

**New 1,2,3-triazole-containing hydroxamic acids
as HDACs inhibitors with chemosensitizing properties**

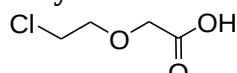
Yulia R. Aleksandrova, Valery K. Brel and Margarita E. Neganova

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1. General Methods and Materials

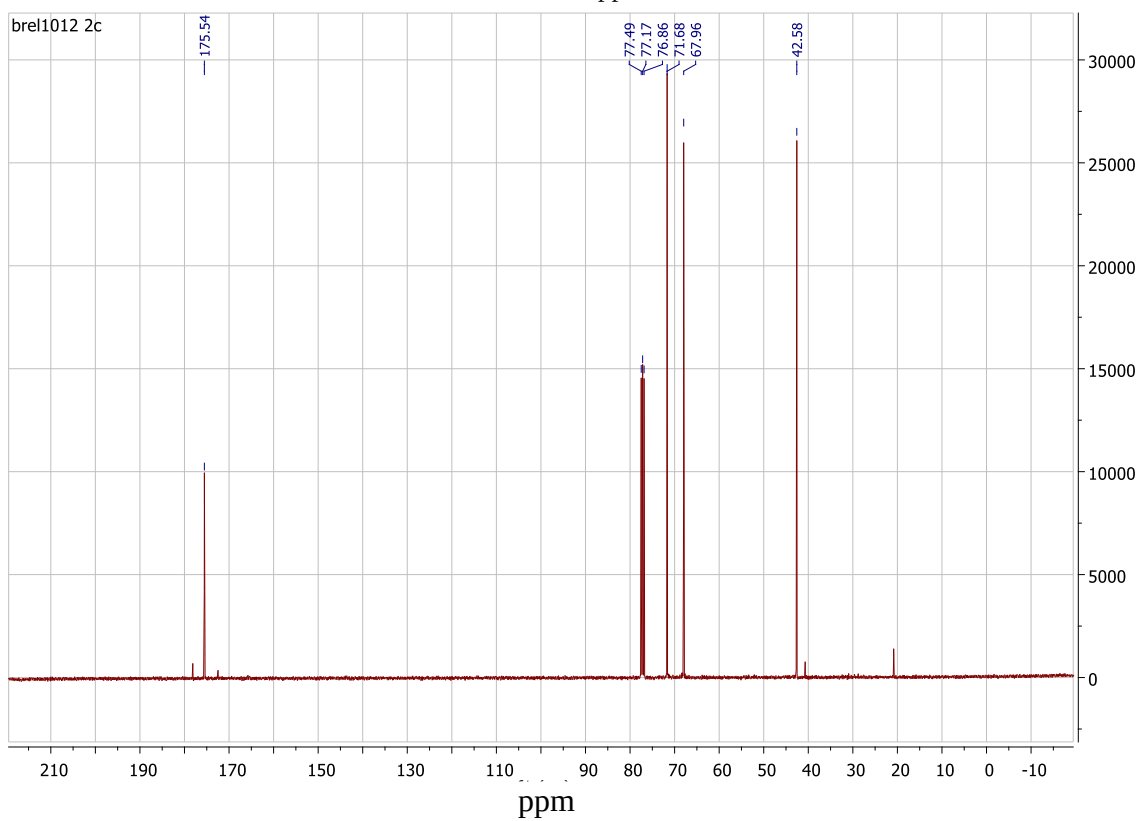
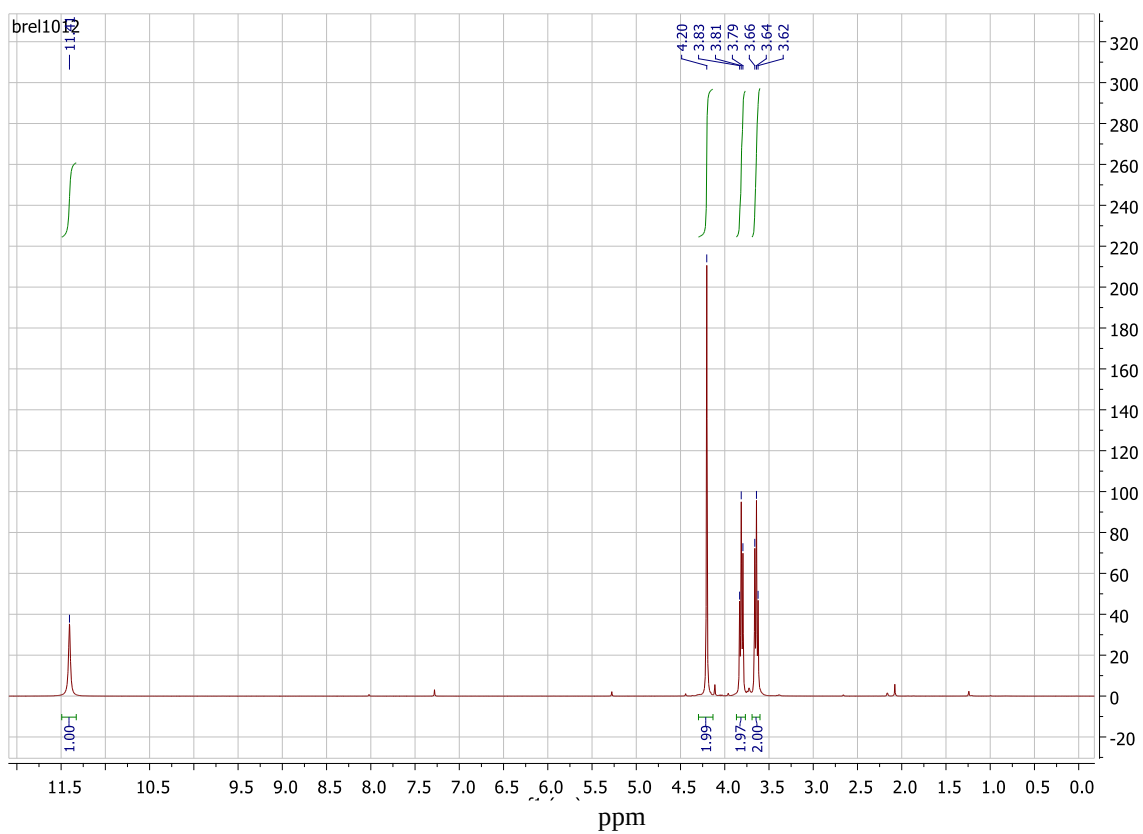
All solvents were purified before use. ^1H , ^{13}C spectra were recorded on Bruker Advance spectrometers (300 MHz and 400 MHz; Germany). Chemical shifts are reported relative to CDCl_3 or CD_3OD . Elemental analysis on C, H, and N contents were determined on a Carlo Erba 1106 device.

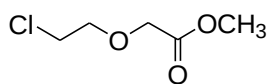
2. Synthesis



2-(2-Chloroethoxy)acetic acid (2) [S1]

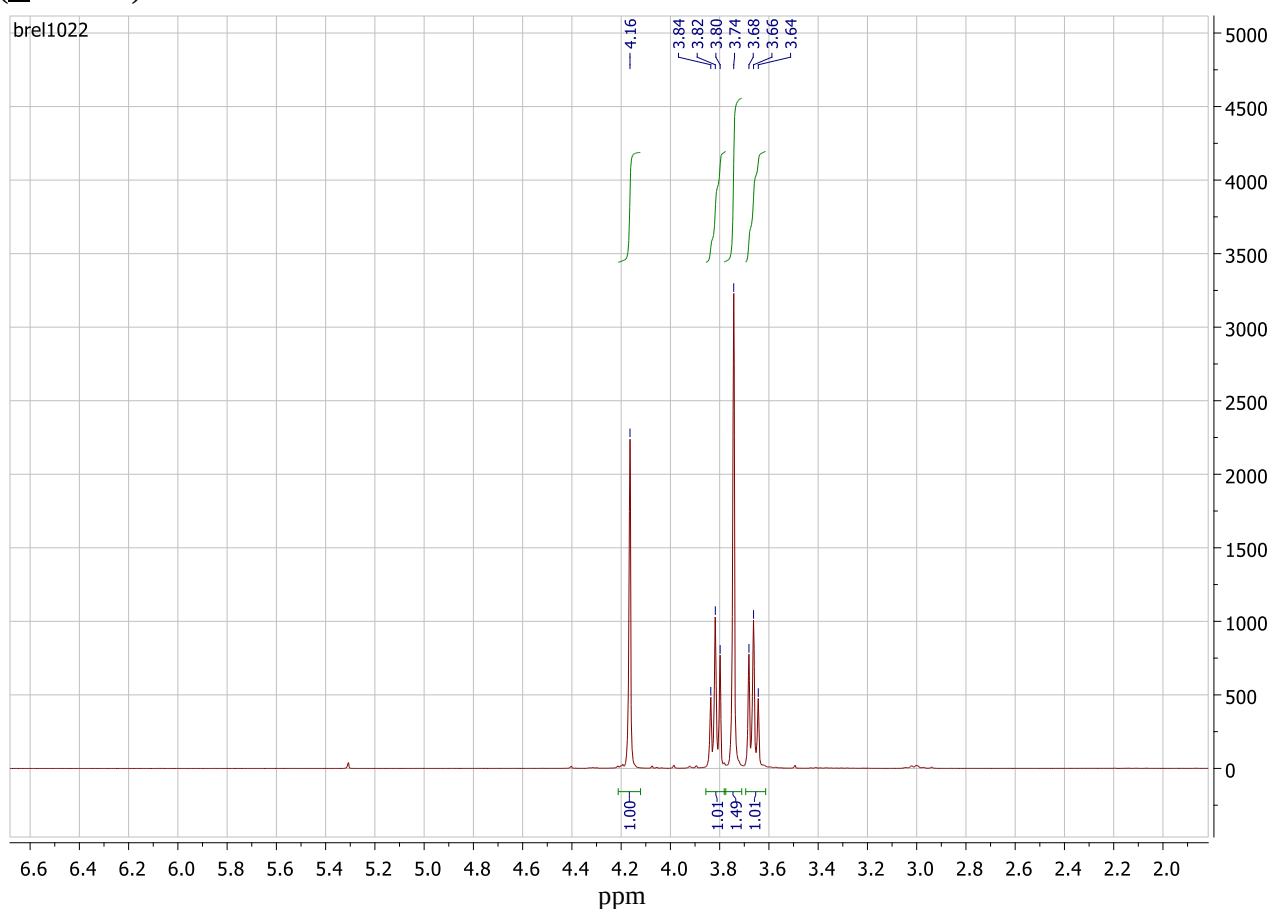
Chromium trioxide (5.94 g, 60 mmol) was dissolved in 60 mL of 1.5 M H_2SO_4 and was cooled down to 0 °C. A solution of 2-(2-chloroethoxy)ethanol (1.24 g, 10 mmol) in acetone (30 mL) was added dropwise, and the resulting mixture was allowed to warm up to room temperature followed by an overnight stirring. The acetone by evaporation and extracted the aqueous phase with dichloromethane (DCM, 3 × 50 mL). Subsequent drying of the collected organic layer was performed by Na_2SO_4 under vacuum. Yield 1.2 g (95%). The crude product was used in the following stages without additional purification. ^1H NMR (300 MHz, CDCl_3): δ 3.65 (t, 2H, J = 6.0 Hz, ClCH_2), 3.82 (t, 2H, J = 6.0 Hz, OCH_2), 4.21 (s, CH_2COOH), 11.37 (s, 1H, COOH). ^{13}C NMR (126 MHz, CDCl_3): 42.6 (CH_2Cl), 68.0 (CH_2O), 71.7 (CH_2COOH), 175.4 (COOH).

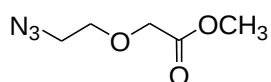
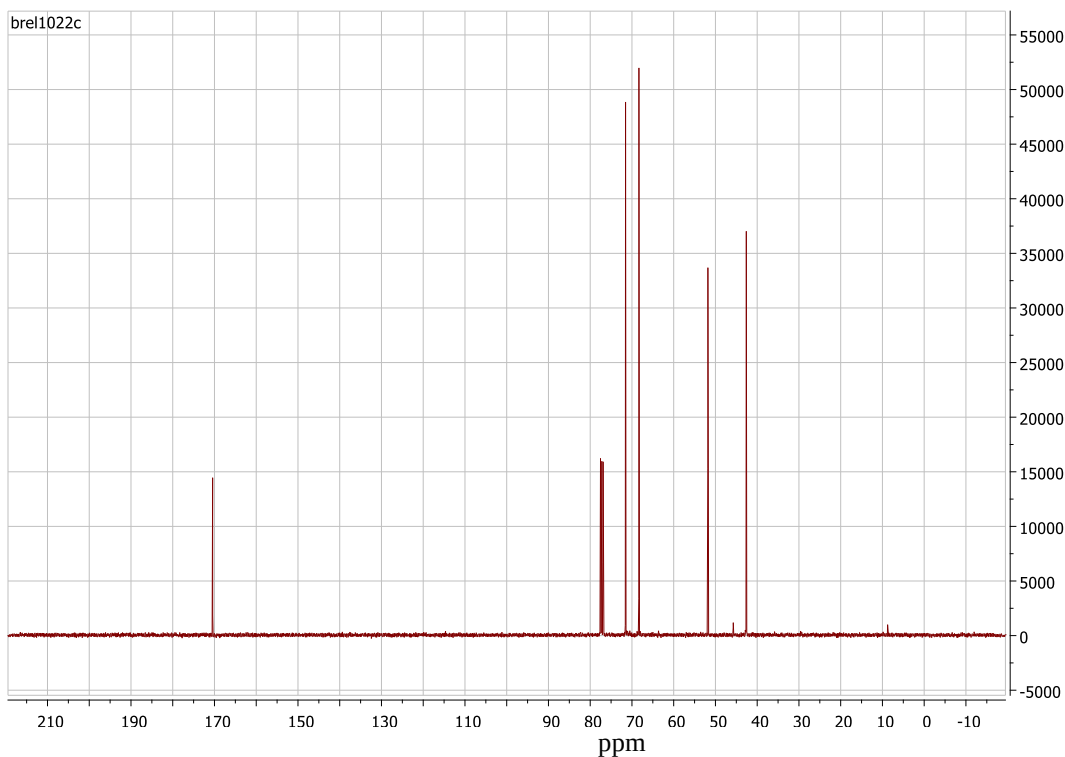




