Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Design of non-cytotoxic 6,7-dihydroxycoumarin-5-carboxylates with antibiofilm activity against *Staphylococcus aureus* and *Candida albicans*

Robert Zscherp,[a,b†] Aishi Chakrabarti,[b,d†] Anna P. Lehmann,[a,b] Hedda Schrey,[e,f] Hoaxuan Zeng,[e,f] Wera Collisi,[e,f] and Philipp Klahn*[b,c,d]

[a] Institute of Organic Chemistry, Technische Universität Braunschweig, Hagenring 30, 38106 Braunschweig, Germany

[b] Laboratories of Natural Product and Conjugation Chemistry (naconLabs) – A Technology Transfer Center of iTUBS mbH, Wilhelmsgarten 3, 38100 Braunschweig, Germany.

[c] Division of Organic and Medicinal Chemistry, Department of Chemistry and Molecular Biology, University of Gothenburg, Kemigården 4, 41296 Göteborg, Sweden

[d] Centre for Antibiotic Resistance Research in Gothenburg (CARe)

[e] Department of Microbial Drugs, Helmholtz Centre for Infection Research (HZI), Inhoffenstrasse 7, 38124 Braunschweig, Germany.

[e] German Centre for Infection Research (DZIF), Partner Site Hannover-Braunschweig, 38124 Braunschweig, Germany.

† Authors contributed equally.

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General methods

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. (*N,N*-Dimethylformamid (DMF): *Acros Organics*, puriss., extra dry, over molesieve (water ≤ 0.005%), Ethanol (EtOH): *Acros Organics*, puriss., absolut, extra dry (water ≤ 0.005%), Pyridin (Pyr): *Acros Organics*, puriss., extra dry, over molesieve (water ≤ 0.005%), Dimethylsulfoxid (DMSO): *Acros Organics*, puriss., extra dry, over mol sieve (water ≤ 0.005%), Methanol (MeOH): *Acros Organics*, puriss., extra dry (water ≤ 0.005%)).
Moisture sensitive reactions were performed under argon atmosphere in dried glassware. Dry dichloromethane, diethyl ether, toluene and tetrahydrofuran for moisture sensitive reactions have been taken from a MB-SPS-800 (MBraun) solvent purifications system and stored under argon. All solvents used for workup and purification were of HPLC grade. Reactions were monitored by TLC, LCMS or NMR.
Solution of compounds in organic solvents were concentrated using rotary evaporators at a water bath temperature of max. 30°C. Solvent residues were removed in high vacuum at pressure of appr. 10^{-2} mbar. Unless otherwise noted solvents were degassed either by a continuous argon flow over minimum of 15 min or using the freeze-pump-thaw (FTP) technique.[1]

Flash chromatography[2] was done using appropriate glass columns filled with silicagel (Merck Millipore, Geduran® Si60, 1.11567.9025, 40-63 µm) or using the Biotage Select® chromatography system with a DAD detector and cartridges packed with silicagel (Merck Millipore, Geduran® Si60, 1.11567.9025, 40-63 µm) using a Cartridger® C-670 from the company Büchi.

Preparative reversed phase high pressure liquid chromatography (prep. HPLC RP) was performed on either a Hypersil GOLD C18 RP-column (Part No. 25005-259270), 5 µm, 250 mm×21.2 mm (10 mL/min) or a Hypersil GOLD C18 RP-column (Part No. 25005-259070A), 5 µm, 250 mm×10.0 mm (5 mL/min) each equipped with a guard column of the same material using a Thermo Fisher Scientific Dionex Ultimate 3000 HPLC system. Eluents, gradients and additives are given in parentheses. As eluents HPLC grade acetonitrile and water (VWR Chemicals, HPLC grade) with or without 0.1% of TFA (Carl Roth, 6957.1, 99.9%) or buffer added were used. Appropriate reaction mixtures were filtered through CHROMAFIL® PET-45/15 MS filters (45 µm) before injected. Product containing fractions were combined, diluted with dest. H_2O (min. 1:1/solvent:H_2O), frozen and lyophilized using a VaCo2® Freeze dryer from Zirbus (-80°C, 0.05 mbar).

Thin-layer chromatography (TLC) was performed on pre-coated glass plates (Merck TLC Silicagel 60 F_{254}, 1.15341.0001, 2.5x7.5 cm) and components were visualized by observation under UV light (λ = 254 nm [UV^{254}] or λ = 366 nm [UV^{366}]) or visible light, treatment of developed plates in an iodine chamber or by treating the plates with TLC staining solutions (for preparation see list below) followed by heating. Eluent or eluent-mixtures used are reported in parentheses.

CAM staining solution [CAM]: 1 g $Ce(IV)(SO_4)_2$, 2.5 g $(NH_4)_6Mo_4O_7$ in 100 mL 10% H_2SO_4

Ninhydrin staining solution [Ninhydrin]: 1.5 g Ninhydrin in 100 mL *abs.* EtOH and 3.0 mL HOAc.

Preparative thin-layer chromatography was performed on pre-coated glass plates (Merck TLC Silicagel 60 F_{254}, 1.05715.0001, 20x20 cm, max. 10-15 mg/plate and Analtech Uniplate Silica gel GF Z51305-9, 20x20 cm x 2 mm, max 100-150 mg/plate). Eluent or eluent-mixtures used and number of developments are reported in parentheses. Compounds were visualized by observation under UV light

(λ = 254 or 366 nm). Compound containing silica gel fractions were scratched from the plate with a scalpel, crushed to small pieces and compounds were eluated by appropriate solvent mixtures.

NMR spectra were recorded on a Bruker AV-300, AVIII400 und AVIIIHD500 with cryoprobe system at 293.15 K. ^{1}H NMR spectra were recorded at 300 MHz, 400 MHz and 500 MHz. ^{13}C NMR spectra were recorded at 76 MHz, 100 MHz and 126 MHz. Chemical shifts are reported in ppm relative to solvent signal. Multiplicity is indicated as follows: s (singlet); bs (broad singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublets), etc.. For the processing of the raw data the software MestReNova (Version 9.0.1-13254) from MestreLab Research S.L. was utilized.

IR spectra were recorded on a Bruker Tensor 27 IR spectrometer with ATR-technique. Only the wave numbers of observed absorption peaks are given.

Low resolution mass spectrometry (LRMS) data were recorded using an LC-MS system consisting of an Accela HPLC (Thermo Scientific) equipped with an Accela photodiode array (PDA) Detector, Accela autosampler, and Accela 1250 pump which was coupled to an LTQ XL mass spectrometer (Thermo Scientific) for HPLC/HESI-MS analyses. Heated electrospray ionization was used with an enhanced scan range of 120 to 2000 amu. Gradient HPLC solvent programs consisted of LCMS-grade H_2O, CH_3CN, and 2% formic acid in H_2O. An Agilent Zorbax Eclipse Plus C18 (3.5µm, 2.1×150 mm) column was used, which was kept at 30°C. The PDA detector was set to a scanning range from 190 to 600 nm with 1 nm wavelength steps.

High resolution mass spectrometry (HRMS) data were recorded on a Finnigan MAT 95 (EI, 70eV) mass spectrometer and a Finnigan MAT 95 XL (ESI) mass spectrometer.

UV-Vis spectroscopy data were recorded on a Cary 100 Bio (Varian).

Fluorescence Emission Spectroscopy data were recorded on a Cary Eclipse (Varian).

Hydrogenation reactions were performed in a laboratory high pressure autoclave HR-100 (Berghof).

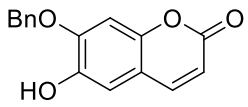
Microwave reactions were performed in an Initiator+ (Biotage)

Thin-layer chromatography mass spectrometry (TLC-MS) data were recorded on an Advion Expression compact mass spectrometer equipped with an Advion Plate Express automated TLC plate reader.

Imaging of bacteria (microscopy) was performed using a DMi8 inverted microscope equipped with a 40x/1.30 oil immersion objective. Images were captured in bright field mode (BF) or in fluorescence mode (F) using a GFP filter set (excitation filter: 480 nm, 20 nm bandwidth; emission filter: 527 nm, 15 nm).

Synthesis of the compounds

7-(Benzyloxy)-6-hydroxy-2*H*-Chromen-2-one (7)

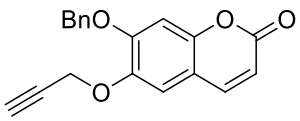


Chemical Formula: $C_{16}H_{12}O_4$
Molecular Weight: 268.27

Na_2CO_3 (892 mg, 8.42 mmol, 3.0 equiv) was added to a solution of Esculetin (**2**) (500 mg, 2.81 mmol, 1.0 equiv) in dry DMF (5.0 mL, 0.56 M) under argon atm. at 0 °C and the mixture was stirred for 40 min at 0 °C. Benzyl bromide (1.0 mL, 8.42 mmol, 3.0 equiv) was added and the mixture was stirred for 14 h at -10 °C. The mixture was diluted with EtOAc (50 mL) and quenched by addition of HCl_{aq} (1 M, 10 mL). The phases were separated, and the organic phase was washed with H_2O (3x 50 mL) and brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5) yielding 7-(benzyloxy)-6-hydroxy-2*H*-chromen-2-one (**7**) (548 mg, 2.04 mmol, 73%) as an amorphous, white solid.

TLC (CH_2Cl_2:MeOH/95:5) R_f: 0.78 [UV^{366}, CAM]. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ [ppm]: 9.45 (s, 1H), 7.90 (d, *J* = 9.5 Hz, 1H), 7.58 – 7.25 (m, 5H), 7.07 (d, *J* = 15.7 Hz, 2H), 6.24 (d, *J* = 9.5 Hz, 1H), 5.23 (s, 2H).**^{13}C-NMR:** (76 MHz, DMSO-d_6) δ [ppm]: 160.6, 150.6, 148.1, 144.2, 143.9, 136.4, 128.5, 128.0, 127.9, 112.7, 112.2, 111.7, 101.4, 70.1. The analytical data were in accordance with the literature.[3]

7-(Benzyloxy)-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one (8)

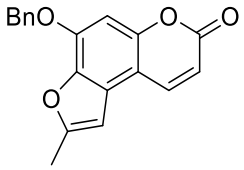


Chemical Formula: $C_{19}H_{14}O_4$
Molecular Weight: 306.32

Propargyl bromide (80% in toluene, 295 µL, 2.65 mmol, 1.3 equiv) was added to a mixture of 7-(benzyloxy)-6-hydroxy-2H-chromen-2-one (**7**) (548 mg, 2.04 mmol, 1.0 equiv) and K_2CO_3 (365 mg, 2.65 mmol, 1.3 equiv) in dry acetone (11 mL, 0.19 M) under argon atm. at 23 °C and the mixture was heated up to 55 °C for 16 h. The mixture was diluted with H_2O (30 mL) and extracted with EtOAc (3x 20 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99:1) yielding 7-(benzyloxy)-6-(prop-2-yn-1-yloxy)-2H-chromen-2-one (**8**) (565 mg, 1.85 mmol, 91%) as an amorphous, white solid.

TLC (CH_2Cl_2:MeOH/99:1) R_f: 0.70 [UV^{366}, CAM]. **IR** (ATR) [cm^{-1}]: 3279, 3065, 2933, 2873, 1718, 1614, 1561, 1509, 1437, 1381, 1273, 1243, 1200, 1170, 1142, 1097, 1016, 932, 856, 821, 744, 697, 604, 582, 546. **HRMS** (ESI) [m/z]: 329.07865, calculated 329.07843 for $[C_{19}H_{14}O_4Na]^+$, err [ppm] 0.67; 635.16816, calculated 635.16764 for $[C_{38}H_{28}O_8Na]^+$, err [ppm] 0.82. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ [ppm]: 7.95 (d, *J* = 9.5 Hz, 1H), 7.56 – 7.22 (m, 6H), 7.20 (s, 1H), 6.30 (d, *J* = 9.5 Hz, 1H), 5.23 (s, 2H), 4.85 (d, *J* = 2.3 Hz, 2H), 3.60 (s, 1H).**^{13}C-NMR:** (76 MHz, DMSO-d_6) δ [ppm]: 160.4, 151.9, 149.8, 144.1, 143.6, 136.1, 128.5, 128.2, 128.0, 113.0, 112.1, 111.3, 101.6, 78.9, 78.8, 70.3, 56.6.

4-(Benzyloxy)-2-methyl-7*H*-furo[3,2-f]chromen-7-one (9)

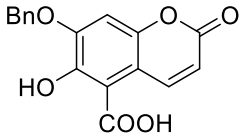


Chemical Formula: $C_{19}H_{14}O_4$
Molecular Weight: 306.32

CsF (705 mg, 4.65 mmol, 5.0 equiv) was added to a solution of 7-(benzyloxy)-6-(prop-2-yn-1-yloxy)-2H-chromen-2-one (**8**) (285 mg, 0.93 mmol, 1.0 equiv) in *N*,*N*-diethyl aniline (3.25 µL, 0.29 M) under argon atm. at 23 C and the mixture was heated up to 216°C in the microwave reactor for 3 h. The mixture was diluted with EtOAc (50 mL), washed with $HCl_{(aq.)}$ (1 M,3 x 40 mL) and brine (10 mL), dried over Na_2SO_4 and filtered. The solution was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99:1) yielding 4-(benzyloxy)-2-methyl-7H-furo[3,2-f]chromen-7-one (**9**) (203 mg, 0.66 mmol, 71%) as an amorphous, beige solid.

TLC (CH_2Cl_2:MeOH/99:1) R_f: 0.70 [UV^{366}, CAM]. **IR** (ATR) [cm^{-1}]: 3066, 2922, 1720, 1606, 1507, 1453, 1410, 1345, 1290, 1244, 1181, 1143, 1115, 1075, 1005, 963, 941, 897, 812, 741, 699, 671, 602, 555. **HRMS** (ESI) [m/z]: 329.07859, calculated 329.07843 for $[C_{19}H_{14}O_4Na]^+$, err [ppm] 0.49; 635.16807, calculated 635.16764 for $[C_{38}H_{28}O_8Na]^+$, err [ppm] 0.68. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ [ppm]: 8.28 (d, *J* = 9.5 Hz, 1H), 7.60 – 7.52 (m, 2H), 7.49 – 7.35 (m, 4H), 7.04 (d, *J* = 1.2 Hz, 1H), 6.35 (d, *J* = 9.5 Hz, 1H), 5.37 (s, 2H), 2.50 (d, 3H), .**^{13}C-NMR:** (76 MHz, DMSO-d_6) δ [ppm]: 160.6, 158.0, 152.0, 146.4, 141.5, 139.5, 135.9, 128.6, 128.3, 128.2, 127.2, 111.9, 104.5, 102.0, 96.3, 70.6, 13.9.

7-(Benzyloxy)-6-hydroxy-2-oxo-2H-chromene-5-carboxylic acid (11b)



Chemical Formula: $C_{17}H_{12}O_6$
Molecular Weight: 312.28

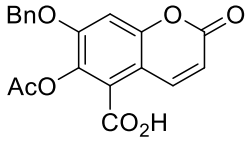
Ozone (0.2 L/h O_2, 0.5 A) was bubbled through a solution of 4-(benzyloxy)-2-methyl-7H-furo[3,2-f]chromen-7-one (**9**) (90 mg, 0.29 mmol, 1.0 equiv) in MeOH (5.0 mL, 0.06 M) and CH_2Cl_2 (5.0 mL, 0.06 M) at -78°C for 10 min. Argon was bubbled through the reaction mixture for 5 min at -78°C, before PPh_3 (63 µL, 0.87 mmol, 3.0 equiv) was added. The solution was allowed to warm up to 23 °C and stirred for 30 min at 23 °C. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99:1 →95:5).

The residue was dissolved in tBuOH (2.0 mL, 0.15 M), H_2O (2.0 mL, 0.15 M) and MeCN (1.0 mL, 0.29 M) and 2-methylbut-2-ene (441 µL, 3.48 mmol, 12.0 equiv) was added. The mixture was cooled to 0°C and $NaH_2PO_4{\cdot}H_2O$ (360 mg, 2.61 mmol, 9.0 equiv) and $NaClO_2$ (235 mg, 2.61 mmol, 9.0 equiv) were added. The mixture was stirred for 1 h at 23 °C. The mixture was acidified by addition of citric acid (10wt%, 20 mL) and extracted with CH_2Cl_2 (4 x 20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/9:1→7:3) yielding 7-

(benzyloxy)-6-hydroxy-2-oxo-2H-chromene-5-carboxylic acid (**11**) (43 mg, 0.14 mmol, 48%) as an amorphous, pale yellow solid.

TLC (CH_2Cl_2:MeOH/8:2) R_f: 0.20 [UV^{366}, CAM]. **IR** (ATR) [cm^{-1}]: 3411, 3092, 2934, 1768, 1722, 1662, 1600, 1445, 1372, 1284, 1256, 1200, 1135, 1104, 1017, 960, 894, 838, 754, 701, 668, 629, 594, 543. **HRMS** (ESI) [m/z]: 311.05608, calculated 311.05611 for $[C_{17}H_{11}O_6]^-$, err [ppm] -0.10. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ [ppm]: 9.64 (d, *J* = 9.9 Hz, 1H), 7.56 – 7.47 (m, 2H), 7.43 – 7.22 (m, 4H), 7.01 (s, 1H), 6.20 (d, *J* = 9.9 Hz, 1H), 5.18 (s, 2H).**^{13}C-NMR:** (76 MHz, DMSO-d_6) δ [ppm]: 170.9, 160.4, 153.5, 151.7, 146.6, 144.4, 136.6, 128.4, 128.3, 127.8, 127.8, 127.0, 111.4, 102.3, 69.8.

6-Acetoxy-7-(benzyloxy)-2-oxo-2H-chromene-5-carboxylic acid (11a)



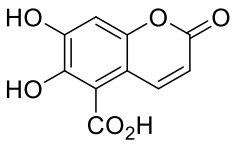
Chemical Formula: $C_{19}H_{14}O_7$
Molecular Weight: 354.31

4-(Benzyloxy)-2-methyl-7H-furo[3,2-f]chromen-7-one (0.10 g, 0.34 mmol, 1.0 eq) was dissolved in CH_2Cl_2 (3 mL) and methanol (0.6 mL) and cooled to -78 ° C. Then ozone was bubbled through the solution for 5 min followed by argon for 5 min. PPh_3 (98.07 mg, 0.37 mmol, 1.1 eq) was added, the mixture was stirred at -78°C for 15 min and then warmed to 23 ° C. The solution was concentrated, diluted with EtOAc (10 mL), washed with saturated NaCl solution (1x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. This was followed by a coarse purification via column chromatography (CH_2Cl_2:MeOH/1:99).

The crude product was reduced with 2-methyl-2-butene (0.5 mL, 4.08 mmol, 12.0 eq) and a mixture of t-BuOH:H_2O:CH_3CN (3: 3: 1, 2.1 mL: 2.1 mL: 0.7mL) at 0° C. NaH_2PO_4 (0.42 g, 3.06 mmol, 9.0 eq) and $NaClO_2$ (0.27 g, 3.06 mmol, 9.0 eq) were added and the ice bath was removed after 5 min. The mixture was stirred vigorously at 23 °C for 2h and then quenched with $Na_2S_2O_3$ solution. The solution was brought to pH = 6 with citric acid solution, extracted with EtOAc (3x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/3:7) to yield 6-acetoxy-7-(benzyloxy)-2-oxo-2H-chromene-5-carboxylic acid (**11a**) (32 mg, 0.090 mmol, 27%) as a beige solid.

TLC (MeOH:CH_2Cl_2/1.5:8.5) R_f: 0.20 [CAM, UV^{258}, UV^{366}]. **^{1}H NMR** (300 MHz, MeOD-d_4) δ 8.02 (d, *J* = 9.7 Hz, 1H), 7.54 – 7.27 (m, 7H), 7.02 (d, *J* = 10.6 Hz, 1H), 6.22 (dd, *J* = 9.8, 7.2 Hz, 1H), 5.18 (s, 2H), 2.22 (s, 3H). **^{13}C NMR** (75 MHz, MeOD-d_4) δ 170.4, 162.9, 155.2, 154.5, 144.3, 137.4, 134.5, 129.6, 129.3, 128.8, 128.55, 113.9, 110.3, 101.7, 72.2, 20.5.

6,7-Dihydroxy-2-oxo-2H-chromene-5-carboxylic acid (DHCou)



Chemical Formula: $C_{10}H_6O_6$
Molecular Weight: 222.15

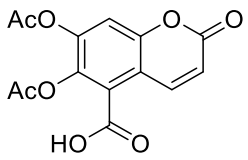
6-Acetoxy-7-(benzyloxy)-2-oxo-2H-chromene-5-carboxylic acid (32 mg, 0.09 mmol, 1.0 eq) was dissolved in methanol (0.25 mL) and Palladium on charcoal (10%, 5 mg) was added under a hydrogen

atmosphere and stirred for 2.5 h at 23°C. 1 M HCl (0.25 mL) was added, and the mixture was stirred at 23°C for 24 h under argon atm.. The crude was filtered through celite, washed with methanol, and concentrated under reduced pressure. The residue was dissolved in 1.0 mL of H_2O/MeCN (7:3), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×21.0 mm (10 mL/min), Eluents: H_2O (0.1% TFA):MeCN (0.1% TFA)/95:5 → 75:25), t_R = 25 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding 6,7-dihydroxy-4-methyl-2-oxo-2H-chromene-5-carboxylic acid (**DHCou**) (14.4 mg, 0.0648 mmol, 72%) as a yellowish solid.

Alternatively, when 7-(Benzyloxy)-6-hydroxy-2-oxo-2H-chromene-5-carboxylic acid (**11b**) was reacted in the presence of Palladium on charcoal (10%, 5 mg) under a hydrogen atmosphere and stirred for 2.5 h at 23°C 6,7-dihydroxy-4-methyl-2-oxo-2H-chromene-5-carboxylic acid (**DHCou**) was obtained in 82% following the work up and purification shown above.

TLC (MeOH: CH_2Cl_2/3:7) R_f: 0.15 [CAM, UV^{258}, UV^{366}]. **LRMS** (ESI) [m/z]: 221.1, calculated: 221.0 for $[C_{10}H_5O_6]^-$. **HRMS** (ESI) [m/z]: 245.00574, calculated 245.00566 for $[C_{10}H_6O_6Na]^+$, err [ppm] 0.32. **^{1}H NMR** (300 MHz, MeOD-d_4) δ 8.85 (d, J = 10.0 Hz, 1H), 6.85 (s, 1H), 6.19 (d, J = 10.0 Hz, 1H).

6,7-Diacetoxy-2-oxo-2*H*-chromene-5-carboxylic acid (12)

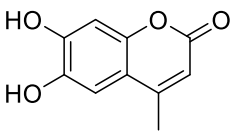


Chemical Formula: $C_{14}H_{10}O_8$
Molecular Weight: 306.23

Palladium on charcoal (10w% Pd, 4.5 mg) was added to a solution of 7-(benzyloxy)-6-hydroxy-2-oxo-2H-chromene-5-carboxylic acid (**11a**) (45 mg, 0.12 mol, 1.0 equiv) in MeOH (1.0 mL, 0.12 M) under argon atmosphere. A pressure of 1 bar H_2 atm. was applied, and the mixture was stirred for 3 h at 23 °C. The mixture was filtered through celite® and concentrated under reduced pressure. The residue was dissolved in dry DMF (0.8 mL, 0.15 M) and Ac_2O (29 µL, 0.31 mmol, 2.5 equiv) and pyridine (25 µL, 0.31 mmol, 2.5 equiv) were added at 0 °C. The mixture was stirred for 90 min at 23 °C. EtOAc (10 mL) was added, and the mixture was washed with H_2O (3 x 10 mL) and brine (5 mL). The solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5→7:3) yielding 6,7-Diacetoxy-2-oxo-2*H*-chromene-5-carboxylic acid (**12**) (23.5 mg, 0.0768 mmol, 64%) as an amorphous, pale yellow solid.

TLC (CH_2Cl_2:MeOH/75:25) R_f: 0.38 [UV^{366}, CAM]. **LRMS** (ESI) [m/z]: 307.0 $[C_{14}H_{11}O_8]^+$. **HRMS** (ESI) [m/z]: 351.00882, calculated 351.00873 for $[C_{14}H_{10}O_8Na_2]^+$, err [ppm] 0.26.

6,7-Dihydroxy-4-methyl-2*H*-chromen-2-one (14)

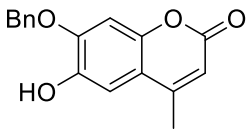


Chemical Formula: $C_{10}H_8O_4$
Molecular Weight: 192.17

Ethyl 3-oxobutanoate (25 mL, 0.2 mol, 5.0 eq) was added to a solution of hydroxyquinol **13** (5.0 g, 40 mmol, 1.0 equiv) in TFA (50 mL; 0.8 M) and the mixture was heated up to 100 °C for 3 h. The mixture was cooled to 23 °C and *n*-hexane (200 mL) was added. The resulting precipitate was filtered and washed with *n*-hexane yielding 6,7-Dihydroxy-4-methyl-2*H*-chromen-2-one (**14**) (7.2 g, 37 mmol, 94%) as a beige, amorphous solid.

TLC (EtOAc:Hex/1:4) R_f: 0.38 [UV^{254}, CAM]. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ [ppm]: 10.18 (1H, s), 9.34 (1H, s), 7.00 (1H, s), 6.73 (1H, s), 6.10 (1H, s), 2.31 (3H, s). **^{13}C-NMR:** (76 MHz, DMSO-d_6) δ [ppm]: 160.9, 153.5, 150.4, 148.0, 143.1, 111.8, 110.7, 109.7, 103.0, 18.5. The analytical data were in accordance with the literature.[4]

7-(Benzyloxy)-6-hydroxy-4-methyl-2*H*-chromen-2-one (15)

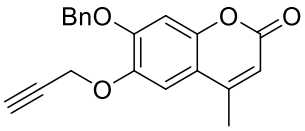


Chemical Formula: $C_{17}H_{14}O_4$
Molecular Weight: 282.29

Na_2CO_3 (830 mg, 7.8 mmol, 3.0 equiv) was added to a solution of 6,7-dihydroxy-4-methylcoumarin (**14**) (500 mg, 2.6 mmol, 1.0 equiv) in dry DMF (4.6 mL, 0.53 M) under argon atm. at 0°C and the mixture was stirred for 40 min at 0 °C. BnBr (930 µL, 7.8 mmol, 3.0 equiv) was added dropwise over a period of 10 min and the mixture was stirred for 14 h at -4 °C. Et_2O (50 mL) was added and the reaction was quenched by addition of aq. HCl (1 M) and acidified to pH 2. The mixture was filtered, and the precipitate was washed with H_2O. The precipitate was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99.5:0.5) and the resulting residue was recrystallized from EtOAc:Hex/1:2 yielding 7-(Benzyloxy)-6-hydroxy-4-methyl-2*H*-chromen-2-one (**15**) (300 mg, 1.1 mmol, 40%) as a pale pink, amorphous solid.

TLC (CH_2Cl_2:MeOH/99.5:0.5) R_f: 0.27 [UV^{254}, CAM]. **IR** (ATR) [cm^{-1}]: 3326, 3033, 1692, 1610, 1565, 1532, 1442, 1377, 1286, 1251, 1228, 1204, 1160, 1069, 974, 943, 856, 820, 745, 690, 666, 618, 579, 548. **HRMS** (ESI-IT) [m/z]: 283.09669, calculated 283.09649 for $[C_{17}H_{15}O_4]^+$, err [ppm] 0.71. **^{1}H-NMR** (500 MHz, DMSO-d_6) δ [ppm]: 9.42 (1H, bs), 7.51-7.49 (2H, m), 7.42-7.38 (2H, m), 7.35-7.32 (1H, m), 7.08 (1H, s), 7.07 (1H, s), 6.17 (1H, m), 5.24 (2H, m), 2.33 (3H, m). **^{13}C-NMR** (126 MHz, DMSO-d_6) δ [ppm]: 160.4, 153.0, 150.4, 147.4, 143.8, 136.4, 128.4, 128.0, 127.8, 112.5, 111.3, 109.4, 101.4, 70.0, 18.2. **UV-Vis/fluorescence emission** λ_{Ex}= 339 nm, λ_{Em}= 406 nm, ε = 11480.19 L*mol^{-1}*cm^{-1} (c = 0.060 mg/10 mL in CH_2Cl_2).

7-(Benzyloxy)-4-Methyl-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one (16)



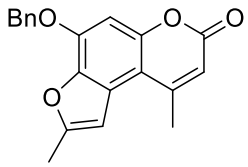
Chemical Formula: $C_{20}H_{16}O_4$
Molecular Weight: 320.34

Propargyl bromide (80% in toluene, 0.9 mL, 8.4 mmol, 1.3 equiv) was added to a mixture of 7-benzyloxy-6-hydroxy-4-methylcoumarin (**15**) (1.8 g, 6.4 mmol, 1.0 equiv) and K_2CO_3 (1.2 g, 8.4 mmol, 1.3 equiv) in dry acetone (34 mL, 0.19 M) under argon atm. and the mixture was heated up to 56 °C

for 16 h. The reaction was quenched by addition of H_2O (60 mL) and the solution was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99.5:0.5) yielding 7-(Benzyloxy)-4-Methyl-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one (**16**) (1.7 g, 5.4 mmol, 85%) as a yellow, amorphous solid.

TLC (CH_2Cl_2:MeOH/99.5:0.5) R_f: 0.44 [UV^{254}, CAM]. **IR** (ATR) [cm^{-1}]: 3287, 3064, 2925, 2871, 2306, 2121, 2061, 1706, 1613, 1561, 1508, 1428, 1385, 1272, 1224, 1155, 1066, 1017, 988, 925, 849, 789, 733, 694, 588, 566, 546. **HRMS** (ESI-IT) [m/z]: 321.11237, calculated 321.11214 for $[C_{20}H_{17}O_4]^+$, err [ppm] 0.72. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ [ppm]: 7.47 (m, 2H), 7.41 (m ,2H), 7.35 (m, 1H), 7.32 (s, 1H), 7.18 (s,1H), 6.23 (s, 1H), 5.24 (s, 2H), 4.91 (d, *J* = 2.4 Hz, 2H), 3.59 (t, *J* = 2.4 Hz, 1H), 2.40 (d, *J* = 1.2 Hz, 3H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ [ppm]: 160.2, 153.2, 151.9, 149.1, 143.5, 136.1, 128.5, 128.2, 127.9, 112.1, 111.6, 109.7, 101.7, 78.8, 70.3, 56.9, 18.3. **UV-Vis/fluorescence emission** λ_{Ex}= 332 nm, λ_{Em}= 403 nm, ε = 12971.33 L*mol^{-1}*cm^{-1}, (c = 0.061 mg/10 mL in CH_2Cl_2).

