Approaches towards the synthesis of 7-halo-1,2-dihydroxyindolizidines (7-halolentiginosines) thwarting Grob fragmentation processes

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Supplementary data:

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Biological assays:

Cell culture

The human acute lymphoblastic monocytoid cell line U937 (Zooprofilactic Institute, Brescia, Italy) was cultured in Roswell Park Memorial Institute (RPMI) 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 2 mM glutamine (Hyclone, Cramlington, England, UK), 50 U/mL penicillin and 50 U/mL streptomycin (Hyclone). U937 was cultured at 37 °C under humidified 5% CO_2 atmosphere, in the presence or absence of **27**, **28**, **29a**, **29b** at the concentrations. of 10, 100, 250, 500 μM. The **SN38** (a metabolite of irinotecan, a topoisomerase I inhibitor) at the concentration of 10 μM was used as positive control in MTS and apoptosis assays.

Evaluation of apoptosis

Apoptosis was evaluated, after 18 h of incubation, by flow cytometry analysis of hypodiploid events following treatment of the cells with detergent and PI staining, using a method that distinguishes nuclei from apoptotic, necrotic or viable cells, as previously described.[1] Isolated nuclei were analyzed using a FACScan flow cytometry (BD Biosciences, San Josè, CA). Detectors and amplifier gains for forward and orthogonal scatter were adequately selected in order to simultaneously detect nuclei from viable, apoptotic and necrotic cells. Events were gated on forward versus orthogonal scatter in such a way that degraded DNA from cell debris or from doublets was excluded, and nuclei from viable, apoptotic and necrotic cells were assayed. Data acquisition and analysis were performed using CellQuestTM software on a minimum of 5000 events for each sample (BD Biosciences, San Josè, CA).

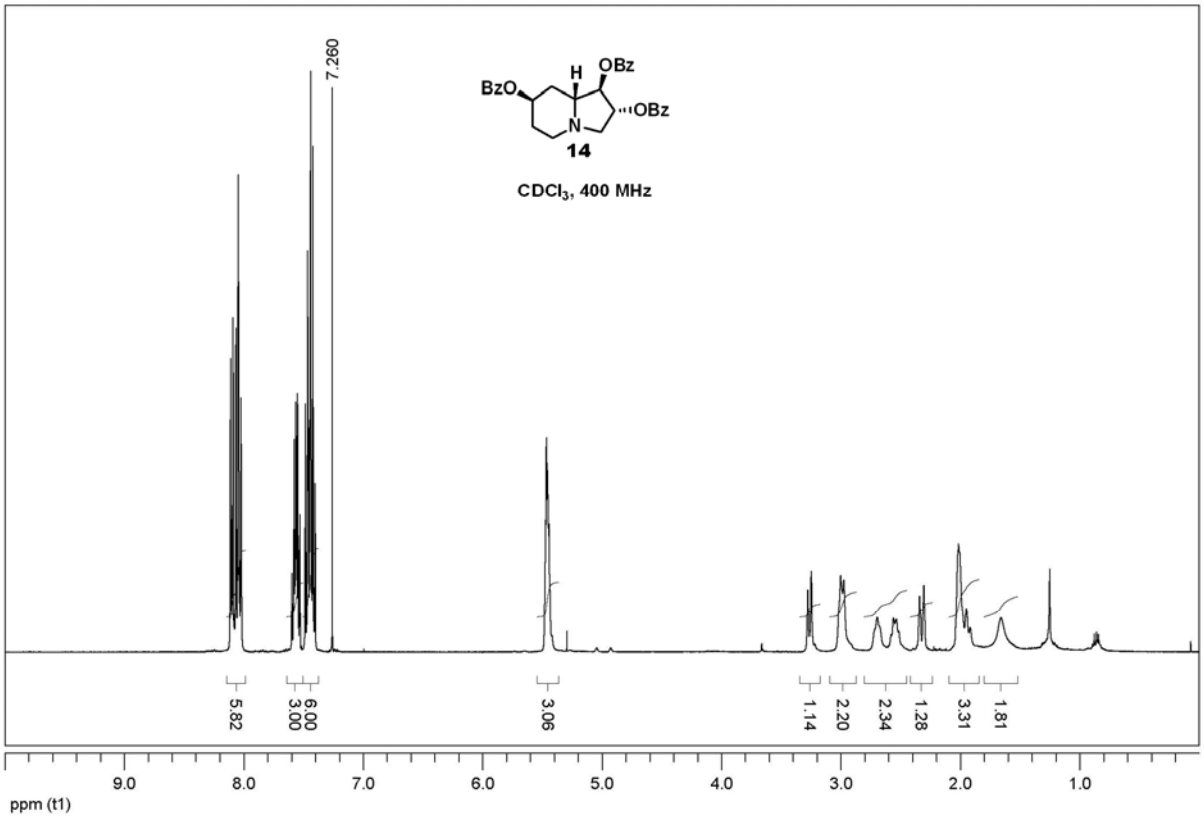
MTS assay

Cell metabolic activity, measured by reduction of MTS to formazan, was evaluated using a colorimetric commercial kit, MTS (Cell Titer 96 Aqueous One Solution, Promega). The assay was performed by seeding 1×10^4 U937 cells, in 100 μL in the presence or absence of **27**, **28**, **29a**, and **29b** at seven different concentrations, from 1 to 1000 μM in complete medium RPMI supplemented with 5% FBS. Twenty microliters of "Cell Titer 96 Aqueous One Solution Reagent" was added directly to culture wells at the end of the culture, incubated for 1–4 h and then absorbance was read at 490 nm.

Calculation of inhibitory concentration

Cumulative results from at least three different determinations were used to calculate the drug concentration required to inhibit mitochondrial enzyme activity by 50% (CC_{50}), as evaluated by MTS assay in all cell lines. The CC_{50} were calculated according to the best-fit curve, y value versus log x, where y is the value of the examined function and x is the drug concentration.

