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Novel bivalent positive allosteric AMPA receptor modulator of bis-amide series

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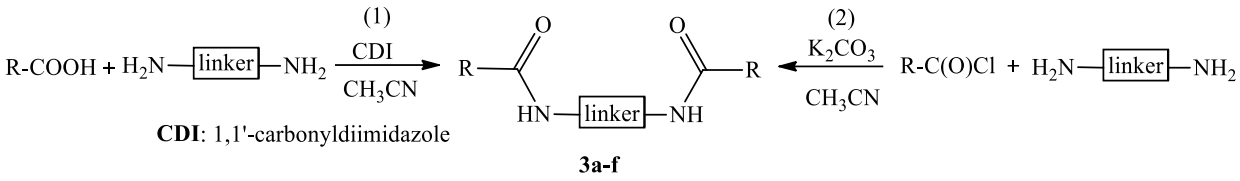
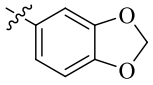
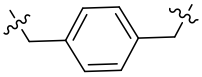
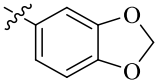
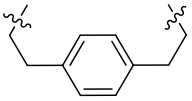
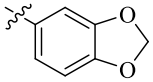
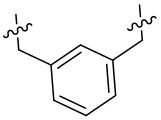
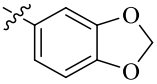
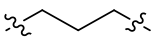
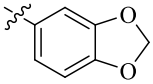
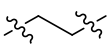
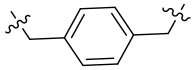
1. General

NMR spectra were recorded on the Agilent 400-MR spectrometer (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts (δ) were measured with reference to the solvents, $CDCl_3$ (δ = 7.26 ppm for ^{1}H, δ = 77.16 ppm for ^{13}C), $(CD_3)_2SO$ (δ = 2.50 ppm for ^{1}H, δ = 39.52 ppm for ^{13}C), CD_3OD (δ = 3.31 ppm for ^{1}H, δ = 49.00 ppm for ^{13}C). Chemical shifts (δ) are given in ppm; *J* values are given in Hz. When necessary, assignments of signals in NMR spectra were made using 2D techniques. Accurate mass measurements (HRMS) were performed on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measurements were done in a positive ion mode (interface capillary voltage 4500 V) or in a negative ion mode (3200 V). Analytical thin layer chromatography was carried out with Silufol silica gel plates (supported on aluminum); the detection was done by UV lamp (254 and 365 nm) and chemical staining (5% aqueous solution of $KMnO_4$). Column chromatography was performed on silica gel (230–400 mesh, Merck). All starting materials were commercially available. All reagents except commercial products of satisfactory quality were purified by literature procedures prior to use.



Scheme S1 Synthesis of bis-amides **3a**–**f**.

Compound	R	Linker	Yield, %, (procedure)
3a			80 (1)
3b			72 (1)
3c			56 (2)
3d			51 (2)
3e			76 (2)
3f			58 (2)

2. Experimental procedures and characterization of products

General procedures for the synthesis of compounds 3a,b

To a solution of piperonylic acid (0.33 g, 2 mmol) in 16 ml anhydrous acetonitrile, 1,1'-carbonyldiimidazole (CDI) (0.36 g, 2.2 mmol) was added and the mixture was stirred for 2 h at room temperature. Then the corresponding diamine (1 mmol) was added in several portions and the resulting mixture was stirred vigorously at room temperature for 1.5 h (TLC control). After completion of the reaction, the mixture was filtered, washed with cold acetonitrile (2×20 ml) and concentrated *in vacuo*. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 50/1).

N,N'-*[1,4-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide),* ***(3a)***

Yield 0.50 g (80%); colorless solid, mp 253-254 °C; R_f=0.81 (CH_2Cl_2/CH_3OH, 5/1); δ_H (400 MHz, DMSO-d_6): 4.41 (d, 4H, 2CH_2NH, *J* 5.7 Hz), 6.08 (s, 4H, 2CH_2O), 6.98 (d, 2H, 2CH, *J* 8.1 Hz), 7.25 (s, 4H, 4CH), 7.41 (d, 2H, 2CH, *J* 1.5 Hz), 7.47 (dd, 2H, 2CH, *J* 8.1, 1.5 Hz), 8.87 (br. t, 2H, 2NH, *J* 5.7 Hz); δ_C (101 MHz, DMSO-d_6): 42.5 ($2CH_2NH$), 101.7 ($2CH_2O$), 107.4 (2CH), 107.9 (2CH), 122.3 (2CH), 127.3 (4CH), 128.4 (2C), 138.3 (2C), 147.4 (2C), 149.7 (2C),

165.4 (2C=O). Found (%): C, 66.02; H, 4.81; N, 6.41. Calc. for $C_{24}H_{20}N_2O_6$ (%): C, 66.66; H, 4.66; N, 6.48. HRMS $[M + K]^+$: calcd. for $C_{24}H_{20}N_2O_6K$ 471.0953, found 471.0953.

N,N'-*[1,4-Phenylenebis(ethane-1,2-diyl)]bis(1,3-benzodioxole-5-carboxamide),* ***(3b)***

Yield 0.22 g (72%); colorless solid, mp 249-251 °C; R_f=0.81 (CH_2Cl_2/CH_3OH, 5/1); δ_H (400 MHz, DMSO-d_6): 2.78 (br. t, 4H, 2C*H*$_2$CH$_2$NH, *J* 7.7 Hz), 3.38-3.46 (m, 4H, 2CH$_2$C*H*$_2$NH), 6.09 (s, 4H, 2CH$_2$O), 6.97 (d, 2H, 2CH, *J* 8.1 Hz), 7.16 (s, 4H, 4CH), 7.36 (br. s, 2H, 2CH), 7.42 (br. d, 2H, 2CH, *J* 8.1 Hz), 8.42 (br. t, 2H, 2NH, *J* 5.5 Hz); δ_C (101 MHz, DMSO-d_6): 34.8 (2*C*H$_2$CH$_2$NH), 41.0 (2CH$_2$*C*H$_2$NH), 101.6 (2CH$_2$O), 107.2 (2CH), 107.8 (2CH), 122.1 (2CH), 128.6 (2C), 128.7 (4CH), 137.3 (2C), 147.3 (2C), 149.6 (2C), 165.2 (2C=O). Found (%): C, 67.33; H, 4.88; N, 5.92. Calcd.for $C_{26}H_{24}N_2O_6$ (%): C, 67.82; H, 5.25; N, 6.08. HRMS $[M + K]^+$: calcd. for $C_{26}H_{24}N_2O_6K$ 499.1266, found 499.1269.

General procedures for the synthesis of compounds 3c–f

To a solution of acyl chloride (2 mmol) in anhydrous acetonitrile (20 ml), anhydrous potassium carbonate (0.35 g, 2.5 mmol) was added and the mixture was stirred for 15 min at room temperature. Then the corresponding diamine (1 mmol) was added in several portions and the resulting mixture was stirred vigorously at room temperature for 1.5 h (TLC control). After completion of the reaction, the mixture was filtered, washed with cold acetonitrile (2×20 ml) and concentrated *in vacuo*. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 50/1).

N,N'-*[1,3-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide),* ***(3c)***

Yield 0.17 g (56%); colorless solid, mp 174-177 °C; R_f=0.80 (CH_2Cl_2/CH_3OH, 5/1); δ_H (400 MHz, $CDCl_3$+CD_3OD): 4.48 (d, 4H, 2C*H*$_2$NH, *J* 5.7 Hz), 5.96 (s, 4H, 2CH$_2$O), 6.74 (d, 2H, 2CH, *J* 8.1 Hz), 7.14-7.22 (m, 4H, 4CH), 7.23 (d, 2H, 2CH, *J* 1.6 Hz), 7.29 (dd, 2H, 2CH, *J* 8.1, 1.6 Hz), 7.65 (br. t, 2H, 2NH, *J* 5.7 Hz); δ_C (101 MHz, $CDCl_3$+CD_3OD): 43.8 (2CH$_2$NH), 101.7 (2CH$_2$O), 107.6 (2CH), 107.9 (2CH), 122.0 (2CH), 126.5 (CH), 126.6 (2CH), 128.1 (2C), 128.9 (CH), 138.8 (2C), 147.9 (2C), 150.4 (2C), 167.7 (2C=O). Found (%): C, 66.20; H, 4.65; N, 6.23. Calcd. for $C_{24}H_{20}N_2O_6$ (%): C, 66.66; H, 4.66; N, 6.48. HRMS $[M+K]^+$: calcd. for $C_{24}H_{20}N_2O_6K$ 471.0953, found 471.0953.

