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Synthesis and X-ray structure of potent anticancer 4-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)isoxazole

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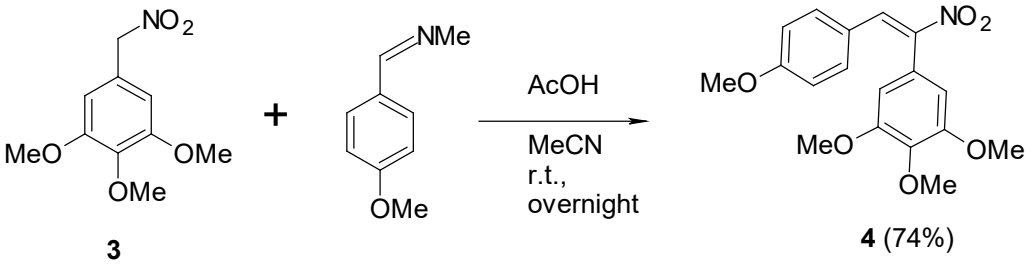
Table of content:

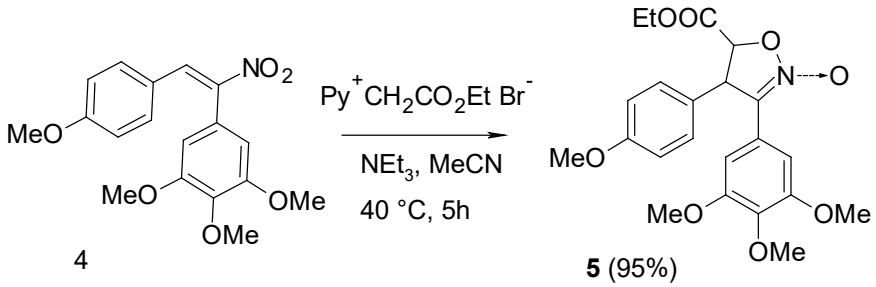
Chemistry. Materials and methods. Melting points were measured using a Boetius melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument [working frequencies of 500.13 MHz (^{1}H) and of 125.76 MHz (^{13}C), respectively]. Chemical shift values are reported in parts per million (ppm) and referenced to the appropriate NMR solvent peaks. Spin–spin coupling constants (*J*) are reported in Hertz (Hz). NMR spectra were processed using original software designed at N. D. Zelinsky Institute of Organic Chemistry RAS (Moscow, Russian Federation). Low resolution mass spectra (*m*/*z*) were recorded on a Finnigan MAT/INCOS 50 mass spectrometer at 70 eV using direct probe injection. High resolution mass spectra (HRMS) were measured on a Bruker micrOTOF II instrument using electrospray ionization (ESI). Elemental analysis was performed on the automated Perkin-Elmer 2400 CHN microanalyzer. Thin layer chromatography (TLC) analysis was performed using Merck 60 F_{254} plates. Flash chromatography was accomplished using Silica (Acros, 0.035– 0.070 mm, 60 Å). Solvents and reagents were purified by standard procedures.

***2-(4-Methoxyphenyl)-1-nitro-1-(3,4,5-trimethoxyphenyl)ethylene* (4)**



Acetic acid (48 mmol, 2.9 mL) was added to a solution of nitromethylarene **3** (4.14 g, 12 mmol) and anisaldehyde *N*-methylimine (1.94 g, 13 mmol) in MeCN (6.2 mL), and this was stirred at room temperature overnight (TLC control). The volatile substances were evaporated, the residue was diluted with CH_2Cl_2 (20 mL), washed with water (20 mL), and the organic layer was dried over $MgSO_4$. Then the solvent was evaporated, and the residue was triturated with minimal amount of hot MeOH. Crystals of nitroethylene **4** were filtered, washed with cold methanol and dried *in vacuo*. Yellow crystals (3.067 g, 74 %); mp. 142–146 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (s, 1H, HC=), 7.09 (d, J = 7.1 Hz, 2H, H-2′,6′), 6.78 (d, J = 6.8 Hz, 2H, H-3′,5′), 6.54 (s, 2H, H-2,6), 3.94 (s, 3H, OMe), 3.81 (s, 6H, 2 × OMe), 3.80 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 154.1 (2C), 147.2, 139.3, 134.9, 133.4 (2C), 126.2, 123.5, 114.4 (2C), 107.5 (2C), 61.0, 56.3 (2C), 55.4; HRMS (ESI/QTOF) *m*/*z*: $[M+H]^+$ Calc. for $C_{18}H_{20}NO_6$ 346.1285; Found 346.1277; $[M+Na]^+$ Calc. for $C_{18}H_{19}NO_6Na$ 368.1105; Found 368.1098.

