Facile Derivatization of Azide Ion Using Click Chemistry for Its Sensitive Detection with LC-MS

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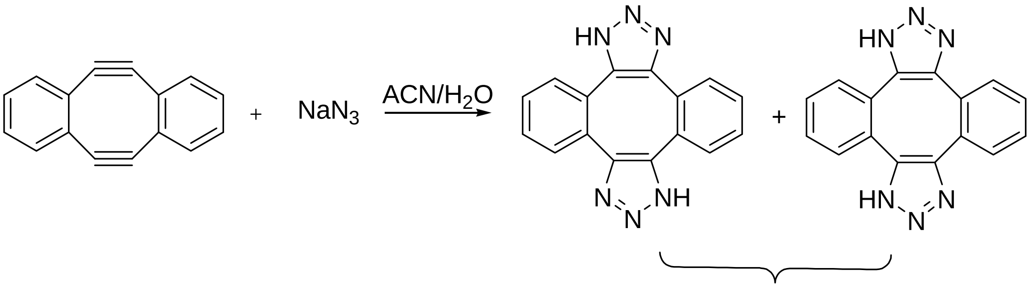
General methods and materials

Solvents and reagents for synthesis were obtained from Sigma-Aldrich. Thin layer chromatography (TLC) was run on Sorbtech W/UV254 plates (0.25 mm thick), and visualized under UV-light. Flash chromatography was performed using Fluka silica gel 60 (mesh size 0.040-0.063 mm). ^{1}H, ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer in deuterated DMSO-d_6 with either tetramethylsilane (TMS) (0.00 ppm) or the NMR solvent as the internal reference. HRMS characterization was performed using a Micromass (Q-Tof micromass^{TM}) mass spectrometer.

Synthesis

DBA 1 was synthesized following literature procedures.[1, 2]

DCTA 2

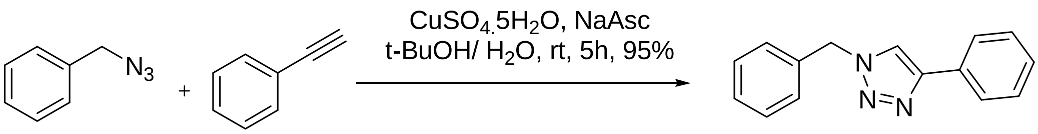


DBA, 1

DCTA, 2

To a solution of NaN_3 (33 mg, 0.5 mmol) in 1 mL water, was added DBA (11 mg, 0.05 mmol) dissolved in 1 mL acetonitrile. The mixture was stirred at room temperature for 0.5 h and then solvent was evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 10:1) to give a white solid (12 mg, yield 84%). ^{1}H NMR (DMSO-d_6), 7.54 (brs, 8H) and 15.44 (brs, 2H). ^{13}C NMR (DMSO-d_6), 129.7, 130.5, 131.0 and 142.2. HRMS (+ESI): calcd for $[C_{16}H_{11}N_6]^+$ $[M+H]^+$ m/z 287.1045, Found 287.1059.

ITSD 3 was synthesized following literature procedures.[3]



ITSD, **3**

Scheme S2, Synthesis of 1-benzyl-4-phenyl-1H-1,2,3-triazole (**3**) as internal standard (ITSD) for LC/MS

