dried under reduced pressure overnight then dissolved in dry THF, and cooled to −78 °C. This cold solution was stirred and a solution of NaHMDS (1.25 eq, 1M in THF) was added drop-wise. The resulting yellow suspension was stirred at −78 °C for 1 h, allowed to warm in an ice bath for 40 min, and then allowed to warm to rt and stirred for 1 h. The reaction mixture was quenched with pH 7 buffer solution and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting residue was purified on a silica gel column chromatography with hexane-EtOAc to give corresponding olefin.

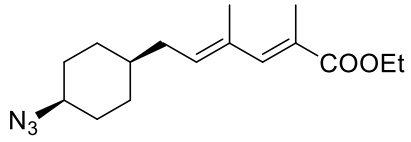
General procedure 3. Removal of TBS protecting group. Synthesis of compounds S4, 15a, 15b, S6 and 22:

A solution of TBS protected alcohol (1 eq) in THF was cooled in an ice-bath and TBAF (1.3 eq, 1.0 M in THF) was slowly added using a syringe during a period of 5 minutes. The light yellow solution was allowed to stir for 1 h at 0 ˚C and after for an additional 2 h at rt. The reaction time was monitored by TLC until the starting material was consumed. The solution was diluted with EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, washed with brine (50 ml) then dried over $MgSO_4$ and concentrated. The crude residue was purified on silica gel column chromatography with hexane-EtOAc to give corresponding alcohol.

General procedure 4. Ester formation with isobutyric anhydride. Synthesis of compounds 1-4, 5a and 5b:

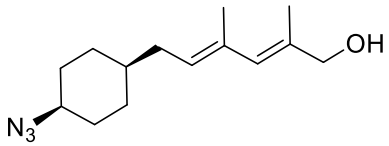
A stirred solution of alcohol (1 eq) and DMAP (0.5 eq) in CH_2Cl_2 (2 ml) was cooled at 0 ˚C and Et_3N (5 eq) was added. After 5-10 min isobutyric anhydride (3.3 eq) was added and the stirring was maintained for 90 min at 0 ˚C. The reaction time was monitored by TLC until the starting material was consumed. Water was added to the reaction mixture and diluted with CH_2Cl_2. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. Purification of the residue on silica gel column chromatography with hexane-EtOAc gave corresponding ester.

Ethyl (2*E*,4*E*)-6-((1S,4S)-4-azidocyclohexyl)-2,4-dimethylhexa-2,4-dienoate (S1)



A solution of *t*-BuOK (1 ml, 1mmol) 1M in THF was slowly added to a cooled (-78 ˚C) suspension of (1-ethoxy-1-oxopropan-2-yl)triphenylphosphonium bromide[5] (0.72 g, 1.06 mmol) in CH_2Cl_2 (3 ml). After, the reaction mixture was warmed to 0 ˚C and stirred 30 min. After this time, a solution of **9**[4] (0.11 g, 0.53 mmol) in CH_2Cl_2 (2 ml) was added via cannula and the solution was stirred over 1 h at 0 ˚C and 5 h at rt. Then, the reaction was quenched with saturated aqueous NH_4Cl, diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over $MgSO_4$ and the solvent was evaporated. The residue was purified on silica gel column chromatography with hexane-EtOAc (95:5) to give the title compound (75 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.1 Hz, 3H), 1.62 – 1.48 (m, 9H), 1.83 (s, 3H), 2.00 (s, 3H), 2.11 – 2.06 (m, 2H), 3.82 – 3.78 (m, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 5.60 (t, *J* = 7.5 Hz, 1H), 7.11 (s, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 14.2 (q), 14.4 (q), 16.6 (q), 27.5 (t), 29.4 (t), 35.2 (t), 37.0 (d), 57.9 (d), 60.7 (q), 125.3 (s), 133.2 (s), 134.8 (d), 143.0 (d), 169.3 (s). IR (NaCl film) 2099, 1708, 1383, 1250, 1119 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{16}H_{26}N_3O_2$: 292.20195 [M+H]; found: 292.20235

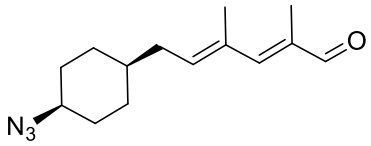
(2*E*,4*E*)-6-((1*S*,4*S*)-4-Azidocyclohexyl)-2,4-dimethylhexa-2,4-dien-1-ol (S2)



A solution of DIBALH (0.6 ml, 0.6 mmol) 1 M in heptane was slowly added to a solution of **S1** (70 mg, 0.24 mmol) in CH_2Cl_2 (2 ml) cooled at -78 ˚C. The mixture was stirred over 2.5h. The reaction was quenched with MeOH (0.3 ml) and saturated aqueous potassium sodium tartrate (2.5 ml) at -78 ˚C. After the mixture was warmed to rt and extracted with AcOEt (3 x 8 ml). The organic layers were washed with brine and dried with $MgSO_4$ and evaporated. The residue was purified on silica gel column chromatography with hexane-EtOAc (9:1) to give **S2** (55 mg, 93%). ^{1}H

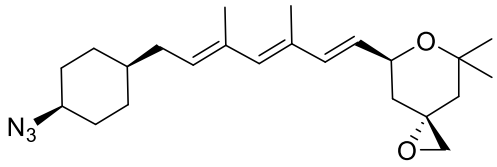
NMR (400 MHz, $CDCl_3$) δ 1.36 – 1.23 (m, 4H), 1.58 – 1.50 (m, 5H), 1.73 (s, 3H), 1.80 (s, 3H), 2.09 – 1.95 (m, 2H), 3.80 – 3.76 (m, 1H), 4.02 (s, 2H), 5.31 (t, *J* = 7.5 Hz, 1H), 5.88 (s, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 15.5 (q), 17.1 (q), 27.5 (t), 29.4 (t), 34.9 (t), 37.1 (d), 58.0 (d), 69.6 (t), 128.9 (d), 129.7 (d), 133.2 (s), 134.2 (s). IR (NaCl film) 3564-3083, 2099, 1383, 1254 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{14}H_{25}N_3O$: 250.1919 [M+H]; found: 250.1914

(2*E*,4*E*)-6-((1*S*,4*S*)-4-Azidocyclohexyl)-2,4-dimethylhexa-2,4-dienal (10)



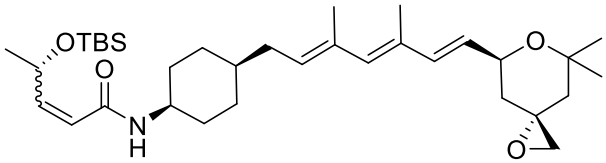
Dess−Martin periodinate (112 mg, 0.26 mmol) was added portionwise over a mixture of **S2** (55 mg, 0.22 mmol) and solid $NaHCO_3$ (22 mg, 0.26 mmol) in dry CH_2Cl_2 (2 ml) cooled at 0 °C. The resulting solution was stirred for 1 h at rt. After this time the reaction mixture was diluted with CH_2Cl_2, saturated aqueous $NaHCO_3$ (2 ml) and saturated aqueous sodium thiosulfate (2 ml). The mixture was allowed to stir for 1 h at rt. The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 5 ml). The combined organic fractions were dried over $MgSO_4$ and concentrated. The resulting residue was purified on a silica gel column chromatography with hexane-AcOEt (95:5 to 90:10) to give **10** (38 mg, 70%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 – 1.14 (m, 1H), 1.34 – 1.25 (m, 2H), 1.53 – 1.46 (m, 4H), 1.79 – 1.73 (m, 2H), 1.89 (s, 3H), 1.90 (s, 3H), 2.12 – 2.05 (m, 2H), 3.84 – 3.69 (m, 1H), 5.82 (t, *J* = 7.5 Hz, 1H), 6.67 (s, 1H), δ 9.32 (s, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 10.8 (q), 16.2 (q), 27.5 (t), 29.4 (t), 35.5 (t), 36.9 (d), 57.8 (d), 134.1 (s), 135.5 (s), 139.5 (d), 155.1 (d), 196.3 (d). IR (NaCl film) 2099, 1678, 1619, 1384, 1261, 1023 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{14}H_{24}N_3O$: 248.17574 [M+H]; found: 248.17708

(3*R*,7*S*)-7-((1*E*,3*E*,5*E*)-7-((1*S*,4*R*)-4-Azidocyclohexyl)-3,5-dimethylhepta-1,3,5-trien-1-yl)-5,5-dimethyl-1,6-dioxaspiro[2.5]octane (12)



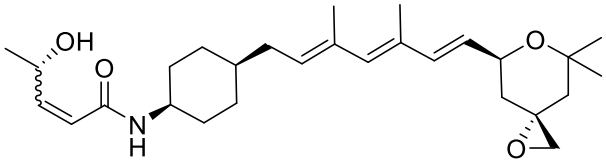
Following the general procedure 2, sulfone **11**[4] (65 mg, 0.18 mmol) and aldehyde **10** (35 mg, 0.14 mmol) led to compound **12** (E/Z ratio 96:4). Compound **12** was obtained by purification with silica gel column chromatography with hexane-EtOAc (85:15) to give triene as an oil (30 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 – 1.12 (m, 2H), 1.28 (s, 3H), 1.38 – 1.31 (m, 2H), 1.40 (s, 3H), 1.63 – 1.50 (m, 5H), 1.74 (s, 3H), 1.83 – 1.77 (m, 2H), 1.88 (s, 3H), 1.99 – 1.89 (m, 2H), 2.04 (dd, *J* = 7.5, 6.5 Hz, 2H), 2.57 (s, 2H), 3.82 – 3.76 (m, 1H), 4.52-4.45 (m, 1H), 5.33 (t, *J* = 7.5, 1H), 5.62 (dd, *J* = 15.6, 6.7 Hz, 1H), 5.90 (s, 1H), 6.28 (d, *J* = 15.6, Hz, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 14.1 (q), 17.2 (q), 23.9 (q), 27.5 (t), 29.4 (t), 31.7 (q), 35.1 (t), 37.1 (d), 38.8 (t), 42.6 (t), 51.2 (t), 55.8 (s), 58.1 (d), 69.7 (d), 73.1 (s), 128.3 (d), 130.1 (d), 132.2 (s), 133.6 (s), 136.5 (d), 137.1 (d).). IR (NaCl film) 2099, 1383, 1327, 1259, 1063 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{23}H_{36}N_3O_2$: 386.28075 [M+H]; found: 386.28075

4-((*tert*-Butyldimethylsilyl)oxy)-*N*-((1*R*,4*S*)-4-((2*E*,4*E*,6*E*)-7-((3*R*,5*S*)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3,5-dimethylhepta-2,4,6-trien-1-yl)cyclohexyl)pent-2-enamide (S3)



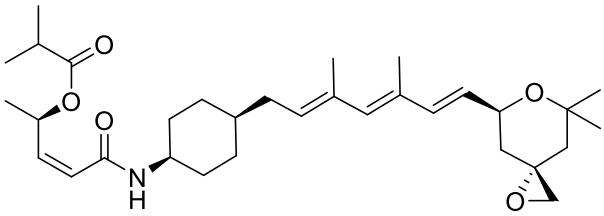
Following the general procedure 1, azide **12** (33 mg, 0.085 mmol) and (*S*,*Z*)-4-(TBS-oxy)pent-2-enoic acid[6] led to amide **S3**. Purification of the compound on silica gel column chromatography with hexane-EtOAc (90:10 to 85:15) gave **S3 (**Z/E: 7:3, 33 mg, 68%, 2 steps) as a colorless oil. This mixture was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.87 (s, 9H), 1.20 – 1.13 (m, 4H), 1.27 – 1.25 (m, 3H), 1.29 – 1.27 (m, 3H), 1.41 (s, 3H), 1.69 – 1.55 (m, 8H), 1.74 (s, 3H), 1.87 (s, 3H), 2.02 – 1.92 (m, 2H), 2.09 – 2.04 (m, 2H), 2.57 (s, 2H), 4.07 – 4.01 (m, 1H), 4.51 – 4.45 (m, 1H), 5.34 (t, *J* = 7.5 Hz, 1H), 5.57 – 5.49 (m, 2H), 5.66 – 5.60 (m, 1H), 5.90 (s, 1H), 5.99 (dd, *J* = 11.5, 7.9 Hz, 1H), 6.28 (d, *J* = 15.7 Hz, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 14.1 (q), 17.3 (q), 23.9 (q), 26.0 (q), 28.1 (t), 29.5 (t), 29.8 (t), 31.7 (q), 34.3 (t), 36.4 (d), 38.8 (t), 42.6 (t), 45.5 (d), 51.2 (t), 55.8 (s), 65.6 (d), 69.8 (d), 73.2 (s), 120.0 (d), 128.4 (d), 130.1 (d), 132.3 (s), 133.6 (s), 136.4 (d), 137.1 (d), 150.2 (d), 165.0 (s). IR (NaCl film) 1623, 1533, 1384, 2352, 2075 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{34}H_{58}NO_4Si$: 572.41296 [M+H]; found: 572.41382

***N*-((1*R*,4*S*)-4-((2*E*,4*E*,6*E*)-7-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3,5-dimethylhepta-2,4,6-trien-1-yl)cyclohexyl)-4-hydroxypent-2-enamide (S4)**



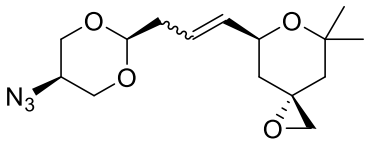
Following the general procedure 3, TBS protected alcohol **S3** (30 mg, 0.052 mmol) led to alcohol **S4.** Purification on silica gel with hexane-EtOAc (7:3) gave **S4** (Z/E 7:3, 21 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 – 1.17 (m, 4H), 1.38 (s, 3H), 1.39 (s, 2H), 1.45 (s, 3H), 1.72 – 1.64 (m, 8H), 1.78 (s, 3H), 1.92 (s, 3H), 2.05 – 1.96 (m, 2H), 2.13 – 2.09 (m, 2H), 2.61 (s, 2H), 4.13 – 4.07 (m, 1H), 4.57 – 4.47 (m, 1H), 4.86 – 4.76 (m, 1H), 5.38 (t, *J* = 7.4 Hz, 1H), 5.67 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.78 (d, *J* = 12.0, 1H), 5.9 (bs, 1H), 5.94 (s, 1H), 6.19 (dd, *J* = 12.0, 5.4 Hz, 1H), 6.32 (d, *J* = 15.7 Hz, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 14.1 (q), 17.3 (q), 22.9 (q), 23.9 (q), 28.0 (t), 28.1 (t), 29.4 (t), 31.7 (q), 34.3 (t), 36.5 (d), 38.8 (t), 42.6 (t), 46.0 (d), 51.2 (t), 55.8 (s), 64.7 (d), 69.7 (d), 73.2 (d), 123.2 (d), 128.5 (d), 129.9 (d), 132.3 (s), 133.7 (s), 136.3 (d), 137.0 (d), 150.2 (d), 165.9 (s). IR (NaCl film) 3540-3200, 1657, 1623, 1536, 1382, 1062 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{28}H_{43}NO_4$: 458.32649 [M+H]; found: 458.32669.

(*S*,*Z*)-5-(((1*R*,4*R*)-4-((2*E*,4*E*,6*E*)-7-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3,5-dimethylhepta-2,4,6-trien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl isobutyrate (1)



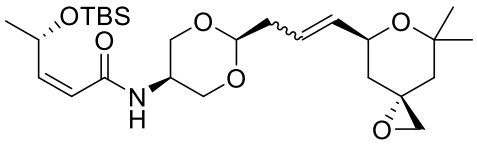
Following the general procedure 4, alcohol **S4** (18 mg, 0.039 mmol) led to ester **1**. The purification on silica gel column chromatography with hexane-EtOAc (8:2) gave a diasteromeric mixture. This mixture was purificated again by semi-preparative RP-HPLC to give **1** (5 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 – 1.13 (m, 2H), 1.15 (s, 3H), 1.17 (s, 3H), 1.29 (s, 3H), 1.36 (d, *J* = 6.3 Hz, 3H), 1.41 (s, 3H), 1.65 – 1.57 (m, 9H), 1.74 (s, 3H), 1.88 (s, 3H), 2.03 – 1.91 (m, 2H), 2.09 – 2.04 (m, 2H), 2.56 – 2.50 (m, 1H), 2.57 (s, 2H), 4.17 – 4.10 (m, 1H), 4.52 – 4.45 (m, 1H), 5.35 (t, *J* = 7.5 Hz, 1H), 5.67 – 5.59 (m, 2H), 5.82 – 5.75 (m, 2H), 5.90 (s, 1H), 6.28 (d, *J* = 15.7 Hz, 1H), 7.27 – 7.25(m, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 14.1 (q), 17.2 (q), 19.0 (q), 19.1 (q), 20.4 (q), 23.9 (q), 27.8 (t), 29.7 (t), 29.7 (t), 31.7 (q), 34.1 (d), 36.8 (t), 38.8 (t), 42.6 (t), 45.4 (d), 51.2 (t), 55.8 (s), 69.1 (d), 69.8 (d), 73.2 (s), 125.8 (d), 128.4 (d), 130.4 (d), 132.2 (s), 133.4 (s), 136.5 (d), 137.1 (d), 137.7 (d), 165.0 (s), 177.5 (s). IR (NaCl film) 1735, 1689, 1628, 1533, 1450, 1368, 1241, 1050 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{32}H_{50}NO_5$: 528.36835 [M+H]; found: 528.36835.

(3*R*,7*S*)-7-(3-((2*S*,5*R*)-5-Azido-1,3-dioxan-2-yl)prop-1-en-1-yl)-5,5-dimethyl-1,6-dioxaspiro[2.5]octane (14)



Following the general procedure 2, sulfone **11** (309 mg, 0.877 mmol) and **13**[7] (165 mg, 0.964 mmol) led to compound **14**. Purification was carried out on a silica gel column chromatography with CH_2Cl_2-EtOAc (95:5) to give 127 mg (42%) of the product as a diasteromeric mixture (E/Z ratio 1:1). This mixture was used without further purification.

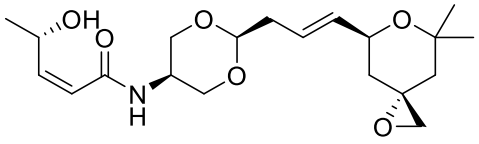
(*S*,2*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)-*N*-((2*R*,5*R*)-2-(3-((3*R*,5*S*)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)allyl)-1,3-dioxan-5-yl)pent-2-enamide (S5)



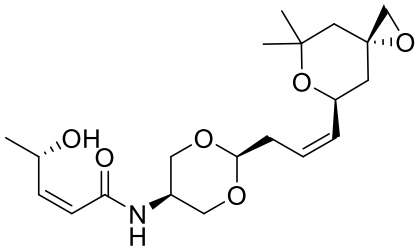
Following the general procedure 1, azide **14** (114 mg, 0.369 mmol) and (*S*,*Z*)-4-(TBS-oxy)pent-2-enoic acid led to compound **S5** The purification was carried out on silica gel column chromatography with hexane-EtOAc (9:1 to 7:3) to give 147 mg (81%, 2 steps) of desired amide as a colorless oil with ratio Z/E: 1:1. This mixture was used without further purification.

(*S,Z*)-*N*-((2*R*,5*R*)-2-((*E*)-3-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)allyl)-1,3-dioxan-5-yl)-4-hydroxypent-2-enamide (15a) and (*S,Z*)-*N*-((2*R*,5*R*)-2-((*Z*)-3-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)allyl)-1,3-dioxan-5-yl)-4-hydroxypent-2-enamide (15b)

Following the general procedure 3, TBS protected alcohol **S5** (140 mg, 0.282 mmol) led to alcohols **15a** and **15b**. The purification was carried out on silica gel column chromatography with hexane-EtOAc (1:1 to 0:1) to give 83 mg (77%) of mixture 1:1 E/Z. This mixture was separated by RT-HPLC semi-preparative and both alcohols were collected.

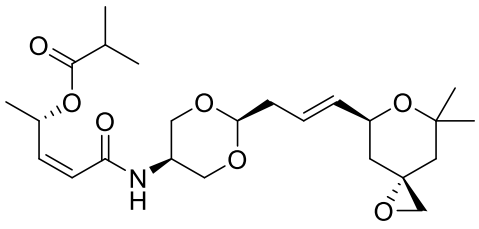


15a ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 – 1.09 (m, 2H), 1.26 (s, 3H), 1.36 – 1.32 (m, 3H), 1.38 (s, 3H), 1.92 – 1.82 (m, 1H), 2.01 – 1.93 (m, 1H), 2.43 – 2.34 (m, 2H), 2.56 (s, 2H), 3.99 – 3.90 (m, 5H), 4.38 (dd, *J* = 11.4, 5.7, 1H), 4.61 (t, *J* = 5.1, 1H), 4.79 (m, 1H), 5.74 – 5.50 (m, 2H), 5.82 (d, *J* = 12.0, 1H), 6.19 (dd, *J* = 12.0, 5.6, 1H), 6.89 – 6.70 (m, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 22.9 (q), 23.9 (q), 31.6 (q), 38.1 (t), 38.3 (t), 42.5 (t), 44.1 (d), 51.2 (t), 55.7 (s), 64.7 (d), 69.1 (d), 70.2 (t), 73.2 (s), 102.2 (d), 122.7 (d), 125.5 (d), 134.5 (d), 150.7 (d), 166.1 (s). IR (NaCl film) 3628-3096, 1635, 1440, 1385, 1233, 1158 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{20}H_{31}NaNO_6$: 404.2044 [M+Na]; found: 404.2044.



15b ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (d, *J* = 13.6, 2H), 1.25 (s, 3H), 1.35 (d, *J* = 6.6, 3H), 1.40 (s, 3H), 1.88 (m, 1H), 1.98 (m, 1H), 2.47 (m, 2H), 2.57 (s, 2H), 4.03 – 3.88 (m, 5H), 4.62 (t, *J* = 5.2, 1H), 4.72 (ddd, *J* = 11.5, 6.0, 2.5, 1H), 4.83 – 4.76 (m, 1H), 5.59 – 5.50 (m, 2H), 5.86 (d, *J* = 12.0, 1H), 6.18 (dd, *J* = 12.0, 5.6, 1H), 6.99 (d, *J* = 7.5, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 23.0 (q), 23.8 (q), 31.6 (q), 33.7 (t), 38.1 (t), 42.5 (t), 44.1 (d), 51.2 (t), 55.9 (s), 64.6 (d), 65.0 (d), 70.0 (t), 70.1 (t), 73.2 (s), 101.9 (d), 123.2 (d), 125.6 (d), 133.5 (d), 150.0 (d), 166.1 (s). IR (NaCl film) 3630-3120, 1631, 1440, 1385, 1233, 1158 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{20}H_{31}NaNO_6$: 404.2044 [M+Na]; found: 404.2044.

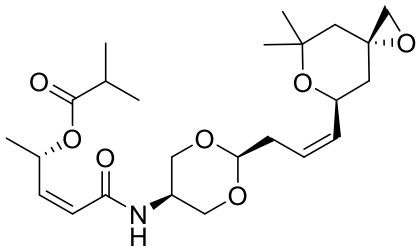
(S,Z)-5-(((2R,5R)-2-((E)-3-((3R,5S)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)allyl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl isobutyrate (2)



Following the general procedure 4, alcohol **15a** (10 mg, 0.026 mmol) led to ester **2**. The purification was carried out on silica gel column

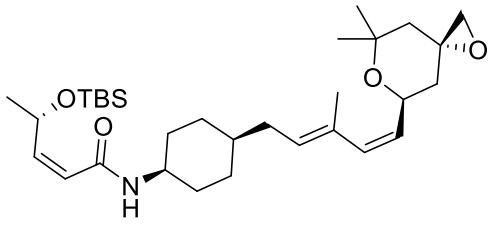
chromatography with hexane-EtOAc (1:9) to give 9.5 mg (79%) of pure ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 (s, 3H), 1.16 (s, 3H), 1.21 – 1.18 (m, 2H), 1.26 (s, 3H), 1.39 – 1.35 (m, 6H), 1.96 – 1.85 (m, 2H), 2.44 – 2.32 (m, 2H), 2.54 – 2.46 (m, 1H), 2.56 (s, *J* = 4.8, 2H), 3.99 – 3.89 (m, 5H), 4.38 (ddd, *J* = 11.6, 6.1, 2.3, 1H), 4.59 (t, *J* = 5.3, 1H), 5.62 – 5.53 (m, 1H), 5.71 – 5.63 (m, 1H), 5.88 – 5.77 (m, 2H), 6.13 (dq, *J* = 13.2, 6.5, 1H), 7.18 (d, *J* = 7.9, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 19.0 (q), 19.1 (q), 20.1 (q), 23.9 (q), 29.8 (t), 31.6 (d), 34.1 (d), 38.2 (t), 38.3 (t), 42.6 (t), 43.8 (s), 51.2 (t), 55.7 (s), 68.7 (d), 69.1 (d), 70.3 (t), 73.2 (s), 102.2 (d), 123.2 (d), 125.8 (d), 134.3 (d), 142.8 (d), 164.9 (s), 176.8 (s). IR (NaCl film) 1725, 1386, 1194, 1145, 1117, 1040 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{24}H_{38}NO_7$: 452.2643 [M+H]; found: 452.2641.

(*S,Z*)-5-(((2*R*,5*R*)-2-((*E*)-3-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)allyl)-1,3-dioxan-5-yl)amino)-5-oxopent-3-en-2-yl isobutyrate (3)



Following the general procedure 4, alcohol **15b** (10 mg, 0.026 mmol) led to ester **3**. The purification was carried out on silica gel column chromatography with hexane-EtOAc (1:9) to give 9 mg (76%) of pure ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 – 1.09 (m, 2H), 1.14 (s, 3H), 1.16 (s, 3H), 1.25 (s, 3H), 1.37 (d, *J* = 6.5, 3H), 1.40 (s, 3H), 2.01 – 1.82 (m, 2H), 2.49 – 2.44 (m, 2H), 2.54 – 2.50 (m, 1H), 2.56 (s, 2H), 4.00 – 3.88 (m, 5H), 4.60 (t, *J* = 5.2, 1H), 4.70 (ddd, *J* = 11.5, 7.2, 2.4, 1H), 5.64 – 5.47 (m, 2H), 5.92 – 5.75 (m, 2H), 6.14 (dq, *J* = 13.1, 6.5, 1H), 7.17 (d, *J* = 8.0, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 19.0 (q), 19.1 (q), 20.1 (q), 23.8 (q), 31.7 (q), 33.7 (t), 34.1 (d), 38.2 (t), 42.5 (t), 43.8 (s), 51.1 (t), 55.7 (s), 64.9 (d), 68.7 (d), 70.3 (t), 70.3 (t), 73.2 (s), 102.0 (d), 123.2 (d), 126.0 (d), 133.3 (d), 142.8 (d), 165.0 (s), 176.8 (s). IR (NaCl film) 1738, 1614, 1521, 1456, 1373, 1123, 1066 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{24}H_{38}NO_7$: 452.2643 [M+H]; found: 452.2646.

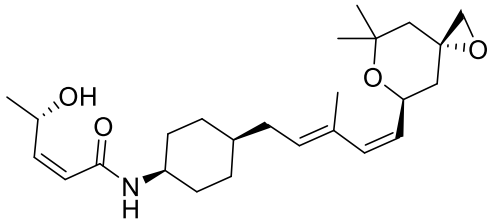
(*S,Z*)-4-((*tert*-Butyldimethylsilyl)oxy)-*N*-((1*R*,4*R*)-4-((2*E*,4*Z*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)pent-2-enamide (17)



Following the general procedure 1, azide **16** (118 mg, 0.344 mmol, mixture E/Z, 9:1) led to compound **17** (145 mg, 75%, 2 steps) as a diastereomeric mixture with ratio E/Z = 9:1. This mixture was purified on silica gel column chromatography (40 cm long column) with hexane-Et_2O (9:1) and 11 mg of pure *Z* diastereomer was collected. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.01 (s, 3H), 0.83 (s, 9H), 1.18 – 1.07 (m, 3H), 1.23 (m, 3H), 1.33 (s, 3H), 1.49 – 1.42 (m, 1H), 1.52 (d, *J* = 2.1, 3H), 1.67 – 1.56 (m, 7H), 1.71 (s, 3H), 2.08 –

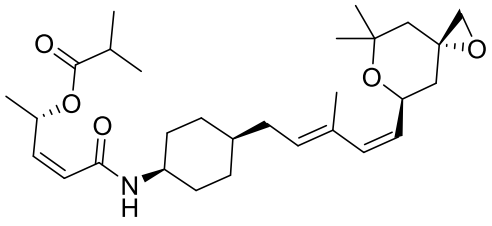
1.79 (m, 4H), 2.52 (s, 2H), 4.09 – 3.96 (m, 1H), 4.86 (ddd, *J* = 11.3, 8.7, 2.0, 1H), 5.25 (dd, *J* = 11.7, 8.7, 1H), 5.37 (t, *J* = 7.5, 1H), 5.59 – 5.43 (m, 2H), 5.72 (d, *J* = 7.8, 1H), 5.96 – 5.89 (m, 2H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ -4.6 (q), -4.5 (q), 16.8 (q), 18.3 (q), 23.9 (q), 24.0 (q), 26.0 (q), 28.0 (t), 28.2 (t), 29.7 (t), 29.8 (t), 31.8 (t), 34.4 (t), 36.6 (d), 38.9 (t), 42.5 (d), 45.3 (d), 51.2 (t), 55.9 (s), 65.3 (d), 65.6 (d), 73.2 (s), 120.1 (d), 129.2 (d), 130.0 (d), 133.0 (s), 136.0 (d), 150.1 (d), 165.0 (s). IR (NaCl film) 1737, 1668, 1632, 1529, 1448, 1244, 1049 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{31}H_{54}NO_4Si$: 532.3822 [M+H]; found: 532.3827.

(*S,Z*)-*N*-((1*R*,4*R*)-4-((2*E*,4*Z*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)-4-hydroxypent-2-enamide (S6)



Following the general procedure 3, TBS protected alcohol **17** (9 mg, 0.015 mmol) led to alcohol **S6**. The purification on silica gel column chromatography with hexane-EtOAc (3:7) gave 5.5 mg (80%) of desired alcohol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.14 – 1.02 (m, 3H), 1.20 (s, 3H), 1.28 (d, *J* = 6.7, 3H), 1.31 (s, 3H), 1.48 – 1.40 (m, 1H), 1.66 – 1.52 (m, 7H), 2.14 – 1.78 (m, 4H), 1.68 (s, 3H), 2.50 (s, 2H), 4.06 – 3.98 (m, 1H), 4.74 – 4.63 (m, 1H), 4.88 – 4.79 (m, 1H), 5.26 – 5.20 (m, 1H), 5.37 – 5.31 (m, 1H), 5.70 (d, *J* = 12.0 1H), 5.88 (d, *J* = 11.6, 1H), 6.02 (d, *J* = 7.1, 1H), 6.07 (dd, *J* = 12.0, 5.4, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 16.9 (q), 22.9 (q), 24.0 (q), 27.8 (t), 28.0 (t), 29.7 (t), 31.8 (d), 34.4 (t), 36.7 (d), 38.9 (t), 42.5 (t), 45.7 (d), 51.2 (t), 56.0 (s), 64.7 (d), 65.3 (d), 73.2 (s), 123.4 (d), 129.2 (d), 129.7 (d), 133.1 (s), 135.9 (d), 150. 0(d), 166.0 (s). IR (NaCl film) 3580-3096, 1660, 1540, 1440, 1380, 1183, 1108 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{25}H_{40}NO_4$: 418.2957 [M+H]; found: 418.2955.

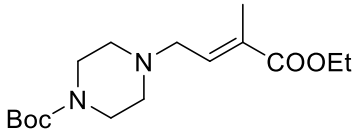
(*S,Z*)-5-(((1*R*,4*R*)-4-((2*E*,4*Z*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl isobutyrate (4)



Following the general procedure 4, alcohol **S6** (5.5 mg, 0.013 mmol) led to ester **4**. The purification on silica gel with hexane-EtOAc (7:3) gave 5 mg (80%) of pure ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.17 (s, 3H), 1.24 – 1.18 (m, 2H), 1.26 (s, 3H), 1.39 – 1.34 (m, 6H), 1.50 – 1.43 (m, 1H), 1.69 – 1.54 (m, 8H), 1.75 (s, 3H), 2.12 – 1.86 (m, 4H), 2.57 – 2.48 (m, 3H), 4.18 – 4.09 (m, 1H), 4.95 – 4.84 (m, 1H), 5.28 – 5.22 (m, 1H), 5.43 (t, *J* = 7.5, 1H), 5.63 (dd, *J* = 11.6, 9.0, 1H), 5.84 – 5.73 (m, 2H), 5.96 (d, *J* = 11.6, 1H), 7.28 (d, *J* = 7.8, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 16.7 (q), 19.0 (q), 19.1 (q), 20.4 (q), 23.9 (q), 27.7 (t), 28.1 (t), 29.7 (t), 29.8 (t), 31.8 (q), 34.1 (d), 34.7 (t), 36.7 (d), 39.0 (t), 42.6 (t), 45.4 (d), 51.1 (t), 55.8 (s), 65.3 (d), 69.1 (d), 73.2 (d),

125.7 (d), 129.1 (d), 130.6 (d), 132.8 (s), 136.1 (d), 137.9 (d), 165.0 (s), 177.5 (s). IR (NaCl film) 1730, 1685, 1625, 1536, 1192, 1050 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{29}H_{46}NO_5$: 488.3376 [M+H]; found: 488.3368.

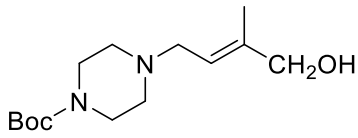
tert-Butyl (_E_)-4-(4-Ethoxy-3-methyl-4-oxobut-2-en-1-yl)piperazine-1-carboxylate (S7)



Dry DMSO (6.15 ml, 86.7 mmol) was added to a solution of oxalyl chloride (3.73 ml, 43.5 mmol) in dry CH_2Cl_2 (50 ml) cooled at -78 ˚C and the mixture was stirred 10 min. After, a solution of commercial **18** (5 g, 21.7 mmol) in CH_2Cl_2 (10 ml) was added via cannula and the solution was stirred over 80 min at -78 ˚C. Et_3N (18 ml, 130 mmol) was added and the reaction mixture was stirred for 10 min at -78 ˚C and 1 h at rt. After this time, the suspension was filtered off and the organic layer was washed with H_2O (15 ml), dried with $MgSO_4$ and evaporated. The resulting aldehyde intermediate was used in the next step without further purification.

A solution of *t*-BuOK (22 ml, 22 mmol) 1M in THF was slowly added to as suspension of (1-ethoxy-1-oxopropan-2-yl)triphenylphosphonium bromide [5] (10.2 g, 23 mmol) in CH_2Cl_2 (40 ml) at -78 ˚C. After the addition the reaction mixture was warmed to 0 ˚C and stirred 30 min. After this time a solution of aldehyde *tert*-butyl 4-(2-oxoethyl)piperazine-1-carboxylate (3.5 g, 15.3 mmol) in CH_2Cl_2 (10 ml) was added via cannula and the solution was stirred over 1 h at 0 ˚C and 1 h at rt. The reaction was quenched with saturated aqueous NH_4Cl, diluted with CH_2Cl_2, washed with brine and the organic layer was dried over $MgSO_4$. The solvent was evaporated the residue was purified on silica gel column chromatography with hexane-EtOAc (4:6 to 3:7) to give 2.44 g (65%) of desired olefin. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.1 Hz, 3H), 1.46 (s, 9H), 1.85 (d, *J* = 1.3 Hz, 3H), 2.44 – 2.38 (m, 4H), 3.14 (d, *J* = 6.5, Hz, 2H), 3.48 – 3.43 (m, 4H), 4.19 (q, *J* = 7.1 Hz, 2H), 6.80 (tq, *J* = 6.5, 1.3 Hz, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 12.6 (q), 14.1 (q), 28.2 (q), 53.0 (t), 56.1 (t), 60.4 (t), 79.4 (s), 130.0 (s), 137.4 (d), 154.5 (s), 167.3 (s). IR (NaCl film) 1699, 1419, 1365, 1245, 1172, 1135, 1004 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{16}H_{29}N_2O_4$: 313.21218 [M+H]; found: 313.21231.

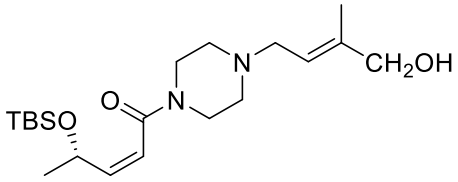
tert-Butyl (_E_)-4-(4-Hydroxy-3-methylbut-2-en-1-yl)piperazine-1-carboxylate (19)



A solution of DIBALH (15.5 ml, 15.5 mmol) 1 M in heptane was slowly added to a solution of **S7** (2.2 g, 7.05 mmol) in CH_2Cl_2 (15 ml) cooled at -78 ˚C. The solution was stirred over 5 min at -78 ˚C and 1 h at rt. The reaction was quenched with MeOH (8 ml) and saturated aqueous potassium sodium tartrate (10 ml). The mixture was allowed to stir over 30 min and extracted with CH_2Cl_2 (3 x 10 ml). The organic layers were washed with brine and dried with $MgSO_4$ and evaporated. The residue was purified on silica gel column chromatography with EtOAc - MeOH (9:1) to give **19** (1.5 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.66 (s, 3H), 2.47 – 2.34 (m, 4H), 3.01 (d, *J* = 7.0 Hz, 2H), 3.45 – 3.35 (m, 4H), 4.01 (s, 2H), 5.66 – 5.41 (m, 1H). ^{13}C

NMR (100.6 MHz $CDCl_3$) δ 14.1 (q), 28.5 (q), 53.1 (t), 55.7 (t), 68.2 (t), 79.8 (t), 120.9 (d), 139.0 (s), 154.8 (s). IR (NaCl film) 3550-3064, 1693, 1421, 1245, 1171, 1128, 999 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{14}H_{27}N_2O_3$: 271.20162 [M+H]; found: 271.20142.

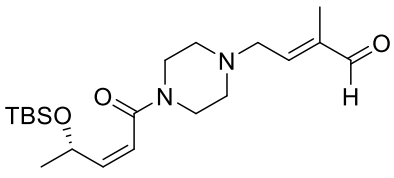
(*S,Z*)-4-((*tert*-Butyldimethylsilyl)oxy)-1-(4-((*E*)-4-hydroxy-3-methylbut-2-en-1-yl)piperazin-1-yl)pent-2-en-1-one (S8)



TFA (15 ml) was added to a solution of **19** (0.55 g, 2.03 ml) in CH_2Cl_2 (15 ml) cooled at 0 ˚C. The solution was stirred over 10 min at 0 ˚C. and 1 h at rt. Then the volatiles were evaporated and the residue was dissolved in 10 ml of CH_2Cl_2 and carefully statured aqueous $NaHCO_3$ was added to *p*H = 10 and shacked over 10 min. Then water was evaporated, the residue was taken up in MeCN, the solids separated by centrifugation, and the solution concentrated. The resulting amine intermediate was used in the next step without further purification.

i-Pr_2EtN (2,12 ml, 12.18 mmol) and a solution of (*S,Z*)-4-(*tert*-butyldimethylsilyloxy)pent-2-enoic acid (0.51 g, 2.23 mmol) in MeCN (3 ml) were added over a stirred solution of crude amine in dry MeCN (3 ml) and the mixture was cooled in an ice-bath. HBTU (0.723 g, 2.23 mmol) was added portion wise to the reaction solution and after the addition the reaction suspension warmed to rt and stirred 2 h. Then the solution was diluted with EtOAc (12 ml) and successively washed with saturated aqueous $NaHCO_3$ (2ml), saturated aqueous NH_4Cl (5 ml), saturated $NaHCO_3$ (3 x 5 ml) and brine (5ml). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified on silica gel column chromatography with EtOAc-MeOH (9:1) to give **S8** (738 mg, 90%, 2 steps) . ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03 (s, 3H), 0.05 (s, 3H), 0.87 (s, 9H), 1.27 (d, *J* = 6.3 Hz, 3H), 1.69 (s, 3H), 2.54 – 2.41 (m, 4H), 3.07 (d, *J* = 6.9 Hz, 2H), 3.72 – 3.50 (m, 4H), 4.05 (s, 2H), 5.03 – 4.95 (m, 1H), 5.58 – 5.52 (m, 1H), 5.98 – 5.87 (m, 2H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ -4.5 (q), -4.3 (q), 14.02 (q), 19.0 (s), 24.3 (q), 26.4 (q), 42.1 (t), 47.0(t), 56.1(t), 67.4(d), 68.1(t), 120.0(d), 120.3(d), 141.3(s), 148.0(d), 167.5(s). IR (NaCl film) 3550-3200, 1618, 1462, 1249, 1075 996 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{20}H_{39}N_2O_3Si$: 383.27245 [M+H]; found: 383.27245.

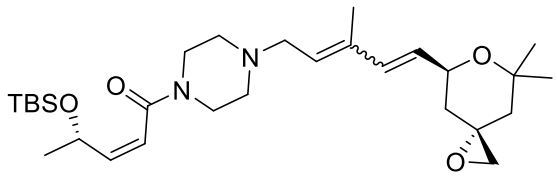
(*E*)-4-(4-((*S,Z*)-4-((*tert*-Butyldimethylsilyl)oxy)pent-2-enoyl)piperazin-1-yl)-2-methylbut-2-enal (20)



Dess−Martin periodinate (728 mg, 1.71) was portionwise added to a mixture of **S8** (597 mg, 1.56 mmol) and solid $NaHCO_3$ (145 mg, 1.71 mmol) in dry CH_2Cl_2 (20 ml) cooled at 0 °C. The resulting solution was stirred for 1 h at 0 ˚C and 1 h at rt, then was diluted with CH_2Cl_2 and added over a 1:1

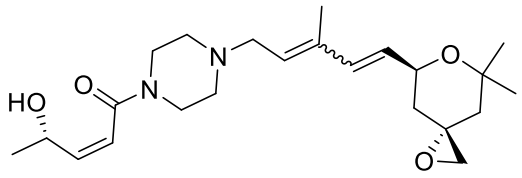
mixture (20 ml) of a saturated aqueous $NaHCO_3$ and saturated aqueous sodium thiosulfate. The mixture was stirred for 1 h at rt. The aqueous layer was separated and washed with CH_2Cl_2 (2 × 20 ml). The combined organic fractions were dried over $MgSO_4$ and concentrated. The residue was purified on a silica gel column chromatography with AcOEt-MeOH (97:3) to give **20** (460 mg, 78%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ), -0.10 - 0.06 (m, 6H), 0.84 (s, 9H), 1.24 (d, *J* = 6.3 Hz, 3H), 1.73 (s, 3H), 2.50 – 2.38 (m, 4H), 3.28 (d, *J* = 6.4 Hz, 2H), 3.72 – 3.47 (m, 4H), 5.01 – 4.92 (m, 1H), 5.96 – 5.80 (m, 2H), 6.54 – 6.49 (m, 1H), 9.41 (s, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ -4.6 (q), -4.5 (q), 9.6 (q), 18.2 (s), 24.0 (q), 25.9 (q), 41.2 (t), 46.0 (t), 53.2 (t) 56.1 (t), 66.2 (d), 118.4 (d), 141.0 (s), 147.8 (d), 149.3 (d), 165.5 (s), 194.5 (s). IR (NaCl film) 2808, 1692, 1649, 1437, 1075, 837 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{20}H_{37}N_2O_3Si$: 381.25680 [M+H]; found: 381.25668

(*S*,2*Z*)-4-((*tert*-Butyldimethylsilyl)oxy)-1-(4-(5-((3*R*,5*S*)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)piperazin-1-yl)pent-2-en-1-one (21)



Following the general procedure 2, sulfone **11** (60 mg, 0.17 mmol) and aldehyde **20** (50 mg, 0.13 mmol) led to compound **21**. The purification of the compound was carried out on a silica gel column chromatography with EtOAc-MeOH (98:2) to give 46 mg (70%) of a desired diene (E/Z ratio 8:2) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ) -0.00 (s, 3H), 0.01 (s, 3H), 0.83 (s, 9H), 1.18 – 1.10 (m, 2H), 1.26 – 1.23 (m, 6H), 1.37 (s, 3H), 1.72 (s, 3H), 2.00 – 1.85 (m, 2H), 2.44 – 2.35 (m, 4H), 2.54 (s, 2H), 3.07 (d, *J* = 7.1 Hz, 2H), 3.69 – 3.43 (m, 4H), 4.44 (ddd, *J* = 11.6, 6.6, 2.0 Hz, 1H), 4.99 – 4.92 (m, 1H), 5.53 – 5.47 (m, 1H), 5.61 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.93 – 5.86 (m, 2H), 6.25 (d, *J* = 15.7 Hz, 1H). NMR (400 MHz, $CDCl_3$) δ), -4.5 (q), -4.4 (q), 12.8 (q), 18.3 (s), 23.9 (q), 24.1 (q), 26.0 (q), 31.6 (q), 38.7 (t), 41.4 (t), 42.5 (t), 46.3 (t), 51.1 (t), 53.0 (s), 53.4 (t), 55.7 (t), 66.4 (d), 69.5 (d), 73.2 (s), 118.7 (d), 128.2 (d), 129.0 (d), 135.4 (d), 136.5 (s), 147.6 (d), 165.5 (s). IR (NaCl film) 1632, 1461, 1382, 1249, 1074, 836 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{29}H_{51}N_2O_4Si$: 519.36126 [M+H]; found: 519.36144

(*S*,2*Z*)-1-(4-(5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)piperazin-1-yl)-4-hydroxypent-2-en-1-one (22)

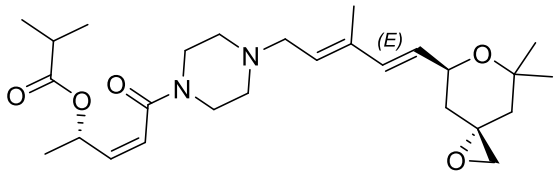


Following the general procedure 3, TBS protected alcohol **21** (230 mg, 0.444 mmol) led to compound **22**. Purification of the product on a silica gel column chromatography with EtOAc-MeOH (1:0 to 9:1) gave 129 mg (67%) of desired alcohol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 – 1.13 (m, 2H), 1.27 (s, 3H), 1.31 (d, *J* = 6.5 Hz, 3H), 1.39 (s, 3H), 1.75 (s, 3H), 2.01 – 1.86 (m, 2H),

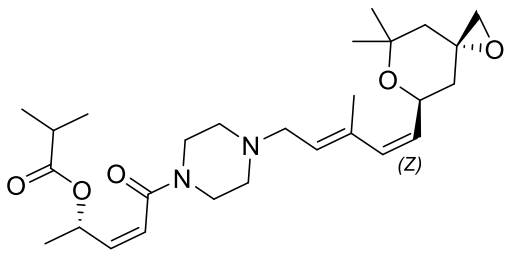
2.49 – 2.38 (m, 4H), 2.59 – 2.53 (m, 2H), 3.10 (d, *J* = 7.0 Hz, 2H), 3.55 – 3.45 (m, 2H), 3.73 – 3.58 (m, 2H), 4.47 (ddd, *J* = 11.5, 6.5, 2.1 Hz, 1H), 4.65 – 4.56 (m, 1H), 5.52 (t, *J* = 7.0 Hz, 1H), 5.64 (dd, *J* = 15.7, 6.5 Hz, 1H), 6.11 – 6.04 (m, 2H), 6.28 (d, *J* = 15.7 Hz, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 12.8 (q), 22.5 (q), 23.9 (q), 31.6 (q), 38.7 (t), 41.8 (t), 42.5 (t), 46.6 (t), 51.2 (t), 52.8 (t), 53.3 (t), 55.7 (t), 64.9 (d), 69.5 (d), 73.2 (s), 121.4 (d), 128.2 (d), 129.0 (d), 135.4 (d), 136.6 (s), 147.7 (d), 166.5 (s). IR (NaCl film) 3628-3096, 1616, 1439, 1383, 1236, 1062, 833 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{23}H_{37}N_2O_4$: 405.27428 [M+H]; found: 405.27525

(*S,Z*)-5-(4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)piperazin-1-yl)-5-oxopent-3-en-2-yl isobutyrate (5a) and (*S,Z*)-5-(4-((2*E*,4*Z*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)piperazin-1-yl)-5-oxopent-3-en-2-yl isobutyrate (5b)

Following the general procedure 4, alcohol **22** (42 mg, 0.10 mmol) led to mixture of **5a** and **5b.** Purification on silica gel column chromatography with hexane-EtOAc (8:2) gave 45 mg (98%) of diasteromeric mixture. This mixture was separated by semi-preparative RP-HPLC to give 24 mg of *E* and 4 mg of *Z* (60%) of pure diastereomers:



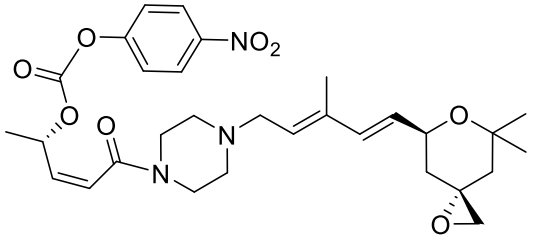
5a ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (d, *J* = 2.4 Hz, 3H), 1.14 (d, *J* = 2.4 Hz, 3H), 1.25 – 1.16 (m, 2H), 1.28 (s, 3H), 1.40 (s, 3H), 1.38 (d, *J* = 6.3 Hz, 3H), 1.75 (s, 3H), 2.02 – 1.88 (m, 2H), 2.52 – 2.39 (m, 5H), 2.59 – 2.55 (m, 2H), 3.12 (d, *J* = 6.9 Hz, 2H), 3.87 – 3.39 (m, 4H), 4.47 (ddd, *J* = 11.9, 6.6, 2.3 Hz, 1H), 5.54 (t, *J* = 6.9 Hz, 1H), 5.64 (dd, *J* = 15.7, 6.6 Hz, 1H), 5.83 – 5.69 (m, 2H), 6.04 (d, *J* = 11.1 Hz, 1H), 6.28 (d, *J* = 15.7 Hz, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 12.7 (q), 18.9 (q), 18.9 (q), 20.0 (q), 23.7 (q), 31.5 (q), 34.9 (d), 38.5 (t), 41.2 (t), 42.4 (t), 46.1 (t), 51.0 (t), 52.9 (t), 55.6 (t), 55.8 (t), 68.7 (d), 69.4 (d), 73.1 (s), 122.5 (d), 128.1 (d) 128.9 (d), 135.3 (d), 140.1 (d), 165.2 (s), 176.1 (s). IR (NaCl film) 1730, 1631, 1462, 1383, 1233, 1158 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{27}H_{43}N_2O_5$: 475.31665 [M+H]; found: 475.31680.



