Scalable synthesis of a key intermediate for the production of pleuromutilin-based antibiotics

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Supplementary Material

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General Experimental Procedures.

All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula, or were handled in a nitrogen-filled drybox (working oxygen level <5 ppm). Organic solutions were concentrated under reduced pressure at 20–35 °C. Flash-column chromatography was performed as described by Still *et al.*[1] using silica gel (60 Å, 40–63 μm particle size) purchased from SiliCycle. Analytical thin-layered chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV) and/or submersion in aqueous *p*-anisaldehyde solution (PAA) or aqueous potassium permanganate solution ($KMnO_4$), followed by brief heating with a heat gun.

Materials.

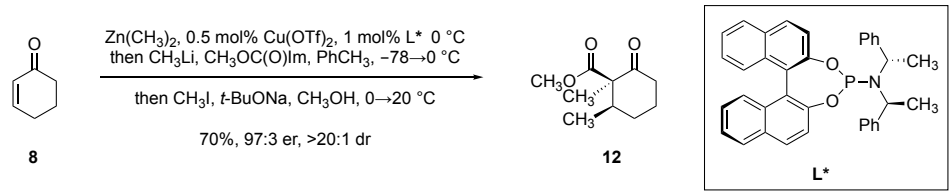
Benzene, dichloromethane, tetrahydrofuran, and toluene were purified according to the method of Pangborn *et al*.[2] The phosphoramidite ligand **L***,[3] *N*-(carbomethoxy)imidazole (the Heller–Sarpong reagent),[4] and the eneimide **5** (**19**→**5**, Scheme 2 of manuscript)[5] were prepared according to literature procedures. Potassium *tert*-butoxide was stored in a nitrogen-filled drybox. All other reagents and solvents were used as received.

Equipment.

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400, 500, or 600 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26). Data are represented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), integration. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 101 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonance of the solvent ($CDCl_3$, δ 77.0). Data are represented as follows: chemical shift (δ ppm). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were obtained using a Thermo Electron Corporation Nicolet 6700 FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). High-resolution mass spectrometry (HRMS) data were obtained on a Waters UPLC/HRMS instrument equipped with an ESI high-resolution mass spectrometry detector.

Synthetic Procedures and Characterization Data.

*Synthesis of the α-methyl-β-ketoester **12**:*

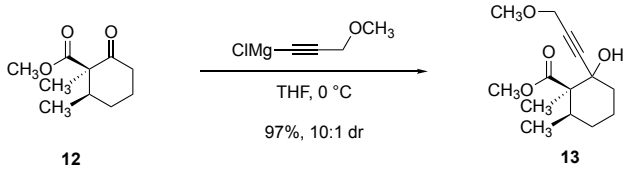


A suspension of copper(II) bis(trifluoromethansulfonate) (94.0 mg, 260 μmol, 0.500 mol%) and **L*** (281 mg, 520 μmol, 1.00 mol%) in toluene (70 mL) was stirred for 30 min at 20 °C. The resulting solution was cooled to 0 °C for 20 min and then cyclohex-2-ene-1-one (**8**, 5.04 mL, 52.0 mmol, 1 equiv) was added. A solution of dimethylzinc in toluene (1.2 M, 46.8 mL, 56.2 mmol, 1.08 equiv) was then added dropwise over 20 min and the resulting mixture was stirred for an additional 20 min at 0 °C. The stirred solution was cooled to –78 °C for 20 min and then a solution of methyllithium in ether (1.6 M, 35.1 mL, 56.2 mmol, 1.08 equiv) was added dropwise over 10 min. After stirring an additional 5 min at –78 °C, a solution *N*-carbomethoxyimidazole in toluene (4.33 M, 15.0 mL, 65.0 mmol, 1.25 equiv) was added dropwise over 10 min. The resulting solution was stirred for 10 min at –78 °C and then was allowed to warm to –30 °C over a period of 2 h. The mixture was then further warmed to 0 °C over a period of 2 h. The mixture was slowly diluted with methanol (100 mL) and the diluted mixture was stirred for 20 min at 0 °C. Iodomethane (16.2 mL, 260 mmol, 5.00 equiv) and sodium *tert*-butoxide (9.97 g, 104 mmol, 2.00 equiv) were then added in sequence. The resulting solution was allowed to warm to 20 °C over a period of 14 h. The warmed product mixture was diluted with aqueous citric acid solution (10% w/v, 400 mL) and the resulting mixture was extracted with ether (3 × 150 mL). The organic layers were combined and the combined layers were dried over magnesium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 5% ether–hexanes) to provide the *α*-methyl *β*-ketoester **12** as a colorless oil (6.71 g, 70%). The purity of the *α*-methyl *β*-ketoester **12** was determined to be >95% by quantitative ^{1}H NMR analysis. The spectroscopic data for the *α*-methyl *β*-ketoester **12** obtained in this way were in agreement with those previously reported.[6] For determination of the enantiomeric excess of the *α*-methyl *β*-ketoester **12**, see ref. 6.

R_f = 0.30 (5% ethyl acetate–hexanes; $KMnO_4$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 3H), 2.72 (td, *J* = 14, 6.8 Hz, 1H), 2.47–2.39 (m, 1H), 2.08–1.97 (m, 1H), 1.97–1.83 (m, 1H), 1.72–1.58 (m, 3H), 1.34 (s, 3H), 1.14 (d, *J* = 6.4 Hz, 3H).

