Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information

Re-Engineering 6-Thiopurine: Development of New Methods to Assess Toxicity and the Discovery of New Positive Small Molecule Potentiators

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Biological

All standard chemicals used this study were the highest grades available and were purchased through Sigma Aldrich (Saint Louis, MO, USA), VWR (Radnor, PA, USA), Fisher Scientific (Denver, CO, USA), or AK Scientific (Union City, CA, USA). Specialized reagents were purchased through specific vendors. Glycylglycine (gly-gly: G1127), β-nicotinamide adenine dinucleotide (NAD^+: N1636), uridine 5'-diphosphoglucose (UDPG disodium salt: U4625), uridine 5'-diphosphoglucuronic acid (UDPGA: U6751), uridine 5'-diphosphoglucose dehydrogenase (UDPGDH: U6885), 6-thiopurine monohydrate (6TP), bilirubin (including three mixed isomer, B4126), alamethicin (A5361), pooled rat liver microsomes, Williams' Medium E Formulation (WME: W1878),AND (M9066) were purchased from Sigma Aldrich. 6-Thiouric acid (6TU: SC-213040), and doxorubicin (SC-280681) was purchased from Santa Cruz Biotechnology. HPLC-grade water was obtained by passing distilled water through a reverse osmosis system followed by treatment with a Thermo Scientific Barnstead Smart2Pure 3UV purification system (Fisher: 10-451-045), herein referred to as nanopure water. Trypsin (30-2101), RPMI-1640 media (30-2001), 100 μg/mL penicillin & 100 U/mL streptomycin (30-2300), and Fetal Bovine Serum (FBS: 30-2020) was purchased from American Tissue Culture Collection (ATCC). Sprague-Dawley male rats, 8-10 weeks old, were purchased from Taconic.

All standard consumable supplies used in this study were purchased from VWR or Fisher Scientific. All materials used in biological evaluation was purchased in sterile packaging. Specific equipment utilized in this work are: 1) Hewlett-Packard 8452 Diode Array UV/Vis spectrophotometer (Palo Alto, CA, USA) equipped with a Lauda Brinkman

Ecoline RE 106 E100 circulating water bath (VWR), 2) HPLC system consisting of an CBM-20A/20Alite system controller, SIL-20AHT Autosampler, SPD-20A SPD-20AVUV-Vis detector, LC-20AT Solvent delivery module, CTO-20A column oven, DGU-20A3R Degassing unit and LC-20AD/20AT Gradient Value Kit purchased from Shimadzu Scientific Instruments (Kyoto, Japan), 3) all incubated reactions were performed with a Labcare American PRECISION water bath model 25 purchased from Fisher Scientific, and 4) BioTek Synergy H1 Hybrid Multi-Mode reader purchased from Fisher Scientific. All HPLC separations were performed on a Discovery C18 analytical column, 4.6 mm x 100 mm, 5 μm particle size (504955-30) along with the respective guard column (59576) purchased from Sigma Aldrich. Data was processed and all figures and tables constructed via the program Prism 7.02 for Mac, GraphPad Software (La Jolla, CA, USA). All chemical structures were prepared with ChemDraw Professional 16.0 by Perkim Elmer (Waltham, MA). All statistical calculations within this body of work was performed by the treatment of two-way factorials (positive and negative controls, designed structure of RCBD, and T-tests).

UDP-Glucose Dehydrogenase Inhibition Assay

All protocols used for the formation of UDPGA via the enzymatic conversion by UDPGDH was performed by previously published procedures by our laboratory.[1]

UDP-Glucose Dehydrogenase HPLC Method

Figure S1: Mobile phase composition for the separation of substrate and products from UDPGDH for the HPLC method developed, and shown in Figure S2.

