Supplementary Information

Development of Novel Silyl Cyanocinnamic Acid Derivatives as Metabolic Plasticity Inhibitors for Cancer Treatment

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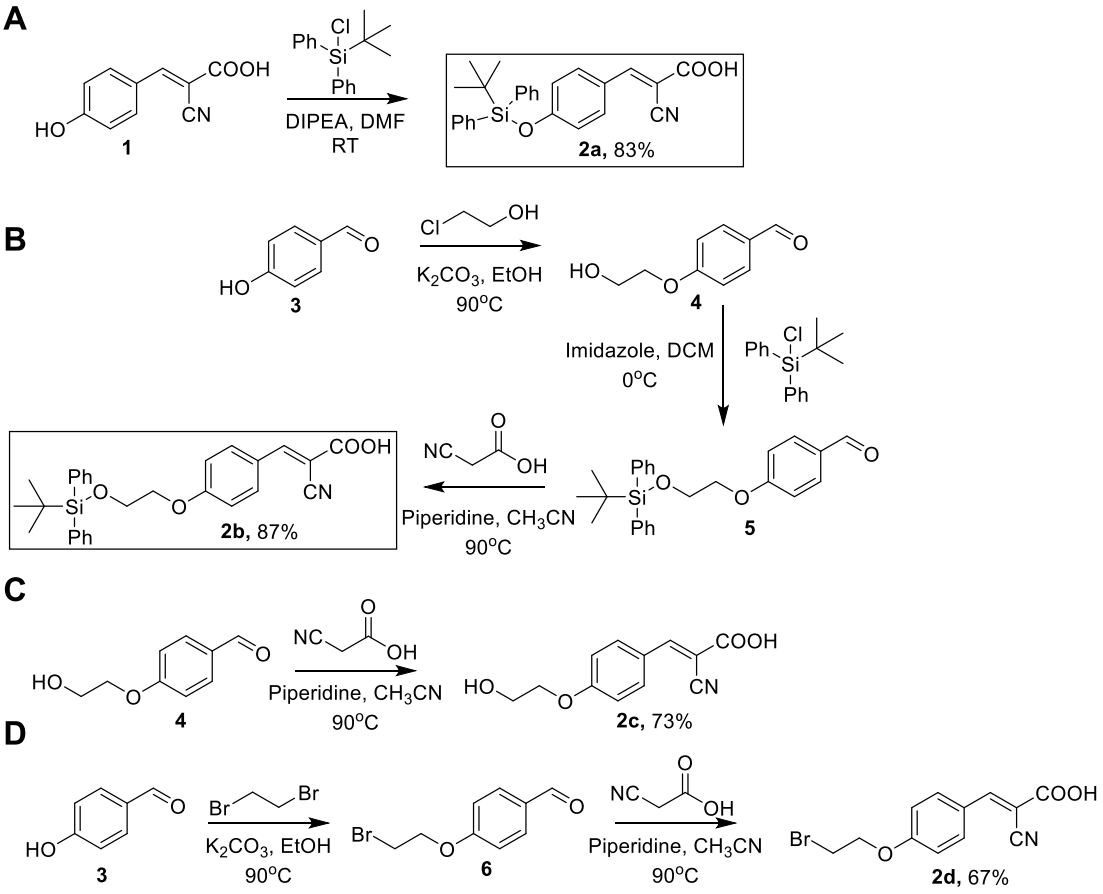
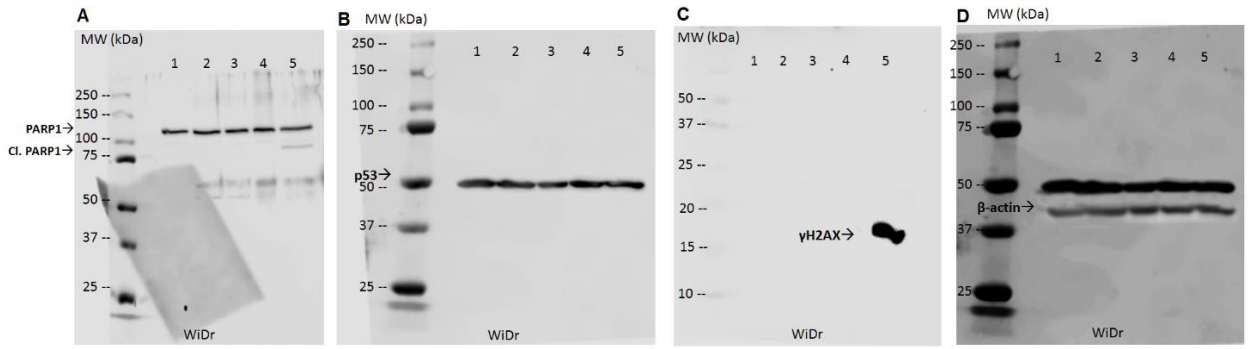
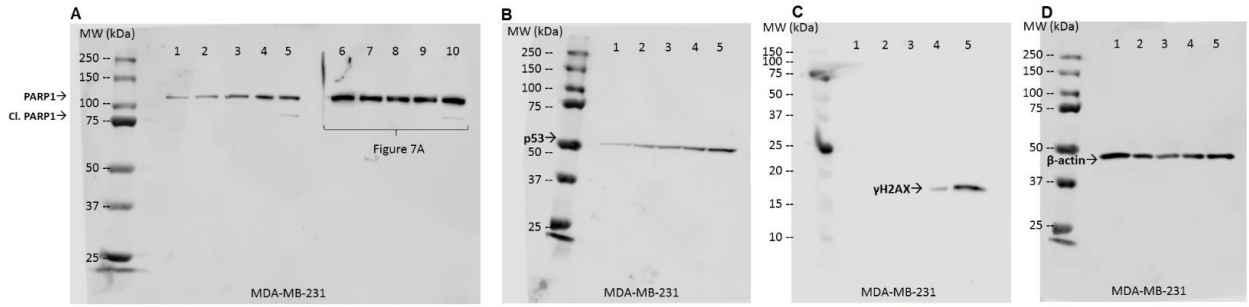