4-(Benzyloxy)-2,9-Dimethyl-7*H*-furo[3,2-f]chromen-7-one (17)

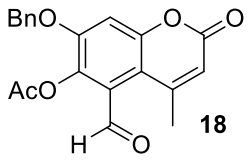


Chemical Formula: $C_{20}H_{16}O_4$
Molecular Weight: 320.34

CsF (2.4 g, 15.5 mmol, 5.0 equiv) was added to a mixture of 7-benzyloxy-6-propargyloxy-4-methylcoumarin (**16**) (1.0 g, 3.1 mmol, 1.0 equiv) in degassed $PhNEt_2$ (7.3 mL, 0.42 M) and the mixture was heated up to 170 °C for 14 h. The mixture was cooled to 23 °C and EtOAc (30 mL) was added. The mixture was washed with aq. HCl (1 M, 3 x 40 mL) and brine (40 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99.5:0.5) yielding 4-(benzyloxy)-2,9-Dimethyl-7*H*-furo[3,2-f]chromen-7-one (**17**) (470 mg, 1.5 mmol, 47%) as a beige, amorphous solid.

TLC (CH_2Cl_2:MeOH/99.5:0.5) R_f: 0.45 [UV^{254}, CAM]. **IR** (ATR) [cm^{-1}]: 3066, 3036, 2957, 2924, 2874, 1711, 1597, 1504, 1444, 1397, 1338, 1282, 1227, 1157, 1111, 1060, 996, 965, 941, 891, 845, 813, 735, 698, 596, 541. **HRMS** (ESI-IT) [m/z]: 321.11236, calculated 321.11214 for $[C_{20}H_{17}O_4]^+$, err [ppm] 0.69. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ [ppm]: 7.52 (m, 2H), 7.44 (m, 2H), 7.38 (m, 1H), 7.07 (s, 1H), 7.02 (m, 1H), 6.20 (m, 1H), 5.36 (s, 2H), 2.56 (s, 3H), 2.49 (s, 3H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ [ppm]: 160.2, 157.6, 153.9, 151.8, 146.2, 140.1, 135.9, 128.6, 128.3, 128.2, 125.8, 110.9, 105.9, 104.3, 96.6, 70.5, 21.6, 13.7. **UV-Vis/fluorescence emission** λ_{Ex}= 334 nm, λ_{Em}= 395 nm, ε = 17516.36 L*mol^{-1}*cm^{-1}, (c = 0.047 mg/10 mL in CH_2Cl_2).

7-(Benzyloxy)-5-formyl-4-methyl-2-oxo-2H-chromen-6-yl acetate (18)



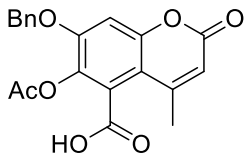
18

Chemical Formula: $C_{20}H_{16}O_6$
Molecular Weight: 352.34

To trifluoroacetic acid anhydride (230 µL, 1.7 mmol, 26.8 eq) was added H_2O_2 (30%ig, 32 µL, 0.31 mmol, 4.97 eq) dropwise at 0°C and the resulting solution was added to 4-(benzyloxy)-2,9-Dimethyl-7*H*-furo[3,2-f]chromen-7-one (**17**) (20 mg, 62 µmol, 1.0 eq) at -2 °C and stirred for 1 h. Afterwards water (250 µL) and 10% aqueous $NaHSO_3$ solution (250 µL) were added at 0°C and the mixture was adjusted to the pH 9 with saturated $NaHCO_3$ solution. The mixture was extracted with EtOAc (3x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in methanol (2.7 mL) and water (0.8 mL), $NaIO_4$ (26 mg, 0.12 mmol, 1.9 eq) were added and the mixture was stirred for 1 h 20 min at 23°C. Then the mixture was diluted with water (15 mL) and extracted with $CHCl_3$ (3x 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography through silica gel (CH_2Cl_2:MeOH/95:5) to yield 7-(Benzyloxy)-5-formyl-4-methyl-2-oxo-2H-chromen-6-yl acetate **(18)** (7.6 mg, 22 µmol, 35%) as yellow amorphous solid.

TLC: (CH_2Cl_2:MeOH/99:1) R_f = 0.26 [UV^{254}, CAM], **^{1}H NMR** (400 MHz, DMSO-d_6) δ [ppm]: 10.63 (1H, s), 7.42 (6H, m), 6.37 (1H, s), 5.32 (2H, s), 2.30 (3H, s), 2.24 (3H, s).

6-Acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (19)



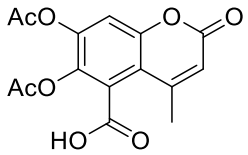
Chemical Formula: $C_{20}H_{16}O_7$
Molecular Weight: 368.34

Ozone (0.2 L/h O_2, 0.5 A) was bubbled through a solution of 4-(benzyloxy)-2,9-Dimethyl-7*H*-furo[3,2-f]chromen-7-one (**17**) (200 mg, 0.62 mmol, 1.0 equiv) in MeOH (8.5 mL, 0.07 M) and CH_2Cl_2 (42 mL, 0.15 M) at -78°C for 2 min. Argon was bubbled through the reaction mixture for 5 min at -78°C, before PPh_3 (330 mg, 1.3 mmol, 2.0 equiv) was added. The solution was allowed to warm up to 23 °C and stirred for 30 min at 23 °C. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/99:1 →95:5). The residue was dissolved in tBuOH (3.2 mL, 0.19 M), H_2O (3.2 mL, 0.19 M) and MeCN (1.1 mL, 0.56 M) and 2-methylbut-2-ene (590 µL, 5.5 mmol, 12.0 equiv) was added. The mixture was cooled to 0°C and $NaH_2PO_4 \cdot H_2O$ (500 mg, 4.1 mmol, 9.0 equiv) and $NaClO_2$ (370 mg, 4.1 mmol, 9.0 equiv) were added. The mixture was stirred for 1.5 23 °C. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (20 mL) and acidified with aq. citric acid (10 wt%) to pH 6. The mixture was extracted with EtOAc (3 x 25 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4 and filtered. The solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5→7:3) yielding 6-acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (**19**) (115 mg, 0.31 mmol, 50%) as an amorphous, pale yellow solid.

TLC (CH_2Cl_2:MeOH/77:23) R_f: 0.25 [UV^{254}, CAM]. **IR** (ATR) [cm^{-1}]: 3406, 2925, 1717, 1602, 1497, 1440, 1372, 1290, 1214, 1174, 1088, 1028, 910, 841, 733, 692, 644, 601, 555. **HRMS** (ESI-IT) [m/z]: 367.08296, calculated 367.08233 for $[C_{20}H_{15}O_7]^-$, err [ppm] 1.70. **^{1}H-NMR** (500 MHz, CD_3OD) δ [ppm]: 7.43 (m, 2H), 7.38 (m, 2H), 7.33 (m, 1H), 7.01 (s, 1H), 6.16 (s, 1H), 5.20 (s, 2H), 2.63 (s, 3H), 2.24 (s, 3H) **^{13}C-NMR** (126 MHz, CD_3OD) δ [ppm]: 173.3, 170.3, 162.8, 156.3, 154.7, 154.4, 137.3, 135.3, 134.5, 129.6, 129.3,

128.5, 113.6, 110.0, 101.4, 72.1, 20.5, 20.5. **UV-Vis/fluorescence emission** λ_{Ex}= 324 nm, λ_{Em}= 390 nm, ε = 9152.81 L*mol^{-1}*cm^{-1}, (c = 0.066 mg/10 mL in MeOH).

6,7-Diacetoxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (20)

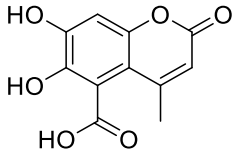


Chemical Formula: $C_{15}H_{12}O_8$
Molecular Weight: 320.25

Palladium on charcoal (10w% Pd, 5.1 mg) was added to a solution of 6-acetoxy-7-benzyloxy-5-carboxy-4-methylcoumarin (**19**) (51 mg, 0.14 mol, 1.0 equiv) in MeOH (1.0 mL, 0.14 M) under argon atmosphere. A pressure of 1 bar H_2 atm. was applied, and the mixture was stirred for 2.5 h at 23 °C. The mixture was filtered through celite® and concentrated under reduced pressure. The residue was dissolved in dry DMF (1 mL, 0.14 M) and Ac_2O (16 µL, 0.17 mmol, 1.2 equiv) and pyridine (23 µL, 0.28 mmol, 2.0 equiv) were added at 0 °C. The mixture was stirred for 3 h at 23 °C. EtOAc (10 mL) was added, and the mixture was washed with H_2O (3 x 10 mL) and brine (5 mL). The solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography through silica gel (CH_2Cl_2:MeOH/95:5→7:3) yielding 6,7-Diacetoxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (**20**) (20.2 mg, 63 µmol, 45%) as an amorphous, pale yellow solid.

TLC (CH_2Cl_2:MeOH/77:23) R_f: 0.33 [UV^{254}, CAM]. **^{1}H-NMR** (400 MHz, CD_3OD) δ [ppm]: 7.26 (s, 1H), 6.30 (d, *J* = 1.3 Hz, 1H), 2.64 (d, *J* = 1.3 Hz, 3H), 2.29 (s, 3H), 2.29 (s, 3H). **^{13}C-NMR** (100 MHz, CD_3OD) δ [ppm]: 172.9, 169.9, 169.0, 161.9, 155.4, 152.9, 146.6, 136.6, 135.6, 116.2, 114.7, 111.7, 20.6, 20.5, 20.3.

6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (4-MeDHCou)



Chemical Formula: $C_{11}H_8O_6$
Molecular Weight: 236.18

Palladium on charcoal (10w% Pd, 1.2 mg) was added to a solution of 6-acetoxy-7-benzyloxy-5-carboxy-4-methylcoumarin (**18**) (9.9 mg, 27 µmol, 1.0 equiv) in MeOH (250 µL, 0.14 M) under argon atmosphere. A pressure of 1 bar H_2 atm. was applied, and the mixture was stirred for 2.5 h at 23 °C. The mixture was filtered through celite® and concentrated under reduced pressure. The residue was dissolved in a solution of HCl in MeOH (0.5 M, 1 mL, 0.03 M) and stirred for 2.5 d at 23 °C. The solvent was removed under reduced pressure and the residue was dissolved in 1.0 mL of H_2O/MeCN (7:3), filtered through a CHROMAFIL® 45 µm filter and directly purified by preparative HPLC (Hypersil GOLD C18 RP-column, 5 µm, 250 mm×21.0 mm (10 mL/min), Eluents: H_2O (0.1% TFA):MeCN (0.1% TFA)/95:5 → 75:25), t_R = 19.5 min). Product containing fractions were diluted with H_2O, frozen with liquid N_2 at -196°C and lyophilized yielding 6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (**4-MeDHCou**) (4.6 mg, 19 µmol, 72%) as a white, amorphous solid.

TLC (CH_2Cl_2:MeOH/77:23) R_f: 0.10 [UV^{254}, CAM]. **IR** (ATR) [cm^{-1}]: 3312, 2922, 2851, 1673, 1601, 1560, 1397, 1338, 1280, 1227, 1167, 1072, 1026, 981, 943, 850, 763, 688, 595, 572, 547. **HRMS** (ESI-IT) [m/z]:

259.02136, calculated 259.02131 for $[C_{11}H_8O_6Na]^+$, err [ppm] 0.19. **^{1}H-NMR** (400 MHz, CD_3OD) δ [ppm]: 6.79 (s, 1H), 6.12 (s, 1H), 2.51 (s, 3H) **^{13}C-NMR** (126 MHz, CD_3OD) δ [ppm]: 172.5, 163.2, 155.3, 151.1, 149.8, 141.7, 121.8, 113.1, 109.6, 103.6, 20.5. **UV-Vis/fluorescence emission** λ_{Ex}= 349 nm, λ_{Em}= 433 nm, ε = 5375.03 $L*mol^{-1}*cm^{-1}$, (c = 0.058 mg/10 mL in MeOH).

Evaluation of the biological activity

Description of the MIC assay

Serial dilutions of compounds were prepared as triplicates in sterile U-bottom shaped 96-well plates (Corning™, USA)**.** Mueller-Hinton broth (MHB) media was used for bacteria and YMG media was used for filamentous fungi and yeasts. The selected organisms represent a broad spectrum of pathogens of clinical interest, as well as sensitive indicator strains (Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus, Mycolicibacterium smegmatis*; Gram-negative bacteria: *Acinetobacter baumannii, Chromobacterium violaceum*, *Escherichia coli*, *Pseudomonas aeruginosa*; filamentous fungi: *Mucor hiemalis*; yeasts: *Candida albicans*, *Pichia anomala, Rhodotorula glutinis, Schizosaccharomyces pombe*). The compounds were dissolved in MeOH (1mg/mL), added to the bacterial suspension and diluted to the final concentrations. The plate was incubated at 37°C in static conditions. Growth inhibition was assessed after 24 h. MeOH was used as negative control. Kanamycin (1.0 mg/mL; 2 µL [*M. smegmatis*]), gentamicin (1.0 mg/mL; 2 µL [*P. aeruginosa*]), ciprobay (2.54 mg/ml; 2 µL [*A. baumannii*]), nystatin (1.0 mg/mL; 20 µL [*S. pombe, P. anomala, M. hiemalis, C. albicans, R. glutinis*]), and oxytetracycline (1.0 mg/mL; 2 µL [*C. violaceum*, *E. coli*, *S. aureus*] and 20 µL [*B. subtilis*]) were used as positive controls.[5] The highest concentration tested were 66.7 µg/mL for DHCou, 4-MeDHCou, Esculetin and 4-Methylesculetin.

Table S1: MIC [µg/mL] assay data

		Antimicrobial activity MIC [µg/mL]				
Organisms	Strain No.	**DHCou**	**4-MeDHCou**	**Esculetin**	**4-Me-Esculetin**	Ref.
Bacteria						
B. subtilis	DSM 10	–	–	–	–	8.3[a]
S. aureus	DSM 346	–	–	–	–	1.7[a]
M. smegmatis	ATCC 700084	–	–	–	–	1.7[b]
A. baumannii	DSM 30008	–	–	–	–	0.3[c]
C. violaceum	DSM 30191	–	–	–	–	0.4[a]
E. coli	DSM 1116	–	–	–	–	1.7[a]
P. aeruginosa	PA14	–	–	–	–	0.4[d]
Fungi						
M. hiemalis	DSM 2656	–	–	–	–	4.2[e]
P. anomala	DSM 6766	–	–	–	–	8.3[e]
R. glutinis	DSM 10134	–	–	–	–	2.1[e]
C. albicans	DSM 1665	–	–	–	–	8.3[e]
S. pombe	DSM 70572	–	–	–	–	4.2[e]

References: [a] oxytetracycline; [b] kanamycin; [c] ciprobay; [d] gentamicin; [e] nystatin; – : not active.

Description of the biofilm assays

Table S2: Biofilms and preformed biofilm inhibition of *S. aureus* and biofilm inhibition of *C. albicans*.

Compound	Biofilm inhibition [% ± SD]	
	***S. aureus* (DSM 1104)**	***C. albicans* (DSM 11225)**
Esculetin (**2**)	93 ± 2 (250 µg/mL)[a] 33 ± 6 (125 µg/mL)[a]	77 ± 7 (250 µg/mL)[c] 58 ± 17 (125 µg/mL)[c]
4-Methylesculetin (**14**)	94 ± 1 (250 µg/mL)[a] 48 ± 8 (125 µg/mL)[a]	76 ± 7 (250 µg/mL)[c] 48 ± 14 (125 µg/mL)[c]
DHCou	-[b]	62 ± 9 (250 µg/mL)[d]
4-MeDHCou	75 ± 5 (250 µg/mL)[b] 43 ± 11 (125 µg/mL)[b] 31 ± 15 (62.5 µg/mL)[b]	60 ± 2 (250 µg/mL)[d]

(–): no activity, SD: standard deviation, References [%]: [a] Microporenic acid A (MAA): 93 ± 0.3 (250 µg/mL), 93 ± 1 (62.5 µg/mL), 62 ± 6 (7.8 µg/mL); [b] MMA: 82 ± 6 (250 µg/mL), 81 ± 8 (62.5 µg/mL), 73 ± 17 (7.8 µg/mL); [c] Farnesol: 87 ± 3 (250 µg/mL), 79 ± 14 (31.3 µg/mL), 67 ± 11 (15.6 µg/mL). [d] Farnesol: 75 ± 6 (250 µg/mL), 58 ± 15 (31.3 µg/mL), 46 ± 14 (15.6 µg/mL)

Staphylococcus aureus DSM 1104 was taken from −20 °C stock and cultured in 25 mL CASO (casein-peptone soymeal-peptone) medium in a 250 mL flask at 100 rpm at 37°C for 20 h. The OD_{600} of the culture solution was measured and adjusted to 0.001 McFarland standard. The serial compounds (250–2 µg/mL) were diluted in CASO with 4 % glucose broth to 150 µL and incubated in 96 well microtiter plates (TPP tissue culture ref.no 92196) for 24 h at 37 °C. The biofilm inhibition was assessed by using 150 µL 0.1% crystal violet (CV) (Thermo Fisher, Waltham, USA) as following previously established protocols.[6–9] The bacterial solution of 96 well plate was discarded and wells were washed by using PBS (phosphate-buffered saline) buffer. In the next step, the biofilms were stained by 0.1% CV at room temperature for 15 min and then washed three times by using PBS buffer. The biofilm was dissolved in 150 µL ethanol (95%), and the absorbance was finally quantified using a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 530 nm. Methanol (2.5%) and microporenic acid A (250–2 µg/mL) were used as a negative control and a positive control, respectively. Standard deviations (SD) of two repeats with duplicates each were 15% or less.

For the preformed biofilm assay, the cultured bacterial suspension of *S. aureus* DSM 1104 was adjusted to 0.001 McFarland standard at OD_{600} and incubated in 96-well tissue microtiter plates for 24 h in 150 µL CASO with 4% glucose broth. The supernatant of 96 well plate was removed and washed with 150 µL PBS buffer. Serially compounds were diluted in 150 µL of the fresh media (CASO with 4% glucose broth) at the concentration (250–2 µg/mL) and added into the plates. The plates were incubated for 24 h at 37 °C. Staining of the preformed biofilm and controls was carried out as mentioned above. Methanol (2.5%) and microporenic acid A (250–2 µg/mL) were used as a negative control and a positive control, respectively.[7] SD of two repeats with duplicates each were 15 % or less. All experiments were accomplished in duplicates with two repetitions.

Candida albicans DSM 11225 was cultured in 25 mL YPED (Yeast extract Peptone Dextrose) medium in a 250 mL flask at 30 °C at 100 rpm for 18 h. The OD_{600} of the bacterial suspension was measured and adjusted to 0.05 McFarland standard in RPMI 1640 medium. The 150 µL bacterial solution was added in 96 well non-tissue microtiter plates (Falcon non-tissue plate ref.no 351172) at 37 °C at 150 rpm.

After 90 min the supernatant was removed and washed two times by using PBS buffer. Serially compounds were diluted in 150 µL of the fresh media (RPMI 1640) at the concentration (250–2 µg/mL) and added into the wells. Methanol (2.5 %) and farnesol (250–2 µg/mL) were separately used as a negative control and positive control, respectively. The plates were further incubated at 37 °C at 150 rpm for 24 h. Then, the supernatant was discarded, and biofilms were stained by 150 µL 0.1% CV at room temperature for 25 min after once washed by PBS buffer. Afterwards, the plates were washed four times by using PBS buffer. The biofilms were dissolved in 150 µL ethanol (95%) and the absorbance was finally quantified using a plate reader (Synergy 2, BioTek, Santa Clara, USA) at 610 nm. SD of two repeats with duplicates each were 17% or less.

Description of the cell proliferation assay

The corresponding cells were cultivated at 37 °C and 10 % CO_2 in the medium given in Table S8. 60 µL of serial dilutions of the test compound were given to 120 µL of suspended cells (50.000/mL) in wells of 96-well plates. After 5 days of incubation growth inhibition (IC_{50}) was determined using an MTT assay.[10] The compounds were dissolved in MeOH (1 mg/mL), MeOH itself was used as negative control, epothilon (1 mg/mL) was used as positive control.

Table S3: Medium conditions for different cell types.

Cell type	**Medium**	**Additives**
L-929 (DSMZ ACC 2)	DME medium (high glucose) (Gibco)	10% fetal calf serum (Gibco)
SKOV-3 DSMZ ATCC HTB 77)	Mc Coys-Medium (Gibco)	10% fetal calf serum (Gibco)
MCF-7 (DSMZ ACC 115)	RPMI-Medium (Gibco)	10% fetal calf serum (Gibco), 1% MEM NEAA, 0,25% Human Insulin (Gibco)
A549 (DSMZ ACC 107)	DME medium (high glucose) (Gibco)	10% fetal calf serum (Gibco)
A431(DSMZ ACC 91)	DME medium (high glucose) (Gibco)	10% fetal calf serum (Gibco)
KB3.1 (DSMZ ACC 158)	DME medium (high glucose) (Gibco)	10% fetal calf serum (Gibco)

Table S4: Cytotoxic activity against several selected mammalian cell lines

	Cytotoxic activity IC_{50} [µM]				
Cell line	**DHCou**	**4-MeDHCou**	**Esculetin**	**4-Me-Esculetin**	**Epothilon B**
KB3.1 (ACC158)	–	–	29.8	30.2	$3.3 \cdot 10^{-5}$
L929 (ACC2)	–	–	41.5	33.8	$4.7 \cdot 10^{-4}$
A549 (ACC107)	n.t.	n.t.	18.5	21.9	$6.7 \cdot 10^{-5}$
A431 (ACC91)	n.t.	n.t.	41.5	38.5	$5.1 \cdot 10^{-5}$
PC-3 (ACC465)	n.t.	n.t.	46.0	38.0	$9.5 \cdot 10^{-5}$
SKOV-3 (ATTCC HTB 77)	n.t.	n.t.	45.5	42.7	$2.6 \cdot 10^{-4}$
MCF-7 (A115)	n.t.	n.t.	19.6	27.1	$3.0 \cdot 10^{-5}$

(–): no cytotoxicity or changed cells observed (max. concentration 1 mg/mL = 4.5 mM for **4-MeDHCou** and 4.2 mM for **DHCou**), n.t.: not tested.

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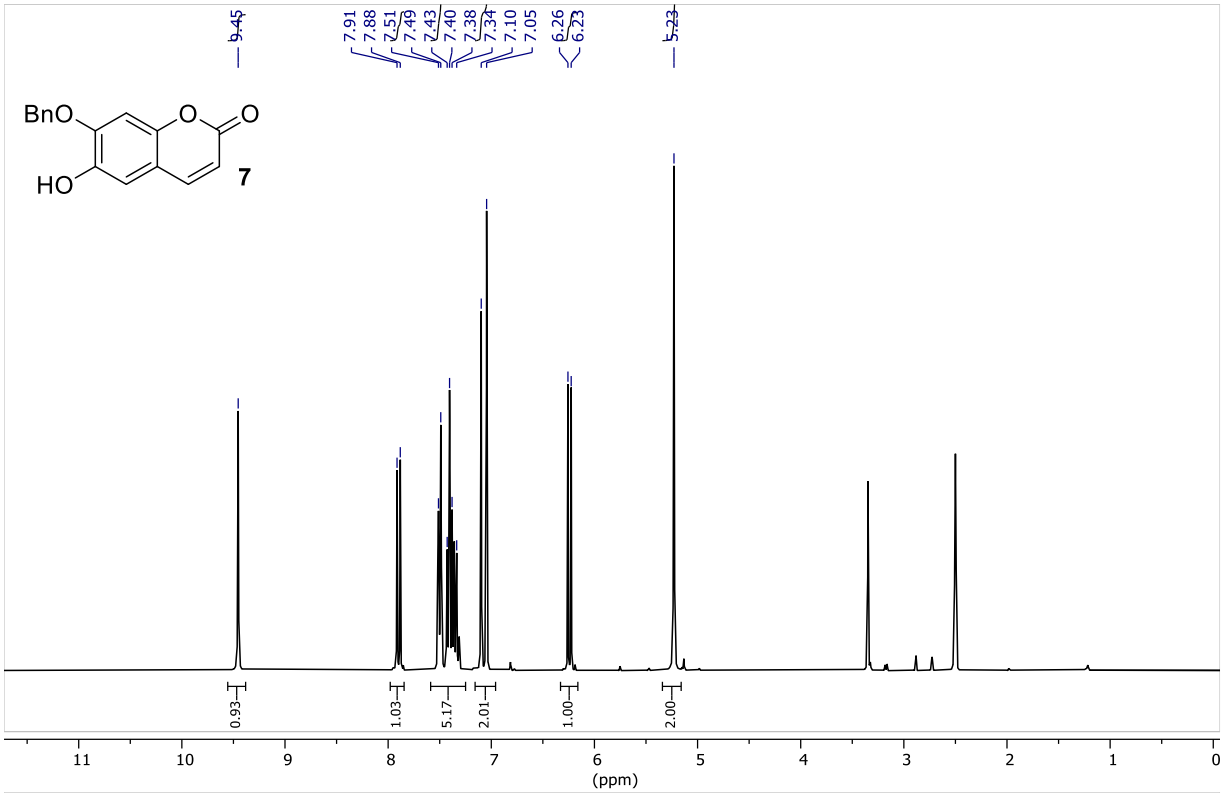
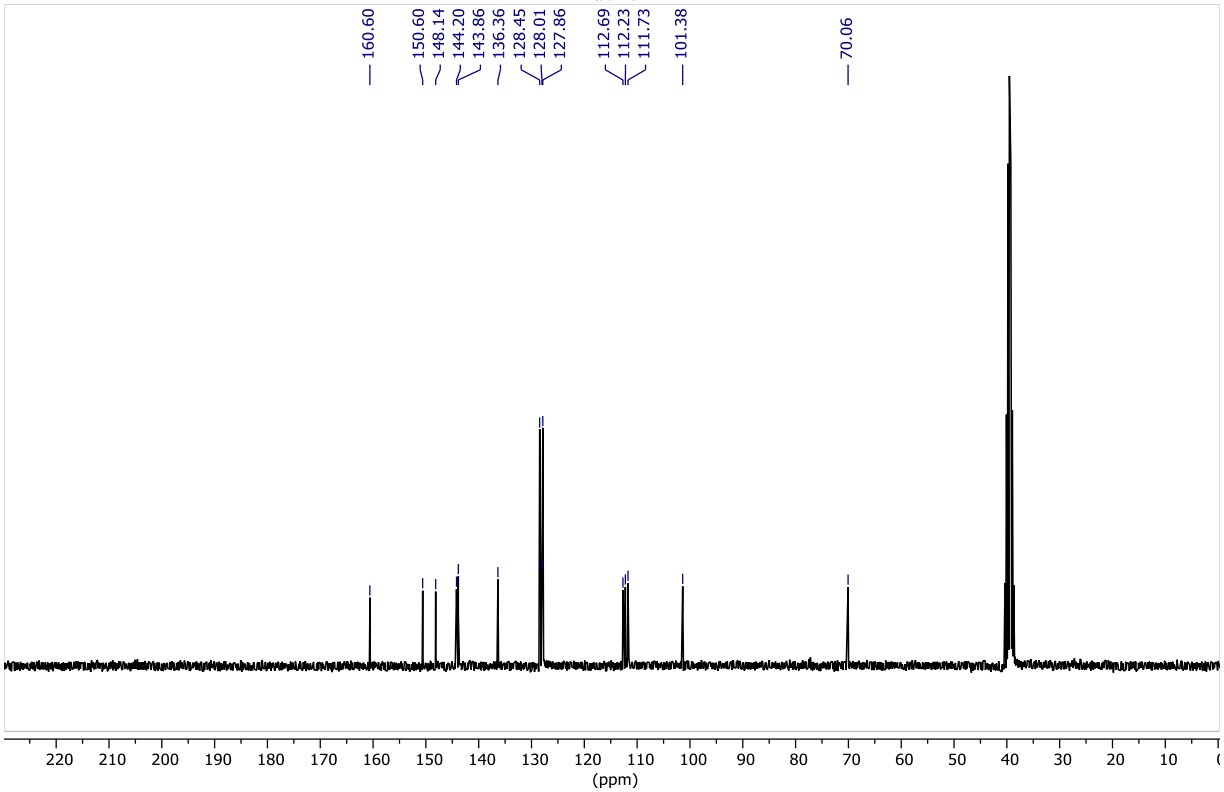
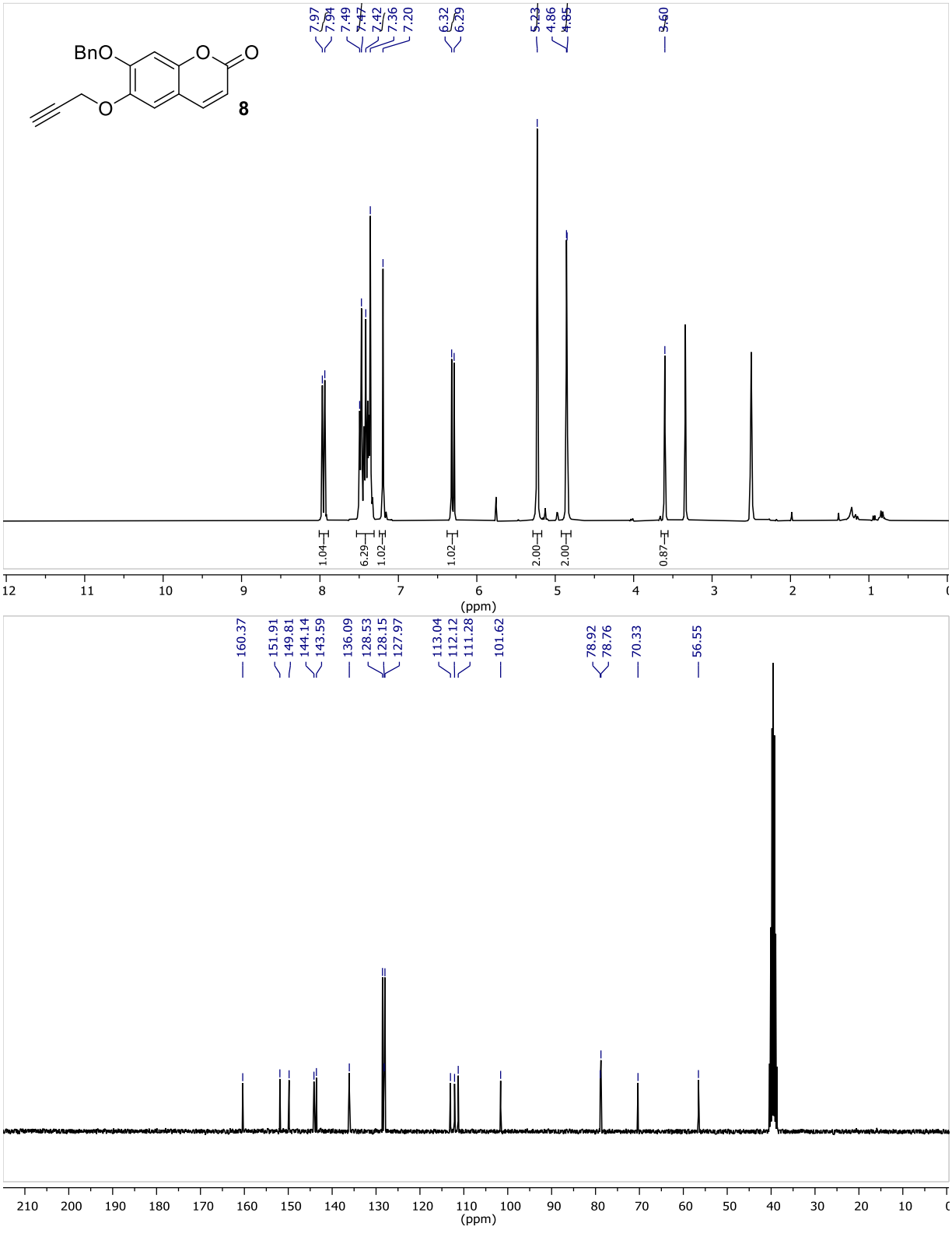
Spectra Annex

