Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

Molecular replication using covalent base-pairs with traceless linkers

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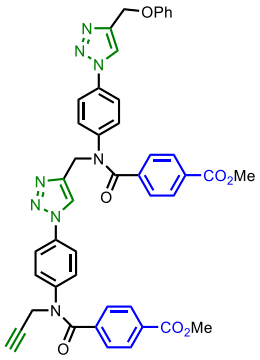
General experimental details.

All the reagents and materials used in the synthesis of the compounds described below were bought from commercial sources, without prior purification. Dry THF and CH_2Cl_2 were obtained from a solvent purification system (Pure Solv™, Innovative Technology, Inc.). Anhydrous DMF was purchased from Sigma-Aldrich. Thin layer chromatography was carried out using with silica gel 60F (Merck) on glass plates. Flash chromatography was carried out on an automated system (Combiflash Rf+ or Combiflash Rf Lumen) using prepacked cartridges of silica (25µ PuriFlash® columns). All NMR spectroscopy was carried out on a Bruker 400 MHz DPX400, 400 MHz AVIII400, 500 MHz DCH cryoprobe or 500 MHz TCI Cryoprobe spectrometer using the residual solvent as the internal standard. All chemical shifts (δ) are quoted in ppm and coupling constants given in Hz. Splitting patterns are given as follows: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). FT-IR spectra were measured on a PerkinElmer Spectrum One spectrometer equipped with an ATR cell. Melting points were measured in a Mettler Toledo MP50 Melting Point System. UPLC analysis of samples was performed using Waters Acquity H-class UPLC coupled with a single quadrupole Waters SQD2. ACQUITY UPLC CSH C18 Column, 130Å, 1.7 µm, 2.1 mm X 50 mm was used as the UPLC column. The conditions of the UPLC method are as follows: gradients of water +0.1% formic acid (solvent A) and acetonitrile +0.1% formic acid (solvent B=) as specified in each case. Flow rate: 0.6 ml/min; Column temperature of 40^{o}C; Injection volume of 2 µL. The signal was monitored at 254 nm. HRMS analysis was performed in a Waters LCT Premier equipped with a TOF mass analyser and W optics for enhanced resolution, using 50% aqueous acetonitrile with 0.25% formic acid as mobile phase.

Synthesis and characterization of described compounds

Compound 3



Compound **1**[S1] (0.131 g, 0.28 mmol), compound **2**[S2] (0.120 g, 0.30 mmol), $Cu(CH_3CN)_4PF_6$ (0.010 g, 0.03 mmol) and TBTA (0.015 g, 0.03 mmol) were mixed in a round-bottom flask and, under N_2, THF (5 mL) was added. The reaction was stirred overnight at room temperature. Once the reaction was completed, TBAF (1M in THF, 0.280 mL, 0.280 mmol) was added dropwise. The solution was stirred for 10 min at room temperature, quenched with 0.1N HCl soln. and extracted with EtOAc (3x) followed by washing with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrate under vacuum. The crude was purified by flash column chromatography on silica gel (gradient from 10% to 80% of EtOAc in Pet. Ether) to afford compound **3** (0.204 g, 93%) as a foam.

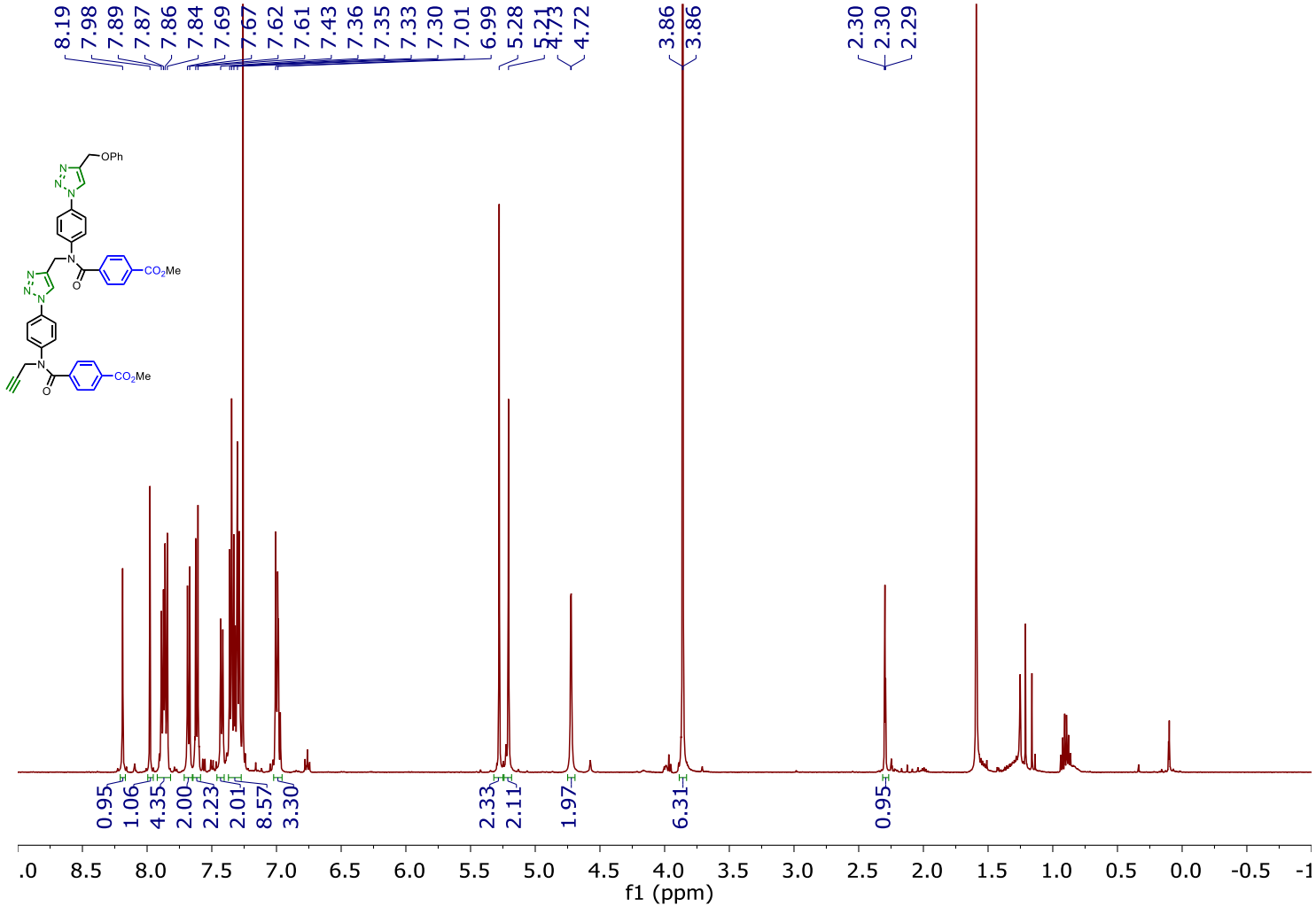
^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 8.19 (s, 1H), 7.98 (s, 1H), 7.88 (d, 2H, *J* = 8.5 Hz), 7.85 (d, 2H, *J* = 8.5 Hz), 7.68 (d, 2H, *J* = 9.0 Hz), 7.62 (d, 2H, *J* = 9.0 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 7.36-7.29 (m, 8H), 6.99 (m, 3H), 5.28 (s, 2H), 5.21 (s, 2H), 4.72 (d, 2H, *J* = 2.5 Hz), 3.86 (s, 3H), 3.86 (s, 3H), 2.30 (t, 1H, *J* = 2.5 Hz).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 169.5, 169.3, 166.2, 166.2, 158.2, 145.5, 144.5, 143.9, 142.6, 139.2, 139.1, 135.8, 135.5, 131.8, 131.7, 129.8, 129.5, 129.5, 129.2, 128.8, 128.8, 122.2, 121.6, 121.5, 121.2, 120.8, 114.9, 78.4, 73.3, 62.0, 52.5, 52.5, 46.4, 39.8.

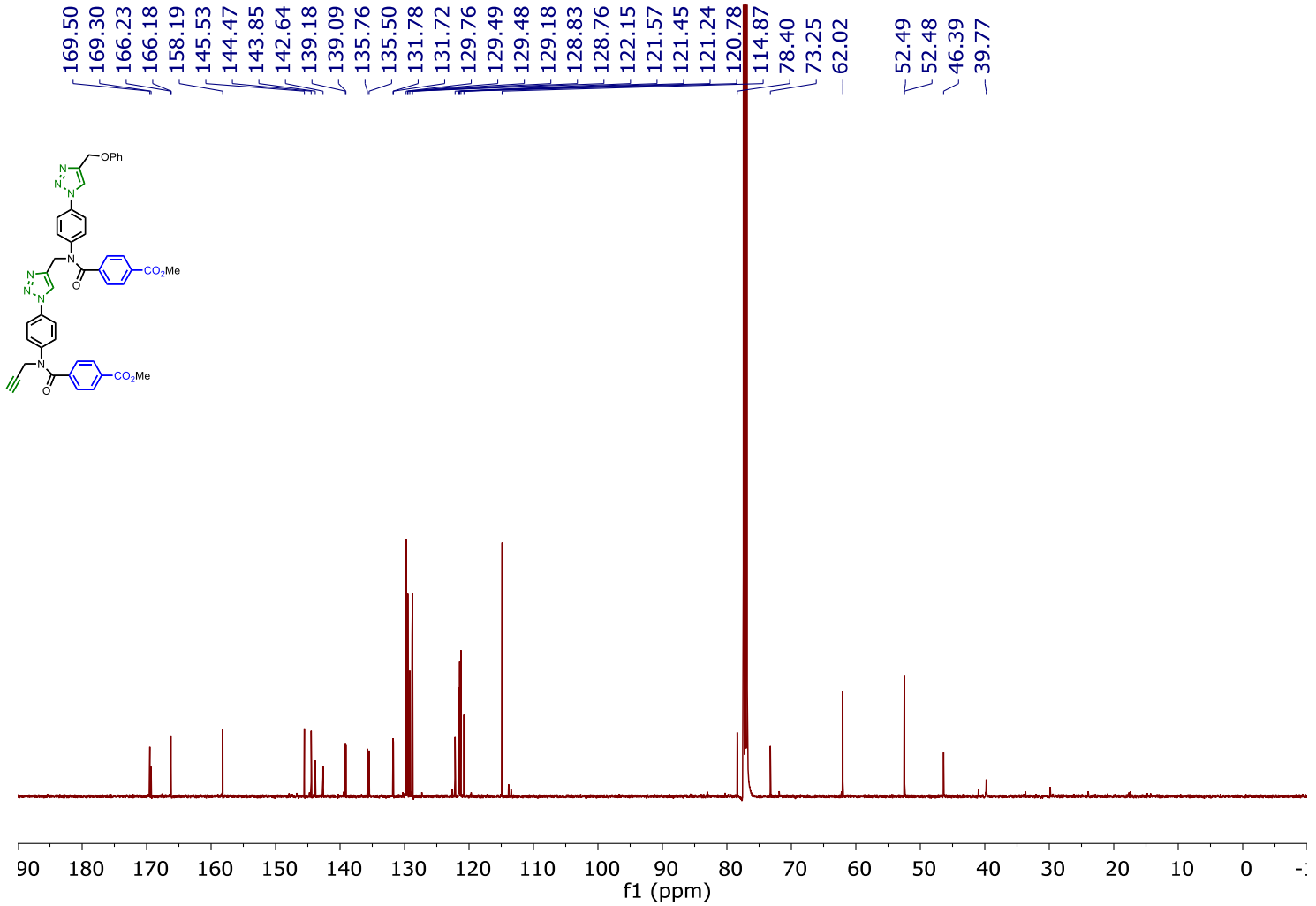
HRMS (ES+): calcd for $C_{45}H_{37}N_8O_7$ 801.2785 $[M+H]^+$, found 801.2778 $[M+H]^+$.

FT-IR (ATR): ν_{max} 2951, 1720, 1650, 1519, 1279, 1240, 1109, 755 and 732 cm^{-1}.

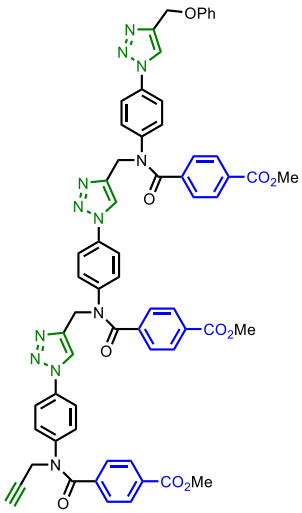
^{1}H-NMR (400 MHz, $CDCl_3$) compound 3



^{13}C NMR (100.6 MHz, $CDCl_3$) compound 3



Compound 4



Compound **3** (0.215 g, 0.25 mmol), Compound **2** (0.109 g, 0.27 mmol), $Cu(CH_3CN)_4PF_6$ (0.009 g, 0.03 mmol) and TBTA (0.013 g, 0.03 mmol) were mixed in a round-bottom flask and, under N_2, THF (5 mL) was added. The reaction was stirred overnight at room temperature. Once the reaction was completed, TBAF (1M in THF, 0.260 mL, 0.260 mmol) was added dropwise. The solution was stirred for 10 min at room temperature, quenched with 0.1N HCl soln. and extracted with EtOAc (3x) followed by washing with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrate under vacuum. The crude was purified by flash column chromatography on silica gel (gradient from 15% to 90% of EtOAc in Pet. Ether) to afford compound **4** (0.282 g, 98%) as a foam.

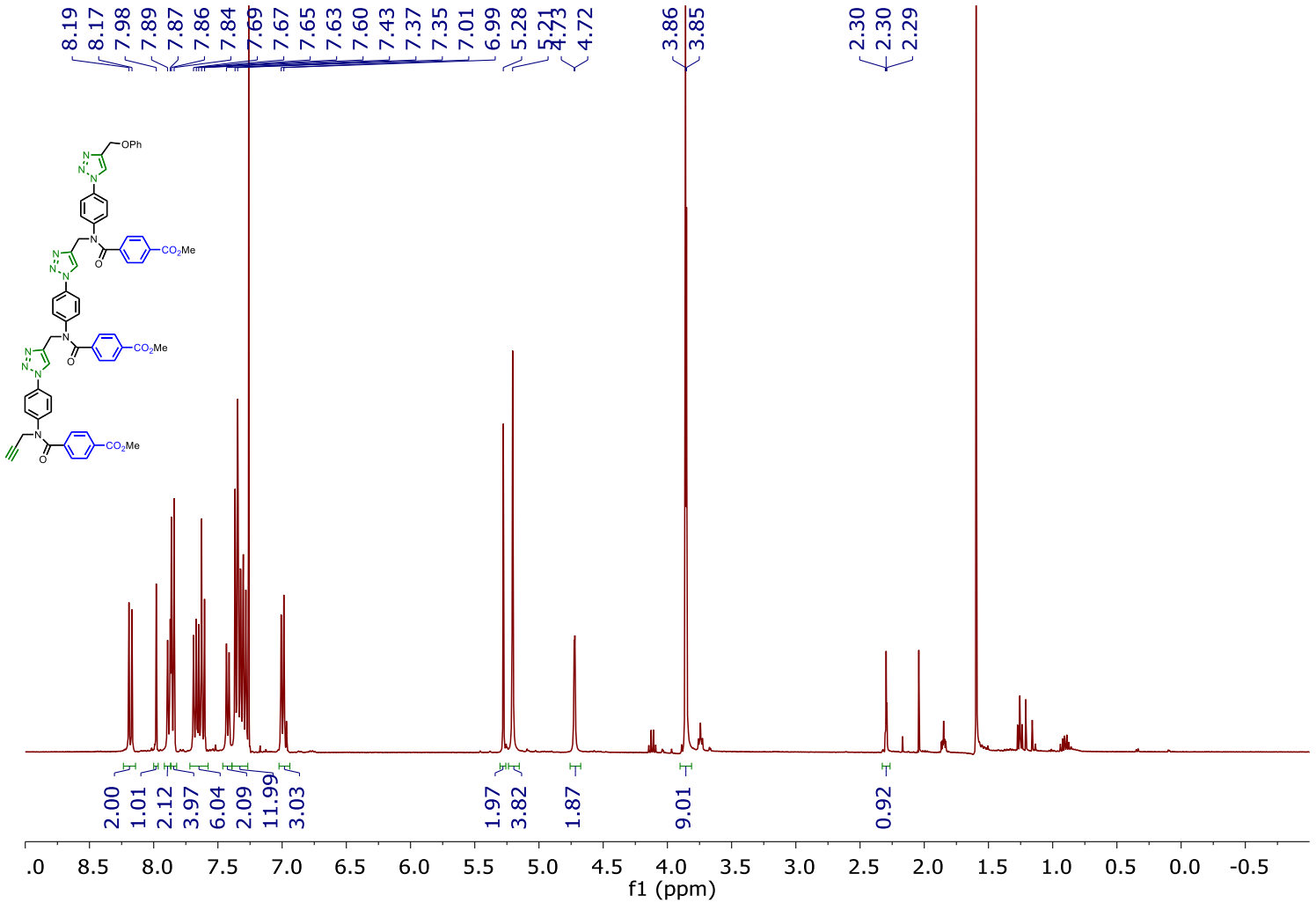
^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 8.19 (s, 1H), 8.17 (s, 1H), 7.98 (s, 1H), 7.88 (d, 2H, *J* = 8.5 Hz), 7.85 (d, 4H, *J* = 8.5 Hz), 7.68 (d, 2H, *J* = 9.0 Hz), 7.64 (d, 2H, *J* = 9.0 Hz), 7.62 (d, 2H, *J* = 9.0 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 7.37-7.28 (m, 12H), 6.99 (m, 3H), 5.28 (s, 2H), 5.21 (s, 4H), 4.72 (d, 2H, *J* = 2.5 Hz), 3.86 (s, 6H), 3.85 (s, 3H), 2.30 (t, 1H, *J* = 2.5 Hz).