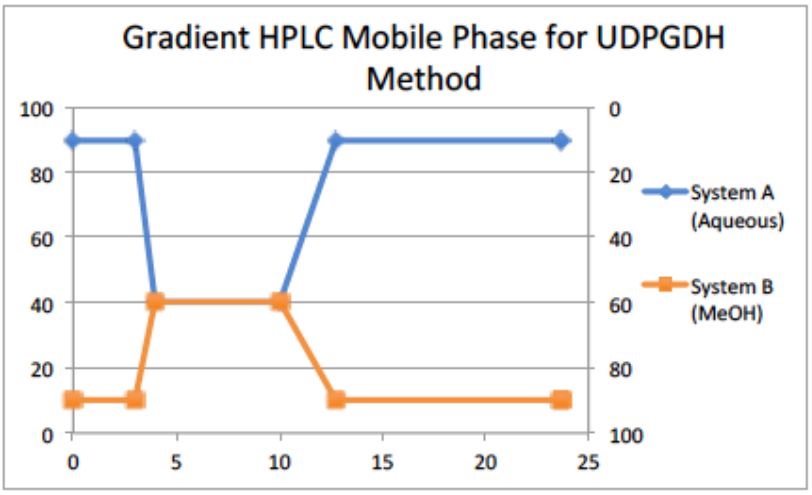


Figure S2: Chromatogram for the separation of substrates and products for the UDPGDH reaction via the mobile phase outlined in Figure S1.

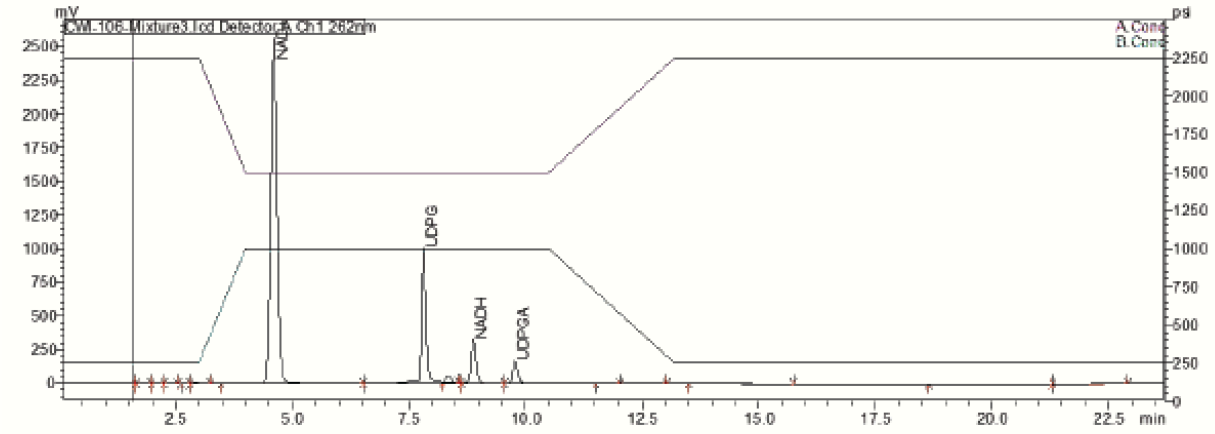
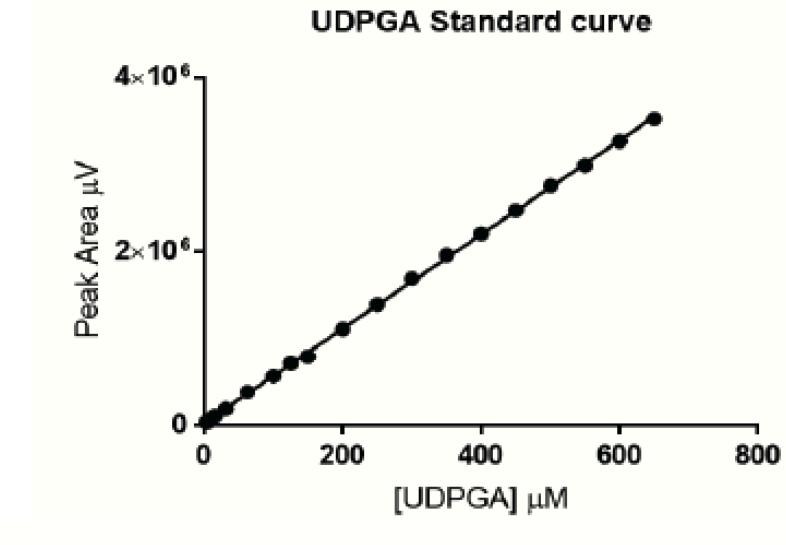
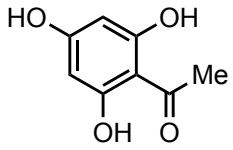