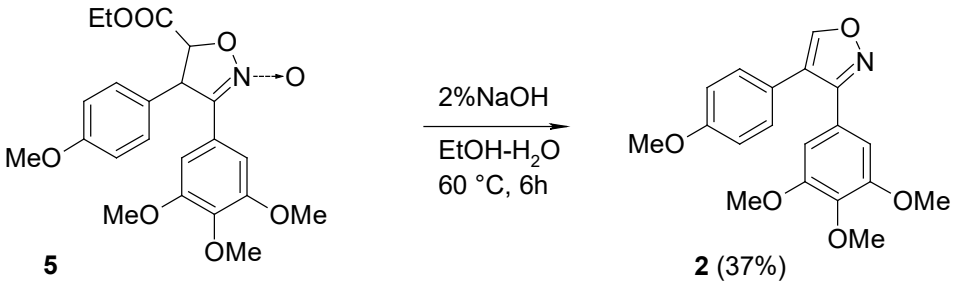
***5-Ethoxycarbonyl-4-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)- 4,5-dihydroisoxazole N-oxide* (5)**



Pyridinium salt (0.738 g, 3 mmol) was added to a solution of nitrostilbene 4 (0.518 g, 1.5 mmol) in MeOH (10 mL) at room temperature and stirring, then Et_3N (1.075 g, 4.35 mmol) was added, and this was stirred for 1 h (TLC control). The solvent was evaporated *in vacuo*, the residue was washed with water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give 4,5-dihydroisoxazole *N*-oxide **5.** Yellow oil (95%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 8.2 Hz, 2H, H-2′6′), 7.14 (s, 2H, H-2,6), 6.91 (d, J = 8.7 Hz, 2H, H-3′,5′), 4.99 (d, J = 2.8 Hz, 1H, H-4iso), 4.81 (d, J = 2.6 Hz, 1H, H-5iso), 4.38 – 4.28 (m, 2H, OCH_2), 3.82 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.76 (s, 6H, 2 × OMe), 1.35 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.1, 159.8, 153.1(2C), 139.2, 130.4, 128.3(2C), 128.2, 120.7, 115.0(2C), 104.4(2C), 78.7, 62.5, 60.8, 56.1,

55.3(2C), 54.2, 14.1. Calc for $C_{22}H_{25}NO_8$ C, 61.25; H, 5.84; N, 3.25. Found C, 61.35; H, 5.71; N, 3.46.

4-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)isoxazole (**2**)



4,5-Dihydroisoxazole *N*-oxide **5** was added to a 2% solution of NaOH in EtOH (10 mL) and water (2 mL), and this was stirred at 60°C for 5–6 h. Ethanol was evaporated *in vacuo*, the residue was suspended in CH_2Cl_2 (60 mL) and washed with water. The organic layer was dried with $MgSO_4$, evaporated. Isoxazole **2** was obtained as a yellowish oil (37%) after purification by column chromatography (benzene-benzene/ethyl acetate gradient 1:10). Analytical sample was obtained by crystallisation from MeOH. Yellow crystals; mp 93-94 °C (MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (s, 1H, H-5 iso), 7.23 (d, J = 7.2 Hz, 2H, H-2′,6′), 6.91 (d, J = 6.9 Hz, 2H, H-3′,5′), 6.76 (s, 2H, H-2,6), 3.87 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.70 (s, 6H, 2OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.8, 159.6, 156.0(2C), 153.2, 139.1, 130.5 (2C), 123.9, 121.2, 119.8, 114.2(2C), 105.8(2C), 60.9, 56.0(2C), 55.4. HRMS (ESI/QTOF) *m*/*z*: $[M+H]^+$ Calc.for $C_{19}H_{20}NO_5$ 342.1338; Found 342.1336.

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X-ray crystallographic data and refinement details of (2)

X-ray diffraction data were collected at 100K on a Bruker Quest D8 diffractometer equipped with a Photon-III area-detector (shutterless φ- and ω-scan technique), using graphite-monochromatized Mo K_a-radiation. The intensity data were integrated by the SAINT program[S1] and were corrected for absorption and decay using SADABS.[S2] The structure was solved by direct methods using SHELXT[S3] and refined on F^2 using SHELXL-2018.[S4] All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in ideal calculated positions and refined as riding atoms with relative isotropic displacement parameters. The Mercury program suite[S5] was used for molecular graphics. Crystal data, data collection and structure refinement details for **2** is summarized in Table S1.