N,N'-*Propane-1,3-diylbis(1,3-benzodioxole-5-carboxamide),* ***(3d)***

Yield 0.25 g (51%); colorless solid, mp 169-171 °C; R_f=0.79 (CH_2Cl_2/CH_3OH, 5/1); δ_H (400 MHz, CD_3OD + $CDCl_3$): 1.82 (quint, 2H, CH_2, *J* 6.4 Hz), 3.42 (t, 4H, 2C*H*$_2$NH, *J* 6.4 Hz), 6.00 (s, 4H, 2CH$_2$O), 6.82 (d, 2H, 2CH, *J* 8.2 Hz), 7.30 (d, 2H, 2CH, *J* 1.7 Hz), 7.39 (dd, 2H, 2CH, *J* 8.2, 1.7 Hz); δ_C (101 MHz, CD_3OD + $CDCl_3$): 29.8 (CH_2), 37.6 (2CH$_2$NH), 102.5 (2CH$_2$O), 108.1 (2CH), 108.5 (2CH), 122.7 (2CH), 128.8 (2C), 148.7 (2C), 151.3 (2C), 169.0 (2C=O). Found (%): C, 60.19; H, 4.62; N, 7.31. Calcd. for $C_{19}H_{18}N_2O_6$ (%): C, 61.62; H, 4.90; N, 7.56. HRMS $[M + K]^+$: calcd. for $C_{19}H_{18}N_2O_6K$ 409.0796, found 409.0797.

N,N'-*Ethane-1,2-diylbis(1,3-benzodioxole-5-carboxamide),* ***(3e)***

Yield 0.46 g (76%); colorless solid, mp 238-241°C; R_f=0.77 (CH_2Cl_2/CH_3OH, 5/1); δ_H (400 MHz, DMSO-d_6 + $CDCl_3$): 3.39-3.43 (m, 4H, 2C*H*$_2$NH), 6.03 (s, 4H, 2CH$_2$O), 6.85 (d, 2H,

2CH, *J* 8.1 Hz), 7.35 (d, 2H, 2CH, *J* 1.5 Hz), 7.42 (dd, 2H, 2CH, *J* 8.1, 1.5 Hz), 8.32-8.37 (br. m, 2H, 2NH); δ_C (101 MHz, DMSO-d_6 + $CDCl_3$): 39.2 ($2CH_2NH$), 101.3 ($2CH_2O$), 107.3 (2CH), 107.4 (2CH), 121.9 (2CH), 128.4 (2C), 147.1 (2C), 149.5 (2C), 165.7 (2C=O). Found (%): C, 60.49; H, 4.35; N, 7.78. Calcd. for $C_{18}H_{16}N_2O_6$ (%): C, 60.67; H, 4.55; N, 7.86. HRMS [M + K]$^+$: calcd. for $C_{18}H_{16}N_2O_6K$ 395.0640, found 395.0643.

N,N'-*[1,4-Phenylenebis(methylene)]bis(2-methylpropanamide),* ***(3f)***

Yield 0.22 g (58%); colorless solid, mp 218-221 °C; R_f=0.75 (CH_2Cl_2/CH_3OH, 5/1); δ_H (400 MHz, $CDCl_3$): 1.18 (d, 12H, $4CH_3$, *J* 6.9 Hz), 2.38 (sept, 2H, 2CH, *J* 6.9 Hz), 4.41 (d, 4H, $2CH_2$, *J* 5.7 Hz), 5.72 (br. s, 2H, 2NH), 7.23 (s, 4H, 4CH); δ_C (101 MHz, $CDCl_3$): 19.8 ($4CH_3$), 35.9 (2CH), 43.3 ($2CH_2NH$), 128.2 (4CH), 138.0 (2C), 176.9 (C=O). Found (%): C, 69.38; H, 8.99; N, 10.0. Calcd. for $C_{16}H_{24}N_2O_2$ (%): C, 69.53; H, 8.75; N, 10.14. HRMS [M+NH_4]$^+$: calcd. for $C_{16}H_{28}N_3O_2$ 294.2176, found 294.2174.

3. Patch clamp studies

In vitro electrophysiological experiments were carried out using a patch clamp technique with local fixation of potential as described earlier.[16,17] Freshly isolated single Purkinje neurons from the cerebellum of 12-16 day old Wistar rats were used as a test system. Transmembrane currents were induced by the activation of AMPA receptors with a solution of their partial agonist kainic acid using fast superfusion of solutions, where 30 μL of the agonist buffer were added to the neuron washing buffer at a constant rate, and the agonist concentration was varied in the range of 10^{-6}–10^{-4} M. The transmembrane currents for individual neurons were recorded using 2.5–5.5 MΩ borosilicate microelectrodes in a whole-cell configuration with an EPC-9 device from HEKA, Germany. The data were processed by a Pulsfit program from HEKA, Germany. Cyclothiazide (CTZ) as a well-known positive allosteric modulator of AMPA receptors was used as a reference ligand. The experimental results for compounds **3a**–**f** are presented in Table 1.

4. Investigation of the neuroprotective effect in the oxidative stress model

Materials. DMEM (HyClone, USA), FBS (Gibco, USA), L-glutamine (ICN Pharmaceuticals, USA), poly-D-lysine (BD Biosciences, USA), H_2O_2 (PFC Obnovlenie, Russia), DMSO (AppliChem, Panreac, Germany) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (AppliChem, Panreac, Germany). 75 cm^2 Culture flasks (TPP, Switzerland), 96-well plates (TPP, Switzerland), pipettes 2 mL, 5 mL, 10 mL (TPP, Switzerland).

Cell cultivation. The experiments were carried out on mouse hippocampal neuronal HT-22 cells. The cells were cultured in DMEM medium supplemented with 5% fetal bovine serum (FBS) in a CO_2 incubator at 37 ºC in an atmosphere containing 5% CO_2 until a monolayer was formed.

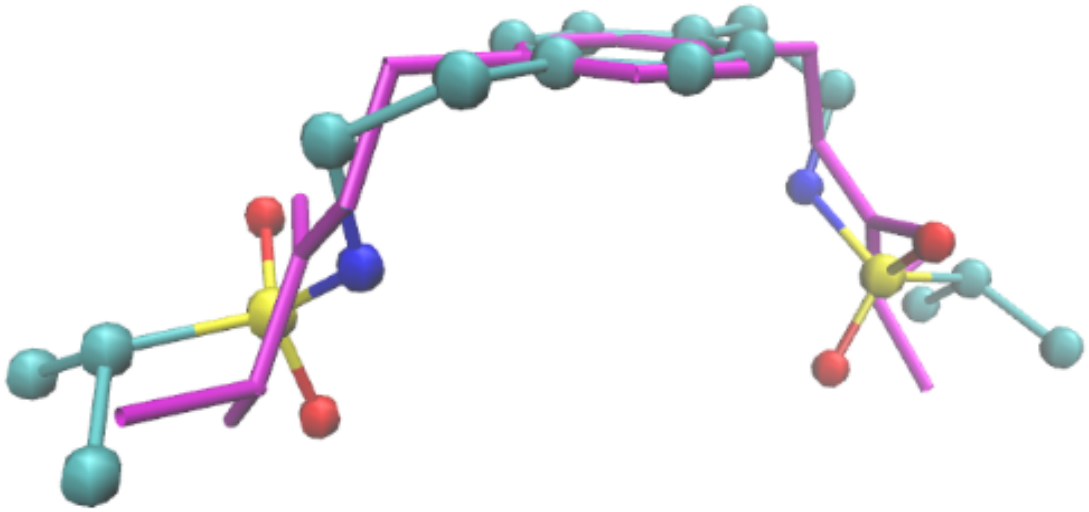
Oxidative stress model. The test compound dissolved in deionized water supplemented with 3% DMSO was introduced 24 h before or immediately after H_2O_2 at final concentrations of 10^{-5}-10^{-11}M. To create oxidative stress, HT-22 hippocampal cells were incubated in the presence of H_2O_2 at a final concentration of 1.5 mM in the atmosphere of 5% CO_2 for 30 min at 37 °C in

DMEM medium containing 5% FBS and 2 mM of L-glutamine.[21] Further, the H_2O_2-containing culture medium was replaced with a normal one, and after 4 h the cell viability was determined using the MTT test.

MTT test. At the end of the experiment, the culture medium was replaced with an MTT solution (0.5 mg/ml) and incubated for 30 min at 37 °C.[22] Then, the MTT solution was taken from the wells, and DMSO (dimethyl sulfoxide) was added to dissolve formazan. After 15 min, the light absorption was measured on a Multiscan spectrophotometer (Thermo) at a wavelength of 600 nm.

Statistical data processing. The Kruskal-Wallis test followed by Dunn's test (ANOVA) was used to assess intergroup differences when comparing the two groups. The data are presented as m ± s.d. The differences were considered significant at $p \leq 0.05$.

