Supplementary Material

Design, Synthesis and Evaluation of a Low-Molecular-Weight ^{11}C-Labeled Tetrazine for Pretargeted PET Imaging applying Bioorthogonal *in Vivo* Click Chemistry

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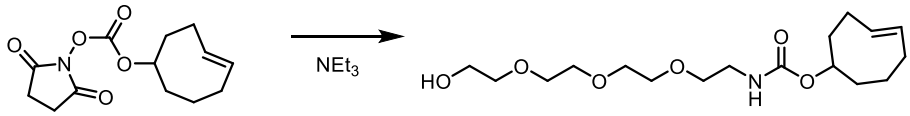
1. Materials and Methods

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. DCM and 1,4-dioxane were dried using PURESOLV-columns (Innovative Technology Inc.). Dry acetonitrile (Sigma Aldrich) was commercially obtained and stored under argon. Boc protected aminoacetonitrile and dienophiles *s*-TCO **4** and s-TCO-OPNP were prepared following known procedures.[1,2] Anhydrous hydrazine was prepared by the vacuum thermolysis of hydrazine cyanurate.[3] All other solvents were distilled prior to use. Drying of organic solvents after extraction was performed using anhydrous Na_2SO_4 or $MgSO_4$ (Sigma Aldrich) and subsequent filtration. Human Plasma for research purposes was purchased from the Austrian Red Cross blood donation service. Reactions were carried out under an atmosphere of argon in air-dried glassware with magnetic stirring. Sensitive liquids were transferred via syringe. Thin layer chromatography was performed using TLC alumina plates (Merck, silica gel 60, fluorescence indicator F254, or Merck, aluminium oxide neutral, fluorescence indicator F254). Detection in radio-TLC was performed by placing the TLC plates on multisensitive phosphor screens (Perkin-Elmer Life Sciences, Waltham, MA). The screens were scanned at 300 dpi resolution using a PerkinElmer Cyclone® Plus Phosphor Imager (Perkin-Elmer Life Sciences). Preparative column chromatography was performed using a Büchi Sepacore Flash System (2 x Büchi Pump Module C-605, Büchi Pump Manager C-615, Büchi UV Photometer C-635, Büchi Fraction Collector C-660) using silica gel 60 (40-63 µm) as obtained from Merck and distilled or redistilled solvents. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker Avance IIIHD 600 MHz spectrometer equipped with a Prodigy BBO cryo probe or on a Bruker Avance UltraShield 400 spectrometer at 20 °C. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and calibrated using solvent residual peaks. Data are shown as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad signal) and integration. UV/VIS spectrophotometry was done on a UV-1800 Spectrophotometer (Shimadzu) and temperature-controlled Applied Photophysics 05-19 and SX20 (535 nm LED light source) stopped flow spectrophotometer systems (Applied Photophysics, Surrey, UK) were used for stopped flow measurements. Fluorescence measurements were done on an Edinburgh FLS920 system (Livingston, UK) using Suprasil glass cuvettes. ESI-MS was performed on an HCT ion trap mass spectrometer (Bruker, Germany). A Thermo Fischer Exactive Plus Orbitrap (LC-ESI+) Mass Spectrometer was used for high-resolution mass spectrometry. [^{11}C]Methane was produced *via* the $^{14}N(p, \alpha)^{11}C$ nuclear reaction by irradiating nitrogen gas containing 10% hydrogen using a PETtrace cyclotron equipped with a methane target system (GE Healthcare, Uppsala, Sweden). [^{11}C]Methyl iodide was prepared *via* the gas-phase method in a TracerLab FXC Pro synthesis module (GE Healthcare) and converted into [^{11}C]methyl triflate by passage through a column containing silver-triflate impregnated graphitized carbon.[4,5] HPLC analysis was performed on a 1200 series system (Agilent Technologies) using a Phenomenex Luna SCX (5 µm, 4.6 x 250 mm) column and 9:1 50 mM phosphate buffer pH=2.4:EtOH (flow rate: 1.5 mL/min). For radio-HPLC a GABI* radioactivity detector (Raytest) was used. Retention times were 12.3 min for precursor **8** and 16 min for [^{11}C]-**1**. Preparative HPLC separation was done on the built in HPLC system on the TracerLab FXC pro synthesis module using a Phenomenex Luna SCX (5 µm, 4.6 x 250 mm) column and 9:1 50 mM phosphate buffer pH=2.4:EtOH (flow rate: 1.5 mL/min) in combination with a K-2001 UV detector (Knauer) and radioactivity detector.

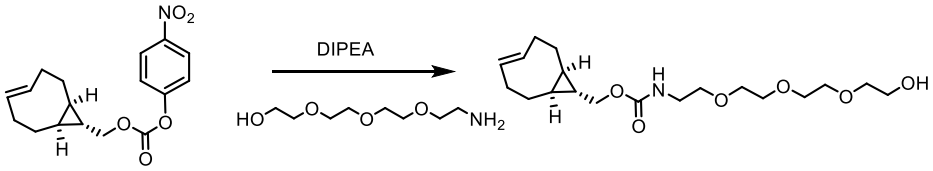
2. Chemical Synthesis and Radiolabeling

***(E)*-cyclooct-4-en-1-yl (2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)carbamate (6)**



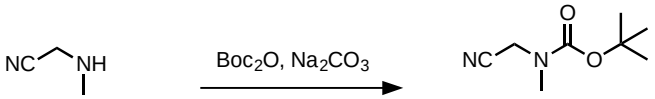
2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethan-1-ol (27 mg, 0.14 mmol, 1.5 eq.) in 200 µl anhydrous DMF was treated with triethyl amine (39 µl, 0.28 mmol, 3 eq.), followed by TCO-O(CO)NHS [6] (25 mg, 0.093 mmol, 1 eq.) in 800 µl anhydrous DMF. The mixture was stirred at room temperature for 22 hours, diluted with 1.25 mL DMSO and 1.25 mL water and loaded onto 30 g C18-silica. The column was eluted with a gradient of acetonitril in water (5-80%). Freeze drying of the product containing fraction yielded 28 mg of title compound as colorless oil (87% of theory). ^{1}H NMR (400 MHz, D_2O) ^{1}H NMR (400 MHz, D_2O) d = 1.54 - 1.71 (m, 3 H) 1.84 - 2.07 (m, 4 H) 2.22 - 2.37 (m, 3 H) 3.25 (t, *J*=5.27 Hz, 2 H) 3.55 (t, *J*=5.27 Hz, 2 H) 3.58 - 3.62 (m, 2 H) 3.63 - 3.72 (m, 10 H) 4.25 (d, *J*=8.20 Hz, 1 H) 5.45 - 5.58 (m, 1 H) 5.59 - 5.74 (m, 1 H) ppm; ^{13}C NMR (100 MHz, D_2O) □ = 30.6, 32.1, 33.7, 37.9, 40.4, 60.4, 69.4, 69.5, 69.6, 69.7, 71.7, 81.9, 133.4, 135.7, 158.3 ppm; ESI-MS $[M+H]^+$ calcd. 346.2 for $C_{17}H_{32}NO_6^+$, found 346.3.

((1R,8S,9r,E)-bicyclo[6.1.0]non-4-en-9-yl)methyl (2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl) carbamate (7)



2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethan-1-ol (50 mg, 0.259 mmol, 1.1 eq.) in anhydrous DMF was treated with s-TCO-OC(O)PNP[1] (75 mg, 0.235 mmol, 1 eq.) in one portion followed by diisopropyl ethyl amine (76 mg, 0.59 mmol, 2.5 eq.) causing intense yellow coloration. The mixture was stirred at room temperature for 2 hours, diluted with 1.25 mL DMSO and 1.25 mL water and loaded onto a preparative HPLC column (Phenomenex Luna C18, 21.2x250 mm, 10 µm). Elution with a gradient from 10 to 90% MeCN in water followed by freeze drying of the product fraction yielded 55 mg of title compound as colorless oil (63% of theory). ^{1}H NMR (400 MHz, D_2O) δ = 0.38 - 0.49 (m, 2 H) 0.49 - 0.63 (m, 2 H) 0.77 - 0.90 (m, 1 H) 1.82 - 1.96 (m, 2 H) 2.11 - 2.27 (m, 3 H) 2.32 (d, *J*=12.10 Hz, 1 H) 3.28 (t, *J*=5.27 Hz, 2 H) 3.55 (t, *J*=5.46 Hz, 2 H) 3.58 - 3.71 (m, 13 H) 3.91 (d, *J*=5.07 Hz, 2 H) 5.08 - 5.18 (m, 1 H) 5.81 - 5.93 (m, 1 H); ^{13}C NMR (100 MHz, D_2O) δ = 20.8, 21.9, 24.4, 27.5, 32.3, 33.6, 38.4, 40.12, 60.5, 69.5, 69.6, 69.7, 69.8, 71.9, 131.6, 138.7, 158.1 ppm; ESI-MS $[M+Na]^+$ calcd. 394.2 for $C_{19}H_{33}NNaO_6^+$, found 394.2.

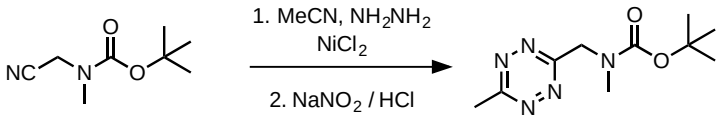
***tert*-butyl N-cyanomethyl-N-methylcarbamate (8)**



8

N-Methylaminoacetonitrile hydrochloride (1 eq., 1.44 g, 13.51 mmol) was dissolved in a mixture of MeOH (12 mL) and water (4 mL). The solution was treated with sodium carbonate (1.15 eq., 1.64 g, 15.5 mmol). Afterwards Boc_2O (1.5 eq., 4.42 g, 20.2 mmol) was added in one portion. The reaction mixture was stirred for 3 h at room temperature and concentrated. The residue was dissolved in dichloromethane and washed with water. The aqueous phase was re-extracted twice with DCM. The combined organic layer was dried over sodium sulfate and concentrated. Purification by column chromatography (40 g SiO_2, 25% ethyl acetate in hexanes + 0.1% Et_3N) yielded the title compound (2.3 g, 13.5 mmol, 99%) as a white solid. ^{1}H NMR data matched that previously reported.[7]

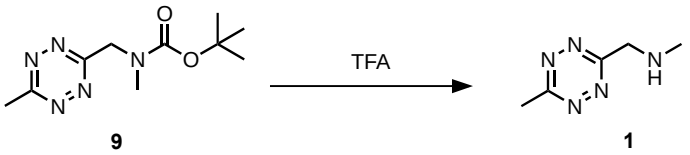
***tert*-butyl N-methyl-N-((6-methyl-1,2,4,5-tetrazin-3-yl)methyl)carbamate (9)**



8 **9**

To a stirred mixture of Boc-protected N-methylaminoacetonitrile **8** (1 eq., 0.6 g, 3.5 mmol), dry acetonitrile (7 eq., 0.98 g, 24 mmol) and nickel chloride (0.4 eq., 0.18 g, 1.4 mmol) was carefully added anhydrous hydrazine (36 eq., 4 g, 125 mmol) while cooling using an ice/salt bath. After finished addition the mixture was heated to 42°C for 18 hours. Afterwards the mixture was cooled to 0°C and a ~10% solution of sodium nitrite (2 eq., 0.5 g, 7 mmol) in water was added. While cooling 1N HCl was added slowly until gas evolution ceased. The mixture was extracted ten times with diethyl ether, combined extracts dried over Na_2SO_4, and concentrated. Column chromatography (90 g SiO_2, 50% Et_2O in hexanes + 1% Et_3N) afforded **9** (238 mg, 28%) as a pink oil that crystallized in the freezer.^{1}H NMR (600 MHz, C_6D_6) δ 4.74 (s, 1.2 H), 4.53 (s, 0.8 H), 2.91 (s, 1.3 H), 2.81 (s, 1.7 H), 2.47 (s, 1.3 H), 2.39 (s, 1.7 H), 1.42 (s, 5 H), 1.30 (s, 4 H); ^{13}C NMR (151 MHz, C_6D_6) δ 168.9, 168.7, 167.6, 167.4, 156.4, 155.4, 80.2, 80.1, 52.2, 52.0, 36.1, 36.0, 28.7, 28.6, 21.0 ppm (NMR spectra show a mixture of rotamers); Attempted HR-ESI-FTMS resulted in fragmentation and detection of deprotected substance **1** as $[M+H]^+$ ion (for MS data of **1** see below).

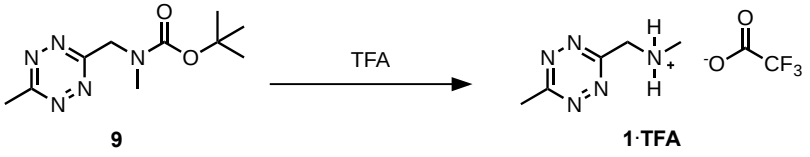
N-methyl-1-(6-methyl-1,2,4,5-tetrazin-3-yl)methanamine (1)



9 **1**

To a stirred solution of **9** (1 eq., 137 mg, 0.573 mmol) in dry DCM (5 mL) was added trifluoroacetic acid (11.4 eq., 0.5 mL, 6.5 mmol) at 0°C. The mixture was stirred at 0°C until TLC indicated complete consumption of starting material (3h). The reaction mixture was partitioned between water and DCM. The organic layer was extracted twice with water and the combined aqueous phases were made alkaline by addition of NaOH. The mixture was extracted several times with DCM and the combined organic layer was washed with water and dried over sodium sulfate. Removal of solvent afforded **1** (40 mg, 51%) as a pink solid. ^{1}H NMR (600 MHz, CD_2Cl_2) δ 4.32 (s, 2 H), 3.03 (s, 3 H), 2.49 (s, 3 H), 1.93 (br. s, 1 H); ^{13}C NMR (151 MHz, CD_2Cl_2) δ 168.8, 168.4, 54.3, 36.2, 21.6 ppm; HR-ESI-FTMS $[M+H]^+$ calcd. 140.0931 for $C_5H_{10}N_5^+$, found 140.0926.

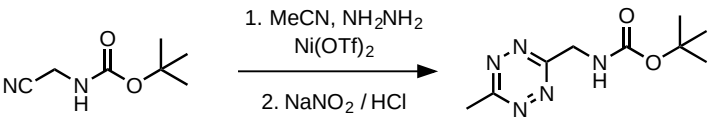
N-methyl-1-(6-methyl-1,2,4,5-tetrazin-3-yl)methanamine trifluoroacetate (1·TFA)



9 **1·TFA**

To a stirred solution of **9** (1 eq., 36 mg, 0.15 mmol) in dry DCM (1 mL) was added trifluoroacetic acid (8.7 eq., 0.1 mL, 1.3 mmol) at 0°C. The mixture was stirred at 0°C until TLC indicated complete consumption of starting material. The reaction mixture was evaporated to dryness on the rotary evaporator. The dark red residue was dissolved in 5 mL water, frozen and lyophilized to obtain **1·TFA** as red oil in quantitative yield. No signs of degradation were noted even after prolonged storage at room temperature. ^{1}H NMR (400 MHz, D_2O) δ 4.90 (s, 2 H), 3.02 (s, 3 H), 2.90 (s, 3 H); ^{13}C NMR (101 MHz, D_2O) δ 169.3, 162.9 (q, J = 35.3 Hz), 162.1, 116.3 (q, J = 292 Hz), 49.6, 33.2, 20.4; ^{19}F NMR (376.5 MHz, D_2O) δ -75.6 ppm.

