Supporting information

Highly Reactive Bis-Cyclooctyne-Modified Diarylethene for SPAAC-mediated Cross-Linking

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1. General Information.

1.1 Solvents and reagents

Tetrahydrofuran was dried over sodium wire and distilled from a mixture of calcium hydride and lithium aluminium hydride with triphenylmethane as an indicator. Diethyl ether was distilled from a mixture of calcium hydride and lithium aluminium hydride. Dichloromethane, methanol, hexane, acetonitrile and toluene were distilled from calcium hydride. Petroleum ether refers to the fraction of petroleum ether boiling in the range of 40-60 °C. All other reagents and solvents were used as supplied, without prior purification.

1.2 Chromatography

Flash column chromatography was carried out using Kieselgel 60 silica (230-400 mesh) with distilled solvents under a positive pressure of nitrogen. TLC was carried out on glass Merck Kieselgel 60 F254 plates, visualised by ultraviolet irradiation (254 and 365 nm)

1.3 NMR Spectroscopy

NMR spectra were recorded on a Bruker Ultrashield 400 (^{1}H: 400 MHz and ^{13}C: 101MHz) or 500 (^{1}H: 500 MHz and ^{13}C: 126 MHz) spectrometers. Chemical shifts are quoted in ppm and rereferenced to the residual non-deuterated solvent peak, and are reported based on appearance rather than interpretation. ^{1}H spectra are reported as follows: δ_H (*spectrometer frequency, solvent*): *ppm* (*no. of protons, multiplicity, J-coupling constant*(*s*), *assignment*). ^{13}C spectra are reported as follows: δ_C (*spectrometer frequency, solvent*)*: ppm* (*assignment*). Spectral assignment was aided by the results of DEPT, COSY, HMBC and HSQC experiments where appropriate.

1.4 IR Spectroscopy

IR spectra were recorded neat on a Perkin Elmer Spectrum One FT-IR spectrophotometer fitted with an attenuated total reflectance (ATR) sampling accessory. Absorption maxima are reported in wavenumbers (cm^{-1}).

1.5 High Resolution Mass Spectrometry

Accurate masses were recorded on a Waters LCT Premier Time of Flight mass spectrometer or Micromass Quadrupole-Time of Flight mass spectrometer. Reported mass values are within the error limits of ±5 ppm.

1.6 Liquid Chromatography-Mass Spectrometry

LCMS chromatograms were obtained on an Agilent 1200 series LC using a Supelcosil ABZ+PLUS column (33 mm x 4.6 mm, 3 μm), together with an ESCi Multi-Mode IonisationWaters ZQ spectrometer using Mass Lynx 4.1 software. Chromatograms were monitored by absorbance using diode array detection at a wavelength range of 190-600 nm.

1.7 High-performance Liquid Chromatography

Analytical HPLC chromatograms were obtained on an Agilent 1260 Infinity (Supelcosil C18 column), eluting with a linear gradient of MeCN (with 0.05% TFA) in water (with 0.1% TFA) over 15 minutes. Semi-preparative HPLC was run on an Agilent 1260 Infinity (Supelcosil C18 column), eluting with a linear gradient of MeCN (with 0.05% TFA) in water (with 0.1% TFA) over 20 minutes. Retention times are reported to the nearest 0.01 min.

1.8 Melting points

All melting points were measured on a Büchi B545 melting point apparatus and are uncorrected.

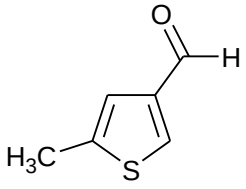
1.9 Naming and numbering of compounds

Where given, systematic compound names are those generated by ChemBioDraw Ultra 13.0 following IUPAC conventions. The numbering of atoms for spectral assignment purposes is consistent with the IUPAC name.

Synthetic procedures

Peptide synthesis general procedure

Standard Fmoc-based solid-phase peptide synthesis (SPPS) and commercially available reagents were used for the peptide synthesis. Rink amide 4 methylbenzhydrylamine resin pre-loaded with an appropriate amino acid with loading of 0.67 mmol/g (150 mg, 1 equiv) was used. Coupling of the amino acids was performed using the following molar ratios of the reagents: an Fmoc-amino acid (4 equiv), HOBt (4 equiv), HBTU (3.9 equiv), DIPEA (8 equiv). The coupling time in all cases was 40 min. N-Fmoc deprotection was carried out by treating the resin with 20% piperidine in dimethyl formamide for 20 min. After completing the synthesis, the resin was washed with dichloromethane and dried under vacuum for 24 h. The peptides (Table 1) were cleaved from the resin with a cleavage cocktail (trifluoroacetic acid, triisopropylsilane and water, 92.5:2.5:5 v/v, 10 ml, 30 min). The volatile products were blown off from the filtered solutions by argon. Residual materials were dissolved in an acetonitrile-water (1:1) mixture and lyophilized. The crude peptides were purified on a preparative (22 x 250 mm) Vydac C18 column with a linear A:B gradient of 5% B/min slope. The purity of the peptides was determined on an analytical (4.6 x 250 mm) Vydac C18 column with a linear A:B gradient of 1% B/min slope.



5-methylthiophene-3-carbaldehyde

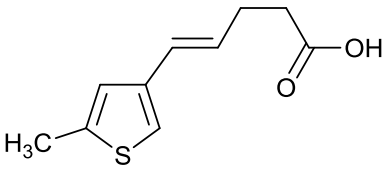
(From Gol'dfarb et al. - J. Gen. Chem. USSR (Engl. Transl.), 1964, vol. 34, p. 969)

n-BuLi solution (26.4 ml, 1.6M in hexanes, 42.4 mmol, 1.5 eq.) was added dropwise during 15 min to a stirred solution of 4-bromo-2-methylthiophene **8** (5 g, 28.2 mmol, 1 eq.) in ether (35 ml) at -78°C. The mixture was stirred at -78°C during additional 90 min and solution of DMF (4.36 ml, 56.4 mmol, 2 eq.) in ether (20 ml) was added rapidly with vigorous stirring at -78°C, the mixture was kept at this temperature for 15 min and allowed to warm to room temperature. After quenching with saturated aqueous citric acid (100 ml) the resulting biphasic mixture was stirred for 30 min. The aqueous layer was extracted with 3×25 ml of ether, combined organic layers were dried over $MgSO_4$, evaporated and applied to flash chromatography column (silica, hexane:ethyl acetate 11:1). Pale yellow liquid, yield 2.97 g (84%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.90 (d, J = 1.2 Hz, 1H), 7.21 (t, J = 1.2 Hz, 1H), 2.53 (d, J = 1.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 185.17, 143.29, 142.25, 135.79, 122.92, 15.39.

HRMS (ESI+): $[M+H]^+$ $C_6H_7OS^+$ Calculated – 127.0212, Found – 127.0208.



(4E/Z)-5-(5-methylthiophen-3-yl)pent-4-enoic acid

t-BuOK (6.62 g, 59 mmol, 2.5 eq.) was added portionwise to a stirred suspension of (3-carboxypropyl)(triphenyl)phosphonium bromide (12.15 g, 28.32 mmol, 1.2 eq.) in dichloromethane (50 ml) at 0 °C. After 15 min solution of 5-methylthiophene-3-carbaldehyde (2.97 g, 23.60 mmol, 1 eq.) in dichloromethane (10 ml) was added dropwise, the reaction mixture was stirred at 0°C for one hour and allowed to warm to the room temperature while stirring overnight. Water (200 ml) was added, the

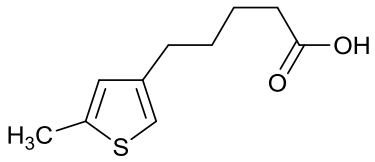
layers were separated and the aqueous layer was extracted with 2×50 ml of dichloromethane. Organic layers were discarded, the aqueous layer was acidified with 10% hydrochloric acid to pH 2 and the product was extracted with 3×50 ml of dichloromethane. After drying over Na_2SO_4 and solvent evaporation the product was purified in form of E/Z mixture (9:1) by flash column chromatography (silica, hexane:ethyl acetate 1:1 with 1% of acetic acid). White solid, yield 3.64 g (79%). An analytical sample of pure (E)-isomer was obtained after crystallization from hexane. M.p. 116 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (t, J = 1.3 Hz, 1H), 6.83 (d, J = 1.3 Hz, 1H), 6.40 – 6.32 (m, 1H), 6.04 – 5.94 (m, 1H), 2.55 – 2.47 (m, 4H), 2.45 (d, J = 1.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 179.35, 140.43, 139.83, 127.49, 125.96, 123.09, 119.48, 33.94, 27.91, 15.53.

IR ν_{max} (neat, cm^{-1}): 3200-2300 (broad), 1688, 1424, 1405, 1297, 1272, 1207, 1125, 970.

HRMS (ESI+): $[M+H]^+C_{10}H_{13}O_2S^+$ Calculated – 197.0631, Found – 197.0627.



5-(5-methylthiophen-3-yl)pentanoic acid (**7**)

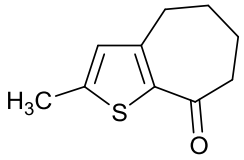
(4E/Z)-5-(5-methylthiophen-3-yl)pent-4-enoic acid (3.64 g, 18.58 mmol) and 10% Pd on charcoal (500 mg) in THF (100 ml) were stirred under H_2 atmosphere (balloon pressure) overnight at room temperature. The mixture was filtered through zeolite pad and solvent was removed. White solid, yield 3.68 g (100%). M.p. 48 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (q, J = 1.1 Hz, 1H), 6.59 (s, 1H), 2.61 – 2.51 (m, 2H), 2.45 (d, J = 1.1 Hz, 3H), 2.41 – 2.32 (m, 2H), 1.75 – 1.59 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 180.02, 142.33, 139.80, 126.63, 117.90, 33.98, 30.23, 29.88, 24.41, 15.46.

IR ν_{max} (neat, cm^{-1}): 3200-2500 (broad), 2945, 1705, 1428, 1205, 925, 825, 721.

HRMS (ESI+): $[M+H]^+ C_{10}H_{15}O_2S^+$ Calculated – 199.0787, Found – 199.0781.



2-methyl-4,5,6,7-tetrahydro-8H-cyclohepta[b]thiophen-8-one (**6**)

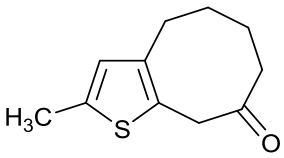
Oxalyl chloride (2.081 ml, 24.26 mmol, 4.5 eq.) was added dropwise to the stirred solution of 5-(5-methylthiophen-3-yl)pentanoic acid **7** (1.068 g, 5.39 mmol, 1 eq.) in dichloromethane (20 ml). Initial gas evolution ceased after 10 min, then the mixture was stirred for 45 min at room temperature. Volatiles were removed under reduced pressure, 1,1,1,3,3,3-hexafluoropropan-2-ol (20 ml) was added in one portion and the resulting mixture was stirred overnight at room temperature. After evaporation, the residue was applied to column flash chromatography (silica, hexane:ethylacetate 9:1). Yellow liquid, yield 590 mg (70%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.60 (d, J = 1.1 Hz, 1H), 2.92 – 2.85 (m, 2H), 2.77 – 2.71 (m, 2H), 2.46 (d, J = 1.0 Hz, 3H), 1.95 – 1.84 (m, 4H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 195.47, 149.51, 148.29, 138.93, 130.09, 41.60, 29.69, 25.51, 21.82, 16.01.

IR ν_{max} (neat, cm^{-1}): 2933, 2864, 2355, 1637, 1449, 1286, 1201, 919, 831.

HRMS (ESI+): $[M+H]^+$ $C_{10}H_{13}OS^+$ Calculated – 181.0682, Found – 181.0677.



2-methyl-4,6,7,9-tetrahydrocycloocta[b]thiophen-8(5H)-one (**10**)

t-BuOK (1.57 g, 14 mmol, 1.2 eq.) was added to a stirred suspension of methyltriphenylphosphonium bromide (4.98 g, 14 mmol) in THF at 0 °C and the resulting mixture was stirred at that temperature for 15 min. 2-methyl-4,5,6,7-tetrahydro-8H-cyclohepta[b]thiophen-8-one **6** (2.09 g, 11.6 mmol) was added at 0 °C and the mixture was allowed to warm to the room temperature upon overnight stirring. After filtration through zeolite and solvent removal crude 2-methyl-8-methylidene-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene **9** was obtained. This compound is highly sensitive to the traces of acids and rearranges easily during flash chromatography giving corresponding endocyclic alkene, 2,8-dimethyl-5,6-dihydro-

4H-cyclohepta[b]-thiophene, so it was used for the next step without further purification.

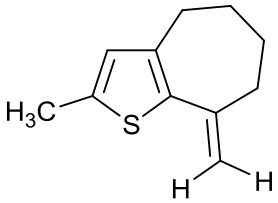
The crude product **9** after the previous step was dissolved in MeOH:H_2O (95:5, 75 ml total), hydroxy{[(4-methylphenyl)sulfonyl]oxy}phenyl-λ^3-iodane (4.78 g, 12.18 mmol, 1.05 eq.) was added at 20°C with ice bath cooling to prevent overheating due to exothermic reaction. After 3 min the cooling bath was removed and the mixture was stirred at room temperature for 20 min. All volatiles were removed under reduced pressure and the residue was purified by flash column chromatography. Pale yellow oil, yield 1.46 g (65% in two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.45 (d, J = 1.4 Hz, 1H), 3.70 (s, 2H), 2.59 – 2.51 (m, 2H), 2.45 – 2.36 (m, 5H), 1.84 – 1.75 (m, 2H), 1.74 – 1.64 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 211.75, 139.02, 137.70, 128.35, 127.73, 42.88, 40.81, 29.14, 27.68, 25.51, 15.21.

IR ν_{max} (neat, cm^{-1}): 2921, 2856, 1701, 1441, 1137, 1104, 828.

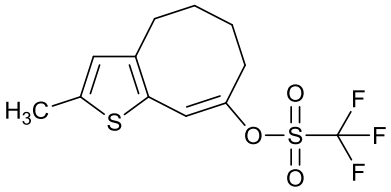
HRMS (ESI+): $[M+H]^+$ $C_{11}H_{15}OS^+$ Calculated – 195.0838, Found – 195.0833.



2-methyl-8-methylidene-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene (**9**)

This compound was isolated in crude form only as a mixture with triphenylphosphinoxide by-product. During purification attempted by flash column chromatography on silica the title compound partly or completely isomerized into endocyclic alkene, 2,8-dimethyl-5,6-dihydro-4H-cyclohepta[b]-thiophene *via* acid-catalyzed alkene isomerization. Two compounds came out of the column in one spot, so purification of **8** is not feasible. It is important to use non-acidic CD_3CN instead of $CDCl_3$ for NMR to prevent undesirable alkene migration.

^{1}H NMR (400 MHz, CD_3CN) δ 6.45 (d, J = 1.1Hz, 1H), 5.00 (d, J = 1.6 Hz, 1H), 4.85 (d, J = 1.6 Hz, 1H), 2.68 – 2.62 (m, 2H), 2.53 – 2.46 (m, 2H), 2.34 (d, J = 1.1 Hz, 3H), 1.84 – 1.69 (m, 4H).



(8E)-2-methyl-4,5,6,7-tetrahydrocycloocta[b]thiophen-8-yl trifluoromethanesulfonate (**11**)

Solution of 2-methyl-4,6,7,9-tetrahydrocycloocta[b]thiophen-8(5H)-one **10** (400 mg, 2.06 mmol, 1 eq.) in THF (4 ml) was added dropwise to a stirred solution of LDA (1.0M in THF/hexanes, 2.47 ml, 2.47 mmol, 1.2 eq.) in THF (15 ml) at -78°C. After 1h of stirring at this temperature a solution of 1,1,1-trifluoro-N-phenyl-N-[(trifluoromethyl)sulfonyl]-methanesulfonamide (883 mg, 2.47 mmol, 1.2 eq.) in THF (6ml) was added dropwise, the resulting mixture was stirred for 30 min at -78 °C and allowed to warm to the room temperature overnight. After quenching with saturated aqueous NH_4Cl followed by CH_2Cl_2 extraction (3×30 ml) and drying the combined organic layers (Na_2SO_4) the solvent was evaporated. The residue was purified by flash chromatography (silica, hexane). Colourless oil, yield 503 mg (75%).

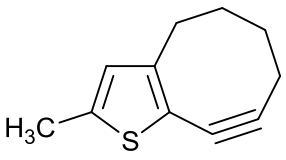
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.47 (s, 1H), 6.44 (s, 1H), 2.74 – 2.67 (m, 2H), 2.59 – 2.51 (m, 2H), 2.43 (s, 3H), 1.75 – 1.69 (m, 2H), 1.69 – 1.62 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 149.61, 141.28, 140.45, 127.99, 126.83, 118.56 (q, $^1J_{C\text{-}F}$ = 320.2 Hz), 114.75, 32.37, 27.18, 26.30, 21.26, 15.35.

^{19}F NMR (376 MHz, $CDCl_3$) δ -73.95.

IR ν_{max} (neat, cm^{-1}): 2932, 2861, 1412, 1244, 1202, 1139, 1008, 926, 873.

HRMS (ESI+): $[M+H]^+C_{12}H_{14}F_3O_3S_2^+$ Calculated – 327.0331, Found – 327.0326.

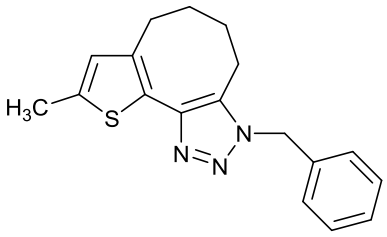


2-methyl-8,9-didehydro-4,5,6,7-tetrahydrocycloocta[b]thiophene (**12**)

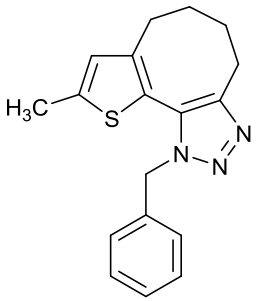
Solution of *t*-BuOK (83 mg, 0.736 mmol, 1.5 eq.) in THF (1 ml) was added to a stirred solution of (8E)-2-methyl-4,5,6,7-tetrahydrocycloocta[b]thiophen-8-yl trifluoro-methanesulfonate **11** (160 mg, 0.491 mmol, 1 eq.) in THF (1 ml) at -78°C

and the resulting solution was stirred at indicated temperature for one hour. The excess of *t*-BuOK was quenched by adding 0.25 ml of water at -78°C. This solution was used immediately for click reaction with benzylazide to obtain the triazoles **13a** and **13b**.

In series of experiments, we tried to isolate and purify the title compound but it appeared to be stable only in diluted solution for several hours at room temperature. In one experiment, the resulting THF solution was diluted with 10-fold volume of water and product was extracted with ether (3×20 ml). All operations were performed at 0 °C as quick as possible. Any attempt to concentrate the resulting ether solution led to product decomposition, attempt to purify product by chromatography on silica or distillation were also unsuccessful. The obtained ether solution reacted with benzylazide also giving the triazoles **13a** and **13b**, but the yield in this case is 10-15% less than using fresh THF solution without any workup.

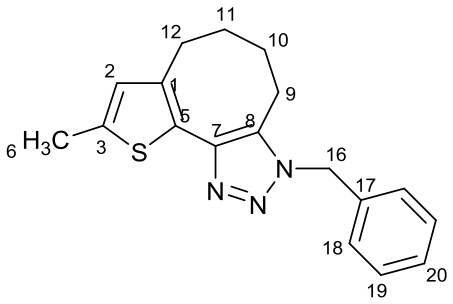


13b



13a

Benzylazide (123 μl, 0.982 mmol, 2 eq. calculating on **10**) was added to a stirred THF solution of **12** obtained at the previous step at 0 °C. After 30 min the mixture was diluted with 10×fold volume of water, product was extracted with dichloromethane (3×30 ml). Solvent removal and flash column chromatography (silica, hexane:ethylacetate 3:1) yielded **13b** and **13a** (the molar ratio 74:26, 78% total yield after two steps). Isomers were separated by preparative HPLC (Supelcosil column) using linear gradient of MeCN in 0.1% TFA/H_2O.



3-benzyl-9-methyl-4,5,6,7-tetrahydro-3H-thieno[2',3':3,4]cycloocta[1,2-d][1,2,3]triazole (**13b**)

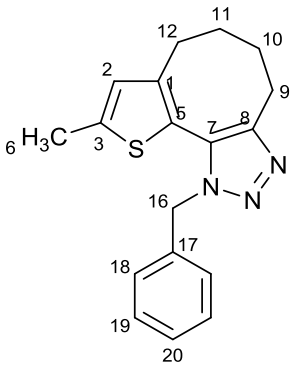
Colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 3H, H19 and H20), 7.19 – 7.14 (m, 2H, H18), 6.49 (q, J = 1.2 Hz, 1H, H2), 5.46 (s, 2H, H16), 2.66 – 2.60 (m, 2H, H9), 2.59 – 2.53 (m, 2H, H12), 2.45 (d, J = 1.2 Hz, 3H, H6), 1.77 – 1.63 (m, 4H, H10 and H11).

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.70 (C7), 139.60 (C3), 137.91 (C5), 134.65 (C17), 133.09 (C8), 129.03 (C19), 128.33 (C20), 127.48 (C2), 127.16 (C18), 126.03 (C1), 52.00 (C16), 28.96 (C10 or C11), 26.35 (C12), 22.85 (C9), 21.29 (C10 or C11), 15.37 (C6).

IR ν_{max} (neat, cm^{-1}): 2934, 2858, 1454, 1427, 1353, 1243, 1156, 1032, 725, 697

HRMS (ESI+): $[M+H]^+C_{18}H_{20}N_3S^+$ Calculated – 310.1372, Found – 310.1369.



1-benzyl-9-methyl-4,5,6,7-tetrahydro-1H-thieno[2',3':3,4]cycloocta[1,2-d][1,2,3]triazole (**13a**)

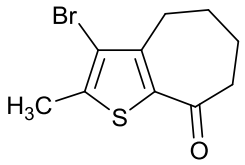
Colourless crystals, m.p. 110°C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.22 (m, 3H, H19 and H20), 7.11 – 7.05 (m, 2H, H18), 6.57 (q, J = 1.3 Hz, 1H, H2), 5.61 (s, 2H, H16), 2.99 – 2.77 (m, 2H, broad,

H9), , 2.65 – 2.21 (m, 2H, broad, H12, overlapped with signal δ 2.44), 2.44 (d, J = 1.3 Hz, 3H, H6), 1.83 – 1.46 (m, 4H, broad, H10 and H11).

^{13}C NMR (101 MHz, $CDCl_3$) δ 147.09, 144.48, 142.17, 135.74, 128.79, 128.53, 128.12, 128.05, 127.17, 118.22 (C1), 52.15 (C16), 28.65, 27.73, 25.74, 24.05 (broad), 15.50 (C6).

HRMS (ESI+): $[M+H]^+C_{18}H_{20}N_3S^+$ Calculated – 310.1372, Found – 310.1371.



3-bromo-2-methyl-4,5,6,7-tetrahydro-8H-cyclohepta[b]thiophen-8-one (**16**)

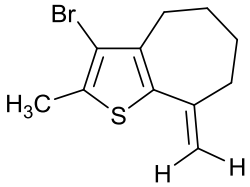
2-methyl-4,5,6,7-tetrahydro-8H-cyclohepta[b]thiophen-8-one **6** (478 mg, 2.66 mmol, 1 eq.) was dissolved in $CHCl_3$ (9 ml) at 0 °C, anhydrous $AlCl_3$ (886 mg, 6.65 mmol, 2.5 eq.) was added followed by Br_2 (150 μl, 467 mg, 2.92 mmol, 1.1 eq.) under stirring. The mixture was warmed to the room temperature and stirred for 20 min. The reaction mixture was quenched with ice/1M HCl mixture (20 ml, 1:1). CH_2Cl_2/H_2O workup and subsequent flash column chromatography (SiO_2, hexane/ethylacetate 9:1) yielded the title compound as white solid (595 mg. 86%), m.p. 72 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.98 – 2.91 (m, 2H), 2.81 – 2.76 (m, 2H), 2.44 (s, 3H), 2.00 – 1.86 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 194.75, 146.95, 143.23, 137.66, 114.22, 42.04, 30.80, 25.47, 21.78, 16.43.

IR ν_{max} (neat, cm^{-1}): 2918, 2866, 1618, 1453, 1277, 1195, 774.

HRMS (ESI+): $[M+H]^+C_{10}H_{12}BrOS^+$ Calculated – 258.9787, Found – 258.9786.



3-bromo-2-methyl-8-methylene-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene (**17**)

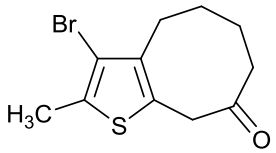
t-BuOK (52 mg, 0.463mmol, 1.2 eq.) was added to a stirred suspension of methyltriphenylphosphonium bromide (165 mg, 0.463mmol, 1.2 eq.) in THF (2 ml) at 0 °C and the resulting mixture was stirred at that temperature for 15 min. 3-Bromo-2-methyl-4,5,6,7-tetrahydro-8H-cyclohepta[b]thiophen-8-one **16** (100 mg, 0.386 mmol, 1 eq.) was added at 0 °C and the mixture was allowed to warm to the room temperature overnight with stirring. After filtration through zeolite, solvent removal and flash column chromatography (Al_2O_3 basic Brockmann 1, hexane/ethylacetate 9:1) the title compound was obtained as colourless oil. Yield 91 mg, 92%.

^{1}H NMR (400 MHz, CD_3CN) δ 5.08 (d, J = 1.3 Hz, 1H), 4.94 (d, J = 1.3 Hz, 1H), 2.75 – 2.66 (m, 2H), 2.54 – 2.47 (m, 2H), 2.32 (s, 3H), 1.86 – 1.71 (m, 4H).

^{13}C NMR (101 MHz, CD_3CN) δ 144.60, 139.15, 137.51, 132.11, 113.57, 113.01, 36.56, 30.10, 29.33, 27.30, 15.41.

IR ν_{max} (neat, cm^{-1}): 2925, 2855, 1701, 1618, 1435, 878.

HRMS (ESI+): $[M+H]^+C_{11}H_{14}BrS^+$ Calculated – 256.9994, Found – 256.9989.



3-bromo-2-methyl-4,6,7,9-tetrahydrocycloocta[b]thiophen-8(5H)-one (**18**)

This compound was synthesized analogously to 2-methyl-4,6,7,9-tetrahydrocycloocta [b]thiophen-8(5H)-one **10**, starting from 3.144 g (12.1 mmol) of 3-bromo-2-methyl-4,5,6,7-tetrahydro-8H-cyclohepta[b]thiophen-8-one **16**, without purification of the intermediate alkene **17**. Purified by flash column chromatography (SiO_2, hexane/ethylacetate 9:1). Pale yellow crystals, yield 2.108 g, 75% in two steps.

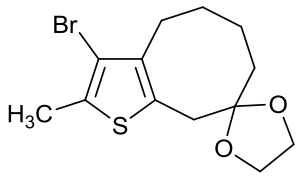
M.p. 64°C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 2H), 2.70 – 2.60 (m, 2H), 2.42 – 2.38 (m, 2H), 2.37 (s, 3H), 1.86 – 1.75 (m, 2H), 1.74 – 1.65 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 210.99, 137.62, 132.28, 127.26, 112.43, 43.62, 40.55, 27.69, 27.42, 25.64, 15.23.

IR ν_{max} (neat, cm^{-1}): 2932, 1700, 1441, 1226, 1105, 940.

HRMS (ESI+): $[M+H]^+C_{11}H_{14}BrOS^+$ Calculated – 272.9943, Found – 272.9939.



3-bromo-2-methyl-4,6,7,9-tetrahydro-5H-spiro[cycloocta[b]thiophene-8,2'-[1,3]dioxolane] (**19**)

3-bromo-2-methyl-4,6,7,9-tetrahydrocycloocta[b]thiophen-8(5H)-one **18** (818 mg, 3.0 mmol, 1 eq.), ethyleneglycol (1 ml, 1.11 g, 18 mmol, 6 eq.) and *p*-toluenesulfonic acid monohydrate (57 mg, 0.3 mmol, 10% mol) in benzene (50 ml) were refluxed during 6h with Dean-Stark adapter. After cooling, the mixture was washed with saturated $NaHCO_3$, the aqueous layer was extracted 2×20 ml CH_2Cl_2. Combined organic layers were dried over Na_2SO_4 and evaporated. Purified by flash column chromatography (SiO_2, hexane/ethylacetate 9:1). White solid, yield 796 mg, 84%.

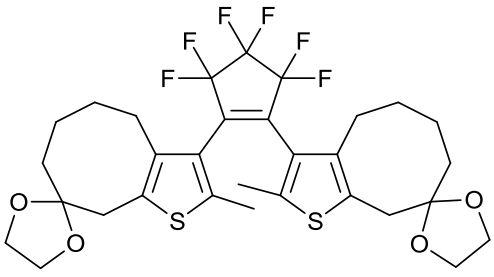
M.p. 111°C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 – 3.91 (m, 4H), 3.02 (s, 2H), 2.76 – 2.67 (m, 2H), 2.36 (s, 3H), 1.68 – 1.48 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 137.65, 131.46, 129.81, 112.27, 111.56, 64.78, 36.56, 35.13, 28.99, 28.01, 21.84, 15.18.

IR ν_{max} (neat, cm^{-1}): 2934, 1441, 1339, 1267, 1128, 1074, 1040, 985, 942, 735.

HRMS (ESI+): $[M+H]^+C_{13}H_{18}BrO_2S^+$ Calculated – 317.0205, Found – 317.0200.



3,3''-(perfluorocyclopent-1-ene-1,2-diyl)bis(2-methyl-4,6,7,9-tetrahydro-5H-spiro[cycloocta[b]thiophene-8,2'-[1,3]dioxolane]) (**20**)

To a stirred solution of 3-bromo-2-methyl-4,6,7,9-tetrahydro-5H-spiro[cycloocta-[b]thiophene-8,2'-[1,3]dioxolane] **19** (796 mg, 2.51 mmol, 1 eq.) in THF (15 ml) *n*-

BuLi (1.6 M in hexane, 1.88 ml, 3.01 mmol, 1.2 eq.) was added dropwise with stirring at -78°C. After stirring at indicated temperature for 1.5 h, octafluorocyclopentene (168 μl, 266 mg, 1.26 mmol, 0.5 eq.) was added dropwise and the mixture was stirred at -78 °C for additional 1.5 h. The reaction mixture was then allowed to warm to the room temperature (30 min), saturated NH_4Cl was added and the product was extracted with CH_2Cl_2 (3×50 ml). Purified by flash column chromatography (SiO_2, hexane/ethylacetate 4:1), white solid, yield 494 mg, 61%.

M.p. 205-206°C.

Compound **20** exists as a mixture of two fast-interconverting *syn-anti* rotamers, which have separate sets of signals in NMR spectra.

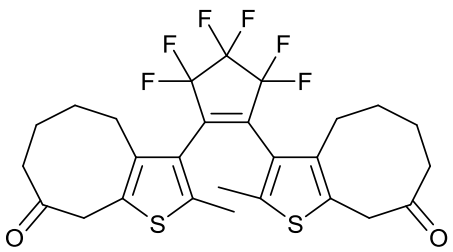
^{1}H NMR (500 MHz, $CDCl_3$) δ 4.05 – 3.87 (m, 8H), 3.07 (d, J = 14.6 Hz, 1H), 3.03 (d, J = 14.6Hz, 1H), 2.79 (d, J = 8.9 Hz, 1H), 2.76 (d, J = 8.9 Hz, 1H), 2.55 – 2.43 (m, 2H), 2.38 – 2.26 (m, 5H), 2.24 (d, J_{H-F} = 1.4 Hz, 3H), 1.69 – 1.39 (m, 12H).

^{13}C NMR (126 MHz, $CDCl_3$, signals of CF_2-groups are omitted due to low intensity and complex C-F couplings) δ 141.92 (t, J_{C-F} = 24.3 Hz), 138.58 (d, J_{C-F} = 29.0 Hz), 138.28 (d, J_{C-F} = 34.2 Hz), 131.61 (d, J_{C-F} = 7.8 Hz), 124.25 (d, J_{C-F} = 37.1 Hz), 112.61, 112.55, 64.79, 64.76, 35.52 (d, J_{C-F} = 9.2 Hz), 35.17, 30.46, 30.33, 27.78, 27.34, 15.23 (d, J_{C-F} = 5.0 Hz), 15.11 (d, J_{C-F} = 4.1 Hz).

^{19}F NMR (376 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ -107.42, -107.45, -108.13, -108.16, -110.34, -110.35, -111.03, -111.04, -111.06, -111.45, -112.15, -114.95, -114.98, -115.66, -115.69, -132.56, -132.59, -132.63, -133.20, -133.23, -133.26, -134.54, -134.55, -134.57, -134.59, -134.60, -136.41, -136.43, -136.46, -137.04, -137.07, -137.10.

IR ν_{max} (neat, cm^{-1}): 2945, 1443, 1338, 1266, 1127, 1039, 987, 935, 728.

HRMS (ESI+): $[M+H]^+C_{31}H_{35}F_6O_4S_2^+$ Calculated – 649.1875, Found – 649.1872.



3,3'-(perfluorocyclopent-1-ene-1,2-diyl)bis(2-methyl-4,6,7,9-tetrahydrocycloocta[b]thiophen-8(5H)-one) (**21**)

3,3''-(perfluorocyclopent-1-ene-1,2-diyl)bis(2-methyl-4,6,7,9-tetrahydro-5H-spiro-[cycloocta[b]thiophene-8,2'-[1,3]dioxolane] **20** (494 mg, 0.762 mmol) was dissolved in mixture of 1M HCl (40 ml) and dioxane (40 ml). The resulting solution was heated at 70 °C for 1 h and all volatiles were removed in vacuum. The residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$, brine and dried over Na_2SO_4. Solvent removal and flash column chromatography (SiO_2, hexane/ethylacetate 2:1) afforded title compound **21** as a pale yellow oil. Yield 323 mg, 76%.

Compound **21** exists as a mixture of two fast-interconverting *syn-anti* rotamers, which have separate sets of signals in NMR spectra.

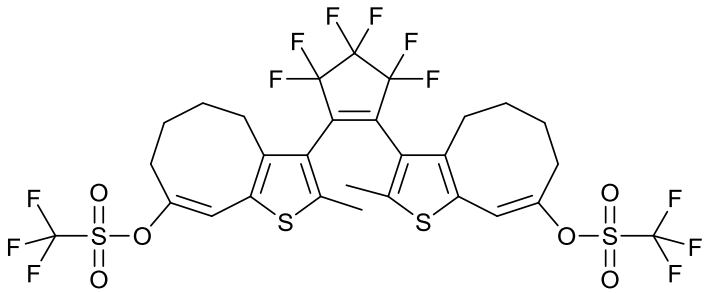
^{1}H NMR (500 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ 3.81 – 3.68 (m, 2H), 3.65 – 3.59 (m, 2H), 2.56 – 2.18 (m, 14H), 1.91 – 1.63 (m, 6H), 1.55 – 1.47 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$, signals of CF_2-groups are omitted due to low intensity and complex C-F couplings) δ 210.15, 142.35 – 141.45 (m), 139.29, 139.05, 137.64, 137.34, 129.15, 124.78, 124.55, 42.43 (d, J_{C-F} = 9.2 Hz), 41.55 (d, J_{C-F} = 7.6 Hz), 29.02 (d, J_{C-F} = 3.7 Hz), 28.69 (d, J_{C-F} = 2.7 Hz), 26.96, 26.42, 24.63, 24.53, 15.17 (d, J_{C-F} = 5.8 Hz), 14.97 (d, J_{C-F} = 4.1 Hz).

^{19}F NMR (376 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ -106.27, -106.29, -106.97, -107.00, -109.09, -109.10, -109.12, -109.14, -109.80, -109.81, -109.83, -110.29, -110.99, -113.87, -113.90, -114.58, -114.61, -131.54, -131.57, -131.60, -132.18, -132.21, -132.24, -133.58, -133.60, -133.62, -133.63, -133.65, -135.38, -135.41, -135.44, -136.02, -136.05, -136.08.

IR ν_{max} (neat, cm^{-1}): 2932, 1703, 1442, 1342, 1273, 1142, 997, 729.

HRMS (ESI+): $[M+H]^+C_{27}H_{27}F_6O_2S_2^+$ Calculated – 561.1351, Found – 561.1345.



(8E,8'E)-(perfluorocyclopent-1-ene-1,2-diyl)bis(2-methyl-4,5,6,7-tetrahydrocycloocta[b]thiophene-3,8-diyl) bis(trifluoromethanesulfonate) (**22**)

LDA (1.0 M in THF/hexanes, 493 μl, 0.493 mmol, 2.64 eq.) was added to a solution on *bis*-ketone **21** in THF (4 ml) at -78 °C and the mixture was stirred at this temperature for 1 h. A solution of 1,1,1-trifluoro-N-phenyl-N-[(trifluoromethyl) sulfonyl]-methanesulfonamide (176 mg, 0.493 mmol, 2.64 eq.) in THF (1 ml) was added and the resulting solution was allowed to warm to the room temperature overnight with stirring. After usual workup with saturated NH_4Cl/CH_2Cl_2 the title compound **22** was purified by flash column chromatography (SiO_2, hexane/ethylacetate 9:1). Colourless oil, yield 79 mg, 51%.

Compound **22** exists as a mixture of two fast-interconverting *syn-anti* rotamers, which have separate sets of signals in NMR spectra.

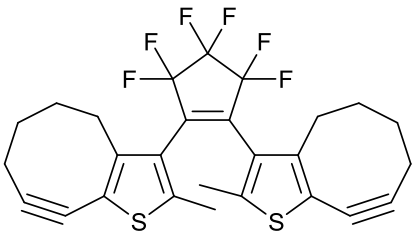
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.35 (s, 1H), 6.35 (s, 1H), 2.58 – 2.33 (m, 11H), 2.29 (d, J = 1.4 Hz, 3H), 1.80 – 1.43 (m, 8H).

^{13}C NMR (126 MHz, $CDCl_3$, signals of CF_2-groups are omitted due to low intensity and complex C-F couplings) δ 150.91, 150.74, 142.70, 142.45, 142.19 – 141.46 (m), 139.05, 138.81, 128.48, 128.36, 124.92, 124.46, 118.62 (q, $^1J_{C\text{-}F}$ = 319.9 Hz), 114.15, 114.07, 32.60, 32.47, 26.57, 25.93, 25.70 (broad, two overlapped signals), 21.31, 21.11, 15.63 (d, $J_{C\text{-}F}$ = 6.2 Hz), 15.46 (d, $J_{C\text{-}F}$ = 3.9 Hz).

^{19}F NMR (376 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ -74.96, -75.02, -105.88, -105.91, -106.59, -106.62, -109.86, -109.88, -109.89, -109.91, -110.57, -110.59, -110.61, -111.42, -111.44, -111.45, -112.12, -112.13, -112.15, -112.17, -116.33, -116.37, -117.04, -117.08, -132.03, -132.07, -132.10, -132.67, -132.70, -132.74, -134.63, -134.64, -134.66, -134.68, -134.68, -137.29, -137.33, -137.36, -137.93, -137.96, -137.99.

IR ν_{max} (neat, cm^{-1}):2940, 1415, 1204, 1135, 895, 732.

HRMS (ESI+): $[M+H]^+C_{29}H_{25}F_{12}O_6S_4^+$ Calculated – 825.0337, Found – 825.0333.

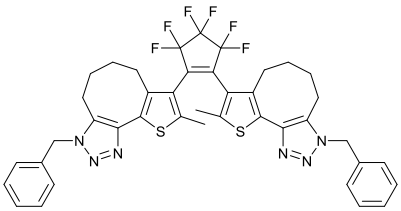


3,3'-(3,3,4,4,5,5-hexafluorocyclopent-1-ene-1,2-diyl)bis(2-methyl-8,9-didehydro-4,5,6,7-tetrahydrocycloocta[b]thiophene) (**5**)

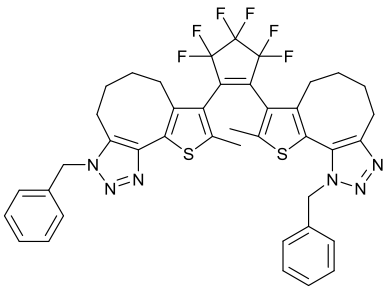
Compound **22** (120 mg, 0.145 mmol, 1 eq.) was dissolved in THF (4 ml), cooled to -78 °C and a solution of *t*-BuOK (65 mg, 0.58 mmol, 4 eq.) in THF (1 ml) was added. The mixture was stirred at -78 °C for 1 h. Saturated aqueous NH_4Cl (0.1 ml) was added, the resulting solution of compound **5** was warmed to 0°C and used immediately for click reactions with benzylazide and azidopeptide (see below). The contentration of compound **5** in the obtained solution was calculated assuming 100% yield in elimination reaction, equal to 0.029 M.

Any attempts of isolation and purification of title compound by column chromatography were unsuccessful.

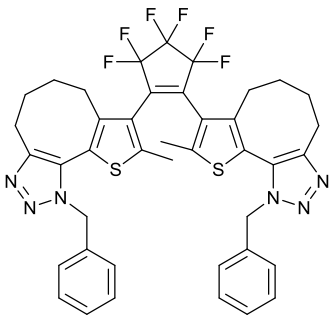
Mixture of isomers



23a



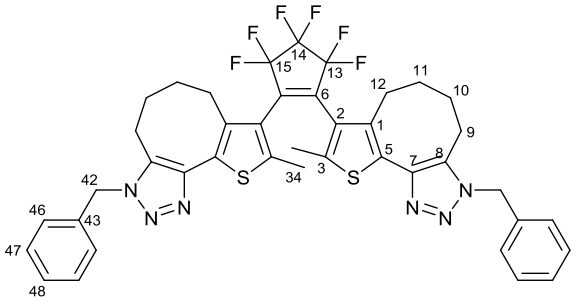
23b



23c

An aliquote of **5** stock solution (3 ml, 0.087 mmol of **5**) prepared as described above was diluted with MeOH (84 ml) to gain 1 mM concentration of compound **5**. Benzyl azide (21.7 μl, 23 mg, 0.174 mmol, 2 eq.) was added and the resulting mixture was stirred for 1h at room temperature. Samples for LCMS analysis were taken after 5

and 30 min of the reaction commencement. Solvent removal and flash column chromatography (SiO_2, hexane/ethylacetate 1:1) yielded a mixture of isomeric compounds **23a**-**23c** as colorless oil (56 mg, 82%). MS-ESI(+) $[M+H]^+$ 791.5. Three isomeric triazoles were separated by preparative HPLC (Supelcosil column) using linear gradient of MeCN in 0.1% TFA/H_2O.



8,8'-(perfluorocyclopent-1-ene-1,2-diyl)bis(3-benzyl-9-methyl-4,5,6,7-tetrahydro-3H-thieno[2',3':3,4]cycloocta[1,2-d][1,2,3]triazole) (**23a**)

This compound was isolated as trifluoroacetate after preparative HPLC, colourless oil.

Compound **23a** exists as a mixture of two fast-interconverting *syn-anti* rotamers, which have separate sets of signals in NMR spectra.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 6H, H47 and 48), 7.20 – 7.15 (m, 4H, H46), 5.58 – 5.36 (m, 4H, H42), 2.73 – 2.64 (m, 2H, H9), 2.55 – 2.43 (m, 2H, H9), 2.42 – 2.35 (m, 5H, H12 and H34), 2.33 (d, J = 1.4 Hz, 3H, H34), 2.32 – 2.22 (m, 2H, H12), 1.80 – 1.46 (m, 8H, H10 and 11).