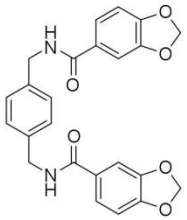
5. Molecule superposition



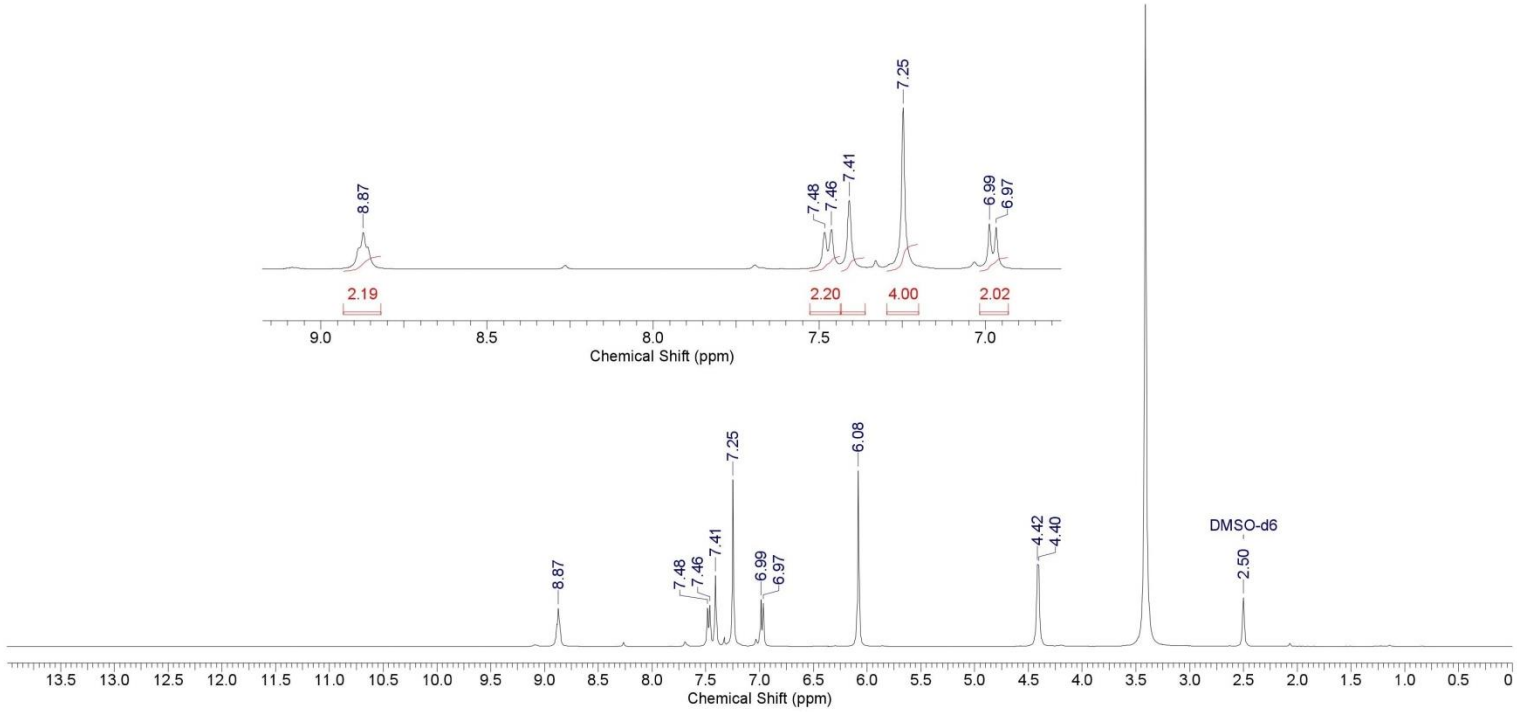
FigureS1 Superposition of molecule **2** (teal) with molecule **3f** (magenta).

6. NMR Spectra

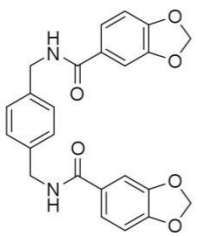
***N,N'*-[1,4-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3a)**



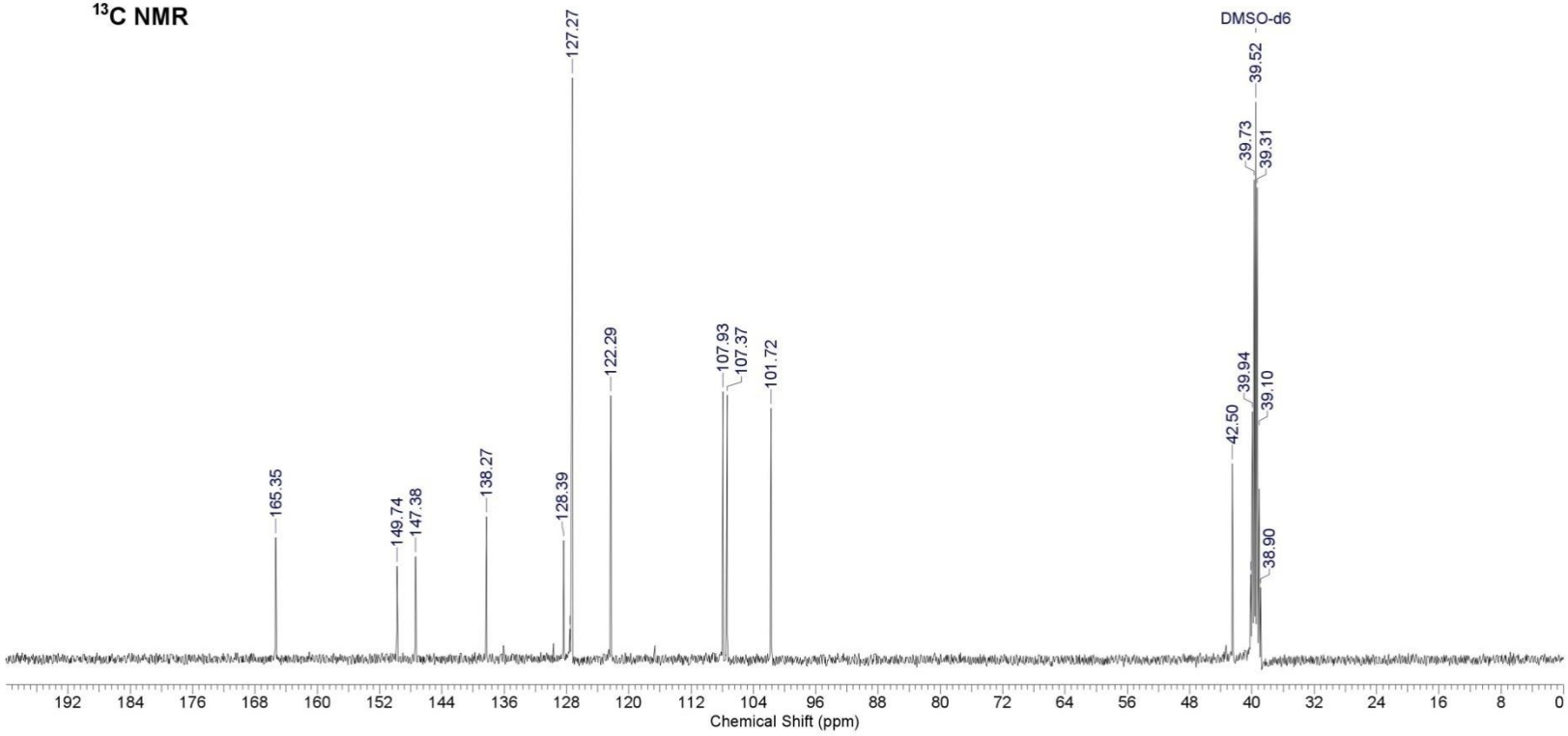
^{1}H NMR



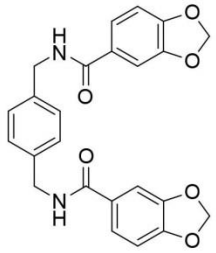
***N,N′*-[1,4-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3a)**



