

Supporting information

Synthesis of 4*H*-1,4-oxazines as transthyretin amyloid fibril inhibitors

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Table of contents

General information

Methods and Materials

Preparation of the receptor and ligands using GAUSSVIEW

Docking of ligands using AutoDock 4.0

Analysis of results using AutoDockTools or Discovery Studio

Fibril formation assay.....2

General procedure for the synthesis of *N,N*-bis(phenacyl)anilines (2) and characterization data.....4

General procedure for the synthesis of 2,4,6-triaryl-4*H*-1,4-oxazines (1) and chracterization data.....7

References.....10

NMR spectra of final compounds 1.....12

General information

EXPERIMENTAL SECTION

Methods and Materials

All chemicals were used as purchased, without further purification. Thin-layer chromatography (TLC) was conducted on silica gel 60 F254 plates (Merck KGaA). Melting points were determined on an XT-5A digital melting point apparatus and are uncorrected. IR spectra were obtained using KBr pellets on a Bruker VERTEX 70 spectrometer. ¹H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer in CDCl₃ or Acetone-d₆, with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra were obtained on a VG 70SE mass spectrometer (Manchester, UK), which was operated in electron impact or electrospray ionization modes.

Preparation of the receptor and ligands using GAUSSVIEW

The three-dimensional structures of ligands were constructed using standard bond lengths and angles using GAUSSVIEW 3.09 software. Geometry optimizations were carried out with the semi-empirical AM1 method, and output files were minimized by using the density functional (DFT) method, applying the B3LYP (Becke, Lee, Yang and Parr) correlation function in the second optimization. Gasteiger partial charges were assigned using AutoDock Tools. The crystal structure of the TTR receptor in a complex with T4 was retrieved from the Protein Data Bank (PDB entry code 1ICT). After removing the inhibitor from the complex, polar hydrogen atoms and Kollman-united charges were added to the macromolecule.

Docking of ligands using AutoDock 4.0²⁷

Docking was carried out using one of several search methods. The most efficient method is a Lamarckian genetic algorithm (LGA), which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first builds a population of individuals, each 'gene' being a different random conformation of the docked compound. The local search algorithm then performs energy minimizations on a user-specified proportion of the population of individuals. If the energy of the new individual is lower than that of the old individual, the new one is automatically accepted as the next step in docking. However, traditional genetic algorithms and simulated annealing are also available. For typical systems, AutoDock is run several times to produce several docked

conformations. Analysis of the predicted energy and the consistency of results reveals the best solution. For docking, a grid spacing of 0.375 Å and 40×40×40 points were used. Given the known location of the T4 binding site, the cubic grid box was centred in and encompassed the catalytic active site. The grid centre was (x, y and z) 15.743, -45.392, and 42.712. Docked conformations were generated using LGA, with an initial population of 150 structures. Other parameters were left at their default values. For the assessment, the same docking protocol was used on the reference inhibitor T4. The first-ranked docked conformation and the lowest-energy conformation of the most populated cluster were selected as the binding conformation.

Analysis of results using AutoDockTools or Discovery Studio

AutoDockTools includes a number of methods for analysing the results of docking simulations, including tools for clustering results by conformational similarity, visualizing conformations, and visualizing interactions between ligands and proteins. In this study, model analyses were performed using ACCELRY'S DS VISUALIZER 3.1 software.

Fibril formation assay

Each compound was dried and then dissolved at 7.2 mM in spectroscopic grade DMSO as a primary stock solution. For assays in which the final inhibitor concentration was 7.2 μM and 3.6 μM, the initial 7.2mM inhibitor stock solution was diluted to 1.4mM and 720 μM solutions respectively with DMSO. A typical sample for the measurement of fibril inhibition was prepared by micropipetting 5 μL of inhibitor solution into an Eppendorf tube and adding 500 μL of TTR at 0.4 mg/mL in 10 mM sodium phosphate, 100 mM KCl and 1 mM EDTA (pH 7.6). The inhibitor-TTR solutions were vortexing and incubated for 30 min at 37°C to allow plenty of time for potential inhibitors to bind to TTR. Then, the solutions were rendered acidic and amyloidogenic by the addition of 495uL of 200mM acetate buffer, 100mM KCl, 1mM EDTA (pH 4.2), which was added to each solution to yield a final pH of 4.4. These solutions were incubated for 72h to evaluate inhibitor efficacy. The Eppendorf tube was then vortexed again to ensure a uniform suspension, and the turbidity of the suspension at 400 nm was measured. All compounds were soluble and none absorbed appreciably at 400nm, ensuring that turbidity was the result of TTR amyloid formation. All samples were done in triplicate. The ratio of the turbidities of the sample of interest to that of a sample prepared in the same way but lacking any inhibitor multiplied by 100% gave the percent fibril formation. In this study, inhibiting ratio is one hundred

percent minus percentage fibril formation.

General procedure for the synthesis of *N,N*-bis(phenacyl)anilines (2**) and characterization data**

A mixture of phenacyl bromide (12 mmol), Na₂CO₃ (12 mmol), and aniline (6.0 mmol) was stirred under reflux at 110°. The progress of the reaction was monitored by TLC. After the indicated reaction time, the solid products **2** were recrystallized from ethanol.

Diphenacylaniline (**2a**)¹⁶

Yield 65%; mp 196.2-197.4 °C. Lit. 198 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.02-8.04 (d, J=7.2Hz, 4H, Ar-H), 7.60-7.64 (t, J=7.6Hz, 2H, Ar-H), 7.49-7.53 (t, J=7.6Hz, 4H, Ar-H), 7.14-7.18 (t, J=7.6Hz, 2H, Ar-H), 6.71-6.75 (t, J=7.6Hz, 1H, Ar-H), 6.54-6.56 (d, J=8.4Hz, 2H, Ar-H), 4.95 (s, 4H, N-CH₂).

N,N-Bis(4-methylphenacyl)anilines (**2b**)¹⁶

Yield 62%; mp 103.7-104.5 °C. Lit. 103 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.92-7.94 (d, J=8.0Hz, 4H, Ar-H), 7.29-7.31 (d, J=8.0Hz, 4H, Ar-H), 7.12-7.16 (t, J=7.6Hz, 2H, Ar-H), 6.72-6.74 (d, J=7.2Hz, 1H, Ar-H), 6.51-6.53 (d, J=8.4Hz, 2H, Ar-H), 2.43 (s, 6H, CH₃) 4.92 (s, 4H, N-CH₂).

N,N-Bis(4-chlorophenacyl)anilines (**2c**)¹⁶

Yield 76%; mp 108.1-109.8 °C. Lit. 110 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.95-7.97 (d, J=8.4Hz, 4H, Ar-H), 7.47-7.49 (d, J=8.4Hz, 4H Ar-H), 7.15-7.19(t, J=8.0Hz, 2H, Ar-H), 6.74-6.78 (t, J=7.2Hz, 1H, Ar-H), 6.51- 6.53 (d, J=8.0Hz, 2H, Ar-H), 4.90 (s, 4H, N-CH₂).

N,N-Bis(4-methoxyphenacyl)anilines (**2d**)¹⁶

Yield 60%; mp 150.9-151.2 °C. Lit. 152 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.00-8.02 (d, J=8.8Hz, 4H, Ar-H), 7.12-7.16 (t, J=8.0Hz, 2H, Ar-H), 6.95-6.98 (d, J=8.8Hz, 4H, Ar-H), 6.69-6.73 (t, J=7.6Hz, 1H, Ar-H), 6.52-6.54 (d, J=8.4Hz, 2H Ar-H), 3.88 (s, 6H, OCH₃) 4.89 (s, 4H, N-CH₂).

N,N-Bis(phenacyl)-3-methylanilines (**2e**)

Yield 72%; mp 133.2-134.5 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.00-8.05 (m, 4H, Ar-H), 7.66-7.68 (m, 2H, Ar-H), 7.44-7.57 (m, 4H, Ar-H), 7.10-7.12 (d, J=8.8Hz, 2H, Ar-H), 6.46-6.48 (d, J=8.4, 2H, Ar-H), 4.95 (s, 4H, N-CH₂), 2.21 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 196.5, 148.8, 140.5, 138.9, 133.5, 130.1, 129.5, 129.1, 118.3, 115.9, 112.5, 58.2, 21.4; HRMS (EI): (m/z) calcd for C₂₃H₂₁NO₂: 343.1632 [M]⁺; found 343.1653.