Methyl 2-(2-chloroethoxy)acetate (3) [S1]

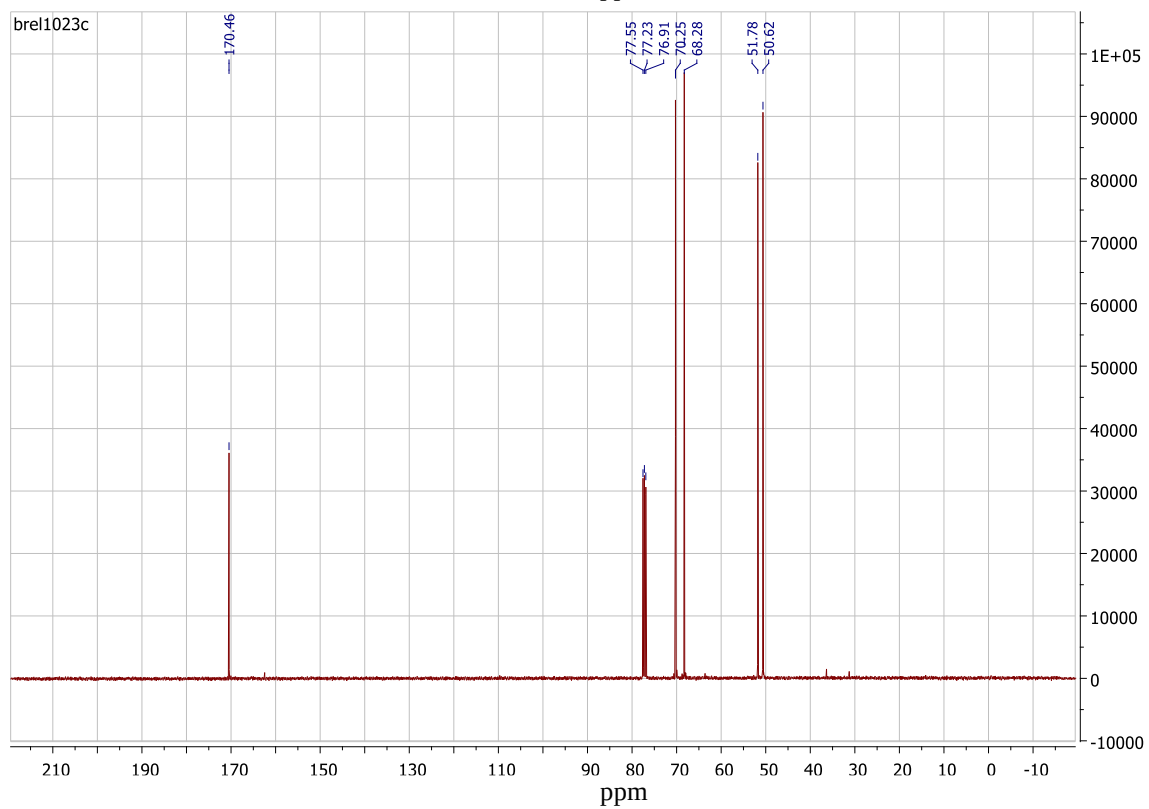
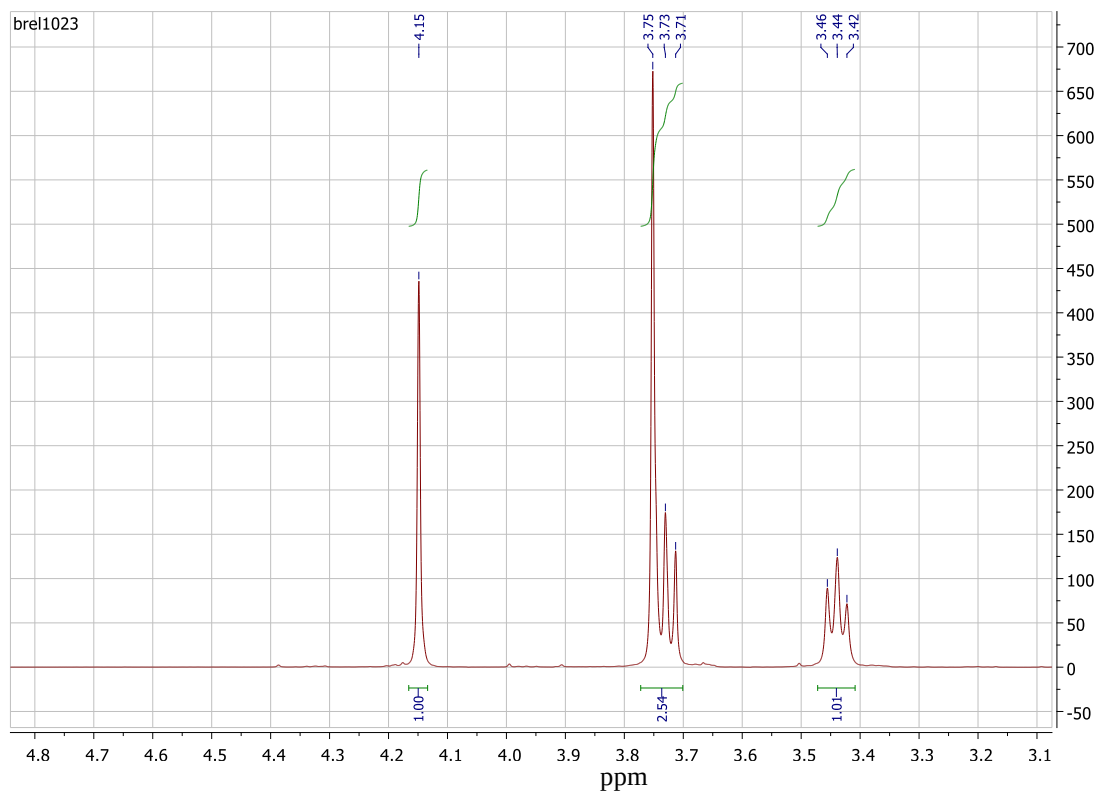
A solution of 2-(2-chloroethoxy)acetic acid 1.0 g (0.008 mol) in 10 ml anhydrous CH_2Cl_2 was treated with oxalyl chloride (5 g, 0.04 mol), and one drop of DMF was added. The mixture was stirred at room temperature for 6 h., the excess of the oxalyl chloride was removed on a rotary evaporator and the brown viscous oil residue was dissolved anhydrous CH_2Cl_2 . Reaction mixture cooled at 0°C and added mixture methanol 0.32 g (0.01 mol), Et_3N 1.01 g (0.01 mol) in anhydrous CH_2Cl_2 10 ml. Reaction mixture was allowed to warm up to room temperature followed by an overnight stirring. To reaction mixture was added 20 ml H_2O and extracted CH_2Cl_2 . The combined organic phase was dried over anhydrous Na_2SO_4 , and the solvent was removed by evaporation. The crude product was used in the following stages without additional purification. Yield 1.3 g (96%). ^1H NMR (300 MHz, CDCl_3): δ 3.66 (t, 2H, $J = 6.0$ Hz, ClCH_2), 3.74 (s, 3H, OCH_3), 3.82 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.16 (s, $\text{CH}_2\text{COOCH}_3$). ^{13}C NMR (126 MHz, CDCl_3): 42.6 (CH_2Cl), 51.8 (CH_3O), 68.3 (OCH_2), 71.5 ($\text{CH}_2\text{COOCH}_3$), 170.4 (COOCH_3).

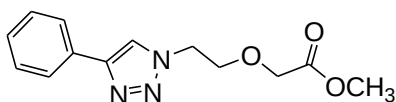




Methyl 2-(2-azidoethoxy) acetate (4) [S2]

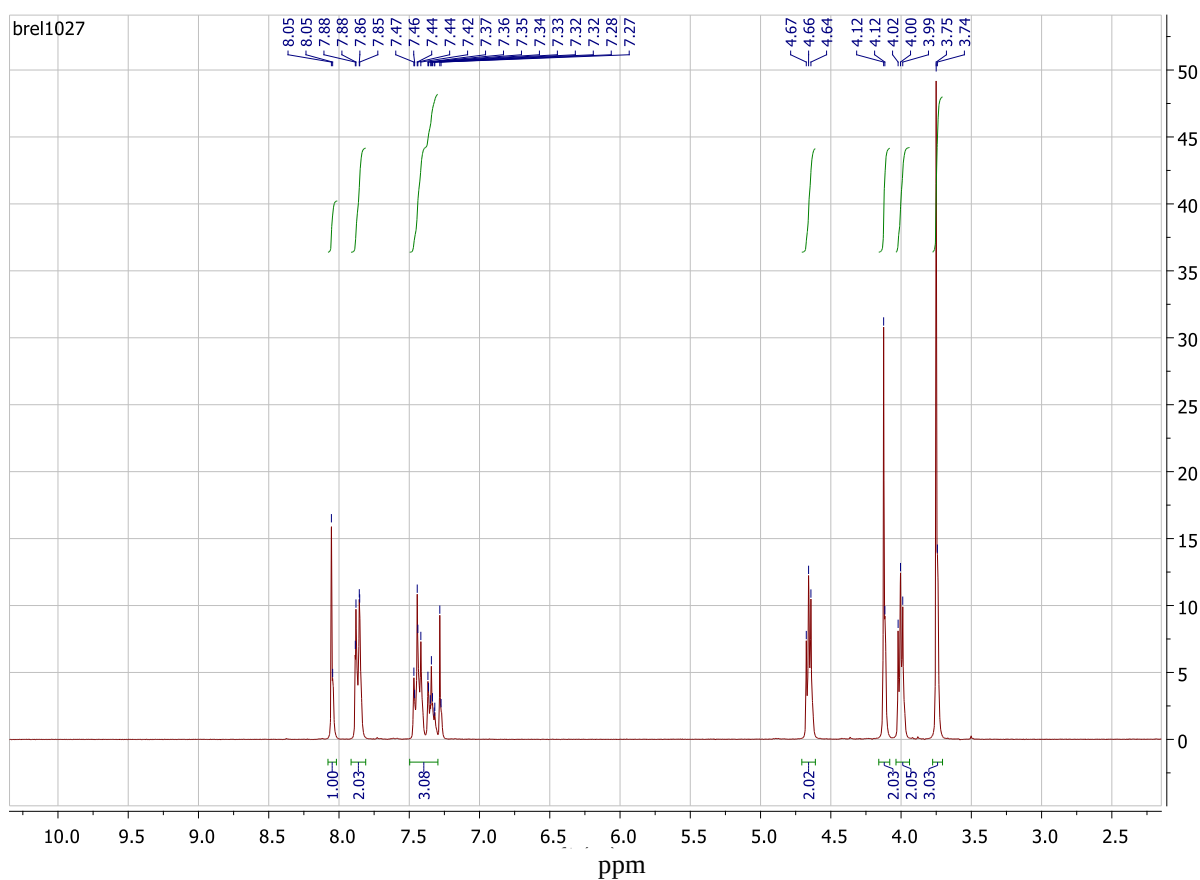
Methyl 2-(2-chloroethoxy) acetate (**3**) 2 g (0.015 mol) and sodium azide 4 g (0.06 mol), were dissolved in DMF 20 ml. The solution was stirred 12 h at 60 °C and the solvent was evaporated in a vacuum. The crude product washed by 1N-HCl, NaHCO₃ in H₂O, sat. NaCl in H₂O and was used in the following stages without additional purification. Yield 2.3 g (95%). ¹H NMR (300 MHz, CDCl₃): δ 3.44 (t, 2H, *J* = 6.0 Hz, N₃CH₂), 3.73 (t, 2H, *J* = 6.0 Hz, OCH₂), 3.75 (s, 3H, OCH₃), 4.15 (s, CH₂COOCH₃). ¹³C NMR (126 MHz, CDCl₃): 50.6 (N₃CH₂), 51.8 (OCH₃), 68.3 (CH₂O), 70.3 (CH₂COOCH₃), 170.5 (COOCH₃).