Table S1 Crystal data and structure refinement for **2**

Compound	**2**
Empirical formula	C19 H19 N O5
Formula weight	341.35
Temperature, K	100.00(10)
Wavelength, Å	0.71073
Crystal system	Triclinic
Space group	P-1
a, Å	7.60340(10)
b, Å	10.6503(2)
c, Å	10.8878(2)
α, °	74.5640(10)
β, °	89.5210(10)
γ, °	75.7160(10)
Volume, Å^3	822.05(2)
Z, Z′	2, 1
ρ_{calc}, g/cm^3	1.379
μ, mm^{-1}	0.100
F(000)	360
Crystal size, mm^3	0.394 × 0.389 × 0.239
θ for data collection, °	2.411 -32.828
Index range	-11<=h<=11, -16<=k<=16, -16<=l<=16
Reflections collected	40048
Independent reflections [R(int)]	6112 [0.0485]
Reflections with $I > 2\sigma(I)$	4657
T_{max} / T_{min}	0.7465 / 0.6974
Data / restraints / parameters	6112 / 0 / 230
Goodness-of-fit on F^2	1.014
R_1 / wR_2 [I>2σ(*I*)]	0.0447 / 0.1089
R_1 / wR_2 (all reflections)	0.0670 / 0.1231
ρ_{max}/ρ_{min} (eÅ^{-3})	0.437 / -0.311
CCDC number	2335715

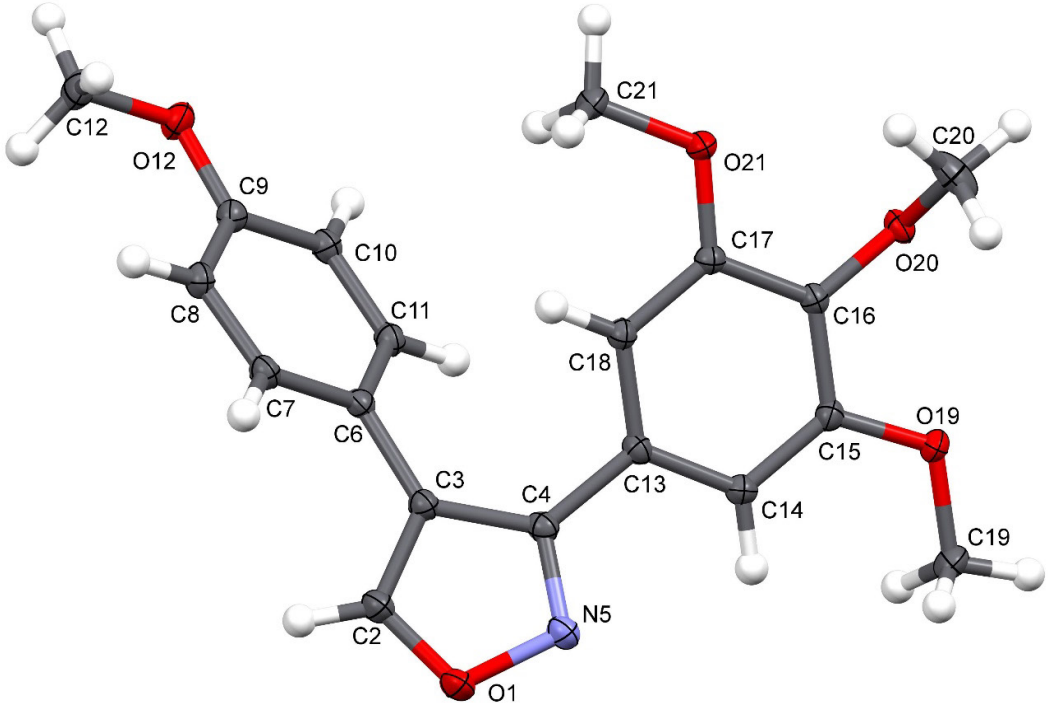

Figure S1.

Table S2. Selected bond lengths in 2, Å.

O(1)-N(5)	1.4102(12)	C(9)-O(12)	1.3671(12)
O(1)-C(2)	1.3505(14)	C(15)-O(19)	1.3618(12)
C(2)-C(3)	1.3553(14)	C(16)-O(20)	1.3740(12)
C(3)-C(4)	1.4417(14)	C(17)-O(21)	1.3625(12)
C(4)-N(5)	1.3186(13)		
C(3)-C(6)	1.4706(14)		
C(4)-C(13)	1.4743(14)		

Compound **2** crystallize in the $P\bar{1}$ space group (No. 2) with one molecule in the asymmetric part of unit cell without solvent in the crystal structure. The geometry of the molecule with the numbering of atoms is shown in Figure S1. Conformation of the molecule is characterized by dihedral angles, showing the turning of aromatic fragments relative to the central isoxazole ring. The values of these dihedral angles are 45.83° and 27.51° for methoxyphenyl and trimethoxyphenyl fragments, correspondently. The methoxy groups O12–C12, O19–C19 and O21–C21 are located in the plane of the aromatic fragments, while the O20–C20 group moves out of this plane, which is due to the steric hindrance of the location of this substituent. The corresponding torsion angles are -2.56(15)° (C8–C9–O12–C12), 2.81(15)° (C14–C15–O19–C19), 74.63(13)° (C17–C16–O20–C20), -5.55(15)° (C18–C17–O21–C21).