*Synthesis of the propargylic alcohol **13**:*



A solution of *iso*-propylmagnesium chloride in tetrahydrofuran (2.0 M, 24.7 mL, 49.4 mmol, 1.30 equiv) was added dropwise over 10 min to a solution of methylpropargyl ether (4.17 mL, 49.4 mmol, 1.30 equiv) in tetrahydrofuran (25 mL) at 0 °C. The resulting solution was stirred for 20 min at 0 °C. The solution of the Grignard reagent was added dropwise over 10 min to a solution of the *α*-methyl *β*-ketoester **12** (7.00 g, 38.0 mmol, 1 equiv) in tetrahydrofuran (190 mL) at 0 °C. The resulting mixture was stirred for 20 min at 0 °C. The cold product mixture was diluted sequentially with saturated aqueous ammonium chloride solution (20 mL) and water (80 mL). The diluted product mixture was warmed to 20 °C over a period of 10 min. The warmed mixture was extracted with ether (3 × 100 mL). The organic layers were combined and the combined organic layers were dried over magnesium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the propargylic alcohol **13** [9.37 g, 97%, 10:1 dr (stereochemistry not assigned)]. The purity of the propargylic alcohol **13** obtained in this way was determined to be >95% by quantitative ^{1}H NMR analysis. An analytically-pure sample of the product **13** was obtained by preparative thin-layered chromatography (eluting with 35% ethyl acetate–hexanes).

Major diastereomer:

R_f = 0.48 (35% ethyl acetate–hexanes, PAA, stains green).

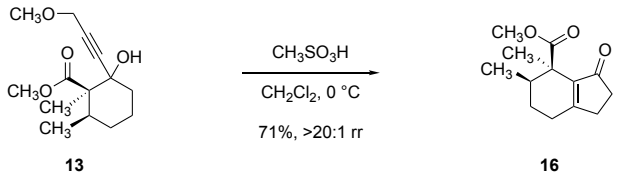
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (s, 2H), 3.70 (s, 3H), 3.61–3.41 (br s, 1H), 3.37 (s, 3H), 2.19 (ddd, *J* = 12.8, 11.4, 4.2 Hz, 1H), 1.94–1.43 (m, 6H), 1.46 (s, 3H), 0.98 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.8, 88.4, 81.5, 74.4, 60.1, 57.7, 54.4, 51.7, 38.9, 36.8, 29.7, 21.7, 20.6, 17.5.

IR (ATR-FTIR), cm^{-1}: 3448 (m, br), 2937 (s), 1725 (s), 1457 (m).

HRMS: calculated for $[C_{14}H_{22}O_4Na]^+$ 277.1416, found 277.1411.

*Synthesis of the cyclopentenone **16**:*

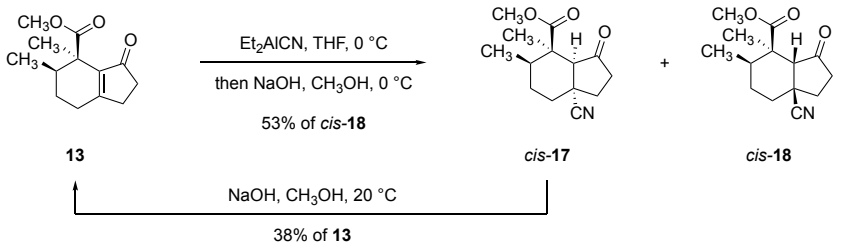


Methanesulfonic acid (10.9 mL, 167 mmol, 5.00 equiv) was added dropwise over 20 min to a solution of the propargylic alcohol **13** (8.50 g, 33.4 mmol, 1 equiv) in dichloromethane (30 mL) at 0 °C. The resulting mixture was stirred for 1 h at 0 °C and then was allowed to warm to 20 °C over 2 h. The warmed product mixture was diluted sequentially with ether (100 mL), water (100 mL), and aqueous sodium hydroxide solution (3 M, 60 mL). The diluted product mixture was extracted with ether (3 × 100 mL). The organic layers were combined and the combined organic layers were dried over magnesium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 10% ethyl acetate–hexanes initially, grading to 35% ethyl acetate–hexanes, linear gradient) to provide the cyclopentenone **16** as a yellow solid (5.26 g, 71%). The purity of the cyclopentenone **16** obtained in this way was determined to be >95% by quantitative ^{1}H NMR analysis. Spectrocopic data for the cyclopentenone **16** obtained in this way were in agreement with those previously reported.[5]

R_f = 0.38 (30% ethyl acetate–hexanes; UV).

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.61 (s, 3H), 2.51 (t, J = 4.7 Hz, 2H), 2.42–2.27 (m, 4H), 1.72–1.62 (m, 3H), 1.41 (s, 3H), 0.90 (d, J = 6.4 Hz, 3H).

*Synthesis of nitrile cis-**18**:*



A solution of diethylaluminum cyanide in toluene (1.0 M, 16.6 mL, 16.6 mmol, 3.00 equiv) was added dropwise over 10 min to a solution of the cyclopentenone **13** (1.23 g, 5.53 mmol, 1 equiv) in tetrahydrofuran (37 mL) at 0 ºC. The reaction mixture was stirred for 1 h at 0 ºC. The product mixture was diluted sequentially with saturated aqueous sodium bicarbonate solution (50 mL) and ether (50 mL) and the resulting mixture was warmed to 22 ºC. Water (30 mL) was added to the warmed mixture and the resulting mixture was stirred vigorously for 30 min at 22 ºC. The organic layer was separated and the aqueous layer was extracted with ether (3 × 100 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was dissolved in methanol (30 mL) and the resulting solution was cooled to 0 ºC for 5 min. Aqueous sodium hydroxide solution (100 mM, 9 mL) was added to the cooled solution. After stirring the resulting mixture for 1 h at 0 ºC, saturated aqueous ammonium chloride solution (50 mL) was added and the product mixture was extracted with ether (3 × 50 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 100% toluene initially, grading to 15% ethyl acetate–toluene, four steps) to provide the nitrile *cis*-**18** as white solid (727 mg, 53%). The spectroscopic data for the nitrile *cis*-**18** obtained in this way were in agreement with those previously reported.[5]