***tert*-butyl N-((6-methyl-1,2,4,5-tetrazin-3-yl)methyl)carbamate** (**10**)

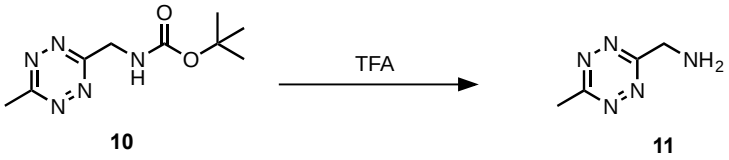


10

To a stirred mixture of tert-butyl (cyanomethyl)carbamate (1 eq., 0.62 g, 4 mmol), dry acetonitrile (7 eq., 1.46 mL, 28 mmol) and nickel trifluoromethanesulfonate (0.52 eq., 0.74 g, 2.08 mmol) was carefully added anhydrous hydrazine (36 eq., 4.5 mL, 144 mmol) while cooling using an ice/salt bath. After finished addition the mixture was heated to 65°C for 20 hours. Afterwards the mixture was cooled to 0°C and a ~10% solution of sodium nitrite (2 eq., 0.55 g, 8 mmol) in water was added. While cooling 1N HCl was added slowly until gas evolution ceased (pH 3). The mixture was extracted three

times with diethyl ether, combined extracts dried over Na_2SO_4, and the solvent removed. Column chromatography (90 g SiO_2, 50% Et_2O in hexanes) afforded **10** (260 mg, 29%) as a pink oil. ^1H and ^{13}C NMR data matched that previously reported.[8]

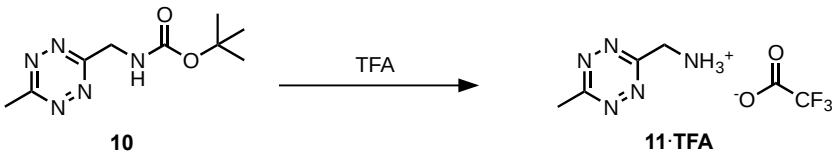
(6-methyl-1,2,4,5-tetrazin-3-yl)methanamine (11)



10 **11**

To a stirred solution of **10** (1 eq., 315 mg, 1.4 mmol) in dry DCM (8 mL) was added trifluoroacetic acid (4.6 eq., 0.5 mL, 6.5 mmol) at 0°C. The mixture was stirred at room temperature for 26 h. The reaction mixture was diluted with DCM (40 mL) and 25% NH_4OH (30 mL). Brine (~40 mL) was added, the organic phase was separated and the aqueous phase extracted five times with DCM. The combined organic layer was dried over sodium sulfate and concentrated to yield 156 mg **11** (89%) as a pink-red solid. Even though purity of the raw product was high according to ^1H-NMR the product was further purified (column chromatography over SiO_2 using 5% MeOH in DCM) prior to ^{11}C labeling and NMR measurements. ^1H NMR (600 MHz, $CDCl_3$) δ 4.51 (s, 2 H), 3.08 (s, 3 H), 1.85 (br. s, 2 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.3, 168.1, 45.3, 21.2 ppm; HR-ESI-FTMS $[M+H]^+$ calcd. 126.0774 for $C_4H_8N_5^+$, found 126.0772.

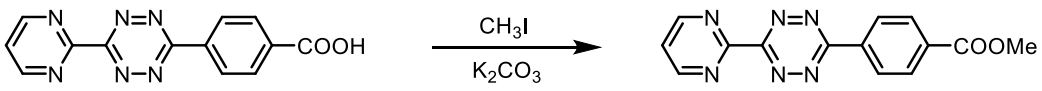
(6-methyl-1,2,4,5-tetrazin-3-yl)methanamine trifluoroacetate (11·TFA)



10 **11·TFA**

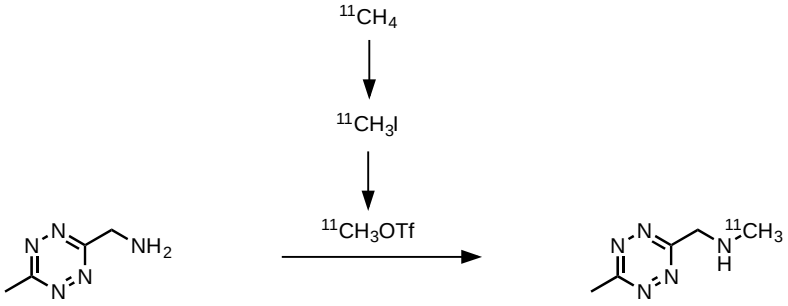
To a stirred solution of **10** (1 eq., 20.1 mg, 0.089 mmol) in dry DCM (1 mL) was added trifluoroacetic acid (14.6 eq., 0.1 mL, 1.3 mmol) at 0°C. The mixture was stirred at 0°C until TLC indicated complete consumption of starting material. The reaction mixture was evaporated to dryness on the rotary evaporator. The dark red residue was redissolved in 5 mL water, frozen and lyophilized to obtain **11·TFA** as a pink solid. No signs of degradation were noted even after prolonged storage at room temperature. ^1H NMR (400 MHz, D_2O) δ 4.91 (s, 2 H), 3.08 (s, 3 H); ^{13}C NMR (101 MHz, D_2O) δ 169.2, 163.0 (q, J = 35.3 Hz), 162.8, 116.3 (q, J = 292 Hz), 41.0, 20.4; ^{19}F NMR (376.5 MHz, D_2O) δ -75.6 ppm

methyl 4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoate



4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoic acid [9] (1 eq., 0.56 g, 2 mmol) and potassium carbonate (2 eq., 0.55 g, 4 mmol) were stirred in 37 mL anhydrous DMF under an argon atmosphere. Methyl iodide (1.1 eq., 0.311 g, 2.20 mmol) was added in one portion, and the reaction was stirred at room temperature for 16 h after which TLC showed full conversion. The reaction mixture was diluted with 300 mL EtOAc, washed twice with H_2O and brine, dried over $MgSO_4$, and the solvent was removed on the rotary evaporator. Flash chromatography with 5% MeOH in DCM over a pad of silica yielded the title compound (520 mg, 88.3% of theory) as a deep purple solid. Rf (5% MeOH in DCM) = 0.76, ^{1}H NMR (600 MHz, DMSO-d_6) d = 3.94 (s, 3 H) 7.85 (t, *J*=4.84 Hz, 1 H) 8.26 - 8.29 (m, 2 H) 8.72 - 8.74 (m, 2 H) 9.21 (d, *J*=4.69 Hz, 2 H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) d = 52.6, 123.1, 128.5, 130.2, 133.2, 135.8, 158.6, 159.0, 162.9, 163.1, 165.7 ppm; FT-MS [M+H$^+$] calcd. 295.0938, found 295.0946

Radiosynthesis of N-[^{11}C]methyl-1-(6-methyl-1,2,4,5-tetrazin-3-yl)methanamine ([^{11}C]-1)



11 [^{11}C]-**1**

[^{11}C]methyl triflate was bubbeled through a solution of compound **11** (0.5 mg, 4 µmol) dissolved in anhydrous MeCN (400 µL) using a TracerLab FXC Pro synthesis module. The reaction mixture was heated for 5 min to 75°C, followed by cooling to 25°C and dilution with HPLC eluent (500 µL, 50 mM phosphate buffer pH = 2.4/ EtOH, 9/1, v/v) and injected into a built-in HPLC system. A Phenomenex Luna SCX 250 x 4.6 mm was eluted with 50 mM phosphate buffer pH=2.4 (solvent A) and EtOH (solvent B), A:B= 9:1 (v/v) at a flow rate of 1.5 mL/min. The HPLC eluate was monitored in series for radioactivity and ultraviolet (UV) absorption at a wavelength of 280 nm. On this system, radiolabeling precursor **11** and product [^{11}C]-**1** eluted with retention times of 11.5—13.5 min and 16-18 min, respectively.

The product fraction from HPLC was collected and evaporated to dryness on a rotary evaporator, followed by reformulation in 0.1 M Na_2HPO_4 at an approximate concentration of 37 MBq/mL for intravenous (IV) injection into animals. The formulated product had a pH of 6 and an osmolarity of 485 mmol/kg.

[^{11}C]-**1** ready for IV injection was obtained in a decay-corrected radiochemical yield based on $^{11}CH_3OTf$ of 52 ± 6% (*n* = 3) in a total synthesis time of 30 min. Radiochemical purity of [^{11}C]-**1** was

determined by analytical radio-HPLC using a Phenomenex Luna SCX column (5 µm, 4.6 x 250 mm) and elution with 50 mM phosphate buffer pH=2.4 (solvent A) and EtOH (solvent B), A:B= 9:1 (v/v) at a flow rate of 1.5 mL/min. UV detection was performed at a wave-length of 530 nm. The retention time of [^{11}C]-**1** was 16 min on this HPLC system. The identity of [^{11}C]-**1** was confirmed by HPLC-comparison injecting unlabeled **1**. Radiochemical purity of [^{11}C]-1 was > 95%. The specific activity of [^{11}C]-**1** was determined by measuring radioactivity of a 1 mL aliquot (using a Capintec Dose calibrator) and by HPLC quantification of **1** (conditions described above ; dilution series**:** 0.01 mM, 0.1 mM, 0.5 mM). The Specific activity was determined to be 10 ± 5.4 GBq/µmol (n=4).

Radiosynthesis of N-[^{11}C]methyl-1-(6-methyl-1,2,4,5-tetrazin-3-yl)methanamine ([^{11}C]-1) using 8·TFA as precursor

The general labeling procedure was followed using 1.2 mg **11·TFA** (5.3 µmol) in 400 µl anhydrous MeCN. Prior to labeling 1 µl diisopropylethyl amine (5.75 µmol, 1.08 eq) was added to the reaction mixture. [^{11}C]-**1** was obtained in 34.6% (n = 1) decay corrected radiochemical yield and in >98% radiochemical purity.

Synthesis of mesoporous silica nanoparticles (MSNs) [10]

1 g (2.74 mmol) cetyltrimethylammonium bromide (CTAB) was suspended in 490 mL 15 mM NaOH. The mixture was stirred using a mechanical stirrer at 300 rpm and heated to 80°C which resulted in a homogenous solution. Tetraethylorthosilicate (TEOS, 6.7 ml, 30.23 mmol) was added within 10 seconds and the turbid mixture was stirred at 80°C for additional 2 hours. The mixture was allowed to reach room temperature, the stirring was turned off and the precipitate was allowed to settle. The supernatant was decanted off and the precipitate was collected by centrifugation (10 m, 3000 x g). The pellet was resuspended in 50 mL distilled water, vortexed for 60 seconds followed by centrifugation (10 m, 3000 x g). This washing cycle was repeated twice with water and once with methanol. Drying in vacuo yielded 1.5 g MSNs as white powder (83% of theory). The surfactant was removed by heating in air using the following temperature program: 20°C → 300°C (2°C/min), 300°C (2 h), 300°C → 550°C (2°C/min), 550°C (16 h). The size of the particles was in the range of 120-145 nm (determined by SEM, Fig. S1).

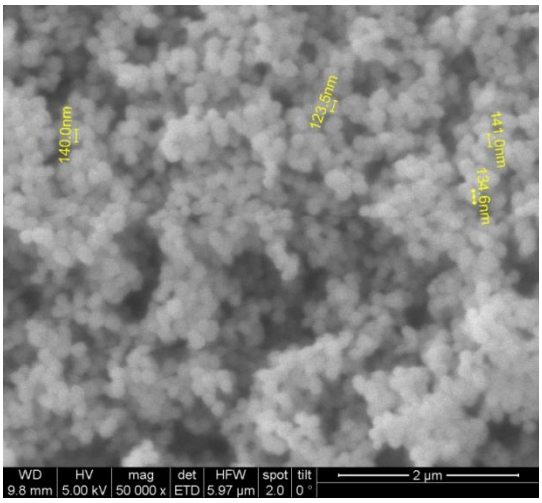


Figure S1: *Electron microscopy of mesoporous silica nanoparticles*

Amino-functionalization of MSNs[11]

357 mg of MSNs were suspended in 20 mL anhydrous toluene and heated on a dean stark trap for 2 h to remove traces of moisture. The dean stark trap was removed and 3-aminopropyltrimethoxysilane (APTMS, 80 µl, 0.458 mmol) was added. The mixture was refluxed for additional 3 h and allowed to cool and settle. The majority of toluene was decanted off and the solids were collected by centrifugation (5 min, 3000 x g). The pellet was resuspended in MeOH, and the solids again collected by centrifugation. Drying in vacuo at 60°C afforded 223 mg amino-grafted MSNs as white powder. The modification did not influence particle size as determined by SEM (Fig. S2).

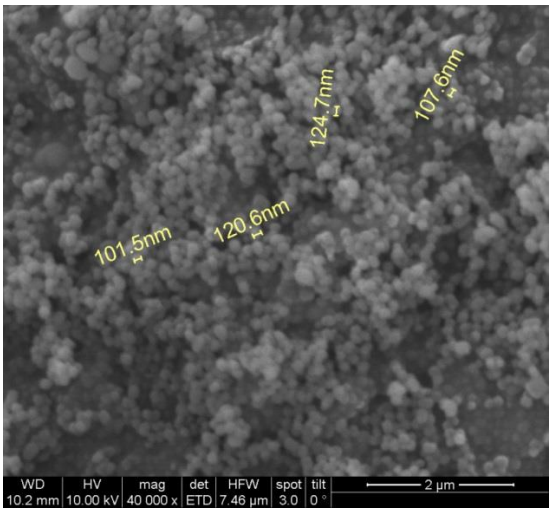


Figure S2: *Electron microscopy of amino-functionalized MSNs*

Determination of amine surface modification

The Fluram (4'-phenylspiro[2-benzofuran-3,2'-furan]-1,3'-dione) assay was chosen for analysis of the degree of surface functionalization with amines.[12] Fluram forms highly fluorescent adducts with primary amines. A stock solution was made by dissolving 14.98 mg native MSNs in 30 g 0.02 N NaOH

followed by the addition of 6.07 mg APTMS (2.26 mmol amine/g MSNs). This stock solution was diluted 1:1, 1:9, 1:99 with a matrix solution (containing 0.5 mg/g native MSNs in 0.02N NaOH) giving solutions corresponding to 1.13 mmol/g, 0.226 mmol/g and 0.0226 mmol/g amine surface modification). The analyte (amino modified MSNs) was also dissolved in dilute NaOH (0.495 mg/g 0.02N NaOH; total 11.22 mg). The blank contained only matrix solution. 100 µl of standard, blank or sample solution were treated with 2 mL 200 mM phosphate buffer pH 8 in a quartz cuvette followed by 1 mL 1 mM fluram solution in acetone. The samples were measured 5 min after fluram addition at 22°C in an Edinburgh FLS920 fluorometer (excitation: 366 nm, emission: 480 nm). Results are summarized in Table S1.

Table S1: *Dilution series concentrations and measured fluorescence intensities*

mmol NH_2/g	cts
blank	1.21E+05
0.022586	1.24E+05
0.22586	1.81E+05
1.1293	3.82E+05
2.2586	6.04E+05

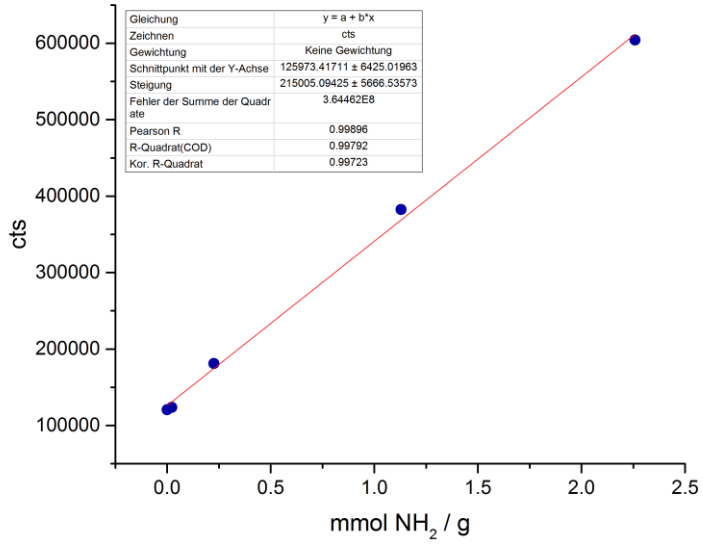
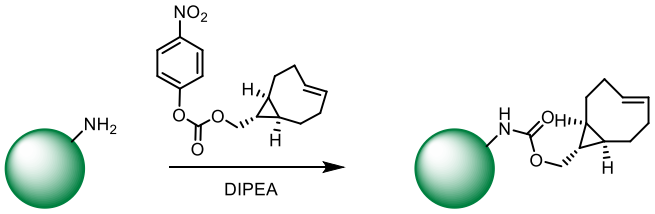


Figure S3: *Linear regression of dilution series data points*

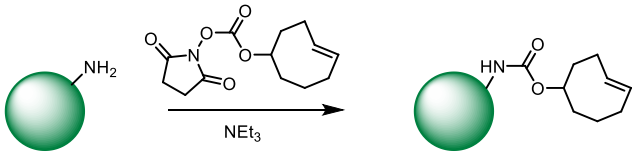
Results of standard and blank measurements were fitted by a linear regression. The sample was found to have **a functionalization degree of 0.92 mmol NH_2/g MSNs**. (3.24E+05 cts; calculated from linear regression shown in Fig. S3).

s-TCO modification of amino-MSNs



600 µl anhydrous DMF and 30 µl diisopropyl ethyl amine (0.172 mmol) were added to 8 mg of amino-MSNs (7.36 µmol amine). 5 mg of s-TCO-OC(O)PNP[1] (15.75 µmol, 2.14 eq) were added in one portion resulting in intense yellow coloration. The reaction mixture was shaken for 16 hours at room temperature and the particles harvested by centrifugation (13.4krpm, 3 min). The supernatant was discarded and the pellet resuspended in 1.5 mL DMF by vortexing. The solvent was again removed following centrifugation. These washing cycles were repeated three times with DMF and once with EtOH. The particles were dried in high vacuum at ambient temperature using a speedvac system.

