Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information

Insight into the complete substrate-binding pocket of ThiT by chemical and genetic mutations

L. J. Y. M. Swier, L. Monjas, F. Reeßing, R. C. Oudshoorn, A. Sachrap, T. Primke, M. M. Bakker, E. van Olst, T. Ritschel, I. Faustino, S. J. Marrink, A. K. H. Hirsch and D. J. Slotboom

Synthesis of thiamine analogues 7 and 8

General procedure for the synthesis of 12 and 13 (GP-A). To a solution of the corresponding aldehyde (1.0 eq) in MeOH at room temperature, $NaBH_4$ (0.5 eq) was added (no dry conditions needed). The reaction mixture was stirred at room temperature for 30–60 min. The reaction was quenched with ice, extracted 3 times with CH_2Cl_2, and the combined organic layers were washed once with water, once with a saturated aqueous solution of NaCl, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography.

General procedure for the synthesis of aldehydes 14 and 15 (GP-B). To a solution of the corresponding bromide (1.0 eq) in anhydrous THF at 0 °C, ^{i}PrMgCl (2.0 M in THF, 1.1 eq) was added dropwise. The reaction mixture was stirred at 0 °C for 10 min, then cooled to –78 °C, and ^{n}BuLi (2.5 M in hexanes, 2.2 eq) was added dropwise. The reaction mixture was left to warm up from –78 to –40 °C for 1 h, then cooled down to –78 °C, anhydrous DMF (18 eq) was added and it was left to warm up from –78 °C to room temperature for 18 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl, extracted 3 times with Et_2O, and the combined organic layers were washed once with water, once with a saturated aqueous solution of NaCl, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography.

General procedure for the synthesis of enamines 16 and 17 (GP-C). To a solution of the corresponding aldehyde (1.0 eq) and 3-anilinopropionitrile (1.2 eq) in anhydrous DMSO, NaOMe in anhydrous MeOH (2.0 M, 1.2 eq) was added. The reaction mixture was stirred in the microwave at 70 °C and 100 W for 30 min. The reaction mixture was diluted with water, extracted 3 times with EtOAc, and the combined organic layers were washed once with water, once with a saturated aqueous solution of NaCl, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography.

General procedure for the formation of the aminopyrimidinyl ring in compounds 7 and 8 (GP-D). To a solution of the corresponding enamine (1.0 eq) in anhydrous MeOH, acetamidine·HCl (hygroscopic: before use co-evaporate with toluene 3 times, 2.0 eq) and NaOMe in anhydrous MeOH (2.0 M, 4.0 eq) were added. The reaction mixture was stirred at reflux (90 °C, pre-heated oil bath) in a pressure tube for 2 days. Then, the reaction mixture was cooled down, and the solvent evaporated under reduced pressure. The crude was purified by flash column chromatography.

(3-Bromo-5-methyl-phenyl)methanol (12): This compound was synthesized according to **GP-A**, using 3-bromo-5-methylbenzaldehyde (**10**, 2.20 g, 11.0 mmol), MeOH (35 mL) and $NaBH_4$ (0.208 g, 5.50 mmol). The crude was purified by flash column chromatography (pentane/Et_2O 4:1), to afford **12** as a white solid (2.01 g, 10.0 mmol, 91%). M.p. 46–48 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.30 (s, 1H), 7.25 (s, 1H), 7.08 (s, 1H), 4.61 (s, 2H), 2.32 (s, 3H), 1.92 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 142.9 (C), 140.5 (C), 131.3 (CH), 127.0 (CH), 126.3 (CH), 122.5 (C), 64.6 (CH_2), 21.2 (CH_3). IR (cm^{-1}) 3306, 2914, 2865, 1605, 1573, 1443, 1253, 1024, 843, 804, 678, 628. HRMS (ESI+) calculated for C_8H_8Br [M – OH]$^+$ 182.9804, found 182.9804.

3-(Hydroxymethyl)-5-(methyl)benzaldehyde (14): This compound was synthesized according to **GP-B**, using **12** (1.0 g, 4.97 mmol), anhydrous THF (40 mL), ^{i}PrMgCl (2.0 M in THF, 2.74 mL, 5.47 mmol), ^{n}BuLi (2.5 M in hexanes, 4.37 mL, 10.93 mmol) and anhydrous DMF (7 mL, 90.8 mmol). The crude was purified by flash column chromatography (pentane/Et_2O 1:1), to afford **14** as a yellow oil (0.487 g, 3.24 mmol, 65%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.68 (s, 1H), 7.62 (s, 1H), 7.47 (s, 1H), 4.76 (s, 2H), 2.44 (s, 3H), 1.74 (br s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 192.7 (CHO), 142.0 (C), 139.4 (C), 136.8 (C), 133.8 (CH), 129.5 (CH), 125.5 (CH), 64.6 (CH_2), 21.3 (CH_3). IR (cm^{-1}) 3379, 2921, 2869, 2734, 1690, 1599, 1294, 1142, 1039, 856, 682, 631. HRMS (ESI+) calculated for $C_9H_{11}O_2$ [M + H]$^+$ 151.0754, found 151.0753.

2-(3-(Hydroxymethyl)-5-methylbenzyl)-3-(phenylamino)acrylonitrile (16): This compound was synthesized according to **GP-C**, using **14** (0.450 g, 2.99 mmol), 3-anilinopropionitrile (0.524 g, 3.59 mmol), anhydrous DMSO (4.2 mL) and NaOMe (2.0 M in MeOH, 1.79 mL, 3.59 mmol). The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 99:1), to afford **16** as a pale yellow solid (0.230 g, 0.826 mmol, 28%). ^{1}H-NMR (400 MHz, CD_3OD) δ 7.53 (s, 1H), 7.30–7.26 (m, 2H), 7.11–7.08 (m, 3H), 7.06–7.02 (m, 2H), 6.99–6.95 (m, 1H), 4.56 (s, 2H), 3.59 (s, 2H), 2.33 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 143.0 (C), 142.9 (C), 142.5 (CH), 139.7 (C), 139.4 (C), 130.6 (2 CH), 129.1 (CH), 127.1 (CH), 125.2 (CH), 124.1 (CN), 123.3 (CH), 116.5 (2 CH), 83.4 (C), 65.2 (CH_2), 32.8 (CH_2), 21.4 (CH_3). IR (cm^{-1}) 3020, 2913, 2865, 2473, 2445, 2189, 1630, 1597, 1501, 1347, 1240, 748, 685, 504. HRMS (ESI+) calculated for $C_{18}H_{19}N_2O$ [M + H]$^+$ 279.1492, found 279.1491.

(3-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-methylphenyl)-methanol (7): This compound was synthesized according to **GP-D**, using **16** (0.230 g, 0.826 mmol), anhydrous MeOH (2.2 mL), acetamidine·HCl (0.156 g, 1.65 mmol) and NaOMe (2.0 M in MeOH, 1.65 mL, 3.30 mmol). The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 95:5), to afford **7** as a yellow solid (0.136 g, 0.560 mmol, 87% based on recovered starting material). M.p. 157–159 °C. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.74 (s, 1H), 7.06 (s, 1H), 7.00 (s, 1H), 6.95 (s, 1H), 4.53 (s, 2H), 3.75 (s, 2H), 2.39 (s, 3H), 2.31 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 165.5 (C), 163.9 (C), 152.7 (CH), 143.2 (C), 139.7 (C), 138.7 (C), 129.4 (CH), 127.3 (CH), 125.5 (CH), 115.6 (C), 65.0 (CH_2), 34.2 (CH_2), 24.2 (CH_3), 21.4 (CH_3). IR (cm^{-1}) 3336, 3154, 3069, 3024, 2788, 2674, 1661, 1594, 1569, 1464, 1434, 1065, 835, 774, 604, 519. HRMS (ESI+) calculated for $C_{14}H_{18}N_3O$ [*M* + H]$^+$ 244.1444, found 244.1446.

(3-Bromo-5-(trifluoromethyl)phenyl)methanol (13): This compound was synthesized according to **GP-A**, using 3-bromo-5-(trifluoromethyl)benzaldehyde (**11**, 0.50 g, 1.98 mmol), MeOH (8.0 mL) and $NaBH_4$ (0.038 g, 0.99 mmol). The crude was purified by flash column chromatography (pentane/Et_2O 9:1 to 8:2), to afford **13** as a white solid (0.438 g, 1.72 mmol, 87%). M.p. 50–51 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.68 (s, 1H), 7.56 (s, 1H), 4.75 (s, 2H), 1.80 (br s, 1H) ^{13}C-NMR (101 MHz, $CDCl_3$) δ 144.1 (C), 133.1 (q, *J* = 1.0, CH), 132.7 (q, *J* = 33.0, C), 127.6 (q, *J* = 3.8, CH), 123.3 (q, *J* = 272.9, CF_3) 123.0 (C), 122.2 (q, *J* = 3.8, CH), 63.9 (CH_2). ^{19}F-NMR (376 MHz, $CDCl_3$) δ –62.86. IR (cm^{-1}) 3244, 3099, 2932, 2873, 1443, 1315, 1157, 1121, 1097, 1048, 865, 793, 693. HRMS (ESI–) calculated for $C_8H_5BrF_3O$ [*M* – H]$^-$ 252.9470, found 252.9482.

3-(Hydroxymethyl)-5-(trifluoromethyl)benzaldehyde (15): This compound was synthesized according to **GP-B**, using **13** (0.418 g, 1.64 mmol), anhydrous THF (13 mL), ^{i}PrMgCl (2.0 M in THF, 0.90 mL, 1.80 mmol), ^{n}BuLi (2.5 M in hexanes, 1.44 mL, 3.60 mmol) and anhydrous DMF (2.3 mL, 29.8 mmol). The crude was purified by flash column chromatography (pentane/Et_2O 4:1), to afford **15** as a colorless oil (0.221 g, 1.08 mmol, 66%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.06 (s, 1H), 8.04 (s 1H), 7.90 (s, 1H), 4.86 (s, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 191.3 (CHO), 143.6 (C), 136.8 (C), 131.9 (q, *J* = 33.2, C) 130.4 (q, *J* = 1.0, CH), 128.9 (q, *J* = 3.8, CH), 125.5 (q, *J* = 3.8, CH), 123.5 (q, *J* = 272.7, CF_3), 63.5 (CH_2). ^{19}F-NMR (376 MHz, $CDCl_3$) δ –62.82. IR (cm^{-1}) 3392, 2962, 2937, 2872, 1704, 1343, 1216, 1123, 1104, 875, 698, 679. HRMS (ESI–) calculated for $C_9H_6F_3O_2$ [*M* – H]$^-$ 203.0314, found 203.0326.

2-(3-(Hydroxymethyl)-5-(trifluoromethyl)benzyl)-3-(phenylamino)-acrylonitrile (17): This compound was synthesized according to **GP-C**, using **15** (0.190 g, 0.929 mmol), 3-anilinopropionitrile (0.163 g, 1.11 mmol), anhydrous DMSO (1.3 mL) and NaOMe (2.0 M in MeOH, 0.55 mL, 1.11 mmol). The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 99:1), to afford **17** as a yellow, sticky solid (0.041 g, 0.12 mmol, 13%). ^{1}H-NMR (400 MHz, CD_3OD) δ 7.61 (s, 1H), 7.55 (m, 2H), 7.50 (s, 1H), 7.31–7.26 (m, 2H), 7.13–7.07 (m, 2H), 7.01–6.97 (m, 1H), 4.68 (s, 2H), 3.73 (s, 2H). ^{13}C-NMR (101 MHz, CD_3OD) δ 144.8 (C), 143.2 (CH), 142.7 (C), 141.3 (C), 132.0 (q, *J* = 30, C), 131.3 (q, *J* = 1.0, CH), 130.6 (2CH), 124.8 (q, *J* = 3.8, CH), 123.8 (CN), 123.5 (CH), 123.5 (q, *J* = 275, CF_3), 122.7 (q, *J* = 3.8, CH), 116.6 (2CH), 82.1 (C), 64.3 (CH_2), 32.5 (CH_2). ^{19}F-NMR (376 MHz, CD_3OD) δ –64.06. Only the signals corresponding to the major isomer are reported. IR (cm^{-1}) 3358, 2962, 2204, 1651, 1591, 1275, 1218, 1145, 1121, 1106, 1030, 756. HRMS (ESI+) calculated for $C_{18}H_{16}F_3N_2O$ [*M* + H]$^+$ 333.1209, found 333.1212.

(3-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-(trifluoromethyl)-phenyl)-methanol (8): This compound was synthesized according to **GP-D**, using **17** (0.037 g, 0.11 mmol), anhydrous MeOH (0.27 mL), acetamidine·HCl (0.02 g, 0.22 mmol) and NaOMe (2.0 M in MeOH, 0.22 mL, 0.445 mmol). The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 95:5), to afford **8** as a white solid (0.010 g, 0.034 mmol, 30%). M.p. 139–141 °C. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.81 (s, 1H), 7.54 (s, 1H), 7.45 (s, 1H), 7.43 (s, 1H), 4.65 (s, 2H), 3.89 (s, 2H), 2.41 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 166.7 (C), 163.7 (C), 154.8 (CH), 144.9 (C), 140.8 (C), 132.0 (q, *J* = 32.0, C), 131.5 (q, *J* = 1.0, CH), 125.7 (q, *J* = 271.5, CF_3), 125.0 (q, *J* = 3.8, CH), 122.7 (q, *J* = 3.8, CH), 114.4 (C), 64.2 (CH_2), 33.9 (CH_2), 24.7 (CH_3). ^{19}F-NMR (376 MHz, CD_3OD) δ –64.04. IR (cm^{-1}) 3330, 3162, 2924, 1643, 1595, 1563, 1437, 1344, 1220, 1105, 1029, 704. HRMS (ESI+) calculated for $C_{14}H_{15}F_3N_3O$ [*M* + H]$^+$ 298.1162, found 298.1163.

Synthesis of deazathiamine derivatives 19–24

5-((5-((Benzyloxy)methyl)-4-methylthiophen-3-yl)methyl)-2-methyl-pyrimidin-4-amine (**19**): To a solution of NaH (previously washed twice with pentane to remove the oil, in a Schlenk flask under N_2; 14.4 mg, 0.600 mmol) in anhydrous DMF (0.20 mL), **2** (50.0 mg, 0.200 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. Then, benzyl bromide (34.3 mg, 0.200 mmol) and anhydrous DMF (0.2 mL) were added (slow addition of benzyl bromide, exothermic!), and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl, extracted 3 times with CH_2Cl_2, and the

combined organic layers were washed once with water, once with a saturated aqueous solution of NaCl, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 97:3 to 95:5), to afford **19** as a white solid (18.0 mg, 0.0530 mmol, 27%). M.p. 145–147 ºC. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.59 (s, 1H), 7.36–7.31 (m, 4H), 7.31–7.25 (m, 1H), 6.94 (s, 1H), 4.64 (s, 2H), 4.54 (s, 2H), 3.65 (s, 2H), 2.40 (s, 3H), 2.05 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 166.3 (C), 163.7 (C), 154.4 (CH), 139.4 (C), 138.8 (C), 136.3 (C), 129.4 (2 CH), 129.0 (2 CH), 128.8 (CH), 122.4 (CH), 114.1 (C), 109.7 (C), 72.8 (CH_2), 65.6 (CH_2), 28.7 (CH_2), 24.7 (CH_3), 12.3 (CH_3). IR (cm^{-1}) 3277, 3223, 3095, 2922, 2856, 1670, 1597, 1557, 1482, 1427, 1650, 1080, 739, 702, 599. HRMS (ESI+) calculated for $C_{19}H_{22}N_3OS$ [*M* + H]$^+$ 340.1478, found 340.1476. Two side products were isolated: *N*-benzylated (4.60 mg, 0.0135 mmol) and dibenzylated (7.0 mg, 0.016 mmol) compounds.

2-(4-((4-Amino-2-methylpyrimidin-5-yl)methyl)-3-methylthiophen-2-yl)ethyl 1*H*-indole-3-carboxylate (20): To a solution of **26** (25.0 mg, 59.9 µmol) in CH_3CN (0.5 mL), DBU (18.2 mg, 0.119 mmol) was added. Then, a solution of indole-3-carboxylic acid (19.3 mg, 0.119 mmol) in CH_3CN (0.2 mL), and tetrabutylammonium iodide (5.40 mg, 1.46 µmol) were added, and the reaction mixture was stirred at room temperature for 3 days. Subsequently, water and CH_2Cl_2 were added to the reaction mixture, and the phases were separated. The aqueous layer was extracted 3 times with CH_2Cl_2, and the four combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. After a flash column chromatography (CH_2Cl_2/MeOH 97:3), the product was mixed with tetrabutylammonium iodide, and it was purified by HPLC (Shimadzu HPLC-PDA, Column XTerra® Prep MS C18 10 µm 7.8x150 mm; method: 15 min, gradient from H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5 to 5:95, then hold for 3 min at H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 5:95; flow rate 1 mL min^{-1}) to afford **20** as a white solid (1.85 mg, 4.55 µmol, 6%). ^{1}H-NMR (400 MHz, CD_3OD) δ 8.02–7.99 (m, 1H), 7.96 (s, 1H), 7.45–7.43 (m, 2H), 7.23–7.13 (m, 2H), 6.94 (s, 1H), 4.49 (t, *J* = 6.6, 2H), 3.70 (s, 2H), 3.27 (t, *J* = 6.6, 2H), 2.53 (s, 3H), 2.11 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 167.1 (C), 165.9 (C), 161.6 (C), 141.6 (CH), 138.1 (C), 136.8 (C), 136.5 (C), 134.2 (C), 133.4 (CH), 127.2 (C), 123.7 (CH), 122.5 (CH), 122.0 (CH), 121.3 (CH), 116.0 (C), 113.0 (CH), 108.3 (C), 64.7 (CH_2), 29.1 (CH_2), 28.4 (CH_2), 21.4 (CH_3), 12.2 (CH_3). HRMS (ESI+) calculated for $C_{22}H_{23}N_4O_2S$ [*M* + H]$^+$ 407.1536, found 407.1533.

General procedure for the reductive amination in the synthesis of 21, 22 and 23 (GP-E). To a solution of aldehyde **25** (1.0 eq) in anhydrous DMF, the corresponding amine (1.2 eq), MeOH and $MgSO_4$ (added when the reaction mixture is homogenous) were added. The reaction mixture was stirred at 60 °C (pre-heated oil bath) for 20 h. Then, the reaction mixture was cooled down, $NaBH_4$ (0.5–1.0 eq) was added, and the reaction mixture was stirred at room temperature for 20 h. Subsequently, the solution was filtered, and the solvent was evaporated under reduced pressure. The crude was purified by flash column chromatography.

5-((5-(((2,2-Diphenylethyl)amino)methyl)-4-methylthiophen-3-yl)methyl)-2-methylpyrimidin-4-amine (21): This compound was synthesized according to **GP-E**, using aldehyde **25** (80.0 mg, 0.324 mmol), anhydrous DMF (1.8 mL), 2,2-diphenylethylamine (76.5 mg, 0.388 mmol), MeOH (*ca.* 0.1 mL), $MgSO_4$ (*ca.* 50 mg), and later $NaBH_4$ (12.3 mg, 0.324 mmol). The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 95:5), to afford **21** as a white solid (84.8 mg, 0.198 mmol, 61%). M.p. 54–56 ºC. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.58 (s, 1H), 7.29–7.14 (m, 10H), 6.84 (s, 1H), 4.17 (t, *J* = 7.8, 1H), 3.88 (s, 2H), 3.61 (s, 2H), 3.23 (d, *J* = 7.8, 2H), 2.40 (s, 3H), 1.92 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 166.3 (C), 163.6 (C), 154.5 (CH), 144.0 (2 C), 138.7 (C), 137.9 (C), 134.9 (C), 129.7 (4 CH), 129.0 (4 CH), 127.7 (2 CH), 121.3 (CH), 114.0 (C), 54.0 (CH_2), 52.1 (CH), 46.6 (CH_2), 28.7 (CH_2), 24.8 (CH_3), 12.2 (CH_3). IR (cm^{-1}) 3321, 3147, 2919, 2833, 1629, 1590, 1558, 1449, 1116, 970, 740, 698. HRMS (ESI+) calculated for $C_{26}H_{29}N_4S$ [*M* + H]$^+$ 429.2107, found 429.2100.

5-((5-((((Adamantan-1-yl)methyl)amino)methyl)-4-methylthiophen-3-yl)methyl)-2-methylpyrimidin-4-amine (22): This compound was synthesized according to **GP-E**, using aldehyde **25** (45.0 mg, 0.18 mmol), anhydrous DMF (1 mL), 1-adamantane-methylamine (39 µL, 0.22 mmol), MeOH (*ca.* 0.1 mL), $MgSO_4$ (*ca.* 20 mg), and later $NaBH_4$ (3.4 mg, 0.09 mmol) The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 95:5), to afford **22** as a yellow solid (0.023 g, 0.058 mmol, 32%). M.p. 74–76 °C. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.57 (s, 1H), 6.88 (s, 1H), 3.89 (s, 2H), 3.65 (s, 2H), 2.39 (s, 3H), 2.27 (s, 2H), 2.06 (s, 3H), 1.98–1.92 (m, 3H), 1.78–1.65 (m, 6H), 1.56–1.52 (m, 6H). ^{13}C-NMR (101 MHz, CD_3OD) δ 166.2 (C), 163.7 (C), 154.4 (CH), 139.4 (C), 138.5 (C), 134.4 (C), 121.1 (CH), 114.2 (C), 62.6 (CH_2), 48.0 (CH_2), 41.9 (3 CH_2), 38.2 (3 CH_2), 34.4 (C), 29.9 (3 CH), 28.7 (CH_2), 24.7 (CH_3), 12.3 (CH_3). IR (cm^{-1}) 3322, 3168, 2898, 2845, 1635, 1592, 1560, 1435, 1237, 1107, 1034, 973, 734. HRMS (ESI+) calculated for $C_{23}H_{33}N_4S$ [*M* + H]$^+$ 397.2420, found 397.2418.

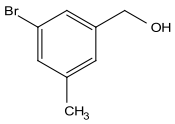
2-Methyl-5-((4-methyl-5-(((pyridin-2-ylmethyl)amino)methyl)-thiophen-3-yl)methyl)pyrimidin-4-amine (23): This compound was synthesized according to **GP-E**, using aldehyde **25** (16.0 mg, 0.065 mmol), DMF (0.3 mL), 2-(aminomethyl)pyridine (8.0 µL, 0.078 mmol), MeOH (*ca.* 0.05 mL), $MgSO_4$ (*ca.* 10 mg), and later $NaBH_4$ (13.0 mg,

0.033 mmol). The crude was purified by flash column chromatography (CH_2Cl_2/MeOH 9:1), to afford **23** as a white solid (0.015 g, 0.043 mmol, 66%). M.p. 136–138 °C. ^{1}H-NMR (400 MHz, CD_3OD) δ 8.50 (m, 1H), 7.80 (td, J = 7.8, 1.8, 1H), 7.59 (s, 1H), 7.48 (d, J = 7.8, 1H), 7.30 (m, 1H), 6.89 (s, 1H), 3.93 (s, 4H), 3.64 (s, 2H), 2.39 (s, 3H), 2.01 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 166.3 (C), 163.7 (C), 160.1 (C), 154.4 (CH), 149.8 (CH), 138.7 (CH), 138.6 (C), 138.0 (C), 135.1 (C), 124.1 (CH), 123.8 (CH), 121.5 (CH), 114.1 (C), 54.2 (CH_2), 46.3 (CH_2), 28.8 (CH_2), 24.7 (CH_3), 12.2 (CH_3). IR (cm^{-1}) 3328, 3294, 3148, 2923, 2860, 2819, 1656, 1591, 1563, 1471, 1430, 1321, 1135, 976, 767, 733, 594. HRMS (ESI+) calculated for $C_{18}H_{22}N_5S$ [M + H]$^+$ 340.1590, found 340.1589.

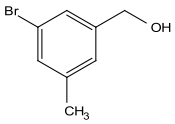
(**2*R*,3*R*,4*S*,5*R*,6*S*)-2-(Acetoxymethyl)-6-((2-(4-((4-amino-2-methylpyrimidin-5-yl)methyl)-3-methylthiophen-2-yl)ethyl)thio)-tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (27):** To a solution of **26** (62 mg, 0.15 mmol) in anhydrous CH_3CN (5 mL), 1-thio-β-D-glucose tetraacetate (81 mg, 0.22 mmol), DBU (33 µL, 0.22 mmol) and tetrabutylammonium iodide (5.5 mg, 0.015 mmol) were added and the reaction mixture was stirred for 24 h. Then, another 0.22 mmol of 1-thio-β-D-glucose tetraacetate, 0.44 mmol of DBU and 0.03 mmol of tetrabutylammonium iodide were added, and the mixture was stirred for 72 h. The solvent was evaporated under reduced pressure, and the crude purified by flash column chromatography (CH_2Cl_2/MeOH 97:3), to afford **27** as a yellow thick oil (29 mg, 0.048 mmol, 32%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 6.65 (s, 1H), 5.18 (t, J = 9.4, 1H), 5.09–4.92 (m, 4H), 4.40 (d, J = 10.0, 1H), 4.22 (dd, J = 12.4, 5.0, 1H), 4.12 (dd, J = 12.4, 2.3, 1H), 3.66 (ddd, J = 10.0, 5.0, 2.3, 1H), 3.60 (s, 2H), 3.06–2.79 (m, 4H), 2.50 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 170.7 (CO), 170.3 (CO), 169.52 (CO), 169.48 (CO), 166.3 (C), 161.8 (C), 155.6 (CH), 137.8 (C), 137.1 (C), 132.8 (C), 119.1 (CH), 111.8 (C), 83.5 (CH), 76.1 (CH), 73.9 (CH), 70.0 (CH), 68.4 (CH), 62.3 (CH_2), 31.0 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 25.5 (CH_3), 20.86 (CH_3), 20.84 (CH_3), 20.71 (CH_3), 20.70 (CH_3), 12.5 (CH_3). HRMS (ESI+) calculated for $C_{27}H_{36}N_3O_9S_2$ [M + H]$^+$ 610.1888, found 610.1884.

(2*S*,3*R*,4*S*,5*S*,6*R*)-2-((2-(4-((4-Amino-2-methylpyrimidin-5-yl)methyl)-3-methylthiophen-2-yl)ethyl)thio)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (24): To a solution of **27** (20 mg, 0.033 mmol) in MeOH (3 mL), NaOMe (2.0 M in MeOH, *ca.* 2 mL) was added and the reaction mixture was stirred for 17 h. Then, the solvent was evaporated under reduced pressure, and the crude purified by flash column chromatography (CH_2Cl_2/MeOH 90:10 to 70:30), to afford **24** as a white solid (6.6 mg, 0.015 mmol, 45%). M.p. 50–52 °C. ^{1}H-NMR (400 MHz, CD_3OD) δ 7.59 (s, 1H), 6.79 (s, 1H), 4.35 (d, J = 9.7, 1H), 3.86 (dd, J = 12.1, 2.1, 1H), 3.69–3.63 (m, 3H), 3.37–3.25 (m, 3H, signal overlaps with CD_3OD), 3.24–3.18 (m, 1H), 3.12–3.07 (m, 2H), 3.03–2.84 (m, 2H), 2.40 (s, 3H), 2.04 (s, 3H). ^{13}C-NMR (101 MHz, CD_3OD) δ 166.0 (C), 163.7 (C), 153.7 (CH), 138.9 (C), 138.3 (C), 133.9 (C), 120.2 (CH), 114.4 (C), 87.1 (CH), 82.0 (CH), 79.5 (CH), 74.4 (CH), 71.4 (CH), 62.8 (CH_2), 32.3 (CH_2), 30.5 (CH_2), 28.8 (CH_2), 24.5 (CH_3), 12.4 (CH_3). IR (cm^{-1}) 3336, 3172, 3089, 2920, 2850, 1660, 1596, 1558, 1427, 1356, 1278, 1025, 983, 874, 810, 777, 736, 575. HRMS (ESI+) calculated for $C_{19}H_{28}N_3O_5S_2$ [M + H]$^+$ 442.1465, found 442.1458.

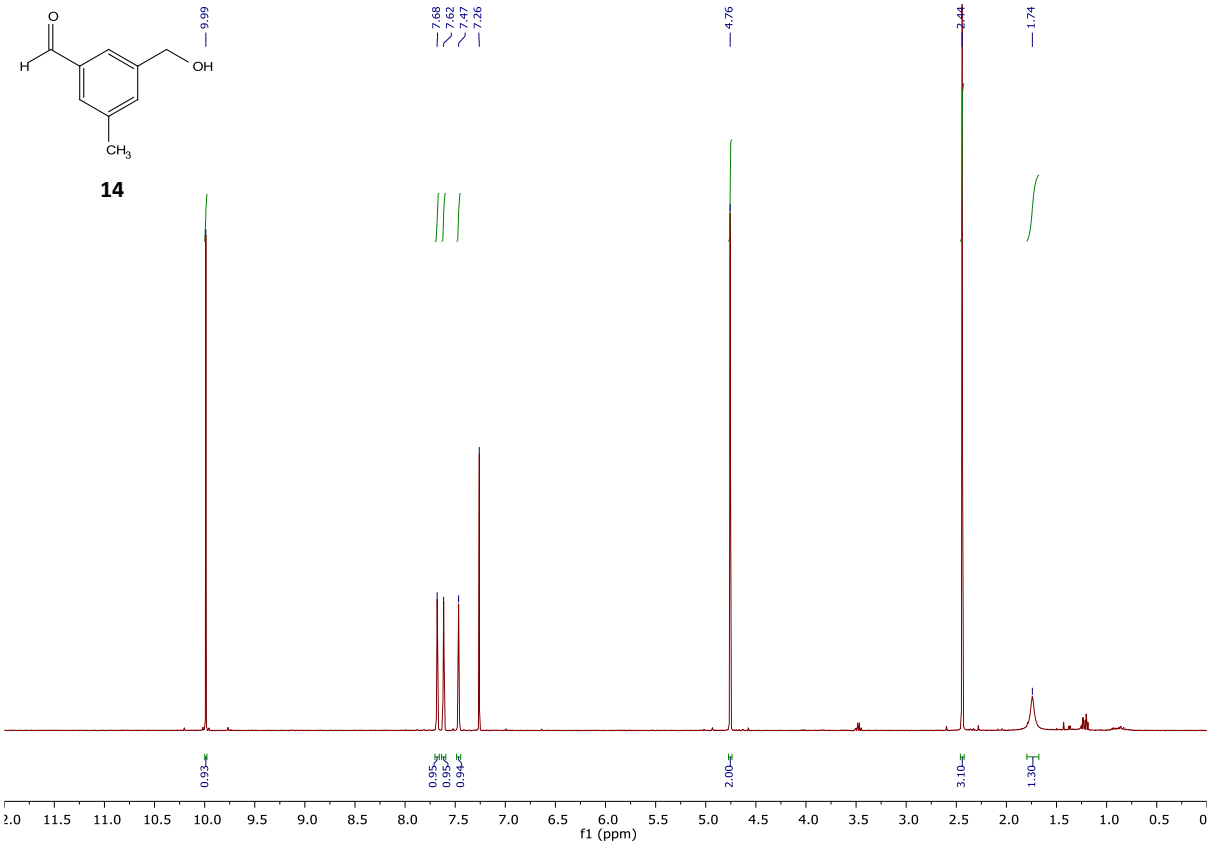
NMR spectra



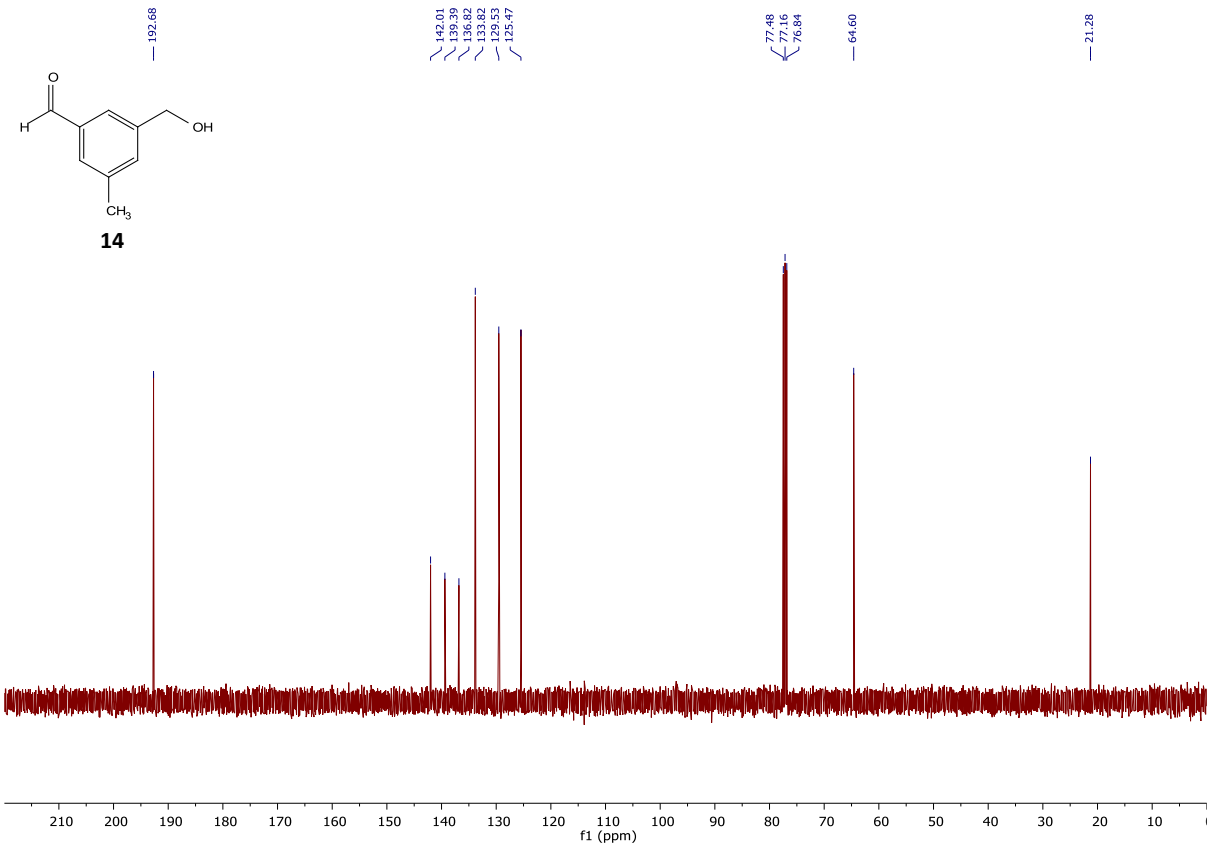
12



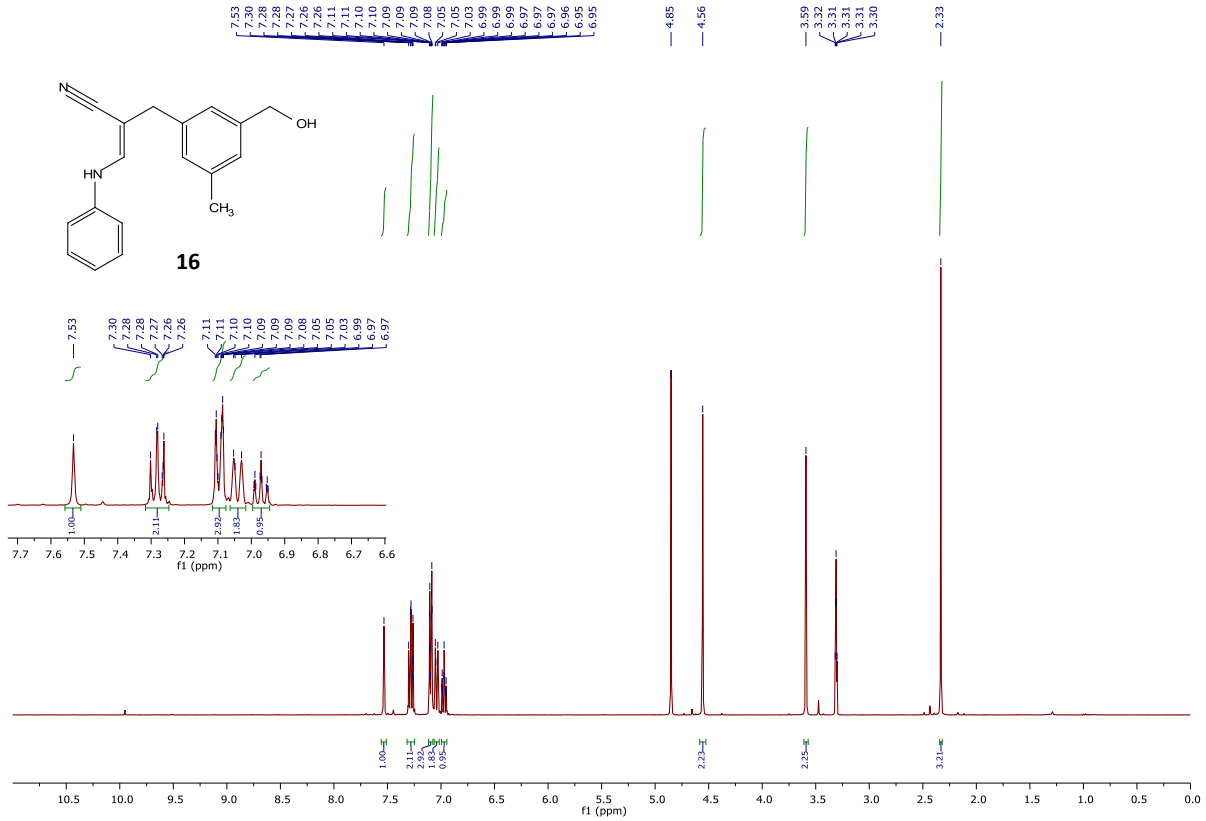
12

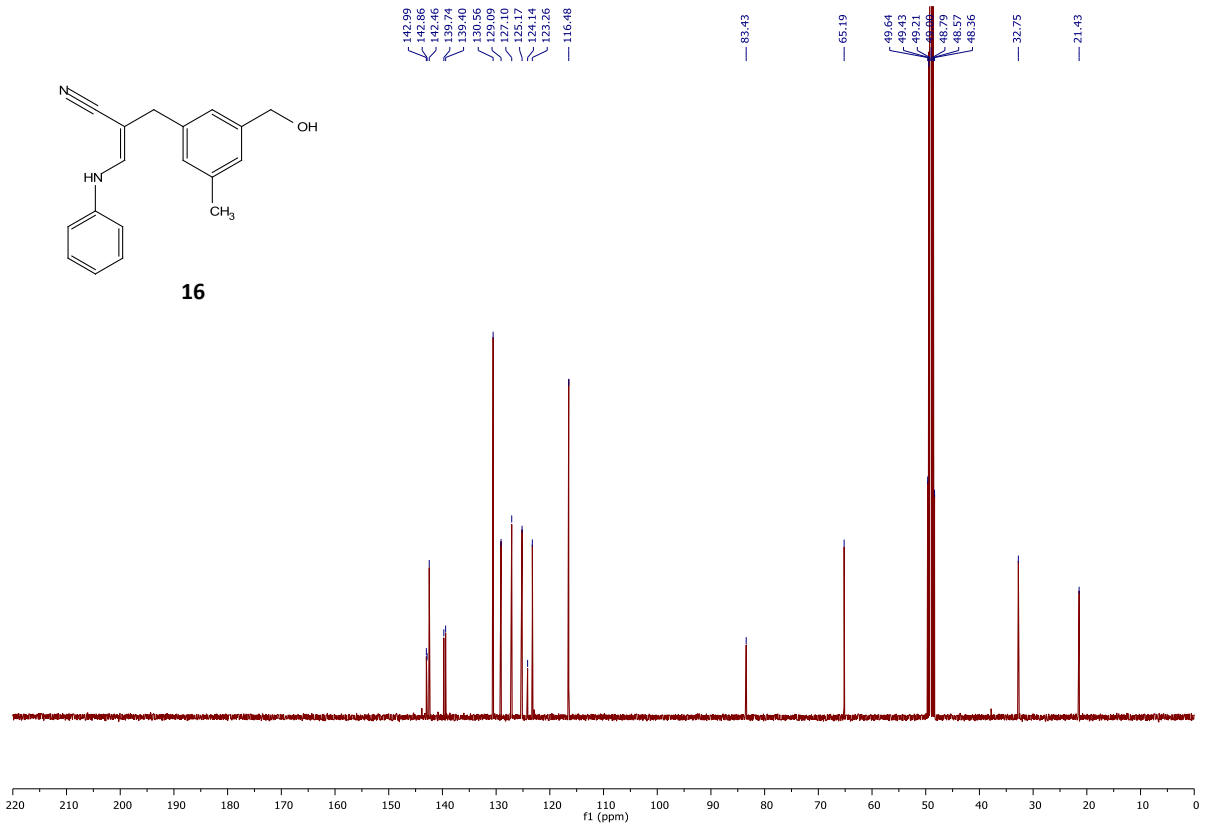

O
H
OH
CH3
9.99
7.68
7.62
7.47
7.26
4.76
2.44
1.74
0.93
0.95
0.95
0.94
2.00
3.10
1.30
f1 (ppm)

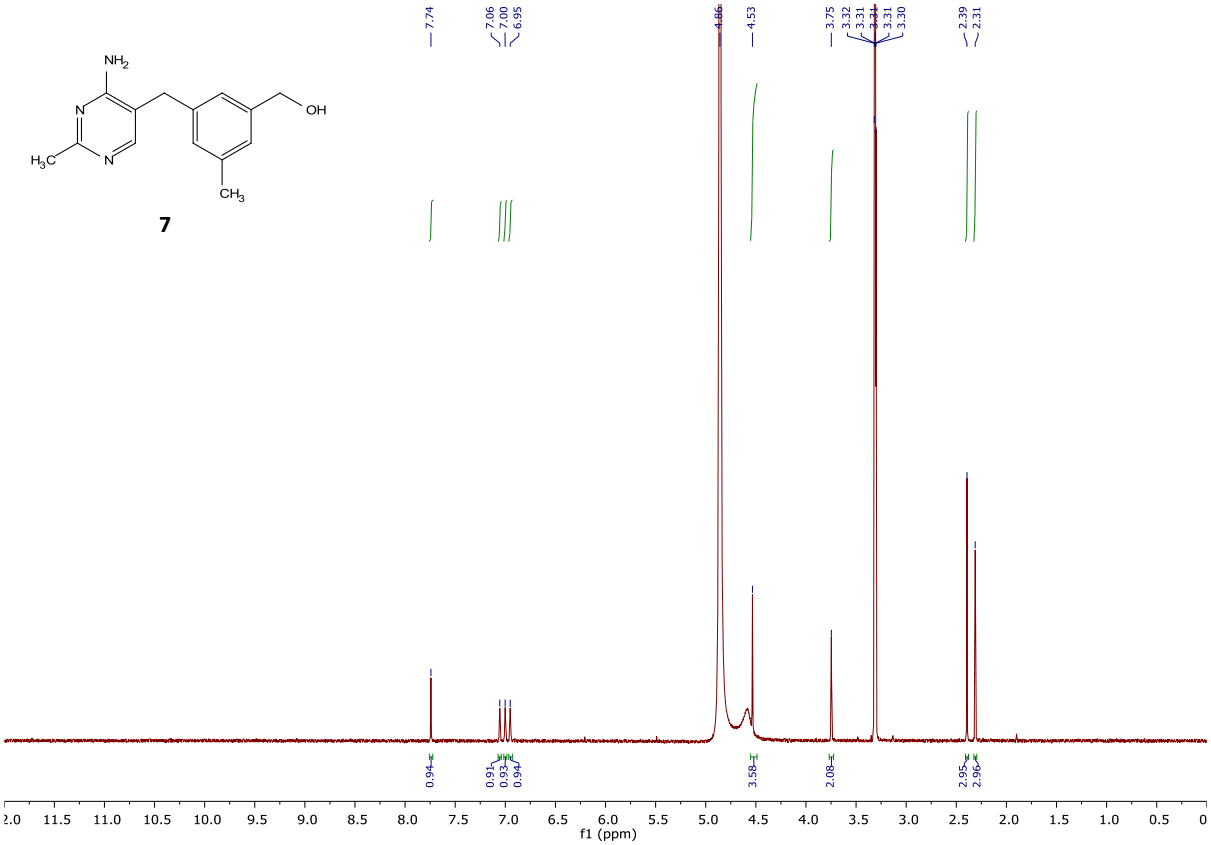
14

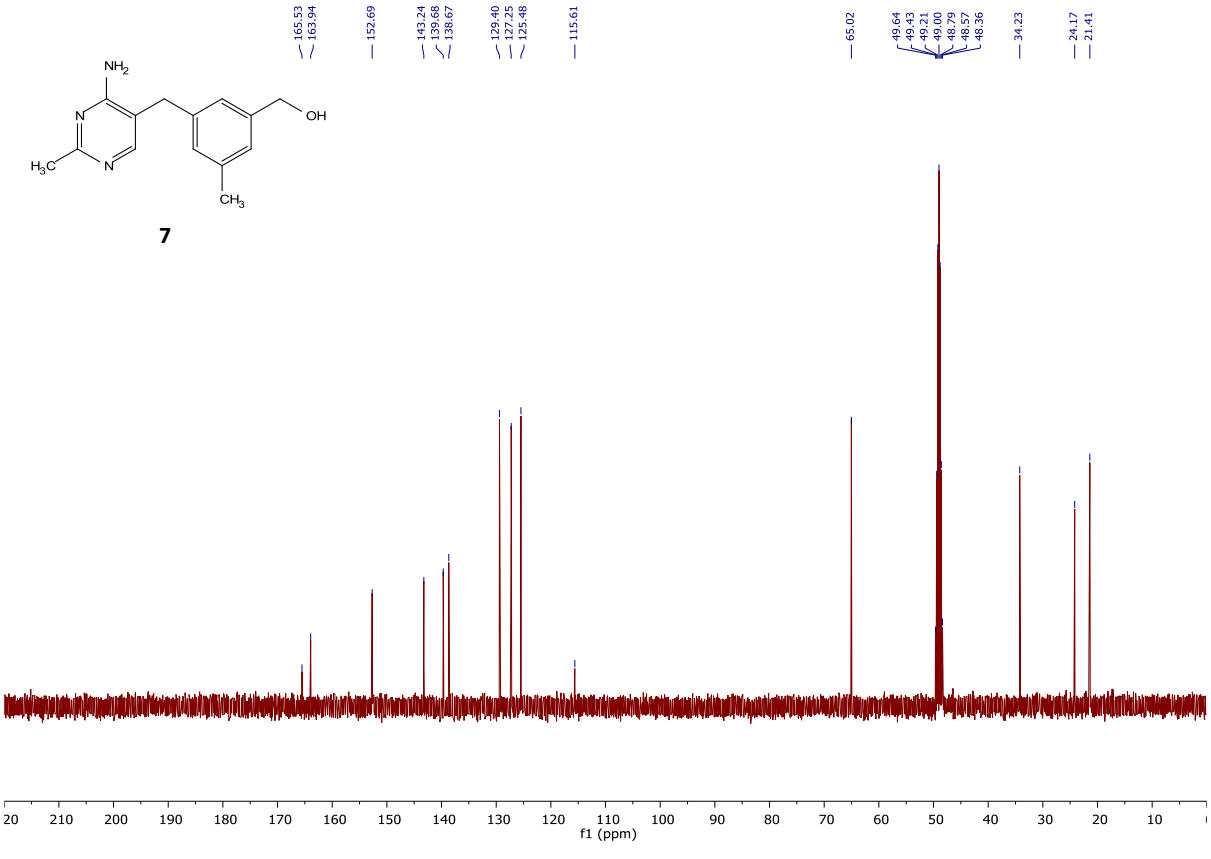

O
H
OH
CH3
192.68
142.01
139.39
136.82
133.82
129.53
125.47
77.48
77.16
76.84
64.60
21.28
f1 (ppm)

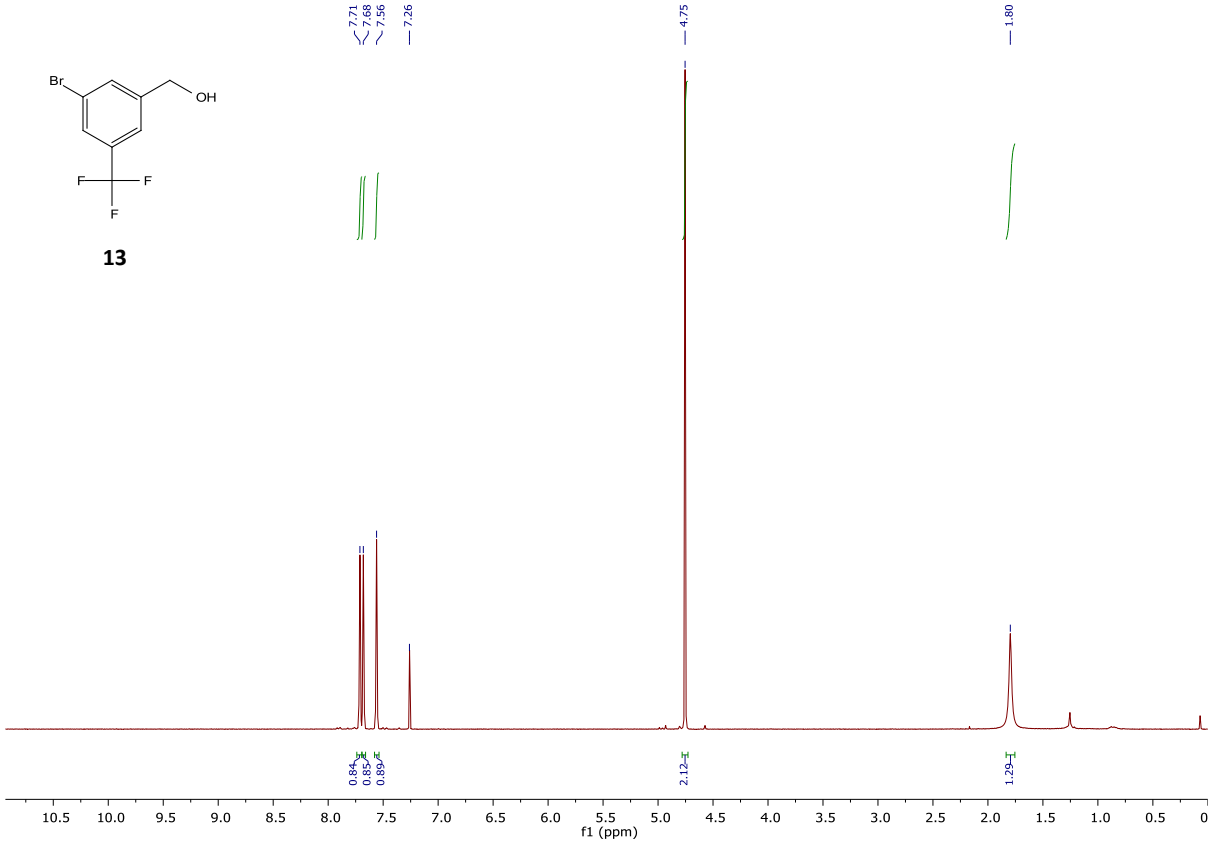
14

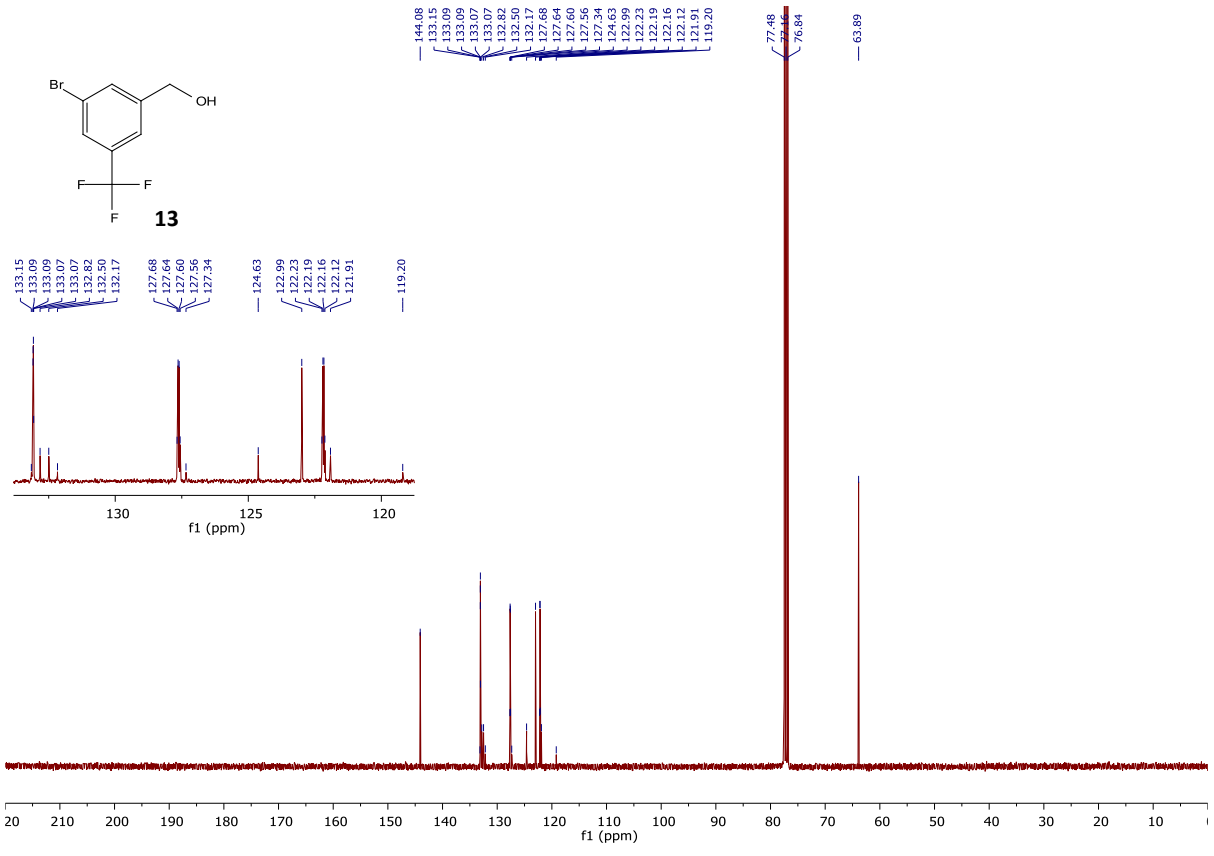

N
OH
HN
CH3
16
f1 (ppm)

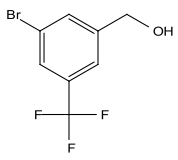

N
OH
HN
CH3
16
f1 (ppm)


NH2
N
H3C
N
OH
CH3
7
7.74
7.06
7.00
6.95
4.86
4.53
3.75
3.32
3.31
3.31
3.31
3.30
2.39
2.31
0.94
0.91
0.93
0.94
3.58
2.08
2.95
2.96
f1 (ppm)


NH2
N
H3C
N
OH
CH3
7
165.53
163.94
152.69
143.24
139.68
138.67
129.40
127.25
125.48
115.61
65.02
49.64
49.43
49.21
49.00
48.79
48.57
48.36
34.23
24.17
21.41
f1 (ppm)

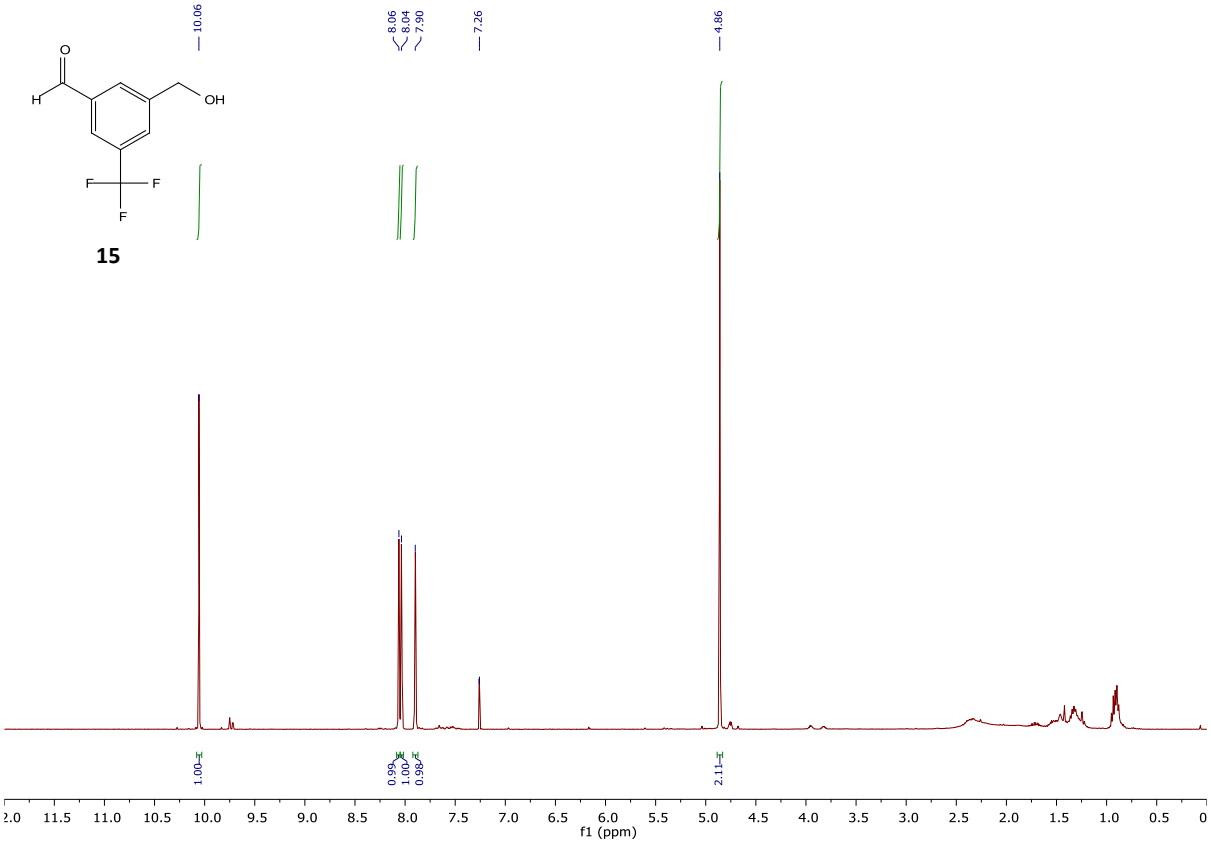

Br
OH
F
F
F
13
7.71
7.68
7.56
7.26
4.75
1.80
0.84
0.85
0.89
2.12
1.29
f1 (ppm)

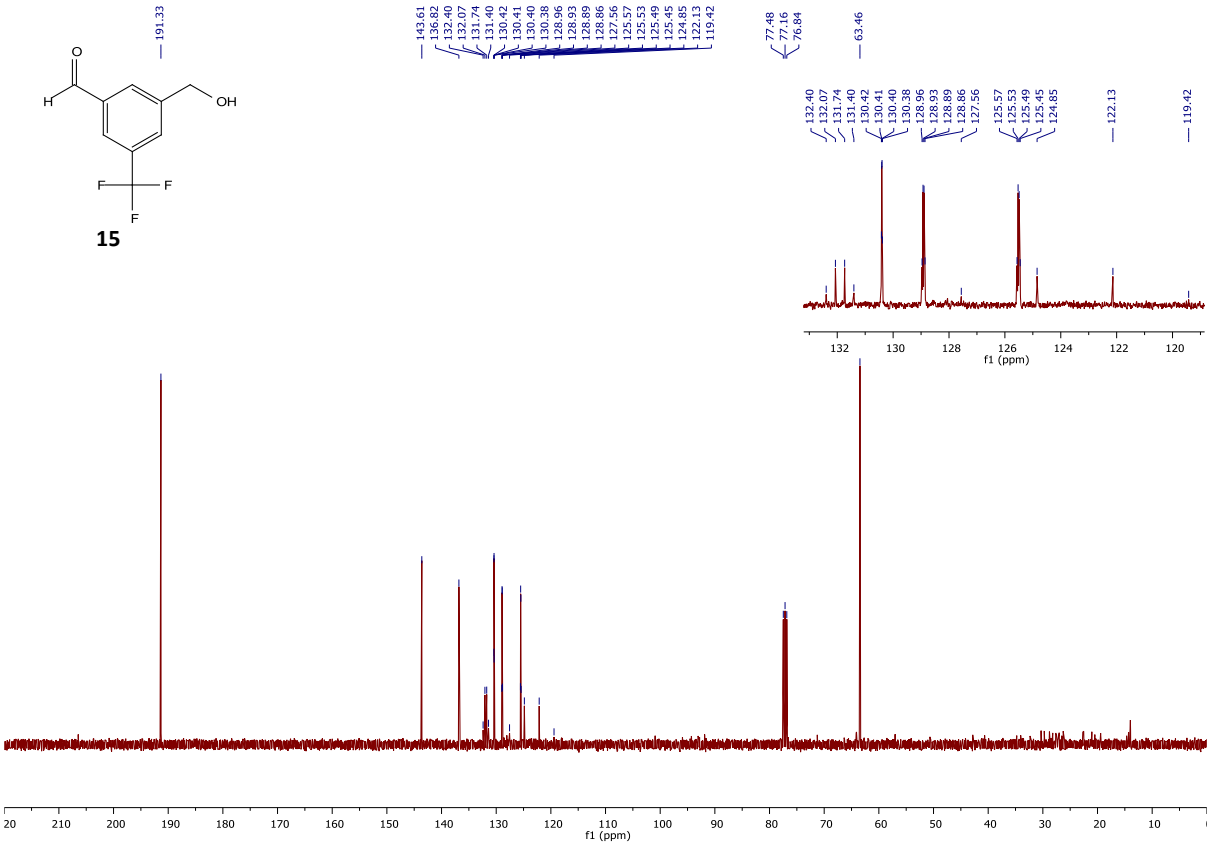

Br
OH
F
F
F
13
144.08
133.15
133.09
133.09
133.07
133.07
132.82
132.50
132.17
127.68
127.64
127.60
127.56
127.34
124.63
122.99
122.23
122.19
122.16
122.12
121.91
119.20
77.48
77.16
76.84
63.89
f1 (ppm)

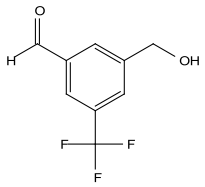

Br
OH
F
F
F

13

-62.86
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

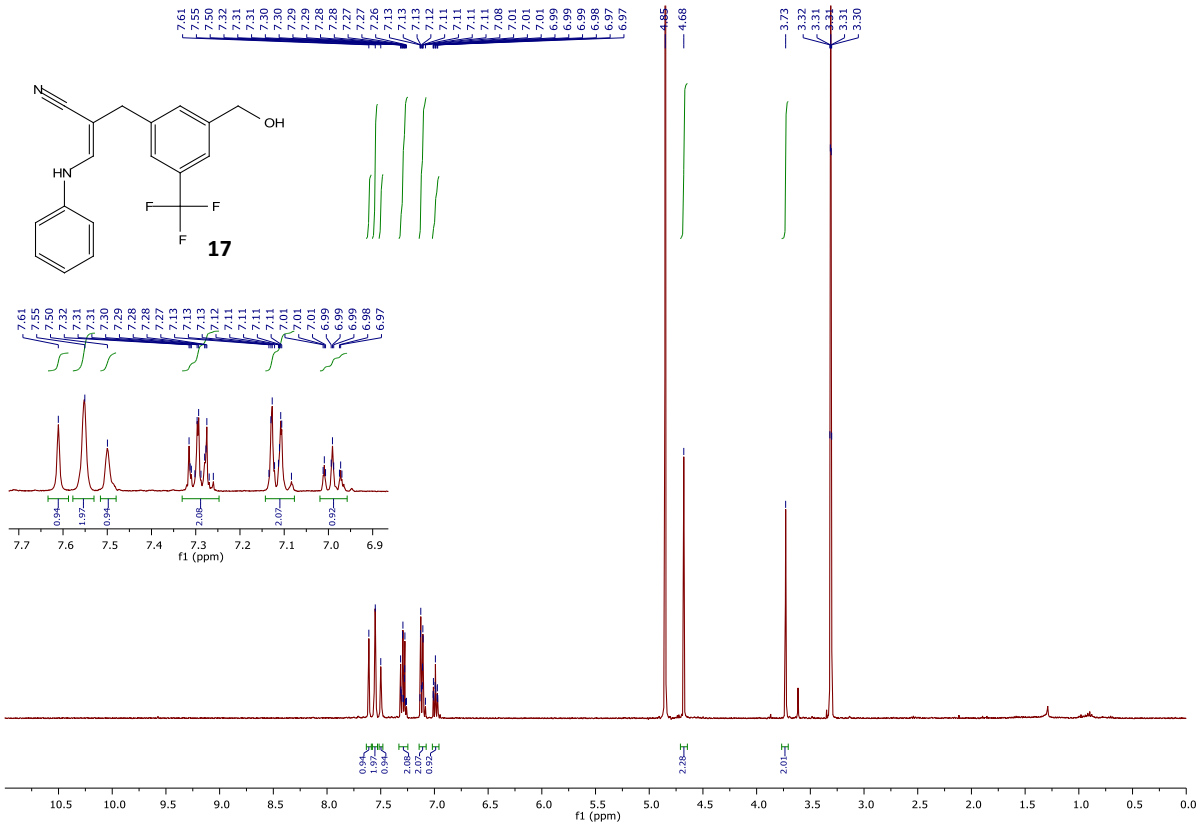

10.06
8.06
8.04
7.90
7.26
4.86
O
H
OH
F
F
F
15
1.00
0.99
1.00
0.98
2.11
f1 (ppm)

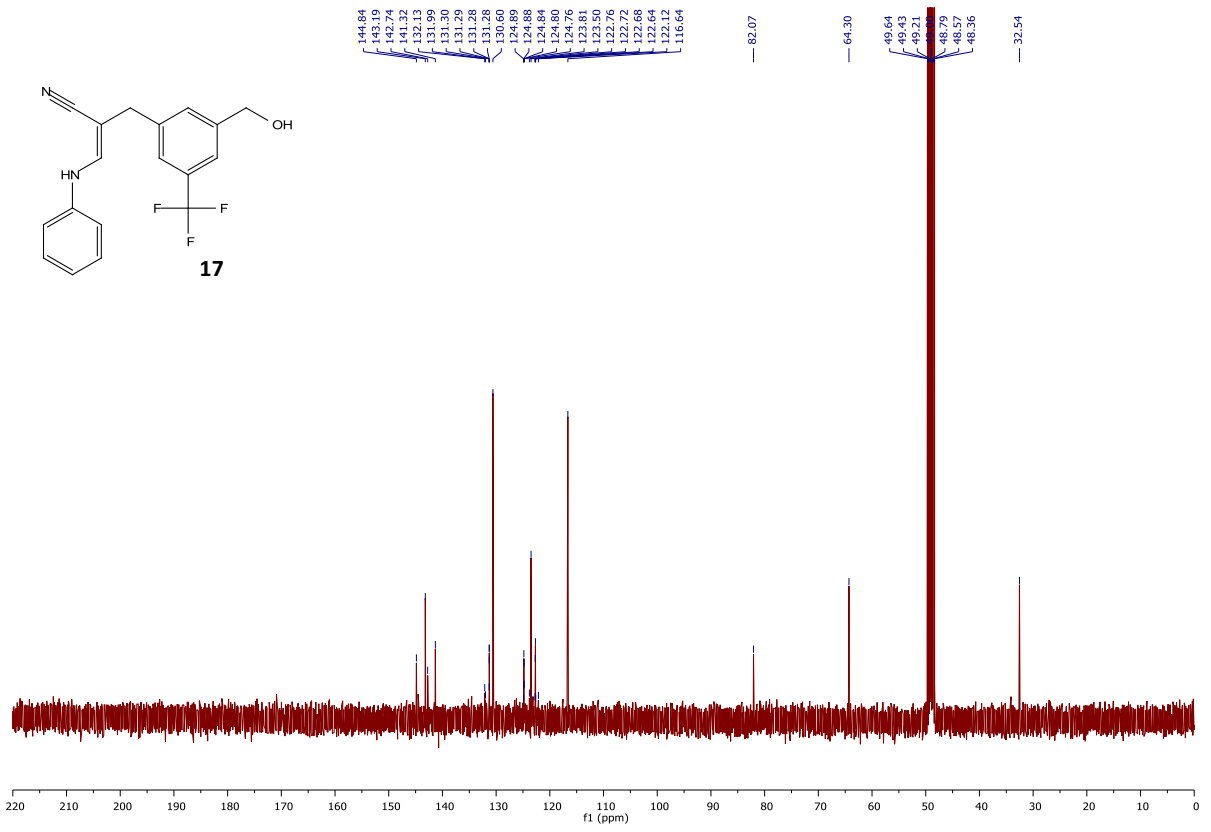

191.33
143.61
136.82
132.40
132.07
131.74
131.40
130.42
130.41
130.40
130.38
128.96
128.93
128.89
128.86
127.56
125.57
125.53
125.49
125.45
124.85
122.13
119.42
77.48
77.16
76.84
63.46
O
H
OH
F
F
F
15
f1 (ppm)

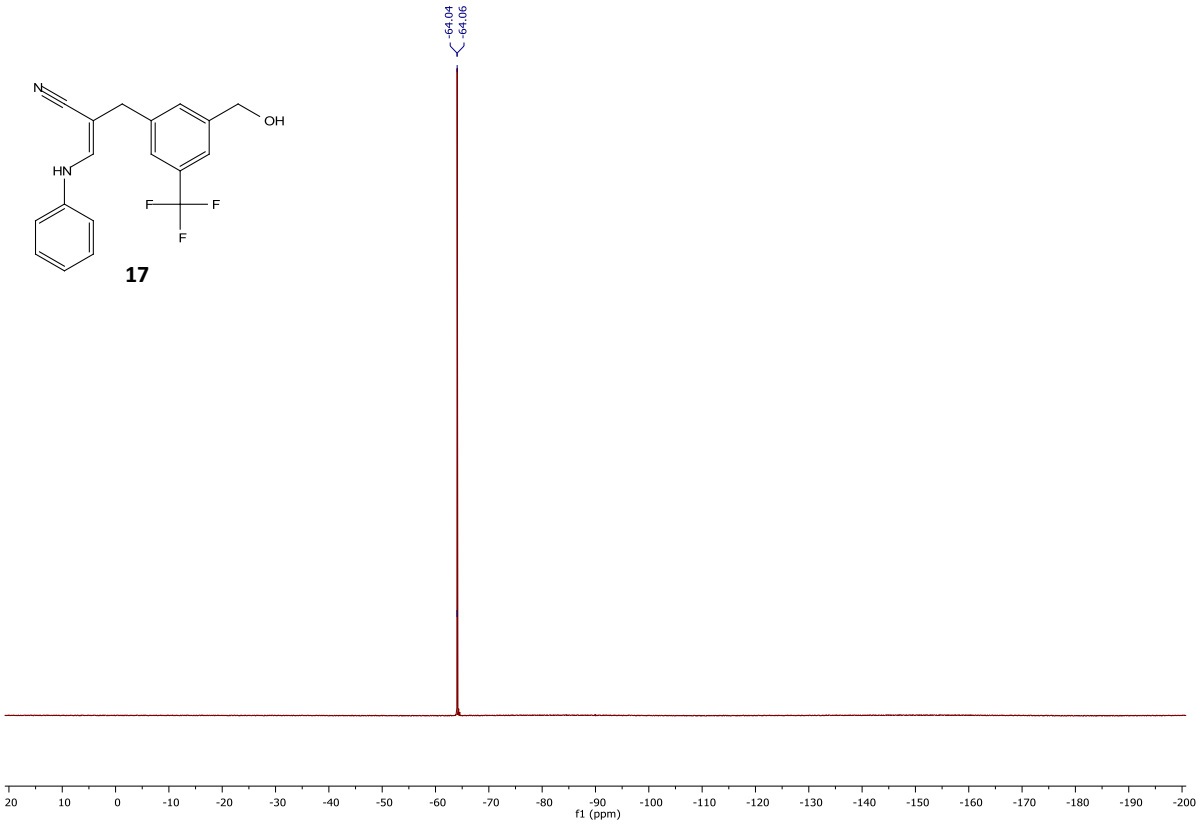

O
H
OH
F
F
F

15

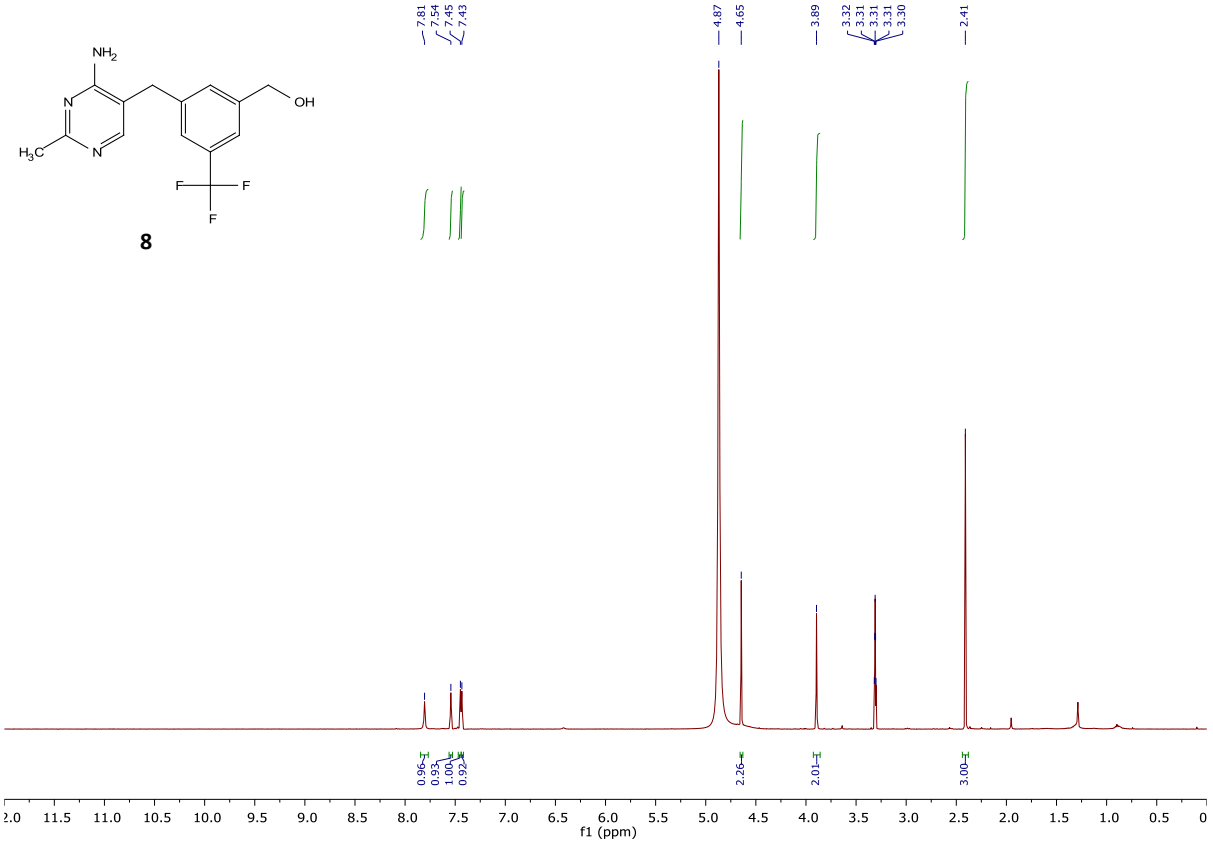
-62.82
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

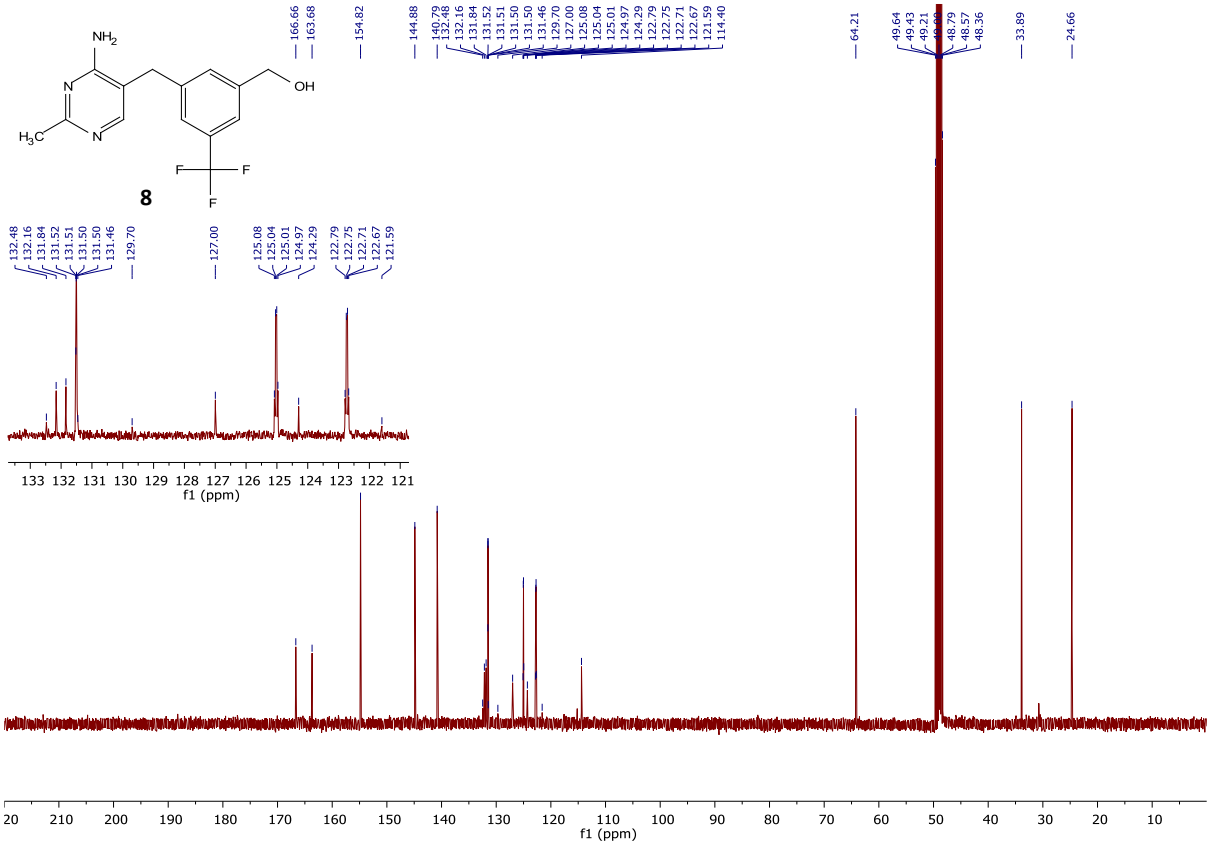

N
OH
HN
F
F
F
17
f1 (ppm)

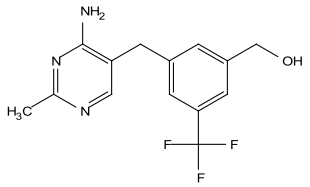

N
OH
HN
F
F
F
17
f1 (ppm)


-64.04
-64.06
N
OH
HN
F
F
F
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

17

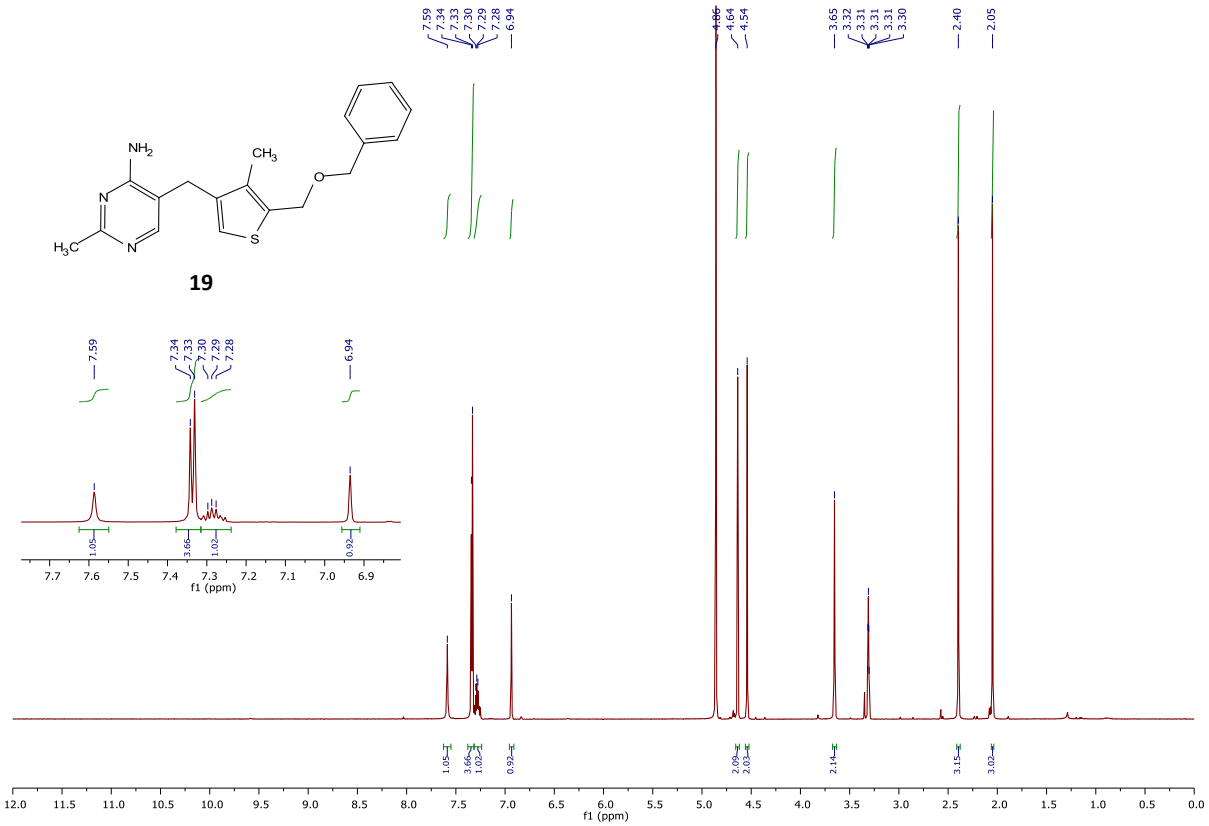

NH2
N
OH
H3C
N
F
F
F
8
7.81
7.54
7.45
7.43
4.87
4.65
3.89
3.32
3.31
3.31
3.31
3.30
2.41
0.96
0.93
1.00
0.92
2.26
2.01
3.00
f1 (ppm)

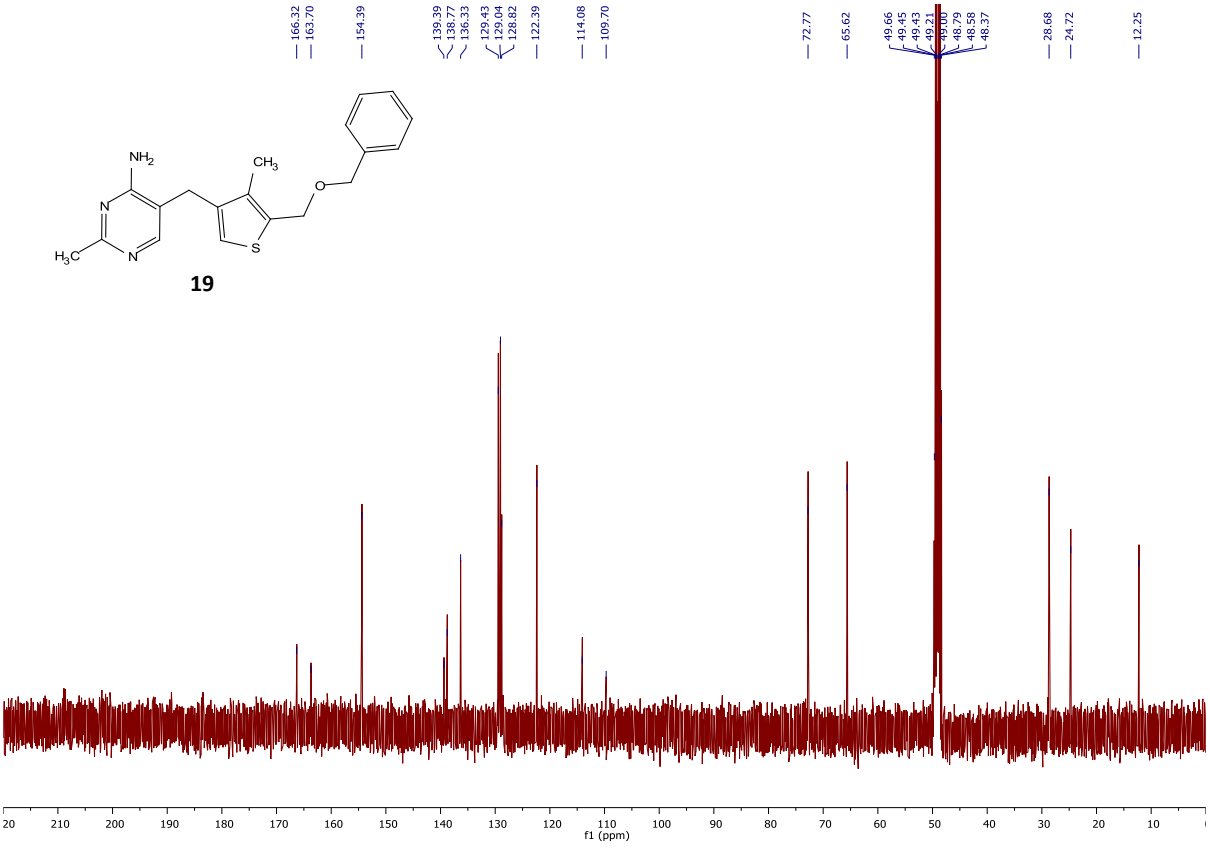

NH2
N
OH
H3C
N
F
F
F
8
166.66
163.68
154.82
144.88
140.79
132.48
132.16
131.84
131.52
131.51
131.50
131.50
131.46
129.70
127.00
125.08
125.04
125.01
124.97
124.29
122.79
122.75
122.71
122.67
121.59
114.40
64.21
49.64
49.43
49.21
48.79
48.57
48.36
33.89
24.66
f1 (ppm)

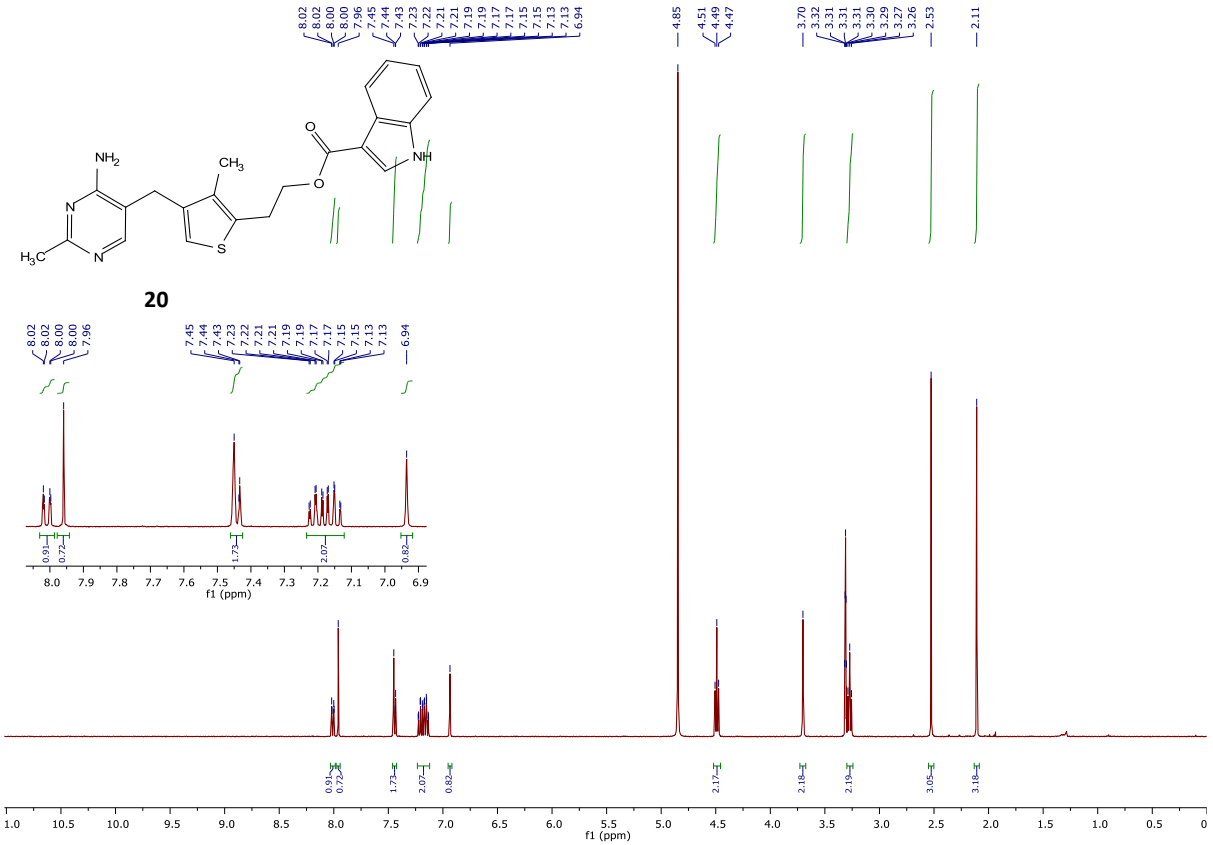

NH2
N
H3C
N
OH
F
F
F

8

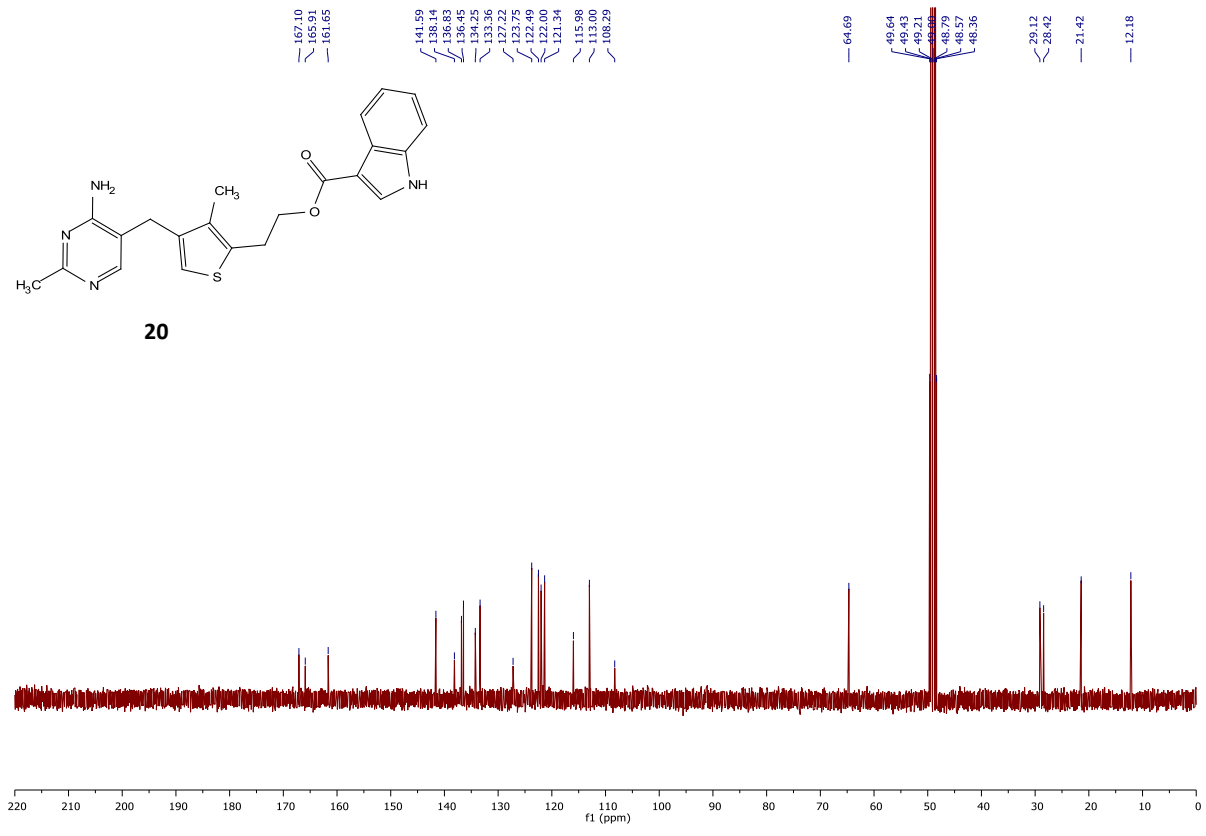
-64.04
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)


7.59
7.34
7.33
7.30
7.29
7.28
6.94
4.66
4.64
4.54
3.65
3.32
3.31
3.31
3.31
3.30
2.40
2.05
NH2
CH3
N
O
H3C
N
S
19
f1 (ppm)

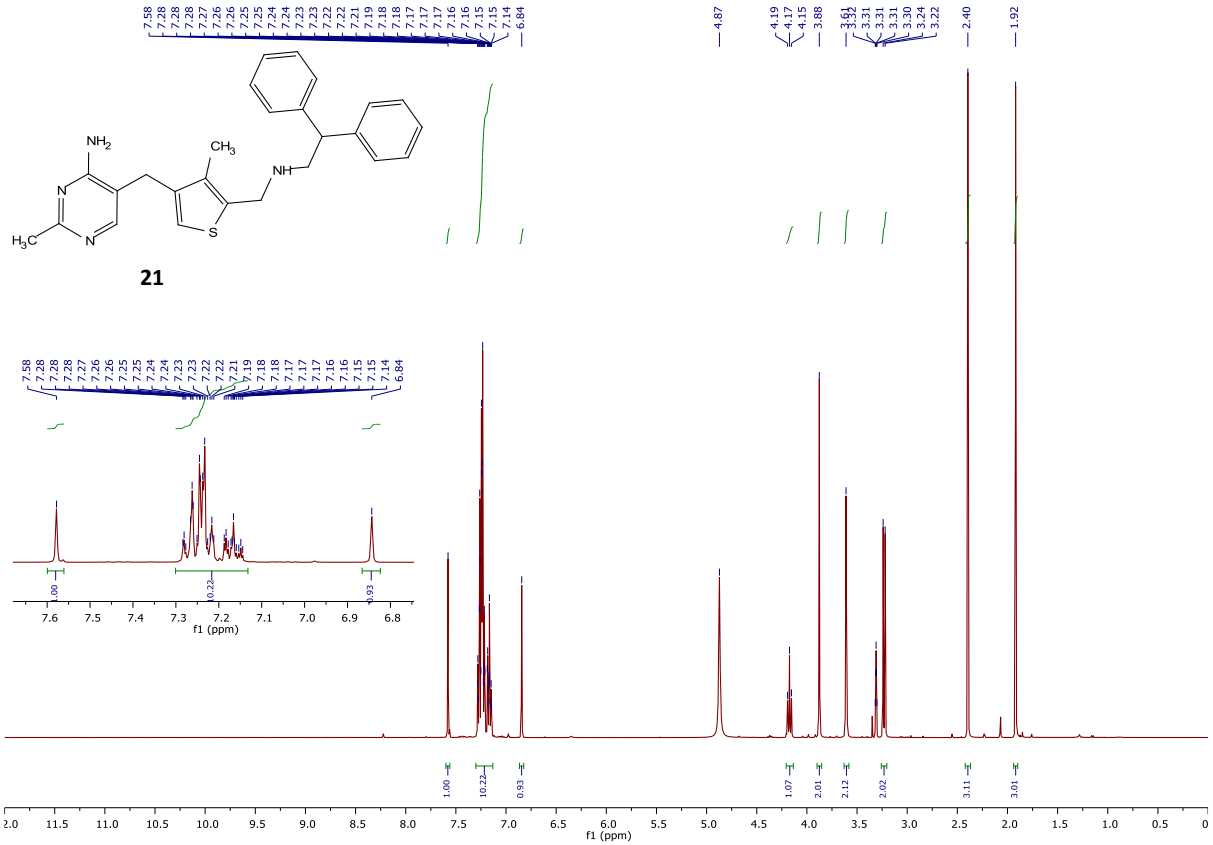

166.32
163.70
154.39
139.39
138.77
136.33
129.43
129.04
128.82
122.39
114.08
109.70
72.77
65.62
49.66
49.45
49.43
49.21
49.00
48.79
48.58
48.37
28.68
24.72
12.25
NH2
CH3
N
O
H3C
N
S
19
f1 (ppm)

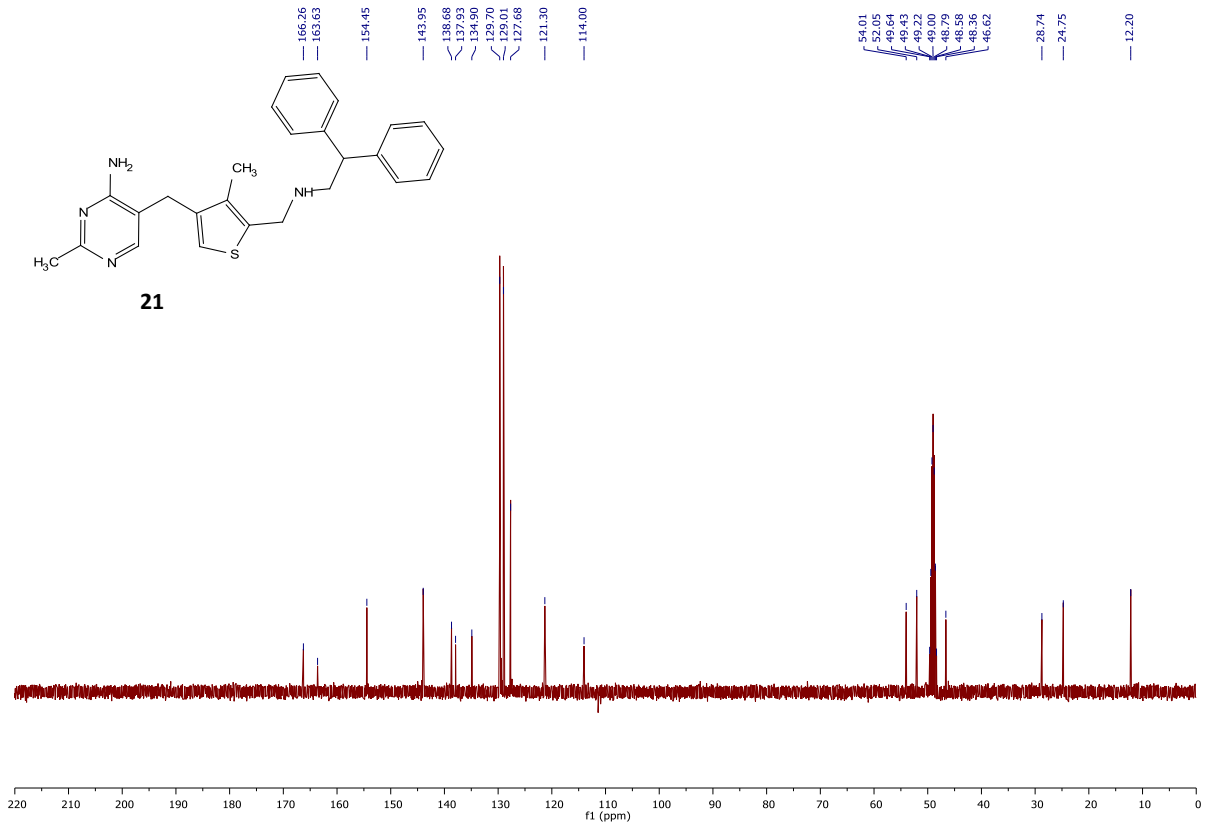

8.02
8.02
8.00
8.00
7.96
7.45
7.44
7.43
7.23
7.22
7.21
7.21
7.19
7.19
7.17
7.17
7.15
7.15
7.13
7.13
6.94
4.85
4.51
4.49
4.47
3.70
3.32
3.31
3.31
3.31
3.30
3.29
3.27
3.26
2.53
2.11
NH2
CH3
N
H3C
N
S
O
O
NH
0.91
0.72
1.73
2.07
0.82
2.17
2.18
2.19
3.05
3.16
f1 (ppm)

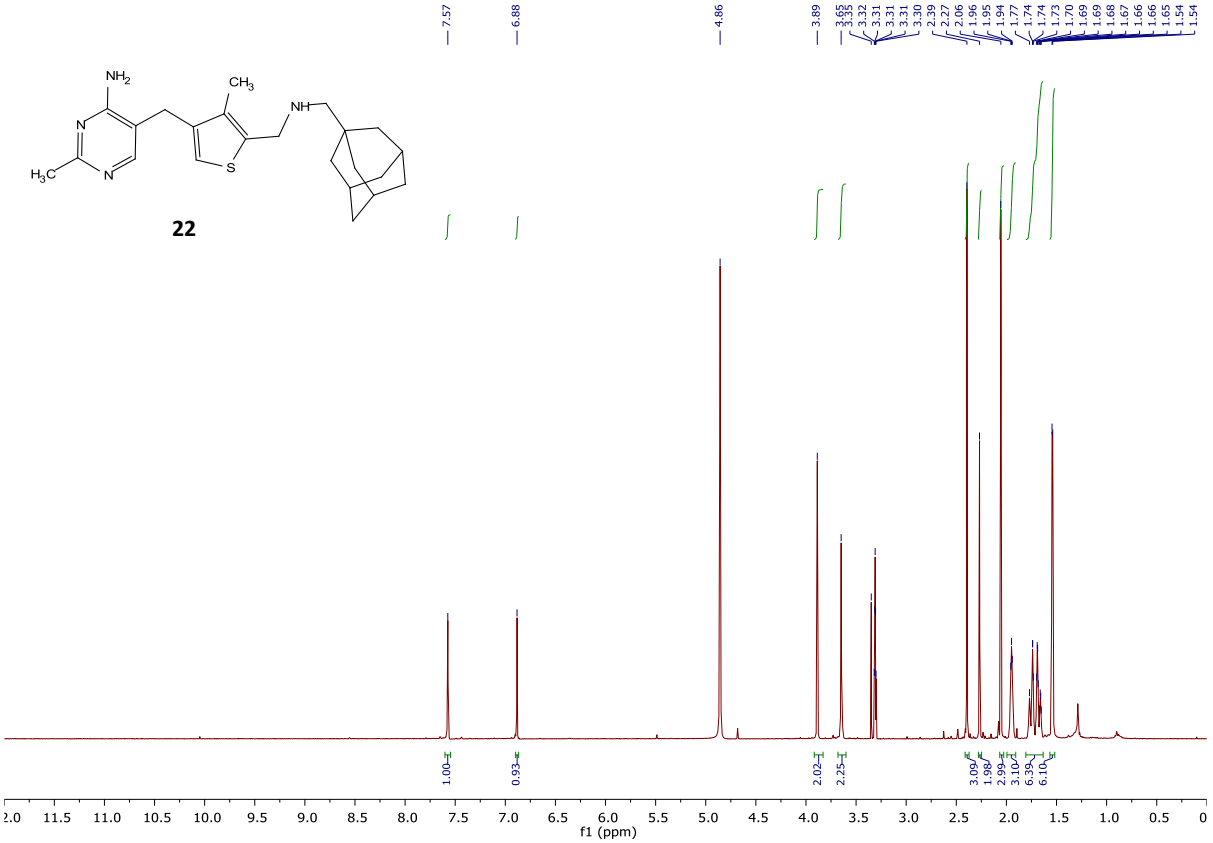
20

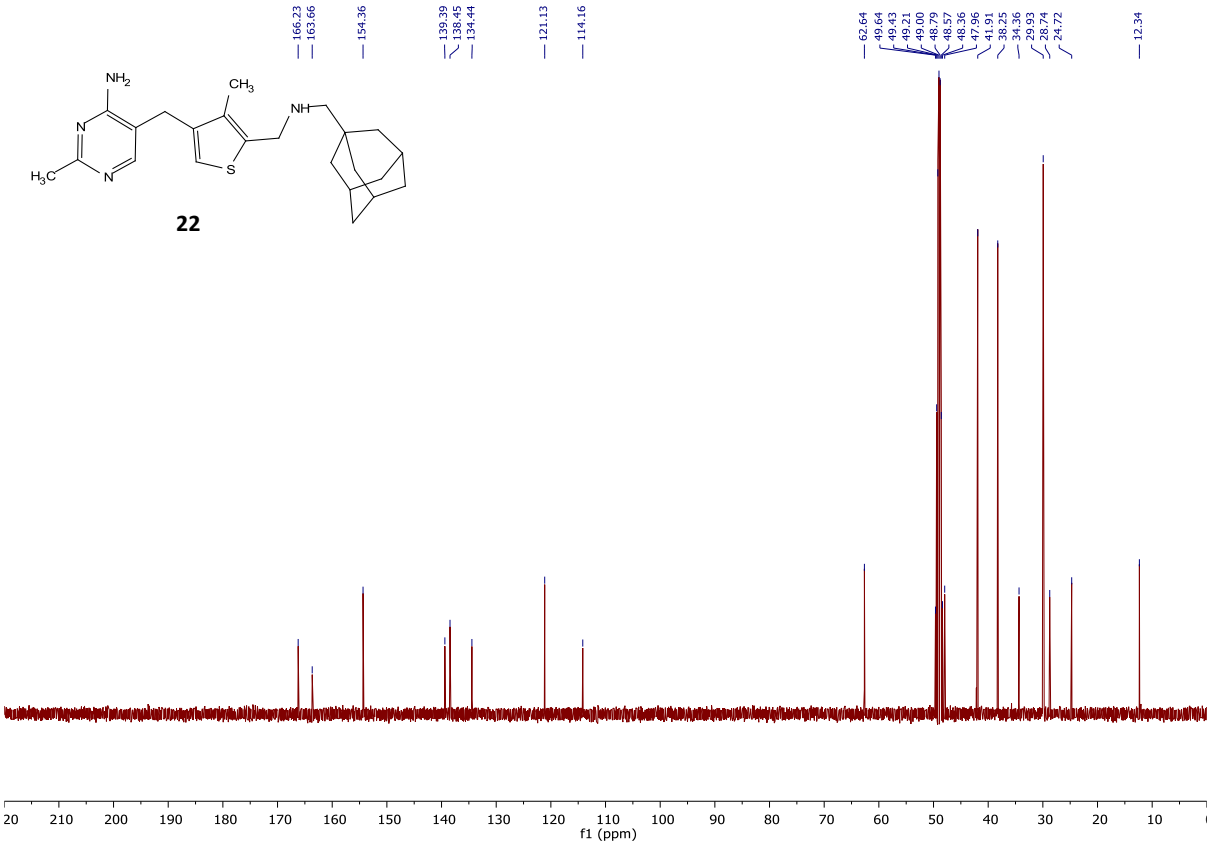

167.10
165.91
161.65
141.59
138.14
136.83
136.45
134.25
133.36
127.22
123.75
122.49
122.00
121.34
115.98
113.00
108.29
64.69
49.64
49.43
49.21
48.79
48.57
48.36
29.12
28.42
21.42
12.18
NH2
CH3
N
H3C
N
S
O
O
NH
f1 (ppm)

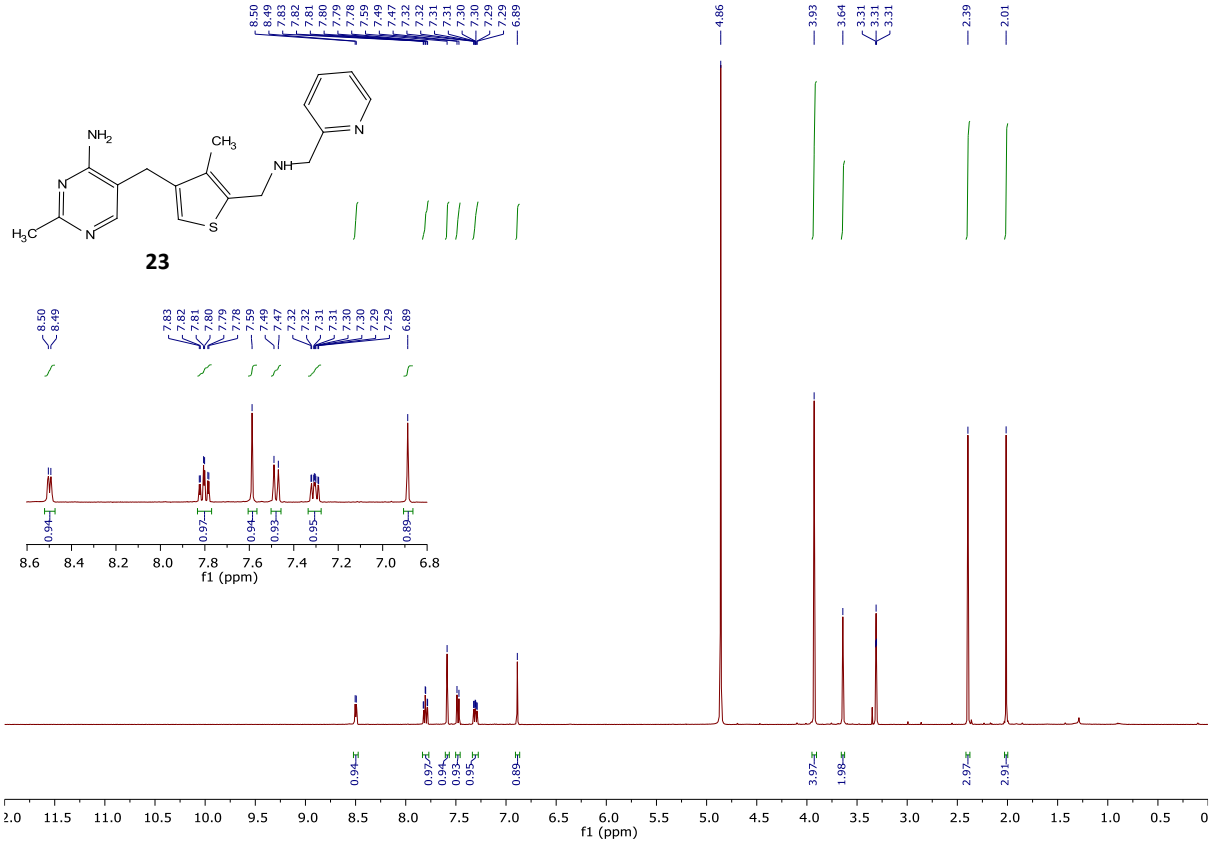
20

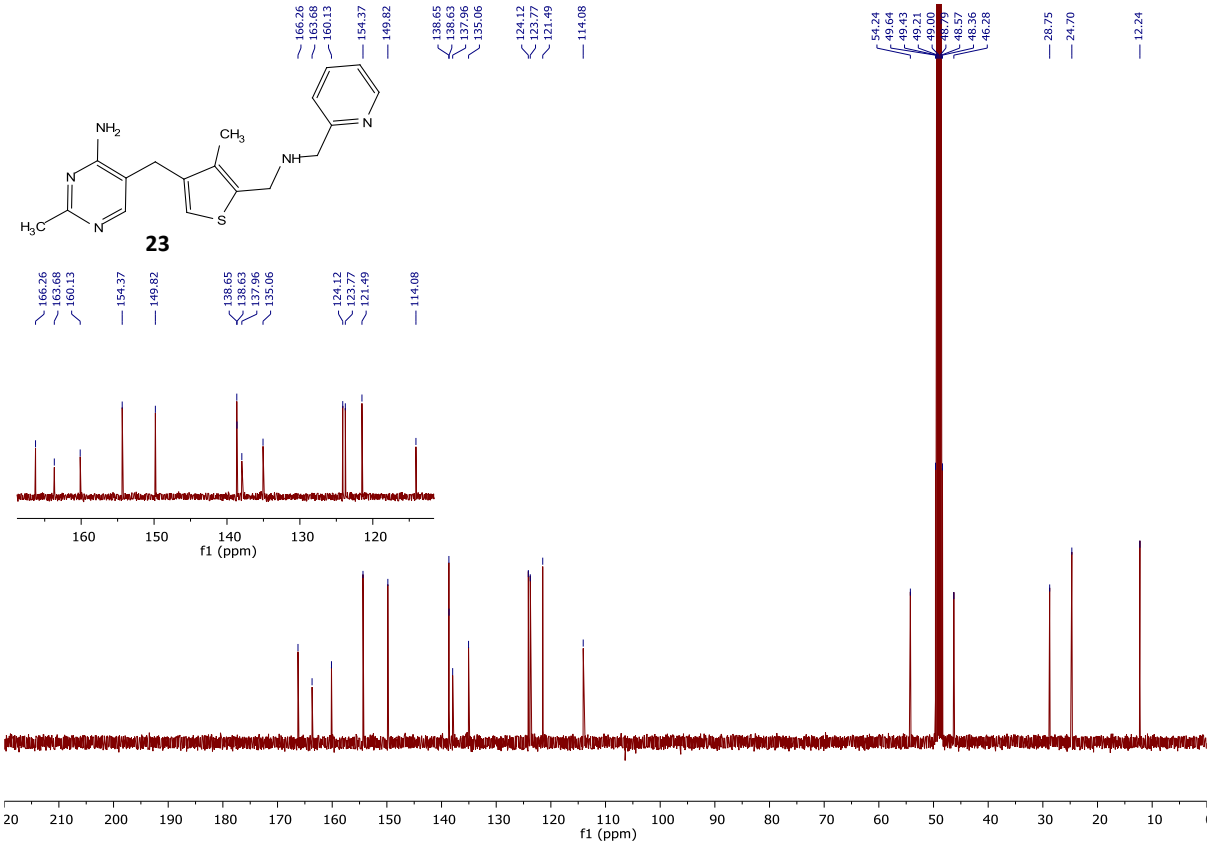

NH2
CH3
NH
N
H3C
N
S
21
f1 (ppm)

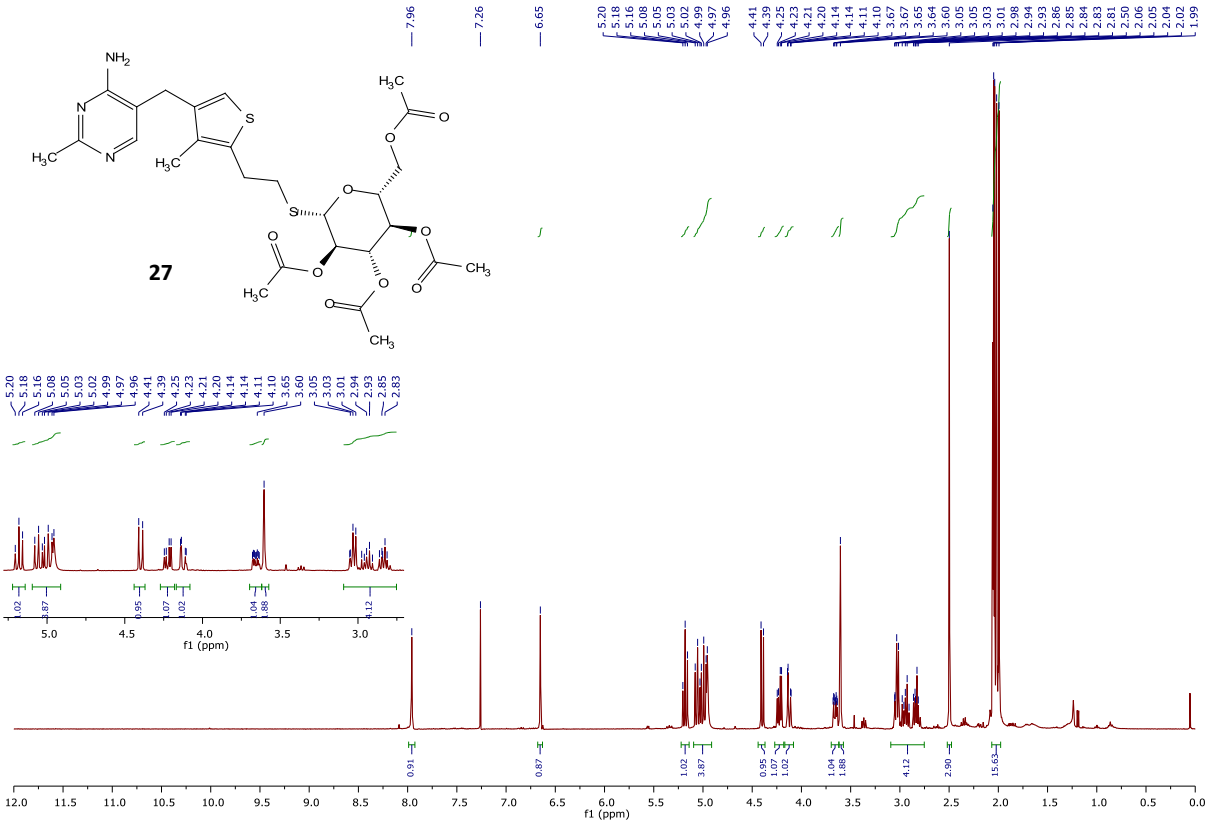

NH2
CH3
NH
N
H3C
N
S
21
f1 (ppm)

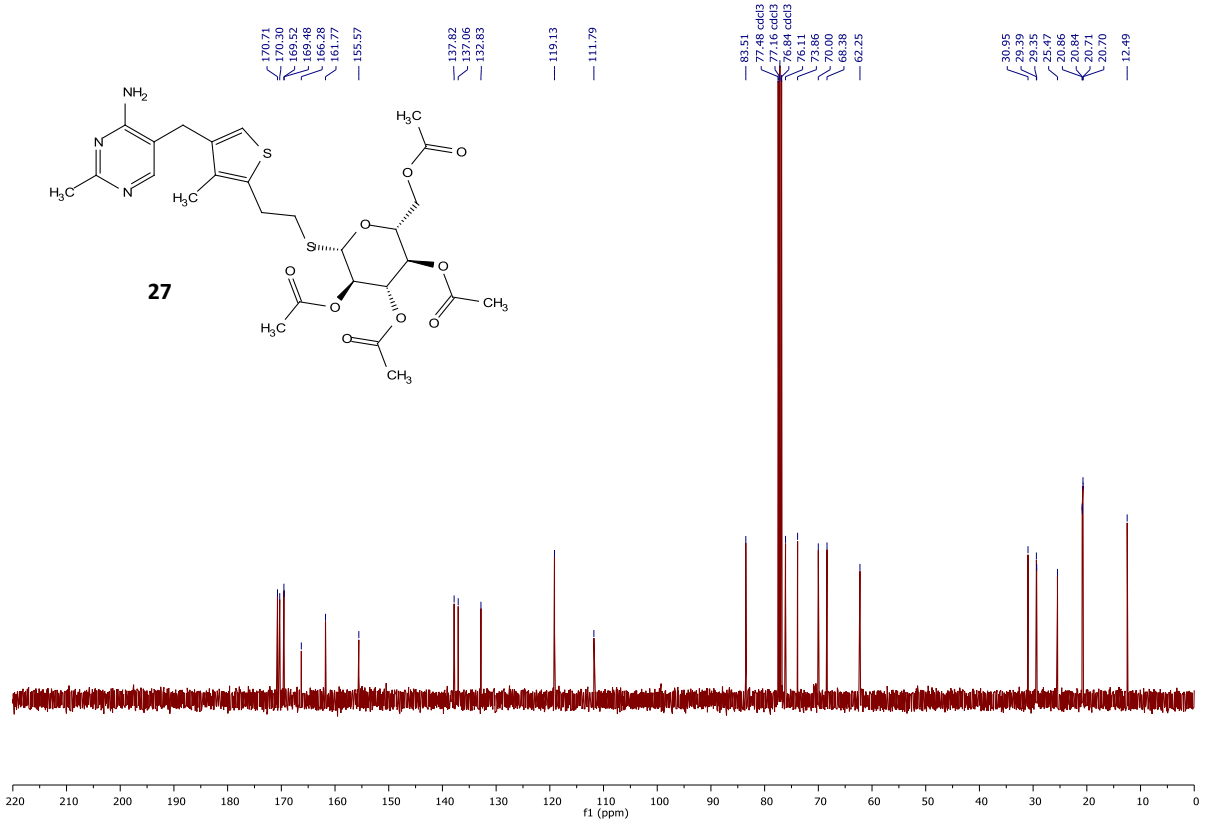

NH2
CH3
NH
N
H3C
N
S
22
7.57
6.88
4.86
3.89
1.00
0.93
2.02
2.25
3.09
1.98
2.99
3.10
6.39
6.10
f1 (ppm)

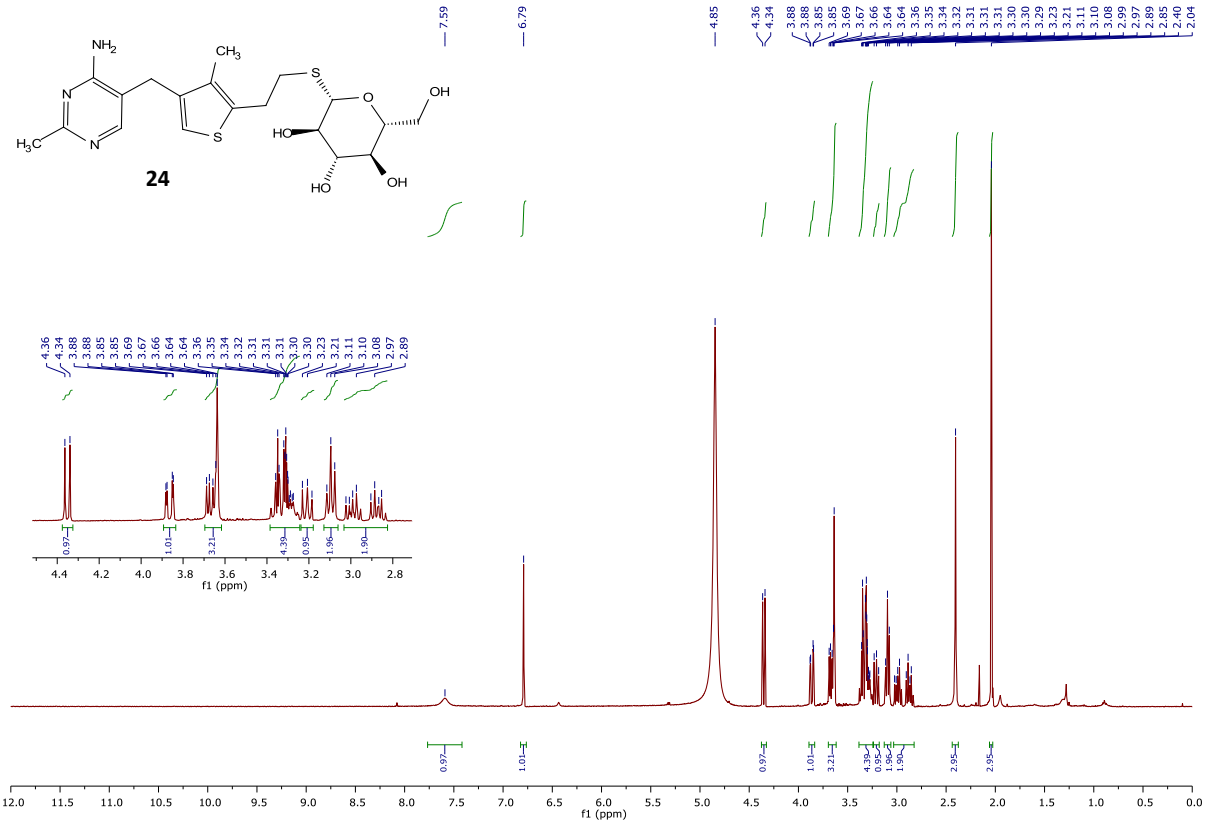

NH2
CH3
NH
N
H3C
N
S
22
166.23
163.66
154.36
139.39
138.45
134.44
121.13
114.16
62.64
49.64
49.43
49.21
49.00
48.79
48.57
48.36
47.96
41.91
38.25
34.36
29.93
28.74
24.72
12.34
f1 (ppm)

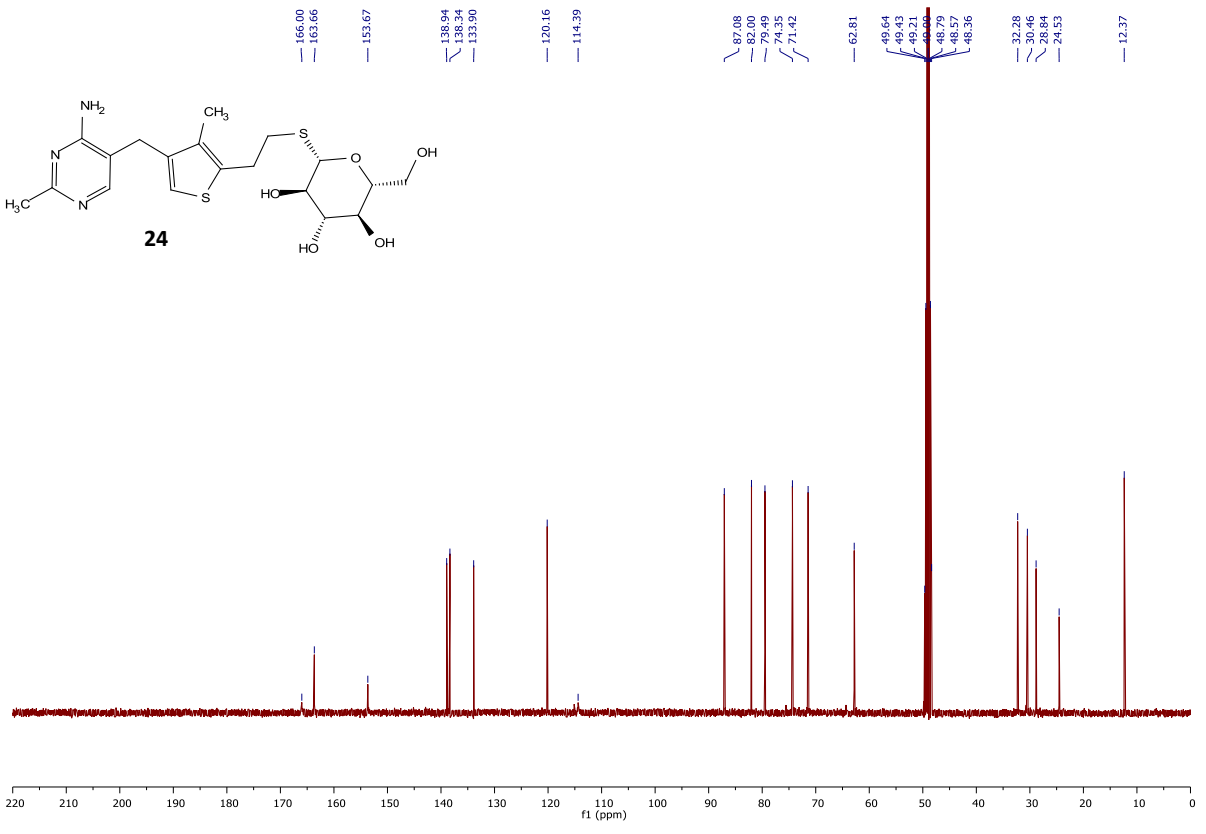

23
f1 (ppm)


23
f1 (ppm)


27
f1 (ppm)


27
f1 (ppm)


24
7.59
6.79
4.85
4.36
4.34
3.88
3.85
3.69
3.67
3.66
3.64
3.36
3.35
3.34
3.32
3.31
3.30
3.23
3.21
3.11
3.10
3.08
2.99
2.97
2.89
2.85
2.40
2.04
f1 (ppm)


24
166.00
163.66
153.67
138.94
138.34
133.90
120.16
114.39
87.08
82.00
79.49
74.35
71.42
62.81
49.64
49.43
49.21
48.79
48.57
48.36
32.28
30.46
28.84
24.53
12.37
f1 (ppm)