^{13}C NMR (126 MHz, $CDCl_3$) δ 141.58 (thiophene Ar), 141.36 (t, $^2J_{C-F}$ = 22.2 Hz, C6), 140.82 (thiophene Ar), 138.99 (triazole Ar), 138.94 (triazole Ar), 136.52 (thiophene Ar), 136.03 (thiophene Ar), 134.42 (C43), 134.36 (C43), 133.60 (triazole Ar), 133.52 (triazole Ar), 129.14 (C47 or 48), 129.09 (C47 or 48), 128.46 (C47 or 48), 128.41 (C47 or 48), 127.85 (thiophene Ar), 127.68 (thiophene Ar), 127.26 (C46), 127.18 (C46), 124.41 (thiophene Ar), 124.08 (thiophene Ar), 115.95 (triplet of quartets, $^1J_{C-F}$ = 256.3 Hz, $^2J_{C-F}$ = 24.5 Hz, C13), 111.15 (triplet of quintets, $^1J_{C-F}$ = 268.8 Hz, $^2J_{C-F}$ = 25.7 Hz, C14), 52.17 (C42), 52.13 (C42), 28.12 (C10 or 11), 28.06 (C10 or 11), 25.55

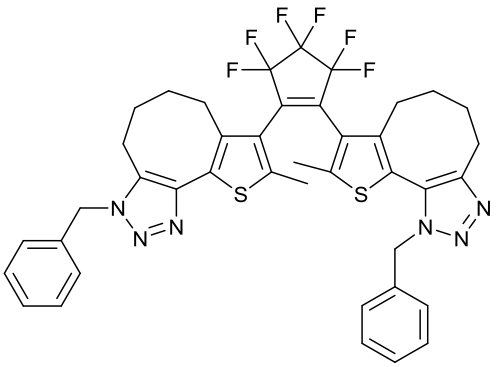
(C12), 24.93 (C12), 22.92 (C9), 22.81 (C9), 21.64 (C10 or 11), 21.45 (C10 or 11), 15.50 (d, J = 6.9 Hz, C34), 15.35 (d, J = 4.0 Hz, C34).

^{19}F NMR (376 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ -75.84 (trifluoroacetate anion), -104.21, -104.25, -104.92, -104.95, -108.42, -108.44, -108.45, -109.15, -110.59, -111.29, -111.31, -111.32, -115.80, -115.84, -116.51, -116.55, -131.00, -131.04, -131.07, -131.63, -131.67, -131.70, -133.67, -133.69, -133.71, -136.81, -136.85, -136.88, -137.44, -137.48, -137.51.

Structure assignment for compound **21a** (anti-isomer) was based on NOESY correlations between H9 (two multiplets2.73-2.64 and 2.55-2.43 from two rotamers) and both H42 (m, 5-58-5.36) and H46 (m, 7.20-7.15).

IR ν_{max} (neat, cm^{-1}): 2933, 1454, 1343, 1266, 1189, 1143, 725.

HRMS (ESI+): $[M+H]^+C_{41}H_{37}F_6N_6S_2^+$ Calculated – 791.2420, Found – 791.2415.



3-benzyl-8-(2-(1-benzyl-9-methyl-4,5,6,7-tetrahydro-1H-thieno[2',3':3,4]cycloocta[1,2-d][1,2,3]triazol-8-yl)-3,3,4,4,5,5-hexafluorocyclopent-1-en-1-yl)-9-methyl-4,5,6,7-tetrahydro-3H-thieno[2',3':3,4]cycloocta[1,2-d][1,2,3]triazole (**23b**)

Was isolated as trifluoroacetate after preparative HPLC, colourless oil.

Compound **23b** exists as a mixture of two fast-interconverting *syn-anti* rotamers, which have separate sets of signals in NMR spectra. Unsymmetrical structure caused by *syn-anti* position of triazoles leads to further duplication of signals in NMR spectra comparing to the symmetrical compound **23a**.

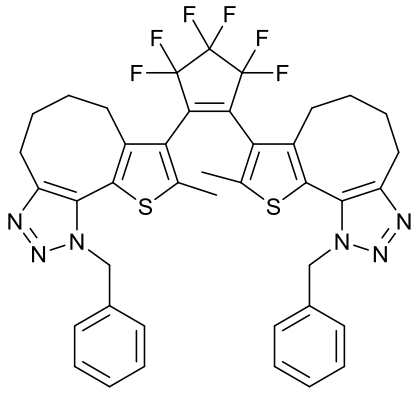
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.30 (m, 2H), 7.31 – 7.18 (m, 5H), 7.14 (t, J = 7.7 Hz, 1H), 7.05 (d, J = 8.0 Hz, 0.5H), 6.97 – 6.91 (m, 1.5H), 5.68 – 5.36 (m, 4H),

2.86 – 2.63 (m, 4H), 2.60 – 2.40 (m, 1H), 2.33 (dd, J = 3.2, 1.6 Hz, 3H), 2.26 (s, 2H), 2.19 – 1.31 (m, 12H).

^{13}C NMR (126 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ 147.88, 147.68, 143.70, 143.47, 143.26, 142.64, 142.45, 142.30, 142.10, 141.42, 141.12, 140.94, 140.79, 139.14, 139.03, 136.18, 136.03, 135.28, 135.22, 134.49, 134.38, 133.78, 129.31, 129.28, 129.00, 128.85, 128.77, 128.69, 128.42, 128.37, 128.25, 128.18, 127.59, 127.47, 126.85, 126.82, 125.22, 125.17, 124.16, 124.03, 119.57, 118.11, 117.90, 117.72, 116.07, 115.87, 115.65, 114.07, 113.85, 113.63, 113.42, 111.48, 111.27, 111.07, 109.31, 109.10, 108.91, 52.55, 52.47, 52.33, 28.52, 28.39, 28.03, 27.04, 26.99, 25.87, 25.65, 25.36, 24.52, 23.19, 21.72, 21.54, 15.53, 15.50, 15.43, 15.15.

^{19}F NMR (376 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ -75.96 (trifluoroacetate anion), -104.79, -104.82, -105.01, -105.50, -105.71, -109.56, -110.09, -110.26, -110.67, -111.37, -115.00, -115.47, -115.50, -115.70, -116.17, -116.20, -131.30, -131.33, -131.36, -131.93, -131.96, -132.00, -133.90, -133.92, -133.93, -133.95, -133.97, -136.40, -136.43, -136.46, -137.03, -137.06, -137.09.

HRMS (ESI+): $[M+H]^+C_{41}H_{37}F_6N_6S_2^+$ Calculated – 791.2420, Found – 791.2418.



8,8'-(perfluorocyclopent-1-ene-1,2-diyl)bis(1-benzyl-9-methyl-4,5,6,7-tetrahydro-1H-thieno[2',3':3,4]cycloocta[1,2-d][1,2,3]triazole) (**23c**)

This compound was isolated as trifluoroacetate after preparative HPLC in minute quantities (less than 1 mg).

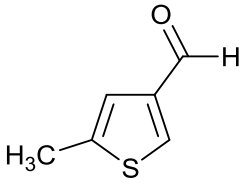
^{19}F NMR (376 MHz, $CDCl_3$, peaks are indicated without multiplet analysis) δ -75.62 (trifluoroacetate anion), -103.86, -104.58, -108.64, -110.31, -115.70, -116.39, -131.16, -131.80, -133.91, -137.06, -137.69.

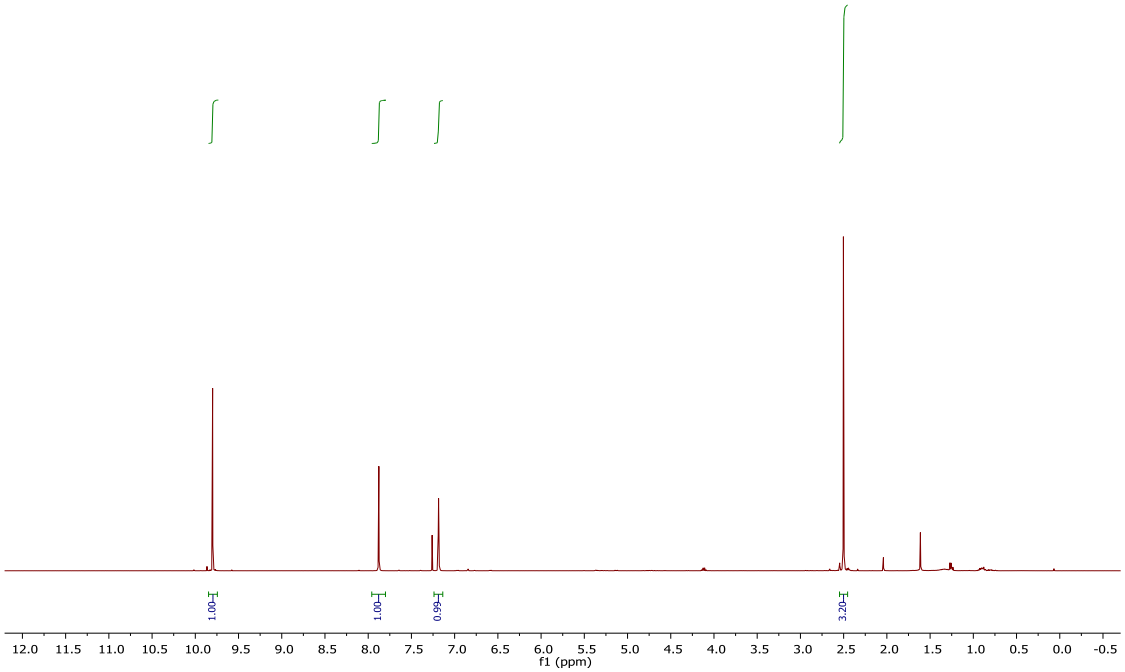
HRMS (ESI+): $[M+H]^+C_{41}H_{37}F_6N_6S_2^+$ Calculated – 791.2420, Found – 791.2412.

Reaction of **5** with linear peptide **24** forming stapled peptide **26**.

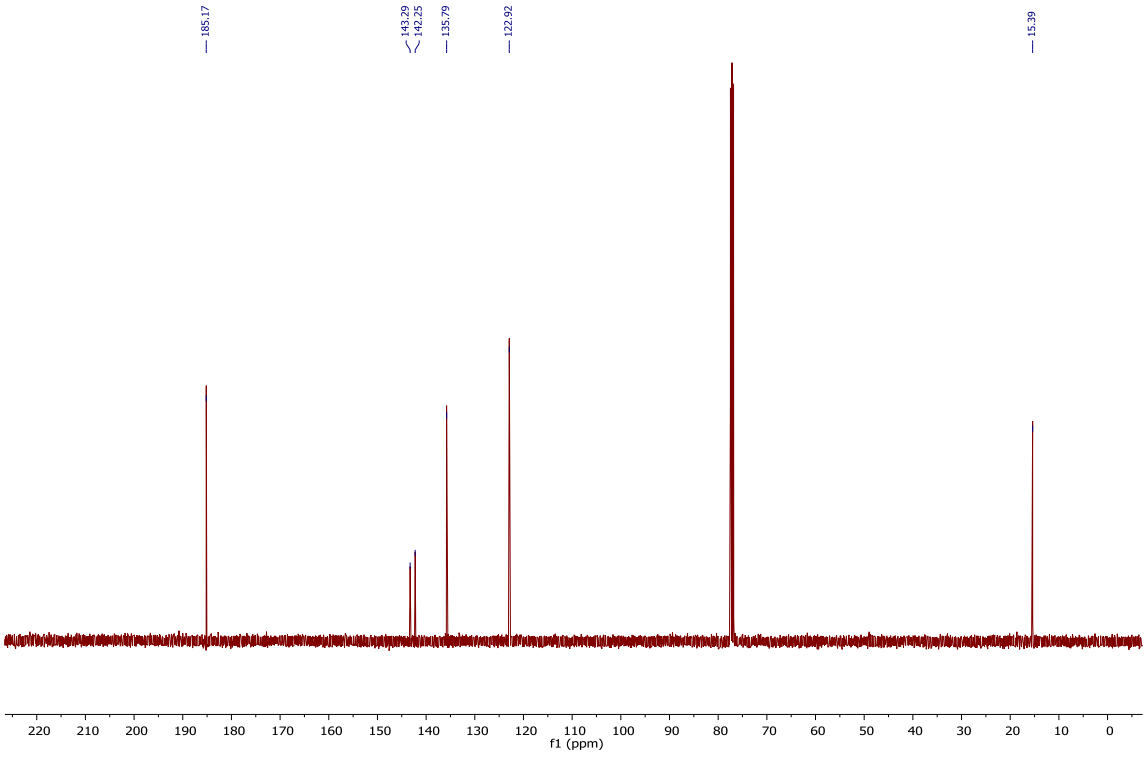
An aliquot of the solution of linker which contained 5.5 μmol of **5** was added to solution of linear peptide **24** (7.88 mg, 5 μmol) in H_2O/MeCN 1:3 mixture(15,76 ml, peptide concentration 0.5 mM). The resulting mixture was stirred for 1h at room temperature. The solution was then freeze-dried and the residue was purified by preparative HPLC yielding four different fractions of stapled peptide **26**: fraction 1 (0.67 mg), fraction 2 (0.18 mg), fraction 3 (0.79 mg) and major fraction 4 (4.76 mg, 45%). Total 6.4 mg, 61%

Copies of the spectral and analytical data for key intermediate and target compounds.

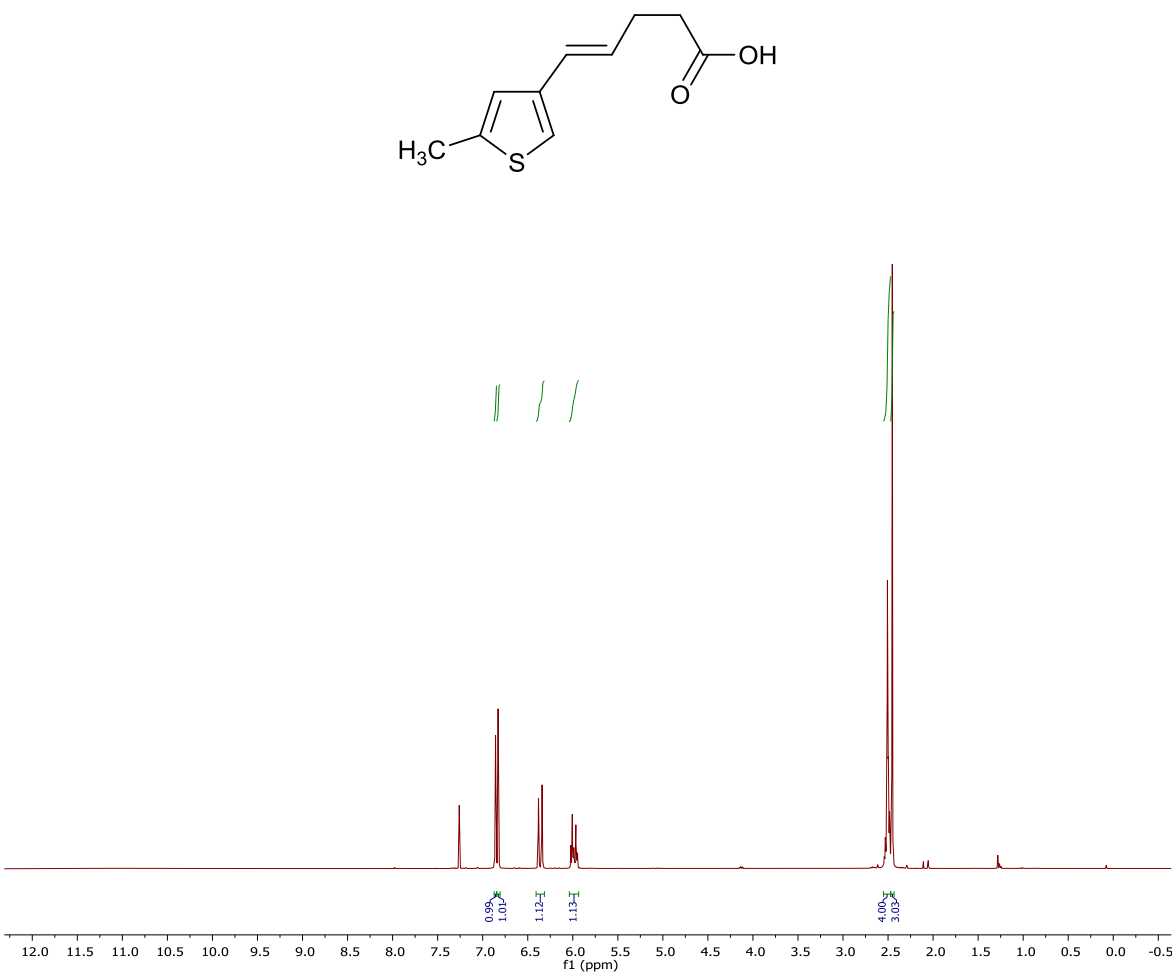




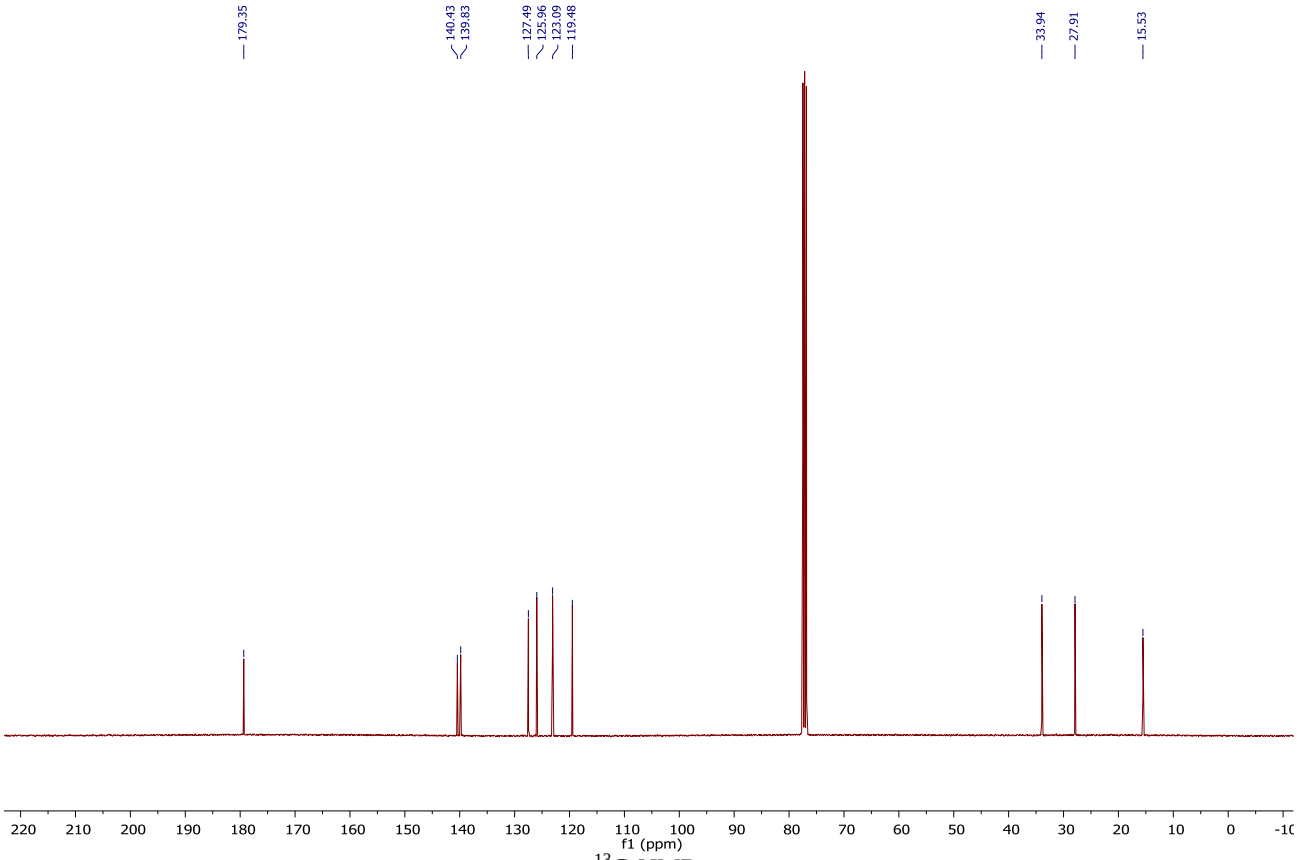
^{1}H-NMR



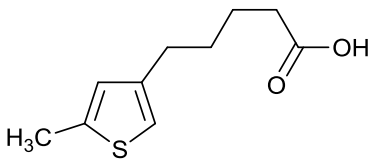
^{13}C-NMR


OH
O
H3C
S
0.99
1.01
1.12
1.13
4.00
3.03
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

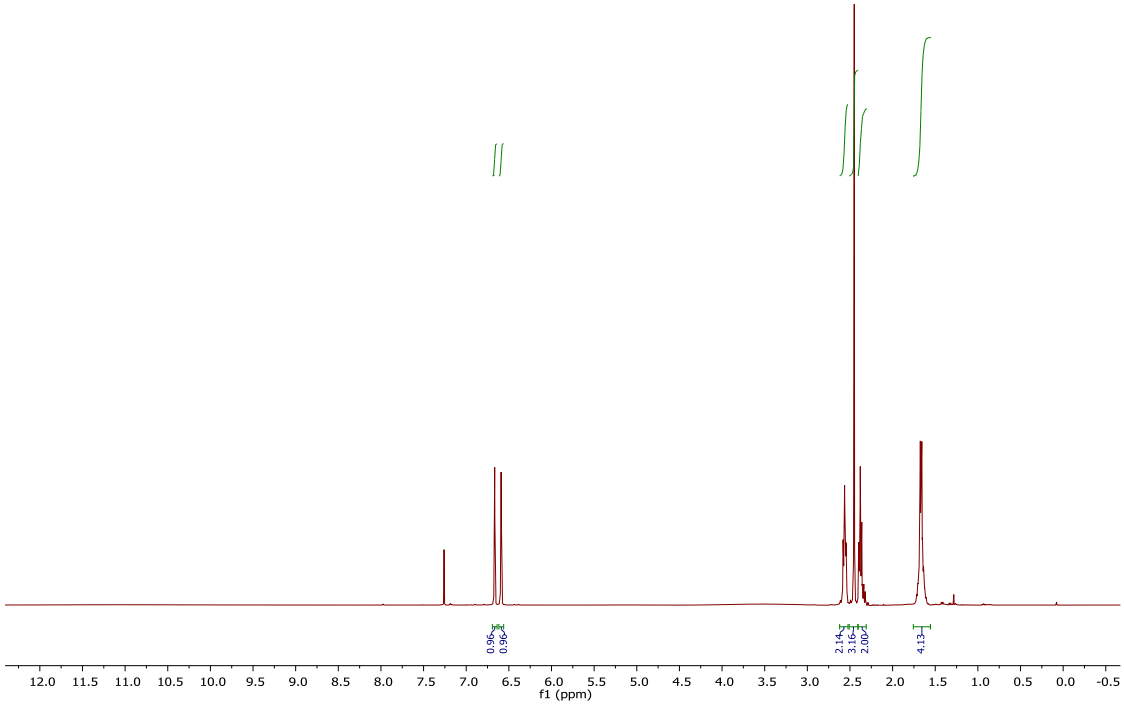
^{1}H-NMR


179.35
140.43
139.83
127.49
125.96
123.09
119.48
33.94
27.91
15.53
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

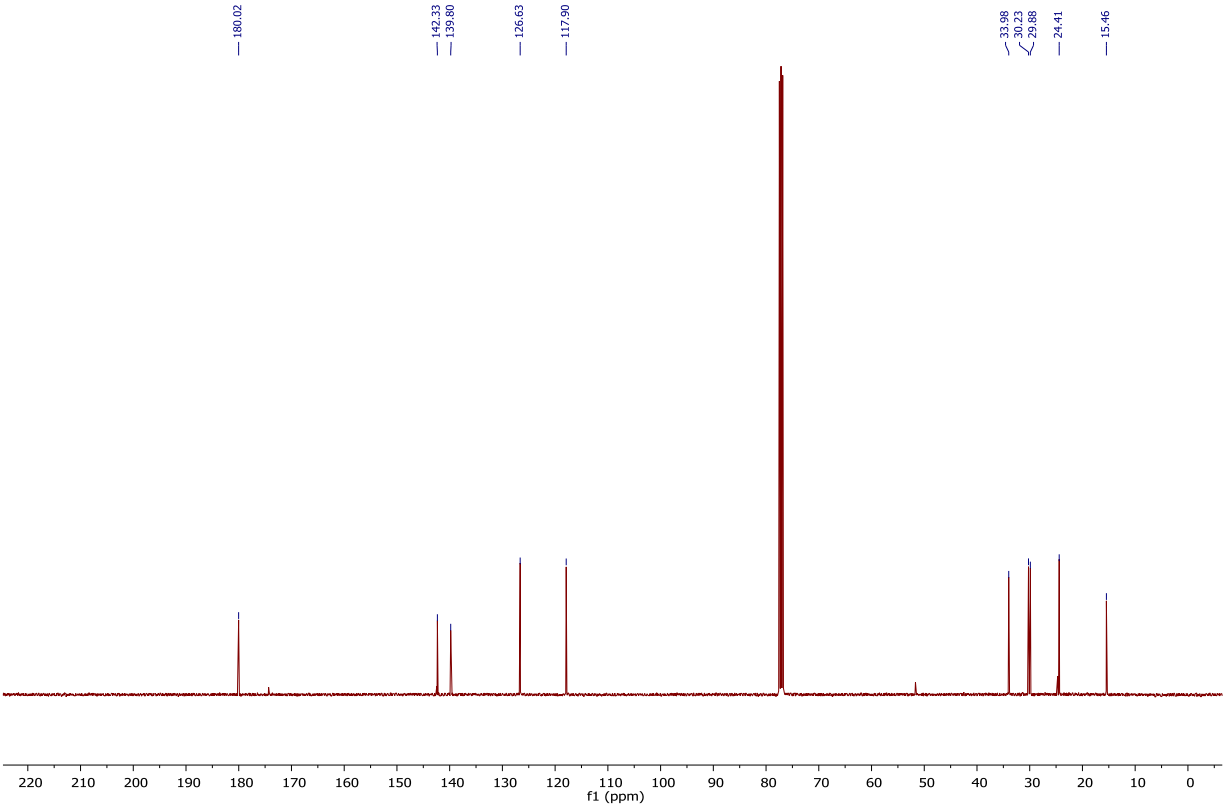
^{13}C-NMR



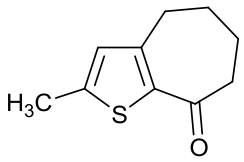
7



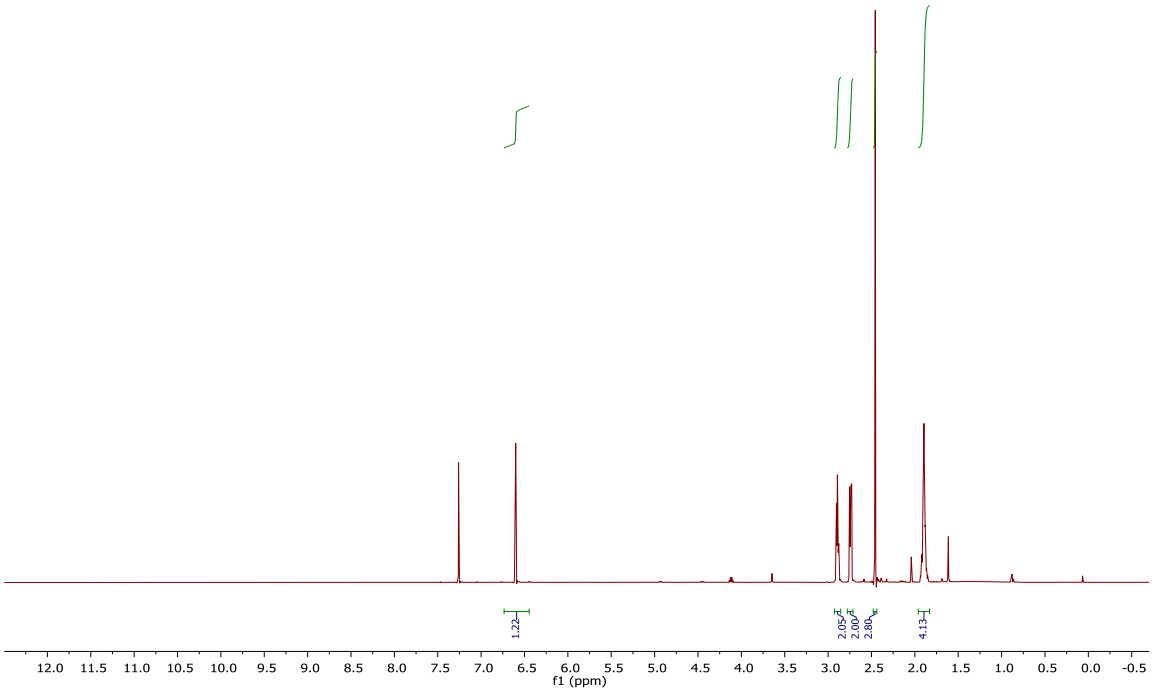
^{1}H-NMR



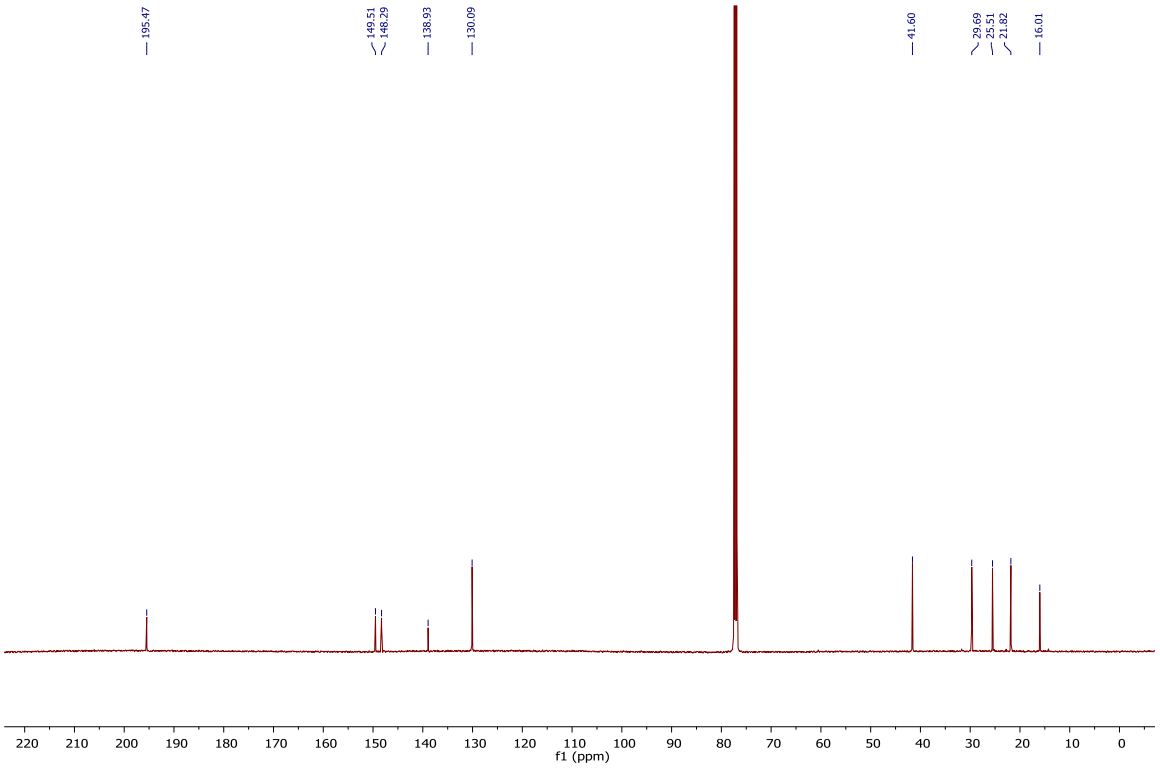
^{13}C-NMR



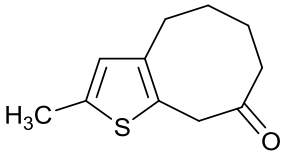
6



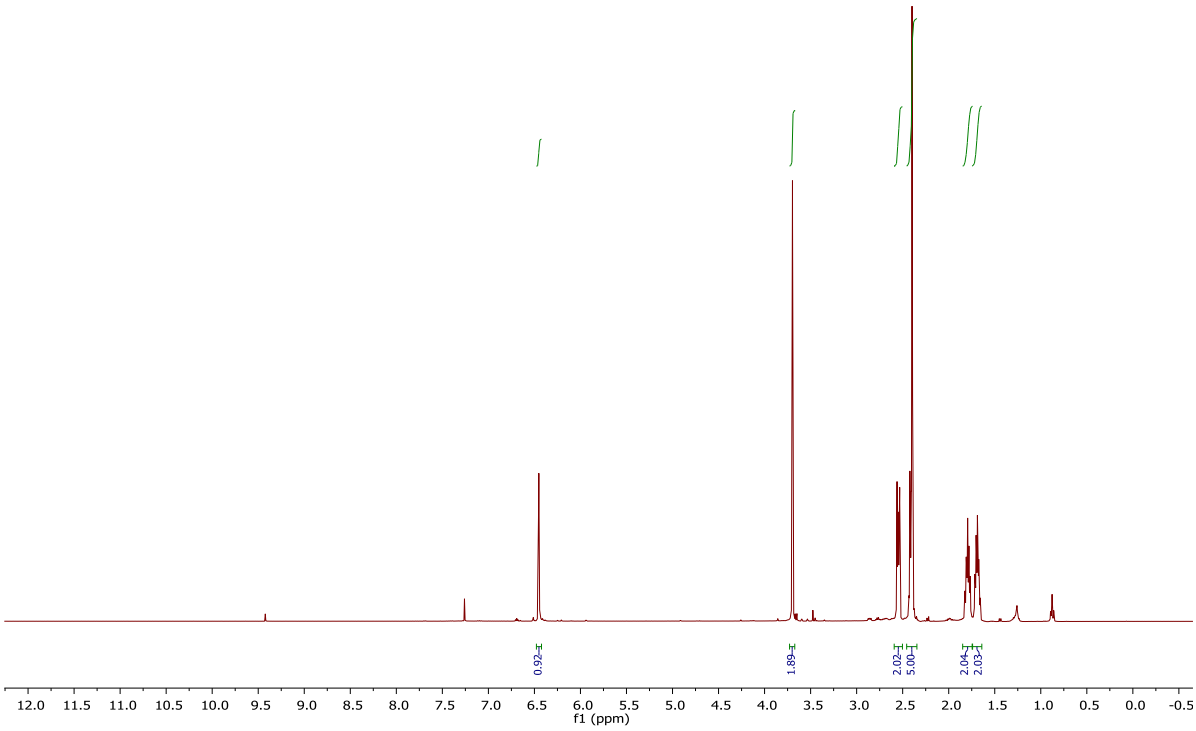
^{1}H-NMR



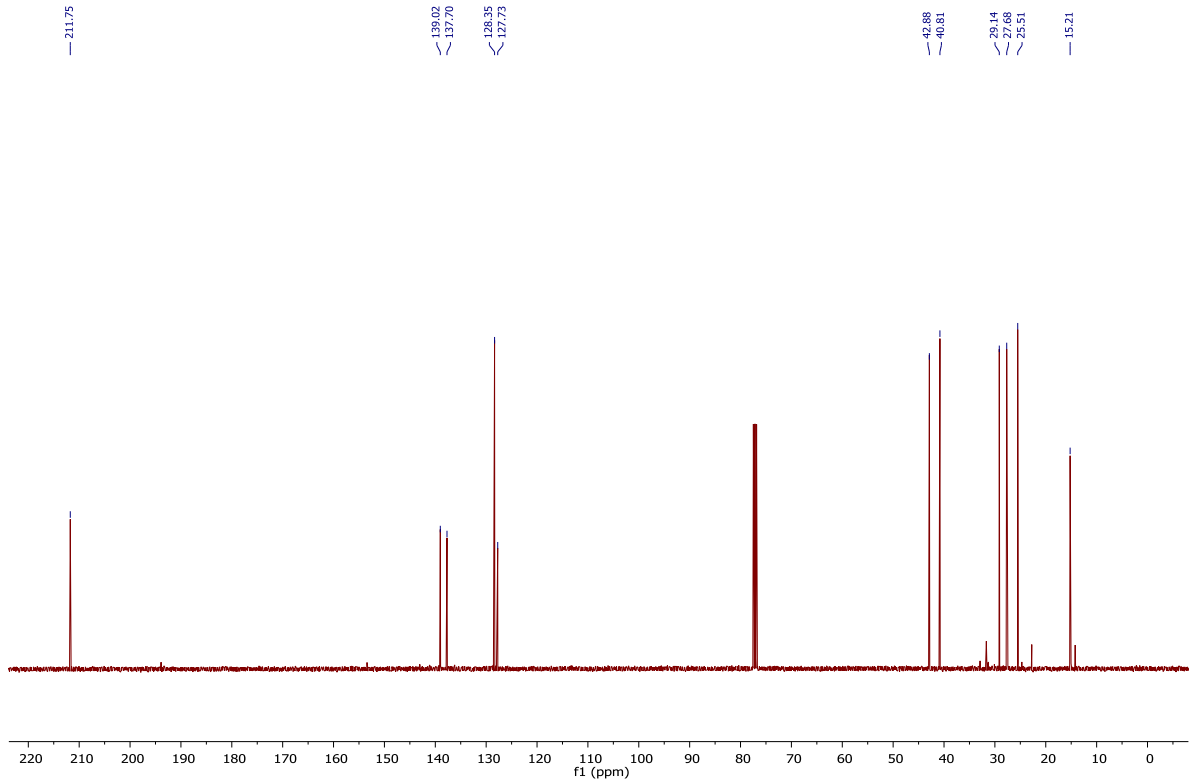
^{13}C-NMR



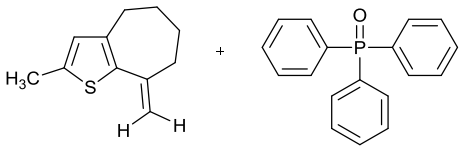
10



^{1}H-NMR

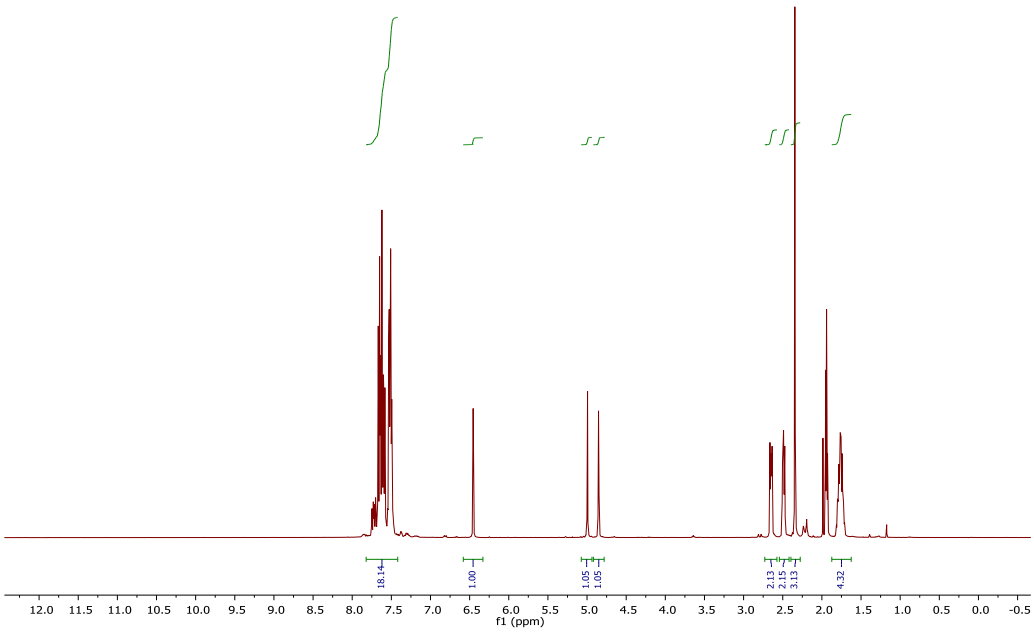


^{13}C-NMR

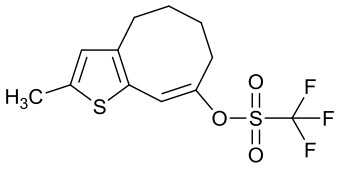


crude mixture

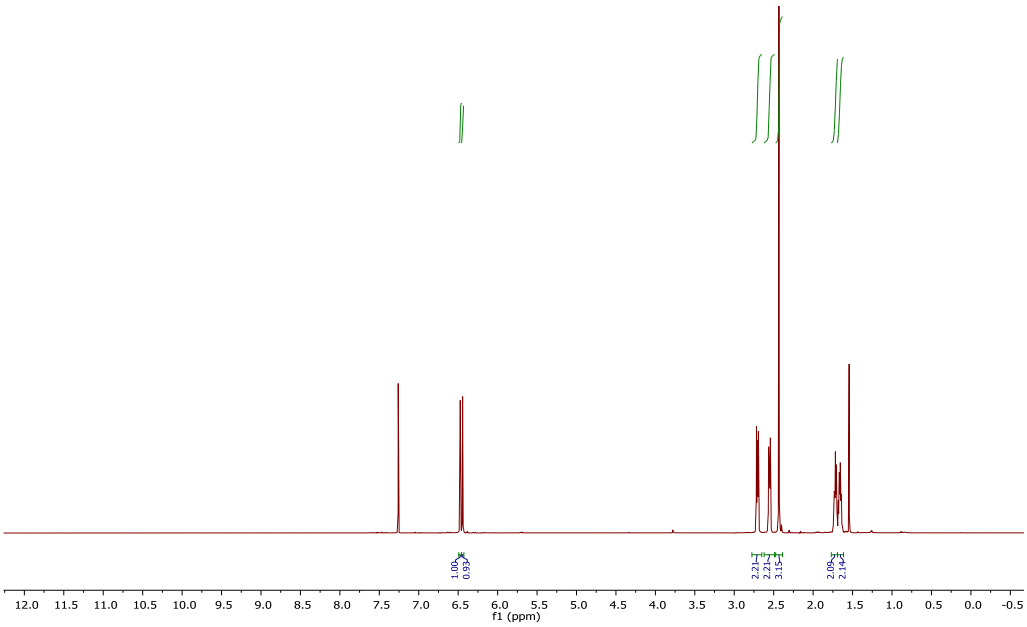
9



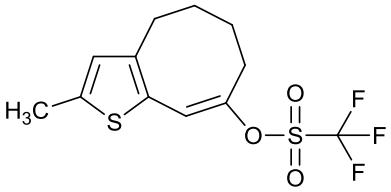
^{1}H-NMR



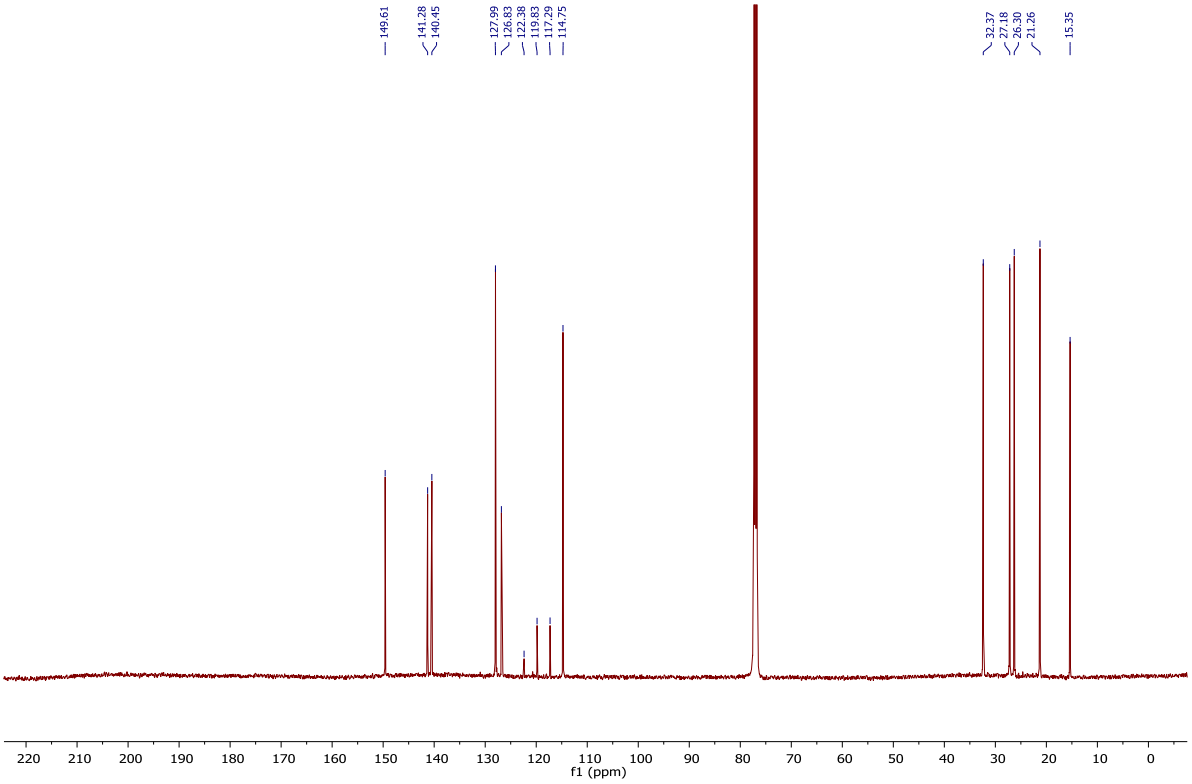
11


1.00
0.93
2.21
2.21
3.15
2.09
2.14
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

