

Supplementary Materials

Photo-induced porcine gelatin cross-linking by homobi- and homotrifunctional tetrazoles

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Table of Contents

S2-S3. Procedures for the synthesis of 1-5.

S4-S11. ¹H NMR, ¹³C NMR and ATR-FTIR spectra of synthesized compounds.

S12-S19. Video frames of thermal stability test at 37 °C.

Synthetic procedures

General information

All reagents and solvents were purchased from commercial sources (Fluorochem Co.; Tokyo Chemical Industry Co. and Aldrich Chemical Co.) and used as received. Chromatographic purifications were performed using Merck 9385 silica gel, pore size 60 Å (230–400 mesh). Melting points were measured with a Stanford Research Systems Optimelt apparatus. IR spectra were recorded with a PerkinElmer Spectrum 100 FT-IR spectrometer equipped with universal ATR sampling accessory. ^1H and ^{13}C spectra were recorded with a Bruker AVANCE III HD 400 MHz spectrometer (^1H : 400 MHz, ^{13}C : 101 MHz), chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants are given in Hz. Splitting patterns are indicated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Elemental analyses were obtained with an Elementar vario MICRO cube instrument. UV λ_{max} were determined with a PerkinElmer Lambda 900 spectrophotometer.

1,4-bis(2-phenyl-2H-tetrazol-5-yl)benzene (1). A mixture of terephthalaldehyde (1.00 g, 7.5 mmol) and *p*-Toluenesulfonyl hydrazide (2.92 g, 15 mmol) in EtOH (35 mL) was heated at reflux under stirring for 5 h. After cooling to r.t., the mixture was poured into cold water. The precipitate formed was recovered by filtration and dried at 80°C overnight. The precipitate was then dissolved in 30 mL of pyridine to give solution A. In parallel, a solution of NaNO_2 (1.03 g, 15 mmol) in water (4 mL) was added dropwise to a cooled (0 °C) mixture of aniline (1.40 g, 15 mmol), concentrated HCl (37% in H_2O , 3.75 mL, 45 mmol), H_2O (5 mL) and EtOH (5 mL) to give solution B. Solution A was cooled with an ice bath and solution B was then slowly added. The mixture was stirred overnight at r.t.. The mixture was then poured into an aqueous HCl solution (200 mL, 3 M) and the precipitate was recovered by filtration. Crystallization (EtOAc) afforded **1** in a pure form (1.51 g, 55%). Pink solid; m.p.: 184–185 °C dec; ^1H NMR (400 MHz, CDCl_3) δ 8.44 (s, 4H), 8.26 – 8.22 (m, 4H), 7.64 – 7.58 (m, 4H), 7.57 – 7.50 (m, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 164.6, 149.7, 136.8, 136.1, 129.8, 129.7, 127.6, 123.8, 119.9. IR (ATR): 3191, 1596, 1560, 1492, 1471, 1450, 1425, 1361, 1322, 1300, 1277, 1213, 1187, 1165, 1093, 1053, 1011, 994, 954, 913, 854, 835, 811, 759, 739, 702, 677, 571, 553 cm^{-1} ; Anal. Calcd. for $\text{C}_{20}\text{H}_{14}\text{N}_8$: C, 65.56; H, 3.85; N, 30.58. Found: C, 65.67; H, 3.89; N, 30.45; UV (DMSO) λ_{max} , nm: 296.

4,4'-bis(2-phenyl-2H-tetrazol-5-yl)-1,1'-biphenyl (2). A mixture of (1,1'-biphenyl)-4,4'-dicarbaldehyde (2.50 g, 12 mmol) and *p*-Toluenesulfonyl hydrazide (4.43 g, 24 mmol) in EtOH (80 mL) was heated at reflux under stirring for 5 h. After cooling to r.t., the mixture was poured into cold water. The precipitate formed was recovered by filtration and dried at 80°C overnight. The precipitate was then dissolved in 90 mL of pyridine to give solution A. In parallel, a solution of NaNO_2 (1.65 g, 24 mmol) in water (6 mL) was added dropwise to a cooled (0 °C) mixture of aniline (2.22 g, 24 mmol), concentrated HCl (37% in H_2O , 6 mL, 72 mmol), H_2O (10 mL) and EtOH (10 mL) to give solution B. Solution A was cooled with an ice bath and solution B was then slowly added. The mixture was stirred overnight at r.t.. The mixture was then poured into an aqueous HCl solution (400 mL, 3 M) and the precipitate was recovered by filtration. Crystallization (MeOH) afforded **2** in a pure form (2.39 g, 45%). Pink solid; m.p.: 186–187 °C dec; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.34 (d, J = 8.0 Hz, 4H), 8.21 (d, J = 7.6 Hz, 4H), 8.07 (d, J = 8.0 Hz, 4H), 7.73 (t, J = 7.5 Hz, 4H), 7.67 (t, J = 7.2 Hz, 2H); IR (ATR): 3065, 3032, 2050, 1697, 1615, 1597, 1535, 1494, 1458, 1431, 1409, 1374, 1360, 1318, 1293, 1253, 1210, 1184, 1165, 1139, 1106, 1087, 1075, 1033, 1013, 992, 909, 863, 825, 748, 731, 715, 692, 675, 575 cm^{-1} ; Anal. Calcd. for $\text{C}_{26}\text{H}_{18}\text{N}_8$: C, 70.58; H, 4.10; N, 25.32. Found: C, 70.70; H, 4.07; N, 25.21; UV (DMSO) λ_{max} , nm: 312.

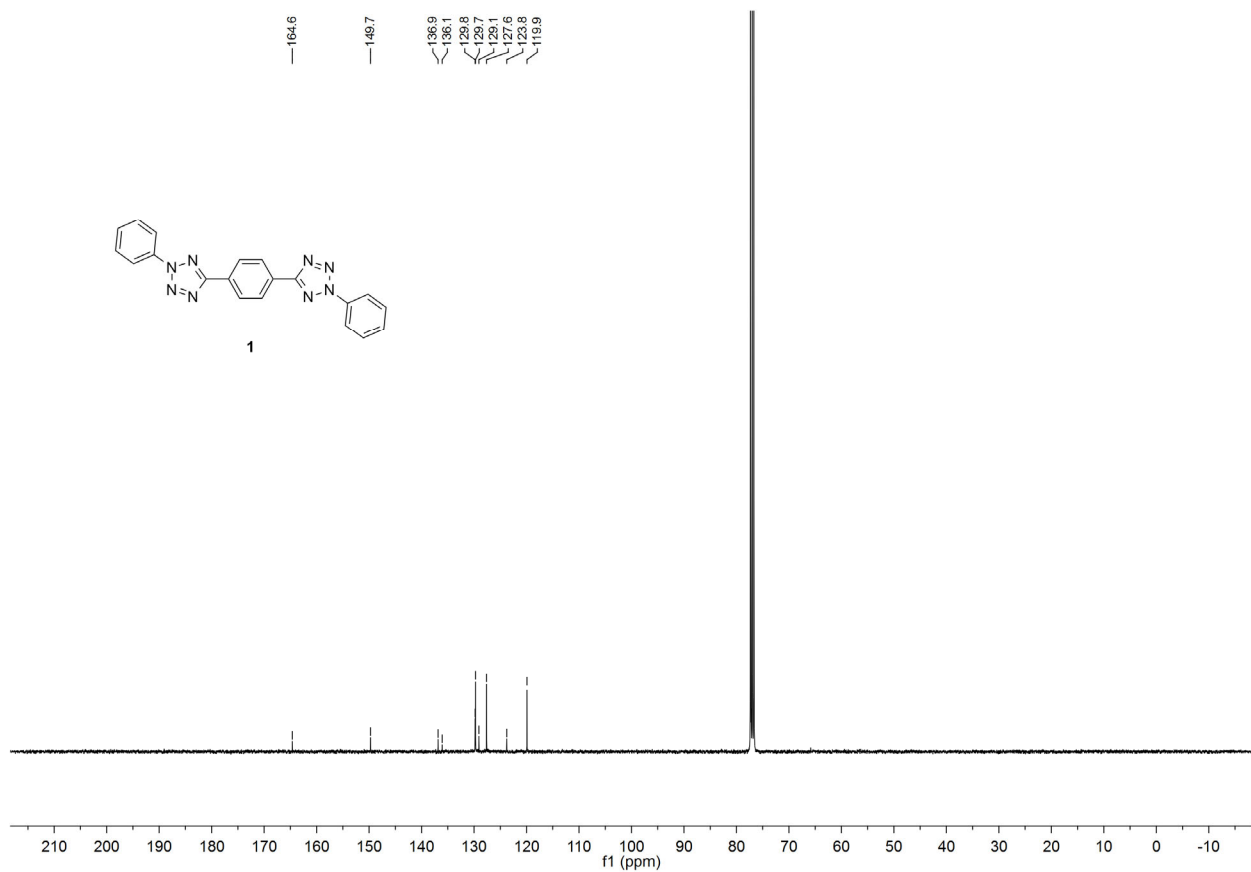
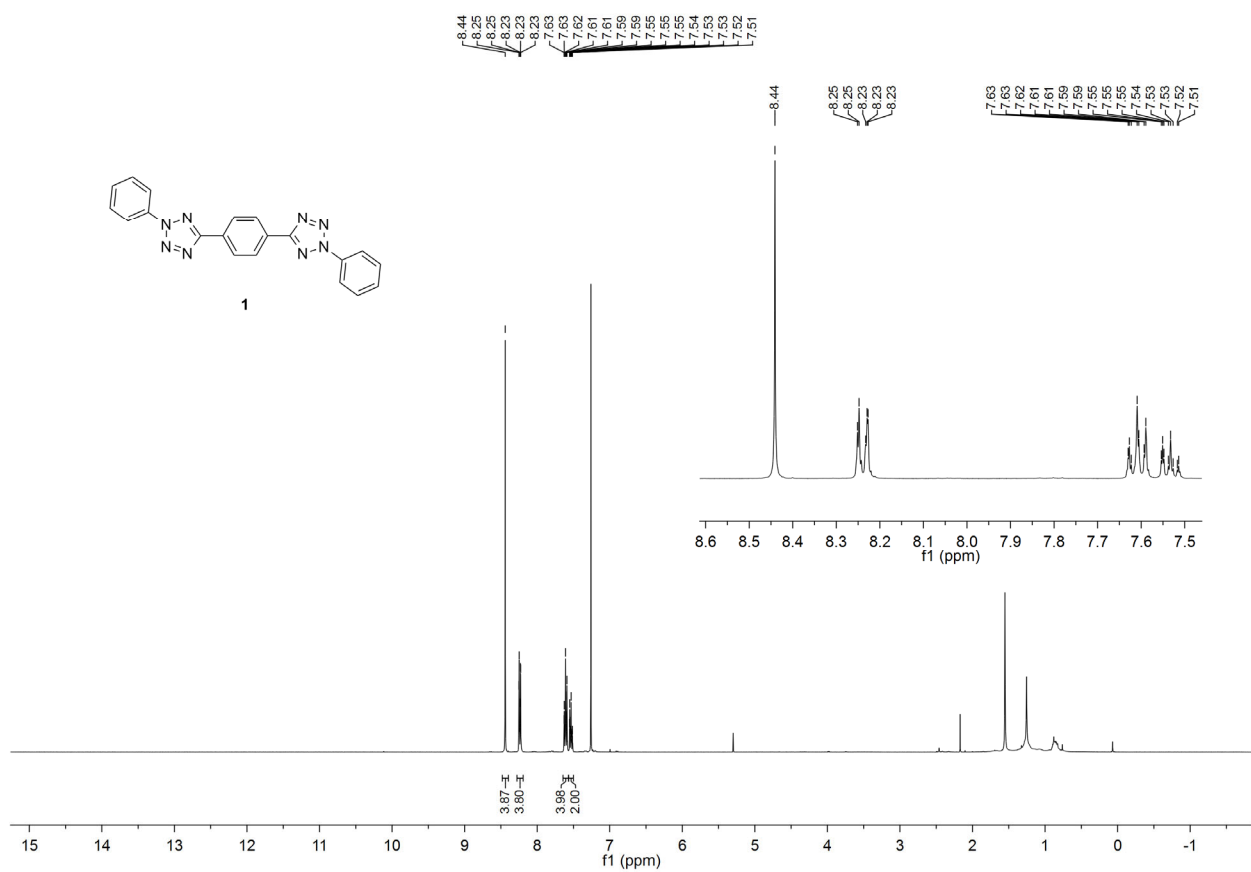
5-phenyl-2H-tetrazole (6). A mixture of Benzonitrile (2.50 g, 24 mmol), sodium azide (1.73 g, 24 mmol) and zinc bromide (5.46 g, 24 mmol) in H_2O (50 mL) was heated at reflux under stirring for 24 h. The mixture was then cooled with an ice bath and acidified with concentrated HCl (37% H_2O) until pH \approx 1. The mixture was extracted with EtOAc (3X 20 mL), the organic layer was dried (Na_2SO_4), and the solvent eliminated under reduced pressure. The crude was dissolved aqueous NaOH (200 mL, 0.25 M). The zinc salts formed were filtered off and the aqueous phase was acidified with aqueous HCl (400 mL, 3 M). The precipitate was recovered by filtration and dried at 80°C overnight to afford **6** in a pure form (2.64 g, 76 %). White solid; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.08 – 8.04 (m, 2H), 7.64 – 7.57 (m, 3H); IR (ATR): 3130, 3056, 2980, 2905, 2834, 2794, 2763, 2684, 2649, 2601, 2543, 2480, 2450, 1898, 1857, 1824, 1765, 1713, 1609, 1563, 1485, 1466, 1439, 1409, 1288, 1256, 1084, 1055, 1035, 1015, 989, 956, 925, 840, 790, 784, 725, 703, 685 cm^{-1} . The physical and spectroscopic data corresponded to those reported in the literature [64].

