Supporting Information

A trifunctional linker suitable for conducting three orthogonal click chemistries in one pot

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Experimental

Materials and Methods

All chemicals for the synthesis of monomers, precursors as well as solvents and auxiliary materials were purchased from commercial sources (Sigma Aldrich, VWR, Carl Roth, Fluka, Alfa Aesar) and used without further purification unless stated otherwise. Reactions were monitored by TLC analysis, using silica gel 60 F254 on aluminium sheets (Merck) was used. Visualization was done by exposure with UV light and / or dipping into an aqueous solution of $KMnO_4$ (0.1 %) or sulphuric solution of cerium sulphate /ammonium molybdate. Silica gel 60 (220-440 mesh ASTM) was used for column chromatography.

NMR spectroscopy (1H, proton-decoupled ^{13}C-APT, ^{13}C, COSY, HSQC) was done on a Bruker Avance 300 MHz spectrometer. NOE experiments were done on a Varian Inova 500 MHz spectrometer. Deuterated solvents (Chloroform-d, DMSO-d^6, D_2O) were obtained from Cambridge Isotope Laboratories Inc. and remaining peaks were referenced according to literature. Peak shapes are specified as follows: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), m (multiplet). FT-IR spectra were recorded on a Bruker Alpha-P infrared spectrometer, equipped with an attenuated total reflection (ATR) accessory using a diamond crystal.

Electron impact (EI, 70 eV) mass spectra were recorded on a Waters GCT Premier equipped with direct insertion (DI). MALDI-TOF mass spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer.

Synthesis

Literatur: Benzylazide,[1] and pyTz[2] were prepared following procedures from the respective references. 1-(2-hydroxyethyl)-1H-pyrrole-2,5-dione was prepared in a three-step synthesis from furan, maleic anhydride and 2-aminoethanol.[3] *cis*-5-Norbornene-*endo*-2,3-dicarboxylic anhydride [4] and *cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride [5] were prepared from Diels-Alder reactions of maleic anhydride and freshly distilled cyclopentadiene.

Synthesis of *endo, endo* 3-((prop-2-yn-1-yloxy)carbonyl)bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (*endo*-**2**)[6]

A 100 mL Schlenk flask equipped with magnetic stir bar was filled with 1.12g (6.822 mmol, 1 equiv.) *cis*-5-Norbornene-*endo*-2,3-dicarboxylic anhydride. After addition of 20 mL dry DCM and 50 µL triethylamine, 887 µL (15.01 mmol, 2.2 equiv.) propargyl alcohol was added dropwise at room temperature. The mixture was heated to reflux for 48 h. Afterwards the mixture was concentrated *in vacuo* and the colourless residue was recrystallized from cyclohexane.

Yield: 1.306 g (5.93 mmol, 87%), white solid

IR (cm^{-1}): 3280 ($\nu_{C\equiv C-H}$), 3170 - 2470 (ν_{COOH}), 2180 ($\nu_{C\equiv C}$), 1742 ($\nu_{C=O}$), 1694 ($\nu_{C=O}$)

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.33 – 1.36 (d, $^3J_{HH}$ = 8.6 Hz , 1H, H-7a), 1.49 – 1.51 (d, $^3J_{HH}$ = 8.6 Hz, 1H, 7b), 2.46– 2.47 (t, 1H, -C≡C-H), 3.19 – 3.21 (2H, H-1, H-4), 3.31 – 3.32 (dd, $^3J_{HH}$ =10.2, 2.9, 1H, H-3), 3.35 – 3.36 (dd, $^3J_{HH}$ =10.1, 3.1 Hz, 1H, H-2), 4.50 – 4,56 (dd, $^3J_{HH}$ =15.6 Hz, 2.3 Hz, 1H, -O-CH_2-C≡), 4,64 – 4.69 (dd, $^3J_{HH}$ =15.6 Hz, 2.3 Hz, 1H, -O-CH_2-C≡), 6.20 – 6.22 (m, 1H, H-5), 6.33 – 6.35 (m, 1H, H-6), 9.41 (bs, 1H, -COO*H*)

APT (100 MHz, $CDCl_3$,) δ: 46.23, 46.80, 48.03, 48.31, 48.88, 52.05, 74.96, 134.49, 135.75, 171.73, 178.19