Supplemental Figure 1: Synthetic scheme of silylated CHC analogs (**A**) **2a** and (**B**) **2b** and non-silylated analogs (**C**) **2c** and (**D**) **2d**.

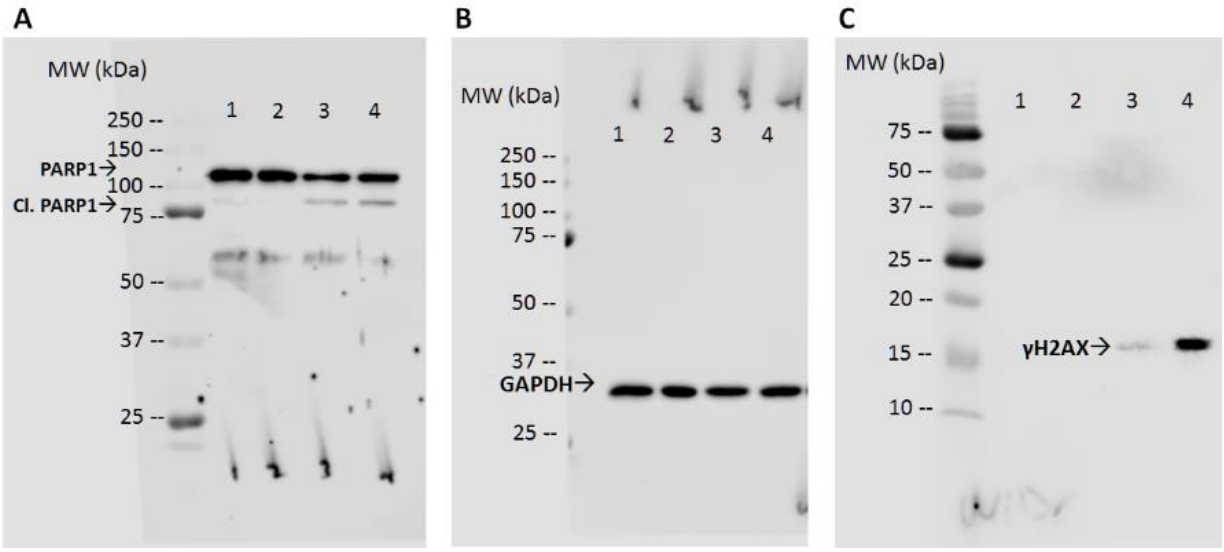
Synthesis of directly attached TBDPS-CHC **2a** was accomplished by reacting commercially available CHC **1** with TBDPS-Cl in the presence of DIPEA. Upon completion, the reaction mixture was poured over 3N HCl and the resulting precipitate was filtered and washed with hexanes to obtain pure **2a** (Fig 1A). Synthesis of Ex-TBDPS-CHC **2b** was achieved first by reacting *p*-hydroxybenzaldehyde **3** with chloroethanol under basic and heated conditions to obtain the intermediate *p*-hydroxyethylbenzaldehyde **4**, which was subsequently reacted with TBDPS-Cl in the presence of imidazole. The resulting TBDPS substituted aldehyde **5** was condensed with cyanoacetic acid in the presence of piperidine and refluxed in acetonitrile for 10 hours. Upon completion of the reaction, the solution was poured over ice and 3N HCl. The resulting precipitate was then filtered and washed with diethyl ether to obtain pure **2b** (Fig 1B). To demonstrate the importance of the silyl group in providing biological activity, compound **2c** and **2d** were synthesized. Specifically, compound **2d** was synthesized as a homolog of **2b** to demonstrate that hydrophobicity offered by TBDPS template is necessary for biological activity. Similarly, the products of hydroxyethyl-CHC **2c** and bromoethyl-CHC **2d** were obtained via a standard Knovenagel condensation of the corresponding aldehydes (**4** and **6**, respectively) with cyanoacetic acid in the presence of piperidine (Fig 1C & 1D).