[1] C. Matteucci, A. Minutolo, E. Balestrieri, F. Marino-Merlo, P. Bramanti, E. Garaci, B. Macchi, A. Mastino, *Cell Death Dis.* **2010**, *1*, e81. doi: 10.1038/cddis.2010.58.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H
OBz
OBz
N
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6.00
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2.34
1.28
3.31
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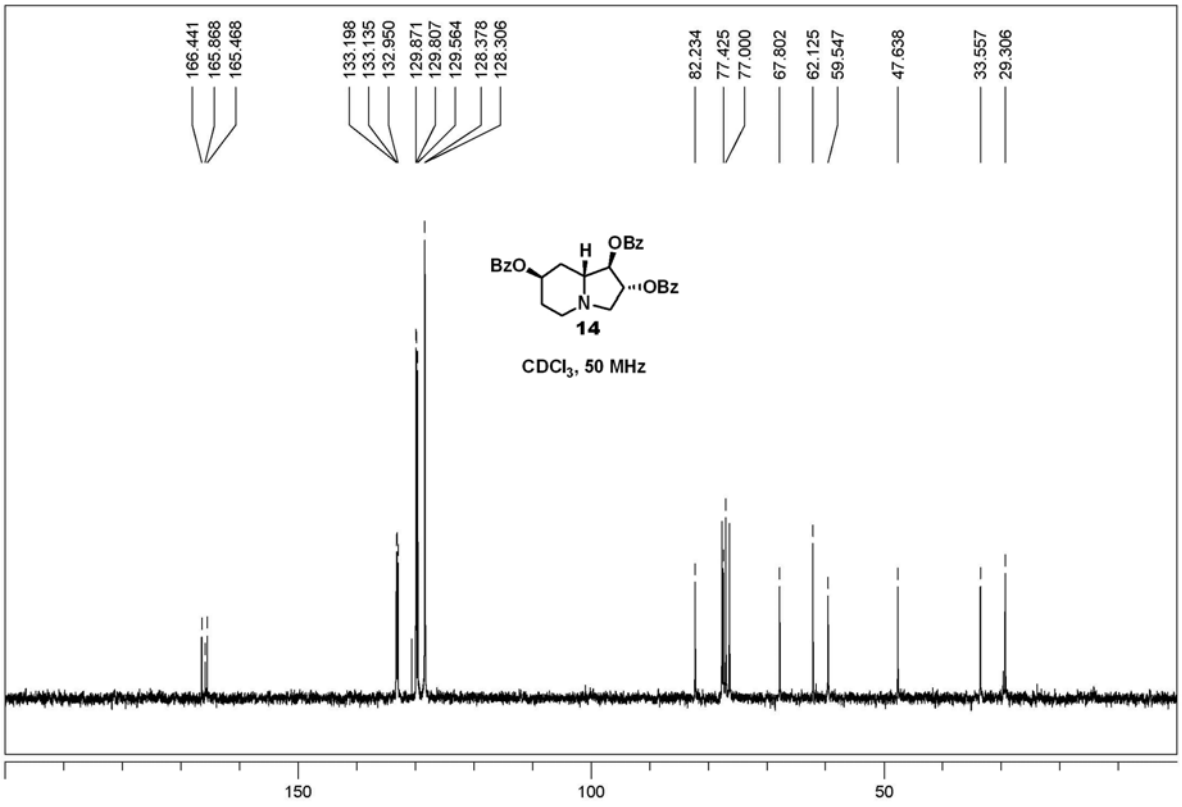
5.0
4.0
3.0
2.0
1.0
ppm (t1)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77.000
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62.125
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29.306
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OBz
OBz
N
14
CDCl3, 50 MHz
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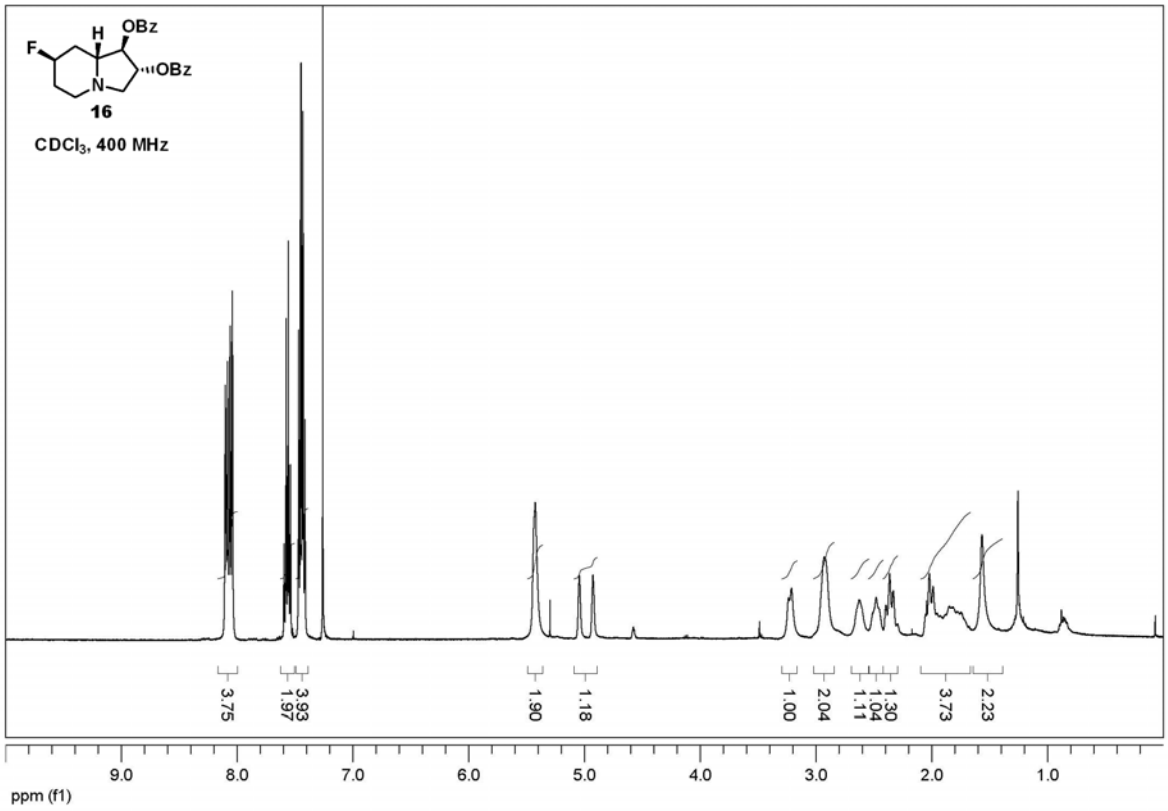
100
50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F
OBz
N