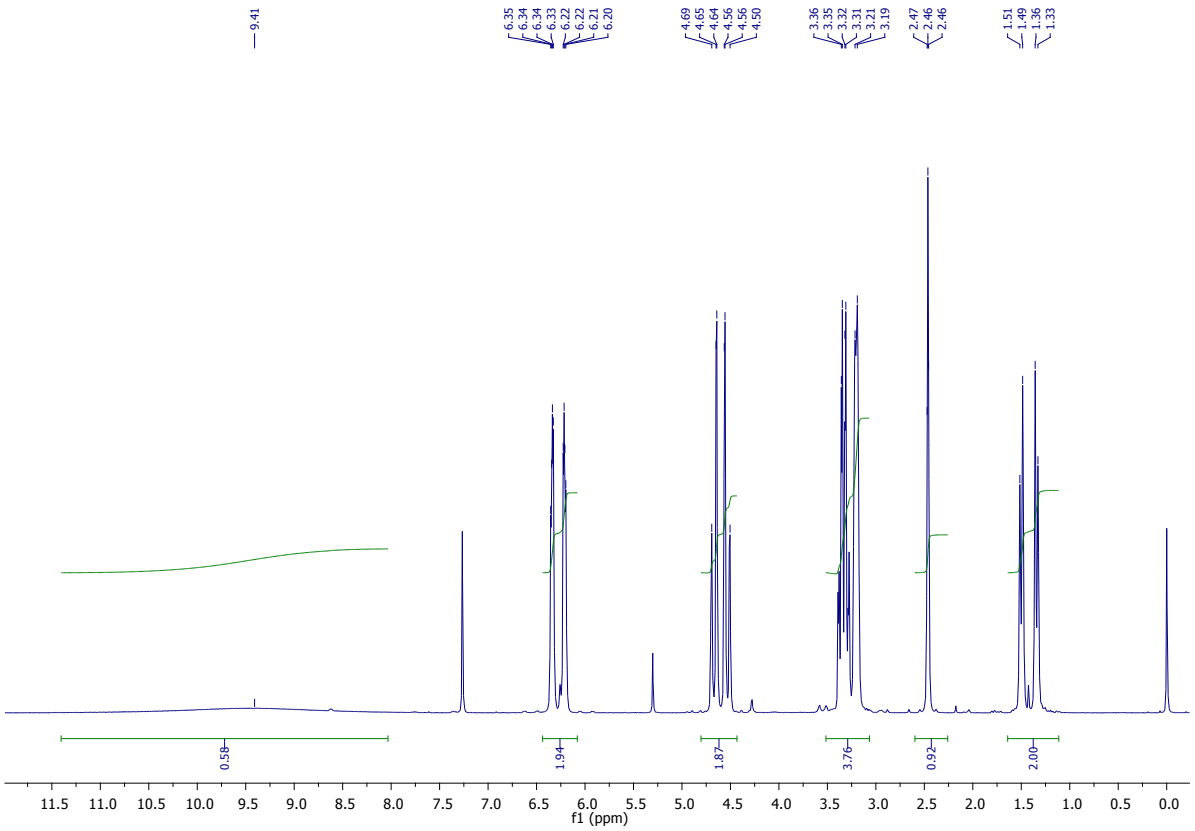

Figure S1: ^{1}H-NMR spectrum of *endo*-**2**

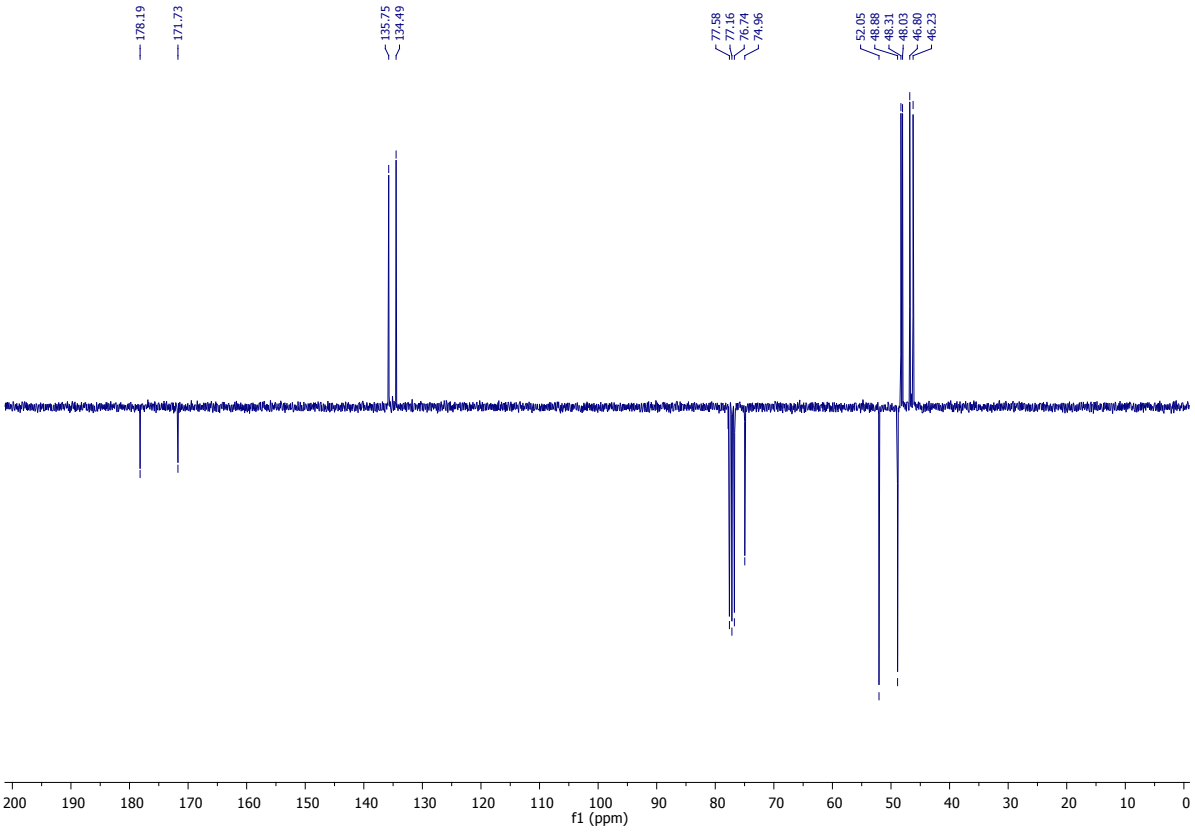

Figure S2: ^{13}C-NMR spectrum of *endo*-**2**

Synthesis of *exo, exo* 3-((prop-2-yn-1-yloxy)carbonyl)bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (*exo*-**2**)

To a solution of 1.5 g (9.14 mmol, 1 equiv.) of *cis*-5-Norbornene-*exo*-2,3-dicarboxylic anhydride in 30 mL dry DCM, 66 µL triethylamine were added followed by dropwise addition of 1.17 mL (20.1 mmol, 2.2 equiv.) propargyl alcohol at room temperature. Then, the reaction mixture was heated to reflux for 48 h (45°C bath temperature). Afterwards, the mixture was concentrated *in vacuo* and the colourless residue was recrystallized from cyclohexane. Yield: 1.7 g (7.72 mmol, 84.5%) of a white solid.

$C_{12}H_{12}O_4$ [220.22 g mol^{-1}] EI DI MS for $C_{12}H_{12}O_4$ calc'd 220.0736; found 220.0742

TLC: (DCM/MeOH 10/1) R_f = 0.23

IR (cm^{-1}): 3280 (νC≡C-H), 3170 - 2470 (νCOOH), 2180 (ν-C≡C-), 1742 (νC=O), 1694 (νC=O)

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.49 – 1.52 (d, $^3J_{HH}$ =9.5 Hz, 1H, H-7b), 2.07 – 2. 10 (d, $^3J_{HH}$ = 9.5 Hz, 1H, 7a), 2.48 – 2.49 (t, $^3J_{HH}$ = 2.6 Hz, 1H, C≡C-*H*), 2.67 (s, 2H, H-2, H-3), 3.14 (s, 2H, H-1, H-4) 4.50 – 4,56 (dd, $^3J_{HH}$ =15.6 Hz, 2.3 Hz, 1H, -O-C*H*$_2$-C≡), 4.58 – 4.74 (dd, $^3J_{HH}$ = 15.6 Hz, 2.4 Hz, 1H, -O-C*H*$_2$-C≡) 6.22 (s, 2H, H-5, H-6), 9,9 (bs, 1H, -COO*H*)

APT (100 MHz, $CDCl_3$) δ: 45.10, 45.39, 45.51, 45.93, 47.03, 47.57, 52.23, 75.00, 137.98, 138.04, 172. 74, 179.21

