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

Plasma Induced Acceleration and Selectivity in Strain-Promoted Azide-Alkyne Cycloadditions

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Supplementary Information

Table of content

Material and methods

Experimental procedures

Unless otherwise indicated, reactions were carried out under an argon atmosphere in flame-dried glassware with magnetic stirring. Air and/or moisture-sensitive liquids were transferred *via* syringe. When required, solutions were degassed by argon bubbling through a needle. Organic solutions were concentrated by rotary evaporation at 25-80 °C and 15-30 torr. Volume ratios are indicated when referring to mixtures of solvents (e.g. DCM/MeOH 95:5).

Material

All reagents used in the experiments were purchased from Sigma-Aldrich, Alfa Aesar, Acros or TCI and were used without any further purification. Anhydrous solvents used in experiments were obtained from Sigma-Aldrich or Alfa Aesar. Analytical thin layer chromatography (TLC) was performed using plates cut from aluminium sheets (ALUGRAM Xtra SIL G/UV254 from Macherey-Nagel). Visualization was achieved under a 254 or 365 nm UV light and by immersion in an appropriate staining solution. Silica gel for column chromatography was purchased from Merck (Geduran® Si 60, 40-63 µm). Column flash chromatography was carried out using silica gel G-25 (40- 63 µm) from Macherey-Nagel or Büchi. Human plasma was obtained from *Établissement Français du Sang Alsace – Lorraine – Champagne – Ardenne* (*EFS* – French Blood Agency) within the framework of a service contract for the transfer of products derived from blood or its components for non-therapeutic and research purposes, in accordance with French laws and regulations.

Instrumentation and methods associated

^{1}H and ^{13}C NMR spectra were recorded at 23 °C on a Bruker 400 spectrometer. Recorded shifts are reported in parts per million (δ) and calibrated using residual non-deuterated solvent. Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad, app = apparent), coupling constant (*J*, Hz) and integration.

HPLC analyses were conducted on a Shimadzu UFLC system, composed of two LC-20AC pumps, one DGU 20 A3 degasser, a SPD 20A detector and a SIL 20AC HT autosampler. We used a Waters Sunfire® C18 5 µm, 4.6 x 150 mm column. Elution was performed with a mixture of mQ water (with 0.05% TFA v/v) and acetonitrile (ACN), under a flow rate of 1 mL.min^{-1} with two different gradients (see table S1 and S2 in kinetic experiments section for details). Samples were kept at 15 °C in autosampler before injection. 50 µL of each sample were injected and the different compounds were detected by UV absorbance at 256 nm.

High-resolution mass spectra were obtained using an Agilent Q-TOF (time of flight) 6520.

LC-MS/MS analyses for plasma proteins binding studies were performed using a Triple Quadrupole Liquid Chromatograph Mass Spectrometer (LCMS 8030, Shimadzu) in multiple reaction-monitoring mode (MRM). See appropriate section for detailed procedure.

Purification by preparative HPLC were carried out on a Waters 600 controller system (pumps: Waters Delta 600; detector: Waters 2489 UV/Vis) equipped with a SunFire™ Prep C18 OBD 5 µM 19×150 mm column (Waters), using water (0.1% TFA, solvent A) and acetonitrile (solvent B) as a solvent system with a flow rate of 17 mL.min^{-1} and a linear gradient of B going from 5% to 95% over 25 min.

Synthesis

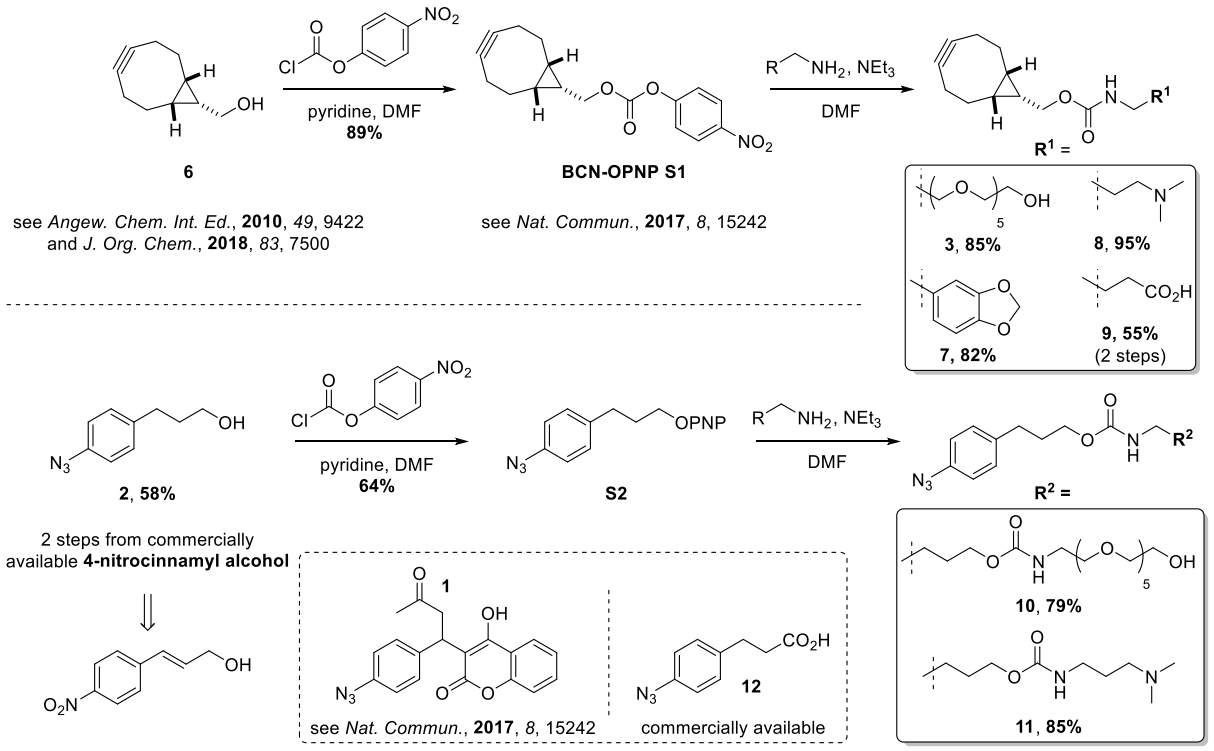
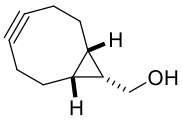


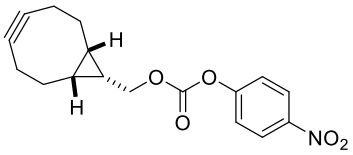
Figure S1. Synthetic overview of all BCN and azide compounds used in this work.

(Bicyclo[6.1.0]non-4-yn-9-yl)methanol (BCN-OH) 6



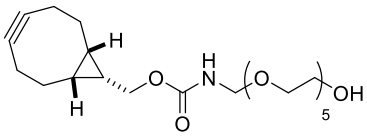
BCN-OH 6 was prepared following a procedure adapted from Dommerholt *et al*.[1] In order to favour the formation of the *endo* isomer over the *exo* at the cyclopropanation step, we followed a procedure developed by O'Brien *et al.* where diRhodium tetraacetate was replaced with diRhodium tetra S-BHTL.[2] All other steps followed the initial report from Dommerholt *et al*.

(Bicyclo[6.1.0]non-4-yn-9-yl)methyl (4-nitrophenyl) carbonate (BCN-OPNP) S1



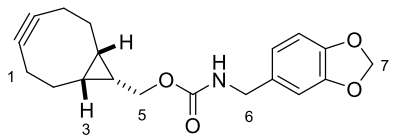
BCN-OPNP S1 was synthesized according to a reported procedure.[3]

(Bicyclo[6.1.0]non-4-yn-9-yl)methyl (16-hydroxy-2,5,8,11,14-pentaoxahexadecyl)carbamate 3



BCN derivative **3** was synthesized according to a reported procedure.[3]

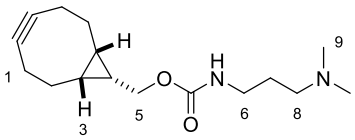
(Bicyclo[6.1.0]non-4-yn-9-yl)methyl (benzo[d][1,3]dioxol-5-ylmethyl)carbamate 7



Piperonylamine (2.0 equiv., 79.2 µL, 0.63 mmol) and triethylamine (3.0 equiv., 0.13 mL, 0.95 mmol) were dissolved in DMF (1.6 mL). This solution was then added dropwise to a solution of **BCN-OPNP S1** (1.0 equiv., 100.0 mg, 0.32 mmol) in DMF (1.6 mL). The mixture was stirred for 16 h at 25 °C before being concentrated to dryness. The crude was then suspended in water and extracted three times with ethyl acetate. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The product was finally

purified by flash column chromatography (cHex/EtOAc 8:2 to 6:4 over 40 min) to give the title product (89.2 mg, 0.26 mmol, 82%) as a yellow oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 – 6.75 (m, 3H, HAr), 5.94 (s, 2H, H7), 4.93 (br s, 1H, NH), 4.27 (d, *J* = 6.0 Hz, 2H, H6), 4.19 (d, *J* = 8.4 Hz, 2H, H5), 2.32 – 2.19 (m, 6H, 4xH1 + 2xH2), 1.63 – 1.53 (m, 2H; 2xH2), 1.37 (app quint, *J* = 8.4 Hz, 1H, H4), 0.99 – 0.90 (m, 2H, H3).
^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 147.9, 147.0, 132.5, 126.1, 120.8, 115.6, 108.3, 108.2, 101.1, 98.9, 63.0, 44.9, 29.1, 21.4, 20.2, 17.8
HRMS (ESI$^+$, m/z) calculated for $C_{19}H_{22}NO_4$ $(M+H)^+$: 328.1549, found 328.1544.

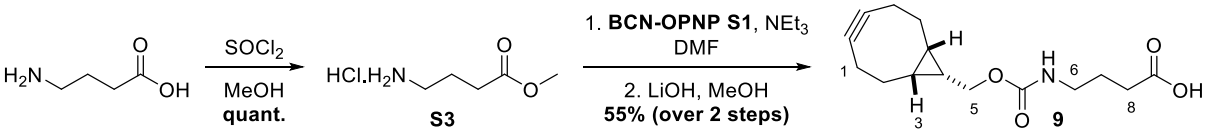
(Bicyclo[6.1.0]non-4-yn-9-yl)methyl (3-(dimethylamino)propyl)carbamate 8



A solution of 3-dimethylpropylamine (1.1 equiv., 44.0 µL, 0.35 mmol) and triethylamine (3.0 equiv., 0.13 mL, 0.95 mmol) in DMF (1.60 mL) was added dropwise to a solution of **BCN-OPNP S1** (1.0 equiv., 100.0 mg, 0.32 mmol) in DMF (1.60 mL). The mixture was stirred for 16 h at 25 °C before being concentrated to dryness. The crude was then purified by flash column chromatography (DCM/MeOH 10:0 to 8:2 over 25 min) to give the title product (84.2 mg, 0.30 mmol, 95%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (br s, 1H, NH), 4.13 (d, *J* = 8.1 Hz, 2H, H5), 3.28 – 3.25 (m, 2H, H6), 2.48 – 2.46 (m, 2H, H8), 2.32 – 2.18 (m, 12H, H9 + 4xH1 + 2xH2), 1.76 – 1.71 (m, 2H, H7), 1.63 – 1.53 (m, 2H; 2xH2), 1.35 (app quint, *J* = 8.6 Hz, 1H, H4), 0.99 – 0.92 (m, 2H, H3).
^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 98.8, 62.6, 57.7, 53.4, 45.2, 40.0, 29.1, 27.0, 21.4, 20.1, 17.8.
HRMS (ESI$^+$, m/z): calculated for $C_{16}H_{27}N_2O_2$ $[M+H]^+$: 279.2067; found: 279.2062

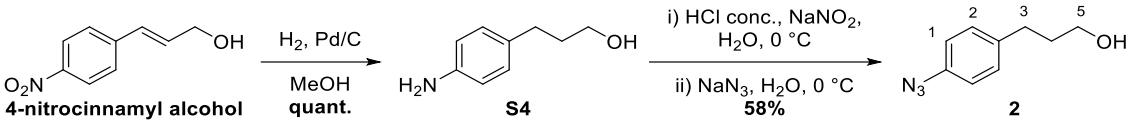
4-((((Bicyclo[6.1.0]non-4-yn-9-yl)methoxy)carbonyl)amino)butanoic acid 9



BCN **9** was obtained in three steps from commercially available γ-aminobutyric acid.
*Step 1 – Formation of methyl 4-aminobutanoate hydrochloride **S3***. 4-Aminobutyric acid (1.0 equiv., 200.0 mg, 1.94 mmol) was dissolved in MeOH (10.0 mL) and cooled to 0°C. Thionyl chloride (1.5 equiv., 0.21 mL, 2.91 mmol) was then added dropwise. The mixture was warmed back to room temperature and stirred for 4 h before being evaporated to dryness. The product was engaged in step 2 without further purification.
Step 2 – Carbamate formation. Methyl 4-aminobutanoate hydrochloride **S3** (3.0 equiv., 177.0 mg, 1.16 mmol) and triethylamine (3.0 equiv., 0.16 mL, 1.16 mmol) were dissolved in DMF (1.95 mL). This solution was then added dropwise to a solution of **BCN-OPNP S1** (1.0 equiv., 122.0 mg, 0.39 mmol) in DMF (1.95 mL). The mixture was stirred for 1.5 h at 25 °C before being concentrated to dryness. The crude was then suspended in water and extracted three times with ethyl acetate. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The crude was finally purified by flash column chromatography (cHex/EtOAc 8:2 to 6:4 over 40 min) to give the title product (71.6 mg, 0.24 mmol, 63%) as a colorless oil.
*Step 3 – Saponification / formation of 4-((((-bicyclo[6.1.0]non-4-yn-9-yl)methoxy)carbonyl)amino) butanoic acid **9.*** Previously obtained BCN (71.6 mg, 0.24 mmol, 1.0 equiv.) was dissolved in MeOH (0.86 mL) and water (0.57 mL) before LiOH (89.5 mg, 3.66 mmol, 15.0 equiv.) was added in one portion. The resulting solution was stirred for 16 h at room temperature before water (5.0 mL) was added to the reaction mixture. The reaction mixture was extracted three times with DCM (15 mL), before the aqueous layer was acidified with a 5% aqueous solution of $KHSO_4$ until pH~2 (aqueous layer turns from yellow to white upon acidification). The aqueous layer was extracted six times with DCM (30 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated. The resulting crude was finally purified by flash column chromatography (DCM/MeOH 95:5 + 0.2‰ formic acid) to give the title product (60.0 mg, 0.21 mmol, 88%) as a colorless oil.
^{1}H NMR (400 MHz, CD_3OD) δ 4.16 (d, *J* = 8.1 Hz, 2H, H5), 3.16 (t, *J* = 6.9 Hz, 2H, H6), 2.34 (t, *J* = 7.4 Hz, 2H, H8), 2.30 – 2.17 (m, 6H, 4xH1 + 2xH2), 1.79 (app quint., *J* = 7.2 Hz, 2H, H7), 1.67 – 1.59 (m, 2H, 2xH2), 1.40 (app quint., *J* = 8.8 Hz, 1H, H4), 1.00-0.92 (m, 2H, H3).

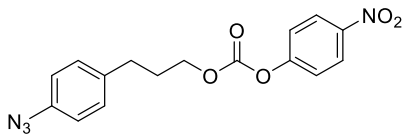
^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 158.0, 127.3, 98.1, 62.2, 39.7, 30.7, 29.3, 28.8, 25.0, 20.5, 20.0, 17.6.
HRMS (ESI$^+$, m/z): calculated for $C_{15}H_{22}NO_4$ $[M+H]^+$: 280.1543; found: 280.1540.

3-(4-Azidophenyl)propan-1-ol 2



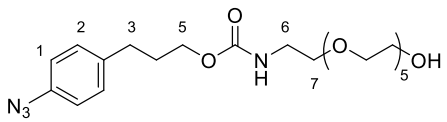
Azide **2** was obtained in two steps from commercially available 4-nitrocinnamyl alcohol:
Step 1 – Catalytic hydrogenation. 4-Nitrocinnamyl alcohol (1.0 equiv., 500.0 mg, 2.79 mmol) was dissolved in MeOH (5.0 mL) under an atmosphere of argon. Palladium on activated charcoal (10 wt%, 0.05 equiv., 81.9 mg, 0.14 mmol) was then added to the solution. The reaction medium was placed under an atmosphere of hydrogen by three consecutive purge-and-refill cycles stirred for 16 h at 25 °C. The suspension was then filtered over Celite® and the filtrate was concentrated under vacuum to afford the title compound, which was used in the next step without further purification.
Step 2 – Azide formation. 3-(4-Aminophenyl)propan-1-ol **S4** (1.0 equiv., 303.0 mg, 2.0 mmol) was dissolved in water (9.0 mL) and cooled at 0 °C. HCl 37% in water (5.0 equiv., 0.83 mL, 10.0 mmol) was added to this solution followed by a solution of $NaNO_2$ (1.0 equiv., 138.0 mg, 2.0 mmol) in water (2.8 mL). The resulting mixture was stirred at 0 °C for 10 min before NaN_3 (1.8 equiv., 234.0 mg, 3.6 mmol) was added. After stirring at 0 °C for 1.5 h, the aqueous medium was extracted three times with EtOAc. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford the title product (205 mg, 1.16 mmol, 58%) as a colorless oil.
^{1}H NMR (400 MHz, CD_3OD) δ 7.22 – 7.20 (m, 2H, H2), 7.00 – 6.96 (m, 2H, H1), 3.57 (t, *J* = 6.5 Hz, 2H, H5), 2.68 (br t, *J* = 7.5 Hz, 2H, H3), 1.83 – 1.76 (m, 2H, H4).
^{13}C NMR (100 MHz, CD_3OD) δ 146.1, 133.3, 130.0, 117.0, 62.3, 35.8, 32.2.
HRMS (ESI$^+$, m/z): calculated for $C_9H_{12}NO$ $[M-N_2+H]^+$: 150.0919; found: 150.0920

3-(4-Azidophenyl)propyl (4-nitrophenyl) carbonate S2



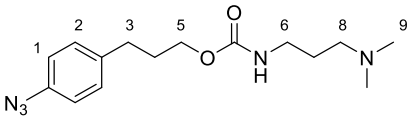
4-Nitrophenol chloroformate (1.5 equiv., 332.0 mg, 1.65 mmol) and pyridine (10.0 equiv., 0.89 mL, 11.0 mmol) were sequentially added to a solution of 3-(4-azidophenyl)propan-1-ol **2** (1.0 equiv., 195.0 mg, 1.1 mmol) in dichloromethane (3.98 mL). The reaction mixture was stirred for 5 h at 25 °C before being quenched with a saturated solution of NH_4Cl and extracted three times with dichloromethane. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The crude was quickly flushed through a short pad of silica (cHex/AcOEt 8:2) to afford the title product, which was used in the next steps with no further purification.

3-(4-Azidophenyl)propyl (17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)carbamate 10



A solution of 17-amino-3,6,9,12,15-pentaoxaheptadecan-1-ol (1.1 equiv., 219.7 mg, 0.71 mmol) and triethylamine (3.0 equiv., 0.30 mL, 2.13 mmol) in DMF (1.0 mL) was added to a solution of 3-(4-azidophenyl)propyl (4-nitrophenyl) carbonate **S2** (1.0 equiv., 243.0 mg, 0.71 mmol) in dichloromethane (3.1 mL). The mixture was stirred for 16 h at 25 °C before being concentrated to dryness. The crude was then suspended in water and extracted three times with ethyl acetate. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The crude was finally purified by flash column chromatography (100% DCM to DCM/MeOH 85:15) to afford the title compound (273.0 mg, 0.56 mmol, 79%) as a yellowish oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 – 7.15 (m, 2H, H2), 6.95 – 6.93 (m, 2H, H1), 4.06 (t, *J* = 6.6 Hz, 2H, H5), 3.73 – 3.71 (m, 2H, H7), 3.66 – 3.59 (m, 18 H, HOEG), 3.55 (t, *J* = 4.9 Hz, 2H, HOEG), 3.36 (br t, *J* = 4.8 Hz, 2H, H6), 2.68 (t, *J* = 7.5 Hz, 2H, H3), 1.94 – 1.87 (m, 2H, H4).
^{13}C NMR (100 MHz, $CDCl_3$) δ 216.9, 156.8, 138.2, 137.7, 129.7, 72.6, 70.6 (3C), 70.5 (3C), 70.3, 70.2 (2C), 63.9, 61.7, 40.8, 31.5, 30.7
HRMS (ESI$^+$, m/z): calculated for $C_{22}H_{36}N_4O_8$ $[M+H]^+$: 485.2606; found: 485.2600

3-(4-Azidophenyl)propyl (3-(dimethylamino)propyl)carbamate 11



A solution of 3-dimethylaminopropylamine (1.1 equiv., 20.3 µL, 0.16 mmol) and triethylamine (3.0 equiv., 60.9 µL, 0.44 mmol) in DMF (0.21 mL) was added to a solution of 3-(4-azidophenyl)propyl (4-nitrophenyl) carbonate **S2** (1.0 equiv., 50.0 mg, 0.15 mmol) in DMF (0.21 mL). The mixture was stirred for 16 h at 25 °C before being concentrated to dryness. The crude was then suspended in water and extracted three times with ethyl acetate. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The crude was finally purified by flash column chromatography (100% DCM to DCM/MeOH 85:15) to afford the title compound (37.7 mg, 0.12 mmol, 85%) as a yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.16 (m, 2H, H2), 6.96 – 6.93 (m, 2H, H1), 5.66 (br s, 1H, NH), 4.05 (t, *J* = 6.5 Hz, 2H, H5), 3.34 – 3.30 (m, 2H, H6), 2.77 (br t, *J* = 6.2 Hz, 2H, H8), 2.66 (t, *J* = 7.5 Hz, 2H, H3), 2.56 (s, 6H, H9), 1.94 – 1.87 (m, 4H, H4 + H7).

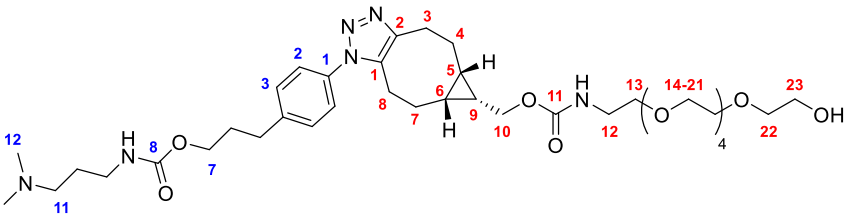
^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 138.2, 137.7, 129.8, 119.0, 77.2, 64.1, 56.4, 53.4, 44.0, 38.6, 31.5, 30.6, 25.8.

HRMS (ESI$^+$, m/z): calculated for $C_{16}H_{27}N_2O_2$ $[M+H]^+$: 306.1930 ; found: 306.1936.

*General procedure for the synthesis of SPAAC products **13**, **14**, and **S3-S15***

A solution of BCN (1.0 equiv.) and azide (1.1 equiv.) in MeOH (0.1 mM) was stirred at room temperature until complete conversion to the expected triazole was observed (typically 16 h). The mixture was then concentrated to dryness under vacuum and the resulting crude dissolved in 4 mL of a 1:1 mixture of acetonitrile and water containing 0.1% of TFA and purified by preparative HPLC (see Material and methods).

(1-(4-(3-(((3-(dimethylamino)propyl)carbamoyl)oxy)propyl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)carbamate 13



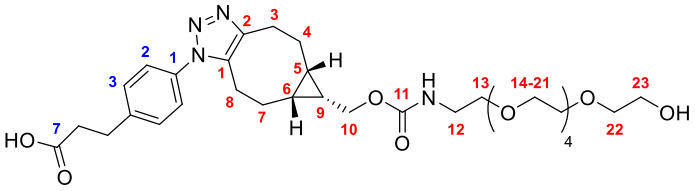
^{1}H NMR (700 MHz, MeOD) δ 7.46 (2H, d, *J* = 8.1 Hz, **H3**), 7.37 (2H, d, *J* = 8.1 Hz, **H2**), 4.19-4.14 (2H, m, **H10**), 4.09 (2H, t, *J* = 6.4 Hz, **H7**), 3.67-3.59 (18H, m, **H14-21** + **H23**), 3.57-3.55 (2H, m, **H22**), 3.52 (2H, t, *J* = 5.4 Hz, **H13**), 3.27 (2H, t, *J* = 5.4 Hz, **H12**), 3.21 (2H, t, *J* = 6.4 Hz, **H9**), 3.20-3.15 (1H, m, **H8**), 3.17-3.14 (2H, m, **H11**), 2.97-2.93 (1H, m, **H3**), 2.94-2.91 (1H, m, **H8**), 2.89 (6H, s, **H12**), 2.82 (2H, t, *J* = 7.5 Hz, **H5**), 2.69 (1H, ddd, *J* = 13.6, 10.4, 3.4 Hz, **H3**), 2.27 (1H, dddd, *J* = 15.3, 11.9, 8.2, 4.3 Hz, **H7**), 2.16 (1H, dddd, *J* = 14.8, 10.4, 7.3, 3.9 Hz, **H4**), 2.00 (2H, app. quint., *J* = 6.9 Hz, **H6**), 1.91 (2H, tt, *J* = 7.9, 6.4 Hz, **H10**), 1.69-1.62 (2H, m, **H4** + **H7**), 1.27 (1H, app. quint., *J* = 8.5 Hz, **H9**), 1.15-1.09 (1H, m, **H6**), 1.10-1.04 (1H, m, **H5**).

^{13}C NMR (176 MHz, MeOD) δ 159.4 (**C8**), 159.2 (**C11**), 146.0 (**C1**), 145.2 (**C4**), 136.8 (**C2**), 135.7 (**C1**), 130.7 (**C3**), 127.2 (**C2**), 117.8, 73.6 (**C23**), 71.6-71.3 (8C, **C14-C22**)*, 71.0 (**C13**), 65.2 (**C7**), 63.5 (**C10**), 62.2 (1C, **C14-C22**)*, 56.7 (**C11**), 43.5 (**C12**), 41.7 (**C12**), 38.4 (**C9**), 32.7 (**C5**), 31.7 (**C6**), 26.5 (2C, **C10** + **C8**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.8 (**C6**), 19.1 (**C9**).

*: undistinguishable from each other.

HRMS (ESI$^+$, m/z) calculated for $C_{38}H_{64}N_6O_{10}$ $(M+2H)^{2+}$: 382.2343, found 382.2348.

3-(4-(-6-(21-hydroxy-3-oxo-2,7,10,13,16,19-hexaoxa-4-azahenicosyl)-5,5a,6,6a,7,8-hexahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-1(4H)-yl)phenyl)propanoic acid 14



^{1}H NMR (700 MHz, MeOD) δ 7.48 (2H, d, *J* = 8.2 Hz, **H3**), 7.38 (2H, d, *J* = 8.2 Hz, **H2**), 4.16 (2H, d, *J* = 8.0 Hz, **H10**), 3.66-3.59 (18H, m, **H14-21** + **H23**), 3.55 (2H, t, *J* = 5.1 Hz, **H22**), 3.52 (2H, t, *J* = 5.4 Hz, **H13**), 3.27 (2H, t, *J* = 5.4 Hz, **H12**), 3.17

(1H, dddd, *J* = 15.4, 10.9, 7.4, 3.3 Hz, **H8**), 3.04 (2H, t, *J* = 7.5 Hz, **H5**), 2.97-2.92 (1H, m, **H3**), 2.95-2.91 (1H, m, **H8**), 2.72 (2H, t, *J* = 7.5 Hz, **H6**), 2.71-2.66 (1H, m, **H3**), 2.27 (1H, dddd, *J* = 15.4, 11.7, 8.1, 4.3 Hz, **H7**), 2.16 (1H, dddd, *J* = 14.4, 10.3, 6.9, 3.7 Hz, **H4**), 1.69-1.62 (2H, m, **H4** + **H7**), 1.27 (1H, app. quint., *J* = 8.5 Hz, **H9**), 1.14-1.05 (2H, m, **H6** + **H5**).

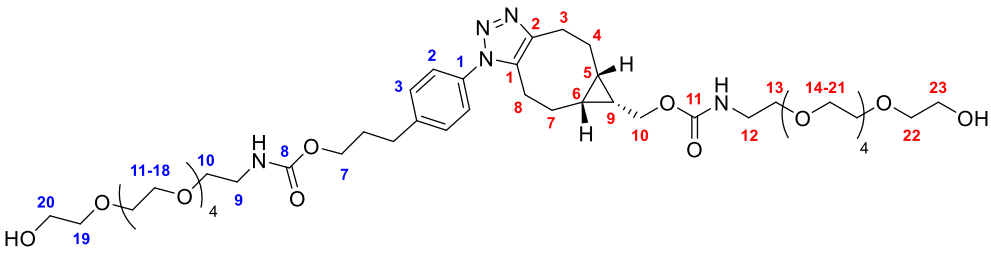
^{13}C NMR (176 MHz, MeOD) δ 176.1 (**C7**), 159.1 (**C11**), 145.8 (**C1**), 144.5 (**C4**), 137.0 (**C2**), 135.8 (**C1**), 130.7 (**C3**), 127.2 (**C2**), 73.7 (**C23**), 71.6-71.2 (8C, **C14**-**C22**)*, 71.0 (**C13**), 63.5 (**C10**), 62.2 (1C, **C14**-**C22**)*, 41.7 (**C12**), 36.1 (**C6**), 31.4 (**C5**), 26.4 (**C8**), 24.4 (**C3**), 23.6 (**C7**), 23.2 (**C4**), 21.0 (**C5**), 20.8 (**C6**), 19.2 (**C9**).

*: undistinguishable from each other.

Remark. Peaks corresponding to the deuterated methyl ester derivative of **14** can be seen in the ^{1}H and ^{13}C NMR spectra – this compound likely results from an *in-situ* esterification between **14** and deuterated methanol catalysed by the residual TFA from the preparative HPLC purification. This was confirmed by the HRMS analysis of **14**, where m/z 666.3799 $[M+H]^+$ and m/z 685.3414 $[M+Na]^+$ are also visible. Similar side products were consistently observed with carboxylic acid derivatives, which should not be considered as impurities.

HRMS (ESI$^+$, m/z) calculated for $C_{32}H_{49}N_4O_{10}$ $(M+H)^+$: 649.3457, found 649.3458.

(1-(4-(1-hydroxy-19-oxo-3,6,9,12,15,20-hexaoxa-18-azatricosan-23-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)carbamate S3



^{1}H NMR (700 MHz, MeOD) δ 7.47 (2H, d, *J* = 8.2 Hz, **H3**), 7.38 (2H, d, *J* = 8.2 Hz, **H2**), 4.16 (2H, d, *J* = 8.0 Hz, **H10**), 4.08 (2H, t, *J* = 6.3 Hz, **H7**), 3.70-3.59 (36H, m, **H14**-**21** + **H23** + **H11**-**18** + **H20**), 3.56-3.52 (8H, m, **H22** + **H13** + **H10** + **H19**), 3.30-3.26 (4H, m, **H12** + **H9**), 3.17 (1H, dddd, *J* = 15.6, 10.9, 7.4, 3.4 Hz, **H8**), 2.97-2.93 (1H, m, **H3**), 2.94-2.91 (1H, m, **H8**), 2.83 (2H, t, *J* = 7.5 Hz, **H5**), 2.70 (1H, ddd, *J* = 13.7, 10.3, 3.2 Hz, **H3**), 2.27 (1H, dddd, *J* = 15.6, 11.7, 8.0, 4.2 Hz, **H7**), 2.16 (1H, dddd, *J* = 14.5, 10.3, 7.0, 3.8 Hz, **H4**), 2.00 (2H, app quint, *J* = 6.9 Hz, **H6**), 1.70-1.63 (2H, m, **H4** + **H7**), 1.27 (1H, app quint, *J* = 8.5 Hz, **H9**), 1.15-1.04 (2H, m, **H6** + **H5**).

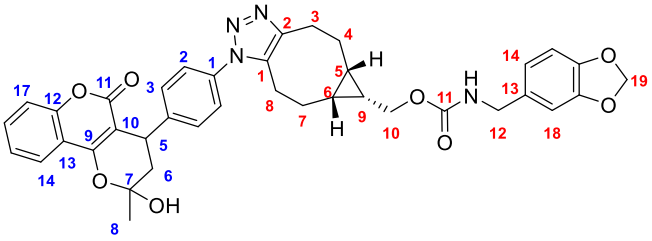
^{13}C NMR (176 MHz, MeOD) δ 159.1 (**C8**), 159.0 (**C11**), 145.7 (**C1**), 145.4 (**C4**), 137.0 (**C2**), 135.5 (**C1**), 130.8 (**C3**), 127.1 (**C2**), 73.6 (2C, **C23** + **C20**), 71.5-71.2 (16C, **C14**-**C22** + **C11**-**C19**)*, 70.9 (2C, **C10** + **C13**), 64.9 (**C7**), 63.5 (**C10**), 62.2 (2C, **C14**-**C22** + **C11**-**C19**)*, 41.7 (2C, **C12** + **C9**), 32.7 (**C5**), 31.7 (**C6**), 26.4 (**C8**), 24.4 (**C3**), 23.6 (**C7**), 23.2 (**C4**), 21.0 (**C5**), 20.8 (**C6**), 19.1 (**C9**).

*: undistinguishable from each other.

HRMS (ESI$^+$, m/z) calculated for $C_{45}H_{76}N_5O_{16}$ $(M+H)^+$: 942.5266, found 942.5245.

(1-(4-(2-hydroxy-2-methyl-5-oxo-3,4-dihydro-2H,5H-pyrano[3,2-c]chromen-4-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (benzo[d][1,3]dioxol-5-ylmethyl)carbamate S4

Compound **S4** was isolated as a mixture of lactol diastereomers (1:0.56 ratio major/minor; C7 diastereomers).

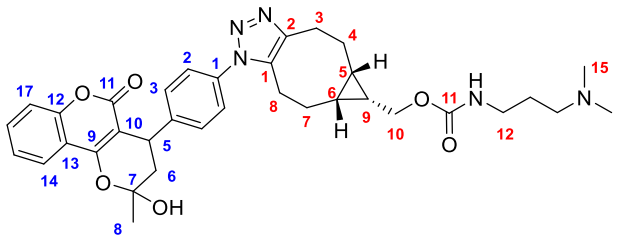


Major diastereomer

^{1}H NMR (700 MHz, MeOD) δ 7.94 (1H, dd, *J* = 8.0, 1.0 Hz, **H14**), 7.63-7.60 (1H, m, **H16**), 7.47 (2H, d, *J* = 8.2 Hz, **H3**), 7.40-7.37 (1H, m, **H15**), 7.37-7.35 (2H, m, **H2**), 7.34-7.32 (1H, m, **H17**), 6.78-6.75 (1H, m, **H18**), 6.75-6.70 (2H, m, **H14** + **H15**), 5.90-5.87 (2H, m, **H19**), 4.24 (1H, dd, *J* = 11.8, 6.9 Hz, **H5**), 4.19-4.14 (4H, m, **H10** + **H12**), 3.20-3.13 (1H, m, **H8**), 2.99-2.94 (1H, m, **H3**), 2.94-2.90 (1H, m, **H8**), 2.72-2.68 (1H, m, **H3**), 2.48 (1H, dd, *J* = 13.9, 6.9 Hz, **H6**), 2.29-2.24 (1H, m, **H7**), 2.18-2.13 (1H, m,

H4), 2.03 (1H, app t, *J* = 12.8, H6), 1.77 (3H, s, H8), 1.69-1.62 (2H, m, H4 + H7), 1.31-1.26 (1H, m, H9), 1.13-1.03 (2H, m, H6 + H5).
^{13}C NMR (176 MHz, MeOD) δ 163.6 (C9), 161.7 (C11), 159.3 (C11), 154.2 (C12), 149.2 (C17), 148.2 (C4), 148.1 (C16),145.3 (C1), 137.6 (C2), 135.5 (C1), 134.7 (C13), 133.2 (C16), 129.7 (C3), 127.2 (C2), 125.3 (C15), 124.2 (C14), 121.6 (C14), 117.4 (2C, C17 + C13), 109.0 (C15), 108.8 (C18), 104.4 (C10), 102.3 (C19), 100.9 (C7), 63.6 (C10), 45.3 (C12), 43.8 (C6), 36.9 (C5), 27.7 (C8), 26.2 (C8), 24.4 (C3), 23.5 (C7), 23.2 (C4), 21.1 (C5), 20.9 (C6), 19.2 (C9).
Minor diastereomer
^{1}H NMR (700 MHz, MeOD) δ 7.96 (1H, dd, *J* = 8.0, 1.1 Hz, H14), 7.65-7.63 (1H, m, H16), 7.48 (2H, d, *J* = 8.3 Hz, H3), 7.40-7.37 (1H, m, H15), 7.37-7.34 (1H, m, H17), 7.33-7.31 (2H, m, H2), 6.78-6.75 (1H, m, H18), 6.75-6.70 (2H, m, H14 + H15), 5.90-5.87 (2H, m, H19), 4.23-4.20 (1H, m, H5), 4.19-4.14 (4H, m, H10 + H12), 3.20-3.13 (1H, m, H8), 2.99-2.94 (1H, m, H3), 2.94-2.90 (1H, m, H8), 2.72-2.68 (1H, m, H3), 2.47-2.43 (1H, m, H6), 2.37 (1H, dd, *J* = 14.1, 5.4 Hz, H6), 2.29-2.24 (1H, m, H7), 2.18-2.13 (1H, m, H4), 1.70 (3H, s, H8), 1.69-1.62 (2H, m, H4 + H7), 1.31-1.26 (1H, m, H9), 1.13-1.03 (2H, m, H6 + H5).
^{13}C NMR (176 MHz, MeOD) δ 164.2 (C9), 162.2 (C11), 159.3 (C11), 154.2 (C12), 149.2 (C17), 148.1 (C16), 147.9 (C4), 145.3 (C1), 137.6 (C2), 135.1 (C1), 134.7 (C13), 133.4 (C16), 130.1 (C3), 126.7 (C2), 125.4 (C15), 124.2 (C14), 121.6 (C14), 117.5 (C17), 117.2 (C13), 109.0 (C15), 108.8 (C18), 102.7 (C10), 102.3 (2C, C7 + C19), 63.6 (C10), 45.3 (C12), 41.8 (C6), 37.2 (C5), 26.9 (C8), 26.2 (C8), 24.4 (C3), 23.5 (C7), 23.2 (C4), 21.1 (C5), 20.9 (C6), 19.2 (C9).
Remark. Peaks corresponding to the products resulting from the hydrolysis of the carbamate group – probably in the course of the purification by preparative HPLC or by prolonged exposure to the residual TFA – can also be seen in the ^{1}H and ^{13}C NMR spectra.
HRMS (ESI^{+}, m/z) calculated for $C_{34}H_{37}N_4O_8$ $(M+H)^+$: 629.2606, found 629.2624.

