ELECTRONIC SUPPLEMENTARY INFORMATION

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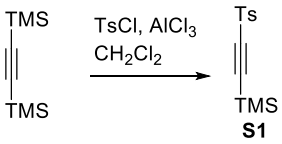
1. General methods.

Chemicals were purchased and used without further purification. All solvents were purified by distillation over drying agents. Optical rotations were measured in a 1.0 cm or 1.0 dm tube with a Jasco P-2000 spectropolarimeter. Infrared spectra were recorded with a Jasco FTIR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX300, AV300, AV500 and AVIII500 for solutions in $CDCl_3$, CD_3OD and DMSO-d_6 at room temperature except when indicated. All the assignments were confirmed by COSY and HSQC experiments. . Chemical shifts are quoted in parts per million from residual solvent peak ($CDCl_3$: ^{1}H - 7.26 ppm and ^{13}C - 77.16 ppm) and coupling constants (*J*) given in Hertz. Multiplicities are abbreviated as: b (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or combinations thereof. Mass spectra (CI and ESI) were recorded on Micromass AutoSpeQ and QTRAP spectrometers. Calculated and exact m/z values are reported in Daltons. NMR and mass spectra were registered in CITIUS (University of Seville). TLC was performed on silica gel HF_{254} (Merck), with detection by UV light charring with H_2SO_4, *p*-anisaldehyde, vanillin, ninhydrin, $KMnO_4$ or with Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. Silica gel 60 (Merck, 63–200 µm) was used for preparative chromatography.

2. Synthesis of known compounds.

p-*Tolyl [(2-trimethylsilyl)ethynyl] sulfone S1.*

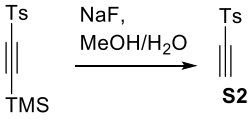


S1

The synthesis of this compound was performed by the procedure previously described.[1] A mixture of $AlCl_3$ (14.7 g, 110 mmol) and tosyl chloride (21.9 g, 114 mmol) was dissolved in dry dichloromethane (100 mL) and stirred under argon atmosphere for 20 minutes at room temperature. After this time, the crude was filtered through celite and the resultant liquid was added dropwise to a solution of commercial bis(trimethylsilyl) acetylene (23 mL, 102 mmol) in dry dichloromethane (100 mL) at 0 °C for 1 hour. After the addition, the reaction was allowed to warm at room temperature overnight. Once the reaction had finished, the crude was poured into a solution of hydrogen chloride in cold water (20%). The organic layer was washed with an aqueous solution of HCl, with brine, and was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resultant solid was recrystallized from cyclohexane affording the desired product as a grey powder (14.1 g, 55%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 0.12 (s, 9H, $Si(CH_3)_3$); 2.47, (s, 3H, CH_3); 7.37 (d, 2H, *J* = 8.5, Ar*H*); 7.88 (d, 2H, Ar*H*).

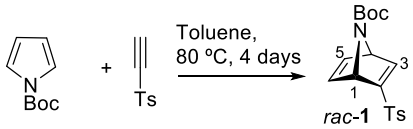
[1] Z. Chen, Zhengming, M. L. Trudell, *Synth. Commun*. 1994, **24**, 3149-3155.

Synthesis of ethynyl p-tolyl sulfone S2.



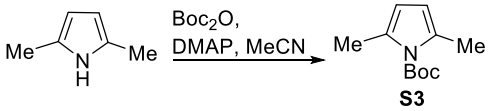
The synthesis of this compound was performed by the procedure previously described.[1] To a solution of *p*-tolyl [(2-trimethylsilyl)ethynyl] sulfone (2.0 g, 8.1 mmol) in methanol (16 mL), a solution of NaF (504 mg, 12.0 mmol) in water (8 mL) was added dropwise at 0 °C, and the reaction was stirred at this temperature for 30 minutes. Then, the reaction was diluted with diethyl ether and it was washed with an aqueous saturated solution of $NaHCO_3$. The organic layer was separated, dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure, affording the desired pure product as a white powder (1.4 g, 94%) without the need of a further purification step. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 2.47 (s, 3H, CH_3 of Ts); 3.45 (s, 1H, Alkynyl-*H*); 7.38 (d, 2H, *J* = 8.6, Ar*H*); 7.89 (d, 2H, Ar*H*).

rac-N-*Boc-2-p-toluenesulfonyl-7-azanorbornadiene (1).*



The synthesis of this compound was performed by the procedure previously described.[2] To a solution of ethynyl *p*-tolyl sulfone (1.4 g, 7.7 mmol) in dry toluene (6 mL) under argon atmosphere, commercial *N*-Boc-pyrrole (6.4 mL, 38.3 mmol) was added. The mixture was heated at 80 °C for 4 days. After this time, the solvent was removed and the crude was purified by silica gel column chromatography (AcOEt/Cy 1:6), affording **1** as a yellow syrup (2.0 g, 75%).^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 1.28 (m, 9H, $C(CH_3)_3$); 2.47 (s, 3H, CH_3 of Ts); 5.18 (bs, 1H, H1or H4); 5.39 (bs, 1H, H1 or H4); 6.87-6.96 (m, 2H, H5 & H6); 7.35 (d, 2H, *J* = 8.1, Ar*H*); 7.57 (bs, 1H, H3); 7.76 (d, 2H, Ar*H*).

N-*Boc-2,5-dimethylpyrrole (S3).*



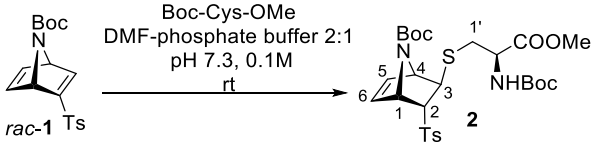
The synthesis of this compound was performed by a different procedure than the previously reported one.[3] To a stirred solution of commercial 2,5-dimethyl-pyrrole (1.9 mL, 21 mmoles) in acetonitrile (20 mL), 4-dimethylaminopyridine (260 mg, 2.1 mmoles) and Boc_2O (4.82 g, 22 mmoles) were added. After 24 hours at room temperature, all the starting material had been consumed (TLC) and the mixture was diluted with diethyl ether and washed with a 1M solution of $NaHSO_4$, water, and a 1M solution of $NaHCO_3$. The organic layer was dried over anhydrous

[2] R. Leung-Toung, Y. Liu, J. M. Muchowski, Y.-L. Wu, *J. Org. Chem*. 1998, **63**, 3235-3250.
[3] H.-P. Kaiser, J. M. Muchowski, *J. Org. Chem*. 1984, **49**, 4203-4209.

Na_2SO_4 and the solvent was removed under reduce pressure. The compound was purified by silica gel column chromatography (AcOEt/Cy 1:40) affording the desired product (3.0 g, 73%) as a brown oil.

3. Synthesis of new compounds.

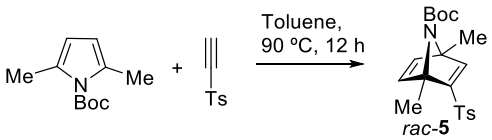
N-*Boc-azanorbornene-Cys adduct (2)*.



To a solution of bicyclic vinyl sulfone **1** (150 mg, 0.43 mmol) in DMF (33 mL) a solution of *N*-Boc-L-cysteine methyl ester (151 mg, 0.64 mmol) in DMF (10 mL) and a phosphate buffer solution (pH 7.3, 0.1 M, 21.5 mL) were added simultaneously, and the mixture was stirred for 10 min. Then, the crude was diluted with diethyl ether, washed with water and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting crude was purified by silica gel column chromatography (AcOEt/Cy 1:4 → 1:3) to afford **2** (215 mg, 86%, mixture of diasteroisomers) as a white foam. IR $\bar{\upsilon}$ 3358, 2978, 2932, 1707 (C=O), 1597,1556, 1497, 1343, 1317, 1286, 1146, 1016, 916, 812, 775, 733, 655 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz, mixture of diasteroisomers and rotamers)* δ 1.29-1.50 (m, 18H,C(CH_3)$_3$); 2.42, 2.43, 2.47 (3 s, 3H, CH_3 of Ts); 2.88-3.15 (broad m, 3H, H1'a, H1'b, H3); 3.50, 3.58 (2 bs, 1H, H2'); 3.73, 3.76, 3.77, 3.78 (3 bs, 3H, CH_3CO); 4.53-4.92 (broad m, 3 H, H1, H2, H4); 5.43 (bs, 1H, N*H*Boc); 6.43, 6.51, 6.63 (3 bs, 2H, H5, H6); 7.30-7.41 (m, 2H, Ar*H*); 7.72-7.86 (m, 2H, Ar*H*). ^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm, mixture of diasteroisomers and rotamers)* δ 21.5, 21.6, 21.7 (*C*H$_3$ of Ts); 27.9, 28.0, 28.1, 28.11, 28.12, 28.2, 28.26, 28.28 (C(*C*H$_3$)$_3$); 34.3, 35.2, 35.4 (C1'); 45.9, 48.0 (C2', broad); 52.6, 52.7 (CH_3CO); 52.9 (C1 or C4); 62.5 (C2 or C3, broad); 67.1, 67.3 (C1 or C4); 70.7 (C2', broad); 73.7 (C2 or C3, broad); 80.2, 80.8, 81.20, 81.23 (*C*(CH$_3$), broad); 127.4, 128.1,129.3, 129.9, 130.1 (*C*HAr); 136.36, 136.42 (C5 & C6, broad); 138.0, 145.0, 145.2, 145.3 (*C*Ar, broad); 1525.0 (C=O Boc, broad); 171.0, 171.1, 171.2 (*C*OOMe, broad). ESI-HRMS *m/z*, calcd. for $C_{27}H_{38}O_8N_2NaS_2$ $(M+Na)^+$: 605.1962; found: 605.1955.

*Note: The heating of the sample favours the retro-Diels-Alder transformation of the compound. Thus, the sample could not be heated, what complicated the assignment of the ^{1}H and ^{13}C-NMR spectra (broad and duplicate signals) due to the presence of N-Boc rotamers.

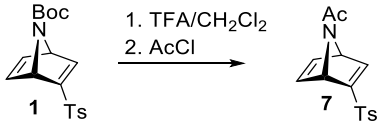
rac*-N-*Boc-1,4-dimethyl-2*-p-*toluenesulfonyl-7-azanorbornadiene (5).



To a solution of *N*-Boc-2,5-dimethylpyrrole (3.0 g, 15 mmol) in dry toluene under Ar atmosphere, ethynyl *p*-tolyl sulfone (570 mg, 3.1 mmol) was added. The mixture was heated at 90 °C overnight. Then, toluene was removed under reduced pressure and the crude was purified by silica gel column chromatography (AcOEt/Cy 1:5) to afford **5** (970 mg, 85%) as a yellowish powder. IR $\bar{\upsilon}$ 3082, 2978, 1695, 1603, 1556, 1456, 1307, 1143, 822, 790 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 1.32 (s, 9 H, C(CH_3)$_3$); 1.83 (s, 3 H, CH_3); 1.97 (s, 3 H, CH_3); 2.44 (s, 3 H, CH_3 of Ts); 6.59 (d, 1 H, $J_{5,6}$ = 5.2; H-5 or H-6); 6.72 (d, 1 H, H-5 or H-6); 7.33, 7.75 (2 d, 2 H, *J* = 8.1, H

arom.); 7.49 (s, 1 H, H-3). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 16.3 (CH_3); 17.4 (CH_3); 21.8 (CH_3 of Ts); 28.3 (-C(CH_3)$_3$); 76.6, 77.7 (C-1, C-4); 81.4 (-C(CH_3)$_3$); 128.3, 129.9 (CH arom.); 136.6, 144.8 (C arom.); 146.9, 149.3 (C-5, C-6); 154.7 (C2); 159.7 (C-3); 160.5 (C=O). ESI-HRMS m/z calcd. for $C_{20}H_{25}NO_4SNa$ $(M+Na)^+$: 398.1402; found: 398.1391.

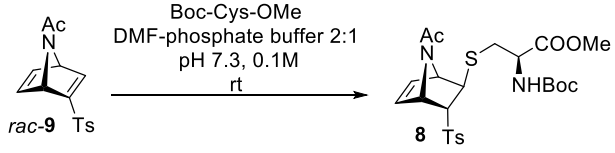
N-*acetyl-2*-p-*toluenesulfonyl-7-azanorbornadiene (7).*



To a solution of **1** (300 mg, 086 mmol) in dichloromethane (14 mL), trifluoroacetic acid (3.5 mL) was added. The reaction was stirred at room temperature for 25 min and the solvent was removed under *vacuum*. The resulting crude was dissolved in dry dichloromethane (16 mL) under Ar atmosphere, and acetyl chloride (120 µL, 1.72 mmol) followed by triethylamine (240 µL, 1.72 mmol), were added at 0 °C. After the addition, the reaction was allowed to warm at room temperature for x hours. Then, the mixture of reaction was diluted with dichloromethane, washed with an aqueous 1M solution of HCl and brine. The organic layer was separated, dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The compound was purified by silica gel column chromatography (AcOEt/Cy, 2:1) to afford **7** (196 mg, 79%) as a brown syrup. IR $\bar{\upsilon}$ 3087; 3063; 2362; 2341; 2249; 1660 (C=O); 1595; 1381; 1315; 1292; 1148; 1084; 1013; 913; 845; 815; 727; 663 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, *δ* ppm, *J* Hz)* *δ* 1.68, 1.86 (s, 3H, C*H*$_3$CO); 2.46 (s, 3H, C*H*$_3$ of Ts), 5.25-5.30, 5.44-5.45, 5.45-5.50, 5.71, 5.74 (m, 2H, H1 &H4); 6.90-699, 7.09-7.11 (m, 2H, H5 & H6); 7.34-7.39 (m, 2H, Ar*H*); 7.57-7.58, 7.61-7.62 (m, 1H, H3); 7.73-7.76 (m, 2H, Ar*H*). ^{13}C-RMN (75 MHz, $CDCl_3$, *δ* ppm) 21.2, 21.5(*C*H$_3$CO); 21.8 (*C*H$_3$ of Ts); 64.0, 65.3, 66.3, 67.6 (C1 & C4); 128.17, 128.21, 130.2, 130.3 (*C*HAr); 135.3 (*C*Ar), 141.4, 142.6 (C5 or C6), 143.0, 145.6 (C5 or C6), 145.3, 145.5, (*C*Ar); 150.7, 153.1 (C3); 159.0, 160.2 (C2); 167.6 (*C*=O). ESI-HRMS *m/z*, calcd. for $C_{15}H_{15}O_3NNaS$ $(M+Na)^+$: 312.0672; found: 312.0665.

*Note: All the signals in ^{1}H-NMR are duplicated due to the existence of two rotamers.

N-*Ac-azanorbornene-Cys adduct (8)*.

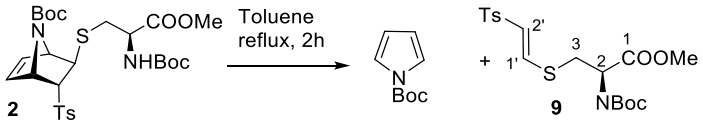


Compound **8** (98.2 mg, 58 %, white foam) was prepared according to the procedure described for compound **2**. IR (ν, cm^{-1}) 3361, 2977, 1709 (C=O), 1645, 1496, 1437, 1392, 1215, 1146, 1086, 1052, 1017, 913, 729, 660. ^{1}H-NMR (500 MHz, CDCl3, *δ* ppm, *J* Hz, mixture of diasteroisomers and rotamers)* *δ* 1.44-1.46 (broad m, 9H, (C(C*H*$_3$)$_3$); 1.92-2.02 (broad m, 3H, C*H*$_3$CO); 2.78-3.20 (broad m, 3H, H1'a, H1'b, H3); 3.35-3.37 (m, 1H, H2'); 3.71-3.78 (broad m, 3H, C*H*$_3$CO); 4.43-5.42 (broad m, 4H, H1, H4, H2, N*H*Boc); 6.40-6.56 (m, 2H, H5 & H6); 7.37-7.41 (m, 2H, Ar*H*); 7.73-7.80 (m, 2H, Ar*H*). ^{13}C-NMR (125 MHz, CDCl3, *δ* ppm, mixture of diasteroisomers and rotamers)* *δ* 21.26, 21.29, 21.80, 21.83, 28.3, 28.40, 28.42, 34.3, 34.6, 35.2, 35.3, 35.8, 45.2, 45.9, 46.3, 46.6,

47.1, 49.3, 49.6, 52.79, 52.84, 52.87, 52.90, 52.96, 52.99, 53.05, 53.09, 53.2, 53.4, 53.5, 53.8, 54.1, 58.4, 59.6, 59.7, 59.9, 60.1, 60.5, 61.2, 61.3, 61.4, 61.5, 63.1, 63.2, 67.2, 67.4, 69.6, 69.8, 71.7, 71.8, 72.1, 72.5, 72.9, 73.3, 80.5, 80.7, 128.2, 128.3, 128.4, 128.6, 128.7, 129.4, 129.5, 130.0, 130.20, 130.27, 130.32, 130.5, 134.3, 134.4, 134.5, 135.0, 135.3, 135.4, 135.6, 135.9, 136.0, 136.3, 136.46, 136.54, 136.9, 137.0, 137.2, 138.0, 138.07, 138.13, 138.7, 137.8, 144.3, 145.35, 135.40, 145.47, 145.6, 145.7, 145.8, 155.1, 165.6, 166.72, 166.82, 167.2, 170.3, 170.8, 170.9, 171.0, 171.10, 171.19, 171.22, 171.27, 171.53. ESI-HRMS *m/z*, calcd. for $C_{24}H_{32}O_7N_2NaS_2$ $(M+Na)^+$: 547.1536; found: 547.1543.

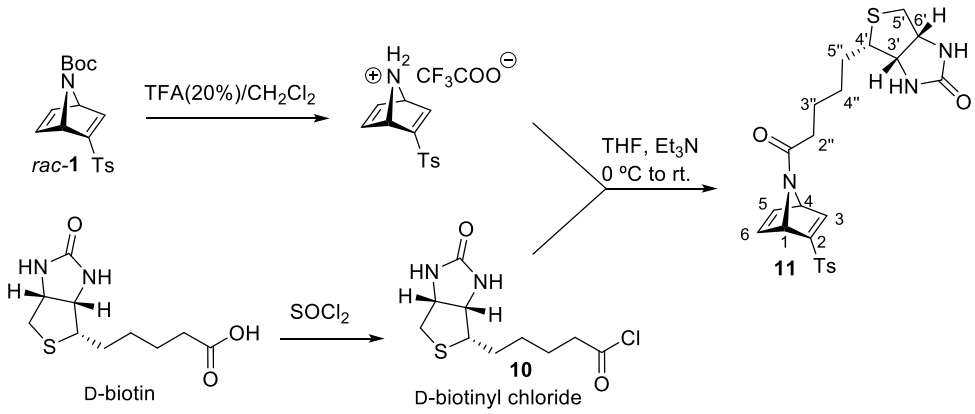
*Note: The heating of the sample favours the retro-Diels-Alder transformation of the compound. Thus, the sample could not be heated, what complicated the assignment of the ^{1}H and ^{13}C-NMR spectra (broad and duplicate signals) due to the presence of N-Boc and N-Ac rotamers.

(E)-N-*(*tert-*Butoxycarbonyl)*-S-*(2-tosylvinyl)-L-cysteine methyl ester (9).*



A solution of **2** (78.5 mg, 0.13 mmol) in toluene (1 mL) was heated at reflux for 2 h. Then, the solvent was removed under reduced pressure and the crude was purified by silica gel column chromatography (AcOEt/Cy 1:7→1:2) to afford first *N*-Boc-pyrrole (21 mg, quant.) and then alkene **9** (57 mg, quant.) as a white foam. Characterization for **9** $[\alpha]_D^{27}$: +46.0 (*c* 0.8, CH_2Cl_2). IR $\bar{u}$ 3356; 3290; 3046; 2925; 2852; 1712 (C=O); 1555; 1505; 1436; 1364; 1314; 1141; 1084; 812; 651 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6, 353 K, δ ppm, *J* Hz) δ 1.38 (s, 9H, $(C(CH_3)_3)$); 2.41 (s, 3H, CH_3 of Ts); 3.17 (dd, 1H, $^2J_{3a,3b}$ =13.7, $J_{3a,2}$ = 8.9, H3a); 3.31 (dd, 1H, $J_{3a,2}$ = 5.1, H3b); 3.66 (bs, 3H, $COOCH_3$); 4.33-4.19 (m, 1H, H2), 6.68 (d, 1H, $J_{1',2'}$ = 14.8, H2'); 7.00-7.21 (bs, 1H, N*H*Boc); 7.42 (d, 2H, *J* = 8.4, Ar*H*); 7.76-7.71 (m, 3H, Ar*H* & H1'). ^{13}C-NMR (75 MHz, $CDCl_3$, 353 K, δ ppm) δ 20.5 (CH_3 of Ts); 27.7 ($C(CH_3)_3$); 33.2 (C3); 51.6 ($COOCH_3$); 52.9 (C2); 78.5 ($C(CH_3)_3$); 122.9 (C2');126.4, 129.4 (*C*HAr); 138.3, 143.3 (*C*Ar); 144.6 (C1'); 170.1 (C=O). ESI-HRMS *m/z* calcd. for $C_{18}H_{25}O_6NNaS_2$ $(M+Na)^+$: 438.1016; found: 438.1012.