Figure S3: UDPGA Standard Curve



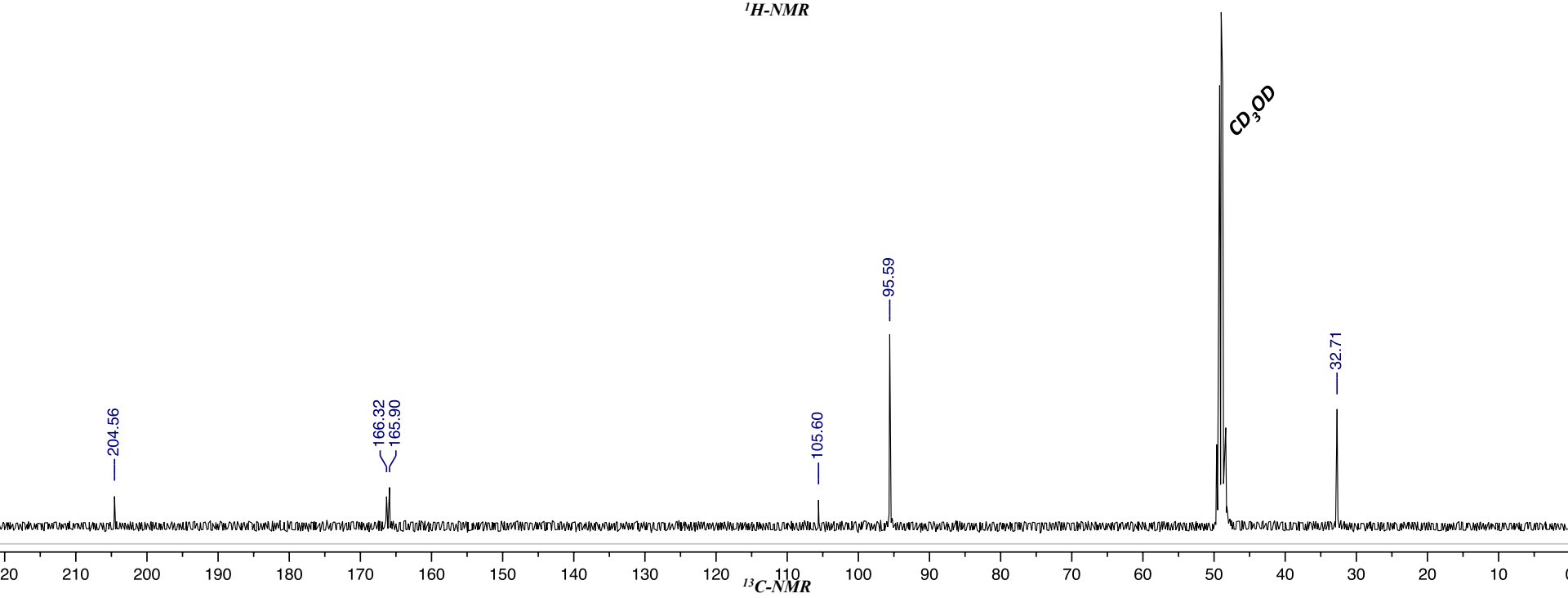
UDP-glucuronosyltransferase activity assay

All protocols used for the quantification of UCB, BMG, BDG via the enzymatic conversion by UGT-1A, and for purine inhibition, was performed by previously published procedures by our laboratory.[1-2]



