Supporting Information

Fragment-based Design of Selective Nanomolar Ligands of the CREBBP Bromodomain

Andrea Unzue,[†] Min Xu,[‡] Jing Dong,[‡] Lars Wiedmer,[‡] Dimitrios Spiliotopoulos,[‡] Amedeo Caflisch[‡,*] and Cristina Nevado[†,*]

[†] Department of Chemistry and [‡] Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland

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*To whom correspondence should be addressed. Phone: (41) 446353945. Fax: (41) 446353948.

E-mail: cristina.nevado@chem.uzh.ch, caflisch@bioc.uzh.ch

† Dept. of Chemistry

‡ Dept. of Biochemistry

1. Fragment-based high-throughput docking

The ALTA (anchor-based library tailoring) procedure was used for fragment-based high-throughput docking (Figure S1). The details of the procedure are presented in the preceding paper.

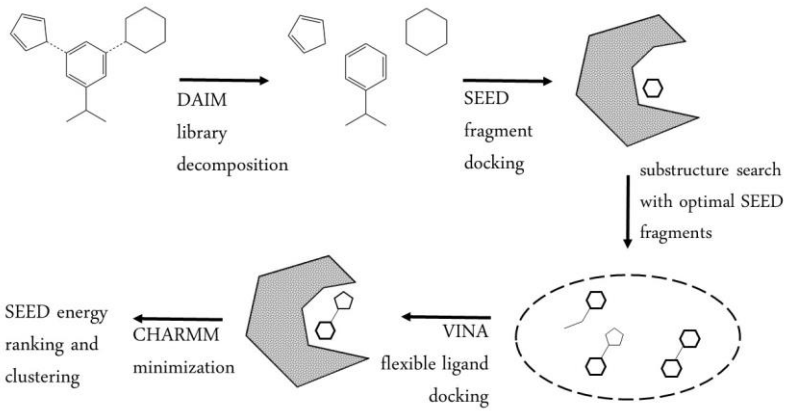


Figure S1. Application of the ALTA (anchor-based library tailoring) procedure[1-2] to the CREBBP bromodomain. The program DAIM automatically decomposes molecules into fragments by cutting at rotatable bonds.[3] CHARMM atom types and non-bonding parameters used in SEED[4-6] were generated by MATCH.[7] The program for fragment docking (called SEED[4-6]) requires about 5 seconds per fragment on a single core of an i7 CPU at 2.8 GHz. CHARMM minimization of the fragment with rigid CREBBP took about 2 seconds per fragment.

2. Finite-difference Poisson calculations

The electrostatic contribution to the binding free energy was evaluated by numerical solution of the Poisson equation using the finite-difference method as implemented in the *PBEQ* module[8] of the program CHARMM.[9] The solute/solvent dielectric discontinuity surface was delimited by the molecular surface spanned by the surface of a rolling probe of 1.4 Å. The dielectric constant of the solute and the solvent were set to 2.0 and 78.5, respectively. The six conserved water molecules were considered explicitly as part of the protein, i.e., they were assigned a dielectric constant of 2.0 as for the protein because they are essentially fixed in space and do not contribute to screening.The ionic strength was set to zero, and the temperature was set to 300 K. The size of the initial grid was determined by considering a layer of at least 20 Å around the solute. The partial charges of the solute were distributed on the grid points by the trilinear interpolation algorithm. First the linearized Poisson equation was solved on a grid of 1.0 Å spacing, which was followed by a focused calculation with a grid encompassing all of the solute and a grid spacing of 0.3 Å. For both calculations an iterative procedure (successive over-relaxation) was used. All calculations were carried out independently on the crystal structure (PDB code 4TQN) and the minimized crystal structure. The latter was obtained

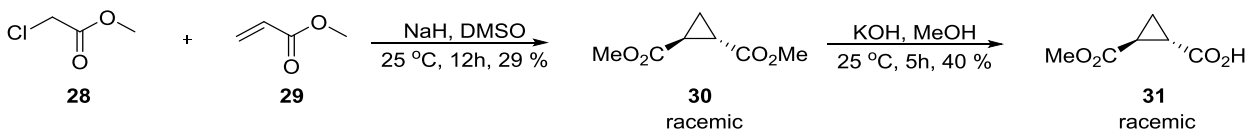
by conjugate gradient minimization with the CHARMM program (version 38b1), and the CHARMM param36 force field for CREBBP and the CHARMM general force field (which is compatible with CHARMM param36) for compound **6**.

3. Synthetic methods

All reactions, unless otherwise stated, were carried out under inert gas atmosphere using standard Schlenk-techniques. All reagents were used as received unless otherwise noted. Solvents were purchased in the best quality available, degassed by purging thoroughly with nitrogen and dried over activated molecular sieves of appropriate size. Alternatively, they were purged with argon and passed through alumina columns in a solvent purification system (Innovative Technology). Reactions were monitored by thin layer chromatography (TLC) using Merck TLC silica gel 60 F_{254}. Flash column chromatography was performed over silica gel (230-400 mesh). NMR spectra were recorded on AV 300, AV2 400 or AV2 500 MHz Bruker spectrometers. Chemical shifts are given in ppm. The spectra are calibrated to the residual ^{1}H and ^{13}C signals of the solvents. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), doublet-doublet (dd), quintet (quint), septet (sept), multiplet (m), and broad (br). Melting points were determined on a Mettler Toledo MP70 melting point instrument. High-resolution electrospray ionization mass spectrometry was performed on a Finnigan MAT 900 (Thermo Finnigan, San Jose, CA, USA) double-focusing magnetic sector mass spectrometer. Ten spectra were acquired. A mass accuracy ≤2 ppm was obtained in the peak matching acquisition mode by using a solution containing 2 µL PEG200, 2 µL PPG450, and 1.5 mg NaOAc (all obtained from Sigma-Aldrich, Buchs, Switzerland) dissolved in 100 mL MeOH (HPLC Supra grade, Scharlau, E-Barcelona) as internal standard. The purity of all tested compounds was determined by HPLC on a Waters Acquity UPLC (Waters, Milford, MA) Top spectrometer using an Acquity BEH C18 HPLC column (1.7 µm, 1× 50 mm, Waters) with a mixture of H_2O + 0.1% HCOOH (A) and CH_3CN + 0.1% HCOOH (B) solvent (0.1 mL flow rate, linear gradient from 5% to 98% B within 4 min followed by flushing with 98% B for 1 min). Unless otherwise stated, all compounds showed ≥ 95 % purity.

The following compounds were prepared according to previously reported procedures: **30**,[10] **31**,[11] **33**,[12] **38**,[13] **39**,[14] **40**,[14] **42**,[15] **44**,[16] **46**,[17] **50**[18] and **51**.[19]

3.1 Synthesis of non-commercially available carboxylic acids (31-51)



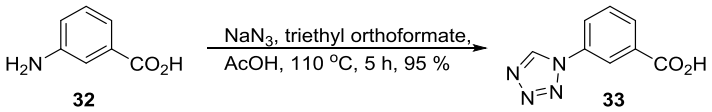
28 **29** **30** racemic **31** racemic

Dimethyl cyclopropane-*trans*-1,2-dicarboxylate (30)[10]

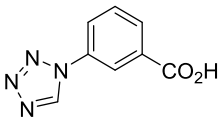
Colourless oil; Yield: 29 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.70 (s, 6H), 2.22 – 2.12 (m, 2H), 1.48 – 1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.2, 52.1, 22.2, 15.3; IR (neat): ῡ = 2955, 1724, 1437, 1399, 1332, 1270, 1197, 1169, 1026, 904, 839, 753, 661, 444, 419, 407 cm^{-1}; MS (ESI), *m/z*: calcd for $C_7H_{10}NaO_4^+$, 181.1; found, 180.9.

2-(Methoxycarbonyl)cyclopropane-1-carboxylic acid (31)[11]

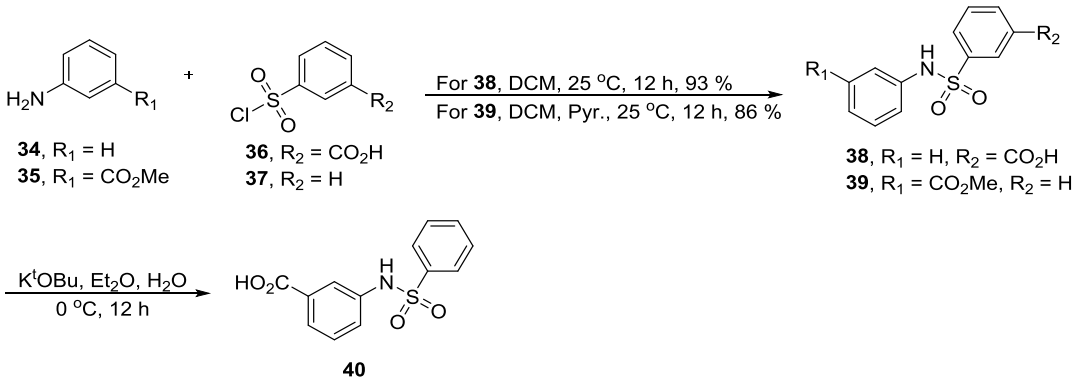
Colourless oil; Yield: 40 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.72 (s, 3H), 2.27 – 2.22 (m, 1H), 2.21 – 2.16 (m, 1H), 1.50 (tdd, *J* = 9.9, 6.0, 4.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.5, 171.8, 52.3, 22.7, 22.0, 15.8; IR (neat): ῡ = 2958, 1704, 1438, 1309, 1176, 913, 743, 415 cm^{-1}; MS (ESI), *m/z*: calcd for $C_6H_7O_4^-$, $[M-H]^-$, 143.0; found, 142.9.



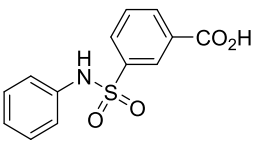
3-(1*H*-Tetrazol-1-yl)benzoic acid (33)[12]



White solid; Yield: 95 %; mp 174-176 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.48 (br, 1H), 10.21 (s, 1H), 8.42 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.1, 142.4, 134.0, 132.7, 130.6, 130.2, 125.2, 121.6; IR (neat): ῡ = 3454, 3397, 3137, 2898, 2788, 2621, 2518, 1704, 1636, 1592, 1500, 1479, 1453, 1289, 1277, 1217, 1195, 1103, 1063, 900, 816, 758, 707 cm^{-1}; MS (ESI), *m/z*: calcd for $C_8H_5N_4O_2^-$, $[M-H]^-$, 189.0; found, 188.8.



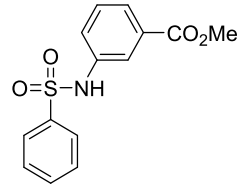
3-(*N*-Phenylsulfamoyl)benzoic acid (38)[13]



White solid; Yield: 93 %; mp 193-195 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.36 (s, 1H), 8.29 (t, *J* = 1.7 Hz, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 7.9 Hz, 2H), 7.11 – 7.01 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.9, 140.0, 137.3, 133.3, 131.8, 130.6, 129.9, 129.2, 127.3, 124.5, 120.5; IR (neat): ῡ = 3246, 2980, 2868, 1686, 1598, 1493, 1444, 1408, 1339, 1294, 1218,

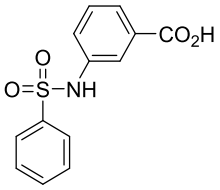
1207, 1173, 1159, 1135, 1075, 1025, 928, 899, 854, 823, 749, 735 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{13}H_{10}NO_4S^-$, $[M-H]^-$, 276.0; found, 275.9.

Methyl 3-(phenylsulfonamido)benzoate (39)[14]

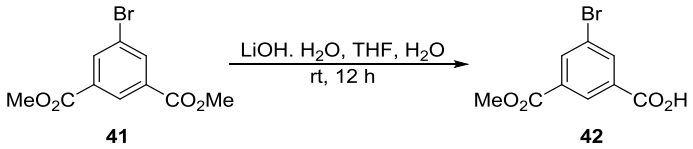


White solid; Yield: 86 %; mp 161-163 °C; ^1H NMR (400 MHz, DMSO-d_6): δ = 10.55 (s, 1H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.70 (s, 1H), 7.61 (d, *J* = 7.0 Hz, 2H), 7.57 – 7.54 (m, 2H), 7.42 – 7.34 (m, 2H), 3.81 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 165.6, 139.2, 138.2, 133.1, 130.5, 129.8, 129.4, 126.6, 124.7, 124.4, 120.3, 52.3; IR (neat): ῡ = 3412, 3227, 3070, 1701, 1605, 1591, 1474, 1446, 1438, 1401, 1337, 1296, 1216, 1175, 1156, 1122, 1114, 1088, 1000, 984, 948, 901, 853, 752, 714 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{14}H_{13}NNaO_4S^+$, 314.1; found, 314.0.

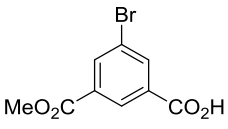
3-(Phenylsulfonamido)benzoic acid (40)[14]



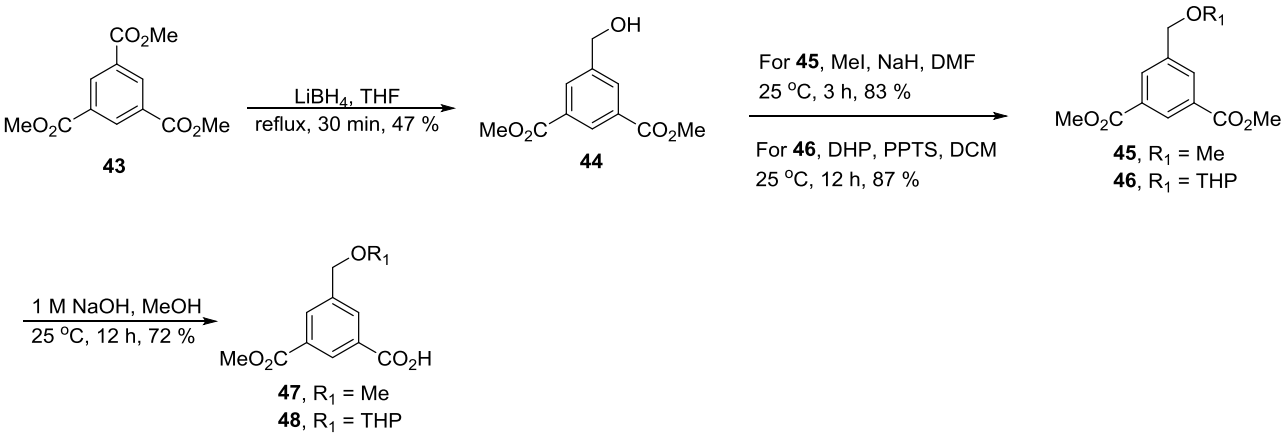
Acid **40** was prepared using the reported procedure and used without further purification. The presence of the acid was confirmed by IR and MS. IR (neat): ῡ = 3250, 3064, 2963, 2825, 1682, 1587, 1447, 1424, 1405, 1333, 1297, 1263, 1158, 1089, 948, 906, 882, 823, 762, 749, 722 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{13}H_{10}NO_4S^-$, $[M-H]^-$, 276.0; found, 276.0.



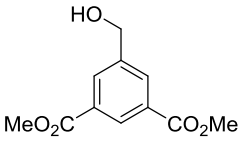
3-Bromo-5-(methoxycarbonyl)benzoic acid (42)[15]



Acid **42** was prepared using the reported procedure and used without further purification. The presence of the acid was confirmed by IR and MS. IR (neat): ῡ = 3079, 2859, 2647, 2522, 1684, 1600, 1574, 1540, 1448, 1391, 1267, 1199, 1147, 905, 797, 754, 727, 712 cm^{-1}; MS (ESI), *m/z*: calcd for $C_9H_6BrO_4^-$, $[M-H]^-$, 256.9; found, 256.8.

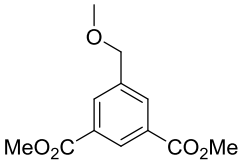


Dimethyl 5-(hydroxymethyl)isophthalate (44)[16]



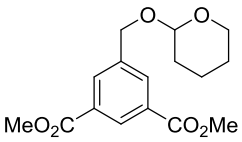
White solid; Yield: 47 %; mp 106-108 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.60 (s, 1H), 8.31 – 8.15 (m, 2H), 4.81 (s, 2H), 3.95 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 166.2, 141.9, 132.0, 130.9, 129.8, 64.2, 52.4; IR (neat): ῡ = 3490, 2954, 1719, 1603, 1437, 1247, 1183, 1107, 1002, 969, 921, 888, 788, 749, 719 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{11}H_{12}NaO_5^+$, 247.1; found, 246.9.

Dimethyl 5-(methoxymethyl)isophthalate (45)



To a solution of dimethyl 5-(hydroxymethyl)isophthalate (**44**, 150 mg, 0.669 mmol) in DMF (1.50 mL) NaH (53.5 mg, 1.34 mmol) was added and stirred at 25 °C for 30 minutes. Methyl iodide (83.3 µL, 1.34 mmol) was added and the reaction mixture was stirred for 3 hours at 25 °C. The reaction was quenched by addition of 1 M HCl solution and extracted with EtOAc three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was then purified by flash column chromatography (hexane: EtOAc, 3:1) affording the desired product in pure form as a white solid (133 mg, 0.558 mmol, 83 % yield). mp 83-85 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.60 (s, 1H), 8.28 – 8.13 (m, 2H), 4.54 (s, 2H), 3.95 (s, 6H), 3.42 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.2, 139.4, 132.8 130.8, 130.0, 73.6, 58.5, 52.4; IR (neat): ῡ = 3490, 2954, 1719, 1603, 1437, 1247, 1183, 1107, 1002, 969, 921, 888, 788, 749, 719 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{12}H_{14}NaO_5^+$, 261.1; found, 261.0.

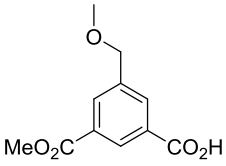
Dimethyl 5-((tetrahydro-2*H*-pyran-2-yl)methyl)isophthalate (46)[17]



White solid; Yield: 87 %; mp 45-48 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (t, *J* = 1.6 Hz, 1H), 8.25 – 8.19 (m, 2H), 4.85 (dd, *J* = 12.4, 0.4 Hz, 1H), 4.73 (t, *J* = 3.5 Hz, 1H), 4.57 (d, *J* = 12.4 Hz, 1H), 3.94 (s, 6H), 3.93 – 3.85 (m, 1H), 3.60 – 3.51 (m, 1H), 1.93 – 1.81 (m, 1H), 1.81 – 1.51 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 166.2, 139.5, 132.9, 130.7, 129.8, 98.2, 67.9, 62.2, 52.3, 30.4, 25.4, 19.2; IR (neat): ῡ = 2952, 2927,

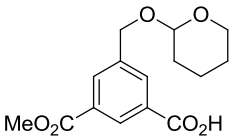
2882, 2846, 1719, 1603, 1453, 1434, 1331, 1318, 1242, 1214, 1203, 1108, 1055, 1015, 965, 911, 869, 811, 751, 720, 712 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{16}H_{20}NaO_6^+$, 331.1; found, 331.1.

3-(Methoxycarbonyl)-5-(methoxymethyl)benzoic acid (47)[20]

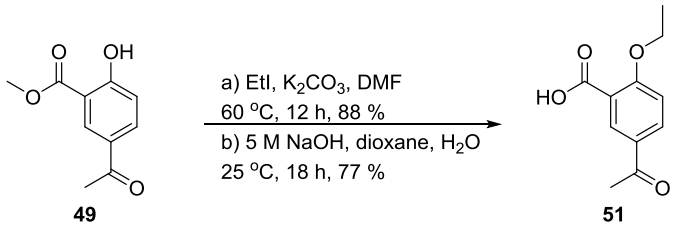


Acid **47** was prepared using the reported procedure and used without further purification. The presence of the acid was confirmed by IR and MS. IR (neat): ῡ = 2918, 2861, 2824, 2626, 1694, 1605, 1458, 1440, 1417, 1314, 1263, 1209, 1115, 1103, 1003, 968, 922, 751, 704 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{11}H_{11}O_5^-$, $[M-H]^-$, 223.1; found, 222.9.

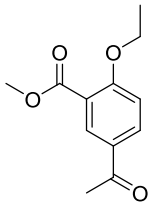
3-(Methoxycarbonyl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)benzoic acid (48)



To a solution of dimethyl 5-((tetrahydro-2*H*-pyran-2-yl)methyl)isophthalate (**46**, 150 mg, 0.486 mmol) 1M NaOH solution (0.486 mL, 0.486 mmol) was added and stirred at 25 °C for 12 hours. The pH of the reaction mixture was then adjusted to 3-4 by adding 10 % citric acid solution and extracted with EtOAc three times and brine. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was then purified by flash column chromatography (5 % MeOH in DCM) affording the desired product in pure form as a white solid (102 mg, 0.347 mmol, 72 %). mp 87-93 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.92 (br, 1H), 10.92 (s, 1H), 8.30 (s, 1H), 8.28 (s, 1H), 4.88 (d, *J* = 12.5 Hz, 1H), 4.76 (t, *J* = 3.3 Hz, 1H), 4.60 (d, *J* = 12.5 Hz, 1H), 3.95 (s, 3H), 3.94 – 3.87 (m, 1H), 3.63 – 3.53 (m, 1H), 1.88 (dt, *J* = 13.0, 4.3 Hz, 1H), 1.83 – 1.49 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 166.1, 139.7, 133.7, 133.4, 130.9, 130.5, 130.0, 98.2, 67.9, 62.2, 52.4, 30.4, 25.3, 19.2; IR (neat): ῡ = 2943, 2873, 1727, 1687, 1606, 1458, 1436, 1421, 1354, 1310, 1252, 1202, 1121, 1072, 1034, 1017, 975, 942, 912, 869, 814, 752, 703 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{15}H_{18}NaO_6^+$, 317.0996; found, 317.0994.



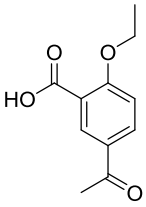
Methyl 5-acetyl-2ethoxybenzoate (50)[18]



White solid; Yield: 88 %; mp 50-53 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.38 (t, *J* = 2.2 Hz, 1H), 8.08 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 4.20 (q, *J* = 7.0 Hz, 2H), 3.91 (s, 3H), 2.57 (d, *J* = 2.0 Hz, 3H), 1.49 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 196.1, 166.0, 162.0, 133.6, 132.6, 129.4, 120.1, 112.6, 64.9, 52.1, 26.3, 14.5; IR (neat): ῡ = 2986, 2947, 1697, 1676, 1597, 1496, 1468, 1439, 1352, 1266,

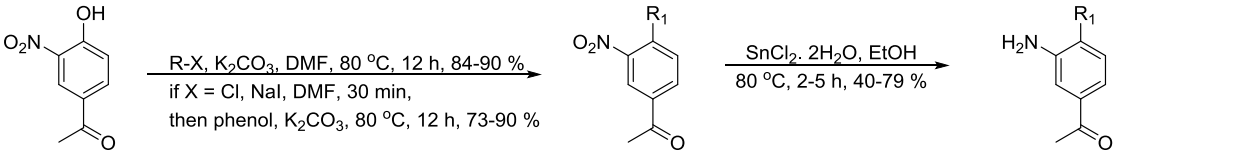
1249, 1233, 1164, 1110, 1101, 1074, 1036, 958, 929, 859, 841, 816, 786, 718 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{12}H_{14}NaO_4^+$, 245.1; found, 244.9.

5-Acetyl-2-ethoxybenzoic acid (51)[19]



Pale yellow solid; Yield: 77 %; mp 118-121 °C; ^{1}H NMR (400 MHz, MeOD): δ = 8.39 (d, *J* = 2.4 Hz, 1H), 8.13 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 1H), 4.24 (q, *J* = 7.0 Hz, 2H), 2.57 (s, 3H), 1.46 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 198.5, 169.0, 163.3, 135.1, 133.4, 130.6, 121.9, 114.0, 66.2, 26.4, 14.8; IR (neat): ῡ = 2989, 2938, 2810, 2615, 1681, 1666, 1598, 1497, 1403, 1354, 1293, 1263, 1224, 1167, 1110, 1076, 1027, 968, 928, 806 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{11}H_{11}NaO_4^+$, 230.1; found, 230.9.

3.2 Synthesis of non-commercially available anilines (60-66)



52

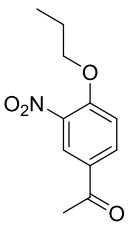
53, R_1 = propoxy
54, R_1 = isobutoxy
55, R_1 = cyclopropylmethoxy
56, R_1 = benzyloxy
57, R_1 = methoxy (commercially available)
58, R_1 = methyl (commercially available)
59, R_1 = fluoro (commercially available)

60, R_1 = propoxy
61, R_1 = isobutoxy
62, R_1 = cyclopropylmethoxy
63, R_1 = benzyloxy
64, R_1 = methoxy
65, R_1 = methyl
66, R_1 = fluoro

General procedure for ether synthesis (53-56)

To a solution of 1-(4-hydroxy-3-nitrophenyl)ethan-1-one (**52**, 1.0 eq) in DMF (0.30 M), the alkyl iodide (1.2 eq) and K_2CO_3 (4.0 eq) were added. The reaction mixture was stirred at 80 ºC for 12 h. A saturated solution of NH_4Cl was added and it was extracted with Et_2O three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (2:1, hexane:EtOAc) affording the desired products in pure form. In the case of alkyl chlorides (products **54** and **55**), the alkyl chloride (2.0 eq) was first stirred in DMF (0.30 M) in the presence of NaI (2.0 eq) for 30 min. The phenol (1.0 eq) and K_2CO_3 (4.0 eq) were then added and the same procedure as the one described above was followed.

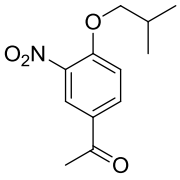
1-(3-Nitro-4-propoxyphenyl)ethan-1-one (53)



White solid; Yield: 84 %; mp 57-60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (d, *J* = 2.1 Hz, 1H), 8.13 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 4.15 (t, *J* = 6.4 Hz, 2H), 2.59 (s, 3H), 2.17 – 1.53 (m, 2H), 1.08 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 194.8, 155.8, 139.5, 133.8, 129.3, 126.1, 114.0, 71.6, 26.3, 22.2, 10.3; IR (neat): ῡ =

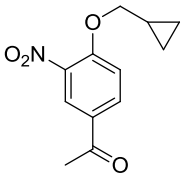
2981, 2972, 2927, 2884, 1666, 1608, 1524, 1499, 1462, 1399, 1356, 1282, 1258, 1156, 1077, 1056, 965, 907, 899, 828, 774 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{11}H_{14}NO_4^+$, 224.0917; found, 224.0918.

1-(4-Isobutoxy-3-nitrophenyl)ethan-1-one (54)



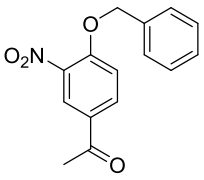
Pale yellow solid; Yield: 90 %; mp 61-63 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.41 (d, *J* = 2.2 Hz, 1H), 8.13 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 3.94 (d, *J* = 6.4 Hz, 2H), 2.59 (s, 3H), 2.17 (dp, *J* = 13.3, 6.6 Hz, 1H), 1.07 (s, 3H), 1.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 194.8, 155.9, 139.3, 133.8, 129.2, 126.2, 113.9, 76.1, 28.2, 26.3, 19.0; IR (neat): ῡ = 2966, 2876, 1677, 1609, 1568, 1531, 1470, 1419, 1355, 1273, 1235, 1166, 1066, 1004, 988, 976, 957, 910, 824, 815, 762, 739 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{16}NO_4^+$, 238.1074; found, 238.1077.

1-(4-(Cyclopropylmethoxy)-3-nitrophenyl)ethan-1-one (55)



Yellow solid; Yield: 73 %; mp 74-77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (d, *J* = 2.2 Hz, 1H), 8.12 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 4.06 (d, *J* = 6.8 Hz, 2H), 2.59 (s, 3H), 1.39 – 1.25 (m, 1H), 0.73 – 0.66 (m, 2H), 0.45 – 0.39 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 194.8, 155.7, 139.6, 133.7, 129.4, 126.1, 114.3, 74.6, 26.3, 9.8, 3.4; IR (neat): ῡ = 3275, 3092, 3011, 1673, 1607, 1566, 1527, 1497, 1412, 1360, 1271, 1171, 1066, 1024, 977, 911, 886, 835, 826, 809 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{14}NO_4^+$, 236.0917; found, 236.0918.

1-(4-(Benzyloxy)-3-nitrophenyl)ethan-1-one (56)

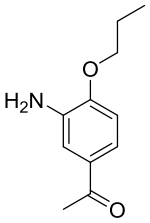


Pale yellow solid; Yield: 90 %; mp 132-135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.42 (d, *J* = 2.2 Hz, 1H), 8.11 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.45-7.32 (m, 5H), 7.18 (d, *J* = 8.8 Hz, 1H), 5.31 (s, 2H), 2.58 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 194.7, 155.1, 139.7, 134.7, 133.7, 129.8, 128.8, 128.5, 126.9, 126.1, 114.6, 71.4, 26.3; IR (neat): ῡ = 2923, 1679, 1611, 1569, 1531, 1493, 1417, 1346, 1267, 1237, 1178, 1066, 1018, 979, 911, 890, 828, 732 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{15}H_{14}NO_4^+$, 272.0917; found, 272.0917.

General procedure for the reduction of nitro phenyls (60-66)

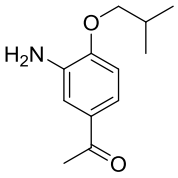
To a mixture of nitrophenyl (1 eq) in EtOH (0.3 M) $SnCl_2.2H_2O$ (4 eq) was added. The reaction mixture was heated to 80 °C for 2-5 h, cooled, and concentrated under reduced pressure. The pH was basified to pH 5 by the addition of a 5 M NaOH solution. The resulting precipitate was filtered off and washed with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (hex: EtOAc, 3:1-1:1) affording the desired anilines in pure form.

1-(3-Amino-4-propoxyphenyl)ethan-1-one (60)



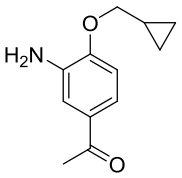
Brown solid; Yield: 40%; mp 53-55 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 – 7.37 (m, 2H), 6.79 (d, *J* = 8.1 Hz, 1H), 4.03 (t, *J* = 6.5 Hz, 2H), 2.52 (s, 3H), 1.98 – 1.78 (m, 2H), 1.07 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.2, 150.9, 135.8, 130.5, 120.8, 114.3, 110.1, 70.0, 26.3, 22.5, 10.5; IR (neat): ῡ = 3485, 3369, 2961, 2934, 2877, 1671, 1615, 1580, 1513, 1474, 1439, 1350, 1295, 1253, 1219, 1148, 1063, 1039, 1013, 977, 916, 886, 787, 770 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{11}H_{16}NO_2^+$, 194.1176; found, 194.1179.

1-(3-Amino-4-isobutoxyphenyl)ethan-1-one (61)



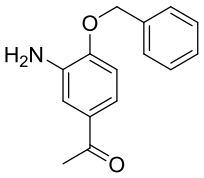
Brown-red oil; Yield: 53 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.34 (m, 3H), 6.77 (d, *J* = 8.3 Hz, 1H), 3.90 (br, 2H), 3.82 (d, *J* = 6.5 Hz, 1H), 2.52 (s, 3H), 2.15 (dp, *J* = 13.3, 6.7 Hz, 1H), 1.06 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.3, 150.7, 136.2, 130.5, 120.4, 113.9, 110.0, 74.7, 28.2, 26.2, 19.2; IR (neat): ῡ = 3472, 3363, 2959, 2928, 2873, 1667, 1613, 1583, 1513, 1470, 1440, 1359, 1296, 1210, 1153, 1064, 1022, 880, 795 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{18}NO_2^+$, 208.1332; found, 208.1334.

1-(3-Amino-4-(cyclopropylmethoxy)phenyl)ethan-1-one (62)



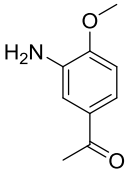
Brown solid; Yield: 61 %; mp 44-46 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 – 7.34 (m, 2H), 6.74 (d, *J* = 8.9 Hz, 1H), 3.94 (br, 2H), 3.90 (d, *J* = 6.9 Hz, 2H), 2.52 (s, 3H), 1.34 – 1.25 (m, 1H), 0.72 – 0.56 (m, 2H), 0.40 – 0.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.3, 150.7, 136.3, 130.6, 120.4, 114.0, 110.2, 73.2, 26.3, 10.2, 3.2; IR (neat): ῡ = 3471, 3359, 3082, 2995, 2925, 1659, 1614, 1582, 1512, 1444, 1409, 1351, 1299, 1250, 1216, 1153, 1058, 1020, 1002, 979, 941, 877, 837, 794 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{16}NO_2^+$, 206.1176; found, 206.1175.

1-(3-Amino-4-(benzyloxy)phenyl)ethan-1-one (63)



Yellow solid; Yield: 53 %; mp 119-122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.44 – 7.34 (m, 7H), 6.87 (d, *J* = 9.0 Hz, 1H), 5.16 (s, 2H), 3.93 (br, 2H), 2.52 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.2, 150.3, 136.4, 136.3, 130.9, 128.7, 128.3, 127.5, 120.3, 114.1, 110.7, 70.5, 26.3; IR (neat): ῡ = 3457, 3355, 2997, 2921, 1659, 1614, 1580, 1510, 1444, 1386, 1358, 1301, 1248, 1211, 1160, 1065, 1023, 997, 981, 927, 891, 857, 792, 749, 705 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{15}H_{16}NO_2^+$, 242.1176; found, 242.1176.

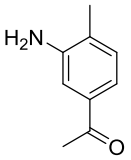
1-(3-Amino-4-methyoxyphenyl)ethan-1-one (64)



Light yellow solid; Yield: 48 %; mp 96-99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.36 (d, *J* = 2.1 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 3.92 (s, 3H),

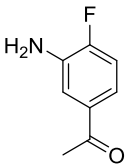
2.53 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 197.3, 151.3, 136.1, 130.7, 120.5, 114.0, 109.2, 55.6, 26.2; IR (neat): ῡ = 3454, 3370, 2935, 1667, 1584, 1514, 1440, 1298, 1219, 905, 729, 648, 406 cm^{-1}; MS (ESI), *m/z*: calcd for $C_9H_{11}NNaO_2^+$, 188.1; found, 187.9.

1-(3-Amino-4-methylphenyl)ethan-1-one (65)

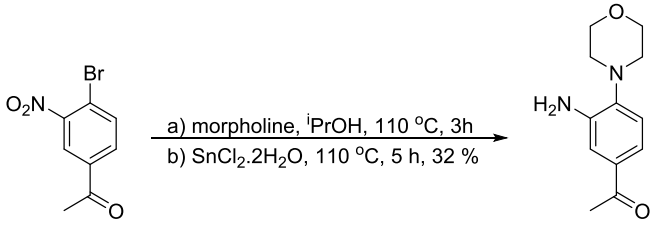


Light brown solid; Yield: 79 %; mp 77-78 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (d, *J* = 1.6 Hz, 1H), 7.28 (s, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 3.77 (br, 2H), 2.54 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.2, 144.8, 136.3 130.4, 128.0, 119.0, 113.8, 26.5, 17.5; IR (neat): ῡ = 3433, 3347, 3229, 1664, 1633, 1602, 1567, 1417, 1357, 1303, 1287, 1237, 1199, 1141, 955, 856, 834 cm^{-1}; MS (ESI), *m/z*: calcd for $C_9H_{12}NO^+$, 150.1; found, 149.9.

1-(3-Amino-4-fluorophenyl)ethan-1-one (66)

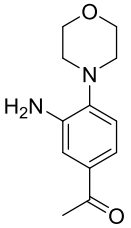


White solid; Yield: 69 %; mp 65-68 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.41 (dd, *J* = 8.7, 2.2 Hz, 1H) 7.31 (ddd, *J* = 8.4, 4.6, 2.2 Hz, 1H), 7.03 (dd, *J* = 10.6, 8.4 Hz, 1H), 3.68 (br, 2H), 2.54 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.0, 154.5 (d, *J* = 247.7 Hz), 134.8 (d, *J* = 13.1 Hz), 134.0 (d, *J* = 3.1 Hz), 119.7 (d, *J* = 7.9 Hz), 116.5 (d, *J* = 5.3 Hz), 115.1 (d, *J* = 19.7 Hz), 26.4; ^{19}F NMR (376 MHz, $CDCl_3$): δ = -127.87; IR (neat): ῡ = 3396, 3325, 3217, 1665, 1607, 1589, 1510, 1422, 1309, 1282, 1244, 1196, 1137, 1099, 1060, 964, 878, 802, 713 cm^{-1}; MS (ESI), *m/z*: calcd for $C_8H_8FNNaO^+$, 176.2; found, 178.8.



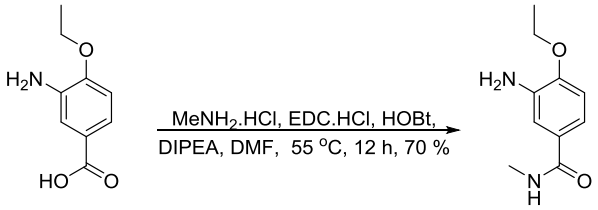
67 **68**

1-(3-Amino-4-morpholinophenyl)ethan-1-one (68)



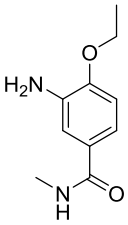
A solution of 1-(4-Bromo-3-nitrophenyl)ethanone (**67**, 500 mg, 2.05 mmol) in isopropanol (5.00 mL) was heated at 110 ºC for 3 h. $SnCl_2.2H_2O$ (1.80 g, 7.98 mmol) was then added and heated at 110 ºC for 5 h. The reaction mixture was concentrated under reduced pressure and the pH was basified to pH 5 by the addition of a 5 M NaOH solution. The resulting precipitate was filtered off and washed with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (hex: EtOAc, 1:1) affording the desired aniline in pure form as a brown solid (145 mg, 0.579 mmol, 32 % yield over two steps). mp 141-144 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.34 (d, *J* = 1.6 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 4.05 (br, 2H), 3.88 – 3.84 (m, 4H), 3.01 – 2.96 (m, 4H), 2.53 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.7, 143.4, 141.0, 133.4, 120.0, 118.7, 114.5, 67.3, 50.7, 26.5; IR (neat): ῡ = 3380, 3311, 2962, 2924, 2824, 1671, 1627, 1591, 1566, 1505, 1445, 1424, 1370, 1363,

1301, 1288, 1255, 1213, 1205, 1106, 1065, 1041, 966, 941, 920, 902, 861, 845, 821, 728 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{17}N_2O_2^+$, 221.1285; found, 221.1283.



69 **70**

3-Amino-4-ethoxy-*N*-methylbenzamide (70)



To a solution of 3-amino-4-ethoxybenzoic acid (**69**, 100 mg, 0.552 mmol) in DMF (1.80 mL), methylamine hydrochloride (150 mg, 2.22 mmol), HOBt (89.4 mg, 0.662 mmol), EDC.HCl (1.104 mmol, 212 mg) and DIPEA (385 µL, 2.21 mmol) were added. The solution was stirred at 55 °C for 12 h, it was concentrated and purified by flash column chromatography (5 % MeOH in DCM) affording the desired amide as a white solid in pure form (75.0 mg, 0.384 mmol, 70 % yield). mp 142-145 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18 (d, *J* = 2.2 Hz, 1H), 7.06 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 5.98 (br, 1H), 4.10 (q, *J* = 7.0 Hz, 2H), 3.90 (br, 2H), 2.98 (d, *J* = 4.9 Hz, 3H), 1.45 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.2, 149.0, 136.3, 127.3, 117.0, 113.6, 110.4, 63.9, 26.7, 14.8; R (neat): ϑ = 3461, 3361, 3282, 2985, 2930, 1610, 1593, 1577, 1550, 1508, 1473, 1390, 1314, 1282, 1223, 1146, 1112, 1040, 886, 823, 777 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{10}H_{15}N_2O_2^+$, 195.1128; found, 195.1129.

3.3 Amide coupling reactions

General procedure A for amide formation

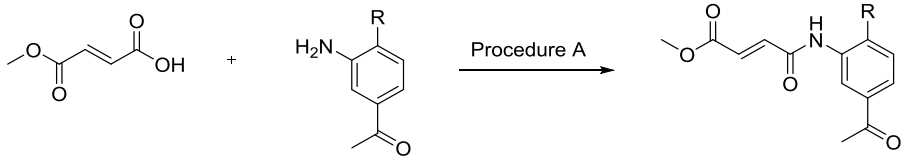
The desired aniline (1.0 eq) and carboxylic acid (1.2 or 1.3 eq) were dissolved in DMF (0.10 M) and EDC.HCl (1.5-2.0 eq), DIPEA (1.5 eq) and HOBt (1.5-2 eq) were added at 25 ºC. In the case of **7**, **16**, **73**, **74**, **78, 90** and **91**, EDC (1.5-2.0 eq) and no DIPEA was used. The reaction mixture was stirred for 12-48 h at 25 ºC, it was concentrated and redissolved in EtOAc. The organic phase was extracted with saturated $NaHCO_3$ solution, 1M HCl and brine. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (using 1:1 hex: EtOAc, EtOAc or 2:1 EtOAc:hex as eluent), affording the desired amides in pure form. This method was used to obtain **7**, **15-17**, **73**, **74, 76**, **78**, **87** and **89-92**.

General procedure B for amide formation

To a solution of the carboxylic acid (1.0 eq) in toluene (1.0 M) thionyl chloride (2.0 eq) and one drop of DMF were added. The solution was refluxed for 3 h, concentrated and dissolved in DCM (0.50 M). The corresponding aniline (1.2 eq) was added and the reaction mixture was stirred at 25 °C for 12h. The reaction mixture was concentrated and purified by flash column chromatography (hex:EtOAc,

2:1) obtaining the desired amides in pure form. This method was used to obtain intermediates **81-86** and **91**.

3.3.1 Synthesis of fumaric acid derivatives

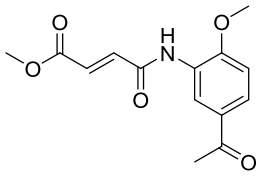


71 (commercial)

64, R = methoxy
65, R = methyl
66, R = fluoro
72, R = H (commercially available)

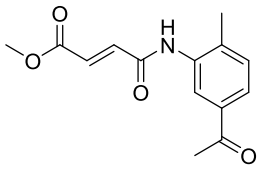
73, R = methoxy
74, R = methyl
75, R = fluoro
76, R = H

Methyl (*E*)-4-((5-acetyl-2-methoxyphenyl)amino)-4-oxobut-2-enoate (73)



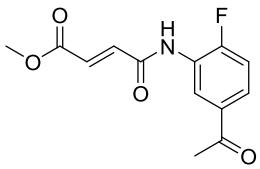
White solid; Yield: 35 %; mp 191-194 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.11 (d, *J* = 1.8 Hz, 1H), 8.05 (s, 1H), 7.81 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.14 – 6.91 (m, 3H), 3.99 (s, 3H), 3.84 (s, 3H), 2.60 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.0, 165.8, 161.4, 151.5, 136.6, 131.2, 130.6, 126.8, 125.4, 120.7, 109.7, 56.2, 52.3, 26.5; IR (neat): ῡ = 3379, 2959, 1718, 1686, 1676, 1645, 1591, 1537, 1493, 1423, 1366, 1312, 1298, 1271, 1257, 1159, 1130, 1021, 976, 885, 824, 801, 763 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{14}H_{16}NO_5^+$, 278.1023; found, 278.1022.

Methyl (*E*)-4-((5-acetyl-2-methylphenyl)amino)-4-oxobut-2-enoate (74)



White solid; Yield: 57%; mp 130-133 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.64 (s, 1H), 8.45 (s, 1H), 8.01 (d, *J* = 7.7 Hz, 1H), 7.63 – 7.49 (m, 2H), 7.35 – 7.15 (m, 1H), 4.14 (s, 3H), 2.88 (s, 3H), 2.88 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 197.1, 165.4, 161.7, 137.6, 137.0, 135.9, 135.1, 130.8, 129.3, 125.4, 123.8, 52.1, 26.6, 18.0; IR (neat): ῡ = 3353, 2954, 1715, 1685, 1670, 1652, 1605, 1529, 1499, 1439, 1355, 1316, 1274, 1226, 1201, 1161, 1139, 1016, 1003, 985, 822, 795, 768 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{14}H_{16}NO_4^+$, 262.1074; found, 262.1072.

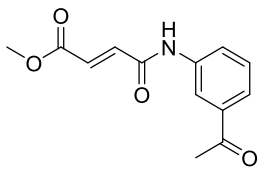
Methyl (*E*)-4-((5-acetyl-2-fluorophenyl)amino)-4-oxobut-2-enoate (75)



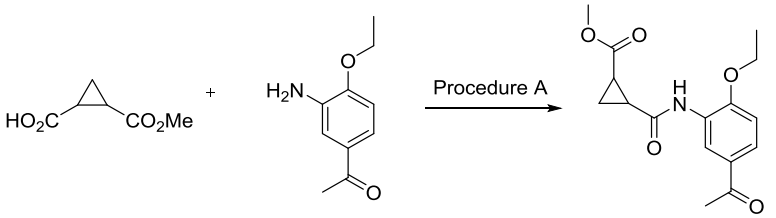
To a solution of mono methyl fumarate (**71**, 127 mg, 0.980 mmol) at 0 °C in DCM (1.00 mL) oxalyl chloride (84.1 µL, 0.980 mmol) and one drop of DMF were added. The solution was stirred at 0 ºC for 20 min and at 25 °C for 30 min. The aniline, 1-(3-amino-4-fluorophenyl)ethan-1-one (**66**, 50.0 mg, 0.327 mmol), was slowly added at 0 ºC, followed by Et_3N (209 µL, 1.50 mmol) and DMAP (12.0 mg, 0.0980 mmol). The reaction mixture was stirred at 25 ºC for 12 h, it was quenched with a saturated solution of NH_4Cl and extracted with EtOAc three times. The combined organic layers were dried

over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (1:1 hex: EtOAc) affording the desired amide in pure form as a white solid (37.0 mg, 0.140 mmol, 43 % yield). mp 176-179 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.06 (d, *J* = 6.7 Hz, 1H), 7.79 (ddd, *J* = 8.5, 5.1, 2.2 Hz, 2H), 7.21 (dd, *J* = 10.3, 8.7 Hz, 1H), 7.13 (d, *J* = 15.3 Hz, 1H), 7.00 (d, *J* = 15.3 Hz, 1H), 3.85 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.5, 165.6, 161.6, 135.7, 134.1 (d, *J* = 3.2 Hz), 132.1, 126.0 (d, *J* = 10.8 Hz), 125.6 (d, *J* = 8.2 Hz), 122.6, 115.4, 115.2, 52.42, 26.63; IR (neat): ῡ = 3354, 2963, 2924, 1715, 1686, 1650, 1614, 1600, 1540, 1487, 1441, 1418, 1357, 1320, 1293, 1261, 1215, 1183, 1153, 1113, 974, 877, 818, 766 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{13}H_{13}FNO_4^+$, 266.0823; found, 266.0823.

Methyl (*E*)-4-((3-acetylphenyl)amino)-4-oxobut-2-enoate (76)

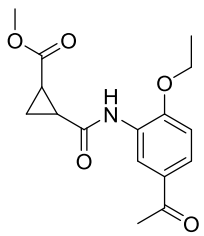


White solid; Yield: 50 %; mp 177-178 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.64 (s, 1H), 8.21 (d, *J* = 7.5 Hz, 1H), 8.14 (s, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 7.49 (t, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 14.3 Hz, 1H), 7.01 (d, *J* = 15.3 Hz, 1H), 3.87 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.4, 166.2, 161.8, 138.4, 137.6, 136.9, 131.2, 129.6, 125.0, 124.7, 119.2, 52.5, 26.7; IR (neat): ῡ = 3332, 1723, 1687, 1673, 1607, 1551, 1485, 1440, 1360, 1334, 1299, 1275, 1223, 1204, 1154, 1003, 973, 898, 878, 811, 711 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{13}H_{14}NO_4^+$, 248.0917; found, 248.09144.



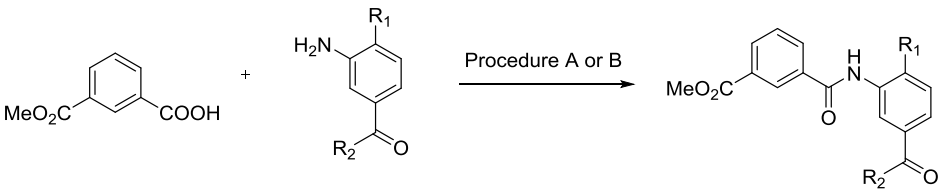
31 **77** **78**

Methyl 2-((5-acetyl-2-ethoxyphenyl)carbamoyl)cyclopropane-1-carboxylate (78)



White solid; Yield: 55 %; mp 166-168 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.99 (s, 1H), 8.05 (s, 1H), 7.74 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 3.74 (s, 3H), 2.56 (s, 3H), 2.35 – 2.26 (m, 1H), 2.20 – 2.11 (m, 1H), 1.63 – 1.57 (m, 1H), 1.52 (t, *J* = 7.0 Hz, 3H), 1.49 – 1.44 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.0, 173.0, 168.4, 150.5, 130.4, 127.2, 124.6, 120.4, 110.4, 64.7, 52.2, 26.5, 25.5, 22.0, 15.5, 14.7; IR (neat): ῡ = 3289, 2986, 2951, 2925, 1723, 1682, 1653, 1601, 1586, 1544, 1500, 1423, 1362, 1344, 1277, 1231, 1203, 1172, 1131, 1039, 956, 925, 875, 815, cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{16}H_{19}NNaO_5^+$, 328.1155; found, 328.1152.

3.3.2 Synthesis of benzoic acid derivatives. Scope on the acyl benzene

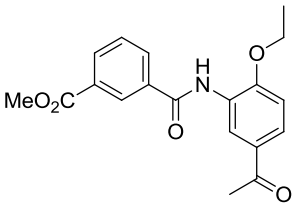


79

77, R_1 = OEt, R_2 = Me (commercially available)
80, R_1 = Br, R_2 = Me (commercially available)
60, R_1 = OPr, R_2 = Me
61, R_1 = O^{i}Bu, R_2 = Me
62, R_1 =  , R_2 = Me
63, R_1 = OBenzyl, R_2 = Me
68, R_1 = morpholine, R_2 = Me
70, R_1 = OEt, R_2 = NHMe

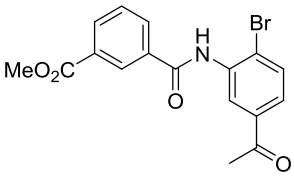
7, R_1 = OEt, R_2 = Me
81, R_1 = Br, R_2 = Me
82, R_1 = OPr, R_2 = Me
83, R_1 = O^{i}Bu, R_2 = Me
84, R_1 =  , R_2 = Me
85, R_1 = OBenzyl, R_2 = Me
86, R_1 = morpholine, R_2 = Me
87, R_1 = OEt, R_2 = NHMe

Methyl 3-((5-acetyl-2-ethoxyphenyl)carbamoyl)benzoate (7)



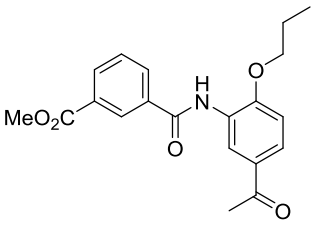
White solid; Yield: 80%; mp 137-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.14 (d, *J* = 2.2 Hz, 1H), 8.65 (s, 1H), 8.53 (t, *J* = 1.5 Hz, 1H), 8.28 – 8.18 (m, 1H), 8.12 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.77 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.60 (td, *J* = 7.8, 0.5 Hz, 1H), 6.96 (d, *J* = 8.6 Hz, 1H) 4.24 (q, *J* = 7.0 Hz, 2H), 3.96 (s, 3H), 2.60 (s, 3H), 1.54 (t, *J* = 7.0 Hz, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.0, 166.1, 164.1, 151.1, 135.1, 132.8, 131.5, 130.8, 130.4, 129.2, 127.7, 127.2, 124.9, 120.5, 110.4, 64.8, 52.4, 26.5, 14.7; IR (neat): ῡ = 3430, 2979, 2954, 1720, 1678, 1590, 1537, 1468, 1433, 1294, 1261, 1228, 1205, 1145, 1033, 988, 907, 797, 728 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{20}NO_5^+$, 342.1336; found, 342.1331.

Methyl 3-((5-acetyl-2-bromophenyl)carbamoyl)benzoate (81)



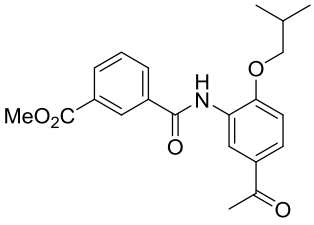
White solid; Yield: 60 %; mp 133-135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.14 (d, *J* = 1.9 Hz, 1H), 8.61 (s, 1H), 8.54 (br, 1H), 8.28 (dd, *J* = 7.8, 1.3 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 7.75 – 7.60 (m, 3H), 3.98 (s, 3H), 3.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.1, 166.0, 164.5, 137.4, 135.9, 134.5, 133.3, 132.7, 131.6, 131.1, 129.4, 128.0, 124.7, 123.0, 119.0, 52.5, 26.7; IR (neat): ῡ = 3242, 2923, 2853, 1726, 1691, 1649, 1572, 1524, 1436, 1414, 1315, 1295, 1245, 1213, 1092, 1083, 1027, 932, 817, 732, 724, 706 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{17}H_{14}BrNNaO_4^+$, 397.9998; found, 397.9996.

Methyl 3-((5-acetyl-2-propoxyphenyl)carbamoyl)benzoate (82)



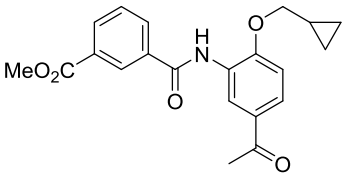
White solid; Yield: 40 %; mp 132-135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.18 (d, *J* = 2.1 Hz, 1H), 8.69 (s, 1H), 8.54 (t, *J* = 1.5 Hz, 1H), 8.28 – 8.21 (m, 1H), 8.15 (ddd, *J* = 7.8, 1.9, 1.2 Hz, 1H), 7.80 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 4.15 (t, *J* = 6.5 Hz, 2H), 3.97 (s, 3H), 2.63 (s, 3H), 2.03 – 1.87 (m, 2H), 1.14 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): 197.2, 166.1, 164.1, 151.2, 135.1, 132.9, 131.7, 130.9, 130.5, 129.3, 127.6, 127.3, 124.9, 120.5, 110.5, 70.6, 52.4, 26.6, 22.5, 10.5; IR (neat): ϑ = 3321, 2970, 2923, 1726, 1681, 1655, 1602, 1585, 1537, 1498, 1461, 1422, 1361, 1331, 1300, 1272, 1202, 1137, 1096, 1074, 975, 887, 805, 728, 719 cm^{-1};HRMS (ESI), *m/z*: calcd for $C_{20}H_{22}NO_5^+$, 356.1493; found, 356.1493.

Methyl 3-((5-acetyl-2-isobutoxyphenyl)carbamoyl)benzoate (83)



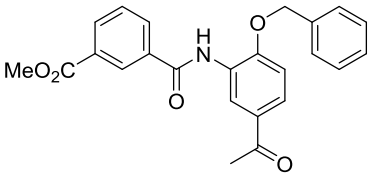
Beige solid; Yield: 89 %; mp 116-119 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.19 (d, *J* = 2.1 Hz, 1H), 8.71 (s, 1H), 8.54 (d, *J* = 1.6 Hz, 1H), 8.29 – 8.21 (m, 1H), 8.19 – 8.12 (m, 1H), 7.79 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 3.94 – 3.96 (m, 5H), 2.62 (s, 3H), 2.24 (dp, *J* = 13.3, 6.7 Hz, 1H), 1.13 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.1, 166.0, 164.0, 151.2, 135.1, 132.9, 131.7, 130.9, 130.5, 129.3, 127.4, 127.3, 124.9, 120.3, 110.5, 75.2, 52.4, 28.3, 26.6, 19.2; IR (neat): ϑ = 3317, 2959, 1725, 1678, 1652, 1601, 1583, 1535, 1497, 1468, 1422, 1360, 1331, 1301, 1277, 1258, 1201, 1140, 1094, 1075, 1018, 885, 809, 727 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{21}H_{24}NO_5^+$, 370.1649; found, 370.1649.

Methyl 3-((5-acetyl-2-(cyclopropylmethoxy)phenyl)carbamoyl)benzoate (84)



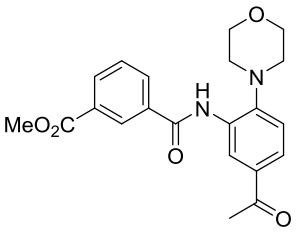
Pale yellow solid; Yield: 91 %; mp 141-142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.17 (d, *J* = 2.1 Hz, 1H), 8.78 (s, 1H), 8.56 (t, *J* = 1.5 Hz, 1H), 8.27 – 8.21 (m, 1H), 8.21 – 8.13 (m, 1H), 7.77 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 8.6 Hz, 1H), 4.02 (d, *J* = 7.1 Hz, 2H), 3.96 (s, 3H), 2.61 (s, 3H), 1.44 – 1.30 (m, 1H), 0.78 – 0.66 (m, 2H), 0.46 – 0.42 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.0, 166.0, 164.0, 151.2, 135.1, 132.8, 131.7, 130.8, 130.5, 129.2, 127.6, 127.5, 124.8, 120.4, 110.8, 74.0, 52.4, 26.5, 10.1, 3.2; IR (neat): ϑ = 3426, 2923, 2853, 1722, 1672, 1587, 1531, 1473, 1462, 1432, 1340, 1305, 1295, 1267, 1254, 1228, 1204, 1146, 1098, 1077, 1026, 990, 921, 905, 804, 729, 720 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{21}H_{22}NO_5^+$, 368.1493; found, 368.1495.

Methyl 3-((5-acetyl-2-(benzyloxy)phenyl)carbamoyl)benzoate (85)



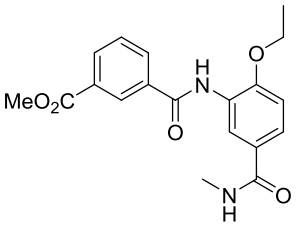
White solid; Yield: 74 %; mp 149-152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.20 (d, *J* = 2.1 Hz, 1H), 8.67 (s, 1H), 8.51 (d, *J* = 1.6 Hz, 1H), 8.26 – 8.18 (m, 1H), 8.07 (ddd, *J* = 7.7, 1.8, 1.2 Hz, 1H), 7.80 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.48 – 7.37 (m, 5H), 7.08 (d, *J* = 8.6 Hz, 1H), 5.27 (s, 2H), 3.94 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.0, 166.0, 164.1, 150.9, 135.5, 135.0, 132.8, 131.5, 130.9, 130.8, 129.1, 128.9, 128.6, 127.7, 127.6, 127.3, 124.8, 120.6, 111.3, 71.2, 52.4, 26.5; IR (neat): ῡ = 3303, 3064, 2954, 2921, 1732, 1717, 1681, 1651, 1602, 1585, 1536, 1498, 1425, 1332, 1282, 1202, 1146, 1078, 1024, 973, 885, 832, 797, 728, 719 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{24}H_{21}NNaO_5^+$, 426.1312; found, 426.1307.

Methyl 3-((5-acetyl-2-morpholinophenyl)carbamoyl)benzoate (86)



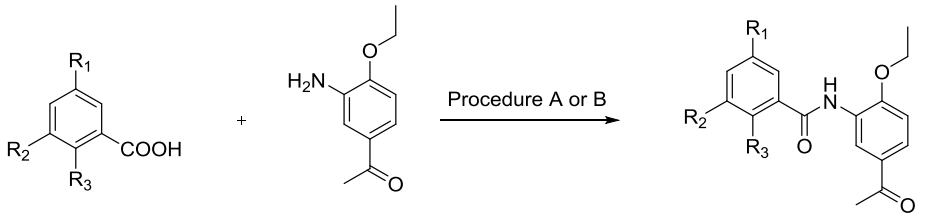
Pale yellow solid; Yield: 95 %; mp 152-155 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.51 (s, 1H), 9.17 (s, 1H), 8.58 (d, *J* = 1.1 Hz, 1H), 8.26 (t, *J* = 8.7 Hz, 2H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 4.00 (s, 3H), 3.99-3.97 (m, 4H), 3.01 (s, 4H), 2.65 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 197.5, 166.0, 163.7, 145.3, 134.8, 134.6, 133.2, 133.0, 132.0, 130.8, 129.5, 127.1, 124.3, 120.6, 120.1, 67.5, 52.5, 52.3, 26.7; IR (neat): ῡ = 3327, 2958, 2582, 1731, 1673, 1579, 1525, 1427, 1356, 1292, 1258, 1234, 1217, 1109, 932, 918, 814, 801, 719 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{21}H_{23}N_2O_5^+$, 383.1602; found, 383.1602.

Methyl 3-((2-ethoxy-5-(methylcarbamoyl)phenyl)carbamoyl)benzoate (87)



White solid; Yield: 66 %; mp 161-163 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.81 (d, *J* = 2.1 Hz, 1H), 8.65 (s, 1H), 8.49 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 8.12 – 8.01 (m, 1H), 7.70 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 6.45 (d, *J* = 4.2 Hz, 1H), 4.17 (q, *J* = 7.0 Hz, 2H), 3.94 (s, 3H), 2.97 (d, *J* = 4.8 Hz, 3H), 1.50 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.5, 166.0, 164.1, 149.7, 135.0, 132.7, 131.4, 130.8, 129.1, 127.7, 127.1, 126.9, 124.7, 117.1, 110.7, 64.6, 52.4, 26.7, 14.6; IR (neat): ῡ = 3432, 3305, 2935, 1723, 1683, 1655, 1590, 1537, 1490, 1467, 1437, 1316, 1302, 1260, 1235, 1216, 1143, 1113, 1040, 1023, 974, 902, 815, 802, 718 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{20}N_2NaO_5^+$, 379.1264; found, 379.1262.