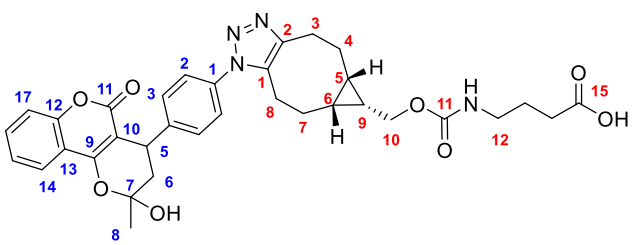
(1-(4-(2-hydroxy-2-methyl-5-oxo-3,4-dihydro-2H,5H-pyrano[3,2-c]chromen-4-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (3-(dimethylamino)propyl)carbamate S5
Compound **S5** was isolated as a mixture of lactol diastereomers (1:0.49 ratio major/minor; C7 diastereomers).



Major diastereomer
^{1}H NMR (700 MHz, MeOD) δ 7.94 (1H, br d, *J* = 7.9 Hz, H14), 7.62-7.59 (1H, m, H16), 7.46 (2H, d, *J* = 8.2 Hz, H3), 7.40-7.37 (1H, m, H15), 7.37-7.35 (2H, m, H2), 7.32 (1H, d, *J* = 8.1 Hz, H17), 4.23 (1H, dd, *J* = 11.9, 6.8 Hz, H5), 4.17 (2H, d, *J* = 7.9 Hz, H10), 3.19 (2H, d, *J* = 6.1 Hz, H12), 3.18-3.13 (1H, m, H8), 3.14-3.12 (2H, m, H14), 2.98-2.93 (1H, m, H3), 2.92-2.89 (1H, m, H8), 2.87 (6H, s, H15), 2.72-2.67 (1H, m, H3), 2.47 (1H, dd, *J* = 13.7, 6.8 Hz, H6), 2.27-2.23 (1H, m, H7), 2.17-2.12 (1H, m, H4), 2.03 (1H, app t, *J* = 12.3 Hz, H6), 1.92-1.87 (2H, m, H13), 1.77 (3H, s, H8), 1.67-1.59 (2H, m, H4 + H7), 1.29-1.23 (1H, m, H9), 1.14-1.04 (2H, m, H6 + H5).
^{13}C NMR (176 MHz, MeOD) δ 163.6 (C9), 161.7 (C11), 159.5 (C11), 154.2 (C12), 147.9 (C4), 145.9 (C1), 136.9 (C2), 135.7 (C1), 133.2 (C16), 129.6 (C3), 127.2 (C2), 125.4 (C15), 124.2 (C14), 117.4 (2C, C17 + C13), 104.4 (C10), 100.9 (C7), 63.8 (C10), 56.7 (C14), 43.8 (C6), 43.5 (C15), 38.4 (C12), 36.8 (C5), 27.7 (C8), 26.5 (C13), 26.4 (C8), 24.3 (C3), 23.8 (C7), 23.4 (C4), 21.1 (C5), 20.9 (C6), 19.1 (C9).
Minor diastereomer
^{1}H NMR (700 MHz, MeOD) δ 7.96 (1H, br d, *J* = 8.3 Hz, H14), 7.65-7.62 (1H, m, H16), 7.47 (2H, d, *J* = 8.3 Hz, H3), 7.40-7.37 (1H, m, H15), 7.37-7.34 (1H, m, H17), 7.31 (2H, d, *J* = 8.3 Hz, H2), 4.23-4.19 (1H, m, H5), 4.17 (2H, d, *J* = 7.9 Hz, H10), 3.19 (2H, d, *J* = 6.1 Hz, H12), 3.18-3.13 (1H, m, H8), 3.14-3.12 (2H, m, H14), 2.98-2.93 (1H, m, H3), 2.92-2.89 (1H, m, H8), 2.87 (6H, s, H15), 2.72-2.67 (1H, m, H3), 2.47-2.43 (1H, m, H6), 2.37 (1H, dd, *J* = 14.1, 5.3 Hz, H6), 2.27-2.23 (1H, m, H7), 2.17-2.12 (1H, m, H4), 1.92-1.87 (2H, m, H13), 1.70 (3H, s, H8), 1.67-1.59 (2H, m, H4 + H7), 1.29-1.23 (1H, m, H9), 1.14-1.04 (2H, m, H6 + H5).
^{13}C NMR (176 MHz, MeOD) δ 164.1 (C9), 162.2 (C11), 159.5 (C11), 154.2 (C12), 147.5 (C4), 145.8 (C1), 136.9 (C2), 135.4 (C1), 133.4 (C16), 130.1 (C3), 126.7 (C2), 125.4 (C15), 124.2 (C14), 117.5 (C17), 117.2 (C13), 102.7 (C10), 102.3 (C7), 63.8 (C10), 56.7 (C14), 43.5 (C15), 41.8 (C6), 38.4 (C12),

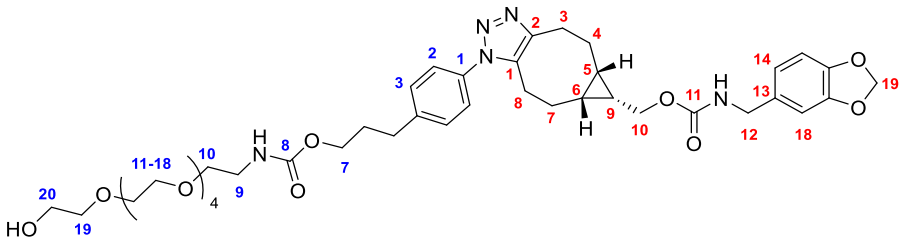
37.2 (**C5**), 26.9 (**C8**), 26.5 (**C13**), 26.4 (**C8**), 24.3 (**C3**), 23.8 (**C7**), 23.4 (**C4**), 21.1 (**C5**), 20.9 (**C6**), 19.1 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{35}H_{42}N_5O_6$ $(M+H)^+$: 628.3130, found 628.3158.

4-((((-1-(4-(2-hydroxy-2-methyl-5-oxo-3,4-dihydro-2H,5H-pyrano[3,2-c]chromen-4-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methoxy)carbonyl)amino)butanoic acid S6
Compound **S6** was isolated as a mixture of lactol diastereomers (1:0.48 ratio major/minor; C7 diastereomers).



Major diastereomer
^{1}H NMR (700 MHz, MeOD) δ 7.93 (1H, dd, *J* = 7.9, 1.0 Hz, **H14**), 7.61-7.59 (1H, m, **H16**), 7.45 (2H, d, *J* = 8.3 Hz, **H3**), 7.40-7.37 (1H, m, **H15**), 7.37-7.35 (2H, m, **H2**), 7.33-7.30 (1H, m, **H17**), 4.23 (1H, dd, *J* = 11.8, 6.7 Hz, **H5**), 4.15 (2H, d, *J* = 7.5 Hz, **H10**), 3.19-3.13 (1H, m, **H8**), 3.12 (2H, t, *J* = 6.8 Hz, **H12**), 2.99-2.94 (1H, m, **H3**), 2.94-2.90 (1H, m, **H8**), 2.72-2.67 (1H, m, **H3**), 2.47 (1H, dd, *J* = 13.7, 6.7 Hz, **H6**), 2.31 (2H, d, *J* = 7.3 Hz, **H14**), 2.28-2.24 (1H, m, **H7**), 2.18-2.13 (1H, m, **H4**), 2.02 (1H, dd, *J* = 13.7, 11.8 Hz, **H6**), 1.78-1.74 (2H, m, **H13**), 1.76 (3H, s, **H8**), 1.69-1.62 (2H, m, **H4** + **H7**), 1.26 (1H, app. quint., *J* = 8.2 Hz, **H9**), 1.13-1.03 (2H, m, **H6** + **H5**).
^{13}C NMR (176 MHz, MeOD) δ 176.9 (**C15**), 163.6 (**C9**), 161.7 (**C11**), 159.2 (**C11**), 154.2 (**C12**), 148.0 (**C4**), 145.5 (**C1**), 137.4 (**C2**), 135.5 (**C1**), 133.2 (**C16**), 129.6 (**C3**), 127.2 (**C2**), 125.3 (**C15**), 124.2 (**C14**), 117.4 (2C, **C17** + **C13**), 104.4 (**C10**), 100.9 (**C7**), 63.4 (**C10**), 43.8 (**C6**), 41.1 (**C12**), 36.8 (**C5**), 32.0 (**C14**), 27.7 (**C8**), 26.3 (**C8**), 26.2 (**C13**), 24.4 (**C3**), 23.5 (**C7**), 23.2 (**C4**), 21.0 (**C5**), 20.8 (**C6**), 19.2 (**C9**).
Minor diastereomer
^{1}H NMR (700 MHz, MeOD) δ 7.95 (1H, dd, *J* = 8.0, 1.2 Hz, **H14**), 7.64-7.62 (1H, m, **H16**), 7.48 (2H, d, *J* = 8.4 Hz, **H3**), 7.40-7.37 (1H, m, **H15**), 7.37-7.34 (1H, m, **H17**), 7.33-7.31 (2H, m, **H2**), 4.23-4.20 (1H, m, **H5**), 4.15 (2H, d, *J* = 7.5 Hz, **H10**), 3.19-3.13 (1H, m, **H8**), 3.12 (2H, t, *J* = 6.8 Hz, **H12**), 2.99-2.94 (1H, m, **H3**), 2.94-2.90 (1H, m, **H8**), 2.72-2.67 (1H, m, **H3**), 2.47-2.43 (1H, m, **H6**), 2.37 (1H, dd, *J* = 14.1, 5.1 Hz, **H6**), 2.31 (2H, d, *J* = 7.3 Hz, **H14**), 2.28-2.24 (1H, m, **H7**), 2.18-2.13 (1H, m, **H4**), 1.78-1.74 (2H, m, **H13**), 1.69 (3H, s, **H8**), 1.69-1.62 (2H, m, **H4** + **H7**), 1.26 (1H, app. quint., *J* = 8.2 Hz, **H9**), 1.13-1.03 (2H, m, **H6** + **H5**).
^{13}C NMR (176 MHz, MeOD) δ 176.9 (**C15**), 164.1 (**C9**), 162.1 (**C11**), 159.2 (**C11**), 154.2 (**C12**), 147.7 (**C4**), 145.4 (**C1**), 137.5 (**C2**), 135.2 (**C1**), 133.4 (**C16**), 130.1 (**C3**), 126.7 (**C2**), 125.4 (**C15**), 124.2 (**C14**), 117.5 (**C17**), 117.2 (**C13**), 102.7 (**C10**), 102.3 (**C7**), 63.4 (**C10**), 41.8 (**C6**), 41.1 (**C12**), 37.2 (**C5**), 32.0 (**C14**), 26.9 (**C8**), 26.3 (**C8**), 26.2 (**C13**), 24.4 (**C3**), 23.5 (**C7**), 23.2 (**C4**), 21.0 (**C5**), 20.8 (**C6**), 19.2 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{34}H_{37}N_4O_8$ $(M+H)^+$: 629.2606, found 629.2624.

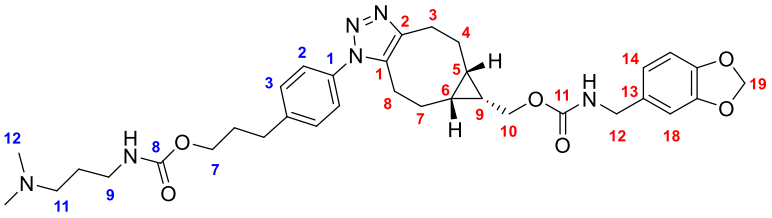
(1-(4-(1-hydroxy-19-oxo-3,6,9,12,15,20-hexaoxa-18-azatricosan-23-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (benzo[d][1,3]dioxol-5-ylmethyl)carbamate S7



^{1}H NMR (500 MHz, MeOD) δ 7.47 (2H, d, *J* = 8.3 Hz, **H3**), 7.36 (2H, d, *J* = 8.3 Hz, **H2**), 6.77 (1H, s, **H18**), 6.75-6.71 (2H, m, **H14** + **H15**), 5.89 (2H, s, **H19**), 4.18 (2H, d, *J* = 8.2 Hz, **H10**), 4.16 (2H, s, **H12**), 4.08 (2H, t, *J* = 6.1 Hz, **H7**), 3.66-3.61 (18H, m, **H11-18** + **H20**), 3.56-3.52 (4H, m, **H10** + **H19**), 3.30-3.27 (2H, m, **H9**), 3.20-3.12 (1H, m, **H8**), 2.97-2.93 (1H, m, **H3**), 2.94-2.89 (1H, m, **H8**), 2.83 (2H, t, *J* = 7.5 Hz, **H5**), 2.71-2.64 (1H, m, **H3**), 2.31-2.22 (1H, m, **H7**), 2.19-2.11 (1H, m, **H4**), 2.05-1.97 (2H, m, **H6**), 1.71-1.61 (2H, m, **H4** + **H7**), 1.28 (1H, app. quint., *J* = 8.6 Hz, **H9**), 1.15-1.02 (2H, m, **H6** + **H5**).
^{13}C NMR (126 MHz, MeOD) δ 159.3 (**C11**), 159.1 (**C8**), 149.2 (**C17**), 148.1 (**C16**), 145.9 (**C1**), 145.3 (**C4**), 136.8 (**C2**), 135.6 (**C1**), 134.7 (**C13**), 130.8 (**C3**), 127.2 (**C2**), 121.6 (**C14**), 109.0 (**C15**), 108.8

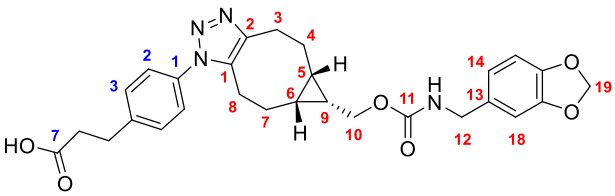
(**C18**), 102.3 (**C19**), 73.6 (**C20**), 71.5-71.3 (8C, **C11**-**C19**)*, 71.0 (**C10**), 65.0 (**C7**), 63.6 (**C10**), 62.2 (**C11**-**C19**)*, 45.2 (**C12**), 41.7 (**C9**), 32.7 (**C5**), 31.7 (**C6**), 26.4 (**C8**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.9 (**C6**), 19.2 (**C9**).
*: undistinguishable from each other.
HRMS (ESI$^+$, m/z) calculated for $C_{41}H_{58}N_5O_{12}$ $(M+H)^+$: 812.4076, found 812.4087.

(1-(4-(3-(((3-(dimethylamino)propyl)carbamoyl)oxy)propyl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (benzo[d][1,3]dioxol-5-ylmethyl)carbamate S8



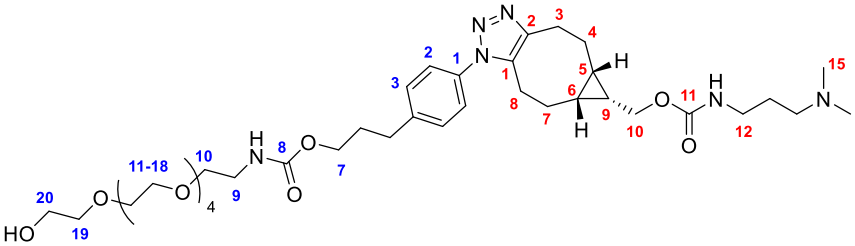
^{1}H NMR (700 MHz, MeOD) δ 7.45 (2H, d, *J* = 8.2 Hz, **H3**), 7.35 (2H, d, *J* = 8.2 Hz, **H2**), 6.77 (1H, s, **H18**), 6.74-6.71 (2H, m, **H14** + **H15**), 5.89 (2H, s, **H19**), 4.18-4.15 (4H, m, **H10** + **H12**), 4.08 (2H, t, *J* = 6.3 Hz, **H7**), 3.21 (2H, t, *J* = 6.5 Hz, **H9**), 3.17-3.13 (3H, m, **H8** + **H11**), 2.94-2.88 (2H, m, **H3** + **H8**), 2.88 (6H, s, **H12**), 2.82 (2H, t, *J* = 7.3 Hz, **H5**), 2.66 (1H, ddd, *J* = 13.5, 9.9, 3.1 Hz, **H3**), 2.25 (1H, dddd, *J* = 15.2, 11.8, 7.9, 4.3 Hz, **H7**), 2.16 (1H, dddd, *J* = 13.5, 10.4, 7.1, 3.8 Hz, **H4**), 2.00 (2H, app. quint., *J* = 6.8 Hz, **H6**), 1.91 (2H, tt, *J* = 7.9, 6.5 Hz, **H10**), 1.67-1.51 (2H, m, **H4** + **H7**), 1.26 (1H, app. quint., *J* = 8.6 Hz, **H9**), 1.13-1.08 (1H, m, **H6**), 1.09-1.03 (1H, m, **H5**).
^{13}C NMR (126 MHz, MeOD) δ 159.4 (**C11**), 159.2 (**C8**), 149.1 (**C17**), 148.1 (**C16**), 146.0 (**C1**), 145.2 (**C4**), 136.7 (**C2**), 135.6 (**C1**), 134.7 (**C13**), 130.7 (**C3**), 127.1 (**C2**), 121.6 (**C14**), 109.0 (**C15**), 108.8 (**C18**), 102.2 (**C19**), 65.2 (**C7**), 63.6 (**C10**), 56.6 (**C11**), 45.2 (**C12**), 43.5 (**C12**), 38.4 (**C9**), 32.7 (**C5**), 31.6 (**C6**), 26.5 (**C8**), 26.4 (**C10**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.9 (**C6**), 19.1 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{34}H_{45}N_6O_6$ $(M+H)^+$: 633.3395, found 633.3421.

3-(4-(-6-((((benzo[d][1,3]dioxol-5-ylmethyl)carbamoyl)oxy)methyl)-5,5a,6,6a,7,8-hexahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-1(4H)-yl)phenyl)propanoic acid S9



^{1}H NMR (700 MHz, MeOD) δ 7.48 (2H, d, *J* = 8.3 Hz, **H3**), 7.37 (2H, d, *J* = 8.3 Hz, **H2**), 6.77 (1H, s, **H18**), 6.74-6.72 (2H, m, **H14** + **H15**), 5.89 (2H, s, **H19**), 4.19 (2H, d, *J* = 8.1 Hz, **H10**), 4.16 (2H, s, **H12**), 3.16 (1H, dddd, *J* = 15.7, 10.7, 7.2, 3.2 Hz, **H8**), 3.04 (2H, t, *J* = 7.5 Hz, **H5**), 2.95-2.89 (2H, m, **H3** + **H8**), 2.73 (2H, t, *J* = 7.5 Hz, **H6**), 2.71-2.66 (1H, m, **H3**), 2.29-2.24 (1H, m, **H7**), 2.17-2.12 (1H, m, **H4**), 1.69-1.62 (2H, m, **H4** + **H7**), 1.28 (1H, app. quint., *J* = 8.7 Hz, **H9**), 1.14-1.05 (2H, m, **H6** + **H5**).
^{13}C NMR (126 MHz, MeOD) δ 176.3 (**C7**), 159.3 (**C11**), 149.2 (**C17**), 148.1 (**C16**), 145.8 (**C1**), 144.5 (**C4**), 137.0 (**C2**), 135.8 (**C1**), 134.7 (**C13**), 130.7 (**C3**), 127.2 (**C2**), 121.6 (**C14**), 109.0 (**C15**), 108.8 (**C18**), 102.3 (**C19**), 63.6 (**C10**), 45.2 (**C12**), 36.1 (**C6**), 31.4 (**C5**), 26.4 (**C8**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.9 (**C6**), 19.2 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{28}H_{31}N_4O_6$ $(M+H)^+$: 519.2238, found 519.2236.

(1-(4-(1-hydroxy-19-oxo-3,6,9,12,15,20-hexaoxa-18-azatricosan-23-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (3-(dimethylamino)propyl)carbamate S10



^{1}H NMR (700 MHz, MeOD) δ 7.47 (2H, d, *J* = 7.9 Hz, **H3**), 7.37 (2H, d, *J* = 7.9 Hz, **H2**), 4.18 (2H, d, *J* = 7.9 Hz, **H10**), 4.07 (2H, t, *J* = 6.2 Hz, **H7**), 3.66-3.61 (18H, m, **H11**-**18** + **H20**), 3.56-3.52 (4H, m, **H10** + **H19**), 3.29

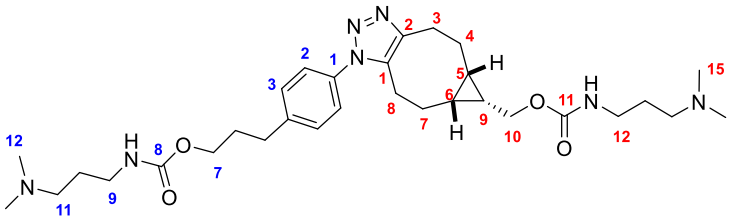
(2H, t, *J* = 5.4 Hz, **H9**), 3.20 (2H, t, *J* = 6.3 Hz, **H12**), 3.18-3.13 (1H, m, **H8**), 3.16-3.12 (2H, m, **H14**), 2.99-2.95 (1H, m, **H3**), 2.97-2.93 (1H, m, **H8**), 2.88 (6H, s, **H15**), 2.83 (2H, t, *J* = 7.4 Hz, **H5**), 2.70 (1H, ddd, *J* = 13.2, 10.2, 2.9 Hz, **H3**), 2.27 (1H, dddd, *J* = 14.7, 11.2, 7.6, 3.9 Hz, **H7**), 2.16 (1H, dddd, *J* = 14.4, 10.2, 7.0, 3.6 Hz, **H4**), 2.03-1.98 (2H, m, **H6**), 1.91 (2H, app quint, *J* = 6.7 Hz, **H13**), 1.69-1.61 (2H, m, **H4** + **H7**), 1.27 (1H, app. quint., *J* = 8.4 Hz, **H9**), 1.15-1.06 (2H, m, **H6** + **H5**).

^{13}C NMR (126 MHz, MeOD) δ 159.5 (**C11**), 159.1 (**C8**), 145.9 (**C1**), 145.3 (**C4**), 136.9 (**C2**), 135.6 (**C1**), 130.8 (**C3**), 127.2 (**C2**), 73.6 (**C20**), 71.5-71.3 (8C, **C11**-**C19**)*, 71.0 (**C10**), 65.0 (**C7**), 63.8 (**C10**), 62.2 (**C11**-**C19**)*, 56.7 (**C14**), 43.5 (**C15**), 41.7 (**C9**), 38.4 (**C12**), 32.7 (**C5**), 31.7 (**C6**), 26.5 (**C13**), 26.4 (**C8**), 24.4 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.9 (**C6**), 19.1 (**C9**).

*: undistinguishable from each other.

HRMS (ESI$^+$, m/z) calculated for $C_{38}H_{63}N_6O_{10}$ $(M+H)^+$: 763.4606, found 763.4581.

(1-(4-(3-(((3-(dimethylamino)propyl)carbamoyl)oxy)propyl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methyl (3-(dimethylamino)propyl)carbamate S11

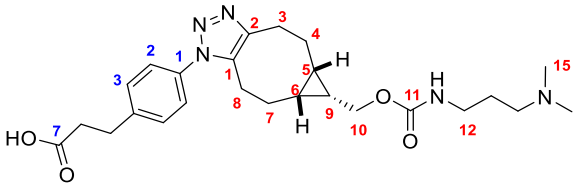


^{1}H NMR (700 MHz, MeOD) δ 7.46 (2H, d, *J* = 8.0 Hz, **H3**), 7.37 (2H, d, *J* = 8.0 Hz, **H2**), 4.20-4.15 (2H, m, **H10**), 4.09 (2H, t, *J* = 6.2 Hz, **H7**), 3.23-3.18 (4H, m, **H12** + **H9**), 3.18-3.13 (1H, m, **H8**), 3.17-3.13 (4H, m, **H14** + **H11**), 2.99-2.90 (2H, m, **H3** + **H8**), 2.89 (6H, s, **H15** / **H12**), 2.88 (6H, s, **H12** / **H15**), 2.83 (2H, t, *J* = 7.5 Hz, **H5**), 2.70 (1H, ddd, *J* = 13.6, 10.4, 3.3 Hz, **H3**), 2.27 (1H, dddd, *J* = 15.2, 11.8, 8.0, 4.3 Hz, **H7**), 2.16 (1H, dddd, *J* = 14.5, 10.4, 7.0, 3.8 Hz, **H4**), 2.01 (2H, app. quint., *J* = 6.8 Hz, **H6**),1.94-1.88 (4H, m, **H10** + **H13**), 1.69-1.62 (2H, m, **H4** + **H7**), 1.27 (1H, app. quint., *J* = 8.3 Hz, **H9**), 1.15-1.06 (2H, m, **H6** + **H5**).

^{13}C NMR (126 MHz, MeOD) δ 159.5 (**C11**), 159.4 (**C8**), 146.0 (**C1**), 145.3 (**C4**), 136.8 (**C2**), 135.7 (**C1**), 130.7 (**C3**), 127.2 (**C2**), 65.2 (**C7**), 63.8 (**C10**), 56.7 (2C, **C11** + **C14**), 43.5 (2C, **C12** + **C15**), 38.4 (**C9** + **C12**), 32.7 (**C5**), 31.7 (**C6**), 26.5 (**C10** + **C13**), 26.4 (**C8**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.8 (**C6**), 19.1 (**C9**).

HRMS (ESI$^+$, m/z) calculated for $C_{31}H_{50}N_7O_4$ $(M+H)^+$: 584.3919, found 584.3927.

3-(4-(-6-((((3-(dimethylamino)propyl)carbamoyl)oxy)methyl)-5,5a,6,6a,7,8-hexahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-1(4H)-yl)phenyl)propanoic acid S12

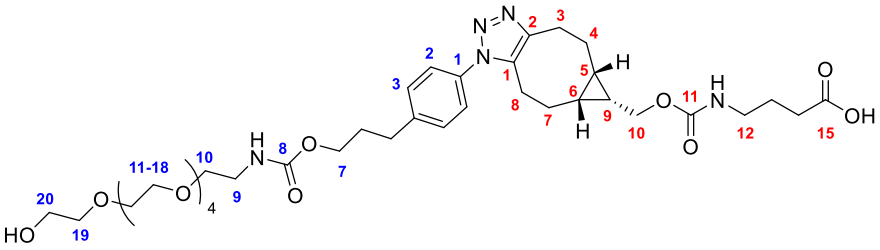


^{1}H NMR (700 MHz, MeOD) δ 7.47 (2H, d, *J* = 8.0 Hz, **H3**), 7.37 (2H, d, *J* = 8.0 Hz, **H2**), 4.17 (2H, d, *J* = 8.1 Hz, **H10**), 3.20 (2H, t, *J* = 6.3 Hz, **H12**), 3.18-3.13 (1H, m, **H8**), 3.16-3.13 (2H, m, **H14**), 3.04 (2H, t, *J* = 7.4 Hz, **H5**), 2.99-2.90 (2H, m, **H3** + **H8**), 2.88 (6H, s, **H15**), 2.72 (2H, t, *J* = 7.4 Hz, **H6**), 2.70-2.65 (1H, m, **H3**), 2.27 (1H, dddd, *J* = 15.1, 11.7, 7.8, 4.1 Hz, **H7**), 2.15 (1H, dddd, *J* = 14.6, 10.4, 7.0, 3.5 Hz, **H4**), 1.93-1.88 (2H, m, **H13**), 1.68-1.60 (2H, m, **H4** + **H7**), 1.26 (1H, app. quint., *J* = 8.3 Hz, **H9**), 1.14-1.04 (2H, m, **H6** + **H5**).

^{13}C NMR (126 MHz, MeOD) δ 176.2 (**C7**), 159.5 (**C11**), 146.0 (**C1**), 144.7 (**C4**), 136.7 (**C2**), 135.8 (**C1**), 130.7 (**C3**), 127.1 (**C2**), 63.8 (**C10**), 56.6 (**C14**), 43.5 (**C15**), 38.4 (**C12**), 36.2 (**C6**), 31.5 (**C5**), 26.5 (**C13**), 26.4 (**C8**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.8 (**C6**), 19.1 (**C9**).

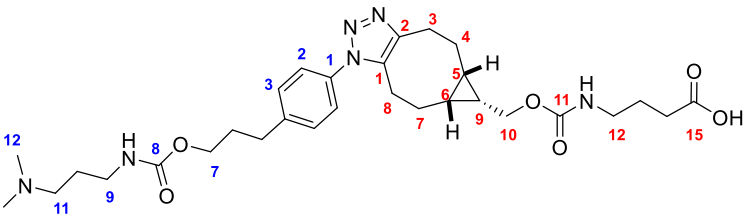
HRMS (ESI$^+$, m/z) calculated for $C_{25}H_{36}N_5O_4$ $(M+H)^+$: 470.2762, found 470.2779.

4-((((1-(4-(1-hydroxy-19-oxo-3,6,9,12,15,20-hexaoxa-18-azatricosan-23-yl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methoxy)carbonyl)amino)butanoic acid S13



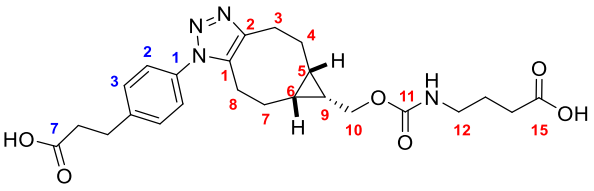
^{1}H NMR (700 MHz, MeOD) δ 7.47 (2H, d, *J* = 7.8 Hz, **H3**), 7.38 (2H, d, *J* = 7.8 Hz, **H2**), 4.16 (2H, d, *J* = 7.7 Hz, **H10**), 4.08 (2H, t, *J* = 6.2 Hz, **H7**), 3.66-3.61 (18H, m, **H11-18** + **H20**), 3.56-3.52 (4H, m, **H10** + **H19**), 3.30-3.28 (2H, m, **H9**), 3.18-3.13 (1H, m, **H8**), 3.12 (2H, t, *J* = 7.1 Hz, **H12**), 2.98-2.91 (2H, m, **H3** + **H8**), 2.83 (2H, t, *J* = 7.4 Hz, **H5**), 2.72-2.66 (1H, m, **H3**), 2.35 (2H, t, *J* = 7.3 Hz, **H14**), 2.29-2.24 (1H, m, **H7**), 2.19-2.14 (1H, m, **H4**), 2.02-1.98 (2H, m, **H6**), 1.80-1.75 (2H, m, **H13**), 1.70-1.63 (2H, m, **H4** + **H7**), 1.26 (1H, app. quint., *J* = 8.1 Hz, **H9**), 1.14-1.05 (2H, m, **H6** + **H5**).
^{13}C NMR (126 MHz, MeOD) δ 176.9 (**C15**), 159.2 (**C11**), 159.1 (**C8**), 145.8 (**C1**), 145.4 (**C4**), 137.0 (**C2**), 135.6 (**C1**), 130.8 (**C3**), 127.2 (**C2**), 73.6 (**C20**), 71.5-71.2 (8C, **C11-C19**)*, 71.0 (**C10**), 65.0 (**C7**), 63.4 (**C10**), 62.2 (**C11-C19**)*, 41.7 (**C9**), 41.0 (**C12**), 32.7 (**C5**), 31.9 (**C14**), 31.7 (**C6**), 26.4 (**C8**), 26.2 (**C13**), 24.4 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.8 (**C6**), 19.1 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{37}H_{58}N_5O_{12}$ $(M+H)^+$: 764.4076, found 764.4062.

4-((((-1-(4-(3-(((3-(dimethylamino)propyl)carbamoyl)oxy)propyl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methoxy)carbonyl)amino)butanoic acid S14



^{1}H NMR (700 MHz, MeOD) δ 7.46 (2H, d, *J* = 8.0 Hz, **H3**), 7.37 (2H, d, *J* = 8.0 Hz, **H2**), 4.19-4.12 (2H, m, **H10**), 4.09 (2H, t, *J* = 6.2 Hz, **H7**), 3.22-3.19 (2H, m, **H9**), 3.18-3.13 (1H, m, **H8**), 3.17-3.14 (2H, m, **H11**), 3.15-3.11 (2H, m, **H12**), 2.98-2.91 (2H, m, **H3** + **H8**), 2.89 (6H, s, **H12**), 2.83 (2H, t, *J* = 7.5 Hz, **H5**), 2.72-2.66 (1H, m, **H3**), 2.32 (2H, t, *J* = 7.5 Hz, **H14**), 2.30-2.25 (1H, m, **H7**), 2.19-2.14 (1H, m, **H4**), 2.01 (2H, app. quint., *J* = 6.9 Hz, **H6**), 1.91 (2H, app. quint., *J* = 6.8 Hz, **H10**), 1.79-1.74 (2H, m, **H13**), 1.69-1.63 (2H, m, **H4** + **H7**), 1.27 (1H, app. quint., *J* = 8.5 Hz, **H9**), 1.15-1.05 (2H, m, **H6** + **H5**).
^{13}C NMR (126 MHz, MeOD) δ 177.0 (**C15**), 159.5 (**C11**), 159.3 (**C8**), 146.1 (**C1**), 145.2 (**C4**), 136.8 (**C2**), 135.7 (**C1**), 130.7 (**C3**), 127.2 (**C2**), 65.3 (**C7**), 63.5 (**C10**), 56.7 (2C, **C11**), 43.5 (**C12**), 41.1 (**C12**), 38.4 (**C9**), 32.7 (**C5**), 32.1 (**C14**), 31.6 (**C6**), 26.5 (2C, **C8** + **C10**), 26.3 (**C13**), 24.3 (**C3**), 23.8 (**C7**), 23.4 (**C4**), 21.1 (**C5**), 20.9 (**C6**), 19.2 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{30}H_{45}N_6O_6$ $(M+H)^+$: 585.3395, found 585.3399.

4-((((-1-(4-(2-carboxyethyl)phenyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methoxy)carbonyl)amino)butanoic acid S15



^{1}H NMR (700 MHz, MeOD) δ 7.47 (2H, d, *J* = 8.3 Hz, **H3**), 7.36 (2H, d, *J* = 8.3 Hz, **H2**), 4.15 (2H, d, *J* = 7.9 Hz, **H10**), 3.18-3.14 (1H, m, **H8**), 3.15-3.11 (2H, m, **H12**), 3.04 (2H, t, *J* = 7.5 Hz, **H5**), 2.96-2.90 (2H, m, **H3** + **H8**), 2.72 (2H, t, *J* = 7.5 Hz, **H6**), 2.72-2.66 (1H, m, **H3**), 2.34 (2H, t, *J* = 7.3 Hz, **H14**), 2.28-2.23 (1H, m, **H7**), 2.17-2.12 (1H, m, **H4**), 1.80-1.74 (2H, m, **H13**), 1.70-1.62 (2H, m, **H4** + **H7**), 1.25 (1H, app. quint., *J* = 8.5 Hz, **H9**), 1.13-1.03 (2H, m, **H6** + **H5**).
^{13}C NMR (126 MHz, MeOD) δ 176.9 (**C15**), 176.2 (**C7**), 159.2 (**C11**), 145.8 (**C1**), 144.7 (**C4**), 136.9 (**C2**), 135.7 (**C1**), 130.7 (**C3**), 127.1 (**C2**), 63.4 (**C10**), 41.0 (**C12**), 36.1 (**C6**), 32.0 (**C14**), 31.4 (**C5**), 26.4 (**C8**), 26.2 (**C13**), 24.3 (**C3**), 23.7 (**C7**), 23.3 (**C4**), 21.1 (**C5**), 20.8 (**C6**), 19.1 (**C9**).
HRMS (ESI$^+$, m/z) calculated for $C_{24}H_{31}N_4O_6$ $(M+H)^+$: 471.2238, found 471.2242.