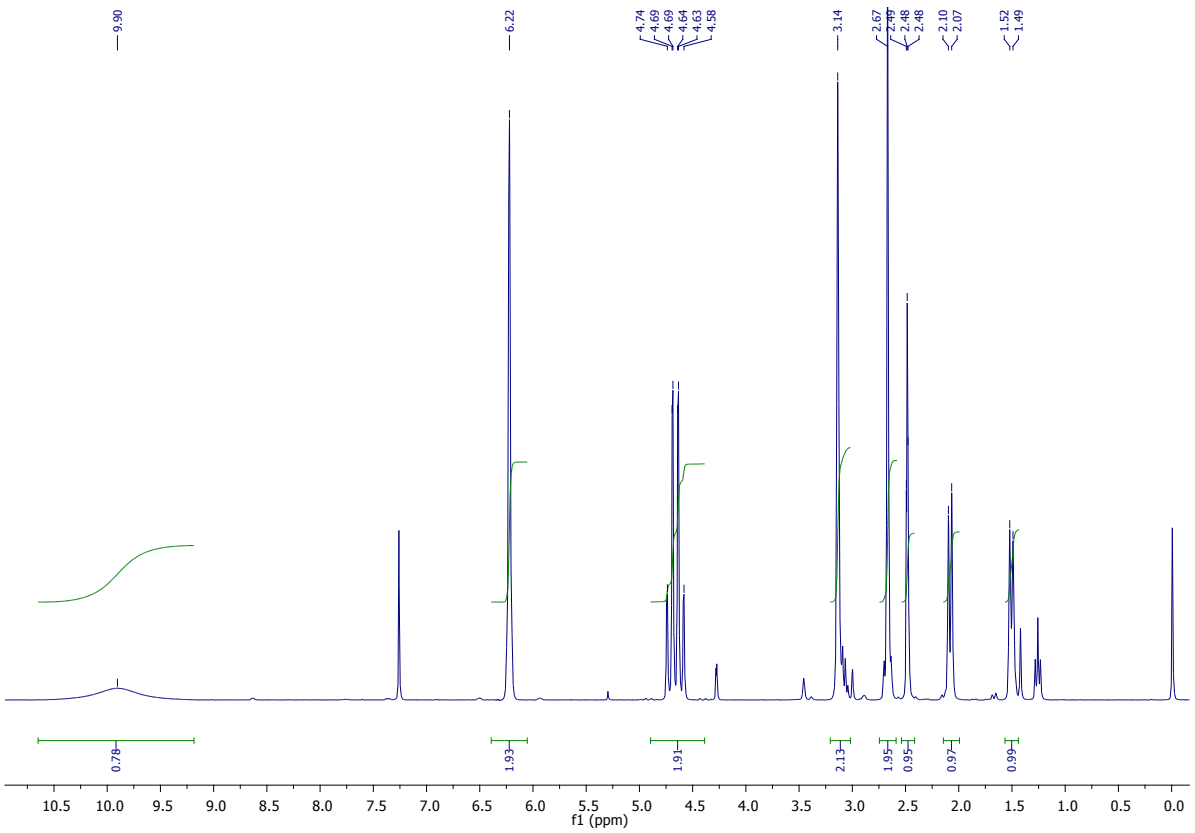

Figure S3: ^{1}H-NMR spectrum of *exo*-**2**

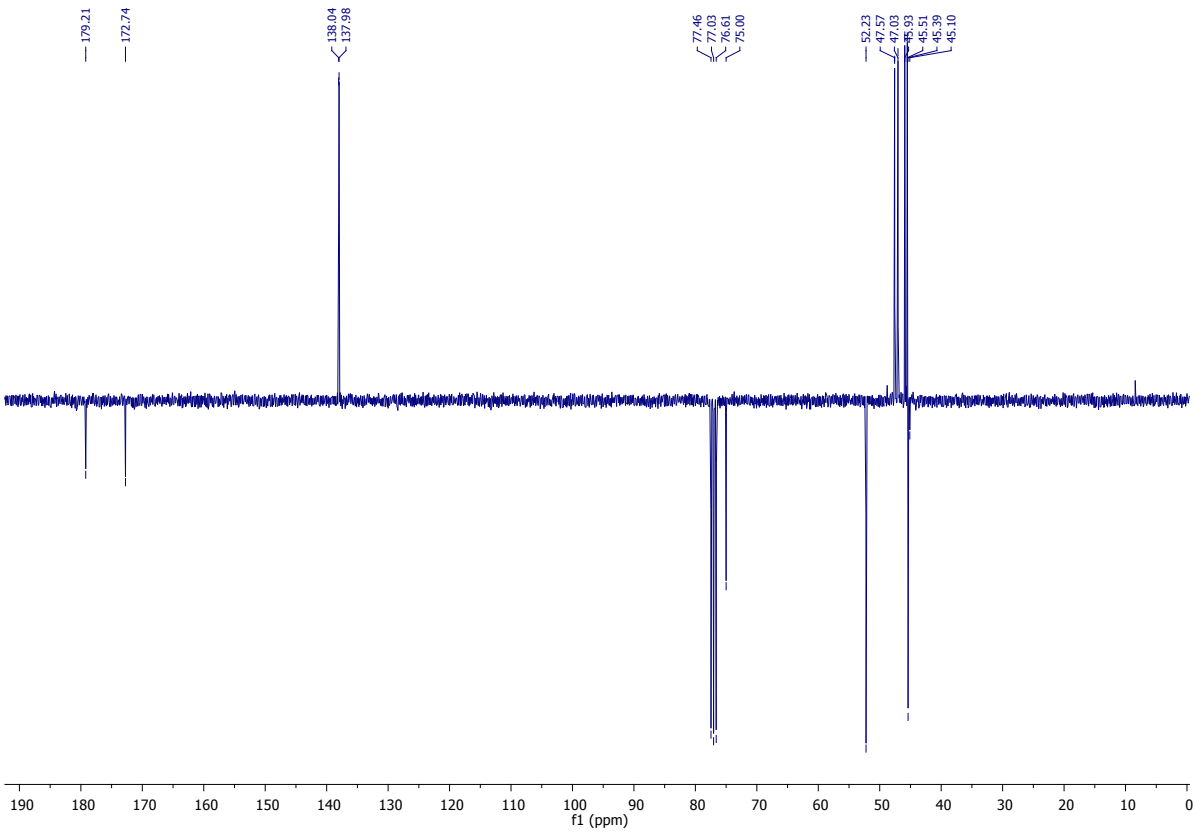

Figure S4: ^{13}C-NMR spectrum of *exo*-**2**

Synthesis of *endo, endo* 2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl) 3-prop-2-yn-1-yl bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (*endo*-**3**)

750 mg (3.41 mmol, 1 equiv.) of 5a were dissolved in 3.49 mL (40.9 mmol, 12 equiv.) oxalyl chloride (DMF?) and stirred at room temperature for 3.5 h before the residual chlorination agent was removed. Afterwards, the solid residue containing the acid chloride was dissolved in 15 mL dry DCM. After addition of 480 mg (3.4 mmol, 1 equiv.) of compound 3 and 471 µL (3.4 mmol, 1.5 equiv.) Et_3N the red mixture was stirred overnight. On the next day the solution was diluted with 40 mL EtAc and extracted with 40 mL HCl (2M), 40 mL $NaHCO_3$, was washed with 40 mL brine, dried over sodium sulfate and concentrated *in vacuo* to obtain a yellow oil. The product was purified by column chromatography (DCM/MeOH 50/1). Yield: 774.6 mg (2.27 mmol, 67 %)

$C_{18}H_{17}NO_6$ [343.33 g mol^{-1}] EI DI MS for $C_{18}H_{17}NO_6$ calc'd 343.1056; found 343.1056

TLC: (cyclohexane/ethyl acetate 3/1) R_f = 0.15

(DCM/MeOH 10/1) R_f = 0.49

IR (cm^{-1}): 3280 (νC≡C-H), 2970 (νC-H), 2180 (ν-C≡C-), 1700 (νC=O), 1240 (νC-O), 1040 (νC-O)

^{1}H-NMR (300 MHz, $CDCl_3$) δ:1.27 – 1.30 (d, $^3J_{HH}$ = 8.65 Hz, 1H, H-7a), 1.43 – 1.46 (d, $^3J_{HH}$ = 8.68, 1H, H-7b), 2.43 – 2.44 (t, $^3J_{HH}$ = 2.37 Hz, 1H, C≡C-H), 3.13 – 3.16 (2 s, 2H, H-1, H-4), 3.26 – 3.29 (m, 2H, H-2, H-3), 3.74 – 3.79 (m, 2H, -CH_2-CH_2-N), 4.05 – 4.12 (m, 1H, O-CH_2-CH_2), 4.23 – 4.30 (m, 1H, O-CH_2-CH_2), 4.49 – 4.55 (dd, $^3J_{HH}$ = 15.65 Hz, 2.37 Hz, 1H, O-CH_2-C≡), 4.63 – 4.67 (dd, $^3J_{HH}$ = 15.66 Hz, 2.39 Hz, 1H, O-CH_2-C≡), 6.21 – 6.27 (m, 2H, H-5, H-6), 6.73 (s, 2H, -CH=CH-, mal)

APT (100 MHz, $CDCl_3$,) δ: 36.85, 46.26, 47.90, 47.92, 48.60, 51.86, 61.38, 74.71, 134.17, 134.87, 134.95, 170.34, 171.49, 171.97