Biotinyl-azanorbornadiene 11

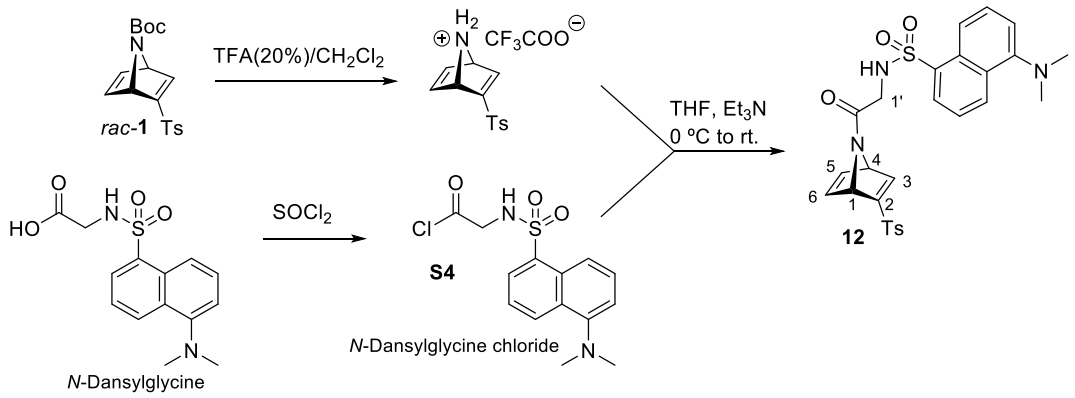


Scheme S1

A round bottom flask was charged with D-biotin (352 mg, 1.44 mmol). Then, thionyl chloride (4.8 mL) was added and the resulting solution was stirred for 2 hours at room temperature under Ar atmosphere. After 2 h of reaction, the excess of thionyl cloride was removed under vacuum to give crude D-biotinyl chloride. In other round bottom flask, a solution of **1** (250 mg, 0.72 mmol) in dichloromethane (10 mL) and TFA (2.5 mL) was stirred for 30 minutes. Then, the solvent was removed under reduced pressure and the crude was dissolved in dry THF (10 mL) under Ar atmosphere. To this mixture, a solution of the crude D-biotinyl chloride **10** (1.44 mmol) in dry THF (15 mL) and trimethylamine (200 µL, 1.44 mmol) were added simultaneously at 0 °C. The reaction was allowed to warm at room temperature for 1 hour. After completion, the solvent was removed under reduced pressure, and the crude was dissolved in dichloromethane, washed with a 1 M solution of aqueous HCl, and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The resulting crude was purified by column chromatography (dichloromethane/methanol 40:1→10:1) to give **11** (256 mg, 75%, mixture of diasteroisomers) as a brown powder. IR $\bar{\upsilon}$ 3245; 3086; 2923; 2854; 1697 (C=O); 1456; 1314; 1148; 1083; 815; 666 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6, 353 K, δ ppm, J Hz, mixture of diasteroisomers and rotamers)* δ 1.21-1.47 (m, 6H, H2''a, H2''b, H3''a, H3''b, H4''a, H-4''b.); 1.57-1.71 (bs, 1H, H5a''.); 2.04-2.09 (bs, 1H, H5b''); 2.43 (bs, 3H, CH_3 of Ts); 2.62 (d, 1H, $^2J_{5'a,5'b}$= 12.4, H5'a); 2.85 (dd, 1H, $J_{5'b,6'}$ = 5.2, H5'b); 3.05 -3. 12 (m, 1H, H4' and H_2O); 4.12-4.16 (m, 1H, H3'); 4.30-4.34 (m, 1H, H6'); 5.41, 5.60, 5.74 (3 bs, 2H, H1 & H4); 6.12 (bs, 2H, NH urea); 6.96-7.05 (m, 2H, H5 & H6); 7.5 (d, 2H, J= 8.0, ArH); 7.74 (bd, 3H, ArH & H3).^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm, mixture of diasteroisomers) δ 20.7 (CH$_3$ of Ts); 23.4, 27.5, 27.6, (C2'', C3'', C4''); 32.3 (C5''); 39.4 (C5'); 54.8 (C4'); 59.0 (C6'); 60.8 (C3'); 63.4, 65.1 (C1 & C4, broad); 127.2, 129.8 (CHAr); 135.1 (CAr); 144.4 (CAr); 142.0, 142.8 (C5 or C6, broad); 152.2 (C3, broad); 154.0 (C2, broad); 162.2 (C=O Urea); 168.9 (C=O Amide, broad). ESI-HRMS m/z calcd. for $C_{23}H_{27}O_4N_3NaS_2$ $(M+Na)^+$: 496.1335; found: 496.1335.

*Note: broad signals in ^{1}H-NMR spectrum due to the presence of rotamers (spectrum registered at 80 °C).

Dansylglycine-azanorbornadiene 12



Scheme S2

A round bottom flask was charged with *N*-Dansylglycine [4] (530.4 mg, 1.72 mmol). Then, thionyl chloride (10 mL) was added and the resulting solution was stirred for 2 hours at room temperature under Ar atmosphere. After 2 h of reaction, the excess of thionyl cloride was removed under vacuum to give crude *N*-Dansylglycine chloride. In other round bottom flask, a solution of **1** (300 mg, 0.86 mmol) in dichloromethane (12 mL) and TFA (3 mL) was stirred for 30 minutes. Then, the solvent was removed under reduced pressure and the crude was dissolved in dry THF (12 mL) under Ar atmosphere. To this mixture, a solution of the crude *N*-Dansylglycine chloride chloride **S4** (1.72 mmol) in dry THF (18 mL) and trimethylamine (240 µL, 1.72 mmol) were added simultaneously at 0 °C. The reaction was allowed to warm at room temperature for 1 hour and a half. After completion, the solvent was removed under reduced pressure, and the crude was dissolved in dichloromethane, washed with a 1 M solution of aqueous HCl, and brine. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The resulting crude was purified by column chromatography (AcOEt/Cy 1:2→1:1) to give **S5** (264.7 mg, 57%) as a green powder. IR $\bar{\upsilon}$ 3266; 2945; 1668(C=O); 1315; 1304; 1146; 1084; 790; 670; 623 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6, 298 K, δ ppm, J Hz, mixture of rotamers 1:1)* δ 2.33, 2.41 (bs, 3H, CH_3 of Ts.); 2.80-2.84 (m, 6H, (CH_3)$_2$N.); 3.13-3.21 (dd, 1H, J= 5.6, $^2J_{1'a\text{-}1'b}$= 17.4, H1'a); 3.54-3.64 (m, 1H, H1'b); 5.34, 5.52, 5.72, 5.77 (4 bs, 2H, H1 and H4); 6.82-6.98 (m, 2H, H5 & H6); 7.23-7.26 (m, 1H, Ar*H*); 7.43 (t, 2H, J= 8.1, Ar*H*); 7.53-7.62 (m, 2H, Ar*H*); 7.68-7.81 (m, 3H, Ar*H* & H3); 8.01-8.08 (m, 1H, Ar*H*); 8.17-8.27 (m, 2H, Ar*H* & N*H*); 8.42-8.46 (m, 1H, Ar*H*).^{13}C-NMR (75 MHz, $CDCl_3$, δ ppm, mixture of rotamers) δ 20.6 (*C*H$_3$ of Ts); 43.9 (C1'); 44.6 ((*C*H$_3$)$_2$NH'); 63.7, 65.6 (C1 & C4, broad); 114.7, 119.0 122.9 (*C*HAr); 127.1, 127.2, 127.4, 128.9, 129.7, 134.9 (*C*HAr); 136.2 (*C*Ar); 141.2, 143.9 (C5 or C6, broad); 144.4 (*C*Ar); 151.0 (C3); 158.1 (C2); 163.9 (C=O Amide, broad). ESI-HRMS *m/z* calcd. for $C_{27}H_{28}O_5N_3S_2$ $(M+H)^+$: 538.1465; found: 538.1457.

*Note: broad signals in ^{1}H-NMR spectrum due to the presence of rotamers.

[4] Prepared as it was described by Herbert Waldmann and coworkers on *Angew. Chem. Int. Ed.* 2010, **49**, 6090 –6095

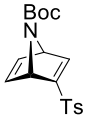
4. Competition experiments of Cys/Lys addition to bicyclic vinyl sulfone 1.

General considerations

All compounds were dissolved in phosphate buffer (pH 7.3, 50 mM) with a 7% of DMSO to a final concentration to 500 µM. Competition assay was performed in the same solvent mixture and in the same concentration at room temperature and after 10 minutes 10 µL aliquot were analysed by HPLC/UV. The amino acid derivatives used for this experiments were only protected in the α amino group letting free carboxilic acid to improve the solubility of the compounds in water.

The HPLC/UV analysis was performed on a Thermo UltiMate 3000 instrument with µBondapak C18 column (125Å, 10 µm, 3.9 mm X 150 mm). The mobile phase consisted of acetonitrile and water. The samples were eluted with a linear gradient (13 minutes 0-50% ACN and 1.5 min 50-100% ACN, 1 mL/min). All chromatograms were registered at 280 nm wavelength.

HPLC Chromatograms



Retention time: 7.26 min.

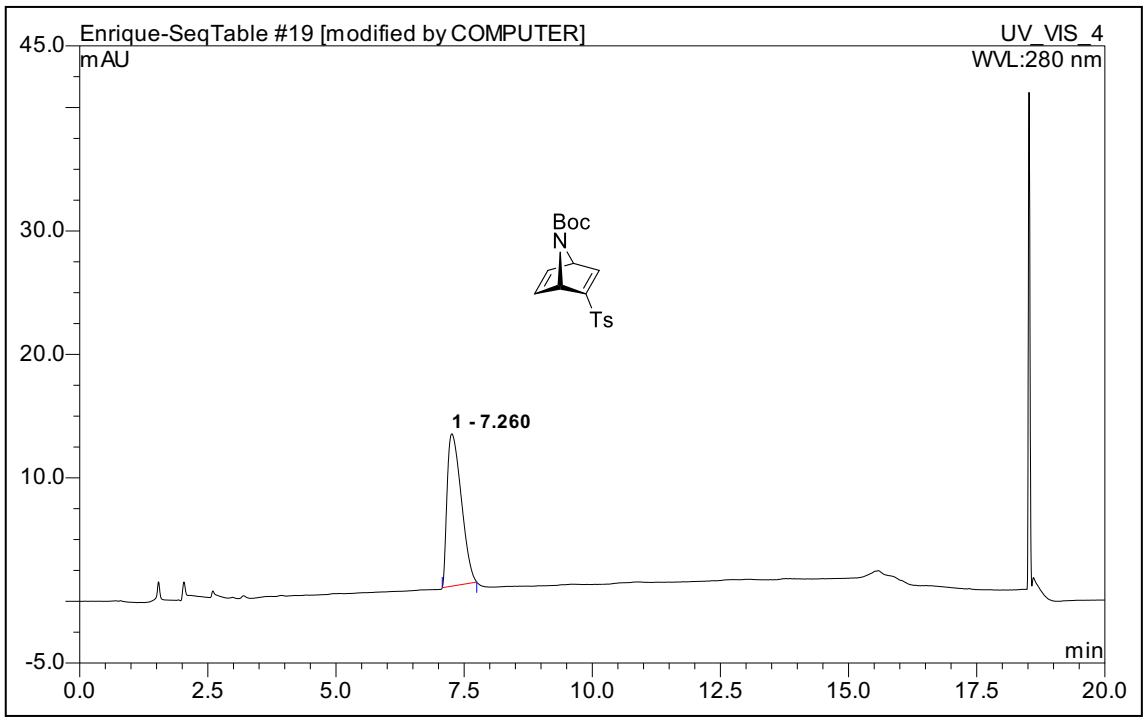
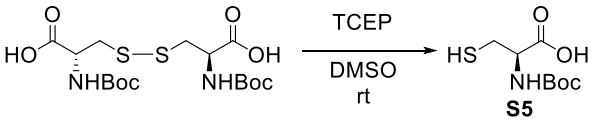


Figure S1. Chromatogram of **1**.



Commercially available (Boc-Cys-OH)$_2$ was diluted in DMSO to a final concetration of 15 mM from 54.5 mM stock solution in the same solvent. Then, a solution of TCEP in the same solvent was added to a final concentration of 150 mM from a stock solution of 360 mM. The reaction was performed at room temperature for 10 min. Ater this time 10 µL aliquot of this solution (previously diluted) was analysed by HPLC/UV and no peak was observed at this wavelength.

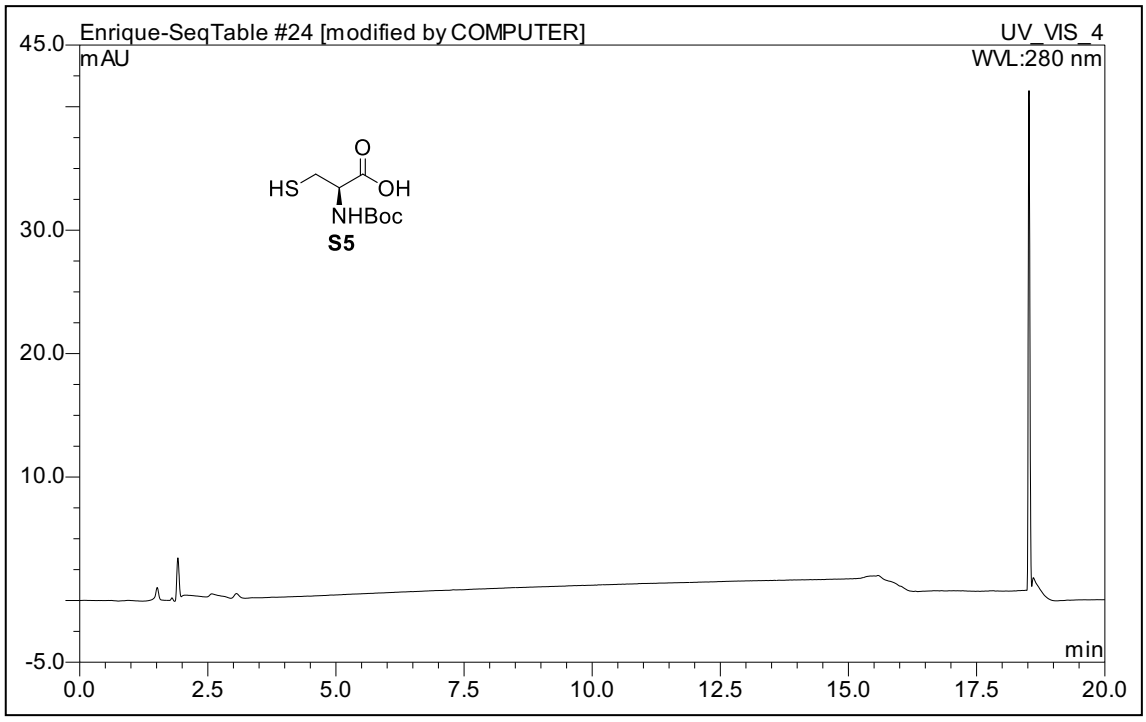
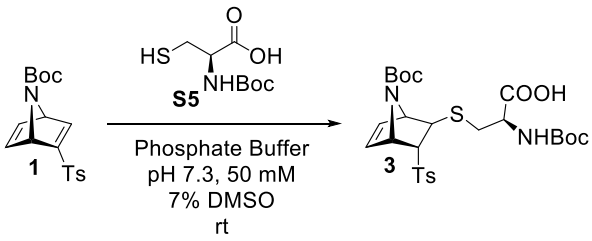


Figure S2. Chromatogram of **S2**.



Compound **1** was diluted in phosphate buffer (pH 7.3, 50 mM) and DMSO to a final concentration of 500 µM from 120 mM stock solution in DMSO. Then, a solution of **S5** in DMSO was added to a final concentration of 500 µM from a stock solution of 10 mM. The reaction was performed at room temperature for 10 min. Ater this time 10 µL aliquot of this solution was analysed by HPLC/UV no peak corresponding to starting materia was detected.

Retention time of 3: 1.56 min.

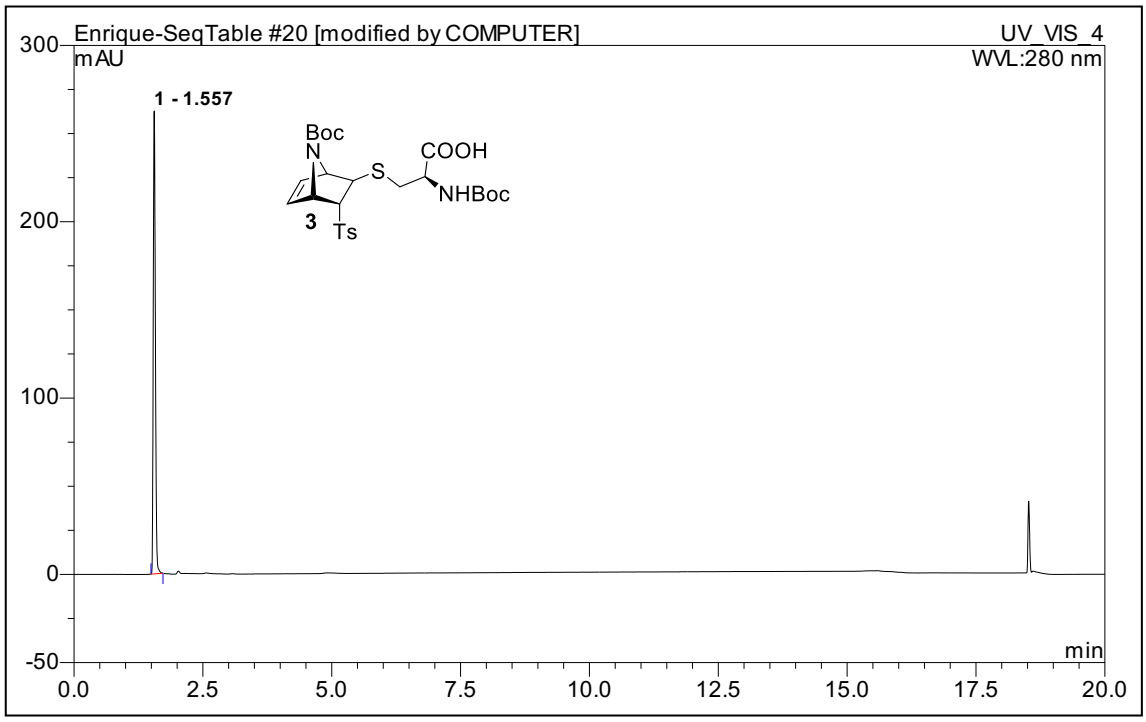
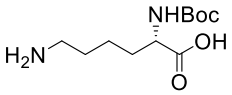


Figure S3. Chromatogram of **5.**



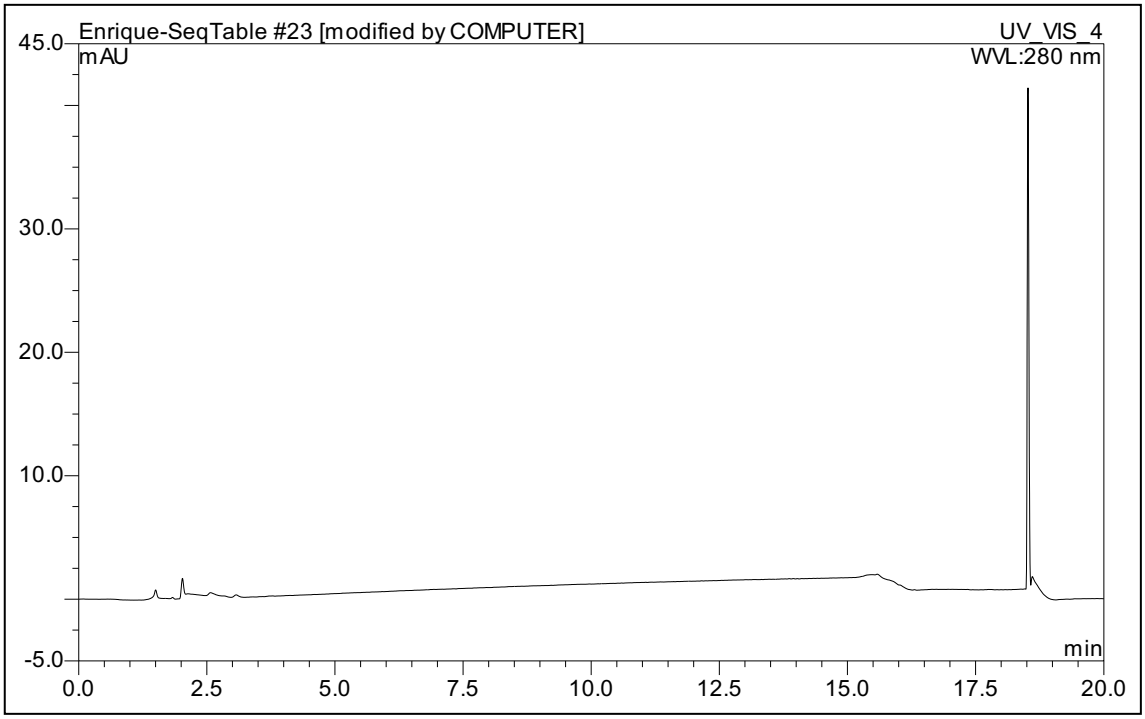
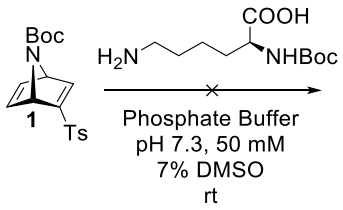


Figure S4. Chromatogram of commercially available *N*-Boc-Lys-OH.