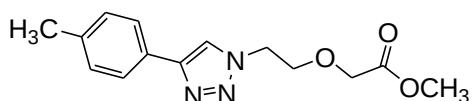
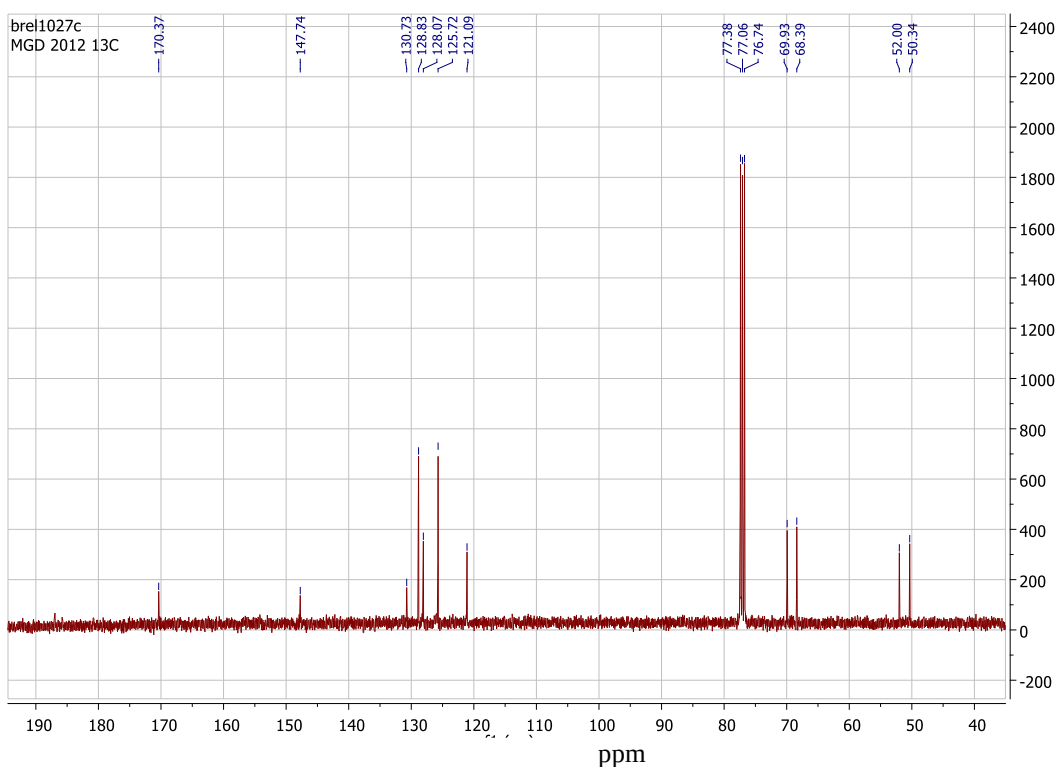




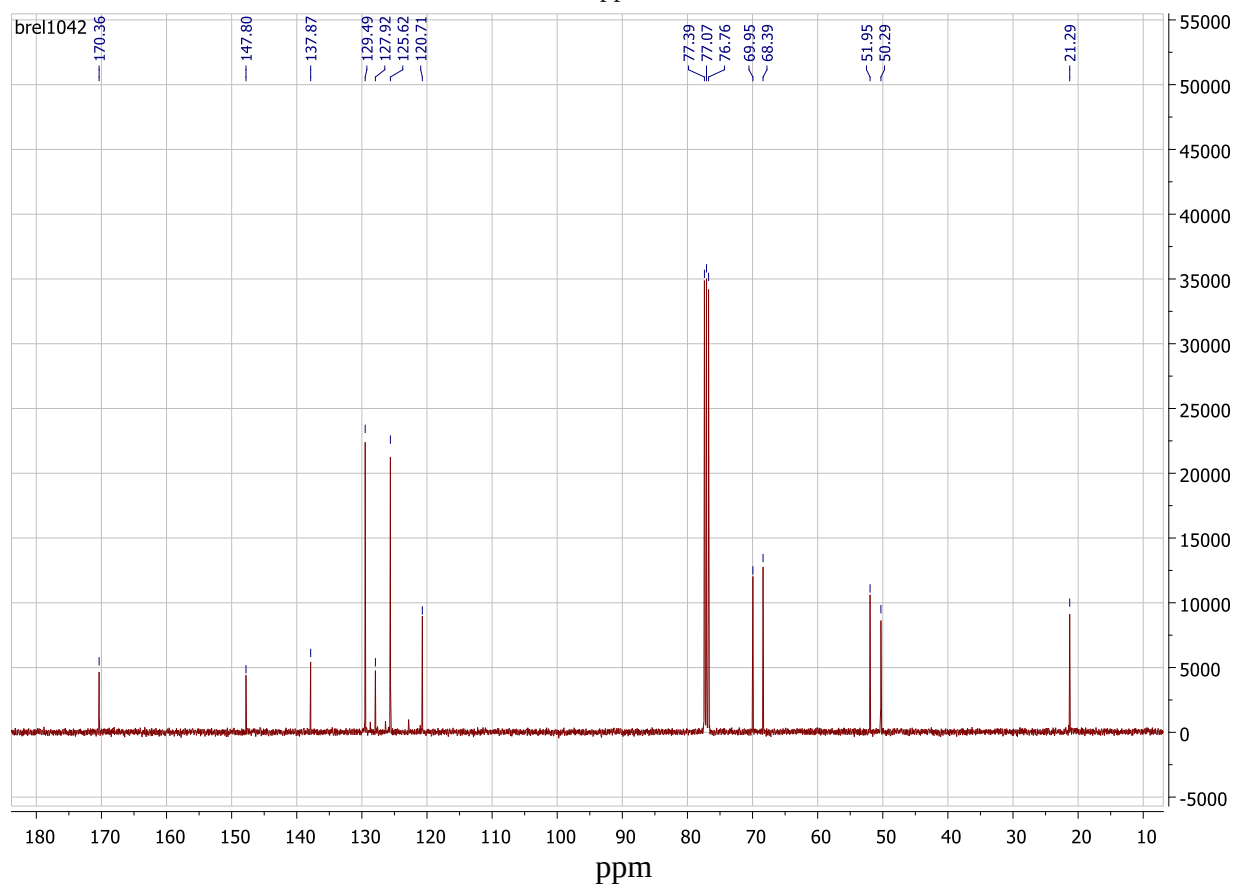
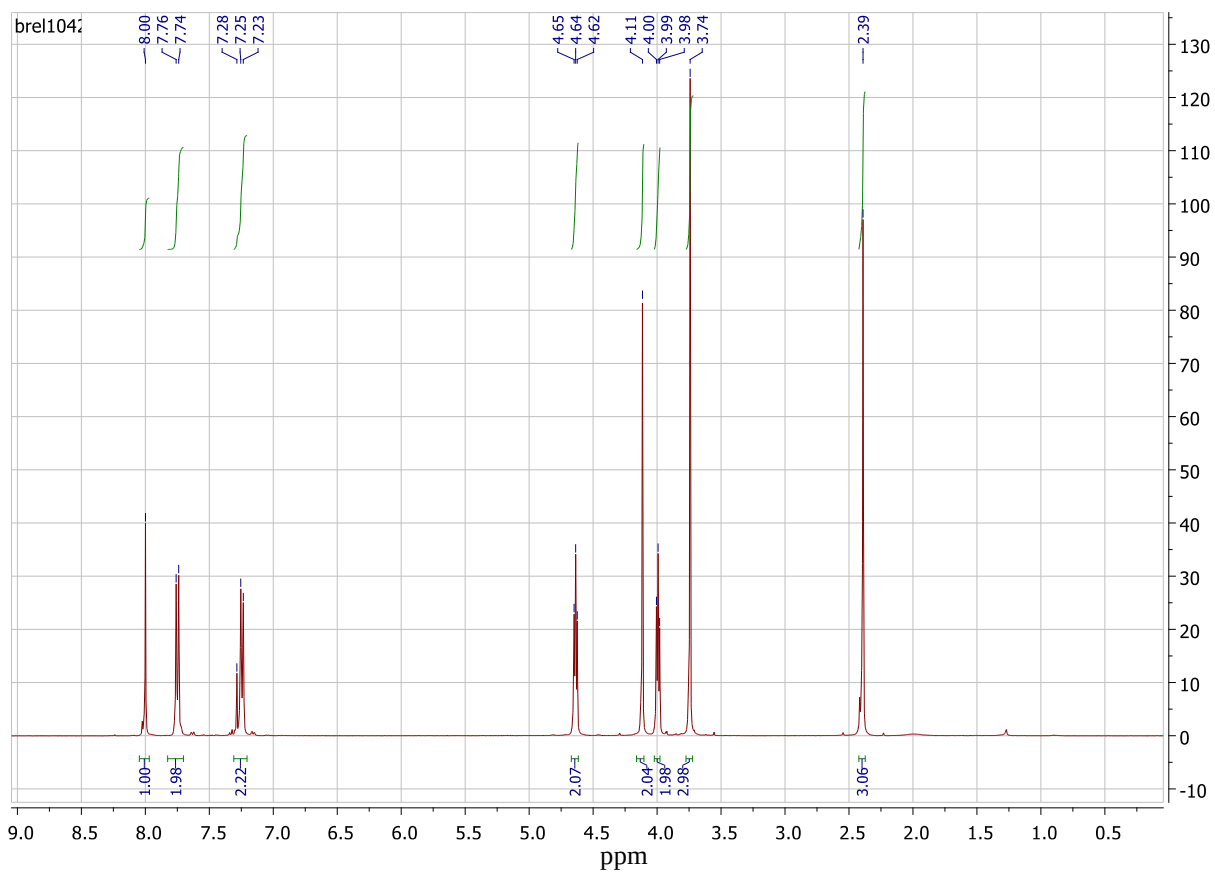
Methyl 2-[4-phenyl-1H-1,2,3-triazol-1-yl]ethoxy acetate (5a)

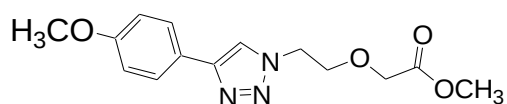
Methyl 2-(2-azidoethoxy)acetate (**4**) 1 g (0.015 mol), 5 mg CuBr, 0.1 ml DIPEA and 15 ml CH₂Cl₂ were stirred 12 h at room temperature. The solution was evaporated in a vacuum. The crude product was purification by column chromatography on SiO₂ (CHCl₃/MeOH 10:1). Yield 2.3 g (65%), yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 3.74 (s, 3H, OCH₃), 3.99 (t, 2H, *J* = 6.0 Hz, OCH₂), 4.12 (s, CH₂COOCH₃), 4.66 (t, 2H, *J* = 6.0 Hz, 1,2,3-triazole-CH₂), 7.23 (m, 1H, *para*-Ph), 7.46 (m, 2H, *meta*-Ph), 7.85 (m, 2H, *ortho*-Ph), 8.05 (s, 1H, =CH). ¹³C NMR (126 MHz, CDCl₃): 50.3 (1,2,3-triazole-CH₂), 52.0 (OCH₃), 68.4 (CH₂O), 69.9 (CH₂COOCH₃), 121.1 (=CH, 1,2,3-triazole), 125.7 (Ph), 128.1 (Ph), 128.8 (Ph), 130.7 (Ph), 147.7 (C=, 1,2,3-triazole), 170.4 (COOCH₃). Elemental analysis calcd. (%) for C₁₃H₁₅N₃O₃: C, 59.77; H, 5.75; N, 16.09; found: C, 59.12; H, 5.63; N, 16.4.