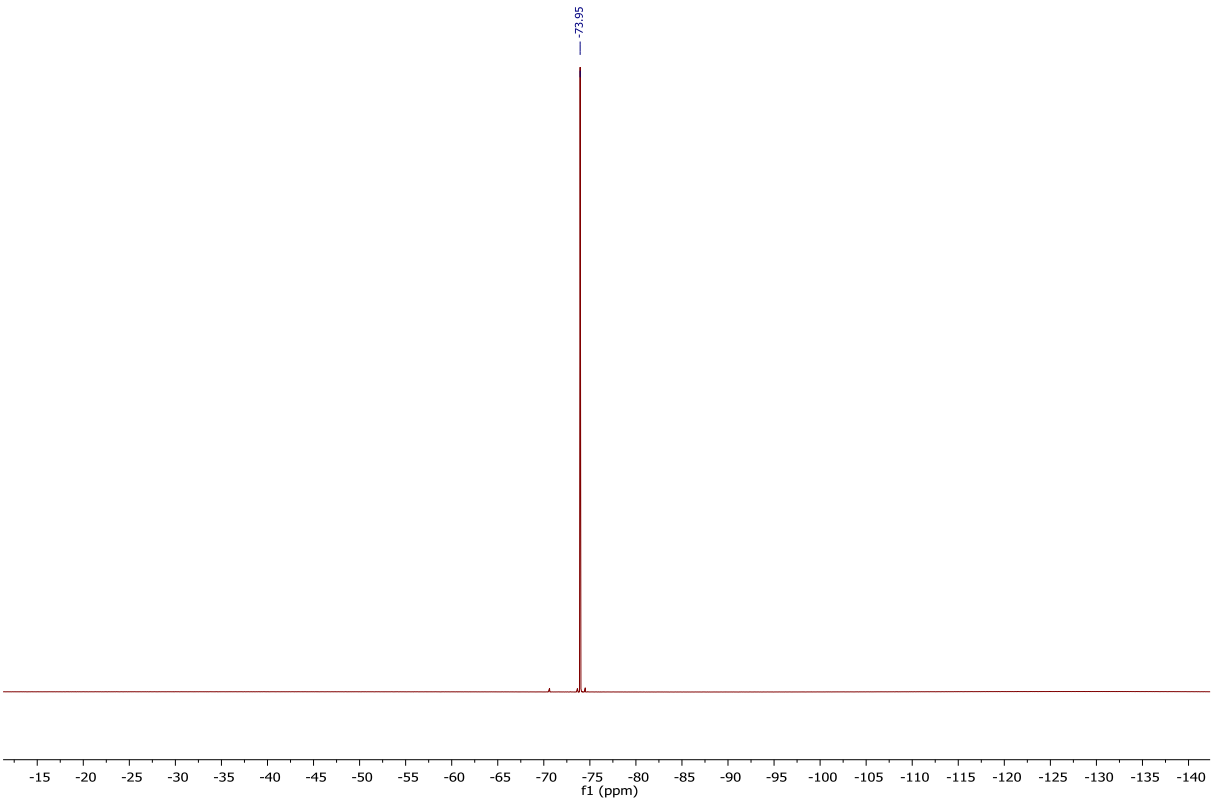
^{1}H-NMR



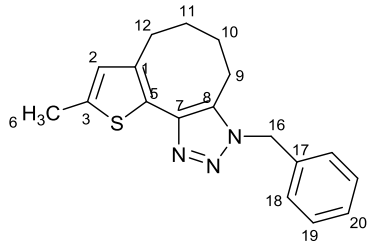
11 (continued)



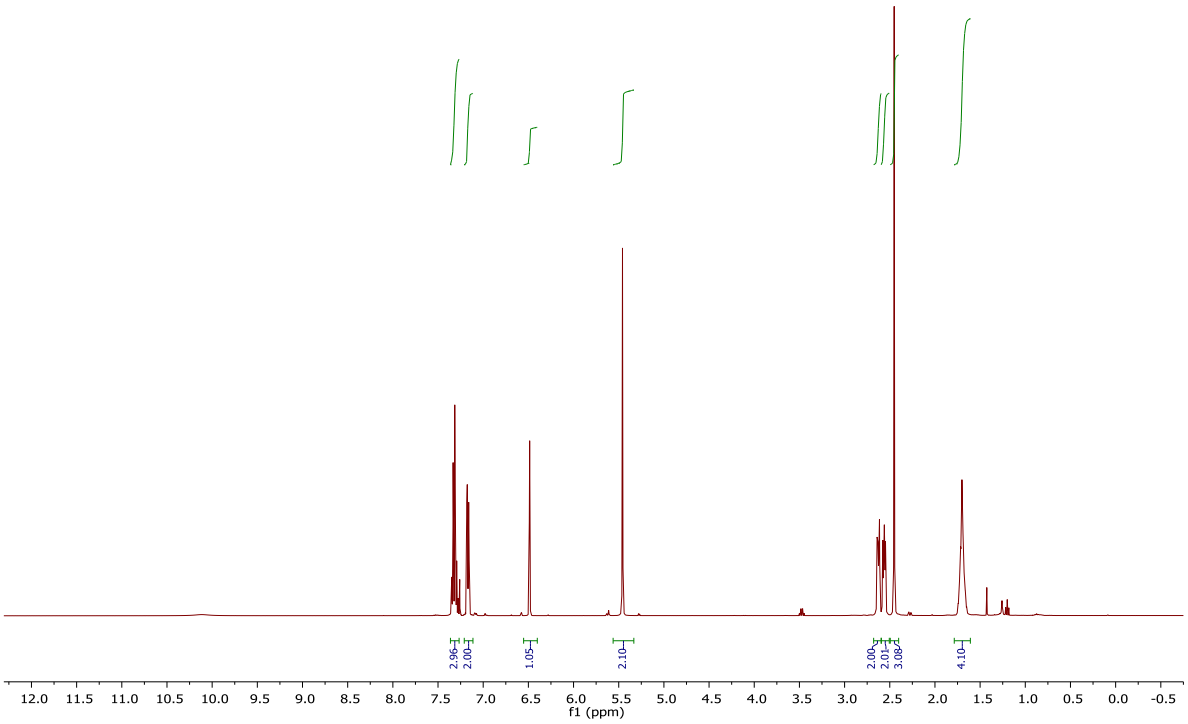
^{13}C-NMR



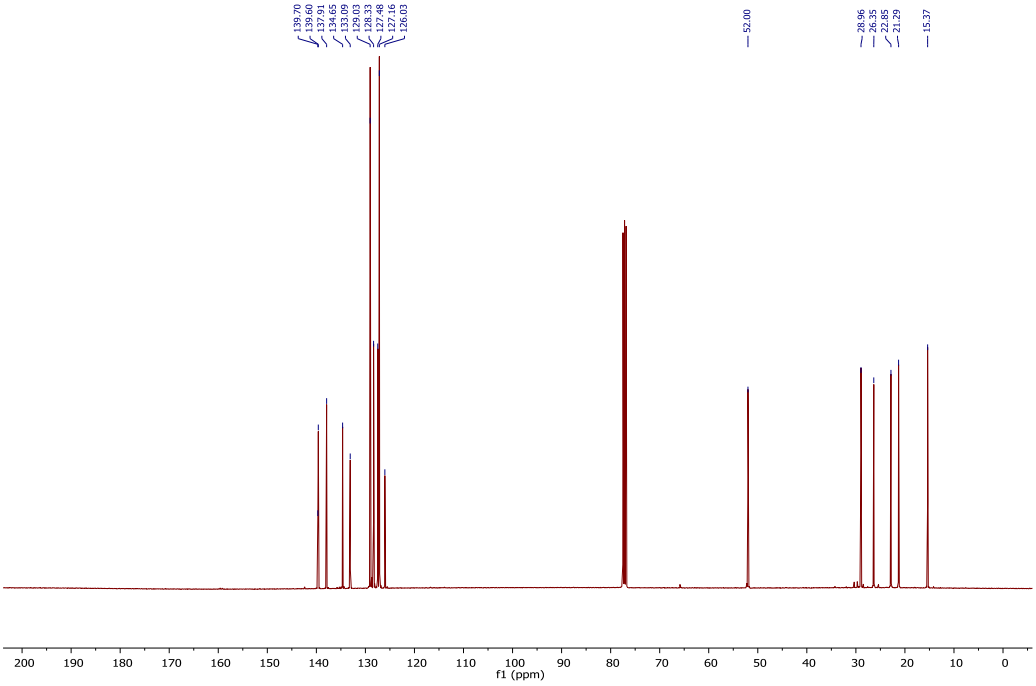
^{19}F-NMR



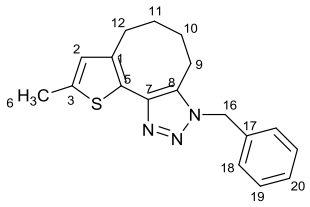
13b



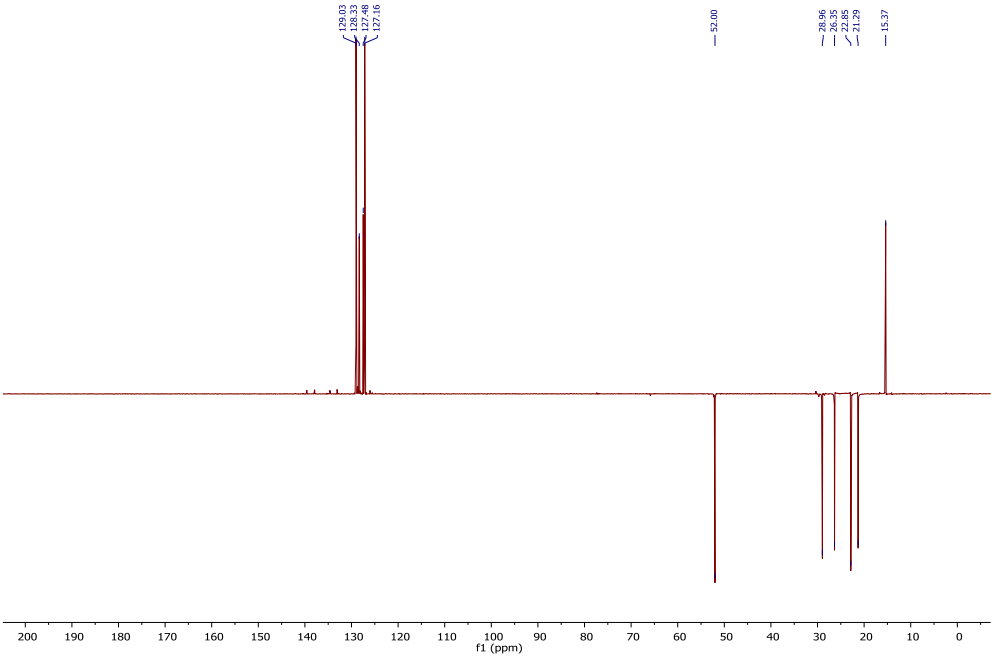
^{1}H-NMR



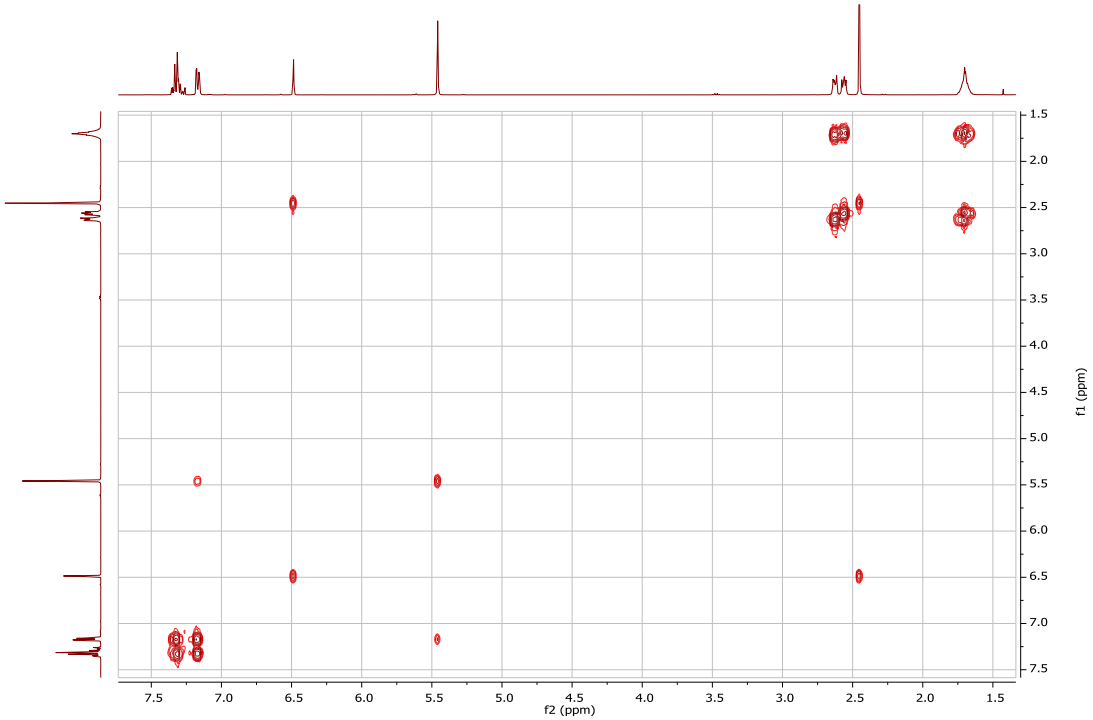
^{13}C-NMR



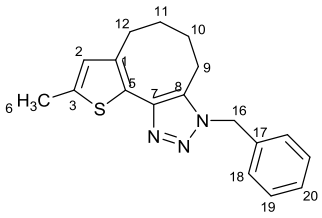
13b (continued)



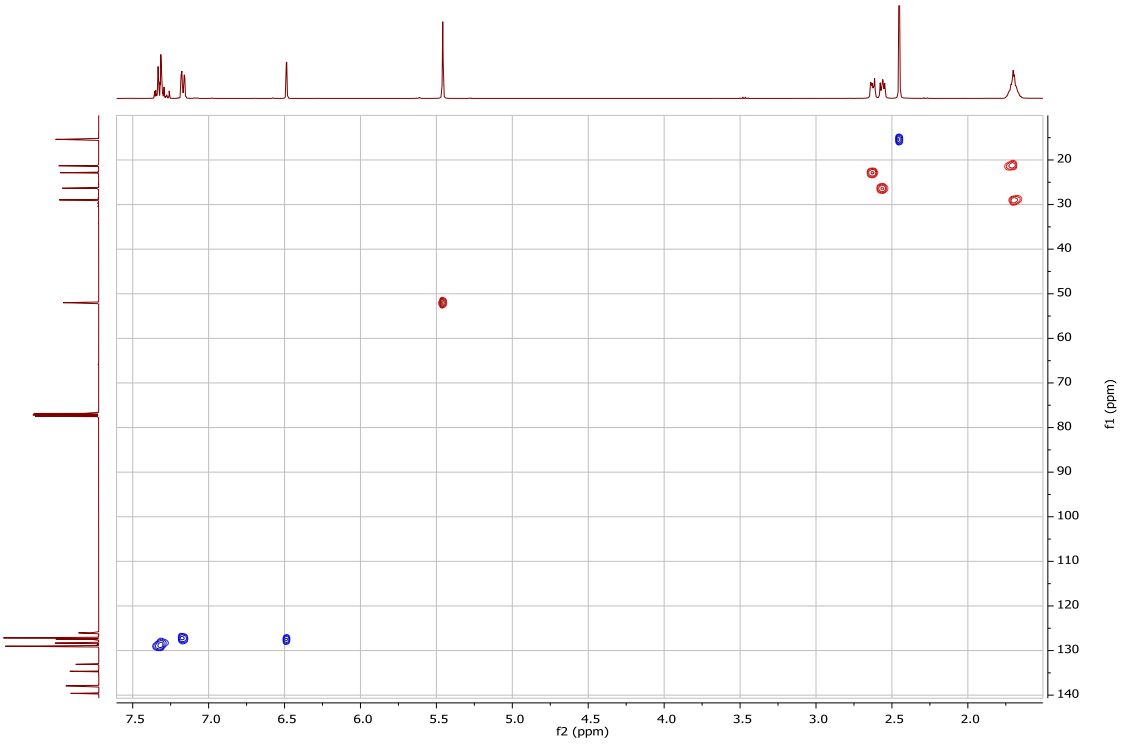
DEPT-135



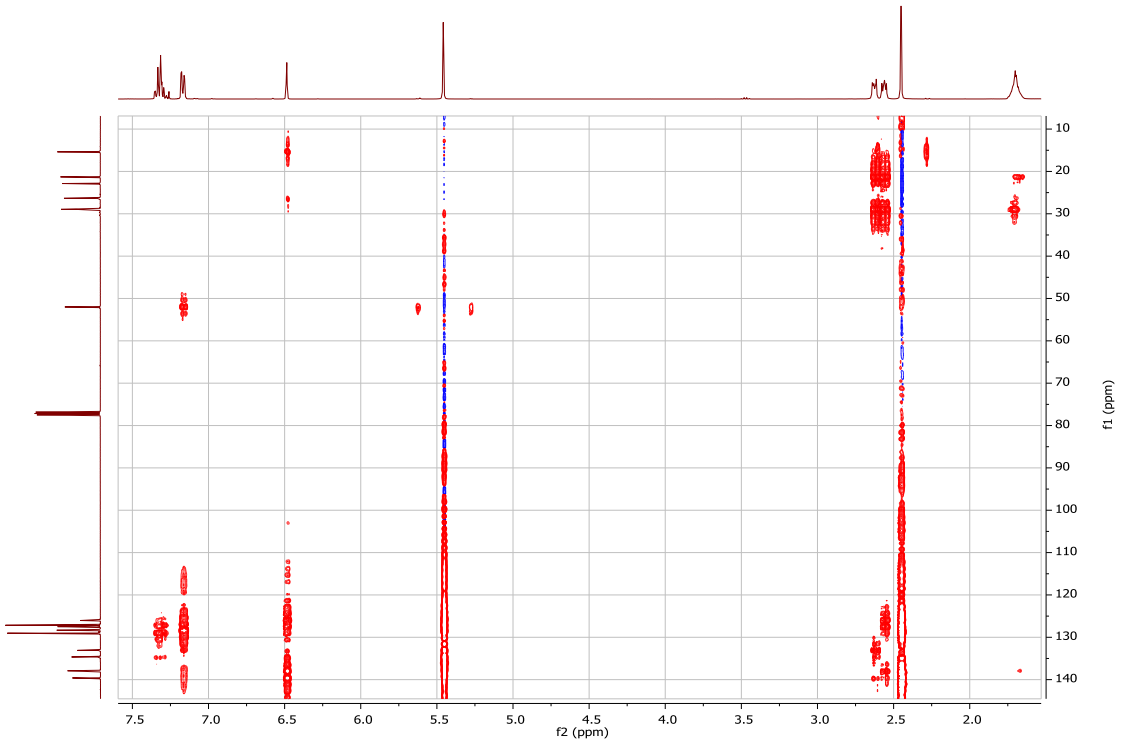
COSY



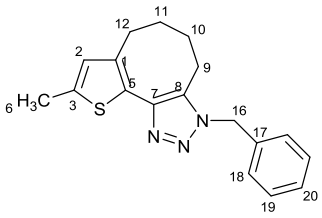
13b (continued)



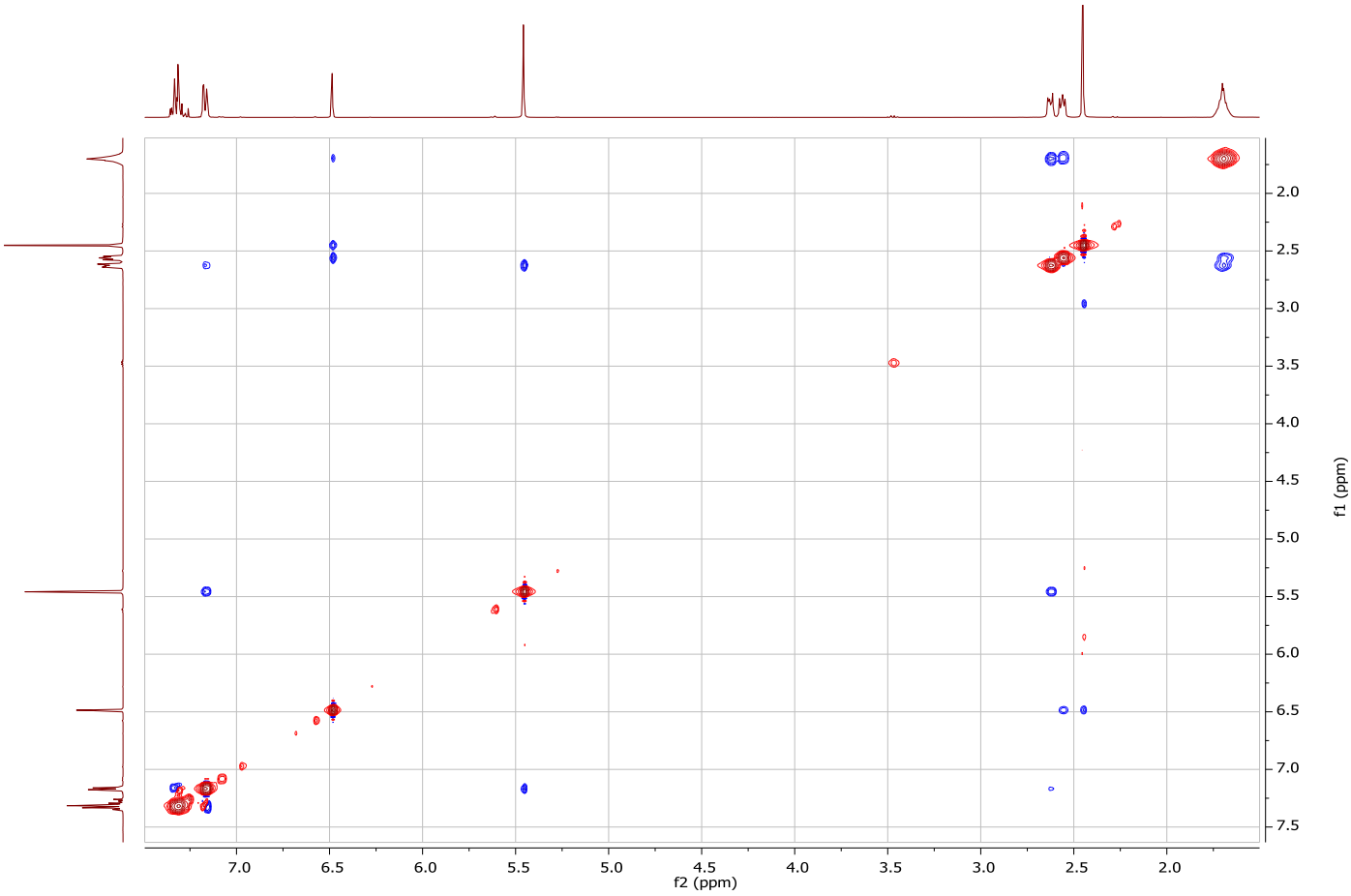
HSQC



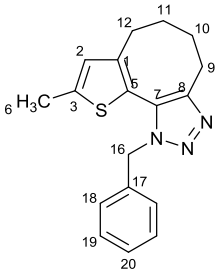
HMBC



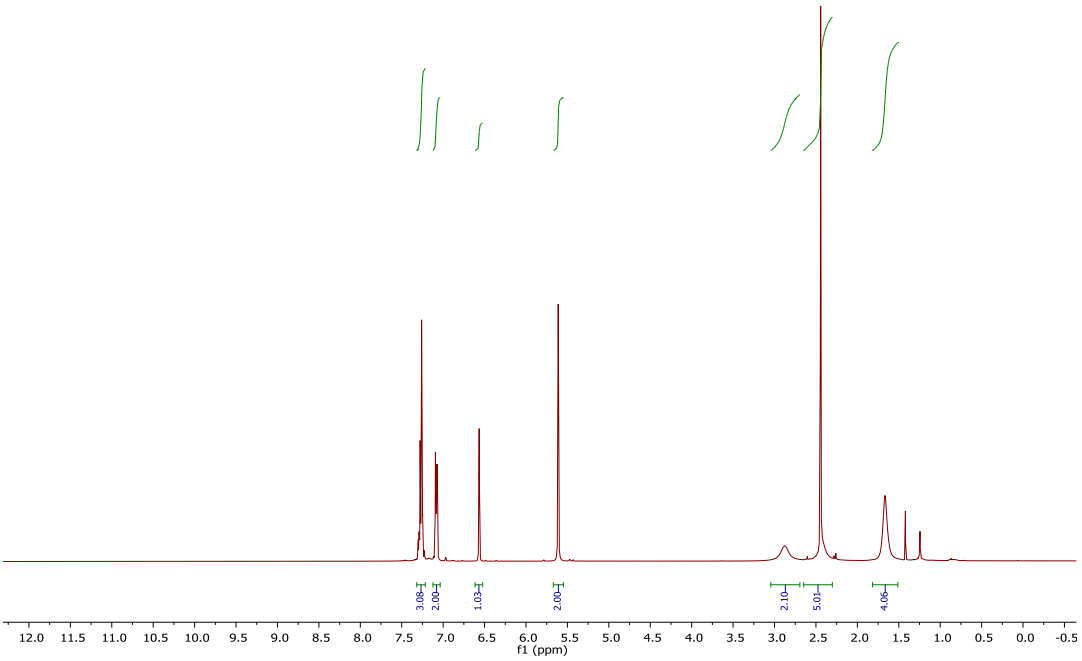
13b (continued)



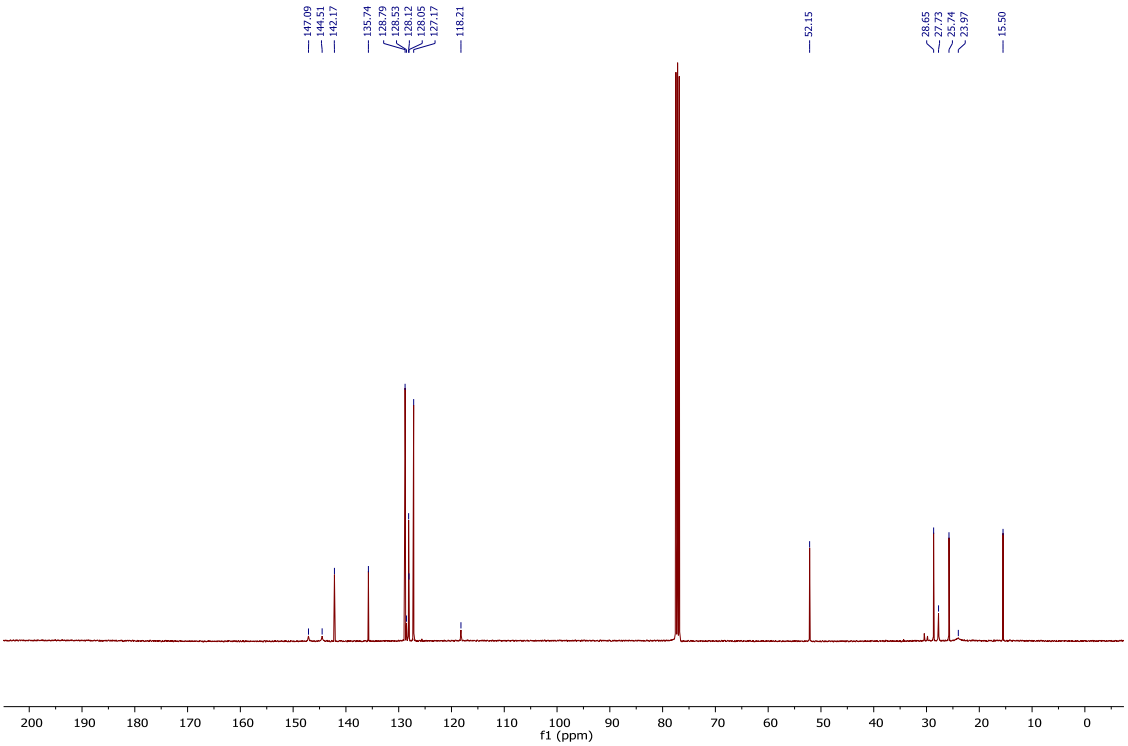
NOESY



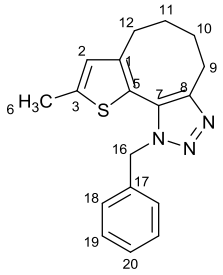
13a



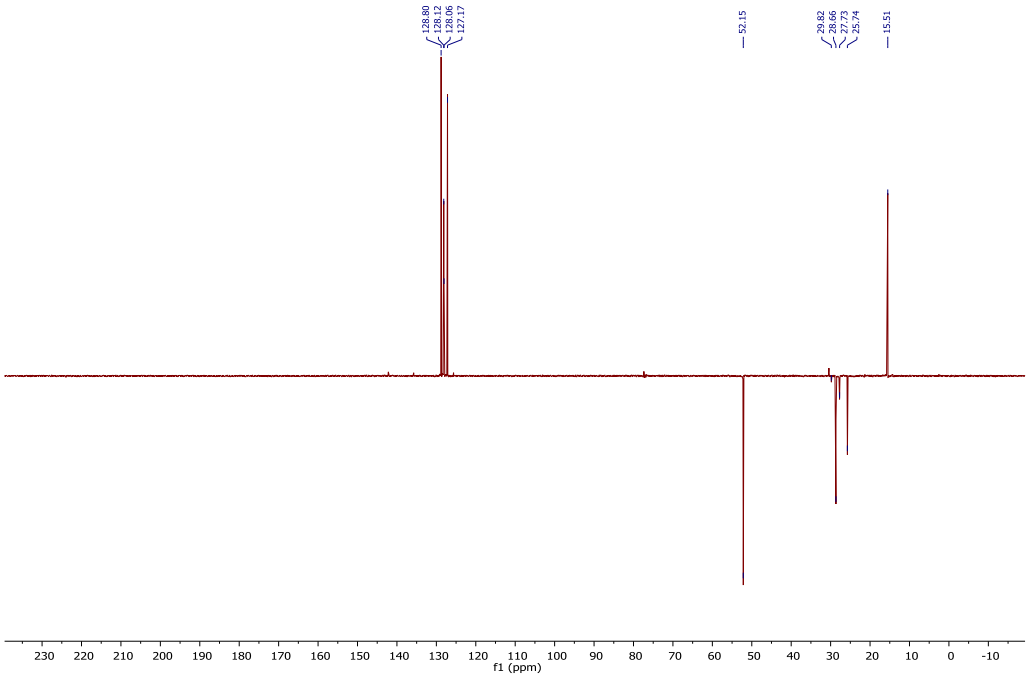
^{1}H-NMR



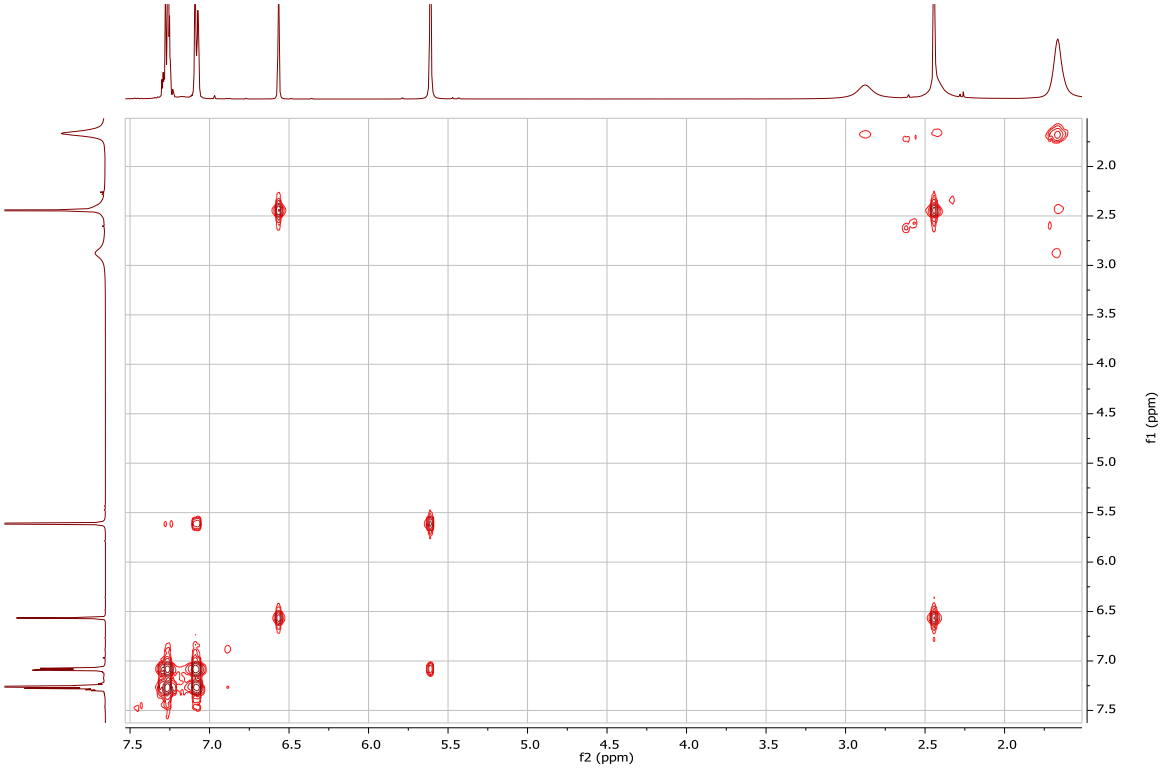
^{13}C-NMR



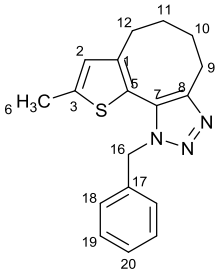
13a (continued)



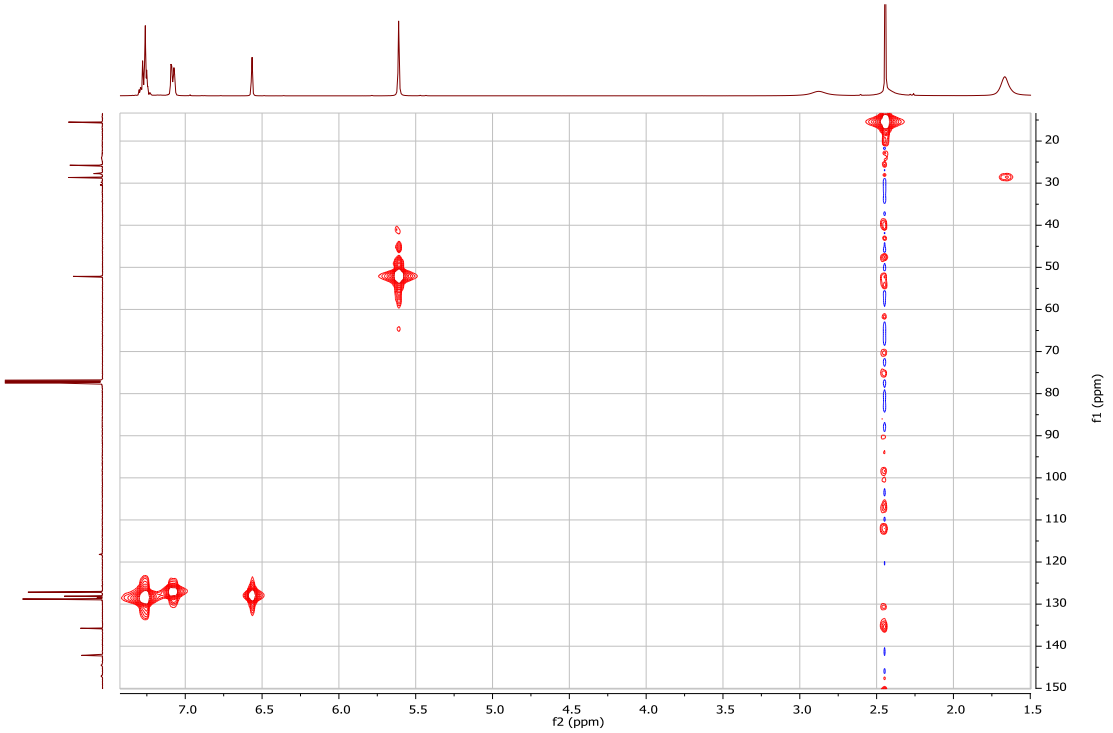
DEPT-135



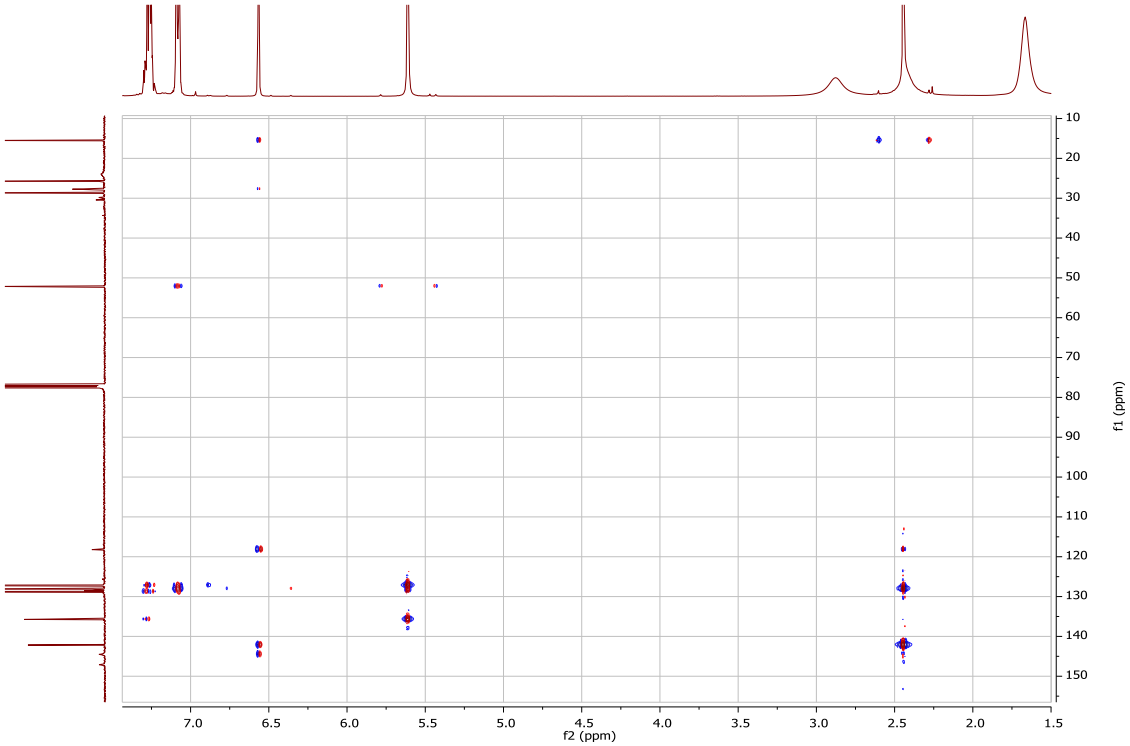
COSY



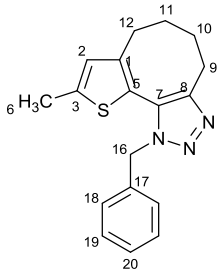
13a (continued)



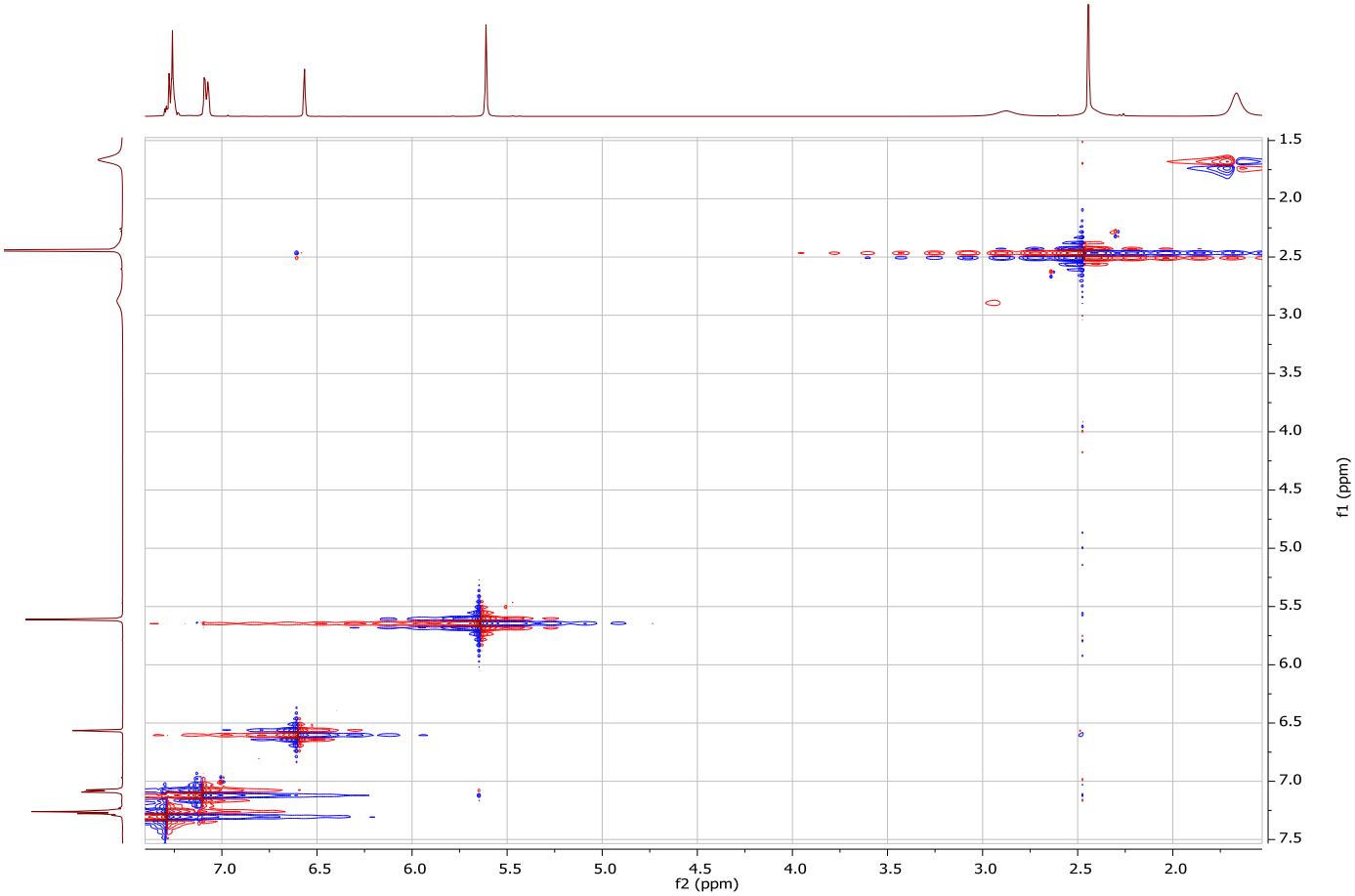
HSQC



HMBC

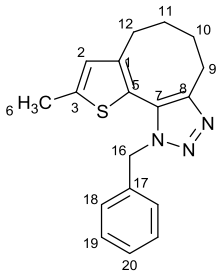


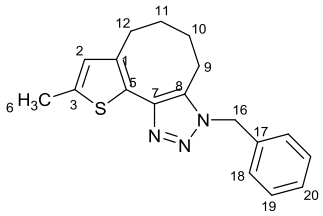
13a (continued)



NOESY

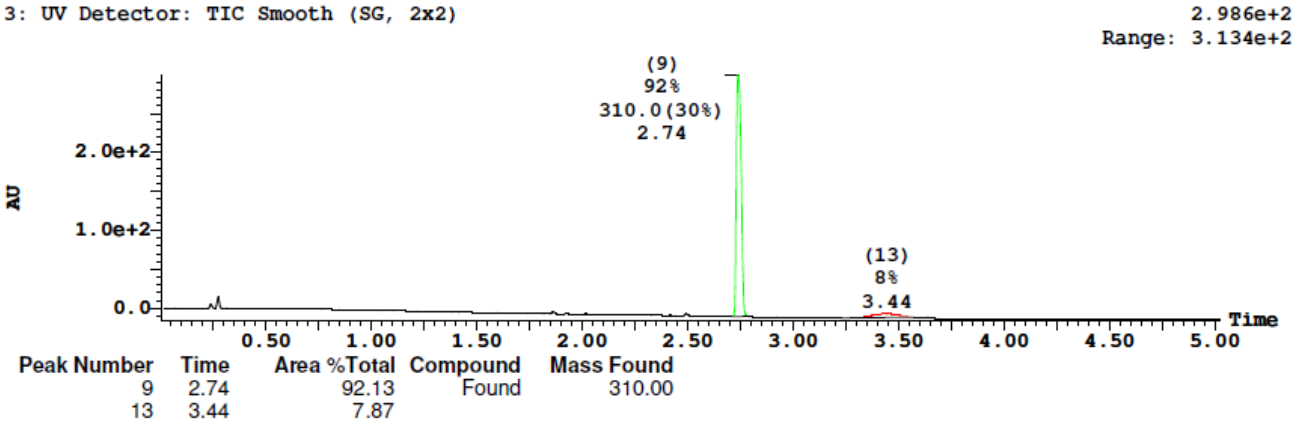
LCMS data of triazoles **13a** and **13b** mixture after flash chromatography.