7-(Benzyloxy)-6-hydroxy-2H-chromen-2-one (7) (^{1}H and ^{13}C NMR)





7-(Benzyloxy)-6-(prop-2-yn-1-yloxy)-2H-chromen-2-one (8) (^{1}H and ^{13}C NMR)



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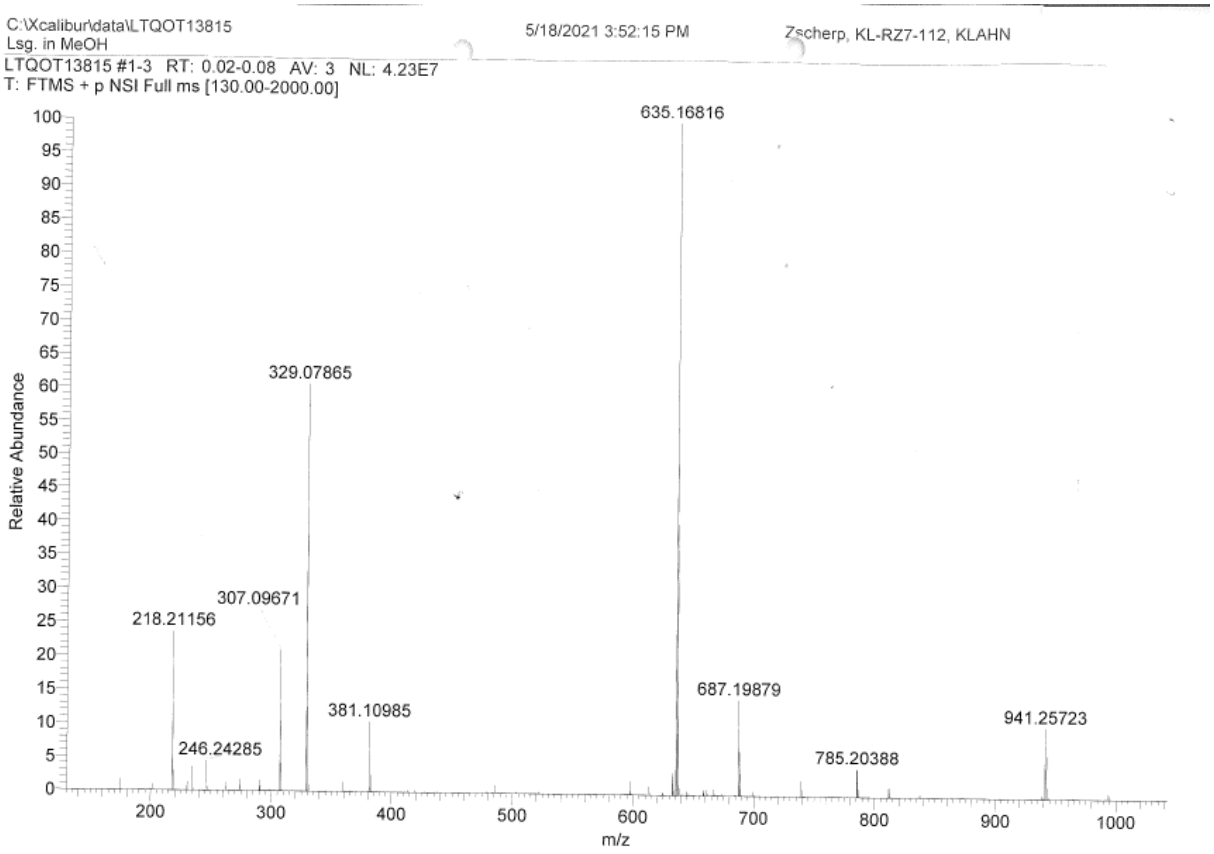

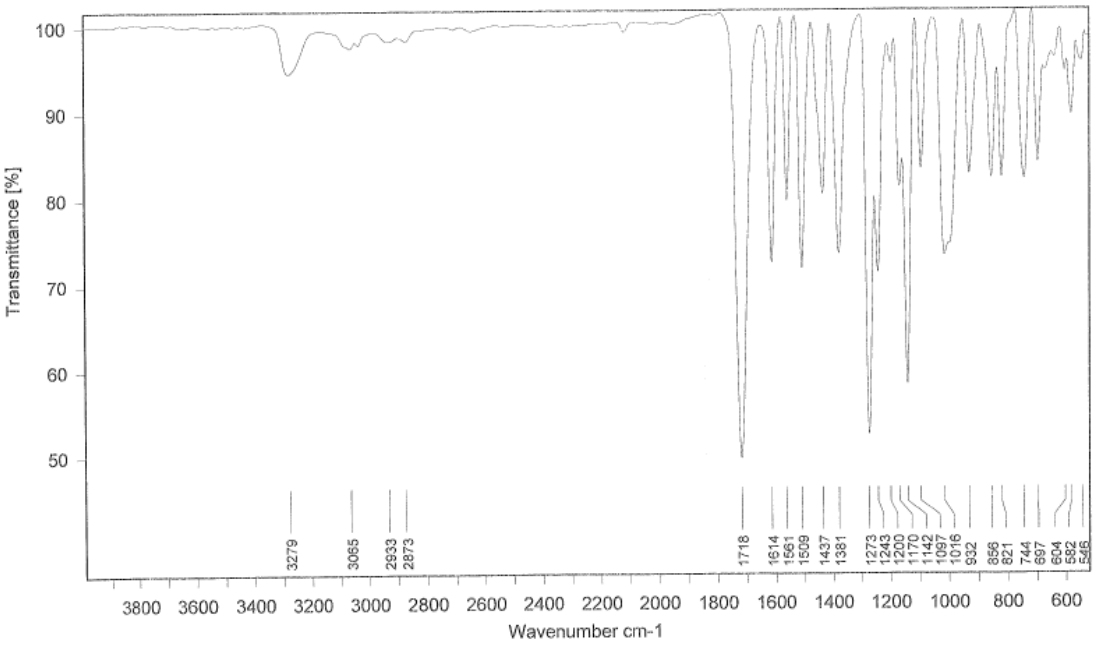

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18.05.2021

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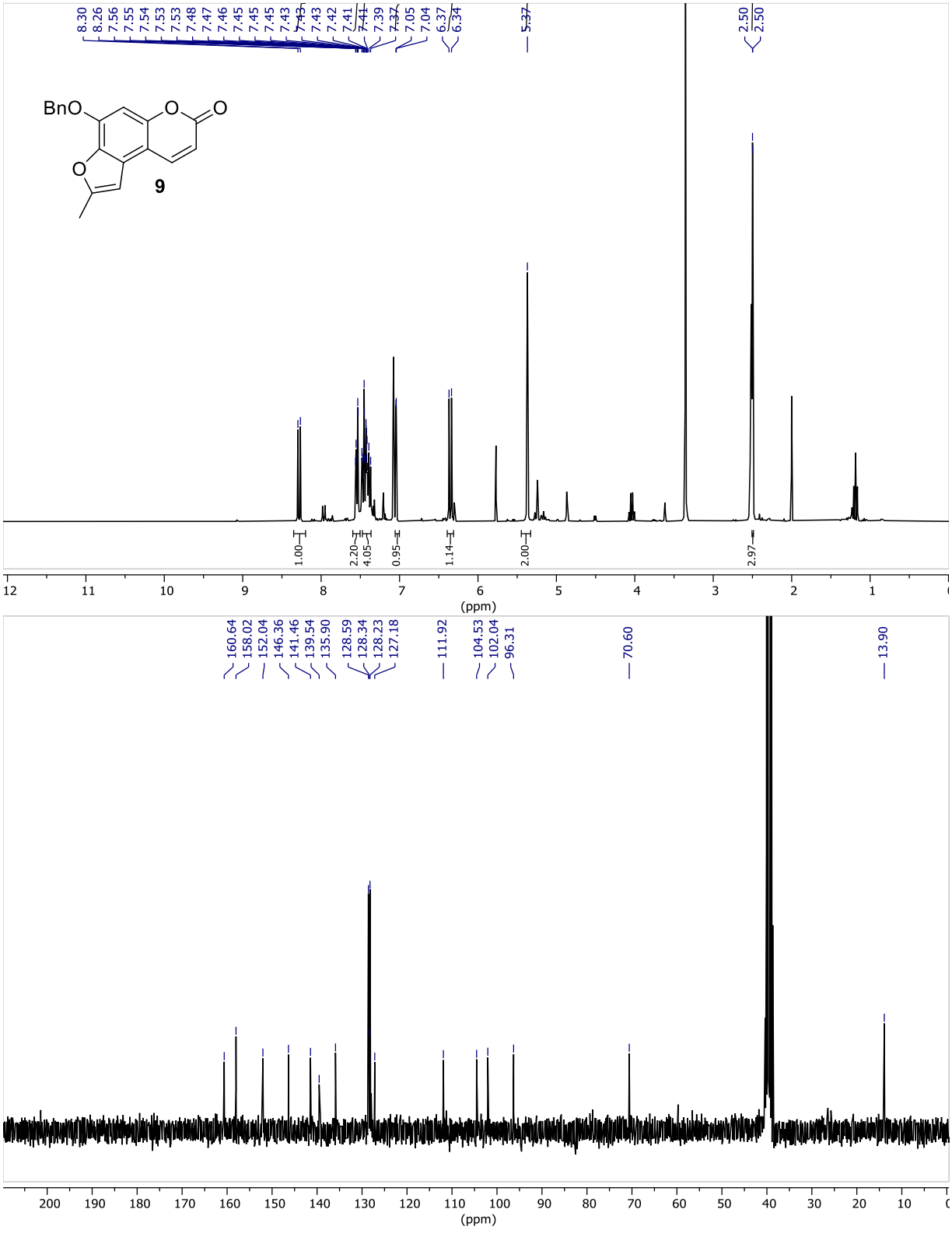

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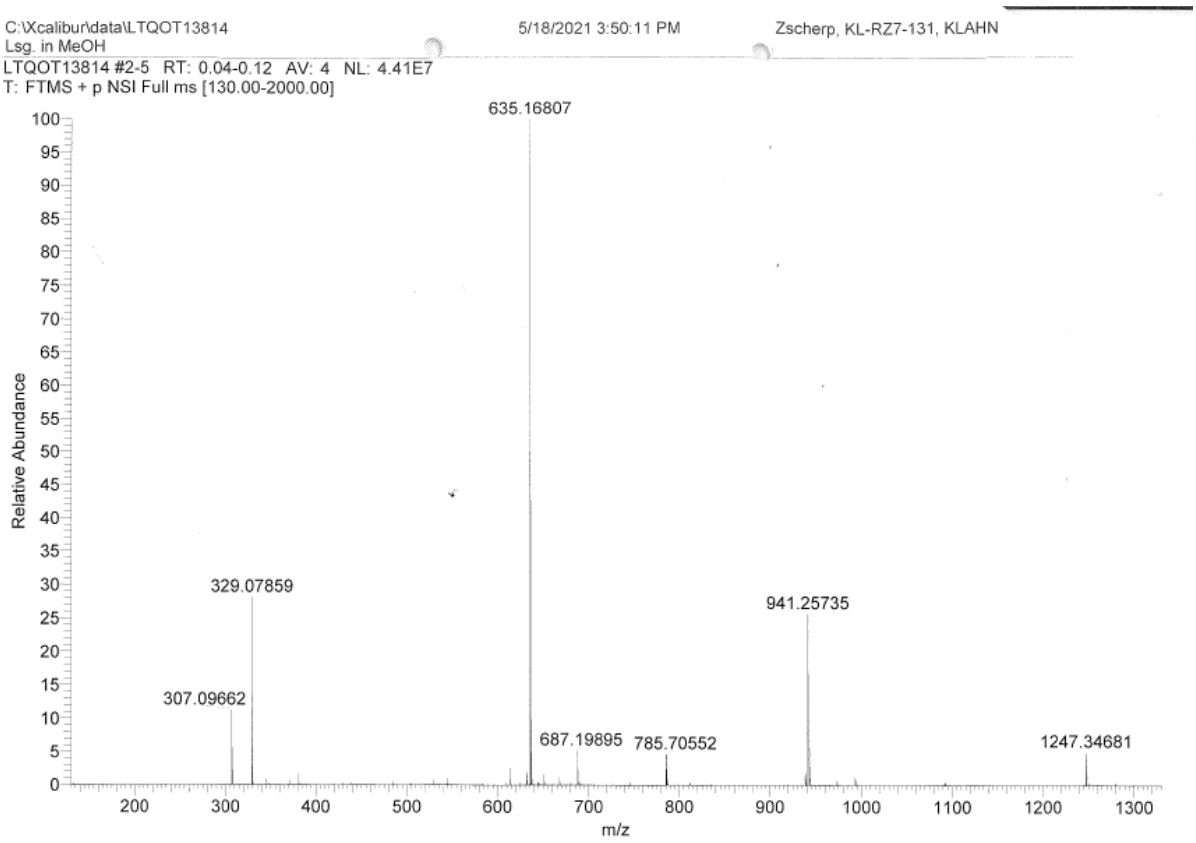

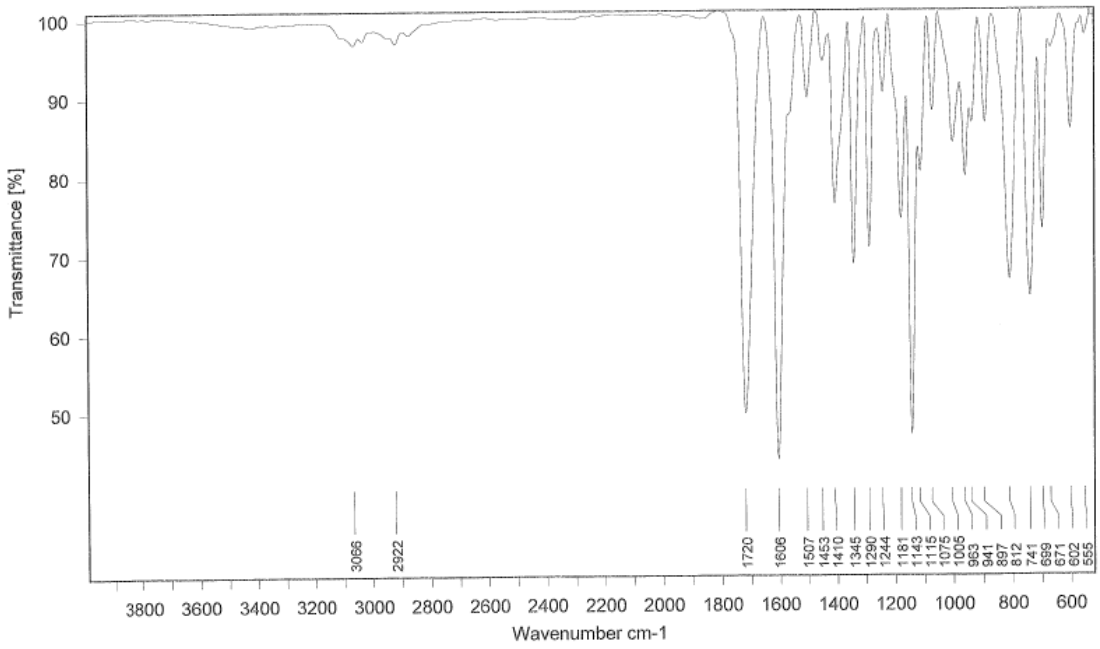

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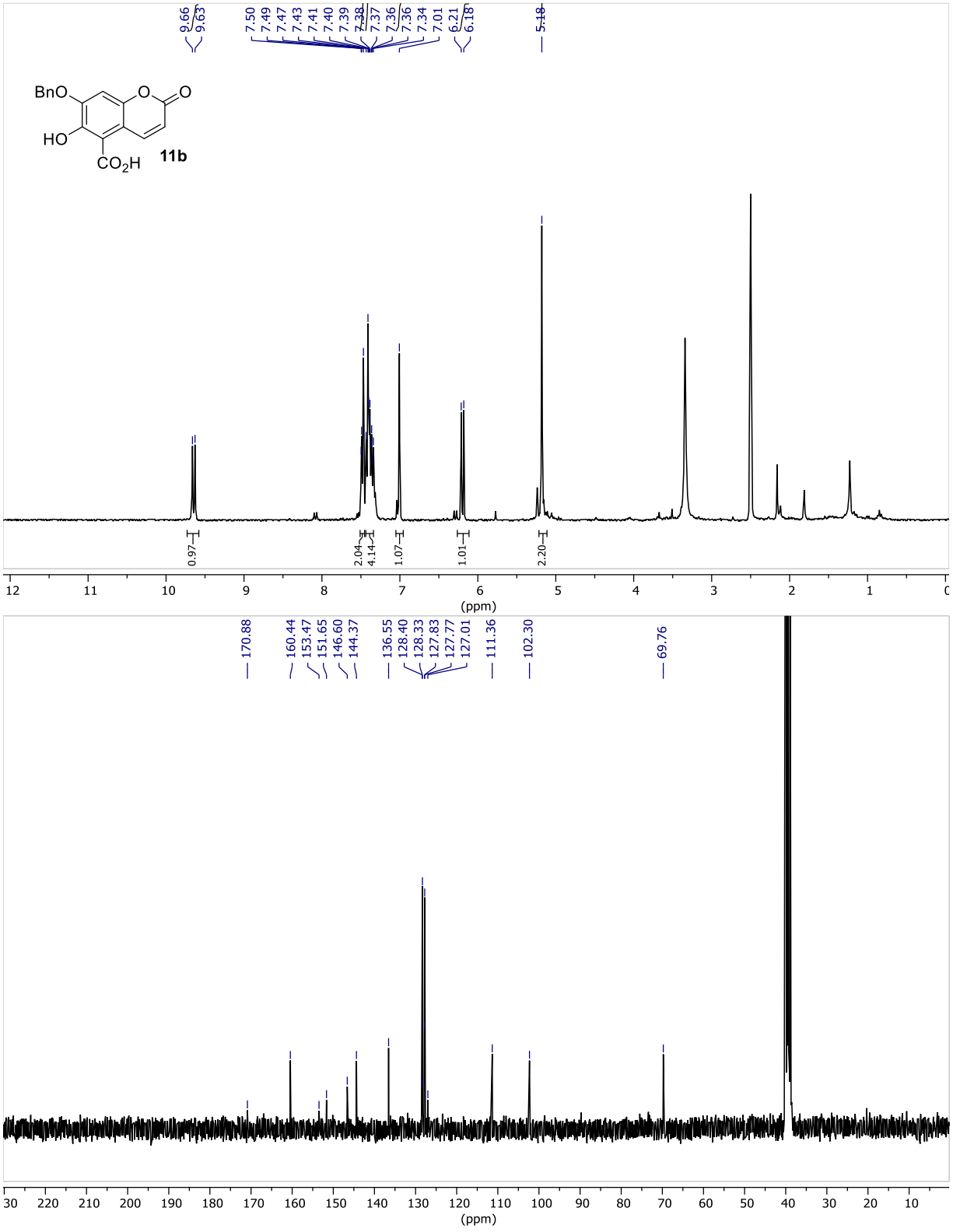

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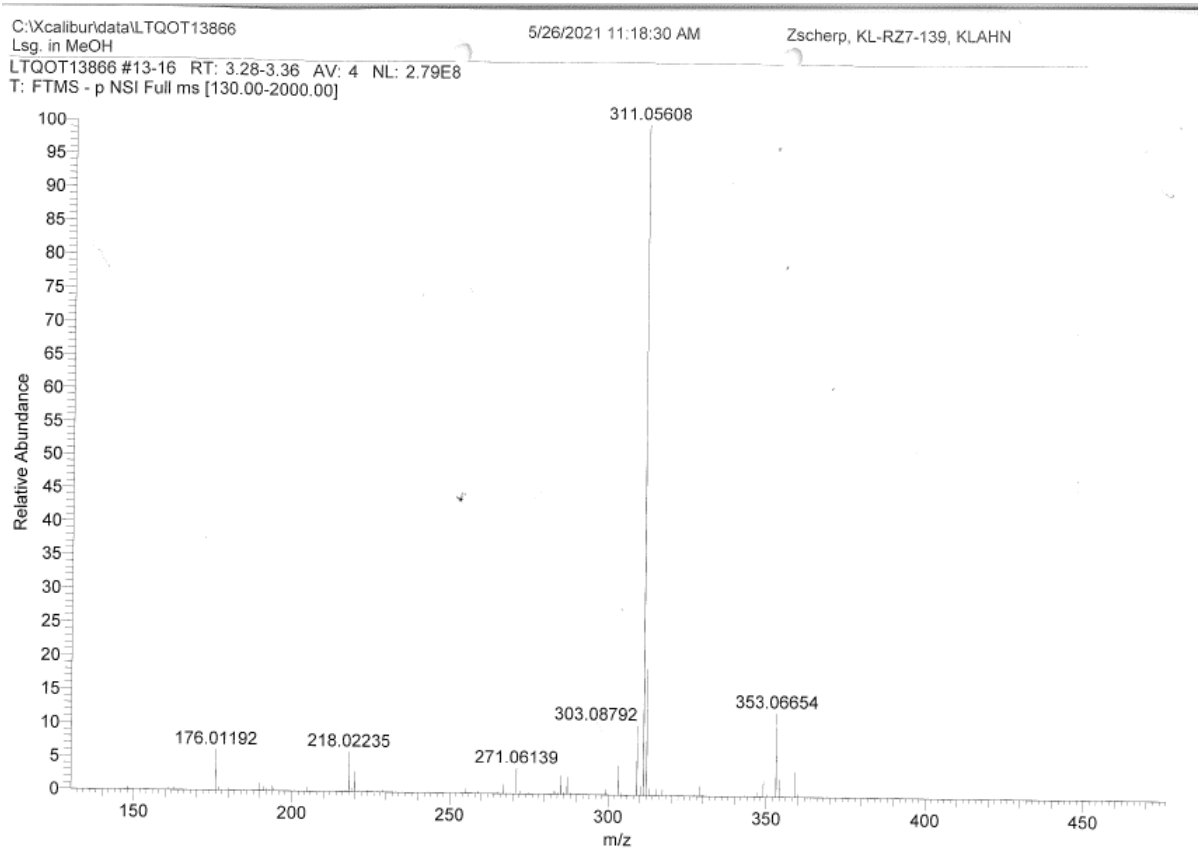

