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Triethylene glycol conjugation of 3,5-bis(benzylidene)piperidin-4-ones – a new approach to hybrid molecules with improved cytotoxic properties

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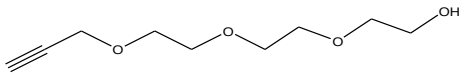
1. General Methods and Materials

All commercial reagents were used as purchased without further purification, all solvents used in the reactions were freshly distilled. Analytical TLC was performed with Merck silica gel 60 F 254 plates (Darmstadt, Germany); visualization was accomplished with UV light or iodine vapors. Column chromatography was carried out using Merck silica gel (Kieselgel 60, 0.063–0.200 mm, Darmstadt, Germany). NMR spectra were recorded on Bruker AV-300 (Bruker, Karlsruhe, Germany) and Bruker AV-400 (Bruker, Rheinstetten, Germany) spectrometers operating at 300 and 400 MHz, respectively, for ^{1}H (TMS reference), at 101 MHz for ^{13}C and at 282 MHz for ^{19}F (CCl_3F reference).

Mono propargyl ether of triethylenglycole [S1] and 1-(2-azidoacetyl)-3,5-bis(benzylidene) -piperidin-4-ones **1a-g** [S2]were prepared by known procedures.

2. Synthesis

Mono propargyl ether of triethylenglycole were prepared by known procedures. [S1]

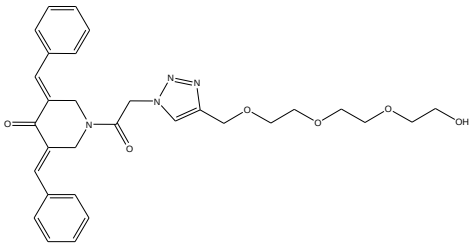


Yellow oily (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 t (1H, HC≡), 2.72 s (1H, OH), 3.59 t (2H, C<u>H</u>$_2$OH), 3.66-3.72 m (10H, 5CH_2), 4.1 d (2H, J = 3Hz, HC≡CCH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 58.3, 61.5, 69.0, 70.2, 70.3, 70.5, 72.5, 74.8, 79.5.

*General Procedure for the (1-(2-(3,5-bis(benzylidene)-4-oxopiperidin-1-yl)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)-(2-(2-ethoxy)ethane-1-ol (**4a-e**).*

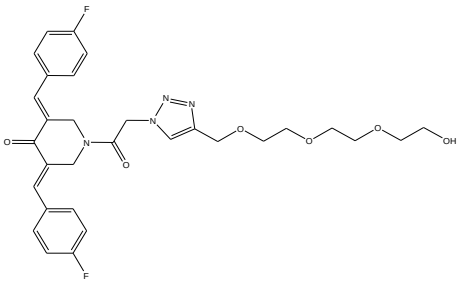
To a stirred mixture of the corresponding mono propargyl ether of triethylenglycole (0.4 mmol, 1.0 eq.) and copper(I) bromide (0.02 mmol, 5 mol.%) in CH_2Cl_2 (5 mL) 1-(2-azidoacetyl)-3,5-bis(benzylidene)-piperidin-4-ones (**3a-e**) (0.42 mmol, 1.05 eq.) was added. The solution obtained was stirred at room temperature for 24 h (TLC monitoring). The solvent was removed *in vacuo*, and the remaining crude product was purified by column chromatography (SiO_2, chloroform/ethanol 100:4) to afford the corresponding product as a yellow oil.

(1-(2-(3,5-bis(benzylidene)-4-oxopiperidin-1-yl)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)-(2-(2-ethoxy)ethane-1-ol (4a)



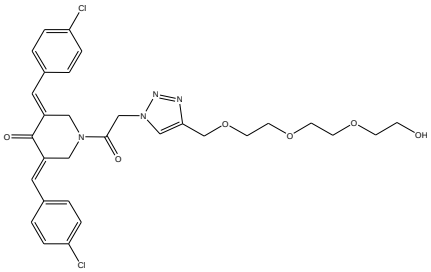
Yellow oil (75 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.64 br.s.(1H, OH), 3.57 q (2H, J = 3Hz, C$\underline{H_2}$CH_2OH), 3.63 m (6H, 3CH_2O), 3.68 q (2H, triazole-CH_2OC$\underline{H_2}$), 4.66 s (2H, C$\underline{H_2}$- triazole), 4.85 d (4H, 2CH_2N), 5.06 s (2H, CH_2-C(O)-), 7.40-7.48 m (10H, 2Ph), 7.68 s (1H, triazole), 7.9 d (2H, 2 HC=).^{13}C NMR (101 MHz, $CDCl_3$) δ 43.9, 46.0 (CH_2N), 50.6 (CH_2OH), 61.5 (CH_2O), 64.3 (CH_2O), 69.4 (CH_2O), 70.2 (CH_2O), 70.4, 70.44 (CH_2O), 72.5 (CH_2-C=O), 124.9 (N-C=), 128.8, 128.8 (C, Ar), 128.8, 129.0 (C, Ar), 129.8, 130.1 (C, Ar), 130,4, 130.5 (C, Ar), 133.9, 134.2 (C=), 138.4, 139.0 (C=), 145.0 (C=), 164.0 (C=O), 185.9 (C=O). Elemental analysis calcd. (%) for $C_{30}H_{34}N_4O_6$: C, 65.88; H, 6.26 N, 10.3; found: C**, 65.93**; H, 6.2**9** N, 10.**7**.

(1-(2-(3,5)-bis((E)-4-fluorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)-(2-(2-ethoxy)ethane-1-ol (4b)