5b ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 – 1.06 (m, 8H), 1.26 (s, 3H), 1.45 – 1.35 (m, 6H), 1.79 (s, 3H), 2.07 – 1.86 (m, 2H), 2.63 – 2.39 (m, 7H), 3.24 – 3.01 (m, 2H), 3.83 – 3.41 (m, 4H), 4.84 (t, *J* = 9.3 Hz, 1H), 5.36 (dd, *J* = 11.5, 9.3 Hz, 1H), 5.50 – 5.44 (m, 1H), 5.83 – 5.69 (m, 2H), 5.96 (d, *J* = 11.5 Hz, 1H), 6.07 (d, *J* = 11.0 Hz, 1H). ^{13}C NMR via HSQC (400 MHz, $CDCl_3$) 140.2 (d), 136.6 (d), 135.2 (d), 130.1 (d), 122.6

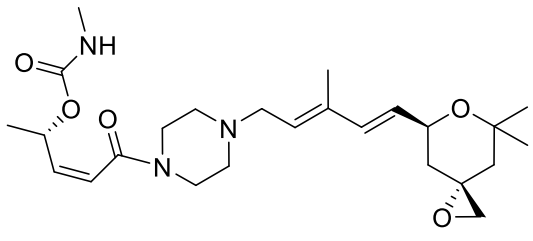
(d), 68.2 (d), 64.9 (d), 56.0 (t), 52.8 (d), 50.5 (t), 45.7 (t), 42.1 (t), 38.4 (t), 33.9 (d), 31.5 (q), 23.7 (q), 20.0 (q), 18.7 (q), 16.7 (q).). IR (NaCl film): = 1731, 1630, 1449, 1368, 1191 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{27}H_{43}N_2O_5$: 475.31665 [M+H]; found: 475.31675.

(*S,Z*)-5-(4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)piperazin-1-yl)-5-oxopent-3-en-2-yl 4-nitrophenyl carbonate (S10)



A solution of alcohol of pure **22** (32 mg, 0.079 mmol) in CH_2Cl_2 (2.5 ml) was stirred at 0 ˚C. Over the cold solution NEt_3 (0.033 ml) and a solution of 4-nitrophenylchloroformate (32 mg, 0.158 mmol) in CH_2Cl_2 (2 ml) were added via cannula. After 40 min of stirring at 0 ˚C, additional 1 eq. of 4-nitrophenyl chloroformate was added and the solution was warmed to rt and stirred for additional 1 h after which TLC showed that most of the starting material had been consumed. Cold water (3 ml) and CH_2Cl_2 was added. The aqueous layer was extracted with CH_2Cl_2 (2x5ml). The combined organic solvents were washed brine (3 ml) and dried over $MgSO_4$ and evaporated. The residue was purified on silica gel column chromatography with AcOEt-MeOH (98:2 to 94:6) to give **S10** (20 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 – 1.11 (m, 2H), 1.27 (s, 3H), 1.39 (s, 3H), 1.53 (d, *J* = 6.3, 3H), 1.71 (s, 3H), 2.01 – 1.82 (m, 2H), 2.46 – 2.37 (m, 4H), 2.57 (s,2H), 3.07 (d, *J* = 7.0, 2H), 3.72 – 3.47 (m, 4H), 4.53 – 4.37 (m, 1H), 5.51 (t, *J* = 7.0, 1H), 5.63 (dd, *J* = 15.7, 6.6, 1H), 5.98 – 5.86 (m, 1H), 6.19 (d, *J* = 10.7, 1H), 6.26 (d, *J* = 15.7, 1H), 7.39 – 7.34 (m, 2H), 8.27 – 8.23 (m, 2H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 12.8 (q), 20.1 (q), 23.9 (q), 31.6 (q), 38.6 (t), 41.6 (t), 42.5 (t), 46.4 (t), 51.2 (t), 52.8 (t), 53.1 (t), 55.7 (t), 55.9 (t), 69.5 (d), 73.3 (d), 74.6 (s), 121.9 (d), 123.7 (d), 125.3 (d), 125.4 (d), 128.2 (d), 129.1 (d), 135.4 (d), 136.6 (s), 139.1(d) 145.4(s), 151.7 (s), 155.7 (s), 164.9 (s). IR (NaCl film) 2943, 2814, 1765, 1719, 1627, 1525, 1215, 1021 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{30}H_{40}N_3O_8$: 570.28154 [M+H]; found: 570.28166.

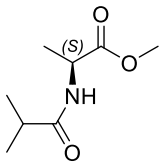
(*S,Z*)-5-(4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-Dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)piperazin-1-yl)-5-oxopent-3-en-2-yl methylcarbamate (6)



A stirred solution of activated carbonate **S10** (20 mg, 0.035 mmol) in $ClCH_2CH_2Cl$ (0.5 ml) was cooled in an ice-bath and the methylamine (0.11 ml, 1M in THF, 0.11 mmol) was added dropwise. The resulting yellow solution was allowed to stir at 0 ˚C for 20 min. Then yellow solid was filtered off volatiles were removed under reduced pressure. The residue was concentrated and purified on silica gel column

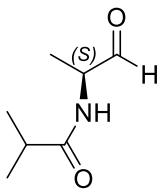
chromatography with AcOEt-Hexane-MeOH(8:2:0.8) to give 12 mg (75%) of the desired carbamate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 – 1.13 (m, 2H), 1.27 (d, *J* = 4.2, 3H), 1.41 – 1.35 (m, 6H), 1.75 (s, 3H), 2.03 – 1.88 (m, 2H), 2.43 (s, 4H), 2.60 – 2.54 (m, 2H), 2.76 (d, *J* = 4.9, 3H), 3.10 (d, *J* = 6.9, 2H), 3.81 – 3.37 (m, 4H), 4.47 (ddd, *J* = 11.0, 6.6, 1.8, 1H), 4.66 (bs, 1H), 5.54 (t, *J* = 6.9, 1H), 5.67 – 5.58 (m, 2H), 5.82 (dd, *J* = 11.7, 7.7, 1H), 6.01 (d, *J* = 11.7, 1H), 6.28 (d, *J* = 15.7, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ12.9 (q), 20.6 (q), 23.9 (q), 27.6 (q), 31.6 (q), 38.7 (t), 41.5 (t), 42.6 (t), 46.4 (t), 51.2 (s), 52.9 (t), 55.8 (t), 56.0 (t), 69.6 (d), 73.3 (s), 122.2 (d), 128.6 (d), 128.9 (d), 135.5 (d), 136.5 (s), 140.5 (d), 156.5 (s), 165.6 (s). IR (NaCl film) 1730, 1631, 1440, 1383, 1233, 1158, 1040 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{27}H_{43}N_2O_5$: 475.31665 [M+H]; found: 475.31680.

Methyl isobutyryl-L-alaninate (24)



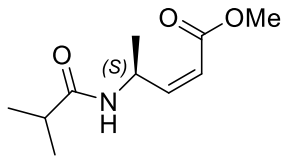
NEt_3 (3.97 ml, 28.6 mmol), isobutyric anhydride (3.57 ml, 21.5 mmol) and 4-DMAP (87 mg, 0.72 mmol) were added to a cooled (0 ˚C) suspension commercial **23** (1.0 g, 7.16 mmol) in CH_2Cl_2 (10 ml) and the mixture was stirred over 15 min. After this time, the ice bath was removed and the solution was allowed to rt and stirred over 3 h. The solution was diluted with CH_2Cl_2 and washed with sat. NH_4Cl and sat. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc, 8:2) gave the desired compound (1.22 g, 99%) as a colorless oil. All analytical data for this compound were identical with the reported in literature [2].

(*S*)-*N*-(1-Oxopropan-2-yl)isobutyramide (25)



A solution of DIBALH (9 ml, 1.0 M in heptane, 9 mmol) was added carefully to a solution of **24** (1.2 g, 6.9 mmol) in toluene (12 ml) cooled at -78 ˚C, over N_2. This mixture was stirred for 2 h at -78 ˚C and then MeOH (5 ml) and Rochelle's salt saturate solution (5 ml) were added. The mixture was diluted with AcOEt, allowed to room temperature and stirred for additional 1 h. After this time the organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (hexane/EtOAc, 1:1) gave the desired compound as colorless oil, 0.7 g (71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, *J* = 6.9, Hz, 6H), 1.37 (d, *J* = 7.4, 3H), 2.43 (hept, *J* = 6.9 Hz, 1H), 4.60 – 4.40 (m, 1H), 6.09 (bs, 1H), 9.56 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.6 (q), 19.6 (q), 19.6 (q), 35.4 (d), 54.4 (d), 177.2 (s), 199.5(s). $[\alpha]_D$=+18.3 (*c* 1.0 in $CHCl_3$); IR (NaCl film) 1725, 1646, 1537, 1200, 1178 cm^{-1}; HRMS (+ESI): m/z calcd for $C_7H_{14}NO_2$: 144.1019 [M+H]; found: 144.1021

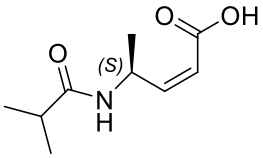
Methyl (*S,Z*)-4-isobutyramidopent-2-enoate (26)



Bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate (0.75 ml, 3.52 mmol) was added to a solution of 18-crown-6 (1.5 g, 5.88 mmol) in THF (12 ml) at room temperature. Then the solution was cooled to -78 ˚C and a 1 M solution of KHMDS in

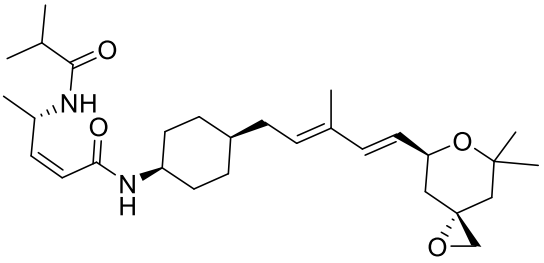
THF (3.52 ml, 3.52 mmol) was added carefully and the resulting mixture was stirred over 5 min. After this time a solution of **25** (0.42 g, 2.94 mmol) in THF (3 ml) was added via cannula and the solution was stirred over 3 h at -78 ˚C. After this time, the reaction was quenched with H_2O (10 ml) and the mixture was allowed to room temperature, diluted with CH_2Cl_2 (12 ml) and the aqueous layer was extracted 2 x CH_2Cl_2 (10 ml). The organic layers were washed with sat. NH_4Cl dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography with hexane-EtOAc (7:3 to 1:1) gave **26** as a colorless oil (0.41 g, 82%) (dr = 92:8). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11 (d, *J* = 6.9, 6H), 1.29 (d, *J* = 6.9 Hz, 3H), 2.35 – 2.26 (m, 1H), 3.71 (s, 3H), 5.34 – 5.20 (m, 1H), 5.77 (dd, *J* = 11.7, 1.1 Hz, 1H), 6.16 (dd, *J* = 11.7, 8.2 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.6 (q), 19.6 (q), 20.0 (q), 35.6 (d), 44.3 (d), 51.6 (q), 119.3 (d), 151.2 (d), 166.6 (s), 176.6 (s). $[\alpha]_D$=+10.8 (*c* 1.0 in $CHCl_3$). IR (NaCl film): =1725, 1646, 1537, 1200, 1178 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{10}H_{18}NO_3$: 200.1281 [M+H]; found: 200.1283.

(*S,Z*)-4-Isobutyramidopent-2-enoic acid (27)



A Me_3SnOH (0.9 g, 5.0 mmol) was added to a solution of methyl **26** (0.2 g, 1.0 mmol) in $ClCH_2CH_2Cl$ and the solution was stirred at 85 ˚C overnight. After this time additional Me_3SnOH (0.54 g, 3.0 mmol) was added and the reaction mixture was stirred overnight. Then, the solvent was evaporated and the residue was dissolved in EtOAc, washed with 5% HCl, brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of residue by silica gel column chromatography with CH_2Cl_2-MeOH (97.5:2.5 to 95:5) gave the desired acid as a white solid (93 mg, 50%). MP 151 – 152 ˚C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 – 1.11 (m, 6H), 1.32 (d, *J* = 6.6 Hz, 3H), 2.44 – 2.33 (m, 1H), 4.81 – 4.63 (m, 1H), 5.69 – 5.55 (m, 1H), 5.81 (bs, 1H), 5.94 (d, *J* = 11.6 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.5 (q), 19.5 (q), 19.9 (q), 35.4 (d), 45.2 (d), 123.7 (d), 140.6 (d), 167.9 (s), 179.0 (s). $[\alpha]_D$=+13.2 (*c* 0.25 in $CHCl_3$). IR (NaCl film) 3460-2517, 1700, 1635, 1536, 1384, 1249, 928 cm^{-1}. HRMS (+ESI): m/z calcd for $C_9H_{16}NO_3$: 186.1247 [M+H]; found: 186.1243

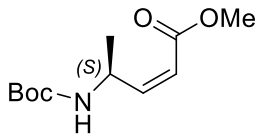
Compound Sudemycin K (7)



Following the general procedure 1, azide **16** [4] (30 mg, 0.085 mmol) and acid 1 led to **Sudemycin K**. The crude was purified on silica gel column chromatography with hexane-EtOAc (1:1 to 1:0) and repurified by semi-preparative RP-HPLC to give the desired amide as a colorless oil (12 mg, 30%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (d, *J* = 6.9, Hz, 6H), 1.19 – 1.11 (m, 2H), 1.24 – 1.19 (m, 6H), 1.34 (s, 3H), 1.57 – 1.50 (m, 7H), 1.73-1.64 (s, 5H), 1.96 – 1.82 (m, 2H), 2.07 – 1.98 (m, 2H), 2.34 – 2.25 (m, 1H), 2.51 (s, 2H), 4.12 – 4.03 (m, 1H), 4.40 (ddd, *J* = 11.7, 6.8, 2.5 Hz, 1H), 4.71 – 4.60 (m, 1H), 5.52 – 5.40 (m, 3H), 5.74 (d, *J* = 11.7 Hz, 1H), 5.88 (d, *J* = 5.9 Hz, 1H), 6.21 (d, *J* = 15.7 Hz, 1H), 8.43 – 8.31 (m, 1H). ^{13}C NMR (100.6 MHz,

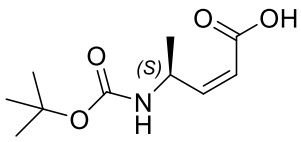
$CDCl_3$) δ 12.7 (q), 19.5 (q), 19.9 (q), 20.6 (q), 23.9 (q), 27.5 (t), 27.9 (t), 29.8 (t), 31.7(q), 34.6(t), 35.5(d), 36.9(d), 38.8(t), 42.6(t), 45.0(d), 45.5(d), 51.2(t), 55.8(s), 69.7(d), 73.2 (s), 125.9 (d), 126.9 (d), 132.4 (s), 133.8 (d), 136.5 (d), 136.5 (d), 137.8 (d), 165.7 (s), 177.4 (s). IR (NaCl film) 3282, 2942, 1653, 1619, 1541, 1483, 1063 928 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{29}H_{47}N_2O_4$: 487.35303 [M+H]; found: 487.35307.

Methyl (*S,Z*)-4-((*tert*-Butoxycarbonyl)amino)pent-2-enoate (29)



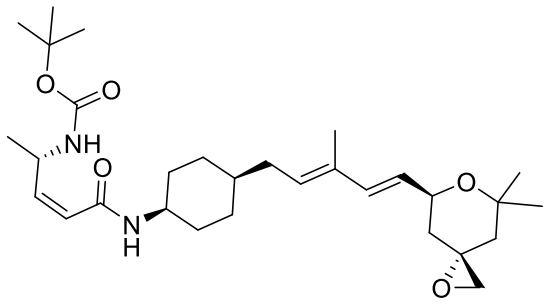
To a solution of 18-crown-6 (0.84 g, 3.18 mmol) in THF (8 ml) bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate (0.401 ml, 1.9 mmol) was added at room temperature. Then the solution was cooled to -78 ˚C and a 1 M solution of KHMDS in THF (1.9 ml, 1.9 mmol) was added carefully and was stirred over 40 min. After this time a solution of commercial **28** (0.275 g, 1.59 mmol) in THF (3 ml) was added via cannula. The solution was stirred over 3 h at -78 ˚C then the reaction was quenched with H_2O (5 ml) and the mixture was allowed to room temperature. The mixture was diluted with CH_2Cl_2 (12 ml) and the aqueous layer was extracted 2 x CH_2Cl_2 (10 ml). The organic layers were washed with sat. NH_4Cl dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography with hexane-EtOAc (7:3) gave **29** (0.27 g, 72%) (dr = 97:3) as a colorless oil. ^{1}H NMR (400 MHz $CDCl_3$) δ 1.27 (d, *J* = 6.8 Hz, 3H), 1.42 (s, 9H), 3.72 (s, 3H), 4.75 (bs, 1H), 5.22 – 5.11 (m, 1H), 5.76 (dd, *J* = 11.6, 1.2 Hz, 1H), 6.18 – 6.05 (m, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 20.2 (q), 28.4 (q), 45.5 (d), 51.4 (q), 79.5 (s), 118.7 (d), 152.3 (d), 155.3 (s), 166.2 (s). IR (NaCl film) 1723, 1629, 1513, 1168, 1049 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{11}H_{19}NNaO_4$: 252.1206 [M+Na]; found: 252.1216

(*S,Z*)-4-((*tert*-Butoxycarbonyl)amino)pent-2-enoic acid (30)



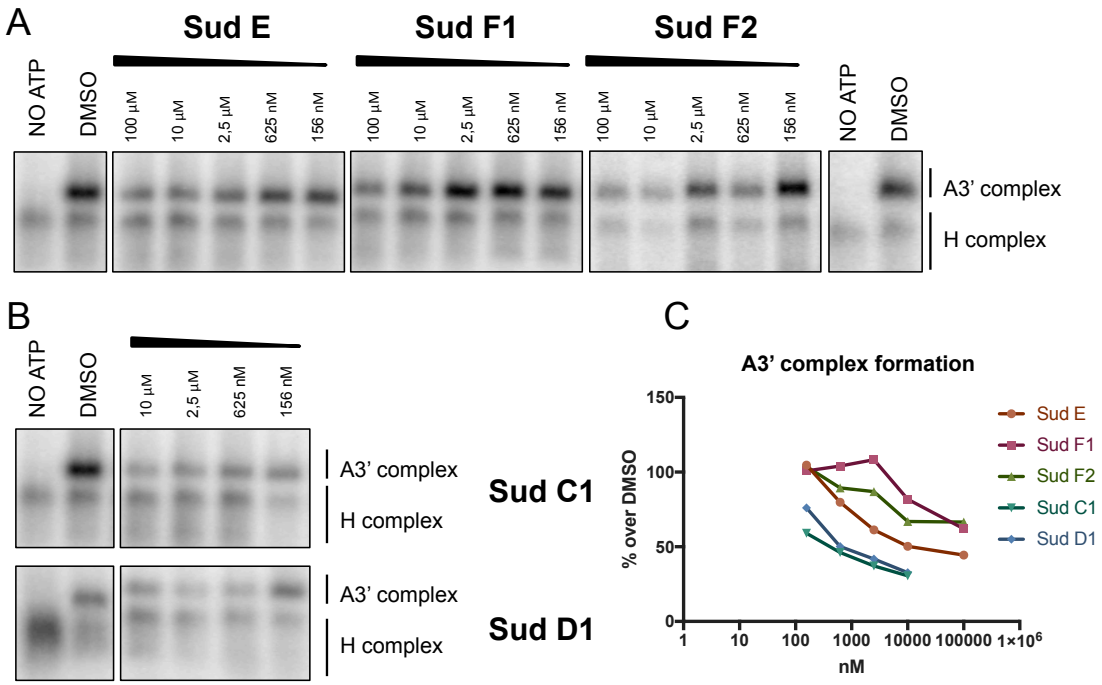
A Me_3SnOH (0.79 g, 4.36 mmol) was added to a solution of **29** (0.125 g, 0.55 mmol) in $ClCH_2CH_2Cl$ (5 ml) and the solution was stirred at 85 ˚C overnight. Then, the solvent was evaporated and the residue was dissolved in EtOAc, washed with HCl 5%, brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with with hexane-EtOAc (8:2 to 7:3) to give the desired acid (83 mg, 75%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.21 (d, *J* = 6.9 Hz, 3H), 1.42 (s, 9H), 5.24 – 5.10 (m, 1H), 5.72 (dd, *J* = 11.6, 1.3 Hz, 1H), 6.14 – 6.03 (m, 1H). ^{13}C NMR (100.6 MHz CD_3OD) δ 20.5(q), 28.7(q), 46.3(d) 80.14(s), 120.05 (d), 152.5 (d), 157.7 (s), 169.4 (s). $[\alpha]_D$=+44.8 (*c* 1.0 in $CHCl_3$). IR (NaCl film) 3474-2872, 1706, 1367, 1166, 1049 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{1o}H_{17}NNaO_4$: 238.1050 [M+Na]; found: 238.1044

***tert*-Butyl ((*S,Z*)-5-(((1*R*,4*R*)-4-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)cyclohexyl)amino)-5-oxopent-3-en-2-yl)carbamate (8)**

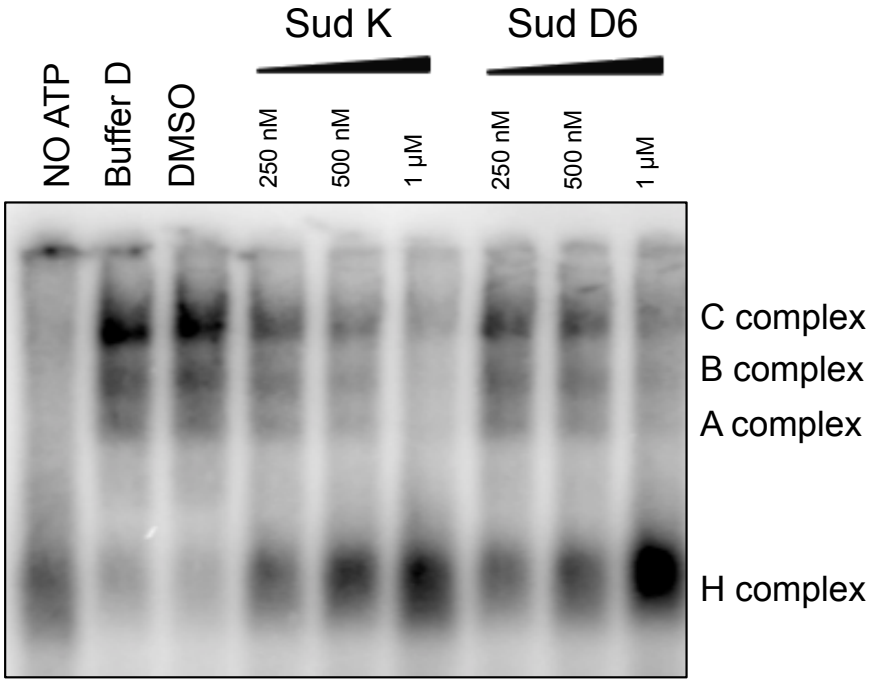


Following the general procedure 1, azide **16** (30 mg, 0.085 mmol) and acid **30** led to carbamate **8**. The product was purified on silica gel column chromatography with hexane-EtOAc (1:1) to give the title compound (25 mg, 50%, 2 steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 – 1.13 (m, 5H), 1.28 (s, 3H), 1.42 – 1.37 (m, 6H), 1.44 (s, 9H), 1.60 – 1.52 (m, 4H), 1.70 (s, 3H), 1.86 – 1.74 (m, 2H), 2.00 – 1.88 (m, 2H), 2.17 – 2.04 (m, 2H), 2.56 (s, 2H), 4.14 – 3.91 (m, 1H), 4.55 – 4.41 (m, 2H), 4.95 – 4.87 (m, 1H), 5.57 – 5.42 (m, 3H), 5.79 (d, *J* = 11.7 Hz, 1H), 6.39 – 6.18 (m, 1H), δ 8.40 (bs, 1H). ^{13}C NMR (100.6 MHz $CDCl_3$) δ 12.6 (q), 20.7 (q), 23.9 (q), 27.3 (t), 27.9 (t), 28.6 (q), 29.6 (t), 29.8 (t), 31.7 (q), 34.6 (t), 37.3 (d), 38.7 (t), 42.6 (t), 45.5 (d), 45.6 (d), 51.1 (t), 55.8 (s), 69.8 (d), 73.3 (s), 80.2 (s), 125.7 (d), 126.8 (d), 132.3 (s), 133.9 (d), 136.8 (d), 137.8 (d), 156.0 (s), 165.9 (s). IR (NaCl film) 2942, 1660, 1622, 1538, 1485, 1072 cm^{-1}. HRMS (+ESI): m/z calcd for $C_{30}H_{49}N_2O_5$: 517.3641 [M+H]; found: 517.3644

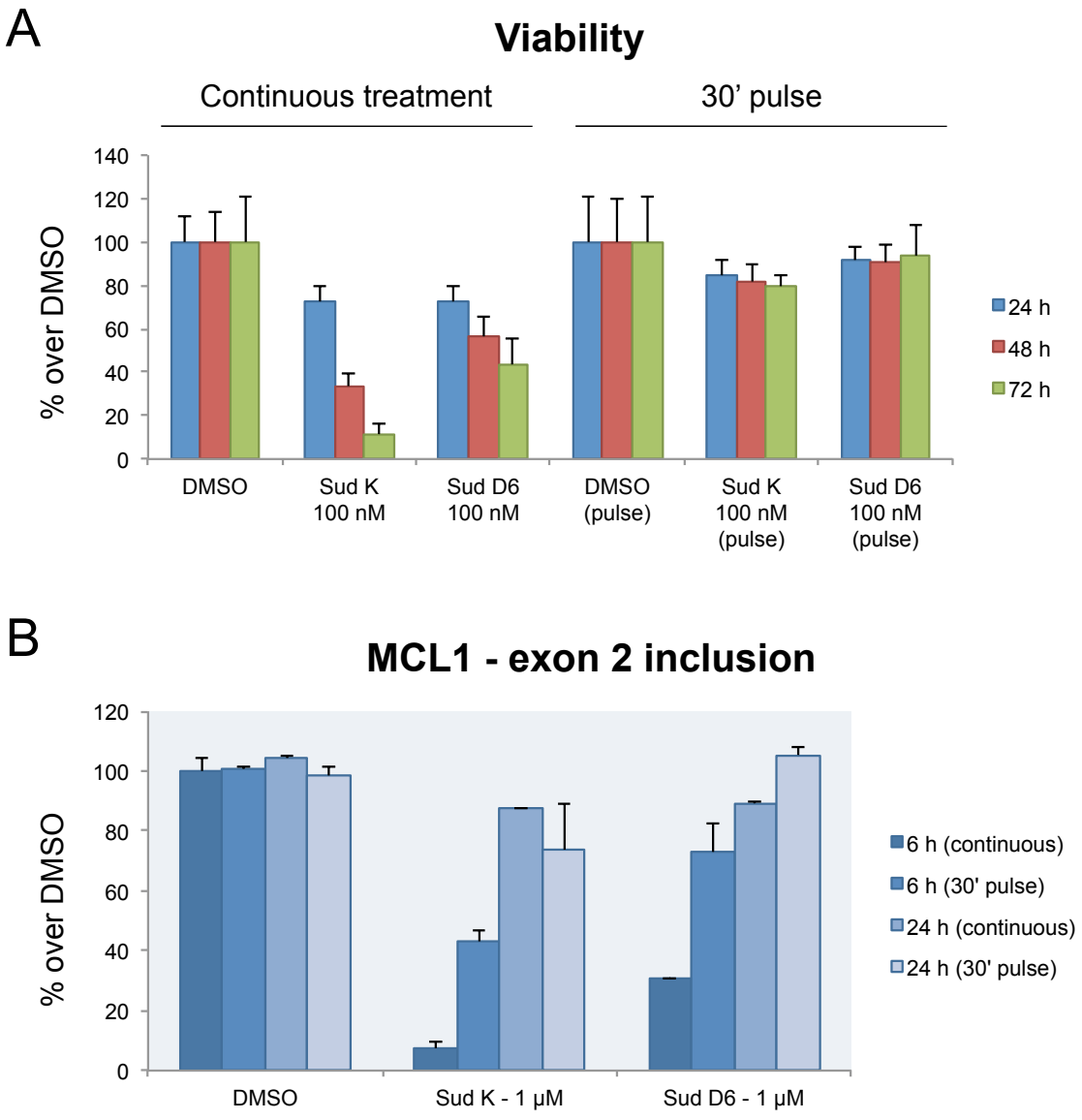
Supplementary Figure 1. *In vitro* spliceosome (A3' complex) formation assays. (A) Representative Phosphoroimager pictures of electrophoretic separation of H and A3' complexes assembled upon incubation of a radioactively labeled adenovirus major late promoter RNA (spanning sequences corresponding to 3' half of intron 1 and part of the following exon) in HeLa nuclear extracts and fractionation on non-denaturing agarose gels. The electrophoretic mobility of A3' and H complexes is indicated, as well as concentrations of the indicated drugs or DMSO as control. Only complex H is formed in the absence of ATP. (B) Assays as in A for the indicated drugs. (C) Quantifications of the results reported in A and B.



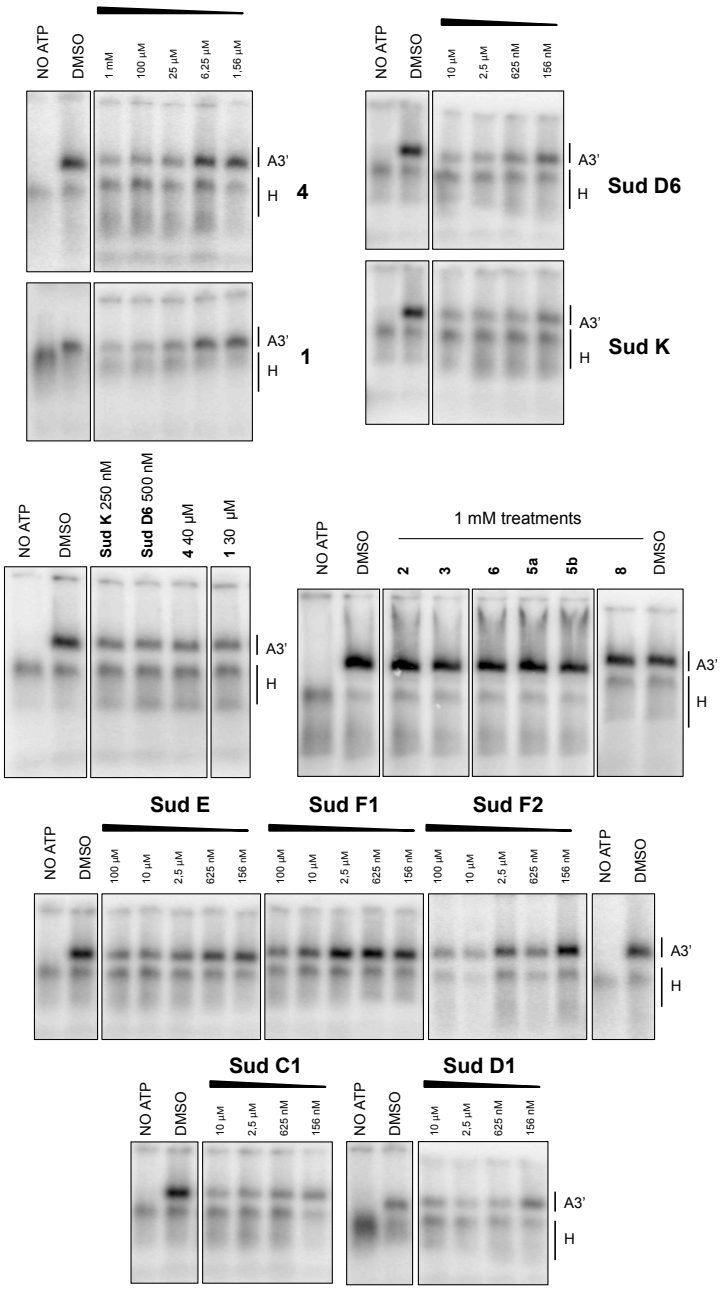
Supplementary Figure 2. Full *in vitro* spliceosome assembly assay. Electrophoretic separation of H, A, B and C complexes assembled upon incubation of a full length adenovirus major late promoter RNA in HeLa nuclear extracts and fractionation on non-denaturing agarose gels. The electrophoretic mobility of the different complexes is indicated, as well as concentrations of the indicated drugs or DMSO as control. Only complex H is formed in the absence of ATP. As expected, Sudemycins K and D6 inhibit the formation of all A, B and C spliceosomal complexes, leading to an increase in complex H.



Supplementary Figure 3. Effects on cytotoxicity and alternative splicing after pulses of drug treatments. (A) Effects of Sud D6 and Sud K on cell viability were evaluated after 24, 48 and 72 h. Cells were either pulsed with drug for 30' or kept in continuous treatment until the first measurement, at 24h. A 30' pulse significantly decreases the effects on cell viability. (B) Effects of Sud D6 and Sud K on *MCL1* alternative splicing upon continuous drug treatment or after a 30' pulse. Exon 2 inclusion levels were measured at 6 or 24 h. Results show reversal of the effects at 24 h and decreased (but not suppressed) effects at 6h upon pulsed treatments compared to continuous treatments.



Supplementary Figure 4. Uncropped images from *in vitro* A3' complex formation assays. The figures correspond to results presented in Figure 2 and Supplementary Figure 1. The top of each panel shows the signal coming from the wells of the gel.



SUPPLEMENTARY REFERENCES

[1] O'Brien, J., Wilson, I., Orton, T., and Pognan, F. (2000) Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity, *Eur J Biochem 267*, 5421-5426.

[2] Corrionero, A., Minana, B., and Valcarcel, J. (2011) Reduced fidelity of branch point recognition and alternative splicing induced by the anti-tumor drug spliceostatin A, *Genes Dev 25*, 445-459.

[3] Effenberger, K. A., James, R. C., Urabe, V. K., Dickey, B. J., Linington, R. G., and Jurica, M. S. (2015) The Natural Product N-Palmitoyl-l-leucine Selectively Inhibits Late Assembly of Human Spliceosomes, *J Biol Chem 290*, 27524-27531.

[4] Lagisetti, C., Palacios, G., Goronga, T., Freeman, B., Caufield, W., and Webb, T. R. (2013) Optimization of antitumor modulators of pre-mRNA splicing, *J Med Chem 56*, 10033-10044.

[5] Shimizu, I., Hayashi, K., Ide, N., and Oshima, M. (1991) Facile synthesis of (—)-serricornin by means of palladium-catalyzed hydrogenolysis of alkenyloxiranes, *Tetrahedron 47*, 2991-2998.

[6] Valverde, S., Martin-Lomas, M., Herradon, B., and Garcia-Ochoa, S. (1987) The reaction of carbohydrate-derived alkoxyaldehydes with methoxycarbonylmethylenetriphenylphosphorane: stereoselective synthesis of β-unsaturated esters, *Tetrahedron 43*, 1895-1901.

[7] Lagisetti, C., Pourpak, A., Goronga, T., Jiang, Q., Cui, X., Hyle, J., Lahti, J. M., Morris, S. W., and Webb, T. R. (2009) Synthetic mRNA splicing modulator compounds with in vivo antitumor activity, *J Med Chem 52*, 6979-6990.

^{1}H-NMR, ^{13}C-NMR and 2D correlation spectra of Synthetic Compound**s**

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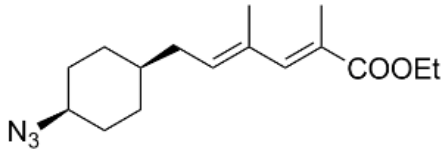

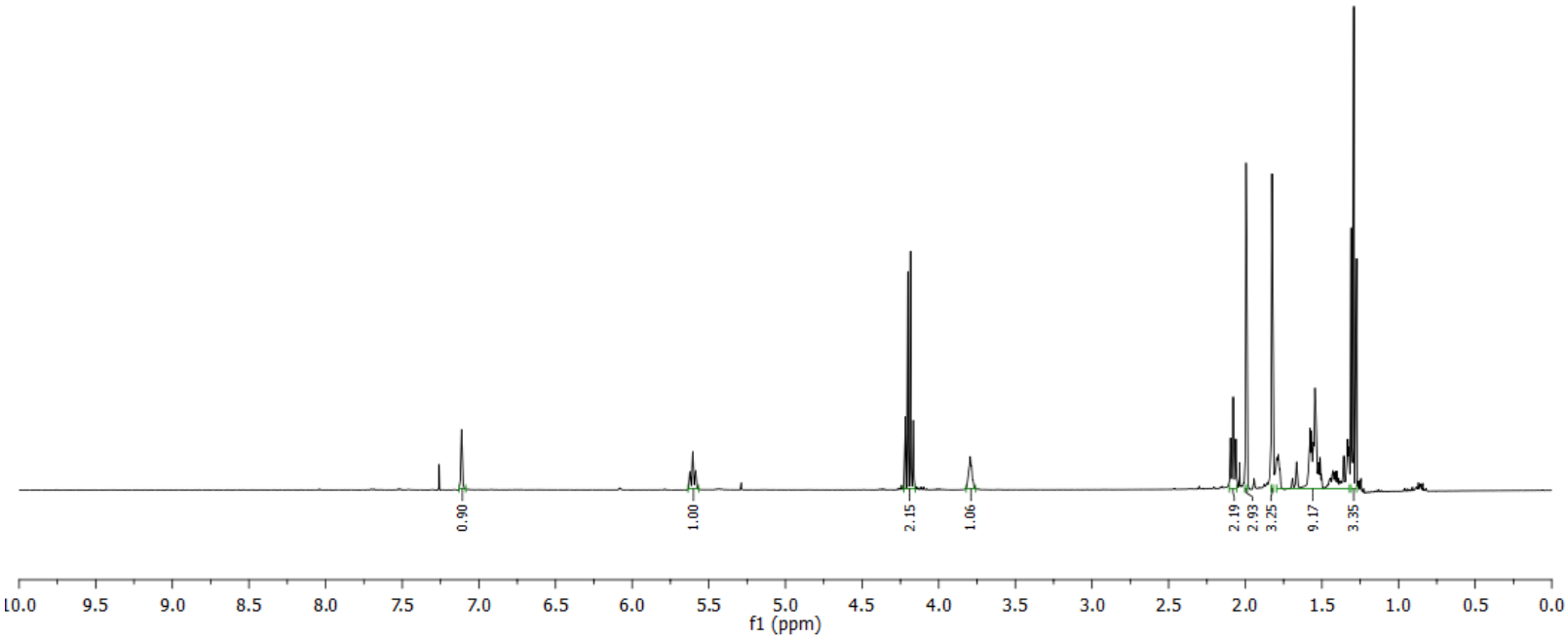

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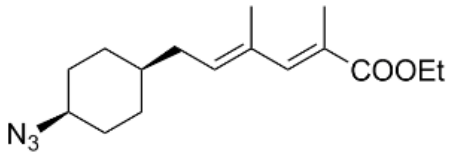

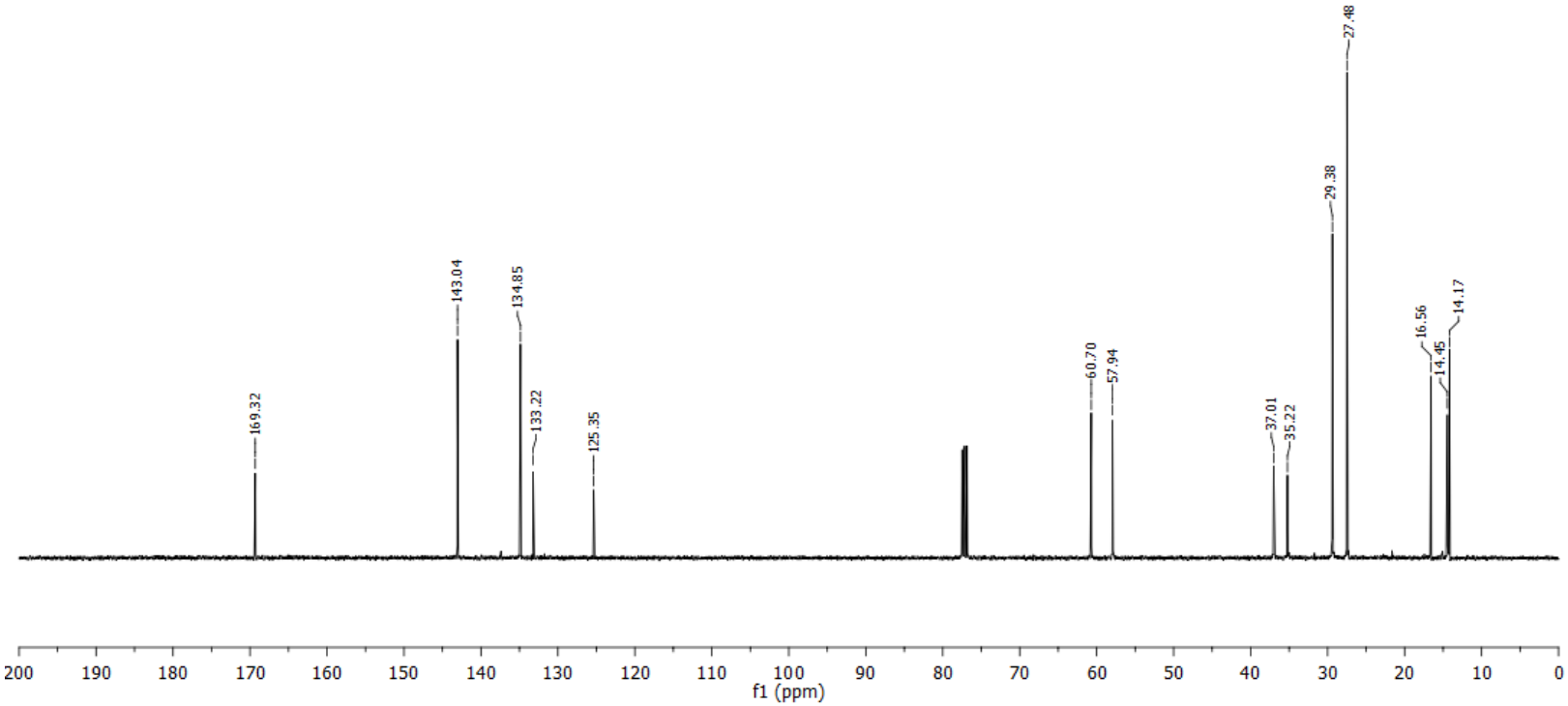

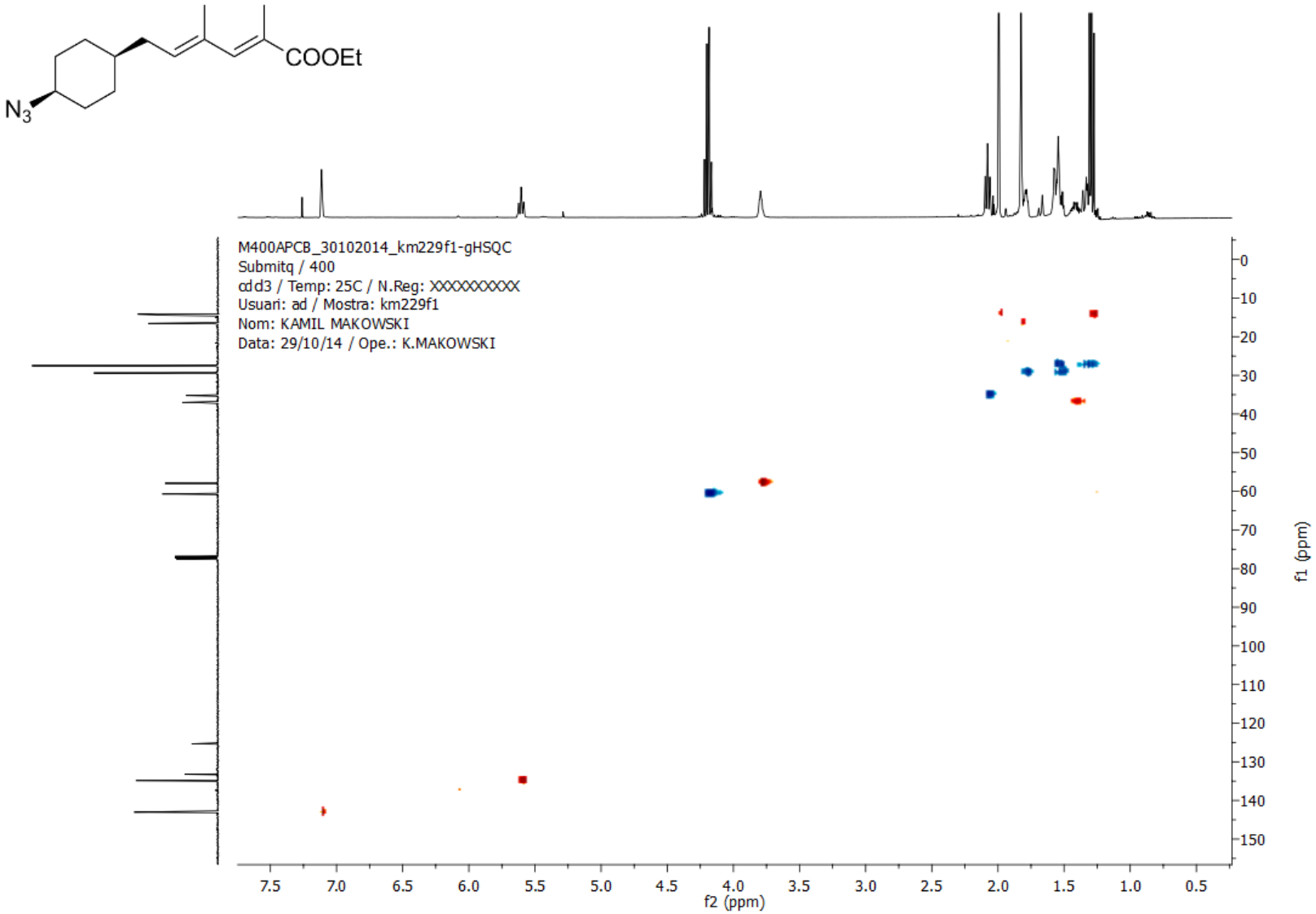
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f2 (ppm)
f1 (ppm)