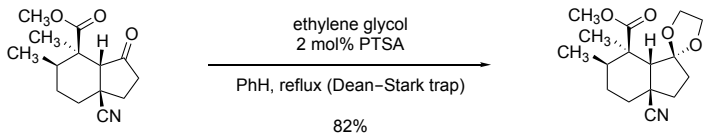
The nitrile *cis*-**17** and the cyclopentenone **13** were isolated separately from the nitrile *cis*-**18**. The mixture of *cis*-**17** and **13** were dissolved in methanol (40 mL) at 22 °C and aqueous sodium hydroxide solution (1 M, 10 mL) was added. The resulting mixture was stirred for 16 h at 22 ºC. The product mixture was concentrated to remove methanol and the concentrated mixture was diluted with saturated aqueous ammonium chloride solution (50 mL). The diluted solution was extracted with ether (3 × 50 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide cyclopentenone **13** (467 mg, 38%). The spectroscopic data for the cyclopentenone **13** obtained in this way were in agreement with those previously reported.[5]

Spectroscopic data for *cis*-**18**:

R_f = 0.33 (25% ethyl acetate–hexanes; $KMnO_4$).

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.68 (s, 3H), 3.12 (s, 1H), 2.49–2.34 (m, 1H), 2.33–2.17 (m, 2H), 2.16–2.07 (m, 1H), 2.06–1.91 (m, 2H), 1.56 (s, 3H), 1.54–1.48 (m, 1H), 1.48–1.40 (m, 1H), 1.36 (td, *J* = 13.7, 4.1 Hz, 1H), 1.16 (d, *J* = 7.0 Hz, 3H).