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1.04
1.30
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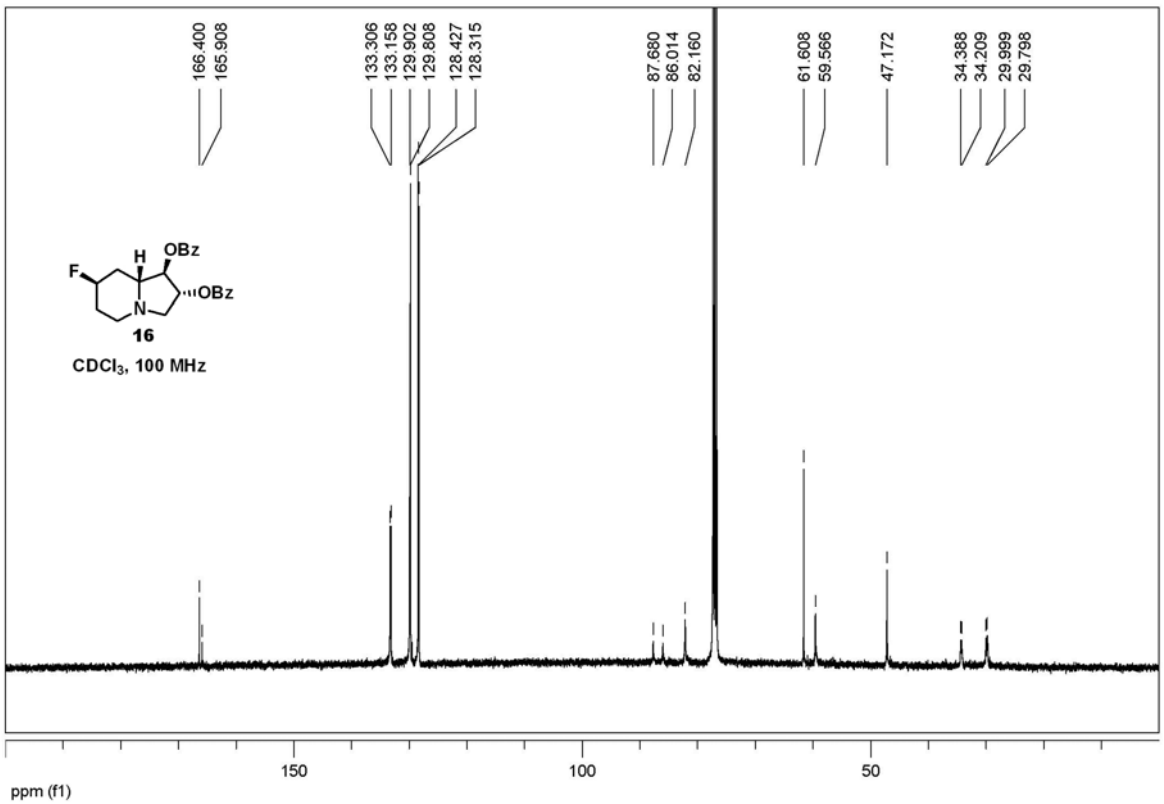
1.0
ppm (f1)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59.566
47.172
34.388
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29.798
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H
F
OBz
N
16
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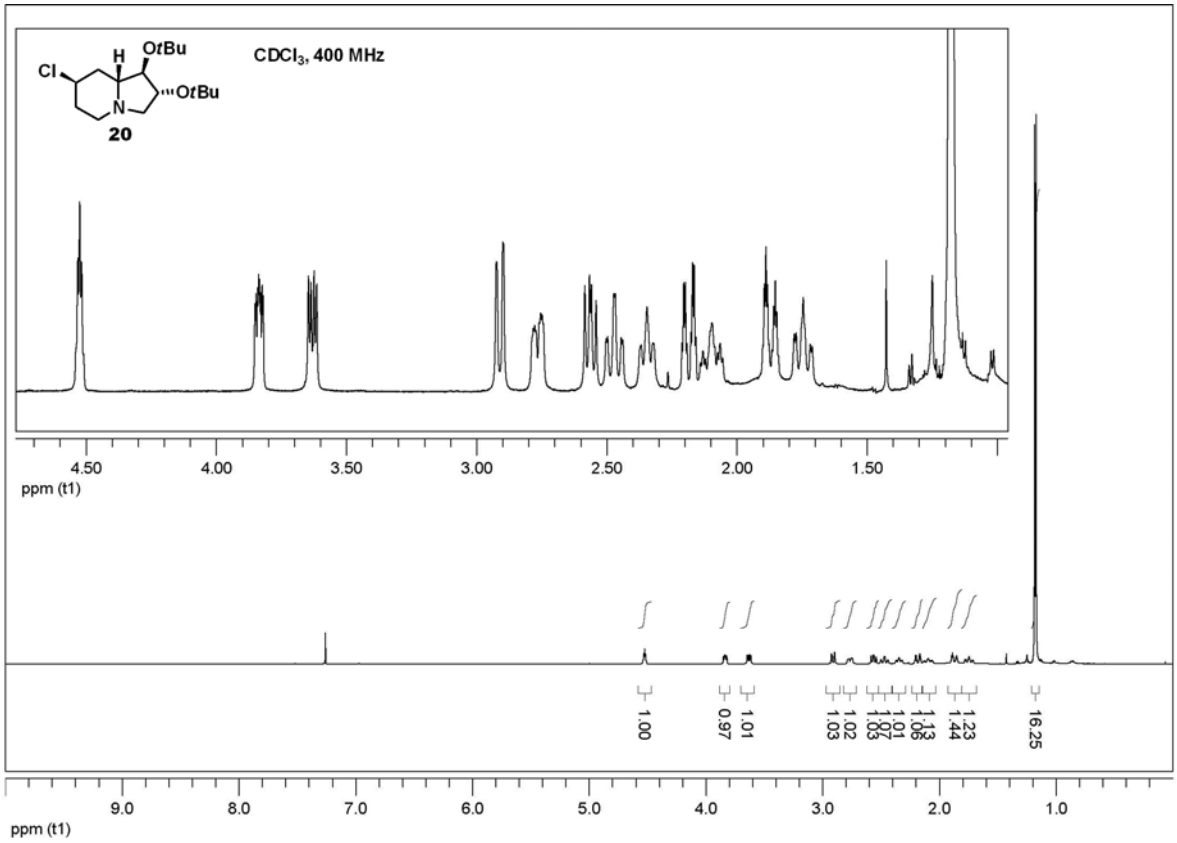
100
50
ppm (f1)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1.50
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1.01
1.03
1.02
1.07
1.01
1.06
1.13
1.23
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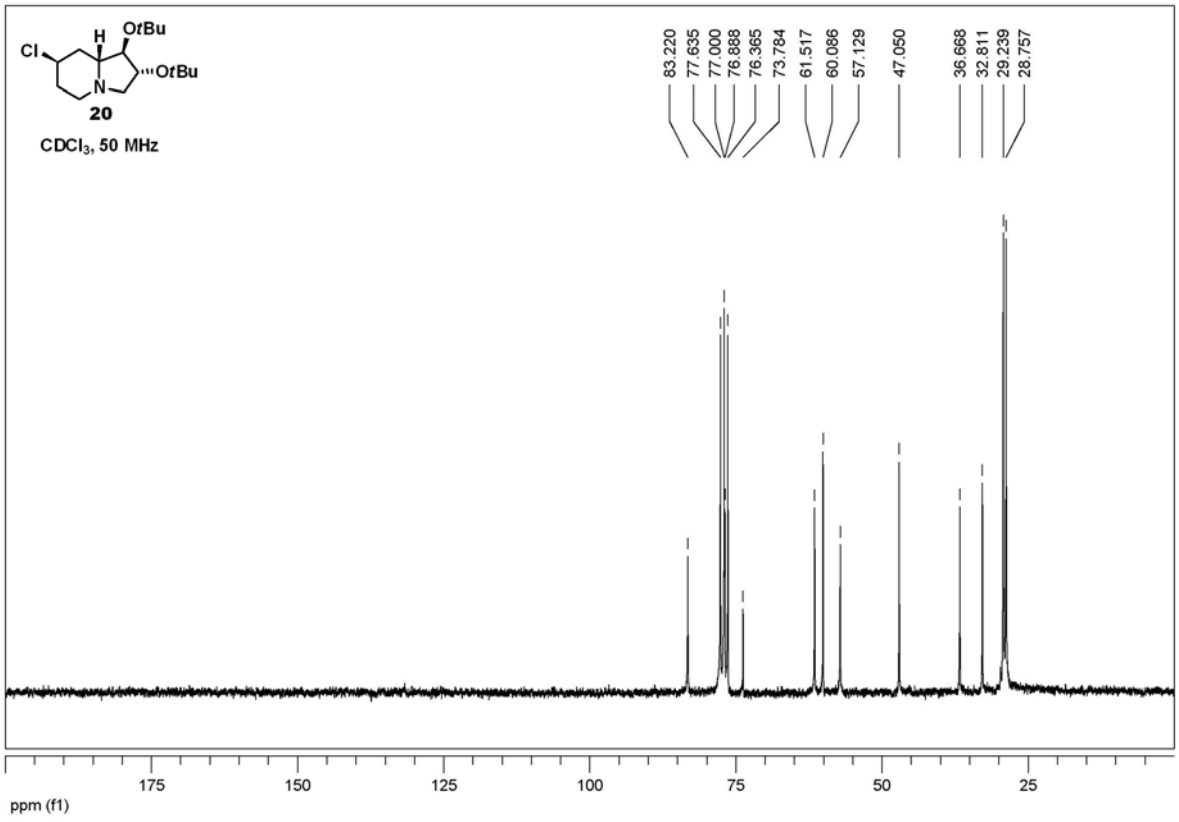
1.0
ppm (t1)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60.086
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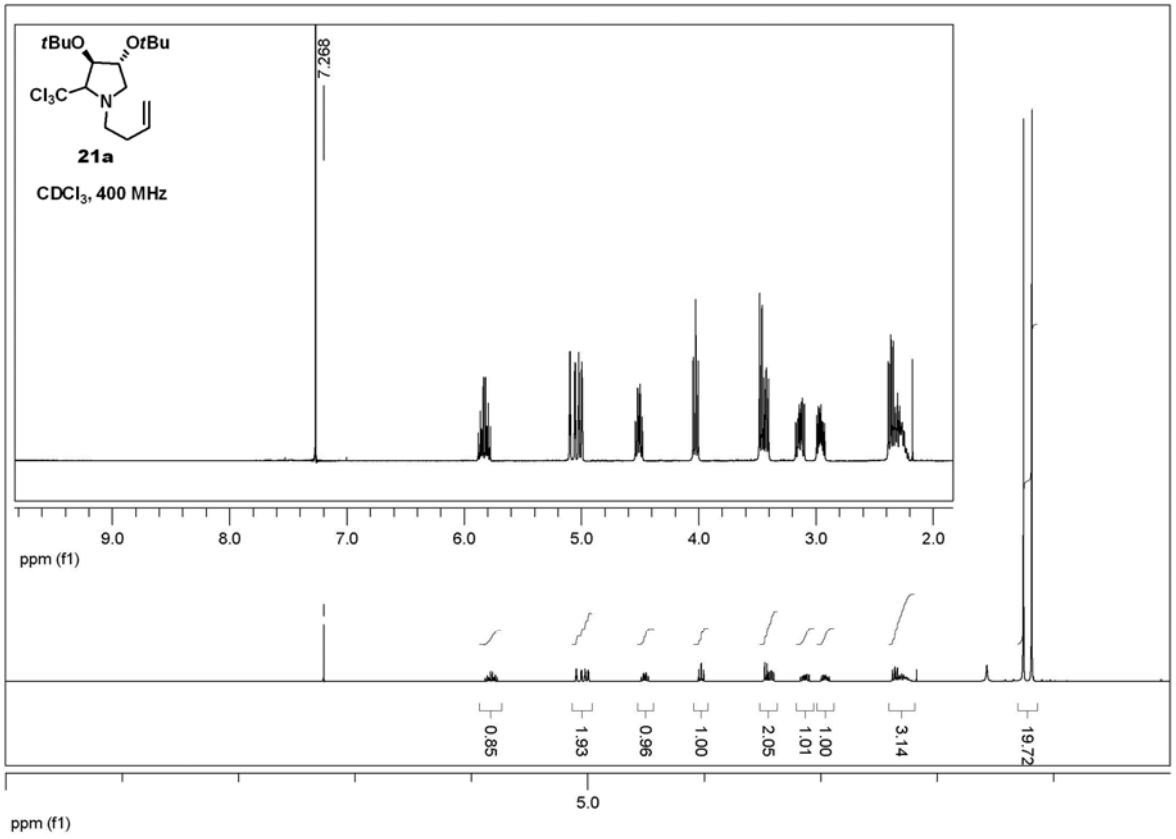
150
125
100
75
50
25