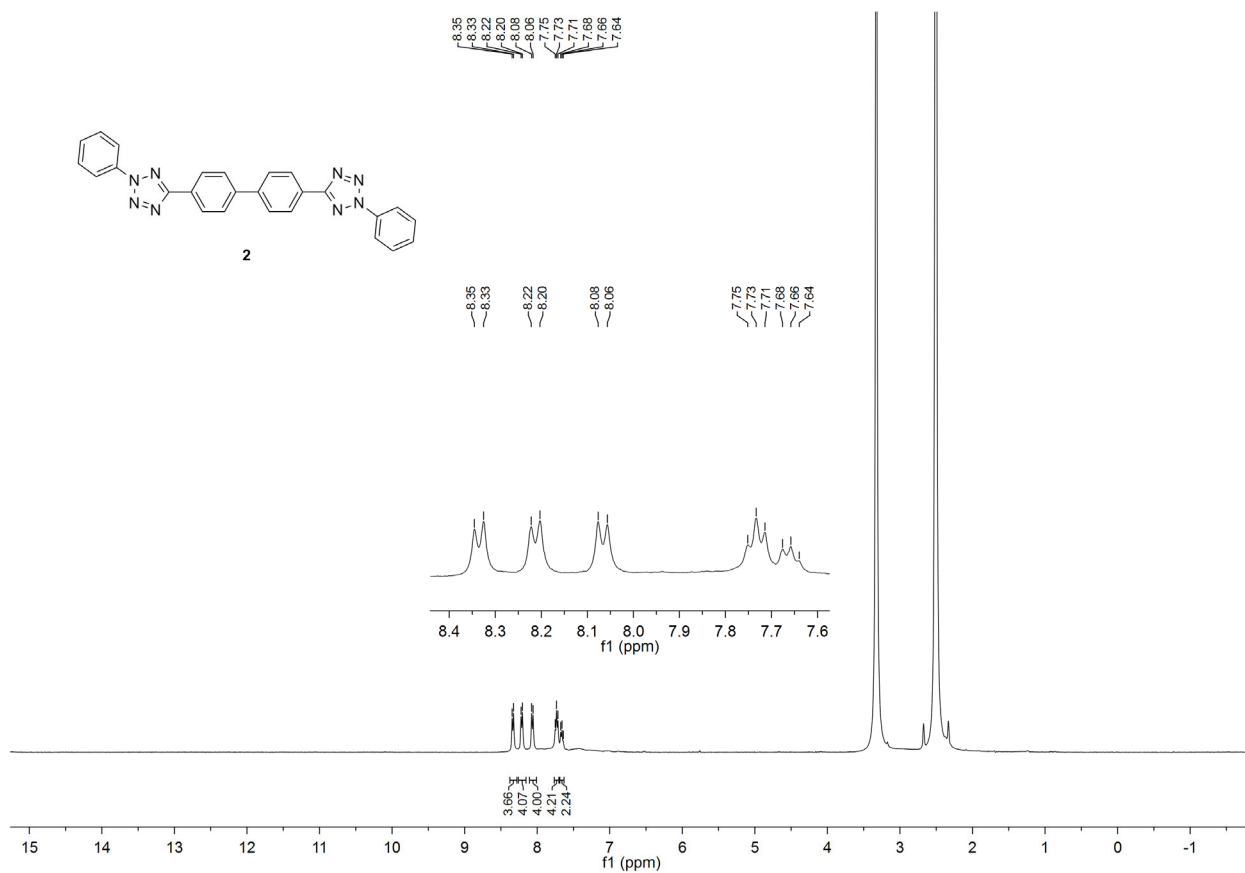
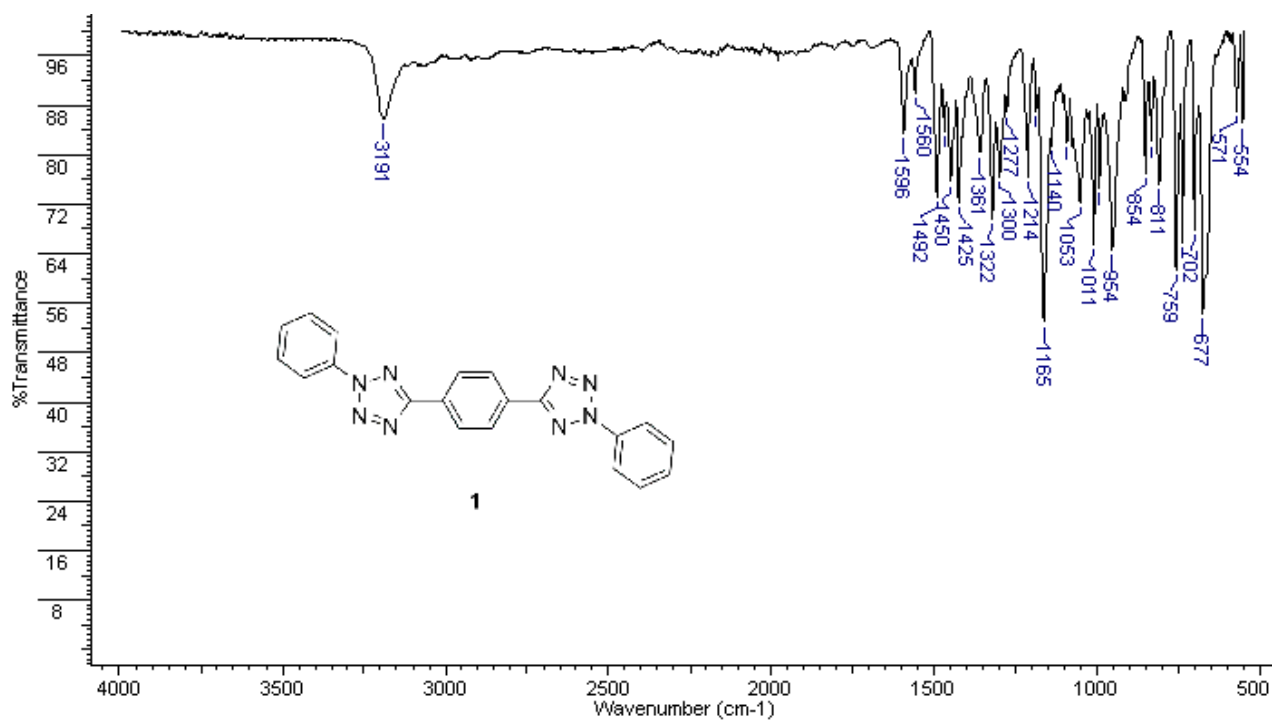
1,6-bis(5-phenyl-2H-tetrazol-2-yl)hexane (3). 5-phenyl-2H-tetrazole **6** (0.76 g, 5.2 mmol) was dissolved in dry DMF (15 mL), under nitrogen atmosphere. K₂CO₃ (0.79 g, 5.7 mmol) was added in one portion and 1,6-dibromohexane (0.40 mL, 2.6 mmol) was added dropwise over a period of 10 min. The mixture was stirred at r.t. for 48 h. The mixture was then extracted with EtOAc (2X 20 mL), the organic layer was washed with brine (2X 20 mL), dried (Na₂SO₄) and the volatiles eliminated under reduced pressure. Flash column chromatography (SiO₂, CH₂Cl₂) afforded **3** in a pure form (0.51 g, 52%). White solid; m.p.: 210-211 °C dec; ¹H NMR (400 MHz, CDCl₃) δ 8.17 – 8.11 (m, 4H), 7.52 – 7.45 (m, 6H), 4.65 (t, *J* = 7.0 Hz, 4H), 2.13 – 2.02 (m, *J* = 7.2 Hz, 4H), 1.49 – 1.42 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 165.1, 130.3, 128.9, 127.4, 126.8, 52.9, 29.1, 25.7; IR (ATR): 3067, 3034, 2947, 2872, 2863, 2165, 1981, 1962, 1895, 1822, 1767, 1716, 1653, 1610, 1585, 1528, 1463, 1449, 1397, 1365, 1352, 1339, 1306, 1286, 1251, 1206, 1177, 1131, 1103, 1070, 1042, 1030, 1000, 997, 921, 855, 787, 759, 729, 689, 617 cm⁻¹; Anal. Calcd. for C₂₀H₂₂N₈: C, 64.15; H, 5.92; N, 29.93. Found: C, 64.36; H, 5.89; N, 29.82; UV (DMSO) λ_{max}, nm: <265.