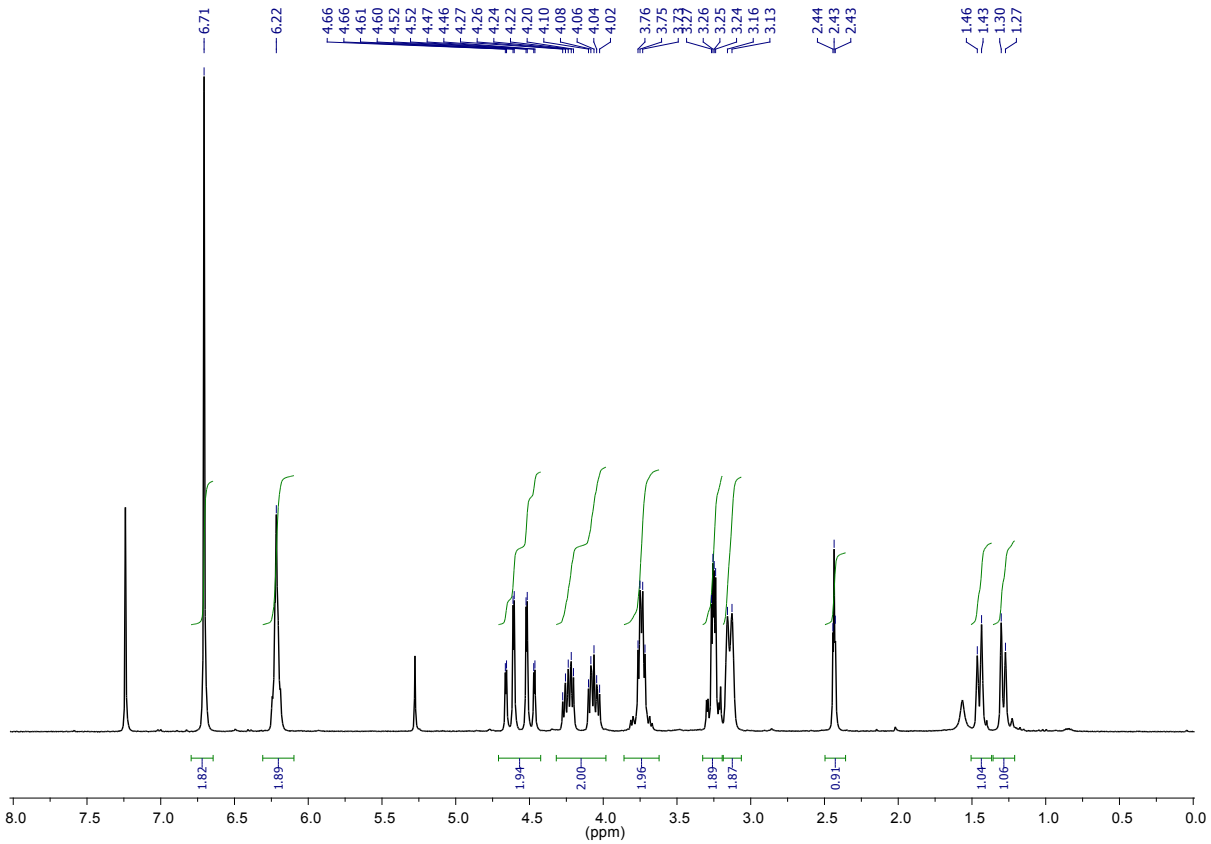

Figure S5: ^{1}H-NMR spectrum of *endo*-**3**

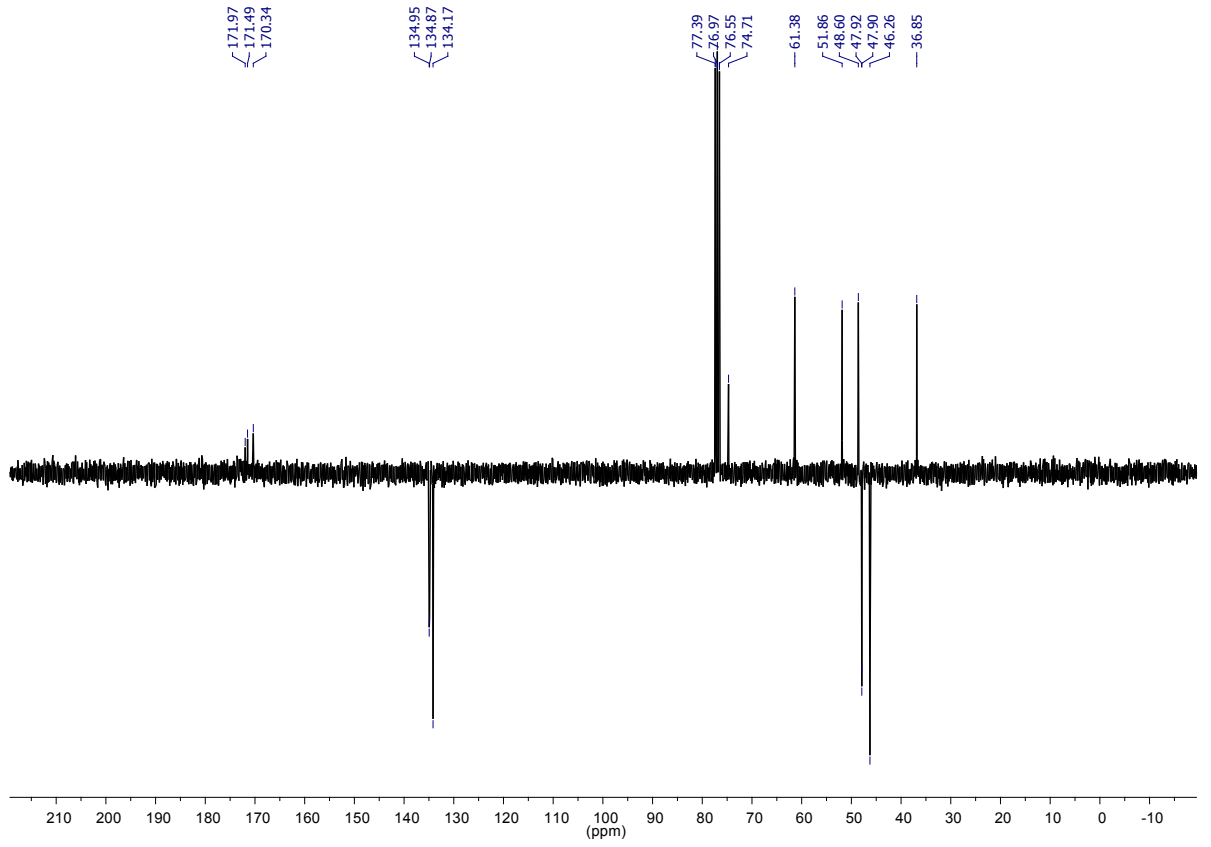

Figure S6: ^{13}C-NMR spectrum of *endo*-**3**

Synthesis of *exo, exo* 2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl) 3-prop-2-yn-1-yl bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (*exo*-**3**)

780 mg (3.54 mmol, 1 equiv.) of carboxylic acid 5b was dissolved in 3.63 mL (42.5 mmol, 12 equiv.) oxalyl dichloride and stirred at room temperature for 2 h before the residual chlorination agent was removed *in vacuo*. Afterwards the residue containing the acid chloride was dissolved in 15 mL dry DCM. After addition of 499 mg (3.54 mmol, 1 equiv.) of compound 3 and 786 µL (5.32 mmol, 1.5 equiv.) Et_3N the red mixture was stirred overnight. On the next day the solution was diluted with 20 mL EtAc and extracted with 20 mL HCl (2M), 20 mL $NaHCO_3$, was washed with brine, dried over sodium sulfate and concentrated *in vacuo* to obtain a yellow oil. The product was purified by column chromatography (DCM/EtAc 10/1).