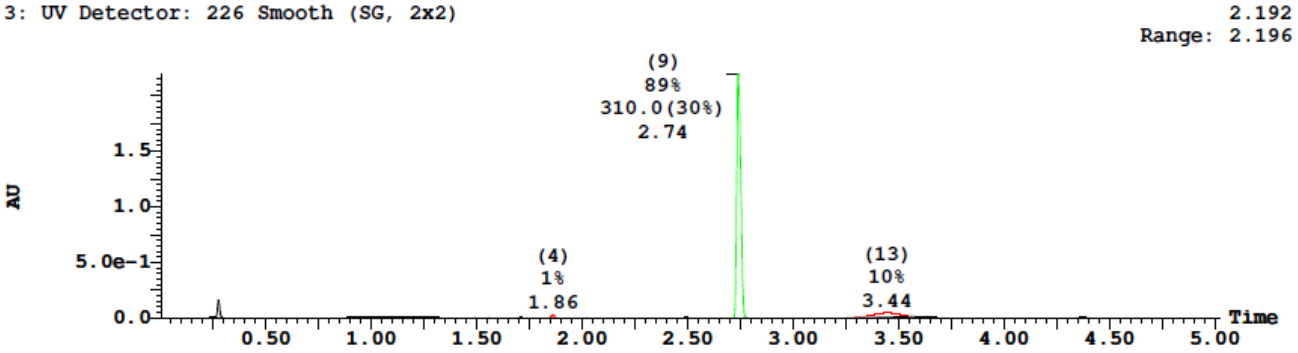


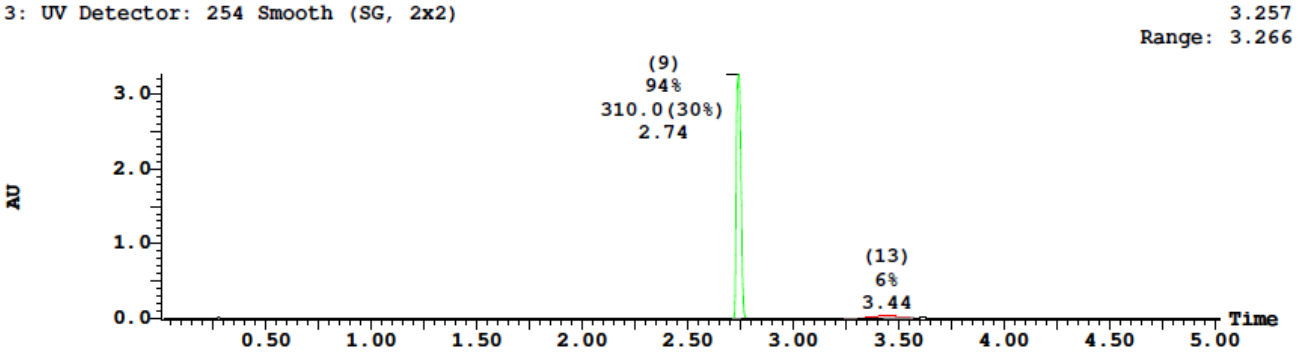
13a 13b

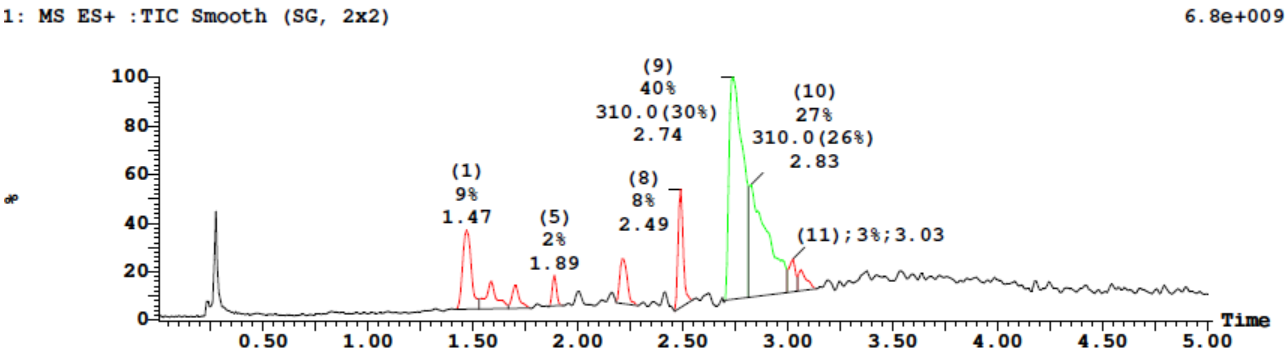
Calculated M=309.1200 g/mol, found $[M+H]^+$=310.1



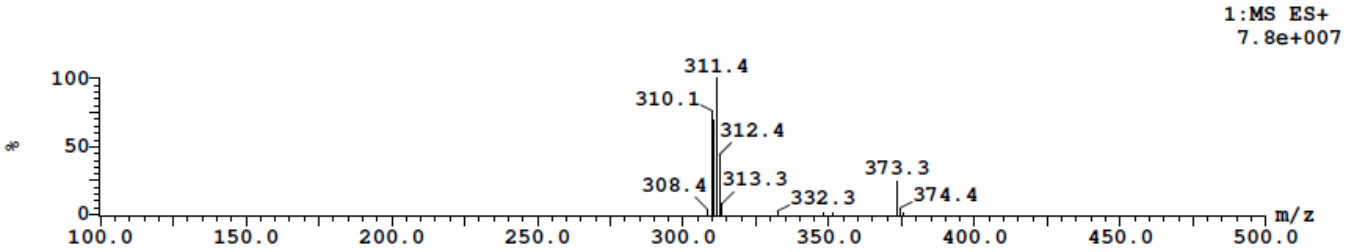
Peak Number	Time	Area %Total	Compound	Mass Found
9	2.74	92.13	Found	310.00
13	3.44	7.87		





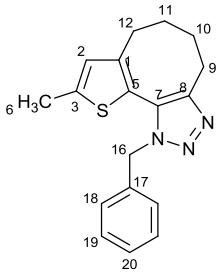


Peak ID	Compound	Time	Mass Found	BPM
9	Found	2.74	311.00	311

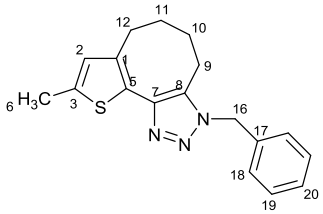


Analytical HPLC chromatogram of triazoles **13b** and **13a** mixture after flash chromatography, before HPLC separation of individual **13a** and **13b**

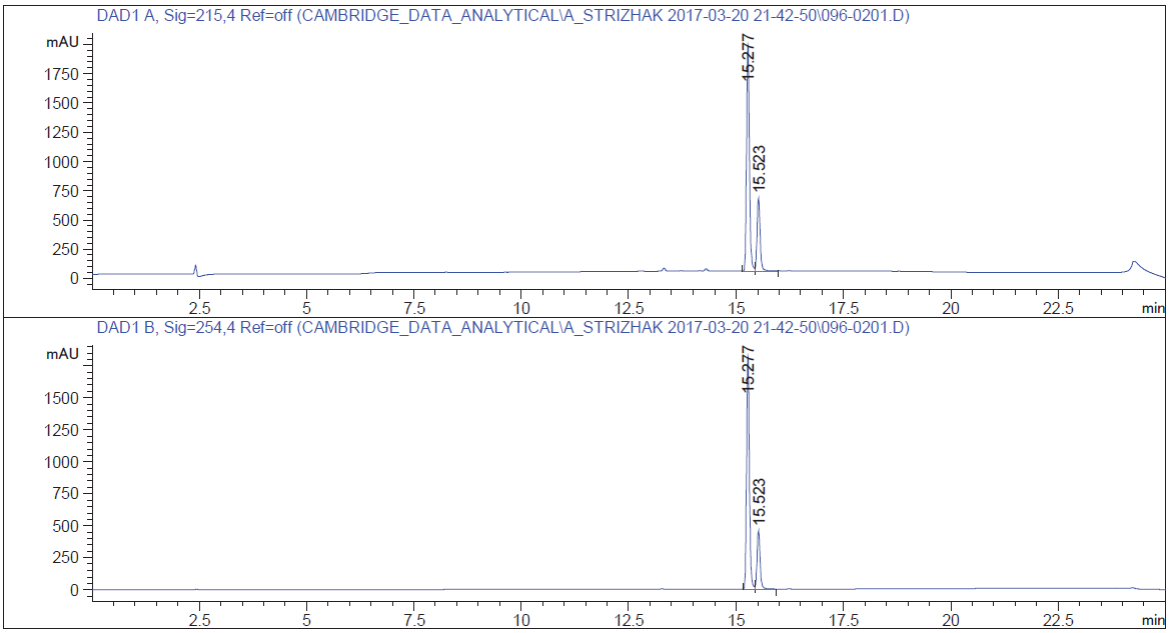
Gradient 5-95% MeCN in 0.1% TFA/H_2O



13a, RT=15.523 min



13b, RT=15.277 min



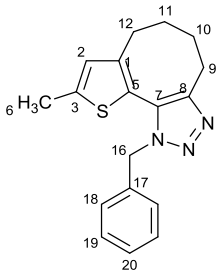
Signal 1: DAD1 A, Sig=215,4 Ref=off

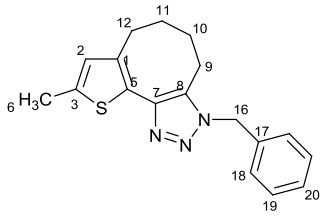
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.277	VV	0.0725	9040.08887	1936.06482	74.6842
2	15.523	VB	0.0753	3064.33057	623.61273	25.3158
Totals :				1.21044e4	2559.67755	

Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.277	BV	0.0711	8287.77539	1821.32764	78.6310
2	15.523	VB	0.0752	2252.31519	459.37827	21.3690
Totals :				1.05401e4	2280.70590	

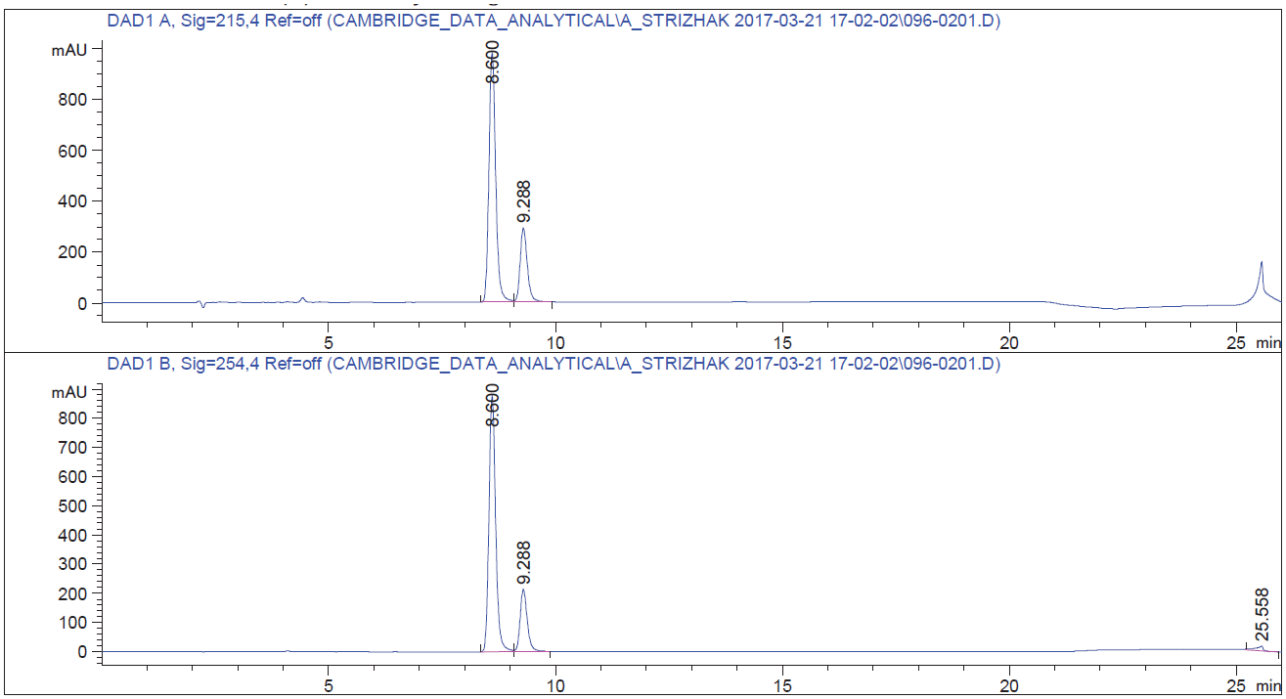
Gradient 45-60% MeCN in 0.1% TFA/H_2O, the same gradient was used for preparative separation.





13a, RT=9.288 min, 24%

13b, RT=8.600 min, 76%



Signal 1: DAD1 A, Sig=215,4 Ref=off

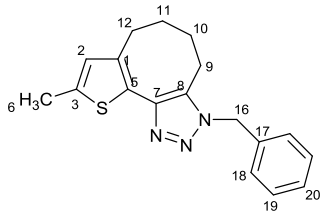
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.600	BV	0.1611	1.01612e4	979.15552	76.0594
2	9.288	VB	0.1662	3198.34619	290.98364	23.9406
Totals :				1.33595e4	1270.13916	

Signal 2: DAD1 B, Sig=254,4 Ref=off

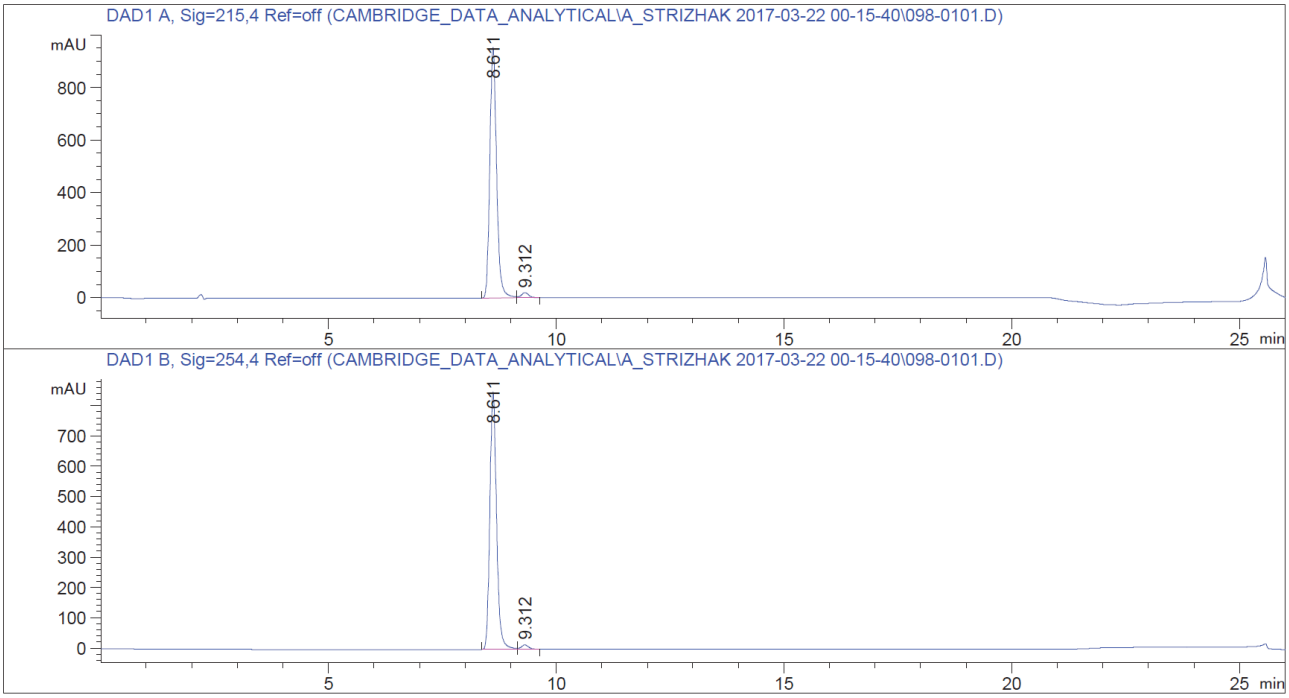
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.600	BV	0.1577	8994.83496	876.62555	78.2323
2	9.288	VB	0.1659	2336.34888	213.17847	20.3203
3	25.558	BB	0.1338	166.41132	16.20544	1.4474
Totals :				1.14976e4	1106.00946	

HPLC chromatograms of individual isomers **13a** and **13b** after preparative HPLC.

Gradient 45-60% MeCN in 0.1% TFA/H_2O



13b, RT=8.611 min



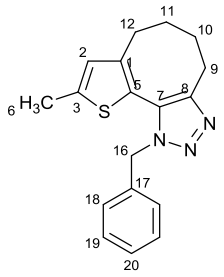
Signal 1: DAD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.611	BV	0.1611	9891.81055	952.83917	97.6632
2	9.312	VB	0.1747	236.68646	20.19464	2.3368
Totals :				1.01285e4	973.03381	

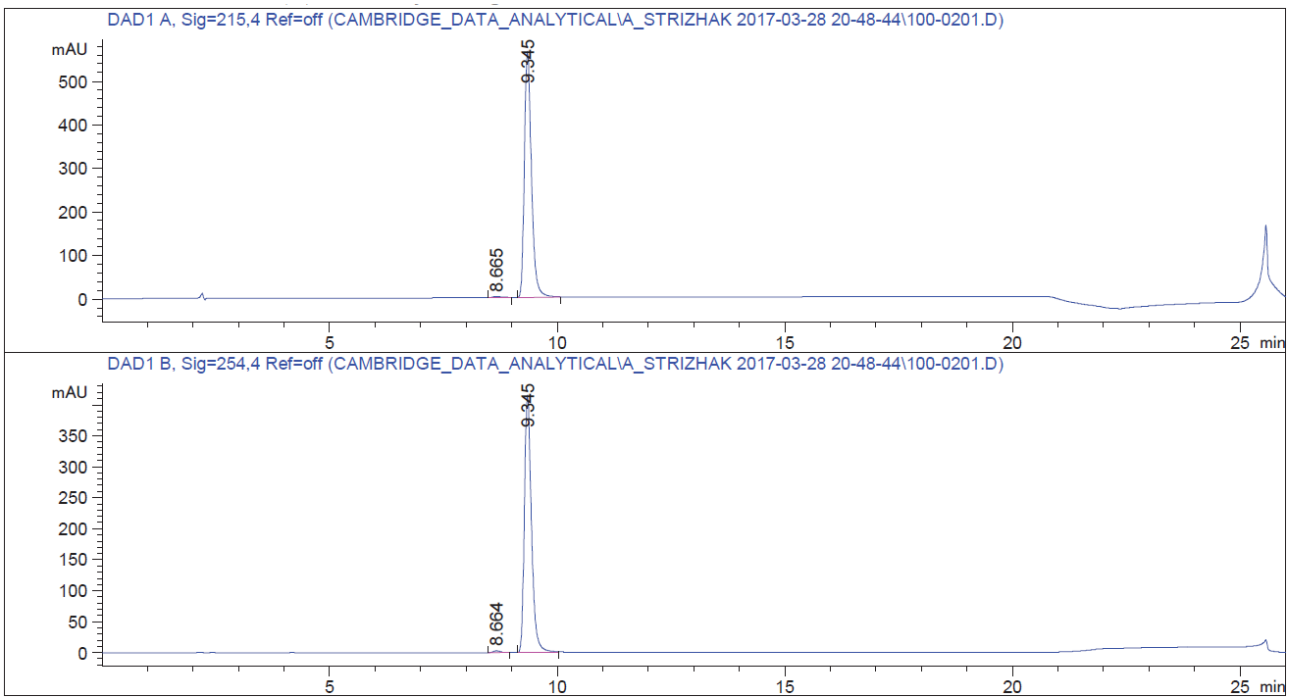
Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.611	BV	0.1579	8729.88770	849.53381	98.0154
2	9.312	VB	0.1766	176.75974	14.88245	1.9846
Totals :				8906.64743	864.41626	

Gradient 45-60% MeCN in 0.1% TFA/H_2O



13a, RT=9.345 min

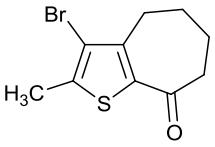


Signal 1: DAD1 A, Sig=215,4 Ref=off

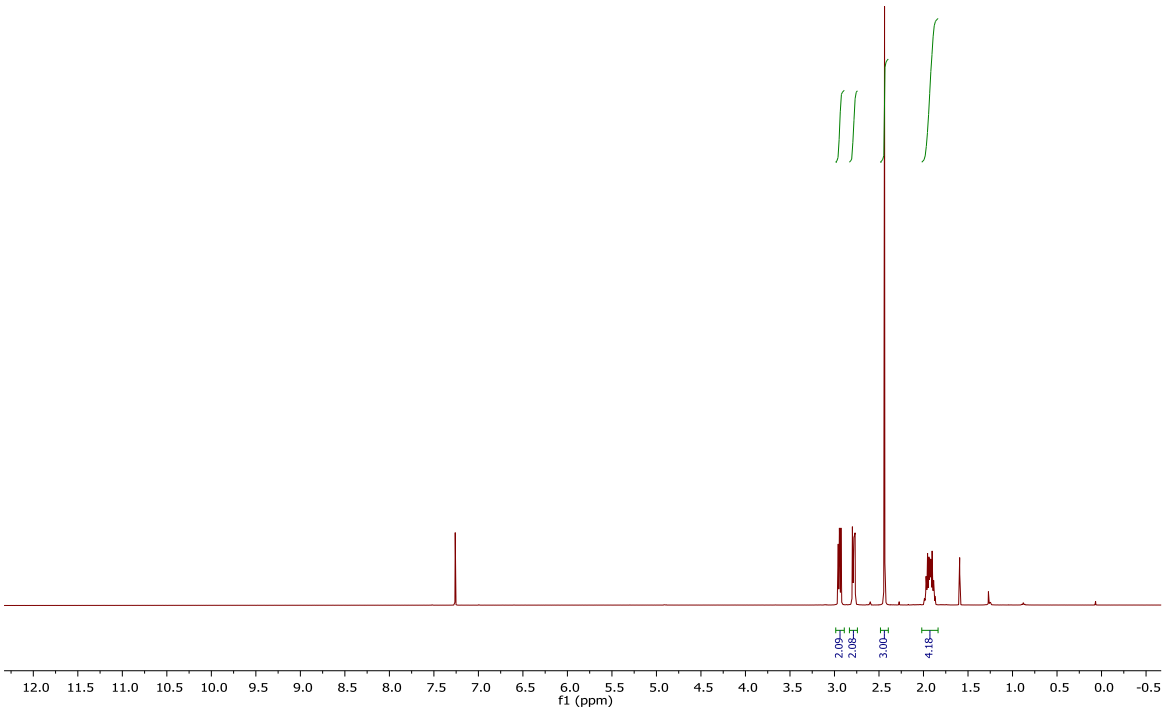
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.665	BB	0.1828	39.50787	3.09756	0.6433
2	9.345	BB	0.1644	6101.79395	563.38007	99.3567
Totals :				6141.30181	566.47762	

Signal 2: DAD1 B, Sig=254,4 Ref=off

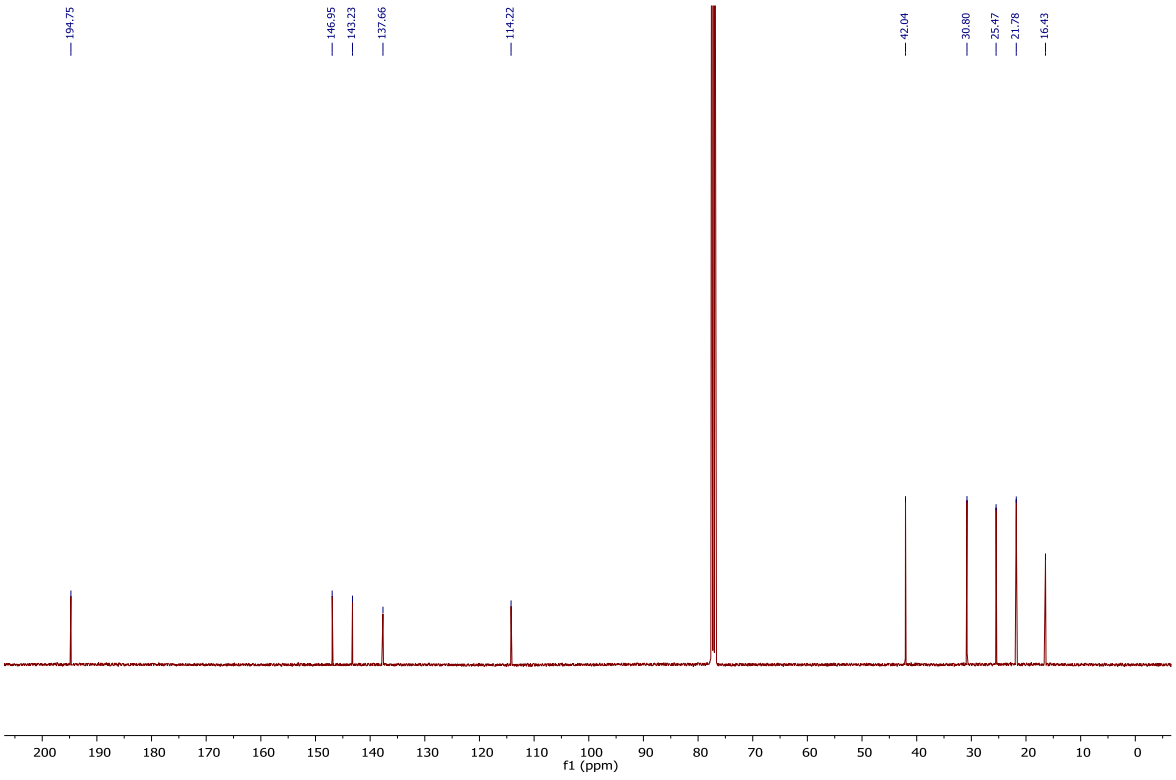
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.664	BB	0.1643	29.55354	2.68802	0.6584
2	9.345	BB	0.1637	4459.10596	413.79846	99.3416
Totals :				4488.65949	416.48649	



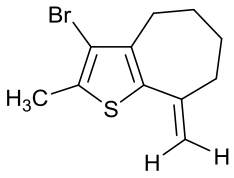
16



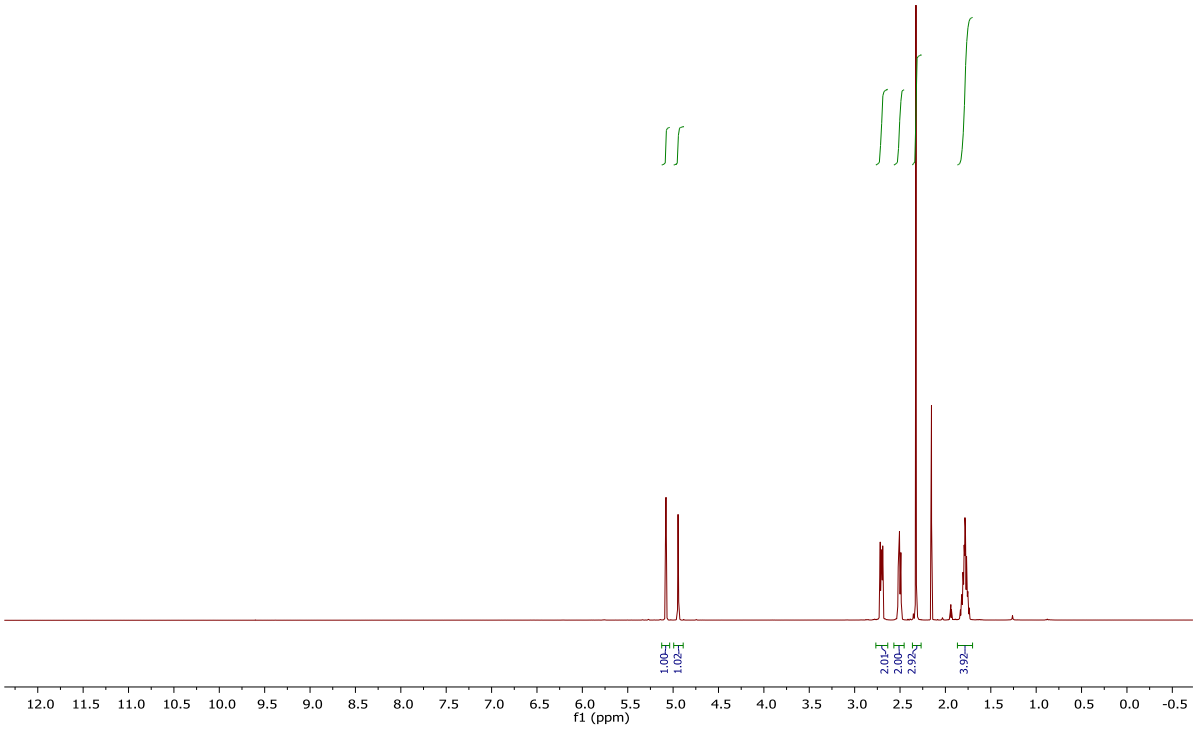
^{1}H-NMR



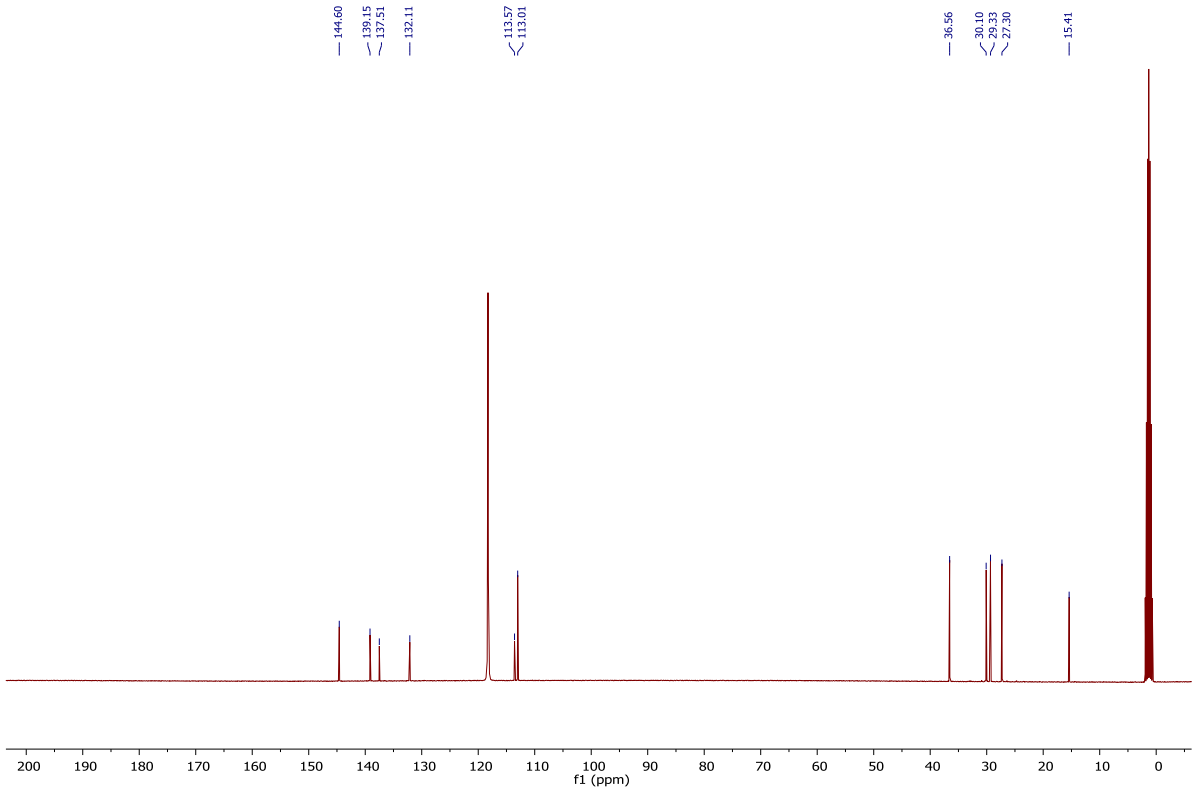
^{13}C-NMR



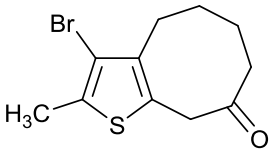
17



^{1}H-NMR



^{13}C-NMR



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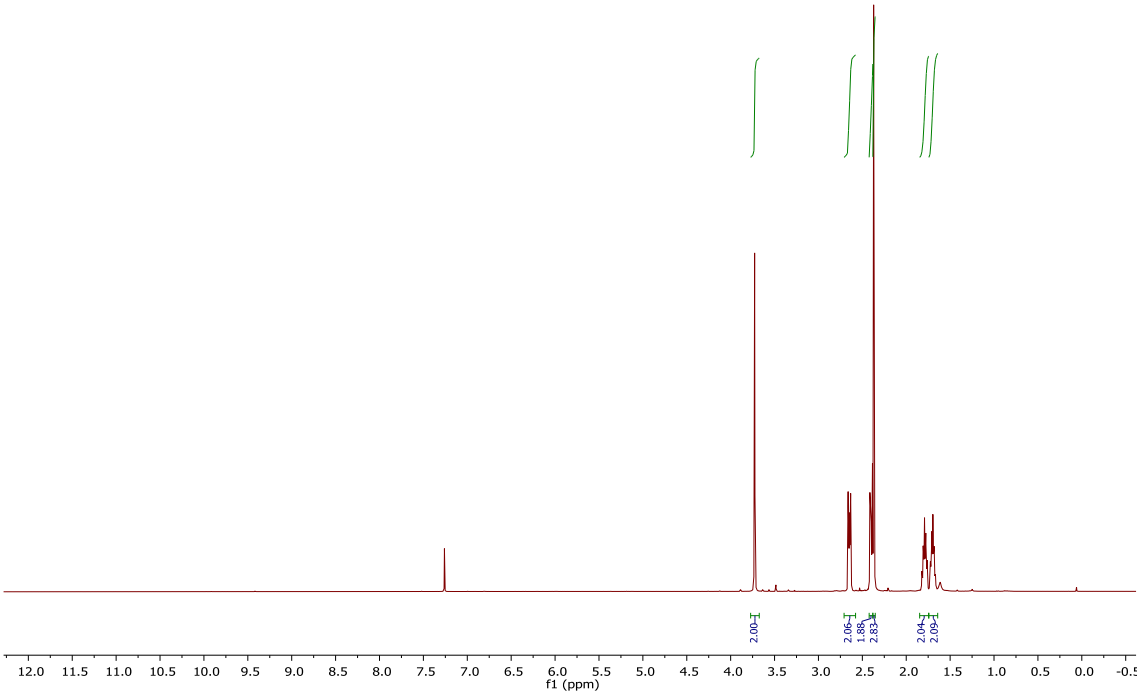

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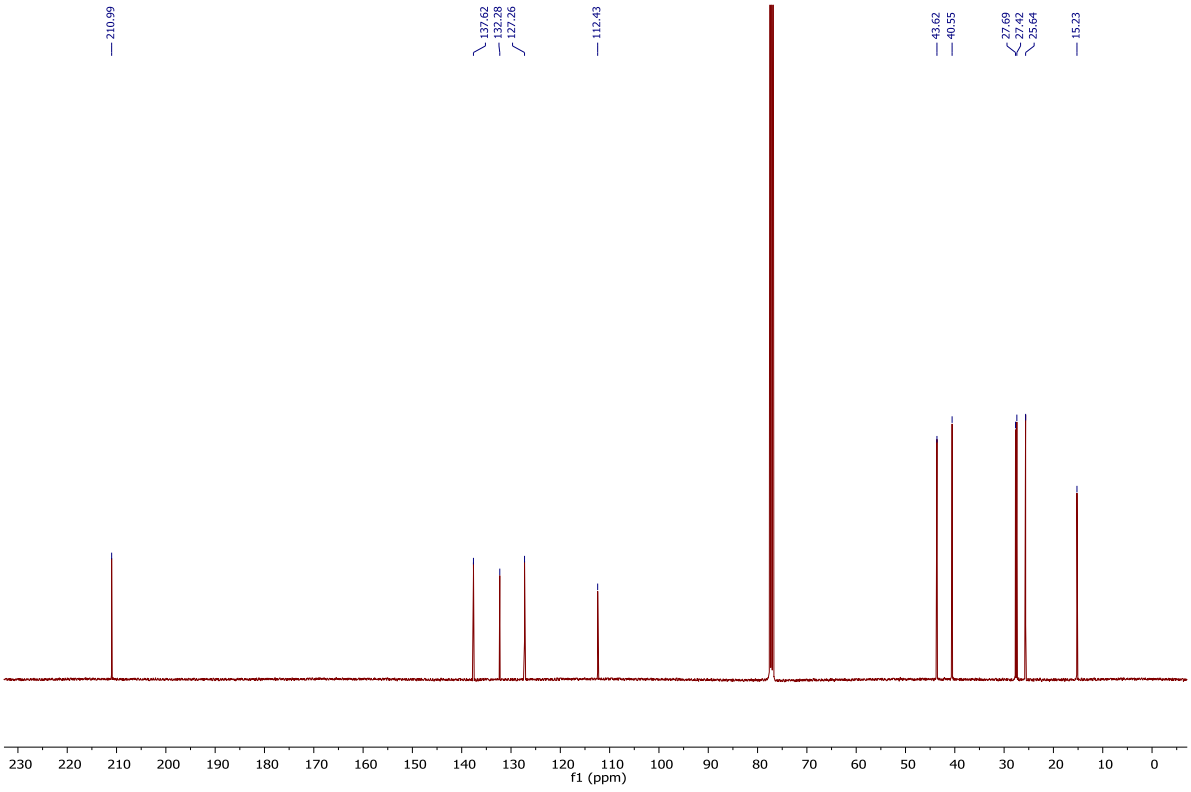
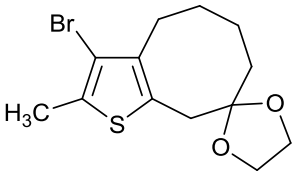