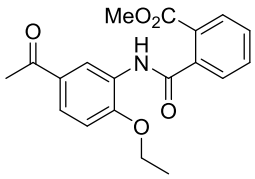
3.3.3 Synthesis of benzoic acid derivatives. Scope on the side chain



88, R_1 = H, R_2 = H, R_3 = CO_2Me (commercially available) **77**
33, R_1 = H, R_2 = tetrazole, R_3 = H
38, R_1 = H, R_2 = N-phenylsulfamide, R_3 = H
40, R_1 = H, R_2 = phenylsulfonamide, R_3 = H
42, R_1 = Br, R_2 = CO_2Me, R_3 = H
47, R_1 = CH_2OMe, R_2 = CO_2Me, R_3 = H
48, R_1 = CH_2OTHP, R_2 = CO_2Me, R_3 = H

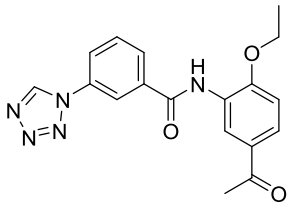
89, R_1 = H, R_2 = H, R_3 = CO_2Me
15, R_1 = H, R_2 = tetrazole, R_3 = H
16, R_1 = H, R_2 = N-phenylsulfamide, R_3 = H
17, R_1 = H, R_2 = phenylsulfonamide, R_3 = H
90, R_1 = Br, R_2 = CO_2Me, R_3 = H
91, R_1 = CH_2OMe, R_2 = CO_2Me, R_3 = H
92, R_1 = CH_2OTHP, R_2 = CO_2Me, R_3 = H

Methyl 2-((5-acetyl-2-ethoxyphenyl)carbamoyl)benzoate (89)



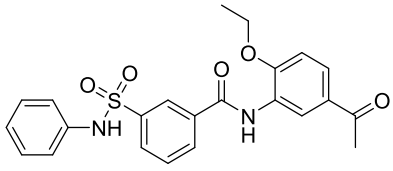
Pale brown solid; Yield: 40 %; mp 130-134 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (s, 1H), 8.09 (s, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.78 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.63 – 7.54 (m, 3H), 6.94 (d, *J* = 8.6 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 3.87 (s, 3H), 2.62 (s, 3H), 1.44 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.2, 167.1, 167.0, 150.9, 138.2, 132.2, 130.5, 130.3, 130.1, 129.3, 127.5, 127.5, 124.8, 120.7, 110.5, 64.7, 52.7, 26.6, 14.6; IR (neat): ῡ = 3274, 2954, 2926, 1727, 1670, 1651, 1600, 1584, 1537, 1497, 1473, 1427, 1357, 1335, 1265, 1202, 1141, 1115, 1071, 1035, 959, 805, 767, 709 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{19}NNaO_5^+$, 364.1155; found, 364.1155.

***N*-(5-Acetyl-2-ethoxyphenyl)-3-(1*H*-tetrazol-1-yl)benzamide (15)**



Brown solid; Yield: 98 %; mp 168-169 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.19 (s, 1H), 9.85 (s, 1H), 8.49 – 8.48 (m, 1H), 8.31 (d, *J* = 2.2 Hz, 1H), 8.17 – 8.11 (m, 2H), 7.87 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.7 Hz, 1H), 4.22 (q, *J* = 7.0 Hz, 2H), 2.55 (s, 3H), 1.38 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 196.2, 164.0, 155.2, 142.5, 136.2, 133.9, 130.5, 129.3, 128.5, 127.6, 126.5, 124.9, 124.1, 120.4, 111.9, 64.4, 26.4, 14.4; IR (neat): ῡ = 3439, 3086, 2970, 2923, 1666, 1591, 1535, 1489, 1430, 1363, 1336, 1260, 1242, 1203, 1133, 1090, 1078, 1032, 1006, 963, 893, 808, 742, 732 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{18}H_{18}N_5O_3^+$, 352.1404; found, 352.1404.

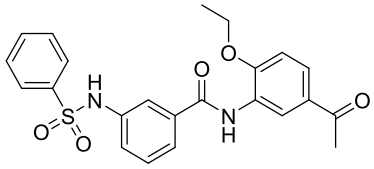
***N*-(5-Acetyl-2-ethoxyphenyl)-3-(*N*-phenylsulfamoyl)benzamide (16)**



White solid; Yield: 63 %; mp 186-189 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.41 (s, 1H), 9.85 (s, 1H), 8.34 (s, 1H), 8.26 (d, *J* = 1.9 Hz, 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H),

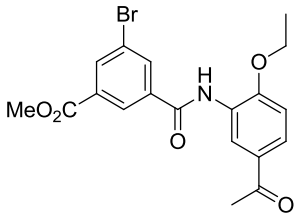
7.86 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.31 – 7.16 (m, 3H), 7.12 (s, 1H), 7.11 (s, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 4.19 (q, *J* = 6.9 Hz, 2H), 2.53 (s, 3H), 1.35 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 196.2, 163.9, 155.3, 140.1, 137.4, 135.3, 131.5, 129.7, 129.5, 129.3, 129.2, 127.6, 126.5, 126.2, 124.9, 124.3, 120.2, 111.9, 64.4, 26.4, 14.4; IR (neat): ῡ = 3423, 3235, 2986, 1682, 1667, 1600, 1589, 1536, 1482, 1469, 1433, 1345, 1258, 1228, 1211, 1159, 1111, 1029, 915, 890, 814, 764, 754, 740 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{23}N_2O_5S^+$, 439.1322; found, 439.1316.

_N_-(5-Acetyl-2-ethoxyphenyl)-3-(phenylsulfonamido)benzamide (17)



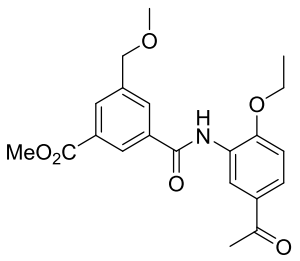
Pale yellow solid; Yield: 52 % over two steps; mp 217-220 °C; Purity: 93 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.56 (br, 1H), 9.44 (s, 1H), 8.37 (d, *J* = 2.0 Hz, 1H), 7.83 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.79 (d, *J* = 7.4 Hz, 2H), 7.68 (s, 1H), 7.63 – 7.60 (m, 2H), 7.58-7.55 (m, 2H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.34 – 7.28 (m, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 2.53 (s, 3H), 1.37 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 196.2, 164.5, 154.5, 139.3, 138.1, 135.4, 133.1, 129.5, 129.4, 127.1, 126.8, 126.6, 123.6, 122.9, 122.7, 119.1, 111.7, 64.4, 26.4, 14.4, 1 C is missing due to overlapping; IR (neat): ῡ = 3393, 3168, 2973, 2908, 1667, 1598, 1584, 1530, 1472, 1423, 1366, 1332, 1273, 1156, 1139, 1089, 1044, 971, 899, 881, 808, 743, 720 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{23}N_2O_5S^+$, 439.1322; found, 439.1324.

Methyl 3-((5-acetyl-2-ethoxyphenyl)carbamoyl)-5-bromobenzoate (90)



Pale yellow solid; Yield: 44 % over two steps; mp 159-161 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.09 (d, *J* = 2.2 Hz, 1H), 8.58 (br, 1H), 8.43 (t, *J* = 1.5 Hz, 1H), 8.38 – 8.33 (m, 1H), 8.27 (t, *J* = 1.8 Hz, 1H), 7.80 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 3.98 (s, 3H), 2.62 (s, 3H), 1.55 (t, *J* = 7.0 Hz, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.0, 164.8, 162.7, 151.2, 136.9, 135.7, 134.8, 132.5, 130.5, 126.9, 126.1, 125.3, 123.3, 120.6, 110.5, 64.9, 52.7, 26.5, 14.7; IR (neat): ῡ = 3414, 2960, 2922, 1739, 1689, 1673, 1588, 1531, 1485, 1437, 1340, 1290, 1259, 1227, 1205, 1150, 1111, 1070, 1029, 981, 931, 889, 800, 750, 733, 723 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{18}BrNNaO_5^+$, 442.0261; found, 442.0264.

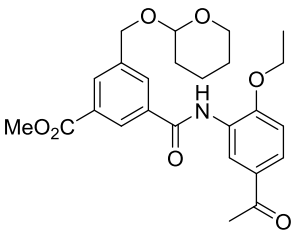
Methyl 3-((5-acetyl-2-ethoxyphenyl)carbamoyl)-5-(methoxymethyl)benzoate (91)



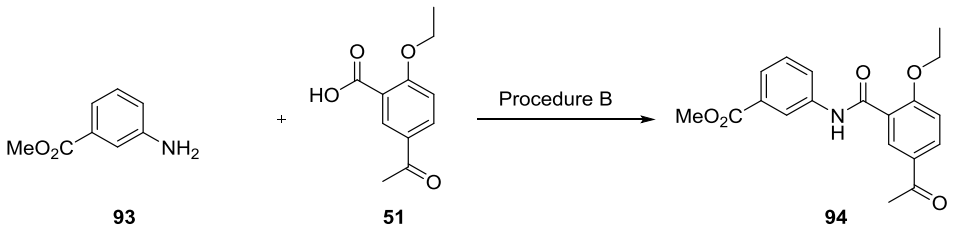
White solid; Yield: 17 % over two steps; mp 123-125 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (d, *J* = 2.1 Hz, 1H), 8.67 (s, 1H), 8.47 (s, 1H), 8.21 (s, 1H), 8.13 (s, 1H), 8.13 (s, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 4.59 (s, 2H), 4.26 (q, *J* = 7.0 Hz, 2H), 3.97 (s, 3H), 3.46 (s, 3H), 2.63 (s, 3H), 1.56 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.1, 166.1, 164.1, 151.2, 140.2, 135.4, 131.7, 131.1, 130.5, 127.2, 126.9, 124.9, 120.6, 110.5, 73.6,

64.8, 58.6, 52.5, 26.6, 14.7, 1 C is missing due to overlapping; IR (neat): ῡ = 3422, 2922, 2851, 1724, 1686, 1669, 1601, 1588, 1531, 1485, 1431, 1262, 1223, 1206, 1146, 1112, 1031, 999, 885, 794, 746, 727 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{21}H_{24}NO_6^+$, 386.1598; found, 386.1596.

Methyl 3-((5-acetyl-2-ethoxyphenyl)carbamoyl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)benzoate (92)

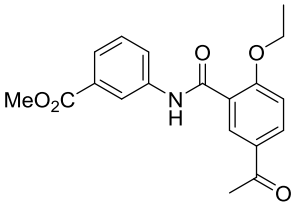


White solid; Yield: 44 %; mp 133-135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (d, *J* = 2.1 Hz, 1H), 8.65 (s, 1H), 8.46 (s, 1H), 8.22 (s, 1H), 8.15 (s, 1H), 7.79 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 4.91 (d, *J* = 12.6 Hz, 1H), 4.76 (t, *J* = 3.5 Hz, 1H), 4.62 (d, *J* = 12.6 Hz, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 3.97 (s, 3H), 3.95 – 3.87 (m, 1H), 3.62 – 3.53 (m, 1H), 2.62 (s, 3H), 1.92 – 1.84 (m, 1H), 1.83 – 1.57 (m, 5H), 1.55 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.1, 166.1, 164.2, 151.1, 140.2, 135.3, 131.8, 131.0, 130.6, 130.5, 127.2, 126.8, 124.9, 120.6, 110.5, 98.3, 68.0, 64.8, 62.2, 52.4, 30.5, 26.5, 25.4, 19.3, 14.7; IR (neat): ῡ = 3437, 2935, 2864, 1721, 1685, 1669, 1593, 1552, 1489, 1441, 1343, 1313, 1256, 1214, 1200, 1182, 1136, 1115, 1022, 974, 906, 871, 802, 740, 716 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{25}H_{29}NNaO_7^+$, 478.1836; found, 478.1835.



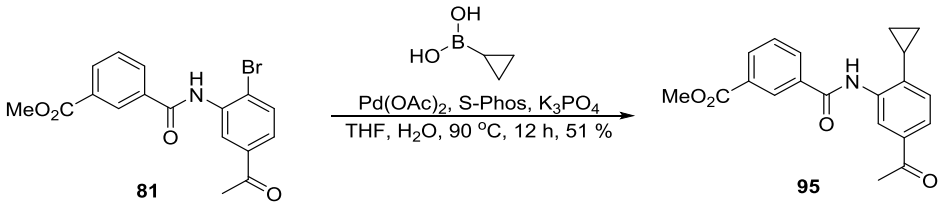
93 **51** **94**

Methyl 3-(5-acetyl-2-ethoxybenzamido)benzoate (94)

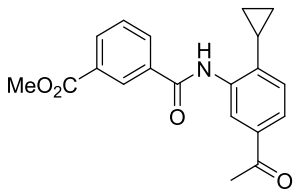


Pale yellow solid; Yield: 34 %; mp 128-130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.04 (s, 1H), 8.87 (d, *J* = 2.4 Hz, 1H), 8.22 (t, *J* = 1.8 Hz, 1H), 8.14 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.98 (ddd, *J* = 8.1, 2.2, 1.0 Hz, 1H), 7.81 (ddd, *J* = 7.7, 1.6, 1.1 Hz, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 4.38 (q, *J* = 7.0 Hz, 2H), 3.93 (s, 3H), 2.63 (s, 3H), 1.69 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.4, 166.7, 162.4, 160.0, 138.5, 133.8, 133.3, 131.0, 130.9, 129.2, 125.4, 124.4, 120.9, 120.9, 112.6, 65.7, 52.2, 26.5, 14.8; IR (neat): ῡ = 3319, 2951, 2925, 1722, 1671, 1599, 1560, 1489, 1435, 1363, 1335, 1291, 1268, 1239, 1213, 1162, 1109, 1071, 1025, 969, 884, 814, 795, 751 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{20}NO_5^+$, 342.1336; found, 342.1336.

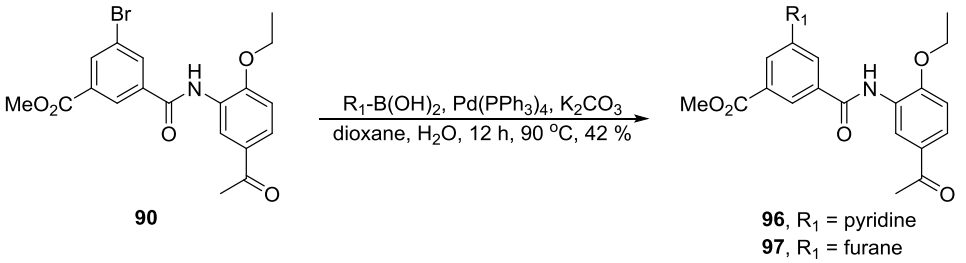
3.4 Suzuki cross-coupling reactions



Methyl 3-((5-acetyl-2-cyclopropylphenyl)carbamoyl)benzoate (95)



To a solution of methyl 3-((5-acetyl-2-bromophenyl)carbamoyl)benzoate (**81**, 33 mg, 0.088 mmol) in THF:H_2O (3:1, 0.22 M) $Pd(OAc)_2$ (2.0 mg, 0.0089 mmol), S-Phos (4.0 mg, 0.0097 mmol) and K_3PO_4 (68 mg, 0.312 mmol) were added. The solution was purged with N_2 and heated to 90 ºC for 12 hours. The reaction mixture was diluted with water and extracted with EtOAc three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (1:1 hex:EtOAc) affording the final product in pure form as a pale brown solid (15 mg, 0.044 mmol, 51 % yield). mp 125-130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.92 (d, *J* = 1.7 Hz, 1H), 8.66 (br, 1H), 8.59 (t, *J* = 1.6 Hz, 1H), 8.28 – 8.23 (m, 1H), 8.22 – 8.16 (m, 1H), 7.72 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 3.97 (s, 3H), 2.63 (s, 3H), 1.93 (tdd, *J* = 10.9, 7.3, 3.6 Hz, 1H), 1.20 – 1.09 (m, 2H), 0.86 – 0.76 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 197.7, 166.1, 164.2, 137.7, 136.8, 136.3, 135.0, 132.9, 131.8, 130.9, 129.4, 128.6, 127.5, 124.1, 121.0, 52.5, 26.7, 11.7, 6.2; IR (neat): ῡ = 3267, 2954, 2924, 1725, 1677, 1643, 1606, 1569, 1525, 1413, 1357, 1319, 1282, 1251, 1221, 1201, 1136, 1098, 1045, 1015, 987, 898, 880, 813, 797, 727 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{20}H_{19}NNaO_4^+$, 360.1206; found, 360.1203.

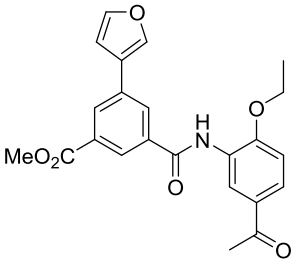


General procedure for Suzuki cross-couplings

To a solution of methyl 3-((5-acetyl-2-ethoxyphenyl)carbamoyl)-5-bromobenzoate (**90**) in dioxane (0.24 M) and water (1 drop), the corresponding boronic acid (1.1 eq), K_2CO_3 (3.0 eq) and $Pd(PPh_3)_4$ (0.25 eq) were added. The reaction was stirred for 12 h at 90 °C. It was diluted with water and extracted with EtOAc three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column

chromatography (hex: EtOAc, 2:1 to pure EtOAc) affording the coupled products. This procedure was used to obtain intermediates **96** and **97**. **96** was extracted and used in the next step without further purification.

Methyl 3-((5-acetyl-2-ethoxyphenyl)carbamoyl)-5-(furan-3-yl)benzoate (97)



Yellow solid; Yield: 42 %; mp 156-163 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.16 (d, *J* = 2.2 Hz, 1H), 8.70 (s, 1H), 8.37 (t, *J* = 1.6 Hz, 1H), 8.32 (t, *J* = 1.6 Hz, 1H), 8.27 (t, *J* = 1.8 Hz, 1H), 7.88 (dd, *J* = 1.4, 0.9 Hz, 1H), 7.80 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.54 (t, *J* = 1.6 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.81 (dd, *J* = 1.9, 0.9 Hz, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 3.98 (s, 3H), 2.62 (s, 3H), 1.57 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.0, 166.0, 164.0, 151.2, 144.3, 139.5, 135.7, 134.1, 131.4, 130.5, 129.9, 129.1, 127.2, 125.5, 125.0, 124.9, 120.6, 110.5, 108.6, 64.8, 52.5, 26.5, 14.7; IR (neat): ῡ = 2927, 2851, 1724, 1682, 1592, 1535, 1434, 1261, 1024, 803, 794, 765, 748 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{21}NNaO_6^+$, 430.1261; found, 430.1260.

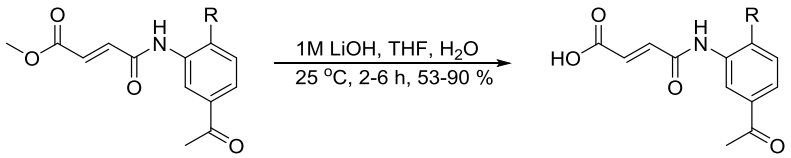
3.5 Methyl ester hydrolysis of the amide coupling products

General procedure for ester hydrolysis

To a solution of the methyl ester (1 eq) in THF (0.1 M) a 1M LiOH solution (5 eq) was added. The reaction mixture was stirred at 25 °C for 2-6 h. It was then concentrated under reduced pressure and 1M HCl was added. The obtained precipitate was washed with hexanes, Et_2O and cold DCM, affording the desired carboxylic acids in pure form. For the synthesis of **18**, the reaction mixture was diluted with water and extracted with DCM and Et_2O three times. The pH of the water phase was then brought to pH 3-4 by addition of 1M HCl and the resulting precipitate was filtered off and washed with DCM.

For the synthesis of **20**, the pH of the reaction mixture was brought to 1 by the addition of 1M HCl solution. It was then concentrated, redissolved in EtOH (0.1 M) and PTSA (0.1 eq) was added. The solution was stirred at 25 °C for 5 h and concentrated. Upon the addition of 1M HCl solution, the final product precipitated. It was filtered out and washed with 1M HCl solution and Et_2O affording the desired alcohol in pure form.

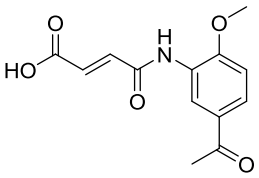
In the case of **22**, the reaction mixture was diluted with water and extracted with hexane. The aqueous phase was then brought to pH 3-4 by addition of a 10% citric acid solution. It was extracted with EtOAc three times, the combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure obtaining the final product **22** in pure form.



73, R = methoxy
74, R = methyl
75, R = fluoro
76, R = H

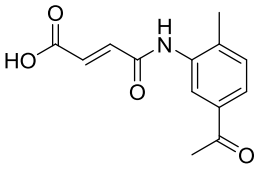
1, R = methoxy
2, R = methyl
3, R = fluoro
4, R = H

(*E*)-4-((5-Acetyl-2-methoxyphenyl)amino)-4-oxobut-2-enoic acid (1)



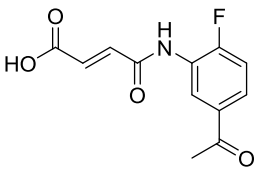
Yellow solid; Yield: 53 %; mp 172-174 °C; ^{1}H NMR (500 MHz, MeOD): δ = 8.82 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.37 (d, *J* = 15.4 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 6.83 (d, *J* = 15.6 Hz, 1H), 4.00 (s, 3H), 2.57 (s, 3H); ^{13}C NMR (125 MHz, MeOD): δ = 199.2, 168.4, 164.6, 155.6, 138.0, 132.6, 131.0, 128.1, 127.8, 123.7, 111.5, 56.8, 26.5; IR (neat): ῡ = 3306, 3068, 2949, 2840, 1702, 1665, 1589, 1542, 1516, 1445, 1422, 1359, 1302, 1269, 1213, 1174, 1134, 1023, 973, 886, 813 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{13}H_{14}NO_5^+$, 264.0867; found, 264.0869.

(*E*)-4-((5-Acetyl-2-methylphenyl)amino)-4-oxobut-2-enoic acid (2)



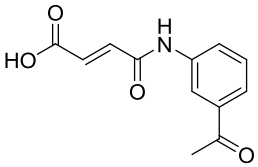
White solid; Yield: 60 %; mp 203-204 °C; ^{1}H NMR (400 MHz, MeOD): δ = 8.14 (s, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 15.3 Hz, 1H), 6.84 (d, *J* = 15.4 Hz, 1H), 6.76 (s, 1H), 2.58 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ = 207.2, 176.3, 172.1, 147.1, 146.9, 146.0, 145.1, 140.9, 140.8, 135.4, 133.9, 36.6, 28.1; IR (neat): ῡ = 3266, 2925, 2604, 1714, 1659, 1607, 1573, 1536, 1417, 1288, 1259, 1231, 1177, 977, 912, 905, 822, 811, 706 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{13}H_{13}NO_4^+$, 248.0917; found, 248.0916.

(*E*)-4-((5-Acetyl-2-fluorophenyl)amino)-4-oxobut-2-enoic acid (3)

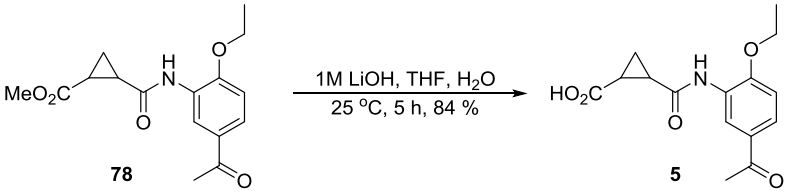


White solid; Yield: 60 %; mp 218-220 °C; ^{1}H NMR (400 MHz, MeOD): δ = 8.76 (dd, *J* = 7.5, 2.0 Hz, 1H), 7.86 (ddd, *J* = 8.5, 4.7, 2.2 Hz, 1H), 7.32 (t, *J* = 9.5 Hz, 1H), 7.30 (d, *J* = 13.9 Hz, 1H), 6.86 (d, *J* = 15.4 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR (125 MHz, MeOD): δ = 198.5, 168.2, 164.8, 164.6, 159.2, 157.2, 155.9, 154.0, 140.8, 137.5, 137.2, 135.0, 133.2, 132.8, 127.9 (d, *J* = 9.0 Hz), 127.3 (d, *J* = 12.3 Hz), 126.3 (d, *J* = 11.6 Hz), 125.6 (d, *J* = 2.2 Hz), 125.5 (d, *J* = 7.8 Hz), 123.8, 116.8 (d, *J* = 20.8 Hz), 116.0 (d, *J* = 20.0 Hz), 102.4, 26.7, presence of rotamers; IR (neat): ῡ = 3446, 3297, 3084, 1707, 1666, 1611, 1601, 1546, 1487, 1417, 1362, 1335, 1293, 1267, 1192, 1112, 990, 925, 887, 825, 758, 721 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{11}FNO_4^+$, 252.0667; found, 252.0669.

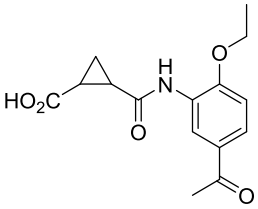
(*E*)-4-((3-Acetylphenyl)amino)-4-oxobut-2-enoic acid (4)



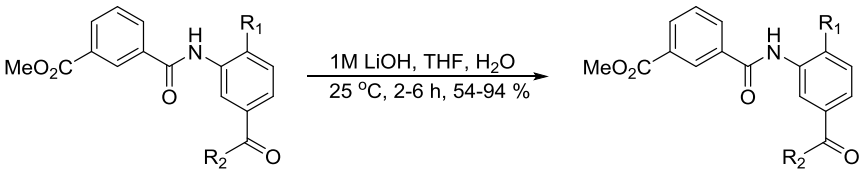
Off white solid; Yield: 90 %; mp 237-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.03 (s, 1H), 10.72 (s, 1H), 8.26 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 15.3 Hz, 1H), 6.69 (d, *J* = 15.4 Hz, 1H), 2.57 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 197.6, 166.3, 161.9, 139.0, 137.4, 136.9, 131.1, 129.4, 124.1, 123.9, 118.6, 26.8; IR (neat): ῡ = 3349, 2835, 2682, 2570, 1703, 1661, 1609, 1552, 1490, 1421, 1363, 1341, 1307, 1281, 1166, 974, 879, 794 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{12}H_{12}NO_4^+$, 234.0761; found, 234.0760.



2-((5-Acetyl-2-ethoxyphenyl)carbamoyl)cyclopropane-1-carboxylic acid (5)



White solid; Yield: 84 %; mp 234-235 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.50 (br, 1H), 9.65 (s, 1H), 8.53 (s, 1H), 7.73 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 4.21 (q, *J* = 6.9 Hz, 2H), 2.71 – 2.62 (m, 1H), 2.48 (s, 3H), 1.94 – 1.87 (m, 1H), 1.41 (t, *J* = 7.0 Hz, 3H), 1.34 – 1.22 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 196.2, 173.1, 169.1, 152.7, 129.2, 127.1, 125.9, 121.9, 111.4, 64.3, 26.3, 23.3, 21.5, 14.4, 14.2; IR (neat): ῡ = 3408, 2992, 2414, 1716, 1642, 1598, 1575, 1517, 1425, 1366, 1320, 1271, 1191, 1130, 1082, 1037, 969, 930, 892, 879, 810 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{15}H_{17}NNaO_5^+$, 314.0999; found, 314.0996.



7, R_1 = OEt, R_2 = Me
82, R_1 = OPr, R_2 = Me
83, R_1 = O^{i}Bu, R_2 = Me

84, R_1 =  , R_2 = Me

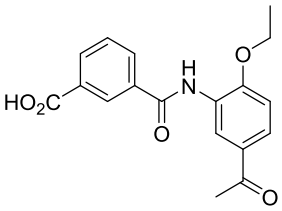
85, R_1 = OBenzyl, R_2 = Me
86, R_1 = morpholine, R_2 = Me
95, R_1 = cyclopropyl, R_2 = Me
87, R_1 = OEt, R_2 = NHMe

6, R_1 = OEt, R_2 = Me
9, R_1 = OPr, R_2 = Me
10, R_1 = O^{i}Bu, R_2 = Me

11, R_1 =  , R_2 = Me

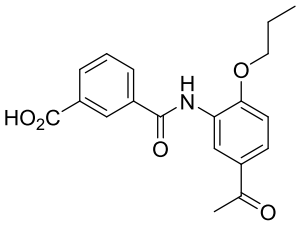
12, R_1 = OBenzyl, R_2 = Me
13, R_1 = morpholine, R_2 = Me
14, R_1 = cyclopropyl, R_2 = Me
24, R_1 = OEt, R_2 = NHMe

3-((5-Acetyl-2-ethoxyphenyl)carbamoyl)benzoic acid (6)



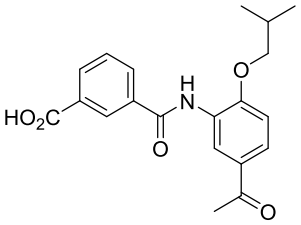
White solid; Yield: 60 %; mp 224-226 °C; ^{1}H NMR (400 MHz, MeOD): δ = 8.65 (d, *J* = 2.2 Hz, 1H), 8.61 – 8.59 (m, 1H), 8.25 (ddd, *J* = 7.8, 1.6, 1.2 Hz, 1H), 8.17 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 7.90 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.67 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 4.27 (q, *J* = 7.0 Hz, 2H), 2.59 (s, 3H), 1.50 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 196.2, 166.8, 164.6, 155.2, 134.8, 132.3, 131.7, 131.2, 129.3, 129.0, 128.5, 127.4, 126.7, 124.7, 111.9, 64.4, 26.4, 14.4; IR (neat): ῡ = 3437, 3412, 2988, 1710, 1684, 1672, 1603, 1585, 1533, 1429, 1301, 1269, 1229, 1205, 1147, 1126, 1077, 1032, 904, 801, 732 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{18}H_{18}NO_5^+$, 328.1180; found, 328.1176.

3-((5-Acetyl-2-propoxyphenyl)carbamoyl)benzoic acid (9)



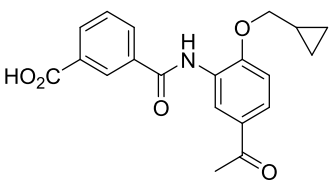
Beige solid; Yield: 77 %; mp 208-211 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.63 (dd, *J* = 15.5, 7.4 Hz, 2H), 8.25 (d, *J* = 7.7 Hz, 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 8.6 Hz, 1H), 4.16 (t, *J* = 6.3 Hz, 2H), 2.59 (s, 3H), 1.96 – 1.84 (m, 2H), 1.10 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 189.7, 159.2, 157.9, 146.7, 126.8, 124.6, 123.3, 121.6, 120.7, 120.1, 119.0, 118.6, 115.3, 103.0, 62.3, 17.0, 14.0, 1.3, 1 C is missing due to overlapping; IR (neat): ῡ = 3427, 2965, 2940, 1719, 1665, 1639, 1590, 1507, 1434, 1404, 1389, 1333, 1262, 1206, 1158, 1070, 1035, 1009, 910, 810, 727 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{20}NO_5^+$, 342.1336; found, 342.1341.

3-((5-Acetyl-2-isobutoxyphenyl)carbamoyl)benzoic acid (10)



White solid; Yield: 94 %; mp 221-223 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.21 (s, 1H), 9.79 (s, 1H), 8.52 (t, *J* = 1.5 Hz, 1H), 8.26 (d, *J* = 2.2 Hz, 1H), 8.20 – 8.16 (m, 1H), 8.16 – 8.12 (m, 1H), 7.86 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.68 (t, *J* = 7.7 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 3.92 (d, *J* = 6.4 Hz, 2H), 2.54 (s, 3H), 2.06 (dt, *J* = 13.2, 4.8 Hz, 1H), 0.99 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 196.2, 166.7, 164.5, 155.7, 134.8, 132.2, 131.6, 131.2, 129.3, 129.0, 128.3, 127.6, 126.7, 124.9, 111.8, 74.5, 27.7, 26.4, 18.9; IR (neat): ῡ = 3375, 2963, 2933, 2877, 2469, 1682, 1644, 1596, 1581, 1510, 1426, 1411, 1356, 1316, 1297, 1270, 1214, 1128, 1107, 1017, 956, 885, 810, 724, 724 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{20}H_{21}NNaO_4^+$, 378.1312; found, 378.1309.

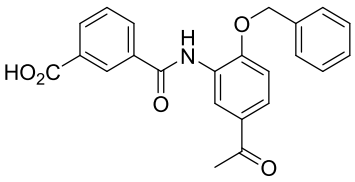
3-((5-Acetyl-2-(cyclopropylmethoxy)phenyl)carbamoyl)benzoic acid (11)



Beige solid; Yield: 54 %; mp 221-223 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.23 (s, 1H), 9.76 (s, 1H), 8.53 (s, 1H), 8.32 (d, *J* = 2.2 Hz,

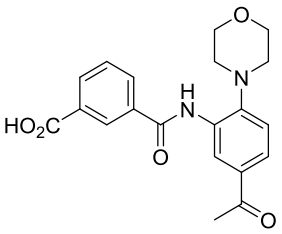
1H), 8.21 – 8.17 (m, 1H), 8.17 – 8.13 (m, 1H), 7.84 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 4.03 (d, *J* = 6.8 Hz, 2H), 2.54 (s, 3H), 1.33 – 1.20 (m, 1H), 0.60 – 0.51 (m, 2H), 0.42 – 0.34 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 196.2, 166.7, 164.4, 155.2, 134.8, 132.3, 131.7, 131.2, 129.4, 129.1, 128.3, 127.4, 126.8, 124.4, 112.3, 72.9, 26.4, 9.9, 3.0; IR (neat): ῡ = 3075, 2957, 2927, 1716, 1670, 1597, 1582, 1510, 1432, 1412, 1396, 1291, 1276, 1262, 1217, 1160, 1111, 1069, 1023, 991, 906, 796, 728 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{20}H_{19}NNaO_5^+$, 376.1155; found, 376.1151.

3-((5-Acetyl-2-(benzyloxy)phenyl)carbamoyl)benzoic acid (12)



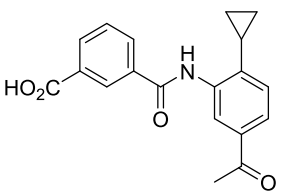
White solid; Yield: 79 %; mp 210-215 °C; Purity: 93 %; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.88 (s, 1H), 8.54 (s, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 8.12 (t, *J* = 8.1 Hz, 2H), 7.85 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.30 (d, *J* = 7.4 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 5.32 (s, 2H), 2.54 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 196.2, 167.1, 165.0, 155.0, 136.5, 134.5, 132.2, 130.7, 129.7, 128.4, 128.3, 127.8, 127.3, 127.1, 125.1, 112.5, 69.9, 26.4, three carbons are missing due to overlapping; IR (neat): ῡ = 3414, 3085, 1713, 1660, 1637, 1593, 1584, 1510, 1434, 1400, 1390, 1323, 1296, 1274, 1221, 1165, 1021, 995, 896, 803, 724, 702 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{20}NO_5^+$, 390.1336; found, 390.1335.

3-((5-Acetyl-2-morpholinophenyl)carbamoyl)benzoic acid (13)



White solid; Yield: 76 %; mp 249-252 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.32 (s, 1H), 9.93 (s, 1H), 8.53 (s, 1H), 8.43 (d, *J* = 1.7 Hz, 1H), 8.21 (d, *J* = 7.7 Hz, 1H), 8.16 (d, *J* = 7.7 Hz, 1H), 7.82 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.71 (t, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 3.78 (t, *J* = 3.9 Hz, 4H), 2.99 (t, *J* = 4.1 Hz, 4H), 2.55 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 196.7, 166.8, 164.2, 149.3, 134.7, 132.5, 131.9, 131.8, 131.3, 131.2, 129.3, 128.2, 126.5, 124.2, 119.7, 66.4, 51.0, 26.6; IR (neat): ῡ = 3346, 2981, 2831, 1723, 1676, 1644, 1598, 1577, 1529, 1457, 1432, 1283, 1234, 1215, 1112, 1069, 935, 918, 894, 848, 824, 726 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{20}H_{21}N_2O_5^+$, 369.1445; found, 369.1444.

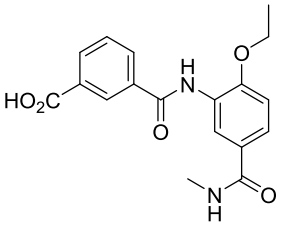
3-((5-Acetyl-2-cyclopropylphenyl)carbamoyl)benzoic acid (14)



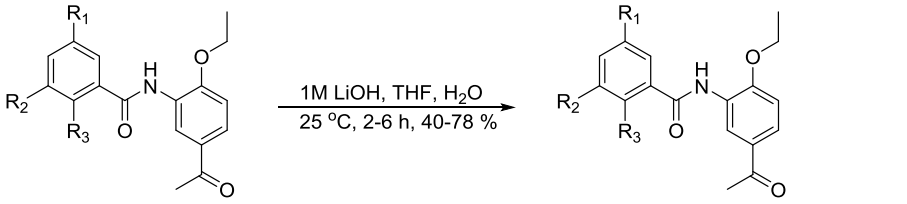
Pale yellow solid; Yield: 57 %; mp 95-100 °C; Purity: 83 %; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.29 (s, 1H), 8.58 (s, 1H), 8.21 – 8.09 (m, 3H), 7.94 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 2.55 (s, 3H), 2.18 – 2.04 (m, 1H), 1.06 – 0.93 (m, 2H), 0.76 – 0.69 (m, 2H);^{13}C NMR (100 MHz, DMSO-d_6): δ = 197.0, 167.1, 165.4, 145.0, 137.1, 134.5, 134.4, 132.2,

130.9, 128.5, 128.5, 126.3, 126.2, 124.9, 26.6, 11.5, 9.0, one C is missing due to overlapping; IR (neat): ῡ = 3282, 2920, 1683, 1638, 1608, 1571, 1532, 1416, 1358, 1294, 1260, 1231, 1101, 1077, 1046, 1022, 882, 819, 797, 728 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{17}NNaO_4^+$, 346.1050; found, 346.1047.

3-((2-Ethoxy-5-(methylcarbamoyl)phenyl)carbamoyl)benzoic acid (24)



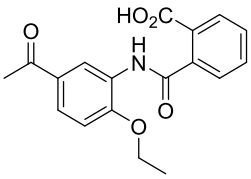
Pale brown solid; Yield: 62 %; mp 226-229 °C; ^{1}H NMR (400 MHz, MeOD): δ = 8.60 (t, *J* = 1.5 Hz, 1H), 8.42 (d, *J* = 2.2 Hz, 1H), 8.27 – 8.22 (m, 1H), 8.19 – 8.15 (m, 1H), 7.73 – 7.60 (m, 2H), 7.14 (d, *J* = 8.7 Hz, 1H), 4.23 (q, *J* = 6.9 Hz, 2H), 2.92 (s, 3H), 1.48 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, MeOD): δ = 170.3, 168.8, 167.4, 154.6, 136.3, 134.0, 132.9, 132.8, 132.7, 130.2, 130.1, 130.0, 129.6, 127.8, 127.7, 126.7, 123.7, 112.6, 65.8, 27.0, 15.0, presence of rotamers; IR (neat): ῡ = 3398, 3081, 2981, 2525, 1689, 1661, 1601, 1573, 1467, 1429, 1395, 1336, 1267, 1234, 1195, 1167, 1123, 1029, 913, 782, 760, 735 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{18}H_{18}N_2O_5^+$, 343.1289; found, 343.1283.



89, R_1 = H, R_2 = H, R_3 = CO_2Me
96, R_1 = pyridine, R_2 = CO_2Me, R_3 = H
97, R_1 = furane, R_2 = CO_2Me, R_3 = H
91, R_1 = CH_2OMe, R_2 = CO_2Me, R_3 = H
92, R_1 = CH_2OTHP, R_2 = CO_2Me, R_3 = H

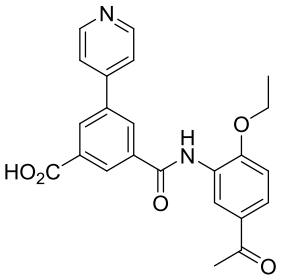
8, R_1 = H, R_2 = H, R_3 = CO_2H
18, R_1 = pyridine, R_2 = CO_2H, R_3 = H
19, R_1 = furane, R_2 = CO_2H, R_3 = H
21, R_1 = CH_2OMe, R_2 = CO_2H, R_3 = H
22, R_1 = CH_2OTHP, R_2 = CO_2H, R_3 = H

2-((5-Acetyl-2-ethoxyphenyl)carbamoyl)benzoic acid (8)



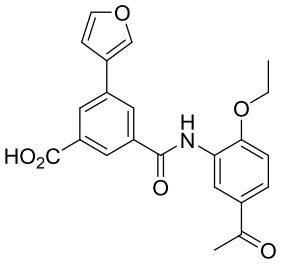
White solid; Yield: 57 %; mp 143-146 °C; ^{1}H NMR (400 MHz, MeOD): δ = 8.75 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 8.9 Hz, 1H), 7.71 – 7.62 (m, 1H), 7.63 – 7.55 (m, 2H), 7.13 (d, *J* = 8.4 Hz, 1H), 4.22 (q, *J* = 6.9 Hz, 2H), 2.59 (s, 3H), 2.59 (s, 3H); ^{13}C NMR (126 MHz, MeOD): δ = 198.7, 197.9, 169.8, 169.1, 168.0, 154.3, 151.3, 138.4, 136.6, 132.7, 131.8, 130.9, 130.6, 130.0, 129.5, 129.5, 128.8, 128.1, 127.4, 126.6, 123.4, 120.4, 114.1, 110.9, 109.8, 64.5, 63.8, 48.2, 48.1, 48.1, 47.9, 47.9, 47.8, 47.7, 47.7, 47.7, 47.7, 47.6, 47.4, 47.3, 47.3, 47.1, 25.1, 24.9, 13.7, 13.4, presence of rotamers; IR (neat): ῡ = 3272, 2970, 2936, 1713, 1662, 1603, 1583, 1540, 1498, 1436, 1275, 1254, 1220, 1159, 1128, 1043, 933, 884, 809, 786, 730, 710, 704 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{18}H_{18}NO_5^+$, 328.1180; found, 328.1181.

3-((5-Acetyl-2-ethoxyphenyl)carbamoyl)-5-(pyridin-4-yl)benzoic acid (18)



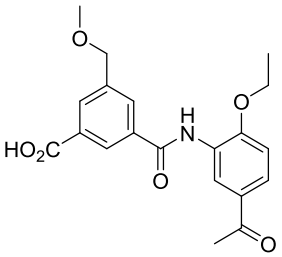
White solid; Yield: 40 % over two steps; mp 270-276 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.91 (s, 1H), 8.70 (d, *J* = 5.7 Hz, 2H), 8.55 (s, 1H), 8.45 (s, 1H), 8.42 (s, 1H), 8.30 (s, 1H), 7.87 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.83 (d, *J* = 4.9 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 1H), 4.21 (q, *J* = 6.9 Hz, 2H), 2.55 (s, 3H), 1.37 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ= 196.2, 166.3, 164.1, 155.6, 145.4, 136.3, 136.0, 132.5, 131.0, 130.8, 130.4, 129.4, 127.8, 126.4, 125.5, 123.6, 112.0, 64.4, 26.4, 14.4, 1 C is missing due to overlapping; IR (neat): ῡ = 3441, 2969, 1682, 1591, 1540, 1432, 1357, 1331, 1260, 1225, 1203, 1066, 1027, 797, 748, 732, 707 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{21}N_2O_5^+$, 405.1445; found, 405.1440.

3-((5-Acetyl-2-ethoxyphenyl)carbamoyl)-5-(furan-3-yl)benzoic acid (19)



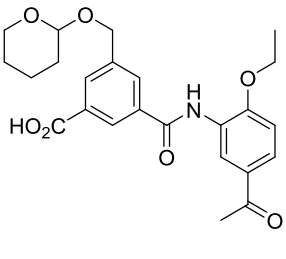
Beige solid; Yield: 55 %; mp 210-214 °C; Purity: 93 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.37 (s, 1H), 9.90 (s, 1H), 8.42 (s, 1H), 8.37 (s, 1H), 8.33 (s, 1H), 8.29 (s, 1H), 7.87 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.83 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.13 (s, 1H), 4.21 (q, *J* = 6.6 Hz, 2H), 2.55 (s, 3H), 1.38 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 196.3, 166.8, 164.5, 155.4, 144.8, 140.6, 135.5, 132.9, 131.9, 129.3, 128.9, 128.6, 127.6, 127.0, 126.6, 125.2, 124.7, 111.9, 108.7, 64.4, 26.5, 14.5; IR (neat): ῡ = 3424, 3147, 2988, 2935, 1719, 1678, 1658, 1588, 1540, 1492, 1432, 1361, 1336, 1266, 1202, 1183, 1141, 1070, 1024, 907, 890, 873, 808, 795, 741 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{22}H_{19}NO_6^+$, 394.1285; found, 394.1279.

3-((5-Acetyl-2-ethoxyphenyl)carbamoyl)-5-(methoxymethyl)benzoic acid (21)



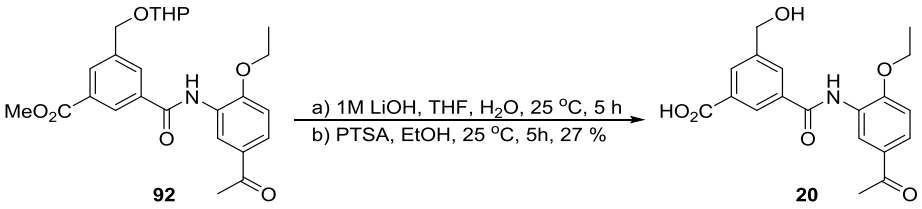
White solid; Yield: 50 %; mp 185-187 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.29 (br, 1H), 9.86 (s, 1H), 8.44 (s, 1H), 8.26 (s, 1H), 8.13 (s, 1H), 8.09 (s, 1H), 7.86 (d, *J* = 8.7 Hz, 1H), 7.21 (d, *J* = 8.6 Hz, 1H), 4.57 (s, 2H), 4.20 (q, *J* = 6.7 Hz, 2H), 3.36 (s, 3H), 2.54 (s, 3H), 1.37 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 196.3, 166.8, 164.6, 155.4, 139.6, 134.9, 131.2, 131.0, 130.6, 129.4, 127.6, 126.7, 125.0, 111.9, 72.7, 64.4, 57.9, 26.5, 14.5; one C is missing due to overlapping; IR (neat): ῡ = 3443, 2986, 2922, 1683, 1592, 1539, 1432, 1361, 1263, 1243, 1190, 1135, 1113, 1035, 893, 809, 742, 708 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{20}H_{21}NNaO_6^+$, 394.1261; found, 394.1263.

3-((5-Acetyl-2-ethoxyphenyl)carbamoyl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)benzoic acid (22)

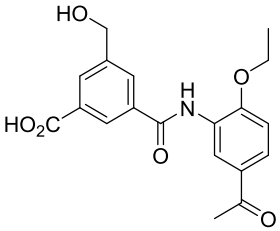


White solid; Yield: 78 %; mp 165-170 °C; Purity: 92 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.23 (br, 1H), 9.80 (s, 1H), 8.44 (s, 1H), 8.31 (d, *J* =

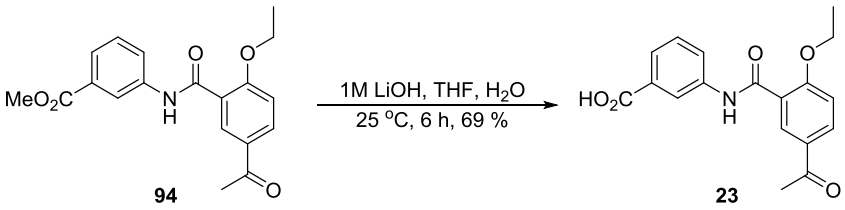
1.9 Hz, 1H), 8.14 (s, 1H), 8.11 (s, 1H), 7.85 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 4.82 (d, *J* = 12.6 Hz, 1H), 4.75 (t, *J* = 3.3 Hz, 1H), 4.62 (d, *J* = 12.6 Hz, 1H), 4.21 (q, *J* = 6.9 Hz, 2H), 3.81 (ddd, *J* = 11.1, 8.3, 2.9 Hz, 1H), 3.53 – 3.49 (m, 1H), 2.54 (s, 3H), 1.84 – 1.65 (m, 2H), 1.62 – 1.44 (m, 4H), 1.37 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 196.4, 166.8, 164.7, 155.3, 139.8, 135.1, 131.4, 131.2, 130.8, 129.5, 127.6, 127.5, 126.9, 124.8, 112.0, 97.8, 67.6, 64.5, 61.6, 30.3, 26.5, 25.1, 19.2, 14.5; IR (neat): ϑ = 3427, 2938, 2873, 1718, 1679, 1647, 1590, 1536, 1436, 1343, 1296, 1260, 12001, 1185, 1121, 1073, 1027, 976, 957, 899, 809, 743 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{24}H_{27}NNaO_7^+$, 464.1680; found, 464.1678.



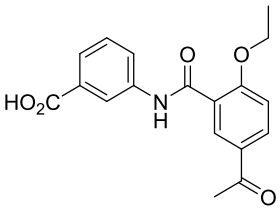
3-((5-Acetyl-2-ethoxyphenyl)carbamoyl)-5-(hydroxymethyl)benzoic acid (20)



White solid; Yield: 27 % over two steps; mp 225-228 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.24 (s, 1H), 9.83 (s, 1H), 8.39 (s, 1H), 8.27 (s, 1H), 8.12 (d, *J* = 4.9 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.6 Hz, 1H), 5.49 (t, *J* = 5.6 Hz, 1H), 4.65 (d, *J* = 5.5 Hz, 2H), 4.20 (q, *J* = 6.9 Hz, 2H), 2.54 (s, 3H), 1.37 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 196.3, 167.0, 164.7, 155.3, 143.8, 134.7, 131.0, 130.1, 129.8, 129.4, 127.5, 126.9, 126.8, 124.9, 111.9, 64.4, 62.2, 26.5, 14.5; IR (neat): ϑ = 3391, 3262, 2975, 2933, 1719, 1646, 1604, 1581, 1541, 1498, 1477, 1437, 1361, 1336, 1298, 1274, 1232, 1206, 1183, 1147, 1054, 1037, 987, 904, 893, 821, 796, 753, 710 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{19}H_{20}NO_6^+$, 358.1285; found, 358.1285.



3-(5-Acetyl-2-ethoxybenzamido)benzoic acid (23)



Pale yellow solid; Yield: 69 %; mp 209-212 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.96 (br, 1H), 10.34 (s, 1H), 8.39 (s, 1H), 8.20 (d, *J* = 1.9 Hz, 1H), 8.09 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 4.26 (q, *J* = 6.9 Hz, 2H), 2.57 (s, 3H), 1.40 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz,

DMSO-d_6): δ = 196.0, 167.1, 164.0, 159.5, 139.1, 132.4, 131.4, 130.2, 129.4, 129.0, 124.9, 124.4, 123.7, 120.3, 112.7, 64.7, 26.5, 14.4; IR (neat): ϑ = 3343, 2981, 1719, 1670, 1596, 1552, 1488, 1409, 1362, 1299, 1265, 1203, 1149, 1110, 1080, 1025, 921, 890, 811, 756 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{18}H_{17}NNaO_5^+$, 350.0999; found, 350.0995.

4. Bromodomain expression and purification

Proteins were purified as described previously.[21] Briefly, His-tagged bromodomains were expressed in *Escherichia coli* BL21(DE3) cells upon induction with isopropyl thio-beta-D-galactoside (IPTG, final concentration 0.1 mM) for 16 h at 18 °C. Bacteria were lysated and (when required) the resulting extract was treated to remove DNA, adding 0.15% polyethylenimine (PEI). The His-tagged proteins were purified on HisTrap columns (GE Healthcare) and eluted using a step gradient of imidazole. The poly-Histidine tags were removed by overnight incubation with His-tagged tobacco etch virus (TEV) protease purified in-house (if required by the purification protocol, in the meantime the sample was exchanged via dialysis). A size-exclusion chromatography step (HiLoad 16/600 Superdex75 column) and a Ni-affinity chromatography step were subsequently performed to finally purify the cleaved bromodomains. Samples were then concentrated, flash frozen and stored at –80 °C.

5. X-ray crystallography

Crystallization, Data Collection, and Structure Determination

Crystals of the CREBBP bromodomain were grown at 4°C using the hanging drop vapor diffusion method. A 50 mM solution of compound **6** (in 100 % DMSO) was added into the CREBBP protein to reach a final DMSO concentration of 1 % (v/v) and the mixture was incubated on ice for 1 hour before crystallization. Then equal volumes of protein (with compound **6**) and reservoir solutions (0.1 M MES pH 6.5, 0.10 $MgCl_2$, 20 % PEG 6000, 10 % ethylene glycol) were mixed and crystals appeared after 1 to 2 days. The crystals were flash-frozen in liquid nitrogen with extra 10% ethylene glycol as cryoprotectant for measurements. Data sets were collected on a PILATUS 6MF detector at the Swiss Light Source beamline X06SA of the Paul Scherrer Institute (Villigen, Switzerland) and indexed, integrated and scaled with the XDS[22] and CCP4 programs.[23] The structures were solved by molecular replacement with PHASER[24] using the CREBBP structure (PDB entry 4NR5) as a search model and refined with PHENIX.[25] The atomic coordinates and structure factors of CREBBP in complex with inhibitor **6** have been deposited with the Protein Data Bank as entry 4TQN.

Table S1

	Compound **6**
Space group	P1 21 1
Unit cell	
a (Å)	24.94
b (Å)	42.94
c (Å)	51.98
alpha	90.00
beta	97.24
gamma	90.00
Resolution range (Å)	42.94 -1.70
Unique reflections	12119(1768)
<I/σ(I)>	15.6(5.3)
R merge	0.068(0.380)
Completeness (%)	99.9(99.2)
Multiplicity	6.5(6.2)
Refinement	
Resolution range (Å)	33.00-1.70
R factor/R free	0.1813/0.1990
Mean B factors (A2)	23.24
RMS bonds (Å)	0.006
RMS angles (°)	1.155

5.1 Composite Omit Map of Ligand 6

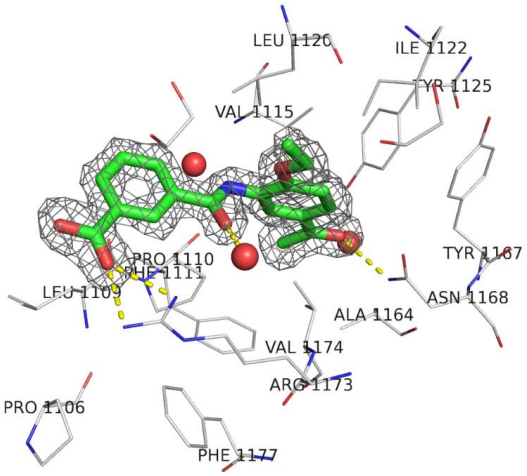


Figure S2. $2mF_o - DF_c$ electron density maps contoured at 1σ (grey mesh) were generated in a region within 1.6 Å for compound **6** using PHENIX and Pymol.

6. Thermal shift measurements

Thermal shift measurements were carried out as previously described[26] with a final volume of 20 µl, ligand and protein concentrations 100 µM (50 µM for compound **18**) and 2 µM, respectively. The reported values (ΔT_m) are calculated as the difference between the transition midpoints of an individual sample and the average of the reference wells (containing the protein and the DMSO only) in the same plate. DMSO concentration was kept at 0.2% (v/v).

6.1 Phylogenetic tree

The bromodomain sequence aligment previously reported by Filippakopoulos *et. al.* was used excluding the residues from the plasmid.[21] The evolutionary history was inferred using the Neighbor-Joining method.[27] The optimal tree with the sum of branch length = 21.33784094 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method[28] and are in the units of the number of amino acid substitutions per site. The analysis involved 61 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 57 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.[29]

6.2 Correlation between IC_{50} and Thermal Shift values with CREBBP

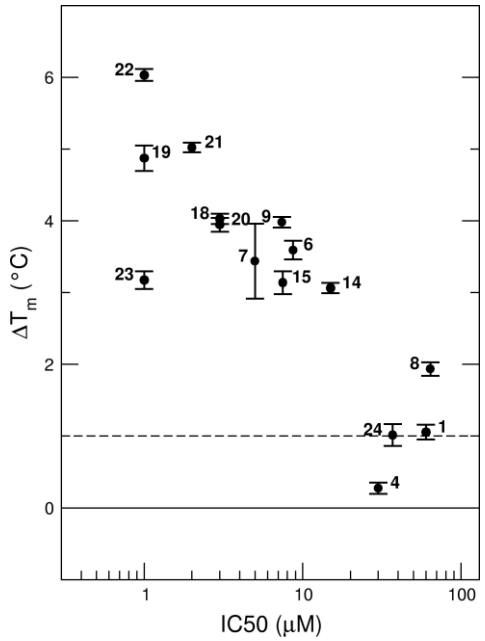


Figure S3. Correlation between IC_{50} values (determined by TR-FRET assay at BPS Bioscience) and thermal shift values (ΔT_m) obtained with CREBBP. Average and SEM of the thermal shift results are shown.

7. TR-FRET assays

TR-FRET assays were carried out in duplicate at BPS Bioscience using a recombinant CBP bromodomain (BPS catalogue #31128) and the BET Ligand (BPS catalogue #33000) as provided in the CREBBP TR-FRET Assay Kit (BPS catalogue #32619). A 10 mM solution of the compound under investigation in DMSO was prepared and shipped to BPS Bioscience, where it was tested at 10 concentrations over the range of 0.001-10 µM (compounds **6**, **7**, **9**-**13**, **15**-**19**, **21**-**23**) or 0.01-100 µM (compounds **1**-**5**, **8**, **14**, **20**, **24**) . Each compound solution was then diluted in water to obtain a 10% DMSO solution. 2 µL of this dilution were added to a 20 µL reaction mixture (12.5 nM CBP, 125 nM BET Ligand, including FRET dyes and the amount of compound needed to reach the indicated concentration in the Table below). The resulting mixture was incubated for 2 hours at room temperature prior to reading the TR (time resolved)-FRET signal using a Tecan Infinite M1000 plate reader. The negative control consisted of the aforementioned mixture in which the buffer was added in place of compound. TR-FRET were recorded as the ratio of the fluorescence of the acceptor and the donor dyes (acceptor/donor).

The TR-FRET data was analyzed using Graphpad Prism software. The percent activity in the presence of each compound was calculated according to the following equation: % activity = [(F- Fb)/(Ft – Fb)]×100, where Ft is the TR-FRET signal in the absence of any compound (100 % activity), Fb the TR-FRET signal in the absence of the bromodomain (0 % activity) and F the TR-FRET signal in the presence of the compound. The percent inhibition was calculated according to the following equation: % inhibition = 100 - % activity. The values of % activity versus a series of compound concentrations were then plotted using non-linear regression analysis of Sigmoidal dose-response curve generated with the equation $Y=B+(T-B)/1+10^{((LogIC50-X)\times Hill\ Slope)}$, where Y=percent activity, B=minimum percent activity, T=maximum percent activity, X= logarithm of compound and Hill Slope=slope factor or Hill coefficient. The IC_{50} value corresponds to the concentration causing a half-maximal percent activity.

8. BROMOscan assays

K_D and % binding of binding affinity determinations by means of BROMOscan technology was carried out at DiscoveRx. E. *coli* derived from BL21 strain was used as host to grow T7 phage strains displaying the bromodomains. E. *coli*, grown to log-phase, were infected with T7 phage (from a frozen stock, being the multiplicity of infection 0.4) and incubated while shaking at 32 °C for 90-150 minutes, until lysis. In order to remove cell debris, lysates were centrifuged at 5,000 x g and filtered (0.2 µm). Affinity resins were obtained by treating streptavidin-coated magnetic beads with biotinylated acetylated peptide ligands for 30 minutes at 25°C. Those beads were then blocked with excess of biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % bovine serum albumin, BSA, 0.05 % Tween20, 1 mM dithiothreitol, (DTT) removing the unbound ligand and reducing non-specific phage binding.

During the experiment, the bromodomain, ligand-bound affinity beads and test compounds were combined in a buffer composed of 17% SeaBlock, 0.33x phosphate-buffered solution, PBS, 0.04% Tween20, 0.02% BSA, 0.004% sodium azide and 7.4 mM DTT. Test compounds were prepared as 50 mM in pure DMSO and diluted to 5 mM with monoethylene gycol, MEG (100× concentrated in respect to the top screening concentration, 50 µM). During the assay a DMSO and MEG final concentration of 0.1% and 0.9% respectively was used. The assays were carried out in polystyrene 96-well plates in a final volume of 0.135 mL. The assay plates were incubated at 25 °C with shaking for 1 hour and the affinity beads were washed with a buffer composed of 0.05% Tween 20 in PBS. The beads were then re-suspended in the elution buffer (1x PBS, 0.05% Tween 20, 2 µM non-biotinylated affinity ligand) and incubated at 25°C with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR. Binding constants (K_d) were calculated with a standard dose-response curve using the Hill equation and curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm. The % of binding interactions are reported as '% Ctrl', where lower numbers indicate stronger hits. The corresponding values were obtained as follows:

$$\%\, ctrl = \frac{test\ compound\ signal - positive\ control\ signal}{negative\ control\ signal - positive\ control\ signal} \times 100$$

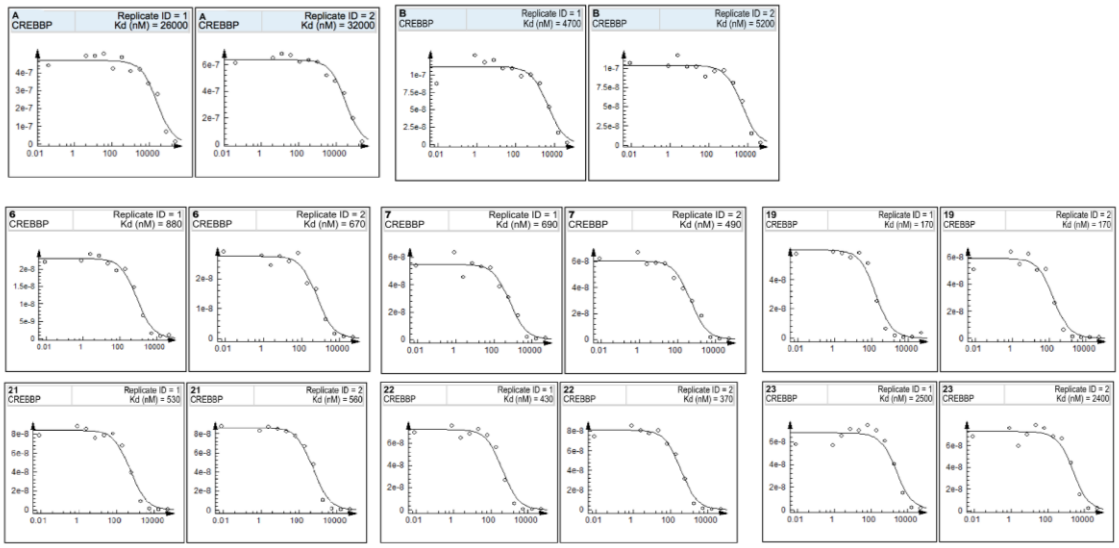


Figure S4. Dose response curves for the binding of the CREBBP bromodomain to compounds **A**, **B**, **6**, **7**, **19** and **21**-**23** performed at DiscoveRx.