9

^{1}H-NMR



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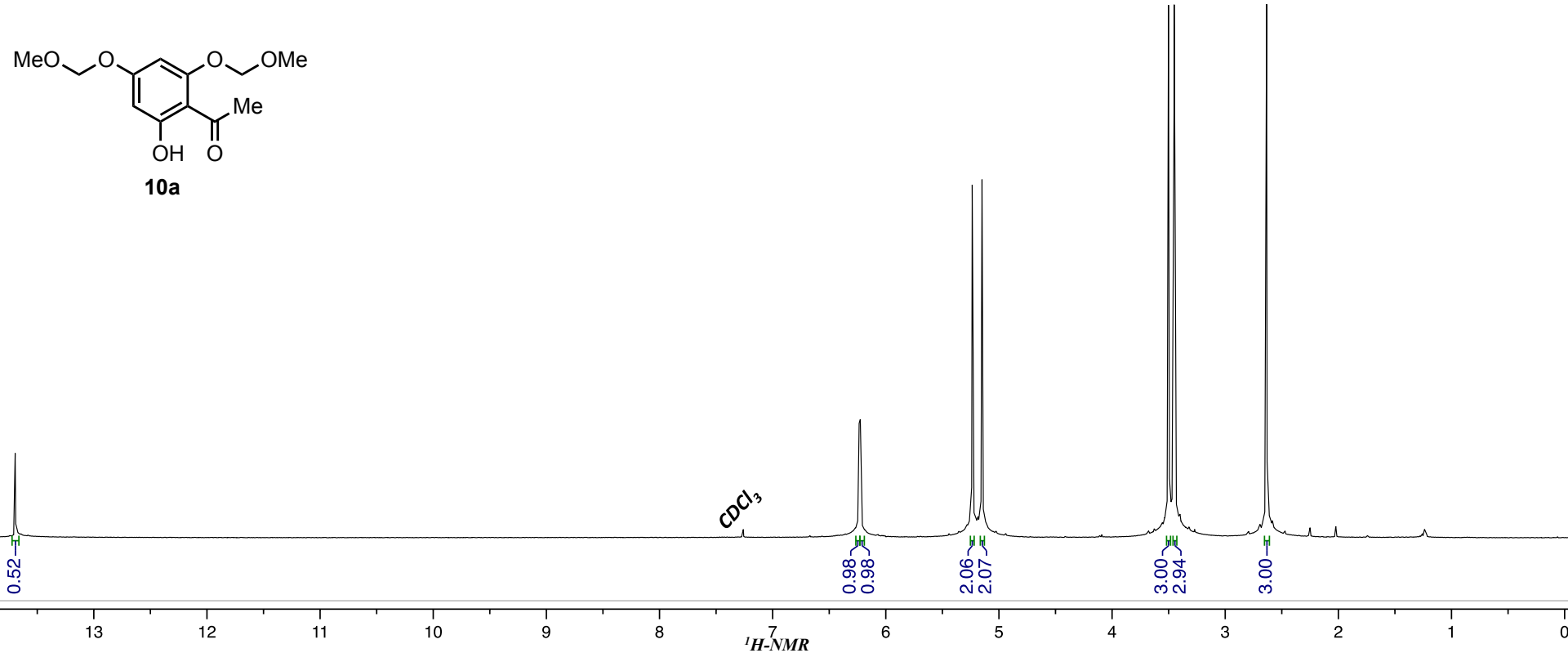
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O
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Me
OH
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10a
CDCl3
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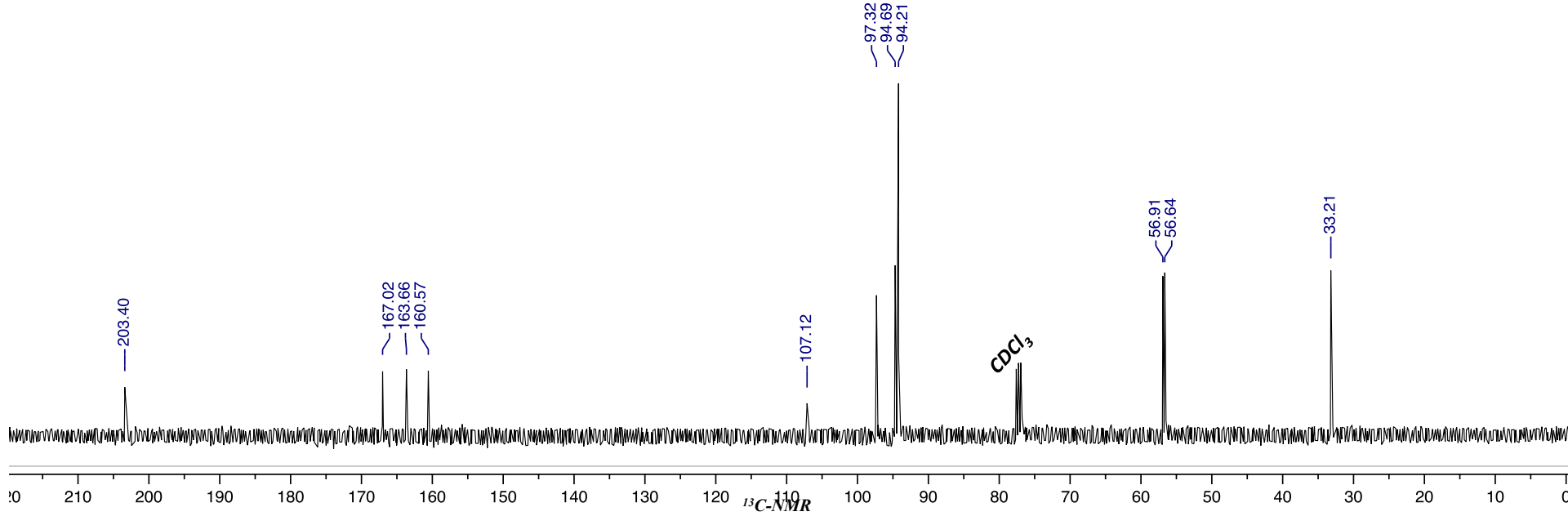
0.98
0.98
2.06
2.07
3.00
2.94
3.00
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1H-NMR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94.21
56.91
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160.57
107.12
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210
200
190
180
170
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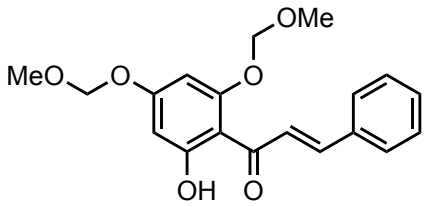
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
13C-NMR