Sample Preparation

DCTA (1 mM) was dissolved in 50% DMSO aqueous solution. This was kept as stock solution. DBA and ITSD were dissolved in DMSO to prepare stock solutions at 10 mM and 1 mM. 1 mM of NaN_3 aqueous solution was prepared. DMSO solution A containing 10 µM ITSD and 200 µM DBA was newly prepared from stock solution each time before reaction. NaN_3 aqueous solution was diluted into different concentrations (0.2 µM, 1 µM, 2 µM, 10 µM, 15 µM, 20 µM, 40 µM, 100 µM, 150 µM, and 200 µM) and was reacted with 200 µM DBA for about 24 h at RT. The NaN_3 aqueous solutions were mixed with DMSO solution A at 1:1 ratio at 0.5 h interval in order to accommodate the delay in analysis of samples in the queue (each run takes about 29 min). The individual reaction mixture was injected into LC/MS without further purification. These solutions were then analyzed by LC/MS in multiple-reaction-monitoring (MRM) mode. The formed DCTA (m/z 287) was detected as precursor ion, and the most abundant fragmentation ion m/z 203 was detected as daughter ion. At the same time, the precursor ion (m/z 236) and corresponding daughter ion (m/z 91) of ITSD was monitored. Each measurement was performed three times. The peak area ratio of DCTA and ITSD was calculated after automatically integrating the peak area of DCTA and ITSD using the mass spectrometer operating system.

Liquid Chromatography/Mass Spectrometry Operating Conditions

API-MS detection was achieved using an AB SCIEX API 3200^{TM} LC/MS/MS triple quadrupole mass spectrometer equipped with an orthogonal Turbo ion spray source and Agilent 1200 Series HPLC System. All organic solvents were of high performance liquid chromatography (HPLC) grade and were obtained from Fisher (Suwanee, GA). MiliQ grade water was produced by using a Millipore system (Millipore Corp., Bedford, MA). Samples were delivered to the ionization source by an autosampler (Agilent 1200 HPLC system). A C18 column (3 µm particle size, 2.1×100 mm, Restek, Bellefonte, PA, USA) was used for the chromatographic separation. The injection volume was 10 uL. A generic chromatographic method was used in this study. The HPLC mobile phase consists of H_2O with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient conditions were: starting at 60% A and 40% B, holding at 40% B for 4 min, changing linearly to 100% B in 4 min, maintaining 100% B for 4 min, decreasing linearly to 40% B within 2 min and equilibrating at 40% B for 15 min. This gives a total analysis time of 29 min. After analysis the column was washed using the above procedure. The flow rate was 0.3 mL/min. Data were processed using the Analyst 1.5 software from AB SCIEX.

Detection was in the positive mode. The MRM mode monitoring the m/z 287 Da of DCTA precursor ion and m/z 203 Da of daughter ion was used. The analyte-dependent and ESI source parameters were specifically optimized for the detection of DCTA using the mobile phase specified. The experimental parameters were set as follows: ion spray voltage 5500 V, nebulizing gas (gas 1) 65 psi, the turbo gas (gas 2) at 50 psi, temperature at 400 °C, and with the interface heater turned ON. The declustering potential (DP) was optimized at 65 V and entrance potential (EP) was 12 V. Collision energy (CE) was up to 44 eV.