Yield: 1.018 mg (2.9 mmol, 81.9 %)

TLC: (DCM/EE 10/1) R_f = 0.72

IR (cm^{-1}): 3280 (νC≡C-H), 2970 (νC-H), 2180 (ν-C≡C-), 1700 (νC=O), 1240 (νC-O), 1040 (νC-O)

^{1}H-NMR (300 MHz, $CDCl_3$) δ:1.47 – 1.50 (d, $^3J_{HH}$ = 9.5 Hz, 1H, H-7b), 2.01 – 2.04 (d, $^3J_{HH}$ = 9.5 Hz, 1H, H-7a), 2.48 (m, 1H, C≡C-H), 2.60 – 2.65 (m, 2H, H-2, H-3), 3.10 (bs, 2H, H-1, H-4), 3.78 – 3.82 (m, 2H, -CH_2-CH_2-N), 4.07 – 4.11 (m, 1H, O-CH_2-CH_2), 4.31 – 4.39 (m, 1H, O-CH_2-CH_2), 4.56 – 4.17 (mult, $^3J_{HH}$ = 2H, O-CH_2-C≡), 6.20 (m, 2H, H-5, H-6), 6.73 (s, 2H, -CH=CH-, mal)

APT (100 MHz, $CDCl_3$,) δ: 36.99, 45.66, 47.19, 47.84, 52.53, 61.68, 75.09, 134.37, 135.45, 137.64, 170.45, 172.91, 173.60

Approximately 26 % of the *trans*-isomer were formed which was estimated using the characteristic signal at 6.07 (m, 1H, H-6/H-5) ppm (Figure S7).

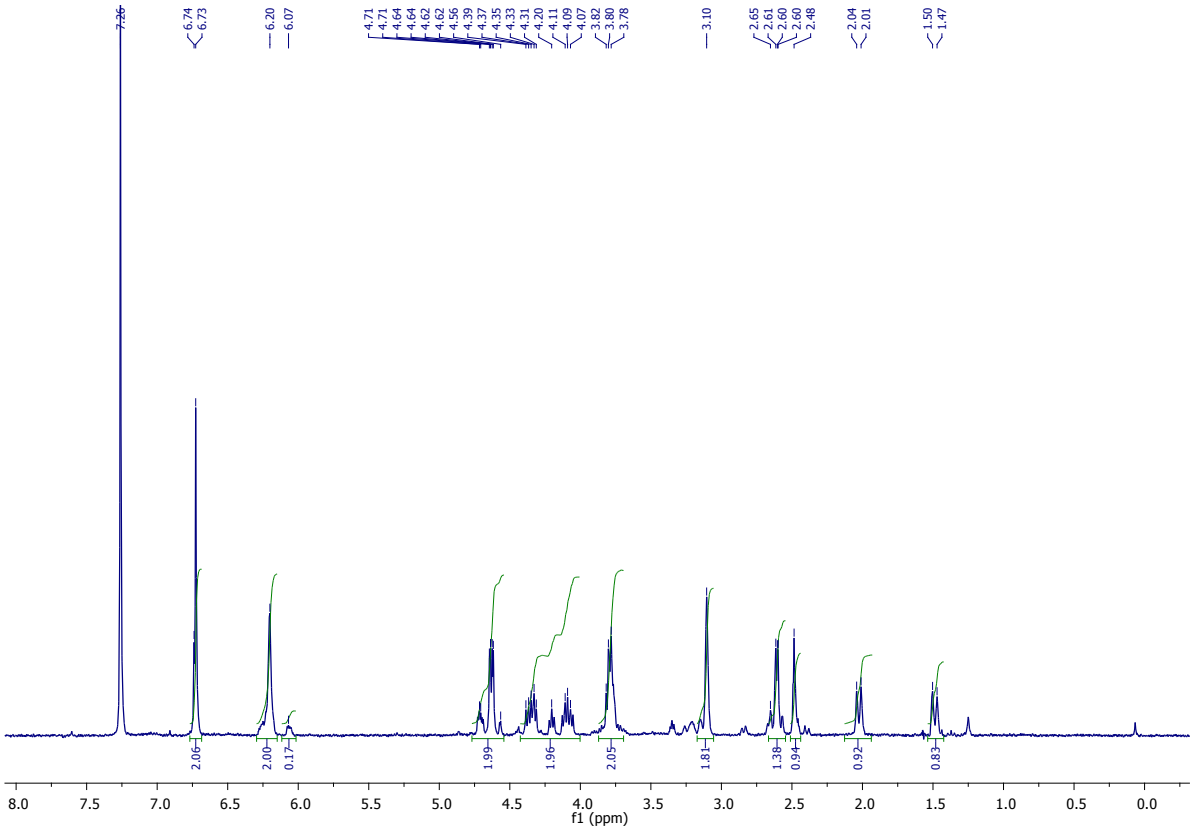

Figure S7: ^{1}H-NMR spectrum of *exo*-**3**

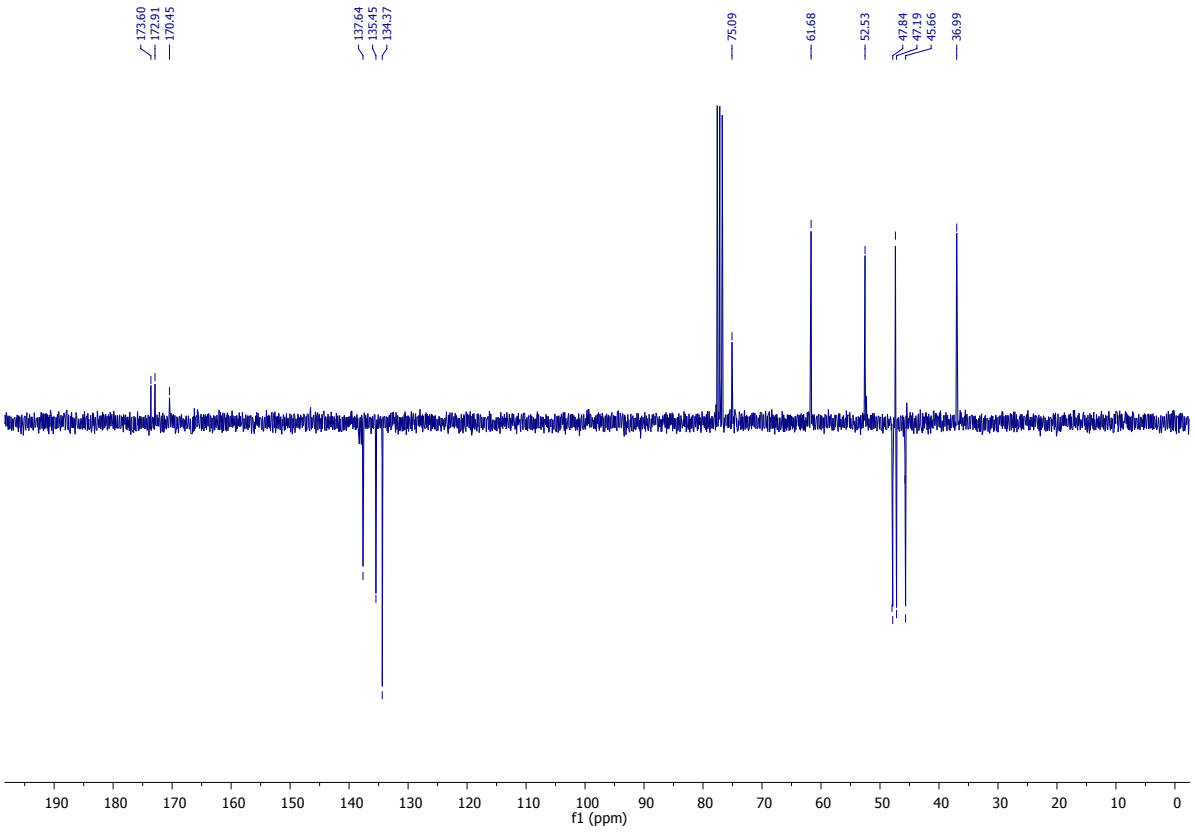

Figure S8: ^{13}C-NMR spectrum of *exo*-**3**

“Click” reactions

Copper-catalyzed azide-alkyne reaction

68 mg (0.198 mmol, 1 equiv.) of *endo*-**4a** were dissolved in 1.5 mL dichloromethane and 750 µl (0.220 mmol, 1.1 equiv.) of a 0.2912 mM solution of (azidomethyl) benzene in DMF (prepared by nucleophilic exchange of benzylbromide with NaN_3) were added, followed by addition of aqueous solutions of copper(II)sulphate (0.05 equiv, 0.01 mmol, 1.82 mg, 0.5 mL) and sodium ascorbate (0.15 equiv, 4.34 mg, 1mL). The reaction progress was monitored by TLC and after full consumption of **4a** was detected, the reaction mixture was worked up by adding 20 mL of H_2O. The aqueous layer was extracted with 2x 20mL dichloromethane and the organic phase was dried over sodium sulphate and evaporated to dryness.