The compound is characterized by multiple weak intermolecular interactions, such as C-H...O and C-H...π types. Despite this, the packing coefficient of molecules in the crystal is 71.7, which is closer to the upper boundary of the value range characteristic of organic compounds (0.65-0.75).[S6]

References

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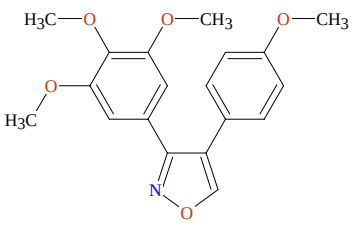

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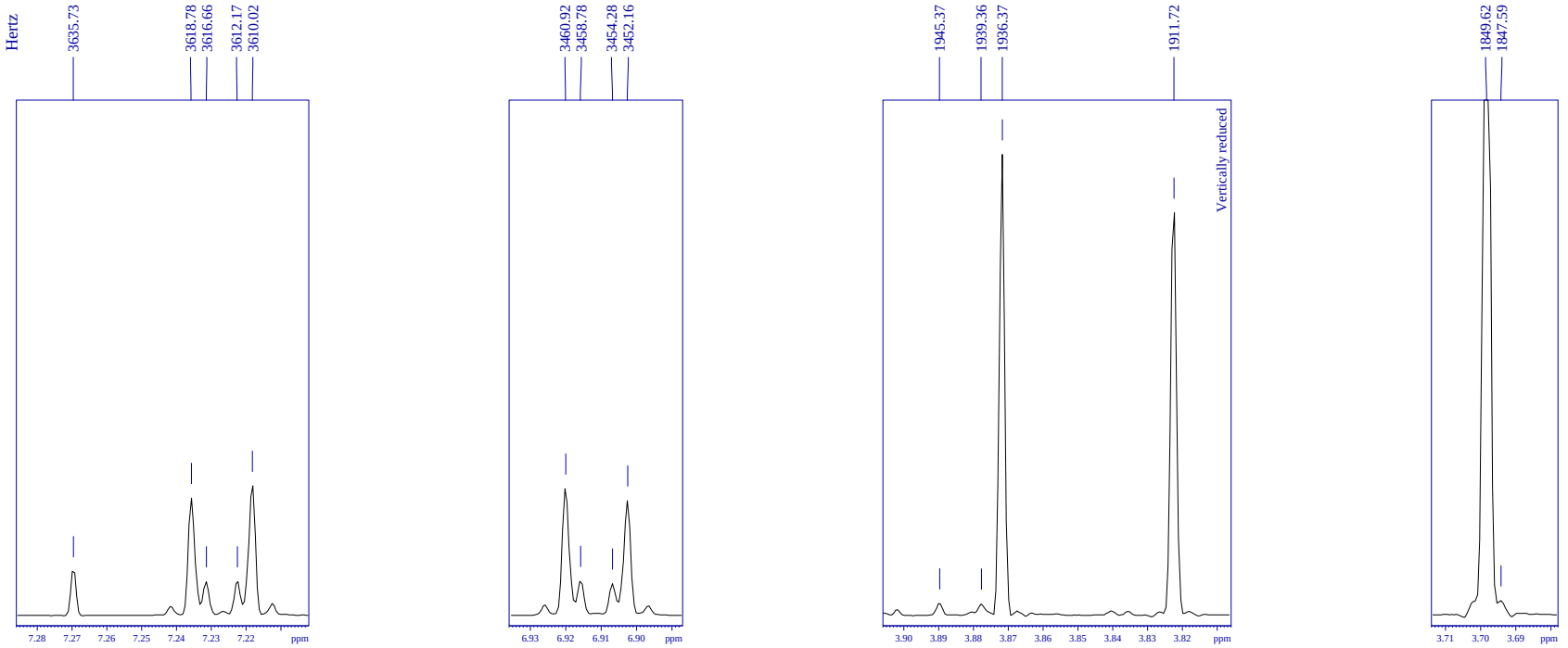
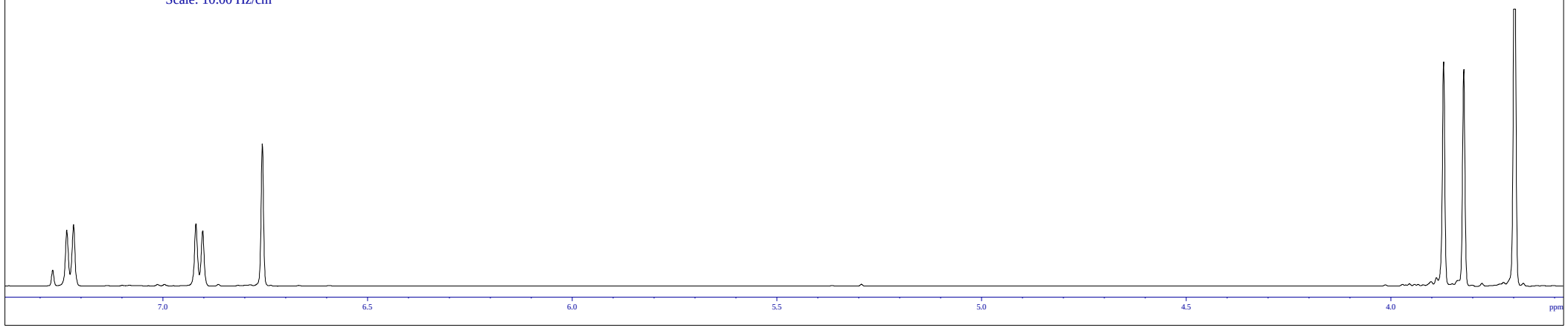
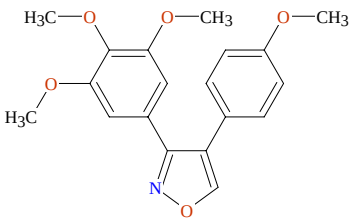