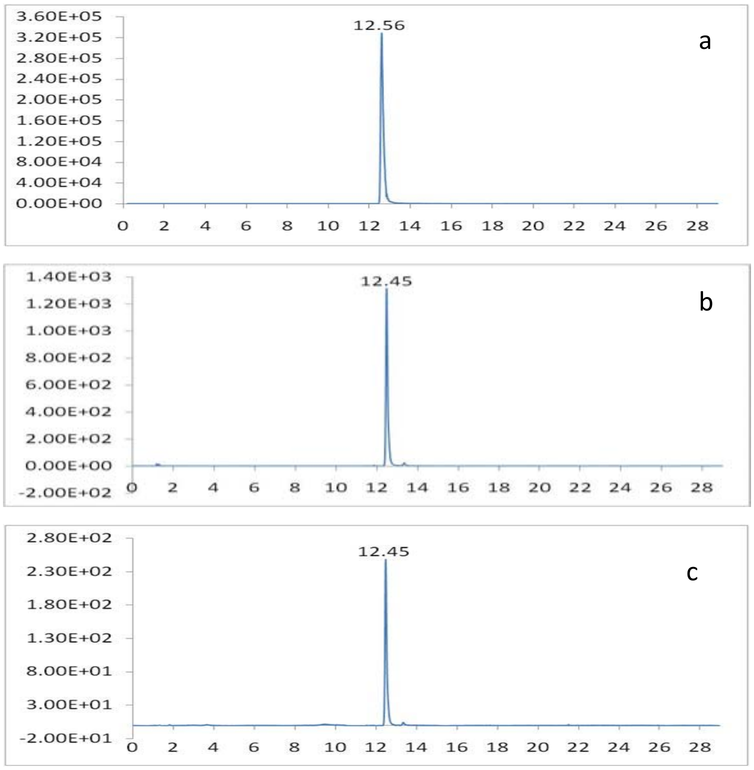


Fig S1 HPLC chromatograms of ITSD: a) LC-MS chromatography, b) UV-based chromatogram at 250 nm, and c) UV-based chromatogram at 270 nm.

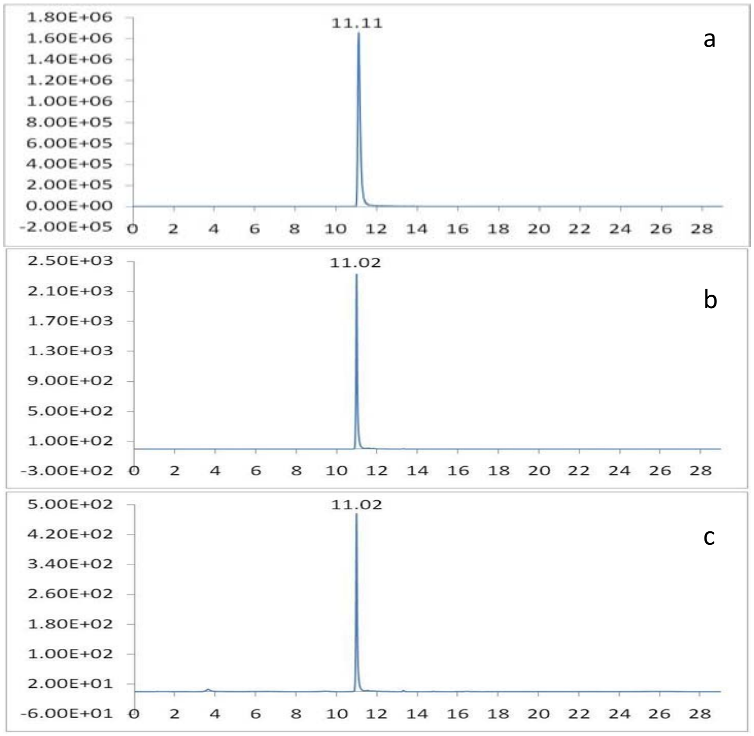


Fig. S2 HPLC chromatograms of DCTA: a) LC-MS chromatography, b) UV-based chromatogram at 250 nm, and c) UV-based chromatogram at 270 nm