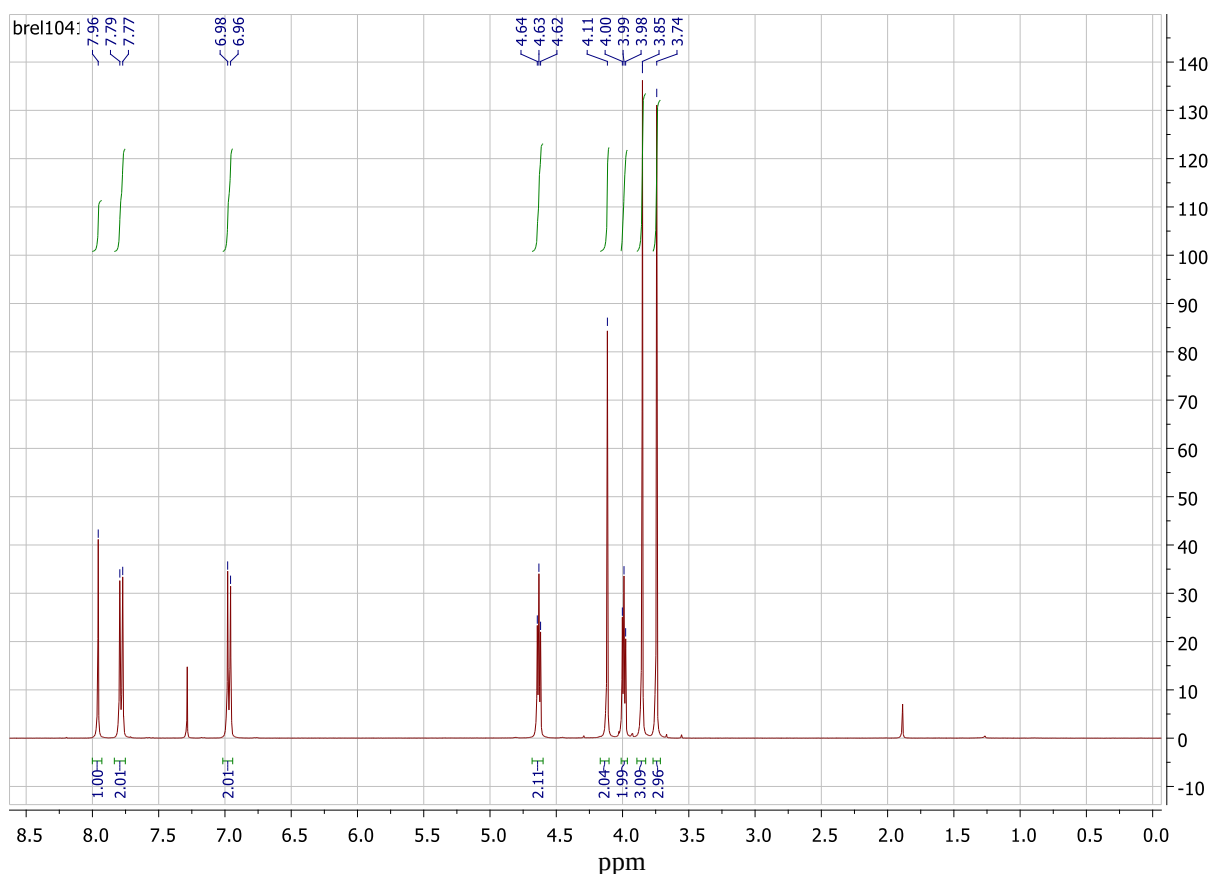


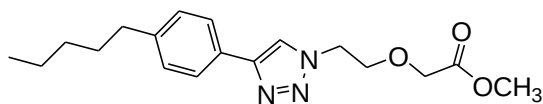
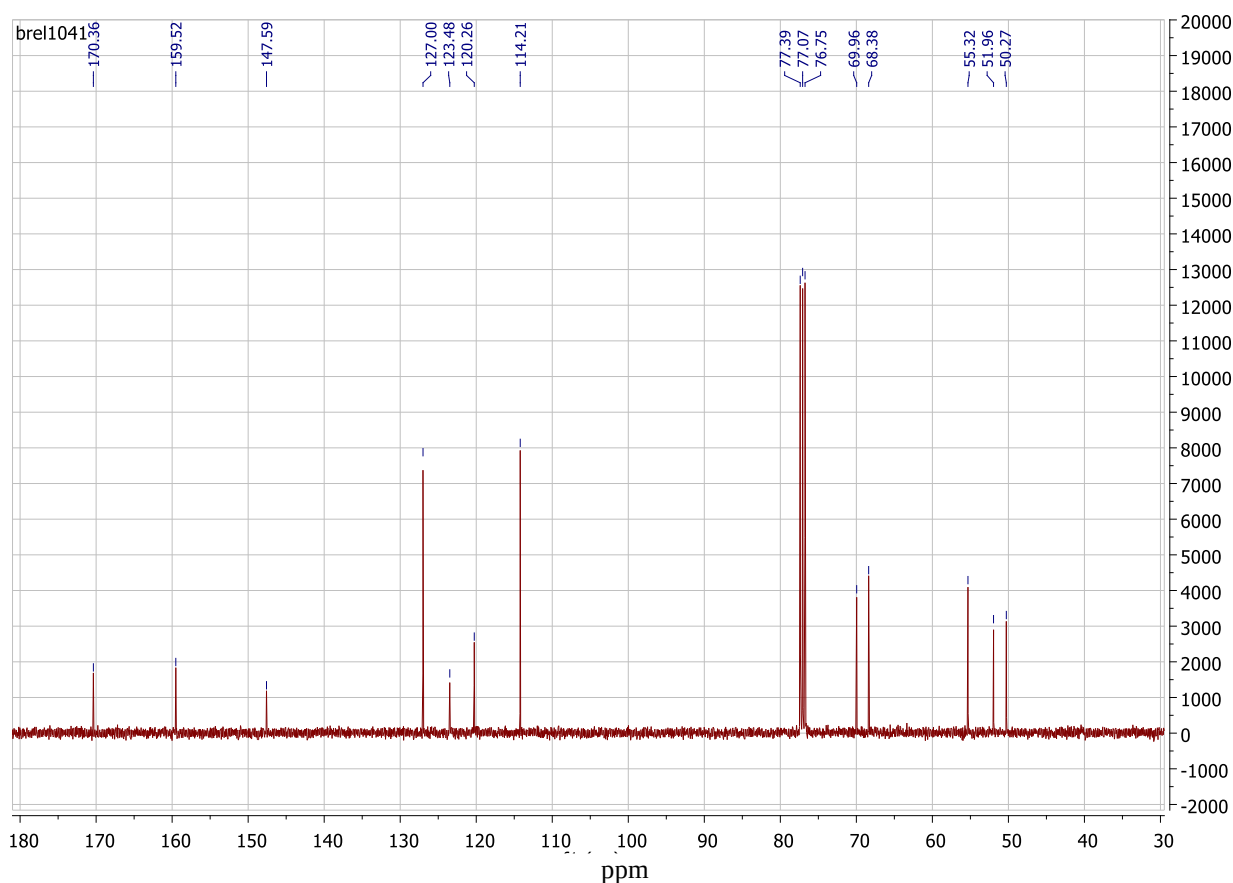
Methyl 2-[4-(4-*p*-tolyl)-1*H*-1,2,3-triazol-1-yl]ethoxy acetate (5b) was prepared similarly of (5a). Yield: 73%, yellow oil. ¹H NMR (300 MHz, CDCl₃): δ 2.39 (s, 3H, CH₃C₆H₄), 3.74 (s, 3H, OCH₃), 3.99 (t, 2H, *J* = 6.0 Hz, OCH₂), 4.12 (s, CH₂COOCH₃), 4.64 (t, 2H, *J* = 6.0 Hz, 1,2,3-triazole-CH₂), 7.24 (d, 2H, *J* = 6.0 Hz, *meta*-Ph), 7.75 (d, 2H, *J* = 6.0 Hz, *ortho*-Ph), 8.0 (s, 1H, =CH 1,2,3-triazole). ¹³C NMR (126 MHz, CDCl₃): 21.3 (CH₃-C₆H₄), 50.3 (1,2,3-triazole-CH₂), 52.0 (OCH₃), 68.4 (CH₂O), 69.9 (CH₂COOCH₃), 120.7 (=CH, 1,2,3-triazole), 125.6 (Ph), 127.9 (Ph), 129.5 (Ph), 137.9 (Ph), 147.8 (C=, 1,2,3-triazole), 170.4 (COOCH₃). Elemental analysis calcd. (%) for C₁₄H₁₇N₃O₃: C, 61.09; H, 6.18; N, 15.27; found: C, 61.56; H, 6.02; N, 15.85.



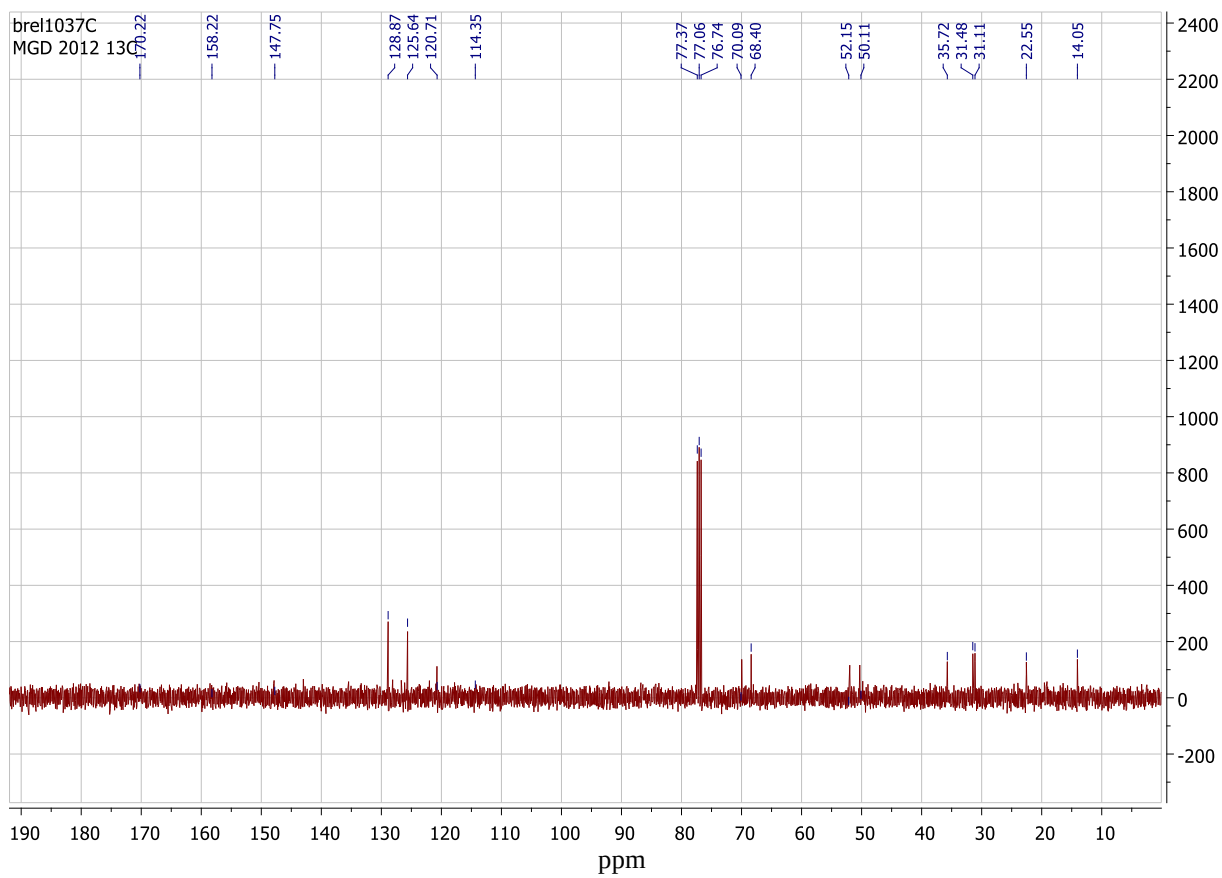
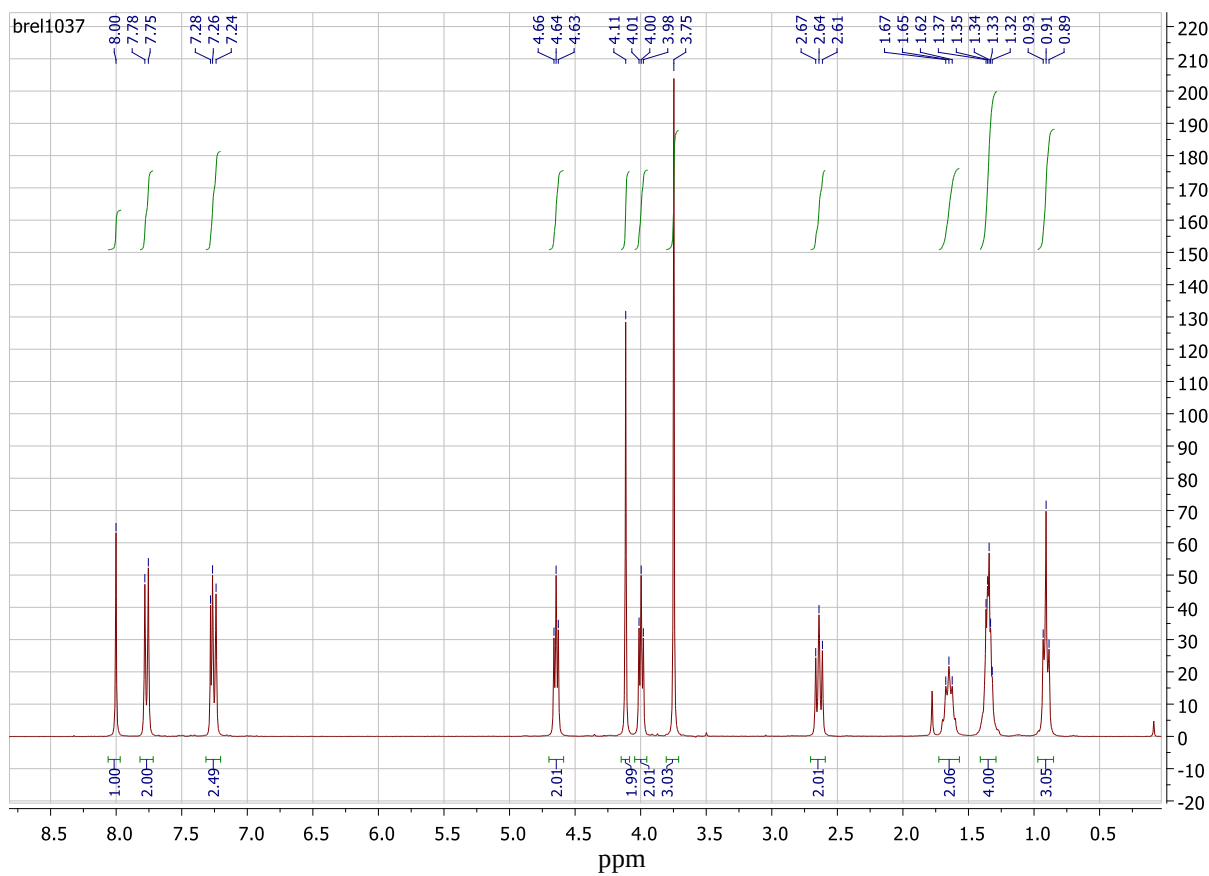


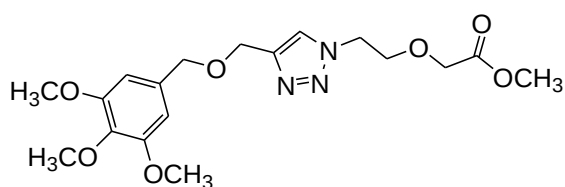
Methyl 2-[4-(4-*p*-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]ethoxy acetate (5c) was prepared similarly to **5a**. Yield: 65%, yellow oil. ^1H NMR (300 MHz, CDCl_3): δ 3.74 (s, 3H, OCH_3), 3.85 (s, 3H, $\text{CH}_3\text{OC}_6\text{H}_4$), 3.99 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.11 (s, $\text{CH}_2\text{COOCH}_3$), 4.63 (t, 2H, $J = 6.0$ Hz, 1,2,3-triazole- CH_2), 6.97 (d, 2H, $J = 6.0$ Hz, *meta*-Ph), 7.76 (d, 2H, $J = 6.0$ Hz, *ortho*-Ph), 7.96 (s, 1H, $=\text{CH}$ 1,2,3-triazole). ^{13}C NMR (126 MHz, CDCl_3): 50.3 (1,2,3-triazole- CH_2), 52.0 (OCH_3), 55.3 ($\text{CH}_3\text{O}-\text{C}_6\text{H}_4$), 68.4 (CH_2O), 70.0 ($\text{CH}_2\text{COOCH}_3$), 114.2 ($=\text{CH}$, 1,2,3-triazole), 120.3 (Ph), 123.5 (Ph), 127.0 (Ph), 147.6 (Ph), 159.5 ($\text{C}=\text{N}$, 1,2,3-triazole), 170.4 (COOCH_3). Elemental analysis calcd. (%) for $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_4$: C, 57.73; H, 5.84; N, 14.43; found: C, 57.12; H, 5.54; N, 14.12.



