Electronic Supplementary Material (ESI) for MedChemComm.

Discovery of Decamidine as a New and Potent PRMT1 Inhibitor

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Supporting Information

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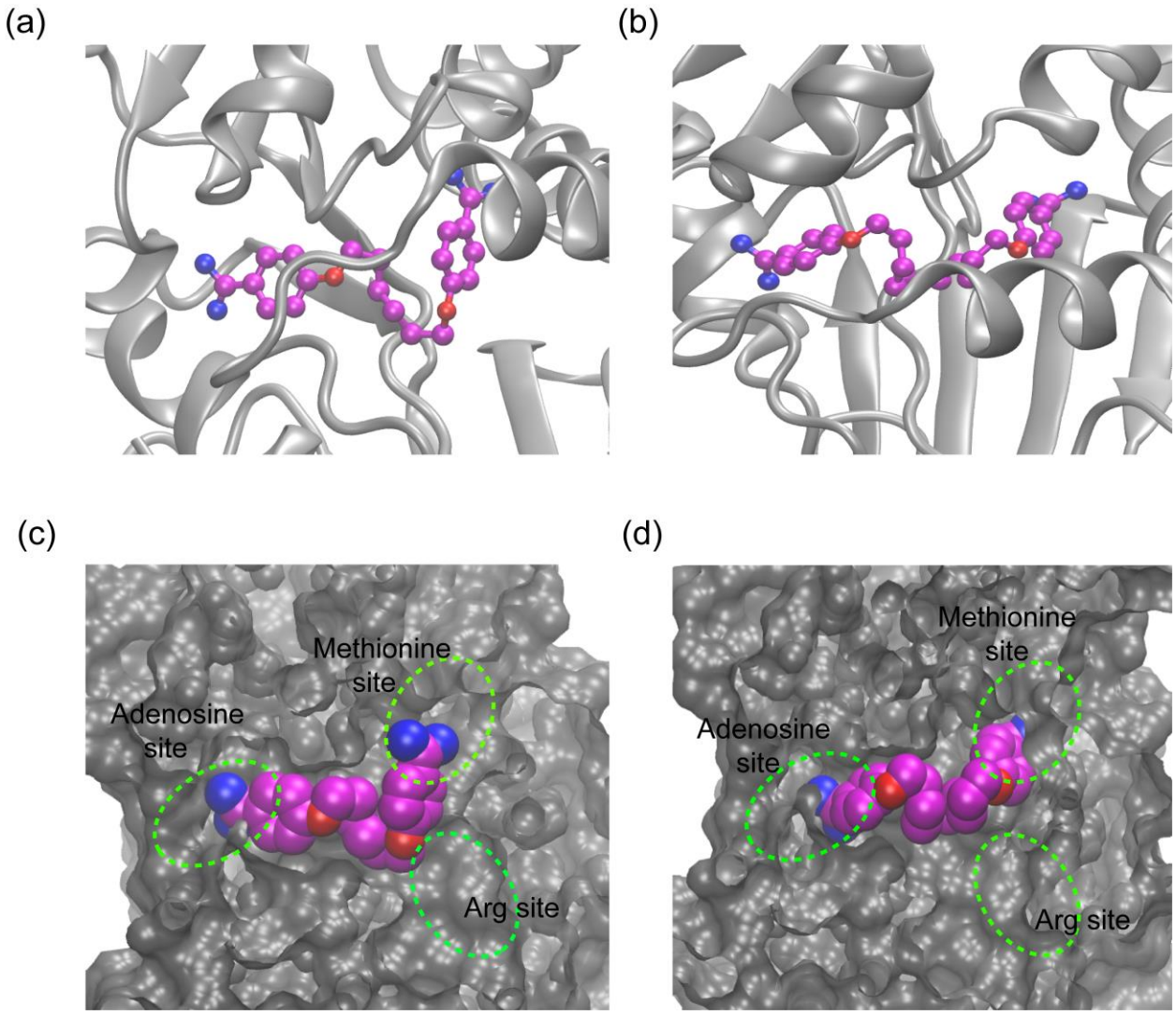


Figure S1. Predicted conformation of **2i** and **2j** bound to the PRMT5 from docking. (a) Overall position of **2i** binding to PRMT5. (b) Overall position of **2j** binding to PRMT5 residues. The human X-ray PRMT5 structure was retrieved from PDB bank (PDB code: 4GQB[1]). Compounds **2i** and **2j** are shown in magenta ball and stick representation. The PRMT5 is shown as cartoon representation. (c) Shape of the binding cavity of PRMT5 (grey) with **2i** (magenta sphere). (d) Shape of the binding cavity of PRMT5 (grey) with **2j** (magenta sphere). The surface in panel (c) and (d) is visualized with VMD.

Reference

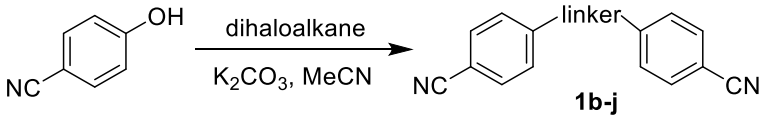
1 S. Antonysamy, Z. Bonday, R. M. Campbell, B. Doyle, Z. Druzina, T. Gheyi, B. Han, L. N. Jungheim, Y. Qian, C. Rauch, M. Russell, J. M. Sauder, S. R. Wasserman, K. Weichert, F. S. Willard, A. Zhang and S. Emtage, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 17960-17965.

Experimental procedure for the synthesis of compounds 2a-2j

General methods

All the chemicals were purchased from Sigma-Aldrich and used as received unless otherwise specified. Compound **2a** was received from National Cancer Institute (NCI), and compound **2h** (pentamidine) was purchased from Sigma-Aldrich. The progress of reactions was monitored by thin-layer chromatography (TLC) on silica gel plates (Selecto scientific, GA). The ^{1}H NMR and ^{13}C NMR spectra of compounds were recorded on either a Varian Unity INOVA 500 MHz or Varian Mercury Plus 400 MHz spectrometers using $CDCl_3$ or d6-DMSO as solvents. The purity of compounds was determined by Shimadzu prominence HPLC system utilizing a column Aeris PEPTIDE 3.6u XB-C18, 250*4.6 mm, a flow of 1 mL/min, solvent A: H_2O with 0.1% trifluoroacetic acid, solvent B: acetonitrile with 0.1% trifluoroacetic acid, and UV-detection at 214 and 260 nm. Preparative HPLC was done on the same system with a column Polaris 5 C18-A with a flow of 5 mL/min, solvent A: H_2O with 0.05% trifluoroacetic acid, solvent B: acetonitrile with 0.05% trifluoroacetic acid. All tested compounds are > 96% pure by analytic HPLC.

General synthetic procedure for compound 1:



For the synthesis of compound **1b**: A mixture of 4-cyanolphenol (2.0 equiv.), dichloromethane (1.0 equiv.) and potassium carbonate (2.0 equiv.) in acetonitrile was heated at 130^{o}C overnight in a sealed tube. Then the mixture was cooled down, concentrated and diluted with a small fraction of ethyl acetate. To the obtained suspension was added 1 M NaOH solution, and the mixture was stirred for 10 min. The suspension was then filtered and washed with H_2O and ethyl acetate. After drying over house vacuum overnight, the desired compound was obtained as a white solid.

For the synthesis of other compounds **1c-1j**: A mixture of 4-cyanolphenol (3.0 equiv.), dibromo alkane (1.0 equiv.) and potassium carbonate (3.0 equiv.) in acetonitrile was refluxed overnight. Then the mixture was cooled down and diluted with EtOAc. The dilute was further washed with H_2O, 1 M NaOH solution, and brine. After that, the desired compound can be obtained either by column chromatography of the concentrated organic layer, or in most cases simply by filtration as an undissolved solid since the desired compounds don't dissolve well in neither organic nor aqueous phase.

Characterization of synthetic intermediates 1:

4,4'-(methylenebis(oxy))dibenzonitrile (**1b**): Yield 44.8%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.8 Hz, 4H), 7.17 (d, *J* = 8.8 Hz, 4H), 5.83 (s, *2*H); MS (ESI) $(M+1)^+$ = 250.9.

4,4'-(ethane-1,2-diylbis(oxy))dibenzonitrile (**1c**): Yield 36.7%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.8 Hz, 4H), 7.01 (d, *J* = 8.8 Hz, 4H), 4.40 (s, 4H); MS (ESI) $(M+K)^+$ = 303.0.

4,4'-(propane-1,3-diylbis(oxy))dibenzonitrile (**1d**): Yield 69.0%. ^{1}H-NMR (400 MHz, d6-DMSO) δ 7.75 (d, *J* = 9.2 Hz, 4H), 7.12 (d, *J* = 9.2 Hz, 4H), 4.21 (t, *J* = 6.4 Hz, 4H), 2.20 (m, 2H); MS (ESI) $(M+K)^+$ = 317.0.

4,4'-(butane-1,4-diylbis(oxy))dibenzonitrile (**1e**): Yield 73.3%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 9.2 Hz, 4H), 6.93 (d, *J* = 9.2 Hz, 4H), 4.07 (m, 4H), 2.00 (m, 4H); (ESI) $(M+1)^+$ = 293.0.

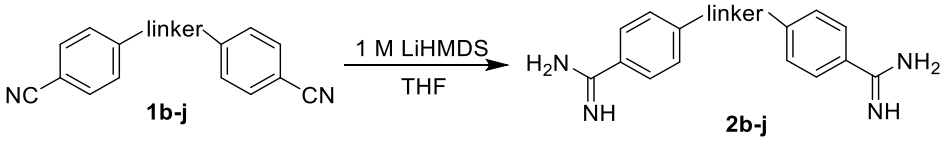
(E)-4,4'-(but-2-ene-1,4-diylbis(oxy))dibenzonitrile (**1f**): Yield 97.0%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 9.2 Hz, 4H), 6.96 (d, *J* = 9.2 Hz, 4H), 6.08 (m, 2H), 4.63 (m, 4H); MS (ESI) $(M+Na)^+$ = 313.1.

4,4'-(pentane-1,4-diylbis(oxy))dibenzonitrile (**1g**): Yield 53.2%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (m, 4H), 6.91 (m, 4H), 4.52 (m, 1H), 4.03 (m, 2H), 1.90 (m, 4H), 1.36 (d, *J* = 6.0 Hz, 3H); MS (ESI) $(M+K)^+$ = 345.1.

4,4'-(hexane-1,6-diylbis(oxy))dibenzonitrile (**1i)**: Yield 86.2%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (m, 4H), 6.93 (m, 4H), 4.01 (t, *J* = 6.0 Hz, 4H), 1.85 (m, 4H), 1.55 (m, 4H); MS (ESI) $(M+1)^+$ = 321.1.

4,4'-(decane-1,10-diylbis(oxy))dibenzonitrile (**1j**): Yield 67.0%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 9.2 Hz, 4H), 6.93 (d, *J* = 8.8 Hz, 4H), 3.99 (t, *J* = 6.4 Hz, 4H), 1.79 (m, 4H), 1.36 (m, 12H); MS (ESI) $(M+K)^+$ = 415.1.

General synthetic procedure for compound 2:



A solution of compound **1** in lithium bis(trimethylsilyl)amide solution (LiHMDS, 1.0 M in THF) was stirred at room temperature overnight. Then it was quenched with 2.0 M HCl solution at ice bath, and the mixture was allowed to stir at room temperature for another 2 h. The THF in the mixture was removed under reduced pressure to give a suspension. After centrifugation of this suspension, the obtained precipitate was purified by preparative reverse-phase HPLC to yield the desired product **2** as a white solid.