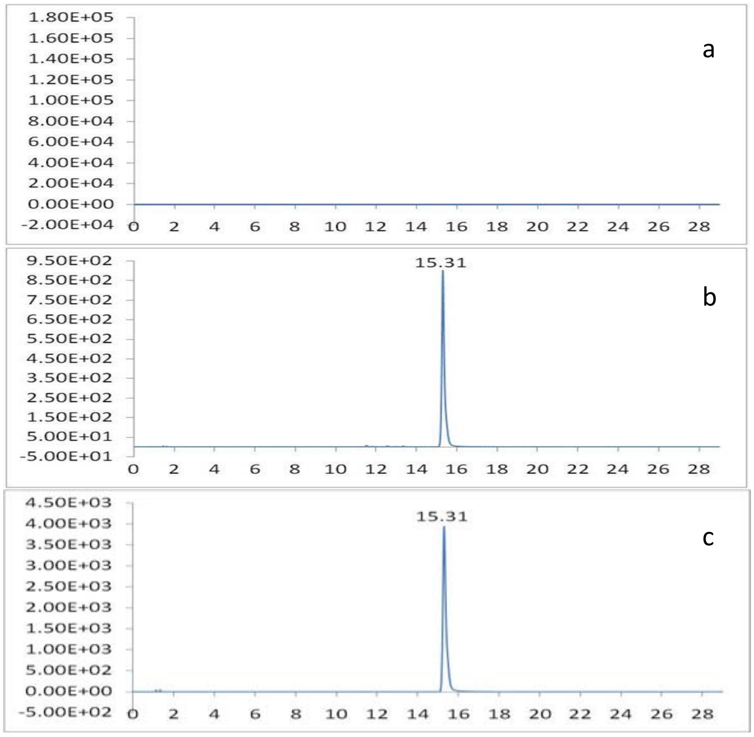


Fig. S3 HPLC chromatograms of DBA: a) LC-MS chromatography, b) UV-based chromatogram at 250 nm, and c) UV-based chromatogram at 270 nm

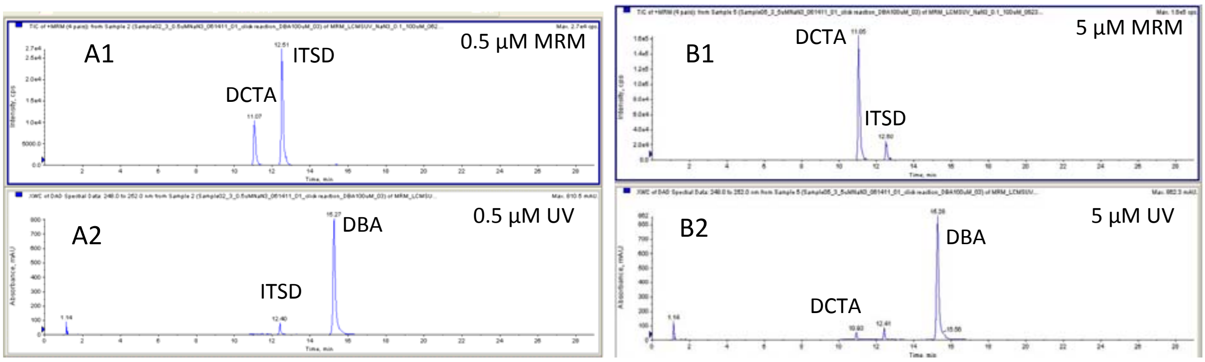


Fig. S4 HPLC chromatograms of the reaction mixture of DBA and NaN_3. A1 and B1: LC-MS chromatograms of the reaction mixtures with NaN_3 at 0.5 and 5 µM, respectively; A2 and B2: UV-based chromatograms of the same reactions detected at 250 nm