ppm (f1)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4.0
3.0
2.0
ppm (f1)
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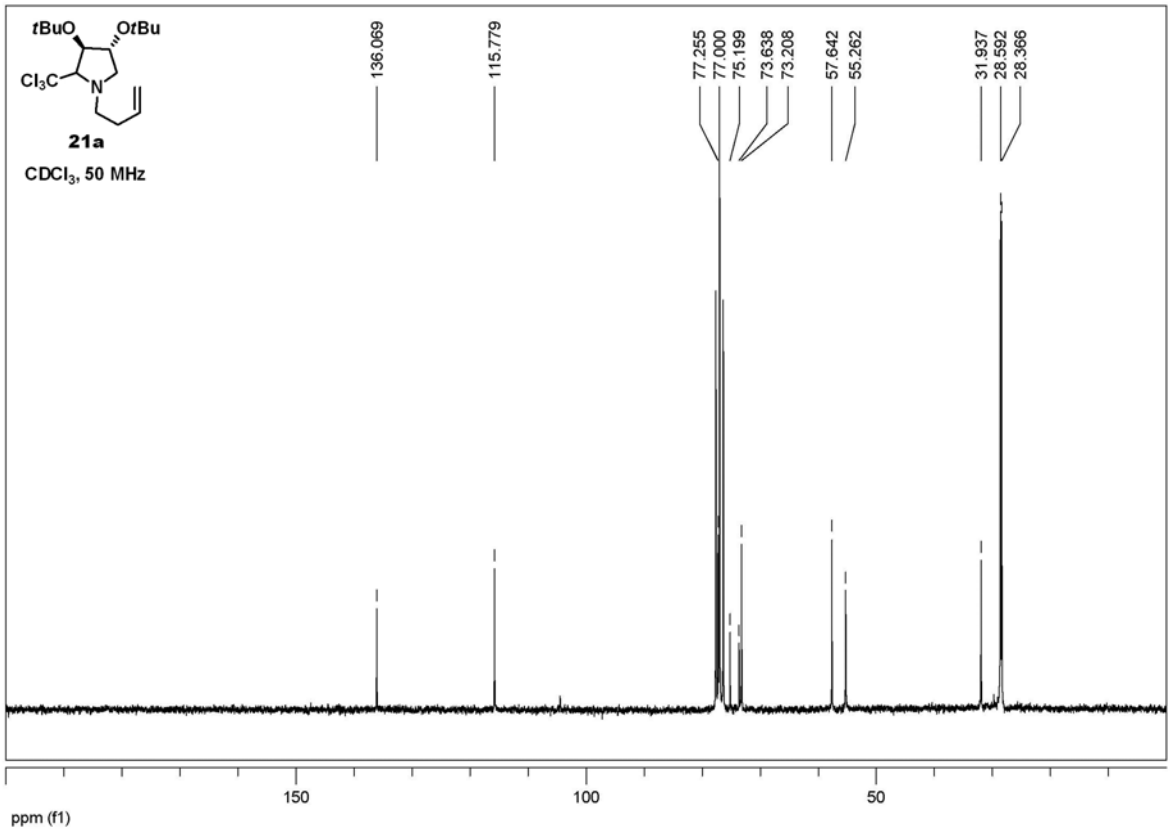
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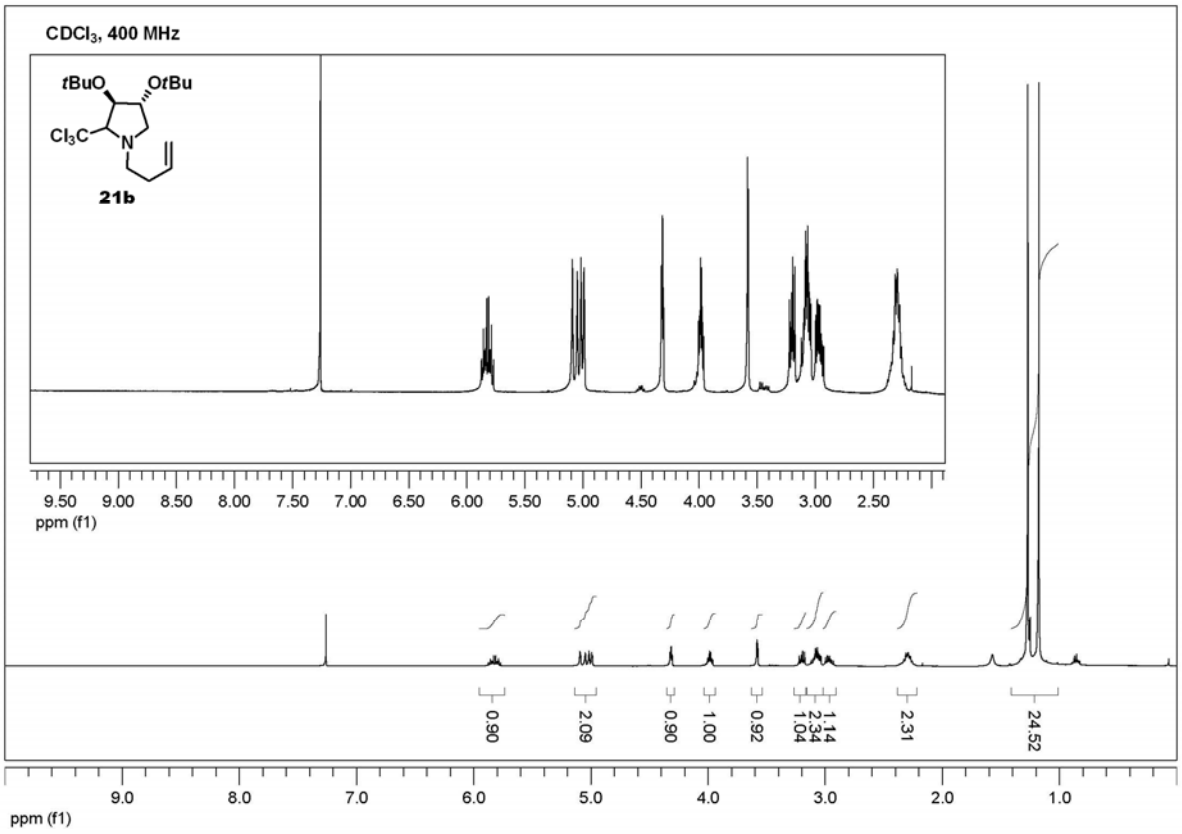
100
50
ppm (f1)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OtBu
Cl3C
N
21b
9.50 9.00 8.50 8.00 7.50 7.00 6.50 6.00 5.50 5.00 4.50 4.00 3.50 3.00 2.50
ppm (f1)
0.90
2.09
0.90
1.00
0.92
2.34
1.04
1.14
2.31
24.52
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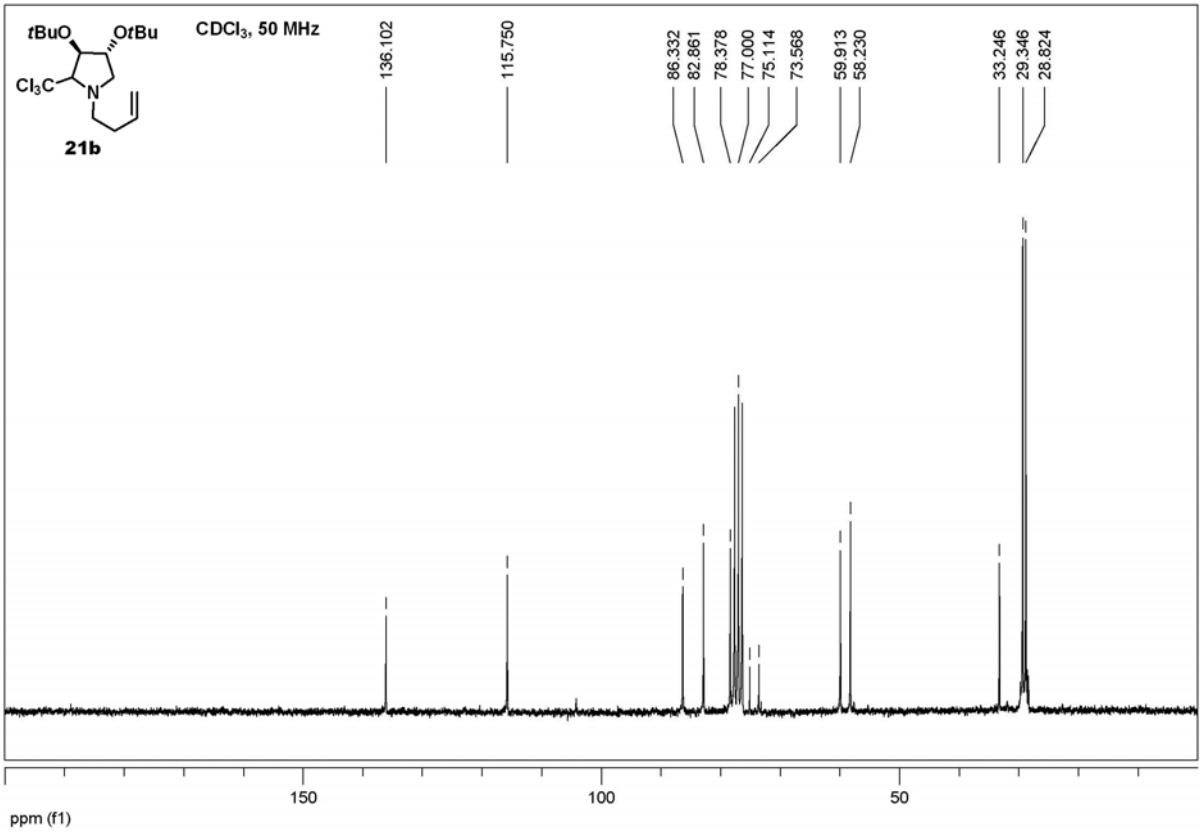
ppm (f1)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Cl3C
N
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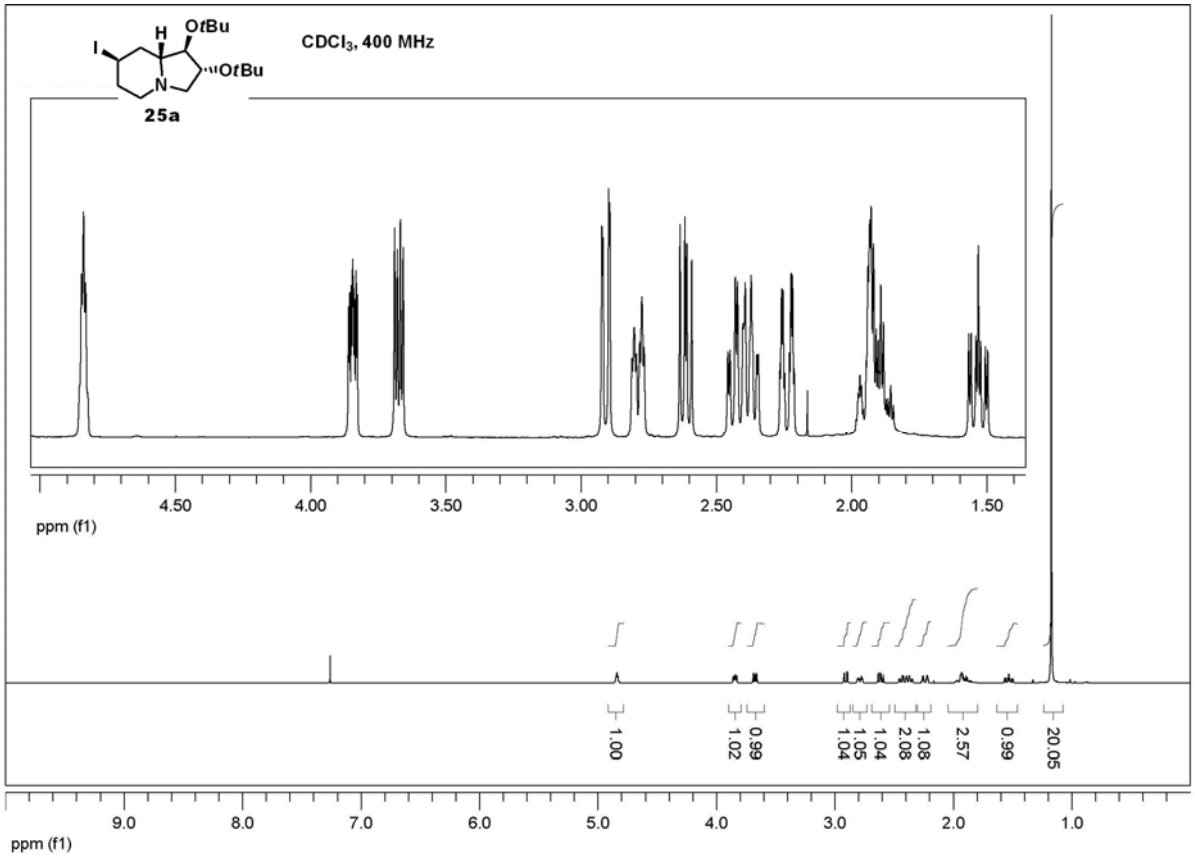
50
ppm (f1)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I
OtBu
N
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3.00
2.50
2.00
1.50
ppm (f1)
1.00
1.02
0.99
1.04
1.05