^{13}C NMR



***N,N'*-[1,4-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3a)**



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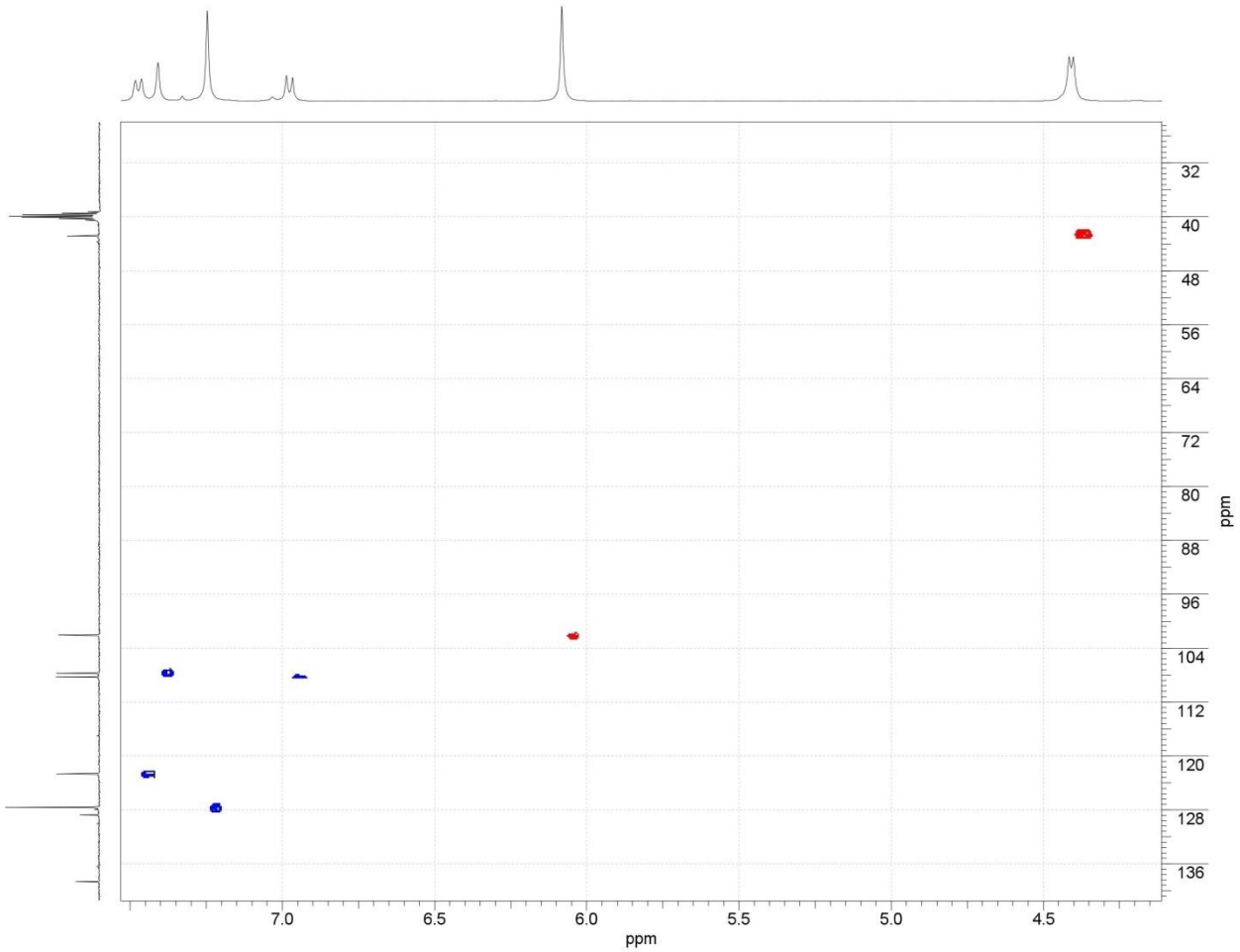
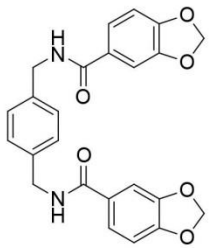
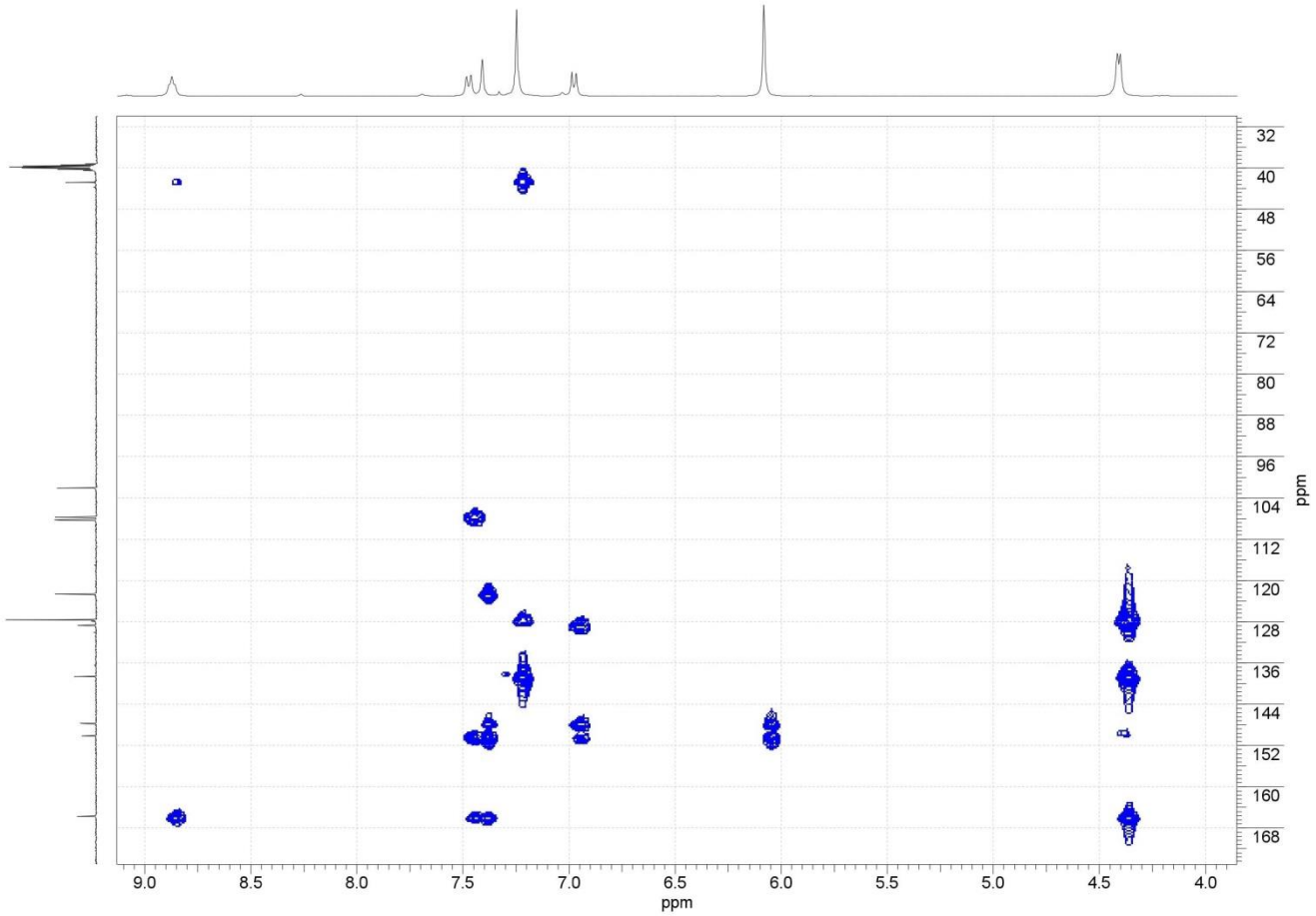


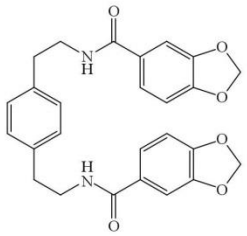
***N,N'*-[1,4-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3a)**