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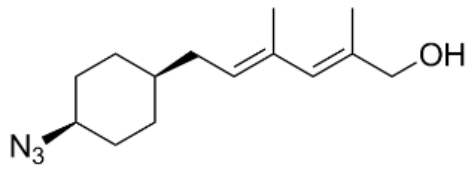

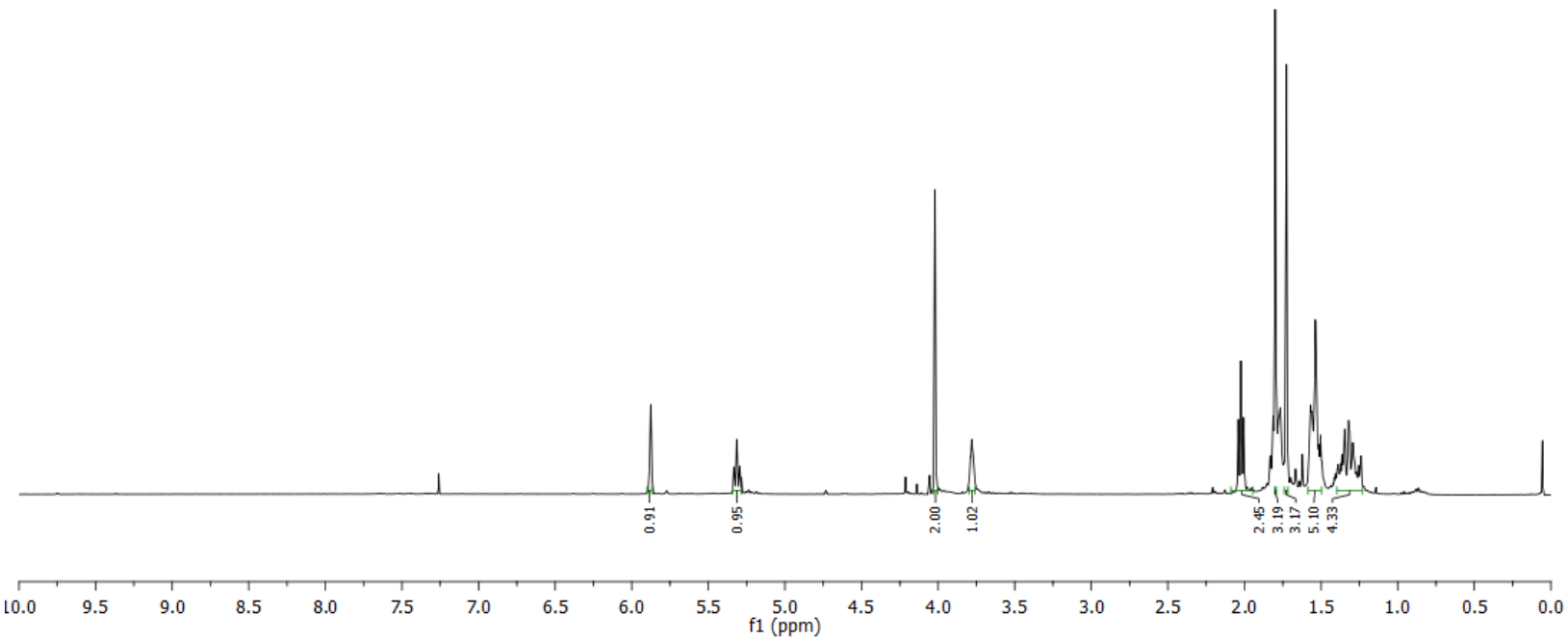

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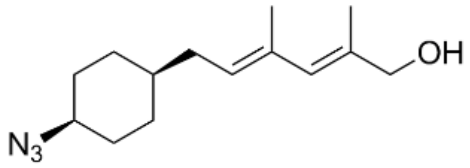

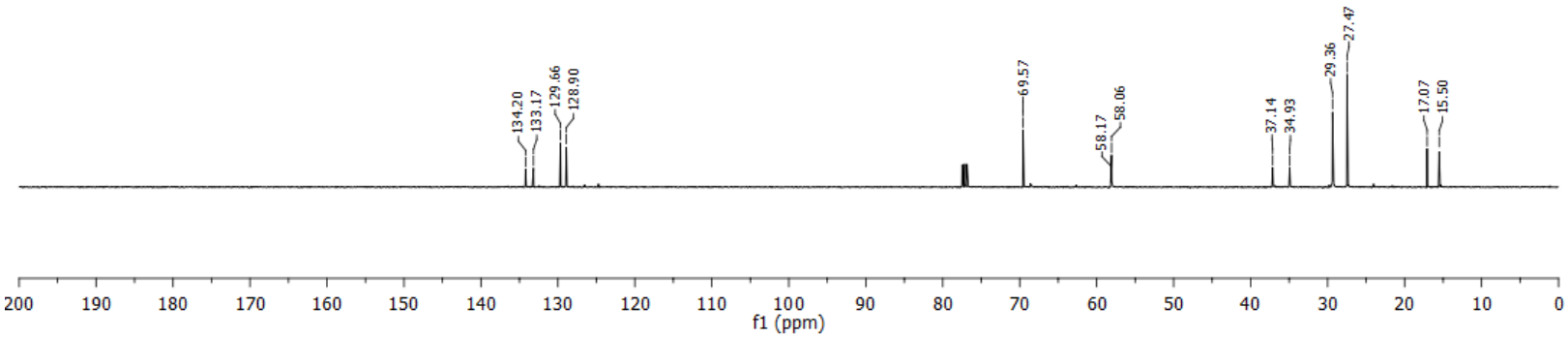

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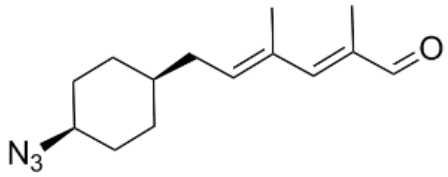
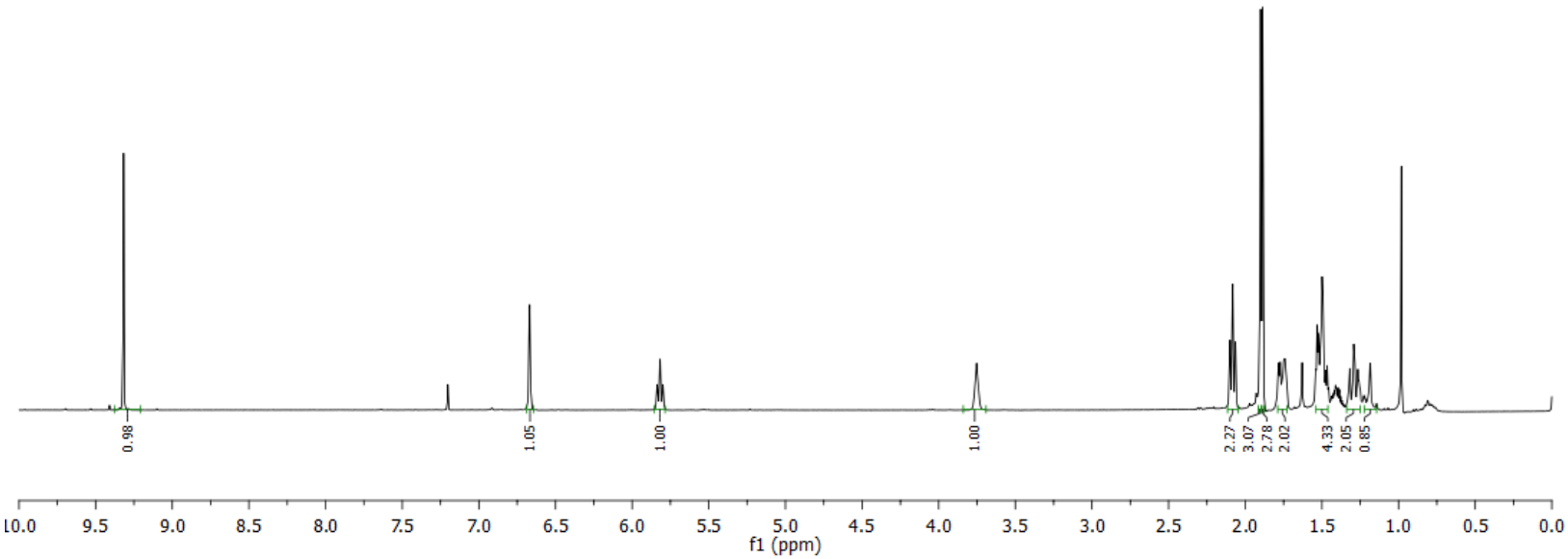
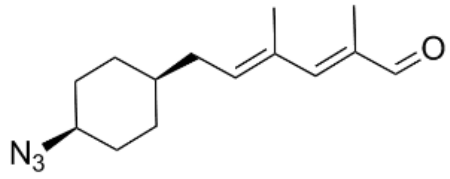
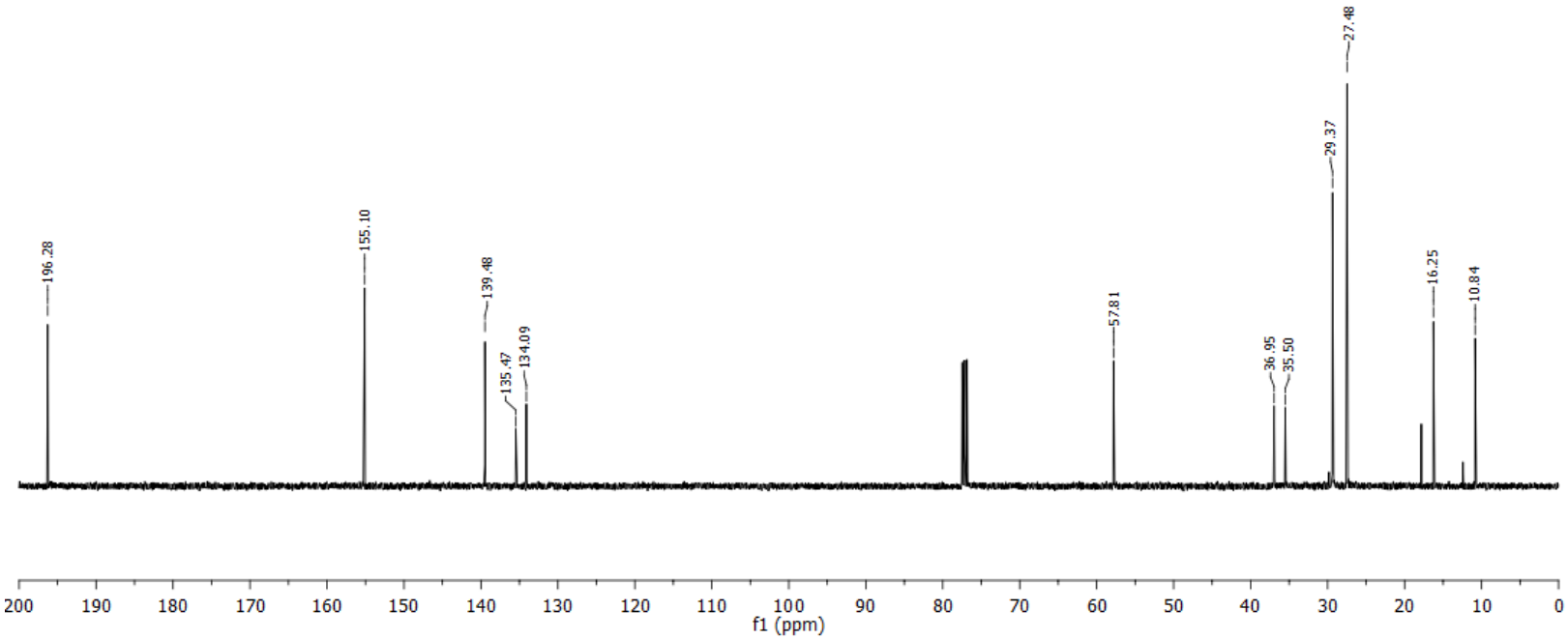
Nom: KAMIL MAKOWSKI
Data: 04/11/14 / Ope.: K.MAKOWSKI



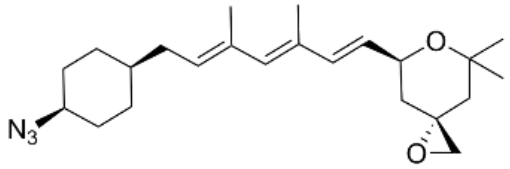


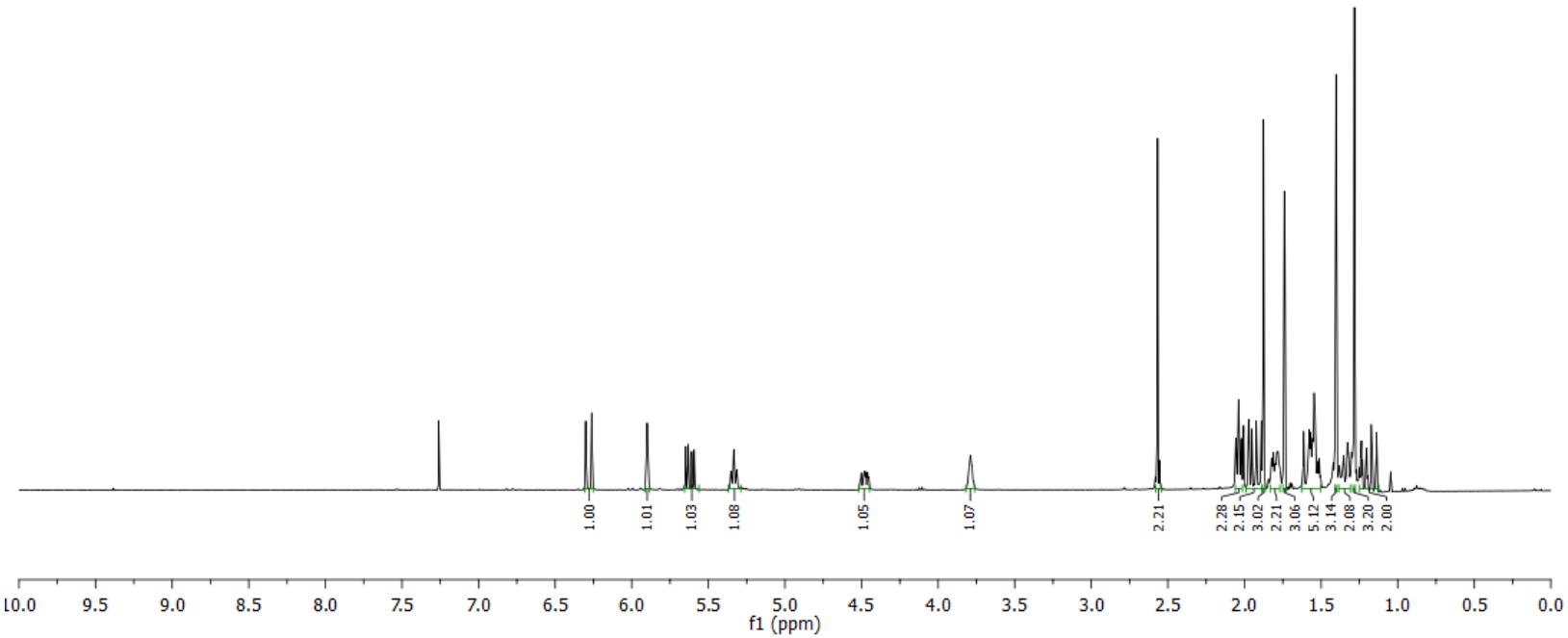
M400APCB_04112014_km235f2-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km235f2
Nom: KAMIL MAKOWSKI
Data: 04/11/14 / Ope.: K.MAKOWSKI



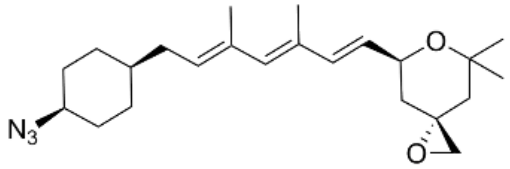


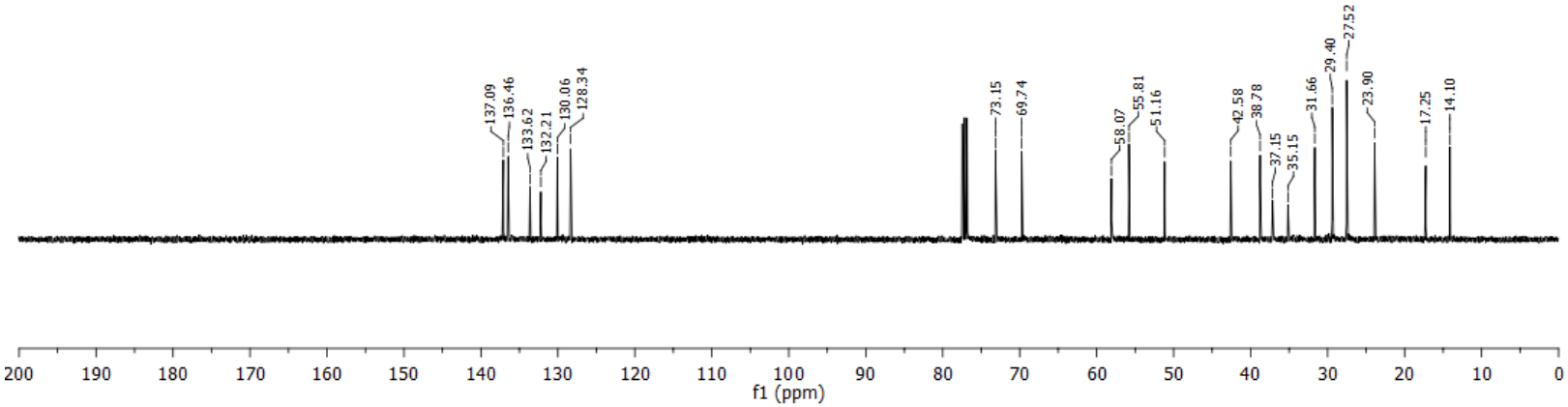
M400APCB_06112014_km236_f2-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km236_f2
Nom: KAMIL MAKOWSKI
Data: 06/11/14 / Ope.: K.MAKOWSKI

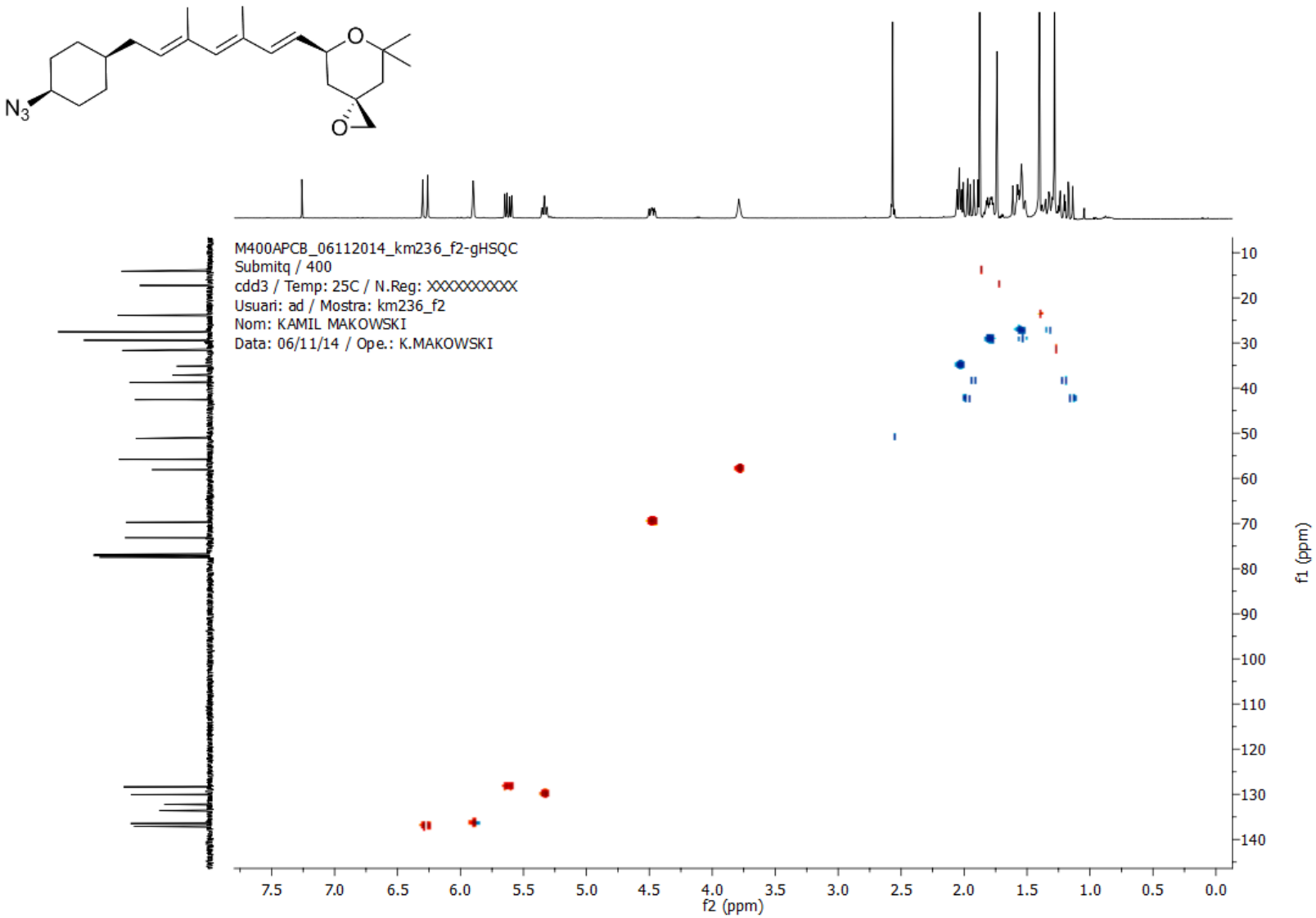




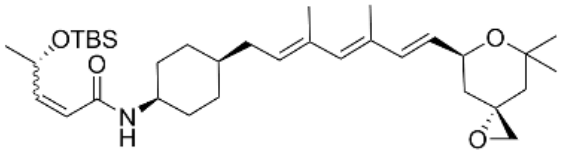
M400APCB_06112014_km236f2-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km236f2
Nom: KAMIL MAKOWSKI
Data: 06/11/14 / Ope.: K.MAKOWSKI



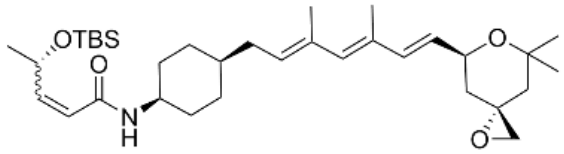


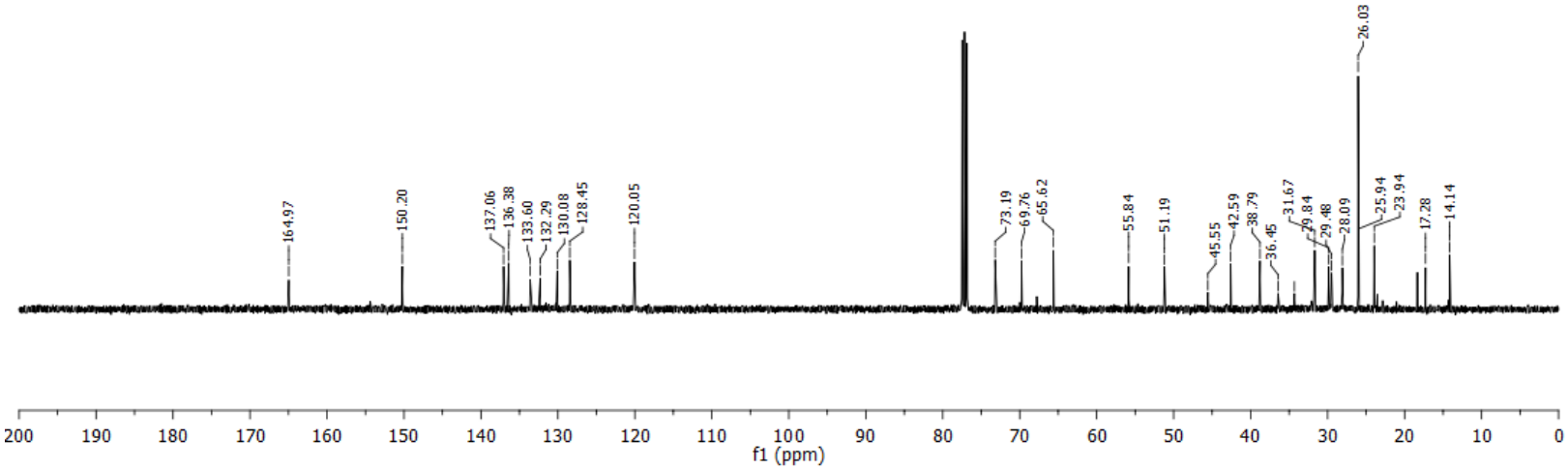

N3
O
O
M400APCB_06112014_km236_f2-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km236_f2
Nom: KAMIL MAKOWSKI
Data: 06/11/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

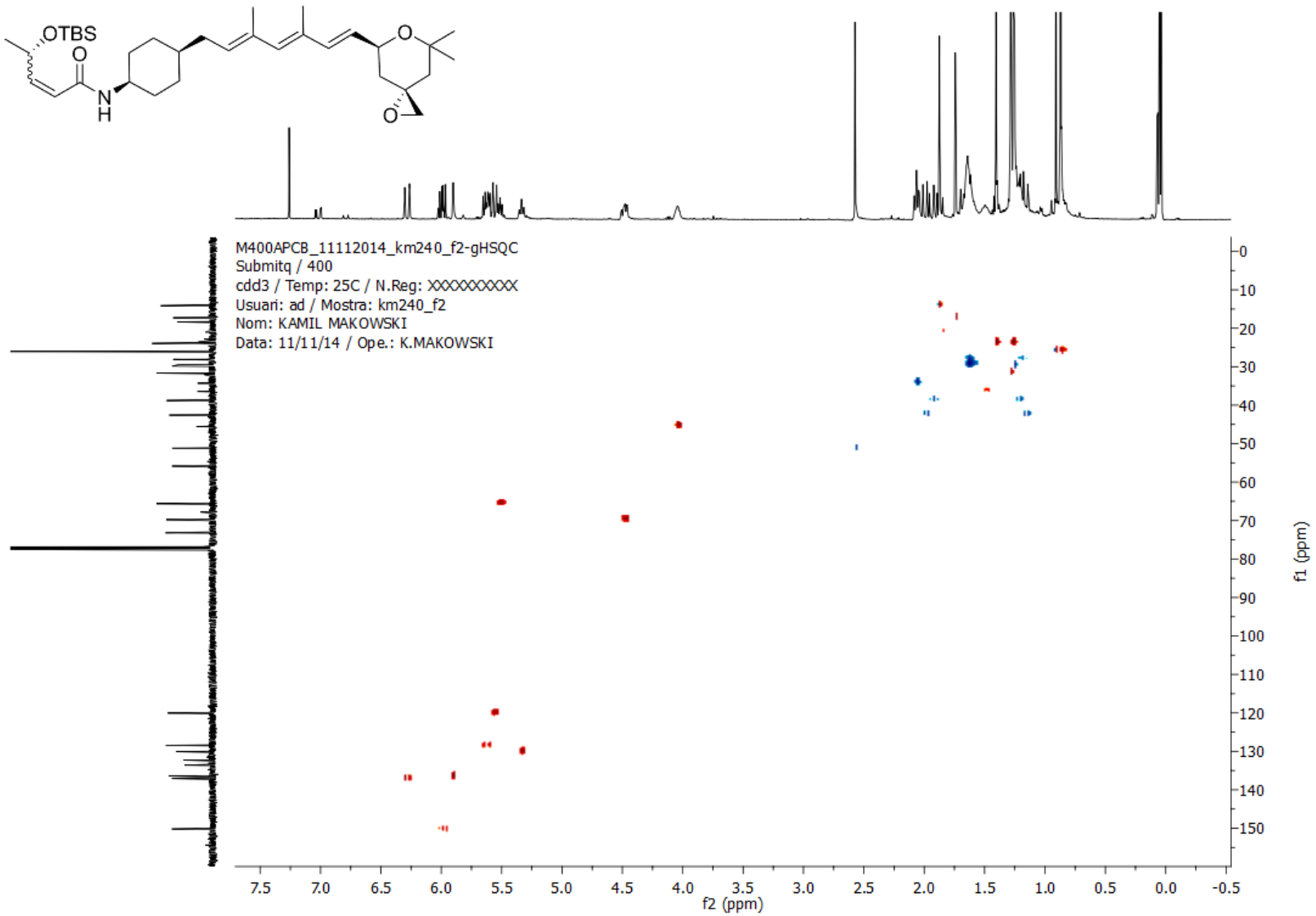
M400APCB_11112014_km240f2-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km240f2
Nom: KAMIL MAKOWSKI
Data: 11/11/14 / Ope.: K.MAKOWSKI

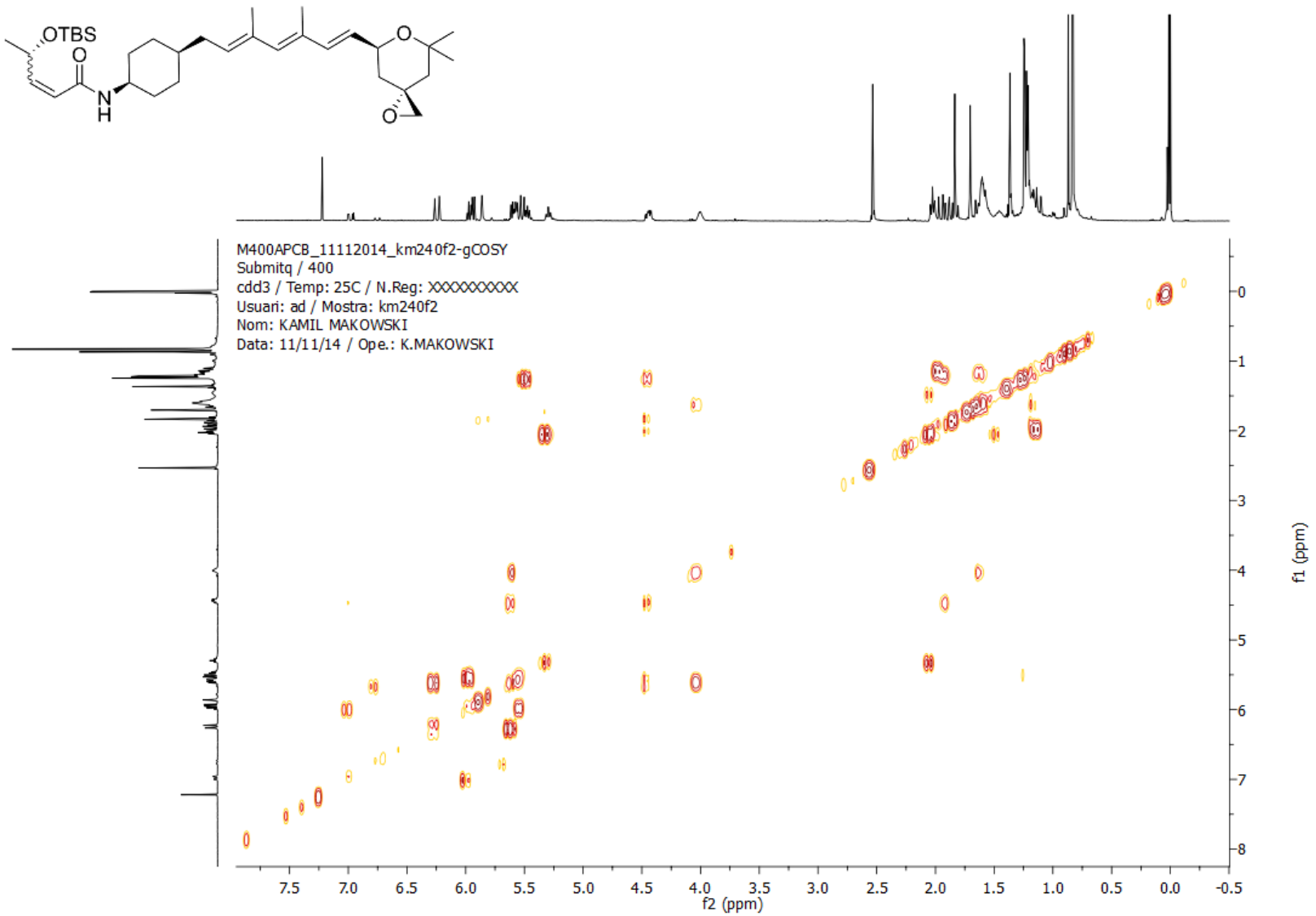


M400APCB_11112014_km240_f2-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km240_f2
Nom: KAMIL MAKOWSKI
Data: 11/11/14 / Ope.: K.MAKOWSKI

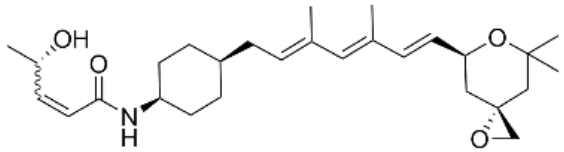


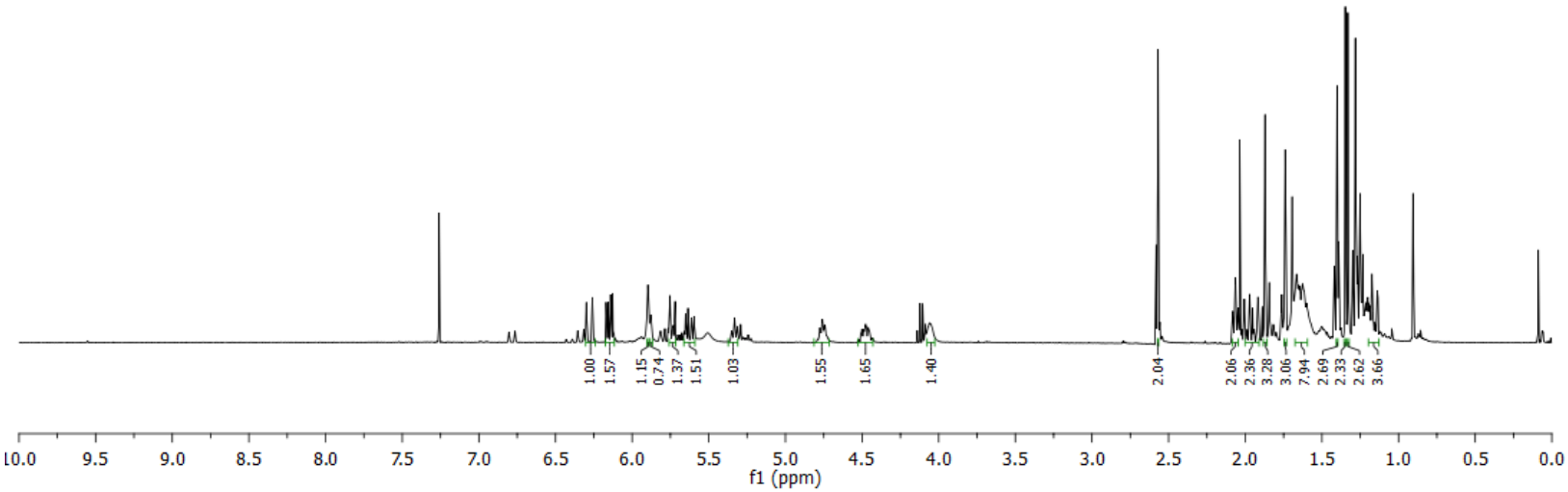



OTBS
O
N
H
O
O
M400APCB_11112014_km240_f2-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km240_f2
Nom: KAMIL MAKOWSKI
Data: 11/11/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

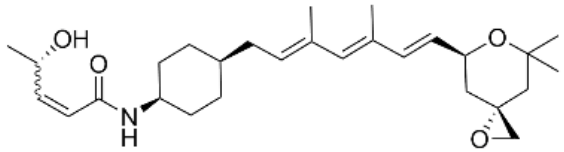

OTBS
O
N
H
O
O
M400APCB_11112014_km240f2-gCOSY
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km240f2
Nom: KAMIL MAKOWSKI
Data: 11/11/14 / Ope.: K.MAKOWSKI
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f2 (ppm)
0
1
2
3
4
5
6
7
8
f1 (ppm)

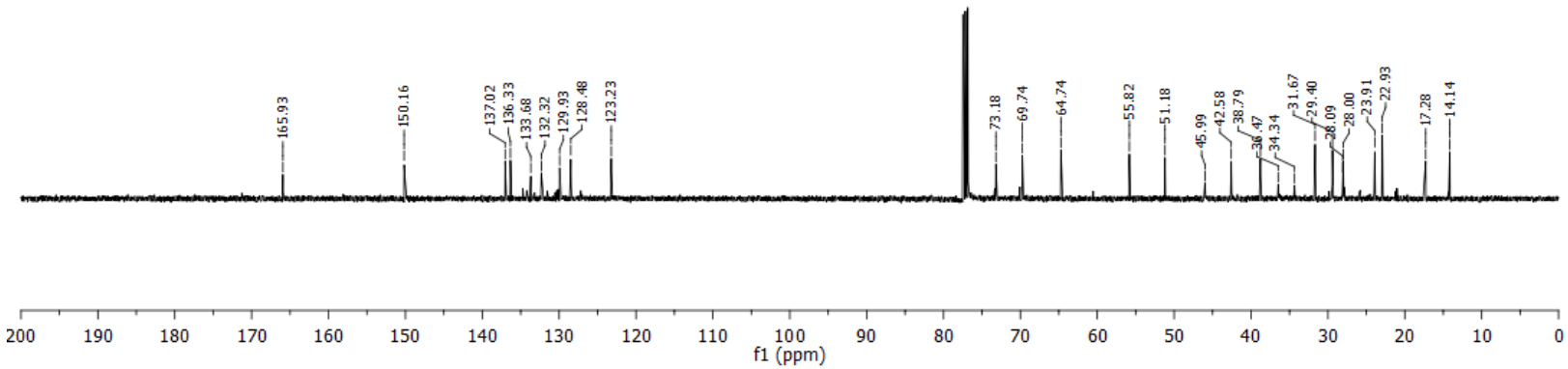
M400APCB_13112014_km241f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km241f1
Nom: KAMIL MAKOWSKI
Data: 13/11/14 / Ope.: K.MAKOWSKI



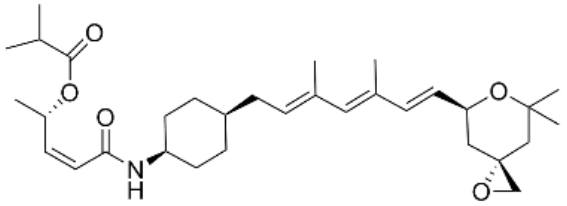


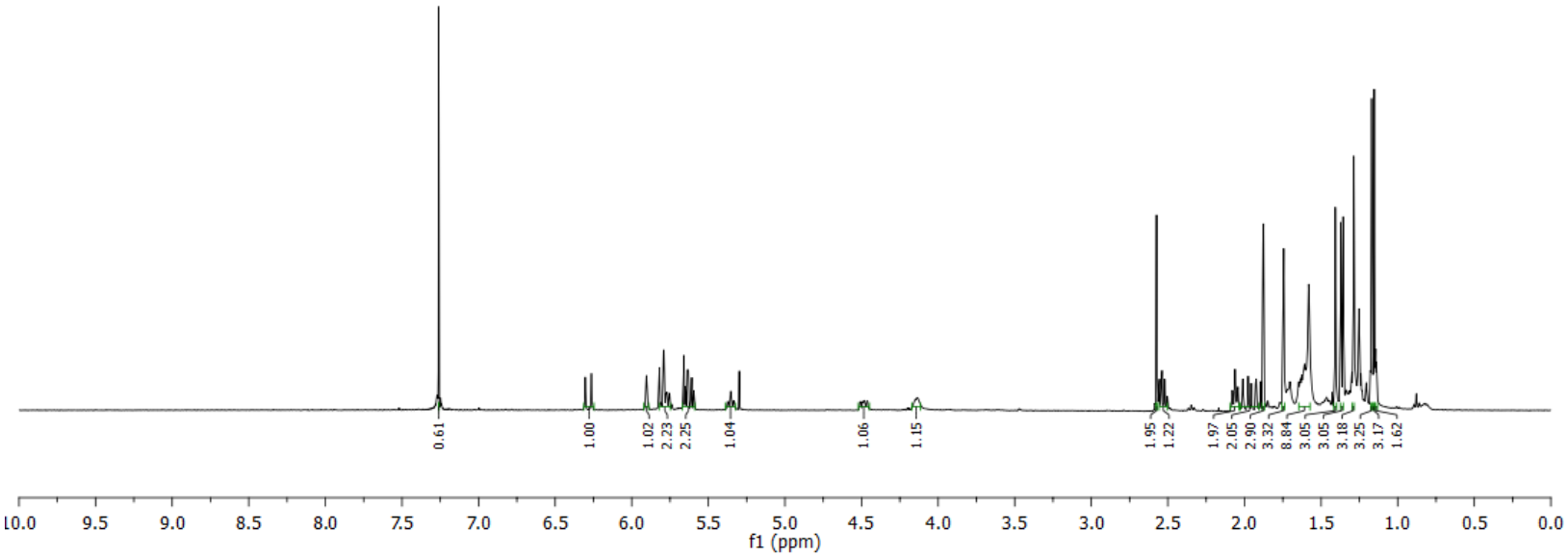
M400APCB_13112014_km241f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km241f1
Nom: KAMIL MAKOWSKI
Data: 13/11/14 / Ope.: K.MAKOWSKI



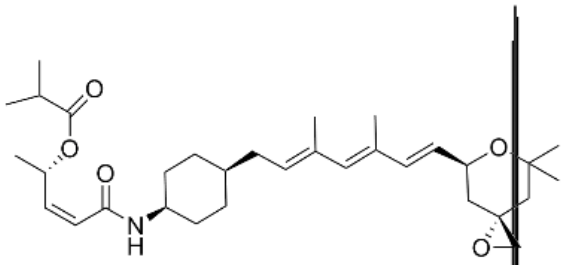


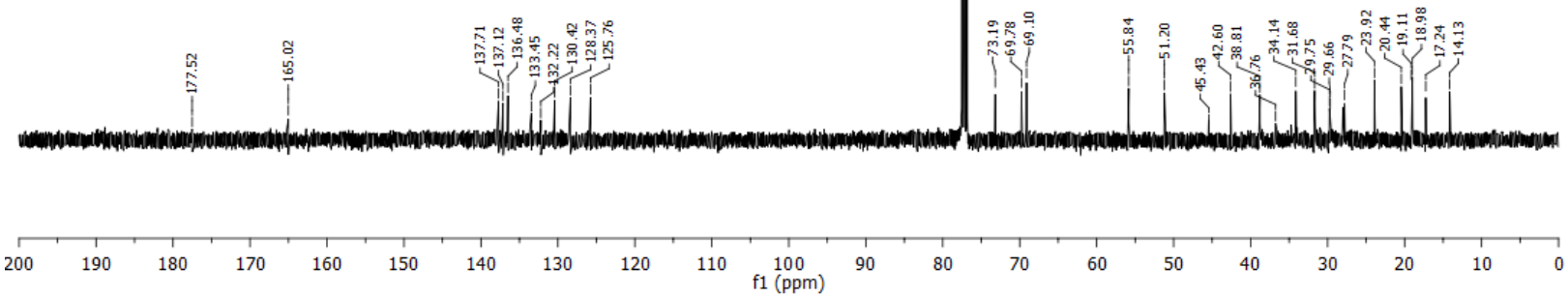
M400APCB_20112014_KM244_PUR_P1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM244_PUR_P1
Nom: KAMIL MAKOWSKI
Data: 20/11/14 / Ope.: K.MAKOWSKI