Kinetic experiments

Kinetics of SPAAC reaction were determined at two concentrations. Reactions were first run at 100 µM in plasma; this concentration was chosen to match that of BCN *in vivo* at the peak of concentration, as determined in our previous work.[3] The same reactions were also run in methanol at the same concentration (i.e.100 µM). However, as the reactivity in methanol was found to be mediocre, a third set of experiments was conducted in methanol at 5 mM, to allow sufficient conversion of azides for an accurate calculation of the second-order kinetic rate in this solvent.
Unless otherwise specified, all experiments were performed in triplicates.

Stock solution preparation
Stock solutions of BCN and azides in DMSO were prepared, at different concentrations.
10 mM azide solutions were prepared by mixing the 100 mM azide stock solution with a 100 mM 1,7-dihydroxynaphthalene (internal standard, IS – see 'Note 1' below) solution in DMSO in a 1:1 ratio (v/v), giving a final 50 mM stock solution of azide and IS in DMSO. This solution was then diluted with DMSO to reach the final concentration of 10 mM for both azide and IS.
10 mM BCN solutions were prepared by simple dilution of the 100 mM solutions.

Note 1. 1,7-Dihydroxynaphthalene was selected as IS as its absorbance is in the same range as the azides' at the same concentration, its retention time is compatible with all other analyzed compounds and it is fully extracted from the plasma under the conditions we used.

Reactions at 100 µM
Reaction mixture was prepared by mixing 980 µL of solvent (i.e. plasma, methanol, or PBS) with 10 µL of the 10-mM azide + IS stock solution and 10 µL of the 10-mM BCN stock solution. The resulting solution was then agitated at 37 °C and 20 µL aliquots were taken at regular intervals (i.e., t = 1, 15, 30, 45 and 60 min). Aliquots were diluted with 80 µL of cold acetonitrile (ACN – see 'Note 2' below), and 50 µL of this final solution were injected in the HPLC system for analysis. Samples at t = 0 min were prepared by replacing the volume of the BCN solution with pure DMSO.

Note 2. For all plasma reactions, precipitation of plasma proteins was achieved by adding a 20-µL sample aliquot to 80 µL of cold acetonitrile (placed in a centrifugation tube and stored in ice for 10 minutes). The mixture was then vortexed for 20 seconds to ensure complete precipitation and then centrifuged for 5 minutes (4000 rpm). 50 µL of the supernatant were finally injected in the HPLC system for analysis.

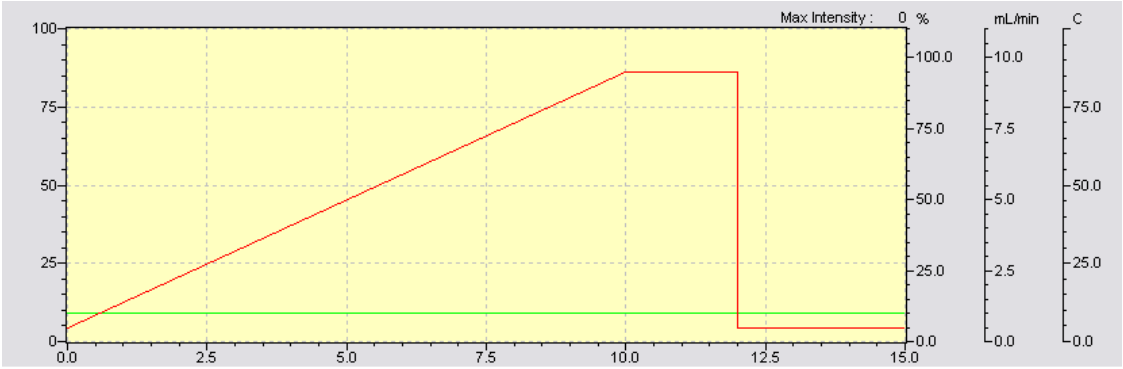
Reactions at 5 mM in MeOH
Reaction mixture was prepared by mixing 85 µL of MeOH with 10 µL of the 50-mM azide + IS stock solution and 5 µL of the 100-mM BCN stock solution. The resulting solution was then agitated at 37 °C and 4 µL aliquots were taken at regular intervals (i.e., t = 1, 15, 30, 45 and 60 min). Aliquots were diluted with 996 µL of cold acetonitrile (ACN – see 'Note 2' below), and 50 µL of this final solution were injected in the HPLC system for analysis. Samples at t = 0 min were prepared by replacing the volume of the BCN solution with pure DMSO.

HPLC analyses.
HPLC analyses were conducted on a Shimadzu UFLC system, composed of two LC-20AC pumps, one DGU 20 A3 degasser, a SPD 20A detector and a SIL 20AC HT autosampler. We used a Waters Sunfire® C18 5 µm, 4.6 x 150 mm column. Elution was performed with a mixture of mQ water (with 0.05% TFA v/v) and acetonitrile (ACN), under a flow rate of 1 $mL.min^{-1}$ with two different methods (see table S1 and S2 below for details). Samples were kept at 15 °C in autosampler before injection. 50 µL of each sample were injected and the different compounds were detected by UV absorbance at 256 nm.
Method 2 was designed to improve peak resolution of compounds eluting with ~60% of ACN, which highly overlap under the linear method 1. Method 1 was utilized to analyze the following pairs: **1-7**, **1-8**, **1-9** and **7-10**. All other pairs were analyzed using method 2.

Table S1. HPLC methods and elution profiles used for sample analysis. Red and green curves correspond to ACN % and flow rate respectively

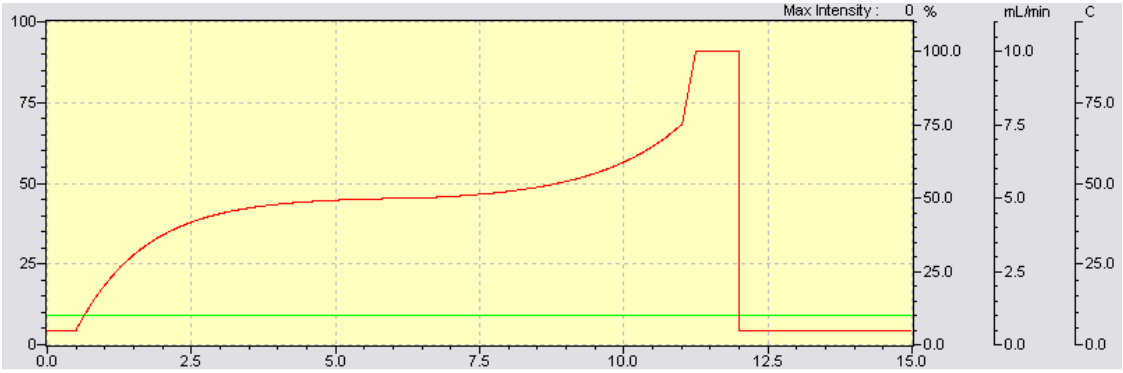
Method 1

cumulated time (min)	% water (0.05% TFA)	% ACN	B curve	flow rate ($mL.min^{-1}$)
0	95	5	0	1
10	5	95	0	1
12	5	95	0	1
12.01	95	5	0	1
15	95	5	0	0



Method 2

cumulated time (min)	% water (0.05% TFA)	% ACN	B curve	flow rate ($mL.min^{-1}$)
0	95	5	0	1
0.5	95	5	-5	1
6.5	50	50	3	1
11	25	75	3	1
11.25	0	100	0	1
12	0	100	0	1
12.01	95	5	0	1
15	95	5	0	0



Calculation of conversion rates

Conversion rate was defined as the percentage of azide that reacted over the considered period (i.e. t = 1, 15, 30, 45 and 60 min). For each chromatogram obtained, the area under the curve of the azide peak was divided by that of the IS and the resulting ratio was normalized against the value obtained at t = 0 min for a given concentration (0.1 mM or 5 mM).

Although this auto-calibration methodology is quite simple, we needed to ensure no bias were introduced during the measurements. We thus checked the stability of azides over a period of 60 min at 37 °C and that the click reaction was leading to a single product. Besides, we also checked the ability of both azides and IS to be fully extracted from plasma as well as the absence of matrix effect, which could be due to the complex composition of plasma. These controls were done to ensure that the decrease in azides concentration was solely due to their reaction with BCN and not to side reactions (see below for further details).

Calculation of second-order kinetic rates.

Second-order kinetic constants of SPAAC reactions between BCN and azides – reacted as equimolar mixtures at either 0.1 mM or 5 mM –were calculated by plotting the evolution of the inverse of azide concentration as a function of time according to the following equation:

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

where $[A]_0$ is the initial molar concentration of the azide reactant; [A], the molar concentration of the azide reactant at a given time; t, the time in seconds; and k, the second-order kinetic constant expressed in $M^{-1}.s^{-1}$. This formula was utilized since the initial concentration of both azide and BCN reactants was identical and that only one single product formed during the reaction (see Figures S22 to S30 for details). Second-order kinetic constants correspond to the slope of curves obtained after linear regression analysis.

Figures S2 to S20 illustrate the conversion rate (left) and second-order kinetic rate plot (right) for all reacting pairs in PBS (when conducted), MeOH and plasma.

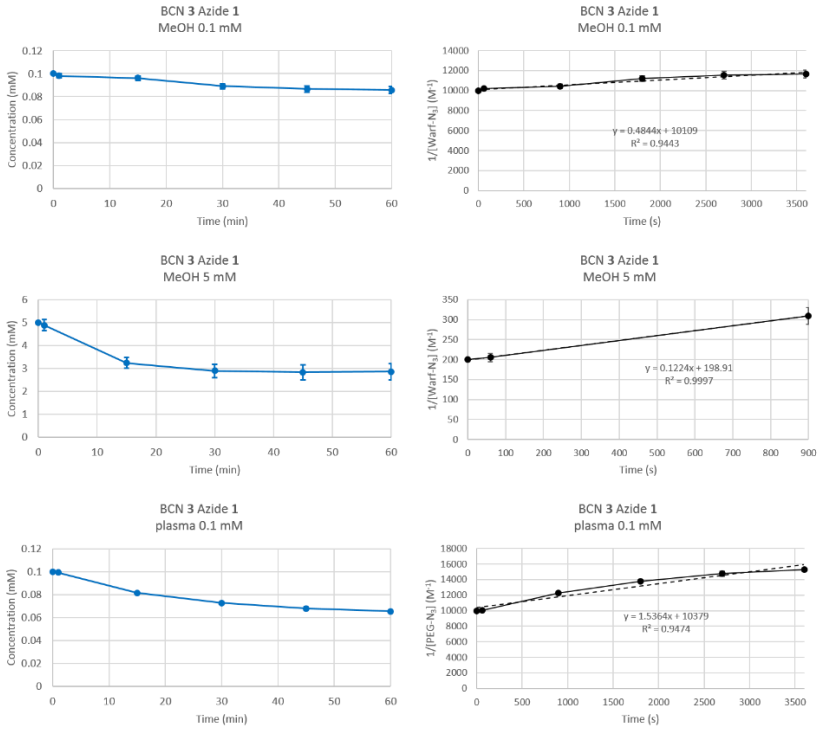


Figure S2. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **3** and azide **1**. Each measurement was done in triplicate (n = 3).

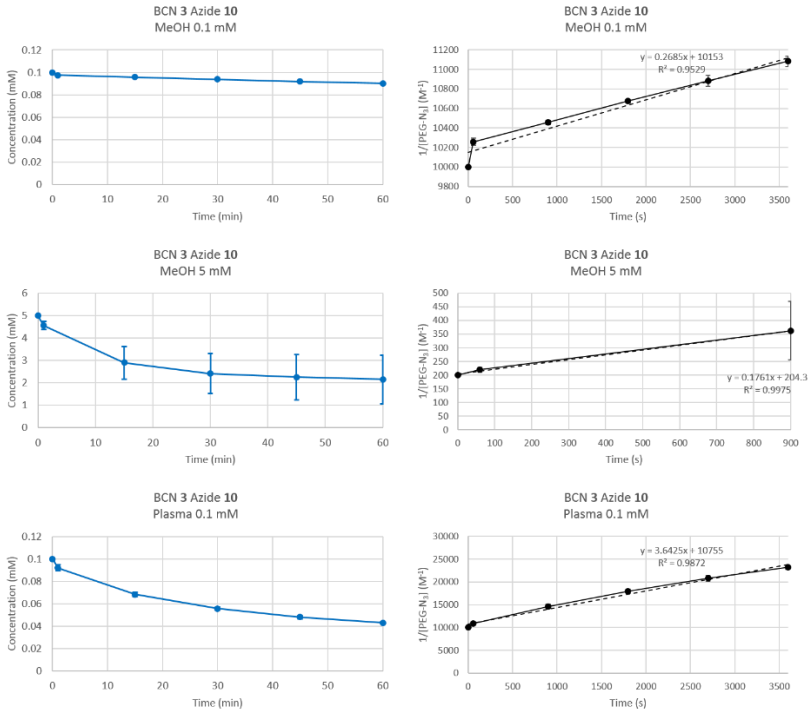


Figure S3. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **3** and azide **10**. Each measurement was done in triplicate (n = 3).

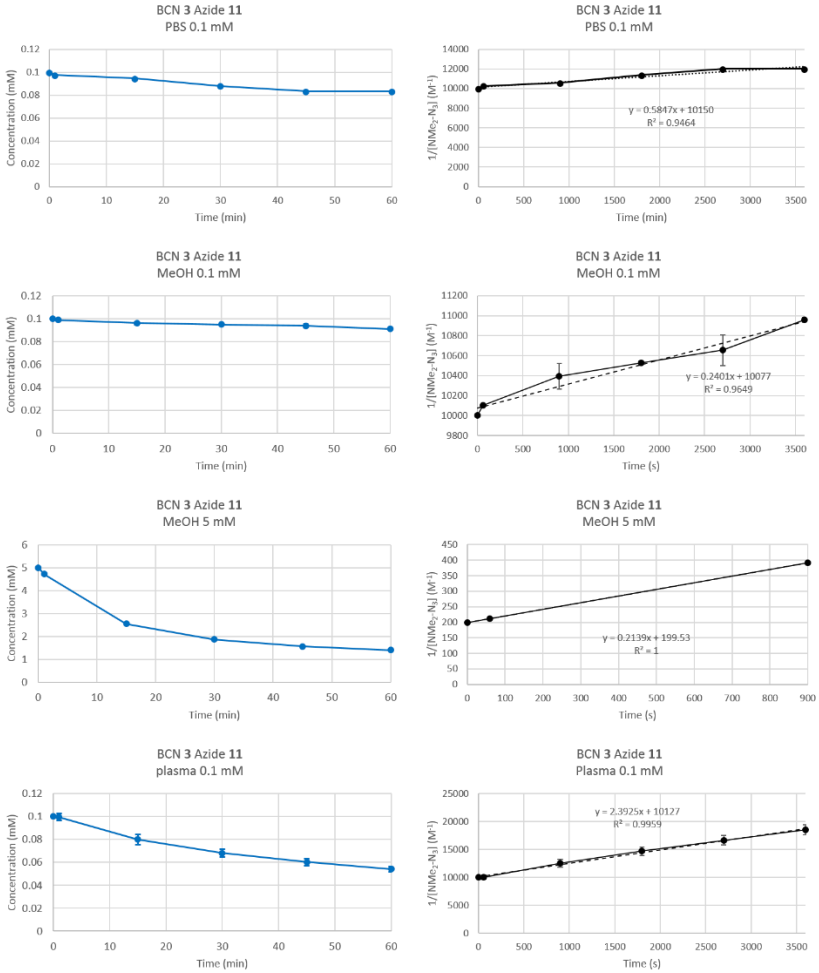


Figure S4. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **3** and azide **11**. Each measurement was done in triplicate (n = 3), except for the reaction in PBS, performed as a single experiment.

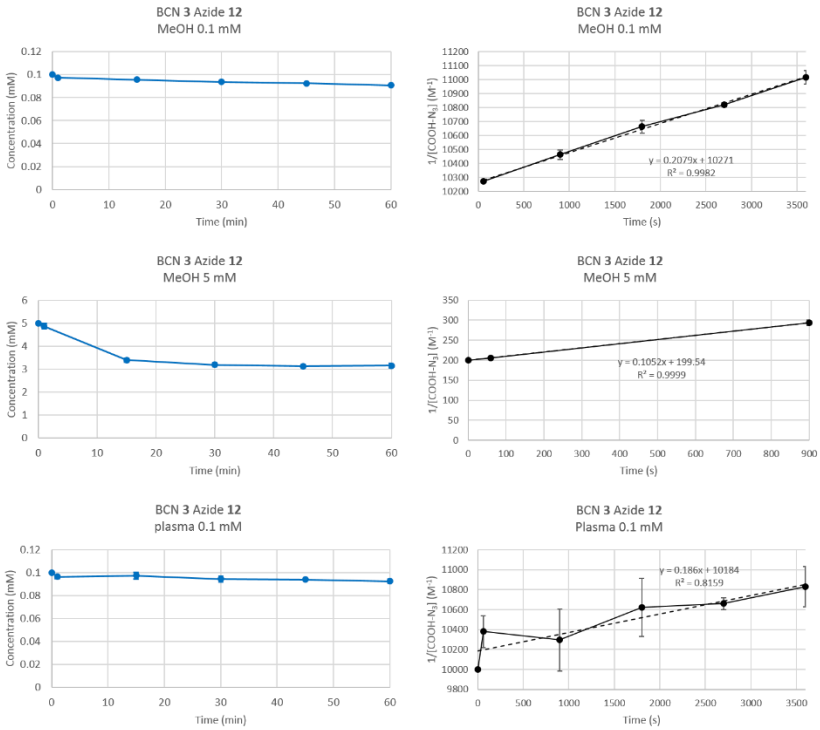


Figure S5. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **3** and azide **12**. Each measurement was done in triplicate (n = 3).

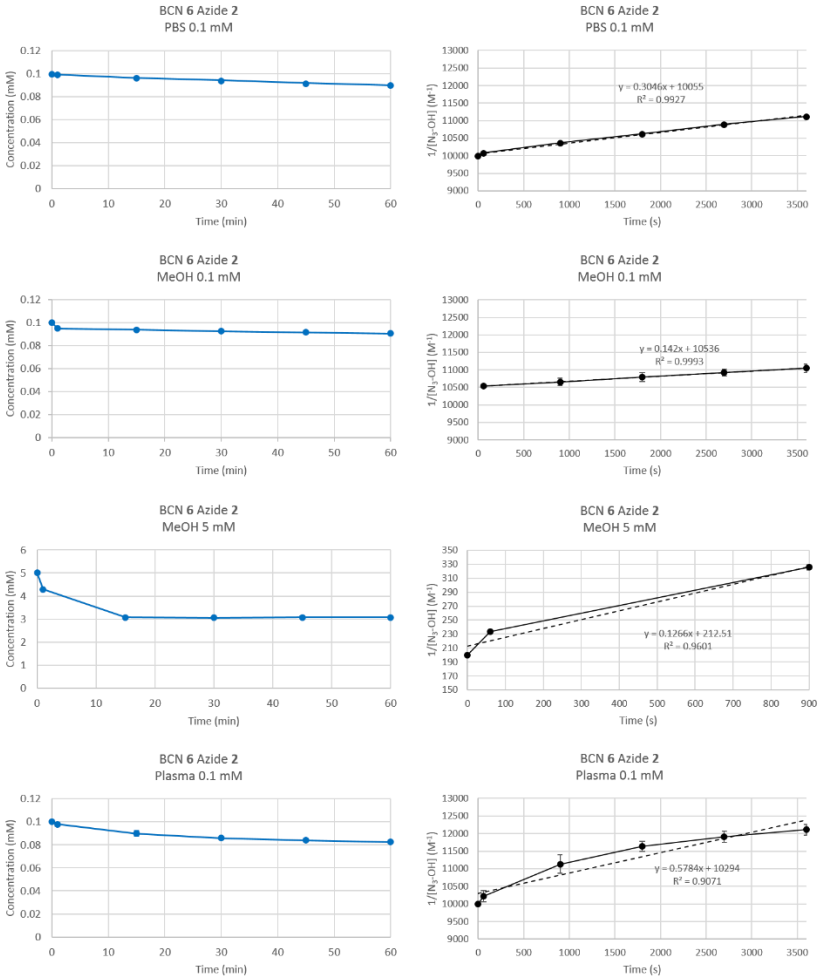


Figure S6. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **6** and azide **2**. Each measurement was done in triplicate (n = 3).

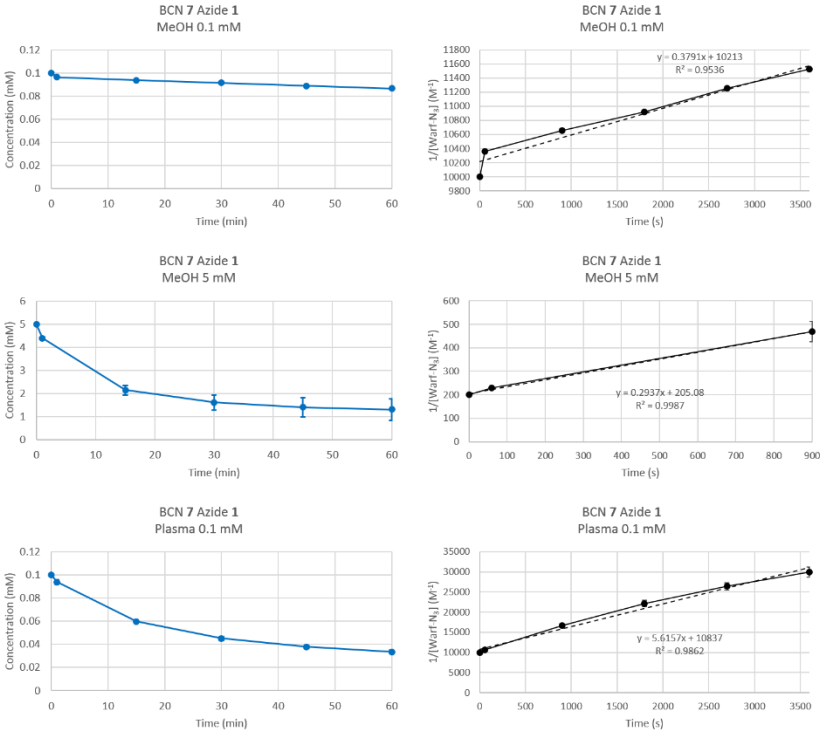


Figure S7. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **7** and azide **1**. Each measurement was done in triplicate (n = 3).

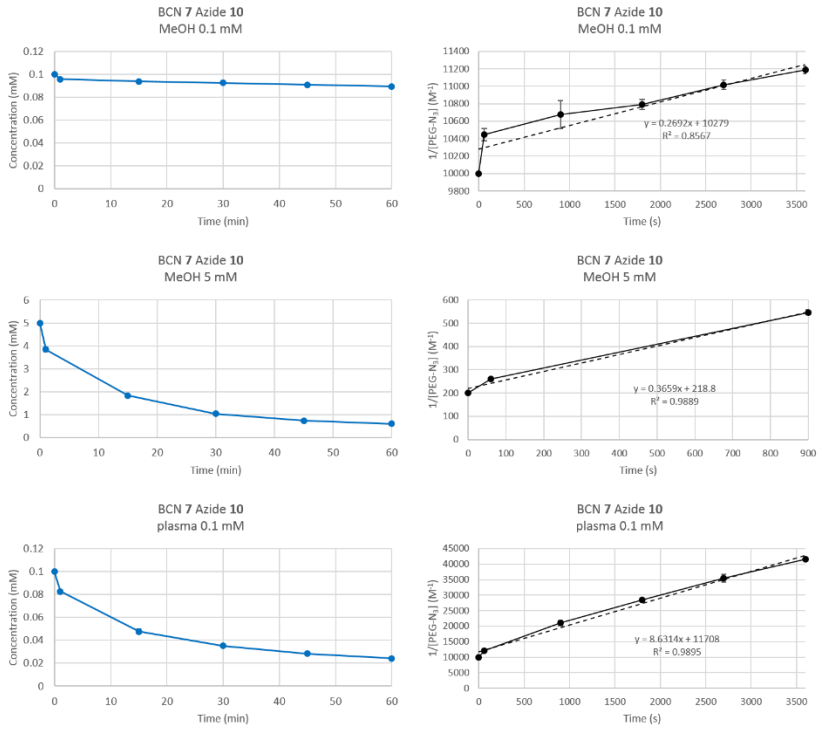


Figure S8. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **7** and azide **10**. Each measurement was done in triplicate (n = 3).

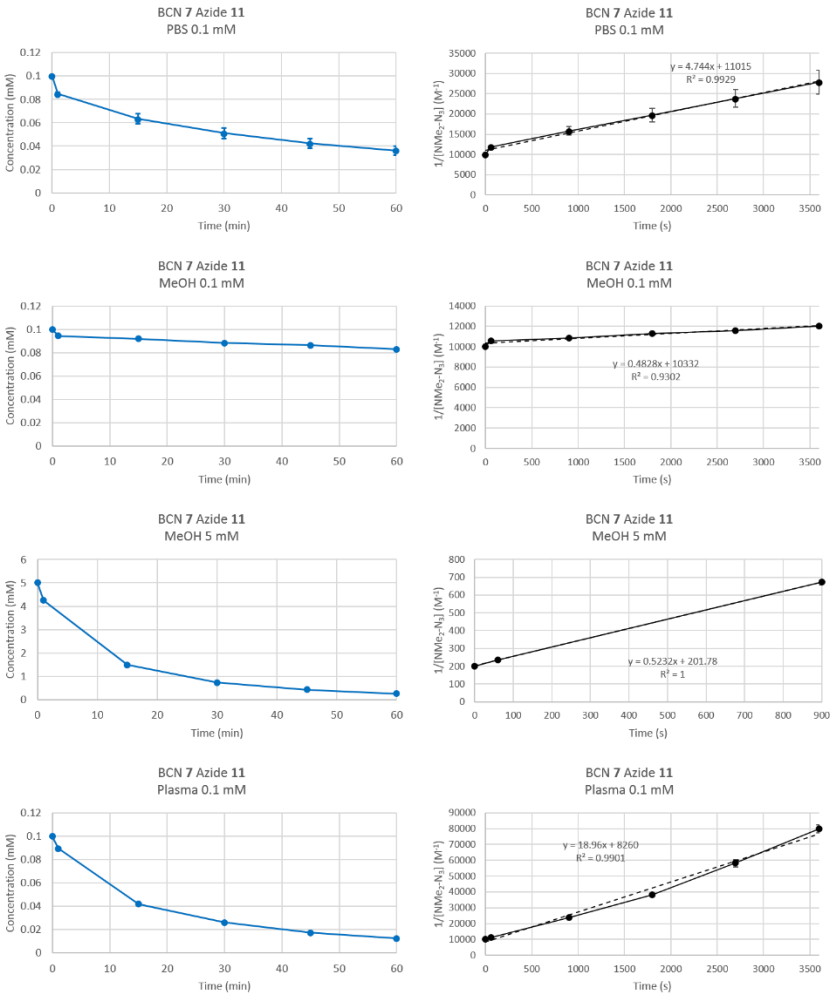


Figure S9. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **7** and azide **11**. Each measurement was done in triplicate (n = 3).

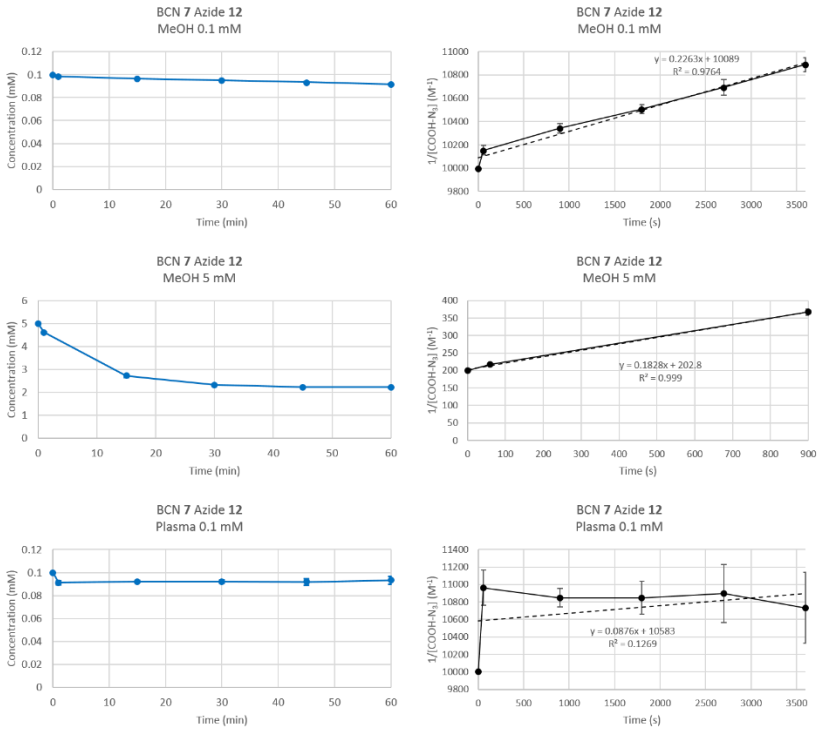


Figure S10. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **7** and azide **12**. Each measurement was done in triplicate (n = 3).

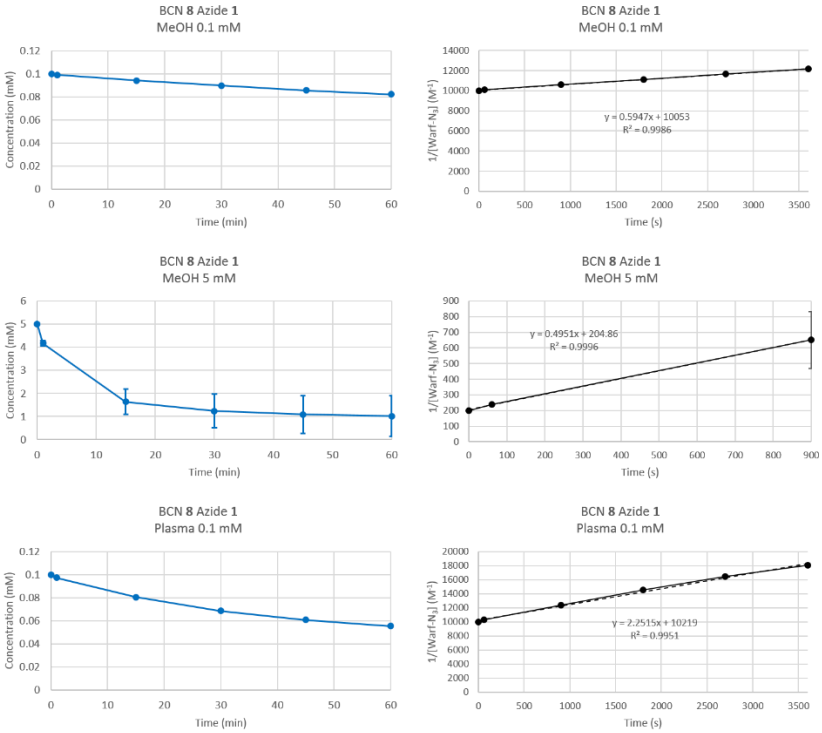


Figure S11. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **8** and azide **1**. Each measurement was done in triplicate (n = 3).

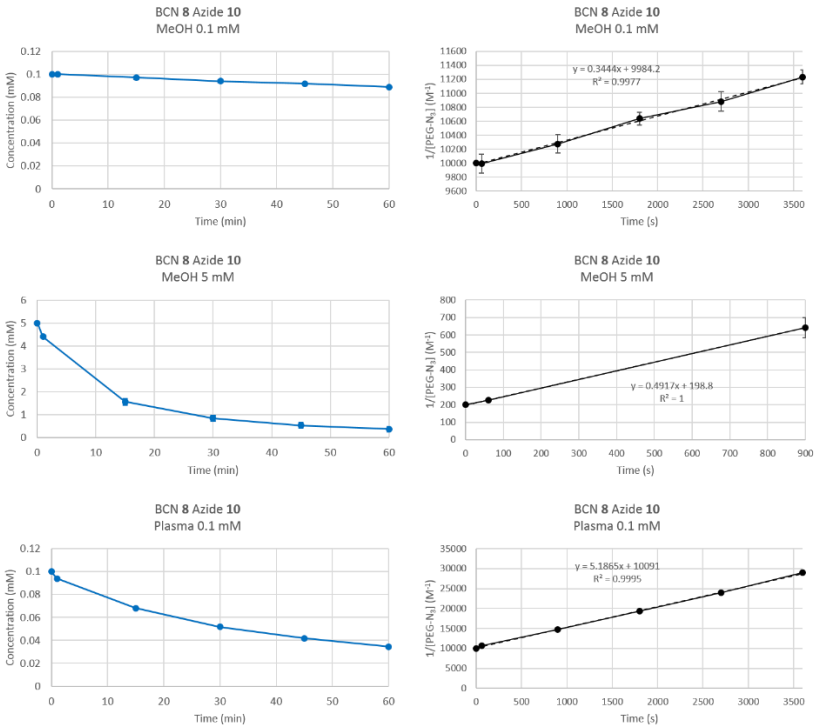


Figure S12. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **8** and azide **10**. Each measurement was done in triplicate (n = 3).

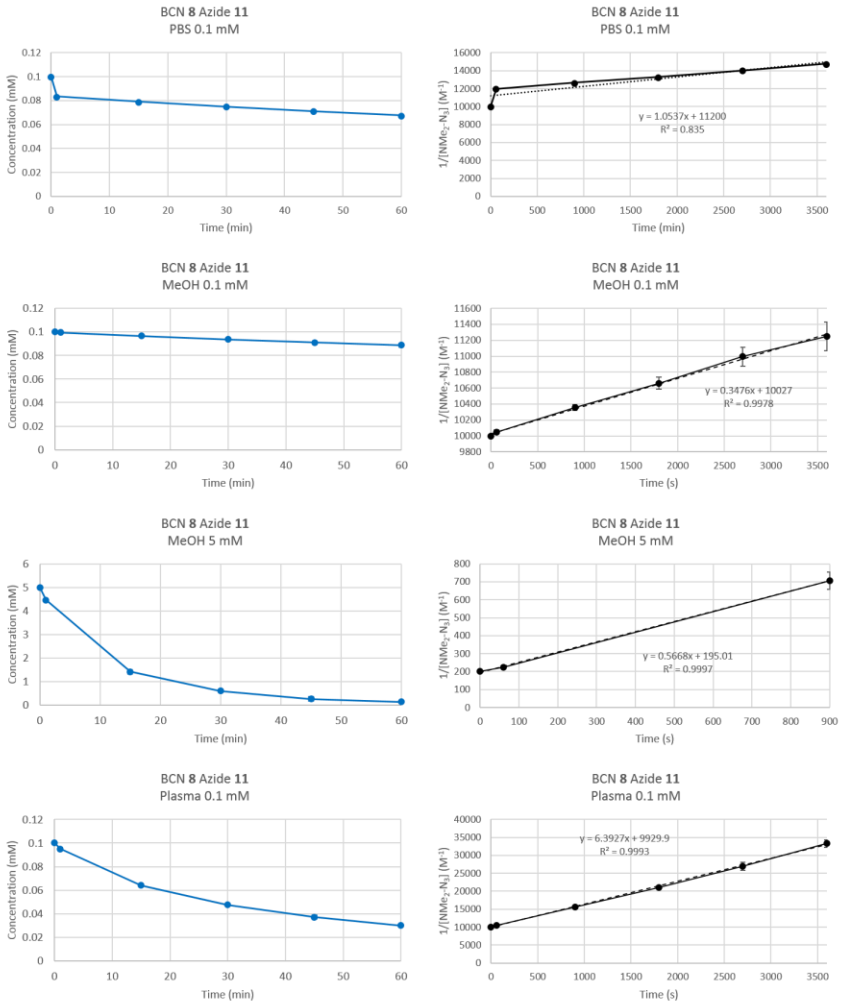


Figure S13. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **8** and azide **11**. Each measurement was done in triplicate (n = 3).