*Synthesis of the ketal **19**:*



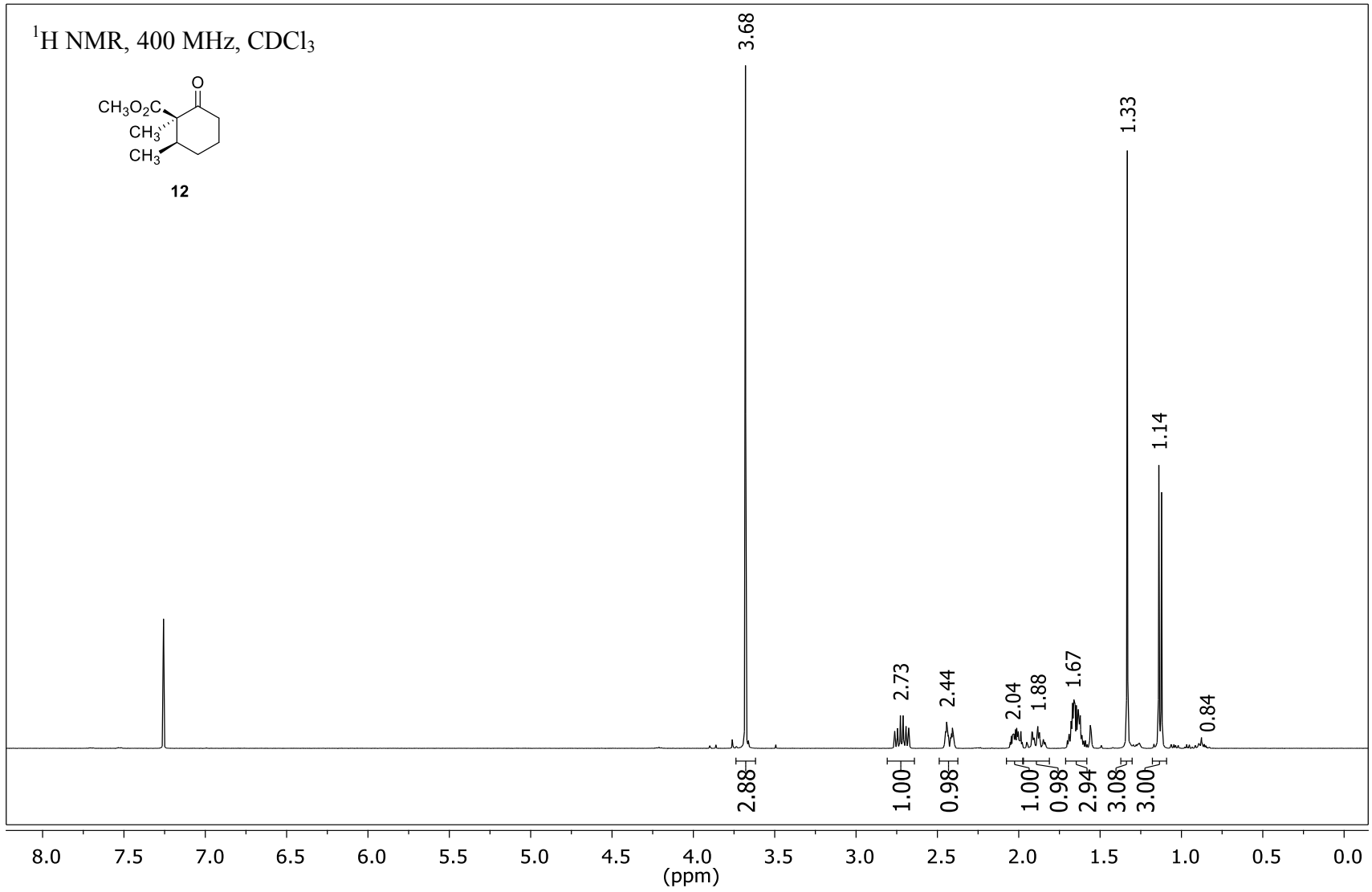
cis-**18** **19**

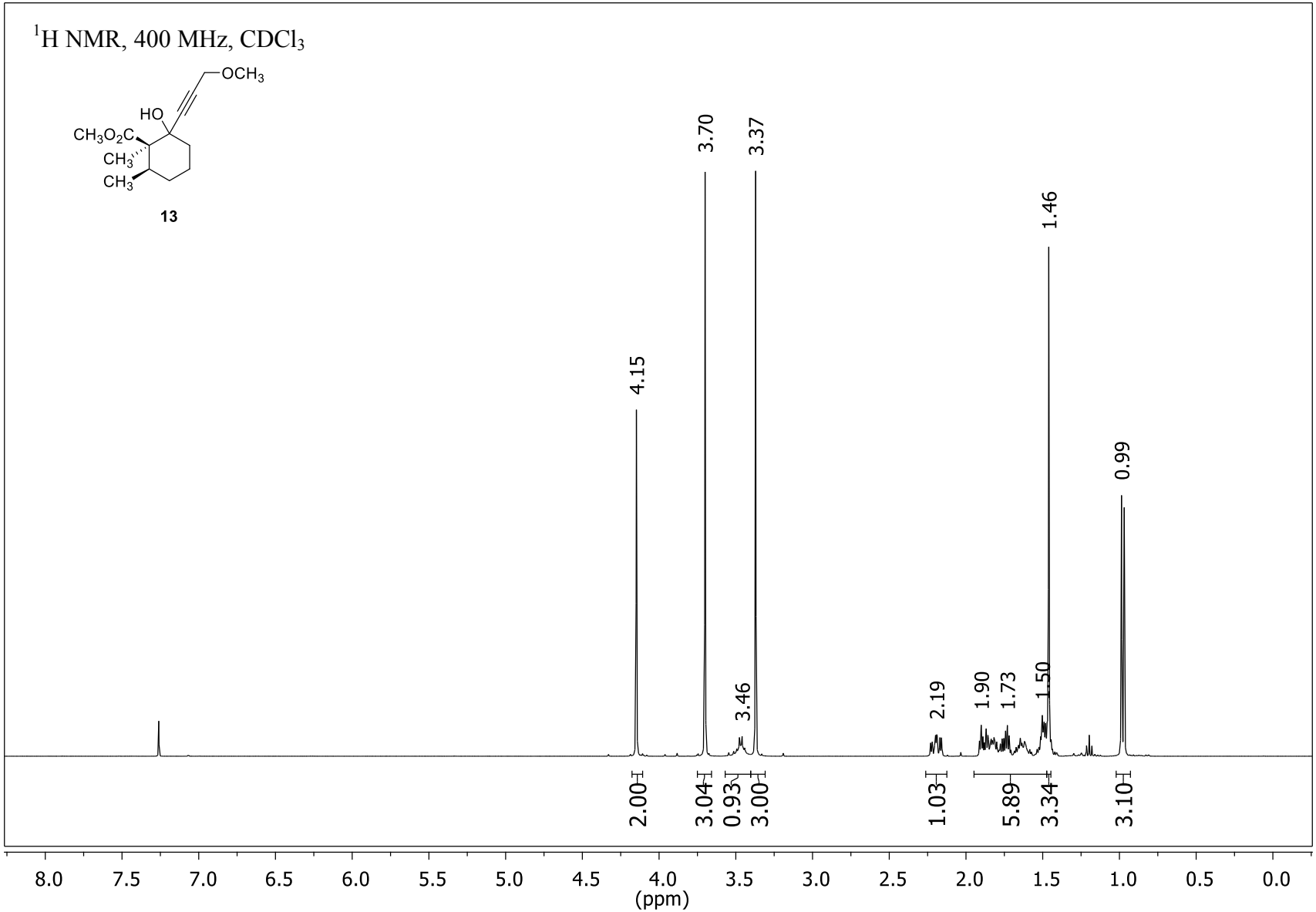
Ethylene glycol (674 μL, 12.1 mmol, 5.00 equiv) and *para*-toluenesulfonic acid (PTSA, 9.2 mg, 48.1 μmol, 2.00 mol%) were added in sequence to a solution of the ketone *cis*-**18** (600 mg, 2.41 mmol, 1 equiv) in benzene (6.0 mL) at 20 ºC. The resulting mixture was stirred and heated at reflux for 72 h with removal of water using a Dean–Stark trap. The product mixture was then allowed to cool to 20 °C. The cooled product mixture was concentrated and the residue obtained was purified by flash-column chromatography (eluting with 5% ethyl acetate–hexanes initially, grading to 40% ethyl acetate–hexanes, linear gradient) to afford the ketal **19** as a white solid (583 mg, 82%). The spectroscopic data for the ketal **19** prepared in this way were in agreement with those previously reported.[5]