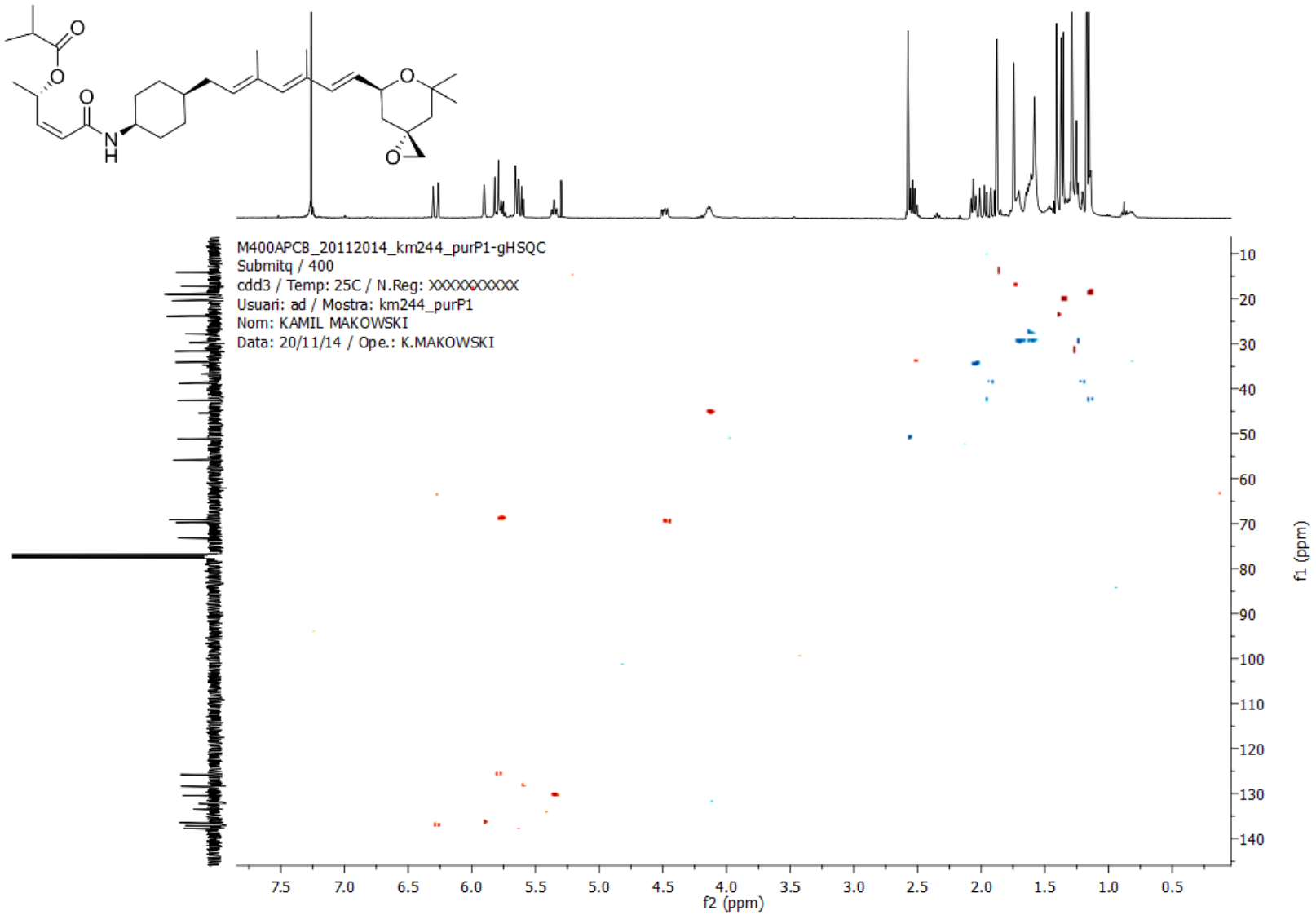


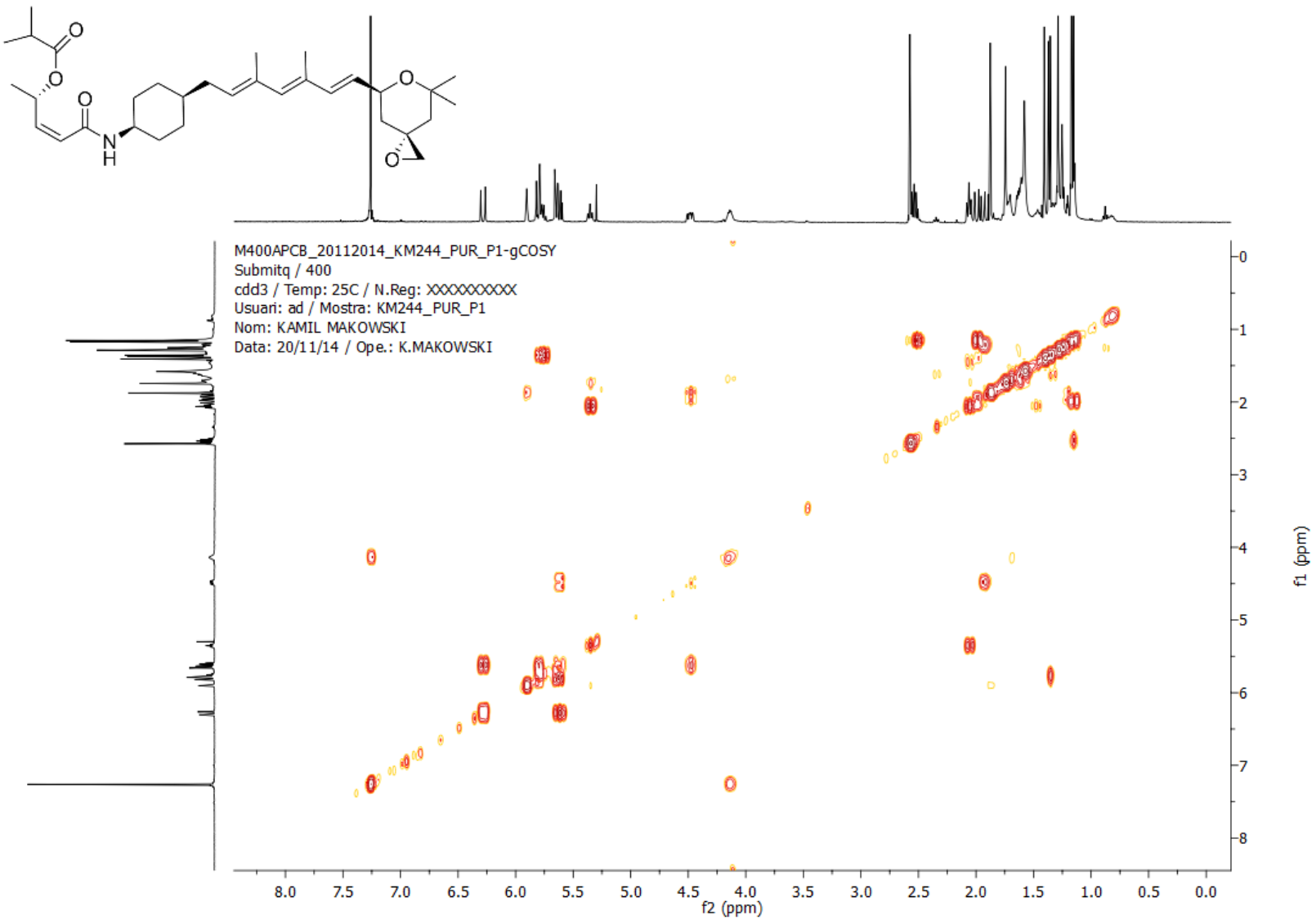


M400APCB_20112014_km244_purP1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km244_purP1
Nom: KAMIL MAKOWSKI
Data: 20/11/14 / Ope.: K.MAKOWSKI

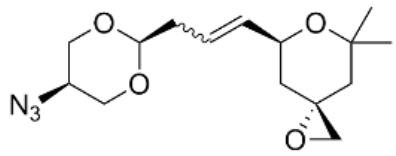




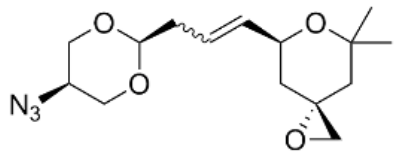

M400APCB_20112014_km244_purP1-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km244_purP1
Nom: KAMIL MAKOWSKI
Data: 20/11/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

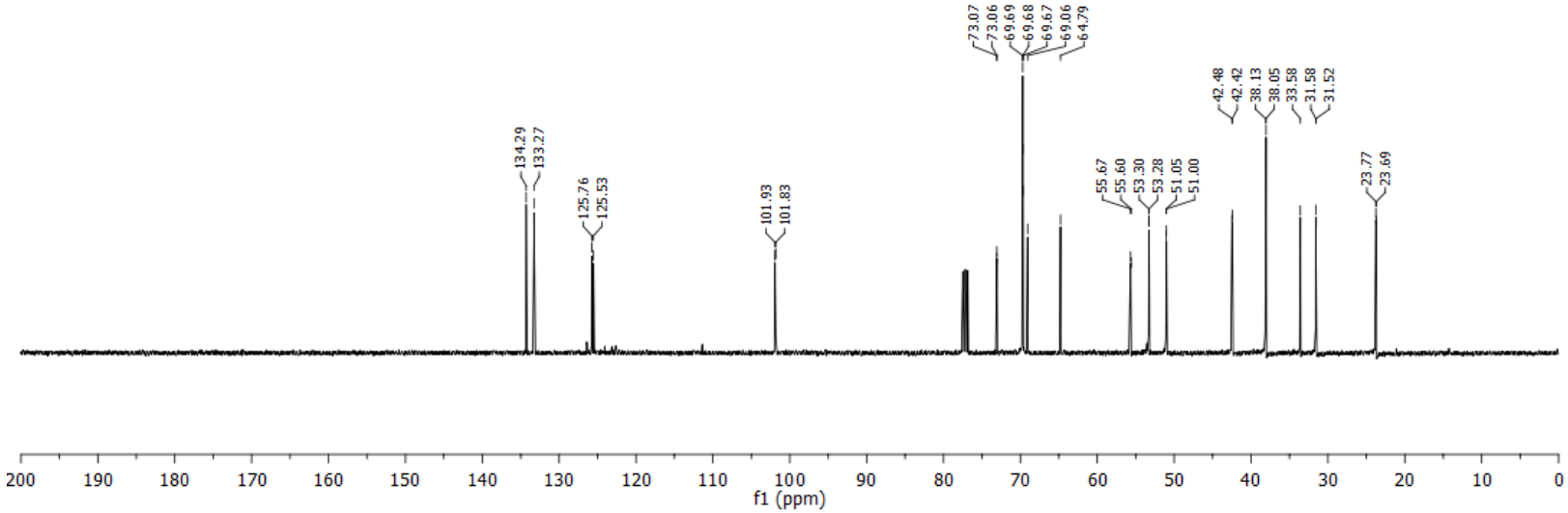

M400APCB_20112014_KM244_PUR_P1-gCOSY
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM244_PUR_P1
Nom: KAMIL MAKOWSKI
Data: 20/11/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

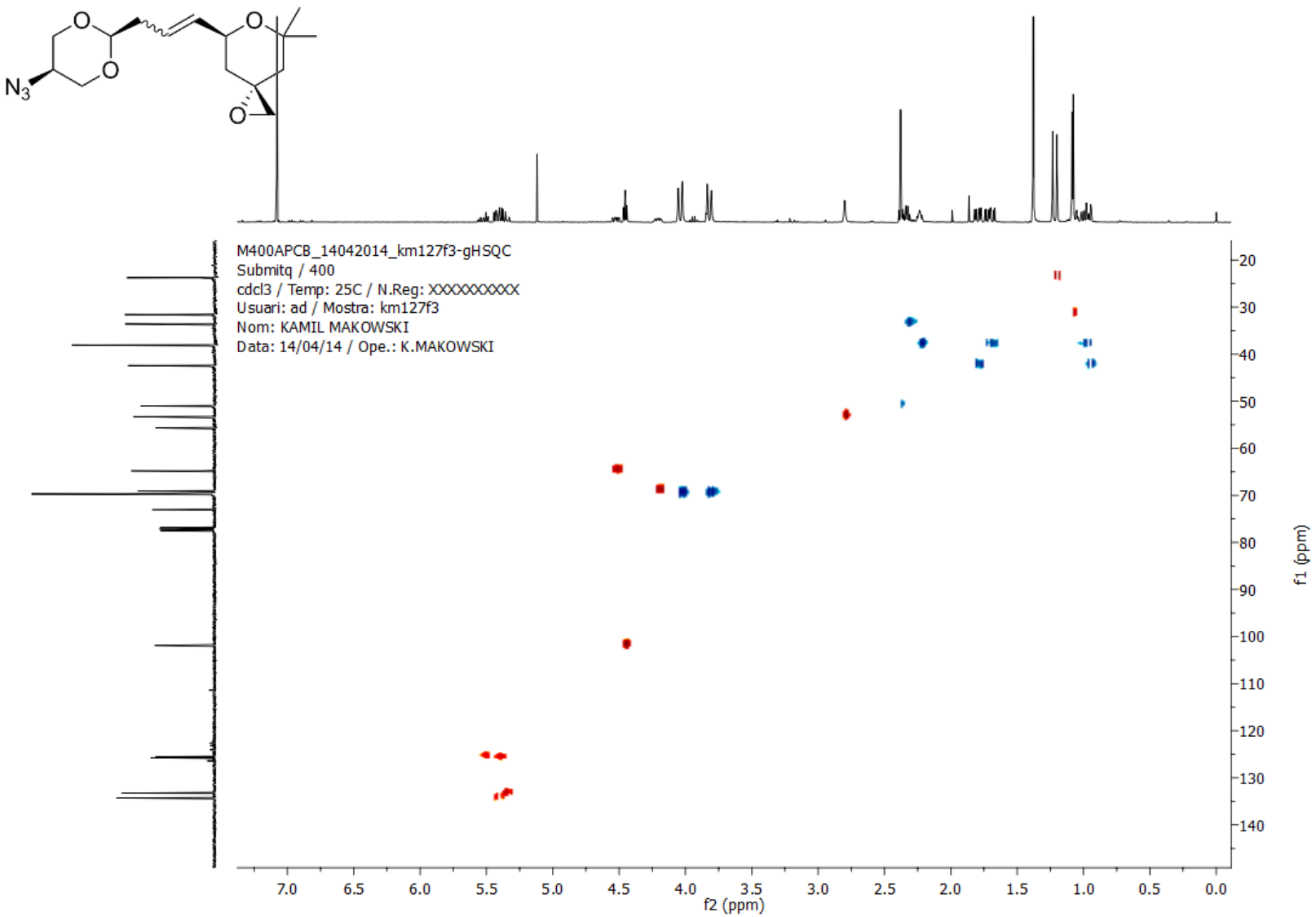
M400APCB_10042014_KM127f3-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM127f3
Nom: KAMIL MAKOWSKI
Data: 10/04/14 / Ope.: K.MAKOWSKI



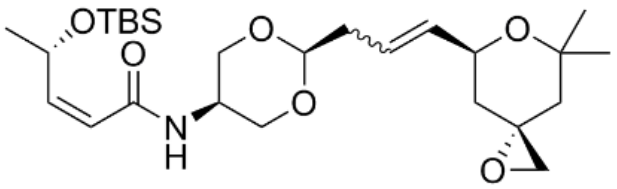
M400APCB_14042014_km127f3-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km127f3
Nom: KAMIL MAKOWSKI
Data: 14/04/14 / Ope.: K.MAKOWSKI



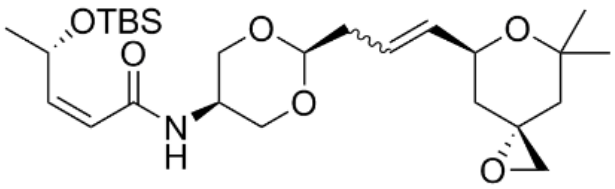


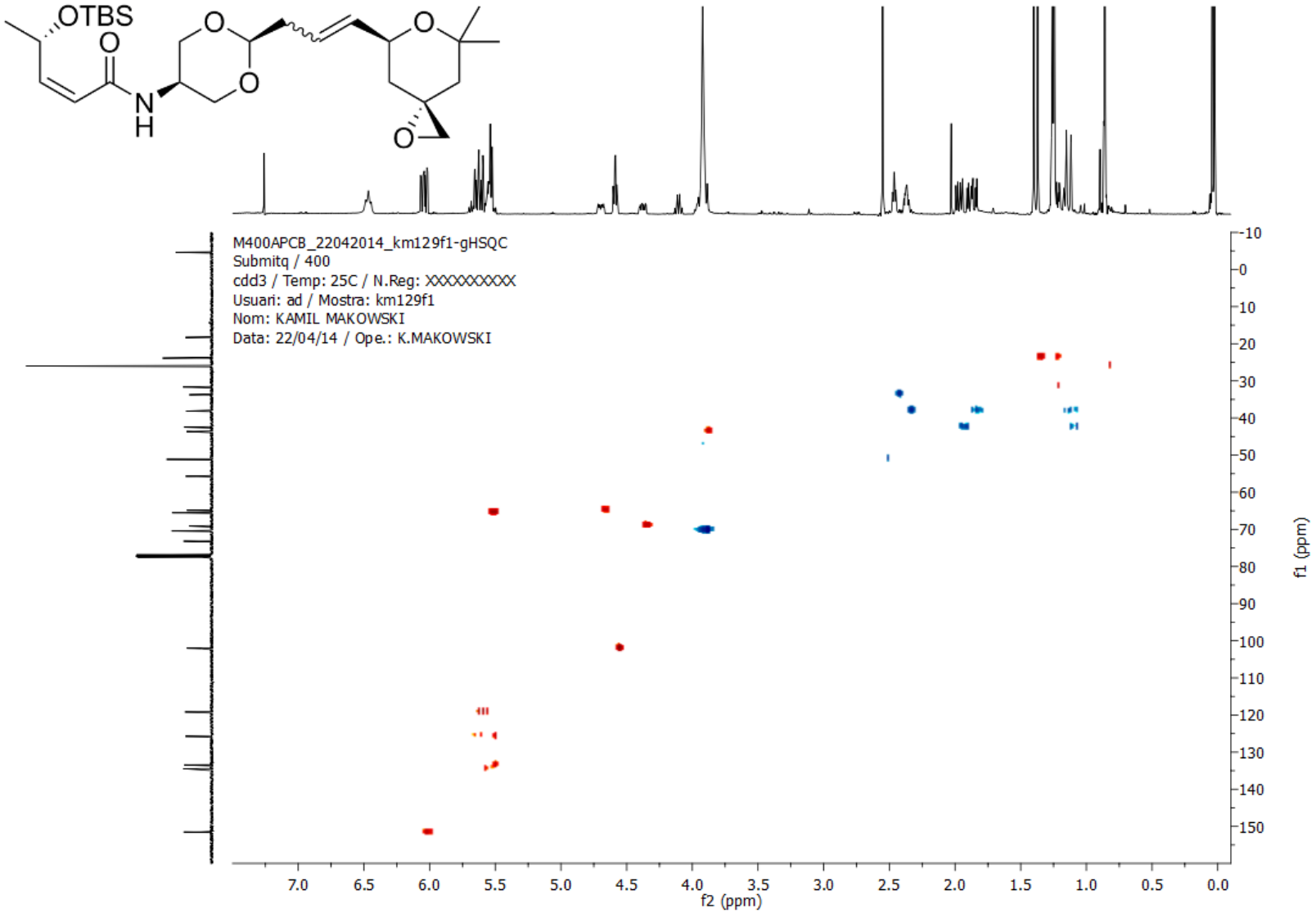

M400APCB_14042014_km127f3-gHSQC
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km127f3
Nom: KAMIL MAKOWSKI
Data: 14/04/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

M400APCB_22042014_km129f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km129f1
Nom: KAMIL MAKOWSKI
Data: 22/04/14 / Ope.: K.MAKOWSKI

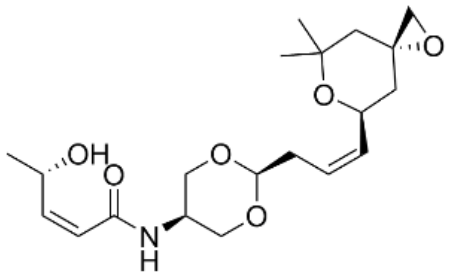


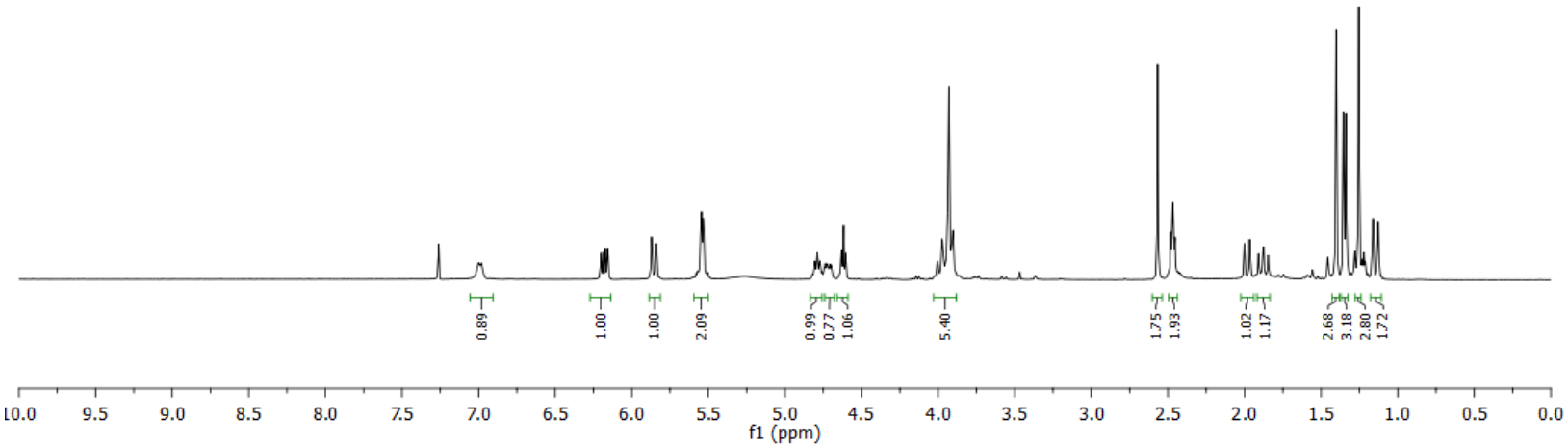
M400APCB_22042014_km129f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km129f1
Nom: KAMIL MAKOWSKI
Data: 22/04/14 / Ope.: K.MAKOWSKI



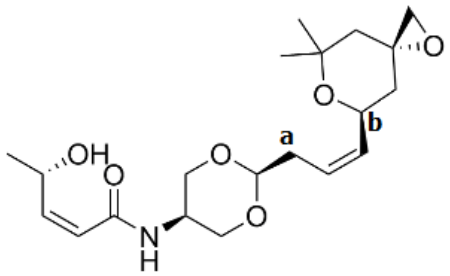

OTBS
O
N
H
O
O
O
O
M400APCB_22042014_km129f1-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km129f1
Nom: KAMIL MAKOWSKI
Data: 22/04/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

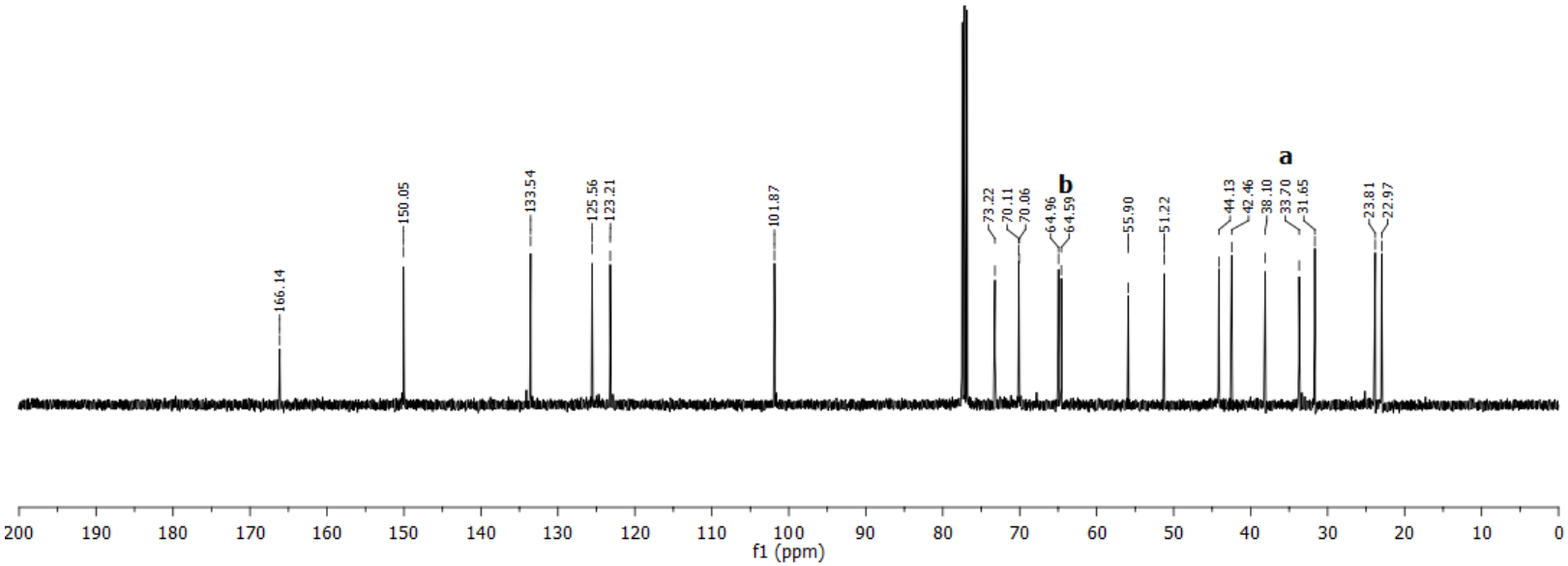
M400APCB_26052014_km133_semi_1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km133_semi_1
Nom: KAMIL MAKOWSKI
Data: 26/05/14 / Ope.: K.MAKOWSKI

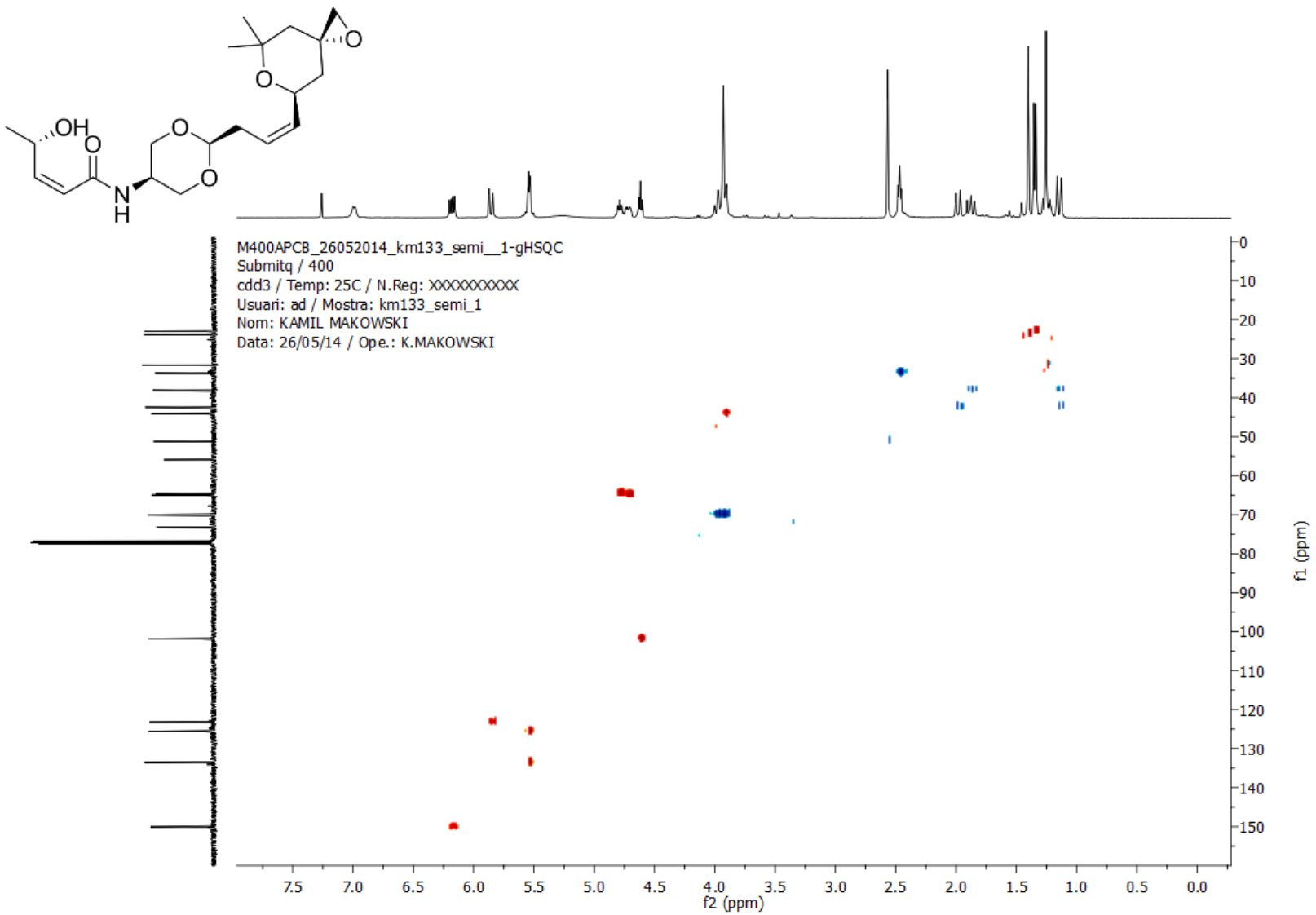




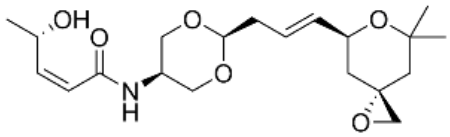
M400APCB_26052014_km133_semi__1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km133_semi_1
Nom: KAMIL MAKOWSKI
Data: 26/05/14 / Ope.: K.MAKOWSKI

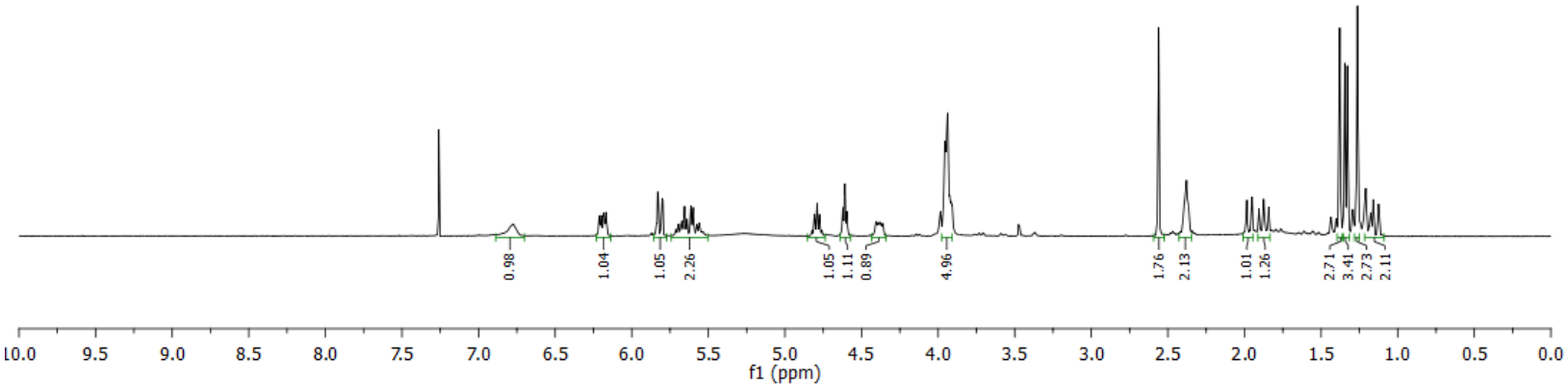




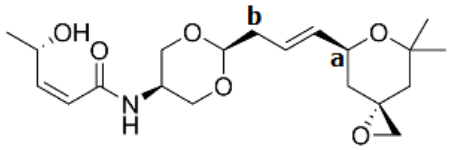

M400APCB_26052014_km133_semi__1-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km133_semi_1
Nom: KAMIL MAKOWSKI
Data: 26/05/14 / Ope.: K.MAKOWSKI
OH
O
O
O
O
N
H
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

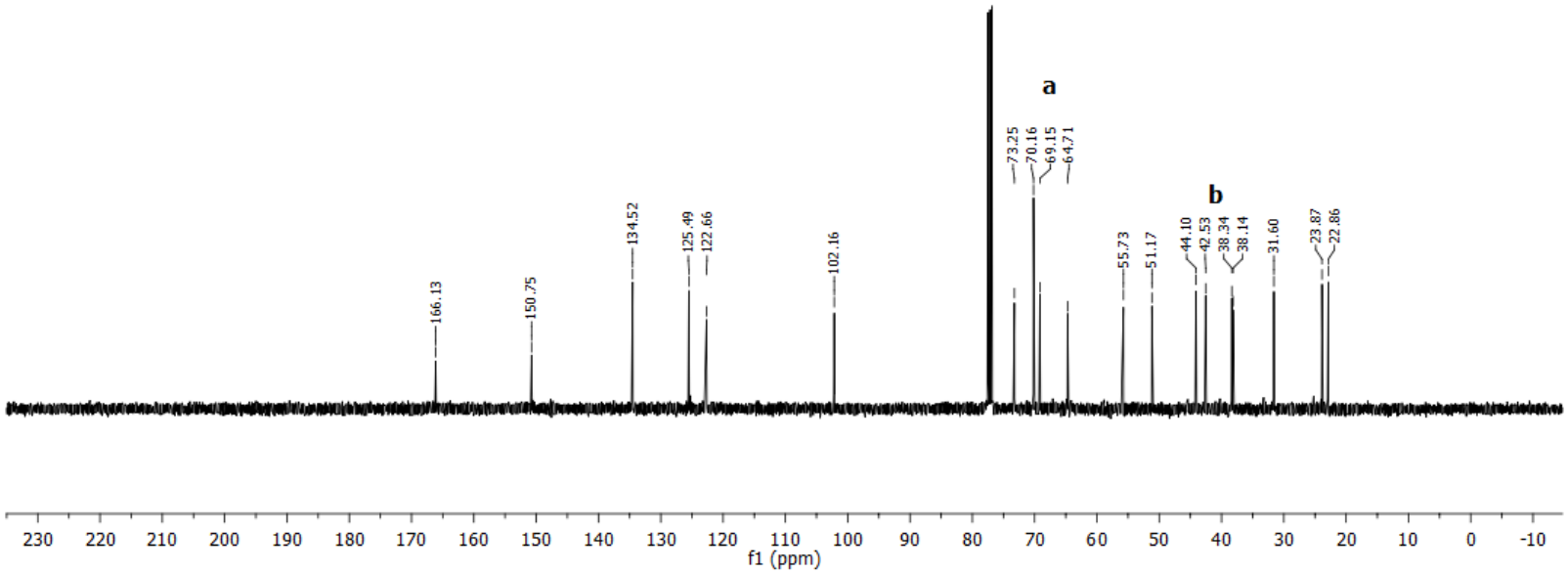
M400APCB_26052014_km133_semi_2-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km133_semi_2
Nom: KAMIL MAKOWSKI
Data: 26/05/14 / Ope.: K.MAKOWSKI

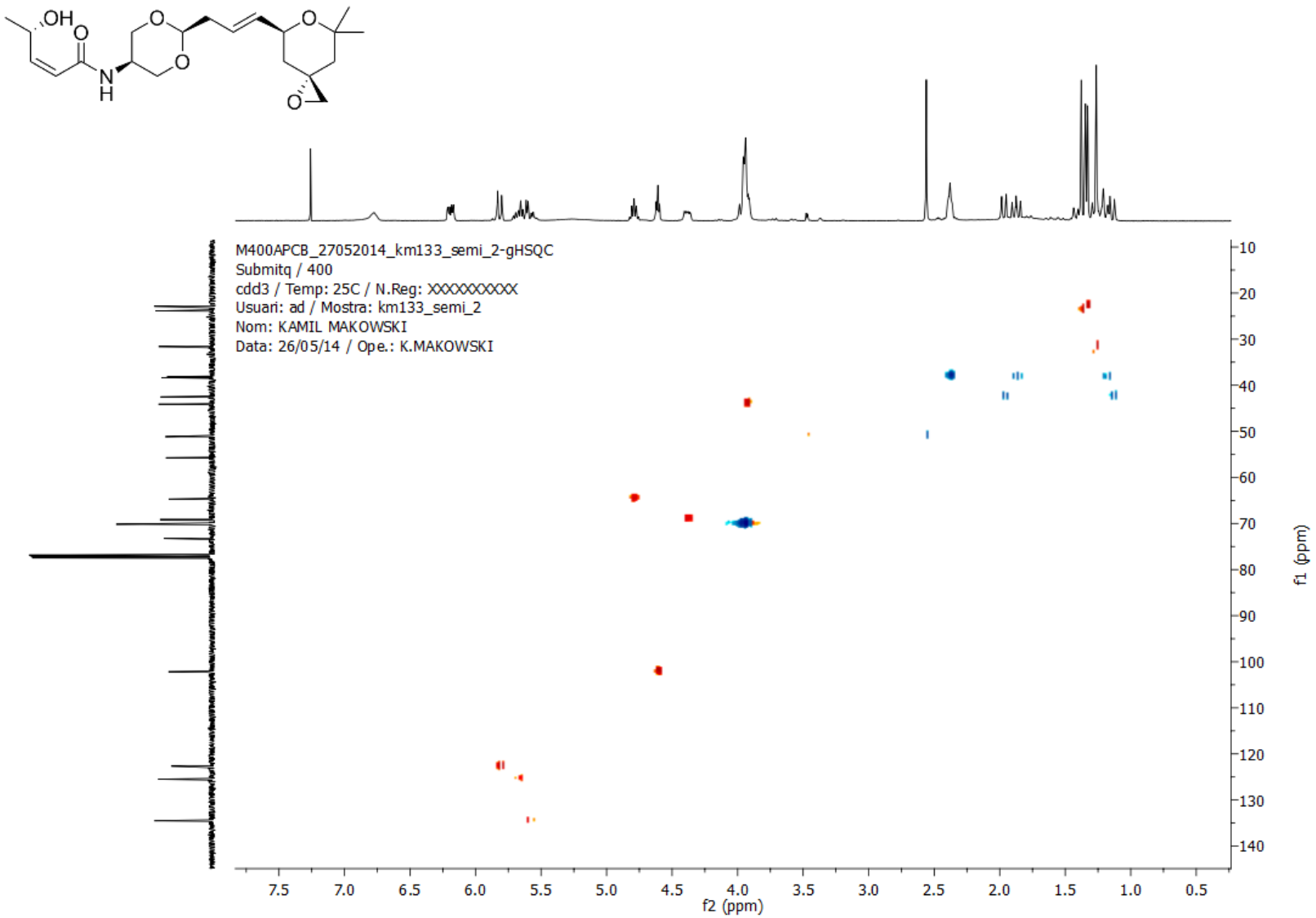




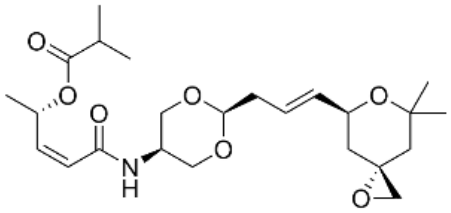
M400APCB_27052014_km133_semi_2-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km133_semi_2
Nom: KAMIL MAKOWSKI
Data: 26/05/14 / Ope.: K.MAKOWSKI

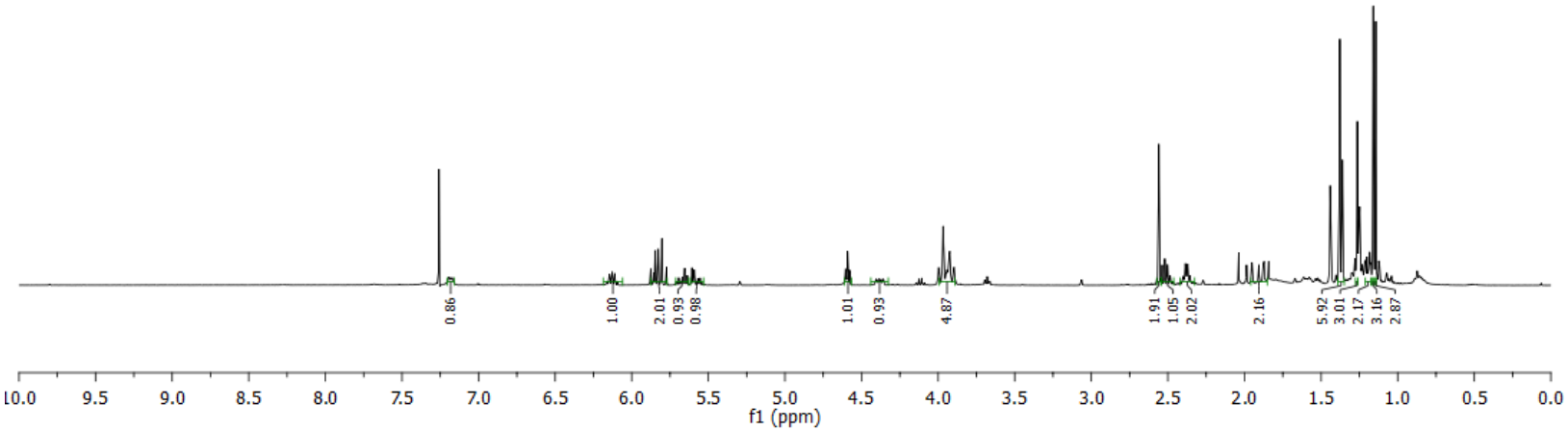




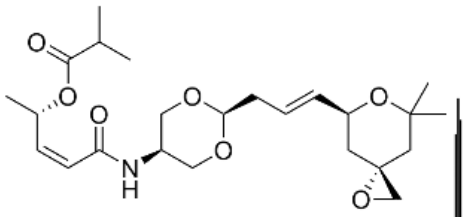

OH
O
N
H
O
O
O
O
M400APCB_27052014_km133_semi_2-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km133_semi_2
Nom: KAMIL MAKOWSKI
Data: 26/05/14 / Ope.: K.MAKOWSKI
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)

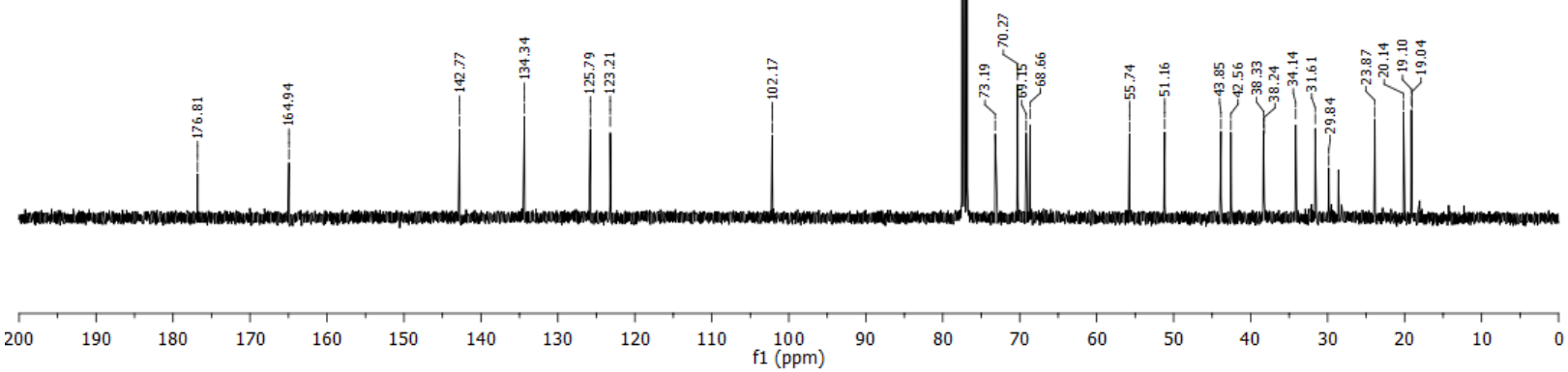
M400APCB_28052014_km145f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km145f1
Nom: KAMIL MAKOWSKI
Data: 28/05/14 / Ope.: K.MAKOWSKI