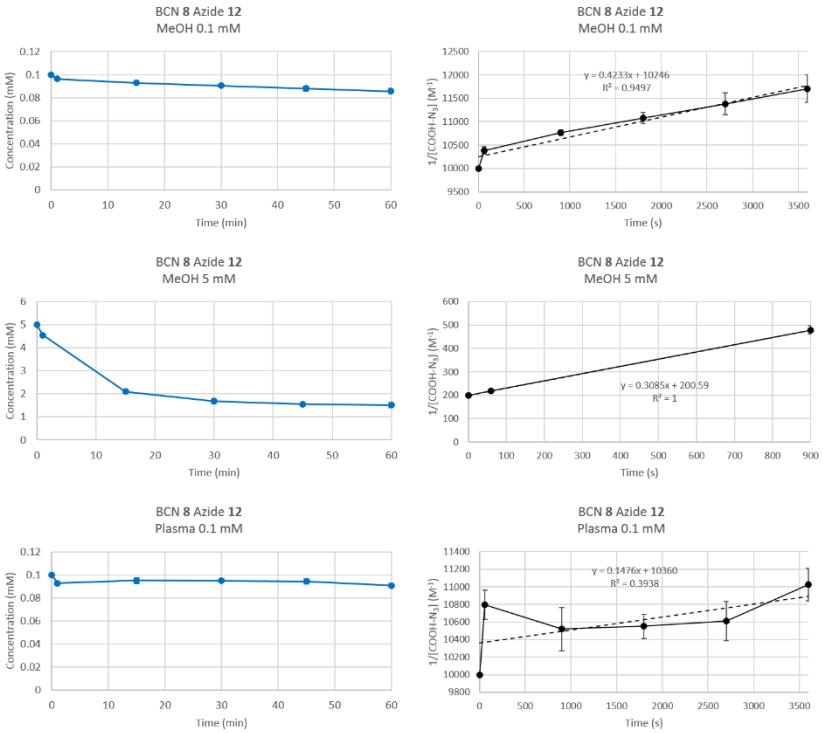


Figure S14. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **8** and azide **12**. Each measurement was done in triplicate (n = 3).

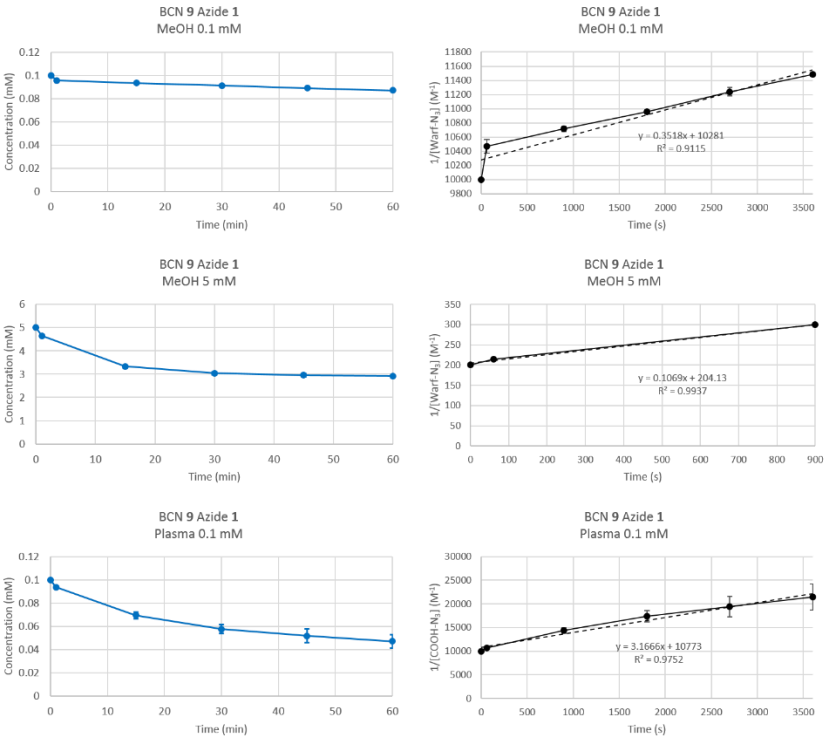


Figure S15. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **9** and azide **1**. Each measurement was done in triplicate (n = 3).

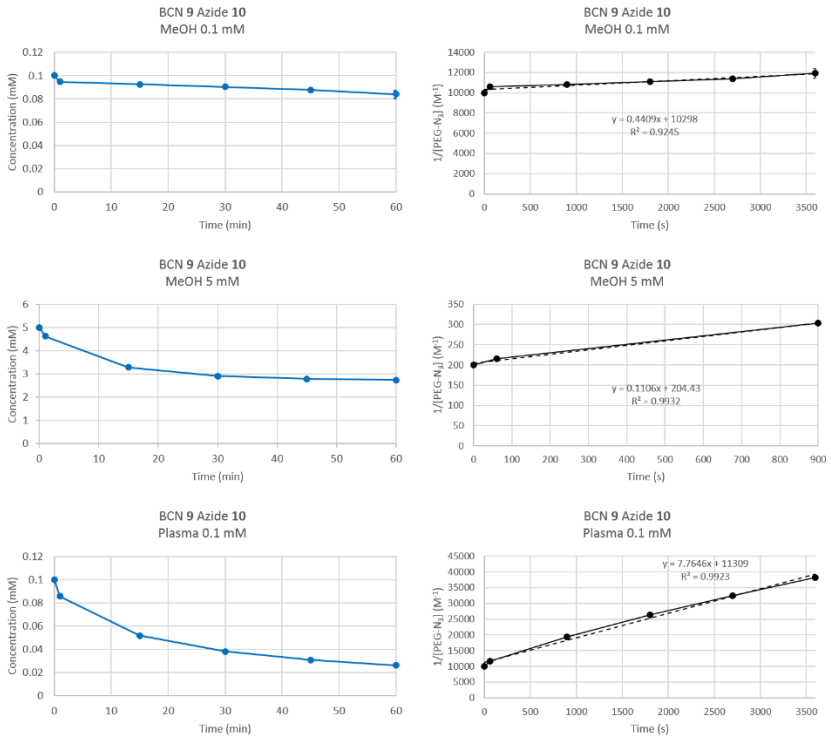


Figure S16. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **9** and azide **10**. Each measurement was done in triplicate (n = 3).

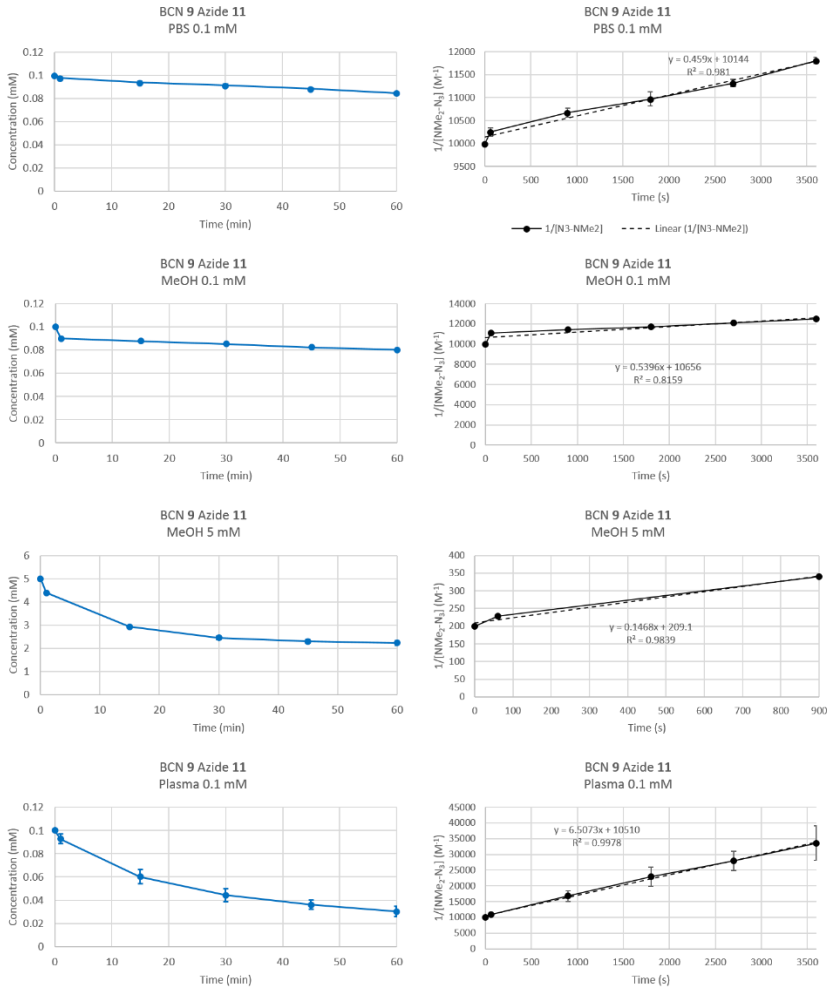


Figure S17. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **9** and azide **11**. Each measurement was done in triplicate (n = 3).

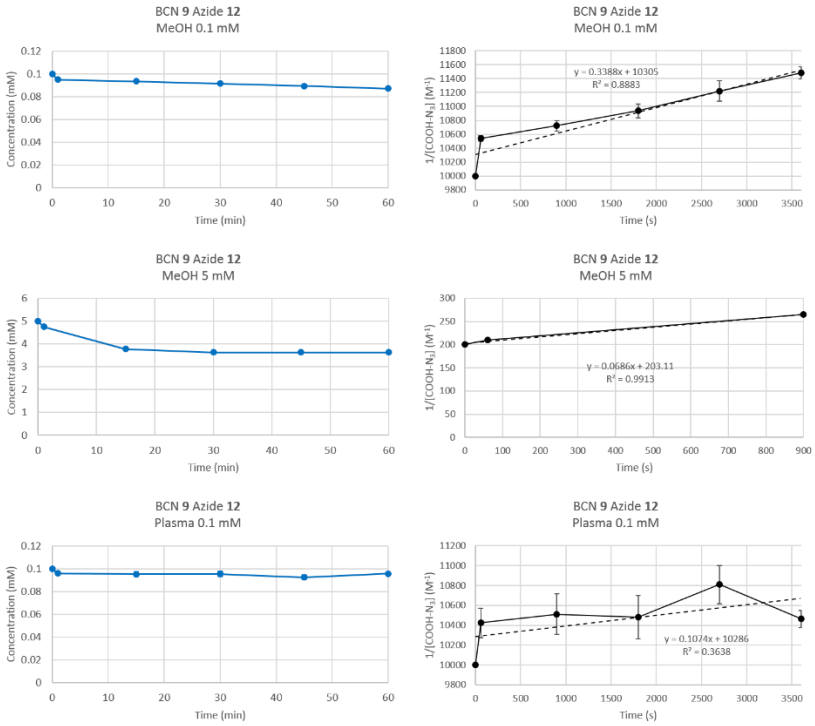


Figure S18. Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction of BCN **9** and azide **12**. Each measurement was done in triplicate (n = 3).

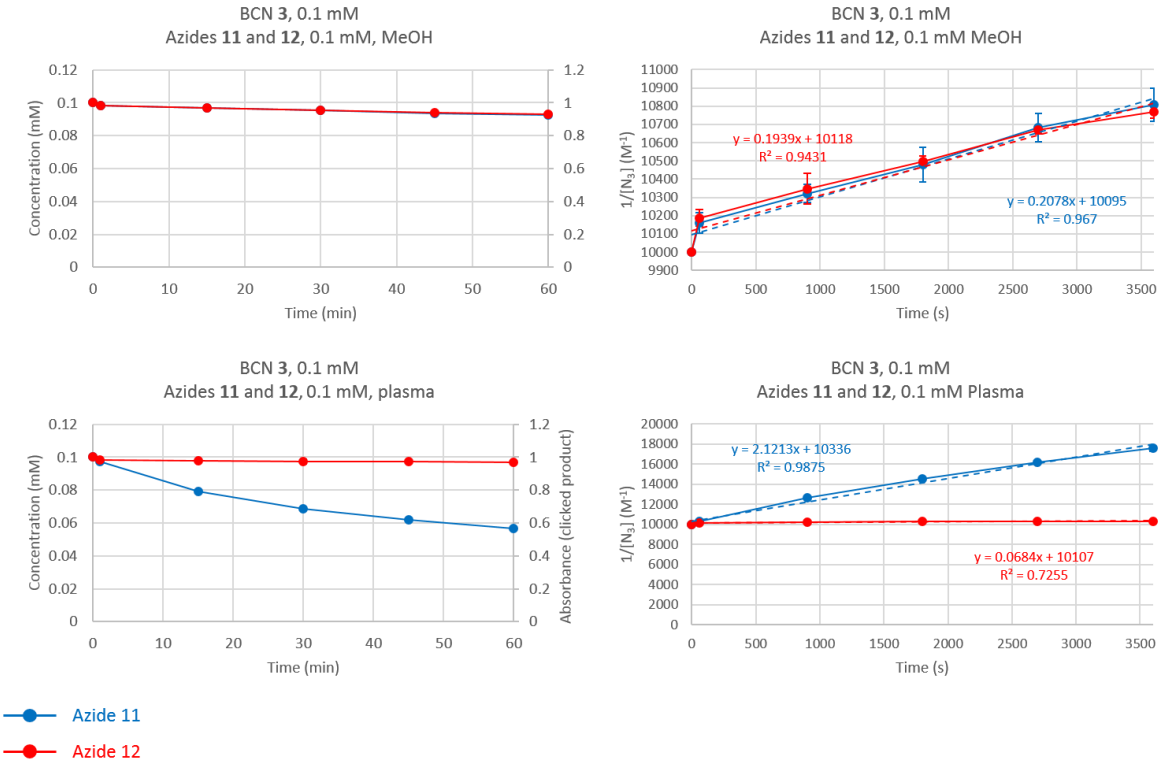


Figure S19. Kinetic profiles (left) and second-order kinetic rates plots (right) for the competition reaction between BCN **3** (0.1 mM) and azides **11** and **12** (0.1 mM) . Each measurement was done in triplicate (n = 3).

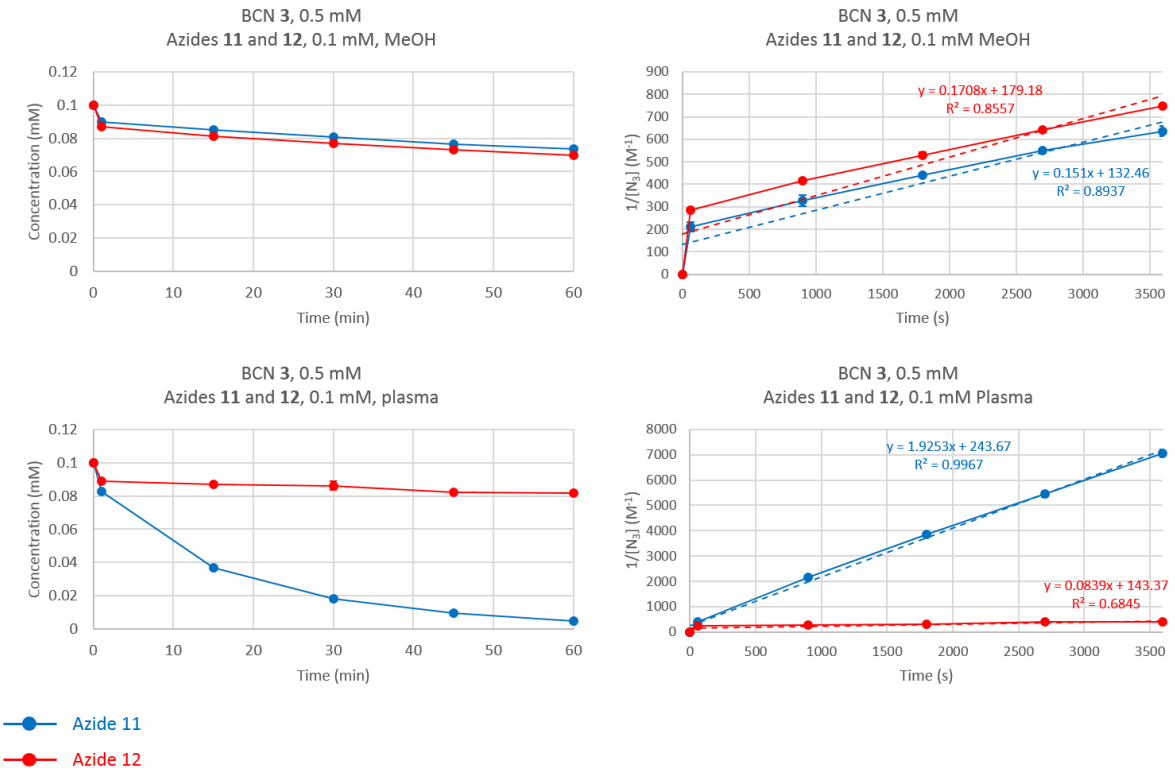


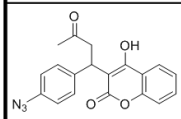
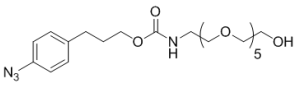
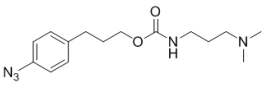
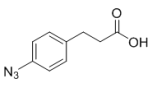
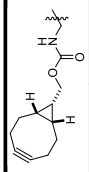
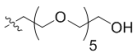
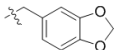
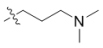
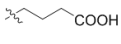
Figure S20. Kinetic profiles (left) and second-order kinetic rates plots (right) for the competition reaction between BCN **3** (0.5 mM) and azides **11** and **12** (0.1 mM) . Each measurement was done in triplicate (n = 3).

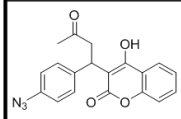
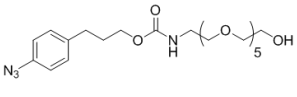
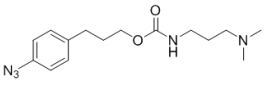
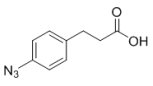
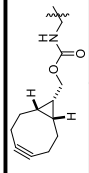
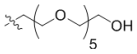
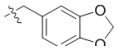
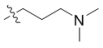
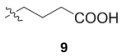
To simplify and make the previous data more visually appealing, conversion rates of azides (i.e. the percentage of reacted azide) in SPAAC reactions in plasma at 0.1 mM and in methanol at 0.1 mM and 5.0 mM are also reported below (Tables S2-S4), at t = 15 min and t = 60 min. These time-points correspond to *in vivo* maximal concentration (15 minutes) and complete disappearance (60 minutes) of azide-modified warfarin **1** in plasma samples, as reported in our previous work.[3] The same color code has been applied to all tables: red corresponds to poor conversion rates, orange to low, blue to good and green to very good conversion rates.

The threshold for poor to very good conversions has been set as follows:

- at t = 15 min, red is <15%, orange is <25%, blue is <50% and green is ≥50%;
- at t = 60 minutes, red is <15%, orange is <50%, blue is <75% and green is ≥75%.

S2. Conversion rates of SPAAC reactions at t = 15 min (top) and t = 60 min (bottom) in plasma at 0.1 mM.

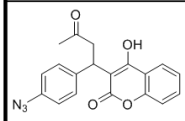
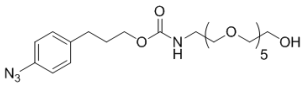
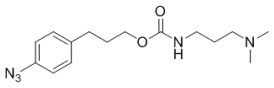
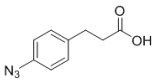
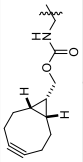
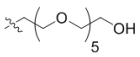
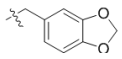
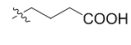
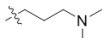
Plasma, 0.1 mM t = 15 min		Azide			
		 1	 10	 11	 12
	 3	18.6 ± 0.9	31.7 ± 1.9	20.1 ± 4.4	2.8 ± 2.9
	 7	40.2 ± 1.2	52.4 ± 1.9	58.0 ± 1.3	7.8 ± 0.8
	 8	19.4 ± 0.3	32.0 ± 0.5	36.0 ± 1.1	4.9 ± 2.2
	 9	30.5 ± 2.8	48.3 ± 0.6	39.9 ± 6.1	4.8 ± 1.8

Plasma, 0.1 mM t = 60 min		Azide			
		 1	 10	 11	 12
	 3	34.6 ± 0.9	56.9 ± 1.0	45.9 ± 2.5	7.7 ± 1.7
	 7	66.5 ± 1.4	75.9 ± 0.2	87.5 ± 0.4	6.7 ± 3.6
	 8	44.5 ± 0.6	65.4 ± 0.5	70.0 ± 0.9	9.3 ± 1.5
	 9	52.9 ± 5.8	70.0 ± 0.3	69.7 ± 4.5	4.4 ± 0.7

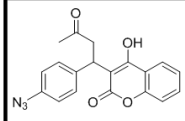
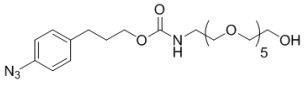
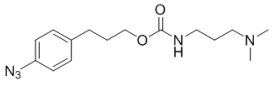
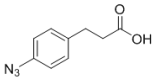
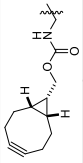
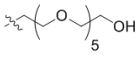
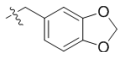
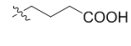
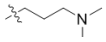
Poor	Low	Good	Very good

Table S3. Conversion rates of SPAAC reactions at t = 15 min (top) and t = 60 min (bottom) in MeOH at 0.1 mM.

MeOH, 0.1 mM
t = 15 min

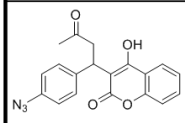
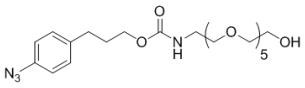
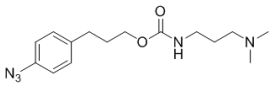
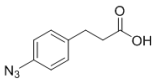
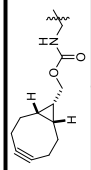
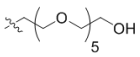
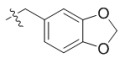
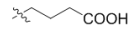
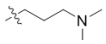
		Azide			
		 1	 10	 11	 12
	 3	0	4.4 ± 0.2	3.8 ± 1.2	4.4 ± 0.4
	 7	6.1 ± 0.2	6.3 ± 1.4	8.0 ± 0.8	3.3 ± 0.4
	 8	6.7 ± 0.3	7.4 ± 0.2	12.3 ± 0.4	6.7 ± 0.7
	 9	5.8 ± 0.7	2.7 ± 1.3	3.4 ± 0.4	7.1 ± 0.5

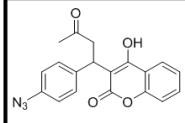
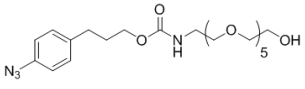
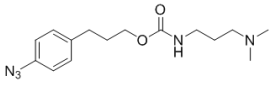
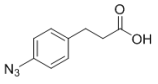
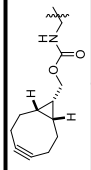
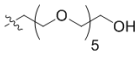
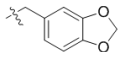
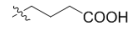
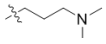
MeOH, 0.1 mM
t = 60 min

		Azide			
		 1	 10	 11	 12
	 3	0	9.8 ± 0.4	8.8 ± 0.2	9.2 ± 0.2
	 7	13.3 ± 0.05	10.6 ± 0.3	16.9 ± 0.2	8.2 ± 0.5
	 8	12.9 ± 0.2	16.0 ± 3.4	19.8 ± 0.2	12.9 ± 0.7
	 9	17.8 ± 0.6	11.0 ± 0.8	11.1 ± 1.4	14.5 ± 2.1

Poor	Low	Good	Very good

Table S4. Conversion rates of SPAAC reactions at t = 15 min (top) and t = 60 min (bottom) in MeOH at 5 mM.

MeOH, 5 mM t = 15 min		Azide			
		 1	 10	 11	 12
	 3	35.1 ± 4.5	42.0 ± 14.6	49.0 ± 0.3	32.0 ± 1.6
	 7	57.1 ± 4.2	63.4 ± 0.8	70.2 ± 0.7	45.5 ± 1.4
	 8	33.3 ± 1.3	34.1 ± 6.0	41.2 ± 1.1	24.4 ± 2.0
	 9	67.2 ± 4.2	68.7 ± 2.7	71.6 ± 1.8	45.5 ± 1.4

MeOH, 5 mM t = 60 min		Azide			
		 1	 10	 11	 12
	 3	42.7 ± 7.0	56.8 ± 21.6	71.8 ± 0.1	36.9 ± 1.9
	 7	74.0 ± 9.4	88.1 ± 1.1	94.8 ± 0.2	55.6 ± 0.6
	 8	41.6 ± 1.2	45.1 ± 6.1	55.4 ± 1.8	27.3 ± 8.4
	 9	79.6 ± 17.6	97.2 ± 2.0	97.0 ± 0.6	69.8 ± 1.9

Poor	Low	Good	Very good

Plasma stability

To assess plasma stability of azides**1**, **2**, **10**-**12**, each t_0 sample (see 'Kinetic experiments', 'Reactions at 100 µM' for sample preparation) was incubated at 37 °C for 60 minutes. Aliquots of 20 µL were then taken and diluted with 80 µL of cold ACN to precipitate plasma proteins. After 5 min centrifugation (4000 rpm), 50 µL of the supernatant were injected in the HPLC system for analysis. Concentrations at t_0 were normalized at 0.1 mM and concentrations at t = 60 min were calculated according to the method described above (see 'Kinetic experiments', 'Calculation of conversion rates') and all experiments were done as triplicates. No significant variation in azide concentration over time was observed (Figure S21).

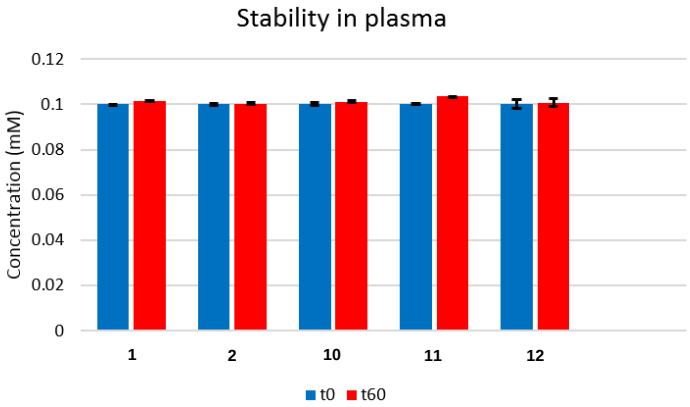


Figure S21. Plasma stability of azides 9, 10, 11 and 12 over 1 h as determined by concentrations at t_0 (blue) and t = 60 min (red). Errors represent the standard variation for triplicates.

Reaction profiles

SPAAC reactions between all azides and BCN consistently led to azide consumption and single product formation, both in plasma at 0.1 mM and in methanol at 5 mM. HPLC elution profiles at t_0, t = 15 min and t = 60 min are reported below, in Figures S22 to S30.

Note 3. The peak at ~6 min corresponds to the internal standard. The peak at ~2 min in all plasma reaction HPLC profiles is the injection peak.

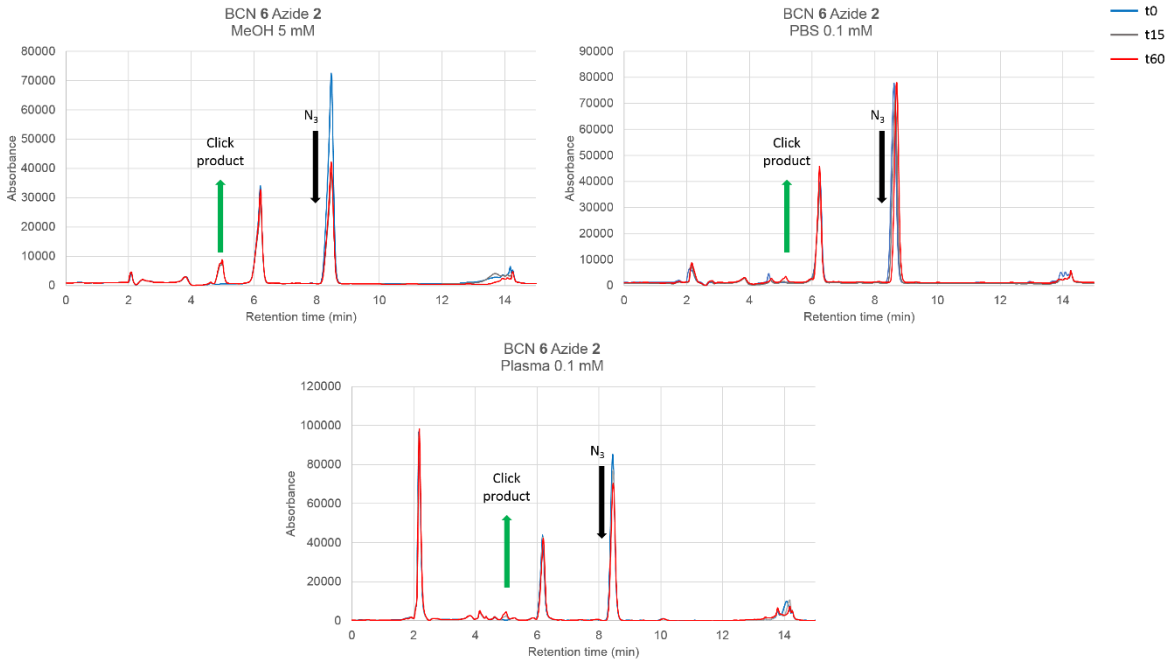


Figure S22. HPLC profile of the SPAAC reaction between BCN **6** and Azide **2** in methanol at 5 mM (top left), PBS at 0.1 mM (top right) and plasma at 0.1 mM (bottom) at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

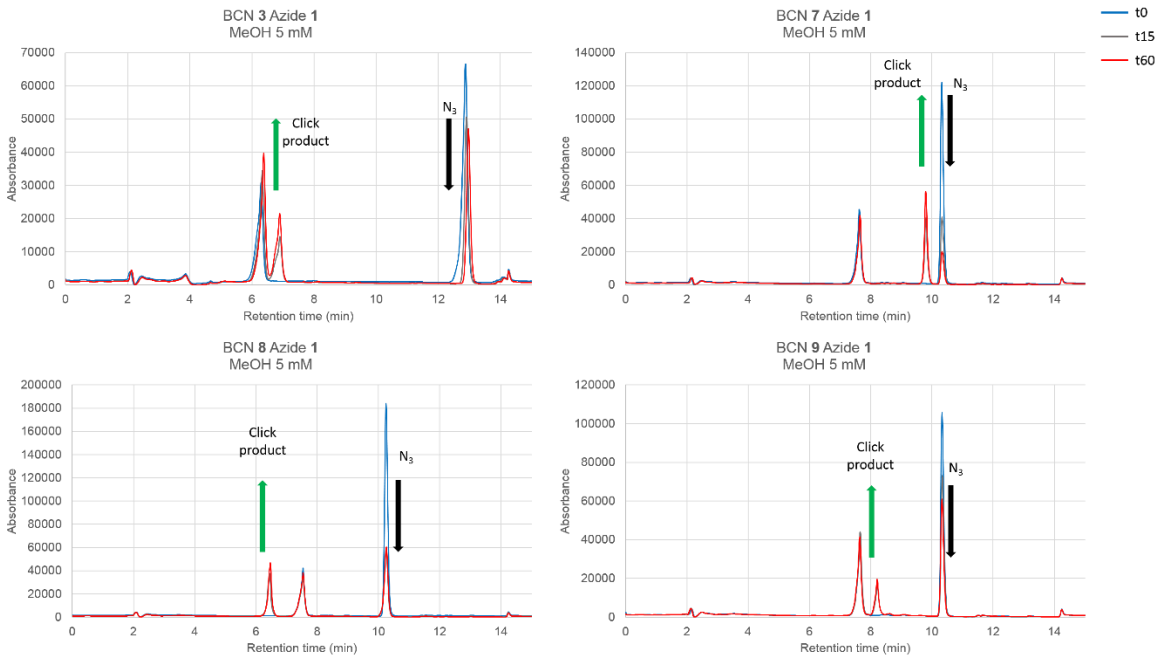


Figure S23. HPLC profile of the SPAAC reaction between azide **1** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in methanol at 5 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

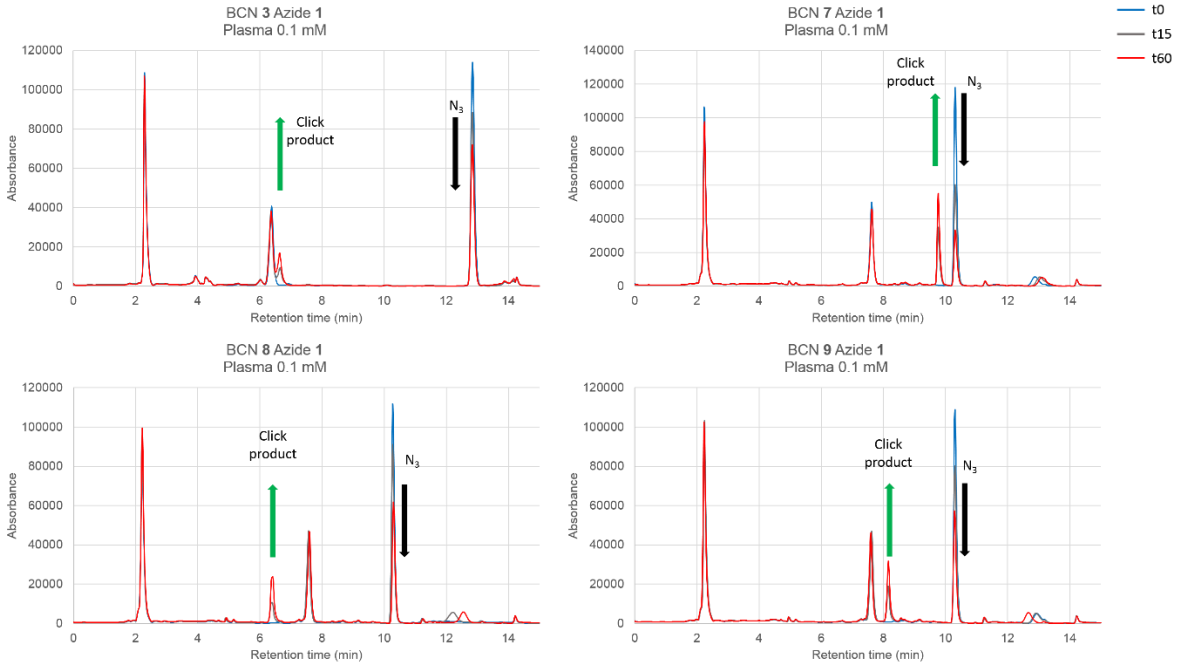


Figure S24. HPLC profile of the SPAAC reaction between azide **1** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in plasma at 0.1 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

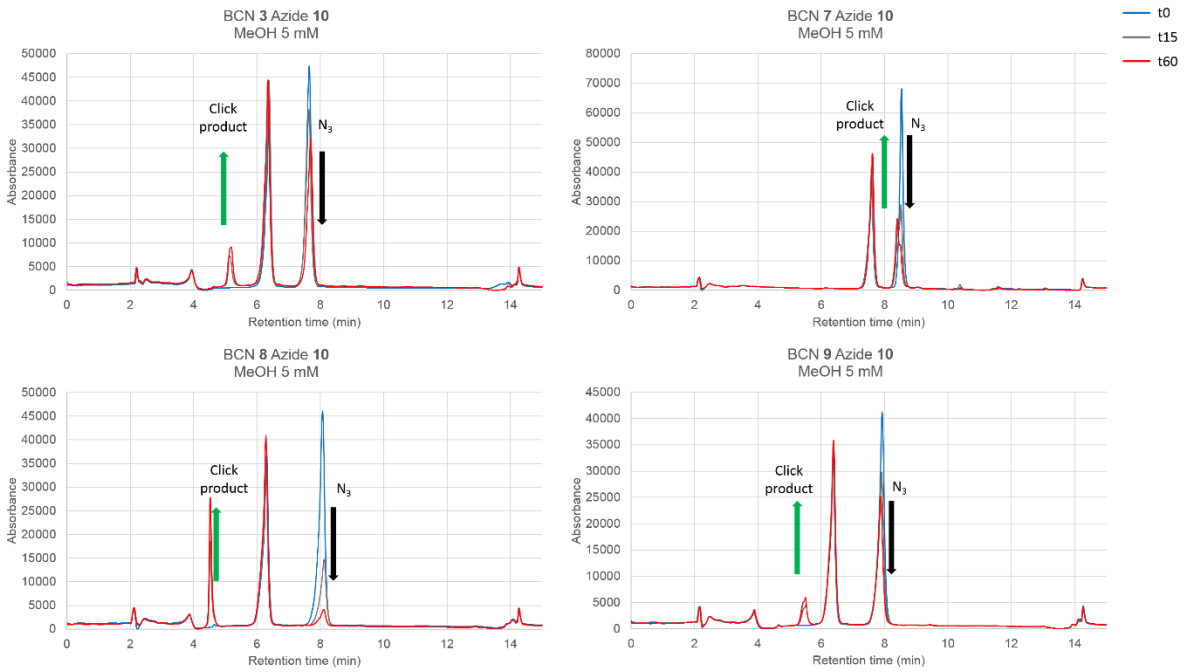


Figure S25. HPLC profile of the SPAAC reaction between azide **10** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in methanol at 5 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