N,N-Bis(4-methylphenacyl)-3-methylanilines (**2f**)

Yield 71%; mp 168.7-169.3 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.00-8.08 (m, 4H Ar-H), 7.44-7.58 (m, 4H Ar-H), 7.09-7.16 (m, 2H, Ar-H), 6.43-6.45 (t, J=8.8Hz, 2H, Ar-H), 4.96 (s, 4H, N-CH₂), 2.43 (s, 6H, CH₃), 2.21 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 196.2, 148.7, 144.5, 139.0, 132.4, 129.6, 129.3, 129.1, 117.9, 115.5, 112.4, 57.5, 21.7, 21.2; HRMS (EI): (m/z) calcd for C₂₅H₂₅NO₂: 371.1923 [M]⁺; found 371.1948.

N,N-Bis(4-chlorinephenacyl)-3-methylanilines (**2g**)

Yield 78%; mp 126.7-127.3 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.90-7.93 (m, 4H Ar-H), 7.43-7.50 (m, 4H Ar-H), 7.05-7.10 (m, 2H, Ar-H), 6.42-6.44 (d, J=8.4Hz, 2H, Ar-H), 4.92 (s, 4H, N-CH₂), 2.31 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 195.6, 148.3, 140.4, 139.1, 133.3, 129.9, 129.3, 129.1, 118.4, 115.6, 112.5, 58.0, 21.5; HRMS (EI): (m/z) calcd for C₂₃H₁₉Cl₂NO₂: 411.0822 [M]⁺; found 411.0853.

N,N-Bis(4-methoxyphenacyl)-3-methylanilines (**2h**)

Yield 64%; mp 153.9-155.5 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.02-8.04 (m, 4H Ar-H), 6.96-6.98 (m, 4H, Ar-H), 6.89-6.91 (m, 1H, Ar-H), 6.41-6.45 (m, 3H, Ar-H), 4.98 (s, 4H, N-CH₂), 3.65 (s, 6H, OCH₃), 2.23 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 194.7, 160.5, 148.5, 139.0, 133.7, 130.0, 129.7, 129.4, 118.6, 115.9, 112.4, 58.1, 57.0, 21.2; HRMS (EI): (m/z) calcd for C₂₅H₂₅NO₄: 403.1831 [M]⁺; found 403.1845.

N,N-Bis(phenacyl)-4-methylanilines (**2i**)¹⁶

Yield 73%; mp 153.2-154.1 °C. Lit. 158 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.00-8.02 (d, J=7.2Hz, 4H, Ar-H), 7.59-7.63 (t, J=7.6Hz, 2H, Ar-H), 7.47-7.51 (t, J=7.6Hz, 4H, Ar-H), 6.76-6.74 (d, 8.8Hz, 2H, Ar-H), 6.56-6.58 (d, J=8.8Hz, 2H, Ar-H), 4.95 (s, 4H, N-CH₂), 2.24 (s, 3H, CH₃).

N,N-Bis(4-methylphenacyl)-4-methylanilines(**2j**)¹⁶

Yield 70%; mp 142.7-144.3 °C. Lit. 128 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.90-7.92 (d, J=8.0Hz, 4H, Ar-H), 7.28-7.30 (d, J=8.0Hz, 4H, Ar-H), 6.94-6.96(d, J=8.0, 2H, Ar-H), 6.46-6.48 (d, J=8.4Hz, 2H, Ar-H), 4.90 (s, 4H, N-CH₂), 2.43 (s, 6H, CH₃), 2.20 (s, 3H, CH₃).

N,N-Bis(4-chlorinephenacyl)-4-methylanilines (**2k**)¹⁶

Yield 80%; mp 137.3-138.1 °C. Lit. 140 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.92-7.94 (d, J=7.6Hz, 4H, Ar-H), 7.32-7.34 (d, J=7.6Hz, 4H, Ar-H), 6.96-6.98 (d, J=8.4Hz, 2H, Ar-H), 6.48-6.50 (d, J=8.4Hz, 2H, Ar-H), 4.88 (s, 4H, N-CH₂), 2.23 (s, 3H, CH₃).

N,N-Bis(4-methoxyphenacyl)-4-methylanilines (**2l**)¹⁶

Yield 66%; mp 169.2-171.1 °C. Lit. 170 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.20-8.22 (d, J=8.8Hz, 4H, Ar-H), 7.95-7.97 (d, J=8.8Hz, 4H, Ar-H), 6.93-6.95 (d, J=8.0Hz, 2H, Ar-H), 6.40-6.42 (d, J=8.4Hz, 2H, Ar-H), 4.80 (s, 4H, N-CH₂), 3.90 (s, 6H, OCH₃), 2.20 (s, 3H, CH₃).

N,N-Bis(phenacyl)-4-chloroanilines (**2m**)

Yield 55%; mp 158.3-160.2 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.01-8.03 (d, J=7.6Hz, 4H Ar-H), 7.65-7.69 (t, J=7.2Hz, 2H, Ar-H), 7.55-7.57 (t, J=7.6Hz, 4H Ar-H), 7.08-7.10 (d, J=8.8Hz, 2H, Ar-H), 6.49-6.51 (d, J=8.8Hz, 2H, Ar-H), 5.03 (s, 4H, N-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 195.4, 148.3, 140.5, 133.2, 130.5, 129.8, 128.5, 123.8, 113.7, 58.5; HRMS (EI): (m/z) calcd for C₂₂H₁₈ClNO₂: 363.1004 [M]⁺; found 363.1035.

N,N-Bis(4-methylphenacyl)-4-chloroanilines (**2n**)

Yield 54%; mp 178.5-179.3 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.89-7.91 (d, J=8.0, 4H Ar-H), 7.28-7.30 (d, J=8.0Hz, 4H Ar-H), 7.07-7.09 (d, J=9.2Hz, 2H, Ar-H), 6.42-6.44 (d, J=9.2Hz, 2H, Ar-H), 4.89 (s, 4H, N-CH₂), 2.43 (s, 6H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 195.7, 147.2, 144.8, 132.5, 129.7, 129.5, 129.3, 123.8, 113.7, 58.6, 21.7; HRMS (EI): (m/z) calcd for C₂₄H₂₂ClNO₂: 391.1345 [M]⁺; found 391.1369.

N,N-Bis(4-chlorinephenacyl)-4-chloroanilines (**2o**)

Yield 67%; mp 181.5-182.3 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.93-7.95 (d, J=8.4, 4H Ar-H), 7.48-7.50 (d, J=8.4Hz, 4H, Ar-H), 7.10-7.12 (d, J=8.4Hz, 2H, Ar-H), 6.42-6.44 (d, J=8.8Hz, 2H, Ar-H), 4.87 (s, 4H, N-CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 195.8, 148.3, 140.5, 133.6, 129.8, 129.5, 129.3, 123.6, 112.8, 57.5; HRMS (EI): (m/z) calcd for C₂₃H₁₆Cl₃NO₂: 443.0247 [M]⁺; found 443.0267.

N,N-Bis(4-chlorinephenacyl)-4-chloroanilines (**2p**)

Yield 49%; mp 168.7-169.3 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.05-8.07 (d, J=8.4Hz, 4H, Ar-H), 7.30-7.32 (d, J=8.4Hz, 4H, Ar-H), 7.02-7.04 (d, J=8.4Hz, 2H, Ar-H), 6.50-6.52 (m, 2H, Ar-H), 4.94 (s, 4H, N-CH₂), 3.63 (s, 6H, OCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 194.7, 160.3, 148.5, 133.8, 129.6, 129.5, 129.3, 123.4, 112.7, 58.2, 57.0; HRMS (EI): (m/z) calcd for C₂₄H₂₂ClNO₄: 423.1208 [M]⁺; found 423.1236.

General procedure for the synthesis of 2,4,6-triaryl-4*H*-1,4-oxazines (**1**) and characterization data

A mixture of *N,N*-bis(phenacyl)anilines **2** (5 mmol) and 0.87 mL (9.4 mmol) of POC₁₃ in 30 mL of pyridine (dried over CaH₂) were heated with occasional swirling at 100 °C for 45 min. The deep red solution was poured onto 50 mL of crushed ice, and the resulting solid was filtered. Crude products were washed twice with a small amount of methanol.

2,4,6-Triphenyl-4*H*-1,4-oxazine (**1a**)¹⁵

Yield 50%; mp 181.7-182.4 °C. Lit. 183 °C-185 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.53-7.77 (d, *J*=6.8 Hz, 4H, Ar-H), 7.29-7.43 (m, 11H, Ar-H), 7.03 (s, 1H, Ar-H), 6.94 (s, 2H, N-CH=).

2,6-Bis(4-methylphenyl)-4-phenyl-4*H*-1,4-oxazine (**1b**)

Yield 53%; mp 113.5-114.2 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.63-7.65 (d, *J*=7.6 Hz, 4H, Ar-H), 6.92-7.35 (m, 9H, Ar-H), 6.91 (s, 2H, N-CH=), 2.35 (s, 6H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 142.0, 138.5, 137.0, 130.1, 129.4, 129.0, 122.8, 120.3, 113.4, 108.6, 20.4; HRMS (ESI): (*m/z*) calcd for C₂₄H₂₁NO: 339.1623 [M]⁺; found 339.1602.