HMBC

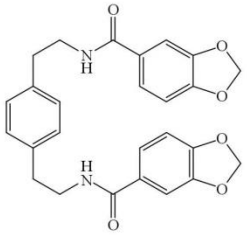


***N,N'*-[1,4-Phenylenebis(ethane-1,2-diyl)]bis(1,3-benzodioxole-5-carboxamide) (3b)**

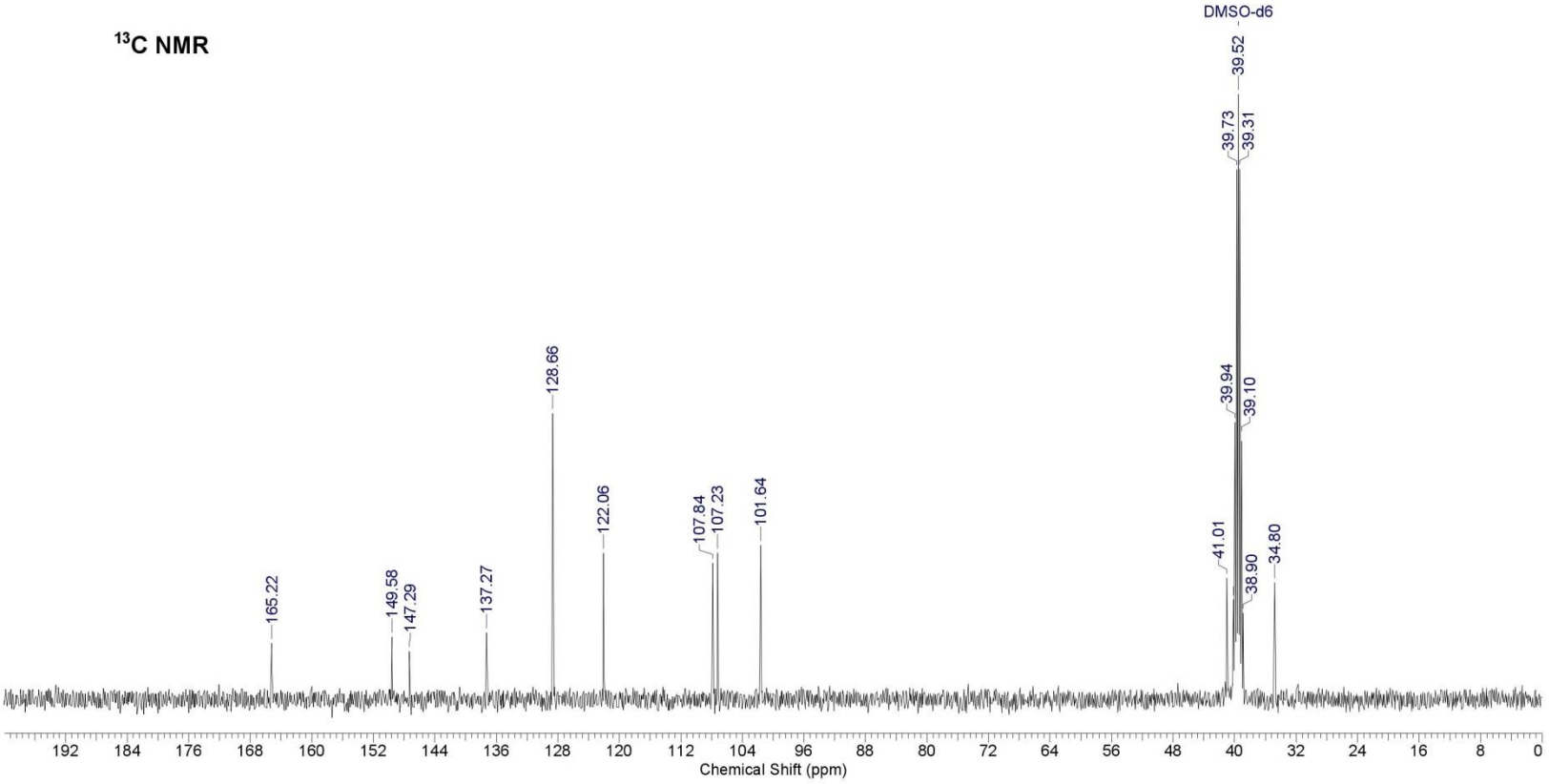


^{1}H NMR

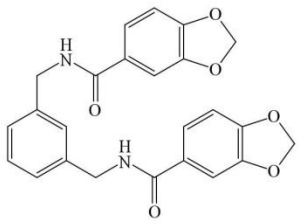
***N,N'*-[1,4-Phenylenebis(ethane-1,2-diyl)]bis(1,3-benzodioxole-5-carboxamide) (3b)**