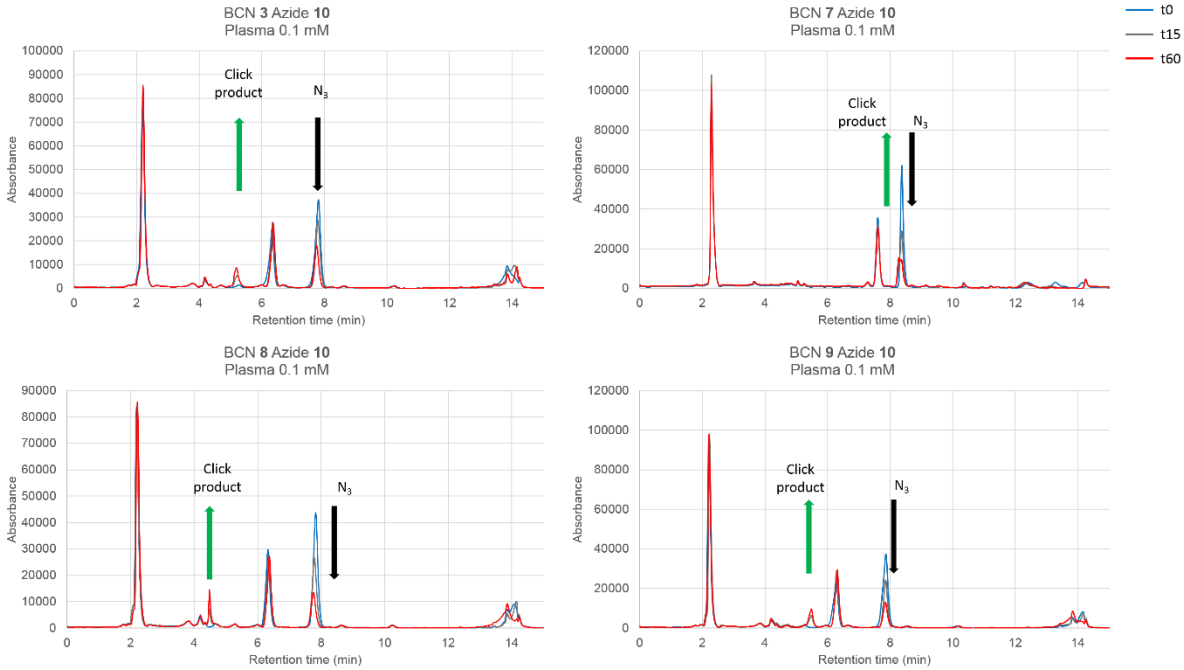


Figure S26. HPLC profile of the SPAAC reaction between azide **10** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in plasma at 0.1 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

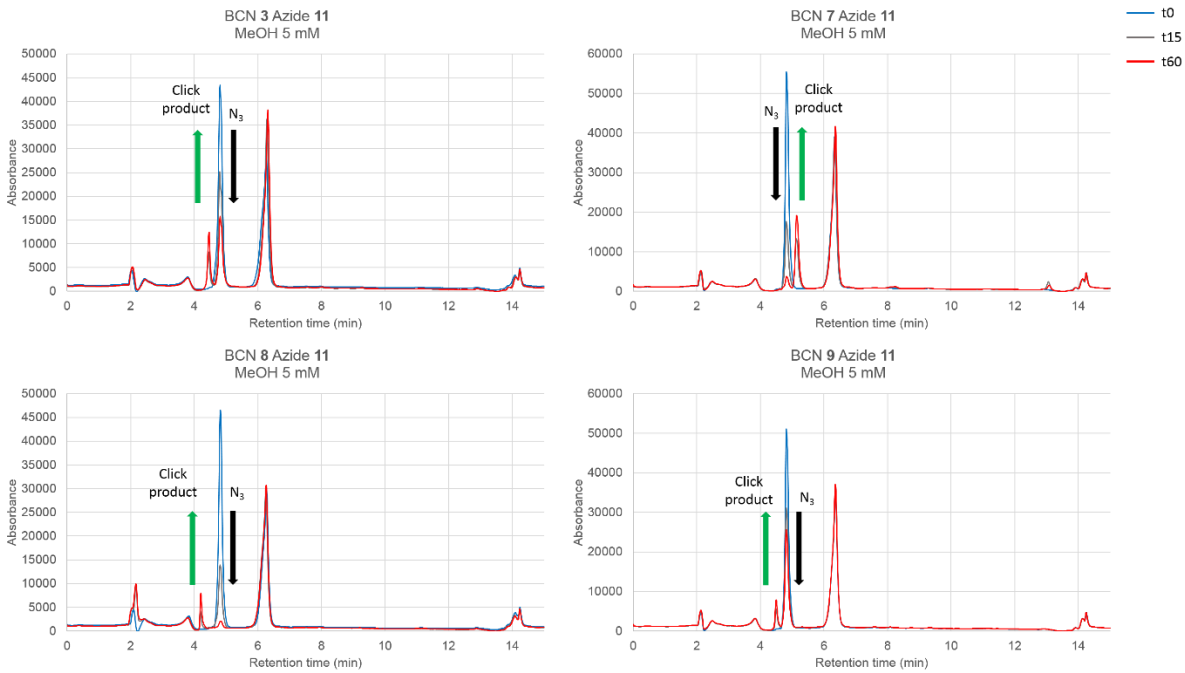


Figure S27. HPLC profile of the SPAAC reaction between azide **11** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in methanol at 5 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

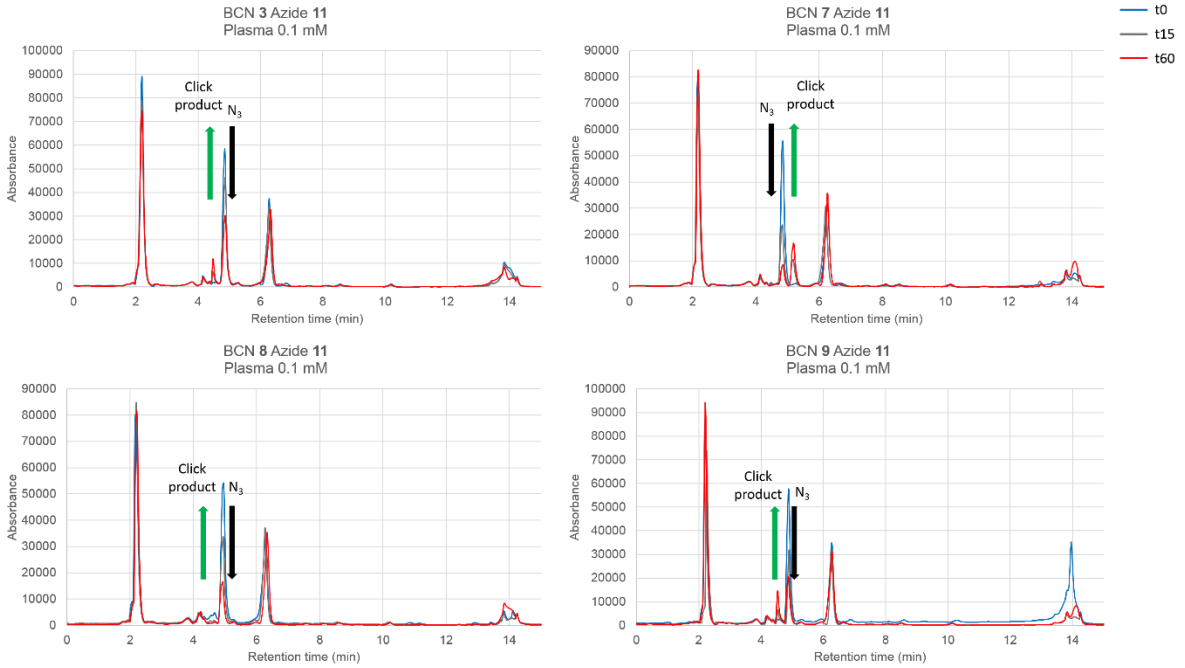


Figure S28. HPLC profile of the SPAAC reaction between azide **11** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in plasma at 0.1 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

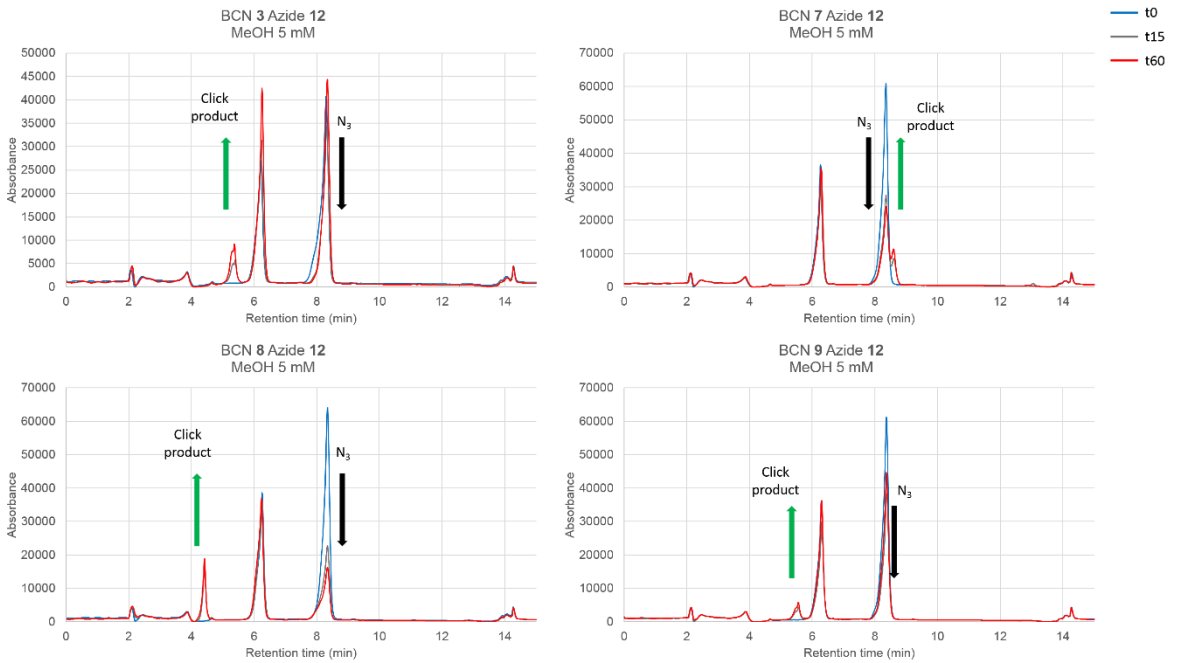


Figure S29. HPLC profile of the SPAAC reaction between azide **12** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in methanol at 5 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

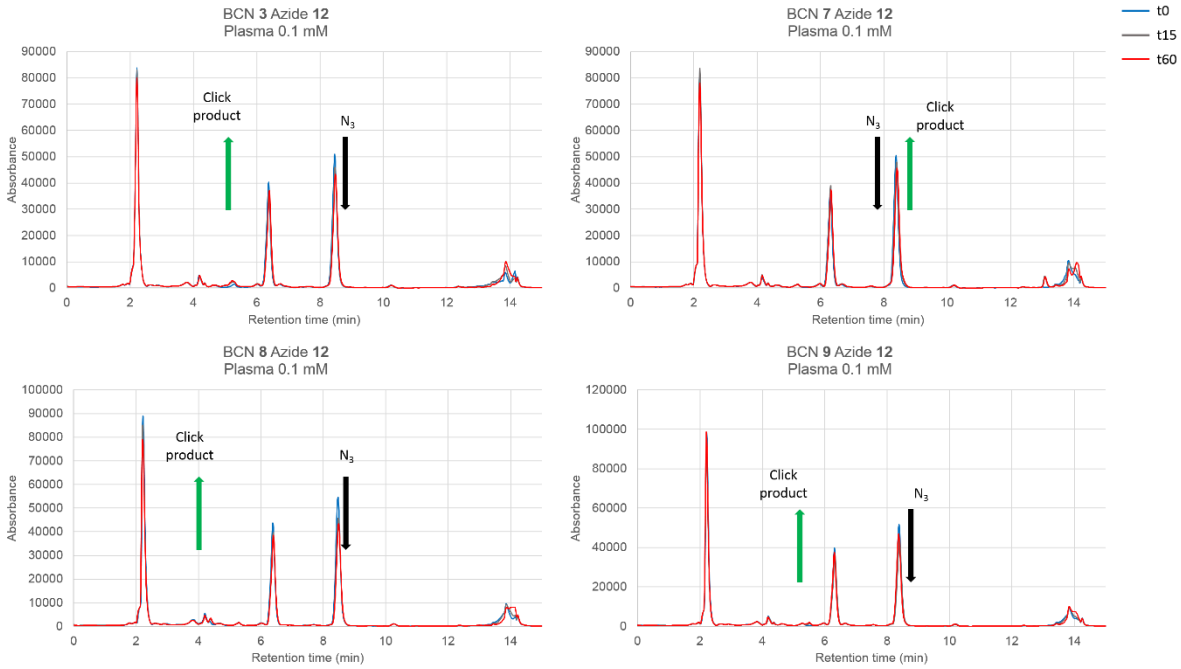


Figure S30. HPLC profile of the SPAAC reaction between azide **12** and BCN **3** (top left), BCN **7** (top right), BCN **8** (bottom left) and BCN **9** (bottom right) in plasma at 0.1 mM at t_0 (blue curve), t = 15 min (grey curve) and t = 60 min (red curve).

Evaluation of azide extraction from plasma

In order to evaluate the extraction percentage of azides from plasma, two sets of azides solutions were prepared, one in plasma and one in methanol, by diluting 5 µL of the 10-mM DMSO stock solutions of azides (also containing IS at 10 mM) with 5 µL of DMSO and 490 µL of either plasma or methanol. 20 µL of these solutions were then added to 80 µL of cold ACN. The resulting suspensions were vortexed for 20 s and centrifuged for 5 min (4000 rpm) before 50 µL of the supernatant were finally injected in the HPLC system for analysis (see above for methods). For each chromatogram obtained, the area under the curve of the azide peak was divided by that of the IS. The ratios obtained for the plasma series were then normalized against those obtained in the MeOH series, set as references. No major differences in concentrations between plasma and methanol samples were observed (Figure S31).

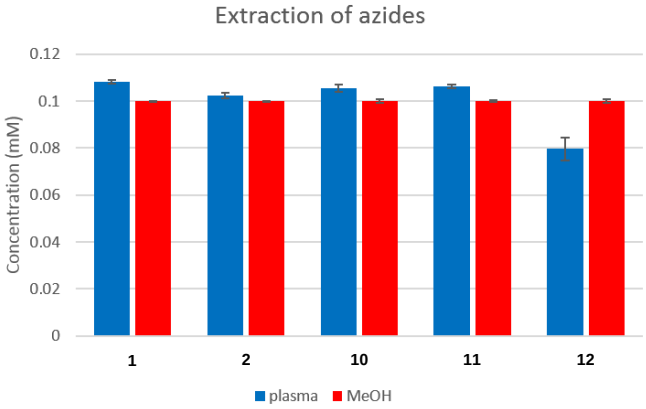


Figure S31. Comparison of azides extraction from human plasma (blue) and MeOH (red). Error bars represent the standard deviation calculated for the triplicate.

Evaluation of matrix effect

As plasma is a complex medium, some of its components may interfere during the extraction and analysis processes and affect the detection of azides, leading to falsely increased results because of this matrix effect. To make sure no such effect was observed, extractions from plasma and PBS were compared, by adding 10 µL of DMSO to 980 µL of either plasma or PBS. The resulting solutions were then transferred in 4 mL of cold ACN, and the resulting suspensions were vortexed for 20 s and centrifuged for 5 min (4000 rpm). Then, 5 µL of the 10-mM DMSO stock solutions of azides (also containing IS at 10 mM) were added to 2495 µL of the previously obtained supernatants and 50 µL of these solutions were finally injected in the HPLC system for analysis (see above for methods). For each chromatogram obtained, the area under the curve of the azide peak was divided by that of the IS. The ratios obtained for the plasma series were then normalized against those obtained in the MeOH series, set as references. No major differences in concentrations between plasma and methanol samples were observed (Figure S32), suggesting no matrix effect.

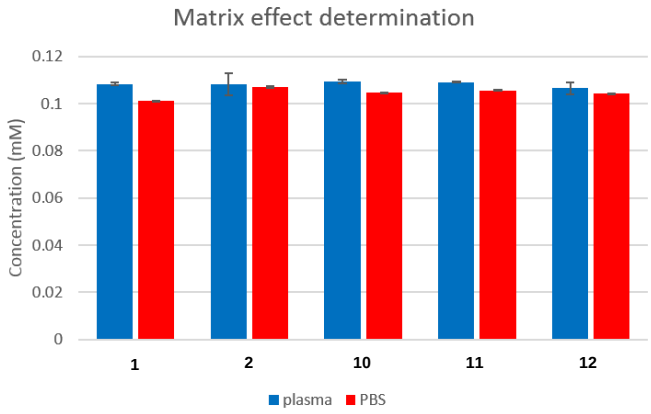


Figure S32. Comparison of concentrations extracted from solutions in plasma (blue) or PBS (red) to evaluate the matrix effect of plasma components. Error bars represent the standard deviation calculated for the triplicate.

Determination of plasma proteins binding

Plasma proteins binding assays were conducted on a RED (Rapid Equilibrium Dialysis) device (90006, ThermoFisher). Samples were prepared (n = 2) as follows: A 100 µM solution of compound was prepared in a mixture of plasma and PBS 1x (1:1 v/v) from a 10 mM DMSO stock solution of the compound. 200 µL of this diluted solution was introduced in the sample chamber and 350 µL of PBS 1x were introduced in the buffer chamber. The unit was covered with sealing tape and incubated for 4 h at 37 °C on an orbital shaker set at 250 rpm. After that, the seal was removed and 70 µL samples of both the buffer and the plasma chambers were collected and placed in separate 1.5 mL microcentrifuge tubes. 70 µL of plasma / PBS (1:1 v/v) and PBS were respectively added to the buffer and the sample chamber. ACN (350 µL) was added to precipitate proteins and solubilize the compound. All samples were frozen before analysis. Before analysis, samples were thawed and vortexed for 5 min before being sonicated in a sonication bath for 1 min and finally centrifuged for 5 min at 15000 g and 16 °C. Supernatants were then analyzed by LC-MS/MS using a Triple Quadrupole Liquid Chromatograph Mass Spectrometer (LCMS 8030, Shimadzu) in multiple reaction-monitoring mode (MRM). Data acquisition and processing were performed using LabSolutions version 5 software.
The percentage of bound compounds was calculated as follows:

$$\% \text{ Free50} = (\text{Peak area of buffer chamber} / \text{Peak area of plasma chamber}) \times 100\%$$
$$\% \text{ Free100} = \% \text{ Free50} / (2 - \% \text{ Free50})$$
$$\% \text{ Bound} = 100\% - \% \text{ Free}$$

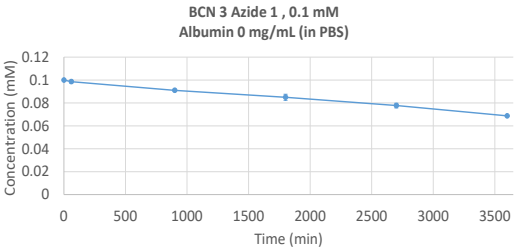
Data are summarized in Table S5 below.

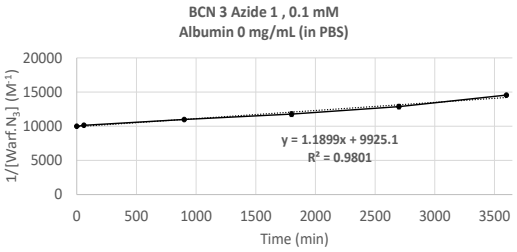
Table S5. Plasma proteins binding values of BCN and azide

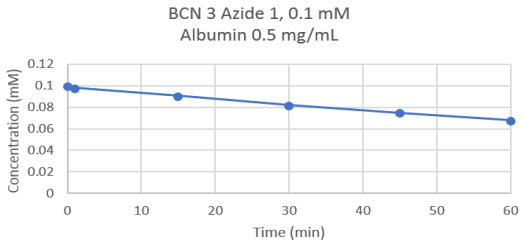
Compound	Volume injected	Plasma chamber, area under the curve		Buffer chamber, area under the curve		% bound
		Experiment 1	Experiment 2	Experiment 1	Experiment 2	
BCN **3**	1 µL	367249	350246	313513	296026	26 ± 1
BCN **6**	1 µL	11692	12740	2056	1846	91 ± 1
BCN **7**	1 µL	34237	30974	822	590	98.9 ± 0.1
BCN **8**	1 µL	785133	748127	682606	618918	26 ± 3
BCN **9**	1 µL	17875	20753	12090	13218	51 ± 2
Azide **2**	1 µL	43426	41099	37647	39754	15 ± 9
Azide **10**	0.2 µL	2974803	3217840	1034854	1161810	78.5 ± 0.5
Azide **11**	0.5 µL	3575740	3705412	1577115	1517177	73 ± 1
Azide **12**	1 µL	43285	43867	35117	39512	25 ± 7

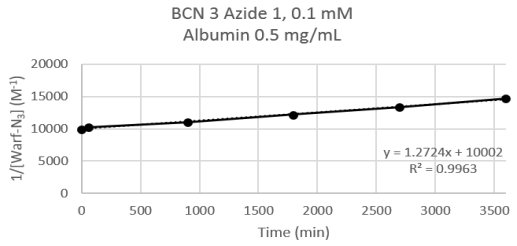
Determination of second-order kinetic rates of SPAAC in the presence of albumin

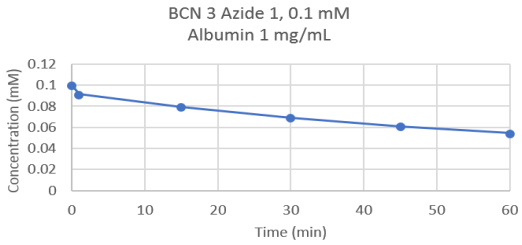
Solutions of human serum albumin (HSA) at six different concentrations (i.e. 0.5, 1, 2.5, 5, 10, and 50 mg/mL) were prepared in PBS and used to study SPAAC between BCN **3** and azide **1**.
Reaction mixtures were prepared by mixing 980 µL of the albumin solution in PBS (0.5, 1, 2.5, 5, 10 or 50 mg/mL) with 10 µL of the 10-mM azide + IS stock solution and 10 µL of the 10-mM BCN stock solution. The resulting solution was then agitated at 37 °C and 20 µL aliquots were taken at regular intervals (i.e., t = 1, 15, 30, 45 and 60 min). Aliquots were diluted with 80 µL of cold acetonitrile (ACN – see 'Note 2' in the Kinetic Experiments section), and 50 µL of this final solution were injected in the HPLC system for analysis. Samples at t = 0 min were prepared by replacing the volume of the BCN solution with pure DMSO. These measurements were done in monoplicate (n = 1).
Kinetic profiles and associated second-order kinetic rates are reported in Figure S33 and Table S6 below.

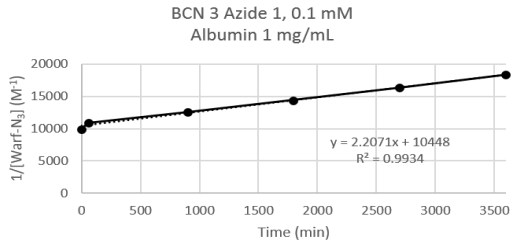

BCN 3 Azide 1 , 0.1 mM
Albumin 0 mg/mL (in PBS)
Concentration (mM)
Time (min)

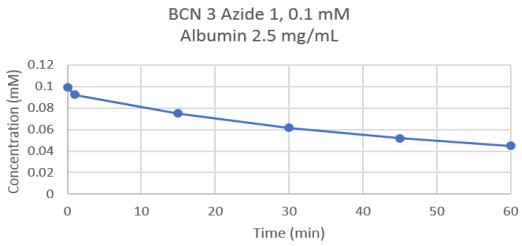

BCN 3 Azide 1 , 0.1 mM
Albumin 0 mg/mL (in PBS)
1/[Warf.N3] (M-1)
y = 1.1899x + 9925.1
R² = 0.9801
Time (min)

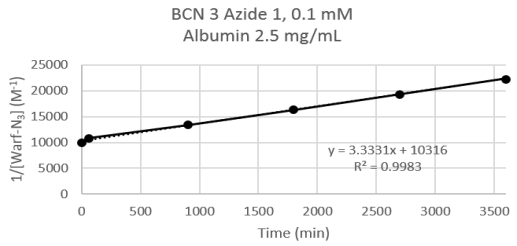

BCN 3 Azide 1, 0.1 mM
Albumin 0.5 mg/mL
Concentration (mM)
Time (min)

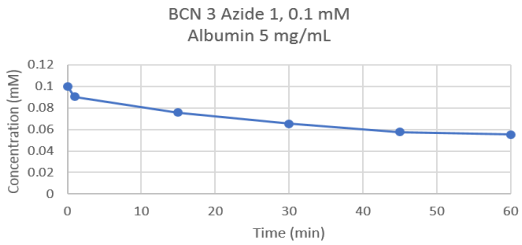

BCN 3 Azide 1, 0.1 mM
Albumin 0.5 mg/mL
1/[Warf-N3] (M-1)
y = 1.2724x + 10002
R² = 0.9963
Time (min)

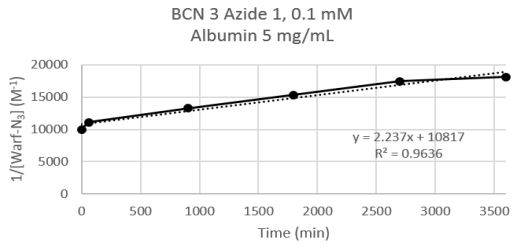

BCN 3 Azide 1, 0.1 mM
Albumin 1 mg/mL
Concentration (mM)
Time (min)

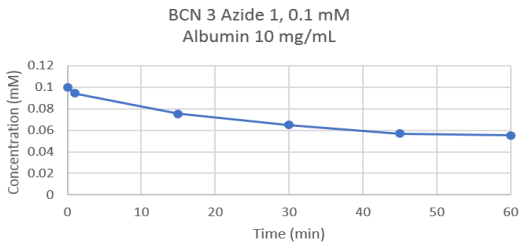

BCN 3 Azide 1, 0.1 mM
Albumin 1 mg/mL
1/[Warf-N3] (M-1)
y = 2.2071x + 10448
R² = 0.9934
Time (min)

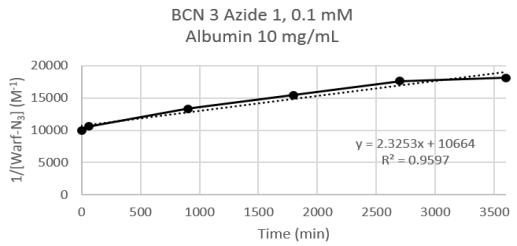

BCN 3 Azide 1, 0.1 mM
Albumin 2.5 mg/mL
Concentration (mM)
Time (min)

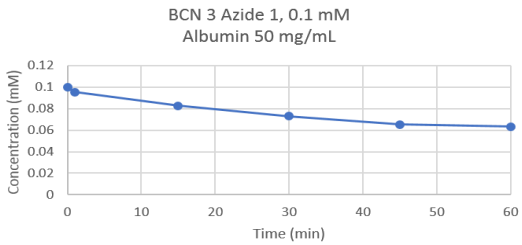

BCN 3 Azide 1, 0.1 mM
Albumin 2.5 mg/mL
1/[Warf-N3] (M-1)
y = 3.3331x + 10316
R² = 0.9983
Time (min)


BCN 3 Azide 1, 0.1 mM
Albumin 5 mg/mL
Concentration (mM)
Time (min)


BCN 3 Azide 1, 0.1 mM
Albumin 5 mg/mL
1/[Warf-N3] (M-1)
y = 2.237x + 10817
R² = 0.9636
Time (min)


BCN 3 Azide 1, 0.1 mM
Albumin 10 mg/mL
Concentration (mM)
Time (min)


BCN 3 Azide 1, 0.1 mM
Albumin 10 mg/mL
1/[Warf-N3] (M-1)
y = 2.3253x + 10664
R² = 0.9597
Time (min)


BCN 3 Azide 1, 0.1 mM
Albumin 50 mg/mL
Concentration (mM)
Time (min)

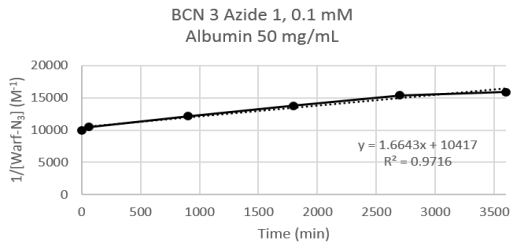

BCN 3 Azide 1, 0.1 mM
Albumin 50 mg/mL
1/[Warf-N3] (M-1)
y = 1.6643x + 10417
R² = 0.9716
Time (min)

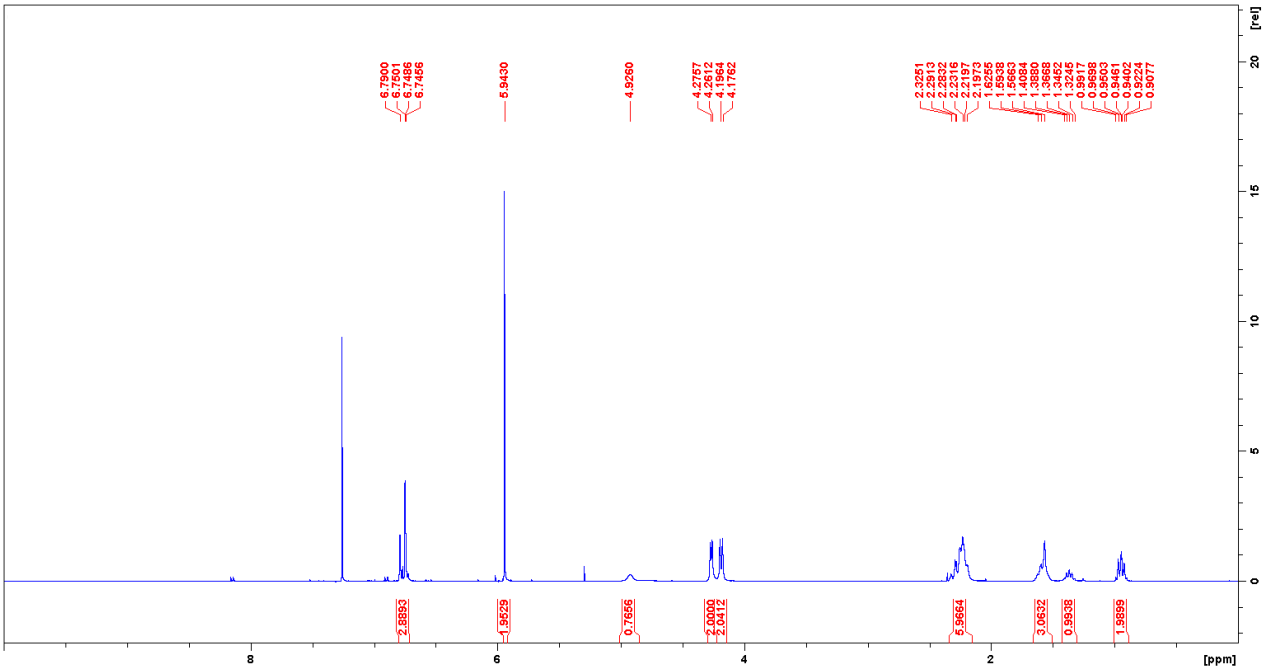
Figure S33 (above). Kinetic profiles (left) and second-order kinetic rates plots (right) for the reaction between BCN **3** and azide **1** in PBS with increasing concentrations in human serum albumin. These measurements were done in monoplicate (n = 1) or duplicate (n = 2)**.**

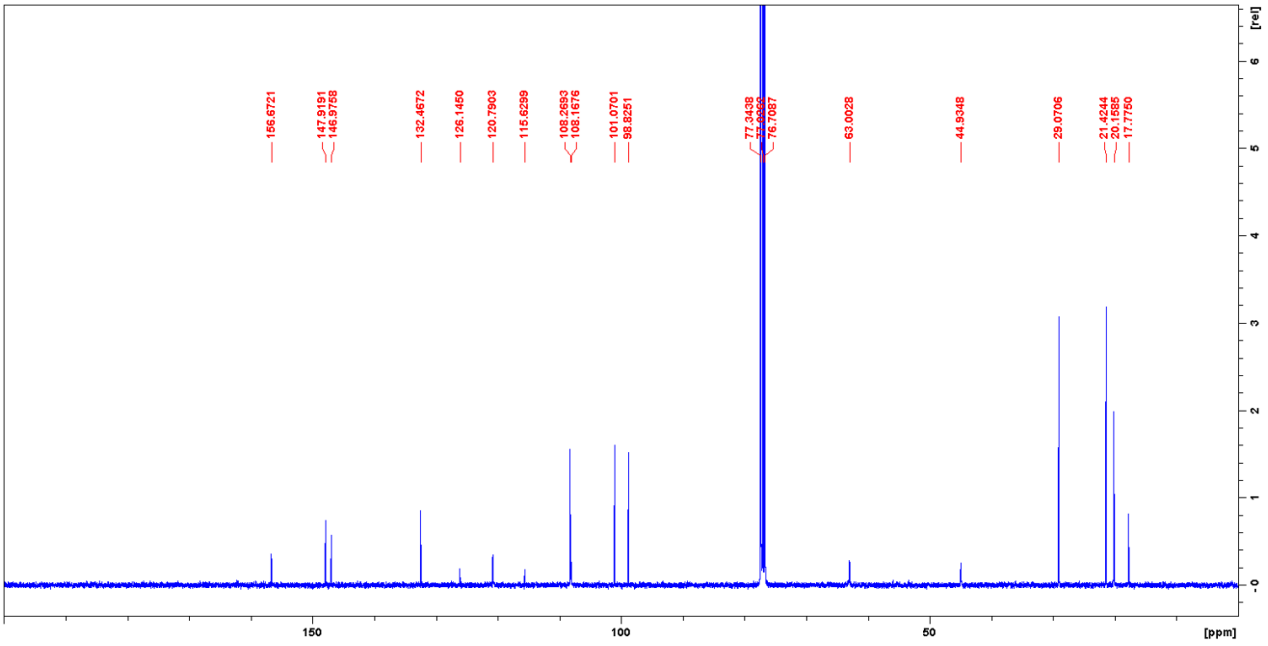
Table S6. Second-order rate constants of SPAAC between BCN **3** and azide **1** in human plasma or HSA solution in PBS at different concentrations.