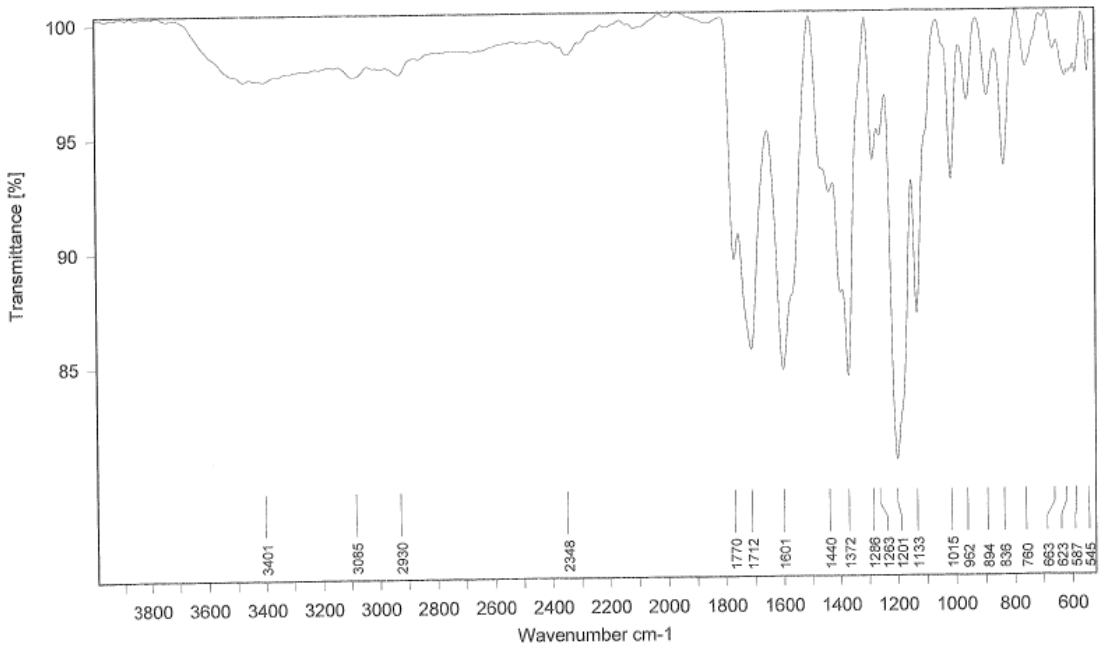

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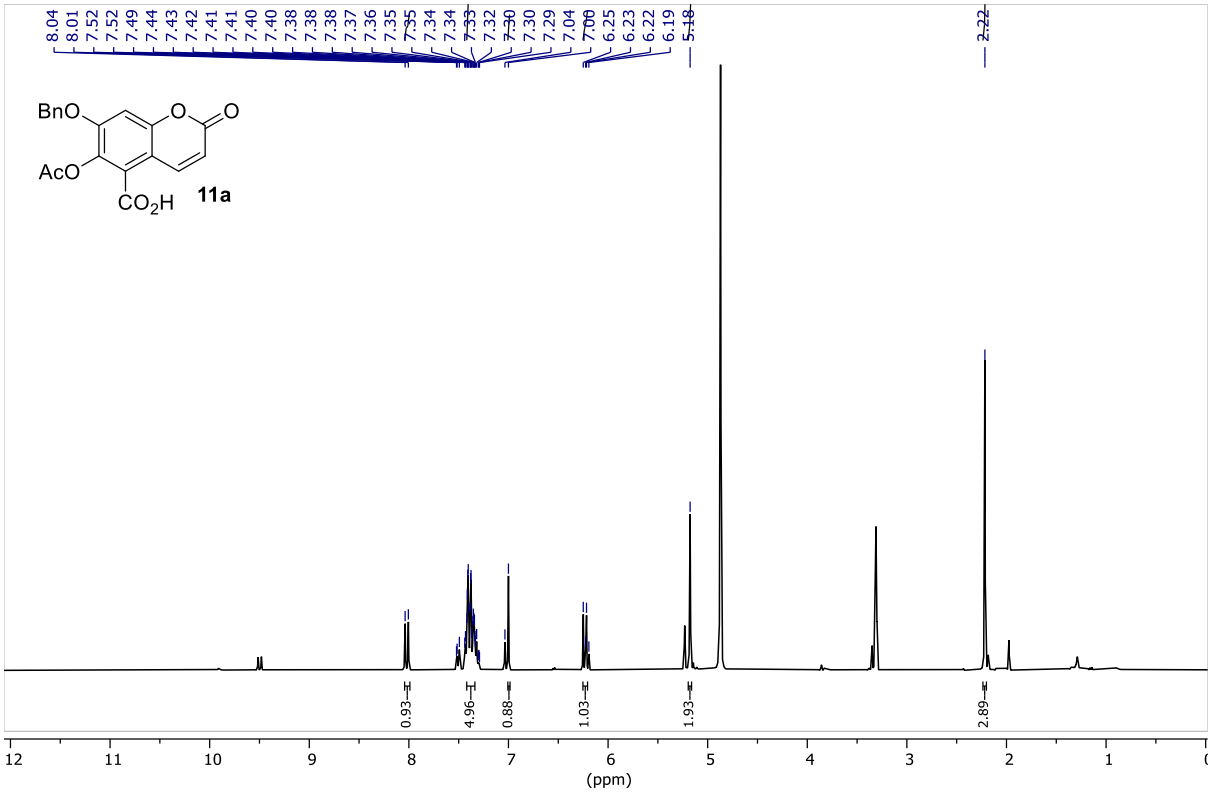
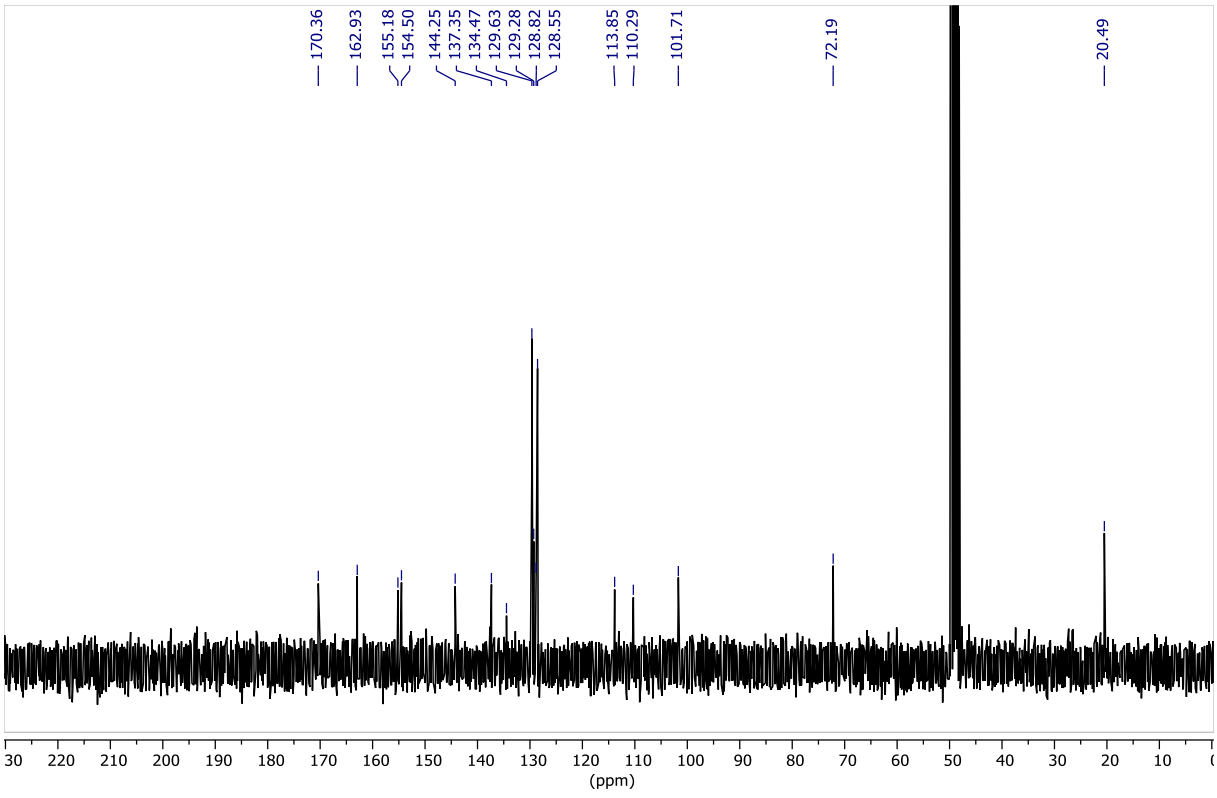
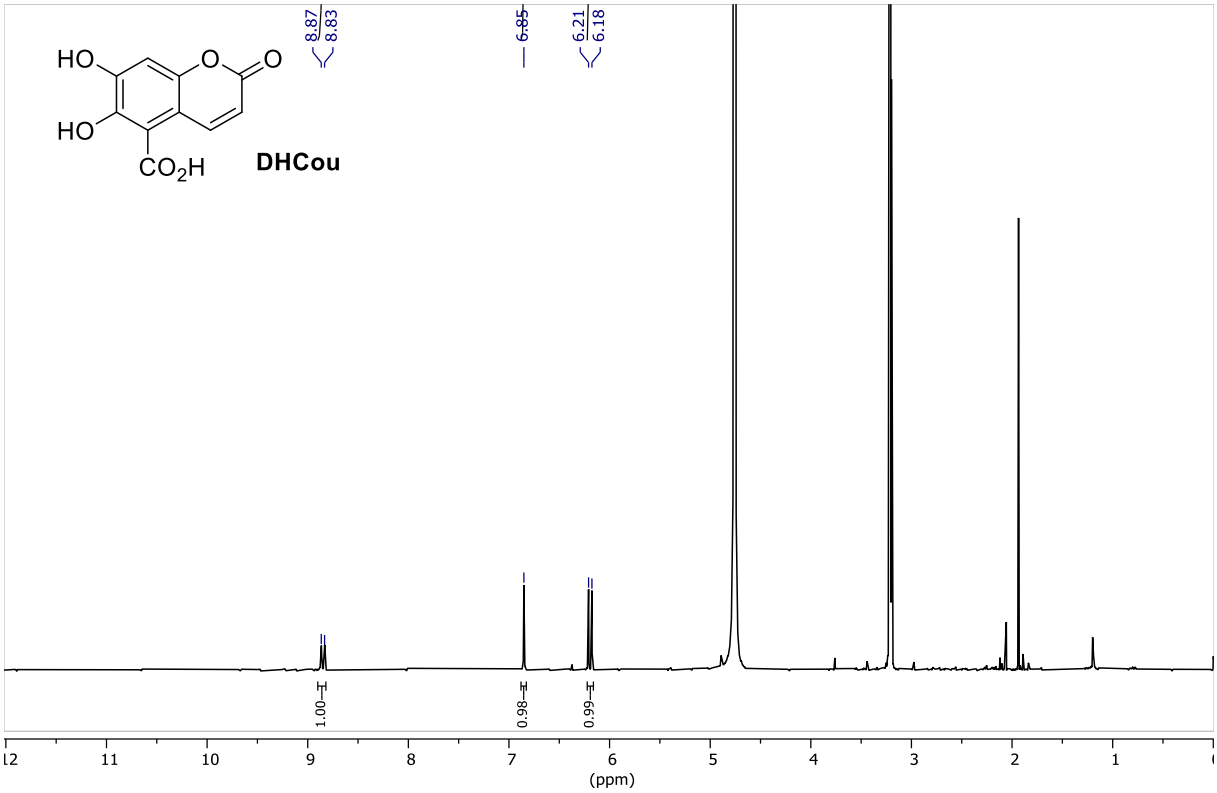
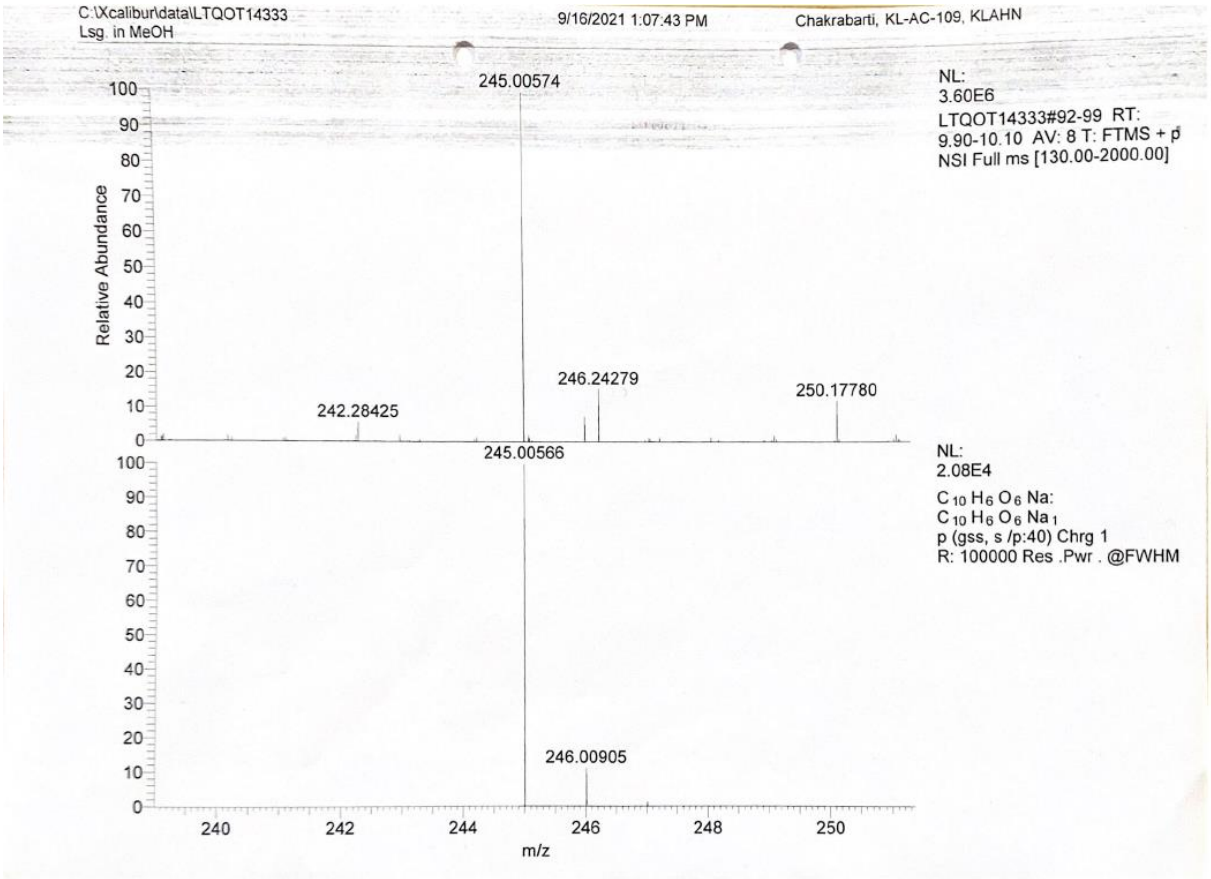




6,7-Dihydroxy-2-oxo-2H-chromene-5-carboxylic acid (DHCou) (^{1}H NMR and HRMS)





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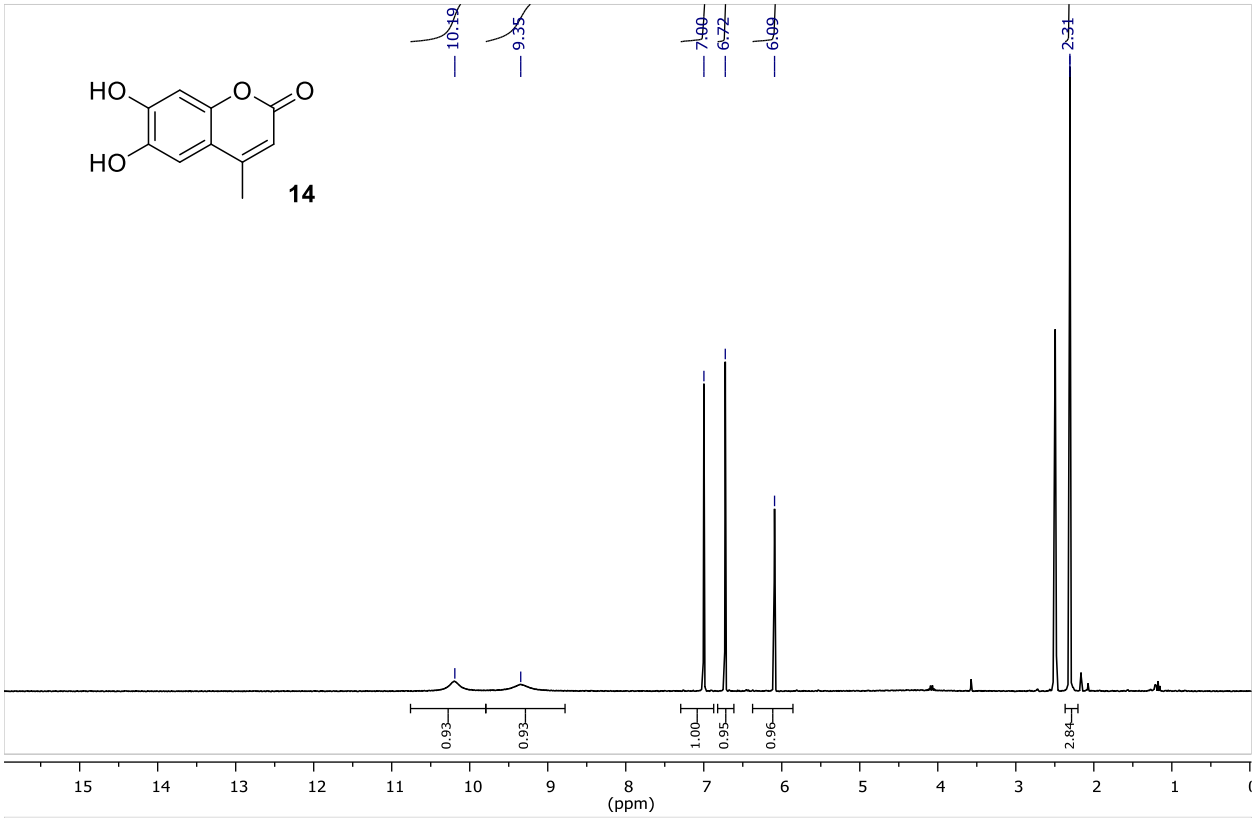
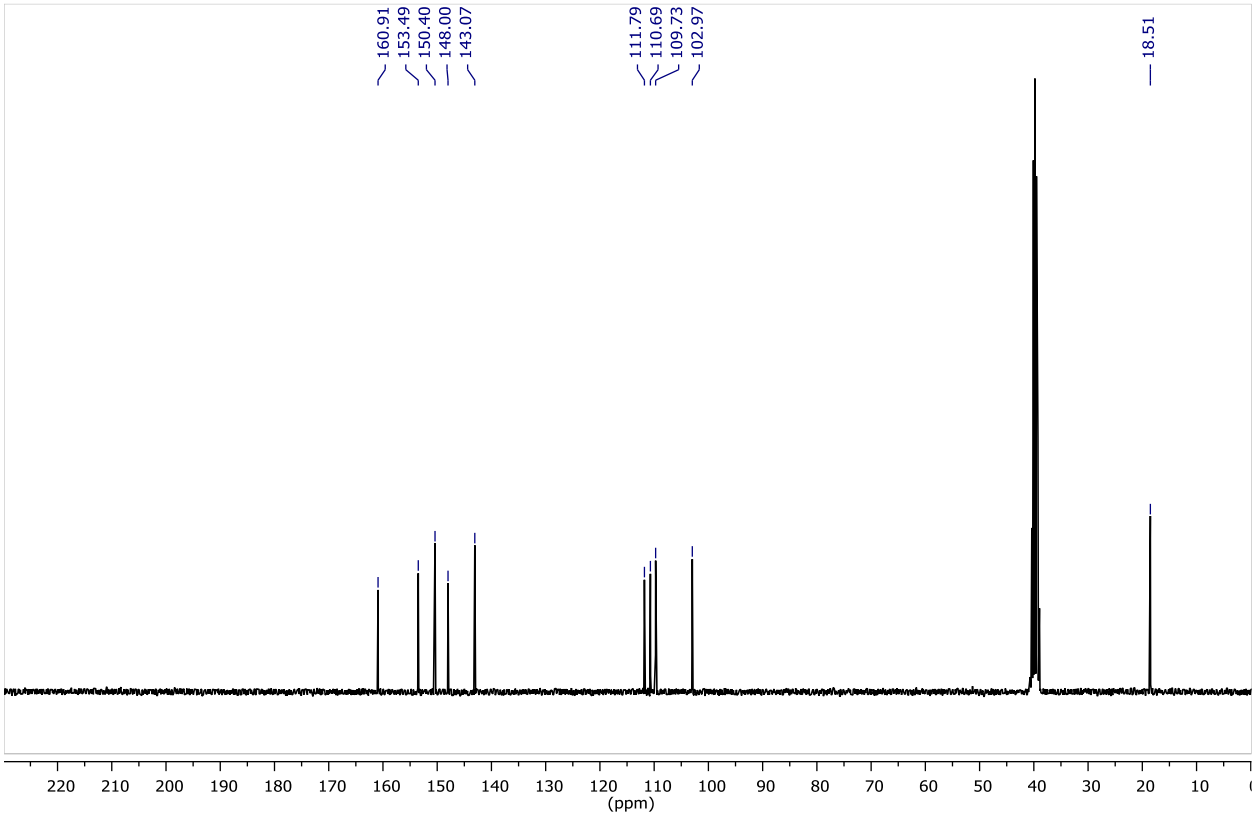
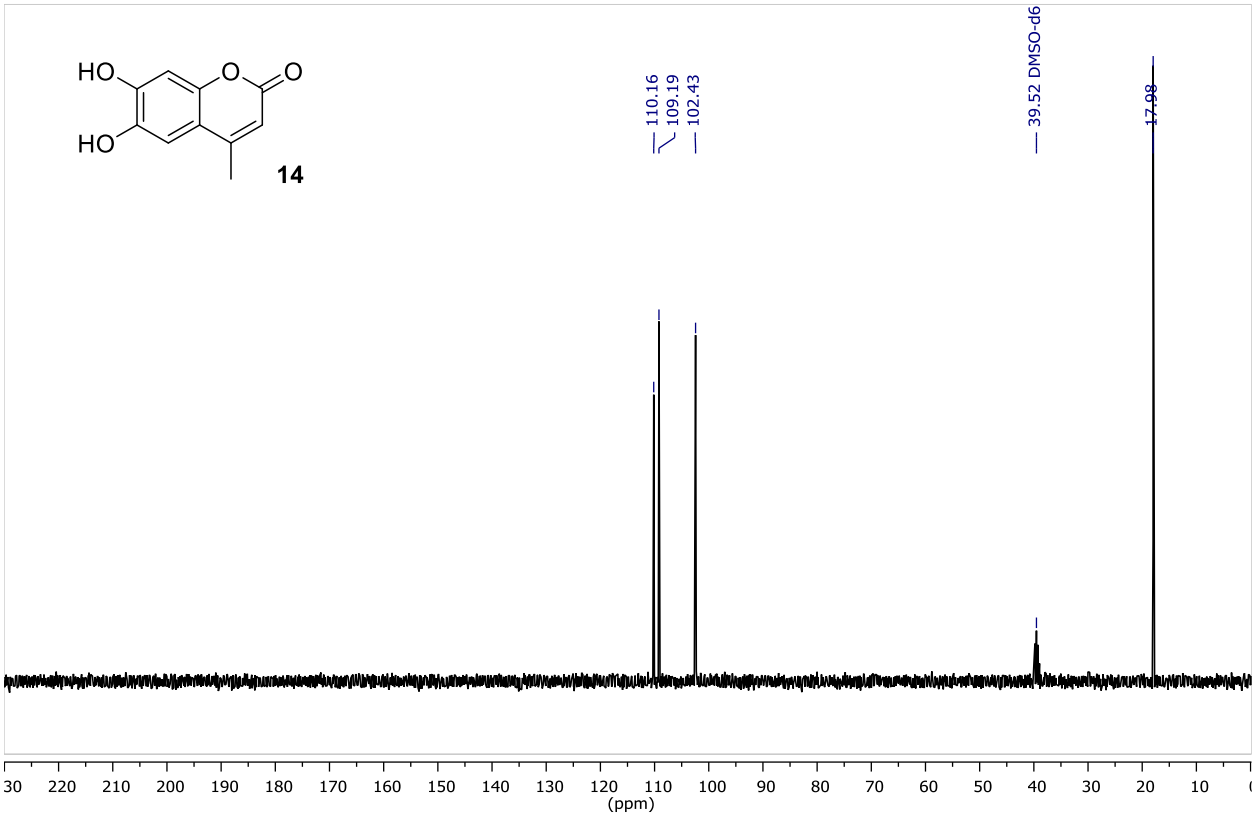
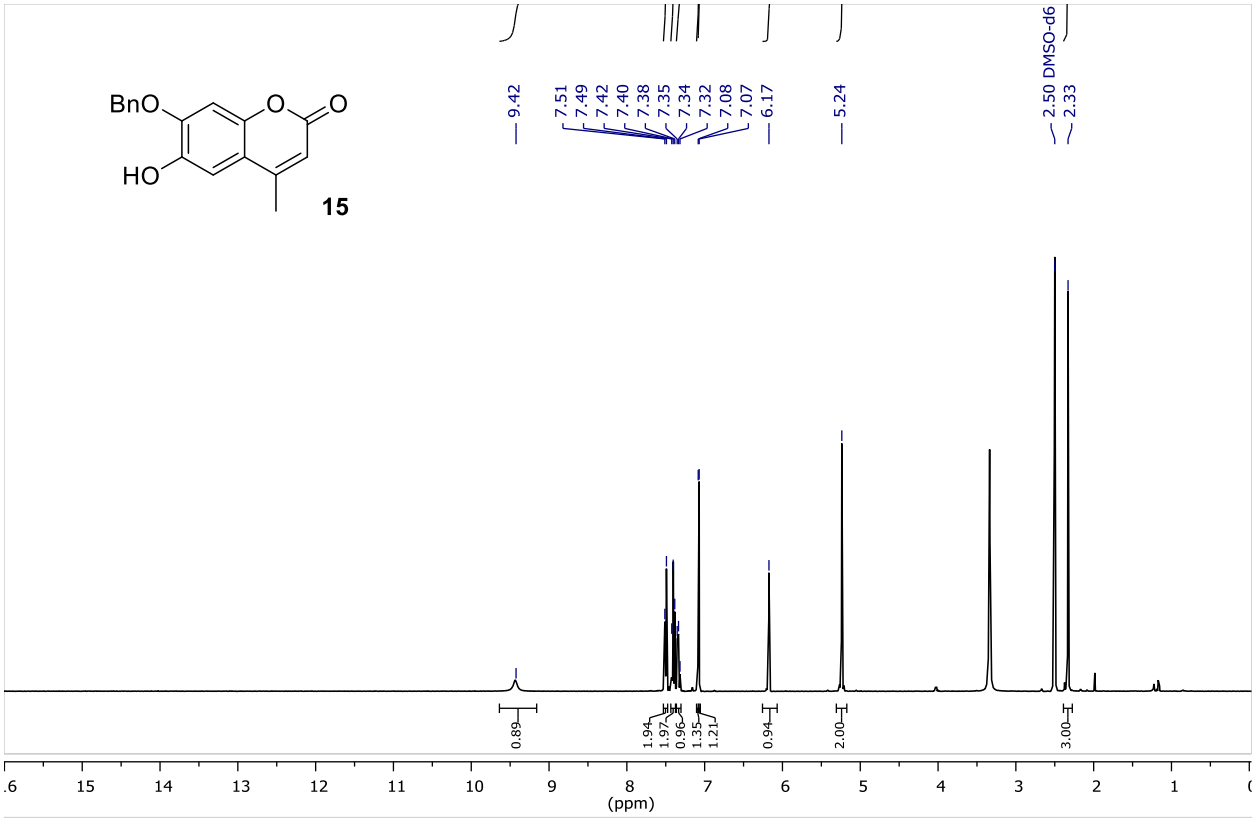


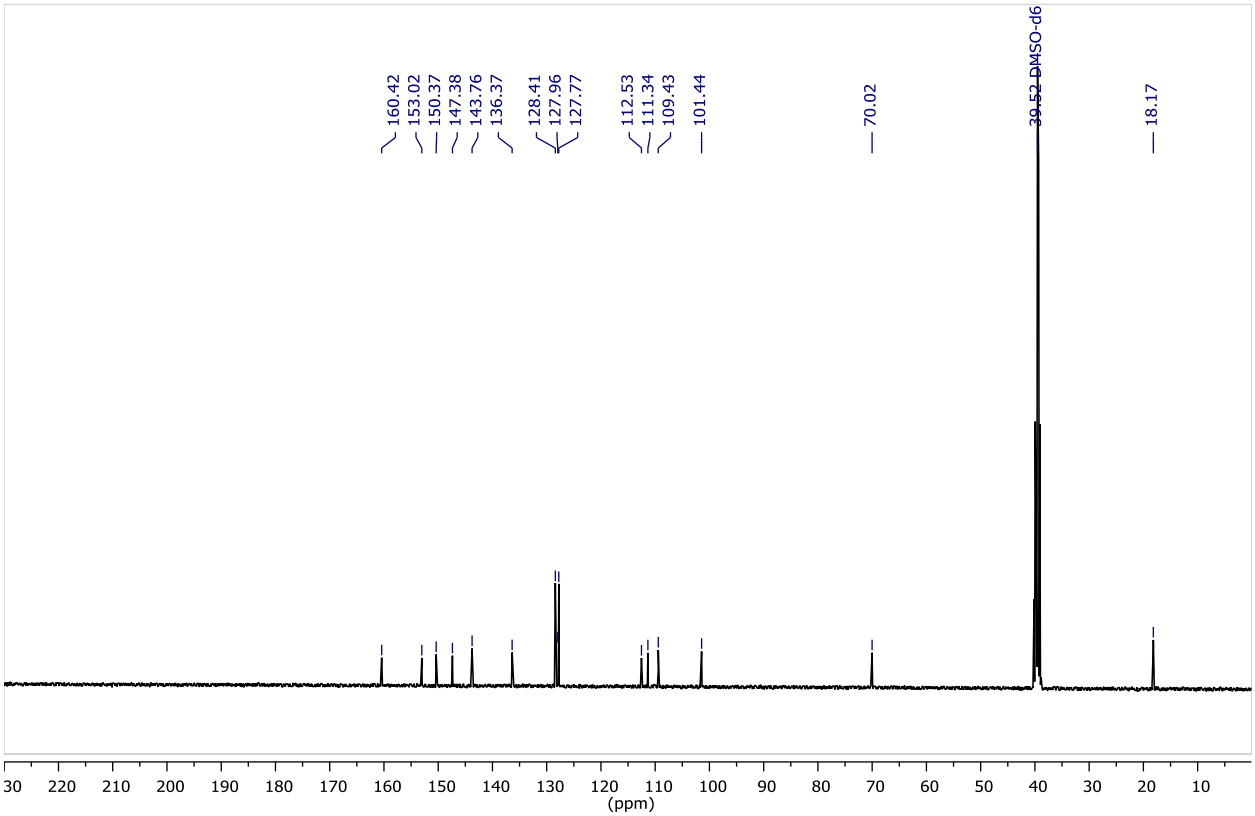


6,7-Dihydroxy-4-methylcoumarin (14) (DEPT NMR)



7-(Benzyloxy)-6-hydroxy-4-methyl-2*H*-chromen-2-one (15) (^{1}H and ^{13}C-NMR)





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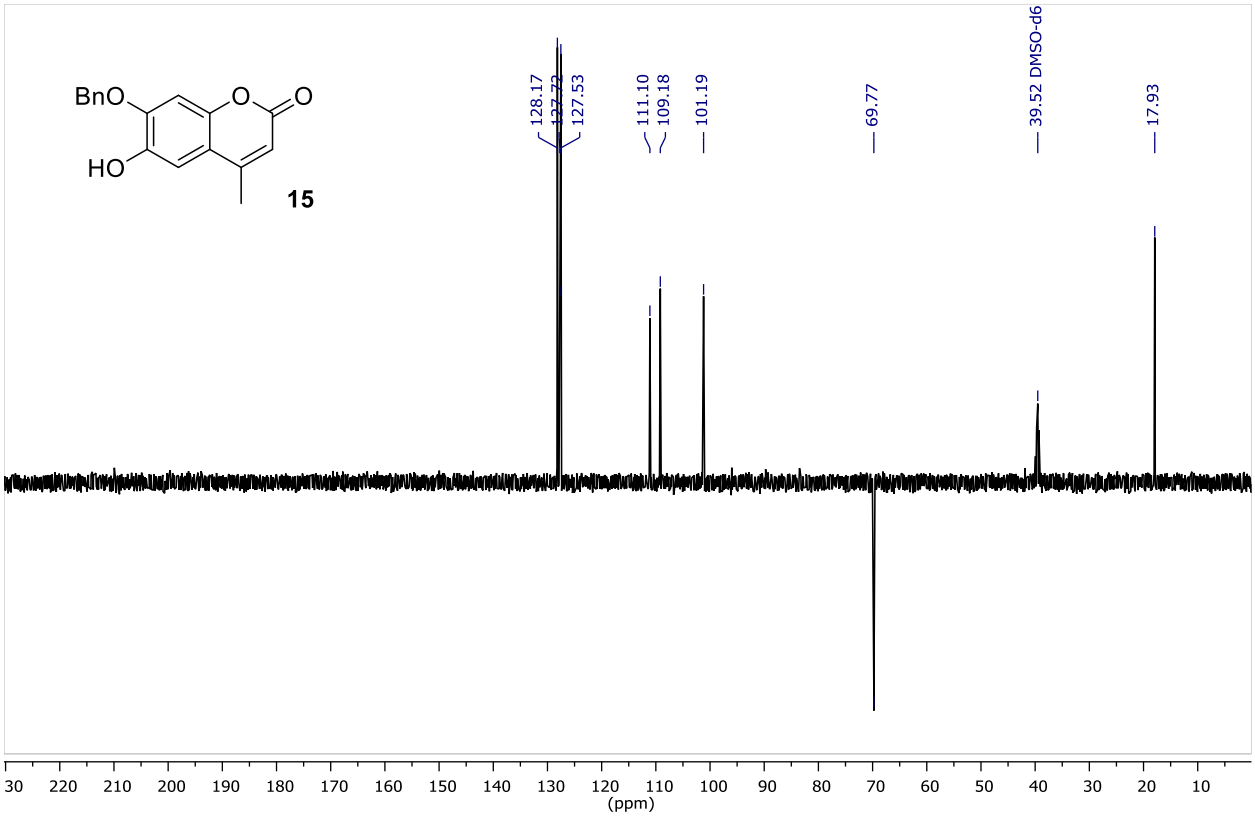