Compound **1** was diluted in phosphate buffer (pH 7.3, 50 mM) and DMSO to a final concentration of 500 µM from 120 mM stock solution in DMSO. Then, a solution of commercially available *N*-Boc-Lys-OH in phosphate buffer (pH 7.3 50 mM) with a 7% DMSO was added to a final concentration of 500 µM from a stock solution of 24 mM. The reaction was performed at room temperature for 10 min. After this time 10 µL aliquot of this solution was analysed by HPLC/UV and only the peak for the starting materia was detected.

Retention time: 7.27 min.

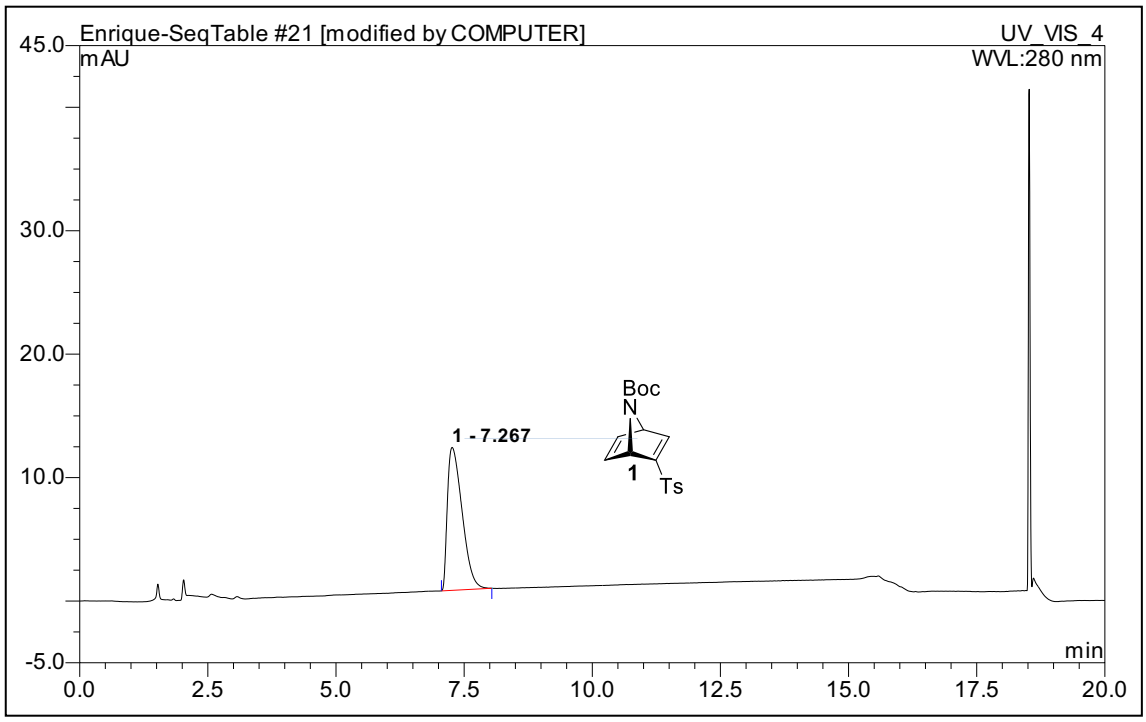
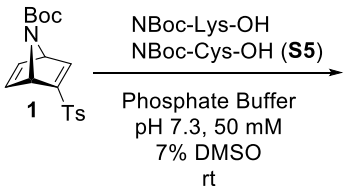


Figure S5. Chromatogram of the reaction between *N*-Boc-Lys-OH and **1**.



Both amino acid derivatives were diluted in phosphate buffer (pH 7.3, 50 mM) and DMSO to a final concentration of 500 µM from 30 mM (Cys) and 24 mM (Lys) stock solution in DMSO. Then, a solution of **1** in DMSO was added to a final concentration of 500 µM from a stock solution of 120 mM. The reaction was performed at room temperature for 10 min. After this time 10 µL aliquot of this solution was analysed by HPLC/UV and only the peak for **3** was detected.

Retention time: 1.54 min.

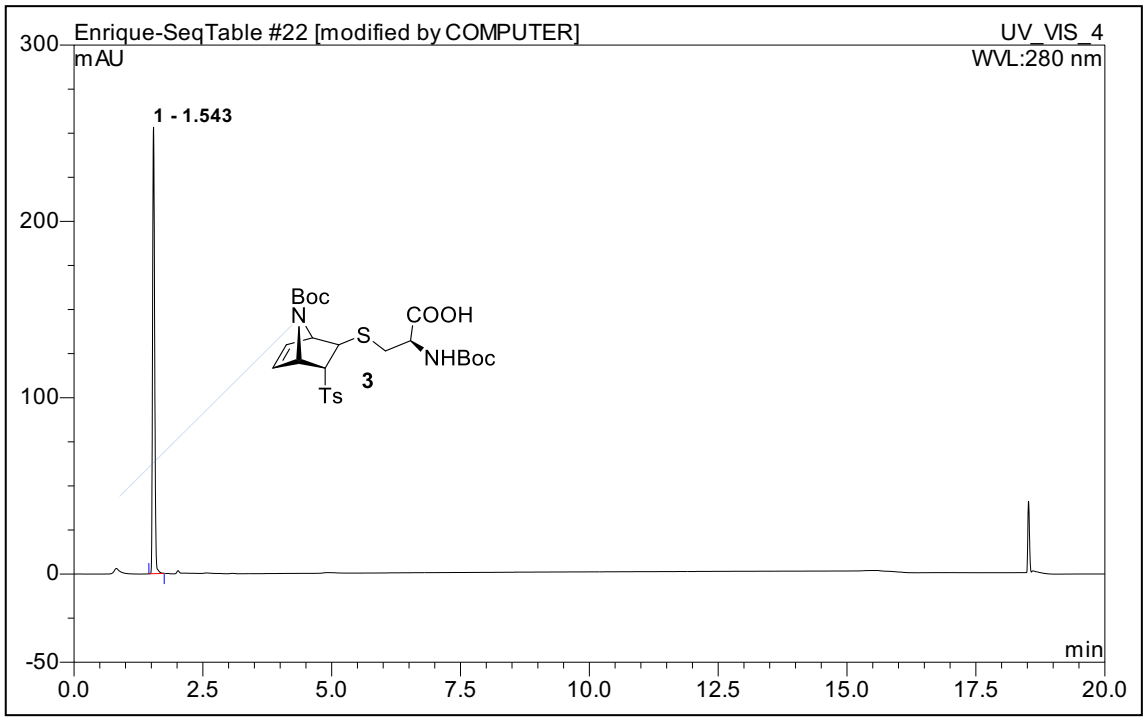
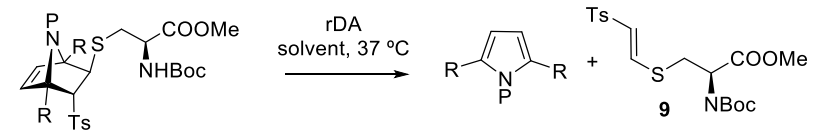


Figure S6. Chromatogram of the competition assay.

5. Determination of $t_{1/2}$ for rDA of azanorbornene-Cys adducts.

Half-life time ($t_{1/2}$) for the retro Diels-Alder (rDA) reactions were determined monitoring the reactions by ^{1}H-NMR in deuterated solvents. Thus, a solution of each substrate (0.04-0.05 mmol) in deuterated solvent (2.4 mL) was heated at 37 °C. A ^{1}H-NMR spectrum was registered at different times of reaction. The integration of NMR signals of the reaction crude affords the % conversion (figure S5). ^{1}H-NMR spectra were obtained by using a Bruker AV300 spectophotometer at room temperature. Samples were prepared either in $CDCl_3$ or DMSO-d_6. Data were plotted with Prism 5 and results fitted to a allosteric sigmoidal curve (Figure S6).



azabicycle-Cys adduct

Azabicycle-Cys adduct	R	P	solvent	half-life
2	H	Boc	$CDCl_3$	16.4 h
8	H	Ac	$CDCl_3$	37 h
6[b]	Me	Boc	$CDCl_3$	<5 min
8	H	Ac	DMSO-d_6	59 h

[b]Azanorbornene **6** is not stable and, once formed, rapidly decomposes into N-Boc-pyrrole and **4**.

Figure S7. Half life times ($t_{1/2}$) for the decomposition of azabicycle-Cys adducts.

An example of the determination of % conversion by ^{1}H-NMR is shown in Figure S8:

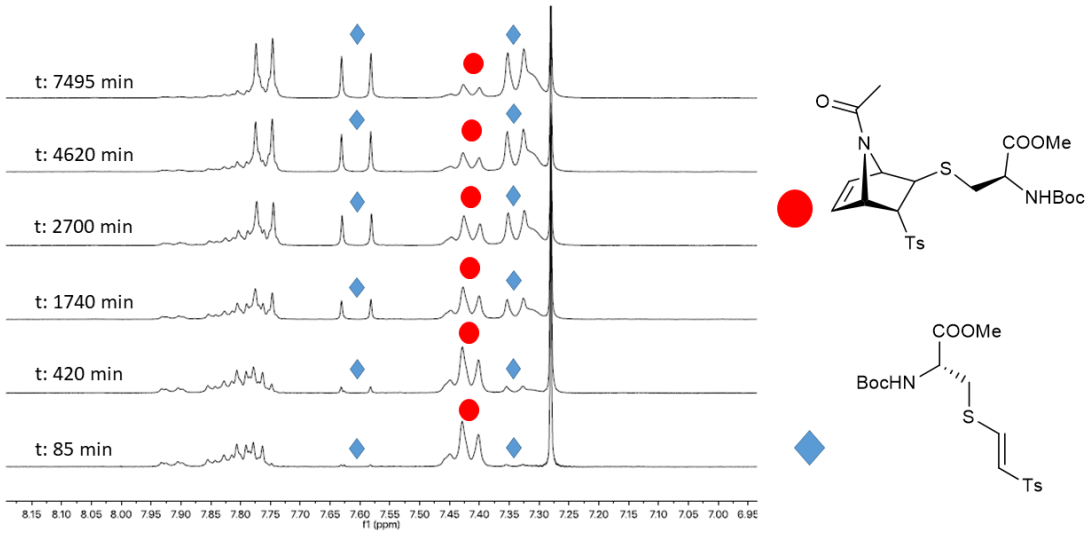


Figure S8. retro Diels-Alder reaction of adduct **10** monitoring in $CDCl_3$ at 37 °C by ^{1}H-NMR.

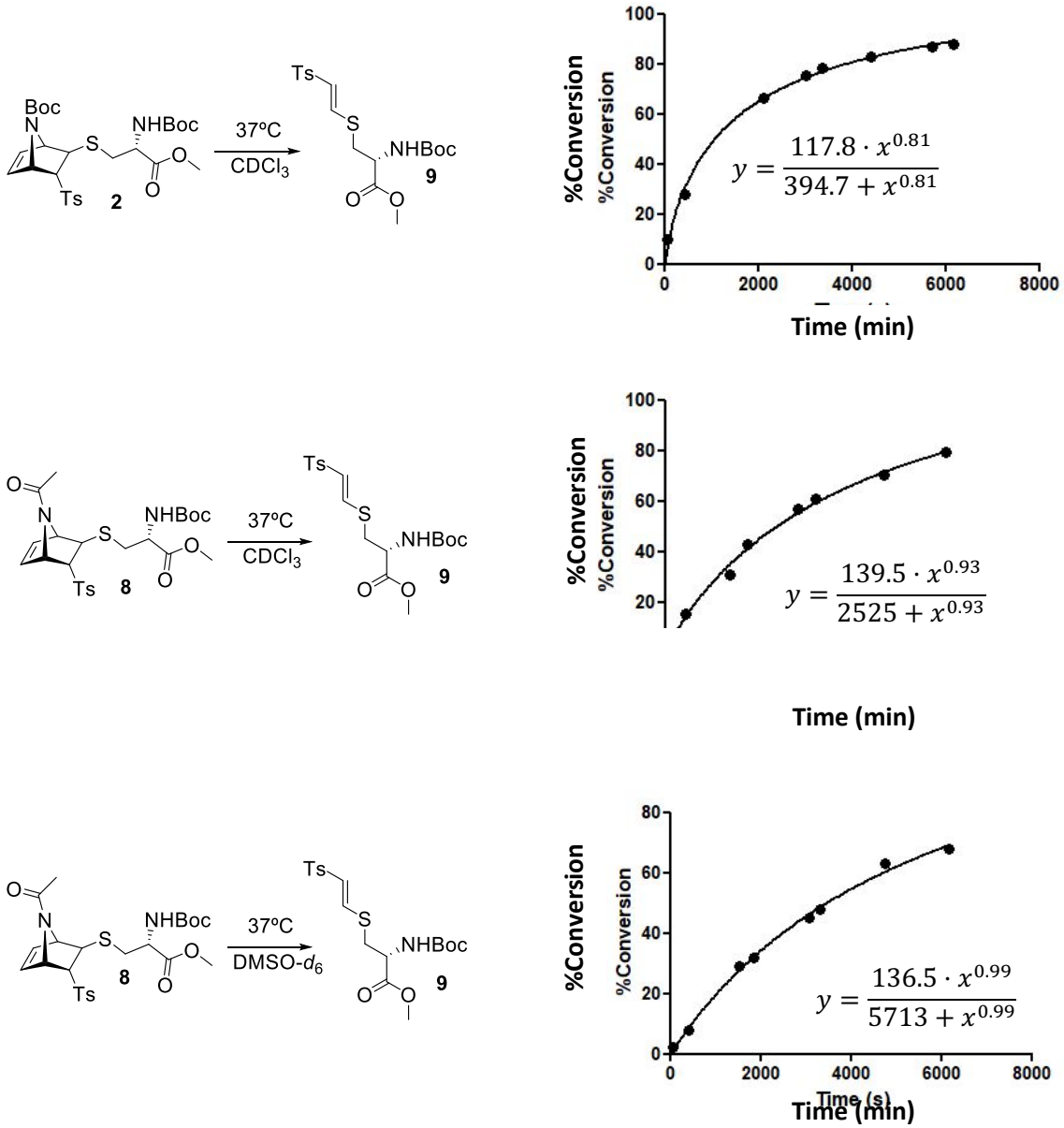


Figure S9. Graphical determination of half-life of azanorbornene-Cys adducts.

In order to determine the differences between DMSO-d_6 and an aqueous media, an assay of this reaction was performed. Compound number **8** (25.5 mg, 0.05 mmol) was dissolved in DMF (1.6 mL) and phosphate buffer (0.8 mL, pH 7.2, 0.1 M). The mixture was stirred at 37 ºC for 59 hours and then, the crude was diluted with ethyl ether, washed firstly with water and then with brine, and the organic layer was dried over Na_2SO_4. After removing the solvent under reduced pressure, an aliquot was taken and analysed by ^{1}HNMR. About 37% of conversion was observed.

6. Determination of kinetic rate constants.

Rate constants were determined under second order conditions. In order to determine the extinction molar coefficient of the tetrazines under the conditions employed, stocks solutions of each tetrazine (1, 2, 3, 4, 5, 6 mM) in 20% H_2O/DMF were prepared. The absorbance was determined in a SpectraMax® i3x Plate Reader (Molecular Devices) with 100 µL of the solutions at 37 °C at 540 nm. The values were plotted against the solution concentrations and fitted to a linear equation to obtain the molar extinction coefficient for the path length used (εl) from the slope.

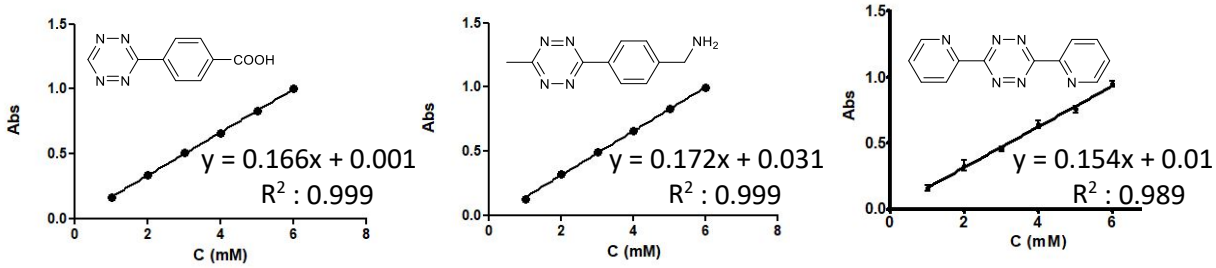
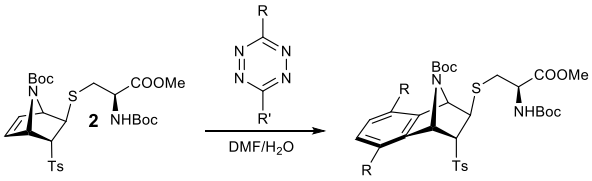


Figure S10. Calibration curve of each tetrazine.

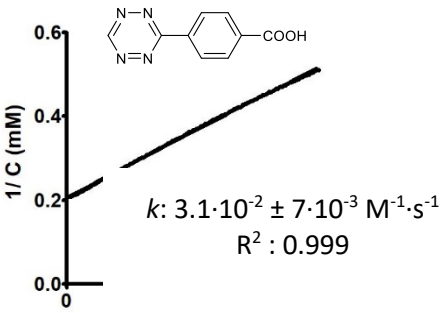
Stocks solutions in 20% H_2O/DMF were prepared for each tetrazine (10 mM) and compound **2** (10 mM). Mixing equal volumes (50 µL) resulted in final concentration of 5 mM of each one in 20% H_2O/DMF. The decay of the UV absortion of the tetrazine at the corresponding wavelength was followed over time at 37 °C. Each measurement was carried out three different times. The concentration at each time were determined using the extinction molar coefficients previously determined and the second rate constant were determined from the slope of a plot 1/C versus time.



a) k: $3.1 \cdot 10^{-2} \pm 1 \cdot 10^{-3}$ $M^{-1} \cdot s^{-1}$

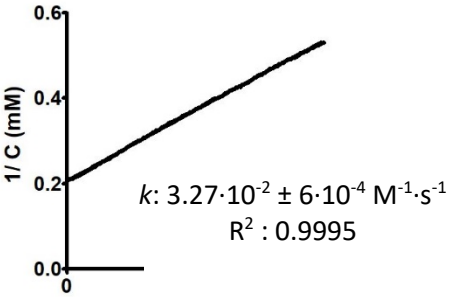
k: $2.87 \cdot 10^{-2} \pm 1 \cdot 10^{-4}$ $M^{-1} \cdot s^{-1}$

R^2 : 0.998



k: $3.1 \cdot 10^{-2} \pm 7 \cdot 10^{-3}$ $M^{-1} \cdot s^{-1}$

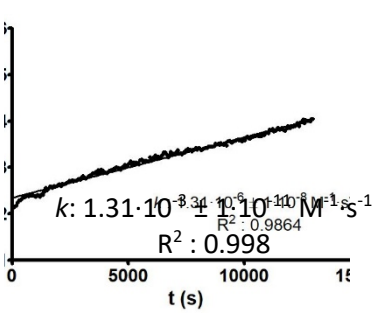
R^2 : 0.999



k: $3.27 \cdot 10^{-2} \pm 6 \cdot 10^{-4}$ $M^{-1} \cdot s^{-1}$

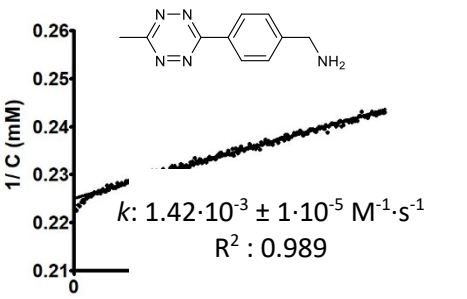
R^2 : 0.9995

b) k: $1.43 \cdot 10^{-3} \pm 5 \cdot 10^{-5}$ $M^{-1} \cdot s^{-1}$



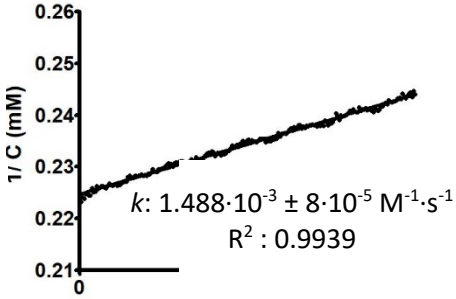
k: $1.31 \cdot 10^{-3} \pm 1 \cdot 10^{-5}$ $M^{-1} \cdot s^{-1}$

R^2 : 0.998



k: $1.42 \cdot 10^{-3} \pm 1 \cdot 10^{-5}$ $M^{-1} \cdot s^{-1}$

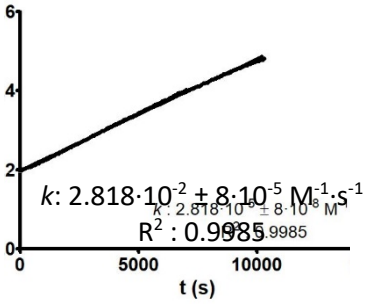
R^2 : 0.989



k: $1.488 \cdot 10^{-3} \pm 8 \cdot 10^{-5}$ $M^{-1} \cdot s^{-1}$

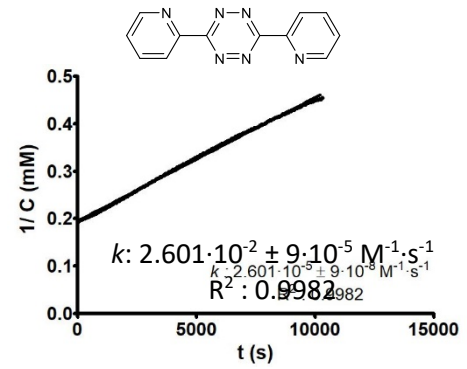
R^2 : 0.9939

c) k: $2.6 \cdot 10^{-2} \pm 1 \cdot 10^{-3}$ $M^{-1} \cdot s^{-1}$



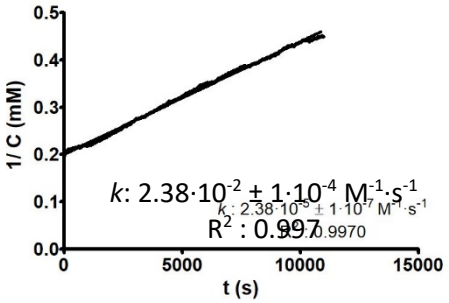
k: $2.818 \cdot 10^{-2} \pm 8 \cdot 10^{-5}$ $M^{-1} \cdot s^{-1}$

R^2 : 0.9985



k: $2.601 \cdot 10^{-2} \pm 9 \cdot 10^{-5}$ $M^{-1} \cdot s^{-1}$

R^2 : 0.9982



k: $2.38 \cdot 10^{-2} \pm 1 \cdot 10^{-4}$ $M^{-1} \cdot s^{-1}$

R^2 : 0.997

Figure S11. Rate constants k obtained from plots of 1/concentration versus time for compound **2** and tetrazines (**13a-c**).

