Supporting Information

to the manuscript:

Balancing Affinity, Selectivity and Cytotoxicity of Hydrazone-based G-Quadruplex Ligands for Activation of Interferon β Gene in Cancer Cells

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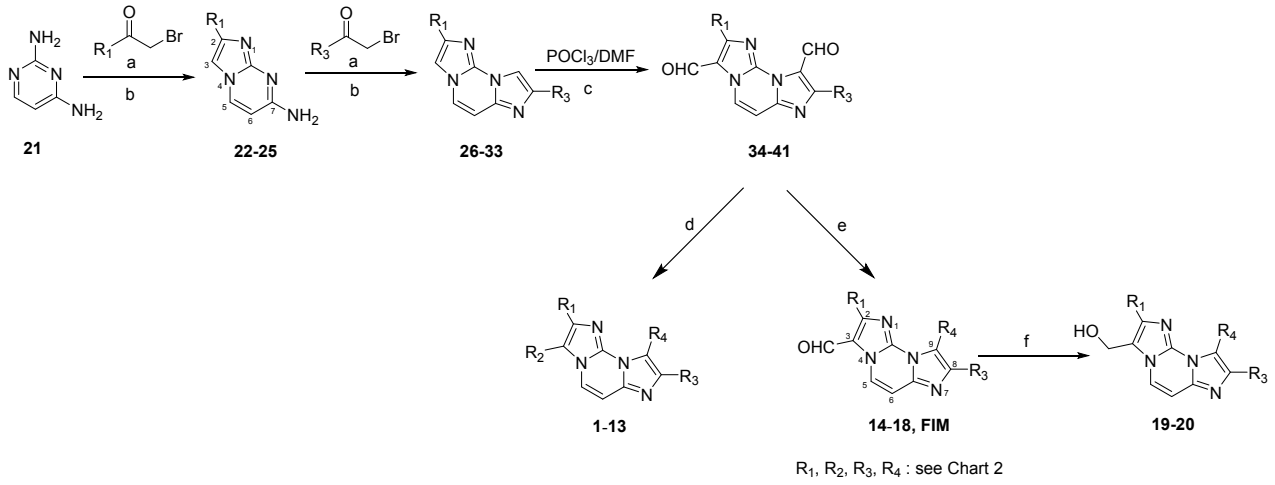
Synthesis of compounds 1-20.

The synthesis of the diimidazo[1,2-*a*:1',2'-*c*]pyrimidine nucleus (Scheme 1) was accomplished in two steps, thus allowing the introduction of different aromatic systems at positions 2 and 8. As previously reported for compounds **22** [1] and **25** [2], the reaction of pyrimidine-2,4-diamine **21** with the appropriate 2-bromo-1-arylethan-1-one led to a first cyclization, involving the nitrogen at position 1 and the amino group at position 2, which allowed to obtain the imidazo[1,2-*a*]pyrimidin-7-amines **22-25** (Scheme S1, Table S1). The structure of compounds **23** and **24** was confirmed by means of NOE experiments. The irradiation of the pyrimidine proton at position 5 (8.35 ppm in compound **23**, 8.37 ppm in compound **24**), provided in both cases a NOE correlation with the imidazole proton, at 7.79 ppm or 7.87 ppm respectively. At the same time, the irradiation of the pyrimidine proton at position 6 (6.28 ppm in compound **23**, 6.27 ppm in compound **24**), showed in both compounds the closeness to the amino group (NOE at 6.89 ppm for compound **23**, NOE at 6.85 ppm for compound **24**), thus confirming the structures assigned. The second cyclization led to the diimidazo[1,2-*a*:1',2'-*c*]pyrimidines **26-33**, which in turn were submitted to a Vilsmeier reaction to obtain the bis-aldehydes **34-41** (Scheme S1, Table S1). The bis-hydrazones **1-13** were prepared by reaction between the appropriate aldehyde (**34-41**) and aminoguanidine hydrochloride or 2-hydrazino-2-imidazoline hydrobromide under reflux. To obtain the mono-hydrazones **14-18**, the same reaction was carried out by adding very slowly the appropriate hydrazine to a solution of the aldehyde in CH_2Cl_2 and keeping the reaction mixture at room temperature. Under these reaction conditions, only the formyl group at position 9 reacted, as described for **FIM**[1] and confirmed by a NOE experiment performed on compound **15**. Indeed, the irradiation of the CHO at 10.08 ppm provided a NOE correlation with the pyrimidine proton at position 5 (9.10 ppm), thus confirming that the unreacted CHO is at position 3. Finally, the alcohols **19** and **20** were obtained by reduction of the formyl group of **FIM** or **15**, respectively.



Reagents and conditions: (a) appropriate 2-bromo-arylethan-1-one, EtOH, reflux; (b) HCl 2N, reflux, then NH_4OH 15%; (c) Vilsmeier reagent ($POCl_3$/DMF), $CHCl_3$, reflux; (d) aminoguanidine hydrochloride or 2-hydrazino-2-imidazoline hydrobromide, EtOH, reflux; (e) aminoguanidine hydrochloride or 2-hydrazino-2-imidazoline hydrobromide, CH_2Cl_2, r.t. (f) $NaBH_4$, EtOH, reflux.

Scheme S1.

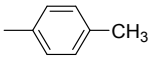
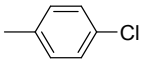
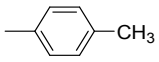
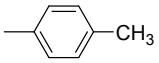
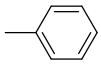
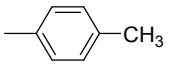
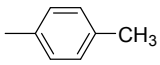
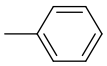
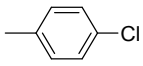
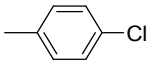
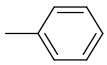
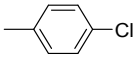
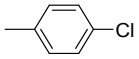
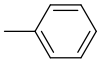
Starting compounds	R_1	R_3
22		–
23		–
24		–
25		–
26, 34		
27. 35		
28, 36		
29, 37		
30, 38		
31, 39		
32, **40**		
33, **41**		

Table S1. Starting compounds.

Methods

Thin layer chromatography was performed on Bakerflex plates (Silica gel IB2-F), the eluent was a mixture of petroleum ether/acetone/methanol in various proportions. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian MR 400 MHz (ATB PFG probe); the chemical shift was expressed in δ (ppm) and referenced to the residual peak of the solvent as the internal standard (DMSO-d_6: δ H = 2.50 ppm; δ C = 39.52 ppm and $CDCl_3$: δ H = 7.26 ppm; δ C = 77.16 ppm). The coupling constant values (*J*) were determined in Hertz (abbreviation: im=imidazole, pym=pyrimidine, th=thiophenyl, ph=phenyl, tol=tolyl, Cl-ph=4-chlorophenyl). High resolution mass spectrometry (HRMS) data were analyzed by flow injection, utilizing electrospray ionization (ESI) on a Waters Xevo G2-XS QTOF instrument in the positive mode. UHPLC−MS analyses were run on a Waters ACQUITY ARC UHPLC/MS system consisting of a QDa mass spectrometer equipped with an ESI interface. Elemental analyses were within ± 0.4% of the theoretical values. UHPLC analysis of compounds **1**, **2**, **8**, **10**, **15**, **19** and **20** were run on a Waters ACQUITY ARC UHPLC/ system consisting of a 2489

UV/Vis detector. Detected wavelength (λ): 254 nm. The analyses were performed on an XBridge BEH C18 column (10 × 2.1 mm i.d., particle size 2.5 μm) with a XBridge BEH C18 VanGuard Cartridge precolumn (5 mm × 2.1 mm i.d., particle size 1.8 μm). The mobile phases were H_2O (0.1% trifluoroacetic acid) (A) and MeOH (0.1% trifuoroacetic acid) (B). Method and gradients used were the following: Linear gradient: 0−3.5 min, 95% A; 3.5 min-4.5 min 30% A – 4.5-7.0 95%A - 5. Flow rate: 0.6 mL/min. Compounds were named relying on the naming algorithm developed by CambridgeSoft Corporation (Perkin Elmer, Milan, Italy) and used in Chem-BioDraw Ultra 14.0 (Perkin Elmer, Milan, Italy). All solvents and reagents, unless otherwise stated, were supplied by Aldrich Chemical Co. Ltd. (Milan, Italy) and were used without further purification. 2,4-Diaminopyrimidine, 2-bromo-1-phenylethan-1-one, 2-bromo-1-(p-tolyl)ethan-1-one, 2-bromo-1-(4-chlorophenyl)ethan-1-one, 2-hydrazino-2-imidazoline hydrobromide and aminoguanidine hydrogencarbonate are commercially available. The following compounds were prepared according to the literature: 2-bromo-1-(thiophen-3-yl)ethan-1-one [3], **22** [1], **25** [2], **32** [2], **33** [1], **40** [2], **41** [4], **FIM** [2].

*General procedure for the synthesis of the bis-hydrazones **1-13***

The appropriate aldehyde (5 mmol) was dissolved in ethanol and treated with two equivalents of aminoguanidine hydrogencarbonat suspended in ethanol and treated with hydrochloridric acid in order to achieve a solution (for compounds **1**, **3**, **5**, **7**, **9**, **11**) or 2-hydrazino-2-imidazoline hydrobromide solubilized in ethanol (to obtain compounds **2**, **4**, **6**, **8**, **10**, **12**, **13**). The reaction mixture was refluxed for 15-30 h according to a TLC test and the resulting precipitate was collected by filtration and crystallized from ethanol.

*2,2'-((2-Phenyl-8-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine-3,9-diyl)bis(methaneylylidene))bis(hydrazine-1-carboximidamide) dihydrochloride (**1**)*

Yield 75%. ^{1}H-NMR (DMSO-d_6): 2.38 (3H, s, CH_3), 7.30 (1H, d, J=8.0, pym), 7.31 (2H, d, J=7.6, tol), 7.55 (3H, m, ph), 7.78 (8H, s broad, NH), 7.88 (2H, d, J=7.6, ph), 7.93 (2H, d, J=7.6, tol), 8.64 (1H, s, =CH), 9.30 (1H, d, J=8.0, pym), 9.55 (1H, s, =CH), 12.12 (1H, s, NNH), 12.46 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 20.99, 103.47, 115.27, 117.83, 128.49, 128.91, 129.08, 129.31, 130.17, 131.73, 138.40, 138.45, 138.57, 139.56, 142.04, 145.73, 146.08, 154.77, 155.18. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{25}H_{24}N_{12}$ $[M+H]^+$: 493.2325, found: 493.2320; calculated for $[M+2H]^{++}$: 247.1202, found: 247.1203. Anal. Calcd for $C_{25}H_{24}N_{12}$·2HCl (565.47): C, 53.10; H, 4.63; N, 29.72. Found: C, 53.14; H, 4.64; N, 29.70.

*3,9-Bis((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2-phenyl-8-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**2**)*

Yield 72%. ^{1}H-NMR (DMSO-d_6): 2.38 (3H, s, CH_3), 3.73 (4H, s, CH_2), 3.81(4H, s, CH_2), 7.30 (2H, d, J=8.0, tol), 7.35 (1H, d, J=7.6, pym), 7.58 (3H, m, ph), 7.85 (2H, d, J=7.6, ph), 8.03 (2H, d, J=8.0, tol), 8.34 (2H, s, NH), 8.66 (1H, s, =CH), 8.90 (2H, s broad, NH), 9.30 (1H, d, J=7.6, pym), 9.49 (1H, s, =CH), 12.34 (1H, s, NNH), 12.72 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 21.00, 42.83, 42.94, 103.62, 115.23, 117.40, 128.43, 128.61, 128.97, 129.08, 129.22, 129.46, 129.77, 131.78, 138.43, 139.75, 140.34, 142.08, 145.74, 146.49, 156.98, 157.61. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{29}H_{28}N_{12}$ $[M+H]^+$: 545.2638, found: 545.2633; calculated for $[M+2H]^{++}$: 273.1358, found:

273.3807. Anal. Calcd for $C_{29}H_{28}N_{12}$·2HBr (706.45): C, 49.31; H, 4.28; N, 23.79. Found: C, 49.28; H, 4.27; N, 23.81.

*2,2'-((2-Phenyl-8-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine-3,9-diyl)bis(methaneylylidene))bis(hydrazine-1-carboximidamide) dihydrochloride (**3**)*
Yield 75%. ^{1}H-NMR (DMSO-d_6): 2.40 (3H, s, CH_3), 7.28 (1H, d, J=7.6, pym), 7.35 (2H, d, J=8.0, tol), 7.48 (3H, m, ph), 7.77 (8H, s broad, NH), 7.78 (2H, d, J=8.0, tol), 8.00 (2H, d, J=6.4, ph), 8.63 (1H, s, =CH), 9.28 (1H, d, J=7.6, pym), 9.54 (1H, s, =CH), 12.27 (1H, s, NNH), 12.60 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 20.93, 103.24, 114.97, 118.08, 127.93, 128.66, 128.85, 128.89, 128.95, 129.42, 129.49, 132.93, 138.27, 138.55, 138.80, 139.42, 142.00, 145.35, 146.16, 154.80, 155.24. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{25}H_{24}N_{12}$ $[M+H]^+$: 493.2325, found: 493.2326; calculated for $[M+2H]^{++}$: 247.1201, found: 247.1205. Anal. Calcd for $C_{25}H_{24}N_{12}$·2HCl (565.47): C, 53.10; H, 4.63; N, 29.72. Found: C, 53.12; H, 4.63; N, 29.74.

*3,9-Bis((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2-phenyl-8-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**4**)*
Yield 65%. ^{1}H-NMR (DMSO-d_6): 2.43 (3H, s, CH_3), 3.72 (4H, s, CH_2), 3.81(4H, s, CH_2), 7.34 (1H, d, J=7.8, pym), 7.40 (2H, d, J=8.0, tol), 7.49 (3H, m, ph), 7.75 (2H, d, J=8.0, tol), 8.11 (2H, d, J=7.2, ph), 8.28 (2H, s, NH), 8.67 (1H, s, =CH), 8.77 (2H, s broad, NH), 9.29 (1H, d, J=7.8, pym), 9.50 (1H, s, =CH), 12.33 (1H, s, NNH), 12.73 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 20.81, 42.75, 42.86, 103.44, 114.91, 117.60, 127.90, 128.35, 128.76, 128.86, 128.88, 129.37, 129.40, 132.59, 138.88, 139.59, 139.78, 140.16, 142.06, 145.62, 146.58, 156.96, 157.58. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{29}H_{28}N_{12}$ $[M+H]^+$: 545.2638, found: 545.2641; calculated for $[M+2H]^{++}$: 273.1358, found: 273.1366. Anal. Calcd for $C_{29}H_{28}N_{12}$·2HBr (706.45): C, 49.31; H, 4.28; N, 23.79. Found: C, 49.29; H, 4.29; N, 23.80.

*2,2'-((2,8-di-p-tolyldiimidazo[1,2-a:1',2'-c]pyrimidine-3,9-diyl)bis(methaneylylidene))bis(hydrazine-1-carboximidamide) dihydrochloride (**5**)*
Yield 78%. ^{1}H-NMR (DMSO-d_6): 2.38 (3H, s, CH_3), 2.41 (3H, s, CH_3), 7.28 (2H, d, J=8.0, tol), 7.29 (1H, d, J=7.6, pym), 7.37 (2H, d, J=8.0, tol), 7.78 (2H, d, J=8.0, tol), 7.79 (8H, s broad, NH), 7.92 (2H, d, J=8.0, tol), 8.63 (1H, s, =CH), 9.28 (1H, d, J=7.6, pym), 9.53 (1H, s, =CH), 12.20 (1H, s, NNH), 12.53 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 20.94, 21.02, 103.44, 114.99, 117.82, 128.39, 128.53, 128.95, 129.00, 129.35, 129.52, 130.31, 138.41, 138.54, 138.75, 138.86, 139.59, 142.15, 145.92, 146.31, 154.69, 155.12. HRMS (ESI/Q-TOF): */z* calculated for $C_{26}H_{26}N_{12}$ $[M+H]^+$: 507.2482, found: 507.2476; calculated for $[M+2H]^{++}$: 254.1280, found: 254.1284. Anal. Calcd for $C_{26}H_{26}N_{12}$·2HCl (579.49): C, 53.89; H, 4.87; N, 29.01. Found: C, 53.91; H, 4.88; N, 28.99.

*3,9-bis((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2,8-di-p-tolyldiimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**6**)*
Yield 65%. ^{1}H-NMR (DMSO-d_6): 2.39 (3H, s, CH_3), 2.42 (3H, s, CH_3), 3.72 (4H, s, CH_2), 3.80 (4H, s, CH_2), 7.30 (2H, d, J=8.0, tol), 7.33 (1H, d, J=7.8, pym), 7.40 (2H, d, J=8.0, tol), 7.72 (2H, d, J=8.0, tol), 8.04 (2H, d, J=8.0, tol), 8.27 (2H, s, NH), 8.60 (1H, s, =CH), 8.70 (2H, s broad, NH), 9.27 (1H, d, J=7.8, pym), 9.47 (1H, s, =CH), 12.46 (2H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 20.92, 21.00,

42.81, 42.93, 103.52, 114.97, 117.43, 128.59, 128.95, 128.99, 129.44, 129.51, 129.89, 138.37, 138.96, 139.72, 139.79, 140.34, 142.10, 145.79, 146.56, 157.13, 157.75. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{30}H_{30}N_{12}$ $[M+H]^+$: 559.2795, found: 559.2793; calculated for $[M+2H]^{++}$: 280.1436, found: 280.1443. Anal. Calcd for $C_{30}H_{30}N_{12}$·2HBr (720.49): C, 49.31; H, 4.28; N, 23.79. Found: C, 49.29; H, 4.29; N, 23.80.

*2,2'-((8-(4-chlorophenyl)-2-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine-3,9-diyl)bis(methaneylylidene))bis(hydrazine-1-carboximidamide) dihydrochloride (**7**)*
Yield 70%. ^{1}H-NMR (DMSO-d_6): 7.29 (1H, d, J=7.8, pym), 7.56 (5H, m, ph+Cl-ph), 7.71 (8H, s broad, NH), 7.88 (2H, d, J=7.6, ph), 8.05 (2H, d, J=8.8, Cl-ph), 8.63 (1H, s, =CH), 9.29 (1H, d, J=7.8, pym), 9.57 (1H, s, =CH), 12.32 (2H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 103.51, 115.33, 118.32, 127.95, 128.56, 128.91, 129.07, 131.09, 131.69, 131.90, 133.43, 138.46, 138.53, 139.51, 142.14, 144.26, 146.04, 154.77, 155.19. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{24}H_{21}ClN_{12}$ $[M+H]^+$: 513.1779, found: 513.1776; calculated for $[M+2H]^{++}$: 257.0929, found: 257.0931. Anal. Calcd for $C_{24}H_{21}ClN_{12}$·2HCl (585.88): C, 49.20; H, 3.96; N, 28.69. Found: C, 49.18; H, 3.97; N, 28.71

*8-(4-Chlorophenyl)-3,9-bis((E)-(2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**8**)*
Yield 78%. ^{1}H-NMR (DMSO-d_6): 3.74 (4H, s, CH_2), 3.81 (4H, s, CH_2), 7.35 (1H, d, J=7.8, pym), 7.55 (5H, m, ph+Cl-ph), 7.85 (2H, d, J=7.2, ph), 8.17 (2H, d, J=8.4, Cl-ph), 8.37 (2H, s, NH), 8.65 (1H, s, =CH), 8.82 (2H, s broad, NH), 9.30 (1H, d, J=7.8, pym), 9.54 (1H, s, =CH), 12.34 (1H, s, NNH), 12.75 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 42.82, 42.95, 103.64, 115.28, 117.99, 128.05, 128.56, 128.96, 129.07, 129.24, 131.23, 131.50, 131.74, 133.55, 139.73, 140.23, 142.21, 144.36, 146.49, 157.00, 157.56. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{28}H_{25}ClN_{12}$ $[M+H]^+$: 565.2092, found: 565.2092; calculated for $[M+2H]^{++}$: 283.1085, found: 283.1090. Anal. Calcd for $C_{28}H_{25}ClN_{12}$·2HBr (726.87): C, 46.27; H, 3.74; N, 23.12. Found: C, 46.30; H, 3.73; N, 23.11.

*2,2'-((8-(4-chlorophenyl)-2-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine-3,9-diyl)bis(methaneylylidene))bis(hydrazine-1-carboximidamide) dihydrochloride (**9**)*
Yield 70%. ^{1}H-NMR (DMSO-d_6): 7.30 (1H, d, J=7.6, pym), 7.48 (3H, m, ph), 7.60 (2H, d, J=8.4, Cl-ph), 7.85 (8H, s broad, NH), 7.95 (2H, d, J=8.4, Cl-ph), 8.00 (2H, d, J=6.8, ph), 8.67 (1H, s, =CH), 9.27 (1H, d, J=7.6, pym), 9.57 (1H, s, =CH), 12.22 (1H, s, NNH), 12.64 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 103.63, 115.54, 118.14, 120.83, 127.94, 128.45, 128.91, 129.38, 130.57, 130.74, 133.02, 133.95, 138.29, 138.33, 139.53, 142.08, 144.51, 145.60, 154.75, 155.18. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{24}H_{21}ClN_{12}$ $[M+H]^+$: 513.1779, found: 513.1776; calculated for $[M+2H]^{++}$: 257.0929, found: 257.0930. Anal. Calcd for $C_{24}H_{21}ClN_{12}$·2HCl (585.88): C, 49.20; H, 3.96; N, 28.69. Found: C, 49.17; H, 3.95; N, 28.72.

*8-(4-Chlorophenyl)-3,9-bis((E)-(2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**10**)*
Yield 73%. ^{1}H-NMR (DMSO-d_6): 3.72 (4H, s, CH_2), 3.80 (4H, s, CH_2), 7.34 (1H, d, J=8.0, pym), 7.48 (3H, m, ph), 7.65 (2H, d, J=8.4, Cl-ph), 7.90 (2H, d, J=8.4, Cl-ph), 8.10 (2H, d, J=7.2, ph), 8.32 (2H, s, NH), 8.69 (1H, s, =CH), 8.85 (2H, s broad, NH), 9.28 (1H, d, J=8.0, pym), 9.50 (1H, s, =CH),

12.44 (1H, s, NNH), 12.84 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 42.82, 42.94, 103.77, 115.48, 117.72, 128.00, 128.46, 128.96, 129.51, 130.61, 130.72, 132.55, 134.07, 139.45, 139.68, 140.09, 142.07, 144.94, 145.60, 156.91, 157.59. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{28}H_{25}ClN_{12}$ $[M+H]^+$: 565.2092, found: 565.2089; calculated for $[M+2H]^{++}$: 283.1085, found: 283.1087. Anal. Calcd for $C_{28}H_{25}ClN_{12}$·2HBr (726.87): C, 46.27; H, 3.74; N, 23.12. Found: C, 46.31; H, 3.74; N, 23.10.

*2,2'-((2,8-bis(4-chlorophenyl)diimidazo[1,2-a:1',2'-c]pyrimidine-3,9-diyl)bis(methaneylylidene))bis(hydrazine-1-carboximidamide) dihydrochloride (**11**)*
Yield 95%. ^{1}H-NMR (DMSO-d_6): 7.32 (1H, d, J=7.6, pym), 7.54 (2H, d, J=8.4, Cl-ph), 7.62 (2H, d, J=8.4, Cl-ph), 7.82 (8H, s broad, NH), 7.94 (2H, d, J=8.4, Cl-ph), 8.08 (2H, d, J=8.4, Cl-ph), 8.64 (1H, s, =CH), 9.28 (1H, d, J=7.6, pym), 9.57 (1H, s, =CH), 12.00 (1H, s broad, NNH), 12.45 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 103.66, 115.57, 118.35, 127.98, 128.50, 128.91, 130.55, 130.73, 131.07, 131.85, 133.47, 133.95, 138.30, 138.41, 139.49, 142.13, 144.27, 144.48, 154.74, 155.17. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{24}H_{20}Cl_2N_{12}$ $[M+H]^+$: 547.1389, found: 547.1387; calculated for $[M+2H]^{++}$: 274.0733, found: 274.0725. Anal. Calcd for $C_{24}H_{20}Cl_2N_{12}$·2HCl (620.32): C, 46.47; H, 3.57; N, 27.10. Found: C, 46.50; H, 3.57; N, 27.11.

*2,8-bis(4-chlorophenyl)-3,9-bis((E)-(2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)diimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**12**)*
Yield 40%. ^{1}H-NMR (DMSO-d_6): 3.74 (4H, s, CH_2), 3.79 (4H, s, CH_2), 7.28 (1H, d, J=8.0, pym), 7.53 (2H, d, J=8.4, Cl-ph), 7.63 (2H, d, J=8.4, Cl-ph), 7.90 (2H, d, J=8.4, Cl-ph), 8.14 (2H, d, J=8.4, Cl-ph), 8.34 (2H, s, NH), 8.68 (1H, s, =CH), 8.90 (2H, s broad, NH), 9.24 (1H, d, J=8.0, pym), 9.53 (1H, s, =CH), 12.62 (2H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 42.82, 42.97, 103.73, 115.47, 117.98, 128.01, 128.53, 128.92, 130.52, 130.72, 131.17, 131.39, 133.51, 134.04, 139.31, 139.54, 140.04, 142.06, 144.28, 144.82, 156.89, 157.56. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{28}H_{24}Cl_2N_{12}$ $[M+H]^+$: 599.1702, found: 599.1713; calculated for $[M+2H]^{++}$: 300.0890, found: 300.0895. Anal. Calcd for $C_{28}H_{24}Cl_2N_{12}$·2HBr (761,31): C, 44.18; H, 3.44; N, 22.08. Found: C, 44.21; H, 3.45; N, 22.11.

*3,9-bis(((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2,8-di(thiophen-3-yl)diimidazo[1,2-a:1',2'-c]pyrimidine dihydrobromide (**13**)*
Yield 33%. ^{1}H-NMR (DMSO-d_6): 3.78, (4H, s, CH_2), 3.81 (4H, s, CH_2), 7.33 (1H, d, J=8.0, pym), 7.66 (1H, dd, J=5.2, J=2.8, th), 7.71 (1H, d, J=5.2, th), 7.81 (1H, d, J=5.2, th), 7.85 (1H, dd, J=5.2, J=2.8, th), 8.16 (1H, d, J=2.8, th), 8.52 (2H, s, NH), 8.57 (1H, d, J=2.8, th), 8.81 (1H, s, =CH), 8.90 (2H, s broad, NH), 9.25 (1H, d, J=8.0, pym), 9.68 (1H, s, =CH), 12.33 (1H, s broad, NNH), 12.80 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 42.83, 42.99, 103.40, 114.65, 117.23, 125.85, 126.09, 127.21, 127.64, 127.81, 128.47, 128.50, 133.00, 133.74, 139.54, 139.77, 140.89, 141.40, 142.10, 142.15, 156.98, 157.54. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{24}H_{22}N_{12}S_2$ $[M+H]^+$: 543.1610, found: 543.1615; calculated for $[M+2H]^{++}$: 272.0844, found: 272.0833. Anal. Calcd for $C_{24}H_{22}N_{12}S_2$·2HBr (704.47): C, 40.92; H, 3.43; N, 23.86. Found: C, 40.96; H, 3.44; N, 23.88.

*General procedure for the synthesis of the mono-hydrazones **14-18***

One equivalent (5 mmol) of aminoguanidine hydrochloride (obtained by treating aminoguanidine hydrogencarbonat, suspended in ethanol, with hydrochloridric acid in order to achieve a solution) or 2-hydrazino-2-imidazoline hydrobromide solubilized in ethanol, was added very slowly (over 48 hours) to the appropriate aldehyde (5 mmol) dissolved in CH_2Cl_2. Then, the reaction mixture was kept at room temperature for 24-48 h. The solution was concentrated under reduced pressure and the resulting precipitate was collected by filtration and crystallized from ethanol/ethyl ether.

*9-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2,8-di-p-tolyldiimidazo[1,2-a:1',2'-c]pyrimidine-3-carbaldehyde hydrobromide (**14**)*

Yield 20%. ^{1}H-NMR (DMSO-d_6): 2.38 (3H, s, CH_3), 2.43 (3H, s, CH_3), 3.73, (4H, s, CH_2), 7.30 (2H, d, J=8.0, tol), 7.42 (2H, d, J=8.0, tol), 7.50 (1H, d, J=7.8, pym), 7.92 (2H, d, J=8.4, tol), 8.03 (2H, d, J=8.4, tol), 8.36 (2H, s, NH), 9.10 (1H, d, J=7.8, pym), 9.45 (1H, s, =CH), 10.07 (1H, s, CHO), 12.72 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 20.98, 20.99, 42.92, 104.88, 117.61, 120.04, 126.23, 128.27, 128.60, 129.44, 129.50, 129.58, 129.73, 138.48, 140.07, 140.09, 140.51, 142.35, 146.01, 153.01, 157.85, 180.31. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{27}H_{24}N_8O$ $[M+H]^+$: 477.2151, found: 477.2150. Anal. Calcd for $C_{27}H_{24}N_8O{\cdot}HBr$ (557.46): C, 58.17; H, 4.52; N, 20.10. Found: C, 58.21; H, 4.52; N, 20.08.

*2-((3-formyl-2,8-diphenyldiimidazo[1,2-a:1',2'-c]pyrimidin-9-yl)methylene)hydrazine-1-carboximidamide hydrochloride (**15**)*

Yield 22%. ^{1}H-NMR (DMSO-d_6): 7.50 (4H, m, ph+pym), 7.52 (4H, s broad, NH), 7.60 (3H, m, ph), 8.01 (2H, m, ph), 8.06 (2H, m, ph), 9.10 (1H, d, J=7.6, pym), 9.54 (1H, s, =CH), 10.09 (1H, s, CHO), 12.47 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 105.07, 118.34, 120.20, 126.20, 127.96, 128.95, 129.01, 129.42, 129.67, 130.15, 130.98, 133.03, 138.13, 140.48, 142.40, 146.04, 152.74, 155.14, 180.43. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{23}H_{18}N_8O$ $[M+H]^+$: 423.1682, found: 423.1679. Anal. Calcd for $C_{23}H_{18}N_8O{\cdot}HCl$ (458.91): C, 60.20; H, 4.17; N, 24.42. Found: C, 60.18; H, 4.17; N, 24.43.

*(E)-2-((3-formyl-2,8-di(thiophen-3-yl)diimidazo[1,2-a:1',2'-c]pyrimidin-9-yl)methylene)hydrazine-1-carboximidamide hydrochloride (**16**)*

Yield 20%. ^{1}H-NMR (DMSO-d_6): 7.47 (1H, d, J=7.6, pym), 7.65 (1H, m, th),7.66 (4H, s broad, NH), 7.77 (1H, d, J=5.2, th), 7.81 (1H, m, th), 7.90 (1H, d, J=4.4, th), 8.47 (1H, d, J=1.6, th), 8.54 (1H, s, th), 9.08 (1H, d, J=7.6, pym), 9.65 (1H, s, =CH), 10.26 (1H, s, CHO), 12.52 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 104.68, 117.72, 119.63, 125.81, 126.34, 127.09, 127.65, 128.18, 128.43, 128.52, 132.37, 133.84, 138.97, 140.56, 141.54, 142.35, 147.83, 155.09, 179.86. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{19}H_{14}N_8OS_2$ $[M+H]^+$: 435.0810, found: 435.0807. Anal. Calcd for $C_{19}H_{14}N_8OS_2{\cdot}HCl$ (470.95): C, 48.46; H, 3.21; N, 23.79. Found: C, 48.50; H, 3.22; N, 23.81.

*2-((3-formyl-8-phenyl-2-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidin-9-yl)methylene)hydrazine-1-carboximidamide hydrochloride (**17**)*

Yield 23%. ^{1}H-NMR (DMSO-d_6): 2.42 (3H, s, CH_3), 7.40 (1H, d, J=7.6, pym), 7.50 (5H, m, ph+tol), 7.51 (4H, s broad, NH), 7.95 (2H, d, J=8.0, tol), 8.00 (2H, d, J=6.8, ph), 9.10 (1H, d, J=7.6, pym),

9.52 (1H, s, =CH), 10.07 (1H, s, CHO), 12.35 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 20.97, 104.93, 118.38, 120.02, 126.20, 127.96, 128.21, 128.93, 129.41, 129.55, 129.59, 133.09, 138.13, 140.06, 140.50, 142.42, 146.02, 152.93, 155.16, 180.34. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{24}H_{20}N_8O$ $[M+H]^+$: 437.1838, found: 437.1845. Anal. Calcd for $C_{24}H_{20}N_8O$·HCl (472.94): C, 60.95; H, 4.48; N, 23.69. Found: C, 61.00; H, 4.47; N, 23.70.

*2-((3-formyl-2,8-di-p-tolyldiimidazo[1,2-a:1',2'-c]pyrimidin-9-yl)methylene)hydrazine-1-carboximidamide hydrochloride (**18**)*
Yield 25%. ^{1}H-NMR (DMSO-d_6): 2.35 (3H, s, CH_3), 2.39 (3H, s, CH_3), 7.31 (2H, d, J=8.0, tol), 7.41 (2H, d, J=8.0, tol), 7.46 (4H, s boad, NH), 7.48 (1H, d, J=7.8, pym), 7.92 (2H, d, J=8.0, tol), 7.94 (2H, d, J=8.0, tol), 9.10 (1H, d, J=7.8, pym), 9.51 (1H, s, =CH), 10.08 (1H, s, CHO), 12.32 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 20.98, 21.00, 104.91, 118.26, 119.99, 126.07, 128.23, 128.51, 129.33, 129.55, 129.60, 130.24, 138.06, 138.46, 140.05, 140.53, 142.31, 146.01, 152.95, 155.38, 180.31. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{25}H_{22}N_8O$ $[M+H]^+$: 451.1995, found: 451.1992. Anal. Calcd for $C_{25}H_{22}N_8O$·HCl (486.96): C, 61.66; H, 4.76; N, 23.01. Found: C, 61.70; H, 4.77; N, 22.99.

*General procedure for the synthesis of the alcohols **19-20***
The appropriate mono-hydrazone (**FIM** or **15**, 5 mmol) was treated with $NaBH_4$ (15 mmol) in methanol 20 mL. The reaction mixture was refluxed for 25 h. After cooling, the precipitate was filtered, suspended in ethanol and treated with HBr 48% to achieve the hydrobromide of compound **19**, or with HCl 36% to achieve the hydrochloride of compound **20**. The crude compounds were crystallized from ethanol/ethyl ether.

*(9-((2-(4,5-dihydro-1H-imidazol-2-yl)hydrazineylidene)methyl)-2,8-diphenyldiimidazo[1,2-a:1',2'-c]pyrimidin-3-yl)methanol hydrobromide (**19**)*
Yield 45%. ^{1}H-NMR (DMSO-d_6): 3.71 (4H, s, CH_2), 4.91 (2H, d, J=4.0, $\underline{CH_2}$OH), 5.63 (1H, t, J=4.0, OH), 7.35 (1H, d, J=7.6, pym), 7.48 (6H, m, ph), 7.90 (2H, d, J=7.6, ph), 8.12 (2H, d, J=7.6, ph), 8.22 (2H, s broad, NH), 8.39 (1H, d, J=7.6, pym), 9.56 (1H, s, =CH), 12.69 (1H, s broad, NNH). ^{13}C-NMR (DMSO-d_6): 42.89, 51.82, 102.49, 117.80, 121.69, 125.41, 127.86, 127.92, 128.66, 129.41, 132.95, 133.07, 137.12, 137.96, 140.38, 142.00, 145.06, 158.04. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{25}H_{22}N_8O$ $[M+H]^+$: 451.1995, found: 451.1996. Anal. Calcd for $C_{25}H_{22}N_8O$·HBr (531.42): C, 56.50; H, 4.36; N, 21.09. Found: C, 56.48; H, 4.36; N, 21.07.

*2-((3-(hydroxymethyl)-2,8-diphenyldiimidazo[1,2-a:1',2'-c]pyrimidin-9-yl)methylene)hydrazine-1-carboximidamide hydrochloride (**20**)*
Yield 40%. ^{1}H-NMR (DMSO-d_6): 4.78 (1H, s broad, OH), 4.91 (2H, s, CH_2), 7.34 (1H, d, J=7.6, pym), 7.48 (6H, m, ph), 7.55 (4H, s broad, NH), 7.94 (2H, d, J=7.2, ph), 8.01 (2H, d, J=6.8, ph), 8.45 (1H, d, J=7.6, pym), 9.62 (1H, s, =CH), 12.50 (1H, s, NNH). ^{13}C-NMR (DMSO-d_6): 51.77, 102.23, 118.09, 121.72, 125.69, 127.87, 127.92, 128.68, 128.80, 129.41, 132.97, 133.11, 136.98, 137.99, 138.52, 141.91, 144.84, 155.22. HRMS (ESI/Q-TOF): *m/z* calculated for $C_{23}H_{20}N_8O$ $[M+H]^+$:

425.1838, found: 425.1839. Anal. Calcd for $C_{23}H_{20}N_8O \cdot HCl$ (460.93): C, 59.93; H, 4.59; N, 24.31. Found: C, 59.89; H, 4.59; N, 24.32.

*General procedure for the synthesis of compounds **23, 24***

2,4-Diaminopyrimidine **21** (10 mmol) was dissolved in 40 mL of acetone and treated with the appropriate 2-bromo-1-(phenyl)ethan-1-one (15 mmol). The reaction mixture was refluxed for 4 h. The resulting precipitate was collected by filtration and treated with HCl 2N 200 mL under reflux for 3 h. Before complete cooling, the solution was cautiously basified by dropwise addition of 15% NH_4OH. The resulting precipitate was collected by filtration and crystallized from ethanol.

*2-(p-tolyl)imidazo[1,2-a]pyrimidin-7-amine (**23**)*

Yield 93%. ^{1}H-NMR (DMSO-d_6): 2.31 (3H, s, CH_3), 6.28 (1H, d, J= 7.2, pym), 6.89 (2H, s, NH_2), 7.20 (2H, d, J=8.2, tol), 7.71 (2H, d, J=8.2, tol), 7.79 (1H, s, im), 8.35 (1H, d, J= 7.2, pym). ^{13}C-NMR (DMSO-d_6): 20.83, 100.22, 105.11, 124.94, 129.11, 130.97, 134.50, 136.41, 140.91, 148.99, 158.36. ESI MS (m/z): calculated for $C_{13}H_{12}N_4$ $[M+H]^+$: 225.11, found 225.31.

*2-(4-Chlorophenyl)imidazo[1,2-a]pyrimidin-7-amine (**24**)*

Yield 97%. ^{1}H-NMR (DMSO-d_6): 6.28 (1H, d, J=7.4, pym), 6.86 (2H, s, NH_2), 7.43 (2H, d, J=8.4, Cl-ph), 7.85 (2H, d, J=8.4, Cl-ph), 7.87 (1H, s, im), 8.36 (1H, d, J=7.4, pym). ^{13}C-NMR (DMSO-d_6): 100.27, 105.91, 126.61, 128.49, 131.27, 133.35, 134.49, 140.59, 149.43, 158.27. ESI MS (m/z): calculated for $C_{12}H_9ClN_4$ $[M+H]^+$: 245.06, found 245.12.

*General procedure for the synthesis of compounds **26-31***

The appropriate imidazopyrimidine (**22**-**25**, 10 mmol) was dissolved in 40 mL of ethanol and treated with the proper 2-bromo-1-(phenyl)ethan-1-one (15 mmol). The reaction mixture was refluxed for 6-12 h according to a TLC test. The resulting precipitate was collected by filtration and treated with HCl 2N 100 mL under reflux for 3 h. Before complete cooling, the solution was cautiously basified by dropwise addition of 15% NH_4OH. The resulting precipitate was collected by filtration and crystallized from ethanol.

*2-Phenyl-8-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine (**26**)*

Yield 18%.^{1}H-NMR (DMSO-d_6): 2.34 (3H, s, CH_3), 7.19 (1H, d, J= 7.8, pym), 7.25 (2H, d, J=8.2, tol), 7.33 (1H, t, J=7.4, ph), 7.47 (2H, t, J=7.4 , ph), 7.93 (2H, d, J=7.4, ph), 7.96 (2H, d, J=8.2, tol), 8.24 (1H, s, im), 8.29 (1H, d, J= 7.8, pym), 8.66 (1H, s, im). ^{13}C-NMR (DMSO-d_6): 20.87, 102.73, 107.01, 109.89, 125.01, 125.09, 125.49, 127.57, 128.78, 129.28, 130.36, 133.07, 137.09, 137.30, 139.82, 141.48, 144.34. ESI MS (m/z): calculated for $C_{21}H_{16}N_4$ $[M+H]^+$: 325.15, found 325.23.

*8-Phenyl-2-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine (**27**)*

Yield 22%. ^{1}H-NMR (DMSO-d_6): 2.34 (3H, s, CH_3), 7.19 (1H, d, J= 7.4, pym), 7.27 (2H, d, J=8,2, tol), 7.33 (1H, t, J=7.6, ph), 7.45 (2H, t, J=7.6 , ph), 7.82 (2H, d, J=8.2, tol), 8.08 (2H, d, J=7.6, ph), 8.18 (1H, s, im), 8.28 (1H, d, J= 7.4, pym), 8.73 (1H, s, im). ^{13}C-NMR (DMSO-d_6): 20.85, 102.61,

107.49, 109.38, 124.96, 125.20, 125.53, 127.77, 128.70, 129.34, 130.29, 133.14, 136.87, 137.18, 139.95, 141.58, 144.17. ESI MS (m/z): calculated for $C_{21}H_{16}N_4$ $[M+H]^+$: 325.15, found 325.23.

*2,8-Di-p-tolyldiimidazo[1,2-a:1',2'-c]pyrimidine (**28**)*
Yield 20%. ^{1}H-NMR (DMSO-d_6): 2.34 (6H, s, 2xCH_3), 7.17 (1H, d, J=7.8, pym), 7.26 (2H, d, J=8.0, tol), 7.27 (2H, d, J=8.0, tol), 7.82 (2H, d, J=8.0, tol), 7.96 (2H, d, J=8.0, tol), 8.17 (1H, s, im), 8.27 (1H, d, J= 7.8, pym), 8.66 (1H, s, im). ^{13}C-NMR (DMSO-d_6): 20.85, 20.87, 102.58, 106.97, 109.34, 124.96, 125.07, 125.48, 129.28, 129.35, 130.31, 130.39, 136.86, 137.08, 137.20, 139.94, 141.47, 144.31. ESI MS (m/z): calculated for $C_{22}H_{18}N_4$ $[M+H]^+$: 339.16, found 339.33.

*8-(4-Chlorophenyl)-2-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine (**29**)*
Yield 15%. ^{1}H-NMR (DMSO-d_6): 7.20 (1H, d, J=7.8, pym), 7.33 (1H, t, J=7.4, ph), 7.35 (2H, t, J=7.4, ph), 7.50 (2H, d, J=8.4, Cl-ph), 7.93 (2H, d, J=7.4, ph), 8.10 (2H, d, J=8.4, Cl-ph), 8,25 (1H, s, im), 8.31 (1H, d, J=7.8, pym), 8.81 (1H, s, im). ^{13}C-NMR (DMSO-d_6): 102.70, 108.06, 110.00, 125.00, 125.46, 127.23, 127.60, 128.73, 128.79, 132.08, 132.20, 133.02, 137.23, 139.86, 141.69, 142.99. ESI MS (m/z): calculated for $C_{20}H_{13}ClN_4$ $[M+H]^+$: 345.09, found 345.23.

*2-(4-Chlorophenyl)-8-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine (**30**)*
Yield 18%. ^{1}H-NMR (DMSO-d_6): 7.22 (1H, d, J=7.6, pym), 7.34 (1H, t, J=7.6, ph), 7.47 (2H, t, J=7.6, ph), 7.54 (2H, d, J=8.8, Cl-ph), 7.95 (2H, d, J=8.8, Cl-ph), 8.08 (2H, d, J=7.6, ph), 8.29 (1H, s, im), 8.30 (1H, d, J=7.6, pym), 8.74 (1H, s, im). ^{13}C-NMR (DMSO-d_6): 102.93, 107.57, 110.40, 125.22, 125.53, 126.68, 127.80, 128.70, 128.82, 131.93, 132.00, 133.08, 137.37, 138.65, 141.57, 144.24. ESI MS (m/z): calculated for $C_{20}H_{13}ClN_4$ $[M+H]^+$: 345.09, found 345.23.

*2,8-Bis(4-chlorophenyl)diimidazo[1,2-a:1',2'-c]pyrimidine (**31**)*
Yield 22%. ^{1}H-NMR (DMSO-d_6): 7.20 (1H, d, J=7.6, pym), 7.50 (2H, d, J=8.4, Cl-ph), 7.53 (2H, d, J=8.4, Cl-ph), 7.94 (2H, d, J=8.4, Cl-ph), 8.09 (2H, d, J=8.4, Cl-ph), 8.28 (1H, s, im), 8.30 (1H, d, J=7.6, pym), 8.80 (1H, s, im). ^{13}C-NMR (DMSO-d_6): 102.87, 108.08, 110.45, 125.44, 126.67, 127.21, 128.72, 128.83, 131.95, 132.03, 132.22, 137.31, 138.68, 141.67, 143.02. ESI MS (m/z): calculated for $C_{20}H_{12}Cl_2N_4$ $[M+H]^+$: 379.05, found 379.24.

*General procedure for the synthesis of the aldehydes **34-39***
The Vilsmeier reagent was prepared at 0-5°C by dropping $POCl_3$ (54 mmol) into a stirred solution of DMF (65 mmol) in $CHCl_3$ (5 mL). The appropriate starting compound (**26-31**, 5 mmol) was suspended in $CHCl_3$ (20 mL). The mixture thus obtained was dropped into the Vilsmeier reagent while maintaining stirring and cooling. The reaction mixture was kept for 3 h at room temperature and under reflux for 20-40 h (according to a TLC test). Chloroform was removed under reduced pressure, the resulting oil was poured onto ice and the precipitate thus obtained was collected by filtration. The crude aldehydes were crystallized from ethanol.

*2-Phenyl-8-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine-3,9-dicarbaldehyde (**34**)*

Yield 90%. ^{1}H-NMR ($CDCl_3$): 2.44 (3H, s, CH_3), 7.32 (2H, d, J=8.0, tol), 7.36 (1H, d, J=7.2, pym), 7.58 (3H, m, ph), 7.88 (2H, m, ph), 8.15 (2H, d, J=8.0, tol), 9.34 (1H, d, J=7.2, pym), 10.15 (1H, s, CHO), 11.51 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): 21.73, 105.09, 120.72, 124.17, 127.88, 128.93, 129.12, 129.33, 129.87, 130.01, 130.83, 130.97, 140.58, 140.94, 143.80, 153.22, 155.36, 180.37, 181.42. ESI MS (m/z): calculated for $C_{23}H_{16}N_4O_2$ $[M+H]^+$: 381.14, found 381.34.

*8-Phenyl-2-(p-tolyl)diimidazo[1,2-a:1',2'-c]pyrimidine-3,9-dicarbaldehyde (**35**)*
Yield 92%. ^{1}H-NMR ($CDCl_3$): 2.48 (3H, s, CH_3), 7.36 (1H, d, J=7.6, pym), 7.39 (2H, d, J=8.0, tol), 7.52 (3H, m, ph), 7.78 (2H, d, J=8.0, tol), 8.23 (2H, m, ph), 9.35 (1H, d, J=7.6, pym), 10.15 (1H, s, CHO), 11.53 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): 21.65, 104.92, 120.57, 124.33, 128.04, 128.12, 128.39, 129.79, 130.08, 130.11, 130.59, 131.80, 140.55, 141.41, 143.82, 152.99, 155.59, 180.42, 181.51. ESI MS (m/z): calculated for $C_{23}H_{16}N_4O_2$ $[M+H]^+$: 381.14, found 381.34.

*2,8-Di-p-tolyldiimidazo[1,2-a:1',2'-c]pyrimidine-3,9-dicarbaldehyde (**36**)*
Yield 95%. ^{1}H-NMR ($CDCl_3$): 2.44 (3H, s, CH_3), 2.47 (3H, s, CH_3), 7.32 (2H, d, J=8.0, tol), 7.36 (1H, d, J=7.8, pym), 7.37 (2H, d, J=8.0, tol), 7.77 (2H, d, J=8.0, tol), 8.15 (2H, d, J=8.0, tol), 9.33 (1H, d, J=7.8, pym), 10.14 (1H, s, CHO), 11.51 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): 21.65, 21.74, 104.91, 120.54, 124.17, 127.96, 128.16, 128.98, 129.13, 129.78, 130.03, 130.07, 140.59, 140.93, 141.37, 143.84, 153.22, 156.59, 180.39, 181.52. ESI MS (m/z): calculated for $C_{24}H_{18}N_4O_2$ $[M+H]^+$: 395.15, found 395.34.

*8-(4-Chlorophenyl)-2-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine-3,9-dicarbaldehyde (**37**)*
Yield 88%. ^{1}H-NMR ($CDCl_3$): 7.35 (1H, d, J=7.8, pym), 7.47 (2H, d, J=8.4, Cl-ph), 7.59 (3H, m, ph), 7.88 (2H, m, ph), 8.24 (2H, d, J=8.4, Cl-ph), 9.35 (1H, d, J=7.8, pym), 10.15 (1H, s, CHO), 11.52 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): 105.06, 120.78, 124.41, 128.13, 128.64, 129.37, 129.88, 130.27, 130.88, 130.92, 131.46, 136.70, 140.47, 143.79, 151.73, 155.38, 180.44, 181.56. ESI MS (m/z): calculated for $C_{22}H_{13}ClN_4O_2$ $[M+H]^+$: 401.08, found 401.24.

*2-(4-Chlorophenyl)-8-phenyldiimidazo[1,2-a:1',2'-c]pyrimidine-3,9-dicarbaldehyde (**38**)*
Yield 93%. ^{1}H-NMR ($CDCl_3$):7.38 (1H, d, J=7.6, pym), 7.52 (3H, m, ph), 7.56 (2H, d, J=8.2, Cl-ph), 7.83 (2H, d, J=8.2, Cl-ph), 8.23 (2H, m, ph), 9.33 (1H, d, J=7.6, pym), 10.13 (1H, s, CHO), 11.48 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): 105.36, 120.79, 124.33, 127.87, 128.41, 129.41, 129.68, 130.10, 130.64, 131.00, 131.74, 137.36, 140.58, 143.78, 153.22, 153.97, 179.89, 181.30. ESI MS (m/z): calculated for $C_{22}H_{13}ClN_4O_2$ $[M+H]^+$: 401.08, found 401.24.

*2,8-Bis(4-chlorophenyl)diimidazo[1,2-a:1',2'-c]pyrimidine-3,9-dicarbaldehyde (**39**)*
Yield 90%. ^{1}H-NMR ($CDCl_3$-d_6): 7.38 (1H, d, J=8.0, pym), 7.49 (2H, d, J=8.4, Cl-ph), 7.57 (2H, d, J=8.4, Cl-ph), 7.83 (2H, d, J=8.4, Cl-ph), 8.25 (2H, d, J=8.4, Cl-ph), 9.35 (1H, d, J=8.0, pym), 10.14 (1H, s, CHO), 11.51 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): 105.30, 120.78, 124.37, 128.06, 128.68, 129.06, 129.72, 130.99, 131.48, 136.77, 137.87, 137.91, 140.52, 143.79, 153.90, 155.48, 179.92, 181.44. ESI MS (m/z): calculated for $C_{22}H_{12}Cl_2N_4O_2$ $[M+H]^+$: 435.04, found 435.15.

^{1}H and ^{13}C NMR spectra are reported below.

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(3) Kipnis, F.; Soloway, H.; Ornfelt, J. 2-Acyloxyacetylthiophenes. *J. Am. Chem. Soc.* **1949**, *71*, 10-11.

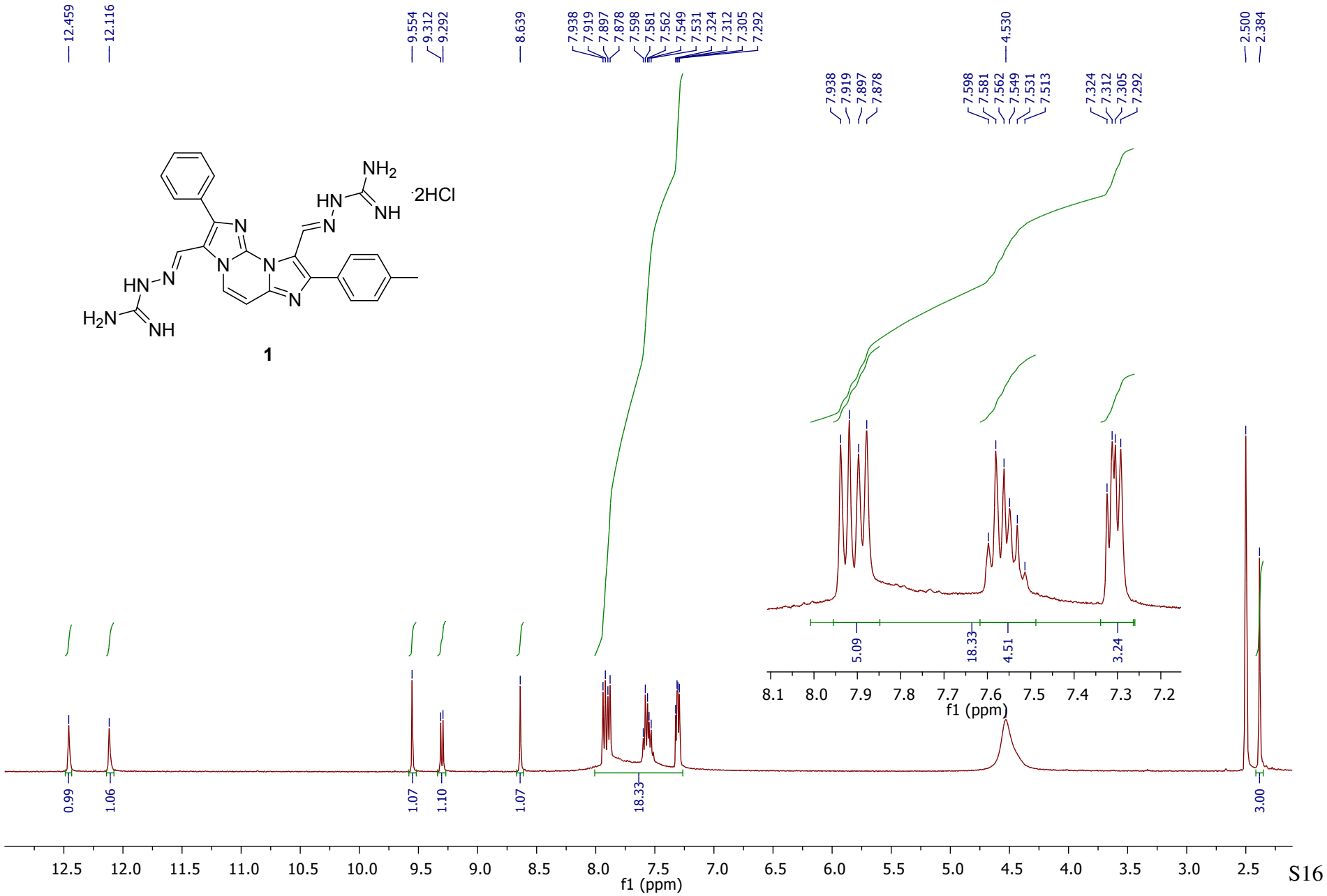
(4) Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Giorgi, G.; Garaliene, V. Potential Antitumor Agents. 34.(1) Synthesis and Antitumor Activity of Guanylhydrazones from Imidazo[2,1-b]Thiazoles and from Diimidazo[1,2-a:1,2-c]Pyrimidine. *Anticancer Res.* **2004**, *24*, 203–212.

NMR spectrometer characteristics

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian MR 400 MHz (ATB PFG probe); the acquisition software was Vnmrj 3.2A; field strength used: 400 MHz ^{1}H, 100 MHz ^{13}C; the chemical shifts were expressed in δ (ppm) and referenced to the residual peak of the solvent as the internal standard (DMSO-d_6: δ H = 2.50 ppm; δ C = 39.52 ppm and $CDCl_3$: δ H = 7.26 ppm; δ C = 77.16 ppm). Data are reported below for the indicated compound.

UHPLC system and conditions

UHPLC analysis were run on a Waters ACQUITY ARC UHPLC/ system consisting of a 2489 UV/Vis detector. Detected wavelength (λ): 254 nm. The analyses were performed on an XBridge BEH C18 column (10 × 2.1 mm i.d., particle size 2.5 μm) with a XBridge BEH C18 VanGuard Cartridge precolumn (5 mm × 2.1 mm i.d., particle size 1.8 μm). The mobile phases were H_2O (0.1% trifluoroacetic acid) (A) and MeOH (0.1% trifuoroacetic acid) (B). Method and gradients used were the following: Linear gradient: 0−3.5 min, 95% A; 3.5 min-4.5 min 30% A – 4.5-7.0 95%A - 5. Flow rate: 0.6 mL/min. UHPLC traces of compounds 1, 2, 8, 10, 15, 19 and 20 are reported from page S56.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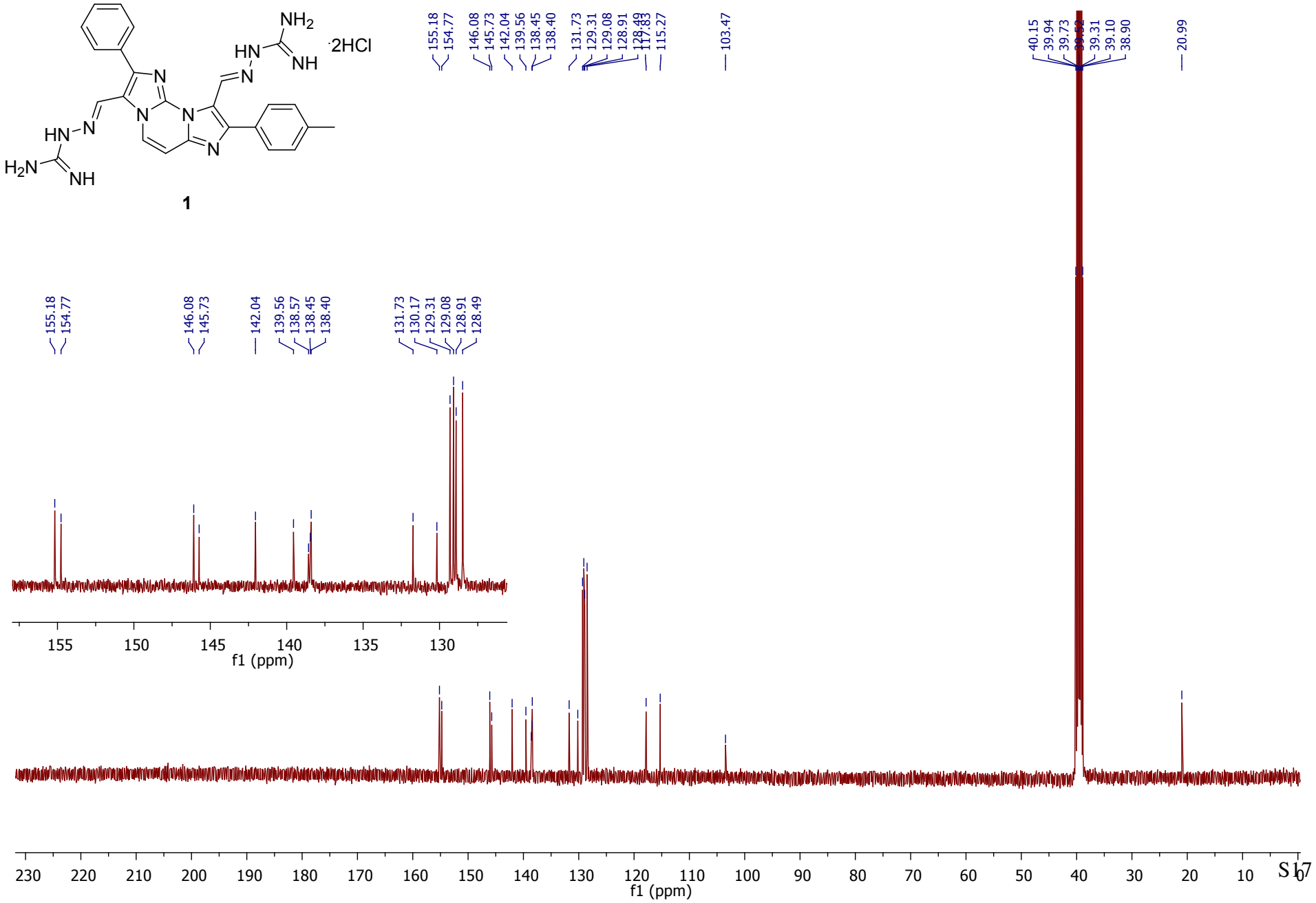
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128.91
128.49
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135
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210
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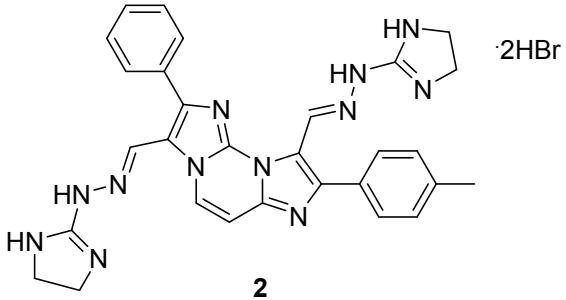
f1 (ppm)



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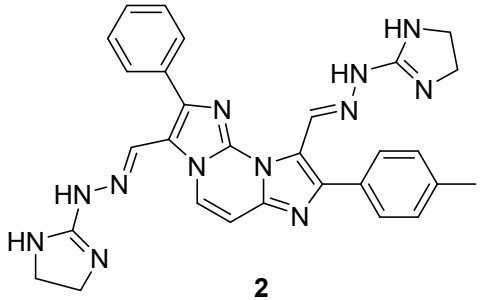
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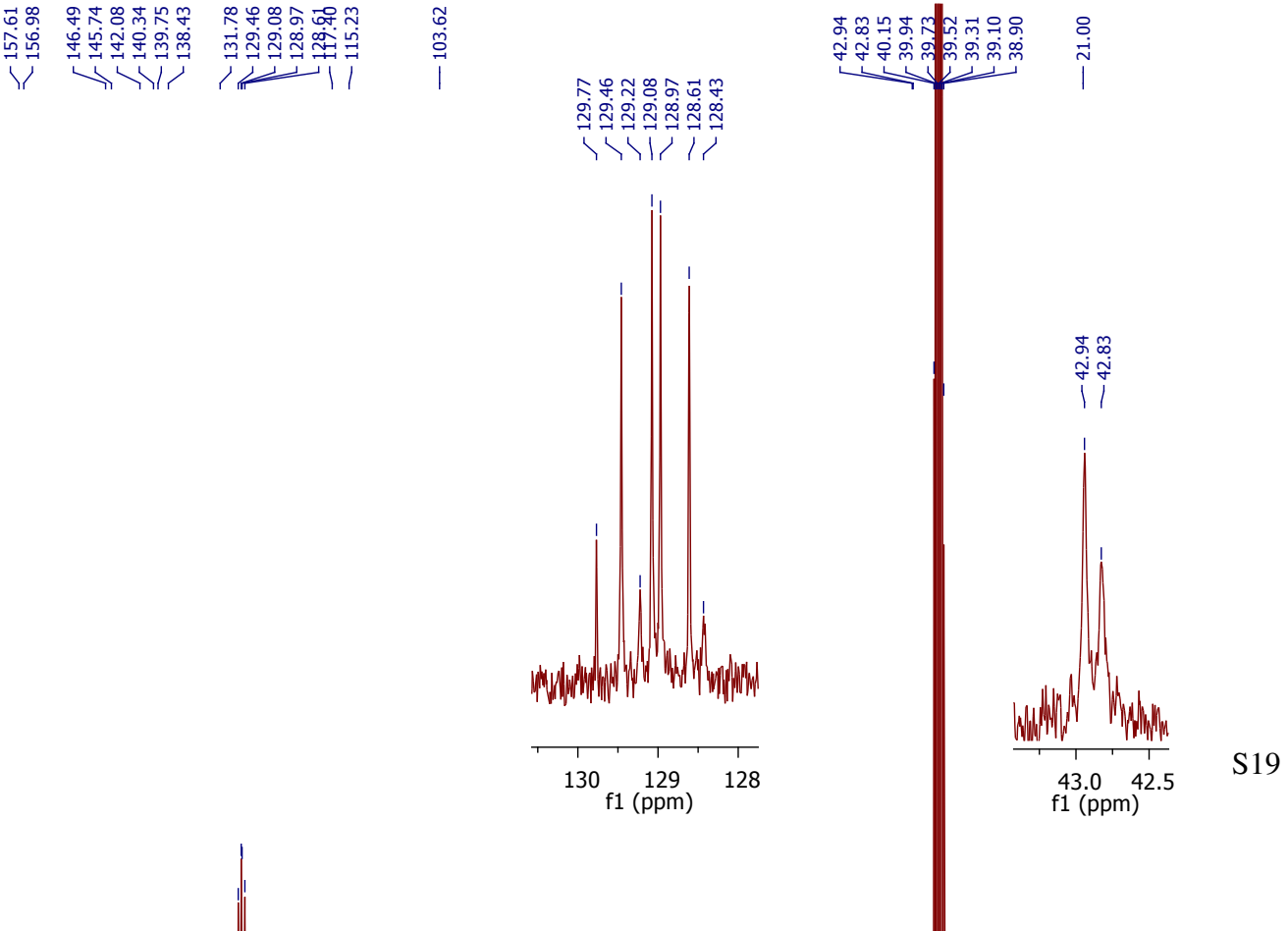
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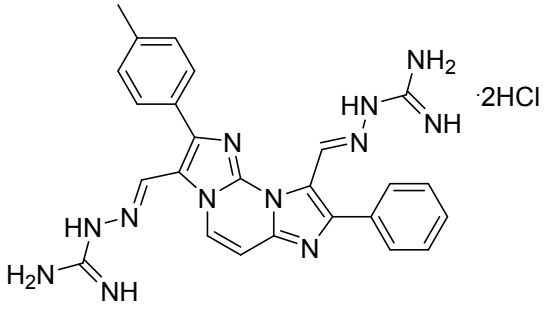
f1 (ppm)



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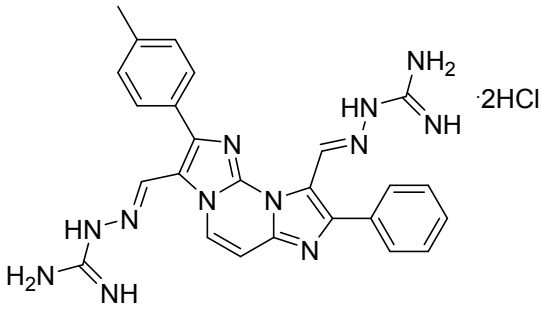
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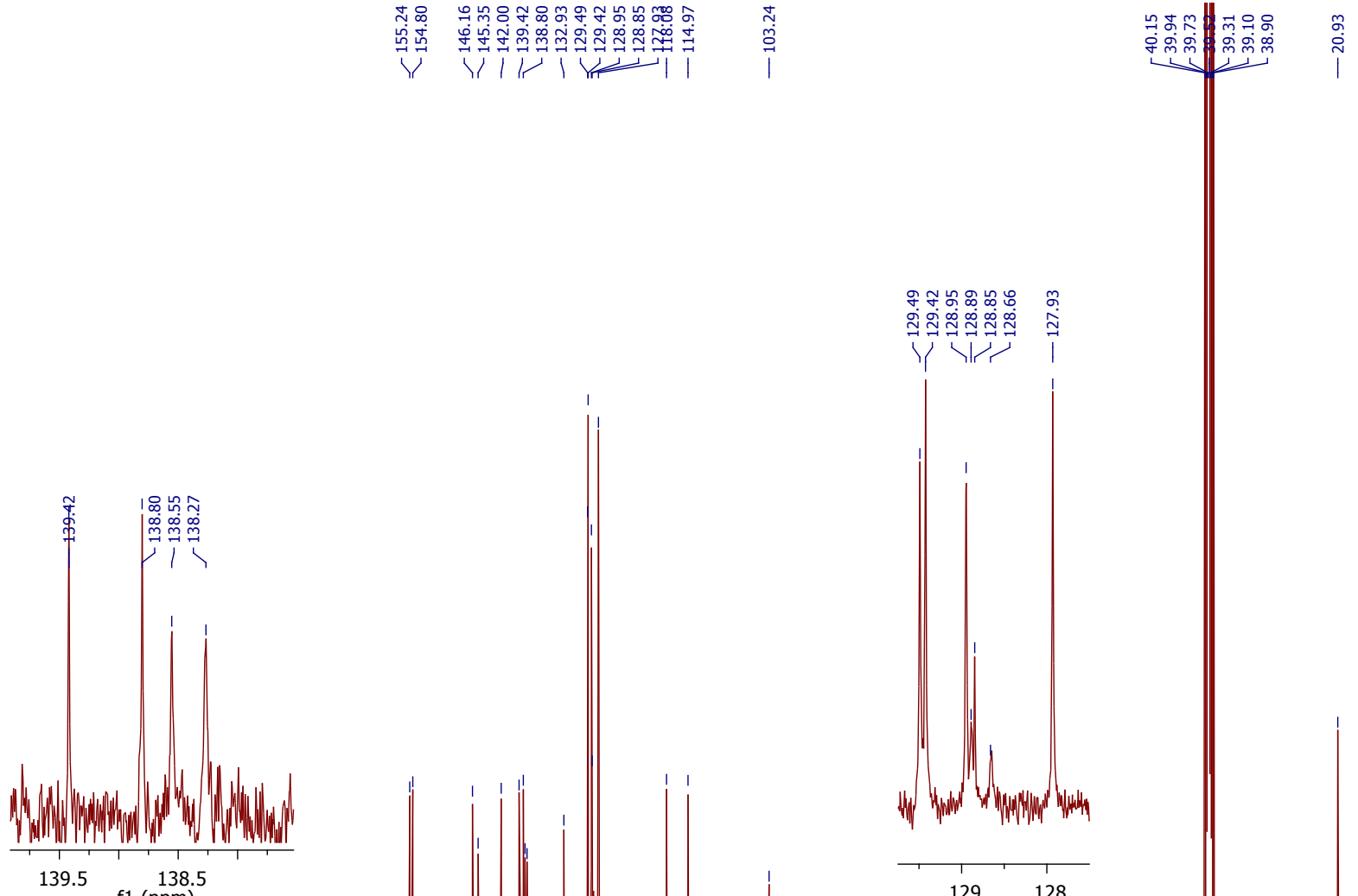
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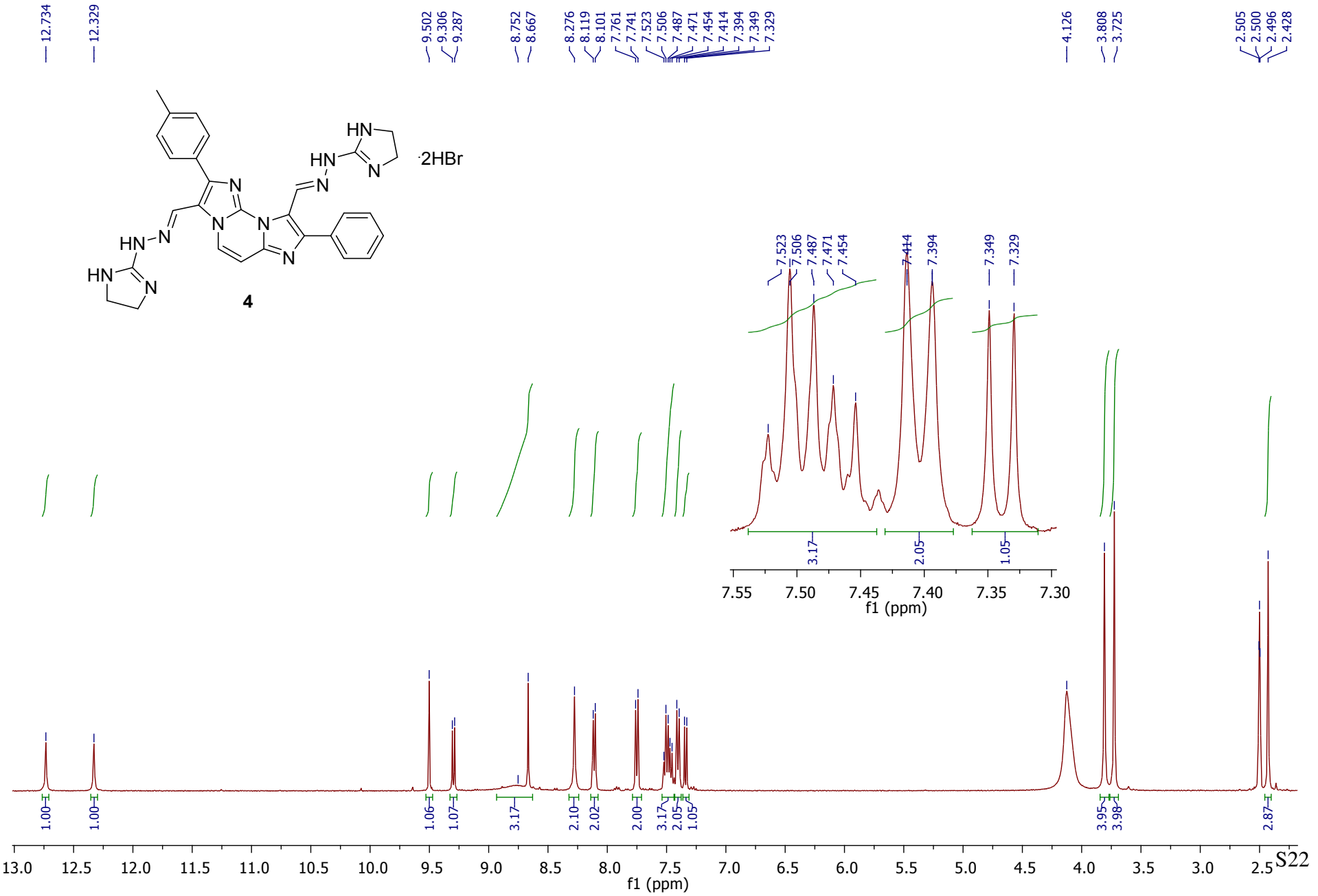
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HN
N
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7.50
7.45
7.40
7.35
7.30
f1 (ppm)
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2.02
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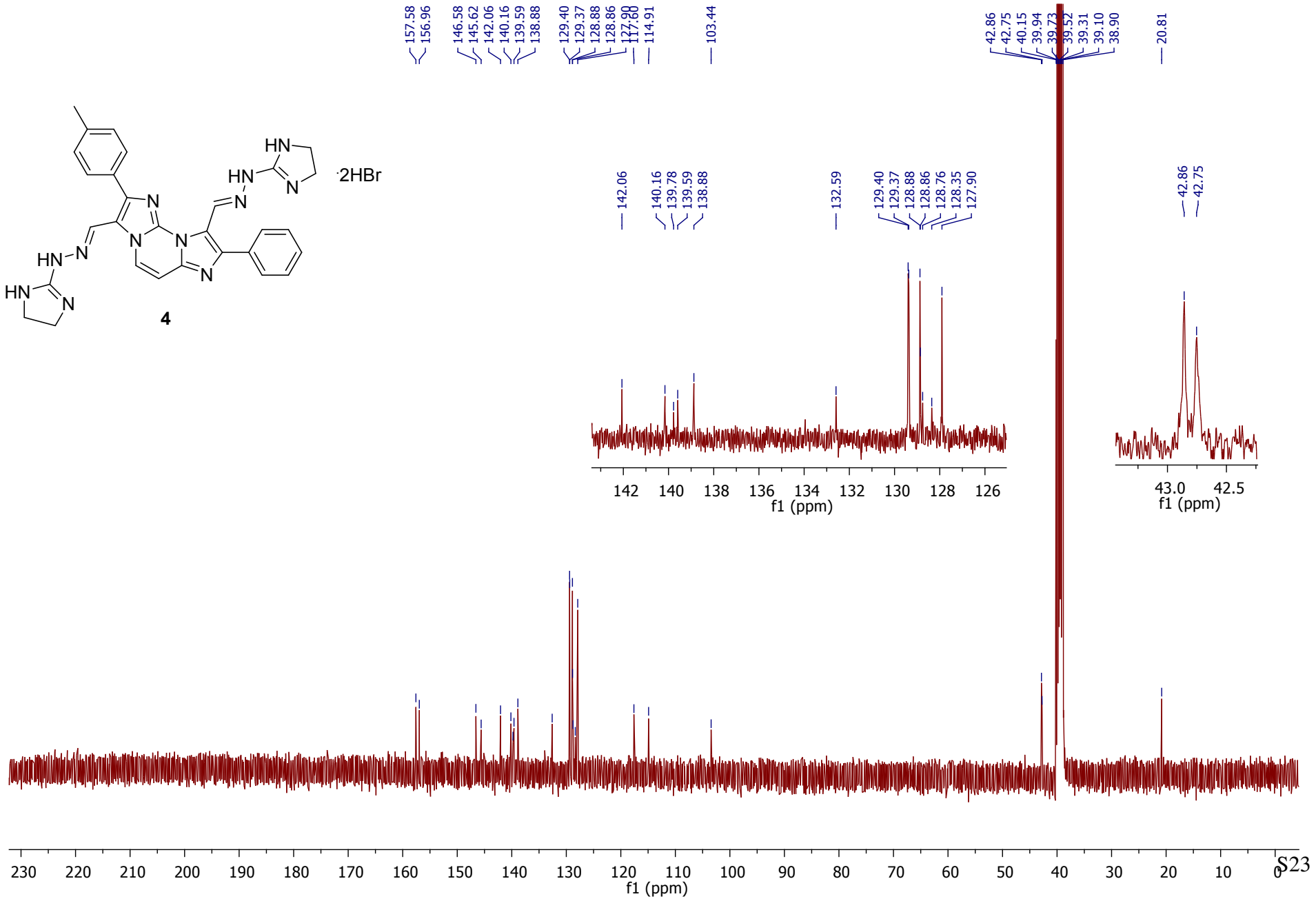
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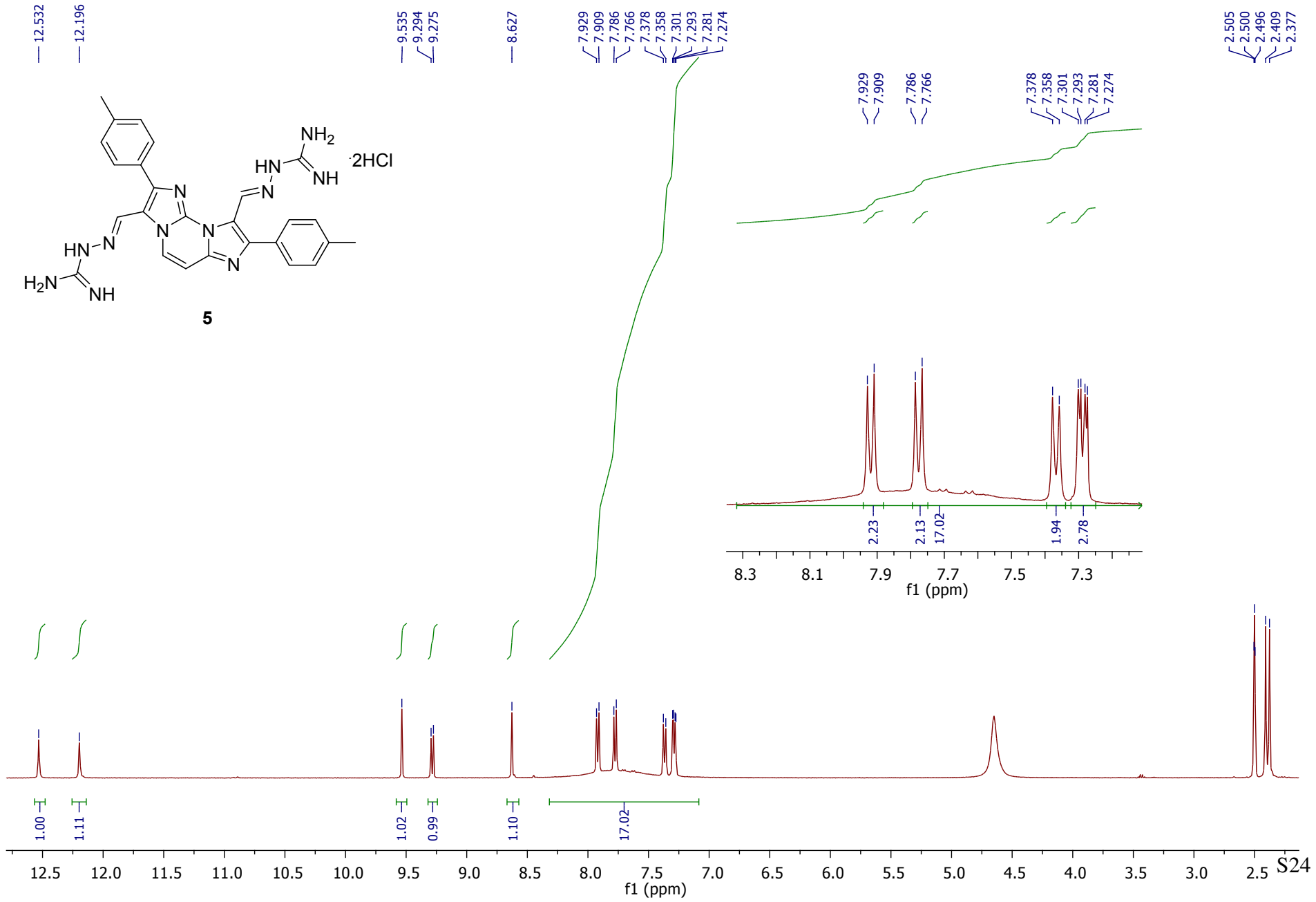
129.37
128.88
128.86
127.90
117.60
114.91
103.44
42.86
42.75
40.15
39.94
39.73
39.52
39.31
39.10
38.90
20.81
HN
HN
N
N
HN
N
N
N
N
HN
N
·2HBr
4
142.06
140.16
139.78
139.59
138.88
132.59
129.40
129.37
128.88
128.86
128.76
128.35
127.90
42.86
42.75
142 140 138 136 134 132 130 128 126
f1 (ppm)
43.0 42.5
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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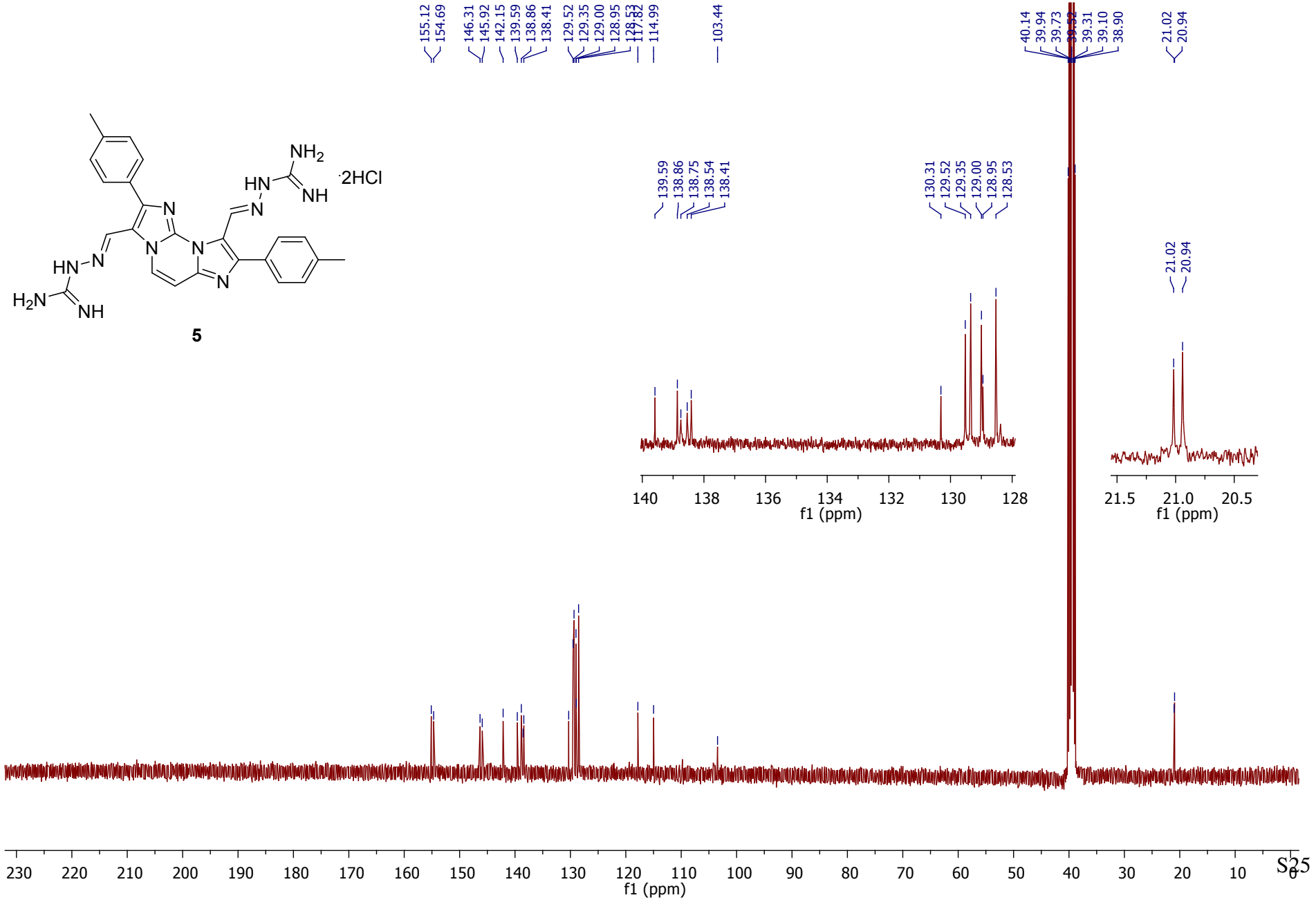
12.196
9.535
9.294
9.275
8.627
7.929
7.909
7.786
7.766
7.378
7.358
7.301
7.293
7.281
7.274
2.505
2.500
2.496
2.409
2.377
NH2
HN
NH
·2HCl
N
HN
N
H2N
NH
5
2.23
2.13
17.02
1.94
2.78
8.3
8.1
7.9
7.7
7.5
7.3
f1 (ppm)
1.00
1.11
1.02
0.99
1.10
17.02
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)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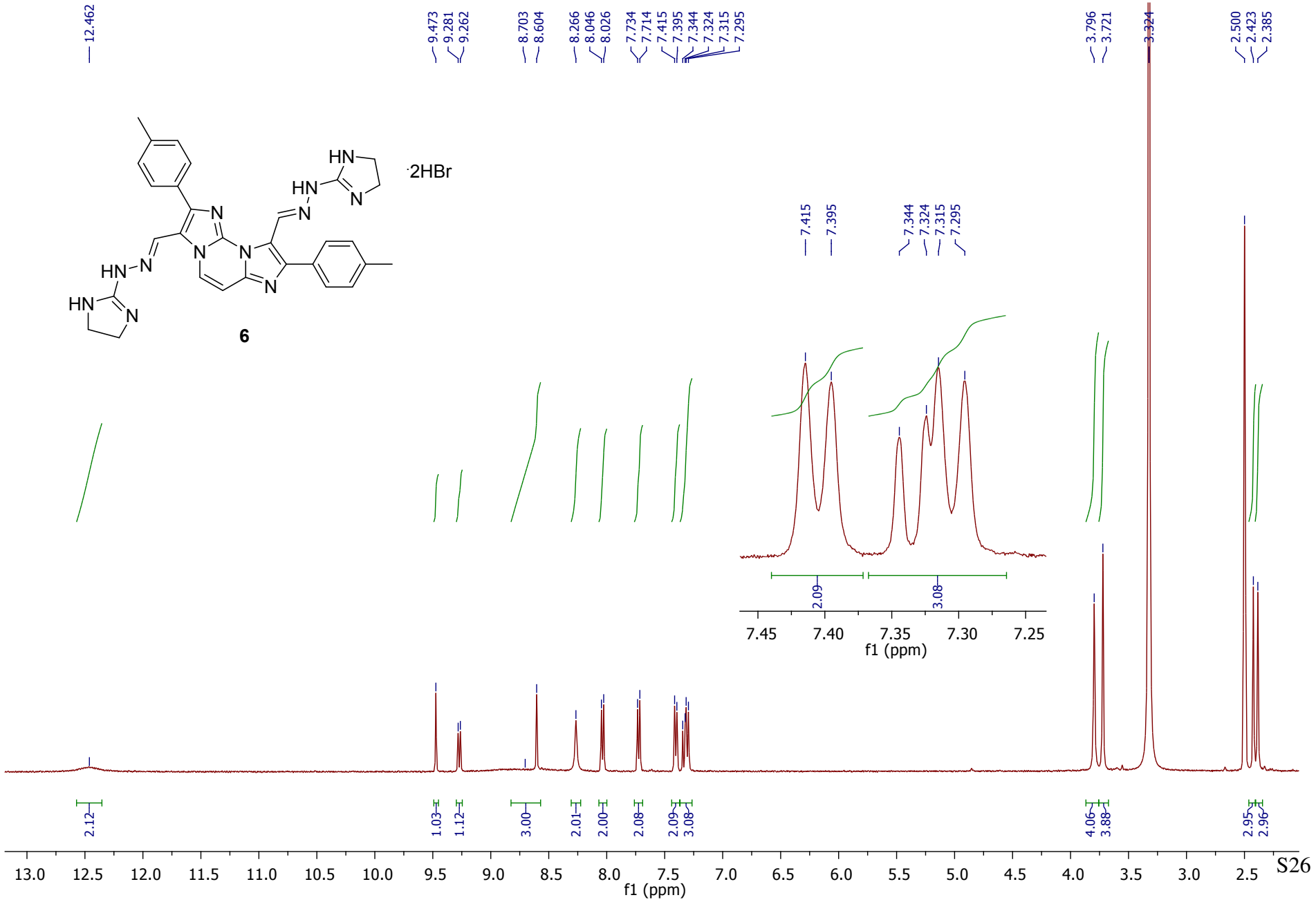
154.69
146.31
145.92
142.15
139.59
138.86
138.41
129.52
129.35
129.00
128.95
114.99
103.44
40.14
39.94
39.73
39.31
39.10
38.90
21.02
20.94
NH2
HN
NH
·2HCl
N
HN
H2N
NH
5
139.59
138.86
138.75
138.54
138.41
130.31
129.52
129.35
129.00
128.95
128.53
140
138
136
134
132
130
128
f1 (ppm)
21.02
20.94
21.5
21.0
20.5
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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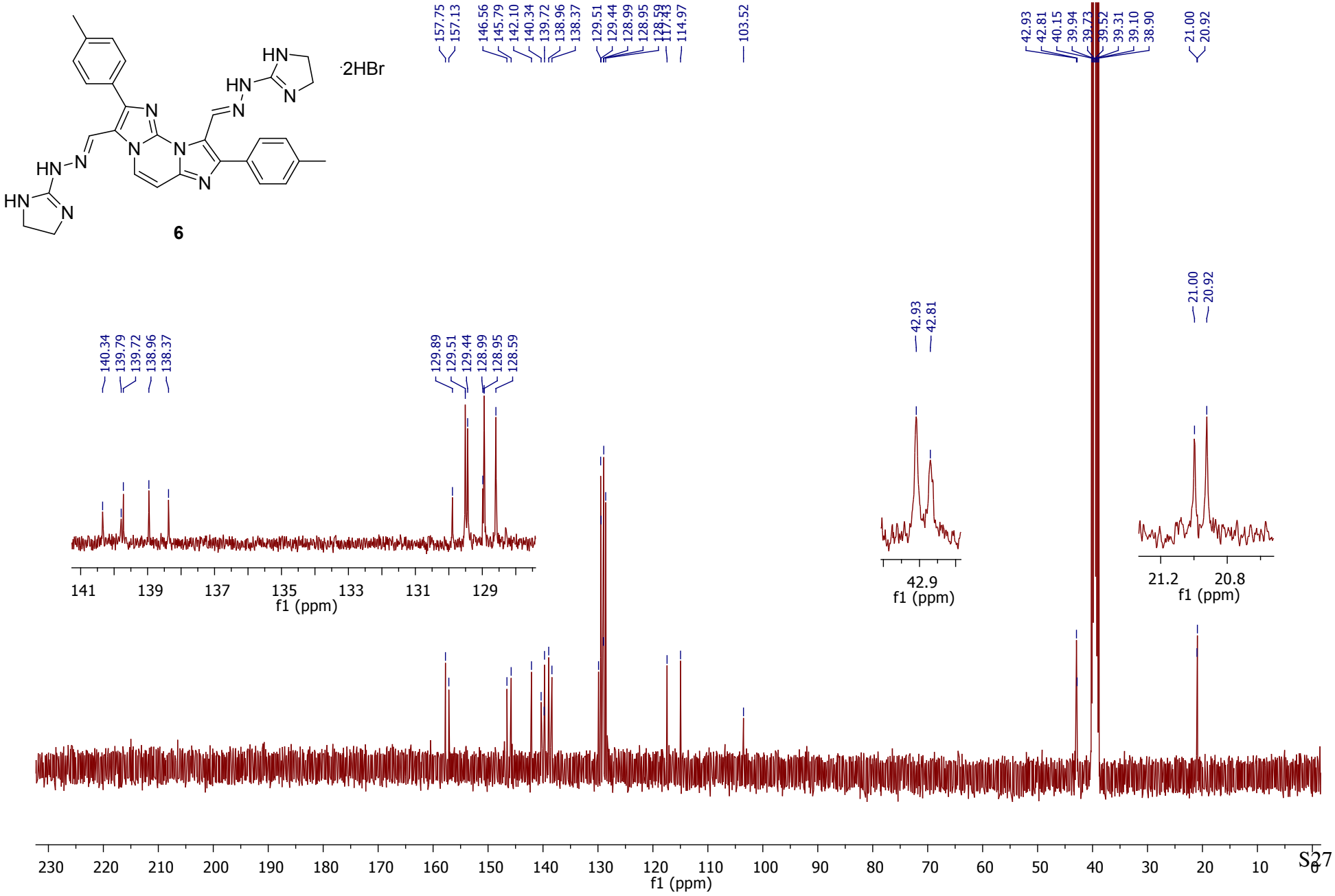
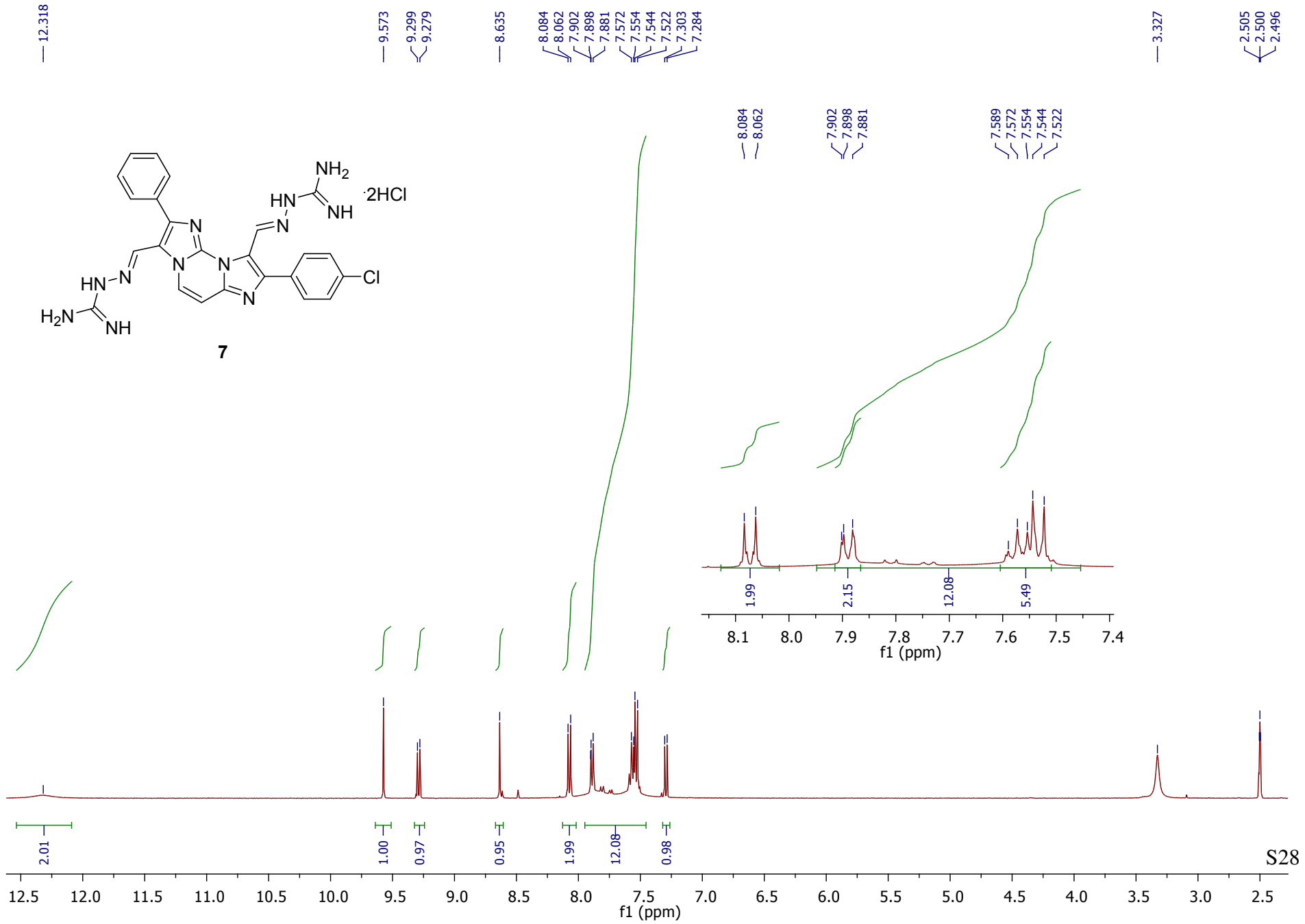
9.473
9.281
9.262
8.703
8.604
8.266
8.046
8.026
7.734
7.714
7.415
7.395
7.344
7.324
7.315
7.295
3.796
3.721
3.324
2.500
2.423
2.385
·2HBr
6
2.12
1.03
1.12
3.00
2.01
2.00
2.08
2.09
3.08
4.06
3.88
2.95
2.96
f1 (ppm)
2.09
3.08
f1 (ppm)


·2HBr
6
157.75
157.13
146.56
145.79
142.10
140.34
139.72
138.96
138.37
129.51
129.44
128.99
128.95
128.59
117.43
114.97
103.52
42.93
42.81
40.15
39.94
39.73
39.52
39.31
39.10
38.90
21.00
20.92
140.34
139.79
139.72
138.96
138.37
129.89
129.51
129.44
128.99
128.95
128.59
141
139
137
135
133
131
129
f1 (ppm)
42.93
42.81
42.9
f1 (ppm)
21.00
20.92
21.2
20.8
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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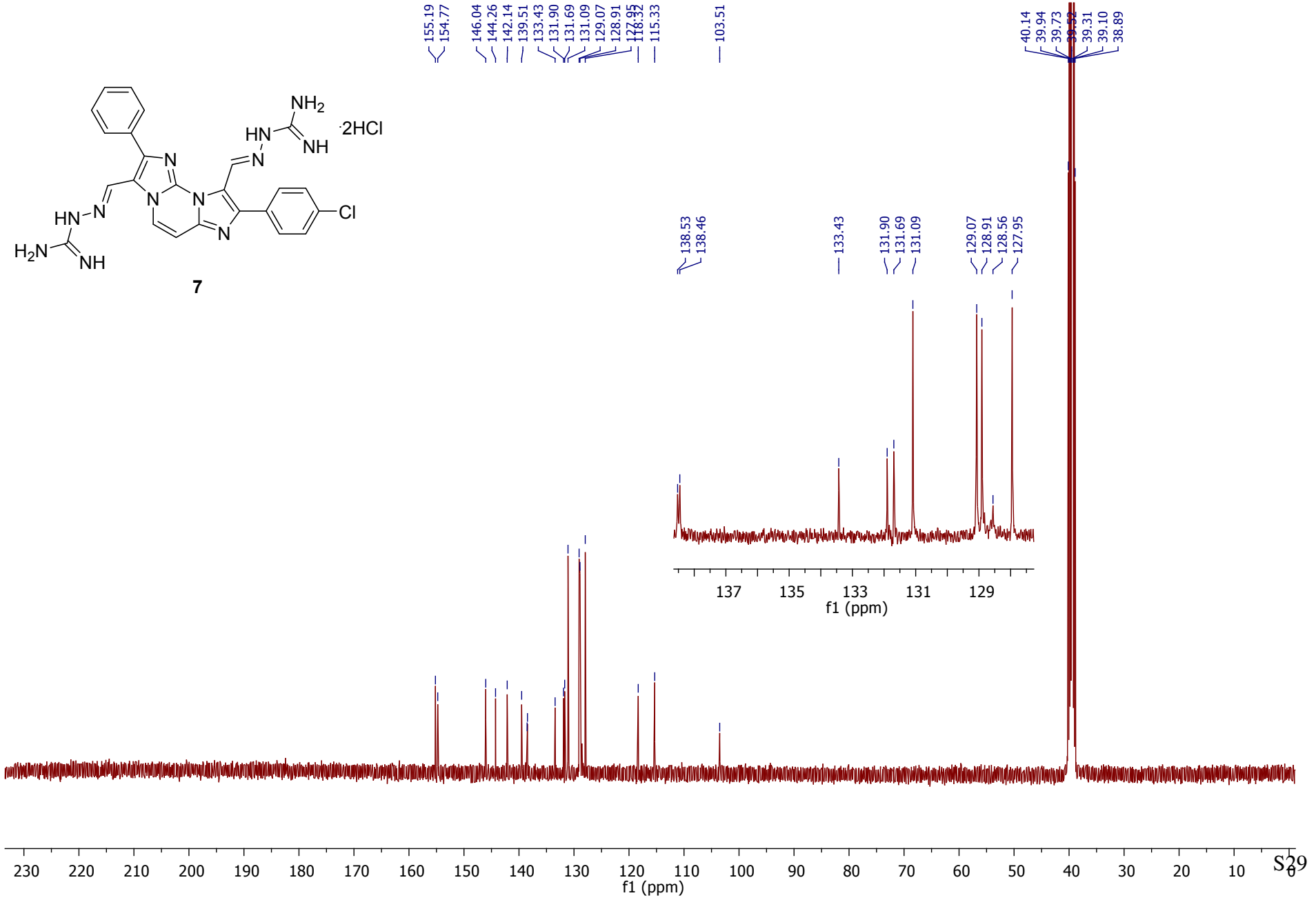
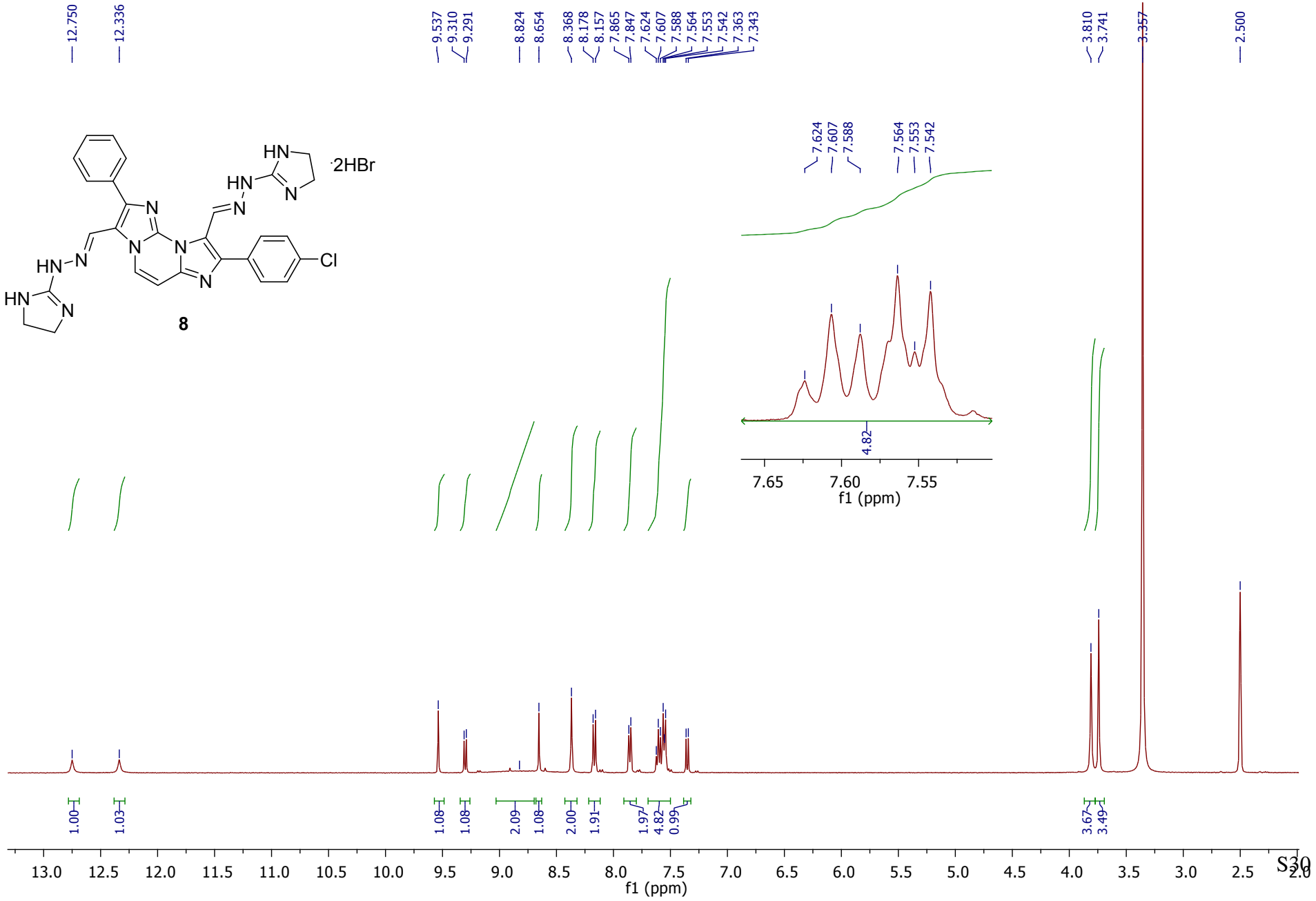
9.299
9.279
8.635
8.084
8.062
7.902
7.898
7.881
7.572
7.554
7.544
7.522
7.303
7.284
3.327
2.505
2.500
2.496
NH2
HN
NH
·2HCl
N
HN
N
H2N
NH
Cl
7
1.99
2.15
12.08
5.49
f1 (ppm)
2.01
1.00
0.97
0.95
1.99
12.08
0.98
f1 (ppm)


NH2
HN
NH
·2HCl
N
HN
N
H2N
NH
Cl
7
155.19
154.77
146.04
144.26
142.14
139.51
133.43
131.90
131.69
131.09
129.07
128.91
127.95
118.32
115.33
103.51
40.14
39.94
39.73
39.52
39.31
39.10
38.89
138.53
138.46
133.43
131.90
131.69
131.09
129.07
128.91
128.56
127.95
137
135
133
131
129
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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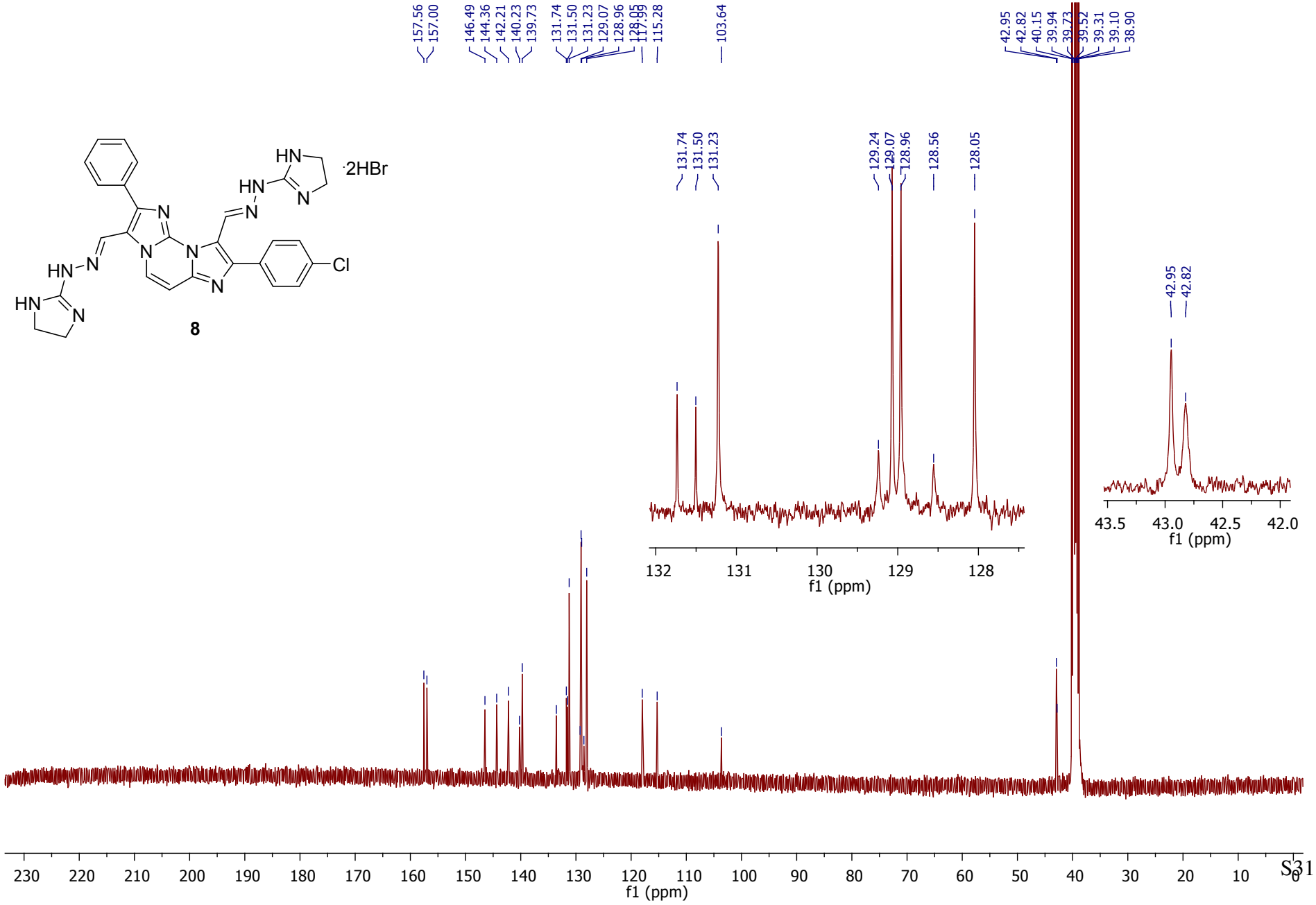
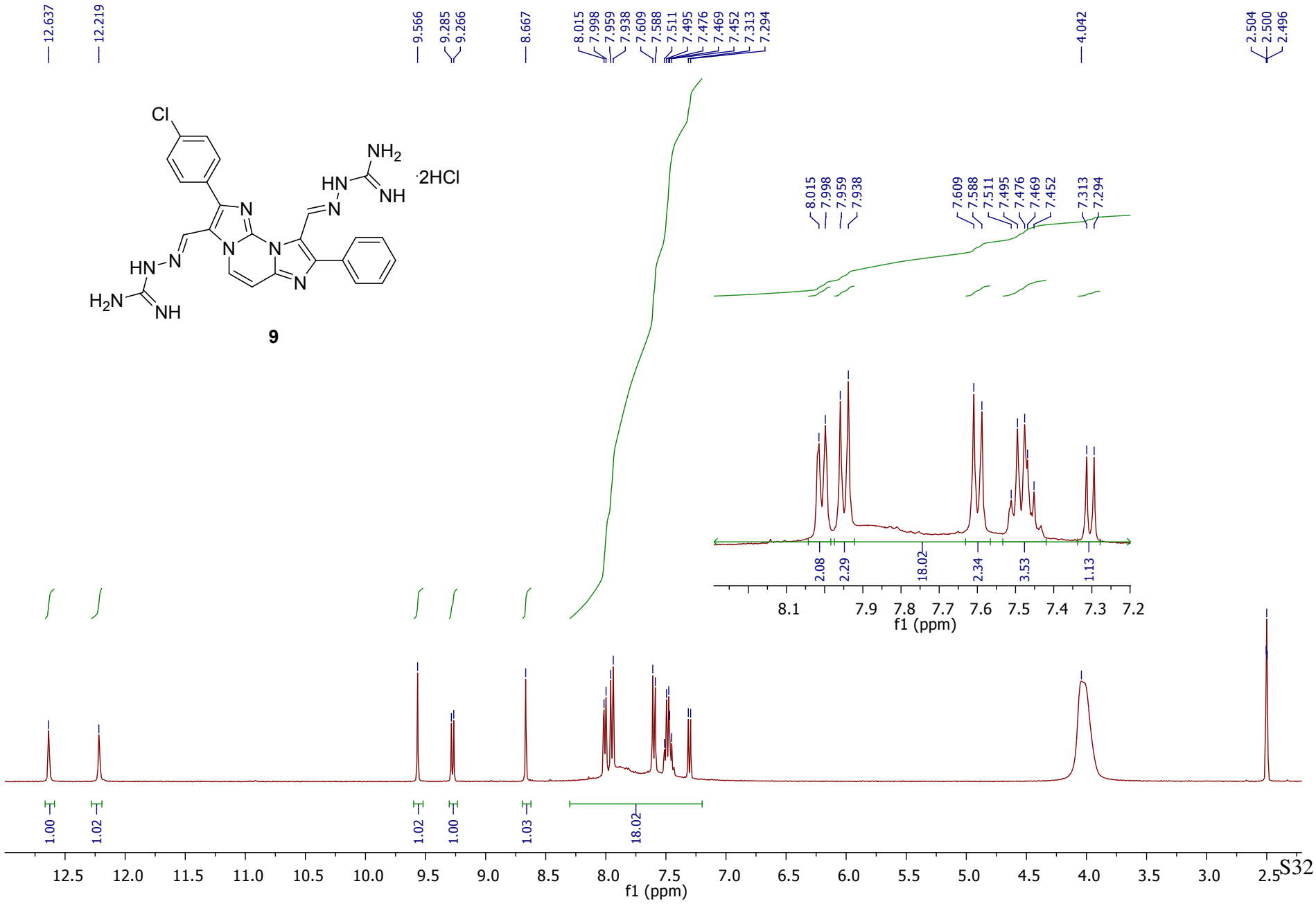
12.336
9.537
9.310
9.291
8.824
8.654
8.368
8.178
8.157
7.865
7.847
7.624
7.607
7.588
7.564
7.553
7.542
7.363
7.343
3.810
3.741
3.357
2.500
HN
HN
N
N
·2HBr
HN
N
N
N
N
HN
N
N
Cl
8
4.82
7.65
7.60
7.55
f1 (ppm)
1.00
1.03
1.08
1.08
2.09
1.08
2.00
1.91
1.97
4.82
0.99
3.67
3.49
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
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
f1 (ppm)


157.56
157.00
146.49
144.36
142.21
140.23
139.73
131.74
131.50
131.23
129.07
128.96
128.05
115.28
103.64
42.95
42.82
40.15
39.94
39.73
39.52
39.31
39.10
38.90
·2HBr
Cl
8
f1 (ppm)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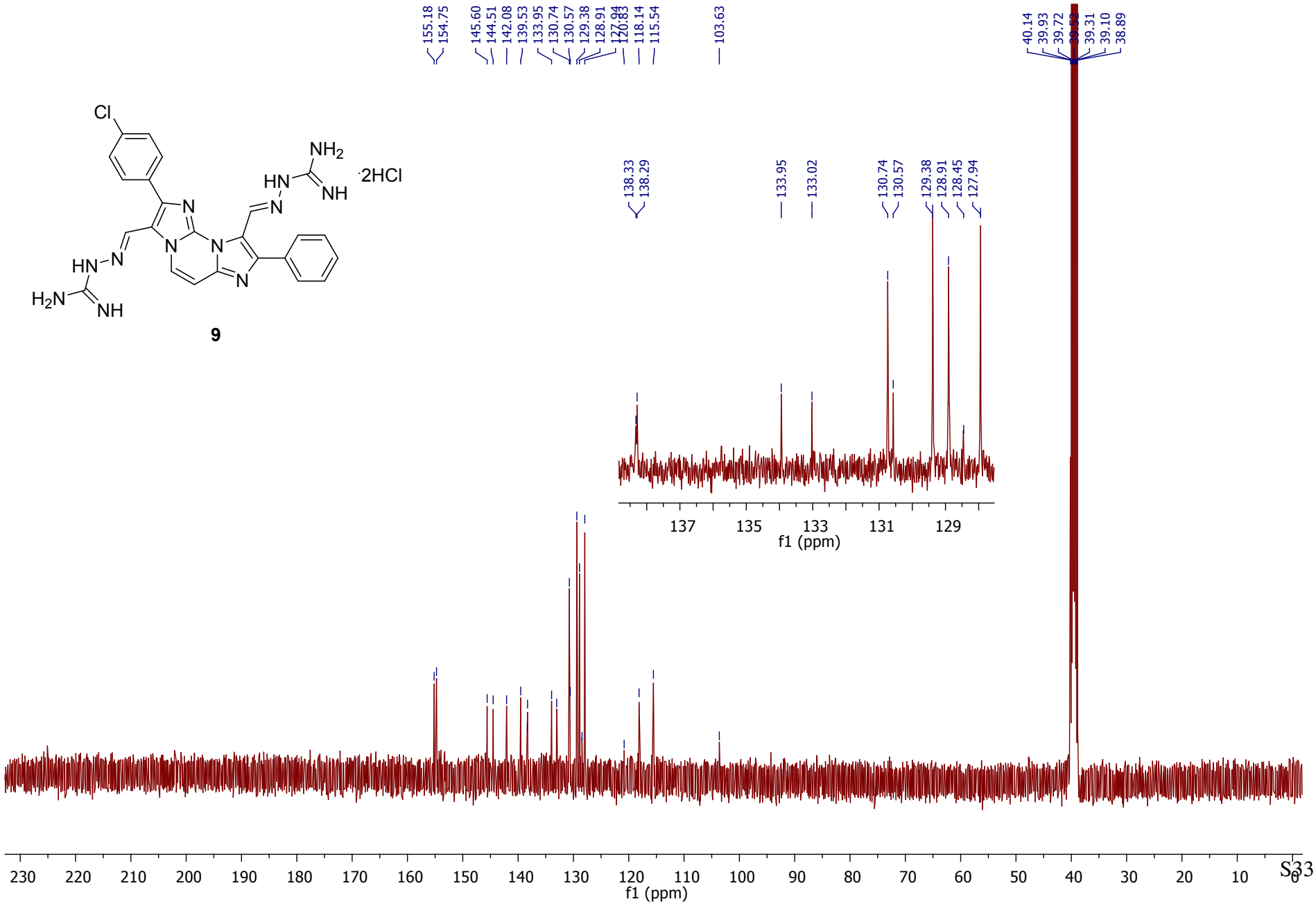
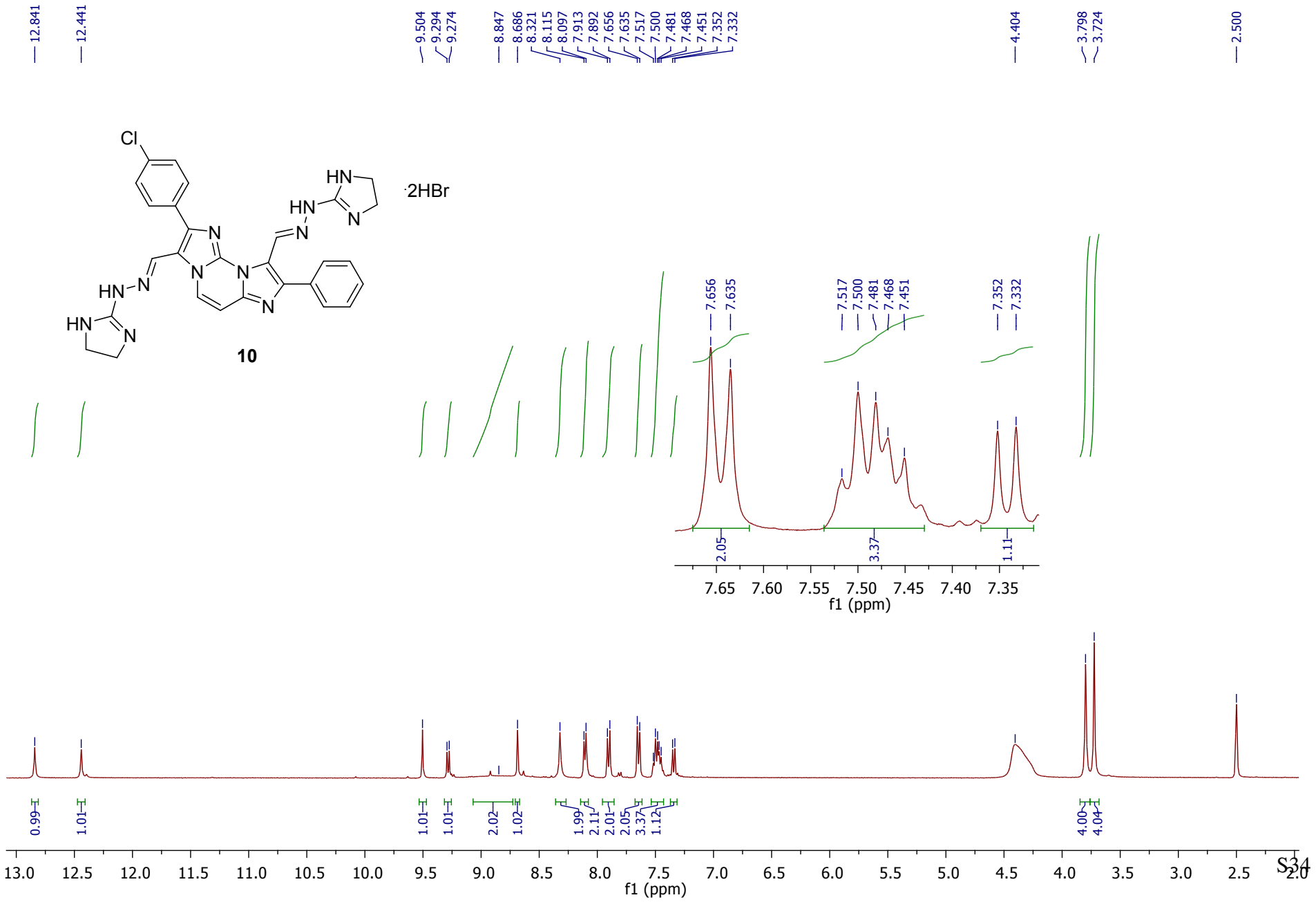
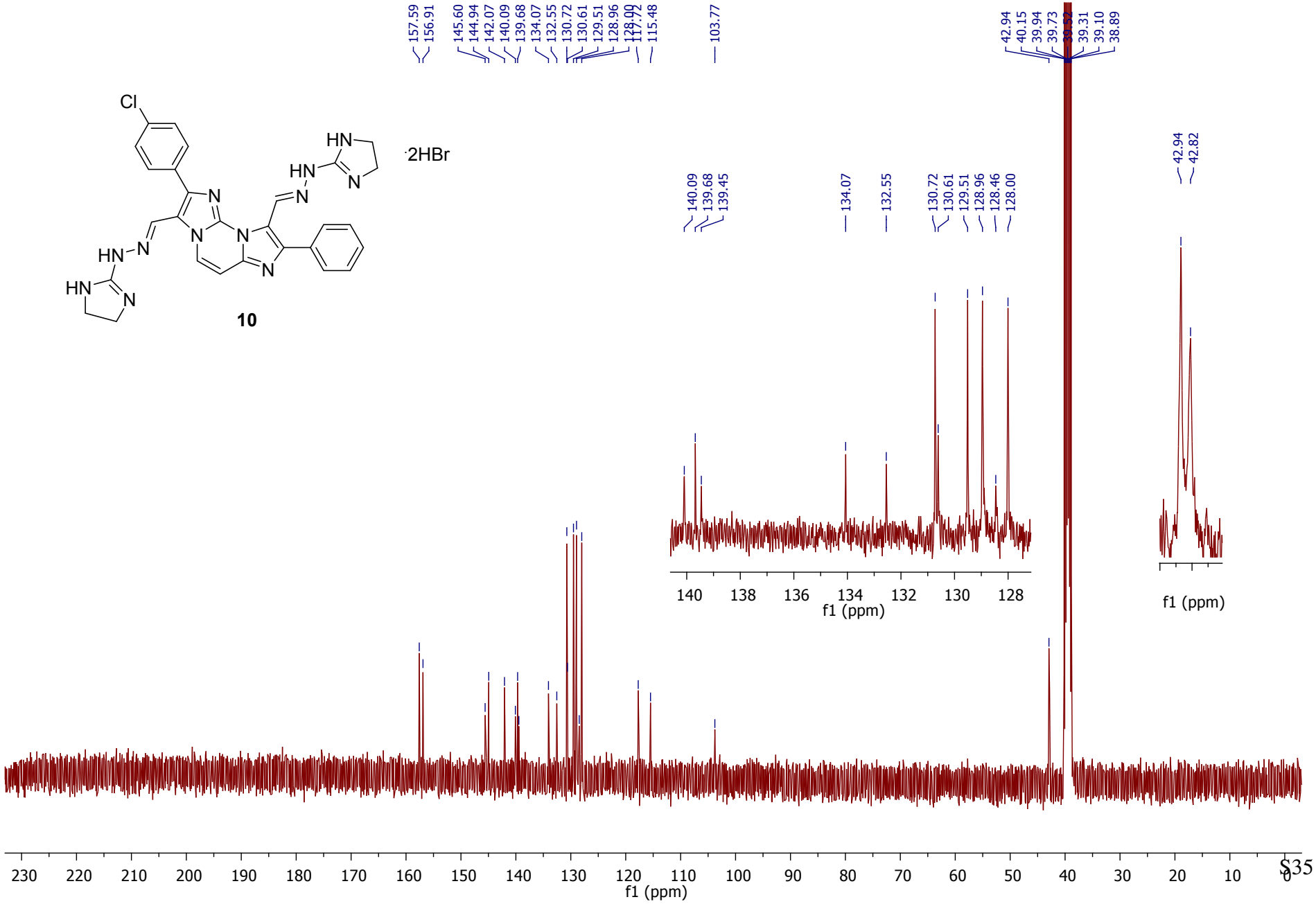
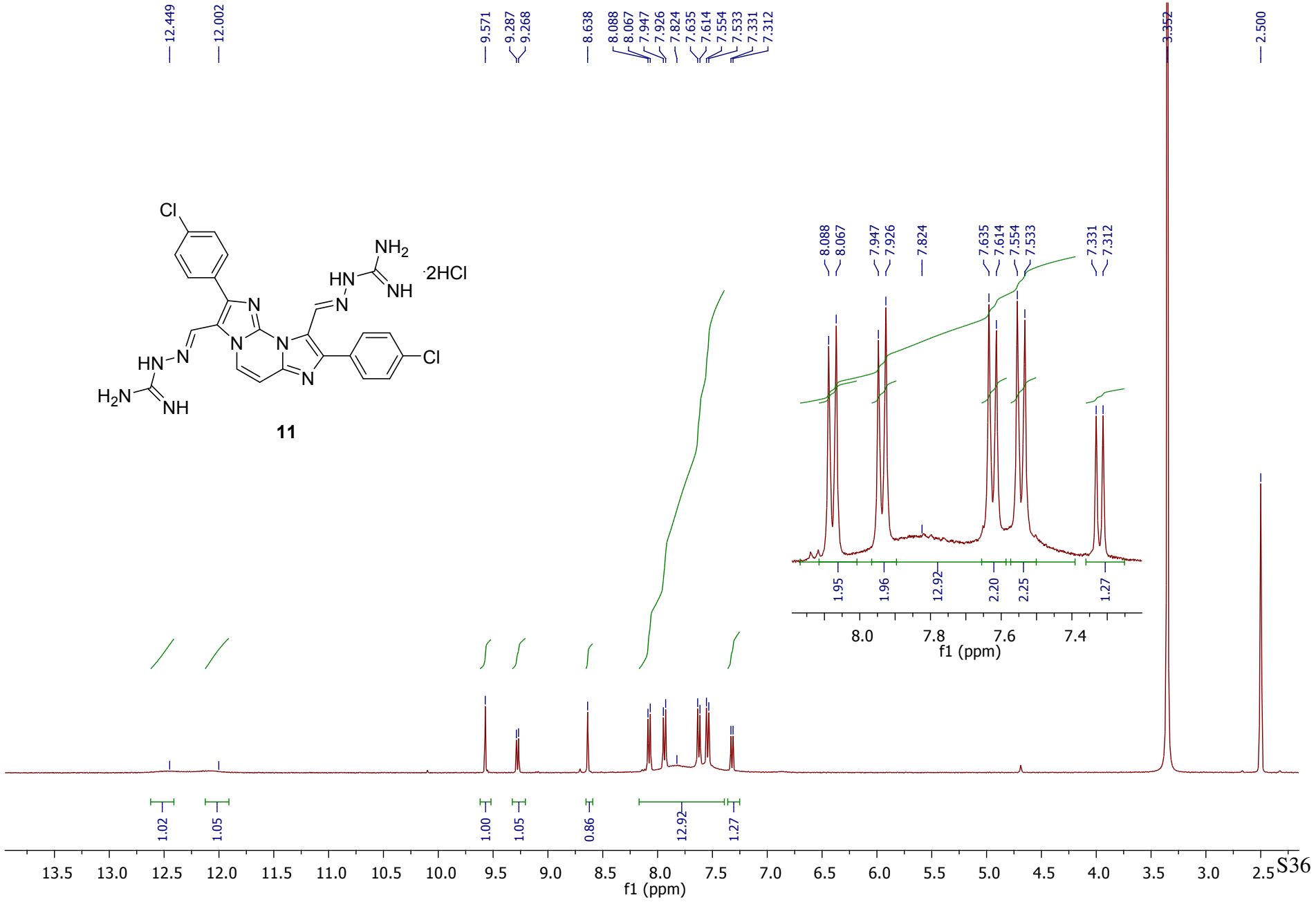
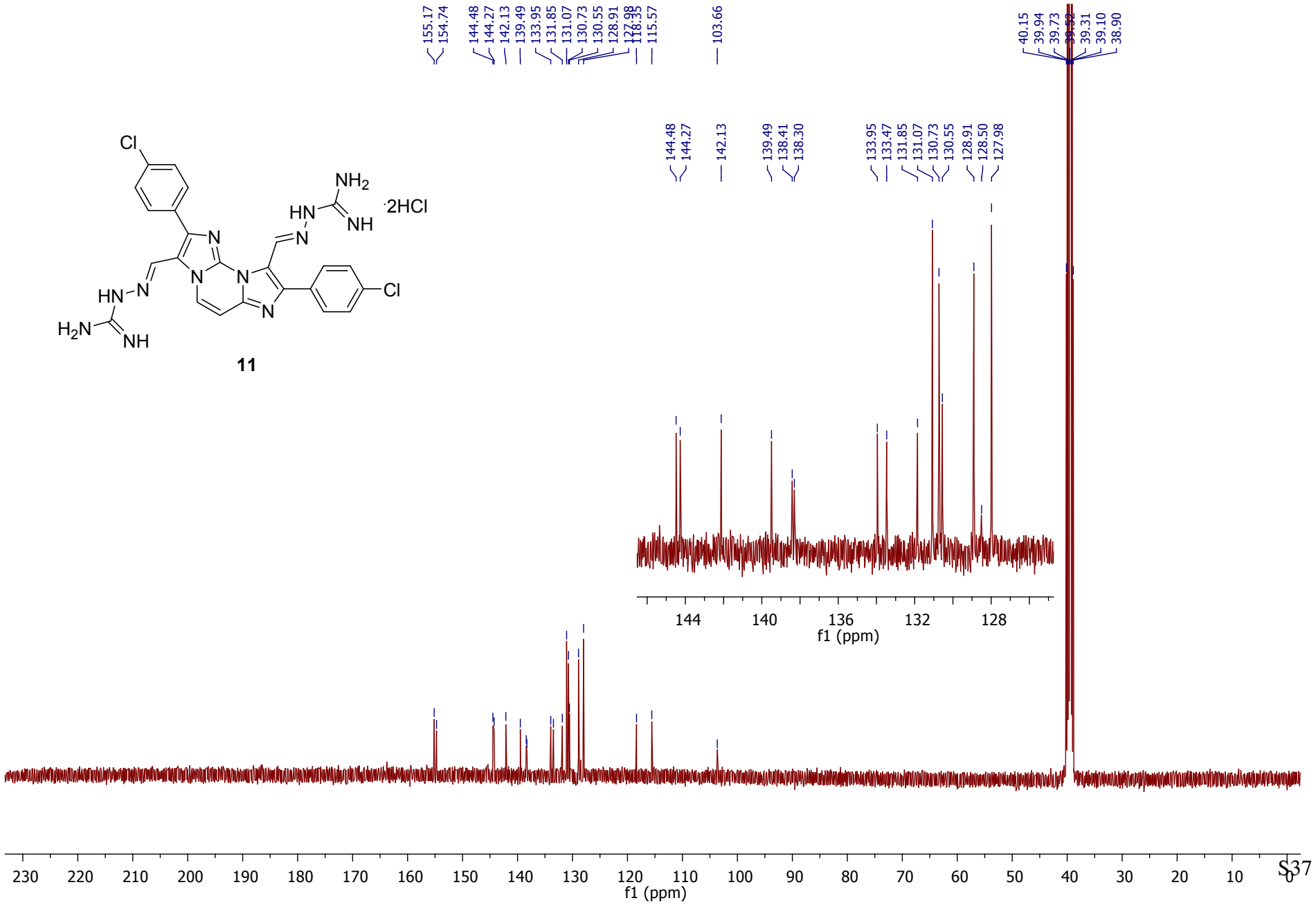
12.219
9.566
9.285
9.266
8.667
8.015
7.998
7.959
7.938
7.609
7.588
7.511
7.495
7.476
7.469
7.452
7.313
7.294
4.042
2.504
2.500
2.496
Cl
NH2
HN
NH
·2HCl
N
HN
H2N
NH
9
1.00
1.02
1.02
1.00
1.03
18.02
2.08
2.29
18.02
2.34
3.53
1.13
f1 (ppm)

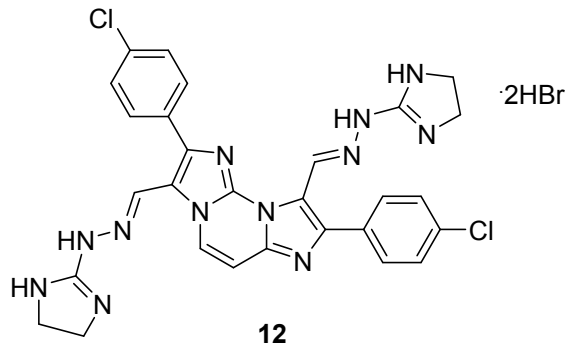

155.18
154.75
145.60
144.51
142.08
139.53
133.95
130.74
130.57
129.38
128.91
118.14
115.54
103.63
40.14
39.93
39.72
39.52
39.31
39.10
38.89
Cl
NH2
HN
NH
·2HCl
N
HN
N
H2N
NH
9
138.33
138.29
133.95
133.02
130.74
130.57
129.38
128.91
128.45
127.94
137
135
133
131
129
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


12.841
12.441
9.504
9.294
9.274
8.847
8.686
8.321
8.115
8.097
7.913
7.892
7.656
7.635
7.517
7.500
7.481
7.468
7.451
7.352
7.332
4.404
3.798
3.724
2.500
Cl
HN
HN
N
N
N
N
HN
N
HN
N
·2HBr
10
2.05
3.37
1.11
7.65 7.60 7.55 7.50 7.45 7.40 7.35
f1 (ppm)
0.99
1.01
1.01
1.01
2.02
1.02
1.99
2.11
2.01
2.05
3.37
1.12
4.00
4.04
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 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
f1 (ppm)


Cl
HN
HN
N
N
HN
N
HN
N
N
N
N
N
·2HBr
10
157.59
156.91
145.60
144.94
142.07
140.09
139.68
134.07
132.55
130.72
130.61
129.51
128.96
128.46
128.00
117.72
115.48
103.77
42.94
40.15
39.94
39.73
39.52
39.31
39.10
38.89
140.09
139.68
139.45
134.07
132.55
130.72
130.61
129.51
128.96
128.46
128.00
42.94
42.82
140 138 136 134 132 130 128
f1 (ppm)
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

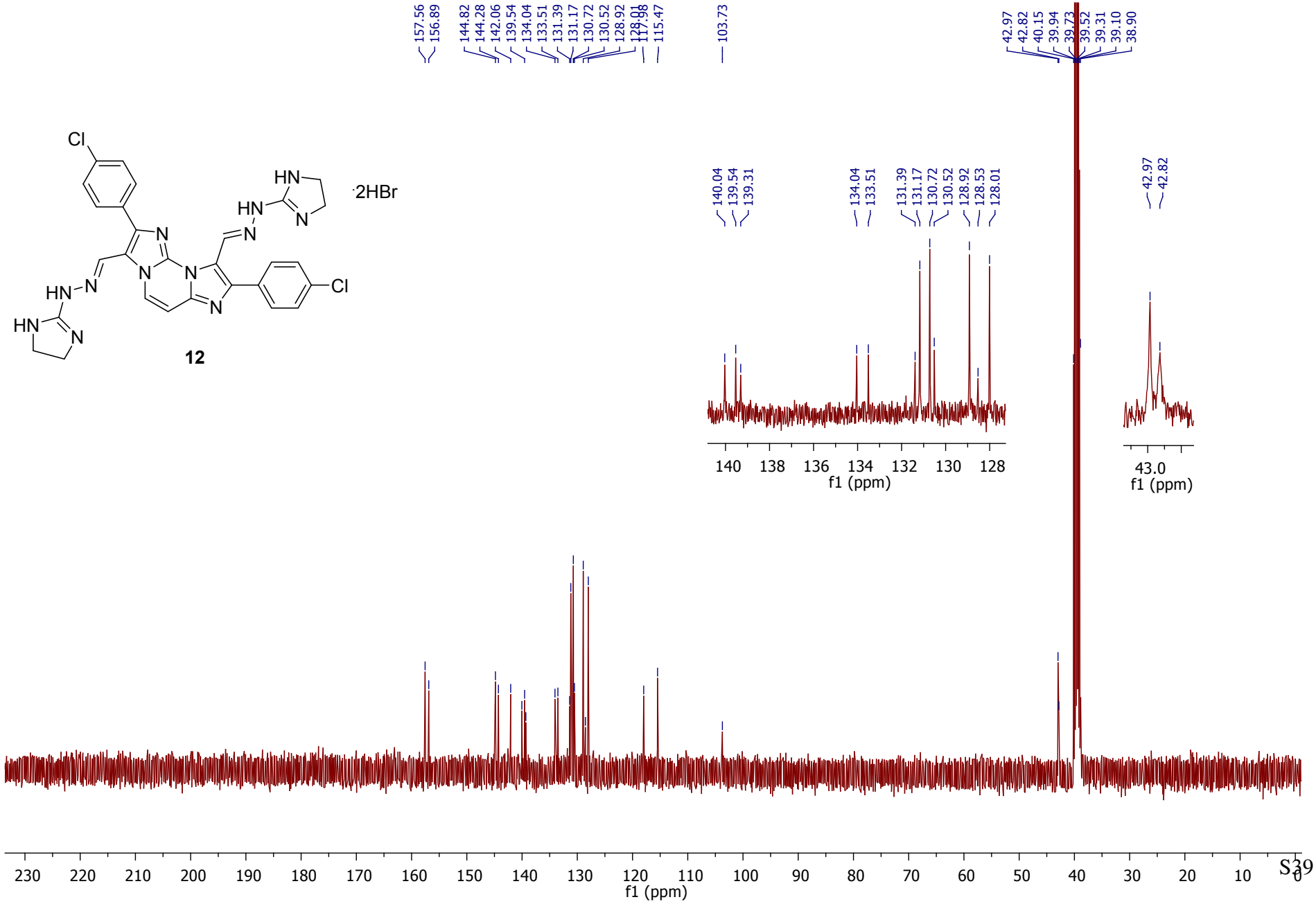

12.449
12.002
9.571
9.287
9.268
8.638
8.088
8.067
7.947
7.926
7.824
7.635
7.614
7.554
7.533
7.331
7.312
3.352
2.500
Cl
NH2
HN
NH
·2HCl
N
HN
N
H2N
NH
Cl
11
1.02
1.05
1.00
1.05
0.86
12.92
1.27
f1 (ppm)
1.95
1.96
12.92
2.20
2.25
1.27


155.17
154.74
144.48
144.27
142.13
139.49
133.95
131.85
131.07
130.73
130.55
128.91
127.98
118.35
115.57
103.66
40.15
39.94
39.73
39.52
39.31
39.10
38.90
Cl
NH2
HN
NH
·2HCl
N
HN
N
H2N
NH
Cl
11
144.48
144.27
142.13
139.49
138.41
138.30
133.95
133.47
131.85
131.07
130.73
130.55
128.91
128.50
127.98
144
140
136
132
128
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Cl
HN
HN
N
·2HBr
N
N
N
HN
N
HN
N
N
Cl

12

12.622
9.526
9.250
9.230
8.898
8.680
8.342
8.149
8.128
7.912
7.891
7.641
7.620
7.538
7.517
7.291
7.271
3.791
3.742
3.365
2.500
1.99
0.99
1.09
1.98
1.00
2.03
2.00
1.97
2.03
1.84
1.00
7.93
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)


157.56
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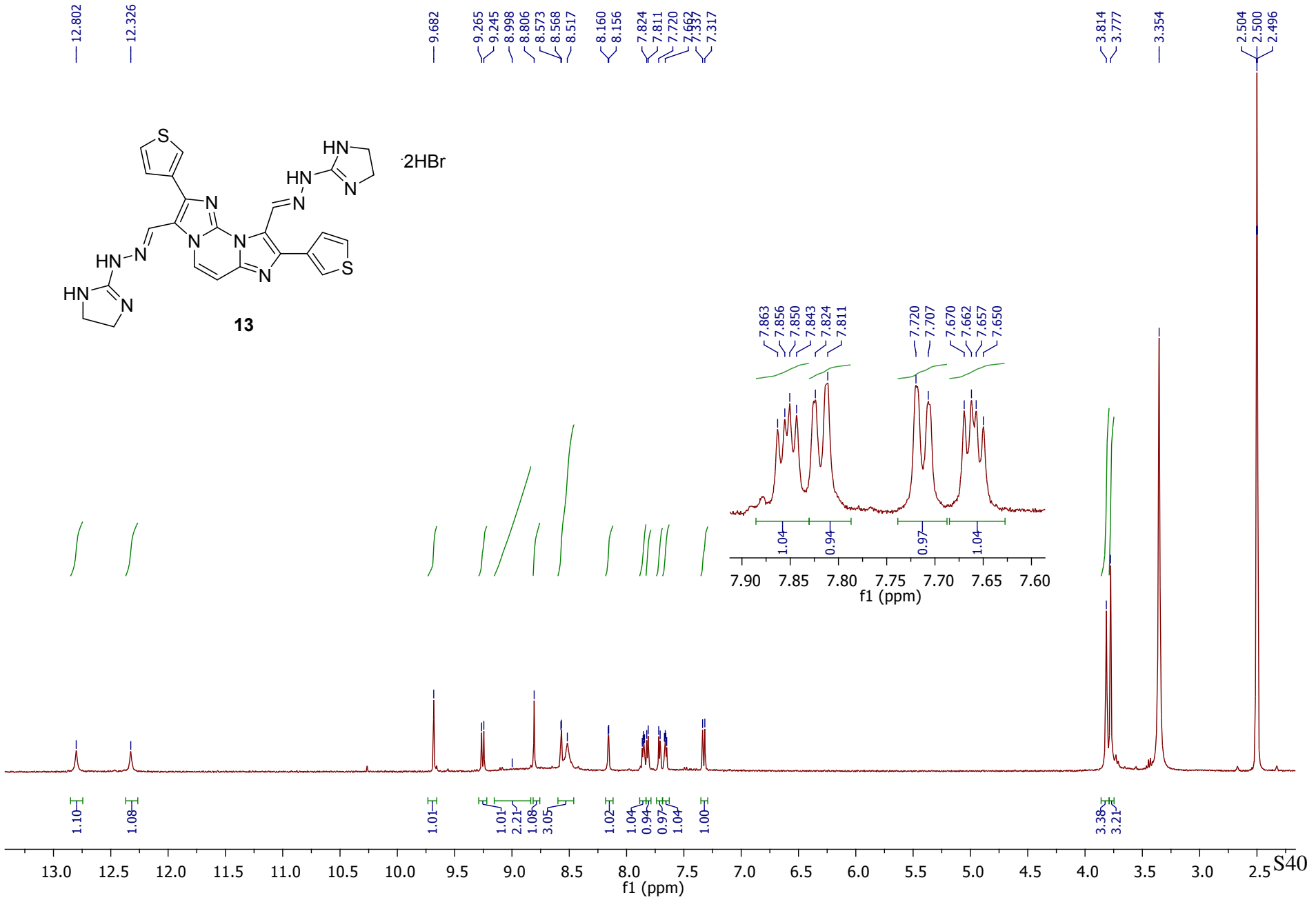
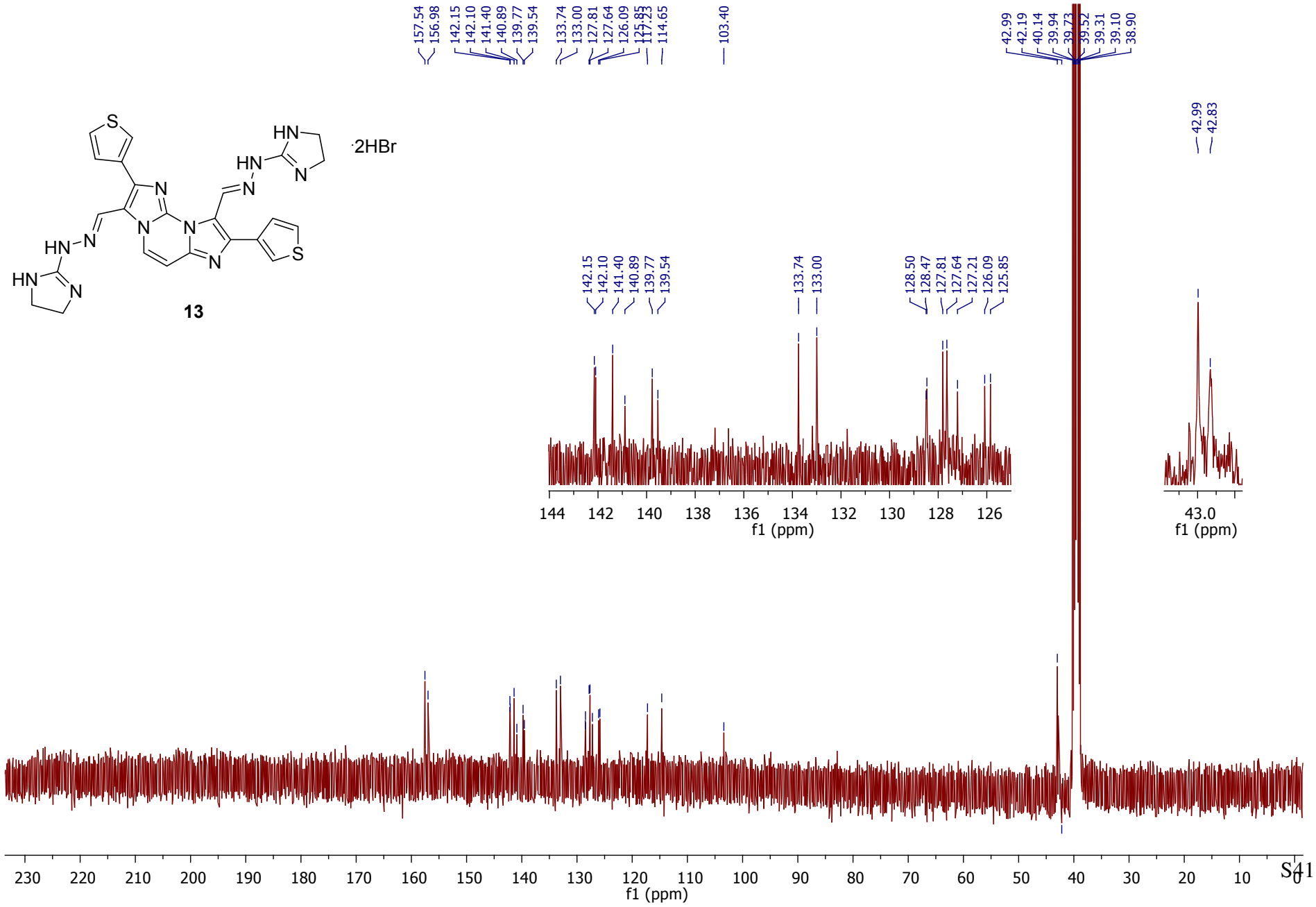
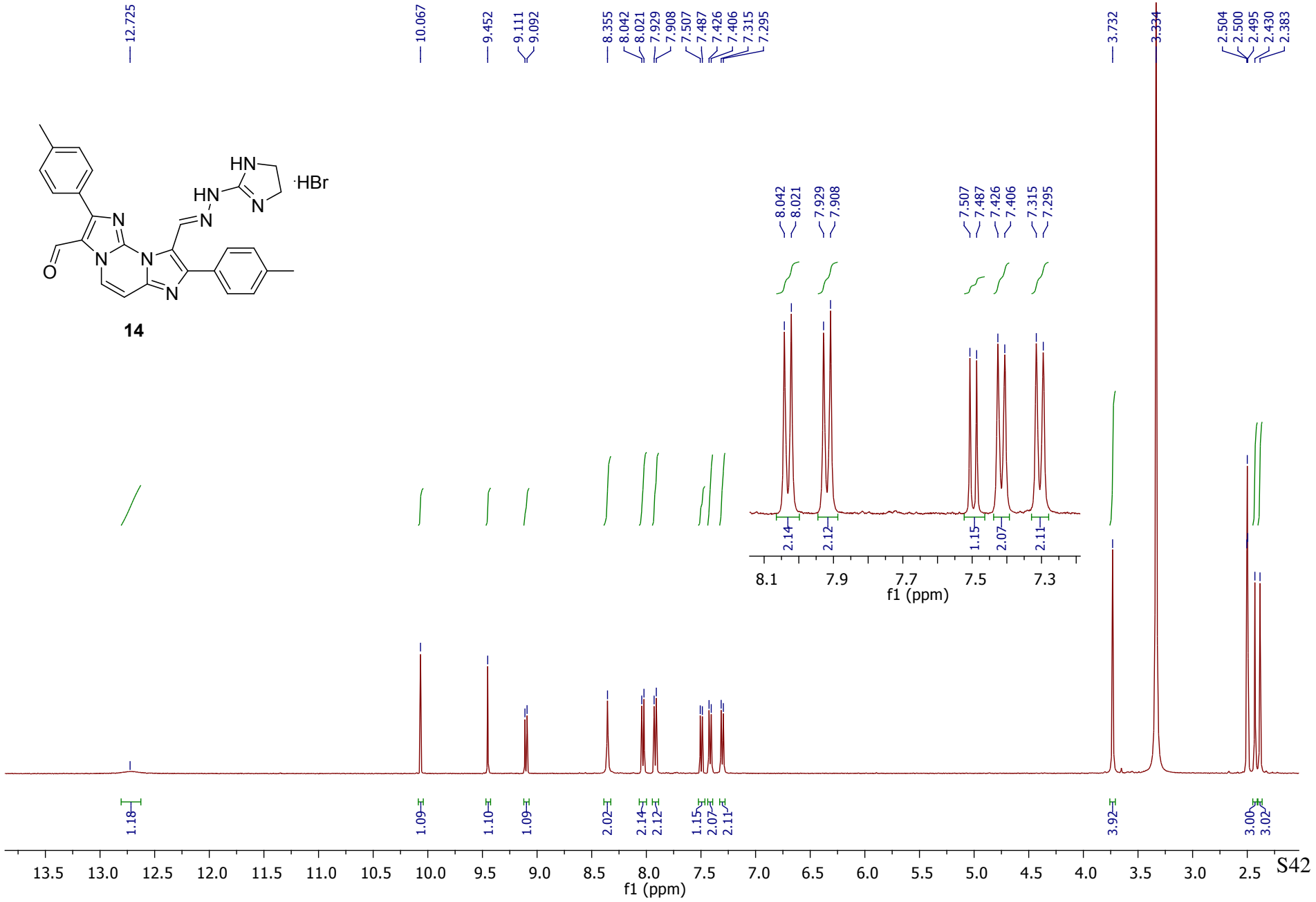
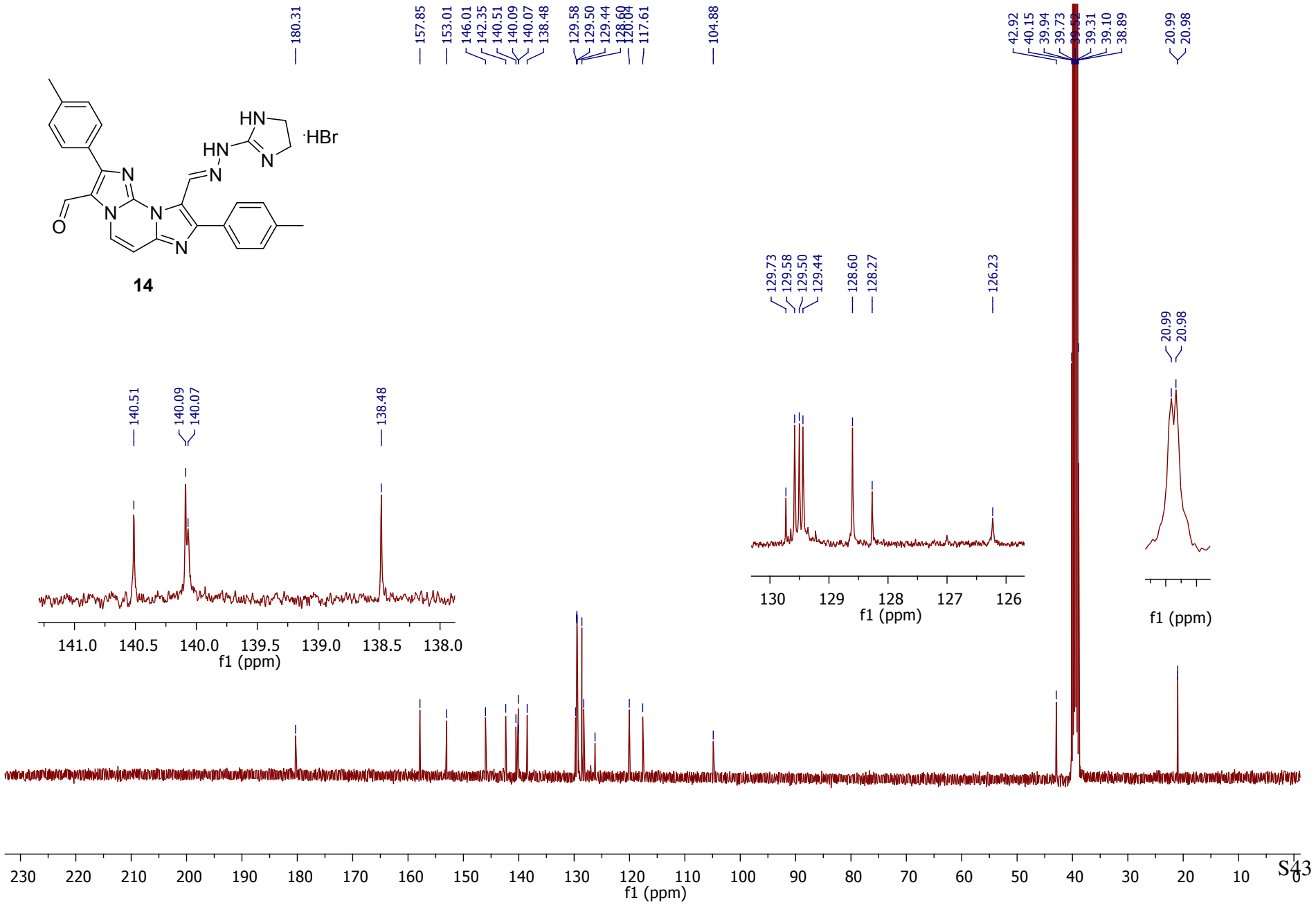
144.82
144.28
142.06
139.54
134.04
133.51
131.39
131.17
130.72
130.52
128.92
128.01
117.98
115.47
103.73
42.97
42.82
40.15
39.94
39.73
39.52
39.31
39.10
38.90
Cl
HN
HN
N
N
N
N
N
HN
N
HN
N
Cl
·2HBr
12
140.04
139.54
139.31
134.04
133.51
131.39
131.17
130.72
130.52
128.92
128.53
128.01
42.97
42.82
140 138 136 134 132 130 128
f1 (ppm)
43.0
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


12.802
12.326
9.682
9.265
9.245
8.998
8.806
8.573
8.568
8.517
8.160
8.156
7.824
7.811
7.720
7.662
7.337
7.317
3.814
3.777
3.354
2.504
2.500
2.496
·2HBr
13
7.863
7.856
7.850
7.843
7.824
7.811
7.720
7.707
7.670
7.662
7.657
7.650
1.04
0.94
0.97
1.04
7.90 7.85 7.80 7.75 7.70 7.65 7.60
f1 (ppm)
1.10
1.08
1.01
1.01
2.21
1.08
3.05
1.02
1.04
0.94
0.97
1.04
1.00
3.38
3.21
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
f1 (ppm)


157.54
156.98
142.15
142.10
141.40
140.89
139.77
139.54
133.74
133.00
127.81
127.64
126.09
125.85
117.23
114.65
103.40
42.99
42.19
40.14
39.94
39.73
39.52
39.31
39.10
38.90
42.99
42.83
·2HBr
13
142.15
142.10
141.40
140.89
139.77
139.54
133.74
133.00
128.50
128.47
127.81
127.64
127.21
126.09
125.85
144 142 140 138 136 134 132 130 128 126
f1 (ppm)
43.0
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


12.725
10.067
9.452
9.111
9.092
8.355
8.042
8.021
7.929
7.908
7.507
7.487
7.426
7.406
7.315
7.295
3.732
3.334
2.504
2.500
2.495
2.430
2.383
HN
HN
·HBr
N
N
N
N
N
O
14
1.18
1.09
1.10
1.09
2.02
2.14
2.12
1.15
2.07
2.11
3.92
3.00
3.02
f1 (ppm)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157.85
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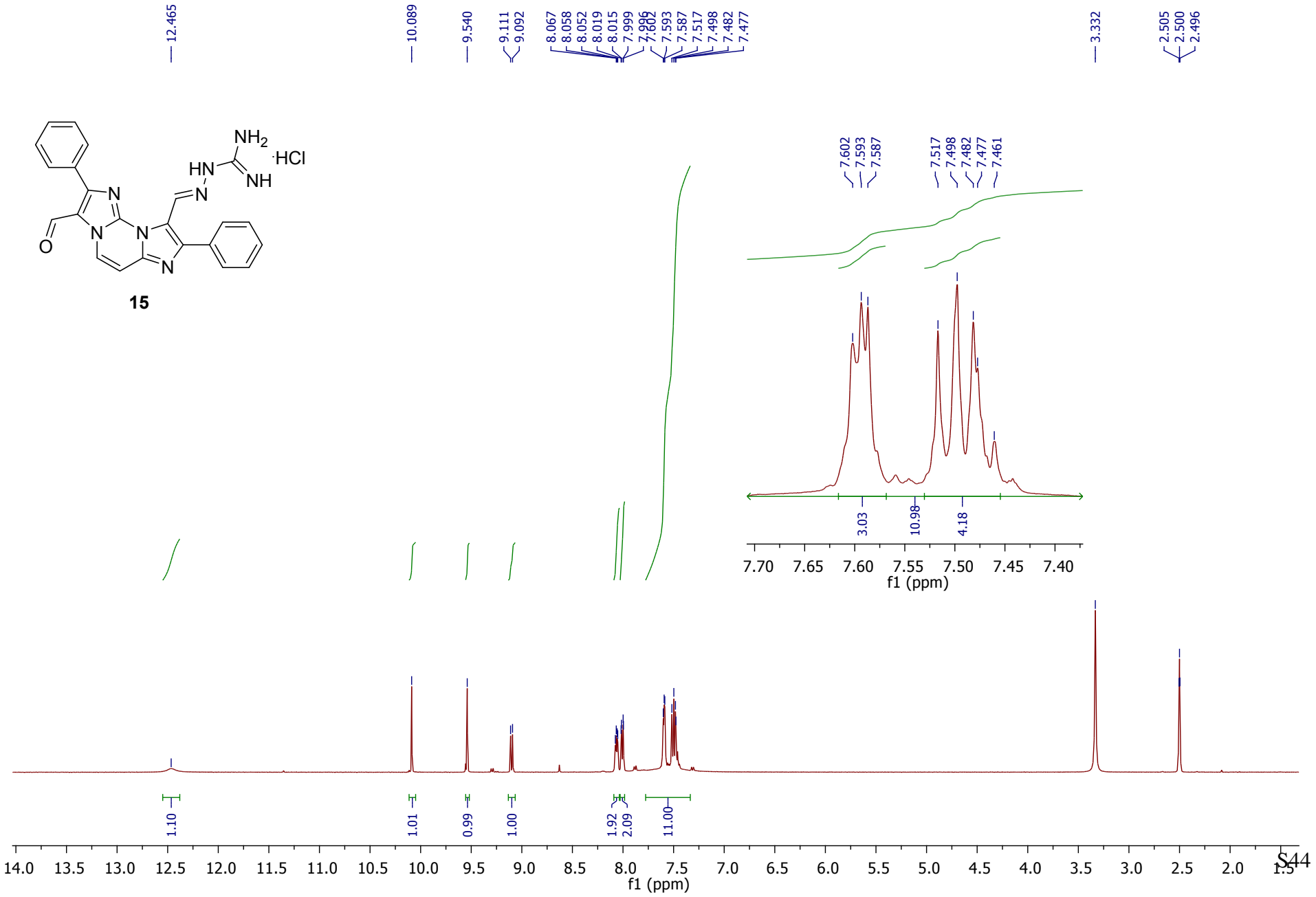
146.01
142.35
140.51
140.09
140.07
138.48
129.58
129.50
129.44
128.60
128.27
120.04
117.61
104.88
42.92
40.15
39.94
39.73
39.52
39.31
39.10
38.89
20.99
20.98
HN
HN
N
N
·HBr
N
N
N
N
O
14
f1 (ppm)
f1 (ppm)
f1 (ppm)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9.540
9.111
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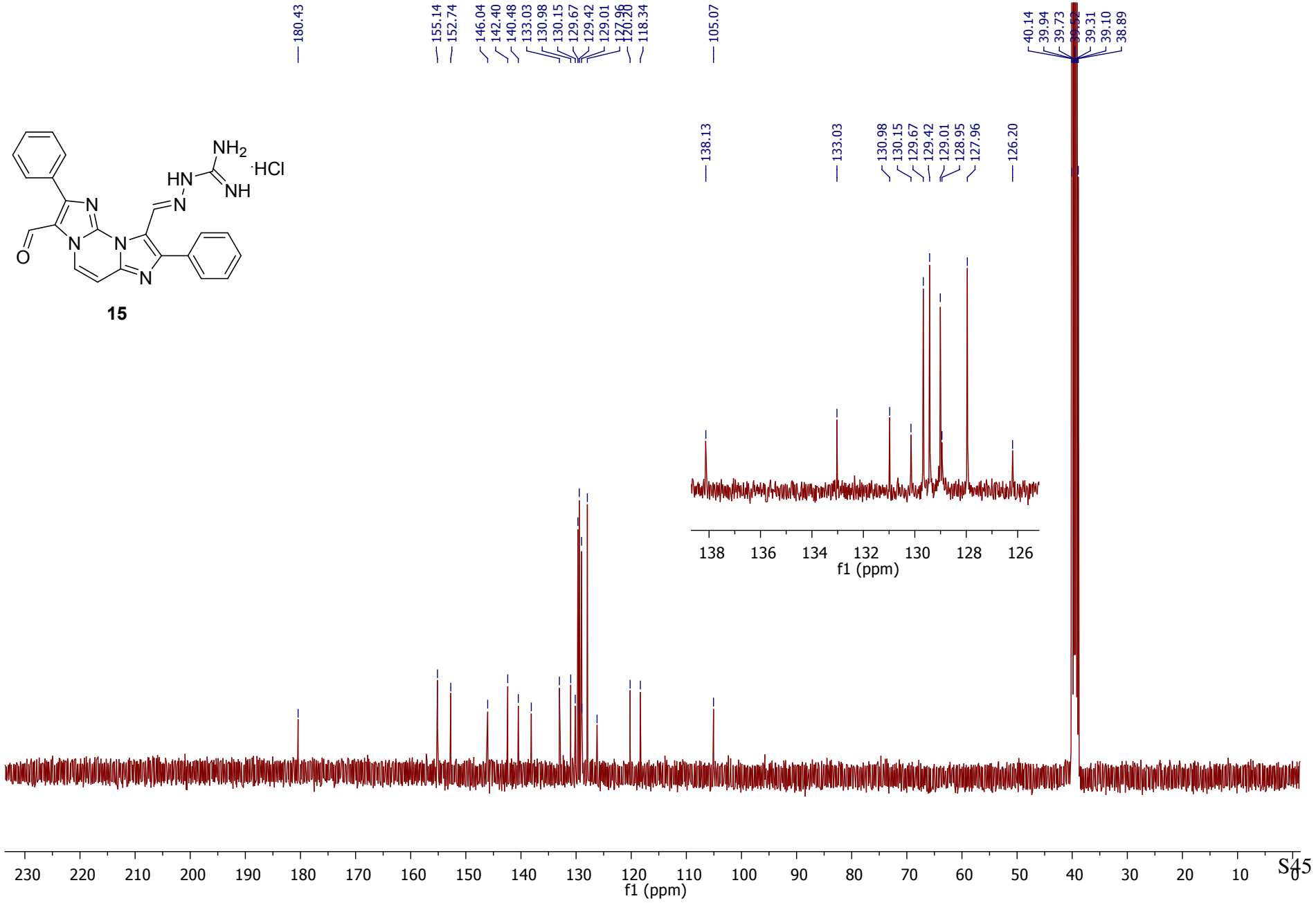
8.067
8.058
8.052
8.019
8.015
7.999
7.996
7.602
7.593
7.587
7.517
7.498
7.482
7.477
7.461
3.332
2.505
2.500
2.496
NH2
·HCl
HN
NH
N
O
15
3.03
10.98
4.18
7.70 7.65 7.60 7.55 7.50 7.45 7.40
f1 (ppm)
1.10
1.01
0.99
1.00
1.92
2.09
11.00
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
f1 (ppm)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140.48
133.03
130.98
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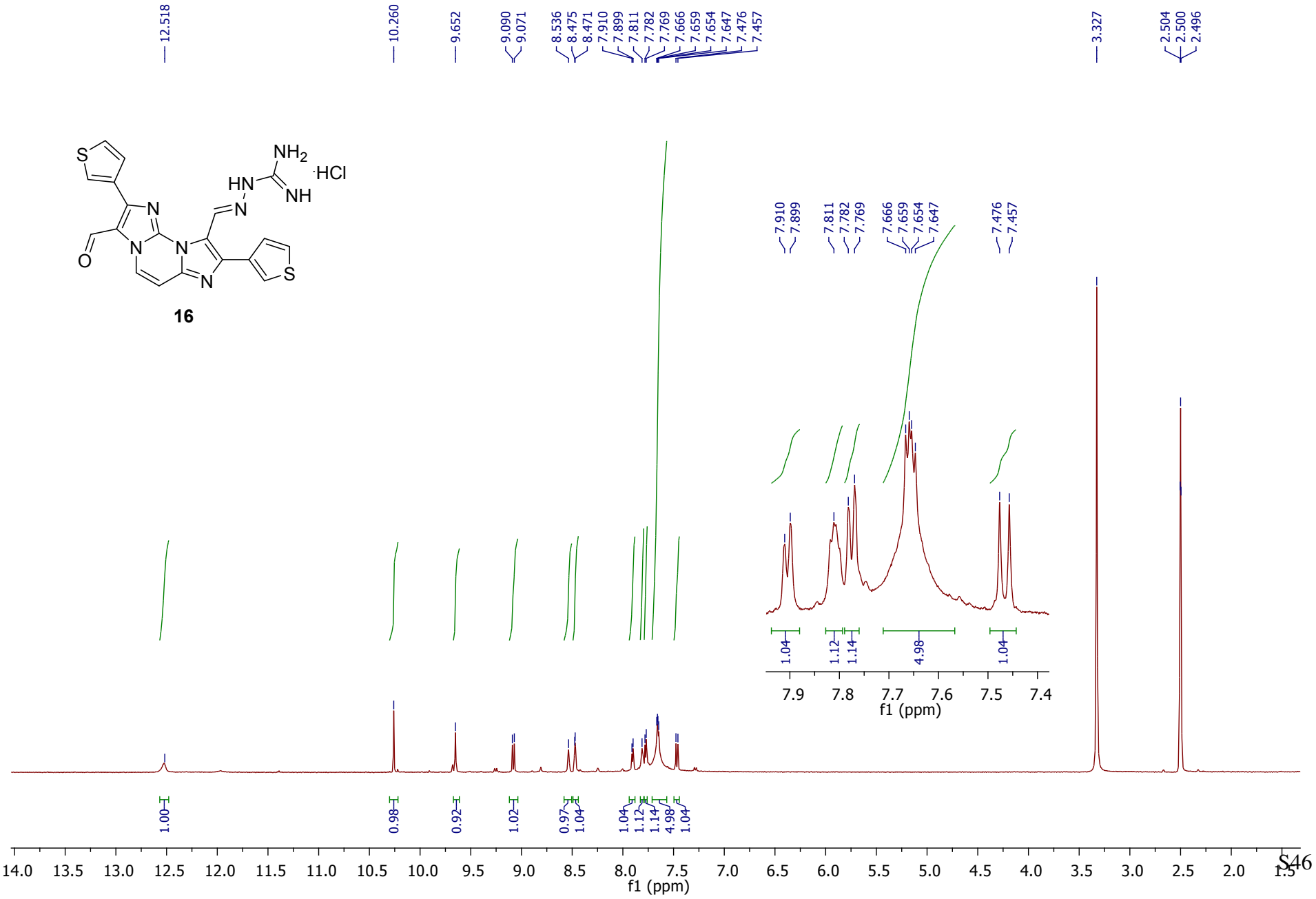
129.67
129.42
129.01
118.34
105.07
40.14
39.94
39.73
39.52
39.31
39.10
38.89
138.13
133.03
130.98
130.15
129.67
129.42
129.01
128.95
127.96
126.20
NH2
HCl
HN
NH
N
O
15
138
136
134
132
130
128
126
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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9.652
9.090
9.071
8.536
8.475
8.471
7.910
7.899
7.811
7.782
7.769
7.666
7.659
7.654
7.647
7.476
7.457
3.327
2.504
2.500
2.496
NH2
HCl
HN
NH
N
O
S
16
1.00
0.98
0.92
1.02
0.97
1.04
1.04
1.12
1.14
4.98
1.04
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
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
f1 (ppm)
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1.12
1.14
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1.04
7.9
7.8
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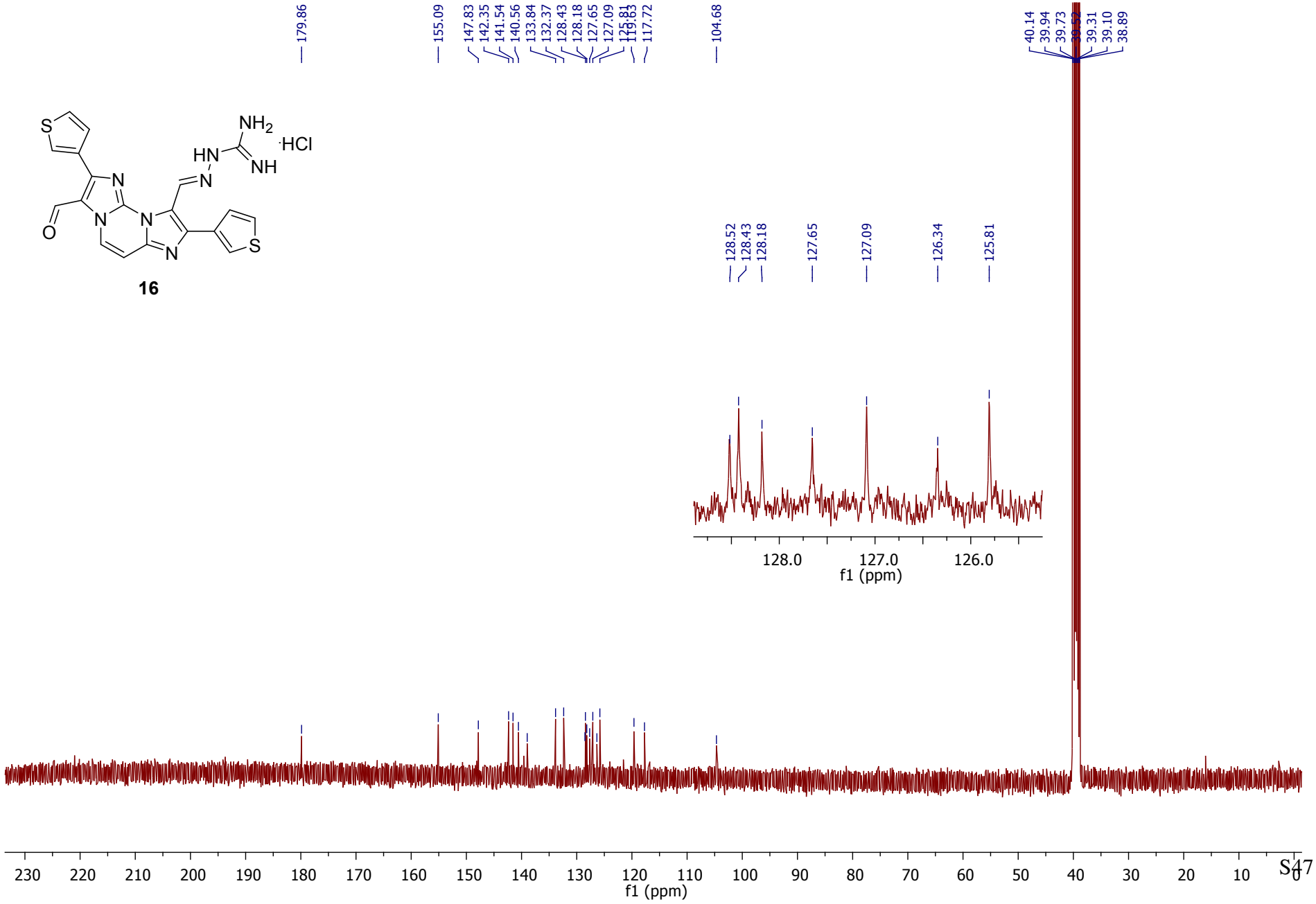
7.6
7.5
7.4
f1 (ppm)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127.09
125.81
119.63
117.72
104.68
40.14
39.94
39.73
39.52
39.31
39.10
38.89
NH2
HCl
HN
NH
N
O
S
16
128.52
128.43
128.18
127.65
127.09
126.34
125.81
128.0
127.0
126.0
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
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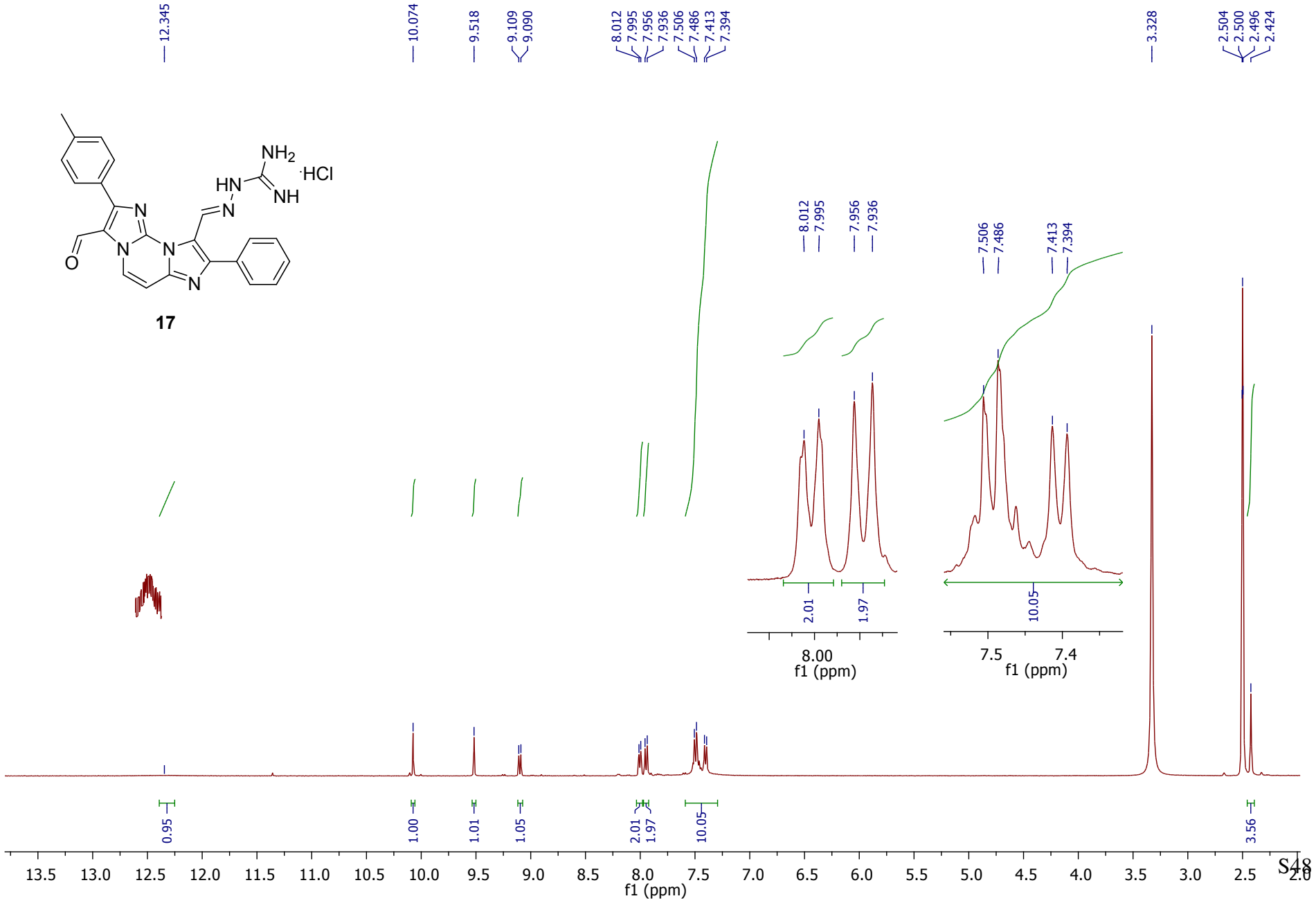
f1 (ppm)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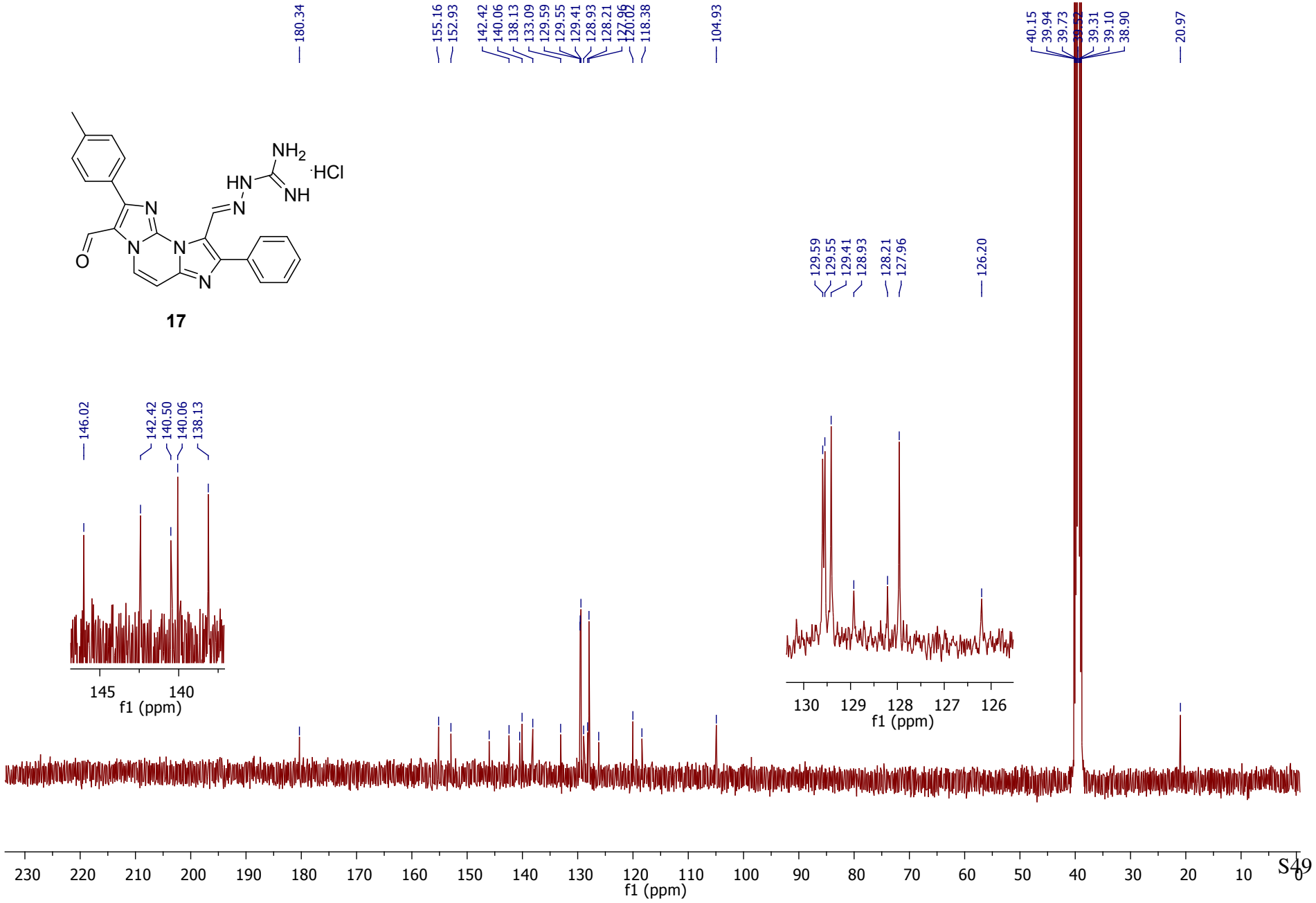
9.090
8.012
7.995
7.956
7.936
7.506
7.486
7.413
7.394
3.328
2.504
2.500
2.496
2.424
NH2
HN
NH
·HCl
N
N
N
N
O
17
0.95
1.00
1.01
1.05
2.01
1.97
10.05
3.56
f1 (ppm)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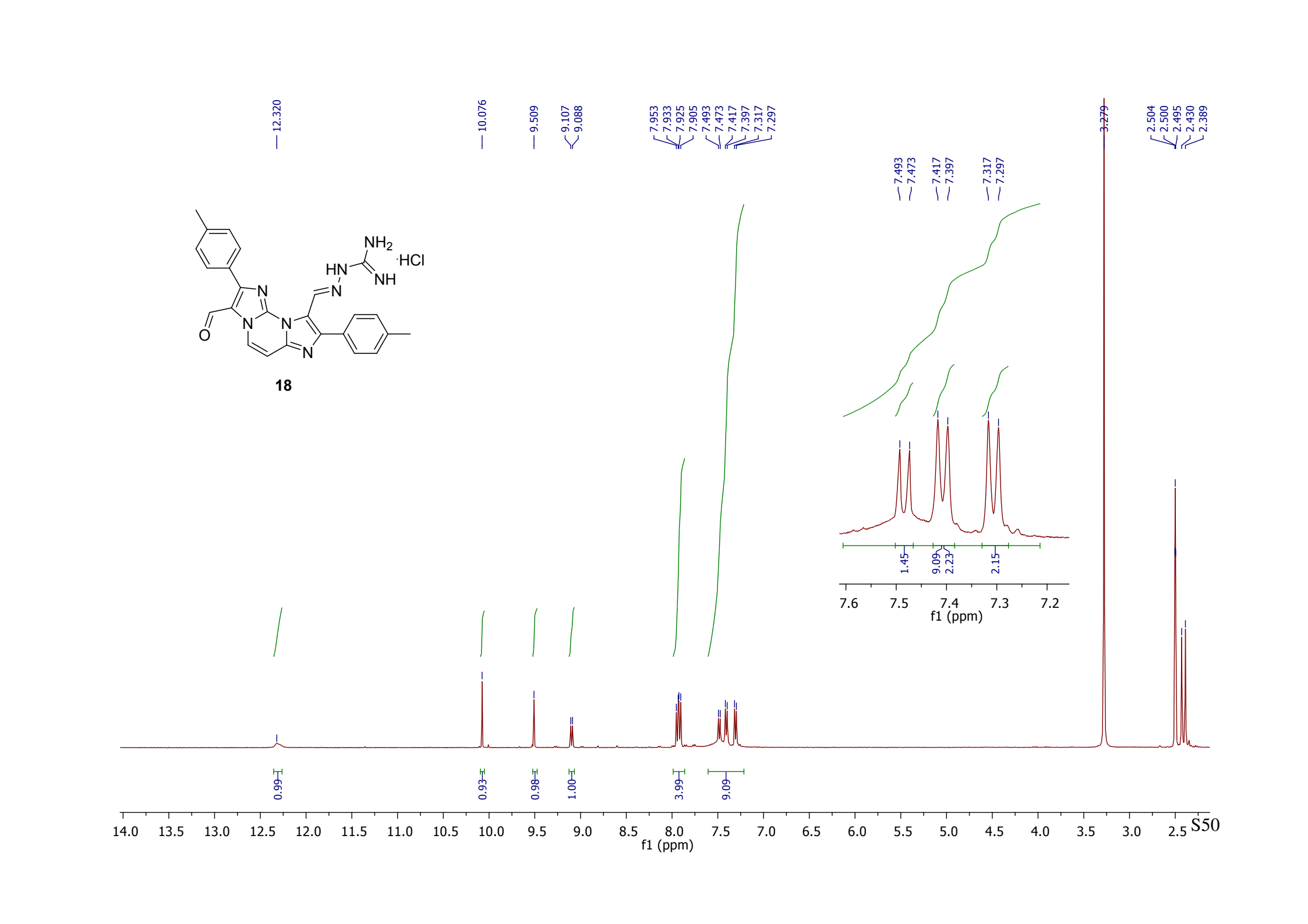
152.93
142.42
140.06
138.13
133.09
129.59
129.55
129.41
128.93
128.21
127.96
120.02
118.38
104.93
40.15
39.94
39.73
39.52
39.31
39.10
38.90
20.97
NH2
HCl
HN
NH
N
N
N
N
O
17
146.02
142.42
140.50
140.06
138.13
145
140
f1 (ppm)
129.59
129.55
129.41
128.93
128.21
127.96
126.20
130
129
128
127
126
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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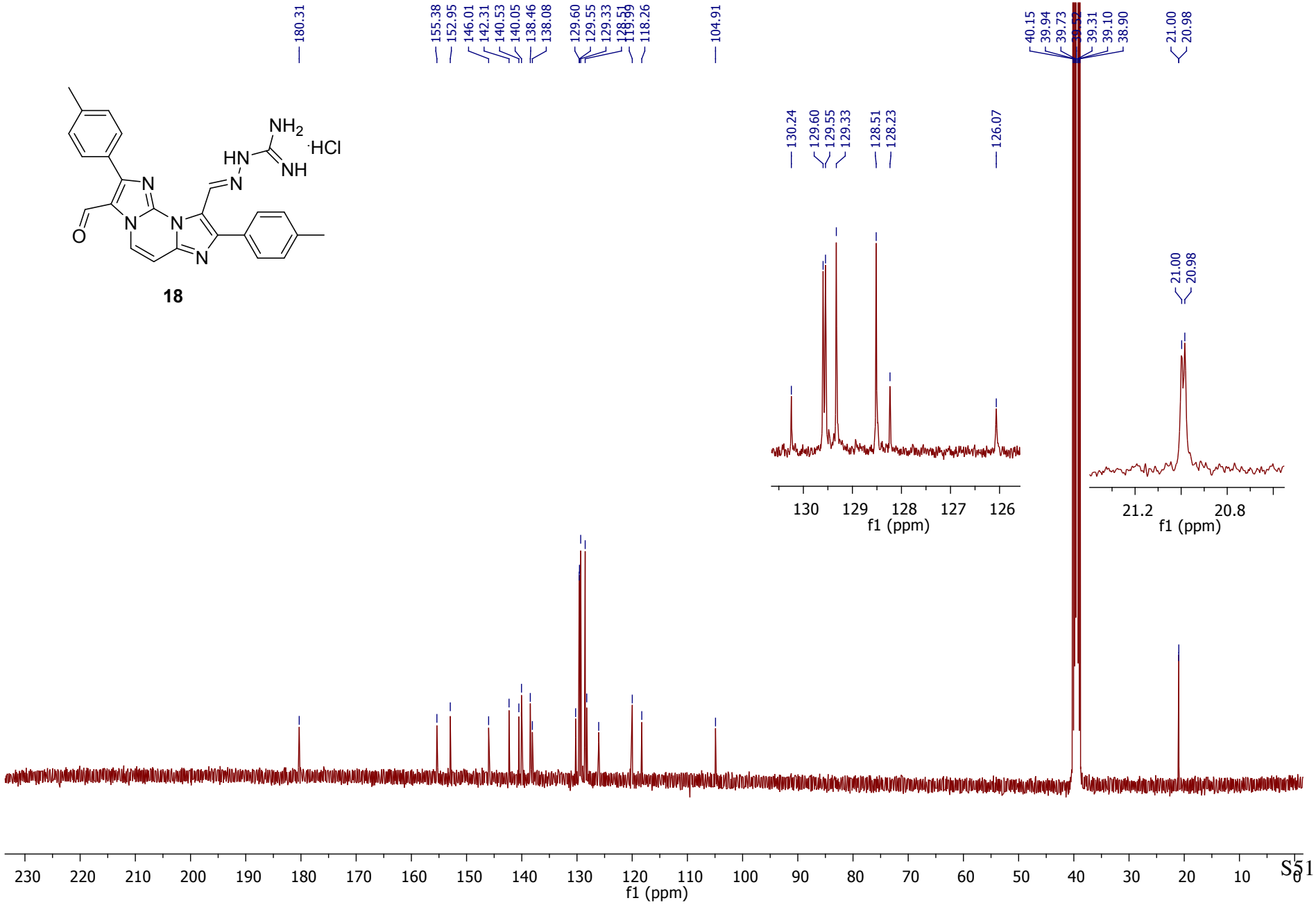
9.107
9.088
7.953
7.933
7.925
7.905
7.493
7.473
7.417
7.397
7.317
7.297
3.279
2.504
2.500
2.495
2.430
2.389
NH2
·HCl
HN
NH
N
O
18
0.99
0.93
0.98
1.00
3.99
9.09
1.45
9.09
2.23
2.15
f1 (ppm)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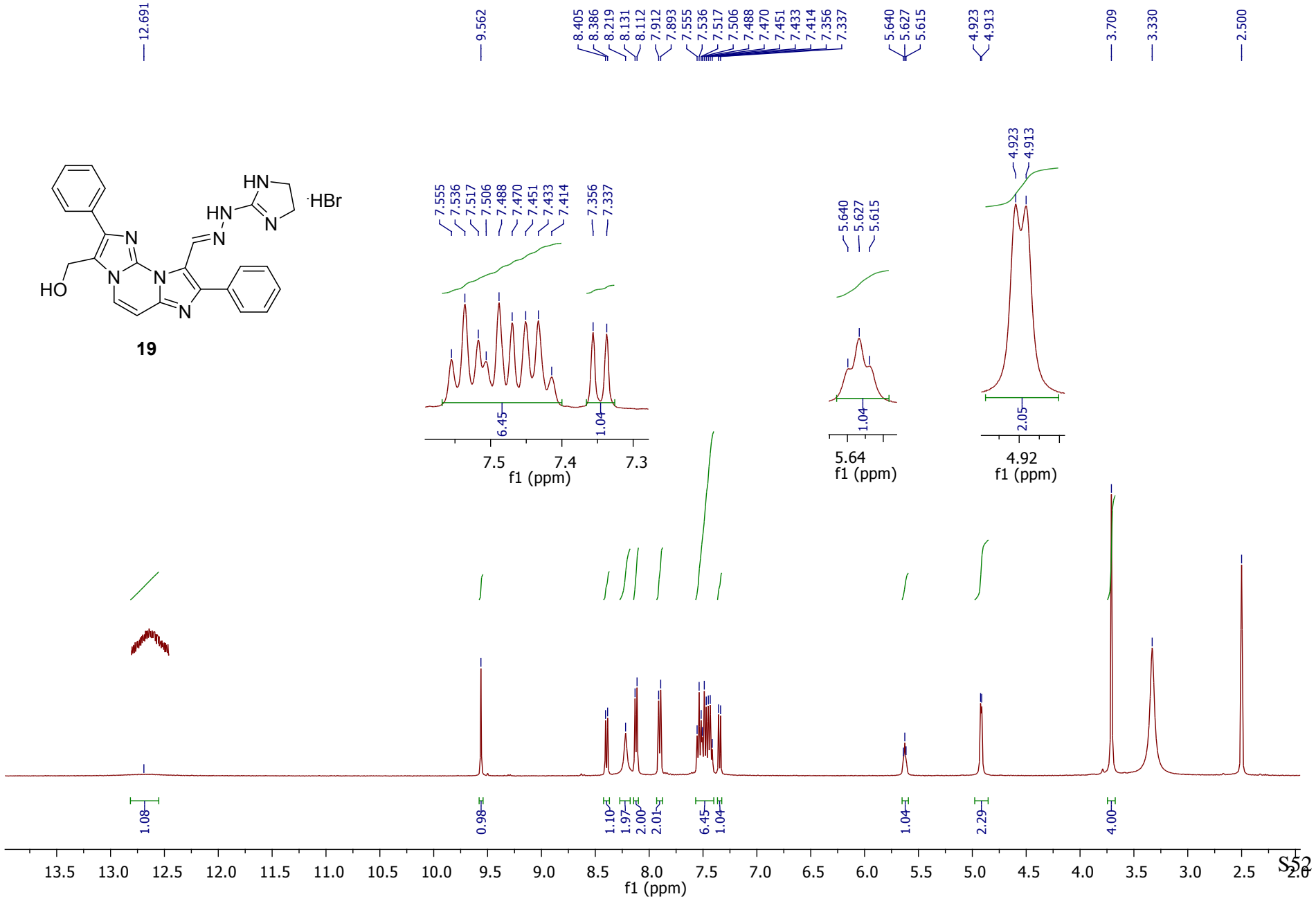
155.38
152.95
146.01
142.31
140.53
140.05
138.46
138.08
129.60
129.55
129.33
128.51
128.23
118.26
104.91
40.15
39.94
39.73
39.52
39.31
39.10
38.90
21.00
20.98
NH2
·HCl
HN
NH
N
O
18
130.24
129.60
129.55
129.33
128.51
128.23
126.07
130
129
128
127
126
f1 (ppm)
21.00
20.98
21.2
20.8
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.912
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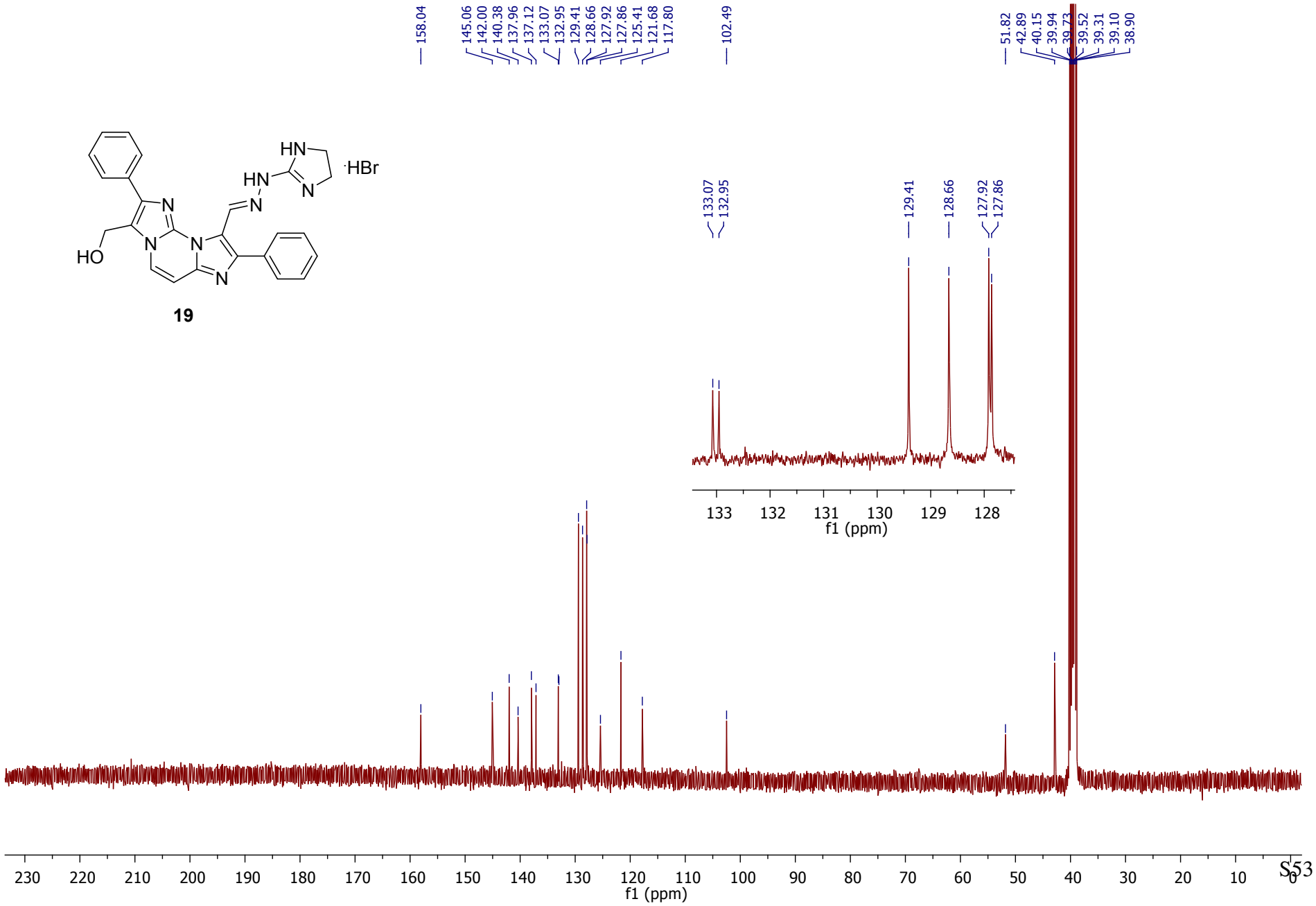
7.536
7.517
7.506
7.488
7.470
7.451
7.433
7.414
7.356
7.337
5.640
5.627
5.615
4.923
4.913
3.709
3.330
2.500
HN
HN
HBr
N
N
N
N
N
HO
19
6.45
1.04
7.5
7.4
7.3
f1 (ppm)
1.04
5.64
f1 (ppm)
2.05
4.92
f1 (ppm)
1.08
0.98
1.10
1.97
2.00
2.01
6.45
1.04
1.04
2.29
4.00
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
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
f1 (ppm)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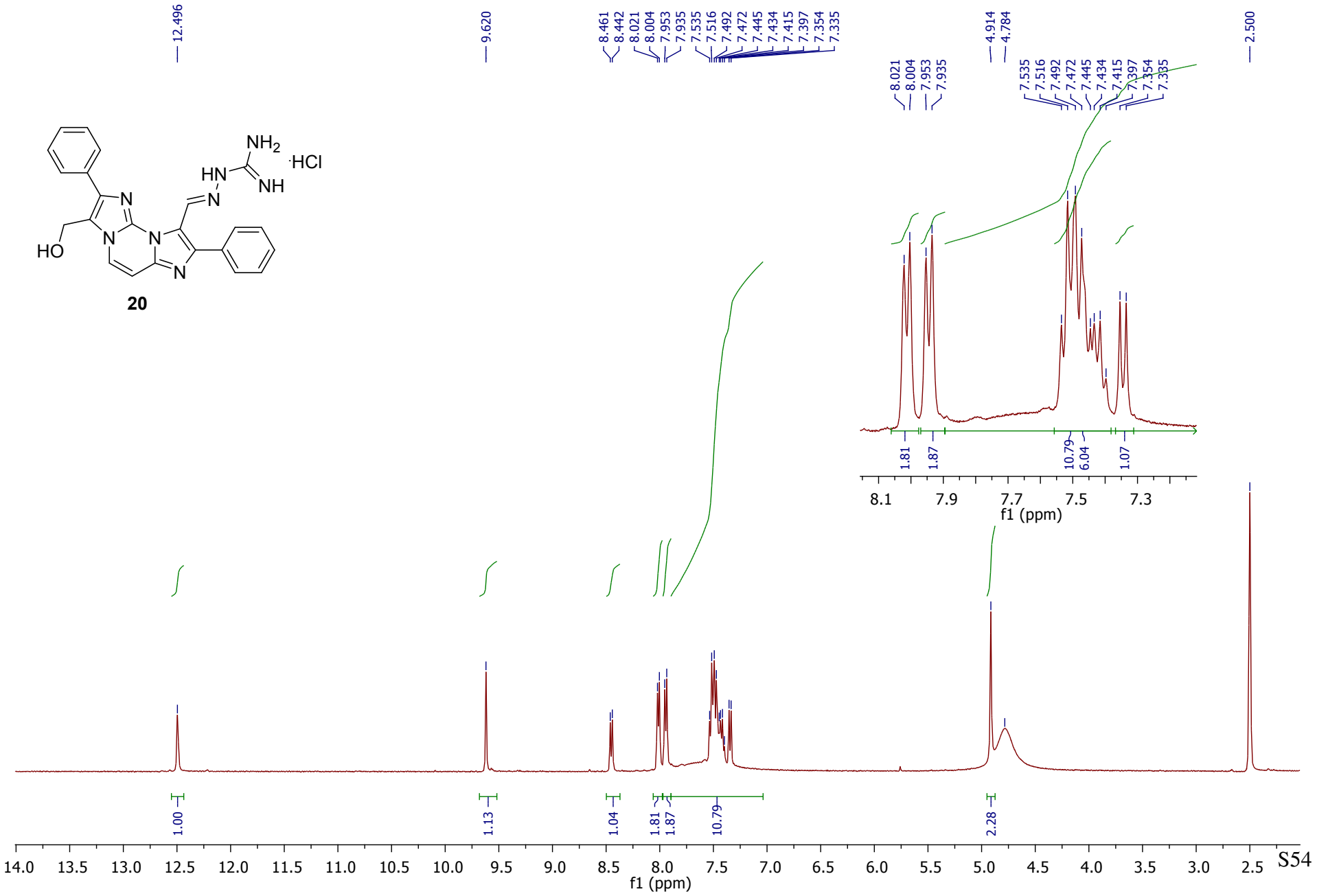
137.12
133.07
132.95
129.41
128.66
127.92
127.86
125.41
121.68
117.80
102.49
51.82
42.89
40.15
39.94
39.73
39.52
39.31
39.10
38.90
HN
HN
N
N
·HBr
N
N
N
HO
N
19
133.07
132.95
129.41
128.66
127.92
127.86
133
132
131
130
129
128
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.021
8.004
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7.935
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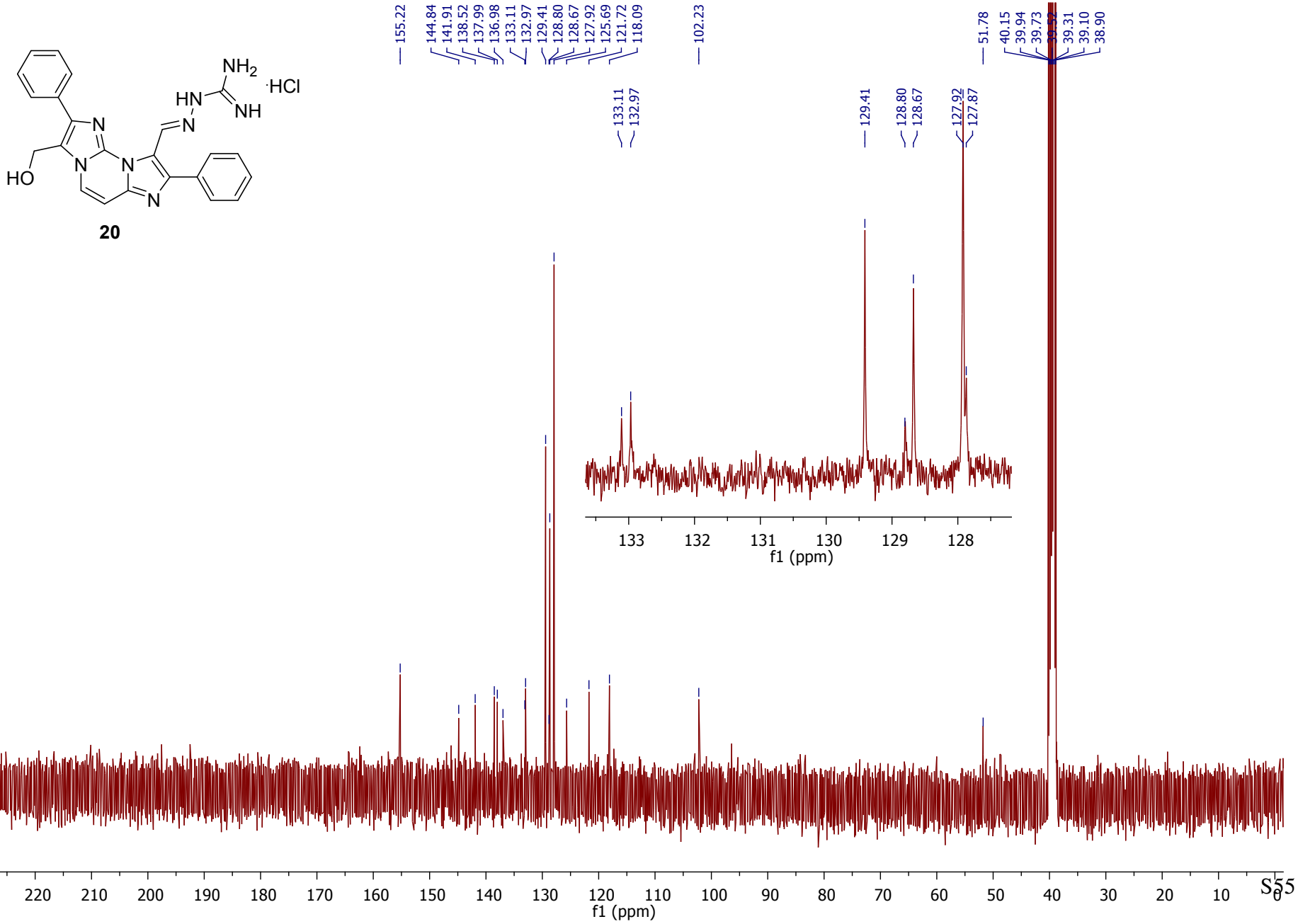
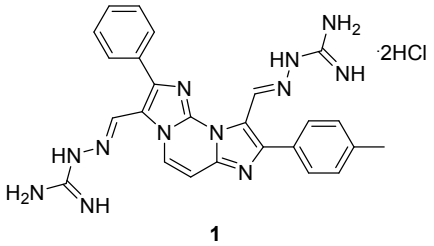
7.516
7.492
7.472
7.445
7.434
7.415
7.397
7.354
7.335
4.914
4.784
2.500
NH2
HCl
HN
NH
N
HO
20
1.00
1.13
1.04
1.81
1.87
10.79
2.28
f1 (ppm)

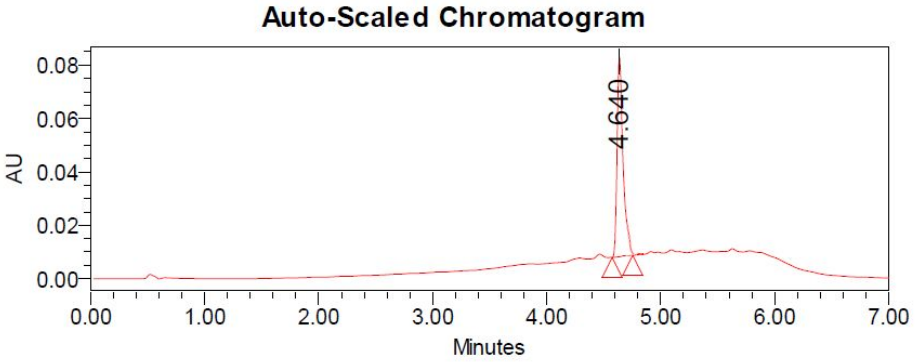

NH2
·HCl
HN
NH
N
N
N
N
HO
N
155.22
144.84
141.91
138.52
137.99
136.98
133.11
132.97
129.41
128.80
128.67
127.92
125.69
121.72
118.09
102.23
51.78
40.15
39.94
39.73
39.52
39.31
39.10
38.90
133.11
132.97
129.41
128.80
128.67
127.92
127.87
133
132
131
130
129
128
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

20

UHPLC traces of compounds 1, 2, 8, 10, 15, 19 and 20.

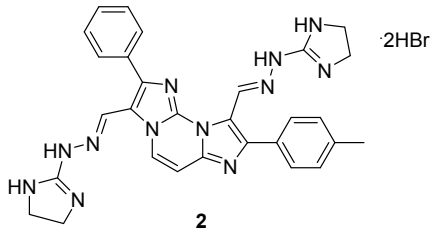


1

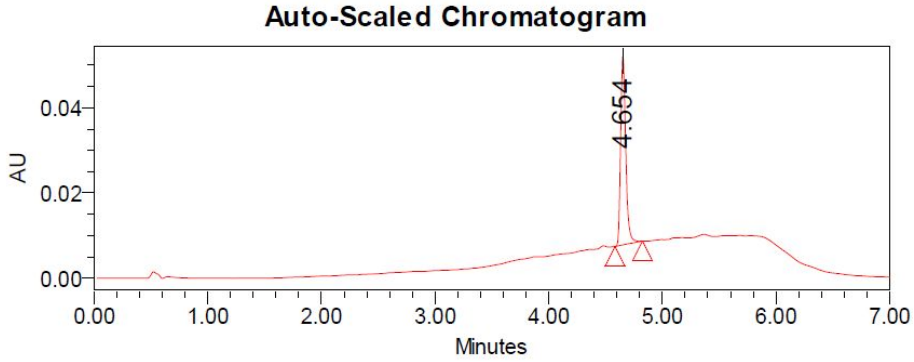


Peak Results

	Name	RT	Area	% Area	Height
1		4.640	287523	100.00	74356

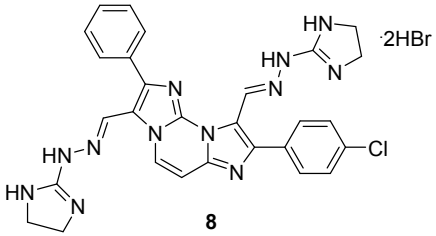


2

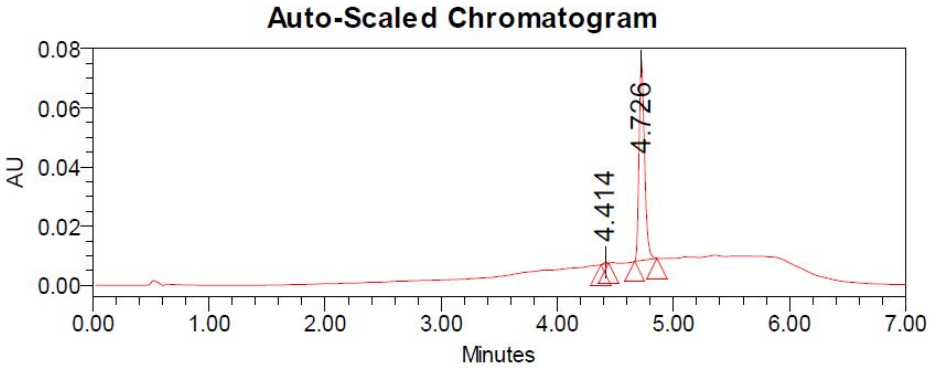


Peak Results

	Name	RT	Area	% Area	Height
1		4.654	137918	100.00	44448

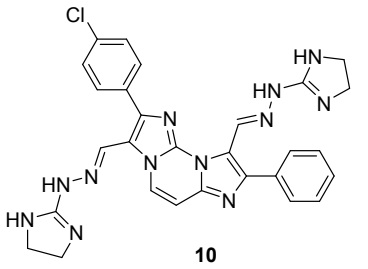


8



Peak Results

	Name	RT	Area	% Area	Height
1		4.414	896	0.41	426
2		4.726	219999	99.59	67621



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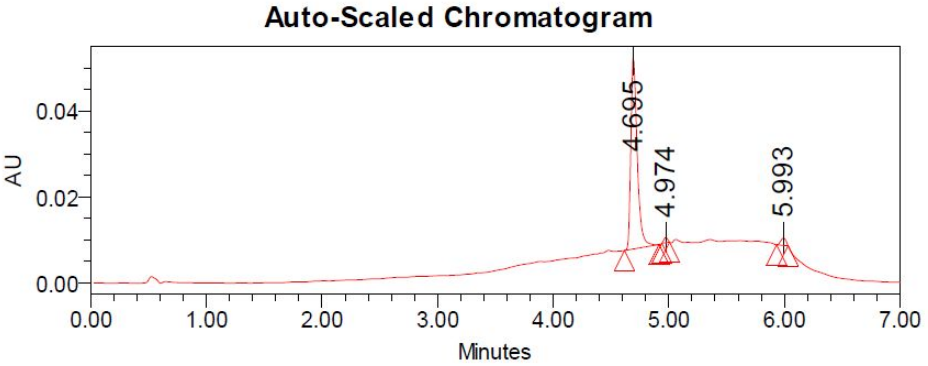
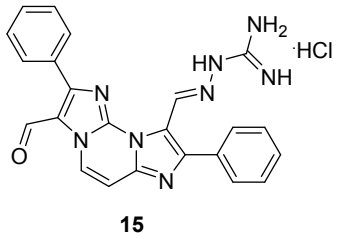
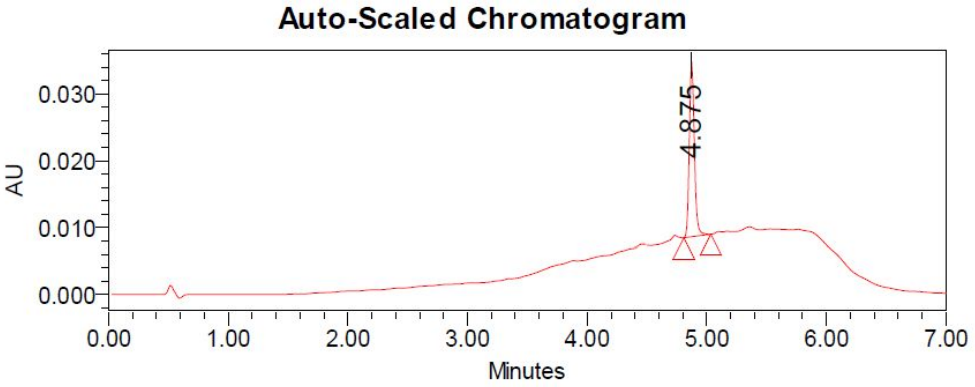


Peak Results

	Name	RT	Area	% Area	Height
1		4.695	169168	95.41	44669
2		4.974	2773	1.56	1245
3		5.993	5368	3.03	1700

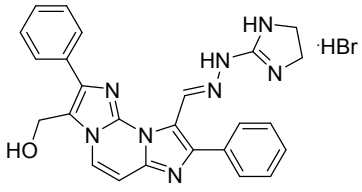


15

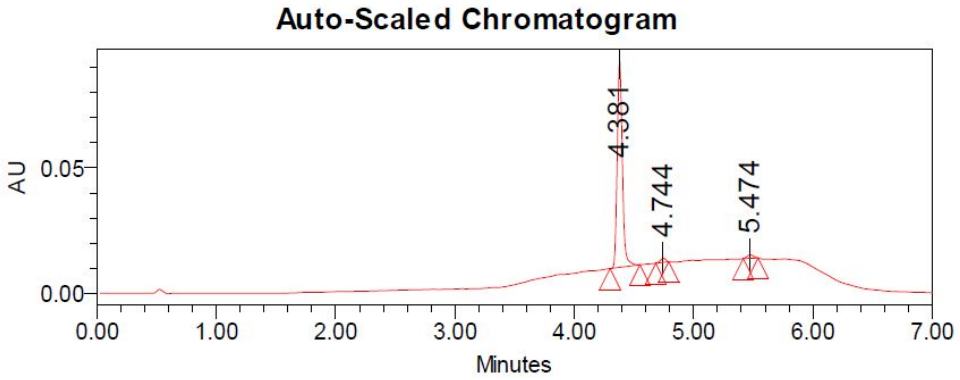


Peak Results

	Name	RT	Area	% Area	Height
1		4.875	76505	100.00	25970

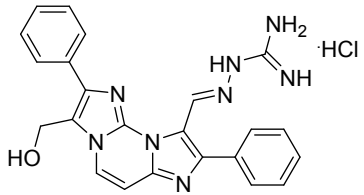


19

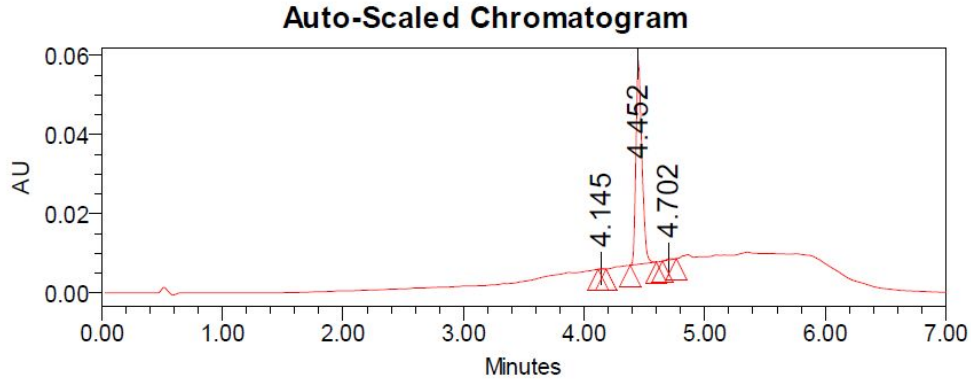


Peak Results

	Name	RT	Area	% Area	Height
1		4.381	244592	95.94	81928
2		4.744	5093	2.00	1601
3		5.474	5259	2.06	1357



20



Peak Results

	Name	RT	Area	% Area	Height
1		4.145	234	0.13	112
2		4.452	177536	99.32	51383
3		4.702	990	0.55	306

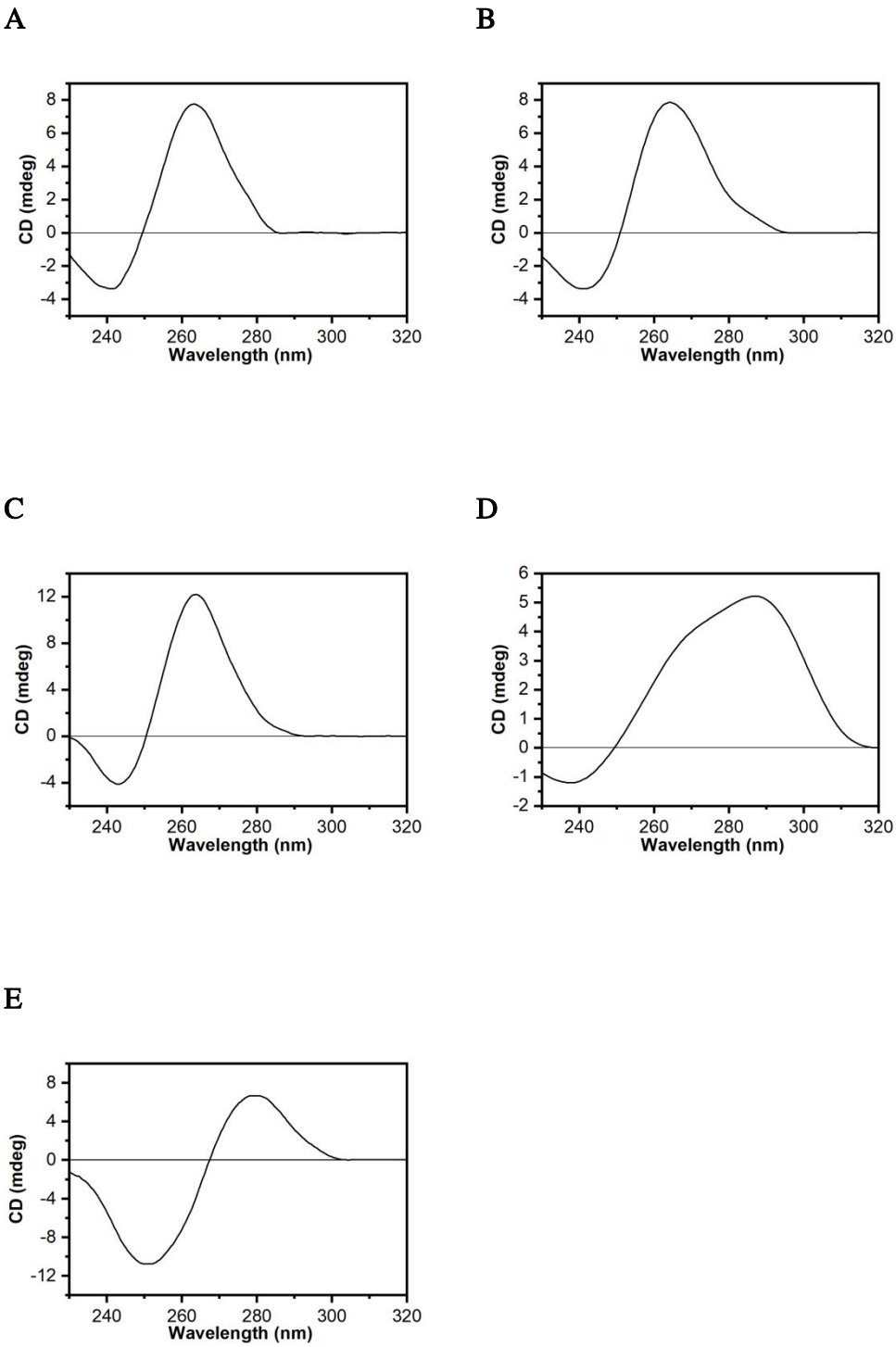


Figure S1. CD spectra of (A) *c-kit1*, (B) *c-kit2*, (C) *c-myc*, (D) tel_{26}, and (E) *hairpin* in 5 mM KH_2PO_4 aqueous buffer (pH 7.0) containing 20 mM KCl at 20 °C.

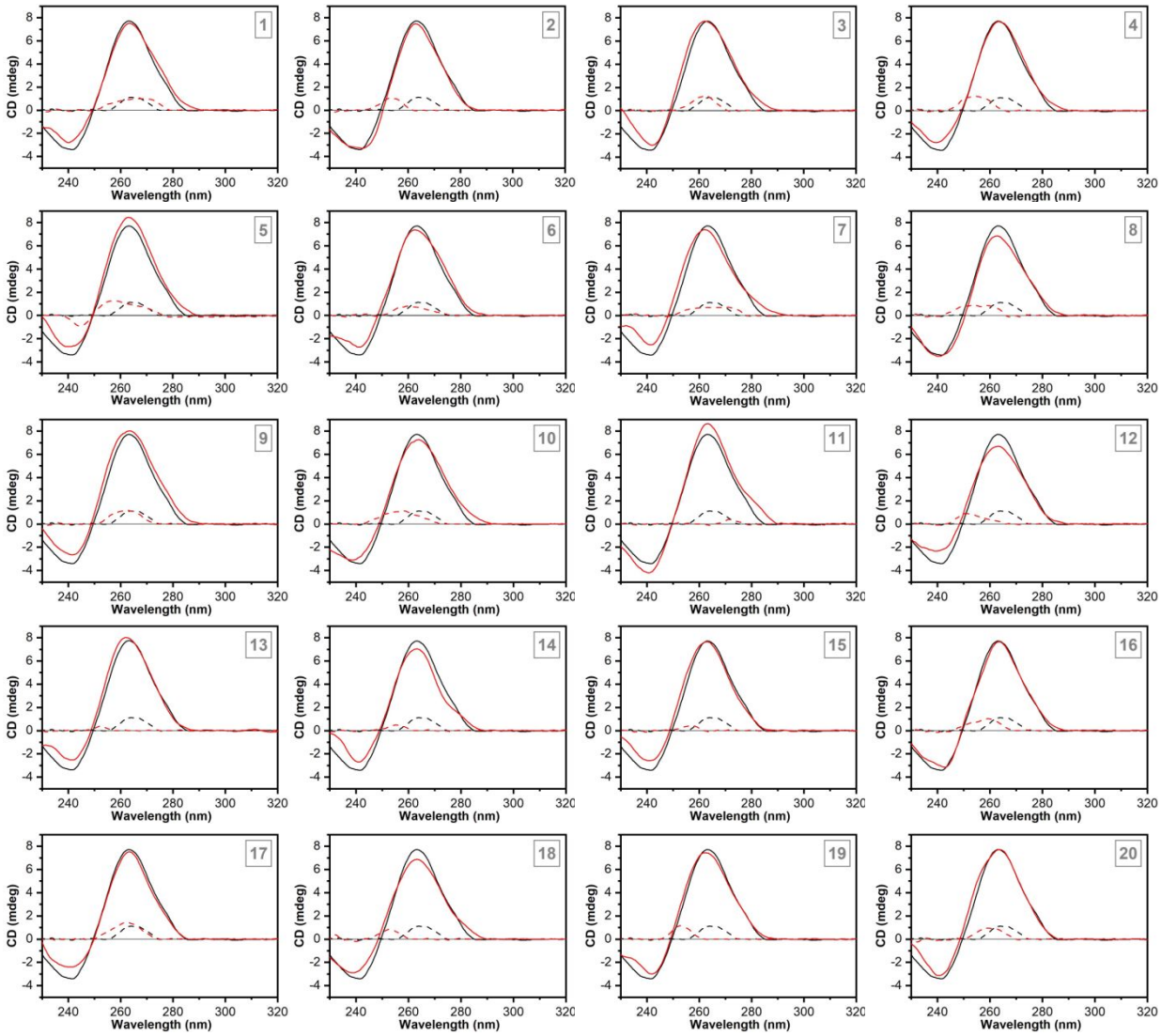


Figure S2. CD spectra of *c-kit1* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20**, recorded at 20 and 100 ˚C (solid and dashed lines, respectively).

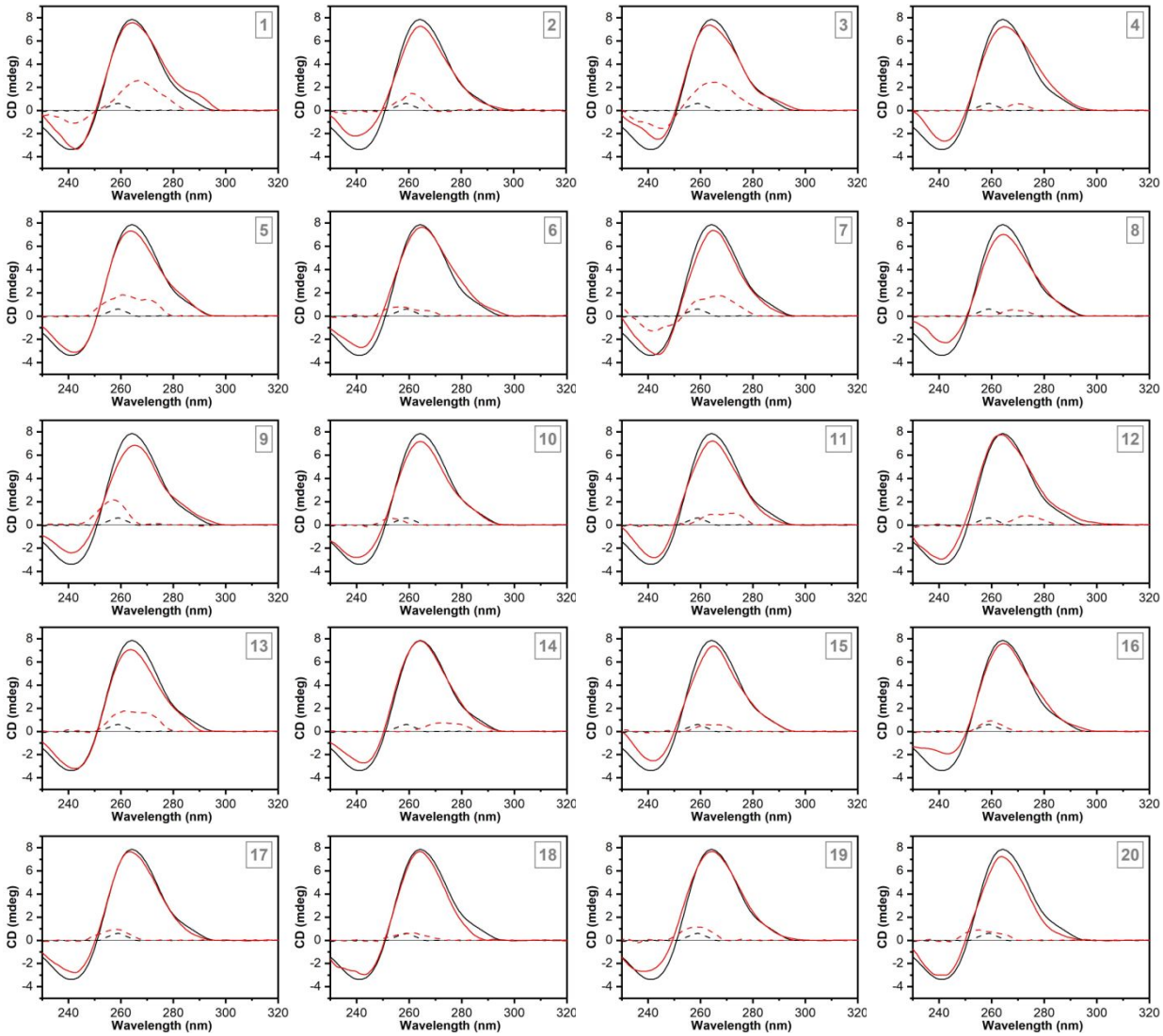


Figure S3. CD spectra of *c-kit2* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20**, recorded at 20 and 100 ˚C (solid and dashed lines, respectively).

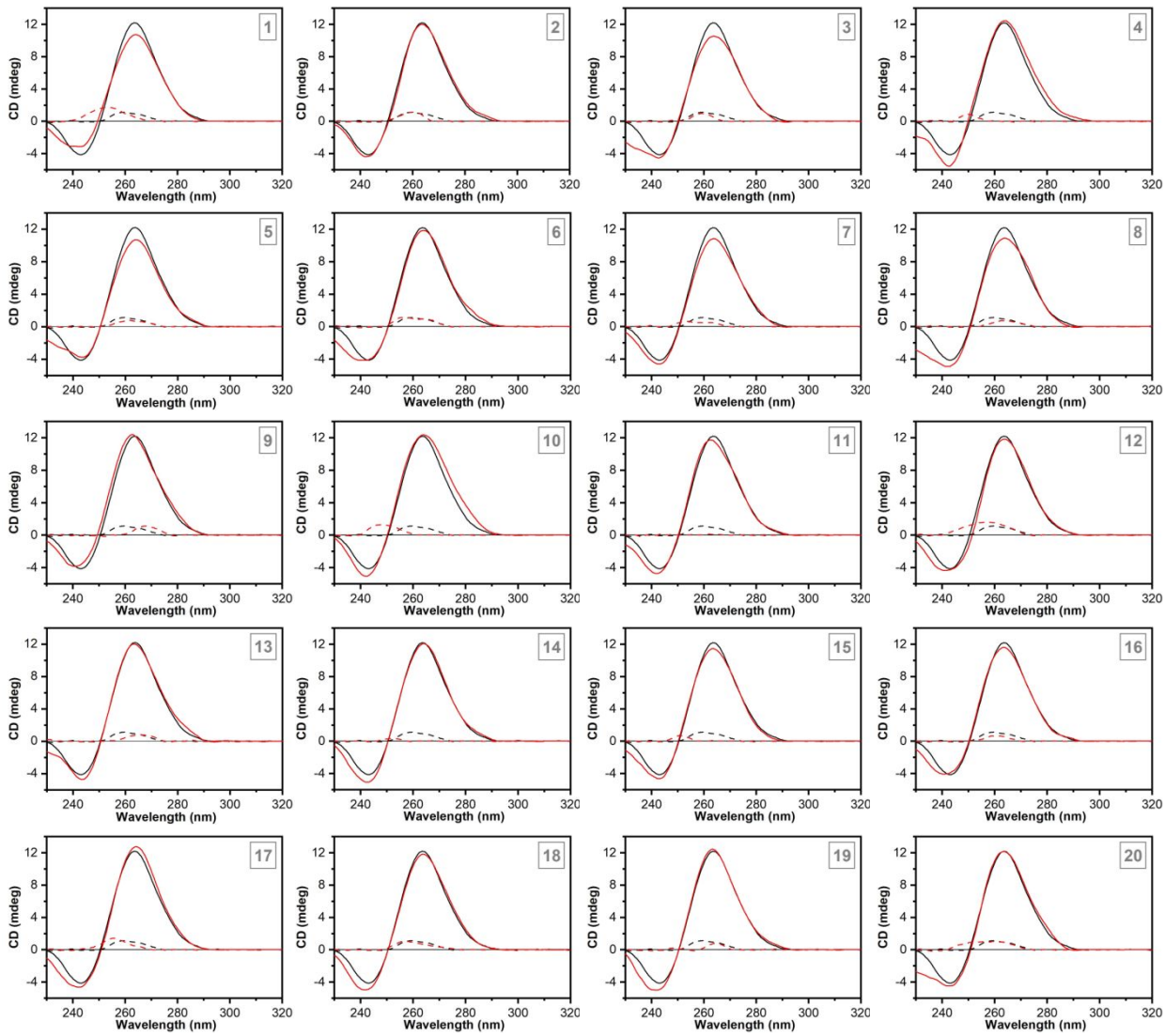


Figure S4. CD spectra of *c-myc* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20**, recorded at 20 and 100 ˚C (solid and dashed lines, respectively).

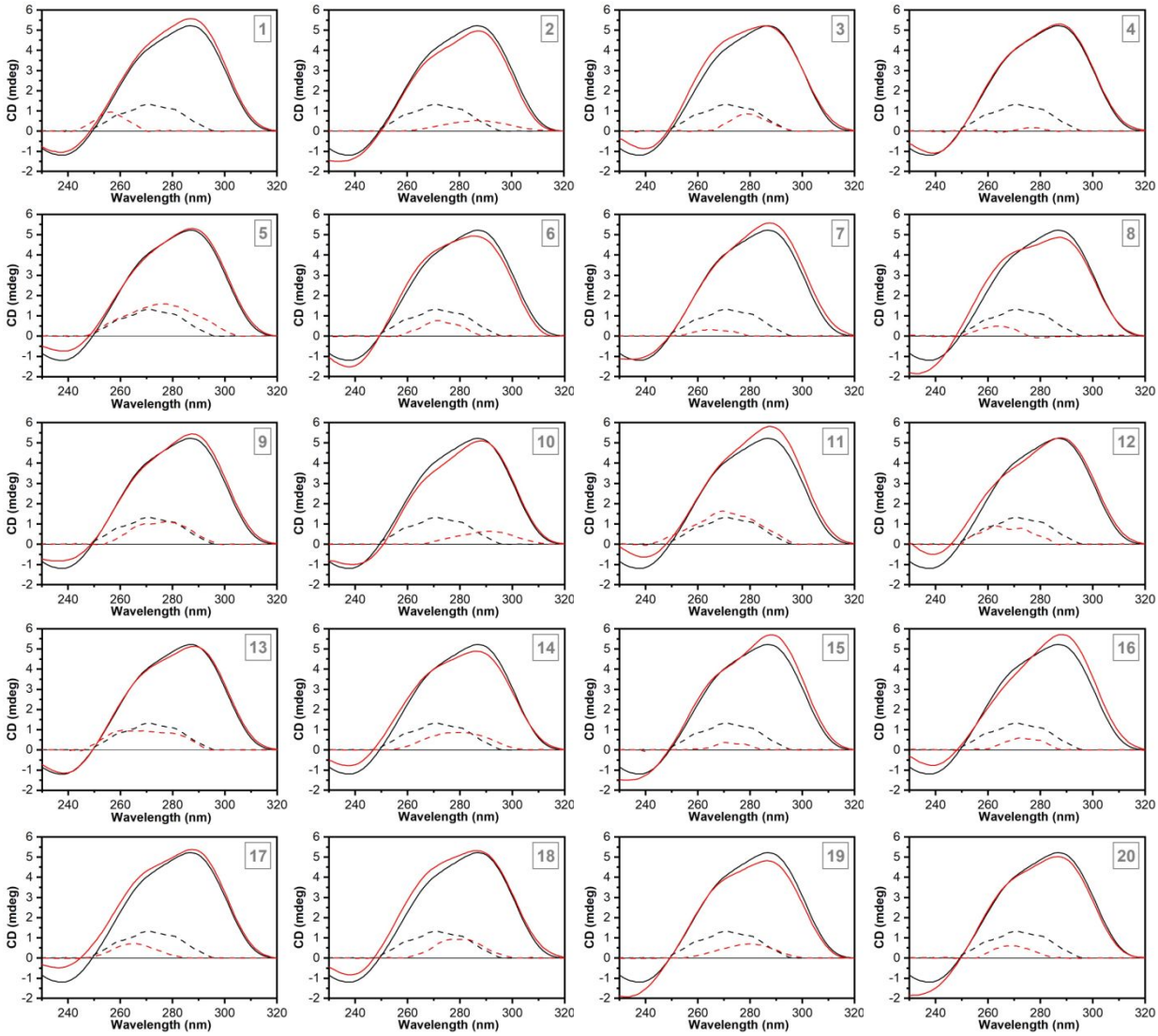


Figure S5. CD spectra of *tel*$_{26}$ in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20**, recorded at 20 and 100 ˚C (solid and dashed lines, respectively).

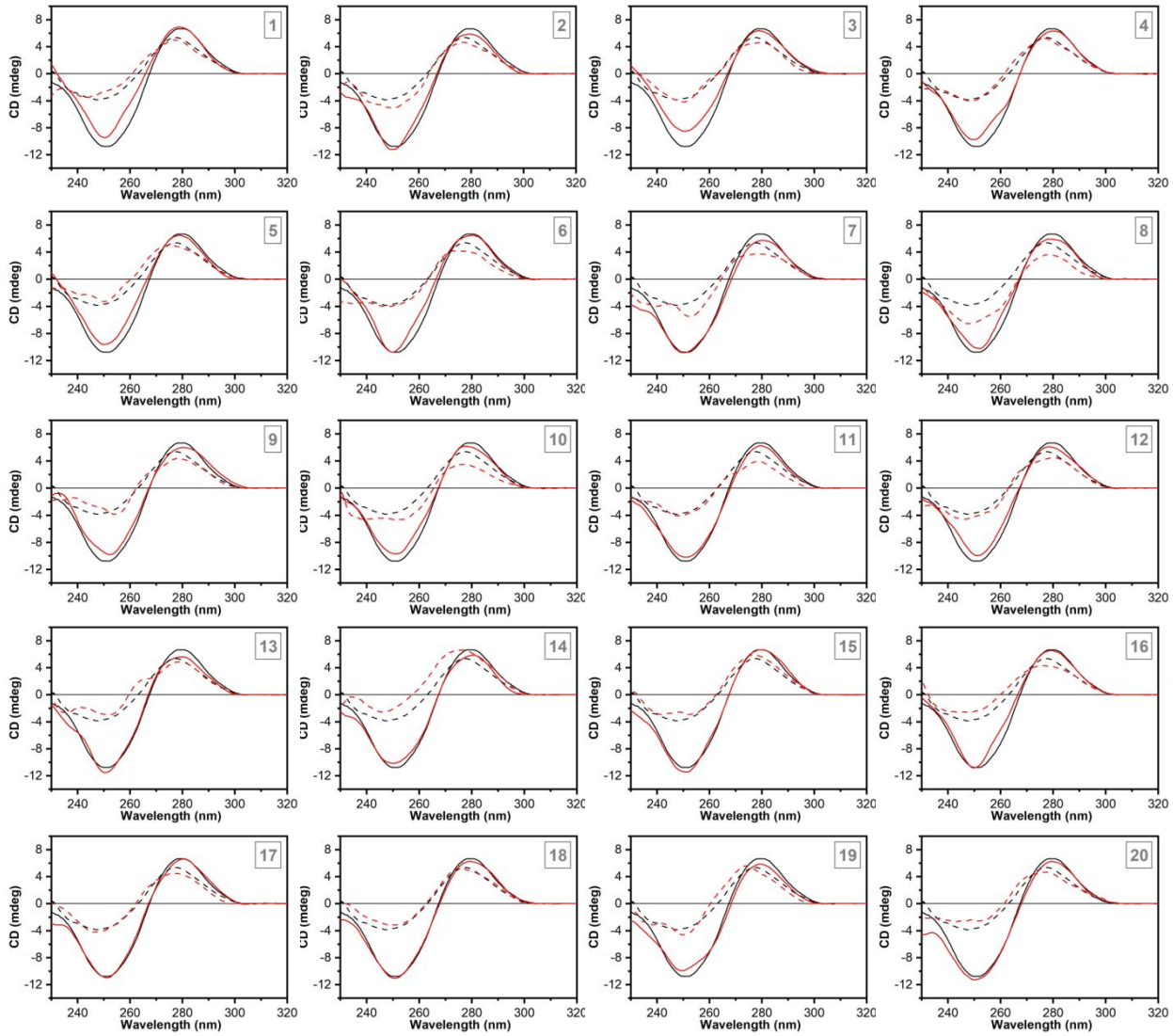


Figure S6. CD spectra of *hairpin* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20**, recorded at 20 and 100 ˚C (solid and dashed lines, respectively).

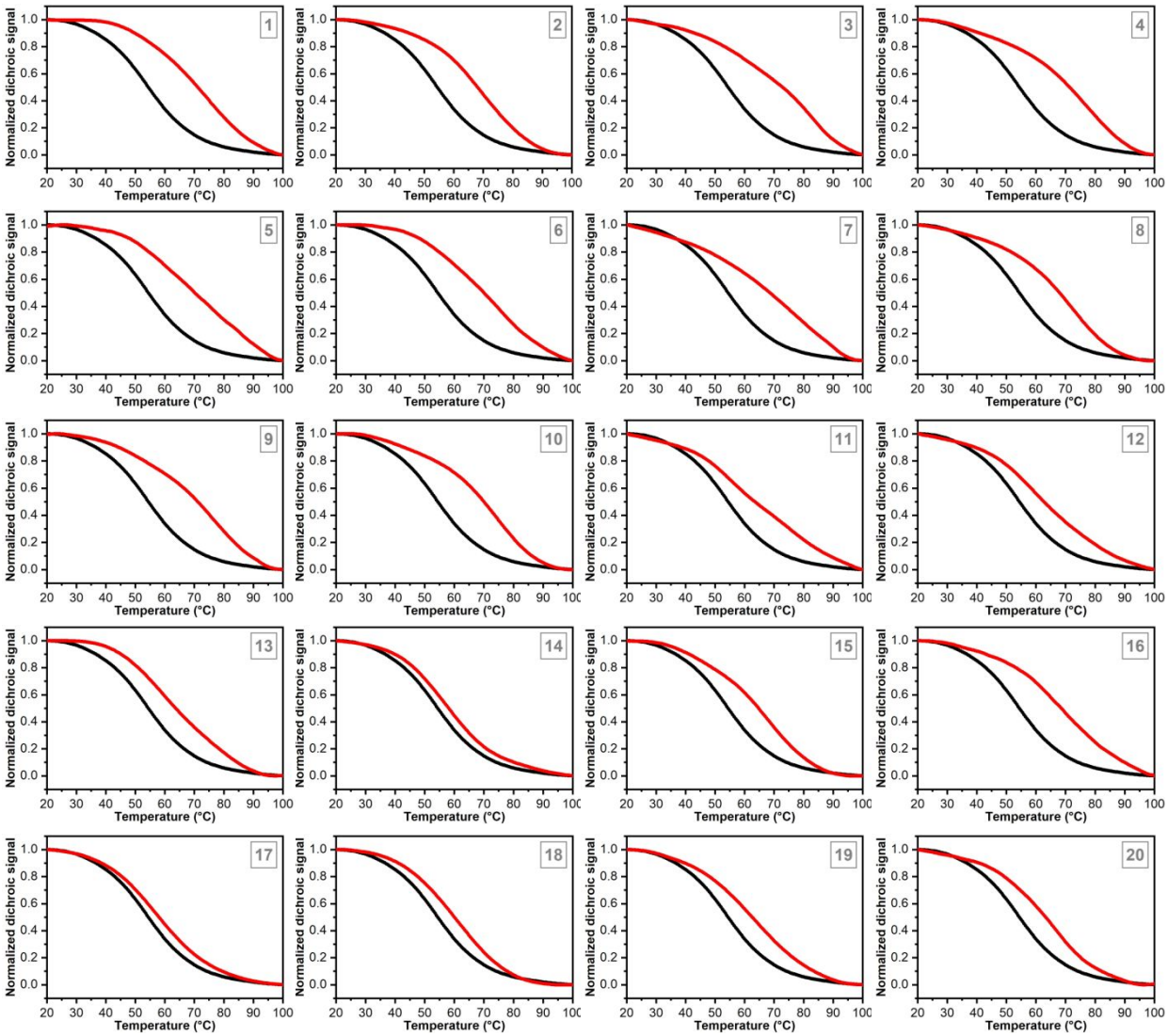


Figure S7. Normalized CD melting curves of *c-kit1* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20** recorded at 1 °C/min heating rate.

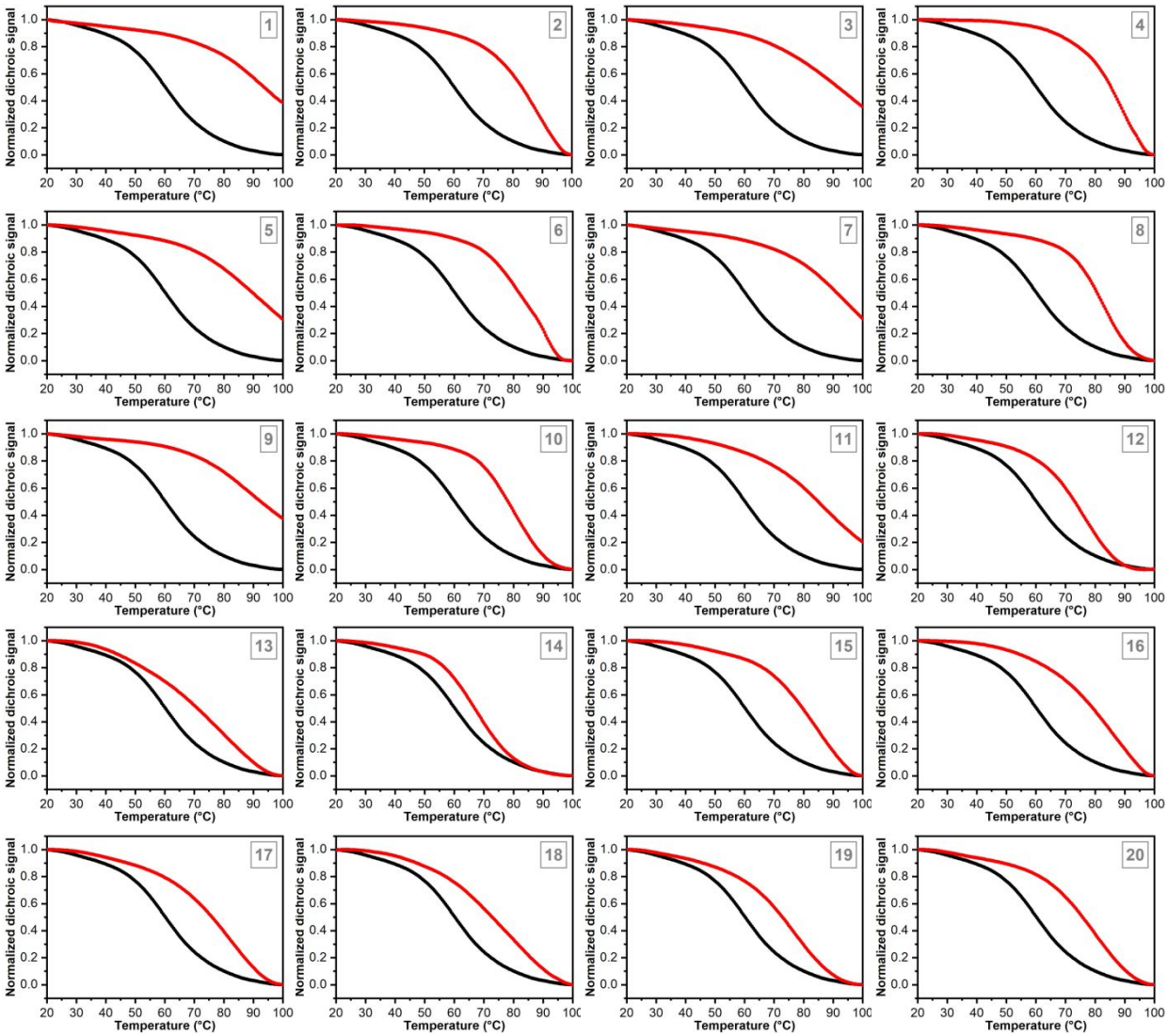


Figure S8. Normalized CD melting curves of *c-kit2* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20** recorded at 1 ˚C/min heating rate. Due to the exceptional ligand-induced thermal stabilization of G-quadruplex observed for compounds **1**, **3**, **5**, **7**, **9**, and **11** (the melting processes are not completed even at 100 ˚C), the corresponding melting curves were normalized by dividing only by the maximum.

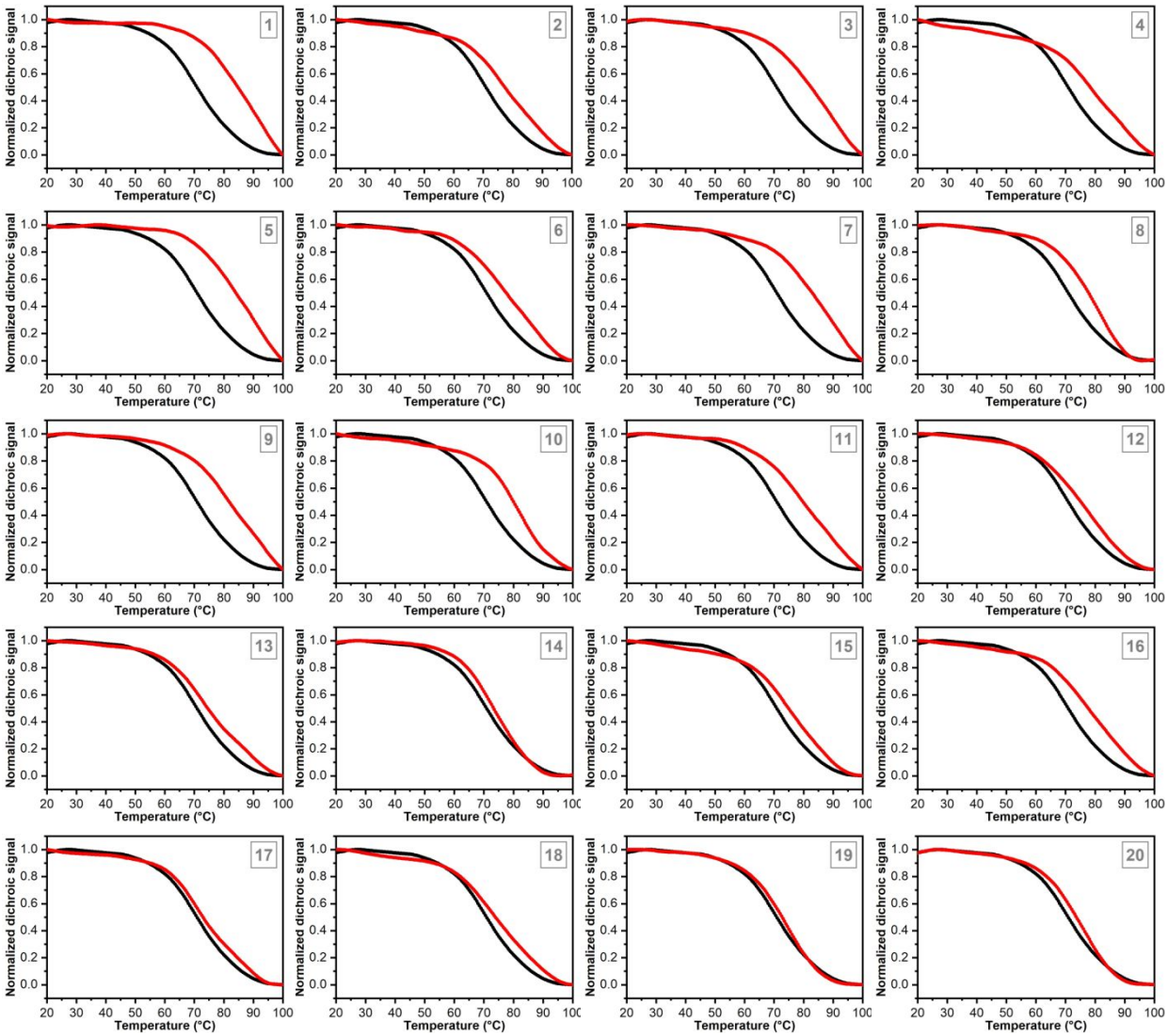


Figure S9. Normalized CD melting curves of *c-myc* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20** recorded at 1 °C/min heating rate.

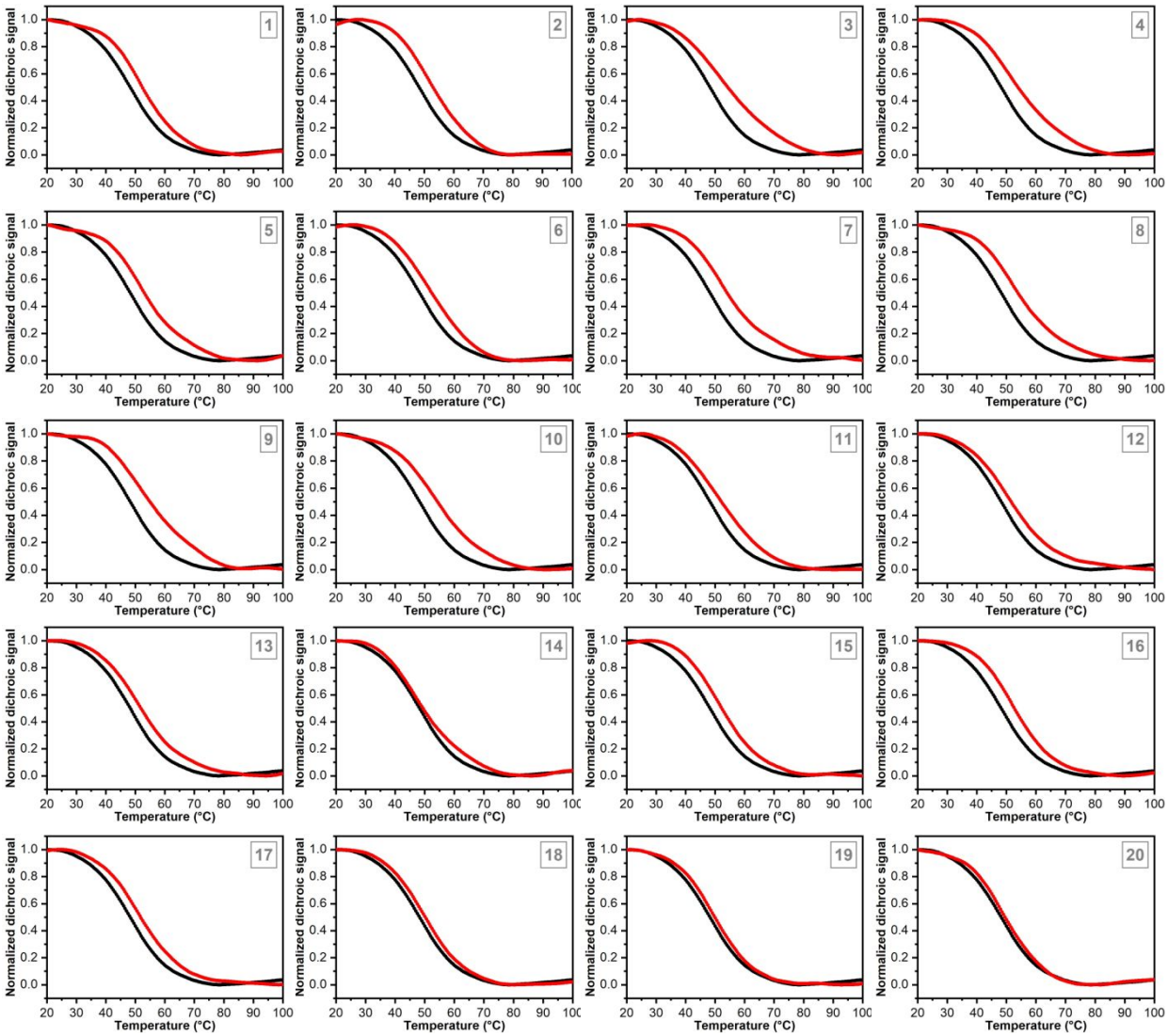


Figure S10. Normalized CD melting curves of tel_{26} in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20** recorded at 1 °C/min heating rate.

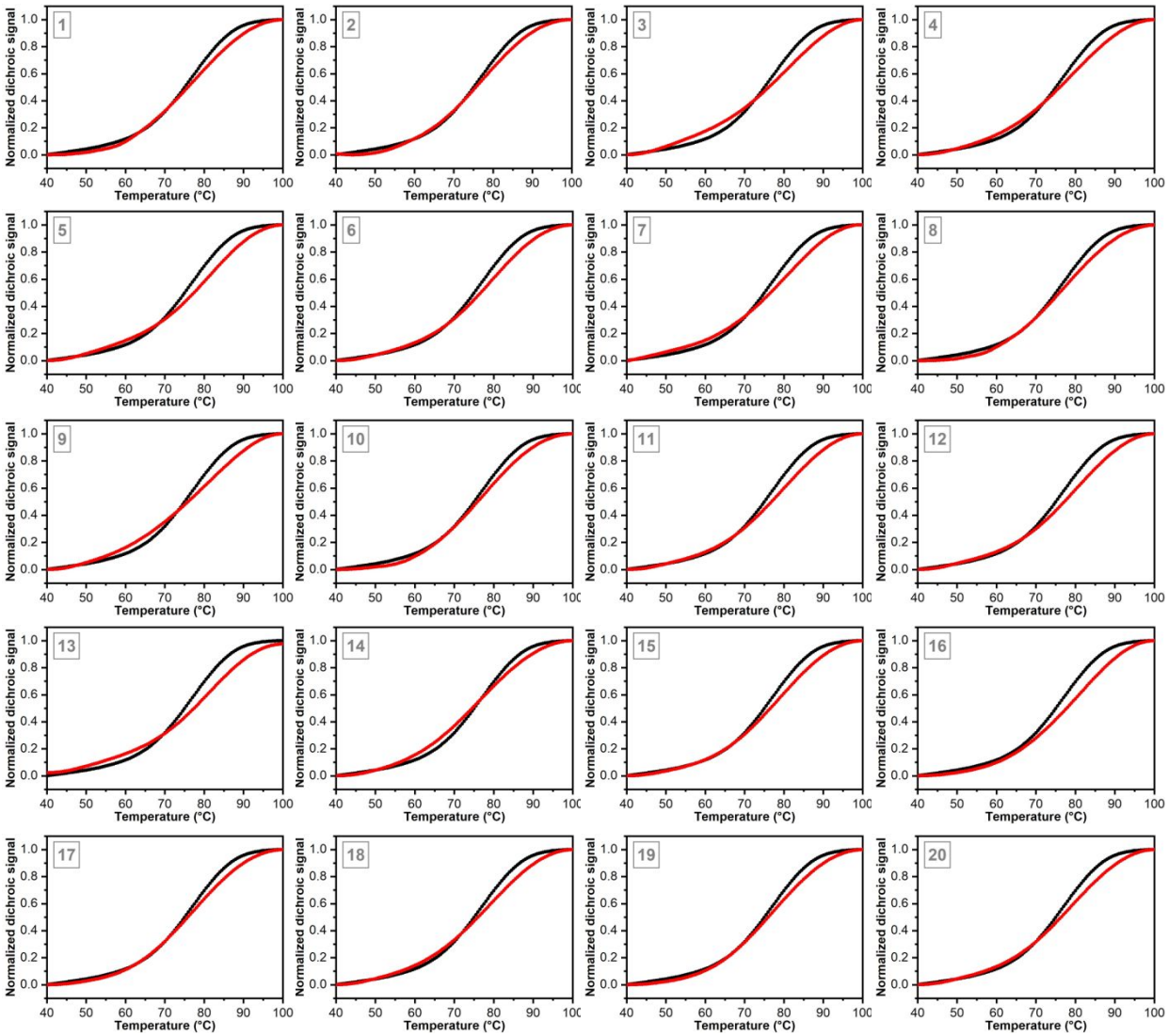


Figure S11. Normalized CD melting curves of *hairpin* in the absence (black lines) and presence (red lines) of 2 molar equiv of compounds **1**–**20** recorded at 1 °C/min heating rate.

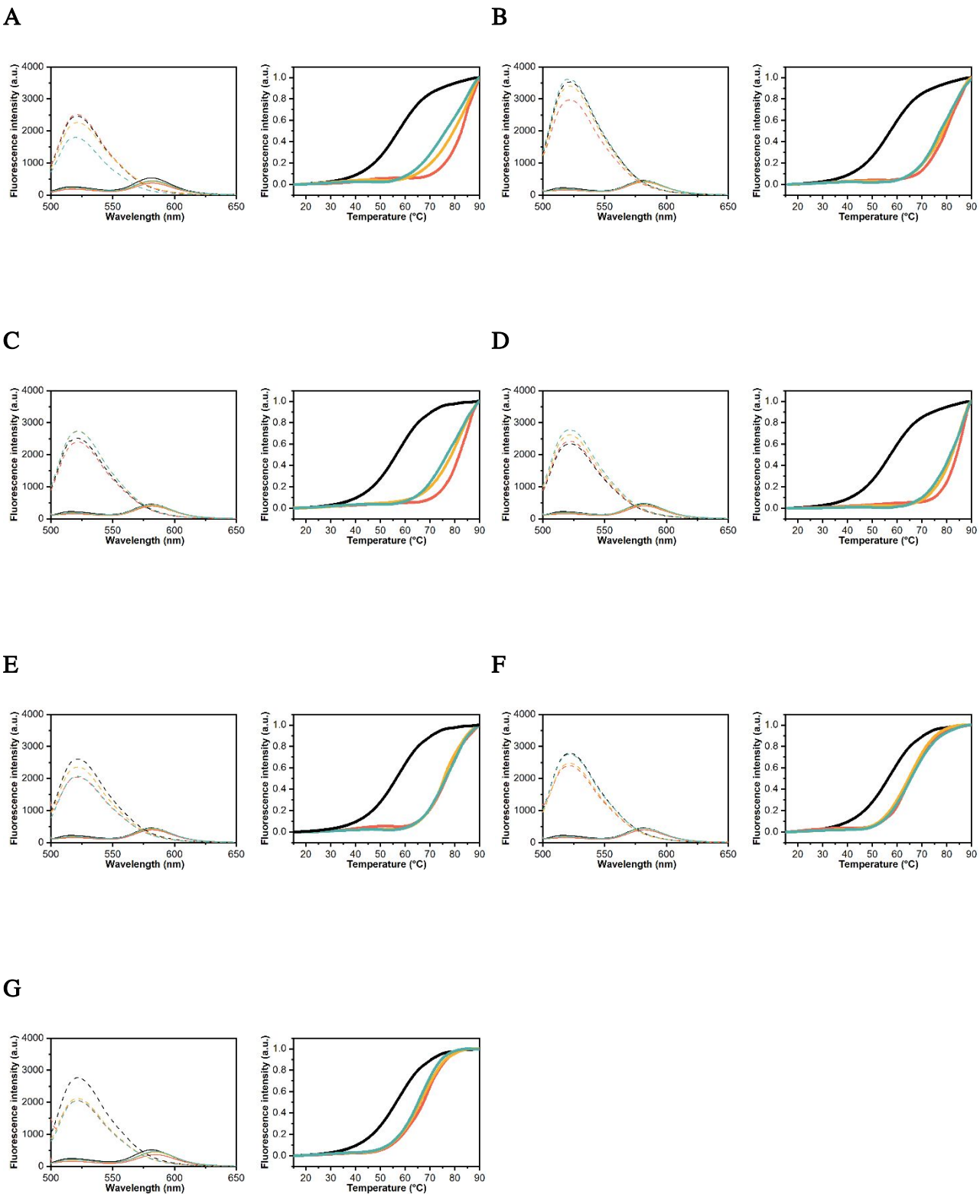
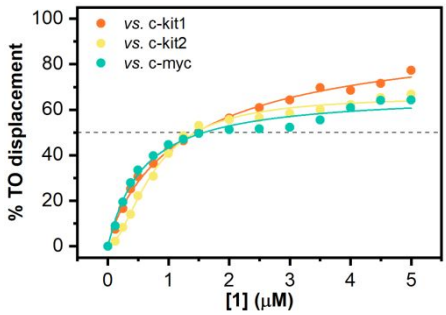


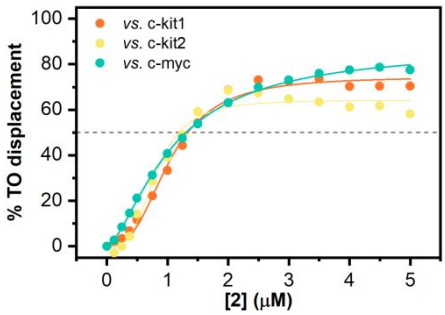
Figure S12. Fluorescence emission spectra (left of each panel) acquired at 15 and 90 °C (solid and dashed lines, respectively), and normalized FRET melting curves recorded at 0.2 °C/min (right of each panel) for *F-c-kit1-T* oligonucleotide (0.2 μM) in the absence (black lines) and presence (red

lines) of (A) **1**, (B) **2**, (C) **8**, (D) **10**, (E) **15**, (F) **19**, and (G) **20** (0.4 μM). Experiments in the presence of compounds were also performed by adding large excesses of *hairpin* duplex competitor, i.e., 15 and 50 molar equiv with respect to G-quadruplex (yellow and green lines, respectively).

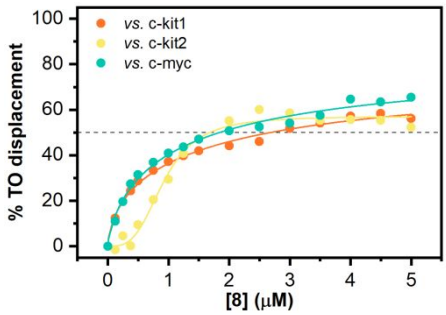
A


vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[1] (μM)

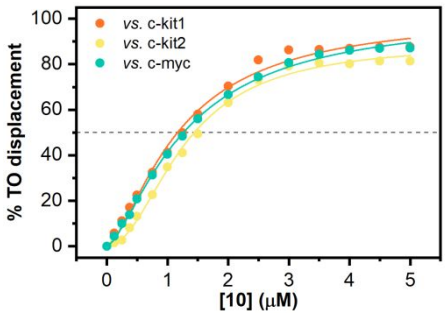
B


vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[2] (μM)

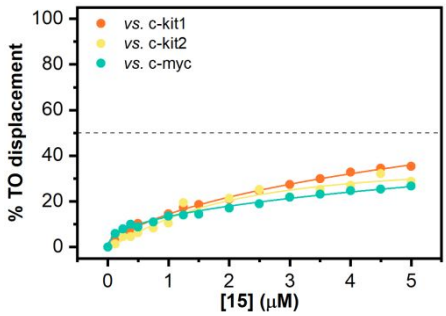
C


vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[8] (μM)

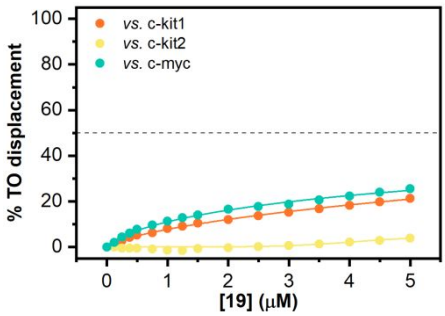
D


vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[10] (μM)

E


vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[15] (μM)

F


vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[19] (μM)

G

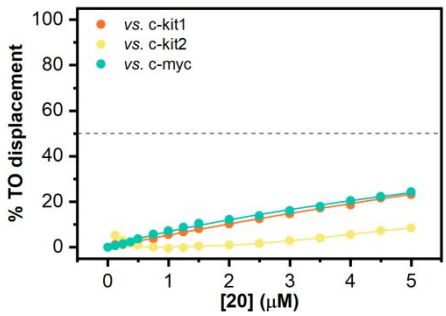

vs. c-kit1
vs. c-kit2
vs. c-myc
% TO displacement
[20] (μM)

Figure S13. Dose–response curves from fluorescent intercalator displacement (G4-FID) assay for *c-kit1*, *c-kit2*, and *c-myc* G-quadruplexes with compounds (A) **1**, (B) **2**, (C) **8**, (D) **10**, (E) **15**, (F) **19**, and (G) **20**.

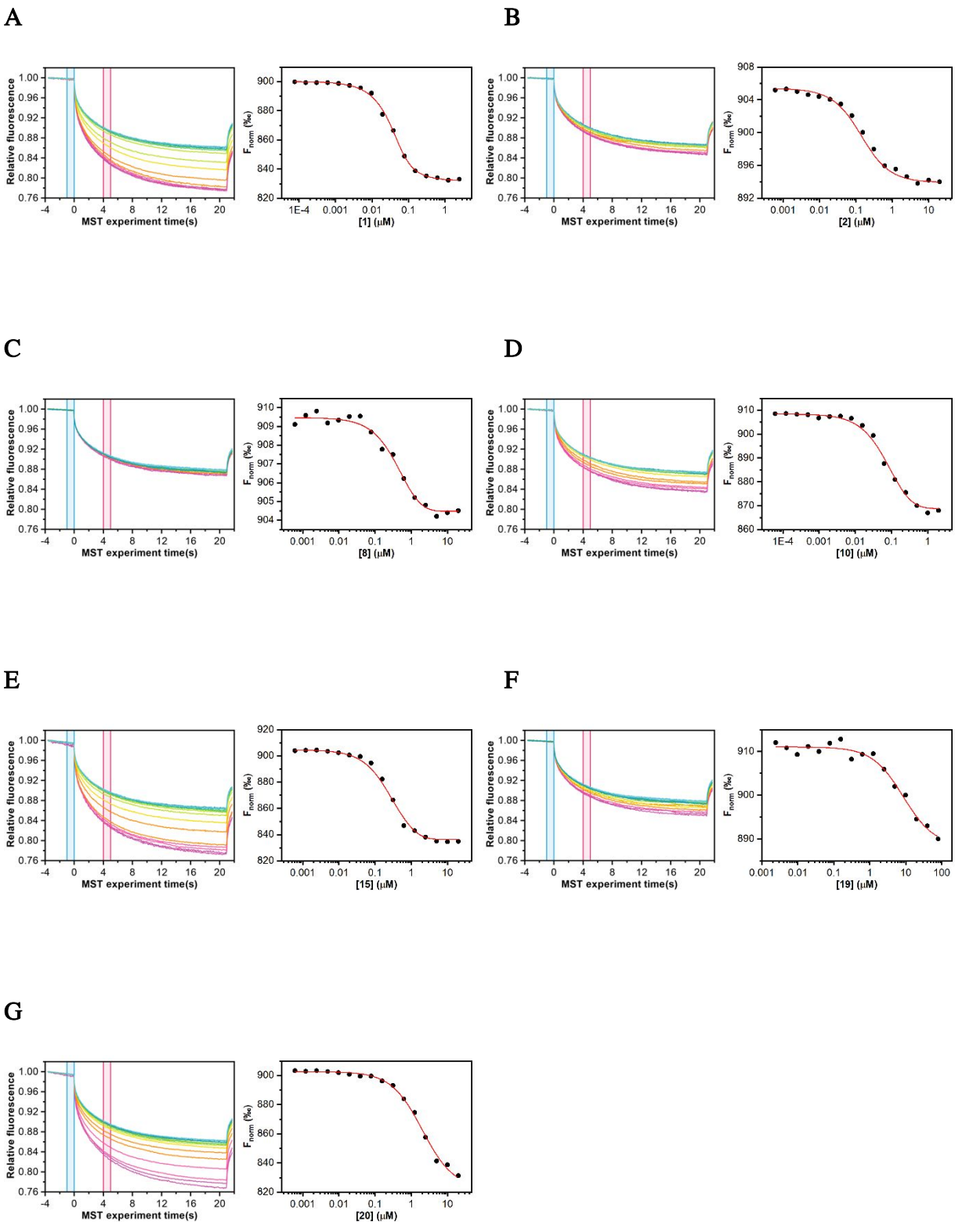


Figure S14. MST experiments for the interaction of (A) **1**, (B) **2**, (C) **8**, (D) **10**, (E) **15**, (F) **19**, and (G) **20** with *Cy5.5-c-kit1 G4*. (Left) Time traces recorded by adding increasing concentrations of compound to the labeled DNA G4 and (right) the corresponding binding curves.

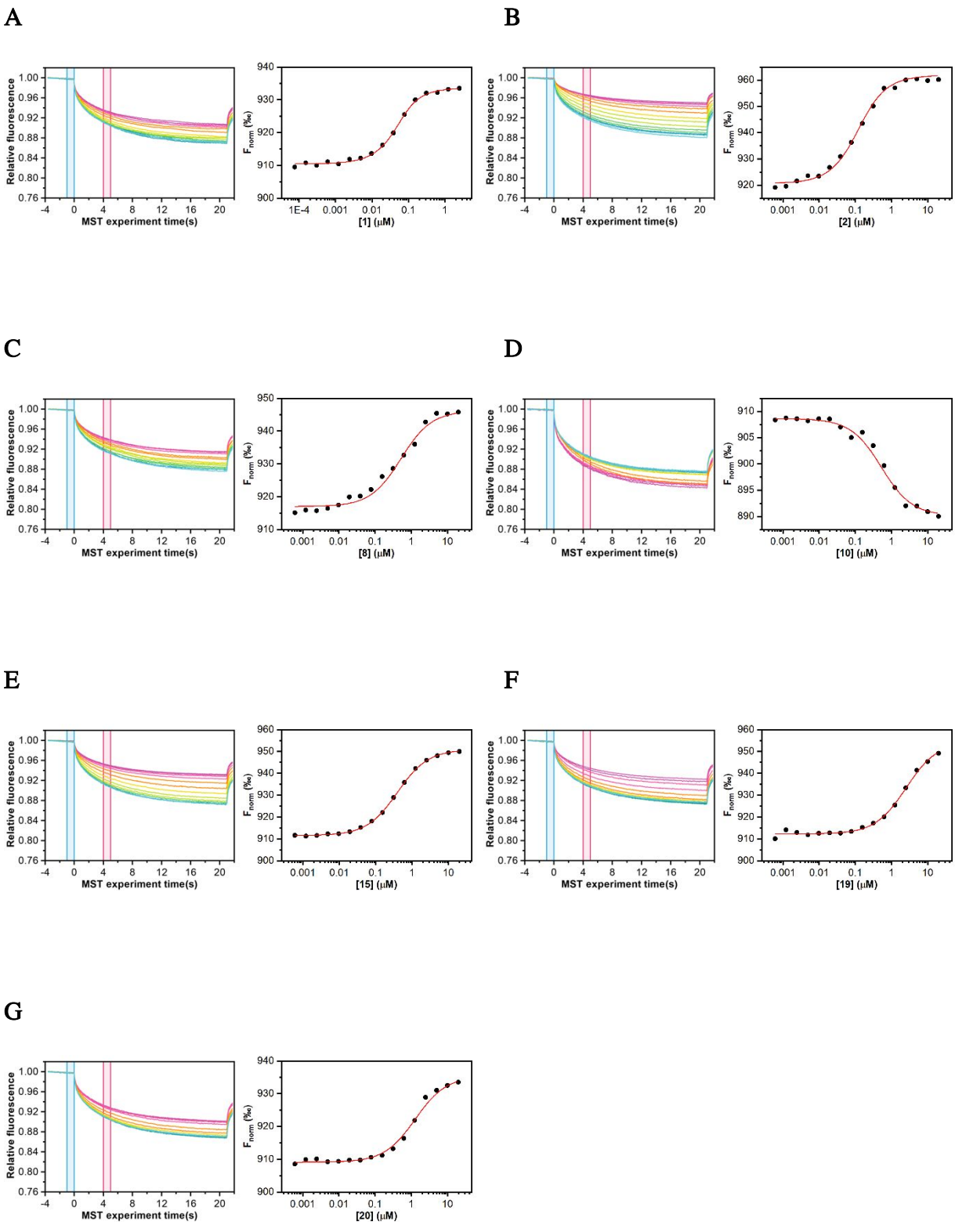


Figure S15. MST experiments for the interaction of (A) **1**, (B) **2**, (C) **8**, (D) **10**, (E) **15**, (F) **19**, and (G) **20** with *Cy5.5-c-kit2 G4*. (Left) Time traces recorded by adding increasing concentrations of compound to the labeled DNA G4 and (right) the corresponding binding curves.

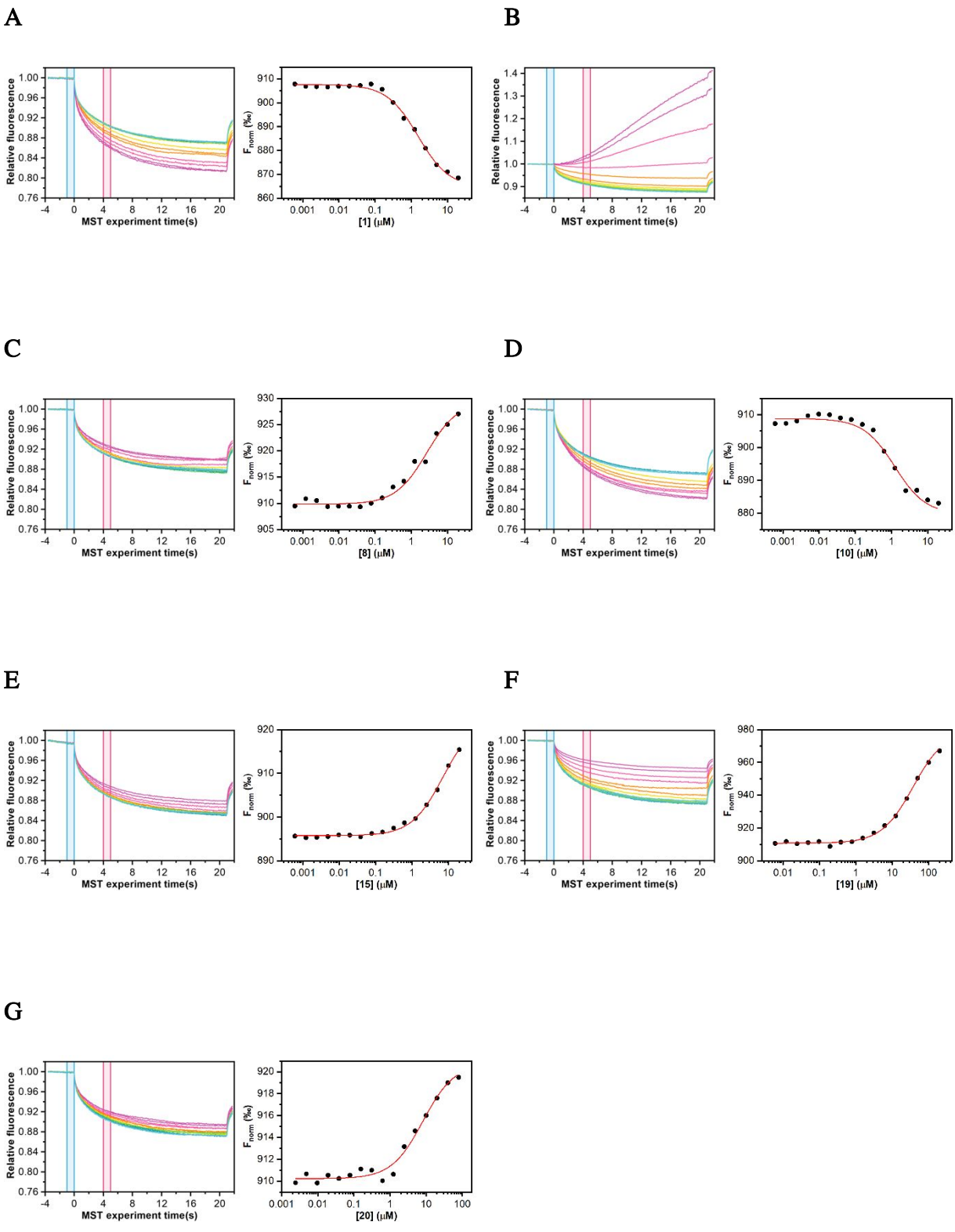


Figure S16. MST experiments for the interaction of (A) **1**, (B) **2**, (C) **8**, (D) **10**, (E) **15**, (F) **19**, and (G) **20** with *Cy5.5-c-myc G4*. (Left) Time traces recorded by adding increasing concentrations of

compound to the labeled DNA G4 and (right) the corresponding binding curves. In the case of compound **2** (B) we were unable to obtain a reliable binding curve.

Table S2

Cytotoxic potency of selected hydrazones derivatives in human MRC5 normal fibroblast line.

Compound	MRC5
FG	n.d.
1	47.58 ± 14.65
2	>200
8	23.22 ± 6.92
FIM	3.55 ± 0.83
15	2.29 ± 0.56
19	5.62 ± 0.93
20	24.52 ± 10.06
PDS	3.16 ± 0.29