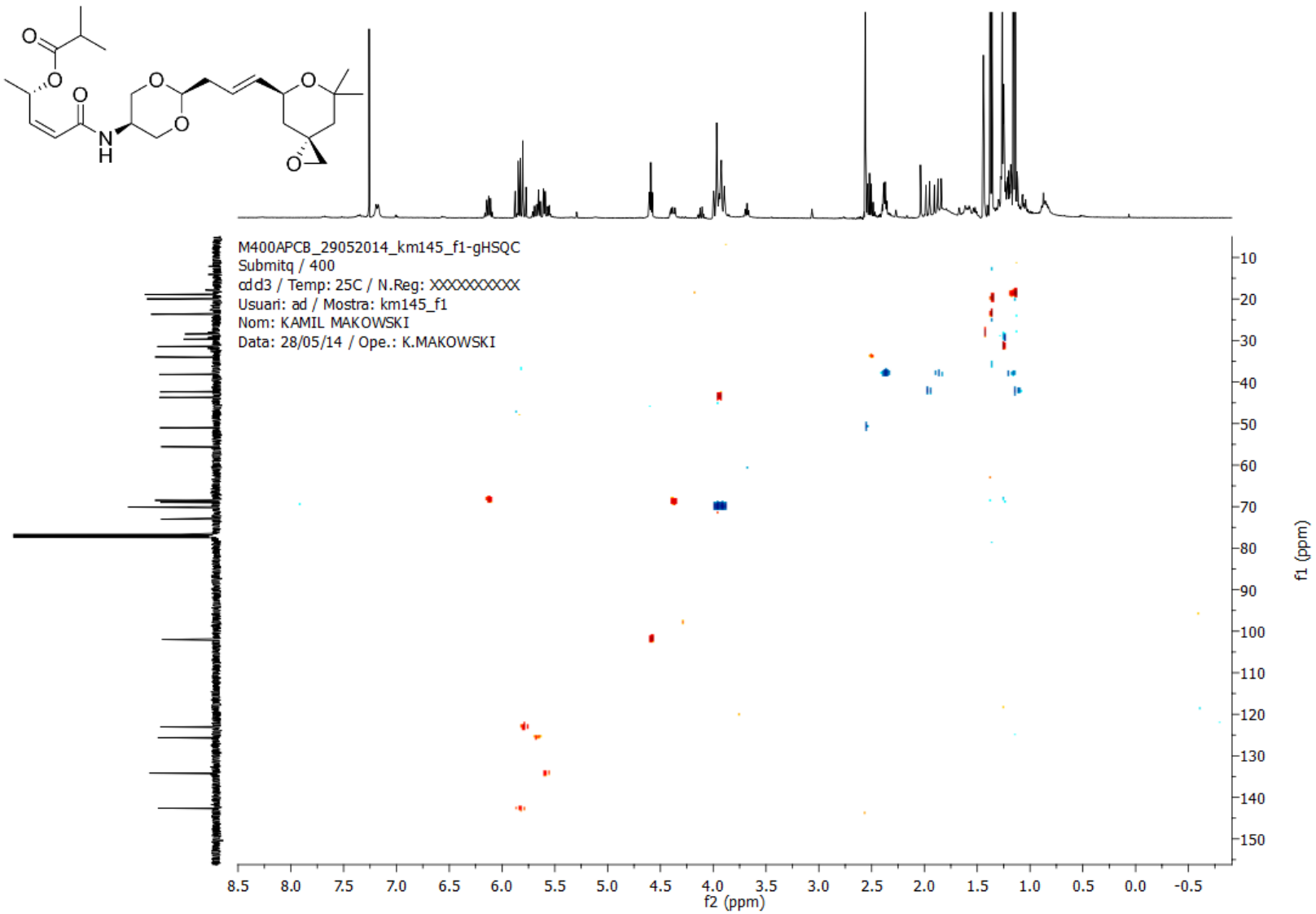




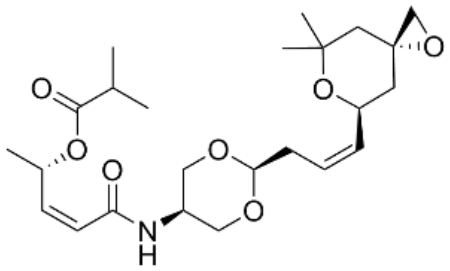
M400APCB_29052014_km145_f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km145_f1
Nom: KAMIL MAKOWSKI
Data: 28/05/14 / Ope.: K.MAKOWSKI

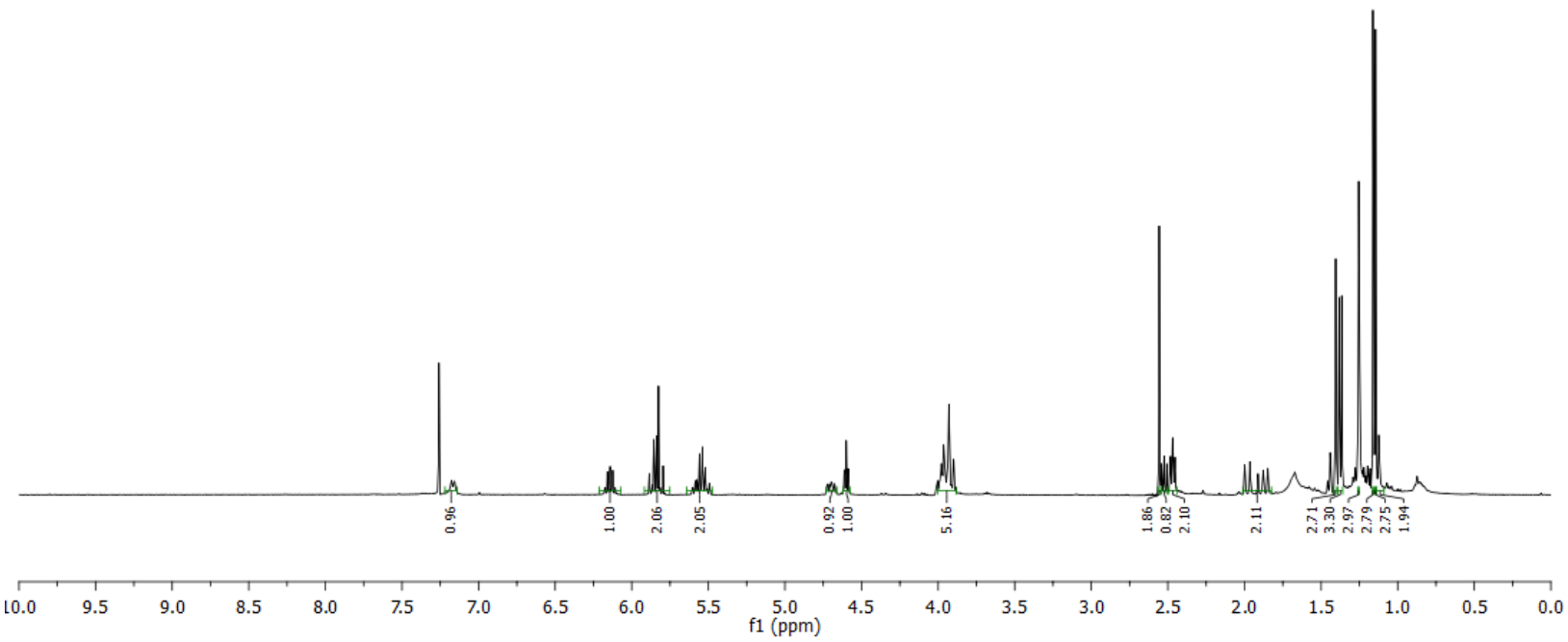




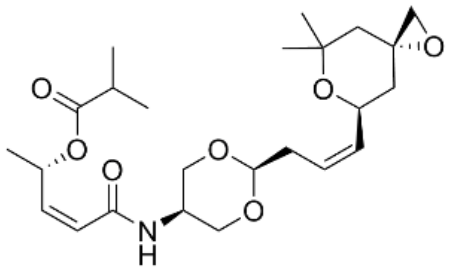

M400APCB_29052014_km145_f1-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km145_f1
Nom: KAMIL MAKOWSKI
Data: 28/05/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

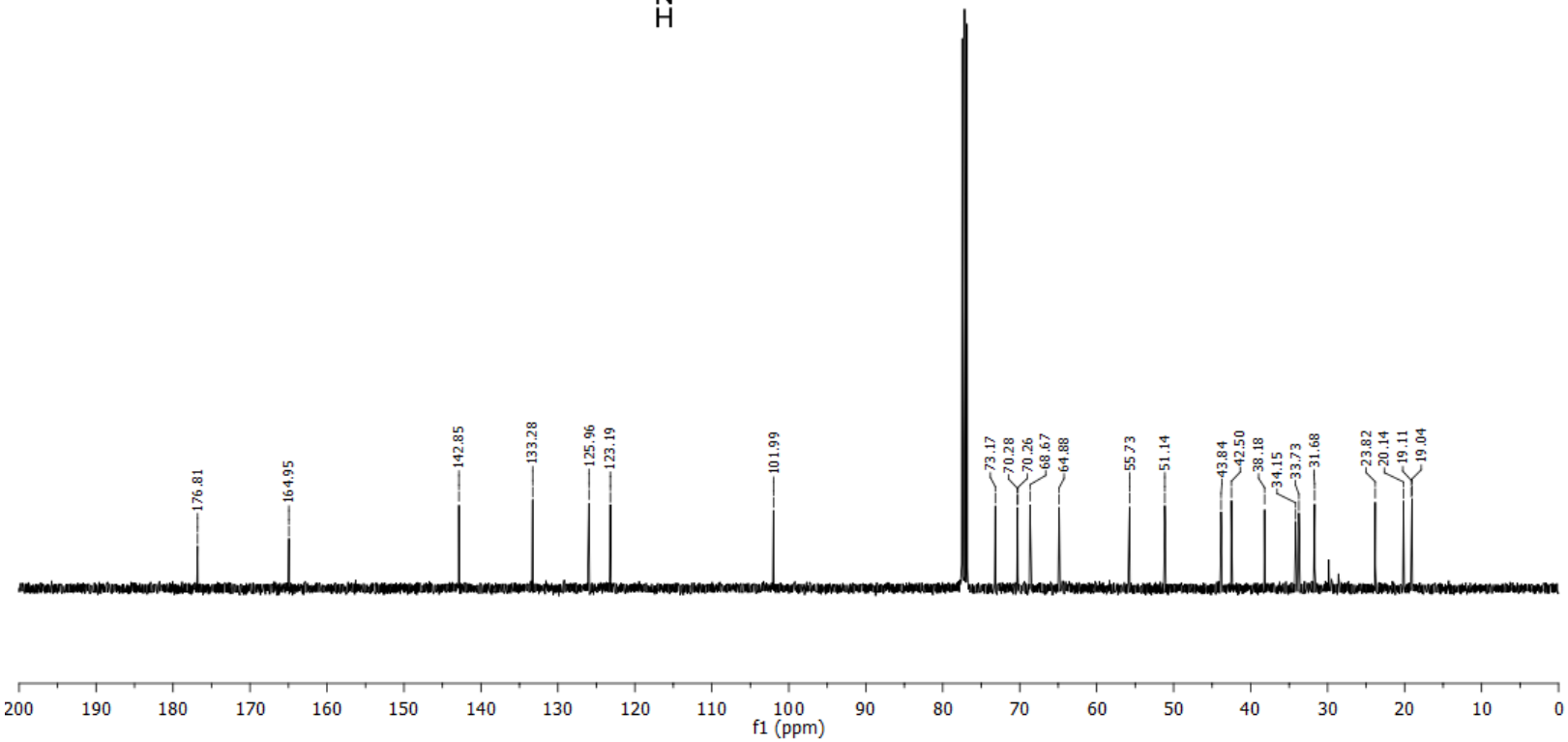
M400APCB_29052014_km146f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km146f1
Nom: KAMIL MAKOWSKI
Data: 29/05/14 / Ope.: K.MAKOWSKI

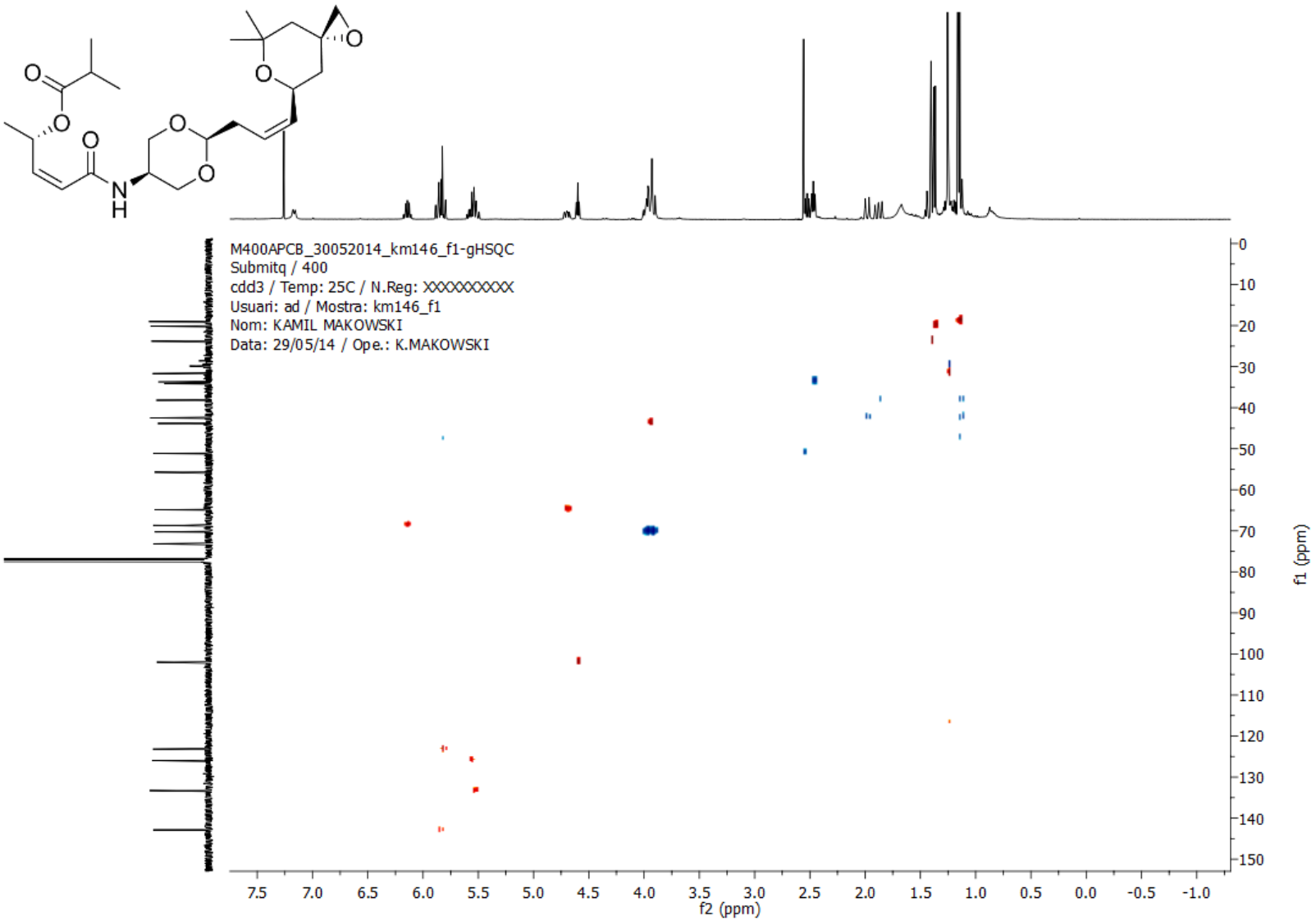


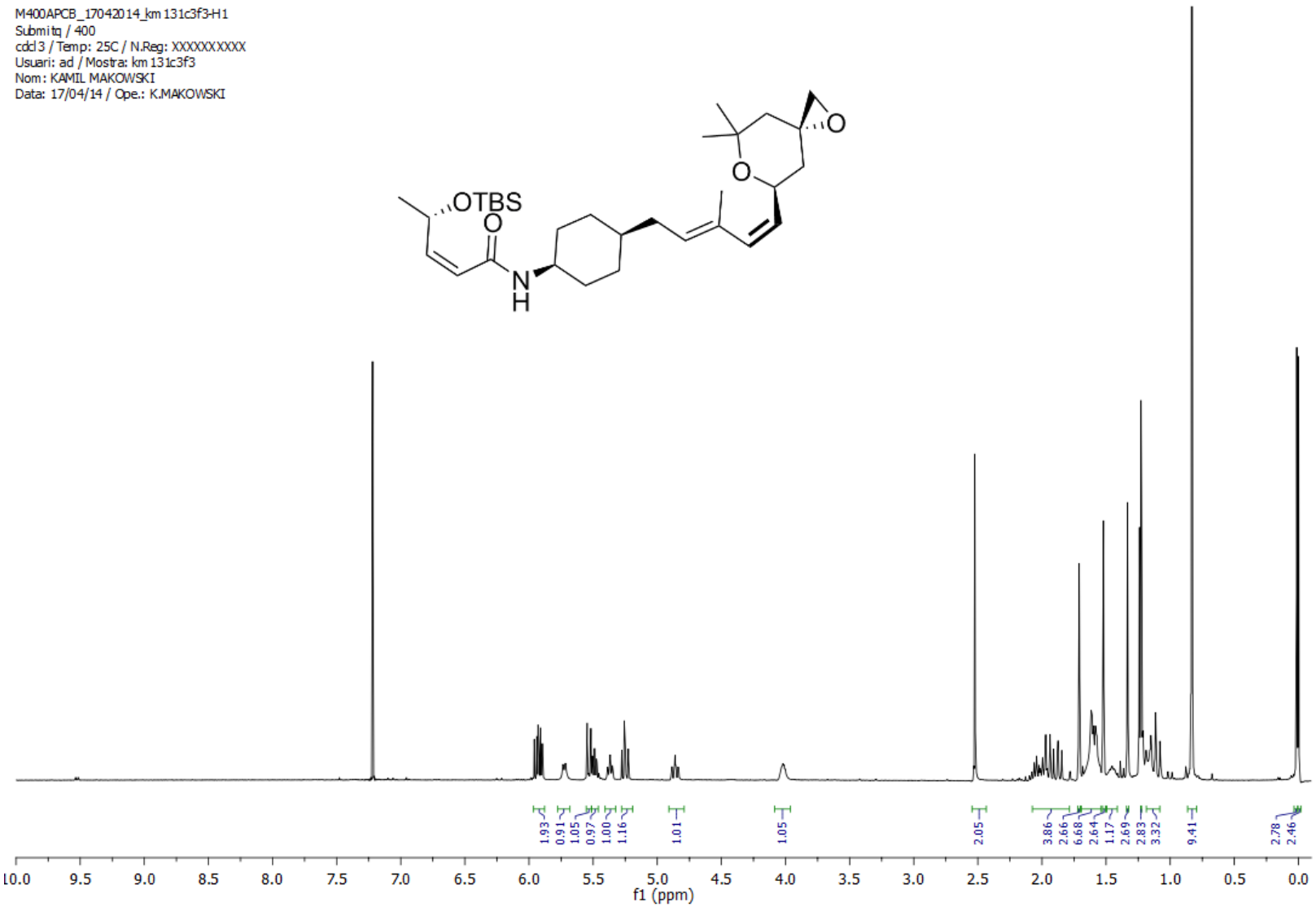


M400APCB_30052014_km146_f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km146_f1
Nom: KAMIL MAKOWSKI
Data: 29/05/14 / Ope.: K.MAKOWSKI

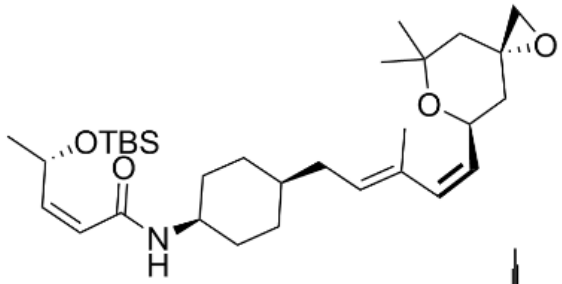


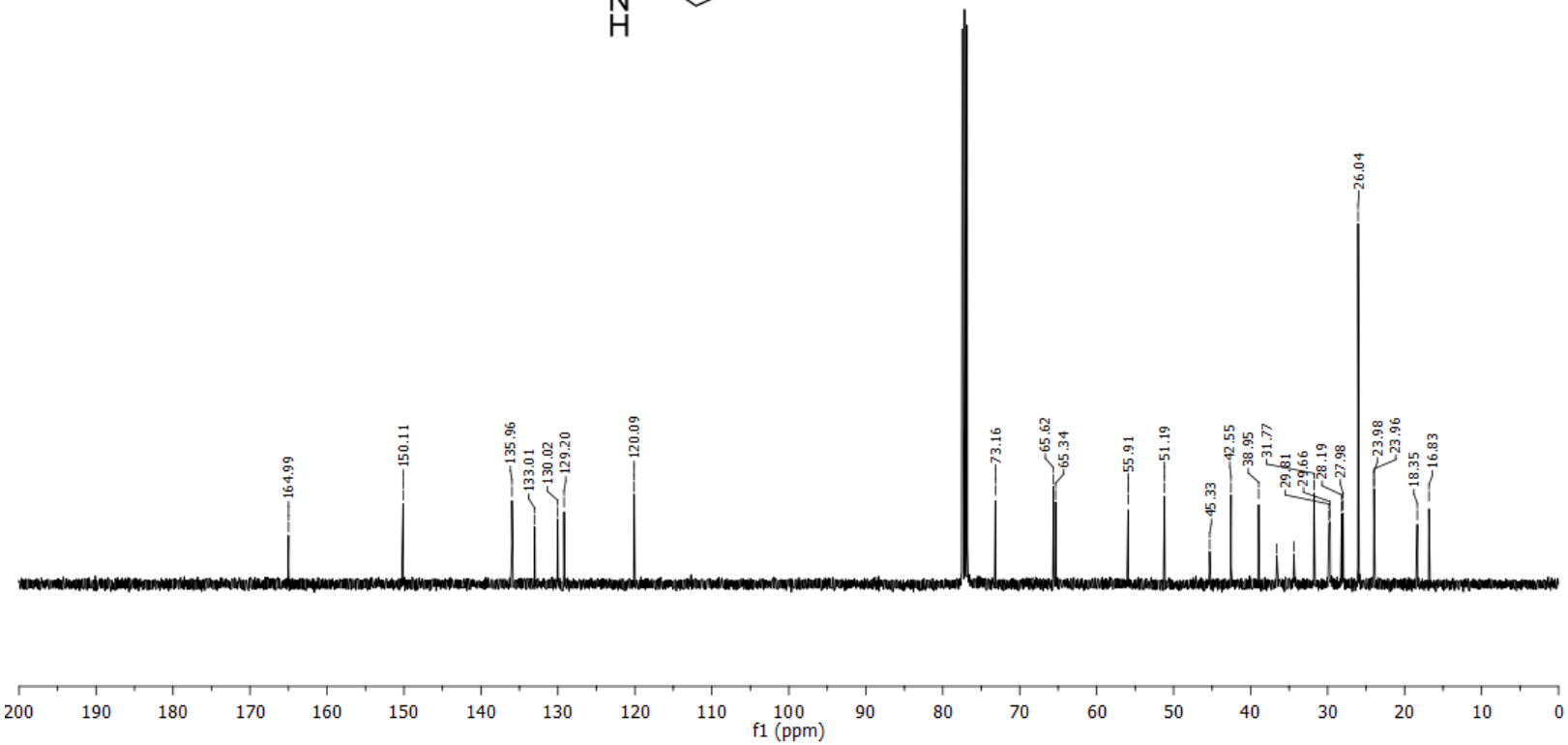


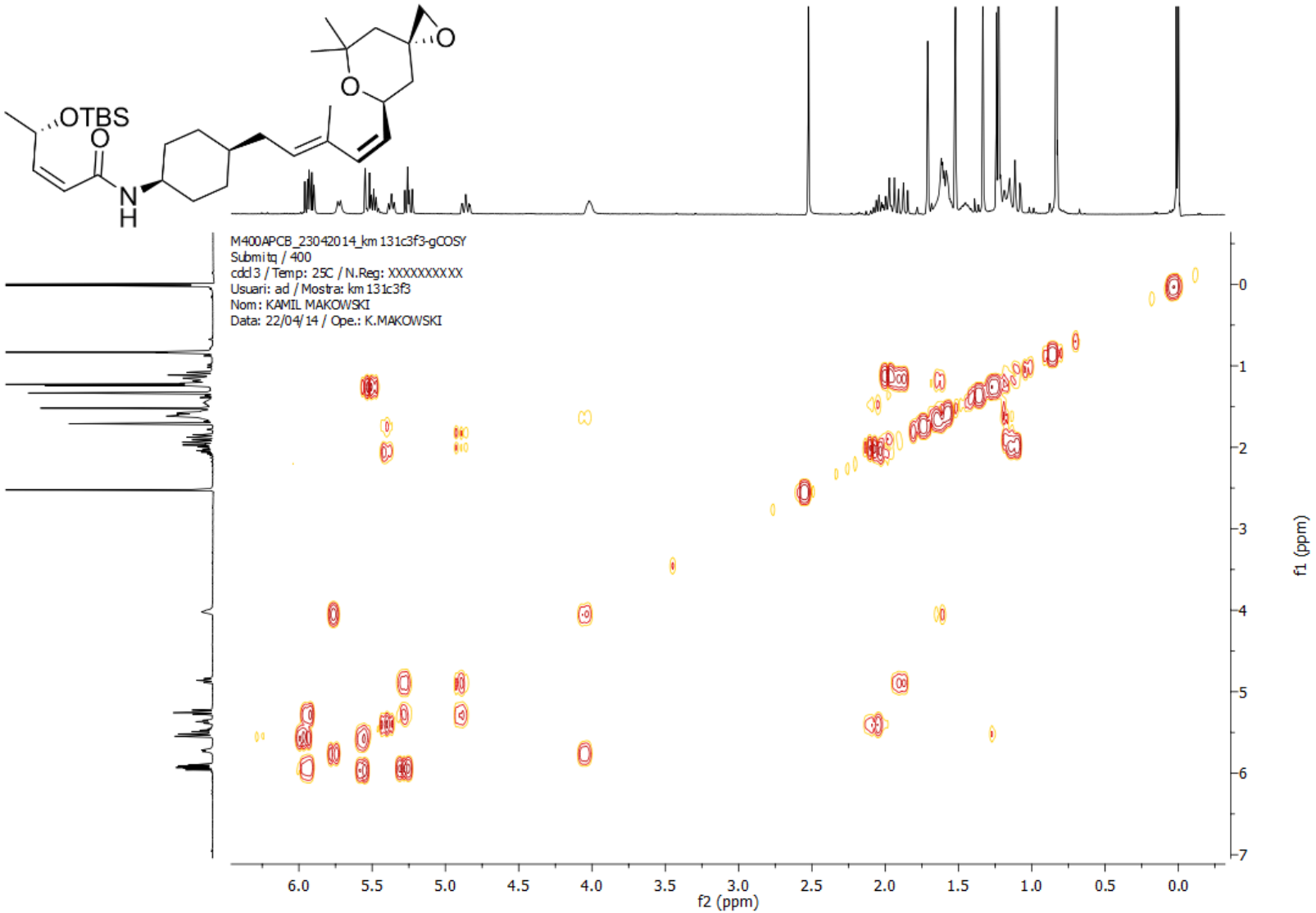

M400APCB_30052014_km146_f1-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km146_f1
Nom: KAMIL MAKOWSKI
Data: 29/05/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

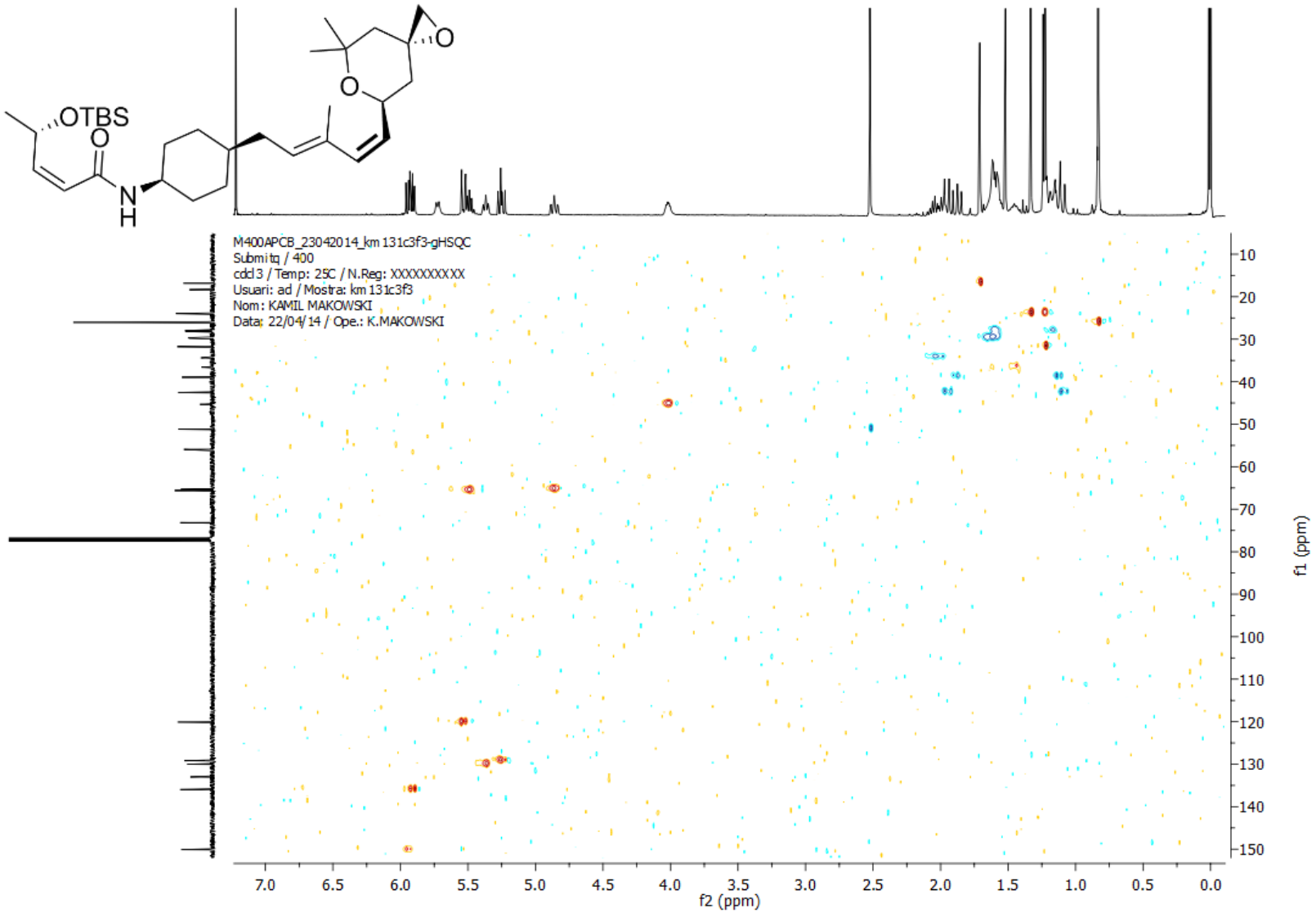

M400APCB_17042014_km131c3f3-H1
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km131c3f3
Nom: KAMIL MAKOWSKI
Data: 17/04/14 / Ope.: K.MAKOWSKI
OTBS
O
N
H
O
O
1.93
0.91
1.05
0.97
1.00
1.16
1.01
1.05
2.05
3.86
2.66
6.68
2.64
1.17
2.69
2.83
3.32
9.41
2.78
2.46
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

M400APCB_23042014_km131c3f3-C13
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km131c3f3
Nom: KAMIL MAKOWSKI
Data: 22/04/14 / Ope.: K.MAKOWSKI

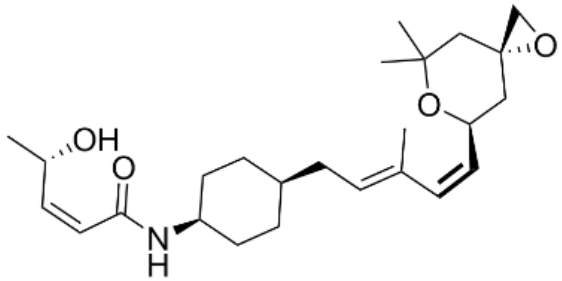


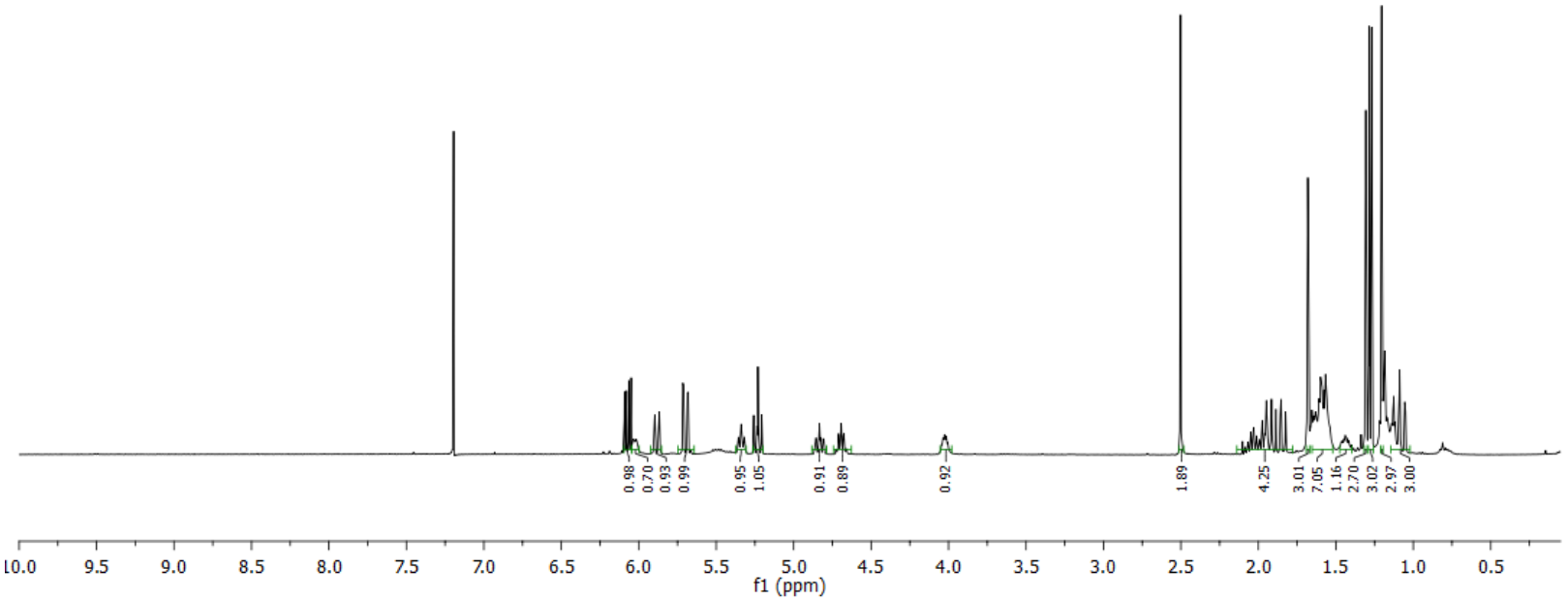



OTBS
O
N
H
O
O
M400APCB_23042014_km131c3f3-gCOSY
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km131c3f3
Nom: KAMIL MAKOWSKI
Data: 22/04/14 / Ope.: K.MAKOWSKI
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
f1 (ppm)

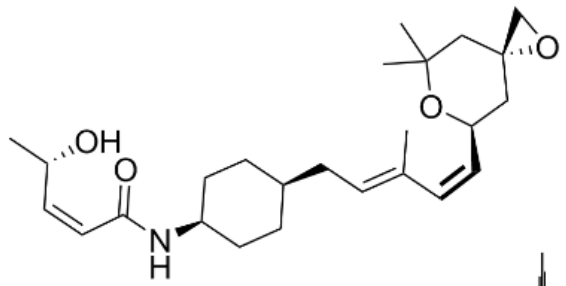

OTBS
O
N
H
O
O
M400APCB_23042014_km131c3f3-gHSQC
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km131c3f3
Nom: KAMIL MAKOWSKI
Data: 22/04/14 / Ope.: K.MAKOWSKI
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

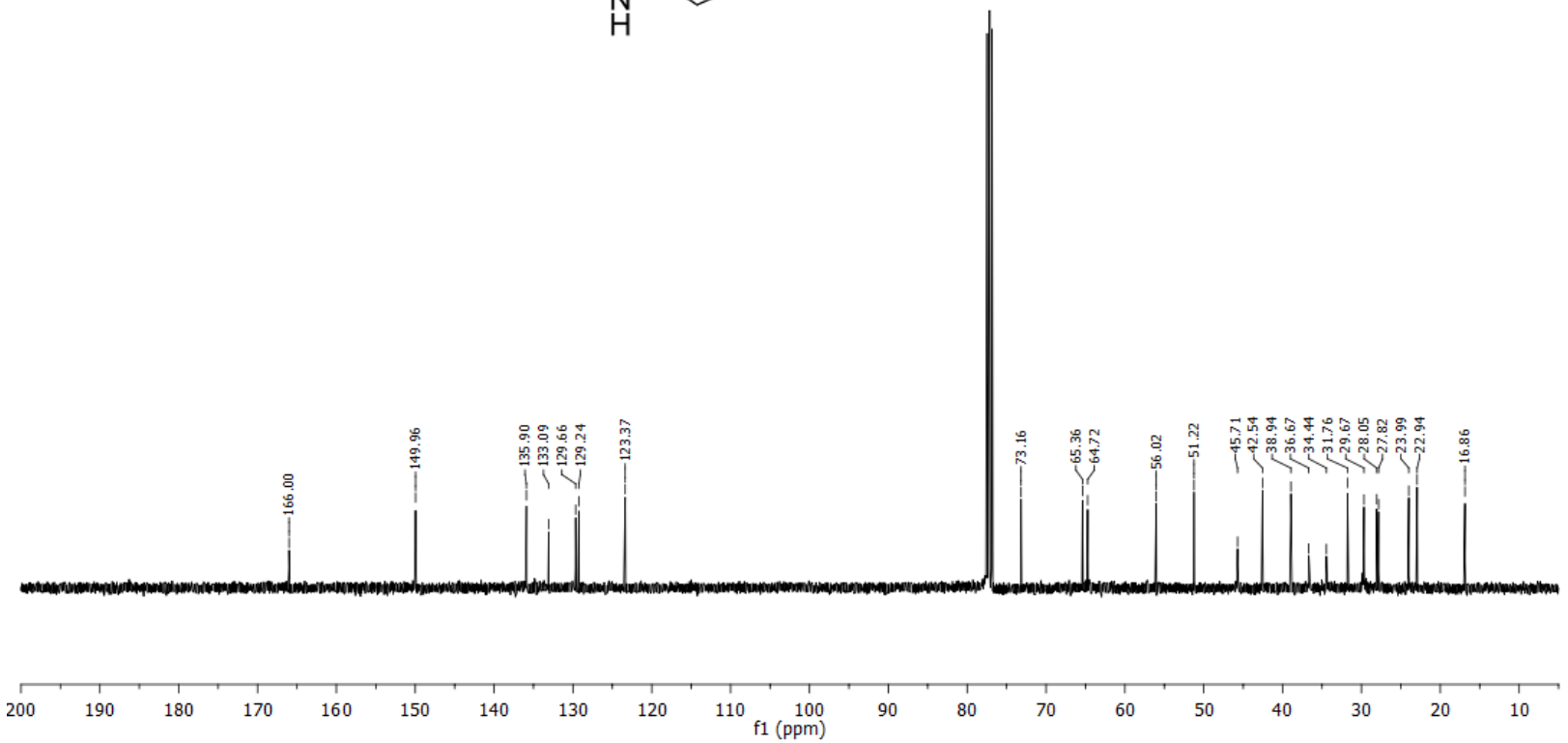
M400APCB_25042014_km134f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km134f1
Nom: KAMIL MAKOWSKI
Data: 24/04/14 / Ope.: K.MAKOWSKI

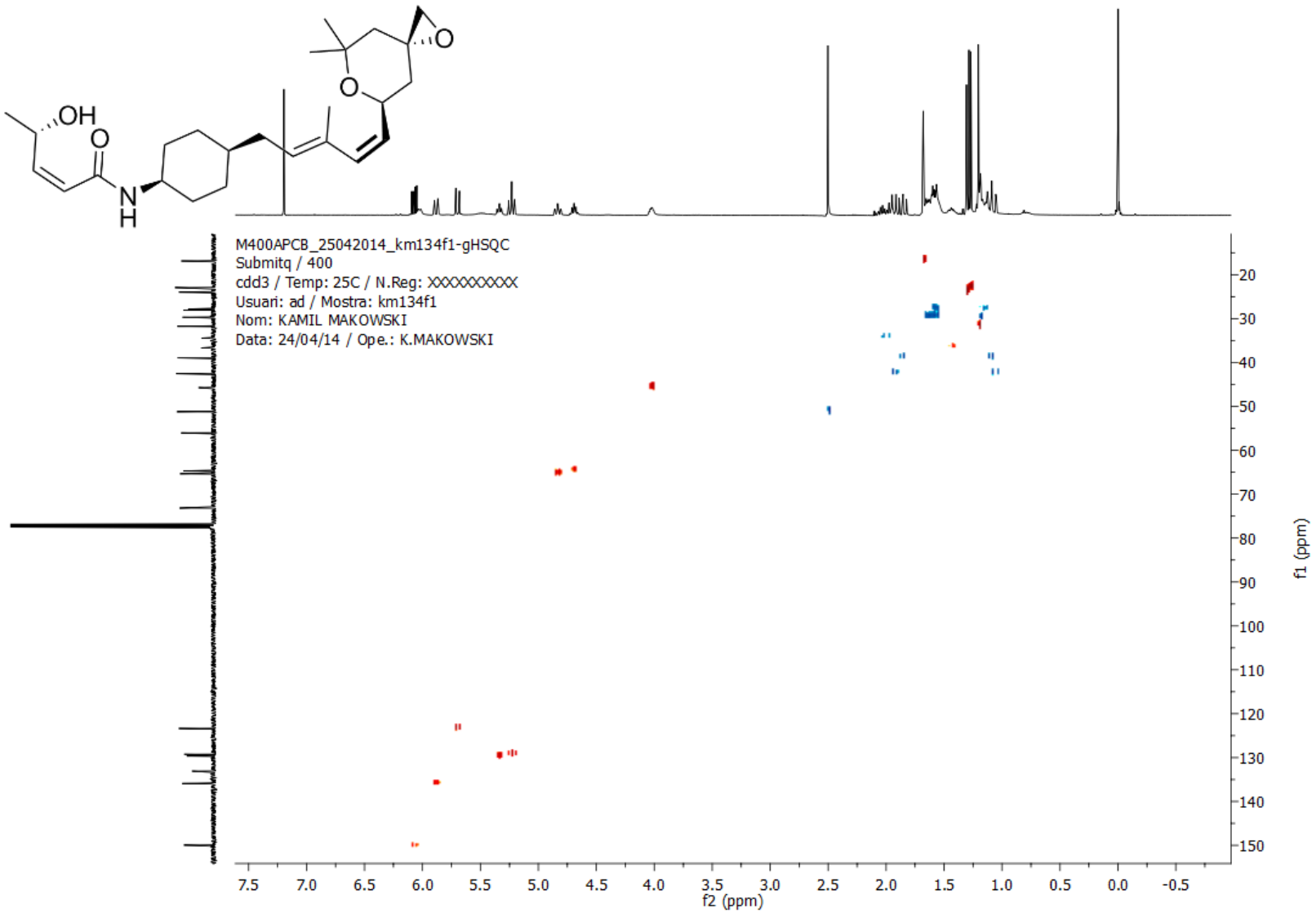


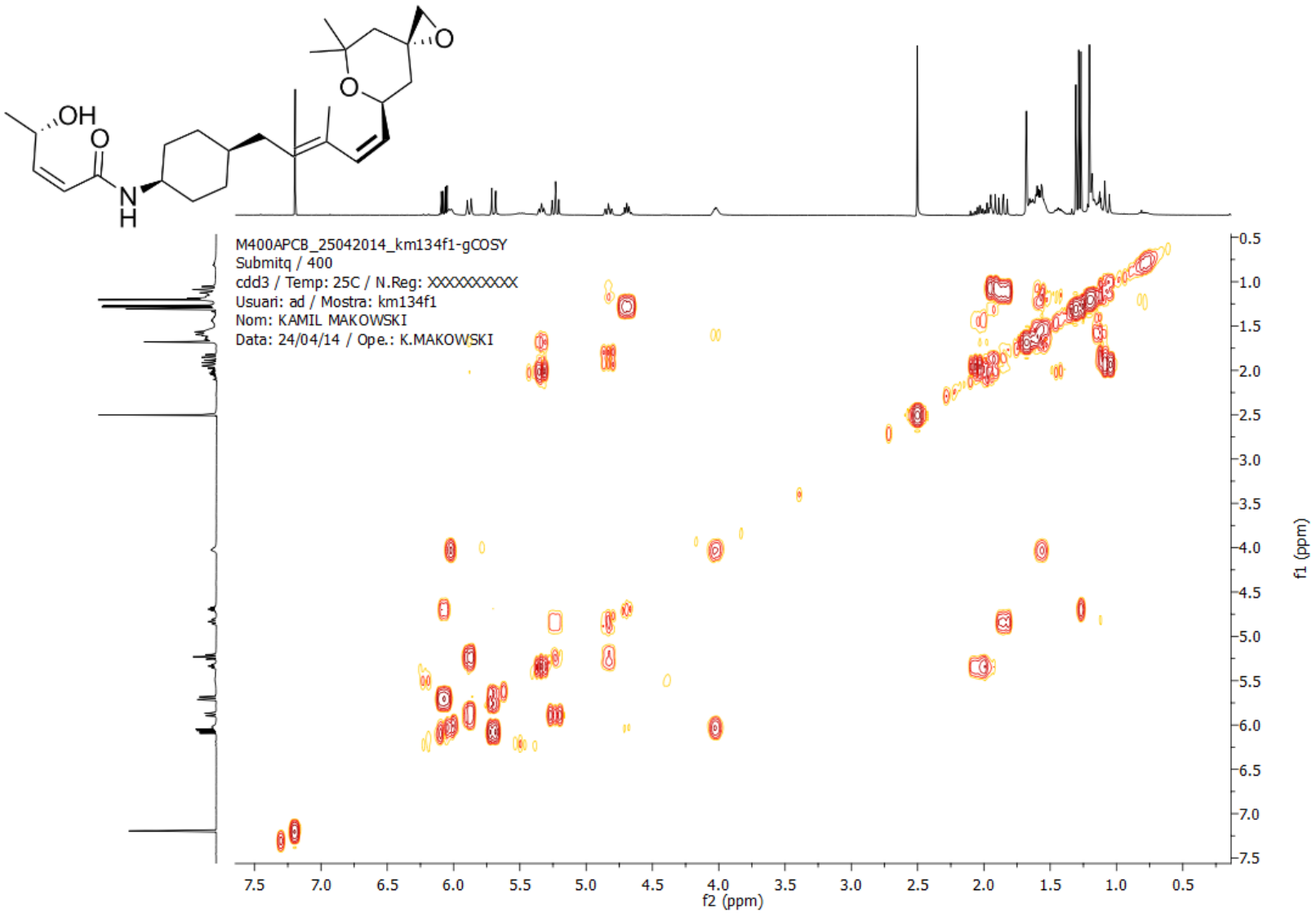


M400APCB_25042014_km134f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km134f1
Nom: KAMIL MAKOWSKI
Data: 24/04/14 / Ope.: K.MAKOWSKI