7. Computational studies.

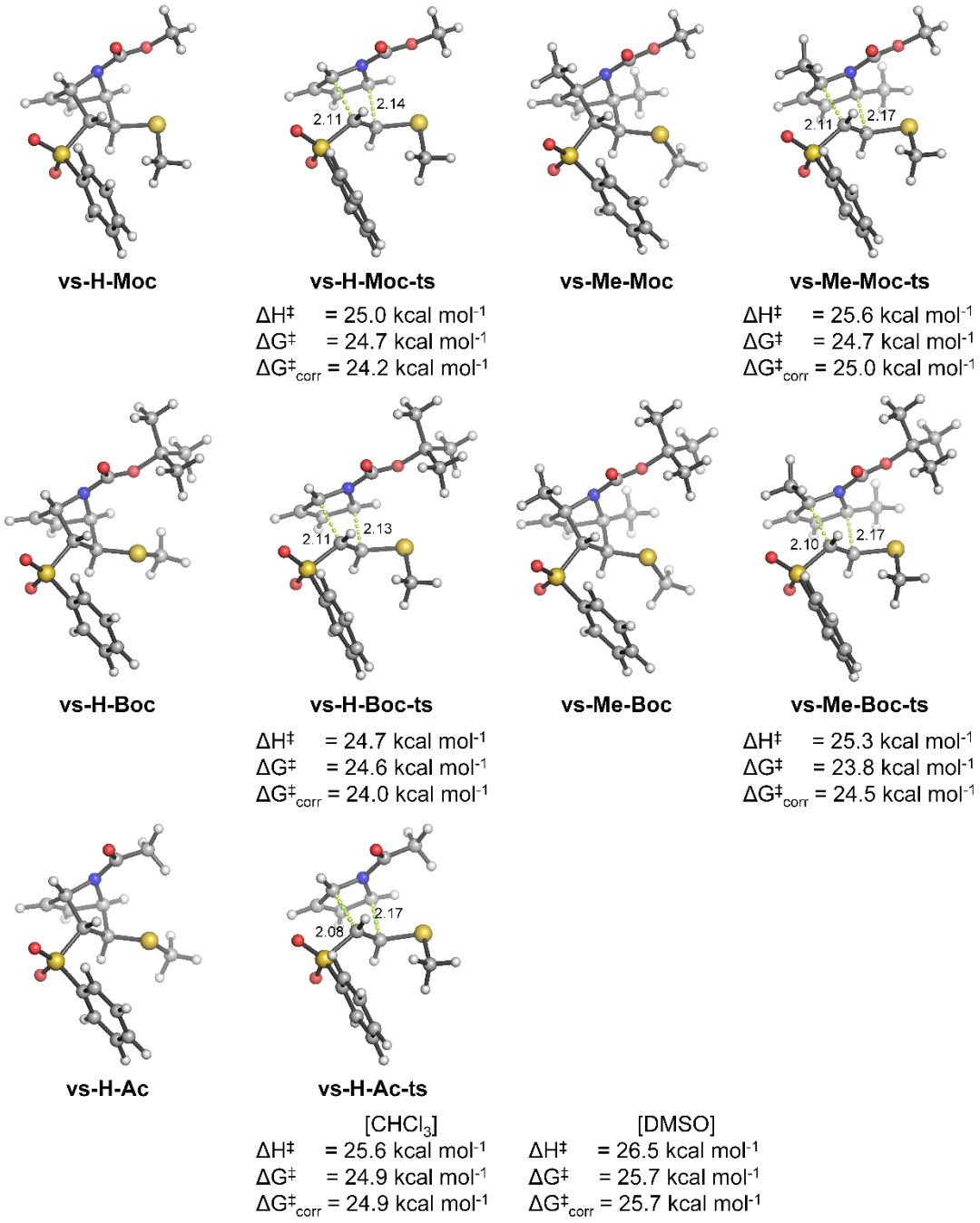


Figure S12. Lowest-energy conformations calculated for *N*-Moc-, *N*-Boc- and *N*-Ac protected (Moc = methyl carbamate; Boc = *tert*-butyl carbamate; Ac = acetyl) bicyclic azanorbornene vinyl sulfones without (**vs-H**) and with (**vs-Me**) methyl groups at the bridgehead carbons, and their retro-Diels Alder (rDA) reactions calculated with $PCM_{CHCl3/DMSO}$/M06-2X/6-31+G(d,p). Distances are given in angstrom and activation enthalpies ($\Delta H^{‡}$), free energies ($\Delta G^{‡}$) and free energies with quasiharmonic corrections to enthalpy and entropy ($\Delta G^{‡}_{corr}$) in kcal mol^{-1}. Calculated kinetics and thermodynamics (not shown) suggest that the absence of such bridgehead alkyl groups should

have little to no effect in the rDA reaction rate. While most of the calculated activation enthalpies ($\Delta H^{‡}$) and free energies with quasi-harmonic corrections ($\Delta G^{‡}_{corr}$) suggest a deceleration of the rDA reaction in analogues of **6** (labelled **vs-Me**) with respect to those of **2** (labelled **vs-H**), only the consideration of nearly complete models incorporating the Boc, phenyl sulfone and thioether groups (**vs-H/Me-Boc**) and uncorrected activation free energies ($\Delta G^{‡}$), allowed reproducing the observed trend .

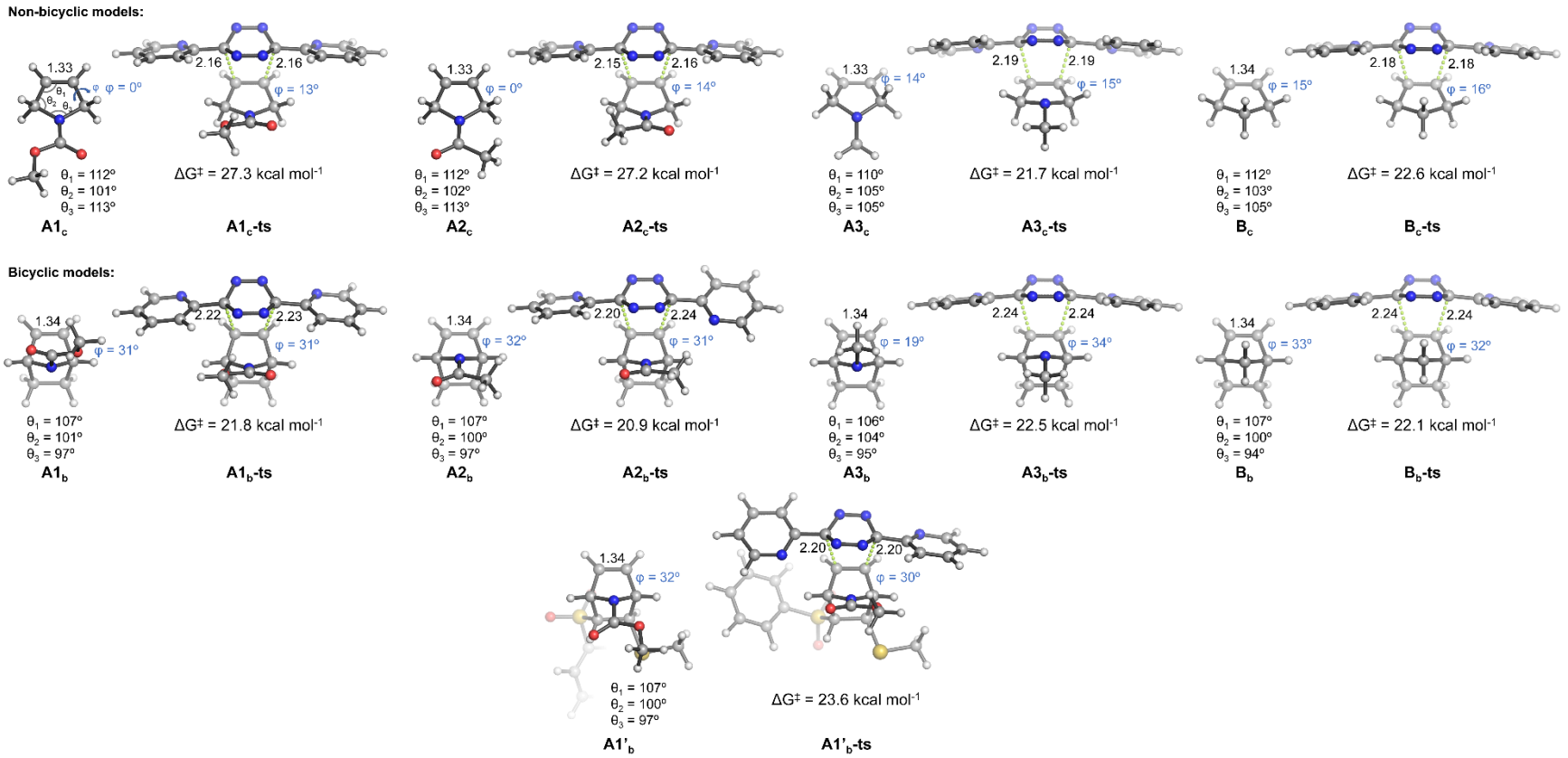


Figure S13. Lowest-energy conformations calculated for cyclic 2,5-dihydro-1*H*-pyrrole (**A1-3$_c$**) and cyclopentene (**B$_c$**) and bicyclic azanorbornene (**A1-3$_b$**) and norbornene (**B$_b$**) models and their iEDDA reactions with tetrazine **13a** calculated with PCM$_{DMF}$/M06-2X/6-31+G(d,p). Distances are given in angstrom and internal angles (θ_1–θ_3) in degrees. Activation free energies ($\Delta G^{‡}$) are calculated at the experimental reaction temperature (37º, 310 K). While most of the substrates are already pre-distorted in a geometry resembling the iEDDA transition structures and show similar activation barriers, *N*-Moc- and *N*-Ac-protected (Moc = methyl carbamate; Ac = acetyl) 2,5-dihydro-1*H*-pyrroles are planar and undergo significant distortion upon cycloaddition, thus increasing the corresponding activation barriers.

Computational Details.

Full geometry optimizations and transition structure (TS) searches were carried out with Gaussian 16[5] using the M06-2X hybrid functional[6] and 6-31+G(d,p) basis set with ultrafine integration grids. Bulk solvent effects in chloroform, dimethyl sulfoxide and *N*,*N*-dimethylformamide were considered implicitly through the IEF-PCM polarizable continuum model.[7] The possibility of different conformations was taken into account for all structures. All stationary points were characterized by a frequency analysis performed at the same level used in the geometry optimizations from which thermal corrections were obtained at the reaction temperatures (298.15 for retro-Diels-Alder and 310.10 K for iEDDA). The quasiharmonic approximation reported by Truhlar *et al.* was occasionally used to replace the harmonic oscillator approximation for the calculation of the vibrational contribution to enthalpy and entropy.[8] Scaled frequencies were not considered. Mass-weighted intrinsic reaction coordinate (IRC) calculations were carried out by using the Gonzalez and Schlegel scheme[9,10] in order to ensure that the TSs indeed connected the appropriate reactants and products. Gibbs free energies (ΔG) were used for the discussion on the relative stabilities of the considered structures. Free energies calculated using the gas phase standard state concentration (1 atm = 1/24.5 M) were converted to reproduce the standard state concentration in solution (1 M) by adding or subtracting 1.89 kcal mol^{-1} for bimolecular additions and decompositions, respectively. The lowest energy conformer for each calculated stationary point was considered in the discussion; all the computed structures can be obtained from authors upon request. Cartesian coordinates, electronic energies, entropies, enthalpies, Gibbs free energies, and lowest frequencies of the calculated structures are summarized below.

[5] Gaussian 16, Revision B.01, M. J. Frisch, *et al.* Gaussian, Inc., Wallingford CT, 2016.
[6] Y. Zhao, D. G. Truhlar, *Theor. Chem. Acc.* 2008, **120**, 215–241.
[7] G. Scalmani, M. J. Frisch, *J. Chem. Phys.* 2010, **132**, 114110.
[8] R. F. Ribeiro, A. V. Marenich, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem. B* 2011, **115**, 14556–14562.
[9] C. Gonzalez, H. B. Schlegel, *J. Chem. Phys.* 1989, **90**, 2154–2161.
[10] C. Gonzalez, H. B. Schlegel, *J. Phys. Chem.* 1990, **94**, 5523–5527.

Table S1. Energies, entropies, and lowest frequencies of the lowest energy calculated structures.

Label	E_{elec} (Hartree)	E_{elec} + ZPE (Hartree)	H (Hartree)	S (cal mol^{-1} K^{-1})	G (Hartree)	G_{corr} (Hartree)	Lowest freq. (cm^{-1})	# of imag freq.
vs-H-Moc[a,d]	–1733.377516	–1733.070594	–1733.049125	151.0	–1733.120879	–1733.117083	23.1	0
vs-H-Moc-ts[a,d]	–1733.334697	–1733.031405	–1733.009360	154.0	–1733.082525	–1733.078504	–516.1	1
vs-Me-Moc[a,d]	–1811.977245	–1811.615104	–1811.590514	163.7	–1811.668289	–1811.663992	20.8	0
vs-Me-Moc-ts[a,d]	–1811.933840	–1811.574838	–1811.549734	166.6	–1811.628905	–1811.624220	–488.7	1
vs-H-Boc[a,d]	–1851.276559	–1850.885601	–1850.86001	172.5	–1850.941947	–1850.935321	10.2	0
vs-H-Boc-ts[a,d]	–1851.234084	–1850.846767	–1850.820709	172.7	–1850.902741	–1850.897127	–512.7	1
vs-Me-Boc[a,d]	–1929.876125	–1929.429901	–1929.401385	181.3	–1929.487539	–1929.48192	22.1	0
vs-Me-Boc-ts[a,d]	–1929.833086	–1929.390263	–1929.361067	186.5	–1929.449683	–1929.442893	–486.8	1
vs-H-Ac *(CHCl$_3$)* [a,d]	–1658.174644	–1657.873544	–1657.852766	149.2	–1657.923645	–1657.919178	19.7	0
vs-H-Ac-ts *(CHCl$_3$)* [a,d]	–1658.130437	–1657.833257	–1657.811937	151.5	–1657.883928	–1657.879503	–514.2	1
vs-H-Ac *(DMSO)* [b,d]	–1658.181519	–1657.880436	–1657.859676	148.7	–1657.930349	–1657.926077	19.9	0
vs-H-Ac-ts *(DMSO)* [b,d]	–1658.135824	–1657.838848	–1657.817522	151.4	–1657.889444	–1657.885121	–512.2	1
13a[c,e]	–790.249006	–790.057529	–790.042481	121.3	–790.102433	–790.098729	24.8	0
A1c[c,e]	–439.093807	–438.944003	–438.933612	93.9	–438.980025	–438.979469	56.4	0
A1c-ts[c,e]	–1229.331674	–1228.987628	–1228.963524	160.3	–1229.042761	–1229.037708	–415.1	1
A2c[c,e]	–363.891751	–363.747548	–363.738208	88.2	–363.781803	–363.781388	65.2	0
A2c-ts[c,e]	–1154.129707	–1153.791109	–1153.768132	154.0	–1153.844230	–1153.839841	–419.8	1
A3c[c,e]	–250.555522	–250.421163	–250.414061	75.9	–250.451561	–250.451561	108.8	0
A3c-ts[c,e]	–1040.799570	–1040.471747	–1040.450684	144.1	–1040.521920	–1040.518794	–380.8	1
Bc[c,e]	–195.239765	–195.122340	–195.116419	69.8	–195.150940	–195.150940	166.2	0
Bc-ts[c,e]	–985.482655	–985.171046	–985.151394	137.7	–985.219435	–985.216657	–372.1	1
A1b[c,e]	–516.464063	–516.278220	–516.267057	97.0	–516.315024	–516.314668	74.8	0
A1b-ts[c,e]	–1306.709708	–1306.330544	–1306.305068	165.6	–1306.386932	–1306.381704	–309.8	1
A2b[c,e]	–441.261413	–441.081301	–441.071064	92.2	–441.116658	–441.116447	80.4	0
A2b-ts	–1231.508205	–1231.134562	–1231.110108	160.2	–1231.189269	–1231.184833	–314.7	1
A3b	–327.937459	–327.767048	–327.759065	80.0	–327.798590	–327.798589	227.4	0
A3b-ts	–1118.180348	–1117.816682	–1117.794381	150.6	–1117.868828	–1117.864472	–298.1	1
Bb	–272.616946	–272.463089	–272.456469	73.9	–272.493006	–272.493006	258.6	0
Bb-ts	–1062.860732	–1062.513073	–1062.492275	144.1	–1062.563498	–1062.559521	–284.7	1
A1'b	–1733.383521	–1733.076593	–1733.053442	156.4	–1733.130758	–1733.125888	17.5	0
A1'b-ts	–2523.627081	–2523.127028	–2523.089447	221.1	–2523.198751	–2523.189973	–341.4	1

[a]Energy values calculated at the PCM($CHCl_3$)/M06-2X/6-31G(d) level. [b]Energy values calculated at the PCM(DMSO)/M06-2X/6-31G(d) level. [c]Energy values calculated at the PCM(DMF)/M06-2X/6-31G(d) level. [d]Thermal corrections at 298.15 K. [e]Thermal corrections at 310.15 K. 1 Hartree = 627.51 kcal mol^{-1}.