TCO-modification of amino-MSNs



100 µl anhydrous DMF and 15 µl triethyl amine (0.108 mmol) were added to 5.8 mg of amino-MSNs (5.3 µmol amine). 1 mg of TCO-O(CO)NHS[6] (3.74 µmol, 0.81 eq) in 200 µl anhydrous DMF was added in one portion. The reaction mixture was shaken for 16 hours at room temperature and the particles harvested by centrifugation (13.4 krpm, 3 min). The supernatant was discarded and the pellet resuspended in 1.5 mL DMF by vortexing. The solvent was again removed following centrifugation. These washing cycles were repeated three times with DMF and twice with water. The pellet was resuspended in 1.2 mL 0.9% saline.

Analysis of reactive dienophile loading on s-TCO-MSNs and TCO-MSNs

Highly reactive tetrazine methyl 4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoate (15.43 mg, 52.43 µmol) was dissolved in MeOH (100 mL) to obtain a 0.524 mM solution. The stock solution and a dilution series (1:1, 1:3, 1:9: 1:19, stock solution diluted with MeOH) were analyzed by HPLC (isocratic, 30% MeCN in 10 mM phosphate buffer pH 6, Agilent Extend-C18 column 3.0 x 100 mm, 3.5 µm, 1.3 ml/min), monitoring the absorption at 530 nm. The tetrazine eluted at 1.6-1.7 min. A linear regression was calculated based on HPLC results (Fig. S4). A defined mass (1-1.45 mg) of nanoparticles (s-TCO-MSNs, TCO-MSNs, Amino-MSNs) was weighted into 1.5 mL tubes and treated with tetrazine stock solution (400-500 µl, corresponding to 210 – 262 nmol). The vials were vortexed for 60 s and shaken for additional 10 min at 37°C to allow for quantitative IEDDA reaction. Following

centrifugation (13.3 krpm, 5 min) excess tetrazine in the supernatant was analyzed by HPLC using the method described above. The calibration factor was obtained by linear regression of HPLC data arising from the dilution series (Table S2, Fig. S4).

Table S2: *Dilution series concentrations and detector response*

Dilution	c [µmol/ml]	Mean area [mAu*s]
Stock	0.524	185.34
1:1	0.262	91.69
1:3	0.131	48.25
1:9	0.052	18.47
1:19	0.026	9.63

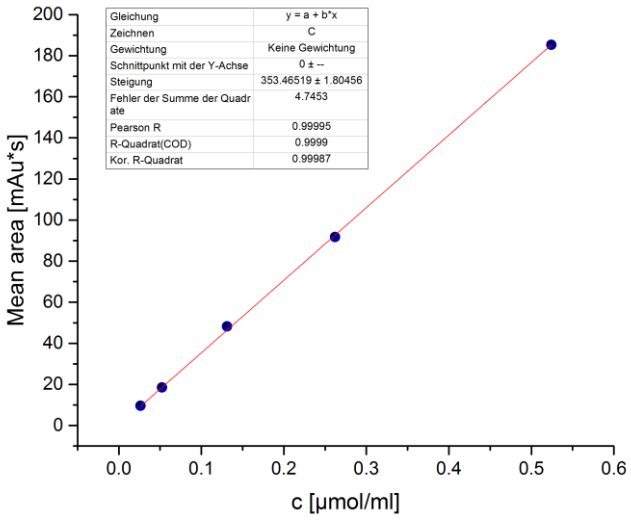


Figure S4: *Linear regression of dilution series data points used for dienophile loading assay*

The dienophile-loading in µmol/g was calculated using the following formula. Results are summarized in Table S3.

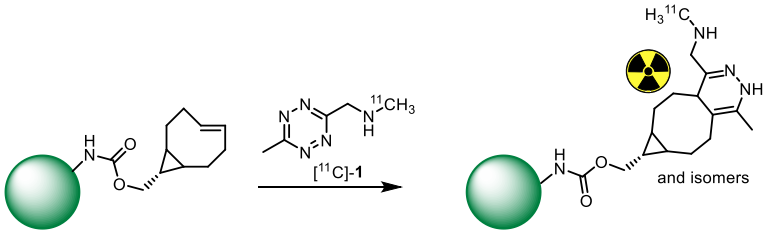
$$TCO\ loading\ \left[\frac{\mu mol}{g}\right] = \frac{1000 * V_{stock}\ [ml]}{m\ NP\ [mg]} * \left(0.52435 - \frac{area\ [mAu * s]}{calibration\ factor}\right)$$

Table S3: *Results of dienophile loading assay*

Particle	mass [mg]	V_{stock} [ml]	Mean area [mAu*s]	Dienophile [µmol/g]

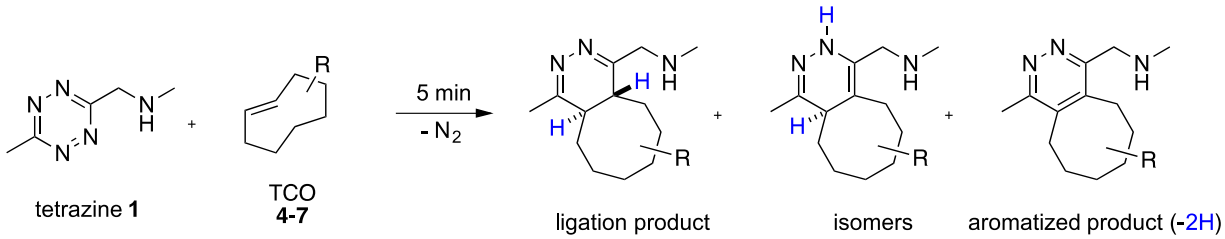
Amino-MSNs	1.450	0.50	184.26	**1**
s-TCO-MSNs	1.160	0.40	63.41	**119**
TCO-MSNs	0.500	0.50	144.21	**116**

Synthesis of ^{11}C-labeled MSNs



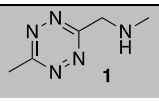
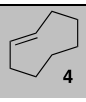
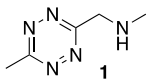
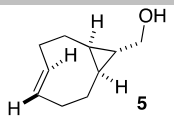
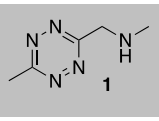
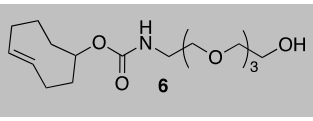
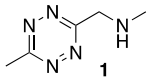
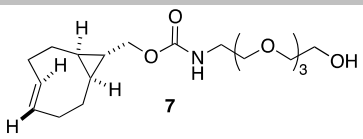
[^{11}C]-**1** (70.3 MBq in 1.5 mL phosphate buffer pH 5.9) was added to 1.48 mg s-TCO-MSNs (176 nmol s-TCO) and vortexed briefly. After 5 min standing at room temperature the suspension was centrifuged (13.3 krpm, 2 min). The supernatant was discarded and the pellet consisting of [^{11}C]MSNs (44.4 MBq, 80% decay-corrected yield) was resuspended in 300 µl 0.9% saline to obtain a suspension ready for i.v. administration.

IEDDA-initiated conjugations

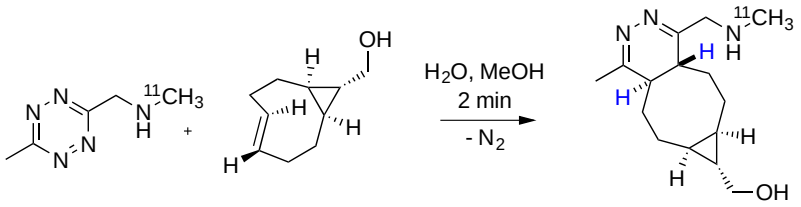


To a 3 mM solution of tetrazine **1** in dry 1,4-dioxane (for **1**+**4** and **1**+**5**) (1 mL) or PBS (for **1**+**6** and **1**+**7**) was added a 3 mM solution of TCO-derivative (1 eq.) in dry 1,4-dioxane (**4** and **5**) or water (**6** and **7**) (1 mL) and the resulting mixture was stirred for 5 min at room temperature. Due to the fast isomerization and aromatization known for the tetrazine/TCO-ligation products, the sample was directly subjected to LCMS analysis for characterization (Table S4).

Table S4. IEDDA-initiated conjugation of tetrazine (**1)** and TCO-derivatives **4**-**7**

Tetrazine	Dienophile	Product formula	Product Calcd. mass	Found mass
 **1**	 **4**	$[M+H]^+$ $C_{13}H_{24}N_3^+$	$[M+H]^+$ 222.2	$[M+H]^+$ 222.0
 **1**	 **5**	$[M+H]^+$ $C_{15}H_{26}N_3O^+$	$[M+H]^+$ 264.2	$[M+H]^+$ 264.2
 **1**	 **6**	$[M+H]^+$ $C_{22}H_{39}N_4O_6^+$ (aromatized)	$[M+H]^+$ 455.3	$[M+H]^+$ 455.3
 **1**	 **7**	$[M+H]^+$ $C_{24}H_{41}N_4O_6^+$ (aromatized)	$[M+H]^+$ 481.3	$[M+H]^+$ 481.5

IEDDA initiated conjugation of [^{11}C]-1 with dienophile 5



tetrazine [^{11}C]-**1**

s-TCO (**5**)

ligation products + isomers [^{11}C]-**12** + [^{11}C]-**13**

To prove IEDDA reactivity of tetrazine [^{11}C]-**1**, a solution of s-TCO (**5**) (0.5 mg, 3.3 µmol) in MeOH (5 µL) was added to 37 MBq of tetrazine [^{11}C]-**1** dissolved in a mixture of 50 mM phosphate buffer pH=2.4 (solvent A) and EtOH (solvent B), A:B= 9:1 (v/v), 200 µL. The reaction was stirred for 2 min and ~0.5 µL were spotted on SiO_2 plates (together with [^{11}C]-**1** as reference) and TLCs were developed with 10% MeOH + 1% triethylamine in DCM. Analysis of radioactivity distribution on the TLC plates using a Cyclone Phospho Imager indicated complete consumption of [^{11}C]-**1** and the formation of more hydrophilic ligation products [^{11}C]-**12** and [^{11}C]-**13** (Manuscript Fig. 2c).

3. Reaction kinetics

Tetrazine **1** was weighted into a volumetric flask and filled with dry 1,4-dioxane to generate a 3 mM solution. Dienophiles TCO **(4)** and s-TCO (**5**) were weighted into a volumetric flask and filled with dry 1,4-dioxane to generate a 30 mM solution. The reactant solutions were transferred into the driver syringes of the stopped-flow apparatus (Applied Photophysics, Surrey, UK). Upon temperature constancy (± 0.1°C of aimed value) triggering of driver syringe pneumatics caused mixing of equal reactant volumes. Depending on reaction rate time intervals between 10 to 200 s were chosen to collect 4000 data points at 530 nm. All measurements were made in duplicate or triplicate. Reactions in 1,4-dioxane were monitored at 20°C, 37 °C and 60°C. The pseudo first order rate constant (k_{obs}) was determined by linearization of the decay curve followed by linear fitting. The second order rate constant (k) was calculated from the pseudo-first order rate constant. Linearization and direct fitting to the Eyring equation (Equation 1) was used to calculate the enthalpy of activation ($\Delta H^{\ddagger}$) and the entropy of activation ($\Delta S^{\ddagger}$). Equation 2 gave rise to free energy of activation at 298.15 K ($\Delta G^{\ddagger}$). Results are summarized in Table S5 (rate constants) and Table S6 (thermodynamic data). Selected plots of kinetic measurements and Eyring linearization are shown in Fig. S5.

$$\ln\left(\frac{k}{T}\right) = -\left(\frac{\Delta H^{\ddagger}}{R}\right) * \left(\frac{1}{T}\right) + \ln\left(\frac{K_b}{h}\right) + \left(\frac{\Delta S^{\ddagger}}{R}\right) \qquad (1)$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - \mathrm{T}\Delta S^{\ddagger} \qquad (2)$$

Table S5. Results of kinetic measurements (rate constants).

Reaction	Solvent	T (°C)	k_{obs} (s^{-1})	k ($M^{-1}s^{-1}$)
1 + **4**	1,4-dioxane	20	$(8.6 \pm 0.1) \cdot 10^{-3}$	0.57 ± 0.01
		37	$(15.6 \pm 0.5) \cdot 10^{-3}$	1.04 ± 0.03
		60	$(29.9 \pm 0.4) \cdot 10^{-3}$	1.99 ± 0.03
1 + **5**	1,4-dioxane	20	0.99 ± 0.03	65.9 ± 2
		37	1.34 ± 0.02	89.5 ± 1.3
		60	2.026 ± 0.03	135.1 ± 2

Table S6. Results of kinetic measurements (thermodynamic data).