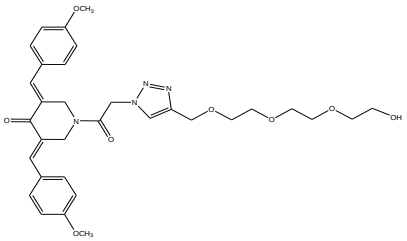
Yellow oil (70 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.75 br.s.(1H, OH), 3.55 q (2H, J = 3Hz, C$\underline{H_2}$CH_2OH), 3.61 m (6H, 3CH_2O), 3.67 q (2H, triazole-CH_2OC$\underline{H_2}$), 4.65 s (2H, C$\underline{H_2}$- triazole), 4.85 d (4H, 2CH_2N), 5.13 s (2H, CH_2-C(O)-), 7.14 dt (4H, J_{HF}=27 Hz, J_{HH}=6 Hz, Ar), 7.40 dd (4H, J_{HF}=9 Hz, J_{HH}=6 Hz, Ar), 7.69 s (1H, triazole), 7.8 d (2H, 2 HC=). ^{13}C NMR (101 MHz) δ 43.7, 46.1 (CH_2N), 50.7 (CH_2OH), 61.6 (CH_2O), 64.3 (CH_2O), 69.5 (CH_2O), 70.2 (CH_2O), 70.4 (CH_2O), 70.5 (CH_2O), 72.5 (CH_2-C=O), 124.6 (N-C=), 130.1 (C=), 130.5 (d, J = 13 Hz, C, Ar), 132.6 (d, J = 5 Hz, =CH), 130.5 (C, Ar), 137.8 (d, J = 55 Hz C, Ar), 145.3 (C=), 163.0 (d, J_{HF} = 250 Hz, C-F) 164.0 (C=O), 185.6 (C=O). ^{19}F NMR (282 MHz, $CDCl_3$) δ -108.6, -109.3. Elemental analysis calcd. (%) for $C_{30}H_{34}F_2N_4O_6$: C, 61.81; H, 5.53 N, 9.67; found: C, 61.87; H, 5.56 N, 9.70.

(1-(2-(*3,5*)-bis((E)-4-chlorobenzylidene)-4-oxopiperidin-1-yl)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)-(2-(2-ethoxy)ethane-1-ol (4c)



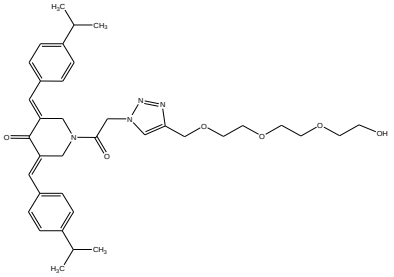
Yellow oil (65 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66 br.s.(1H, OH), 3.58 q (2H, J = 3Hz, C<u>H</u>$_2$CH$_2$OH), 3.63-3.64 m (6H, 3CH$_2$O), 3.68 q (2H, triazole-CH$_2$OC<u>H</u>$_2$), 4.67 s (2H, C<u>H</u>$_2$-triazole), 4.85 d (4H, 2CH$_2$N), 5.12 s (2H, CH$_2$-C(O)-), 7.34-7.49 m (8H, 2Ph), 7.70 s (1H, triazole), 7.83 d (2H, 2 HC=). δ ^{13}C NMR (101 MHz, $CDCl_3$) δ 43.9, 46.0 (CH$_2$N), 50.6 (CH$_2$OH), 61.4 (CH$_2$O), 64.3 (CH$_2$O), 69.4 (CH$_2$O), 70.2 (CH$_2$O), 70.4, 70.44 (CH$_2$O), 72.5 (CH$_2$-C=O), 125.5 (N-C=), 129.2 (HC=), 129.5 (C, Ar), 130.9 (C=), 131.7 (CH, Ar), 132.5 (C, Ar), 136.7 (ClC, Ar), 137.7 (HC=), 163.9 (C=O), 185.4 (C=O). Elemental analysis calcd. (%) for $C_{30}H_{34}Cl_2N_4O_6$: C, 58.51; H, 5.24 N, 9.15; found: C, 58.55; H, 5.27 N, 9.18. $C_{30}H_{34}Cl_2N_4O_6$.

(1-(2-oxo-2-(4-oxo-3,5-bis((*E*)-4-methoxybenzylidene)piperidin-1-yl)ethyl)-1*H*-1,2,3-triazol-4-yl))-(2-(2-ethoxy)ethane-1-ol (4d)



Yellow oil (67 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.98 br.s.(1H, OH), 3.55 q (2H, J = 3Hz, C<u>H</u>$_2$CH$_2$OH), 3.61-3.64 m (6H, 3CH$_2$O), 3.81 q (2H, triazole-CH$_2$OC<u>H</u>$_2$), 3.82 d (3H, OCH$_3$), 4.63 s (2H, C<u>H</u>$_2$- triazole), 4.85 d (4H, 2CH$_2$N), 5.12 s (2H, CH$_2$-C(O)-), 6.89 – 6.99 m (4H, Ar), 7.68-7.83 m (4H, Ar), 7.68 s (1H, triazole), 7.81 d (2H, 2 HC=). ^{13}C NMR (101 MHz, $CDCl_3$) δ 44.9, 46.1 (CH$_2$N), 50.7 (CH$_2$OH), 55.5 (OCH$_3$), 61.6 (CH$_2$O), 64.4 (CH$_2$O), 69.4 (CH$_2$O), 70.2 (CH$_2$O), 70.4 (CH$_2$O), 70.5 (CH$_2$O), 72.5 (CH$_2$-C=O),114.5 (CH, Ar), 126.6 (C, Ar), 128.6 (HC=), 132.7 (HC, Ar), 137.9 (C=), 138.6 (HC=), 145.1 (C=), 160.9 (<u>C</u>OCH$_3$, Ar), 163.9 (C=O), 185.7 (C=O). Elemental analysis calcd. (%) for $C_{32}H_{38}N_4O_8$: C, 63.32; H, 6.31 N, 9.28; found: C, 63.37; H, 6.33 N, 9.30. $C_{32}H_{38}N_4O_8$.

(1-(2-(*3,5*)-bis((E)-4-isopropylbenzylidene)-4-oxopiperidin-1-yl)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)-(2-(2-ethoxy)ethane-1-ol (4e)



Yellow oil (60 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23-1.27 m (12H, 4CH$_3$), 2.91 m (2H, 2C<u>H</u>(CH$_3$)$_2$), 3.18 br.s.(1H, OH), 3.54-3.55 m (2H, C<u>H</u>$_2$CH$_2$OH), 3.54-3.58 m (6H, 3CH$_2$O), 3.62 m (2H, triazole-CH$_2$OC<u>H</u>$_2$), 4.64 s (2H, C<u>H</u>$_2$- triazole), 4.87 d (4H, 2CH$_2$N), 5.12 s (2H, CH$_2$-C(O)-), 7.28-7.35 m (10H, Ar), 7.70 s (1H, triazole), 7.85 d (2H, 2 HC=). ^{13}C NMR (101

MHz, $CDCl_3$) δ 23.6 ($2CH_3$), 34.0 ($HC(CH_3)_2$), 43.9, 46.0 (CH_2N), 50.5 (CH_2OH), 61.5 (CH_2O), 64.3 (CH_2O), 69.4 (CH_2O), 70.2 (CH_2O), 70.4, 70.44 (CH_2O), 72.5 (CH_2-C=O), 126.9 (N-C=), 127.2 (C, Ar), 129.6 (C, Ar), 130.7 (C, Ar), 131.7 (C=), 138,4 (C=), 139.5 (C=), 151.3 (C, Ar), 164.0 (C=O), 185.5 (C=O). Elemental analysis calcd. (%) for $C_{35}H_{44}N_4O_6$: C, 68.13; H, 7.19 N, 9.13; found: C, 68.16; H, 7.22 N, 9.15. $C_{35}H_{44}N_4O_6$.