^{13}C-NMR



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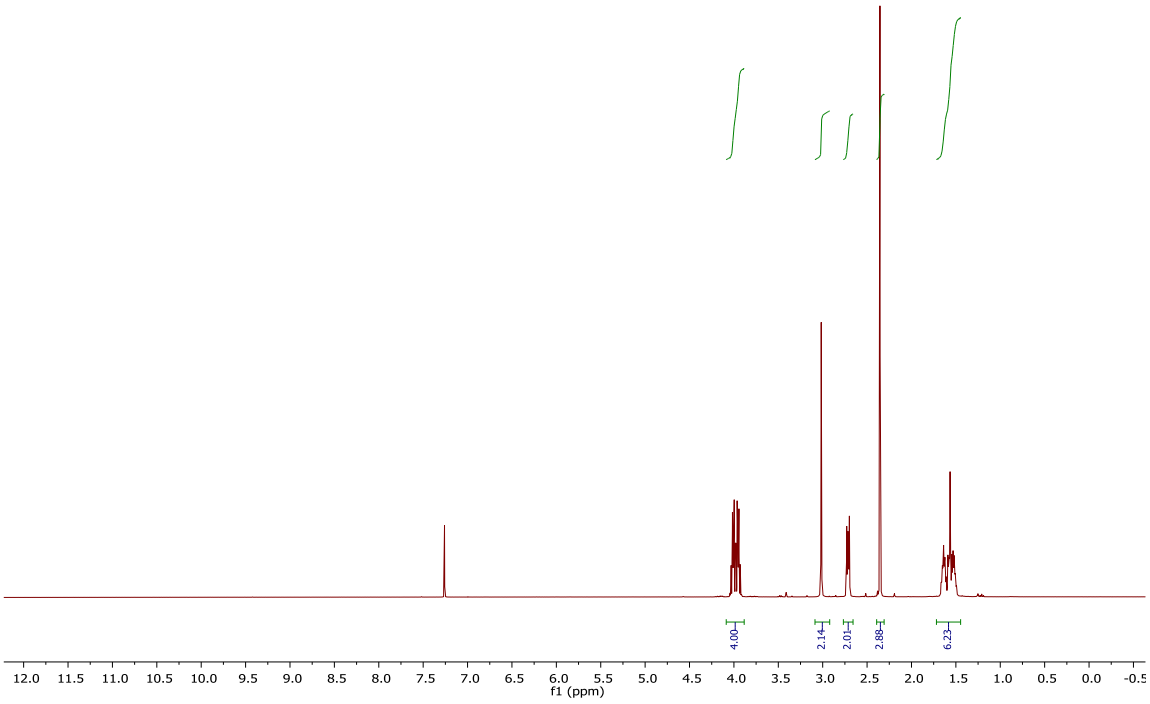
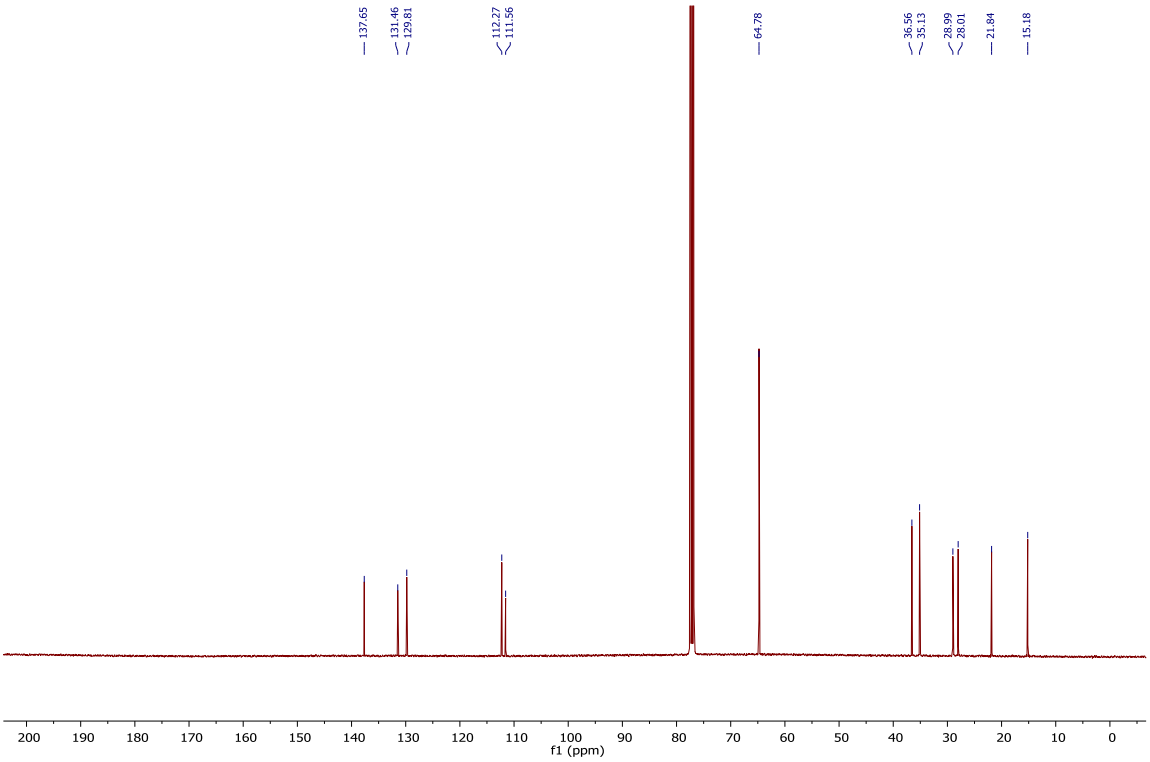
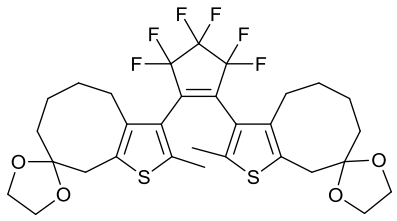


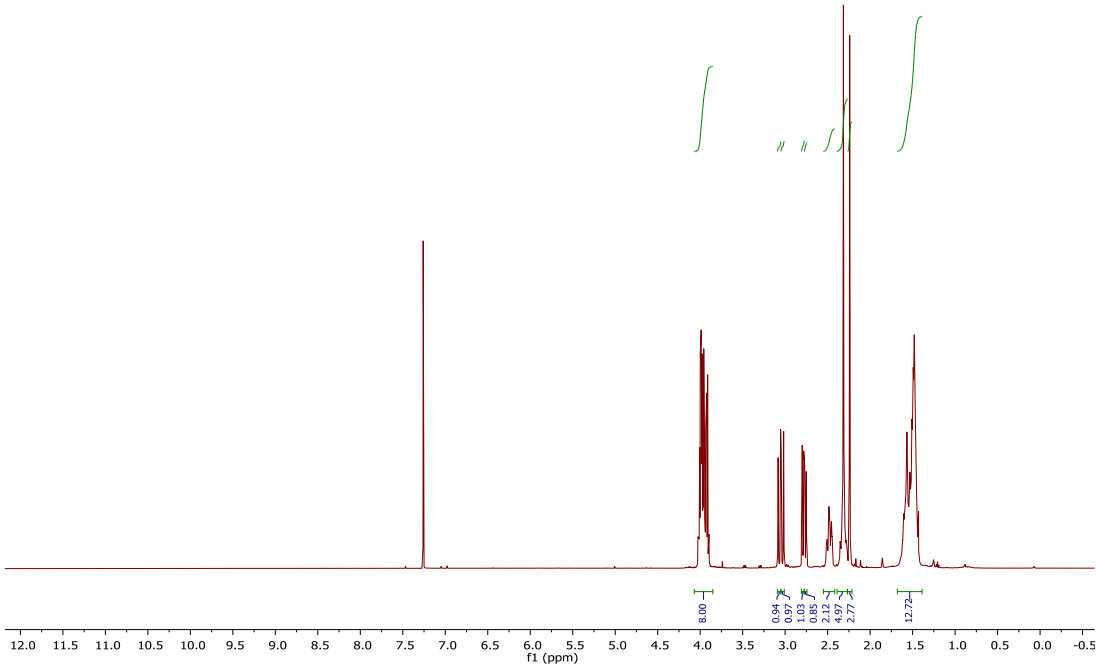
^{1}H-NMR



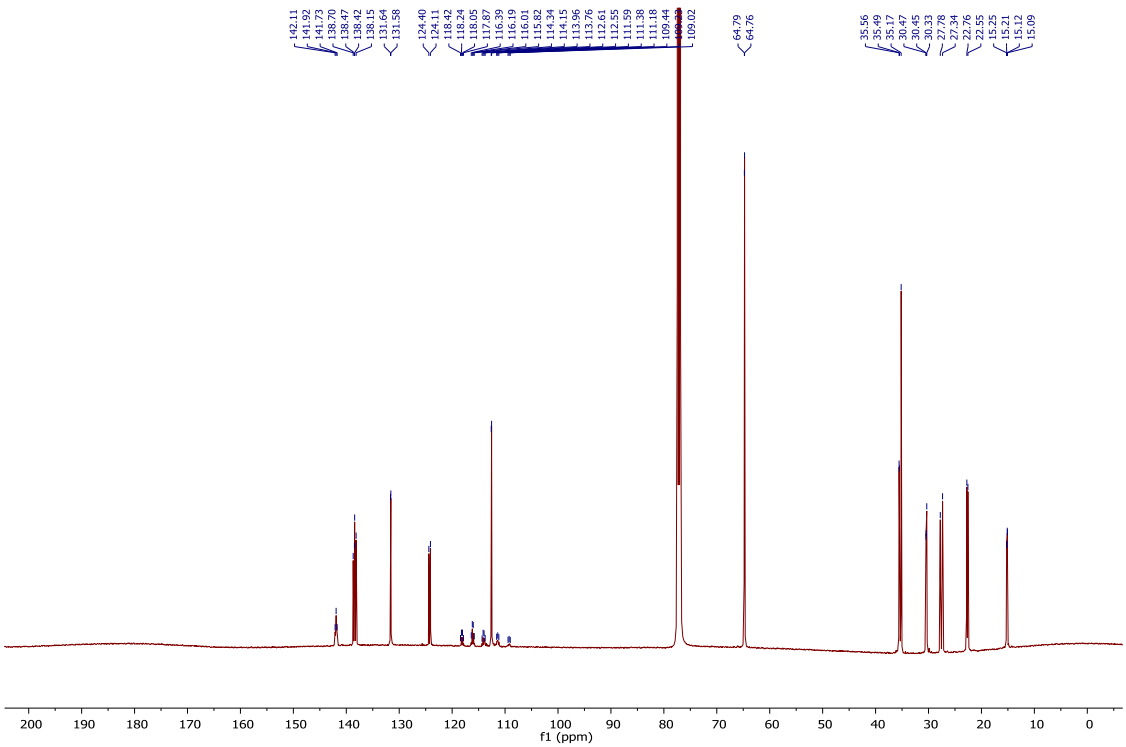
^{13}C-NMR



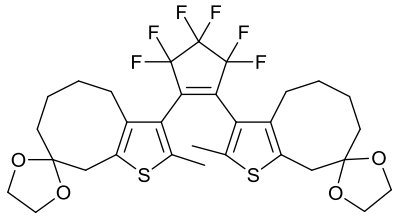
20



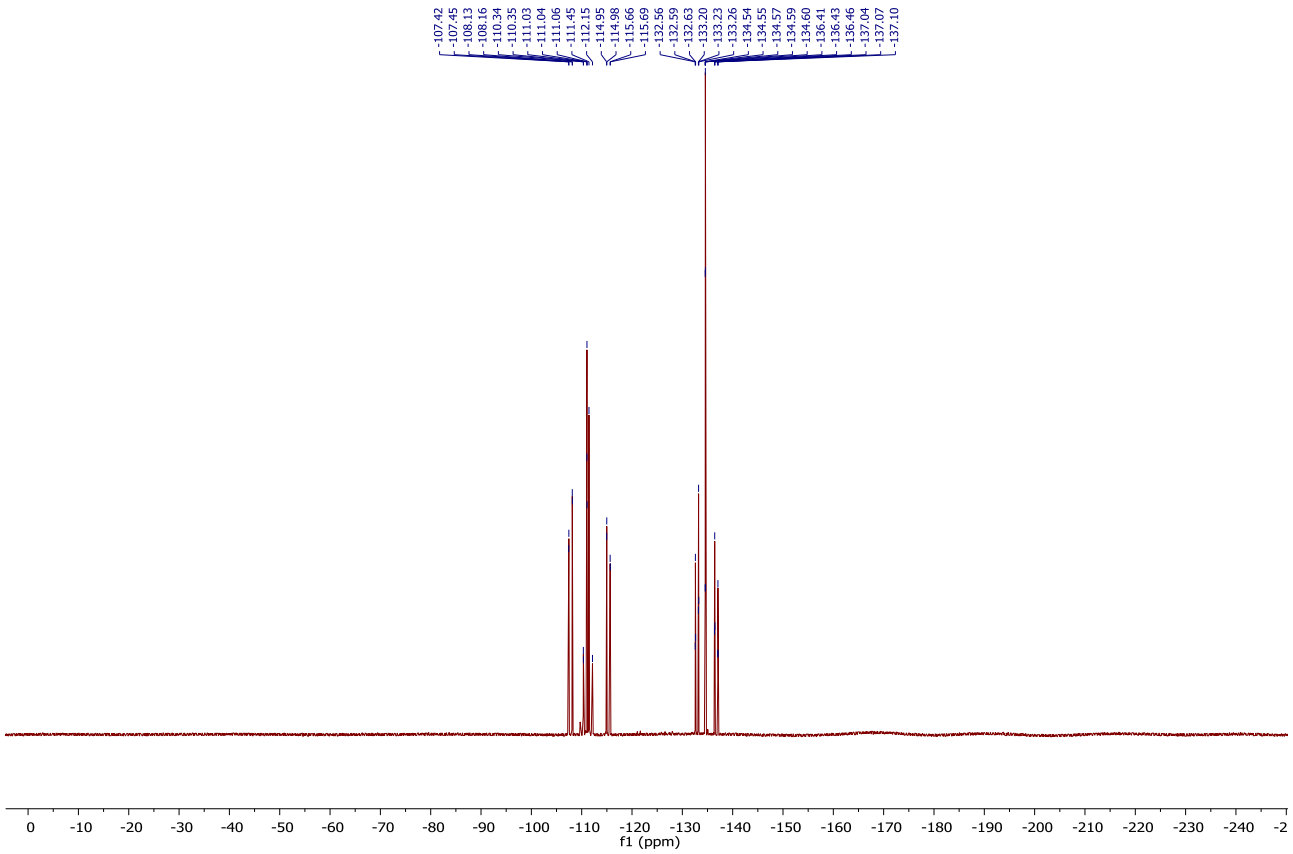
^{1}H-NMR



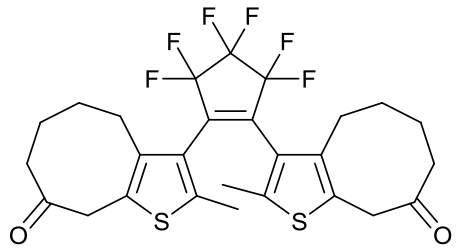
^{13}C-NMR



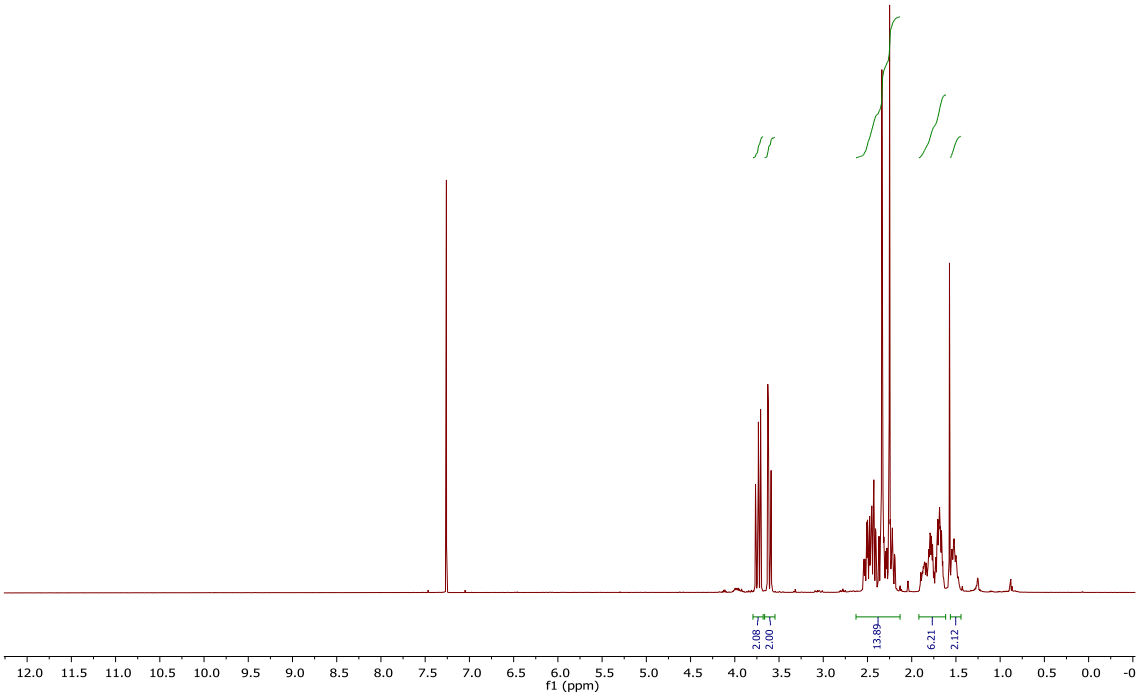
20 (continue)



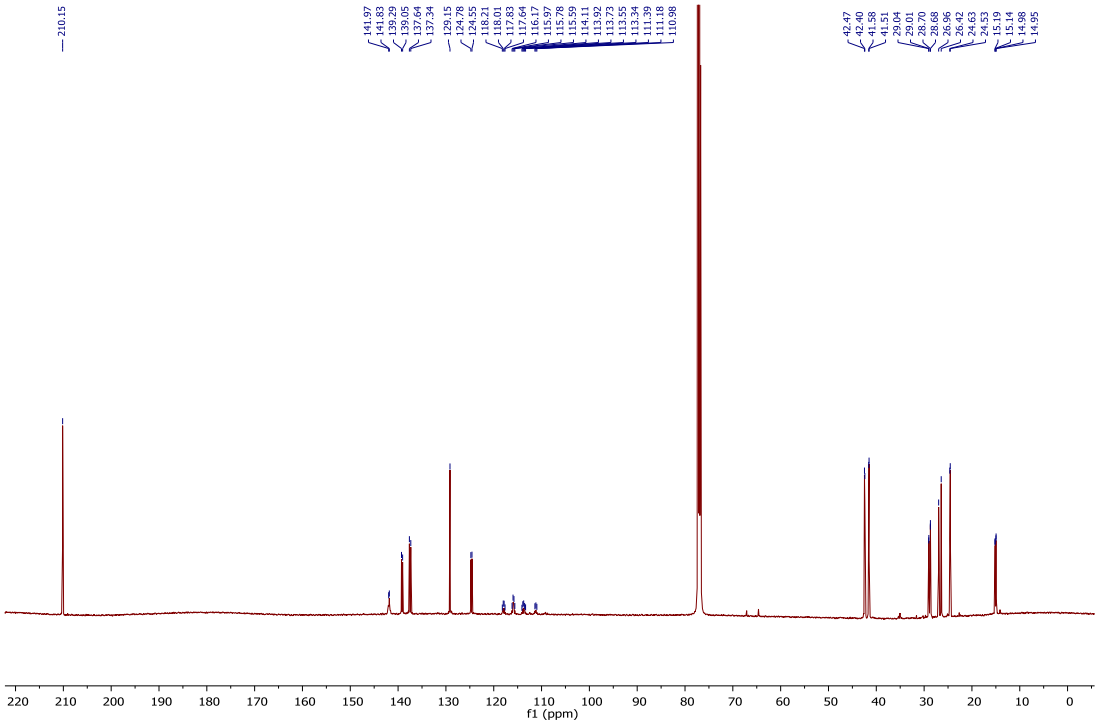
^{19}F-NMR



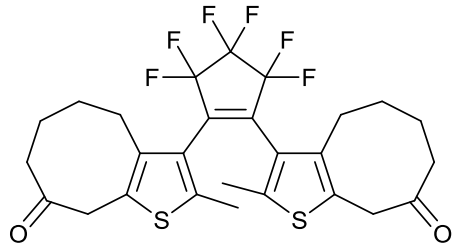
21



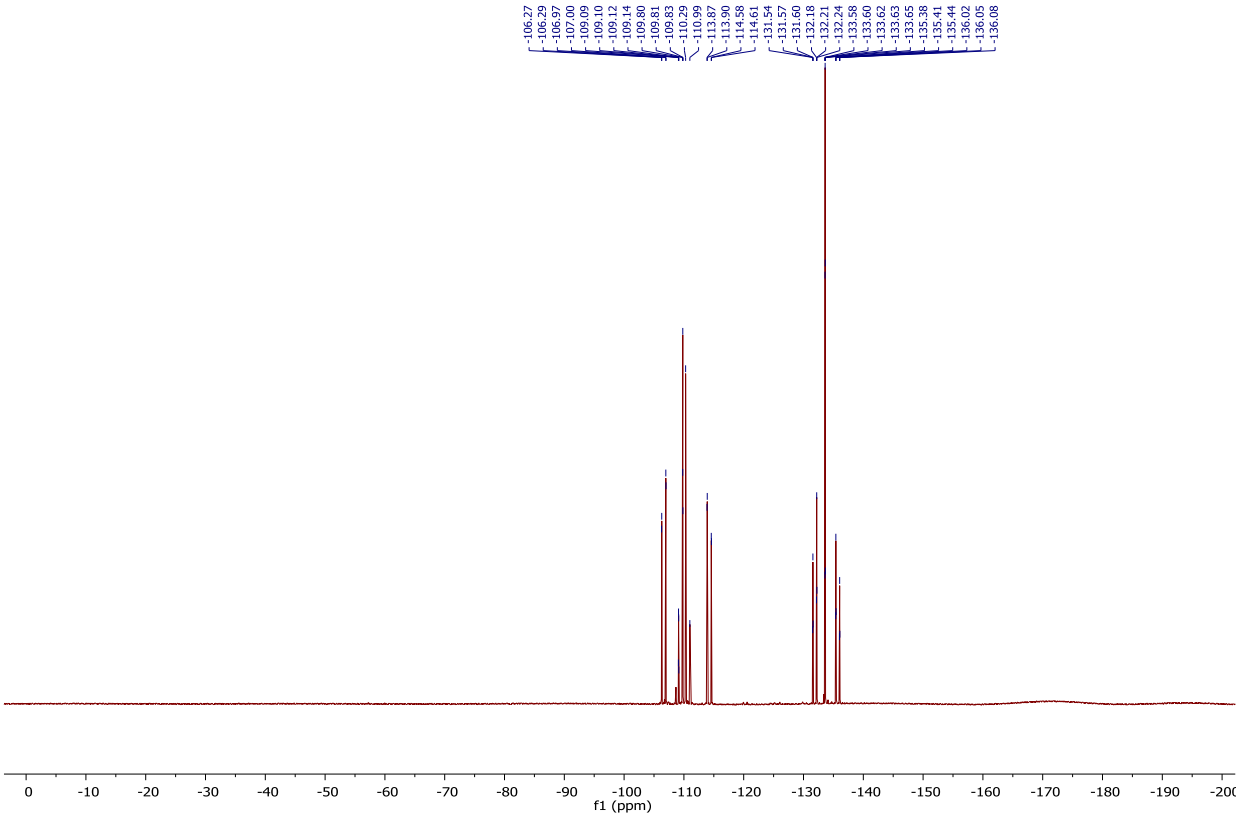
^{1}H-NMR

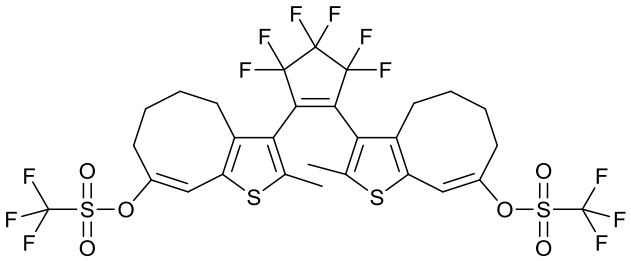


^{13}C-NMR

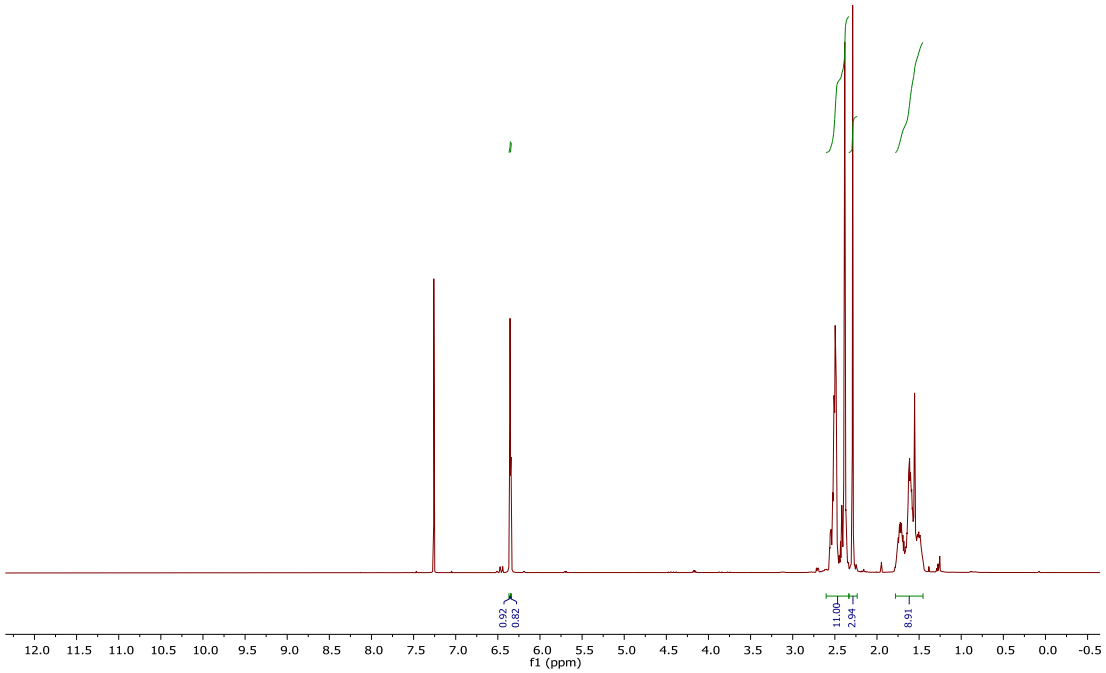

F
F
F
F
F
F
S
S
O
O

21 (continue)

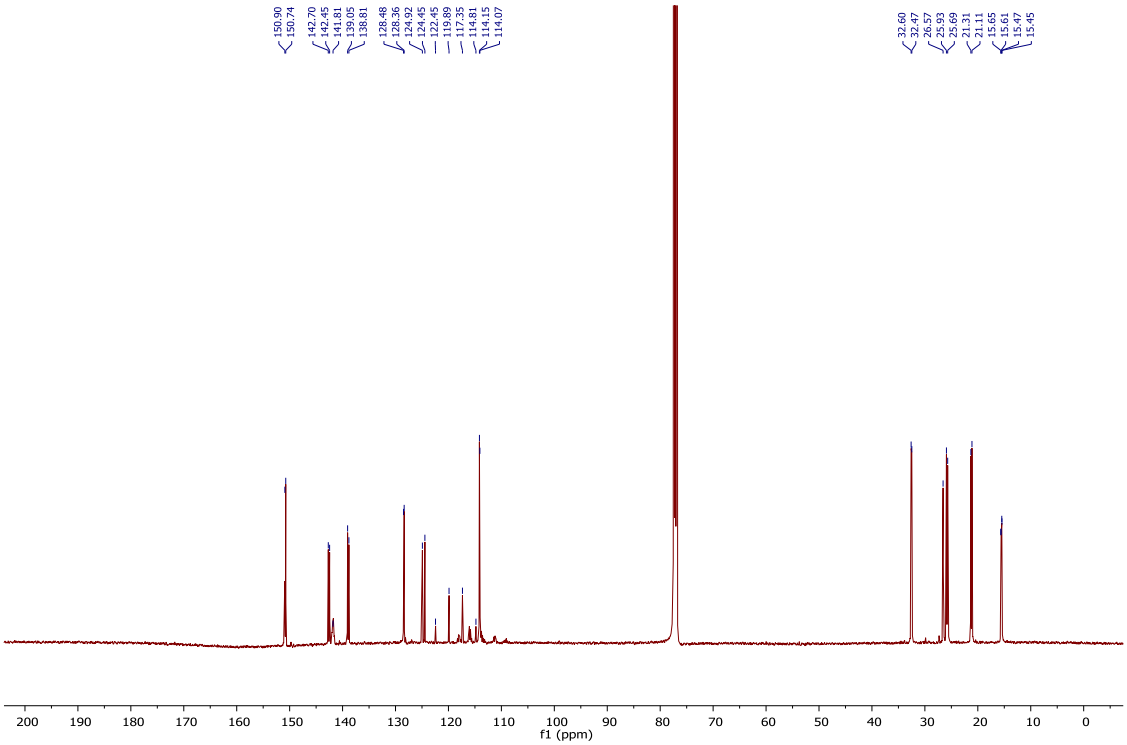

-106.27
-106.29
-106.97
-107.00
-109.09
-109.10
-109.12
-109.14
-109.80
-109.81
-109.83
-110.29
-110.99
-113.87
-113.90
-114.58
-114.61
-131.54
-131.57
-131.60
-132.18
-132.21
-132.24
-133.58
-133.60
-133.62
-133.63
-133.65
-135.38
-135.41
-135.44
-136.02
-136.05
-136.08
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
f1 (ppm)



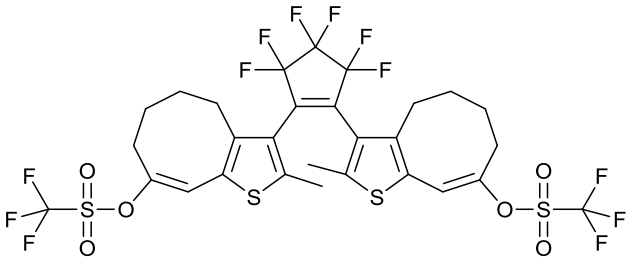
22



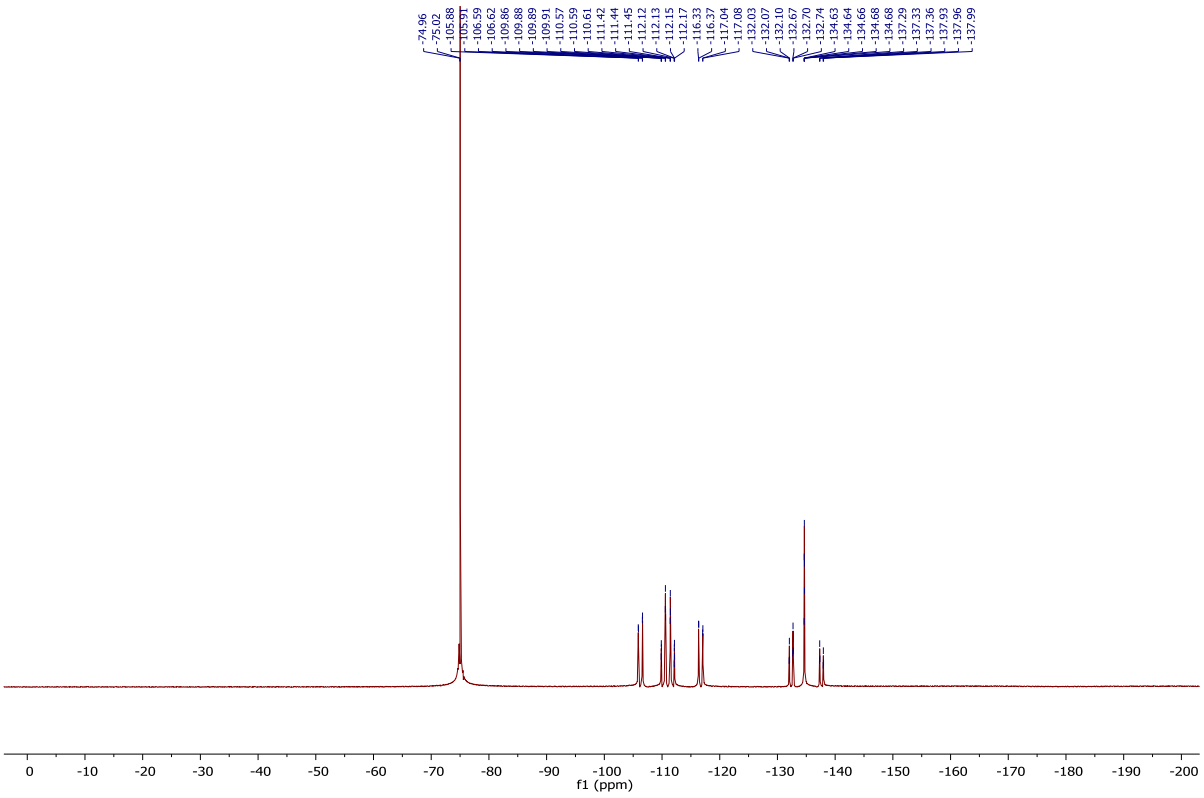
^{1}H-NMR



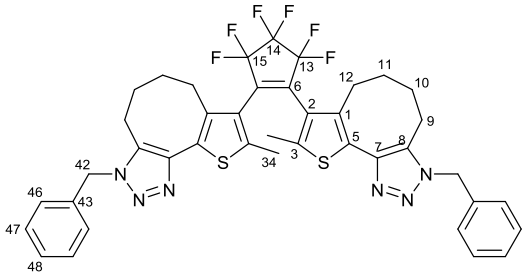
^{13}C-NMR



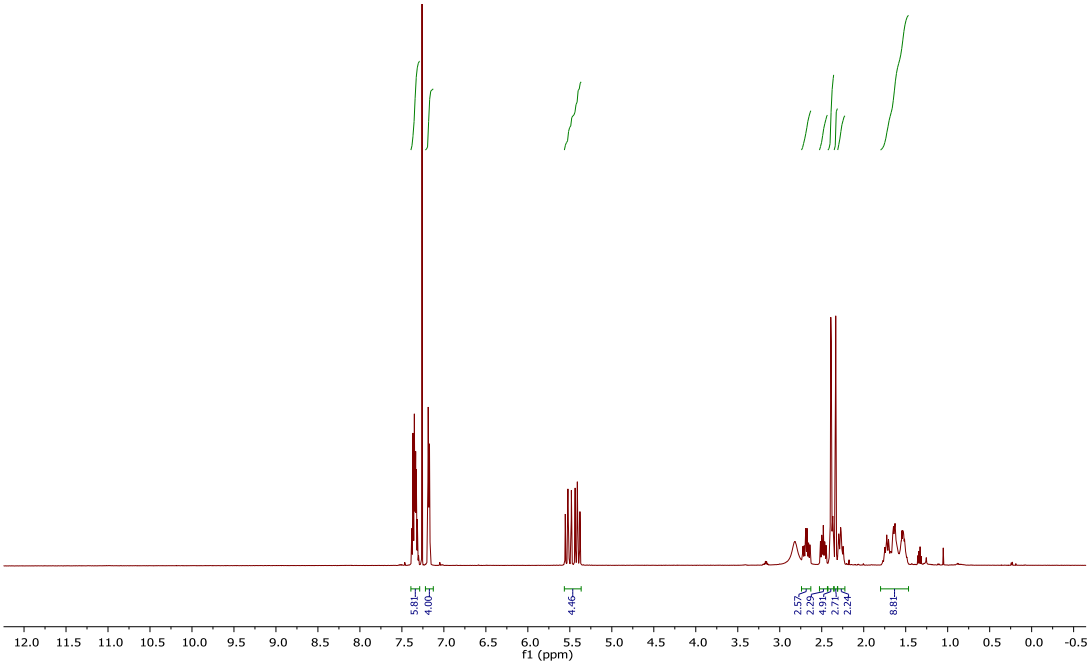
22 (continue)



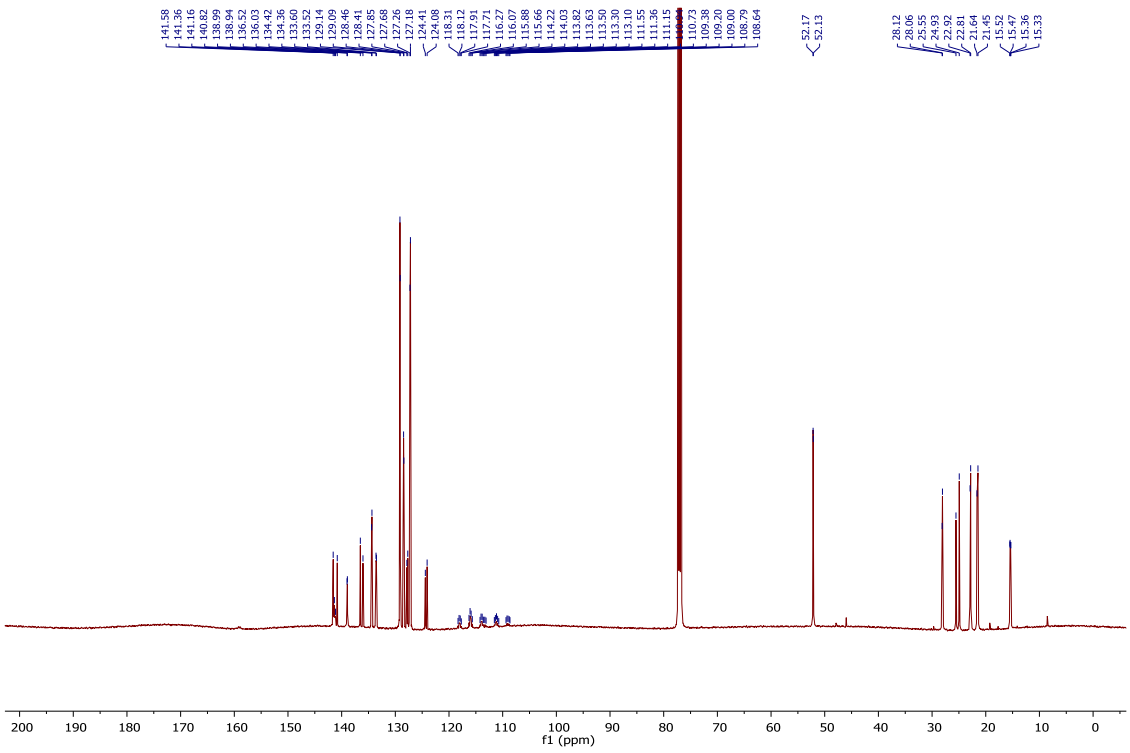
^{19}F-NMR



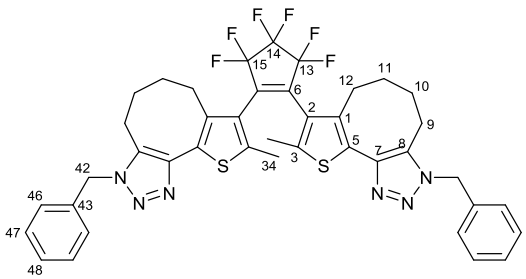
23a



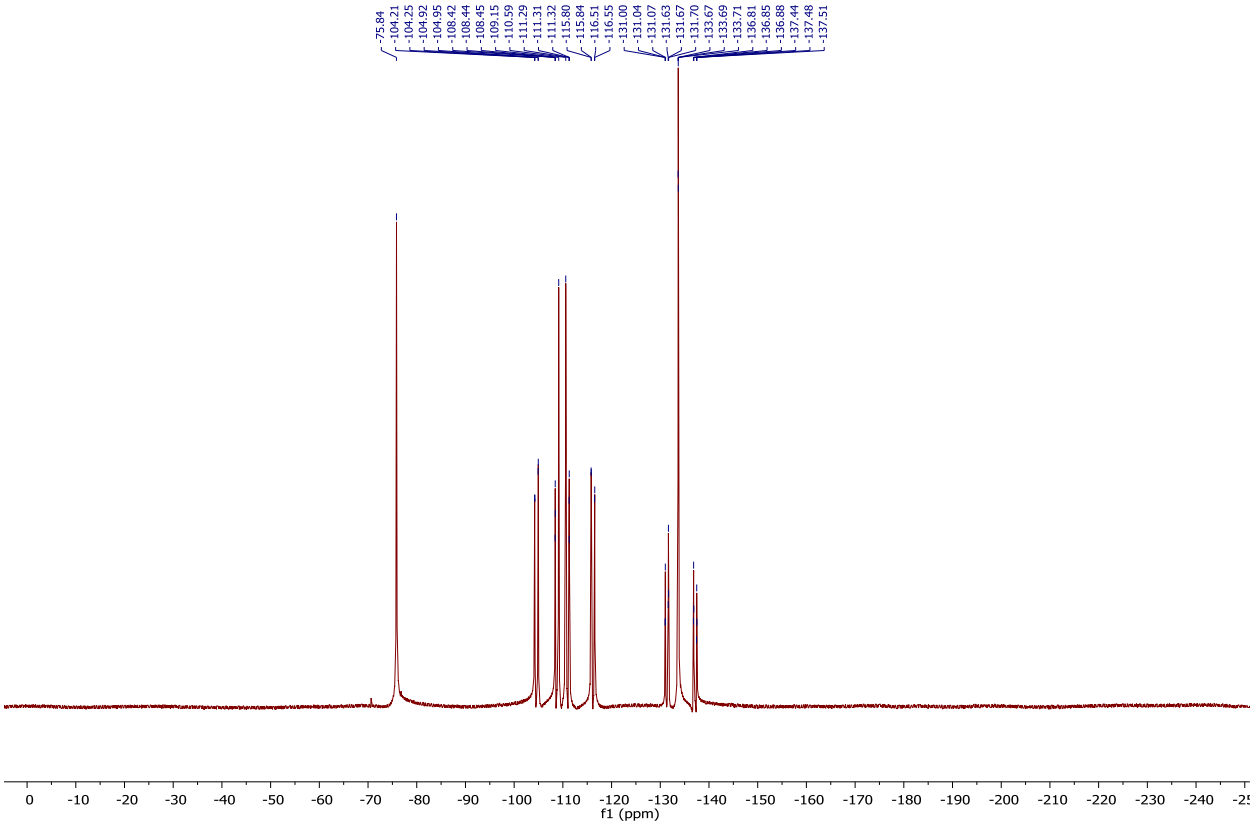
^{1}H-NMR



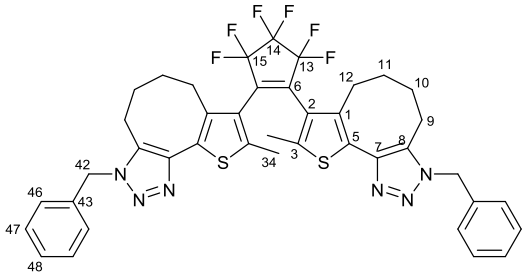
^{13}C-NMR



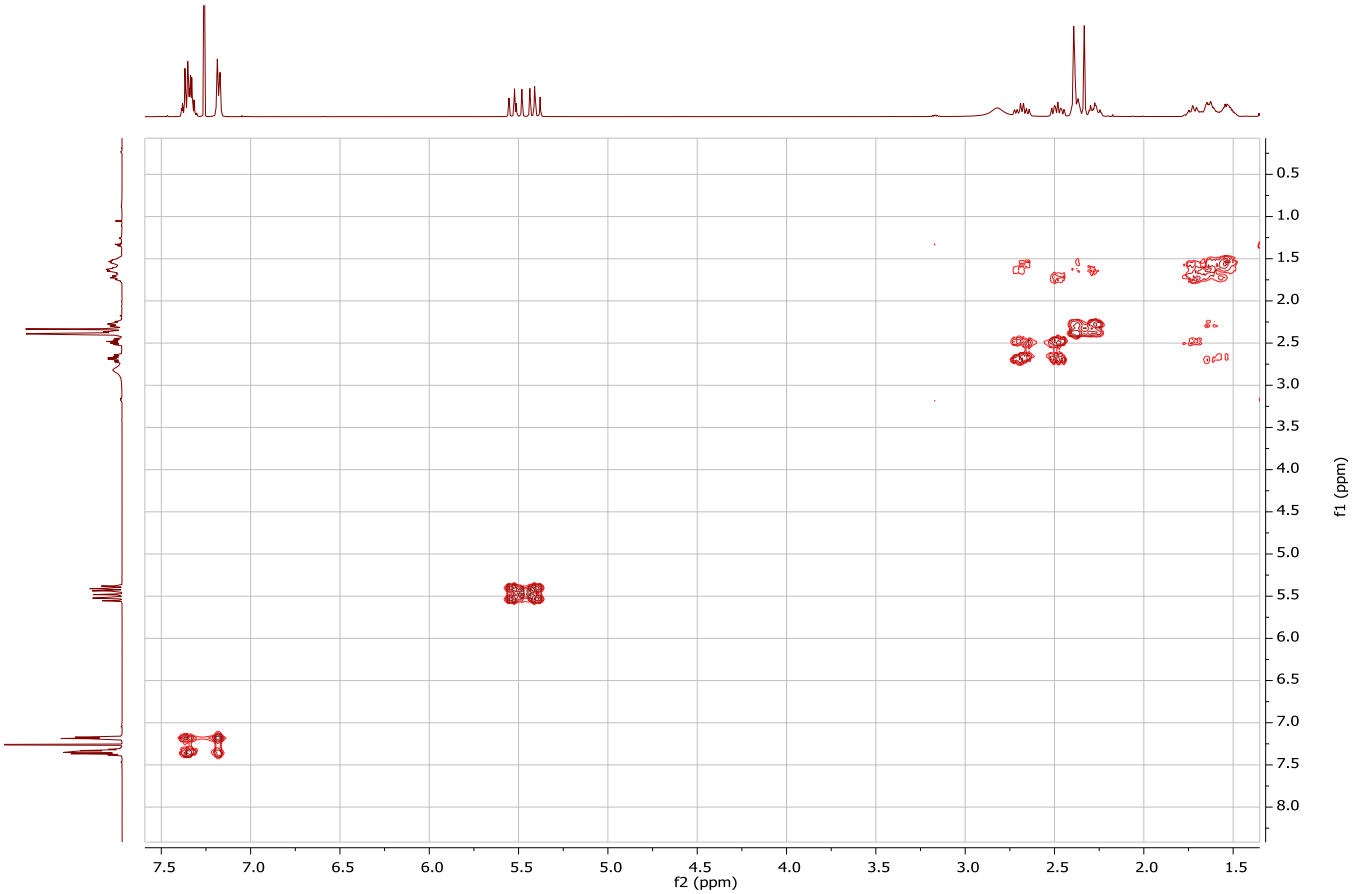
23a (continue)