	Plasma	**HSA solution in PBS (concentration in mg.mL^{-1})**						
		0	0.5	1.0	2.5	5.0	10.0	50.0
Second-order rate constants (M^{-1}.s^{-1})	1.54	1.19	1.27	2.21	3.33	2.24	2.33	1.66

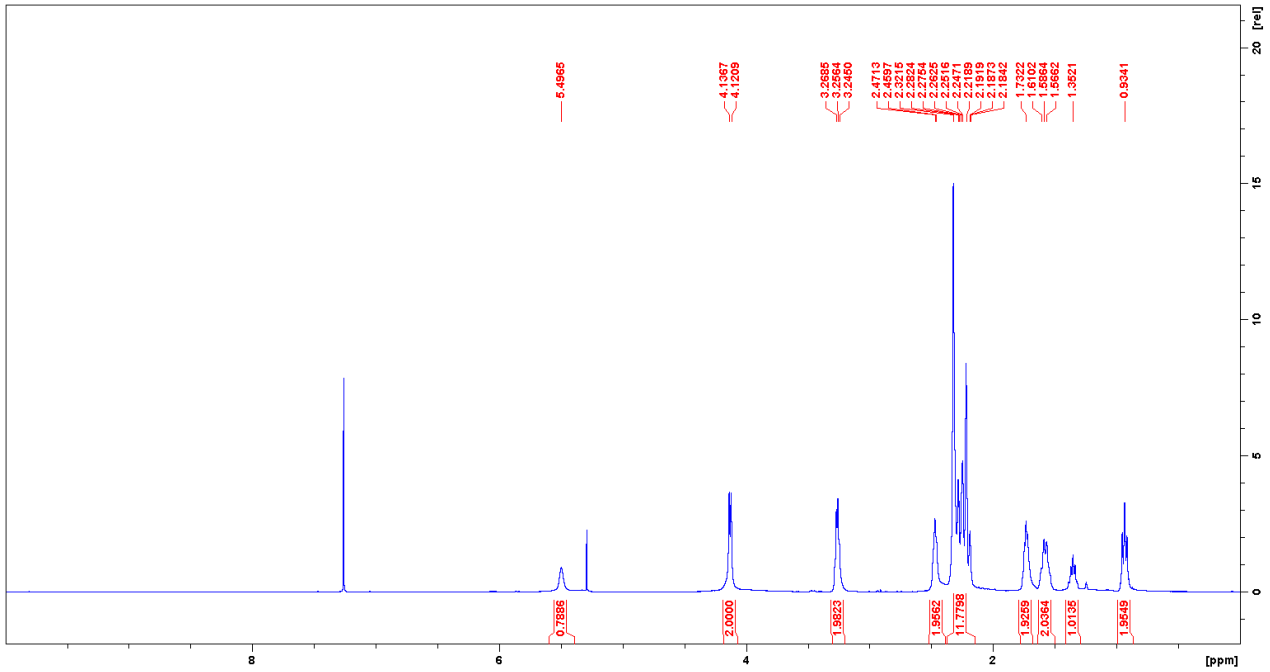
NMR Spectra

BCN **7**





BCN **8**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4.1209
3.2685
3.2564
3.2460
2.4713
2.4597
2.3215
2.2824
2.2754
2.2625
2.2516
2.2471
2.2189
2.1919
2.1773
2.1642
1.7322
1.6102
1.5864
1.5662
1.3521
0.9341
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2.0000
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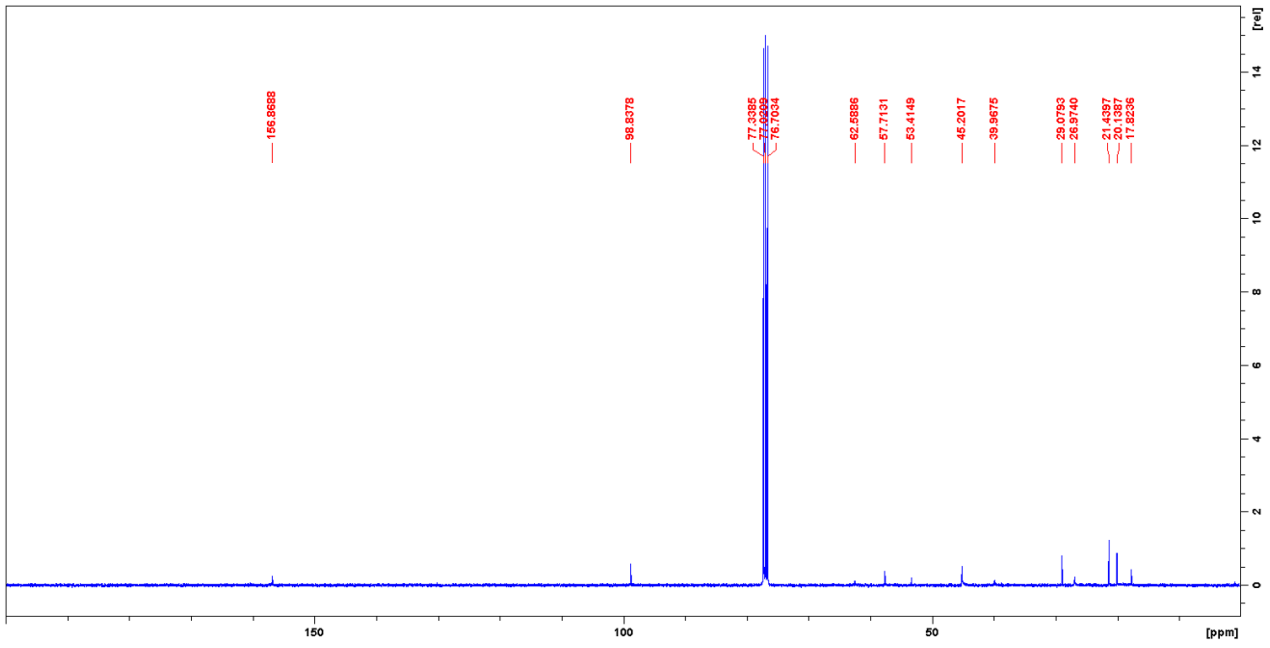
11.7798
1.9259
2.0364
1.0135
1.9549
[rel]
[ppm]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98.8378
77.3385
77.0209
76.7034
62.6886
57.7131
53.4149
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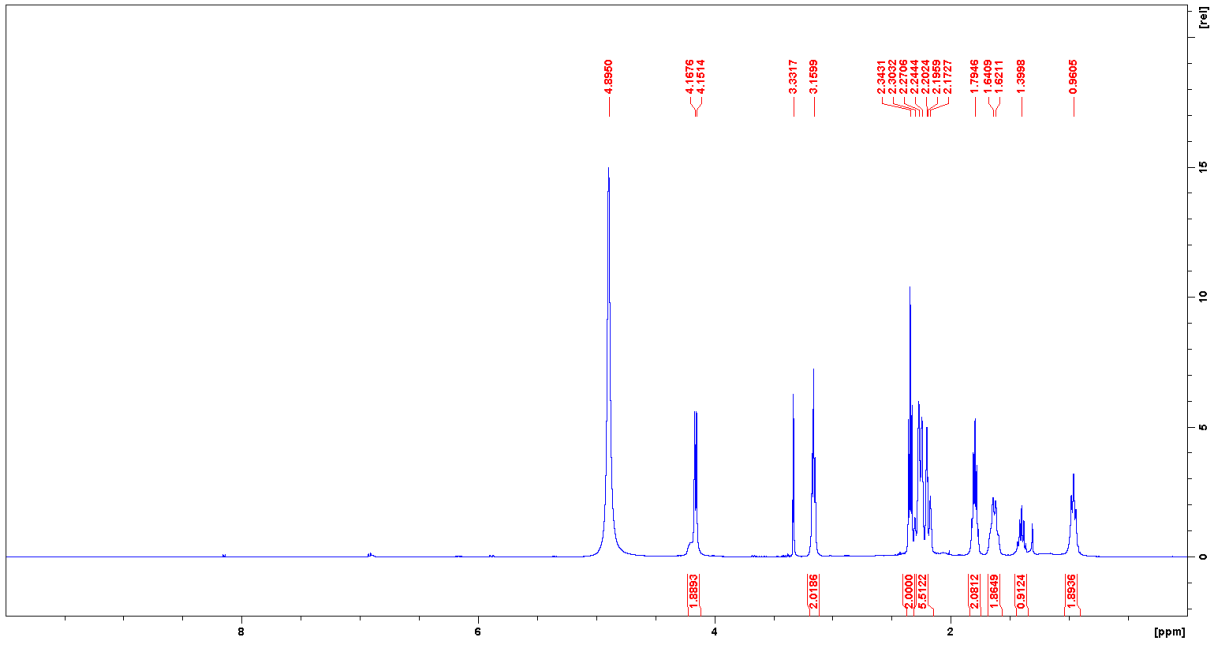
29.0793
25.9740
21.4397
20.1387
17.2236
[rel]
[ppm]

BCN **9**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2.1727
1.7946
1.6409
1.6211
1.3998
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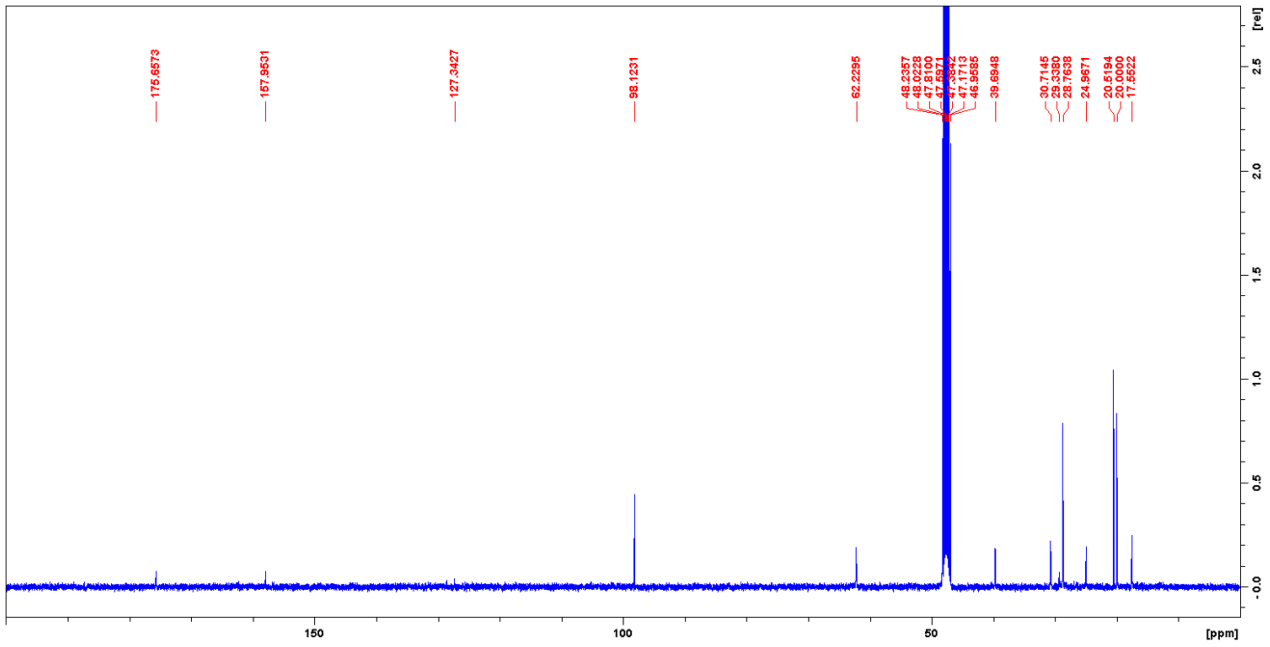
[ppm]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157.9531
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98.1231
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48.2357
48.0228
47.8100
47.6971
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47.1713
46.9585
39.6948
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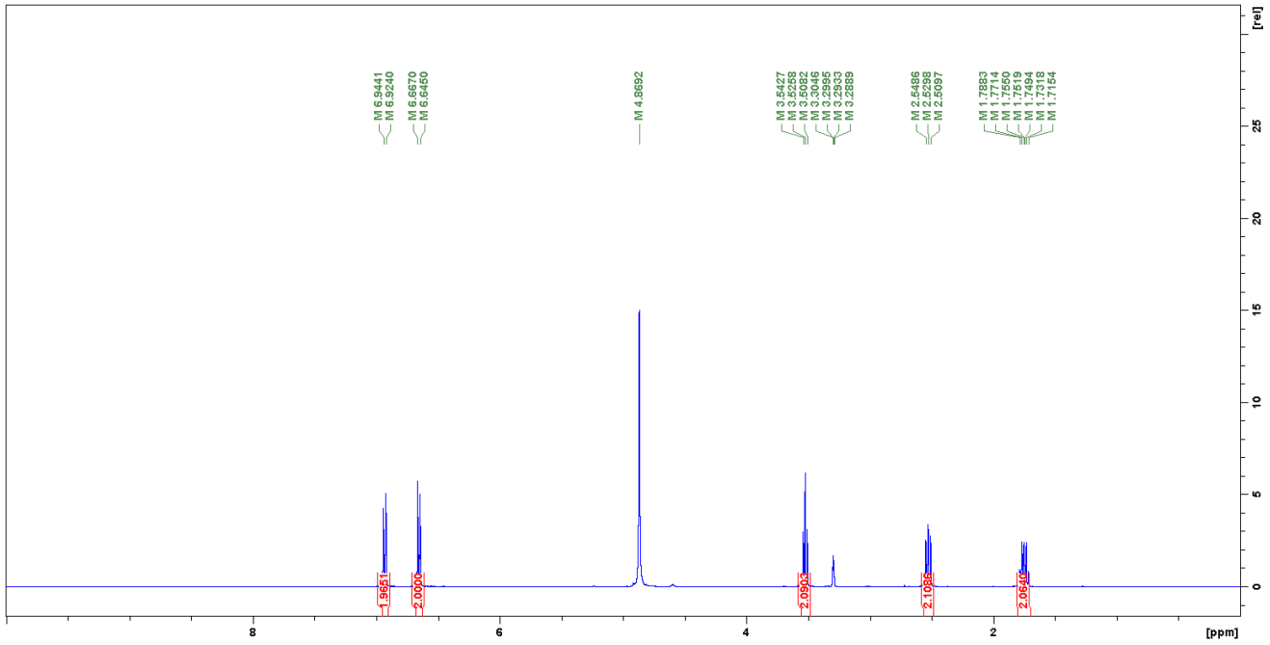
29.3380
28.7638
24.9671
20.5194
20.0000
17.8522
[rel]
[ppm]

Azide **2**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M 6.9240
M 6.6670
M 6.6460
M 4.8692
M 3.5427
M 3.5258
M 3.5082
M 3.3046
M 3.2995
M 3.2883
M 3.2689
M 2.5486
M 2.5298
M 2.5097
M 1.7883
M 1.7714
M 1.7559
M 1.7539
M 1.7494
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2.0000
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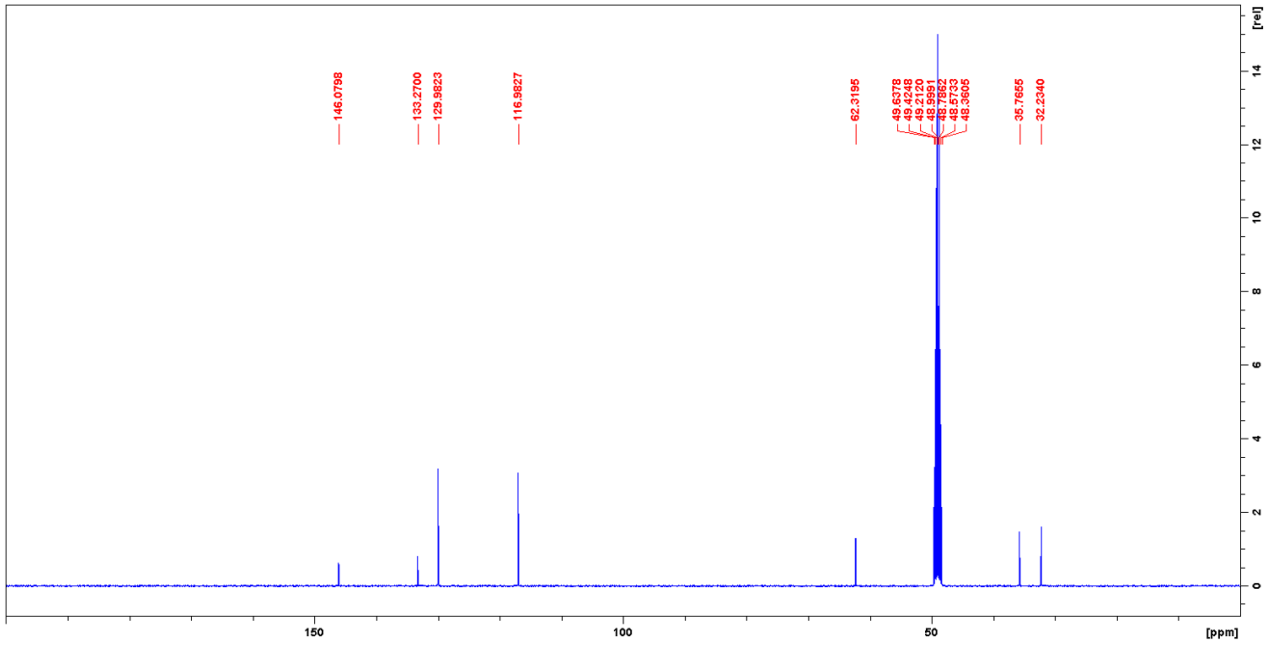
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129.9823
116.9827
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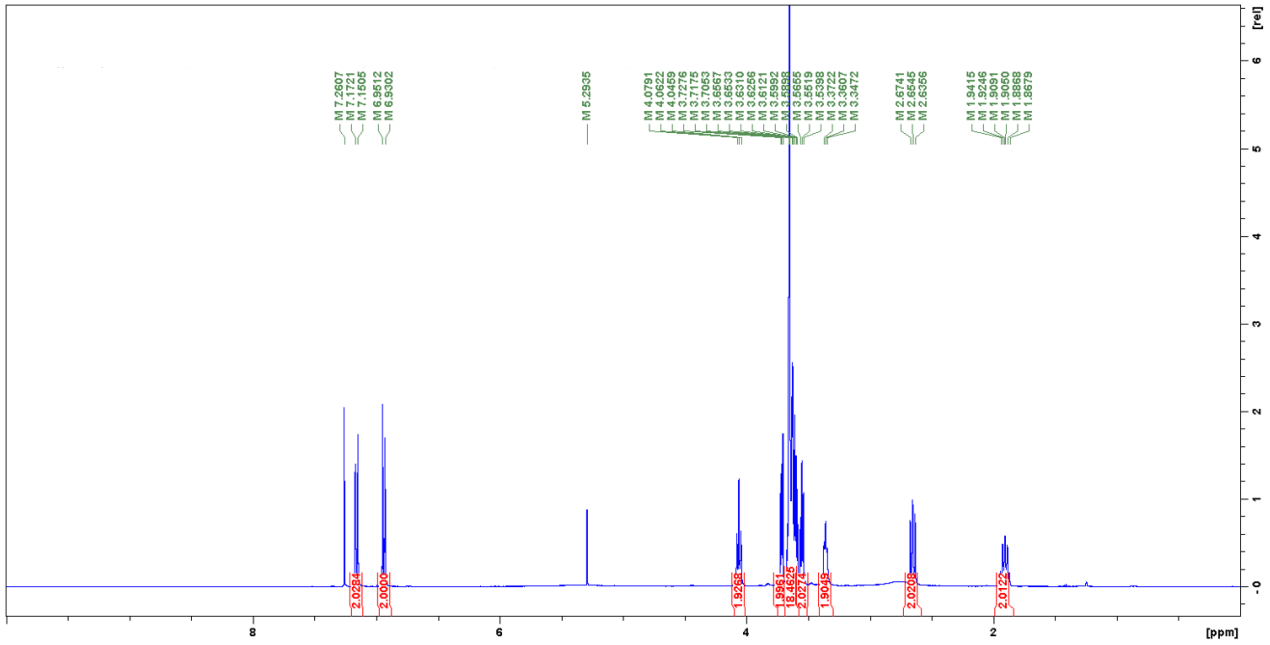
32.2340
[rel]
[ppm]

Azide **10**


M 7.2607
M 7.1721
M 7.1505
M 6.9512
M 6.9302
M 5.2935
M 4.0791
M 4.0622
M 4.0459
M 3.7276
M 3.7175
M 3.7053
M 3.6667
M 3.6533
M 3.6210
M 3.6026
M 3.6121
M 3.5992
M 3.5888
M 3.5665
M 3.5519
M 3.5398
M 3.3722
M 3.3607
M 3.3472
M 2.6741
M 2.6545
M 2.6386
M 1.9415
M 1.9246
M 1.9091
M 1.8960
M 1.8868
M 1.8679
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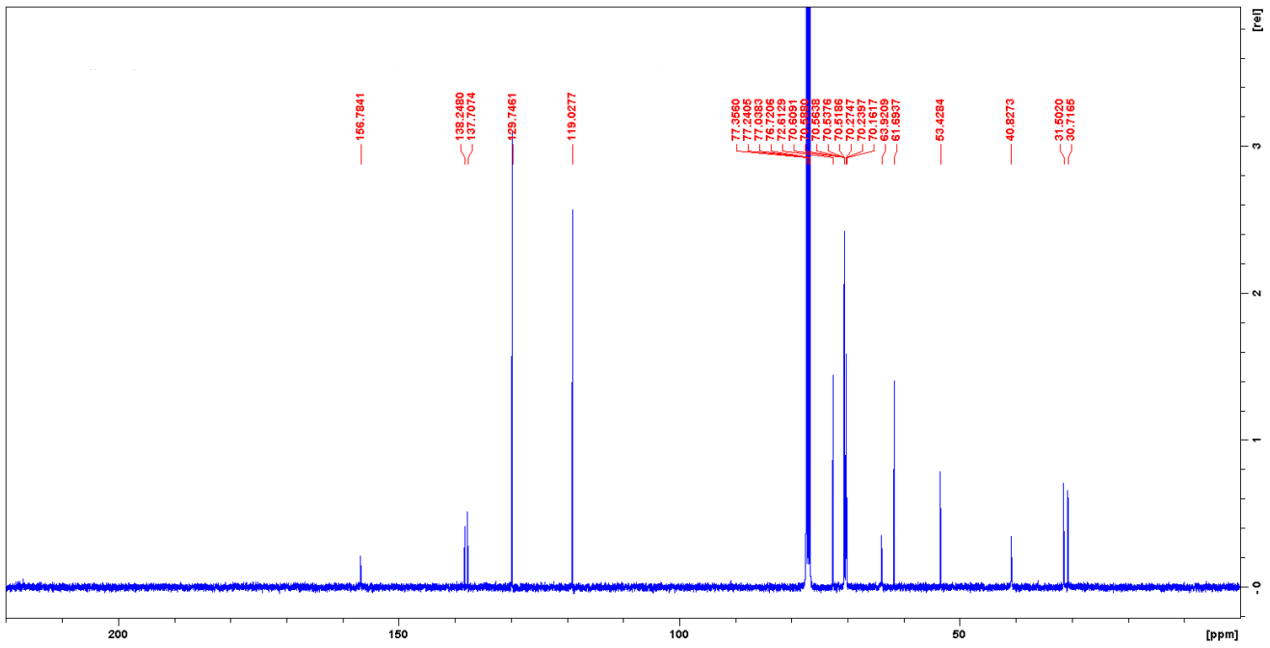
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76.6091
70.6880
70.5638
70.5376
70.5186
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70.2397
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61.6937
53.4284
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31.5020
30.7165
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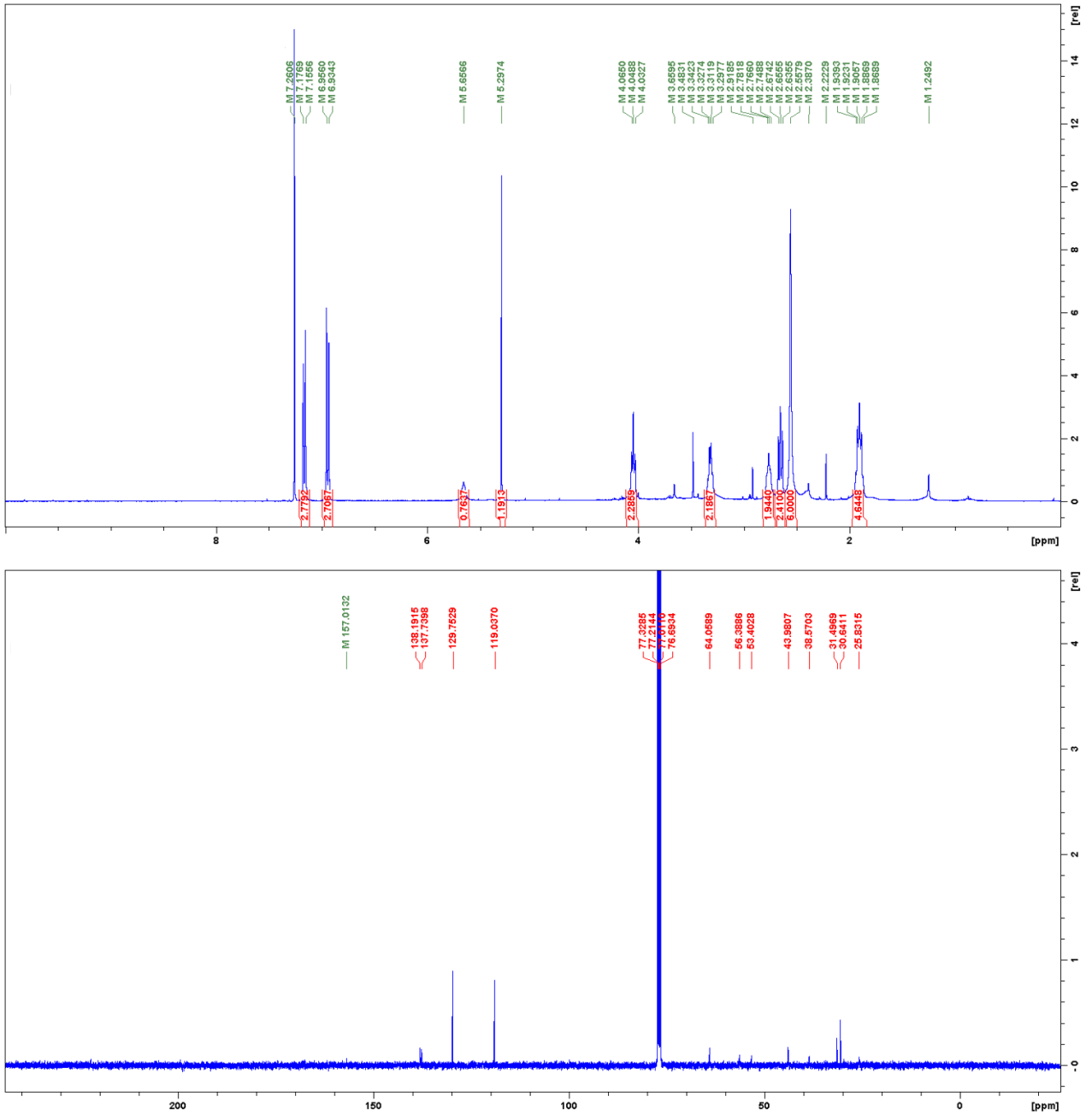
Azide **11**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M 7.1556
M 6.9560
M 6.9348
M 5.6566
M 5.2974
M 4.0660
M 4.0483
M 4.0327
M 3.6595
M 3.4831
M 3.3403
M 3.3274
M 3.3119
M 3.2977
M 2.9185
M 2.7818
M 2.7680
M 2.7488
M 2.6742
M 2.6555
M 2.6395
M 2.6279
M 2.3870
M 2.2229
M 1.9393
M 1.9231
M 1.9067
M 1.8869
M 1.8689
M 1.2492
2.7792
2.7067
0.7637
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2.2856
2.1867
1.9440
2.4100
6.0000
4.6448
[rel]
[ppm]
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137.7398
129.7629
119.0370
77.3285
77.2144
77.0110
76.6934
64.0589
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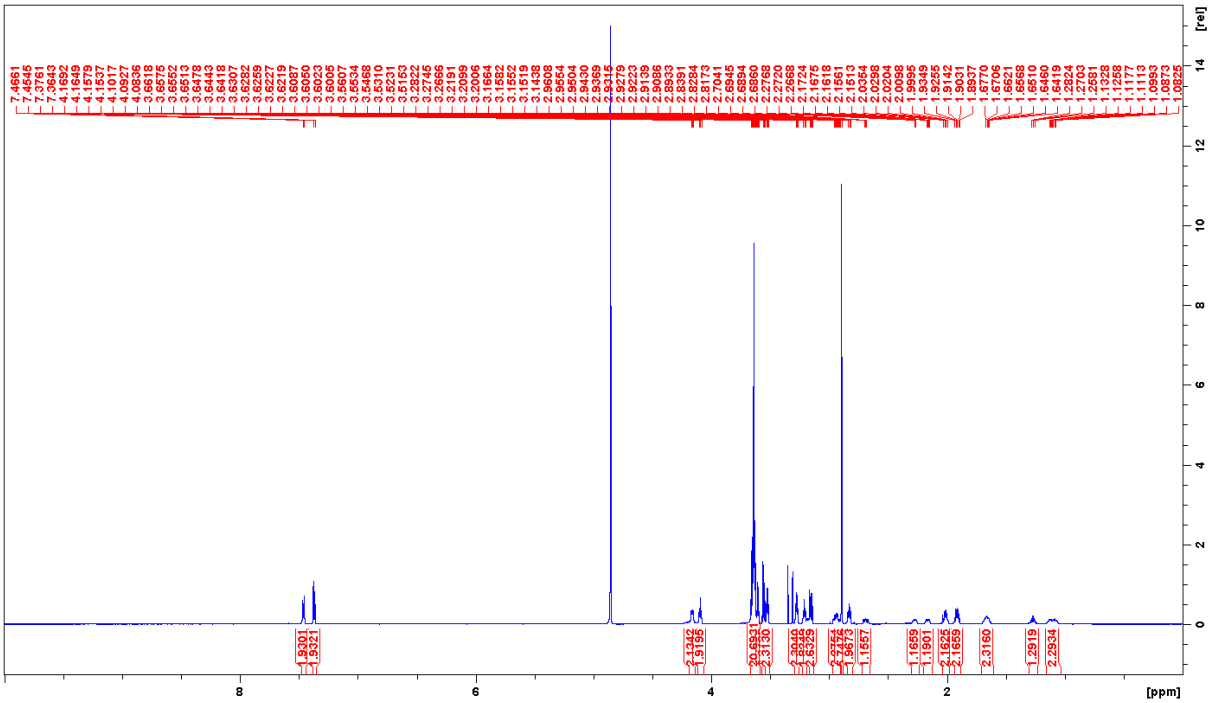
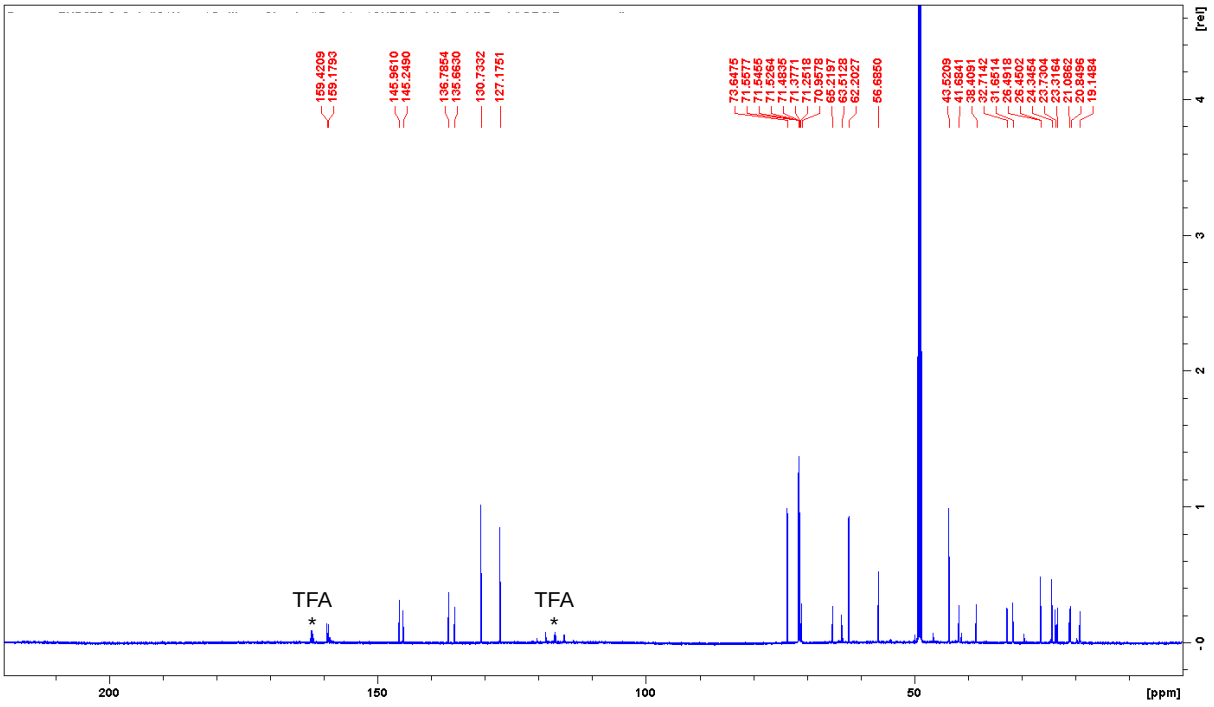
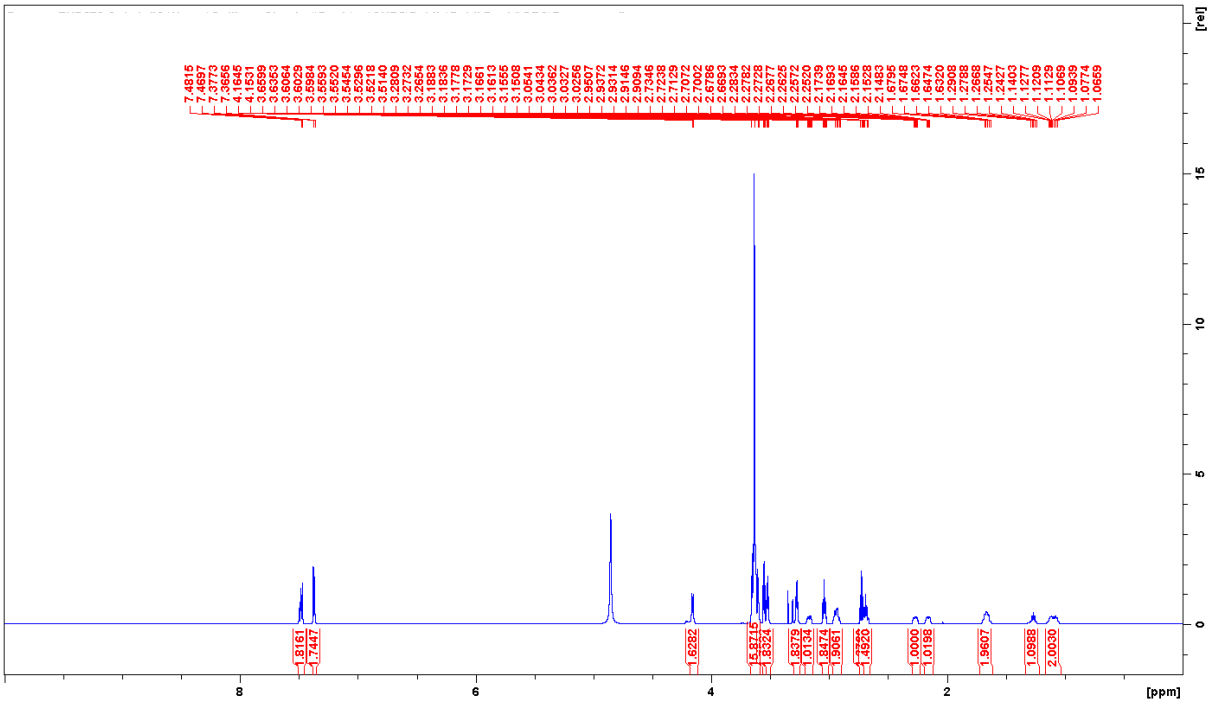
[rel]
[ppm]

Triazole **13**





Triazole **14**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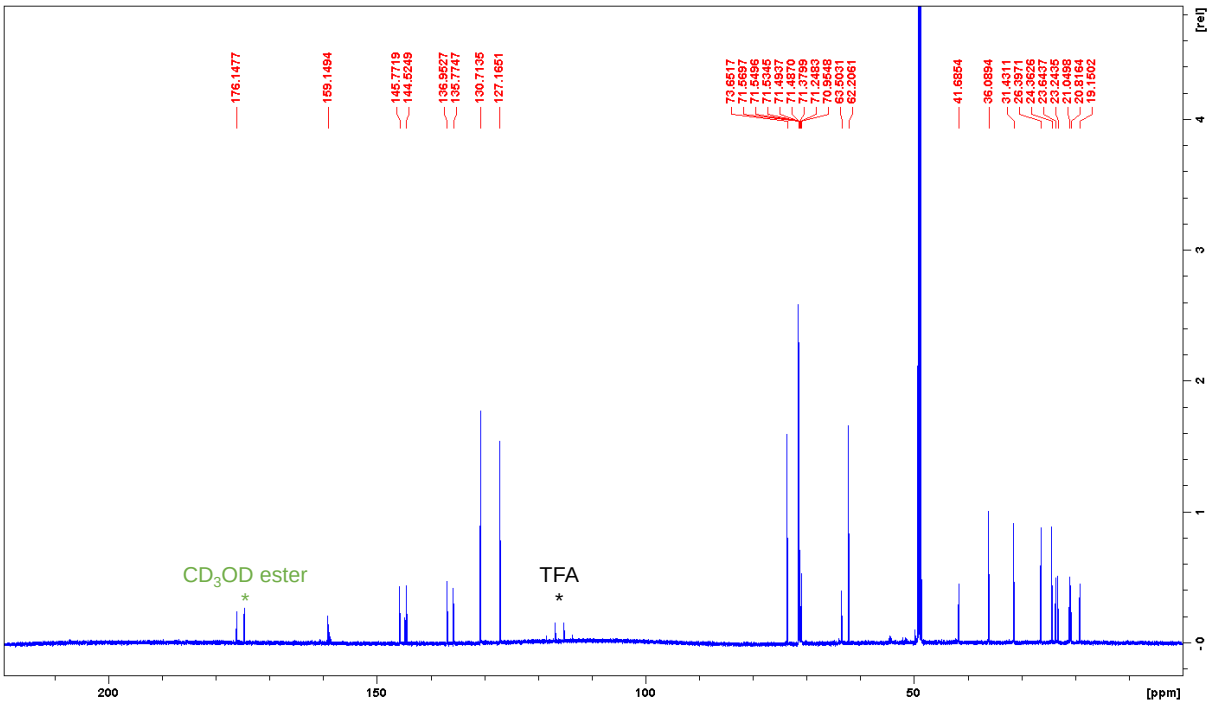
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136.7747
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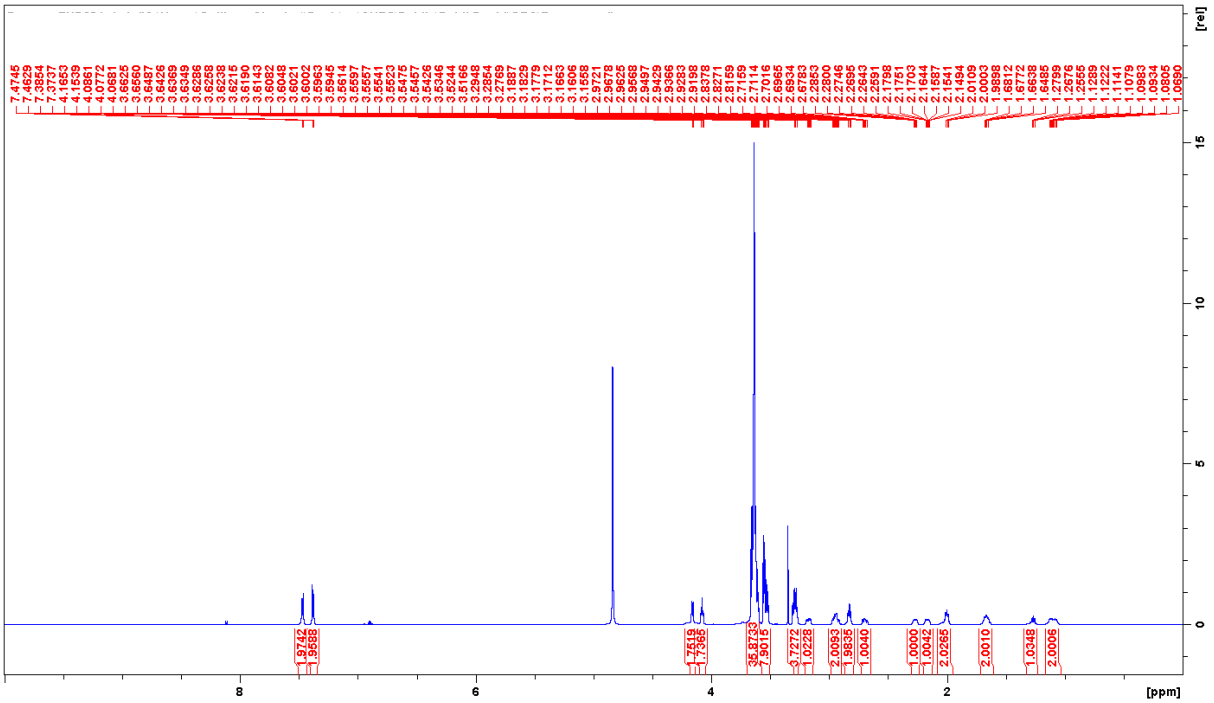
73.6517
71.5697
71.5496
71.5345
71.4937
71.4870
71.3799
71.2483
70.9548
63.5031
62.2061
41.6854
36.0894
31.4311
28.3971
28.3826
23.6437
23.2435
21.0498
20.8164
19.1502
CD3OD ester
*
TFA
*
[rel]
[ppm]