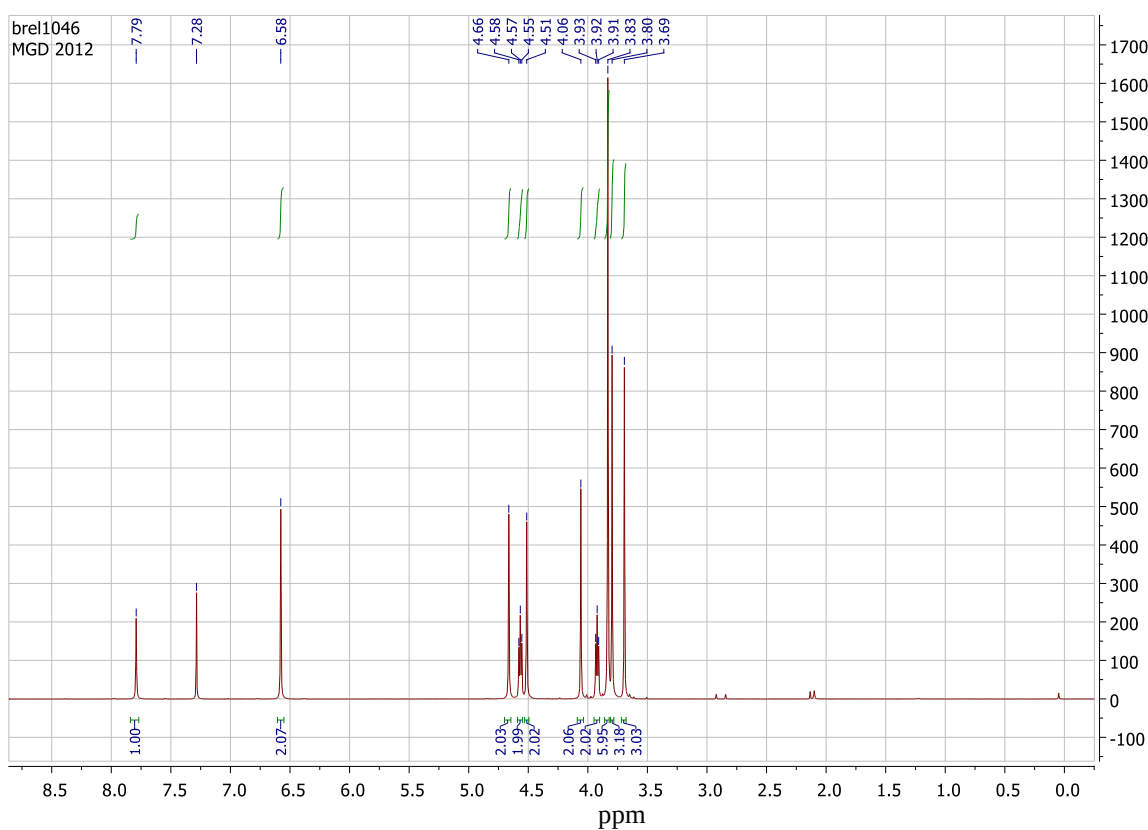


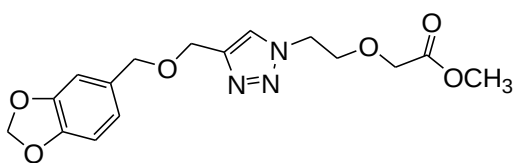
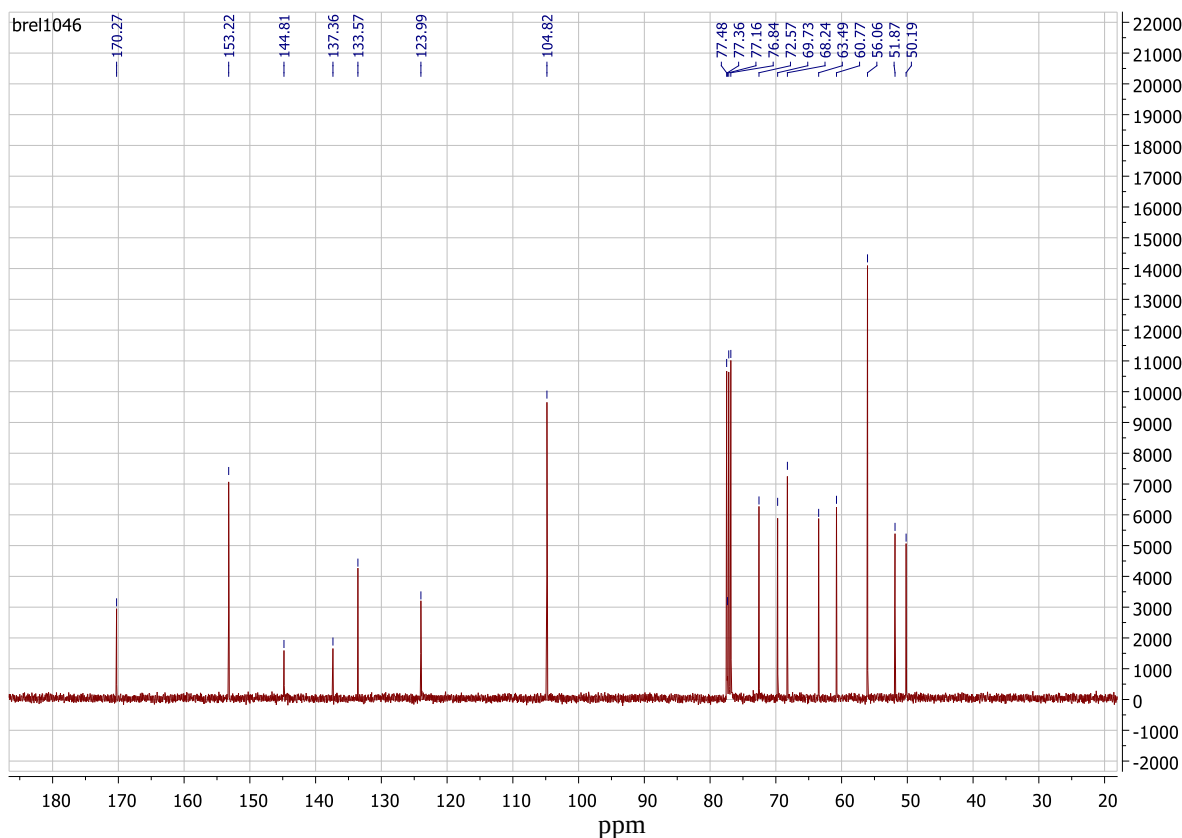
Methyl 2-[4-(4-*p*-pentylphenyl)-1*H*-1,2,3-triazol-1-yl]ethoxy acetate (5d) was prepared similarly to **5a**. Yield: 70%, yellow oil. ^1H NMR (300 MHz, CDCl_3): δ 0.93 (t, 3H, J = 6.0 Hz, CH_3), 1.32–1.37 (m, 4 H, 2 CH_2), 1.60–1.70 (m, 2H, CH_2), 2.64 (t, 2H, J = 9.0 Hz, CH_2), 3.75 (s, 3H, OCH_3), 4.0 (t, 2H, J = 6.0 Hz, OCH_2), 4.11 (s, $\text{CH}_2\text{COOCH}_3$), 4.64 (t, 2H, J = 6.0 Hz, 1,2,3-triazole- CH_2), 7.26 (d, 2H, J = 6.0 Hz, *meta*-Ph), 7.76 (d, 2H, J = 6.0 Hz, *ortho*-Ph), 8.00 (s, 1H, =CH 1,2,3-triazole). ^{13}C NMR (126 MHz, CDCl_3): 14.1 (CH_3), 22.6 (CH_2), 31.1 (CH_2), 31.5 (CH_2), 35.7 (CH_2), 50.3 (1,2,3-triazole- CH_2), 52.0 (CH_3O), 68.4 (CH_2O), 69.9 ($\text{CH}_2\text{COOCH}_3$), 120.7 (=CH, 1,2,3-triazole), 125.6 (Ph), 128.1 (Ph), 128.9 (Ph), 143.0 (Ph), 147.8 ($\text{C}=\text{N}$, 1,2,3-triazole), 170.4 (COOCH_3). Elemental analysis calcd. (%) for $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_3$: C, 65.26; H, 7.55; N, 12.69; found: C, 65.02; H, 7.63; N, 12.56.



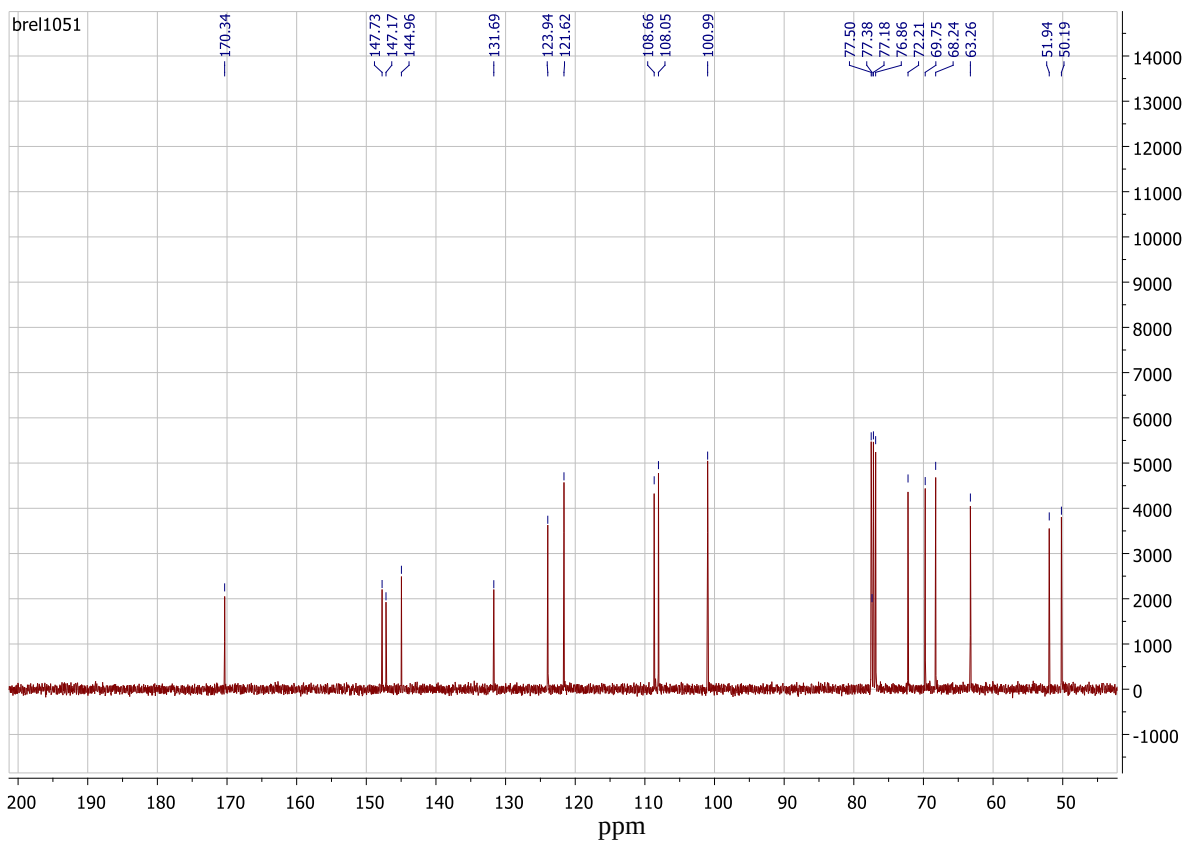
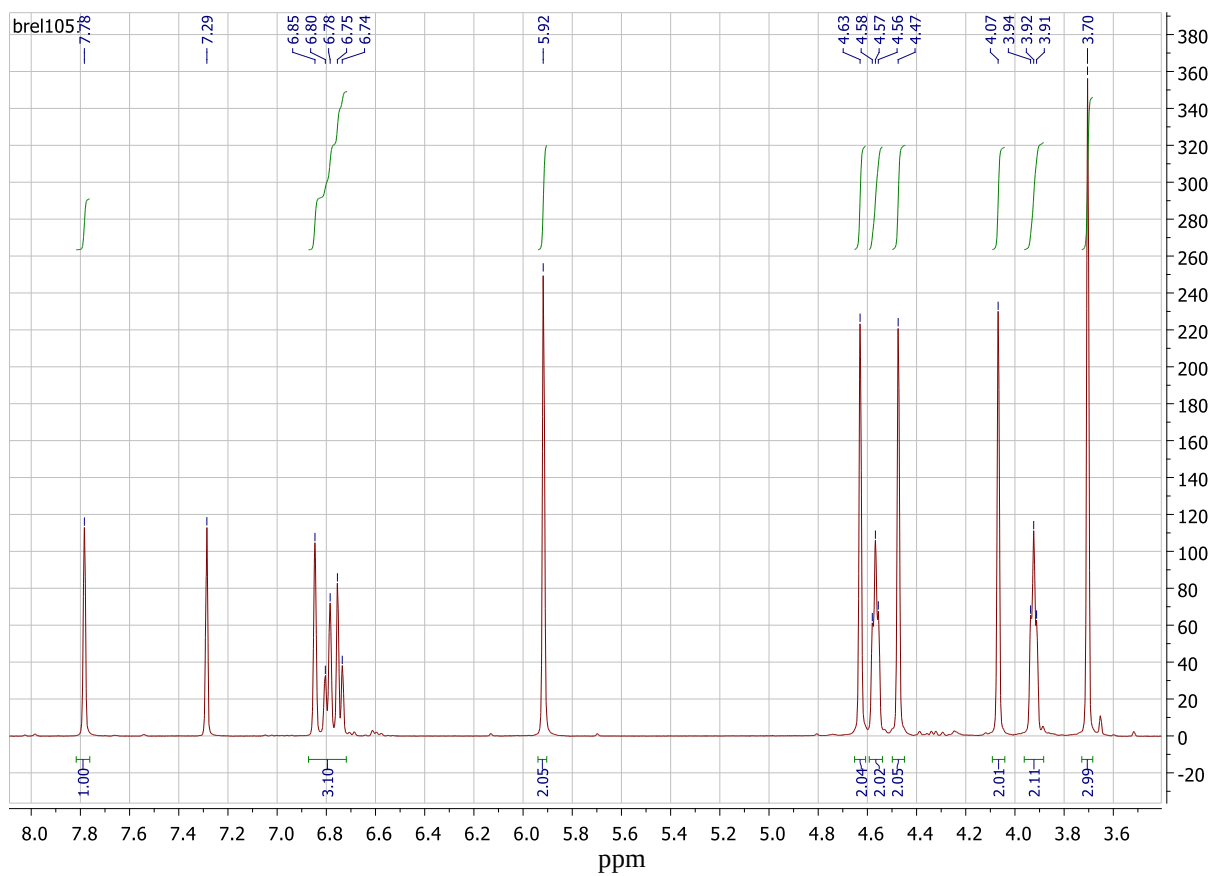