1.04
2.08
1.08
2.57
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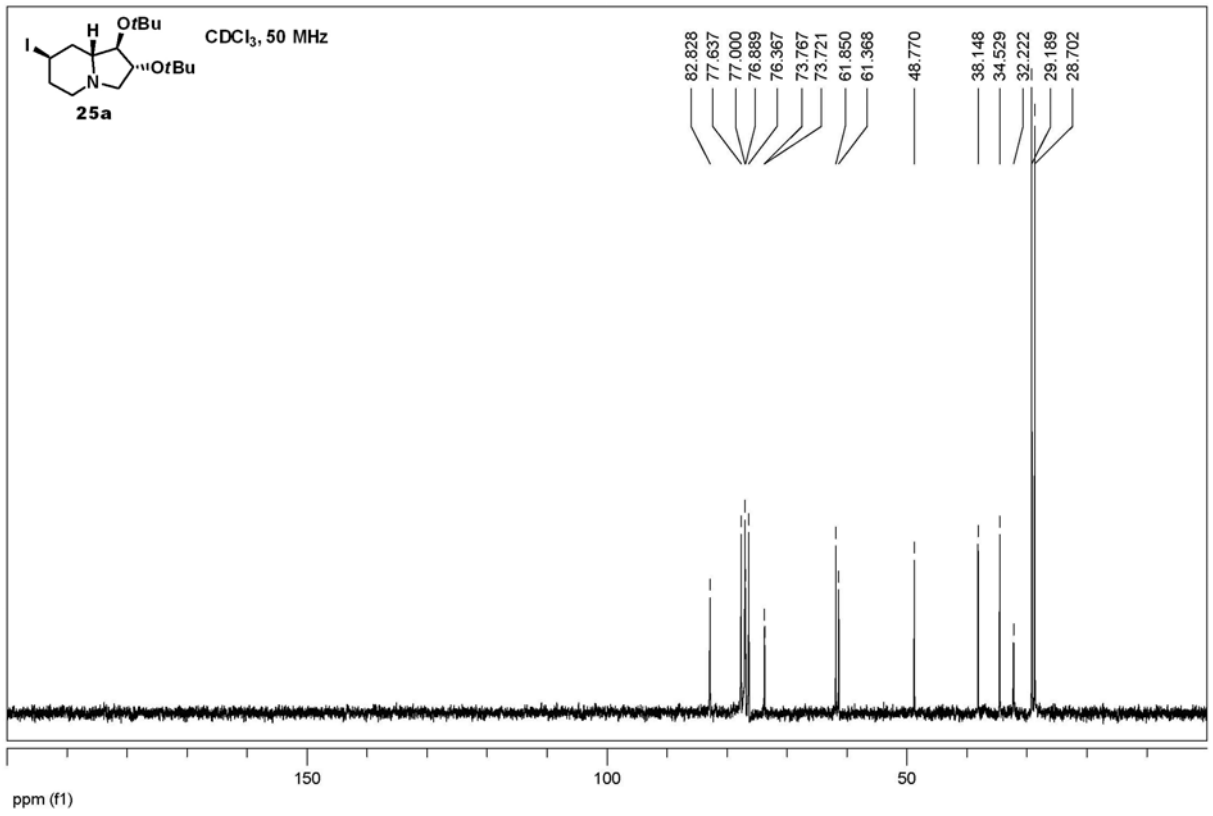
1.0
ppm (f1)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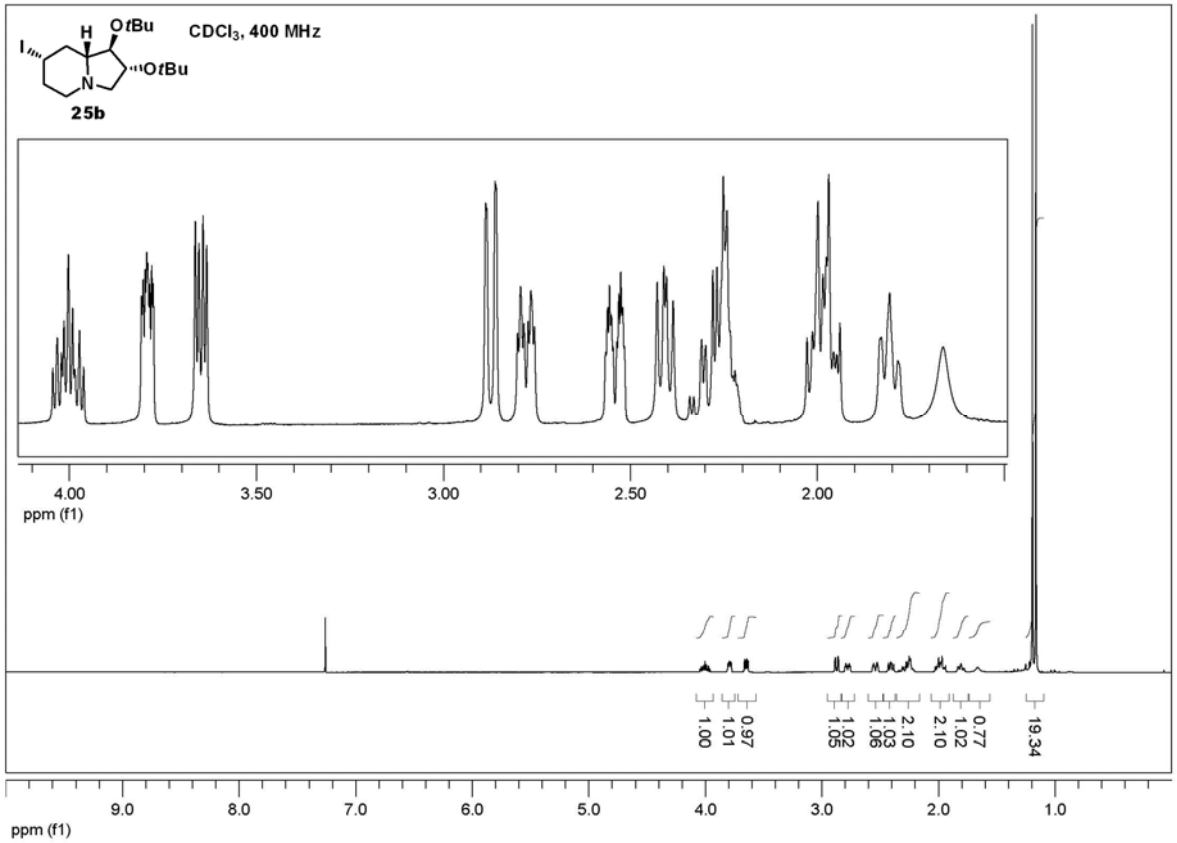
100
50
ppm (f1)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H
I
OtBu
N
25b
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3.50
3.00
2.50
2.00
ppm (f1)
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1.01
0.67
1.05
1.02
1.06
1.03
2.10
2.10
1.02
0.77
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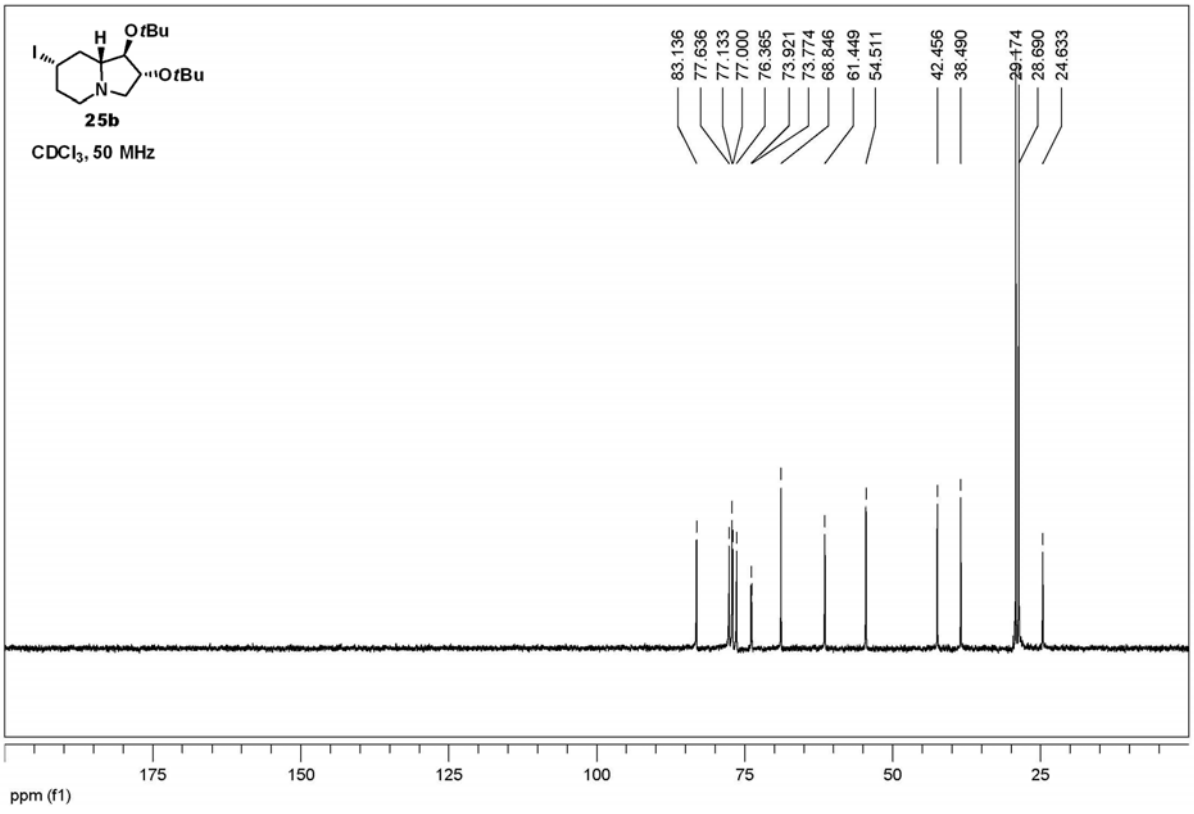
1.0
ppm (f1)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I
OtBu
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77.000
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73.774
68.846
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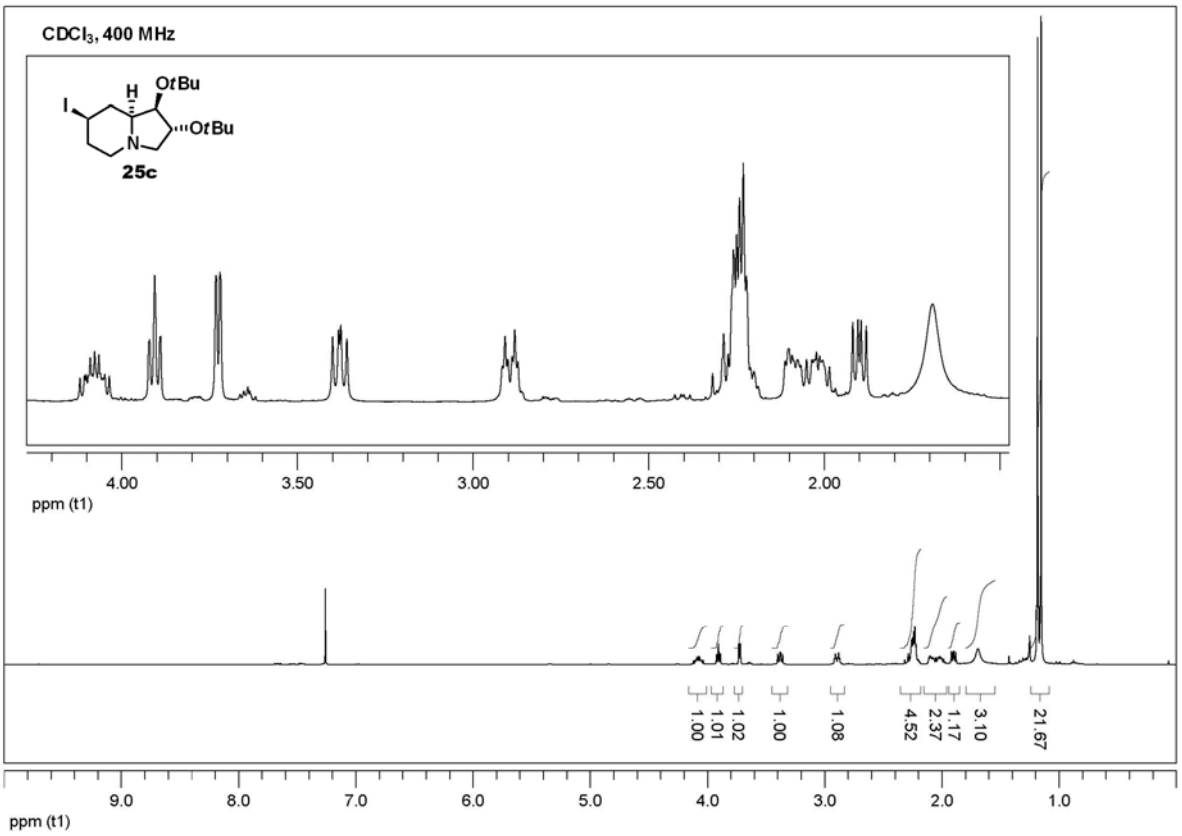
150
125
100
75
50
25