2,6-Bis(4-chlorophenyl)-4-phenyl-4*H*-1,4-oxazine (**1c**)

Yield 60%; mp 132.5-133.6 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.77-7.79 (d, *J*=8.0 Hz, 4H, Ar-H), 7.30-7.54 (m, 9H, Ar-H), 7.05 (s, 2H, N-CH=); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.6, 138.8, 132.6, 129.2, 128.4, 127.5, 124.6, 122.9, 114.9, 109.0; HRMS (ESI): (*m/z*) calcd for C₂₂H₁₅Cl₂NO: 379.0531 [M]⁺; found 379.0416.

2,6-Bisphenyl-4-(3-methylphenyl)-4*H*-1,4-oxazine (**1e**)

Yield 55%; mp 107.6-108.5 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.75-7.77 (d, *J*=8.0 Hz, 4H, Ar-H), 7.09-7.43 (m, 9H, Ar-H), 7.03 (s, 2H, N-CH=), 6.77-6.79 (d, *J*=7.6 Hz, 1H, Ar-H), 2.35 (s, 3H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.1, 137.8, 132.7, 130.1, 129.8, 128.3, 127.1, 122.5, 120.5, 114.2, 112.5, 110.0, 19.7; HRMS (ESI): (*m/z*) calcd C₂₃H₁₉NO: 325.1 [M]⁺; found 324.9.

2,6-Bis(4-methylphenyl)-4-(3-methylphenyl)-4*H*-1,4-oxazine (**1f**)

Yield 58%; mp 112.5-113.2 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.74-7.77 (m, 4H, Ar-H), 7.12-7.44 (m, 7H, Ar-H), 7.03 (s, 2H, N-CH=), 6.81 (d, *J*=7.2 Hz, 1H, Ar-H), 2.25 (s, 6H, Ar-CH₃), 2.21 (s, 3H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 141.2, 138.5, 137.2, 137.0, 129.9, 129.3, 129.0, 122.7, 120.2, 114.3, 113.6, 108.6, 20.3, 19.7; HRMS (ESI): (*m/z*) calcd for C₂₅H₂₃NO:

353.1780 [M]⁺; found 353.1649 [M+H]⁺.

2,6-Bis(4-chlorinephenyl)-4-(3-methylphenyl)-4*H*-1,4-oxazine (**1g**)

Yield 66%; mp 122.5-124.1 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.74-7.77 (m, 4H, Ar-H), 7.10-7.43 (m, 7H, Ar-H), 7.07 (s, 2H, N-CH=), 6.80-6.81 (d, J=7.2Hz, 1H, Ar-H), 2.34 (s, 3H, CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.5, 138.8, 137.0, 132.6, 129.5, 128.5, 127.5, 124.7, 120.4, 114.6, 113.0, 109.0, 19.7; HRMS (ESI): (m/z) calcd for C₂₃H₁₇Cl₂NO: 393.1 [M]⁺; found 392.8.

2,6-Bisphenyl-4-(4-methylphenyl)-4*H*-1,4-oxazine (**1i**)

Yield 53%; mp 115.7-117.2 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.74-7.76 (d, J=7.6Hz, 4H, Ar-H), 7.38-7.42 (t, J=7.6Hz, 4H, Ar-H), 7.27-7.31 (t, J=7.6Hz, 2H, Ar-H), 7.15-7.22 (dd, J=18.0, 8.4Hz, 4H, Ar-H), 6.98 (s, 2H, N-CH=), 2.28 (s, 3H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.0, 137.8, 132.9, 130.0, 129.9, 128.4, 127.1, 122.6, 113.9, 110.0, 19.7; HRMS (ESI): (m/z) calcd for C₂₃H₁₉NO: 325.1 [M]⁺; found 324.9.

2,4,6-Tri(4-methylphenyl)-4*H*-1,4-oxazine (**1j**)

Yield 57%; mp 133.6-134.2 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.13-7.60 (m, 12H, Ar-H), 6.87 (s, 2H, N-CH=), 2.27 (s, 6H, Ar-CH₃), 2.04 (s, 3H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 141.0, 138.4, 137.1, 130.0, 129.5, 129.1, 128.3, 122.7, 113.8, 108.7, 20.5, 19.8; HRMS (ESI): (m/z) calcd for C₂₅H₂₃NO: 353.1780 [M]⁺; found 353.1702.

2,6-Bis(4-chlorinephenyl)-4-(4-methylphenyl)-4*H*-1,4-oxazine (**1k**)

Yield 62%; mp 153.9-154.7 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.73-7.75 (d, J=8.8Hz, 4H, Ar-H), 7.39-7.42 (m, 4H, Ar-H), 7.20-7.22 (d, J=8.4Hz, 2H, Ar-H), 7.15-7.17 (d, J=8.4Hz, 4H, Ar-H), 6.97 (s, 2H, N-CH=), 2.29 (s, 3H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.0, 138.8, 132.3, 130.0, 128.4, 128.3, 127.6, 124.5, 113.8, 110.1, 19.6; HRMS (ESI): (m/z) calcd for C₂₃H₁₇Cl₂NO: 393.1 [M]⁺; found 392.8.

2,6-Bisphenyl-4-(4-chlorinephenyl)-4*H*-1,4-oxazine (**1m**)

Yield 63%; mp 98.6-99.5 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.76-7.78 (d, J=7.6Hz, 4H, Ar-H), 7.29-7.43 (m, 10H, Ar-H), 7.03 (s, 2H, N-CH=); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.7, 138.3, 132.6, 129.2, 128.4, 127.5, 124.6, 122.9, 114.9, 109.0; HRMS (ESI): (m/z) calcd for C₂₂H₁₆ClNO: 345.0920 [M]⁺; found 345.0812 [M+H]⁺.

2,6-Bis(4-methylphenyl)-4-(4-chlorinephenyl)-4*H*-1,4-oxazine (**1n**)

Yield 63%; mp 98.6-99.9 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 6.90-7.47 (m, 12H, Ar-H), 6.44 (s, 2H, N-CH=), 2.38 (s, 6H, Ar-CH₃); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.7, 138.5, 129.4, 129.3, 129.1, 124.2, 122.9, 122.7, 114.7, 109.1, 20.2; HRMS (ESI): (m/z) calcd for C₂₄H₂₀ClNO: 373.1233 [M]⁺; found 373.1126.

2,4,6-Tri(4-chlorinephenyl)-4*H*-1,4-oxazine (**1o**)

Yield 74%; mp 145.3-146.7 °C; ¹H NMR (400 MHz, Acetone-*d*₆) δ 7.64-7.66 (d, J=6.8Hz, 4H, Ar-H), 7.08-7.45 (m, 8H, Ar-H), 6.95 (s, 2H, N-CH=); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 140.6, 138.7, 132.5, 129.2, 128.2, 127.5, 124.6, 122.8, 114.7, 109.0; HRMS (EI): (m/z) calcd for C₂₂H₁₄Cl₃NO: 413.0141 [M]⁺; found 414.9969 [M+H]⁺.