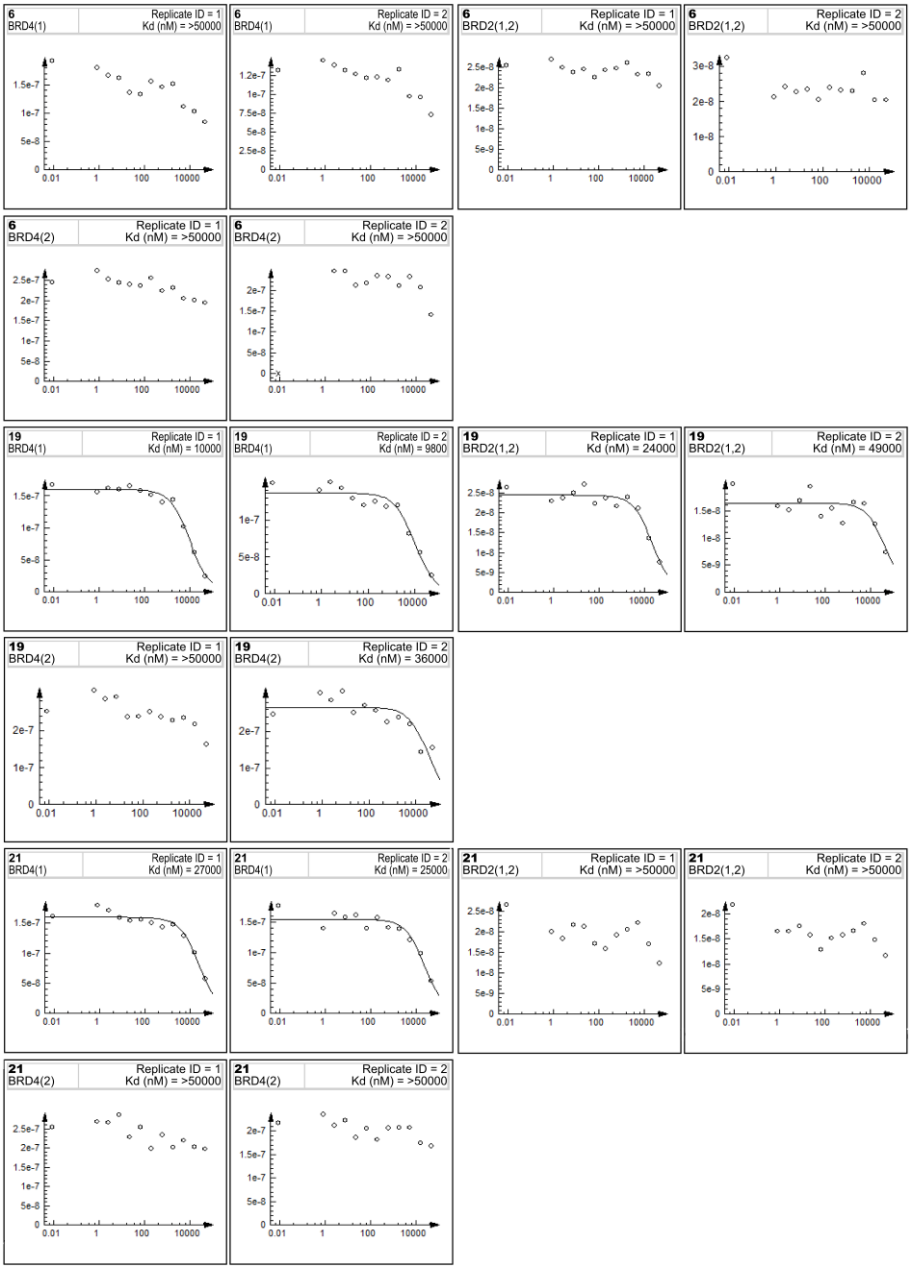


Figure S5. Dose response curves for the binding of the BET bromodomains to compounds **6**, **19** and **21** performed at DiscoveRx.

9. ITC EXPERIMENTS

Isothermal Titration Calorimetry experiments were performed on a VP-ITC instrument (MicroCal, Inc., Northampton, MA). Protein samples thoroughly dialyzed against the same batch of buffer in order to minimize artifacts due to minor differences in buffer composition and the protein concentration was determined after a filtering through a 0.22 μm pore-size filter.

Bromodomains (300-500 μM) were injected into the 1.4-mL sample cell containing the compound (50 μM) dissolved into the ITC buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1% DMSO). The titration experiments were carried out at 15°C while stirring at 300 rpm: after a control injection of 2 μL, 29

10-μL injections (10 s duration, with a 4 min interval between) were performed. The raw data were integrated, normalized for concentration, and analyzed using a single binding site model, supplied with the MicroCal Origin software package to obtain the apparent K_d values (K_d^{app}). The actual K_d values were calculated assuming that DMSO acted as a competing ligand[30] according to the equation:[31]

$$K_d = \frac{K_d^{app}}{1 + \frac{[DMSO]}{K_d^{DMSO}}}$$

where [DMSO] and K_d^{DMSO} indicate the DMSO concentration in the ITC buffer and the dissociation constant of the DMSO for the bromodomain under investigation, respectively (Figure S6).

The K_d^{DMSO} value for CREBBP was calculated titrating the histone H3K56ac peptide (ac-IRRYQ(Kac)STELLY-am, where ac-, -am and Kac, indicate acetylation, amidation and acetylated lysine side chain, respectively, purchased at GenScript) into a CREBBP bromodomain solution in the presence and absence of 0.75% DMSO with protein and peptide concentrations of 688 or 740 μM and 85 μM, respectively (Figure S7).

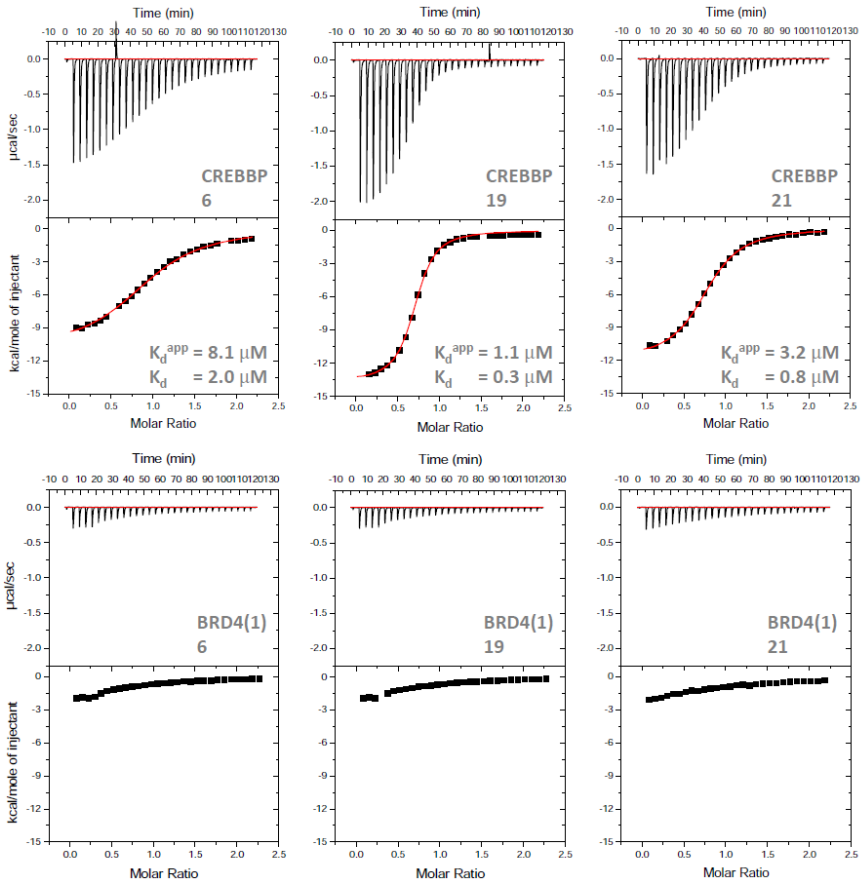
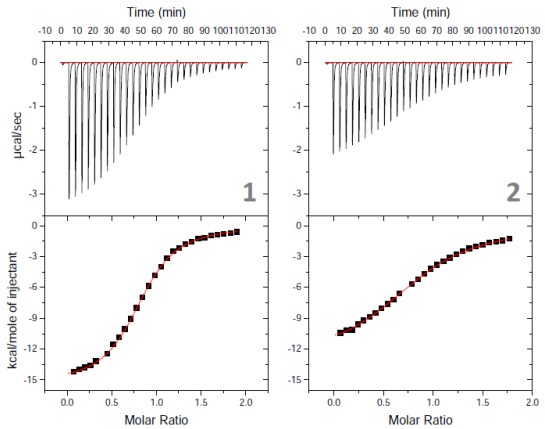


Figure S6. ITC titration curves for the binding of the CREBBP and BRD4(1) bromodomains (upper and lower panels, respectively) to compounds **6**, **19** and **21**.



exp	[DMSO] (M)	app K_d (µM)
1	0.000	4.77
2	0.106	15.71

$$K_{d_{cmpd}} = \frac{K_{d_{cmpd}}^{app}}{1 + \frac{[DMSO]}{K_{d_{DMSO}}}}$$

$$K_{d_{DMSO}} = 46.3\ mM$$

Figure S7. The N-terminally acetylated, C-terminaly amidated IRRYQ(Kac)STELLY peptide was titrated into a CREBBP bromodomain solution in the absence of DMSO (experiment 1) and in the presence of 0.75% DMSO (experiment 2). The K_d for DMSO was calculated as shown in the right panel.

Table S2. Thermodynamic parameters measured by ITC for the CREBBP bromodomain. ΔG and ΔH values are given in kcal/mol, ΔS values are given in cal/mol/T.

Cpd	N	K_d^{app} (μM)	ΔG	ΔH	ΔS
6	0.99 ± 0.01	8.13 ± 0.43	–6.7	–10.9 ± 0.1	–14.5
19	0.70 ± 0.00	1.11 ± 0.08	–7.9	–13.7 ± 0.1	–20.1
21	0.79 ± 0.00	3.25 ± 0.10	–7.2	–11.9 ± 0.0	–16.2

10. Cell culture and cytotoxicity measurements

MDA-MB-231, HT-29 cells (obtained from the UZH Cancer Institute) and HeLa cells (obtained from Dr. Nathan Luedtke, Chemistry Department, UZH) were cultured in DMEM supplemented with 10 % (v/v) fetal bovine serum. K562 cells (obtained from Dr. Silvio Hemmi, Institute of Molecular Life Sciences, UZH), HL-60, ML2 (obtained from Dr. Nathan Luedtke, Chemistry Department, UZH) and HOP-92 (purchased from the NCI) were cultured using RPMI medium supplemented with 10 % (v/v) fetal bovine serum. Finally, AML3, PL-21 and MOLM-13 (obtained from Dr. Nathan Luedtke, Chemistry Department, UZH) were cultured using RPMI medium supplemented with 20 % (v/v) fetal bovine serum. All the media were additionally supplemented with 100 units/mL of penicillin, 100 μg/mL of streptomycin, 4.5 g/L glucose, 0.11g/L sodium pyruvate and 2mM glutamine and the cells were grown at 37 ºC in 5 % CO_2 atmosphere with 80 % relative humidity.

MDA-MB-231, HT-29, HeLa, HOP-92 and PC-3 cells were plated at 10,000 cells per well (100 μL per well) in 96-well culture dishes and allowed to incubate for 24 h. The old media was removed, cells were washed with PBS (phosphate-buffered saline) and fresh medium was added. A 5 mM solution of inhibitor (in 100% DMSO) was serially diluted in the culture media (8 different concentrations were used) and allowed to incubate for 72 h (MDA-MD-231, HT-29, HOP-92, HL-60, ML2, AML3, PL-21, MOLM-13 and PC-3) or 48h (HeLa and K562). Control cells were treated with the same DMSO concentrations. After the incubation period the medium was removed, cells were washed with PBS to be then incubated with fresh medium containing 86 nM resazurin. Resazurin is reduced to the fluorescent resorufin in the mitochondria: the fluorescence intensity upon incubation in the presence of a living cell culture thus directly correlates with the metabolic viability of the cells. Fluorescence was quantified after 4 hours using a fluorescence microplate reader (Biotek, FLx800™, excitation and emission wavelengths 560 and 590 nm, respectively). The measured fluorescence values were corrected from the control samples containing DMSO.

Leukemia cell lines (K562, HL-60, ML2, AML3, PL-21 and MOLM-13) were seeded at a density of 20,000 cells per well in 100 μL of RMPI media in 96 well microtiter plates. After 24 hours, 12.5 μL of a 10 fold concentrated drug (or DMSO solution for the control) in RMPI media was added in every well. After 48 hour incubation, resazurin was added to every well to obtain a final concentration of 86 nM and, after 3 hours, cell viability was assessed by measuring the ability of the

cells to process resazurin by quantifying the fluorescence using a fluorescence microplate reader (Biotek, FLx800^{TM}) as described above.

Table S3. Resazurin reduction (percentage of the control) upon incubation of compounds **6**, **9**, **10**, **15**, **19-23** for 48 or 72 h at a concentration of 50 µM in eleven different cancer cell lines. This preliminary screening of toxicity was done as a single experiment. NM indicates no metabolic activity change in comparison to the DMSO-treated cells.

	MDA-MD-231	**HT-29**	**HeLa**	**HOP-92**	**K562**	**HL-60**	**ML2**	**AML3**	**PL-21**	**MOLM-13**	**PC-3**
6	NM	NM	NM	80.3	98.3	77.1	98.3	86.8	90.2	65.2	87.7
9	90.7	NM	94.8	91.0	NM	62.8	NM	87.3	74.7	15.1	84.3
10	NM	NM	97.6	71.9	NM	87.8	97.3	90.0	86.0	NM	89.1
15	86.7	NM	93.2	97.8	81.3	43.2	64.1	63.0	59.4	50.2	94.1
19	NM	NM	84.5	65.3	84.8	42.1	53.2	68.9	69.2	49.4	65.0
20	84.3	NM	94.2	72.8	NM	90.3	NM	98.7	NM	76.3	99.9
21	94.4	NM	90.4	77.3	NM	84.7	NM	96.9	94.3	73.2	81.8
22	88.2	NM	89.3	78.3	90.8	45.7	26.5	69.9	70.5	43.0	64.1
23	NM	NM	NM	NM	99.2	68.6	NM	84.2	86.3	74.8	69.5

Table S4. Metabolic activity values (%) on three different leukemia cell lines at 50 µM compound concentration.[a]

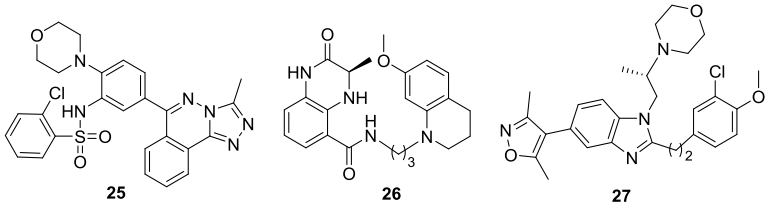
Cmpd	HL-60		ML2		MOLM-13	
	Acid	Ester	Acid	Ester	Acid	Ester
6	80	78	93	75	71	46 (46 µM)[b]
9	78	89	66	97	19 (33 µM)[b]	82
10	89	89	100	86	89	88
11	77	85	88	99	73	76
15	59	-	107	-	57	-
19	59	96	91	85	60	90
20	89	-	102	-	91	-
21	81	74	104	73	85	66
22	65	73	93	36 (14.4 µM)[b]	61	11 (5.3 µM)[b]
23	82	88	66	60	60	26 (37 µM)[b]

[a] Metabolic activity values were determined using resazurin reduction after 72 h incubation with the corresponding compound in comparison to DMSO treated cells at eight different concentrations (100-0.8 µM). The % of metabolic activity at 50 µM compound concentration is shown. The % of metabolic activity is given as the mean of at least three independent experiments. Variability around

the mean value was < 26 % in all cases. [b] Values in parentheses are GI_{50} values as determined by triplicate experiments with less than 30 % standard error.

11. Comparison table to known CREBBP ligands

Table S5. Activity and selectivity of acyl benzene derivatives and comparison with previously reported CREBBP bromodomain ligands.

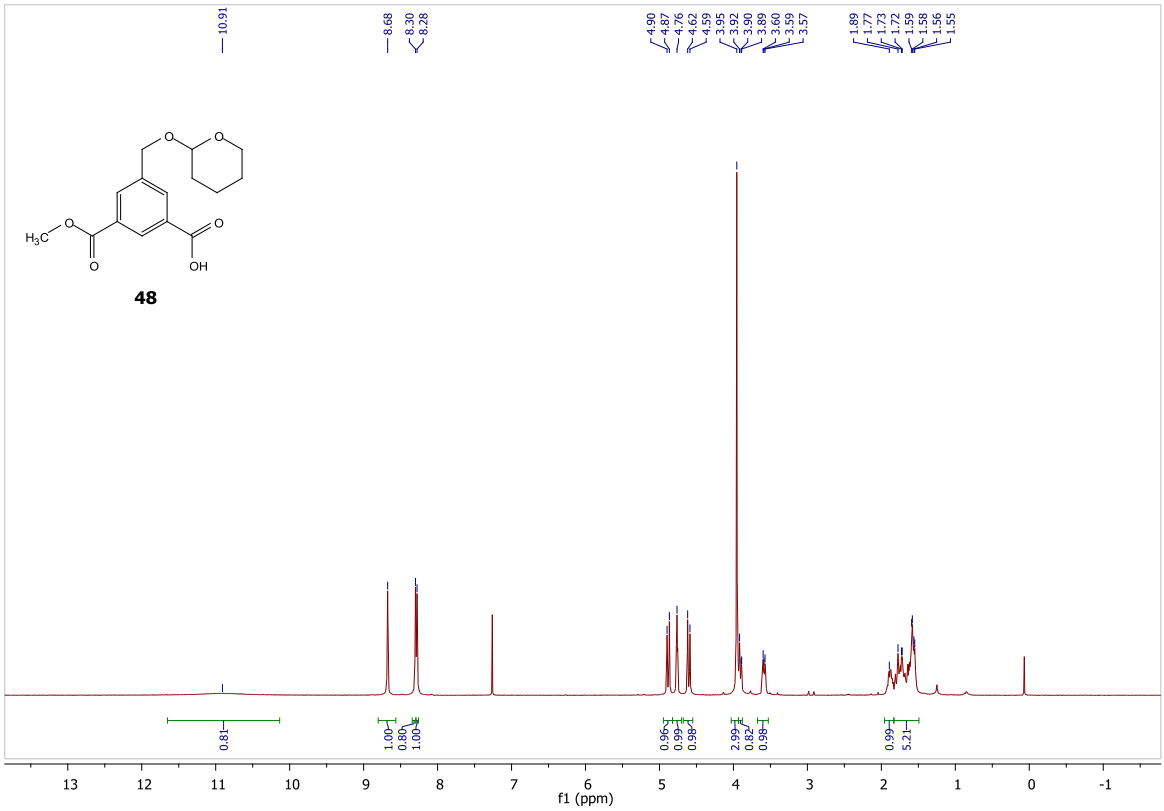


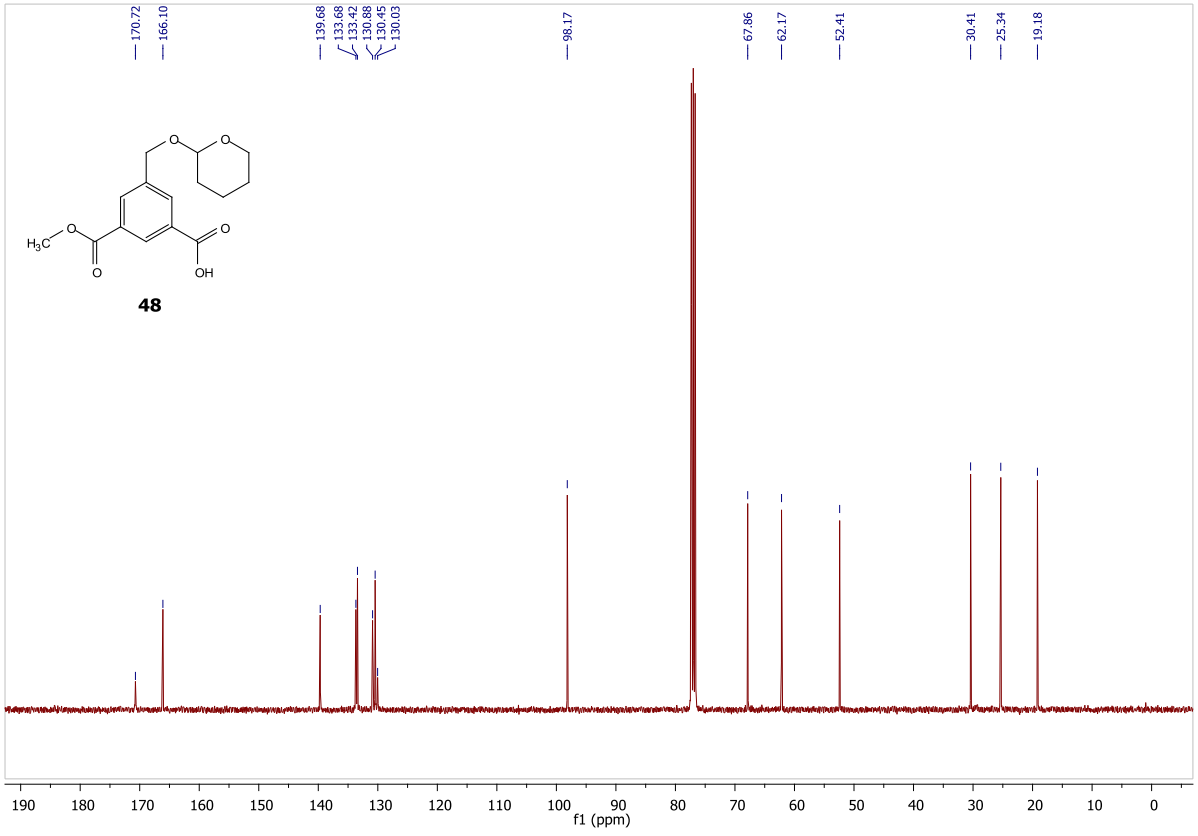
Cmpd	LE[a]	LLE	K_d (μM) ITC	K_d (μM) competition binding assay[32-33]					ΔT_m (°C)[b]		
			CBP	CBP	BRD4(1)	BRD4(2)	BRD2(1,2)	S[c]	CBP	EP300	BRD4(1)
This study											
6	0.35	3.7	2.0	0.77	>50	>50	>50	>65	3.6	3.4	0.5
19	0.32	3.2	0.3	0.17	10	36	36	59	4.9	5.9	1.7
21	0.32	4.0	0.8	0.54	26	>50	>50	48	5.0	4.6	1.3
22	0.28	3.8	–	0.40	-	-	-	-	6.0	5.9	1.6
Reported by others (main text, Figure 3, bottom)											
25[34]	0.25	2.4	–	0.20[d]	0.16[d]	-	-	0.8	7.6	–	4.4
26[35]	0.29	4.3	0.3	–	1.38[e]	-	-	4	5.4	–	–
27[36]	0.27	2.0	0.021	0.080[d]	0.85	5.2	-	40	9.7	9.7	1.8

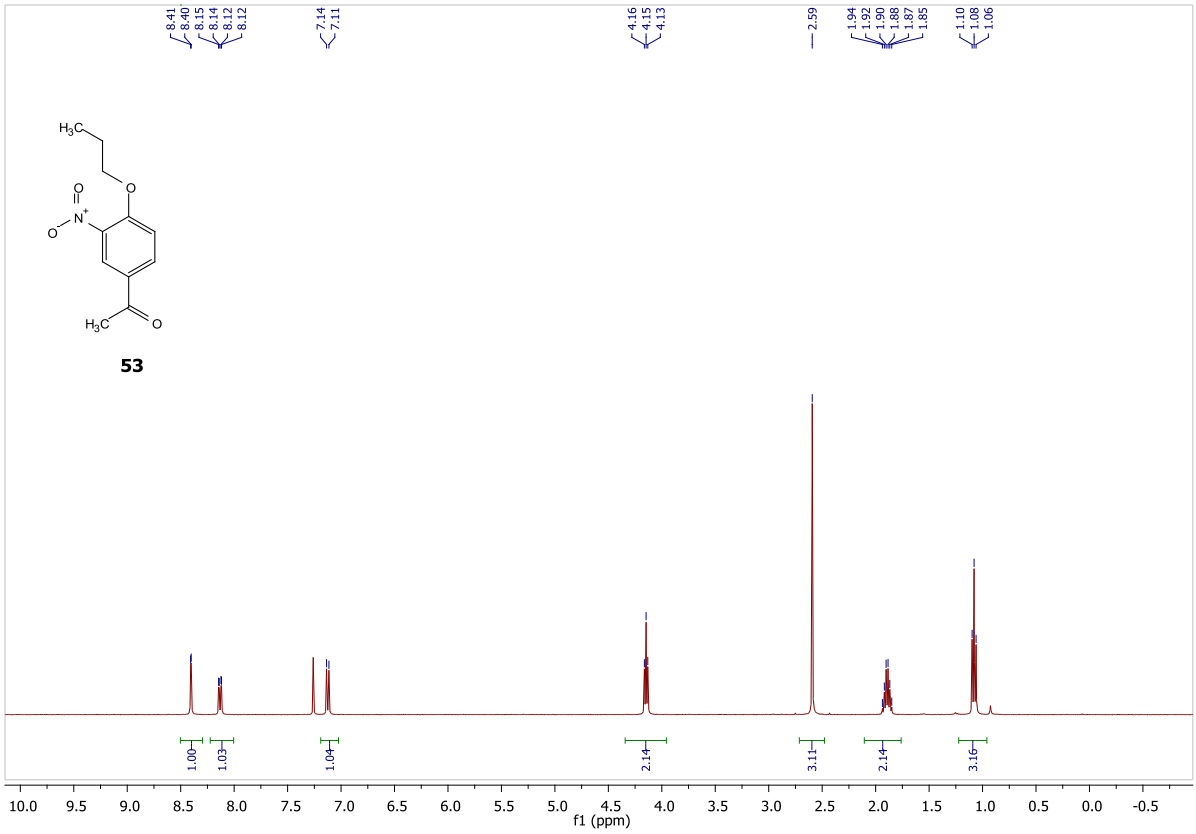
[a] LE = ligand efficiency calculated as (ΔG/number of heavy atoms) is reported in kcal/mol per heavy atom; LLE = lipophilic ligand efficiency (calculated as pK_d-*c*logP),[37-38] clogP was calculated using ChemDraw. [b] Median value of the shift in the melting temperature (number of measurements between 9 and 38 per compound/protein pair). The largest SEM was 0.5 °C and most SEM values were below 0.2 °C. [c] Selectivity (S) between the CREBBP and BRD4(1) bromodomains determined by the ratio of K_d values obtained via the competition binding assay. It was not possible to calculate the selectivity using the K_d values obtained via ITC due to the impossibility to reliably fit the titration curves for the BRD4(1) bromodomain. [d] Potency determined by AlphaScreen. [e] Potency determined by Isothermal Titration Calorimetry (ITC).

12. NMR traces of selected compounds

12.1 Intermediate compounds



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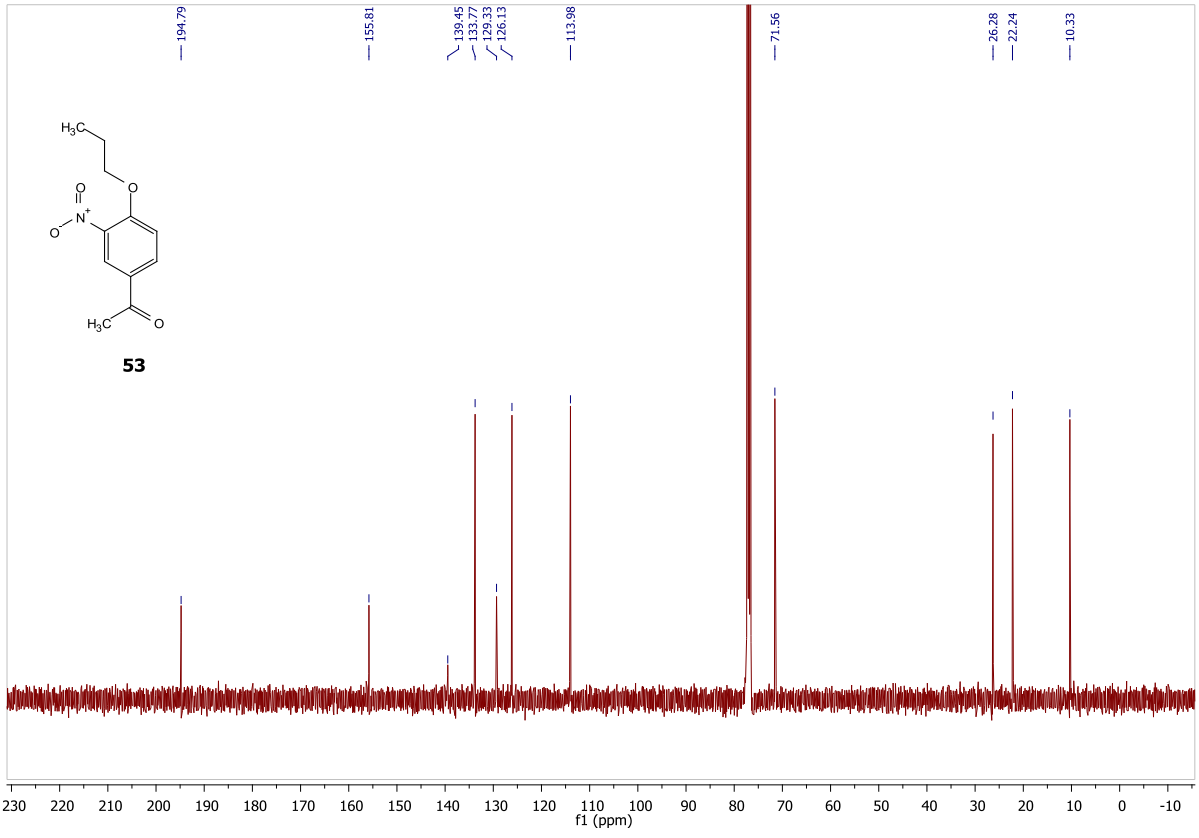
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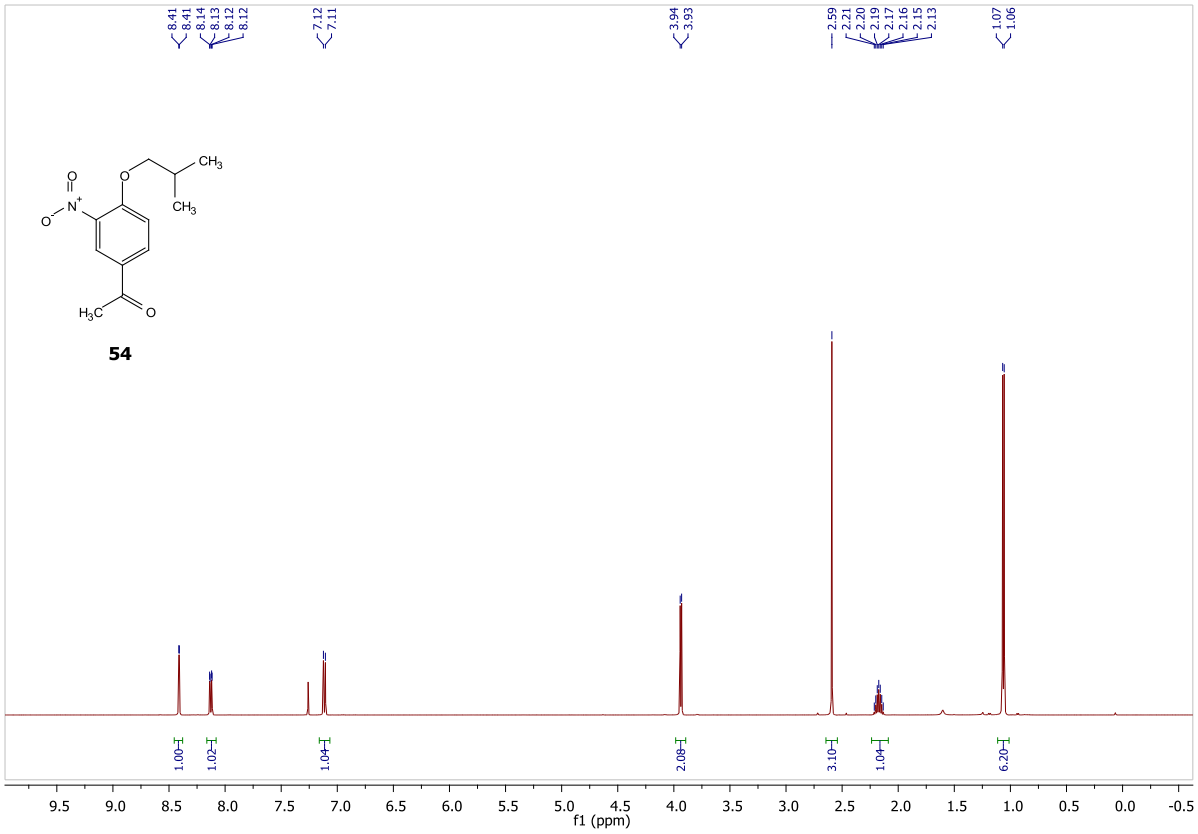
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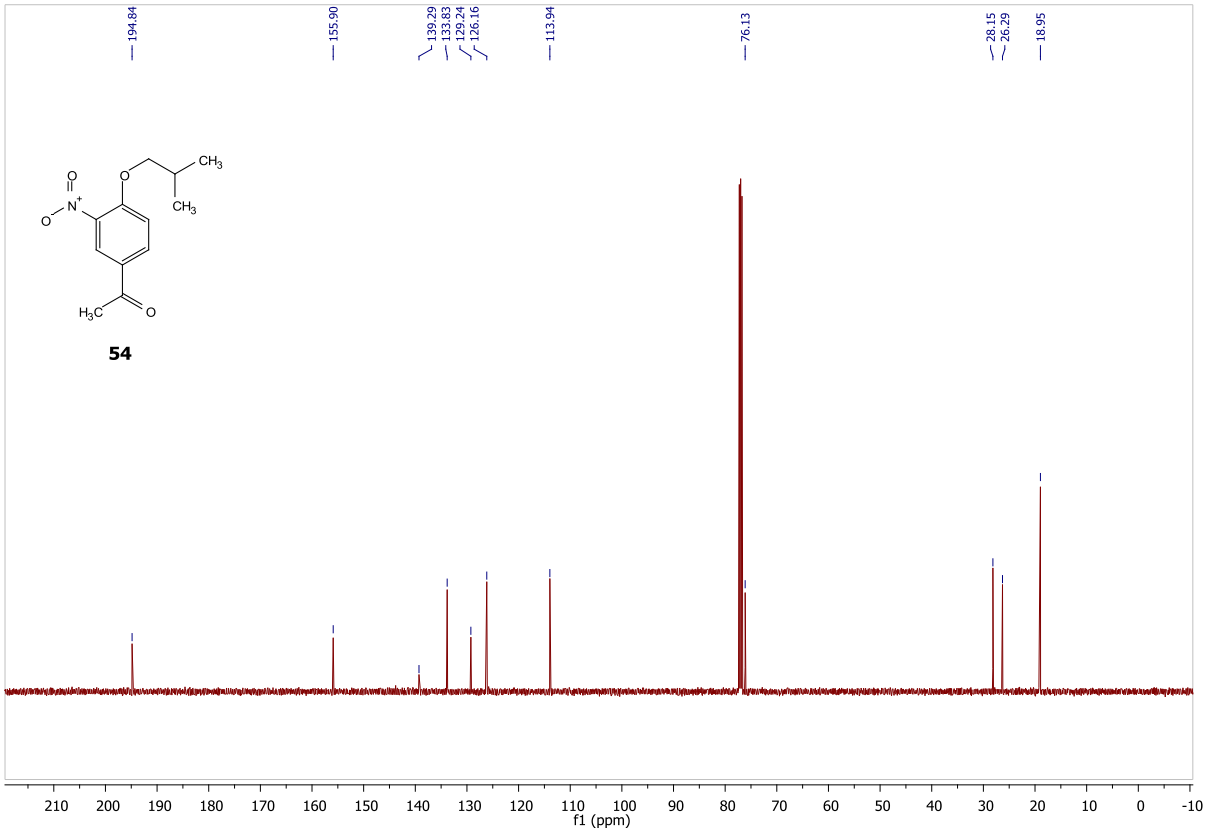
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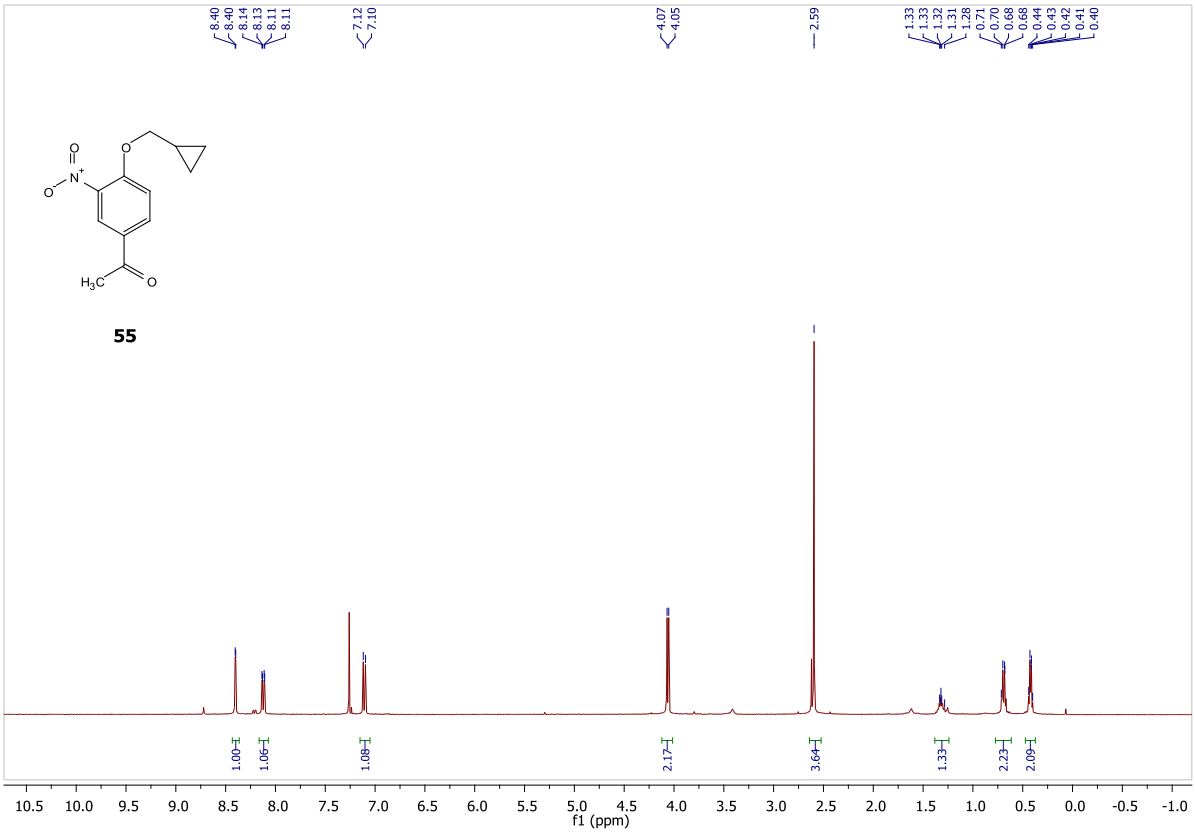
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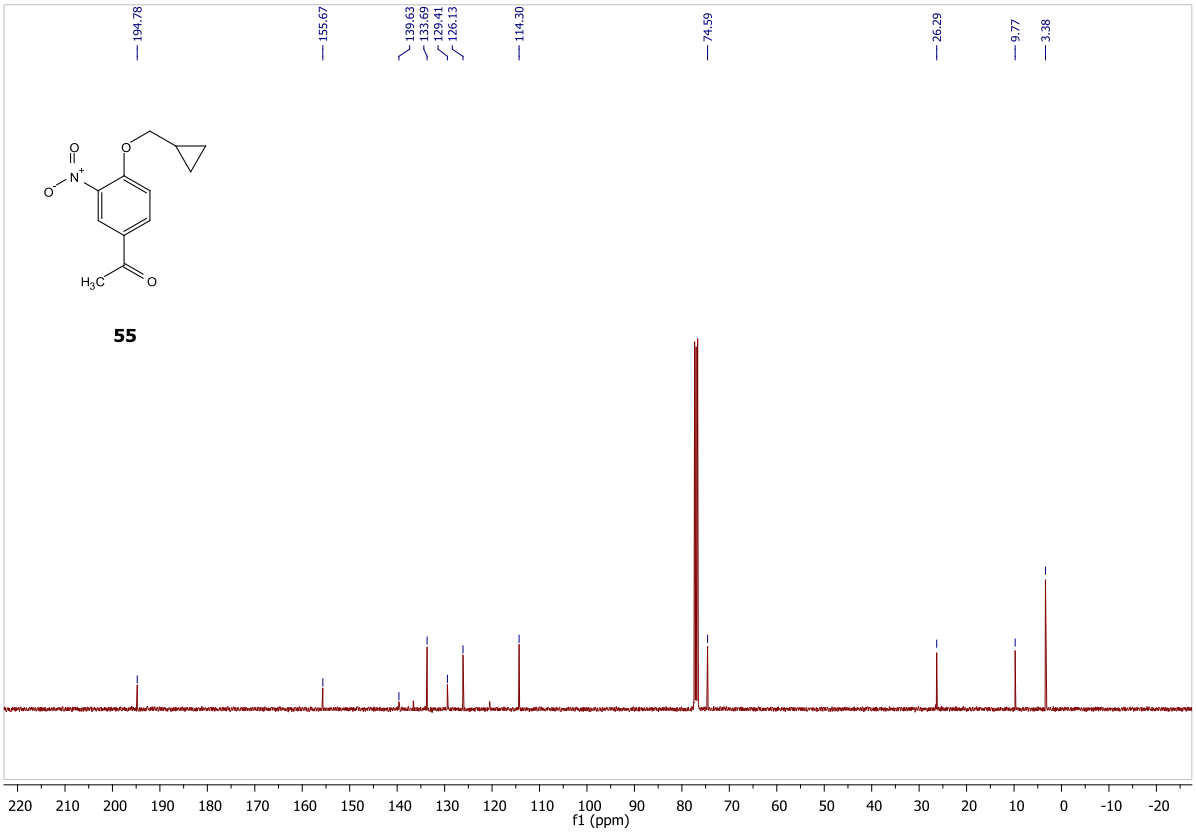
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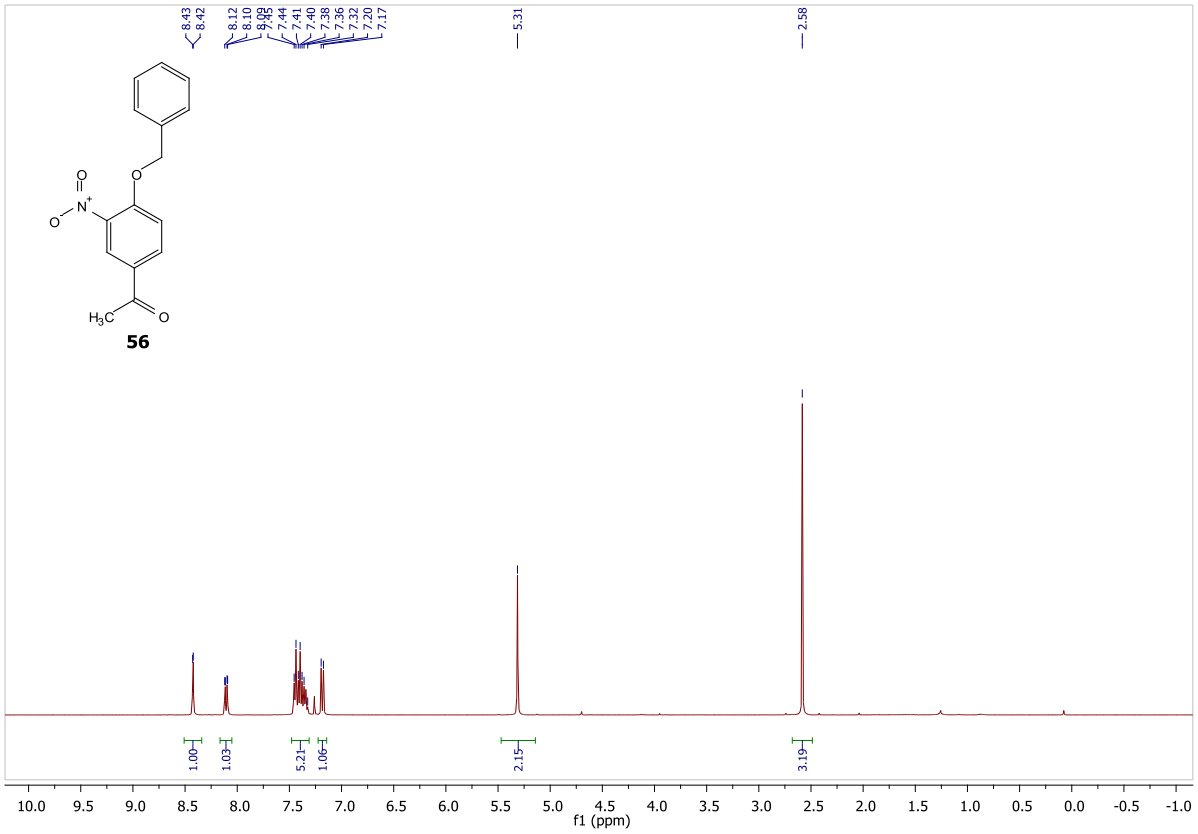
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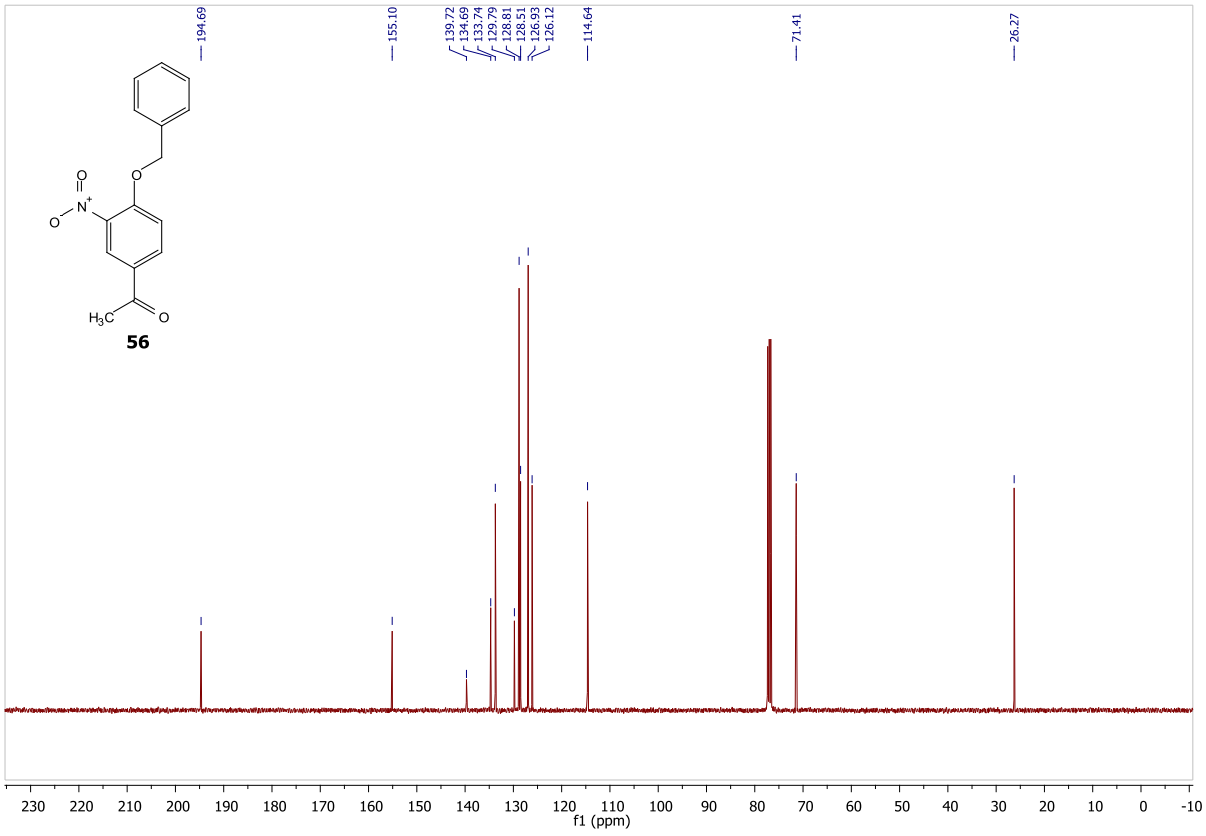
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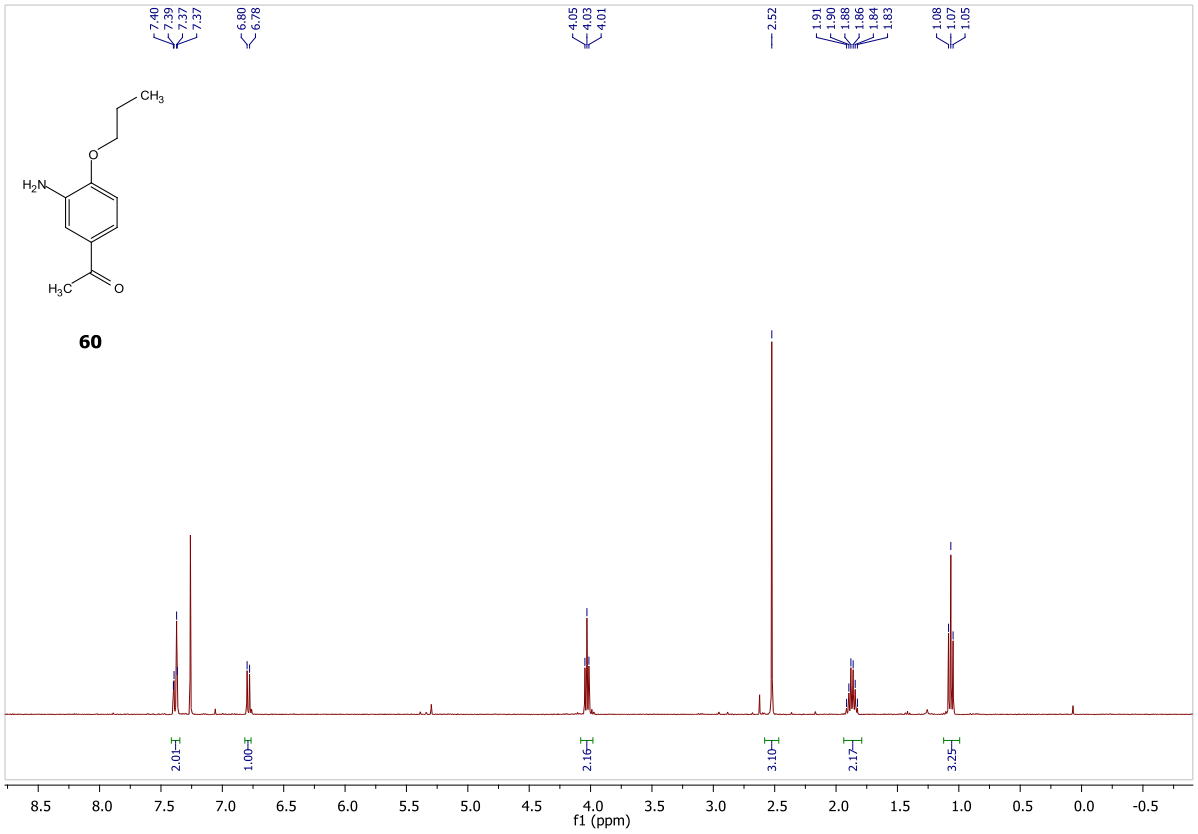
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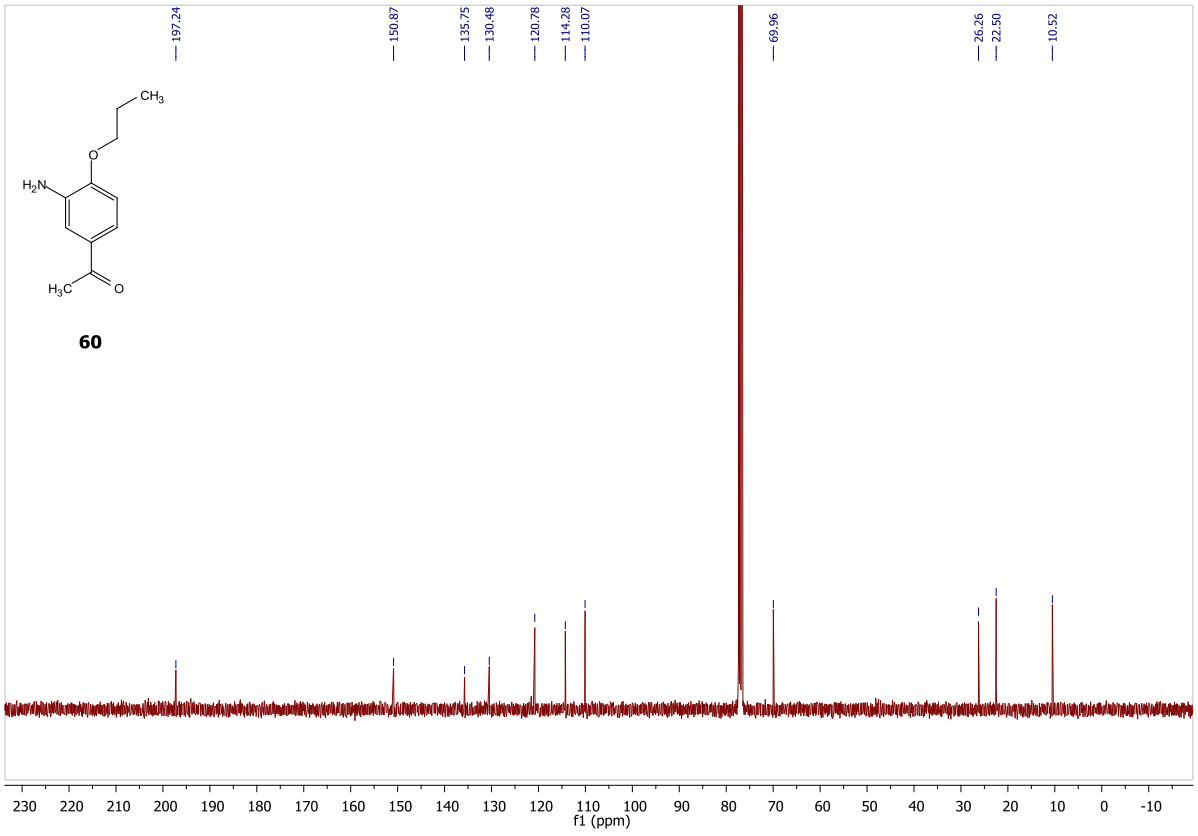
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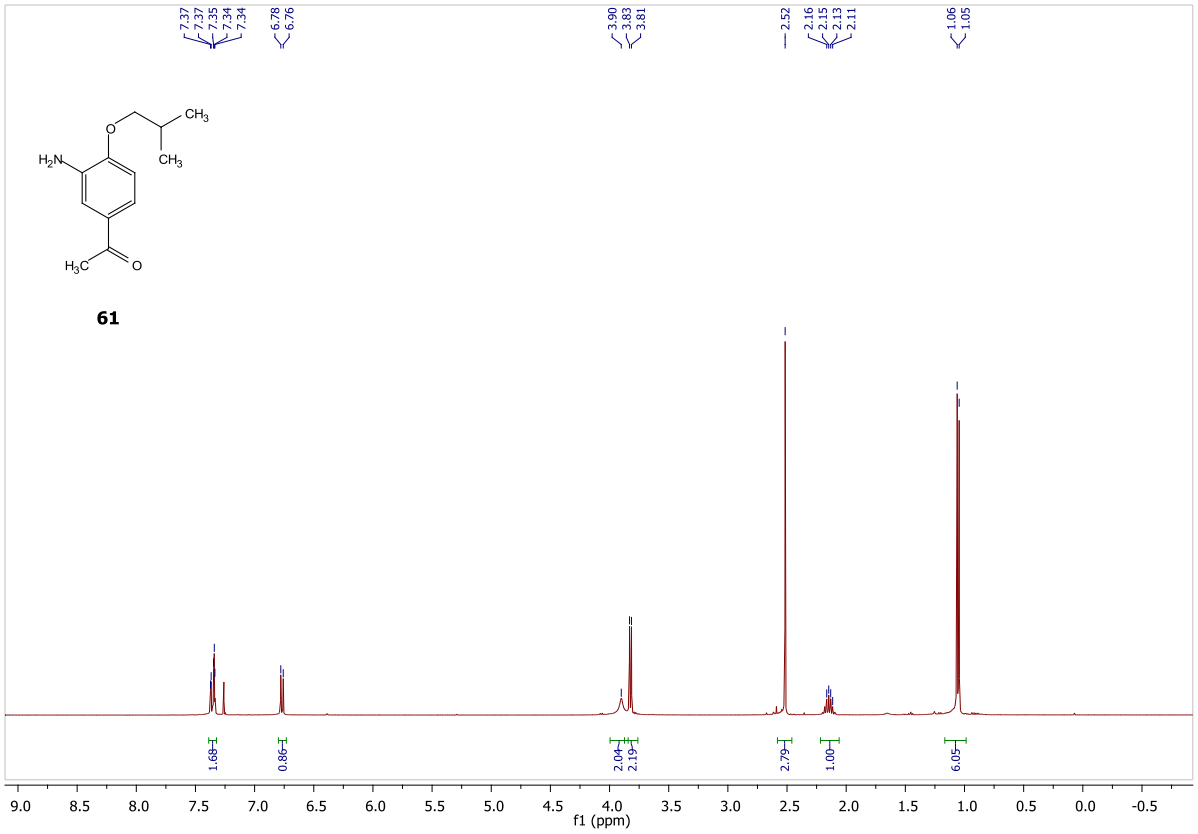
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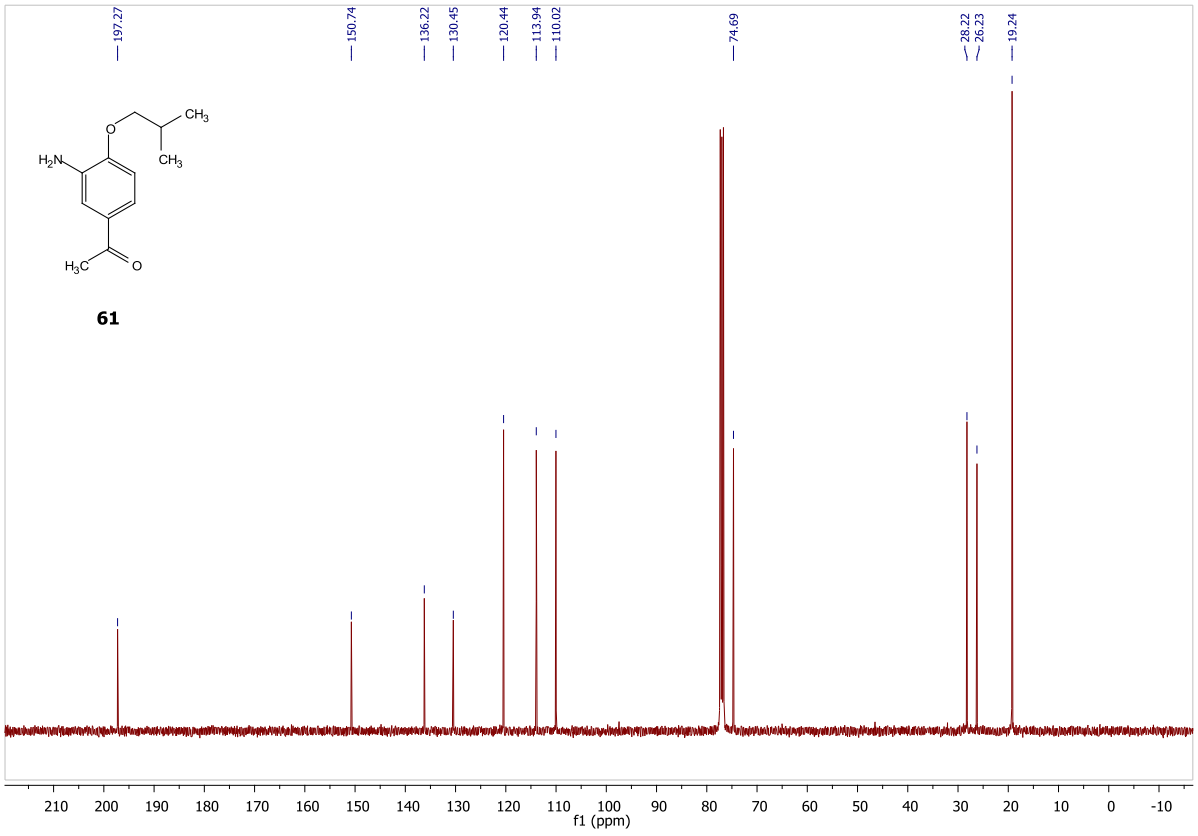
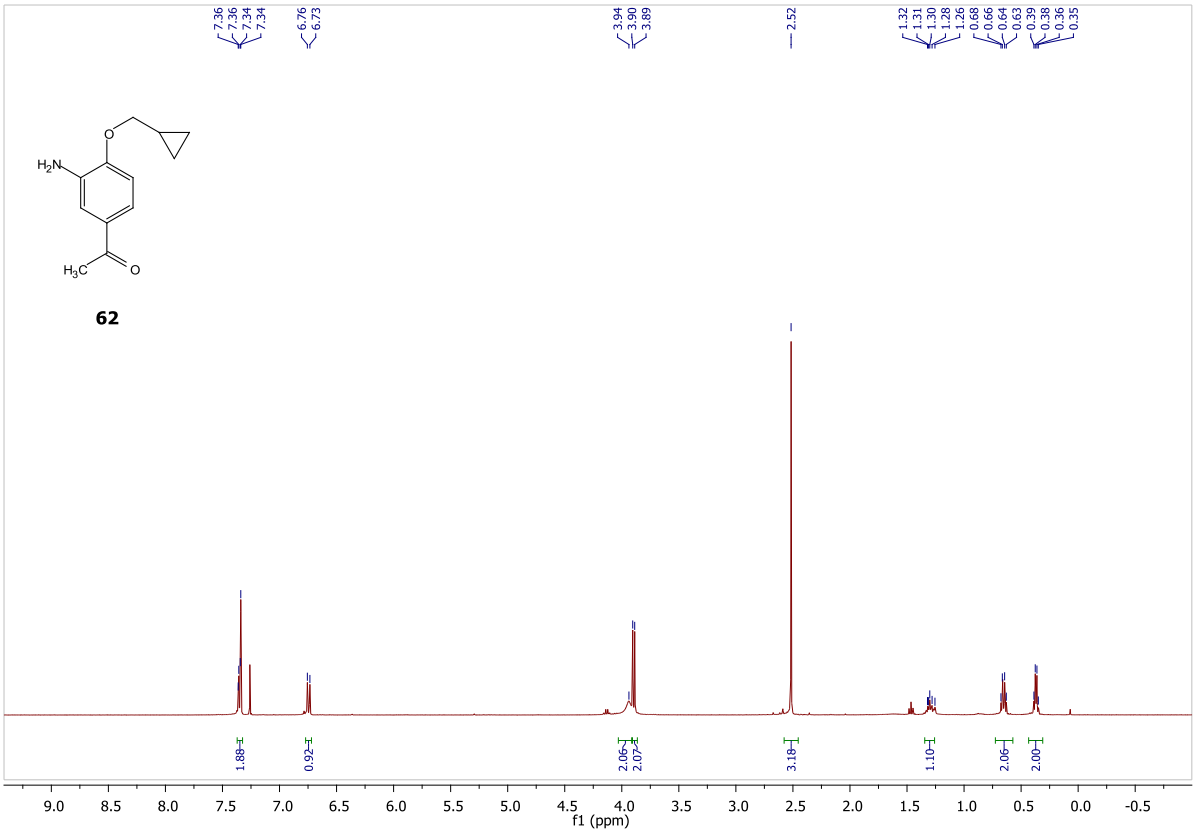
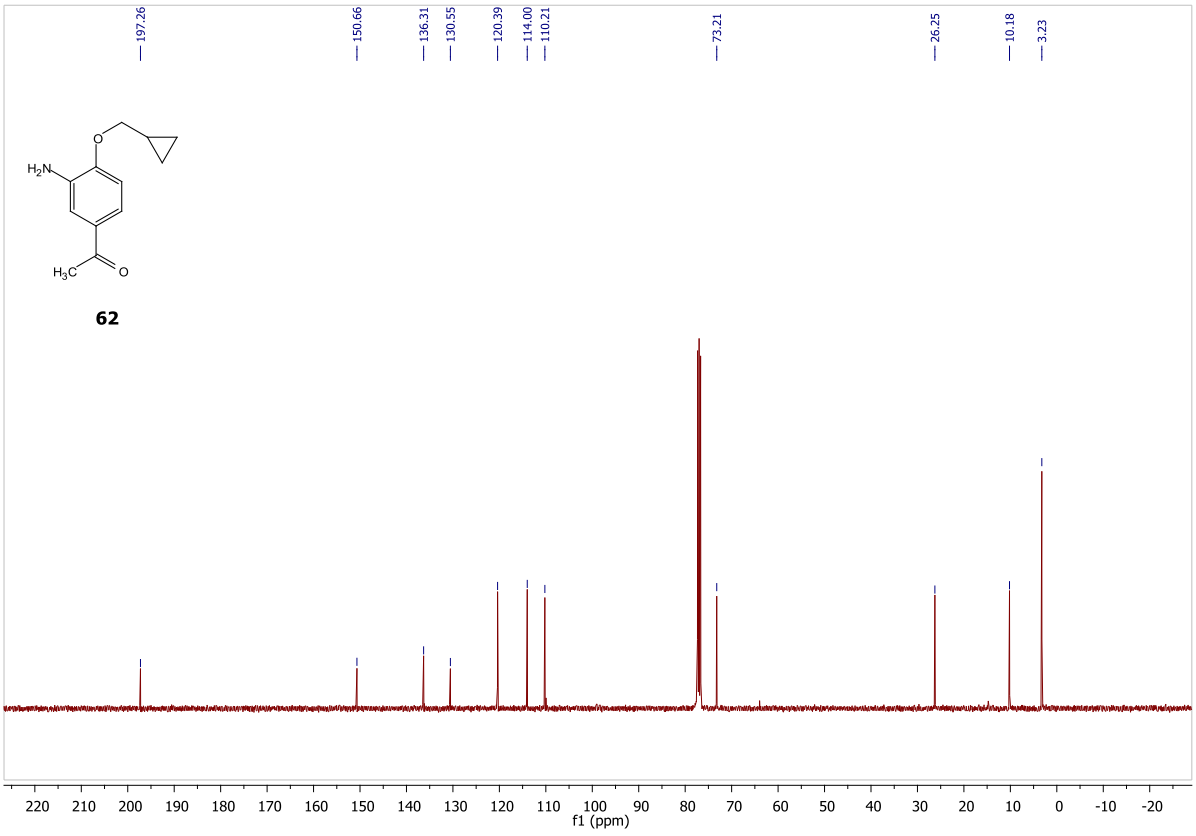
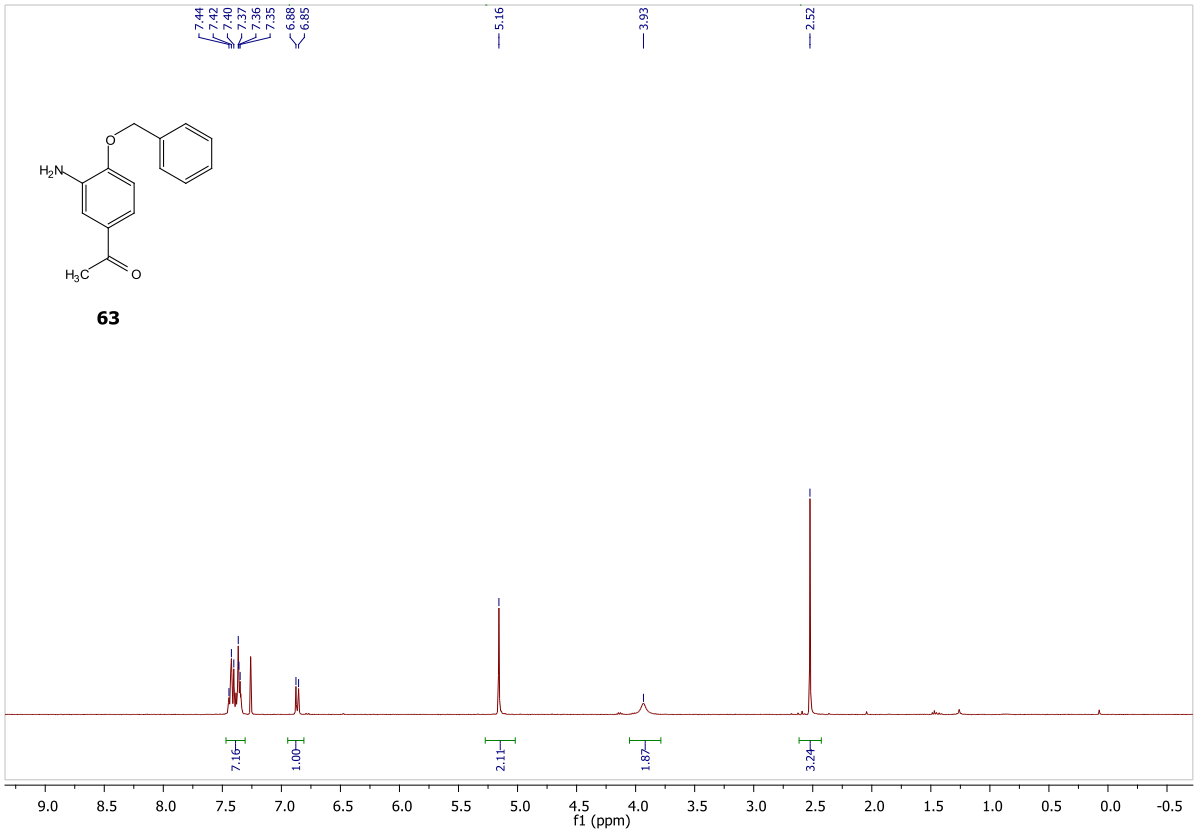