MALDI-TOF HRMS: calcd for $C_{25}H_{24}N_4O_6$ 499.1594 ($M+Na^+$), found 499.1632 ($M+Na^+$)

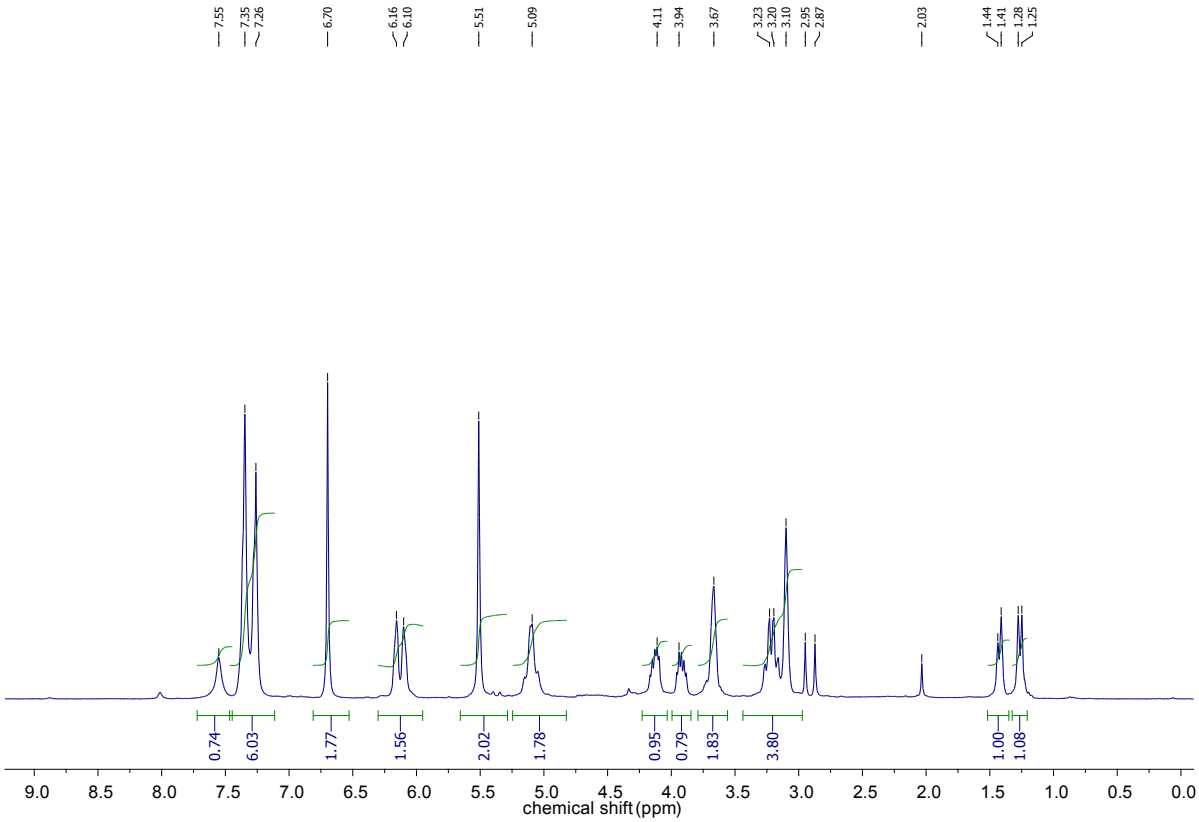

Figure S9: ^{1}H-NMR spectrum of *endo*-**3** treated with benzyl azide (CuAAC)

Michael thiol-ene reaction

For the thiol-ene click reaction, 10 mg (0.03 mmol, 1 equiv) of *endo*-**4a** were dissolved in 1 mL DCM. After addition of 7.24 µL (0.03 mmol, 1 equiv) dodecanethiol and 10 µL Et_3N, the reaction was finished within two hours. The solvent was evaporated and a slightly yellow oil remained which was dried in vacuo and directly transferred into an NMR tube.

MALDI-TOF HRMS: calcd for $C_{30}H_{43}NO_6S$ 568.2709 ($M+Na^+$), found 568.2749 ($M+Na^+$)

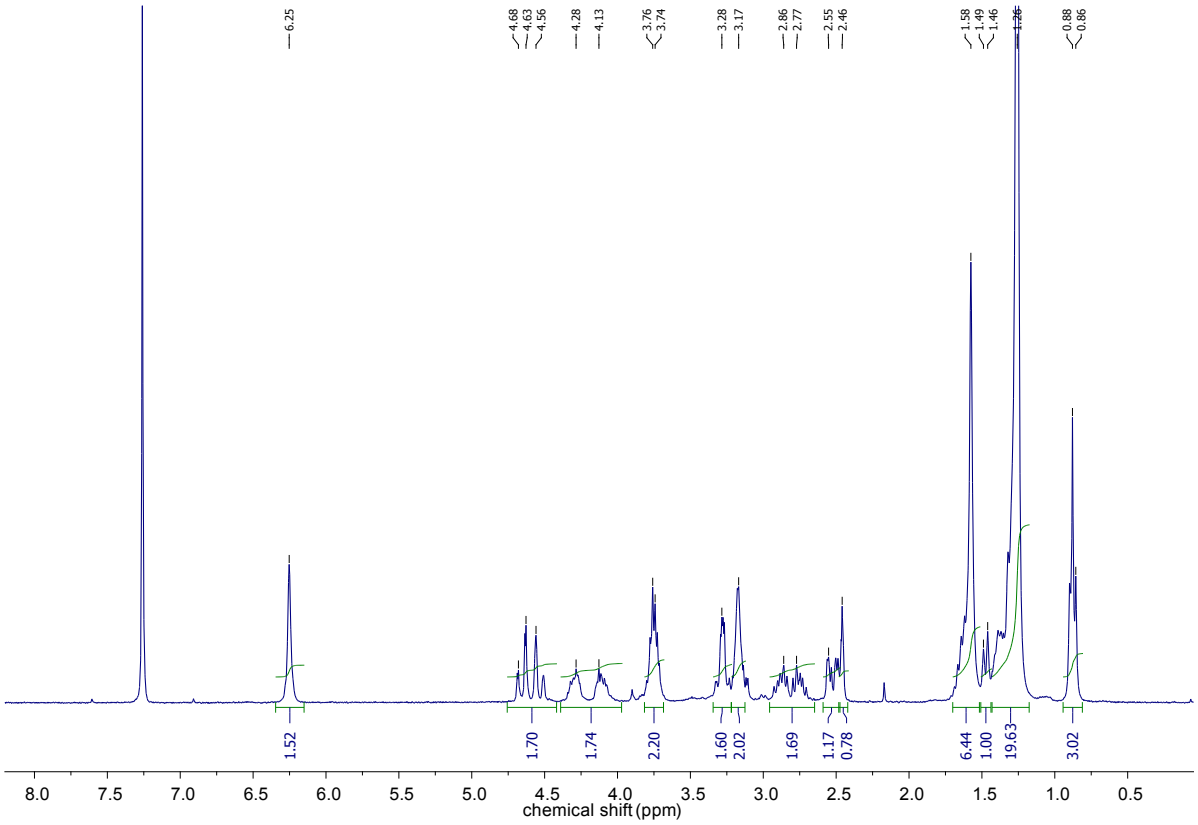

Figure S10: ^{1}H-NMR spectrum of *endo*-**3** treated with dodecanethiol

Inverse electron demand Diels-Alder reaction

10 mg (0.03 mmol, 1 equiv.) of compound *endo*-**3** were dissolved in MeOH and 10% H_2O (1 mL). 7.54 mg (0.03 mmol, 1 equiv.) pyTz were added into the vial and the reaction mixture was stirred at room temperature for 8 days to accomplish full conversion of the norbornene double bond. The mixture turned from a deep violet color (due to pyTz) to pale yellow. The solvent was evaporated and the remaining oil was analyzed by NMR and MALDI-TOF-MS.