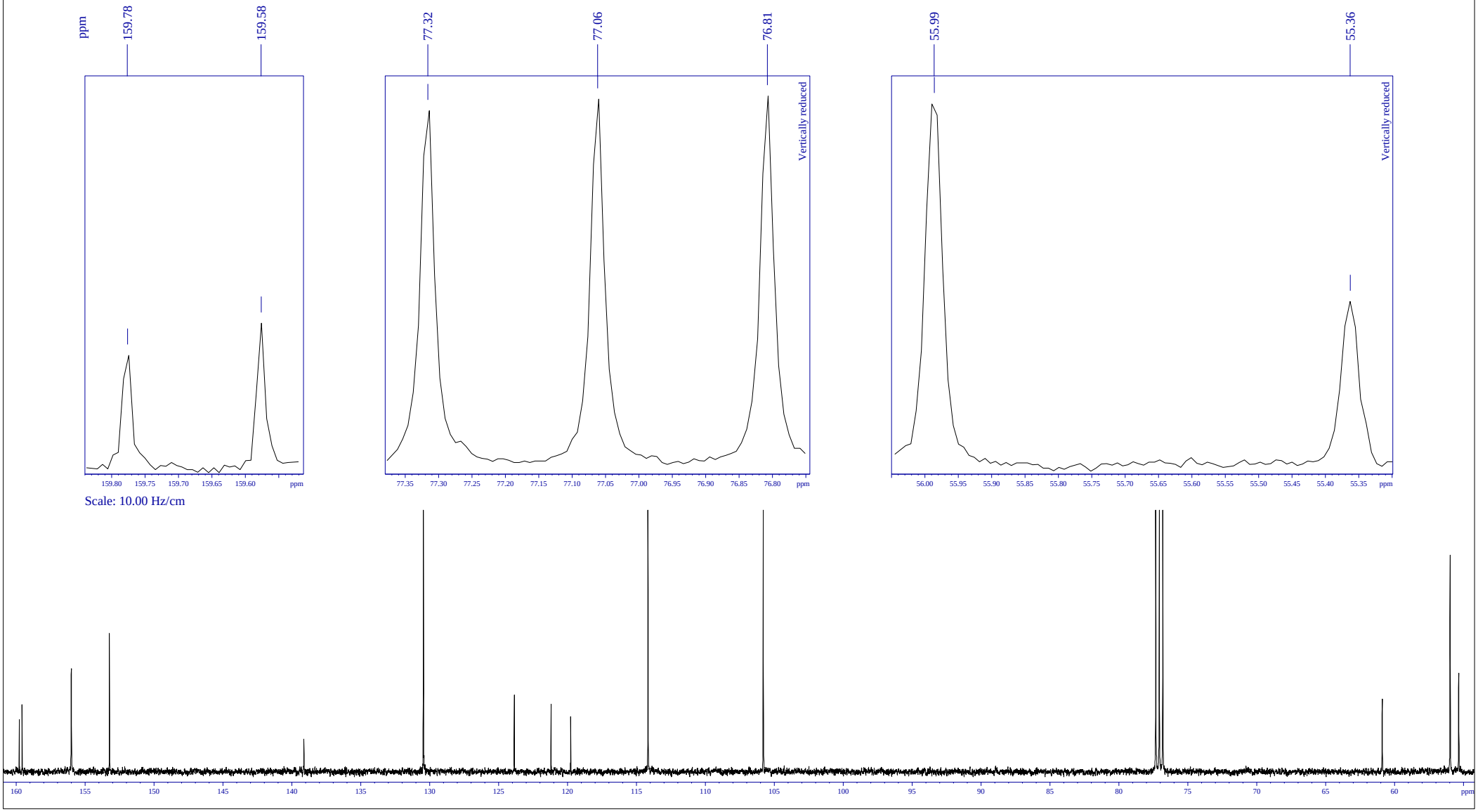
2 (NMR/29330038)