Cartesian coordinates of the lowest energy structures calculated with PCM(H_2O)/M06-2X/6-31G(d)

vs-H-Moc

C	1.465681	2.704075	-0.165463
C	1.407760	1.499185	0.759147
C	1.936915	2.262250	-1.333871
H	1.077505	3.683344	0.081769
C	0.239538	0.577970	0.233130
H	1.403062	1.660540	1.833192
N	2.555922	0.742715	0.257453
C	2.193330	0.777220	-1.170222
H	2.025846	2.797783	-2.270114
H	0.088487	-0.249254	0.934580
C	0.768891	0.091303	-1.142846
C	2.979158	-0.392430	0.906331
H	2.915899	0.303484	-1.830933
H	0.147111	0.435648	-1.973671
O	2.692099	-0.675126	2.054370
O	3.805643	-1.105898	0.129674
C	4.228464	-2.356913	0.681220
H	4.885460	-2.796861	-0.065961
H	4.763199	-2.197154	1.618497
H	3.360460	-2.997208	0.853960
S	0.989230	-1.716374	-1.266813
C	-0.738037	-2.265615	-1.280559
H	-1.291218	-1.782673	-2.088865
H	-0.717251	-3.342412	-1.456486
H	-1.229840	-2.073796	-0.323725
S	-1.330189	1.486746	0.207523
O	-1.358614	2.257063	1.458488
O	-1.489449	2.183546	-1.073219
C	-2.584620	0.226323	0.293078
C	-3.324385	-0.064029	-0.848821
C	-2.769088	-0.459386	1.493544
C	-4.270762	-1.086198	-0.787760
H	-3.154759	0.500575	-1.759788
C	-3.710902	-1.482970	1.537709
H	-2.191080	-0.193380	2.373715
C	-4.455416	-1.796655	0.397678
H	-4.859649	-1.327605	-1.666204
H	-3.867553	-2.031610	2.460152
H	-5.188393	-2.595864	0.438070

vs-H-Moc-ts

C	1.532301	2.686964	-0.304897
C	1.681699	1.691680	0.707039
C	2.017999	2.153161	-1.472735
H	0.986907	3.611283	-0.178204
C	0.105509	0.419729	0.133701
H	1.566457	1.809948	1.775730
N	2.611642	0.781490	0.210246
C	2.475082	0.836644	-1.181574
H	1.936594	2.568270	-2.467673
H	0.149279	-0.213895	1.019738
C	0.542574	-0.076825	-1.106859
C	3.017865	-0.340180	0.931866
H	3.089058	0.198209	-1.802685
H	0.096198	0.330819	-2.009797
O	2.660655	-0.564922	2.065479
O	3.866730	-1.072688	0.219506
C	4.229215	-2.328984	0.809510
H	4.924899	-2.786520	0.110303
H	4.702431	-2.166050	1.778352
H	3.335505	-2.945698	0.925964
S	1.027439	-1.800593	-1.207988
C	-0.602504	-2.586407	-1.010625
H	-1.267446	-2.299704	-1.827537
H	-0.442187	-3.666079	-1.035882
H	-1.050522	-2.306505	-0.053807
S	-1.323647	1.457382	0.187015
O	-1.311967	2.199589	1.455765
O	-1.433788	2.195055	-1.077871
C	-2.672015	0.289760	0.254892
C	-3.274364	-0.110952	-0.934840
C	-3.041235	-0.244146	1.487629
C	-4.276669	-1.077605	-0.884325
H	-2.963364	0.333947	-1.874856
C	-4.041357	-1.213503	1.523550
H	-2.558625	0.101339	2.396636
C	-4.654019	-1.629843	0.340385
H	-4.762719	-1.397966	-1.799924
H	-4.344749	-1.639410	2.474060
H	-5.433405	-2.384288	0.374107

vs-Me-Moc

C	-1.404620	2.252586	1.319919
C	-1.369019	1.680343	-0.090298
C	-1.677878	1.249626	2.148894
H	-1.147800	3.278972	1.551767
C	-0.079591	0.780910	-0.178174
N	-2.370113	0.592082	0.062744
C	-1.825777	-0.011346	1.314402
H	-1.698834	1.251278	3.232441
H	-0.009300	0.393413	-1.201947
C	-0.342636	-0.346965	0.849647
C	-2.724637	-0.173706	-1.032876
H	0.342784	-0.301510	1.703365
O	-2.401570	0.054273	-2.184390
O	-3.543682	-1.173541	-0.680519
C	-3.874024	-2.087082	-1.731030
H	-4.509813	-2.840693	-1.270606
H	-4.407624	-1.570951	-2.530453
H	-2.964951	-2.543091	-2.129261
S	-0.113662	-1.958672	0.028136
C	0.252282	-3.005041	1.463852
H	-0.605637	-3.096572	2.130824
H	0.509275	-3.991393	1.074793
H	1.111980	-2.603669	2.006218
S	1.494288	1.630214	0.071796
O	1.618100	2.630015	-0.995819
O	1.620263	2.051251	1.471657
C	2.678896	0.332560	-0.215982
C	3.182241	-0.372606	0.874302
C	3.014779	0.009722	-1.528557
C	4.043147	-1.441877	0.636669
H	2.910179	-0.078138	1.883293
C	3.877391	-1.060567	-1.751454
H	2.612133	0.589360	-2.353647
C	4.383925	-1.785969	-0.671930
H	4.449563	-2.002668	1.471849
H	4.153841	-1.327324	-2.765881
H	5.053329	-2.621012	-0.851901
C	-1.586280	2.679701	-1.206842
H	-1.406432	2.239703	-2.186257
H	-2.615277	3.047325	-1.163068
H	-0.904892	3.521349	-1.064013
C	-2.602914	-1.125734	1.977388
H	-2.146751	-1.331310	2.951043
H	-3.635771	-0.808753	2.138802
H	-2.605813	-2.036879	1.379495

vs-Me-Moc-ts

C	-1.387670	-2.501382	-0.613758
C	-1.509544	-1.659224	0.536622
C	-1.864231	-1.812354	-1.692153
H	-0.861362	-3.446683	-0.607491
C	0.099893	-0.379526	0.080208
N	-2.427546	-0.657817	0.159865
C	-2.319592	-0.536048	-1.246312
H	-1.796701	-2.090311	-2.735900
H	0.104602	0.111141	1.054111
C	-0.340332	0.323481	-1.057673
C	-2.788778	0.372998	1.033551
H	0.084927	0.058736	-2.021852
O	-2.325515	0.512062	2.142728
O	-3.740790	1.137163	0.506782
C	-4.066487	2.308703	1.266529
H	-4.829368	2.824888	0.688188
H	-4.448847	2.026646	2.248230
H	-3.175987	2.931234	1.376726
S	-0.756933	2.057692	-0.863654
C	0.869783	2.735218	-0.408553
H	1.585471	2.588363	-1.219302
H	0.729036	3.804328	-0.237076
H	1.244182	2.266491	0.504540
S	1.516715	-1.423886	-0.092407
O	1.559664	-2.365164	1.035784
O	1.568002	-1.948315	-1.463588
C	2.884702	-0.292533	0.104534
C	3.462526	0.270238	-1.030160
C	3.296323	0.050266	1.390803
C	4.481880	1.206682	-0.867861
H	3.119097	-0.028398	-2.015625
C	4.311807	0.992316	1.539631
H	2.833847	-0.419851	2.253434
C	4.899601	1.570295	0.412822
H	4.948889	1.651332	-1.740502
H	4.647083	1.270677	2.533180
H	5.691320	2.302607	0.534133
C	-3.187134	0.351200	-2.091908
H	-4.233953	0.046157	-2.002235
H	-3.120449	1.404230	-1.824634
H	-2.875023	0.229238	-3.131678
C	-1.382154	-2.150182	1.951023
H	-0.891244	-1.426658	2.599895
H	-2.375111	-2.356341	2.362549
H	-0.798546	-3.070839	1.941515

vs-H-Boc

C	-0.018411	3.129700	0.591109
C	-0.331205	2.117060	-0.498560
C	-0.458039	2.603109	1.736587
H	0.552615	4.035835	0.438479
C	0.637785	0.896649	-0.288387
N	-1.557601	1.528888	0.046746
C	-1.051416	1.249003	1.395644
H	-0.328363	2.980791	2.742645
H	0.472501	0.183686	-1.104520
C	0.192946	0.325316	1.081359
C	-2.328816	0.657144	-0.690344
H	0.961523	0.435523	1.852560
O	-2.256268	0.564659	-1.902755
O	-3.197596	0.022787	0.105082
S	-0.187702	-1.440398	0.853134
C	-0.955279	-1.821717	2.449560
H	-1.942720	-1.362805	2.530748
H	-1.065695	-2.906333	2.494953
H	-0.316091	-1.497519	3.273793
S	2.390502	1.302839	-0.412023
O	2.576031	1.902839	-1.739240
O	2.827789	2.035916	0.780332
C	3.169206	-0.297294	-0.385081
C	3.679722	-0.775181	0.818941
C	3.198914	-1.050561	-1.557065
C	4.236618	-2.052062	0.845748
H	3.647628	-0.151910	1.706822
C	3.755665	-2.326060	-1.514441
H	2.804641	-0.639454	-2.481487
C	4.269514	-2.823912	-0.315687
H	4.644709	-2.442231	1.772002
H	3.792058	-2.928295	-2.415838
H	4.702349	-3.818714	-0.288680
H	-1.790111	0.829992	2.073631
H	-0.410094	2.456745	-1.527563
C	-4.181171	-0.909635	-0.447292
C	-5.126261	-0.169832	-1.389314
C	-3.478060	-2.080202	-1.126616
C	-4.925686	-1.382783	0.795721
H	-5.554915	0.701117	-0.884384
H	-4.610474	0.156450	-2.292916
H	-5.943662	-0.839712	-1.672101
H	-2.772100	-2.546338	-0.433350
H	-4.227313	-2.823231	-1.415904
H	-2.938791	-1.757668	-2.018092
H	-5.708640	-2.090326	0.510565
H	-4.238278	-1.882828	1.484377
H	-5.388159	-0.536114	1.310620

vs-H-Boc-ts

C	0.278780	3.135374	-0.267091
C	0.701083	2.141360	0.667616
C	0.776985	2.773699	-1.493460
H	-0.432198	3.919341	-0.048794
C	-0.622551	0.601706	0.106403
H	0.645745	2.179568	1.746828
N	1.753731	1.467743	0.056842
C	1.510268	1.564260	-1.315104
H	0.541527	3.212376	-2.453237
H	-0.374865	-0.067747	0.930370
C	-0.200382	0.292674	-1.199065
C	2.493086	0.472553	0.707002
H	2.194948	1.098388	-2.011297
H	-0.791331	0.669976	-2.029474
O	2.313780	0.202190	1.873704
O	3.396931	-0.033510	-0.116144
S	0.594283	-1.286261	-1.489592
C	-0.831640	-2.396107	-1.268481
H	-1.599154	-2.191167	-2.017235
H	-0.464739	-3.416652	-1.396121
H	-1.253873	-2.283415	-0.266539
S	-2.224089	1.306149	0.347042
O	-2.265221	1.919350	1.682267
O	-2.582130	2.112591	-0.826924
C	-3.299125	-0.119072	0.372035
C	-3.902232	-0.526605	-0.814965
C	-3.451044	-0.829787	1.560715
C	-4.679511	-1.683075	-0.807523
H	-3.765831	0.058108	-1.719295
C	-4.226542	-1.987253	1.553307
H	-2.976642	-0.475637	2.470705
C	-4.835587	-2.412938	0.371578
H	-5.162876	-2.013044	-1.721149

H	-4.358457	-2.552958	2.469569
H	-5.439601	-3.314578	0.371413
C	4.327472	-1.081786	0.330690
C	5.247334	-0.509138	1.402787
C	3.558399	-2.306718	0.813246
C	5.099186	-1.397133	-0.943828
H	5.740901	0.394959	1.034676
H	4.695489	-0.273947	2.314193
H	6.017059	-1.248697	1.641498
H	2.843808	-2.626957	0.050512
H	4.271789	-3.117599	0.986980
H	3.023663	-2.105227	1.742015
H	5.846179	-2.168194	-0.739041
H	4.419055	-1.762740	-1.718440
H	5.609415	-0.503629	-1.313623

vs-Me-Boc

C	-0.084342	2.824744	1.206529
C	-0.400865	2.095440	-0.092982
C	-0.440822	2.026715	2.208607
H	0.426626	3.778868	1.243165
C	0.624744	0.906952	-0.208657
N	-1.575705	1.304020	0.352499
C	-0.987223	0.744994	1.601925
H	-0.291387	2.165471	3.273038
H	0.423263	0.382548	-1.150966
C	0.307050	0.022739	1.021063
C	-2.306424	0.557787	-0.557999
H	1.112625	0.040827	1.763570
O	-2.161058	0.622330	-1.766056
O	-3.238925	-0.164400	0.069073
S	0.088427	-1.703199	0.479766
C	0.480434	-2.572295	2.023076
H	-0.258429	-2.373014	2.800237
H	0.477782	-3.638691	1.792308
H	1.478016	-2.287563	2.367288
S	2.362564	1.378511	-0.339810
O	2.515804	2.105926	-1.605600
O	2.804683	2.007197	0.910213
C	3.182666	-0.195067	-0.482043
C	3.702024	-0.790267	0.664775
C	3.214216	-0.823375	-1.724483
C	4.261496	-2.061884	0.560532
H	3.674512	-0.259207	1.611471
C	3.777025	-2.093853	-1.814436
H	2.809646	-0.323722	-2.599435
C	4.292415	-2.711640	-0.673925
H	4.675160	-2.542785	1.440844
H	3.813413	-2.600468	-2.772887
H	4.726913	-3.703268	-0.749126
C	-0.562302	2.981777	-1.310449
H	-0.618710	2.403619	-2.230804
H	-1.475539	3.573954	-1.204562
H	0.292088	3.660326	-1.367346
C	-1.868113	-0.073228	2.518267
H	-1.323486	-0.249557	3.451457
H	-2.781040	0.481180	2.747139
H	-2.142901	-1.029550	2.073000
C	-4.141416	-1.043561	-0.673906
C	-5.050694	-0.211054	-1.572435
C	-3.351314	-2.090991	-1.452392
C	-4.945574	-1.703884	0.440697
H	-5.552750	0.563404	-0.984798
H	-4.486806	0.258629	-2.379037
H	-5.814954	-0.861553	-2.008093
H	-2.652379	-2.603773	-0.785320
H	-4.049596	-2.828127	-1.860032
H	-2.793146	-1.642065	-2.274600
H	-5.686035	-2.383533	0.010786
H	-4.285209	-2.276255	1.098891
H	-5.465518	-0.947560	1.035418

vs-Me-Boc-ts

C	0.230877	2.955157	-0.422031
C	0.623787	2.012279	0.583002
C	0.715218	2.517833	-1.620857
H	-0.461166	3.766660	-0.240142
C	-0.754953	0.518125	0.075915
N	1.669258	1.272343	-0.002919
C	1.446068	1.311861	-1.396106
H	0.489359	2.904243	-2.606242
H	-0.559035	-0.094359	0.956858
C	-0.326971	0.075727	-1.191472
C	2.362771	0.270935	0.697700
H	-0.896108	0.387746	-2.062931
O	2.050102	-0.086610	1.811364
O	3.408618	-0.140332	-0.003901
S	0.409032	-1.552850	-1.306586
C	-1.004055	-2.596141	-0.829198
H	-1.818130	-2.493846	-1.548869
H	-0.646781	-3.628069	-0.828679
H	-1.359518	-2.334377	0.170241
S	-2.354165	1.265839	0.188640
O	-2.457873	1.987960	1.464859
O	-2.646973	1.979231	-1.061770
C	-3.459346	-0.134858	0.278418
C	-4.032484	-0.617281	-0.894951
C	-3.670811	-0.749469	1.510760
C	-4.838318	-1.752419	-0.828914
H	-3.850567	-0.105587	-1.834803
C	-4.472971	-1.887360	1.562483
H	-3.220562	-0.336672	2.408436
C	-5.051561	-2.388082	0.394770
H	-5.298690	-2.139547	-1.731966
H	-4.650221	-2.378915	2.513267
H	-5.676814	-3.273975	0.440377
C	2.368504	0.717966	-2.420653
H	3.342191	1.215597	-2.382363
H	2.534281	-0.349348	-2.281448
H	1.926246	0.883938	-3.405754
C	0.555523	2.291733	2.058293
H	0.282850	1.410269	2.636226
H	1.528722	2.643475	2.414526
H	-0.190663	3.069019	2.224473
C	4.301836	-1.189488	0.505716
C	4.993773	-0.700771	1.773755
C	3.526905	-2.483895	0.722124
C	5.303170	-1.344447	-0.632454
H	5.469583	0.267854	1.594032
H	4.290190	-0.608750	2.601827
H	5.771150	-1.418641	2.050896
H	3.012144	-2.773366	-0.197898
H	4.235198	-3.273560	0.989643
H	2.793353	-2.383151	1.523146
H	6.041497	-2.107007	-0.371557
H	4.793018	-1.650323	-1.550372
H	5.823028	-0.399953	-0.815577

vs-H-Ac *(CHCl3)*

C	-1.952819	-2.090291	-1.104698
C	-1.775080	-1.474765	0.274315
C	-2.234263	-1.086833	-1.939394

H	-1.770132	-3.130622	-1.338300
C	-0.430764	-0.663614	0.263939
N	-2.697443	-0.341062	0.173834
C	-2.220446	0.184931	-1.110116
H	-2.331717	-1.116728	-3.016995
H	-0.272908	-0.250656	1.267138
C	-0.693711	0.443897	-0.785970
C	-3.137344	0.368007	1.261524
H	-0.079912	0.319426	-1.684159
O	-2.978688	-0.058492	2.399627
S	-0.330949	2.062932	-0.044635
C	-0.535479	3.109119	-1.511305
H	-1.562858	3.085609	-1.880396
H	-0.293974	4.129165	-1.209861
H	0.153958	2.798609	-2.299367
S	1.043194	-1.656342	-0.043339
O	1.101984	-2.647468	1.037445
O	1.060472	-2.106396	-1.439106
C	2.361624	-0.481179	0.170061
C	2.902043	0.133193	-0.956793
C	2.773908	-0.161241	1.461806
C	3.886422	1.102794	-0.778491
H	2.563271	-0.155168	-1.946798
C	3.756604	0.811815	1.624725
H	2.340542	-0.671393	2.316703
C	4.306457	1.442871	0.507703
H	4.325526	1.590120	-1.642515
H	4.094365	1.074290	2.621499
H	5.071576	2.200845	0.641047
C	-3.893380	1.641859	0.960536
H	-3.187411	2.434840	0.698567
H	-4.589030	1.503202	0.129754
H	-4.439844	1.934017	1.856372
H	-2.767643	1.047455	-1.486604
H	-1.920409	-2.095555	1.153886

vs-H-Ac-ts *(CHCl3)*

C	-2.028435	-2.274249	-0.524360
C	-2.002058	-1.406904	0.610014
C	-2.496120	-1.538143	-1.584397
H	-1.599851	-3.266117	-0.547962
C	-0.318181	-0.281368	0.130028
H	-1.866372	-1.682056	1.646863
N	-2.830037	-0.330216	0.290868
C	-2.773900	-0.228399	-1.100467
H	-2.514656	-1.823991	-2.626966
H	-0.261652	0.250857	1.080026
C	-0.703938	0.406038	-1.033870
C	-3.171755	0.672016	1.209361
H	-3.323285	0.549472	-1.615341
H	-0.348415	0.034786	-1.990980
O	-2.759575	0.606085	2.349693
S	-0.954735	2.174384	-0.942219
C	0.753092	2.732459	-0.649785
H	1.390284	2.475205	-1.497737
H	0.718412	3.817772	-0.536787
H	1.152013	2.283551	0.263295
S	0.974544	-1.484530	0.023034
O	0.893975	-2.358316	1.201898
O	0.968567	-2.082305	-1.318009
C	2.455591	-0.500762	0.175886
C	3.083439	-0.047172	-0.981123
C	2.905262	-0.152365	1.447727
C	4.196319	0.781958	-0.856260
H	2.705961	-0.346311	-1.953925
C	4.015808	0.681677	1.558222
H	2.398443	-0.535321	2.328117
C	4.656280	1.148598	0.409153
H	4.703597	1.140305	-1.745881
H	4.382910	0.962439	2.539697
H	5.521788	1.796830	0.500939
C	-4.124082	1.728162	0.716181
H	-4.367655	2.381718	1.551965
H	-3.671454	2.320371	-0.081726
H	-5.034410	1.261352	0.330769

vs-H-Ac *(DMSO)*

C	-1.949735	-2.070286	-1.137841
C	-1.781705	-1.472268	0.250531
C	-2.212590	-1.054567	-1.964077
H	-1.779090	-3.110797	-1.379691
C	-0.432066	-0.669537	0.265941
N	-2.692079	-0.328908	0.152593
C	-2.198516	0.208029	-1.121086
H	-2.302021	-1.069133	-3.042656
H	-0.282308	-0.267377	1.274775
C	-0.675225	0.452267	-0.774668
C	-3.174242	0.356602	1.232026
H	-0.052675	0.339275	-1.668069
O	-3.063770	-0.095755	2.369299
S	-0.315452	2.059535	-0.006304
C	-0.500025	3.130859	-1.458010
H	-1.523831	3.116534	-1.836233
H	-0.256800	4.144079	-1.136013
H	0.196095	2.827681	-2.242635
S	1.043935	-1.662445	-0.027718
O	1.103749	-2.644625	1.064002
O	1.064992	-2.138470	-1.415884
C	2.362469	-0.486995	0.172260
C	2.892775	0.125125	-0.961220
C	2.782789	-0.161813	1.460576
C	3.875898	1.098001	-0.792824
H	2.546481	-0.162015	-1.948918
C	3.765399	0.813321	1.612910
H	2.354883	-0.666069	2.321539
C	4.305548	1.442120	0.489467
H	4.305394	1.584963	-1.661667
H	4.109723	1.079844	2.606277
H	5.069643	2.202356	0.614651
C	-3.910867	1.640511	0.929838
H	-3.192856	2.432370	0.699083
H	-4.581856	1.520991	0.076351
H	-4.482544	1.924109	1.812584
H	-2.734799	1.077638	-1.495550
H	-1.943826	-2.102950	1.120088

vs-H-Ac-ts *(DMSO)*

C	-2.037705	-2.271801	-0.539437
C	-2.020018	-1.412841	0.600594
C	-2.487904	-1.525717	-1.600493
H	-1.620390	-3.268473	-0.563553
C	-0.317776	-0.284032	0.137033
H	-1.897011	-1.694354	1.637190
N	-2.834685	-0.327810	0.278899
C	-2.763160	-0.217184	-1.111961
H	-2.499725	-1.804228	-2.645167
H	-0.263965	0.241154	1.090885
C	-0.700564	0.410584	-1.023658
C	-3.189772	0.666124	1.199393
H	-3.305587	0.564054	-1.628819
H	-0.338782	0.050872	-1.982840
O	-2.795696	0.587717	2.347041