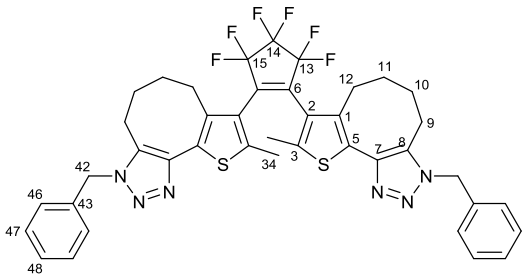
^{19}F-NMR



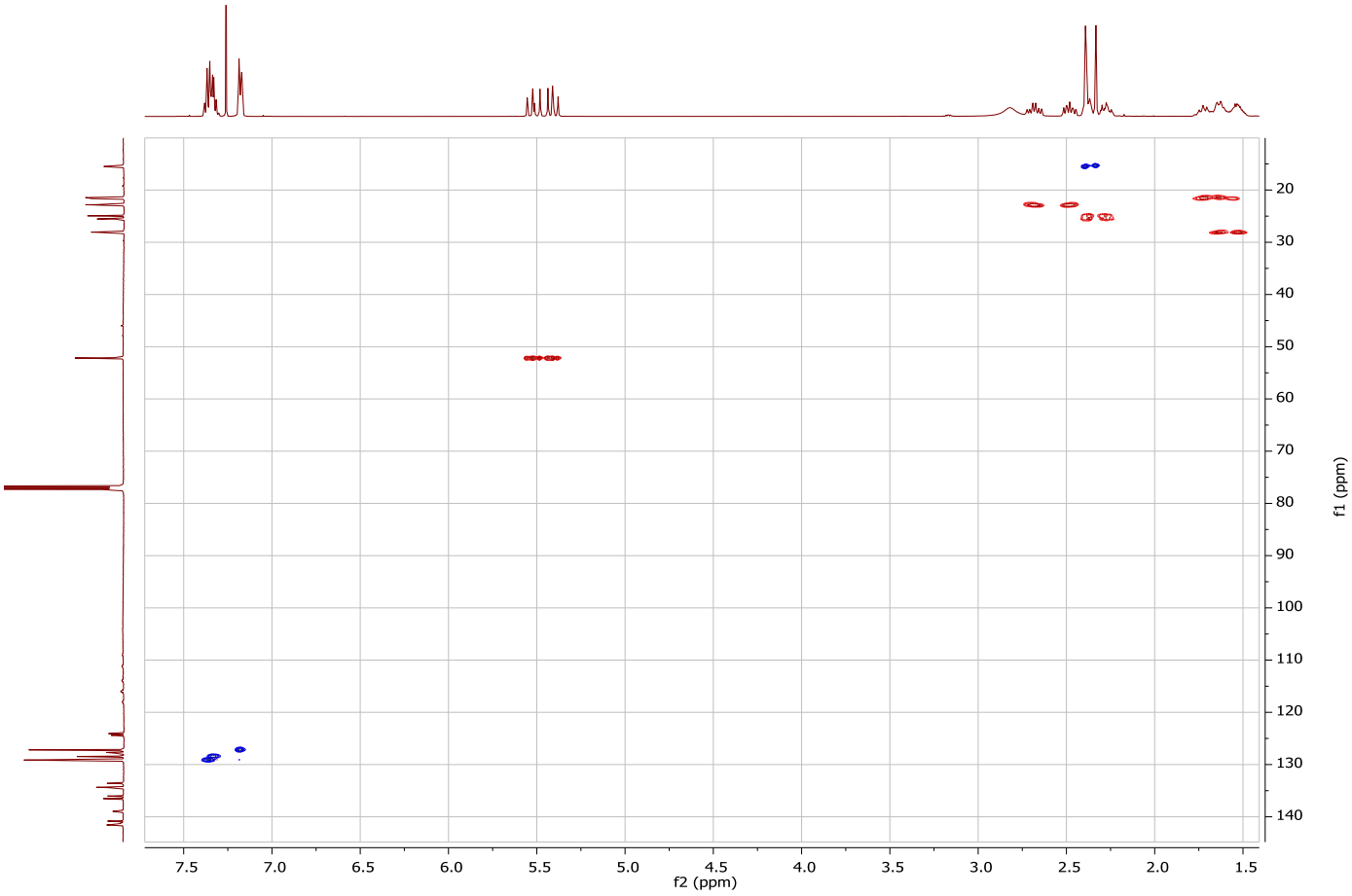
23a (continue)



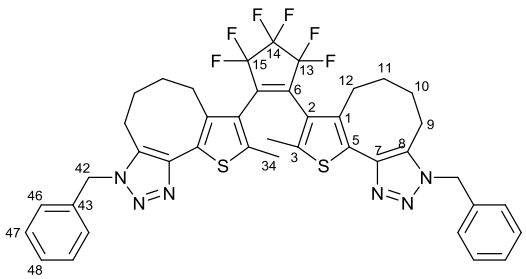
COSY



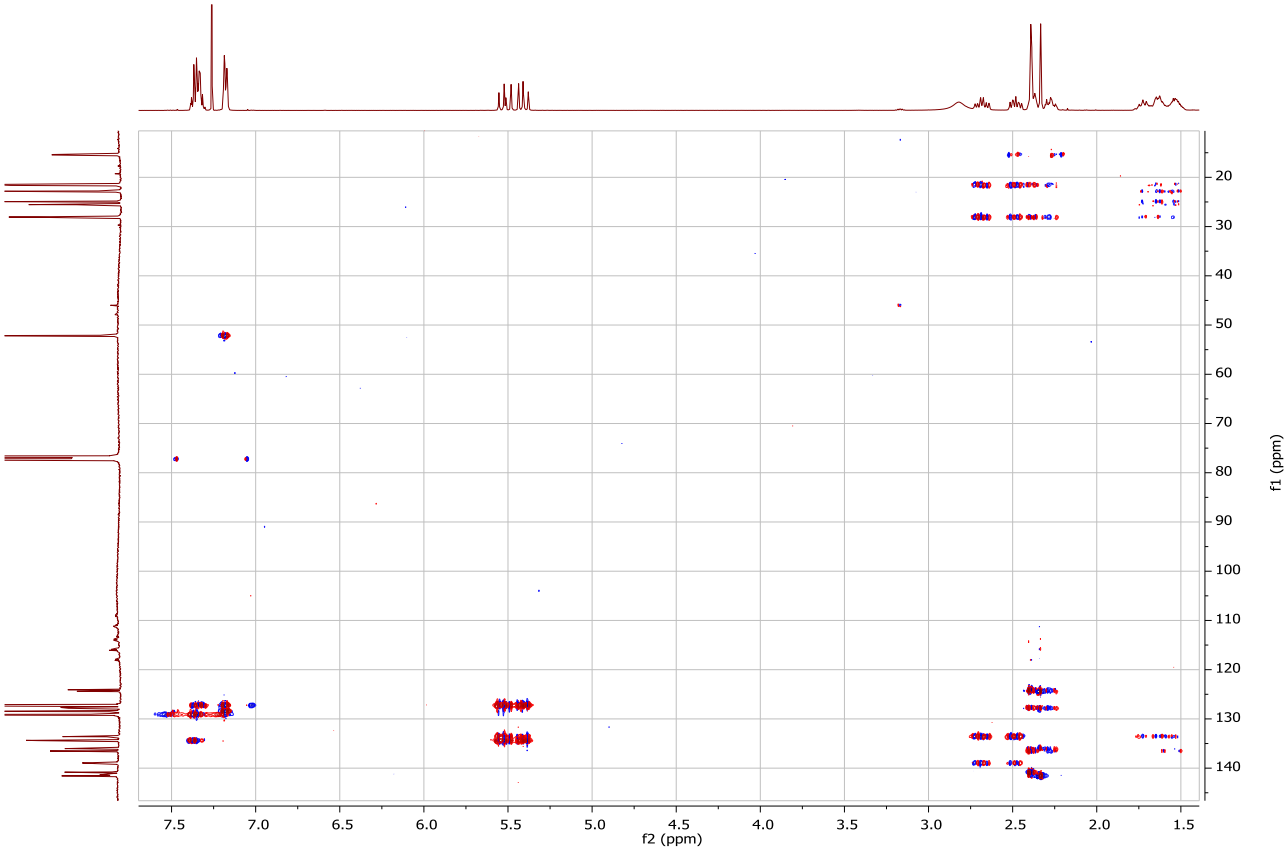
23a (continue)



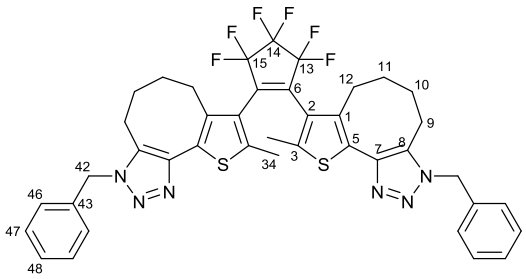
HSQC



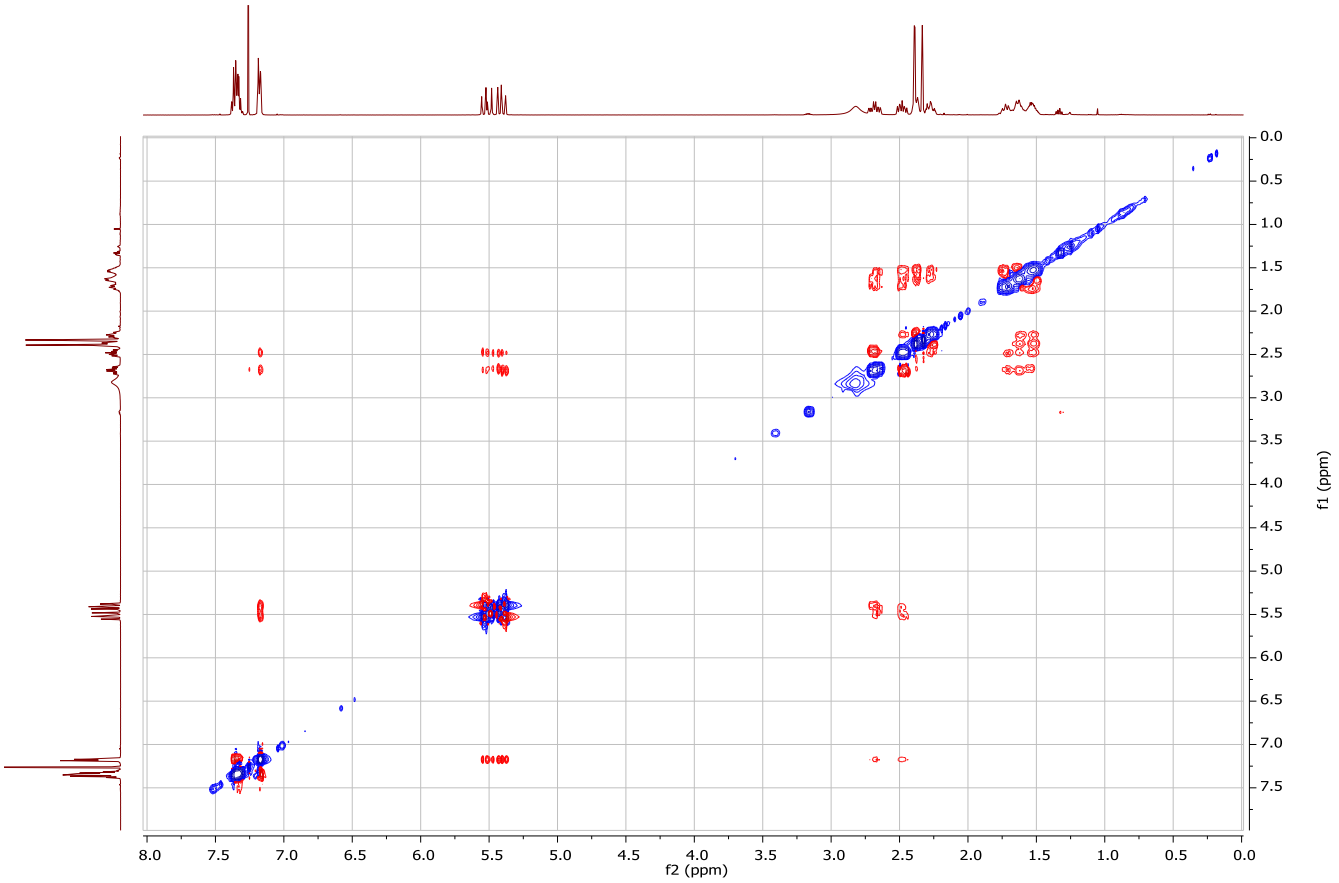
23a (continue)



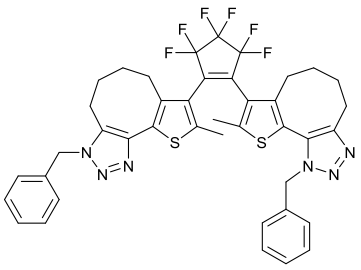
HMBC



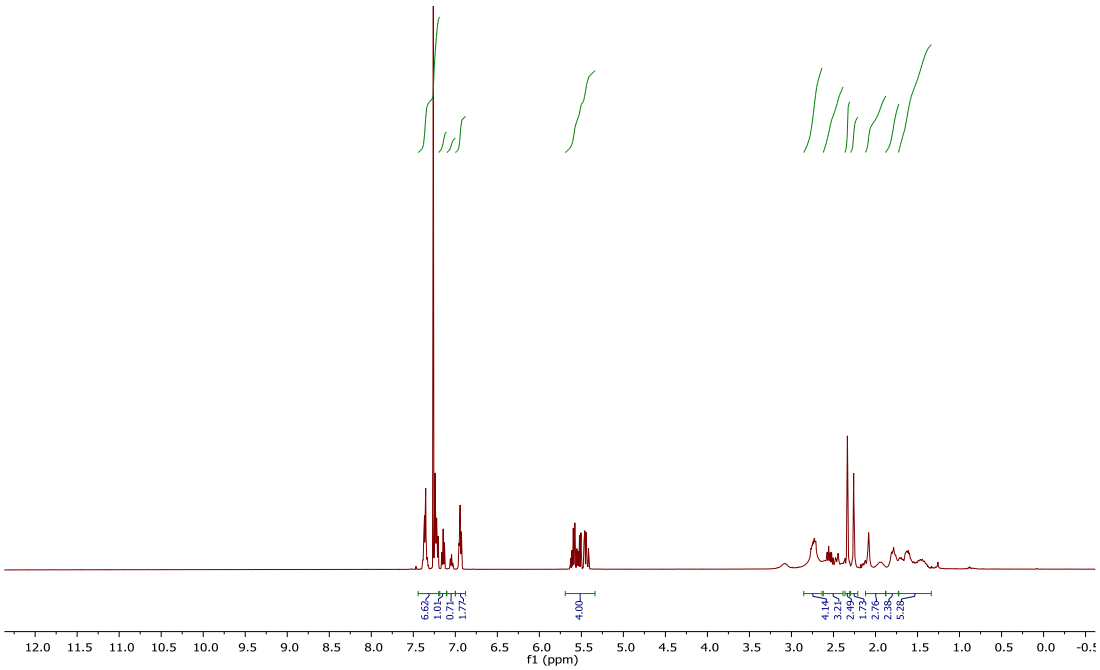
23a (continue)



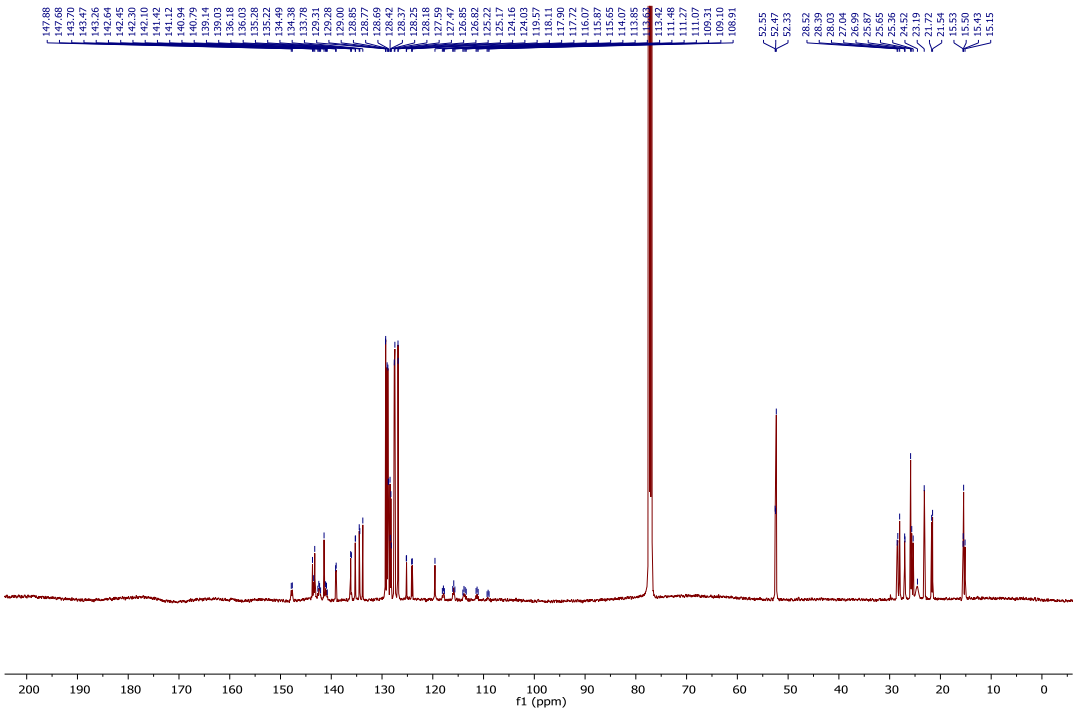
NOESY



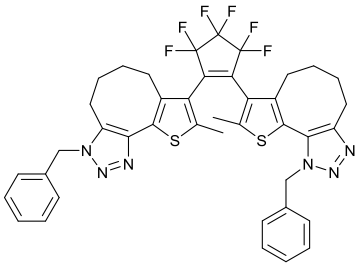
23b



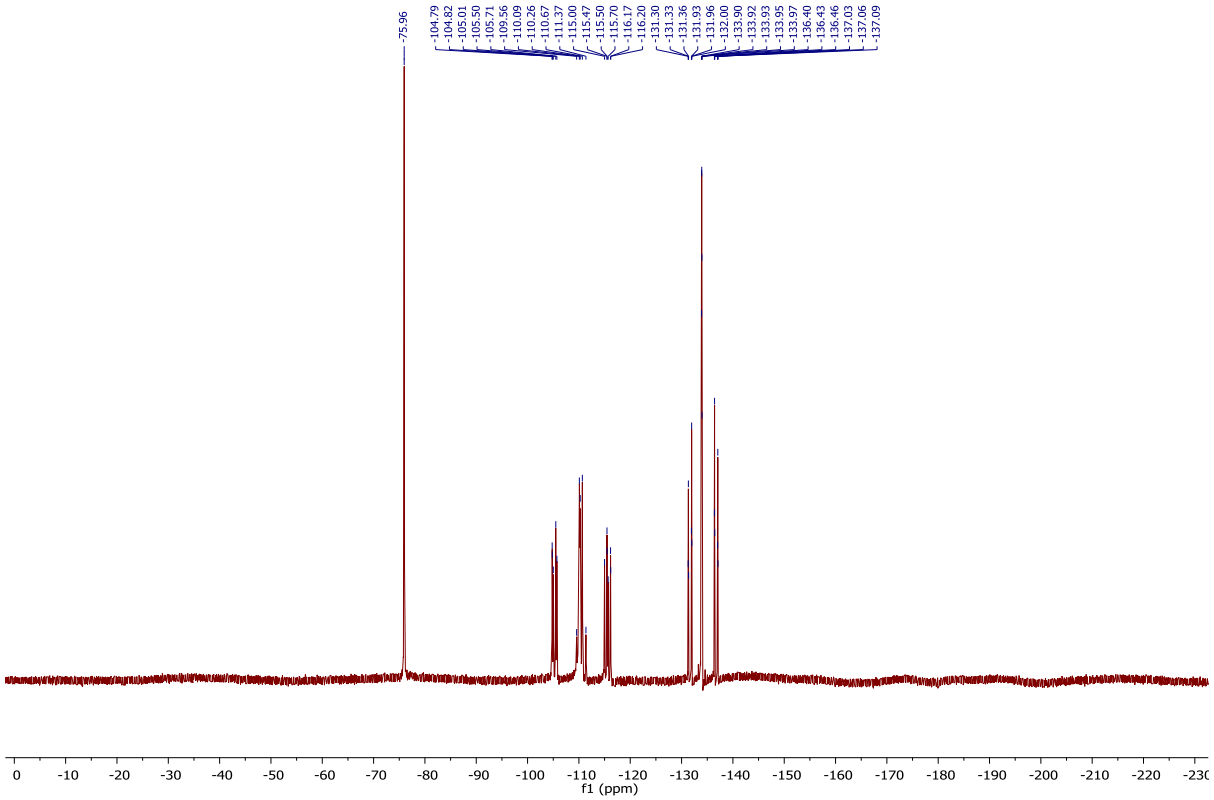
^{1}H-NMR



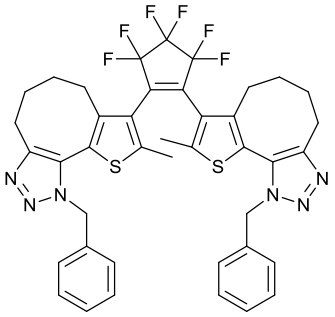
^{13}C-NMR



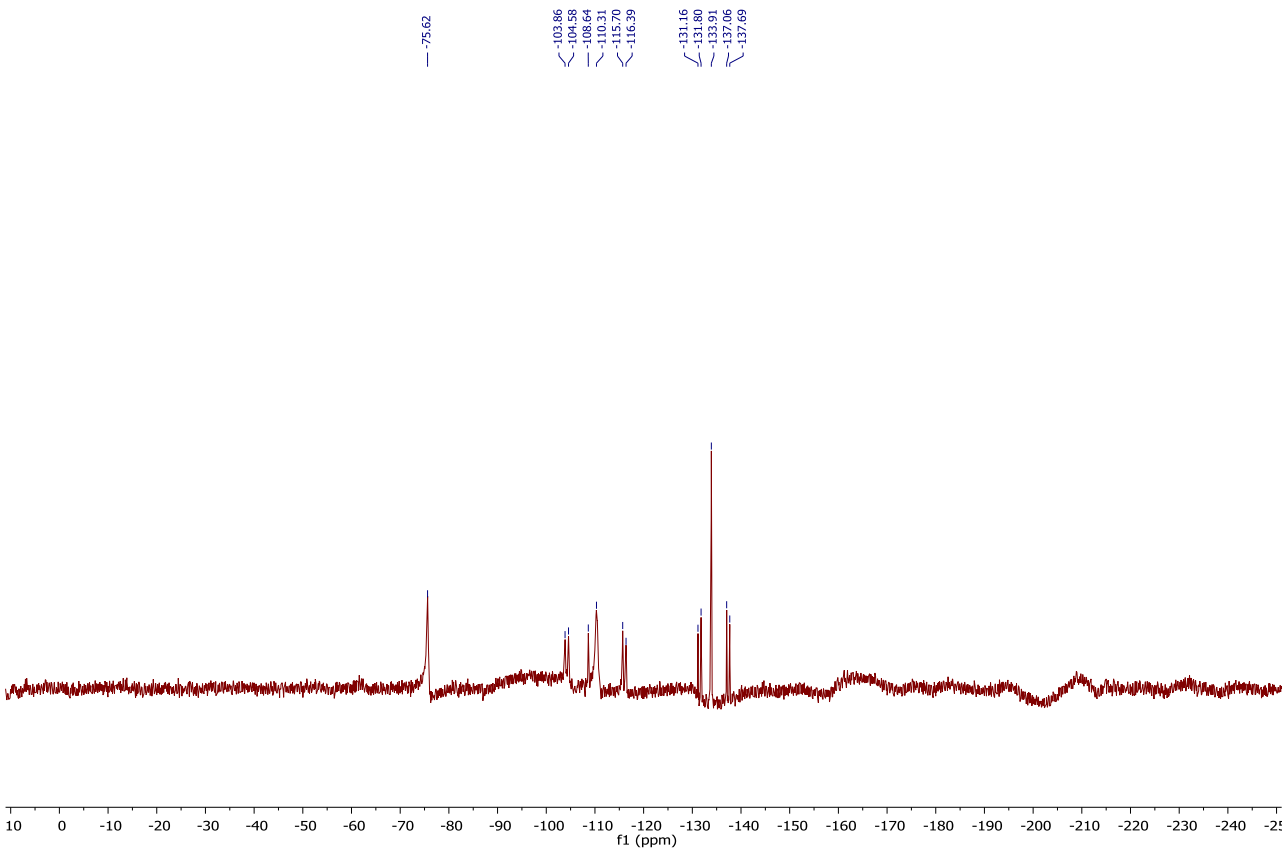
23b (continue)



^{19}F-NMR



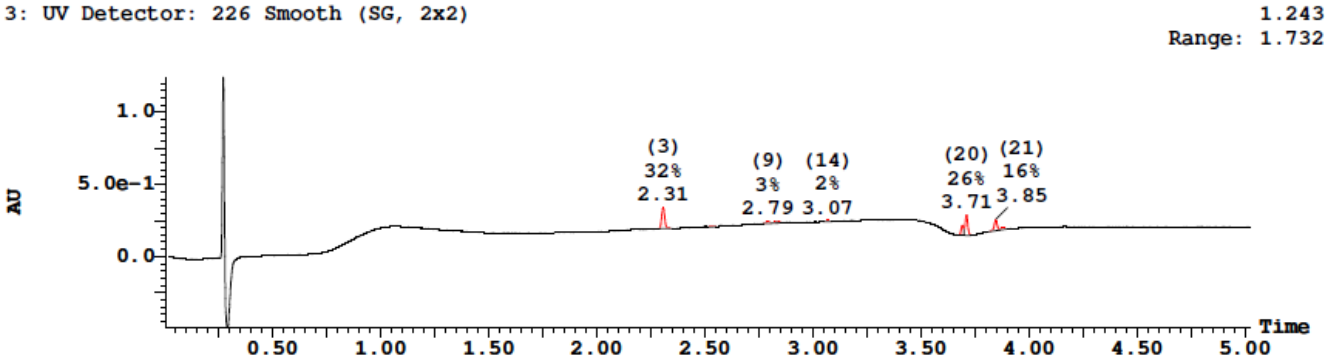
23c

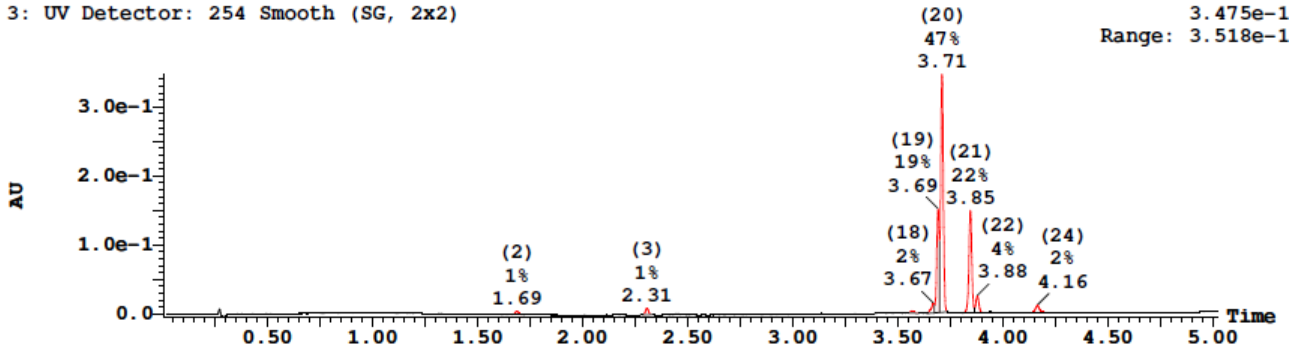

-75.62
-103.86
-104.58
-108.64
-110.31
-115.70
-116.39
-131.16
-131.80
-133.91
-137.06
-137.69
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240
f1 (ppm)

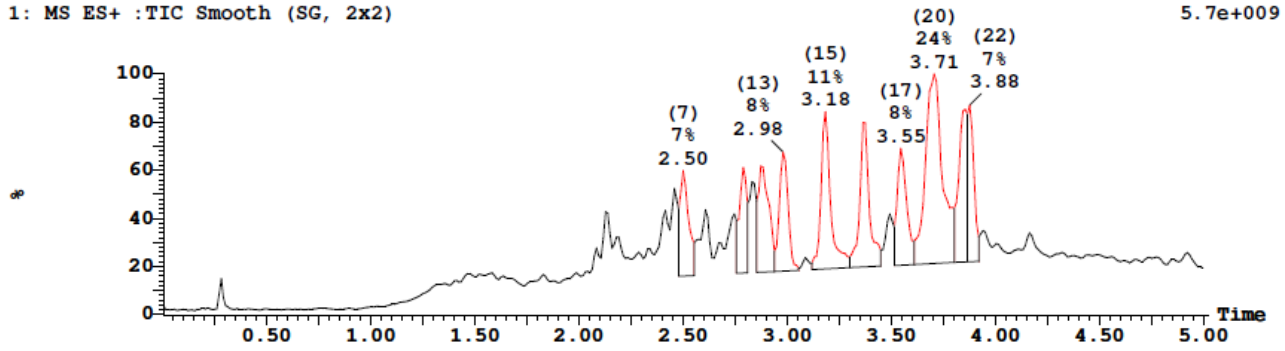
^{19}F-NMR

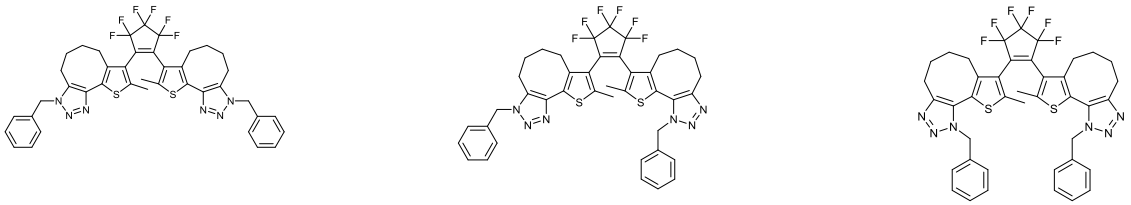
Reaction mixture of linker **5** and benzylazide after 5 min.

Products **23a-c** mixture is observed (RT=3.67 min, 3.69 min, 3.71 min) along with 1:1 adducts **S1** and **S2** (RT=3.85 min, 3.88 min)





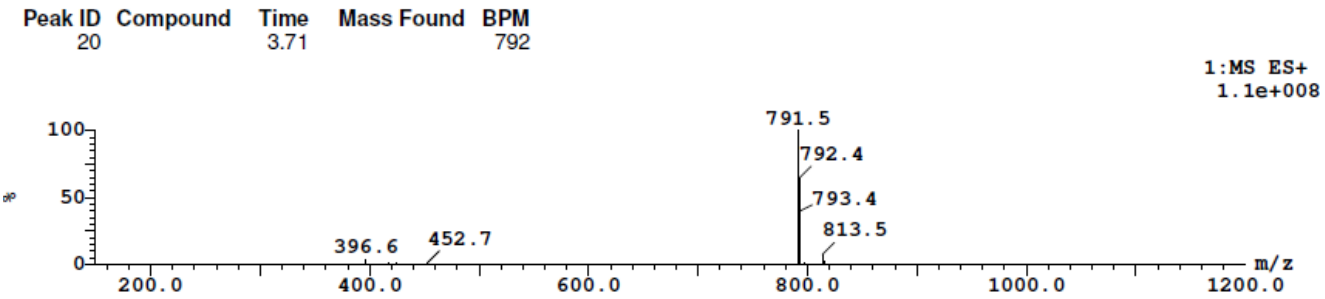


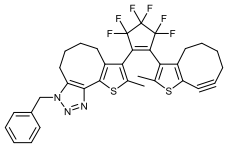


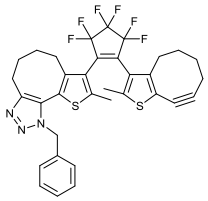
23a **23b** **23c**

M=790.2347

Regioisomers **23a-c**, RT=3.67, 3.69 and 3.71 min, $[M+H]^+$=791.5



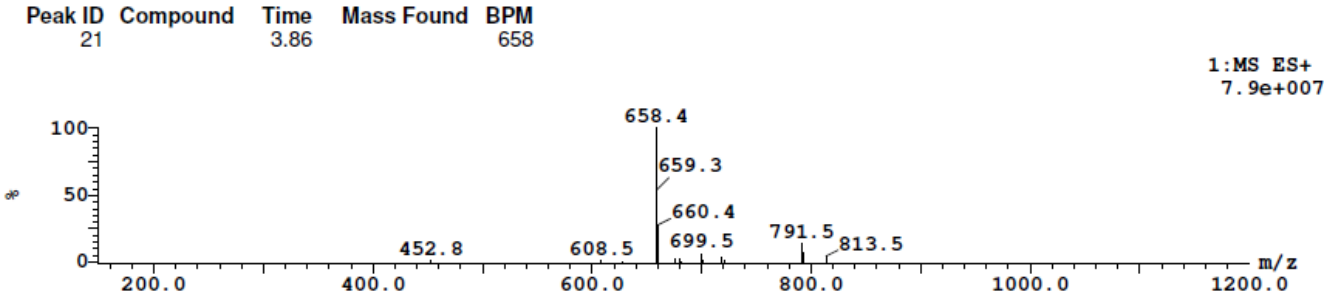




S1 **S2**

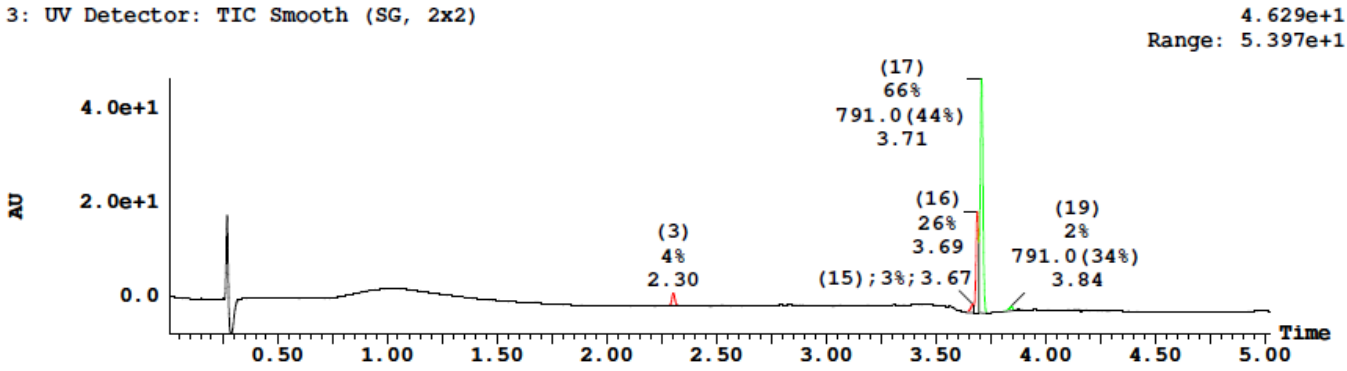
M=657.1707

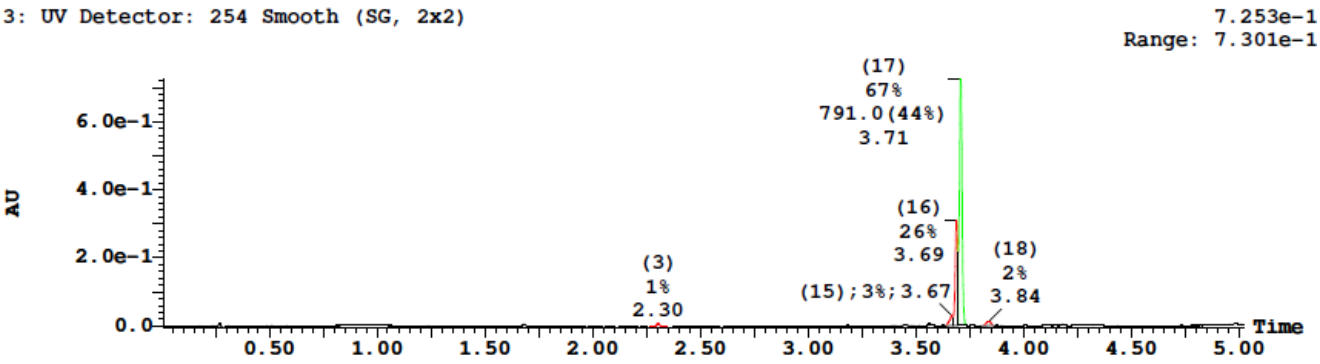
Regioisomers **S1** and **S2**, RT=3.85 and 3.88 min, $[M+H]^+$=658.4

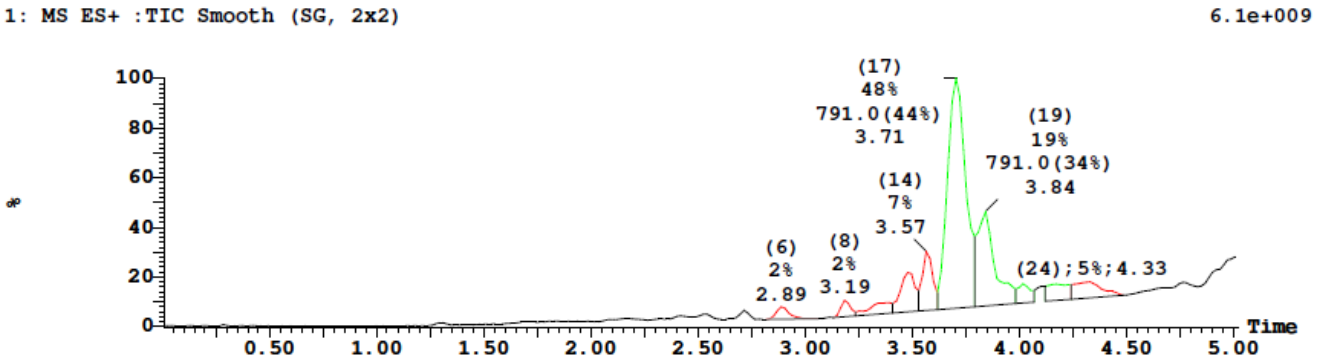


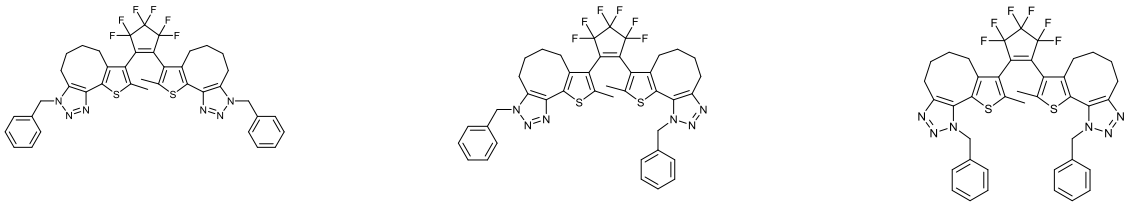
Reaction mixture of linker **5** and benzylazide after 30 min.

Products **23a-c** mixture is observed (RT=3.67 min, 3.69 min, 3.71 min), no 1:1 adducts **S1-2** are present in the reaction mixture.







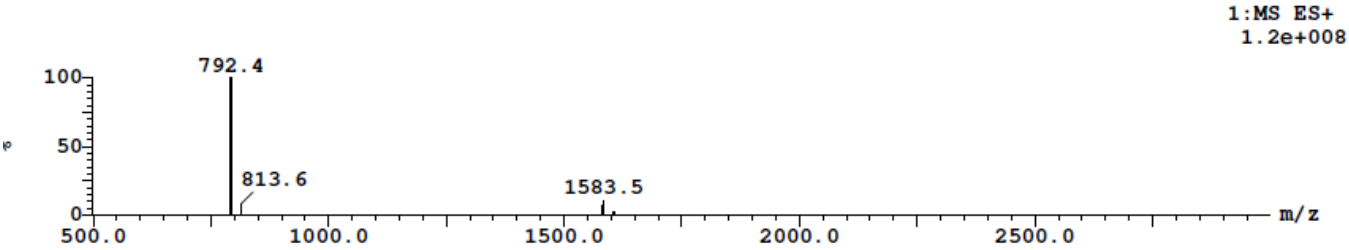


23a **23b** **23c**

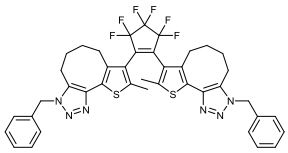
M=790.2347

Regioisomers **23a-c**, RT=3.67, 3.69 and 3.71 min, $[M+H]^+$=792.4

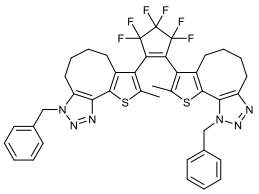
Peak ID	Compound	Time	Mass Found	BPM
17	Found	3.71	814.00,792.00	792



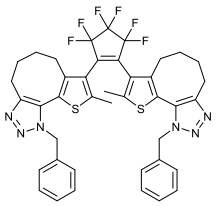
Analytical HPLC chromatogram of isomers mixture **23a**, **23b**, and **23c** after their isolation by flash cromatography. Gradient 70-80% MeCN in 0.1% TFA/H_2O.