159.78
159.58
156.00
153.23
139.12
130.45
123.86
121.19
119.77
114.16
105.80
77.32
77.06
76.81
60.91
55.99
55.36
0.00



180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

2 (NMR/29330038)



The Best Applied NMR!

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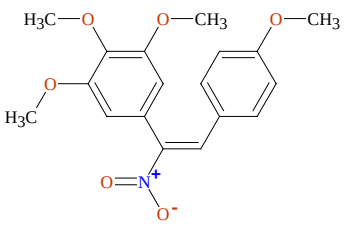

Found protons = 9 impurity* = 0.6 %

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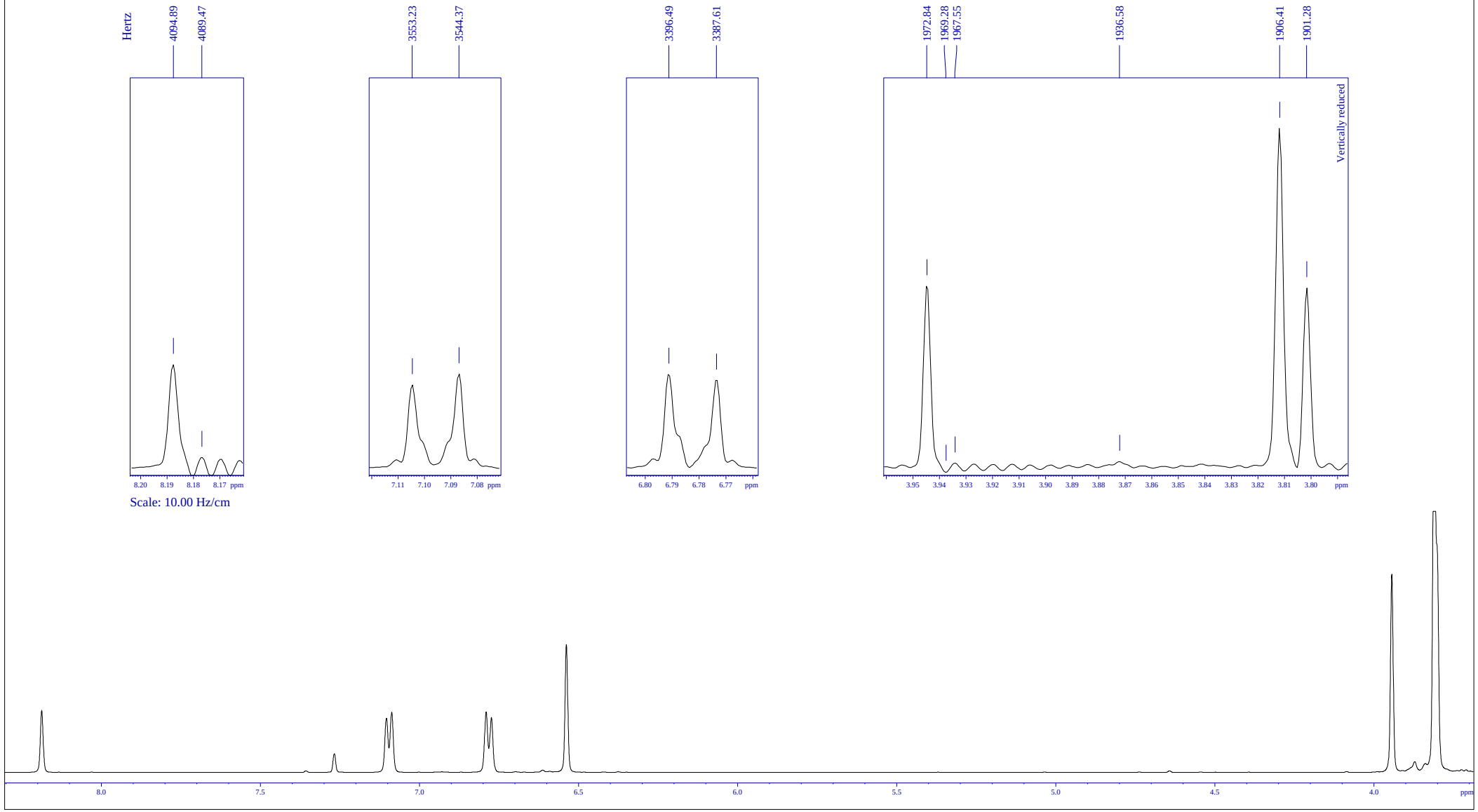