ppm (f1)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H
I
OtBu
N
25c
4.00
3.50
3.00
2.50
2.00
ppm (t1)
1.00
1.01
1.02
1.00
1.08
4.52
2.37
1.17
3.10
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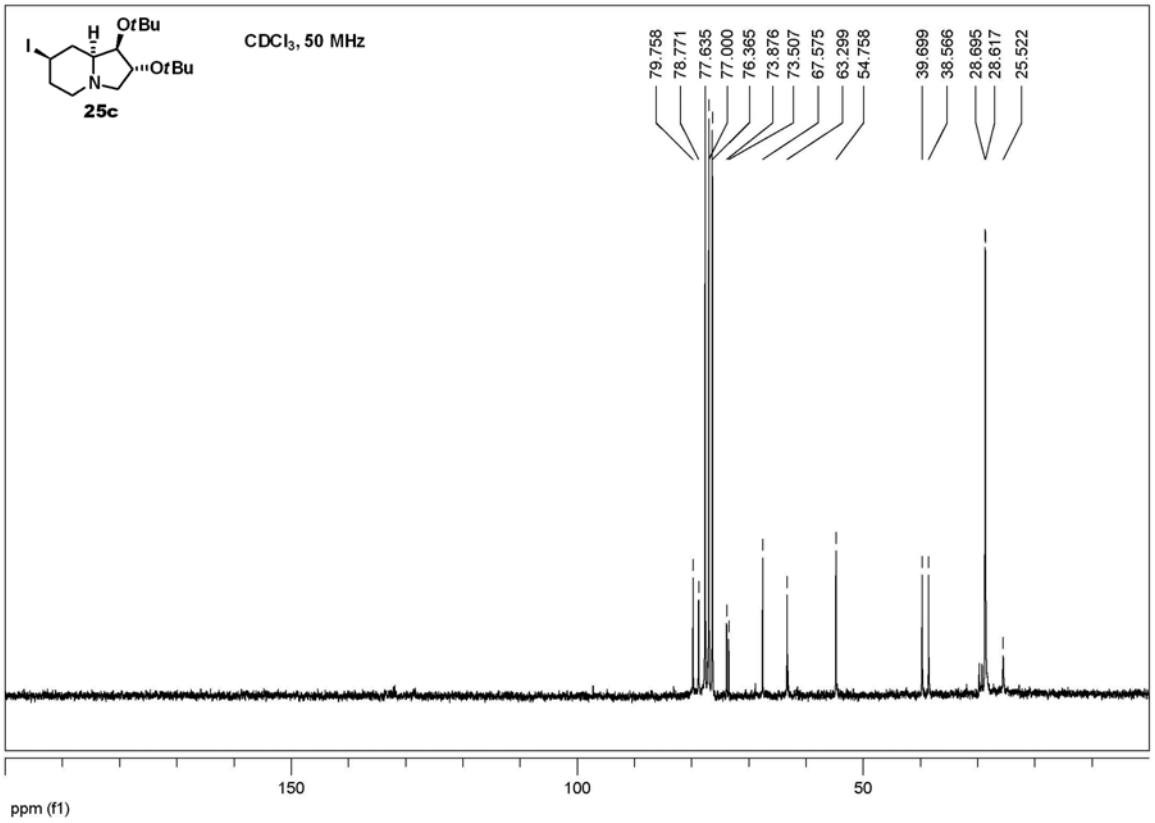
3.0
2.0
1.0
ppm (t1)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I
OtBu
N
25c
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54.758
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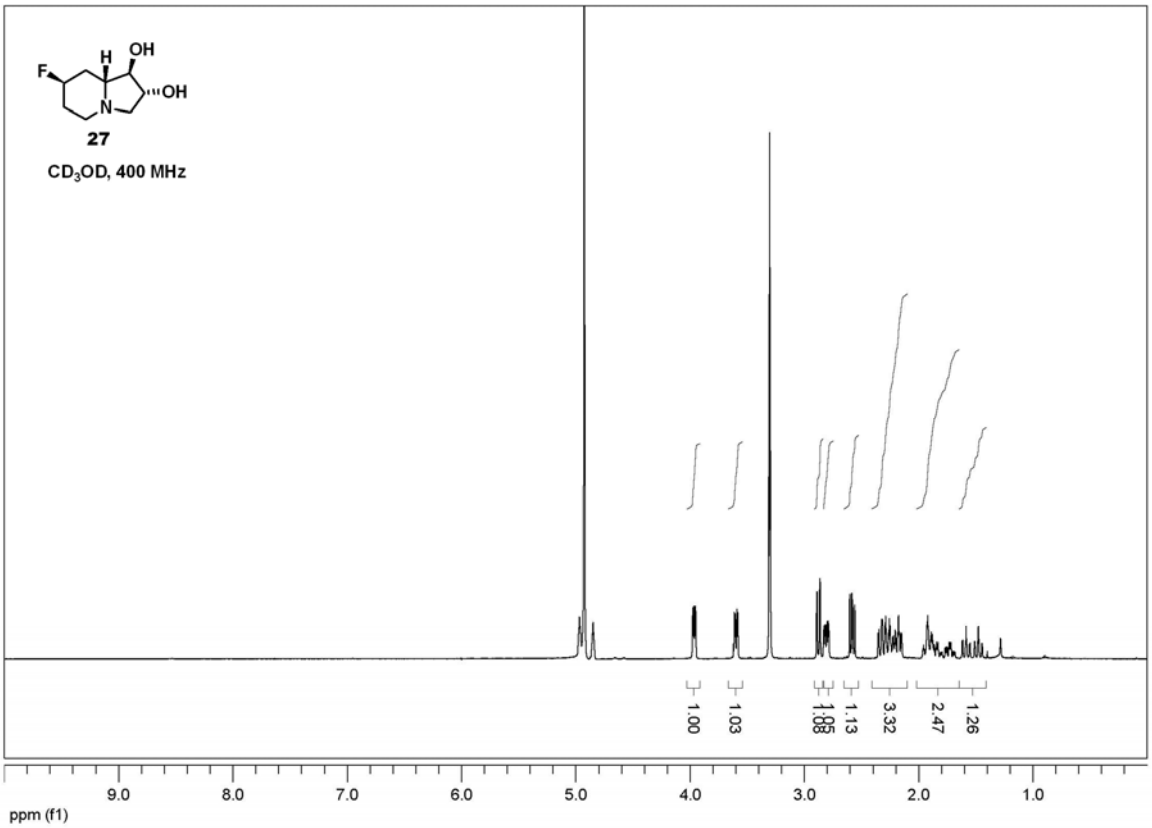
150
100
50
ppm (f1)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1.05
1.08
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3.32
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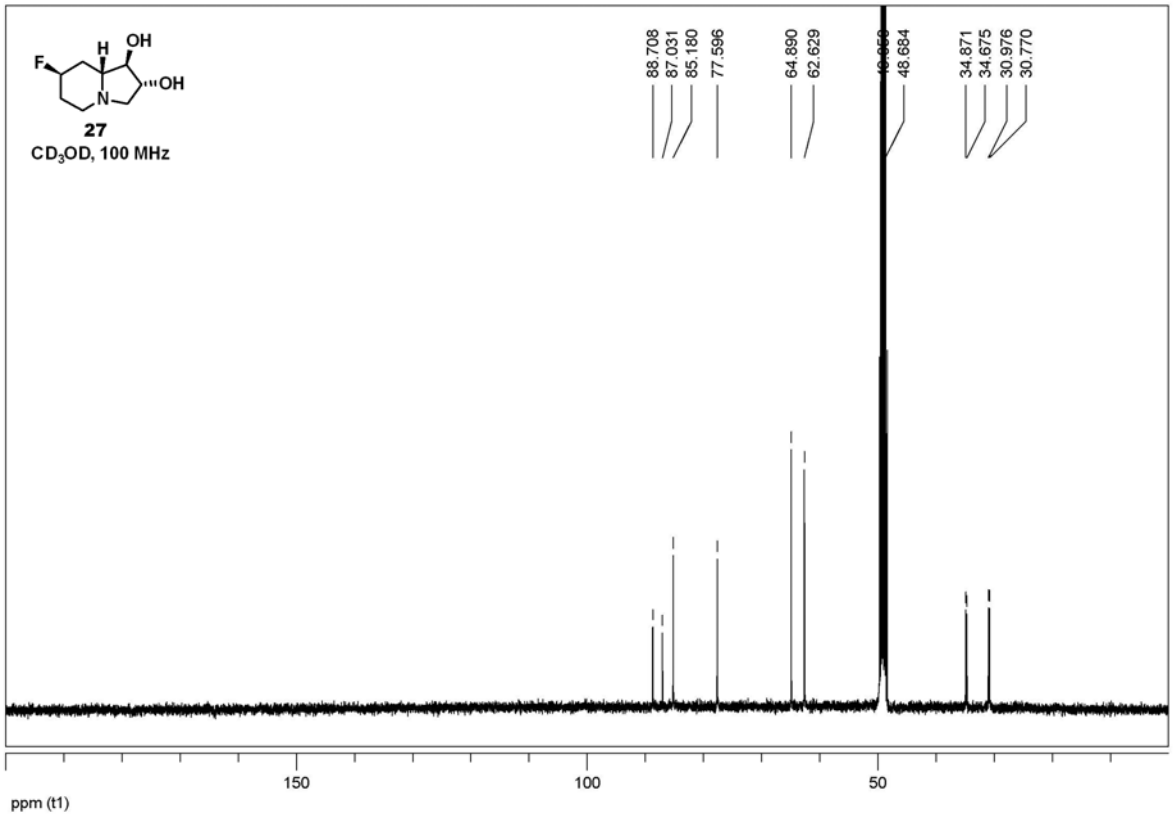
4.0
3.0
2.0
1.0
ppm (f1)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62.629
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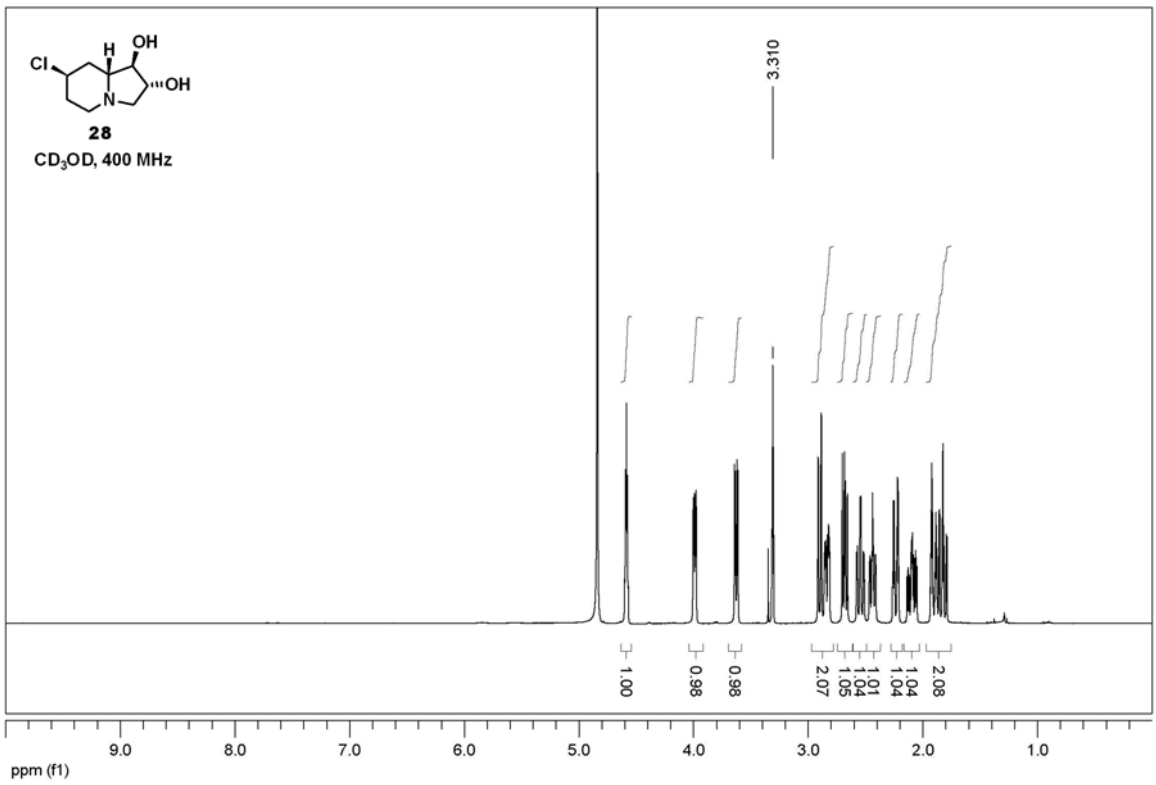
30.976
30.770
150
100
50
ppm (t1)