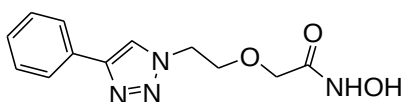
Methyl 2-[(4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl]ethoxy acetate (5e) was prepared similarly to 5a. Yield: 63%, yellow oil. ^1H NMR (300 MHz, CDCl_3): δ 3.69 (s, 3H, COOCH_3), 3.80 (s, 3H, OCH_3), 3.83 (s, 6H, 2OCH_3), 3.92 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.06 (s, $\text{CH}_2\text{COOCH}_3$), 4.51 (s, 2H, CH_2O), 4.55 (t, 2H, $J = 6.0$ Hz, 1,2,3-triazole- CH_2), 4.66 (s, 2H, CH_2O), 6.58 (s, 2H, *ortho*-Ph), 7.79 (s, 1H, $=\text{CH}$ 1,2,3-triazole). ^{13}C NMR (126 MHz, CDCl_3): 50.2 (1,2,3-triazole- CH_2), 52.0 (OCH_3), 56.1 ($3\text{CH}_3\text{O}$, Ar), 60.8 (CH_2O), 63.5 (CH_2O), 68.4 (CH_2O), 68.4 (CH_2O), 72.6 ($\text{CH}_2\text{COOCH}_3$), 104.8 ($=\text{CH}$, 1,2,3-triazole), 124.0 (Ph), 133.6 (Ph), 137.4 (Ph), 144.8 (Ph), 153.2 ($\text{C}=\text{N}$, 1,2,3-triazole), 170.3 (COOCH_3). Elemental analysis calcd. (%) for $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_7$: C, 54.68; H, 6.33; N, 10.63; found: C, 54.32; H, 6.63; N, 10.23.



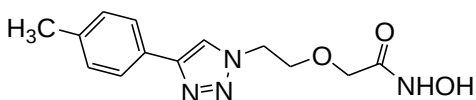
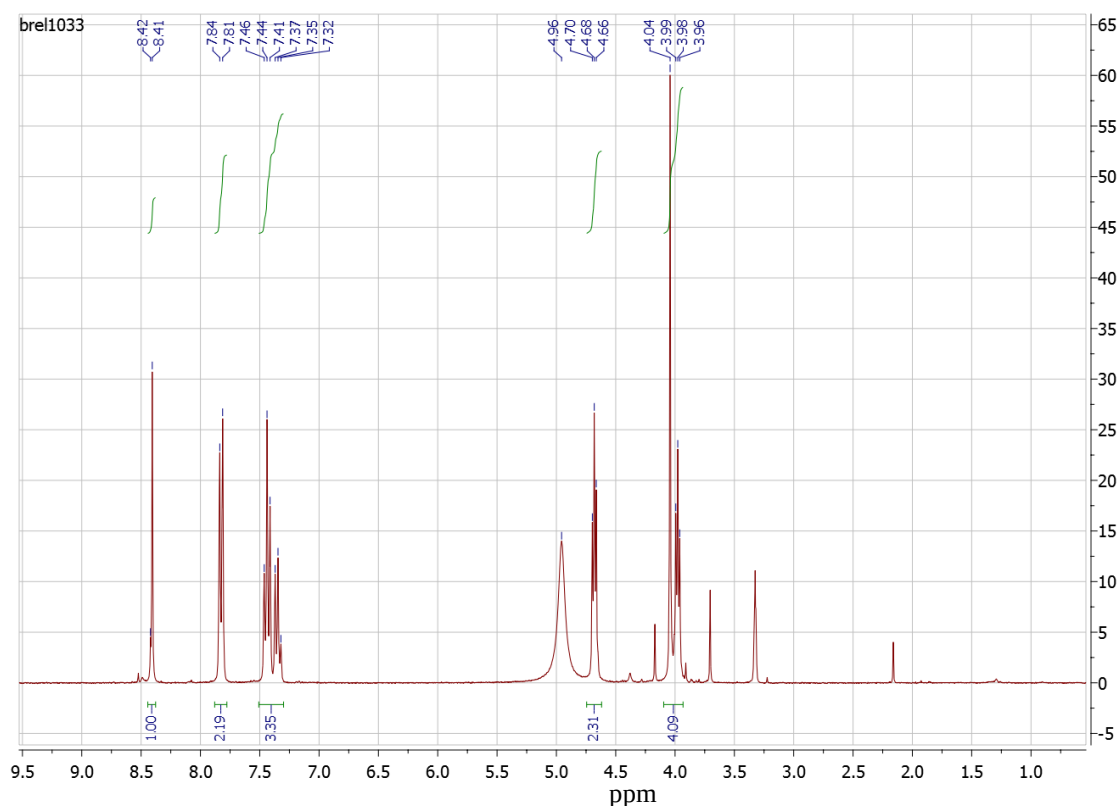


Methyl 2-[(4-((1,3-benzodioxol-5-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl]ethoxyacetate (5f) was prepared similarly to **5a**. Yield: 65%, yellow oil. ^1H NMR (300 MHz, CDCl_3): δ 3.7 (s, 3H, COOCH_3), 3.92 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.07 (s, $\text{CH}_2\text{COOCH}_3$), 4.47 (s, 2H, CH_2O), 4.57 (t, 2H, $J = 6.0$ Hz, 1,2,3-triazole- CH_2), 4.63 (s, 2H, CH_2O), 5.92 (s, 2H, OCH_2O), 6.74 – 6.85 (m, 3H, Ar), 7.78 (s, 1H, $=\text{CH}$ 1,2,3-triazole). ^{13}C NMR (126 MHz, CDCl_3): 50.2 (1,2,3-triazole- CH_2), 52.0 (OCH_3), 63.3 (CH_2O), 68.2 (CH_2O), 69.7 (CH_2O), 72.2 ($\text{CH}_2\text{COOCH}_3$), 101.0 (OCH_2O , 1,2,3-triazole), 108.1 ($=\text{CH}$, 1,2,3-triazole), 108.7 (Ph), 121.6 (Ph), 124.0 (Ph), 132.0 (Ph), 145.0 (Ph), 147.2 (Ph), 147.7 ($\text{C}=\text{N}$, 1,2,3-triazole), 170.3 (COOCH_3). Elemental analysis calcd. (%) for $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_6$: C, 55.01; H, 5.44; N, 12.03; found: C, 54.42; H, 5.64; N, 12.49.

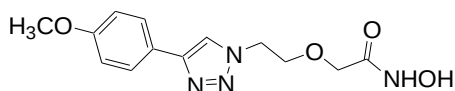
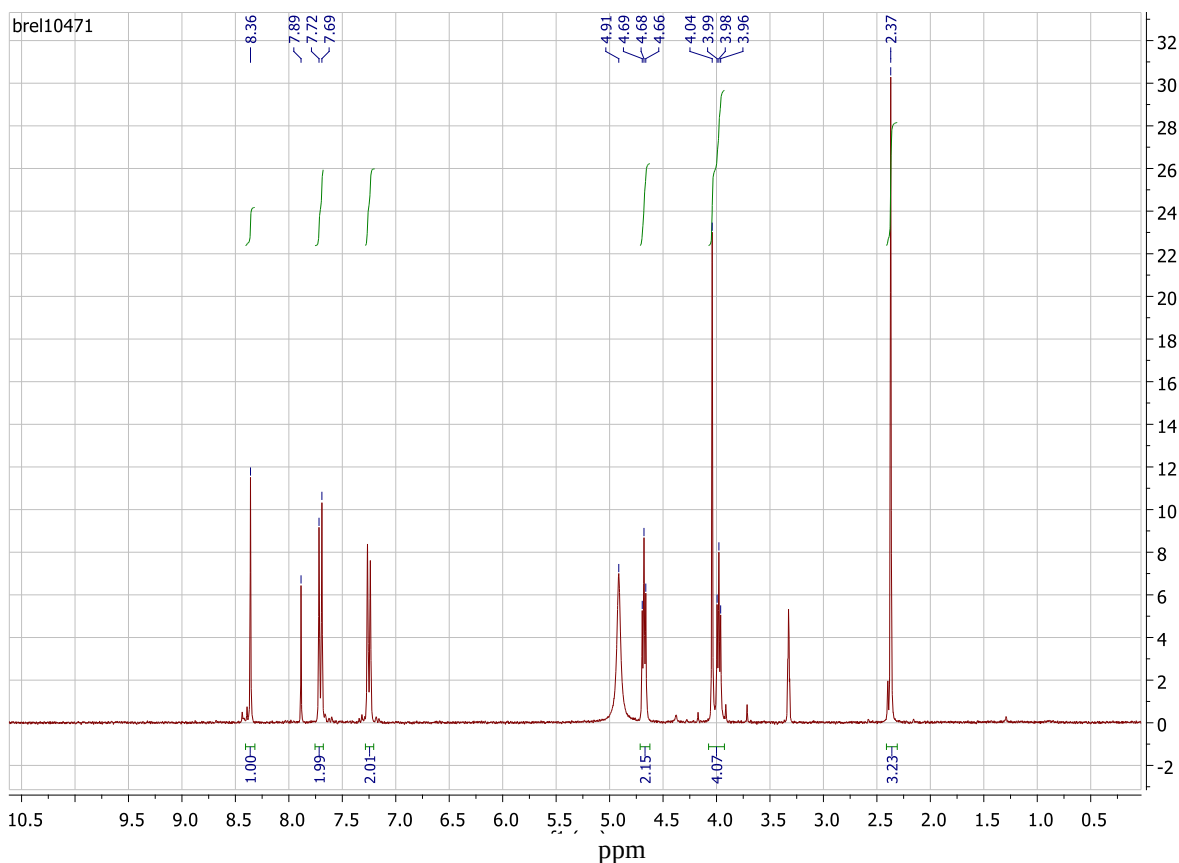




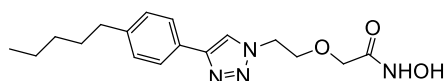
N-Hydroxy-2-[(4-phenyl)-1H-1,2,3-triazol-1-yl]ethoxyacetamide (6a). Methyl 2-[4-phenyl-1H-1,2,3-triazol-1-yl]ethoxy acetate (**5a**) (0.1 g, 0.015 mol), 0.066 g (0.002 mol) H_2NOH in 15 ml MeOH and one drop MeONa in MeOH were stirred 24 h at room temperature. The solution was evaporated in a vacuum. The crude product was recrystallization from hexane, white crystals b.p. 146-147°C, yield: 72%. ^1H NMR (300 MHz, CD_3OD): δ 3.74 (s, 3H, OCH_3), 3.99 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.12 (s, $\text{CH}_2\text{COOCH}_3$), 4.66 (t, 2H, $J = 6.0$ Hz, 1,2,3-triazole- CH_2), 7.23 (m, 1H, *para*-Ph), 7.46 (m, 2H, *meta*-Ph), 7.85 (m, 2H, *ortho*-Ph), 8.05 (s, 1H, =CH). ^{13}C NMR (126 MHz, CD_3OD): 50.3 (1,2,3-triazole- CH_2), 52.0 (OCH_3), 68.4 (CH_2O), 69.9 ($\text{CH}_2\text{COOCH}_3$), 121.1 (=CH, 1,2,3-triazole), 125.7 (Ph), 128.1 (Ph), 128.8 (Ph), 130.7 (Ph), 147.7 ($\text{C}=\text{N}$, 1,2,3-triazole), 170.4 (COOCH_3). Elemental analysis calcd. (%) for $\text{C}_{12}\text{H}_{14}\text{N}_4\text{O}_3$: C, 54.88; H, 5.38; N, 21.44; found: C, 54.12; H, 5.63; N, 21.04.