Characterization of compounds 2:

4,4'-(methylenebis(oxy))dibenzimidamide (**2b**): Yield 61.5%. ^{1}H-NMR (500 MHz, d6-DMSO) δ 9.21 (s, 4H), 9.04 (s, 4H), 7.83 (d, *J* = 9.0 Hz, 4H), 7.32 (d, *J* = 9.0 Hz, 4H), 6.12 (s, 2H); ^{13}C NMR (126 MHz, d6-DMSO) 165.2, 160.4, 130.7, 122.2, 116.6, 89.4; MS (ESI) $(M+H)^+$ = 285.0; HRMS (ESI^+) for $C_{15}H_{16}N_4O_2 \cdot H^+$ [MH^+]: calculated, 285.1352; found, 285.1349.

4,4'-(ethane-1,2-diylbis(oxy))dibenzimidamide (**2c**): Yield 86.7%. ^{1}H-NMR (400 MHz, d6-DMSO) δ 9.14 (s, 4H), 8.81 (s, 4H), 7.81 (d, *J* = 8.8 Hz, 4H), 7.21 (d, *J* = 8.8 Hz, 4H), 4.46 (s, 4H); ^{13}C-NMR (100 MHz, d6-DMSO) δ 165.1, 163.0, 130.6, 120.4, 115.3, 67.2; MS (ESI) $(M+H)^+$ = 299.1; HRMS (ESI^+) for $C_{16}H_{18}N_4O_2 \cdot H^+$ [MH^+]: calculated, 299.1508; found, 299.1501.

4,4'-(propane-1,3-diylbis(oxy))dibenzimidamide (**2d**): Yield 89.0%. ^{1}H-NMR (400 MHz, d6-DMSO) δ 9.12 (s, 4H), 8.92 (s, 4H), 7.88 (d, *J* = 9.2 Hz, 4H), 7.16 (d, *J* = 8.8 Hz, 4H), 4.24 (t, *J* = 6.4 Hz, 4H), 2.23 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, d6-DMSO) δ 165.1, 163.3, 130.7, 120.1, 115.3, 65.3, 28.7; MS (ESI) $(M+H)^+$ = 313.2; HRMS (ESI^+) for $C_{17}H_{20}N_4O_2 \cdot H^+$ [MH^+]: calculated, 313.1665; found, 313.1661.

4,4'-(butane-1,4-diylbis(oxy))dibenzimidamide (**2e**): Yield 47.8%. ^{1}H-NMR (500 MHz, d6-DMSO) δ 9.16 (s, 4H), 8.98 (s, 4H), 7.82 (d, *J* = 9.0 Hz, 4H), 7.16 (d, *J* = 9.0 Hz, 4H), 4.17 (m, 4H), 1.92 (m, 4H); ^{13}C NMR (126 MHz, d6-DMSO) δ 165.1, 163.4, 130.6, 120.0, 115.2, 68.2, 25.6; MS (ESI) $(M+H)^+$ = 327.3; HRMS (ESI^+) for $C_{18}H_{22}N_4O_2 \cdot H^+$ [MH^+]: calculated, 327.1821; found, 327.1817.

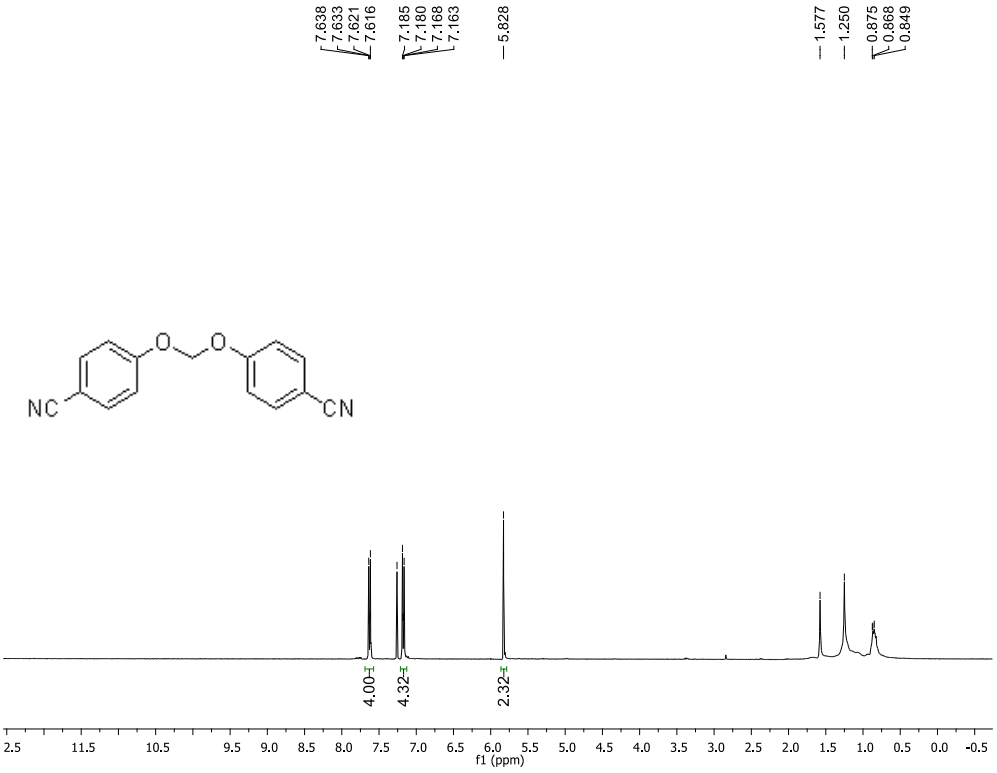
(E)-4,4'-(but-2-ene-1,4-diylbis(oxy))dibenzimidamide (**2f**): Yield 59.0%. ^{1}H-NMR (400 MHz, d6-DMSO) δ 9.12 (br, 4H), 8.83 (br, 4H), 7.80 (d, *J* = 8.8 Hz, 4H), 7.17 (d, *J* = 8.8 Hz, 4H), 6.10 (s, 2H), 4.72 (s, 4H); ^{13}C NMR (126 MHz, d6-DMSO) 165.1, 162.9, 130.6, 128.7, 120.2, 115.4, 68.0; MS (ESI) $(M+2H)^{2+}$ = 163.0; HRMS (ESI^+) for $C_{18}H_{20}N_4O_2 \cdot H^+$ [MH^+]: calculated, 325.1665; found, 325.1659.

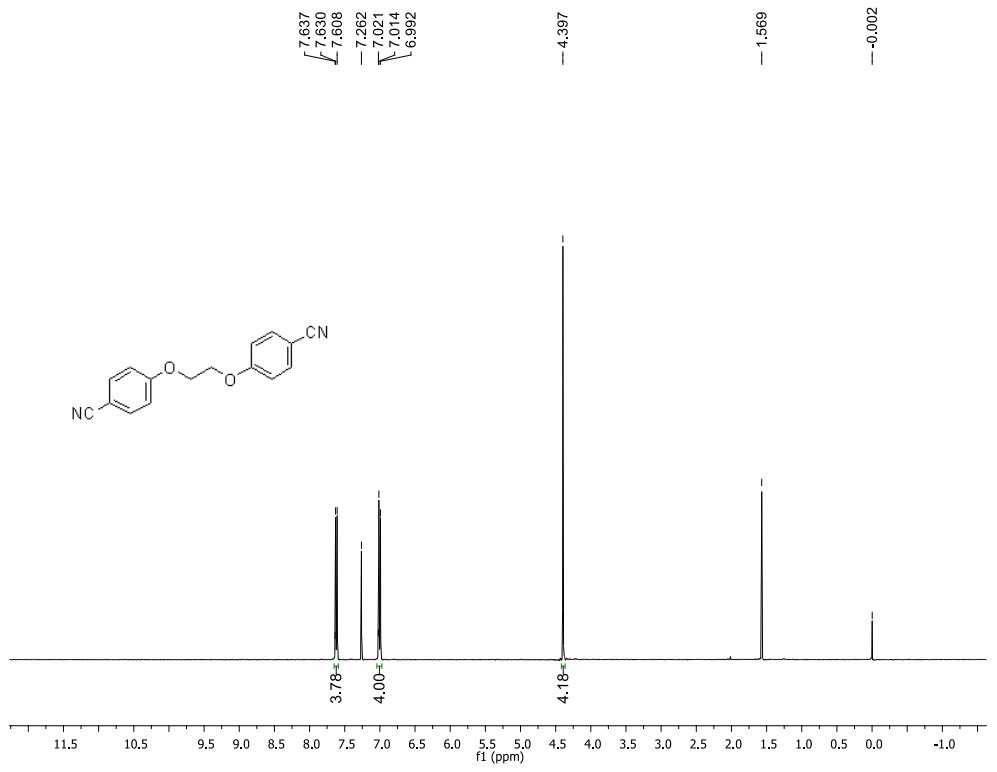
4,4'-(pentane-1,4-diylbis(oxy))dibenzimidamide (**2g**): Yield 49.0%. ^{1}H-NMR (500 MHz, d6-DMSO) δ 9.14 (s, 4H), 8.89 (s, 4H), 7.80 (m, 4H), 7.15 (m, 4H), 4.73 (m, 1H), 4.12 (t, *J* = 6.0 Hz, 2H), 1.83 (m, 4H), 1.30 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (126 MHz, d6-DMSO) δ 165.1, 163.4, 162.7, 130.7, 130.6, 119.9, 119.7, 116.0, 115.2, 73.8, 68.4, 32.6, 25.0, 19.8; MS (ESI) $(M+H)^+$ = 341.1; HRMS (ESI^+) for $C_{19}H_{24}N_4O_2 \cdot H^+$ [MH^+]: calculated, 341.1978; found, 341.1974.