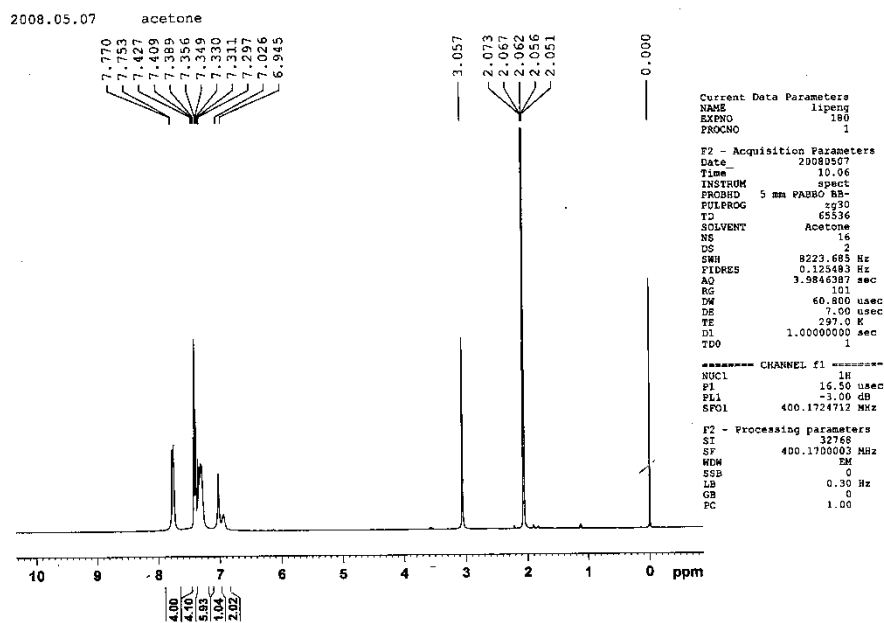
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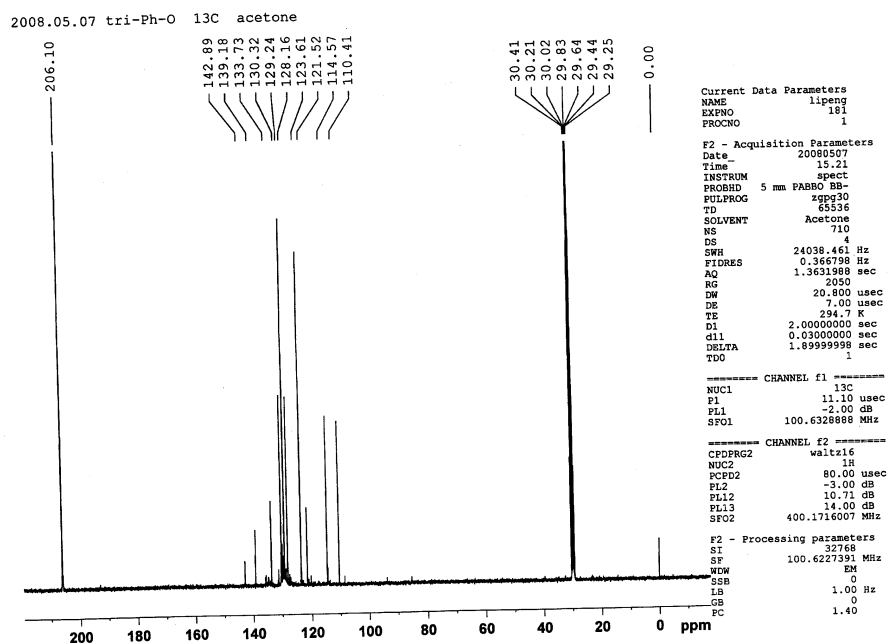
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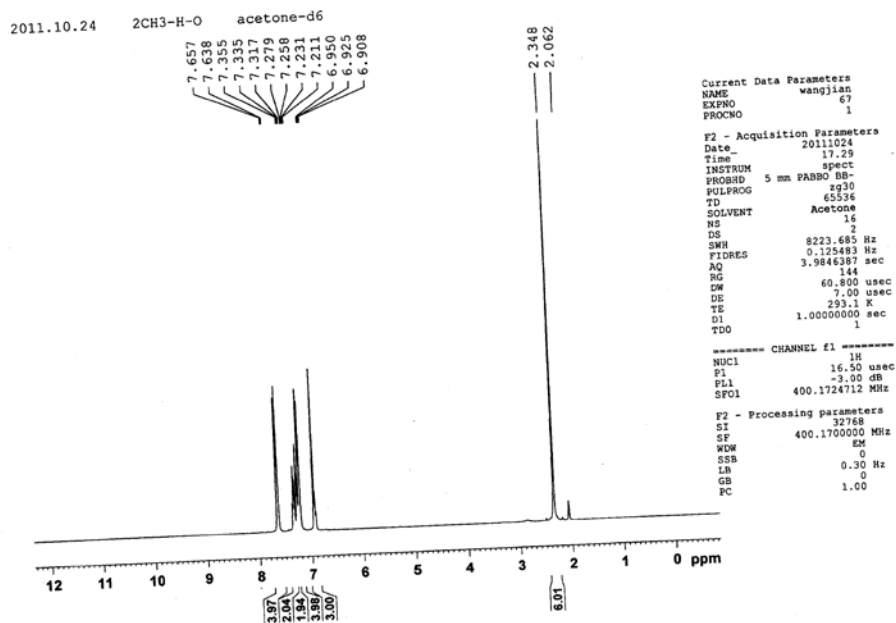
NMR spectra of final compounds 1