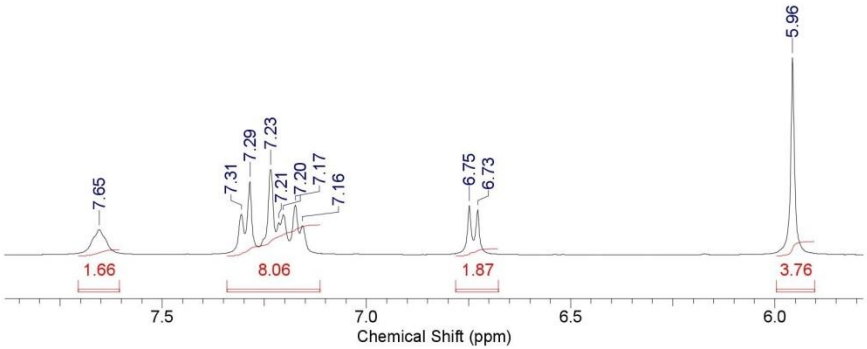
^{13}C NMR

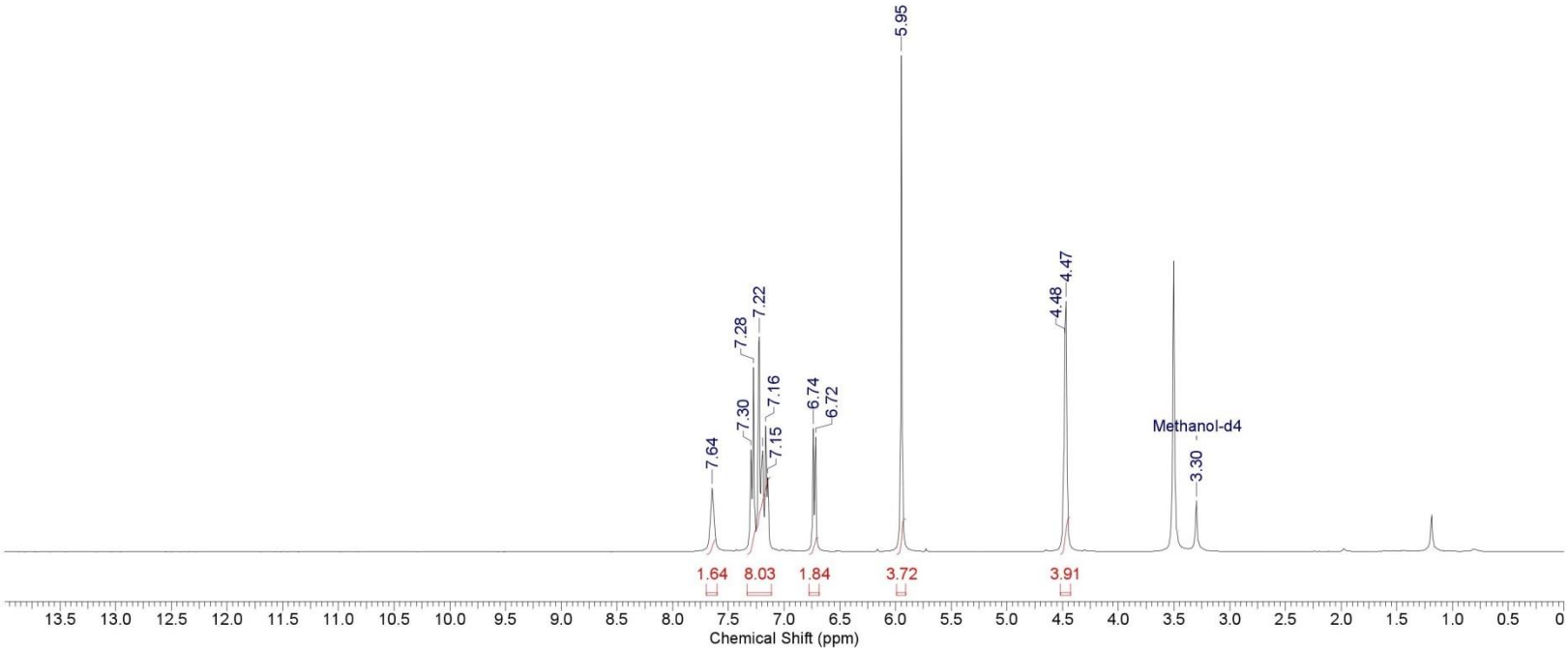


***N,N′*-[1,3-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3c)**

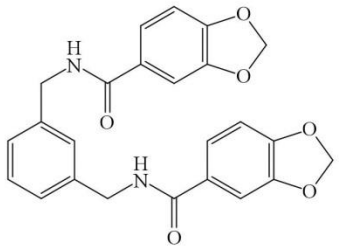


^{1}H NMR

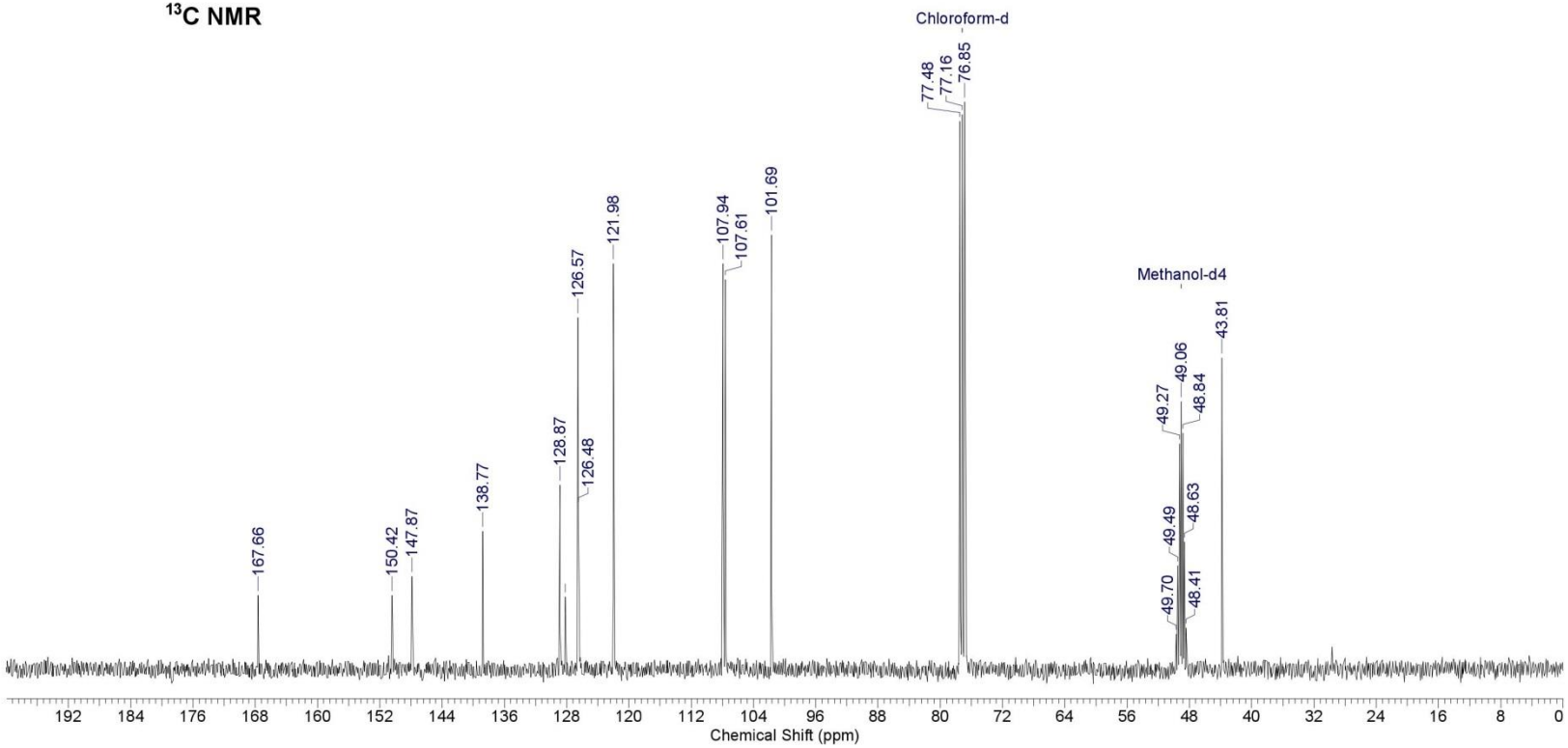




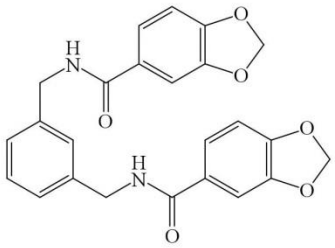
***N,N′*-[1,3-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3c)**



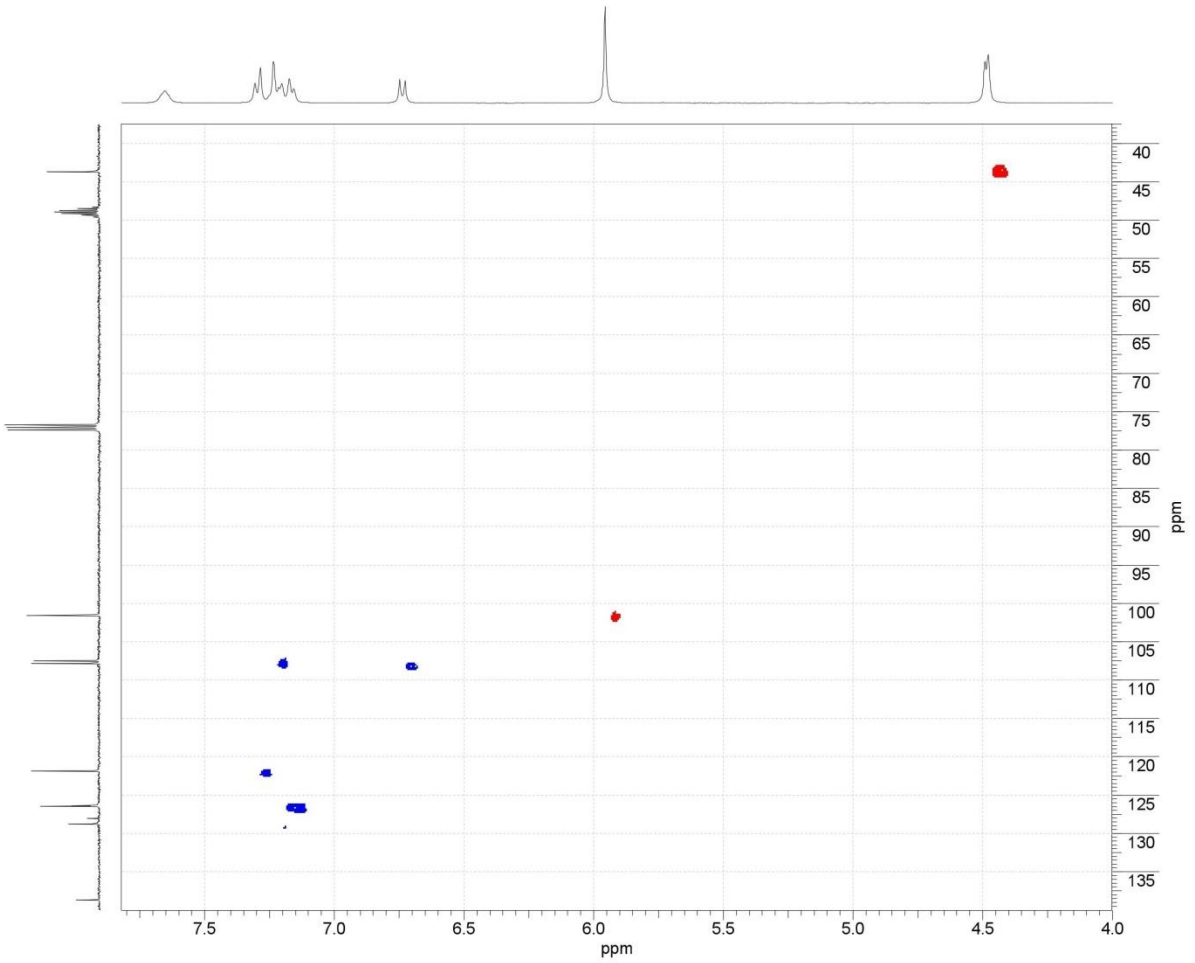
^{13}C NMR



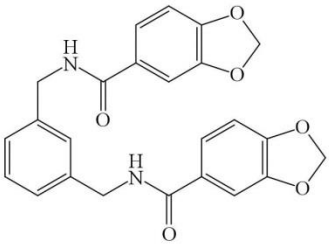
***N,N'*-[1,3-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3c)**



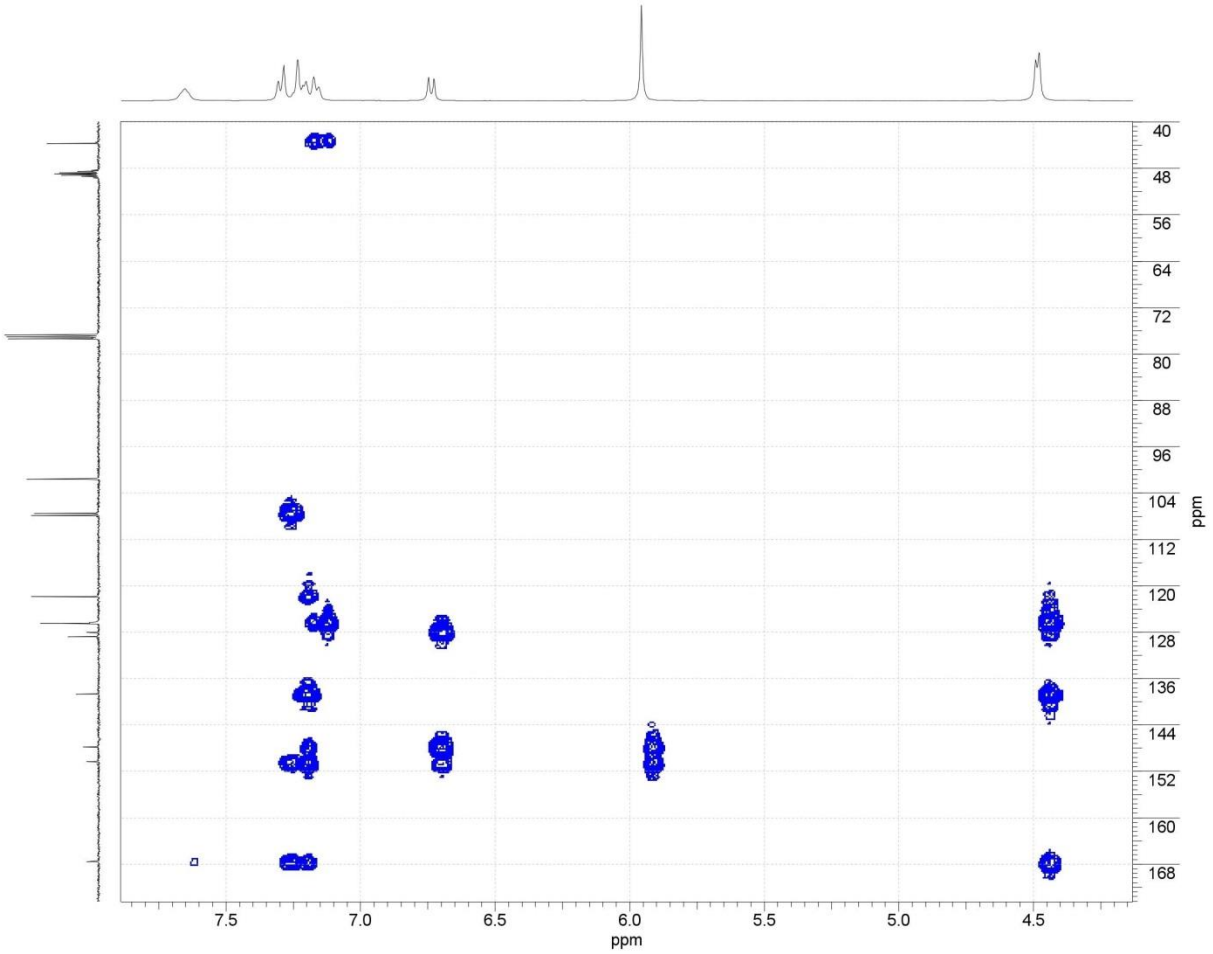
HSQC



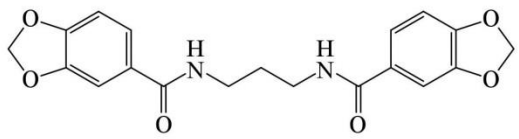
***N,N′*-[1,3-Phenylenebis(methylene)]bis(1,3-benzodioxole-5-carboxamide) (3c)**