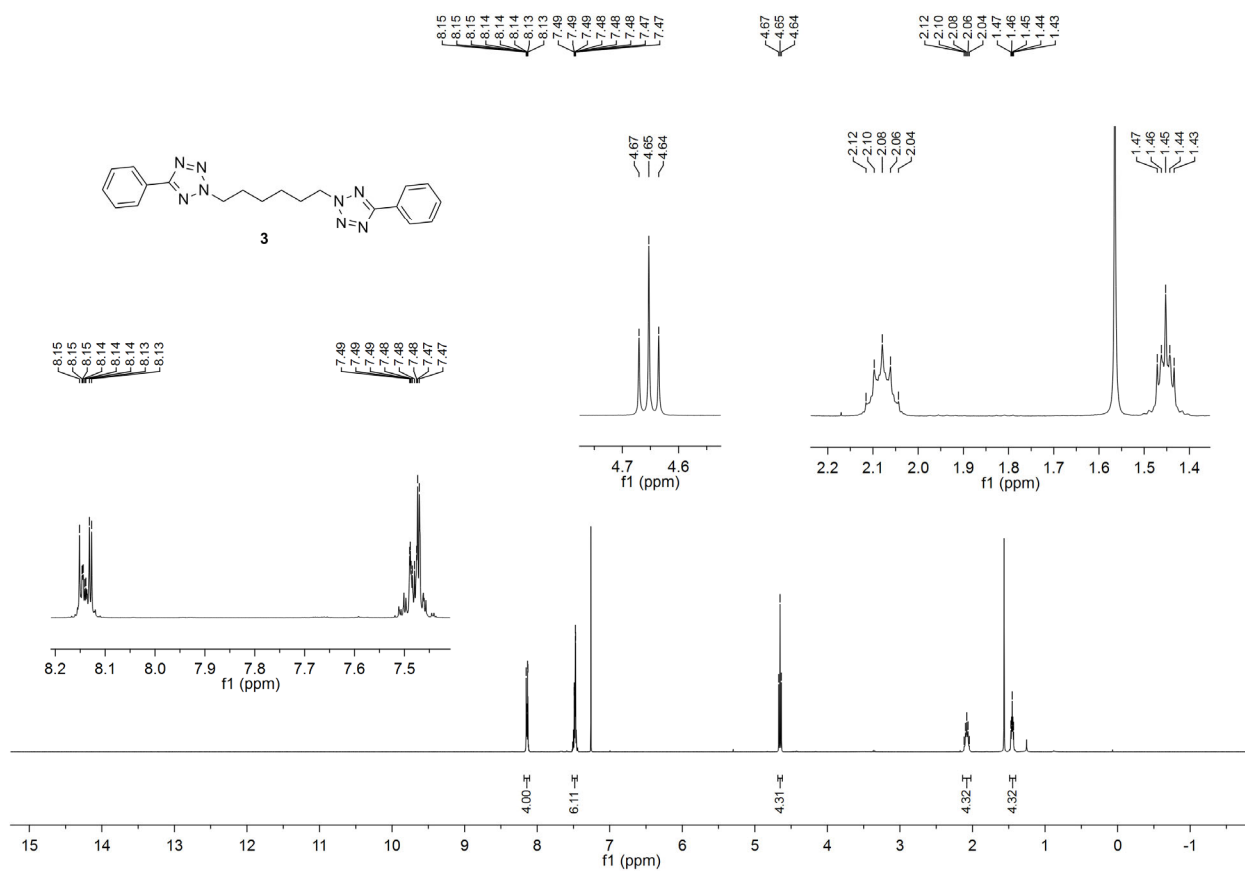
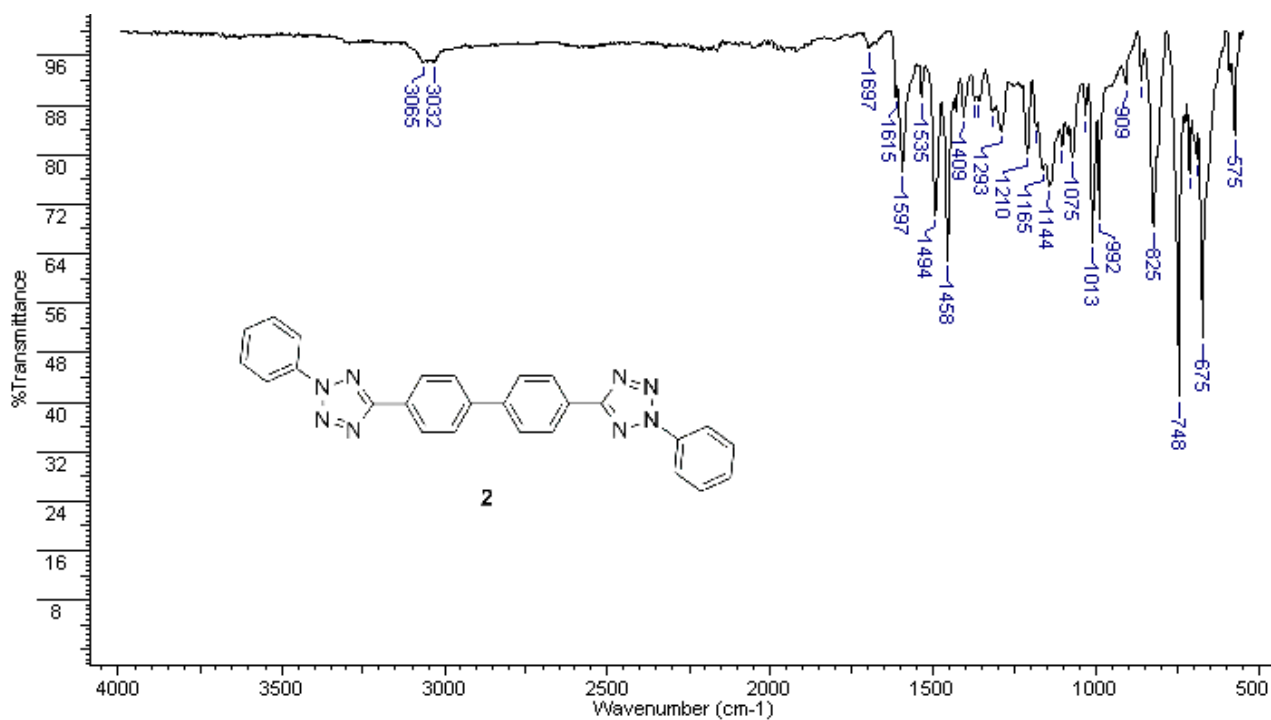
4-(5-(thiophen-2-yl)-2H-tetrazol-2-yl)phenol (7). A mixture of 2-Thiophenecarboxaldehyde (1.00 g, 8.9 mmol) and *p*-Toluenesulfonyl hydrazide (1.66 g, 8.9 mmol) in EtOH (25 mL) was heated at reflux under stirring for 5 h. After cooling to r.t., the mixture was poured into cold water. The precipitate formed was recovered by filtration and dried at 80°C overnight. The precipitate was then dissolved in 90 mL of pyridine to give solution A. In parallel, a solution of NaNO₂ (0.62 g, 8.9 mmol) in water (3 mL) added dropwise to a cooled (0 °C) mixture of 4-aminophenol (0.97 g, 8.92 mmol), concentrated HCl (37% in H₂O, 2 mL, 27 mmol), H₂O (5 mL) and EtOH (5 mL) to give solution B. Solution A was cooled with an ice bath and solution B was then slowly added. The mixture was stirred overnight at r.t.. The mixture was then poured into an aqueous HCl solution (200 mL, 3 M) and the precipitate was recovered by filtration. Flash column chromatography (SiO₂, CH₂Cl₂/EtOAc 95:5) afforded **2** in a pure form (2.39 g, 45%). Yellow solid; m.p. 160-161 °C dec; ¹H NMR (400 MHz, CDCl₃) δ 8.08 – 8.02 (m, 2H), 7.89 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.49 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.19 (dd, *J* = 5.0, 3.7 Hz, 1H), 7.04 – 6.99 (m, 2H), 5.38 (br. s, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 161.3, 156.8, 130.4, 128.9, 128.1, 128.1, 128.0, 121.7, 116.2; IR (ATR): 3177, 3108, 3093, 2983, 2952, 2925, 2847, 2701, 2632, 1883, 1811, 1743, 1680, 1644, 1618, 1600, 1575, 1569, 1516, 1474, 1410, 1368, 1340, 1328, 1281, 1254, 1230, 1221, 1201, 1187, 1168, 1124, 1106, 1089, 1074, 1053, 1018, 1005, 971, 939, 908, 851, 833, 747, 714, 705, 688, 669, 635, 628, 572 cm⁻¹