Reaction	Solvent	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
1 + **4**	1,4-dioxane	5.44	-0.041	17.67
1 + **5**	1,4-dioxane	2.65	-0.041	14.92

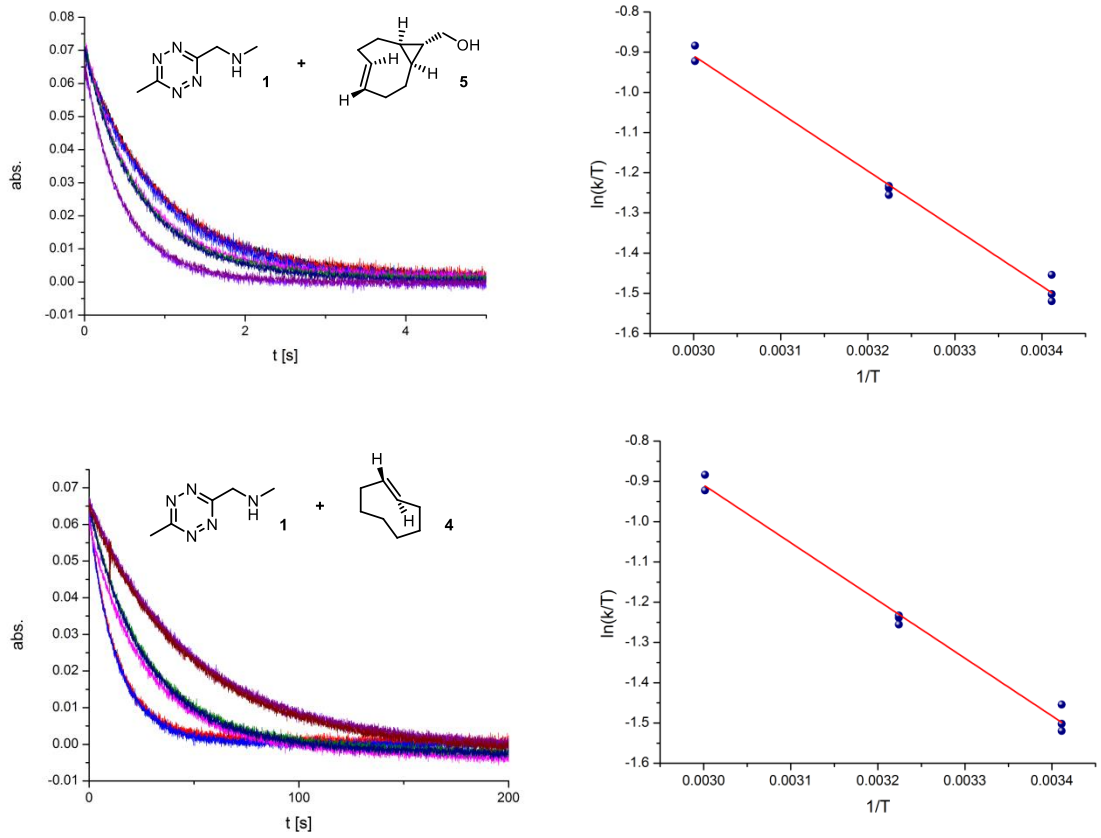


Figure S5. Stopped flow measurements of **1** + **5** and **1** + **4** at 20°C, 37°C and 60°C (left) and Eyring linearization (right)

Reaction rates of **1** with water-soluble dienophiles **6** and **7** were determined in PBS (Fluka, pH 7.4) using a SX20 stopped flow spectrophotometer system (Applied Photophysics, Surrey, UK) equipped with a 535 nm LED light source and a cell temperature of 37.00 ± 0.05 °C. Measurements were done under pseudo-first order conditions with a starting concentration of 1 mM for **1** (as trifluoroacetate), 10.0 mM for **6** and 16.6 mM for **7**. Equal volumes of Tz and TCO solutions were mixed, resulting in concentrations of 0.5 mM for **1**, 5.0 mM for **6** and 8.3 for **7**. Measurements were performed in quintuplicates. The pseudo first order rate constants (k_{obs}) were determined by linearization of the decay curve followed by linear fitting. The second order rate constant (k) was calculated from the pseudo-first order rate constant. Results are summarized in Table S7.

Table S7. Results of kinetic measurements (rate constants).

Reaction	Solvent	T (°C)	k_{obs} (s^{-1})	k ($M^{-1}s^{-1}$)
1 + **6**	PBS	37.00 ± 0.05	0.88 ± 0.01	175.4 ± 1.2
1 + **7**			352.7 ± 25.8	42500 ± 3100

4. *In vitro* and *in vivo* investigations

4.1 Plasma stability of 1

Plasma stability was determined by absorbance measurement. A 5.3 mM solution of **1** in human blood plasma was incubated at 37°C and absorbance at 530 nm was followed over a period of 120 min revealing > 90% recovery (Fig. S6). Identity of remaining **1** was verified by TLC.

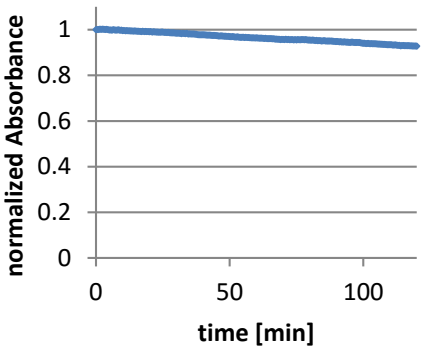


Figure S6. Absorbance of **1** in human blood plasma at 37°C over the course of 120 min

4.2 Animals

For the biodistribution experiments, female BALB/c mice weighting 21.6 ± 5.4 g were purchased from the Division of Laboratory Animal Science and Genetics, Medical University of Vienna, Himberg, Austria. All animals were housed in groups under individual ventilated cage (IVC) conditions in polysulfon type III cages. Environmental conditions were temperature: 22 ±3 °C, humidity: 40% to 70% and a 12-hour light/dark cycle (lights on at 6:00) with free access to standard laboratory diet (ssniff R/m-H, ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. An acclimatization period of at least 1 week was allowed before the animals were used in the experiments. All studies involving laboratory animals were approved by national authorities (*Amt der Niederösterreichischen Landesregierung*) and all study procedures were performed in full accordance to the European Council Directive of September 22, 2010 (2010/63/EU). All efforts were made to comply with the 3R principle.

4.3 PET/MR Imaging – Biodistribution study

Two mice underwent PET scanning on a dual animal bed in a dedicated small-animal PET scanner and one mouse underwent sequential PET/MRI on two stand-alone scanners. Prior imaging, mice were pre-anesthetized in an induction chamber using isoflurane (2.5% in oxygen), placed in prone position on a heated animal bed (38°C) and the lateral tail vein was cannulated using a custom made tail vein catheter. Animal respiratory rate and body temperature were constantly monitored (SA Instruments Inc, Stony Brook, NY, USA) and the isoflurane level was adjusted (1.5-2.5% on oxygen) to achieve a constant depth of anesthesia. Anesthesia was maintained for the whole imaging period. A humidifier was used to moisten the gas mixture before supplying it to the animal(s). For anatomical magnetic resonance imaging (MRI) a single mouse body bed mounted with a mouse whole body RF coil was

used. The whole body coil covers an axial field of view (FOV) of about 8 cm that is very similar to the axial FOV of the PET scanner (7.6 cm). Images were acquired on a 1 Tesla benchtop MRI (ICON, Bruker Biospin GmbH, Ettlingen, Germany) using a modified 3D T_1-weighted gradient echo sequence (T1-FLASH) with the following imaging parameters: echo time (TE) = 5 ms; repetition time (TR) = 25 ms; flip angle (FA) = 25 °; field of view (FOV) = 7.6 x 2.6 x 2.4 cm; matrix = 253 x 93; 32 slices; slice thickness = 750 µm; 5 repetitions; total imaging time = 6 min 15 sec. After MRI, the animal bed was transferred into the gantry of a microPET scanner (Focus 220, Siemens Medical Solutions, Knoxville, TN, USA). Total anesthesia time prior start of PET scanning for mice that underwent PET scanning only was similar to that of the PET/MRI examined animal. Direct after positioning of the animal bed in the gantry of the PET scanner a 10 min transmission scan using a rotating ^{57}Co point source was recorded. Simultaneously with intravenous injection of 16.2 ± 5.9 Mbq [^{11}C]-**1**, dynamic PET imaging was initiated for 60 min using an energy window of 250-750 keV and a timing window of 6 ns. After completion of the imaging procedure, a terminal blood sample was withdrawn under isoflurane anesthesia from the retrobulbar venous plexus and the animals were sacrificed by cervical dislocation. Blood was centrifuged (17000 *g*, 4°C, 4 min) to obtain plasma, and organ samples as well as urine were collected. Aliquots of blood, plasma and urine as well as organ samples were transferred into pre-weighted tubes and measured for radioactivity in a gamma counter (Wizard 1470, Perkin-Elmer, Wellesley, MA, USA). The measured radioactivity data were corrected for radioactive decay and expressed as standardized uptake value ((radioactivity per g/injected radioactivity) x body weight). The 60 min dynamic emission PET data were sorted into 23 frames, which incrementally increased in time length from 5 seconds to 10 min. Images were reconstructed using Fourier rebinning of the 3D sonograms followed by two-dimensional filtered back projection with a ramp filter, resulting in an image voxel size of 0.4 x 0.4 x 0.796 mm^3. A standard data correction protocol (normalization, attenuation and decay correction) was applied to the PET data. The PET units were converted into units of radioactivity concentration by applying a calibration factor derived from imaging of a cylindrical phantom with a known ^{11}C-radioactivity concentration. Corresponding PET/MRI images were aligned using a pre-calculated, fixed transformation matrix. Using the image analysis software Amide,[13] different volumes of interest (brain, liver, kidneys, urinary bladder, heart, lung, bone (left humerus), muscle (right forearm muscle)) were manually outlined on multiple planes and time-radioactivity concentration curves (TACs), expressed in standardized uptake values, were derived.

4.4 Analysis of murine metabolites – *In vivo* stability of [^{11}C]-1

Urine and blood samples were harvested directly after imaging experiments (60 min post administration). Murine plasma was obtained by centrifugation. Proteins were precipitated with acetonitrile and removed by centrifugation. Supernatant solution was spotted on SiO_2 plates and TLCs were developed using 10% MeOH and 1% Et_3N in DCM as mobile phase. [^{11}C]-**1** was spotted as reference. Radioactivity was quantified using a Hewlett Packard Cyclone Phosphor Imager autoradiography system (Figure S7). Analysis showed 50% [^{11}C]-**1** in urine and 38% in plasma together with one main metabolite that is considered to be formed by oxidation of the methyl group at position 6 of the Tz scaffold (similar to the previously reported ^{18}F-labeled Tz[15]).

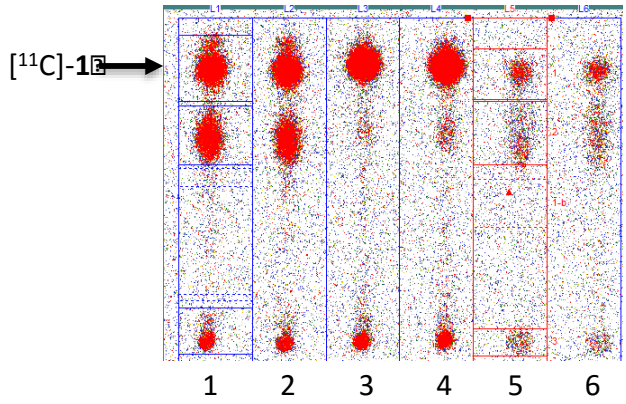


Figure S7. *In vivo* stability of [^{11}C]-**1** as determined by TLC radiochromatography of murine plasma and urine after imaging experiments**:** Lane 1 & 2: Urine metabolites ; Lane 3 & 4: Reference [^{11}C]-**1**; Lane 5 & Lane 6: Plasma metabolites.

4.5 *In vivo* click

For *in vivo* click experiments a group of female BALB/c mice (n=4) was used. A lateral tail vein was used for i.v. administration of water soluble s-TCO derivative **7** (11.8 mg/kg in 0.9% saline, n=2) or 0.9% saline (control group, n=2). After 5 min uptake time the mice were injected with of ^{11}C-[**1**] (mean 8.15 MBq). Retroorbital blood was collected 5 min after administration of ^{11}C-[**1**]. 200 µl of blood was quenched immediately after blood retrieval with 107 µg of tetrazine (4-(1,2,4,5-tetrazin-3-yl)phenyl)methanol in 214 µl water (saturated solution). Murine plasma was obtained by centrifugation and proteins were precipitated with acetonitrile and removed by centrifugation. Supernatant solution was spotted on SiO_2 TLC plates and developed using 20% MeOH in DCM as mobile phase (Fig. S8)

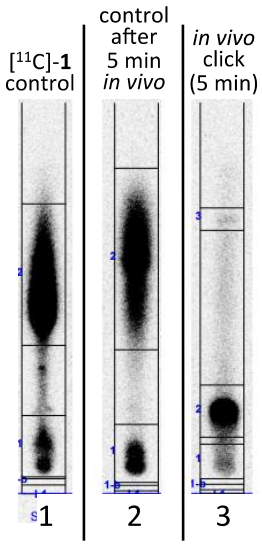


*Figure **S8**. Radio-TLC after in vivo reaction of [^{11}C]-**1** and **7**. Lane 1: [^{11}C]-**1**, Lane 2: control, 5 min biodistribution of [^{11}C]-**1**. Lane 3: after 5 min in vivo reaction .*

Results from this experiment indicate high *in vivo* reactivity between water-soluble s-TCO derivative **7** and [^{11}C]-**1**. After 5 min of *in vivo* reaction time only 1.5 % of total radioactivity originated from [^{11}C]-**1** while >90% of activity was encountered as a single ligation product (Fig. S8, lane 3). Most likely the dihydropyridazine isomers are rapidly oxidized to the appropriate pyridazine resulting in only one detectable aromatized ligation product when the reaction is carried out *in vivo*.

4.6 Biodistribution of [^{11}C]MSNs

The general method for PET data acquisition and reconstruction described under point 4.3 was followed. The nanoparticle suspension was sonicated prior to administration to minimize agglomeration of particles. Female BALB/c mice (n=2) were injected with ^{11}C-labeled MSNs (0.5 mg, 12.9 ± 1.8 MBq) via tail vein catheder and dynamic PET imaging performed for 60 min. PET data revealed rapid and high (18-25 SUV) uptake in the lungs (already after 5 min), while other organs showed much lower activity concentration (Fig. S9). These findings were also confirmed by gamma counter measurements (*ex vivo*).

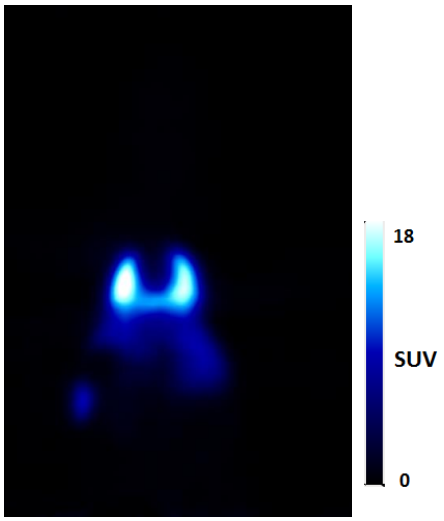


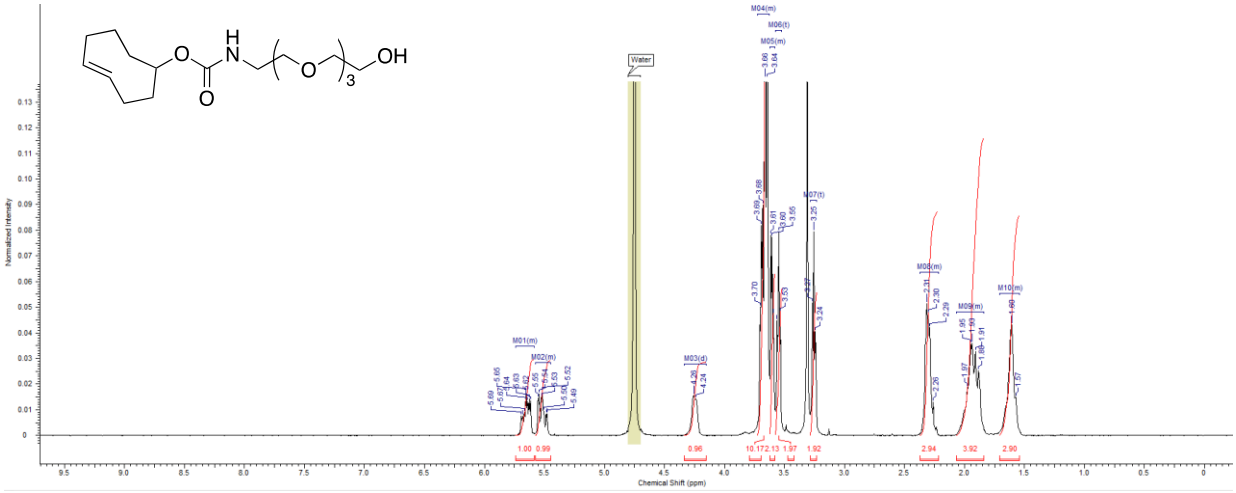
Figure S9. Coronal PET slice (0-60 min) showing high accumulation of [^{11}C]-MSNs in the lung.