MALDI-TOF HRMS: calcd for $C_{30}H_{23}N_5O_6$ (pyridazine) 552.1883 (M+H), found 552.1858 (M+H); calcd for $C_{30}H_{25}N_5O_6$ (dihydropyridazine) 550.1727 (M+H), found 550.1725 (M+H)

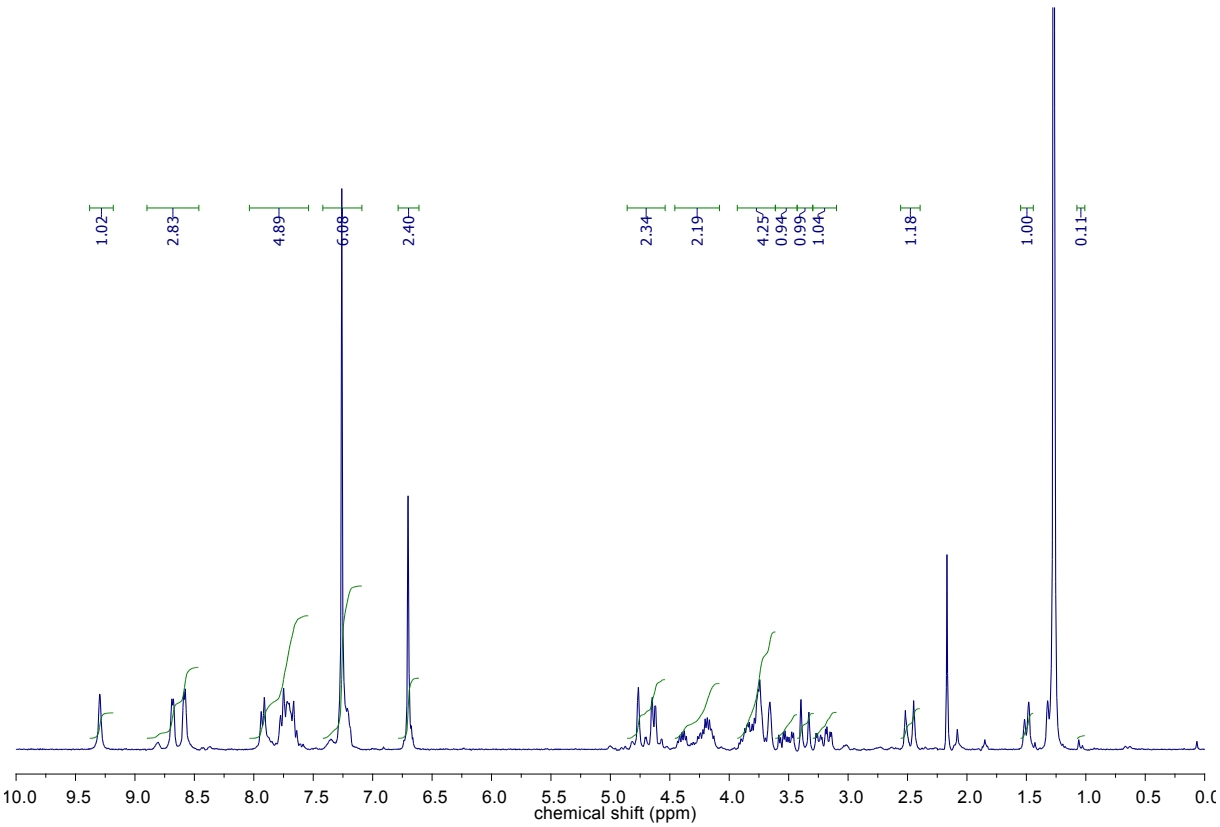

Figure S11: ^{1}H-NMR spectrum of *endo*-**3** treated with di(pyridine-2-yl)tetrazine (1.1 equiv.)

Screening experiments:

20 mg of *endo*-**3**, *exo*-**3** and *±endo,exo*-5-Norbornene-2,3-dicarboxylic acid dimethylester were each placed in small scintillation vials and stirred with 1 equiv. of **pyTz** in 1 mL dichloromethane. The reactions were monitored visually (**pyTz** has a characteristic pink colour) and via TLC. The same procedure was repeated for *endo*-**3** and **pyTz** but performed using methanol with 10% water as solvent.

One-pot click reaction on *endo*-**3**

50 mg (0.150 mmol, 1 equiv.) of *endo*-**3** were dissolved in 1.5 mL dichloromethane and 590 µl (1.1 equiv., 0.160 mmol) of a 0.27 mM solution of (azidomethyl) benzene in DMF (prepared by nucleophilic exchange of benzylbromide with NaN_3) were added, followed by addition of copper(II)sulphate (0.05 equiv, 570 µl aqueous stock solution) and sodium ascorbate (0.15 equiv., as solid). Therefore, the solvent mixture composition was dichloromethane/DMF/H_2O 3/1/1. Immediately after, pyTz (37.8 mg, 0.160 mmol, 1.1 equiv.) and dodecanethiol (38.4 µl, 0,160 mmol, 1.1 equiv.) were added in solid form. The reaction mixture was stirred overnight before being worked up by extracting the reaction mixture with 20 mL H_2O and 2x20 mL dichloromethane. The combined organic layers were dried over $NaSO_4$ and the solvent was evaporated *in vacuo*. The crude product was used directly for ^{1}H-NMR spectroscopy (see Fig. S12 b)) and MALDI-TOF-MS (see Fig. S13).

Sequential click reactions on *endo*-**3** (Synthesis of **7**)

50 mg (0.150 mmol, 1 equiv.) of *endo*-**3** were dissolved in 1.5 mL dichloromethane and 590 µl (1.1 equiv., 0.160 mmol) of a 0.27 mM solution of (azidomethyl) benzene in DMF (prepared by nucleophilic exchange of benzylbromide with NaN_3) were added, followed by addition of an aqueous solutions of copper(II)sulphate (0.05 equiv., 570 µl) and sodium ascorbate (0.15 equiv., as solid). Therefore, the solvent mixture composition was dichloromethane/DMF/H_2O 3/1/1. The reaction progress was monitored by TLC and after full consumption of **3** was detected, pyTz (37.8 mg, 0.160 mmol, 1.1 equiv.) was added. After 1h, the majority of the azide-alkyne clicked product was consumed, therefore dodecanethiol (38.4 µl, 0,160 mmol, 1.1 equiv.) was added. After 2h, 10 µL of trimethylamine were added and the reaction mixture was stirred overnight before being worked up by extracting the reaction mixture with 20 mL H_2O and 2x20 mL dichloromethane. The combined organic layers were dried over $NaSO_4$ and the solvent was evaporated *in vacuo*. The crude product (49.1 mg, 95% yield) was directly used for ^{1}H-NMR spectroscopy and MALDI-TOF-MS.

