Supporting information

Synthesis of acyclic analogues of adenosine sulfonamides and their activity against RNA cap guanine *N*7-Methyltransferase of SARS-CoV-2

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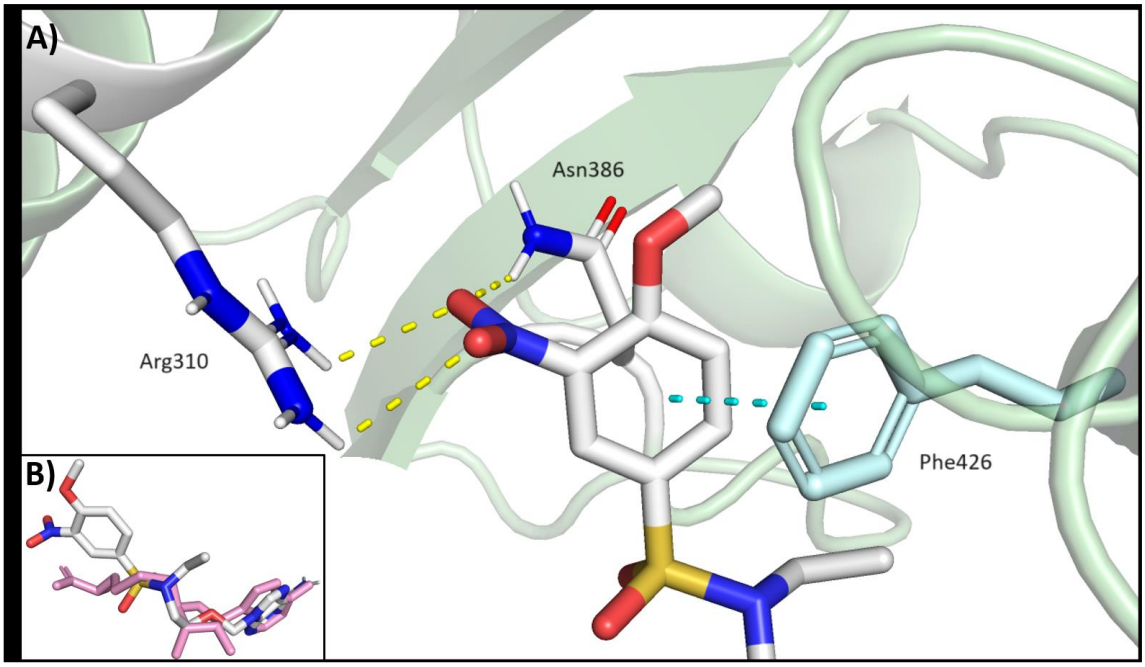
Modeling docking of acyclic nucleoside 15 within the cap-binding pocket of SARS-CoV-2 nsp14



Modeling of the docking of acyclic nucleoside **15** within SARS-CoV-2 nsp14 (PDB ID: 7R2V, resolution 2.53 Å).
A) the arylsulfonamide part of the compound is positioned in the cap-binding pocket of nsp14 and the π–π stacking interaction with Phe426 is shown in cyan (4.6 Å). The hydrogen bonds formed between the *ortho*-nitro, *para*-methoxy substituents of the phenyl ring with Arg310 (2.5 Å, 2.6 Å) and Asn386 (2.3 Å) are shown in yellow.
B) Superposition with **15** and co-crystallized SAH structure (in pink) is shown in bottom left.

Detailed synthetic procedures and spectral characterization data for compounds 1-19

General information

All dry solvents and reagents were purchased from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) analyses were carried out on silica plate 60 F_{254}. Purifications by column chromatography were performed using Biotage Isolera 1 system or Buchi C-815 system with Flash-Pure cartridges (Buchi). NMR experiments were recorded on Bruker 600 MHz spectrometers at 20°C. HRMS analyses were obtained with electrospray ionization (ESI) in positive mode on a Q-TOF Micromass spectrometer. Analytical HPLC was performed on a UHPLC Thermoscientific Ultimate 3000 system equipped with a LPG-3400RS pump, a DAD 3000 detector and an WPS-3000TBRS Autosampler, Column Oven TCC-3000SD. Final compounds **1-19** were stored at -20 °C for several months without any degradation.

General method A for the synthesis of compounds 1 – 13

To a solution at 0 °C under argon of **20** (50 mg, 0.24 mmol, 1.00 eq) or **24** (200 mg, 0.96 mmol, 1.00 eq) in anhydrous DMF (0.1 M) were successively added Et_3N (2.00 eq) and the corresponding arylsulfonyl chloride reactant (1.25 eq) in three portions. After stirring at r.t for 3 hours, the reaction mixture was diluted with AcOEt and H_2O. The aqueous layer was extracted with AcOEt and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, linear gradient 0–6% MeOH in DCM). The fractions were collected, concentrated under vacuum and the resulting solid was resuspended in Et_2O and filtered to give the desired compound as a colorless solid.

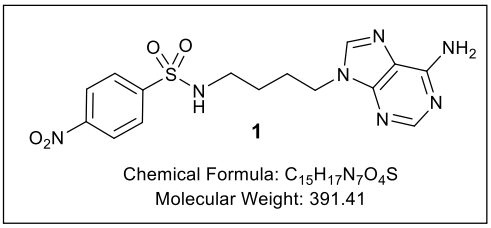
General method B for the synthesis of compounds 14 – 17

A suspension of appropriate *N*-arylsulfonamide-containing acyclic nucleoside **10** – **13** (1.00 eq), ethyl p-toluenesulfonate (1.00 eq), KI (0.10 eq) and K_2CO_3 (3.00 eq) in anhydrous DMF was stirred under argon at 50 °C for 16 hours. After cooling to room temperature, the reaction mixture was diluted with AcOEt and H_2O. The aqueous layer was extracted with AcOEt and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, linear gradient 0–6% MeOH in DCM). The fractions were collected, concentrated under vacuum and the resulting solid was resuspended in Et_2O and filtered to give the desired compound as a colorless solid.

General method C for the synthesis of compounds 18 and 19

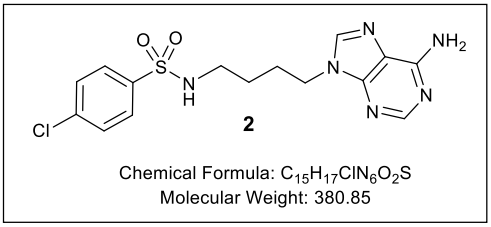
To a solution at room temperature under argon of intermediate **24** (1.0 eq) in 1,4-dioxane (C = 0.02 M) were successively added the suitable propargyl reagent (1.4 eq) and a freshly pre-mixed solution of $CuSO_4$ (0.4 eq, C = 0.06 M) and sodium ascorbate (1.0 eq, C = 0.2 M) in water. After stirring at room temperature for 2 hours, solvents were removed under reduced pressure and the residue was co-evaporated twice with acetonitrile. Then, the residue was re-suspended in a mixture of dichloromethane and methanol (1/1, v : v) and filtered to remove the salts. Silica was added and solvents were removed under vacuum. The residue was purified by flash column chromatography (dry sample, silica gel, linear gradient 0–5% MeOH in DCM). The fractions were collected, concentrated under vacuum and the resulting solid was resuspended in Et_2O and filtered to give the desired compound as a colorless solid.

Synthesis of compounds 1 – 19



Chemical Formula: $C_{15}H_{17}N_7O_4S$
Molecular Weight: 391.41

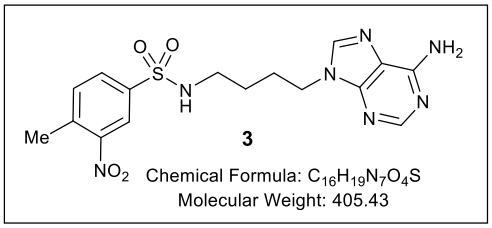
*N-[4-(6-amino-9H-purin-9-yl)butyl]-4-nitrobenzene-1-sulfonamide (**1**).* Following **method A** with 4-nitrobenzenesulfonyl chloride, **1** was obtained as a white solid (74 mg, 78%). Rf 0.48 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.44 – 8.36 (m, 2H, 2 H_{Ar}) ; 8.09 (d, J = 13.1 Hz, 2H, 2 H_{Ar}) ; 8.10 (s, 1H, H_8) ; 8.08 (s, 1H, H_2) ; 8.04 – 7.96 (m, 3H, 2 H_{Ar}, NH) ; 7.17 (s, 2H, NH_2) ; 4.08 (t, J = 6.9 Hz, 2H, CH_2) ; 2.82 (t, J = 7.0 Hz, 2H, CH_2) ; 1.82 – 1.70 (m, 2H, CH_2) ; 1.33 (dq, J = 10.0 Hz, 7.1 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 155.9 (C_6) ; 152.3 (C_2) ; 149.5 (C_4, $C_{q\ Ar}$) ; 146.2 ($C_{q\ Ar}$) ; 140.7 (C_8) ; 128.0 (2 CH_{Ar}) ; 124.6 (2 CH_{Ar}) ; 118.7 (C_5) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.3 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{18}N_7O_4S$ $[M+H]^+$: 392.1135, Found 392.1140.



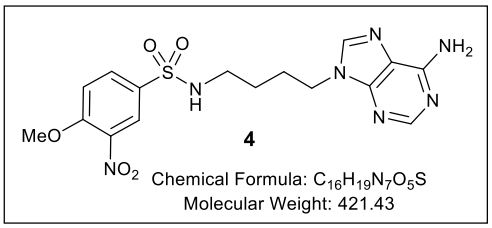
Chemical Formula: $C_{15}H_{17}ClN_6O_2S$
Molecular Weight: 380.85

*N-[4-(6-amino-9H-purin-9-yl)butyl]-4-chlorobenzene-1-sulfonamide (**2**).* Following **method A** with 4-chlorobenzenesulfonyl chloride, **2** was obtained as a white solid (74 mg, 81%). Rf 0.54 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) 8.12 (s, 1H, H_8) ; 8.09 (s, 1H, H_2) ; 7.78 – 7.73 (m, 2H, 2 H_{Ar}) ; 7.70 (br. s, 1H, NH) ; 7.68 – 7.62 (m, 2H, 2 H_{Ar}) ; 7.17 (br. s, 2H, NH_2) ; 4.08 (t, J = 6.9 Hz, 2H, CH_2) ; 2.81 – 2.68 (m, 2H, CH_2) ; 1.83 – 1.70 (m,

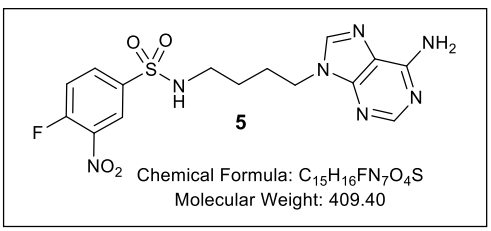
2H, CH_2) ; 1.38 – 1.27 (m, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 155.9 (C_6) ; 152.3 (C_2) ; 149.5 (C_4) ; 140.7 (C_8) ; 139.4 ($C_{q\ Ar}$) ; 137.2 ($C_{q\ Ar}$) ; 129.3 (2 CH_{Ar}) ; 128.4 (2 CH_{Ar}) ; 118.7 (C_5) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.2 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{18}ClN_6O_2S$ $[M+H]^+$: 381.0895, Found 381.0899.



*N-[4-(6-amino-9H-purin-9-yl)butyl]-4-methyl-3-nitrobenzene-1-sulfonamide (**3**).* Following **method A** with 4-methyl-3-nitrobenzenesulfonyl chloride, **3** was obtained as a white solid (77 mg, 78%). Rf 0.54 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.29 (d, J = 2.0 Hz, 1H, H_{Ar}) ; 8.10 (s, 1H, H_8) ; 8.08 (s, 1H, H_2) ; 7.95 (dd, J = 8.1 Hz, 2.0 Hz, 1H, H_{Ar}) ; 7.86 (t, J = 5.7 Hz, 1H, NH) ; 7.70 (d, J = 8.0 Hz, 1H, H_{Ar}) ; 7.17 (br. s, 2H, NH_2) ; 4.08 (t, J = 7.0 Hz, 2H, CH_2) ; 2.80 (t, J = 6.7 Hz, 2H, CH_2) ; 2.58 (s, 3H, CH_3) ; 1.76 (dq, J = 9.8 Hz, 7.0 Hz, 2H, CH_2) ; 1.38 – 1.27 (m, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 155.9 (C_6) ; 152.3 (C_2) ; 149.5 (C_4) ; 148.7 ($C_{q\ Ar}$) ; 140.7 (C_8) ; 139.6 ($C_{q\ Ar}$) ; 137.5 ($C_{q\ Ar}$) ; 134.1 (CH_{Ar}) ; 130.5 (CH_{Ar}) ; 122.5 (CH_{Ar}) ; 118.7 (C_5) ; 117.8 ($C_{q\ Ar}$) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.2 (CH_2) ; 19.8 (CH_3). **HRMS** (ESI+): m/z calc. for $C_{16}H_{20}N_7O_4S$ $[M+H]^+$: 406.1292, Found 406.1291.

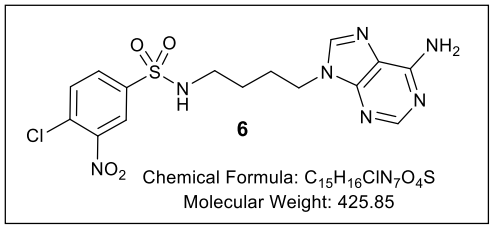


*N-[4-(6-amino-9H-purin-9-yl)butyl]-4-methoxy-3-nitrobenzene-1-sulfonamide (**4**).* Following **method A** with 4-methoxy-3-nitrobenzenesulfonyl chloride, **4** was obtained as a white solid (82 mg, 80%). Rf 0.40 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.23 (d, J = 2.4 Hz, 1H, H_{Ar}) ; 8.11 (s, 1H, H_8) ; 8.09 (s, 1H, H_2) ; 7.99 (dd, J = 9.0 Hz, 2.3 Hz, 1H, H_{Ar}) ; 7.73 (br. s, 1H, NH) ; 7.54 (d, J = 8.9 Hz, 1H, H_{Ar}) ; 7.17 (br. s, 2H, NH_2) ; 4.09 (t, J = 7.0 Hz, 2H, CH_2) ; 4.01 (s, 3H, OMe) ; 2.78 (t, J = 7.0 Hz, 2H, CH_2) ; 1.82 – 1.71 (m, 2H, CH_2) ; 1.38 – 1.27 (m, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 155.9 (C_6) ; 154.7 ($C_{q\ Ar}$) ; 152.3 (C_2) ; 149.5 (C_4) ; 140.7 (C_8) ; 138.5 ($C_{q\ Ar}$) ; 132.5 (CH_{Ar}) ; 132.3 ($C_{q\ Ar}$) ; 123.8 (CH_{Ar}) ; 118.7 (C_5) ; 115.3 ($C_{q\ Ar}$) ; 57.4 (OMe) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.7 (CH_2) ; 26.2 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{16}H_{20}N_7O_5S$ $[M+H]^+$: 422.1241, Found 422.1237.

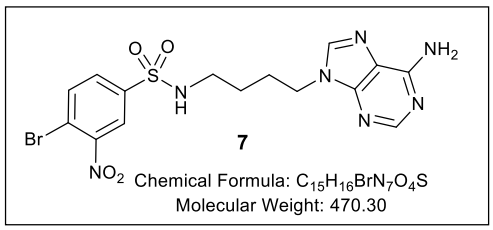


N-[4-(6-amino-9H-purin-9-yl)butyl]-4-fluoro-3-nitrobenzene-1-sulfonamide (**5**). Following **method A** with 4-fluoro-3-nitrobenzenesulfonyl chloride, **5** was obtained as a white solid (55 mg, 56%). Rf 0.51 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.45 (dd, J = 6.9 Hz, 2.4 Hz, 1H, H_{Ar}) ; 8.14 (ddd, J = 8.8 Hz, 3.9 Hz, 2.4 Hz, 1H, H_{Ar}) ; 8.10 (s, 1H, H_8) ; 8.08 (s, 1H, H_2) ; 7.95 (t, J = 5.7 Hz, 1H, NH) ; 7.80 (dd, J = 10.9 Hz, 8.7 Hz, 1H, H_{Ar}) ; 7.17 (br. s, 2H, NH_2) ; 4.09 (t, J = 6.9 Hz, 2H, CH_2) ; 2.81 (t, J = 6.7 Hz, 2H, CH_2) ; 1.82 – 1.72 (m, 2H, CH_2) ; 1.33 (dq, J = 9.7

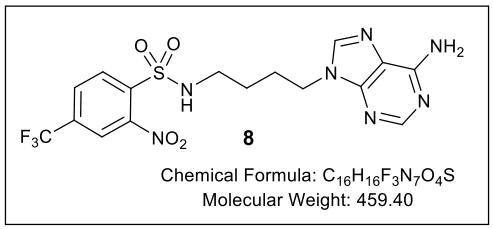
Hz, 7.1 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 157.5, 155.7 (C-F) ; 155.9 (C_6) ; 152.3 (C_2) ; 149.5 (C_4) ; 140.7 (C_8) ; 137.5, 137.4 ($C_{q\,Ar}$) ; 136.9, 136.9 ($C_{q\,Ar}$) ; 134.2, 134.1 (CH_{Ar}) ; 124.9 (CH_{Ar}) ; 120.2, 120.0 (CH_{Ar}) ; 118.7 (C_5) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.2 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{17}FN_7O_4S$ $[M+H]^+$: 410.1041, Found 410.1039.



*N-[4-(6-amino-9H-purin-9-yl)butyl]-4-chloro-3-nitrobenzene-1-sulfonamide (**6**).* Following **method A** with 4-chloro-3-nitrobenzenesulfonyl chloride, **6** (61 mg, 63%) was obtained as a white solid. Rf 0.47 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.41 (d, J = 2.1 Hz, 1H, H_{Ar}) ; 8.11 (s, 1H, H_8) ; 8.09 (s, 1H, H_2) ; 8.04 – 7.96 (m, 3H, 2 H_{Ar}, NH) ; 7.17 (br. s, 2H, NH_2) ; 4.10 (t, J = 6.9 Hz, 2H, CH_2) ; 2.83 (t, J = 7.0 Hz, 2H, CH_2) ; 1.85 – 1.72 (m, 2H, CH_2) ; 1.34 (dq, J = 10.0 Hz, 7.1 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 155.9 (C_6) ; 152.3 (C_2) ; 149.5 (C_4) ; 147.4 ($C_{q\,Ar}$) ; 140.7 (C_8) ; 133.1 (CH_{Ar}) ; 131.2 (CH_{Ar}) ; 129.2 ($C_{q\,Ar}$) ; 123.9 ($C_{q\,Ar}$) ; 118.7 (C_5) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.2 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{17}ClN_7O_4S$ $[M+H]^+$: 426.0746, Found 426.0742.

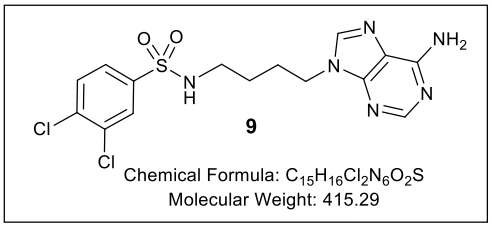


*N-[4-(6-amino-9H-purin-9-yl)butyl]-4-bromo-3-nitrobenzene-1-sulfonamide (**7**).* Following **method A** with 4-bromo-3-nitrobenzenesulfonyl chloride, **7** (71 mg, 62%) was obtained as a white solid. Rf 0.47 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.36 (d, J = 2.1 Hz, 1H, H_{Ar}) ; 8.11 (s, 1H, H_8) ; 8.09 (s, 1H, H_2) ; 8.14 (d, J = 8.5 Hz, 1H, H_{Ar}) ; 7.97 (t, J = 5.8 Hz, 1H, NH) ; 7.91 (dd, J = 8.4 Hz, 2.2 Hz, 1H, H_{Ar}) ; 7.17 (br. s, 2H, NH_2) ; 4.10 (t, J = 6.9 Hz, 2H, CH_2) ; 2.83 (t, J = 6.7 Hz, 2H, CH_2) ; 1.78 (dq, J = 9.7 Hz, 7.1 Hz, 2H, CH_2) ; 1.40 – 1.29 (m, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 155.9 (C_6) ; 152.3 (C_2) ; 149.6 ($C_{q\,Ar}$) ; 149.5 (C_4) ; 141.3 ($C_{q\,Ar}$) ; 140.7 (C_8) ; 136.2 (CH_{Ar}) ; 131.0 (CH_{Ar}) ; 123.5 (CH_{Ar}) ; 118.7 (C_5) ; 117.8 ($C_{q\,Ar}$) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.3 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{17}BrN_7O_4S$ $[M+H]^+$: 470.0240, Found 470.0236.

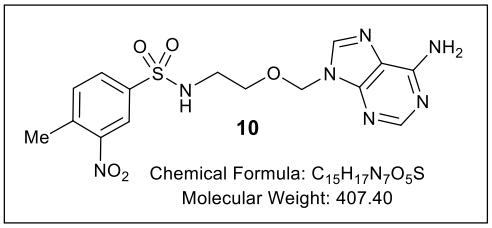


*N-[4-(6-amino-9H-purin-9-yl)butyl]-2-nitro-4-trifluoromethylbenzene-1-sulfonamide (**8**).* Following **method A** with 2-nitro-4-trifluoromethylbenzenesulfonyl chloride, **8** (38 mg, 34%) was obtained as a white solid. Rf 0.47 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.54 (d, J = 1.7 Hz, 1H, H_{Ar}) ; 8.39 (br. s, 1H, NH) ; 8.26 (dd, J = 8.3 Hz, 1.8 Hz, 1H, H_{Ar}) ; 8.17 (d, J = 8.3 Hz, 1H, H_{Ar}) ; 8.11 (s, 1H, H_8) ; 8.09 (s, 1H, H_2) ; 7.18 (br. s, 2H, NH_2) ; 4.10 (t, J = 6.9 Hz, 2H, CH_2) ; 2.95 (t, J = 7.0 Hz, 2H, CH_2) ; 1.79 (dq, J = 9.7 Hz, 7.1 Hz, 2H, CH_2) ; 1.39 (dq, J = 10.0 Hz,

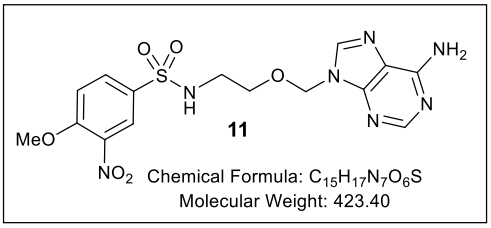
7.2 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-*d*$_6$) δ 155.9 (C_6) ; 152.3 (C_2) ; 149.5 (C_4) ; 147.8 ($C_{q\,Ar}$) ; 140.7 (C_8) ; 136.4 ($C_{q\,Ar}$) ; 133.7, 133.5, 133.2, 133.0 (*C*-CF_3) ; 130.8 (CH $_{Ar}$) ; 129.6, 129.6 (CH $_{Ar}$) ; 125.1, 123.2, 121.4, 119.6 (CF_3) ; 122.0 (CH $_{Ar}$) ; 118.7 (C_5) ; 57.4 (OMe) ; 42.3 (CH_2) ; 42.2 (CH_2) ; 26.6 (CH_2) ; 26.3 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{16}H_{17}F_3N_7O_4S$ $[M+H]^+$: 460.1009, Found 460.1006.



*N-[4-(6-amino-9H-purin-9-yl)butyl]-3,4-dichlorobenzene-1-sulfonamide (**9**).* Following **method A** with 3,4-dichlorobenzenesulfonyl chloride, **9** (70 mg, 70%) was obtained as a white solid. Rf 0.59 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-*d*$_6$) 8.11 (s, 1H, H_8) ; 8.09 (s, 1H, H_2) ; 7.94 (d, J = 2.2 Hz, 1H, H_{Ar}) ; 7.86 (d, J = 8.4 Hz, 1H, H_{Ar}) ; 7.82 (t, J = 5.9 Hz, 1H, NH) ; 7.71 (dd, J = 8.4, 2.2 Hz, 1H, H_{Ar}) ; 7.17 (br. s, 2H, NH_2) ; 4.09 (t, J = 7.0 Hz, 2H, CH_2) ; 2.79 (q, J = 6.7 Hz, 2H, CH_2) ; 1.83 – 1.72 (m, 2H, CH_2) ; 1.38 – 1.29 (m, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-*d*$_6$) δ 156.0 (C_6) ; 152.3 (C_2) ; 149.5 (C_4, $C_{q\,Ar}$) ; 140.9 ($C_{q\,Ar}$) ; 140.7 (C_8) ; 135.4 ($C_{q\,Ar}$) ; 132.1 ($C_{q\,Ar}$) ; 131.7 (CH $_{Ar}$) ; 128.2 (CH $_{Ar}$) ; 126.6 (CH $_{Ar}$) ; 118.7 (C_5) ; 42.3 (CH_2) ; 42.0 (CH_2) ; 26.6 (CH_2) ; 26.2 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{17}Cl_2N_6O_2S$ $[M+H]^+$: 415.0505, Found 415.0504.

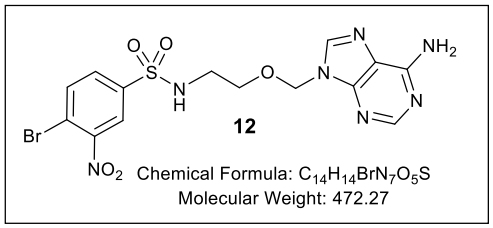


*N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-4-methyl-3-nitrobenzene-1-sulfonamide (**10**).* Following **method A** with 4-methyl-3-nitrobenzenesulfonyl chloride, **10** (289 mg, 74%) was obtained as a white solid. Rf 0.47 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-*d*$_6$) δ 8.31 (d, J = 2.0 Hz, 1H, H_{Ar}) ; 8.20 (s, 1H, H_8) ; 8.14 (s, 1H, H_2) ; 8.04 (br. s, 1H, NH) ; 7.94 (dd, J = 8.0 Hz, 2.0 Hz, 1H, H_{Ar}) ; 7.68 (dd, J = 8.0 Hz, 0.9 Hz, 1H, H_{Ar}) ; 7.28 (br. s, 2H, N*H*$_2$) ; 5.45 (s, 2H, CH_2) ; 3.46 (t, J = 5.6 Hz, 2H, CH_2) ; 2.96 (t, J = 5.5 Hz, 2H, CH_2) ; 2.59 (s, 3H, CH_3). **^{13}C-NMR** (150 MHz, DMSO-*d*$_6$) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 (C_4) ; 148.6 ($C_{q\,Ar}$) ; 141.0 (C_8) ; 139.7 ($C_{q\,Ar}$) ; 137.5 ($C_{q\,Ar}$) ; 134.0 (CH $_{Ar}$) ; 130.5 (CH $_{Ar}$) ; 122.6 (CH $_{Ar}$) ; 118.4 (C_5) ; 71.9 (CH_2) ; 67.5 (CH_2) ; 42.1 (CH_2) ; 19.7 (CH_3). **HRMS** (ESI+): m/z calc. for $C_{15}H_{18}N_7O_5S$ $[M+H]^+$: 408. 1085, Found 408.1086.

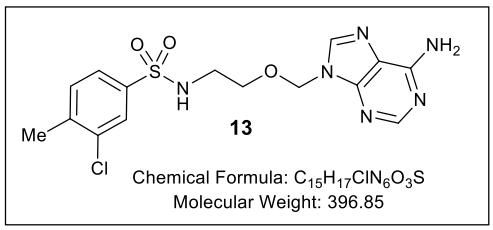


*N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-4-methoxy-3-nitrobenzene-1-sulfonamide (**11**).* Following **method A** with 4-methoxy-3-nitrobenzenesulfonyl chloride, **11** (280 mg, 69%) was obtained as a white solid. Rf 0.40 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-*d*$_6$) δ 8.24 (d, J = 2.3 Hz, 1H, H_{Ar}) ; 8.21 (s, 1H, H_8) ; 8.14 (s, 1H, H_2) ; 7.99 (dd, J = 8.9 Hz, 2.4 Hz, 1H, H_{Ar}) ; 7.90 (br. s, 1H, NH) ; 7.52 (d, J = 9.0 Hz, 1H, H_{Ar}) ; 7.28 (br. s, 2H, NH_2) ; 5.47 (s,

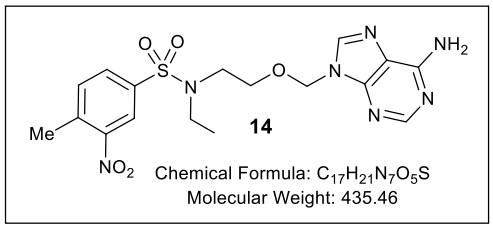
2H, CH_2) ; 4.01 (s, 3H, OMe) ; 3.47 (t, J = 5.6 Hz, 2H, CH_2) ; 2.96 – 2.90 (m, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 154.6 ($C_{q\,Ar}$) ; 152.9 (C_2) ; 149.5 (C_4) ; 141.1 (C_8) ; 138.5 ($C_{q\,Ar}$) ; 132.5 (CH_{Ar}) ; 132.3 ($C_{q\,Ar}$) ; 132.8 (CH_{Ar}) ; 118.4 (C_5) ; 115.2 (CH_{Ar}) ; 71.9 (CH_2) ; 67.6 (CH_2) ; 57.4 (OMe) ; 42.1 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{15}H_{18}N_7O_6S$ $[M+H]^+$: 424.1034, Found 424.1033.



*N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-4-bromo-3-nitrobenzene-1-sulfonamide (**12**).* Following **method A** with 4-bromo-3-nitrobenzenesulfonyl chloride, **12** (277 mg, 61%) was obtained as a white solid. Rf 0.47 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.37 (d, J = 2.1 Hz, 1H, H_{Ar}) ; 8.21 (s, 1H, H_8) ; 8.14 (m, 2H, H_2, H_{Ar}) ; 7.91 (dd, J = 8.4 Hz, 2.2 Hz, 1H, H_{Ar}) ; 7.90 (br. s, 1H, NH) ; 7.28 (br. s, 2H, NH_2) ; 5.46 (s, 2H, CH_2) ; 3.47 (t, J = 5.5 Hz, 2H, CH_2) ; 3.00 (q, J = 5.5 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 ($C_{q\,Ar}$) ; 149.5 (C_4) ; 141.4 ($C_{q\,Ar}$) ; 141.1 (C_8) ; 136.1 (CH_{Ar}) ; 131.0 (CH_{Ar}) ; 123.6 (CH_{Ar}) ; 118.4 (C_5) ; 117.8 ($C_{q\,Ar}$) ; 71.9 (CH_2) ; 67.6 (CH_2) ; 42.2 (CH_2). **HRMS** (ESI+): m/z calc. for $C_{14}H_{15}BrN_7O_5S$ $[M+H]^+$: 472. 0033, Found 472.0033.

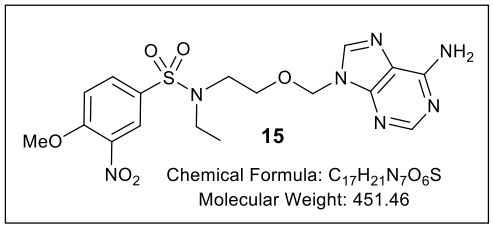


*N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-3-chloro-4-methylbenzene-1-sulfonamide (**13**).* Following **method A** with *3-chloro-4-methyl*benzenesulfonyl chloride, **13** (274 mg, 72%) was obtained as a white solid. Rf 0.52 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.21 (s, 1H, H_8) ; 8.15 (s, 1H, H_2) ; 7.84 (br. s, 1H, NH) ; 7.74 (d, J = 1.8 Hz, 1H, H_{Ar}) ; 7.59 (dd, J = 7.9 Hz, 1.9 Hz, 1H, H_{Ar}) ; 7.52 (dd, J = 7.9 Hz, 0.9 Hz, 1H, H_{Ar}) ; 7.29 (br. s, 2H, NH_2) ; 5.47 (s, 2H, CH_2) ; 3.46 (t, J = 5.6 Hz, 2H, CH_2) ; 2.91 (t, J = 5.6 Hz, 2H, CH_2) ; 2.39 (s, 3H, CH_3). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 (C_4) ; 141.0 (C_8) ; 140.5 ($C_{q\,Ar}$) ; 139.8 ($C_{q\,Ar}$) ; 133.8 ($C_{q\,Ar}$) ; 131.9 (CH_{Ar}) ; 126.5 (CH_{Ar}) ; 125.1 (CH_{Ar}) ; 118.4 (C_5) ; 71.9 (CH_2) ; 67.6 (CH_2) ; 42.1 (CH_2) ; 19.7 (CH_3). **HRMS** (ESI+): m/z calc. for $C_{15}H_{18}ClN_6O_3S$ $[M+H]^+$: 398. 0844, Found 397.0847.

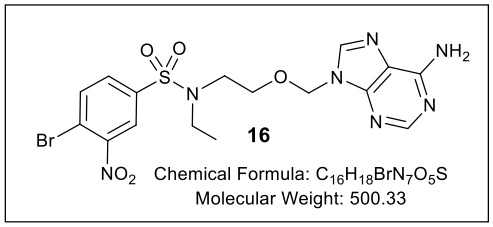


*N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-4-methyl-3-nitro-N-ethylbenzene-1-sulfonamide (**14**).* Following **method B** with **10**, **14** (115 mg, 54%) was obtained as a white solid. Rf 0.52 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.27 (d, J = 2.0 Hz, 1H, H_{Ar}) ; 8.21 (s, 1H, H_8) ; 8.15 (s, 1H, H_2) ; 7.96 (dd, J = 8.1 Hz, 2.0 Hz, 1H, H_{Ar}) ; 7.66 (d, J = 8.3 Hz, 1H, H_{Ar}) ; 7.29 (br. s, 2H, NH_2) ; 5.49 (s, 2H, CH_2) ; 3.61 (t, J = 5.6 Hz, 2H, CH_2) ; 3.31 (m, 2H, CH_2) ; 3.16 (q, J = 7.1 Hz, 2H, CH_2-CH_3) ; 2.58 (s, 3H, CH_3) ; 0.97 (t, J = 7.1 Hz, 3H, CH_3-CH_2). **^{13}C-NMR** (150 MHz,

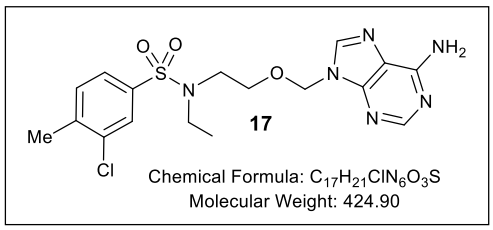
DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 (C_4) ; 148.8 ($C_{q\,Ar}$) ; 141.0 (C_8) ; 138.5 ($C_{q\,Ar}$) ; 137.6 ($C_{q\,Ar}$) ; 134.1 (CH_{Ar}) ; 130.7 (CH_{Ar}) ; 122.8 (CH_{Ar}) ; 118.5 (C_5) ; 118.0 ($C_{q\,Ar}$) ; 71.9 (CH_2) ; 67.2 (CH_2) ; 46.4 ($CH_{2\,Et}$) ; 43.0 (CH_2) ; 19.5 (CH_3) ; 13.9 ($CH_{3\,Et}$). **HRMS** (ESI+): m/z calc. for $C_{17}H_{22}N_7O_5S$ $[M+H]^+$: 436. 1398, Found 436.1400.



N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-4-methoxy-3-nitro-N-ethylbenzene-1-sulfonamide ***(15)****.* Following **method B** with **11**, **15** (113 mg, 53%) was obtained as a white solid. Rf 0.52 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.26 (d, J = 2.4 Hz, 1H, H_{Ar}) ; 8.23 (s, 1H, H_8) ; 8.16 (s, 1H, H_2) ; 8.00 (dd, J = 8.8 Hz, 2.4 Hz, 1H, H_{Ar}) ; 7.48 (d, J = 9.0 Hz, 1H, H_{Ar}) ; 7.29 (br. s, 2H, NH_2) ; 5.51 (s, 2H, CH_2) ; 4.01 (s, 3H, OMe) ; 3.61 (t, J = 5.7 Hz, 2H, CH_2) ; 3.28 (t, J = 5.5 Hz, 2H, CH_2) ; 3.14 (q, J = 7.1 Hz, 2H, CH_2-CH_3) ; 0.96 (t, J = 7.1 Hz, 3H, CH_3-CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 154.6 ($C_{q\,Ar}$) ; 152.9 (C_2) ; 149.7 (C_4) ; 141.1 (C_8) ; 138.8 ($C_{q\,Ar}$) ; 132.6 (CH_{Ar}) ; 131.2 ($C_{q\,Ar}$) ; 124.0 (CH_{Ar}) ; 118.5 (C_5) ; 115.2 (CH_{Ar}) ; 71.9 (CH_2) ; 67.3 (CH_2) ; 57.4 (OMe) ; 46.4 ($CH_{2\,Et}$) ; 43.1 (CH_2) ; 13.9 ($CH_{3\,Et}$). **HRMS** (ESI+): m/z calc. for $C_{17}H_{22}N_7O_6S$ $[M+H]^+$: 452. 1347, Found 452.1346.

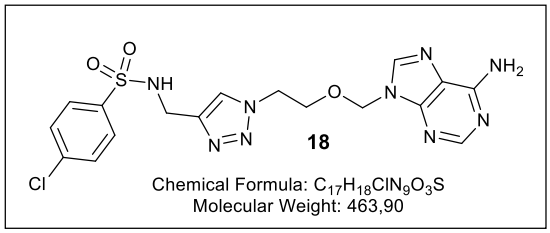


N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-4-bromo-3-nitro-N-ethylbenzene-1-sulfonamide ***(16)****.* Following **method B** with **12**, **16** (100 mg, 47%) was obtained as a white solid. Rf 0.50 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.42 (d, J = 2.2 Hz, 1H, H_{Ar}) ; 8.23 (s, 1H, H_8) ; 8.16 (s, 1H, H_2) ; 8.10 (d, J = 8.5 Hz, 1H, H_{Ar}) ; 7.94 (dd, J = 8.4 Hz, 2.3 Hz, 1H, H_{Ar}) ; 7.29 (br. s, 2H, NH_2) ; 5.50 (s, 2H, CH_2) ; 3.61 (t, J = 5.6 Hz, 2H, CH_2) ; 3.31 (m, 2H, CH_2) ; 3.17 (q, J = 7.1 Hz, 2H, CH_2-CH_3) ; 2.91 (t, J = 5.6 Hz, 2H, CH_2) ; 0.97 (t, J = 7.1 Hz, 3H, CH_3-CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.9 ($C_{q\,Ar}$) ; 149.7 (C_4) ; 141.0 (C_8) ; 140.2 ($C_{q\,Ar}$) ; 136.0 (CH_{Ar}) ; 131.2 (CH_{Ar}) ; 123.8 (CH_{Ar}) ; 118.5 (C_5) ; 118.0 ($C_{q\,Ar}$) ; 71.9 (CH_2) ; 67.1 (CH_2) ; 46.6 ($CH_{2\,Et}$) ; 43.1 (CH_2) ; 13.9 ($CH_{3\,Et}$). **HRMS** (ESI+): m/z calc. for $C_{16}H_{19}BrN_7O_5S$ $[M+H]^+$: 500. 0346, Found 500.0345.

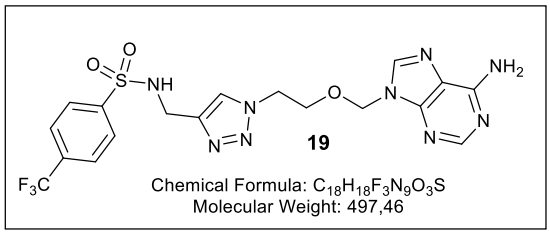


N-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-3-chloro-4-methyl-N-ethylbenzene-1-sulfonamide ***(17)****.* Following **method B** with **13**, **17** (107 mg, 50%) was obtained as a white solid. Rf 0.58 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.23 (s, 1H, H_8) ; 8.16 (s, 1H, H_2) ; 7.75 (d, J = 1.8 Hz, 1H, H_{Ar}) ; 7.60 (dd, J = 8.0 Hz, 2.0 Hz, 1H,

H_{Ar}) ; 7.52 (dd, J = 7.9 Hz, 0.8 Hz, 1H, H_{Ar}) ; 7.30 (br. s, 2H, NH_2) ; 5.52 (s, 2H, CH_2) ; 3.60 (t, J = 5.8 Hz, 2H, CH_2) ; 3.27 (t, J = 5.8 Hz, 2H, CH_2) ; 3.13 (q, J = 7.1 Hz, 2H, CH_2-CH_3) ; 2.39 (s, 3H, CH_3) ; 0.95 (t, J = 7.1 Hz, 3H, CH_3-CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 (C_4) ; 141.0 (C_8) ; 140.9 ($C_{q\ Ar}$) ; 138.7 ($C_{q\ Ar}$) ; 134.0 ($C_{q\ Ar}$) ; 132.1 (CH $_{Ar}$) ; 126.8 (CH $_{Ar}$) ; 125.4 (CH $_{Ar}$) ; 118.5 (C_5) ; 71.9 (CH_2) ; 67.4 (CH_2) ; 46.5 ($CH_{2\ Et}$) ; 43.2 (CH_2) ; 19.7 (CH_3) ; 13.9 ($CH_{3\ Et}$). **HRMS** (ESI+): m/z calc. for $C_{17}H_{22}ClN_6O_3S$ $[M+H]^+$: 425.1157, Found 425.1159.



N-[(1-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-1H-1,2,3-triazol-4-yl)methyl]-4-chlorobenzene-1-sulfonamide (**18**). Following **method C** with **23** (50 mg), **18** (73 mg, 74%) was obtained as a white solid. Rf 0.34 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.28 – 8.14 (m, 3H, H_2, H_8, NH) ; 7.79 (s, 1H, $H_{Triazole}$) ; 7.78 – 7.73 (m, 2H, 2H $_{Ar}$) ; 7.66 – 7.60 (m, 2H, 2H $_{Ar}$) ; 7.75 (d, J = 1.8 Hz, 1H, H_{Ar}) ; 7.60 (dd, J = 8.0 Hz, 2.0 Hz, 1H, H_{Ar}) ; 7.52 (dd, J = 7.9 Hz, 0.8 Hz, 1H, H_{Ar}) ; 7.29 (br. s, 2H, NH_2) ; 5.53 (s, 2H, CH_2) ; 4.44 (t, J = 5.3 Hz, 2H, CH_2) ; 4.03 (d, J = 6.0 Hz, 2H, N-CH_2) ; 3.86 (t, J = 5.8 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 (C_4) ; 142.9 ($C_{q\ Triazole}$) ; 141.0 (C_8) ; 139.3 ($C_{q\ Ar}$) ; 137.2 ($C_{q\ Ar}$) ; 129.2 (CH $_{Ar}$) ; 128.5 (CH $_{Ar}$) ; 123.6 (CH $_{Triazole}$) ; 118.5 (C_5) ; 71.8 (CH_2) ; 67.2 (CH_2) ; 49.0 (CH_2) ; 38.0 (N-CH_2) ; 13.9 ($CH_{3\ Et}$). **HRMS** (ESI+): m/z calc. for $C_{17}H_{19}ClN_9O_3S$ $[M+H]^+$: 464.1015, Found 464.1016.



N-[(1-{2-[(6-amino-9H-purin-9-yl)methoxy]ethyl}-1H-1,2,3-triazol-4-yl)methyl]-4-trifluoromethylbenzene-1-sulfonamide (**19**). Following **method C** with **23** (50 mg), **19** (73 mg, 69%) was obtained as a white solid. Rf 0.34 (98:2 DCM/MeOH). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ 8.41 (t, J = 6.0 Hz, 1H, NH) ; 8.21 (s, 1H, H_8) ; 8.17 (s, 1H, H_2) ; 7.99 – 7.91 (m, 4H, 4H $_{Ar}$) ; 7.79 (s, 1H, $H_{Triazole}$) ; 7.29 (br. s, 2H, NH_2) ; 5.52 (s, 2H, CH_2) ; 4.42 (t, J = 5.2 Hz, 2H, CH_2) ; 4.07 (d, J = 5.8 Hz, 2H, N-CH_2) ; 3.86 (t, J = 5.2 Hz, 2H, CH_2). **^{13}C-NMR** (150 MHz, DMSO-d_6) δ 156.0 (C_6) ; 152.9 (C_2) ; 149.7 (C_4) ; 144.4 ($C_{q\ Ar}$) ; 142.9 ($C_{q\ Triazole}$) ; 141.0 (C_8) ; 132.4, 132.1, 131.9, 131.7 (*C*-CF_3) ; 127.5 (CH $_{Ar}$) ; 126.3, 126.3 (CH $_{Ar}$) ; 126.2, 124.4, 122.6, 120.8 (CF_3) ; 123.6 (CH $_{Triazole}$) ; 118.5 (C_5) ; 71.8 (CH_2) ; 67.2 (CH_2) ; 49.0 (CH_2) ; 38.0 (N-CH_2). **HRMS** (ESI+): m/z calc. for $C_{18}H_{19}F_3N_9O_3S$ $[M+H]^+$: 498.1278, Found 498.1273.

HPLC analysis of final compounds 1-19

HPLC analysis conditions:

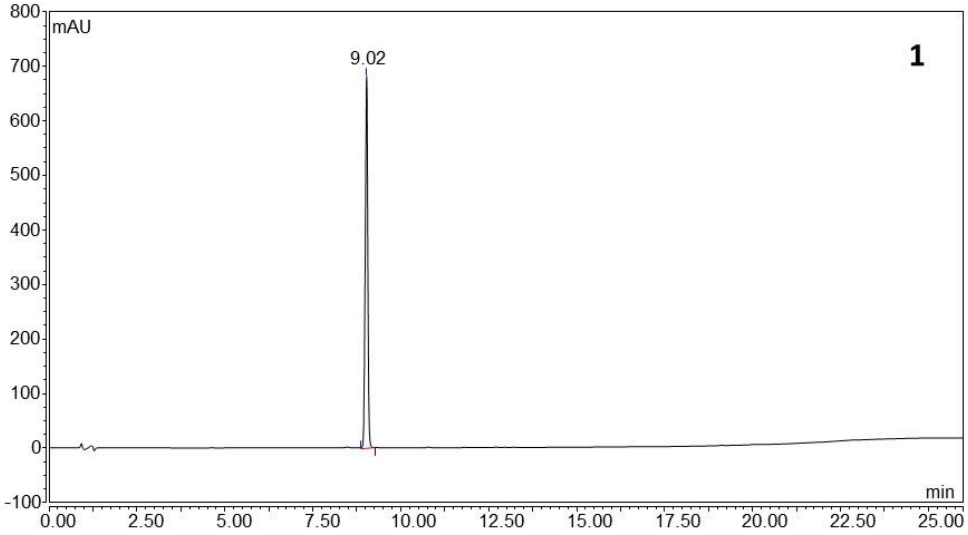
Column: HPLC Column Nucleodur 75/4.6 100-3 C_{18} EC (Macherey Nagel)

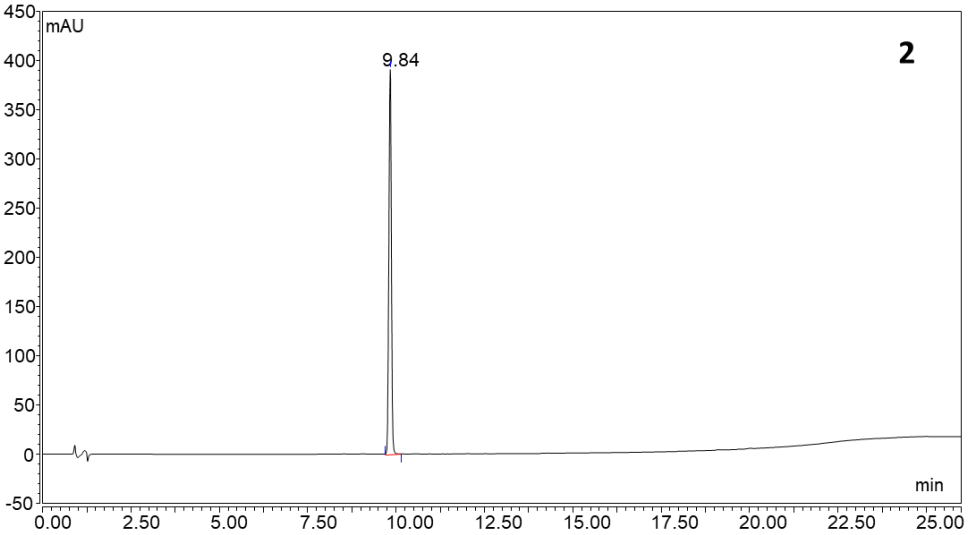
Temperature: 30 °C

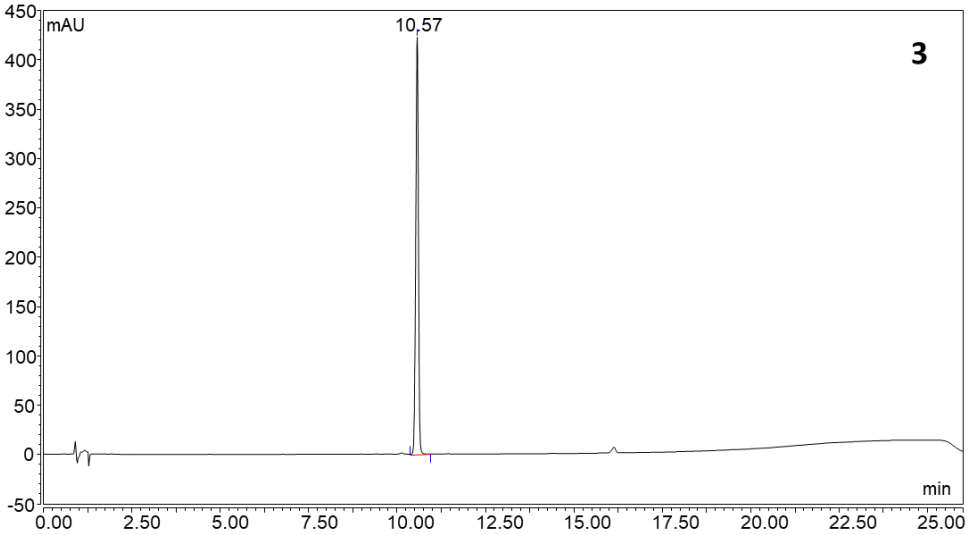
Mobile phase: eluent A: 1% ACN in 12.5 mM TEAAc buffer, pH 7; eluent B: 80% ACN in 12.5 mM TEAAc buffer, pH 7

Gradient: 10% to 100% of eluent B during 20 min. Flow rate: 1.0 mL/min

UV detection at wavelength 260 nm



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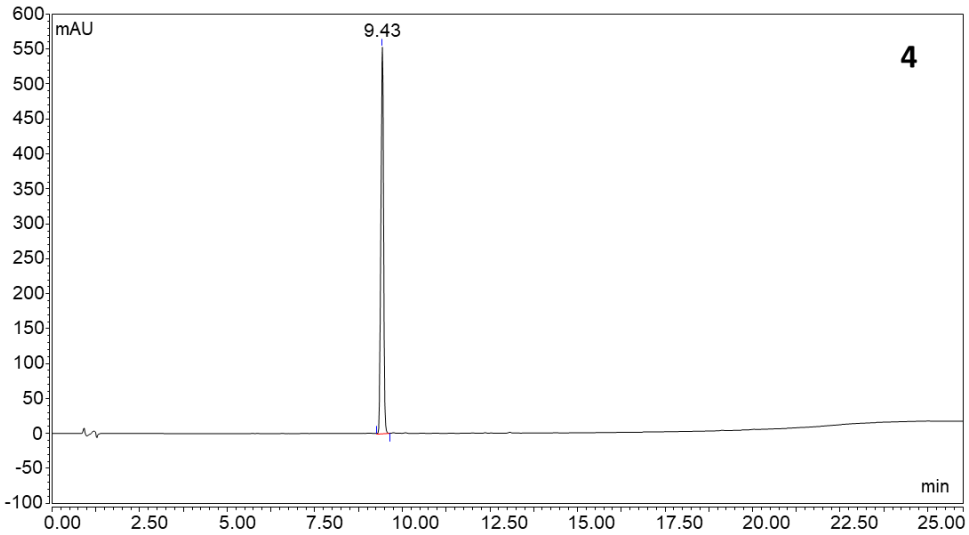
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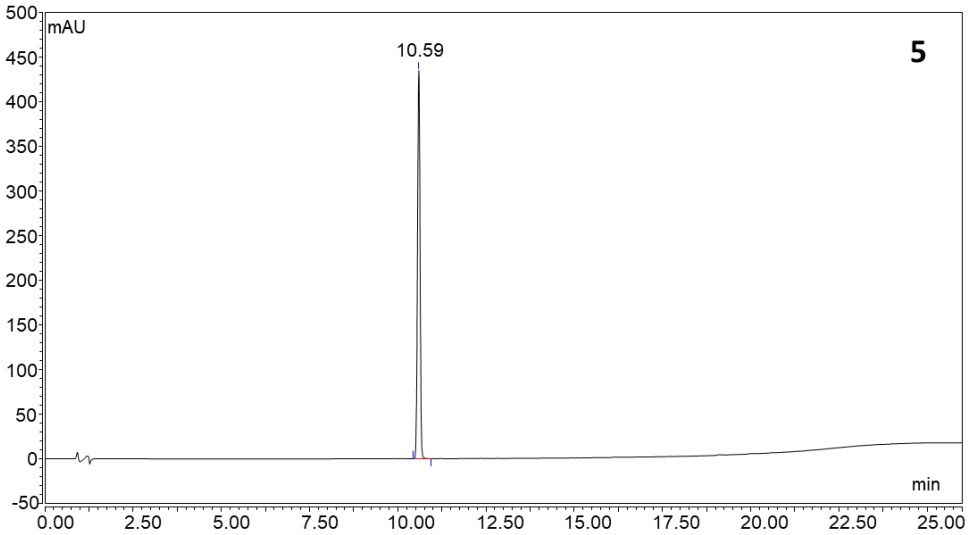
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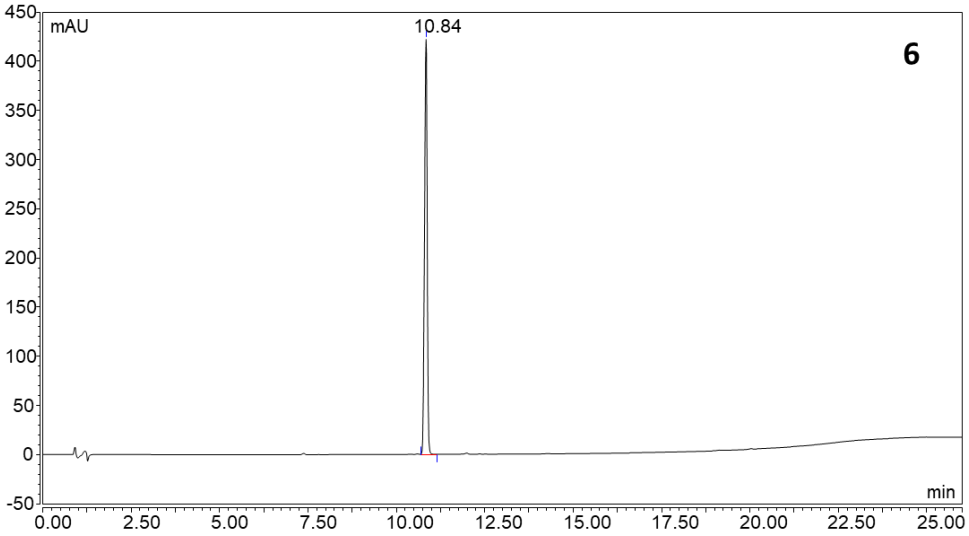
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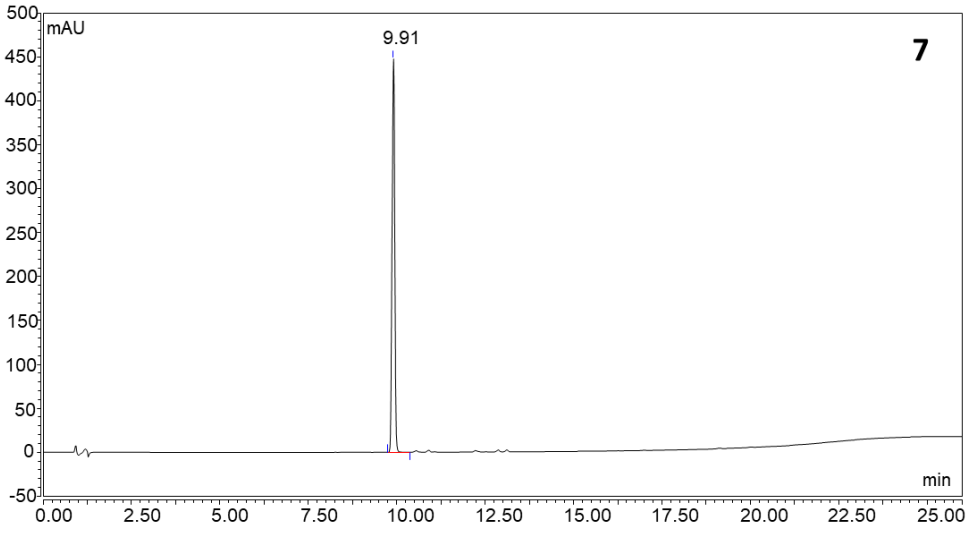
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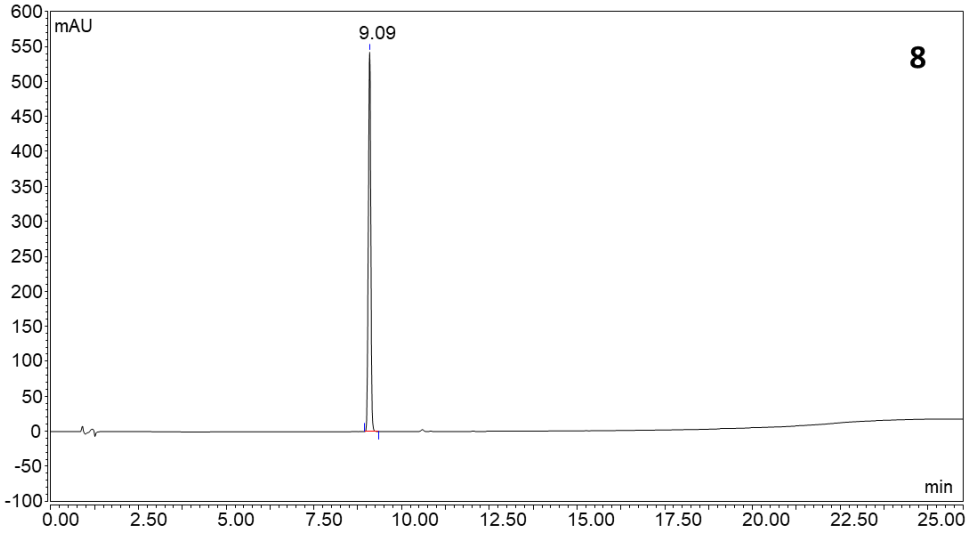
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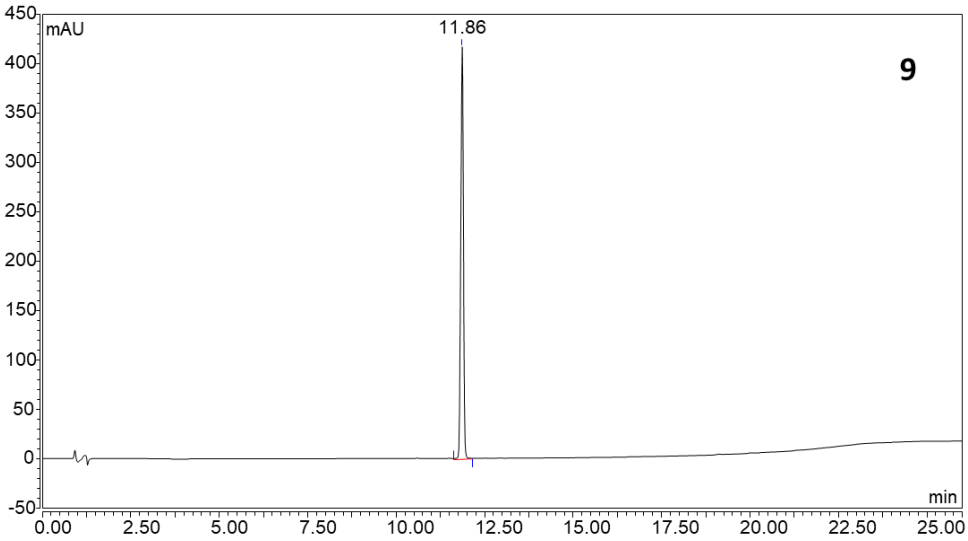
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
0.00
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00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min
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250
200
150
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50
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7.50
10.00
12.50
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20.00
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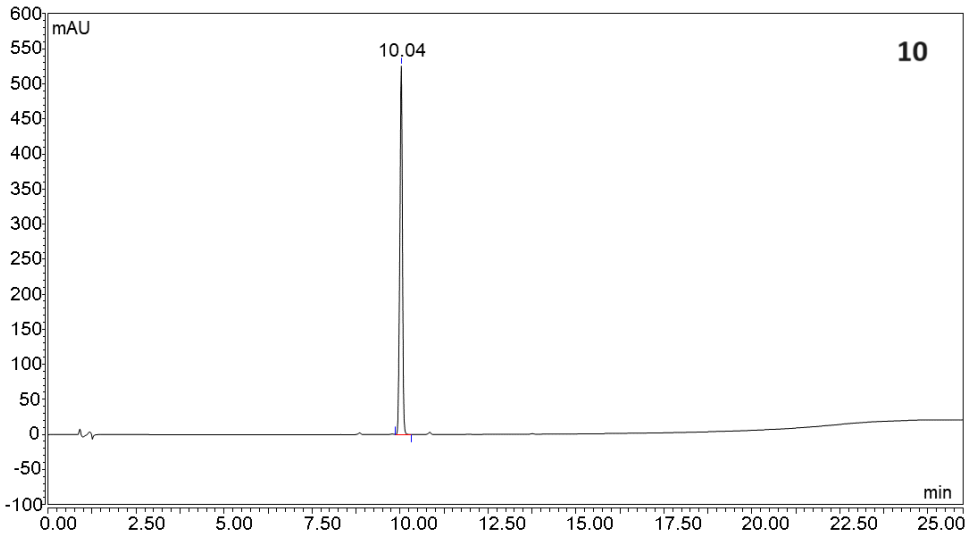
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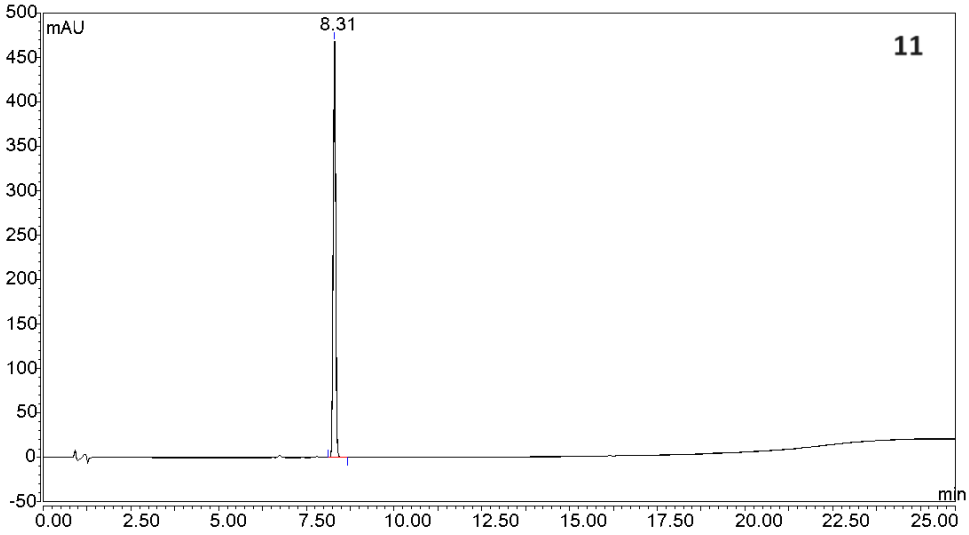
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200
150
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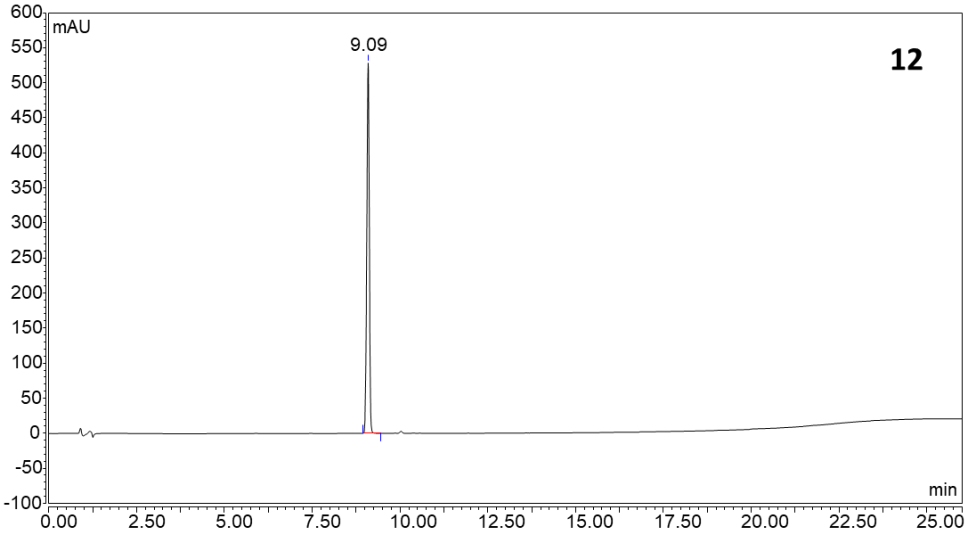
25.00


mAU
9.09
12
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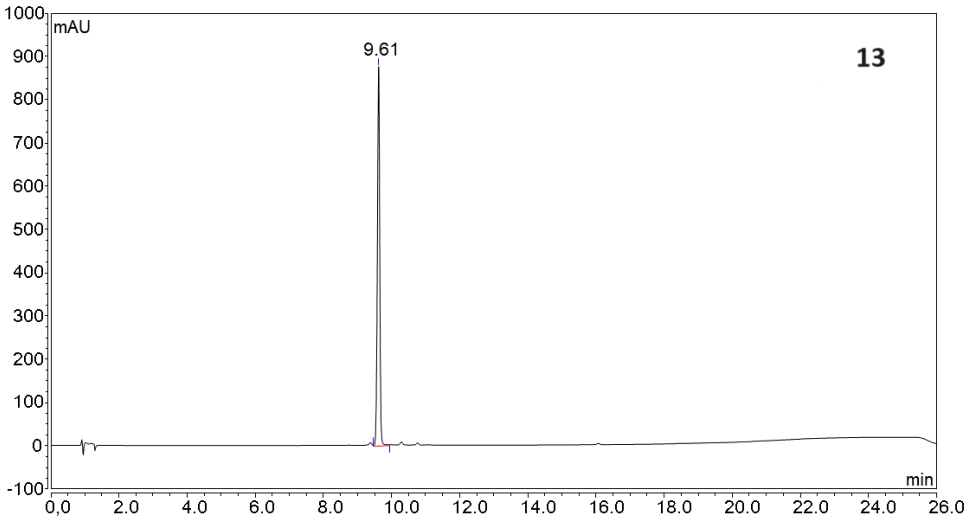
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13
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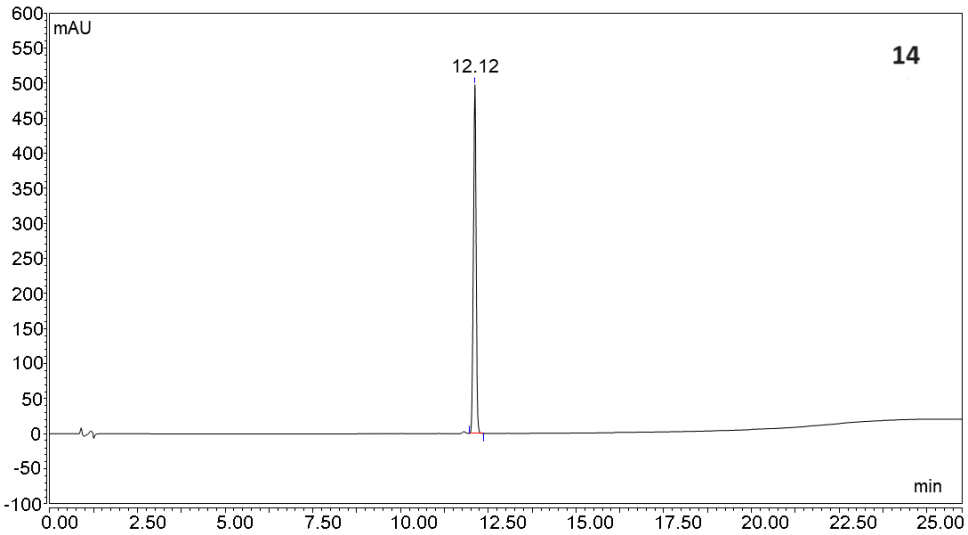
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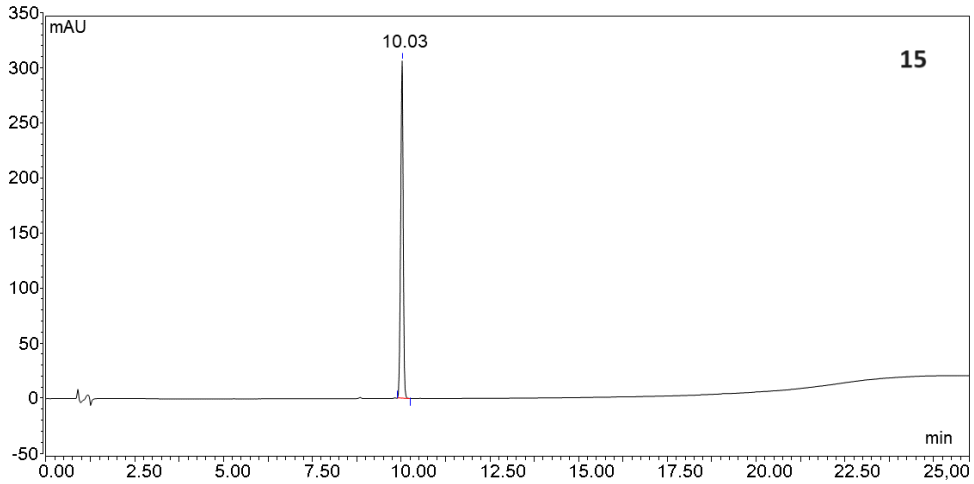
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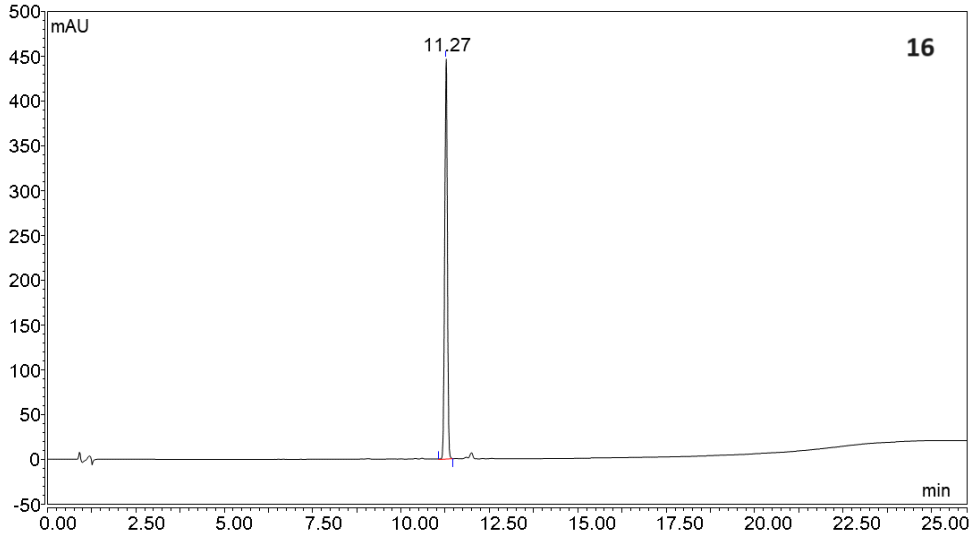
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11.27
16
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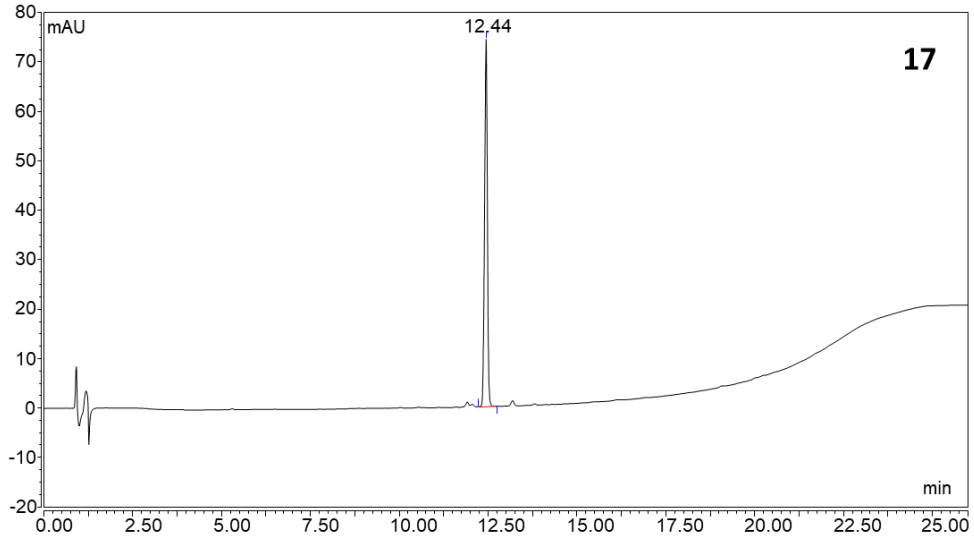
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17
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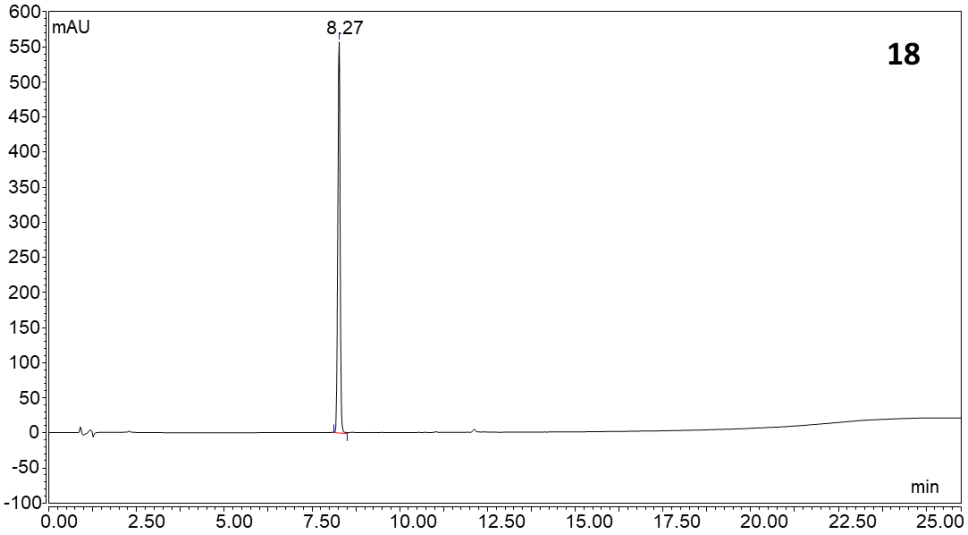
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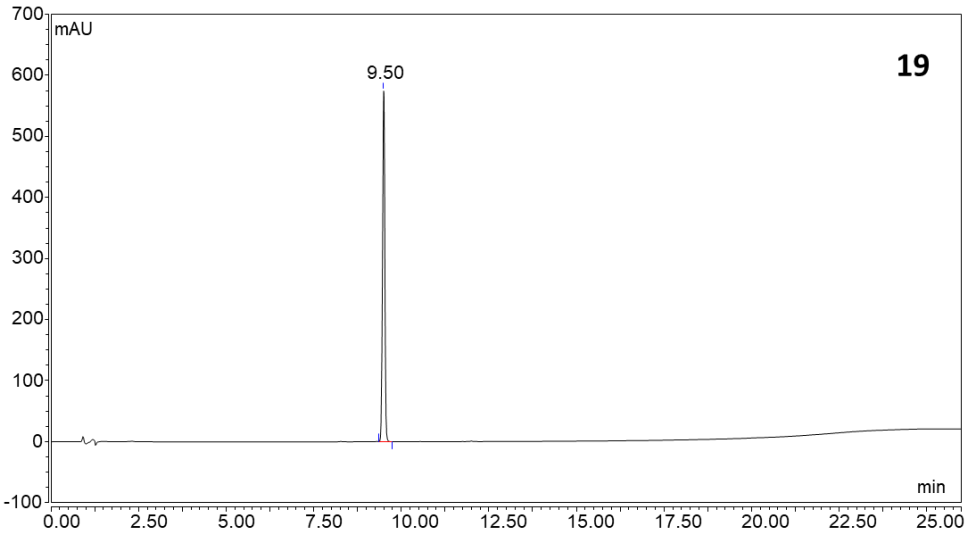
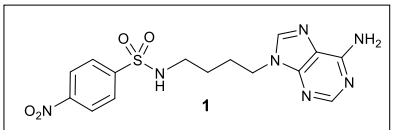
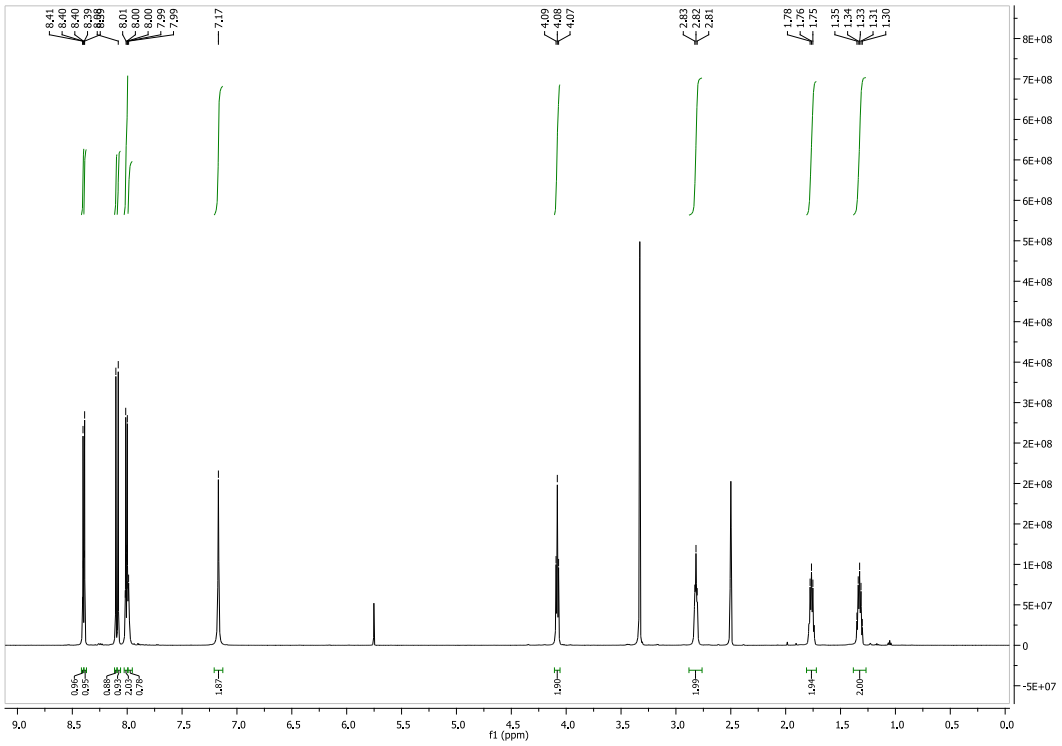

mAU
9.50
19
min

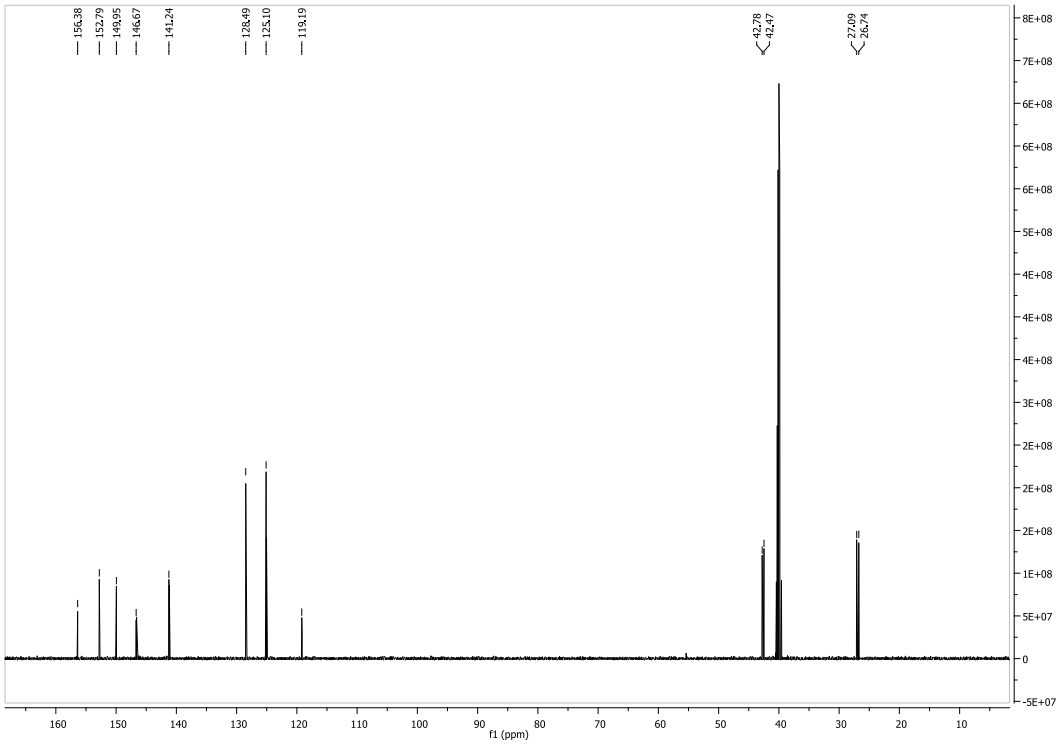
^{1}H-NMR and ^{13}C-NMR spectra of compounds 1-19

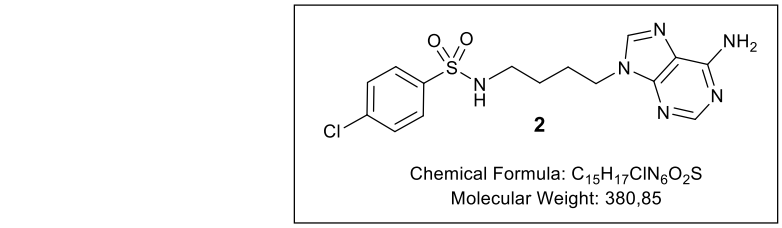


1

Chemical Formula: $C_{15}H_{17}N_7O_4S$
Molecular Weight: 391,41

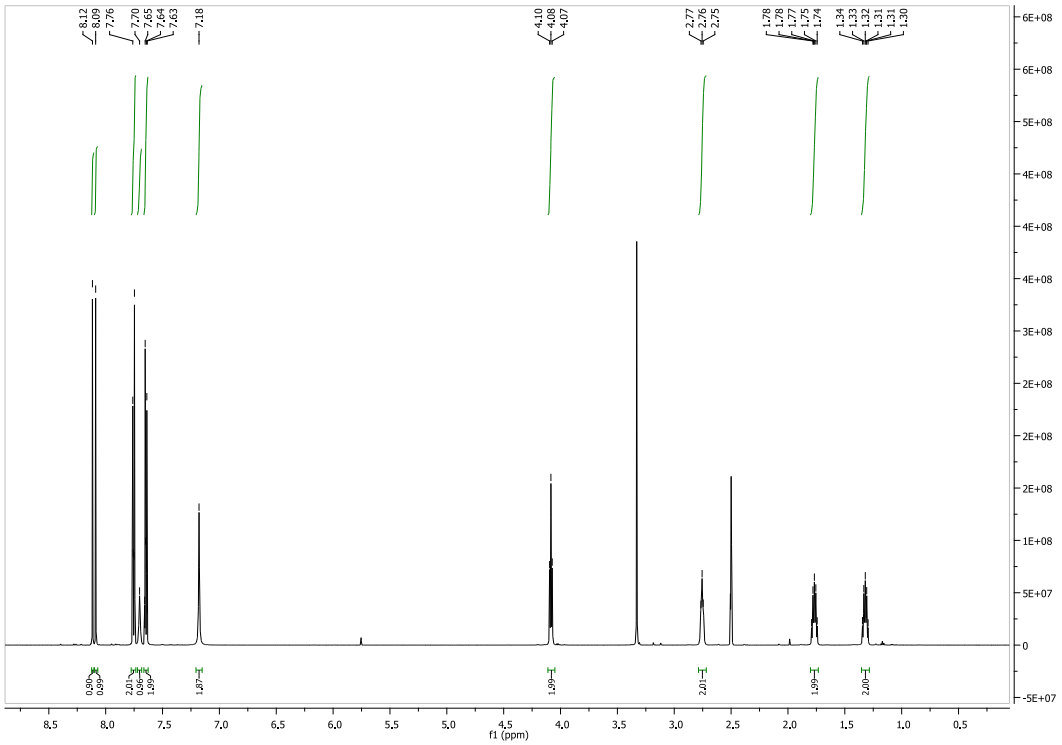


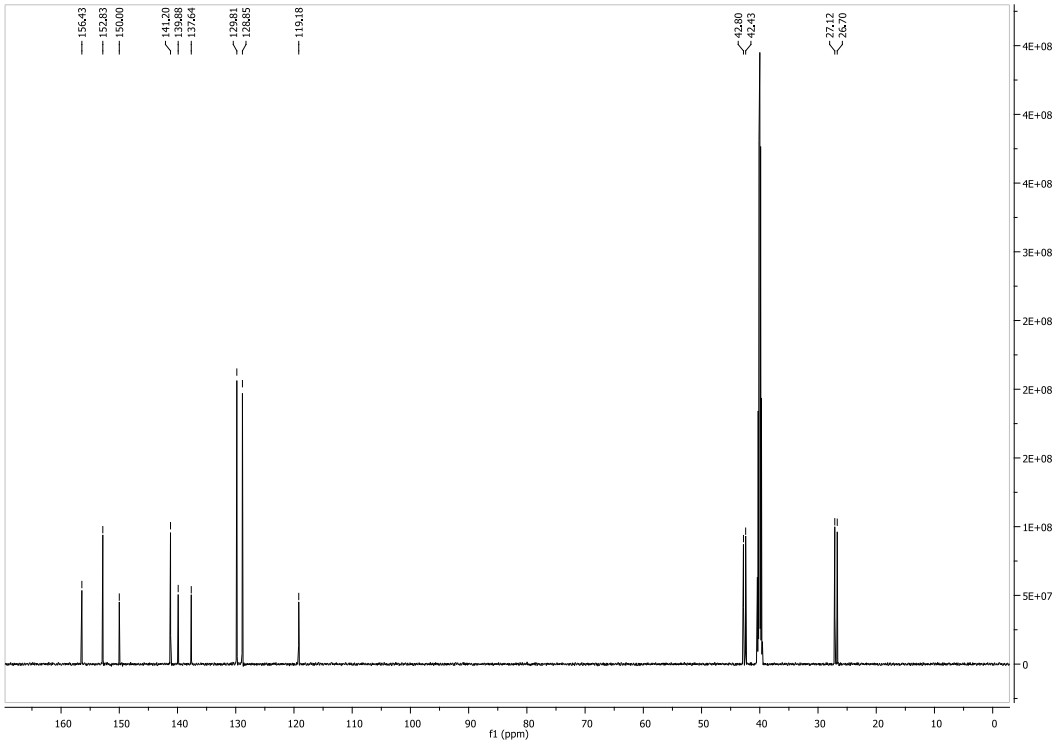


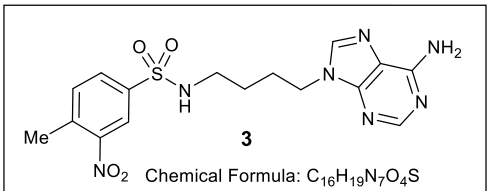


2

Chemical Formula: $C_{15}H_{17}ClN_6O_2S$
Molecular Weight: 380,85

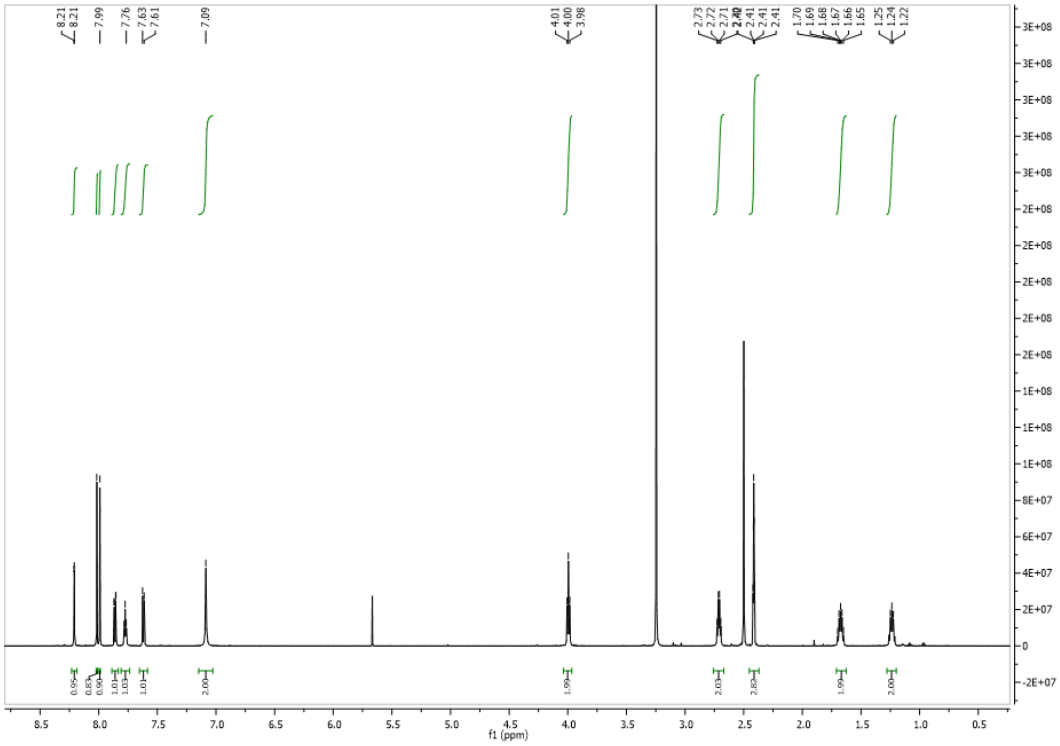


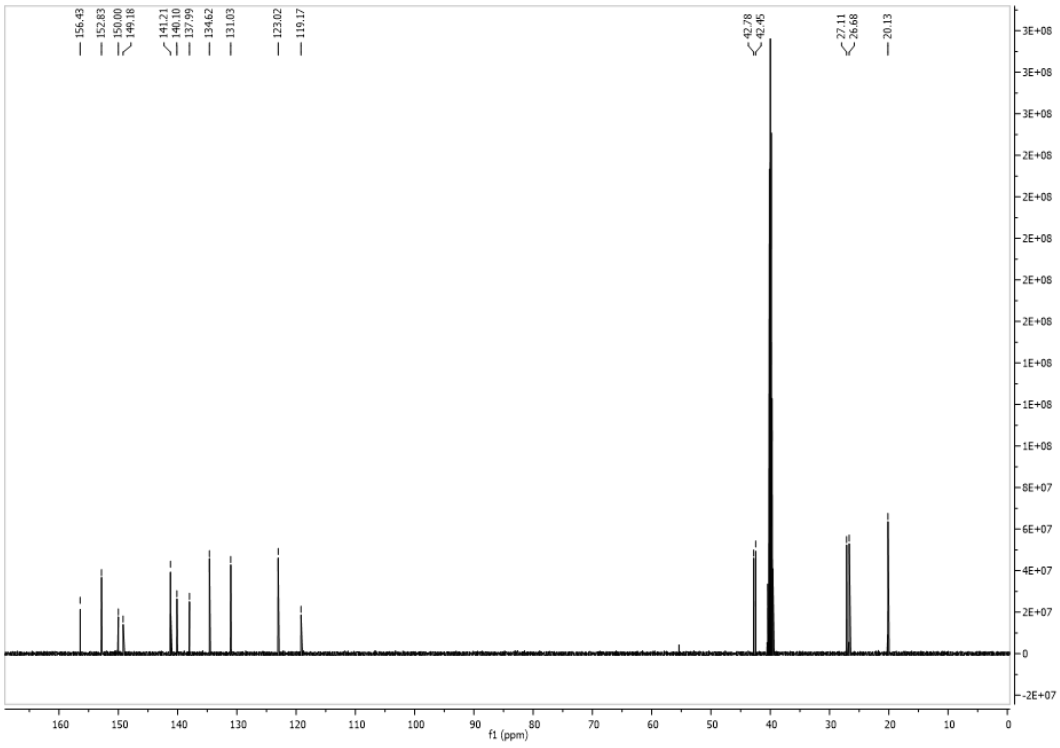


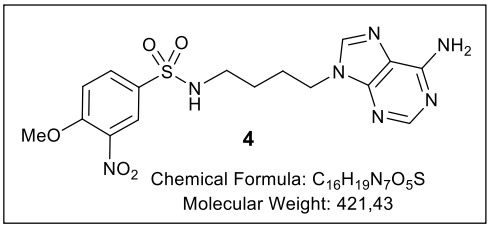


3

Chemical Formula: $C_{16}H_{19}N_7O_4S$
Molecular Weight: 405.43

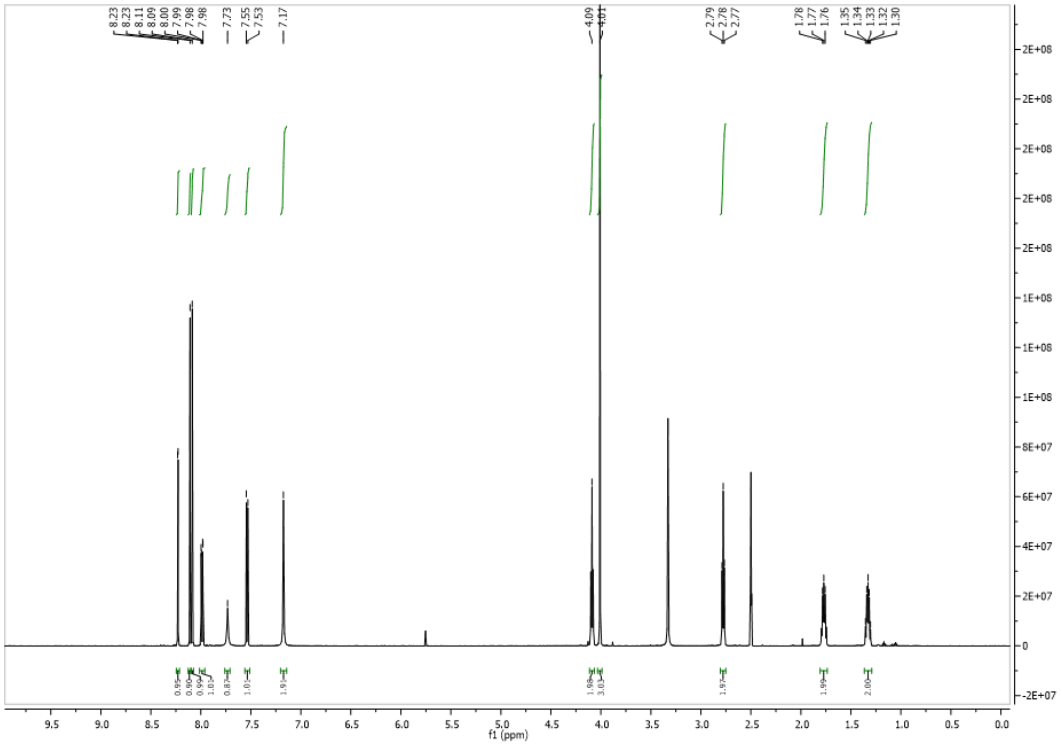


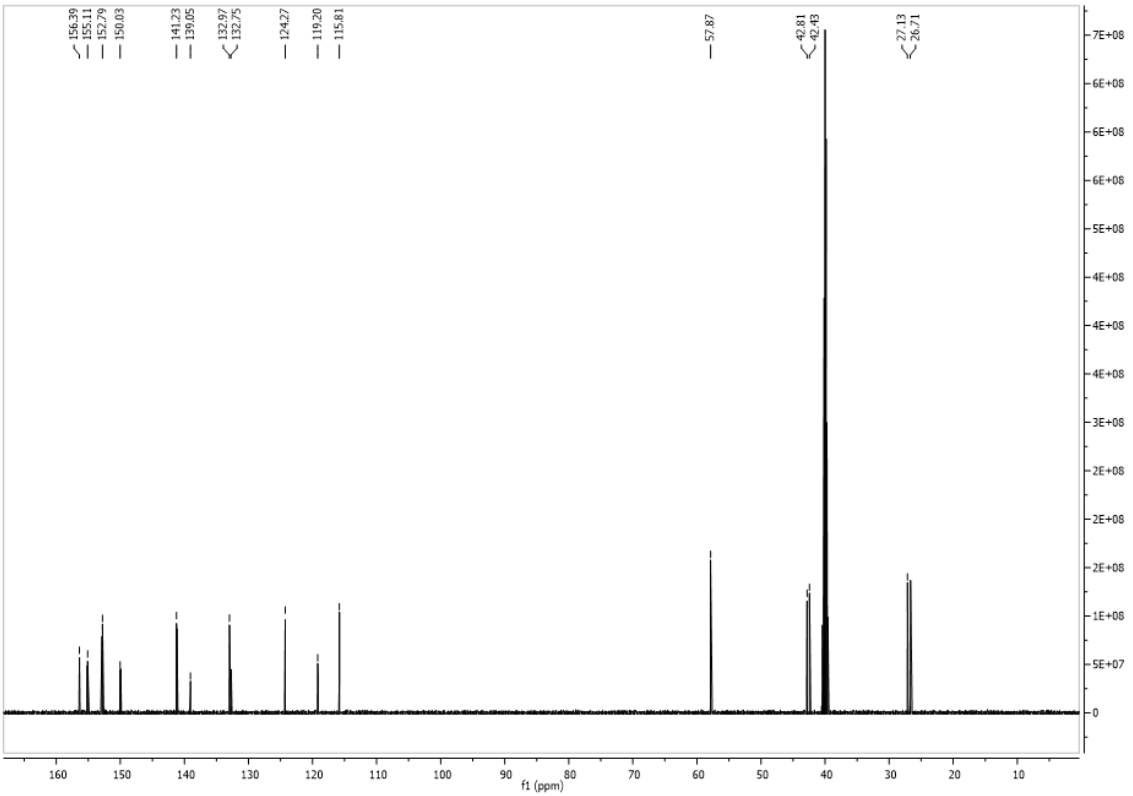


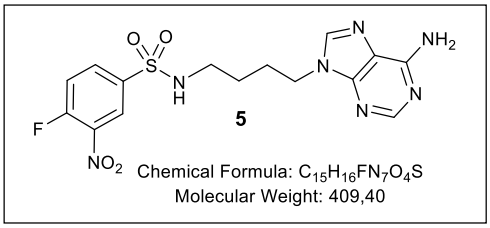


4

Chemical Formula: $C_{16}H_{19}N_7O_5S$
Molecular Weight: 421,43

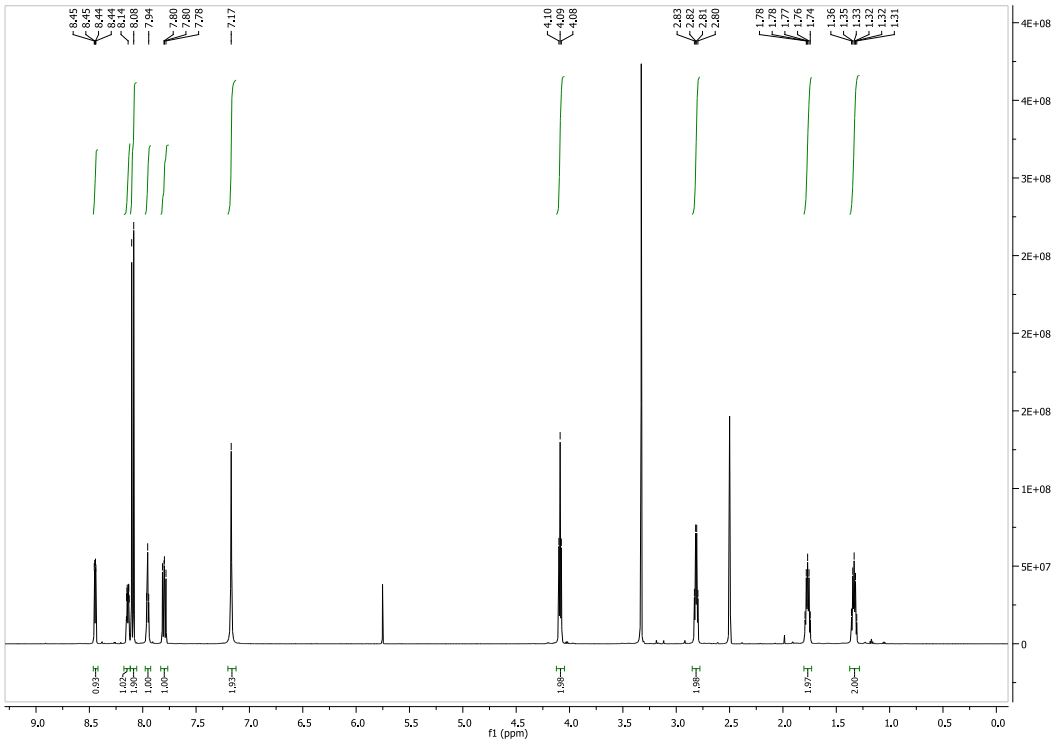


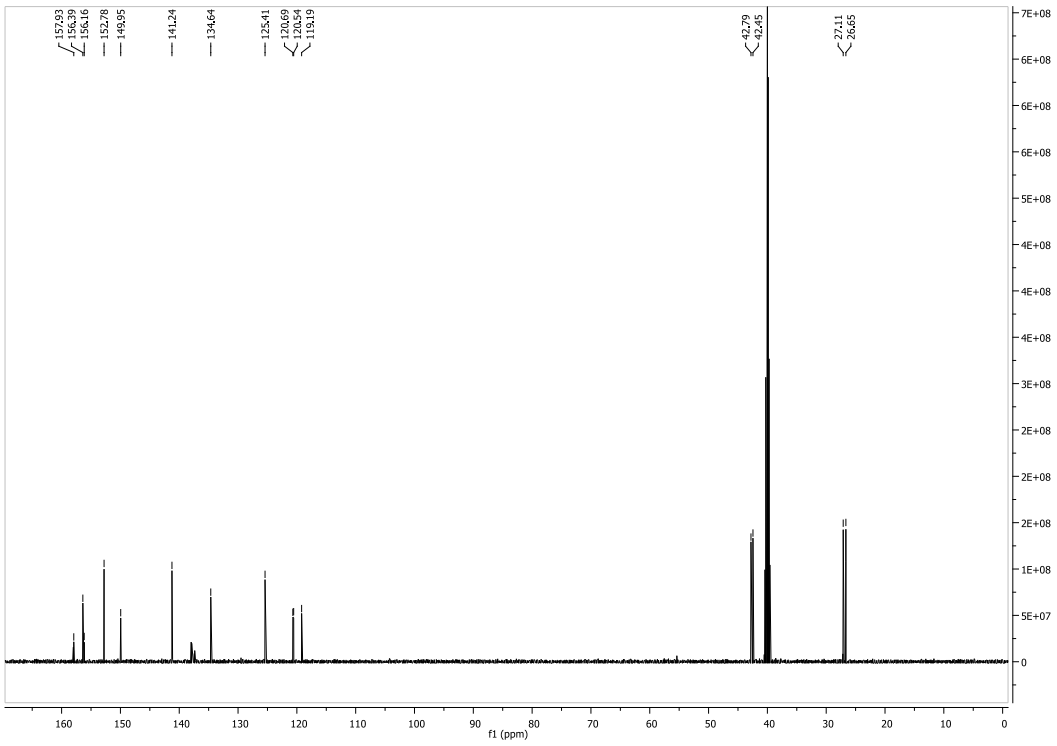


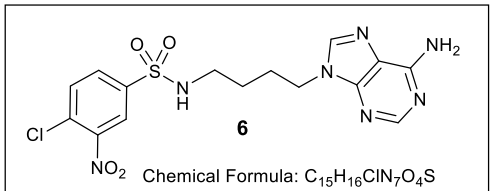


5

Chemical Formula: $C_{15}H_{16}FN_7O_4S$
Molecular Weight: 409,40

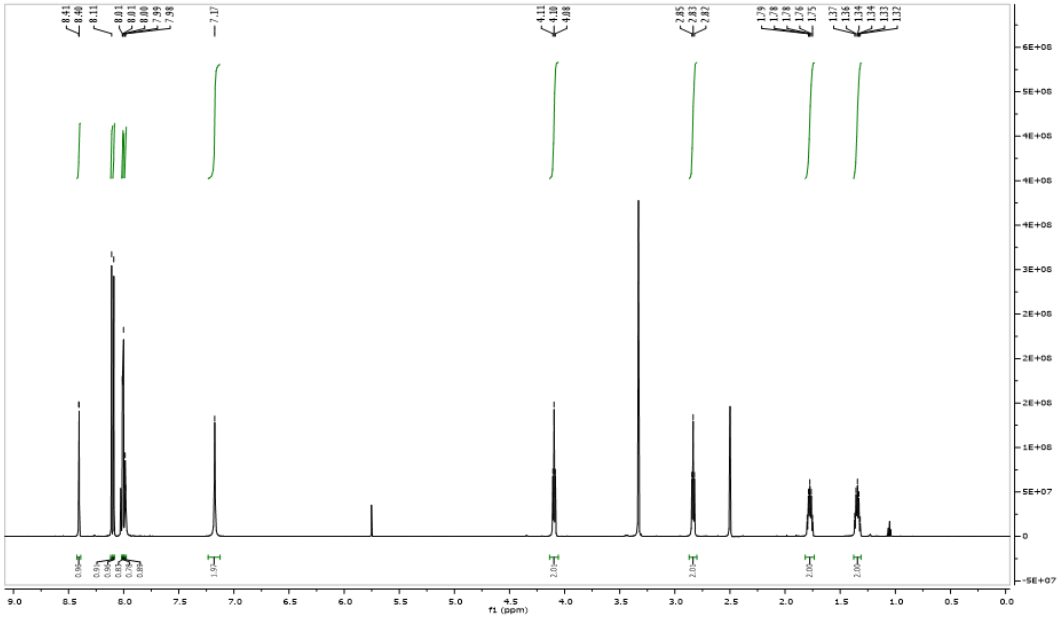


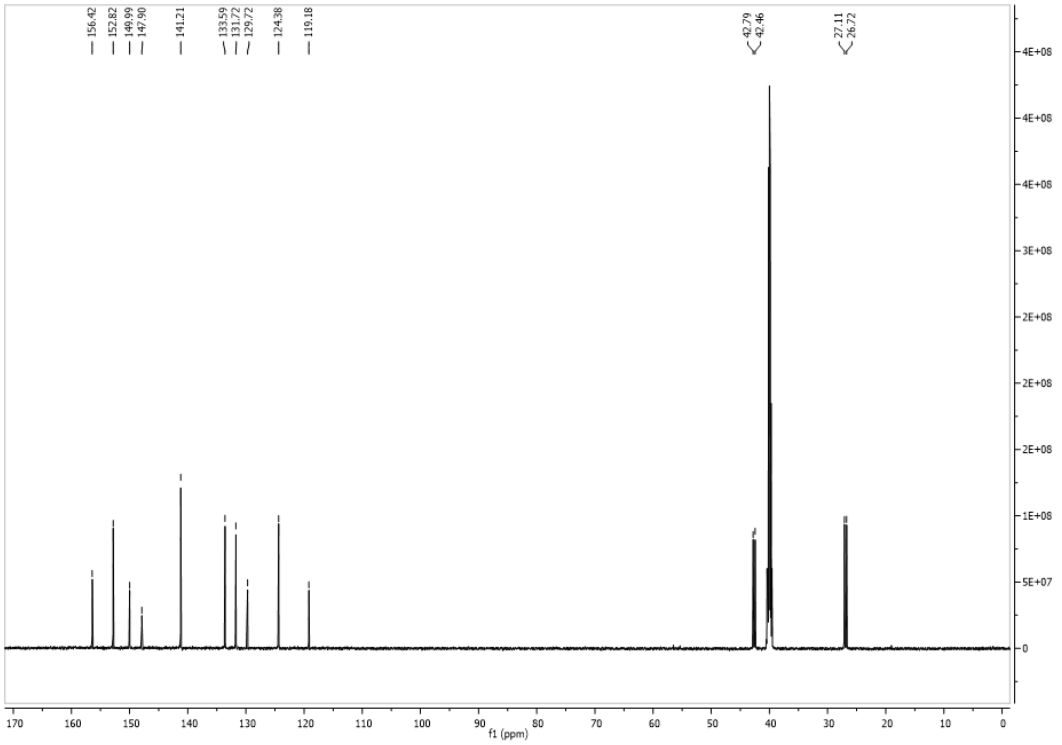


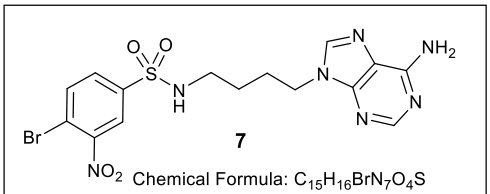


6

Chemical Formula: $C_{15}H_{16}ClN_7O_4S$
Molecular Weight: 425,85

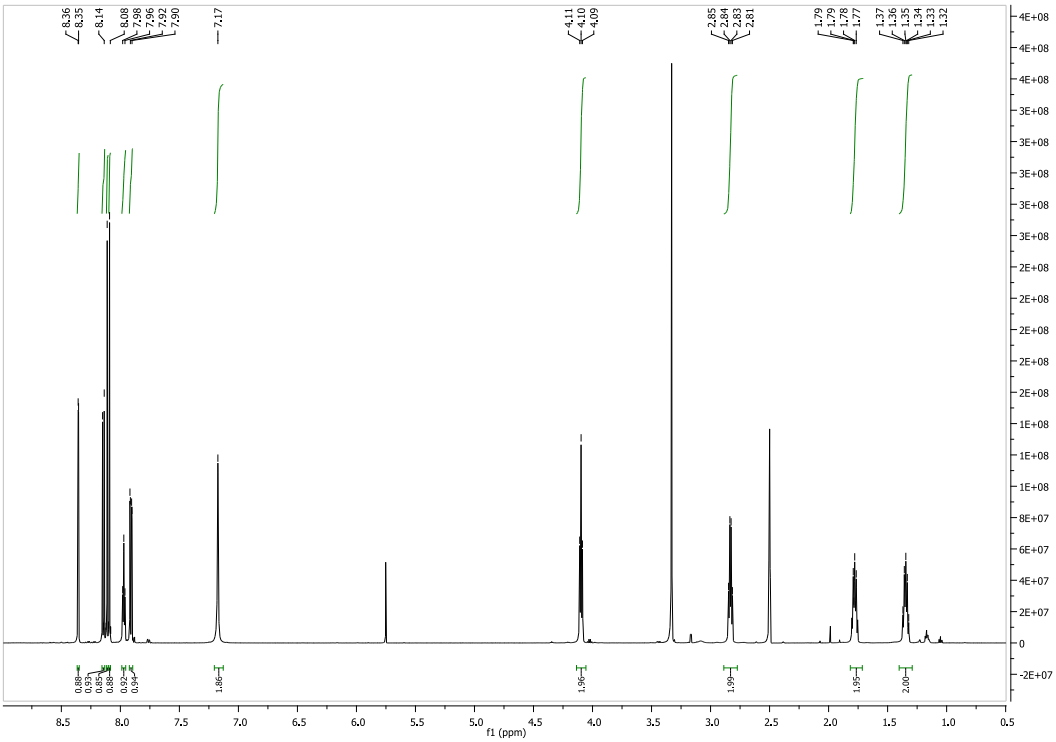


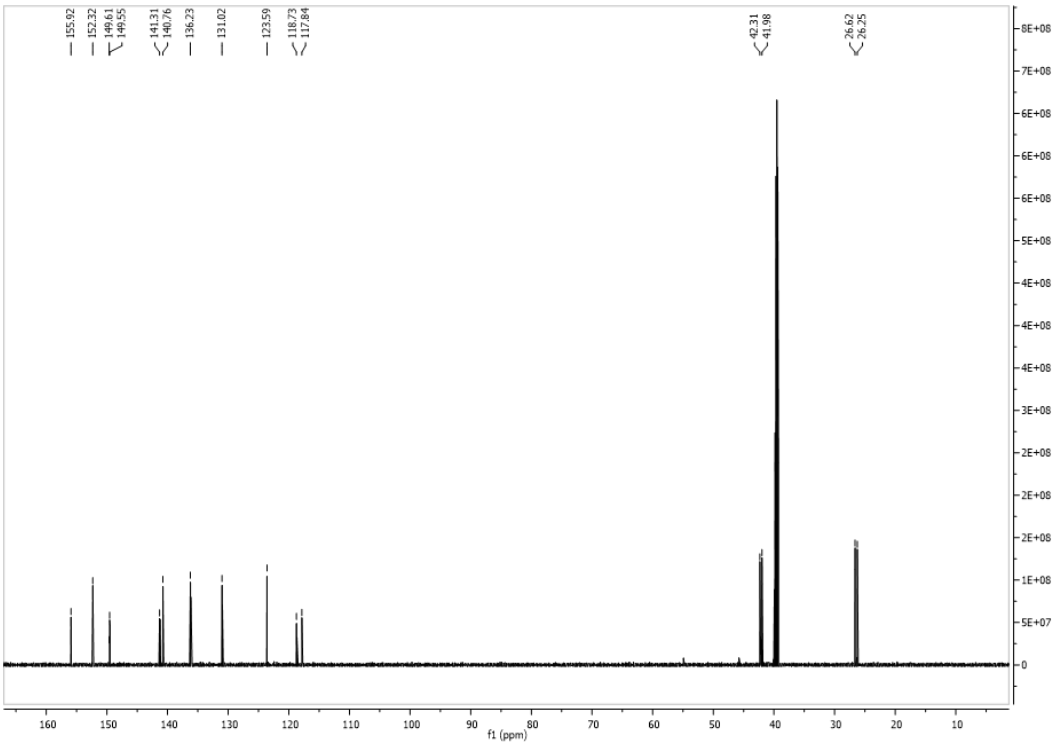


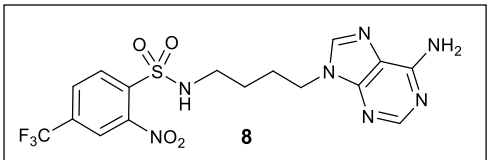


7

Chemical Formula: $C_{15}H_{16}BrN_7O_4S$
Molecular Weight: 470,30

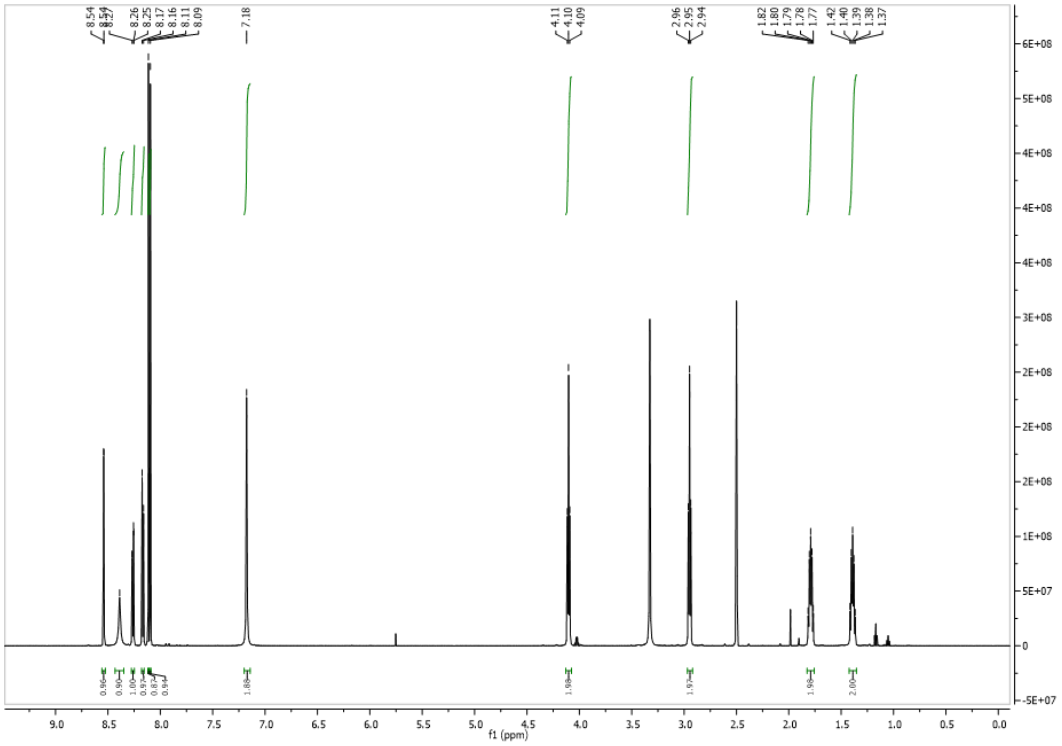


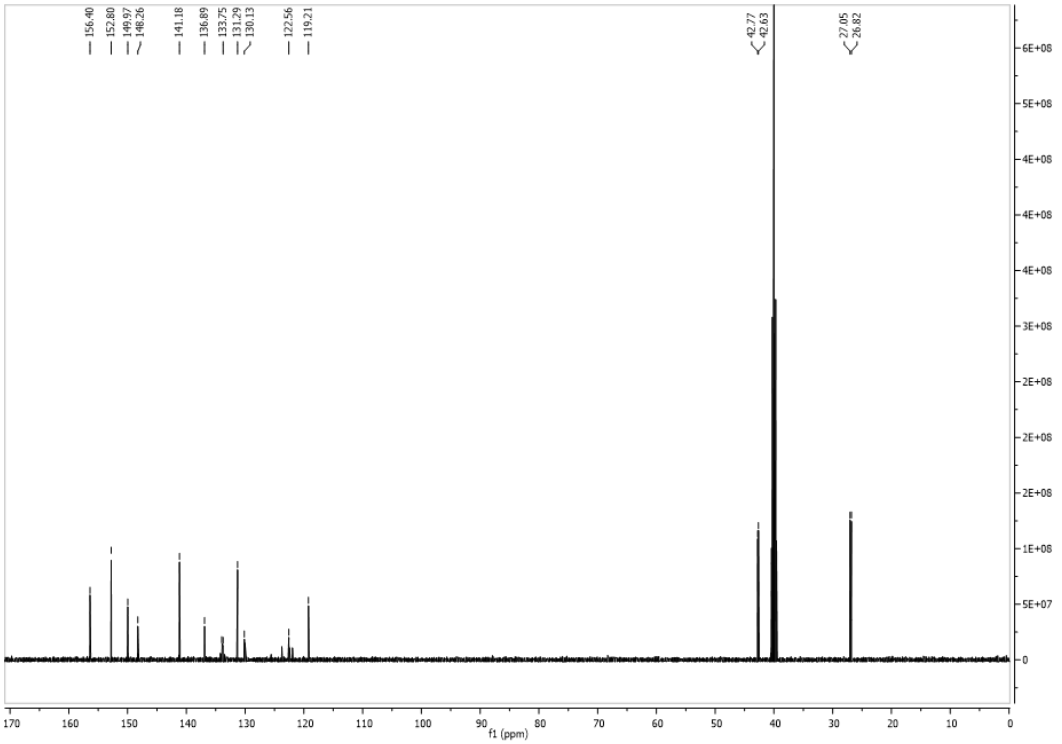


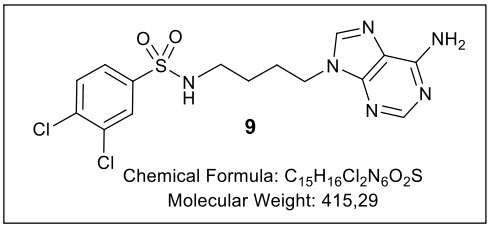


8

Chemical Formula: $C_{16}H_{16}F_3N_7O_4S$
Molecular Weight: 459,40

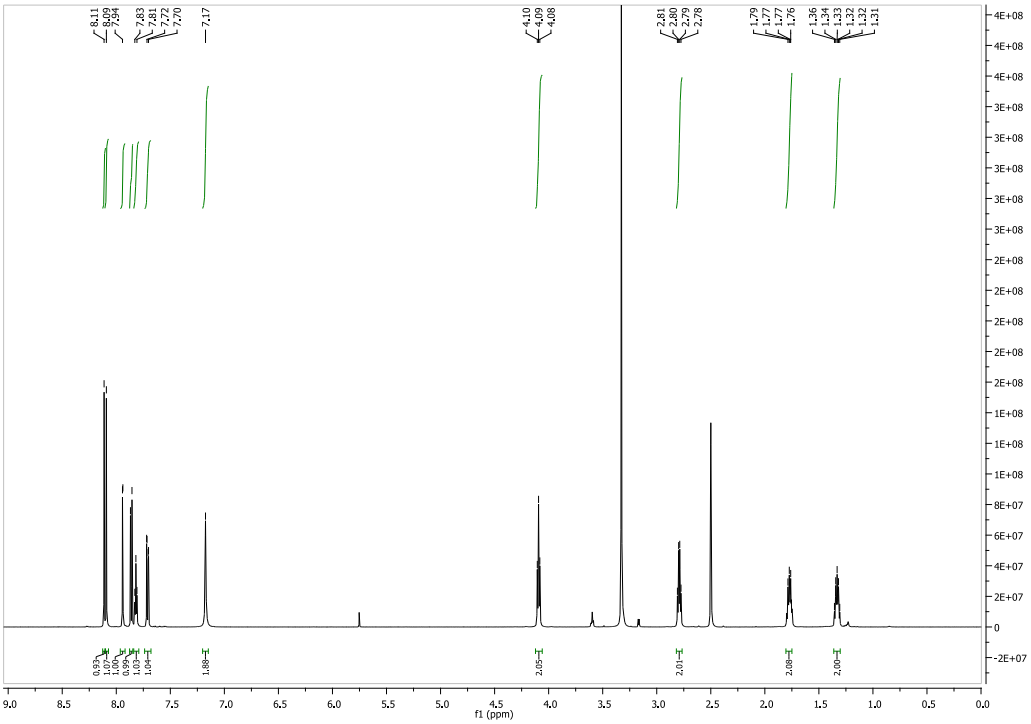


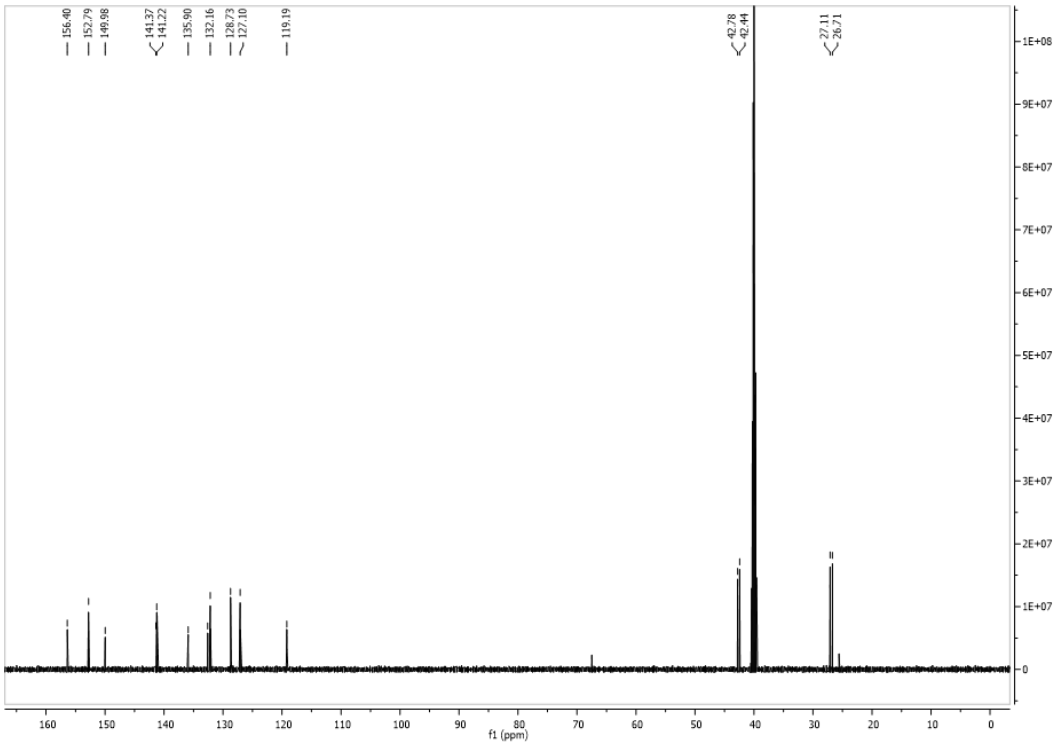


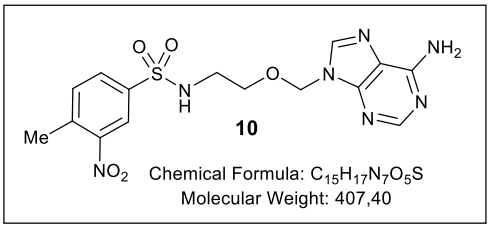


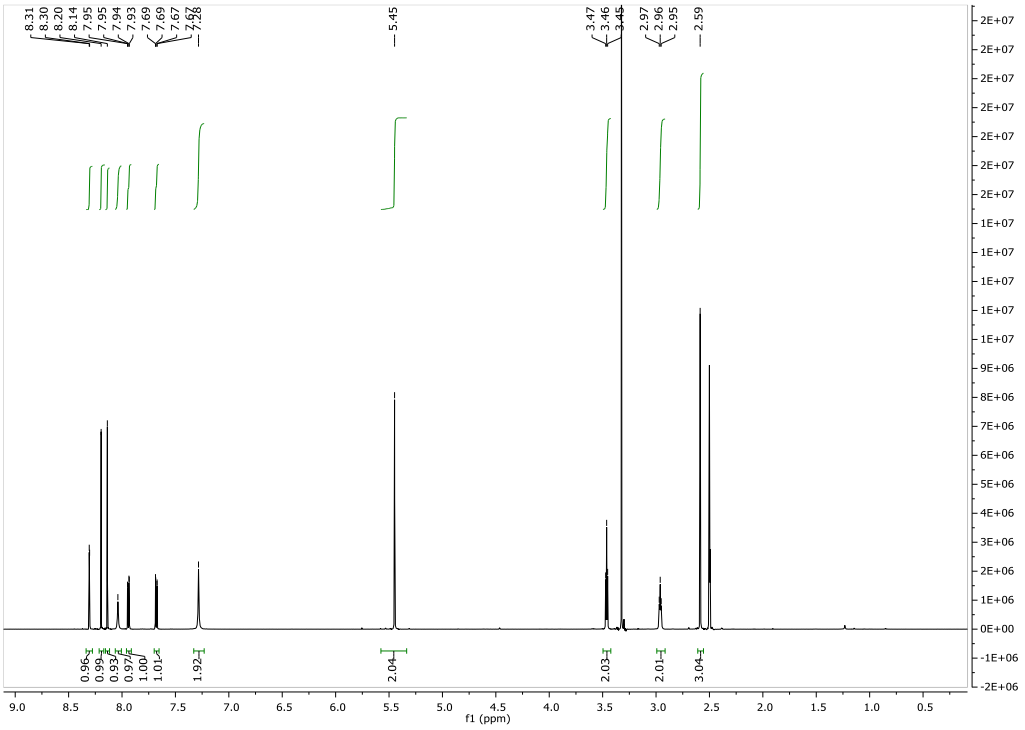
9

Chemical Formula: $C_{15}H_{16}Cl_2N_6O_2S$
Molecular Weight: 415,29






10
Chemical Formula: $C_{15}H_{17}N_7O_5S$
Molecular Weight: 407,40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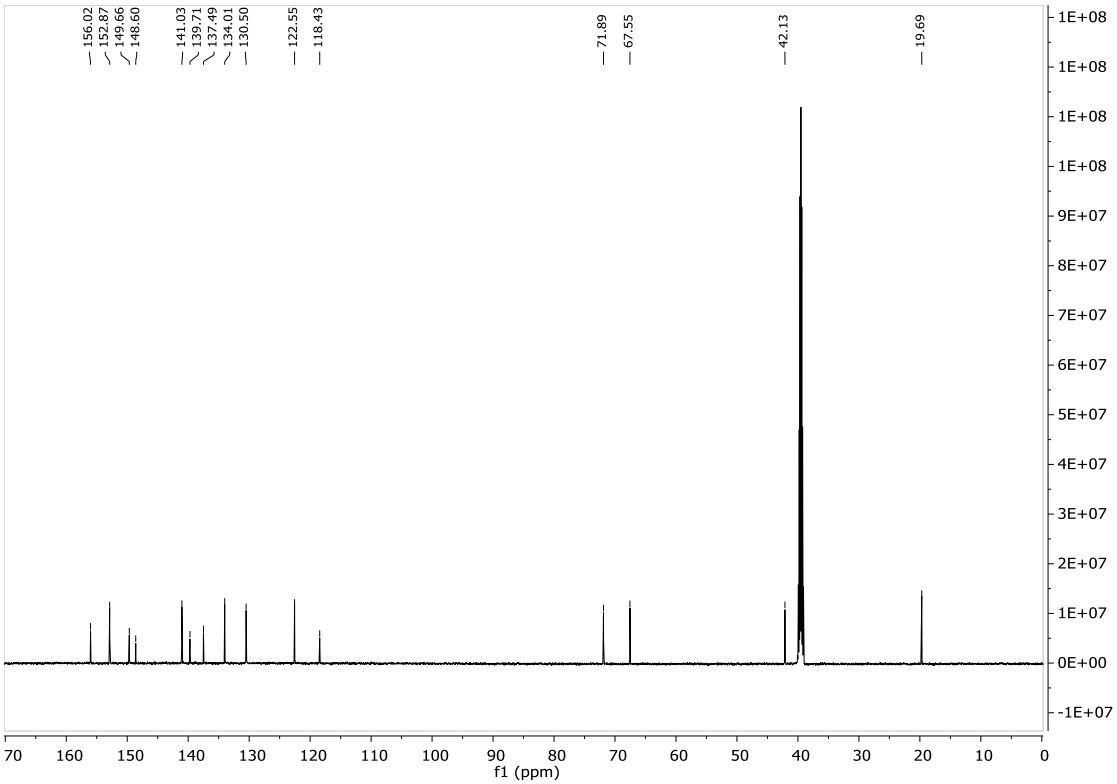
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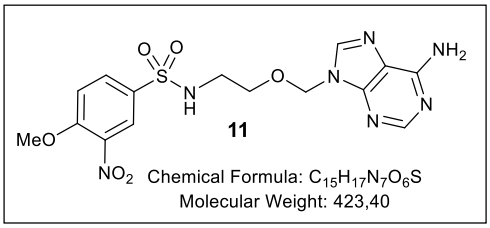
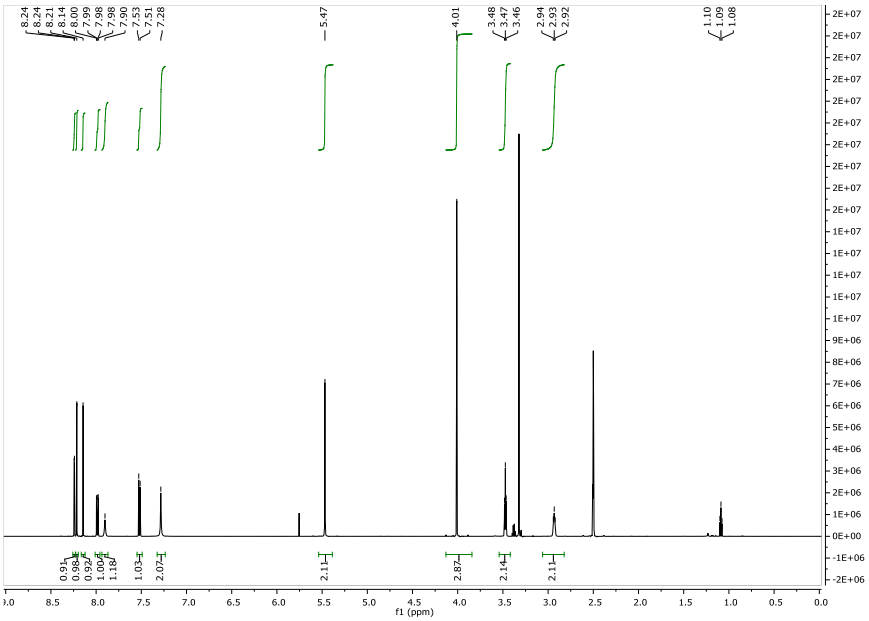

MeO
NO2
NH2
11
Chemical Formula: C15H17N7O6S
Molecular Weight: 423,40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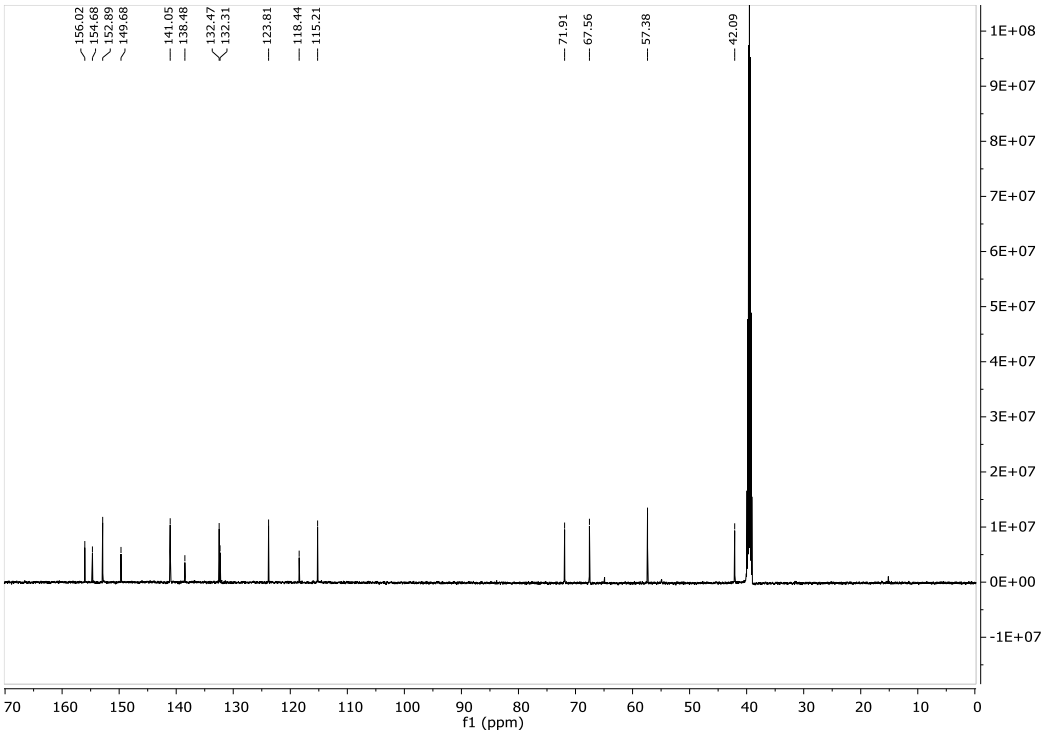
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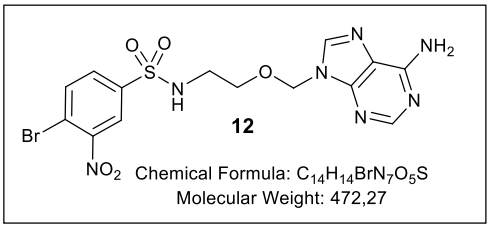
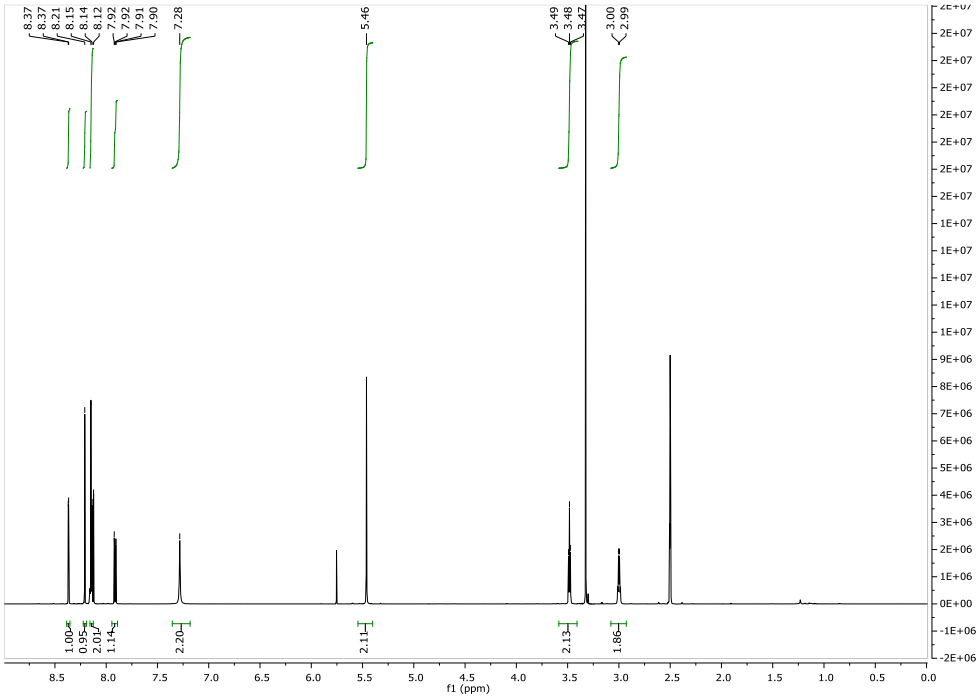
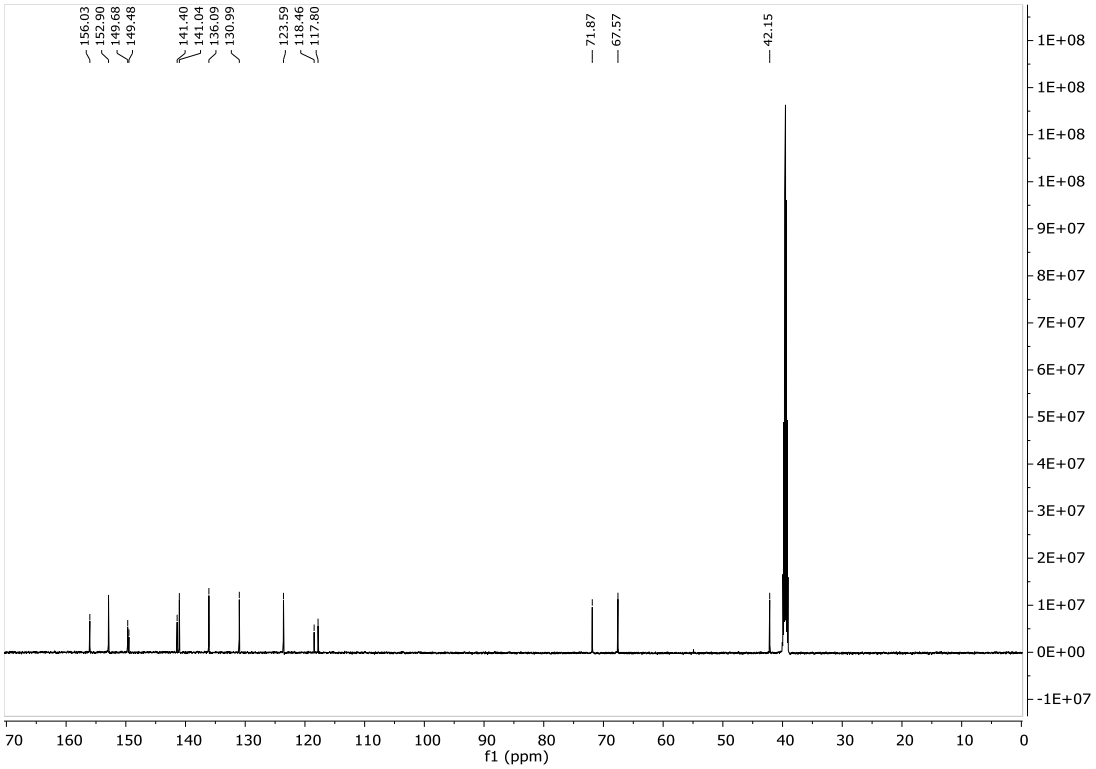
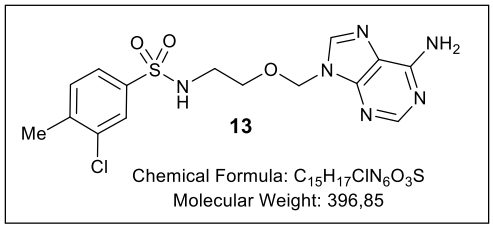


12

Chemical Formula: $C_{14}H_{14}BrN_7O_5S$
Molecular Weight: 472,27

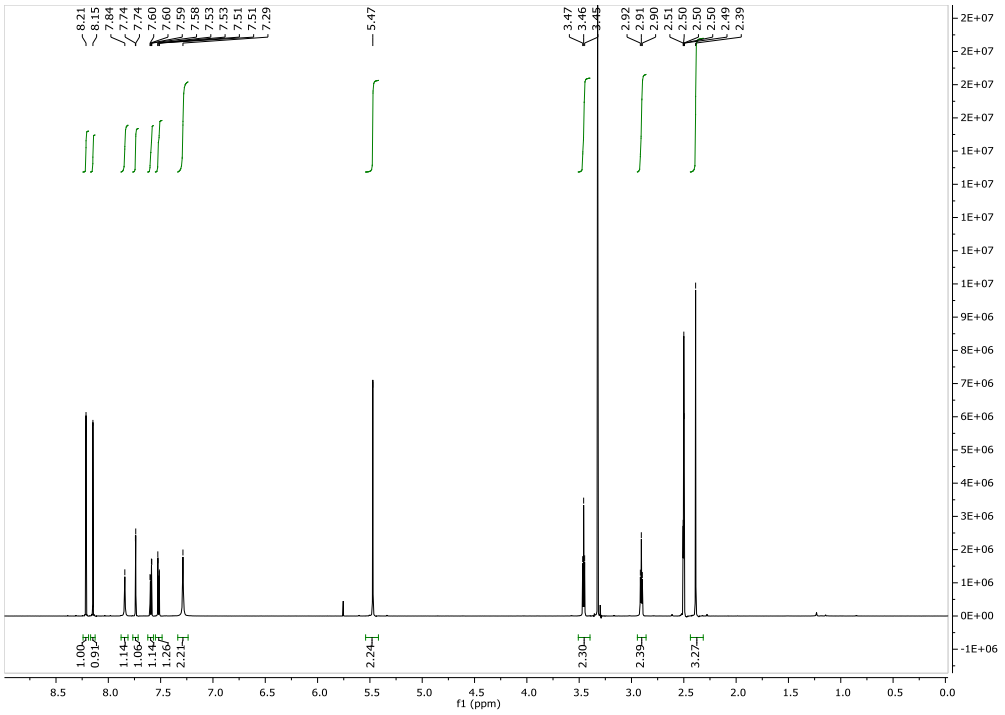


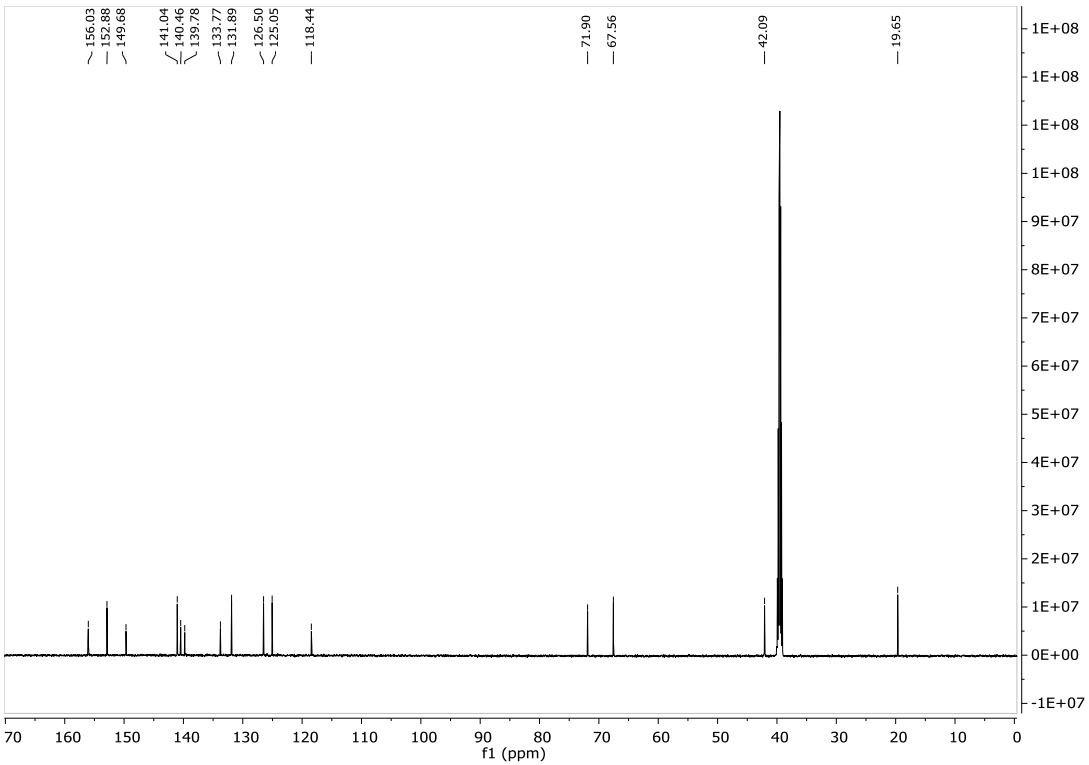


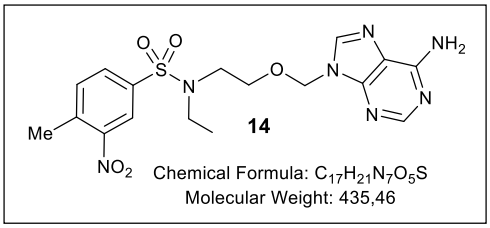


13

Chemical Formula: $C_{15}H_{17}ClN_6O_3S$
Molecular Weight: 396,85

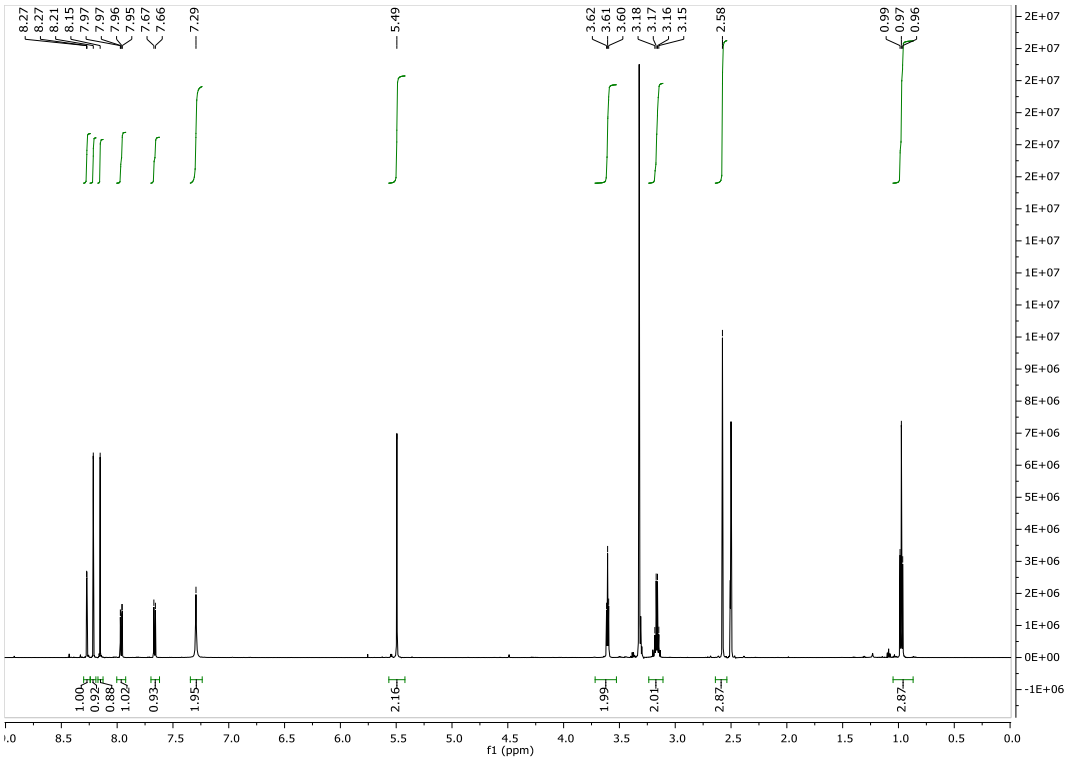


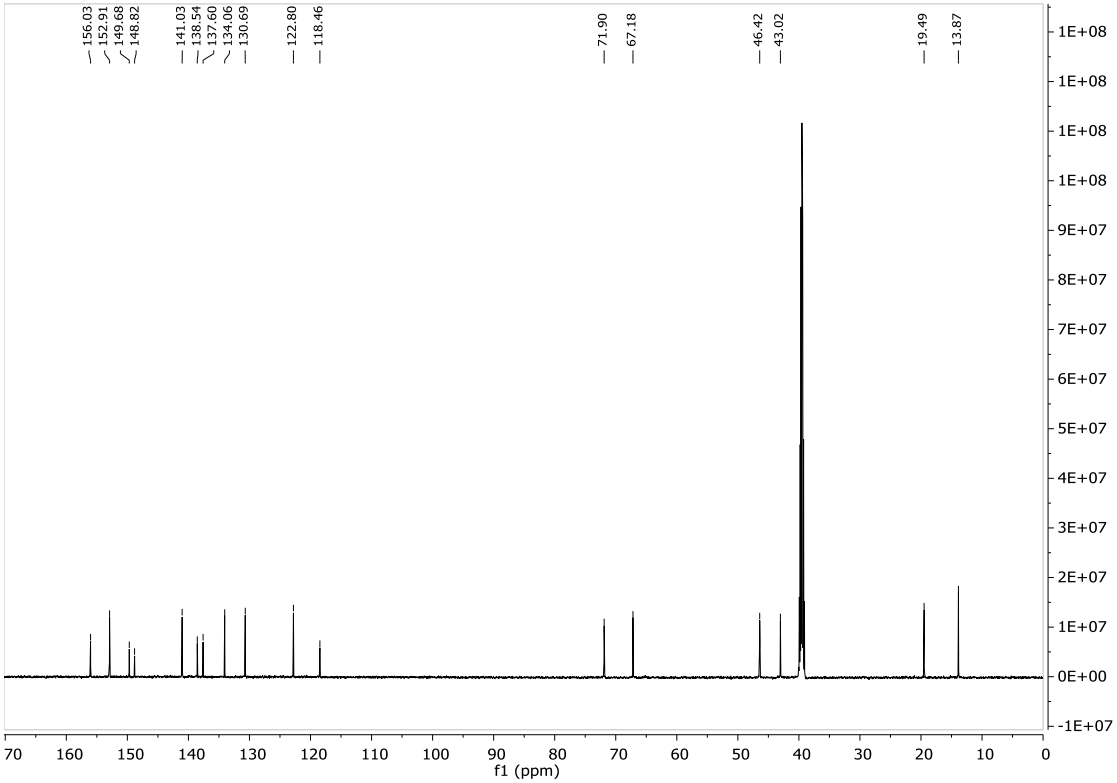


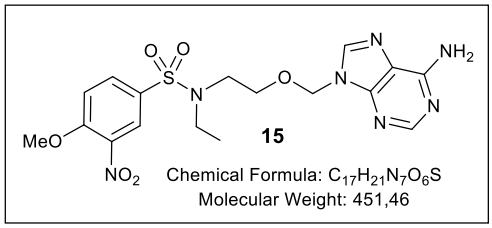


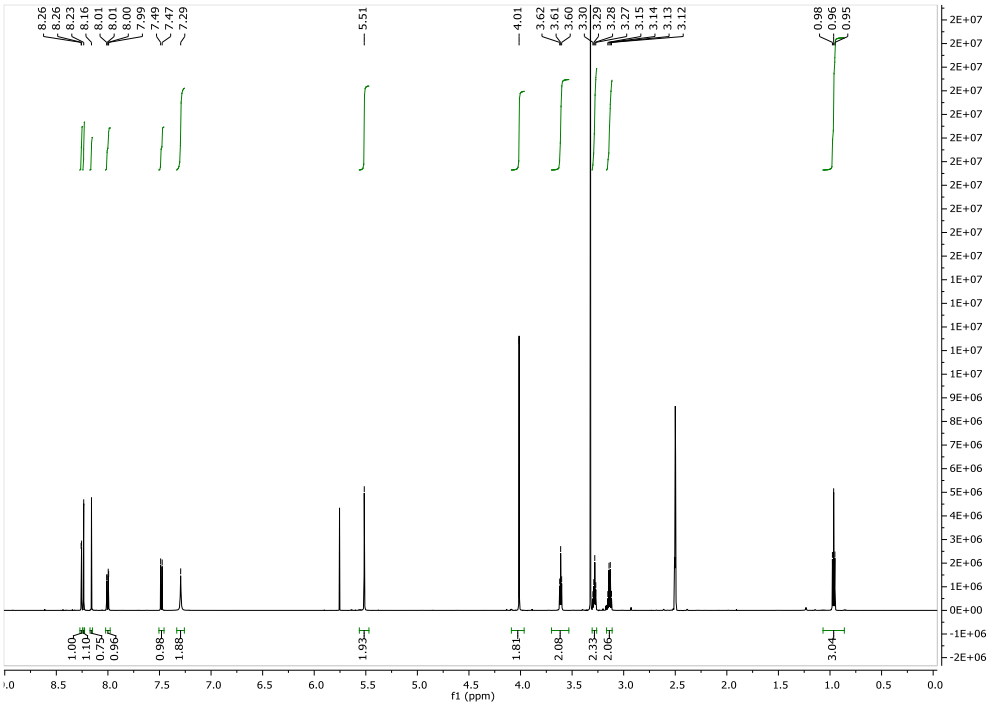
14

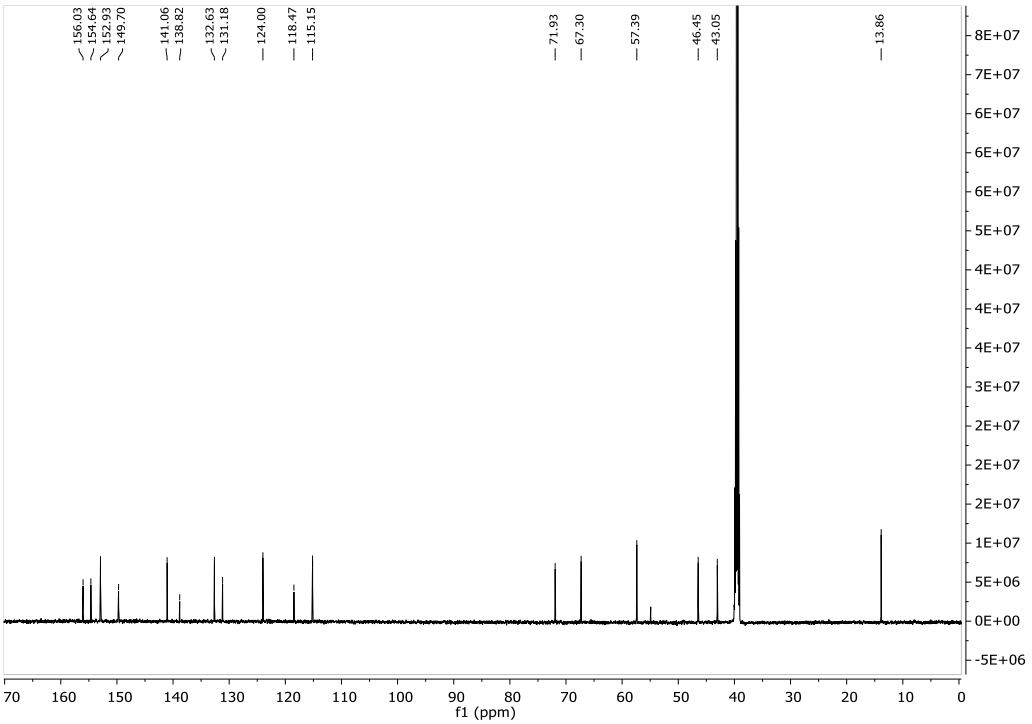
Chemical Formula: $C_{17}H_{21}N_7O_5S$
Molecular Weight: 435,46

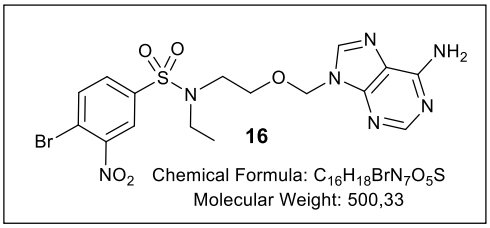


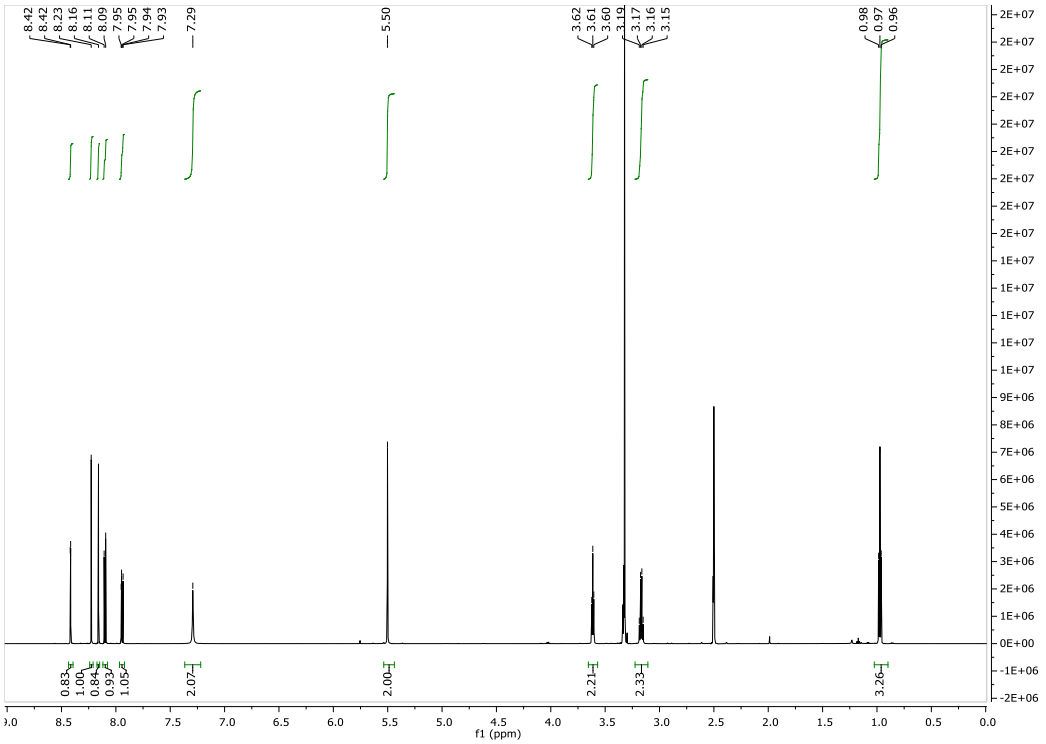



MeO
NO2
NH2
15
Chemical Formula: C17H21N7O6S
Molecular Weight: 451,46


f1 (ppm)


f1 (ppm)


16
Chemical Formula: $C_{16}H_{18}BrN_7O_5S$
Molecular Weight: 500,33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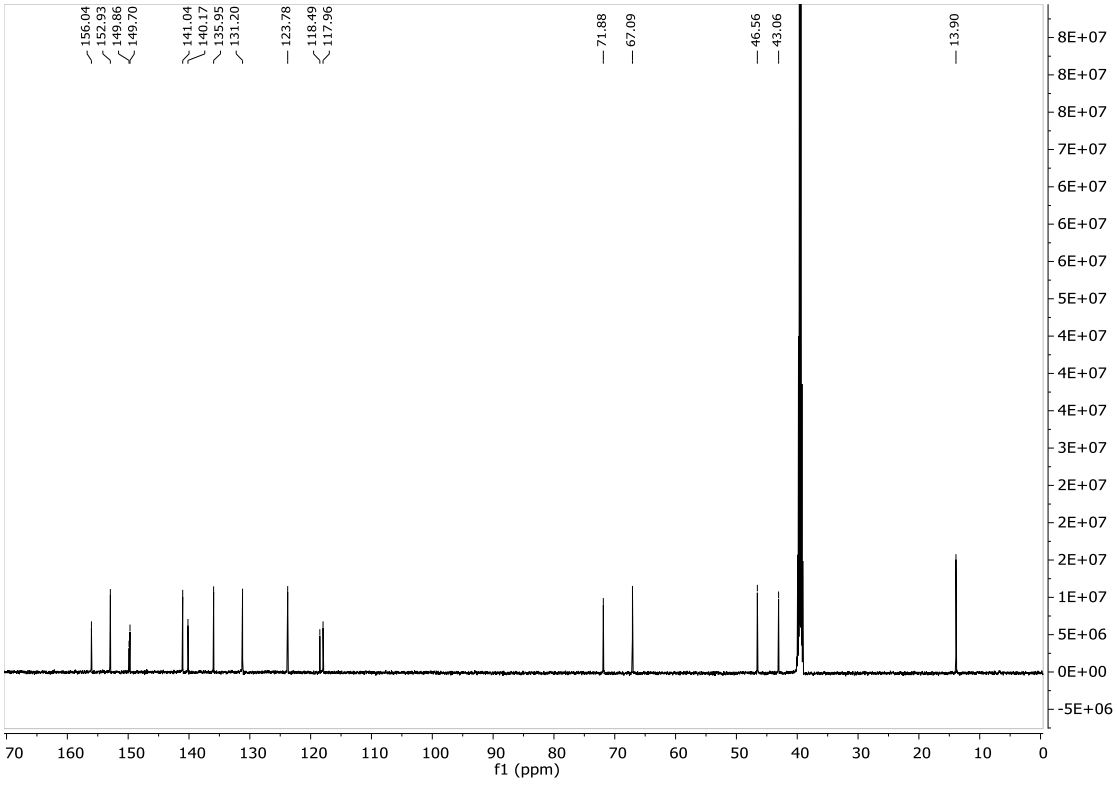
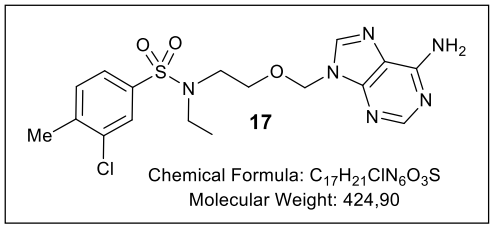
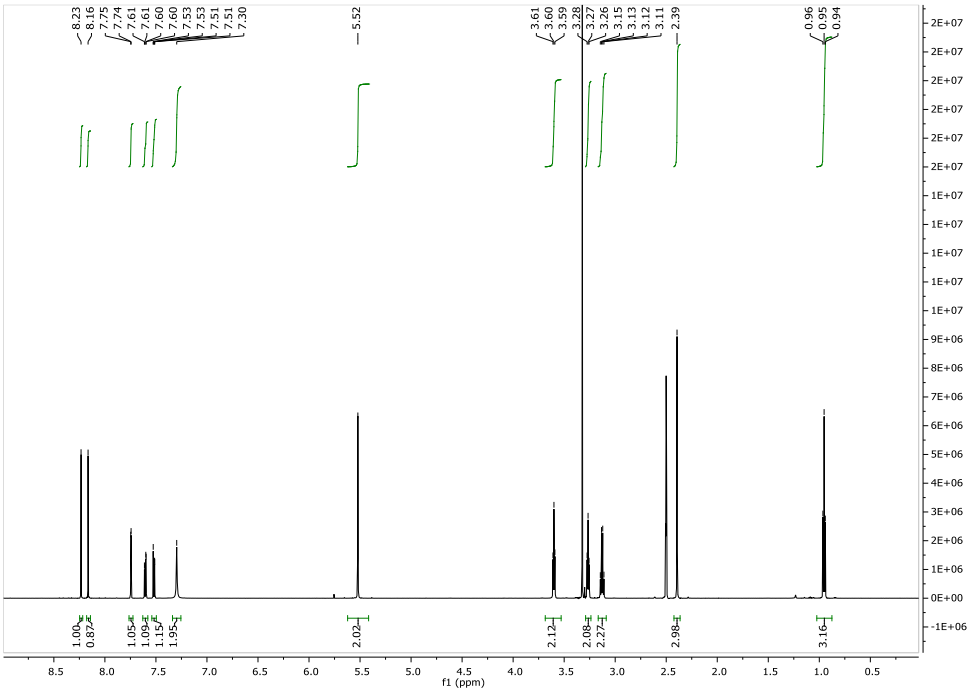

f1 (ppm)


Me
Cl
17
Chemical Formula: $C_{17}H_{21}ClN_6O_3S$
Molecular Weight: 424,90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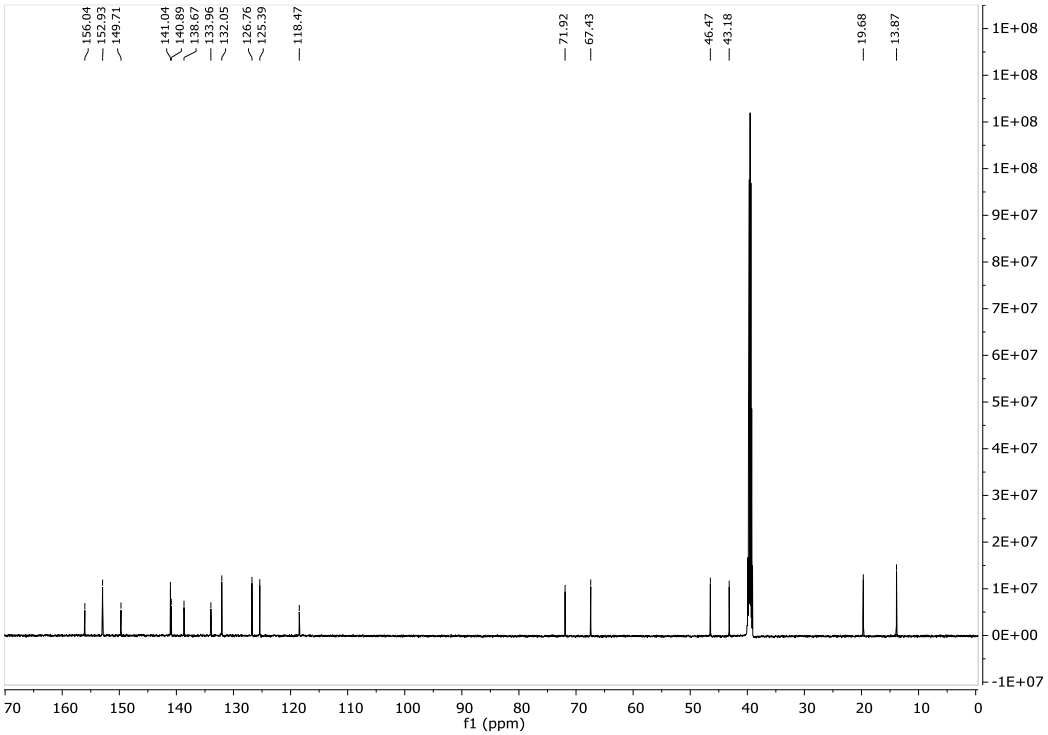
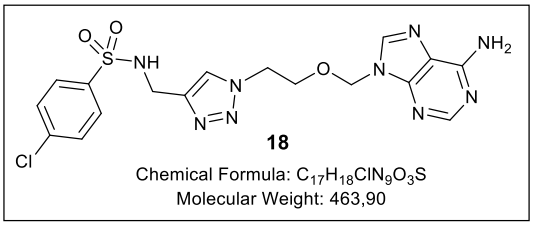
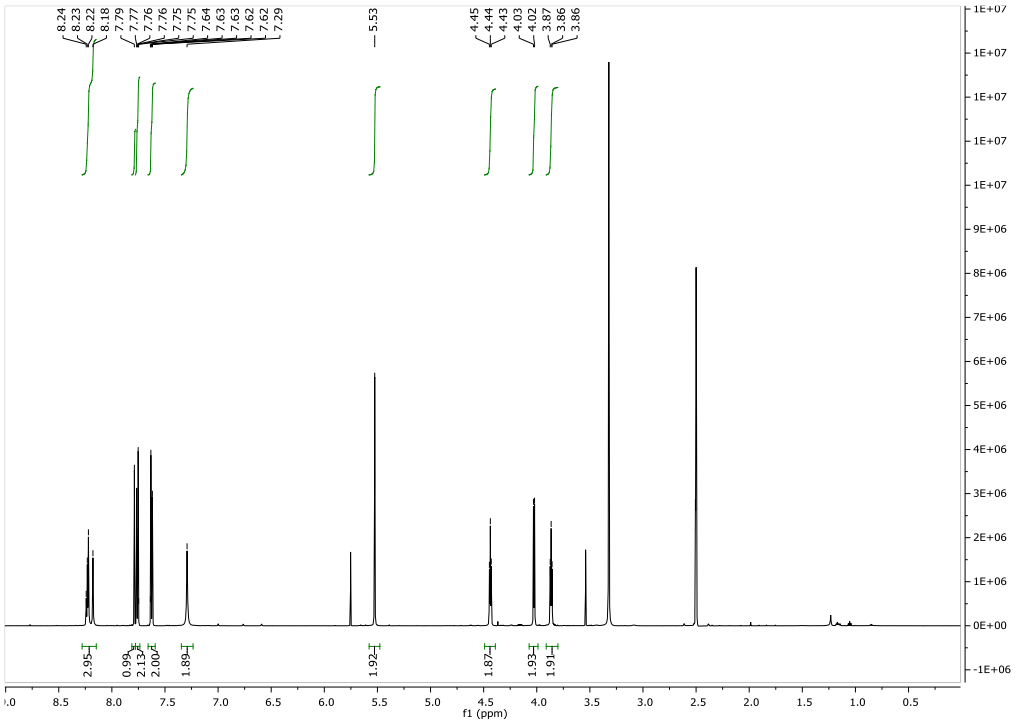

f1 (ppm)

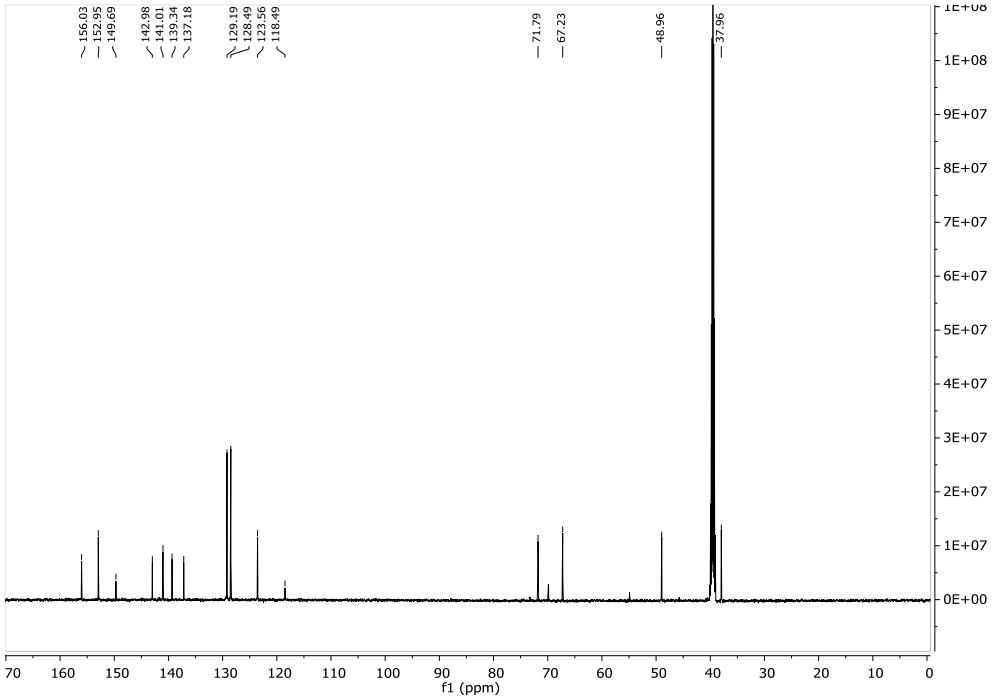


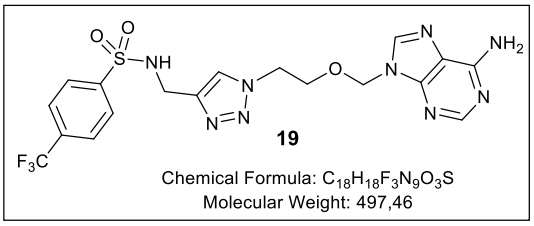
18

Chemical Formula: $C_{17}H_{18}ClN_9O_3S$

Molecular Weight: 463,90

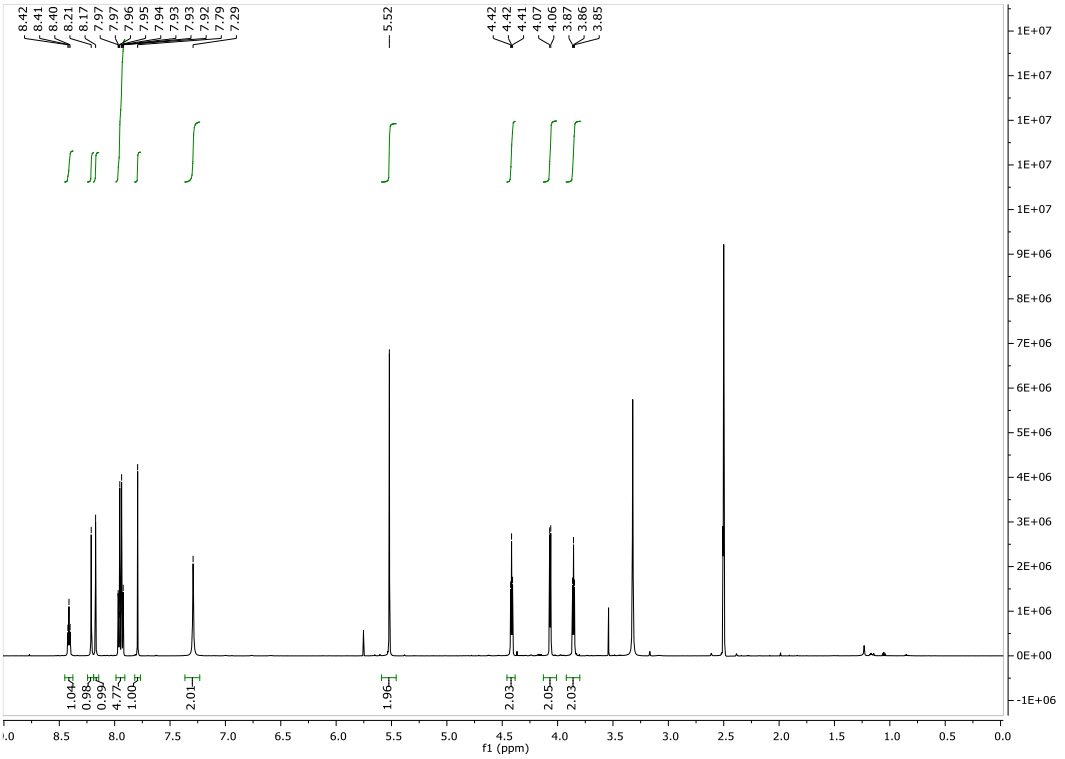


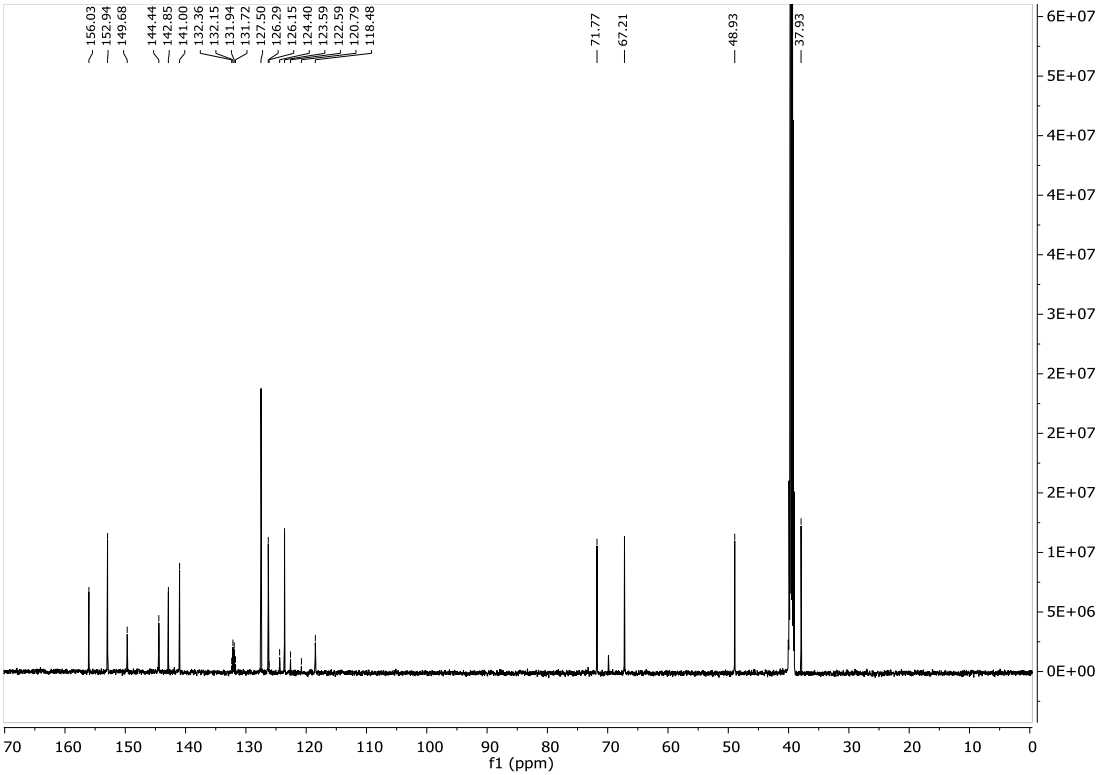




19

Chemical Formula: $C_{18}H_{18}F_3N_9O_3S$
Molecular Weight: 497,46





Expression and purification of recombinant protein

SARS-CoV-2 nsp14 (*N*7-MTase) coding sequence was cloned in fusion with a N-terminus hexa-histidine tag in pET28 plasmids.[6] The protein was expressed in *E.coli* C2566 and purified in a two-step IMAC using cobalt beads. Briefly, cells were lysed by sonication in a buffer containing 50 mM Tris pH 6.8, 300 mM NaCl, 10 mM imidazole, 5 mM $MgCl_2$, and 1 mM BME, supplemented with 0.25 mg/mL lysozyme, 10 µg/mL DNase, and 1 mM PMSF. The protein was next purified through affinity chromatography with HisPur Cobalt resin 480 (Thermo Scientific), washing with an increased concentration of salt (1 M NaCl) and imidazole (20 mM), prior to elution in buffer supplemented with 250 mM imidazole. The second step of purification was performed by size exclusion chromatography (GE Superdex S200) in a final buffer of 50 mM Tris pH 6.8, 300 mM NaCl, 5 mM $MgCl_2$, and 1 mM BME and the protein was subsequently concentrated up to 12.5 µM and conserved at -20 °C in a buffer containing 50% of glycerol.

Determination of the MTase activity by filter binding assay (FBA)

The SARS-CoV-2 nsp14 MTase assay was carried out in reaction mixture [40 mM Tris-HCl (pH 8.0), 1 mM DTT, 1 mM $MgCl_2$, 1.9 µM SAM, and 0.1 µM ^{3}H-SAM (Perkin Elmer)] in the presence of 0.7 µM $GpppAC_4$ synthetic RNA and the MTase (10 nM).[6] Briefly, the enzyme was first mixed with the compound suspended in 50% DMSO (2.5% final DMSO) before the addition of RNA substrate and ^{3}H-SAM and then incubated at 30 °C for 60 min. Reactions were stopped by their 10-fold dilution in ice-cold water. Samples were transferred to diethylaminoethyl (DEAE) filtermat (Perkin Elmer) using a Filtermat Harvester (Packard Instruments). The RNA-retaining mats were washed twice with 10 mM ammonium formate pH 8.0, twice with water and once with ethanol. They were soaked with scintillation fluid (Perkin Elmer), and ^{3}H-methyl transfer to the RNA substrates was determined using a Wallac MicroBeta TriLux liquid scintillation counter (Perkin Elmer).