4.7 Pretargeted imaging using TCO/s-TCO-MSNs

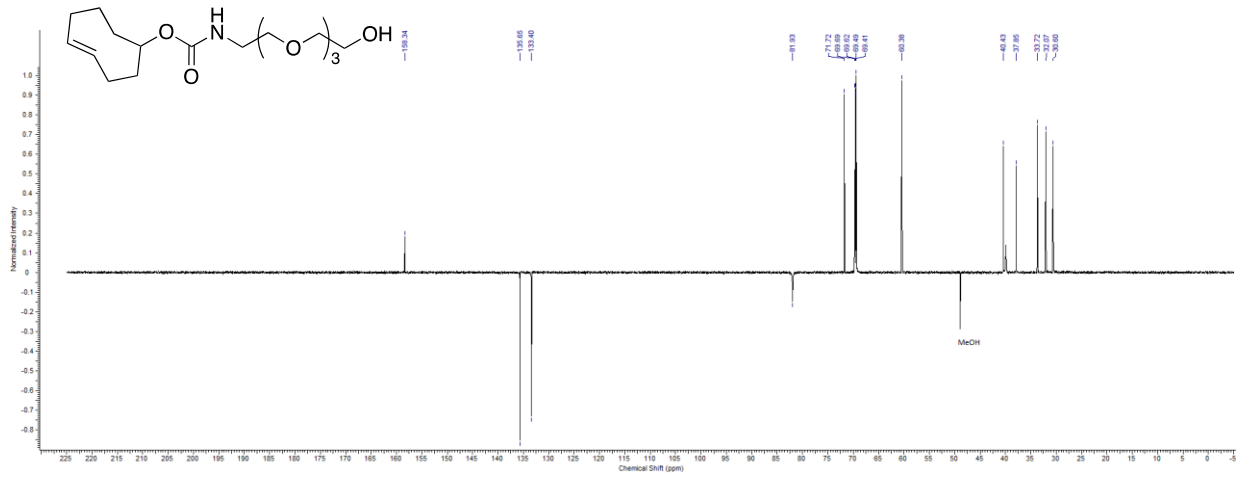
The general method for PET data acquisition and reconstruction described under point 4.3 was followed. Prior to PET imaging functionalized nanoparticles were administered using a tail vein catheter. The nanoparticle suspensions in 0.9% saline were vortexed and sonicated for 60 seconds prior to administration to minimize agglomeration of particles. Animals were divided into two study groups receiving s-TCO-MSNs (n=5, 0.5 mg, 59 nmol s-TCO) or TCO-MSNs (n=2, 0.5 mg, 58 nmol TCO) as suspension in 100 µL physiological saline. 5 min post nanoparticle administration PET data acquisition was started and the animals were dosed with [^{11}C]-**1** (15.4 ± 6.0 MBq, i.v., 100 µl). Dynamic PET scanning was conducted for 60 min. *Ex vivo* measurements were done using a gamma counter.