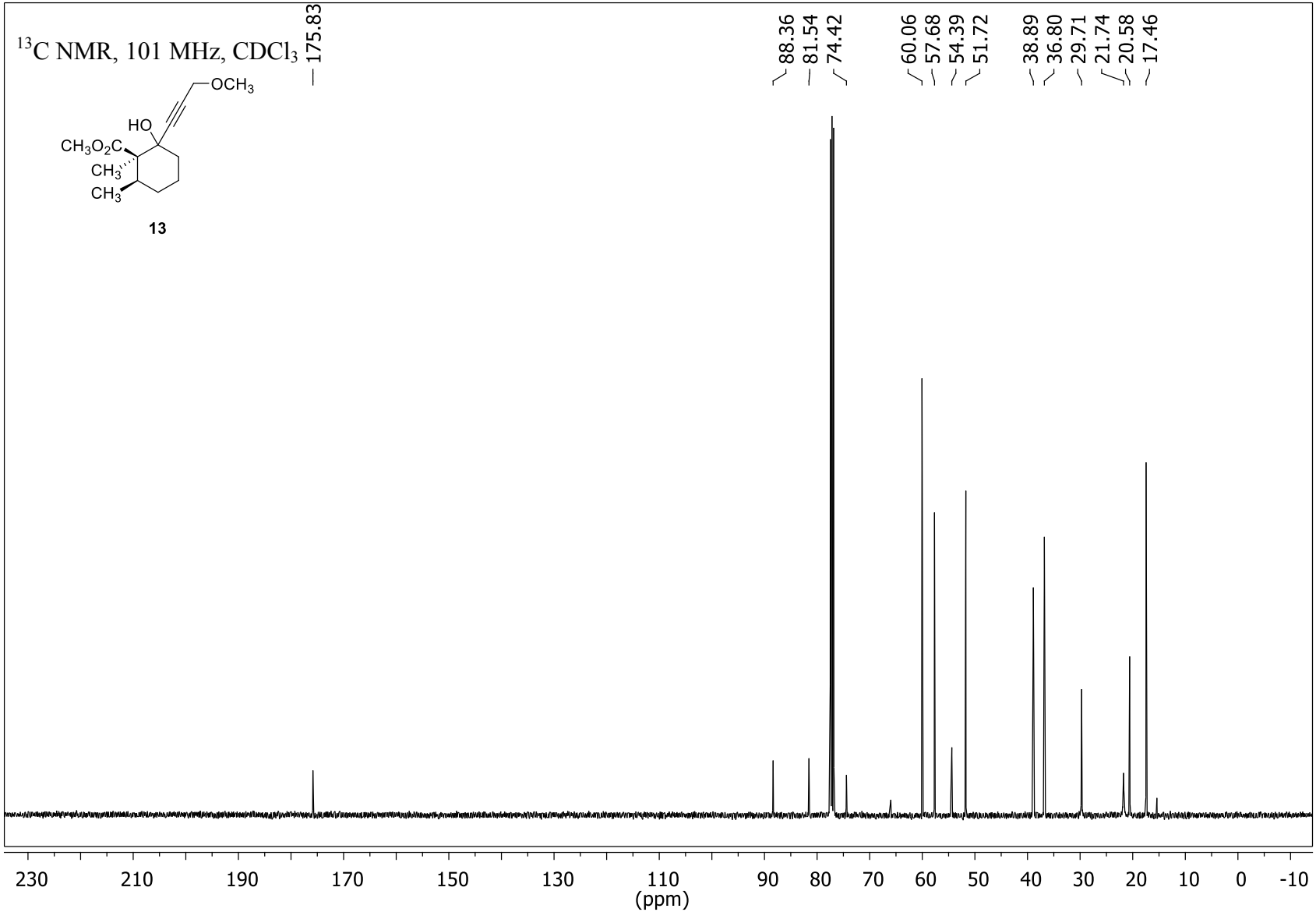
R_f = 0.36 (20% ethyl acetate–hexanes; PAA, stains brown).

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.03–3.96 (m, 1H), 3.94–3.85 (m, 2H), 3.84–3.77 (m, 1H), 3.69 (s, 3H), 3.08 (s, 1H), 2.18–1.72 (m, 8H), 1.58–1.50 (m, 1H), 1.32 (s, 3H), 1.13 (d, *J* = 6.9 Hz, 3H).