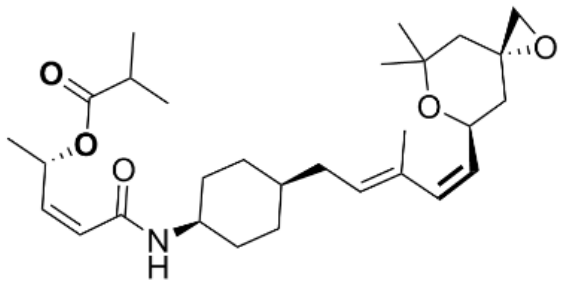


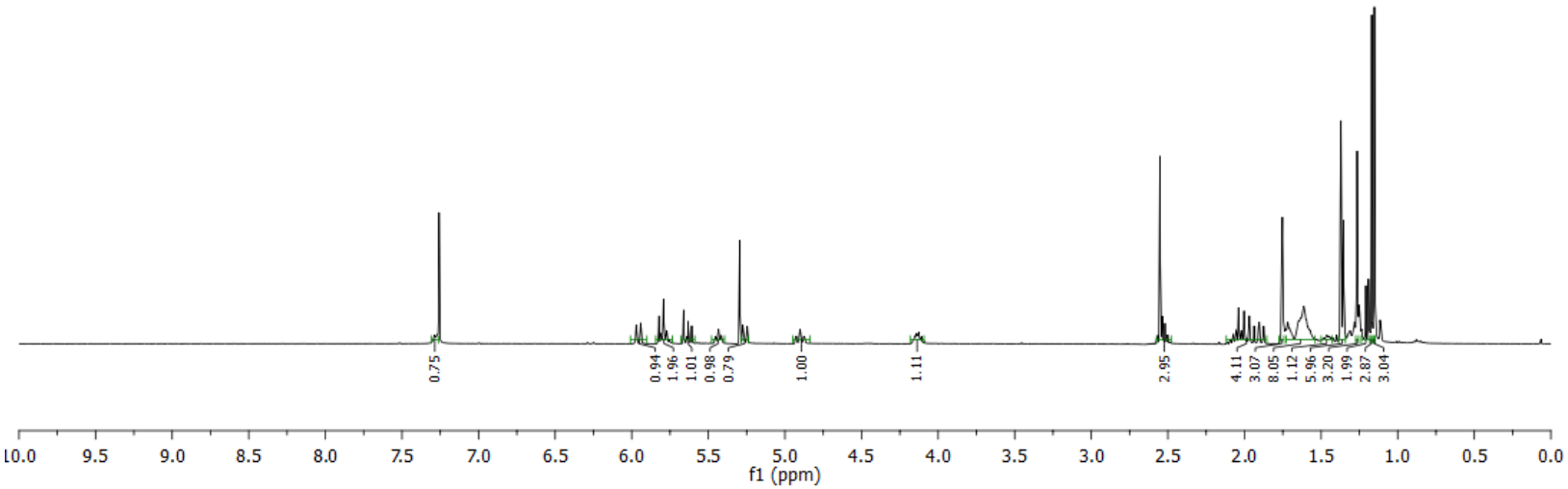



OH
O
N
H
O
O
M400APCB_25042014_km134f1-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km134f1
Nom: KAMIL MAKOWSKI
Data: 24/04/14 / Ope.: K.MAKOWSKI
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

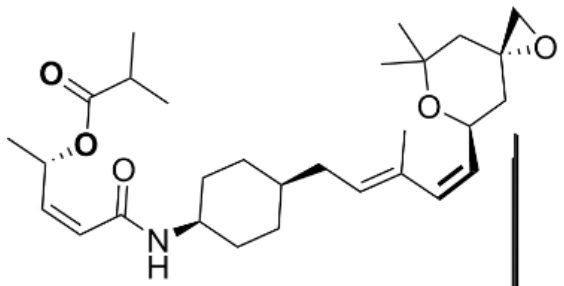

OH
O
N
H
O
O
M400APCB_25042014_km134f1-gCOSY
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km134f1
Nom: KAMIL MAKOWSKI
Data: 24/04/14 / Ope.: K.MAKOWSKI
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
f1 (ppm)

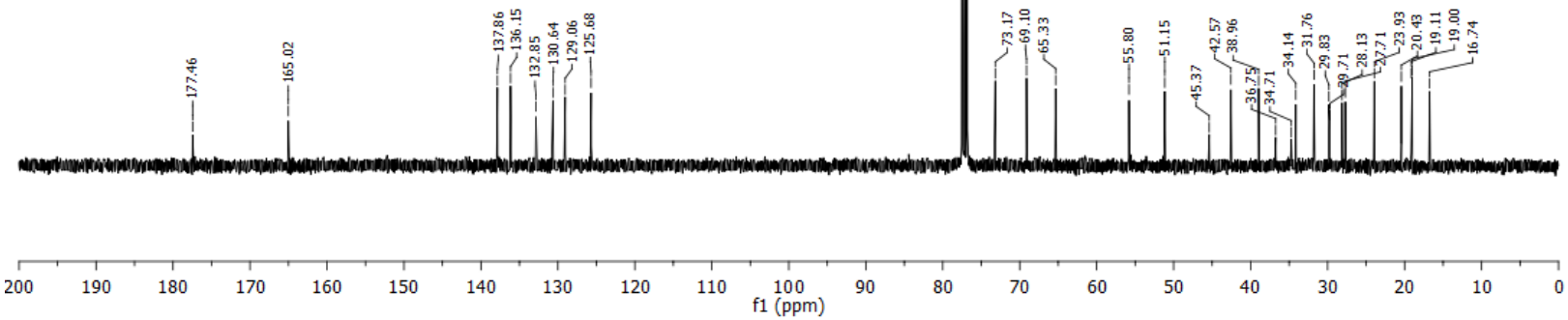
M400APCB_29042014_km136f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km136f1
Nom: KAMIL MAKOWSKI
Data: 28/04/14 / Ope.: K.MAKOWSKI

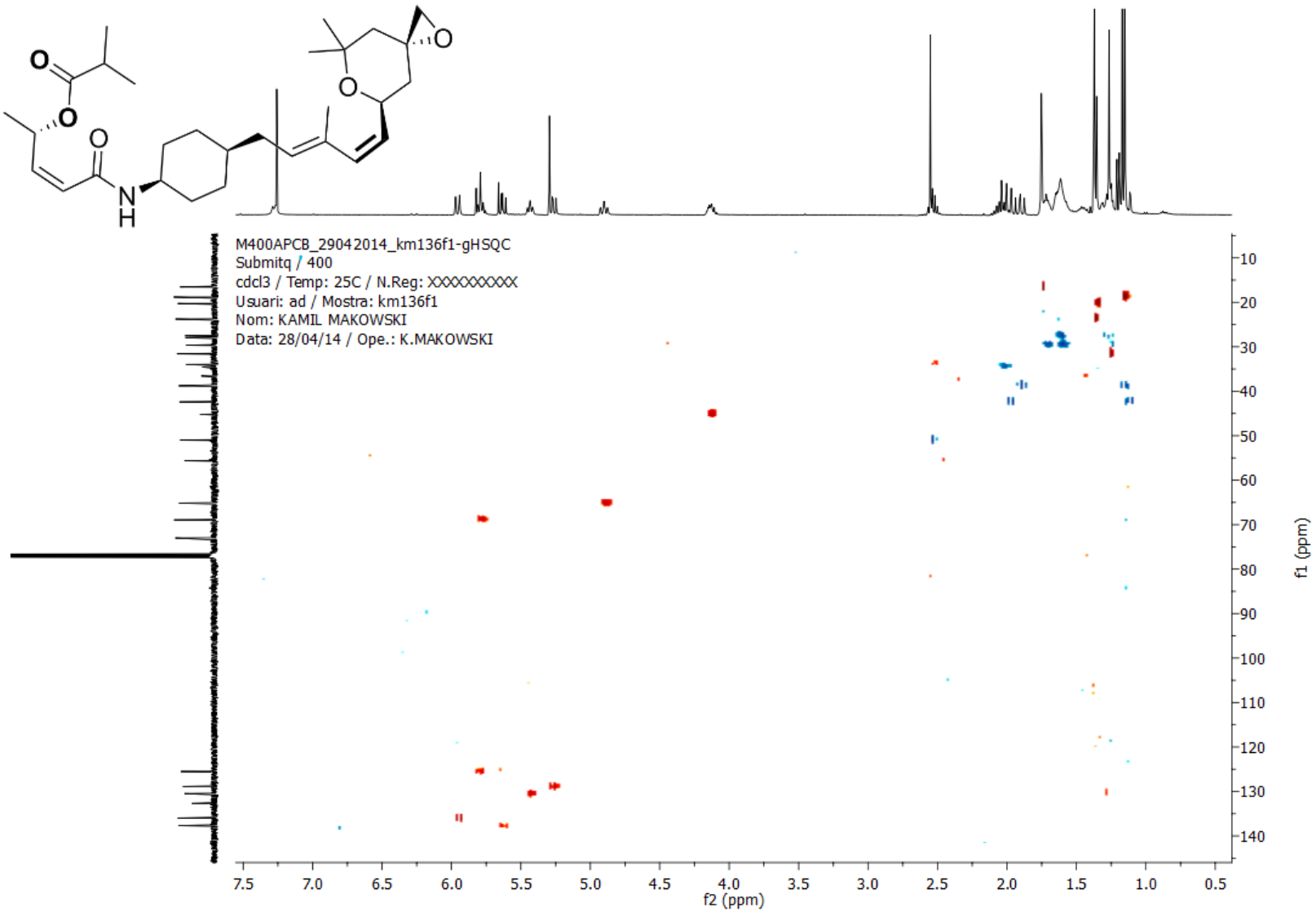




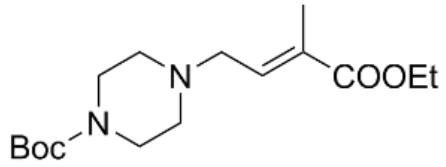
M400APCB_29042014_km136f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km136f1
Nom: KAMIL MAKOWSKI
Data: 28/04/14 / Ope.: K.MAKOWSKI





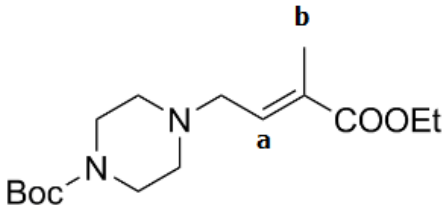

M400APCB_29042014_km136f1-gHSQC
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km136f1
Nom: KAMIL MAKOWSKI
Data: 28/04/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

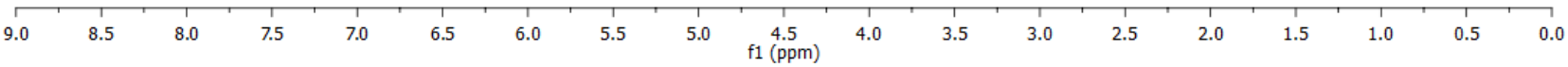
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km259f1
Nom: KAMIL MAKOWSKI
Data: 11/12/14 / Ope.: K.MAKOWSKI



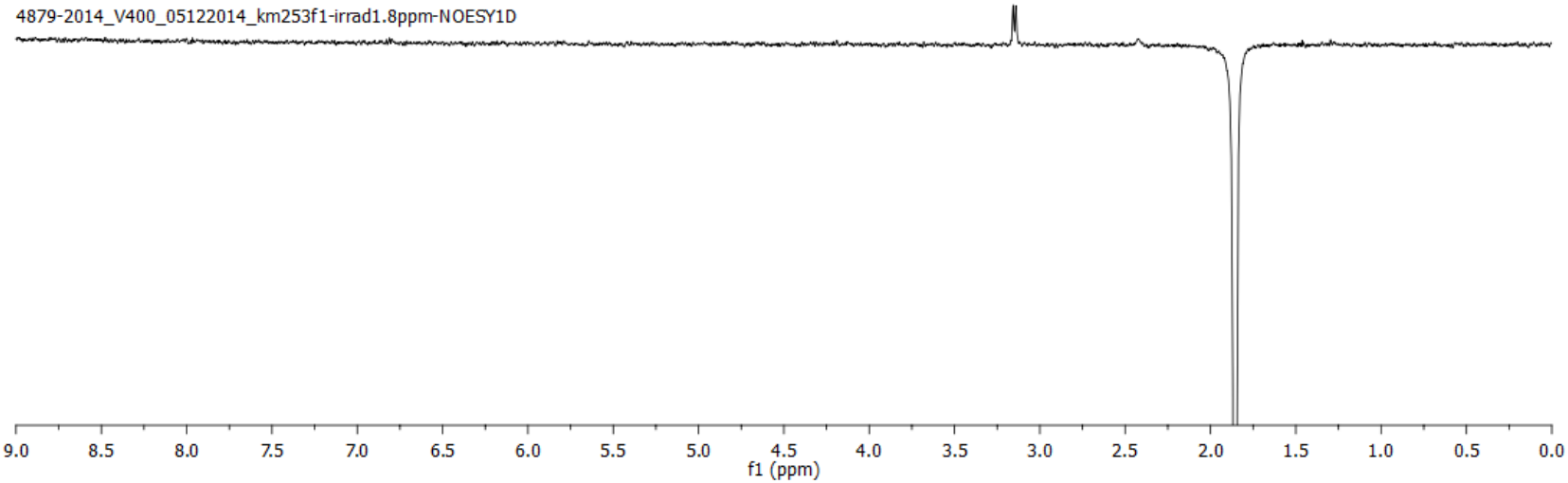
4879-2014_V400_05122014_km253f1-irrad6.8ppm-NOESY1D

400 MHz ($CDCl_3$)

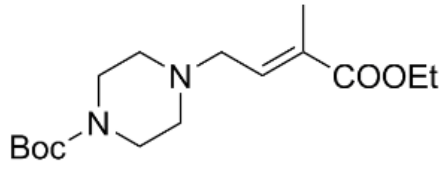


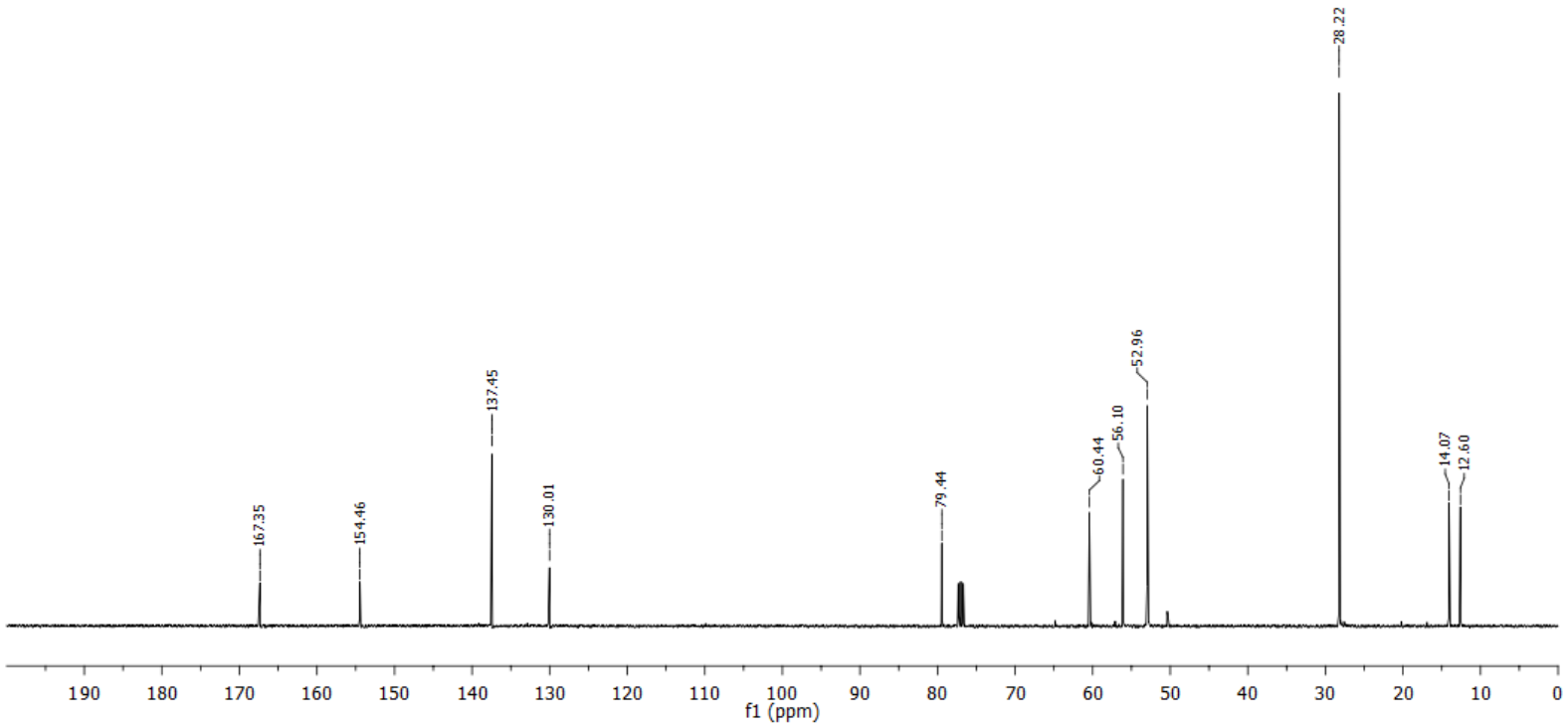


4879-2014_V400_05122014_km253f1-irrad1.8ppm-NOESY1D

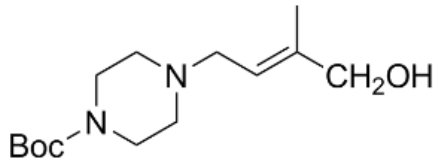


Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km253f1
Nom: KAMIL MAKOWSKI
Data: 02/12/14 / Ope.: K.MAKOWSKI

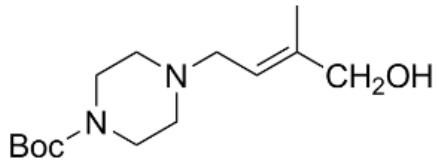


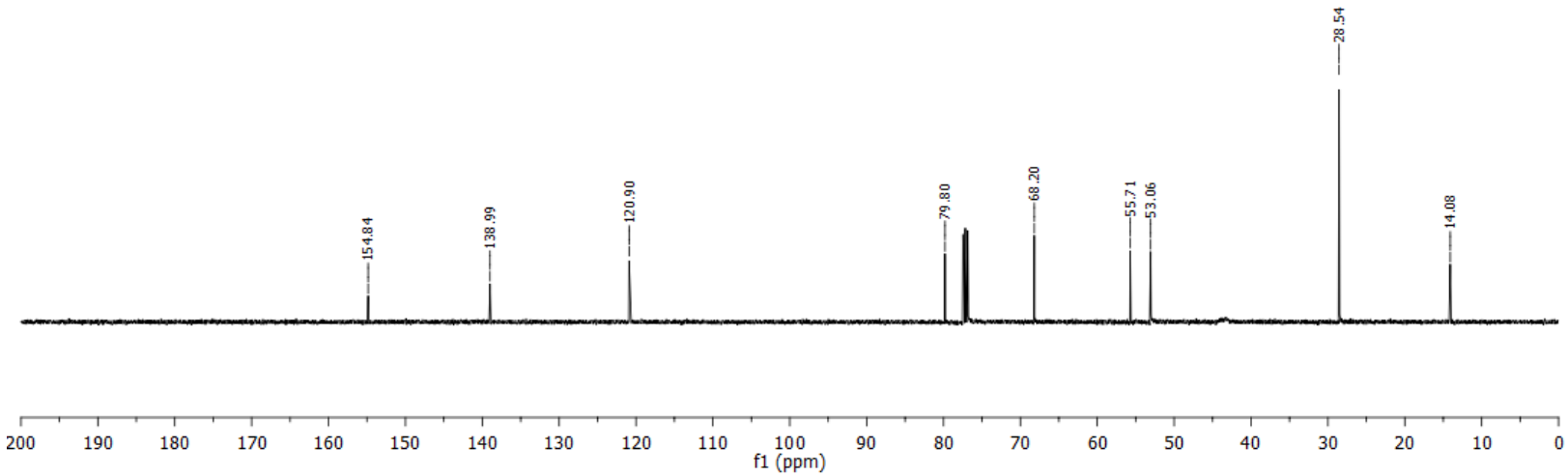


M400APCB_04122014_km255f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km255f1
Nom: KAMIL MAKOWSKI
Data: 04/12/14 / Ope.: K.MAKOWSKI

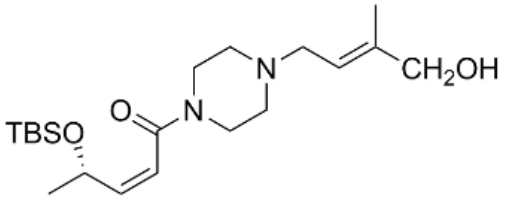


M400APCB_04122014_km255f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km255f1
Nom: KAMIL MAKOWSKI
Data: 04/12/14 / Ope.: K.MAKOWSKI

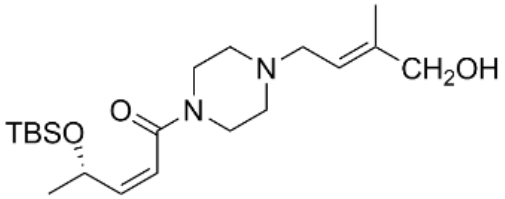


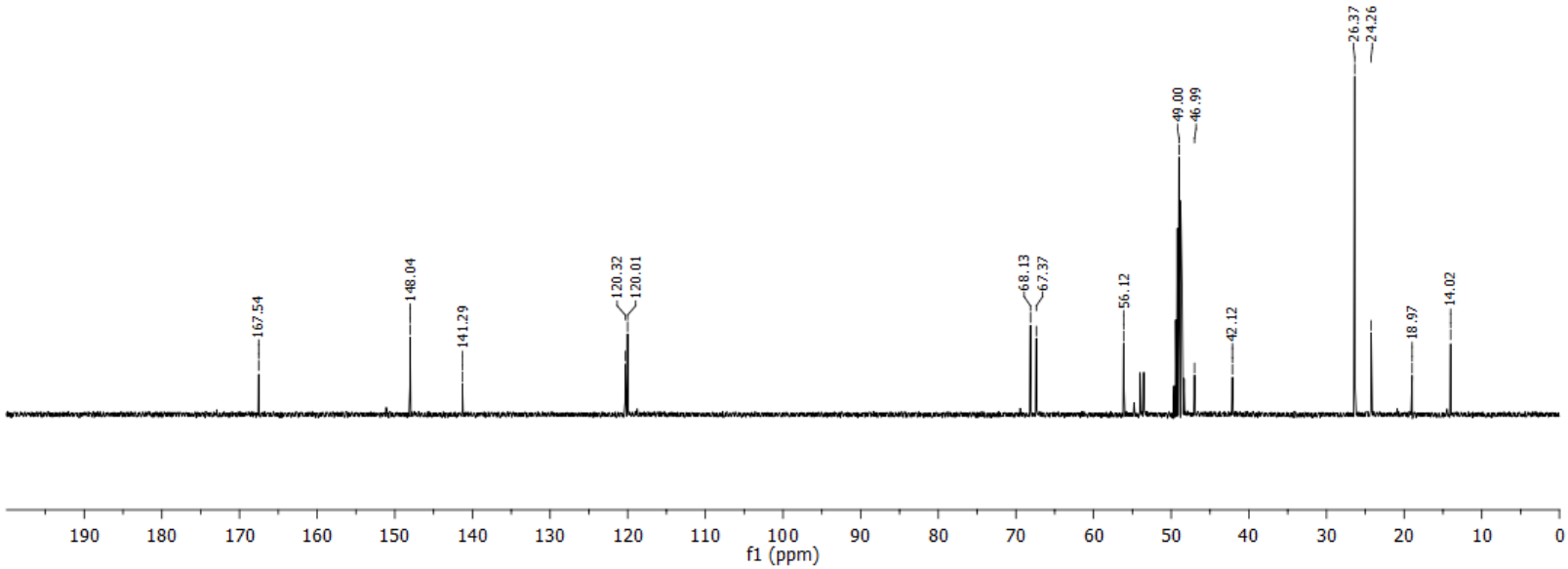


M400APCB_08012015_km278f1-H1
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km278f1
Nom: KAMIL MAKOWSKI
Data: 08/01/15 / Ope.: K.MAKOWSKI

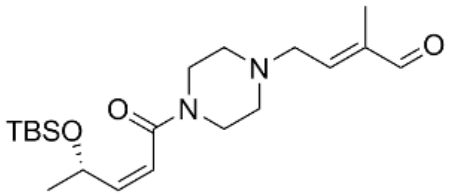


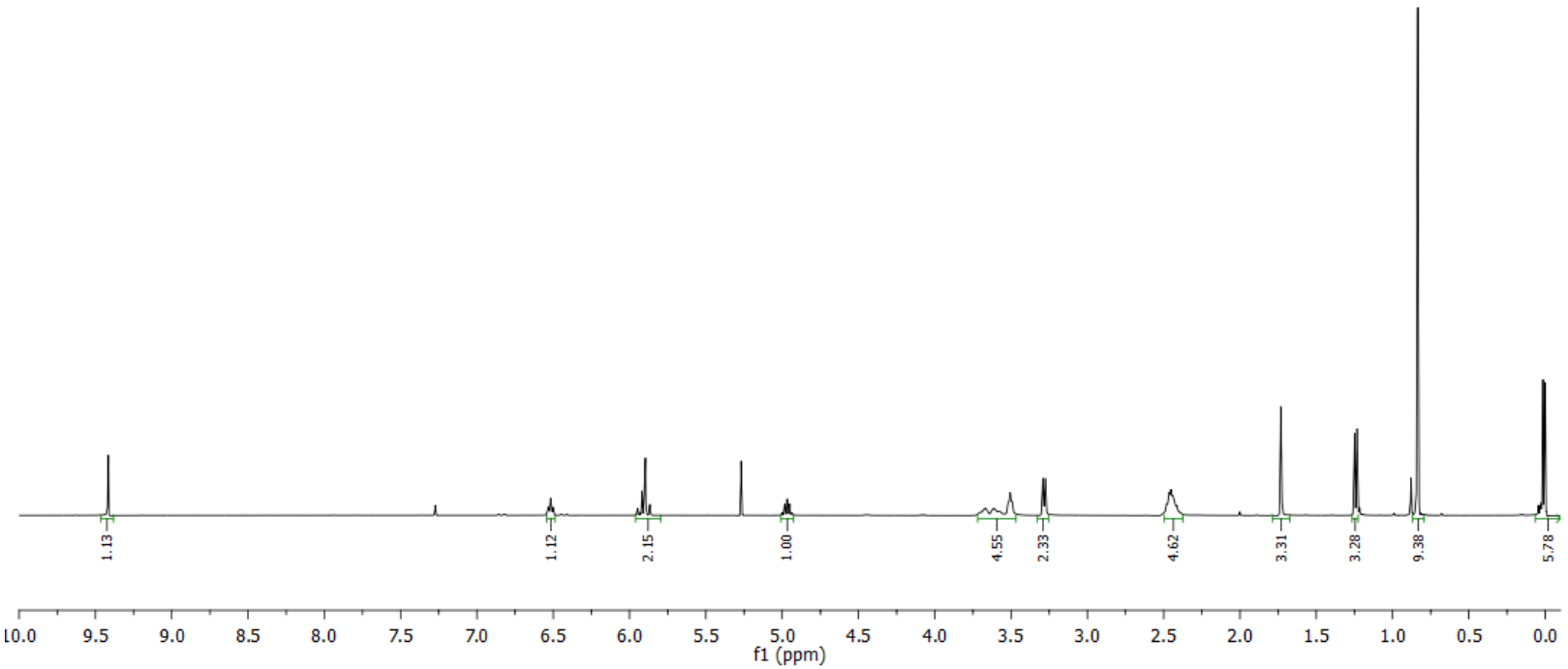
M400APCB_09012015_km278f1-C13
M400PCB /
cd3od / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km278f1
Nom: KAMIL MAKOWSKI
Data: 09/01/15 / Ope.: K.MAKOWSKI



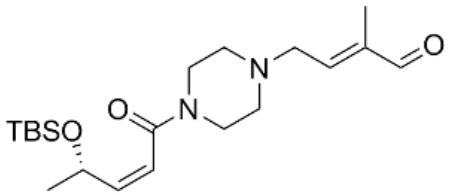


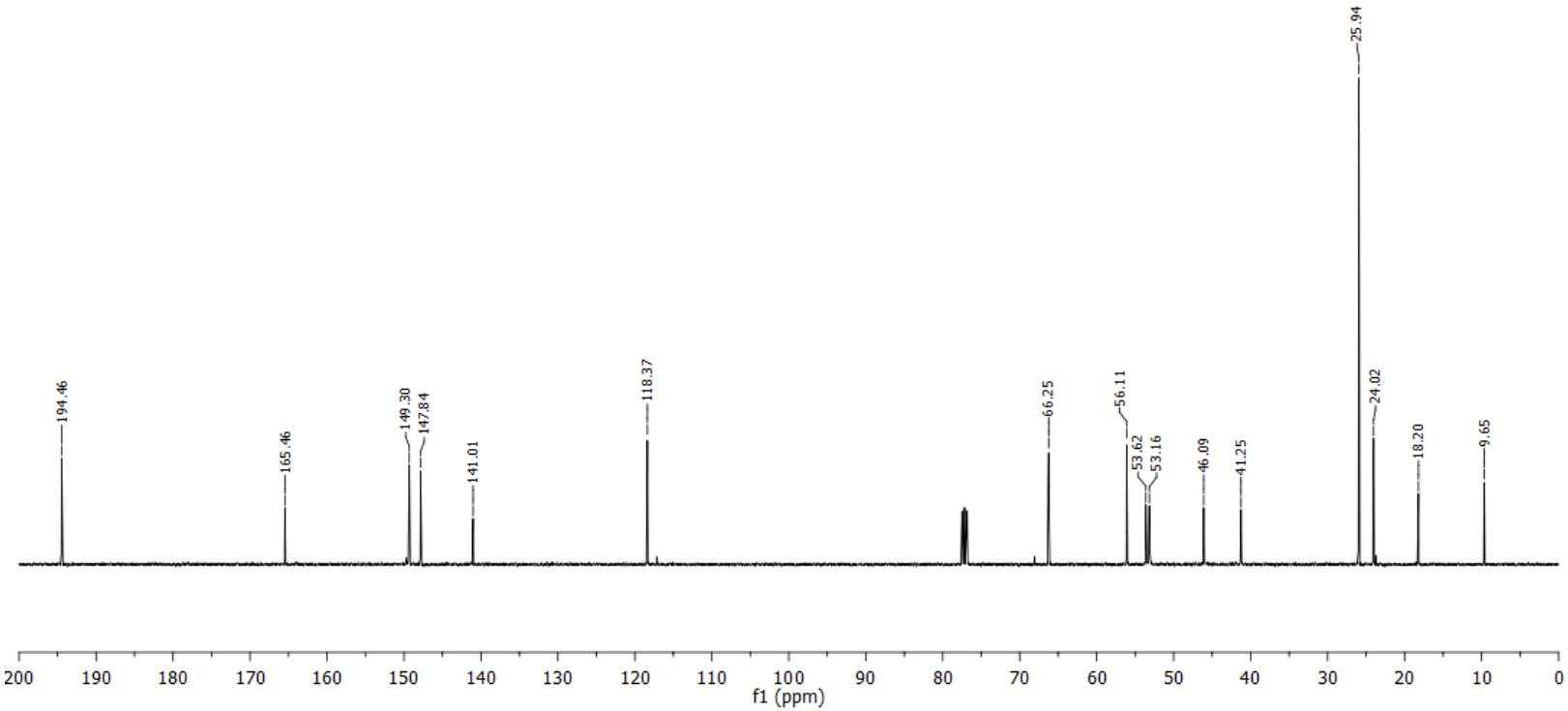
M400APCB_09012015_km280f1-H1
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km280f1
Nom: KAMIL MAKOWSKI
Data: 09/01/15 / Ope.: K.MAKOWSKI



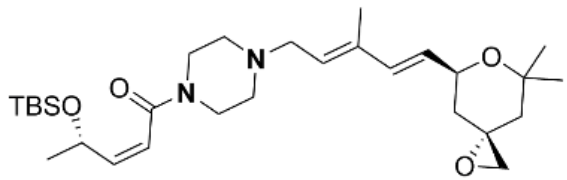


M400APCB_09012015_km280f1-C13
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km280f1
Nom: KAMIL MAKOWSKI
Data: 09/01/15 / Ope.: K.MAKOWSKI

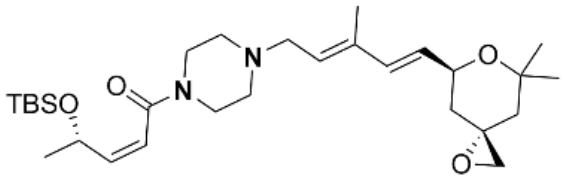


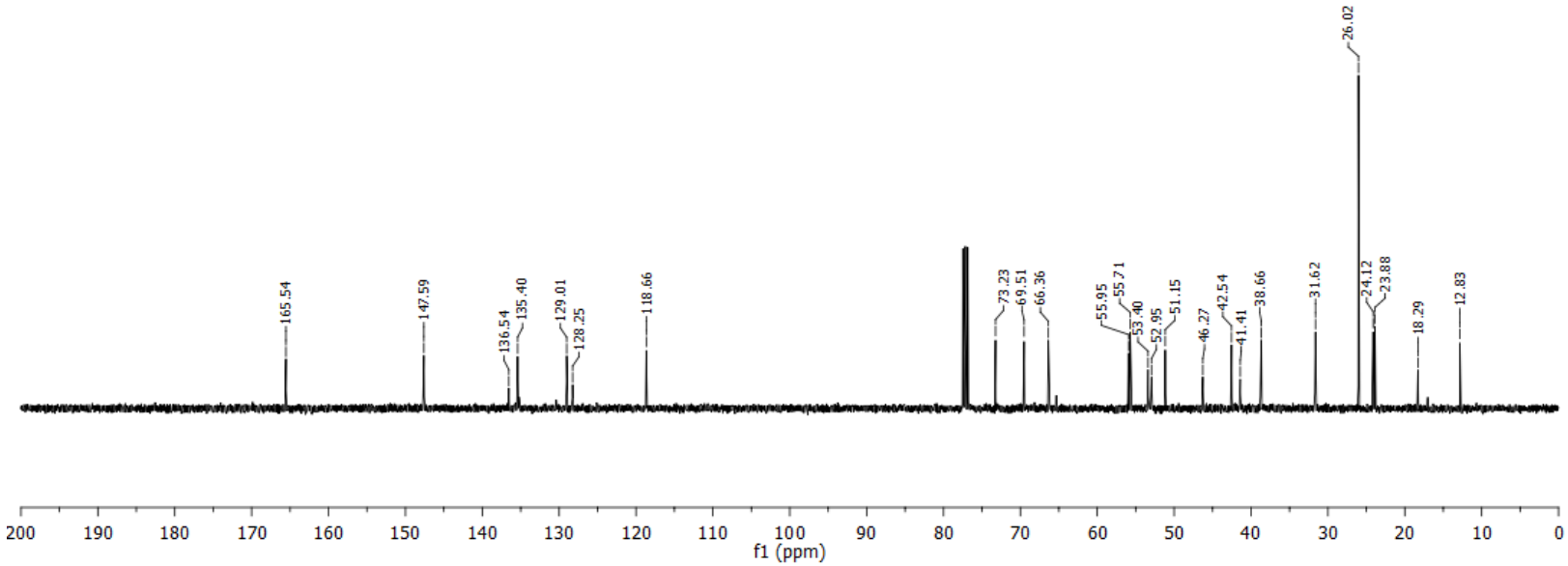


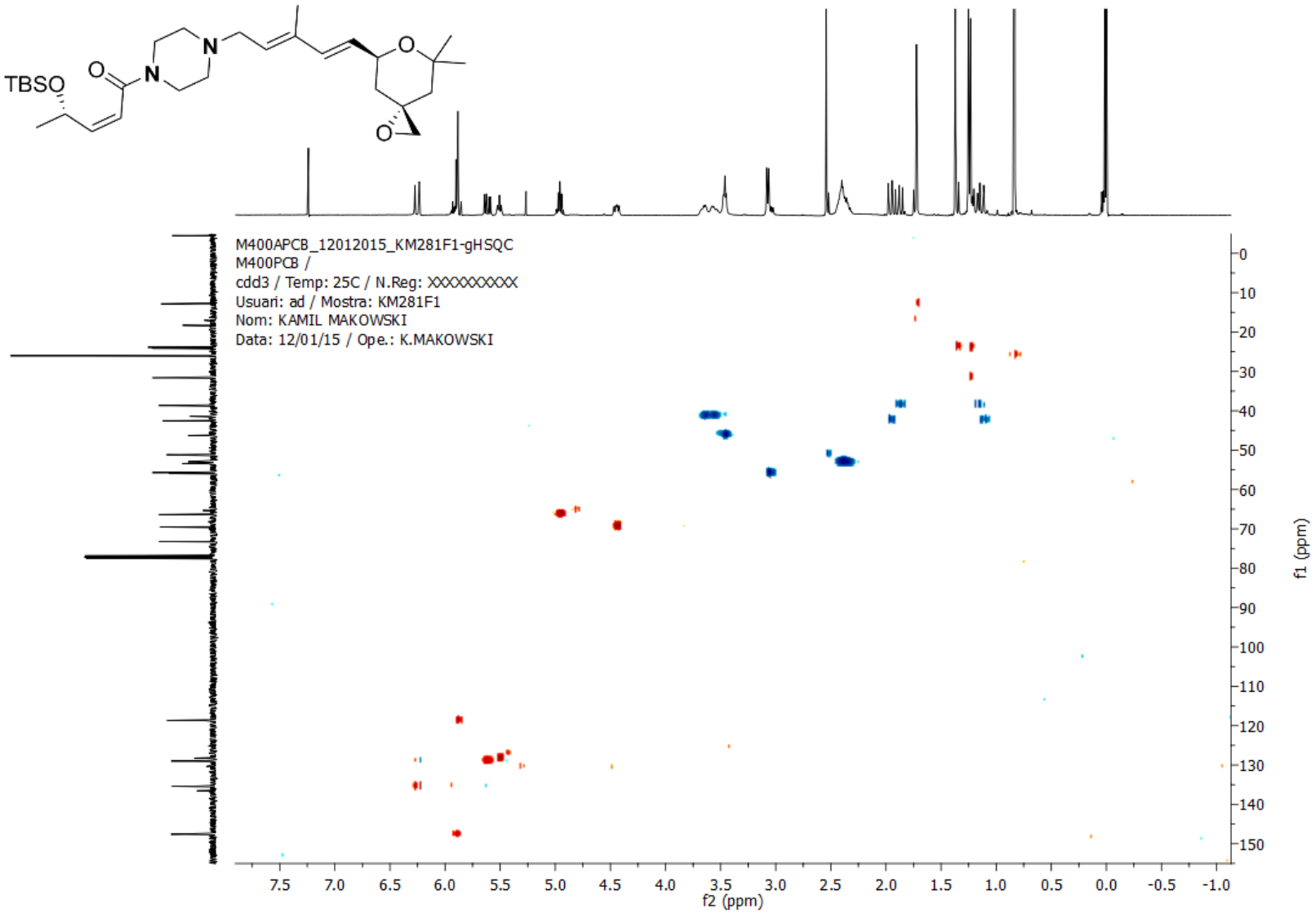
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM281F1
Nom: KAMIL MAKOWSKI
Data: 12/01/15 / Ope.: K.MAKOWSKI



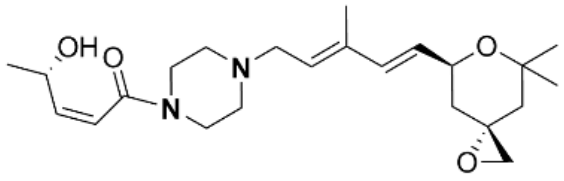
M400APCB_12012015_km281f1-C13
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km281f1
Nom: KAMIL MAKOWSKI
Data: 12/01/15 / Ope.: K.MAKOWSKI

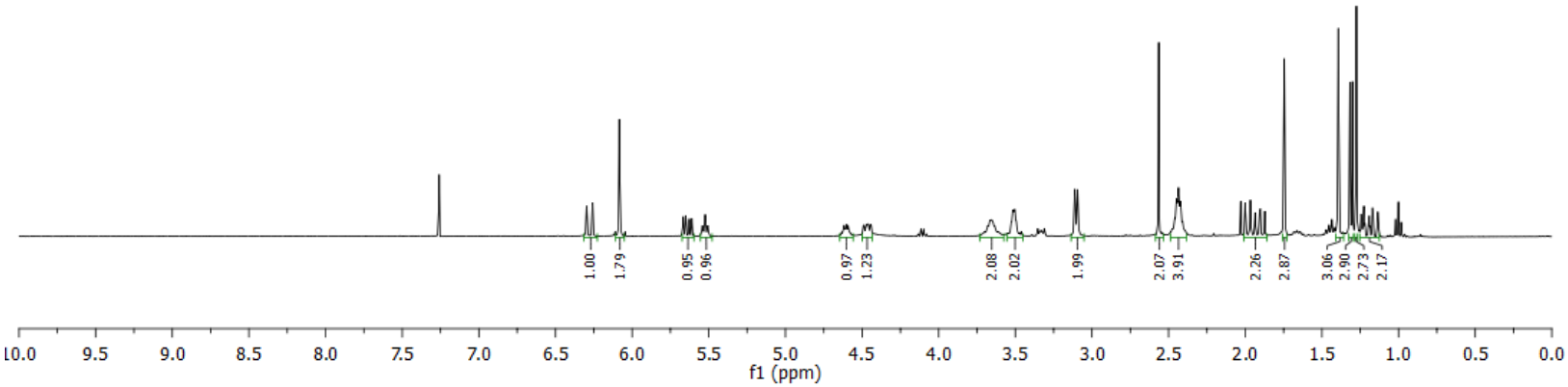




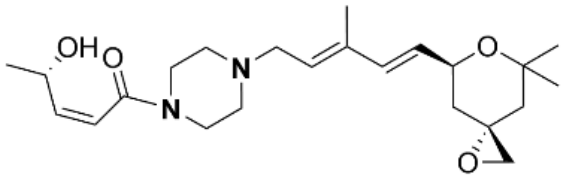

TBSO
O
N
N
O
O
M400APCB_12012015_KM281F1-gHSQC
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM281F1
Nom: KAMIL MAKOWSKI
Data: 12/01/15 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

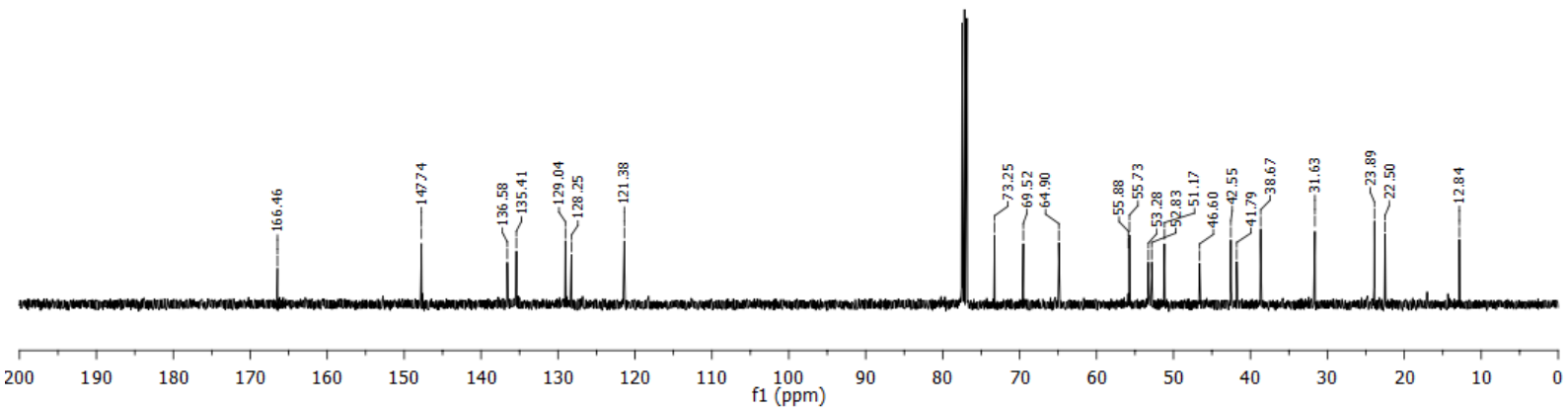
M400APCB_09022015_KM300F1-H1
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM300F1
Nom: KAMIL MAKOWSKI
Data: 09/02/15 / Ope.: K.MAKOWSKI