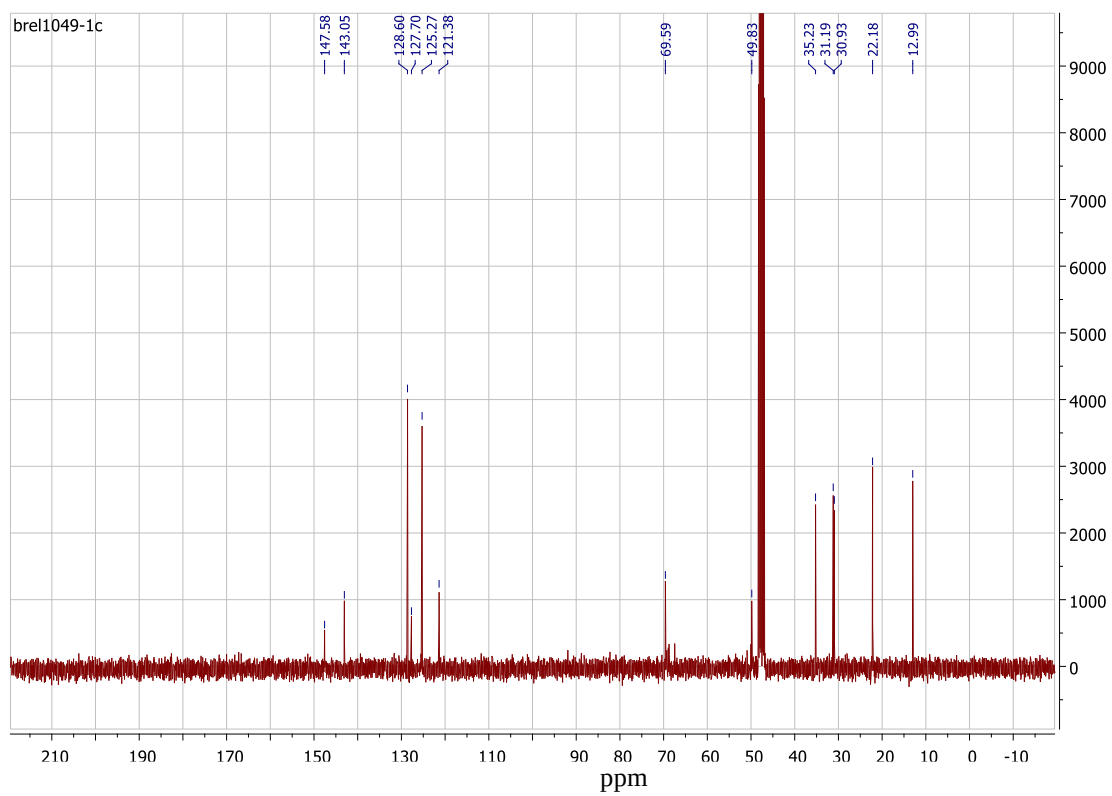
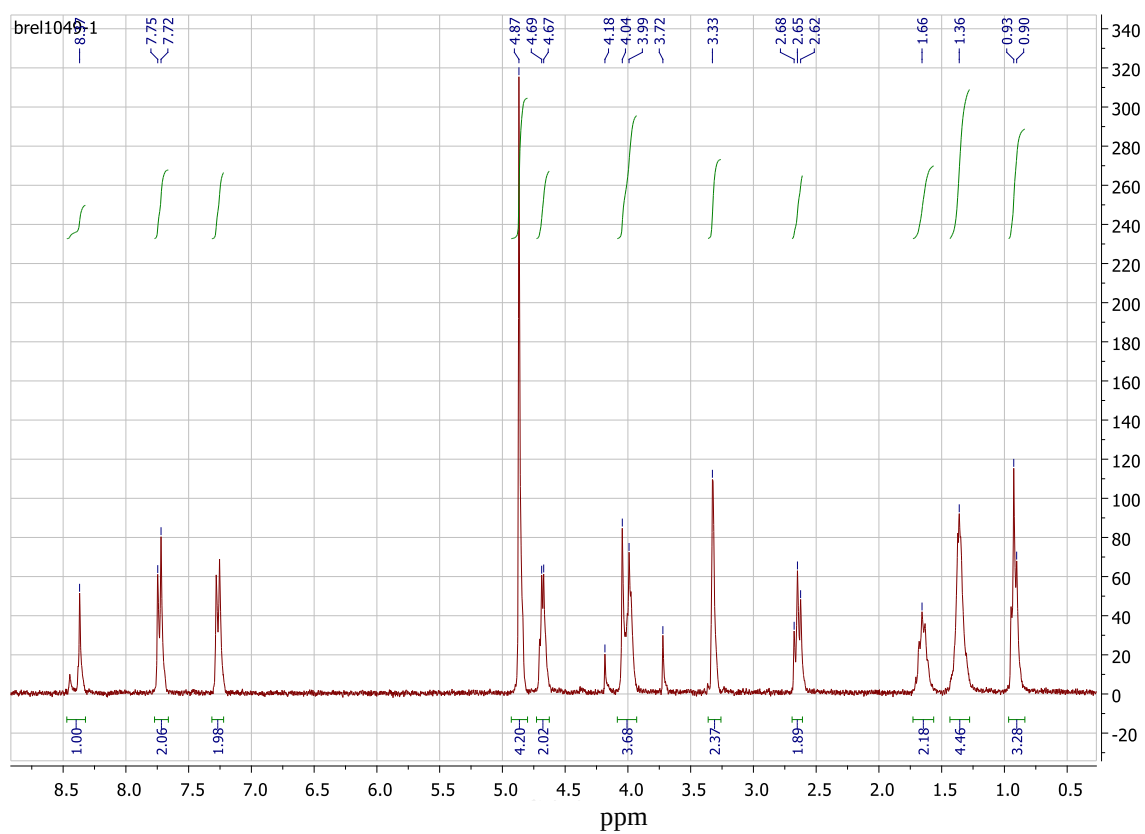
N-Hydroxy-2-[4-(p-tolyl)-1H-1,2,3-triazol-1-yl]ethoxyacetamide (6b) was prepared similarly to **6a**. Yield: 72%, white crystals, b.p. 142-143 °C. ^1H NMR (300 MHz, CD_3OD): δ 2.39 (s, 3H, $\text{CH}_3\text{C}_6\text{H}_4$), 3.74 (s, 3H, OCH_3), 3.99 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.12 (s, $\text{CH}_2\text{COOCH}_3$), 4.64 (t, 2H, $J = 6.0$ Hz, 1,2,3-triazole- CH_2), 7.24 (d, 2H, $J = 6.0$ Hz, *meta*-Ph), 7.75 (d, 2H, $J = 6.0$ Hz, *ortho*-Ph), 8.0 (s, 1H, =CH 1,2,3-triazole). ^{13}C NMR (126 MHz, CD_3OD): 21.3 ($\text{CH}_3\text{-C}_6\text{H}_4$), 50.3 (1,2,3-triazole- CH_2), 52.0 (OCH_3), 68.4 (CH_2O), 69.9 ($\text{CH}_2\text{COOCH}_3$), 120.7 (=CH, 1,2,3-triazole), 125.6 (Ph), 127.9 (Ph), 129.5 (Ph), 137.9 (Ph), 147.8 ($\text{C}=\text{N}$, 1,2,3-triazole), 170.4 (COOCH_3). **Elemental analysis** calcd. (%) for $\text{C}_{13}\text{H}_{16}\text{N}_4\text{O}_3$: C, 56.44; H, 5.83; N, 20.35; found: C, 56.01; H, 5.65; N, 20.34.

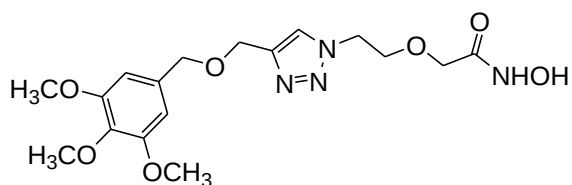


N-Hydroxy-2-[4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl]ethoxyacetamide (6c). Was prepared similarly of (6a). Yield: 65%, white crystals, b.p. 129-132°C. ¹H NMR (300 MHz, CD₃OD): δ 3.7 (s, 3H, OCH₃), 3.85 (s, 3H, CH₃OC₆H₄), 3.99 (t, 2H, *J* = 6.0 Hz, OCH₂), 4.11 (s, CH₂COOCH₃), 4.63 (t, 2H, *J* = 6.0 Hz, 1,2,3-triazole-CH₂), 6.97 (d, 2H, *J* = 6.0 Hz, *meta*-Ph), 7.76 (d, 2H, *J* = 6.0 Hz, *ortho*-Ph), 7.96 (s, 1H, =CH 1,2,3-triazole). ¹³C NMR (126 MHz, CD₃OD): 50.3 (1,2,3-triazole-CH₂), 52.0 (OCH₃), 55.3 (CH₃O-C₆H₄), 68.4 (CH₂O), 70.0 (CH₂COOCH₃), 114.2 (=CH, 1,2,3-triazole), 120.3 (Ph), 123.5 (Ph), 127.0 (Ph), 147.6 (Ph), 159.5 (C=, 1,2,3-triazole), 170.4 (COOCH₃). **Elemental analysis** calcd. (%) for C₁₃H₁₆N₄O₄: C, 53.35; H, 5.51; N, 19.24; found: C, 53.12; H, 5.63; N, 19.43.

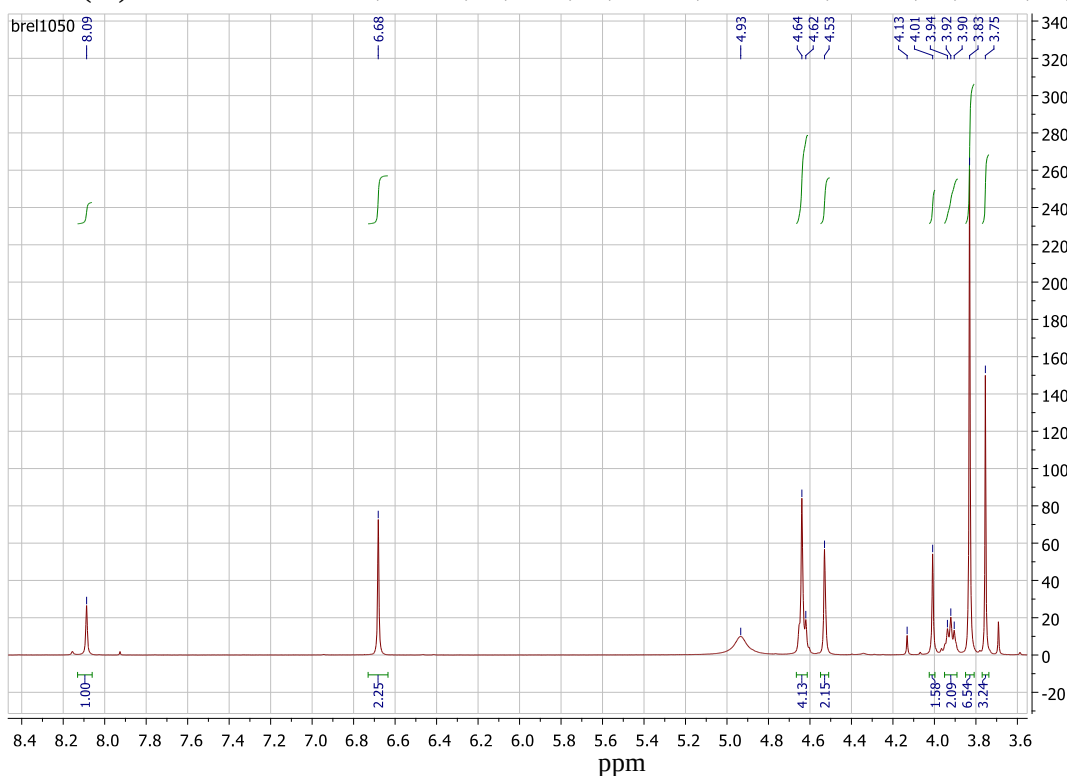


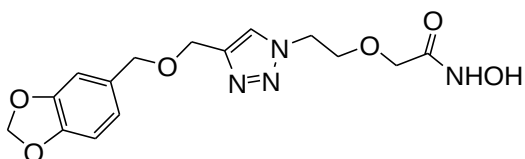
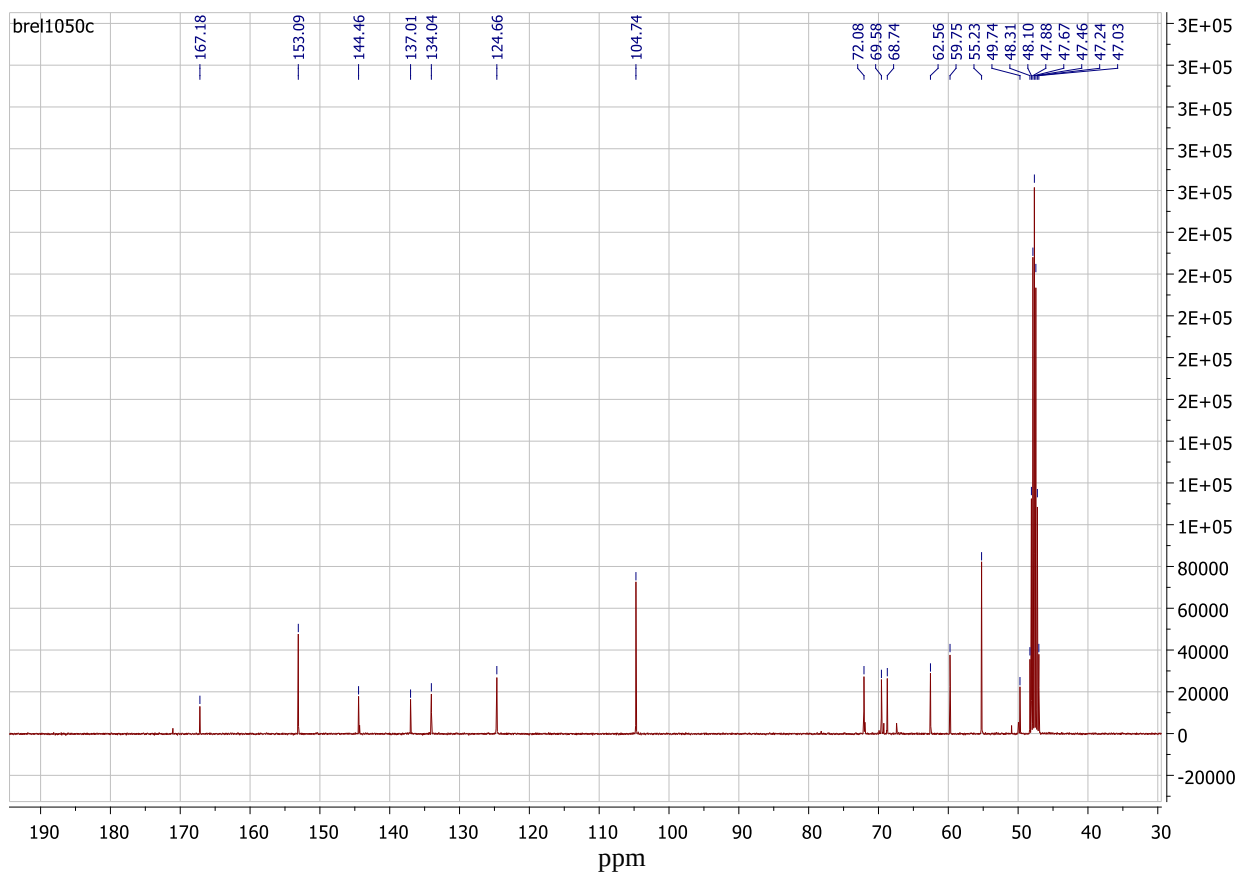
S17