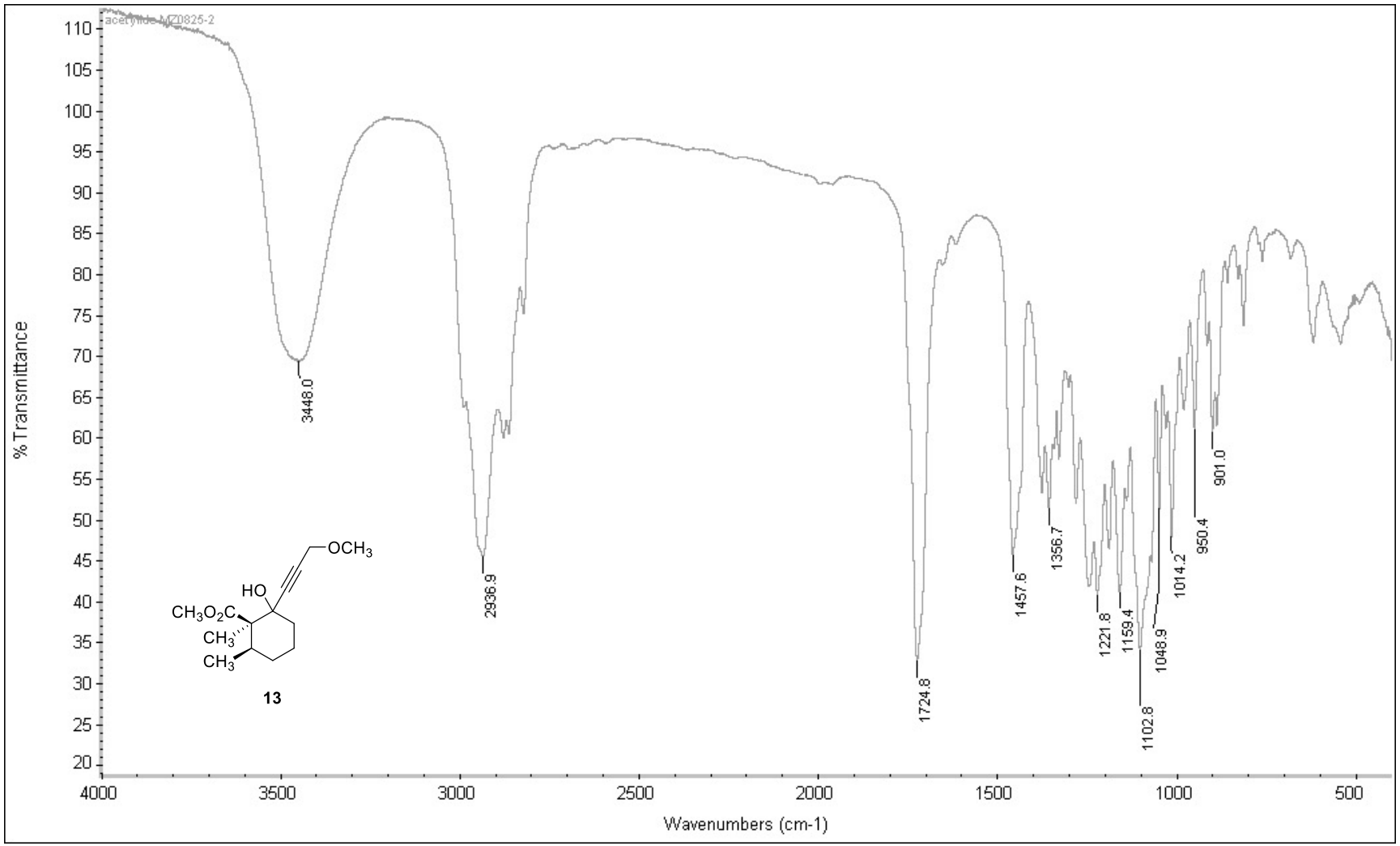
Catalog of Nuclear Magnetic Resonance and Infrared Spectra.