197.27
150.74
136.22
130.45
120.44
113.94
110.02
74.69
28.22
26.23
19.24
CH3
O
H2N
CH3
H3C
O
61
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

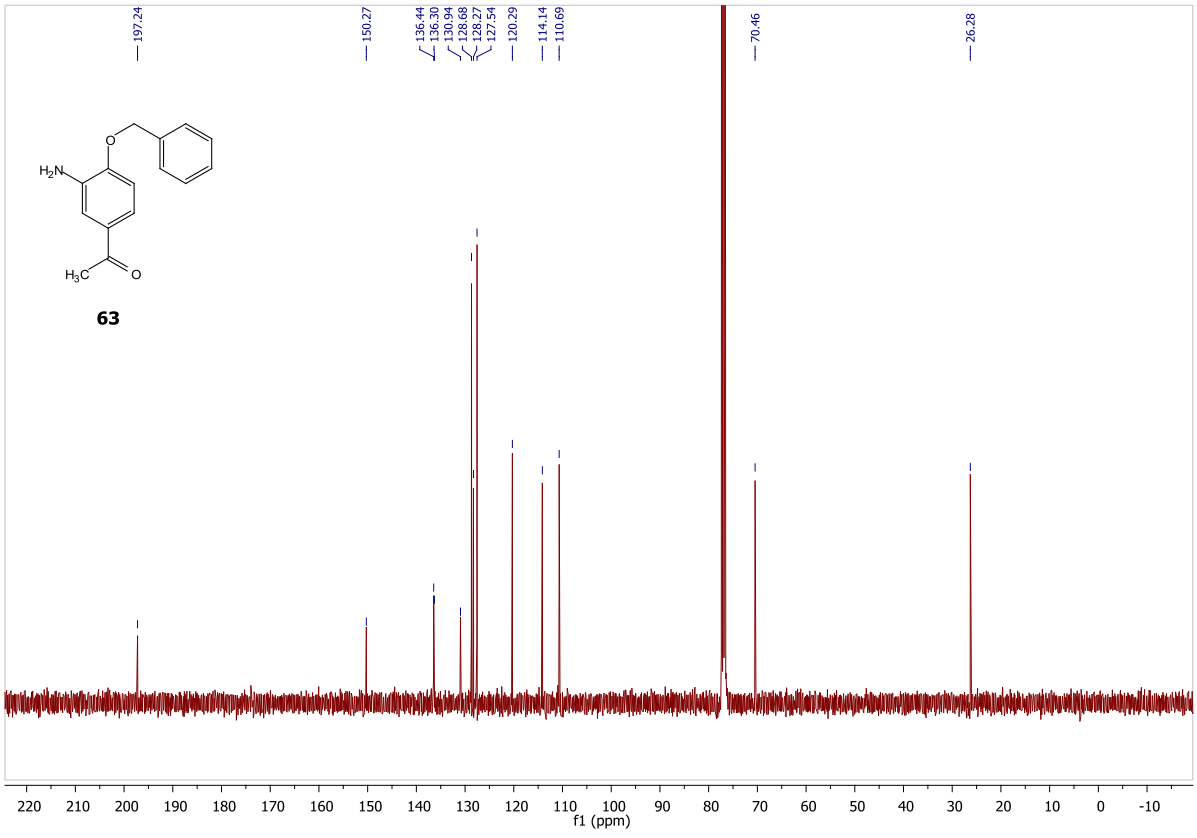

7.36
7.36
7.34
7.34
6.76
6.73
3.94
3.90
3.89
2.52
1.32
1.31
1.30
1.28
1.26
0.68
0.66
0.64
0.63
0.39
0.38
0.36
0.35
O
H2N
H3C
O
1.88
0.92
2.06
2.07
3.18
1.10
2.06
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

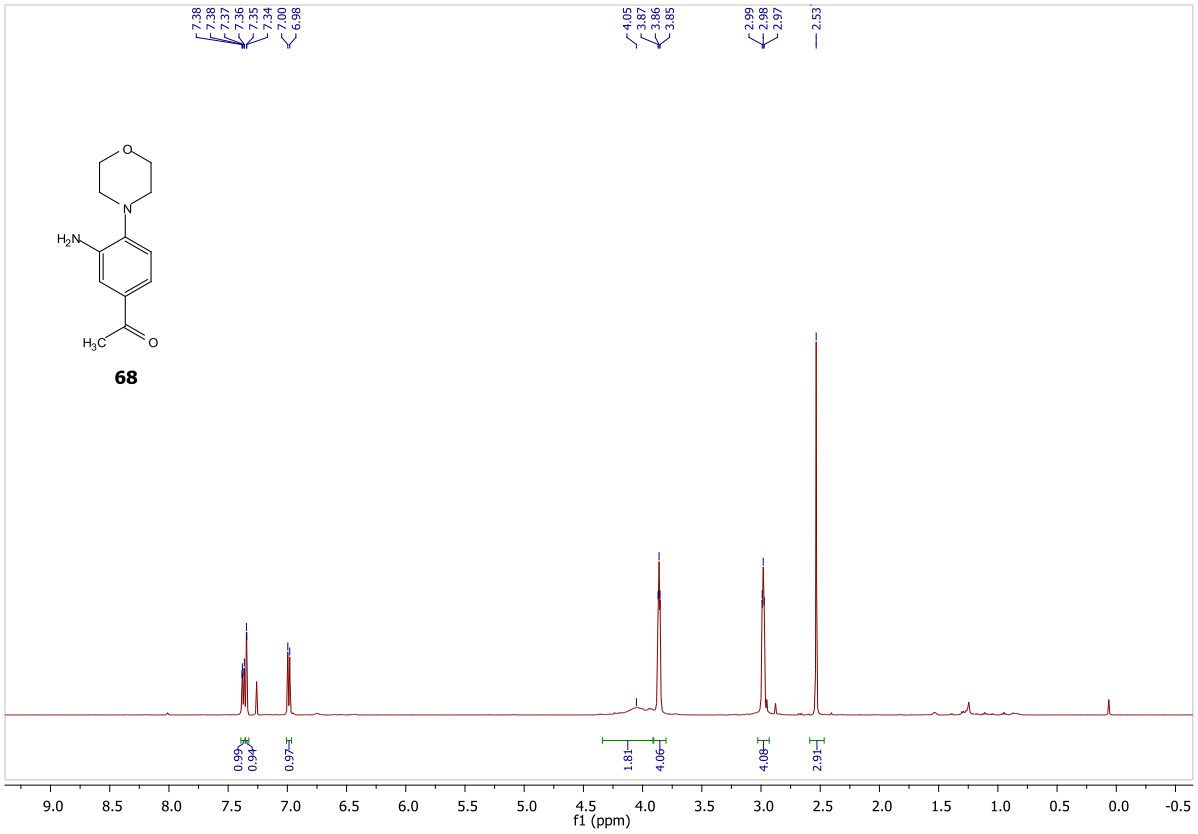
62

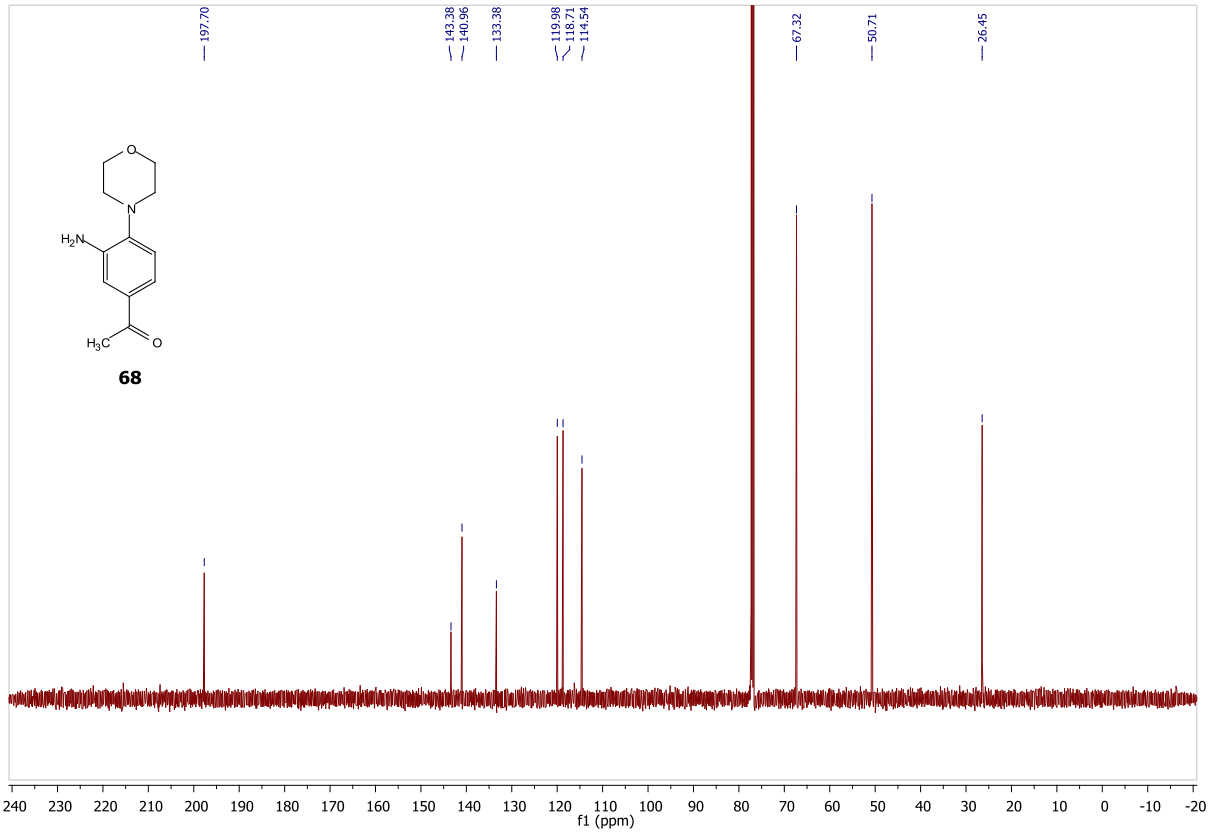

197.26
150.66
136.31
130.55
120.39
114.00
110.21
73.21
26.25
10.18
3.23
O
H2N
H3C
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

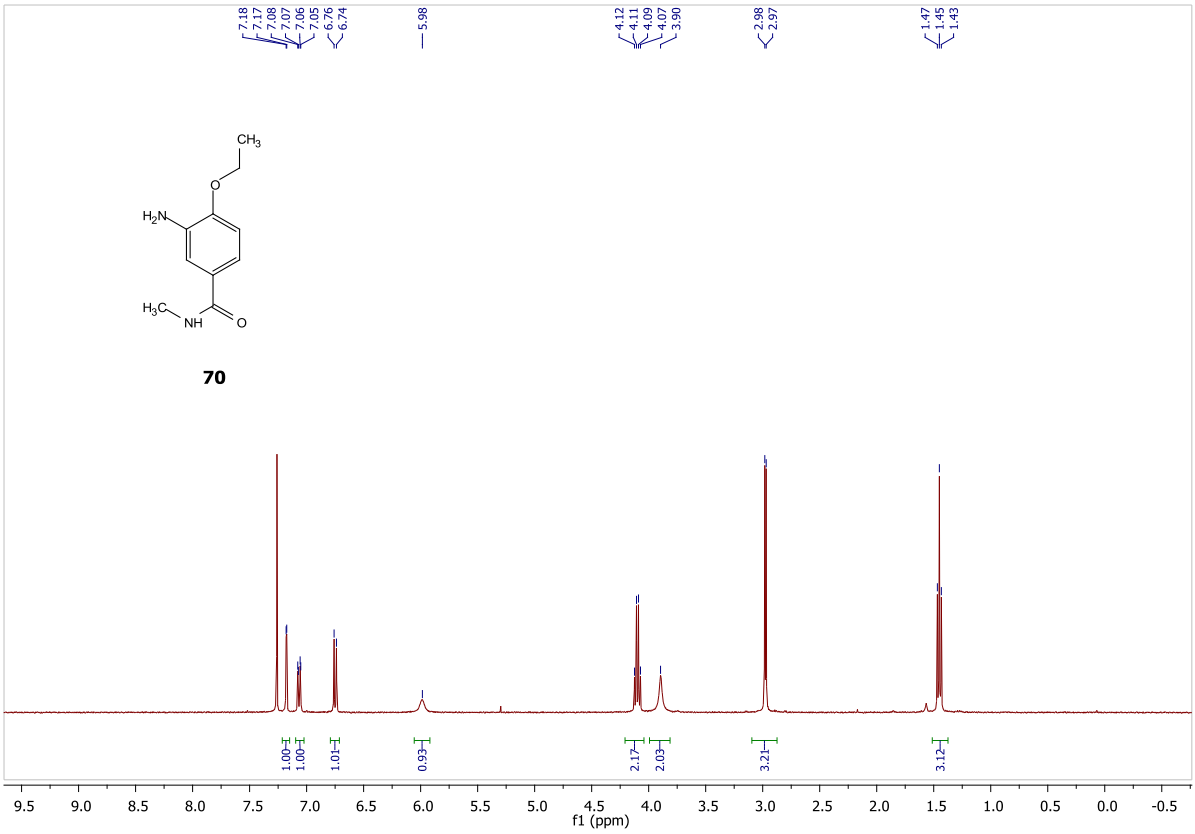
62

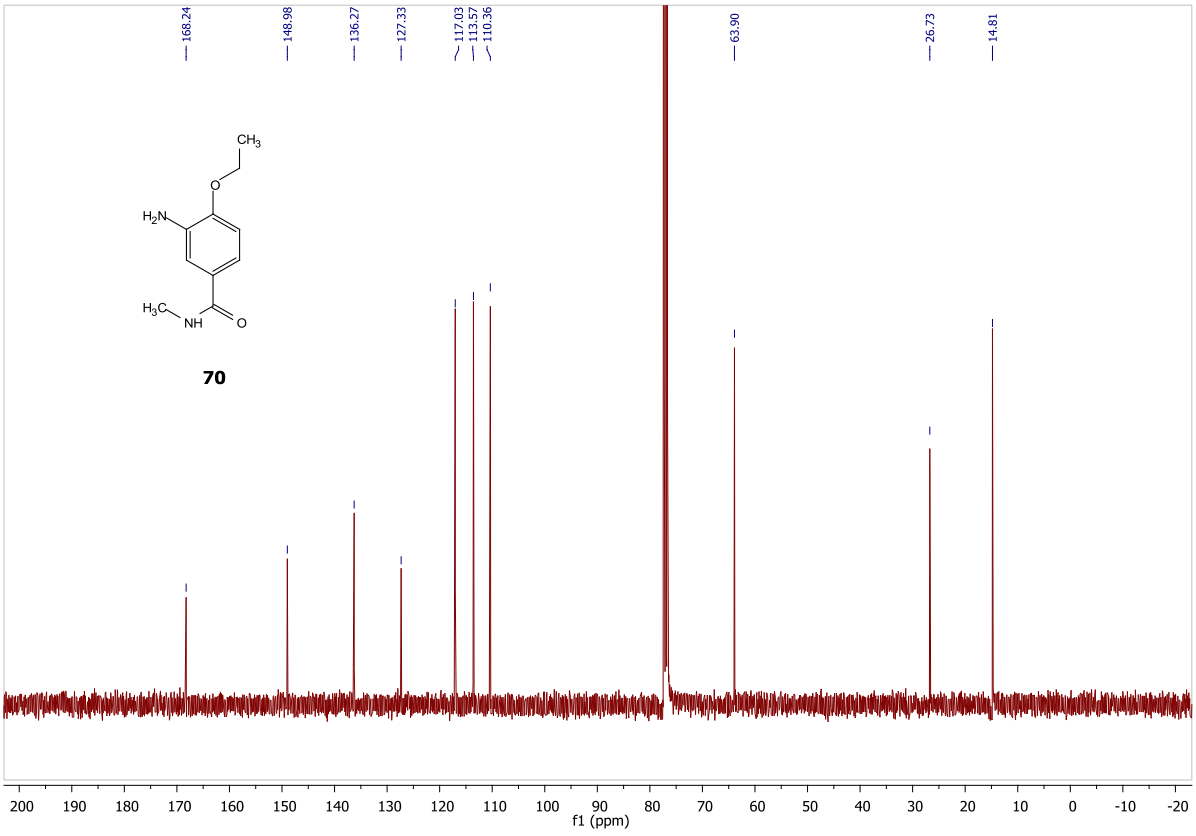

7.44
7.42
7.40
7.37
7.36
7.35
6.88
6.85
5.16
3.93
2.52
O
H2N
H3C
O
63
7.16
1.00
2.11
1.87
3.24
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

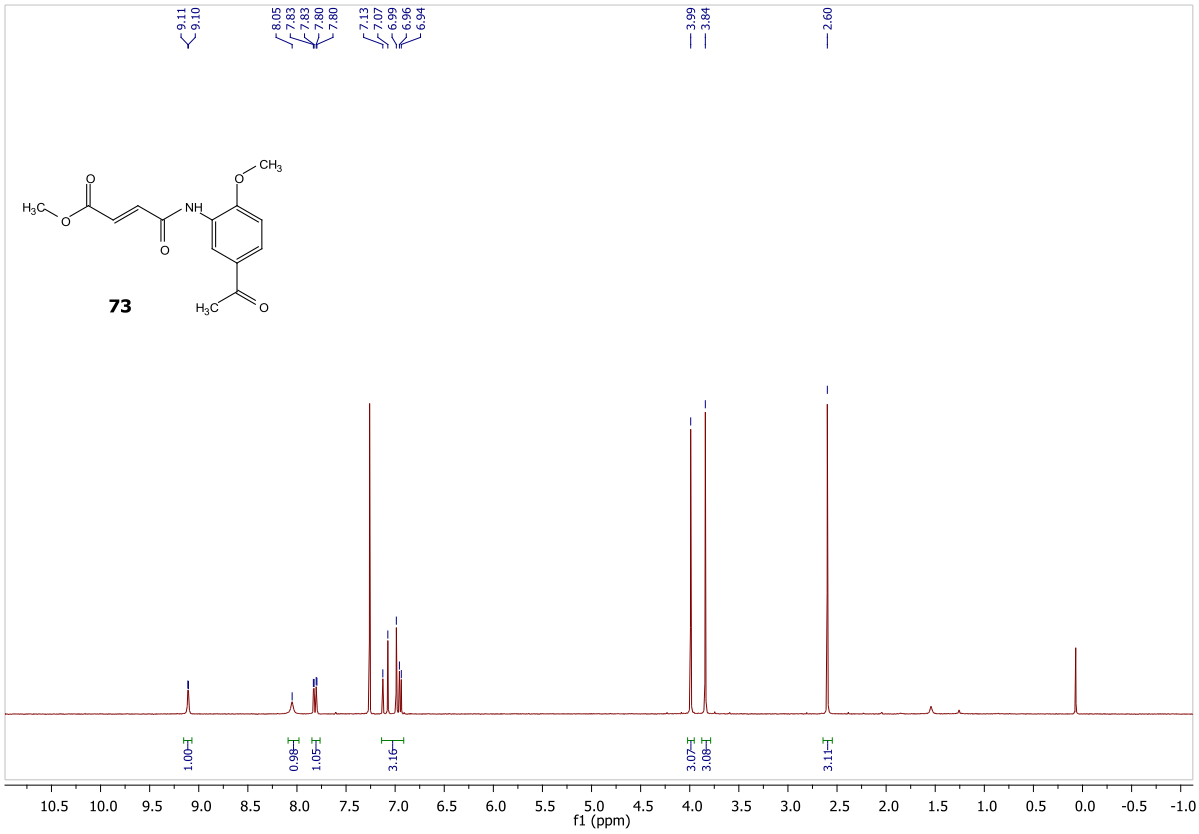

197.24
150.27
136.44
136.30
130.94
128.68
128.27
127.54
120.29
114.14
110.69
70.46
26.28
O
H2N
H3C
O
63
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

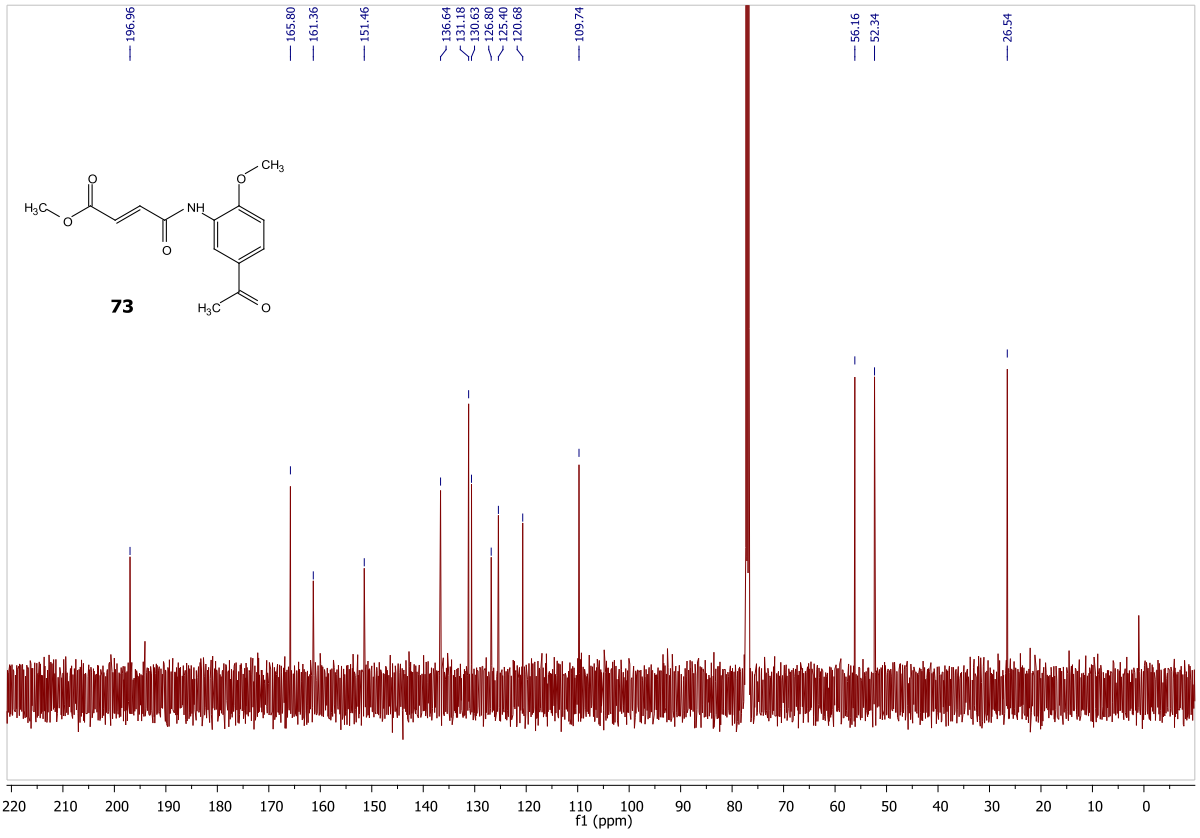

7.38
7.38
7.37
7.36
7.35
7.34
7.00
6.98
4.05
3.87
3.86
3.85
2.99
2.98
2.97
2.53
O
N
H2N
H3C
O
68
0.99
0.94
0.97
1.81
4.06
4.08
2.91
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

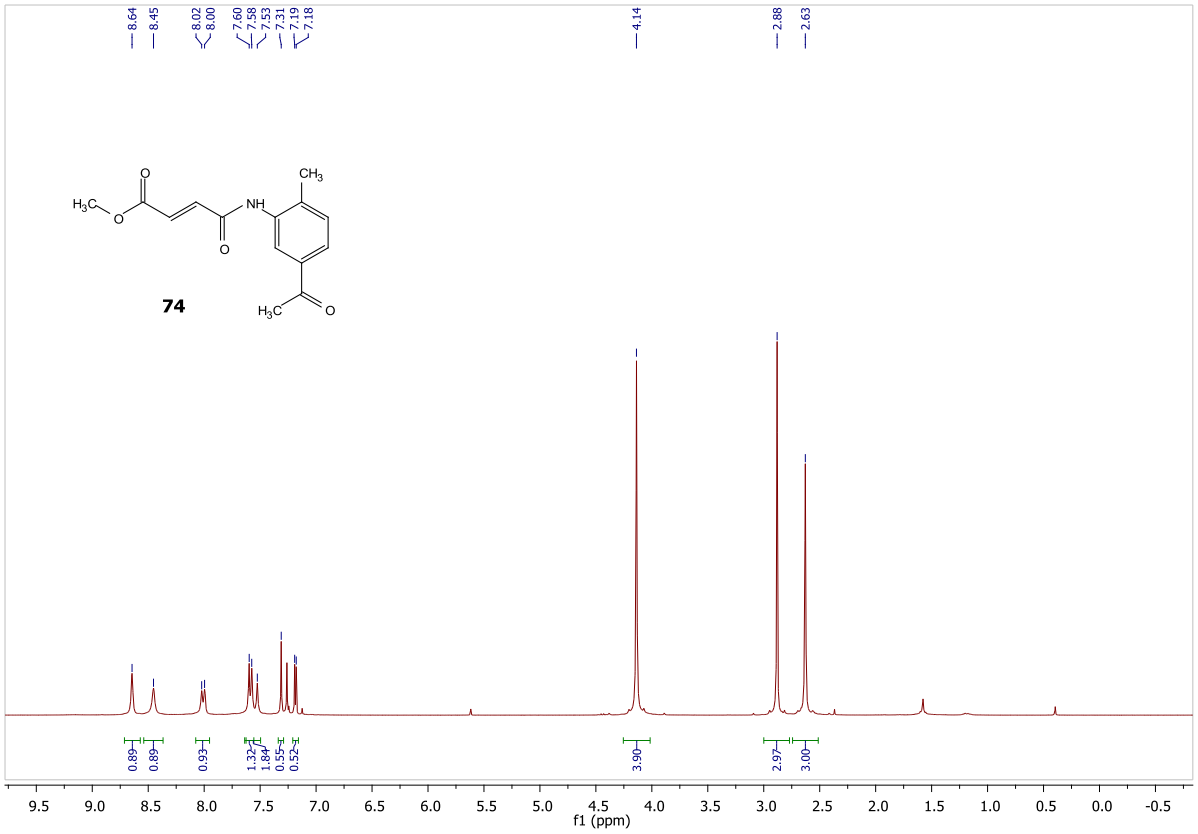

197.70
143.38
140.96
133.38
119.98
118.71
114.54
67.32
50.71
26.45
O
N
H2N
H3C
O
68
240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

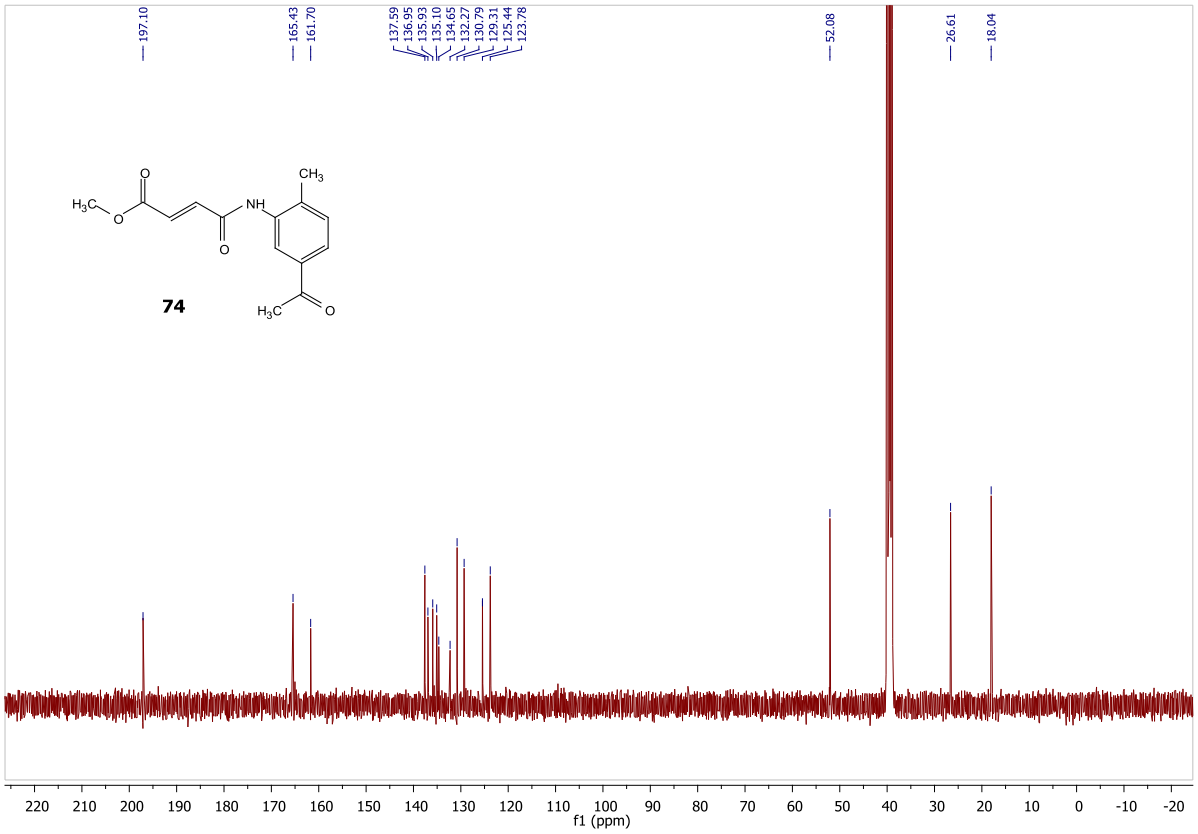

7.18
7.17
7.08
7.07
7.06
7.05
6.76
6.74
5.98
4.12
4.11
4.09
4.07
3.90
2.98
2.97
1.47
1.45
1.43
CH3
O
H2N
H3C
NH
O
70
1.00
1.00
1.01
0.93
2.17
2.03
3.21
3.12
f1 (ppm)

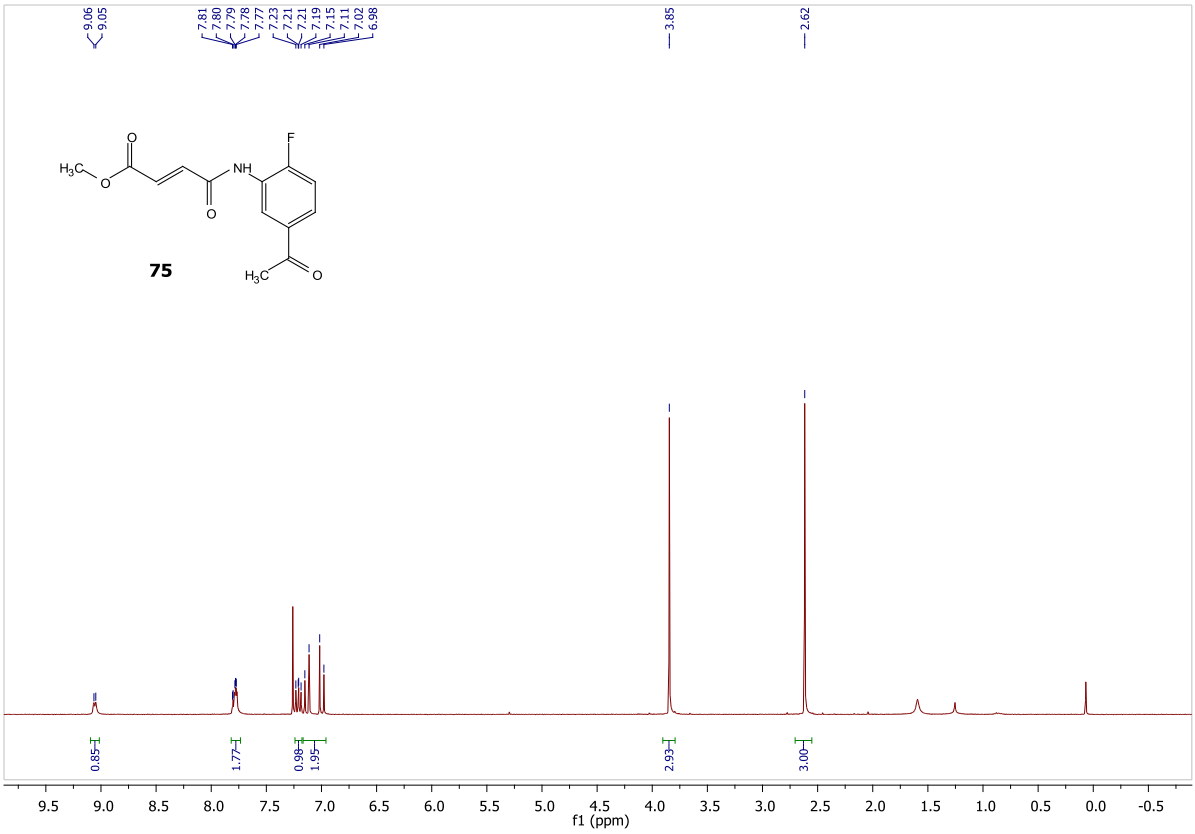

168.24
148.98
136.27
127.33
117.03
113.57
110.36
63.90
26.73
14.81
CH3
O
H2N
H3C
NH
O
70
f1 (ppm)

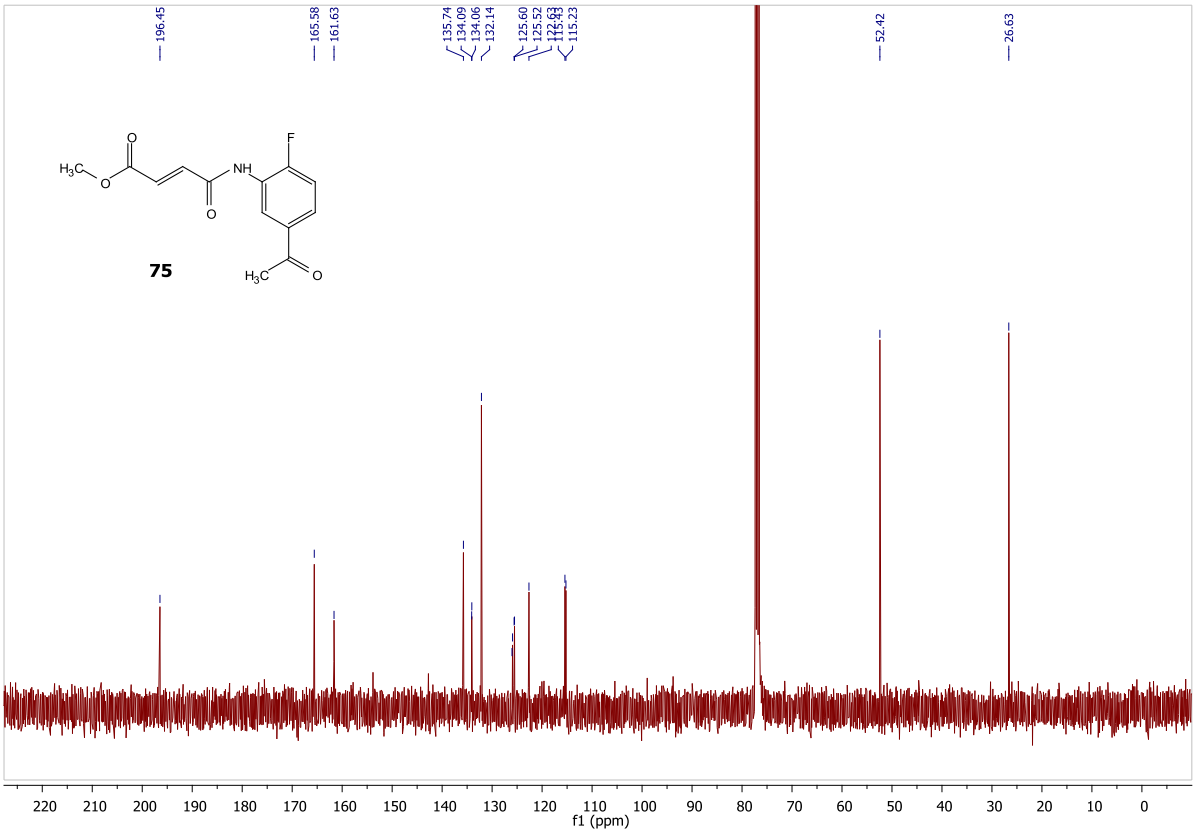

9.11
9.10
8.05
7.83
7.83
7.80
7.80
7.13
7.07
6.99
6.96
6.94
3.99
3.84
2.60
1.00
0.98
1.05
3.16
3.07
3.08
3.11
f1 (ppm)

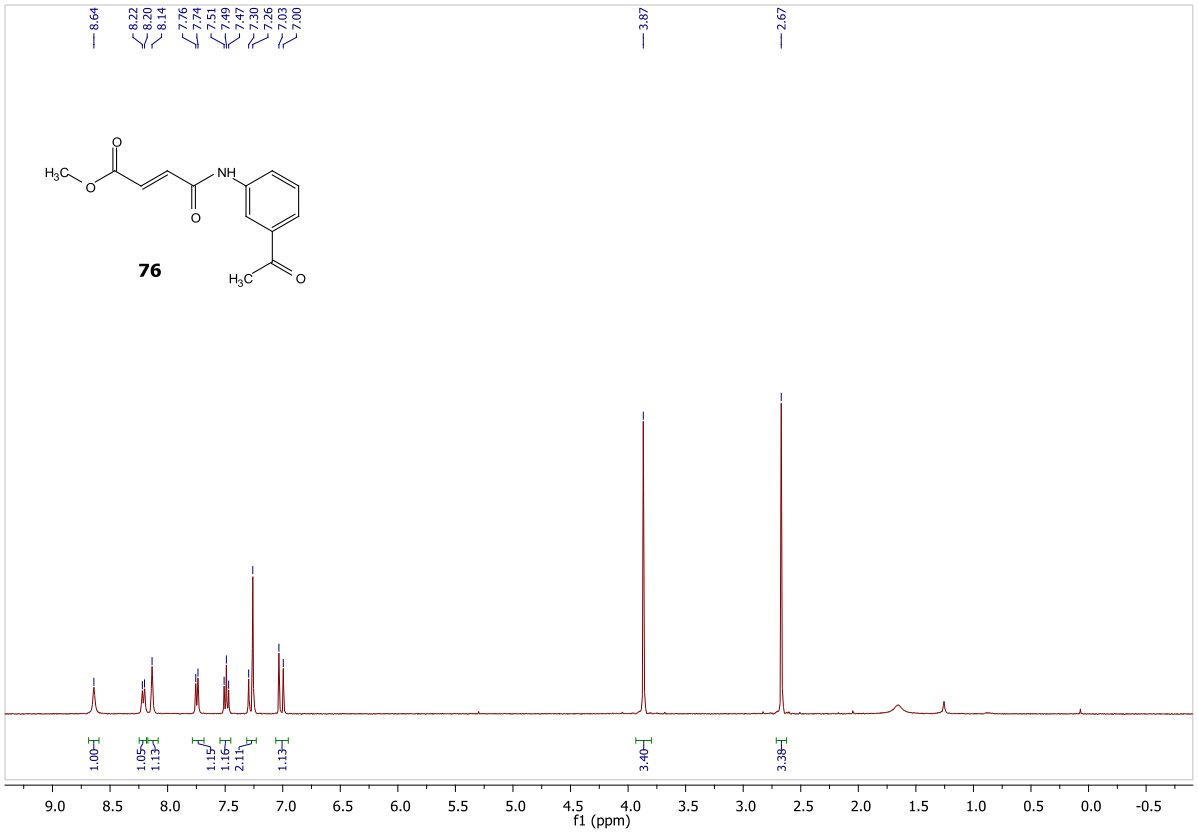

196.96
165.80
161.36
151.46
136.64
131.18
130.63
126.80
125.40
120.68
109.74
56.16
52.34
26.54
f1 (ppm)

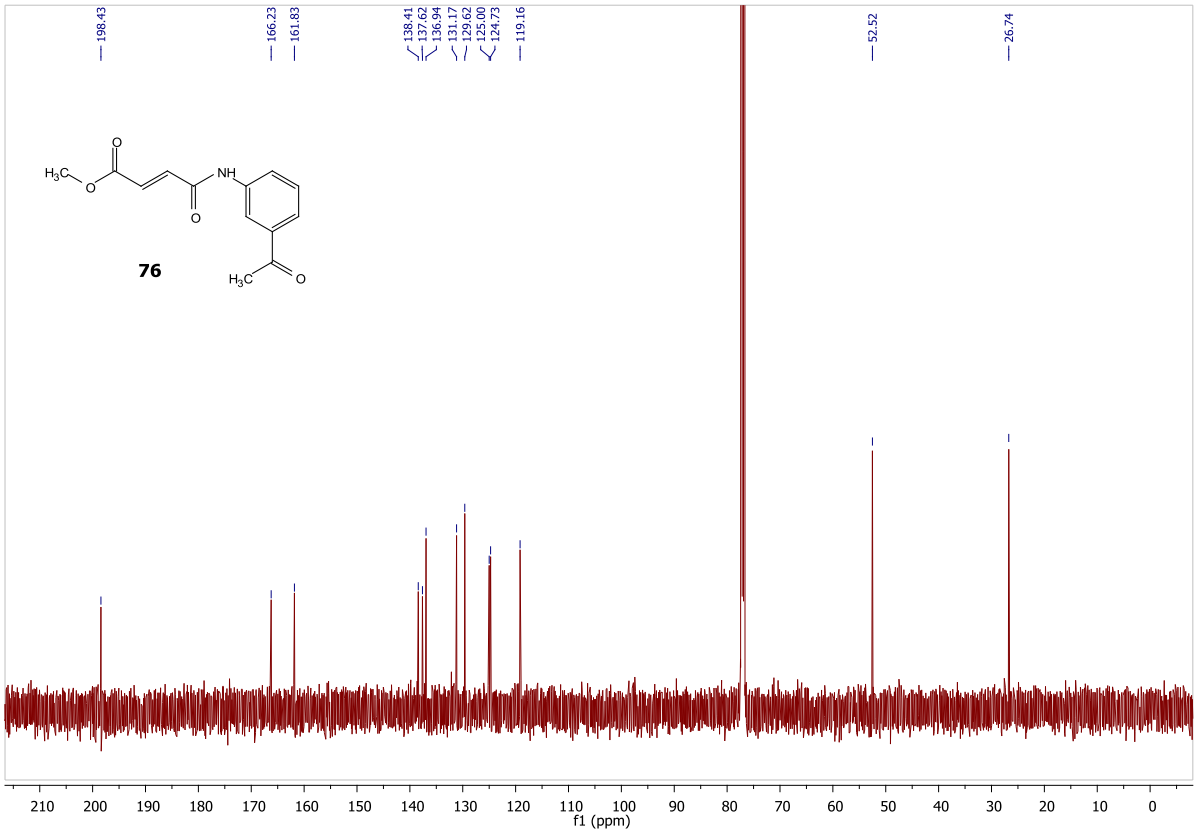

8.64
8.45
8.02
8.00
7.60
7.58
7.53
7.31
7.19
7.18
4.14
2.88
2.63
74
0.89
0.89
0.93
1.32
1.84
0.55
0.52
3.90
2.97
3.00
f1 (ppm)

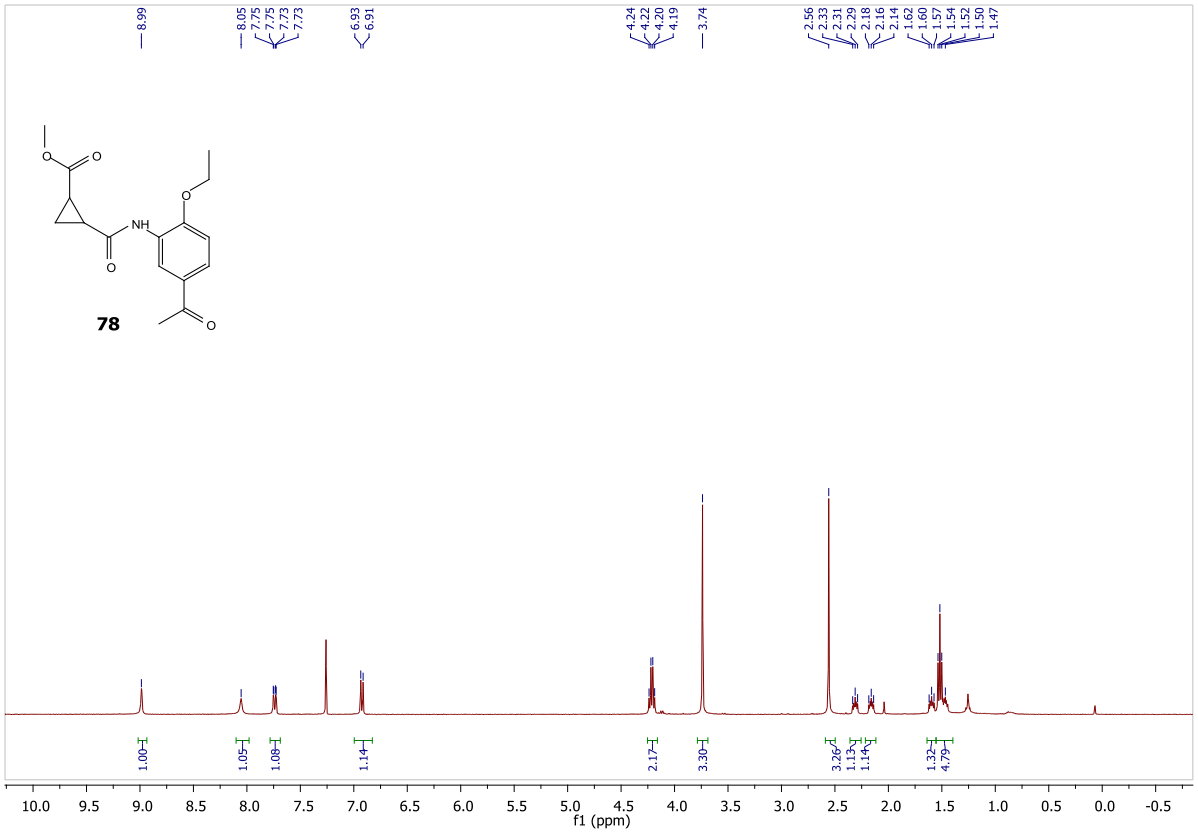

197.10
165.43
161.70
137.59
136.95
135.93
135.10
134.65
132.27
130.79
129.31
125.44
123.78
52.08
26.61
18.04
74
f1 (ppm)

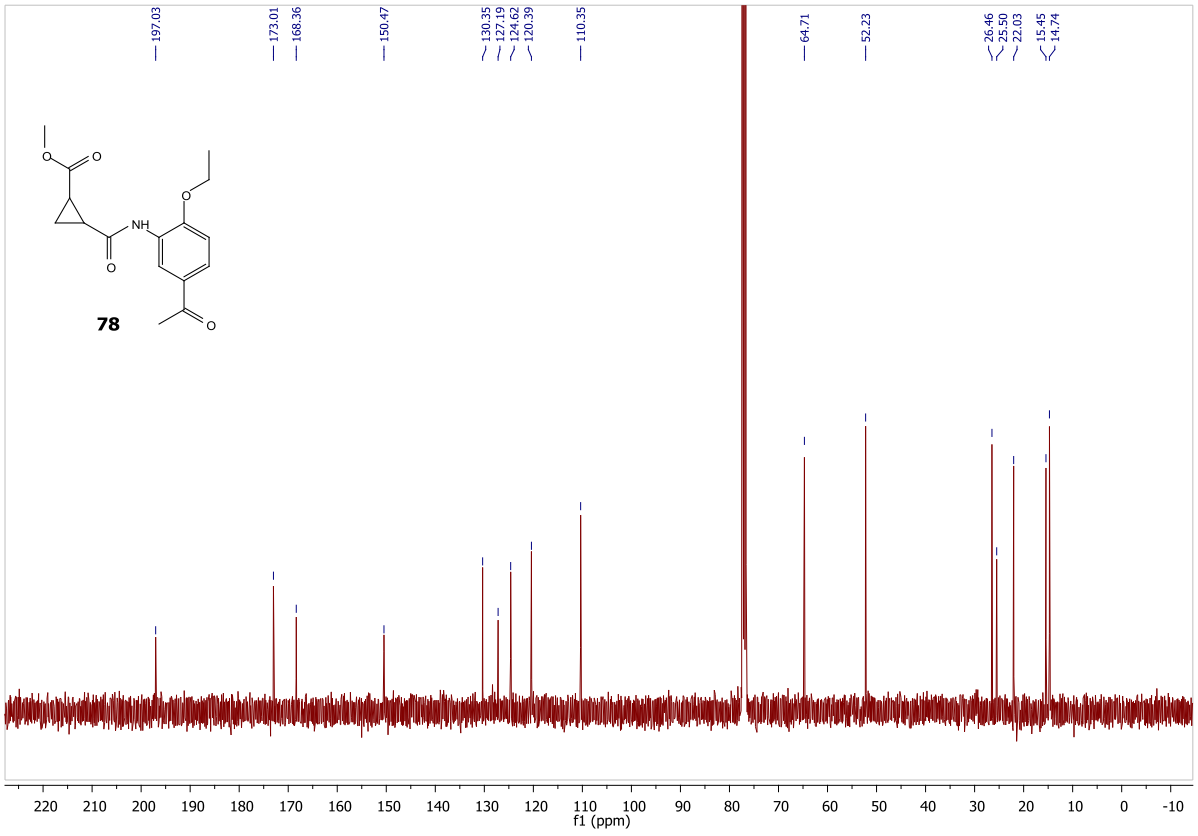

9.06
9.05
7.81
7.80
7.79
7.78
7.77
7.23
7.21
7.21
7.19
7.15
7.11
7.02
6.98
3.85
2.62
O
F
H3C
O
NH
O
O
H3C
O
75
0.85
1.77
0.98
1.95
2.93
3.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

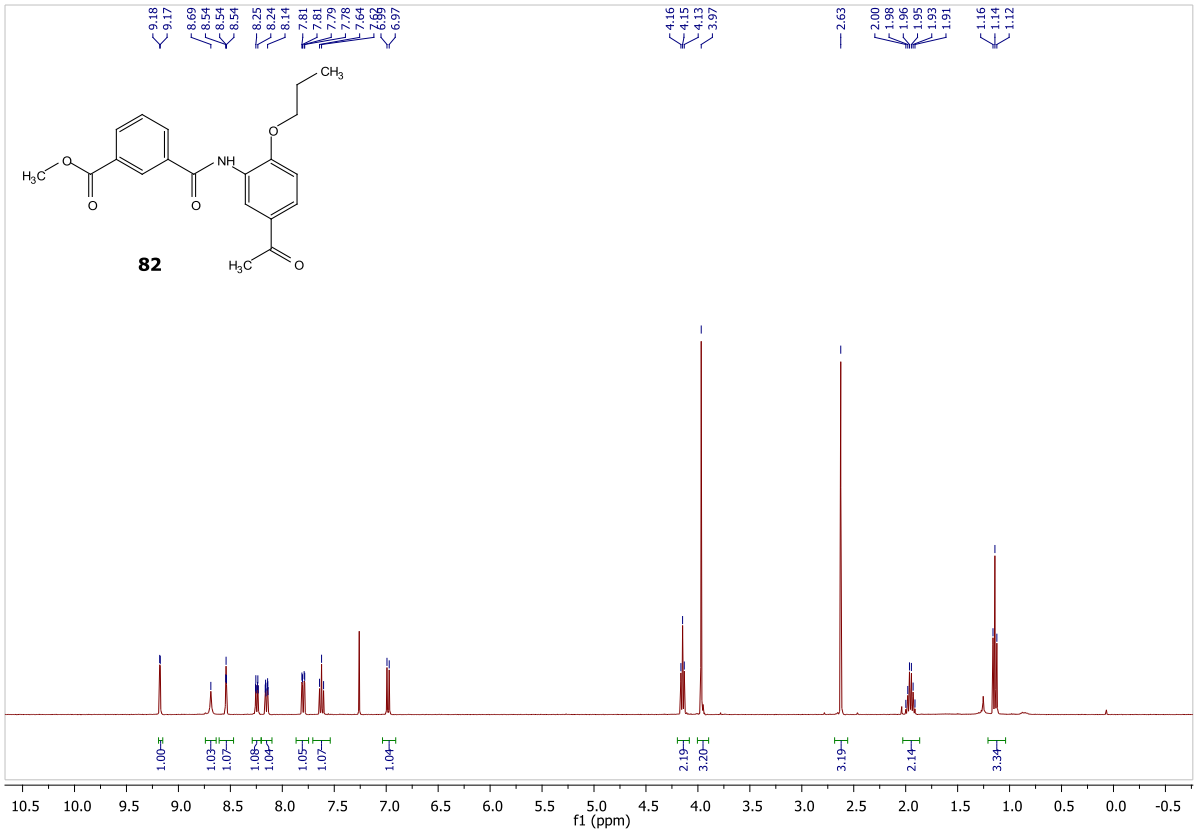

196.45
165.58
161.63
135.74
134.09
134.06
132.14
125.60
125.52
115.23
52.42
26.63
O
F
H3C
O
NH
O
O
H3C
O
75
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


8.64
8.22
8.20
8.14
7.76
7.74
7.51
7.49
7.47
7.30
7.26
7.03
7.00
3.87
2.67
76
1.00
1.05
1.13
1.15
1.16
2.11
1.13
3.40
3.38
f1 (ppm)


198.43
166.23
161.83
138.41
137.62
136.94
131.17
129.62
125.00
124.73
119.16
52.52
26.74
76
f1 (ppm)


8.99
8.05
7.75
7.75
7.73
7.73
6.93
6.91
4.24
4.22
4.20
4.19
3.74
2.56
2.33
2.31
2.29
2.18
2.16
2.14
1.62
1.60
1.57
1.54
1.52
1.50
1.47
78
1.00
1.05
1.08
1.14
2.17
3.30
3.26
1.13
1.14
1.32
4.79
f1 (ppm)