OMe
MeO
O
O
OH
O

11a

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1.11
1.13
2.21
3.07
CDCl3
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0.95
2.36
2.35
3.00
3.00
"Grease"
13
12
11
10
9
8
7
6
5
4
3
2
1
0
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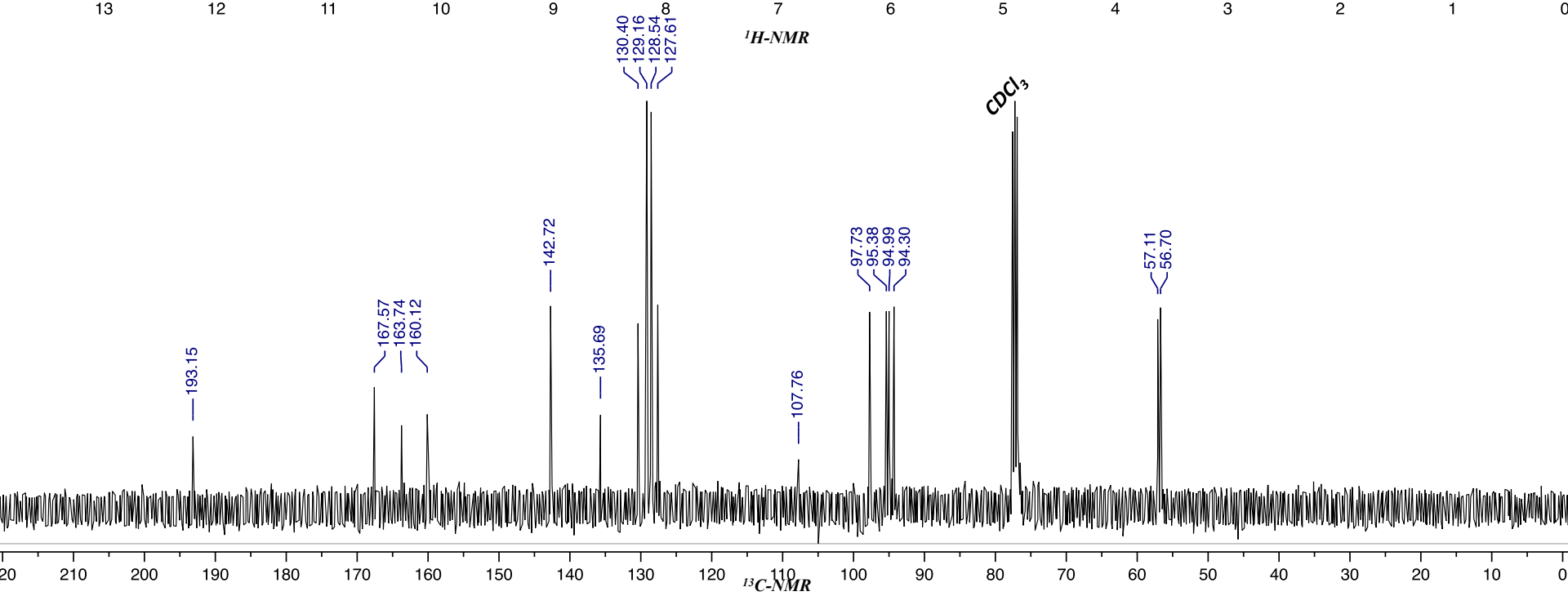
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163.74
160.12
142.72
135.69
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95.38
94.99
94.30
57.11
56.70
210
200
190
180
170
160
150
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130
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100