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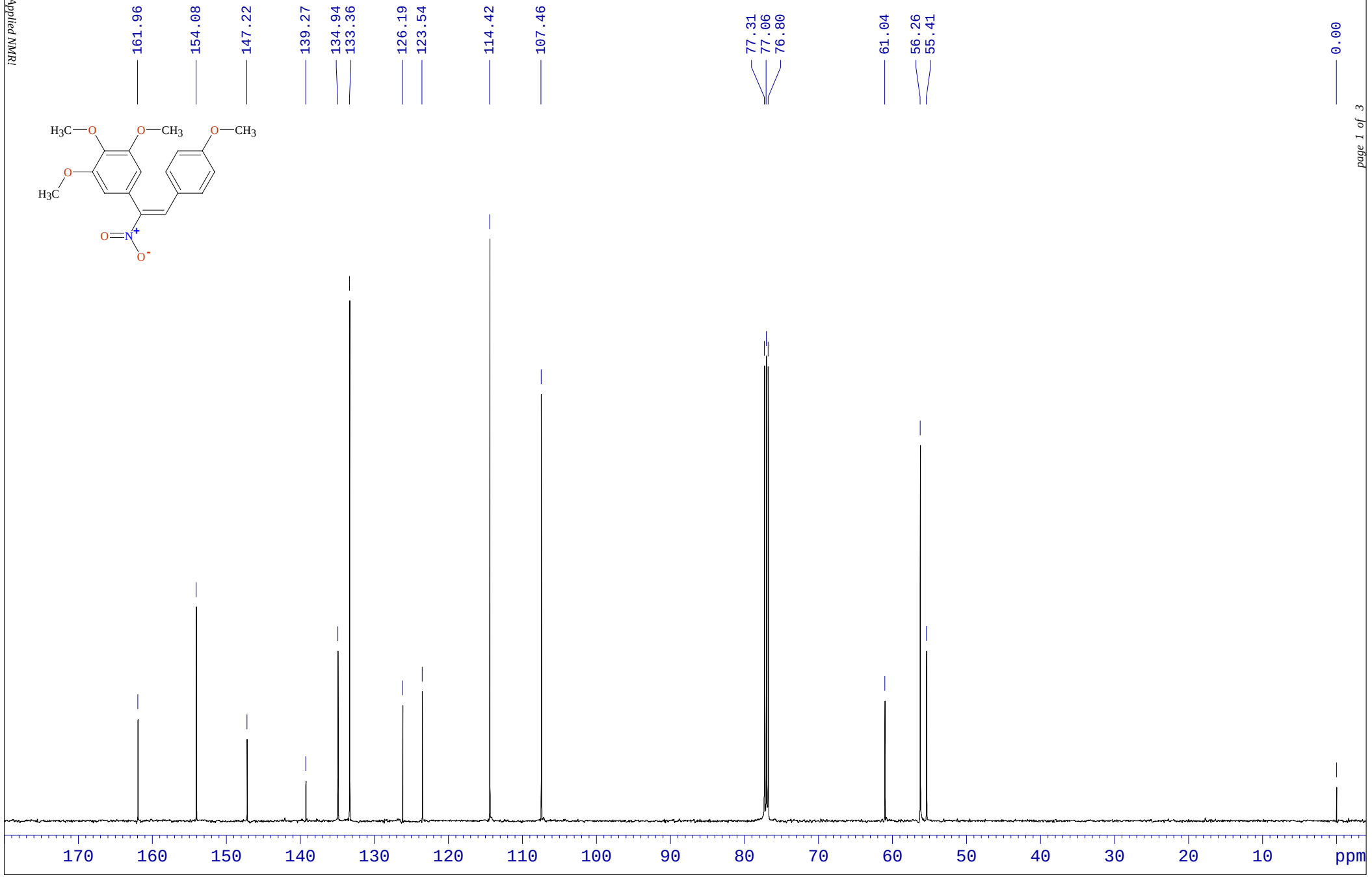
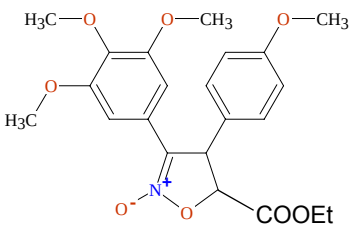


The Best Applied NMR!