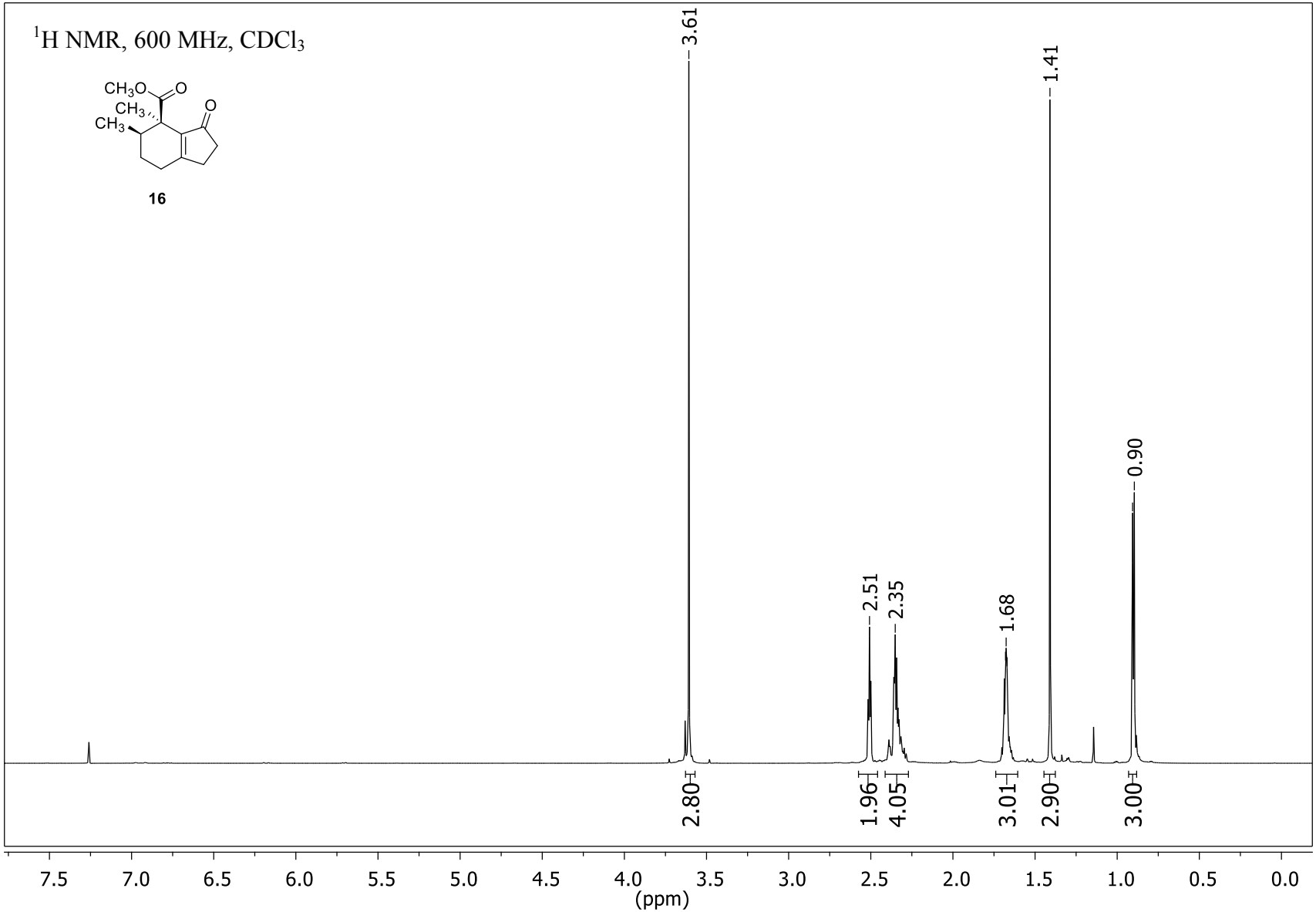


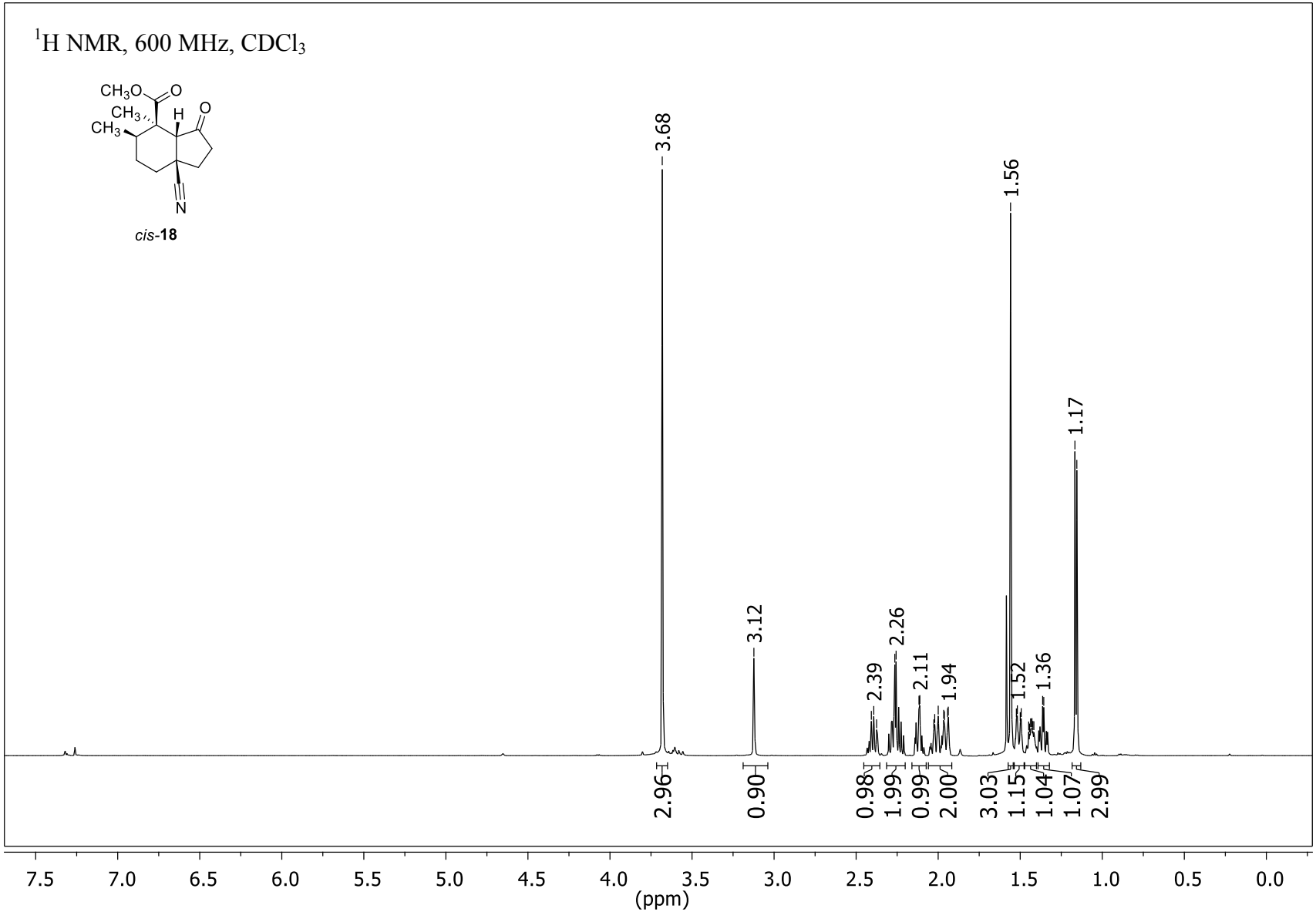

1H NMR, 400 MHz, CDCl3
OCH3
HO
CH3O2C
CH3
CH3
13
4.15
3.70
3.37
3.46
2.19
1.90
1.73
1.50
1.46
0.99
2.00
3.04
0.93
3.00
1.03
5.89
3.34
3.10
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