Triazole **S3**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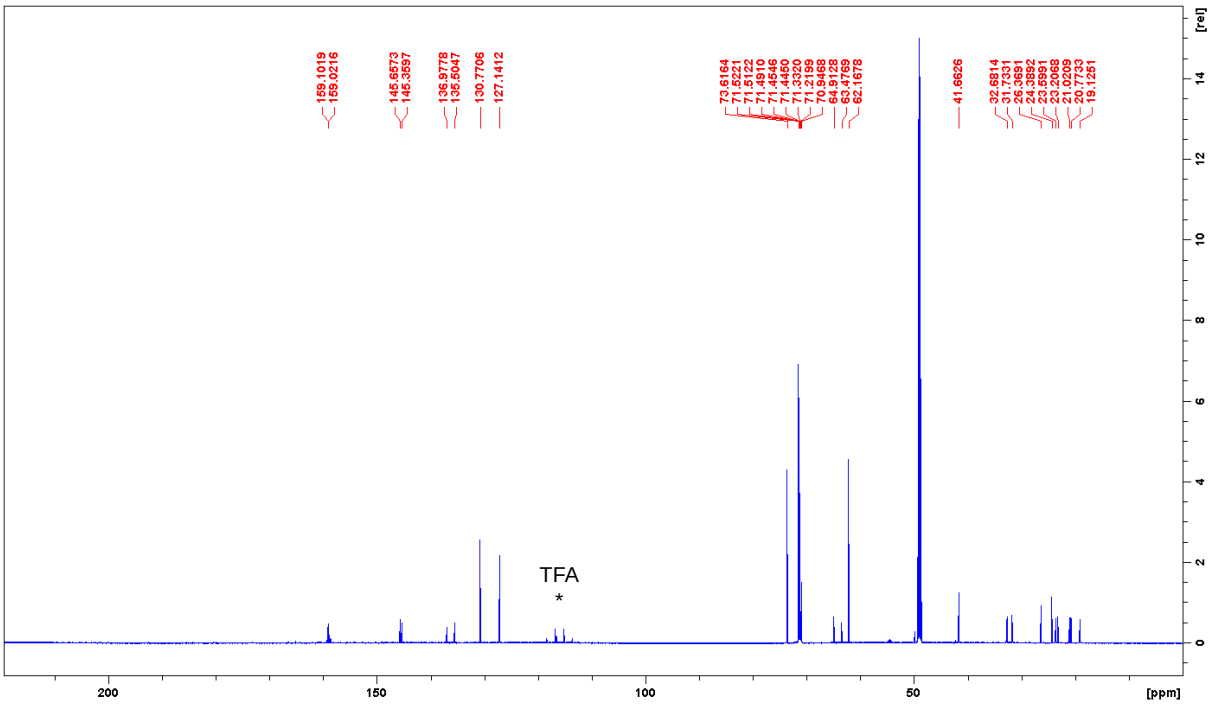
[ppm]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73.6164
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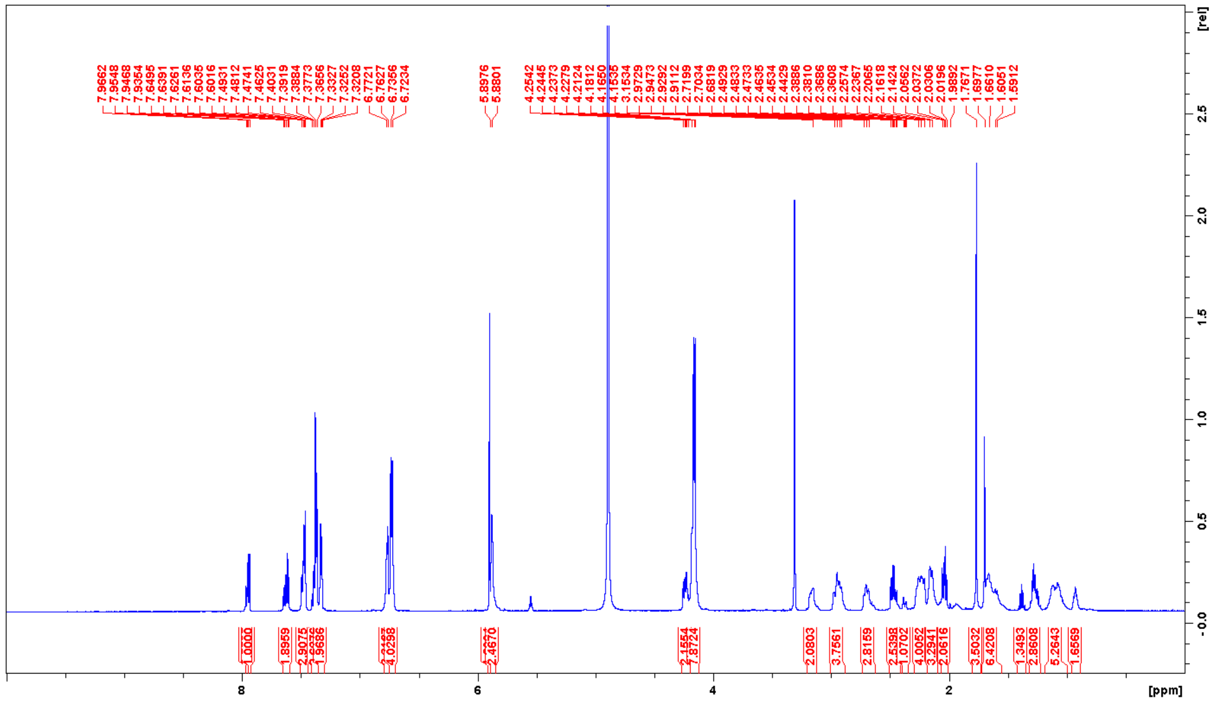
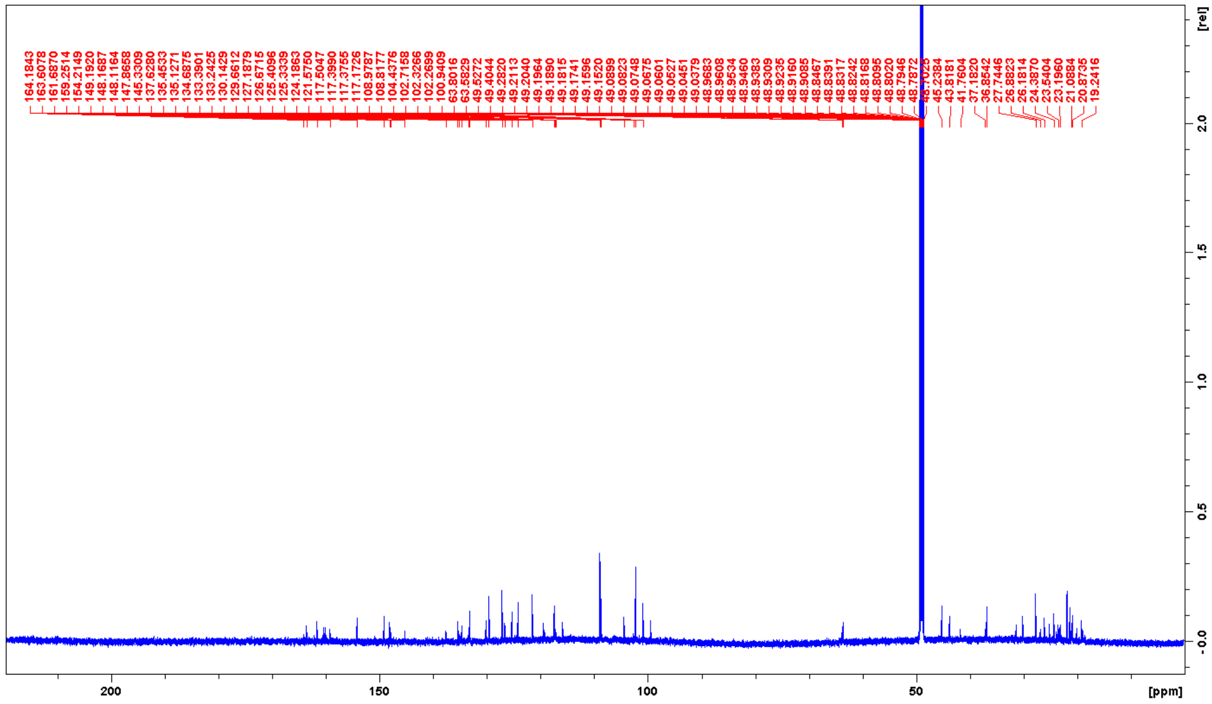
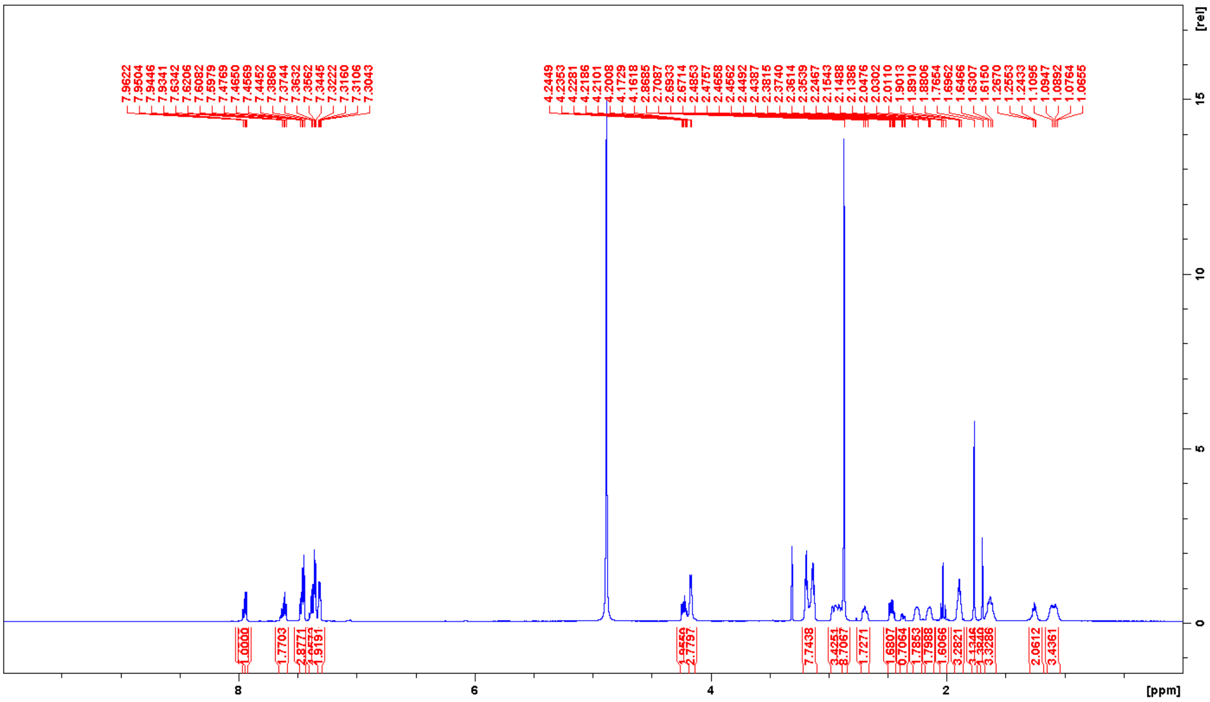
71.4910
71.4546
71.4450
71.3320
71.2199
70.9488
64.9128
63.4769
62.1678
41.6626
32.6814
31.7331
26.3891
25.3892
23.5991
23.2068
21.0209
20.7733
19.1251
TFA
*
[rel]
[ppm]

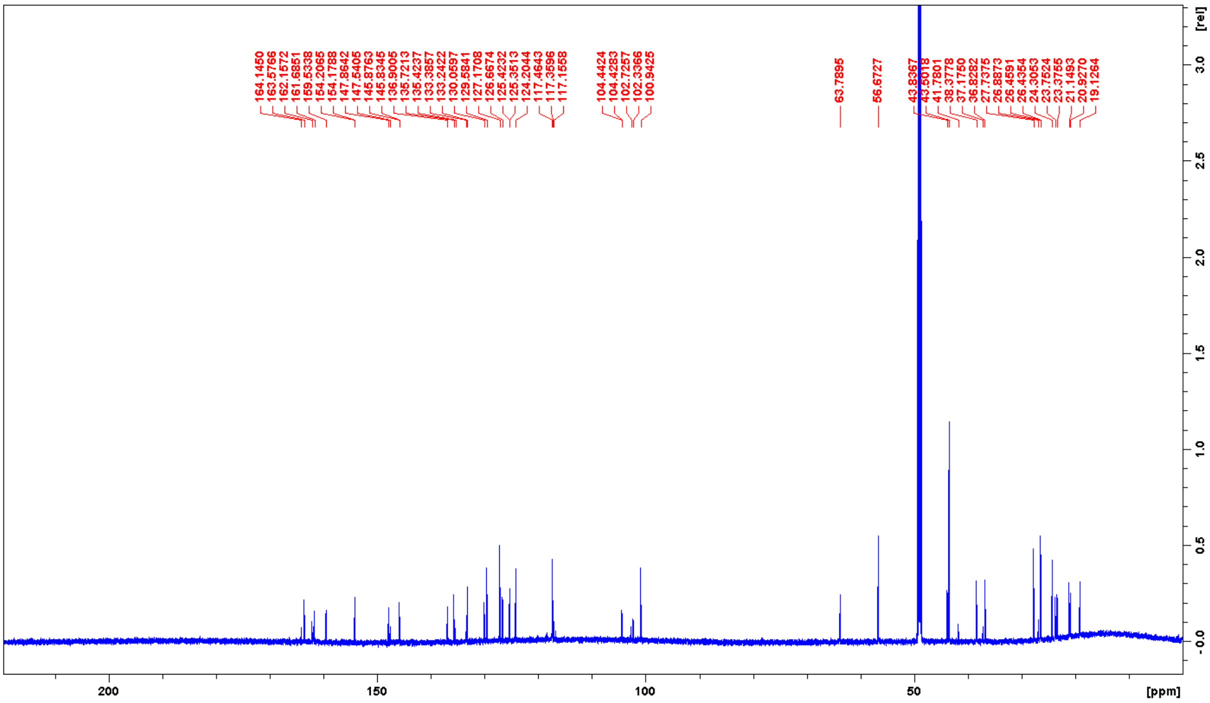
Triazole **S4**



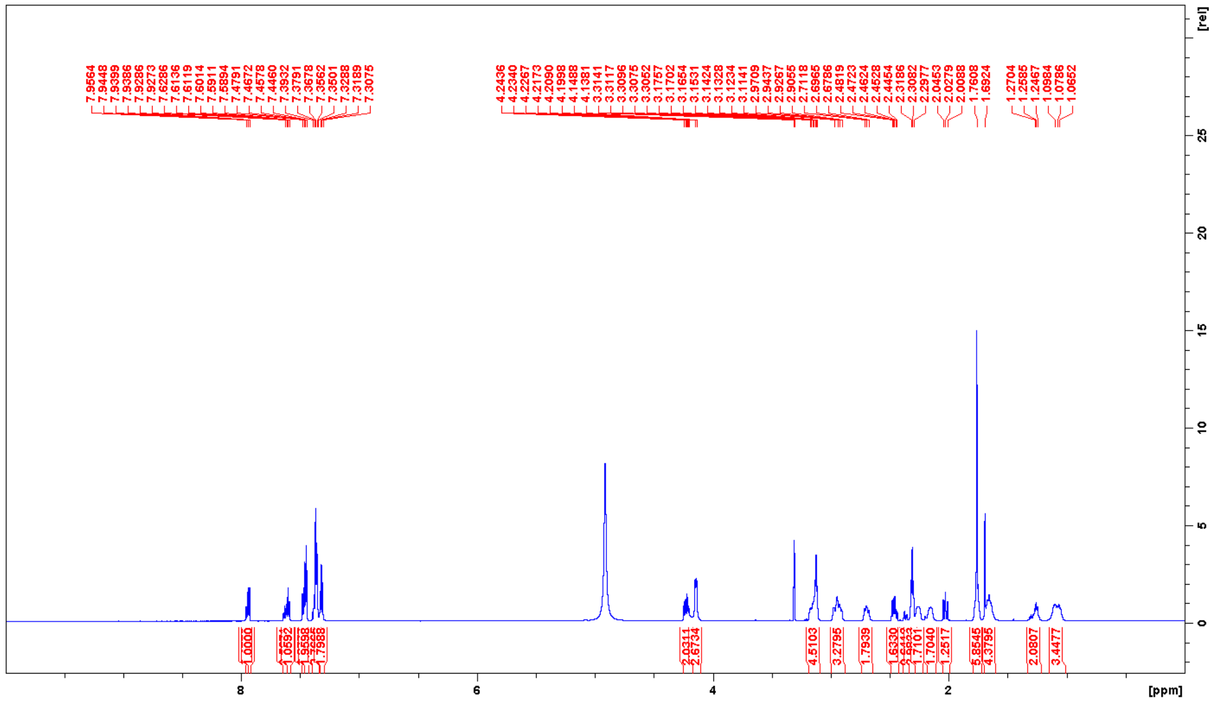


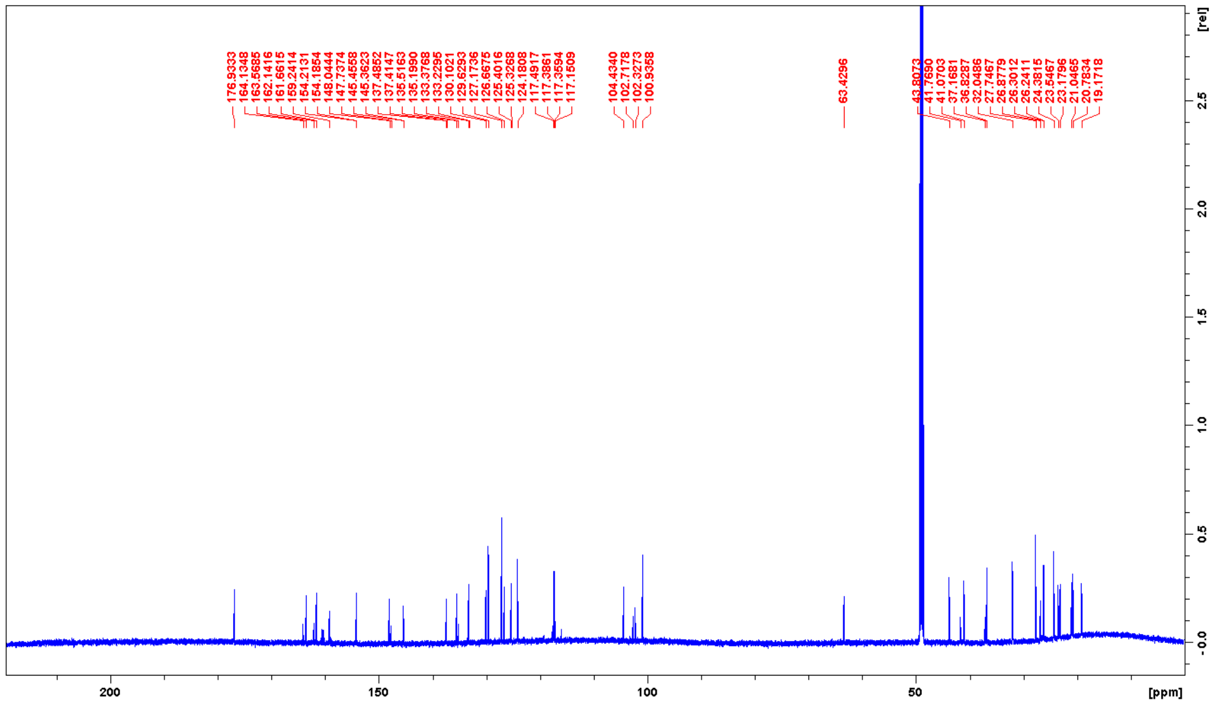
Triazole **S5**



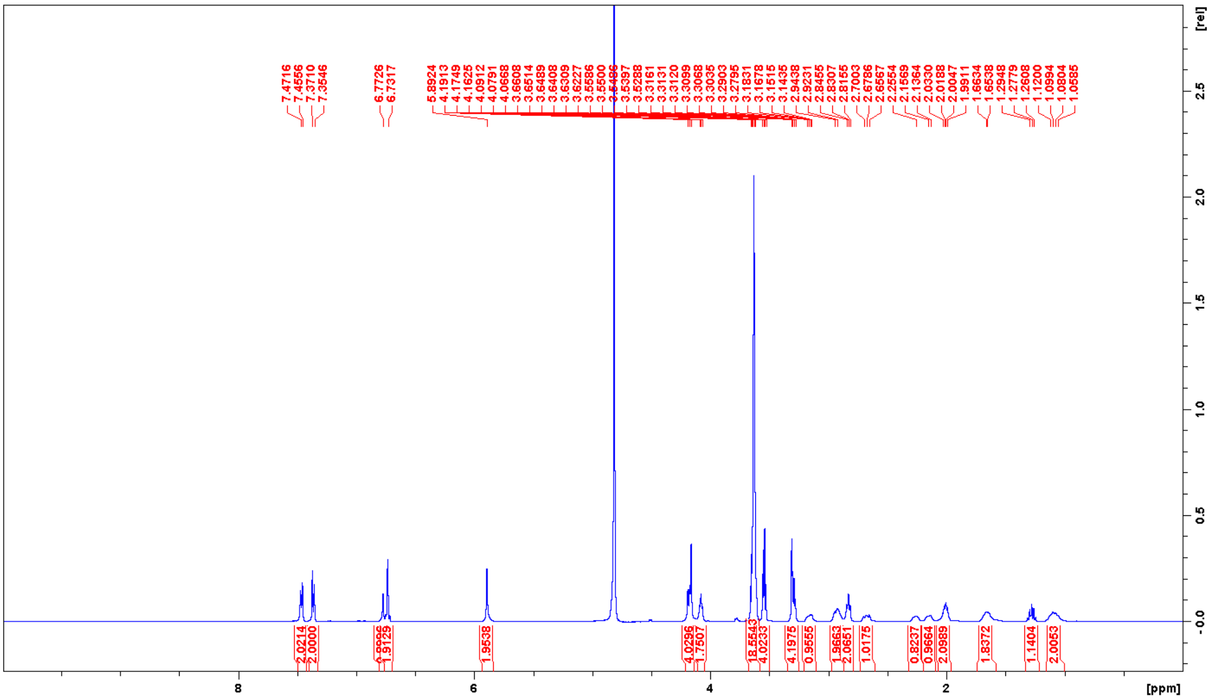


Triazole **S6**





Triazole **S7**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6.7726
6.7317
5.8924
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4.1749
4.1625
4.0912
4.0791
4.0668
3.6608
3.6514
3.6489
3.6408
3.6309
3.6227
3.5586
3.5500
3.5397
3.5288
3.3161
3.3131
3.3120
3.3099
3.3068
3.3035
3.2903
3.2795
3.1831
3.1678
3.1515
3.1435
2.9438
2.9231
2.8455
2.8307
2.8155
2.7003
2.6786
2.6567
2.2554
2.1569
2.1364
2.0330
2.0188
2.0047
1.9911
1.5634
1.5538
1.2948
1.2779
1.2608
1.1200
1.0994
1.0804
1.0585
2.0214
2.0000
1.9129
1.9538
4.0296
1.7507
18.5543
4.0233
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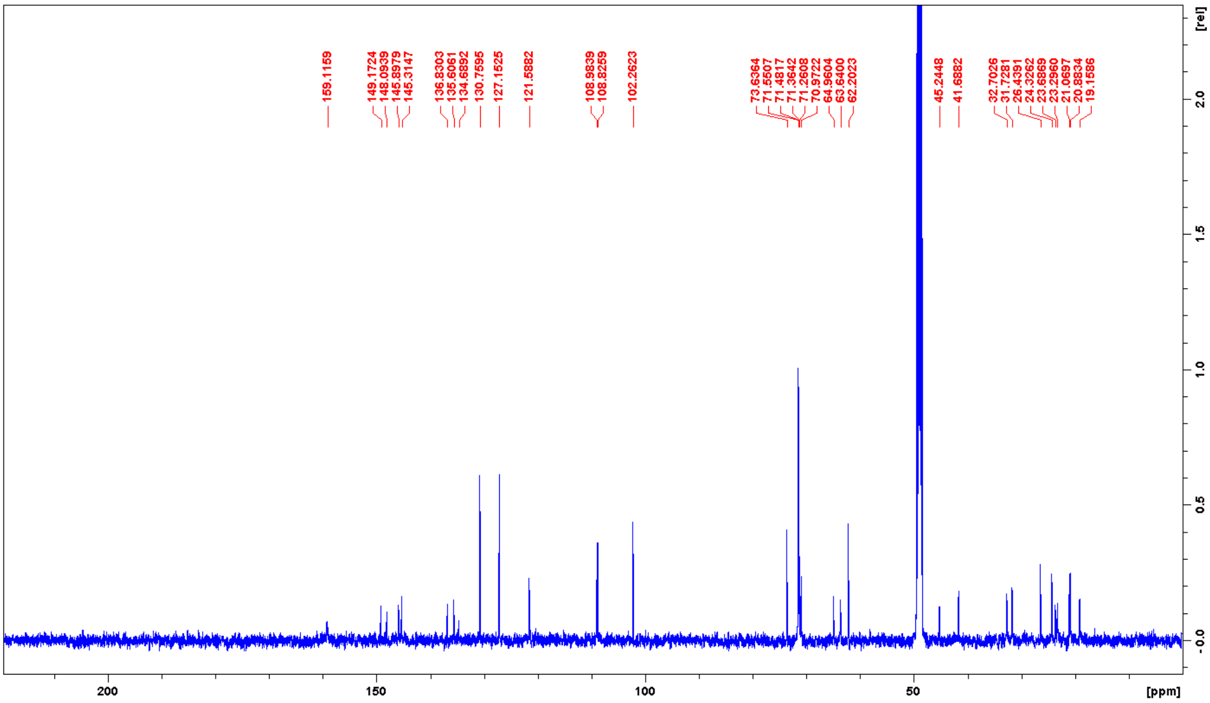
[ppm]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121.5882
108.9839
108.8259
102.2623
73.6364
71.5507
71.4817
71.3642
71.2608
70.9722
64.9604
63.6400
62.2023
45.2448
41.6882
32.7026
31.7281
26.4391
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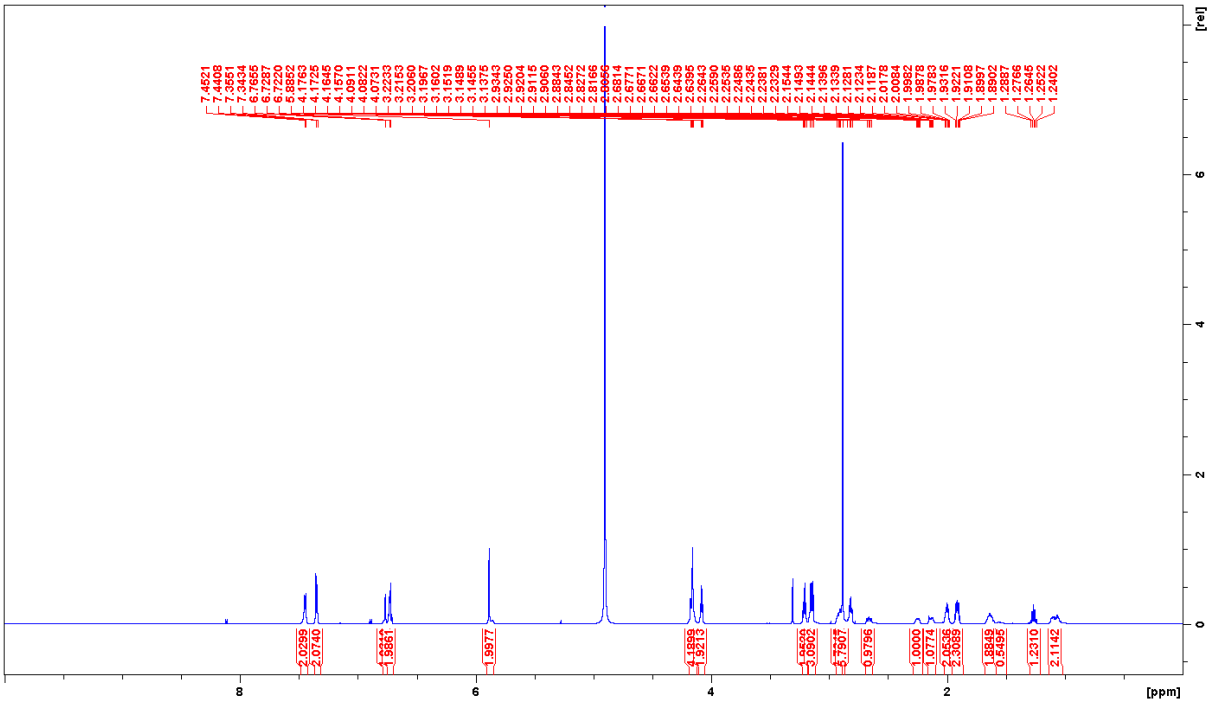
23.6869
23.2960
21.0697
20.8834
19.1586
[rel]
[ppm]