5 (NMR/29330041)

Found protons = 23 impurity* < 0.1 %



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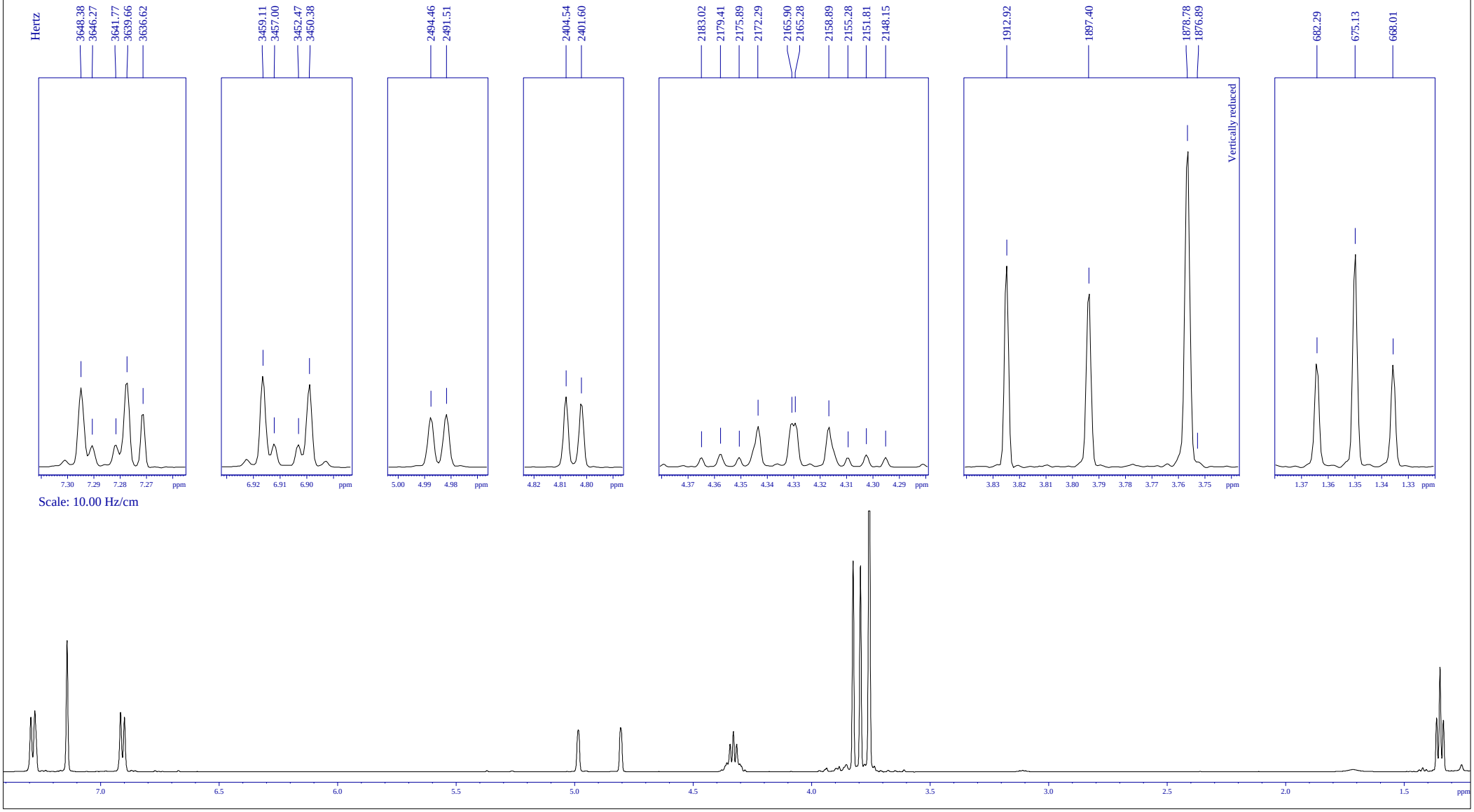

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169.10 159.80 153.07 139.18 130.46 128.31 120.72 114.97 104.40 78.70 77.29 77.04 76.78 62.51 60.83 56.07 55.33 54.21 14.12