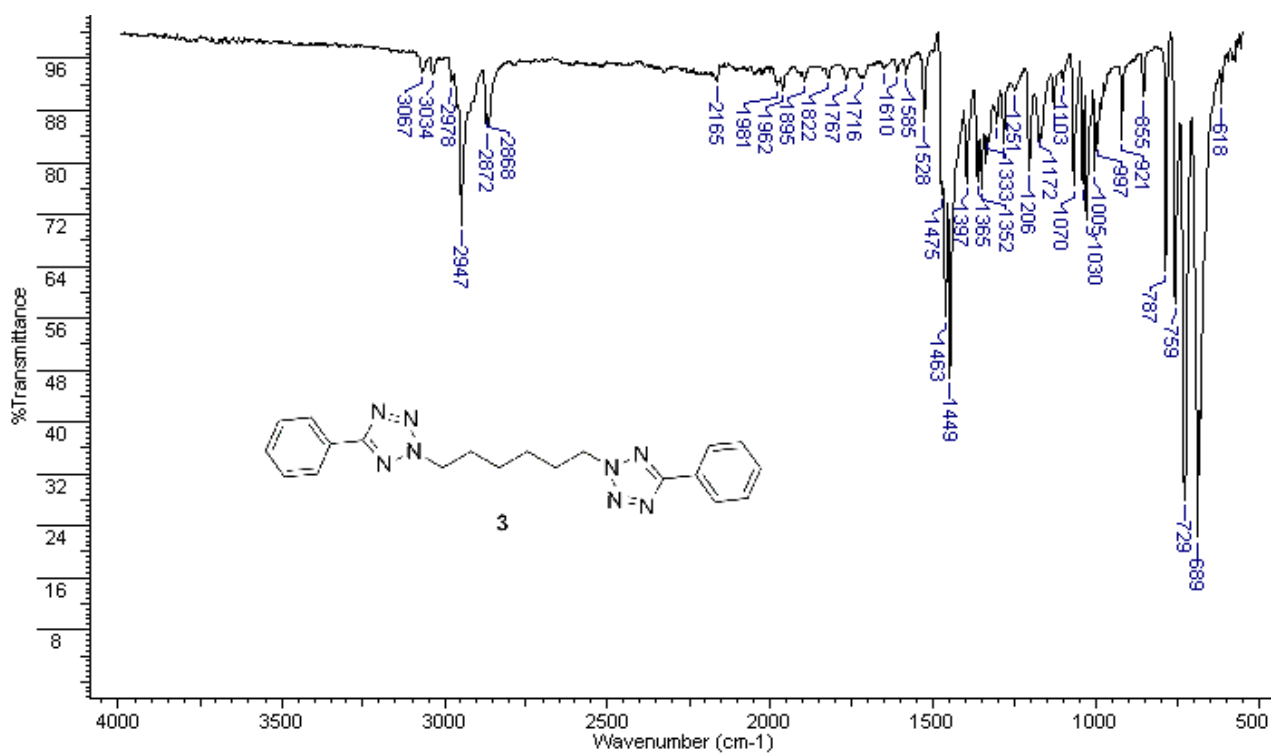
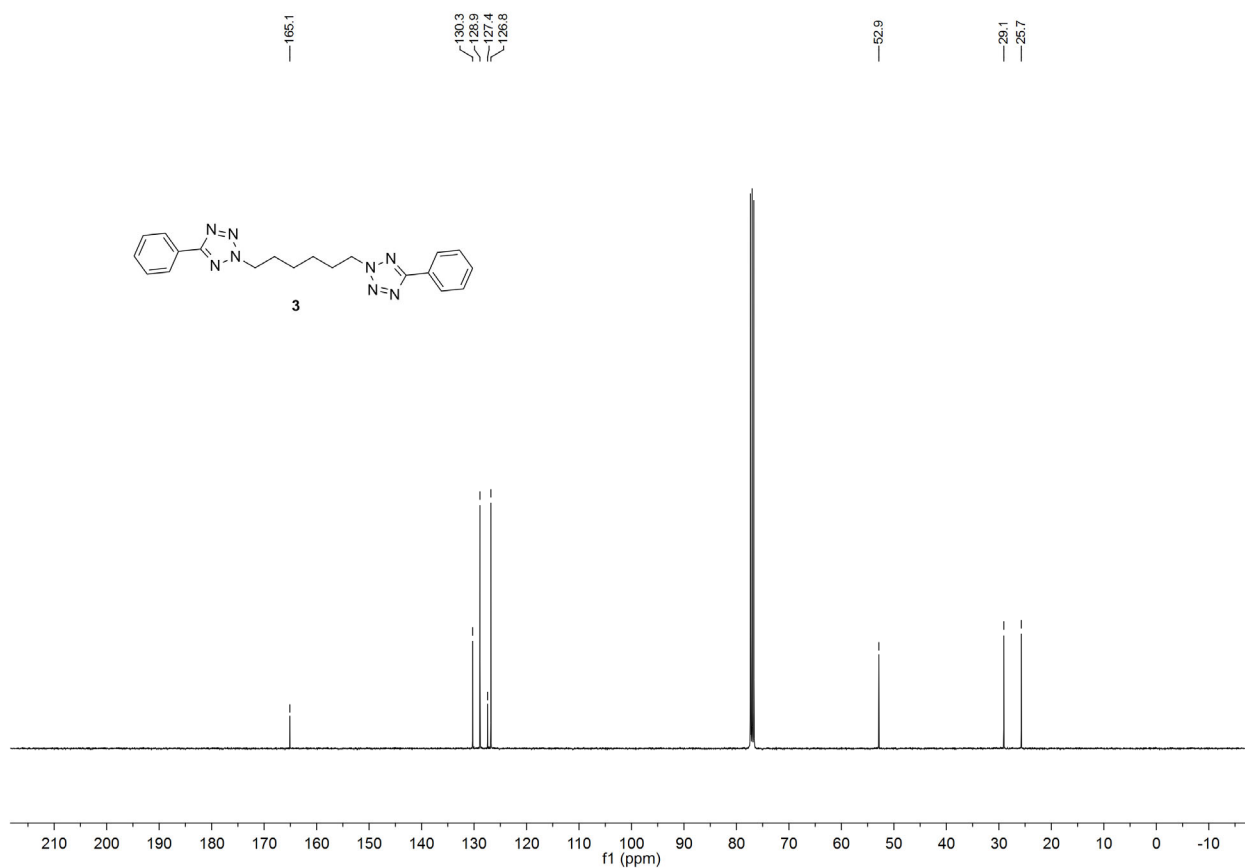
1,4-bis(4-(5-(thiophen-2-yl)-2H-tetrazol-2-yl)phenoxy)butane (4). **7** (0.40 g, 1.7 mmol) was dissolved in dry DMF (10 mL), under nitrogen atmosphere. K₂CO₃ (0.25 g, 1.8 mmol) was added in one portion and 1,4-dibromobutane (0.1 mL, 0.83 mmol) was added dropwise over a period of 10 min. The mixture was stirred r.t. for 72 h. The mixture was then extracted with EtOAc (2X 15 mL), the organic layer was washed with brine (2X 10 mL), dried (Na₂SO₄) and the volatiles eliminated under reduced pressure. Flash column chromatography (SiO₂, CH₂Cl₂) afforded **4** in a pure form (0.39 g, 87%). Yellow solid; m.p.: 190-191 °C dec; ¹H NMR (400 MHz, CDCl₃) δ 8.10 – 8.05 (m, 4H), 7.89 (dd, *J* = 3.7, 1.2 Hz, 2H), 7.49 (dd, *J* = 5.0, 1.2 Hz, 2H), 7.18 (dd, *J* = 5.0, 3.7 Hz, 2H), 7.06 – 7.01 (m, 4H), 4.08 (t, *J* = 6.0 Hz, 4H), 2.04 – 1.96 (m, 4H); ¹³C NMR (101 MHz, CDCl₃) δ 161.1, 159.8, 130.3, 128.9, 128.1, 127.9, 67.3, 27.8; IR (ATR): 3075, 2957, 2939, 2869, 1980, 1888, 1808, 1732, 1609, 1600, 1570, 1517, 1471, 1437, 1431, 1406, 1397, 1376, 1351, 1302, 1276, 1258, 1225, 1204, 1182, 1114, 1066, 1042, 1015, 1001, 965, 852, 843, 827, 788, 748, 734, 704, 686, 682, 654, 638, 575 cm⁻¹; Anal. Calcd. for C₂₆H₂₂N₈O₂S₂: C, 57.55; H, 4.09; N, 20.65. Found: C, 57.22; H, 4.16; N, 20.71; UV (DMSO) λ_{max}, nm: 304.

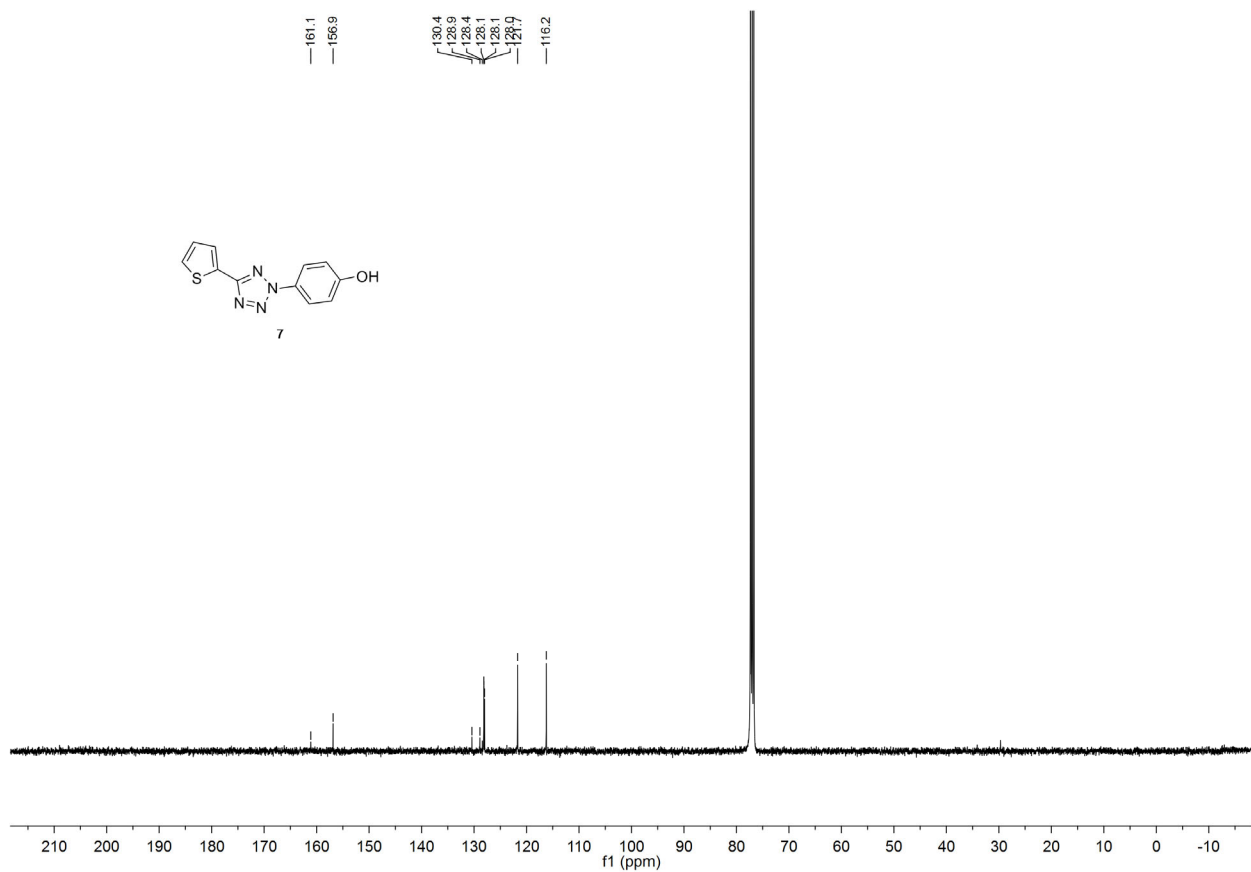
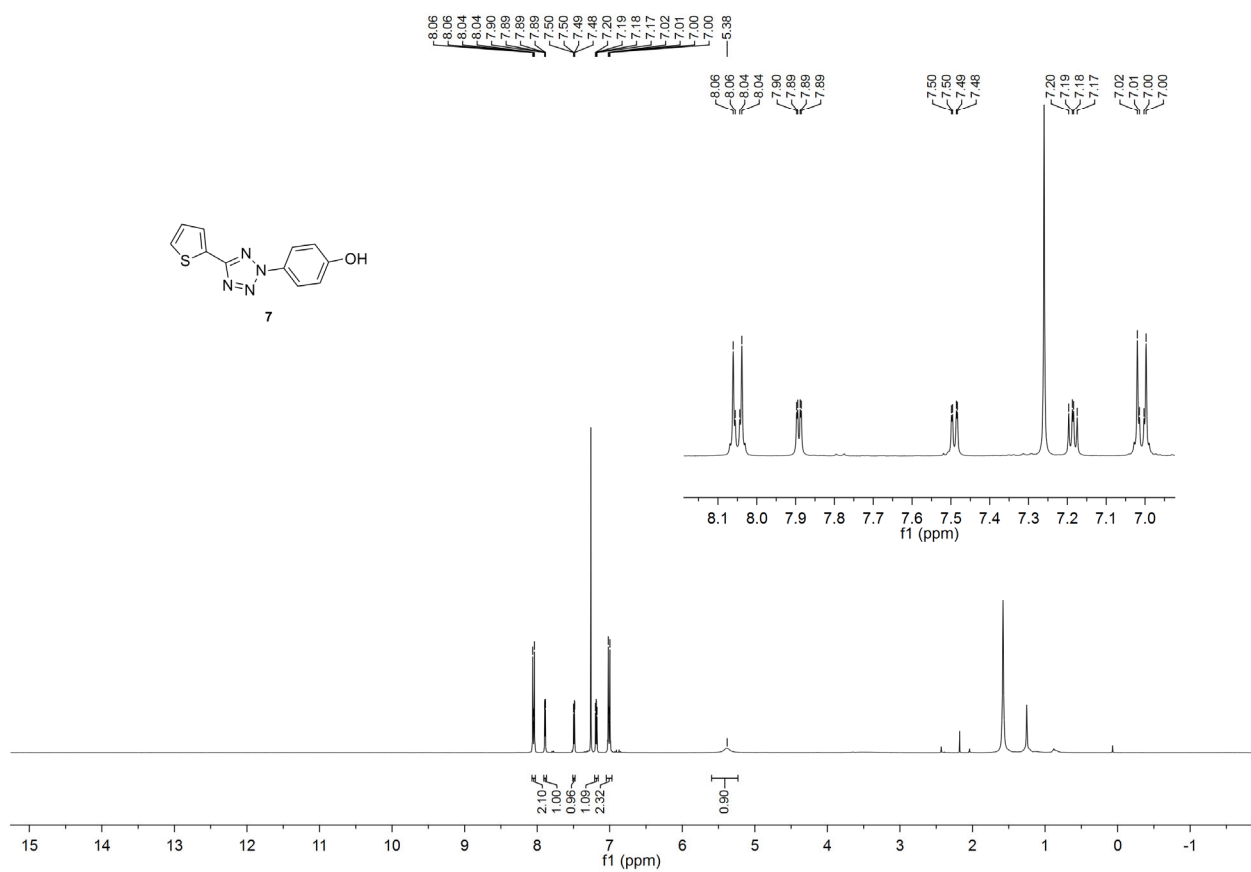
1,3,5-tris((4-(5-(thiophen-2-yl)-2H-tetrazol-2-yl)phenoxy)methyl)benzene (5). **7** (0.40 g, 1.7 mmol) was dissolved in dry DMF (8 mL), under nitrogen atmosphere. K₂CO₃ (0.13 g, 0.94 mmol) was added in one portion and 1,3,5-tris(bromomethyl)benzene (0.83g, 0.23mmol) was added portionwise over a period of 10 min. The mixture was stirred r.t. for 72 h. The mixture was then extracted with EtOAc (2X 15 mL), the organic layer was washed with brine (2X 10 mL), dried (Na₂SO₄) and the volatiles eliminated under reduced pressure. Flash column chromatography (SiO₂, CH₂Cl₂) afforded **5** in a pure form (0.17 g, 87%). White solid; m.p. 192-193 °C dec; ¹H NMR (400 MHz, CDCl₃) δ 8.12 – 8.07 (m, 6H), 7.88 (dd, *J* = 3.6, 1.2 Hz, 3H), 7.55 (s, 3H), 7.49 (dd, *J* = 5.0, 1.1 Hz, 3H), 7.18 (dd, *J* = 5.0, 3.7 Hz, 3H), 7.16 – 7.11 (m, 6H), 5.22 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 161.2, 159.4, 137.5, 130.6, 128.9, 128.1, 128.0, 126.1, 121.5, 115.6, 69.9; IR (ATR): 3107, 3082, 2925, 2854, 2558, 1885, 1805, 1729, 1680, 1608, 1596, 1572, 1509, 1475, 1457, 1440, 1409, 1373, 1300, 1247, 1225, 1203, 1175, 1112, 1066, 1043, 1012, 1002, 964, 893, 867, 850, 835, 807, 747, 701, 688, 673, 632, 587, 574 cm⁻¹; Anal. Calcd. for C₄₂H₃₀N₁₂O₃S₃: C, 59.56; H, 3.57; N, 19.85. Found: C, 59.22; H, 3.51; N, 19.94; UV (DMSO) λ_{max}, nm: 303.