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80
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60
50
40
30
20
10
0
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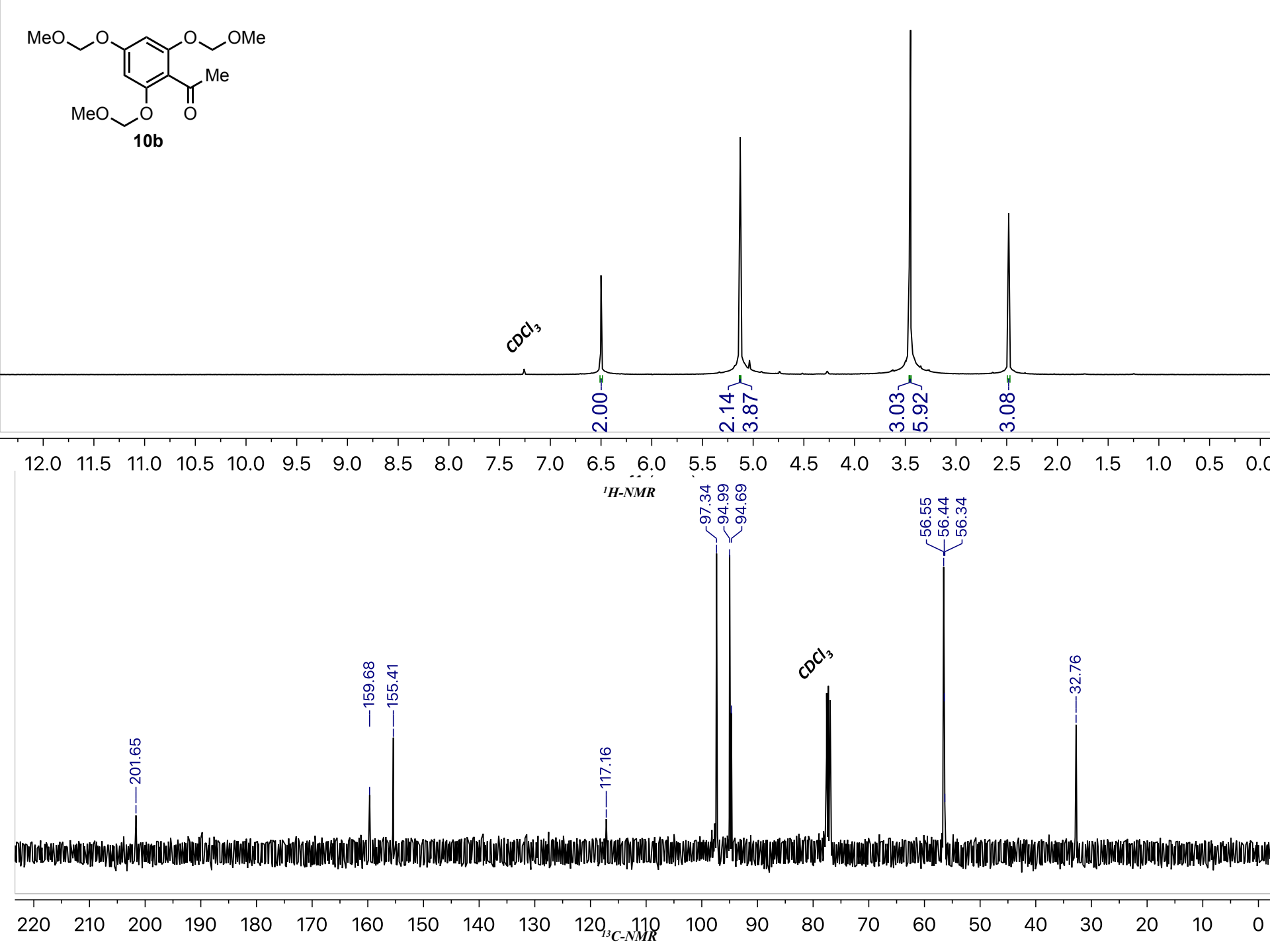
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O
O
OMe
Me
MeO
O
O
10b
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3.03
5.92
3.08
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H-NMR
97.34
94.99
94.69
56.55
56.44
56.34
159.68
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CDCl3
32.76
201.65
117.16