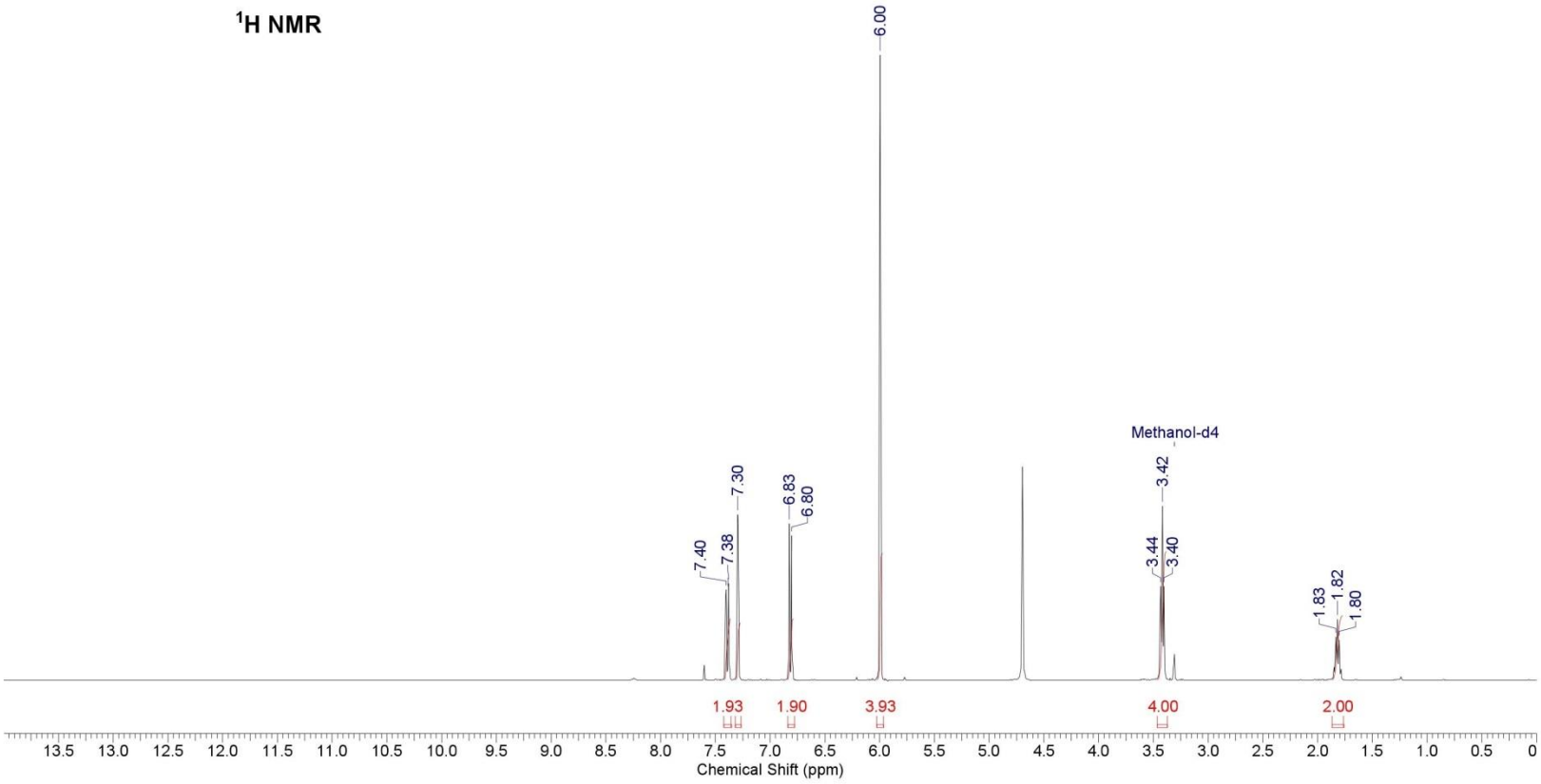
HMBC



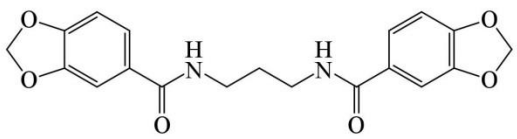
***N,N'*-Propane-1,3-diylbis(1,3-benzodioxole-5-carboxamide) (3d)**



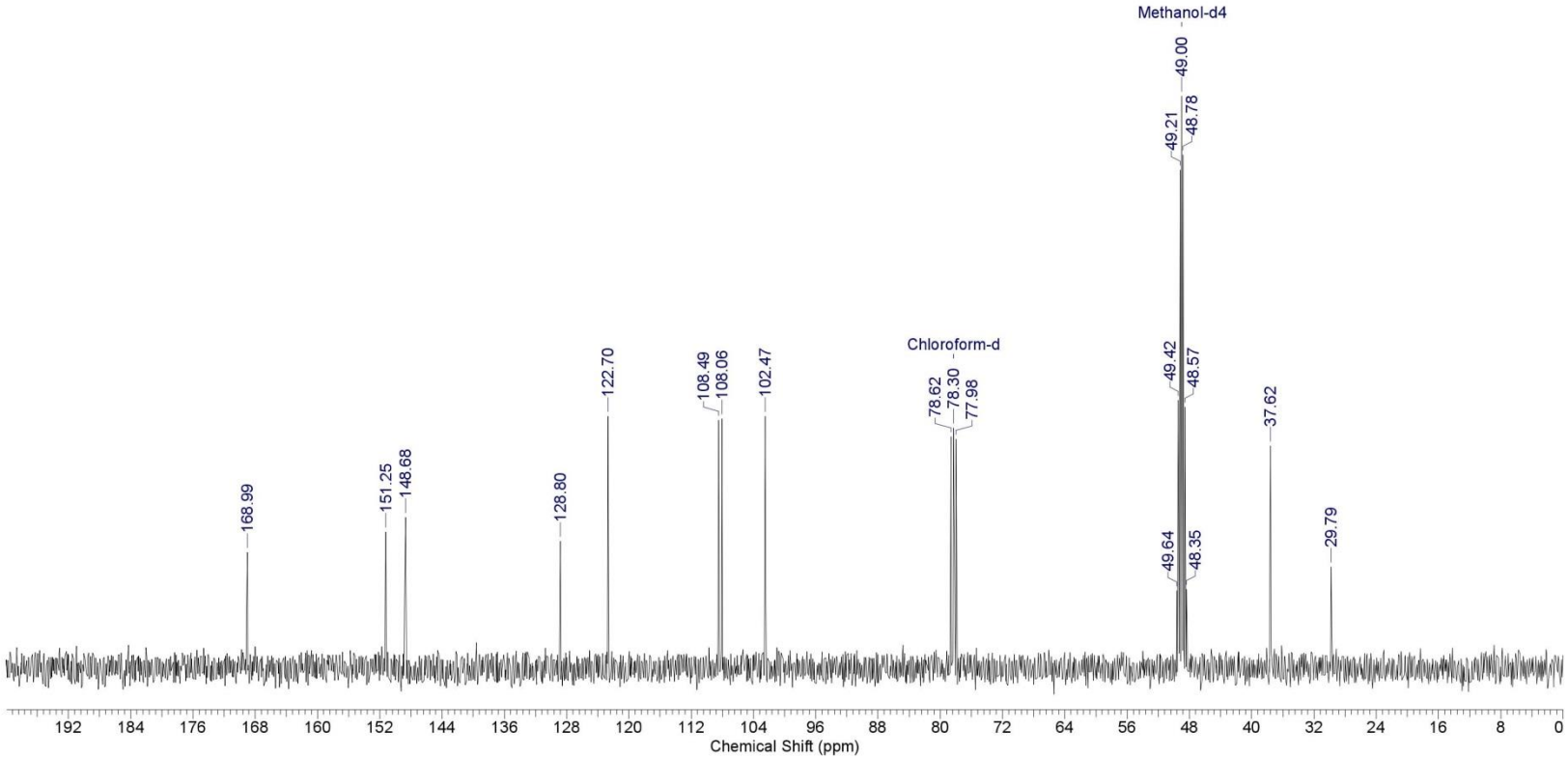
^{1}H NMR



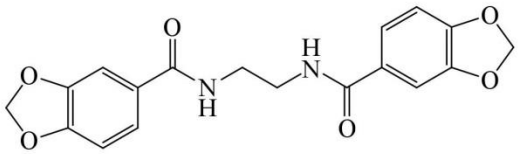
***N,N'*-Propane-1,3-diylbis(1,3-benzodioxole-5-carboxamide) (3d)**