5. NMR Spectra

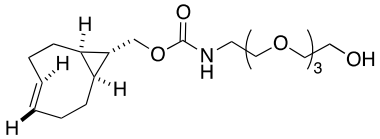
^{1}H spectrum of compound **6**

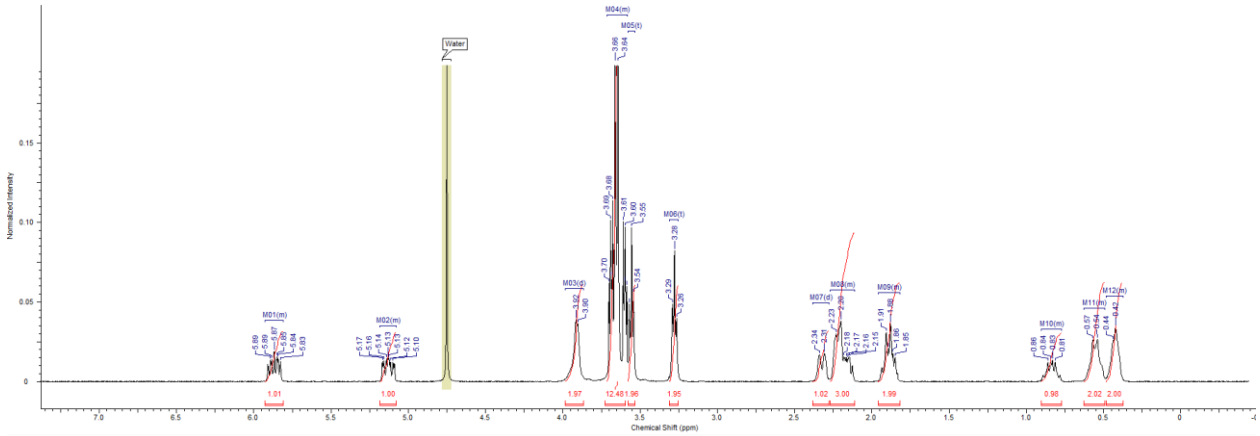


^{13}C spectrum of compound **6**

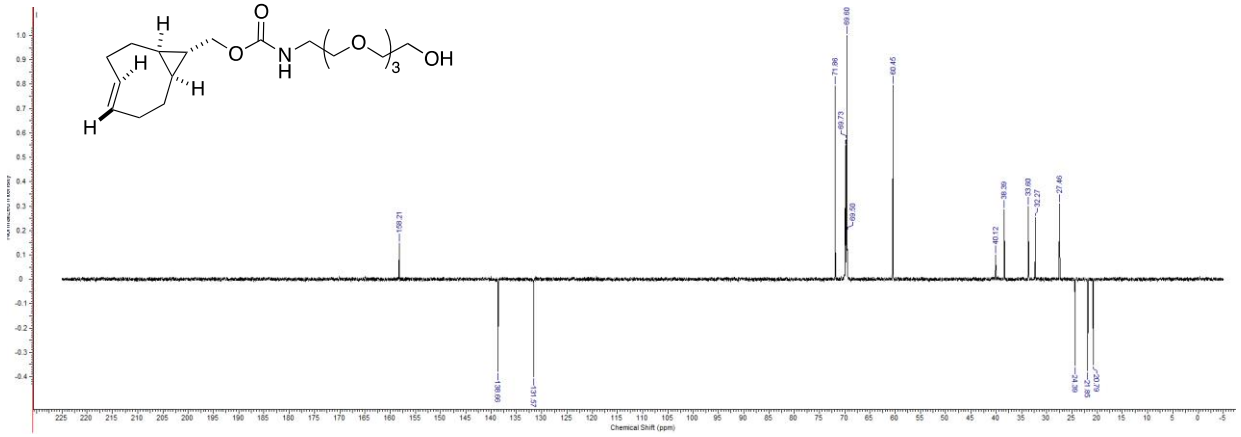


^{1}H spectrum of compound **7**

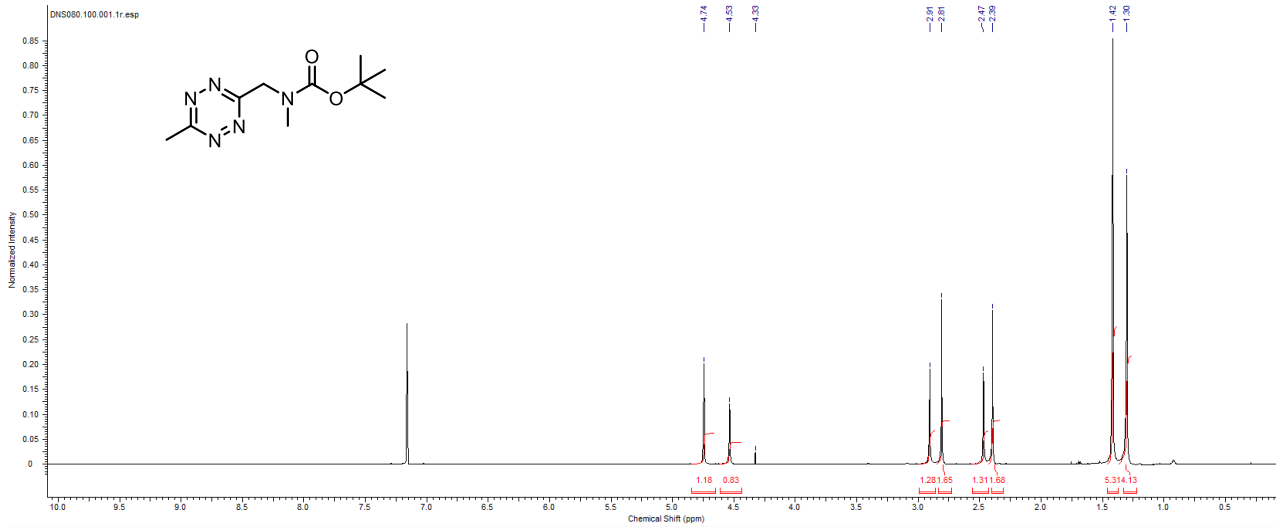




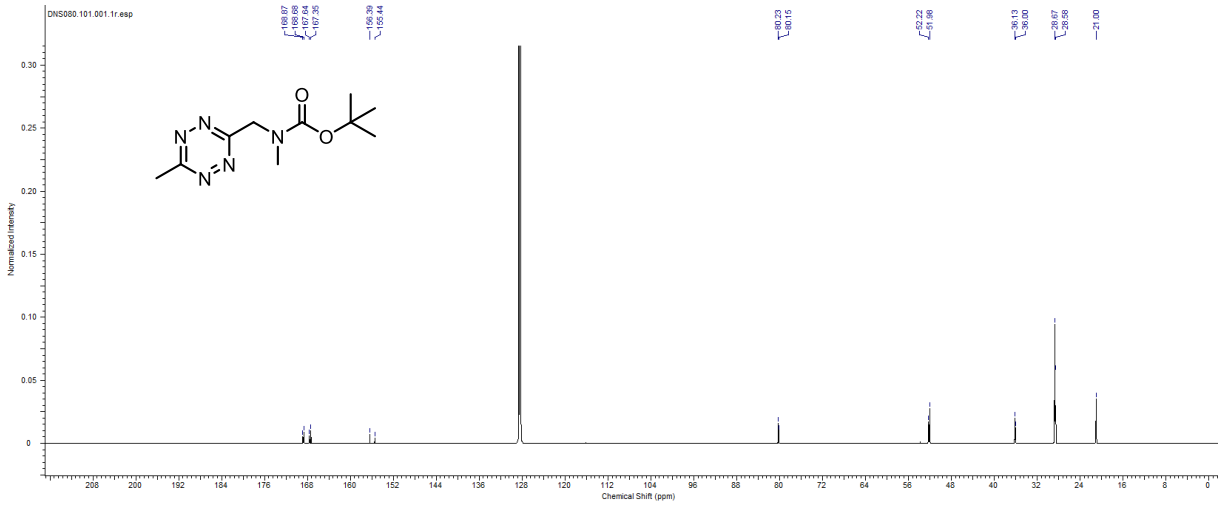
^{13}C spectrum of compound **7**



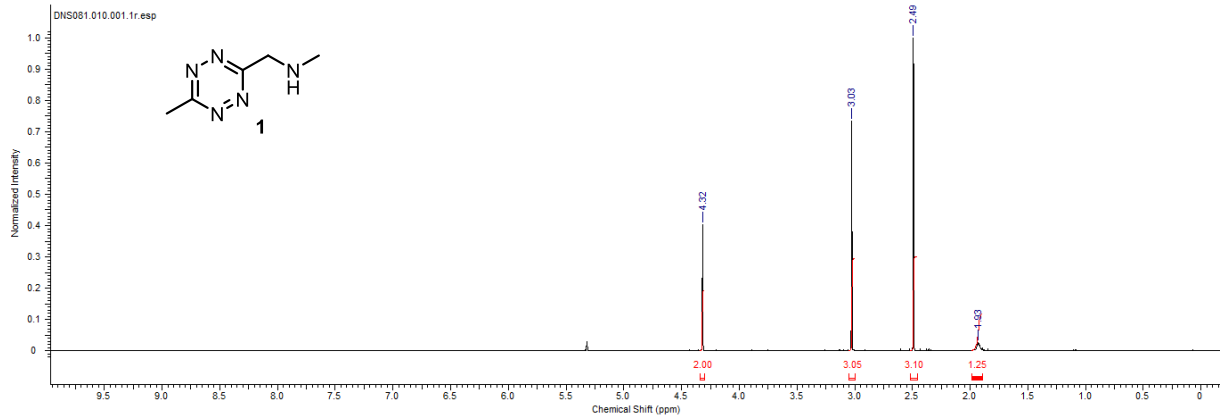
^{1}H spectrum of compound **9**



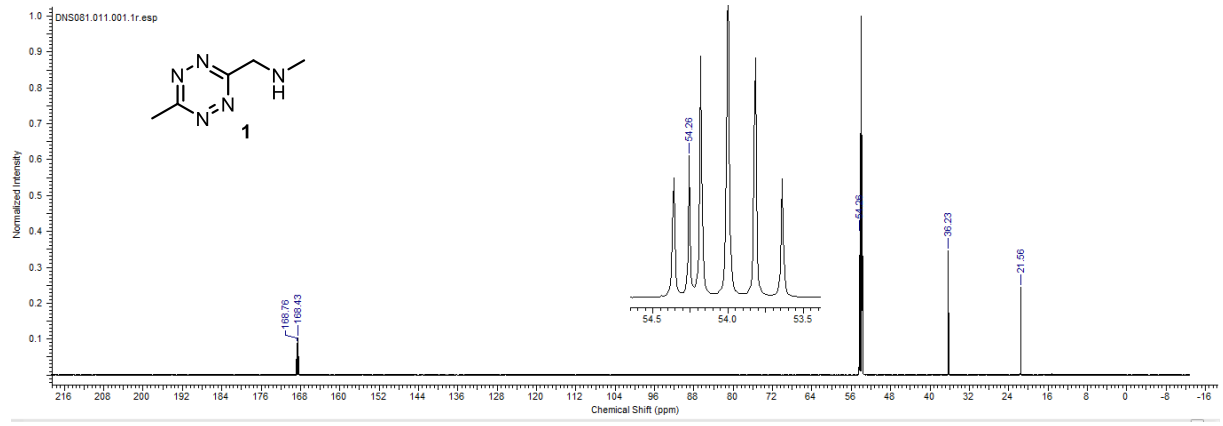
^{13}C spectrum of compound **9**



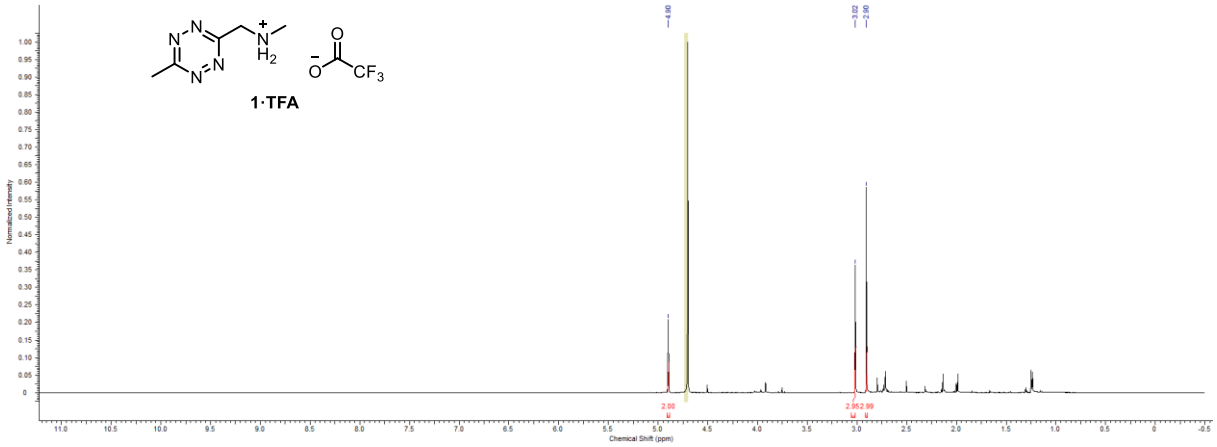
^{1}H spectrum of compound **1**



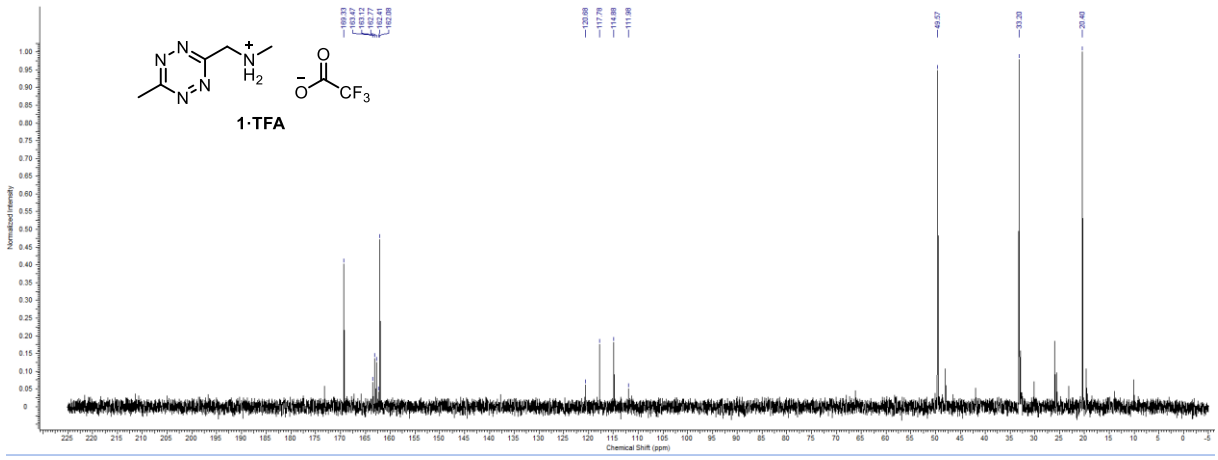
^{13}C spectrum of compound **1**



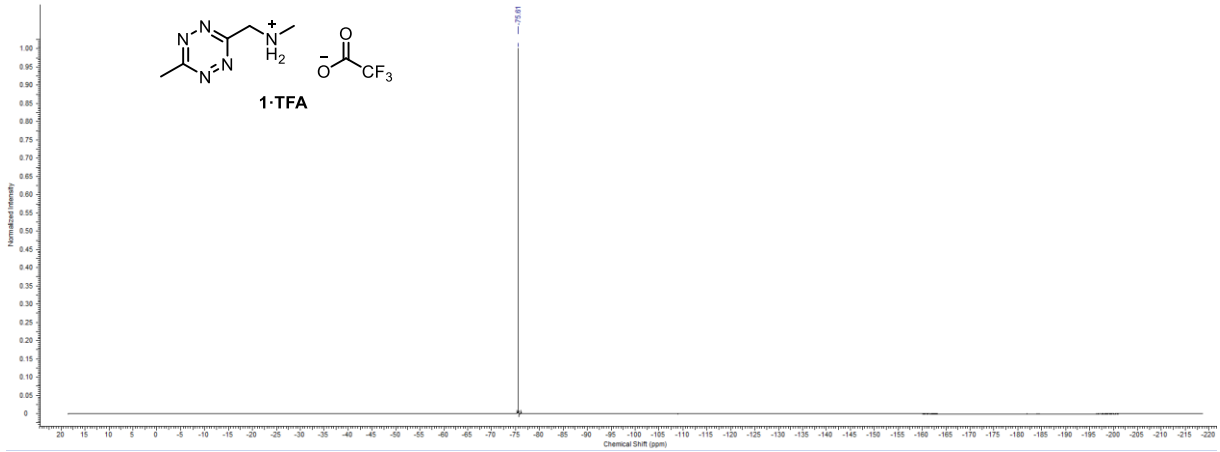
^{1}H spectrum of compound **1·TFA**



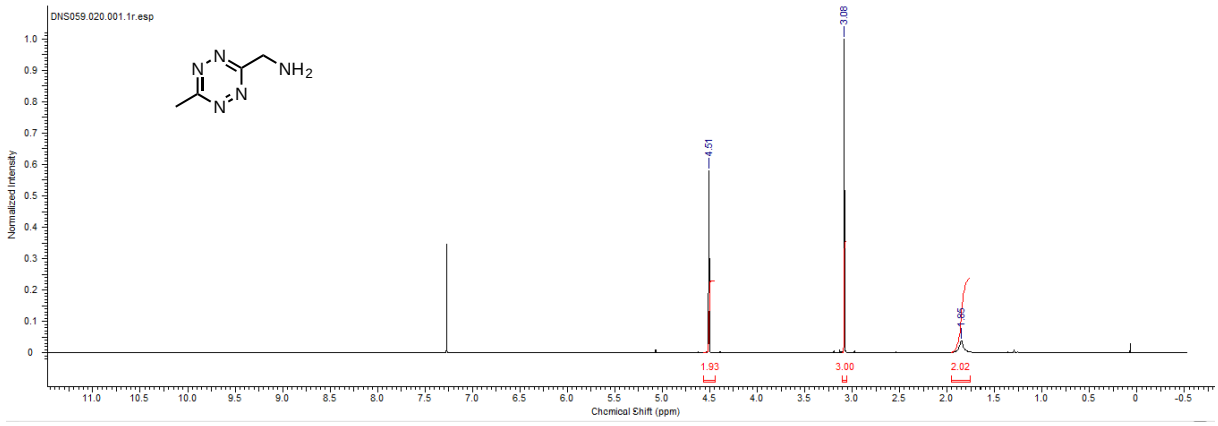
^{13}C spectrum of compound **1·TFA**



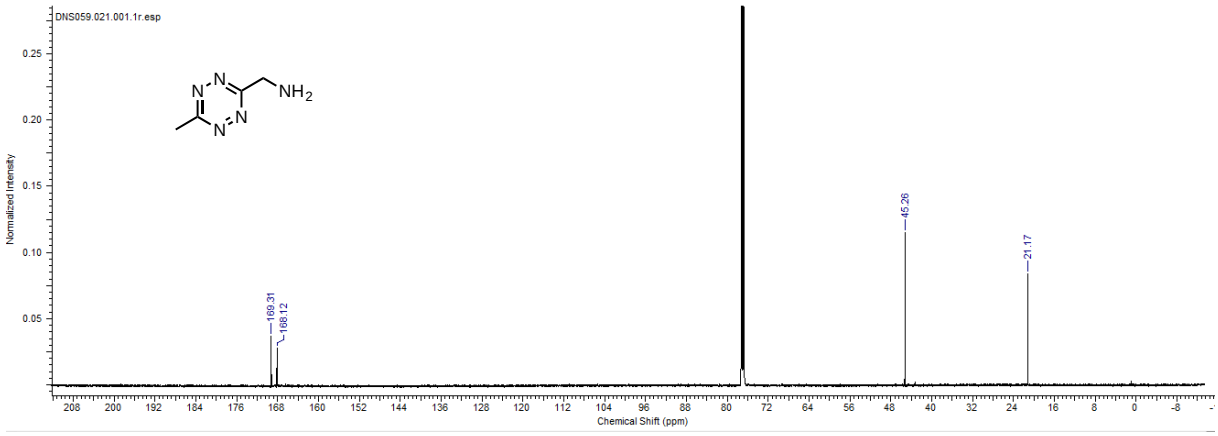
^{19}F spectrum of compound **1·TFA**



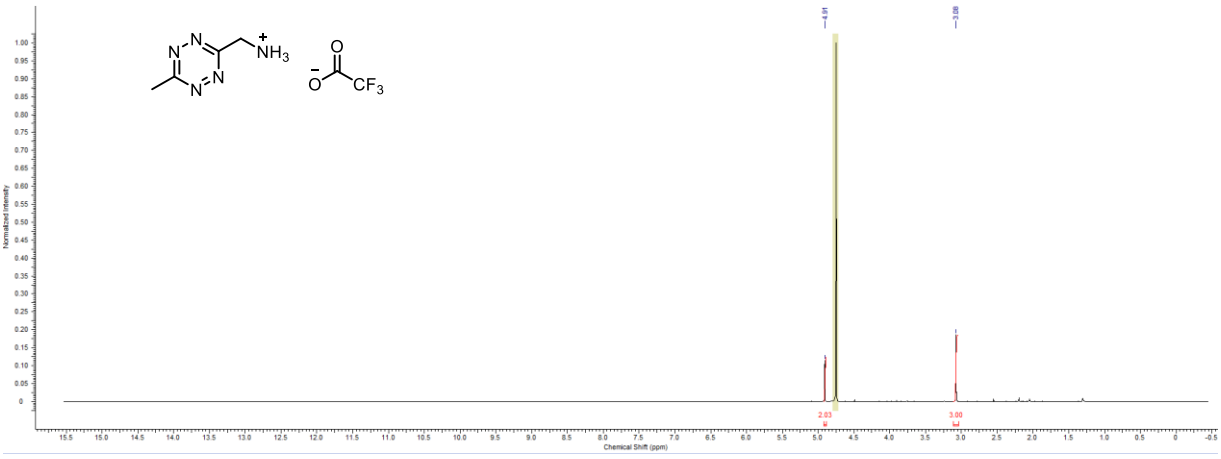
^{1}H spectrum of compound **11**



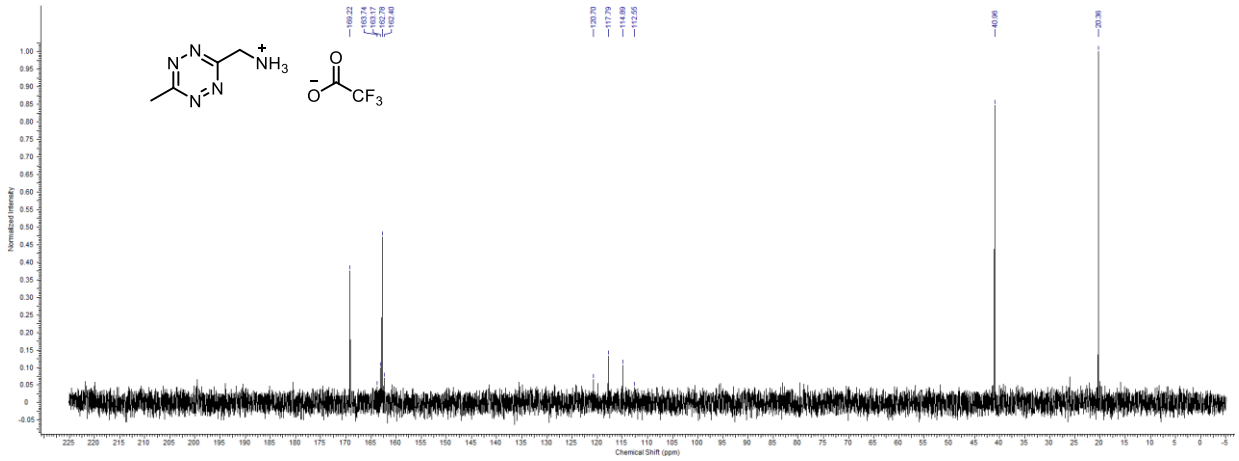
^{13}C spectrum of compound **11**



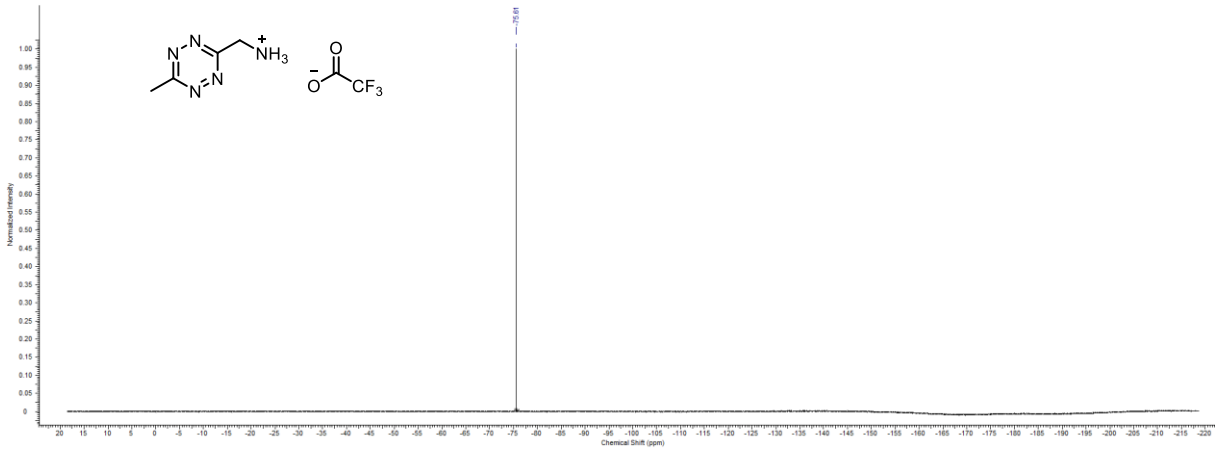
^{1}H spectrum of compound **11·TFA**



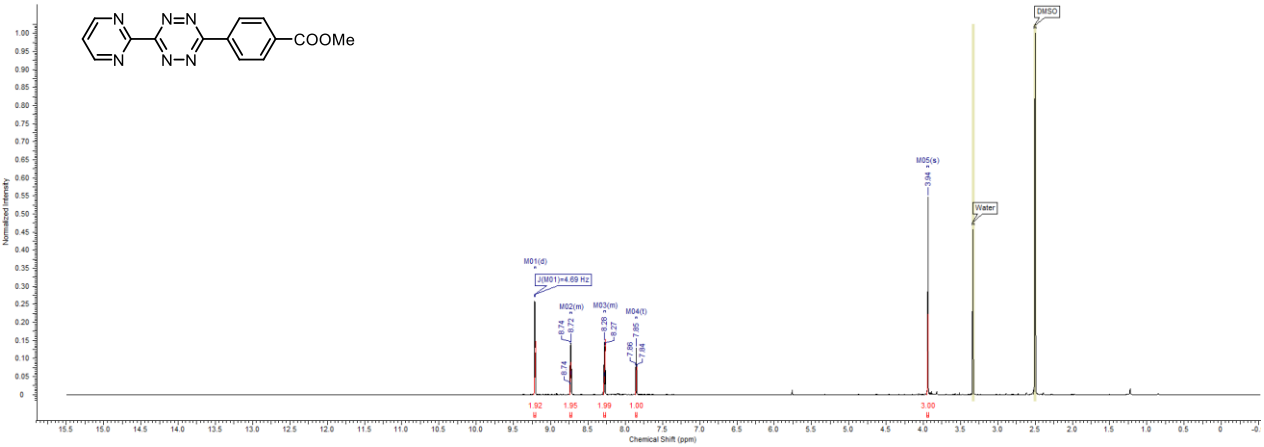
^{13}C spectrum of compound **11·TFA**



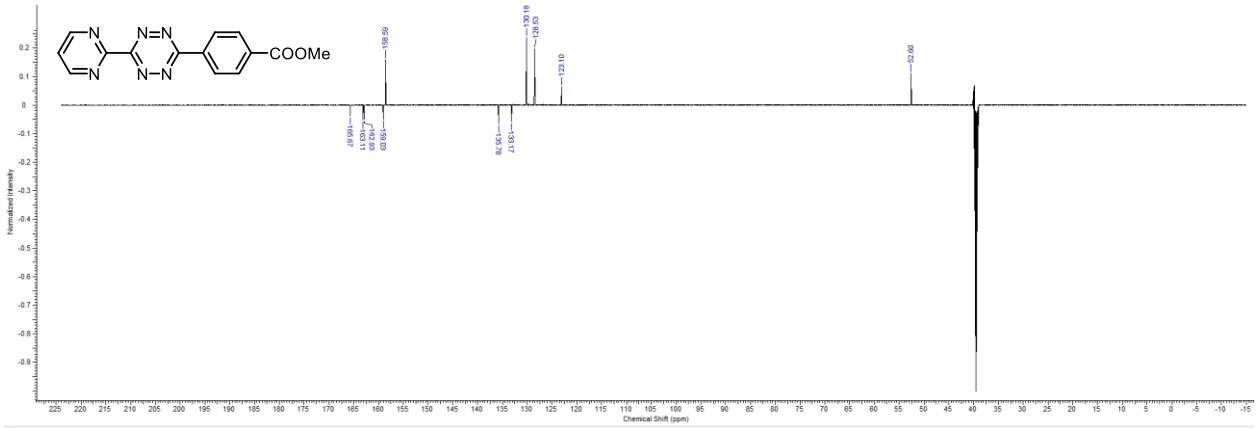
^{19}F spectrum of compound **11·TFA**



^{1}H spectrum of **methyl 4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoate**