Supplemental Figure 2: Full-length blots of cropped images depicted in manuscript Figure 7A. Lanes labeled 1-5 indicate whole-cell lysates of WiDr cells with the following treatments: (1) DMSO, (2) cyanohydroxycinnamic acid (CHC, 100µM), (3) **2a** (25µM), (4) **2a** (50µM), (5) **2a** (50µM). (**A**) WiDr whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for PARP-1. (**B**) WiDr whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for p53. (**C**) Protein from WiDr whole-cell lysates resolved on a 12% SDS-PAGE gel and probed for γH2AX. (**D**) WiDr whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for β-actin and p53 (note arrow indicating β-actin band used in Figure 7A). Due to saturation of p53 band, an independent exposure was performed, as illustrated in panel B, above. Please refer to methods section for details.



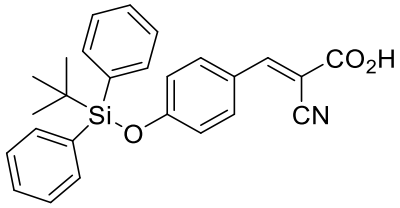
Supplemental Figure 3: Full-length blots of cropped images depicted in manuscript Figure 7A. Lanes labeled 1-5 indicate whole-cell lysates of MDA-MB-231 cells with the following treatments: (1) DMSO, (2) cyanohydroxycinnamic acid (CHC, 100µM), (3) **2a** (25µM), (4) **2a** (50µM), (5) **2a** (50µM). (**A**) MDA-MB-231 whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for PARP-1. Note samples 6-10 indicate the same treatment as 1-5 above, but from independent replicates. PARP-1 from samples 6-10 were used in Figure 7A, as noted. (**B**) MDA-MB-231 whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for p53. (**C**) Protein from MDA-MB-231 whole-cell lysates resolved on a 12% SDS-PAGE gel and probed for γH2AX. (**D**) MDA-MB-231 whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for β-actin. Please refer to methods section for details.



Supplemental Figure 4: Full-length blots of cropped images depicted in manuscript Figure 7B. Lanes labeled 1-4 indicate whole-cell lysates of WiDr cells with the following treatments: (1) DMSO + N-Acetyl cysteine (NAC), (2) DMSO, (3) **2a** (100µM) + NAC, (4) **2a** (100µM). (**A**) Protein from WiDr whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for PARP-1. (**B**) Protein from WiDr whole-cell lysates resolved on an 8% SDS-PAGE gel and probed for GAPDH. (**C**) Protein from WiDr whole-cell lysates resolved on a 12% SDS-PAGE gel and probed for γH2AX. Please refer to methods section for details.

Spectral Characterization

(E)-3-(4-((tert-butyldiphenylsilyl)oxy)phenyl)-2-cyanoacrylic acid (***2a***)



^{1}H NMR (500 MHz, $CDCl_3$):

δ ppm 8.15 (s, 1H), 7.84 (d, *J* = 9 Hz, 2H), 7.67 - 7.72 (m, 4H), 7.43 - 7.50 (m, 2H), 7.37 - 7.43 (m, 4H), 6.86 (d, *J* = 9 Hz, 2H), 1.12 (s, 9H)

^{13}C NMR (126 MHz, $CDCl_3$):

δ ppm 168.0, 161.1, 156.1, 135.3, 133.9, 131.7, 130.4, 128.0, 124.4, 120.8, 115.6, 98.2, 26.4, 19.5

HRMS (ESI) m/z:

calculated for $C_{17}H_{22}N_2O_3$ $[M+Na]^+$: 428.168

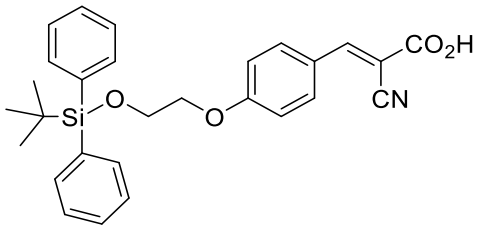
found 428.146

Elemental Analysis C, H, N

Anal. Calcd for $C_{26}H_{25}NO_3Si$ (427.16): C 73.04, H 5.89, N 3.28.