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
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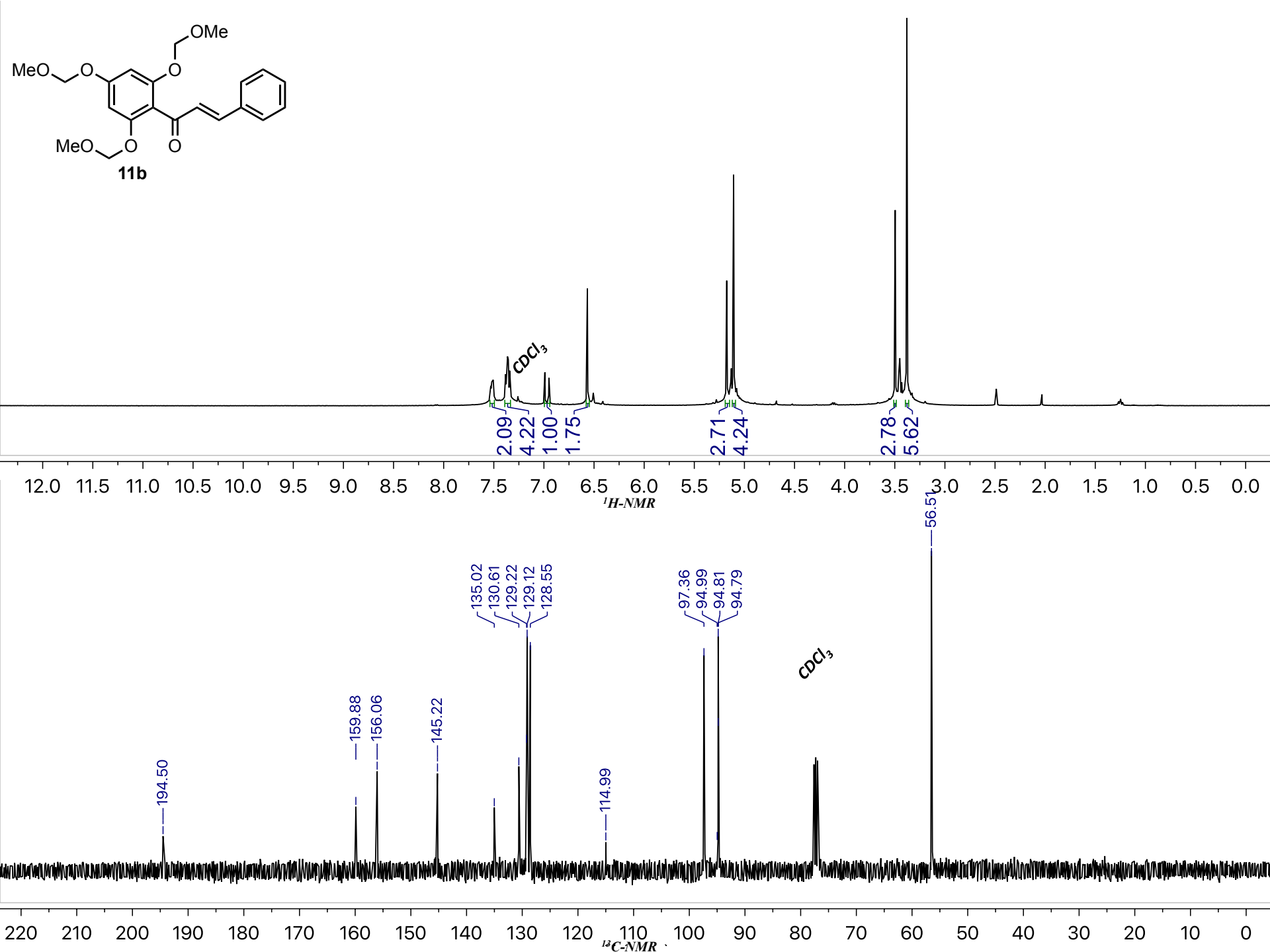
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O
O
MeO
O
O
11b
CDCl3
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4.22
1.00
1.75
2.71
4.24
2.78
5.62
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
1H-NMR
194.50
159.88
156.06
145.22
135.02
130.61
129.22
129.12
128.55
114.99
97.36
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94.79
CDCl3
56.51
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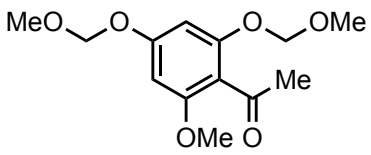
13C-NMR