3. Spectral Data

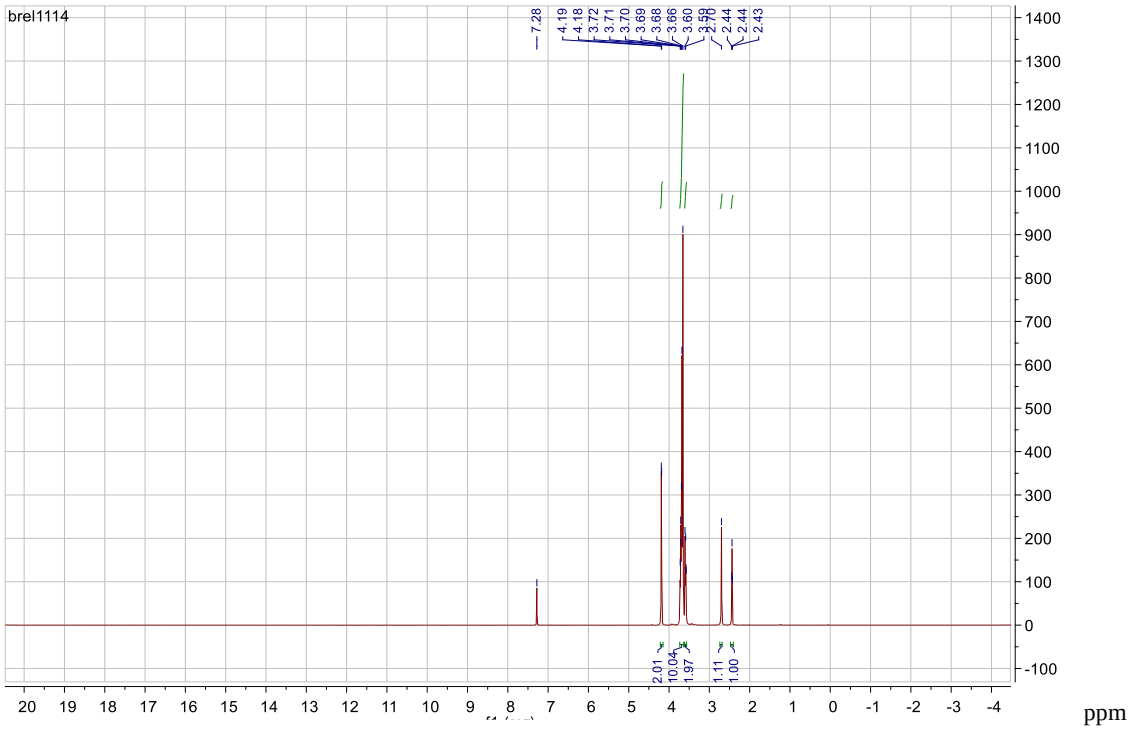


Figure S1. ^{1}H NMR spectrum mono propargyl ether of triethylenglycole ($CDCl_3$)

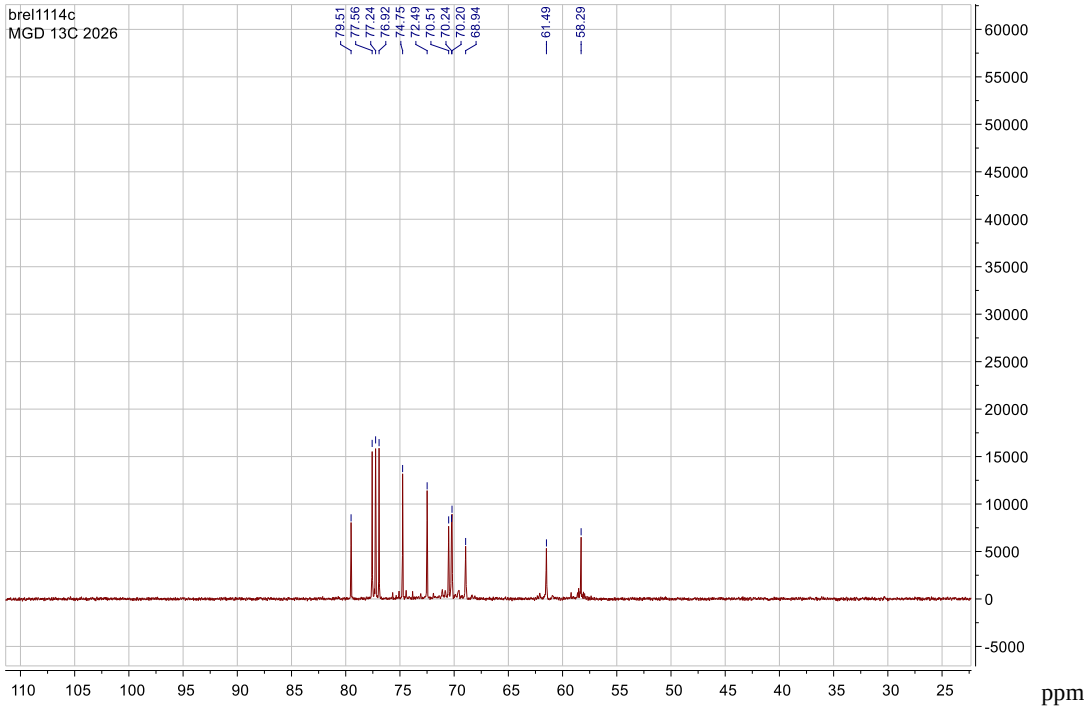


Figure S2. ^{13}C NMR spectrum mono propargyl ether of triethylenglycole ($CDCl_3$