S	-0.948904	2.179458	-0.915477
C	0.762728	2.730207	-0.631185
H	1.392922	2.472152	-1.483973
H	0.732149	3.815304	-0.515919
H	1.164258	2.277611	0.278911
S	0.977298	-1.480695	0.028341
O	0.902876	-2.359639	1.206476
O	0.970473	-2.087972	-1.310163
C	2.460035	-0.500433	0.175081
C	3.076915	-0.038955	-0.985223
C	2.920969	-0.159964	1.445356
C	4.192135	0.787754	-0.865024
H	2.688923	-0.325150	-1.957697
C	4.034288	0.671182	1.550797
H	2.420149	-0.542844	2.329041
C	4.665031	1.144334	0.398689
H	4.689363	1.153698	-1.757044
H	4.409697	0.946350	2.530601
H	5.531641	1.791461	0.486596
C	-4.133725	1.726881	0.703227
H	-4.352978	2.401850	1.528613
H	-3.694664	2.294892	-0.119018
H	-5.057608	1.262431	0.348088

13a

N	-0.643523	-1.191960	-0.059323
C	-1.284777	-0.014644	-0.032617
C	1.284781	0.014623	-0.032634
N	0.661089	-1.172274	-0.050213
N	-0.661081	1.172251	-0.050438
N	0.643527	1.191936	-0.059561
C	-2.771626	-0.006911	-0.003119
C	-3.468660	1.161079	-0.321262
N	-3.371028	-1.154036	0.337427
C	-4.859681	1.128163	-0.285979
H	-2.930233	2.061131	-0.591689
C	-4.703342	-1.168303	0.370496
C	-5.493611	-0.058026	0.066775
H	-5.434866	2.014454	-0.531778
H	-5.165197	-2.109828	0.654998
H	-6.574354	-0.131143	0.109120
C	2.771627	0.006905	-0.003123
C	3.468675	-1.161048	-0.321365
N	3.371007	1.154009	0.337527
C	4.859696	-1.128115	-0.286078
H	2.930259	-2.061083	-0.591876
C	4.703321	1.168292	0.370605
C	5.493607	0.058052	0.066786
H	5.434895	-2.014375	-0.531957
H	5.165163	2.109796	0.655198
H	6.574348	0.131181	0.109141

A1c

C	-0.971545	-1.246587	0.000356
C	-2.446436	-0.946244	-0.000342
C	-2.684383	0.364421	-0.000477
C	-1.408906	1.162710	0.000176
H	-0.657139	-1.810981	0.886146
H	-0.656305	-1.810859	-0.885206
H	-3.192999	-1.731266	-0.000745
H	-3.659480	0.836234	-0.000994
H	-1.309683	1.802372	0.885634
H	-1.308721	1.802379	-0.885156
N	-0.399756	0.103226	0.000744
C	0.912765	0.389565	0.000186
O	1.381137	1.523251	-0.000132
O	1.667746	-0.729485	-0.000012
C	3.081635	-0.512980	-0.000327
H	3.382662	0.038581	0.892008
H	3.528120	-1.505319	-0.001707
H	3.381992	0.040830	-0.891467

A1c-ts

C	-1.190148	0.906415	1.574840
C	-0.592500	-0.485501	1.516762
C	0.792120	-0.400041	1.505448
C	1.213035	1.055450	1.551723
H	-2.006181	1.084357	0.872201
H	-1.561152	1.104003	2.588584
H	-1.135583	-1.342154	1.900804
H	1.441278	-1.182377	1.883292
H	1.979772	1.340686	0.827140
H	1.582177	1.299648	2.556029
N	-0.037662	1.750982	1.260979
C	-0.059129	2.805315	0.410324
O	0.937273	3.376687	-0.010842
O	-1.315192	3.171773	0.096803
C	-1.422156	4.271763	-0.813204
H	-0.944883	4.025016	-1.762976
H	-2.489610	4.429014	-0.952999
H	-0.957178	5.162590	-0.387992
N	-0.576387	-0.098636	-1.190004
C	-1.121451	-1.135240	-0.471927
C	1.365916	-0.995849	-0.487726
N	0.699492	-0.028001	-1.199279
N	-0.445105	-2.337585	-0.471775
N	0.827917	-2.266168	-0.478947
C	-2.606738	-1.191483	-0.371130
C	-3.408436	-0.306658	-1.093839
N	-3.095333	-2.115451	0.464078
C	-4.790211	-0.402226	-0.947086
H	-2.955208	0.425090	-1.752163
C	-4.420582	-2.197546	0.591132
C	-5.310592	-1.366945	-0.091406
H	-5.446101	0.266206	-1.494841
H	-4.790150	-2.956762	1.275024
H	-6.379113	-1.477055	0.054293
C	2.849363	-0.884674	-0.405095
C	3.536633	0.089742	-1.130577
N	3.450044	-1.751998	0.417639
C	4.922119	0.150144	-1.001170
H	2.995516	0.770368	-1.777117
C	4.777503	-1.684181	0.528450
C	5.559218	-0.754548	-0.158947
H	5.490949	0.892184	-1.551481
H	5.239165	-2.400211	1.202947
H	6.635094	-0.743914	-0.026703

A2c

C	-0.689050	1.227744	-0.000280
C	-2.125055	0.777583	0.000103
C	-2.223524	-0.550503	0.000197
C	-0.871450	-1.207776	-0.000091
H	-0.444129	1.825617	-0.887188
H	-0.443759	1.826243	0.886085
H	-2.947843	1.482249	0.000223
H	-3.144265	-1.121358	0.000420
H	-0.701439	-1.834413	-0.884116
H	-0.701133	-1.834784	0.883596
N	0.029172	-0.051996	0.000029
C	1.367750	-0.208869	-0.000004
O	1.883594	-1.333231	-0.000065

C	2.193803	1.057102	0.000166
H	1.972848	1.663887	-0.882734
H	1.973333	1.663202	0.883675
H	3.248583	0.787498	-0.000194

A2c-ts

C	-1.197808	1.322101	1.357437
C	-0.671934	-0.093705	1.481588
C	0.715454	-0.075331	1.479808
C	1.206838	1.351266	1.352496
H	-2.007731	1.432188	0.632501
H	-1.566972	1.662924	2.334073
H	-1.260983	-0.866130	1.963624
H	1.322779	-0.836940	1.956449
H	1.993358	1.508912	0.610197
H	1.579776	1.703829	2.323307
N	-0.004318	2.062588	0.952624
C	0.042163	3.108168	0.091702
O	1.117820	3.595560	-0.264634
N	-0.626202	-0.039484	-1.245057
C	-1.220667	-0.956984	-0.412497
C	1.270115	-0.932943	-0.422720
N	0.651418	-0.027972	-1.250862
N	-0.600339	-2.180151	-0.260566
N	0.674426	-2.167266	-0.264679
C	-2.708357	-0.937549	-0.326518
C	-3.466367	-0.146688	-1.191334
N	-3.242894	-1.702657	0.632096
C	-4.852487	-0.165033	-1.054356
H	-2.978301	0.451988	-1.951344
C	-4.571596	-1.713303	0.748369
C	-5.420181	-0.964314	-0.068320
H	-5.474931	0.433852	-1.710812
H	-4.978653	-2.343514	1.534387
H	-6.493712	-1.009678	0.074929
C	2.756936	-0.881789	-0.341348
C	3.492522	-0.041409	-1.178331
N	3.312692	-1.666259	0.589119
C	4.879094	-0.032511	-1.046555
H	2.987045	0.577108	-1.910402
C	4.641648	-1.649286	0.701343
C	5.469386	-0.853129	-0.091706
H	5.484710	0.605148	-1.681893
H	5.066303	-2.295849	1.464464
H	6.544256	-0.878273	0.046586
C	-1.281139	3.636716	-0.411897
H	-1.739480	2.904868	-1.085860
H	-1.978681	3.829626	0.406596
H	-1.096550	4.558211	-0.961414

A3c

C	0.130509	-1.176943	0.401686
C	1.347901	-0.666132	-0.339297
C	1.347899	0.666134	-0.339299
C	0.130507	1.176942	0.401688
H	-0.389015	-1.990196	-0.115541
H	0.418446	-1.554003	1.392873
H	2.113516	-1.311189	-0.756326
H	2.113511	1.311193	-0.756329
H	-0.389018	1.990195	-0.115537
H	0.418446	1.554000	1.392876
N	-0.756218	-0.000001	0.533623
C	-1.767337	-0.000000	-0.524238
H	-2.400818	-0.884437	-0.421936
H	-2.400818	0.884436	-0.421936
H	-1.327596	0.000000	-1.536740

A3c-ts

C	1.172310	1.968858	-0.707334
C	0.692042	0.639575	-1.241527
C	-0.692042	0.639576	-1.241526
C	-1.172309	1.968859	-0.707332
H	2.040836	1.910117	-0.040894
H	1.455930	2.600400	-1.572837
H	1.295436	0.047761	-1.921036
H	-1.295438	0.047763	-1.921035
H	-2.040833	1.910118	-0.040890
H	-1.455930	2.600402	-1.572834
N	0.000002	2.488119	-0.005750
N	0.639302	-0.252850	1.387714
C	1.246199	-0.815322	0.295147
C	-1.246200	-0.815322	0.295147
N	-0.639303	-0.252850	1.387714
N	0.638316	-1.911289	-0.277957
N	-0.638317	-1.911289	-0.277957
C	2.731746	-0.739524	0.218636
C	3.477717	-0.221080	1.278685
N	3.278659	-1.172331	-0.923366
C	4.862550	-0.165675	1.144115
H	2.979383	0.119054	2.178445
C	4.606704	-1.116035	-1.036334
C	5.443175	-0.623765	-0.033800
H	5.475104	0.227690	1.948631
H	5.023148	-1.474314	-1.973953
H	6.516615	-0.599090	-0.182964
C	-2.731747	-0.739523	0.218636
C	-3.477718	-0.221079	1.278685
N	-3.278659	-1.172330	-0.923366
C	-4.862551	-0.165674	1.144114
H	-2.979383	0.119054	2.178445
C	-4.606705	-1.116034	-1.036334
C	-5.443176	-0.623764	-0.033800
H	-5.475105	0.227690	1.948631
H	-5.023149	-1.474313	-1.973953
H	-6.516616	-0.599089	-0.182964
C	0.000003	3.929121	0.155174
H	-0.885962	4.237236	0.716772
H	0.885968	4.237234	0.716771
H	0.000003	4.456885	-0.815666

Bc

C	0.302715	-1.190703	-0.000000
C	-0.077725	-0.333642	-1.228034
C	-0.077725	1.071214	-0.667756
C	-0.077725	1.071214	0.667756
C	-0.077725	-0.333642	1.228034
H	1.385746	-1.351644	-0.000000
H	-1.077216	-0.587837	-1.606592
H	0.618460	-0.462535	-2.062599
H	-0.120238	1.958612	-1.292200
H	-0.120238	1.958612	1.292200
H	-1.077216	-0.587837	1.606592
H	0.618460	-0.462535	2.062599
H	-0.178650	-2.171485	-0.000000

Bc-ts

C	1.218680	2.200715	-0.613715
C	0.693716	0.902527	-1.196009
C	-0.693825	0.902546	-1.195885
C	-1.218629	2.200756	-0.613483
H	2.094887	2.075747	0.030333
H	1.525694	2.838745	-1.452348

H	1.278546	0.320117	-1.901403
H	-1.278785	0.320200	-1.901223
H	-2.094709	2.075810	0.030743
H	-1.525791	2.838800	-1.452050
N	0.639763	-0.098814	1.385080
C	1.245772	-0.619643	0.271097
C	-1.245770	-0.619586	0.271059
N	-0.639769	-0.098794	1.385073
N	0.637820	-1.697953	-0.336501
N	-0.637834	-1.697932	-0.336515
C	2.731329	-0.545702	0.198467
C	3.478396	-0.066720	1.276324
N	3.277895	-0.939881	-0.957541
C	4.863225	-0.008161	1.143852
H	2.980685	0.242202	2.187681
C	4.606073	-0.881491	-1.068290
C	5.443285	-0.425052	-0.049591
H	5.476247	0.356070	1.961644
H	5.022200	-1.208112	-2.017566
H	6.516736	-0.396305	-0.197976
C	-2.731333	-0.545655	0.198436
C	-3.478406	-0.066784	1.276337
N	-3.277897	-0.939755	-0.957600
C	-4.863238	-0.008251	1.143879
H	-2.980698	0.242073	2.187718
C	-4.606077	-0.881389	-1.068337
C	-5.443294	-0.425056	-0.049594
H	-5.476264	0.355893	1.961708
H	-5.022202	-1.207943	-2.017637
H	-6.516748	-0.396325	-0.197970
C	0.000106	2.799815	0.116304
H	0.000121	3.891301	0.096060
H	0.000201	2.486398	1.163217

A1b

C	-2.378724	0.382238	-0.864068
C	-1.194608	1.051071	-0.191346
C	-2.141818	-0.933148	-0.840014
H	-3.276247	0.898823	-1.180748
C	-1.322160	0.723086	1.334017
N	-0.123294	0.115250	-0.587938
C	-0.802826	-1.124322	-0.151322
H	-2.800587	-1.741305	-1.132796
H	-0.560840	1.273314	1.893565
C	-1.045491	-0.807245	1.362044
C	1.176139	0.398938	-0.280607
H	-1.871879	-1.392003	1.770160
O	1.630185	1.525521	-0.151125
O	1.920780	-0.717371	-0.195379
C	3.318576	-0.508541	0.033849
H	3.757796	-1.502934	0.076444
H	3.754839	0.066684	-0.784511
H	3.473362	0.018516	0.976689
H	-0.245753	-2.032530	-0.366682
H	-0.982989	2.088206	-0.440314
H	-0.142774	-1.036366	1.935325
H	-2.304110	0.995189	1.725152

A1b-ts

C	-0.546006	-0.481308	1.415577
C	-1.084918	0.941183	1.539741
C	0.831120	-0.363508	1.370425
H	-1.079948	-1.337978	1.813123
C	-0.731966	1.363739	3.000398
N	-0.061036	1.686745	0.813431
C	1.125012	1.133870	1.455066
H	1.524434	-1.109244	1.745080
H	-1.205068	2.321935	3.224424
C	0.818276	1.500100	2.940988
C	-0.129972	2.423136	-0.313642
H	1.337605	0.838643	3.637161
O	0.833385	2.892114	-0.901672
O	-1.408664	2.612166	-0.692907
C	-1.578624	3.288074	-1.941432
H	-2.654543	3.352729	-2.093433
H	-1.139834	4.286226	-1.901298
H	-1.114205	2.712345	-2.745118
H	2.064864	1.495122	1.041107
H	-2.103386	1.130575	1.206871
H	1.132454	2.528037	3.132773
H	-1.073652	0.625243	3.728359
N	-0.428463	-2.413245	-0.489101
C	-1.106645	-1.220970	-0.605684
C	1.391057	-1.088517	-0.657477
N	0.848256	-2.345063	-0.512668
N	-0.562952	-0.264362	-1.414487
N	0.717378	-0.199418	-1.443688
C	-2.586072	-1.223746	-0.444717
C	-3.368351	-0.260280	-1.085112
N	-3.087656	-2.158419	0.370160
C	-4.744874	-0.289669	-0.880075
H	-2.899777	0.484207	-1.717831
C	-4.409162	-2.175949	0.553388
C	-5.280918	-1.267832	-0.048577
H	-5.385863	0.441074	-1.362174
H	-4.790157	-2.946550	1.218139
H	-6.346581	-1.328196	0.140773
C	2.867522	-0.929535	-0.555100
C	3.503257	0.149837	-1.172830
N	3.509740	-1.836145	0.189493
C	4.882318	0.270882	-1.026609
H	2.922730	0.866414	-1.742355
C	4.831668	-1.708002	0.317385
C	5.565133	-0.677850	-0.272094
H	5.411880	1.094647	-1.493683
H	5.328768	-2.458923	0.925590
H	6.638509	-0.623132	-0.130575

A2b

C	1.891426	-0.472038	-1.016181
C	0.721327	-1.076318	-0.260816
C	1.784467	0.855076	-0.895056
H	2.700621	-1.043227	-1.454140
C	1.029753	-0.861363	1.258555
N	-0.288602	-0.026228	-0.475029
C	0.544484	1.117841	-0.057910
H	2.484764	1.619361	-1.208523
H	0.274376	-1.372314	1.861275
C	0.911089	0.683106	1.401115
C	-1.611572	-0.202647	-0.220778
H	1.829880	1.161477	1.745526
O	-2.114963	-1.326039	-0.156119
H	0.066432	2.089786	-0.161640
H	0.384706	-2.070328	-0.543676
H	0.102621	0.955751	2.085459
H	2.013436	-1.248408	1.530589
C	-2.436315	1.056679	-0.074563
H	-2.222390	1.769223	-0.874256
H	-3.491233	0.787032	-0.093454
H	-2.211251	1.541549	0.880797

A2b-ts

C	-0.680774	0.081807	1.332071
C	-1.049238	1.521990	0.989111
C	0.701596	0.041426	1.351510
H	-1.328698	-0.554140	1.925744
C	-0.724548	2.331658	2.284268
N	0.091493	1.895812	0.155776
C	1.167262	1.458290	1.037295
H	1.291973	-0.646294	1.947780
H	-1.085682	3.356165	2.169343
C	0.830790	2.281796	2.321044
C	0.193395	2.546390	-1.021073
H	1.227067	1.809052	3.222041
O	1.289449	2.815201	-1.519024
H	2.162429	1.588772	0.616506
H	-2.027369	1.694787	0.542026
H	1.261184	3.281509	2.231848
H	-1.194514	1.891163	3.165970
N	-0.671032	-2.331747	0.060803
C	-1.272723	-1.177905	-0.391292
C	1.227958	-1.210421	-0.417069
N	0.606823	-2.348204	0.046564
N	-0.665854	-0.502735	-1.410222
N	0.616585	-0.519599	-1.423672
C	-2.755234	-1.079210	-0.285034
C	-3.504485	-0.467990	-1.291893
N	-3.295679	-1.591189	0.826640
C	-4.885706	-0.393144	-1.131305
H	-3.014400	-0.080424	-2.177030
C	-4.620475	-1.516730	0.964245
C	-5.459322	-0.927622	0.017406
H	-5.500753	0.071490	-1.894786
H	-5.031800	-1.941188	1.876034
H	-6.529661	-0.891532	0.185052
C	2.711041	-1.121923	-0.323252
C	3.428167	-0.310844	-1.205371
N	3.280182	-1.815214	0.669126
C	4.810840	-0.242718	-1.058373
H	2.907531	0.248566	-1.973684
C	4.606299	-1.741473	0.795259
C	5.416971	-0.974816	-0.042540
H	5.402185	0.374849	-1.726281
H	5.042436	-2.315550	1.608373
H	6.489979	-0.950961	0.109954
C	-1.113086	2.909215	-1.688918
H	-1.678060	2.001212	-1.919346
H	-1.724486	3.539628	-1.037489
H	-0.894946	3.444751	-2.611496

A3b

C	0.029767	1.400860	-0.668307
C	0.029886	-0.069702	-1.088885
C	0.029838	1.400869	0.668316
H	0.134064	2.247799	-1.336905
C	1.462109	-0.592366	-0.777607
N	-0.711063	-0.740335	0.000006
C	0.029915	-0.069686	1.088886
H	0.134115	2.247857	1.336853
H	1.585377	-1.603665	-1.171980
C	1.462118	-0.592381	0.777581
H	2.233378	0.048071	1.210680
H	-0.349180	-0.304158	2.084720
H	-0.349251	-0.304130	-2.084715
H	1.585338	-1.603693	1.171934
H	2.233341	0.048123	-1.210701
C	-2.149591	-0.508909	0.000010
H	-2.584966	-0.981819	-0.884582
H	-2.584739	-0.980218	0.885576
H	-2.424295	0.556071	-0.000888