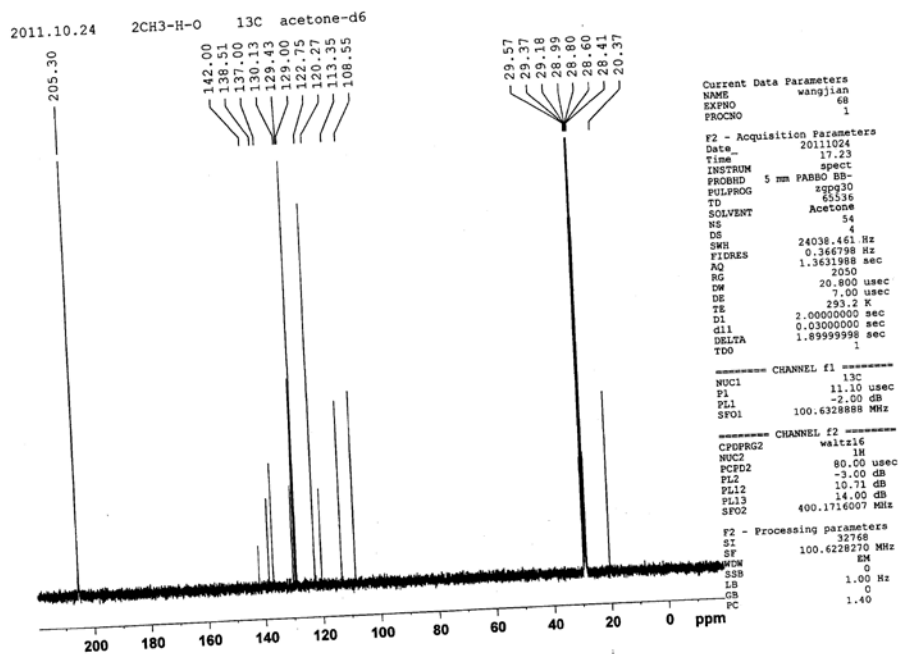
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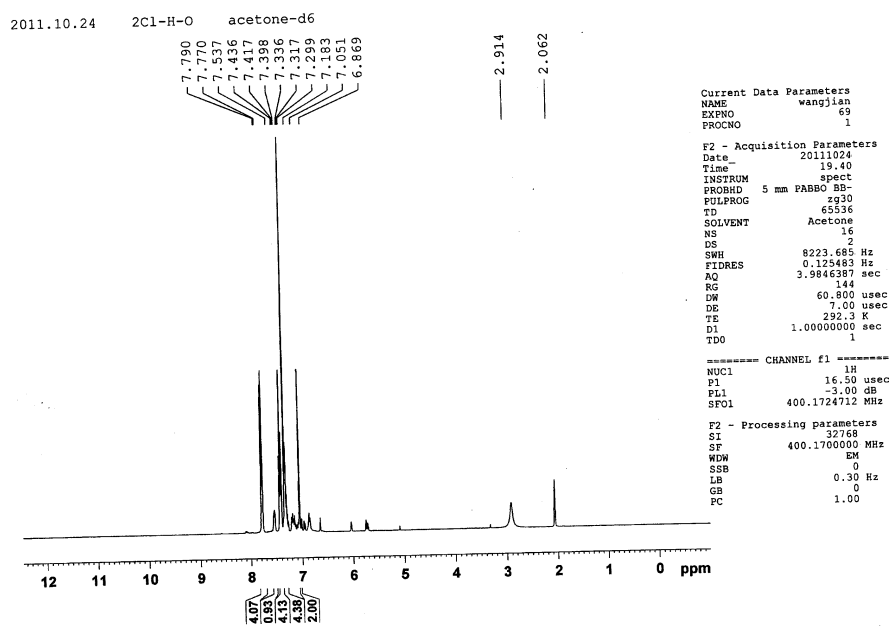
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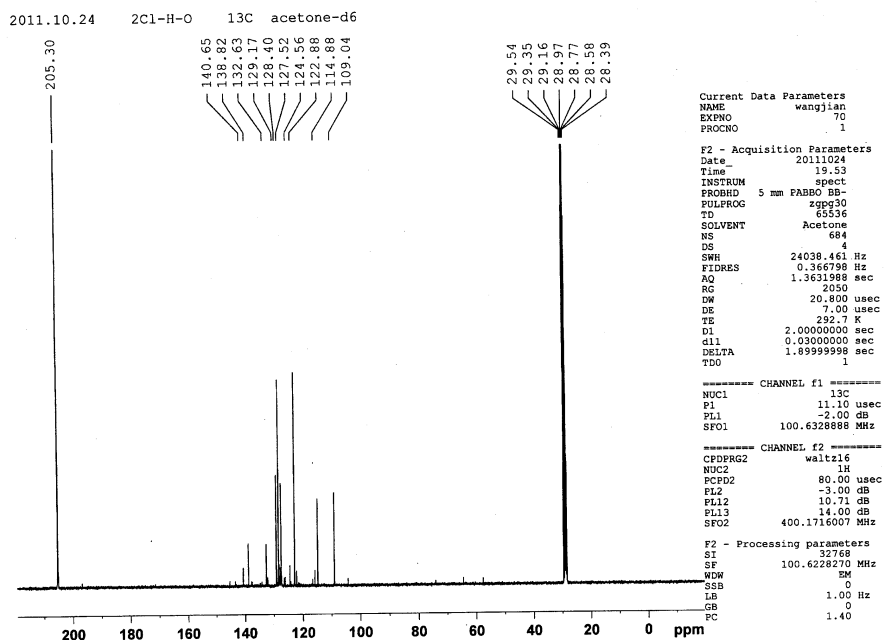
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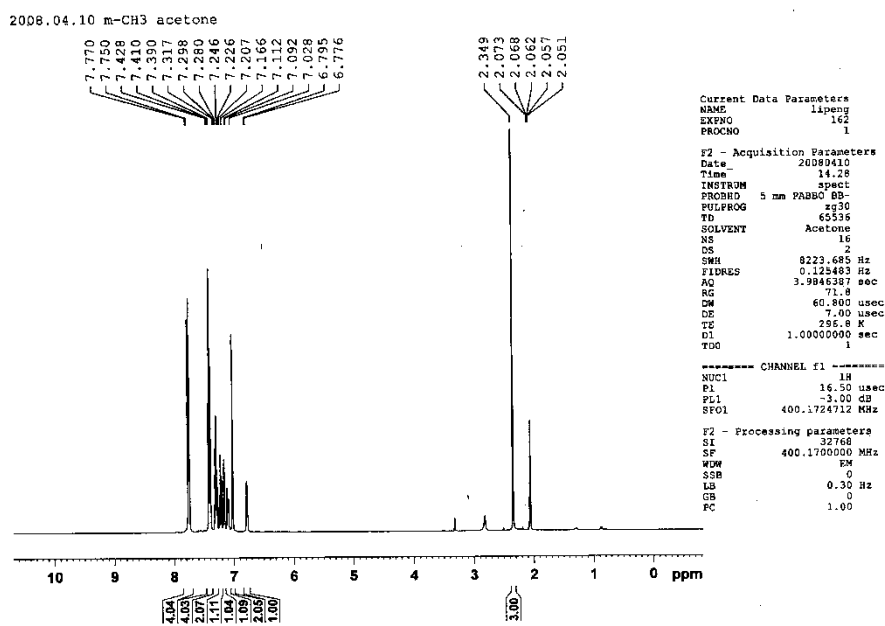
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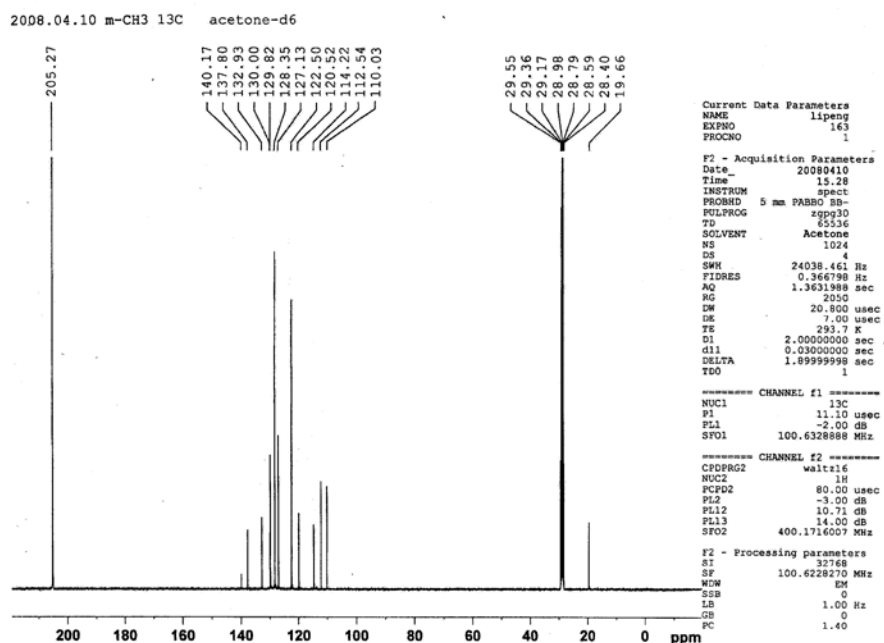
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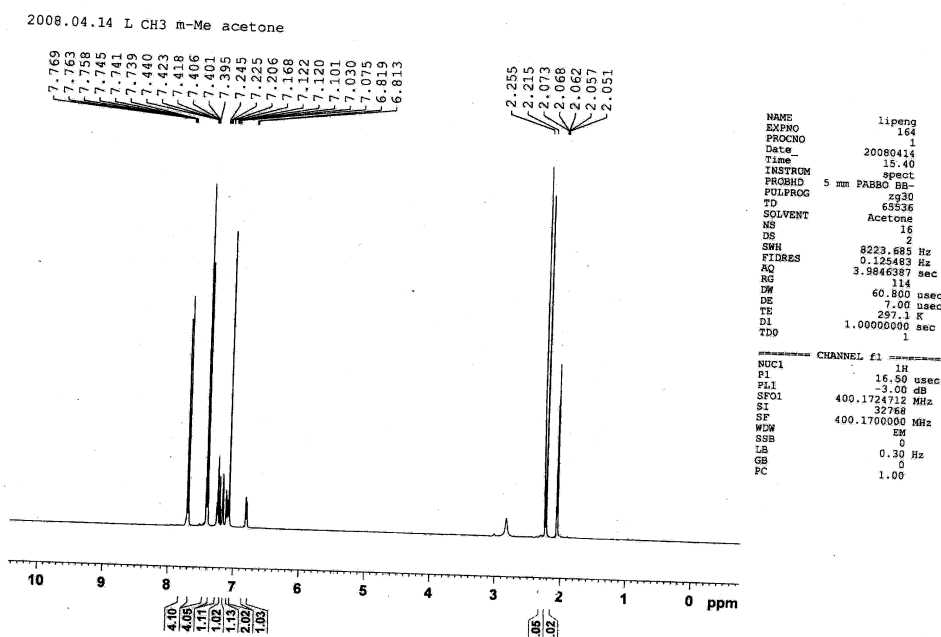
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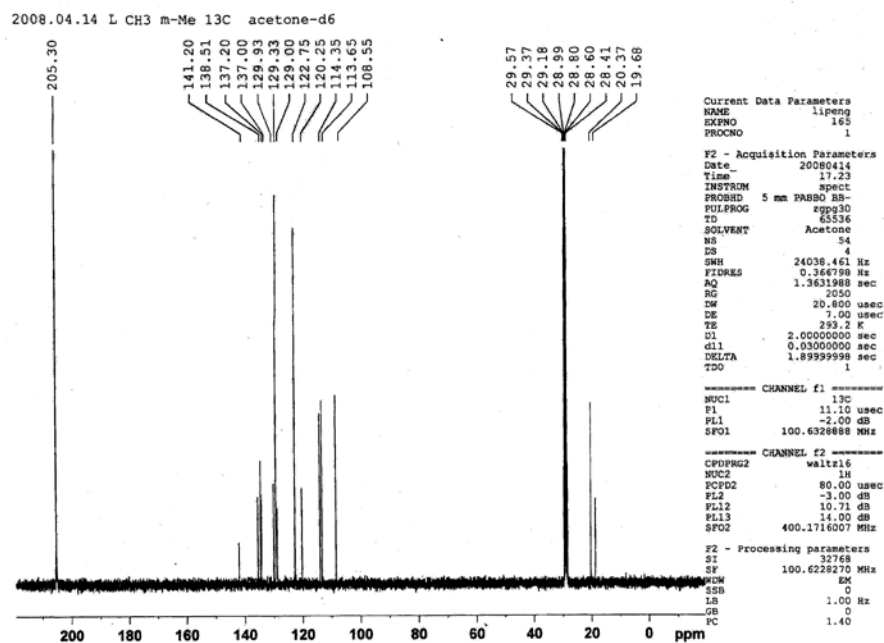
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1e ¹³C NMR