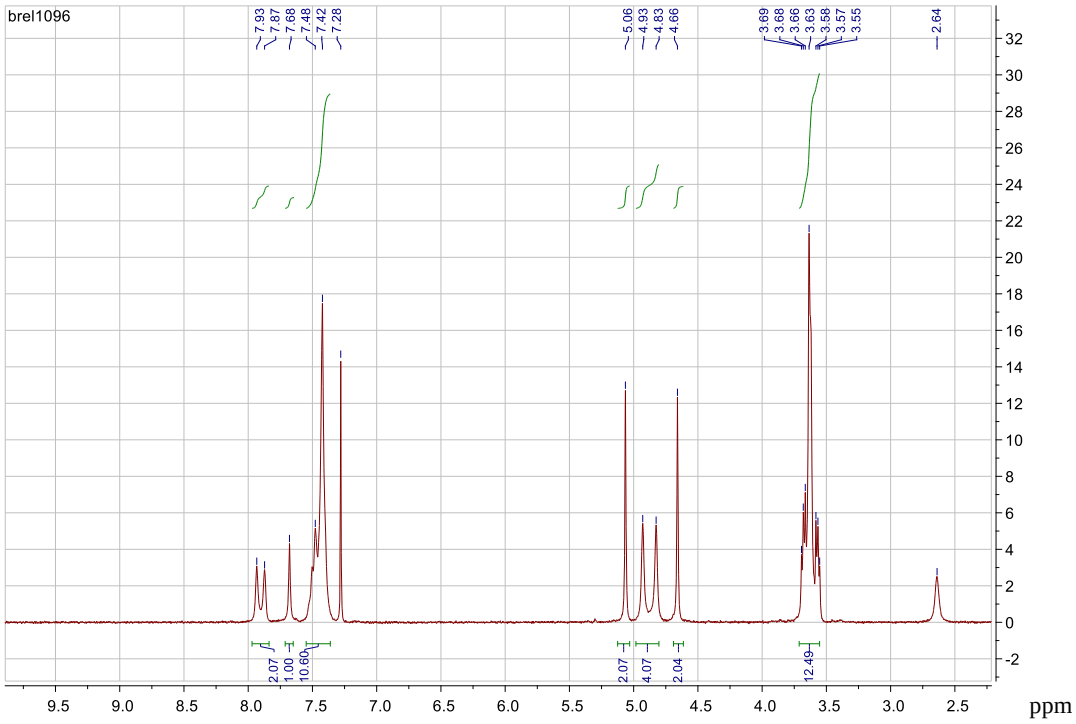


Figure S3. ^{1}H NMR spectrum of compound 4a ($CDCl_3$)

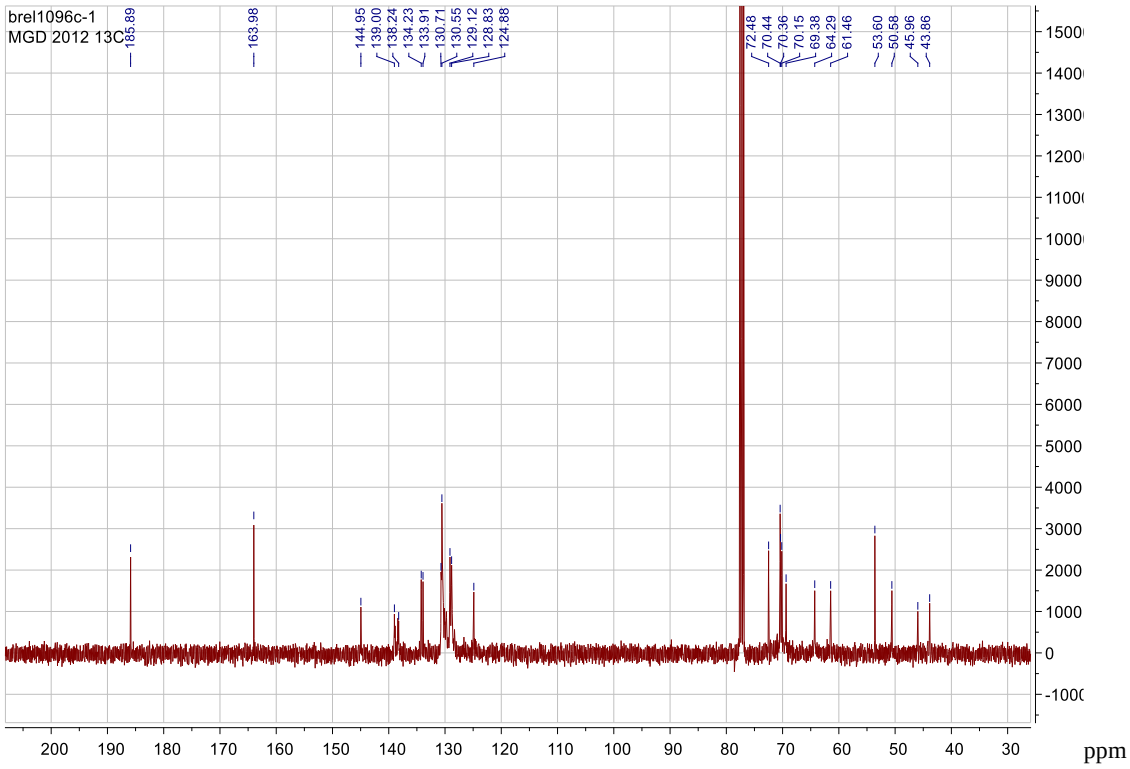


Figure S4. ^{13}C NMR spectrum of compound 4a ($CDCl_3$)