Found: C 72.34, H 5.96, N 3.20

(E)-3-(4-(2-((tert-butyldiphenylsilyl)oxy)ethoxy)phenyl)-2-cyanoacrylic acid (***2b***):



^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.24 (br. s., 1H), 8.01 (d, *J* = 8 Hz, 2H), 7.67 - 7.74 (m, 4H), 7.36 - 7.48 (m, 6H), 6.94 (d, *J* = 8 Hz, 2H), 4.16 (t, *J* = 5 Hz, 2H), 4.02 (t, *J* = 5 Hz, 2H), 1.07 (s, 9H).

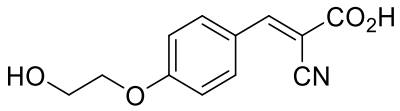
^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 168.8, 163.8, 156.1, 135.6, 134.2, 133.3, 129.9, 127.8, 124.1, 115.4, 98.4, 69.4, 62.3, 26.8, 19.2

Elemental Analysis C, H, N

Anal. Calculated for $C_{28}H_{29}NO_4Si\ Na^+$ (494.18): C 67.99, H 5.91, N 2.83.

Found: C 69.06, H 5.98, N 2.90.

E)-2-cyano-3-(4-(2-hydroxyethoxy)phenyl)acrylic acid (***2c***):



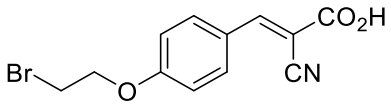
^{1}H NMR (500 MHz, MeOH-d_6):

δ 8.09 (s, 1H), 7.98 (d, *J* = 9 Hz, 2H), 7.12 (d, 2H), 4.20 (t, 2H), 3.97 (t, *J* = 5 Hz, 2H)

^{13}C NMR (126MHz, MeOH-d_6):

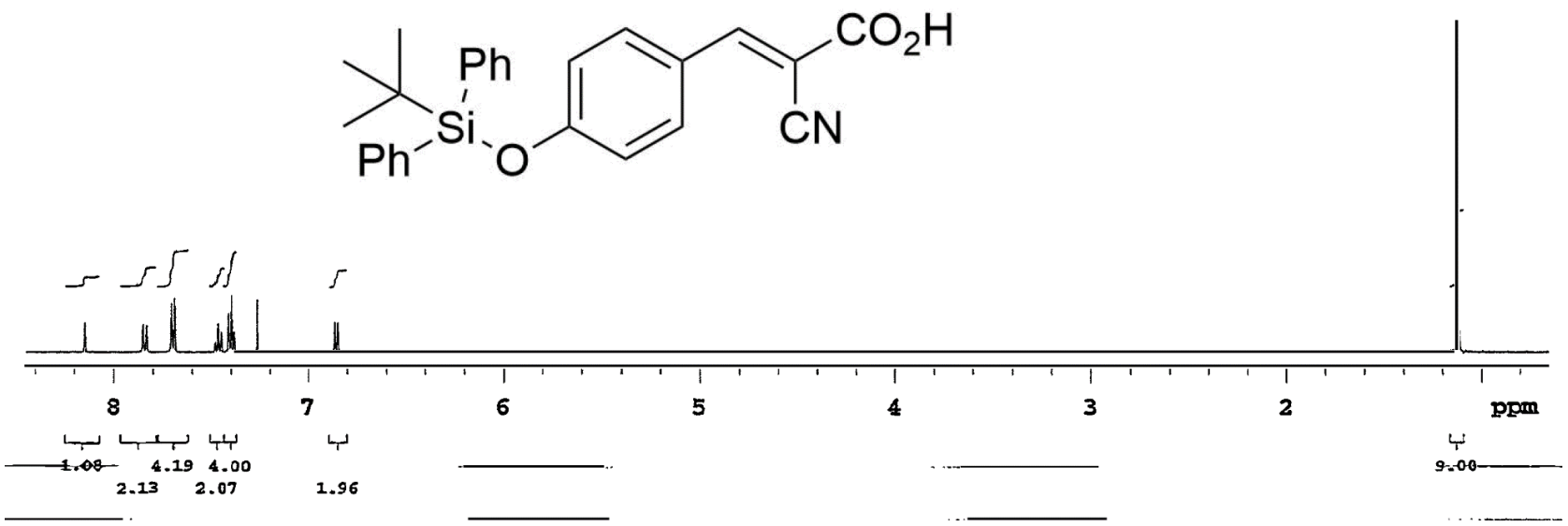
δ 167.5, 161.8, 149.7, 131.8, 125.4, 118.4, 114.6, 107.0, 69.4, 60.1.