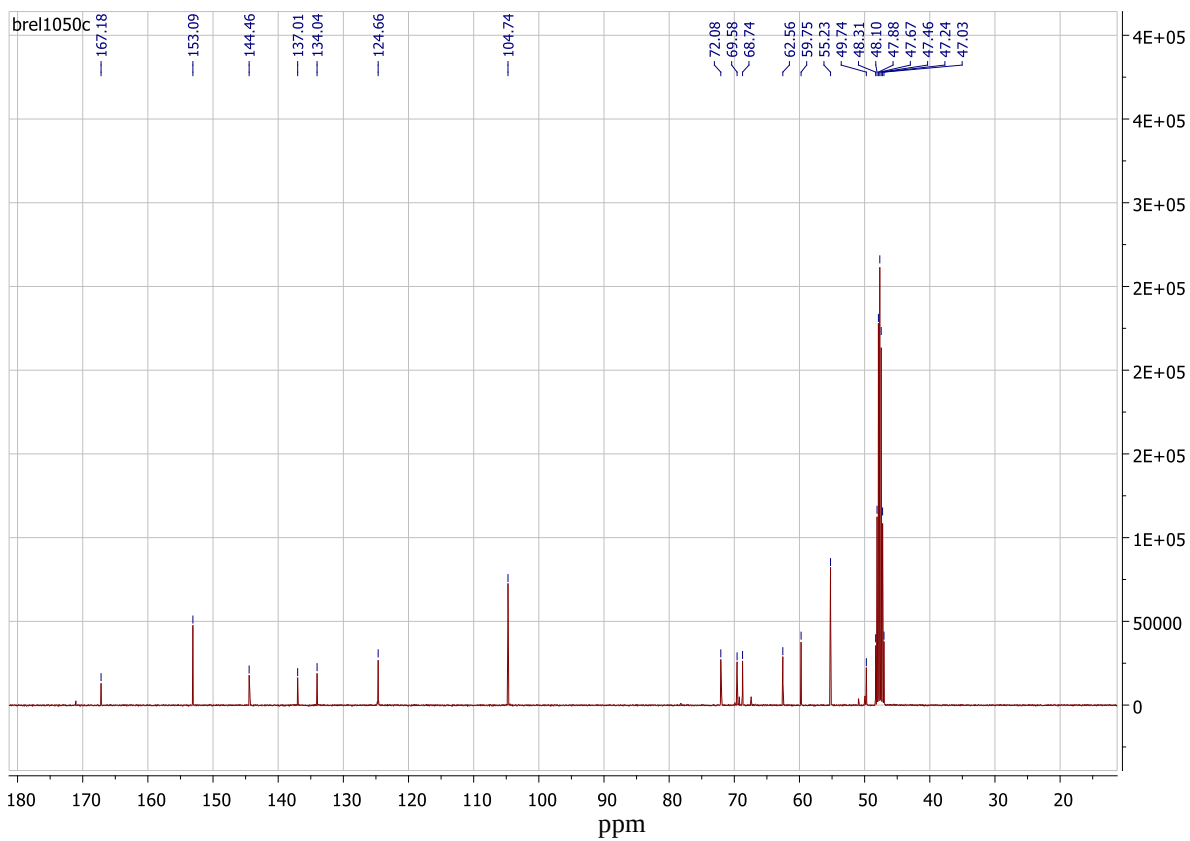
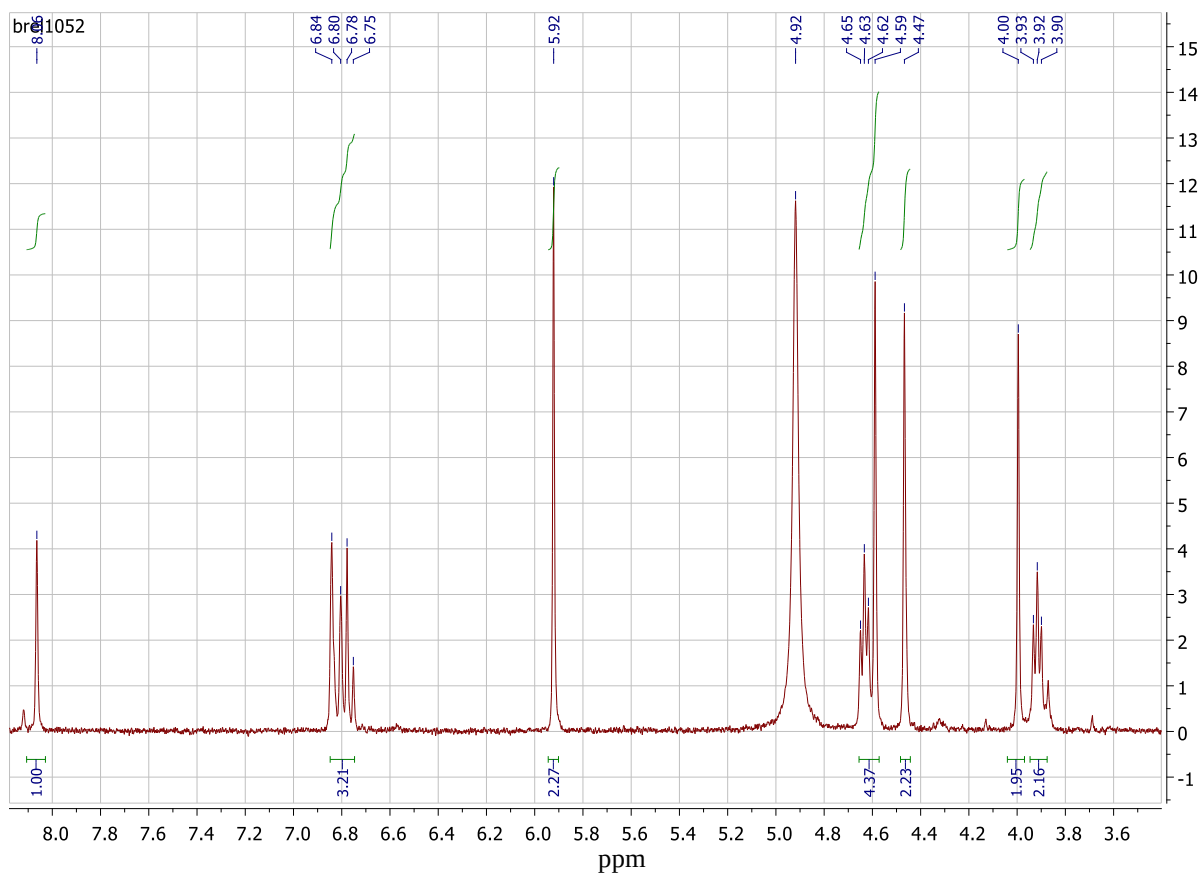


N-Hydroxy-2-[(4-((3,4,5-trimethoxybenzyloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl]-ethoxyacetamide (6e) was prepared similarly to **6a**. Yield: 63%, white crystals, b.p. 116-119 °C. ^1H NMR (300 MHz, CD_3OD): δ 3.80 (s, 3H, OCH_3), 3.83 (s, 6H, 2OCH_3), 3.92 (t, 2H, $J = 6.0$ Hz, OCH_2), 4.06 (s, CH_2CONHOH), 4.51 (s, 2H, CH_2O), 4.55 (t, 2H, $J = 6.0$ Hz, 1,2,3-triazole- CH_2), 4.66 (s, 2H, CH_2O), 6.58 (s, 2H, *ortho*-Ph), 7.79 (s, 1H, $=\text{CH}$ 1,2,3-triazole). ^{13}C NMR (126 MHz, CD_3OD): 49.7 (1,2,3-triazole- CH_2), 55.2 ($3\text{CH}_3\text{O}$, Ar), 59.8 (CH_2O , 62.6 (CH_2O), 68.7 (CH_2O), 69.6 (CH_2O), 72.0 (CH_2CONHOH), 104.7 ($=\text{CH}$, 1,2,3-triazole), 124.7 (Ph), 134.0 (Ph), 137.0 (Ph), 144.3 (Ph), 153.1 ($\text{C}=\text{N}$, 1,2,3-triazole), 171.0 (CONHOH). Elemental analysis calcd. (%) for $\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_7$: C, 51.45; H, 6.01; N, 14.19; found: C, 51.02; H, 5.93; N, 14.41.





***N*-Hydroxy-2-[(4-((1,3-benzodioxal-5-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-methoxy]ethoxyacetamide (6f)** was prepared similarly to **6a**. Yield: 65%, white crystals, b.p. 125-129 °C. ¹H NMR (300 MHz, CD₃OD): δ 3.92 (t, 2H, *J* = 6.0 Hz, OCH₂), 4.07 (s, CH₂COOCH₃), 4.47 (s, 2H, CH₂O), 4.57 (t, 2H, *J* = 6.0 Hz, 1,2,3-triazole-CH₂), 4.63 (s, 2H, CH₂O), 5.92 (s, 2H, OCH₂O), 6.74 – 6.85 (m, 3H, Ar), 7.78 (s, 1H, =CH 1,2,3-triazole). ¹³C NMR (126 MHz, CD₃OD): 49.7 (1,2,3-triazole-CH₂), 62.3 (CH₂O), 68.7 (CH₂O), 69.6 (CH₂O), 71.9 (H₂C₂CONHOH), 101.0 (OCH₂O), 1,2,3-triazole), 107.6 (=CH, 1,2,3-triazole), 108.2 (Ph), 121.6 (Ph), 124.6 (Ph), 131.6 (Ph), 144.3 (Ph), 147.9 (Ph), 147.7 (C=, 1,2,3-triazole), 170.6 (C=O). Elemental analysis calcd. (%) for C₁₅H₁₈N₄O₆: C, 51.39; H, 5.14; N, 16.05; found: C, 51.12; H, 5.34; N, 16.12.



3. Measuring HDACs activity

The activity of histone deacetylases HDAC1, HDAC3, and HDAC8 was assessed using commercially available fluorogenic assay kits (HDAC1, HDAC3, and HDAC8 Fluorogenic Assay Kits; BPS Bioscience, San Diego, USA) according to the manufacturer's instructions. Reactions were conducted in 96-well plates (50 μ L per well), with each well containing assay buffer, recombinant enzyme, fluorogenic substrate, and test compounds (at concentrations ranging from 1 nM to 30 μ M; DMSO $\leq$ 1%). Solvent in an equivalent volume served as the vehicle control, while trichostatin A was employed as the reference inhibitor, with IC₅₀ values for its HDAC-inhibitory effect falling within the range consistent with previously reported data provided in the manufacturer's instructions.

Following incubation of the aforementioned reaction mixture at 37°C for 30 minutes, a developer reagent containing peptidase was added to all wells of the plate to cleave the deacetylated substrate, after which the mixture was incubated for 15 minutes at room temperature, and fluorescence was measured (λ_{ex} = 360 nm, λ_{em} = 460 nm). Experiments were conducted triplicate. IC₅₀ values were determined using nonlinear regression modeling.

4. Cells and cultivation

A-431 (human epidermoid carcinoma) cells were cultured under standard conditions at 37°C in an atmosphere with 5% CO₂ and high humidity. Cells were cultured in DMEM (Biolot, Russia) supplemented with fetal bovine serum (10%, Biolot, Russia), 2 mM L-glutamine, and penicillin-streptomycin. Cells were maintained in logarithmic growth phase by passage every 2-4 days and dissociated using a trypsin-versene solution (at a 1:3 ratio) after reaching 80-90% confluency. Before experiments, cells were adapted in 96-well plates at a concentration of 10⁴ cells/well for 24 hours to allow for adhesion and stabilization of their physiological state.

5. MTT-test

The cytotoxic effect of the compounds was determined using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which is based on the reduction of MTT by active mitochondrial dehydrogenases to insoluble purple formazan.

The test compounds were dissolved in DMSO to prepare stock solutions (10 mM). Working solutions were prepared immediately before experiments by serial dilution.

After 72-hour incubation of cells with the compounds (in the concentration range from 1 to 100 μ M, the DMSO content did not exceed 1%), MTT solution (0.5 mg/ml, Sigma-Aldrich, Saint Louis, MO, USA) was added to the cells and incubated for 2 hours at 37°C. The formed formazan crystals were dissolved by adding DMSO and subsequent gentle mixing. Optical density was measured using a plate spectrophotometer (at λ = 530 nm).

The IC₅₀ values were calculated as parameters of the effectiveness of the cytotoxic effect of the compounds. All experiments were conducted triplicate.

6. References

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