A3b-ts

C	0.691293	0.656266	-0.993460
C	1.091186	1.890742	-0.207051
C	-0.691293	0.656229	-0.993504
H	1.310838	0.235017	-1.778102
C	0.774854	3.079495	-1.184374
N	-0.000102	1.938180	0.774899
C	-1.091291	1.890705	-0.207151
H	-1.310769	0.234934	-1.778176
H	1.181280	4.013612	-0.788310
C	-0.774904	3.079418	-1.184515
H	-1.208381	2.916948	-2.173851
H	-2.089113	1.896236	0.236795
H	2.088971	1.896282	0.236980
H	-1.181527	4.013544	-0.788675
H	1.208560	2.917224	-2.173641
N	0.639987	-2.049399	-0.667164
C	1.249296	-1.160654	0.187539
C	-1.249239	-1.160697	0.187540
N	-0.639901	-2.049421	-0.667163
N	0.640417	-0.914810	1.383940
N	-0.640366	-0.914831	1.383941
C	2.731117	-1.029923	0.127901
C	3.462962	-0.696279	1.269741
N	3.289305	-1.215100	-1.073886
C	4.844005	-0.566858	1.152788
H	2.955831	-0.550561	2.215807
C	4.614339	-1.091328	-1.169426
C	5.436859	-0.770767	-0.089161
H	5.444924	-0.311789	2.019461
H	5.039608	-1.248607	-2.157106
H	6.508211	-0.679942	-0.227401
C	-2.731061	-1.029990	0.127900
C	-3.462888	-0.696189	1.269707
N	-3.289267	-1.215322	-1.073854
C	-4.843932	-0.566778	1.152757
H	-2.955742	-0.550341	2.215744
C	-4.614302	-1.091557	-1.169390
C	-5.436805	-0.770851	-0.089156
H	-5.444837	-0.311587	2.019405
H	-5.039586	-1.248963	-2.157044
H	-6.508158	-0.680039	-0.227393
C	-0.000189	3.089256	1.664425
H	-0.000102	4.063677	1.153700
H	-0.885516	3.038932	2.303944
H	0.884952	3.038894	2.304199

Bb

C	-1.277256	-0.670188	-0.498241
C	-0.085374	-1.125832	0.325570
C	-0.085034	1.126051	0.325096
C	-1.277072	0.670226	-0.498448
C	-0.026445	0.000359	1.377083
H	0.903641	0.000363	1.955973
H	-0.888693	0.000662	2.049450
C	1.179410	-0.778063	-0.523545
H	2.077660	-1.173058	-0.039300
H	1.122760	-1.204622	-1.528063
C	1.179750	0.777472	-0.523832
H	2.078162	1.172295	-0.039691
H	1.123144	1.203740	-1.528438
H	-0.113657	-2.153188	0.691386
H	-0.112936	2.153592	0.690420

H	-1.918811	1.326532	-1.077073
H	-1.919138	-1.326459	-1.076759

Bb-ts

C	0.692931	0.973017	-0.691149
C	1.129709	2.048092	0.287294
C	-0.692921	0.973028	-0.691135
H	1.294450	0.704138	-1.554508
C	0.778418	3.377168	-0.455221
C	-1.129657	2.048118	0.287314
H	-1.294458	0.704170	-1.554488
H	1.175562	4.225944	0.108357
C	-0.778350	3.377186	-0.455205
H	-1.204518	3.410193	-1.461117
H	-2.158772	1.983121	0.645338
H	2.158830	1.983073	0.645296
H	-1.175464	4.225971	0.108381
H	1.204566	3.410164	-1.461142
N	0.638904	-1.743405	-0.975875
C	1.249612	-1.060338	0.053418
C	-1.249630	-1.060312	0.053416
N	-0.638933	-1.743395	-0.975875
N	0.641766	-1.096668	1.273895
N	-0.641787	-1.096657	1.273896
C	2.730560	-0.914384	0.022234
C	3.463193	-0.832470	1.208364
N	3.285988	-0.830540	-1.191925
C	4.842888	-0.670415	1.120214
H	2.958507	-0.899401	2.164578
C	4.609890	-0.679624	-1.260277
C	5.433309	-0.594949	-0.137309
H	5.444751	-0.605313	2.020643
H	5.033254	-0.616032	-2.259136
H	6.503540	-0.467931	-0.253966
C	-2.730579	-0.914353	0.022232
C	-3.463216	-0.832482	1.208362
N	-3.286002	-0.830472	-1.191927
C	-4.842913	-0.670433	1.120212
H	-2.958534	-0.899441	2.164576
C	-4.609905	-0.679562	-1.260279
C	-5.433329	-0.594930	-0.137310
H	-5.444779	-0.605366	2.020642
H	-5.033267	-0.615940	-2.259136
H	-6.503561	-0.467916	-0.253967
C	0.000036	2.018098	1.332291
H	0.000032	1.138967	1.973437
H	0.000052	2.916829	1.956087

A1-b

C	-1.295878	2.724287	0.701738
C	-1.310764	1.786943	-0.493879
C	-1.683141	2.002105	1.755959
H	-0.930331	3.742225	0.675574
C	-0.120991	0.775446	-0.306744
N	-2.420790	0.905564	-0.110212
C	-1.952932	0.598508	1.251318
H	-1.706914	2.291437	2.798589
H	-0.068310	0.136989	-1.196221
C	-0.531809	-0.024144	0.956188
C	-2.882692	-0.065164	-0.966570
H	0.144204	0.134261	1.801634
O	-2.684421	-0.075343	-2.168454
O	-3.645648	-0.950665	-0.312159
C	-4.081068	-2.074091	-1.085732
H	-4.680677	-2.678547	-0.408532
H	-4.678937	-1.742814	-1.935755
H	-3.213702	-2.638469	-1.436134
S	-0.541436	-1.792716	0.509592
C	-1.392790	-2.502571	1.944095
H	-2.461880	-2.284771	1.908190
H	-1.248119	-3.582458	1.891786
H	-0.955949	-2.131413	2.873364
S	1.512960	1.532889	-0.229555
O	1.677052	2.281024	-1.484118
O	1.695926	2.233491	1.046650
C	2.599367	0.124558	-0.243939
C	3.099263	-0.349855	0.966562
C	2.874839	-0.499256	-1.459765
C	3.901752	-1.489034	0.953672
H	2.868986	0.168269	1.891925
C	3.676810	-1.637653	-1.456287
H	2.477625	-0.096255	-2.386456
C	4.184988	-2.130314	-0.252635
H	4.304976	-1.873453	1.884305
H	3.907003	-2.136829	-2.391254
H	4.808928	-3.018105	-0.256223
H	-2.627891	-0.026821	1.829121
H	-1.390861	2.208389	-1.492256

A1′b-ts

C	0.266383	-0.307461	-0.706647
C	0.517923	0.503084	0.567750
C	-1.057192	-0.075444	-1.040584
H	1.043487	-0.507432	-1.433730
C	0.534967	2.015489	0.145586
N	-0.804807	0.487982	1.162395
C	-1.594336	0.887260	0.011701
H	-1.456708	-0.113646	-2.048276
H	0.796369	2.618341	1.021010
C	-0.926155	2.270482	-0.324251
C	-1.157710	0.179804	2.431889
H	-0.986100	2.489502	-1.394778
O	-0.373203	-0.126430	3.313411
O	-2.488884	0.256437	2.587552
C	-2.977259	-0.137055	3.875156
H	-4.057456	-0.016911	3.823400
H	-2.717490	-1.178829	4.072388
H	-2.558550	0.503067	4.653016
H	-2.669331	0.911744	0.179963
H	1.319874	0.179321	1.228289
N	-0.151347	-2.912773	-1.373438
C	0.362877	-2.446484	-0.185415
C	-2.060139	-1.986087	-0.617512
N	-1.386923	-2.682225	-1.598137
N	-0.462892	-2.453933	0.908412
N	-1.701137	-2.215664	0.682108
C	1.822141	-2.636572	0.054943
C	2.602547	-3.370838	-0.839579
N	2.308269	-2.077547	1.170315
C	3.947632	-3.563123	-0.533086
H	2.157518	-3.792656	-1.732466
C	3.599993	-2.263188	1.444566
C	4.458120	-3.007849	0.634495
H	4.582507	-4.140272	-1.197311
H	3.969561	-1.791782	2.351636
H	5.499491	-3.129474	0.911581
C	-3.467089	-1.576577	-0.880385
C	-4.376267	-1.446671	0.171145
N	-3.766289	-1.301160	-2.154754
C	-5.669441	-1.026322	-0.128669
H	-4.067649	-1.666781	1.186255
C	-5.010619	-0.904327	-2.426395

C	-5.998439	-0.752542	-1.452194
H	-6.405394	-0.915009	0.660748
H	-5.227985	-0.689299	-3.469103
H	-6.992239	-0.421667	-1.731509
S	-1.639449	3.655268	0.609149
C	-3.212987	3.822493	-0.278179
H	-3.835790	2.934548	-0.155173
H	-3.729570	4.677951	0.158651
H	-3.034406	4.014703	-1.337602
S	1.743390	2.496613	-1.111095
O	1.935983	3.945386	-0.974394
O	1.322678	1.956542	-2.410313
C	3.232677	1.658606	-0.618669
C	3.904862	2.099754	0.520698
C	3.686519	0.584203	-1.381844
C	5.065475	1.433538	0.906940
H	3.537973	2.951651	1.085368
C	4.855875	-0.062823	-0.989272
H	3.147699	0.282420	-2.274376
C	5.537545	0.357988	0.152743
H	5.603481	1.759035	1.790547
H	5.230189	-0.896624	-1.573436
H	6.443335	-0.156270	0.458112

8. General procedures and characterization methods.

LC–MS method for analysis of protein conjugation.

LC–MS was performed on a Xevo TQD/SQD2 mass spectrometer coupled to an Acquity UPLC system using an Acquity Q6 UPLC BEH300 C4 column (1.7 mm, 2.1 × 50 mm). Solvents A, water with 0.1% formic acid and B, 71% acetonitrile, 29% water and 0.075% formic acid were used as the mobile phase at a flow rate of 0.2 mL min^{-1}. The gradient was programmed as follows: 72% A to 100% B after 25 min then 100% B for 2 min and after that 72% A for 18 min. The electrospray source was operated with a capillary voltage of 3.0 kV and a cone voltage of 40 V. Nitrogen was used as the desolvation gas at a total flow of 700 L h^{-1} . Total mass spectra were reconstructed from the ion series using the MaxEnt algorithm preinstalled on MassLynx software (v. 4.1 from Waters) according to the manufacturer's instructions. To obtain the ion series described, the major peak(s) of the chromatogram were selected for integration and further analysis.

Analysis of protein conjugation by LC–MS.

A typical analysis of a conjugation reaction by LC–MS is described below. The total ion chromatogram, combined ion series and deconvoluted spectra are shown for the product of the reaction. Identical analyses were carried out for all the conjugation reactions performed in this work.

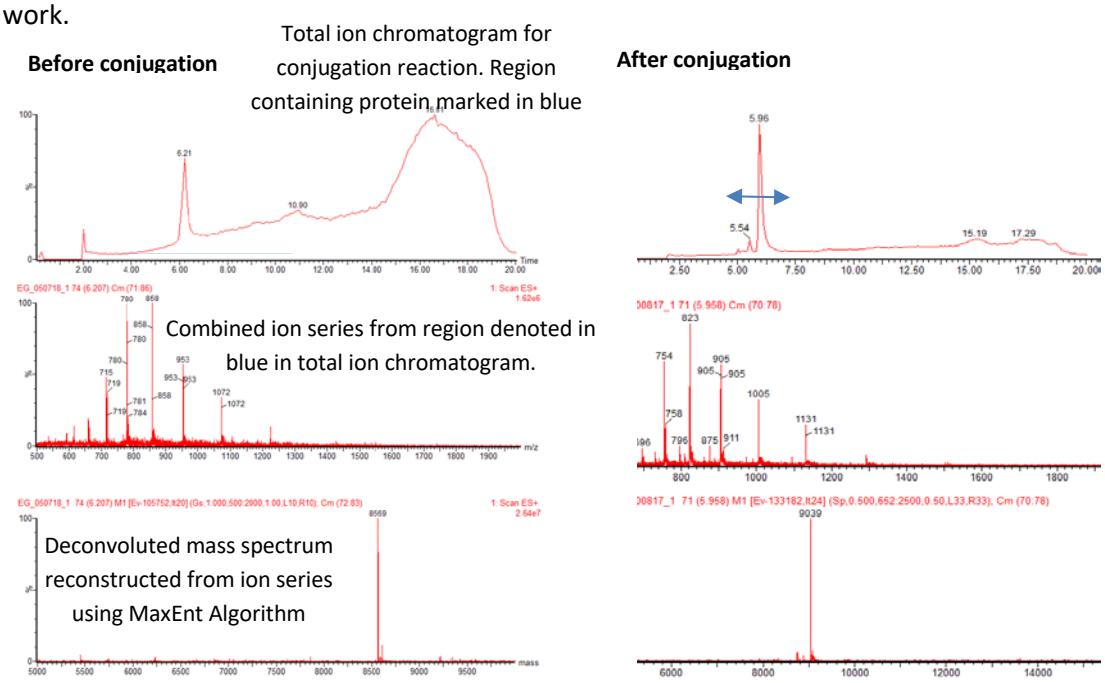


Figure S14. A typical analysis of a conjugation reaction by LC–MS is described for the reaction of ubiquitin K63C protein with the azabicycle derivative **11**. The total ion chromatogram, combined ion series and deconvoluted spectra are shown for the starting material and the product of the reaction of ubiquitin K63C with 1 equiv. of **11**. Identical analyses were carried out for all the conjugation reactions performed in this work.

Protein gels

SDS-PAGE gel electrophoresis was carried out using an XCell SureLock™ Mini-Cell Electrophoresis System from ThermoFisher Scientific (NuPAGE® Bis-Tris Mini Gels, NuPAGE® MES SDS running buffer). Protein molecular weights were approximated by comparison to a protein marker (Precision Plus Protein Standards 10–250 kDa from Bio-Rad). Samples were prepared by mixing 7.5 µL of the mixture to analyse and 2.5 uL of NuPAGE LDS Sample Buffer (4x, 2.5 µL). The solution was then heated at 37 °C for 60 min before being loaded to NuPAGE Bis-Tris mini gel (10x 10 cm) with 4–12% gradient polyacrylamide concentration and subject to electrophoresis at 200 V for 35 min with 1x SDS Running Buffer (NuPAGE MES SDS Running Buffer, 20x, pH 7.3, 50 to 950 mL deionized water). Gels were visualized by fluorescence with a Typhoon TRIO Variable Mode Imaginer at wavelength 555 nm ex and 570 nm em . Then, the gel was stained with Spyro Ruby overnight at 4 °C and washed with a solution 10% MeOH 7% AcOH 83% H20 for 30 minutes and visualized with a Typhoon TRIO Variable Mode Imaginer at wavelength 468 nm ex and 630 nm em.

Stability of bioconjugates in human plasma

A 20 µL aliquot of the corresponding bioconjugate (20 µM) in phosphate buffer (50 mM, pH 7.0) was thawed. 1 µL of reconstituted human plasma was added at room temperature and the resulting mixture vortexed for 10 seconds. The resulting reaction mixture was then incubated at 37 °C overnight. After 1 and 24 h, a 10 µL aliquot of each reaction mixture was analysed by LC–MS.

9. Proteins used in this study

Ubiquitin (Ub-K63C) was expressed and purified as previously described[11]; C2Am was provided by Dr. André Neves and Prof. Kevin Brindle.[12]

Ubiquitin Ub-K63C (6 Lys, 1 free Cys, no disulfides)

Sequence:

SAQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQ**C**ESTLHLVLRLR GG

Isotopically Averaged Molecular Weight = 8566.77 Da

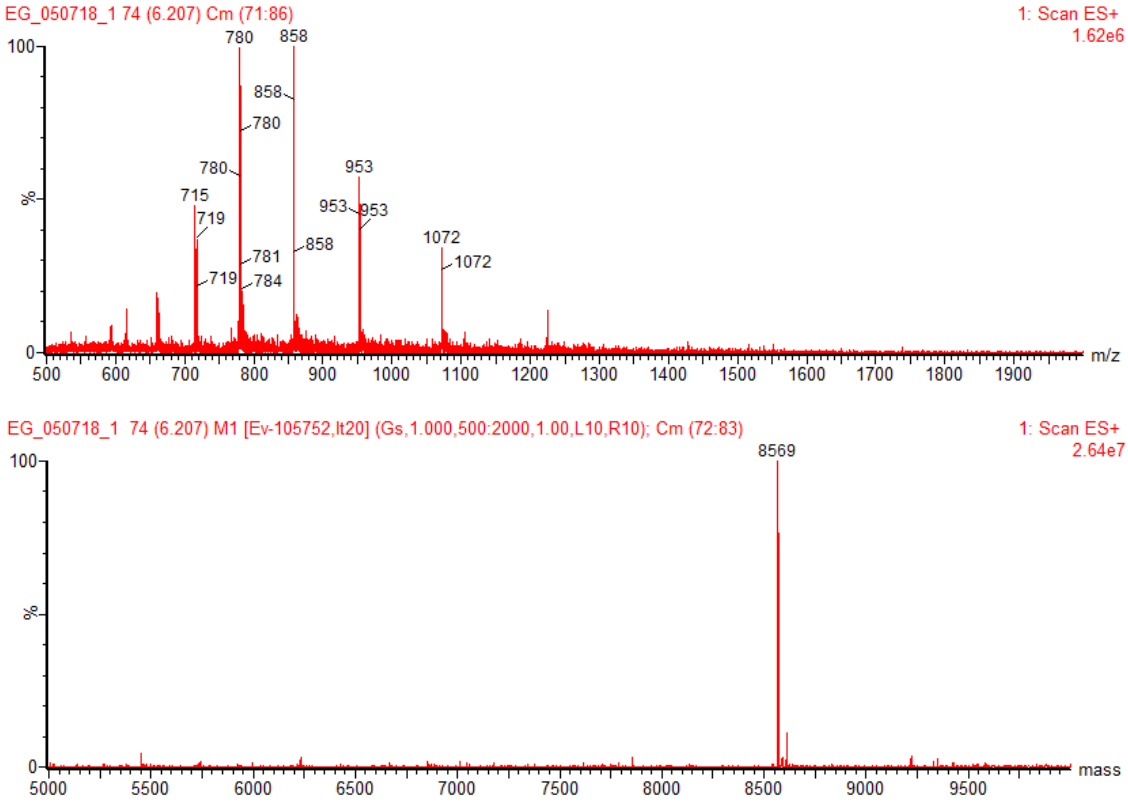


Figure S15. Combined ion series and deconvoluted mass spectrum of Ub-K63C.

[11] Lee B; Sun S; Jiménez-Moreno E; Neves AA; Bernardes GJL, *Bioorg. Med. Chem.* 2018, **26**, 3060–3064.
[12] Alam, I. S.; Neves, A. A.; Witney, T. H.; Boren, J.; Brindle, K. M. *Bioconjug. Chem.* 2010, **21**, 884–891.