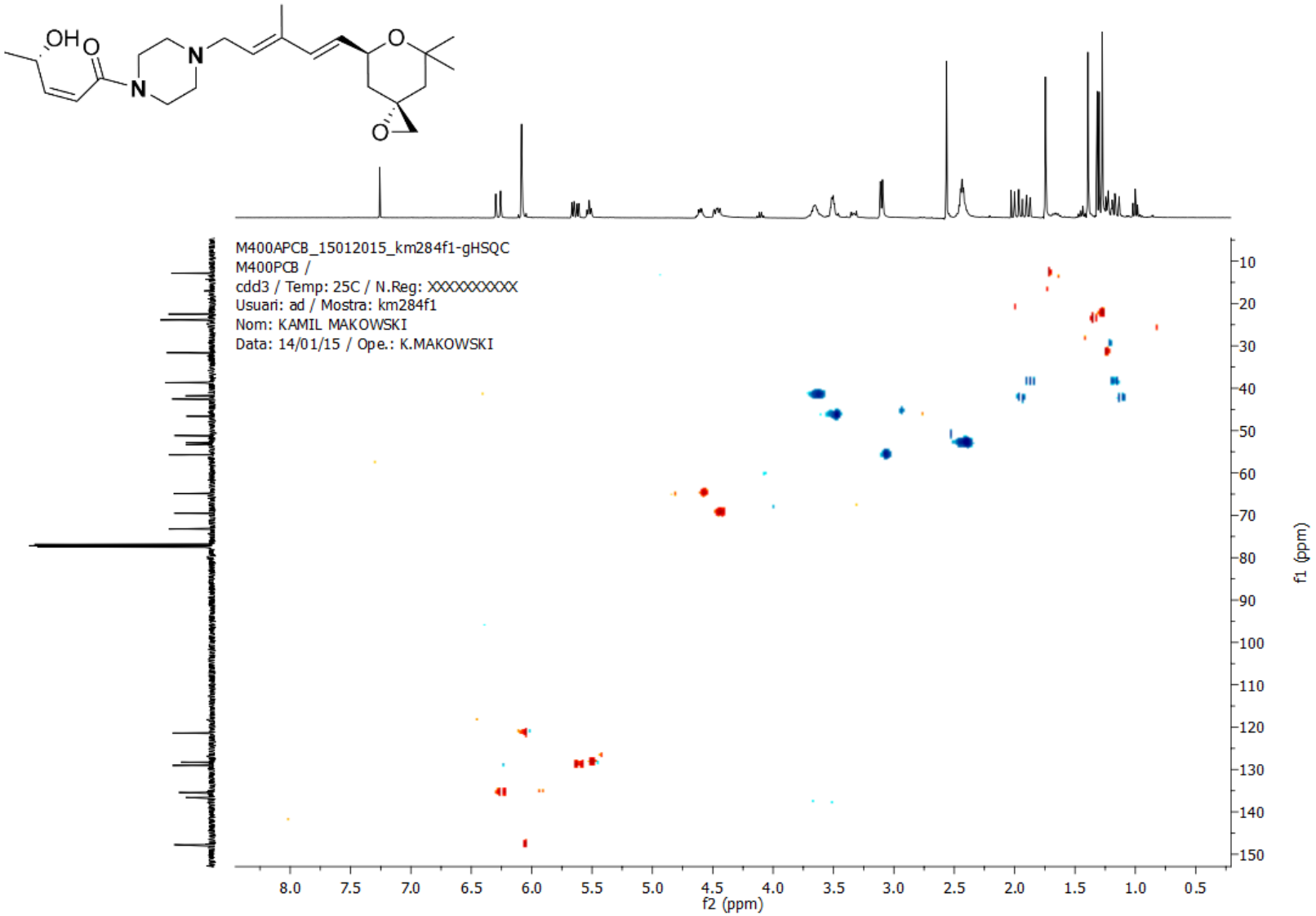




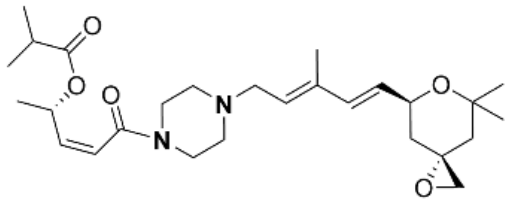
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km284f1
Nom: KAMIL MAKOWSKI
Data: 14/01/15 / Ope.: K.MAKOWSKI

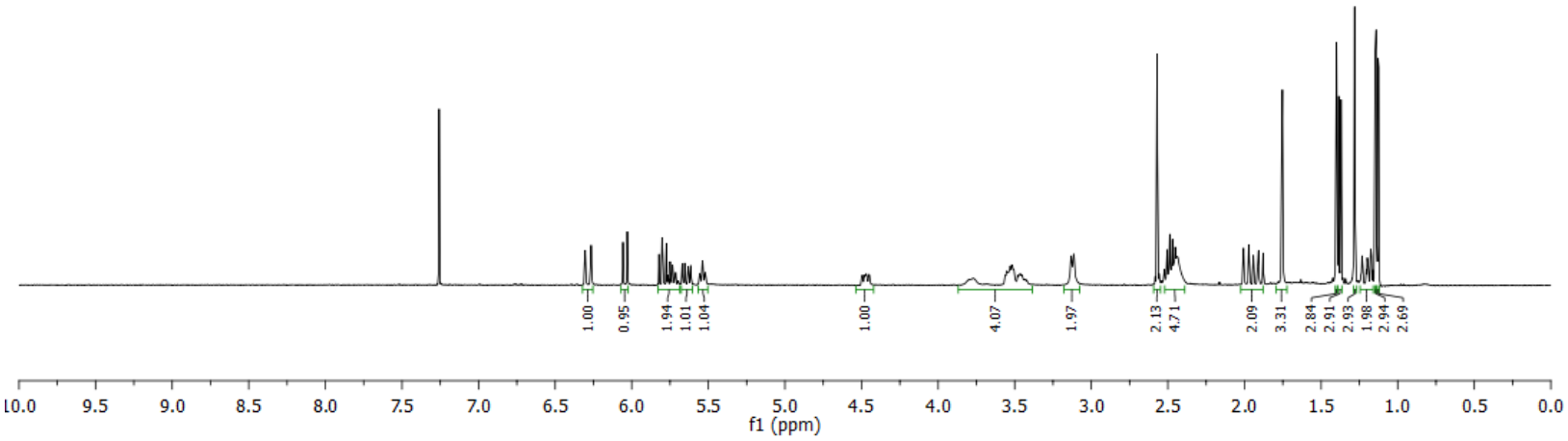




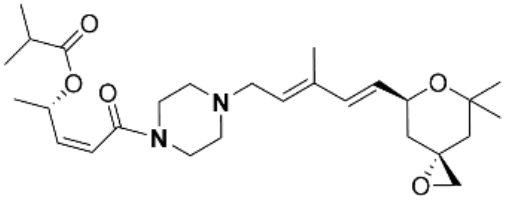

OH
O
N
N
O
O
M400APCB_15012015_km284f1-gHSQC
M400PCB /
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km284f1
Nom: KAMIL MAKOWSKI
Data: 14/01/15 / Ope.: K.MAKOWSKI
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

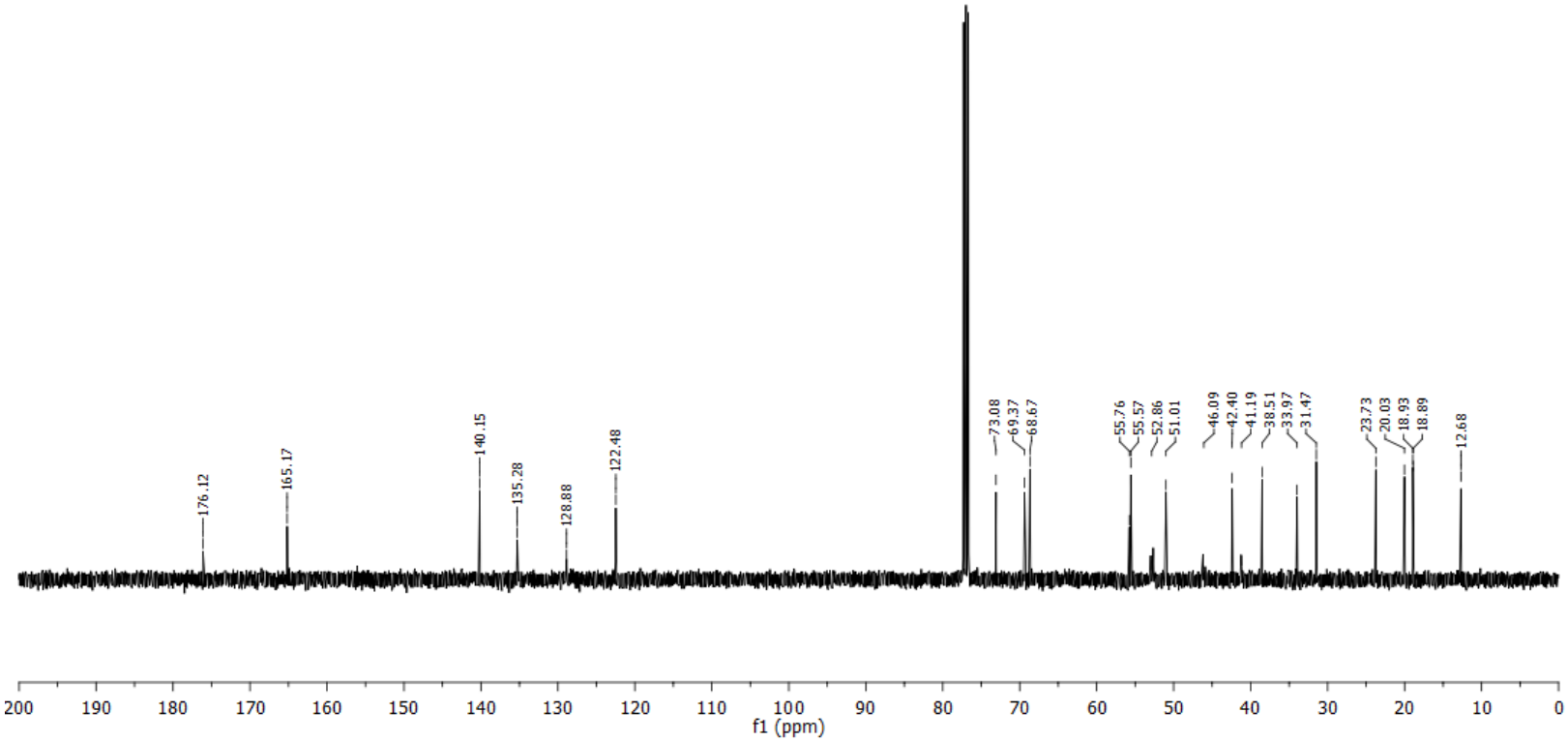
M400APCB_06022015_km291pur_P2-H1
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km291pur_P2
Nom: KAMIL MAKOWSKI
Data: 05/02/15 / Ope.: K.MAKOWSKI

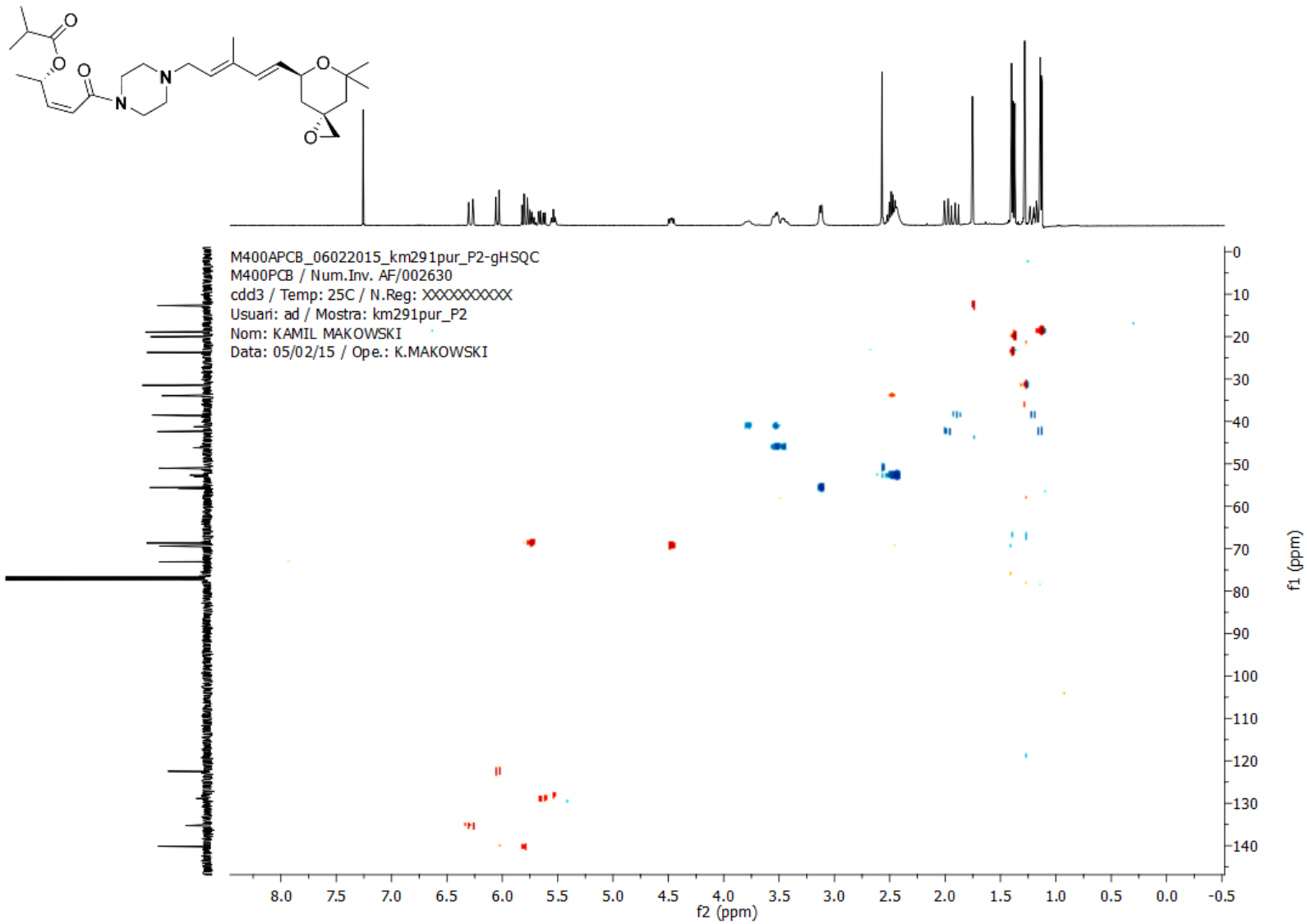


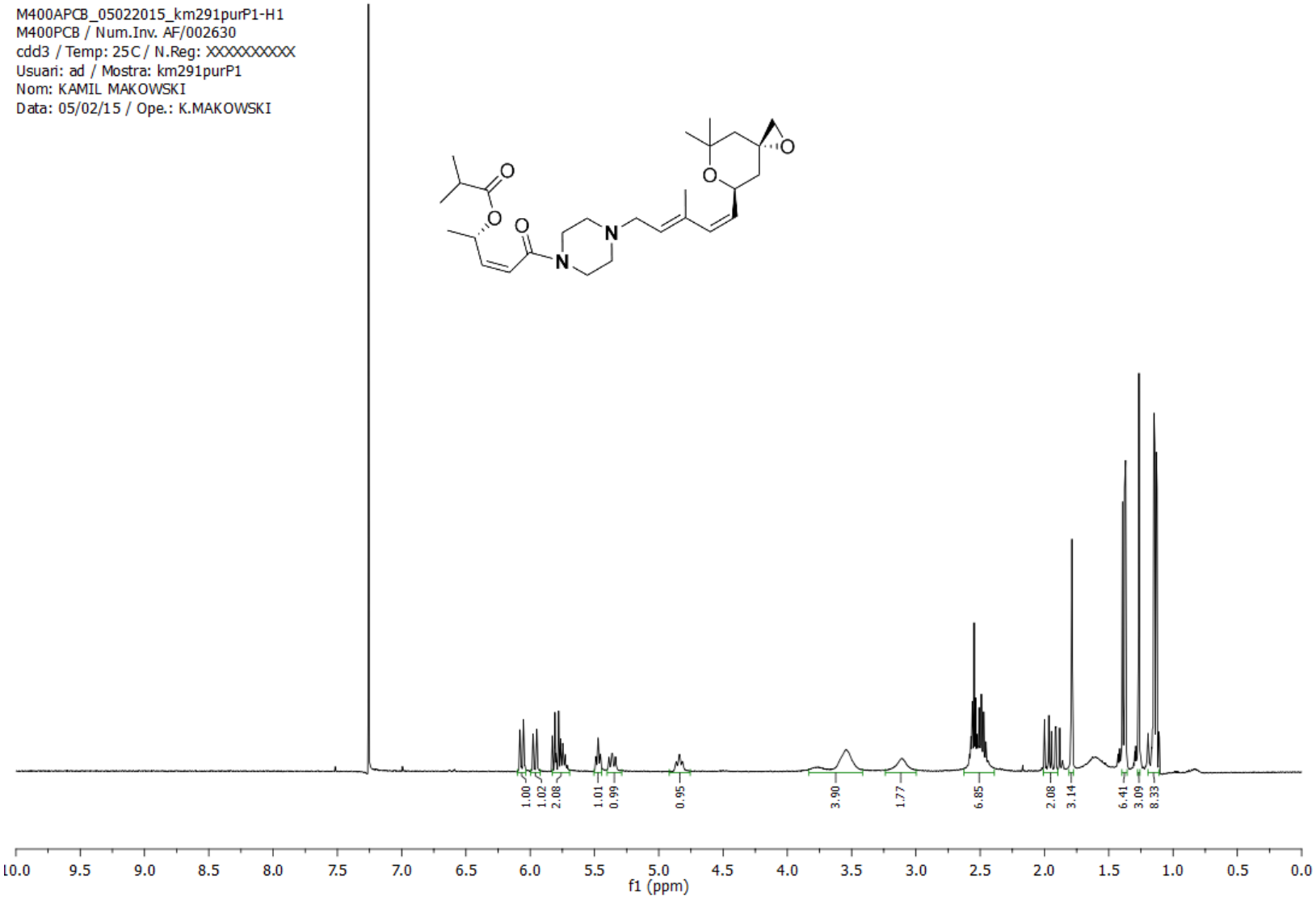


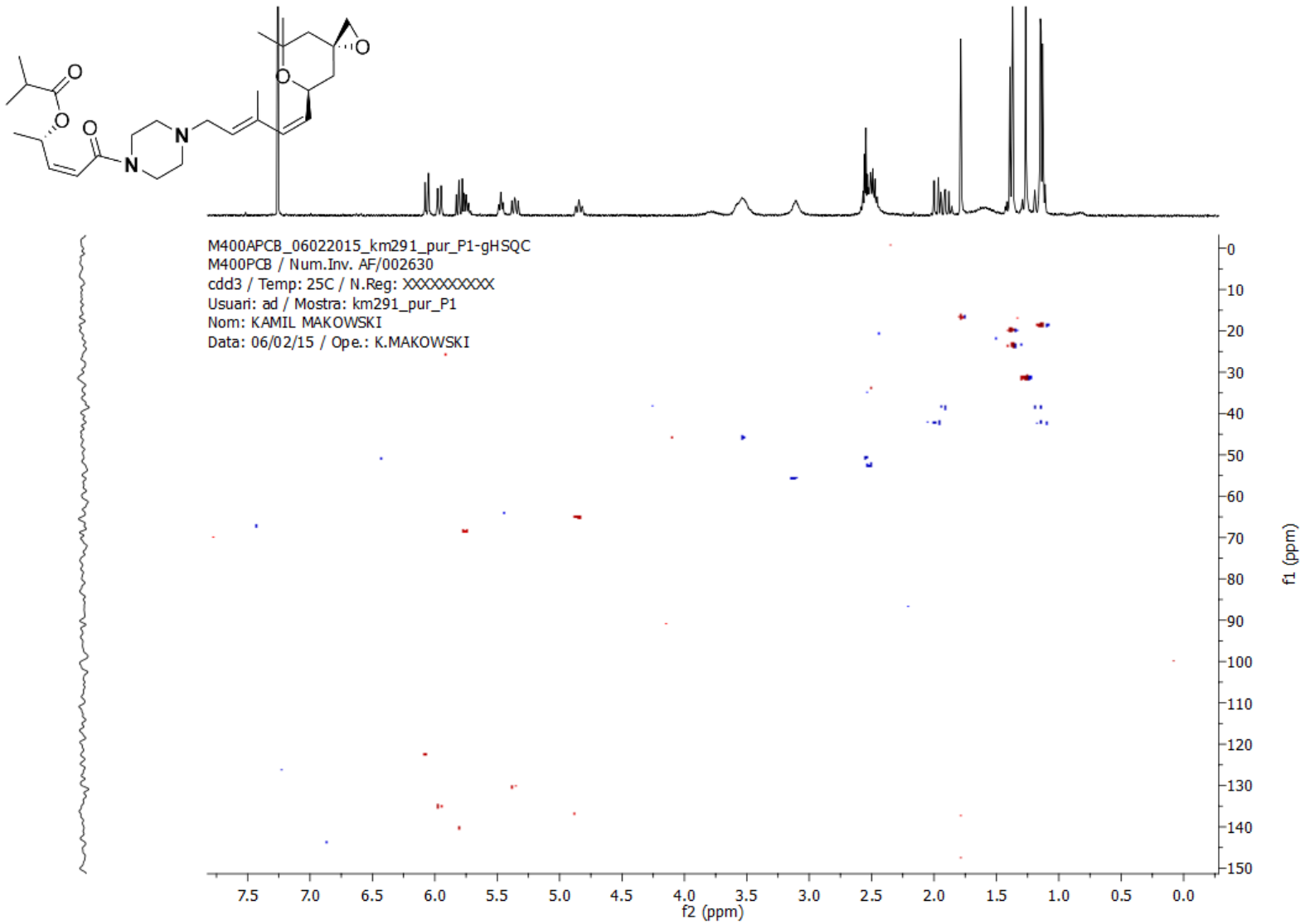
M400APCB_06022015_km291pur_P2-C13
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km291pur_P2
Nom: KAMIL MAKOWSKI
Data: 05/02/15 / Ope.: K.MAKOWSKI



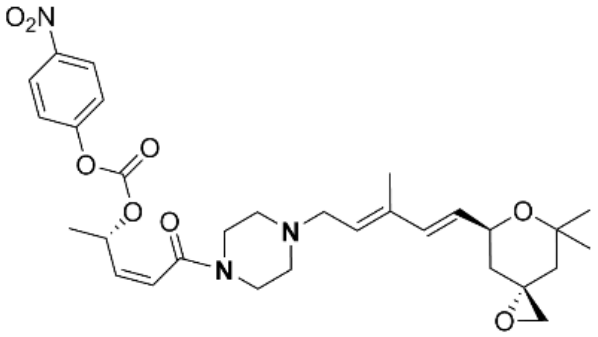


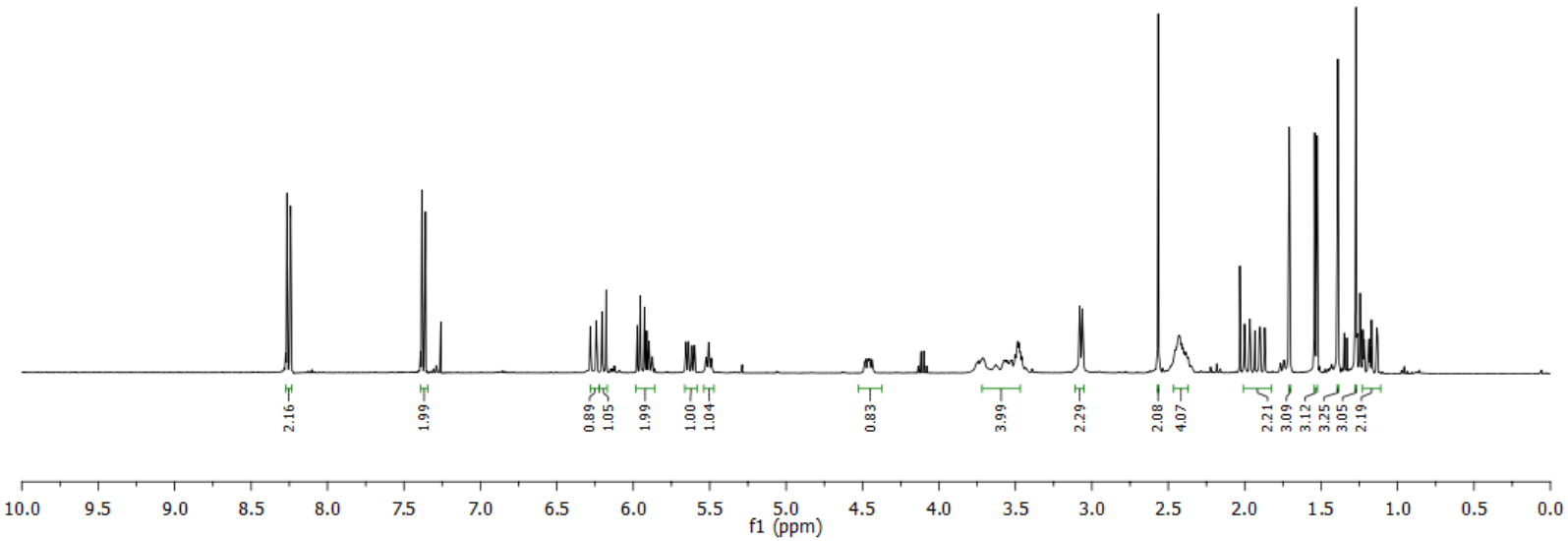

M400APCB_06022015_km291pur_P2-gHSQC
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km291pur_P2
Nom: KAMIL MAKOWSKI
Data: 05/02/15 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)


M400APCB_05022015_km291purP1-H1
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km291purP1
Nom: KAMIL MAKOWSKI
Data: 05/02/15 / Ope.: K.MAKOWSKI
O
O
O
N
N
O
O
1.00
1.02
2.08
1.01
0.99
0.95
3.90
1.77
6.85
2.08
3.14
6.41
3.09
8.33
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

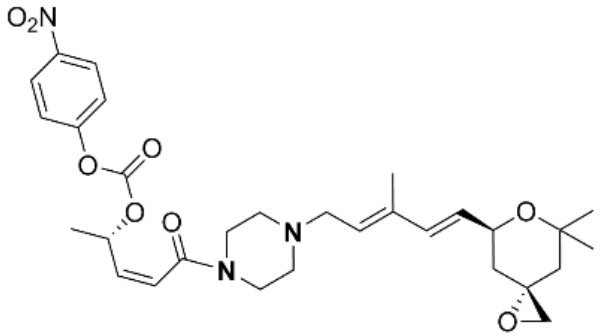

M400APCB_06022015_km291_pur_P1-gHSQC
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km291_pur_P1
Nom: KAMIL MAKOWSKI
Data: 06/02/15 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

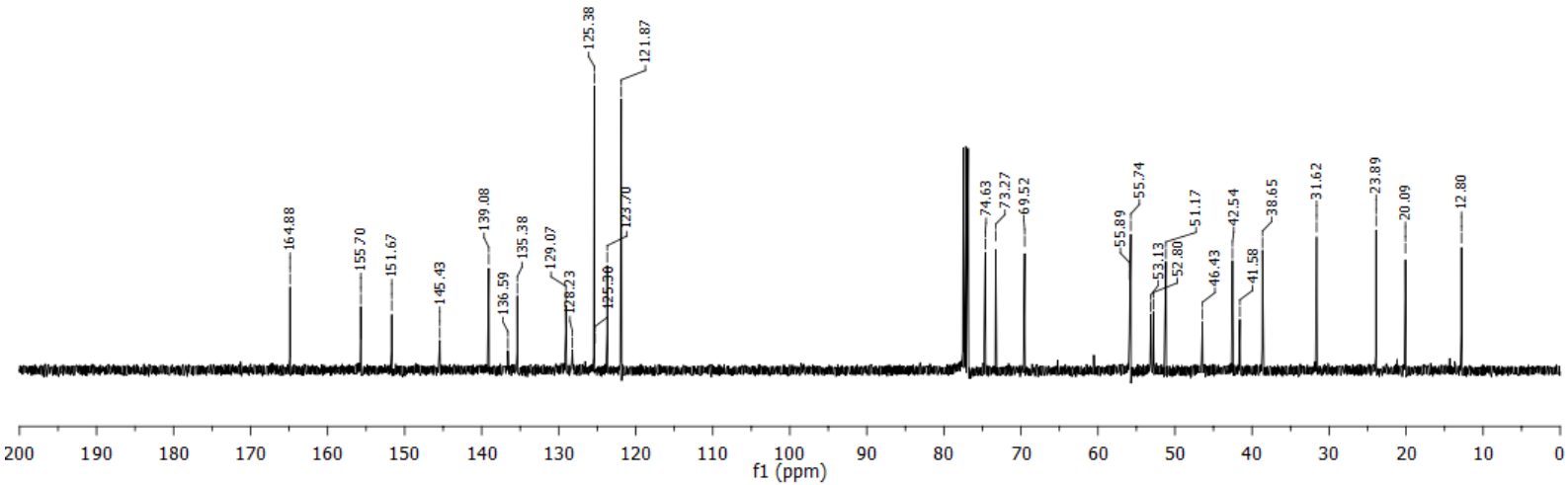
M400PCB / Num.Inv. AF/002630
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km301f1
Nom: KAMIL MAKOWSKI
Data: 10/02/15 / Ope.: K.MAKOWSKI

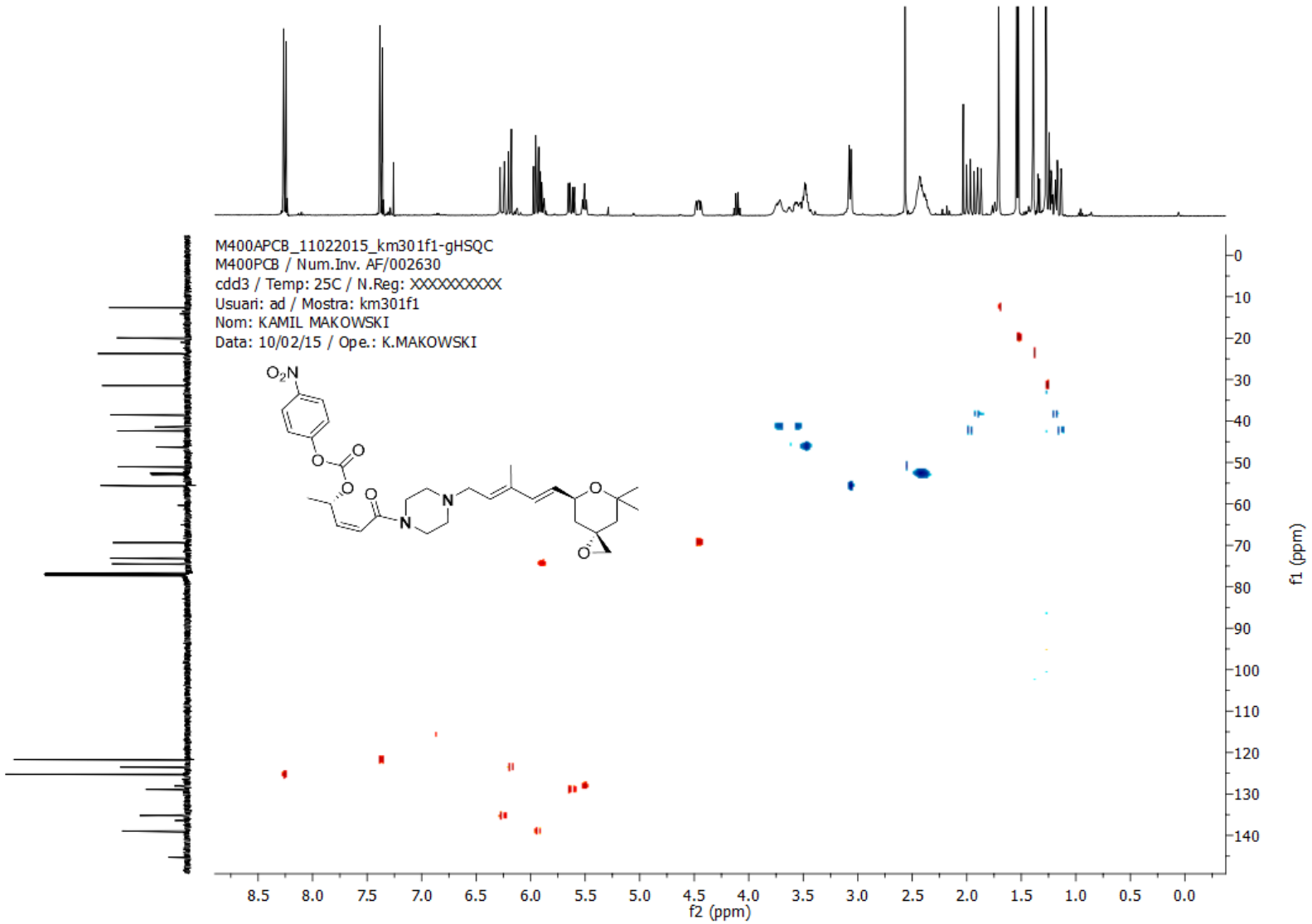




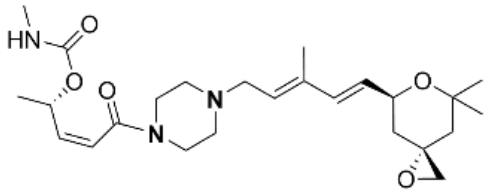
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km301f1
Nom: KAMIL MAKOWSKI
Data: 10/02/15 / Ope.: K.MAKOWSKI



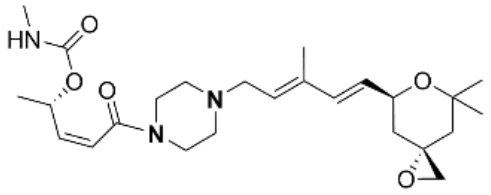


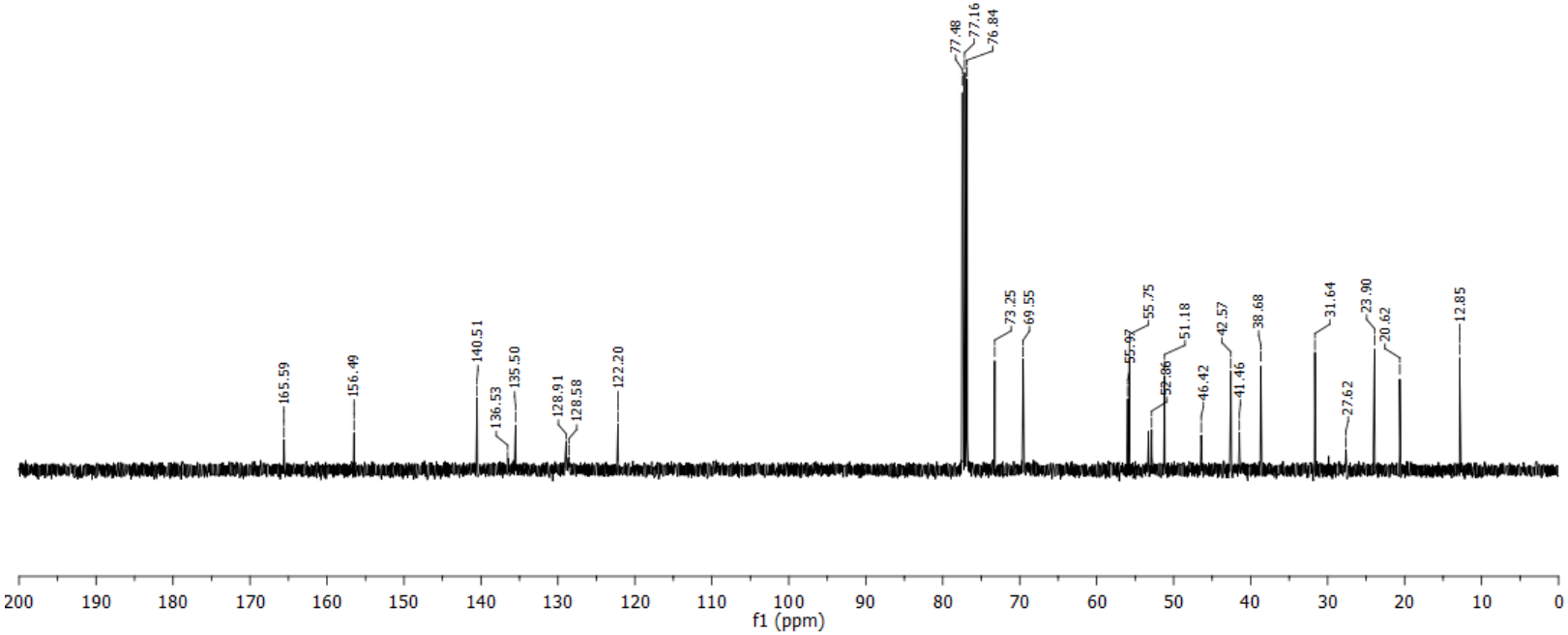

M400APCB_11022015_km301f1-gHSQC
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km301f1
Nom: KAMIL MAKOWSKI
Data: 10/02/15 / Ope.: K.MAKOWSKI
O2N
O
O
O
O
N
N
O
O
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
f1 (ppm)

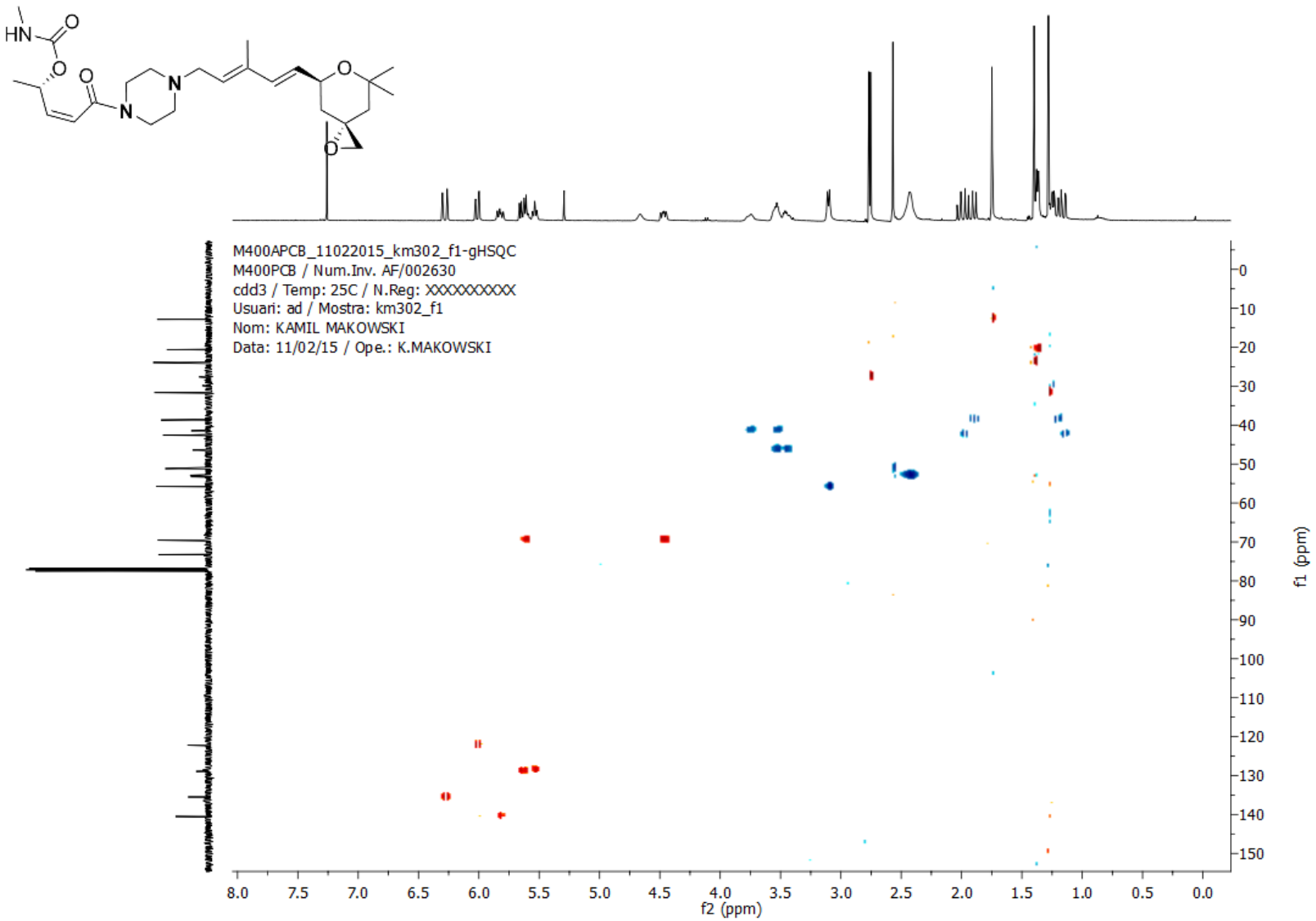
M400APCB_11022015_KM302F1-H1
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: KM302F1
Nom: KAMIL MAKOWSKI
Data: 11/02/15 / Ope.: K.MAKOWSKI



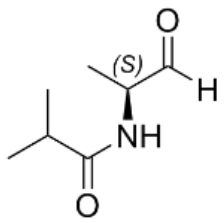
M400APCB_11022015_km302_f1-C13
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km302_f1
Nom: KAMIL MAKOWSKI
Data: 11/02/15 / Ope.: K.MAKOWSKI

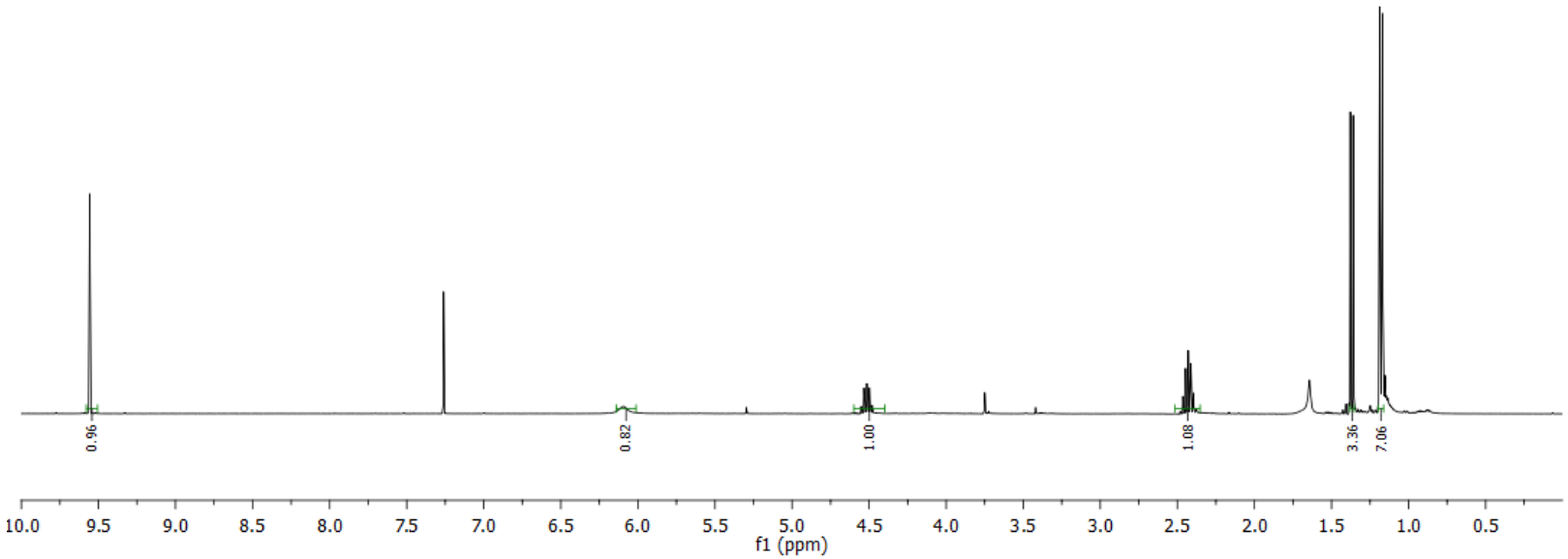




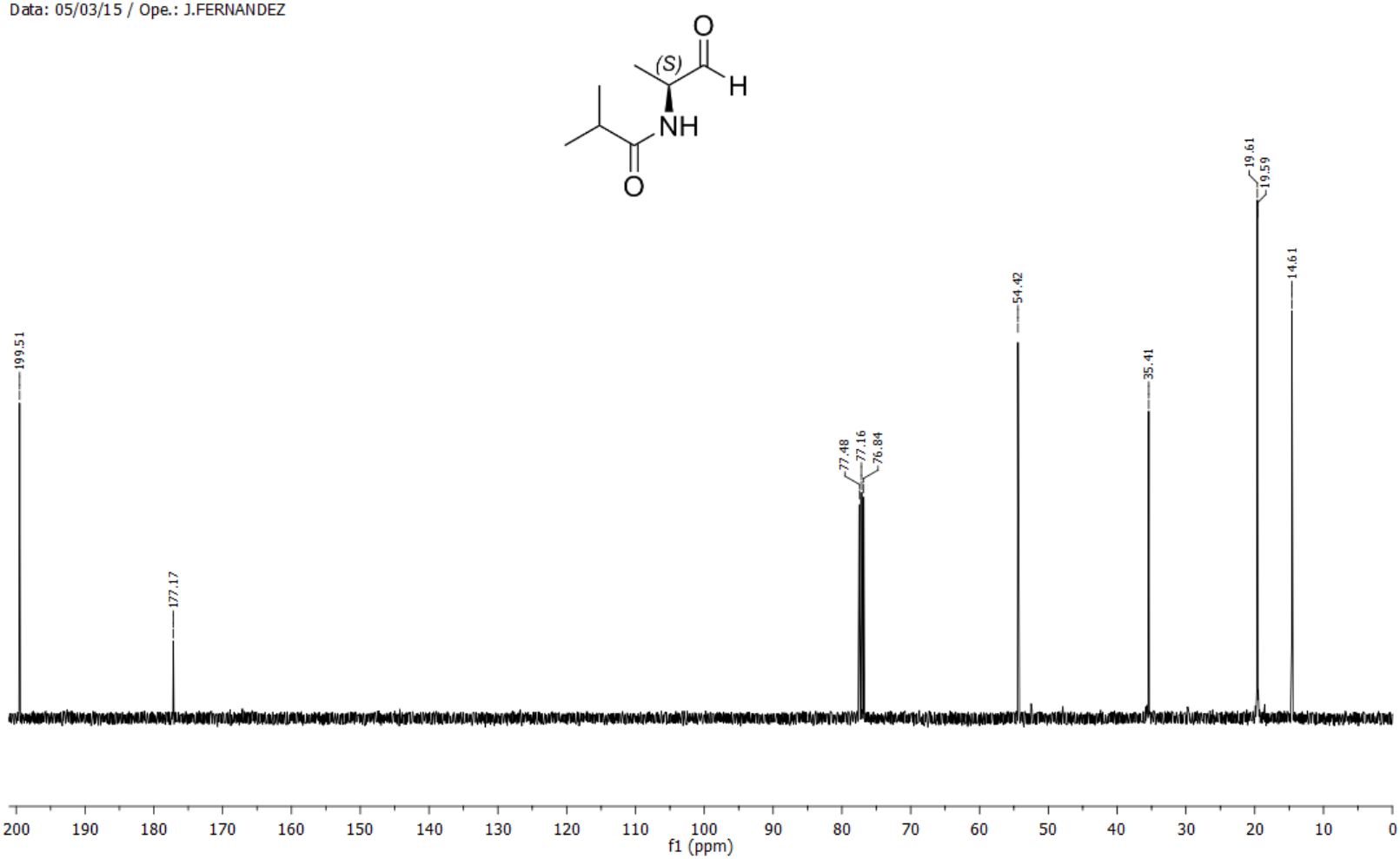

M400APCB_11022015_km302_f1-gHSQC
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km302_f1
Nom: KAMIL MAKOWSKI
Data: 11/02/15 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

M400APCB_09072014_km174f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km174f1
Nom: KAMIL MAKOWSKI
Data: 09/07/14 / Ope.: K.MAKOWSKI