197.03
173.01
168.36
150.47
130.35
127.19
124.62
120.39
110.35
64.71
52.23
26.46
25.50
22.03
15.45
14.74
78
f1 (ppm)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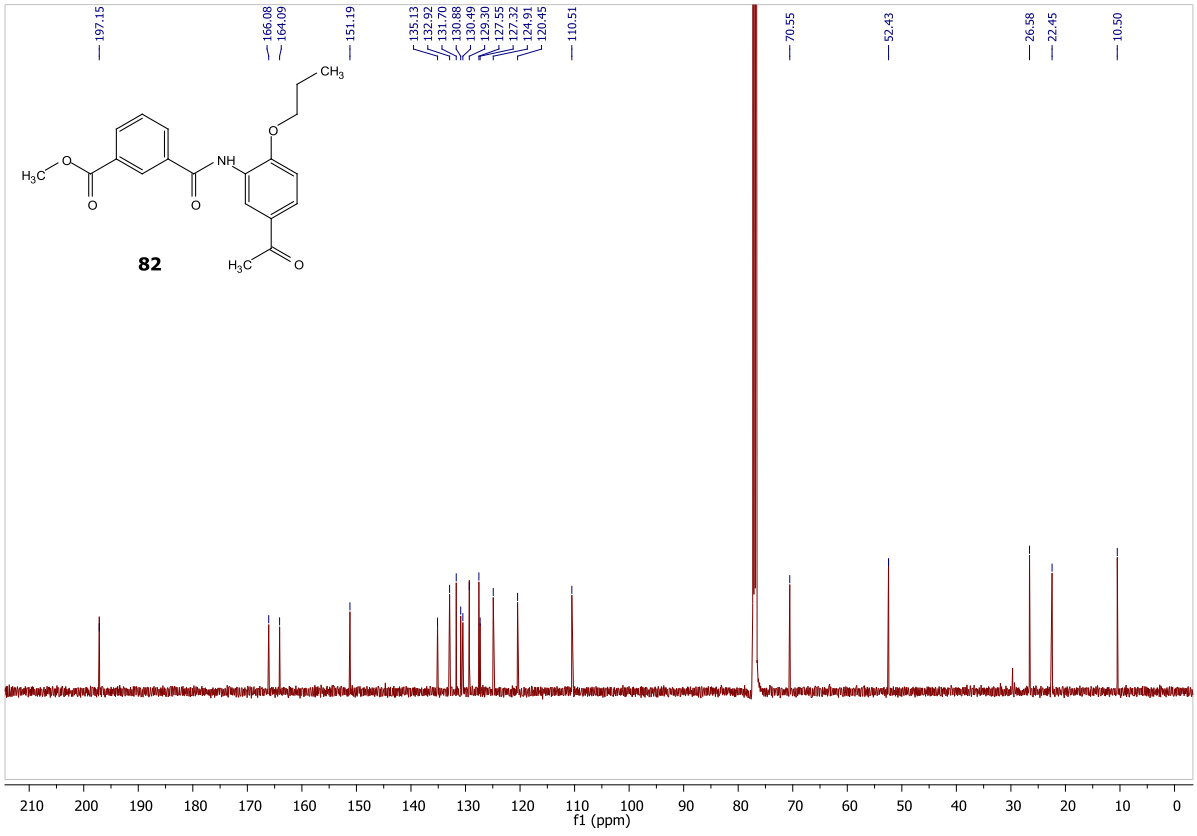
9.17
8.69
8.54
8.54
8.54
8.25
8.24
8.14
7.81
7.81
7.79
7.78
7.64
7.62
7.60
6.99
6.97
4.16
4.15
4.13
3.97
2.63
2.00
1.98
1.96
1.95
1.93
1.91
1.16
1.14
1.12
CH3
O
NH
H3C
O
O
O
H3C
O
82
1.00
1.03
1.07
1.08
1.04
1.05
1.07
1.04
2.19
3.20
3.19
2.14
3.34
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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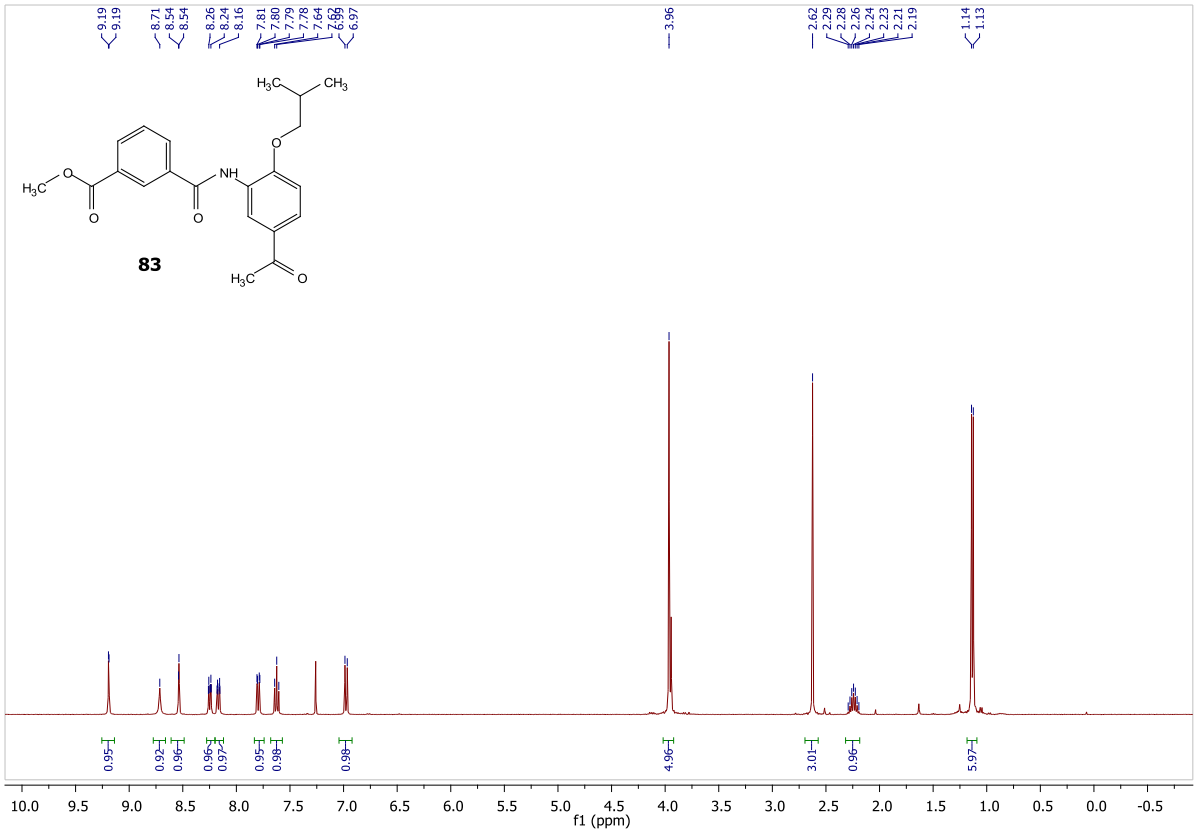
166.08
164.09
151.19
135.13
132.92
131.70
130.88
130.49
129.30
127.55
127.32
124.91
120.45
110.51
70.55
52.43
26.58
22.45
10.50
CH3
O
NH
H3C
O
O
O
H3C
O
82
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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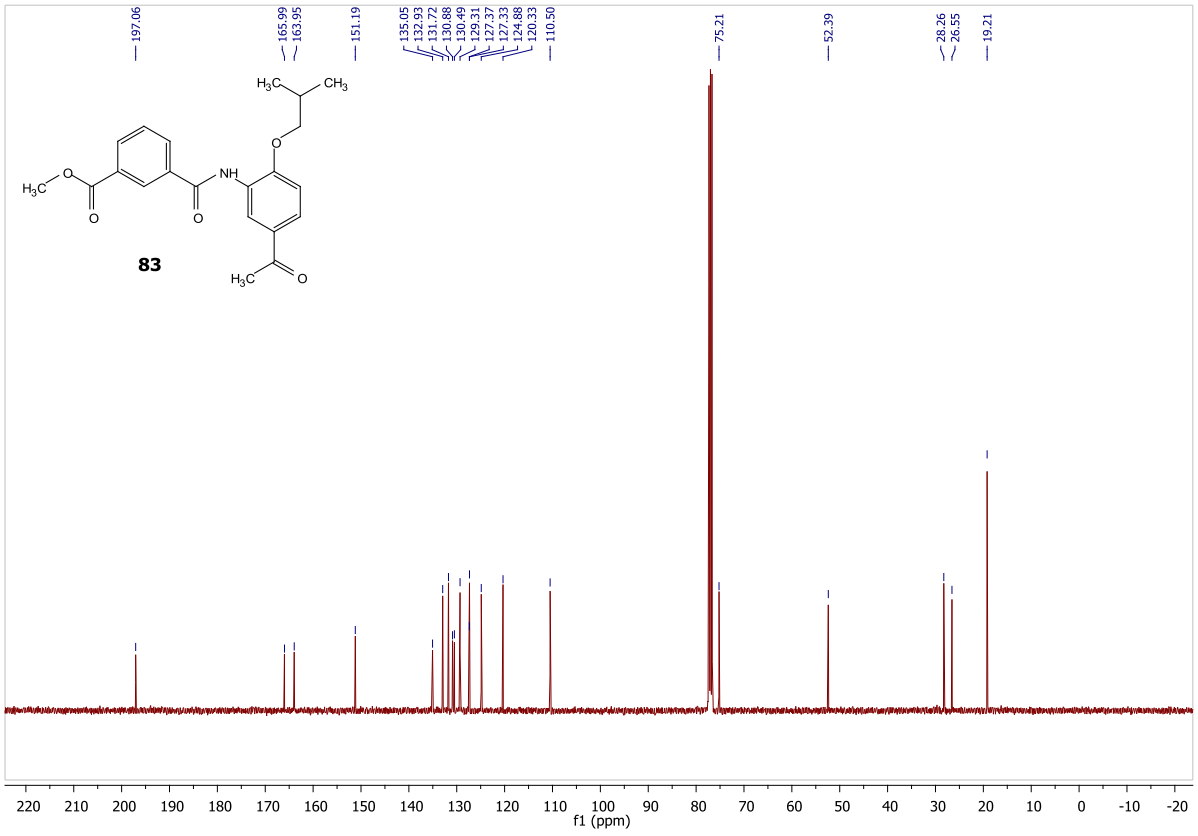
9.19
8.71
8.54
8.54
8.26
8.24
8.16
7.81
7.80
7.79
7.78
7.64
6.99
6.97
3.96
2.62
2.29
2.28
2.26
2.24
2.23
2.21
2.19
1.14
1.13
83
0.95
0.92
0.96
0.96
0.97
0.95
0.98
0.98
4.96
3.01
0.96
5.97
f1 (ppm)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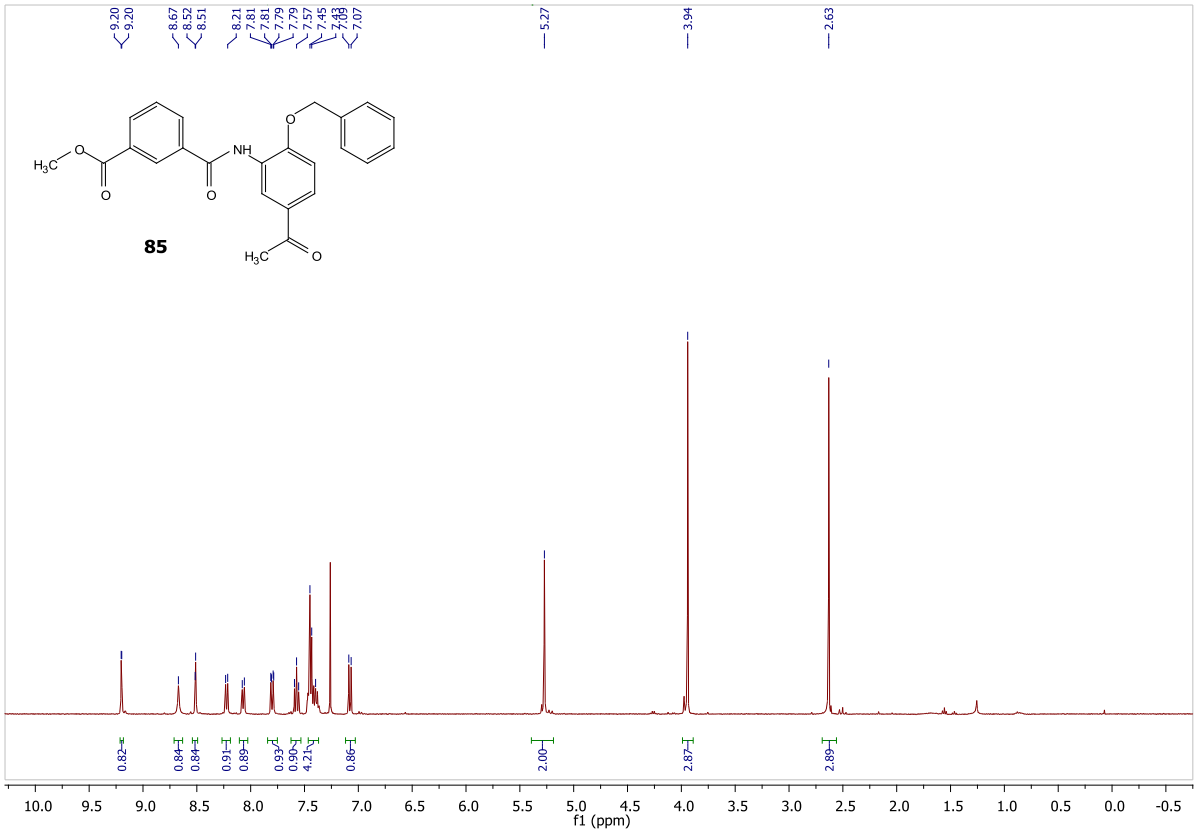
151.19
135.05
132.93
131.72
130.88
130.49
129.31
127.37
127.33
124.88
120.33
110.50
75.21
52.39
28.26
26.55
19.21
83
f1 (ppm)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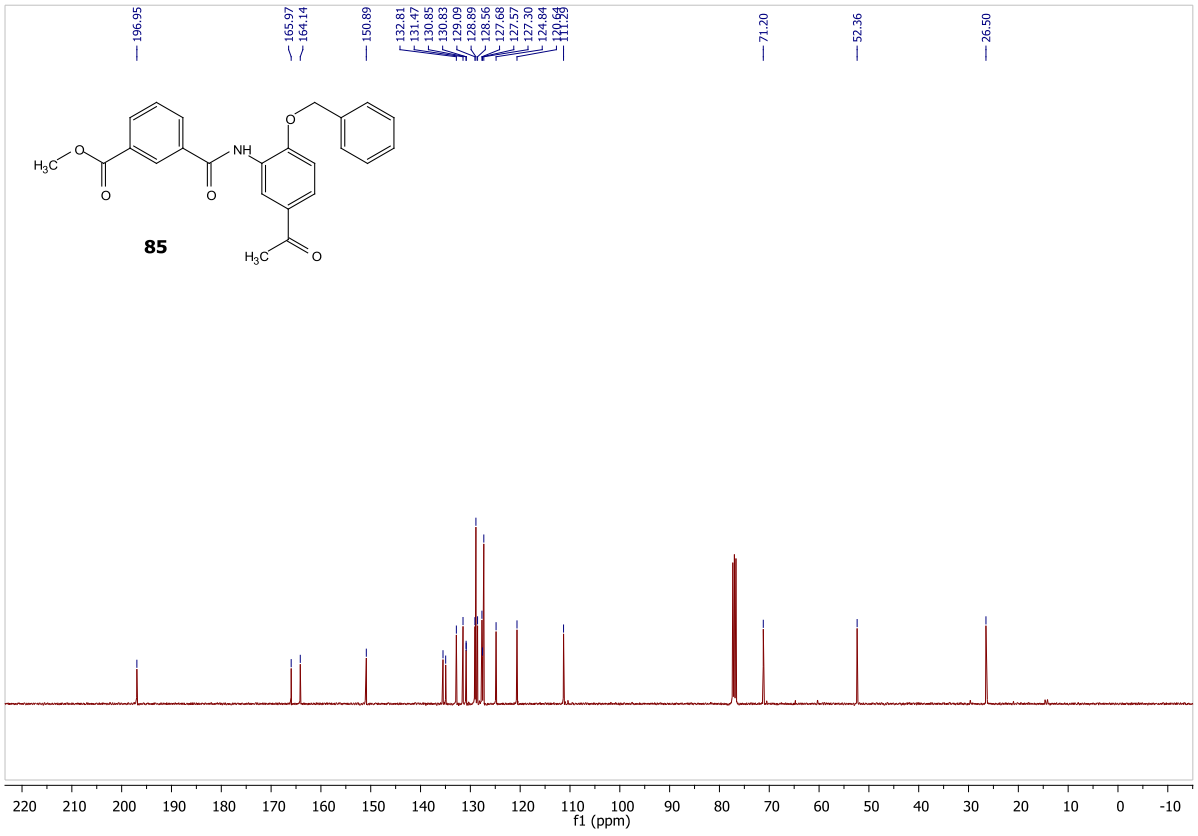
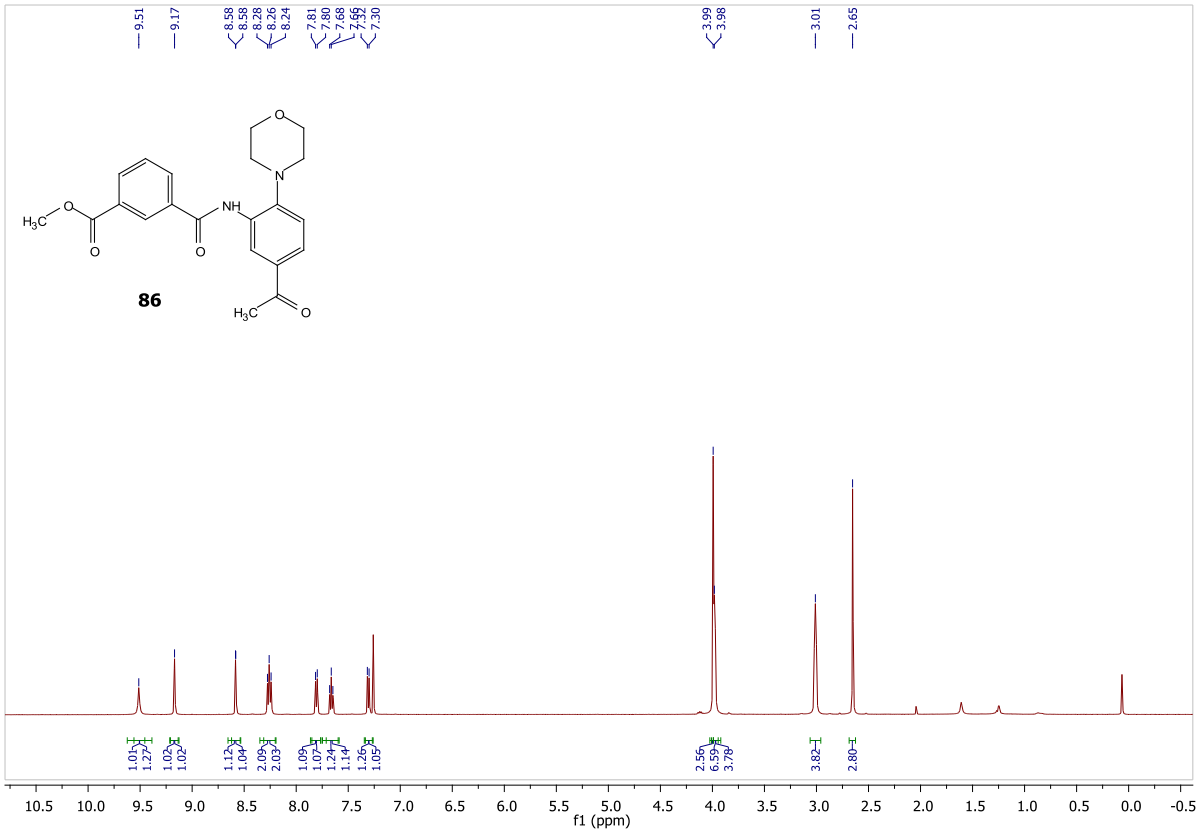
9.20
8.67
8.52
8.51
8.21
7.81
7.81
7.79
7.79
7.57
7.45
7.43
7.08
7.07
5.27
3.94
2.63
O
NH
O
O
H3C
O
O
H3C
O
85
0.82
0.84
0.84
0.91
0.89
0.93
0.90
4.21
0.86
2.00
2.87
2.89
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


196.95
165.97
164.14
150.89
132.81
131.47
130.85
130.83
129.09
128.89
128.56
127.68
127.57
127.30
124.84
120.64
111.29
71.20
52.36
26.50
O
NH
O
O
H3C
O
O
H3C
O
85
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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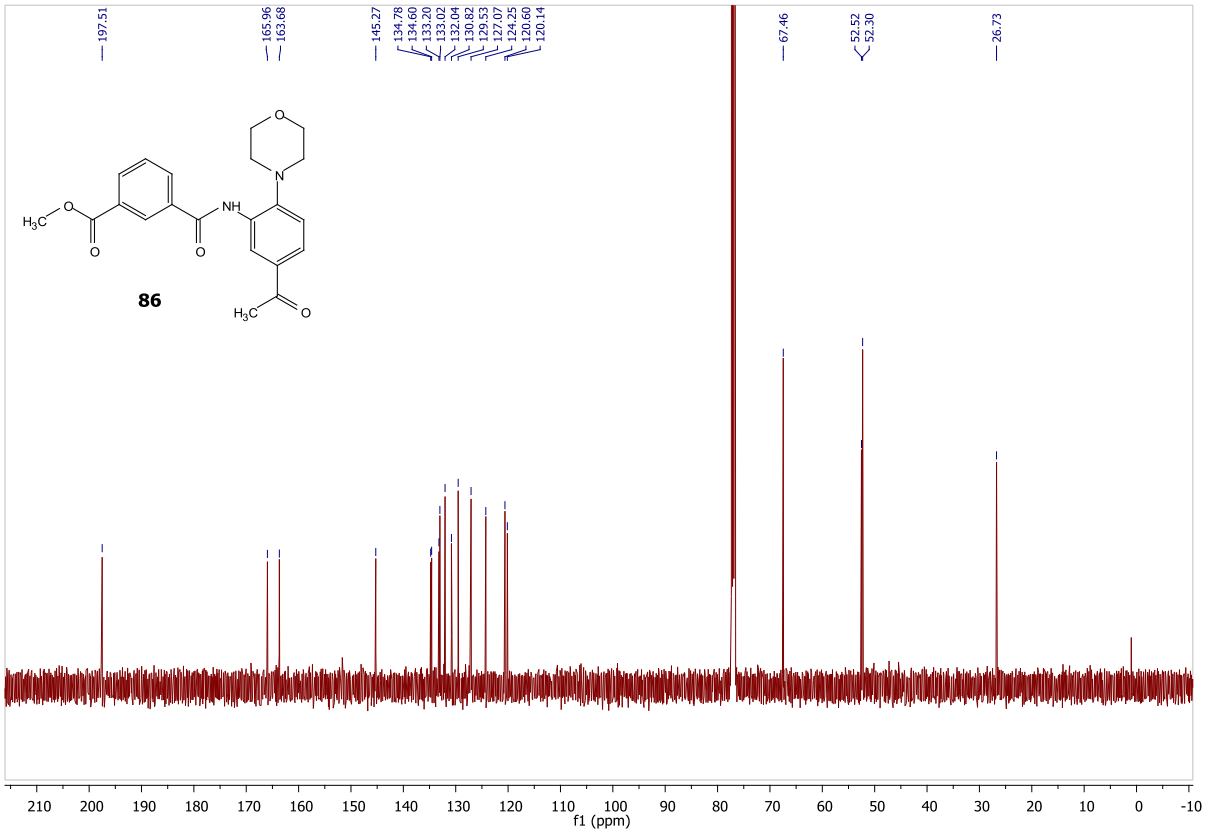
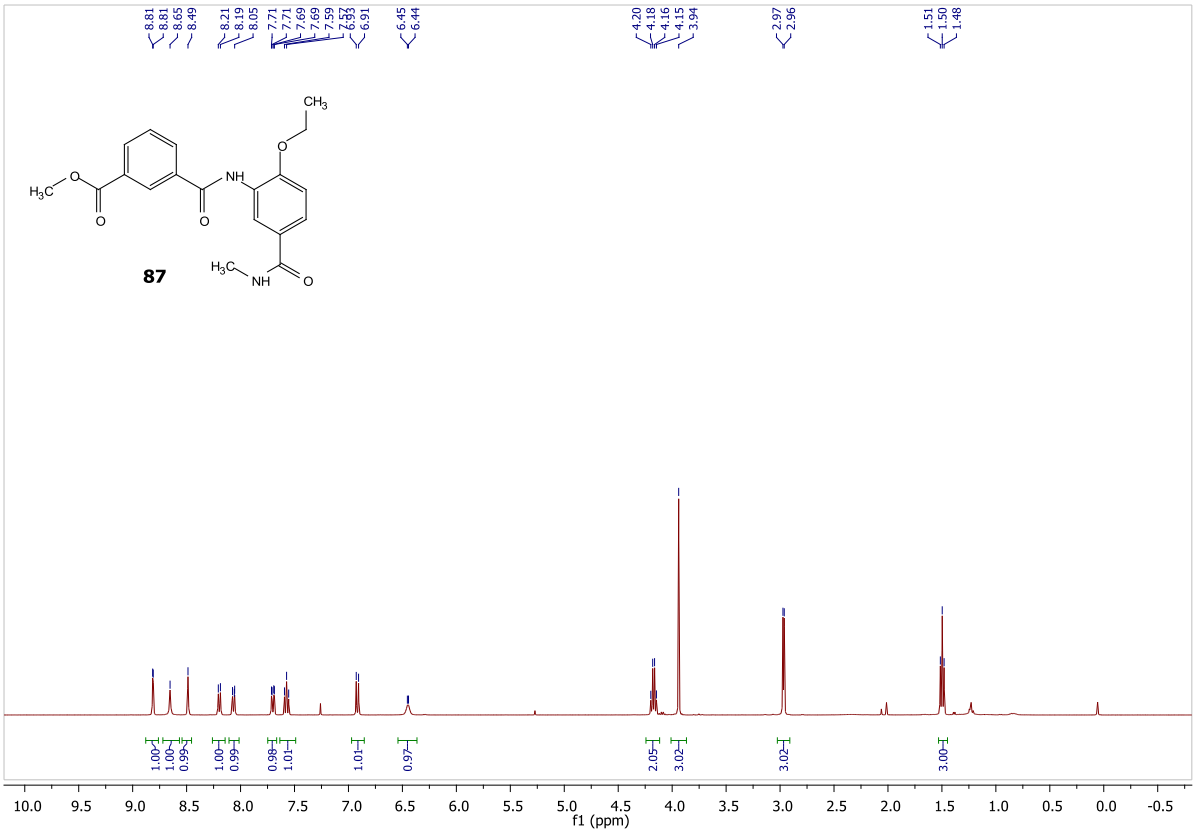
9.17
8.58
8.58
8.28
8.26
8.24
7.81
7.80
7.68
7.30
3.99
3.98
3.01
2.65
86
f1 (ppm)


197.51
165.96
163.68
145.27
134.78
134.60
133.20
133.02
132.04
130.82
129.53
127.07
124.25
120.60
120.14
67.46
52.52
52.30
26.73
86
f1 (ppm)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8.05
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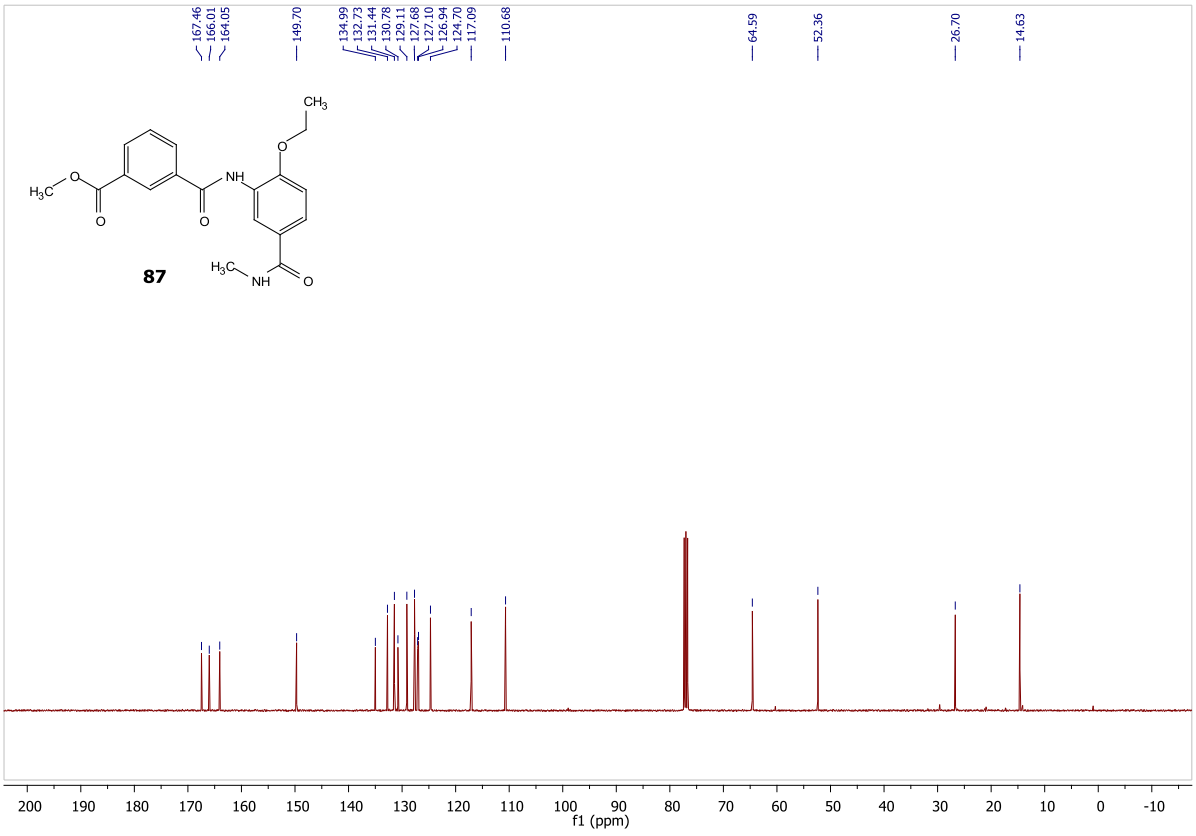
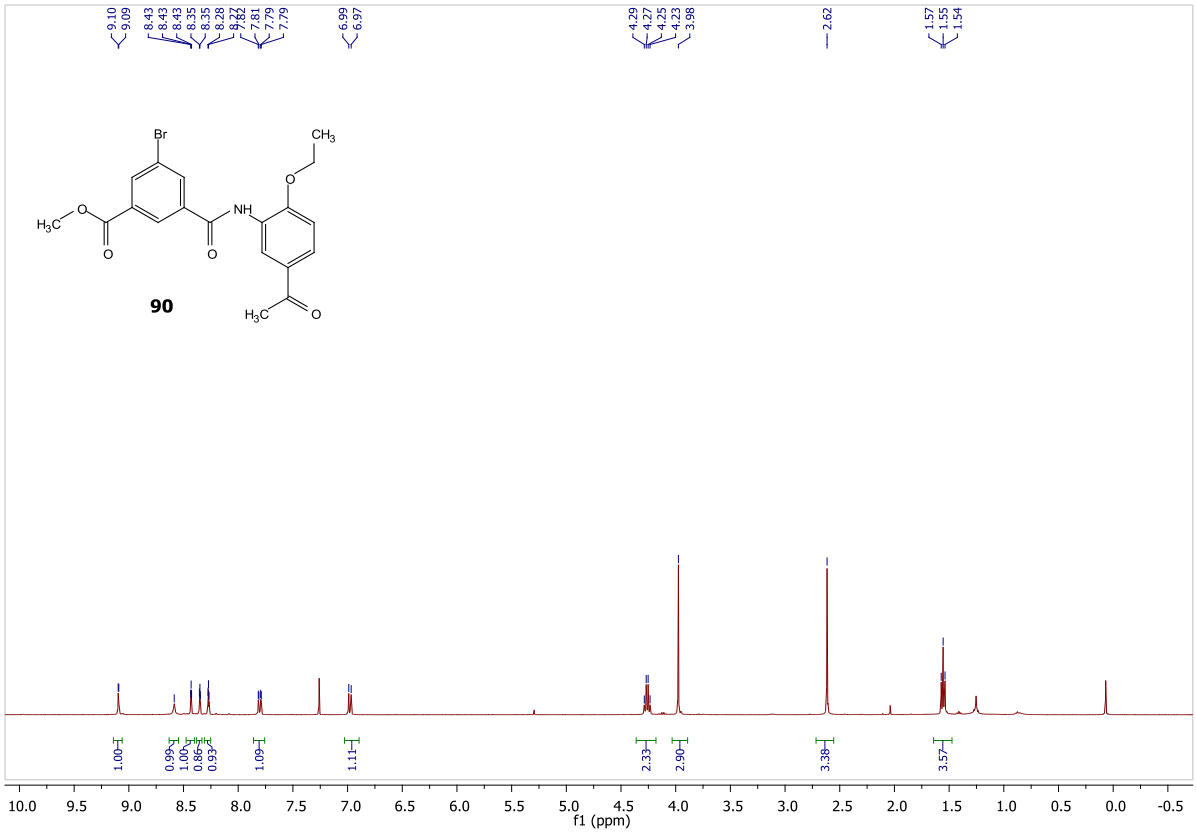
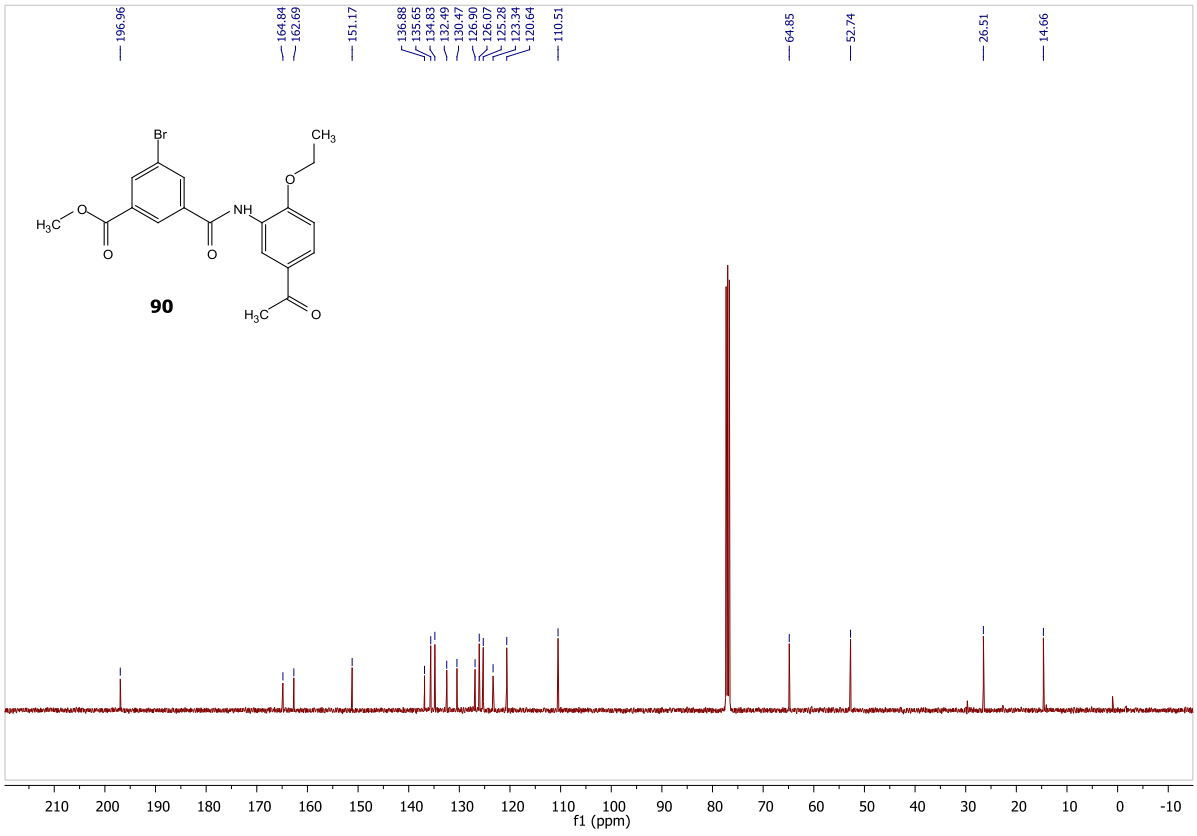
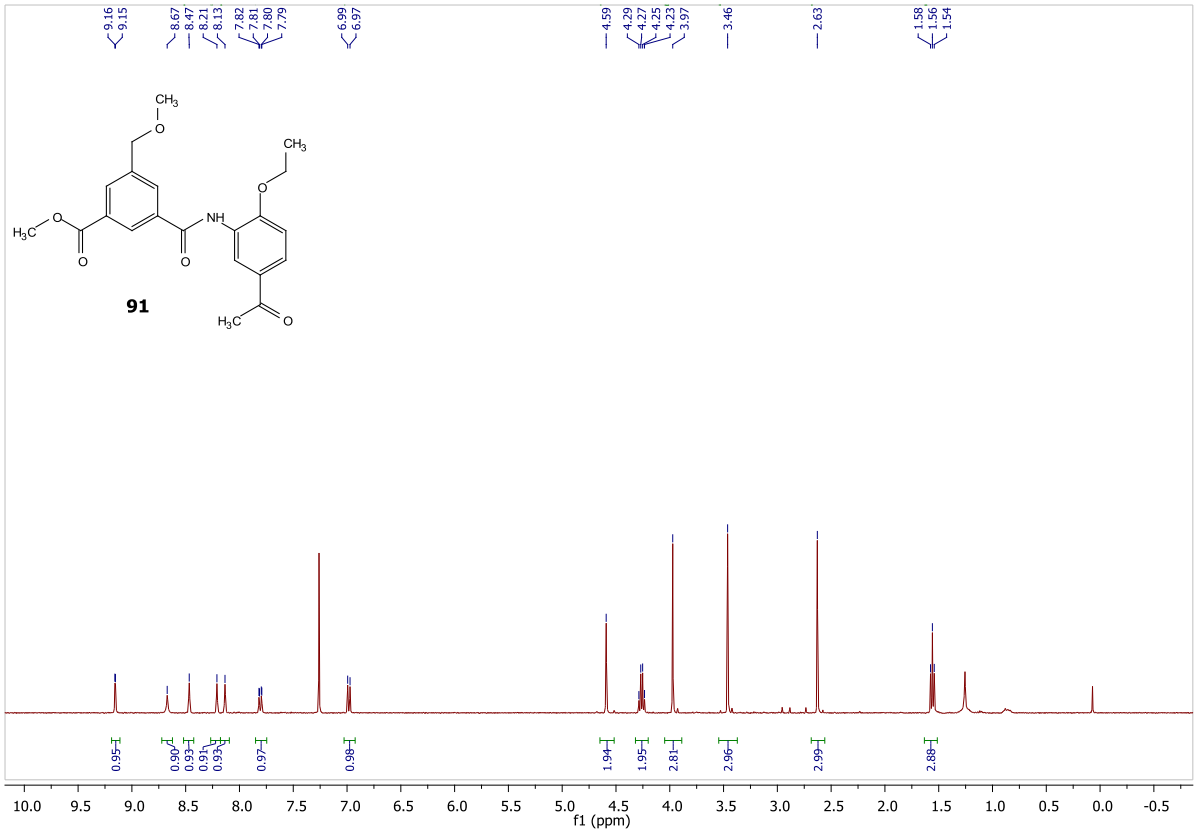
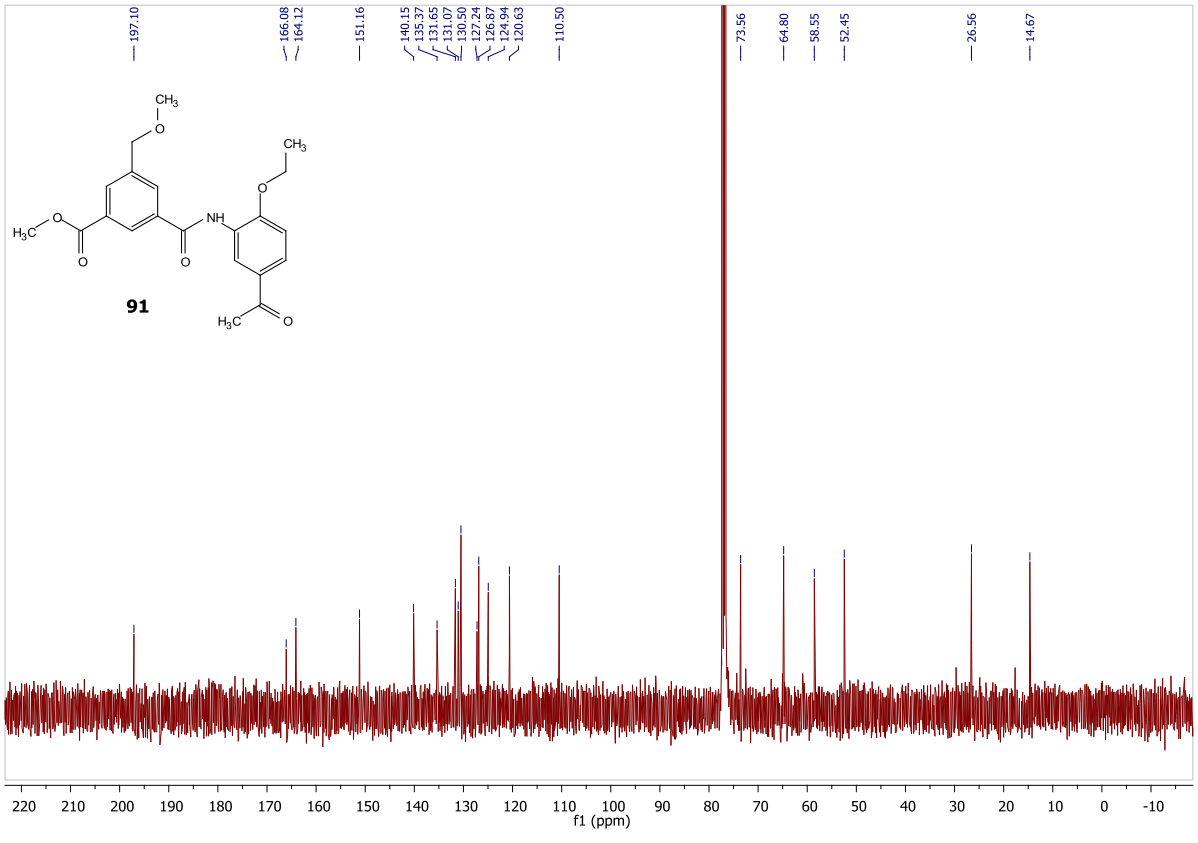
7.71
7.69
7.69
7.59
7.57
6.93
6.91
6.45
6.44
4.20
4.18
4.16
4.15
3.94
2.97
2.96
1.51
1.50
1.48
CH3
O
NH
H3C
O
O
O
H3C
NH
O
87
1.00
1.00
0.99
1.00
0.99
0.98
1.01
1.01
0.97
2.05
3.02
3.02
3.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

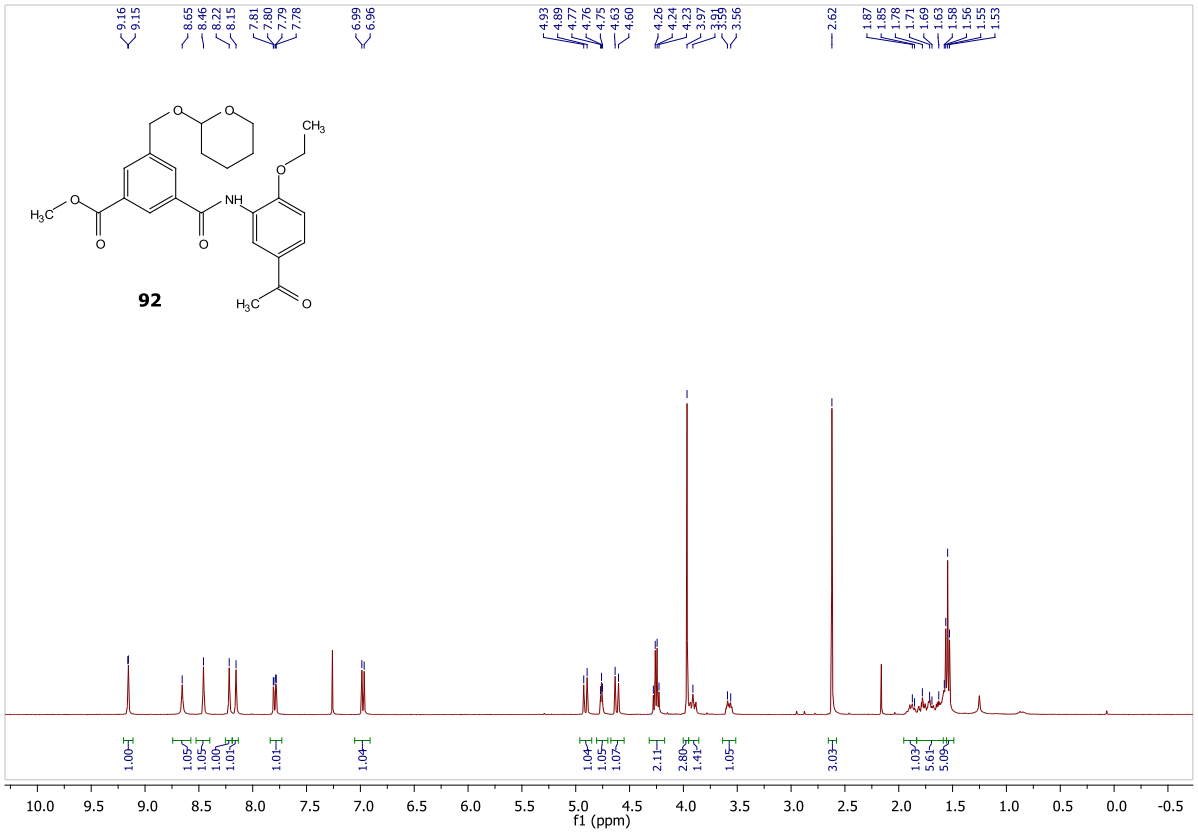

167.46
166.01
164.05
149.70
134.99
132.73
131.44
130.78
129.11
127.68
127.10
126.94
124.70
117.09
110.68
64.59
52.36
26.70
14.63
CH3
O
NH
H3C
O
O
O
H3C
NH
O
87
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

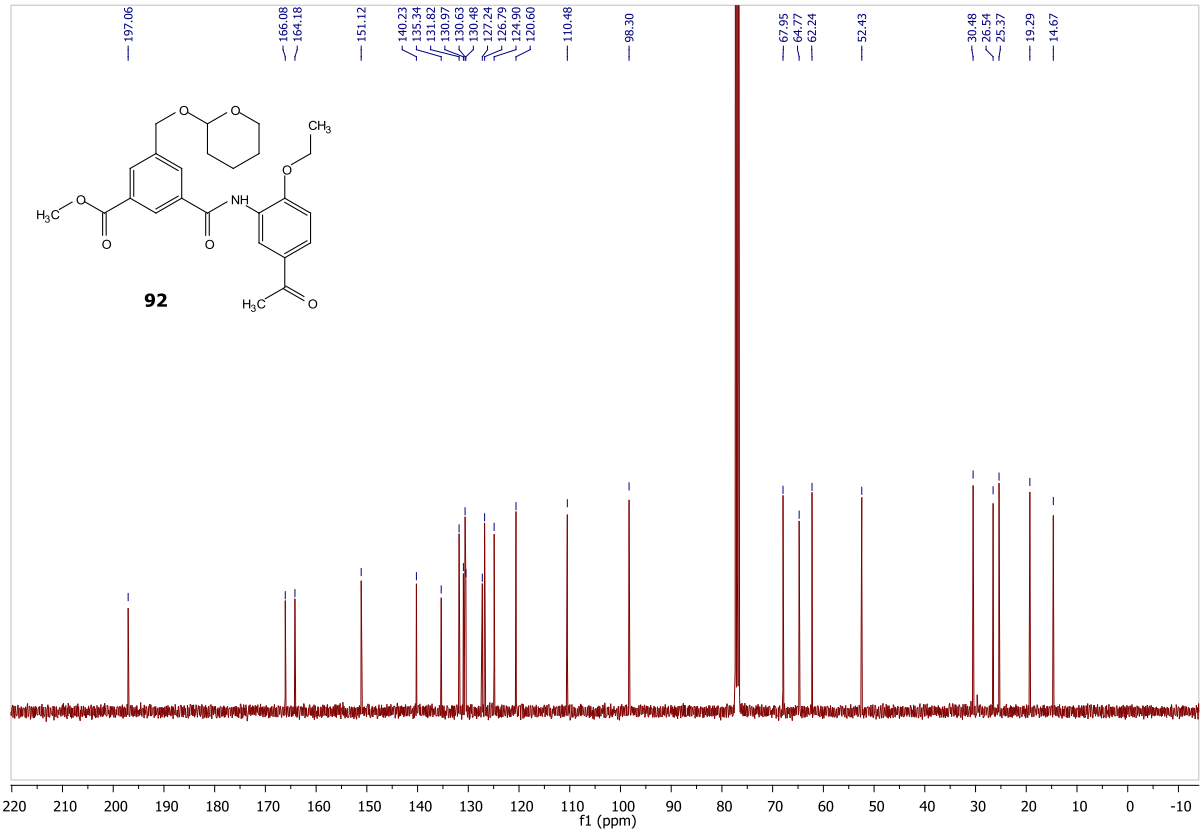

9.10
9.09
8.43
8.43
8.43
8.35
8.35
8.28
8.27
7.82
7.81
7.79
7.79
7.79
6.99
6.97
4.29
4.27
4.25
4.23
3.98
2.62
1.57
1.55
1.54
Br
CH3
O
NH
H3C
O
O
O
H3C
O
90
1.00
0.99
1.06
0.86
0.93
1.09
1.11
2.33
2.90
3.38
3.57
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

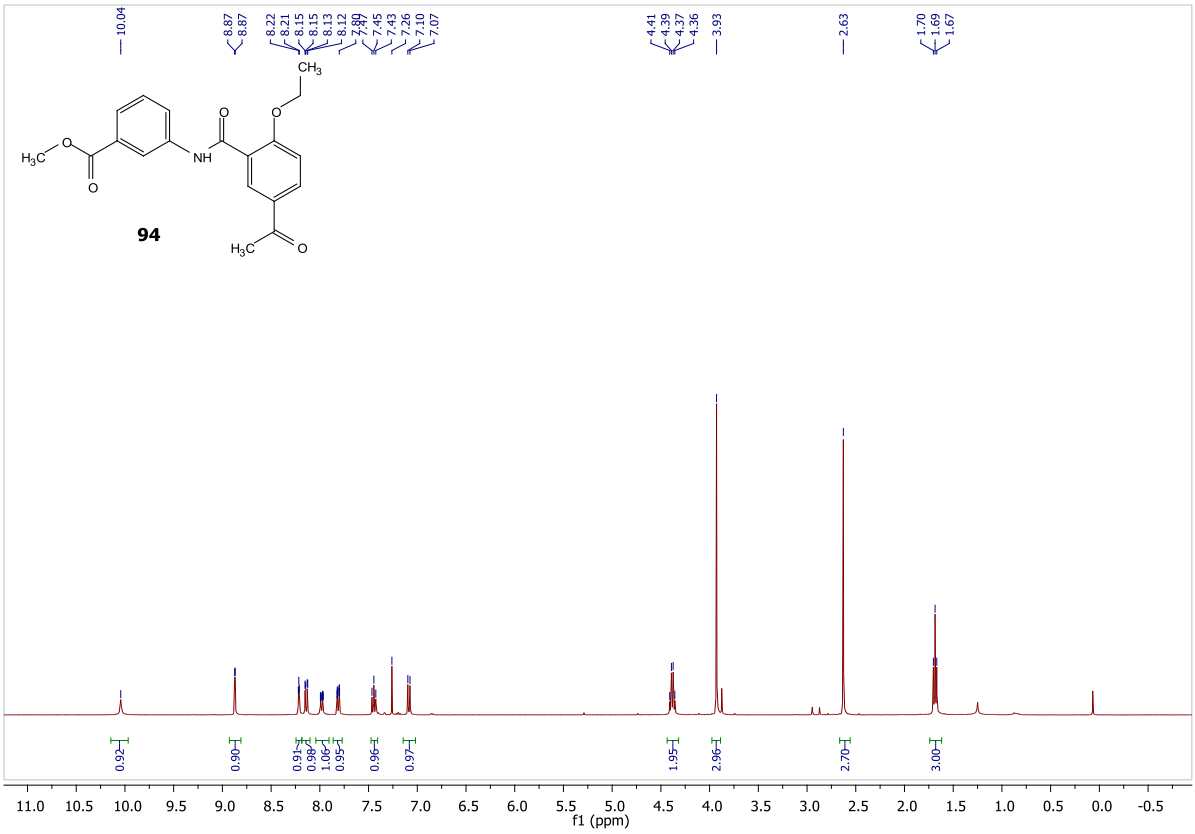

196.96
164.84
162.69
151.17
136.88
135.65
134.83
132.49
130.47
126.90
126.07
125.28
123.34
120.64
110.51
64.85
52.74
26.51
14.66
Br
CH3
O
NH
H3C
O
O
O
H3C
O
90
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

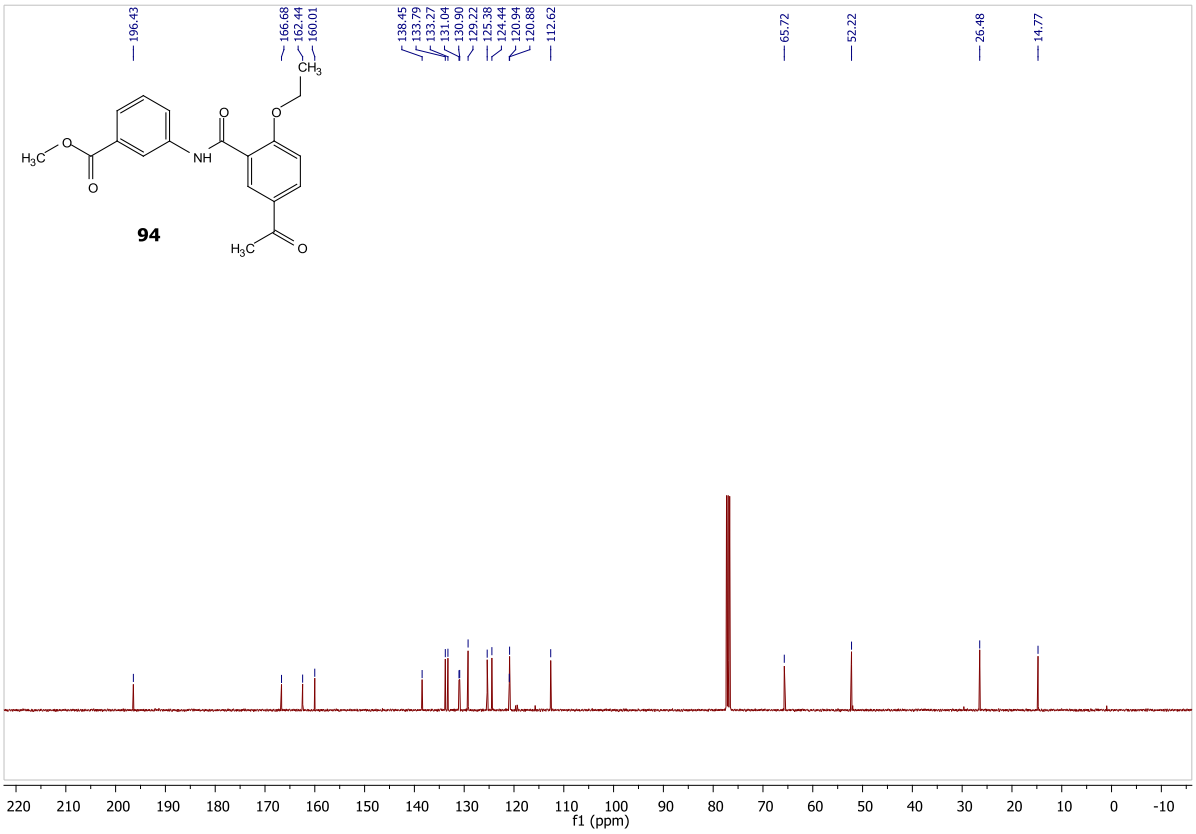

9.16
9.15
8.67
8.47
8.21
8.13
7.82
7.81
7.80
7.79
6.99
6.97
4.59
4.29
4.27
4.25
4.23
3.97
3.46
2.63
1.58
1.56
1.54
91
0.95
0.90
0.93
0.91
0.93
0.97
0.98
1.94
1.95
2.81
2.96
2.99
2.88
f1 (ppm)

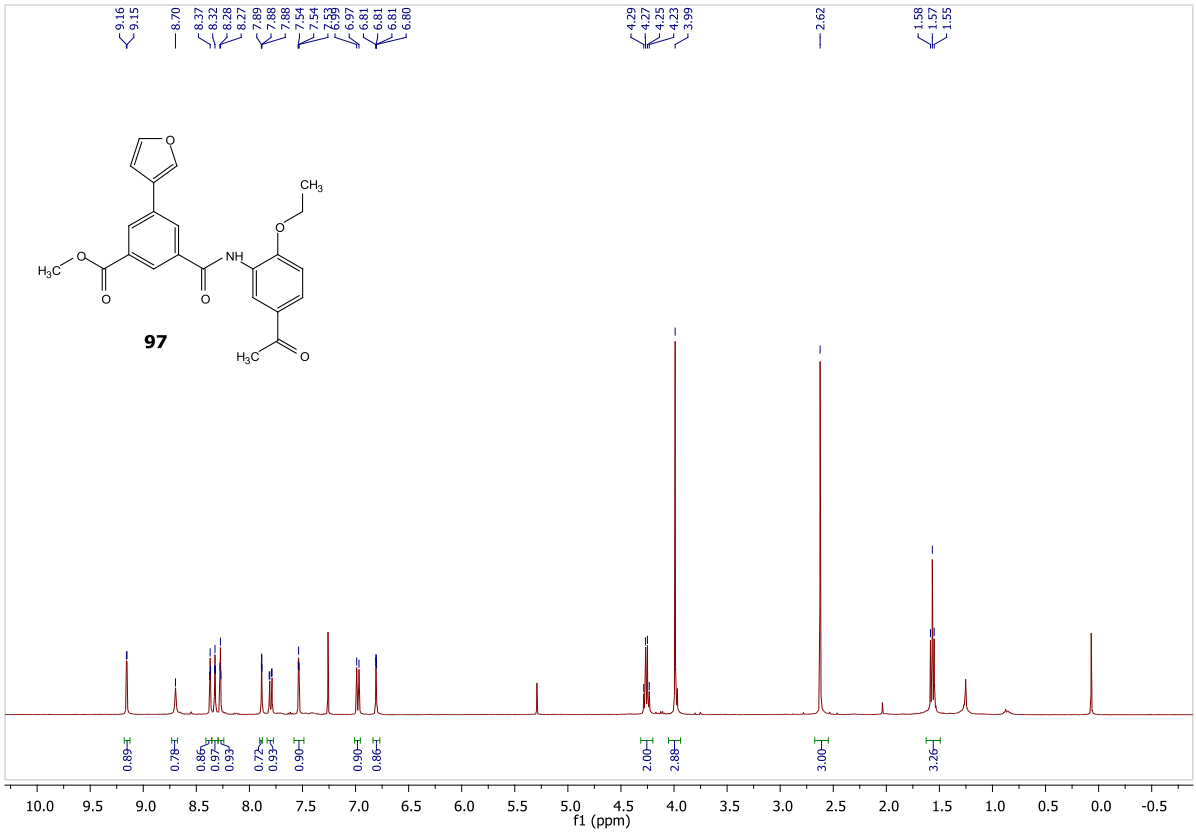

197.10
166.08
164.12
151.16
140.15
135.37
131.65
131.07
130.50
127.24
126.87
124.94
120.63
110.50
73.56
64.80
58.55
52.45
26.56
14.67
91
f1 (ppm)

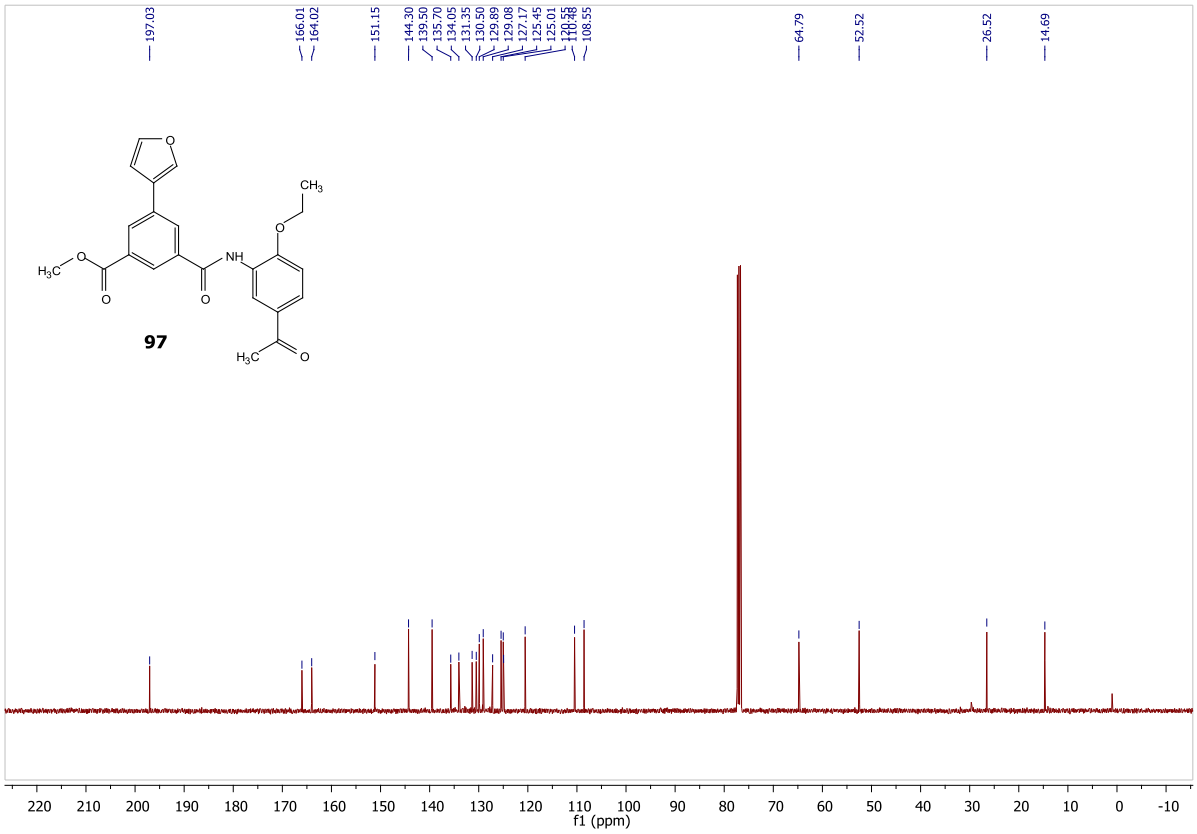

9.16
9.15
8.65
8.46
8.22
8.15
7.81
7.80
7.79
7.78
6.99
6.96
4.93
4.89
4.77
4.76
4.75
4.63
4.60
4.26
4.24
4.23
3.97
3.91
3.59
3.56
2.62
1.87
1.85
1.78
1.71
1.69
1.63
1.58
1.56
1.55
1.53
1.00
1.05
1.05
1.00
1.01
1.01
1.04
1.04
1.05
1.07
2.11
2.80
1.41
1.05
3.03
1.03
5.61
5.09
CH3
O
O
O
NH
O
O
H3C
O
92
H3C
O
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
f1 (ppm)


197.06
166.08
164.18
151.12
140.23
135.34
131.82
130.97
130.63
130.48
127.24
126.79
124.90
120.60
110.48
98.30
67.95
64.77
62.24
52.43
30.48
26.54
25.37
19.29
14.67
CH3
O
O
O
NH
O
O
H3C
O
92
H3C
O
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