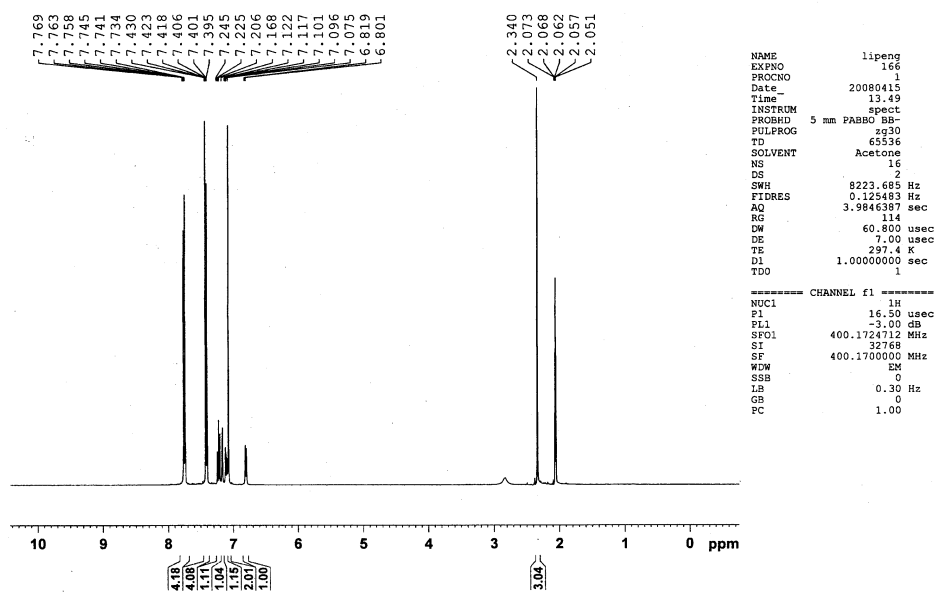


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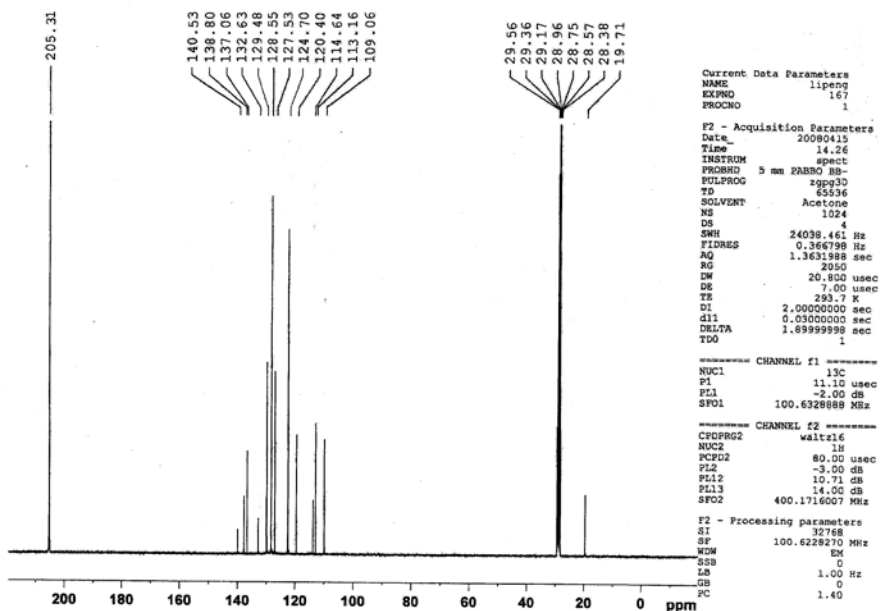
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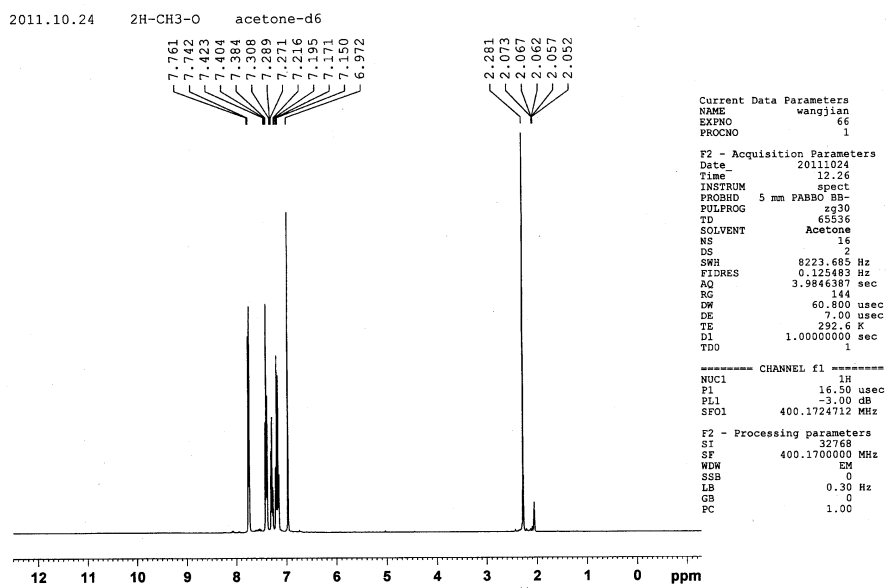