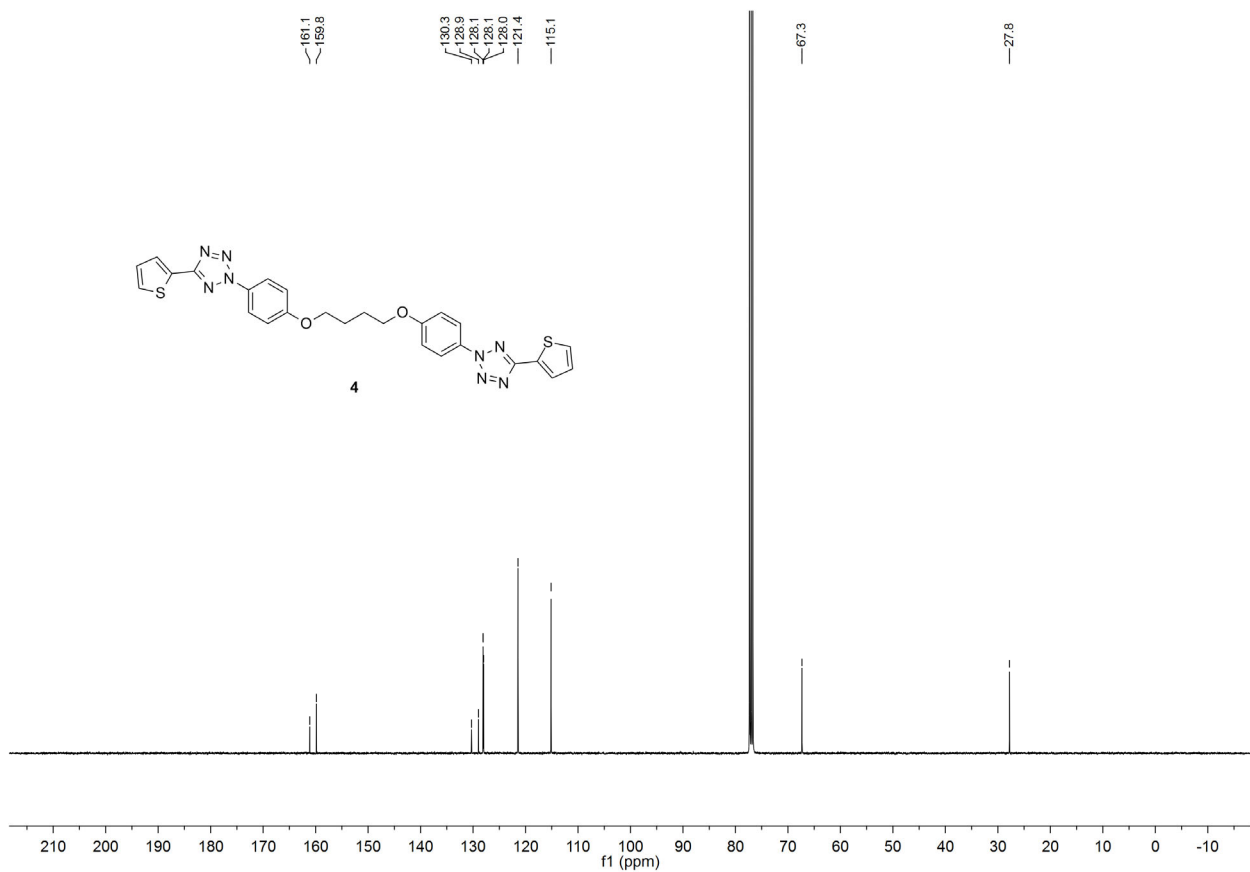
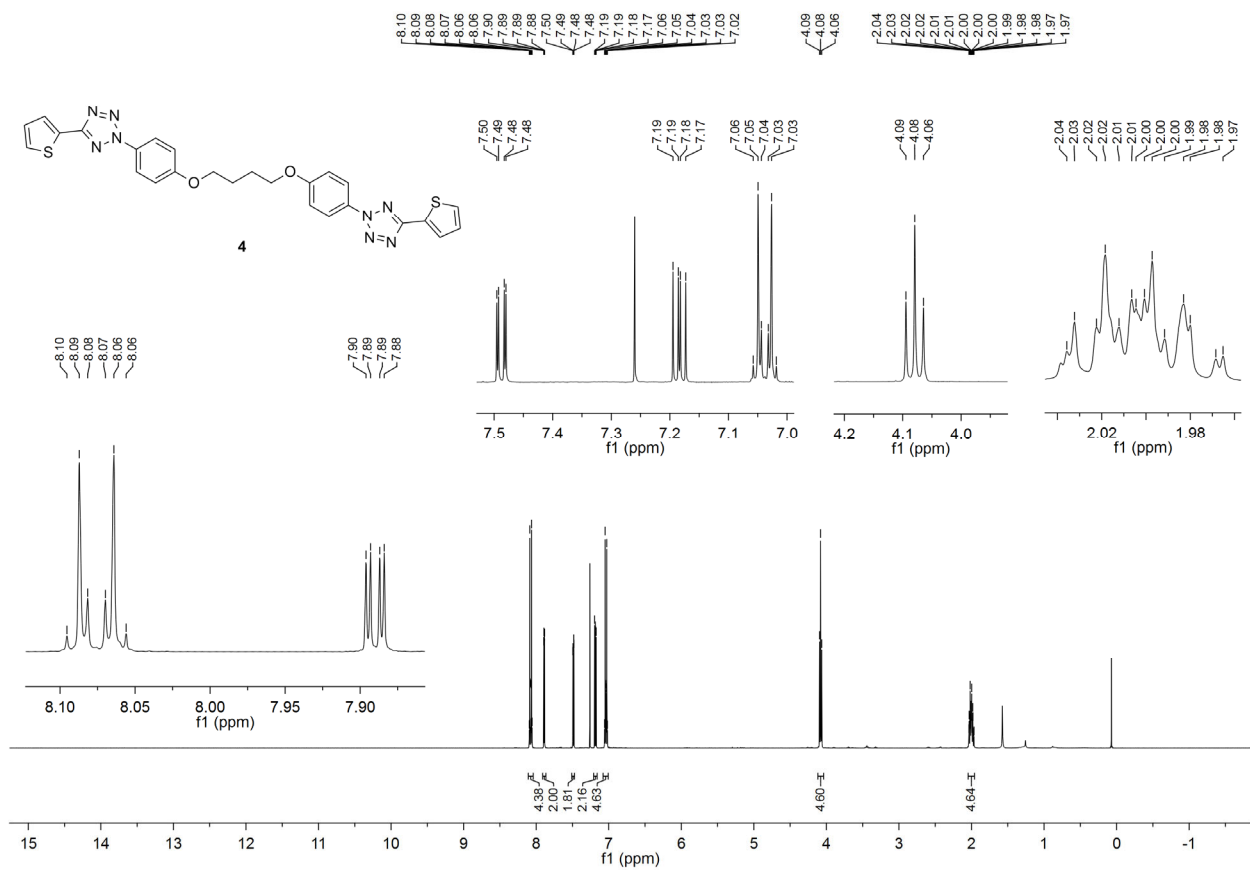


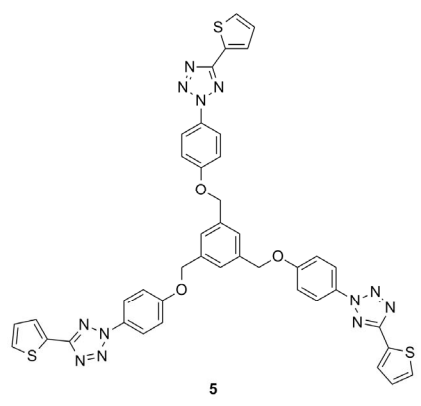
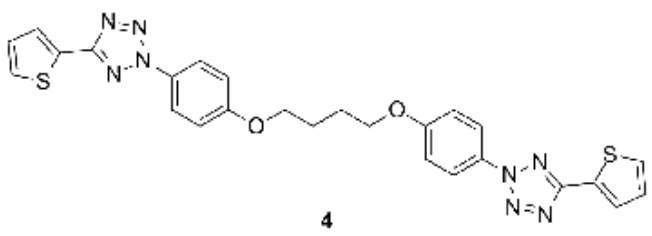