*(E)-3-(4-(2-bromoethoxy)phenyl)-2-cyanoacrylic acid (**2d**)*



^{1}H NMR (500 MHz, $CDCl_3$) d 9.87 (s, 1H), 7.82 (d, J = 8.79 Hz, 2H), 6.99 (d, J = 8.79 Hz, 2H), 4.35 (t, J = 6.10 Hz, 2H), 3.56 - 3.76 (m, 2H)

^{13}C NMR (126 MHz, $CDCl_3$) d 164.7, 161.9, 153.8, 133.4, 125.1, 116.4, 115.2, 100.8, 67.9, 28.5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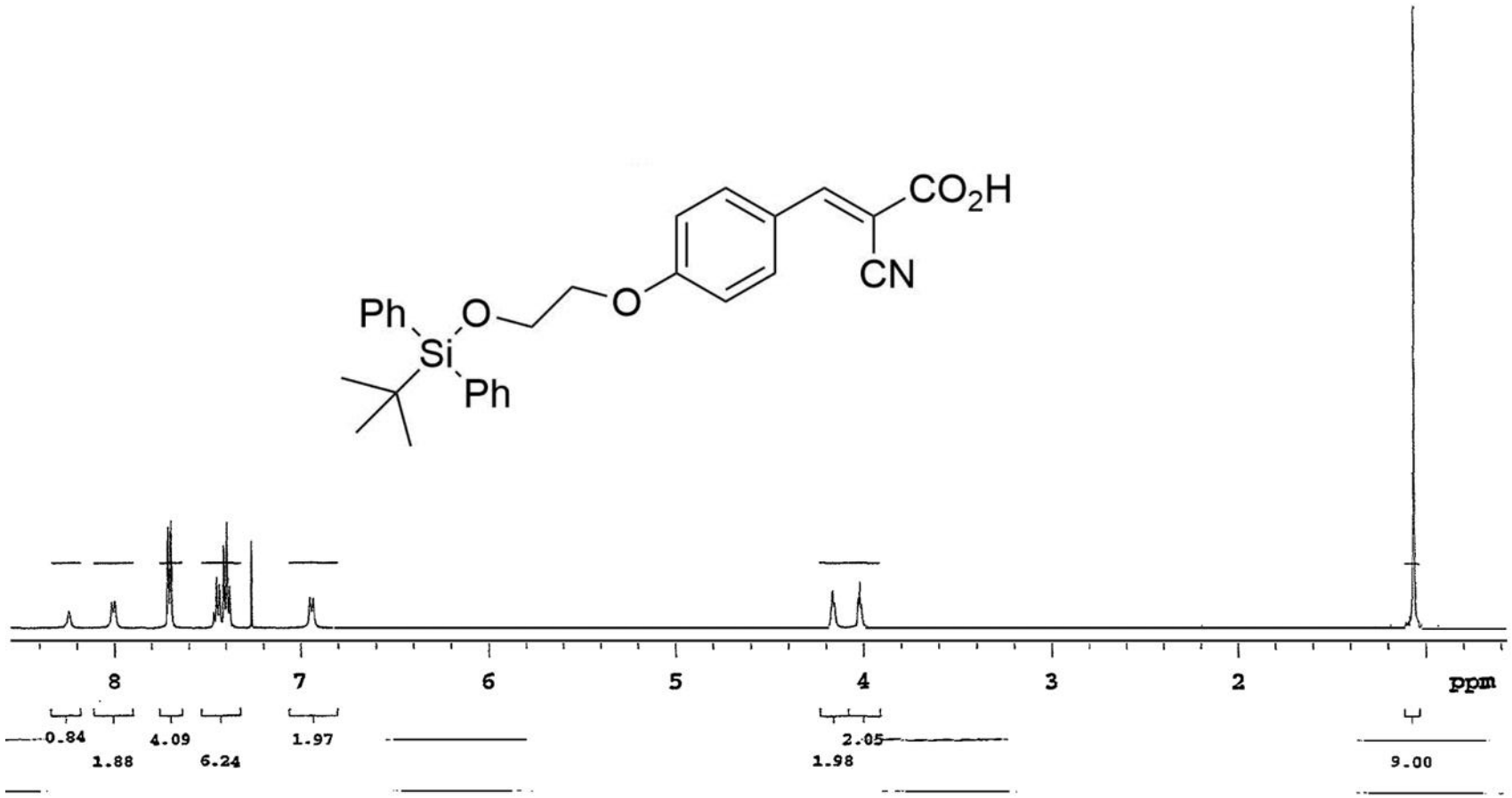
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