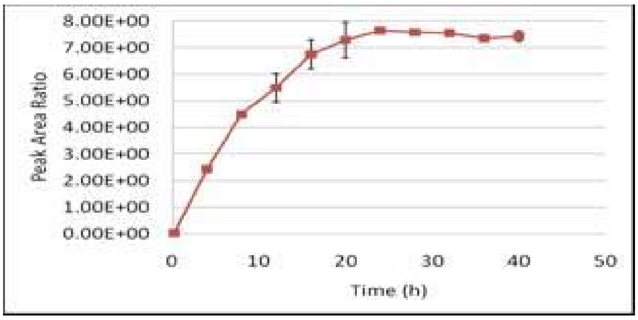
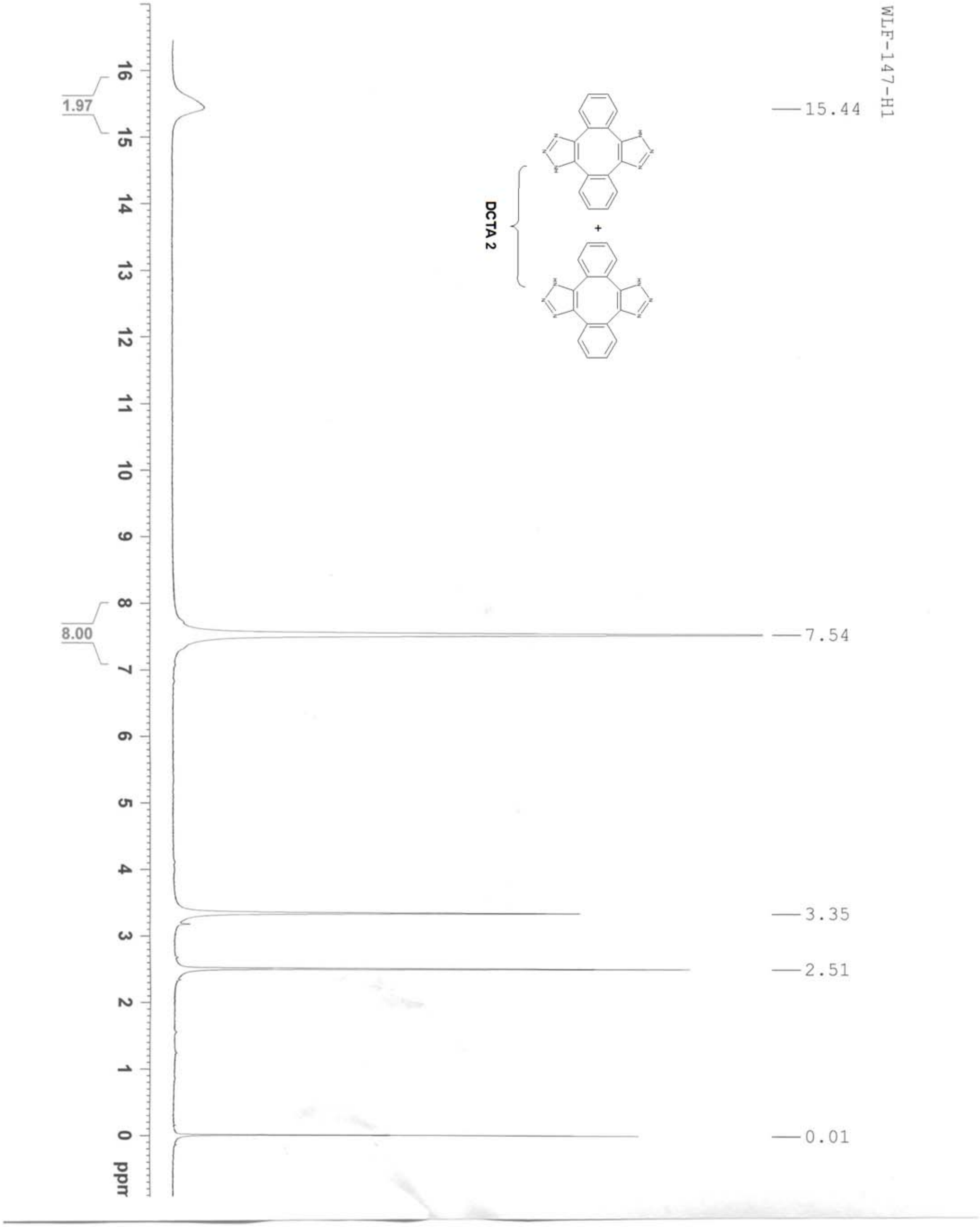


Fig. S5 Time profile of the reaction between10 µM azide and 100 µM DBA monitored at 4-hs intervals