^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 169.5, 169.5, 169.3, 166.2, 166.2, 166.2, 158.2, 145.5, 144.4, 143.8, 142.6, 139.2, 139.2, 139.1, 135.5, 135.4, 131.8, 131.7, 129.8, 129.5, 129.2, 128.9, 128.8, 128.8, 122.2, 122.1, 121.6, 121.4, 121.4, 121.3, 121.2, 120.8, 114.9, 78.4, 73.3, 62.0, 52.5, 52.5, 46.4, 39.8.

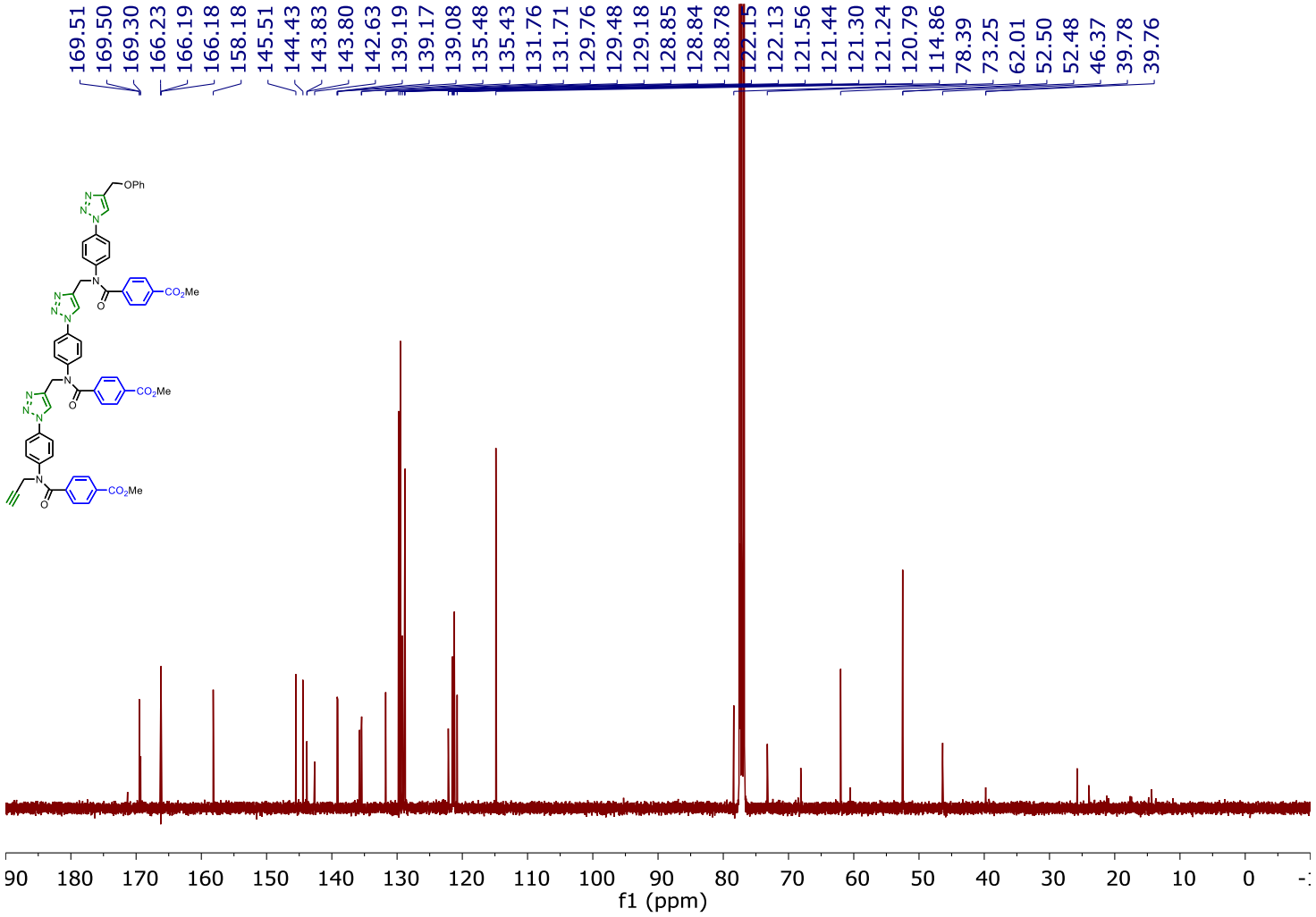
HRMS (ES+): calcd for $C_{63}H_{51}N_{12}O_{10}$ 1135.3851 $[M+H]^+$, found 1135.3859 $[M+H]^+$.

FT-IR (ATR): ν_{max} 2952, 1721, 1649, 1519, 1279, 1241, 1109, 753 and 732 cm^{-1}.

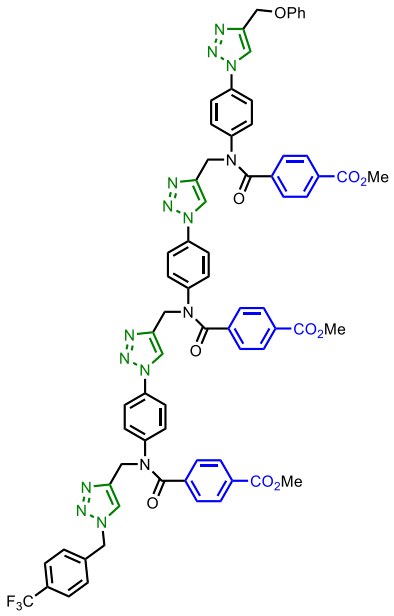
^{1}H-NMR (500 MHz, $CDCl_3$) compound 4



^{13}C-NMR (125 MHz, $CDCl_3$) compound 4



Compound 6



Compound **4** (0.304 g, 0.27 mmol), 1-(azidomethyl)-4-trifluoromethylbenzene **6**[S3] (0.108 g, 0.54 mmol), $Cu(CH_3CN)_4PF_6$ (0.010 g, 0.03 mmol) and TBTA (0.014 g, 0.03 mmol) were mixed in a round-bottom flask and, under N_2, THF (5 mL) was added. The reaction was stirred overnight at room temperature. The solvent was evaporated to dryness and the residue purified by flash chromatography on silica gel (gradient from 0% to 4% of MeOH in EtOAc) to afford compound **6** (0.330 g, 92% containing TBTA as impurity) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ_H = 8.17 (s, 2H), 7.98 (s, 1H), 7.85 (m, 6H), 7.73 (s, 1H), 7.63 (m, 8H), 7.38-7.28 (m, 16H), 6.99 (m, 3H), 5.58 (s, 2H), 5.28 (s, 2H), 5.21 (s, 4H), 5.14 (s, 2H), 3.86 (s, 3H), 3.85 (s, 6H).

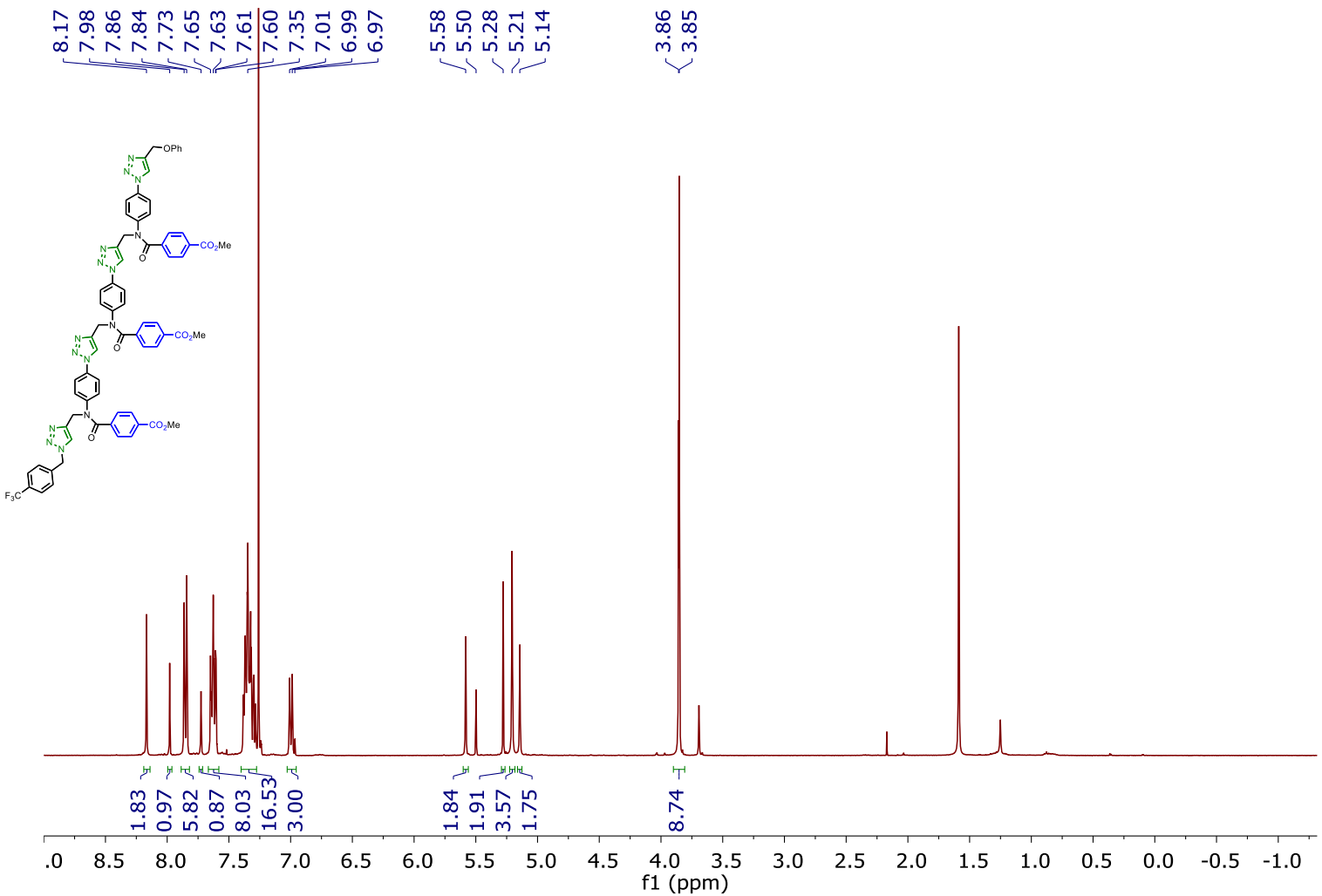
^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 169.5, 169.5, 169.4, 166.2, 158.2, 145.5, 144.4, 144.4, 144.2, 143.8, 143.8, 143.8, 139.3, 139.2, 138.5 (q, J = 1.5 Hz), 135.5, 135.4, 135.4, 131.7, 131.7, 131.4, 131.3 (q, J = 33.0 Hz), 129.8, 129.5, 129.2, 128.9, 128.8, 128.8, 128.8, 128.4, 128.1, 126.3, 124.2, 123.9 (q, J = 272.5), 122.1, 121.6, 121.4, 121.3, 121.2, 120.8, 114.9, 62.0, 53.7, 52.5, 52.5, 46.4, 46.3.

^{19}F NMR (376 MHz, $CDCl_3$): δ_F = -62.8.

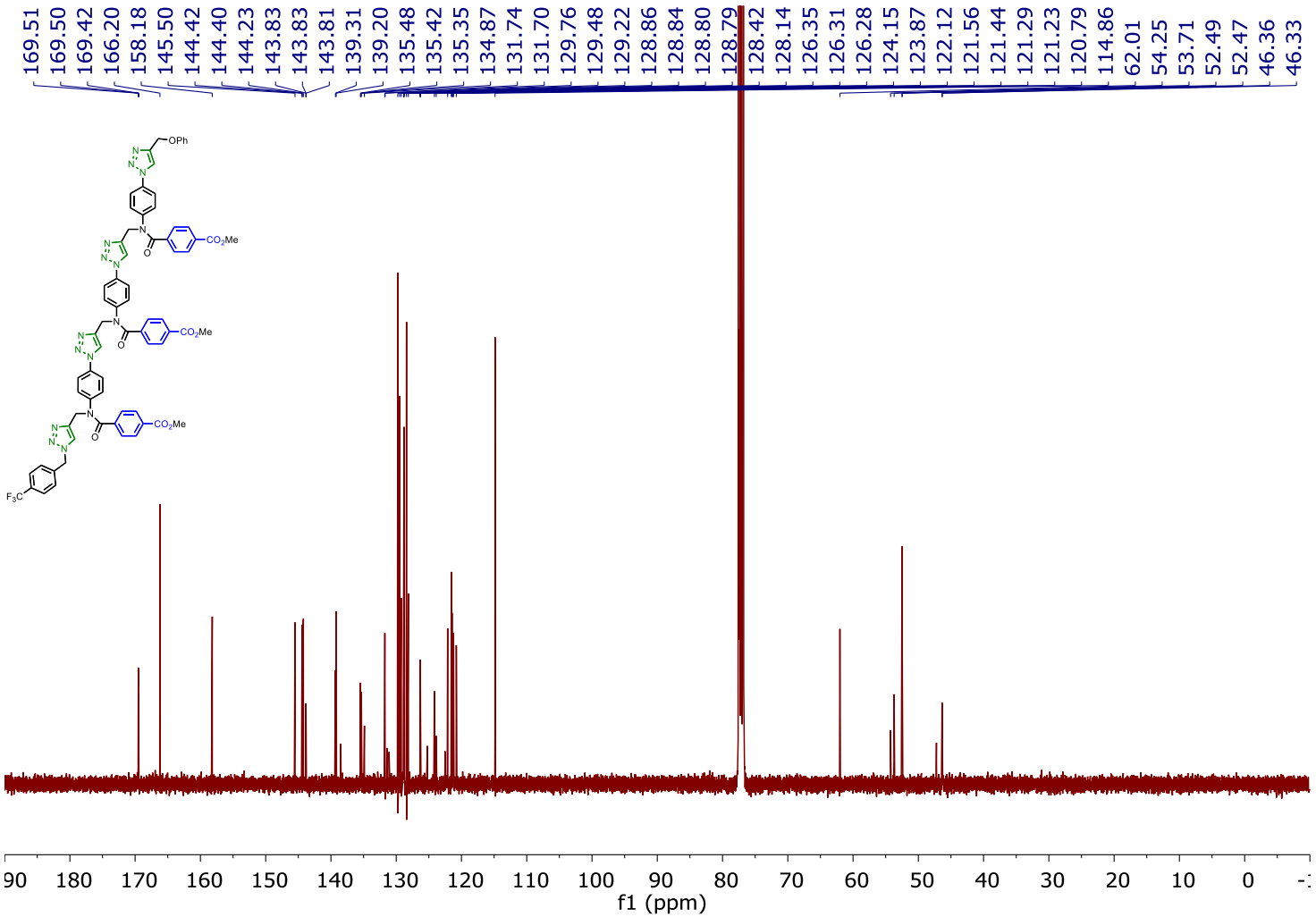
HRMS (ES+): calcd for $C_{71}H_{57}F_3N_{15}O_{10}$ 1336.4365 $[M+H]^+$, found 1336.4363 $[M+H]^+$.

FT-IR (ATR): ν_{max} 1720, 1646, 1518, 1324, 1278, 1240, 1109 and 751 cm^{-1}.

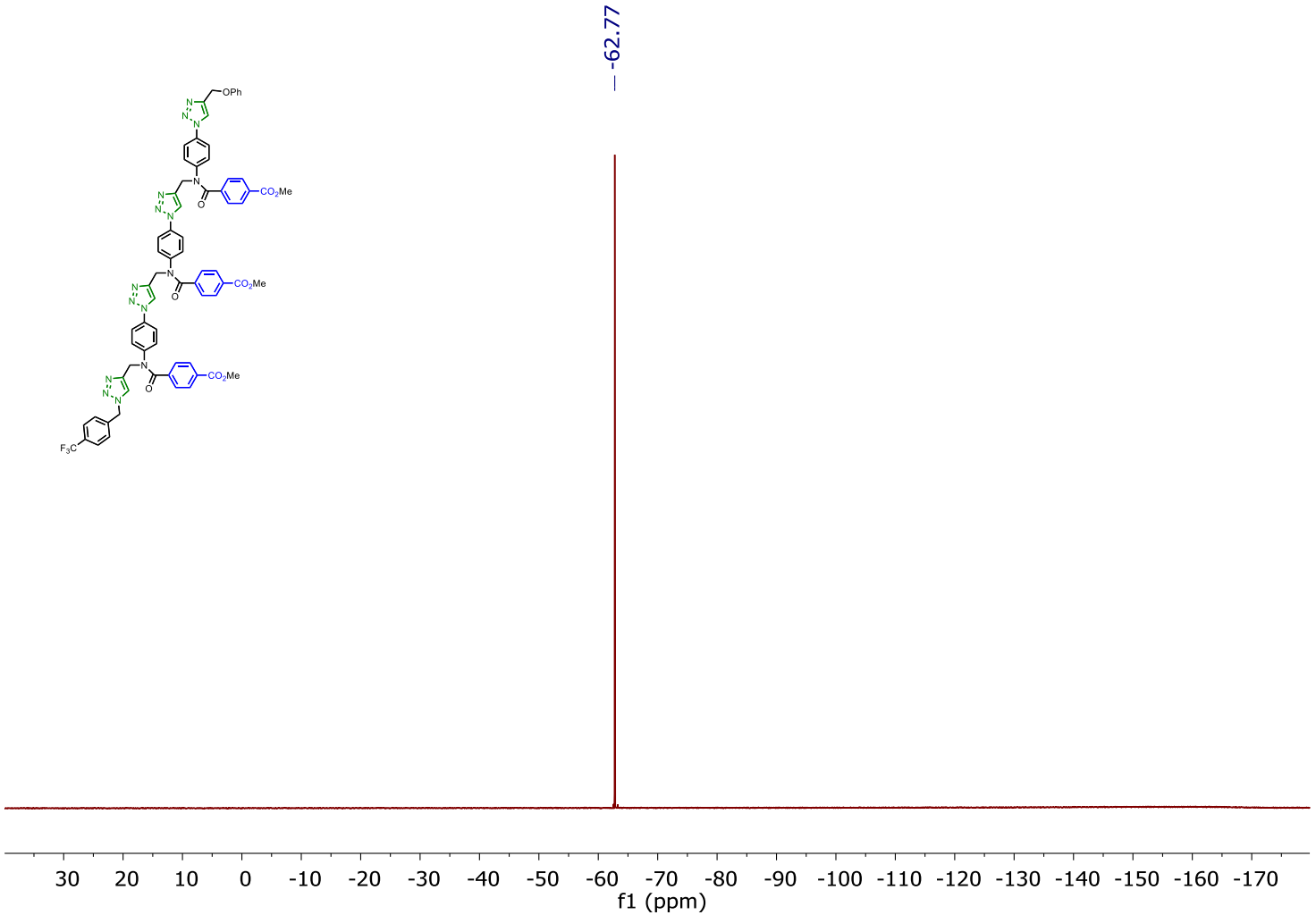
^{1}H-NMR (500 MHz, $CDCl_3$) compound 6



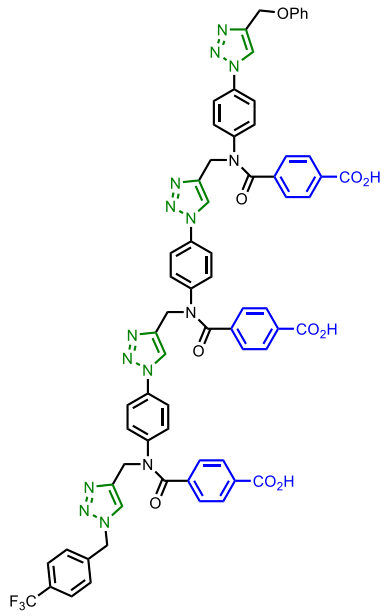
^{13}C-NMR (125 MHz, $CDCl_3$) compound 6



^{19}F NMR (376 MHz, $CDCl_3$) compound 6



Template 7



Compound **6** (0.325 g, 0.24 mmol) was dissolved in THF:H_2O 3:1 (15 mL) and LiOH (0.092 g, 2.19 mmol) was added. The reaction was stirred at room temperature for 2 h. Then, the crude was diluted with H_2O and acidified with 1N HCl soln. to pH 2-3. The aqueous phase was extracted with EtOAc (3x) and the combined organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel using a gradient from 0% to 10% of MeOH (containing 0.05 % aq. HCl) in CH_2Cl_2 to afford template **7** (0.245 g, 78%) as a white solid.

mp: Decomposed before melting.

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 8.85 (s, 1H), 8.74 (s, 1H), 8.73 (s, 1H), 8.19 (s, 1H), 7.78 (m, 12H), 7.68 (d, 2H, *J* = 8.0 Hz), 7.45 (m, 10H), 7.36 (m, 4H), 7.29 (m, 2H), 7.03 (d, 2H, *J* = 7.5 Hz), 6.95 (t, 1H, *J* = 7.5 Hz), 5.68 (s, 2H), 5.20 (s, 4H), 5.17 (s, 2H), 5.16 (s, 2H).

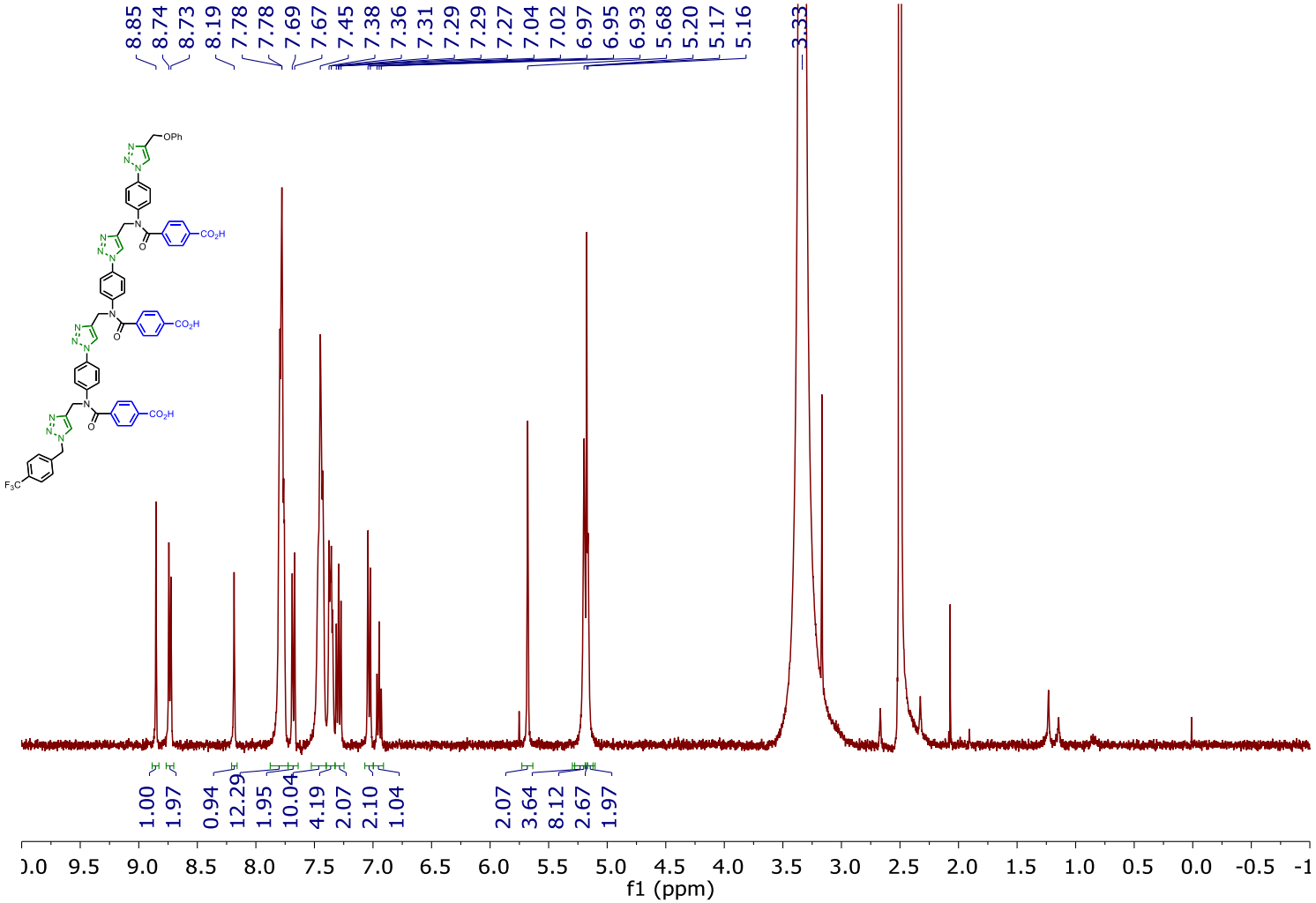
^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ_C = 168.8, 168.8, 168.7, 166.7, 166.7, 166.6, 157.9, 145.5, 144.2, 144.0, 143.1, 142.6, 142.5, 142.3, 140.8 (q, *J* = 1.5 Hz), 139.7, 139. 7, 139.6, 134.7, 134.7, 134.6, 132.0, 132.0, 131.8, 129.5, 129.4, 129.3, 129.3, 129.2, 128.9, 128.8, 128.7, 128.4, 128.4, 128.3, 128.2, 125.6 (q, *J* = 3.5 Hz), 124.5, 124.0 (q, *J* = 272.0 Hz), 122.8, 121.7, 121.6, 121.0, 120.6, 120.5, 120.4, 114.7, 60.9, 54.9, 45.2, 45.1, 44.9.

^{19}F NMR (376 MHz, DMSO-*d*$_6$): δ_F = -61.1.

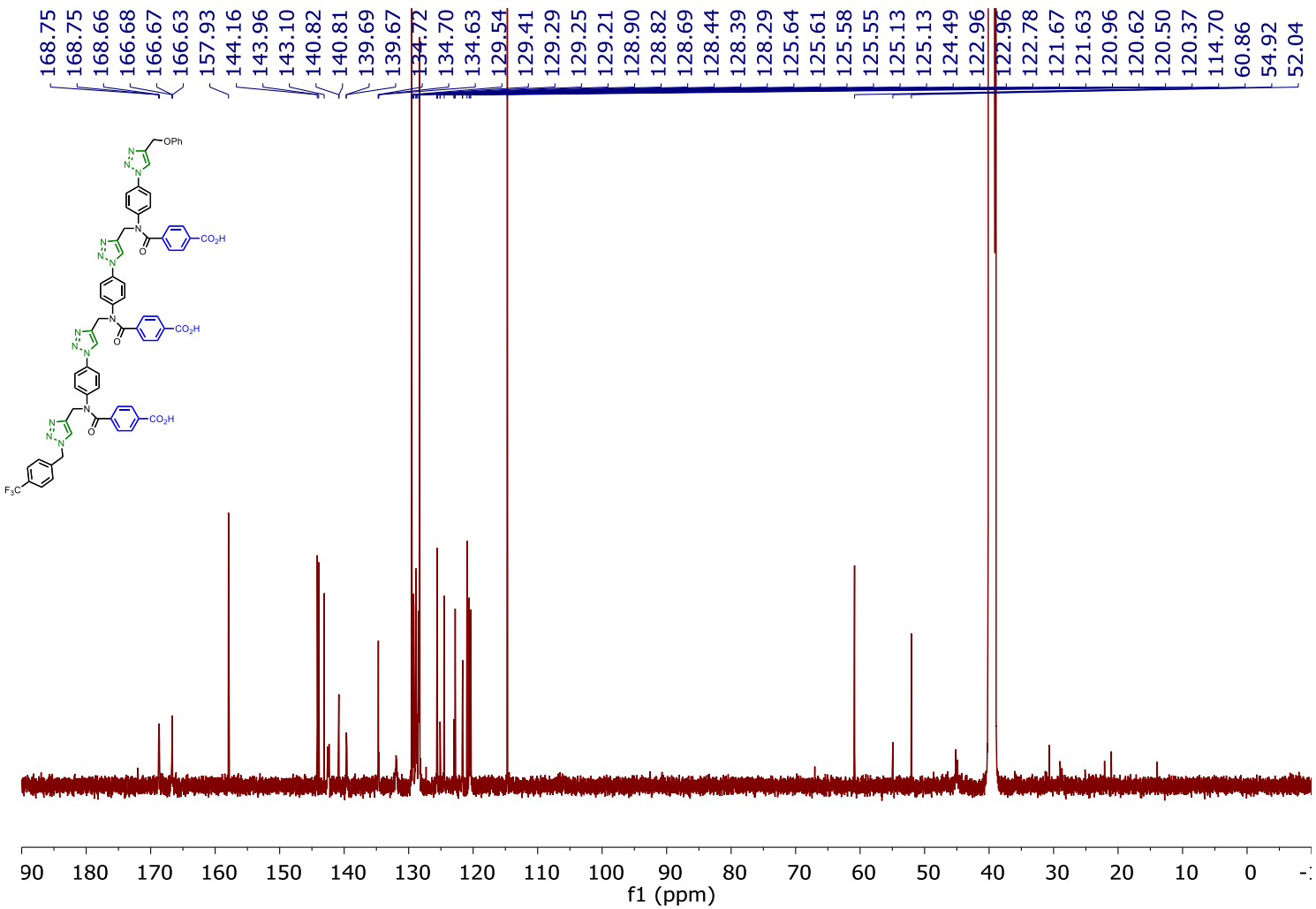
HRMS (ES+): calcd for $C_{68}H_{51}F_3N_{15}O_{10}$ 1294.3895 $[M+H]^+$, found 1294.4263 $[M+H]^+$.

FT-IR (ATR): ν_{max} 3456, 3123, 2929, 1710, 1644, 1519, 1324, 1240 and 735 cm^{-1}.

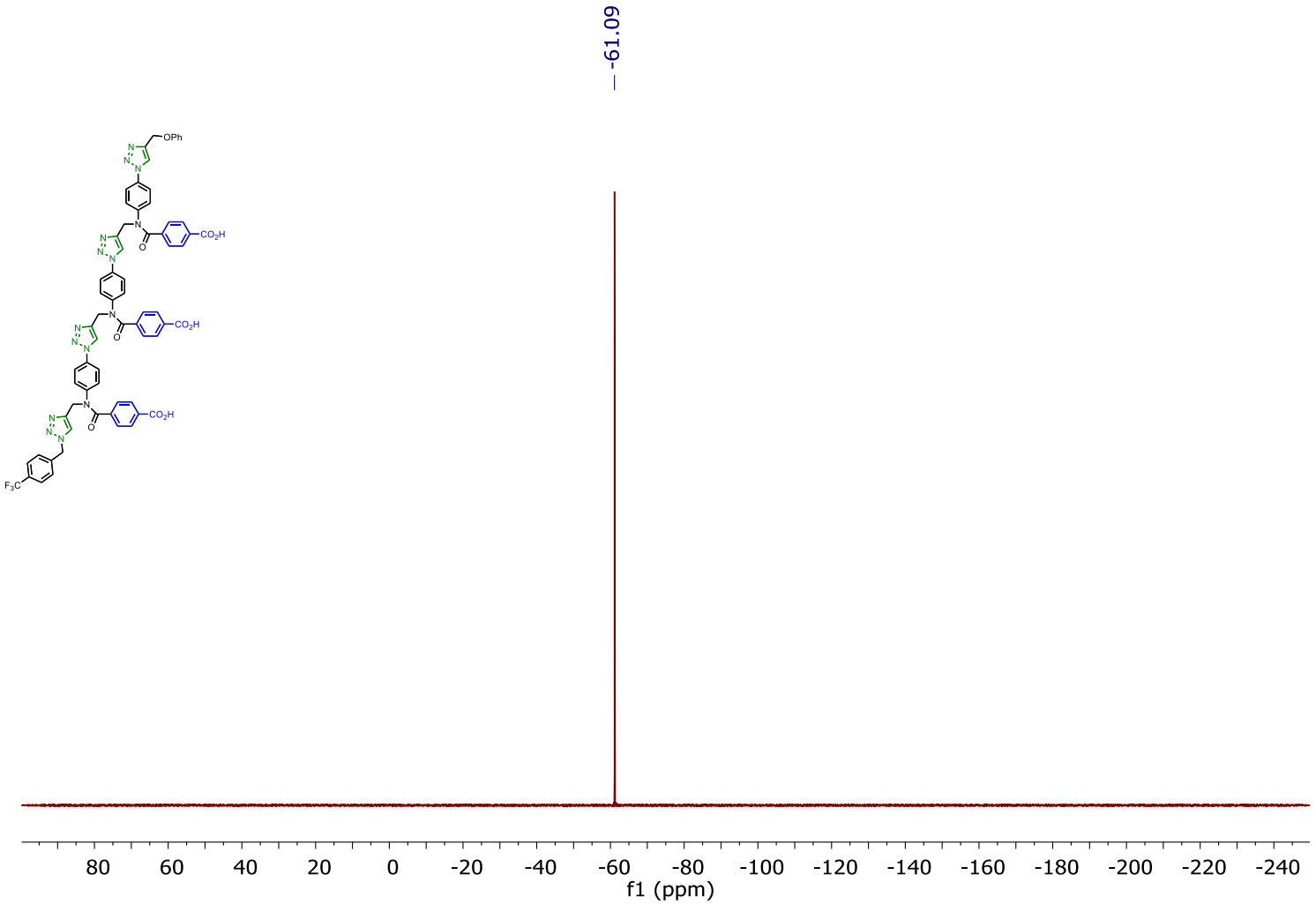
^{1}H-NMR (400 MHz, DMSO-d_6) Compound 7



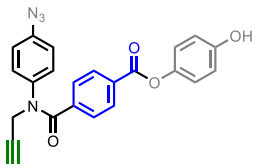
^{13}C-NMR (125 MHz, DMSO-d_6) Compound 7



^{19}F NMR (376 MHz, $CDCl_3$) Compound 7



Compound 10



Compound **8**[S2] (0.079 g, 0.25 mmol), monoprotected benzoquinone **9**[S4] (0.055 g, 0.25 mmol), EDC (0.057 g, 0.30 mmol) and DMAP (0.003 g, 0.025 mmol) were mixed in a round-bottom flask and dissolved in dry CH_2Cl_2 (3 mL). The reaction was stirred overnight at room temperature and then diluted with EtOAc and washed with 0.1N HCl soln. (2x), H_2O (1x) and brine (1x). The solution was dried with anhydrous $MgSO_4$, filtered and the solvent evaporated. The obtained residue was dissolved in dry THF (5 mL) under N_2 atmosphere and, after cooling down the solution to 0 °C, TBAF (1M in THF, 0.250 mL, 0.250 mmol) was added dropwise. After 10 minutes of stirring at 0 °C, the reaction was quenched with 0.1N HCl soln. and diluted with EtOAc. The organic layer was separated and washed with 0.1N HCl soln. (2x), H_2O (1x) and brine (1x). The solution was dried with anhydrous $MgSO_4$, filtered and the solvents evaporated. The obtained residue was purified by flash chromatography (from 0% to 30% of EtOAc in Pet. Ether) to afford compound **10** (0.070 g, 68%) as a solid.

mp: 151-152 °C.

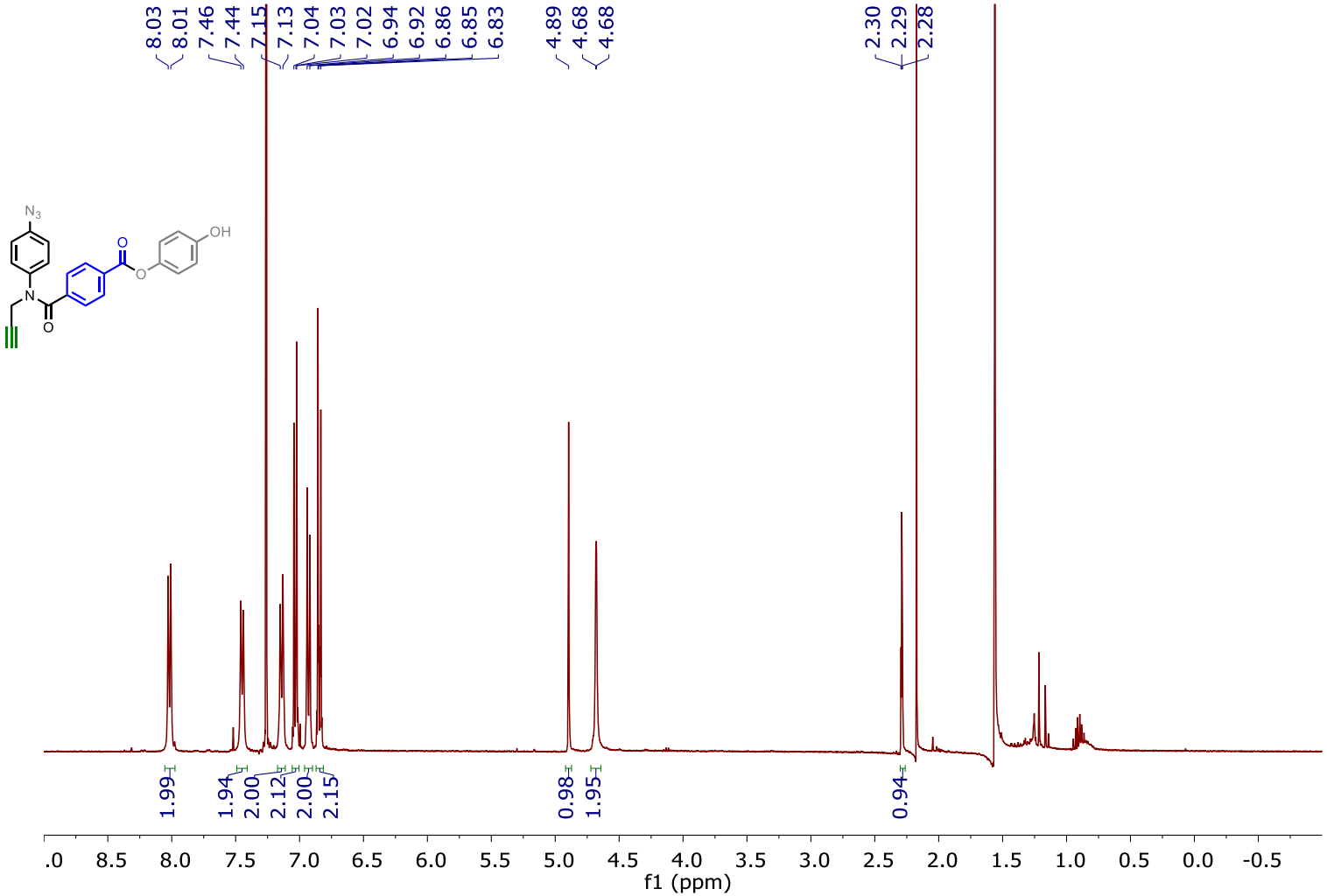
^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 8.02 (d, 2H, *J* = 8.0 Hz), 7.45 (d, 2H, *J* = 8.0 Hz), 7.14 (d, 2H, *J* = 8.5 Hz), 7.03 (d, 2H, *J* = 9.0 Hz), 6.93 (d, 2H, *J* = 8.5 Hz), 6.84 (d, 2H, *J* = 9.0 Hz), 4.89 (s, 1H), 4.68 (d, 2H, *J* = 2.5 Hz), 2.29 (t, 1H, *J* = 2.5 Hz).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 169.3, 164.8, 153.6, 144.4, 140.0, 139.7, 138.9, 130.9, 130.0, 129.4, 128.9, 122.7, 120.1, 116.2, 78.6, 73.0, 39.8.

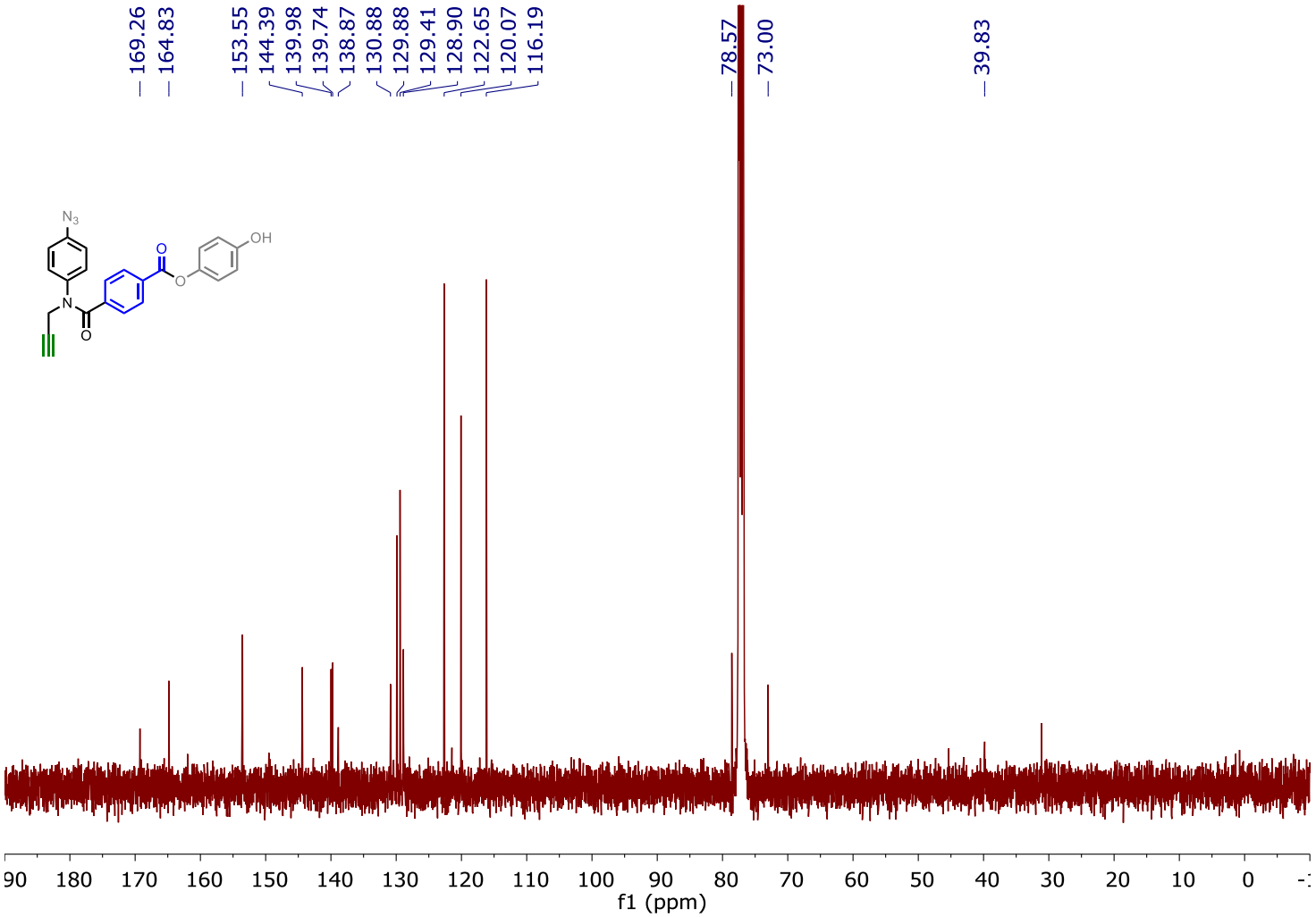
HRMS (ES+): calcd for $C_{23}H_{17}N_4O_4$ 413.1250 $[M+H]^+$, found 413.1269 $[M+H]^+$.

FT-IR (ATR): ν_{max} 3300, 2923, 2126, 2095, 1731, 1634, 1505, 1265, 1191, 1077 and 750 cm^{-1}.

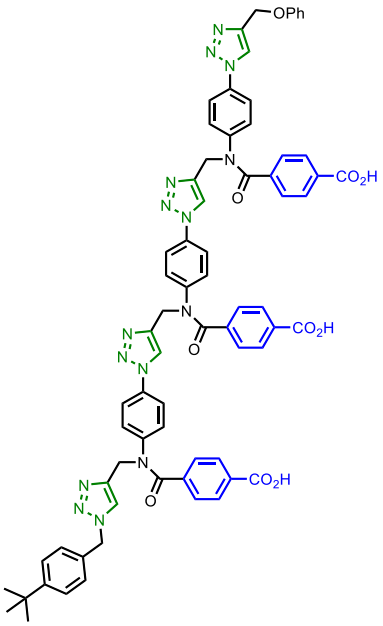
^{1}H-NMR (400 MHz, $CDCl_3$) compound 10



^{13}C-NMR (100.6 MHz, $CDCl_3$) compound 10



Copy (14) – synthesized as reference for covalent template-directed replication



Compound **4** (0.008 g, 0.007 mmol), 1-(azidomethyl)-4-*tert*-butylbenzene[S5] (0.004 g, 0.021 mmol), $Cu(CH_3CN)_4PF_6$ (0.003 g, 0.007 mmol) and TBTA (0.004 g, 0.007 mmol) were mixed in a round-bottom flask and, under N_2, THF (2 mL) was added. The reaction was stirred overnight at room temperature. The solvent was evaporated to dryness and the residue dissolved in THF:H_2O 3:1 (1 mL) and LiOH (~1mg, 0.030 mmol) was added. The reaction was stirred at room temperature for 2 h. Then, the crude was diluted with H_2O and acidified with 1N HCl soln. to pH 2-3. The aqueous phase was extracted with EtOAc (3x) and the combined organic phase was washed with 0.02 M EDTA soln. and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (gradient from 0% to 10% of MeOH with 0.05 % HCl in CH_2Cl_2) to afford **14** (0.004 g, 42%) as a white solid.

mp: Decomposed before melting.

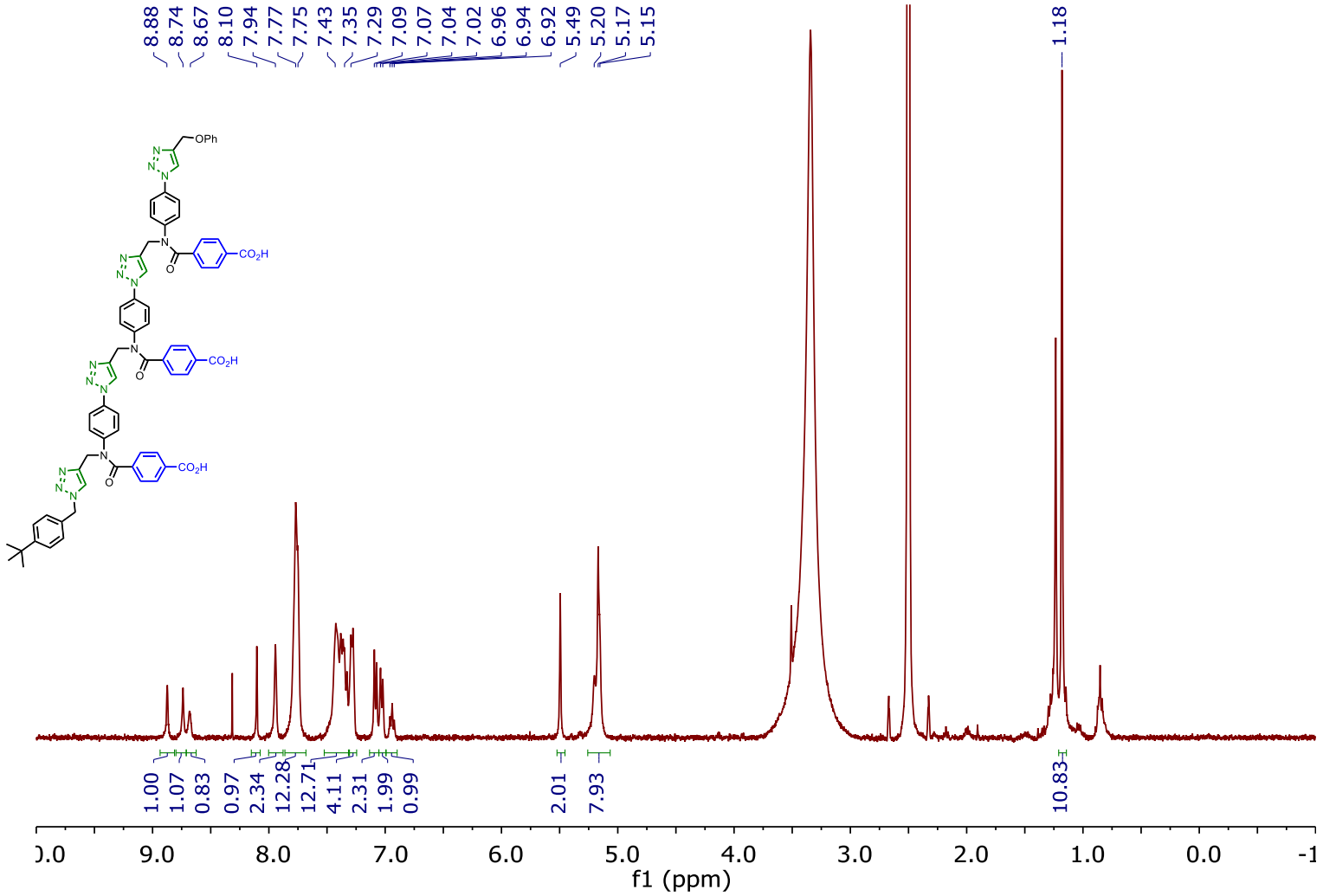
^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 8.88 (s, 1H), 8.74 (s, 1H), 8.67 (s, 1H), 8.11 (s, 1H), 7.77 (m, 12H), 7.44-7.34 (m, 12H), 7.29 (m, 4H), 7.09 (d, 2H, *J* = 8.0 Hz), 7.03 (d, 2H, *J* = 7.5 Hz), 6.94 (t, 1H, *J* = 7.5 Hz), 5.49 (s, 2H, N-CH_2), 5.20 (s, 2H), 5.17 (s, 4H), 5.15 (s, 2H), 1.18 (s, 9H).

^{13}C NMR (125 MHz, DMSO-*d*$_6$): δ_C =169.0, 167.8, 157.9, 150.5, 144.5, 144.3, 143.9, 143.0, 142.5, 142.5, 142.4, 137.8, 134.6, 134.6, 134.5, 133.2, 129.5, 129.2, 129.1, 129.0, 128.7, 128.7, 128.1, 127.3, 125.4, 124.1, 122.8, 121.6, 121.5, 120.9, 120.6, 120.3, 114.7, 60.9, 52.4, 44.9, 44.9, 44.5, 34.2, 31.0.

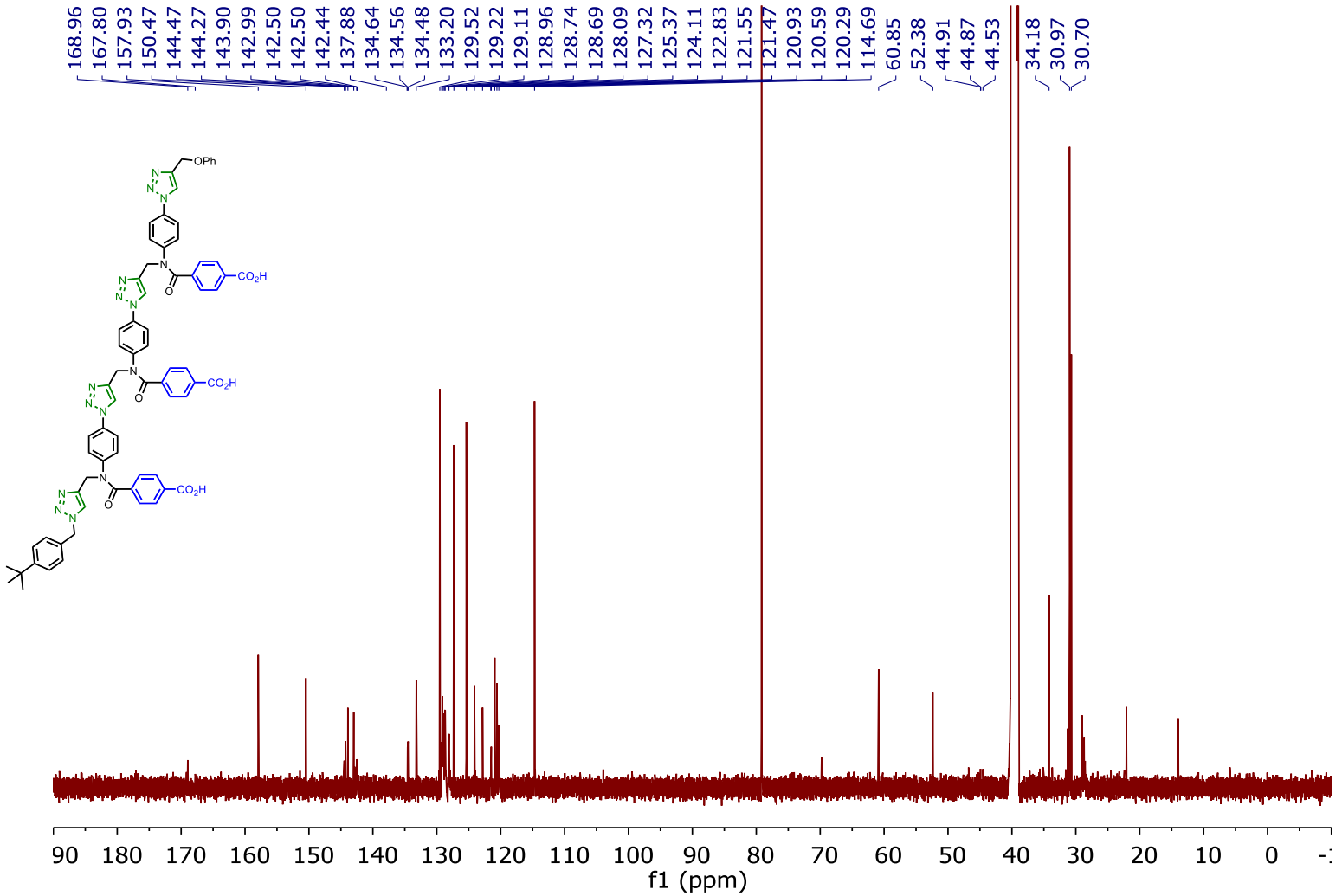
HRMS (ES+): calcd for $C_{71}H_{60}N_{15}O_{10}$ 1282.4648 $[M+H]^+$, found 1282.4642 $[M+H]^+$.

FT-IR (ATR): ν_{max} 2920, 2850, 1643, 1596, 1519, 1389, 1239, 1046 and 737 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6) copy 14



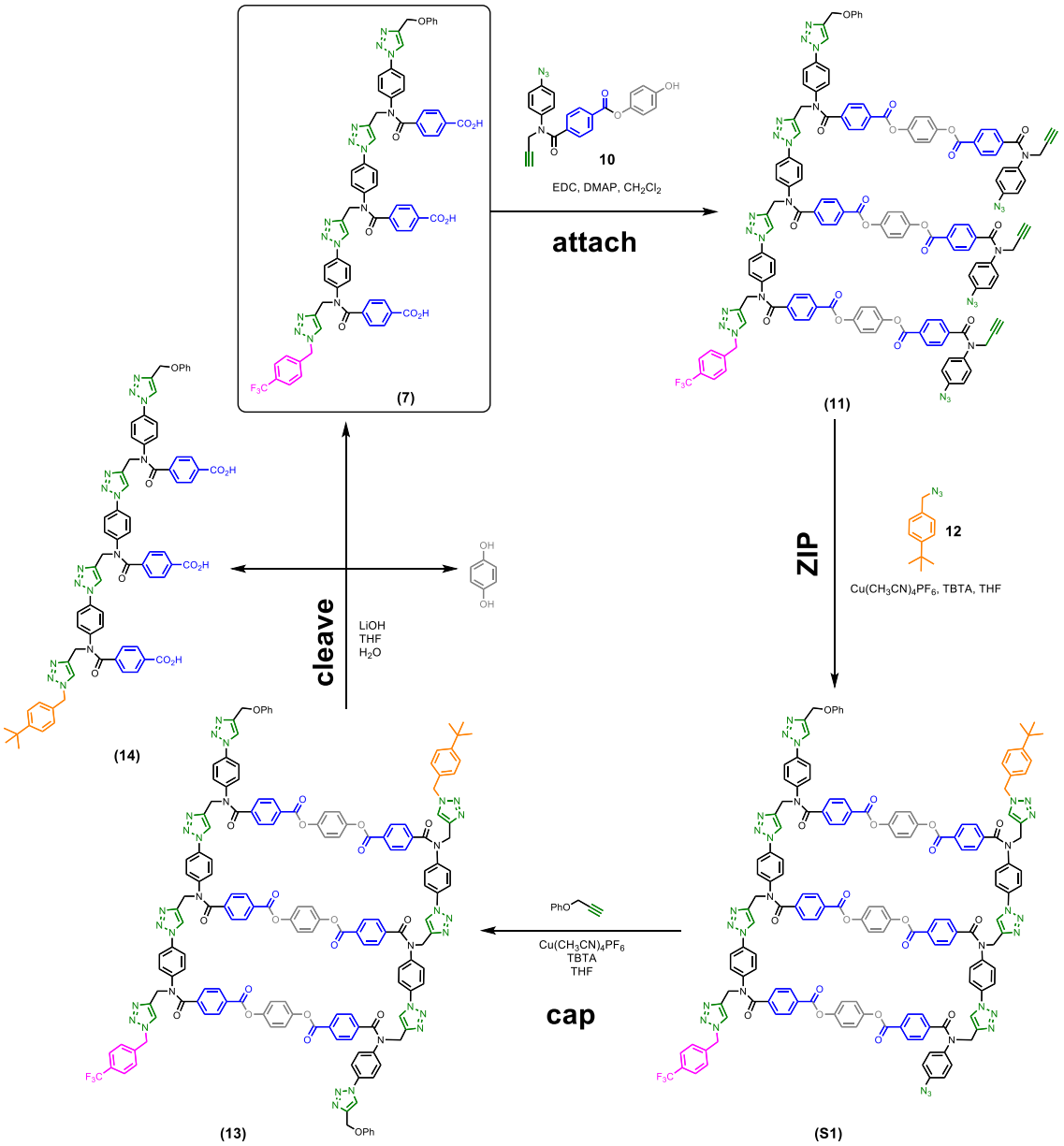
^{13}C NMR (125 MHz, DMSO-d_6) copy 14



Covalent template-directed replication

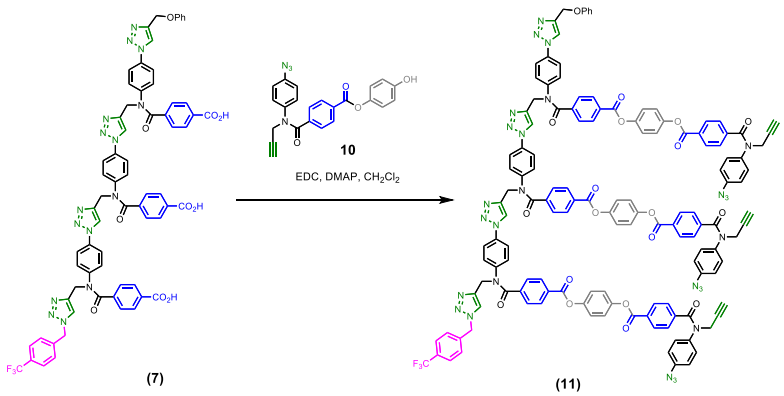
First replication cycle

Scheme S1 shows the synthetic steps for the first replication cycle of template **7**. First step involves the ester coupling between template 7 and excess of monomer **10** to give pre-ZIP intermediate **11**, followed by CuAAC ZIP reaction of **11** using Cu-TBTA in the presence of capping azide **12**. The obtained duplex is capped with phenyl propargyl azide and subsequently hydrolyzed to give template **7** and copy **14**, together with hydroquinone linker.



Scheme S1

Step 1: Monomer attachment



Template **7** (0.021 g, 0.016 mmol), compound **10** (0.023 g, 0.056 mmol), EDC (0.018 g, 0.096 mmol) and DMAP (0.013 g, 0.104 mmol) were mixed in a round-bottom flask and, under N_2, CH_2Cl_2 (1.5 mL) was added. The reaction was stirred overnight at room temperature. The solution was diluted with EtOAc and washed with 0.1N HCl soln. (3x), H_2O (1x) and brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 60% of EtOAc in Pet. Ether and then gradient from 0% to 4% of MeOH in CH_2Cl_2) to afford pre-ZIP **11** (0.034 g, 87%) as a light-yellow foam. Figure S1 shows the UPLC trace of **11**. Full characterization of **11** is shown in the next page.

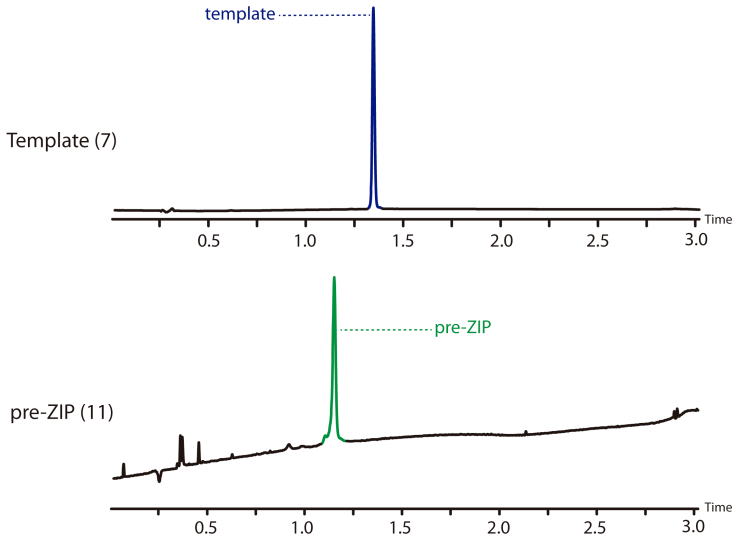


Figure S1. UPLC trace of the starting material **7** (top) and the obtained pre-ZIP **11** (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 5% -100% B + 1 minute 100% B (top) and 0-2 minutes 65% -100% B + 1 minute 100% B (bottom).

Full characterization of pre-ZIP 11.

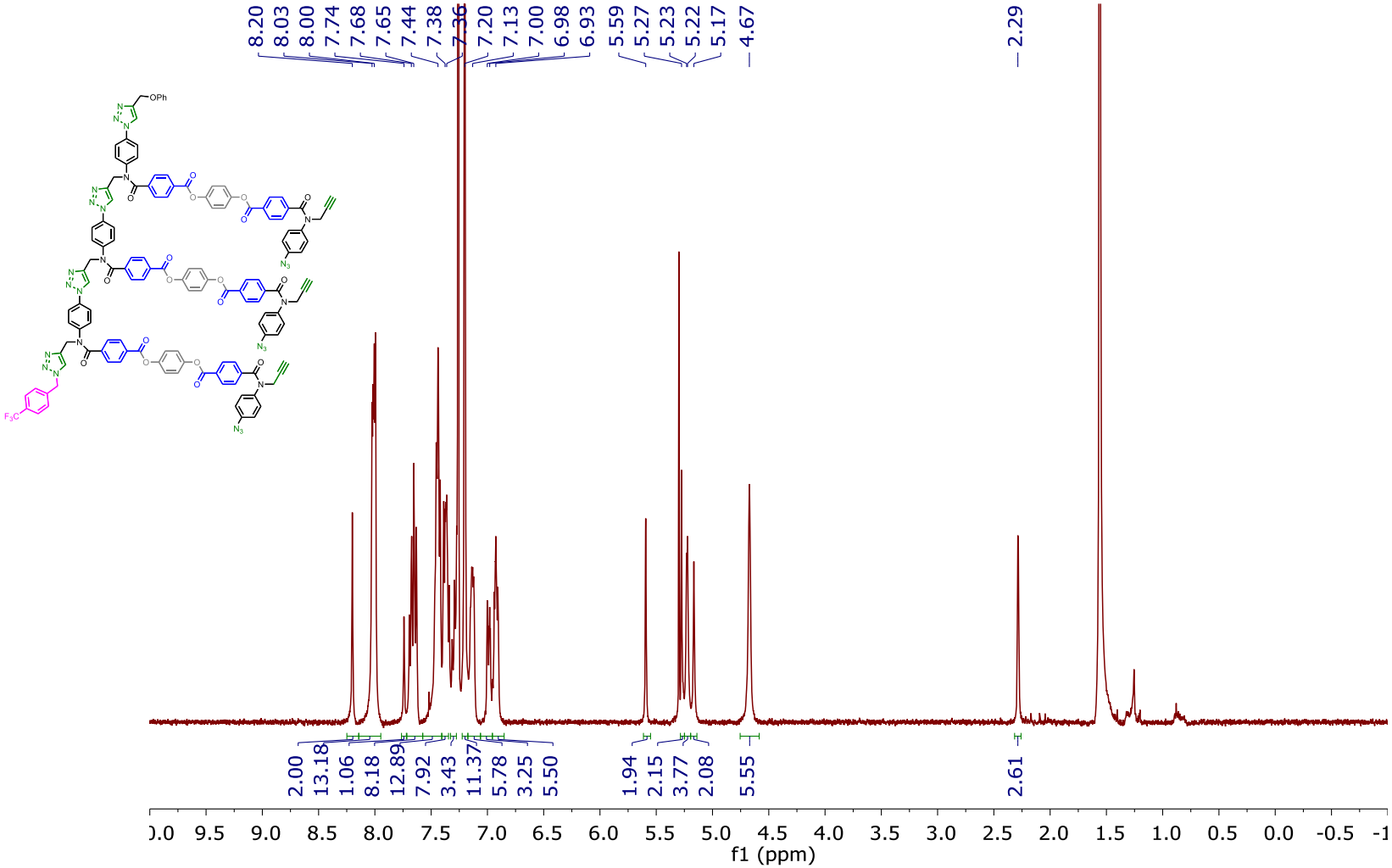
^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 8.20 (s, 2H), 8.01 (m, 13H), 7.74 (s, 1H), 7.66 (m, 8H), 7.44 (m, 12H), 7.37 (m, 8H), 7.30 (m, 2H), 7.13 (m, 6H), 6.99 (m, 3H), 6.92 (m, 6H), 5.59 (s, 2H), 5.27 (s, 2H), 5.23 (s, 2H), 5.22 (s, 2H), 5.17 (s, 2H), 4.67 (s, 6H), 2.29 (m, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 169.3, 169.3, 169.1, 164.3, 164.2, 164.1, 158.2, 148.4, 148.3, 145.5, 144.4, 144.4, 144.1, 143.7, 140.2, 140.1, 140.0, 139.7, 138.8, 138.5, 135.6, 135.5, 1335.4, 131.2 (q, *J* = 33.0 Hz), 130.9, 130.9, 130.8, 130.5, 130.2, 130.1, 129.9, 129.7, 129.4, 129.1, 129.1, 129.0, 128.9, 128.9, 128.9, 128.4, 126.3 (q, *J* = 4.0 Hz), 124.2, 123.9 (q, *J* = 272.5 Hz, 122.7, 122.2, 121.6, 121.5, 121.4, 121.3, 120.8, 120.1, 114.8, 78.6, 73.0, 62.0, 53.7, 46.4, 39.8.

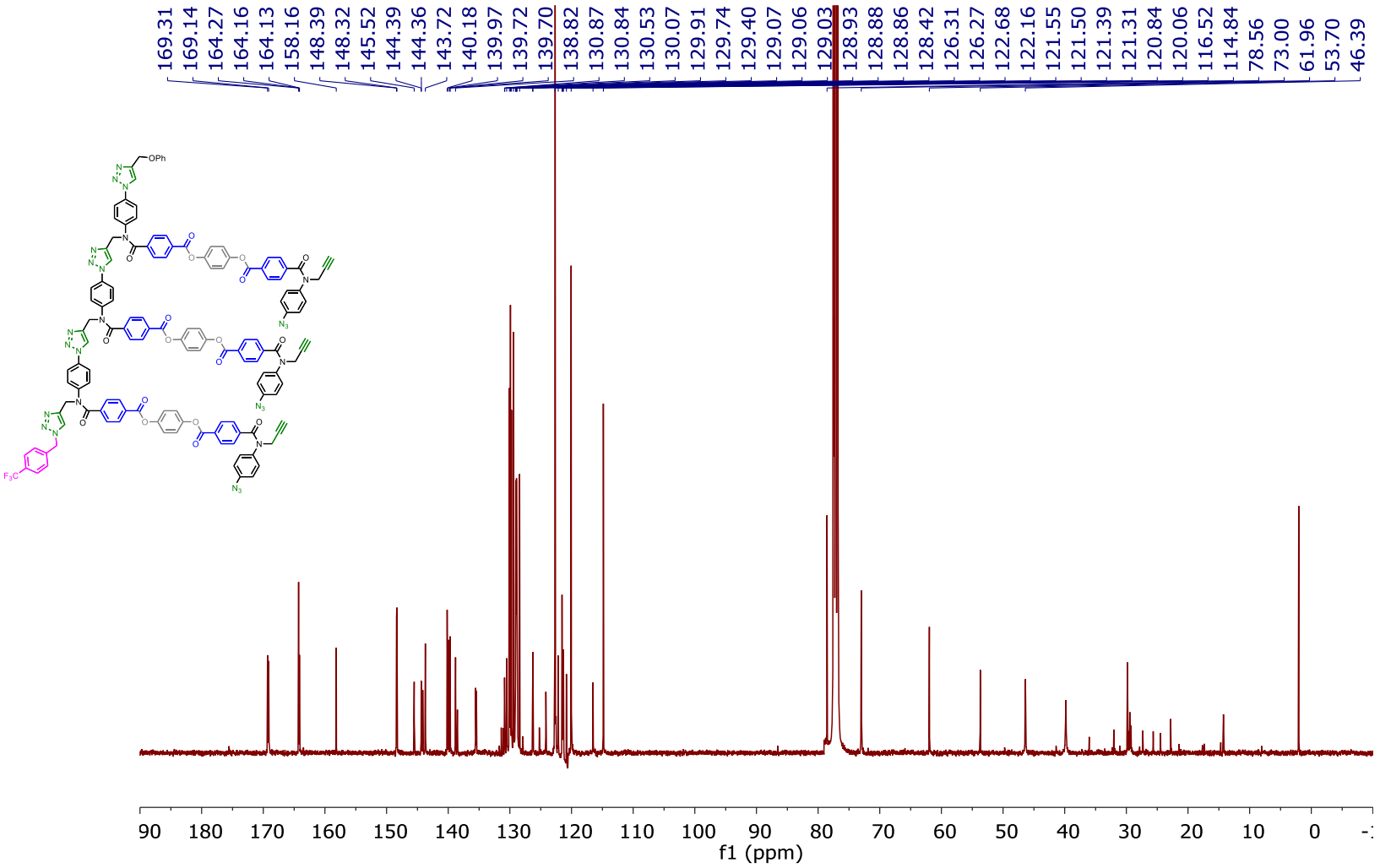
^{19}F NMR (376 MHz, $CDCl_3$): δ_F = -63.7.

FT-IR (ATR): ν_{max} 2125, 2094, 1736, 1649, 1504, 1500, 1260, 1172, 1016 and 752 cm^{-1}.

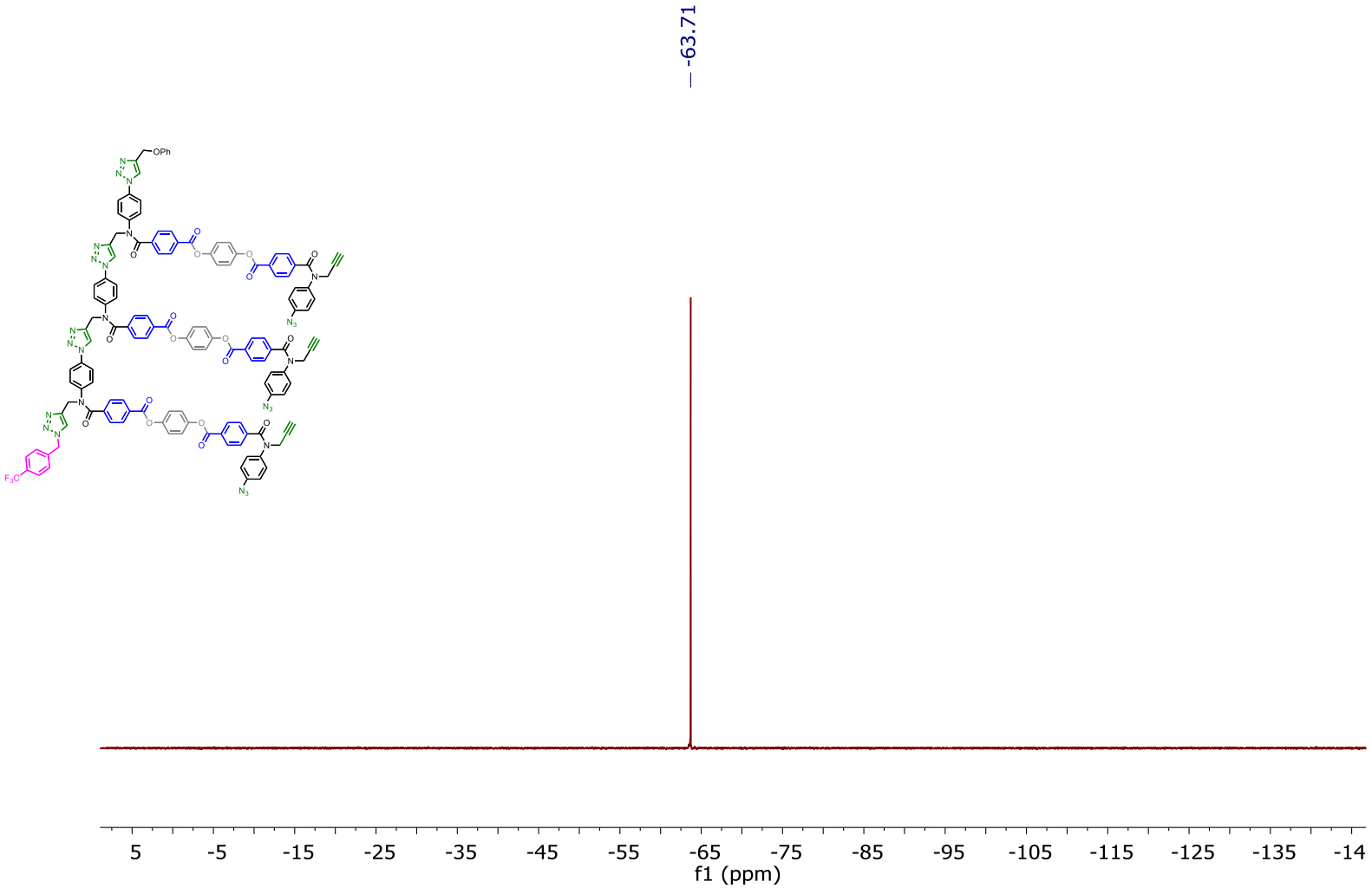
^{1}H NMR (400 MHz, $CDCl_3$) pre-ZIP 11.



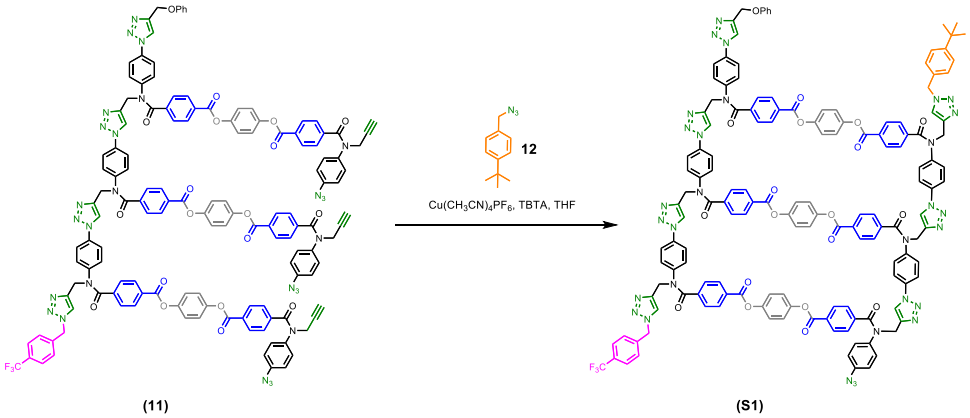
^{13}C NMR (125 MHz, $CDCl_3$) pre-ZIP 11



^{19}F NMR (376 MHz, $CDCl_3$) pre-ZIP 11



Step 2: ZIP



A solution of 1-(azidomethyl)-4-tert-butylbenzene[S5] (0.184 g, 0.97 mmol) in dry and degassed THF (5 mL) was added to a solution of Pre-ZIP **11** (0.032 g, $1.3 \cdot 10^{-2}$ mmol) in dry and degassed THF (855 mL) under N_2 atmosphere. A solution of $Cu(CH_3CN)_4PF_6$ (0.029 g, $7.8 \cdot 10^{-2}$ mmol) and TBTA (0.041 g, $7.8 \cdot 10^{-2}$ mmol) in dry and degassed THF (5 mL) was added to the previous solution and the reaction stirred at room temperature for 3 days. Then, the solvent was evaporated and the crude dissolved in EtOAc and washed with 0.02M EDTA soln. (2x), 0.1M HCl soln. (1x), H_2O (1x) and brine. The organic layer was dried over $MgSO_4$ and concentrate under vacuum. The obtained crude (**S1**) was precipitated with Pet. Ether and centrifuged (repeated three times) in order to remove the excess of capping azide. Figure S2 shows the UPLC trace of the reaction crude.

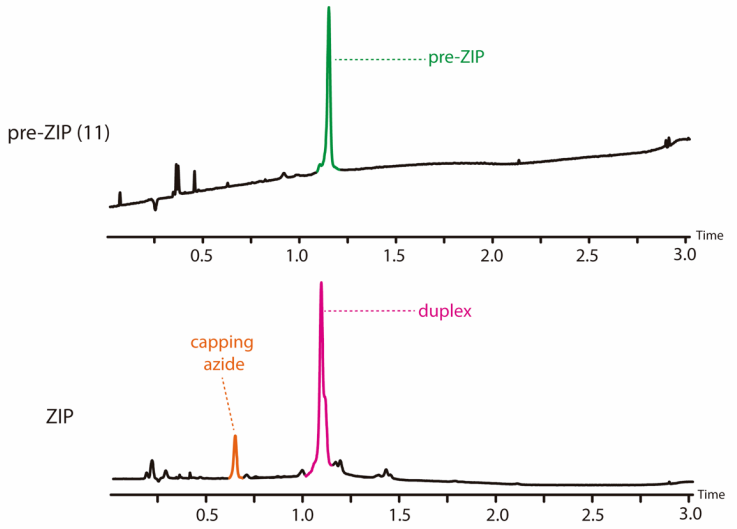
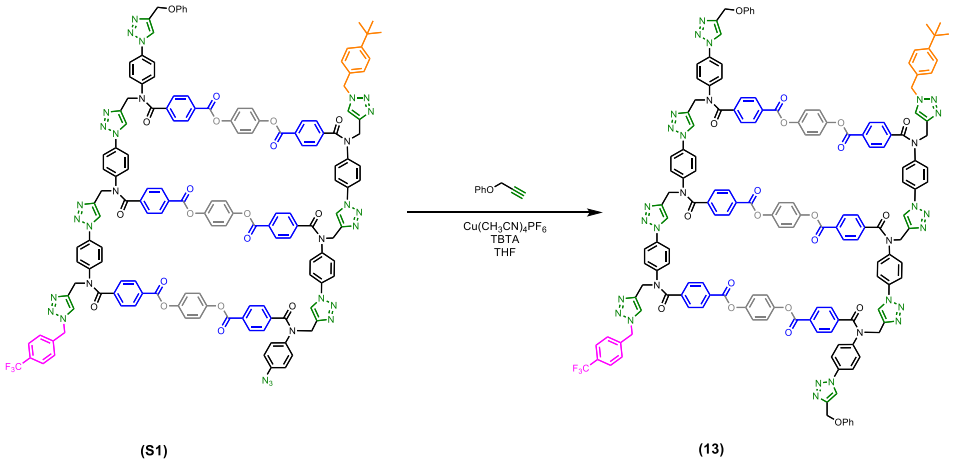


Figure S2. UPLC trace of the pre-ZIP **11** (top) and crude reaction mixture of the ZIP step (bottom). *UPLC Conditions:* C18 column at 40 ºC (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% -100% B + 1 minute 100% B.

Step 3: capping



Phenyl propargyl ether (0.166 mL, 1.30 mmol) was added to a solution of **S1** ($1.3 \cdot 10^{-2}$ mmol) in dry and degassed THF (3 mL). $Cu(CH_3CN)_4PF_6$ (0.005, $1.3 \cdot 10^{-2}$ mmol) and TBTA (0.007 g, $1.3 \cdot 10^{-2}$ mmol) were added to the previous solution and the reaction stirred overnight at room temperature. Then, the reaction was diluted EtOAc and washed with 0.02M EDTA soln. (2x), 0.1M HCl soln. (1x), H_2O (1x) and brine. The organic layer was dried over $MgSO_4$ and concentrate under vacuum. The obtained crude was used without further purification. Figure S3 shows the UPLC trace of the reaction crude.

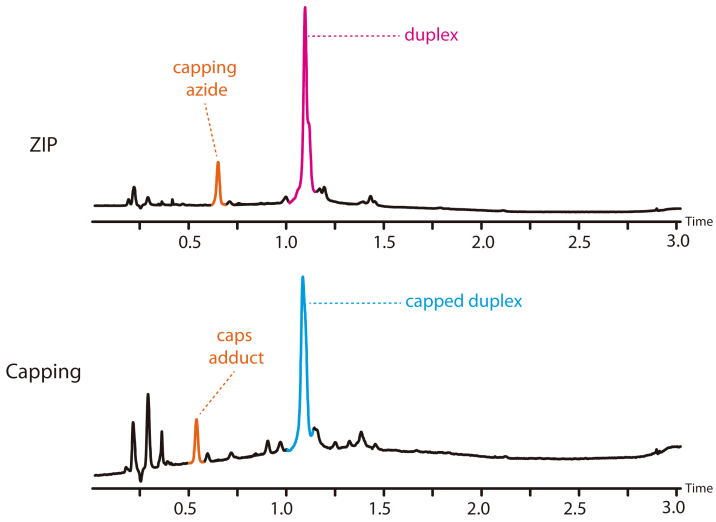
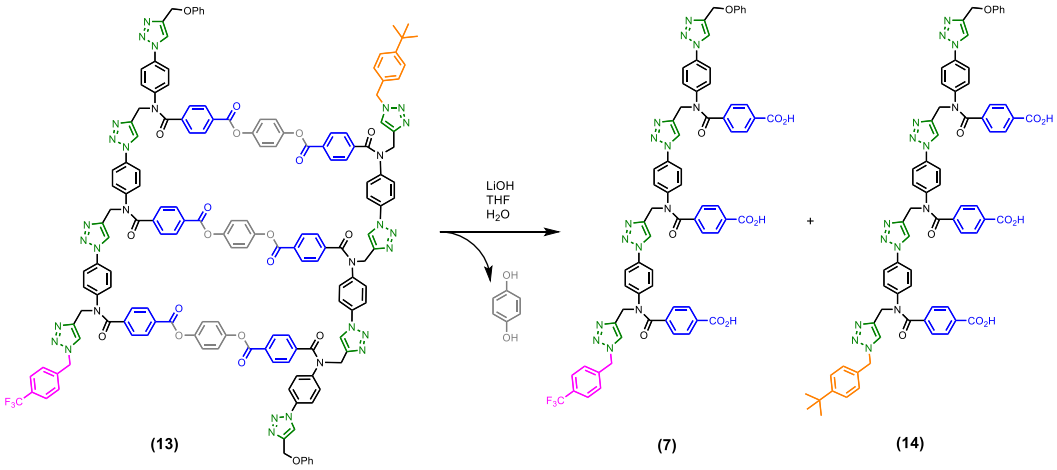


Figure S3. UPLC trace of the crude reaction mixture of the ZIP step (top) and crude reaction mixture of the capping step (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% - 100% B + 1 minute 100% B.

Step 4: cleavage



The crude reaction mixture from previous step ($1.3 \cdot 10^{-2}$ mmol) was dissolved in THF:H_2O 3:1 (1 mL) and 1M LiOH soln. (0.20 mL, 0.20 mmol) was added. The reaction was stirred at room temperature for 45 min. Then, the crude was diluted with H_2O and acidified with 0.1M HCl soln. to pH 2-3. The aqueous phase was extracted with EtOAc (3x) and the combined organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using a gradient from 0% to 20% of MeOH (containing 0.01% of aq. HCl) in CH_2Cl_2 to afford template **7** and copy **14** (0.023 g, 69% over three steps). Figure S4 shows the UPLC trace of the reaction crude and the isolated fraction. Figure S5 shows the 500 MHz ^{1}H NMR spectra of the isolated fraction in comparison with the starting template **7** and the reference of the copy (compound **14**).

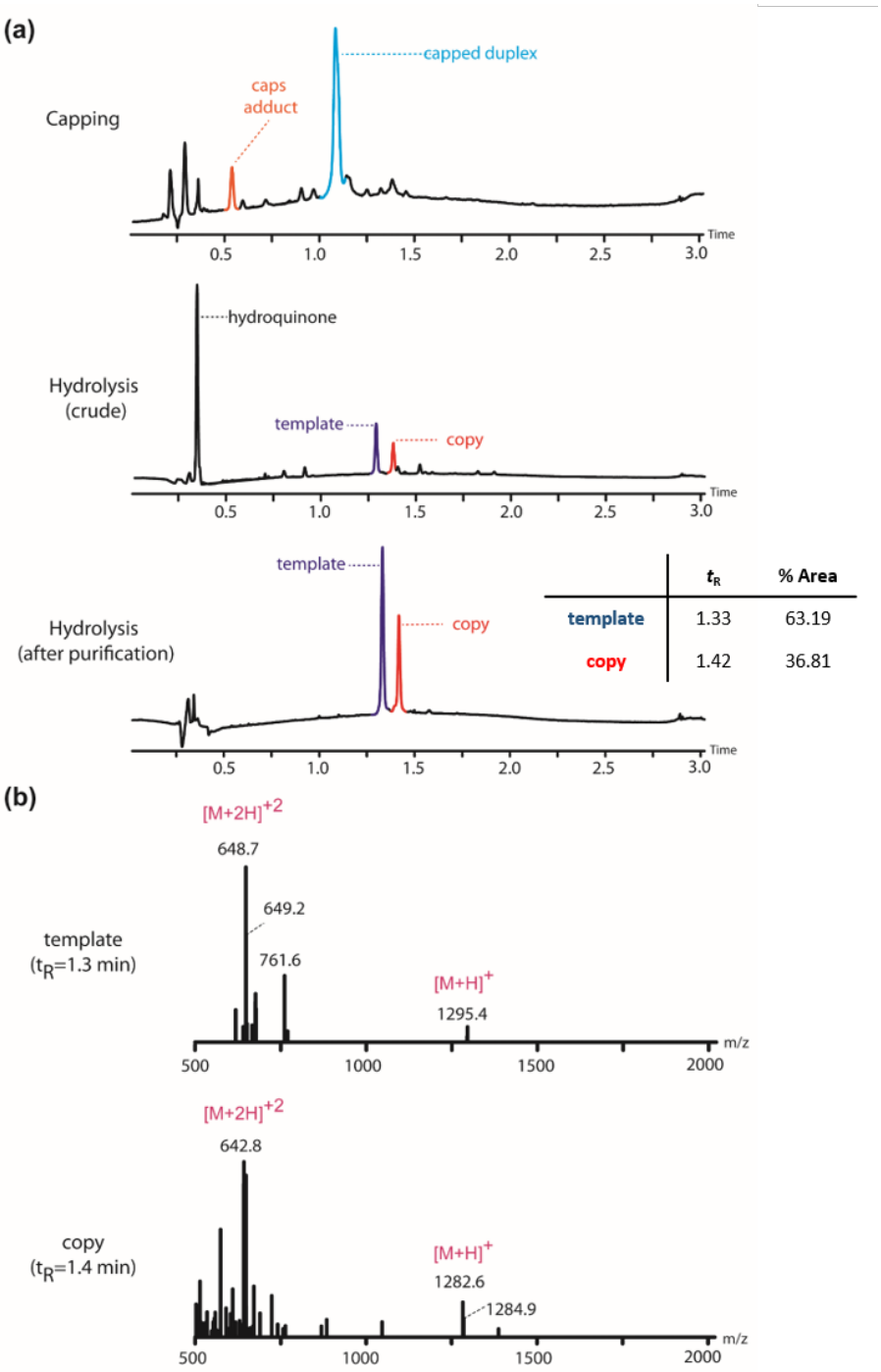


Figure S4. (a) UPLC trace of the crude reaction mixture of the capping step (top), crude reaction mixture of the hydrolysis (middle) and recovered fraction after purification (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% -100% B + 1 minute 100% B (top) and 0-2 minutes 5% - 100% B + 1 minute 100% B (middle and bottom). **(b)** Mass spectra (ESI+) of the two products obtained after the replication cycle: template (MW: 1293.4) and copy (MW: 1281.5).

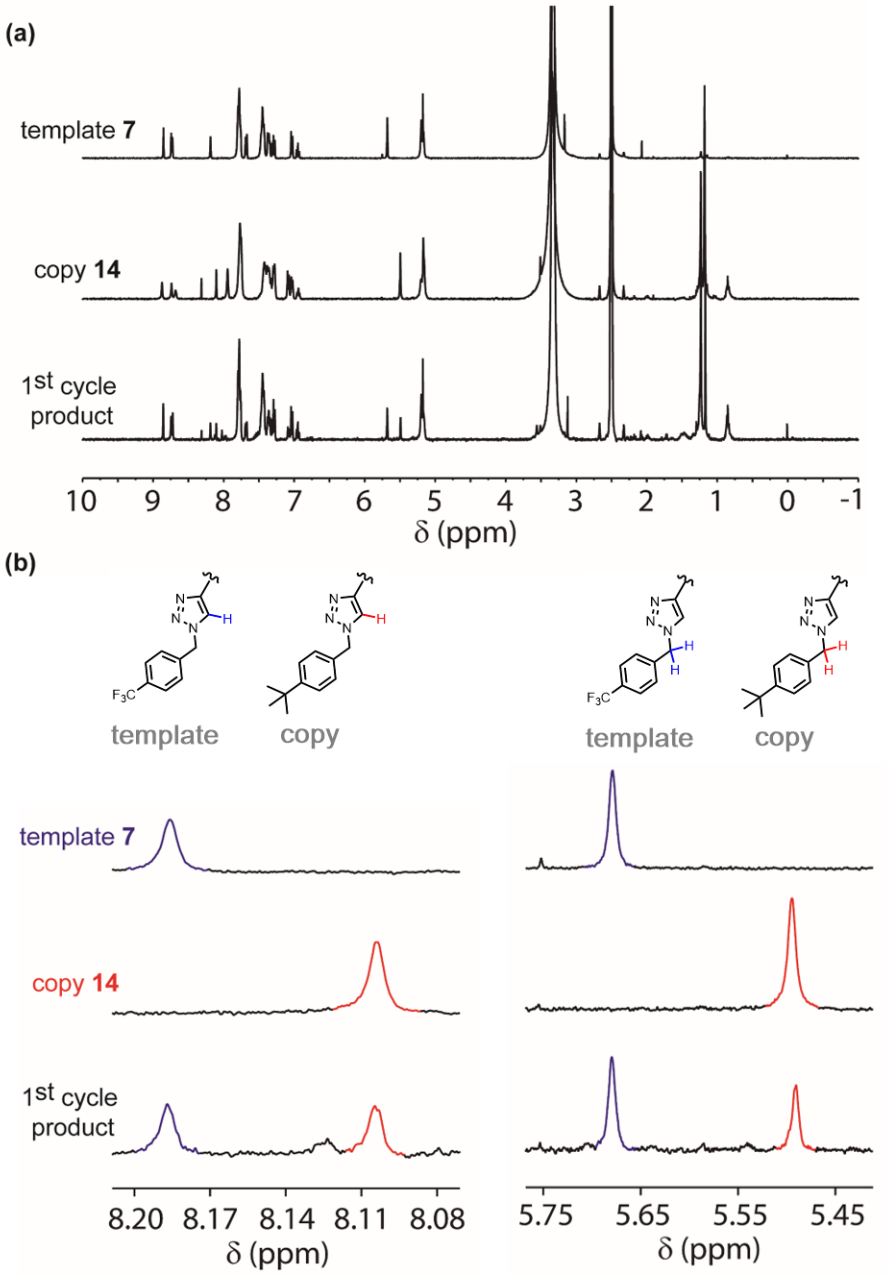
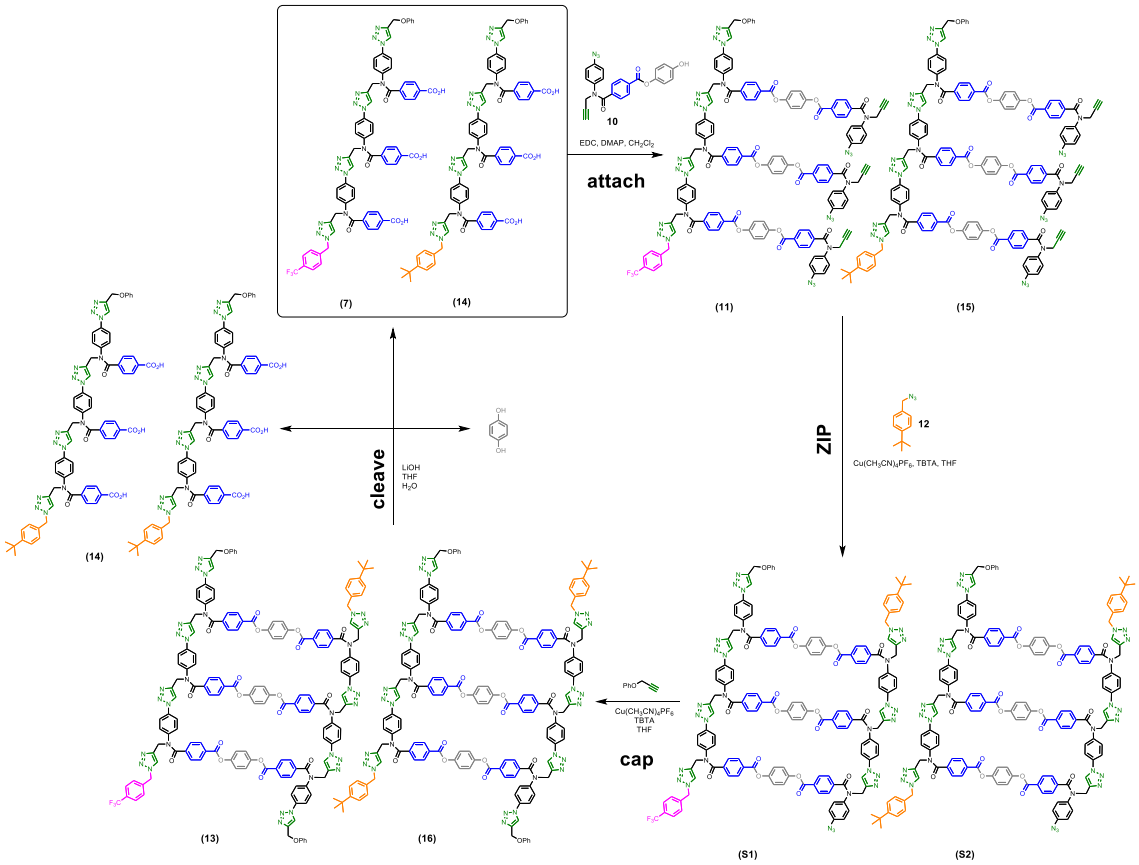


Figure S5. (a) 500 MHz ^{1}H NMR spectra in DMSO-d_6 at 298 K of template **7** (top), reference of the copy **14** (middle) and the recovered fraction after purification (bottom). **(b)** Expansions of key regions and the corresponding proton signals assigned in the structures.

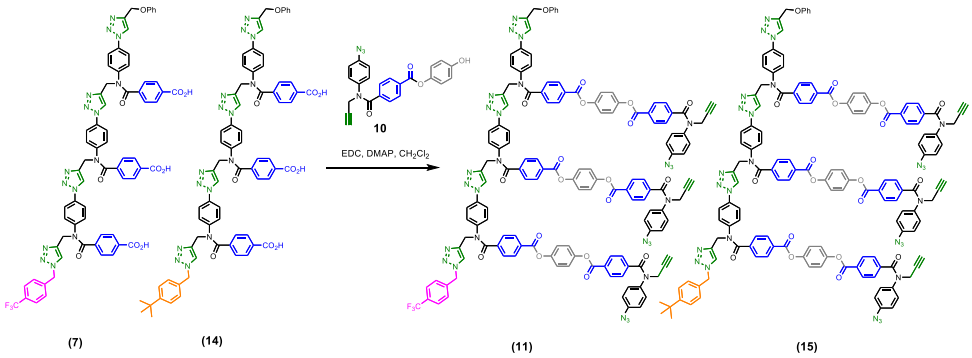
Second replication cycle

Scheme S2 shows the synthetic steps for the second replication cycle of the obtained mixture in the first replication cycle. First step involves the ester coupling of the mixture with excess of monomer **10** to give pre-ZIP intermediates **11** and **15**, followed by CuAAC ZIP reaction of this mixture using Cu-TBTA in the presence of capping azide **12**. The obtained duplexes are capped with phenyl propargyl azide and subsequently hydrolyzed to give template **7** and three molecules of copy **14**, together with hydroquinone linker.



Scheme S2

Step 1: Monomer attachment



The obtained mixture of **7** and **14** from the first replication cycle (0.023 g, $1.8 \cdot 10^{-2}$ mmol), compound **10** (0.046 g, 0.11 mmol), EDC (0.021 g, 0.11 mmol) and DMAP (0.014 g, 0.12 mmol) were mixed in a round-bottom flask and, under N_2, CH_2Cl_2 (2 mL) was added. The reaction was stirred overnight at room temperature. The solution was diluted with EtOAc and washed with 0.1N HCl soln. (3x), H_2O (1x) and brine. The organic phase was dried with $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 60% of EtOAc in Pet. Ether and then gradient from 0% to 4% of MeOH in CH_2Cl_2) to afford a mixture of **11** and **15** (0.034 g, 80%) as a light-yellow foam. Figure S6 shows the UPLC trace of the mixture of **11** and **15**.

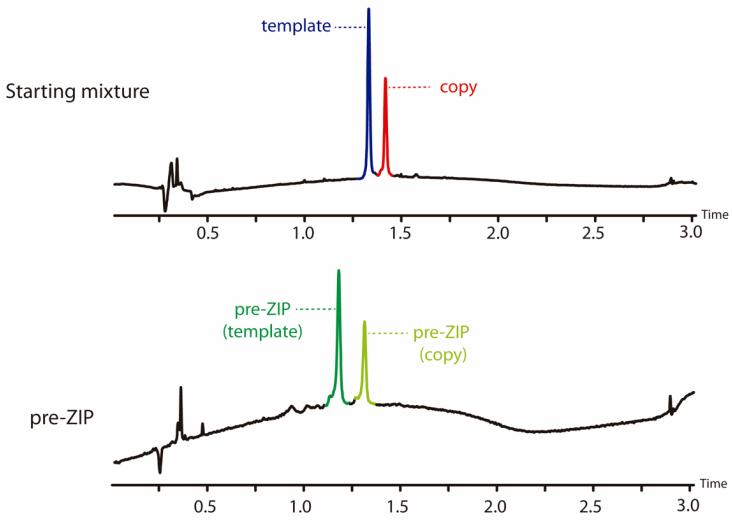
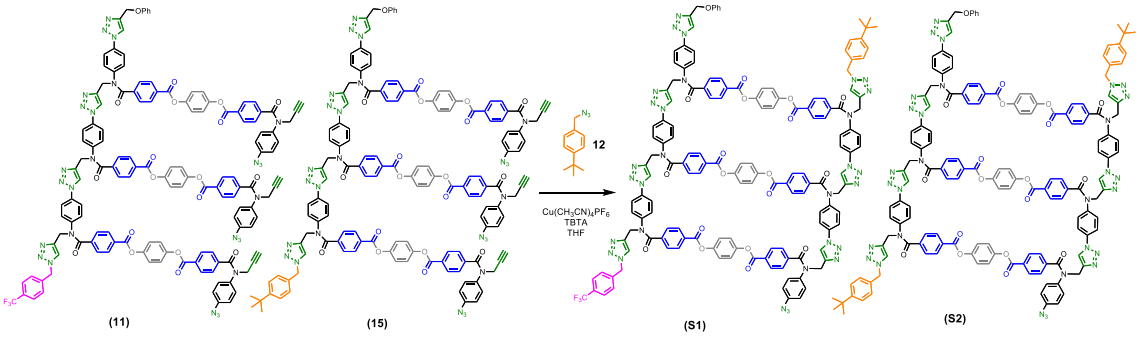


Figure S6. UPLC trace of the starting mixture (top) and the obtained mixture of **11** and **15** (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 5% -100% B + 1 minute 100% B (top) and 0-2 minutes 65% -100% B + 1 minute 100% B (bottom).

Step 2: ZIP



A solution of 1-(azidomethyl)-4-tert-butylbenzene[S5] (0.101 g, 0.53 mmol) in dry and degassed THF (5 mL) was added to a solution of the mixture of **11** and **15** (0.018 g, $7.1 \cdot 10^{-3}$ mmol) in dry and degassed THF (465 mL) under N_2 atmosphere. A solution of $Cu(CH_3CN)_4PF_6$ (0.016 g, $4.3 \cdot 10^{-2}$ mmol) and TBTA (0.023 g, $4.3 \cdot 10^{-2}$ mmol) in dry and degassed THF (5 mL) was added to the previous solution and the reaction stirred at room temperature for 3 days. Then, the solvent was evaporated and the crude dissolved in EtOAc and washed with 0.02M EDTA soln. (2x), 0.1M HCl soln. (1x), H_2O (1x) and brine. The organic layer was dried over $MgSO_4$ and concentrate under vacuum. The obtained crude (mixture of **S1** and **S2**) was precipitated with Pet. Ether and centrifuged (repeated three times) in order to remove the excess of capping azide. Figure S7 shows the UPLC trace of the reaction crude.

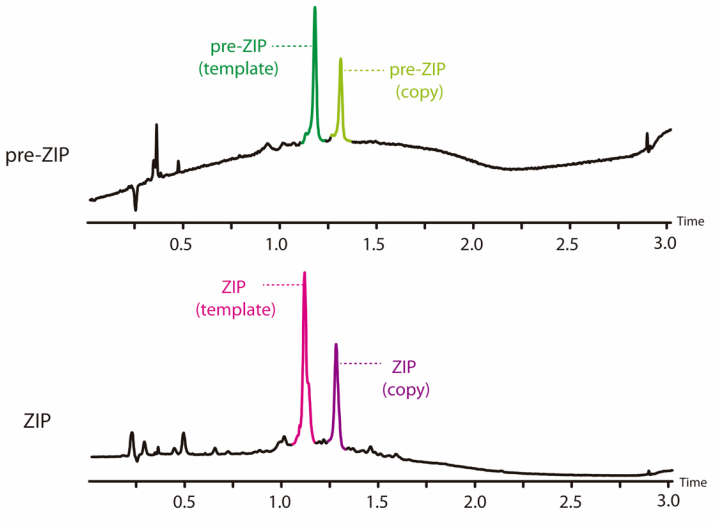
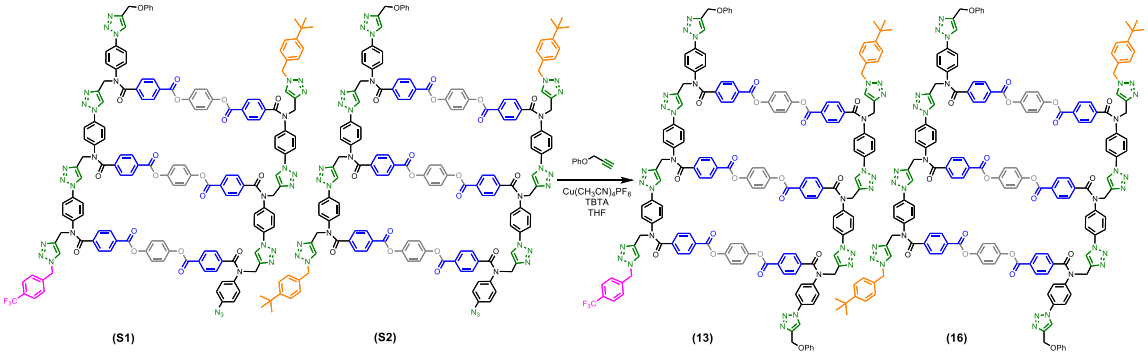


Figure S7. UPLC trace of the mixture of **11** and **15** (top) and crude reaction mixture of the ZIP step (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% -100% B + 1 minute 100%B.

Step 3: capping



Phenyl propargyl ether (0.090 mL, 0.71 mmol) was added to a solution of the mixture of **S1** and **S2** obtained in the previous step ($7.1 \cdot 10^{-3}$ mmol) in dry and degassed THF (3 mL). $Cu(CH_3CN)_4PF_6$ (0.003, $7.1 \cdot 10^{-3}$ mmol) and TBTA (0.004 g, $7.1 \cdot 10^{-3}$ mmol) were added to the previous solution and the reaction stirred overnight at room temperature. Then, the reaction was diluted EtOAc and washed with 0.02M EDTA soln. (2x), 0.1M HCl soln. (1x), H_2O (1x) and brine. The organic layer was dried over $MgSO_4$ and concentrate under vacuum. The obtained crude (mixture of **13** and **16**) was used without further purification. Figure S8 shows the UPLC trace of the reaction crude.

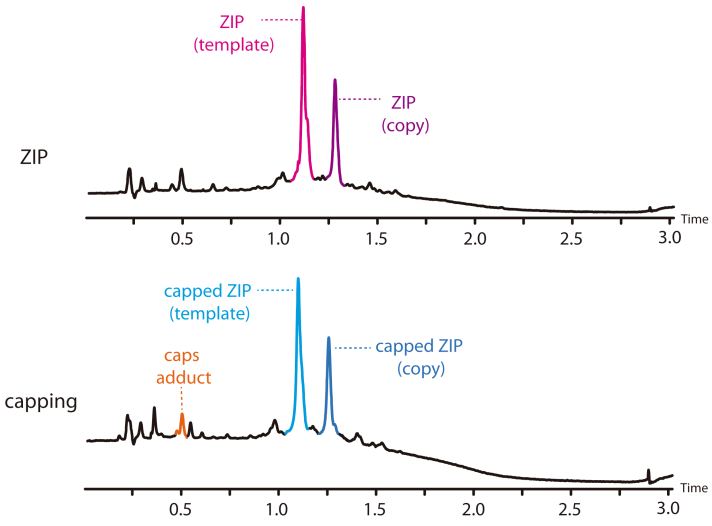
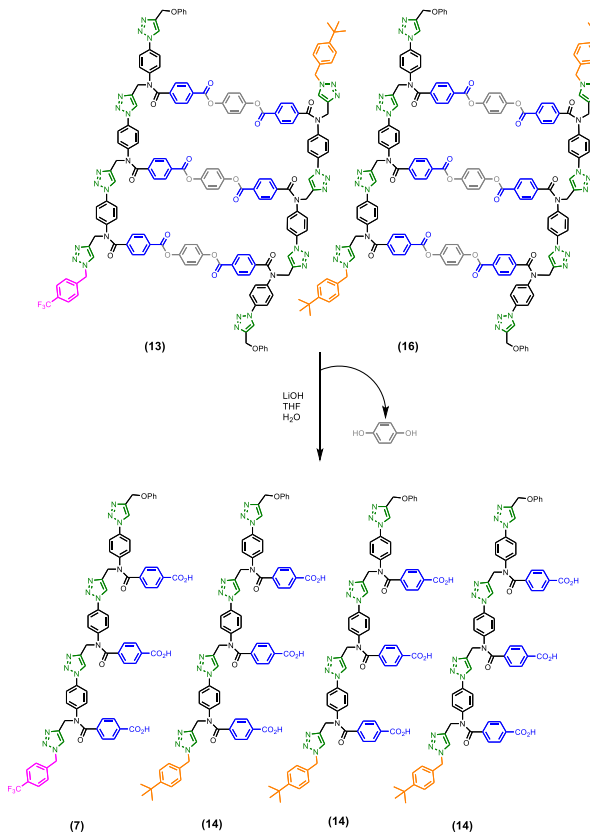


Figure S8. UPLC trace of the crude reaction mixture of the ZIP step (top) and crude reaction mixture of the capping step (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% - 100% B + 1 minute 100% B.

Step 4: cleavage



The crude reaction mixture from previous step ($7.1 \cdot 10^{-3}$ mmol) was dissolved in THF:H_2O 3:1 (1 mL) and 1M LiOH soln. (0.10 mL, 0.10 mmol) was added. The reaction was stirred at room temperature for 45 min. Then, the crude was diluted with H_2O and acidified with 0.1M HCl soln. to pH 2-3. The aqueous phase was extracted with EtOAc (3x) and the combined organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography on silica gel using a gradient from 0% to 20% of MeOH (containing 0.01% of aq. HCl) in CH_2Cl_2 to afford template **7** and copy **14** (0.012 g, 65% over three steps). Figure S9 shows the UPLC trace of the reaction crude and the isolated fraction. Figure S5 shows the 500 MHz ^{1}H NMR spectra of the isolated fraction in comparison with the starting template **7** and the reference of the copy (compound **14**).

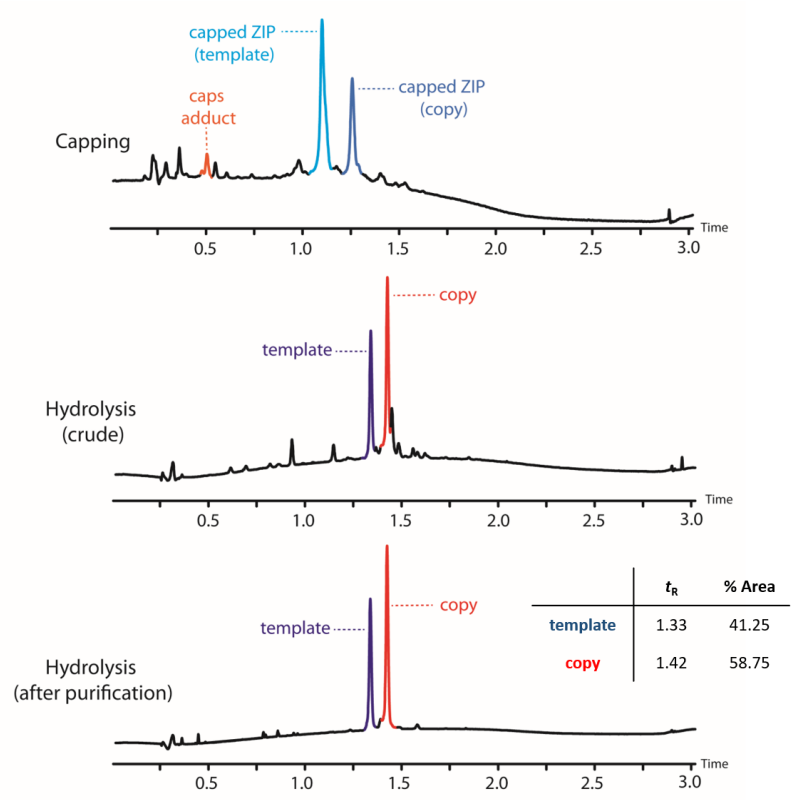


Figure S9. UPLC trace of the crude reaction mixture of the capping step (top), crude reaction mixture of the hydrolysis (middle) and recovered fraction after purification (bottom). *UPLC Conditions:* C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% -100% B + 1 minute 100% B (top) and 0-2 minutes 5% -100% B + 1 minute 100% B (middle and bottom).

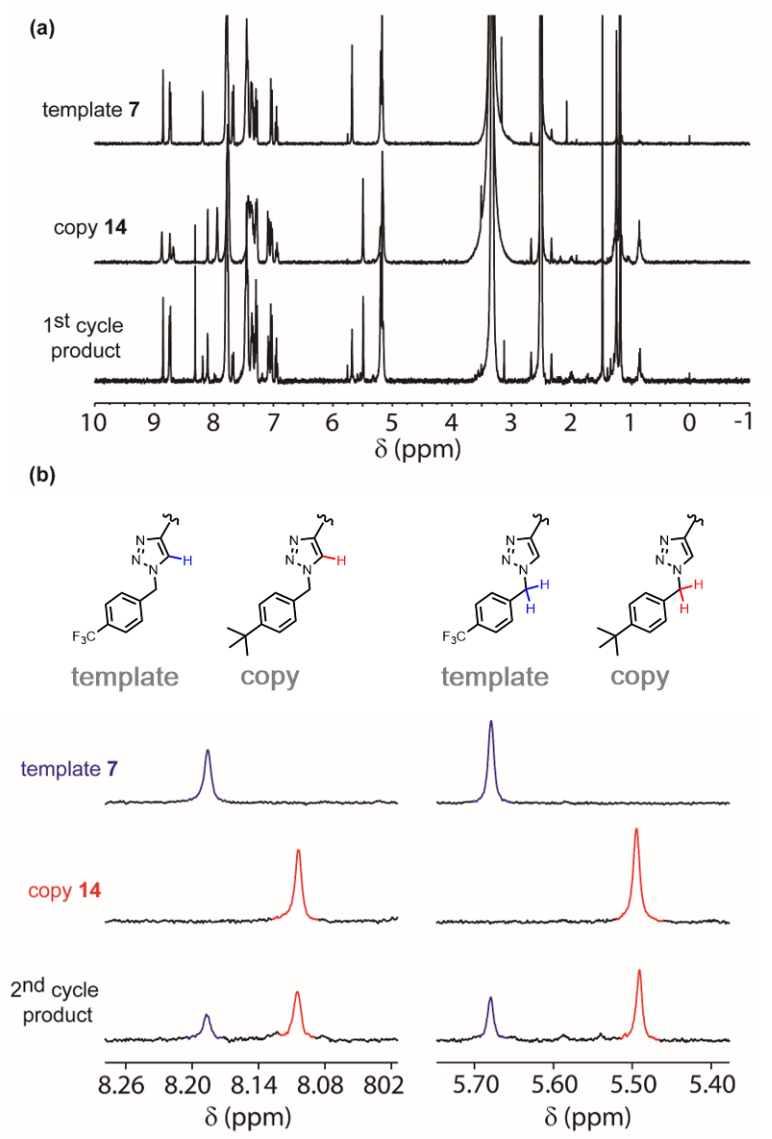


Figure S10. (a) 500 MHz ^{1}H NMR spectra in DMSO-d_6 at 298 K of template **7** (top), reference of the copy **14** (middle) and the recovered fraction after purification (bottom). **(b)** Expansions of key regions and the corresponding proton signals assigned in the structures.

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[S4] D. Núñez-Villanueva, M. A. Jinks, J. Gómez Magenti and C. A. Hunter. *Chem. Commun.*, **2018**, *54*, 10874.

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