MALDI-TOF HRMS: calcd for $C_{30}H_{23}N_5O_6$ (pyridazine) 885.4122 (M+H), found 885.4148 (M+H); calcd for $C_{30}H_{25}N_5O_6$ (dihydropyridazine) 887.4279 (M+H), found 887.4272 (M+H)

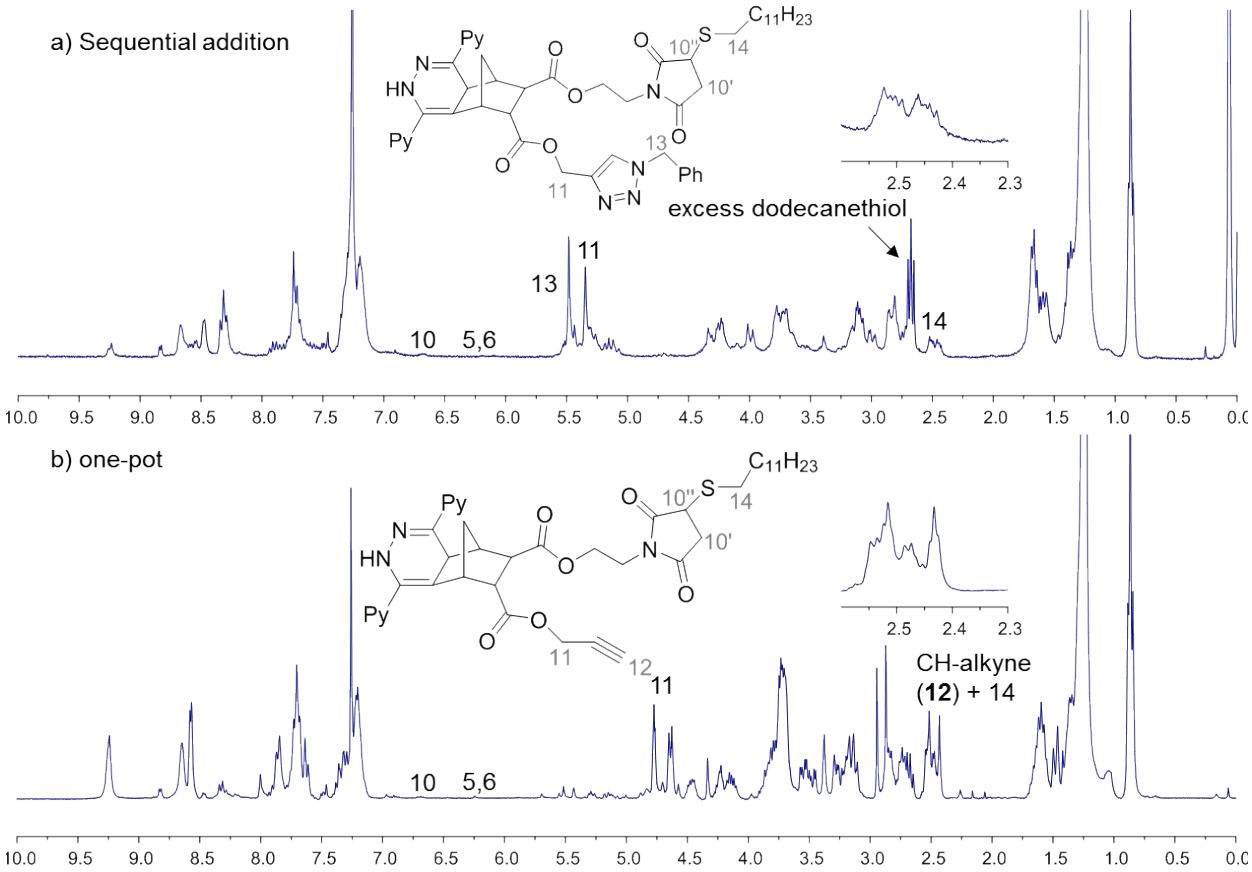

Figure S12: ^{1}H-NMR spectrum of *endo*-**3** treated with benzyl azide (CuAAC), di(pyridine-2-yl)tetrazine and dodecanethiol a) sequentially performed click reactions (**D**) vs. b) "one-pot, triple-click" (**E**)

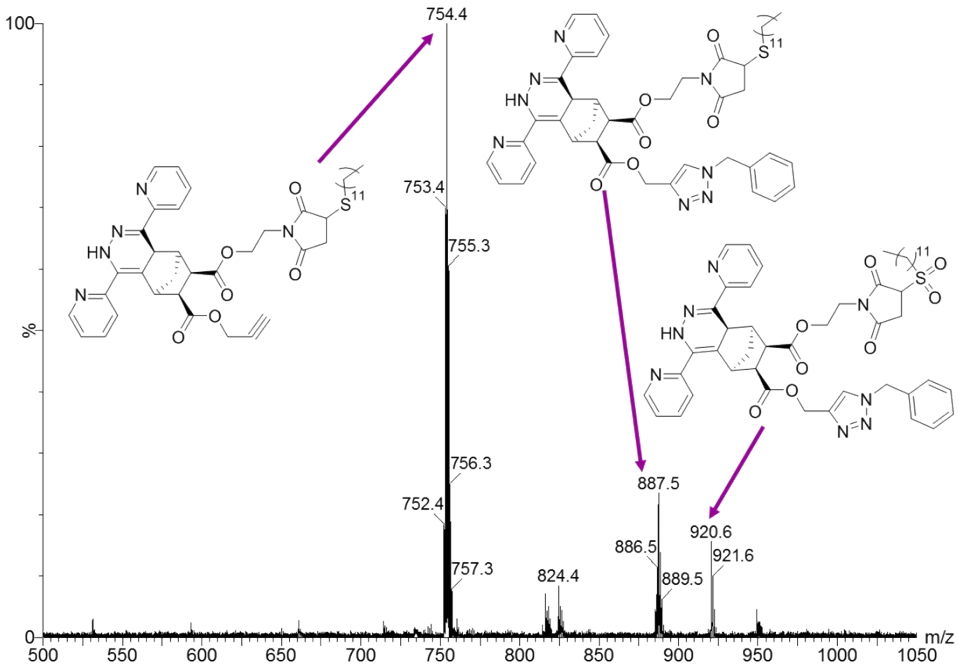

Figure S13: MALDI-TOF MS spectrum of *endo*-**3** treated with benzyl azide (CuAAC), di(pyridine-2-yl)tetrazine and dodecanethiol in a simultaneous "one-pot, triple-click" approach (**E**)

NOESY

Differential ^{1}H NMR NOE measurements were used to confirm the exact configuration of *endo*-**3**. Since in case of *exo*-**3**, partial stereoinversion in the second step occurred resulting in about 26% trans-configured by product, additional ^{1}H NMR NOE measurements were done on *endo*-**2** and *exo*-**2**. In the case of *endo*-**2** and *endo*-**3**, irradiation of H_{7a} at δ 1.34 ppm caused signal enhancements at δ = 3.20 ppm (H_1, H_4) and 3.34 ppm (H_2, H_3), respectively.

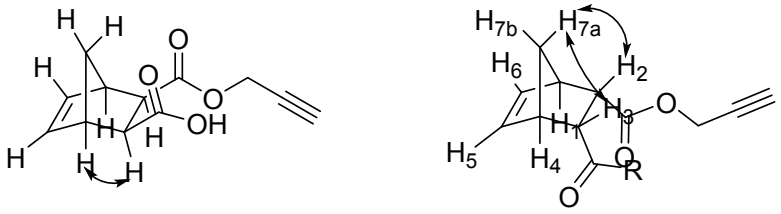

Figure S14: NOE experiments on exo-**2,** endo-**2** (R=OH) and endo-**4** (R=O-$(CH_2)_2$-maleimide)

For *exo*-**2** (Fig. S14, left), only signal enhancement of H_1 and H_4 was observed. These results are consistent with the assumed *endo*-configuration of **3**.

References

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