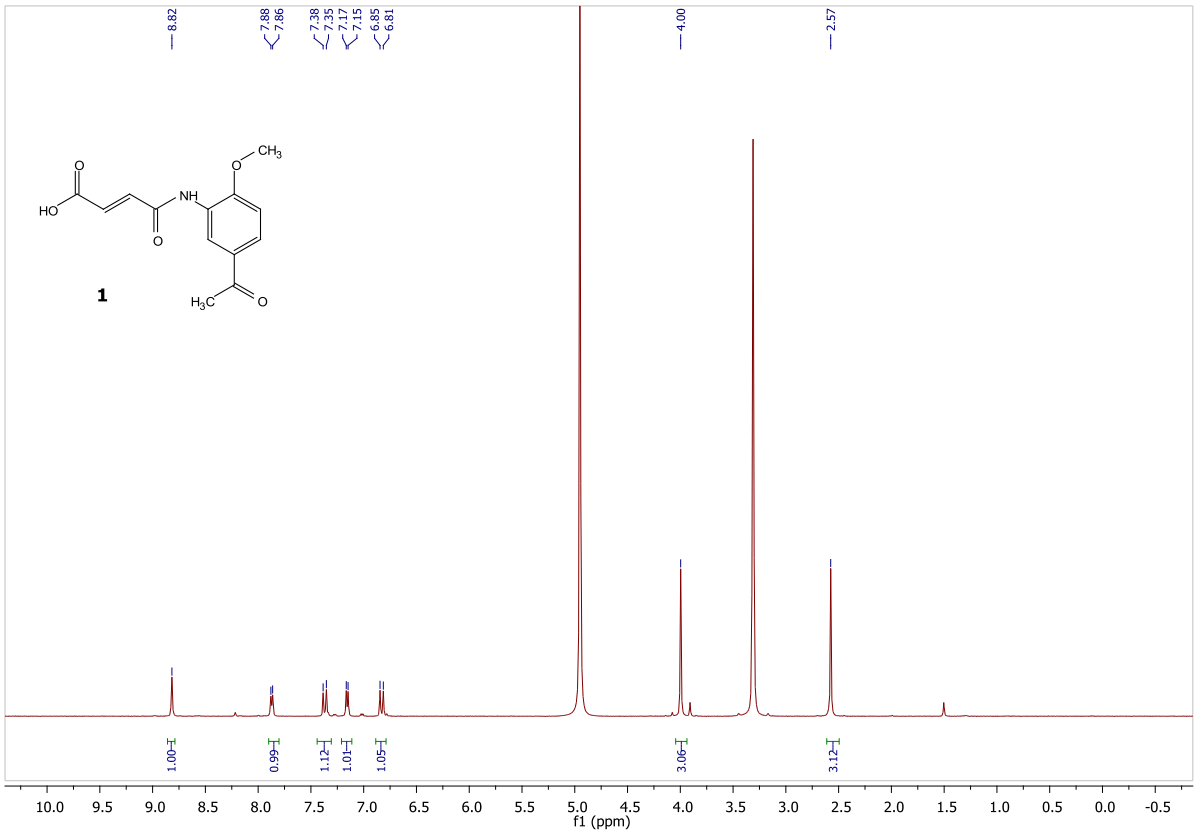

10.04
8.87
8.87
8.22
8.21
8.15
8.15
8.13
8.12
7.89
7.87
7.45
7.43
7.26
7.10
7.07
4.41
4.39
4.37
4.36
3.93
2.63
1.70
1.69
1.67
CH3
O
O
H3C
O
NH
O
94
H3C
O
0.92
0.90
0.91
0.98
1.06
0.95
0.96
0.97
1.95
2.96
2.70
3.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

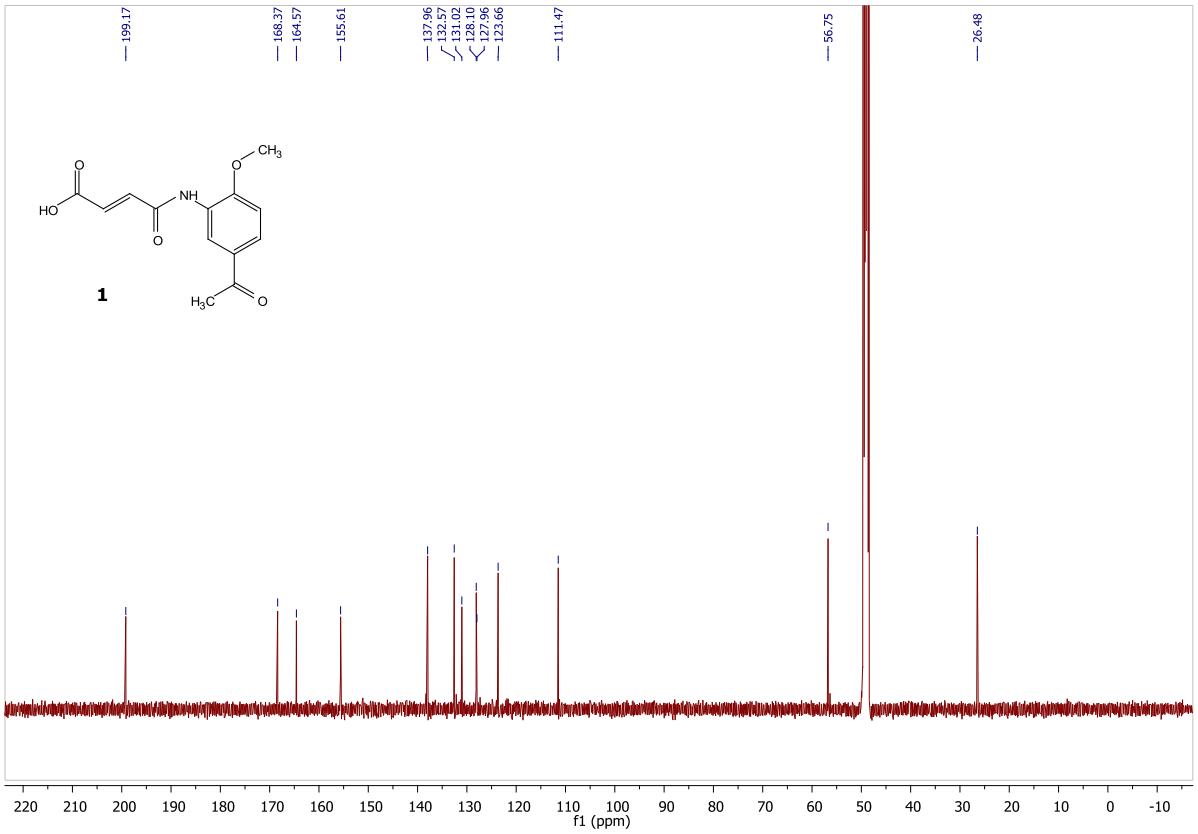

196.43
166.68
162.44
160.01
138.45
133.79
133.27
131.04
130.90
129.22
125.38
124.44
120.94
120.88
112.62
65.72
52.22
26.48
14.77
CH3
O
O
H3C
O
NH
O
94
H3C
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

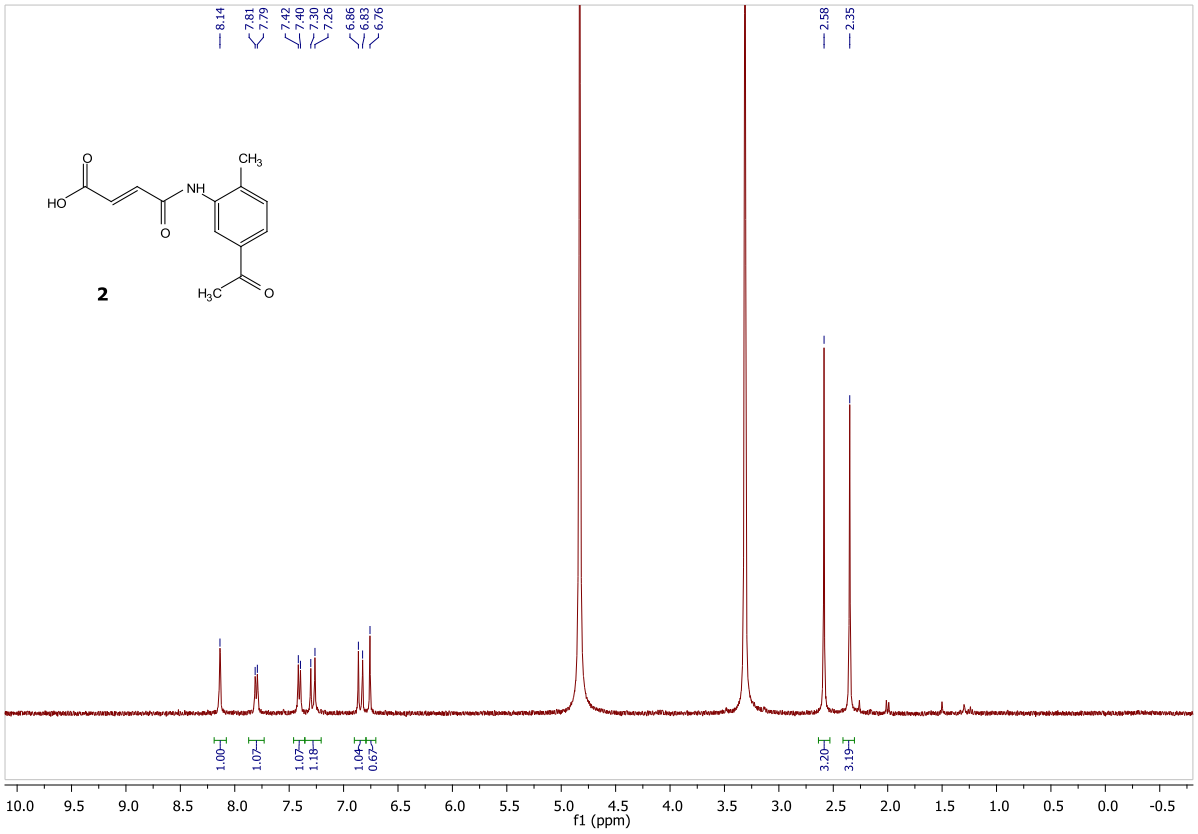

9.16
9.15
8.70
8.37
8.32
8.28
8.27
7.89
7.88
7.88
7.54
7.54
6.97
6.81
6.81
6.80
4.29
4.27
4.25
4.23
3.99
2.62
1.58
1.57
1.55
O
CH3
O
NH
H3C
O
O
O
O
H3C
O
97
0.89
0.78
0.86
0.97
0.93
0.72
0.93
0.90
0.90
0.86
2.00
2.88
3.00
3.26
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

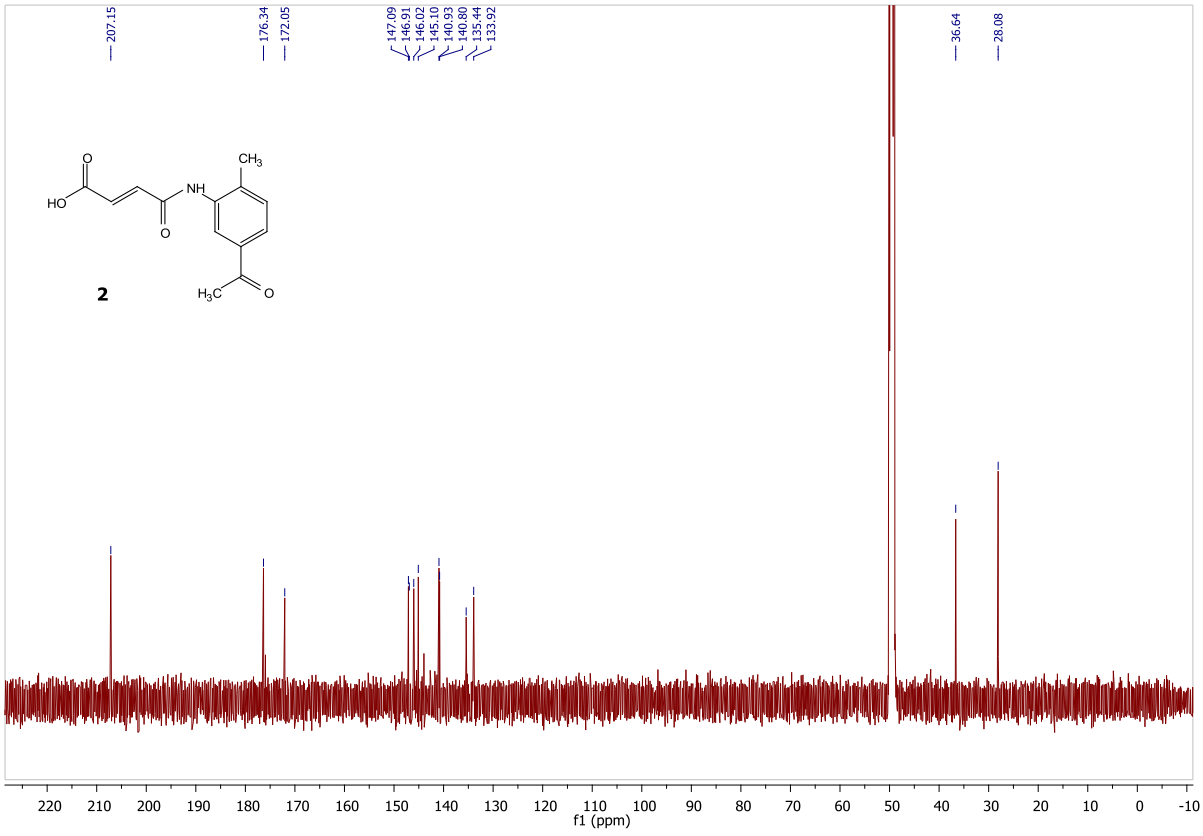

197.03
166.01
164.02
151.15
144.30
139.50
135.70
134.05
131.35
130.50
129.89
129.08
127.17
125.45
125.01
108.55
64.79
52.52
26.52
14.69
O
CH3
O
NH
H3C
O
O
O
O
H3C
O
97
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

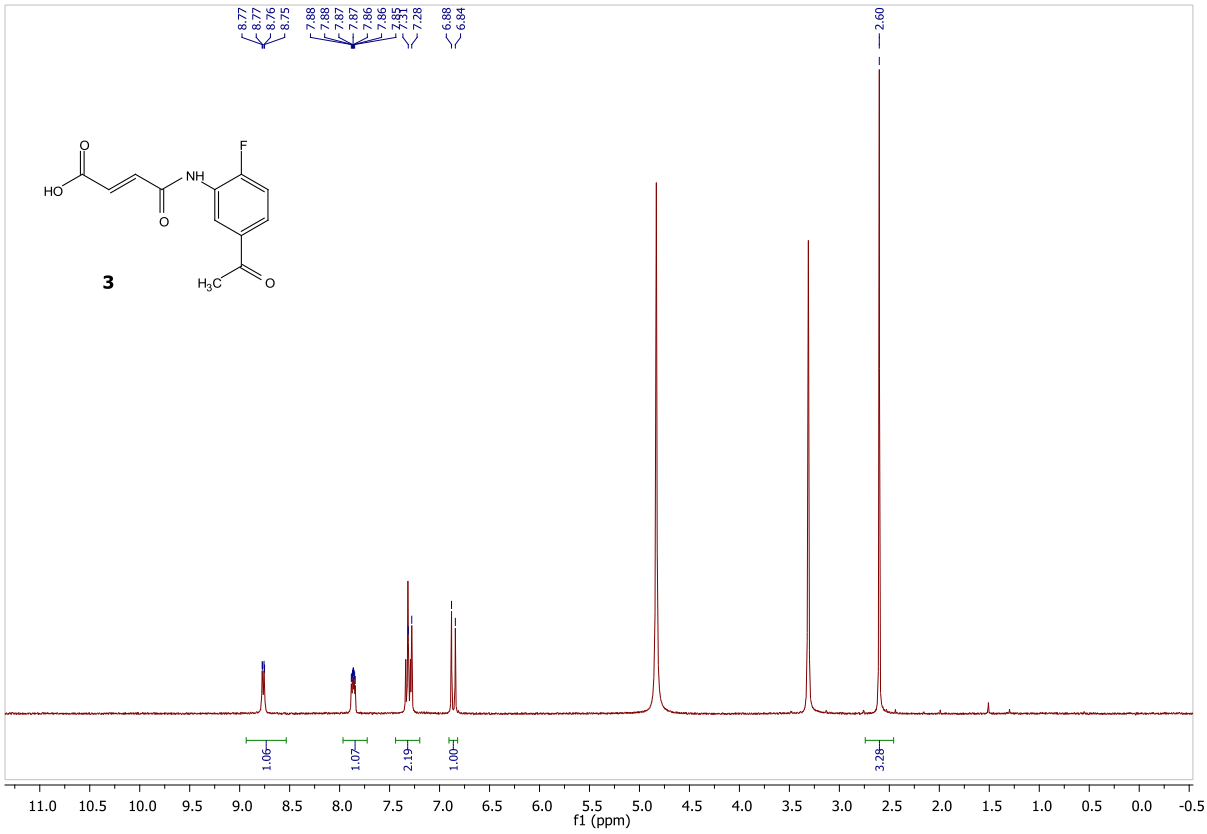
12.2 Final compounds

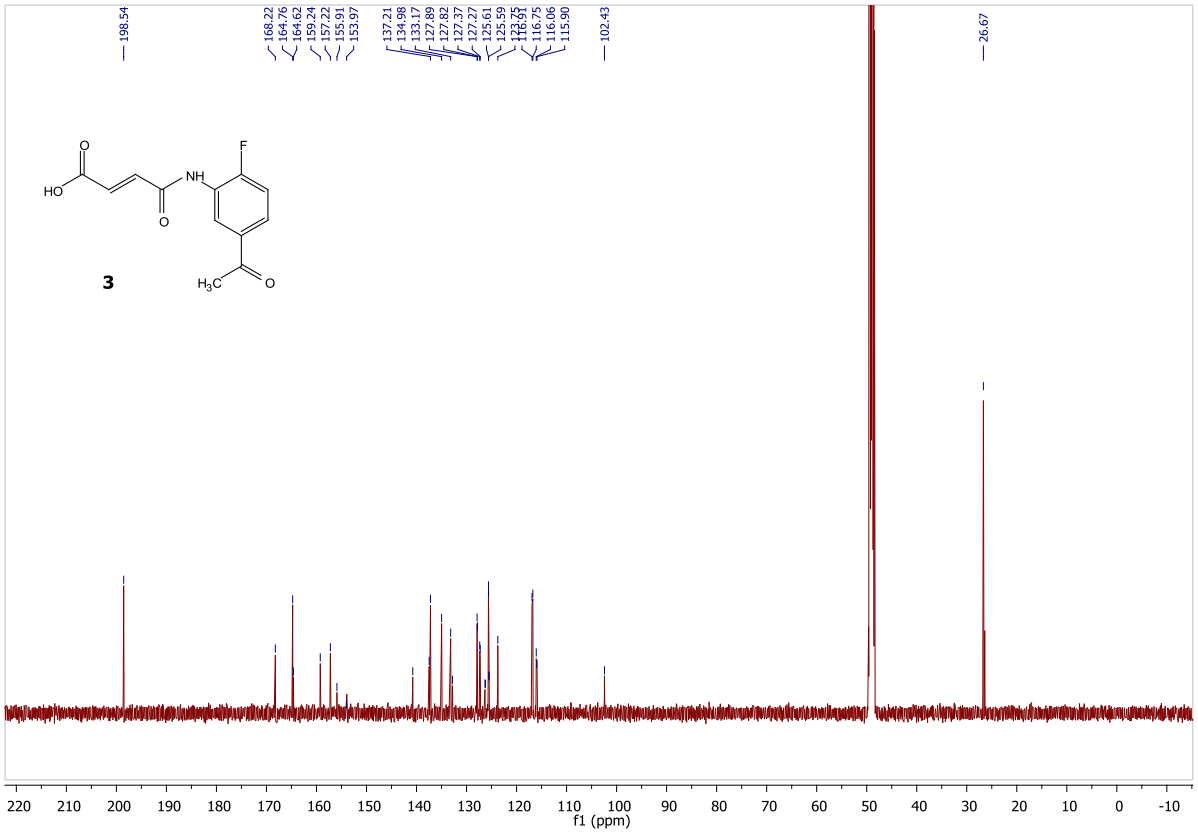


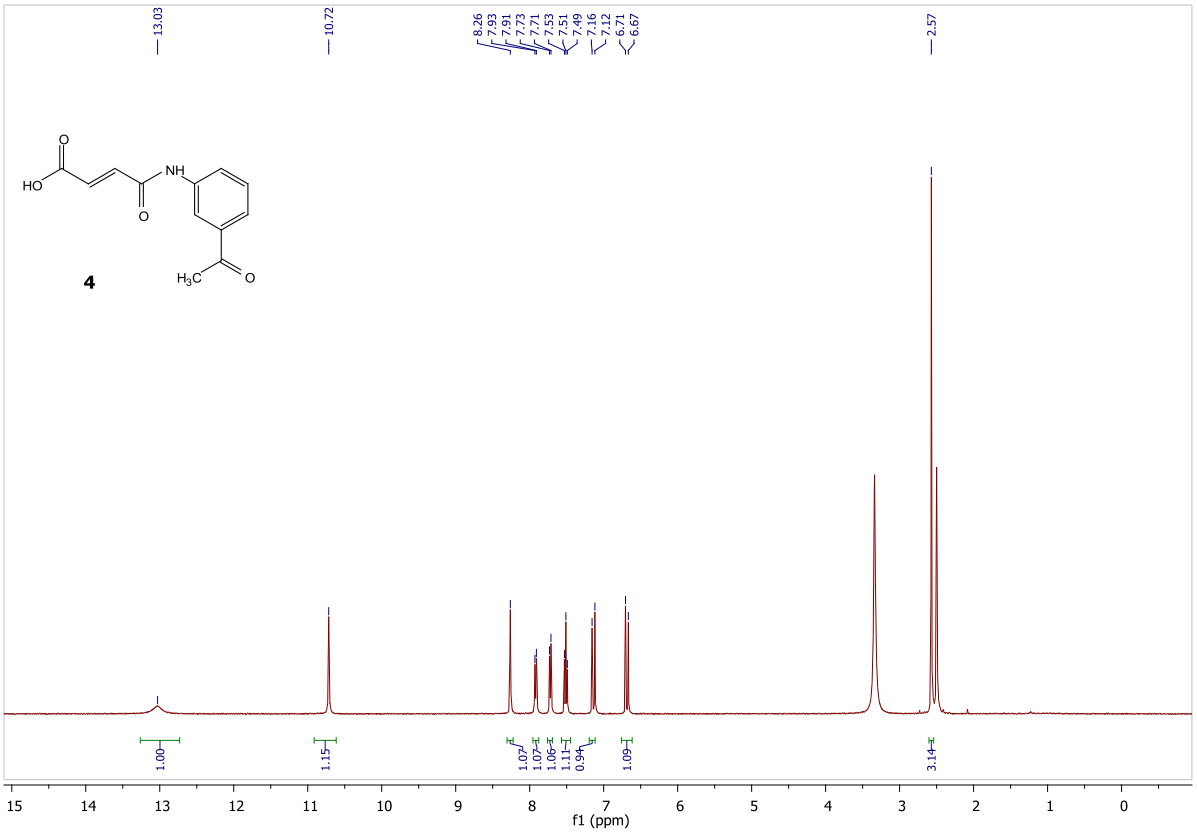


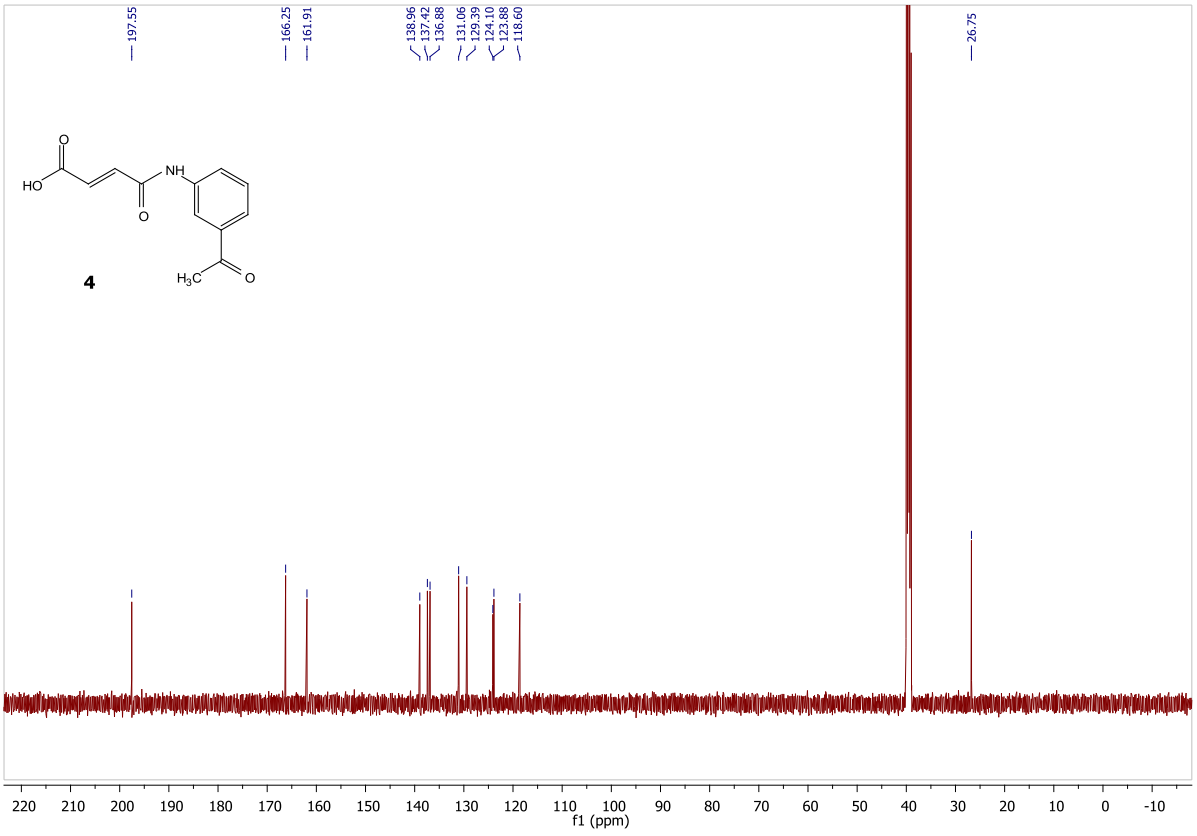

8.14
7.81
7.79
7.42
7.40
7.30
7.26
6.86
6.83
6.76
2.58
2.35
HO
O
NH
O
CH3
H3C
O
2
1.00
1.07
1.07
1.18
1.04
0.67
3.20
3.19
f1 (ppm)

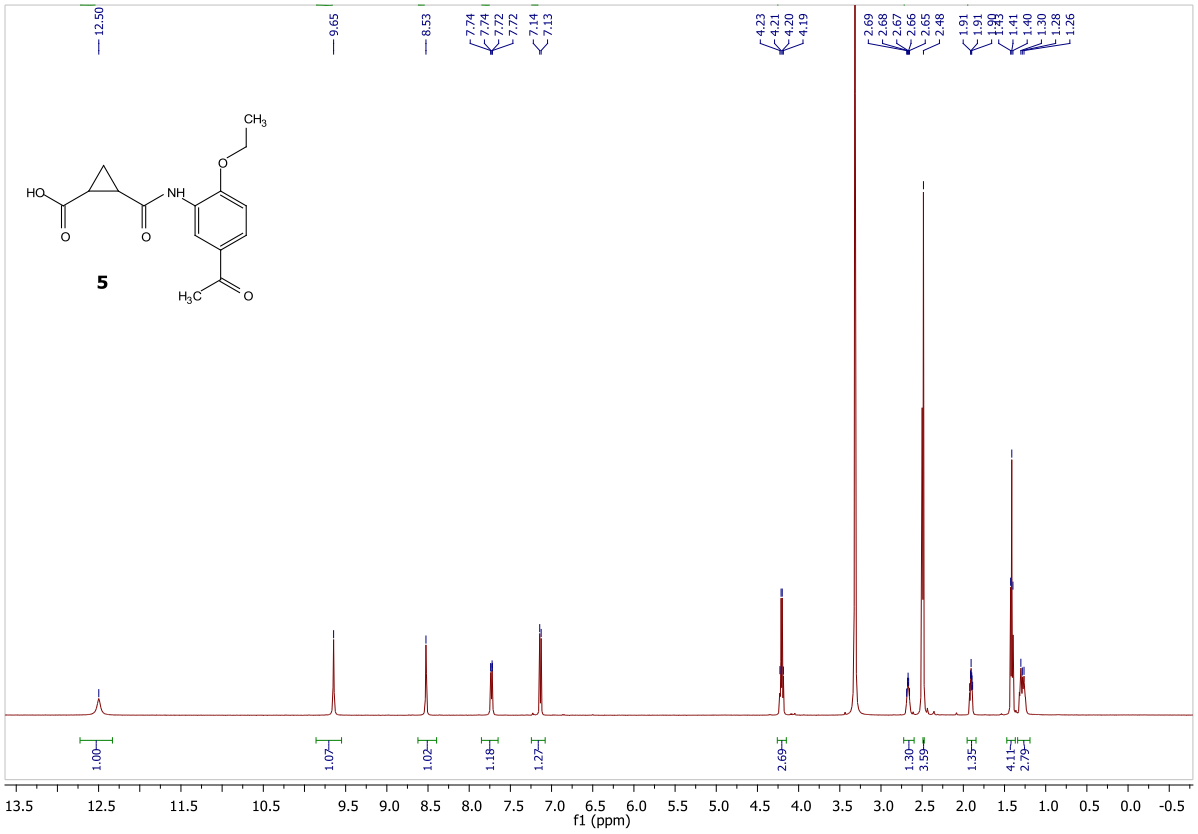

207.15
176.34
172.05
147.09
146.91
146.02
145.10
140.93
140.80
135.44
133.92
36.64
28.08
HO
O
NH
O
CH3
H3C
O
2
f1 (ppm)

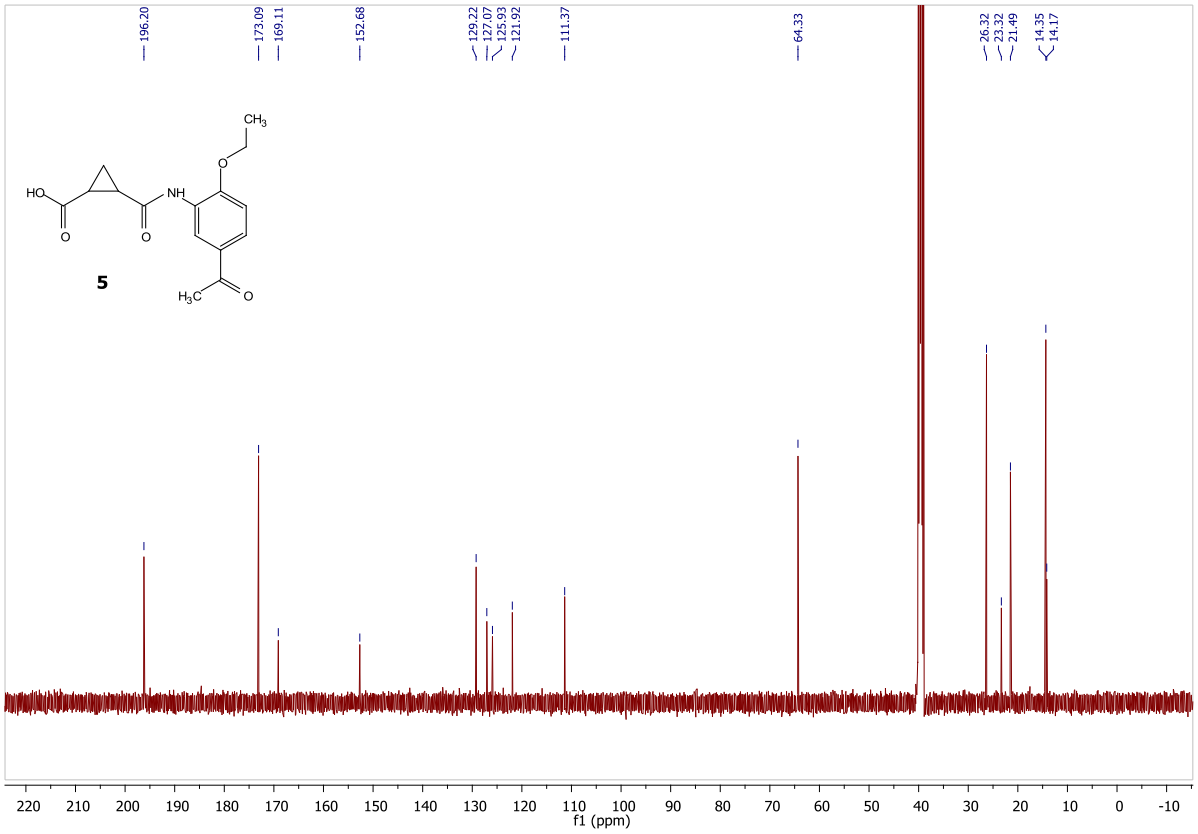

8.77
8.77
8.76
8.75
7.88
7.88
7.87
7.87
7.86
7.86
7.31
7.28
6.88
6.84
2.60
O
HO
NH
F
O
3
H3C
O
1.06
1.07
2.19
1.00
3.28
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

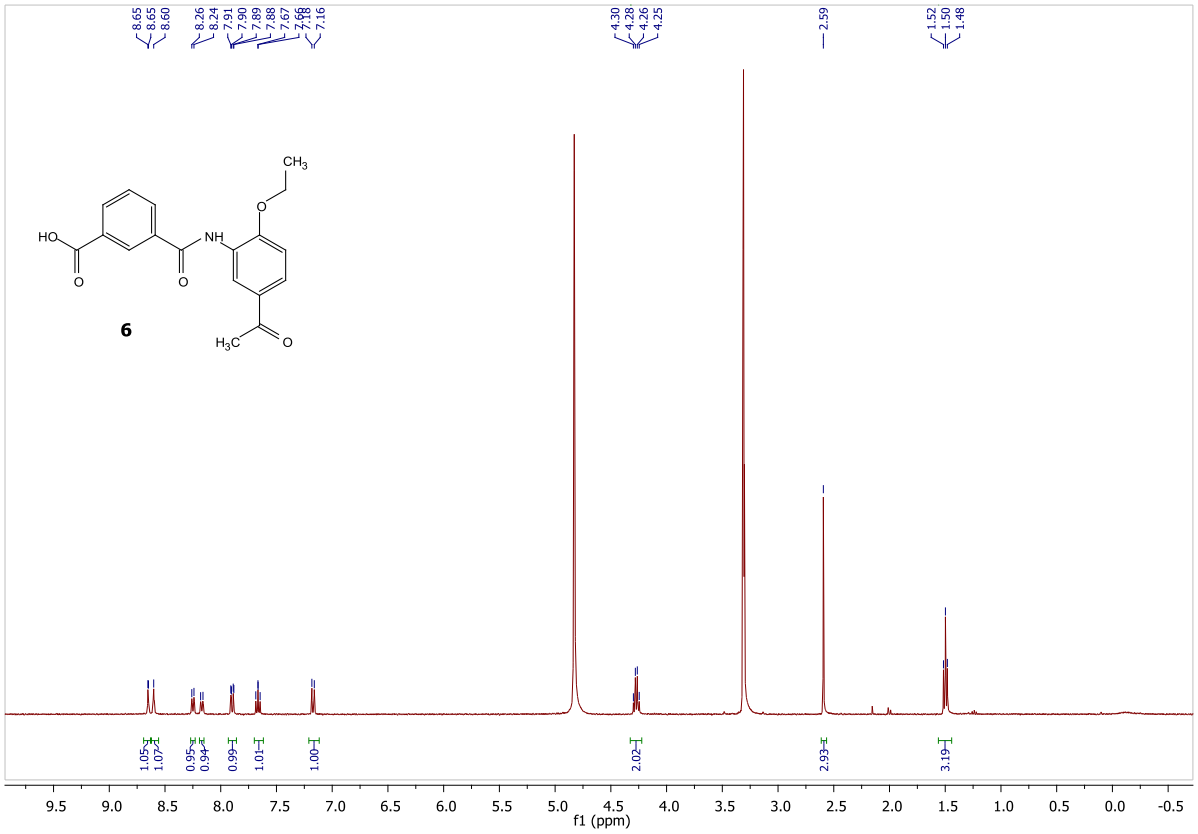

198.54
168.22
164.76
164.62
159.24
157.22
155.91
153.97
137.21
134.98
133.17
127.89
127.82
127.37
127.27
125.61
125.59
123.75
116.91
116.75
116.06
115.90
102.43
26.67
O
HO
NH
F
O
3
H3C
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

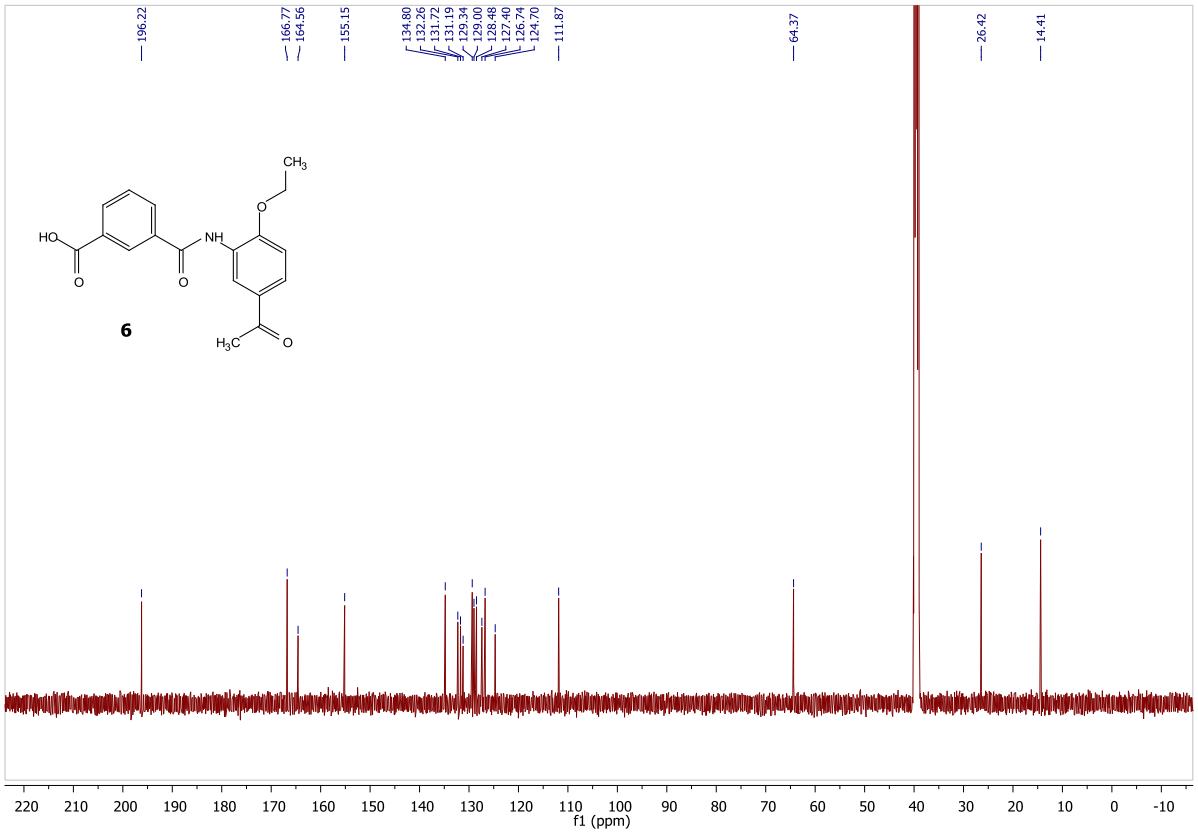

13.03
10.72
8.26
7.93
7.91
7.73
7.71
7.53
7.51
7.49
7.16
7.12
6.71
6.67
2.57
HO
O
NH
O
H3C
O
4
1.00
1.15
1.07
1.07
1.06
1.11
0.94
1.09
3.14
f1 (ppm)

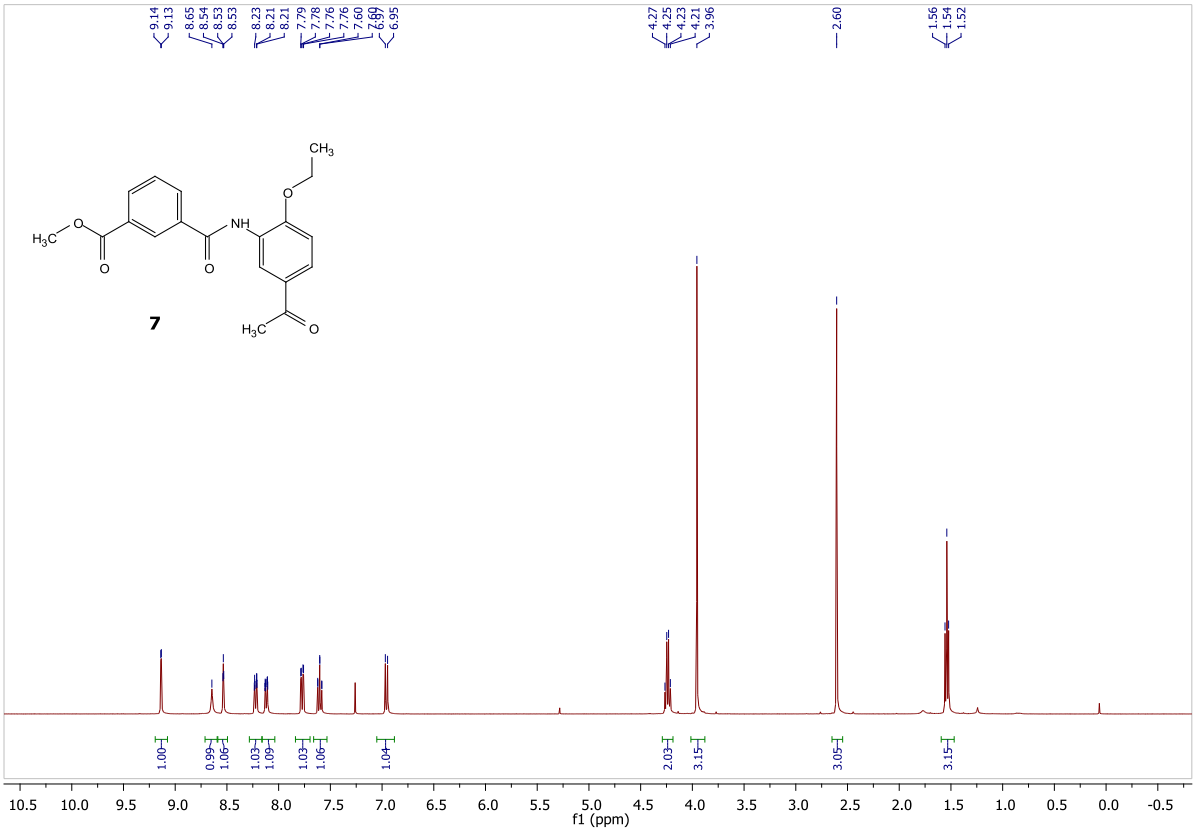

197.55
166.25
161.91
138.96
137.42
136.88
131.06
129.39
124.10
123.88
118.60
26.75
HO
O
NH
O
H3C
O
4
f1 (ppm)

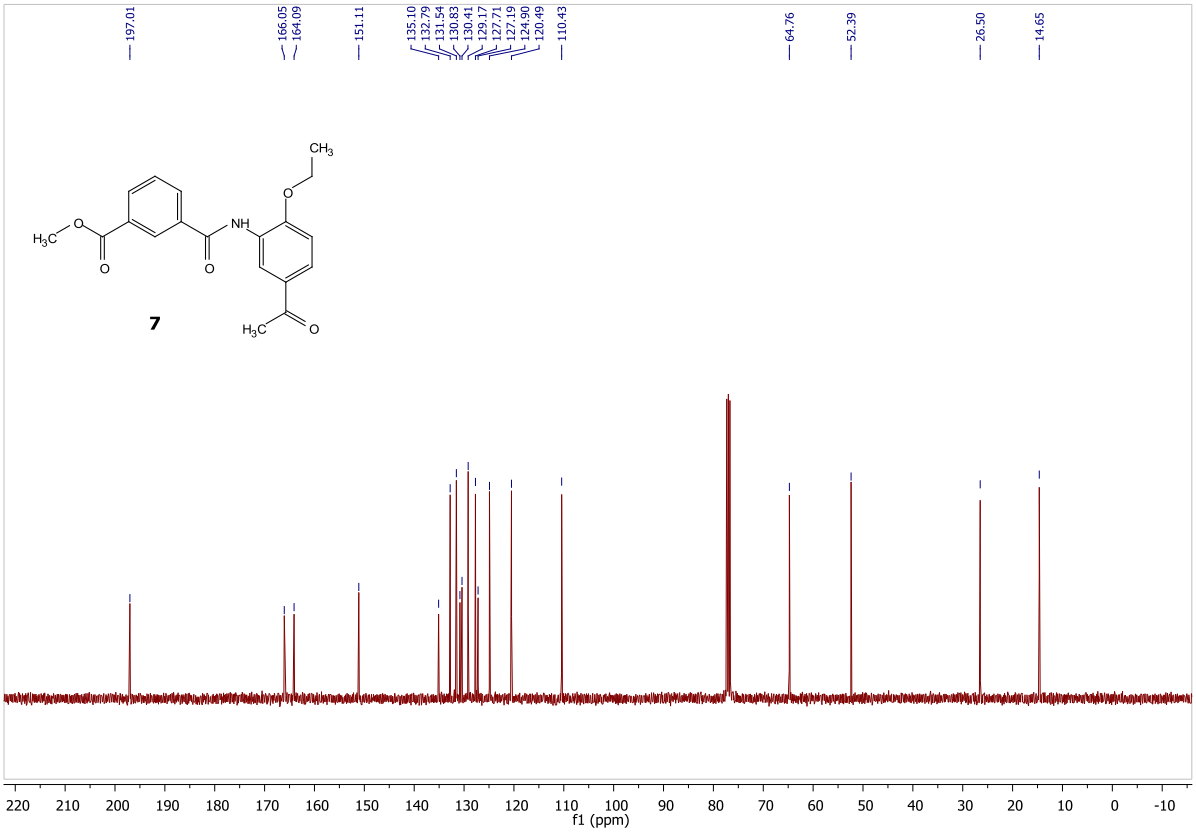

12.50
9.65
8.53
7.74
7.74
7.72
7.72
7.14
7.13
4.23
4.21
4.20
4.19
2.69
2.68
2.67
2.66
2.65
2.48
1.91
1.91
1.90
1.43
1.41
1.40
1.30
1.28
1.26
CH3
O
HO
NH
O
O
5
H3C
O
1.00
1.07
1.02
1.18
1.27
2.69
1.30
3.59
1.35
4.11
2.79
13.5 12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

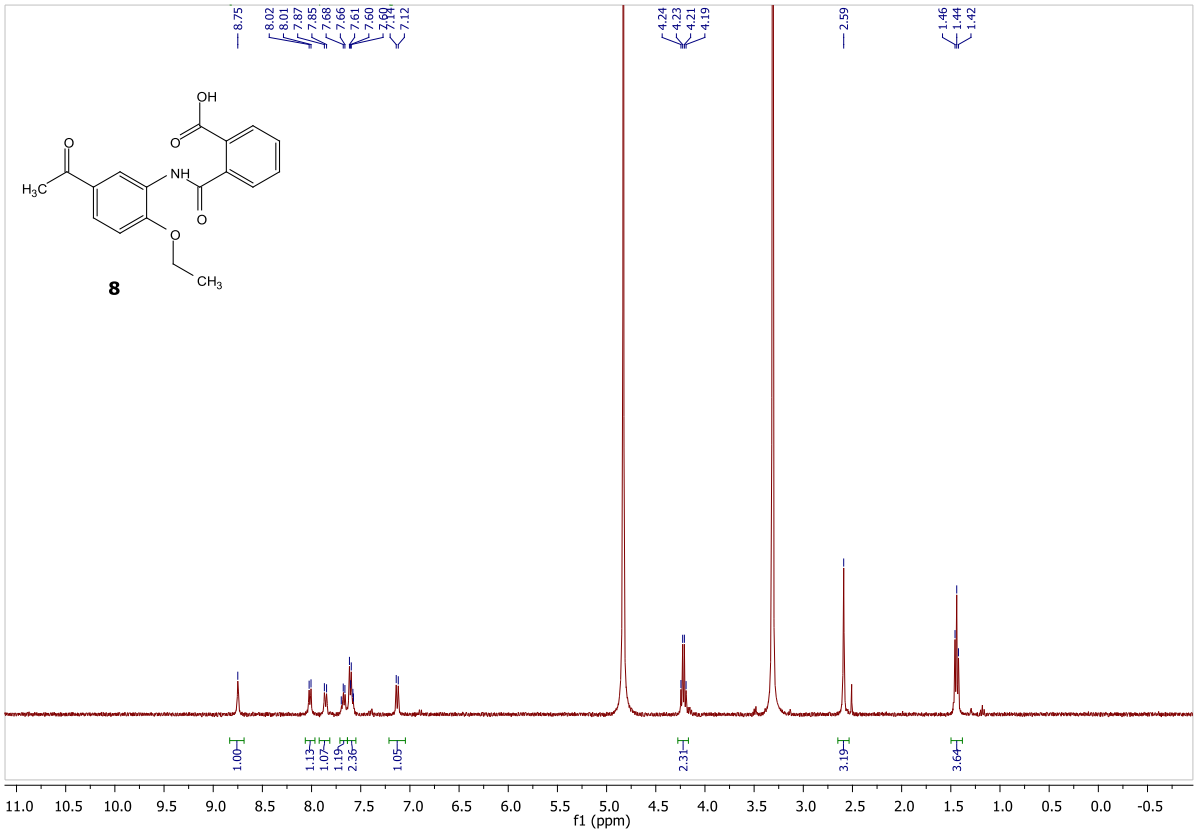

196.20
173.09
169.11
152.68
129.22
127.07
125.93
121.92
111.37
64.33
26.32
23.32
21.49
14.35
14.17
CH3
O
HO
NH
O
O
5
H3C
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


8.65
8.65
8.60
8.26
8.24
7.91
7.90
7.89
7.88
7.67
7.68
7.66
7.16
4.30
4.28
4.26
4.25
2.59
1.52
1.50
1.48
1.05
1.07
0.95
0.94
0.99
1.01
1.00
2.02
2.93
3.19
HO
O
NH
O
CH3
O
H3C
O
6
f1 (ppm)


196.22
166.77
164.56
155.15
134.80
132.26
131.72
131.19
129.34
129.00
128.48
127.40
126.74
124.70
111.87
64.37
26.42
14.41
HO
O
NH
O
CH3
O
H3C
O
6
f1 (ppm)


9.14
9.13
8.65
8.54
8.53
8.53
8.23
8.21
8.21
7.79
7.78
7.76
7.76
7.60
7.60
6.97
6.95
4.27
4.25
4.23
4.21
3.96
2.60
1.56
1.54
1.52
CH3
O
NH
H3C
O
O
O
H3C
O
7
1.00
0.99
1.06
1.03
1.09
1.03
1.06
1.04
2.03
3.15
3.05
3.15
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
f1 (ppm)


197.01
166.05
164.09
151.11
135.10
132.79
131.54
130.83
130.41
129.17
127.71
127.19
124.90
120.49
110.43
64.76
52.39
26.50
14.65
CH3
O
NH
H3C
O
O
O
H3C
O
7
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.01
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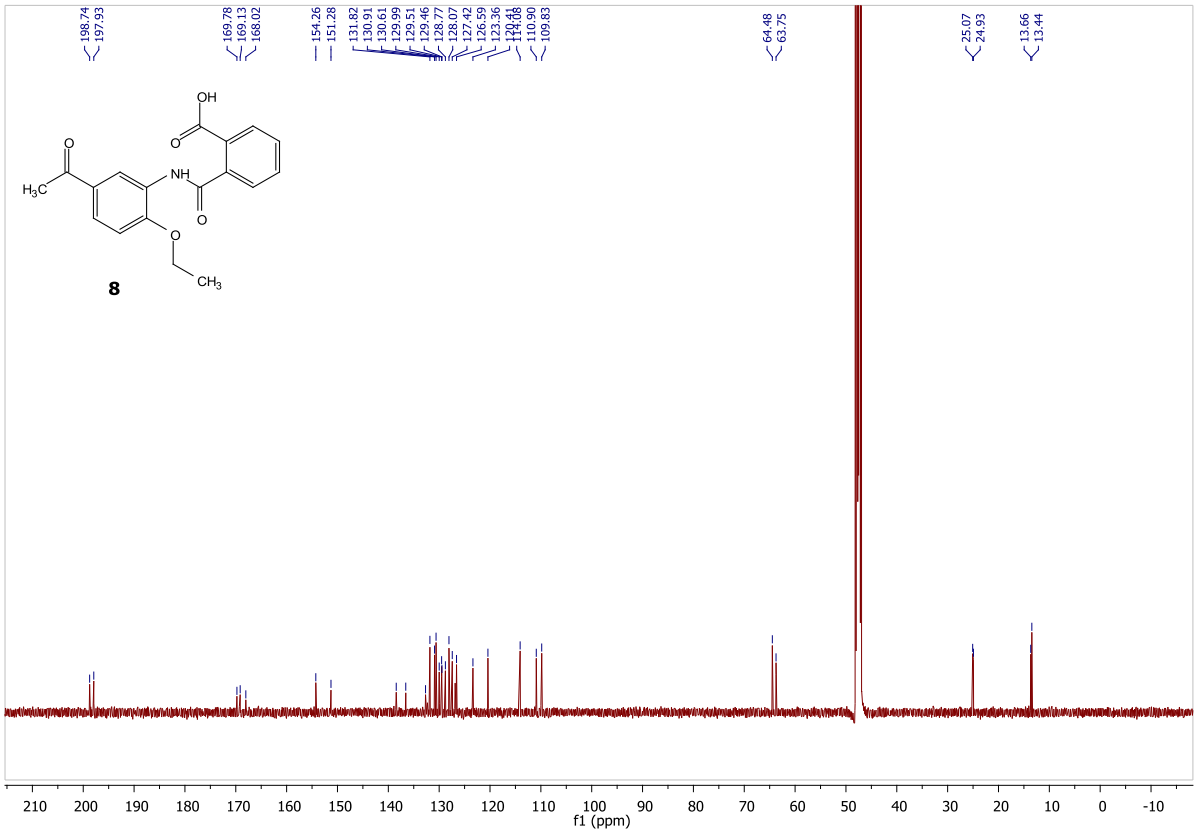
7.85
7.68
7.66
7.61
7.60
7.12
4.24
4.23
4.21
4.19
2.59
1.46
1.44
1.42
OH
O
NH
O
H3C
O
CH3
8
1.00
1.13
1.07
1.19
2.36
1.05
2.31
3.19
3.64
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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197.93
169.78
169.13
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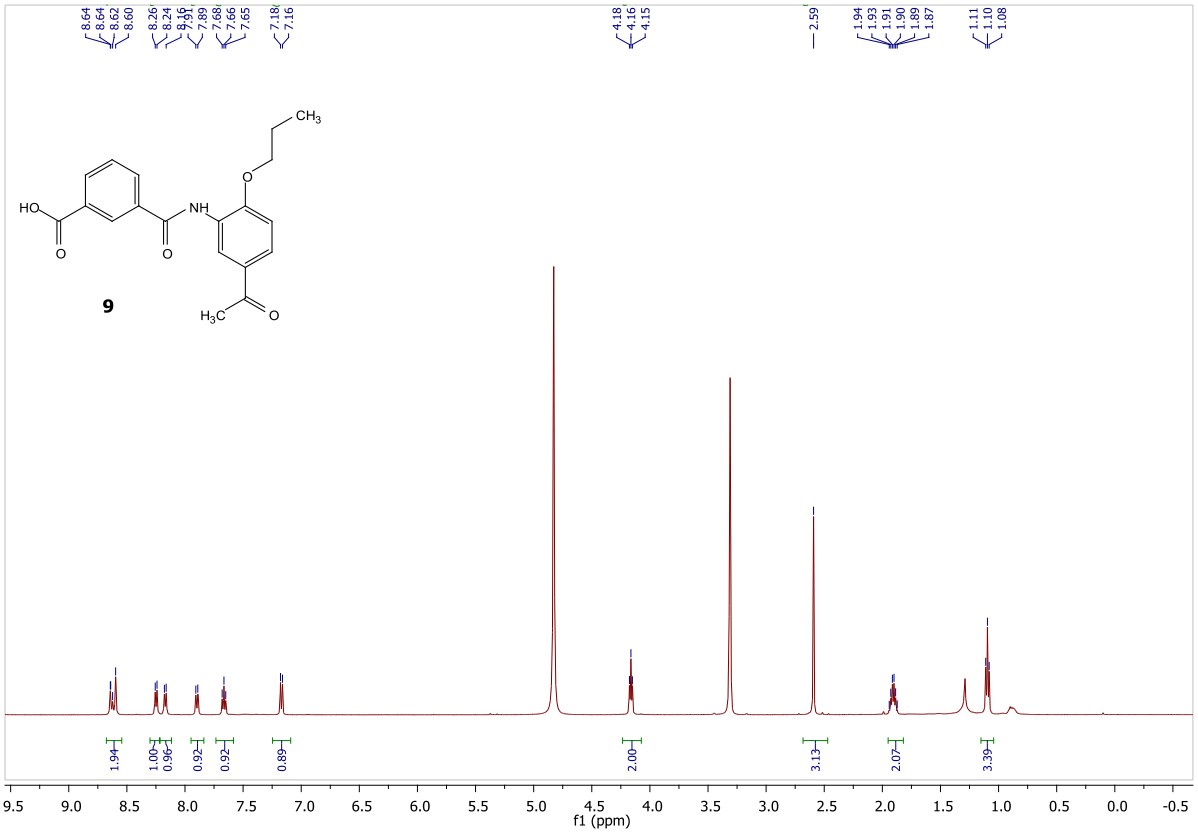
154.26
151.28
131.82
130.91
130.61
129.99
129.51
129.46
128.77
128.07
127.42
126.59
123.36
114.68
110.90
109.83
64.48
63.75
25.07
24.93
13.66
13.44
OH
O
NH
O
H3C
O
CH3
8
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.64
8.62
8.60
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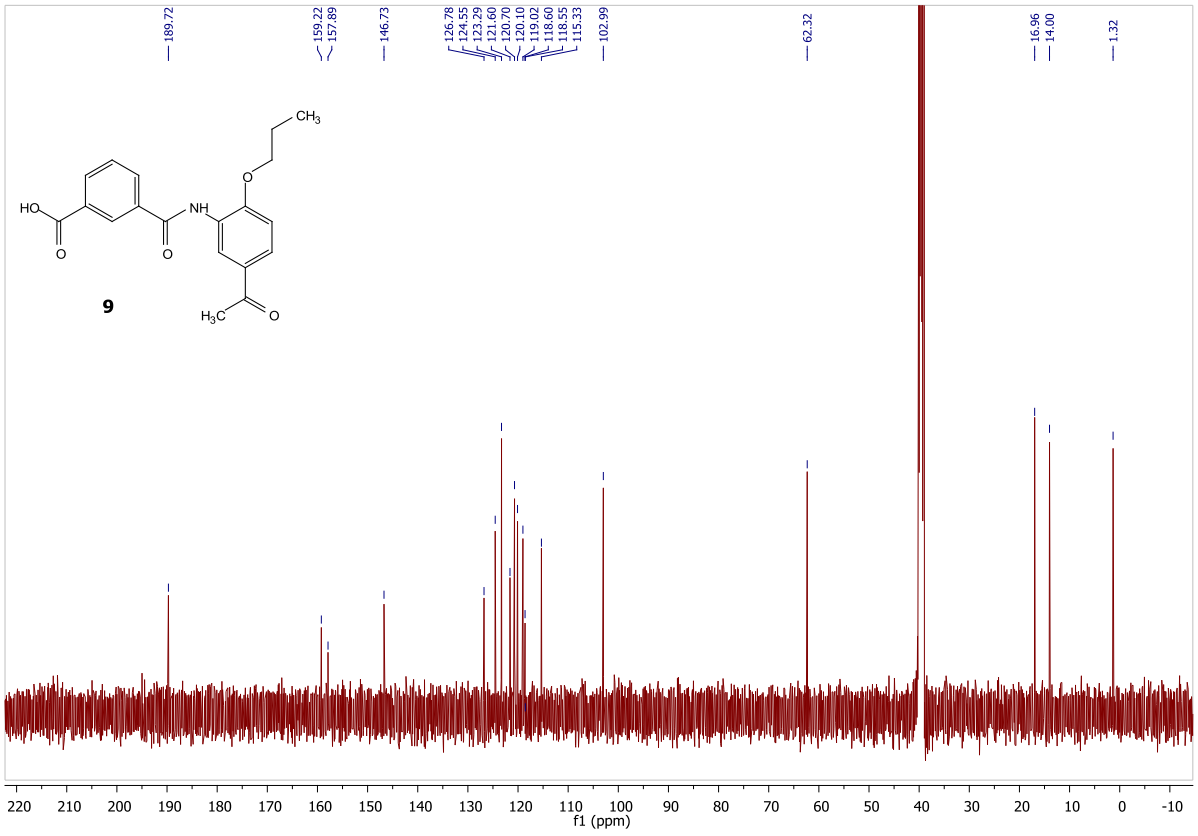
8.24
8.16
7.91
7.89
7.68
7.66
7.65
7.18
7.16
4.18
4.16
4.15
2.59
1.94
1.93
1.91
1.90
1.89
1.87
1.11
1.10
1.08
CH3
O
HO
NH
O
O
9
H3C
O
1.94
1.00
0.96
0.92
0.92
0.89
2.00
3.13
2.07
3.39
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