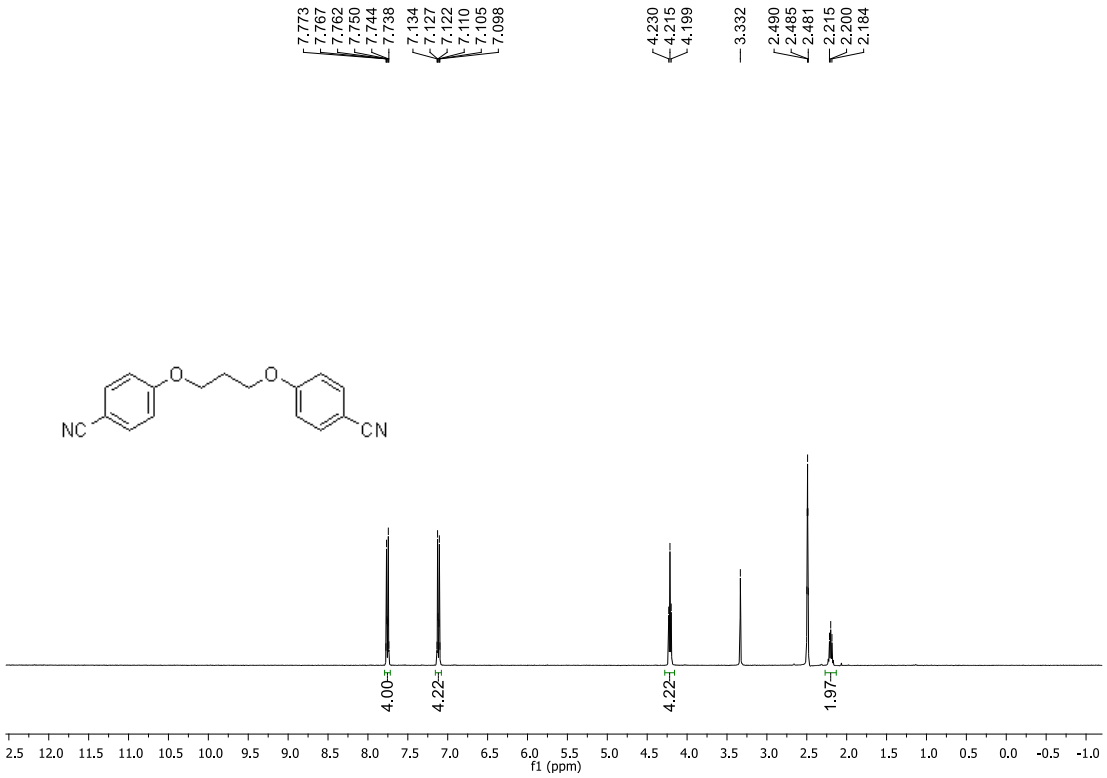
4,4'-(hexane-1,6-diylbis(oxy))dibenzimidamide (**2i**): Yield 78.2%. ^{1}H-NMR (500 MHz, d6-DMSO) δ 9.15 (s, 4H), 8.97 (s, 4H), 7.81 (d, *J* = 8.5 Hz, 4H), 7.15 (d, *J* = 9.0 Hz, 4H), 4.10 (t, *J* = 6.5 Hz, 4H), 1.77 (t, *J* = 6.0 Hz, 4H), 1.49 (m, 4H); ^{13}C NMR (126 MHz, d6-DMSO) 165.1, 163.5, 130.6, 119.9, 115.2, 68.5, 28.9, 25.6; MS (ESI) $(M+H)^+$ = 355.2; HRMS (ESI^+) for $C_{20}H_{26}N_4O_2 \cdot H^+$ $[MH^+]$: calculated, 355.2134; found, 355.2130.

4,4'-(decane-1,10-diylbis(oxy))dibenzimidamide (**2j**): Yield 60.0%. ^{1}H-NMR (500 MHz, d6-DMSO) δ 9.13 (s, 4H), 8.79 (s, 4H), 7.80 (d, *J* = 9.0 Hz, 4H), 7.14 (d, *J* = 9.0 Hz, 4H), 4.08 (t, *J* = 6.5 Hz, 4H), 1.73 (m, 4H), 1.36 (m, 12H); ^{13}C NMR (126 MHz, d6-DMSO) δ 165.0, 163.5, 130.6, 119.8, 115.2, 68.6, 29.4, 29.2, 28.9, 25.9; MS (ESI) $(M+H)^+$ = 411.2; HRMS (ESI^+) for $C_{24}H_{34}N_4O_2 \cdot H^+$ $[MH^+]$: calculated, 411.2760; found, 411.2756.

NMR spectra of synthesized compounds



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2.485
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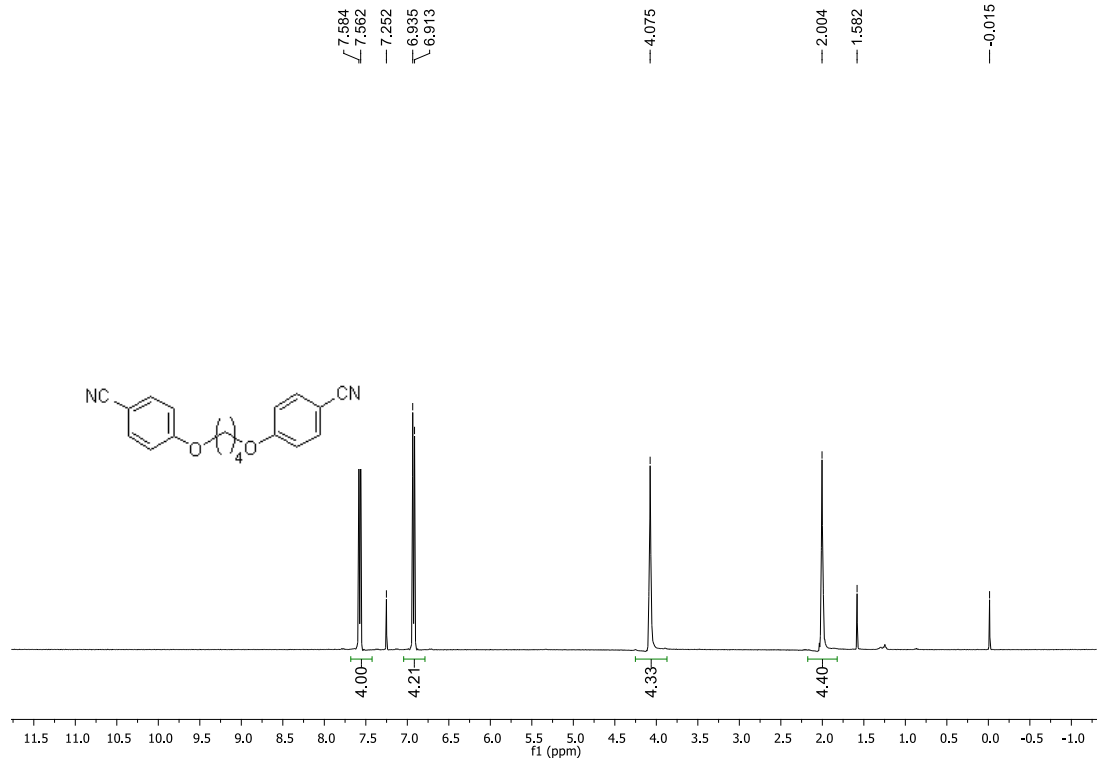
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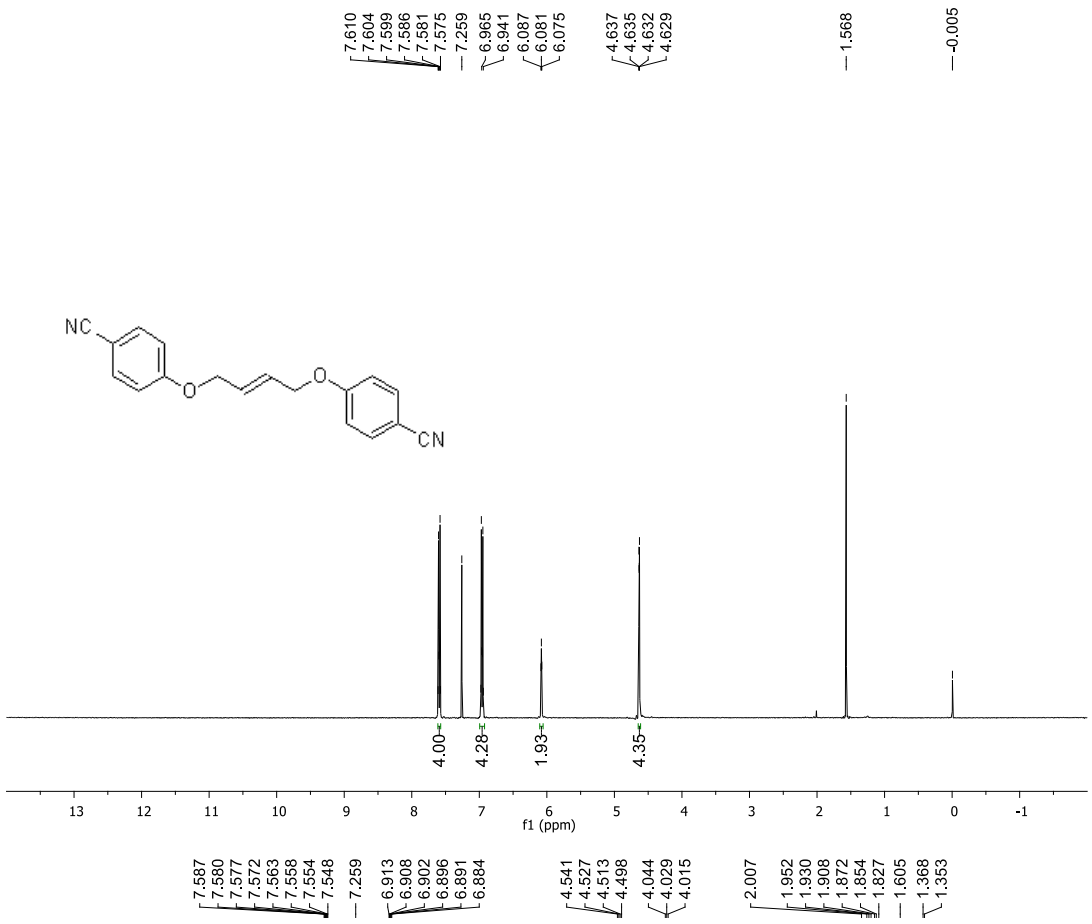
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CN
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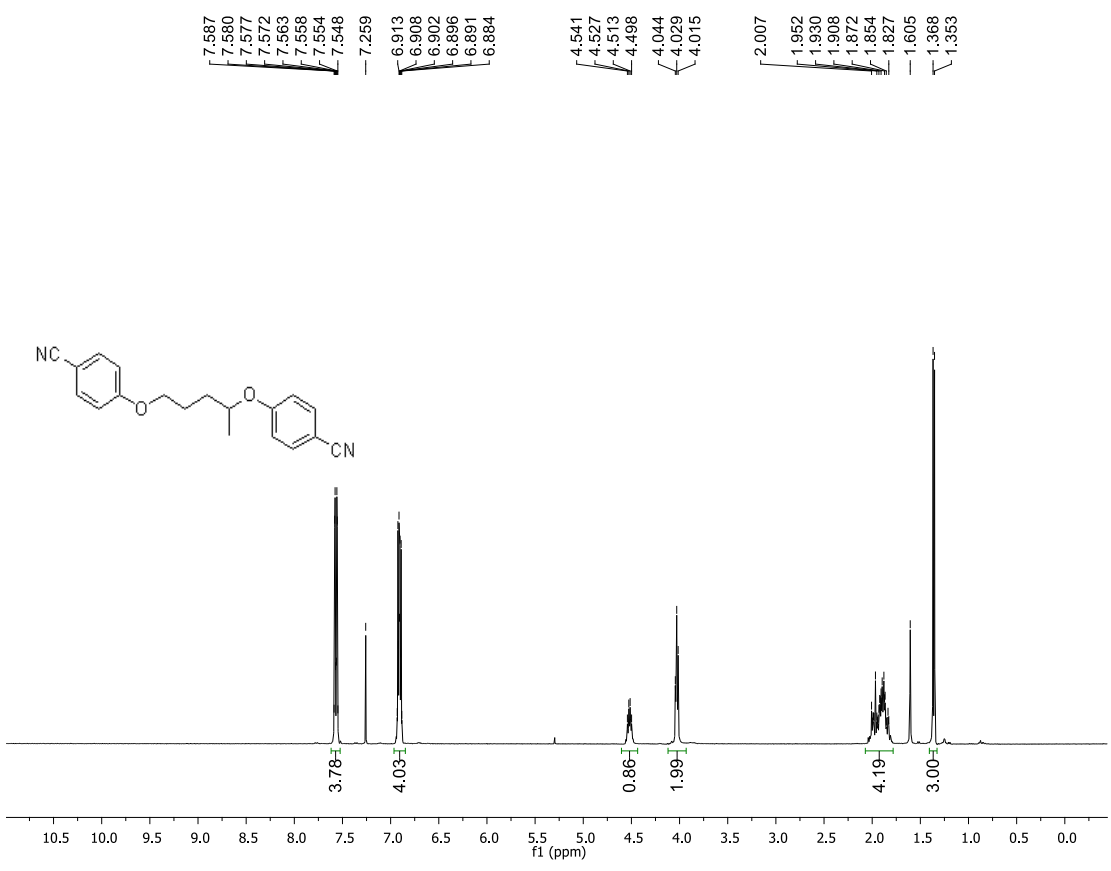
f1 (ppm)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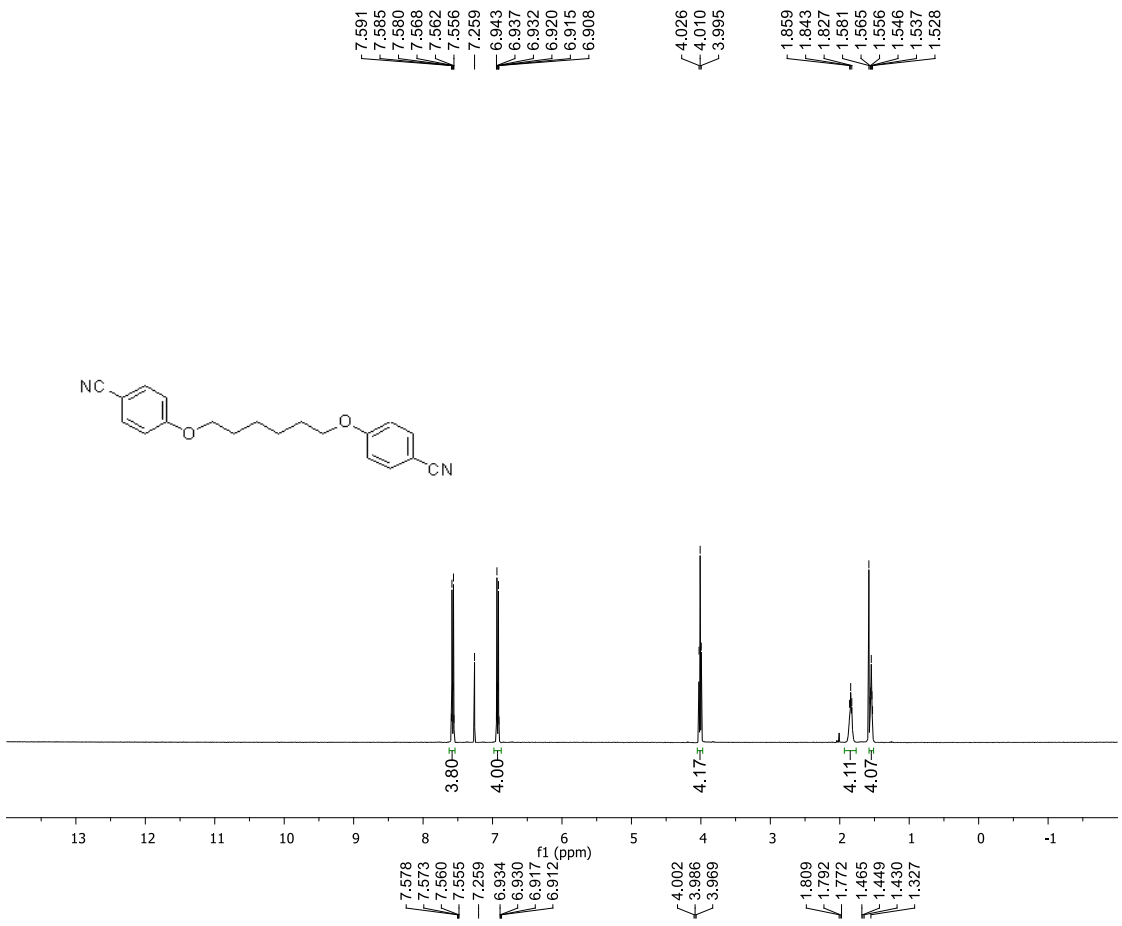
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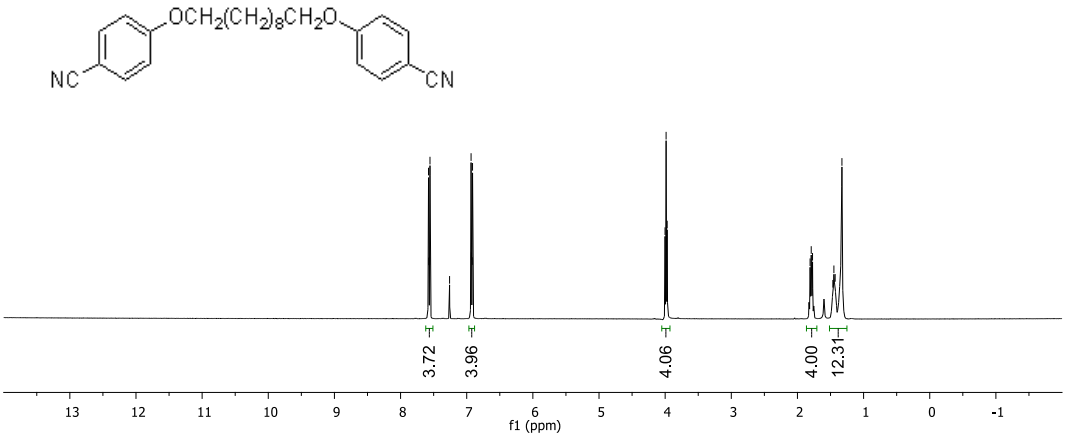
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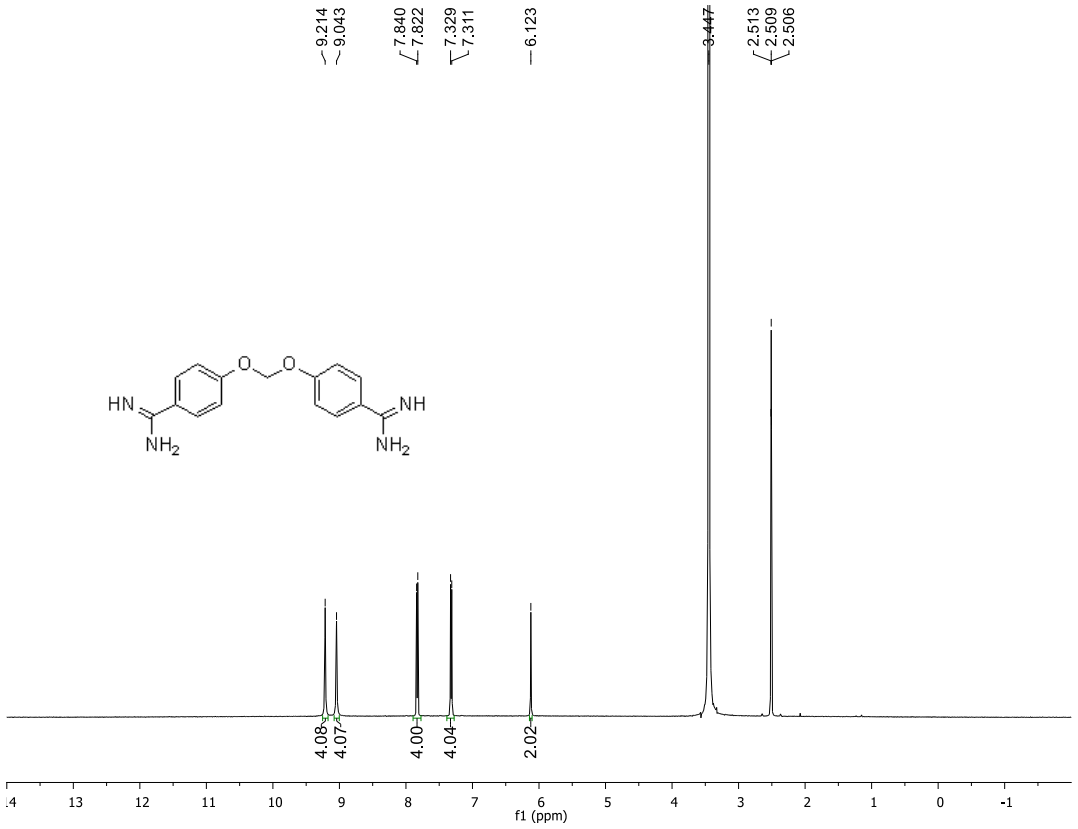
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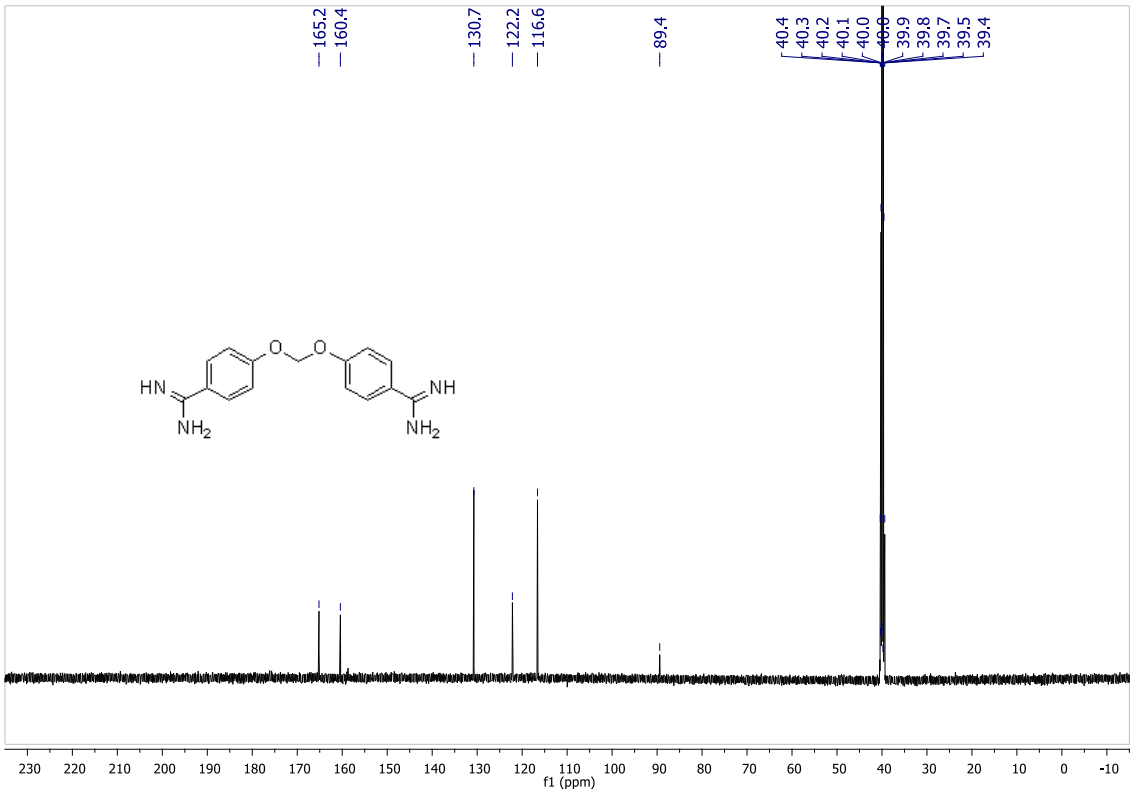
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NH2
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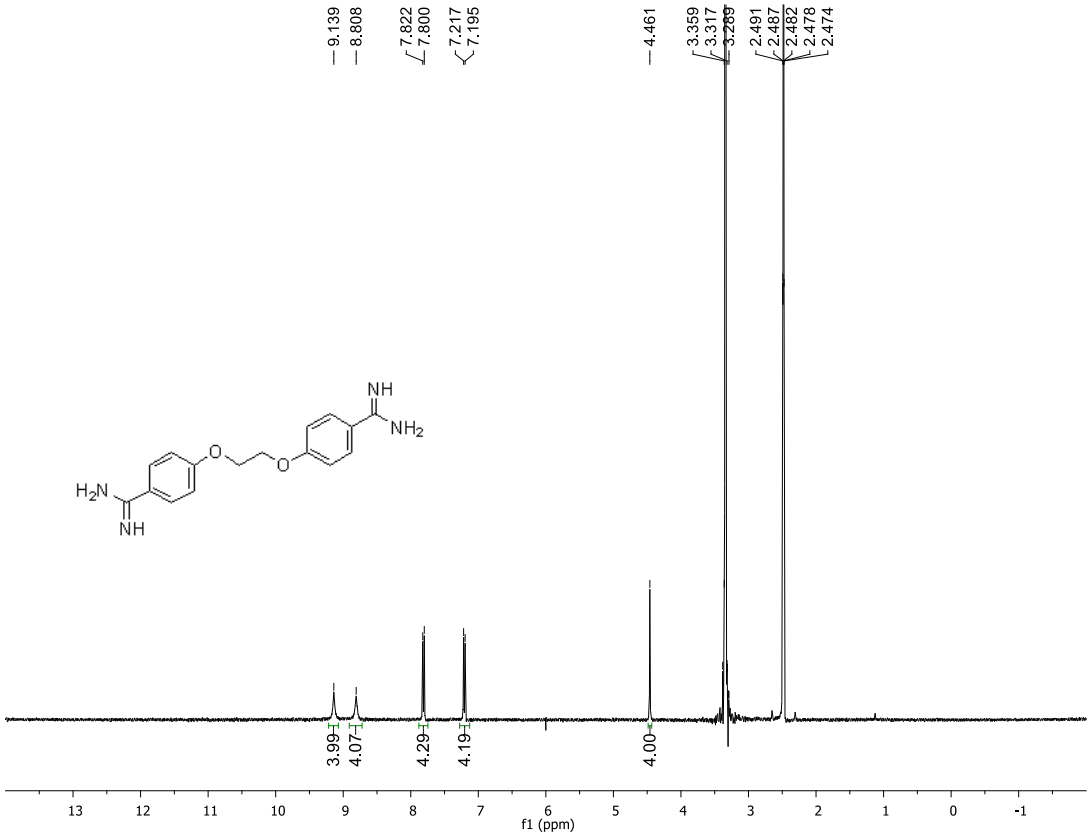
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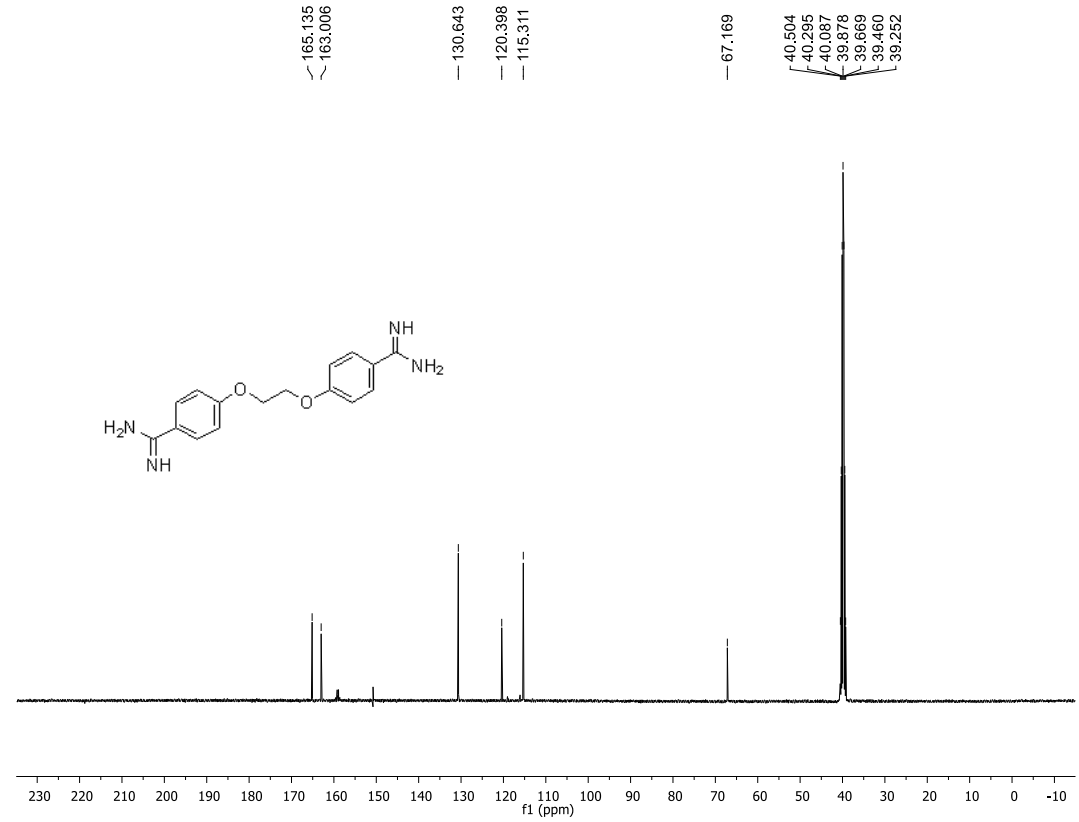
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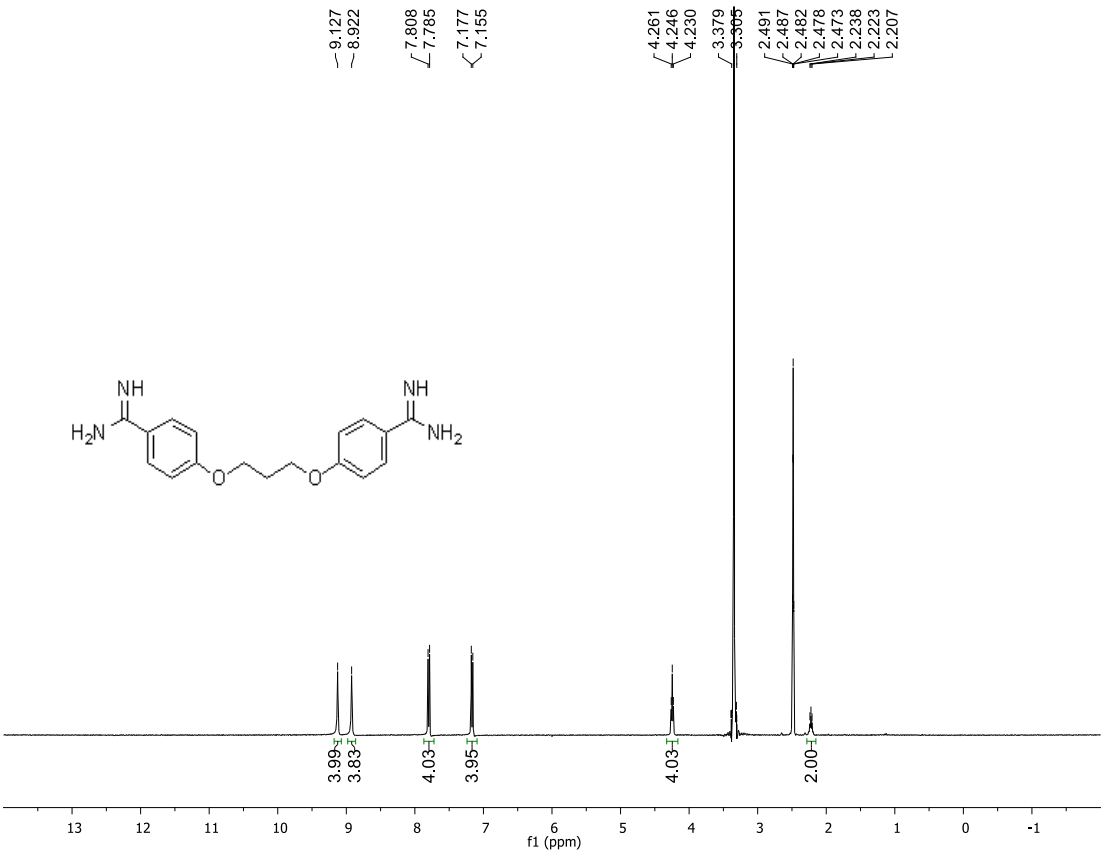
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O
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3.95
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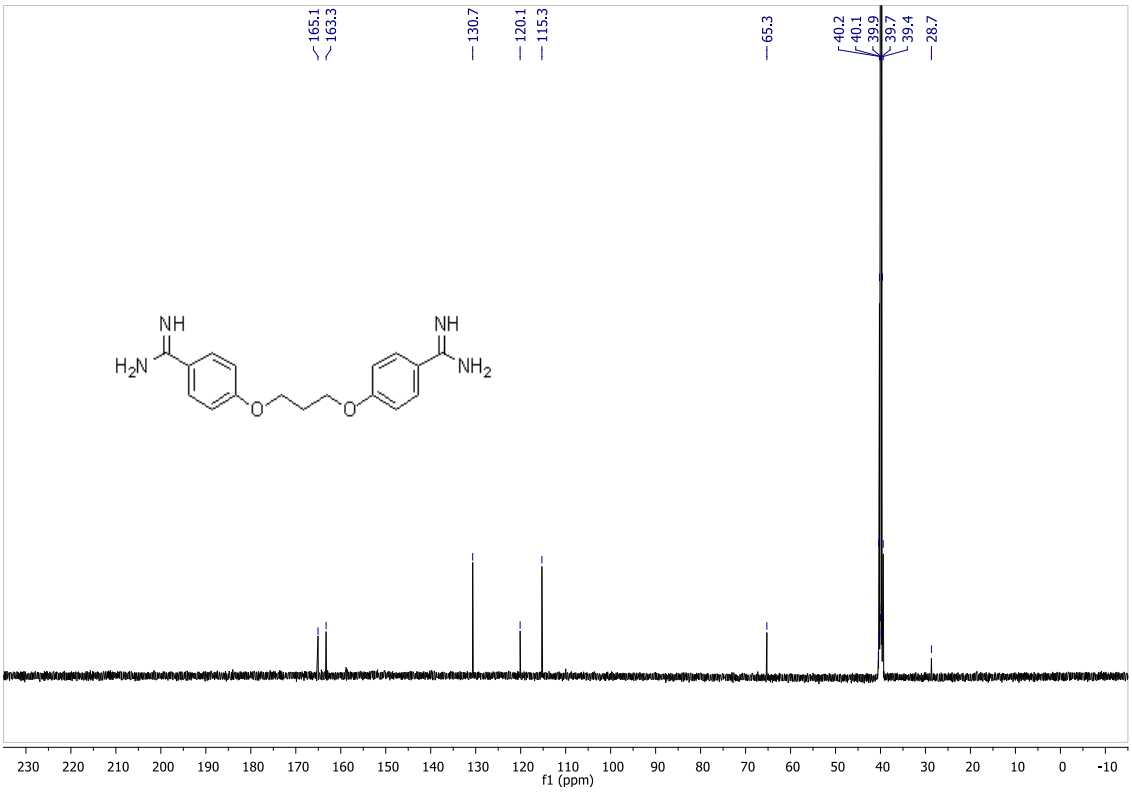
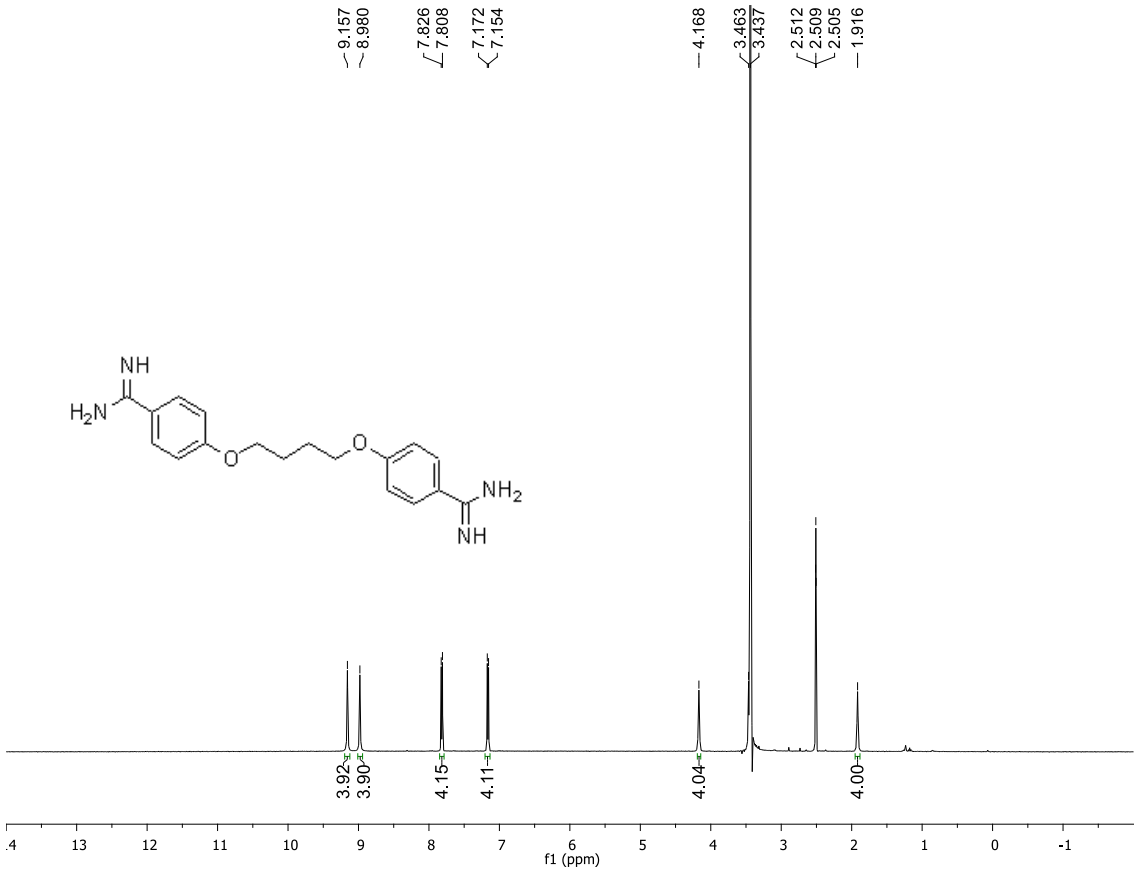
2.00
13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)


165.1
163.3
130.7
120.1
115.3
65.3
40.2
40.1
39.9
39.7
39.4
28.7
NH
H2N
NH
NH2
O
O
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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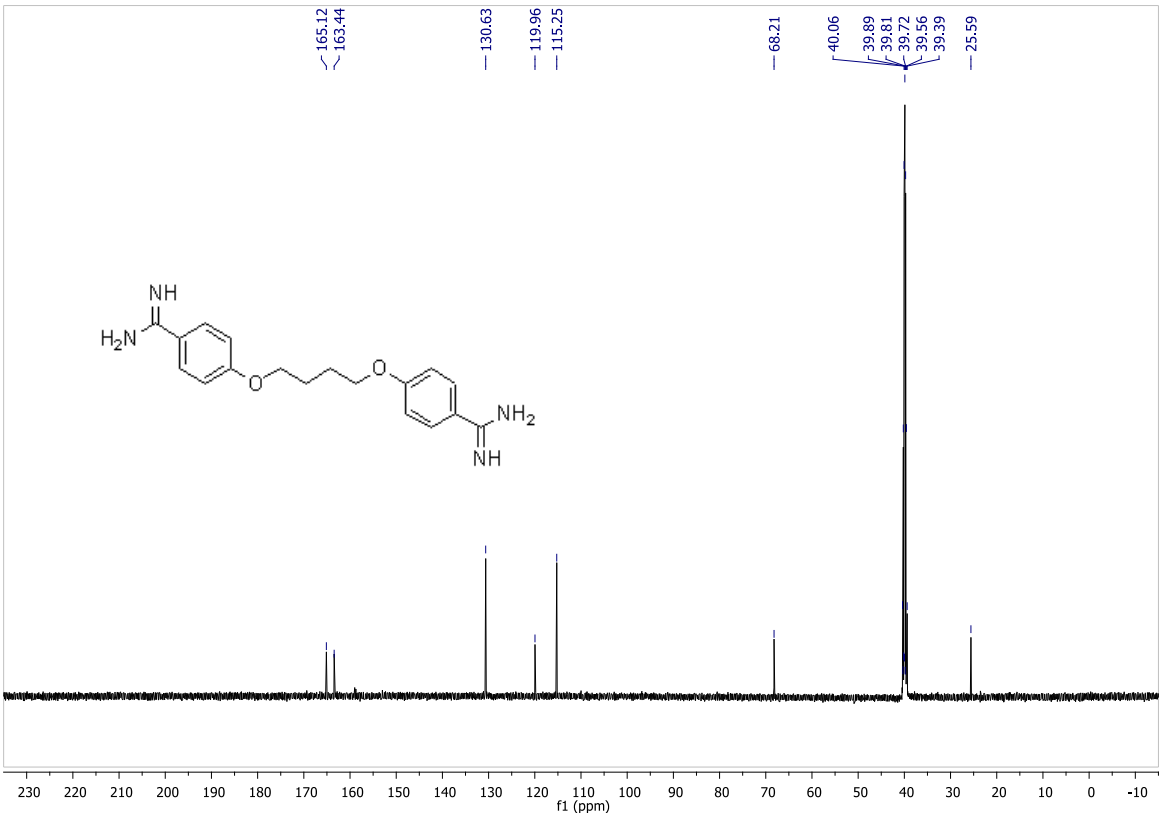
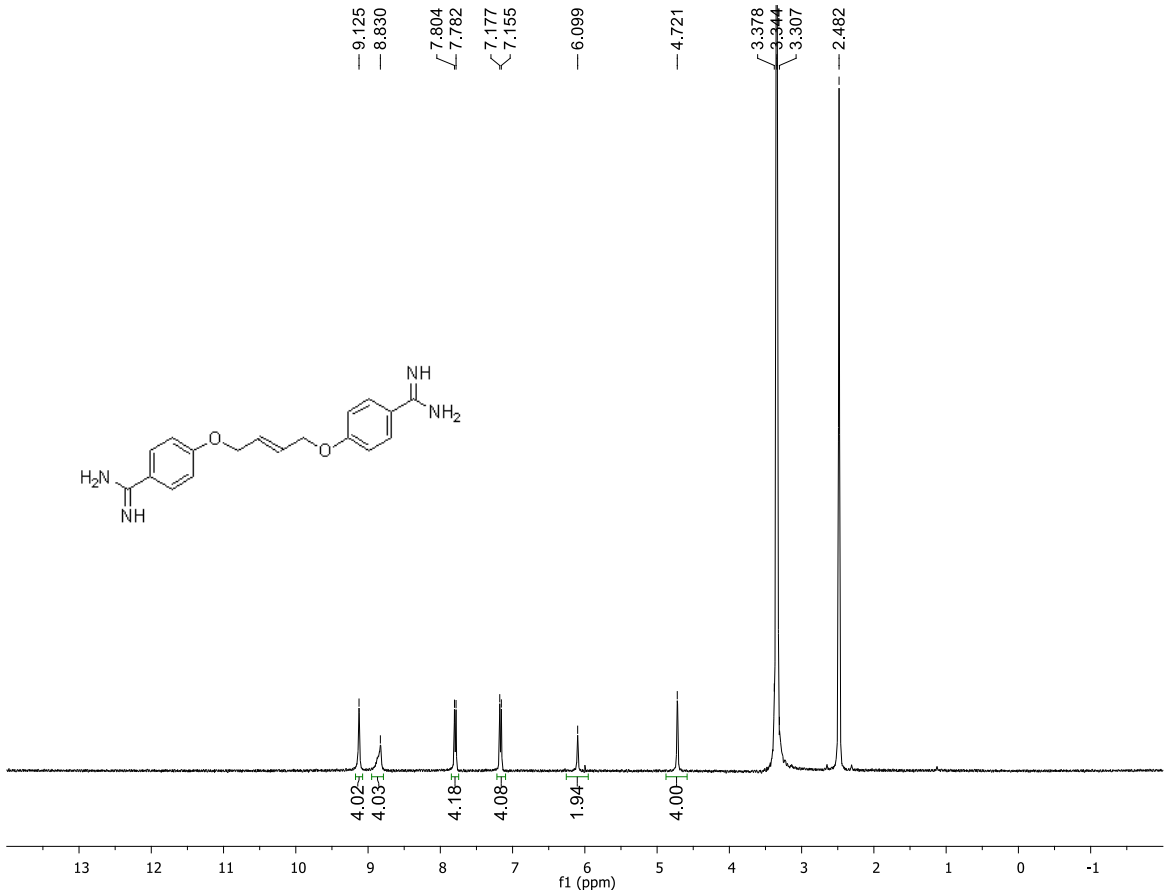
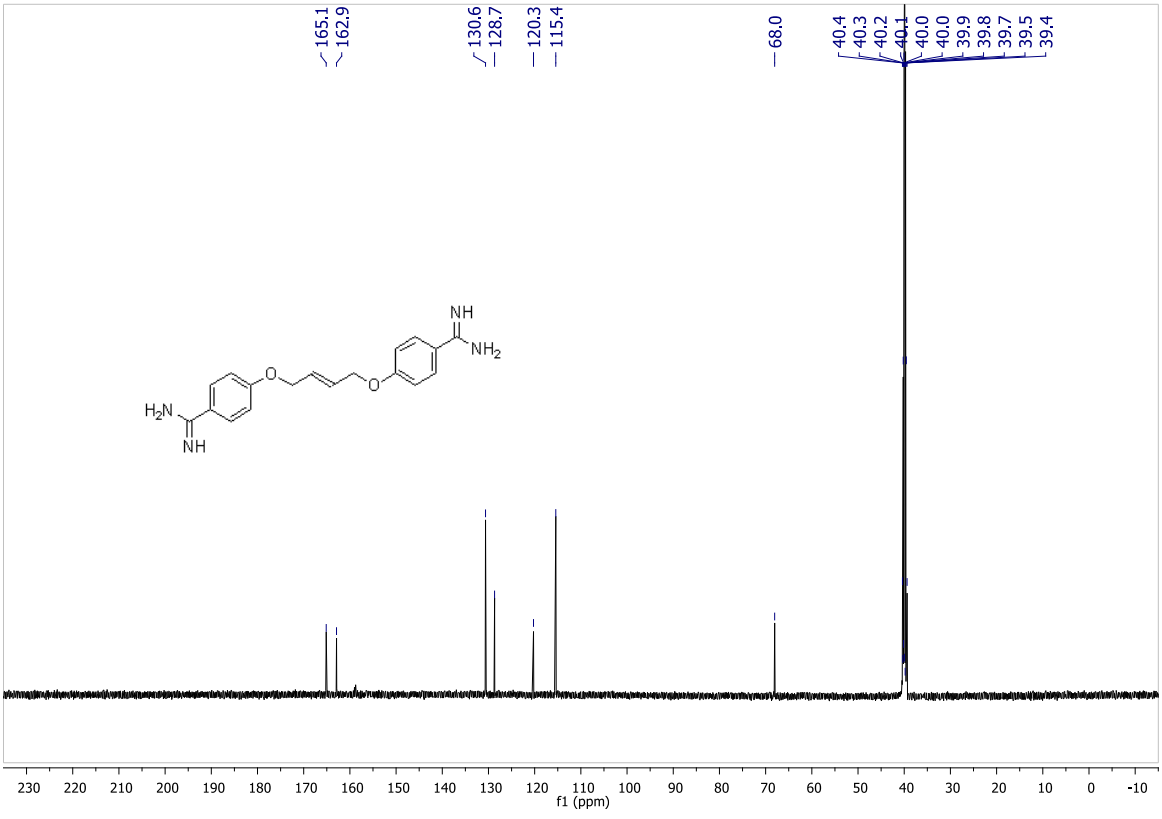
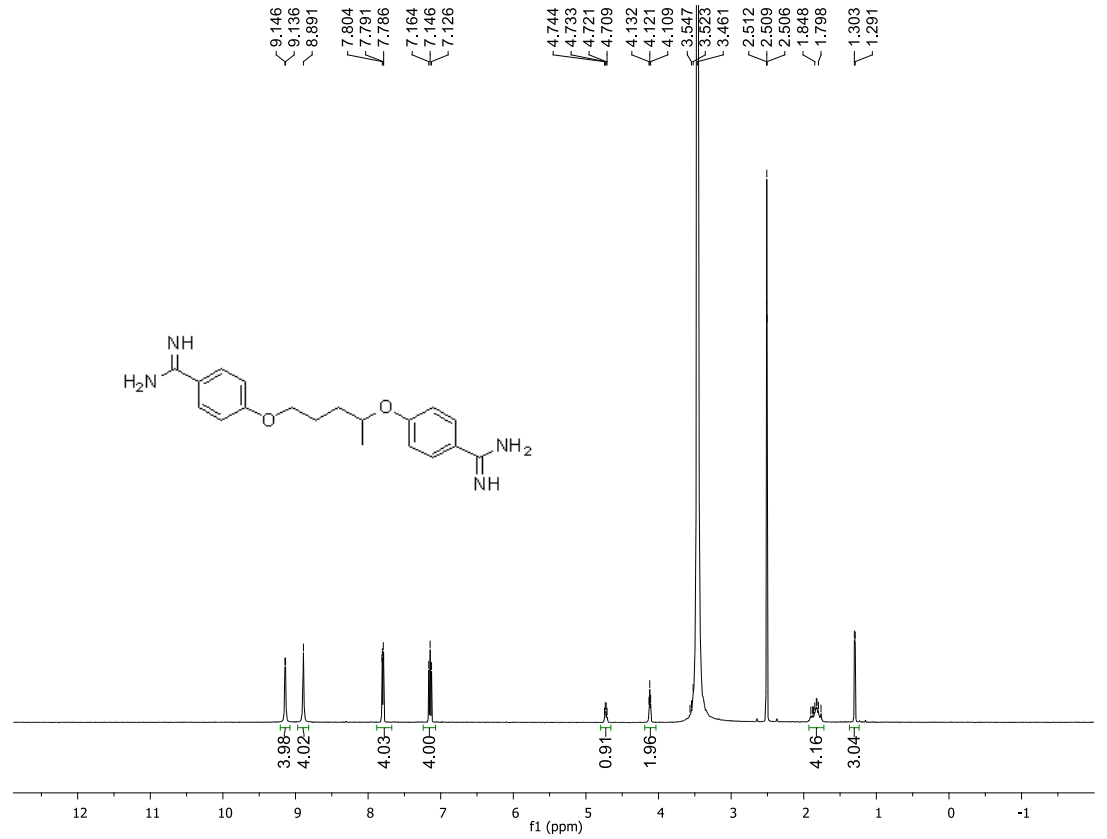
8.980
7.826
7.808
7.172
7.154
4.168
3.463
3.437
2.512
2.509
2.505
1.916
NH
H2N
O
O
NH2
NH
3.92
3.90
4.15
4.11
4.04
4.00
f1 (ppm)