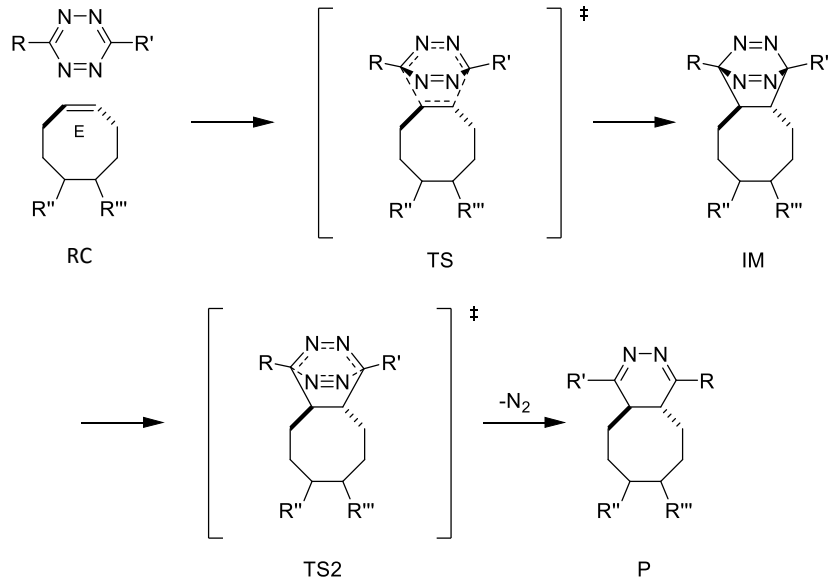
^{13}C spectrum of **methyl 4-(6-(pyrimidin-2-yl)-1,2,4,5-tetrazin-3-yl)benzoate**


COOMe
158.59
130.18
128.83
123.10
52.60
Chemical Shift (ppm)

6. Quantum chemical calculations

All DFT calculations were performed on the Vienna Scientific Cluster 3 using the Gaussian 09 (Rev. D.01) program package.[14] Geometry optimizations to energy minima or transition states as well as subsequent frequency analyses at 298.15 K of all considered compounds and intermediates were achieved using the M06-2X density functional[15] in combination with the 6-31G(d,p)[16] basis set as implemented in Gaussian 09, which has been found to give relatively accurate energy profiles for cycloadditions.[17,18] All calculations were performed in 1,4-dioxane applying the polarizable conductor calculation model (CPCM) as provided in Gaussian 09. Imaginary frequencies corresponding to the desired reaction coordinates were obtained only in the case of transition state calculations. Data analysis was done using GaussView 5 (Gaussian, Inc.).

The reaction of tetrazines with strained dienophiles proceeds in two steps, the first being an inverse electron demand Diels-Alder (IEDDA)-initiated reaction leading to an intermediate that subsequently undergoes retro-Diels-Alder reaction with the loss of N_2 (Scheme S1).



Scheme S1: General mechanism of the tetrazine ligation (RC = reactant complex, TS = transition state, IM = intermediate, P = product).

The first step of this reaction (RC → TS), the inverse electron demand Diels-Alder reaction, is the rate-determining step.[19,20] Therefore, $\Delta G^{\ddagger}$ values of this cycloaddition were considered to determine reaction activation barriers at 298.15 K. Original data is summarized in Table S8 followed by listing of Cartesian coordinates of all relevant structures.

Table S8: Selected original data from Gaussian calculations (E: total electronic energy, ZPE: zero point energy).

Structure	E (hartree)	H (hartree)[a]	G (hartree)[a]	S (Cal/(mol*K))[a]	ZPE (hartree)[a]
TS_{1+4}	-782.533302622	-782.152443	-782.215082	131.835	-782.170739
RC_{1+4}	-782.545771650	-782.164423	-782.234466	147.418	-782.184624
TS_{2+4}	-743.252237286	-742.901448	-742.959890	123.001	-742.918240
RC_{2+4}	-743.272434428	-742.921403	-742.989495	143.313	-742.940619
TS_{3+4}	-865.709502963	-865.323370	-865.388604	137.296	-865.342514
RC_{3+4}	-865.722926843	-865.336327	-865.408282	151.443	-865.357273
TS_{1+5}	-935.088736580	-934.665951	-934.735503	146.384	-934.687298
RC_{1+5}	-935.096619506	-934.673420	-934.749388	159.886	-934.696541
TS_{2+5}	-895.805512643	-895.412924	-895.478989	139.045	-895.432909
RC_{2+5}	-895.820924491	-895.427532	-895.501096	154.829	-895.449600
TS_{3+5}	-1018.26259416	-1017.834645	-1017.908032	154.455	-1017.857081
RC_{3+5}	-1018.27136121	-1017.842855	-1017.922242	167.085	-1017.866880

[a] calculated for T = 298.15

Cartesian Coordinates

TS_{1+4}

C	-0.8947280	2.2034350	-0.1678950
C	-1.8477810	-0.1115990	-0.3334160
C	0.3780830	-0.4376670	-0.2154100
H	0.2098390	-1.0263830	0.6854980
C	0.9026640	0.8240760	-0.0736050
H	1.2853020	1.3073030	-0.9745580
N	-1.8241150	0.6246260	-1.4875910
N	-1.3385720	1.8096960	-1.4014940
N	-2.0102210	0.5343410	0.8588830
N	-1.5150950	1.7180110	0.9481110
C	1.5710320	1.2068610	1.2153040
H	1.5585910	2.2854810	1.3989790
H	1.0536330	0.7190320	2.0498070
C	0.6699980	-1.2370890	-1.4509380
H	-0.1090160	-1.9725360	-1.6667040
H	0.7609670	-0.5667510	-2.3138350
C	3.0381230	0.7226840	1.1567650
H	3.4914380	0.9013430	2.1382270
H	3.5903110	1.3494700	0.4446060
C	2.0034310	-1.9807250	-1.2157500
H	2.2807110	-2.4845420	-2.1485160
H	1.8335500	-2.7725250	-0.4748440
C	3.1743460	-1.0980510	-0.7460670
H	4.0988140	-1.6231450	-1.0068990
H	3.1873730	-0.1734610	-1.3379230
C	3.2282430	-0.7553970	0.7710460
H	4.2071550	-1.0633440	1.1523110
H	2.4974070	-1.3662910	1.3163880
C	-2.4186190	-1.5080600	-0.4525410
C	-0.2702080	3.5631660	-0.0791380
H	0.6468570	3.6121930	-0.6700730
H	-0.0516920	3.8065980	0.9604360
H	-0.9667070	4.3063080	-0.4756250
H	-2.3273870	-1.7968120	-1.5020940
H	-3.4968850	-1.4247340	-0.2272420
N	-1.7572550	-2.5042050	0.3790700
H	-1.9737860	-3.4179540	-0.0051650
C	-2.1746670	-2.4729850	1.7784920
H	-3.2688590	-2.4980280	1.9059700
H	-1.7411870	-3.3303130	2.2982620
H	-1.8091210	-1.5600890	2.2530520

RC_{1+4}

C	-1.6153570	2.0906250	-0.2399240
C	-2.3769350	-0.3532520	-0.2083010
C	0.7691130	-0.3635000	-0.0911220
H	0.6675230	-1.0964170	0.7109460
C	1.1558760	0.8779620	0.2094500
H	1.3067410	1.5872480	-0.6091210
N	-2.0726510	0.1907830	-1.3920760
N	-1.6876250	1.4428200	-1.4100440
N	-2.4208770	0.3316750	0.9434590
N	-2.0260350	1.5778070	0.9295530
C	1.8529210	1.1843950	1.4943950
H	1.7115520	2.2107780	1.8505400
H	1.5088450	0.5022240	2.2808910
C	0.9282680	-0.9162850	-1.4678680
H	0.1925180	-1.6915820	-1.7012500
H	0.8390550	-0.1148030	-2.2116950
C	3.3579570	0.9257630	1.2142460
H	3.9091760	0.9995980	2.1589350
H	3.7406500	1.7279540	0.5700120
C	2.3651940	-1.5016470	-1.5045290
H	2.5978050	-1.7996350	-2.5336270
H	2.3862220	-2.4213180	-0.9053080
C	3.4682520	-0.5486600	-0.9923900
H	4.4131790	-0.8952820	-1.4248950
H	3.3001900	0.4491410	-1.4170940
C	3.6643590	-0.4351030	0.5512020
H	4.7116570	-0.6663140	0.7740530
H	3.0788760	-1.2148360	1.0548380
C	-2.7481470	-1.8116790	-0.1783430
C	-1.0926790	3.4903670	-0.2338490
H	-0.2426850	3.5601590	0.4497290
H	-1.8630220	4.1802050	0.1187980
H	-0.7797400	3.7726810	-1.2384240
H	-3.0276620	-2.0947620	-1.1951140
H	-3.6252590	-1.9281920	0.4793880
N	-1.6131540	-2.6267000	0.2430990
H	-1.7638730	-3.5749420	-0.0847920
C	-1.4308220	-2.6395830	1.6896650
H	-2.3316780	-2.9540090	2.2405910
H	-0.6084920	-3.3127470	1.9429770
H	-1.1671730	-1.6362580	2.0358970

TS_{2+4}

C	-1.1426650	1.7907920	-0.3240800
C	-1.8486690	-0.5978000	-0.0624200
C	0.3379150	-0.5716970	-0.0165180
H	0.3110750	-0.9808160	0.9951420
C	0.6937780	0.7608270	-0.1425640
H	1.0031140	1.0845100	-1.1383150
N	-1.9820530	-0.0580570	-1.3288630
N	-1.6199140	1.1566440	-1.4564530
N	-2.0472570	0.2205230	1.0261490
N	-1.6879390	1.4420110	0.8889290
C	1.4000400	1.4402400	1.0004320
H	1.2619890	2.5254290	0.9946360
H	1.0003240	1.0601330	1.9477840
C	0.6973940	-1.5510590	-1.0926260
H	-0.0071320	-2.3891920	-1.1186290
H	0.6733730	-1.0499290	-2.0678640
C	2.9092180	1.1313580	0.9074000
H	3.3913410	1.5577210	1.7942480
H	3.3275130	1.6660830	0.0447150
C	2.1197850	-2.0830560	-0.8206760
H	2.4079590	-2.7232600	-1.6620640
H	2.0954370	-2.7311440	0.0649080
C	3.1953390	-1.0011420	-0.6165460
H	4.1634760	-1.4626090	-0.8362300
H	3.0669350	-0.2183450	-1.3757190
C	3.2820730	-0.3577370	0.7965560
H	4.3147800	-0.4541640	1.1469460
H	2.6795860	-0.9367250	1.5085750
C	-0.7664060	3.2349790	-0.5046040
H	0.1340060	3.3351550	-1.1155000
H	-0.5987430	3.6993020	0.4673730
H	-1.5782670	3.7624870	-1.0107300
N	-2.1520650	-1.9310730	0.0621450
H	-2.5863140	-2.3087600	-0.7691140
C	-2.6199100	-2.4437910	1.3373120
H	-2.7506730	-3.5223150	1.2462930
H	-1.8706590	-2.2490510	2.1081740
H	-3.5618890	-1.9863170	1.6620290

RC_{2+4}

C	-1.8949710	1.7655720	-0.2336070
C	-2.2085400	-0.7702360	-0.1082070
C	0.7709130	-0.5718850	0.1742720
H	0.7635830	-0.8293680	1.2360780
C	0.9398460	0.7071280	-0.1681490
H	0.9899770	0.9636690	-1.2292030
N	-2.1458880	-0.2056080	-1.3408500
N	-1.9785780	1.0776200	-1.3909920
N	-2.2525910	-0.0807000	1.0452850
N	-2.0880090	1.2174800	0.9661880
C	1.5265810	1.6919650	0.7890210
H	1.2044530	2.7254790	0.6211740
H	1.2651050	1.4185400	1.8180070
C	1.0757840	-1.6789010	-0.7808860
H	0.5041760	-2.5955420	-0.5944230
H	0.8749490	-1.3553980	-1.8094030
C	3.0637110	1.5825770	0.6001400
H	3.5563190	2.1772730	1.3780810
H	3.3357940	2.0427410	-0.3585450
C	2.5931740	-1.9632530	-0.6130300
H	2.9075060	-2.6675440	-1.3920390
H	2.7518670	-2.4726600	0.3461750
C	3.4953710	-0.7110650	-0.6648160
H	4.4990780	-1.0551150	-0.9367560
H	3.1699820	-0.0719380	-1.4952750
C	3.6179890	0.1414240	0.6359530
H	4.6811670	0.2173980	0.8882440
H	3.1560200	-0.3957570	1.4739710
C	-1.6286880	3.2354250	-0.3213170
H	-0.7185970	3.4185100	-0.8984960
H	-1.5097700	3.6461860	0.6812420
H	-2.4521520	3.7482970	-0.8248600
N	-2.2939610	-2.1135440	-0.0602290
H	-2.1639530	-2.5898970	-0.9396200
C	-2.1869570	-2.8427230	1.1868270
H	-1.2334620	-2.6393240	1.6884200
H	-2.9955020	-2.5608280	1.8645730
H	-2.2586240	-3.9076200	0.9696320

TS_{3+4}

C	-0.4510910	2.2505400	0.3467970
C	1.2053330	0.3852900	0.1166910
C	-0.7941460	-0.6229410	0.0086290
H	-0.5329680	-0.9917220	-0.9846920
C	-1.6946110	0.4173940	0.0705840
H	-2.1527470	0.6098010	1.0422490
N	1.0487120	0.9083460	1.3718360
N	0.1960450	1.8602480	1.4898090
N	1.0480830	1.2018490	-0.9705960
N	0.2024760	2.1601050	-0.8534310
C	-2.5380150	0.7432730	-1.1292200
H	-2.8586500	1.7889100	-1.1536310
H	-1.9614140	0.5501560	-2.0412110
C	-0.7641340	-1.6375120	1.1164880
H	0.2059990	-2.1322460	1.2163860
H	-0.9837570	-1.1404860	2.0686660
C	-3.7884950	-0.1630250	-1.0990800
H	-4.3418410	-0.0008530	-2.0306450
H	-4.4476940	0.1704940	-0.2873550
C	-1.8386890	-2.7079450	0.8226660
H	-1.8853400	-3.3800260	1.6866430
H	-1.5055650	-3.3203600	-0.0250240
C	-3.2489510	-2.1702600	0.5206020
H	-3.9516970	-2.9834010	0.7278840
H	-3.4985620	-1.3805980	1.2411130
C	-3.5108230	-1.6673680	-0.9276800
H	-4.3835090	-2.2005210	-1.3179030
H	-2.6769310	-1.9583610	-1.5798420
C	2.1903100	-0.7383220	-0.0327060
C	-1.4747460	3.3365760	0.4950990
H	-2.3413340	2.9877450	1.0611570
H	-1.7945630	3.6846300	-0.4869250
H	-1.0328300	4.1748900	1.0392390
H	1.9083120	-1.3527180	-0.8947530
H	2.1471380	-1.3624450	0.8640870
C	3.6120650	-0.1955140	-0.2139940
H	3.6510160	0.4441350	-1.1007920
H	3.8828830	0.4173730	0.6520390
C	4.6155420	-1.3203620	-0.3605230
H	4.3902160	-1.9365310	-1.2387620
H	4.6205770	-1.9638960	0.5268910
F	5.8843050	-0.7885280	-0.5171400