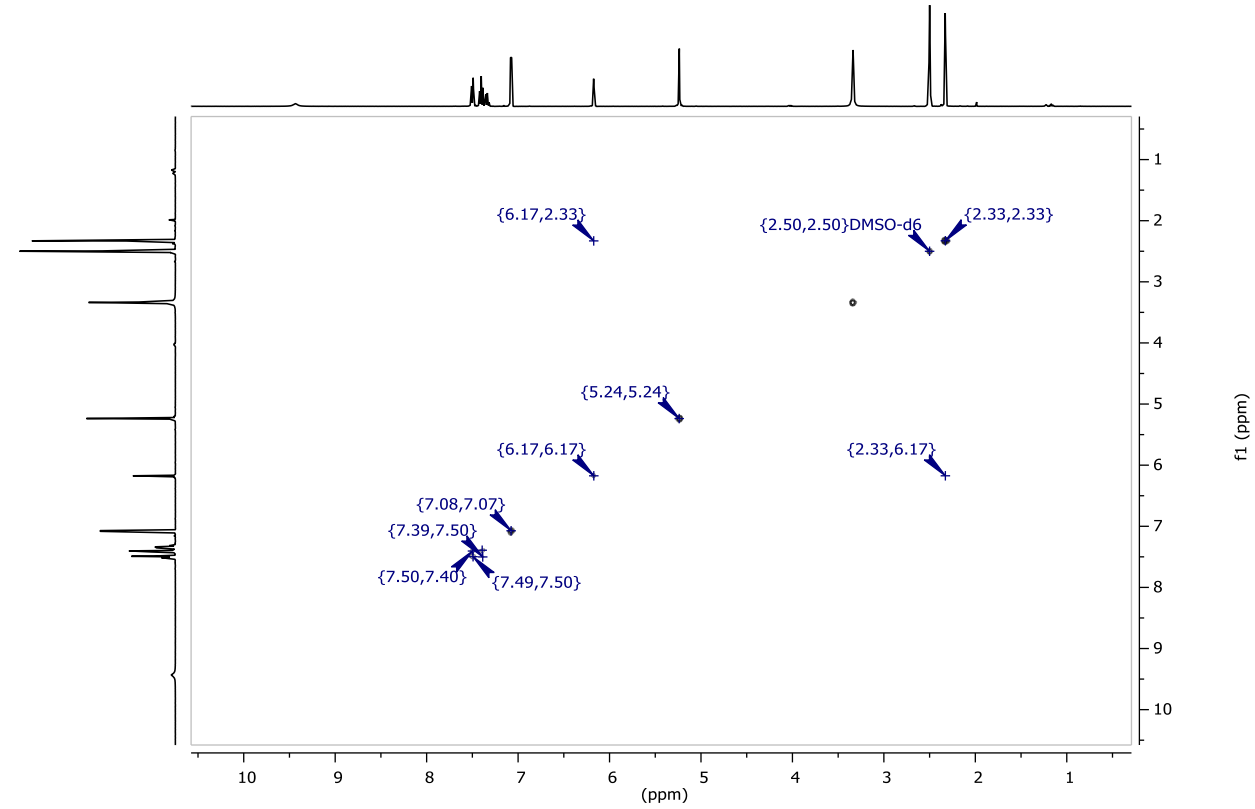

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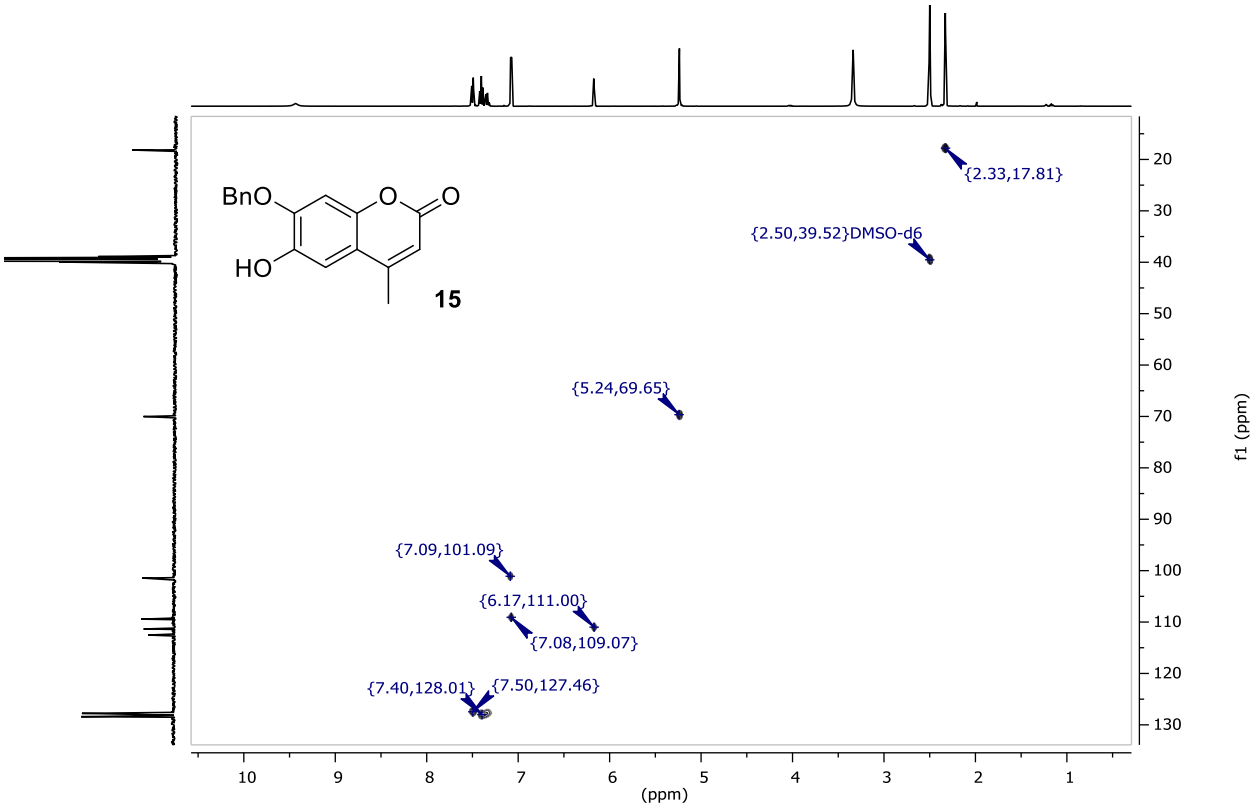

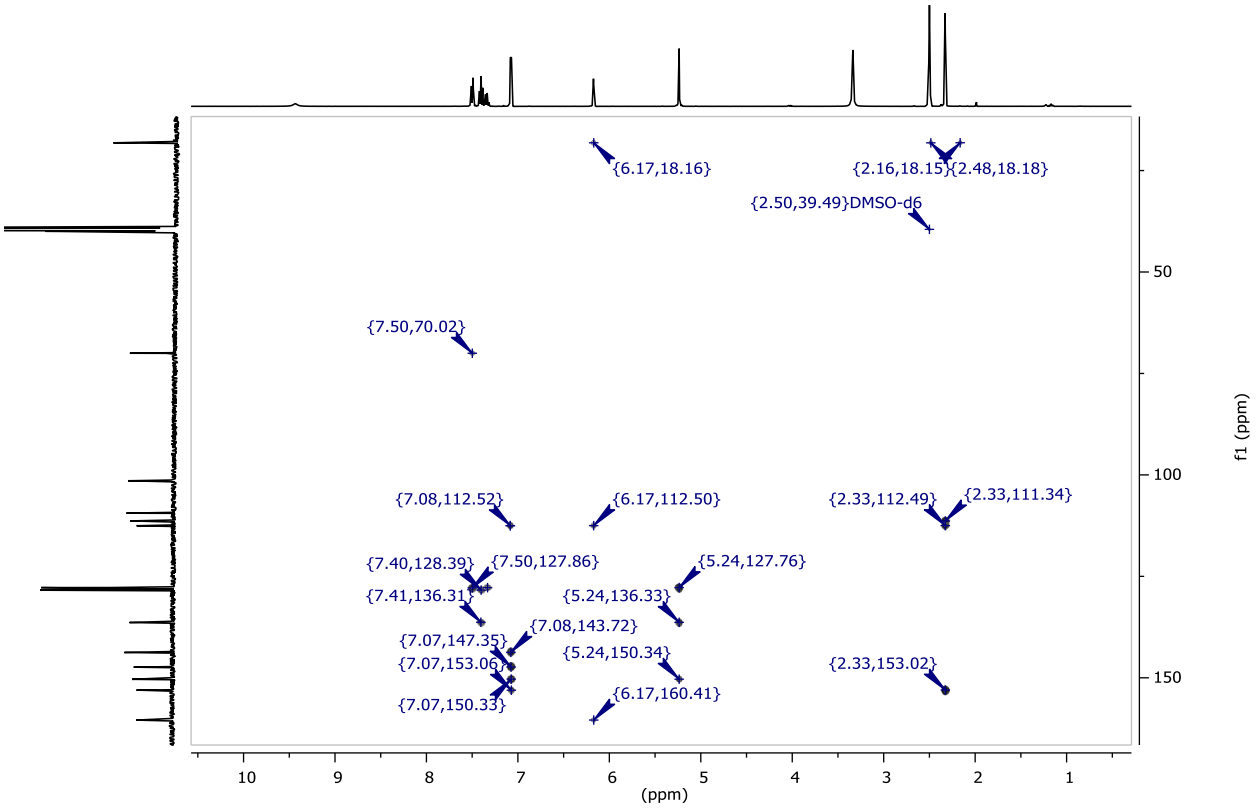

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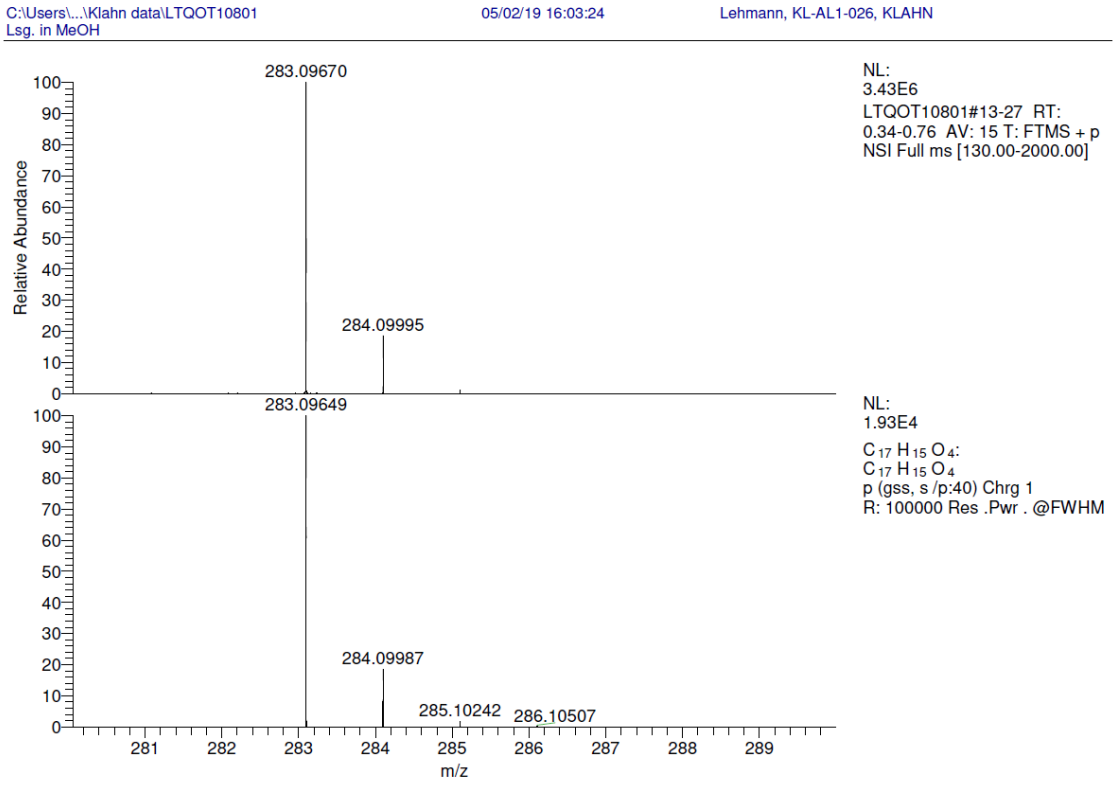

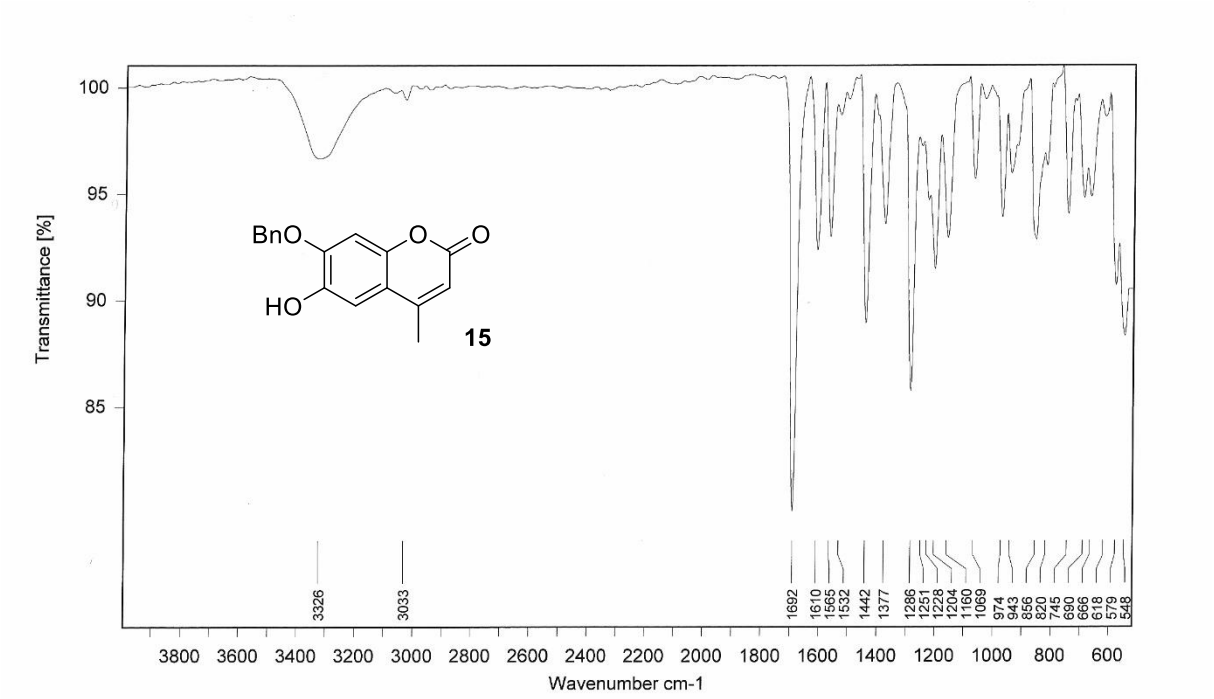

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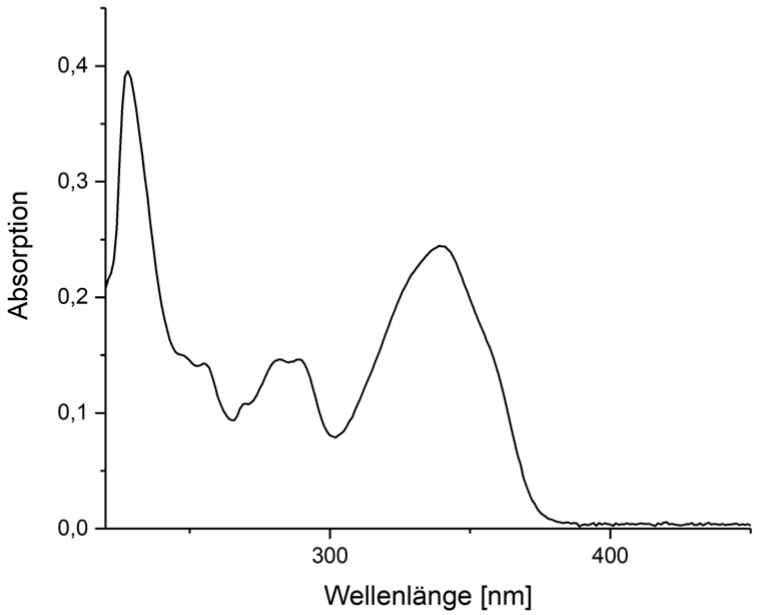
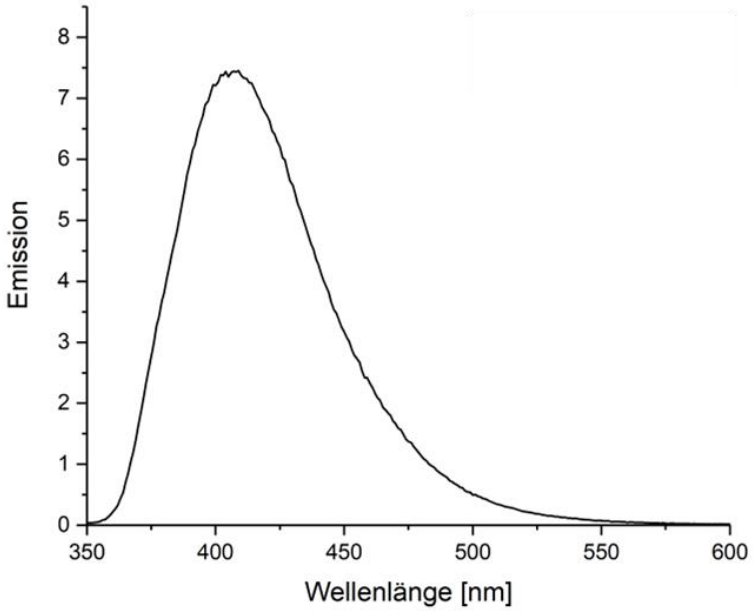
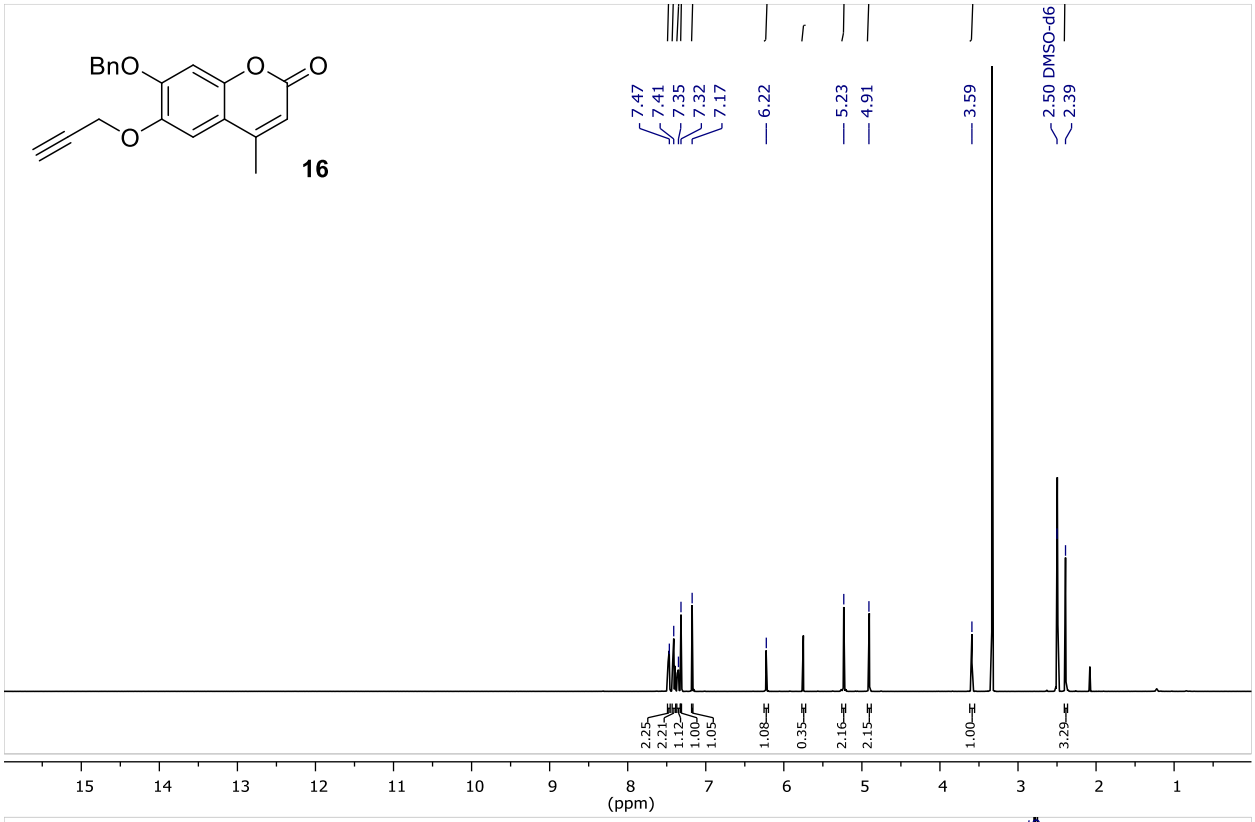
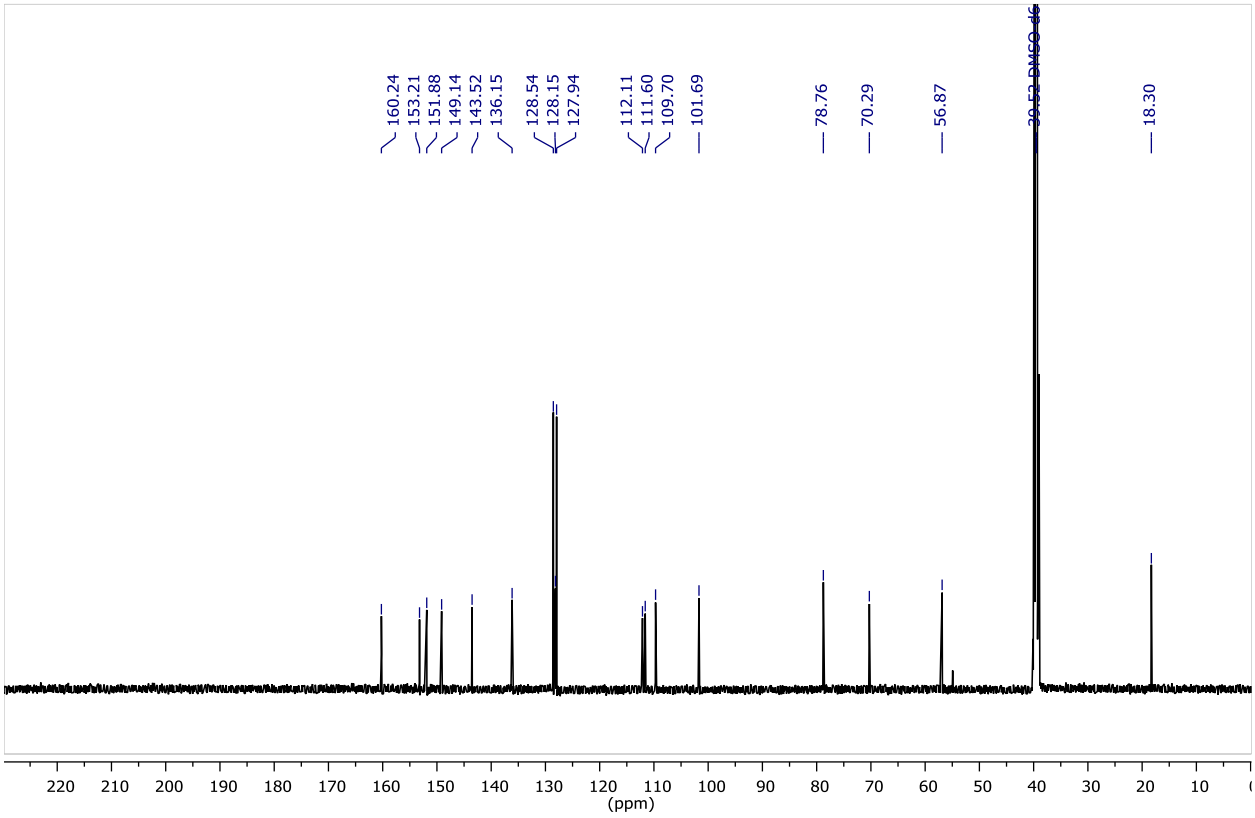


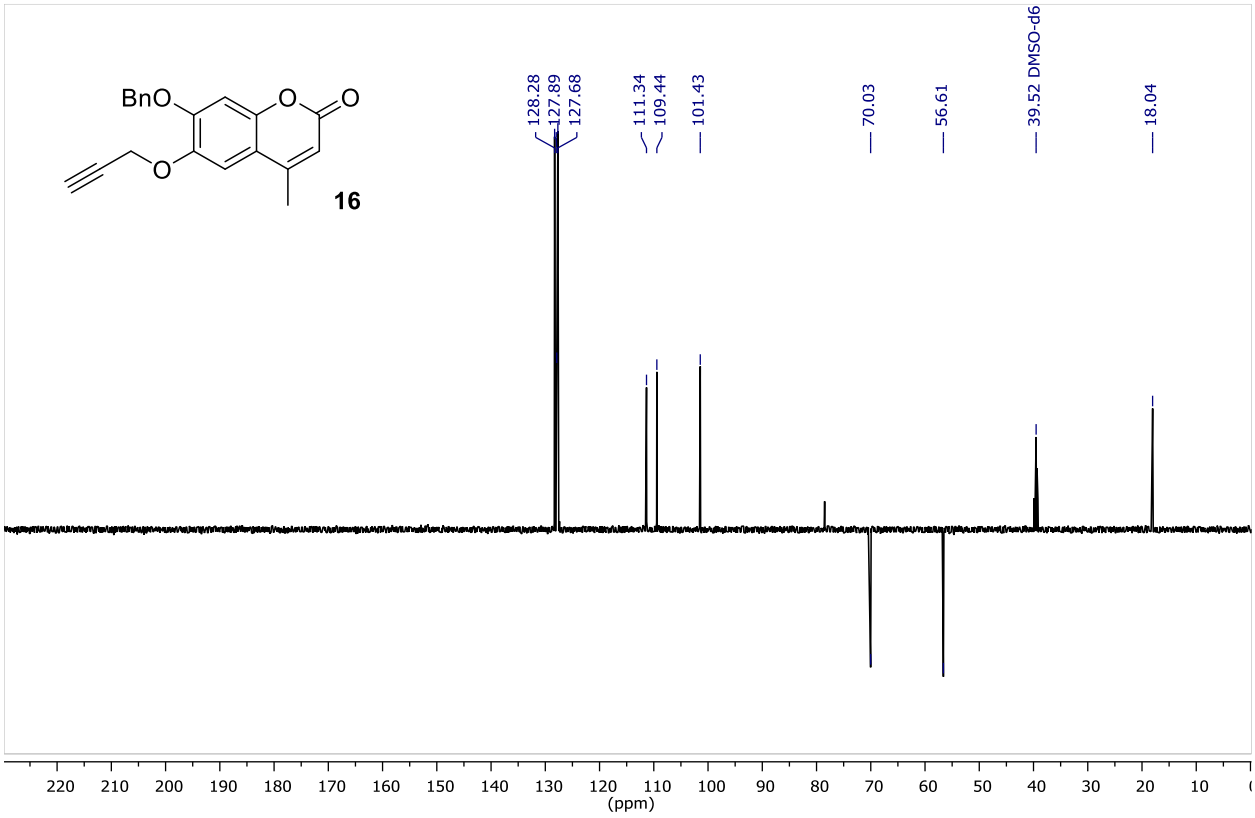


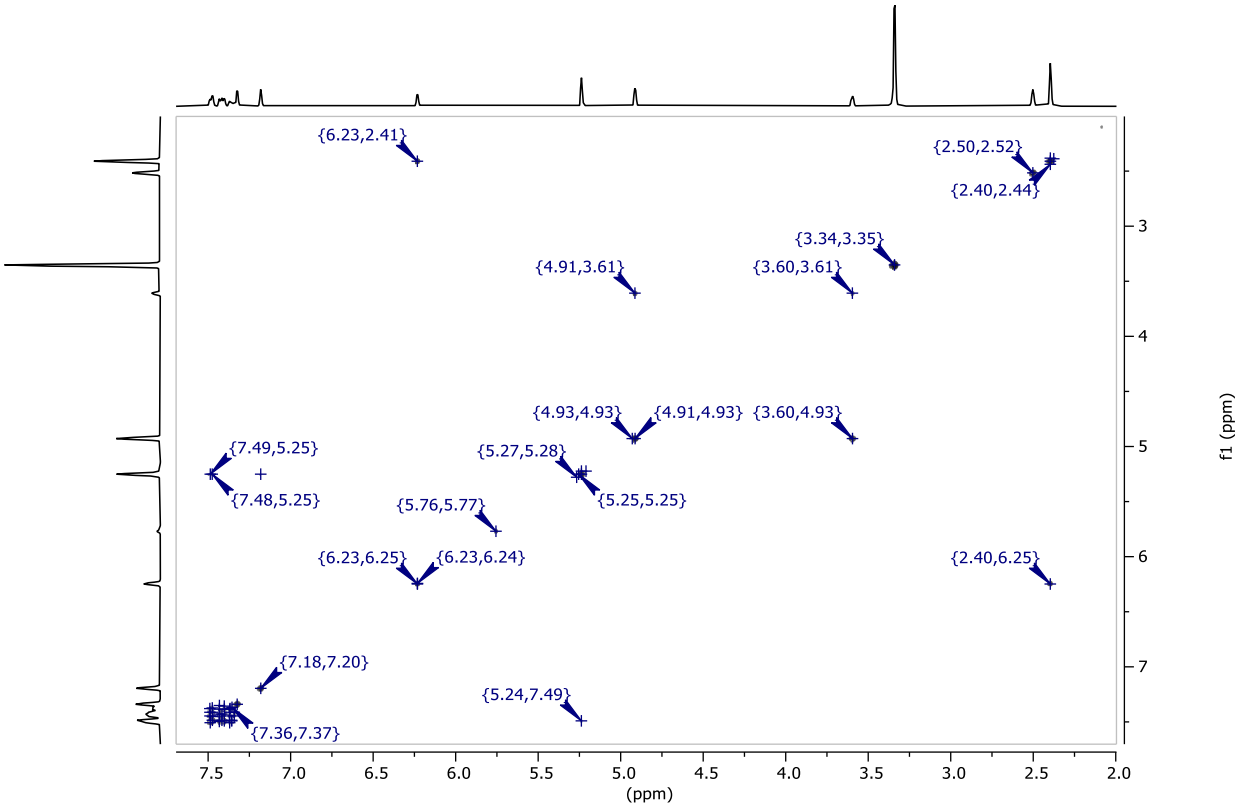
7-(Benzyloxy)-4-Methyl-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one (16) (^{1}H and ^{13}C NMR)



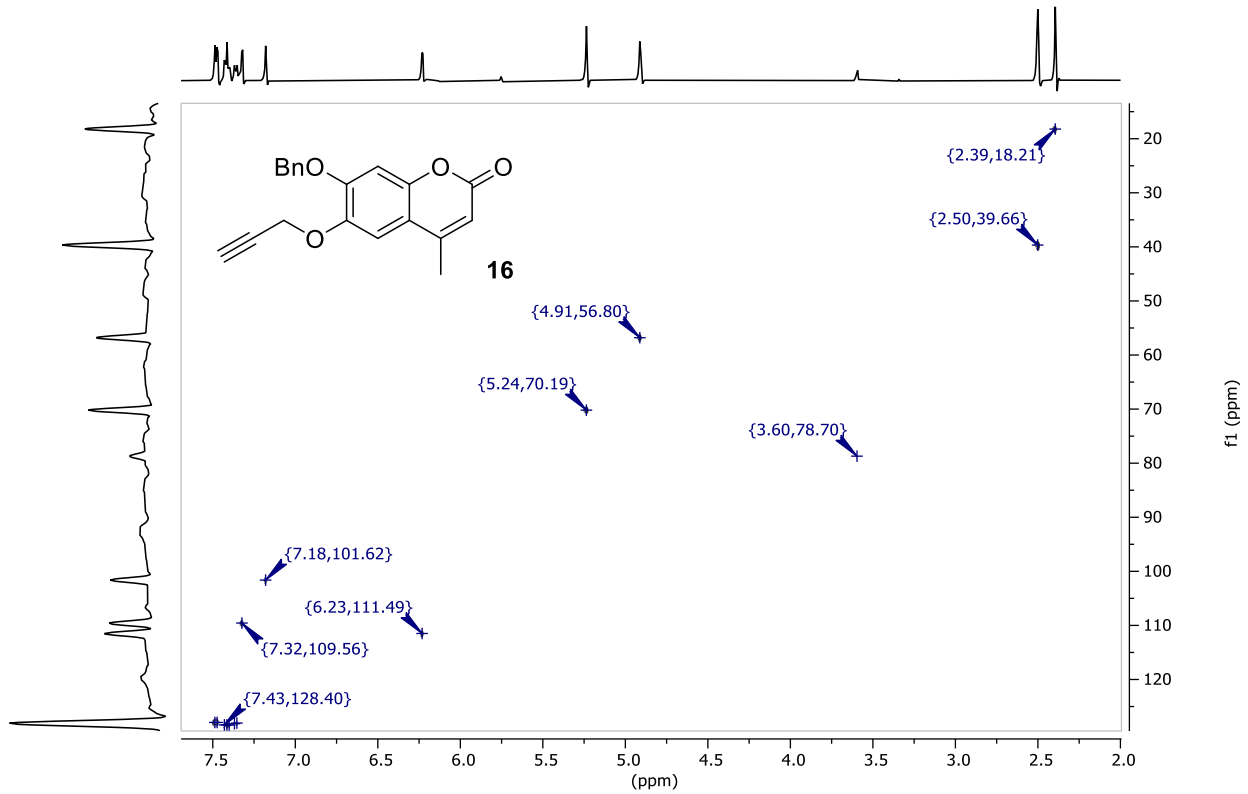


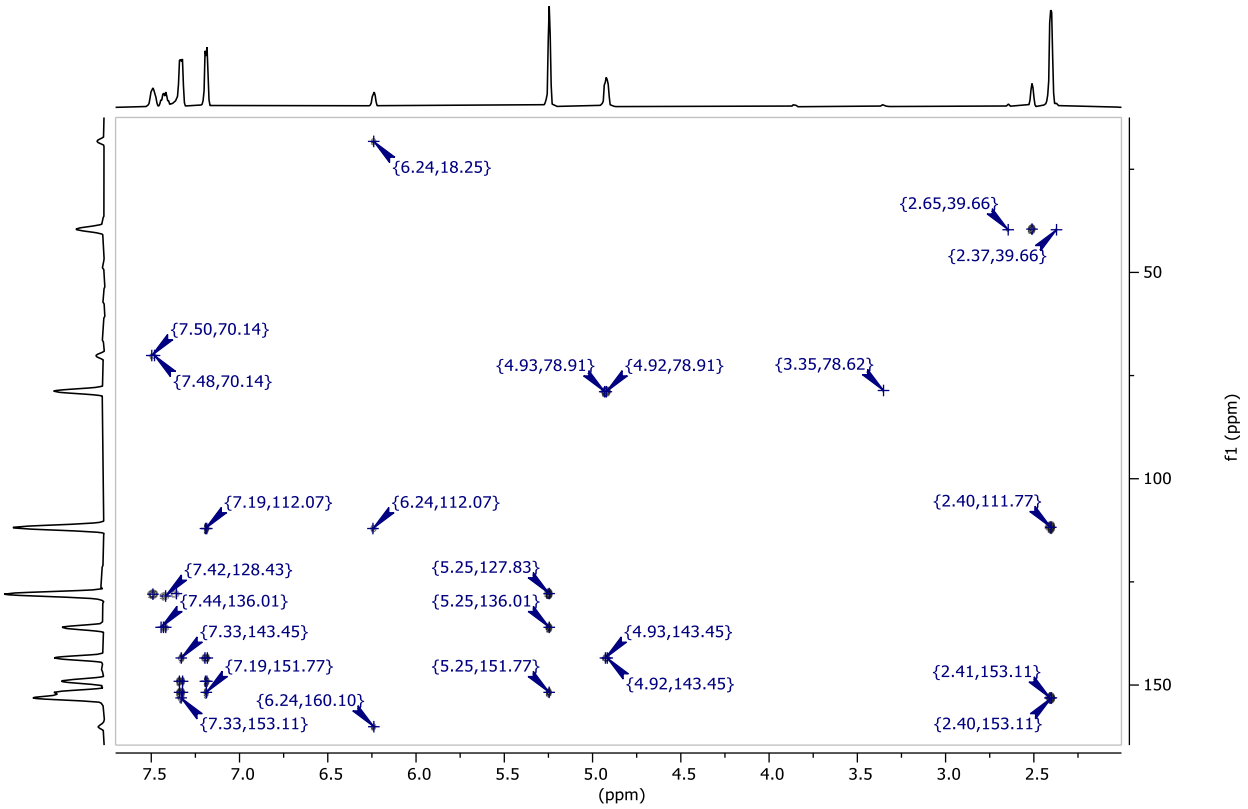
7-(Benzyloxy)-4-Methyl-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one 16) (DEPT and COSY NMR)





7-(Benzyloxy)-4-Methyl-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one (16) (HSQC and HMBC NMR)





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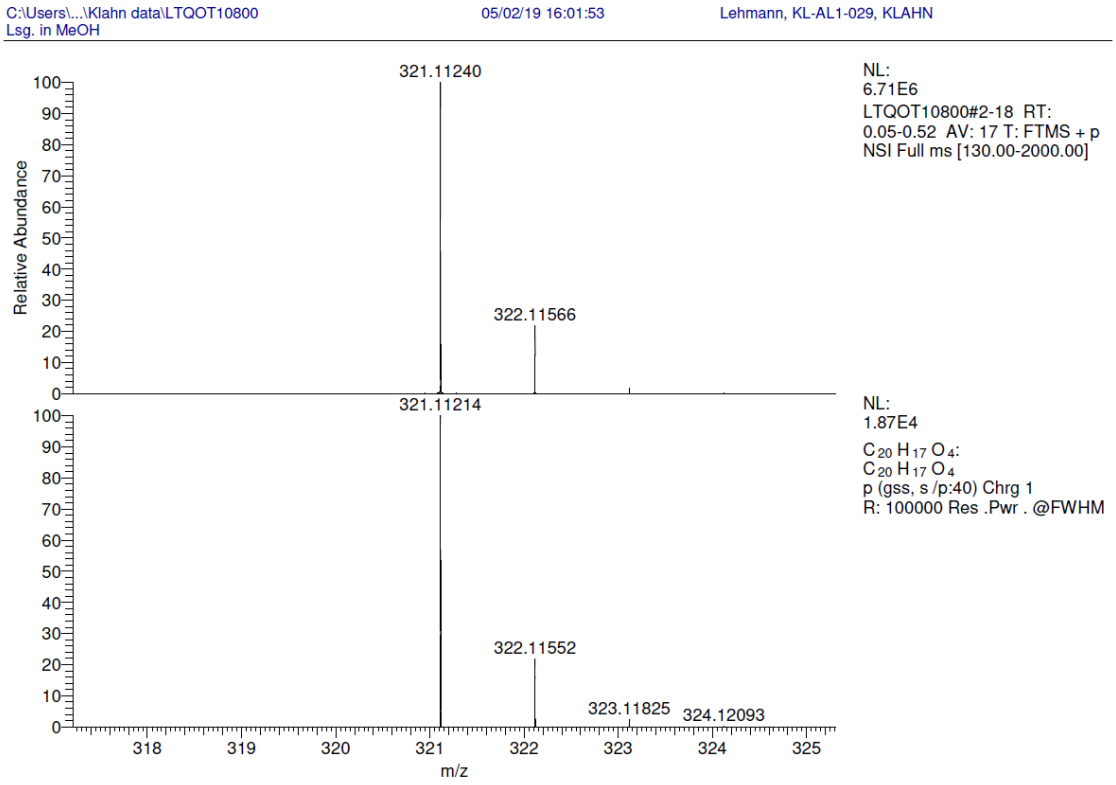
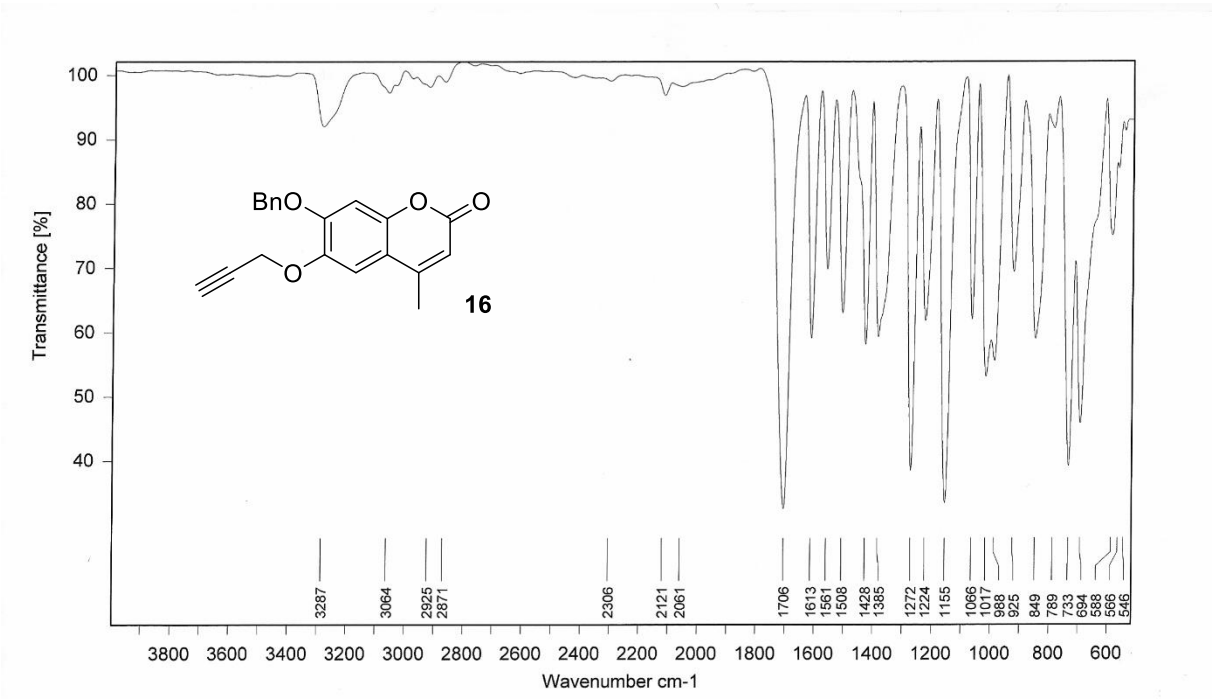
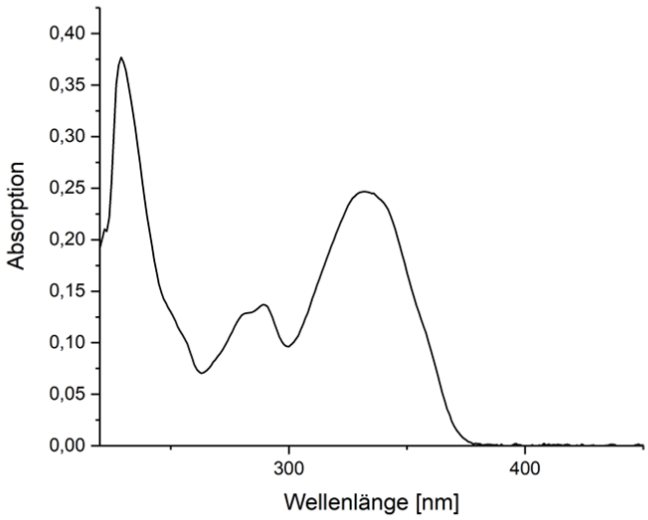
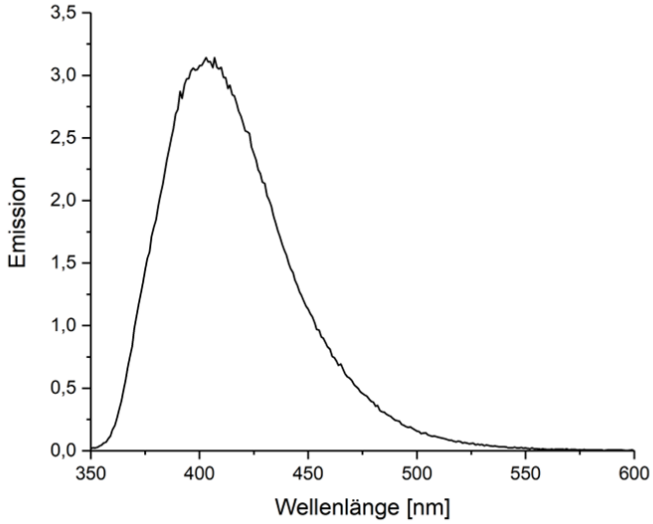


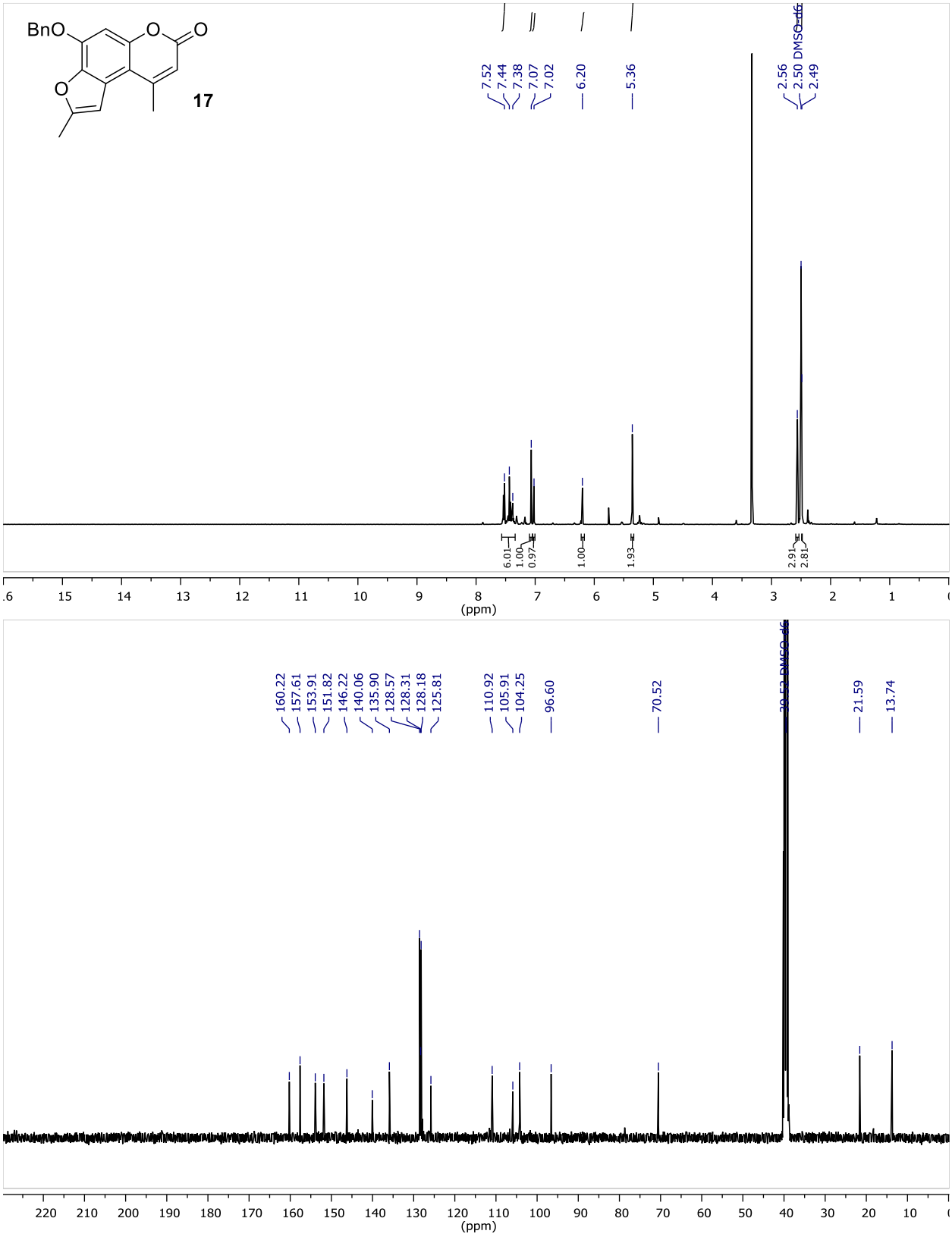


7-(Benzyloxy)-4-Methyl-6-(prop-2-yn-1-yloxy)-2*H*-chromen-2-one (16) (UV/Vis and Fluorescence emission)

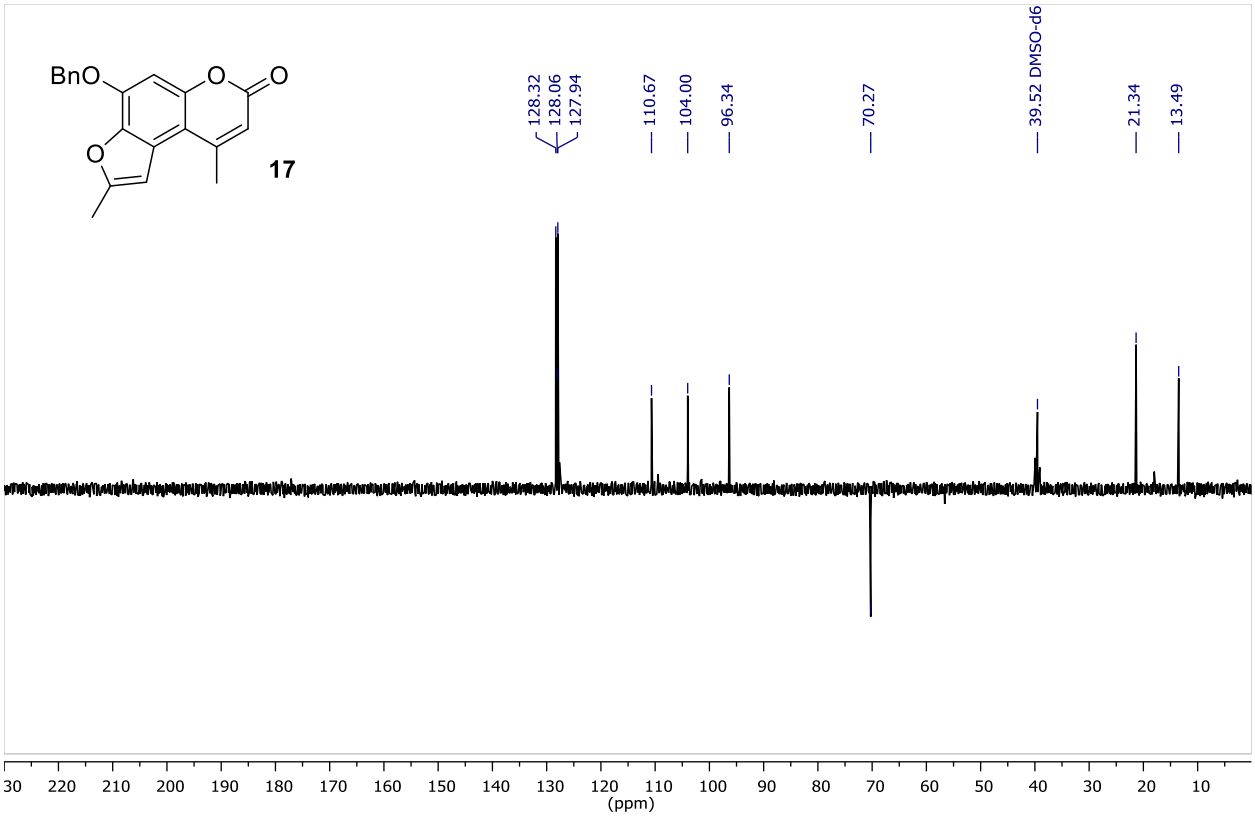


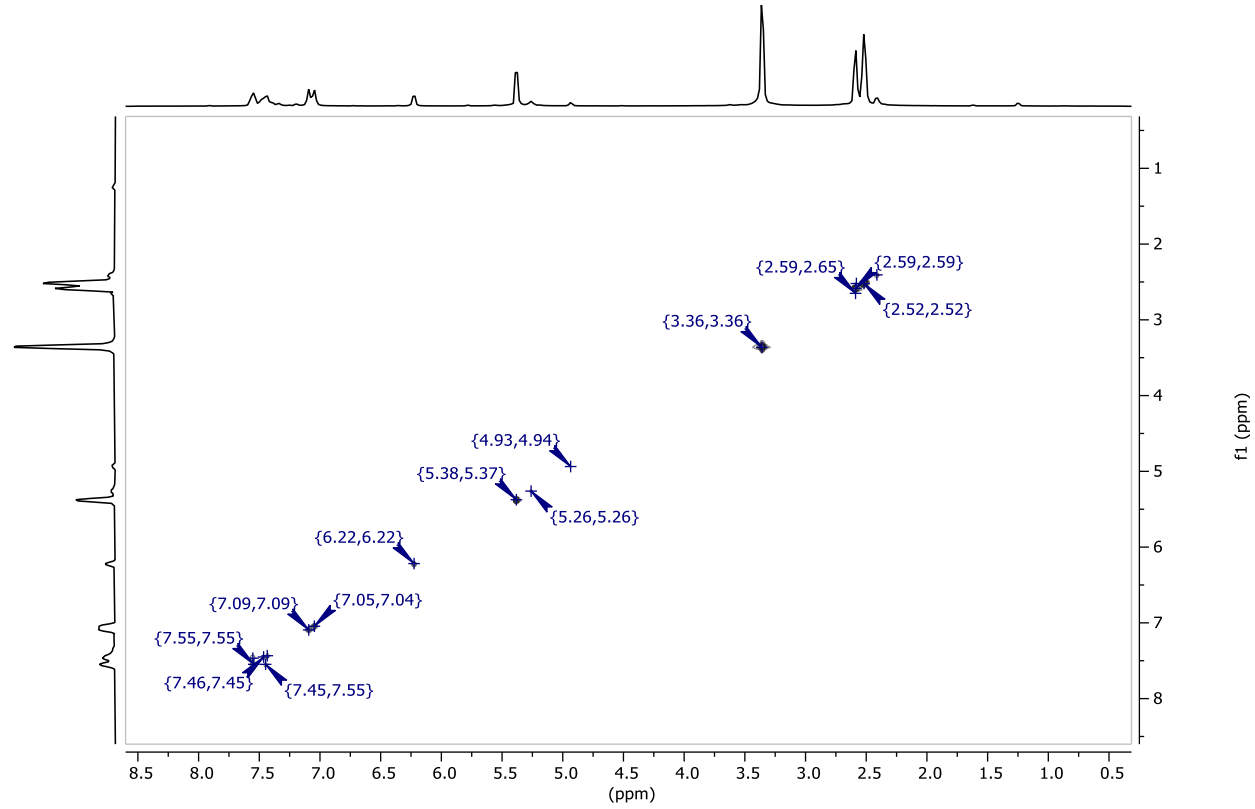


4-Benzyloxy-2,9-dimethyl-7H-furo[3,2-f]-chromen-7-on (17) (^{1}H and ^{13}C NMR)

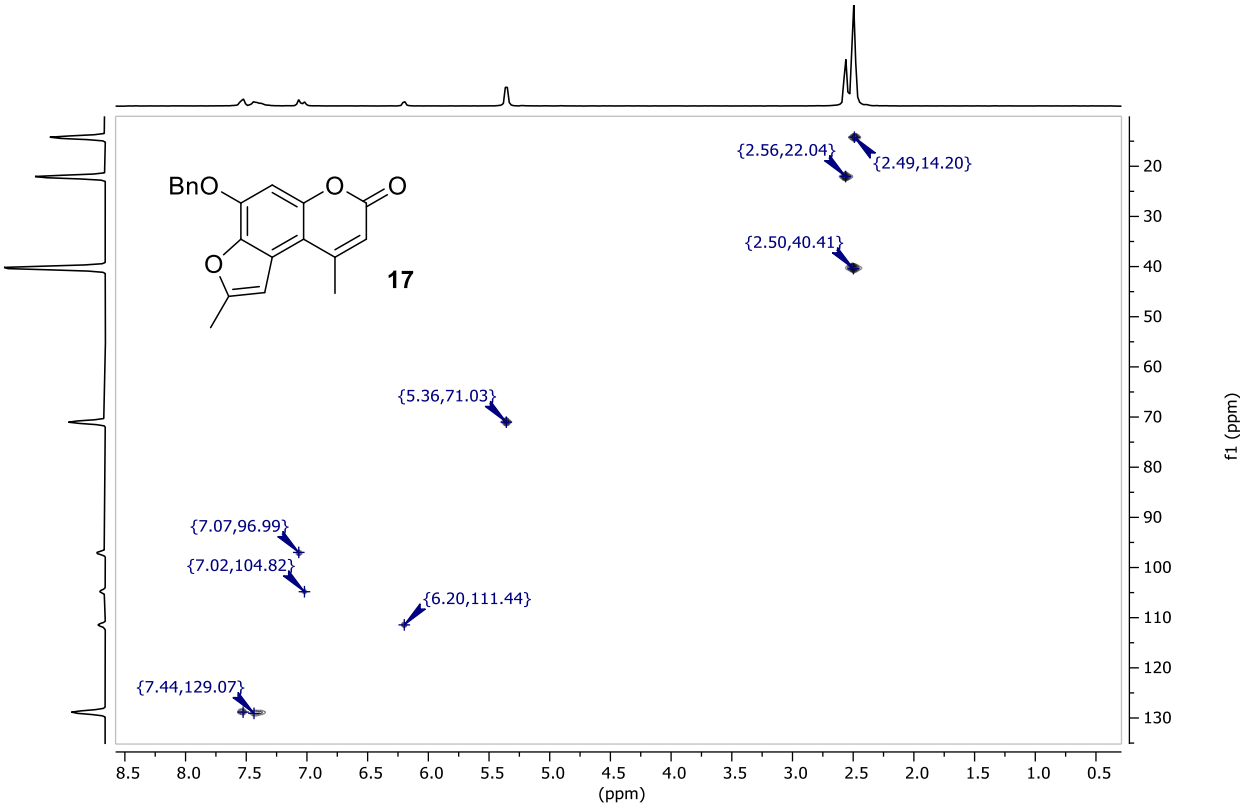


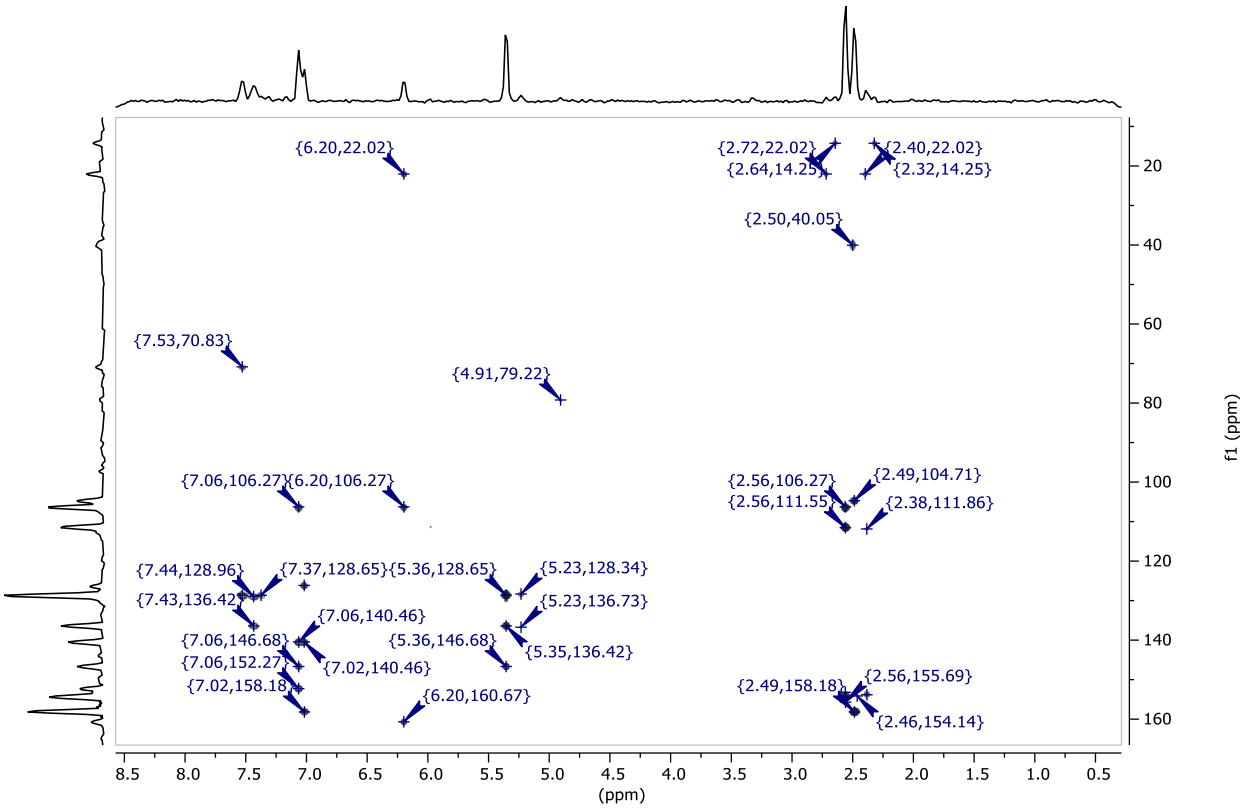
4-Benzyloxy-2,9-dimethyl-7H-furo[3,2-f]-chromen-7-on (17) (DEPT and COSY NMR)



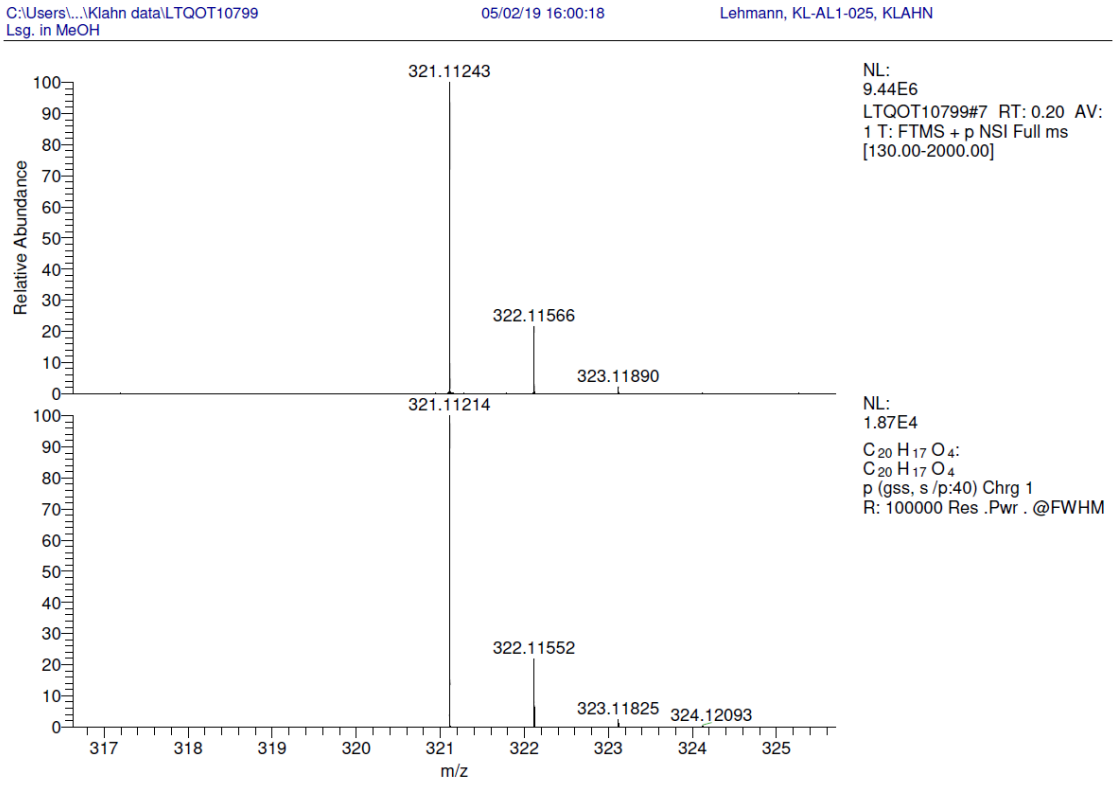


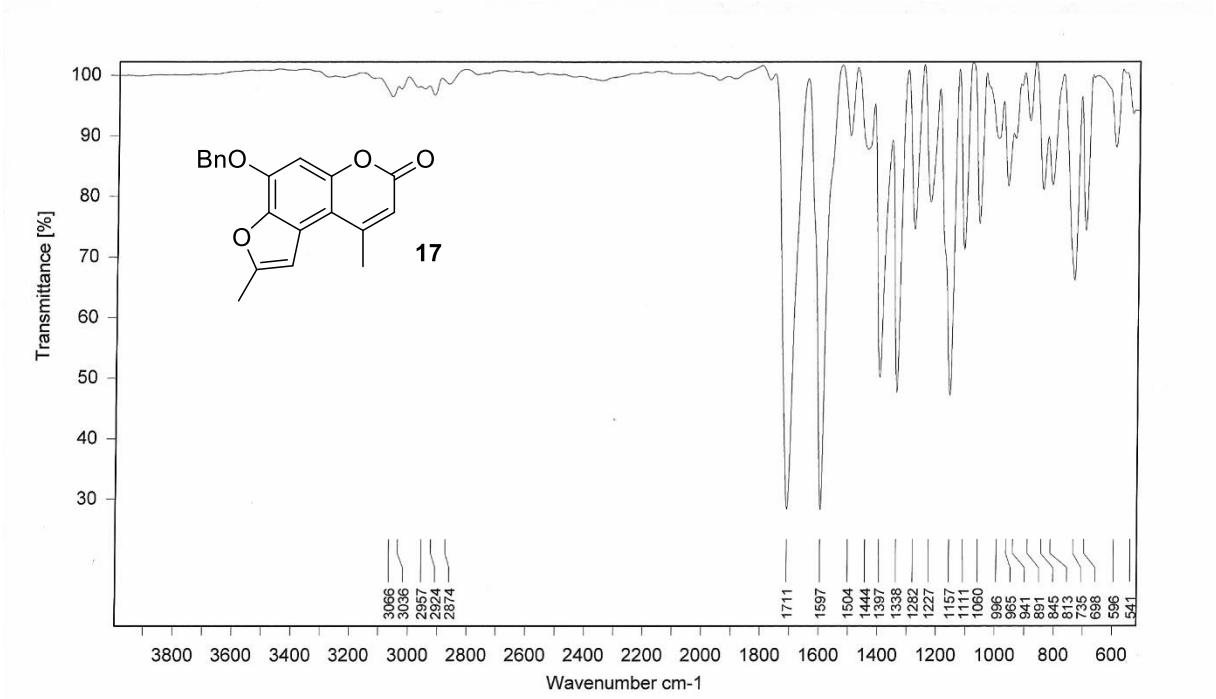
4-Benzyloxy-2,9-dimethyl-7H-furo[3,2-f]-chromen-7-on (17) (HSQC and HMBC NMR)



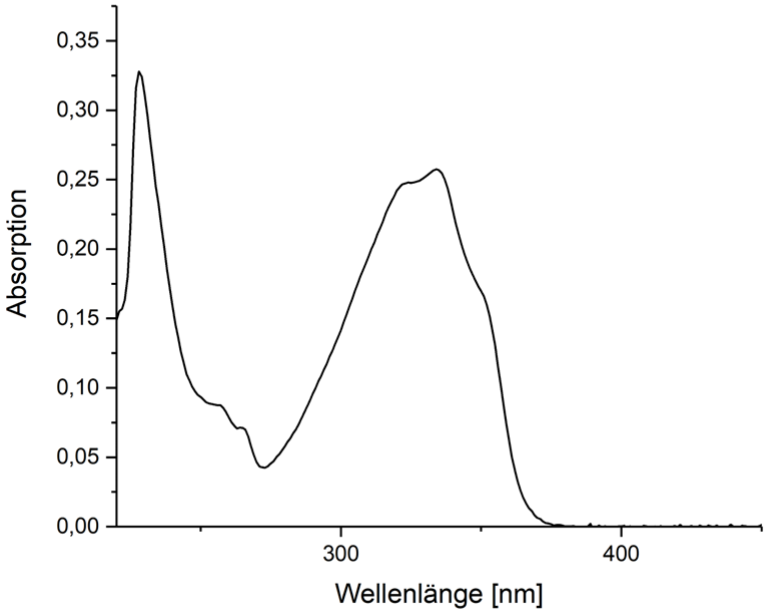


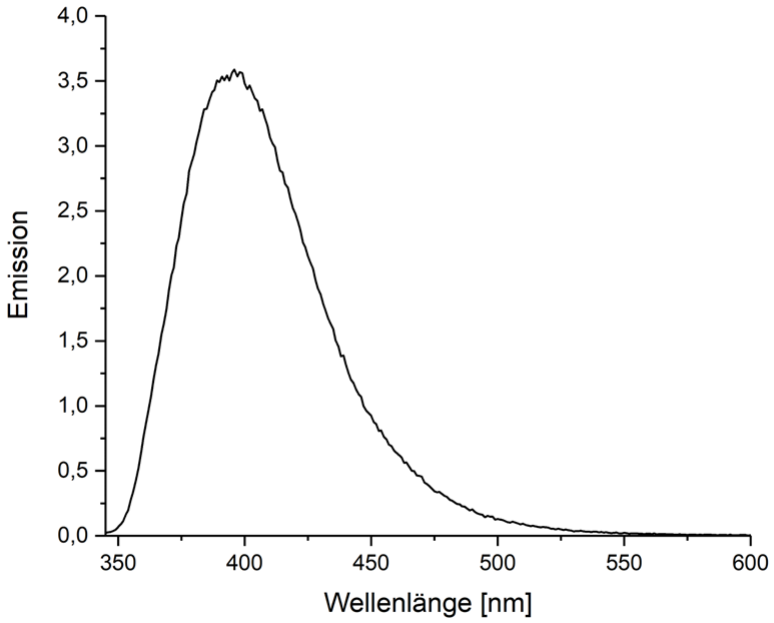
4-Benzyloxy-2,9-dimethyl-7H-furo[3,2-f]-chromen-7-on (17) (HRMS and ATR-IR)



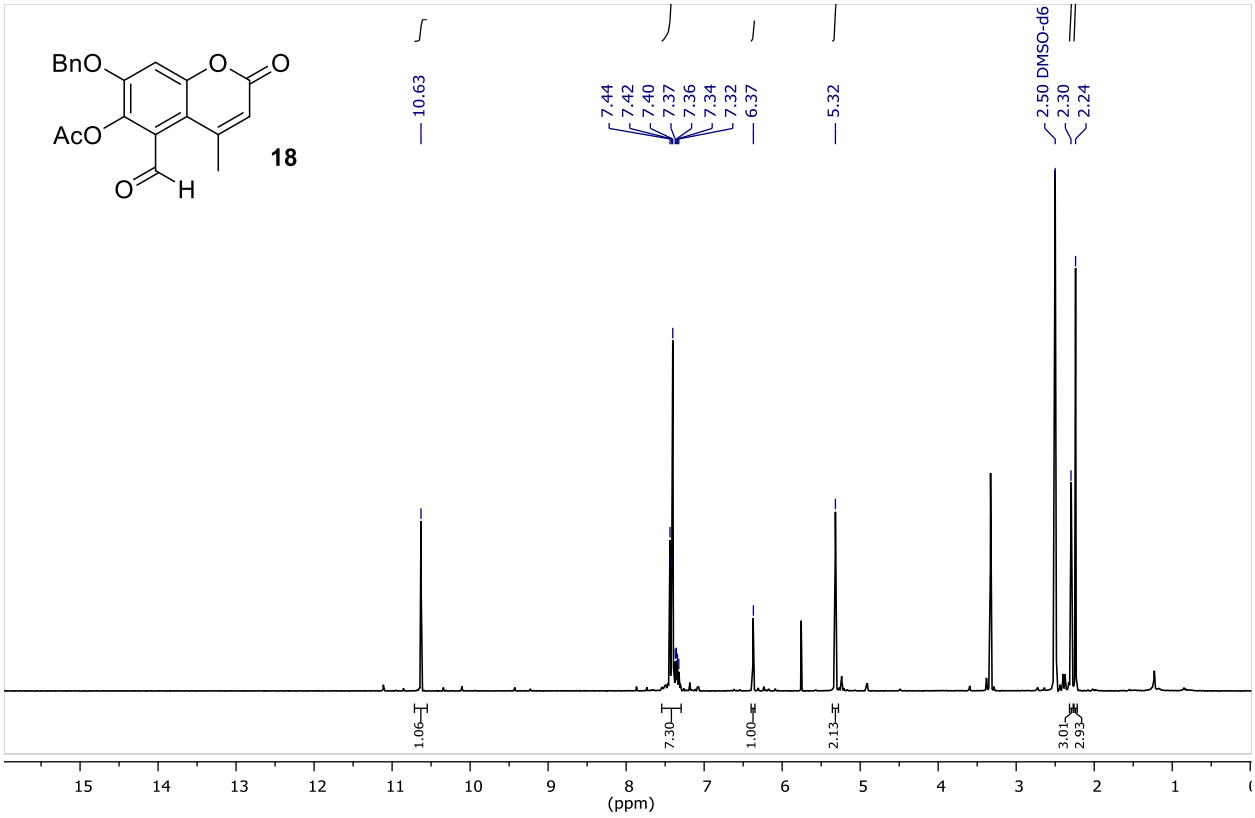


4-Benzyloxy-2,9-dimethyl-7H-furo[3,2-f]-chromen-7-on (17) (UV/Vis and Fluorescence emission)

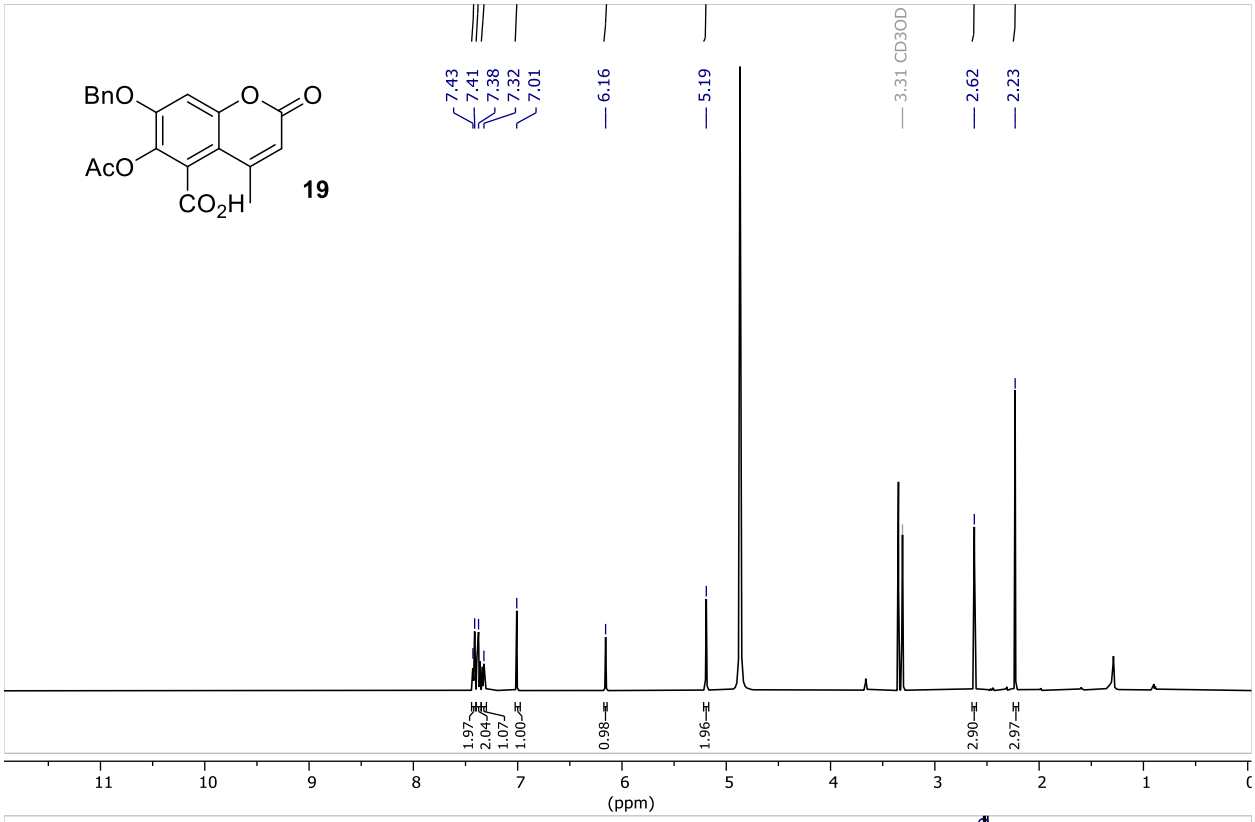


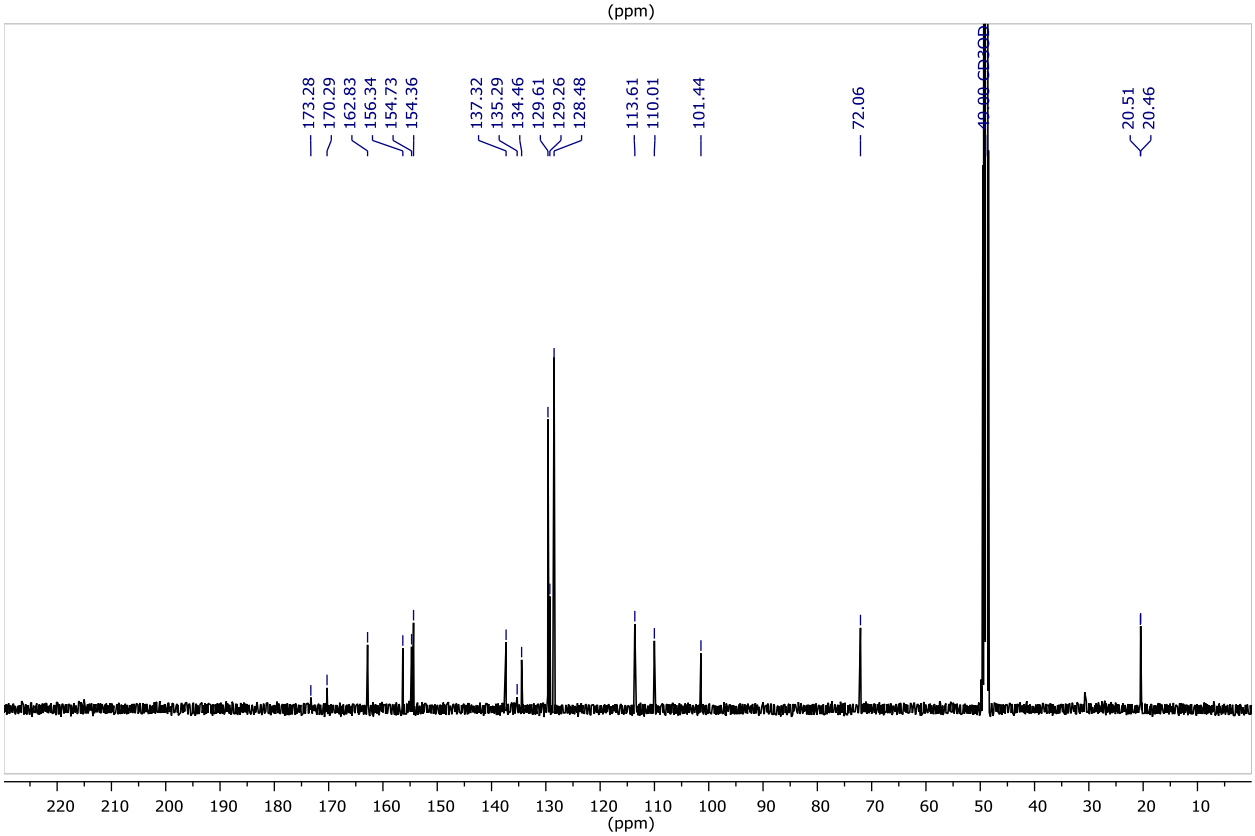


7-(Benzyloxy)-5-formyl-4-methyl-2-oxo-2H-chromen-6-yl acetate (18) (^{1}H NMR)

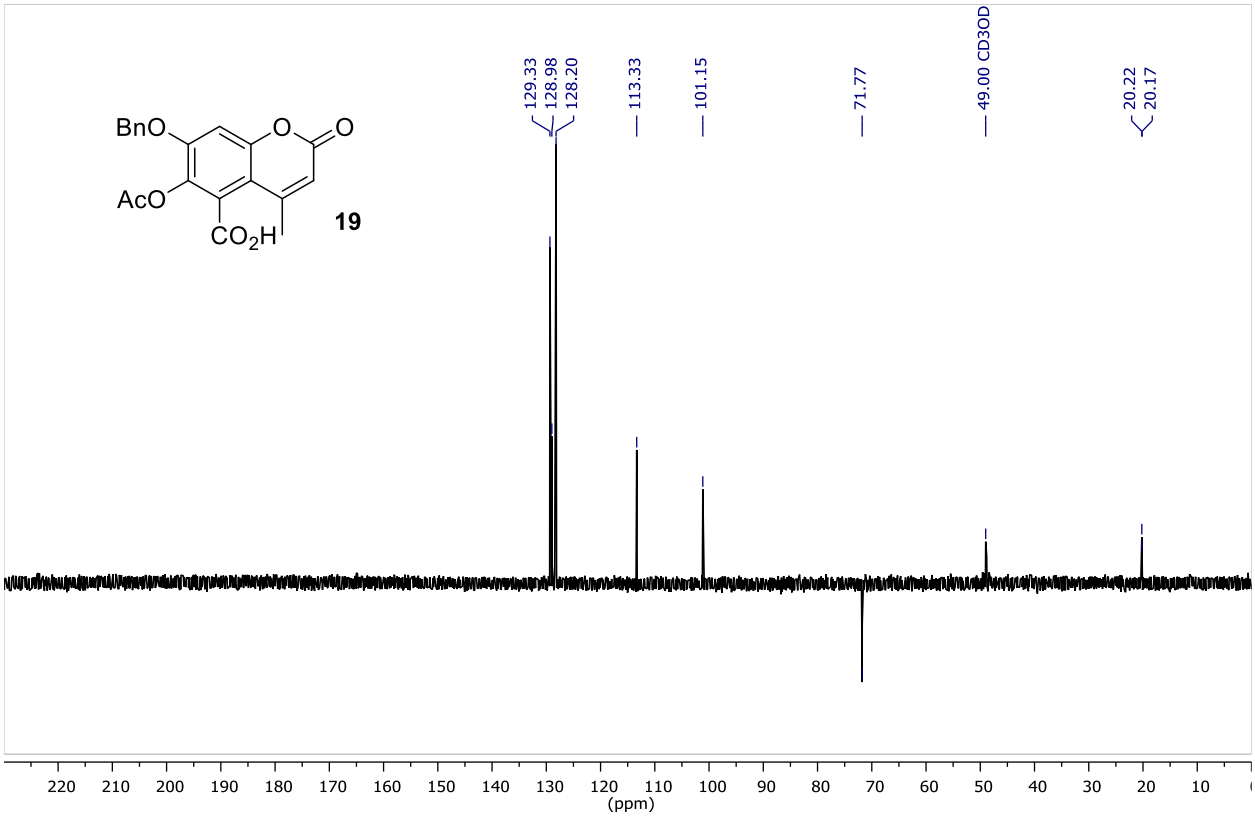


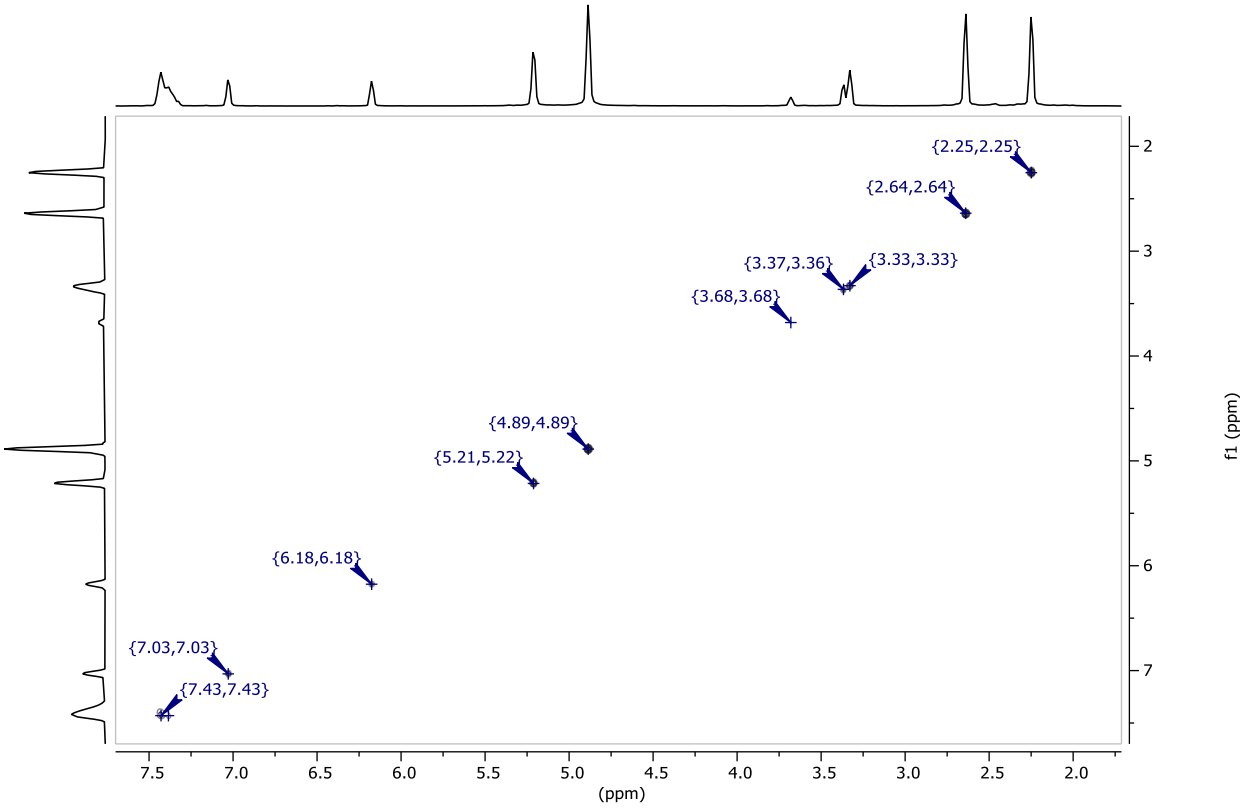
6-Acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (19) (^{1}H and ^{13}C NMR)



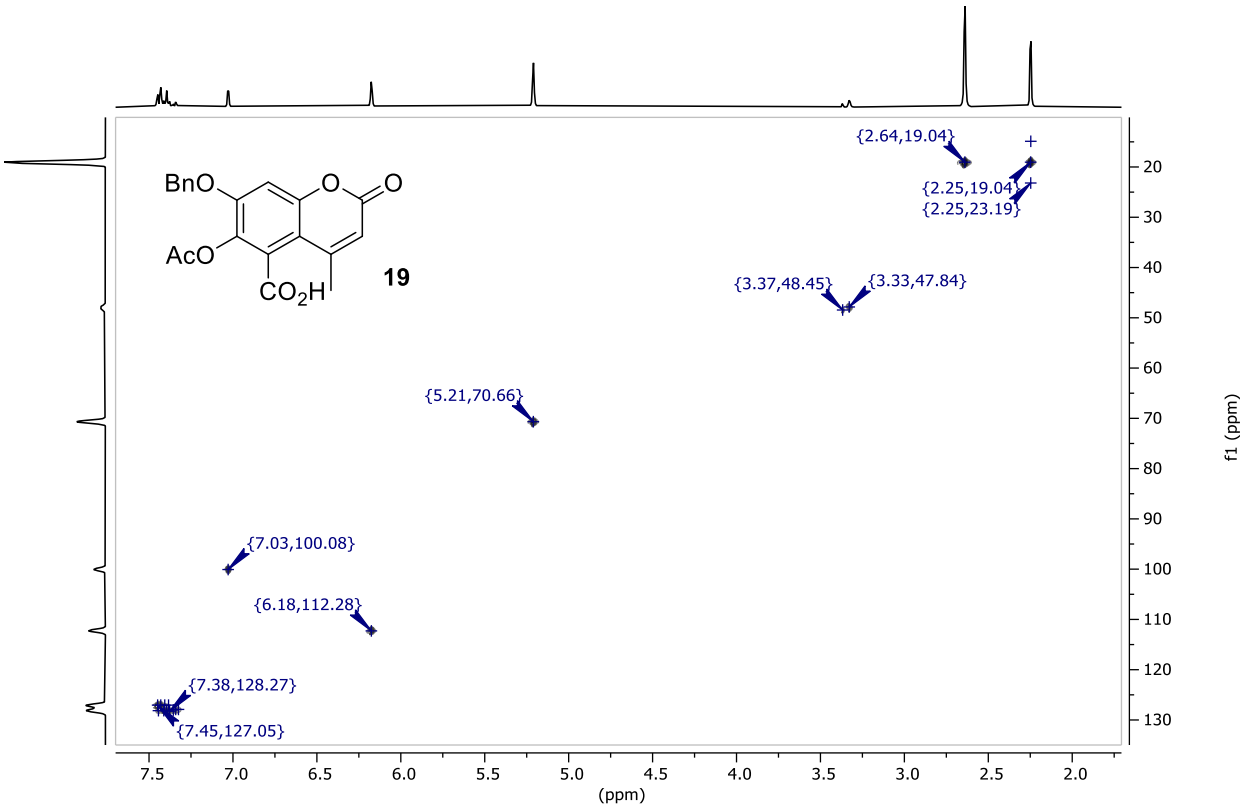


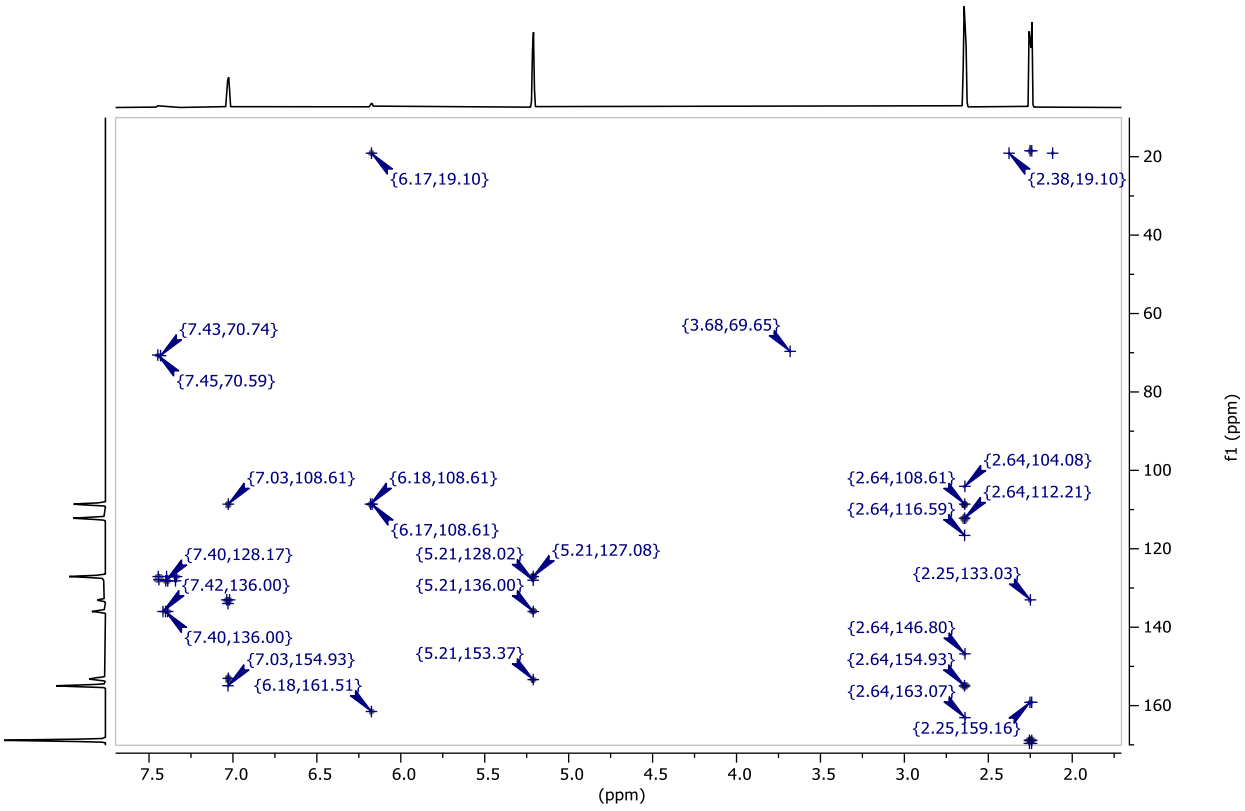
6-Acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (19) (DEPT and COSY NMR)



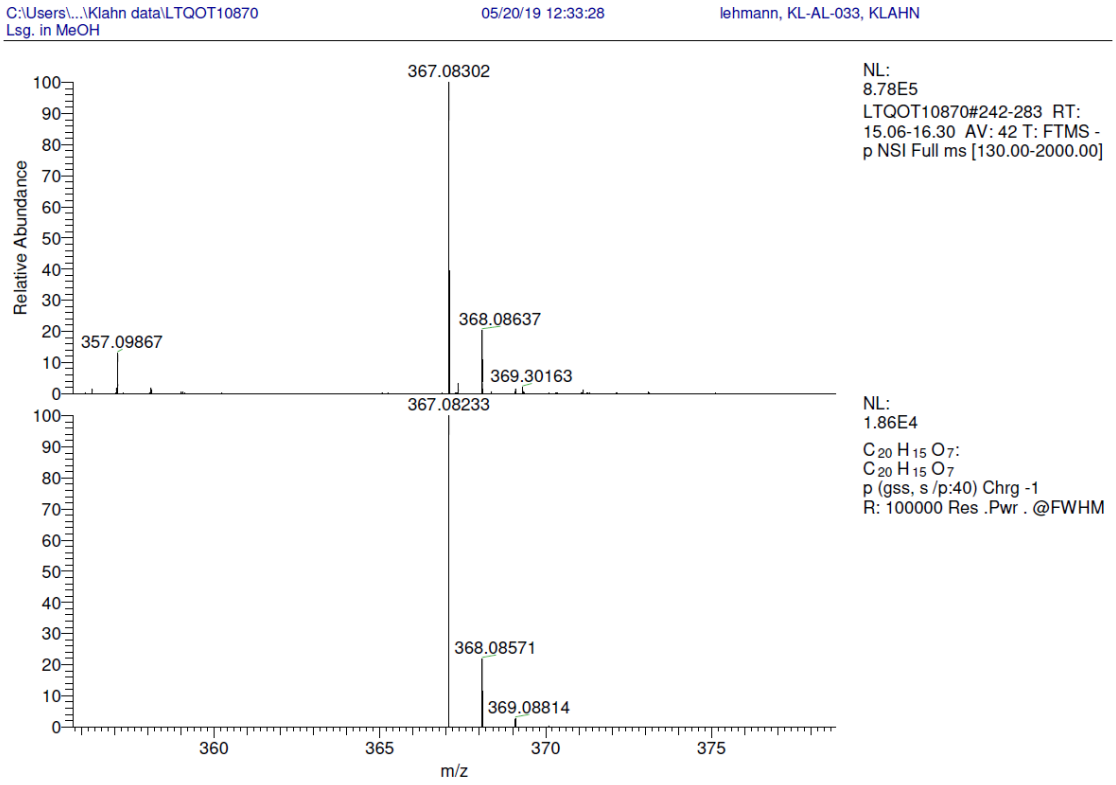


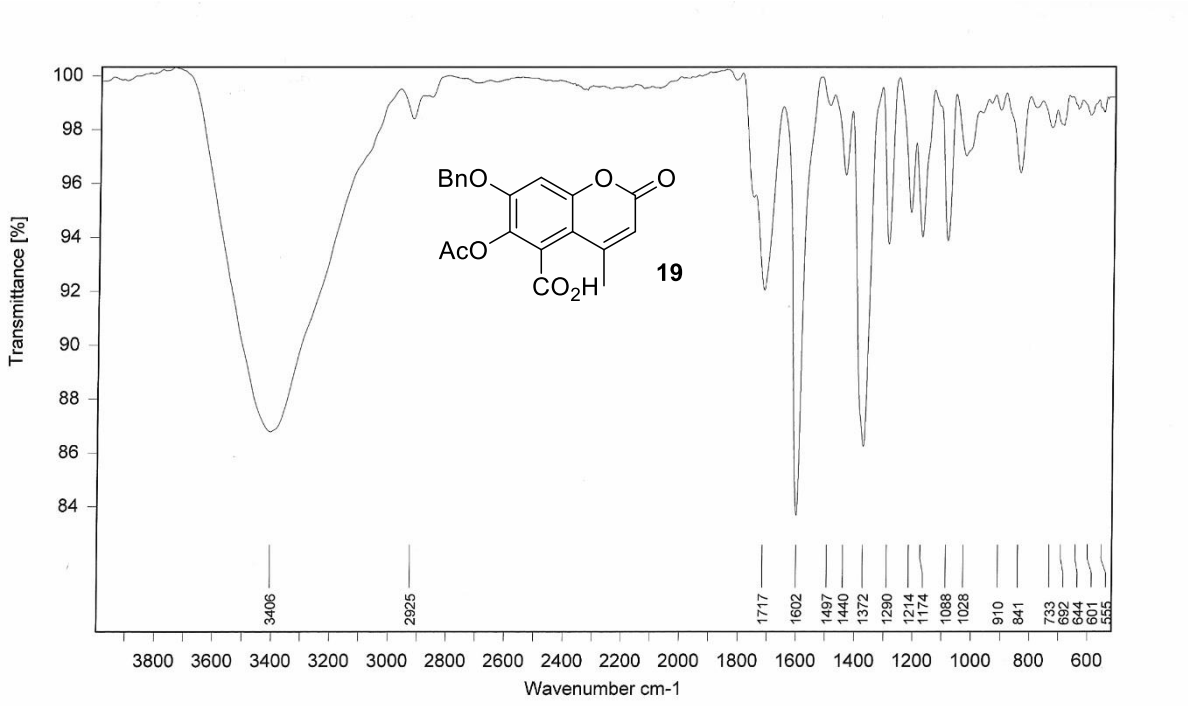
6-Acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (19) (HSQC and HMBC NMR)



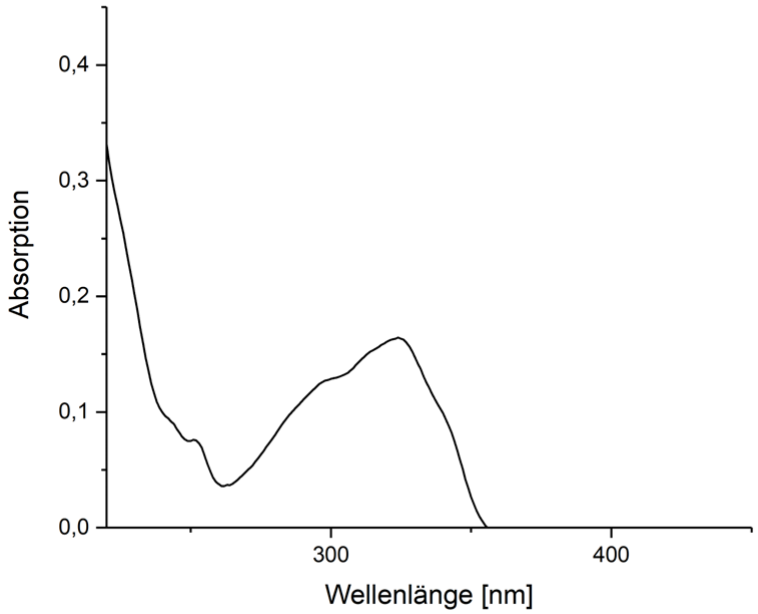


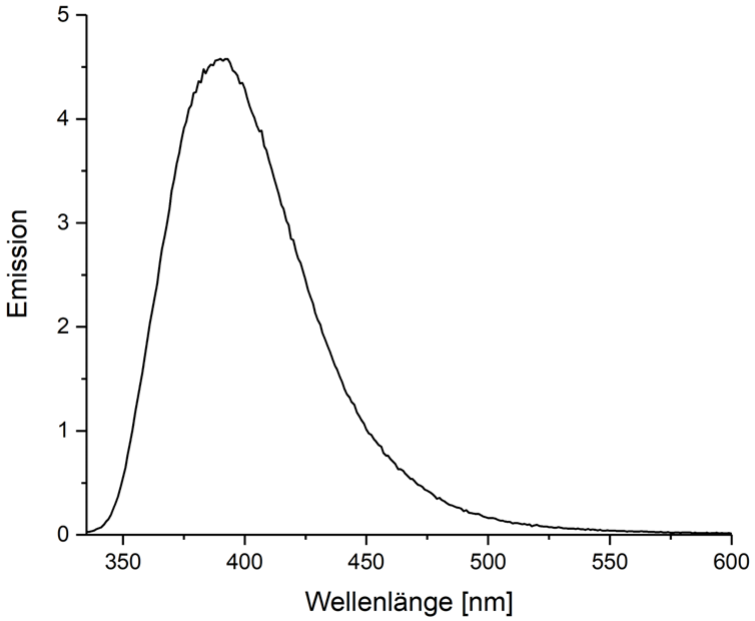
6-Acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (19) (HRMS and ATR-IR)



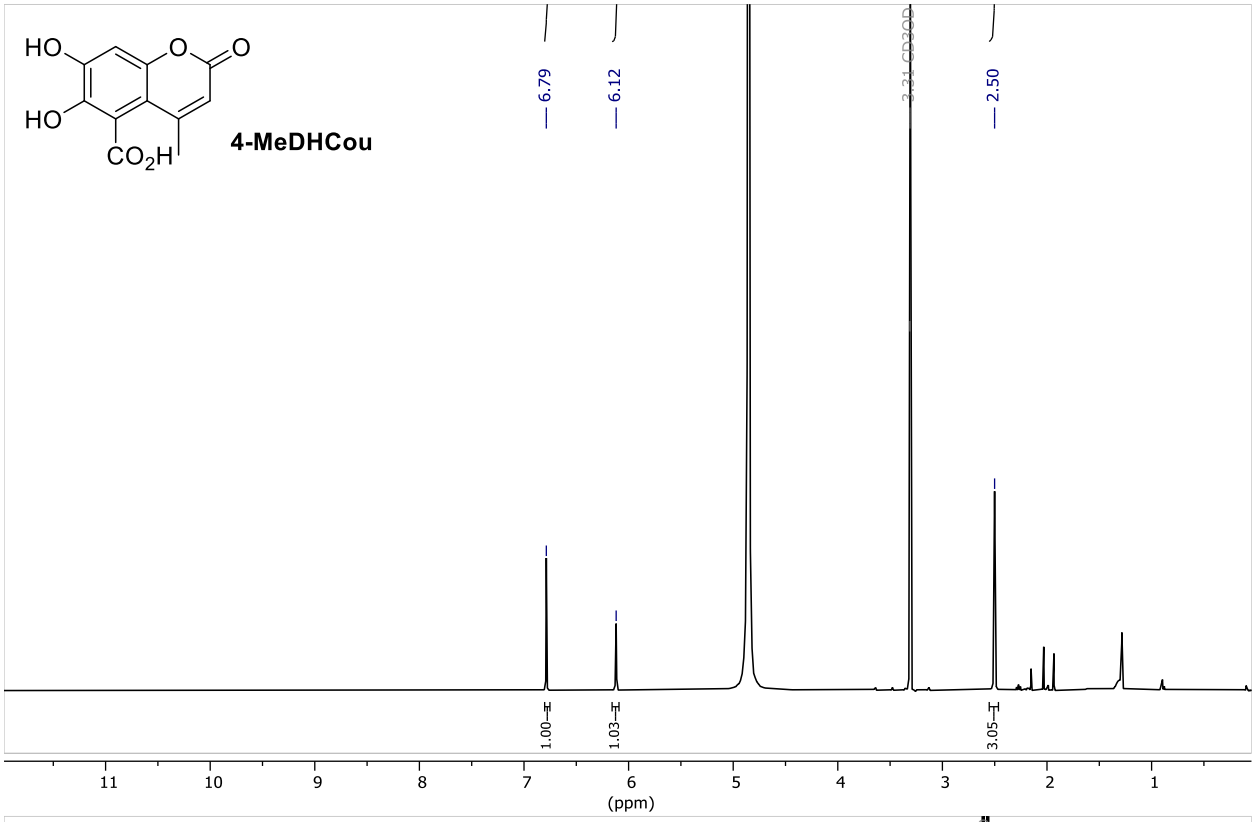


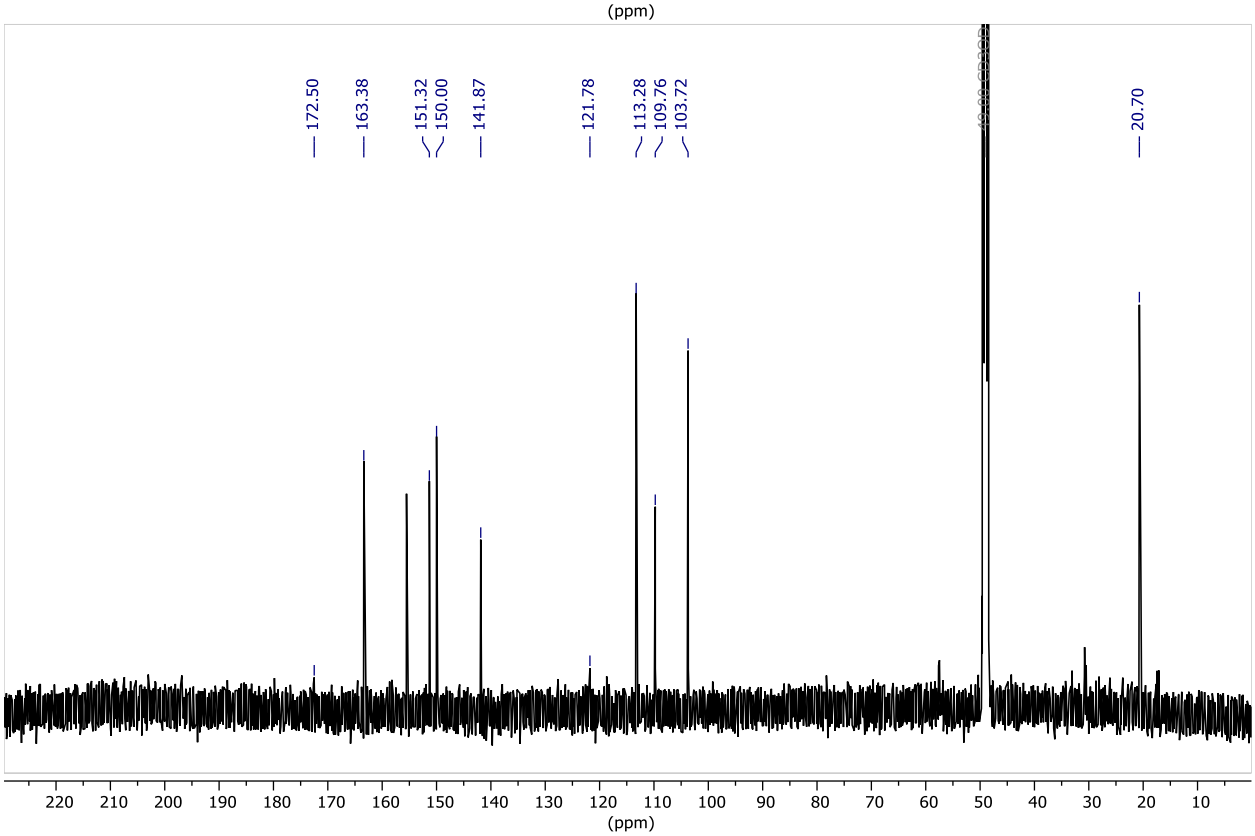
6-Acetoxy-7-(benzyloxy)-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (19) (UV/Vis and Fluorescence emission)



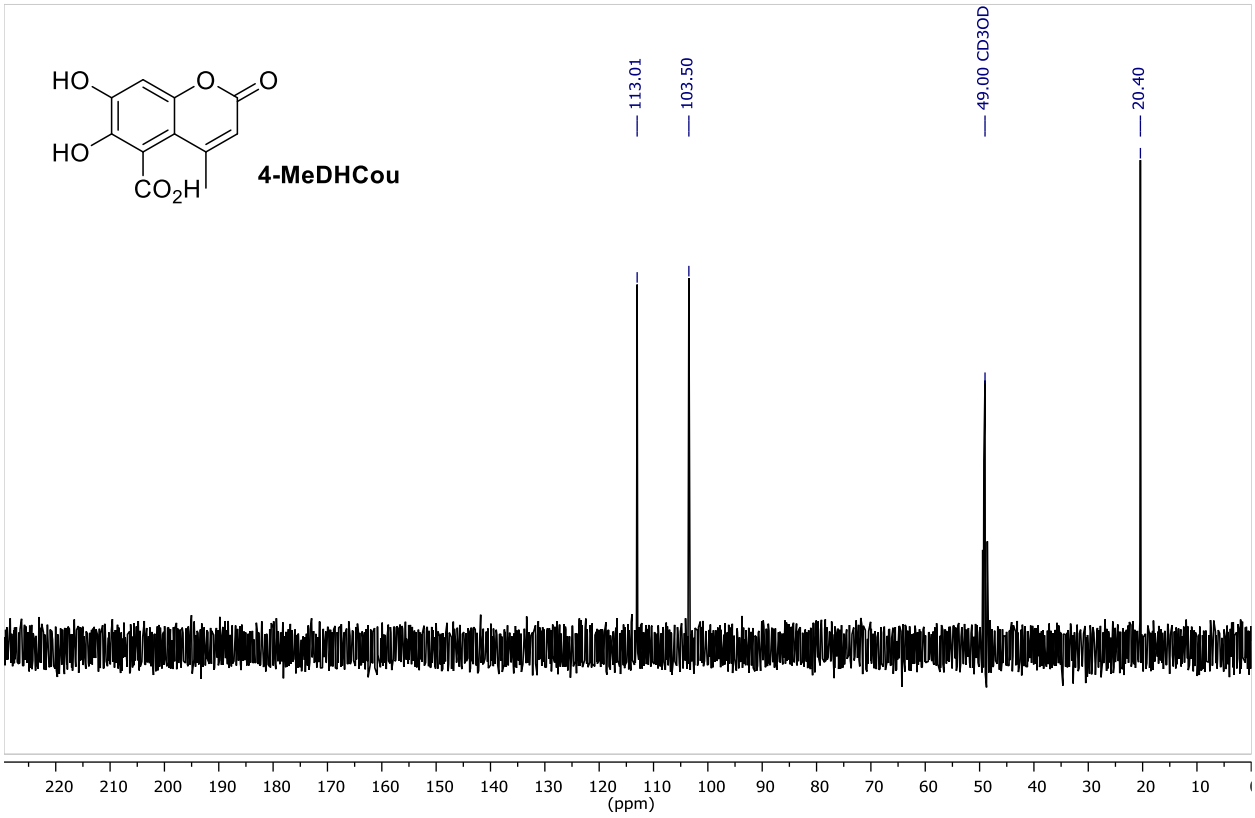


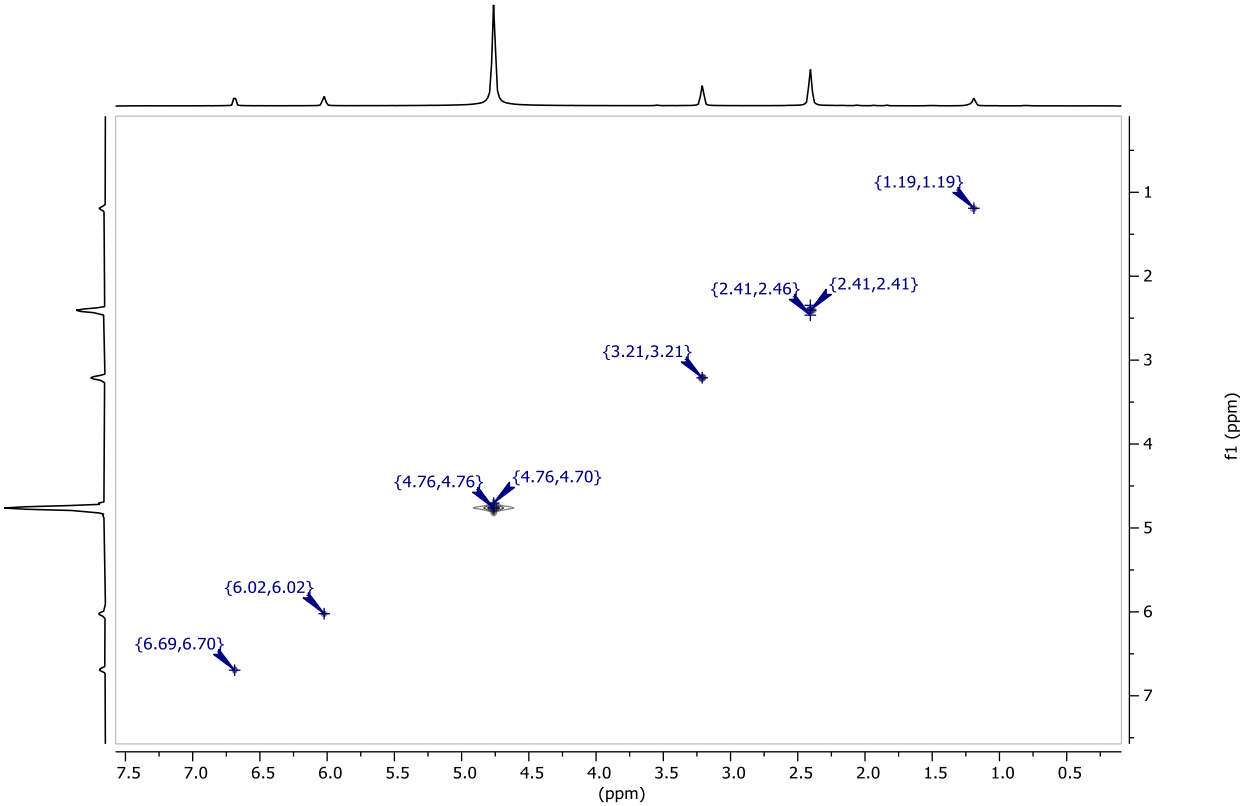
6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (4-MeDHCou) (^{1}H and ^{13}C NMR)



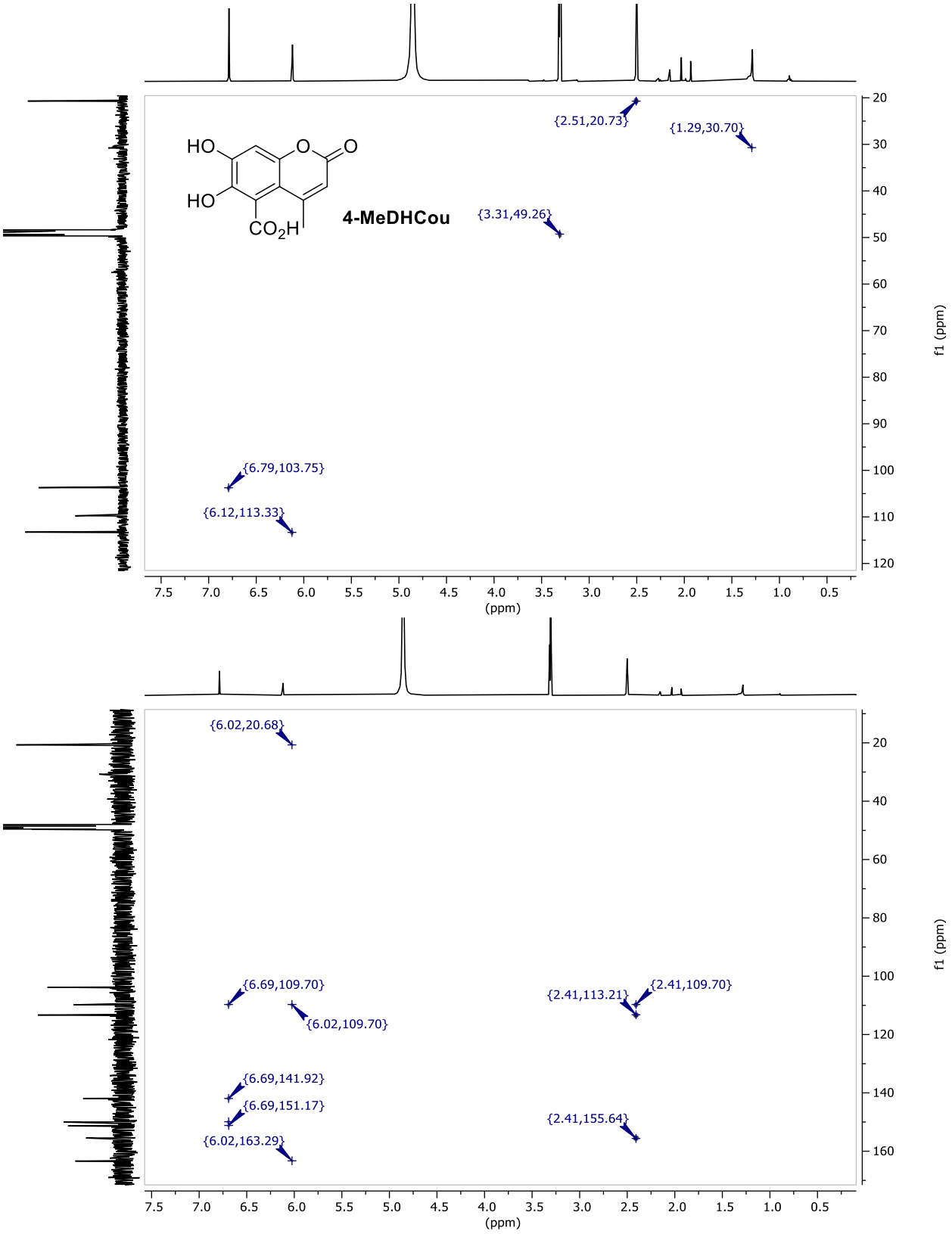


6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (4-MeDHCou) (DEPT and COSY NMR)

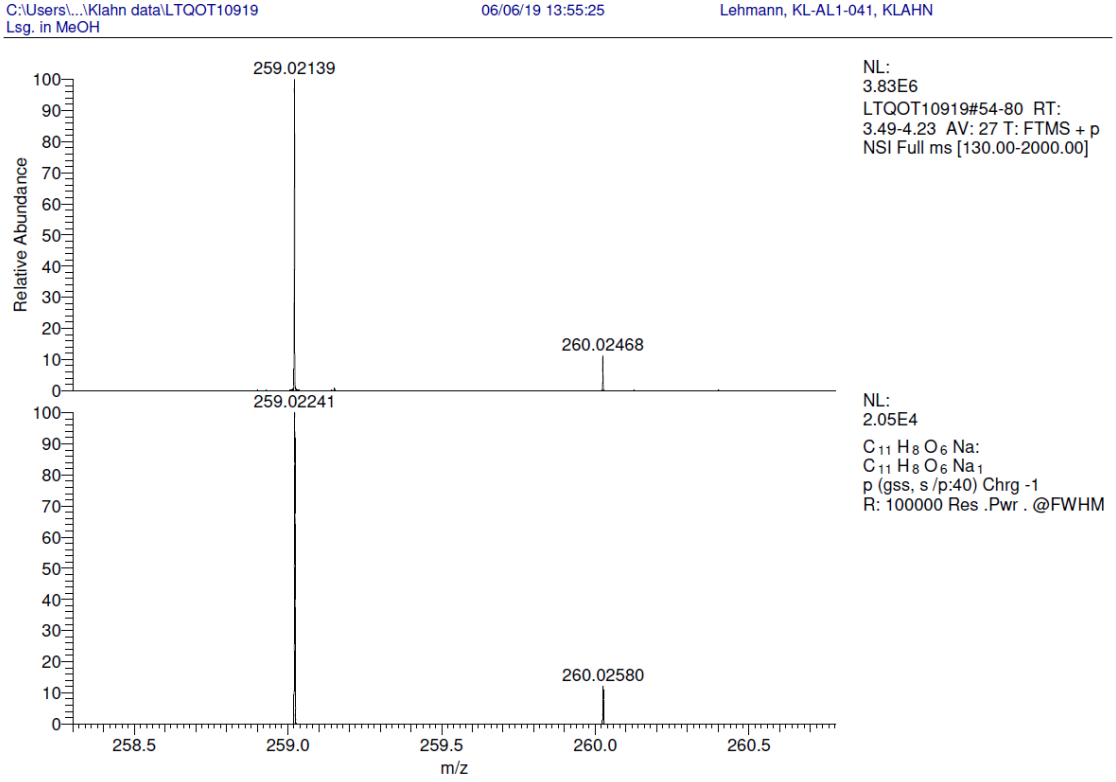


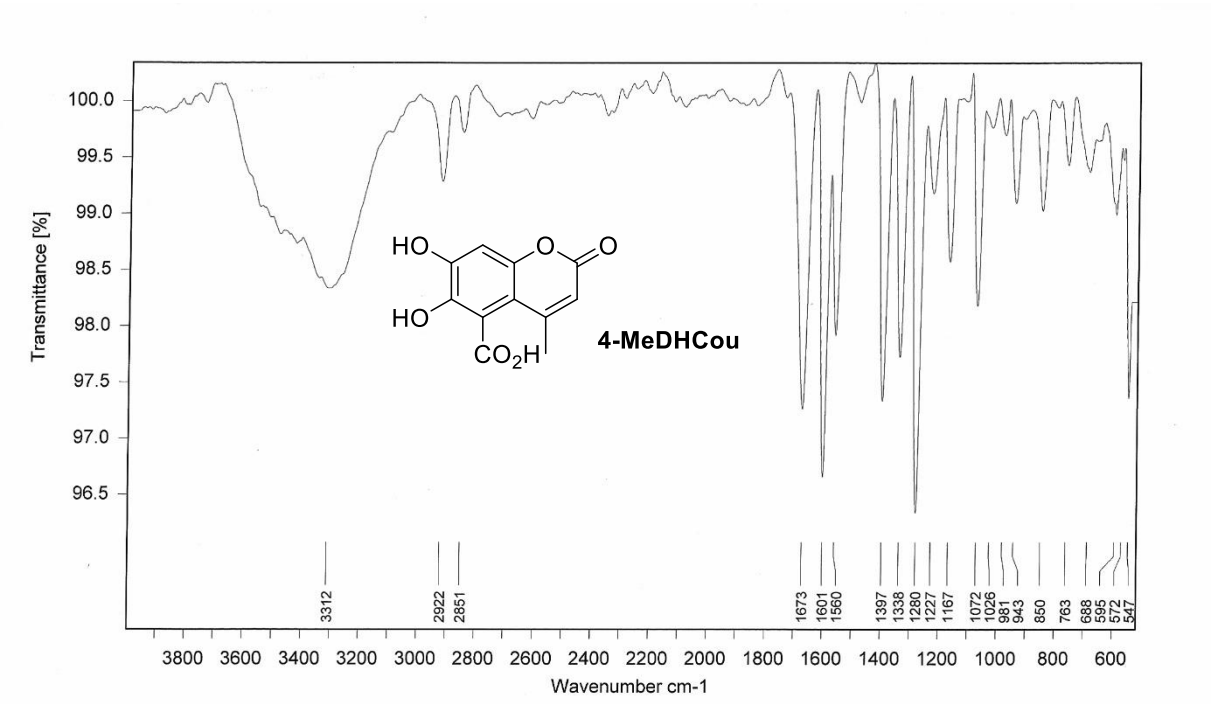


6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (4-MeDHCou) (HSQC and HMBC NMR)



6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (4-MeDHCou) (HRMS and ATR-IR)





6,7-Dihydroxy-4-methyl-2-oxo-2*H*-chromene-5-carboxylic acid (4-MeDHCou) (UV/Vis and Fluorescence emission)