165.12
163.44
130.63
119.96
115.25
68.21
40.06
39.89
39.81
39.72
39.56
39.39
25.59
NH
H2N
O
O
NH2
NH
f1 (ppm)


9.125
8.830
7.804
7.782
7.177
7.155
6.099
4.721
3.378
3.344
3.307
2.482
4.02
4.03
4.18
4.08
1.94
4.00
NH
NH2
O
O
H2N
NH
f1 (ppm)


165.1
162.9
130.6
128.7
120.3
115.4
68.0
40.4
40.3
40.2
40.1
40.0
40.0
39.9
39.8
39.7
39.5
39.4
NH
NH2
O
O
H2N
NH
f1 (ppm)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8.891
7.804
7.791
7.786
7.164
7.146
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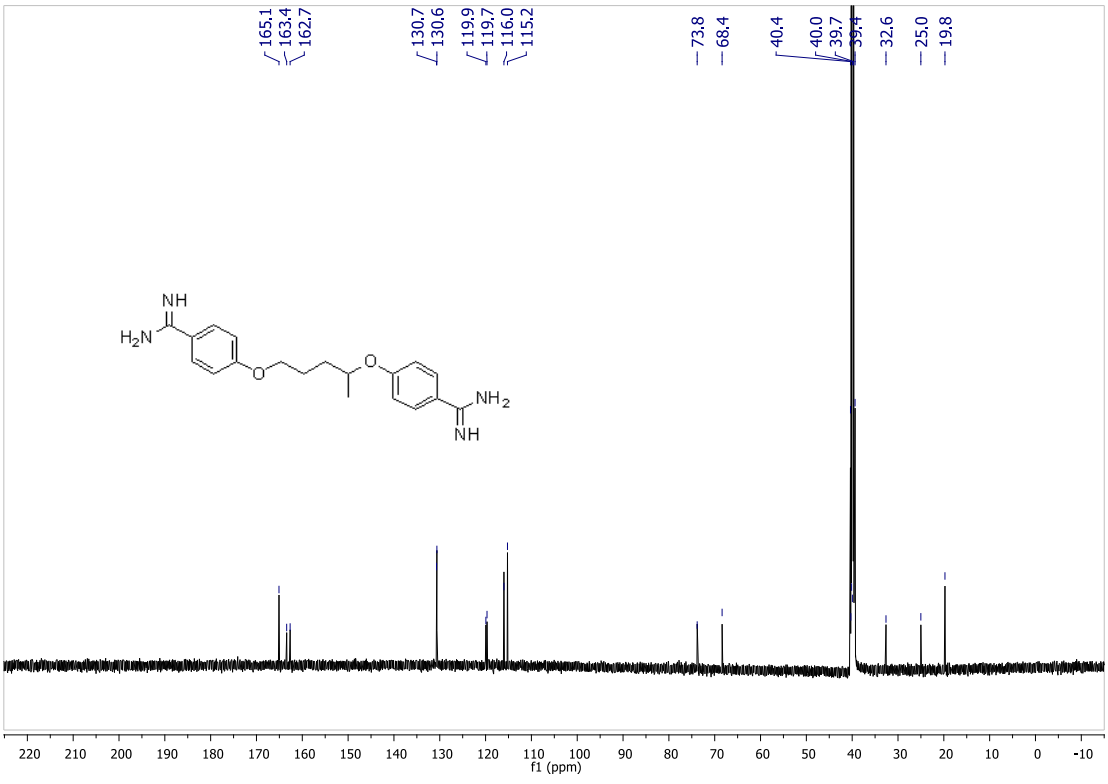
4.744
4.733
4.721
4.709
4.132
4.121
4.109
3.547
3.523
3.461
2.512
2.509
2.506
1.848
1.798
1.303
1.291
NH
H2N
O
O
NH2
NH
3.98
4.02
4.03
4.00
0.91
1.96
4.16
3.04
12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)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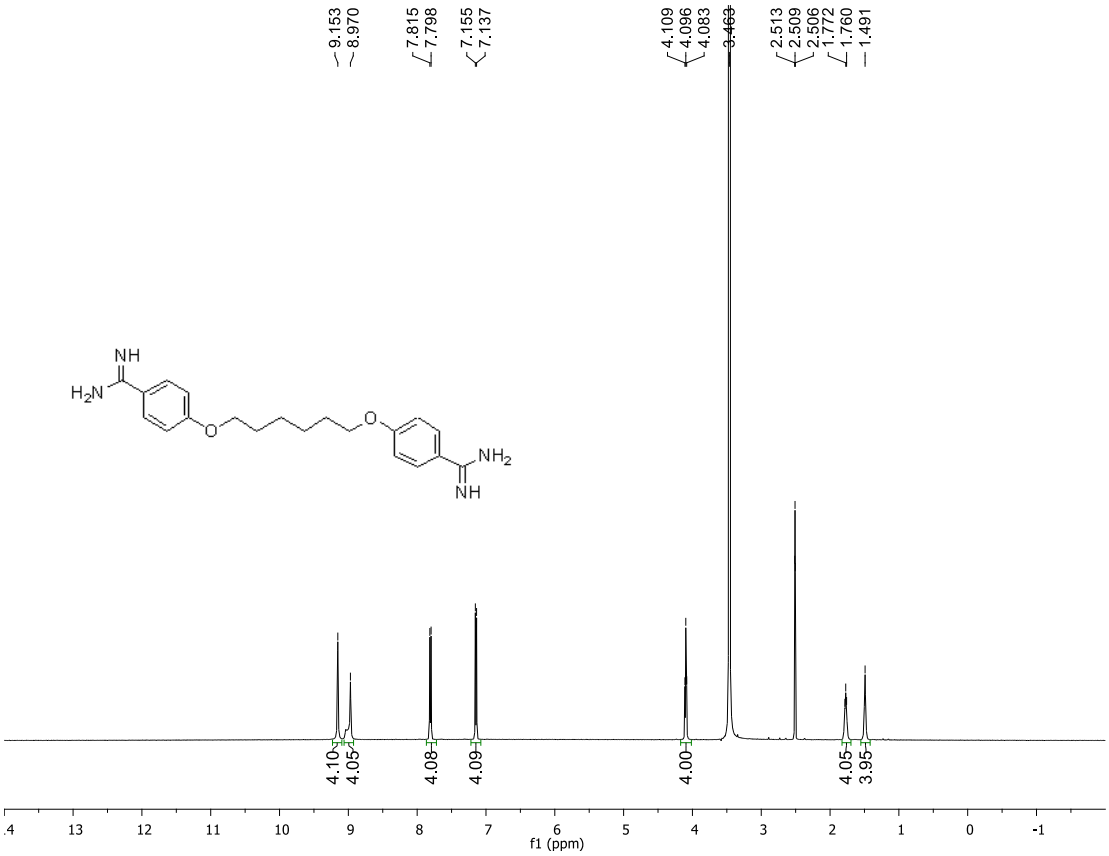
163.4
162.7
130.7
130.6
119.9
119.7
116.0
115.2
73.8
68.4
40.4
40.0
39.7
39.4
32.6
25.0
19.8
NH
H2N
O
O
NH2
NH
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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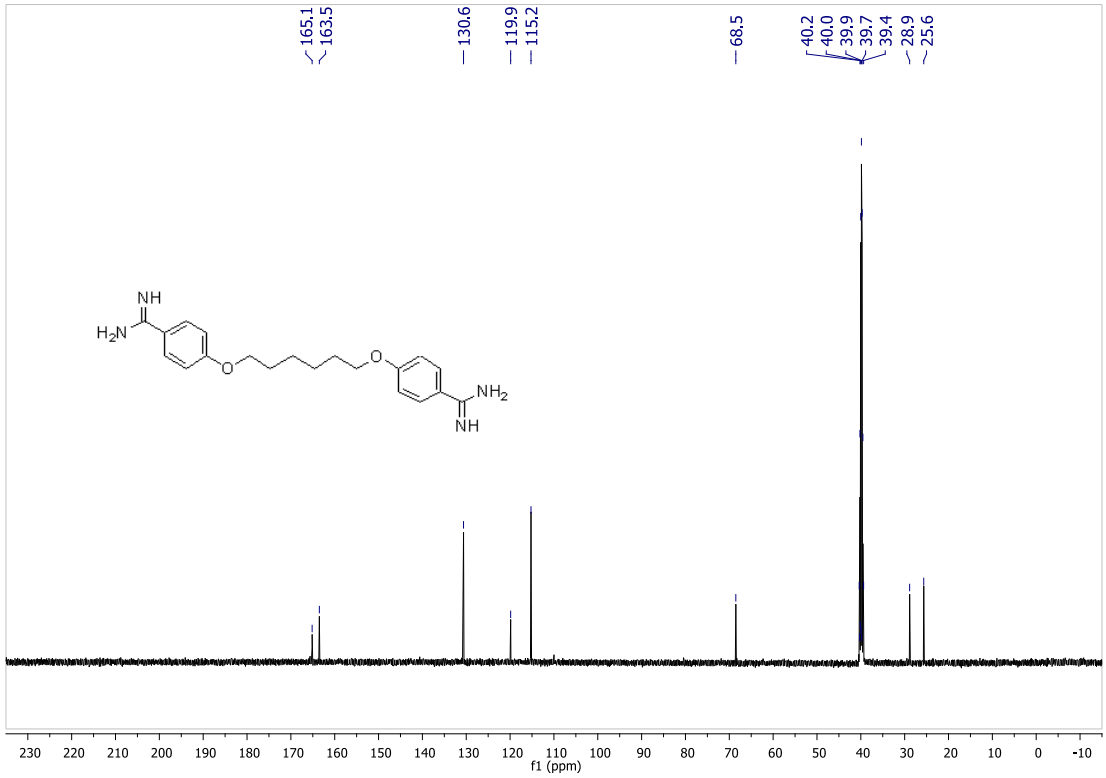
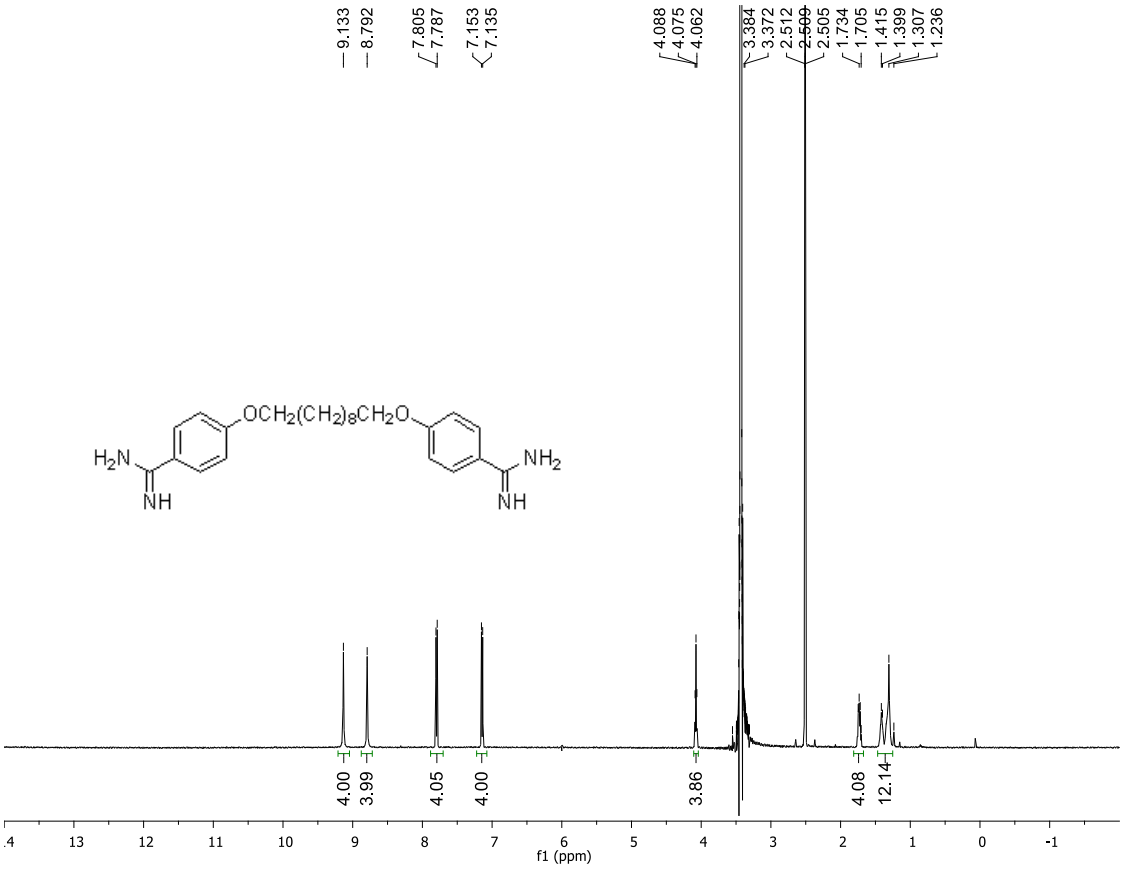
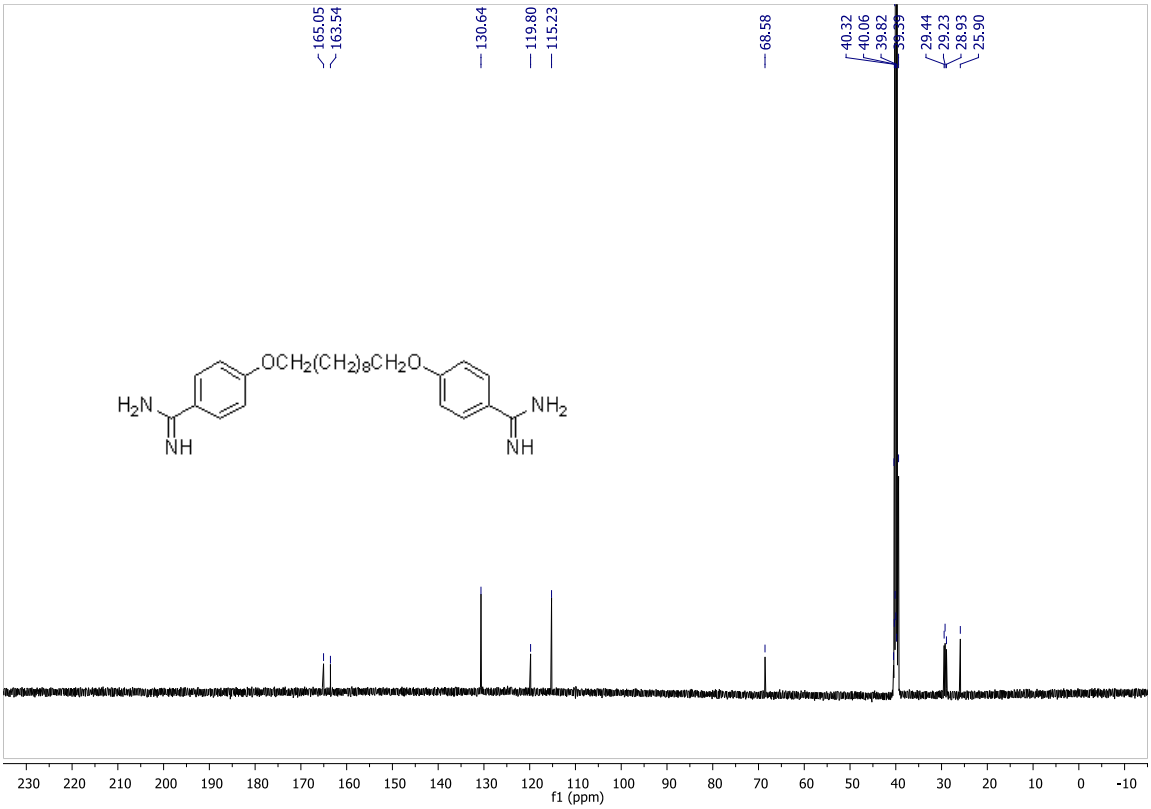
8.970
7.815
7.798
7.155
7.137
4.109
4.096
4.083
3.463
2.513
2.509
2.506
1.772
1.760
1.491
4.10
4.05
4.08
4.09
4.00
4.05
3.95
f1 (ppm)


165.1
163.5
130.6
119.9
115.2
68.5
40.2
40.0
39.9
39.7
39.4
28.9
25.6
f1 (ppm)


9.133
8.792
7.805
7.787
7.153
7.135
4.088
4.075
4.062
3.384
3.372
2.512
2.505
1.734
1.705
1.415
1.399
1.307
1.236
4.00
3.99
4.05
4.00
3.86
4.08
12.14
f1 (ppm)


165.05
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130.64
119.80
115.23
68.58
40.32
40.06
39.82
39.39
29.44
29.23
28.93
25.90
f1 (ppm)

PRMT scintillation proximity assay (SPA)

The SPA was performed on 96-well plate at room temperature. The reaction buffer contains 50 mM HEPES, 10 mM NaCl, 0.5 mM EDTA, 0.5 mM dithiothreitol (DTT), pH 8.0. Firstly, candidate inhibitors in solution (3 μL) were incubated with 9 μL pre-mixture containing H4-20-Biotin peptide, PRMT1 enzyme and 2X reaction buffer for 3 min at room temperature, then [^{3}H]-SAM (3 μL) was added to initiate the reaction. The final concentrations of PRMT1, [^{3}H]-SAM, and H4-20-Biotin are 0.01, 0.5, and 0.5 μM, respectively. The mixtures were incubated at room temperature for 15 min before it was quenched by adding 15 μL isopropanol, and diluted with 30 μL of 50% isopropanol in ddH_2O. After mixing with 5 μL of 20 mg/mL streptavidin-coated SPA beads, the plate was incubated for 30 min in dark, and detected by a Microbeta2 scintillation counter. The positive control was carried out with the corresponding DMSO dilute surrogate under the same condition, and the background control only contained [^{3}H]-SAM and DMSO. For PRMT5 assay, the reaction condition is very similar, except that the reaction time was 45 min. The reported data was based on the average of two experiments.