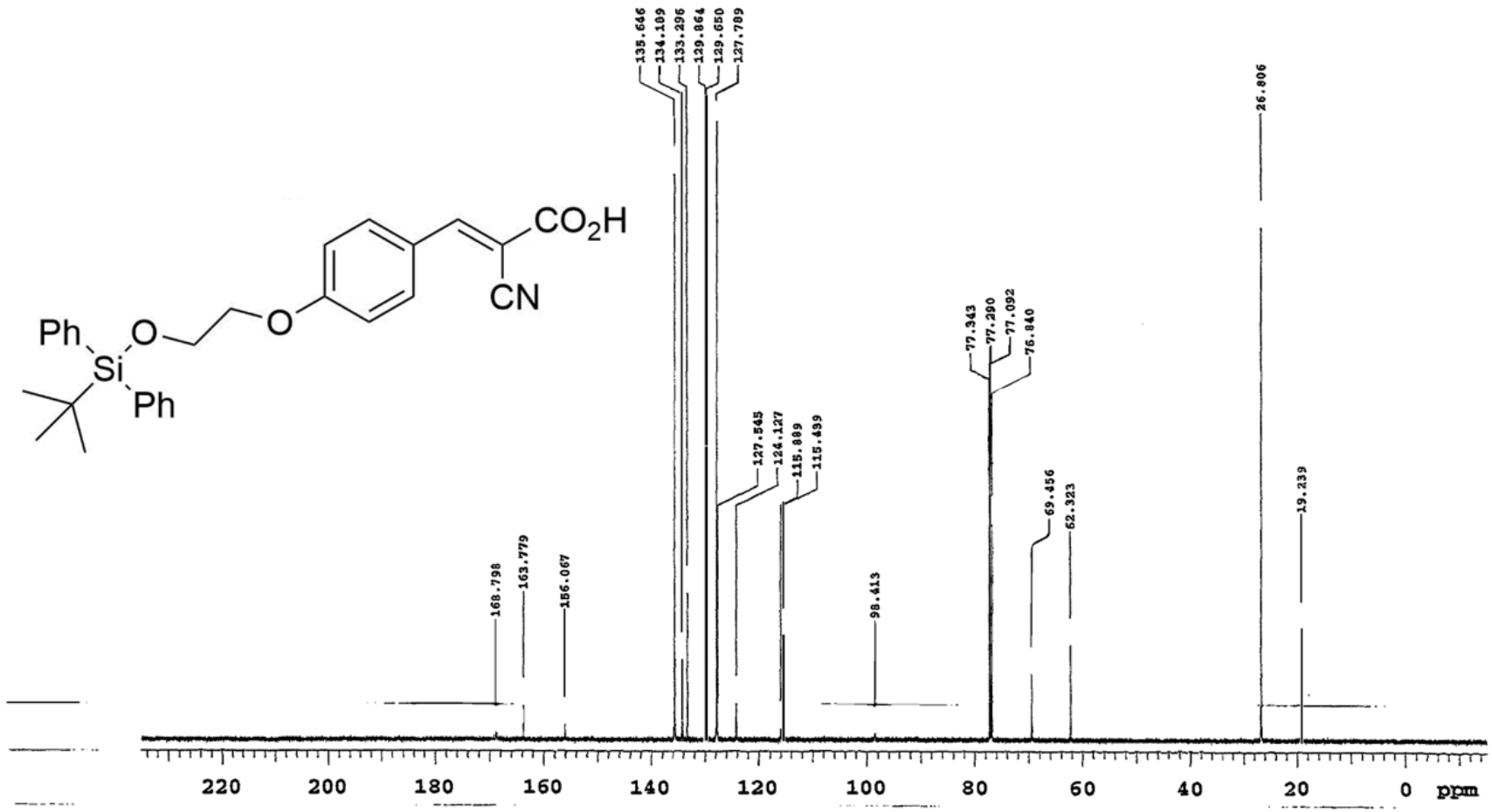
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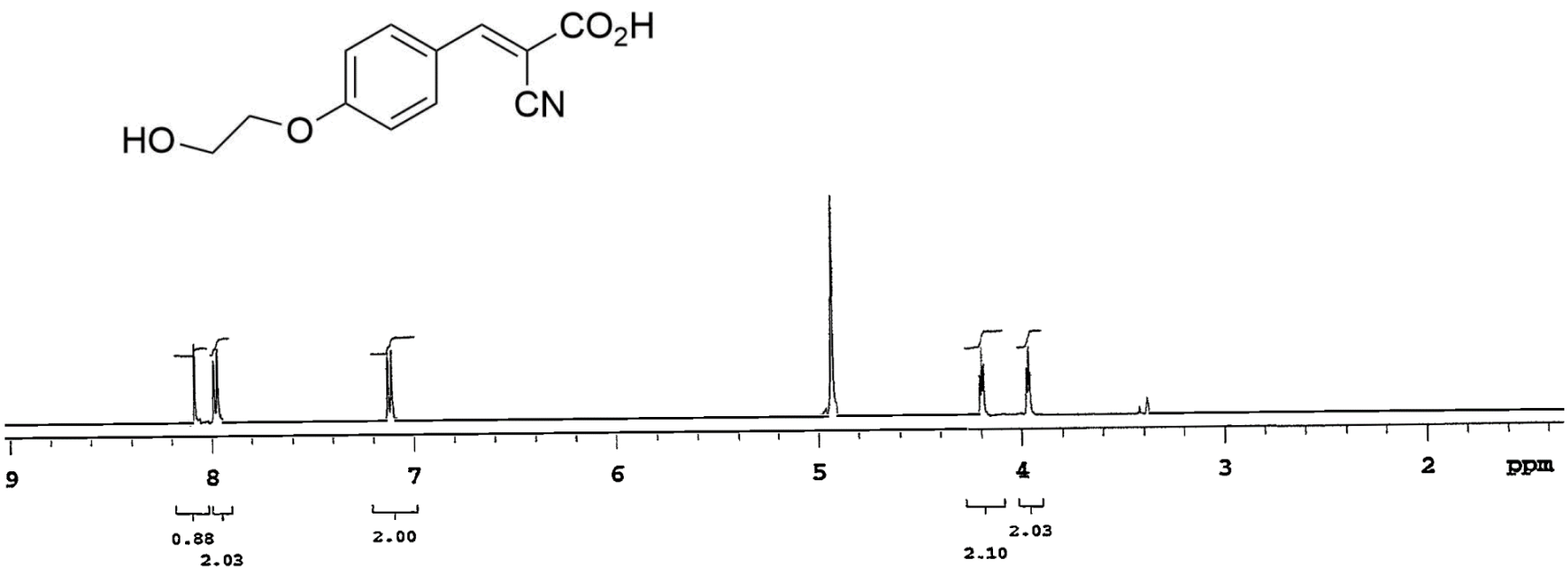
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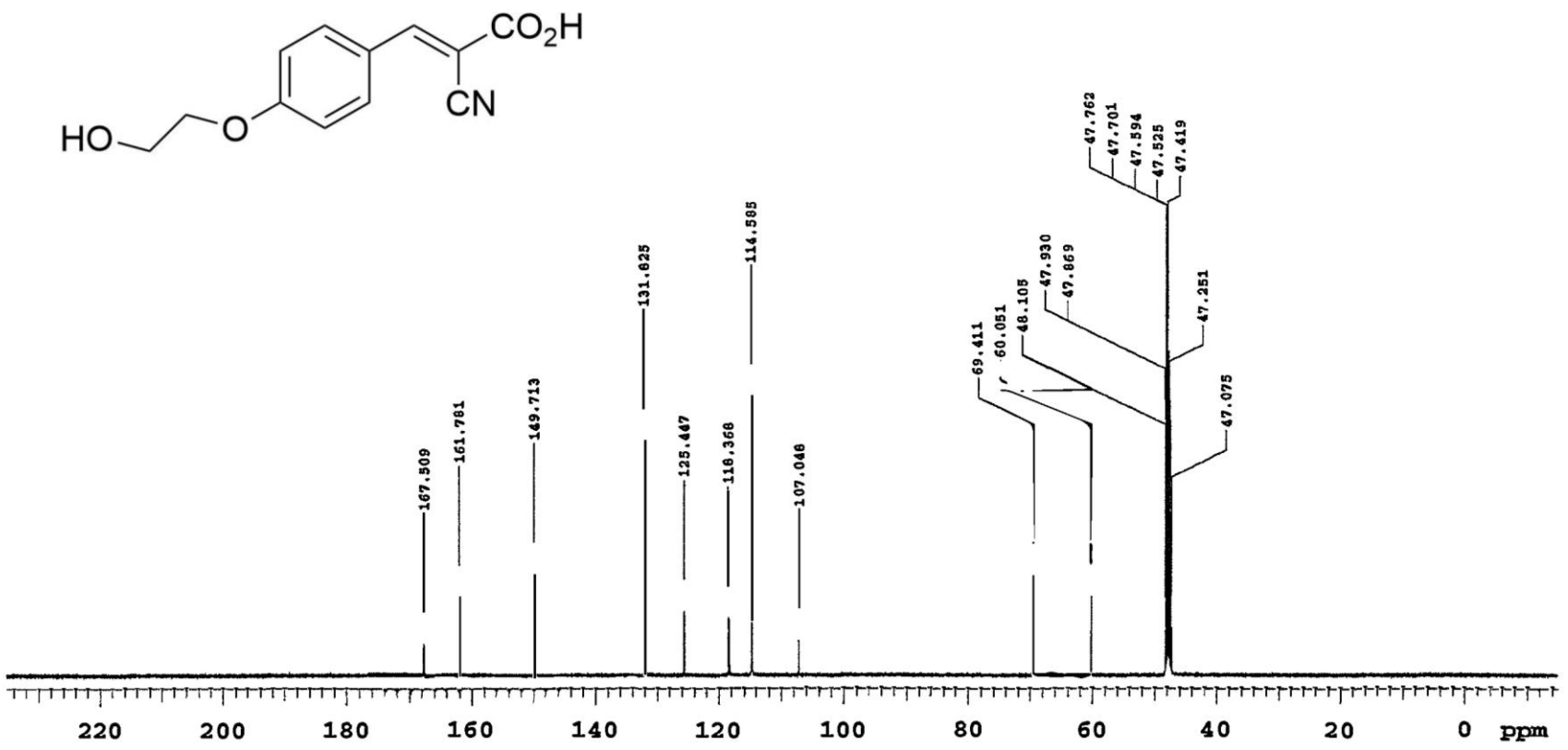