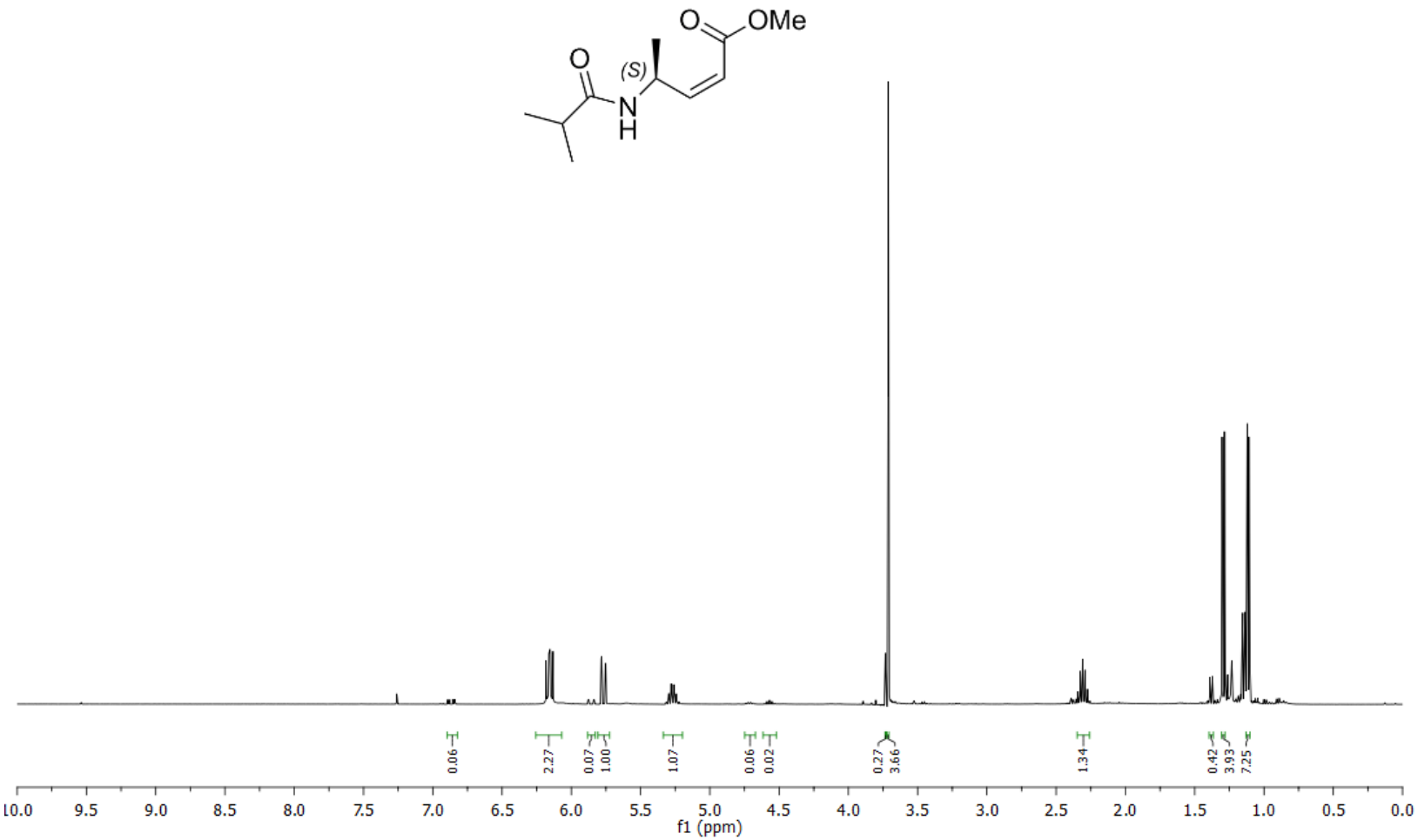




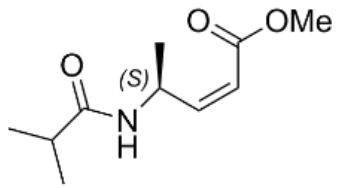
M400APCB_05032015_km174f1-C13
M400PCB / Num.Inv. AF/002630
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km174f1
Nom: JOSE-ANTONIO FERNANDEZ MATEOS
Data: 05/03/15 / Ope.: J.FERNANDEZ

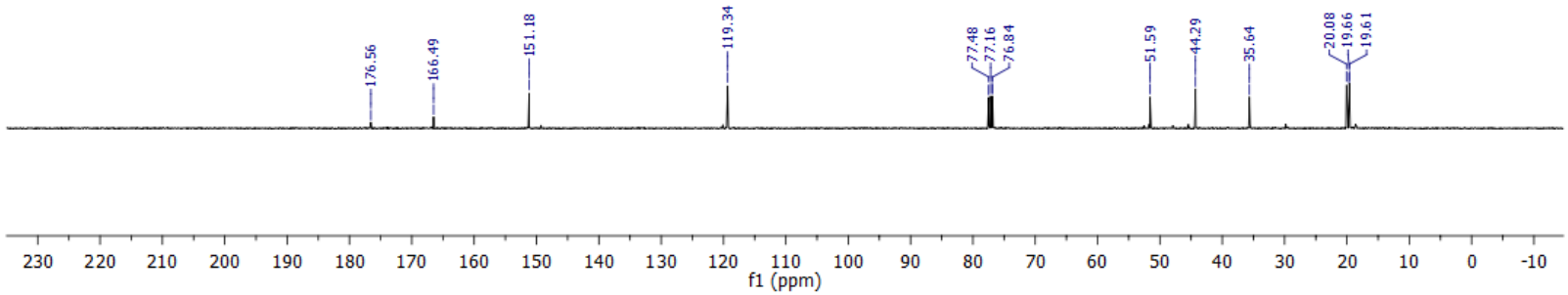


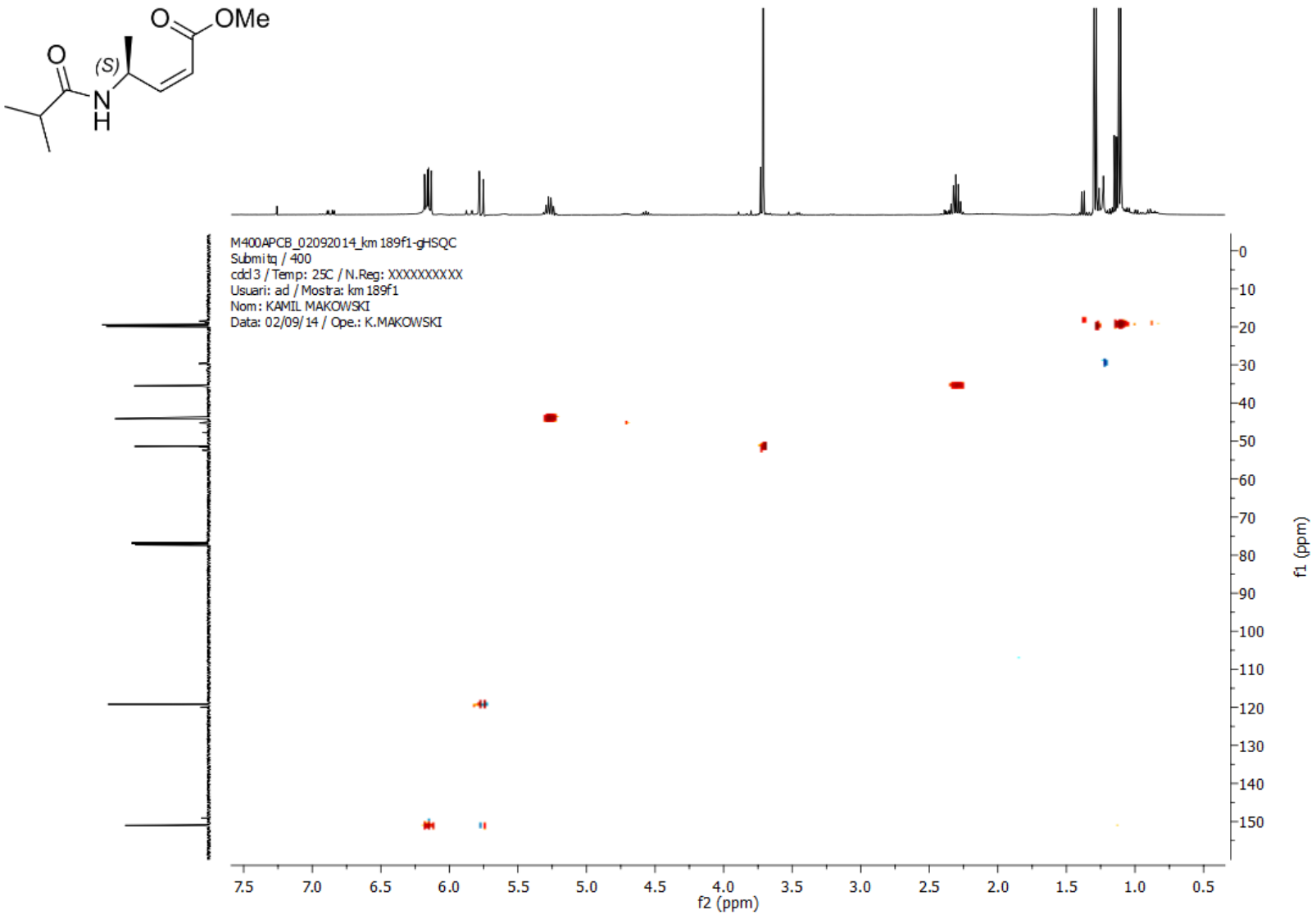
M400APCB_02092014_km189f1-H1
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km189f1
Nom: KAMIL MAKOWSKI
Data: 02/09/14 / Ope.: K.MAKOWSKI



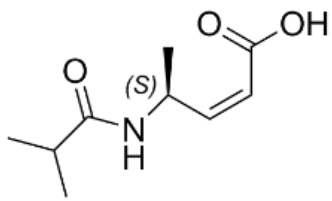
M400APCB_02092014_km189f1-C13
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km189f1
Nom: KAMIL MAKOWSKI
Data: 02/09/14 / Ope.: K.MAKOWSKI



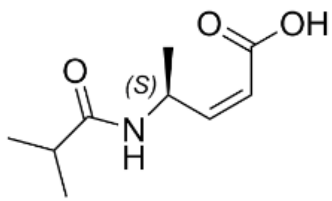



O
OMe
O
(S)
N
H
M400APCB_02092014_km189f1-gHSQC
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km189f1
Nom: KAMIL MAKOWSKI
Data: 02/09/14 / Ope.: K.MAKOWSKI
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

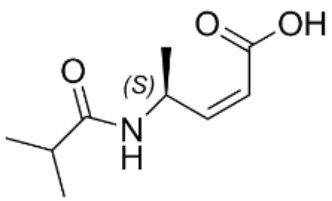
M400APCB_21112014_km246c2f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km246c2f1
Nom: KAMIL MAKOWSKI
Data: 21/11/14 / Ope.: K.MAKOWSKI

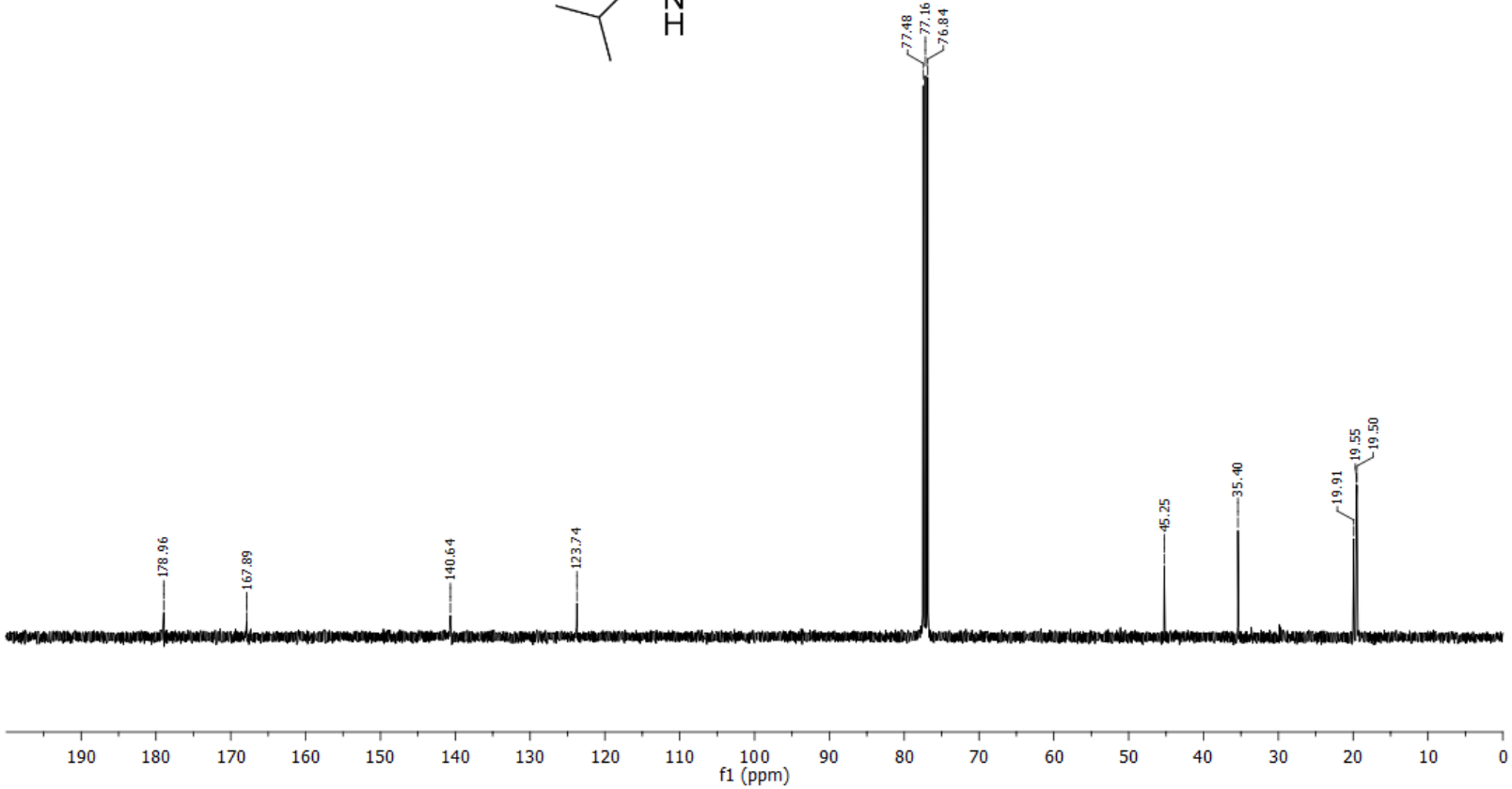


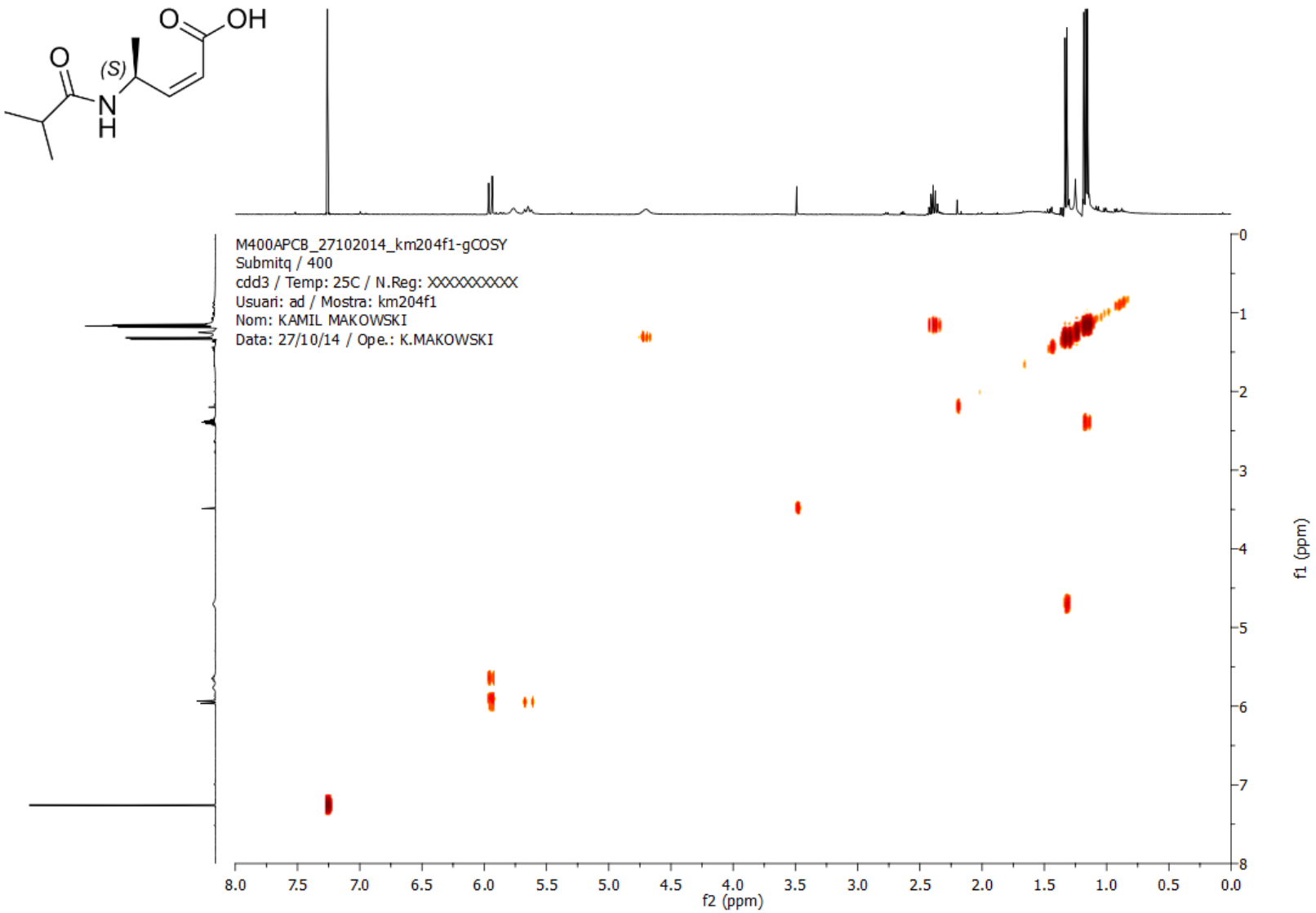
M400APCB_21112014_km246c2f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km246c2f1
Nom: KAMIL MAKOWSKI
Data: 21/11/14 / Ope.: K.MAKOWSKI



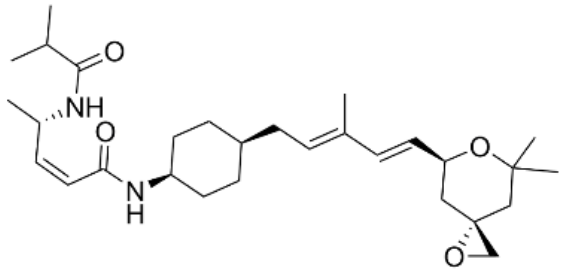
M400APCB_22112014_km246c2f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km246c2f1
Nom: KAMIL MAKOWSKI
Data: 21/11/14 / Ope.: K.MAKOWSKI



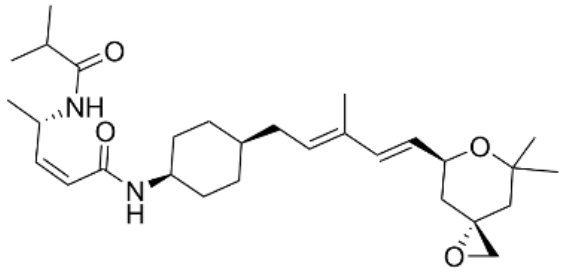


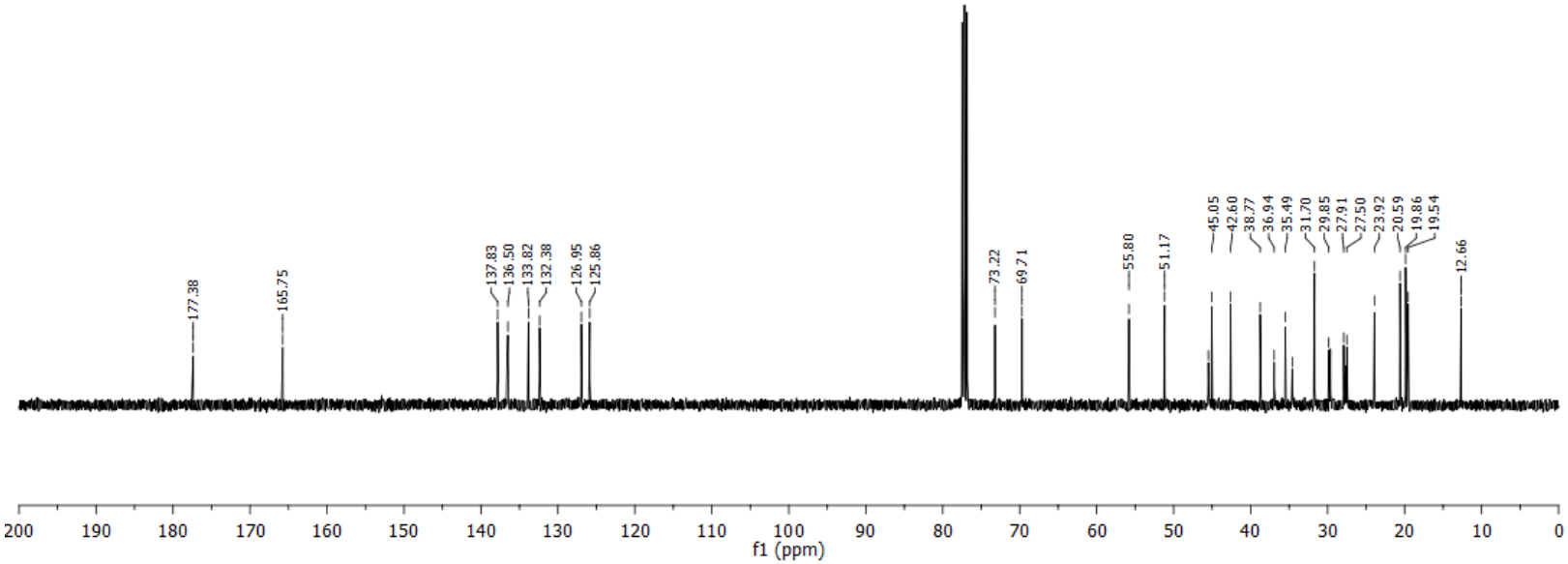

O
OH
O
(S)
N
H
M400APCB_27102014_km204f1-gCOSY
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km204f1
Nom: KAMIL MAKOWSKI
Data: 27/10/14 / Ope.: K.MAKOWSKI
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
f1 (ppm)

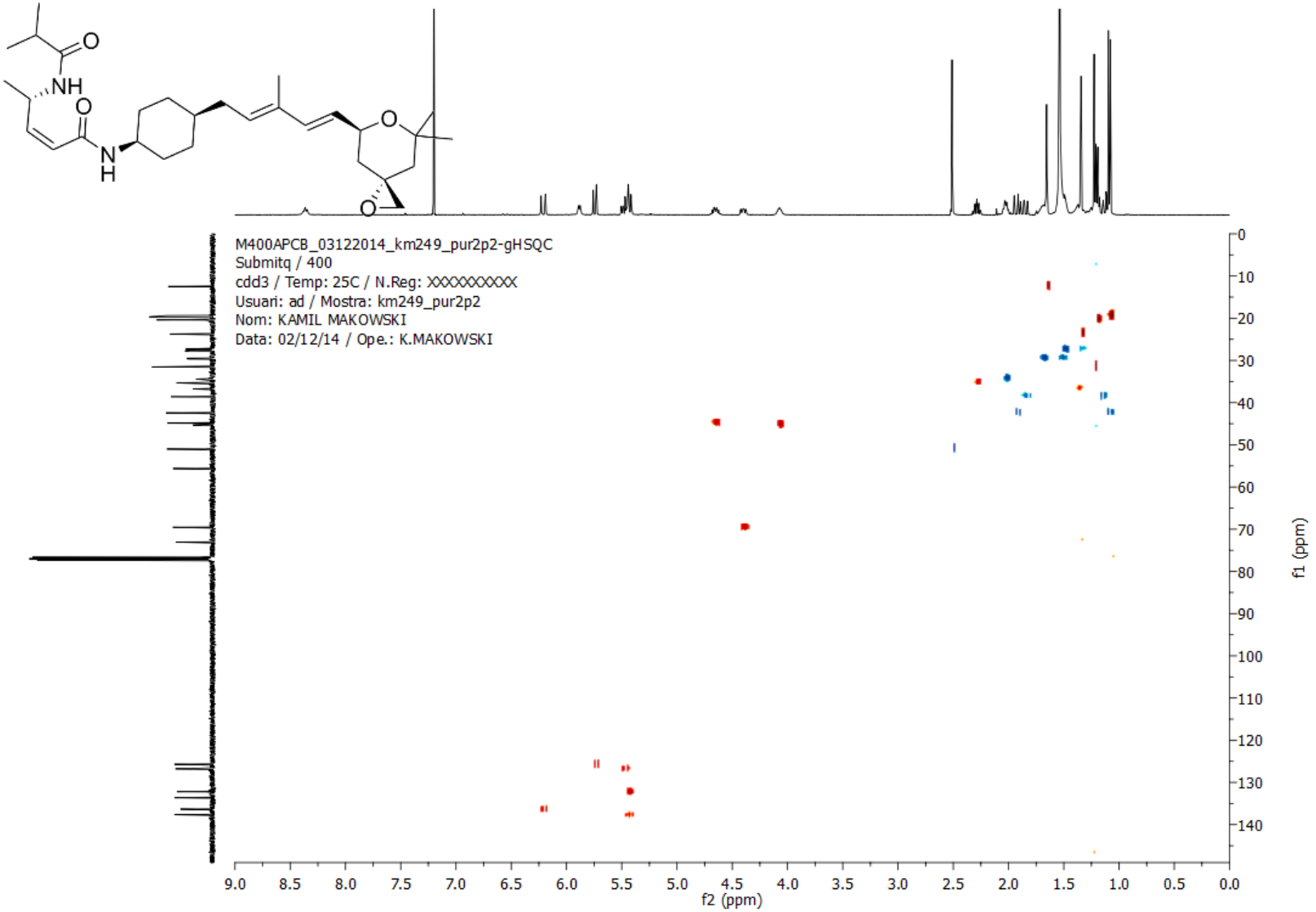
M400APCB_01122014_km249_pur2_p2-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km249_pur2_p2
Nom: KAMIL MAKOWSKI
Data: 01/12/14 / Ope.: K.MAKOWSKI

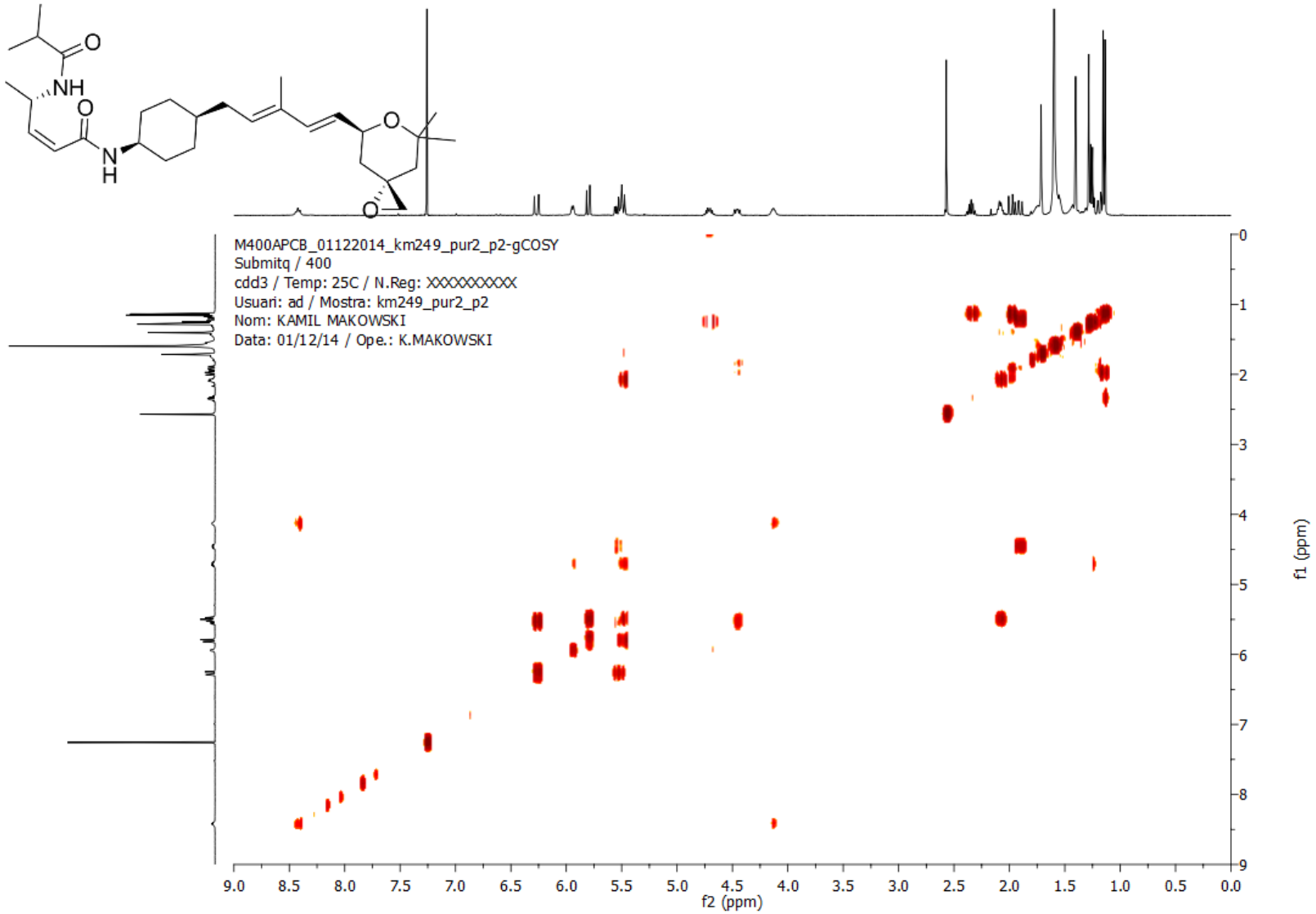


M400APCB_03122014_km249_pur2p2-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km249_pur2p2
Nom: KAMIL MAKOWSKI
Data: 02/12/14 / Ope.: K.MAKOWSKI

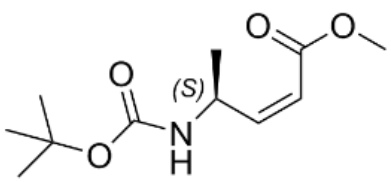




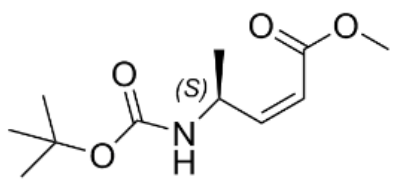

M400APCB_03122014_km249_pur2p2-gHSQC
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km249_pur2p2
Nom: KAMIL MAKOWSKI
Data: 02/12/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

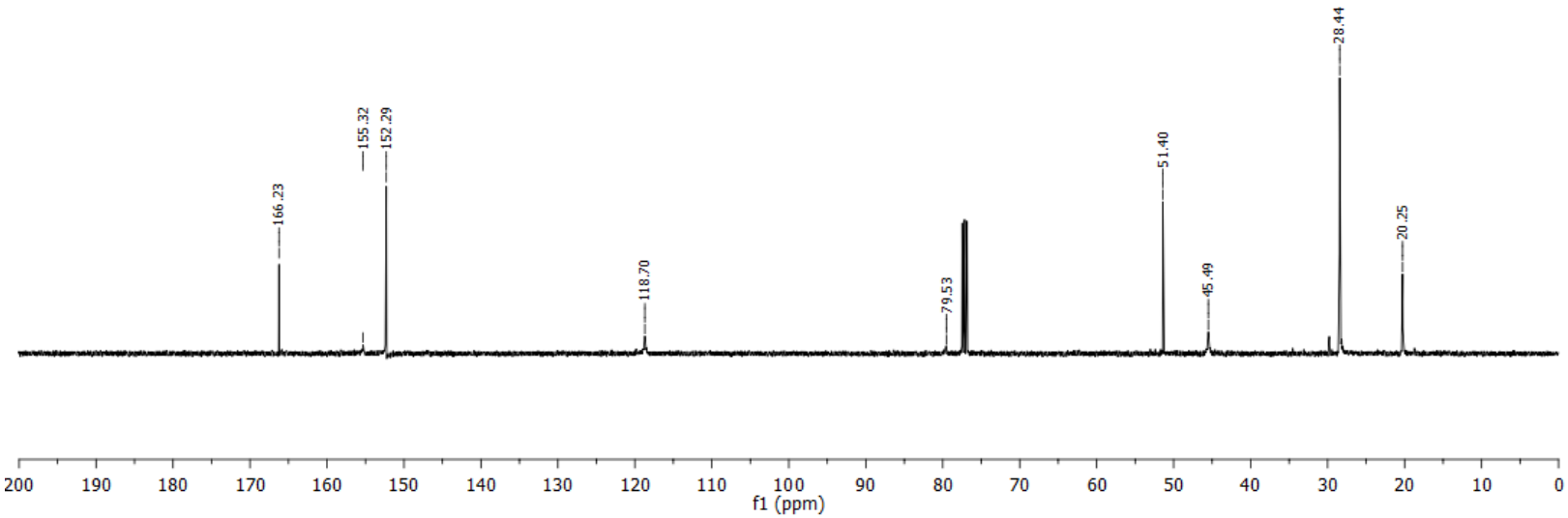

M400APCB_01122014_km249_pur2_p2-gCOSY
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km249_pur2_p2
Nom: KAMIL MAKOWSKI
Data: 01/12/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

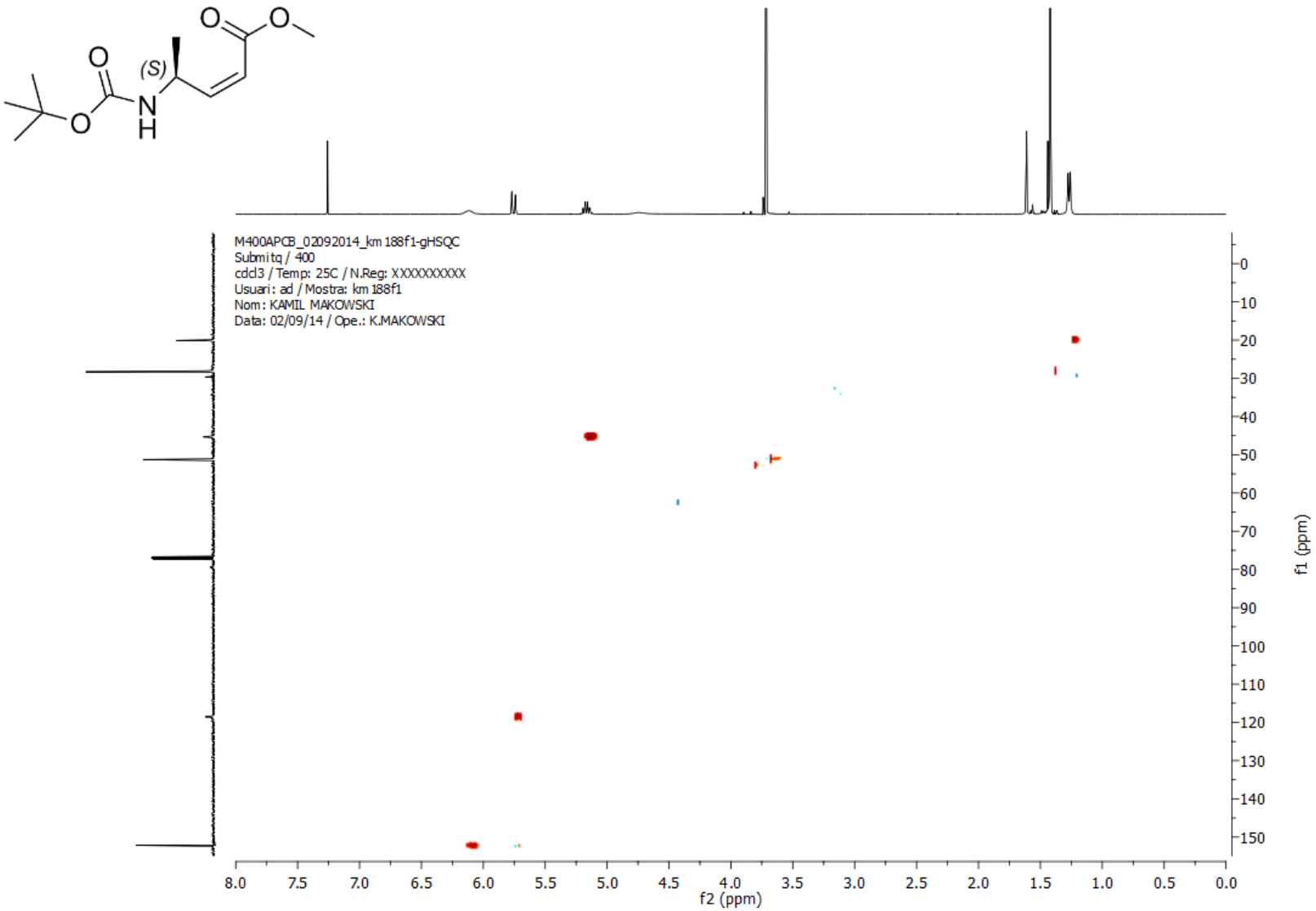
M400APCB_02092014_km192f1-H1
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km192f1
Nom: KAMIL MAKOWSKI
Data: 02/09/14 / Ope.: K.MAKOWSKI



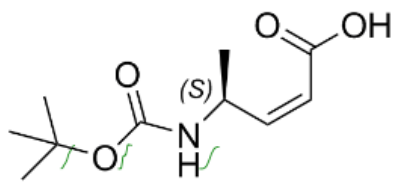
M400APCB_02092014_km188f1-C13
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km188f1
Nom: KAMIL MAKOWSKI
Data: 02/09/14 / Ope.: K.MAKOWSKI



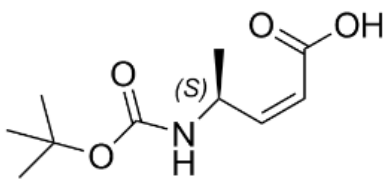



M400APCB_02092014_km188f1-gHSQC
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km188f1
Nom: KAMIL MAKOWSKI
Data: 02/09/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)

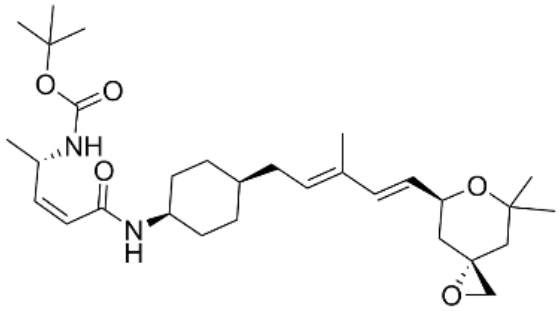
Submitq / 400
cd3od / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km226_f2_metoh
Nom: KAMIL MAKOWSKI
Data: 15/12/14 / Ope.: K.MAKOWSKI



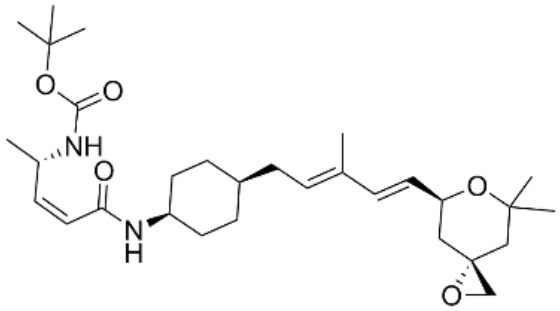
M400APCB_05032015_km226_f2_meoh-C13
M400PCB / Num.Inv. AF/002630
cd3od / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km226_f2_meoh
Nom: JOSE-ANTONIO FERNANDEZ MATEOS
Data: 05/03/15 / Ope.: J.FERNANDEZ

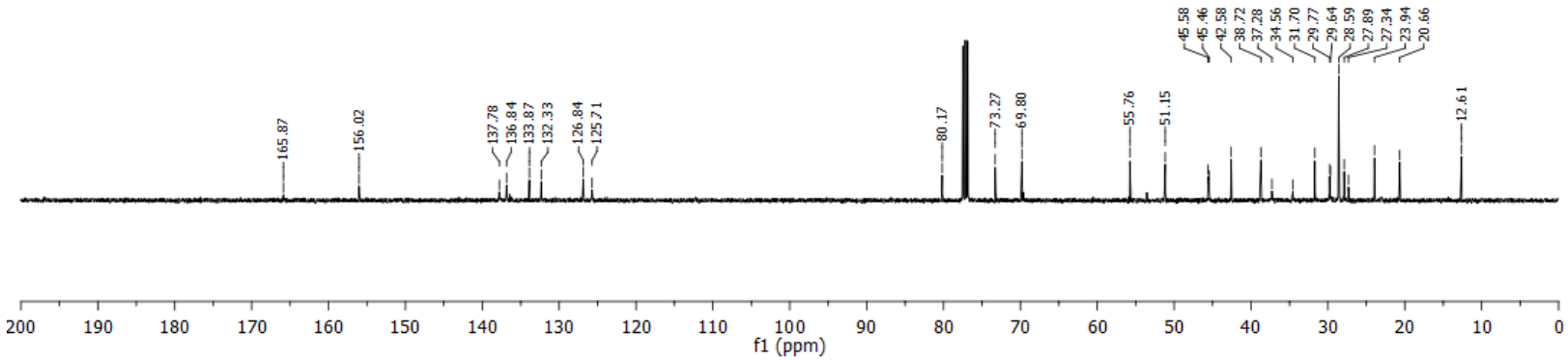


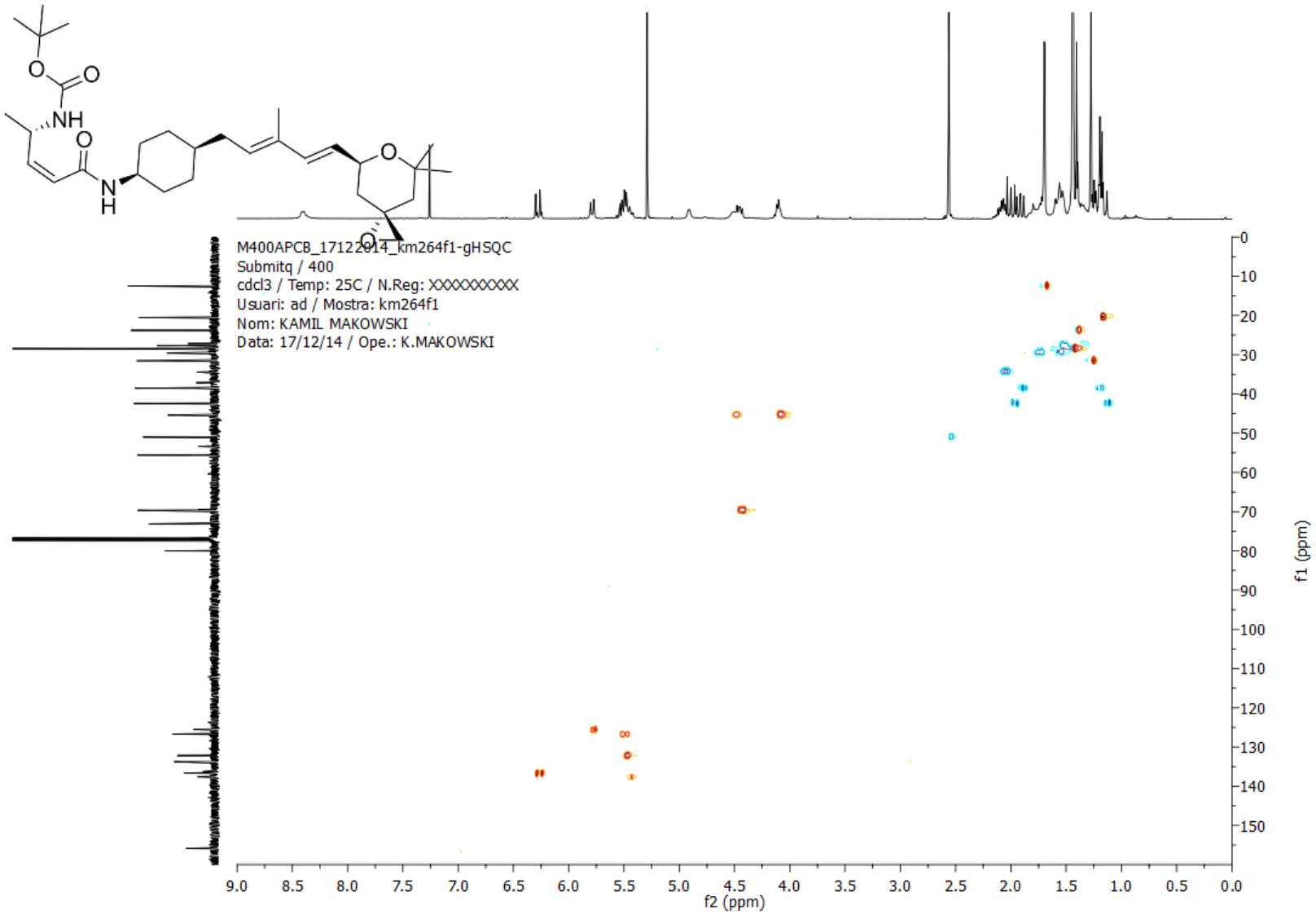
M400APCB_17122014_km264f1-H1
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km264f1
Nom: KAMIL MAKOWSKI
Data: 17/12/14 / Ope.: K.MAKOWSKI



M400APCB_17122014_km264f1-C13
Submitq / 400
cdd3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km264f1
Nom: KAMIL MAKOWSKI
Data: 17/12/14 / Ope.: K.MAKOWSKI






M400APCB_17122014_km264f1-gHSQC
Submitq / 400
cdcl3 / Temp: 25C / N.Reg: XXXXXXXXXX
Usuari: ad / Mostra: km264f1
Nom: KAMIL MAKOWSKI
Data: 17/12/14 / Ope.: K.MAKOWSKI
f2 (ppm)
f1 (ppm)