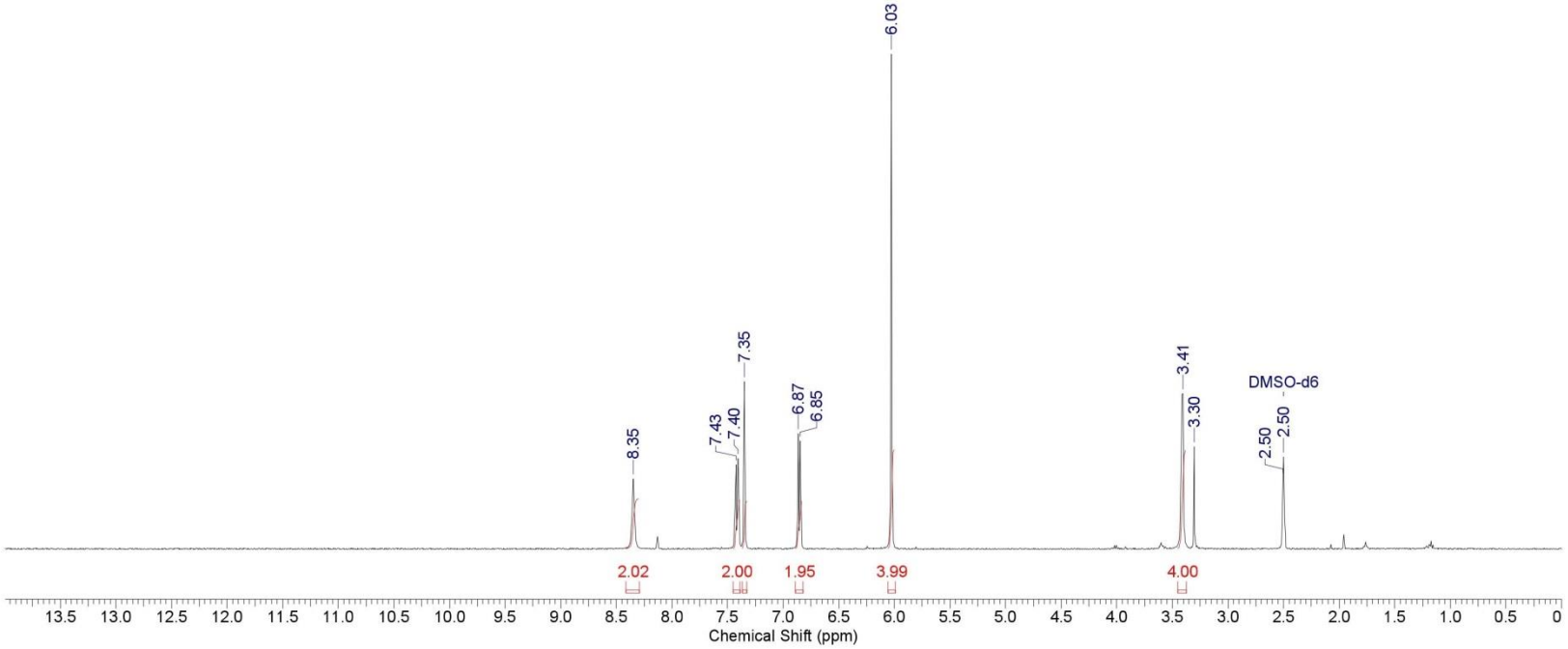
^{13}C NMR



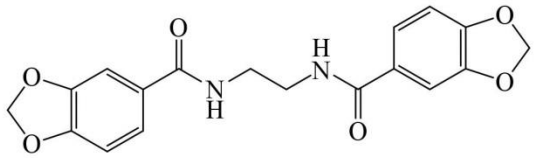
***N,N′*-Ethane-1,2-diylbis(1,3-benzodioxole-5-carboxamide) (3e)**



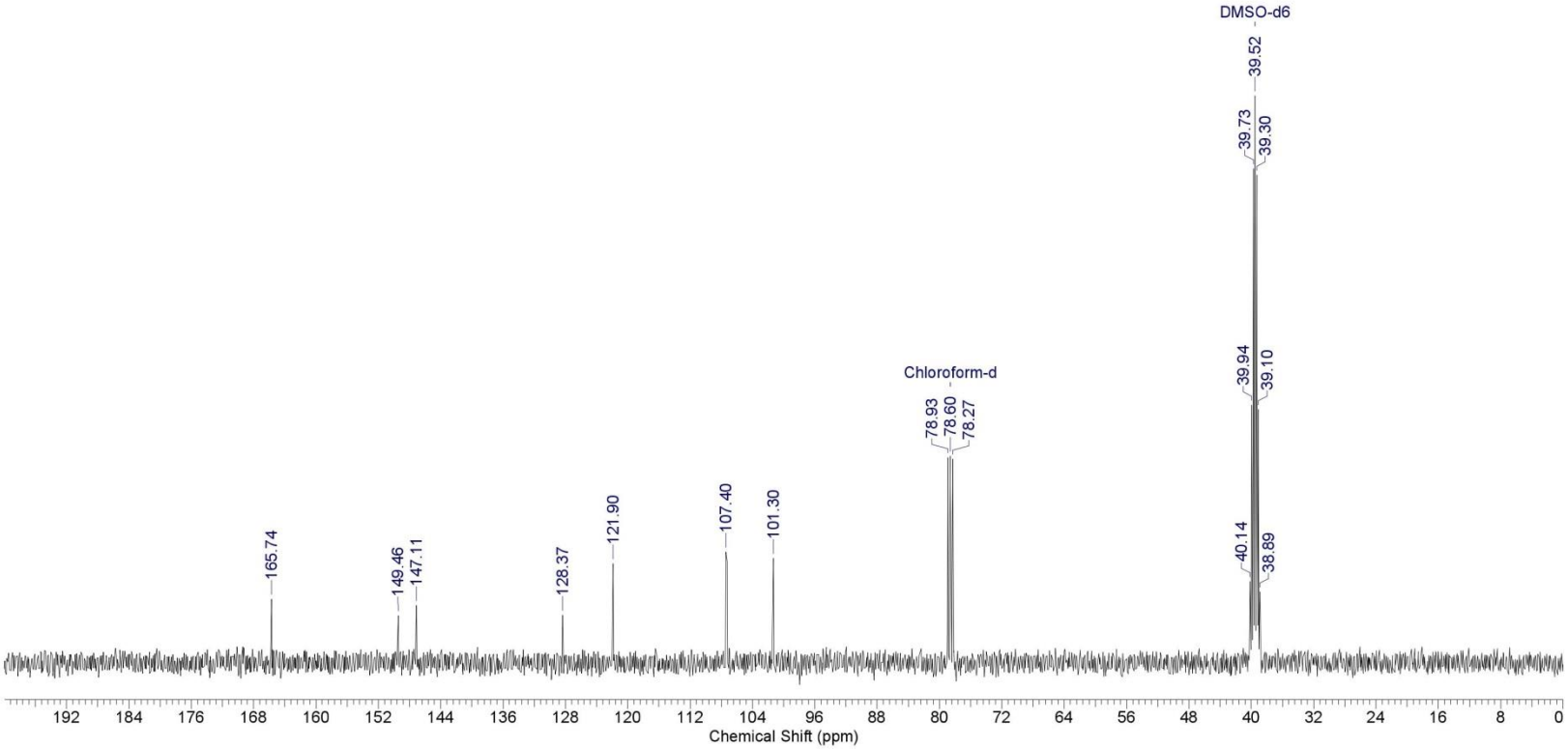
^{1}H NMR



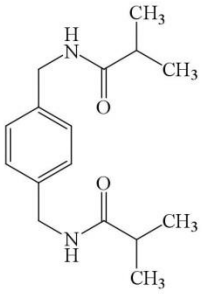
***N,N'*-Ethane-1,2-diylbis(1,3-benzodioxole-5-carboxamide) (3e)**



^{13}C NMR

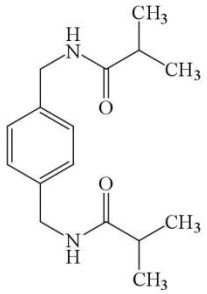


***N,N'*-[1,4-Phenylenebis(methylene)]bis(2-methylpropanamide) (3f)**



^{1}H NMR

***N,N'*-[1,4-Phenylenebis(methylene)]bis(2-methylpropanamide) (3f)**



^{13}C NMR