23a, RT=10.149 min

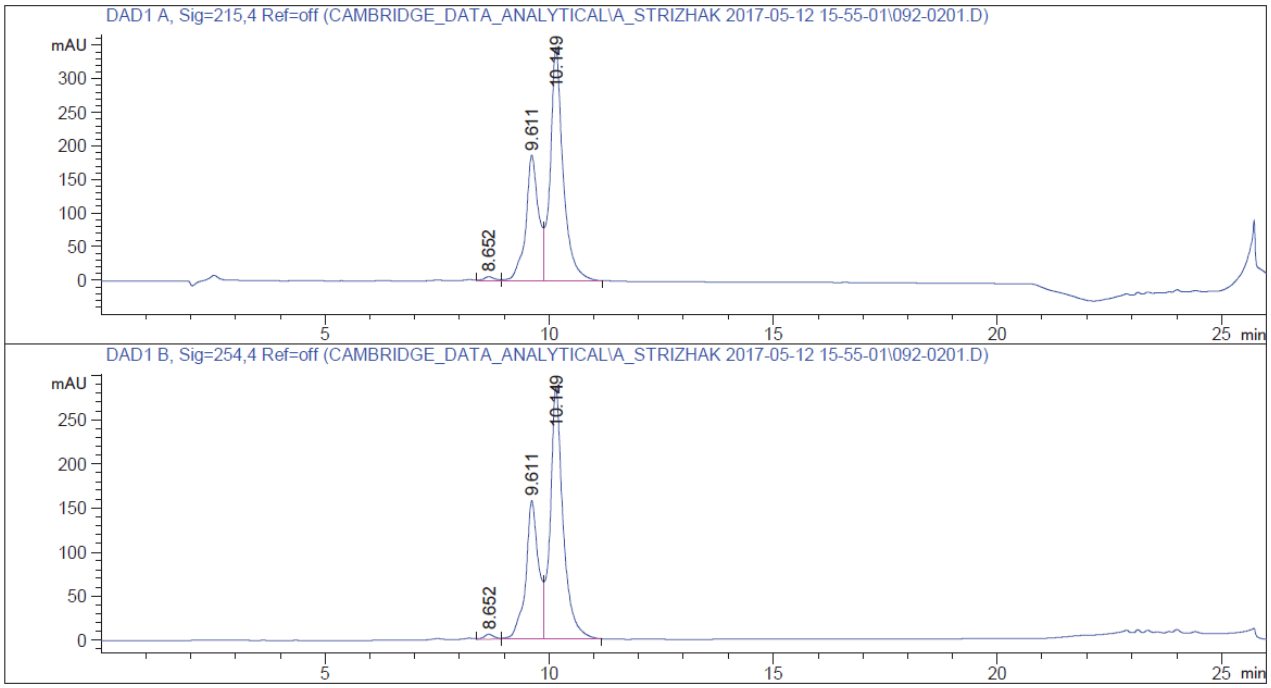


23b, RT=9.611 min



23c, RT=8.652 min

Regioisomers **23a-c**



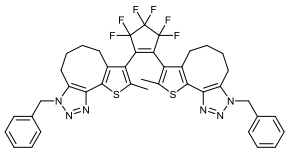
Signal 1: DAD1 A, Sig=215,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.652	VV	0.2397	97.22794	5.91628	0.9037
2	9.611	VV	0.2808	3701.84106	187.12016	34.4066
3	10.149	VB	0.2862	6960.02490	346.73154	64.6897
Totals :				1.07591e4	539.76798	

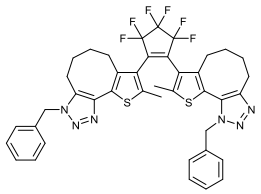
Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.652	VV	0.2354	91.09424	5.67142	1.0219
2	9.611	VV	0.2818	3114.53125	156.76076	34.9387
3	10.149	VB	0.2852	5708.63770	285.55917	64.0394
Totals :				8914.26318	447.99135	

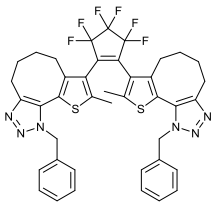
HPLC separation of three diarylethene triazole isomers **21a**, **21b** and **21c**. 70-80% MeCN in 0.1% TFA/H_2O.



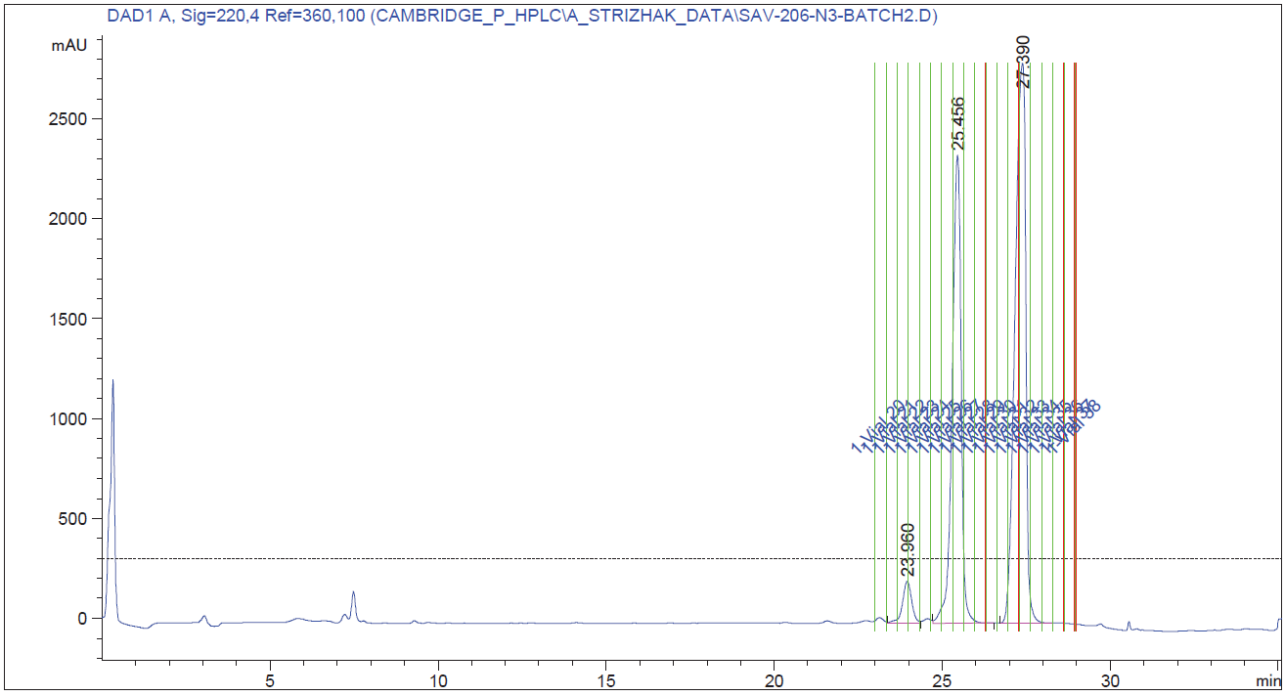
23a, RT=27.390 min, vials 32-33



23b, RT=25.456 min, vials 26-27



23c, RT=23.960 min, vials 22-23



Signal 1: DAD1 A, Sig=220,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	23.960	VV	0.2924	4039.68481	209.98254	3.6807
2	25.456	VB	0.2877	4.33146e4	2342.72437	39.4650
3	27.390	BB	0.3560	6.24002e4	2799.93237	56.8544
Totals :				1.09755e5	5352.63928	

Composition of the mixture: 3% of **21c** (23.960 min), 39% of **21b** (25.456 min) and 57% of major isomer **21a** (27.390 min).

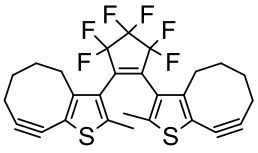
Fraction Information

Fraction collection using a timetable

Frac #	Well #	Location	Volume [µl]	BeginTime [min]	EndTime [min]	Reason	Mass
1	1	1-Vial 20	6604.17	23.0000	23.3302	Time	
2	1	1-Vial 21	6429.17	23.3387	23.6602	Time	
3	1	1-Vial 22	6466.67	23.6668	23.9902	Time	
4	1	1-Vial 23	6475.00	23.9964	24.3202	Time	
5	1	1-Vial 24	6450.00	24.3277	24.6502	Time	
6	1	1-Vial 25	6466.67	24.6568	24.9802	Time	
7	1	1-Vial 26	6425.00	24.9889	25.3102	Time	
8	1	1-Vial 27	6429.17	25.3187	25.6402	Time	
9	1	1-Vial 28	6300.00	25.6552	25.9702	Time	
10	1	1-Vial 29	6450.00	25.9777	26.3002	Time	
11	1	1-Vial 30	6458.33	26.3073	26.6302	Time	
12	1	1-Vial 31	6462.50	26.6370	26.9602	Time	
13	1	1-Vial 32	6475.00	26.9664	27.2902	Time	
14	1	1-Vial 33	6458.33	27.2973	27.6202	Time	
15	1	1-Vial 34	6458.33	27.6273	27.9502	Time	
16	1	1-Vial 35	6454.17	27.9575	28.2802	Time	
17	1	1-Vial 36	6400.00	28.2902	28.6102	Time	
18	1	1-Vial 37	6354.17	28.6225	28.9402	Time	
19	1	1-Vial 38	1037.50	28.9483	29.0002	Time	

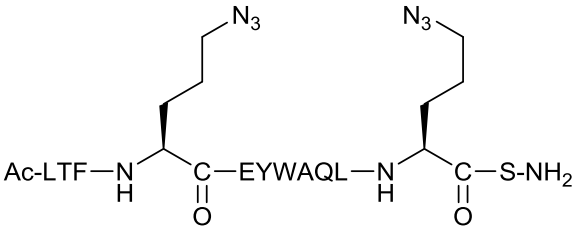
Three fractions were collected: compound **23c** (vials 22-23), compound **23b** (vials 26-27) and compound **23a** (vials 32-33).

Scheme of the reaction between linker **5** and linear azidopeptide **24** forming *i,i+7* stapled peptide **26**.

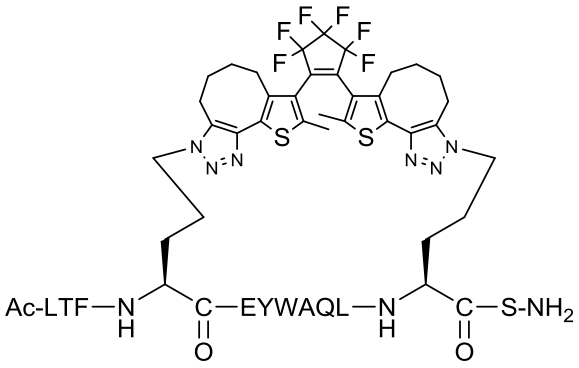


5

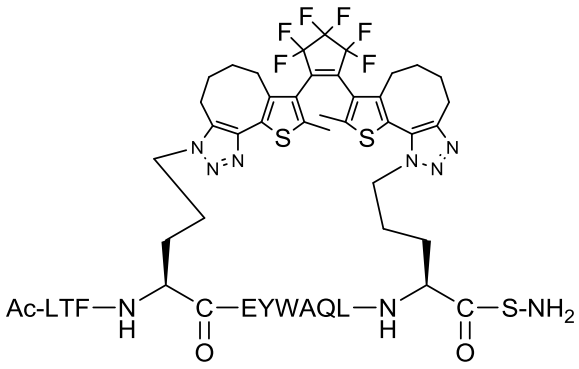
+



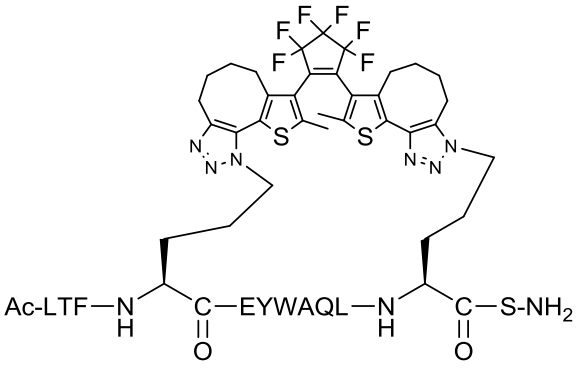
24



26a

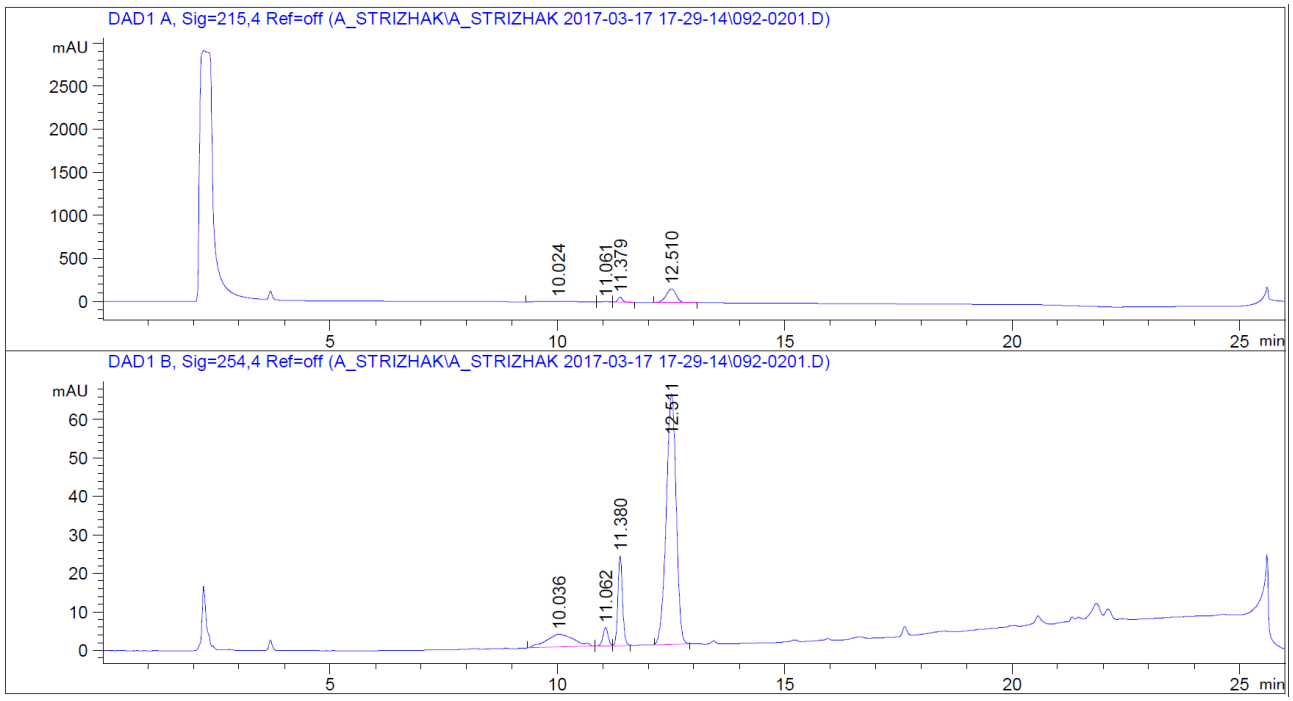


26b



26c

Analytical HPLC chromatogram of reaction mixture between linker **5** and linear pDI-E azidopeptide **22**, 50-90% MeCN in 0.1% TFA/H_2O. Different isomers of stapled peptides at 10.04 min (fraction 1), 11.062 min (fraction 2), 11.380 min (fraction 3) and major isomer at 12.51 min (fraction 4). All four isomers of stapled peptide were separated by semi-preparative HPLC.



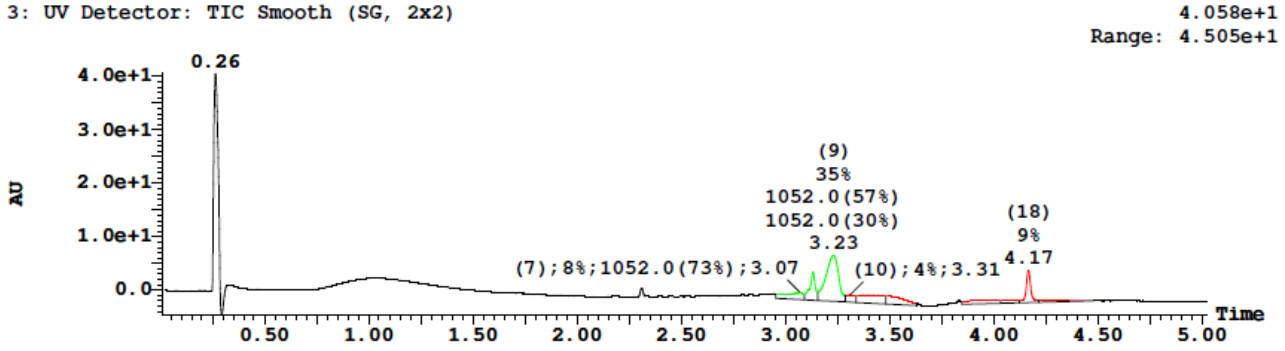
Signal 1: DAD1 A, Sig=215,4 Ref=off

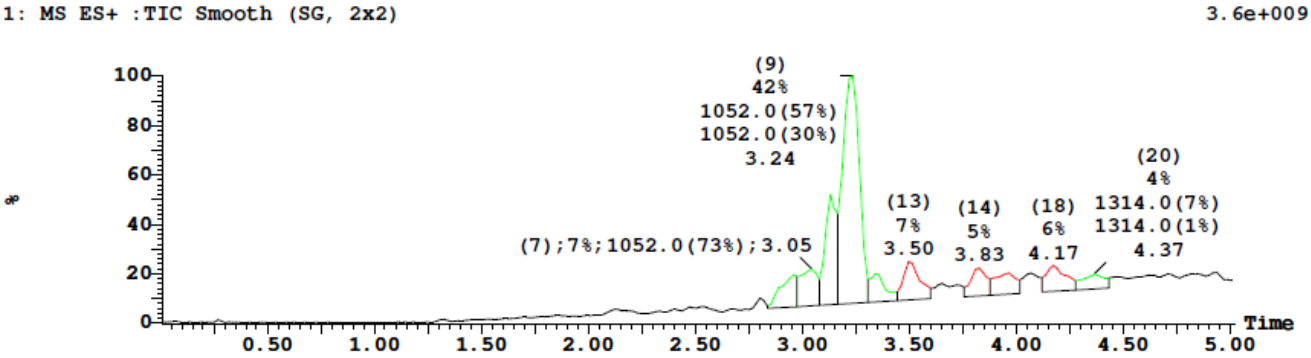
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.024	BV	0.5958	367.71176	8.51487	11.1827
2	11.061	VV	0.1269	89.90922	10.82136	2.7343
3	11.379	VB	0.1116	412.02585	57.47580	12.5304
4	12.510	BB	0.2383	2418.56006	158.27280	73.5526
Totals :				3288.20689	235.08482	

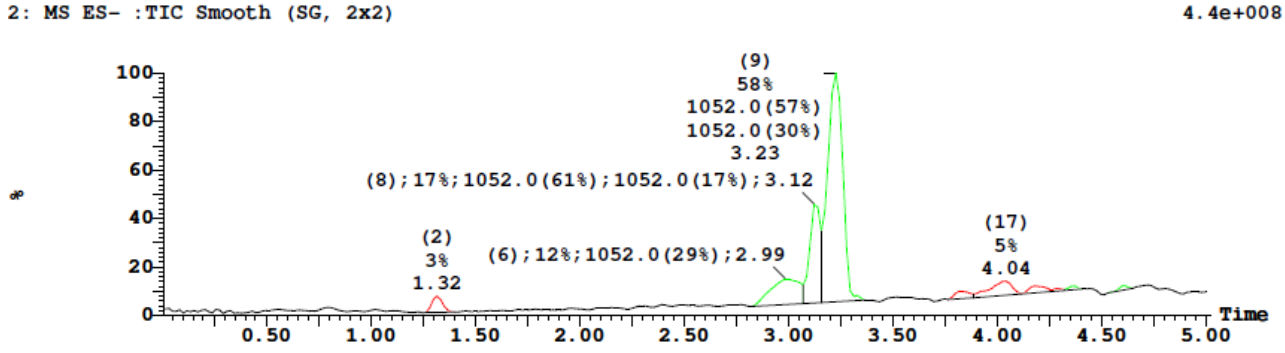
Signal 2: DAD1 B, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.036	BV	0.5566	141.57042	3.33969	10.6303
2	11.062	VV	0.1230	38.14311	4.78330	2.8641
3	11.380	VB	0.1111	166.08116	23.30827	12.4708
4	12.511	BB	0.2368	985.96423	65.06565	74.0347
Totals :				1331.75893	96.49691	

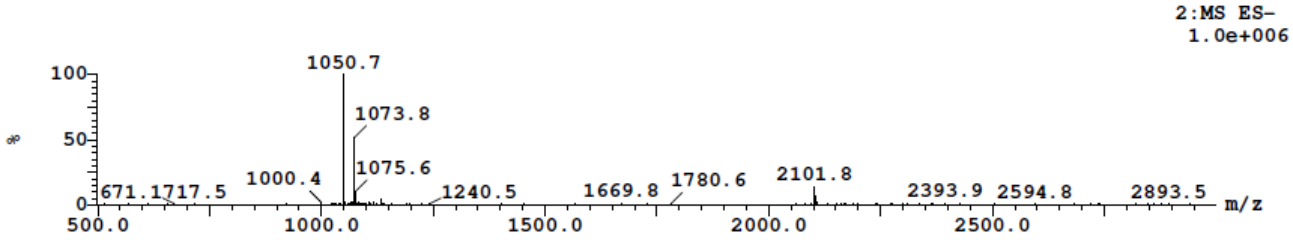
LCMS chromatogram of reaction mixture between linker **5** and linear pDI-E azidopeptide **22**, 5-95% MeCN in 0.1% $HCOONH_4/H_2O$. Different isomers of stapled peptides at 3.07-3.23 min.



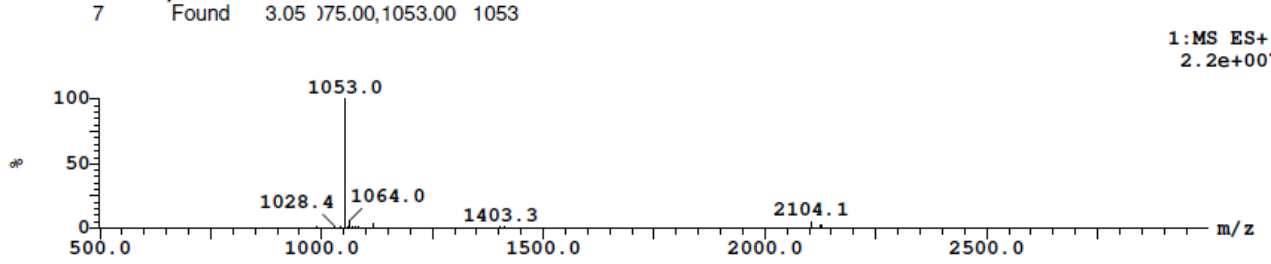




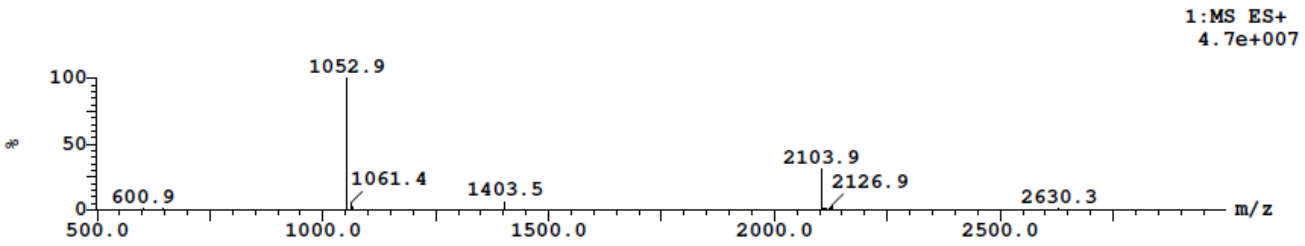
Peak ID	Compound	Time	Mass Found	BPM
6	Found	2.99	1051.00	1051



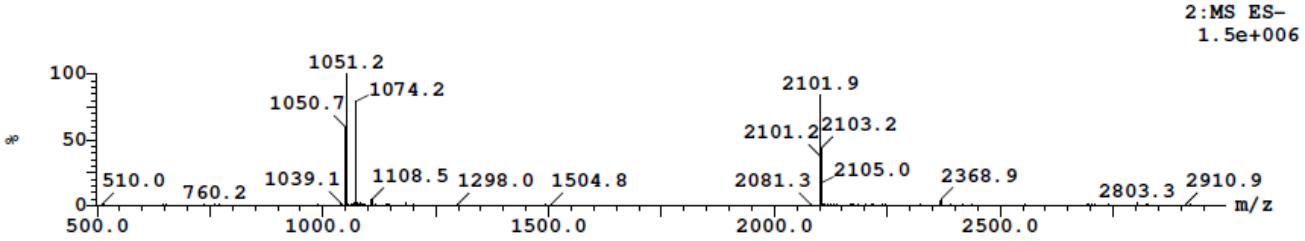
Peak ID	Compound	Time	Mass Found	BPM
7	Found	3.05	075.00,1053.00	1053



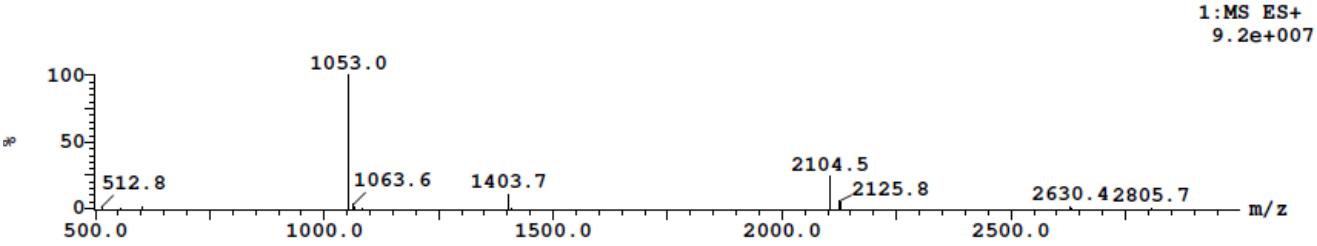
Peak ID	Compound	Time	Mass Found	BPM
8	Found	3.13	1053.00	1053



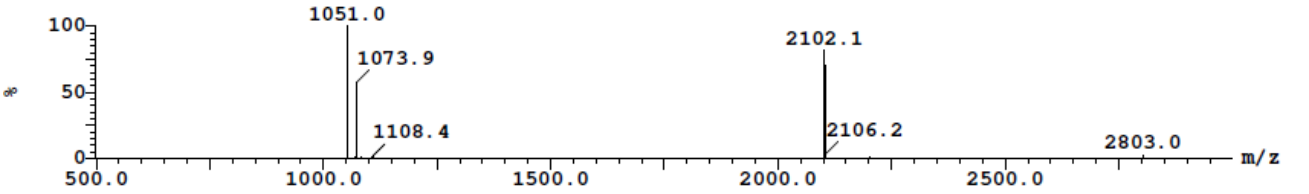
Peak ID	Compound	Time	Mass Found	BPM
8	Found	3.13	1051.00	1051



Peak ID	Compound	Time	Mass Found	BPM
9	Found	3.24	1053.00	1053

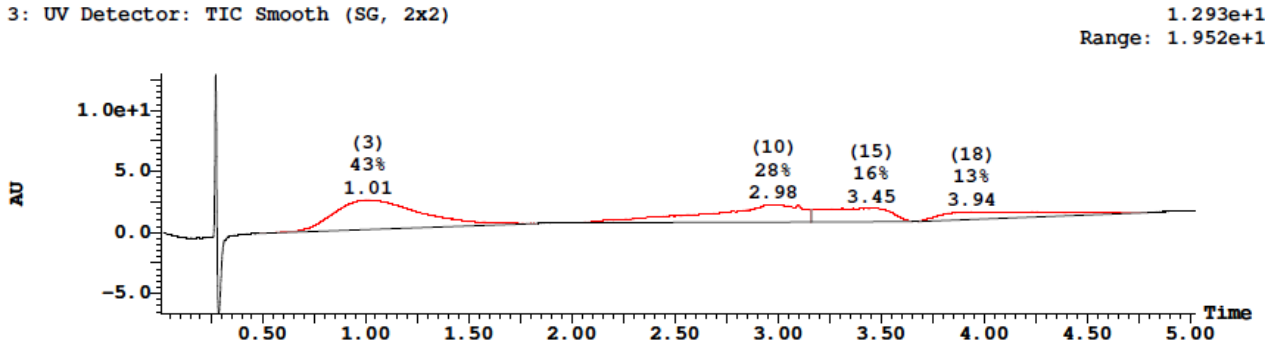


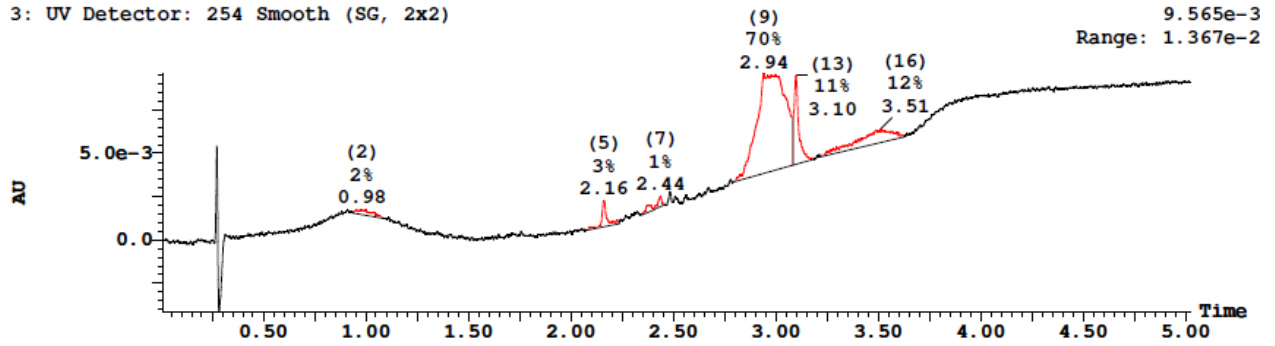
Peak ID	Compound	Time	Mass Found	BPM
9	Found	3.24	1051.00	1051

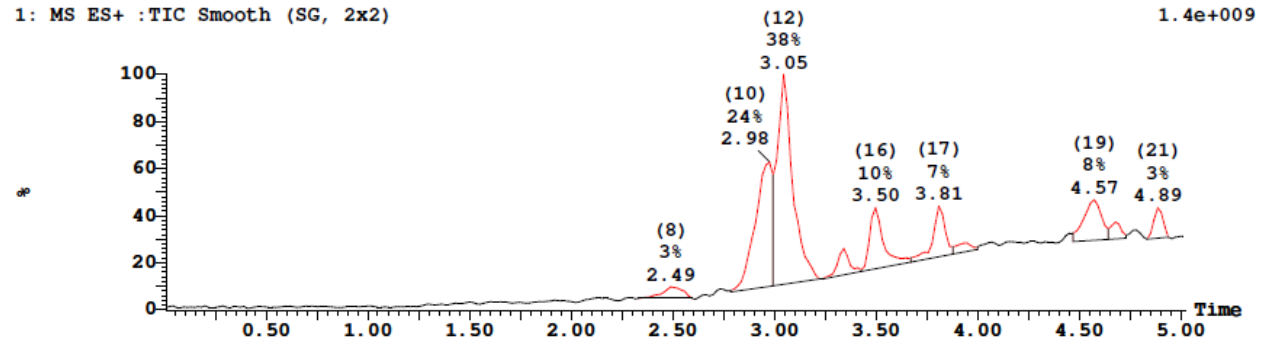


LCMS chromatograms of different stapled peptide isomers obtained after HPLC separation. Calculated M=2103, m/z: $[M+H]^{+}$=2104, $[M+2H]^{2+}$=1052.5.

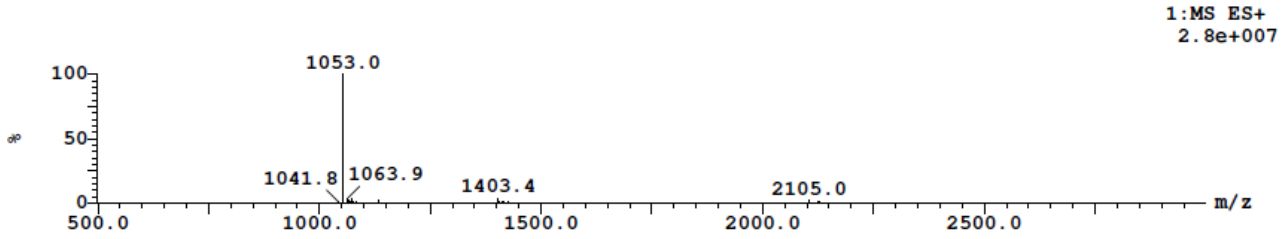
Fraction 1, RT=10.04 min in analytical HPLC chromatogram.



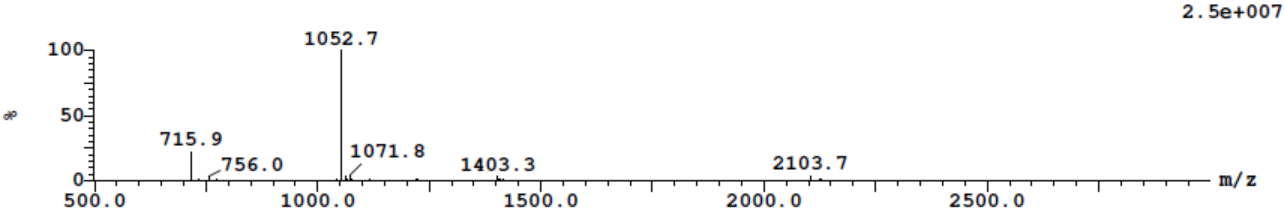




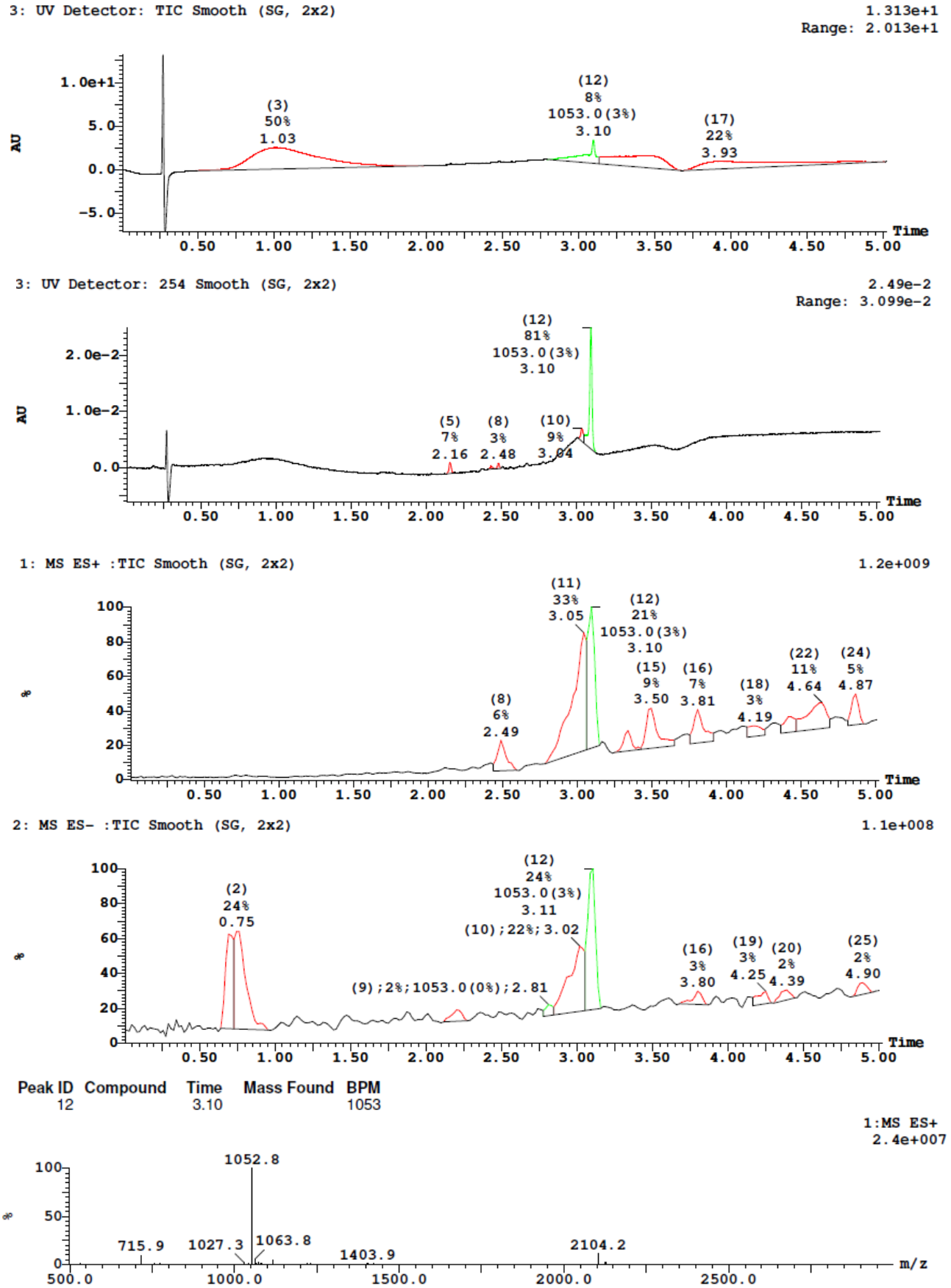
Peak ID Compound Time Mass Found BPM
10 2.98 1053



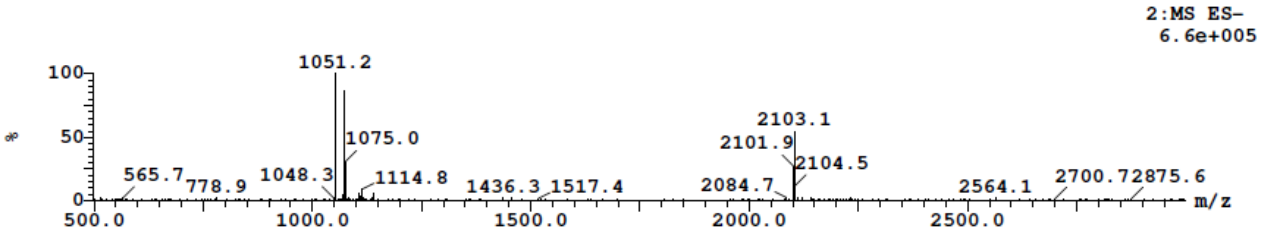
Peak ID Compound Time Mass Found BPM
12 3.05 1053



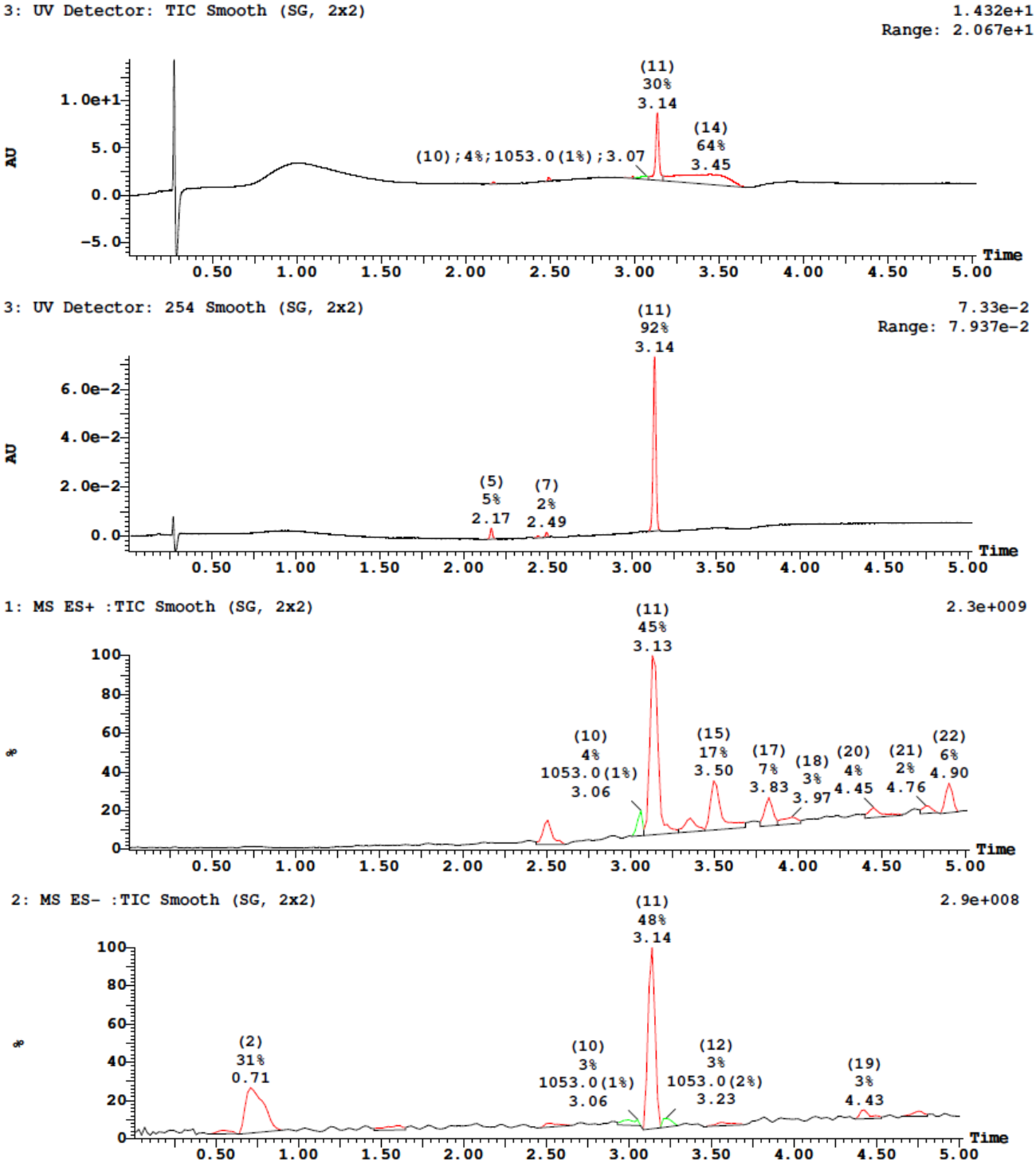
Fraction 2, RT=11.06 min in analytical HPLC chromatogram.



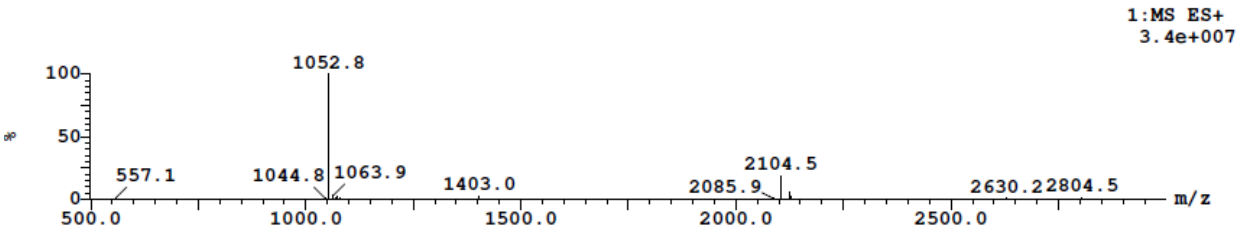
Peak ID	Compound	Time	Mass Found	BPM
12	Tentative	3.10	1052.00	1051



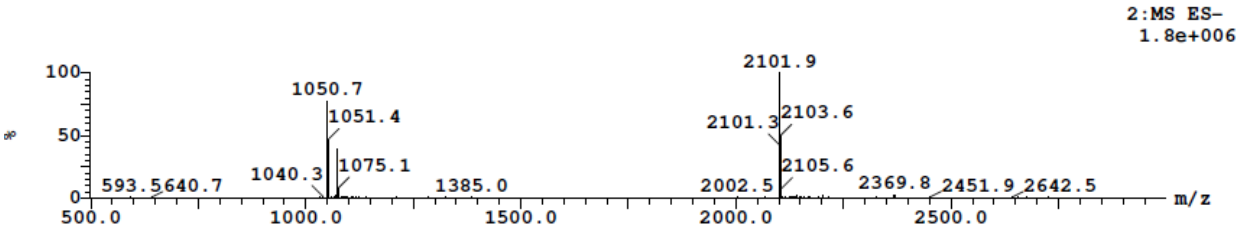
Fraction 3, RT=11.38 min in analytical HPLC chromatogram.