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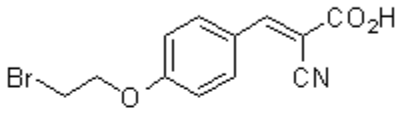
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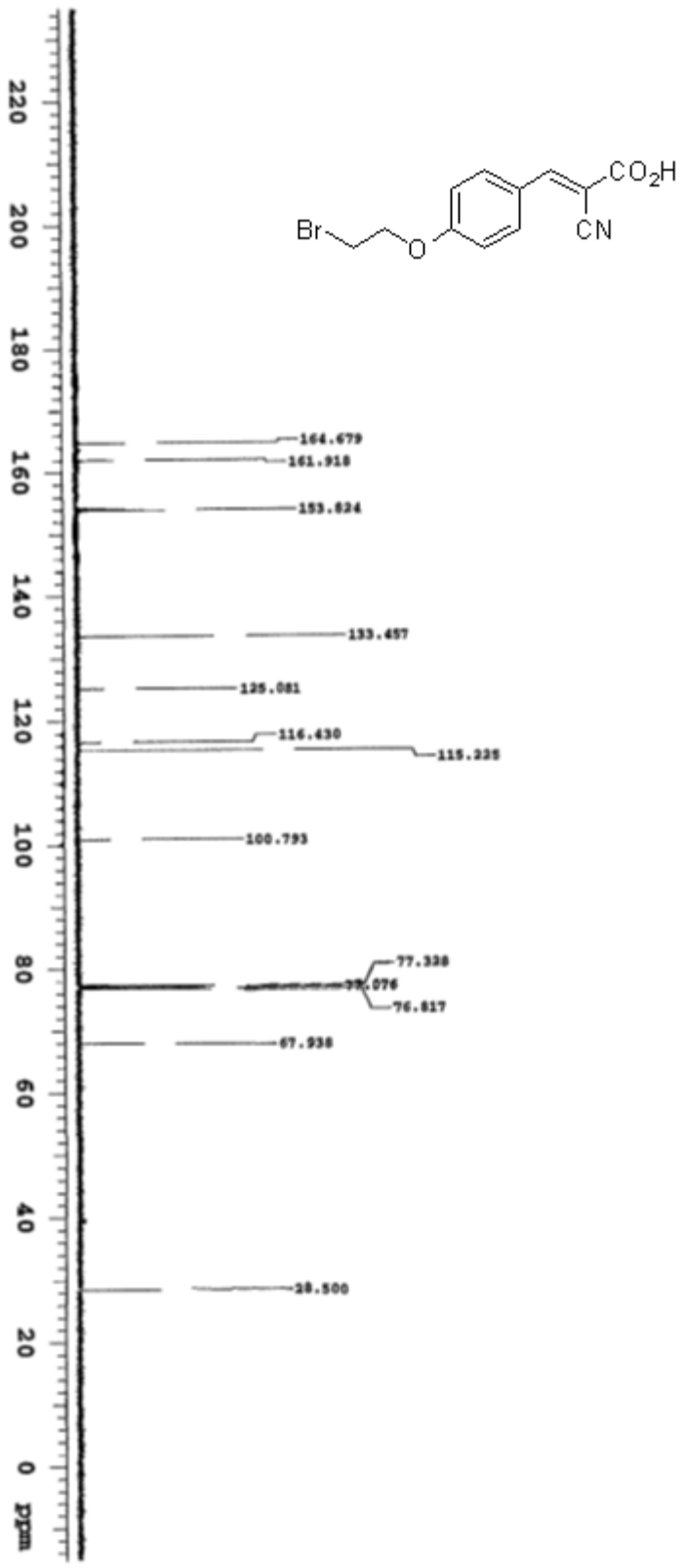
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