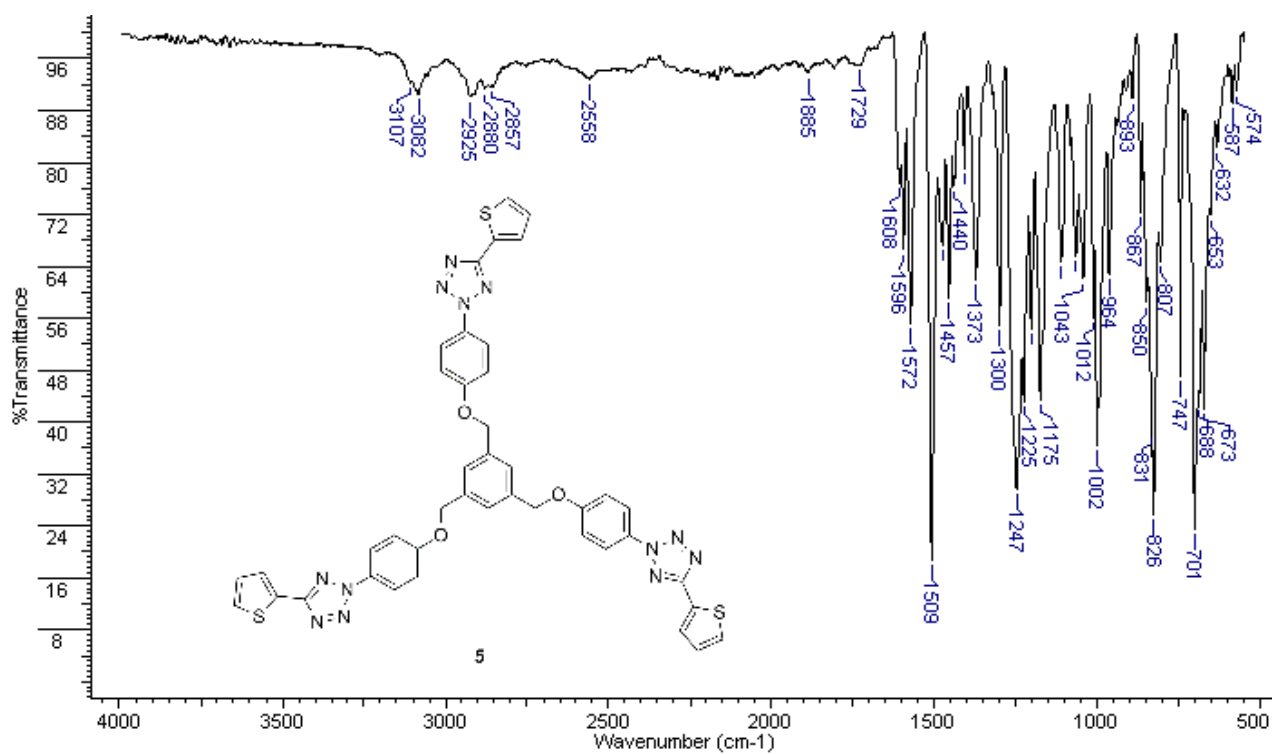
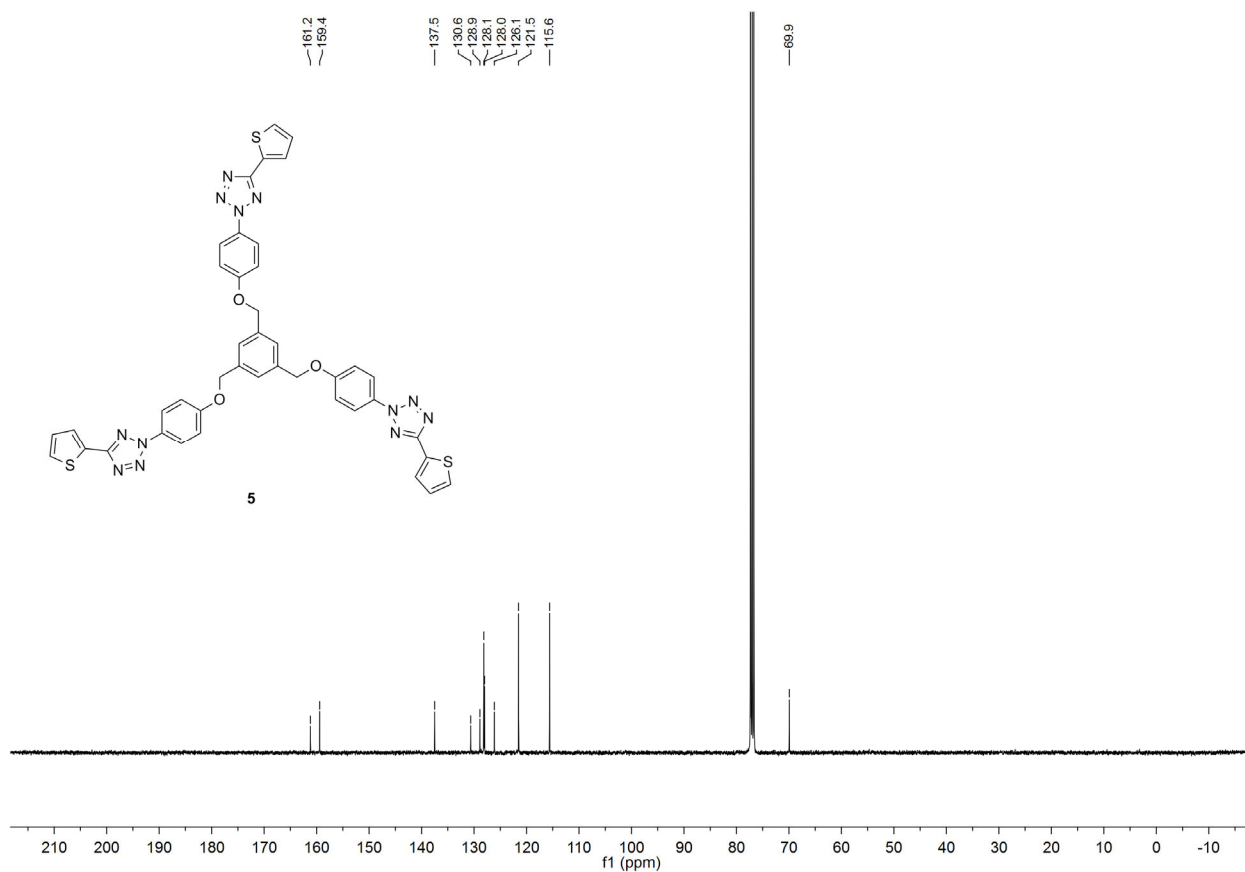












Legend:

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t_0 :