Cl
H
OH
OH
N
28
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0.98
0.96
2.07
1.05
1.04
1.01
1.04
1.04
2.08
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8.0
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5.0
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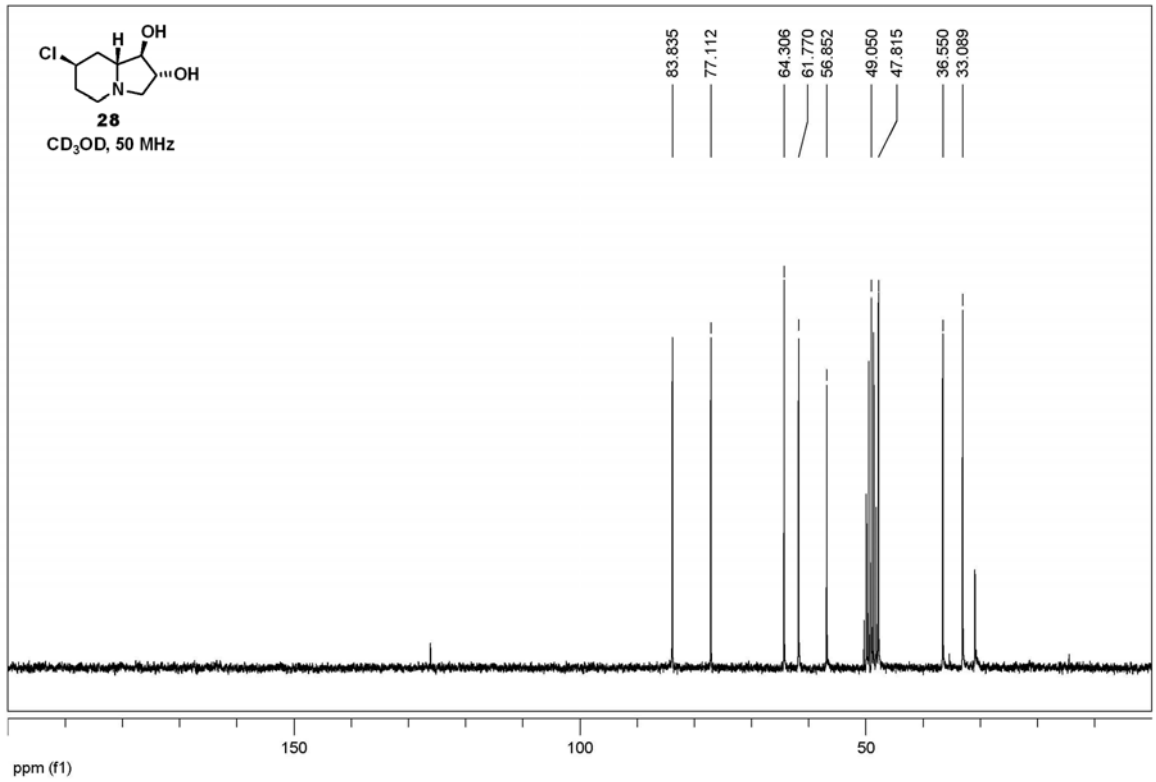
3.0
2.0
1.0
ppm (f1)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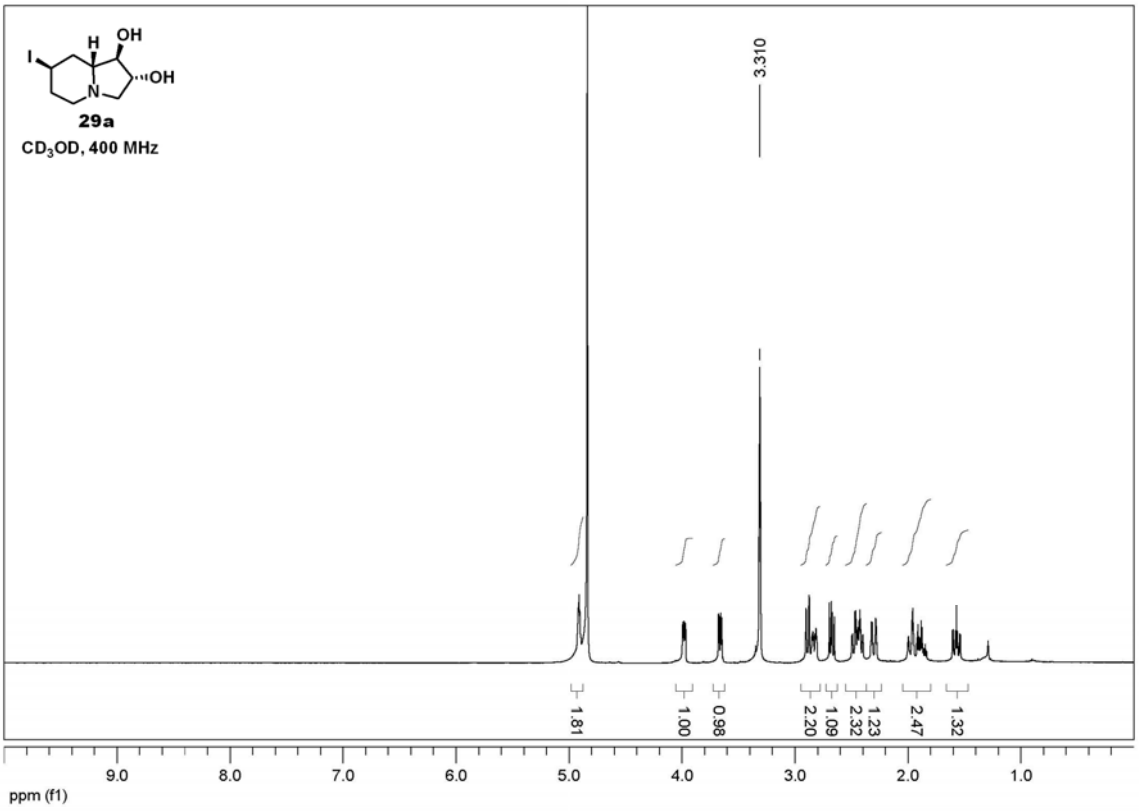
150
100
50
ppm (f1)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1.09
2.32
1.23
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6.0
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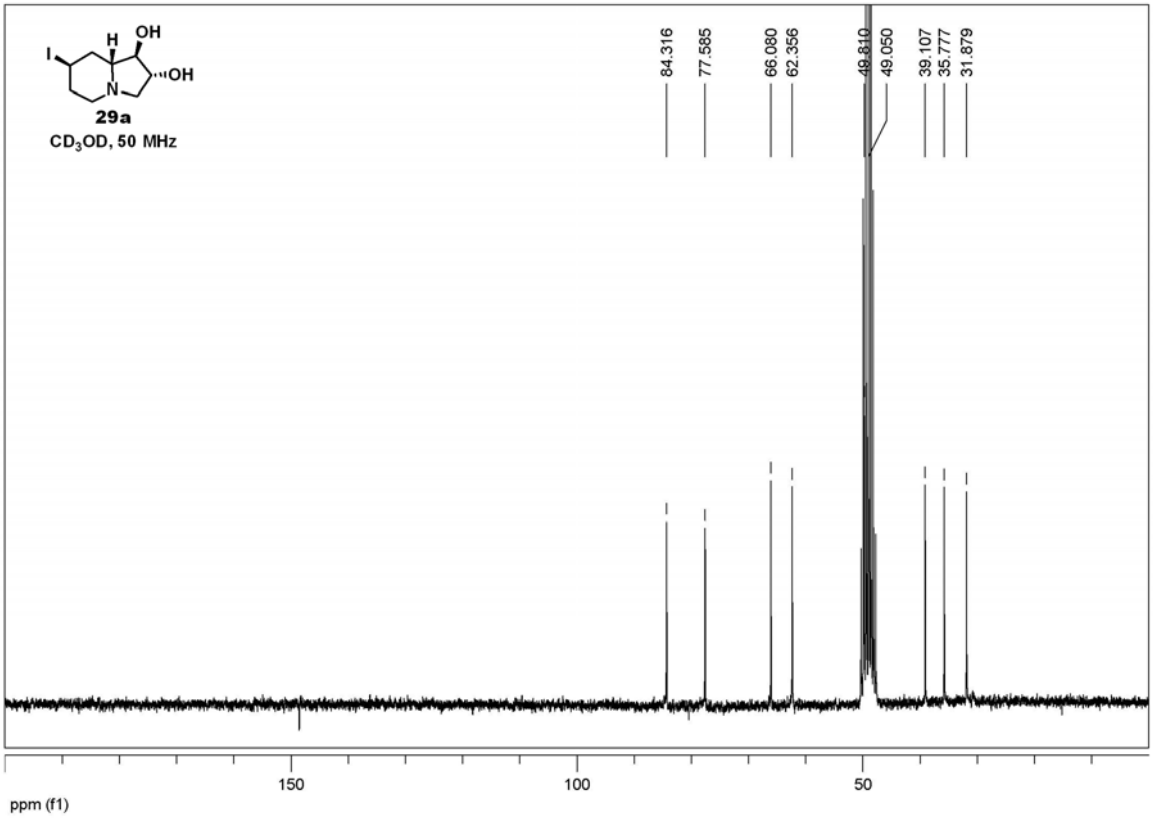
3.0
2.0
1.0
ppm (f1)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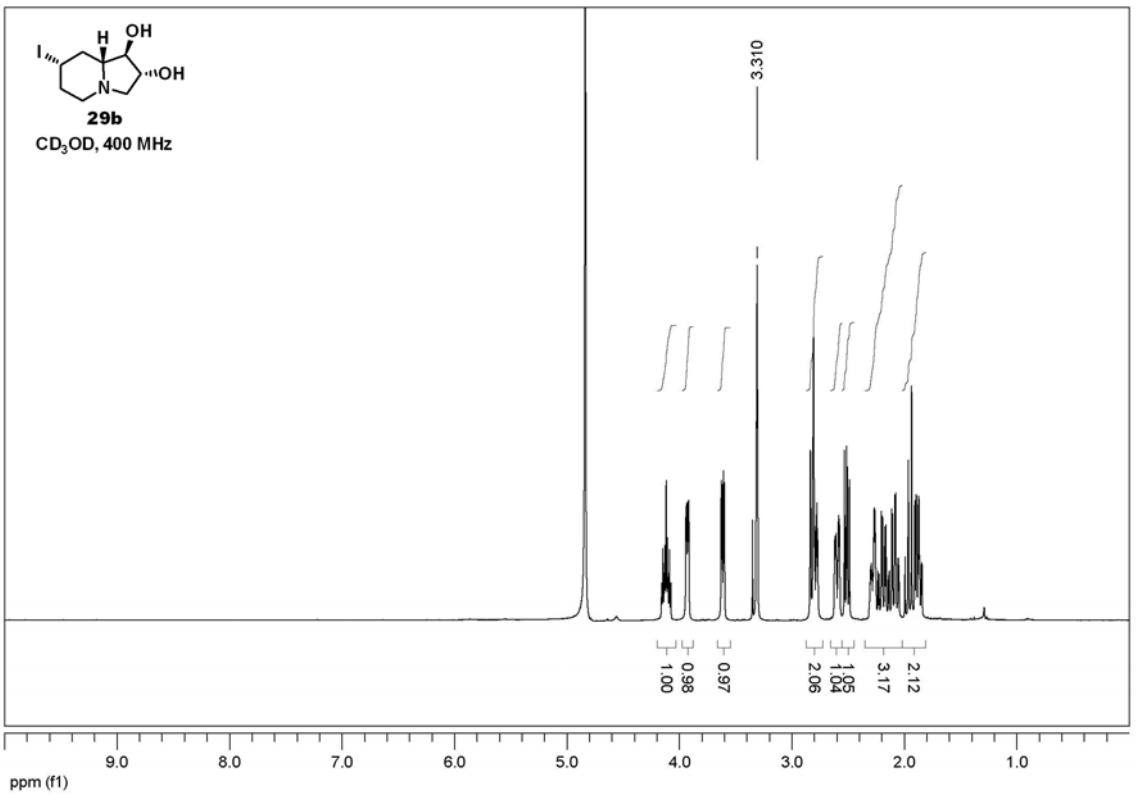
150
100
50
ppm (f1)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1.05
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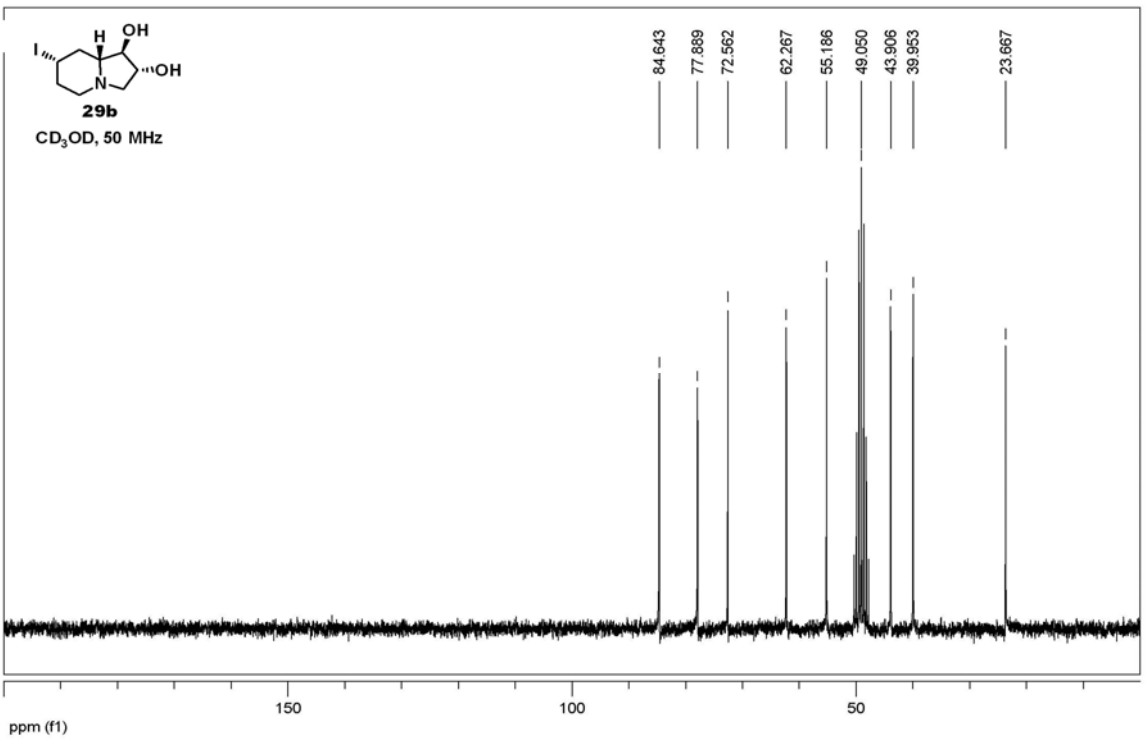
ppm (f1)


29b
CD3OD, 50 MHz
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62.267
55.186
49.050
43.906
39.953
23.667
150
100
50
ppm (f1)

Crystallographic data for compounds 20 and 25c

20:
$C_{16}H_{30}ClNO_2$, M=303.86, Orthorhombic, space group P 21 21 21, *a*=9.6080(1), *b*=10.5870(2), *c*=17.1010(3)Å, V=1739.51(5)Å^3, Z=4 D_c=1.160, μ=1.950 mm^{-1}, F(000)= 664.
4891 reflections were collected with a 4.91<θ< 70.64 range with a completeness to theta 95.8%; 2808 were unique, the parameters were 182 and the final R index was 0.0294 for reflections having I>2σI, and 0.0347 for all data. Flack parameter is -0.002(16) for the exact configuration.
Hydrogen atoms were all assigned in calculated positions.
Absolute configuration was determined by anomalous-dispersion effects in diffraction measurement on the crystal.
A colourless prismatic shaped crystal (0.10x0.06x0.03) was used for collection.
The experiment was carried out at 150°K under nitrogen atmosphere.

25c:
$C_{16}H_{30}INO_2$, M=395.31, Orthorhombic, space group P 21 21 21, *a*=9.035(1), *b*=10.527(1), *c*=19.476(2)Å, V=1852.4(3)Å^3, Z=4 D_c=1.417, μ=1.731 mm^{-1}, F(000)= 808.
10059 reflections were collected with a 4.32<θ< 32.60 range with a completeness to theta 91.1%; 5388 were unique, the parameters were 181 and the final R index was 0.0447 for reflections having I>2σI, and 0.0956 for all data. Flack parameter is -0.03(3) for the exact configuration.
Hydrogen atoms were all assigned in calculated positions.
Absolute configuration was determined by anomalous-dispersion effects in diffraction measurement on the crystal.
A colourless needle shaped crystal (0.10x0.05x0.04) was used for collection.
The experiment was carried out at room temperature.

Data for compound **20** were collected with a Goniometer Oxford Diffraction KM4 Xcalibur2. Cu/Kα radiation (40mA/-40KV), monochromated by an Oxford Diffraction Enhance ULTRA assembly, and an Oxford Diffraction Excalibur PX Ultra CCD were used for cells parameters determination and data collection.
Data for compound **25c** were collected with a Goniometer Oxford Diffraction KM4 Xcalibur2. Graphite-monochromated Mo/Kα radiation (40mA/-40KV) and a KM4 CCD/SAPPHIRE detector were used for cell parameter determination and data collection.
The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects.*

Direct methods of SIR2004** were used in solving the structures and they were refined using the full-matrix least squares on F^2 provided by SHELXL97***.
Multi-scan symmetry-related measurement was used as experimental absorption correction type.
The non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined as isotropic.

CCDC 1004877 for **20** and **CCDC 1004878** for **25c** contain the supplementary crystallographic data for this paper. These data can be obtained, free of charge, from The Crystallographic Data Centre, via www.ccdc.cam.ac.uk/data_request/cif

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