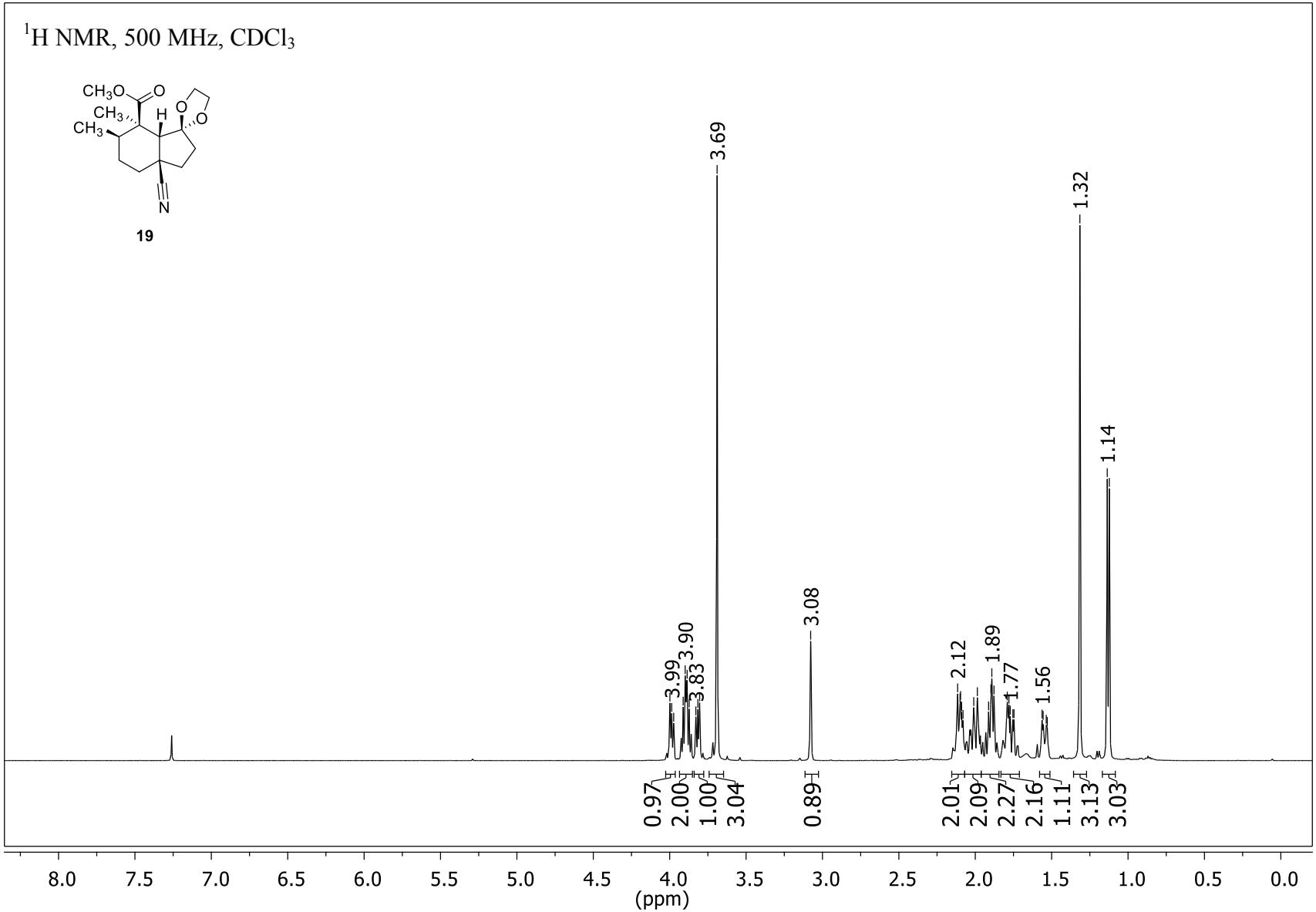

^{13}C NMR, 101 MHz, $CDCl_3$
175.83
88.36
81.54
74.42
60.06
57.68
54.39
51.72
38.89
36.80
29.71
21.74
20.58
17.46
OCH3
HO
CH3O2C
CH3
CH3
13
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
(ppm)

IR (ATR-FTIR)


acetylide-MZ0825-2
% Transmittance
Wavenumbers (cm-1)
3448.0
2936.9
1724.8
1457.6
1356.7
1221.8
1159.4
1102.8
1048.9
1014.2
950.4
901.0
OCH3
HO
CH3O2C
CH3
CH3
13


^{1}H NMR, 600 MHz, $CDCl_3$
CH_3O
O
CH_3
CH_3
O
16
3.61
1.41
0.90
2.51
2.35
1.68
2.80
1.96
4.05
3.01
2.90
3.00
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
(ppm)


1H NMR, 600 MHz, CDCl3
CH3O
O
CH3
H
O
CH3
N
cis-18
3.68
1.56
1.17
3.12
2.39
2.26
2.11
1.94
1.52
1.36
2.96
0.90
0.98
1.99
0.99
2.00
3.03
1.15
1.04
1.07
2.99
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
(ppm)


^{1}H NMR, 500 MHz, $CDCl_3$
CH_3O
O
CH_3
H
O
O
CH_3
N
19
3.69
1.32
1.14
3.08
3.99
3.90
3.83
2.12
1.89
1.77
1.56
0.97
2.00
1.00
3.04
0.89
2.01
2.09
2.27
2.16
1.11
3.13
3.03
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
(ppm)

Bibliography.

1. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
2. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.
3. Feringa, B. L.; Pineschi, M.; Arnold, L. A.; Imbos, R.; de Vries, A. H. M. *Angew. Chem., Int. Ed.* **1997**, *36*, 2620.
4. Heller, S. T.; Sarpong, R. *Org. Lett.* **2010**, *12*, 4572.
5. Murphy, S. K.; Zeng, M.; Herzon, S. B. *Science* **2017**, *356*, 956.
6. Murphy, S. K.; Zeng, M.; Herzon, S. B. *Org. Lett.* **2016**, *18*, 4880.