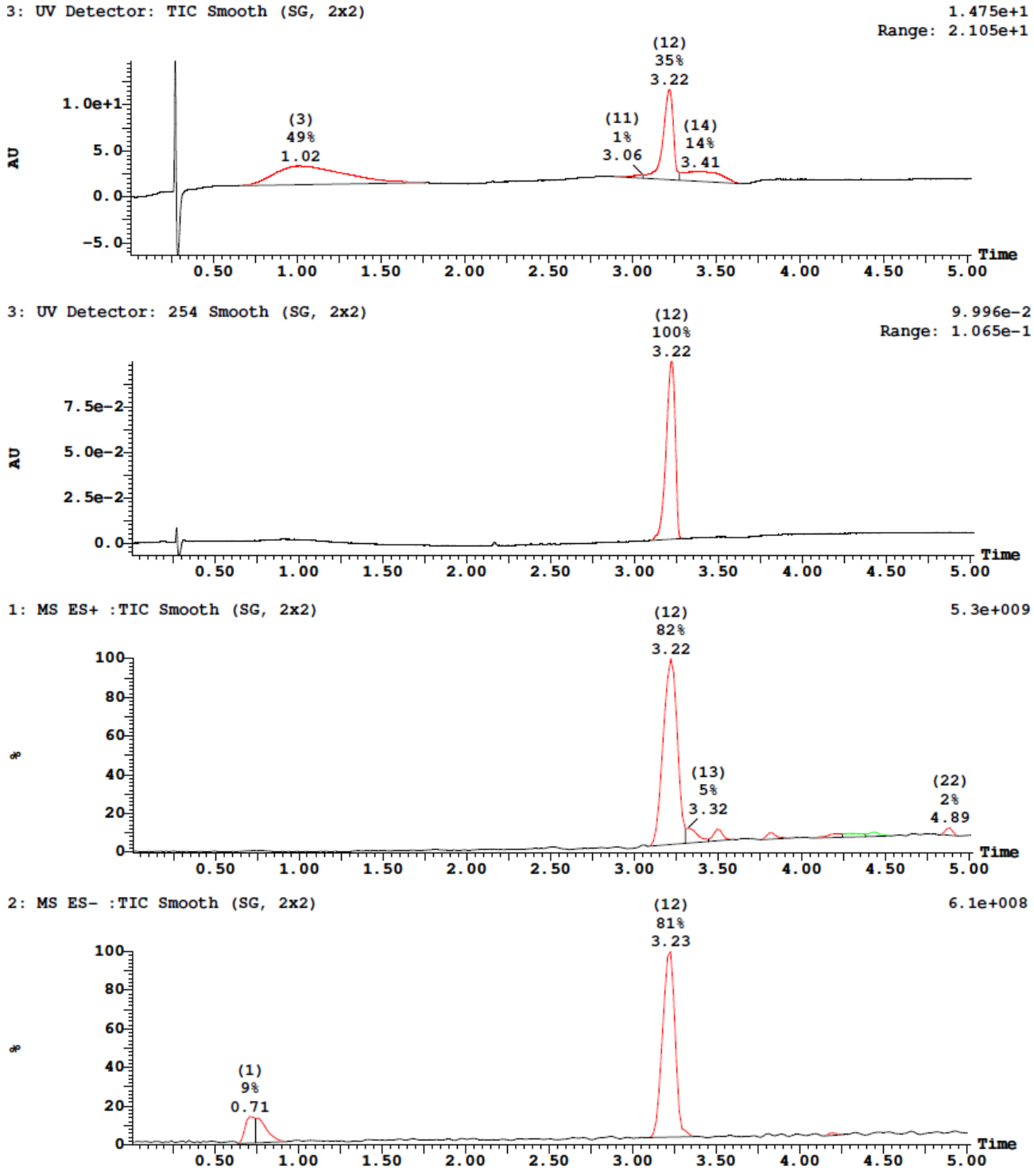
Peak ID	Compound	Time	Mass Found	BPM
11		3.13		1053



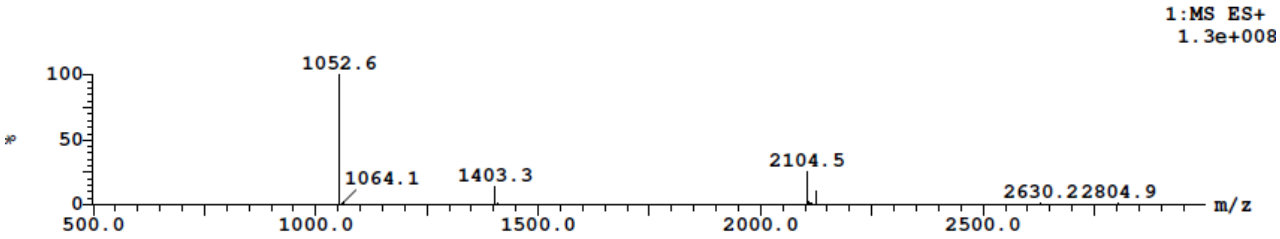
Peak ID	Compound	Time	Mass Found	BPM
11		3.13		2102



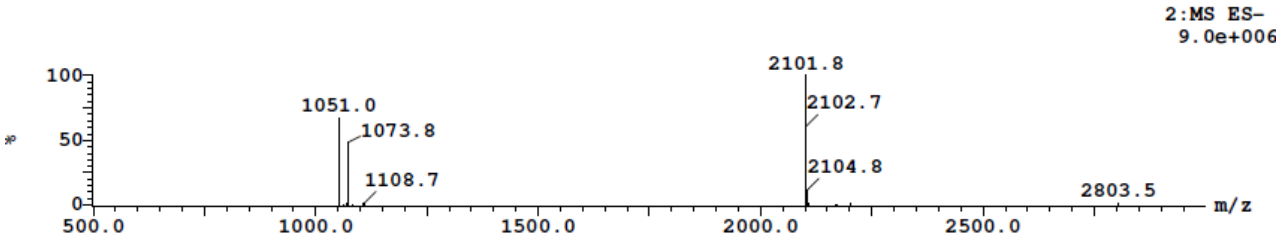
Main fraction 4, RT=12.51 min in analytical HPLC chromatogram.



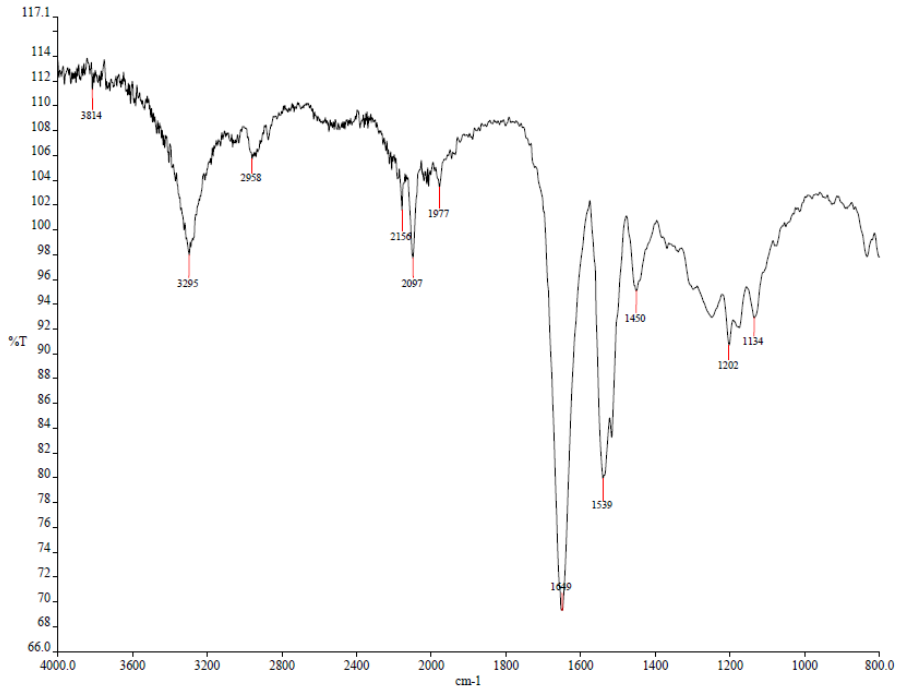
Peak ID	Compound	Time	Mass Found	BPM
12		3.22		1053



Peak ID	Compound	Time	Mass Found	BPM
12		3.22		2102

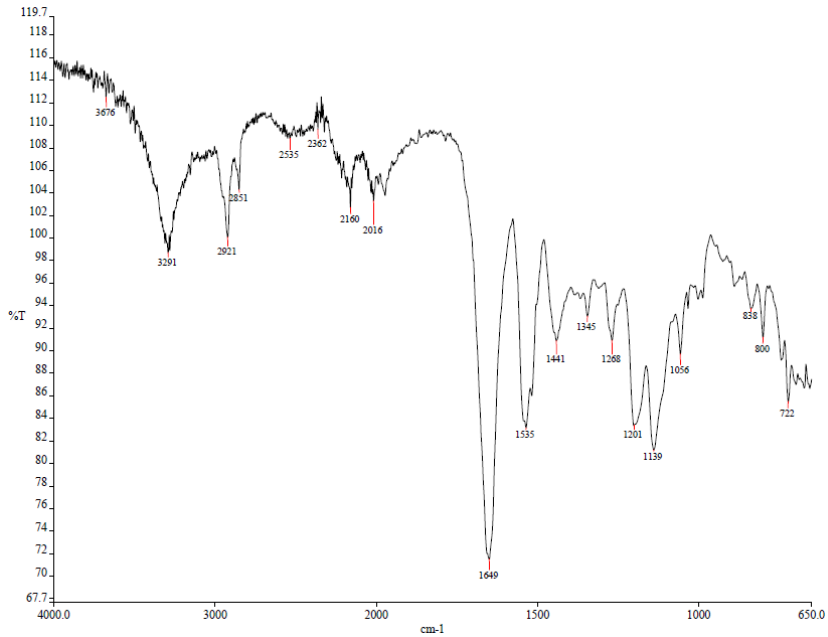


IR spectra of linear peptide **24** and four different isomers of stapled peptide **26**. Absence of N_3-group absorbance in all fraction of stapled peptide **26** proves formation of macrocycle from two linear precursors (both N_3-residues of peptide **24** reacted with two alkyne residues of linker **5)**



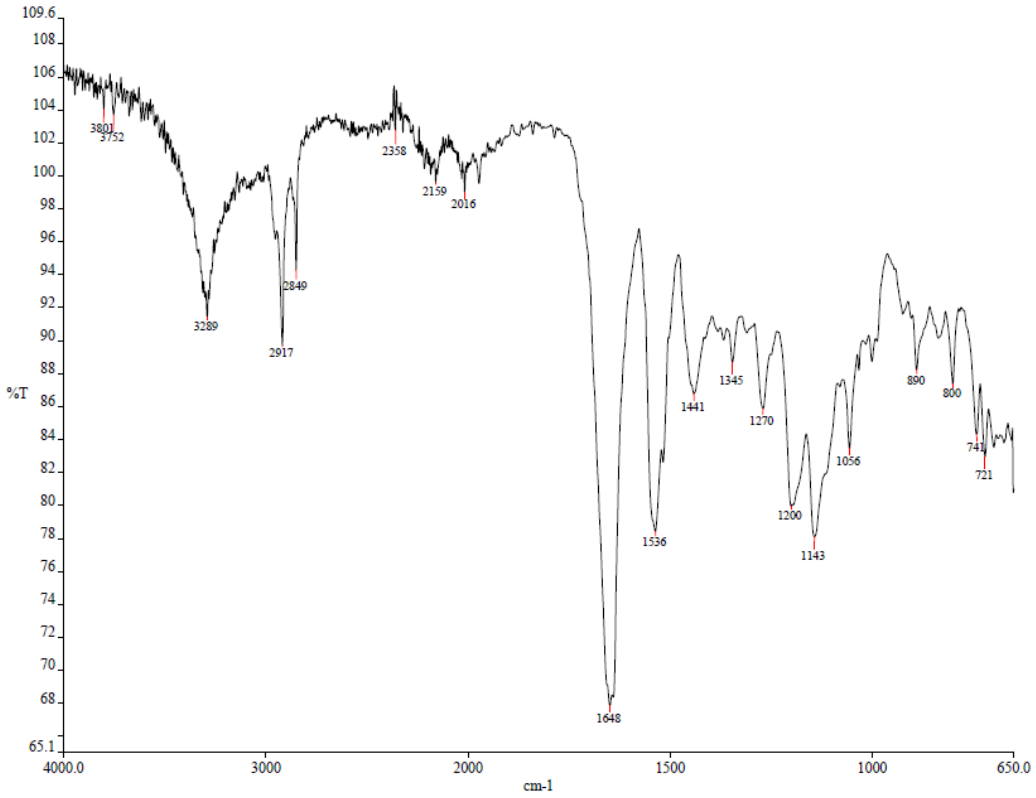
IR spectra of peptide **24**

N_3-group absorbance at 2097 cm^{-1}



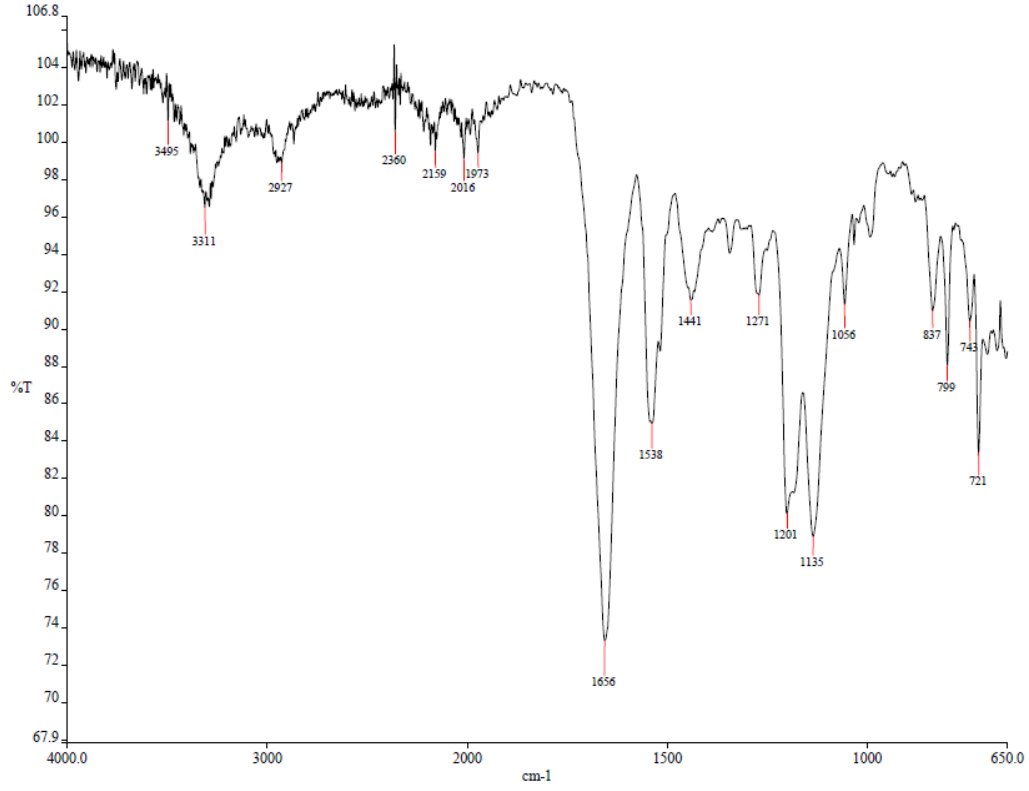
IR spectra of peptide **26** (fraction 1)

No N_3-group absorbance



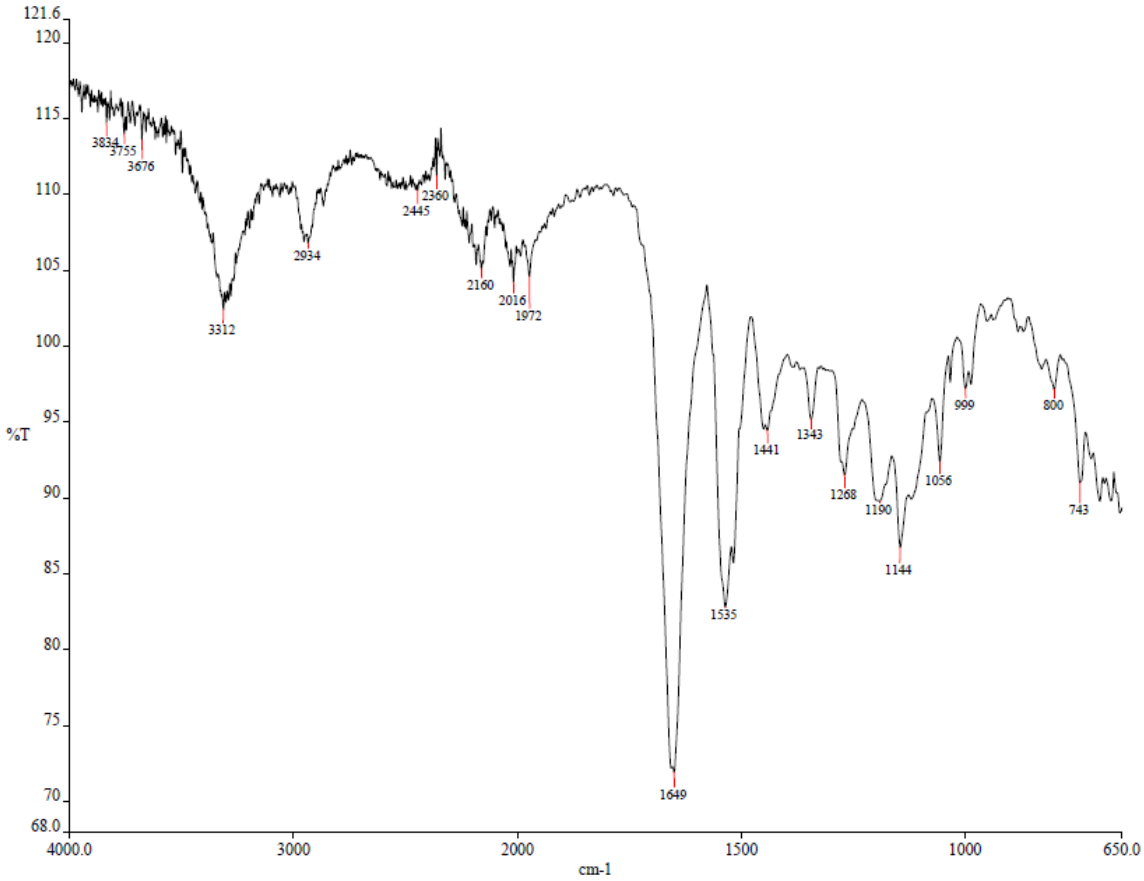
IR spectra of peptide **26** (fraction 2)

No N_3-group absorbance



IR spectra of peptide **26** (fraction 3)

No N_3-group absorbance



IR spectra of peptide **26** (fraction 4)

No N_3-group absorbance

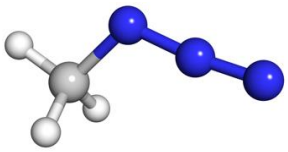
4. Computational data

All calculations were performed with Gaussian 09 Rev D.01[S2] using the B3LYP density functional and the 6-31G(d) basis set within the conductor-like polarizable continuum solvation model (CPCM)[S3-S4] for methanol, using the default integration grid. Vibrational frequencies were computed for all optimized structures to verify that they were either minima (zero imaginary frequencies) or transition states (a single imaginary frequency). The strain-promoted click reaction of **12** with methylazide was modelled at 298.15 K at 1 atm in methanol. Previous computational studies with similar methods provided results in accordance with the experiment.[S5-S6]

4.1 Cartesian coordinates and energetics of B3LYP/6-31G(d)-CPCM-(methanol) stationary points. Absolute energies depicted in hartrees.

4.1.1 Reactants

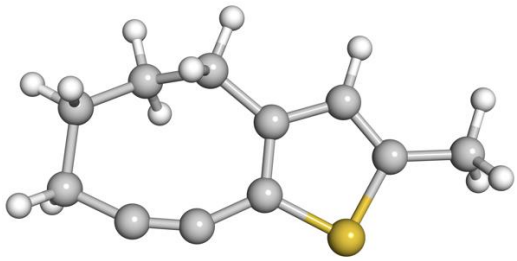
Methylazide



E	*H*	*G*
-204.042207	-204.041262	-204.073388

	X	Y	Z
6	-1.554491	0.283662	0.000005
1	-1.564307	0.916618	-0.894171
1	-1.563690	0.917485	0.893568
7	-0.391263	-0.630461	0.000032
7	0.718117	-0.096148	-0.000071
7	1.801311	0.271227	0.000036
1	-2.442214	-0.348399	0.000593

12

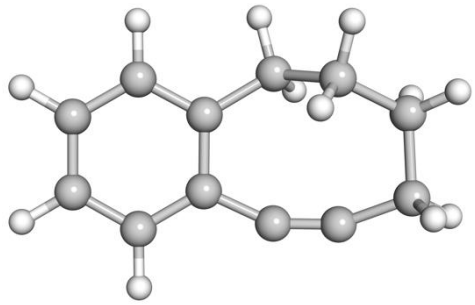


E	*H*	*G*
-824.298001	-824.297056	-824.346758

	X	Y	Z
6	3.465897	-0.945598	0.120803
6	2.112884	-1.438204	-0.119300
6	0.947857	1.531830	-0.394661

6	0.895351	-1.400888	-0.137065
6	-0.305832	0.703523	-0.179139
6	-0.317595	-0.681827	-0.126214
1	4.219476	-1.382529	-0.545293
1	1.284200	1.377252	-1.430759
1	3.774370	-1.186479	1.147635
1	0.648815	2.584457	-0.339955
6	3.416811	0.603137	-0.066852
1	3.457860	0.824508	-1.140577
1	4.335820	1.012389	0.370179
6	2.185040	1.324175	0.548882
1	1.878683	0.822313	1.474171
1	2.521389	2.323158	0.849008
6	-1.628356	1.239311	-0.084762
6	-2.622500	0.302064	0.036080
16	-1.943867	-1.313326	0.041837
6	-4.101042	0.523568	0.152924
1	-4.314799	1.596630	0.136619
1	-4.651607	0.057165	-0.673086
1	-4.504557	0.112952	1.086437
1	-1.838875	2.304847	-0.107949

15

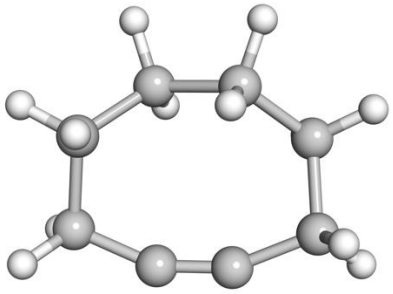


E	*H*	*G*
-464.221431	-464.220487	-464.265976

	X	Y	Z
6	3.071928	-0.976916	0.094986
6	1.712916	-1.455766	-0.150582
6	0.567668	1.382388	-0.459938
6	0.498288	-1.415781	-0.175269
6	-0.733214	0.643367	-0.179190
6	-0.779073	-0.779257	-0.117858

1	3.811675	-1.387254	-0.602633
1	0.927942	1.057974	-1.446383
1	3.389533	-1.283423	1.101114
1	0.318736	2.443609	-0.570116
6	3.040422	0.581359	-0.000789
1	3.164139	0.871786	-1.051458
1	3.919262	0.963845	0.532326
6	1.758769	1.255906	0.555011
1	1.433673	0.743327	1.467892
1	2.034180	2.270860	0.864009
6	-1.938920	1.339422	-0.049213
6	-2.000136	-1.447837	0.050189
1	-1.928286	2.426185	-0.091436
1	-2.009743	-2.533123	0.093120
6	-3.186721	-0.724756	0.169886
6	-3.154741	0.670195	0.125868
1	-4.074577	1.240407	0.223703
1	-4.129654	-1.248137	0.301260

14



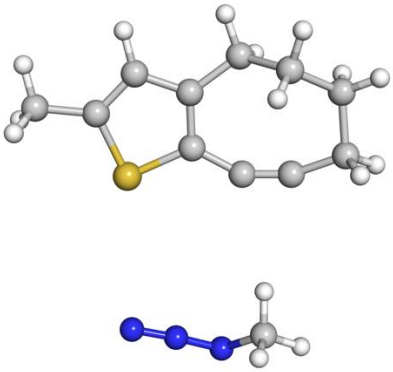
E	*H*	*G*
-311.814183	-311.813239	-311.853855

	X	Y	Z
6	1.952295	-0.922638	0.134150
6	0.604697	-1.483405	0.035668
6	-0.698352	1.380631	-0.352412
6	-0.605111	-1.483389	-0.035920
6	-1.854998	0.570571	0.290124
6	-1.952605	-0.922254	-0.134013
1	2.684166	-1.445972	-0.493838
1	-0.582689	1.078986	-1.402560

1	2.319088	-0.995671	1.167328
1	-1.037945	2.423523	-0.384514
6	1.855239	0.570272	-0.289963
1	1.762113	0.617275	-1.382666
1	2.810778	1.049458	-0.039015
6	0.698709	1.380642	0.352243
1	0.582947	1.079346	1.402487
1	1.038418	2.423509	0.384065
1	-1.761484	0.617482	1.382797
1	-2.319797	-0.995199	-1.167059
1	-2.684400	-1.445416	0.494216
1	-2.810451	1.050096	0.039494

4.1.2 Associated states

A1: *anti*-attack

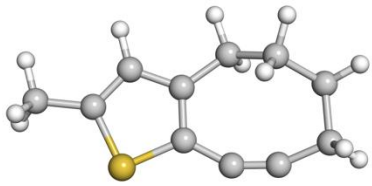


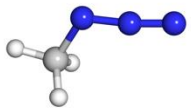
E	*H*	*G*
-1028.338954	-1028.338010	-1028.409401

	X	Y	Z
6	-0.855741	3.443844	0.058330
6	-0.195152	2.164355	-0.180869
6	-2.989935	0.614702	-0.374135
6	-0.071294	0.951920	-0.184878
6	-1.998512	-0.516558	-0.169018
6	-0.622588	-0.345719	-0.147187
1	-0.539086	4.238494	-0.627594
1	-2.905893	0.956625	-1.416568
1	-0.631616	3.795399	1.074915
1	-3.992197	0.180154	-0.290847
6	-2.388707	3.187931	-0.087495
1	-2.641556	3.189040	-1.155046

1	-2.904077	4.049241	0.354576
6	-2.924624	1.878353	0.555080
1	-2.364111	1.649471	1.469016
1	-3.951580	2.082770	0.878524
6	-2.352587	-1.896900	-0.052507
6	-1.289483	-2.756933	0.055001
16	0.221899	-1.872133	0.019272
6	-1.311783	-4.250388	0.188543
1	-2.347384	-4.603725	0.189882
1	-0.787592	-4.743408	-0.639133
1	-0.838978	-4.585836	1.119578
1	-3.381018	-2.246944	-0.050672
6	3.852713	1.875430	0.377342
1	4.082117	1.814979	1.447098
1	2.770994	1.784130	0.228902
7	4.588408	0.842175	-0.386707
7	4.322998	-0.327212	-0.109705
7	4.171116	-1.449917	0.051564
1	4.190094	2.838186	-0.006298

A2: *syn*-attack





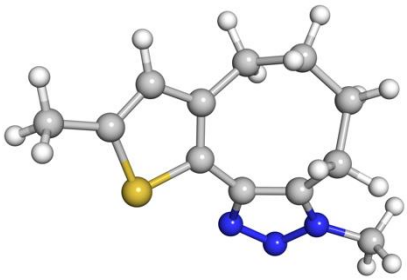
E	*H*	*G*
-1028.338555	-1028.337610	-1028.409573

	X	Y	Z
6	-0.402651	3.382282	-0.049976
6	-0.614921	1.952544	0.156429
6	2.537358	1.408161	0.435853
6	-0.322505	0.770048	0.149608
6	1.979978	0.017651	0.188140
6	0.625829	-0.272150	0.120076

1	-0.987280	4.017438	0.626305
1	2.319993	1.682646	1.479023
1	-0.694577	3.658866	-1.072714
1	3.628680	1.329622	0.377691
6	1.122320	3.642643	0.153885
1	1.323779	3.701758	1.230720
1	1.340278	4.635936	-0.257325
6	2.082295	2.598240	-0.481430
1	1.656861	2.215155	-1.416314
1	2.992111	3.138455	-0.767304
6	2.772460	-1.167011	0.071462
6	2.054954	-2.325980	-0.081584
16	0.336076	-1.987643	-0.092311
6	2.571395	-3.726242	-0.228357
1	3.664979	-3.721442	-0.189212
1	2.207763	-4.382873	0.571399
1	2.269696	-4.176749	-1.181821
1	3.858412	-1.159188	0.102434
6	-4.173401	-2.021345	0.486204
1	-5.116841	-2.138669	-0.058351
1	-4.385300	-1.740810	1.523833
7	-3.292912	-1.030591	-0.171029
7	-3.728986	0.119006	-0.241652
7	-4.009675	1.219701	-0.371277
1	-3.633427	-2.967726	0.474459

4.1.3 Products: triazole

P1: *anti*-attack

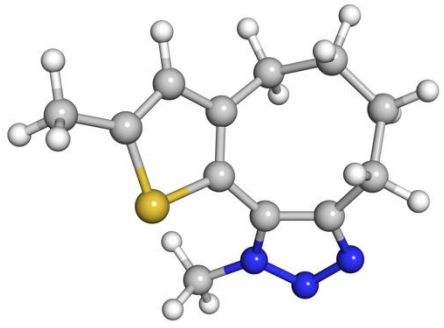


E	*H*	*G*
-1028.468694	-1028.467749	-1028.525021

	X	Y	Z

6	1.867426	1.101022	1.101701
6	1.755155	-0.160756	0.303187
6	-0.442136	1.986009	-1.074464
6	0.650525	-0.831965	-0.210944
6	-1.242156	0.840258	-0.495151
6	-0.737455	-0.400956	-0.174976
1	2.826773	1.096597	1.629537
1	0.267083	1.600144	-1.818658
1	1.090233	1.083520	1.876622
1	-1.141954	2.625869	-1.624191
6	1.748725	2.423492	0.305798
1	2.365538	2.359324	-0.600554
1	2.194194	3.211226	0.925803
6	0.329127	2.876445	-0.066819
1	-0.270043	2.998946	0.845534
1	0.420083	3.876334	-0.509514
6	-2.661689	0.905108	-0.294072
6	-3.233782	-0.254789	0.152564
16	-2.010014	-1.489386	0.355482
6	-4.679798	-0.532806	0.437834
1	-5.273004	0.366174	0.244338
1	-5.073130	-1.339501	-0.192514
1	-4.842506	-0.826509	1.481885
1	-3.244663	1.798072	-0.501715
6	4.216375	-0.810324	0.274171
1	4.729991	-1.678179	-0.138885
1	4.390170	-0.763260	1.352203
7	2.796961	-0.977912	-0.007410
7	2.372526	-2.091080	-0.649938
7	1.074047	-2.005647	-0.773029
1	4.594807	0.099470	-0.197931

P2: *syn*-attack

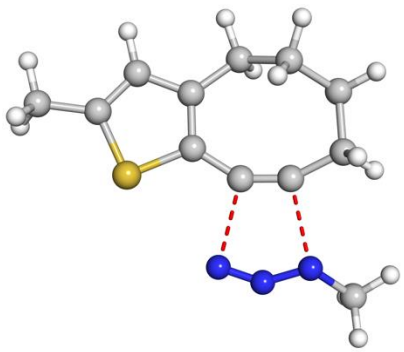


E	*H*	*G*
-1028.467534	-1028.466590	-1028.523618

	X	Y	Z
6	-2.064602	1.355943	-1.185795
6	-2.001639	-0.002009	-0.554150
6	0.043696	1.948478	1.276476
6	-0.904520	-0.696064	-0.053537
6	0.917468	0.925132	0.588726
6	0.478532	-0.270915	0.060408
1	-2.999583	1.395922	-1.755454
1	-0.713651	1.441063	1.887194
1	-1.247477	1.459494	-1.912460
1	0.679361	2.505334	1.974329
6	-2.032688	2.559477	-0.214736
1	-2.725246	2.366691	0.616049
1	-2.435875	3.425248	-0.755556
6	-0.662439	2.970113	0.346611
1	0.011293	3.235799	-0.479236
1	-0.815225	3.890997	0.923479
6	2.335758	1.078750	0.451618
6	2.972127	0.034140	-0.161866
16	1.815543	-1.196949	-0.613073
6	4.436651	-0.134759	-0.437069
1	4.979895	0.749508	-0.090645
1	4.849735	-1.009956	0.078521
1	4.639735	-0.259605	-1.507247
1	2.871279	1.947930	0.822300
6	-0.755437	-3.034161	0.958160
1	-1.527354	-3.674824	1.384425
1	-0.084226	-2.686030	1.745917
7	-1.424934	-1.894746	0.346362
7	-2.750240	-1.941918	0.112556
7	-3.099827	-0.798361	-0.428368
1	-0.183780	-3.595946	0.214615

4.1.4 Transition states

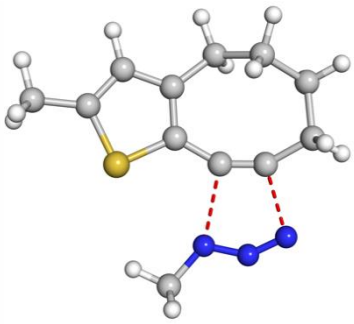
T2:*anti*-attack



E	*H*	*G*
-1028.323567	-1028.322623	-1028.385423

	X	Y	Z
6	-2.423598	1.638603	-0.341208
6	-1.648613	0.414179	-0.100620
6	0.948959	2.343192	0.527804
6	-0.583474	-0.221019	-0.027378
6	1.558658	0.986626	0.243953
6	0.831600	-0.176043	0.046622
1	-3.372502	1.649230	0.208187
1	0.507532	2.323348	1.535537
1	-2.686695	1.689529	-1.407635
1	1.775297	3.060152	0.587114
6	-1.588817	2.886878	0.044557
1	-1.590406	2.990186	1.137242
1	-2.122194	3.761598	-0.347573
6	-0.124005	2.907673	-0.460037
1	-0.050796	2.401127	-1.430108
1	0.137684	3.954881	-0.649668
6	2.967520	0.741241	0.218188
6	3.321226	-0.566362	0.007355
16	1.894519	-1.562085	-0.166003
6	4.698707	-1.153326	-0.078870
1	5.444876	-0.364422	0.057000
1	4.868697	-1.915069	0.691818
1	4.882133	-1.627731	-1.050695
1	3.705732	1.526650	0.355125
6	-4.547451	-1.365315	0.353301
1	-4.531629	-1.525821	1.437086
1	-5.118654	-2.167180	-0.126932
7	-3.198306	-1.262829	-0.210447
7	-2.369162	-2.176170	-0.004299
7	-1.230206	-2.413123	0.060069
1	-5.041944	-0.416225	0.140285

T2:*syn*-attack

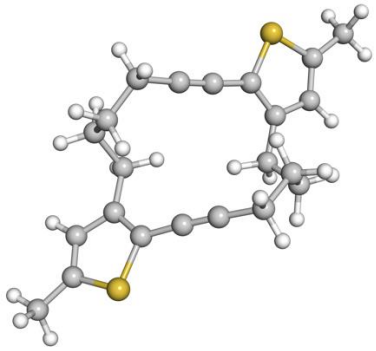


E	*H*	*G*
-1028.322477	-1028.321533	-1028.384043

	X	Y	Z
6	2.797125	-1.557409	-0.462029
6	1.959758	-0.378532	-0.164946
6	-0.418008	-2.509482	0.672880
6	0.819235	0.108690	-0.089529
6	-1.181212	-1.254606	0.309567
6	-0.580945	-0.039691	0.016909
1	3.799998	-1.465671	-0.030219
1	0.088707	-2.344475	1.635047
1	2.936875	-1.620205	-1.551234
1	-1.157104	-3.295877	0.860602
6	2.124564	-2.857763	0.041614
1	2.216873	-2.906295	1.134487
1	2.713431	-3.695167	-0.353312
6	0.638016	-3.051926	-0.346937
1	0.451434	-2.632687	-1.343507
1	0.467199	-4.130434	-0.440667
6	-2.607118	-1.161771	0.269100
6	-3.094731	0.080987	-0.043914
16	-1.777845	1.202025	-0.310088
6	-4.524896	0.515547	-0.162276
1	-5.185379	-0.331891	0.045065
1	-4.769011	1.316157	0.546477
1	-4.758205	0.887795	-1.167182
1	-3.259666	-2.005702	0.474226
6	0.920294	3.778898	0.122876
1	-0.156271	3.767439	0.297291
1	1.110839	4.138306	-0.895001
7	1.390351	2.402662	0.317039

7	2.606074	2.143890	0.184699
7	3.353702	1.251278	0.059290
1	1.382421	4.463834	0.842254

4.1.5 Dimers



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E	*H*	*G*
-1648.650349	-1648.649405	-1648.730766

	X	Y	Z
6	0.006636	-2.719056	1.912284
6	-1.000924	-1.927974	1.200665
6	-1.727376	1.478958	-0.893234
6	-1.868234	-1.298482	0.624218
6	-2.927822	0.585884	-0.698560
6	-2.926843	-0.620449	-0.021550
1	-0.079577	-2.526576	2.990989
1	-0.808196	0.919860	-0.696013
1	-0.234684	-3.783241	1.776759
1	-1.691236	1.789114	-1.945919
6	-1.475773	2.489851	1.484902
1	-1.778301	1.470799	1.753005
1	-2.082823	3.163630	2.100740
6	-1.756888	2.749626	-0.009543
1	-2.737528	3.228089	-0.121422
1	-1.022930	3.465958	-0.401585
6	-4.228052	0.913925	-1.202455
6	-5.205690	-0.004182	-0.930726
16	-4.542308	-1.340753	-0.018980
6	-6.653659	0.033948	-1.318344
1	-6.861003	0.950049	-1.879615
1	-6.930163	-0.819727	-1.948797
1	-7.311284	0.017793	-0.440952

1	-4.426715	1.820702	-1.766835
6	-0.006549	2.719240	1.912079
6	1.000921	1.928070	1.200432
6	1.727375	-1.479025	-0.893340
6	1.868205	1.298517	0.624019
6	2.927808	-0.585933	-0.698651
6	2.926813	0.620430	-0.021689
1	0.234817	3.783402	1.776481
1	0.808170	-0.919907	-0.696286
1	0.079736	2.526811	2.990787
1	1.691344	-1.789315	-1.945989
6	1.475804	-2.489605	1.484946
1	1.778283	-1.470490	1.752872
1	2.082979	-3.163240	2.100819
6	1.756769	-2.749582	-0.009496
1	2.737329	-3.228202	-0.121404
1	1.022658	-3.465833	-0.401399
6	4.228066	-0.914020	-1.202446
6	5.205706	0.004077	-0.930692
16	4.542292	1.340703	-0.019055
6	6.653708	-0.034116	-1.318179
1	6.861061	-0.950217	-1.879447
1	6.930315	0.819557	-1.948589
1	7.311252	-0.018011	-0.440724
1	4.426751	-1.820830	-1.766765

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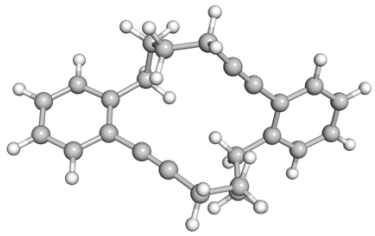
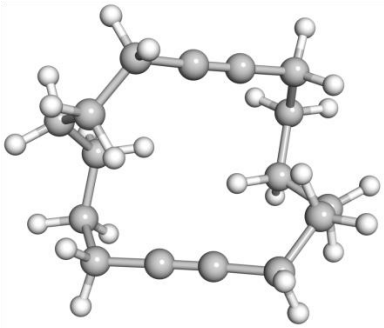


E	*H*	*G*
-928.484906	-928.483962	-928.557763

	X	Y	Z
6	-0.052469	2.591603	1.855126
6	1.009904	1.881763	1.134710
6	1.790874	-1.271133	-0.969933
6	1.926093	1.332150	0.554927
6	3.083794	0.775217	-0.083296

1	0.040964	2.378788	2.929310
1	0.907155	-0.665669	-0.751337
1	0.123222	3.671695	1.747569
1	1.713726	-1.579250	-2.020535
6	1.499708	-2.279855	1.408991
1	1.736594	-1.240167	1.660090
1	2.155941	-2.904716	2.025891
6	1.769739	-2.543717	-0.089251
1	2.727576	-3.064184	-0.209123
1	1.004056	-3.224760	-0.482994
6	0.052348	-2.591938	1.854804
6	-1.009945	-1.882033	1.134331
6	-1.790778	1.271137	-0.969993
6	-1.926054	-1.332374	0.554461
6	-3.048992	0.441047	-0.808840
1	-0.123293	-3.672015	1.747035
1	-0.907103	0.665588	-0.751466
1	-0.041285	-2.379301	2.929004
1	-1.713631	1.579350	-2.020567
6	-1.499772	2.279631	1.409034
1	-1.736757	1.239931	1.659996
1	-2.156059	2.904465	2.025901
6	-1.769553	2.543658	-0.089210
1	-2.727303	3.064274	-0.209155
1	-1.003726	3.224618	-0.482820
6	3.049043	-0.440983	-0.808732
6	4.238786	-0.893631	-1.397288
1	4.218435	-1.822028	-1.964033
6	5.435697	-0.189165	-1.282221
1	6.337541	-0.569368	-1.754175
6	5.465853	1.009761	-0.564172
1	6.390453	1.572053	-0.468224
6	4.300376	1.485132	0.025885
1	4.311104	2.417080	0.583300
6	-3.083762	-0.775261	-0.083597
6	-4.300445	-1.484975	0.025800
1	-4.311224	-2.416938	0.583188
6	-5.465945	-1.009426	-0.564073
1	-6.390612	-1.571590	-0.467995
6	-4.238746	0.893869	-1.397222
1	-4.218384	1.822361	-1.963809
6	-5.435716	0.189501	-1.282114
1	-6.337605	0.569920	-1.753815

28



E	*H*	*G*
-623.667571	-623.666627	-623.729336

	X	Y	Z
6	1.718249	-2.167984	1.394945
6	0.384811	-2.176651	0.778760
6	-2.140698	0.109805	-1.314572
6	-0.735115	-2.219584	0.314737
6	-2.089050	-2.284492	-0.248680
1	1.595297	-2.286819	2.480698
1	-1.107450	0.335480	-1.031555
1	2.277026	-3.054260	1.060451
1	-2.292921	0.573165	-2.298206
6	-2.588950	0.912778	1.143403
1	-2.030177	0.022712	1.454357
1	-3.448584	0.995862	1.820437
6	-3.111959	0.756641	-0.299368
1	-4.040285	0.168465	-0.275424
1	-3.406650	1.751072	-0.661674
6	-1.718066	2.167969	1.395228
6	-0.384770	2.176641	0.778737
6	2.140760	-0.109939	-1.314802
6	0.735057	2.219522	0.314475
6	2.310519	1.407008	-1.503184
1	-2.276842	3.054369	1.061066
1	1.107552	-0.335807	-1.031783
1	-1.594875	2.286525	2.480988
1	2.293108	-0.573421	-2.298358
6	2.588998	-0.912660	1.143264
1	2.030110	-0.022668	1.454241
1	3.448607	-0.995691	1.820336
6	3.112138	-0.756343	-0.299436

1	4.040206	-0.167765	-0.275330
1	3.407336	-1.750653	-0.661661
6	-2.310859	-1.407119	-1.502730
6	2.088921	2.284397	-0.249115
1	-2.315899	-3.326631	-0.513031
1	-3.328115	-1.610630	-1.864477
1	-2.819775	-2.010361	0.524290
1	-1.625868	-1.747101	-2.290045
1	2.315817	3.326525	-0.513471
1	3.327592	1.610762	-1.865297
1	2.819713	2.010198	0.523771
1	1.625152	1.746736	-2.290282

4.2 Calculation of strain energies

Strain energy of **12**, benzo-fused cyclooctyne **15**, and cyclooctyne **14** were calculated by using the following equation:

$$E_{strain} = E_1 - (1/2)*E_2$$

where E_1- single point energy of the strained alkyne

E_2- single point energy of the cyclicdimer of strained alkyne

E_{strain}- strain energy associated with the strained alkyne

Table S4.2. Strain energies (energies in kcal/mol)

Compound	E_1	E_2	E_{strain}
12	-517386.09	-1034809.00	18.40
15	-291437.57	-582904.03	14.45
14	-195784.41	-391596.125	13.65

5. References

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