RC_{3+4}

C	0.0134640	2.5858340	0.2722770
C	1.5415420	0.5365480	0.2078290
C	-1.1564470	-0.7594440	0.0138320
H	-0.8498360	-1.0395950	-0.9971760
C	-1.9447830	0.3075330	0.1652900
H	-2.2890350	0.5649100	1.1700130
N	1.2172750	1.0338060	1.4090100
N	0.4359820	2.0814320	1.4411400
N	1.2373790	1.1255860	-0.9556320
N	0.4544540	2.1729830	-0.9248860
C	-2.7401550	0.8466150	-0.9788480
H	-2.9660710	1.9159690	-0.9034310
H	-2.2047050	0.6791710	-1.9208420
C	-1.0850460	-1.8291850	1.0538610
H	-0.1331140	-2.3712730	1.0725580
H	-1.2512310	-1.3999570	2.0488120
C	-4.0605750	0.0300390	-0.9824430
H	-4.6290310	0.2880730	-1.8832720
H	-4.6741410	0.3464380	-0.1291330
C	-2.2403720	-2.8092660	0.7135090
H	-2.3303890	-3.5399160	1.5256080
H	-1.9674170	-3.3788920	-0.1840790
C	-3.6115250	-2.1385420	0.4794150
H	-4.3720150	-2.9090990	0.6445460
H	-3.7797800	-1.3887860	1.2627980
C	-3.8734690	-1.5012330	-0.9200390
H	-4.7880280	-1.9474300	-1.3251730
H	-3.0746260	-1.7911100	-1.6145300
C	2.3765800	-0.7054310	0.1592460
C	-0.9576700	3.7207790	0.3187200
H	-1.8541810	3.4180090	0.8657590
H	-1.2285930	4.0174670	-0.6940380
H	-0.5176040	4.5716020	0.8444690
H	1.9363030	-1.3898380	-0.5747020
H	2.3342680	-1.1815810	1.1430300
C	3.8248400	-0.3869610	-0.2292240
H	3.8416330	0.1083590	-1.2053670
H	4.2636270	0.3016220	0.5004220
C	4.6669380	-1.6443180	-0.2930860
H	4.2678500	-2.3482750	-1.0324490
H	4.7060390	-2.1436840	0.6817630
F	5.9582810	-1.3107380	-0.6627820

TS_{1+5}

C	-2.5956420	0.4068510	-0.5141960
C	-0.1805810	1.2820370	-0.8509890
C	-0.5201260	-1.7053070	1.2891840
C	0.3846330	0.2920350	0.1267220
C	0.0160180	-1.0222260	0.0559330
C	-1.6291040	1.5699780	-0.3828990
C	-1.8325270	-0.9712180	1.6593670
H	-0.1835370	0.8711470	-1.8679240
H	0.1935450	-1.6403440	2.1183470
H	0.6197690	0.7048770	1.1088520
H	-0.3776910	-1.3739470	-0.8983340
H	0.3829040	2.2189400	-0.8482480
H	-0.7253870	-2.7635220	1.1003820
H	-2.0326950	2.4091840	-0.9616210
H	-1.5751080	0.0008470	2.0957900
H	-1.5918760	1.9109370	0.6593140
H	-2.3768030	-1.5313550	2.4288670
N	2.6815160	-1.6652730	0.7634490
N	2.5325070	-0.4047970	-1.6115620
C	2.0040300	-2.1702830	-0.3050870
C	2.6447930	0.2524150	-0.4243280
N	2.2066130	-1.6480890	-1.5501260
N	3.0112150	-0.4204460	0.6988600
C	-2.7226910	-0.7615480	0.4488690
C	-3.7839340	0.2989780	0.4039760
H	-3.7897230	1.0007720	1.2371340
H	-2.9950520	-1.6951500	-0.0450500
H	-2.8028830	0.1393320	-1.5503490
C	1.5506970	-3.5954330	-0.2291430
C	2.9897860	1.7147480	-0.4800160
C	-5.1395240	-0.0056960	-0.1650490
H	-5.8050610	-0.3852020	0.6240590
H	-5.0378580	-0.7990650	-0.9221100
O	-5.6574960	1.1831260	-0.7382200
H	-6.5532390	1.0032550	-1.0439030
H	2.4724070	2.1619350	-1.3350420
H	4.0721630	1.7920360	-0.6930280
H	2.3337630	-4.2489870	-0.6233120
H	1.3556830	-3.8705610	0.8074580
H	0.6507840	-3.7450060	-0.8288800
N	2.6031760	2.3938960	0.7421820
H	3.0245230	1.8967900	1.5233930
C	3.0382900	3.7830170	0.7477250
H	2.5096300	4.3287410	-0.0404640
H	2.7824430	4.2427520	1.7044230
H	4.1193740	3.9094560	0.5759670

RC_{1+5}

C	-2.7867280	0.4070500	-0.5005950
C	-0.3806880	1.3735680	-0.6227120
C	-0.6891780	-1.9607790	0.9866150
C	0.1059330	0.2486620	0.2351940
C	-0.1871000	-1.0169520	-0.0714450
C	-1.8570320	1.5670840	-0.1748690
C	-2.0562400	-1.3602760	1.4215460
H	-0.3432770	1.1098740	-1.6868870
H	-0.0170440	-2.0093650	1.8515020
H	0.3253340	0.5062770	1.2741630
H	-0.4549560	-1.2478100	-1.1036810
H	0.1803690	2.2999400	-0.4642570
H	-0.8180820	-2.9779690	0.6021010
H	-2.2721380	2.4688660	-0.6411720
H	-1.8492400	-0.4904630	2.0561690
H	-1.8633410	1.7525770	0.9069010
H	-2.6233200	-2.0749850	2.0309510
N	2.9313680	-1.4955290	0.8647600
N	2.8099230	-0.3052590	-1.5605470
C	2.5695480	-2.1411720	-0.2506700
C	2.9714040	0.3723700	-0.4157020
N	2.6042570	-1.5908910	-1.4762720
N	3.1387050	-0.2054610	0.7769220
C	-2.9034990	-0.9206220	0.2366750
C	-3.9857910	0.1126180	0.3619050
H	-4.0134140	0.6531630	1.3075140
H	-3.1506490	-1.7464450	-0.4321000
H	-2.9744530	0.3085180	-1.5701560
C	2.2028870	-3.5862830	-0.1466340
C	3.0843890	1.8679090	-0.4982360
C	-5.3311050	-0.1077390	-0.2671330
H	-5.9900530	-0.6485110	0.4282610
H	-5.2072080	-0.7378360	-1.1621400
O	-5.8782450	1.1562310	-0.6047910
H	-6.7742350	1.0171740	-0.9303190
H	2.4668580	2.2019100	-1.3398070
H	4.1302290	2.1219510	-0.7551430
H	3.0493550	-4.2121450	-0.4432400
H	1.9300680	-3.8256700	0.8812660
H	1.3686080	-3.8050790	-0.8153280
N	2.6222600	2.4952140	0.7235690
H	3.1150830	2.0807450	1.5094060
C	2.8243350	3.9355500	0.6995730
H	2.1961200	4.3749990	-0.0823550
H	2.5167060	4.3627260	1.6560260
H	3.8662400	4.2352520	0.5005690

TS_{2+5}

C	2.5028470	-0.4292780	-0.4649670
C	0.1706670	-1.5102170	-0.7037850
C	0.2512310	1.6630290	1.1613240
C	-0.4862630	-0.5081130	0.1982350
C	-0.2546340	0.8339720	0.0040430
C	1.6345440	-1.6513800	-0.2227870
C	1.6184950	1.0706750	1.5758470
H	0.1516530	-1.1591240	-1.7427200
H	-0.4469070	1.6231660	2.0049570
H	-0.6345220	-0.8425950	1.2280560
H	0.1289850	1.1150430	-0.9774690
H	-0.3350730	-2.4794810	-0.6559660
H	0.3705590	2.7119220	0.8717930
H	2.1035140	-2.5020350	-0.7311320
H	1.4460180	0.1208280	2.0948590
H	1.6301430	-1.9010070	0.8459920
H	2.1157810	1.7373540	2.2902800
N	-2.8617620	1.2518810	0.6903460
N	-2.6504110	-0.2922800	-1.5078480
C	-2.2040900	1.6551570	-0.4405210
C	-2.6689840	-0.8014740	-0.2256040
N	-2.4143280	0.9584050	-1.6102370
N	-3.1013020	-0.0047890	0.8046820
C	2.5236460	0.8310860	0.3828290
C	3.6738590	-0.1325130	0.4329320
H	3.7370680	-0.7490400	1.3289240
H	2.7156830	1.7355540	-0.1957690
H	2.6890040	-0.2426950	-1.5226140
C	4.9998680	0.2357360	-0.1667570
H	5.6226680	0.7508620	0.5793050
H	4.8315080	0.9377470	-0.9986090
O	5.6272930	-0.9521540	-0.6194100
H	6.5066120	-0.7240490	-0.9401600
C	-1.9450390	3.1270110	-0.5871920
H	-2.7986750	3.6072640	-1.0724910
H	-1.8028070	3.5784930	0.3950590
H	-1.0605330	3.3046930	-1.2027760
N	-2.8174730	-2.1571230	-0.1097520
C	-3.3921710	-2.7253000	1.0950270
H	-2.7997840	-2.4242000	1.9620570
H	-4.4261990	-2.4034860	1.2667840
H	-3.3581730	-3.8117380	1.0125120
H	-3.0317790	-2.6092430	-0.9876890

RC_{2+5}

C	-2.6823310	0.4312540	-0.4290070
C	-0.3801880	1.6234710	-0.3375130
C	-0.4258190	-1.8680210	0.9268730
C	0.1808070	0.4720970	0.4378490
C	0.0183060	-0.7756580	-0.0042480
C	-1.8863570	1.6365860	0.0508050
C	-1.8641420	-1.4499010	1.3467470
H	-0.2719070	1.4603990	-1.4168530
H	0.2132230	-1.9403830	1.8142160
H	0.3223970	0.6349890	1.5097310
H	-0.1846980	-0.9231500	-1.0660000
H	0.0756980	2.5881630	-0.0885410
H	-0.4342450	-2.8473520	0.4373470
H	-2.3676790	2.5364250	-0.3511210
H	-1.7753030	-0.6371870	2.0771870
H	-1.9554280	1.7137370	1.1434600
H	-2.3818900	-2.2766740	1.8485140
N	3.2026280	-1.0578600	0.7924960
N	2.8660710	0.3248550	-1.5142310
C	2.8830460	-1.6377430	-0.3647860
C	3.0312800	0.9140630	-0.3035010
N	2.7822130	-0.9678890	-1.5308160
N	3.2853280	0.2491080	0.8380100
C	-2.7000170	-0.9697240	0.1692540
C	-3.8812870	-0.0588620	0.3394070
H	-3.9993690	0.3775850	1.3307220
H	-2.8365840	-1.7409570	-0.5900910
H	-2.8168090	0.4242430	-1.5110030
C	-5.1725230	-0.3357670	-0.3748210
H	-5.8070790	-0.9953650	0.2349550
H	-4.9537560	-0.8644800	-1.3160310
O	-5.8192310	0.9017800	-0.6233690
H	-6.6854320	0.7155700	-1.0017040
C	2.6962520	-3.1222490	-0.3994690
H	1.7927820	-3.3718290	-0.9604060
H	3.5423070	-3.6088730	-0.8925160
H	2.6112120	-3.5050720	0.6177140
N	3.0056310	2.2607590	-0.2727380
H	2.7073370	2.7088660	-1.1257630
C	2.9856280	2.9928330	0.9772290
H	2.9294870	4.0569790	0.7518510
H	2.1255370	2.7071090	1.5945830
H	3.8950210	2.7959400	1.5487320

TS_{3+5}

C	-3.0883090	-0.2794450	-0.5369970
C	-1.5781320	1.8121450	-0.7542370
C	-0.3155960	-0.8436990	1.4703580
C	-0.6282250	1.3275460	0.3048240
C	-0.1749170	0.0384140	0.2548150
C	-2.9659080	1.2269100	-0.3906040
C	-1.8352750	-0.9920400	1.7283360
H	-1.2626900	1.4554410	-1.7418720
H	0.1740700	-0.3958690	2.3425820
H	-0.7577160	1.7722780	1.2946010
H	-0.2355820	-0.4621910	-0.7118770
H	-1.6481460	2.9028890	-0.7914160
H	0.1303790	-1.8283830	1.2995050
H	-3.7284460	1.6891320	-1.0282030
H	-2.2115920	-0.0509120	2.1458050
H	-3.2093630	1.5231950	0.6375900
H	-2.0171100	-1.7688580	2.4801540
N	2.3221780	0.9933550	1.1417190
N	1.6201420	2.0717350	-1.2218680
C	2.1036160	0.2497310	0.0200250
C	1.2701560	2.6181340	-0.0221350
N	2.0501620	0.8583200	-1.1987240
N	1.8919770	2.2065040	1.1204850
C	-2.5961670	-1.3184210	0.4567710
C	-4.0652880	-1.0492450	0.3111490
H	-4.5254830	-0.4799520	1.1179220
H	-2.2547570	-2.2381670	-0.0198080
H	-3.0331590	-0.6120230	-1.5735010
C	2.5620390	-1.1787930	0.0458980
C	0.7042020	4.0046620	-0.0280090
C	-4.9679500	-2.0660700	-0.3257370
H	-5.3442240	-2.7661220	0.4343000
H	-4.3872900	-2.6514950	-1.0560680
O	-6.0347670	-1.3770430	-0.9551540
H	-6.6576510	-2.0308460	-1.2910560
H	0.2501950	4.2176110	-0.9958050
H	-0.0374070	4.1252000	0.7642550
H	1.5070000	4.7253080	0.1491710
H	2.3876330	-1.5812640	1.0480680
H	1.9637200	-1.7577070	-0.6663860
C	4.5183380	-2.7210640	-0.3008740
H	4.3690260	-3.1805910	0.6830040
H	3.9792900	-3.3133990	-1.0495680
C	4.0495620	-1.2809100	-0.3071190
H	4.6375860	-0.7077780	0.4173590
H	4.2236650	-0.8463870	-1.2963890
F	5.8691220	-2.7688100	-0.6001330

RC_{3+5}

C	-3.3779760	-0.2115390	-0.5392920
C	-1.7920550	1.8351970	-0.6793960
C	-0.5006070	-1.0659880	1.2494900
C	-0.8774010	1.1868430	0.3132390
C	-0.4641600	-0.0680620	0.1253420
C	-3.1956040	1.2806270	-0.3039780
C	-2.0135770	-1.2242280	1.5747180
H	-1.5347340	1.5413340	-1.7037020
H	0.0349060	-0.7113890	2.1376620
H	-0.9209290	1.5771050	1.3342520
H	-0.4887870	-0.4690160	-0.8892330
H	-1.8004330	2.9288460	-0.6280680
H	-0.0661030	-2.0274260	0.9557870
H	-3.9687480	1.8059910	-0.8773770
H	-2.3298150	-0.3336370	2.1305260
H	-3.3853950	1.5150280	0.7513470
H	-2.1850440	-2.0879730	2.2288640
N	2.4232140	0.9595480	1.2096830
N	1.8884980	2.1308170	-1.1697400
C	2.4817810	0.3074990	0.0397390
C	1.6613570	2.7271340	0.0090520
N	2.3105520	0.8926790	-1.1524520
N	2.0034910	2.1967760	1.1934900
C	-2.8672240	-1.3587640	0.3233280
C	-4.3290270	-1.0164590	0.3067700
H	-4.7008770	-0.5016820	1.1920750
H	-2.6070210	-2.2375490	-0.2684660
H	-3.4038350	-0.4664860	-1.5990820
C	2.8749070	-1.1368210	0.0544680
C	1.0623050	4.0960940	0.0090770
C	-5.3265530	-1.9284620	-0.3466650
H	-5.6926140	-2.6712920	0.3770890
H	-4.8279080	-2.4770580	-1.1610320
O	-6.3919460	-1.1351100	-0.8426490
H	-7.0703590	-1.7244680	-1.1900550
H	0.7814140	4.3759190	-1.0058050
H	0.1798300	4.1117250	0.6533140
H	1.7774070	4.8221650	0.4035160
H	2.6486980	-1.5444330	1.0441950
H	2.2665450	-1.6665300	-0.6866280
C	4.7588540	-2.7670290	-0.2975170
H	4.5763560	-3.2425440	0.6728890
H	4.1989710	-3.3114050	-1.0666260
C	4.3635670	-1.3051640	-0.2708620
H	4.9681810	-0.7831410	0.4781490
H	4.5783650	-0.8549080	-1.2454840
F	6.1084100	-2.8722560	-0.58543

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