189.72
159.22
157.89
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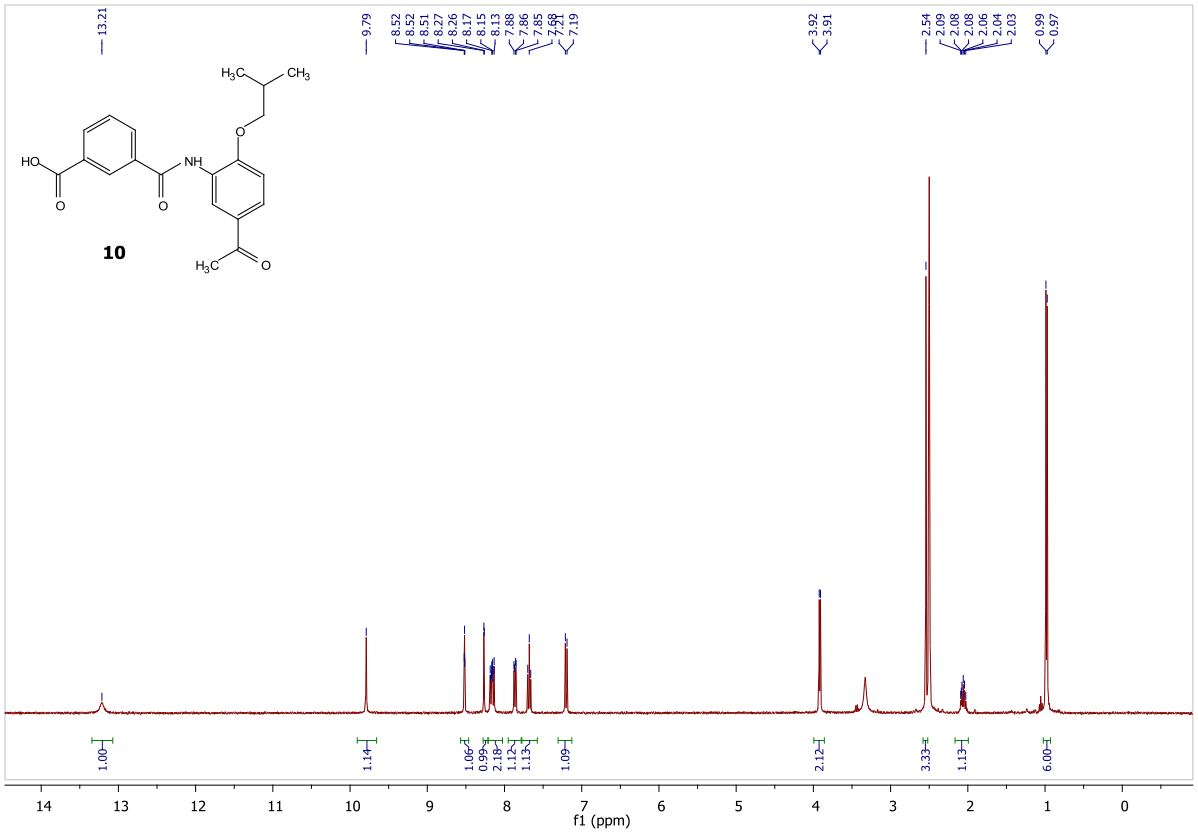
124.55
123.29
121.60
120.70
120.10
119.02
118.60
118.55
115.33
102.99
62.32
16.96
14.00
1.32
CH3
O
HO
NH
O
O
9
H3C
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.52
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8.26
8.17
8.15
8.13
7.88
7.86
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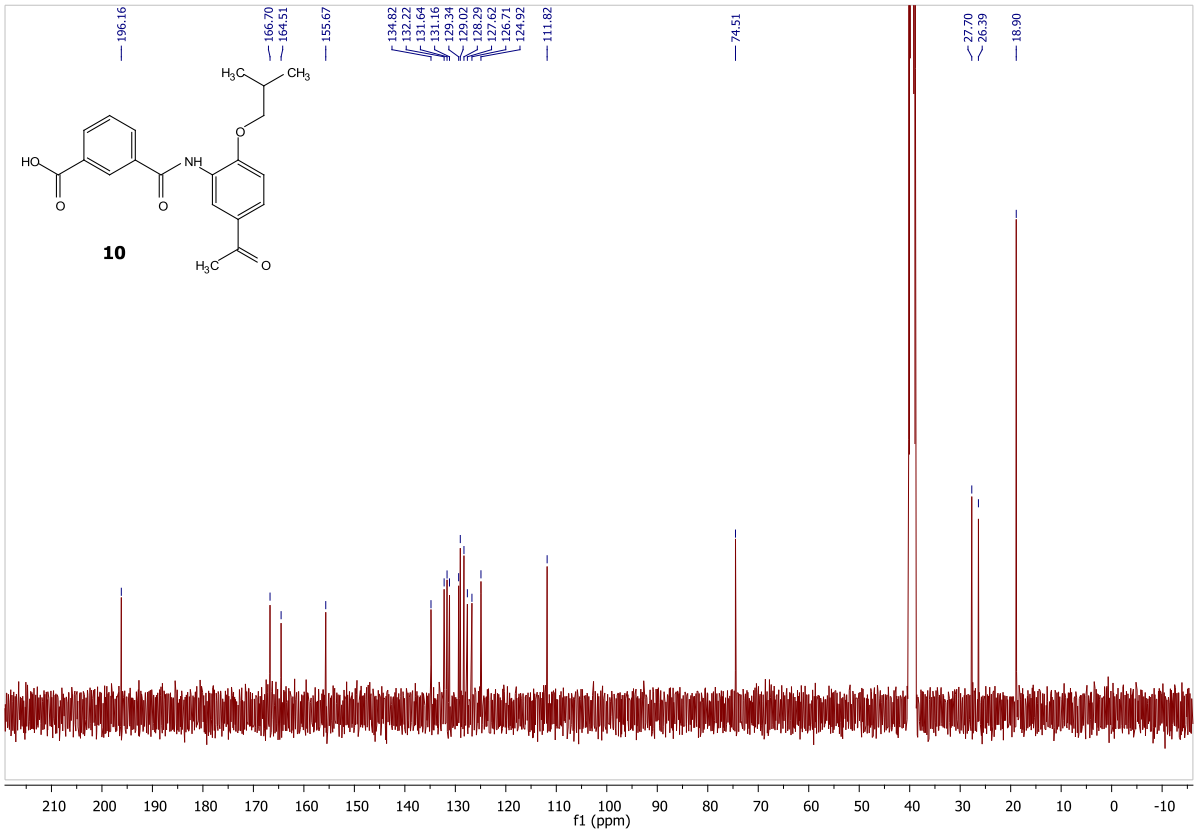
7.68
7.61
7.19
3.92
3.91
2.54
2.09
2.08
2.08
2.06
2.04
2.03
0.99
0.97
10
1.00
1.14
1.06
0.99
2.18
1.12
1.13
1.09
2.12
3.33
1.13
6.00
f1 (ppm)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166.70
164.51
155.67
134.82
132.22
131.64
131.16
129.34
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128.29
127.62
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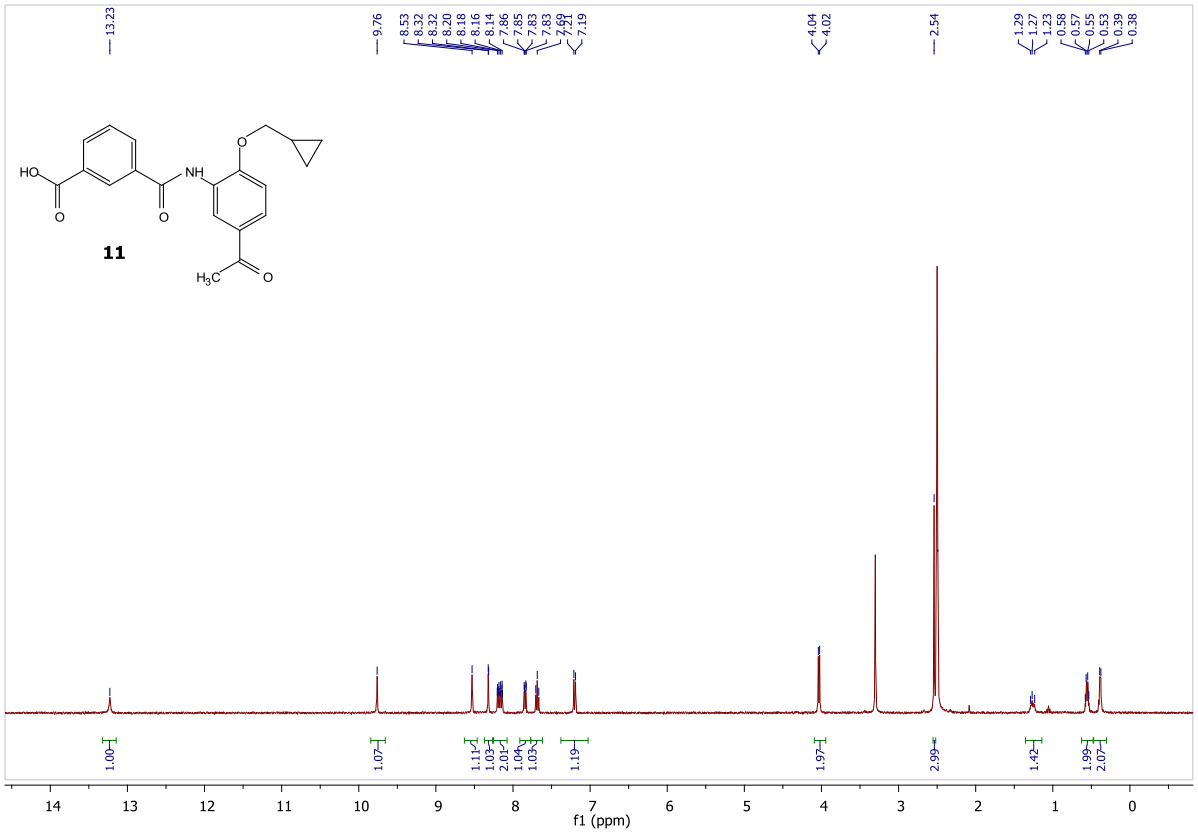
74.51
27.70
26.39
18.90
10
f1 (ppm)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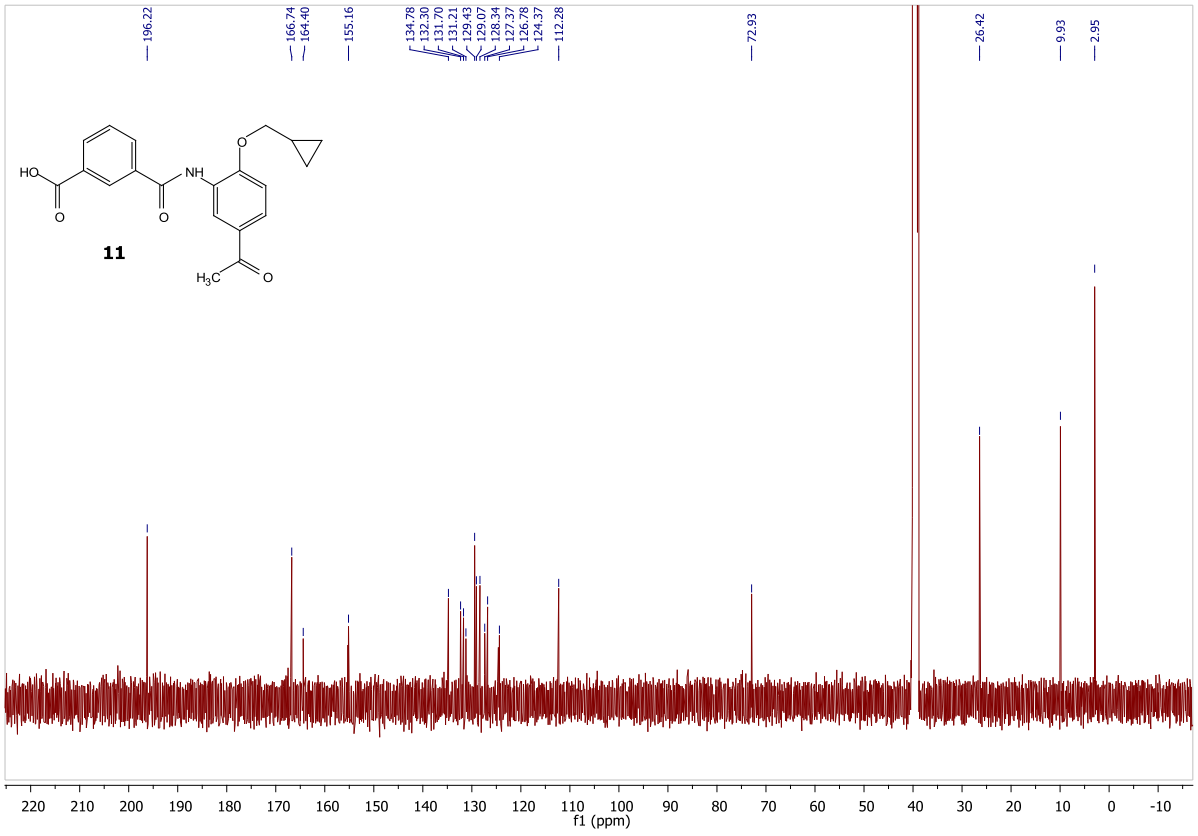
8.18
8.16
8.14
7.86
7.85
7.83
7.83
7.69
7.21
7.19
4.04
4.02
2.54
1.29
1.27
1.23
0.58
0.57
0.55
0.53
0.39
0.38
HO
O
O
NH
O
11
H3C
O
1.00
1.07
1.11
1.03
2.01
1.04
1.03
1.19
1.97
2.99
1.42
1.99
2.07
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)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166.74
164.40
155.16
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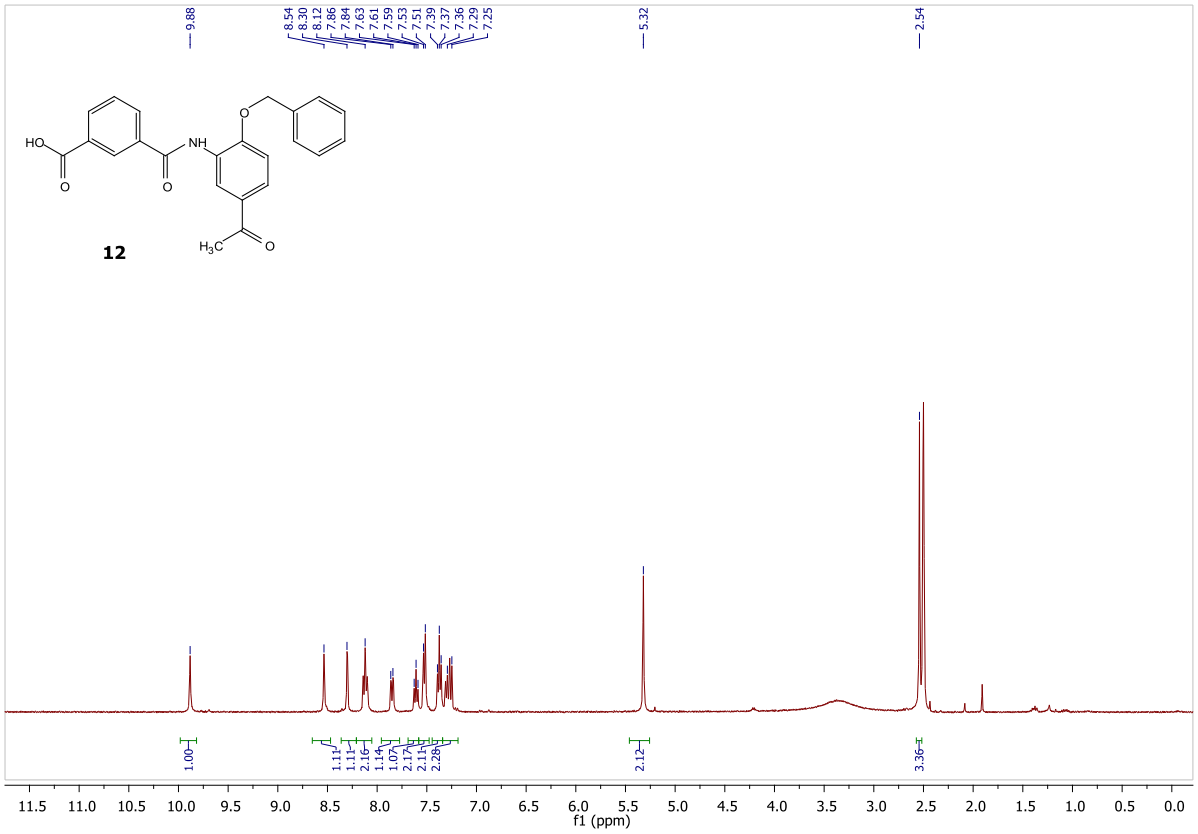
132.30
131.70
131.21
129.43
129.07
128.34
127.37
126.78
124.37
112.28
72.93
26.42
9.93
2.95
HO
O
O
NH
O
11
H3C
O
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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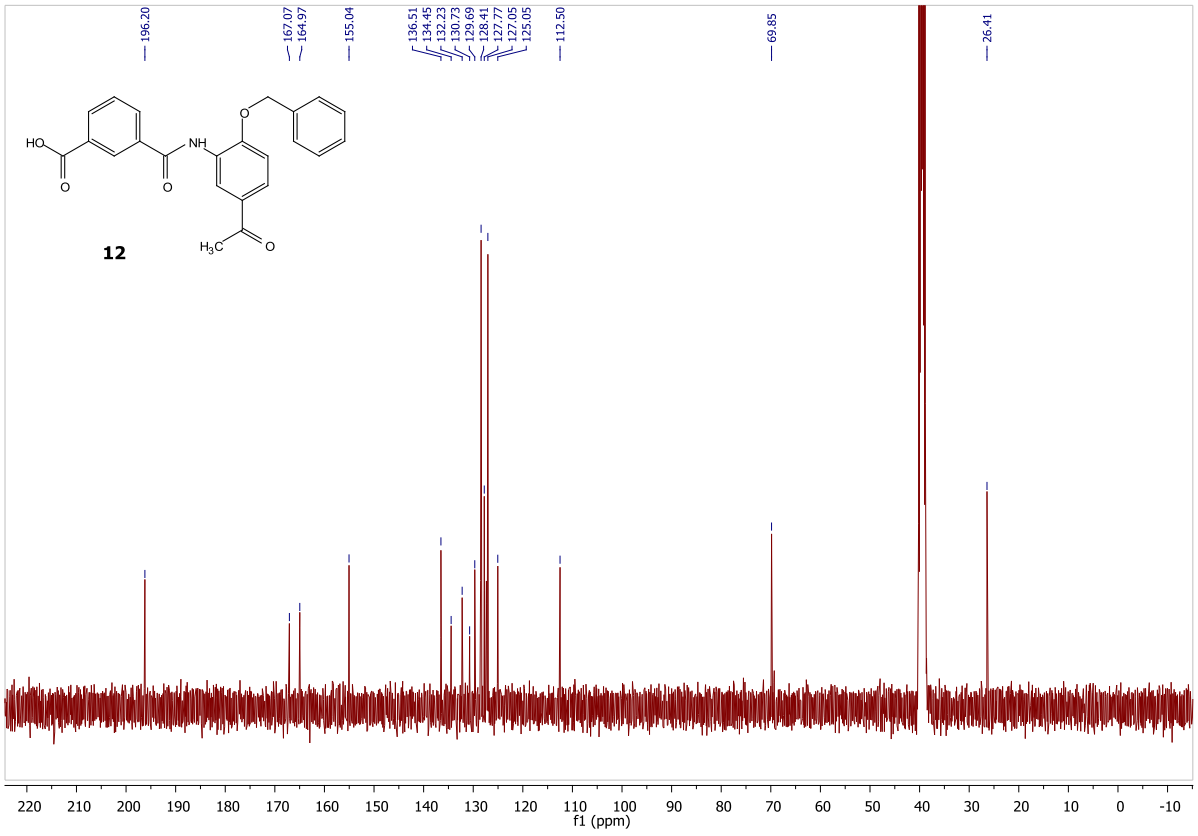
7.84
7.63
7.61
7.59
7.53
7.51
7.39
7.37
7.36
7.29
7.25
5.32
2.54
HO
O
NH
O
O
H3C
O
12
1.00
1.11
1.11
2.16
1.14
1.07
2.17
2.11
2.28
2.12
3.36
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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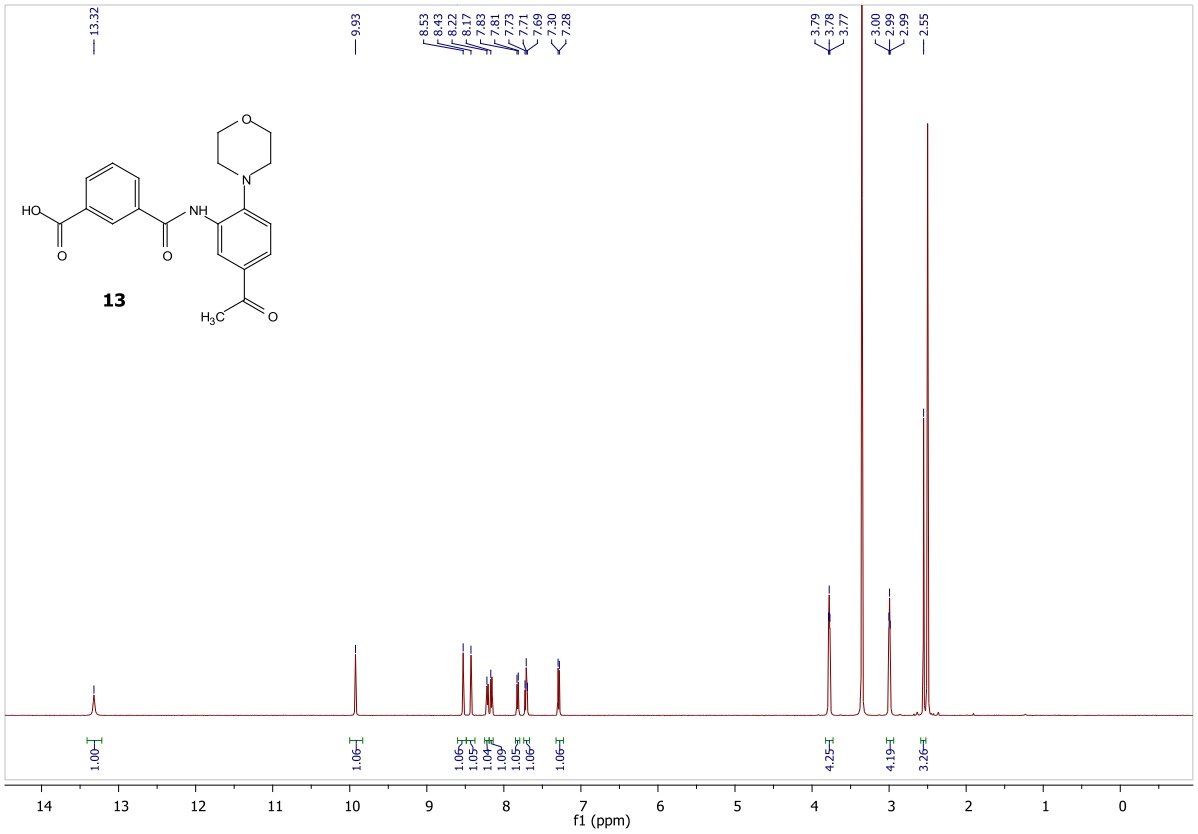
167.07
164.97
155.04
136.51
134.45
132.23
130.73
129.69
128.41
127.77
127.05
125.05
112.50
69.85
26.41
HO
O
NH
O
O
H3C
O
12
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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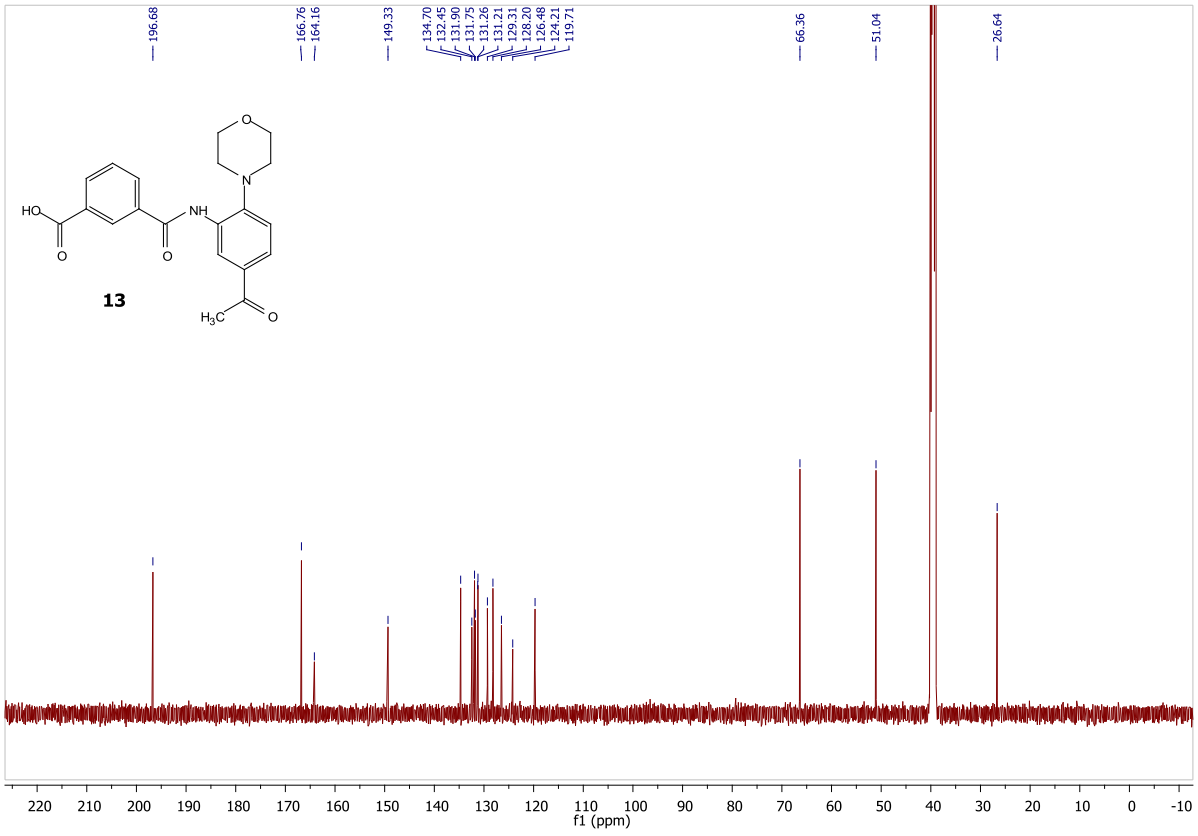
8.53
8.43
8.22
8.17
7.83
7.81
7.73
7.71
7.69
7.30
7.28
3.79
3.78
3.77
3.00
2.99
2.99
2.55
HO
O
O
NH
N
O
H3C
O
13
1.00
1.06
1.06
1.05
1.04
1.09
1.05
1.06
1.06
4.25
4.19
3.26
f1 (ppm)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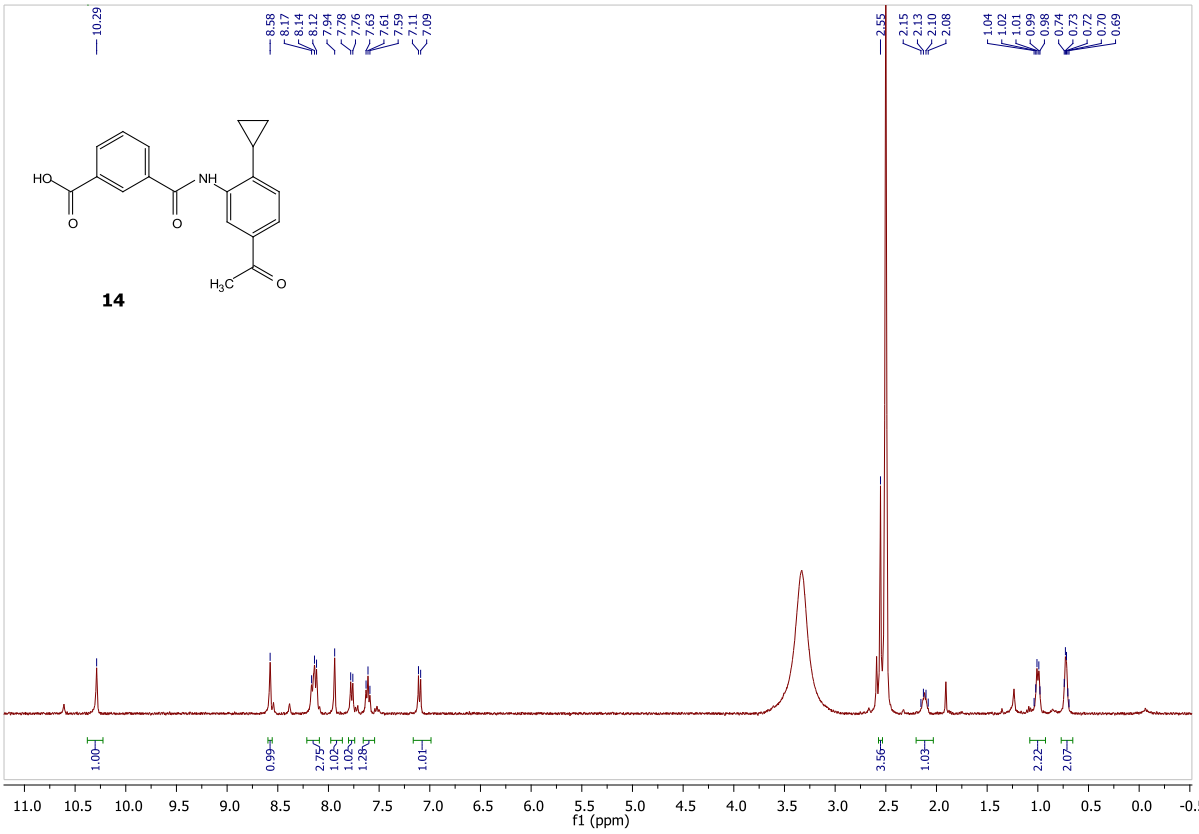
149.33
134.70
132.45
131.90
131.75
131.26
131.21
129.31
128.20
126.48
124.21
119.71
66.36
51.04
26.64
HO
O
O
NH
N
O
H3C
O
13
f1 (ppm)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8.58
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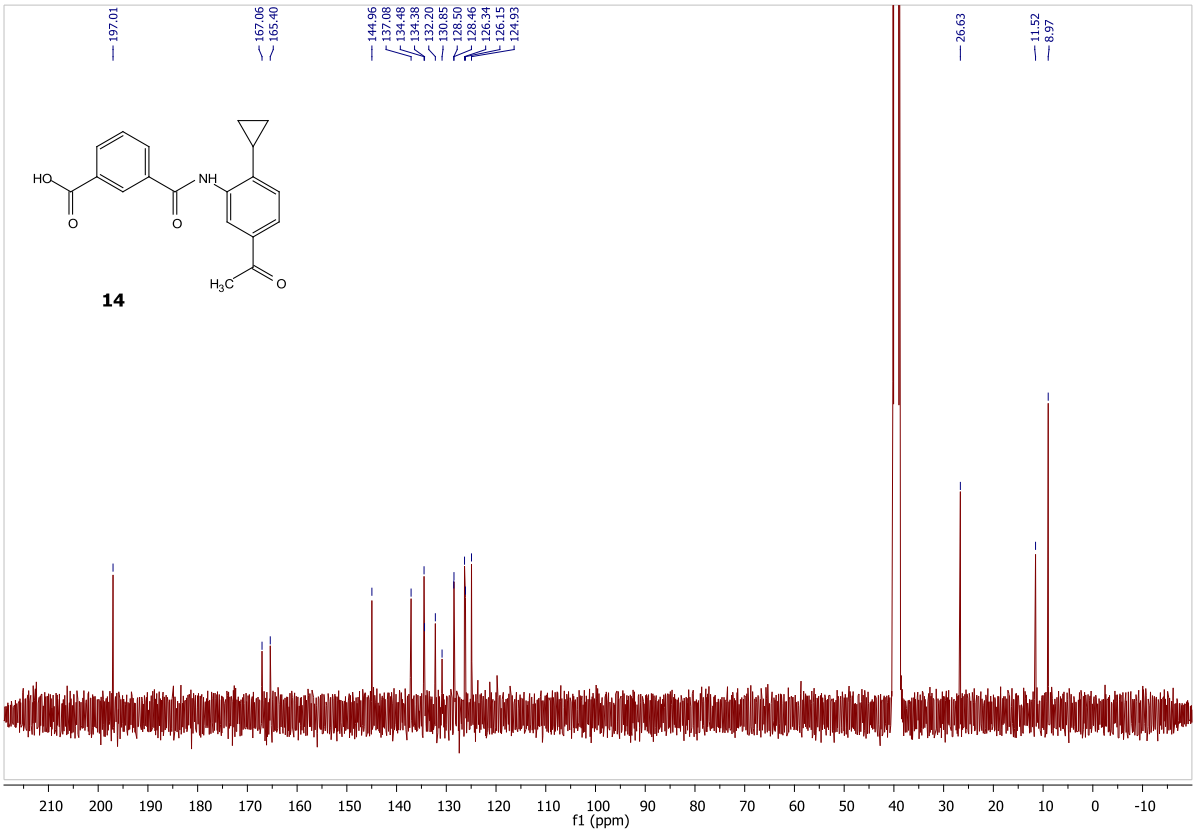
8.14
8.12
7.94
7.78
7.76
7.63
7.61
7.59
7.11
7.09
2.55
2.15
2.13
2.10
2.08
1.04
1.02
1.01
0.99
0.98
0.74
0.73
0.72
0.70
0.69
HO
O
O
NH
H3C
O
14
1.00
0.99
2.75
1.02
1.02
1.28
1.01
3.56
1.03
2.22
2.07
f1 (ppm)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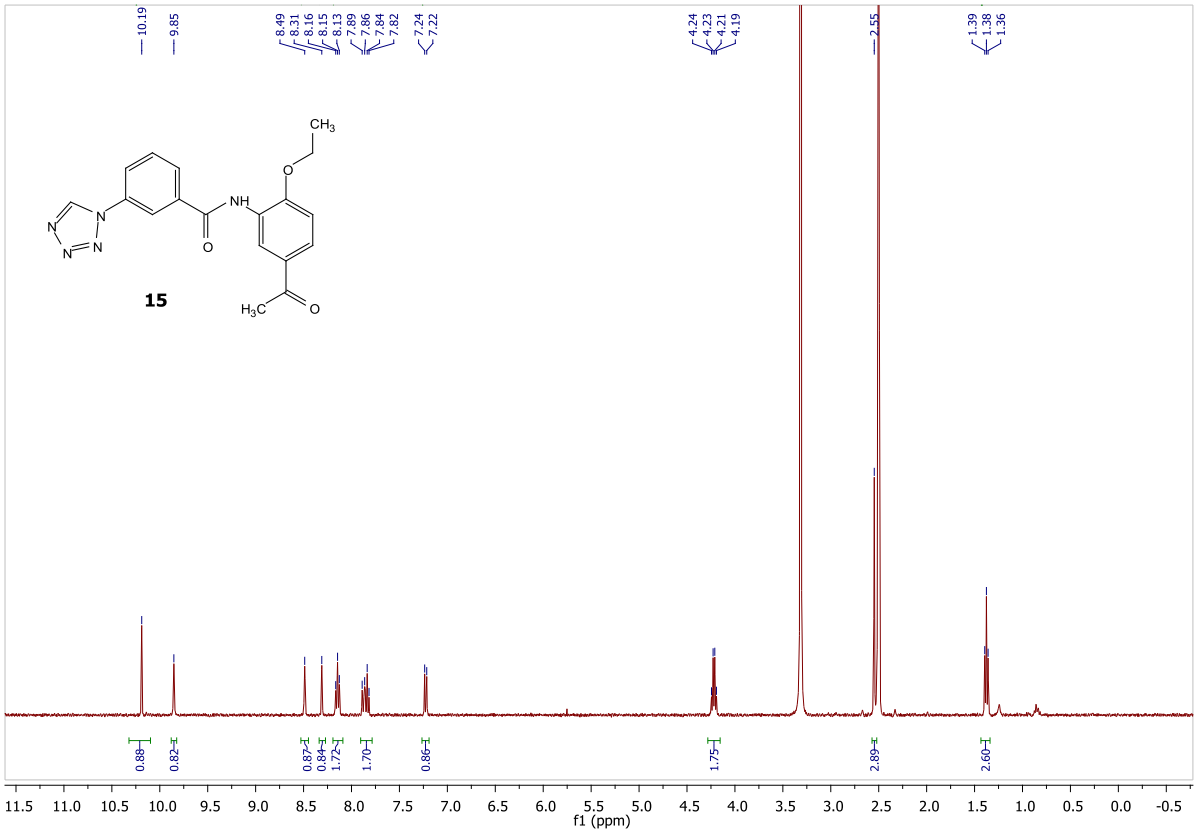
165.40
144.96
137.08
134.48
134.38
132.20
130.85
128.50
128.46
126.34
126.15
124.93
26.63
11.52
8.97
HO
O
O
NH
H3C
O
14
f1 (ppm)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8.15
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7.89
7.86
7.84
7.82
7.24
7.22
4.24
4.23
4.21
4.19
2.55
1.39
1.38
1.36
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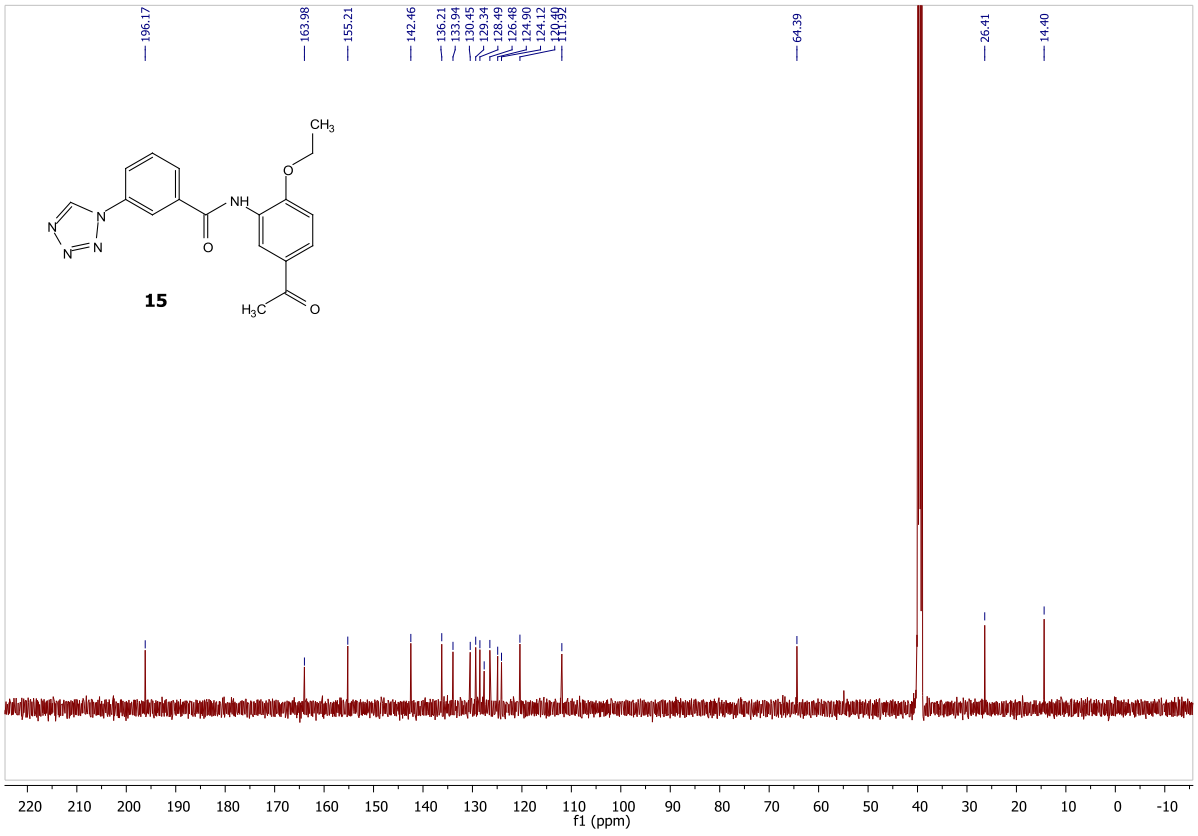
0.82
0.87
0.84
1.72
1.70
0.86
1.75
2.89
2.60
15
f1 (ppm)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136.21
133.94
130.45
129.34
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124.90
124.12
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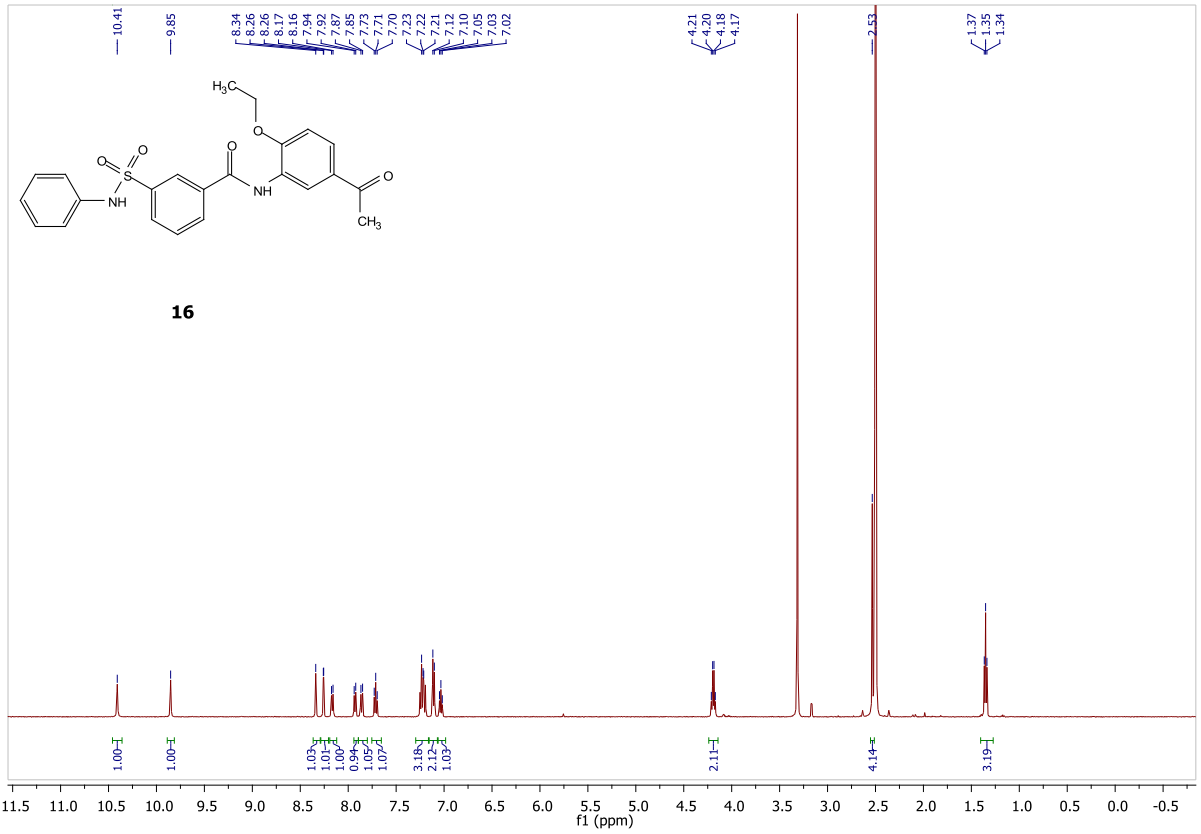
64.39
26.41
14.40
15
f1 (ppm)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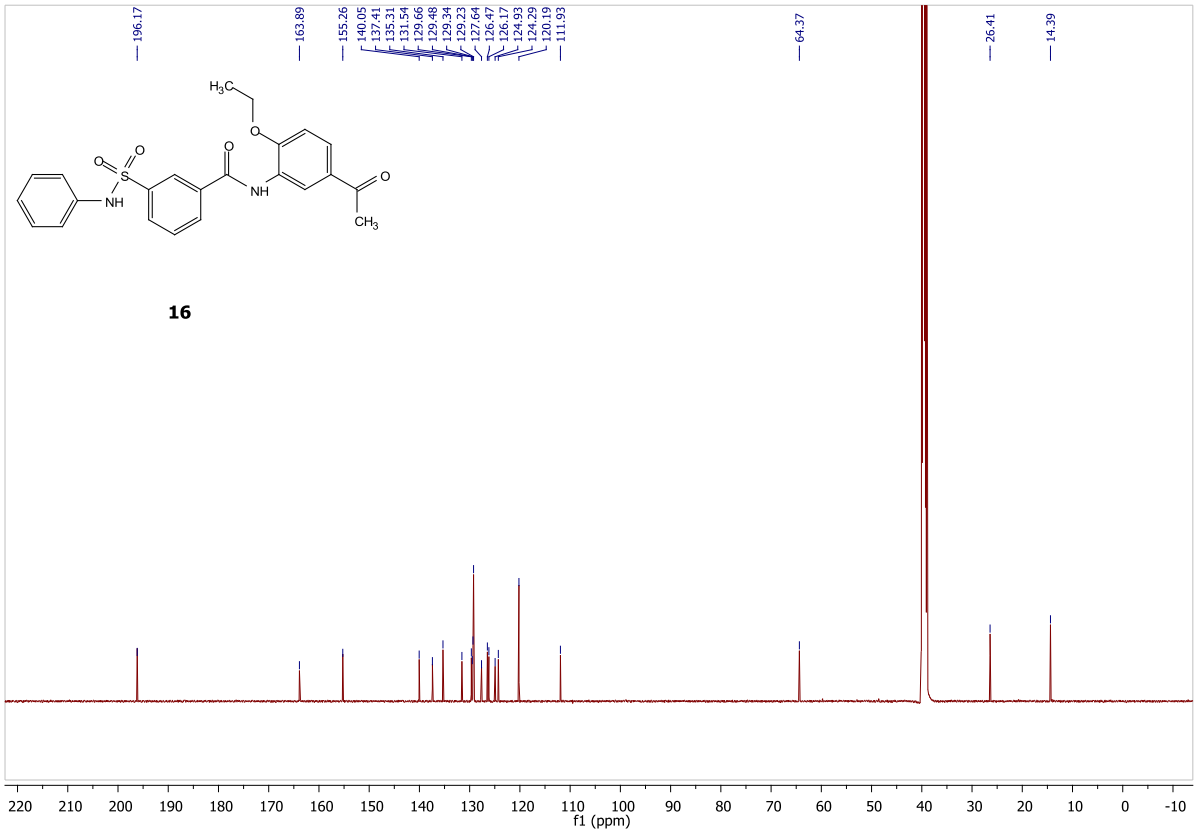
8.26
8.17
8.16
7.94
7.92
7.87
7.85
7.73
7.71
7.70
7.23
7.22
7.21
7.12
7.10
7.05
7.03
7.02
4.21
4.20
4.18
4.17
2.53
1.37
1.35
1.34
H3C
O
O
S
O
NH
O
NH
O
CH3
16
1.00
1.00
1.03
1.01
1.00
0.94
1.05
1.07
3.18
2.12
1.03
2.11
4.14
3.19
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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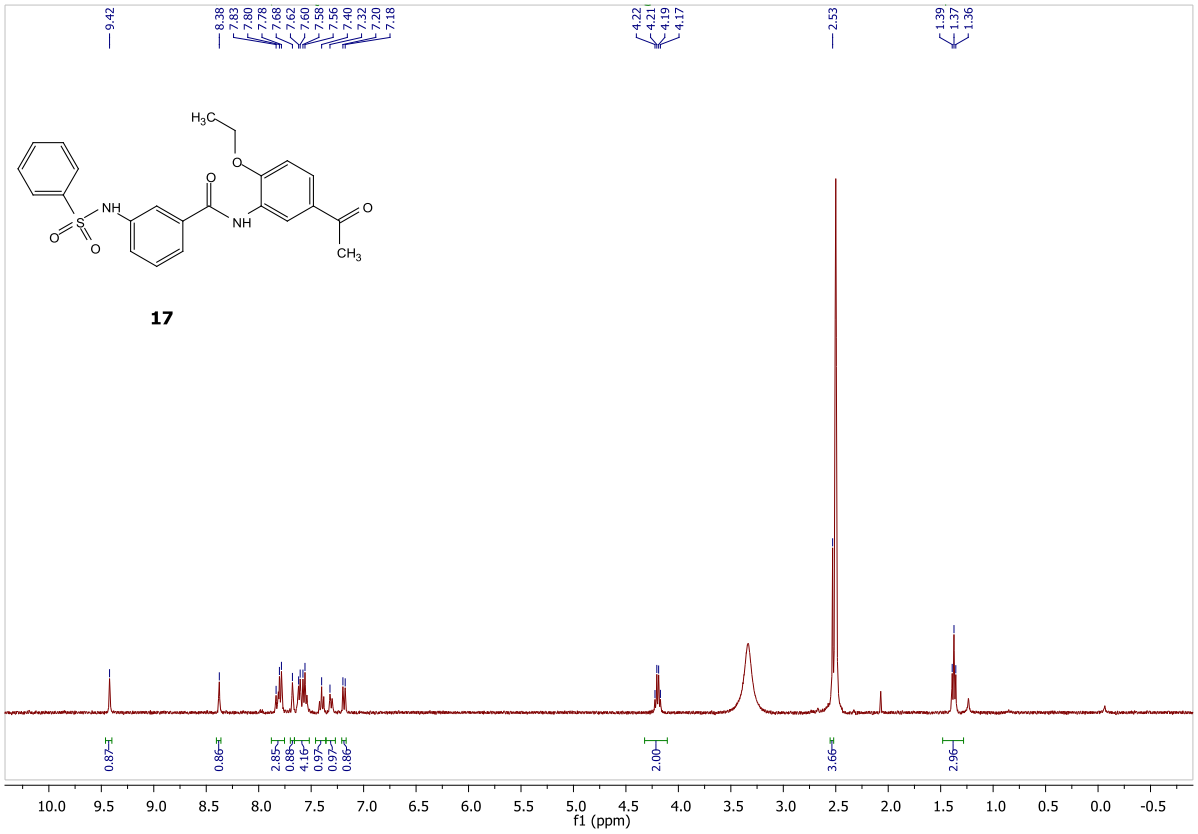
137.41
135.31
131.54
129.66
129.48
129.34
129.23
127.64
126.47
126.17
124.93
124.29
120.19
111.93
64.37
26.41
14.39
H3C
O
O
S
O
NH
O
NH
O
CH3
16
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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7.68
7.62
7.60
7.58
7.56
7.40
7.32
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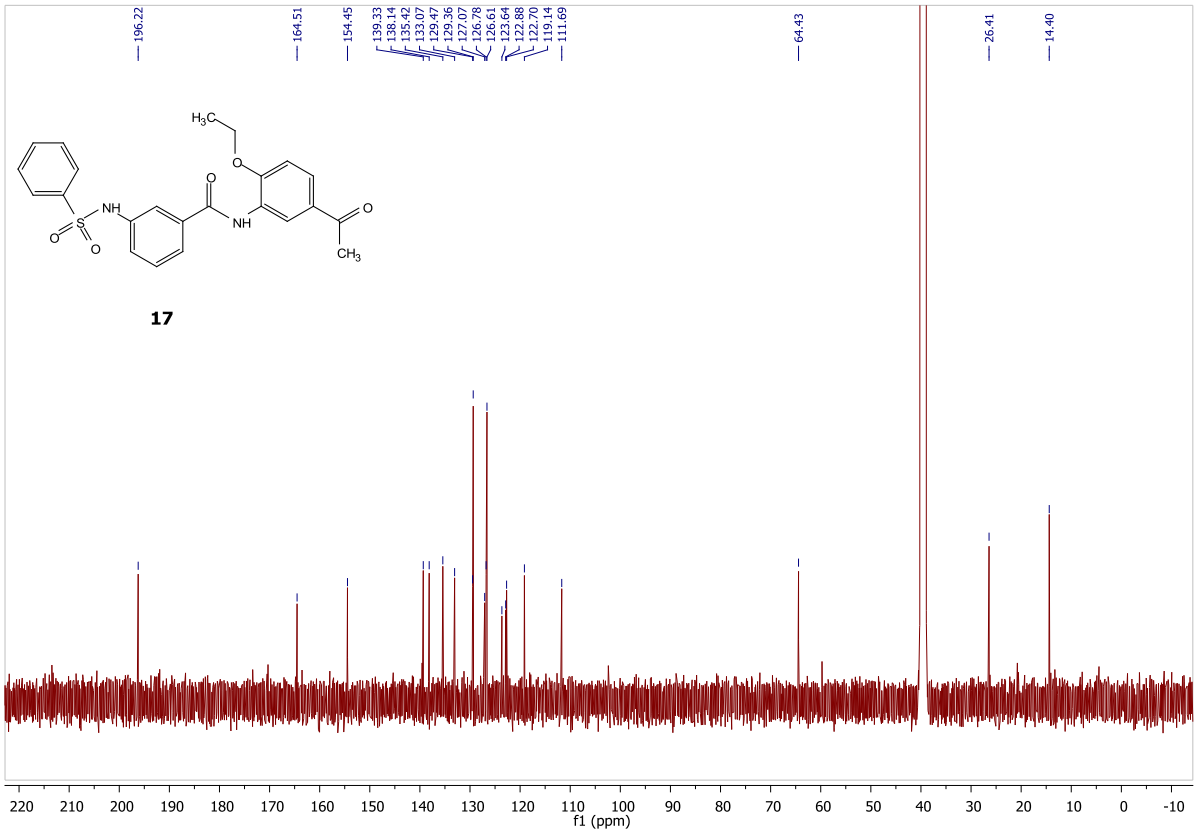
7.18
4.22
4.21
4.19
4.17
2.53
1.39
1.37
1.36
0.87
0.86
2.85
0.88
4.16
0.97
0.97
0.86
2.00
3.66
2.96
f1 (ppm)

17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164.51
154.45
139.33
138.14
135.42
133.07
129.47
129.36
127.07
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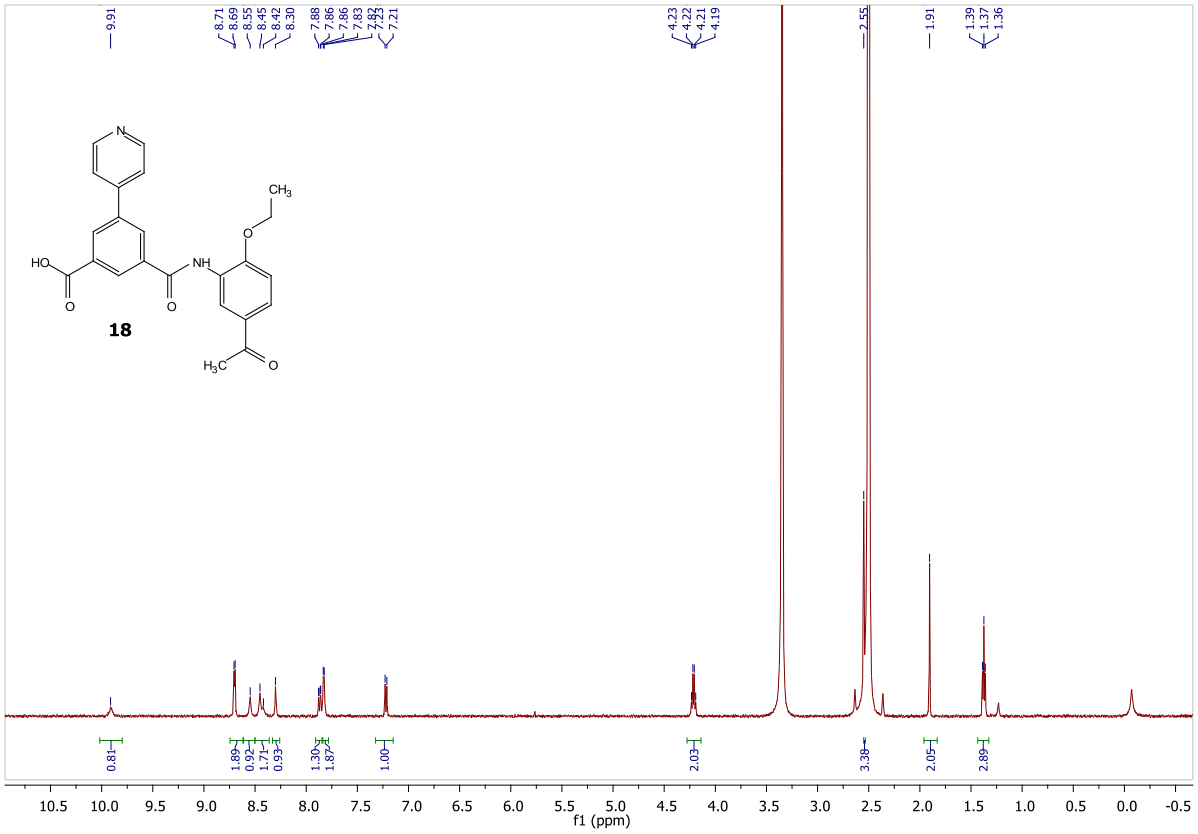
123.64
122.88
122.70
119.14
111.69
64.43
26.41
14.40
f1 (ppm)