Reference and note

1. A. Orita, D. Hasegawa, T. Nakano and J. Otera, *Chem.-Eur. J.*, 2002, **8**, 2000-2004.
2. S. Chaffins, M. Brettreich and F. Wudl, *Synthesis*, 2002, 1191-1194.
3. C. W. Shao, X. Y. Wang, J. M. Xu, J. C. Zhao, Q. Zhang and Y. F. Hu, *J. Org. Chem.*, 2010, **75**, 7002-7005.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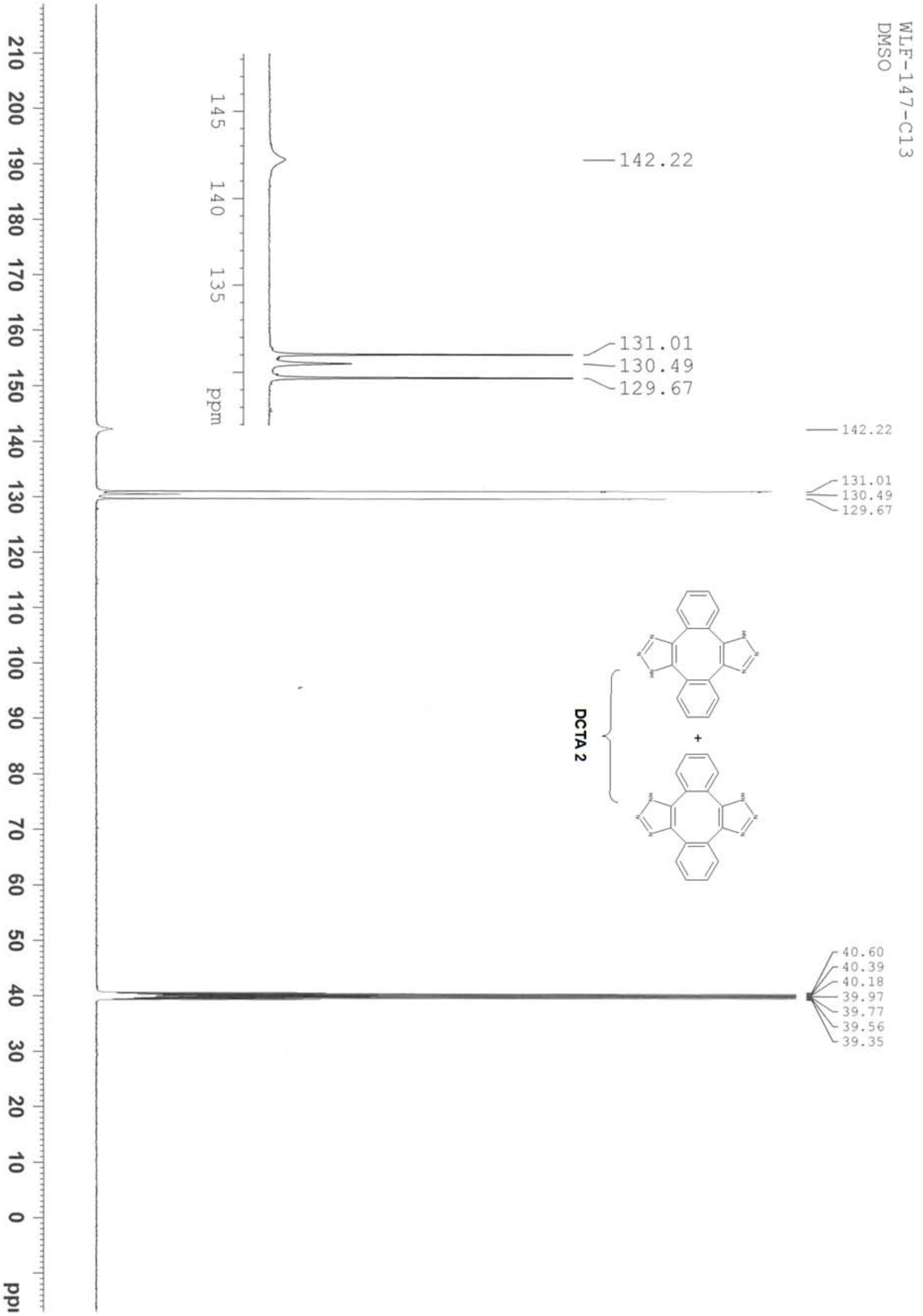
3.35
2.51
0.01
1.97
8.00
DCTA 2
+
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm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140
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ppm
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39.97
39.77
39.56
39.35
DCTA 2
+
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm