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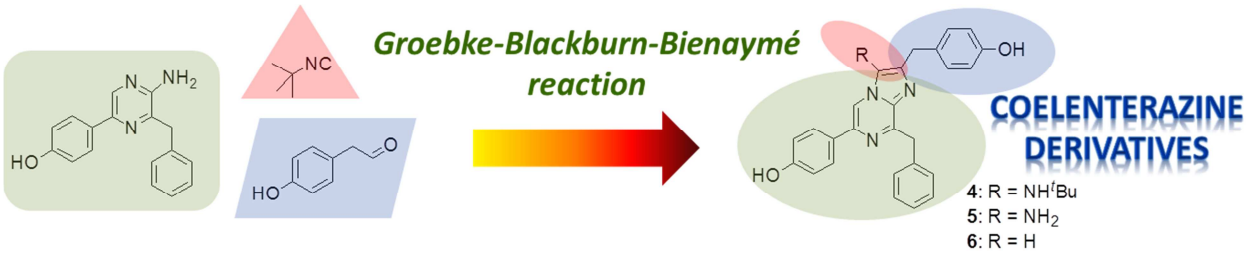

Multicomponent Synthesis of Novel Coelenterazine Derivatives Substituted at the C-3 Position

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KEYWORDS: coelenterazine derivatives; multicomponent reaction; *tert*-butyl group cleavage; azoylidene carbene; hydrogen abstraction.

ABSTRACT: Three novel coelenterazine derivatives substituted at the C-3 position were synthesized through a multicomponent strategy based on Groebke–Blackburn–Bienaymé reaction without using protecting groups. An efficient one-pot *tert*-butyl group cleavage from an aminoimidazopirazine and the first example of direct diazoimidazole derivative hydrogen abstraction in acidified water (traditional Sandmeyer hydroxylation conditions) were described.

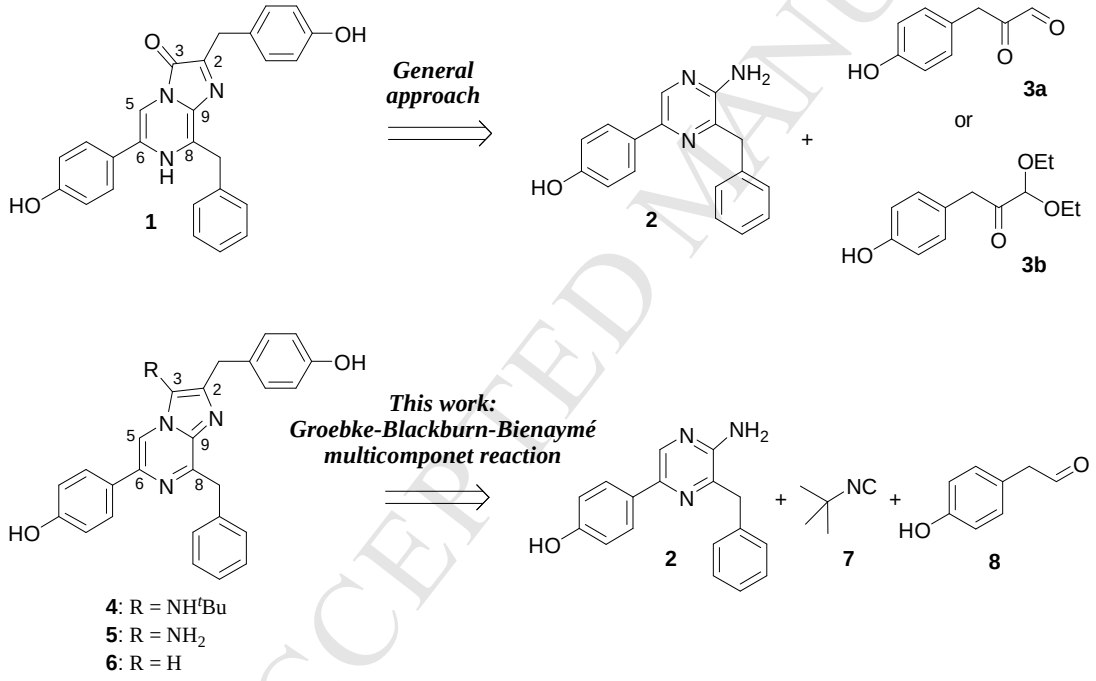
Coelenterazione (**1**), a natural occurring bioluminescent imidazopyrazine,[1] was found in marine organisms such as the jellyfish *Aequoria victoria*,[2] the sea cactus *Cavernularia obesa*,[3] the sea pansy *Renilla reniformis*,[4] the deep sea shrimp *Oplophorus gracilirostris*,[5] obelin *Obelia longissima*[6] and the oceanic squid *Symplectoteuthis oualaniensis*.[7] The luminescent proprieties have shown utility as signaling reagents in biological and clinical research,[8] indeed, coelenterazine is used in new optical technologies for studying the behavior of several cancers.[9]

The bioluminescent and antioxidant[10] proprieties of coelenterazine have encouraged the synthesis of analogues with a view to improving its physical and biological qualities.[11] Coelenterazine is a quite unstable compound, with a half-life of 15 minutes at 37 °C,[12] but its stability can be improved by introducing groups at the C-3 position.[13] K. Kikuchi reported the synthesis of coelenterazine-β-galactose

conjugates to monitor dual gene expression, enzyme activity and high-throughput screening.[13] Despite the high interest in producing new derivatives of coelenterazine, all the syntheses described in literature have in common a final step based on the condensation between coelenteramine **2** (or an analogue) with a free or protected α-keto aldehyde which require several synthetic steps to be achieved.[11,14]

Surprisingly, until now, there is no literature precedence for bioluminescence from any 3-deoxy derivatives of coelenterazine, and also no known synthetic methodology to prepare this class of compounds.

Hereby we present a new approach to obtain three novel coelenterazine derivatives (**4**, **5** and **6**) substituted at the C-3 position by means of Groebke–Blackburn–Bienaymé multicomponent reaction (Scheme 1).



Scheme 1. General and multicomponent approaches in the synthesis of coelenterazine derivatives.

Multicomponent reactions (MCRs) are a powerful and efficient synthetic strategy in modern organic chemistry due to their elegant features to maximize structural complexity and convergence, while saving synthetic procedures.[15] As regards the imidazole chemistry, recently we reported a two-step procedure (Ugi multicomponet reaction followed by Bischler–Napieralski/heterocyclization) to synthesize

dihydroimidazo[1',5':1,2]pyrido[3,4-*b*]indol-2-ium salts and indoles in good yields.[16] Encouraged by these results we aimed our studies at extending the application of MCRs in the synthesis of derivatives of coelenterazine without any protecting group insertion. To the best of our knowledgeable, Groebke–Blackburn–Bienaymé multicomponent reaction[17] was never investigated in the synthesis of coelenterazine derivatives.

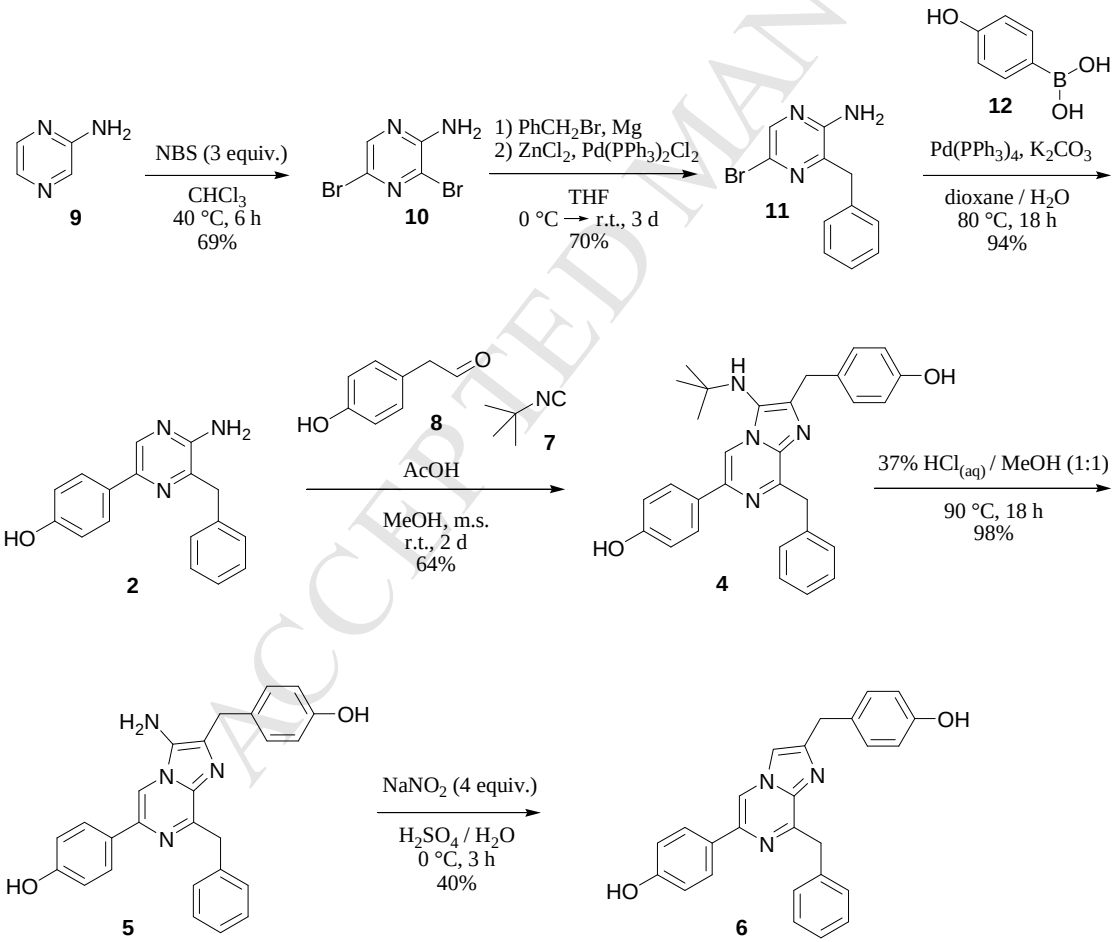
Starting from commercially available aminopyrazine (**9**), bromination with 3.0 equivalents of *N*-bromosuccinimide in chloroform provided 3,5-dibromopyrazin-2-amine (**10**) in 69% yield. Benzylmagnesium bromide, prepared *in situ* by mixing benzylbromide and magnesium turnings in dry THF, was first treated with anhydrous zinc chloride to generate the benzylzinc halide, which was then reacted with **10** at room temperature in the presence of bis(triphenylphosphine)palladium (II) dichloride catalyst to afford 2-amino-3-benzyl-5-bromo-pyrazine (**11**) in 70% yield.[11d] Suzuki coupling of **11** with 4-hydroxyphenylboronic acid **12** gave compound **2** in 94% yield.[11b]

Groebke–Blackburn–Bienaymé three-component reaction was accomplished by mixing in anhydrous methanol **2** with 2-(4-hydroxyphenyl)acetaldehyde[18] (**8**) and *tert*-butylisocyanide (**7**), the reaction was catalyzed by glacial acetic acid and conducted at room temperature. After three days it was observed that a part of the starting material **2** was recovered (60%) and the final yield of the desired product **4** was only 37%. To improve the yield of this reaction, addition portions of aldehyde **8** (1.1 equivalents) and isocyanide **7** (1.1 equivalents) were added after 4 hours and 8 hours. Under these modified conditions, the yield increased to 64% after two days of reaction.

To reach the free amine compound **5**, the cleavage of *tert*-butyl group was investigated. Although several methods to remove a *tert*-butyl group from an aminoimidazole derivative to achieve a primary amine have been described, they required a two-step procedure[19] or a microwave irradiation,[20] and we were interested to find a direct one-pot procedure applicable also to large-scale synthesis without the use of microwave reactors. It was found that a solution of **4** in 1:1 37% aqueous hydrochloric acid solution

and methanol heated to 90 °C for 18 hours afforded the desired product **5**, after neutralization with ammonia solution, in near-quantitative yield (98%).

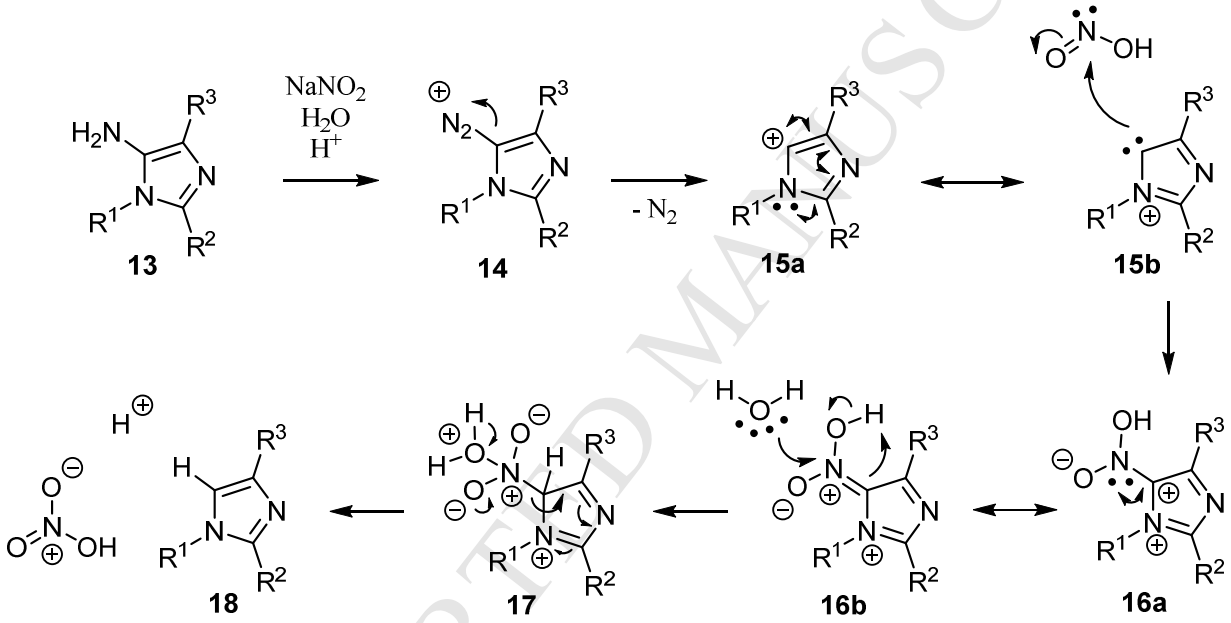
To prepare compound **6**, the reduction of amine **5** to introduce a hydrogen atom in position 3 was investigated. For structure like aminoimidazoles a hydrogen abstraction was observed as main synthetic process during the diazotization in alcoholic solution.[21] As reported, these reactions involve the formation of azoylidene carbenes generated after loss of nitrogen from the diazoimidazole compounds.[22] We found that in classical Sandmeyer-type hydoxylation condition (sodium nitrite, water and sulforic or hydrochloric acid) at 0 °C, amine **5** was subjected to a direct hydrogen abstraction to afford compound **6** as unique isolable product in 40% yield (Scheme 2). Any formation of **1** (tautomer of the hydrated compound) was not detectable by mass spectrometry analysis.



Scheme 2. Synthetic procedure.

Interesting, to the best of our knowledge, hydrogen abstraction of diazoimidazoles in presence of only acidified water and nitrite salt (traditional Sandmeyer hydroxylation conditions) was never described and this reaction is the first of this class. A plausible mechanism is illustrated in Scheme 3.

Diazo-compound **14** spontaneously was converted in carbene **15** after loss of nitrogen. **15** reacted with nitrous acid, generated *in situ* from nitrite salt and acid, to afford intermediate **16** which was able to add a molecule of water to generate intermediate **17**. The final step was the elimination of a molecule of nitric acid and the regeneration of the imidazole aromaticity. In this mechanism nitrous acid plays a role of reducing agent and it is oxidized to nitric acid in the end of the hydrogen abstraction process.



Scheme 3. Proposed mechanism for the hydrogen abstraction.

In conclusion, three novel coelenterazine derivatives substituted at the C-3 position were synthesized by means of Groebke–Blackburn–Bienaymé multicomponent reaction. A very efficient one-pot procedure to remove a *tert*-butyl group from an aminoimidazole derivative and the first example of direct hydrogen abstraction of a diazoimidazole derivative in acidified water (traditional Sandmeyer hydroxylation conditions) are described. The unprecedented strategy described here offers a new and easy way to prepare a wide range of coelenterazine analogues and explore their chemical and luminescent proprieties.

Experimental Section

General Method and Instrument Details. Reactions were carried out in dried glassware and under argon atmosphere. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) on silica gel 60 F254 (Merck) plates using UV light and/or phosphomolybdic acid, *p*-anisaldehyde or 10% H_2SO_4 in ethanol solution to visualize the compounds. Solvents were distilled and dried from sodium benzophenone ketyl for tetrahydrofuran and from calcium hydride for methylene chloride. Dry toluene, *N*,*N*-dimethylformamide and diethyl ether were purchased from commercial suppliers and used without further purification. Flash chromatography was performed on silica gel (40-60mm). Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Melting points were measured on a B-540 Büchi apparatus. ^{1}H and ^{13}C NMR spectra were recorded at 23°C using Bruker Avance DRX300, DRX400. Chemical shifts are reported relative to the residue peaks of the solvent. The following abbreviations are used to denote the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, and br = broad. NMR solvents were purchased from Sigma-Aldrich. Infrared (IR) spectra were recorded with neat liquid or solid samples using a Shimadzu IR Affinity-1 FT-IR spectrometer. HRMS were recorded using a Thermo Finnigan LCQ spectrometer.

3,5-Dibromopyrazin-2-amine (10): In an argon atmosphere, aminopyrazine (600.0 mg, 6.31 mmol, 1.0 equiv.) was dissolved in chloroform (25 mL) and then NBS (3.37 g, 18.93 mmol, 3.0 equiv.) was added over 1h at room temperature. When the addition was completed, the mixture was stirred a further 6 h at 40 °C. The mixture was poured into saturated $Na_2S_2O_3$ solution (100 mL), extracted with EtOAc (3 × 100 mL) and the combined organic layers where washed with saturated brine (100 mL). The organic solution was dried with Na_2SO_4 and concentrated by rotary evaporation. Finally the residue was purified by chromatography on silica gel (gradient elution: PE – EtOAc, 9:1 → 7:3) to give **10** (1.0856g, 4.29 mmol, 69%) as a pale yellow compound. R_f = 0.65 (silica gel, PE – EtOAc, 7:3); mp 114–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 (s, 1H), 5.17 (br, s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.0, 143.2, 124.1, 123.6; IR (neat): ν_{max} = 3447, 3281, 3187, 3154, 2933, 1811, 1714, 1621, 1549, 1506,

1450, 1359, 1333, 1202, 1151, 1133, 1096, 1079, 1040, 908, 877, 801, 755, 696 cm^{-1}; HRMS (ESI): calculated for $C_4H_4{}^{79}Br_2N_3{}^+$ 251.8766, found 251.8779; calculated for $C_4H_4{}^{79}Br^{81}BrN_3{}^+$ 253,8746, found 253.8757; calculated for $C_4H_4{}^{81}Br_2N_3{}^+$ 255.8726, found 255.8735. The spectroscopic data are in good agreement with those reported in the literature.[14a]

3-Benzyl-5-bromopyrazin-2-amine (11): Magnesium turnings (293.9 mg, 12.1 mmol, 3.0 equiv.) and I_2 (153.6 mg, 605 µmol, 0.15 equiv,) were added to a dry 100-mL 2-necked round-bottom flask under an argon atmosphere. Freshly distilled THF (25 mL) was added using a syringe. The mixture was stirred at 0 °C until the brown color of the I_2 disappeared. Then, benzylbromide (1.44 mL, 12.1 mmol, 3.0 equiv.) was slowly added using a syringe. After 10 min $ZnCl_2$ was added and the mixture was stirred for 40 min. To this mixture, bis(triphenylphosphine)palladium (II) dichloride (141.8 mg, 0.20 mmol, 0.05 equiv.) and a solution of 2-amino-3,5-dibromopyrazine (1.02 g, 4.03 mmol, 1.0 equiv) in dry THF (25 mL) were added sequentially at room temperature. The resulting orange-colored reaction mixture was stirred for 3 days at room temperature and then quenched with water (50 mL) at 0 °C. The mixture was diluted with ethyl acetate (300 mL) and water (100 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (4 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated on a rotary evaporator. The residue was dissolved in CH_2Cl_2 and purified by silica gel column chromatography (gradient elution: PE – EtOAc, 85:15 → 65:35) to afford compound **11** (738.2 mg, 2.79 mmol, 70 %) as viscous yellow oil. R_f = 0.46 (silica gel, PE – EtOAc, 7:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.93 (s, 1H), 7.26–7.21 (m, 2H), 7.20–7.15 (m, 1H), 7.15–7.10 (m, 2H), 4.40 (br, s, 2H), 3.99 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.3, 142.6, 141.9, 135.8, 129.2, 128.6, 127.4, 126.4, 40.9; IR (neat): ν_{max} = 3472, 3314, 3200, 3060, 3027, 2919, 2850, 1605, 1555, 1529, 1494, 1424, 1389, 1340, 1261, 1220, 1158, 1117, 1073, 1048, 1028, 1002, 923, 905, 791, 759, 726, 696, 634 cm^{-1}; HRMS (ESI): calculated for $C_{11}H_{11}{}^{79}BrN_3{}^+$ 264.0131, found 264.0133; calculated for $C_{11}H_{11}{}^{81}BrN_3{}^+$ 266.1414, found 266.0113. The spectroscopic data are in good agreement with those reported in the literature.[11d]

4-(5-Amino-6-benzylpyrazin-2-yl)phenol (2): A mixture of 3-benzyl-5-bromopyrazin-2-amine (637.5 mg, 2.41 mmol, 1.0 equiv.), 4-hydroxylphenylboronic acid (398.6 mg, 2.89 mmol, 1.2 equiv.), $Pd(PPh_3)_4$ (277.3 mg, 0.24 mmol, 0.1 equiv.) and K_2CO_3 (1.665 g, 12.0 mmol, 5.0 equiv.) in dioxane (50 mL) and water (10 mL) was heated to 80 °C and stirred for 18 h under Ar. The mixture was cooled to room temperature, diluted with water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (25 mL), dried with Na_2SO_4, filtered, evaporated and the residue subjected to flash silica gel column chromatography (gradient elution: PE – EtOAc, 7:3 → 4:6) to afford compound **2** (625.5 mg, 2.26 mmol, 94%) as a pale brown solid. R_f = 0.65 (silica gel PE – EtOAc, 25:75); mp 202–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.52 (s, 1H), 8.31 (s, 1H), 7.74 (d, J = 8.6 Hz, 2H), 7.34 (d, J = 7.2 Hz, 2H), 7,28 (t, J = 7.2 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 6.81 (d, J = 8.6 Hz, 2H), 6.22 (s, 2H), 4.07 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 157.2, 152.0, 139.7, 139.5, 138.3, 135.9, 128.9, 128.2, 126.2 (× 2), 126.1, 115.5, 38.7; IR (neat): ν_{max} = 3478, 3346, 3216, 3060, 3026, 2912, 2806, 2676, 2597, 2476, 2361, 2339, 1633, 1606, 1546, 1513, 1492, 1443, 1389, 1350, 1264, 1223, 1174, 1156, 1131, 1081,1009, 933, 898, 790, 770 cm^{-1}; HRMS (ESI): calculated for $C_{17}H_{16}N_3O^+$ 278.1288, found 278.1301. The spectroscopic data are in good agreement with those reported in the literature.[14b]

2-(4-Hydroxyphenyl)acetaldehyde (8)

Under anhydrous condition, to a solution of 2-(4-hydroxyphenyl)ethanol (2.00 g, 14.5 mmol, 1.0 equiv.) in DMSO (20 mL) was added diisopropylethylamine (5.29 mL, 30.4 mmol, 2.1 equiv.) and then a solution of SO_3 • pyridine (4.54 g, 28.5 mmol, 2.0 equiv.) in DMSO (20 mL) over 30 min. The mixture was stirred for 1 h at room temperature and quenched by the addition of ice-cold water (200 mL) The aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL) and the organic layers were combined and concentrated under reduced pressure. The crude material was purified using silica flash chromatography (gradient elution: PE – EtOAc, 8:2 → 6:4). Fractions containing the desired product were combined and

concentrated under reduced pressure to yield **8** as a colourless oil (649.2 mg, 4.77 mmol, 33%). R_f = 0.24 (silica gel PE – EtOAc, 8:2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.68 (t, J = 2.4 Hz, 1H), 7.03 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 7.75–6.10 (br, s, 1H), 3.61 (d, J = 2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 201.4, 155.4, 130.8, 123.1, 116.0, 49.6; IR (neat): ν_{max} = 3351 (br), 3022, 2919, 2834, 2730, 2361, 2338, 1886, 1709, 1612, 1596, 1513, 1442, 1359, 1305, 1261, 1218, 1172, 1158, 1105, 1080, 1041, 1014, 957, 935, 827 cm^{-1}; HRMS (ESI): calculated for $[C_8H_8O_2^{\bullet}]^+$ 136.0519, found 136.0522. The spectroscopic data are in good agreement with those reported in the literature.[18]

4-(8-Benzyl-3-(tert-butylamino)-2-(4-hydroxybenzyl)imidazo[1,2-a]pyrazin-6-yl)phenol (4): 4-(5-amino-6-benzylpyrazin-2-yl)phenol (237.6 mg, 857 μmol, 1.0 equiv.) and 2-(4-hydroxyphenyl)acetaldehyde (128.3 mg, 943 μmol, 1.1 equiv.) were dissolved in dry MeOH (4 mL). To this solution molecular sieves 3 Å (240 mg) were added and the mixture was stirred at room temperature. After 20 min *tert*-butylisocyanide (250 μL, 2.22 mmol, 2.6 equiv.) and AcOH (350 μL, 6.11 mmol, 7.1 equiv.) were added. New portions of aldehyde (128.3 mg, 943 μmol, 1.1 equiv.) and isocyanide (107 μL, 943 μmol, 1.1 equiv.) were added after 4 h and after 8 h. The mixture was stirred at room temperature for 48 h, then diluted with water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (25 mL), dried with Na_2SO_4, filtered, evaporated and the residue subjected to flash silica gel column chromatography (gradient elution: PE – EtOAc, 7:3 → 4:6). Fractions containing the desired product were combined and concentrated under reduced pressure to yield **4** as a white solid (260.2 mg, 544 μmol, 64%). R_f = 0.57 (silica gel PE – EtOAc, 6:4); mp 241–245 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.64 (br, s, 1H), 9.14 (br, s, 1H), 8.46 (s, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 7.0 Hz, 2H), 7.25 (t, J = 7.3 Hz, 2H), 7.17 (t, J = 7.3 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 6.67 (d, J = 8.6 Hz, 2H), 4.51 (s, 1H), 4.41 (s, 2H), 4.02 (s, 2H), 1.14 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 157.7, 155.4, 150.9, 142.4, 138.4, 136.7, 134.7, 130.2, 129.7, 129.3, 128.2, 127.7, 127.1, 127.1, 126.3, 115.6, 114.8, 110.0, 55.5, 39.0, 32.0, 30.0; IR (neat):

ν_{max} = 3061 (br), 3027, 2966, 2804, 2677, 2595, 2361, 2339, 2163, 1610, 1546, 1513, 1477, 1452, 1402, 1388, 1363, 1264, 1223, 1203, 1166, 1103, 1084, 1047, 1023, 1003, 931, 911, 821, 785, 751, 726, 667 cm^{-1}; HRMS (ESI): calculated for $C_{30}H_{31}N_4O_2^+$ 479.2442, found 479.2421.

4-(3-Amino-8-benzyl-2-(4-hydroxybenzyl)imidazo[1,2-a]pyrazin-6-yl)phenol (5): Compound **4** (344.1 mg, 720 μmol, 1.0 equiv.) was dissolved in dry MeOH (15 mL), and then 37% aqueous HCl (15 mL) was slowly added. The reaction mixture was warmed to 90 °C for 18 h. The solvent was removed under reduced pressure and the crude was directly purified using silica flash chromatography (dry deposition, gradient elution: CH_2Cl_2 – MeOH, 95:5 → 90:10). Fractions containing the desired product were combined and concentrated under reduced pressure to yield **5** as a pale yellow solid (296.9 mg, 703 μmol, 98%). R_f = 0.59 (silica gel CH_2Cl_2 – MeOH, 9:1); mp 149–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.59 (s, 1H), 9.14 (s, 1H), 8.36 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 7.0 Hz, 2H), 7.25 (t, J = 7.4 Hz, 2H), 7.16 (t, J = 7.4 Hz, 1H), 7.12 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 6.67 (d, J = 8.6 Hz, 2H), 5.44 (s, 2H), 4.36 (s, 2H), 3.98 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 157.5, 155.4, 150.1, 138.8, 136.3, 131.9, 130.6, 130.4, 129.7, 129.4, 129.1, 128.2, 128.1, 126.9, 126.1, 115.5, 114.9, 108.1, 38.9, 31.5; IR (neat): ν_{max} = 3060 (br), 3025, 2922, 2802, 2675, 2591, 2361, 2339, 2050, 1608, 1554, 1512, 1492, 1451, 1402, 1353, 1234, 1168, 1103, 1084, 1043, 1021, 992, 913, 837, 817, 777, 726, 699, 667, 657, 634 cm^{-1}; HRMS (ESI): calculated for $C_{26}H_{23}N_4O_2^+$ 423.1816, found 423.1805.

4-(8-Benzyl-2-(4-hydroxybenzyl)imidazo[1,2-a]pyrazin-6-yl)phenol (6): A solution of **5** (300 mg, 710 μmol, 1.0 equiv.) in a mixture of 98% H_2SO_4 (2.0 mL, 36.0 mmol, 50.7 equiv.) and water (10 mL) was kept at 0 °C whilst a solution of sodium nitrite (195.0 mg, 2.82 mmol, 4.0 equiv.) in water (5 mL) was added dropwise during 20 min. The solution was stirred at 0 °C for 3 h, then warmed to room temperature and neutralized to pH 7 with 25% ammonium hydroxide solution (6 mL). The solvent was removed under reduced pressure and the crude was directly purified using silica flash chromatography (dry deposition, gradient elution: CH_2Cl_2 – MeOH, 98:2 → 96:4). Fractions containing the desired

product were combined and concentrated under reduced pressure to yield **6** as an orange solid (118.7 mg, 281 μmol, 40%). R_f = 0.60 (silica gel CH_2Cl_2 – MeOH, 9:1); mp 238–243 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.75 (br, s, 1H), 9.31 (br, s, 1H), 8.84 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.72 (s, 1H), 7.47 (d, J = 7.0 Hz, 2H), 7.28 (t, J = 7.4 Hz, 2H), 7.19 (t, J = 7.4 Hz, 1H), 7.15 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 8.5 Hz, 2H), 4.48 (s, 2H), 4.05 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 158.1, 155.9, 150.5, 147.5 (br), 138.0 (br), 138.0, 137.2 (br), 129.9, 129.2, 129.1, 128.3, 127.2, 127.0, 126.4, 115.7, 115.3, 113.4, 113.0, 39.2, 33.4; IR (neat): ν_{max} = 3129 (br), 3061, 3027, 2920, 2800, 2679, 2588, 2361, 2339, 1892, 1735, 1663, 1609, 1513, 1481, 1452, 1414, 1351, 1233, 1168, 1104, 1052, 1029, 1004, 920, 816, 783, 733, 700, 667, 660, 649, 635 cm^{-1}; HRMS (ESI): calculated for $C_{26}H_{22}N_3O_2^+$ 408.1707, found 408.1687.

ASSOCIATED CONTENT

Supporting Information

Spectra and characterization data for all new compounds. The Supporting Information is available at DOI:

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Supporting Information

Multicomponent Synthesis of Novel Coelenterazine Derivatives Substituted at the C-3 Position

Vito Vece,* Giuseppina Vuocolo

Imperia-Chemistry, Synthetic Organic Chemistry Laboratory, via Brighei 1, 18100, Imperia, Italy.
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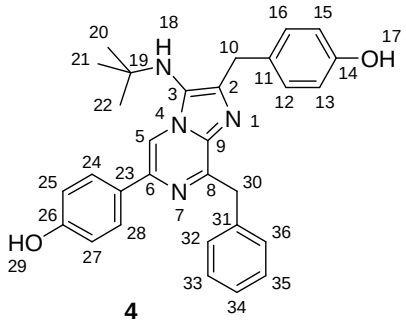
Contents of Supplementary Information

1. ^{1}H (400 MHz) and ^{13}C (100 MHz) Spectal Data of 4, 5 and 6 in DMSO-d_6. Signal Attribution Table



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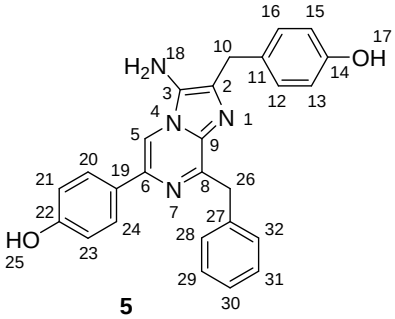

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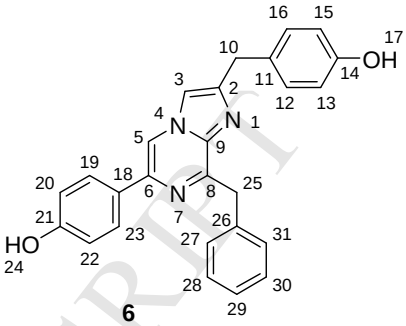

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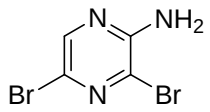
Position	δ ^{13}C^{a}	δ ^{1}H (J in Hz)	Position	δ ^{13}C^{a}	δ ^{1}H (J in Hz)	Position	δ ^{13}C^{a}	δ ^{1}H (J in Hz)
2	127.1		**2**	129.7		**2**	147.5, br	
3	142.4		**3**	130.6		**3**	113.0	7.72, s
5	110.0	8.46, s	**5**	108.1	8.36, s	**5**	113.4	8.84, s
6	136.7		**6**	136.3		**6**	137.2, br	
8	150.9		**8**	150.1		**8**	150.5	
9	134.7		**9**	131.9		**9**	138.0, br	
10	32.0	4.02, s	**10**	31.5	3.98, s	**10**	33.4	4.05, s
11	130.2		**11**	130.4		**11**	129.1	
12, 16	114.8	6.67, d (8.6)	**12, 16**	114.9	6.67, d (8.6)	**12, 16**	115.3	6.73, d (8.5)
13, 15	129.7	7.13, d (8.6)	**13, 15**	129.4	7.12, d (8.6)	**13, 15**	129.9	7.15, d (8.5)
14	155.4		**14**	155.4		**14**	155.9	
17		9.14, br s	**17**		9.14, br s	**17**		9.31, br s
18		4.51, s	**18**		5.44, s			
19	55.5							
20, 21, 22	30.0	1.14, s						
23	127.7		**19**	128.2		**18**	127.0	
24, 28	115.6	6.87, d (8.8)	**20, 24**	115.5	6.86, d (8.8)	**19, 23**	115.7	6.87, d (8.8)
25, 27	127.1	7.82, d (8.8)	**21, 23**	126.9	7.81, d (8.8)	**20, 22**	127.2	7.79, d (8.8)
26	157.7		**22**	157.5		**21**	158.1	
29		9.64, br s	**25**		9.59, br s	**24**		9.75, br s
30	39.0	4.41, s	**26**	38.9	4.36, s	**25**	39.2	4.48, s
31	138.4		**27**	138.8		**26**	138.0	
32, 36	129.3	7.46, d (7.0)	**28, 32**	129.1	7.45, d (7.0)	**27, 31**	129.2	7.47, d (7.0)
33, 35	128.2	7.25, t (7.3)	**29, 31**	128.1	7.25, t (7.4)	**28,30**	128.3	7.28, t (7.4)
34	126.3	7.17, t (7.3)	**30**	126.1	7.16, t (7.4)	**29**	126.4	7.19, t (7.4)

a ^{13}C NMR chemical shift values were assigned on the basis of 2D NMR correlations.

2. ^{1}H, ^{13}C NMR, FT-IR Spectra and HRMS Data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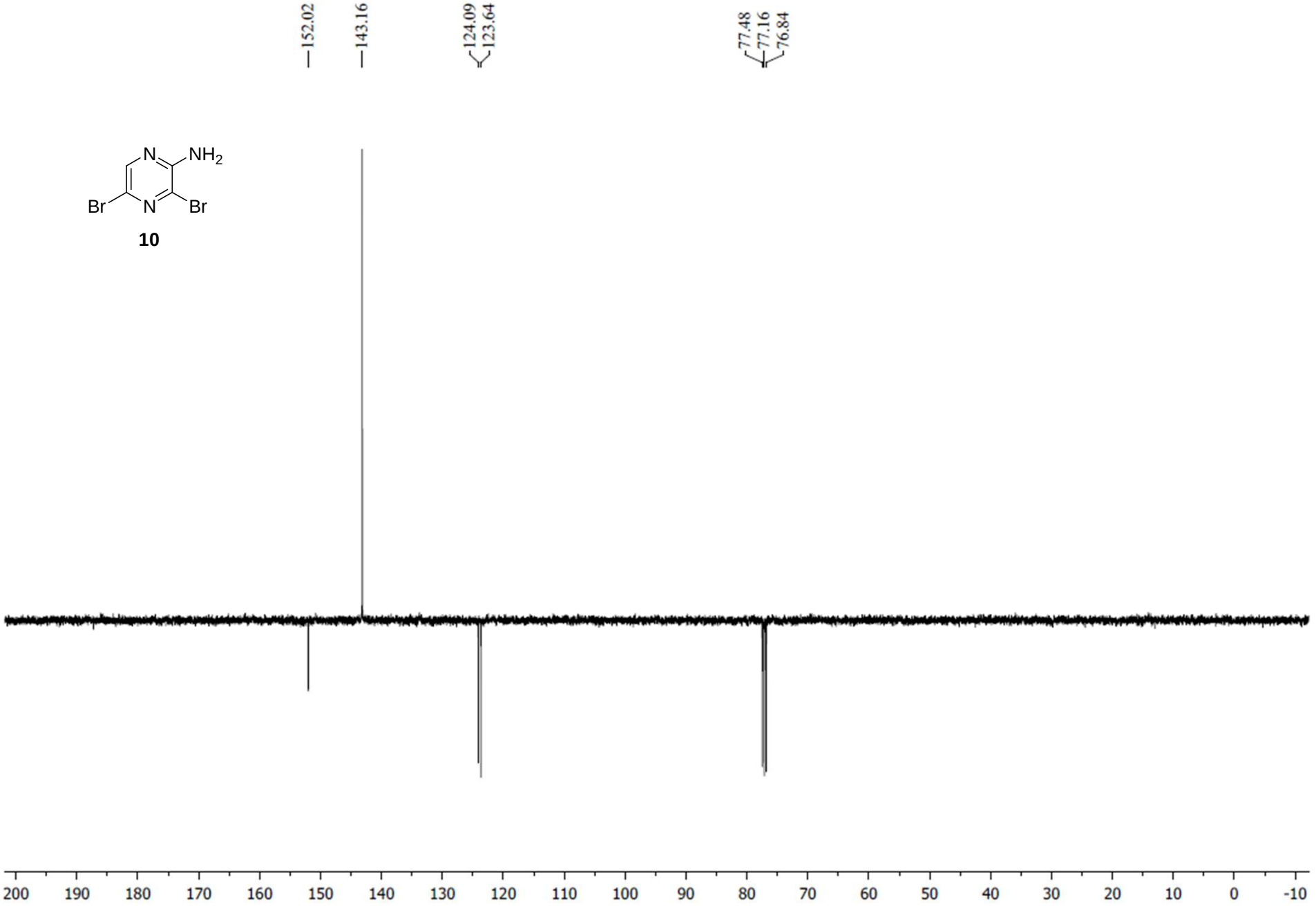
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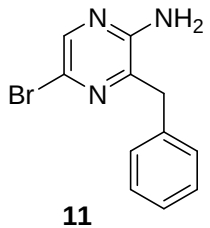
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Br
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11

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7.193
7.190
7.180
7.175
7.140
7.138
7.136
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1.31
2.03
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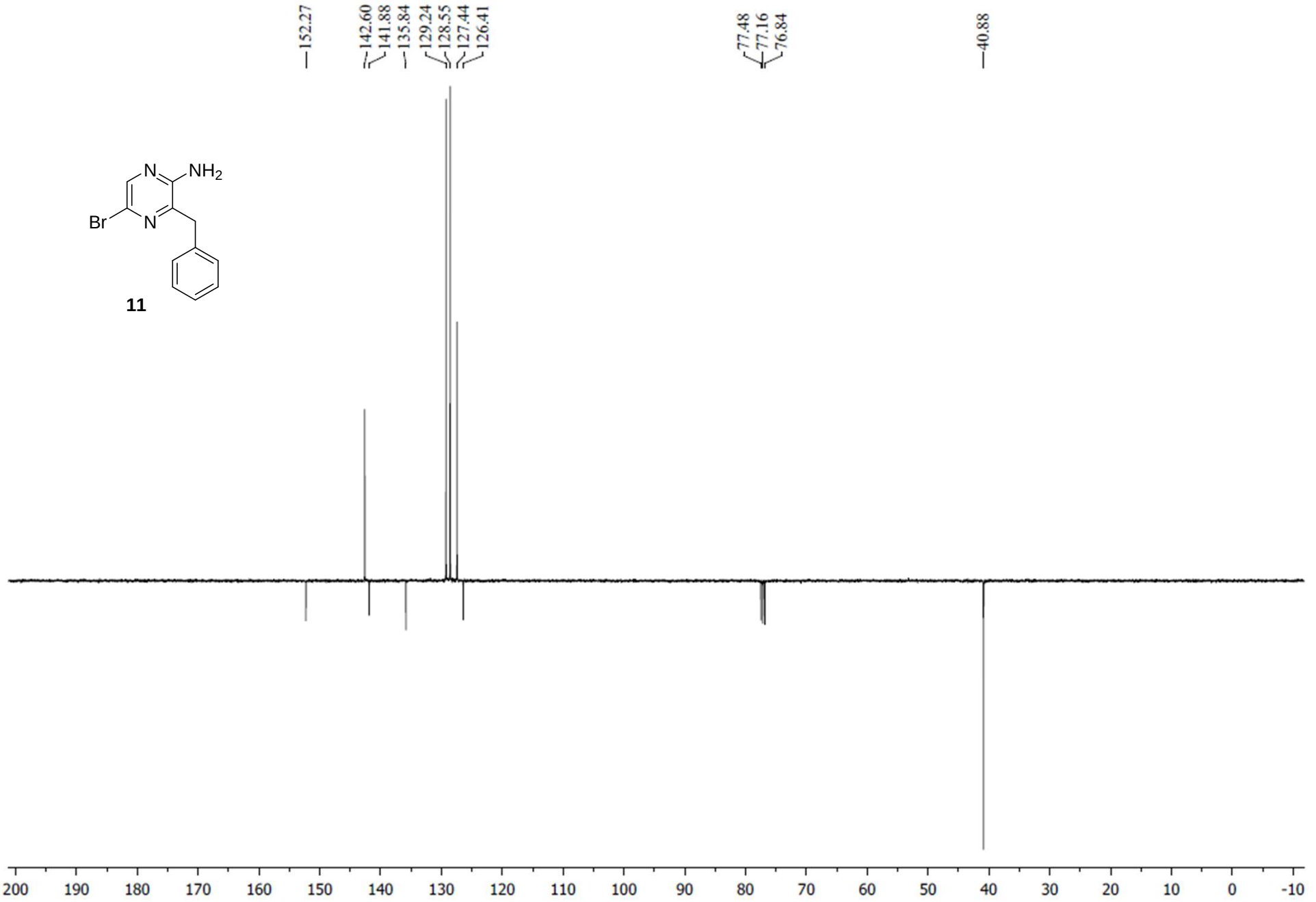
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50
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10
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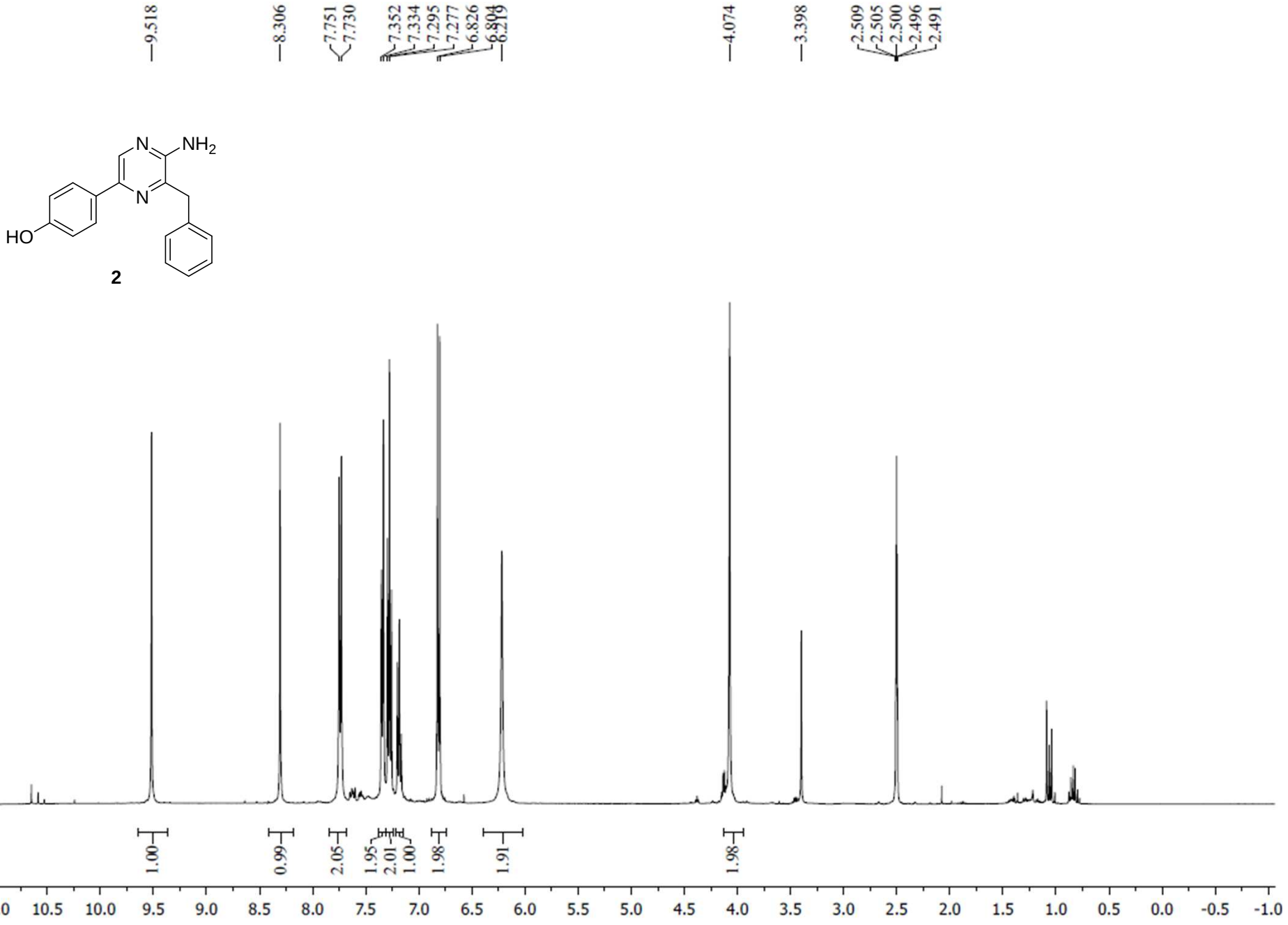
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2.500
2.496
2.491
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N
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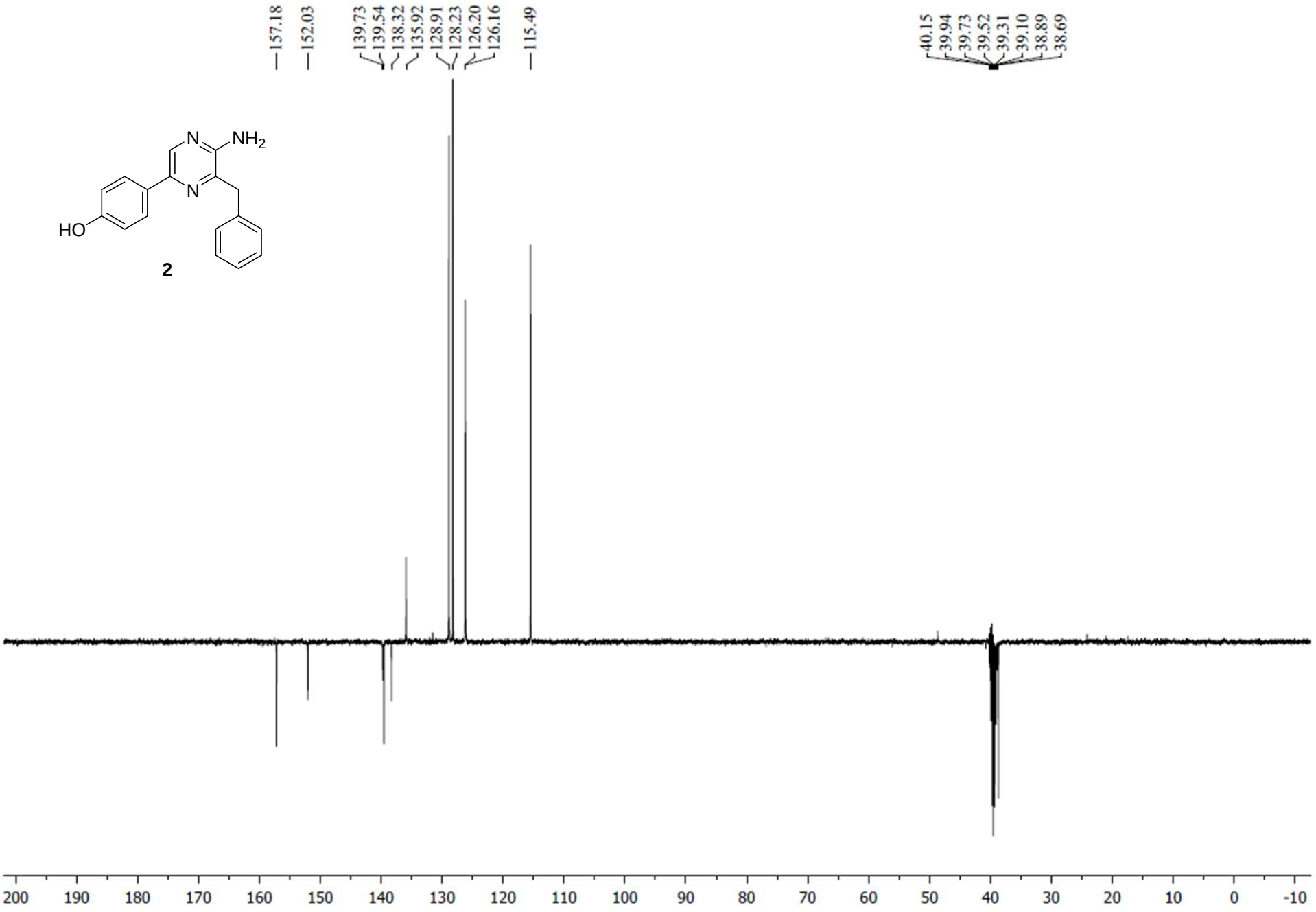
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126.16
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39.94
39.73
39.52
39.31
39.10
38.89
38.69
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NH2
N
HO
2
200
190
180
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140
130
120
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80
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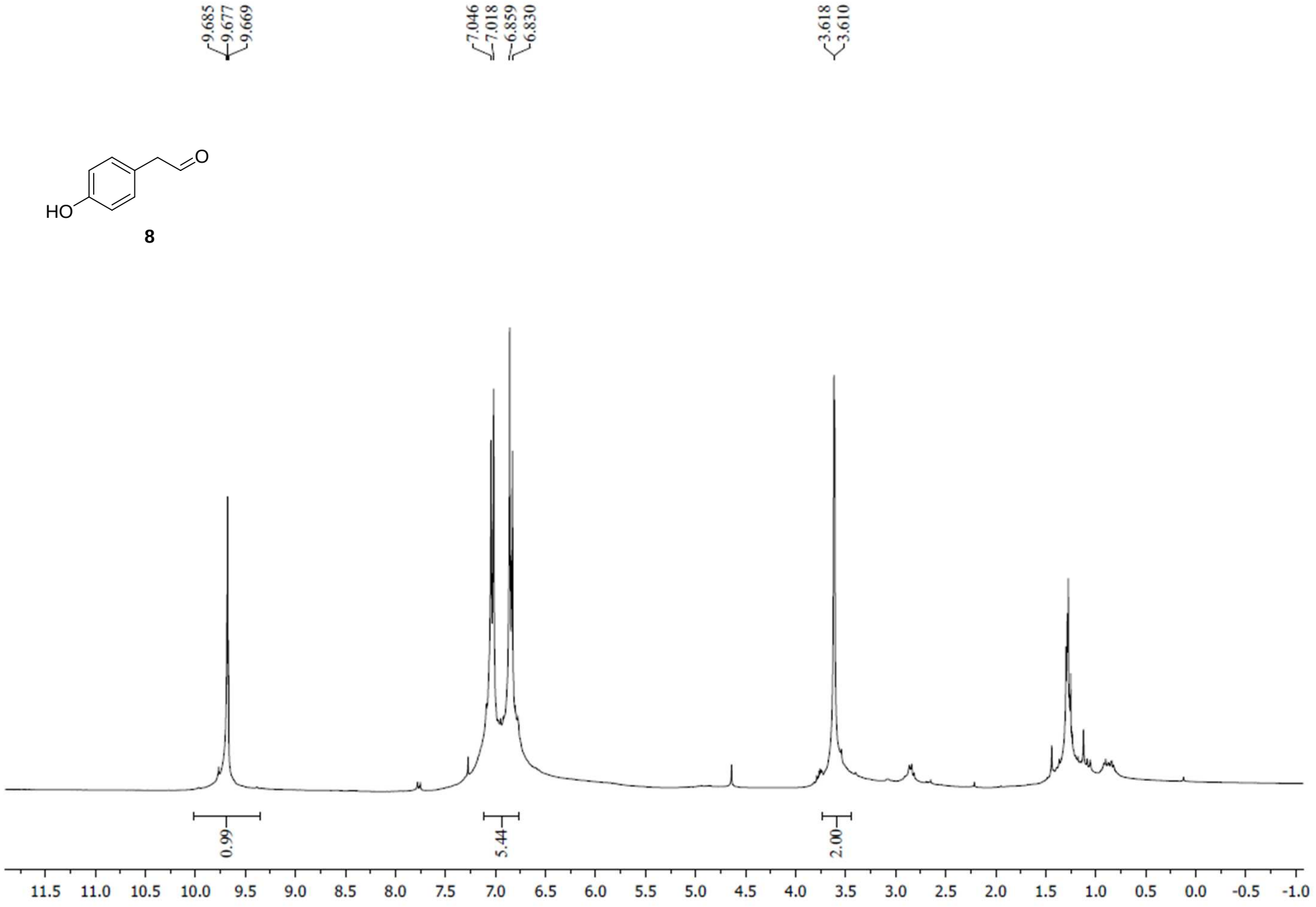
10
0
-10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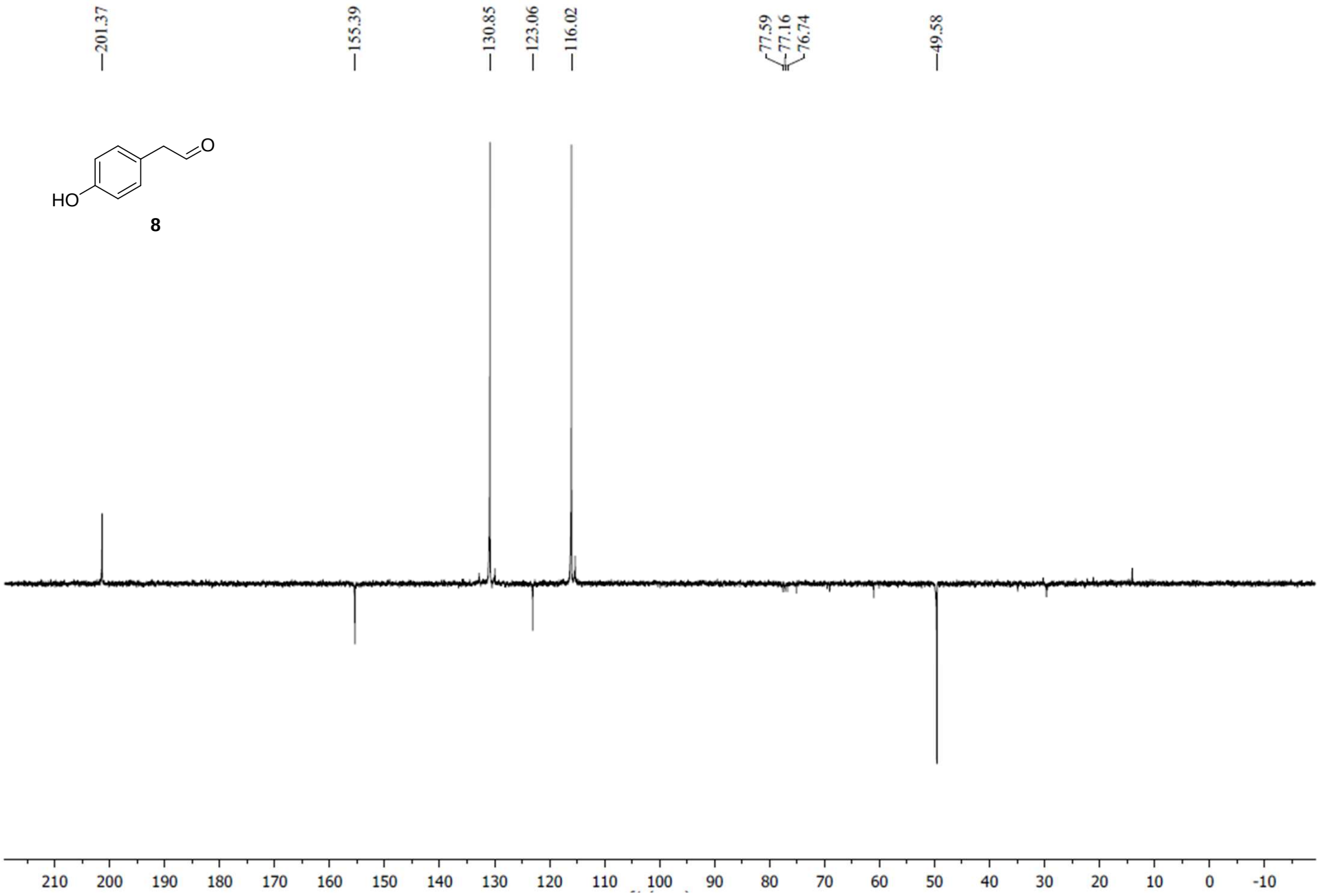
2.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

8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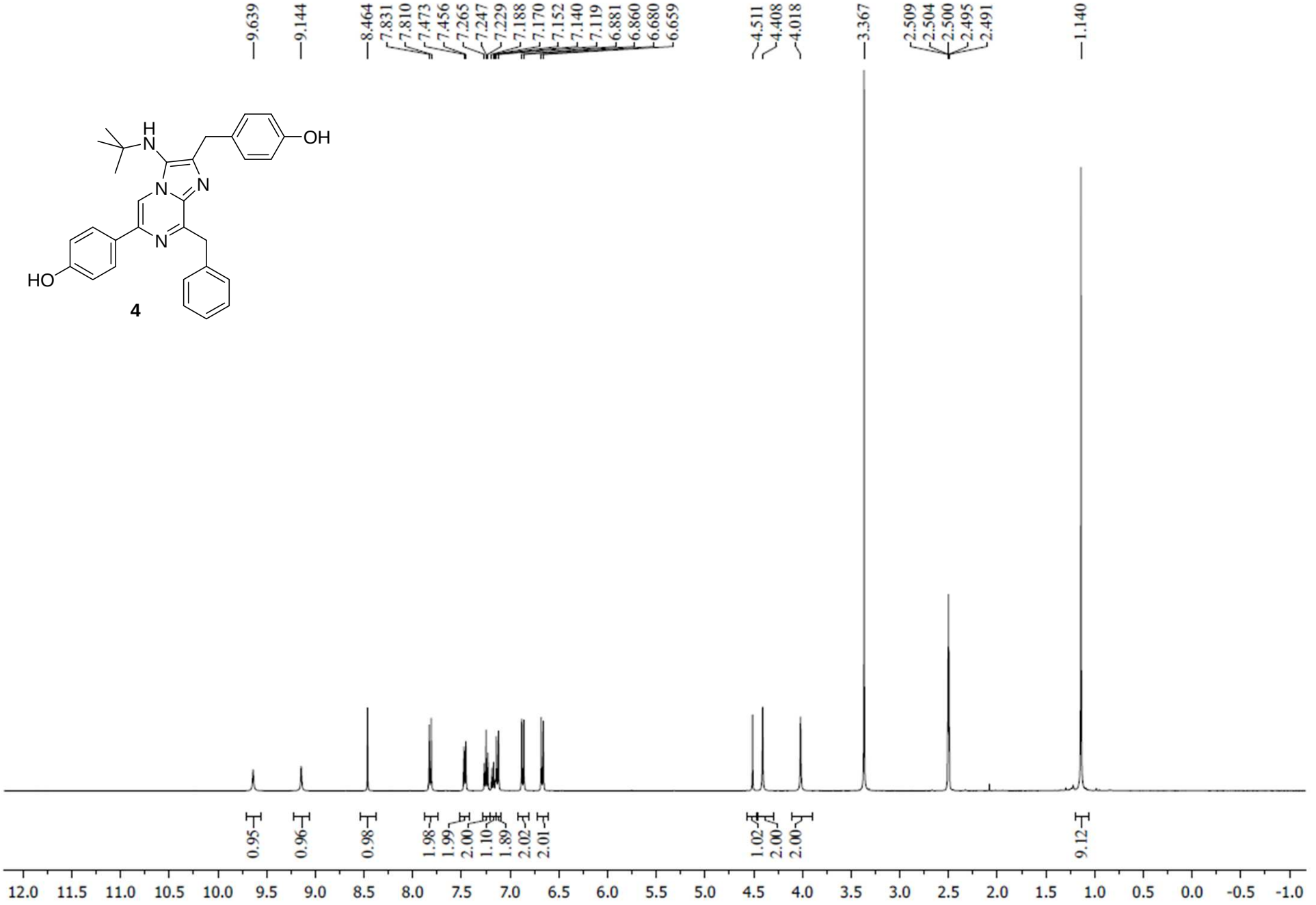
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HO
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1.10
1.89
2.02
2.01
1.02
2.00
2.00
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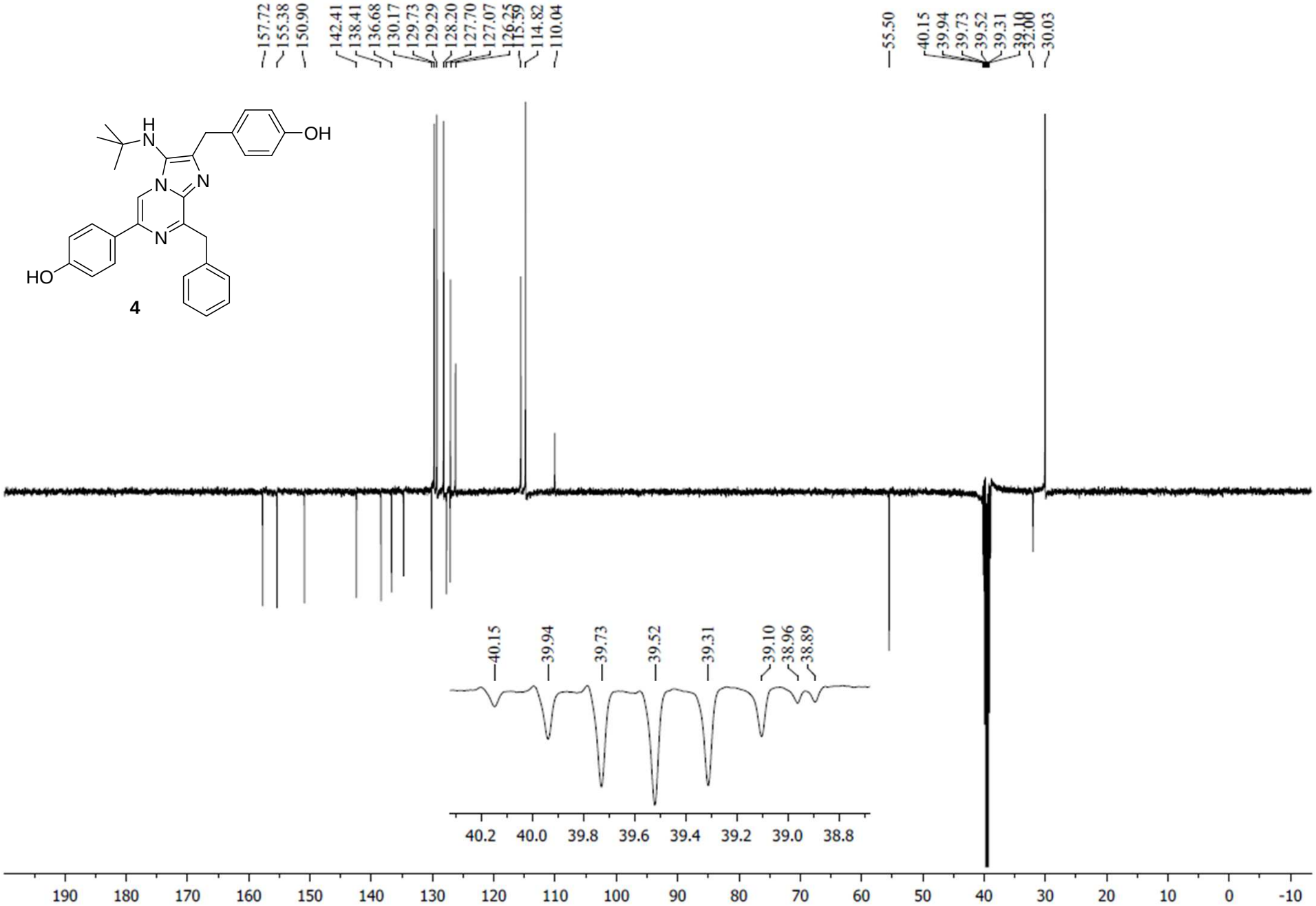
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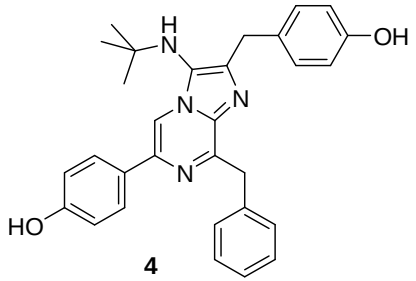
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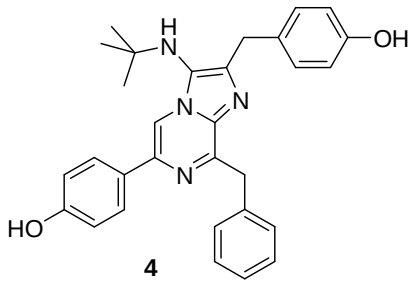
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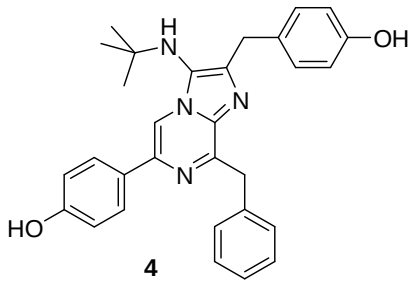
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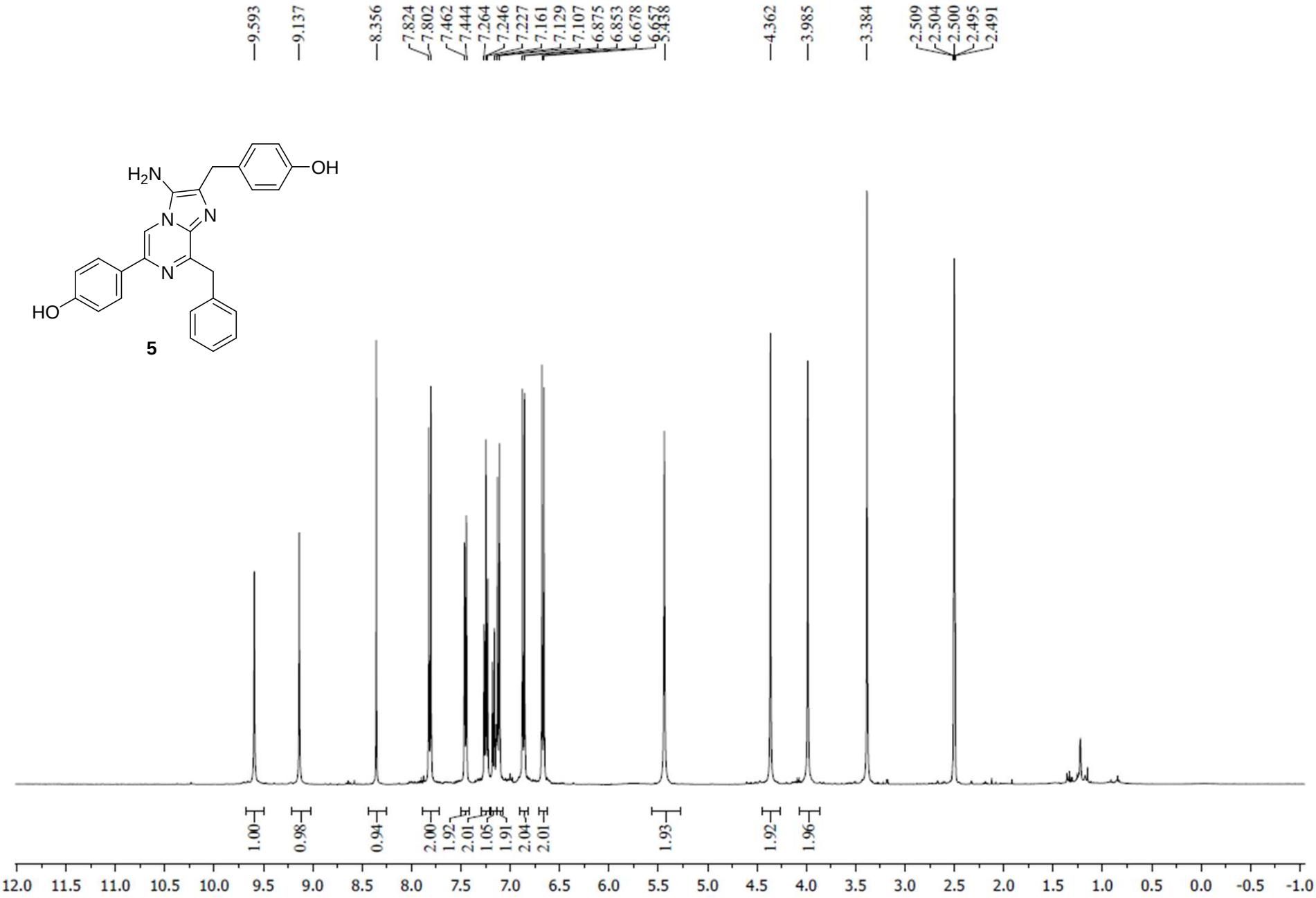
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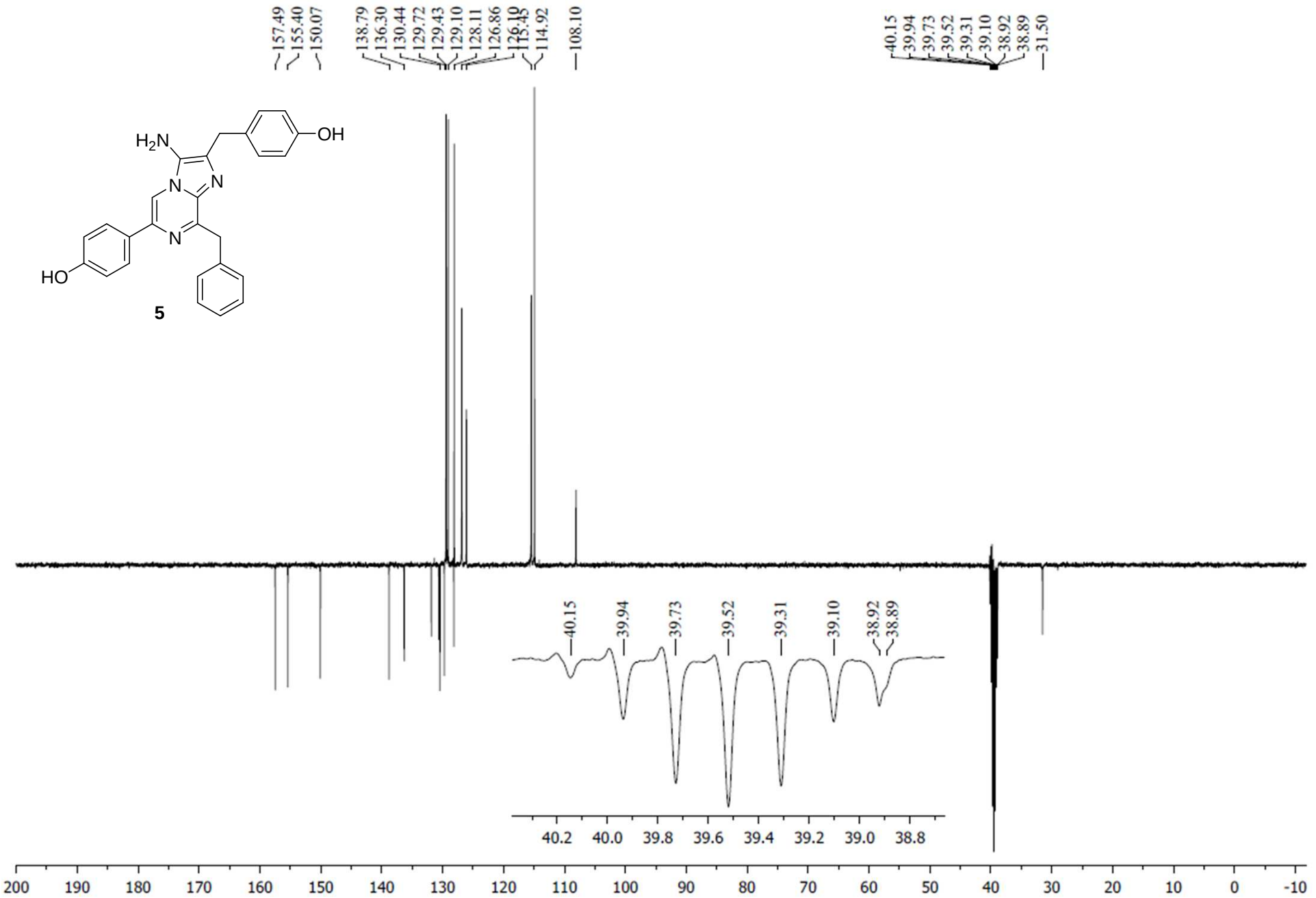
5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39.0
38.8
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150
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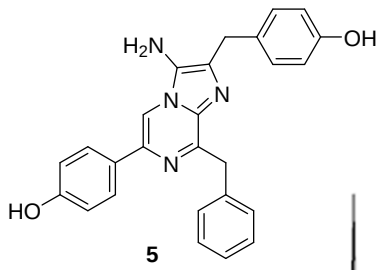

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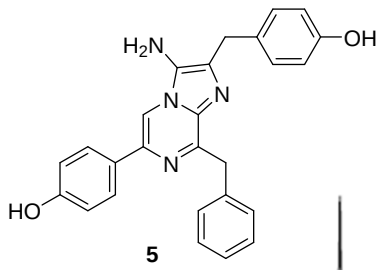
H2N
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N
N
HO

5

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150
160
9.5
9.0
8.5
8.0
7.5
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6.5
6.0
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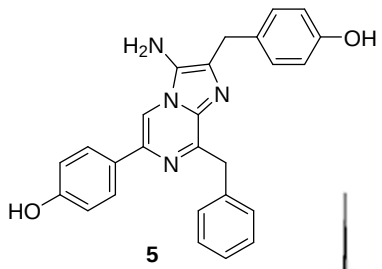
4.5
4.0
3.5
3.0
2.5


H2N
OH
N
N
N
HO

5

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9.5
9.0
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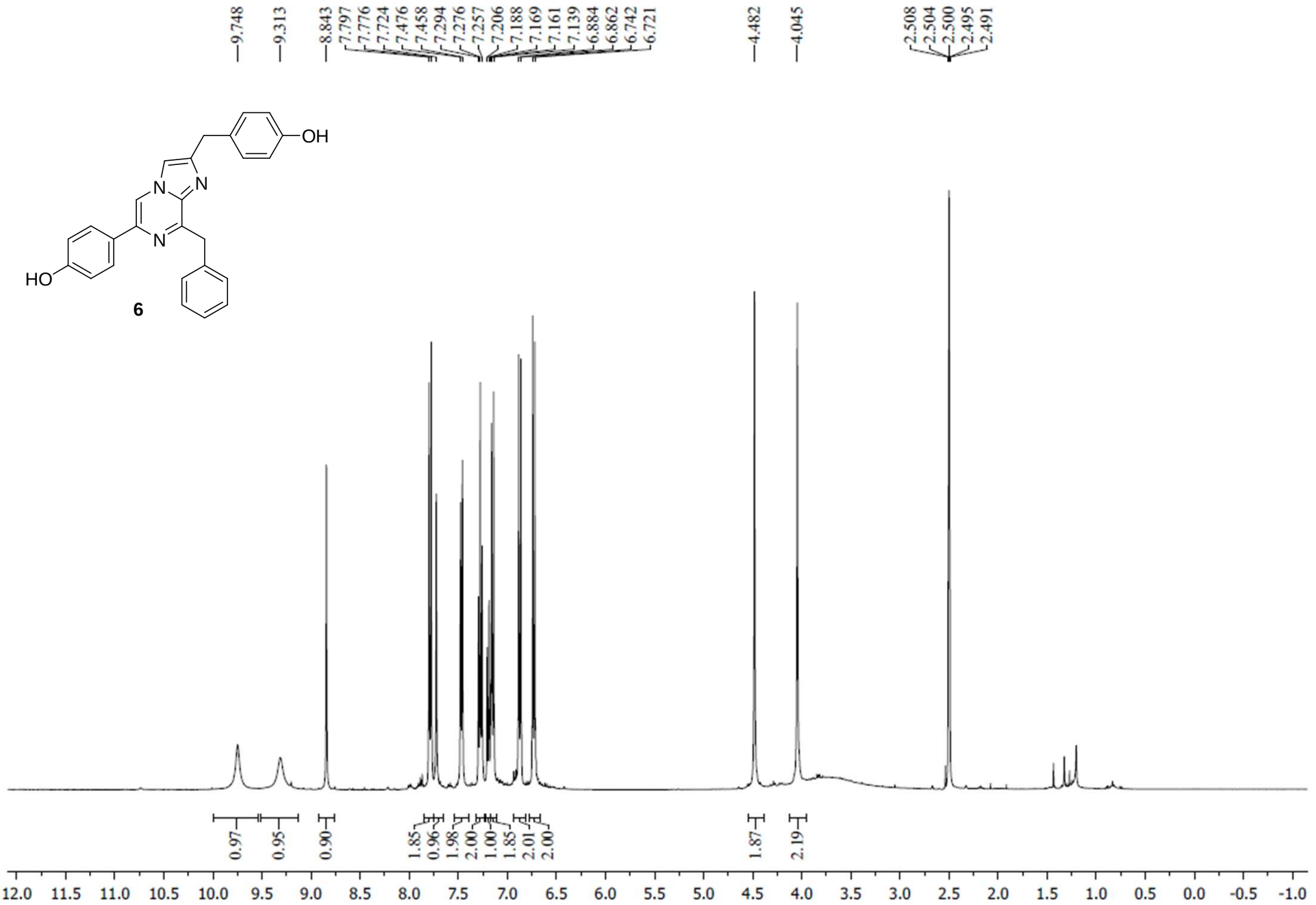
2.5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7.206
7.188
7.169
7.161
7.139
6.884
6.862
6.742
6.721
4.482
4.045
2.508
2.504
2.500
2.495
2.491
OH
HO
N
N
N
6
0.97
0.95
0.90
1.85
0.96
1.98
2.00
1.00
1.85
2.01
2.00
1.87
2.19
12.0
11.5
11.0
10.5
10.0
9.5
9.0
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8.0
7.5
7.0
6.5
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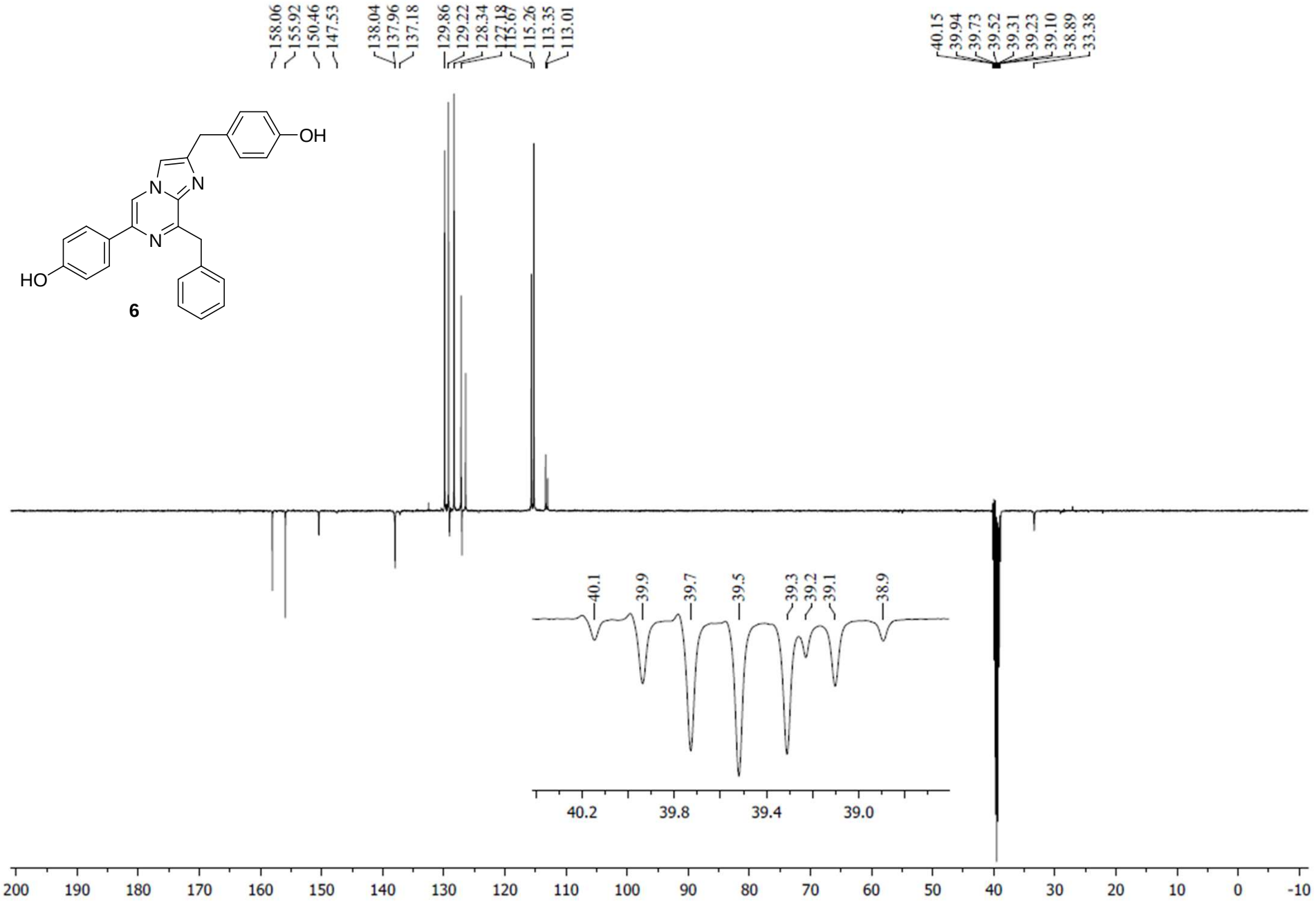
-1.0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129.86
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127.18
115.67
115.26
113.35
113.01
40.15
39.94
39.73
39.52
39.31
39.23
39.10
38.89
33.38
OH
HO
N
N
N
6
40.1
39.9
39.7
39.5
39.3
39.2
39.1
38.9
40.2
39.8
39.4
39.0
200
190
180
170
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140
130
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100
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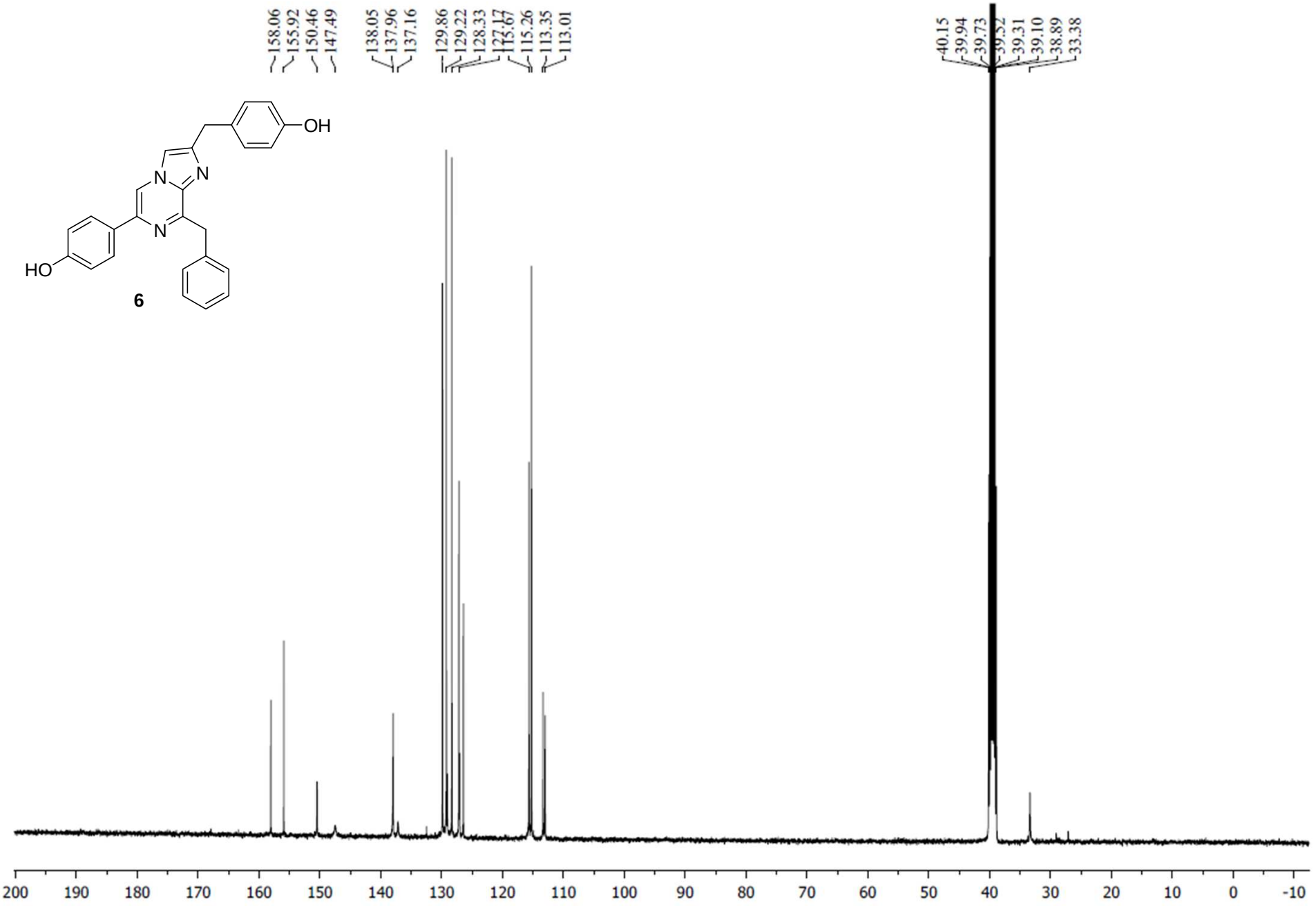
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113.35
113.01
40.15
39.94
39.73
39.52
39.31
39.10
38.89
33.38
OH
HO
N
N
N
6
200
190
180
170
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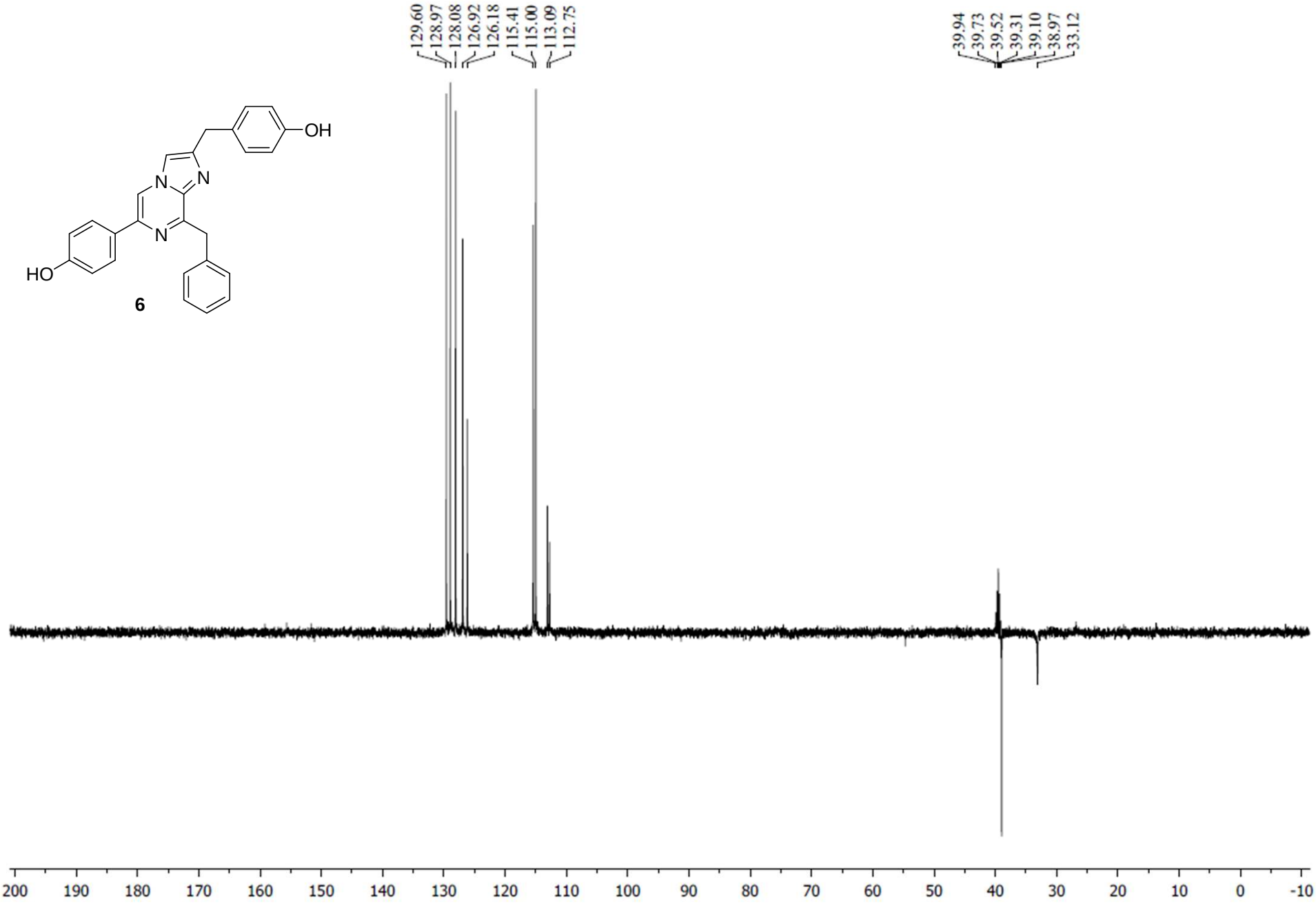
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128.97
128.08
126.92
126.18
115.41
115.00
113.09
112.75
39.94
39.73
39.52
39.31
39.10
38.97
33.12
OH
HO
N
N
N
6
200
190
180
170
160
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130
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100
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80
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60
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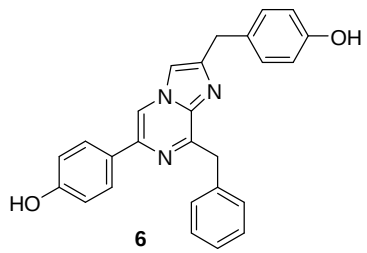
-10


OH
N
N
N
HO

6

2
3
4
5
6
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8
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8.5
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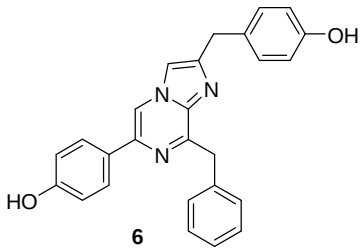
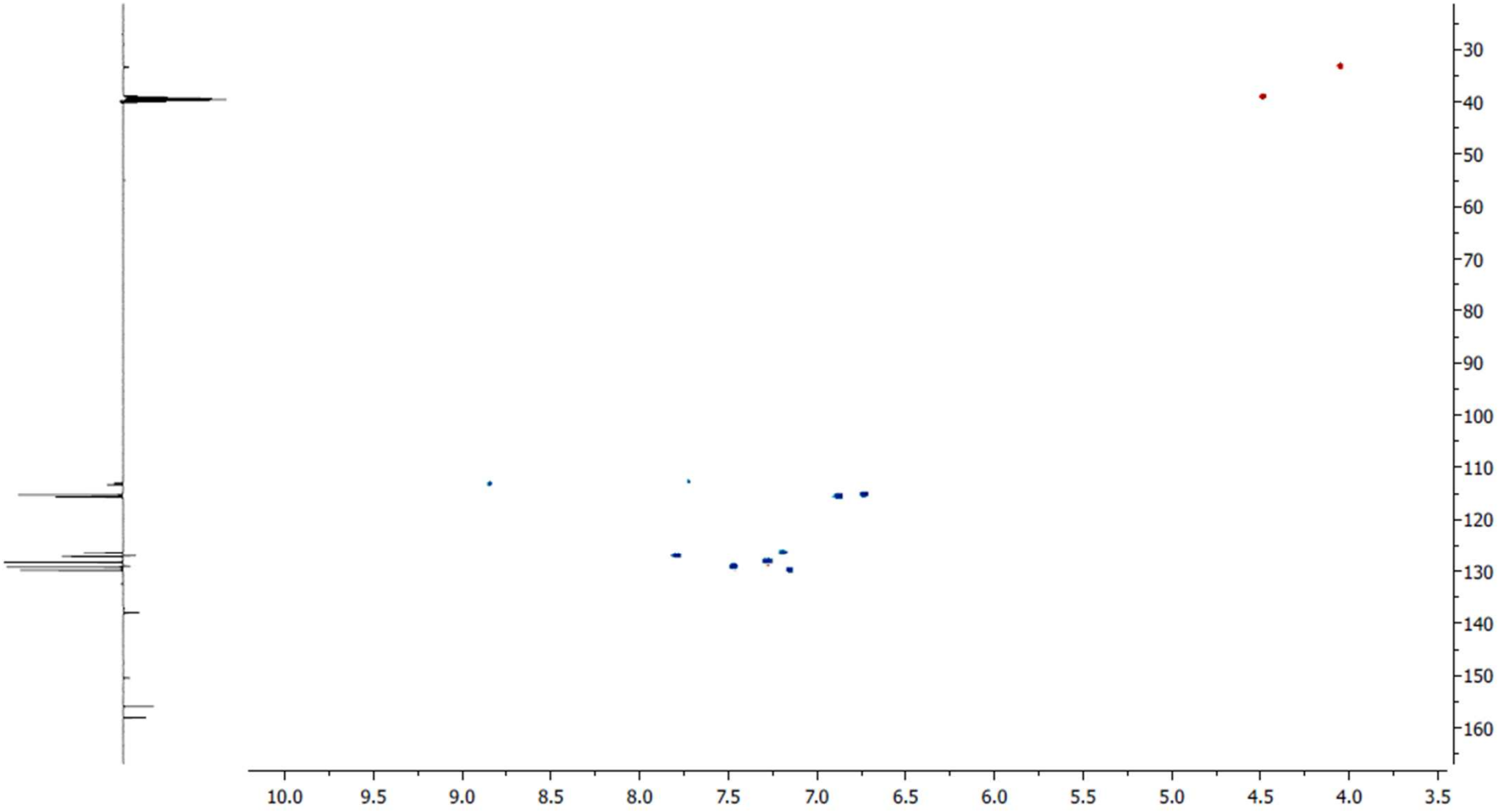
2.5


OH
HO
N
N
N

6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120
130
140
150
160
10.0
9.5
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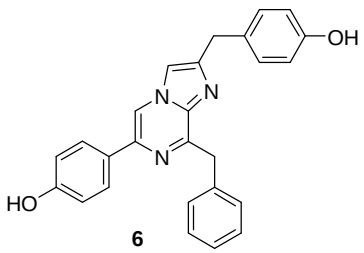
OH
HO
N
N
N

6

30
40
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60
70
80
90
100
110
120
130
140
150
160
170
10.0
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8.0
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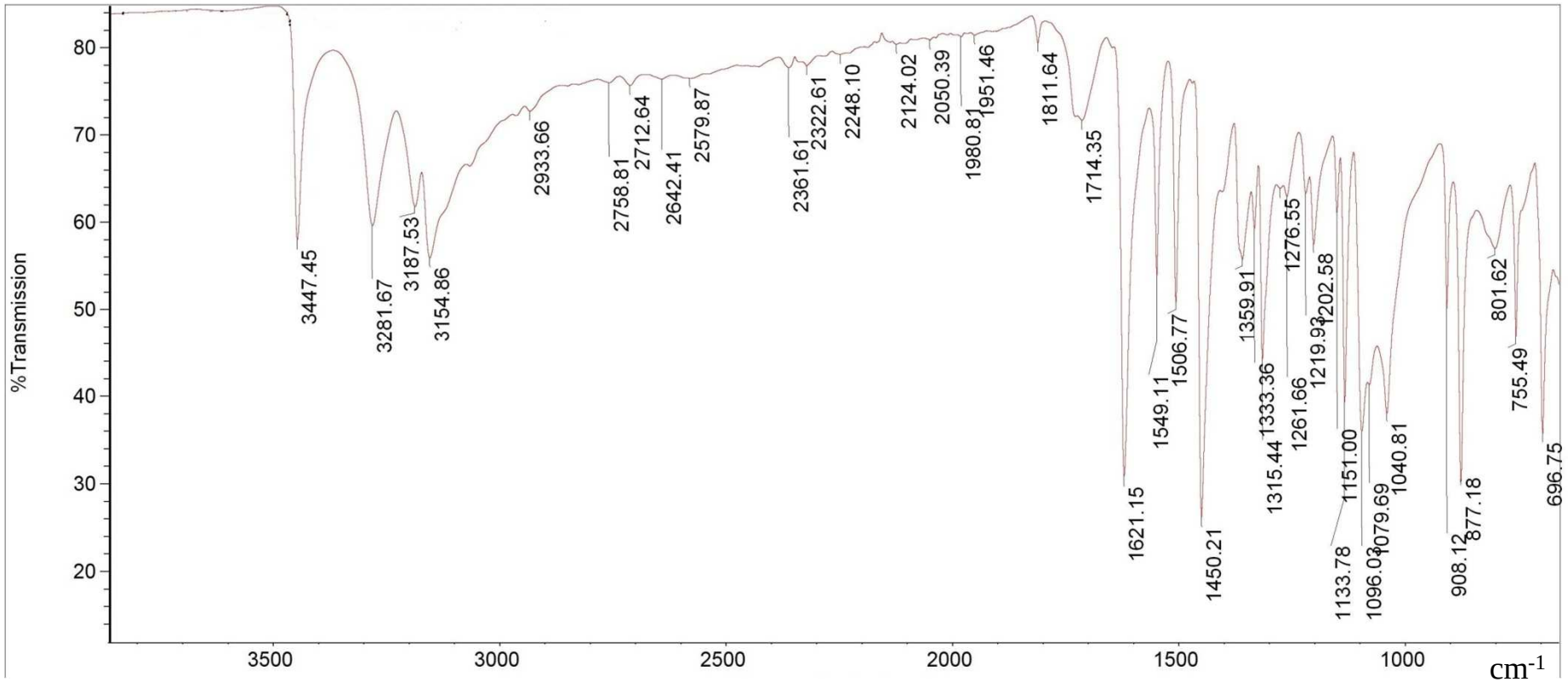
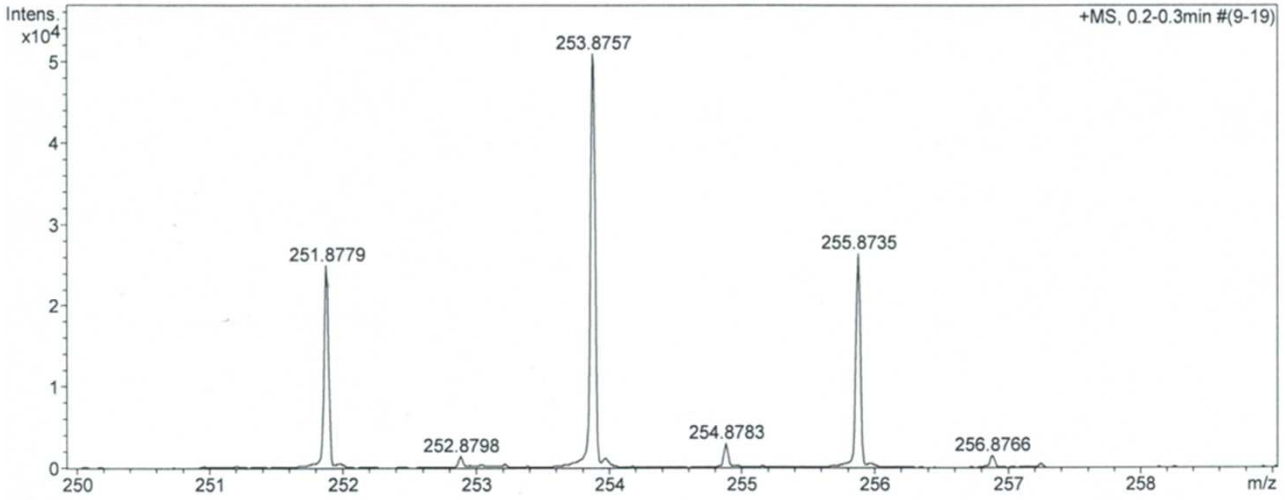
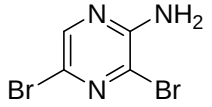
4.5
4.0

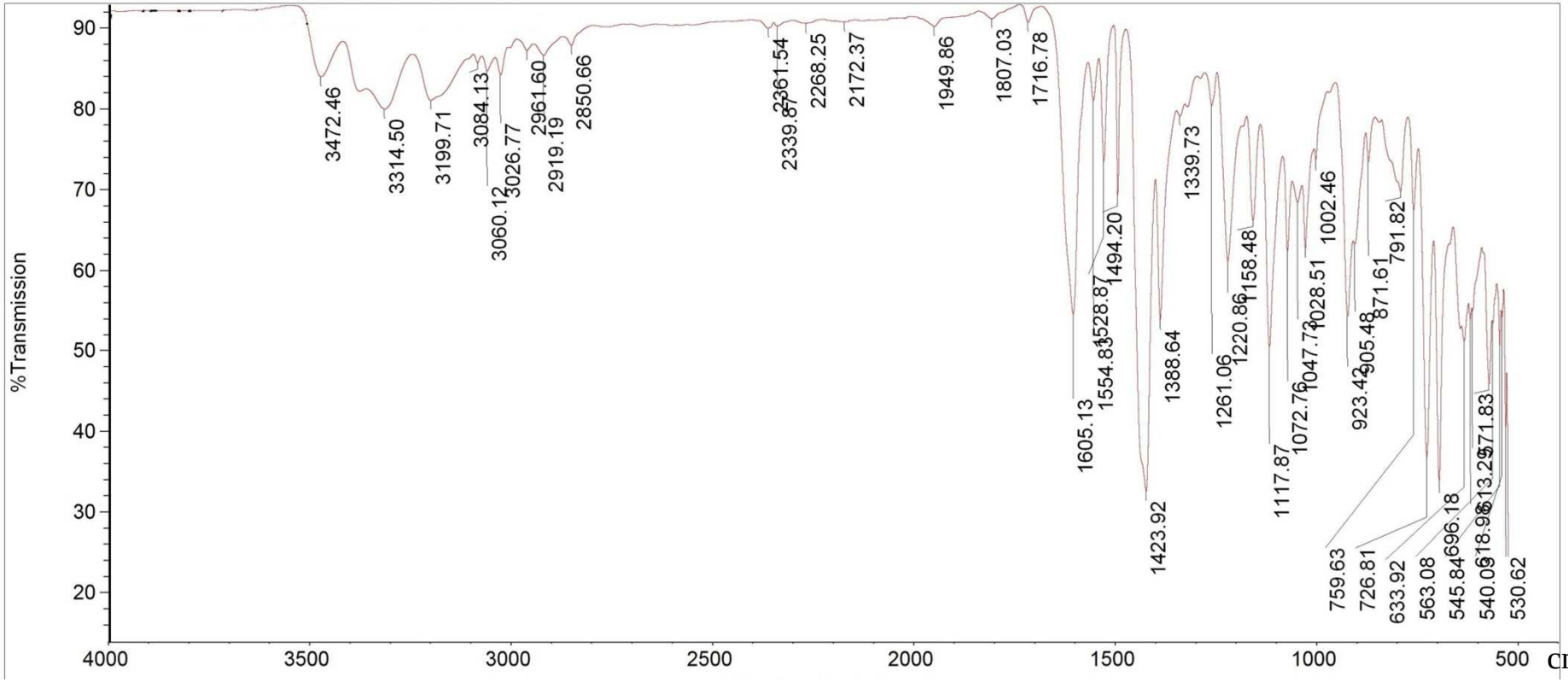


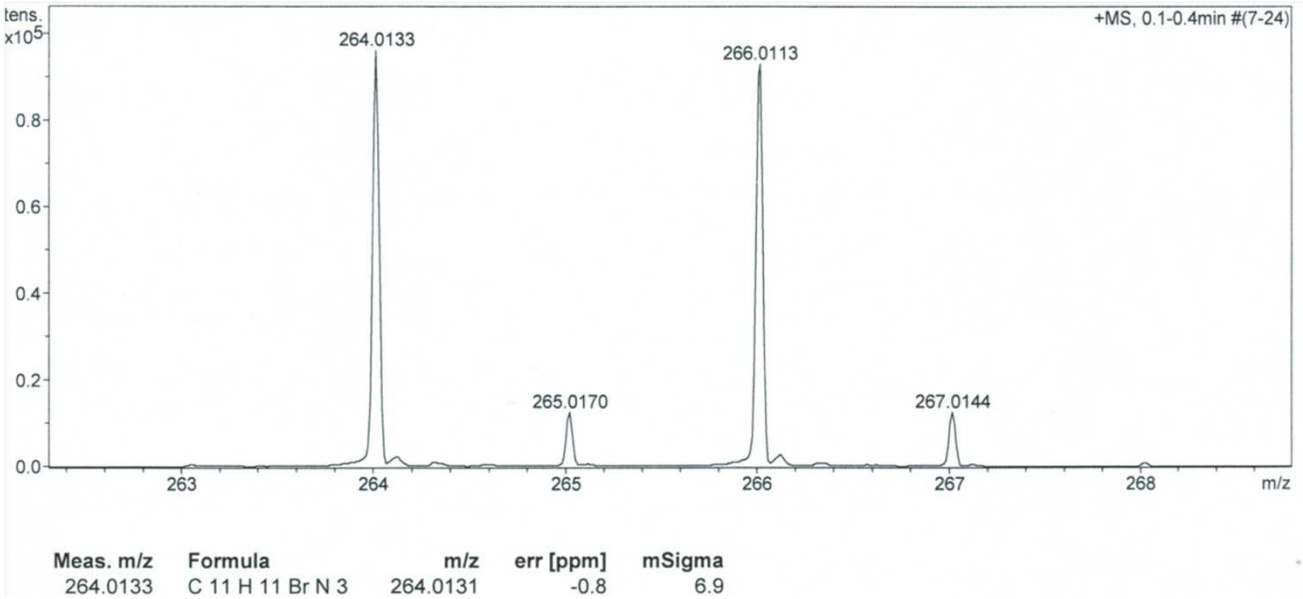


Meas. m/z	Formula	m/z	err [ppm]	mSigma
251.8779	C 4 H 4 Br 2 N 3	251.8766	-4.9	16.3

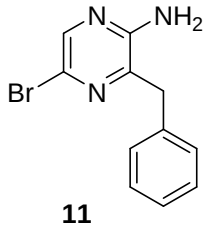


10

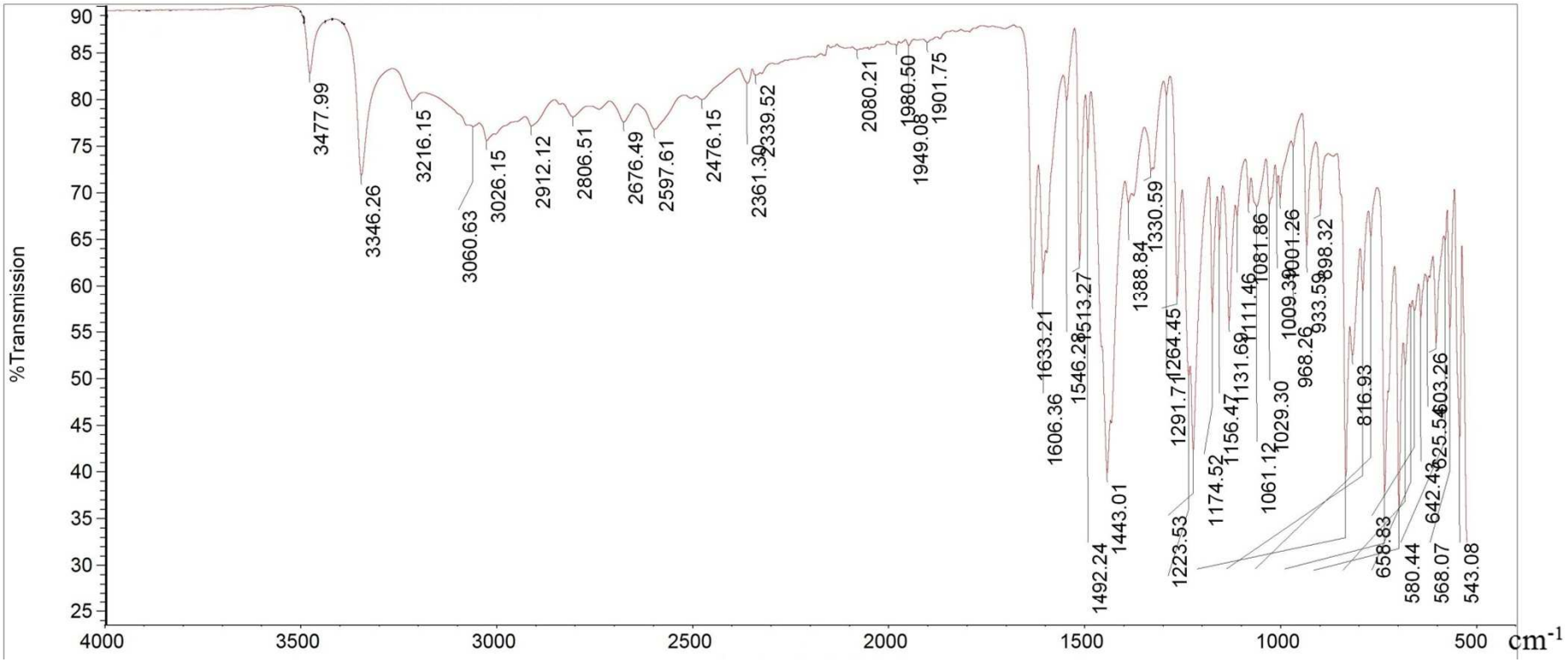


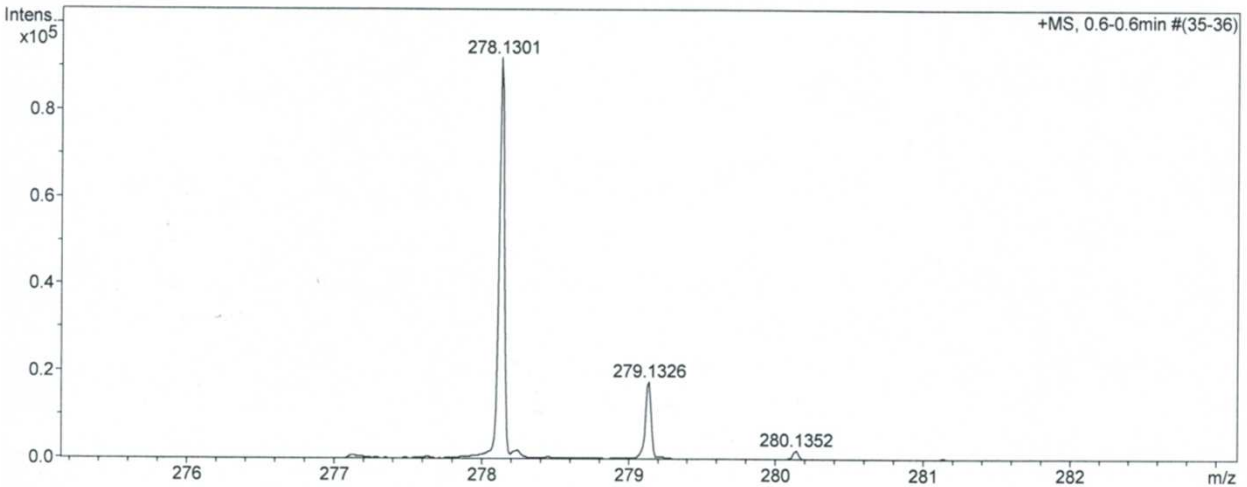


Meas. m/z	Formula	m/z	err [ppm]	mSigma
264.0133	C 11 H 11 Br N 3	264.0131	-0.8	6.9

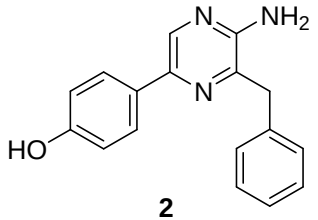


11





Meas. m/z	Formula	m/z	err [ppm]	mSigma
278.1301	C 17 H 16 N 3 O	278.1288	-4.6	2.8



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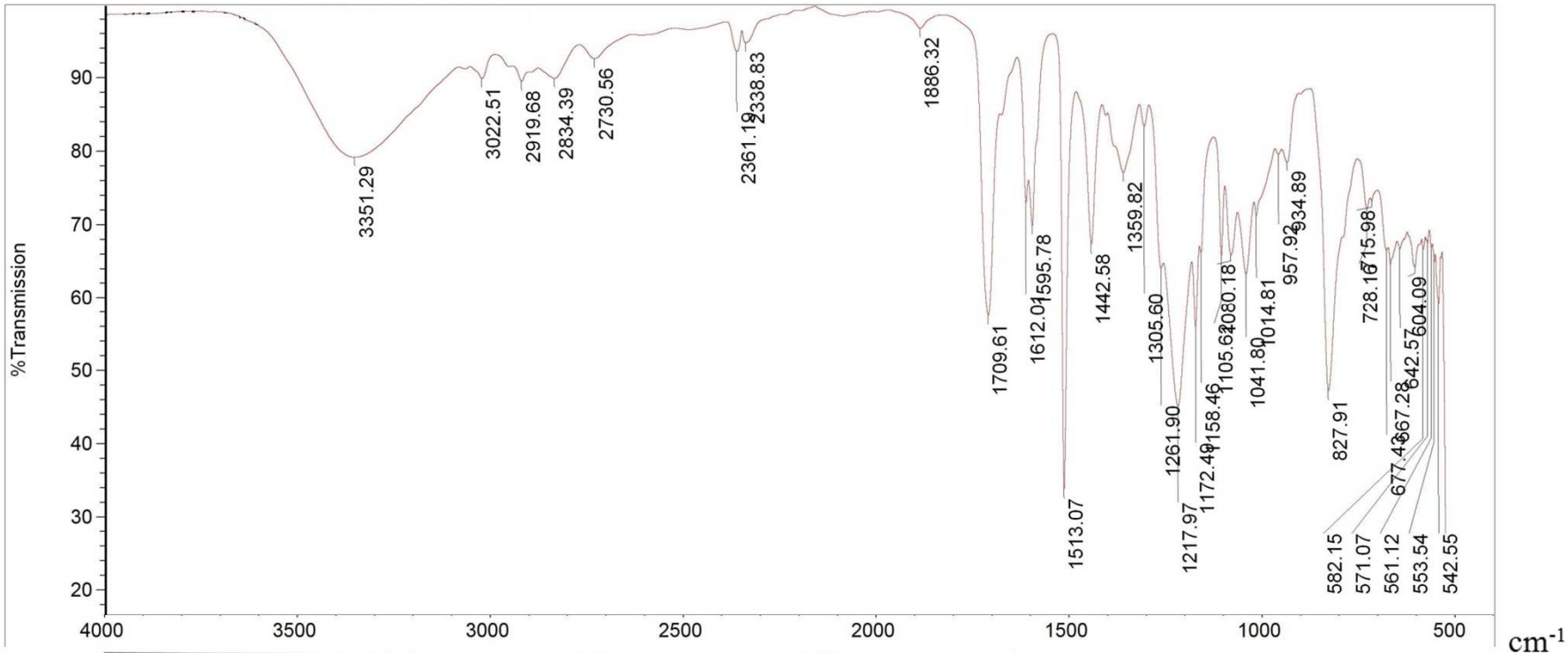
%Transmission
3351.29
3022.51
2919.68
2834.39
2730.56
2361.19
2338.83
1886.32
1709.61
1612.01
1595.78
1513.07
1442.58
1359.82
1305.60
1261.90
1217.97
1172.49
1158.46
1105.62
1080.18
1041.80
1014.81
957.92
934.89
827.91
728.16
715.98
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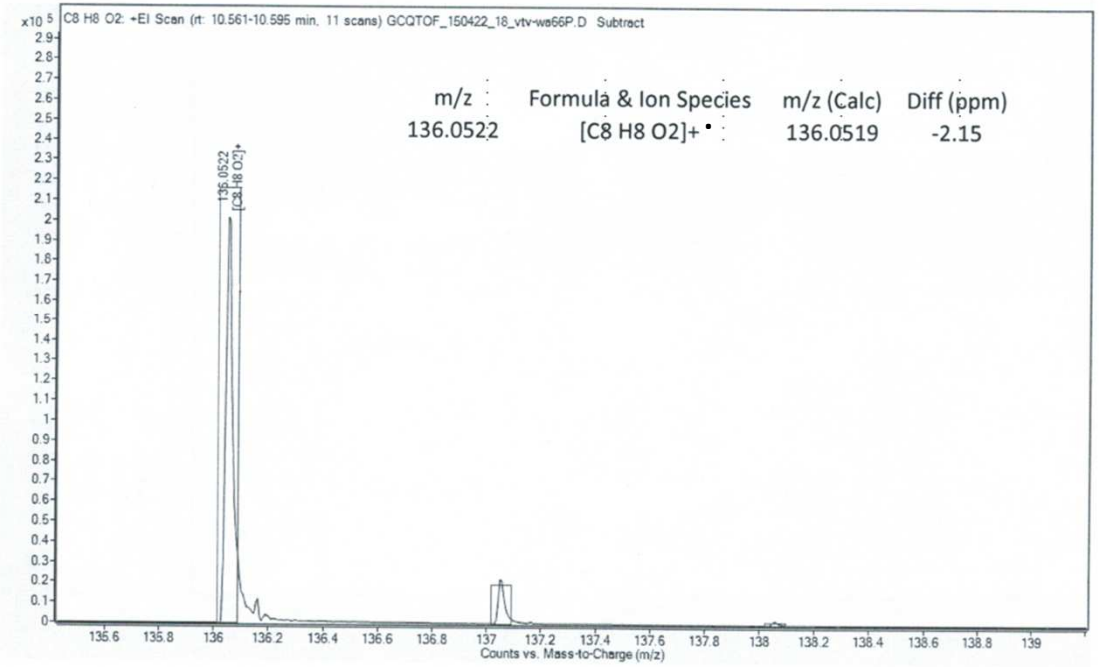
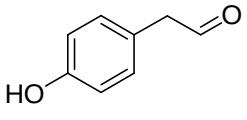
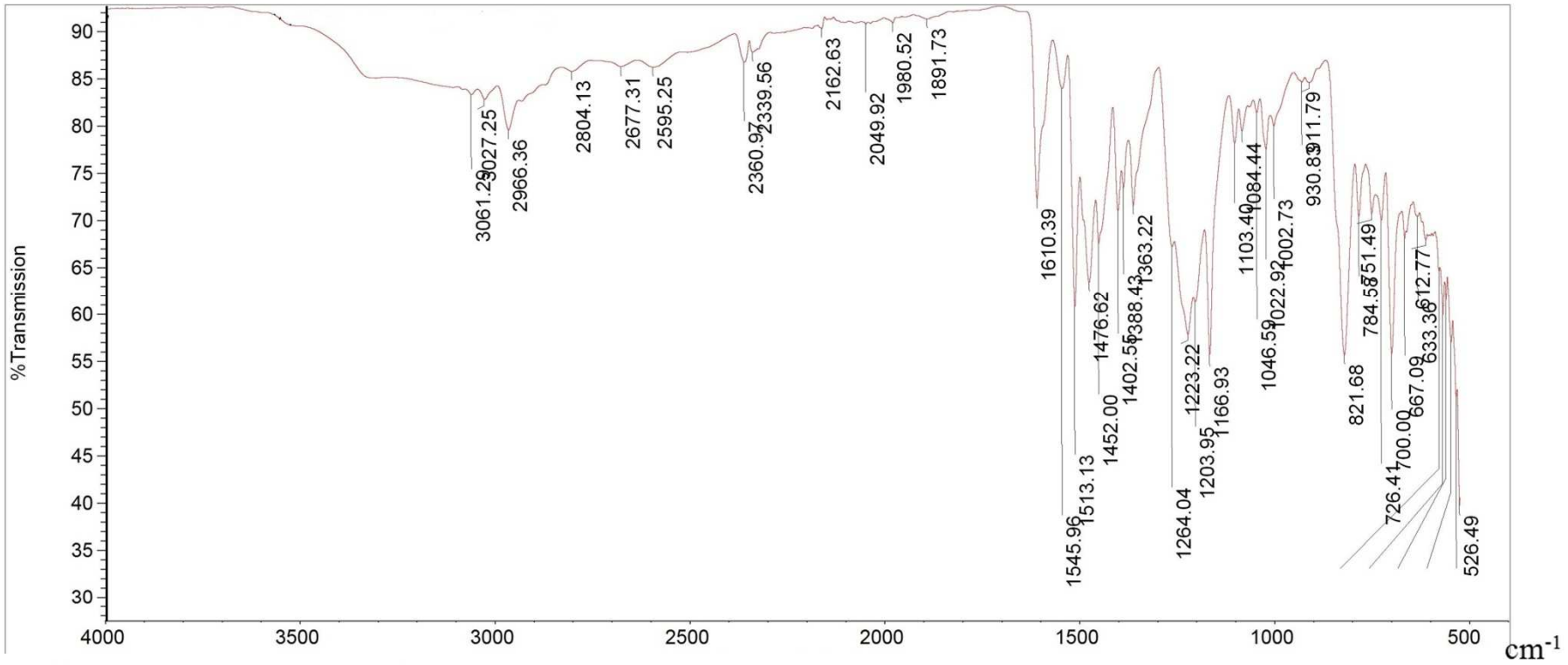
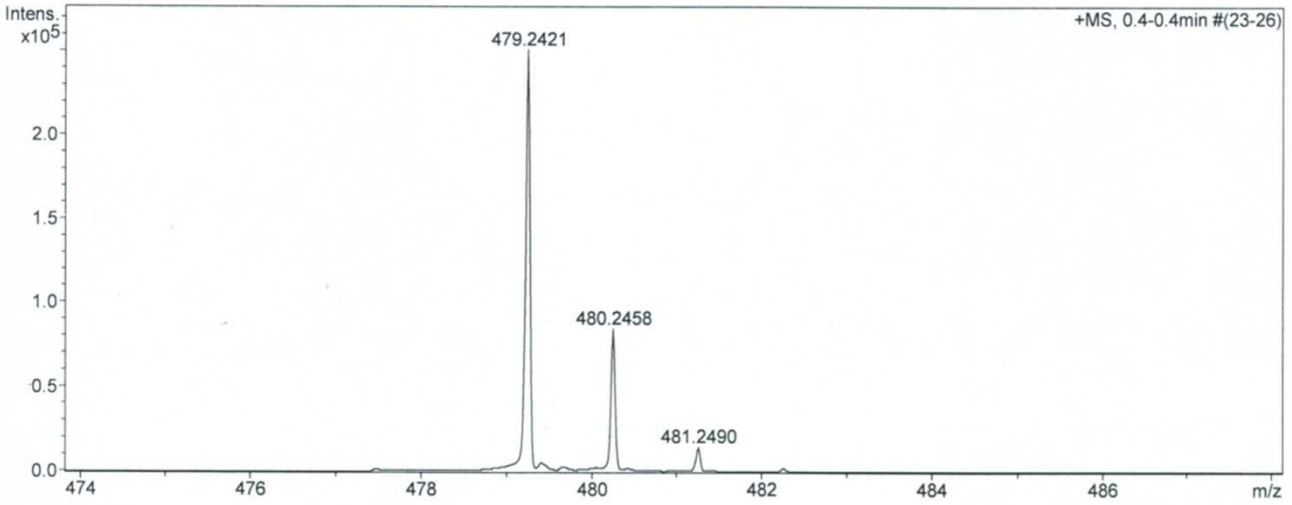
667.28
642.57
604.09
582.15
571.07
561.12
553.54
542.55
cm-1


C8 H8 O2: +EI Scan (rt: 10.561-10.595 min, 11 scans) GCQTOF_150422_18_vtv-wa66P.D Subtract
m/z
136.0522
Formula & Ion Species
[C8 H8 O2]+•
m/z (Calc)
136.0519
Diff (ppm)
-2.15
136.0522
[C8 H8 O2]+•
Counts vs. Mass-to-Charge (m/z)

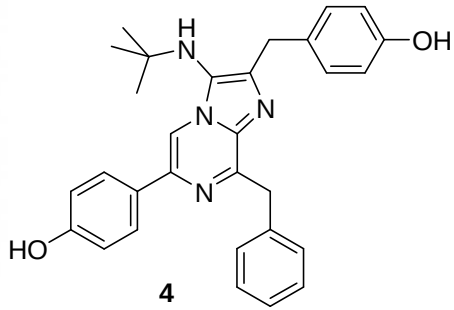

HO
O

8





Meas. m/z	Formula	m/z	err [ppm]	mSigma
479.2421	C 30 H 31 N 4 O 2	479.2442	4.4	3.5



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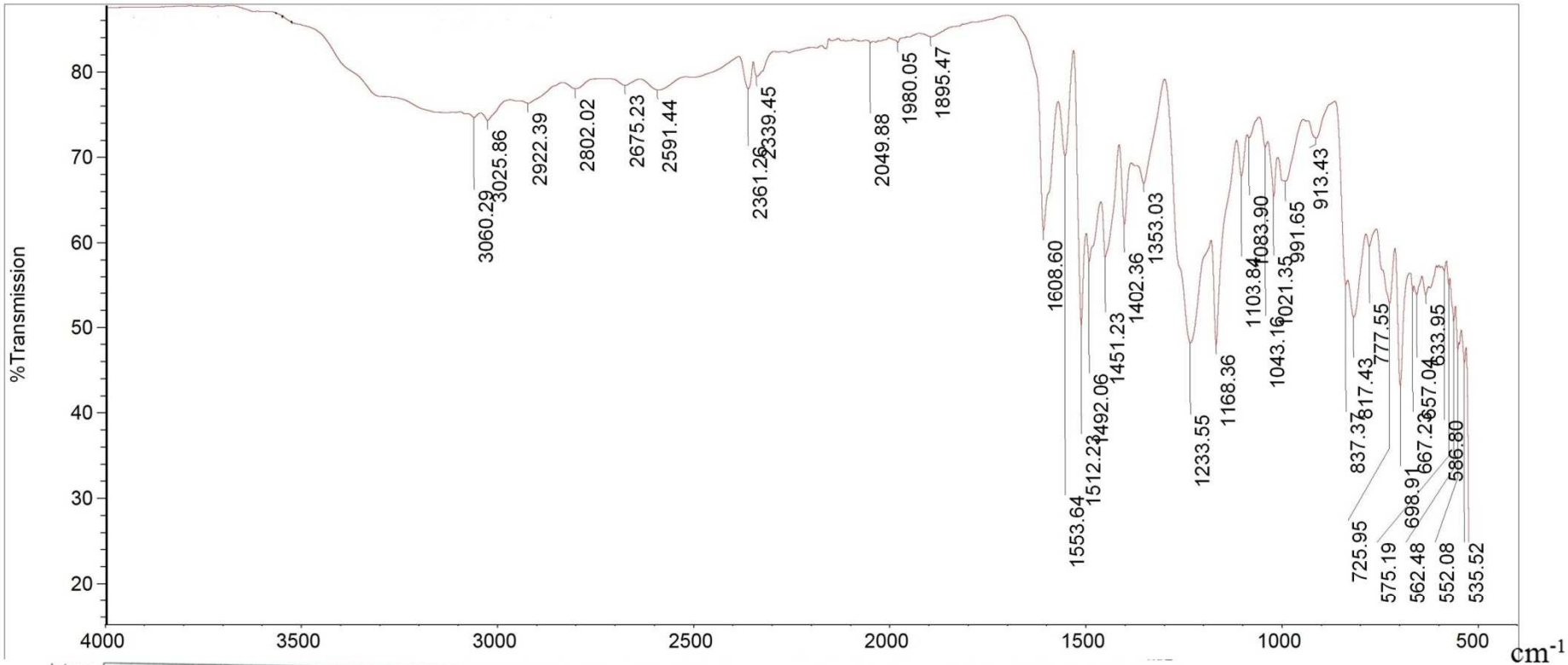
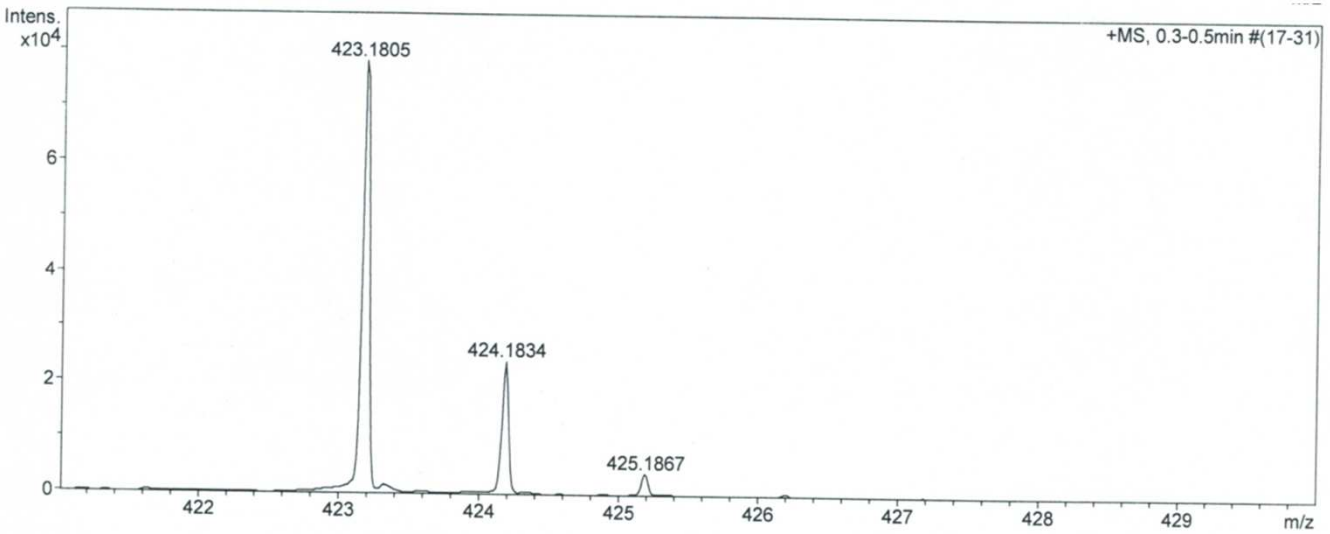
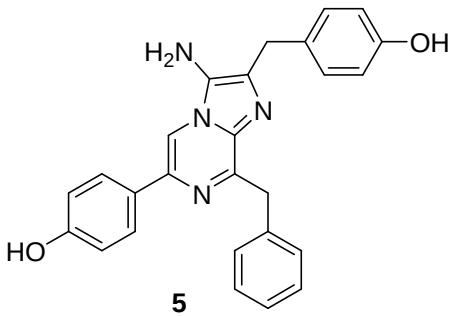




Meas. m/z	Formula	m/z	err [ppm]	mSigma
423.1805	C 26 H 23 N 4 O 2	423.1816	2.5	7.7



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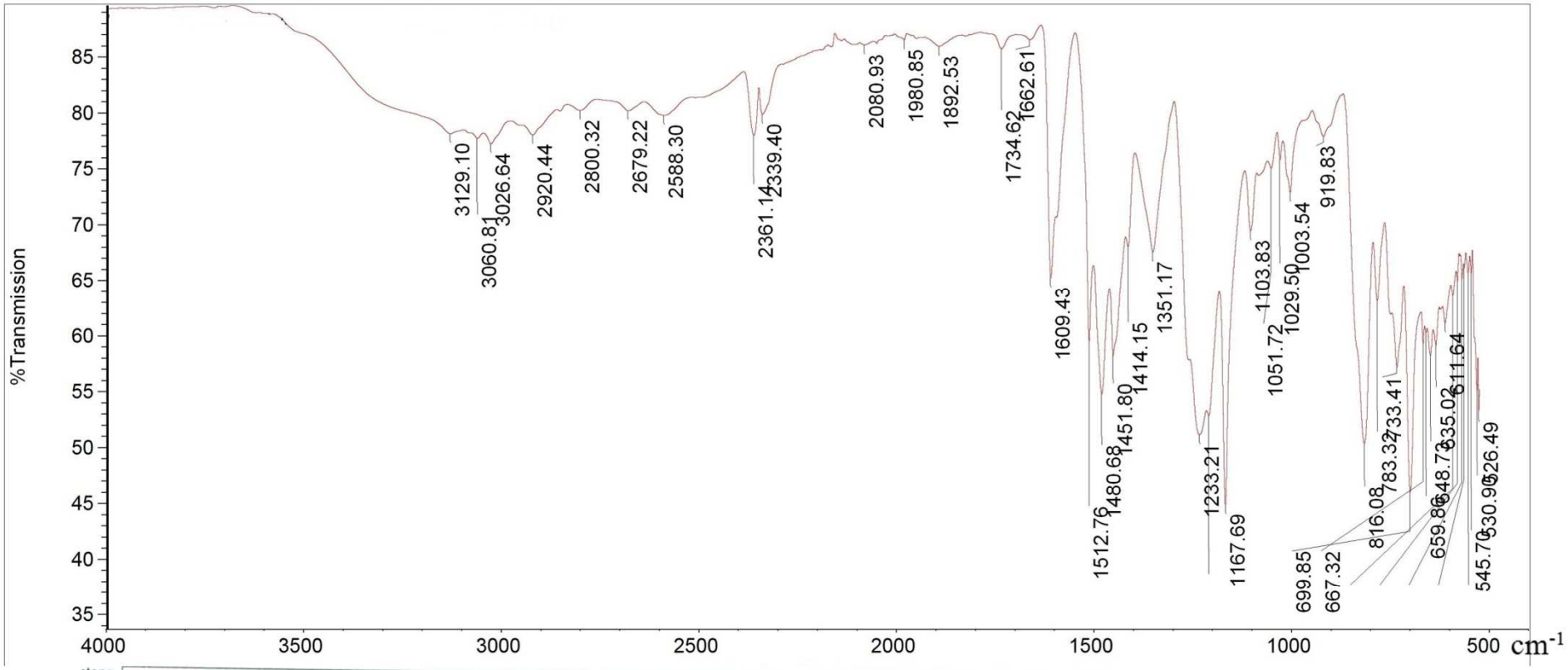
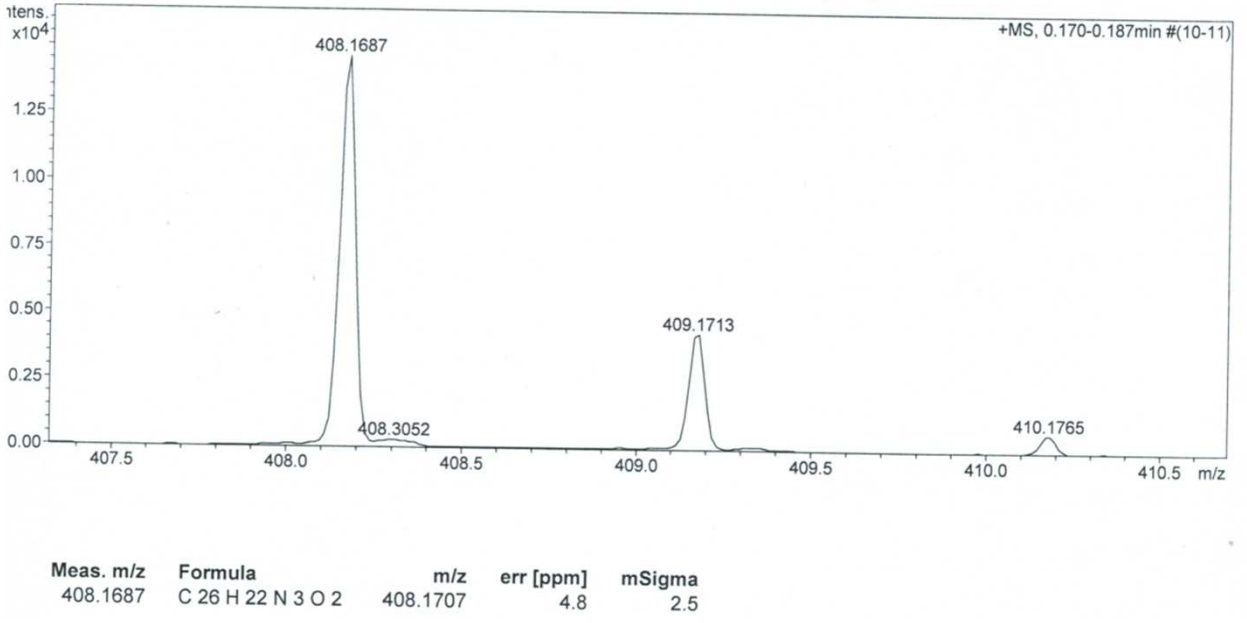
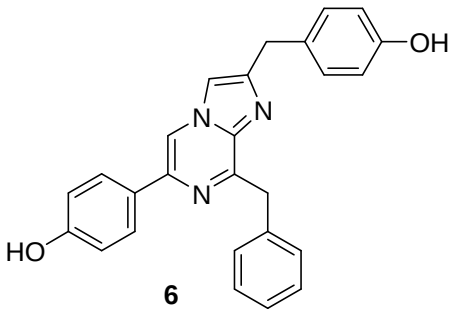




Meas. m/z	Formula	m/z	err [ppm]	mSigma
408.1687	C 26 H 22 N 3 O 2	408.1707	4.8	2.5



6