Triazole **S8**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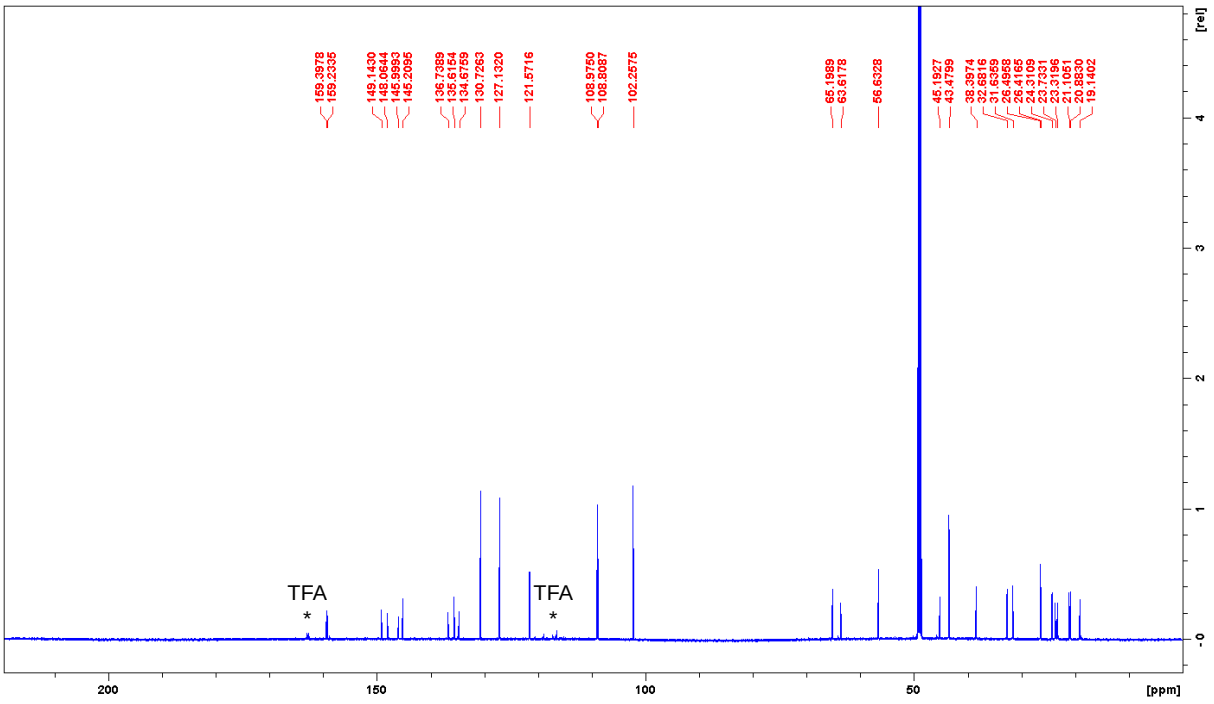
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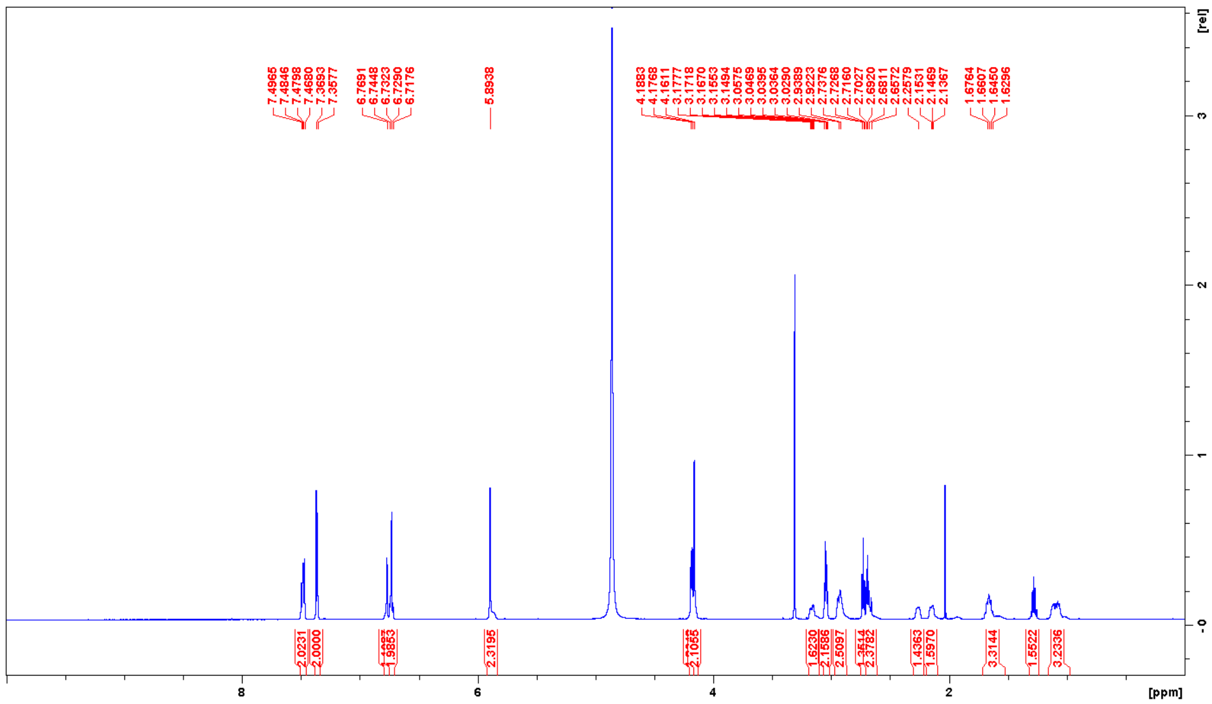
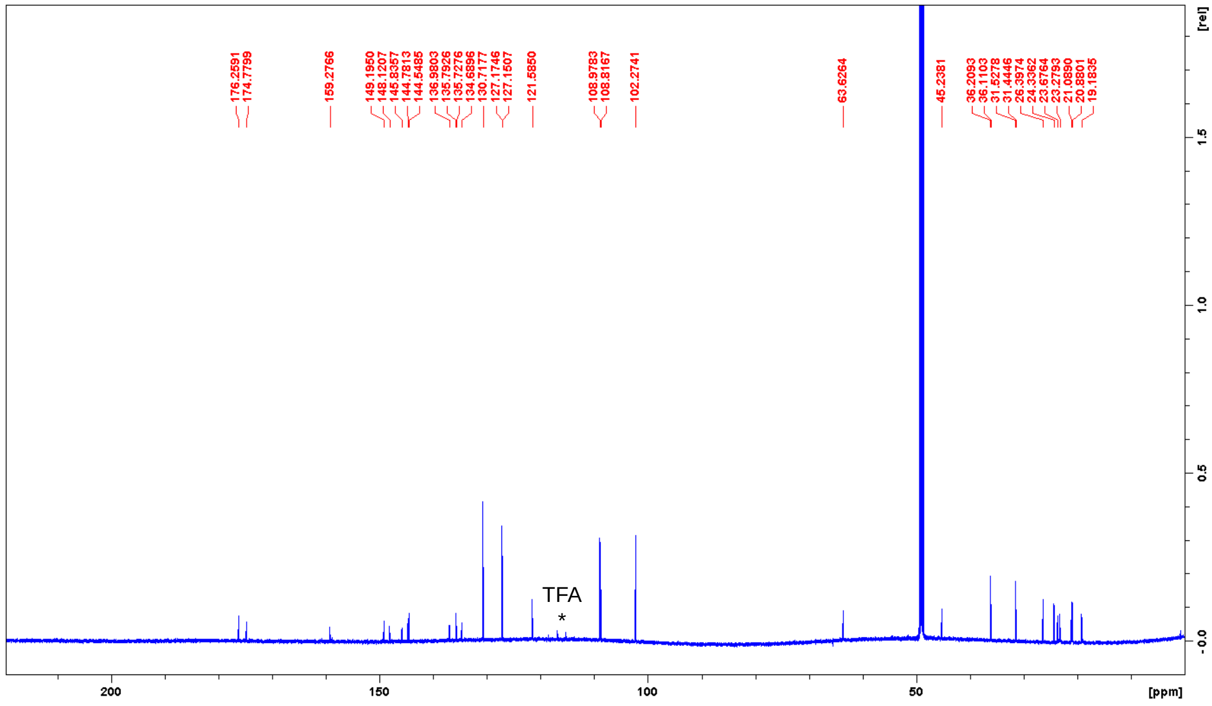
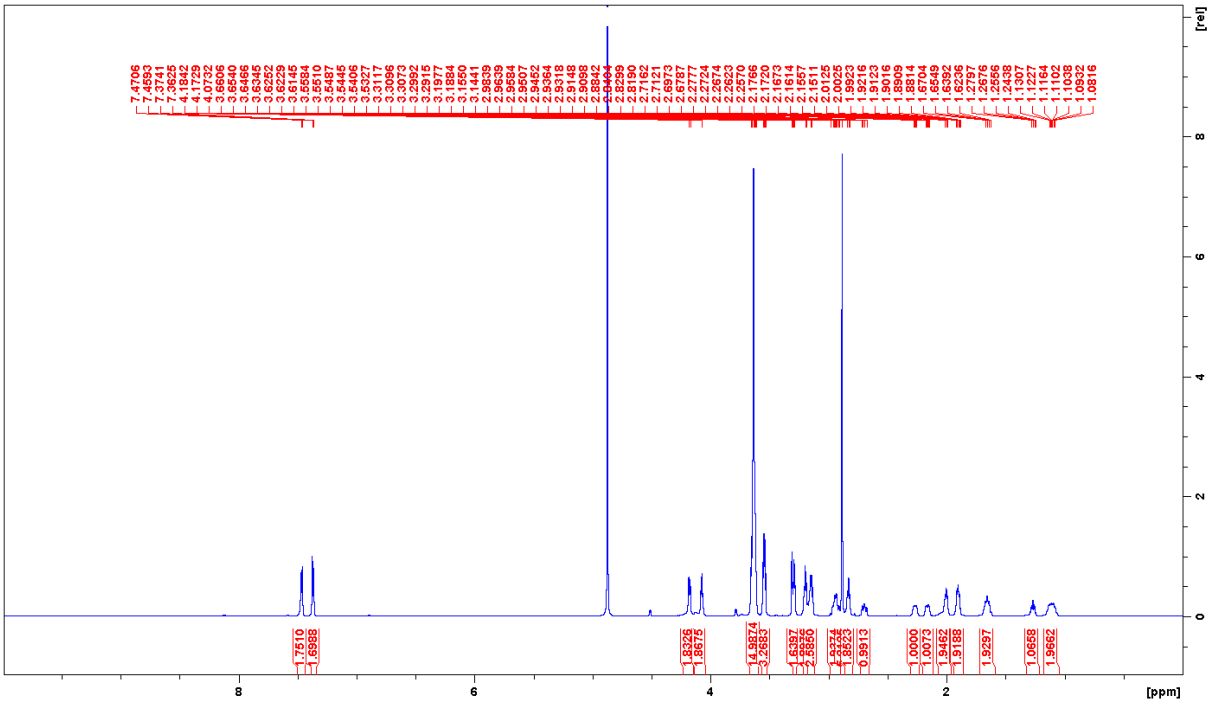
145.9993
145.2095
136.7389
135.6154
134.6759
130.7263
127.1320
121.5716
108.9750
108.8087
102.2575
65.1989
63.6178
56.6328
45.1927
43.4799
38.3974
32.6816
31.6389
26.4988
26.4185
24.7139
23.7331
23.3196
21.1051
20.8830
19.1402
TFA
*
TFA
*
[rel]
[ppm]

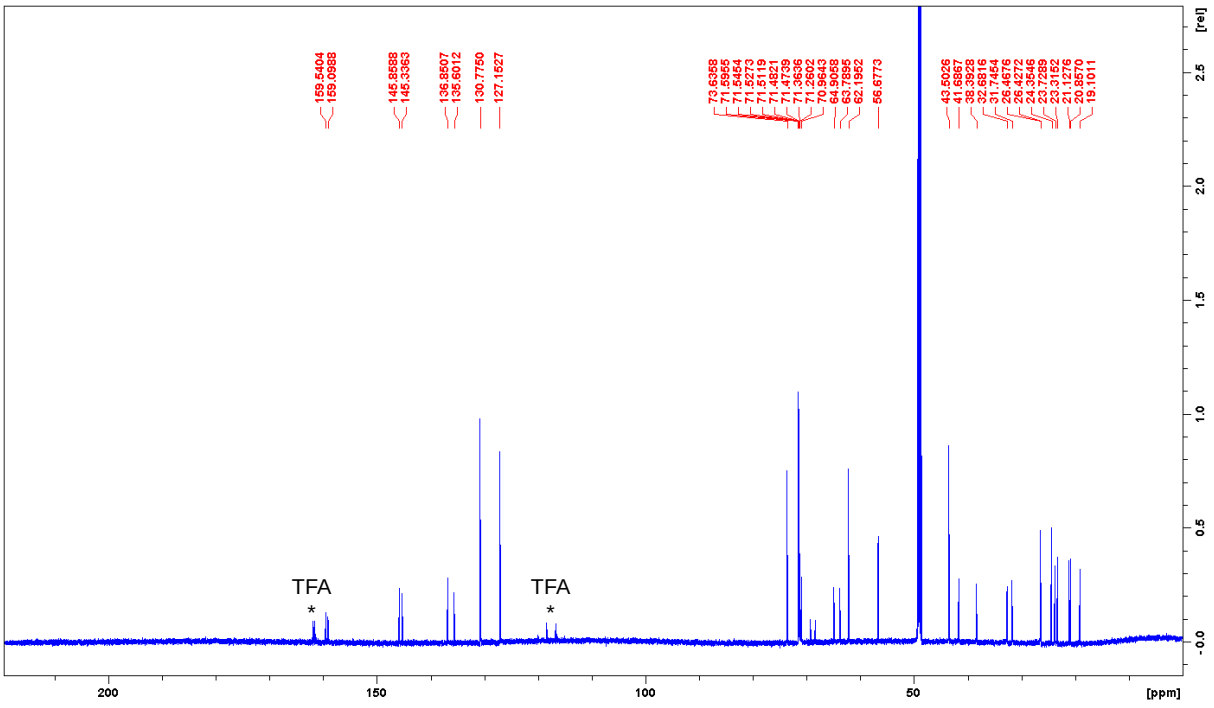
Triazole **S9**



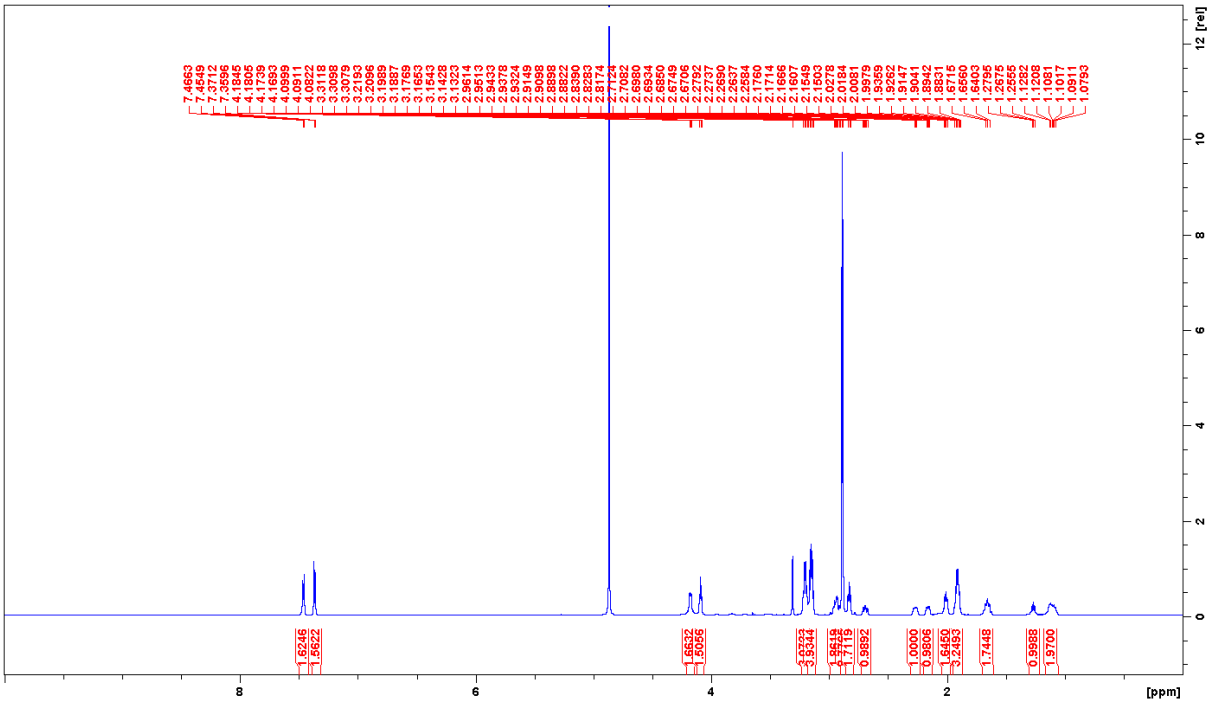


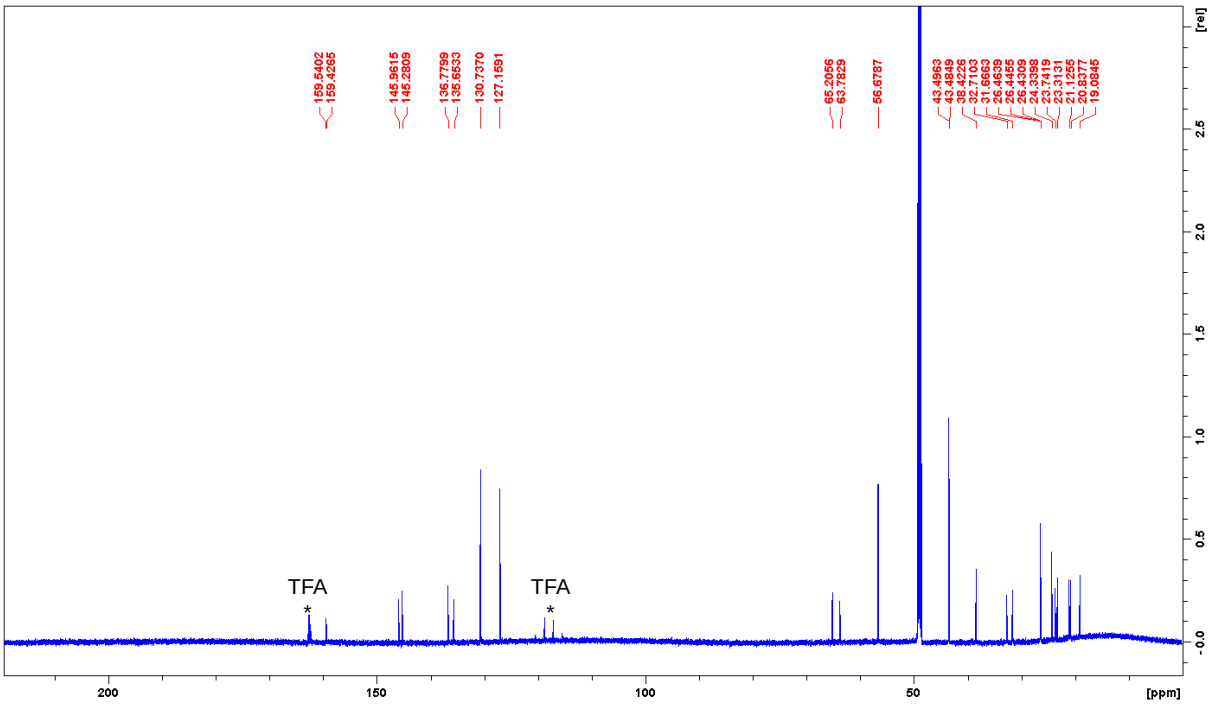
Triazole **S10**



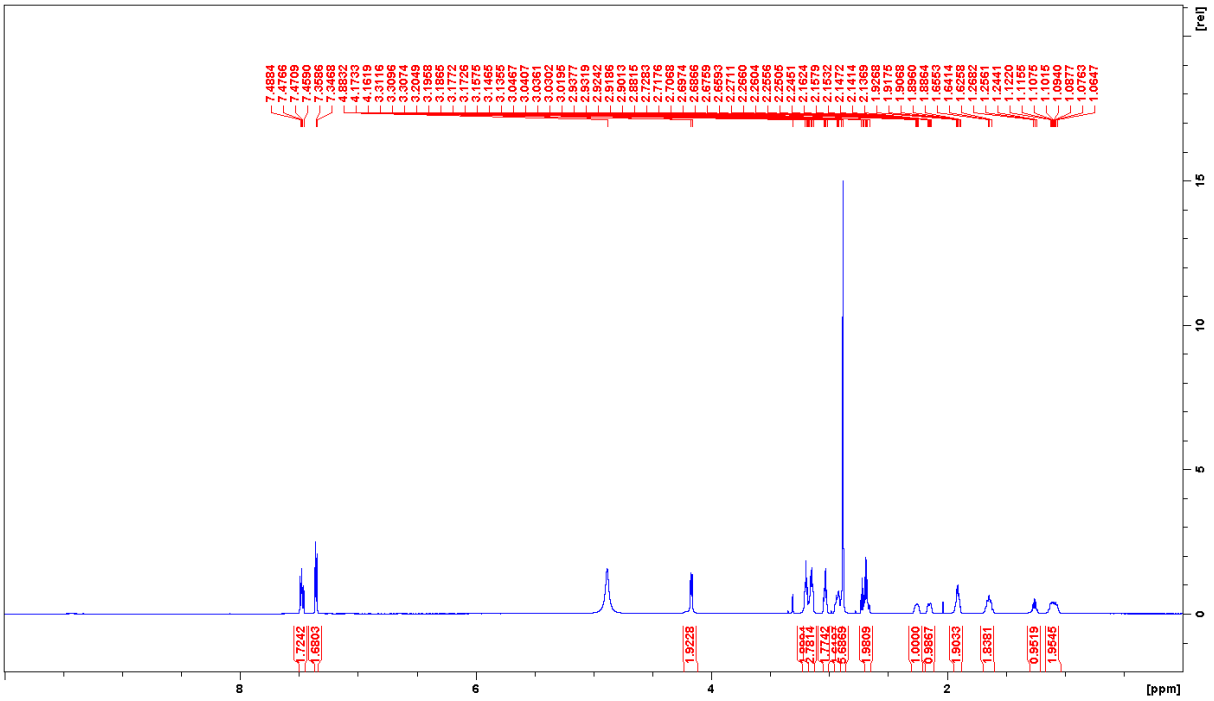


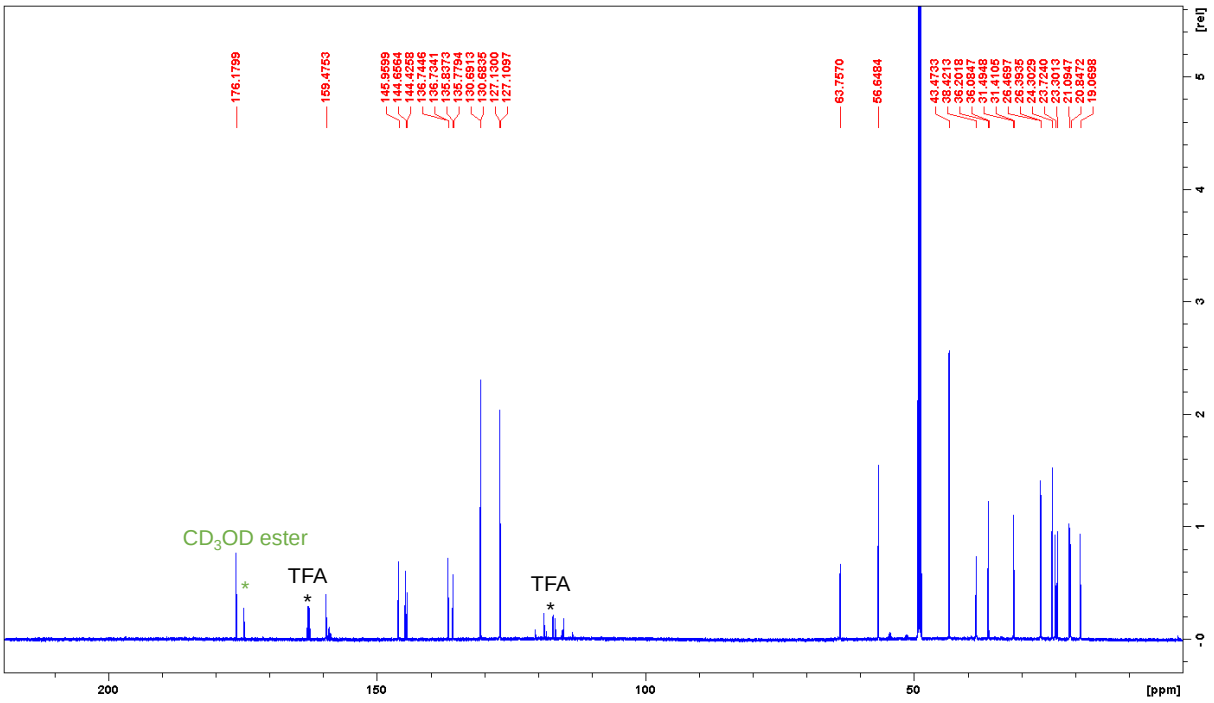
Triazole **S11**



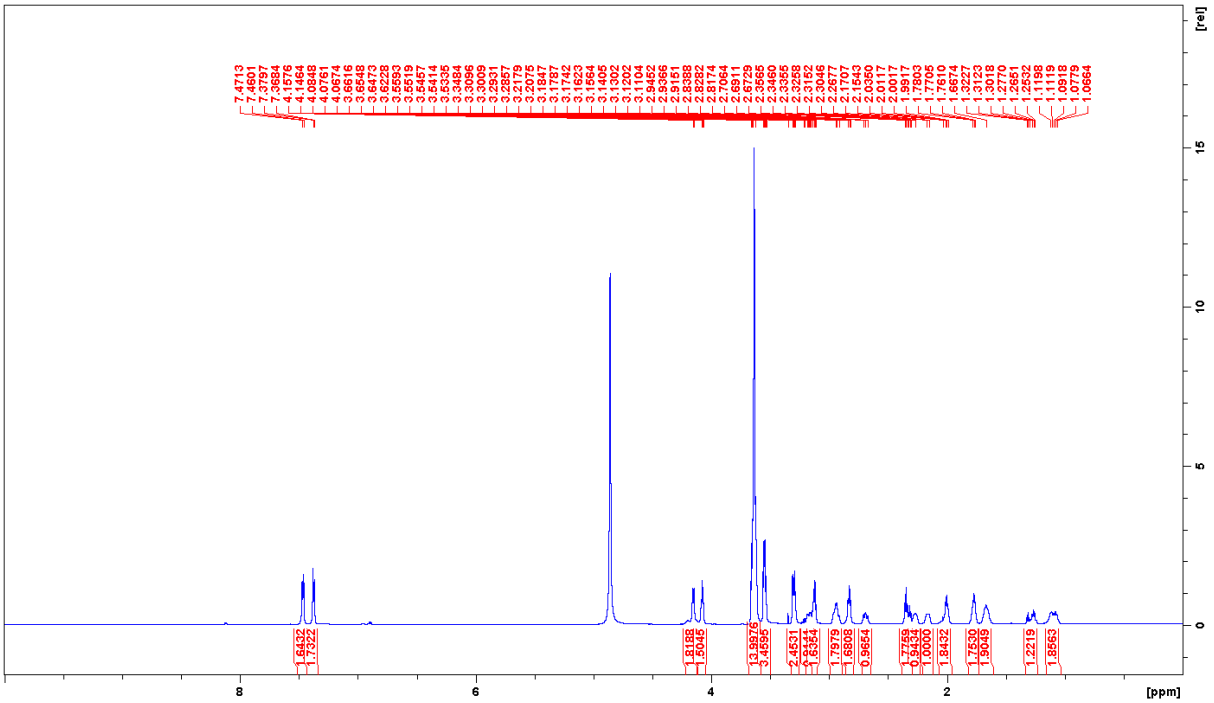


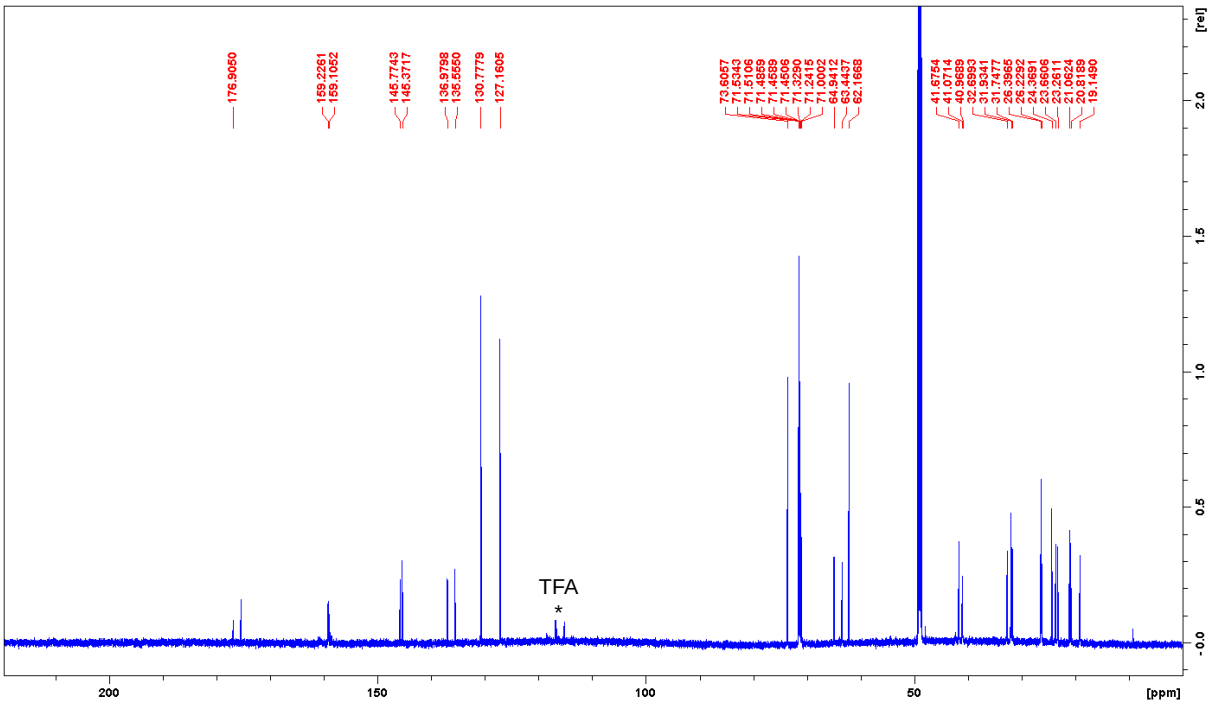
Triazole **S12**



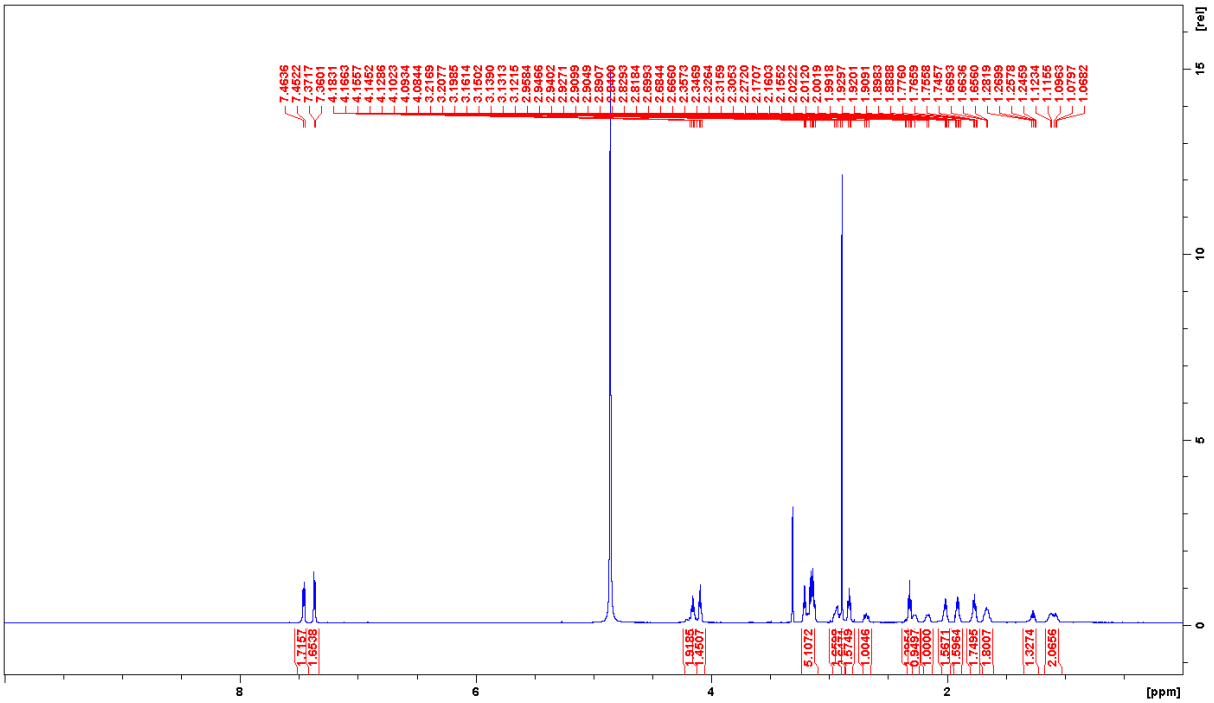


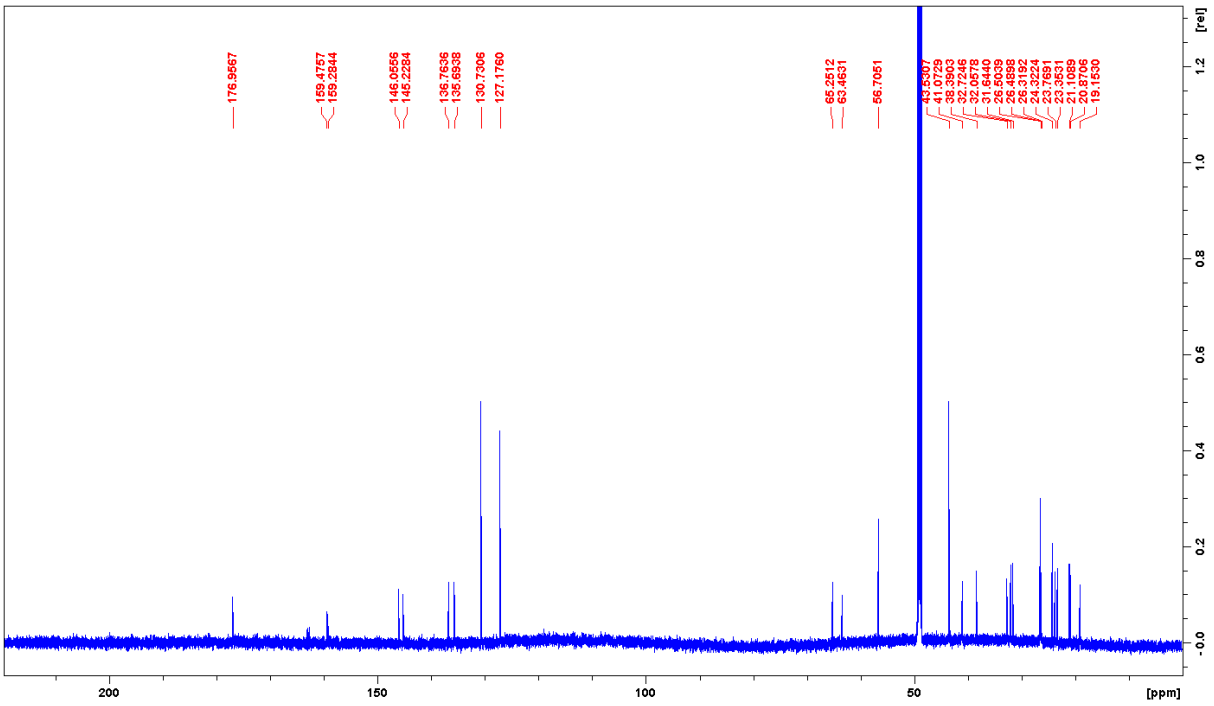
Triazole **S13**



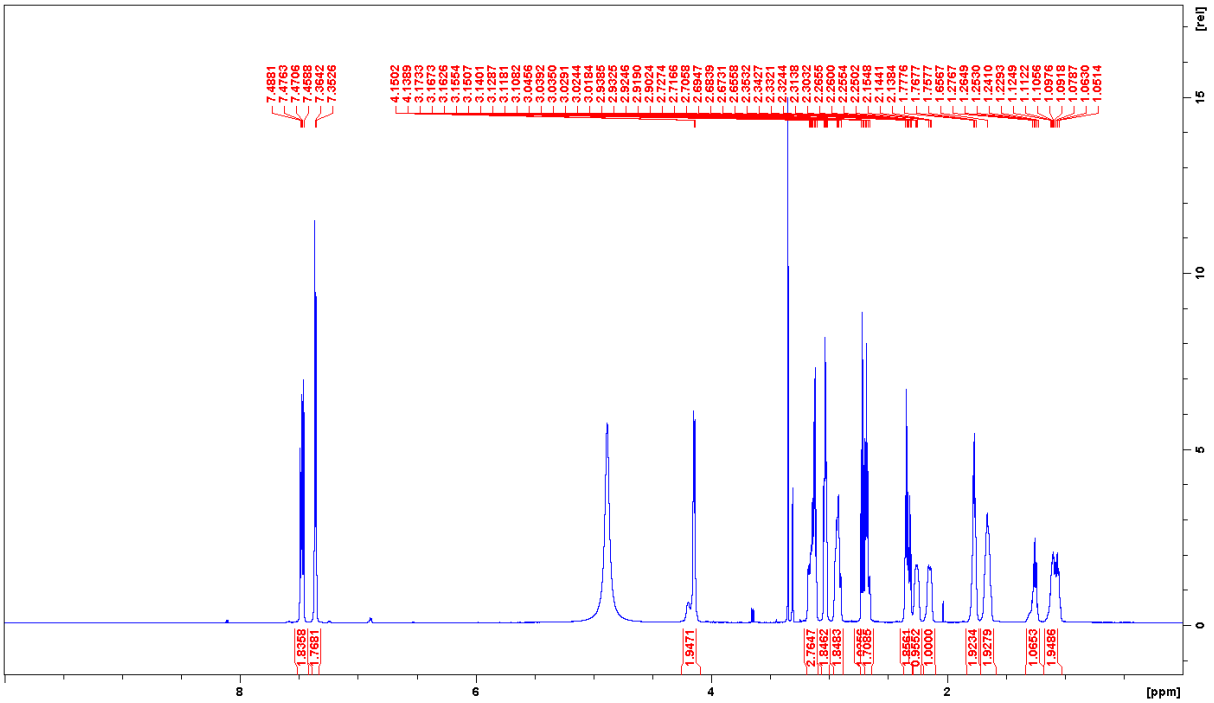


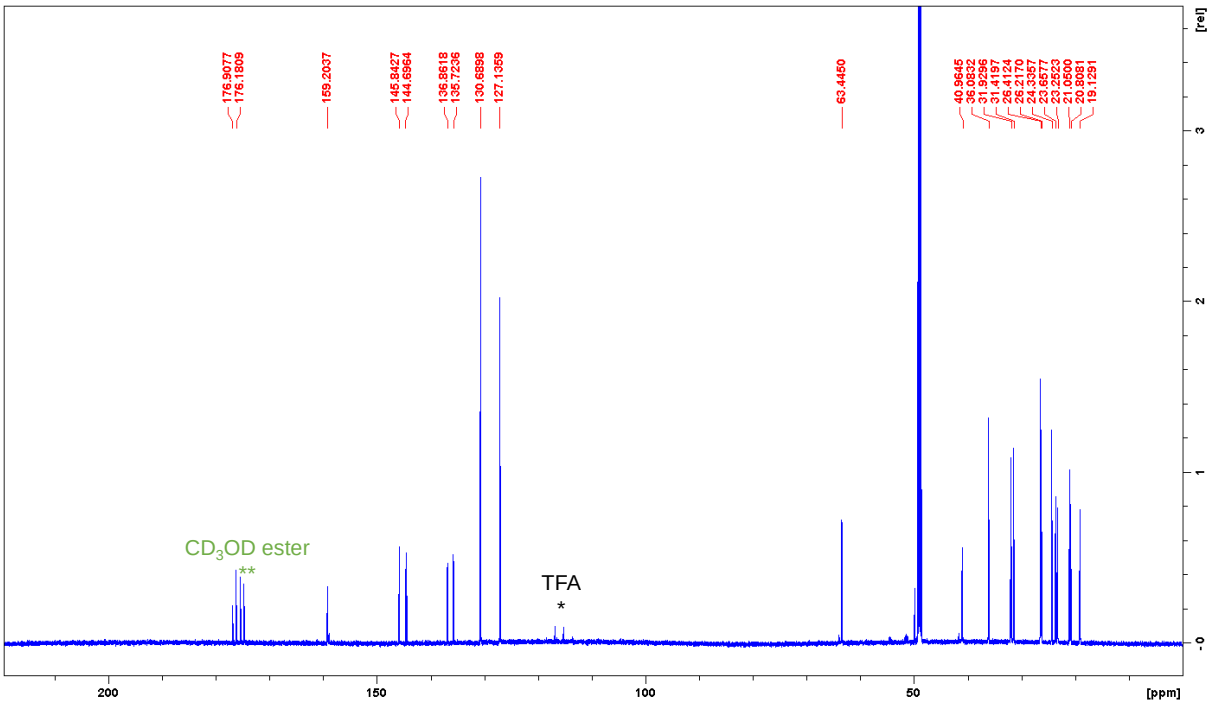
Triazole **S14**





Triazole **S15**





References.

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2 J. G. K. O'Brien, S. R. Chintala and J. M. Fox, *J. Org. Chem.*, 2018, **83**, 7500–7503.
3 S. Ursuegui, M. Recher, W. Krężel and A. Wagner, *Nat Commun*, 2017, **8**, 1–8.