10c

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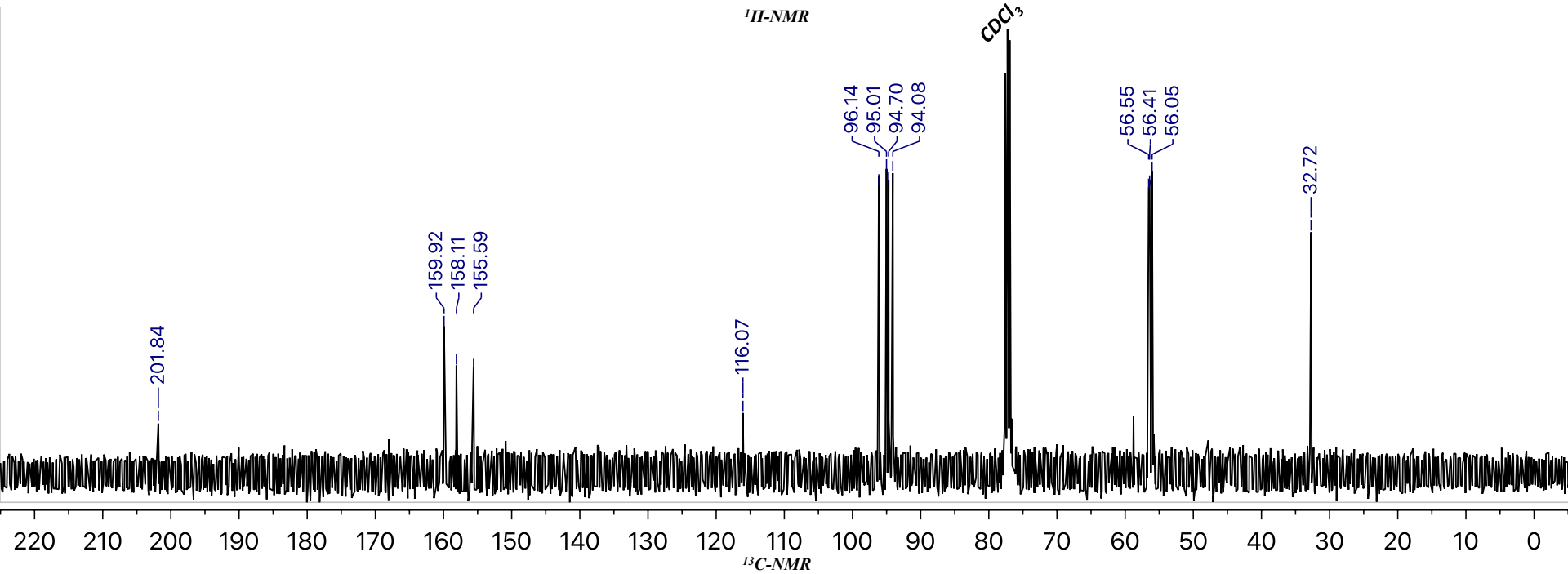

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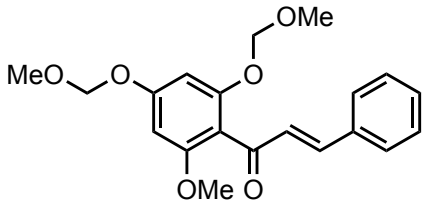

11c

^{1}H-NMR

CDCl$_3$

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12.0 11.5 11.0 10.5 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)

^{13}C-NMR

CDCl$_3$

194.56 160.10 158.77 156.16 144.91 135.13 130.52 129.19 129.07 128.58 96.15 94.84 94.80 94.21 56.53 56.48 56.16

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

References

1. Weeramange, C. J.; Binns, C. M.; Chen, C.; Rafferty, R. J., Inhibition of UDP-glucose dehydrogenase by 6-thiopurine and its oxidative metabolites: Possible mechanism for its interaction within the bilirubin excretion pathway and 6TP associated liver toxicity. *J. Pharm. Biomed. Anal.* **2018,** *151*, 106-115.
2. Torres Hernandez, A. X.; Weeramange, C.; Desman, P.; Fatino, A.; Haney, O.; Rafferty, R. J., Efforts in Redesigning the Antileukemic Drug 6-Thiopurine: Decreasing Toxic Side Effects while Maintaining Efficacy. *MedChemComm* **2018**.