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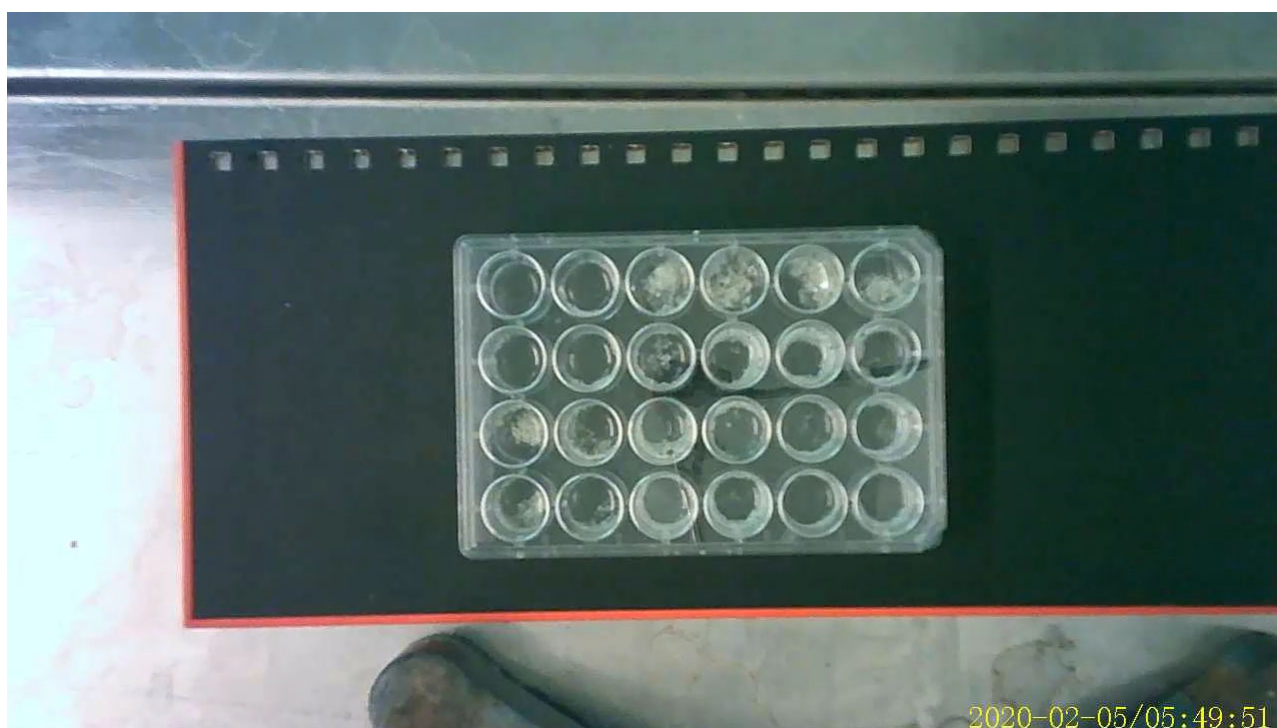
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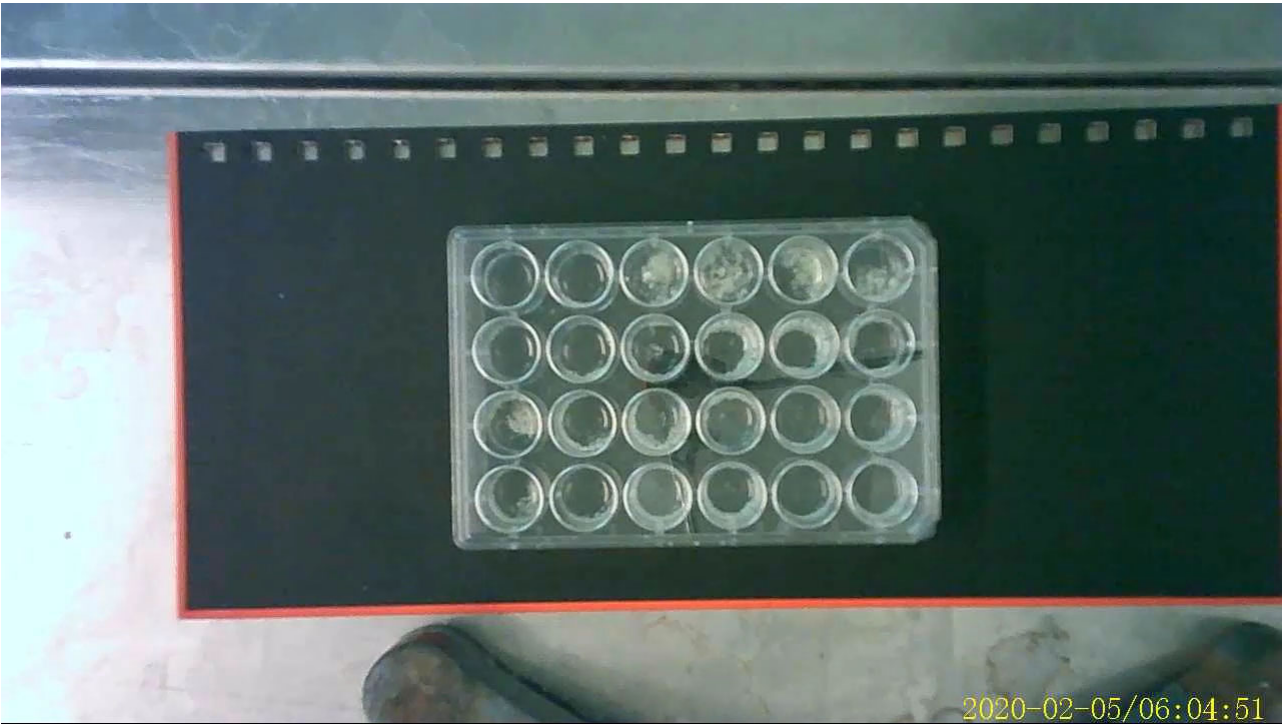
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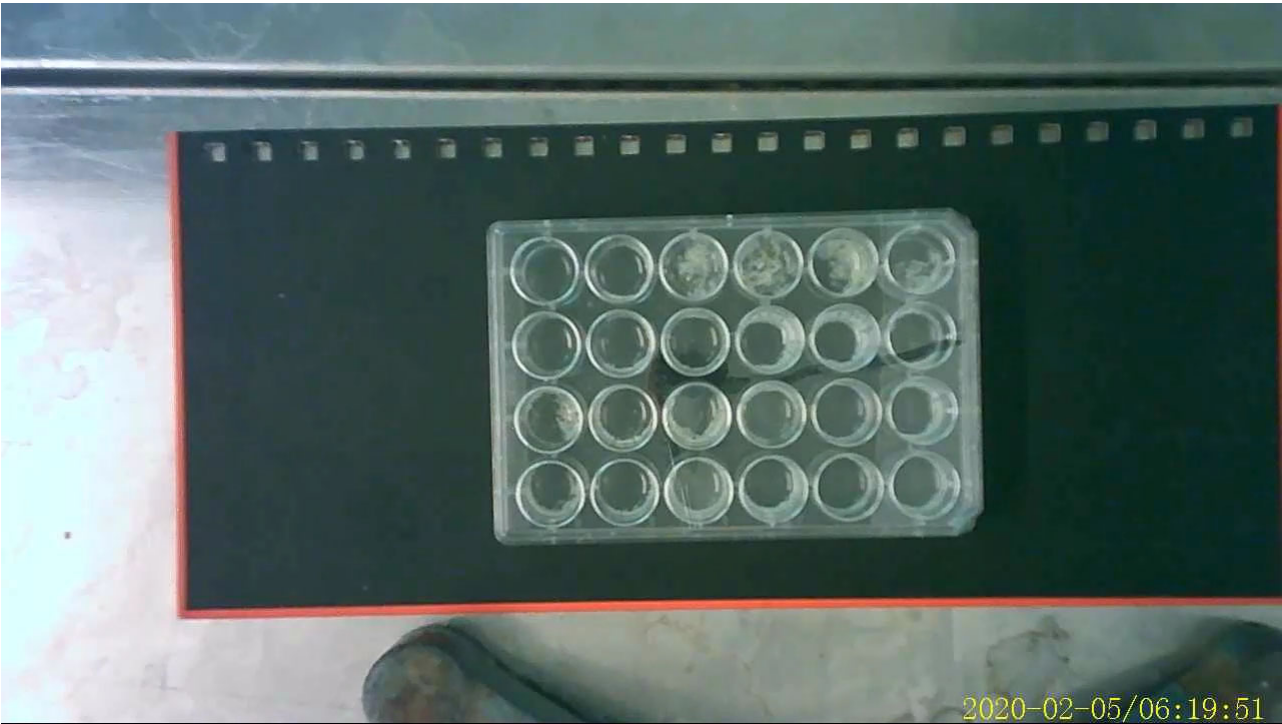
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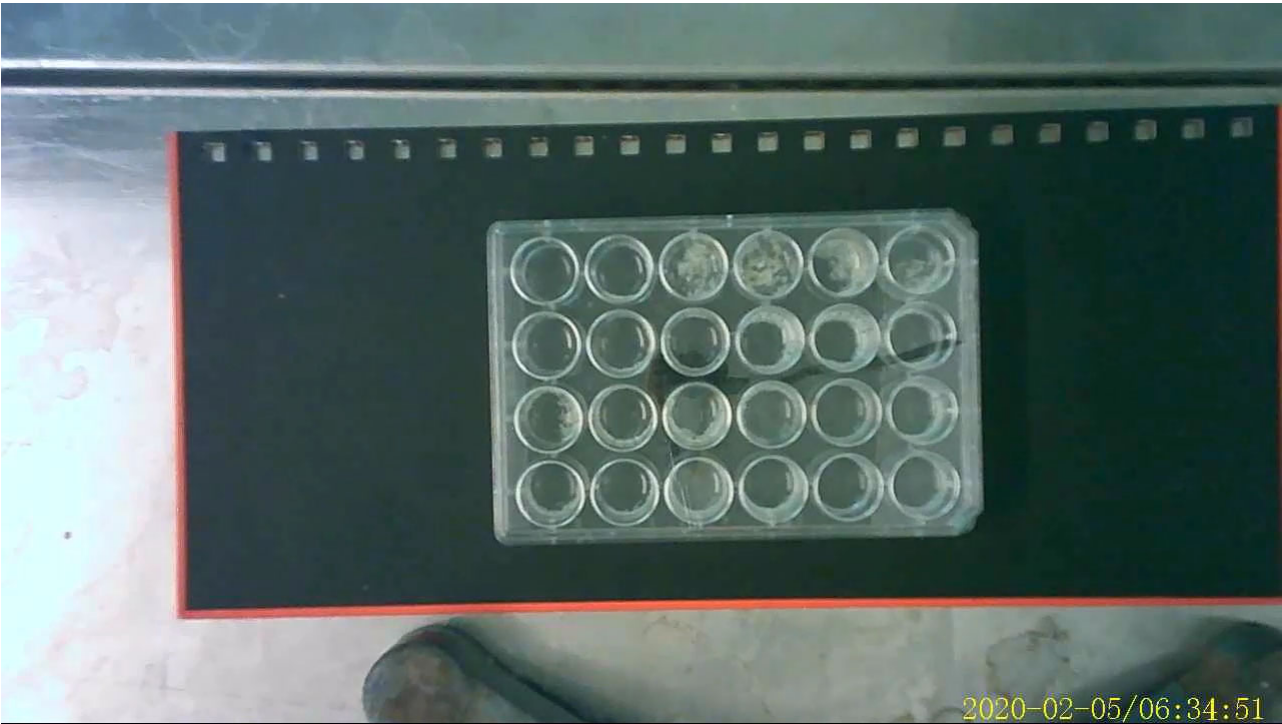
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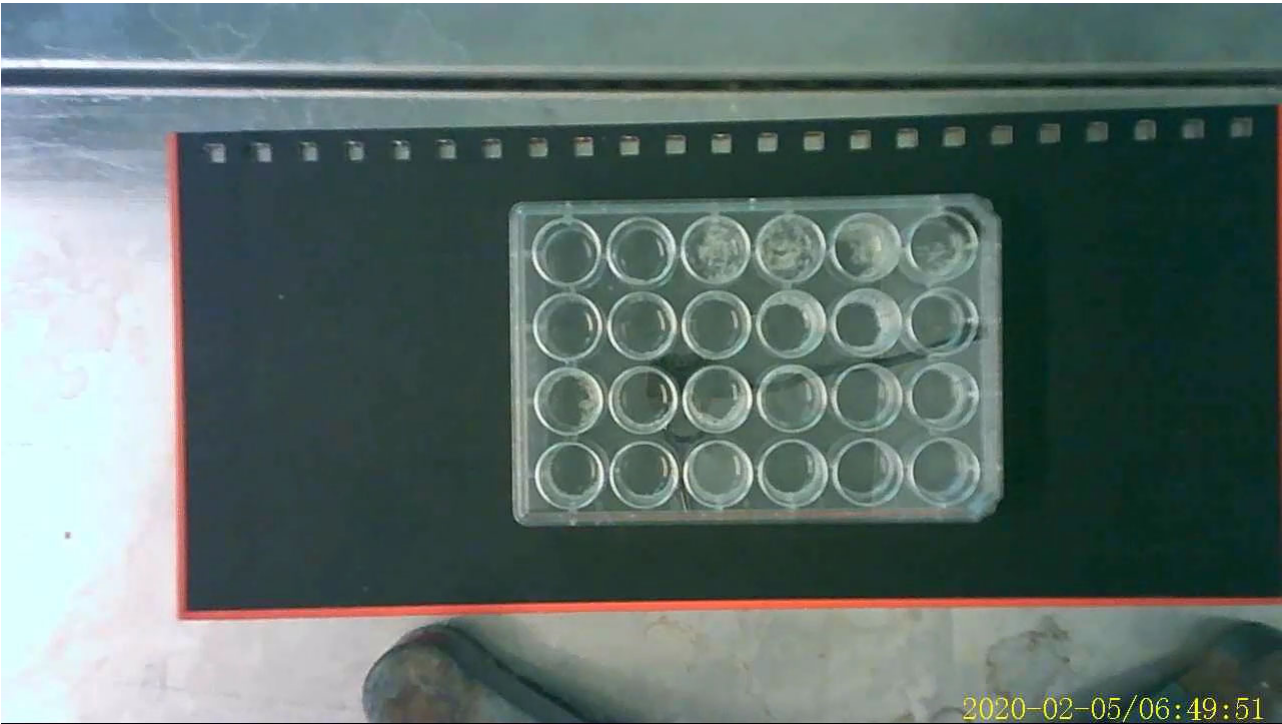