17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7.86
7.83
7.82
7.23
7.21
4.23
4.22
4.21
4.19
2.55
1.91
1.39
1.37
1.36
HO
O
O
NH
O
CH3
H3C
O
18
0.81
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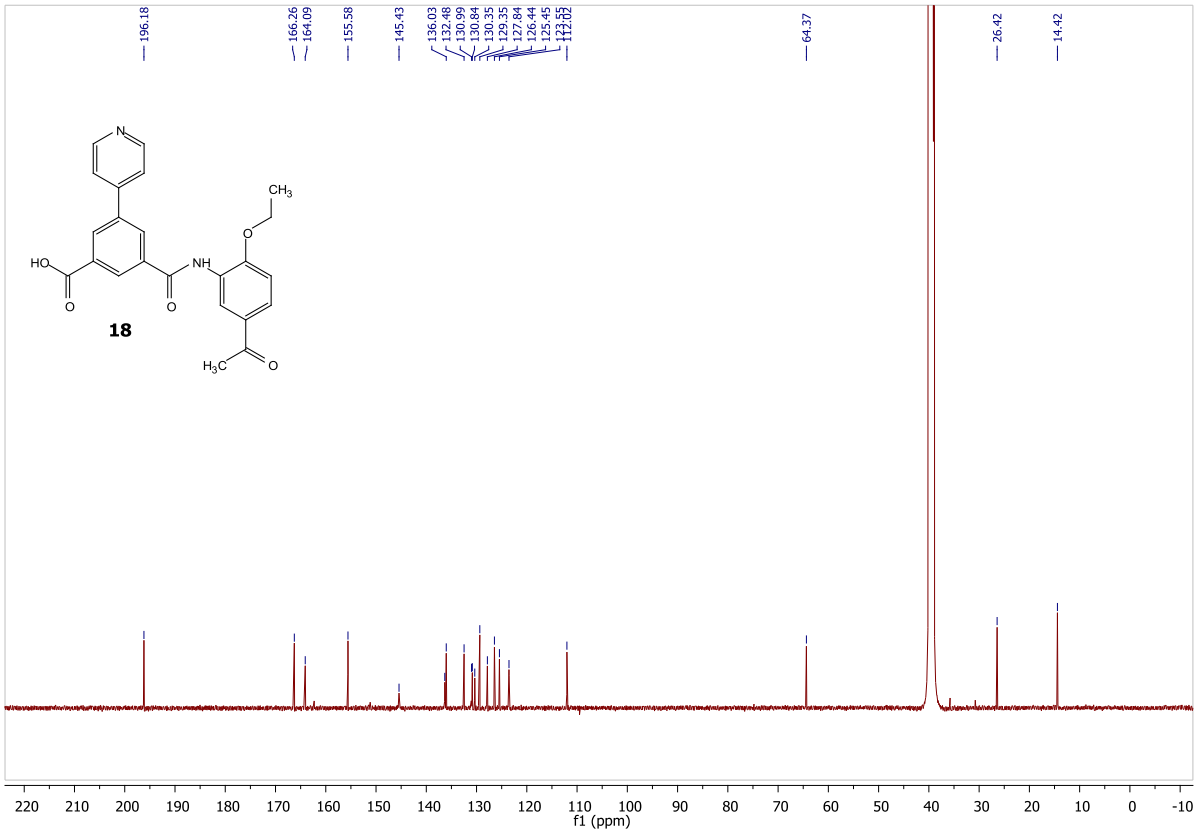
0.92
1.71
0.93
1.30
1.87
1.00
2.03
3.38
2.05
2.89
f1 (ppm)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136.03
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130.99
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130.35
129.35
127.84
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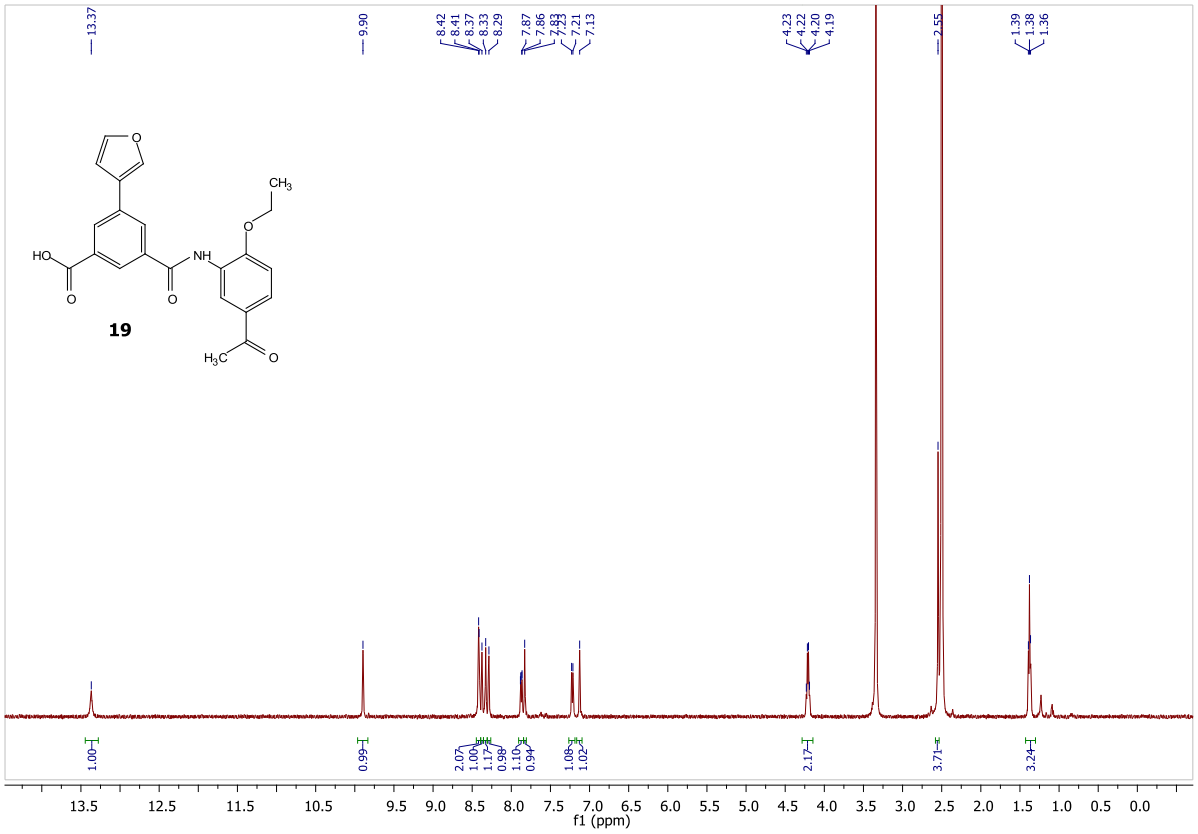
125.45
64.37
26.42
14.42
HO
O
O
NH
O
CH3
H3C
O
18
f1 (ppm)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8.33
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7.87
7.86
7.83
7.25
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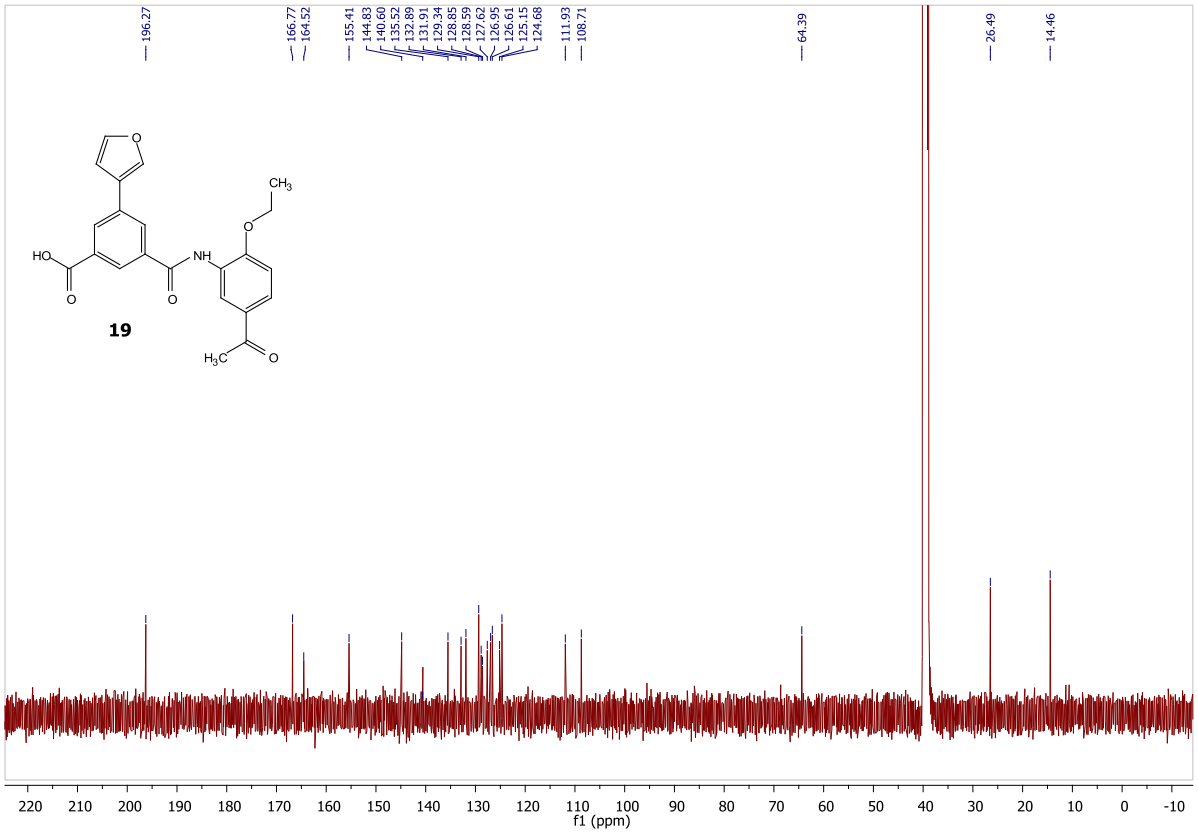
7.13
4.23
4.22
4.20
4.19
2.55
1.39
1.38
1.36
19
f1 (ppm)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129.34
128.85
128.59
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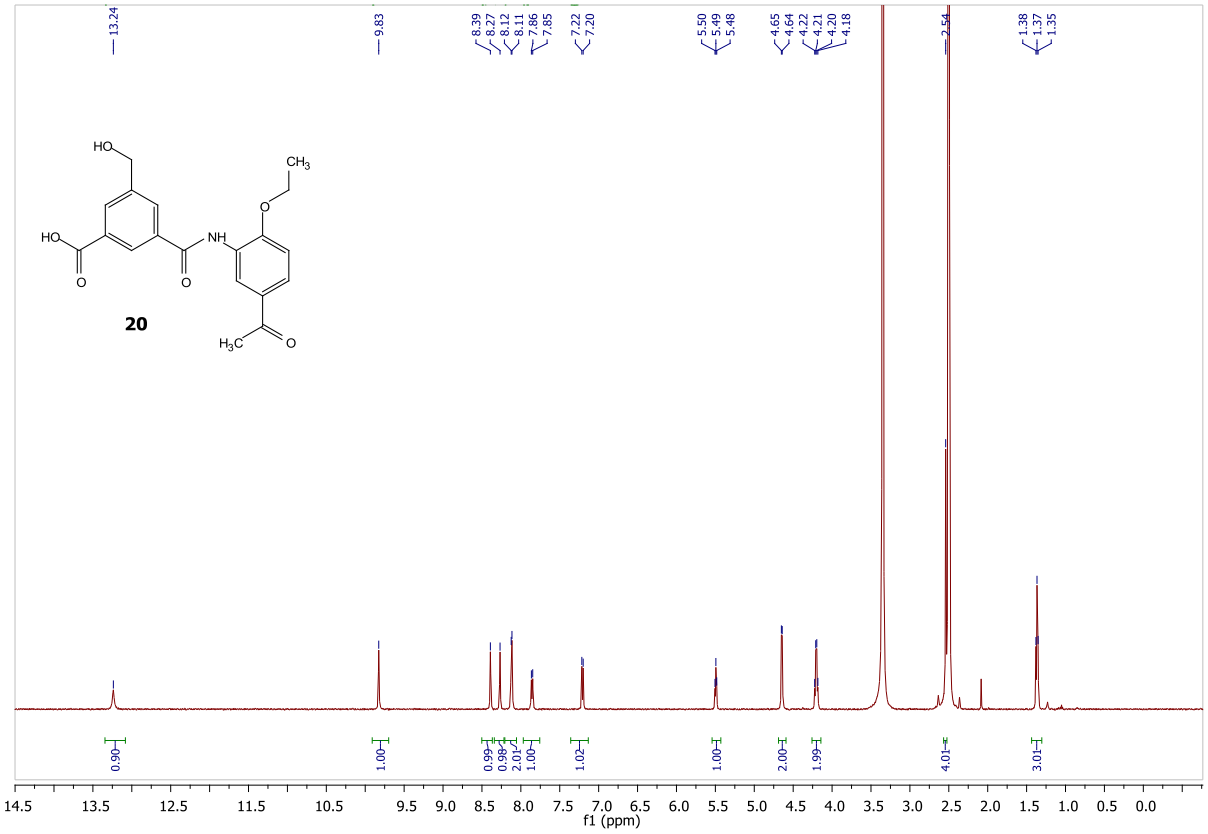
108.71
64.39
26.49
14.46
19
f1 (ppm)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8.11
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7.85
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7.20
5.50
5.49
5.48
4.65
4.64
4.22
4.21
4.20
4.18
2.54
1.38
1.37
1.35
HO
HO
O
O
NH
O
CH3
H3C
O
20
0.90
1.00
0.99
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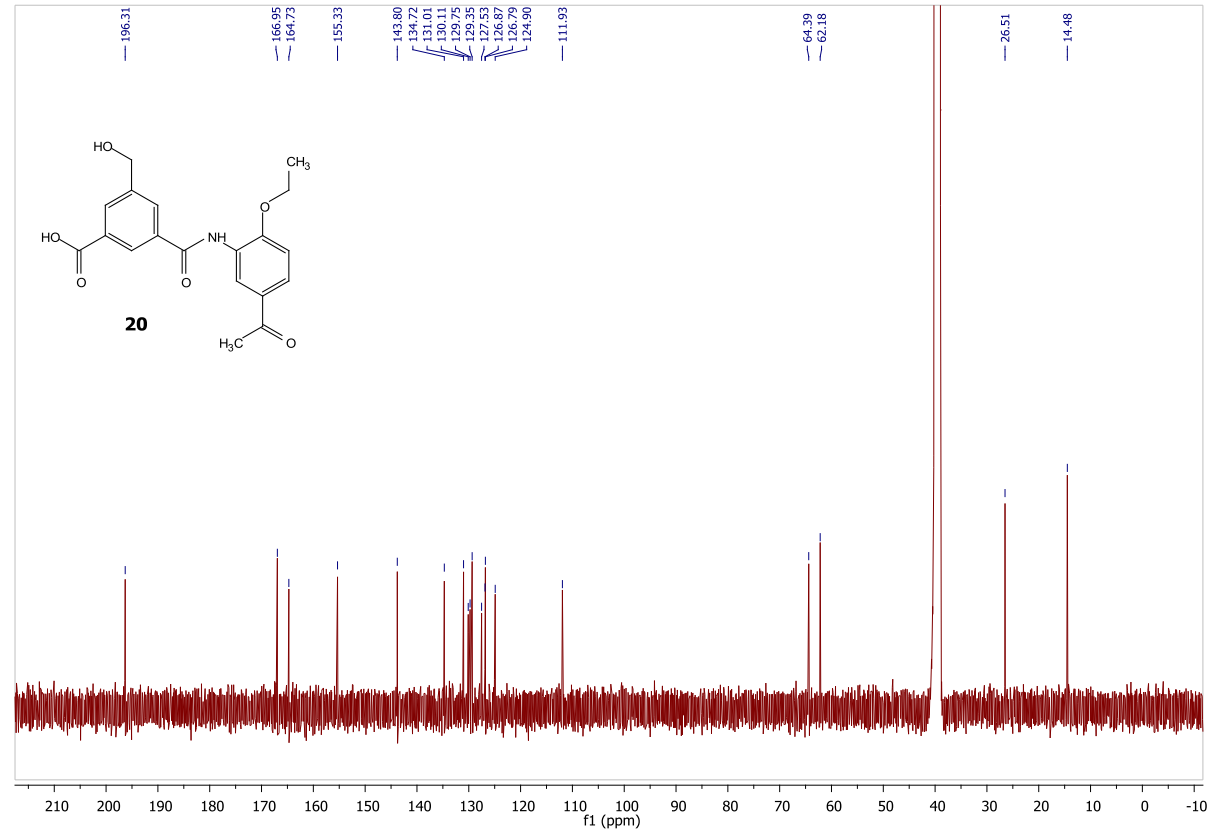
2.01
1.00
1.02
1.00
2.00
1.99
4.01
3.01
f1 (ppm)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129.35
127.53
126.87
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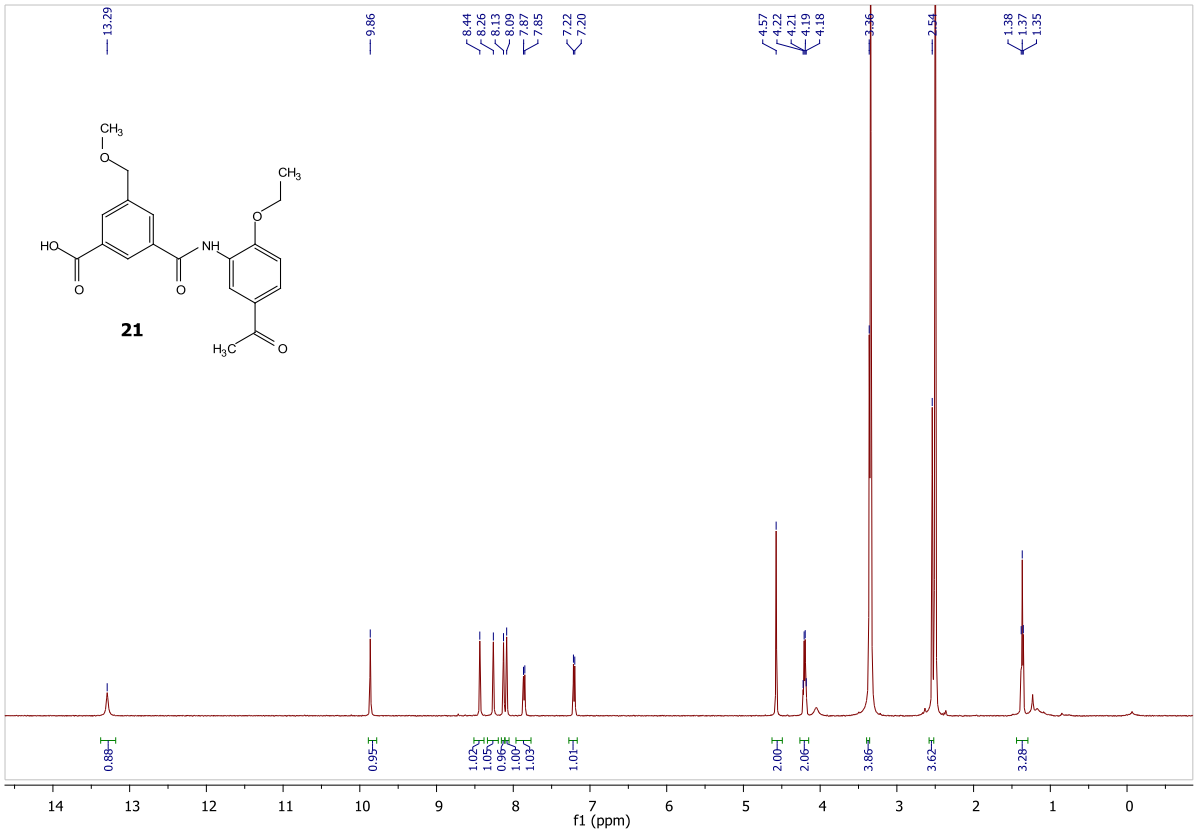
124.90
111.93
64.39
62.18
26.51
14.48
HO
HO
O
O
NH
O
CH3
H3C
O
20
f1 (ppm)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8.09
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7.85
7.22
7.20
4.57
4.22
4.21
4.19
4.18
3.36
2.54
1.38
1.37
1.35
0.88
0.95
1.02
1.05
0.96
1.00
1.03
1.01
2.00
2.06
3.86
3.62
3.28
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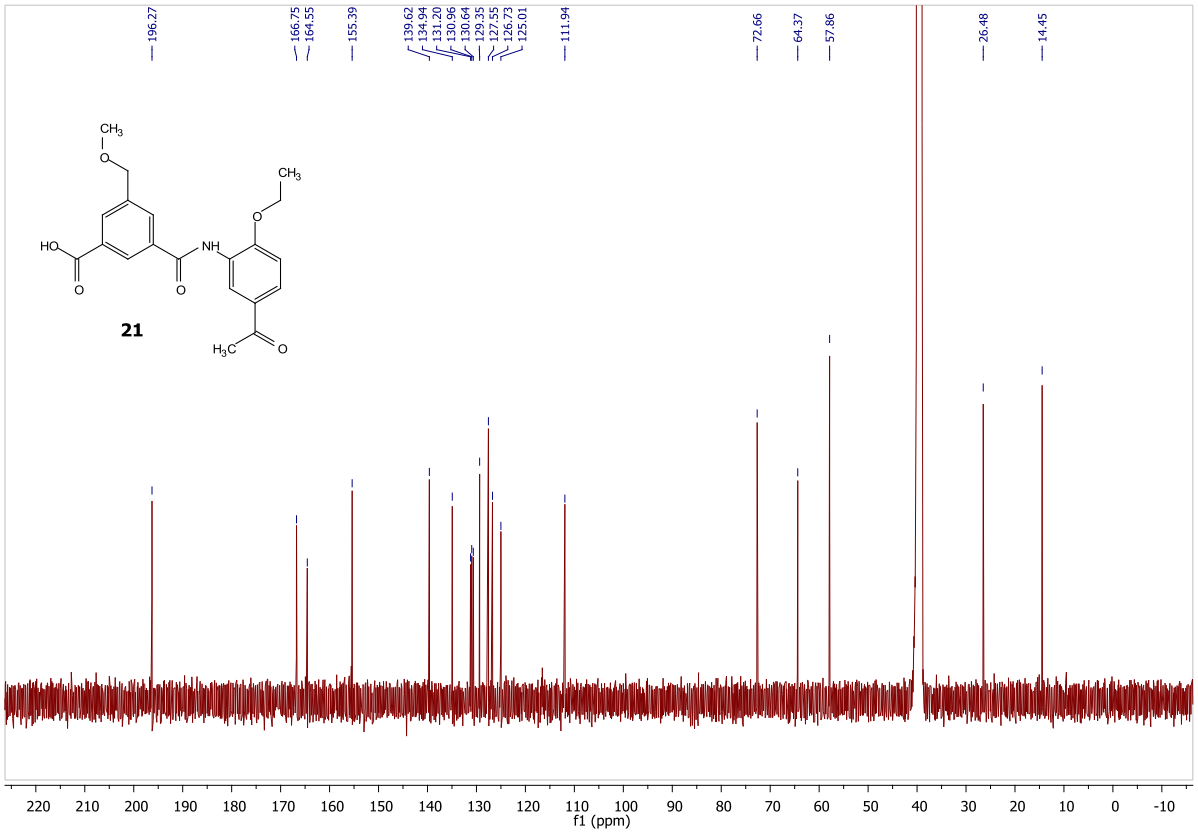
f1 (ppm)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166.75
164.55
155.39
139.62
134.94
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130.96
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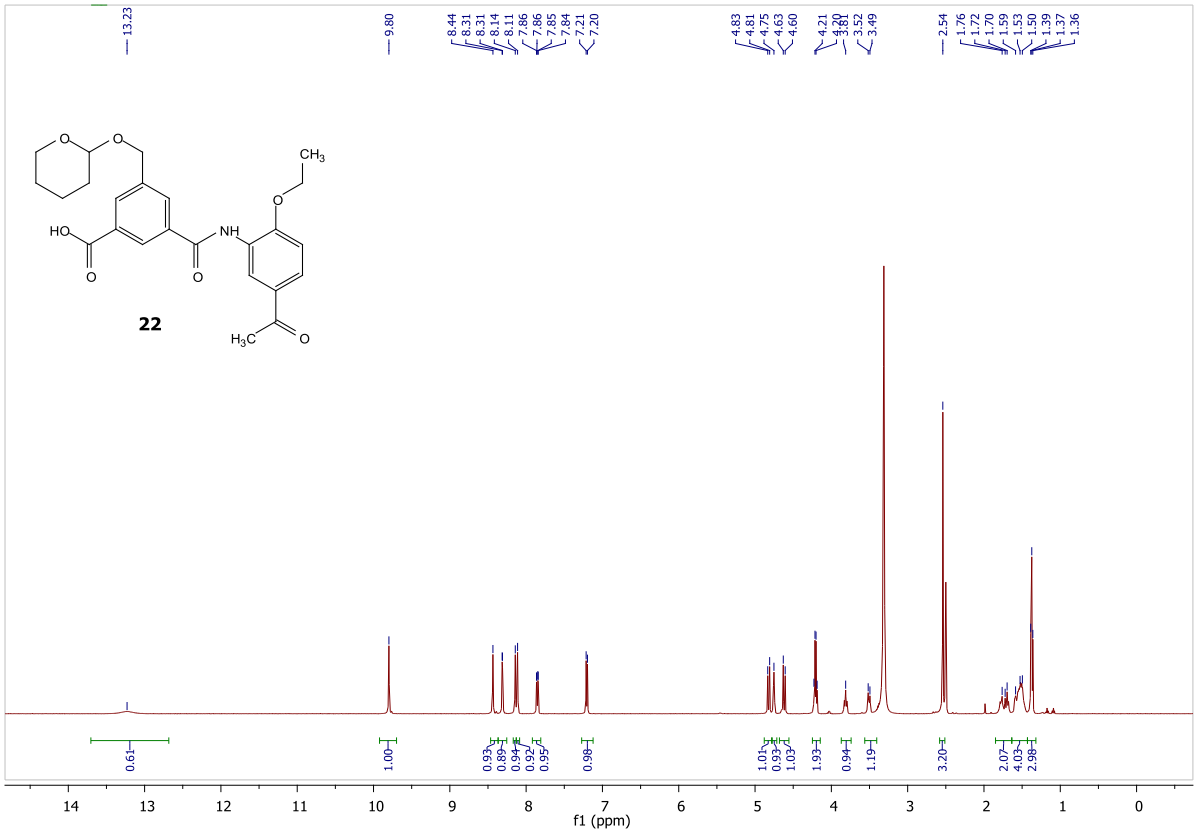
126.73
125.01
111.94
72.66
64.37
57.86
26.48
14.45
21
f1 (ppm)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7.21
7.20
4.83
4.81
4.75
4.63
4.60
4.21
4.20
3.81
3.52
3.49
2.54
1.76
1.72
1.70
1.59
1.53
1.50
1.39
1.37
1.36
0.61
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0.94
0.92
0.95
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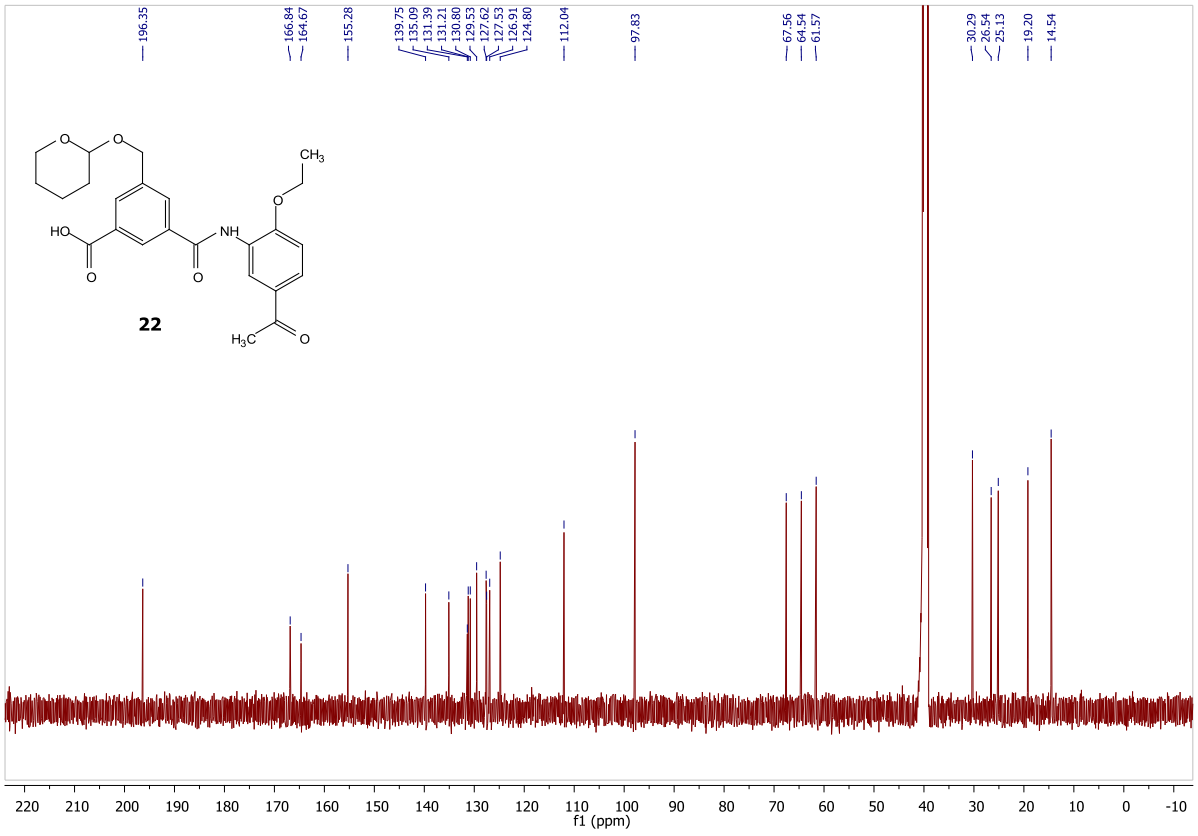
f1 (ppm)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64.54
61.57
30.29
26.54
25.13
19.20
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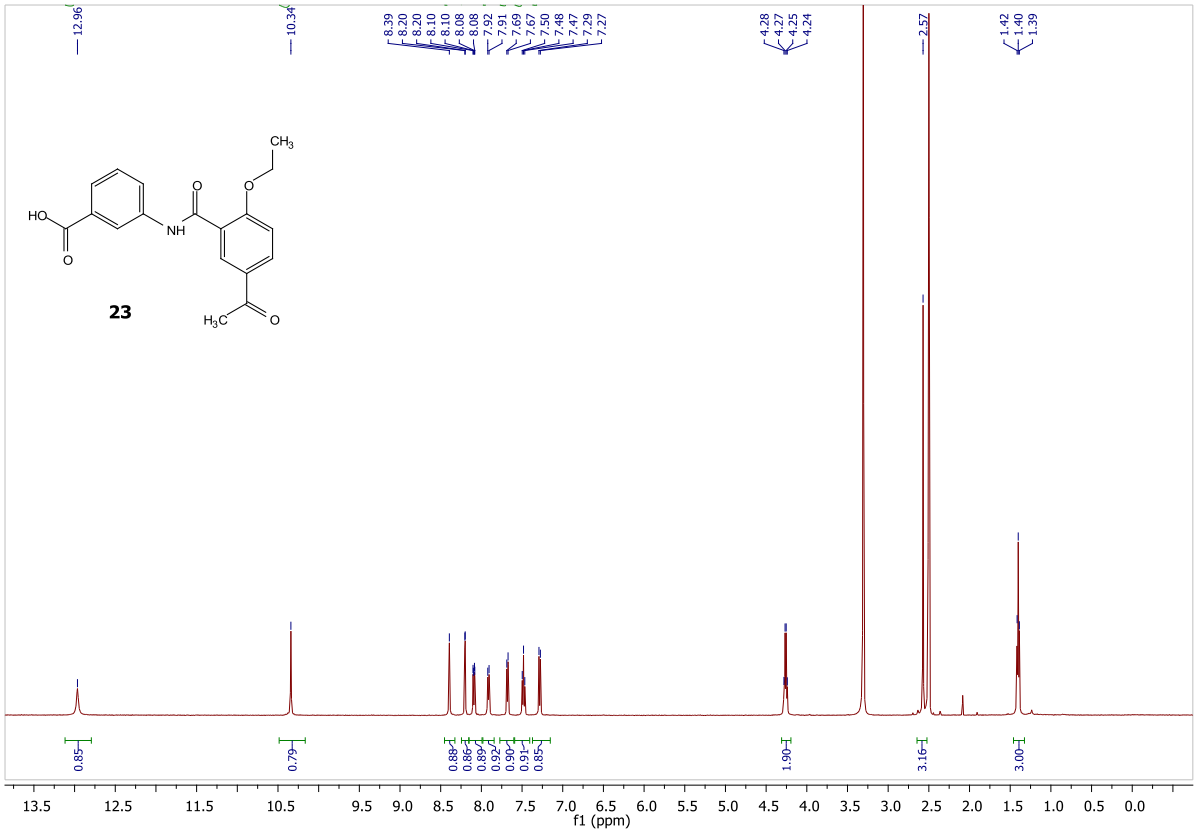
f1 (ppm)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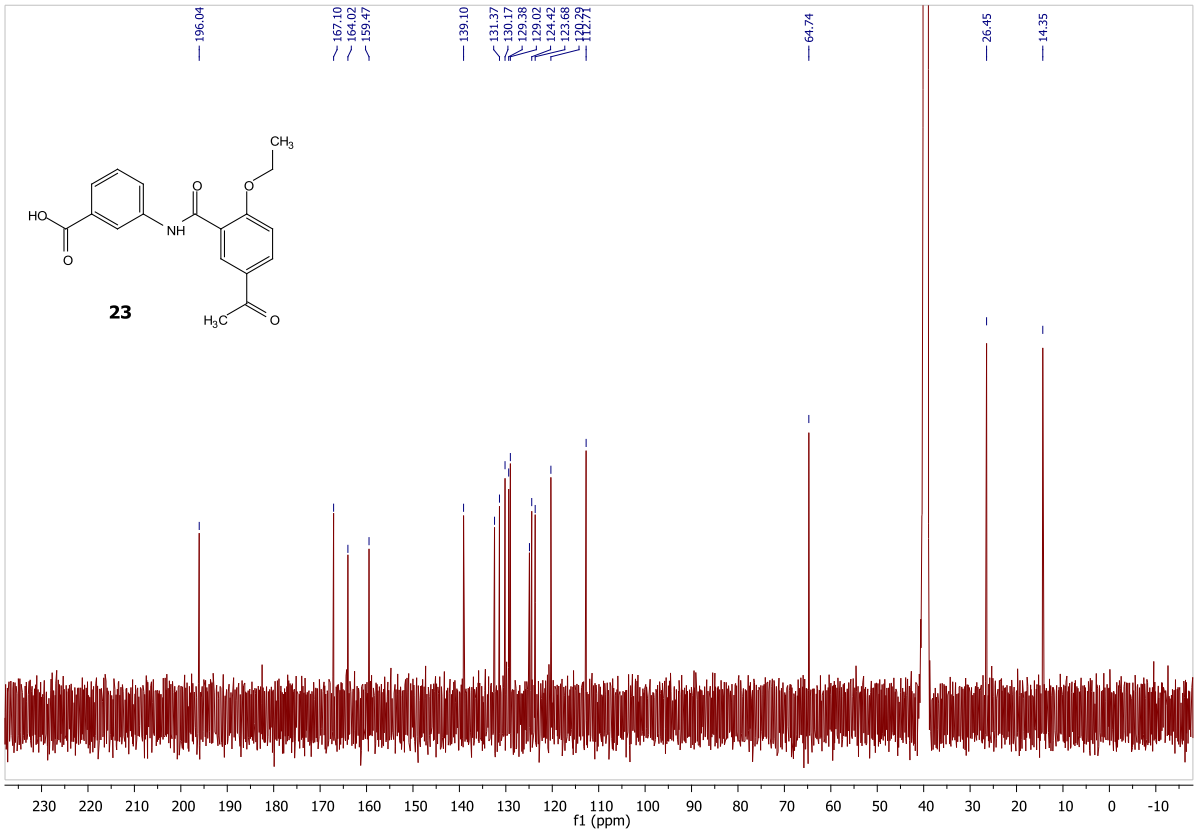
7.48
7.47
7.29
7.27
4.28
4.27
4.25
4.24
2.57
1.42
1.40
1.39
0.85
0.79
0.88
0.86
0.89
0.92
0.90
0.91
0.85
1.90
3.16
3.00
HO
O
NH
O
O
CH3
H3C
O
23
13.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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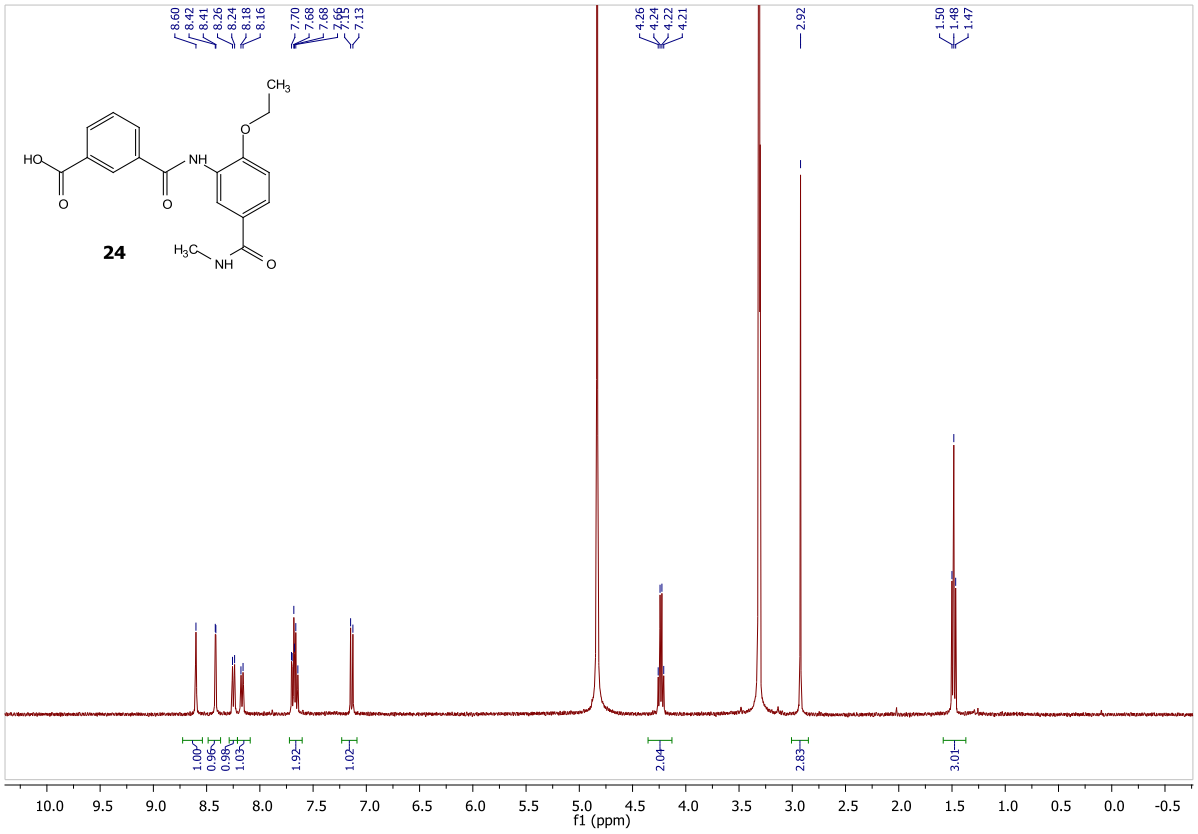
14.35
HO
O
NH
O
O
CH3
H3C
O
23
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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8.18
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7.70
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7.68
7.66
7.15
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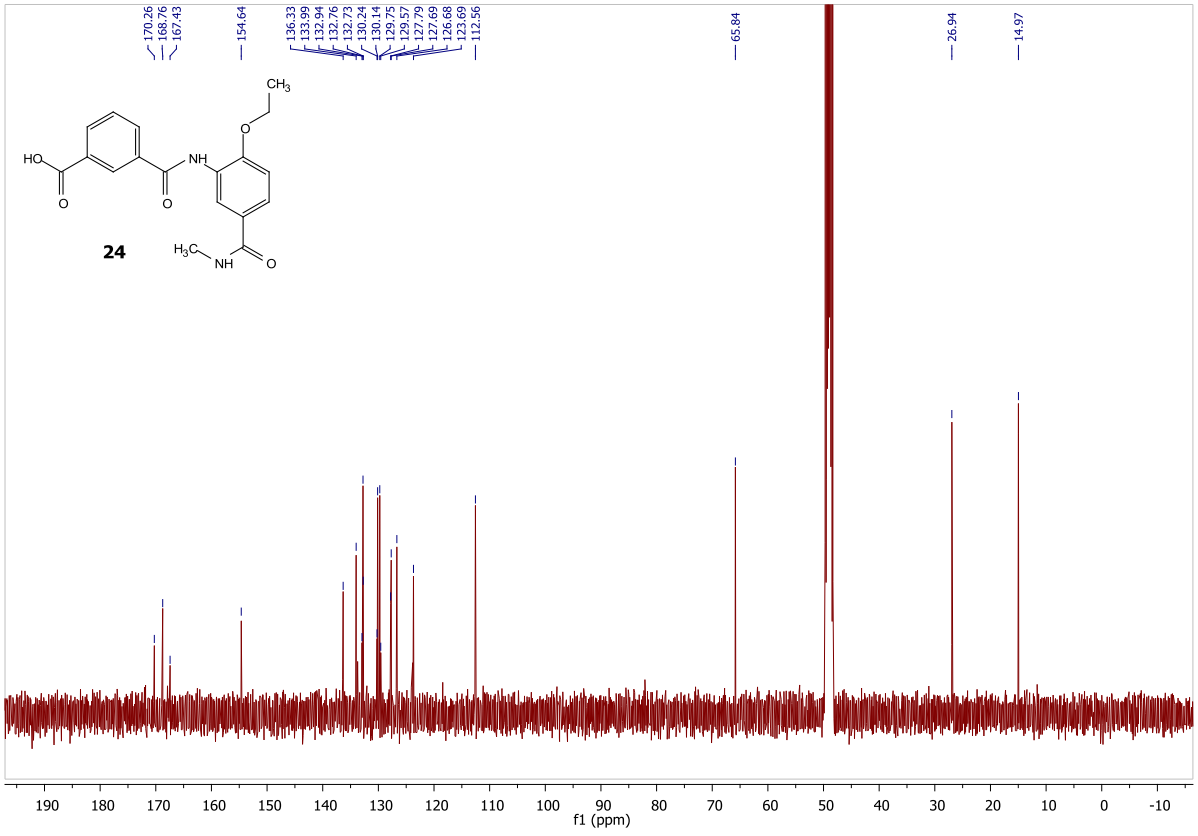
4.24
4.22
4.21
2.92
1.50
1.48
1.47
CH3
O
HO
NH
O
O
24
H3C
NH
O
1.00
0.96
0.98
1.03
1.92
1.02
2.04
2.83
3.01
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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133.99
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130.24
130.14
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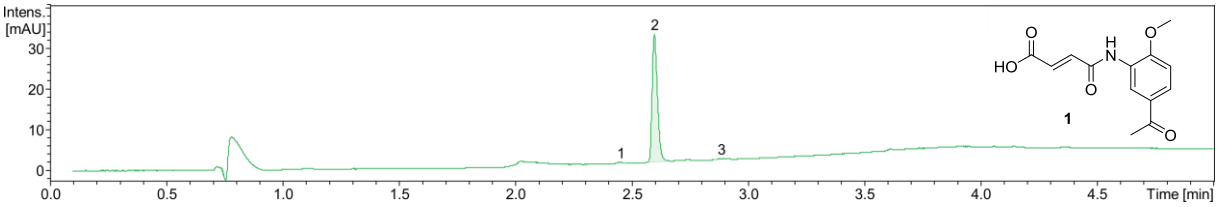
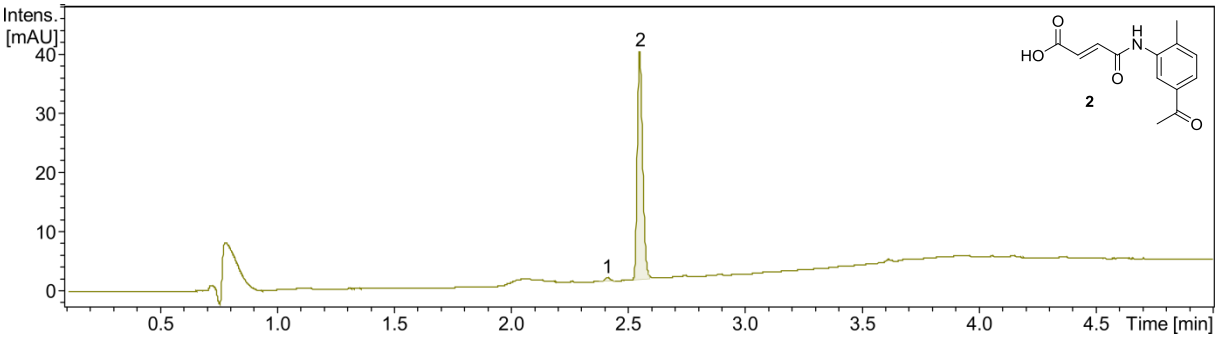
127.69
126.68
123.69
112.56
65.84
26.94
14.97
CH3
O
HO
NH
O
O
24
H3C
NH
O
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

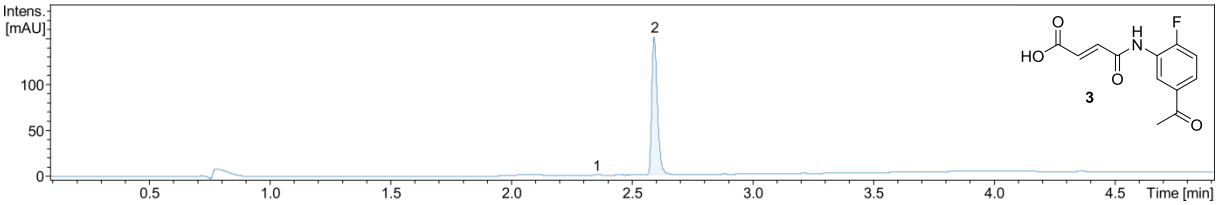
13. HPLC trace (for purity) of tested compounds



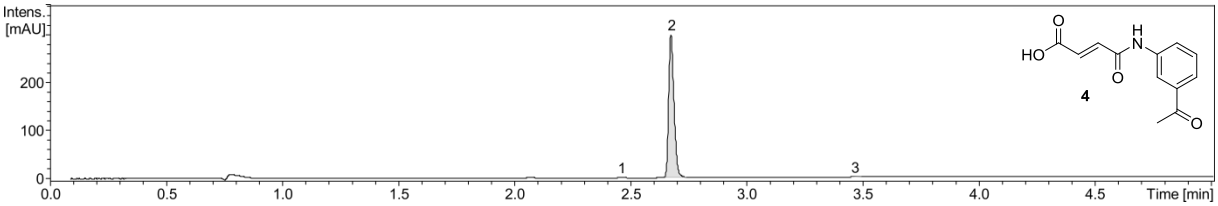
#	RT [min]	Area	Area Frac. %
1	2.5	0.5644	1.11
2	2.6	49.6291	97.56
3	2.9	0.6784	1.33



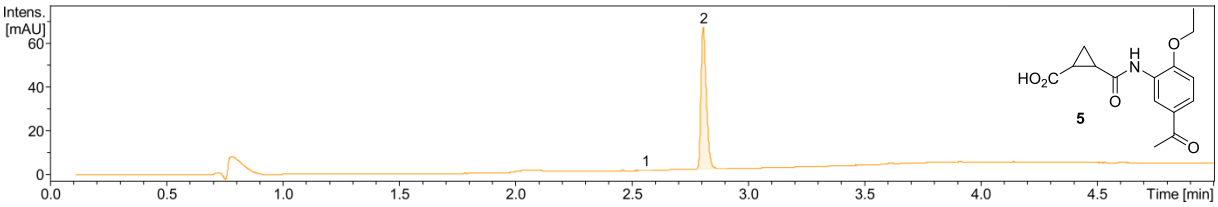
#	RT [min]	Area	Area Frac. %
1	2.4	1.0120	1.65
2	2.6	60.4380	98.35



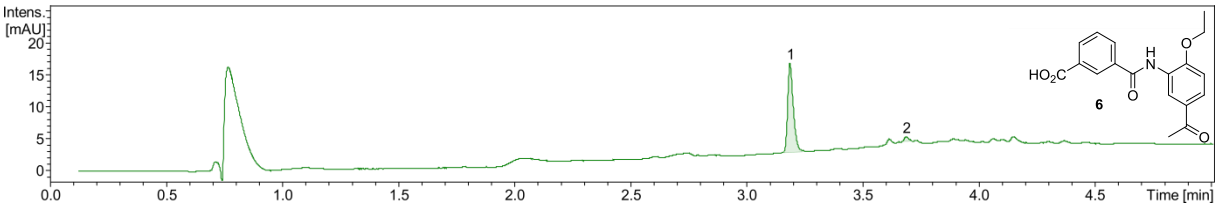
#	RT [min]	Area	Area Frac. %
1	2.4	1.0650	0.45
2	2.6	238.0792	99.55



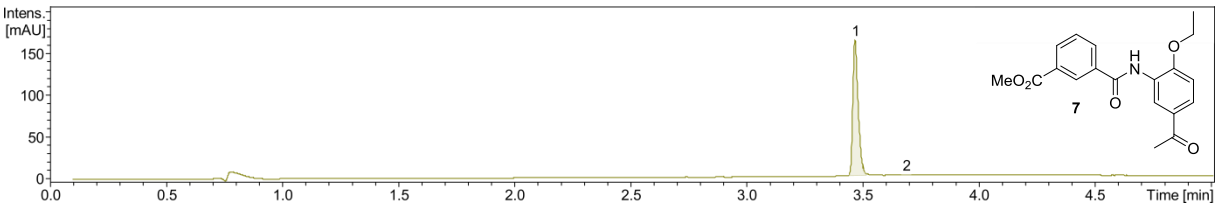
#	RT [min]	Area	Area Frac. %
1	2.5	2.4005	0.50
2	2.7	472.3789	99.29
3	3.5	0.9934	0.32



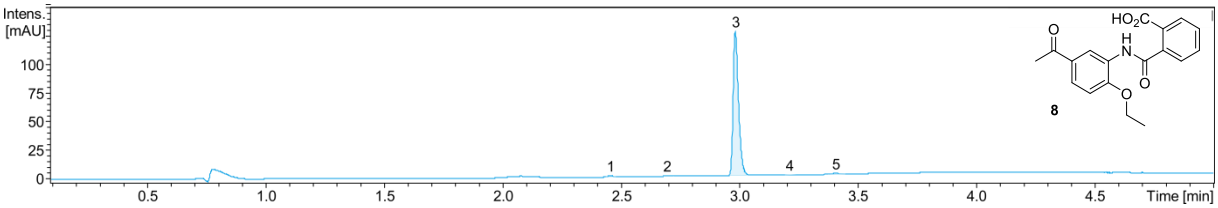
#	RT [min]	Area	Area Frac. %
1	2.6	0.3332	0.32
2	2.8	103.1426	99.68



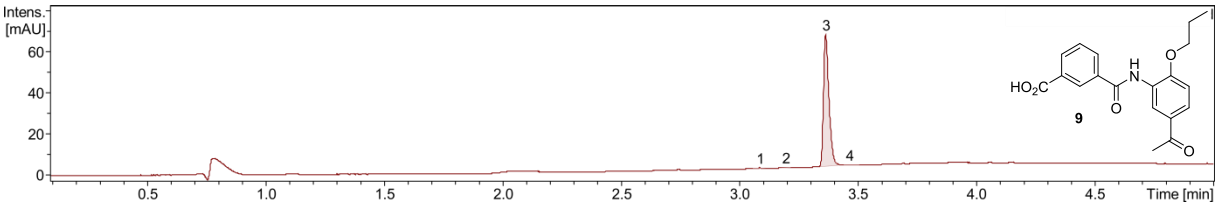
#	RT [min]	Area	Area Frac. %
1	3.2	23.2620	95.83
2	3.7	1.0113	4.17



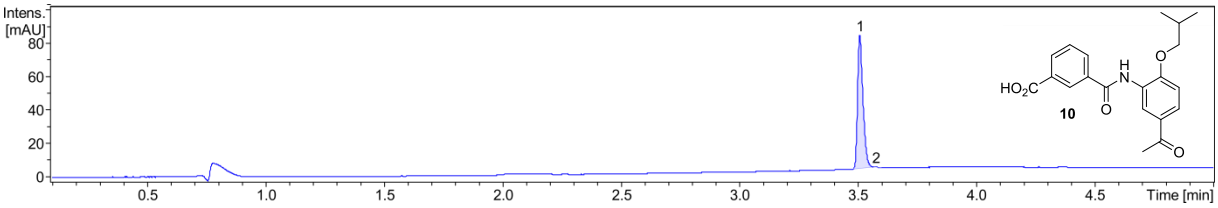
#	RT [min]	Area	Area Frac. %
1	3.5	259.4895	99.76
2	3.7	0.6185	0.24



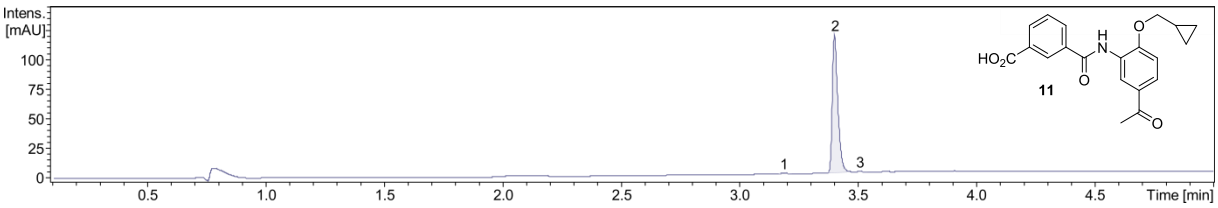
#	RT [min]	Area	Area Frac. %
1	2.5	1.5283	0.75
2	2.7	0.9279	0.46
3	3.0	198.0575	97.77
4	3.2	0.3209	0.16
5	3.4	1.7361	0.88



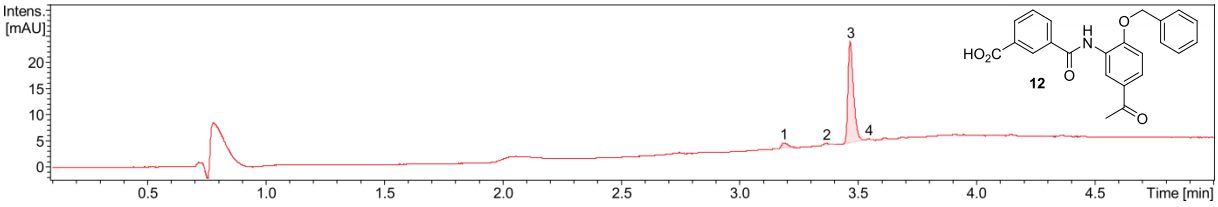
#	RT [min]	Area	Area Frac. %
1	3.1	0.4491	0.44
2	3.2	0.2947	0.29
3	3.4	101.9898	98.83
4	3.5	0.4592	0.45



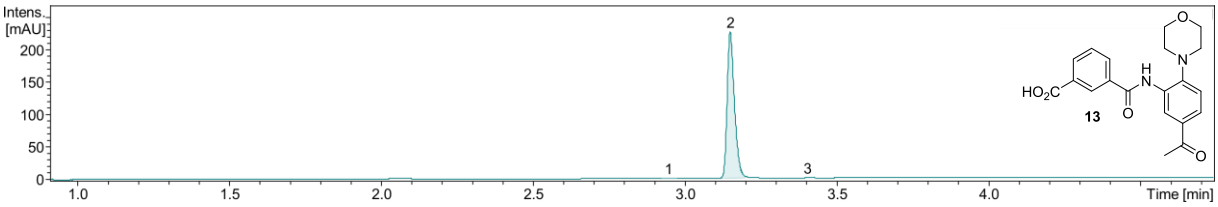
#	RT [min]	Area	Area Frac. %
1	3.5	125.0875	99.83
2	3.6	0.2122	0.17



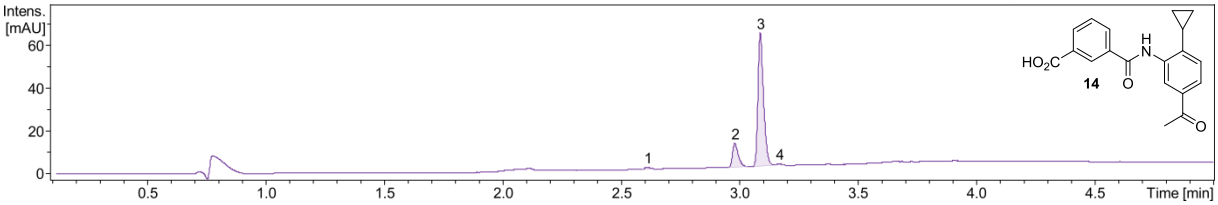
#	RT [min]	Area	Area Frac. %
1	3.2	1.0299	0.54
2	3.4	187.8594	99.16
3	3.5	0.5648	0.30



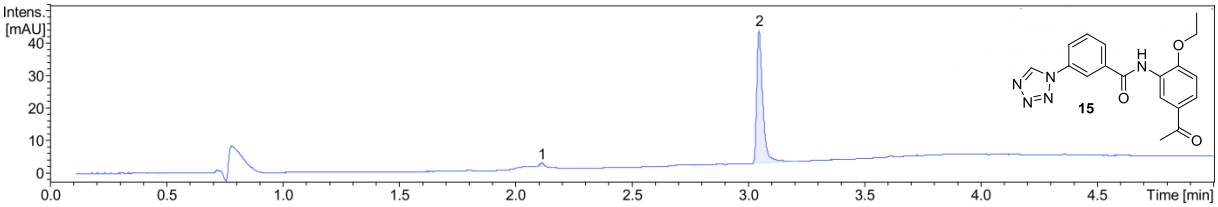
#	RT [min]	Area	Area Frac. %
1	3.2	1.8064	5.37
2	3.4	0.4938	1.47
3	3.5	31.2292	92.77
4	3.5	0.1344	0.40



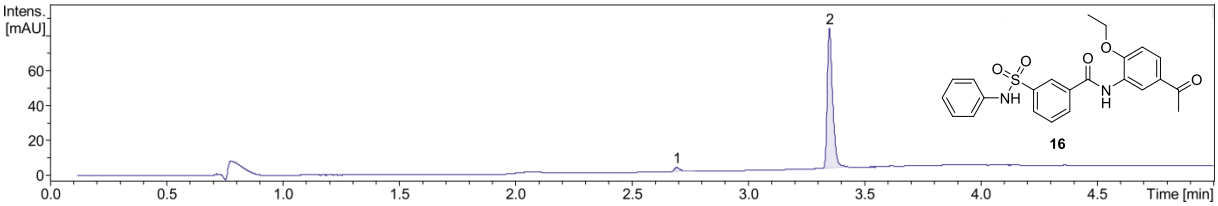
#	RT [min]	Area	Area Frac. %
1	2.9	0.9737	0.26
2	3.1	372.0209	99.62
3	3.4	0.4306	0.12



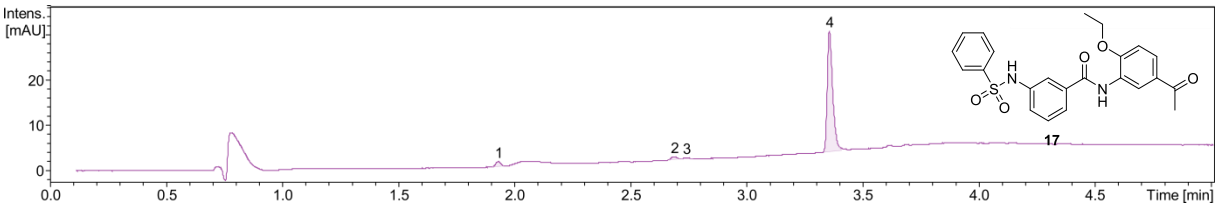
#	RT [min]	Area	Area Frac. %
1	2.6	1.3549	1.11
2	3.0	18.8392	15.46
3	3.1	100.5997	82.54
4	3.2	1.0852	0.89



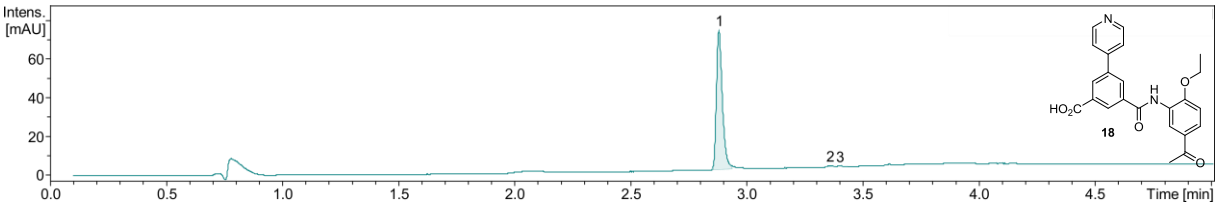
#	RT [min]	Area	Area Frac. %
1	2.1	1.5363	2.03
2	3.0	74.1974	97.97



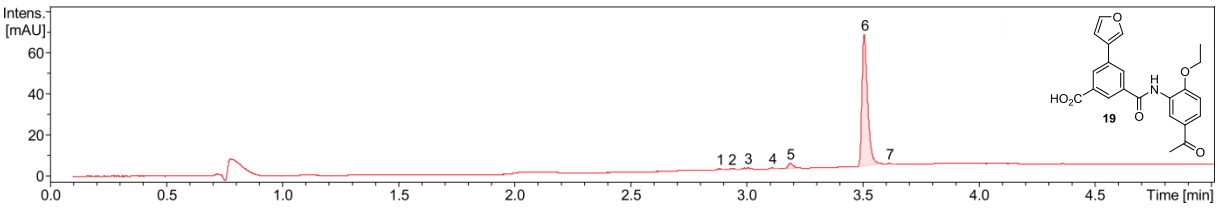
#	RT [min]	Area	Area Frac. %
1	2.7	3.9337	2.97
2	3.3	128.5693	97.03



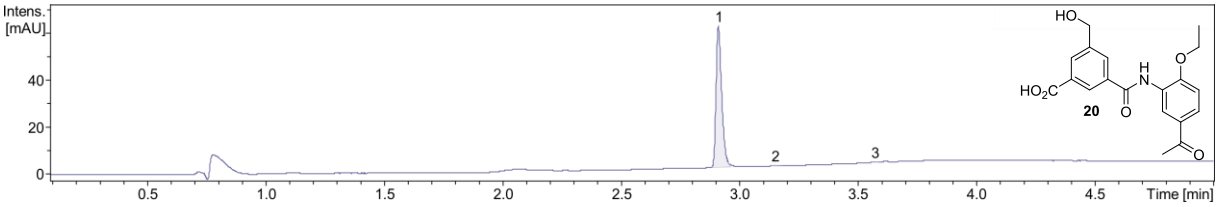
#	RT [min]	Area	Area Frac. %
1	1.9	1.5598	3.41
2	2.7	1.0015	2.19
3	2.7	0.4513	0.99
4	3.4	42.7925	93.42



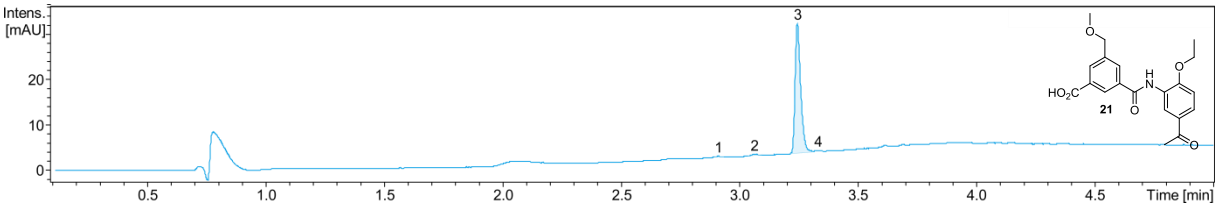
#	RT [min]	Area	Area Frac. %
1	2.9	127.9425	98.95
2	3.4	1.0138	0.78
3	3.4	0.3495	0.27



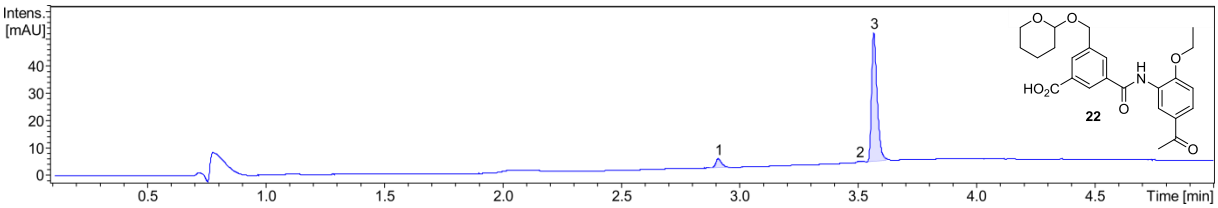
#	RT [min]	Area	Area Frac. %
1	2.9	0.8775	0.76
2	2.9	0.7140	0.62
3	3.0	1.6711	1.45
4	3.1	0.6612	0.57
5	3.2	4.0475	3.52
6	3.5	106.5790	92.61
7	3.6	0.5377	0.47



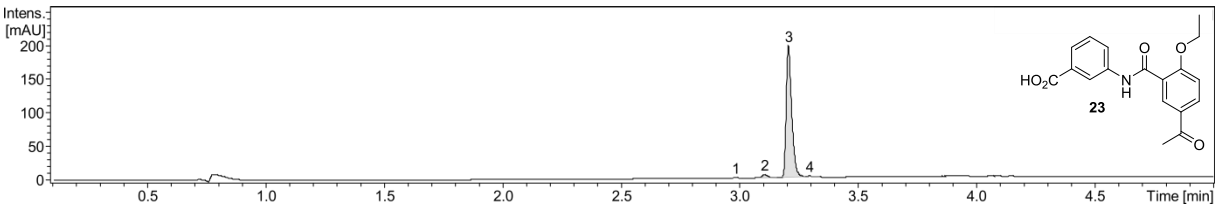
#	RT [min]	Area	Area Frac. %
1	2.9	98.9864	99.32
2	3.1	0.1514	0.15
3	3.6	0.5250	0.53



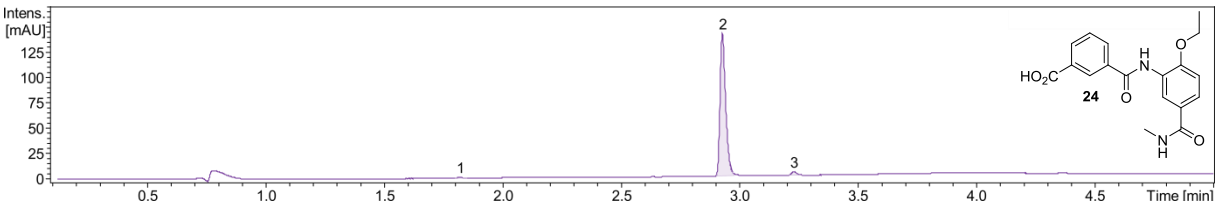
#	RT [min]	Area	Area Frac. %
1	2.9	0.2494	0.52
2	3.1	0.4983	1.05
3	3.2	46.6678	97.96
4	3.3	0.2251	0.47



#	RT [min]	Area	Area Frac. %
1	2.9	5.6436	6.81
2	3.5	0.9238	1.11
3	3.6	76.3489	92.08



#	RT [min]	Area	Area Frac. %
1	3.0	0.6588	0.20
2	3.1	8.4135	2.58
3	3.2	315.5255	96.91
4	3.3	1.0018	0.31



#	RT [min]	Area	Area Frac. %
1	1.8	1.6161	0.68
2	2.9	229.7368	96.95
3	3.2	5.6225	2.37

References

1. Kolb, P.; Kipouros, C. B.; Huang, D.; Caflisch, A. Structure-based tailoring of compound libraries for high-throughput screening: Discovery of novel EphB4 kinase inhibitors. *Proteins* **2008**, *73*, 11-18.
2. Zhao, H.; Dong, J.; Lafleur, K.; Nevado, C.; Caflisch, A. Discovery of a Novel Chemotype of Tyrosine Kinase Inhibitors by Fragment-Based Docking and Molecular Dynamics. *ACS Med. Chem. Lett.* **2012**, *3*, 834-838.
3. Kolb, P.; Caflisch, A. Automatic and Efficient Decomposition of Two-Dimensional Structures of Small Molecules for Fragment-Based High-Throughput Docking. *J. Med. Chem.* **2006**, *49*, 7384-7392.
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5. Scarsi, M.; Apostolakis, J.; Caflisch, A. Continuum Electrostatic Energies of Macromolecules in Aqueous Solutions. *J. Phys. Chem. A* **1997**, *101*, 8098-8106.
6. Scarsi, M.; Apostolakis, J.; Caflisch, A. Comparison of a GB Solvation Model with Explicit Solvent Simulations: Potentials of Mean Force and Conformational Preferences of Alanine Dipeptide and 1,2-Dichloroethane. *J. Phys. Chem. B* **1998**, *102*, 3637-3641.
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