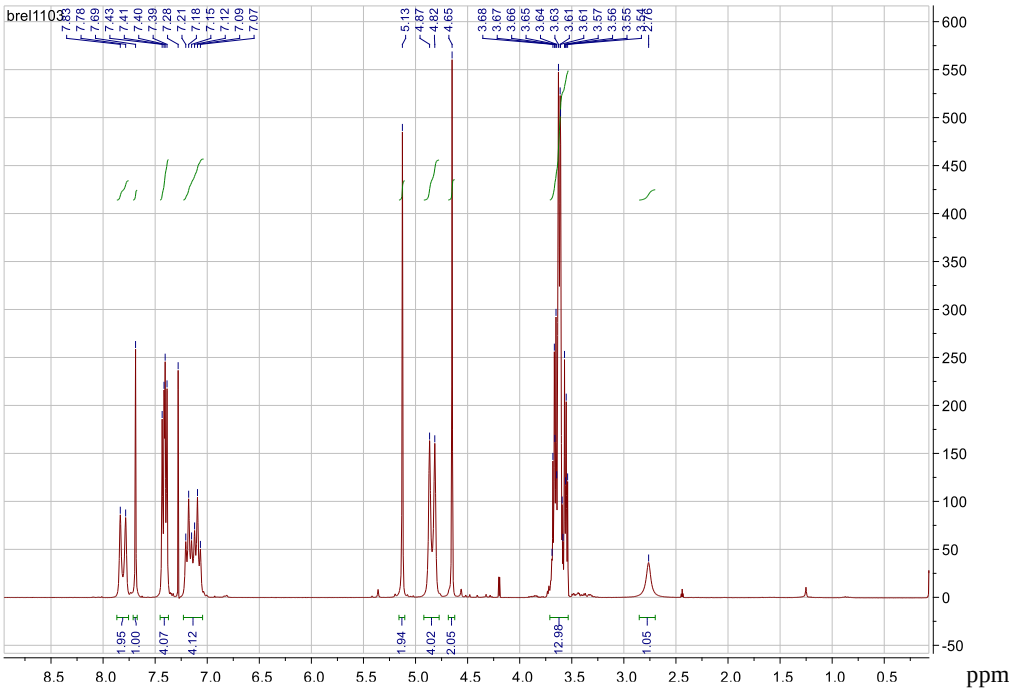


Figure S5. ^{1}H NMR spectrum of compound 4b ($CDCl_3$)

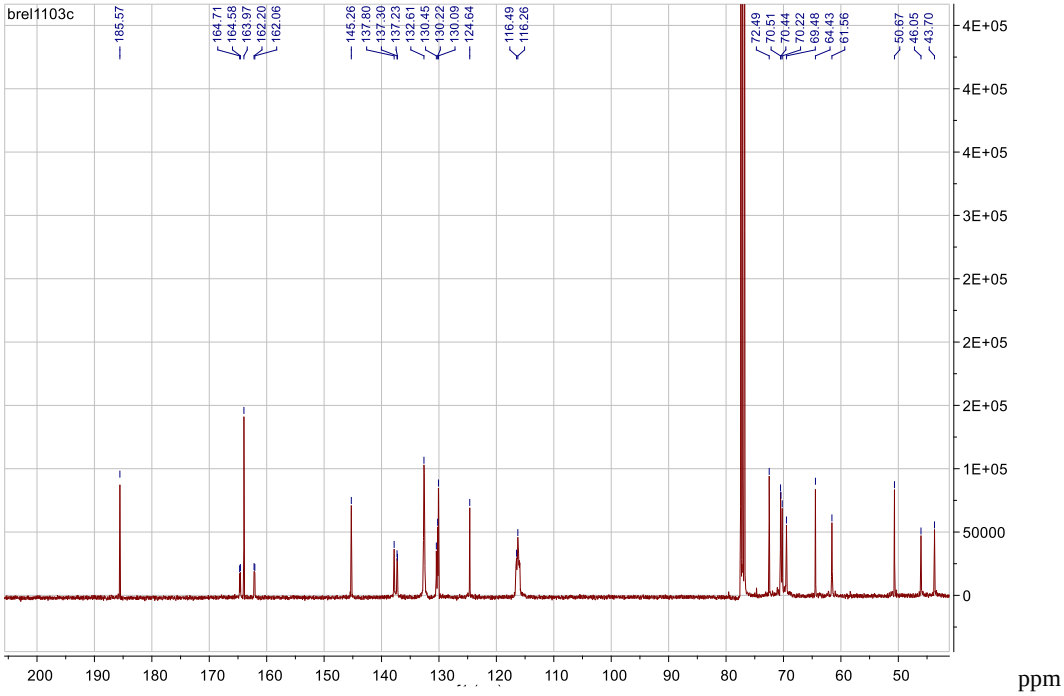


Figure S6. ^{13}C NMR spectrum of compound 4b ($CDCl_3$)

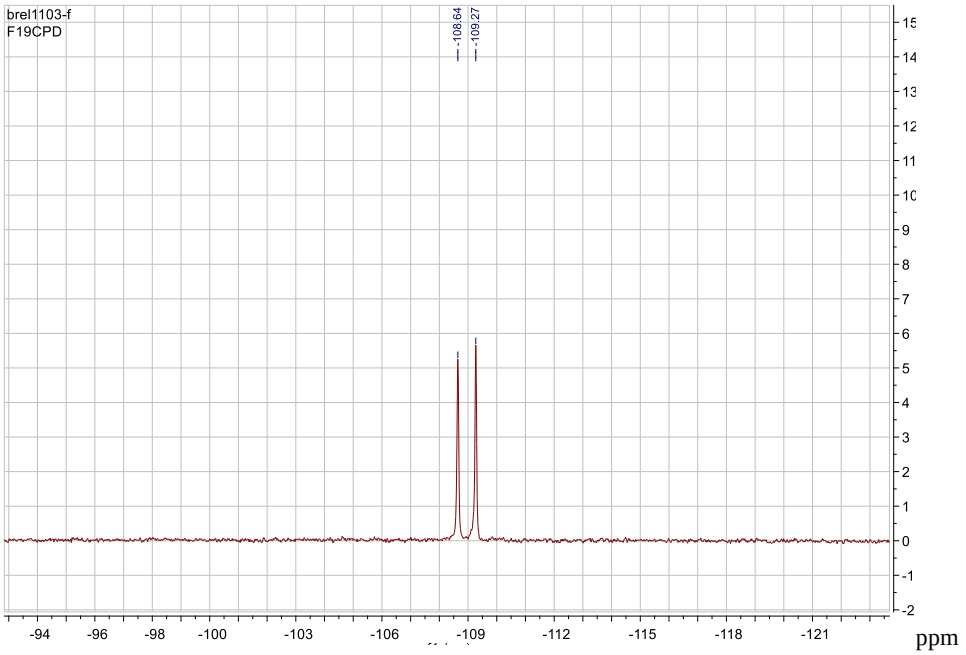


Figure S7. ^{19}F NMR spectrum of compound 4b ($CDCl_3$)

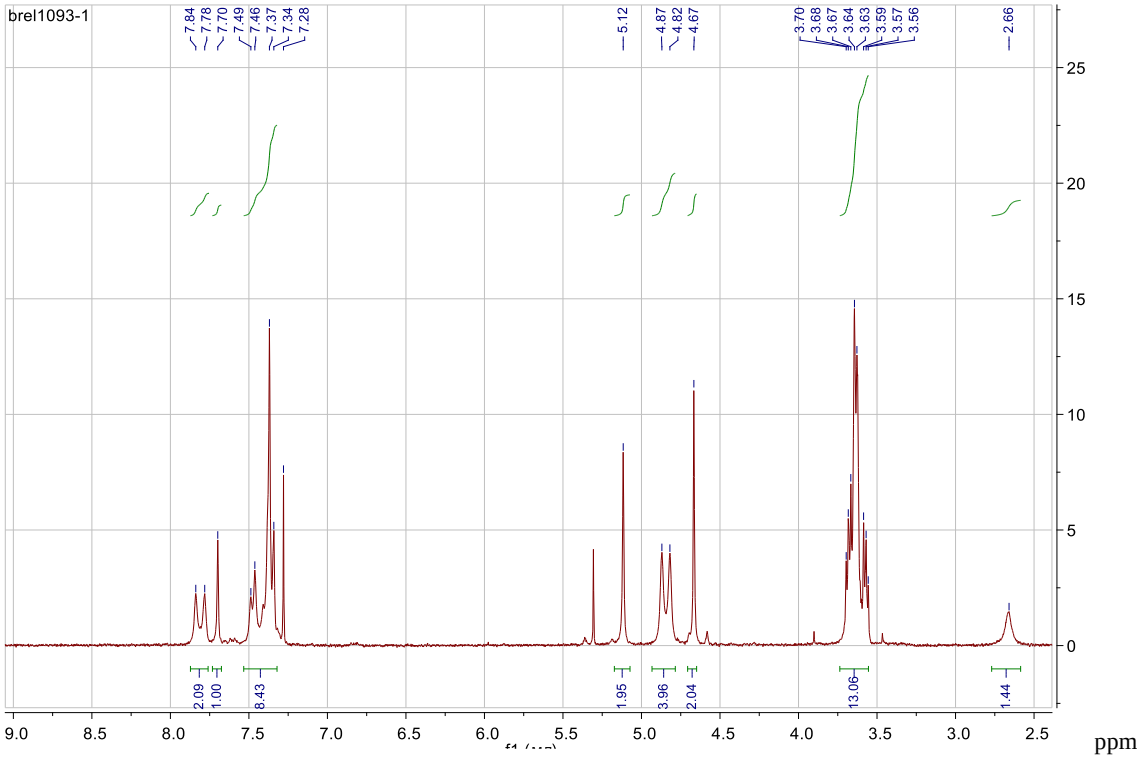


Figure S8. ^{1}H NMR spectrum of compound 4c ($CDCl_3$)

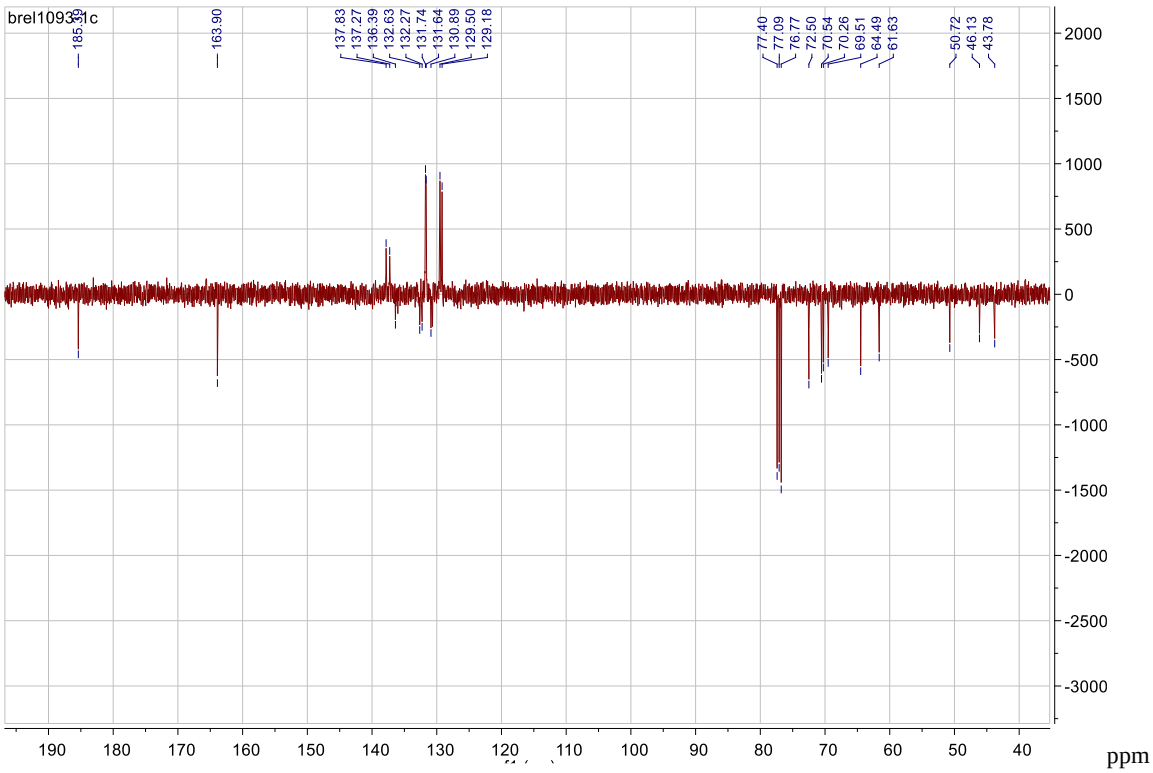


Figure S9. ^{13}C NMR spectrum of compound 4c ($CDCl_3$)

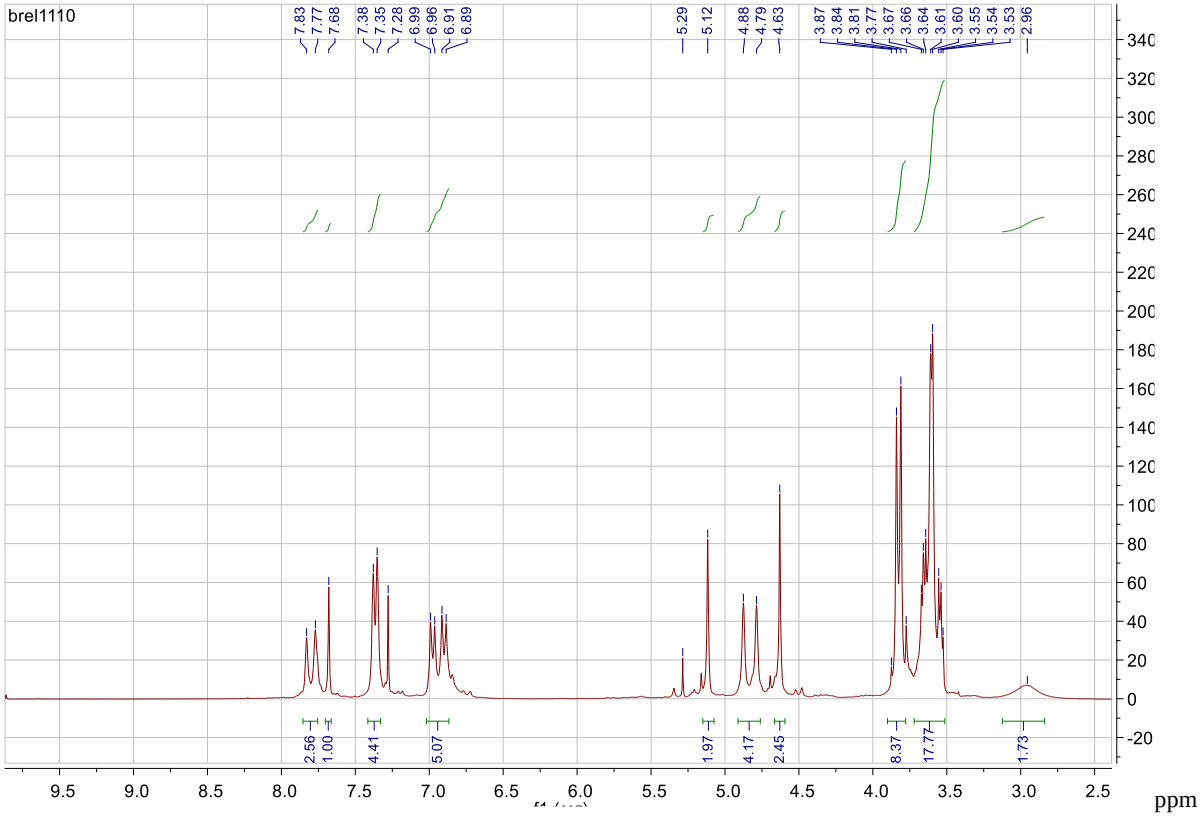


Figure S10. ^{1}H NMR spectrum of compound 4d ($CDCl_3$)

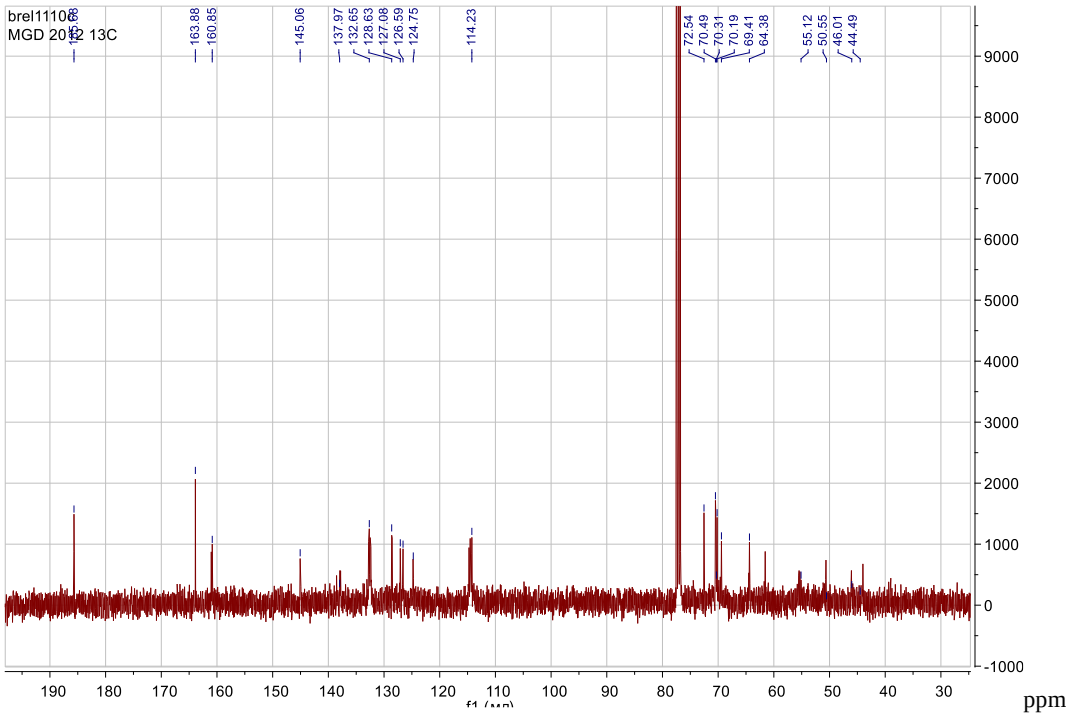


Figure S11. ^{13}C NMR spectrum of compound 4d ($CDCl_3$)