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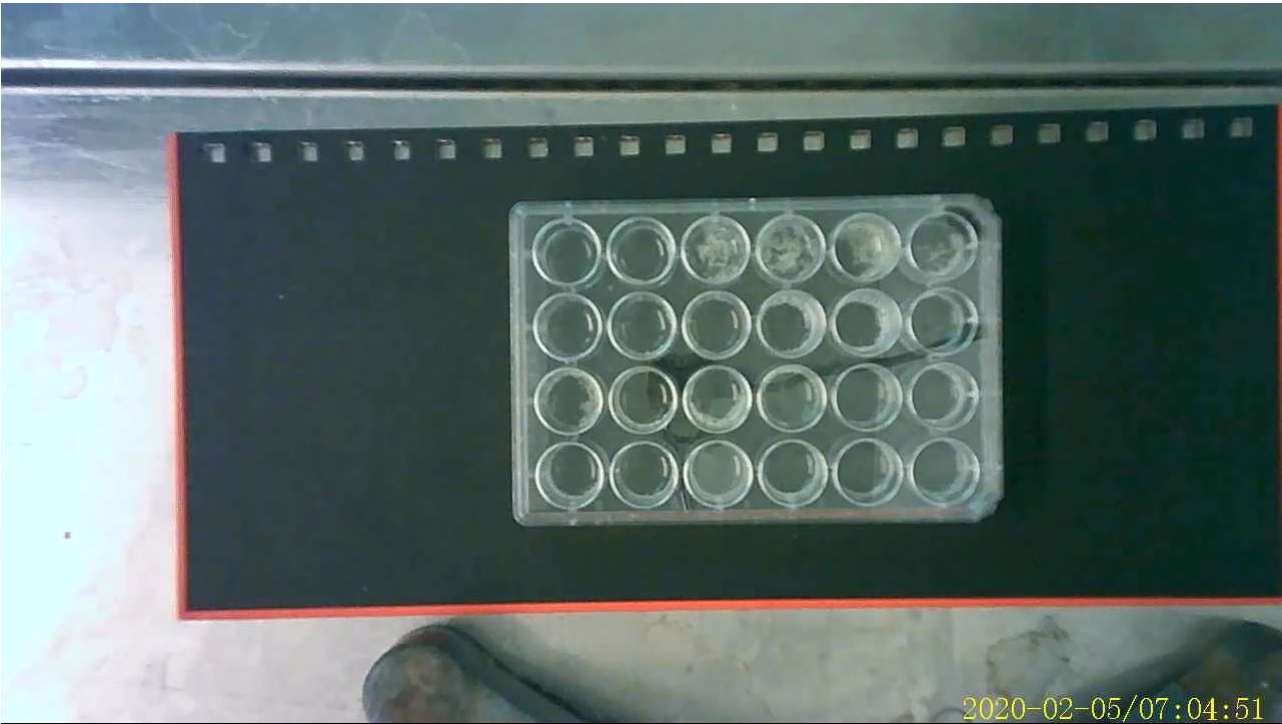
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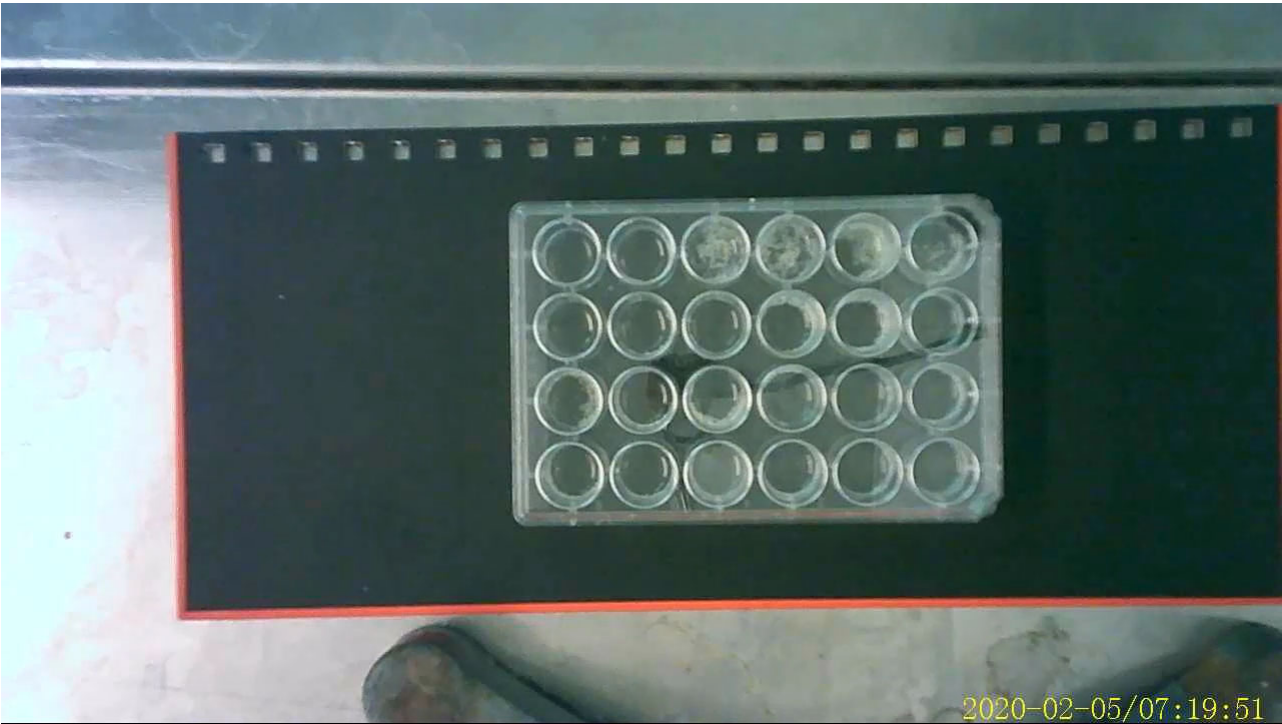
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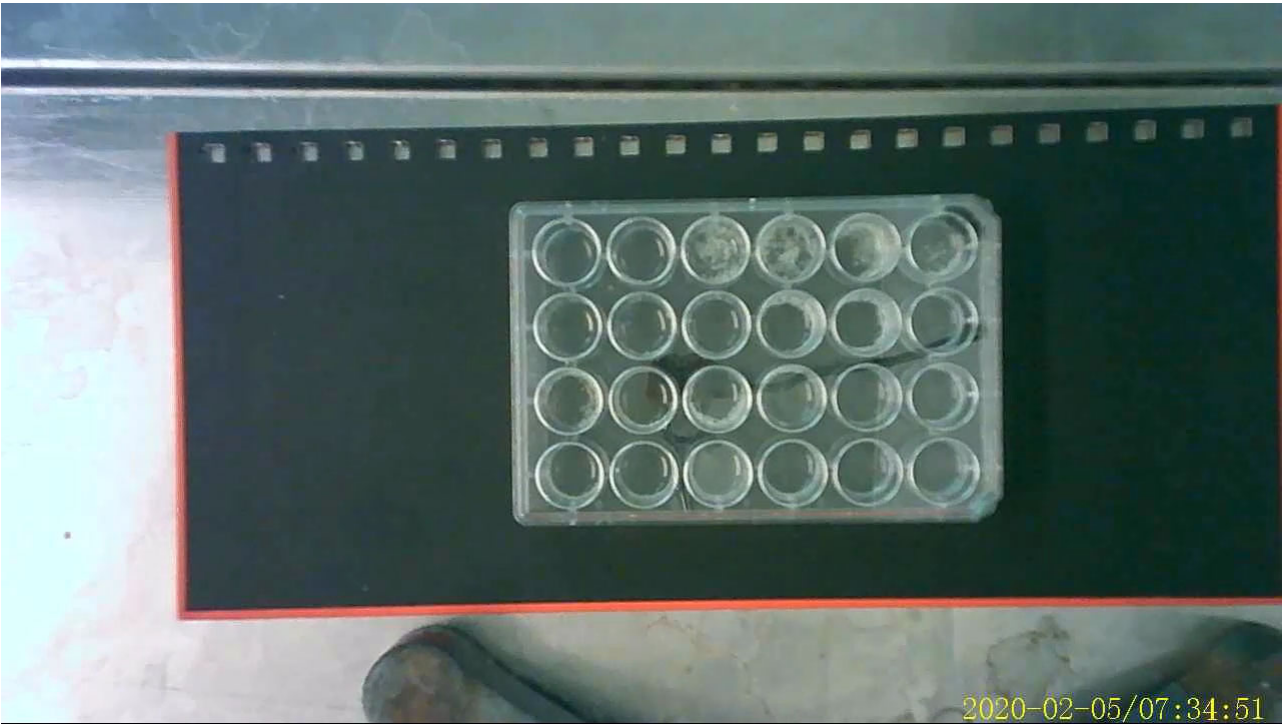
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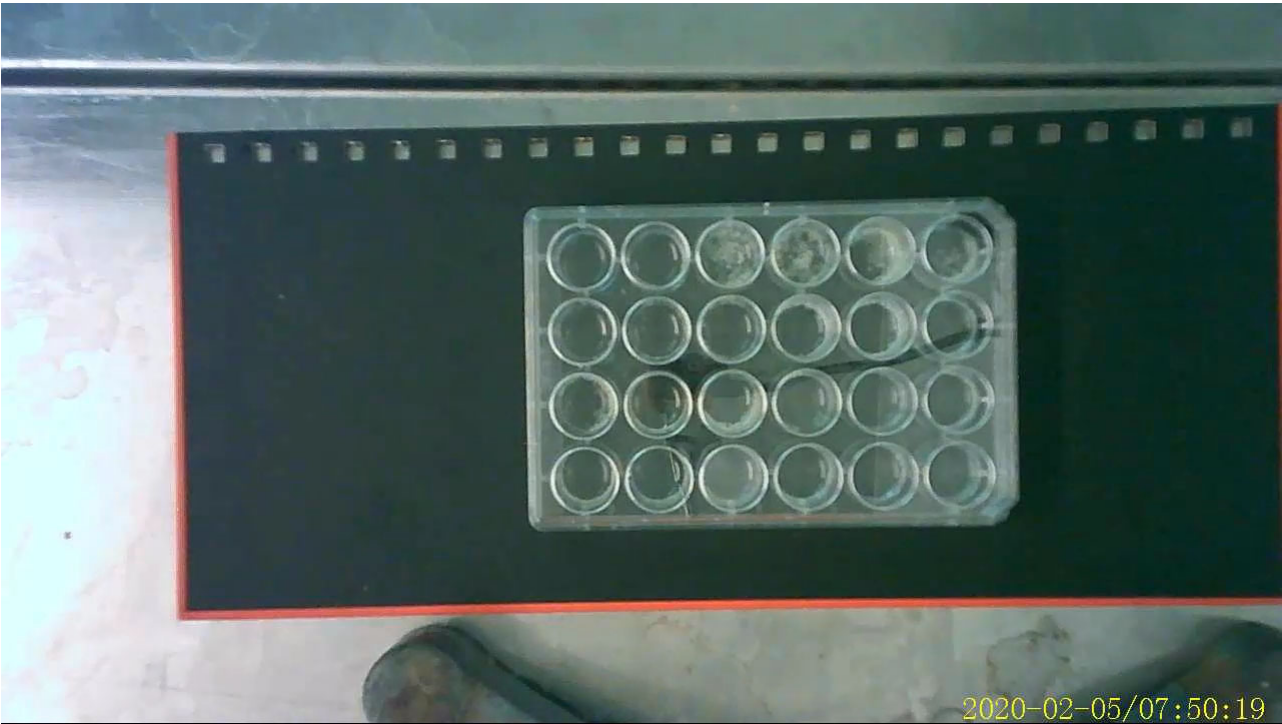
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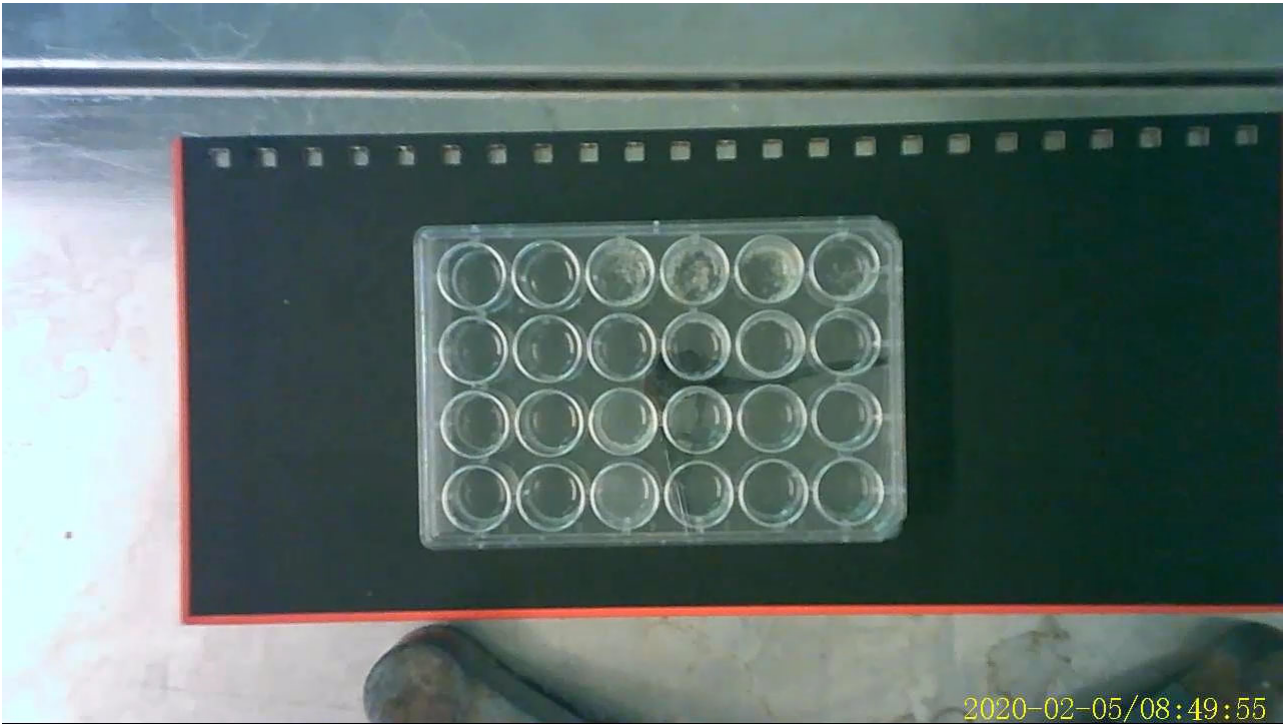
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180 min:



240 min:



300 min:

