Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting information

Hybrid ligands with calixarene and thiodigalactoside groups and their cytotoxicity and galectin binding

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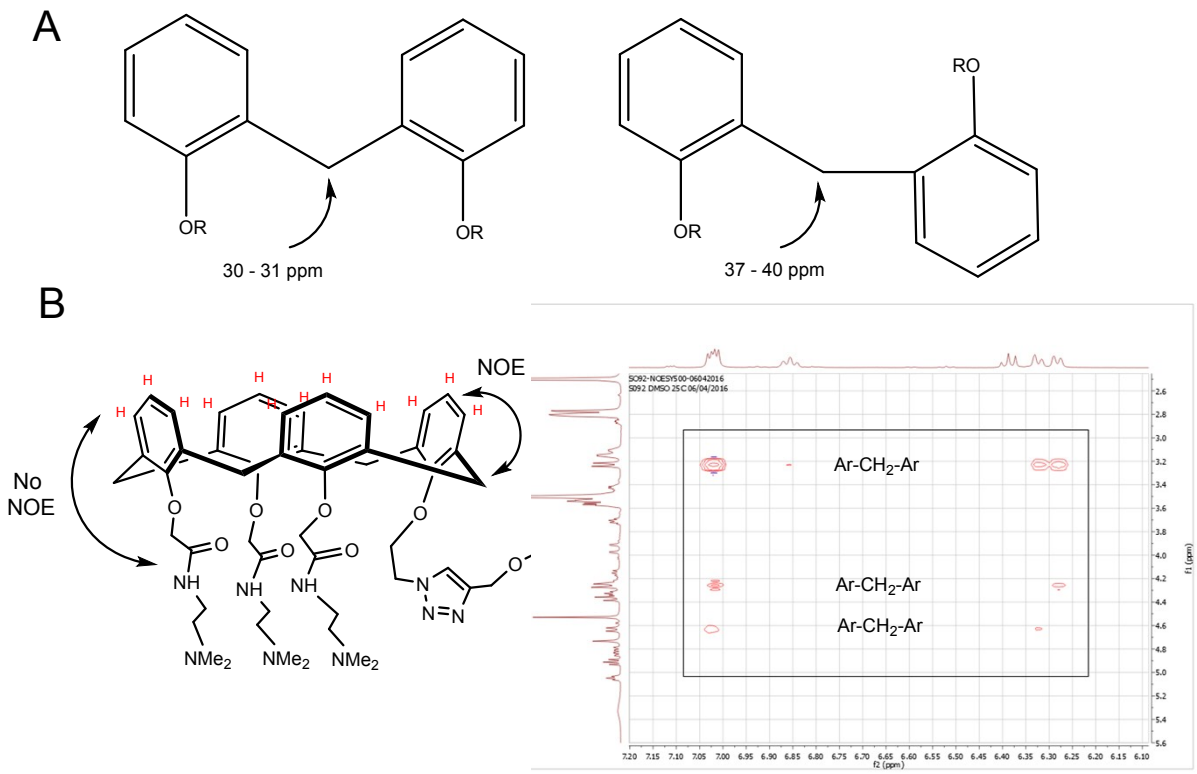


Figure S1. A: ^{13}C-NMR rule for the assignment of the orientation of neighboring aromatic rings in calixarenes; B: cross peaks encountered by aromatic protons (in red) of calixarene in NOESY. For clarity, other protons are not shown. H's are not connected in the structure

Synthetic procedures and compound characterization

Preparation of crude 4: 3-(4-phenyl-1H-1,2,3-triazol)-3'-azido-thiodigalactoside

NaOMe (135 mg, 2.5 mmol) was added to a solution of **18** (120 mg, 0.16 mmol) in CH_3OH (5.0 mL) and the mixture was stirred for 6 h at room temperature. The solution was neutralized with DOWEX-H^+ resin, filtered, and evaporated. Crude **4** (85 mg) was obtained as a white solid and used in the next step without further purification.

25,26,27,28-tetrahydroxycalix[4]arene (6)

P-tert-Butylcalix[4]arene (5.0 g, 7.70 mmol) was suspended in toluene (50 mL), then $AlCl_3$ (8.2 g, 61.60 mmol) and phenol (5.8 g, 61.60 mmol) was added. The reaction mixture was stirred overnight, and then 1 N HCl (50 mL) was added slowly. After 10 min the reaction mixture was extracted by CH_2Cl_2 (3 × 100 mL), and the combined organic phase was concentrated. CH_3OH (50.0 mL) was added to the residues to make a slurry. After filtration, compound **6** (3.2 g, 7.60 mmol, 98 %) was collected as a white solid and used for next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.17 (d, *J* = 1.2 Hz, 4H, OH), 7.04 (dd, J = 7.5, 1.2 Hz, 8H, Ar-H), 6.71 (td, J = 7.6, 1.2 Hz, 4H, Ar-H), 4.23 (m, 4H, CH_2), 3.51 (m, 4H, CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.77, 128.98 , 128.24 , 122.29 , 122.24 , 31.88 , 31.71 , 31.46 , 31.31 .

HRMS (EI, m/z): calculated for$C_{28}H_{24}O_4H^+$([M+H]$^+$): 425.1747, found 425.1729.

25,26,27-Trihydroxy-28-(2'-azidoethoxy) calix[4]arene (7)

To **6** (2.8 g, 6.70 mmol) dissolved in CH_3CN (50 mL) NaOMe (434 mg, 8.03 mmol) was added. The mixture was refluxed for 30 min and 1-azido-2-iodoethane (1.3 g, 6.68 mmol) was added. The mixture was refluxed overnight and the reaction was monitored by TLC. An additional amount of NaOMe (200 mg, 3.70 mmol) was added and the mixture was refluxed for 1 day. Then the mixture was worked up by evaporating the solvent. Then CH_2Cl_2 (100 mL) was added to the residue and the mixture was washed with water (3 × 50 mL). After drying with Na_2SO_4, the organic solvent was evaporated and the residue was purified by silica column chromatography using hexanes: EtOAc = 5:1. The target compound **7** was obtained as a white solid (1.3 g, 2.60 mmol, 39%) and it was used in the next step. Unreacted starting material (1.2 g, 2.80 mmol, 42%) was also retrieved.

^{1}H NMR (400 MHz, CDCl3) δ 9.64 (s, 1H, OH), 9.14 (s, 2H, OH), 7.10 (d, J = 7.7 Hz, 2H, Ar-H), 7.09 – 7.00 (m, 6H, Ar-H), 6.90 (dd, J = 7.9, 7.3 Hz, 1H, Ar-H), 6.70 (tt, J = 7.5, 1.2 Hz, 3H, Ar-H), 4.40 (d, J = 13.0 Hz, 2H, Ar_2CH_2), 4.30 (d, J = 13.8 Hz, 2H, Ar_2CH_2), 4.25 (dd, J = 5.6, 4.3 Hz, 2H, OCH_2), 4.05 (dd, J = 5.6, 4.3 Hz, 2H, CH_2N_3), 3.51 (d, J = 2.5 Hz, 2H, Ar_2CH_2), 3.48 (d, J = 3.2 Hz, 2H, Ar_2CH_2).

^{13}C NMR (101 MHz, $CDCl_3$) δ 151.04 , 150.92 , 149.28 , 134.15 , 129.66 , 129.06 , 128.91 , 128.80 , 128.54 , 128.49 , 128.24 , 126.62 , 122.08 , 121.07 , 74.42 , 51.38 , 32.01 , 31.45 .

HRMS (EI, m/z): calculated for $C_{30}H_{27}N_3O_4H^+$([M+Na]$^+$): 516.1894, found 516.1851.

25,26,27-Tri[(ethoxycarbonyl)methoxy]-28-(2'-azidoethoxy) calix[4]arene (8)

To a solution of **7** (1.3 g, 2.60 mmol) in CH_3CN (50 mL) K_2CO_3 (1.8 g, 13.04 mmol) was added. The mixture was stirred for 2 h and then an excess of ethyl bromoacetate (2.6 g, 15.82 mmol) was added. The mixture was heated to 85 °C and refluxed overnight while monitoring the reaction by TLC. After removing the solvent, the residue was taken up in CH_2Cl_2 (100 mL) and then the organic part was washed with H_2O (3 × 100 mL). After separation and drying with Na_2SO_4, the organic layer was evaporated and the impure compound was purified by column chromatography (PE: EtOAc = 5:1 → 4:1). The product (1.7 g, 2.2 mmol) was obtained as colorless oil. According to ^{1}H NMR, COSY, HSQC and HMBC, this sample contains approx. 75% of the compound **8** (corresponding to 1.275 g, 1.7 mmol, 65 %), which is the "cone" conformation and 25% of other isomers. The solid was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$, extracted from HSQC) δ 6.84 (dd, J = 6.9, 1.5 Hz, 4H), 6.75 (ddd, J = 8.2, 6.6, 4.0 Hz, 2H), 6.53 – 6.44 (m, 6H), 6.30 – 6.22 (m, 1H), 4.82 (s, 1H), 4.80 (d, J = 8.7 Hz, 3H), 4.69 (d, J = 16.0 Hz, 2H), 4.63 (d, J = 13.5 Hz, 2H), 4.52 (d, J = 16.1 Hz, 2H), 4.33 – 4.19 (m, 7H), 4.15 (t, J = 6.4 Hz, 2H), 3.96 (t, J = 6.4 Hz, 2H), 3.25 (d, J = 13.0 Hz, 3H), 3.20 (s, 1H), 1.31 (td, J = 7.1, 5.2 Hz, 9H).

HRMS (EI,m/z): calculated for $C_{42}H_{45}N_3O_{10}Na^+$ ([M+Na]$^+$):774.2997, found 774.2963.

25,26,27-Tris-N-(N,N-dimethyl-2-aminoethyl)carbamoylmethoxy-28-(2'-azidoethoxy) calix[4]arene (12)

To the compound **8** (350 mg) was added under nitrogen N,N-dimethylethylenediamine (7.0 mL). The mixture was stirred for 24 h at 50 °C. The excess of N,N-dimethylethylenediamine was removed by evaporation under reduced pressure. The residue was dissolved in CH_3CN (20 mg/mL) and purified by preparative HPLC using the standard protocol. Fractions containing the product (t_R = 35 min, broad peak) were pooled and the water was removed by freeze-drying to obtain the pure compound **12** as a clear oil (160 mg, 0.18mmol, 39%).

^{1}H NMR (500 MHz, DMSO-d6) δ 6.94 (dd, J = 7.6, 4.3 Hz, 4H), 9.99 – 9.95 (m, 3H), 8.52 (q, J = 7.1, 6.6 Hz, 3H), 6.80 (t, J = 7.5 Hz, 2H), 6.40 (t, J = 7.5 Hz, 2H), 6.36 – 6.28 (m, 3H), 4.66 (d, 2H), 4.59 (d, J = 13.5 Hz, 2H), 4.40 (d, J = 14.0 Hz, 2H), 4.32 (d, J = 13.4 Hz, 2H), 4.28 (d, J = 14.0 Hz, 2H), 4.08 (d, J = 6.2 Hz, 2H), 3.89 (t, J = 6.2 Hz, 2H), 3.55 (dt, J = 13.0, 6.4 Hz, 6H), 3.23 (d, J = 5.7 Hz, 2H), 3.20 (d, J = 6.5 Hz, 8H), 2.81 (d, J = 6.9 Hz, 18H), .

^{13}C NMR (126 MHz, DMSO-d6, extracted from HSQC) δ 129.30, 123.07, 123.03, 128.39, 74.23, 30.90, 74.03, 30.91, 74.03, 71.75, 51.18, 34.42, 30.91, 55.87, 42.50, 39.65

HRMS (EI,m/z): calculated for $C_{48}H_{63}N_9O_7H_3^{3+}$ ([M+3H]$^{3+}$): 880.5069, found 880.5049.

Tetra(ethyleneglycol)-di(2-propinyl) (9)

To a solution of tetraethylene glycol (8.65 mL, 0.050 mol) in THF (50 mL) was added NaH (60% oil dispersion, 2.40 g, 0.060 mol) portionwise at 0 °C, and the reaction was stirred at 0 °C for 1 h. Propargyl

bromide (7.14 g, 0.060 mol) was added to the reaction solution, and the reaction was stirred at room temperature overnight. The reaction was quenched by adding ice cold water, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL), washed with brine, dried over Na_2SO_4. After filtration, the solvent was removed *in vacuo*, the residue was purified by column chromatography (hexanes: acetone = 4: 1→ 3: 1) to afford dipropargylated compound **9** as a colorless oil (2.2 g, 0.008 mmol, 16%). In the meantime, monoproparylated tetraethylene glycol (5.3g, 0.023 mmol, 46%) as a byproduct was isolated for the synthesis of other likers.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (d, J = 2.4, 4H), 3.75 – 3.57 (m, 16H), 2.41 (t, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 79.64, 74.44 , 70.50, 70.56, 70.38, 69.09 , 58.37.

HRMS (EI, m/z): calculated for $C_{14}H_{22}O_5Na^+$ ($[M+Na]^+$): 293.1359, found 293.1349.

Monopropargyl(tetraethylene glycol) tosylate

Monopropargyl-tetraethylene glycol (2.1 g, 0.009 mmol) obtained from the preparation of compound **9** was dissolved in CH_2Cl_2 (15 mL) and then p-toluenesulfonyl chloride (2.2 g, 11.5 mmol) and trimethylamine (2.5 mL, 0.018 mmol) were added. The reaction was stirred at r.t. for 16 h. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (hexanes: EtOAc = 1: 1) to afford glycol tosylate as a yellow oil (2.9 g, 0.0075 mmol, 83 % yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80(d, 2H), 7.36 – 7.30 (m, 2H), 4.19 (d, 2H), 4.18 – 4.14 (m, 2H), 3.70 – 3.51 (m, 14H), 2.45 (s, 3H), 2.40 (t, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 144.91, 133.19, 129.95 , 128.13 , 77.36 , 74.65 , 70.91 , 70.75 , 70.70 , 70.56 , 69.39 , 69.27 , 68.84 , 58.55 , 21.79 .

Octa(ethyleneglycol)-di(2-propinyl) (10)

NaH (60% oil dispersion, 140 mg, 3.50 mmol) was added to a solution of monoproparyl-tetraethylene glycol (270 mg, 1.16 mmol) in THF (10.0 mL) and stirred for 1 h, followed by adding a solution of monopropargyl(tetraethylene glycol) tosylate (448 mg, 1.16 mmol) in THF (2.0 mL). The resulting mixture was stirred at r.t. for 24 h, and quenched by adding ice cold water, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL), washed with brine, dried over Na_2SO_4. After filtration, the solvent was removed *in vacuo*, the residue was purified by column chromatography (EtOAc: CH_3OH = 10: 1) to afford dipropargylated compound **10** as a colorless oil (462 mg, 1.03 mmol, 89 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 – 3.55 (m, 32H), 4.17 (d, J = 2.3 Hz, 4H), 2.41 (t, 1H).

^{13}C NMR (101 MHz, CDCl3) δ 79.71 , 74.64 , 70.64 , 70.61 , 70.60 , 70.43 , 69.15 , 58.44 .

HRMS (EI, m/z):calculated for $C_{22}H_{38}O_9Na^+$ ($[M+Na]^+$): 469.2408, found: 469.2420.

4,7,10,13,16,19,22,25,28,31,34,37,40-tridecaoxatritetraconta-1,42-diyne (11)

NaH (60% oil dispersion,340 mg, 8.50 mmol) was treated to a solution of tetraethylene glycol (276 mg, 1.42 mmol) in THF (10.0 mL) and stirred for 1 h, followed by adding a solution of monopropargyl(tetraethylene glycol) tosylate (1.2 g, 3.11 mmol) in THF (2.0 mL). The resulting mixture was stirred at r.t. for 24 h, and quenched by adding ice cold water, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL), washed with brine, dried over Na_2SO_4. After filtration, the solvent was removed *in vacuo*, the residue was purified by column chromatography (EtOAc: CH_3OH = 10: 1) to afford dipropargylated compound **11** as a colorless oil (620 mg, 1.00 mmol, 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (d, J = 2.4 Hz, 4H), 3.69 – 3.60 (m, 48H), 2.41 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 79.63, 74.50, 70.57 , 70.55 , 70.53, 70.37 , 69.07 , 58.36 .

HRMS (EI, m/z): calculated for $C_{30}H_{54}O_{13}H^+$ ($[M+H]^+$): 623.3637, found: 623.3691.

Bis-{3-deoxy-3[4-(phenyl)-1H-1,2,3-triazol-1-yl]-β-D-galactopyranosyl} sulfane (19)

Compound **18** (10 mg, 0.013 mmol) and phenylacetylene (1.43µl, 0.013 mmol) were dissolved in CH_3CN (2.0 mL) and then CuI (2.50 mg, 0.013 mmol) was added to the solution. The resulting mixture was heated under microwave irradiation at 80 °C for 90 min. After complete conversion of the starting material according to TLC monitoring, the mixture was concentrated *in vacuo*, and then CH_2Cl_2 (5.0 mL) was added. A clear solution was obtained after centrifugation, which was concentrated *in vacuo.* The residue (12 mg) was dissolved in CH_3OH (5 mL) and NaOMe (135 mg, 2.5 mmol) was added. The mixture was stirred for 6 h at room temperature and was neutralized with DOWEX-H^+ resin, filtered, and evaporated. The crude was purified by preparative HPLC using the standard protocol. Fractions containing the product (t_R = 30 min) were pooled and the buffer was removed by freeze-drying to obtain compound **19** as a white solid (2.0 mg, 0.003 mmol, 23 %).

^{1}H NMR (500 MHz, D_2O) δ 8.55 (s, 2H, CH triazole), 7.87 (d, J = 7.8 Hz, 4H, Ar-H), 7.55 (t, J = 7.5 Hz, 4H, Ar-H), 7.48 (t, J = 7.5 Hz, 2H, Ar-H), 5.17 (d, J = 9.7 Hz, 2H, H1), 5.04 (d, J = 10.9 Hz, 2H, H3), 4.49 (t, J = 10.2 Hz, 2H, H2), 4.29 (m, 2H, H4), 4.08 (t, J = 6.2 Hz, 2H, H5), 3.87 (dd, J = 12.0, 7.7 Hz, 2H, H6a), 3.79 (dd, J = 12.0, 4.4 Hz, 2H, H6b).

^{13}C NMR (126 MHz, D_2O, extracted from HSQC) δ 121.43, 126.38, 129.92, 129.53, 84.35, 66.97, 67.38, 68.03, 79.66, 60.41, 60.89

HRMS (EI, m/z) calculated for $C_{28}H_{32}N_6O_8SNa^+$ ($[M+Na]^+$): 635.1895, found 635.1898.

Preparation of compound 20

The compound **18** (30 mg, 0.039 mmol) and compound **11** (42 mg, 0.067 mmol) were dissolved to CH_3CN (2.0 mL) and then CuI (7.50 mg, 0.039 mmol) was added to the solution. The resulting mixture was heated under microwave irradiation at 80 °C for 90 min. After complete conversion of the starting material according to TLC monitoring, the mixture was concentrated *in vacuo*, and then CH_2Cl_2 (5.0 mL) was added. A clear solution was obtained after centrifugation, which was concentrated *in vacuo*. The corresponding compound was obtained by silica chromatography (CH_2Cl_2: CH_3OH = 10: 1→ 5: 1), which was dissolved in

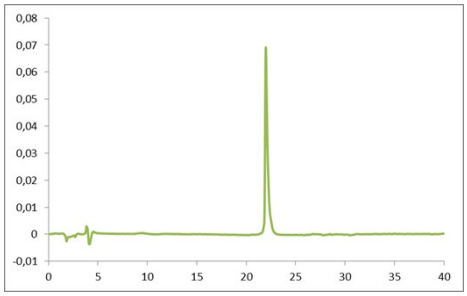
CH_3OH (5 mL) and NaOMe (135 mg, 2.5 mmol) was added. The mixture was stirred for 6 h at room temperature. The solution was neutralized with DOWEX-H^+ resin, filtered, and evaporated. The crude was purified by preparative HPLC using the standard protocol. Fractions containing the product (t_R = 30 min) were pooled and the buffer was removed by freeze-drying to obtain compound **20** as a clear oil (3.0 mg, 0.002 mmol, 5.0 %).

^{1}H NMR (500 MHz, D2O) δ 8.55 (s, 1H), 8.26 (s, 1H), 7.91 – 7.84 (m, 2H), 7.56 (t, J = 7.6 Hz, 2H), 7.49 (d, J = 7.4 Hz, 1H), 5.14 (dd, J = 9.7, 6.9 Hz, 2H), 5.02 (ddd, J = 13.7, 10.6, 3.0 Hz, 2H), 4.74 (s, 2H), 4.55 – 4.39 (m, 2H), 4.30 – 4.21 (m, 4H), 4.06 (td, J = 8.5, 4.3 Hz, 2H), 3.86 (ddd, J = 12.6, 7.8, 5.2 Hz, 2H), 3.81 – 3.62 (m, 50H).

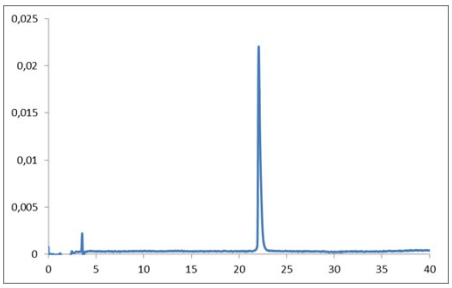
^{13}C NMR (126 MHz, D_2O, extracted from HSQC) δ 121.44, 124.43, 125.77, 125.77, 129.35, 129.35, 128.97, 84.27, 84.29, 66.92, 66.90, 63.05, 69.52, 66.80, 68.02, 57.65, 67.96, 79.66, 60.91, 60.93, 68.90, 69.53.

Analytical HPLC for new compounds

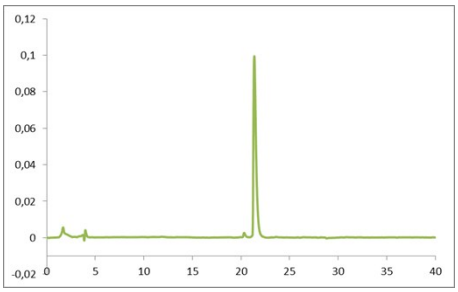
HPLC of Compound **5a**



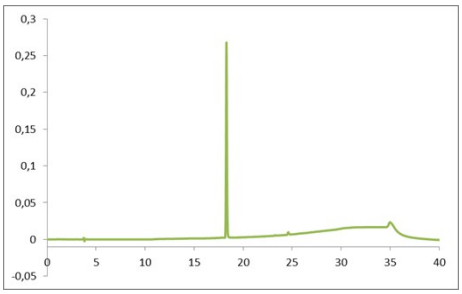
HPLC of Compound **5b**



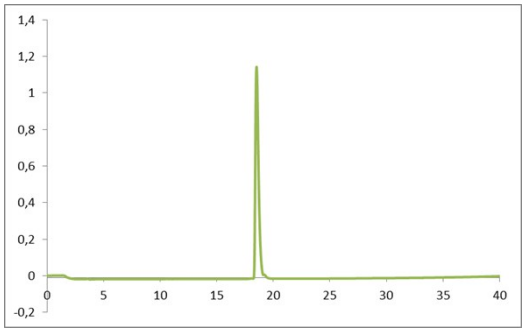
HPLC of Compound **5c**



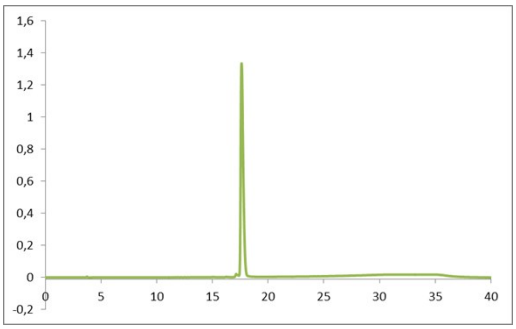
HPLC of Compound **12**



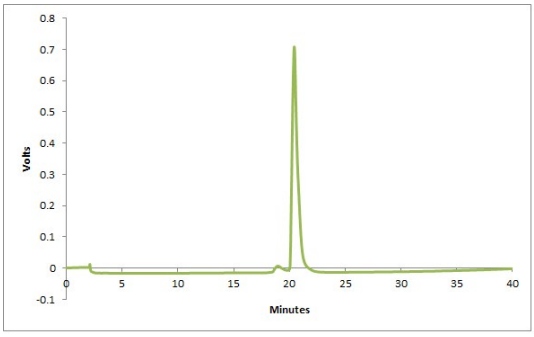
HPLC of Compound **19**



HPLC of Compound **20**



HPLC of hybrid **1**



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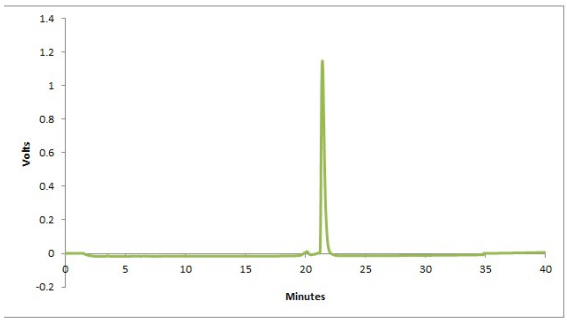
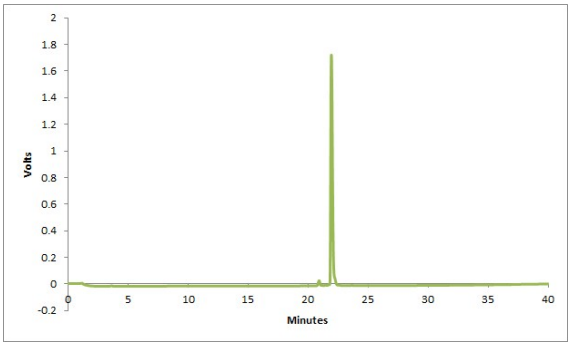
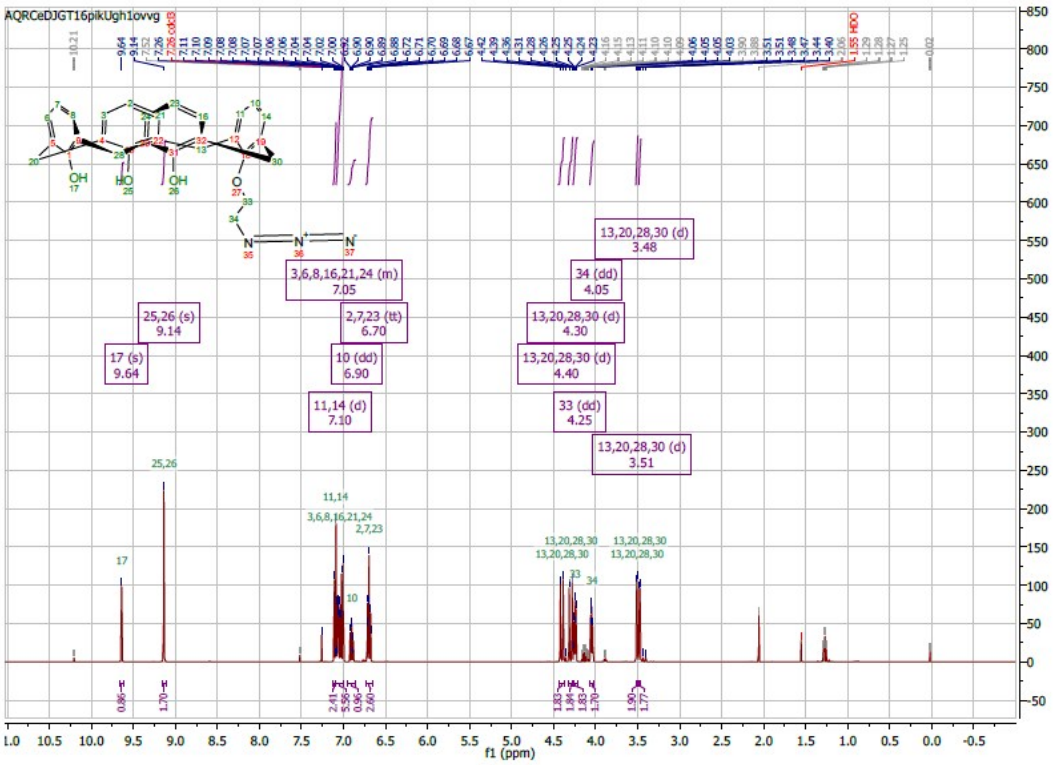


HPLC of hybrid **3**

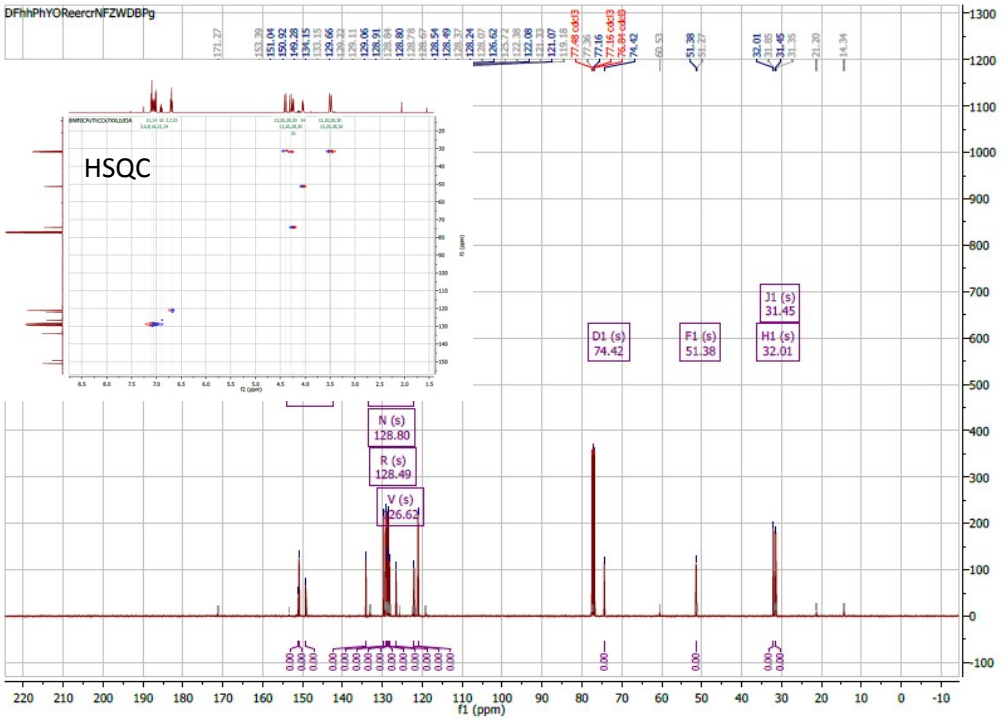


NMR spectra of new compounds

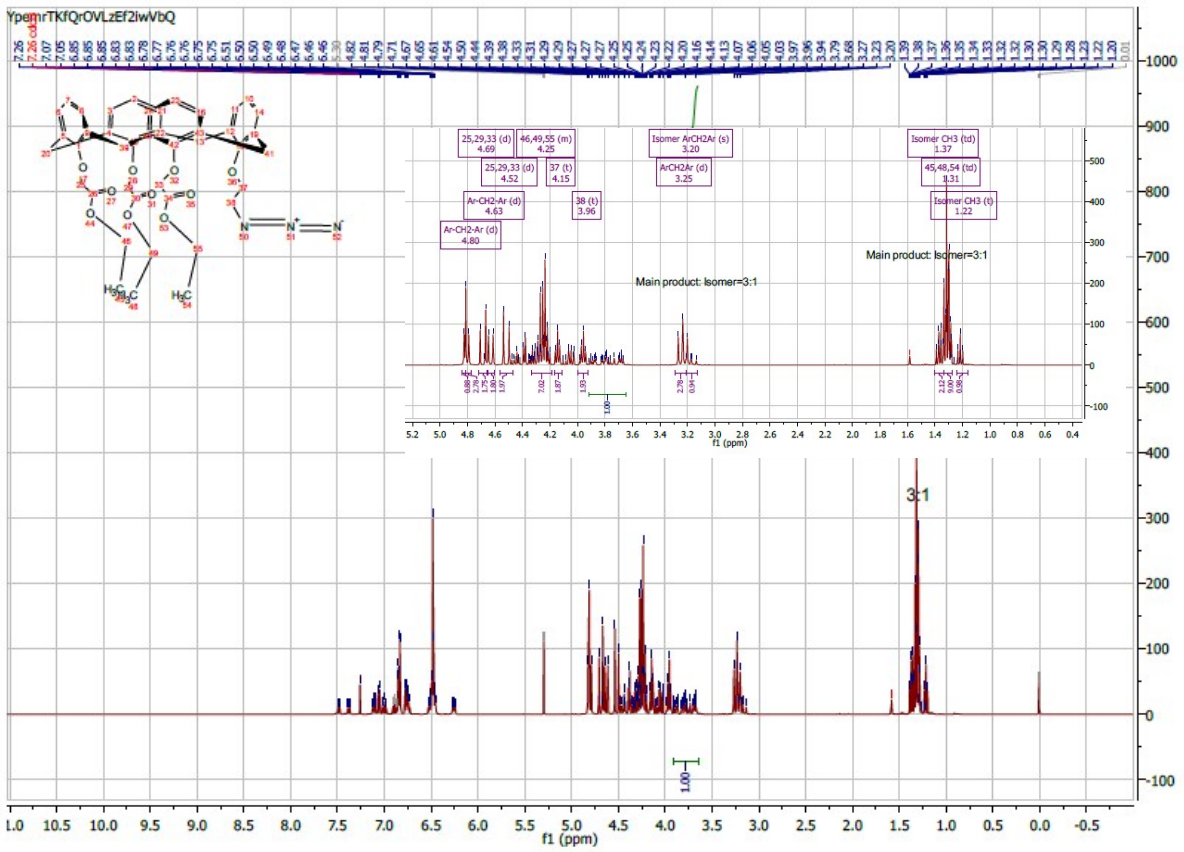
^{1}H NMR of Compound **7**



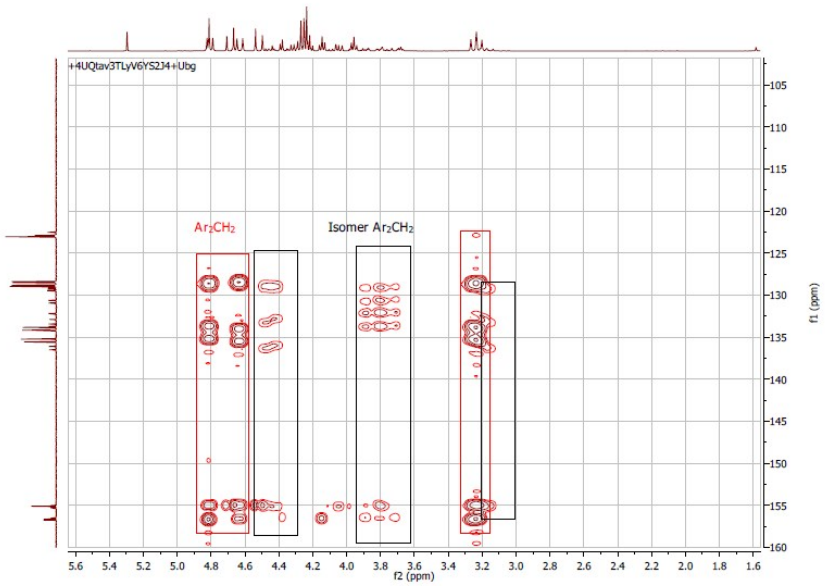
^{13}C NMR of Compound **7**



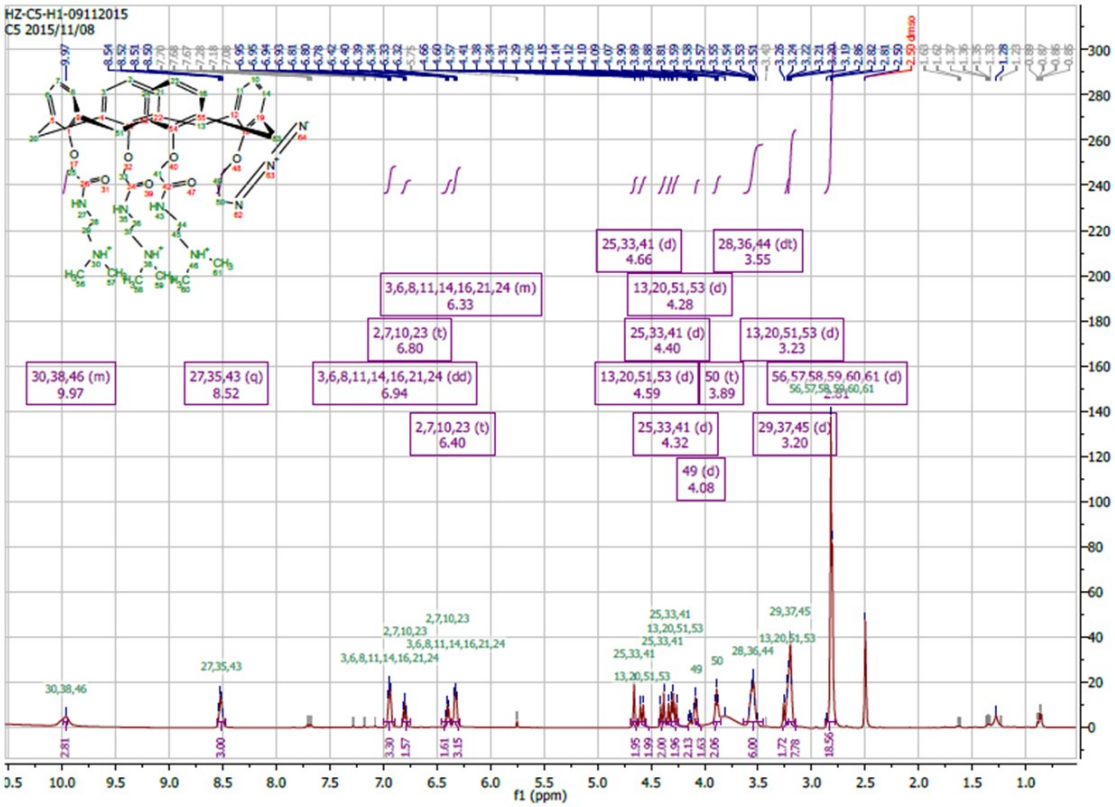
^{1}H NMR of Compound **8** with Isomer



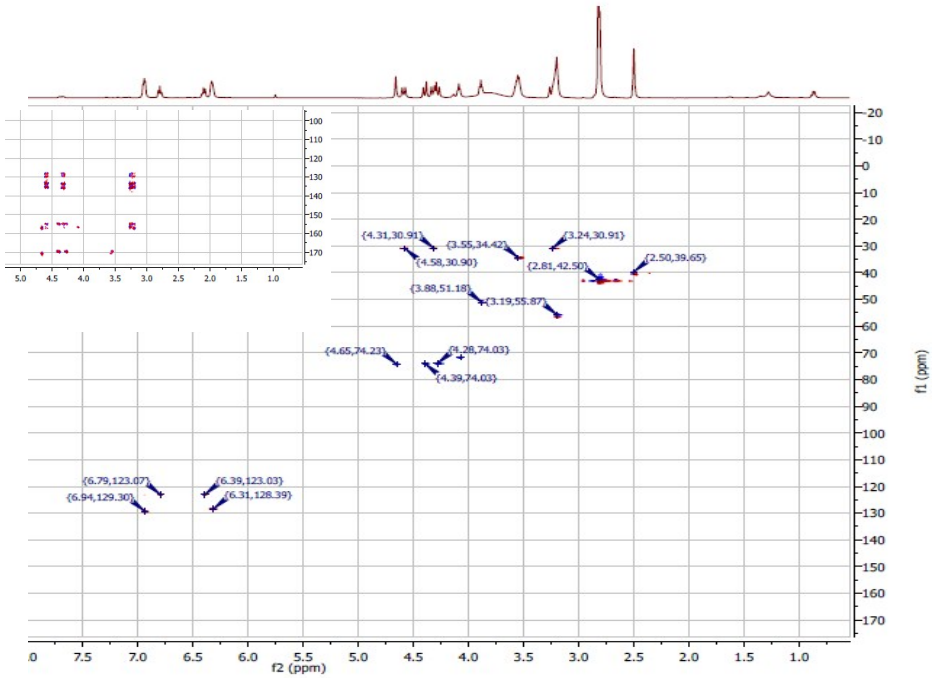
HMBC of Compound **8** with Isomer



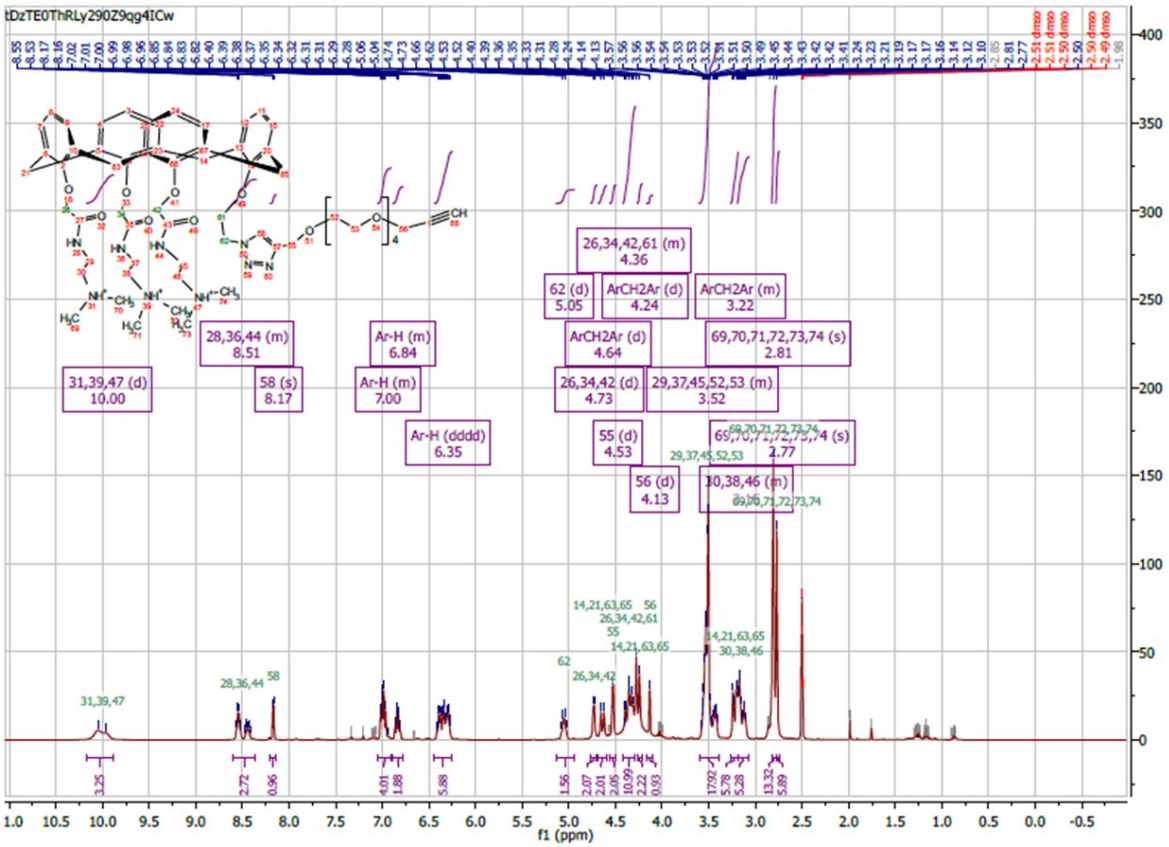
^{1}H NMR of Compound **12**



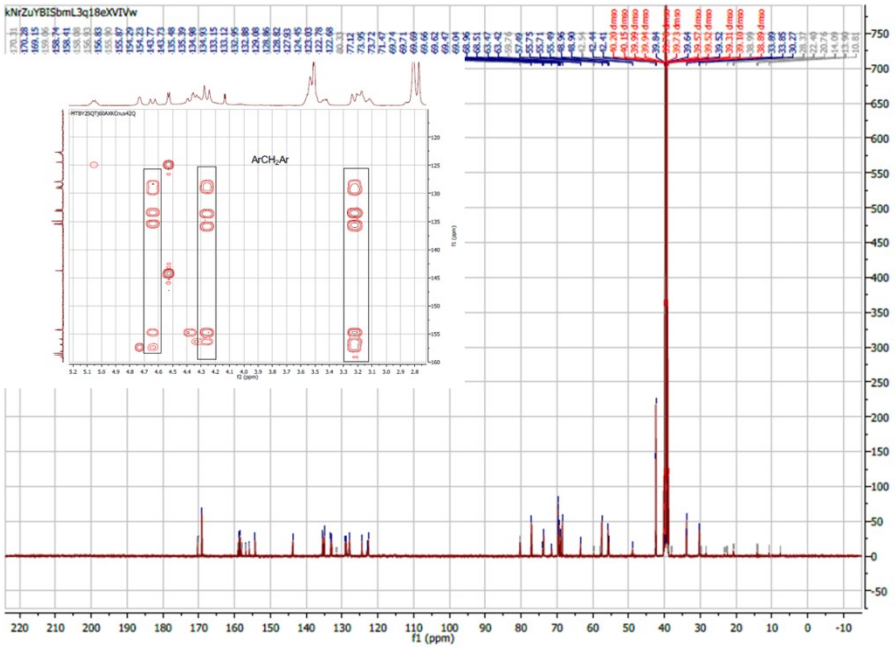
HSQC and HMBC of Compound **12**



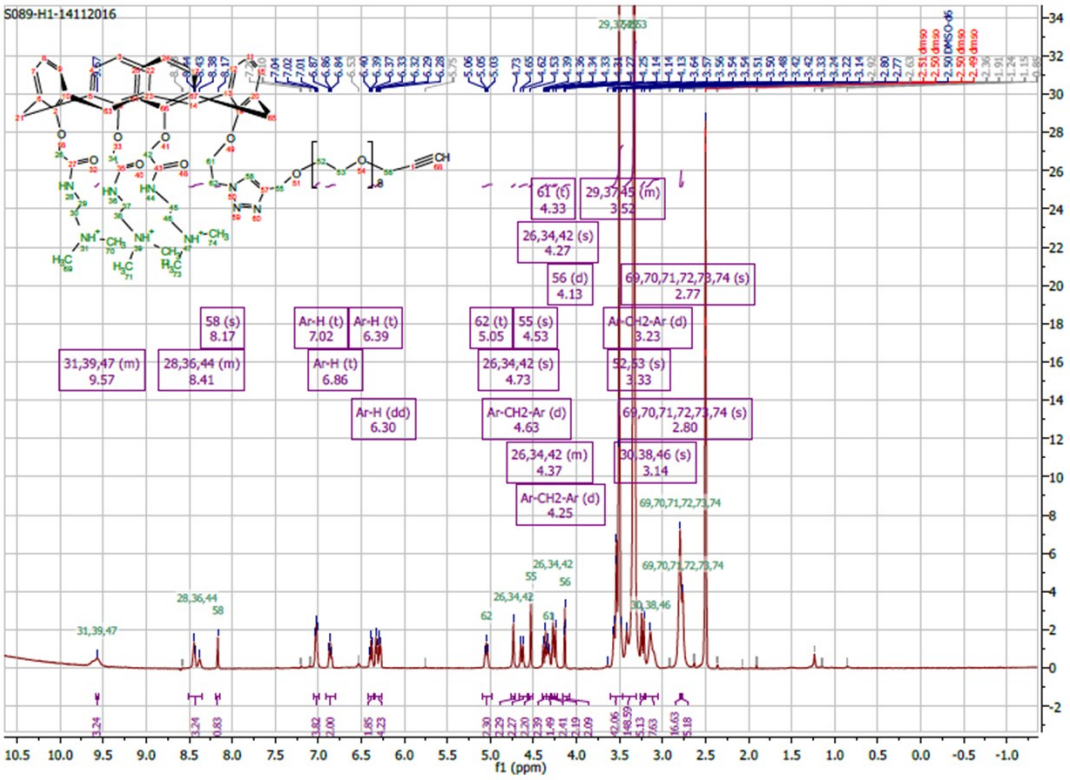
^{1}H NMR of Compound **5a**



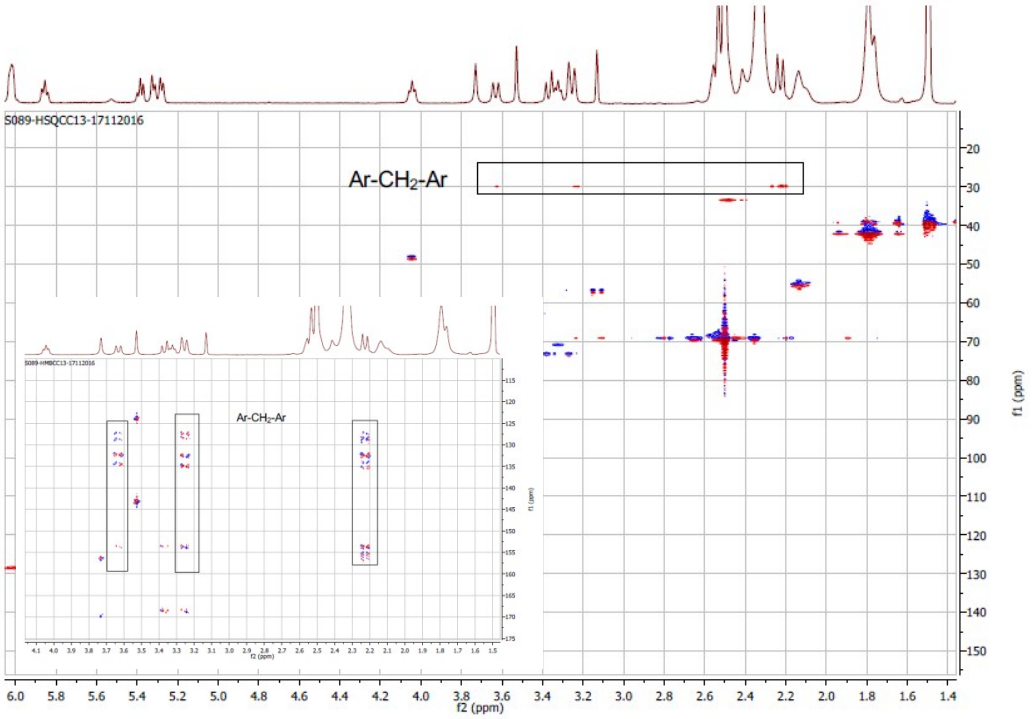
^{13}C NMR and HMBC of Compound **5a**



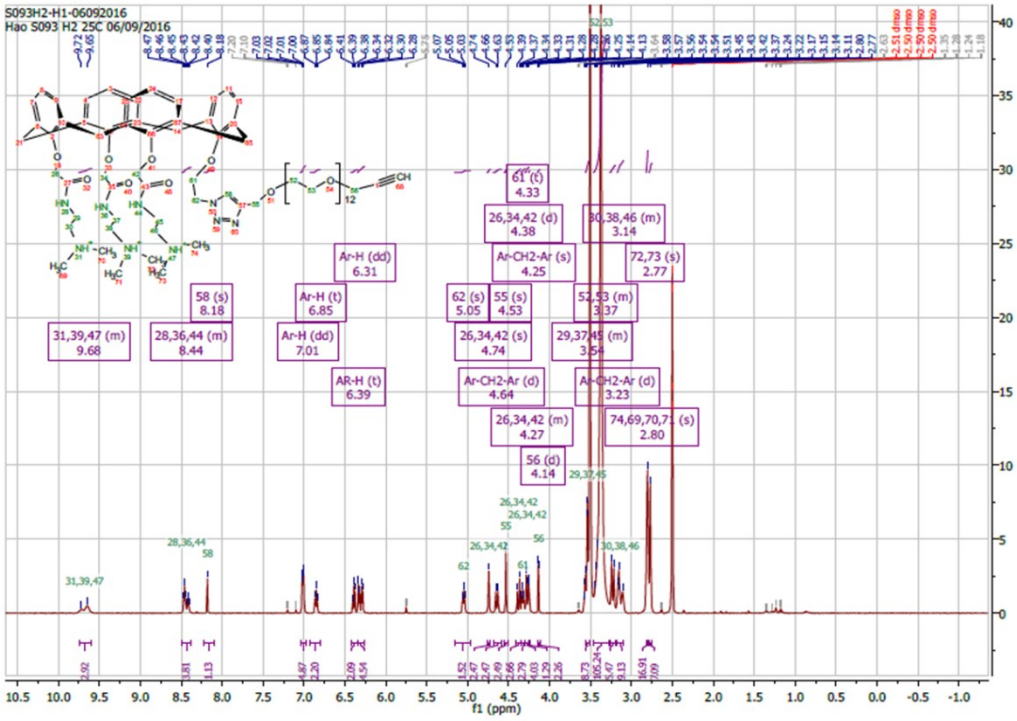
^{1}H NMR of Compound **5b**

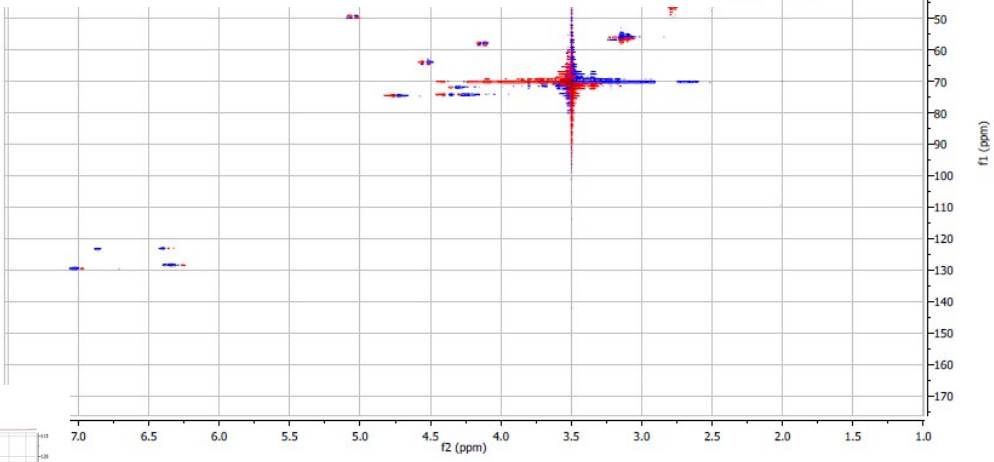


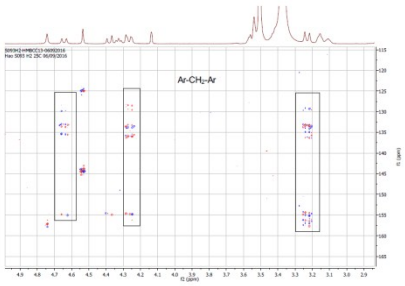
HSQC and HMBC of Compound **5b**



^{1}H NMR of Compound **5c**

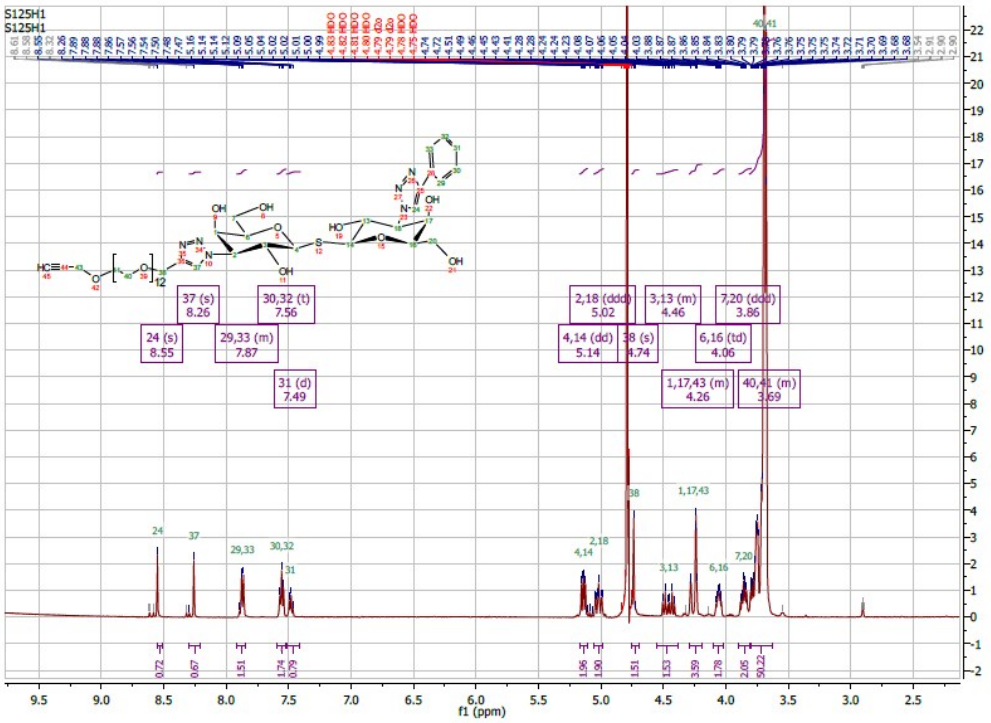




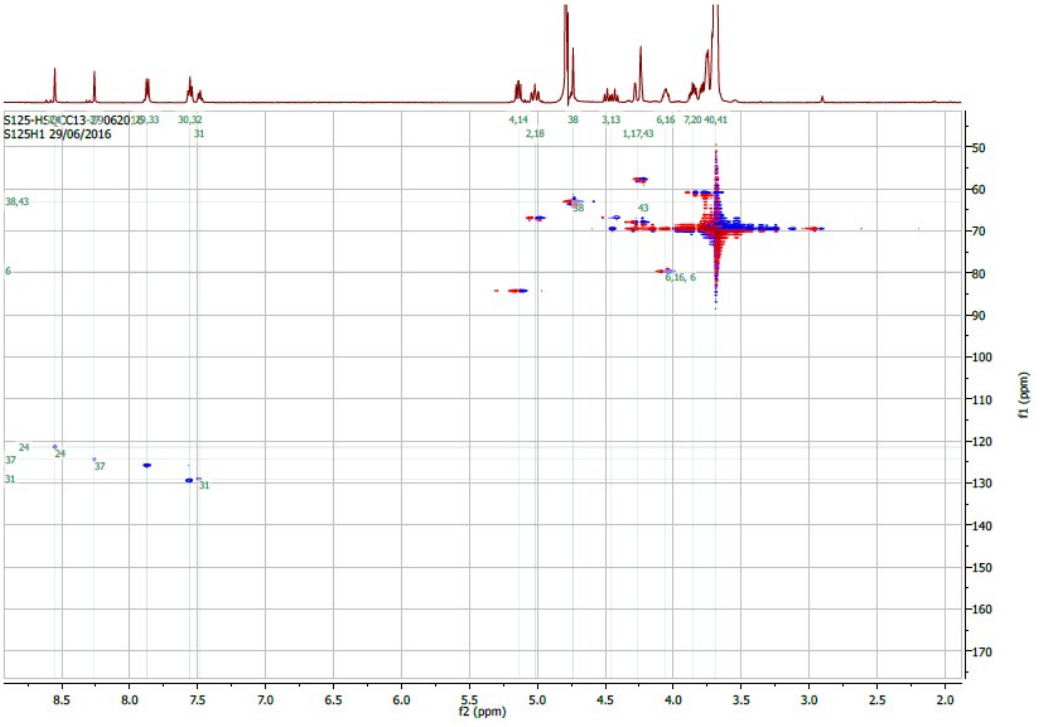


HSQC and HMBC of Compound **5c**

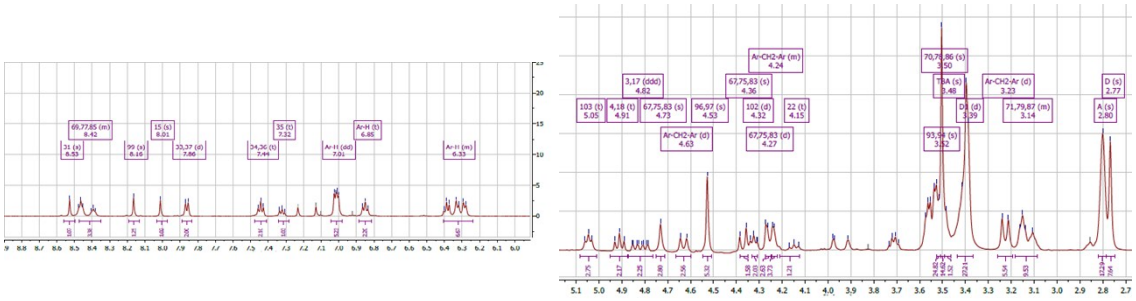
^{1}H NMR of Compound **20**



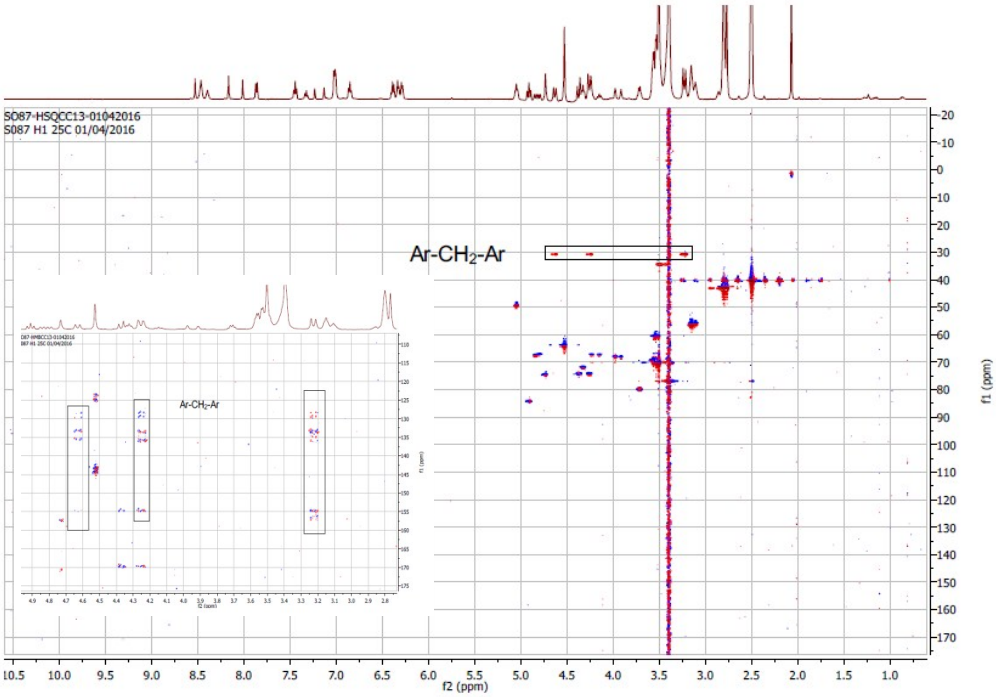
HSQC of Compound **20**



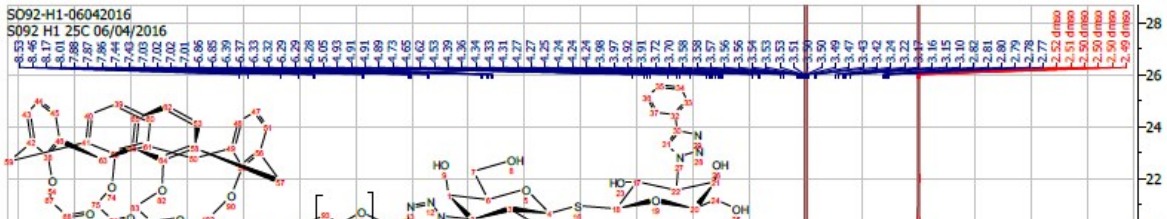
^{1}H NMR of hybrid **1**

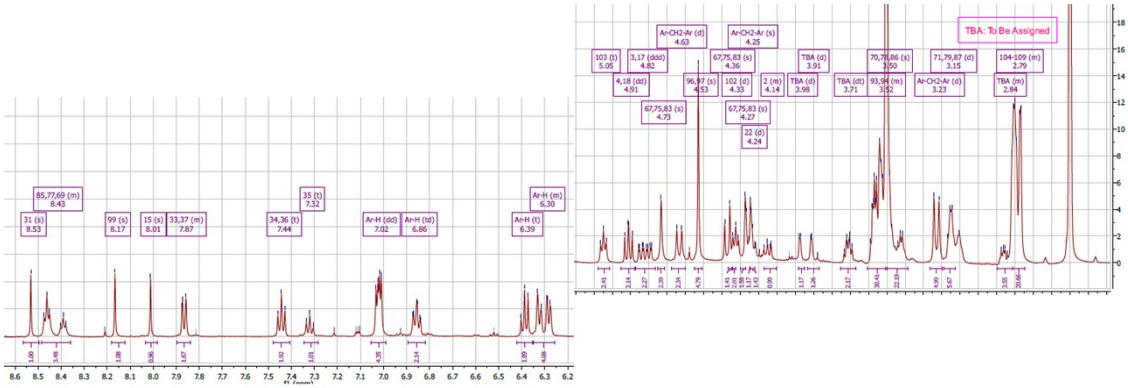


HSQC and hybrid **1**

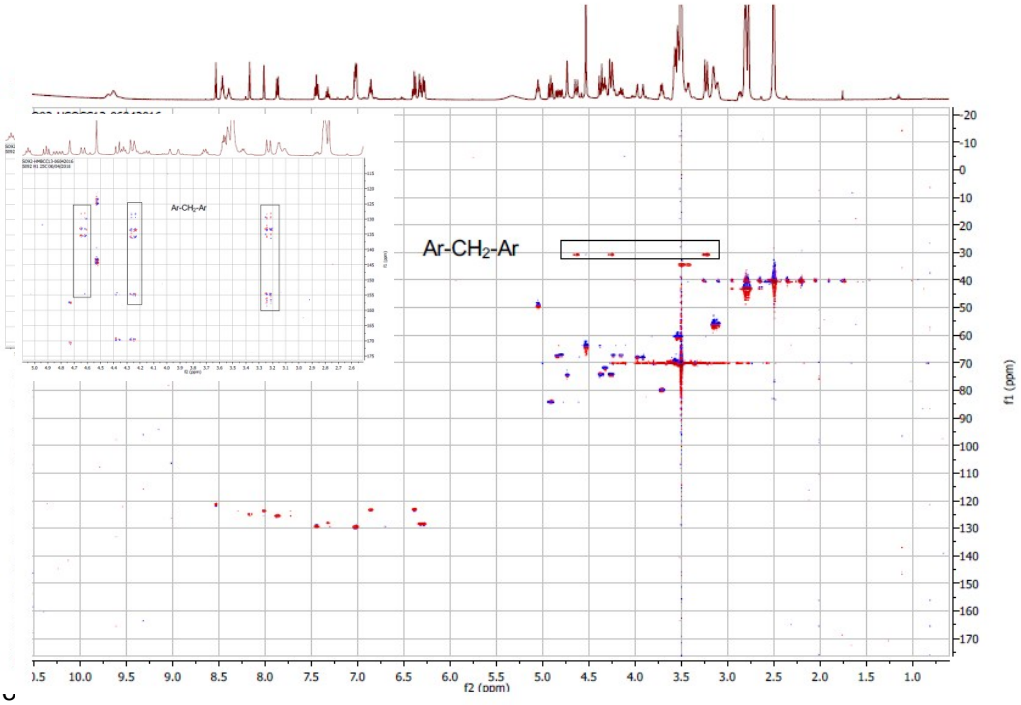


^{1}H NMR of hybrid **2**

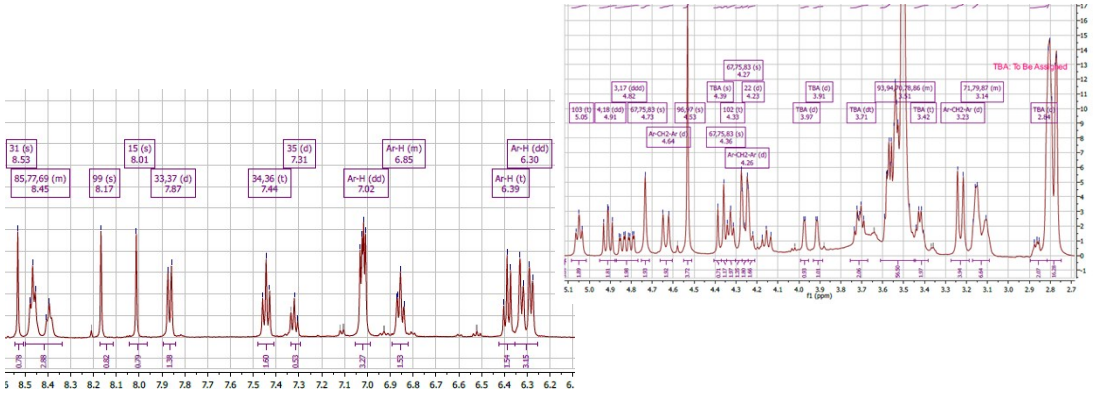




HSQC and HMBC of hybrid **2**



^{1}H NMR S



HSQC of hybrid **3**