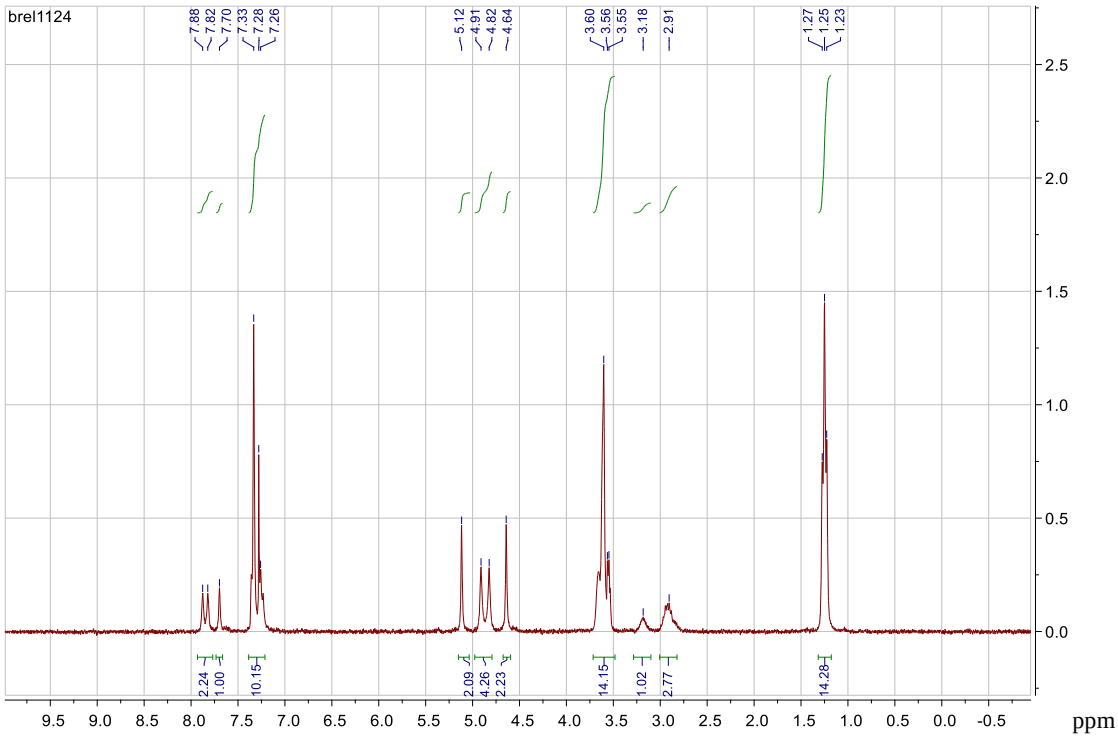


Figure S12. ^{1}H NMR spectrum of compound 4e ($CDCl_3$)

4. RCA

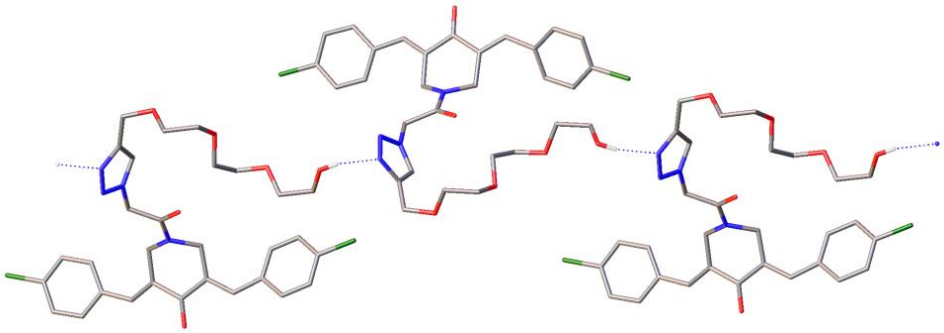


Figure S13 The hydrogen-bonded chain in crystal **4c.**

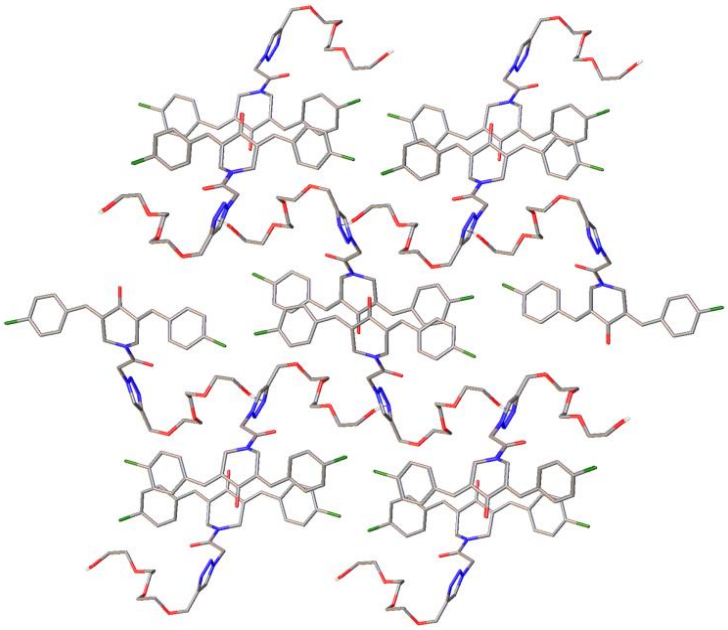


Figure S14 The fragment of crystal packing in crystal **4c** demonstrating the alternating hydrophobic and hydrophilic layers.

(2009), J. Appl. Cryst. 42, 339-341.

Sheldrick, G.M. (2015). Acta Cryst. A71, 3-8.

Sheldrick, G.M. (2015). Acta Cryst. C71, 3-8.


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5. Cell Culture and Cytotoxicity assay

Bladder cancer(T24), glioblastoma (T98G), epidermoid carcinoma (A431), neuroblastoma(SH-SY5Y) and human embryonic kidney (Hek-293) cells cultures were procured from the collection of the Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia. Cell lines T98G and T24 were cultured in Eagle's Minimum Essential Medium (EMEM, Biolot, Russia), A431 and Hek-293 were cultured in Dulbecco's modified Eagle's medium (DMEM, Biolot, Russia), and Human brain neuronal cells (SH-SY5Y) were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12, Biolot, Russia). All of themroutinely with 4.5 g/L L - glutamine and sodium pyruvate supplemented with 10% fetal bovine serum(Biolot, Russia) and 1% penicillin/streptomycin(Paneco, Russia) in a humidified 37°C incubator with 5% CO_2.

Cytotoxicity assay

To determine the cell viability, an MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was conducted. The MTT assay is a method based on the ability of viable cells to metabolize the yellow soluble tetrazolium salt to a blue insoluble formazan product by the mitochondrial succinic dehydrogenase.This experimental procedure of cytotoxicity assay was carried out according to a previously published methodology[S3]. In short, when cells were confluent, they were seeded in 96-well plates ($1Ч10^4$ cells/well, 200 мL) using 0.25 % Trypsin-EDTA (Paneco, Russia) and incubated for 24 h. After that cells were treated with compound (0.01–100 мМ) or 0.1% DMSO (99,9%, Servicebio, Wuhan, China) as control for 72 h. MTT (5 mg/mL in PBS, PanEco, Russia) was added 20 μL/well andincubation for 2 h at 37 °C protected from light. Since then the MTT solution was removed and the resulting formazan crystals were solubilized in 200 μL of DMSO, and absorbance was read at 540 nm. Experiments were performed at least in triplicate, IC_{50} values were calculated using GraphPad Prism 8 and are reported as mean ± SD.

6. References

[S1] M. R. Bockman, R. J. Dalal, R. Kumar and T.M. Reineke, *Polym. Chem.*, 2021, **12**, 28, 4063; https://doi.org/10.1039/d1py00250c**.**

[S2] P. A. Yudaev, Yu. R. Aleksandrova, I. A. Shagina, O.I.Artyushin, E. V. Sharova, A. N. Rodionov, M. E. Neganova and V.K. Brel, *Int. J. Mol. Sci*., 2026, **27**, 4104 ; https://doi.org/10.3390/ijms27094104.