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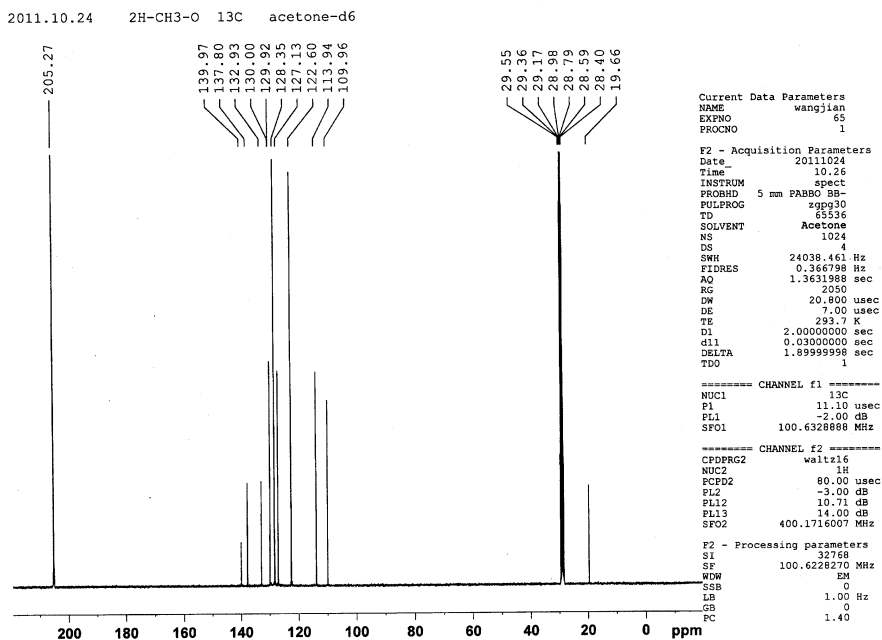
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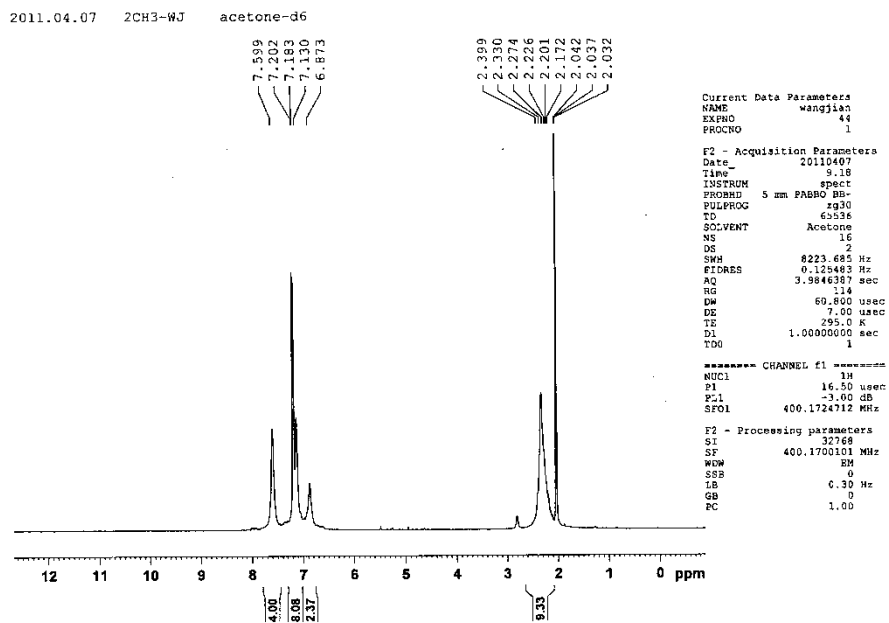
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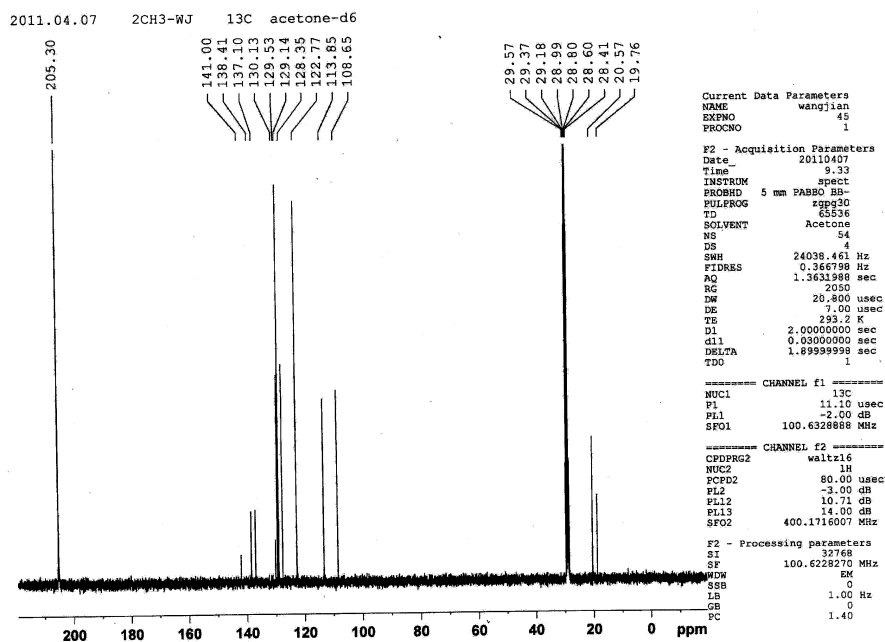
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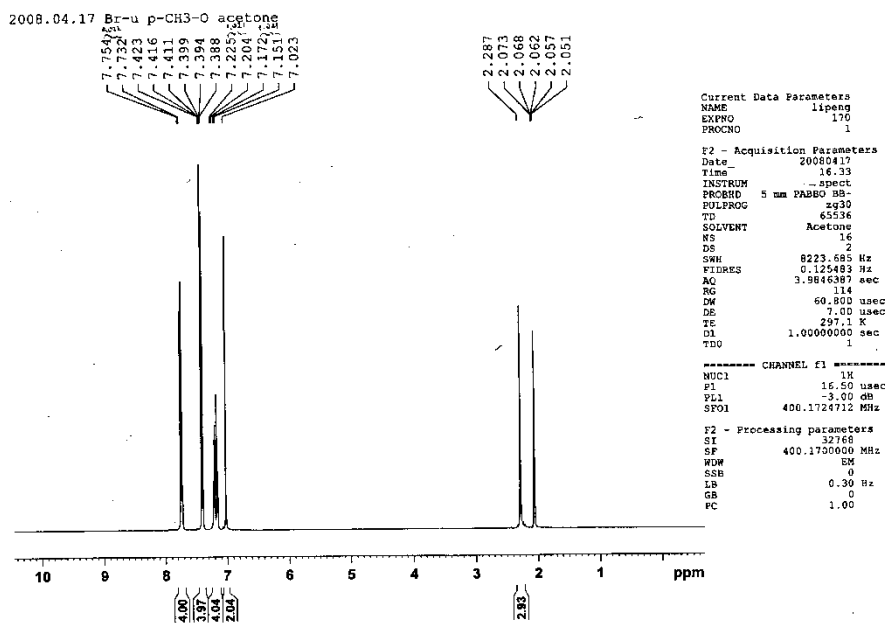
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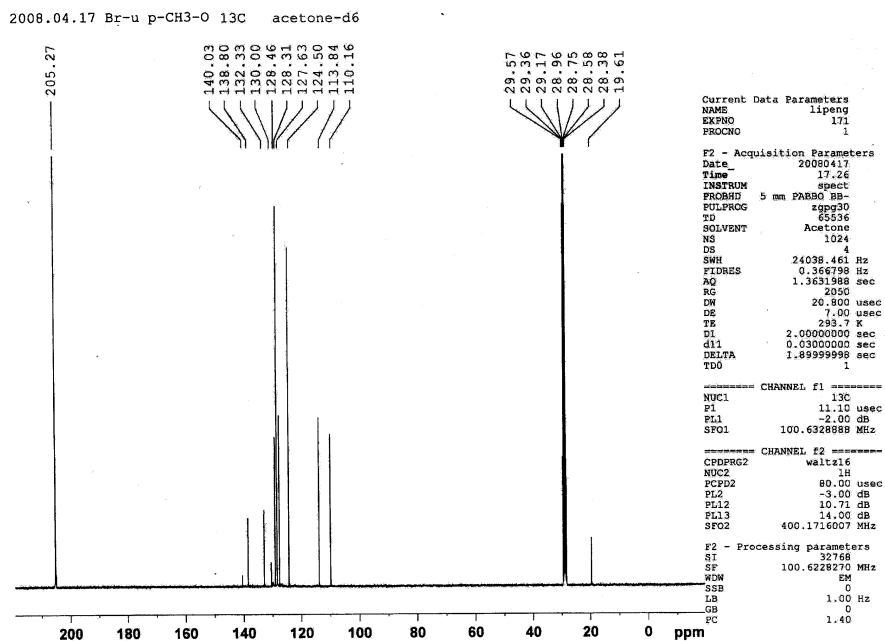
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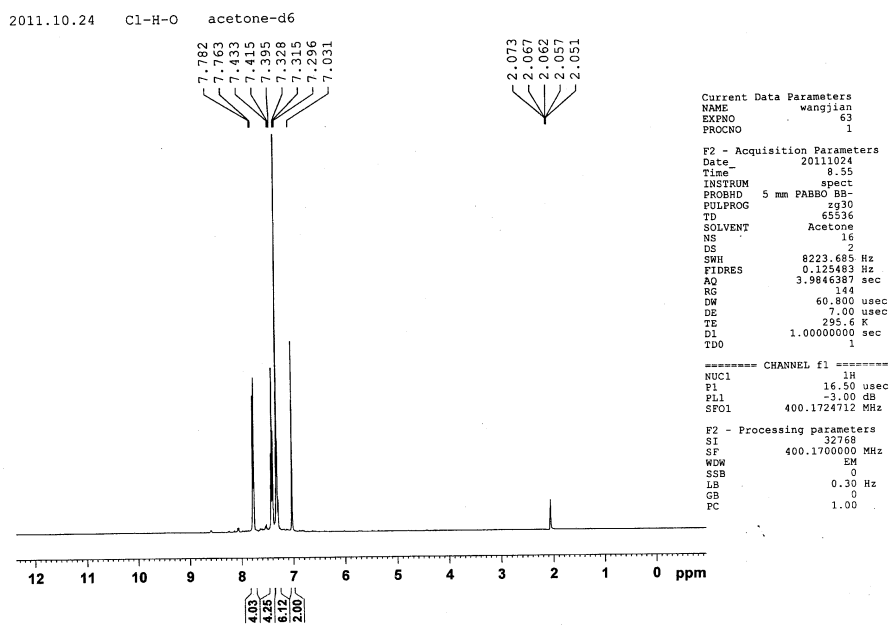
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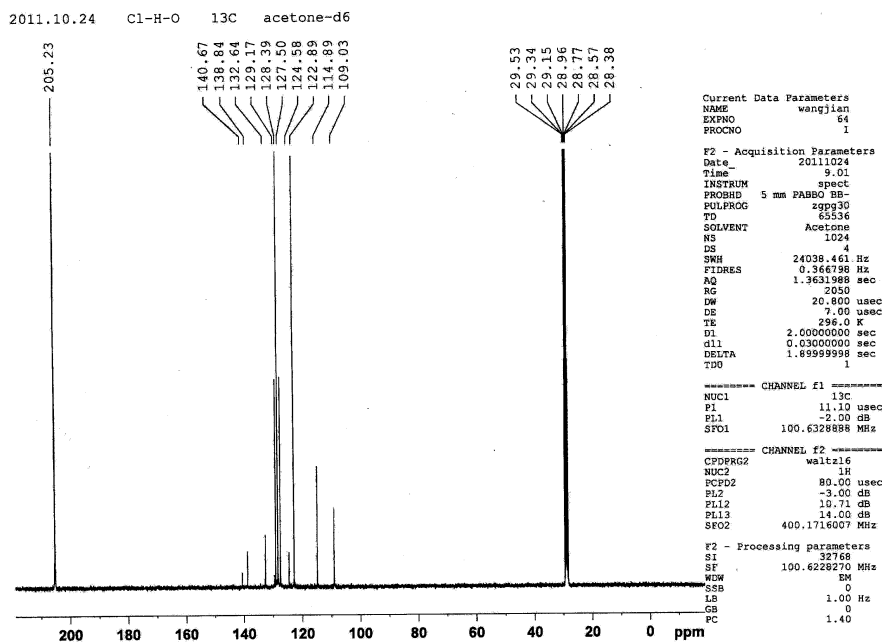
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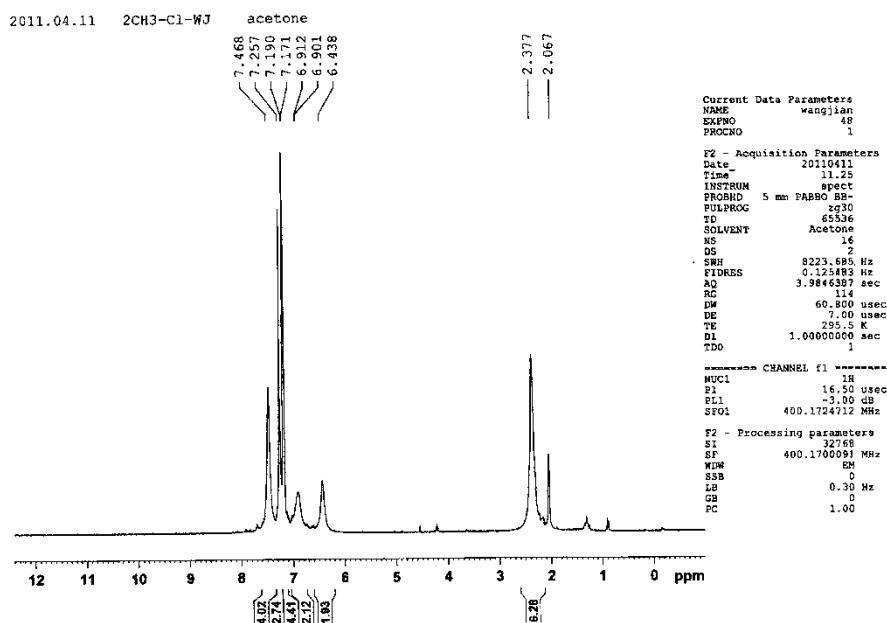
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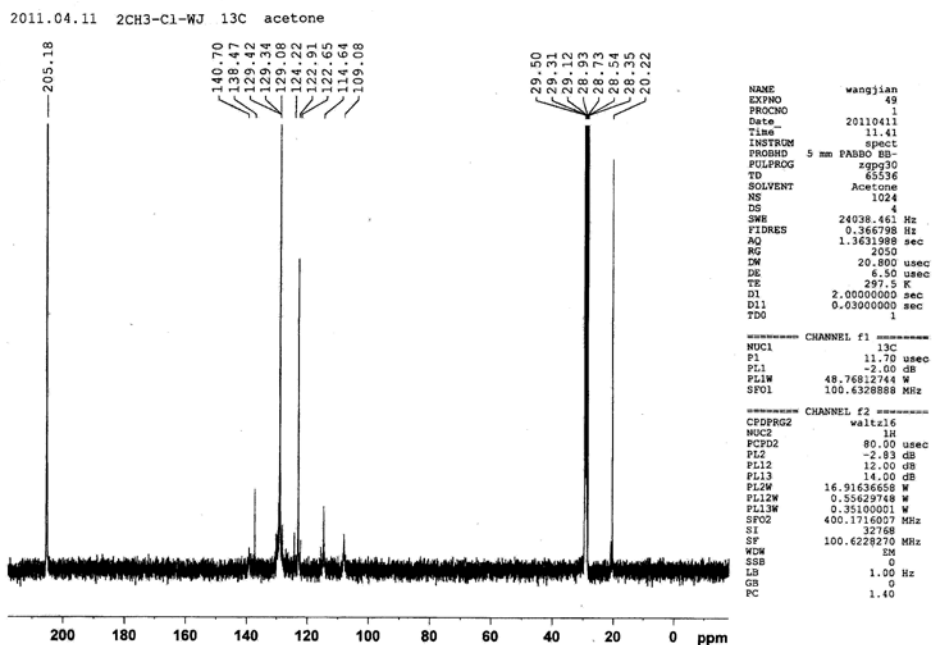
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1m ¹³C NMR

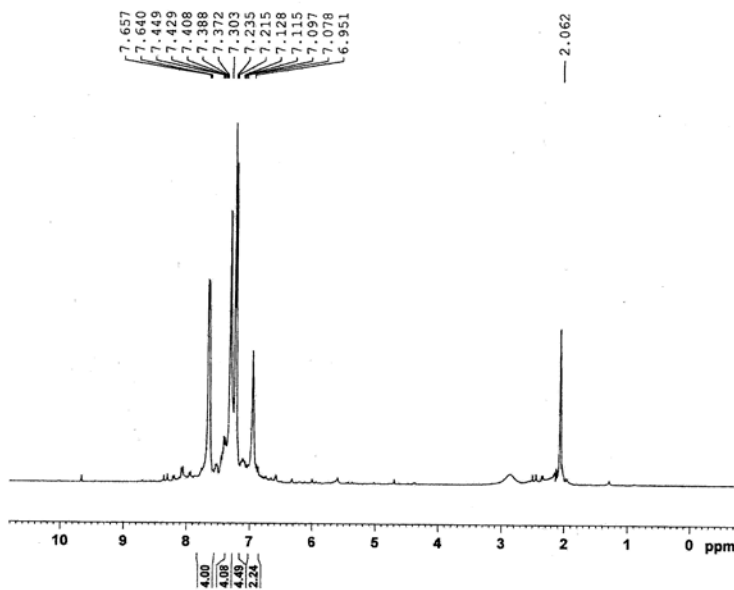


1^{H} NMR



1^{3}C NMR

2012.08.24 LIWEIPENG-WANJIAN acetone



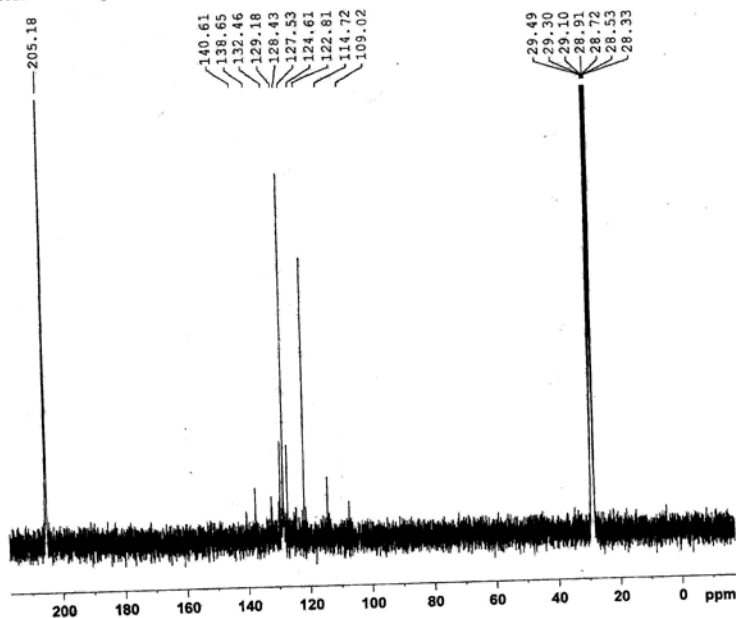
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TD        65536
SOLVENT  Acetone
NS        16
DS        2
SWH       8223.685 Hz
FIDRES   0.125483 Hz
AQ        3.9846387 sec
RG        144
DW        60.800 usec
DE        8.50 usec
TE        297.3 K
D1        1.0000000 sec
TD0       1

===== CHANNEL f1 =====
NUC1      1H
P1        14.50 usec
PL1       -2.83 dB
PL1W     16.91636658 W
SF01     400.127412 MHz
SI        32768
SF        400.1700000 MHz
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
    
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10^1H NMR

2012.08.24 p-Cl 13C Acetone-d6



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PULPROG  zgpg30
TD        65536
SOLVENT  Acetone
NS        1024
DS        4
SWH       24038.461 Hz
FIDRES   0.366798 Hz
AQ        1.3631988 sec
RG        2050
DW        20.800 usec
DE        6.50 usec
TE        299.3 K
D1        2.0000000 sec
D11       0.0300000 sec
TD0       1

===== CHANNEL f1 =====
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P1        11.70 usec
PL1       -2.00 dB
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SF01     100.6288988 MHz

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PCPD2    80.00 usec
PL2       -2.83 dB
PL12     12.00 dB
PL13     14.00 dB
PL2W     16.91636658 W
SF02     0.55629748 MHz
PL12W    0.35100001 W
SF02     400.1716007 MHz
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WDW       EM
SSB       0
LB        1.00 Hz
GB        0
PC        1.40
    
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10^1C NMR