C2Am (14 Lys, 1 free Cys, no disulfides)

Sequence:

GSPGISGGGGGILDSMVEKLGKLQYSLDYDFQNNQLLVGIIQAAELPALDMGGTSDPYVKVFLLPDKKKKFE
TKVHRKTLNPVFNEQFTFKVPY**C**ELGGKTLVMAVYDFDRFSKHDIIGEFKVPMNTVDFGHVTEEWRDLQSA
EK

Isotopically Averaged Molecular Weight = 16222 Da

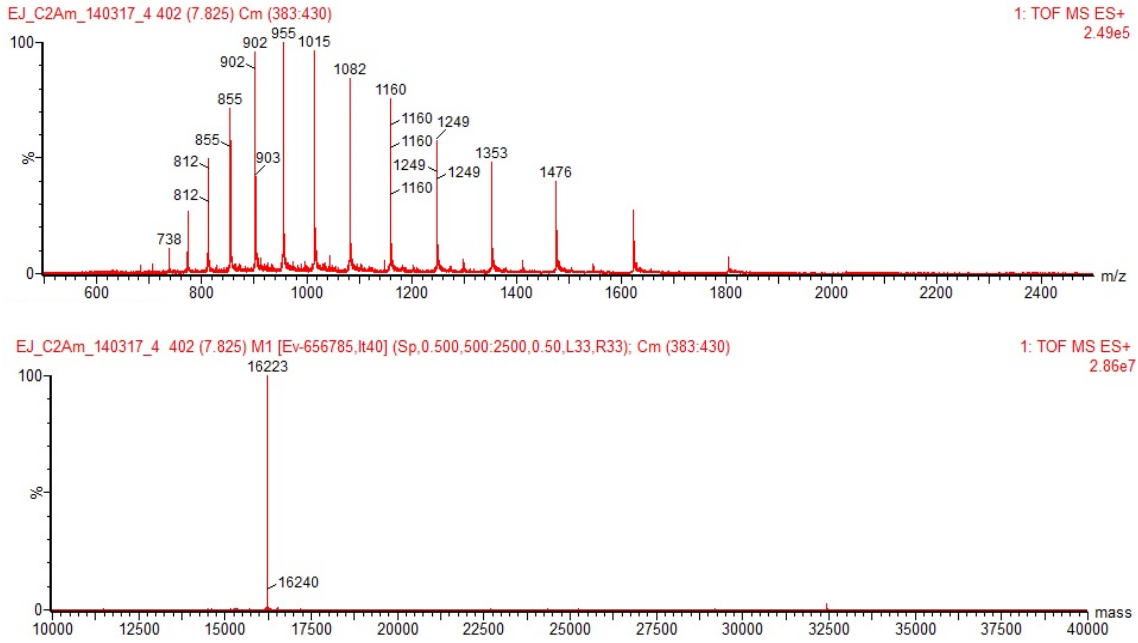
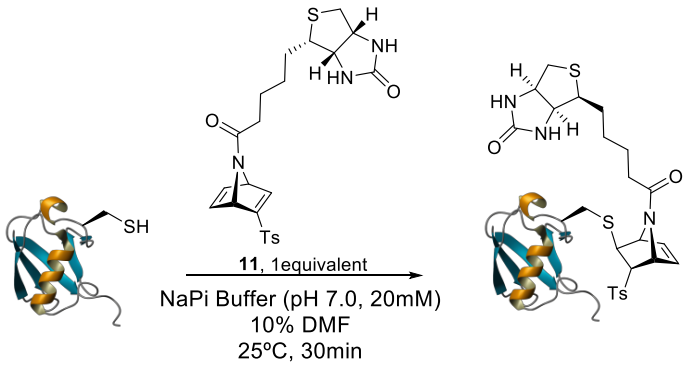


Figure S16. Combined ion series and deconvoluted mass spectrum of C2Am.

10. Reactions and characterization of Ub-K63C-conjugates

Reaction of Ub-K63C with 11



To an eppendorf containing 36.7 µL of NaPi buffer (20 mM, pH 7.0) and 2.5 µL of DMF, 13.3 µL of a stock solution of Ub-K63C (94 µM) was added and the resulting mixture was vortexed for 10 seconds. Afterwards, a 0.5 mM solution of **11** (2.5 µL, 1 equiv.) in DMF was added and the reaction mixed for 30 minutes at room temperature. After this time, a 10 µL aliquot was analysed by LC–MS and complete conversion to the expected product was observed (calculated mass, 9040 Da; observed mass, 9039 Da).

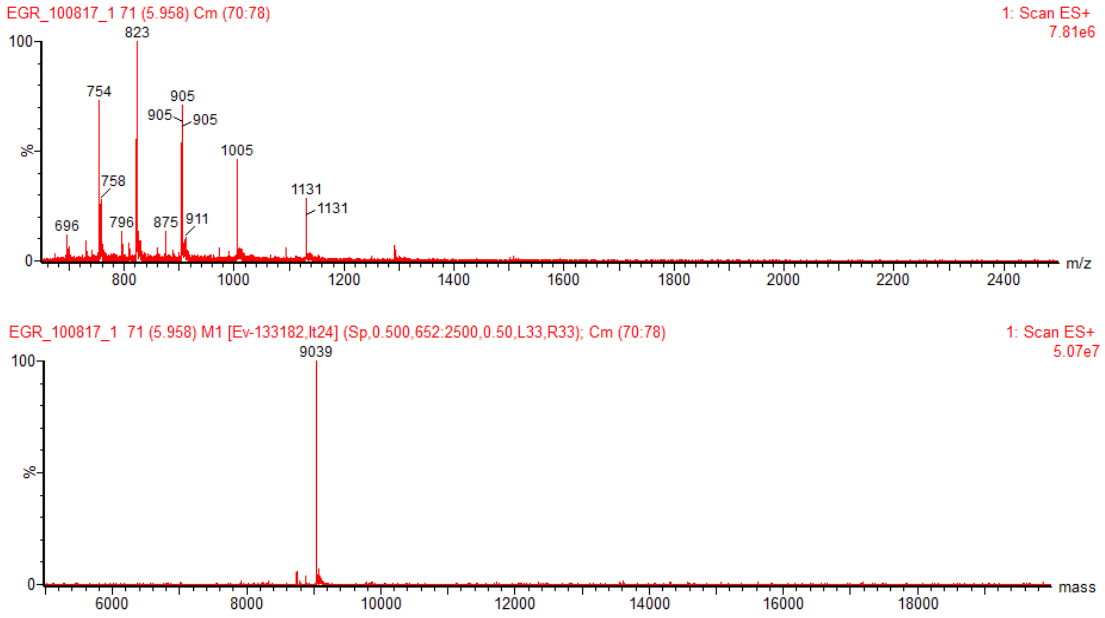
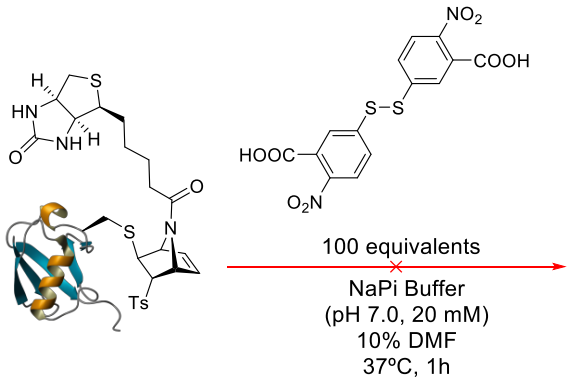


Figure S17. Combined ion series and deconvoluted mass spectrum of the reaction between Ub-K63C and **11** after 30 minutes at room temperature.

Reaction of Ub-K63C–11 with Ellman's reagent



A 15 µL aliquot of **Ub-K63C–11** (25 µM) was transferred to a 0.5 mL eppendorf tube. Ellman's reagent (0.83 µL of a 45.4 mM stock solution in H_2O, 100 equiv.) was added and the resulting mixture vortexed for 10 seconds. After 1 h of additional mixing at 37 °C, a 10 µL aliquot was analysed by LC–MS and the starting protein **Ub-K63C-11** (calculated mass, 9238 Da; observed mass, 9039 Da) was detected unaltered.

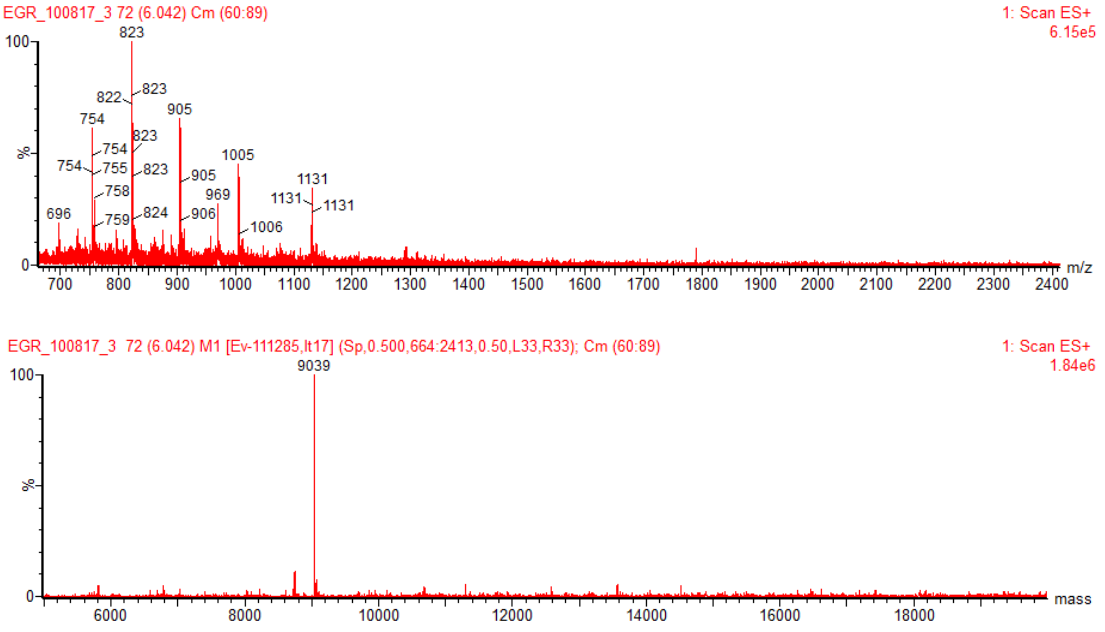


Figure S18. Combined ion series and deconvoluted mass spectrum of the reaction between **Ub-K63C-11** after 60 minutes at 37 °C.

Stability of Ub-K63C–11 in human plasma

A 20 µL aliquot of (20 µM) in 20 mM NaPi buffer at pH 7.0 was thawed. 1 µL of reconstituted human plasma (Sigma-Aldrich) was added at room temperature and the resulting mixture vortexed for 10 seconds. The resulting reaction mixture was then shaken at 37 °C overnight. After 1 and 24 h, a 10 µL aliquot of each reaction mixture was analysed by LC–MS. No significant degradation of the adduct was observed at either time point of protein **Ub-K63C-11** (calculated mass, 9040 Da; observed mass, 9040 Da).

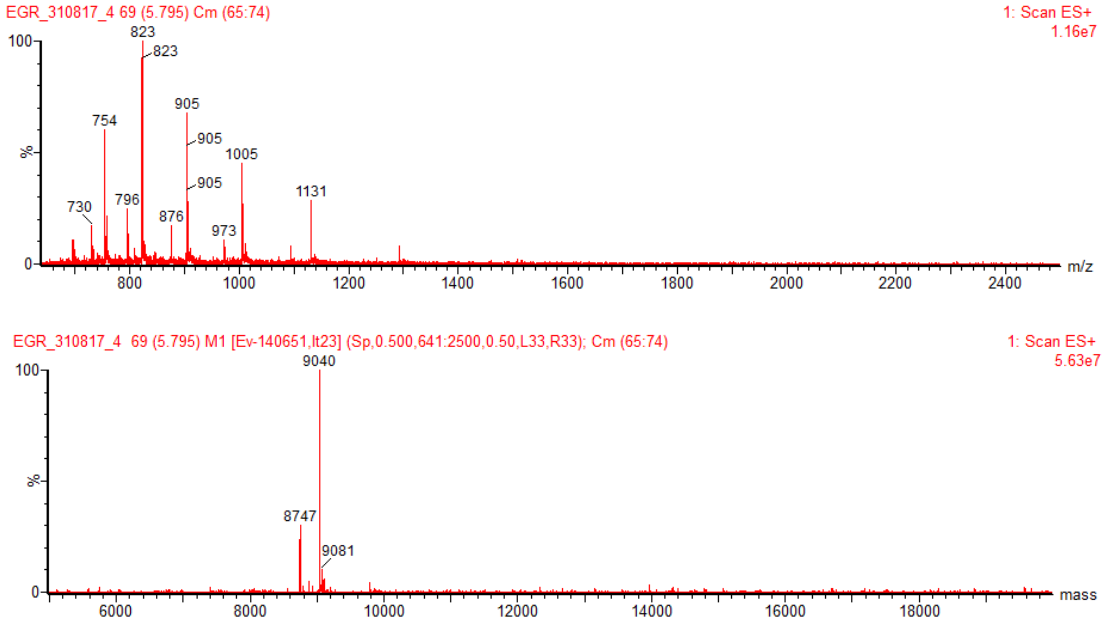
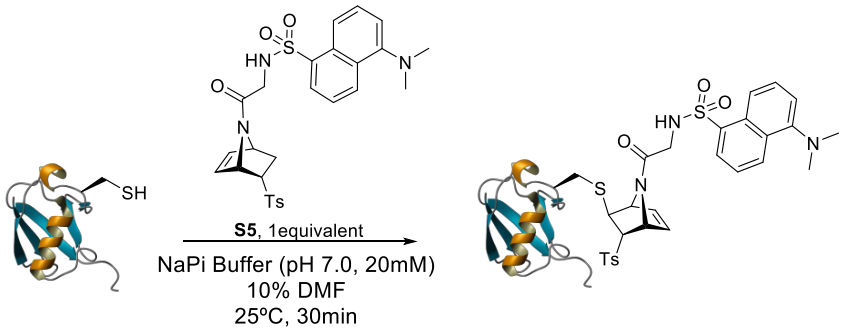


Figure S19. Combined ion series and deconvoluted mass spectrum of **Ub-11** in plasma Incubation of the bioconjugate **Ub-11** in plasma media only shows slight degradation due to rDA reaction (less than 25% after 24h at 37 °C).

Reaction of Ub-K63C with 12



To an eppendorf containing 25 µL of NaPi buffer (20 mM, pH 7.0) and 0.5 µL of DMF, 25 µL of a stock solution of Ub-K63C (90 µM) was added and the resulting mixture was vortexed for 10 seconds. Afterwards, a 0.5 mM solution of **12** (4.5 µL, 1 equiv.) in DMF was added and the reaction mixed for 30 minutes at room temperature. After this time, a 10 µL aliquot was analysed by LC–MS and complete conversion to the expected product was observed (calculated mass, 9104 Da; observed mass, 9105 Da).

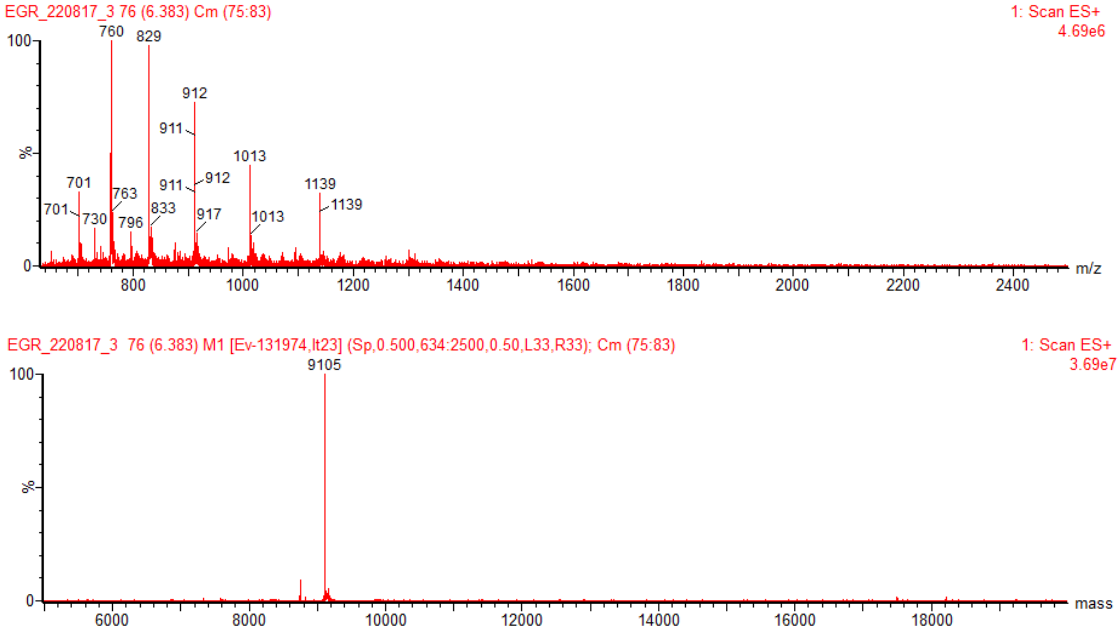
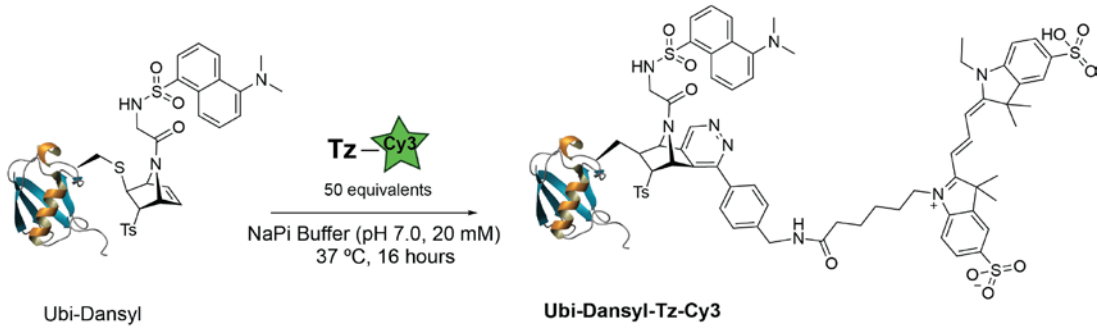


Figure S20. Combined ion series and deconvoluted mass spectrum of the reaction between Ub-K63C and **12** after 30 minutes at room temperature.

6-Methyl-tetrazine-sulfo-cy3 cicloaddition to Ub-K63C-12



A 20 µL aliquot of **Ub-K63C-12** (45 µM) in phosphate Buffer (20 mM, pH 7.0) was thawed. 6-Methyl-tetrazine-sulfo-cy3 (20.5 µL, 5 mM) was added and the resulting mixture was vortexed for 10 seconds and the reaction was stirred overnight at 37 ºC. Analysis of the sample by SDS-PAGE according to the general procedure and also by HPLC showed conversion to the expected product.

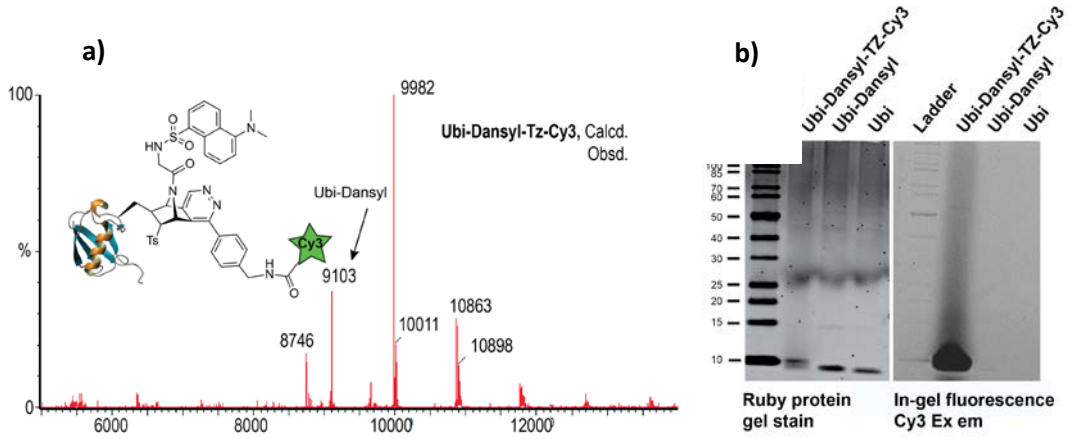
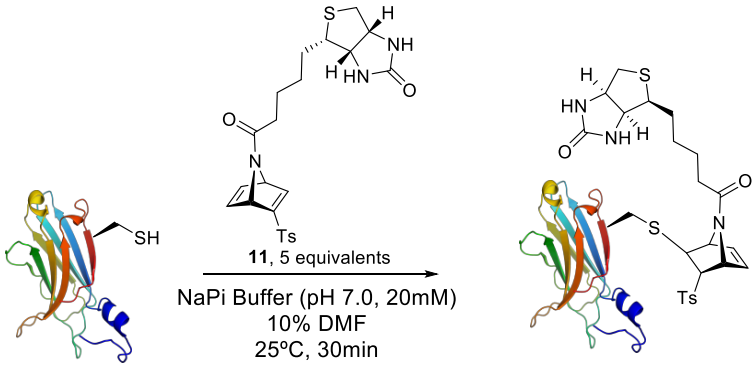


Figure S21. a) Mass spectrum of the reaction between **Ub-12** and the 6-Methyl-tetrazine-sulfo-Cy3. b) Treatment of **Ub-12** with 6-methyl-tetrazine-sulfo-cy3 gave a new fluorescent band as detected by SDS-PAGE that is consistent with the fluorogenic tetrazine bonding to the azanorbornadiene (SDS-PAGE).

11. Reactions and characterization with C2Am-conjugates

Reaction of C2Am with 11



To an eppendorf containing 11.2 µL of NaPi buffer (20 mM, pH 7.0) and 4.5 µL of DMF, 33.3 µL of a stock solution of C2Am (21.75 µM) was added and the resulting mixture vortexed for 10 seconds. Afterwards, 0.5 µL of a 40 mM solution of Tris(2-carboxyethyl)phosphine hydrochloride in water was added and the reaction mixed for 30 min at 37 °C. Then, 0,5 µL of a 10 mM solution of **11** (5 equiv.) in DMF was added. After 30 minutes, a 10 µL aliquot was analysed by LC–MS and complete conversion to the desired product was observed (calculated mass, 16695 Da; observed mass, 16696 Da).

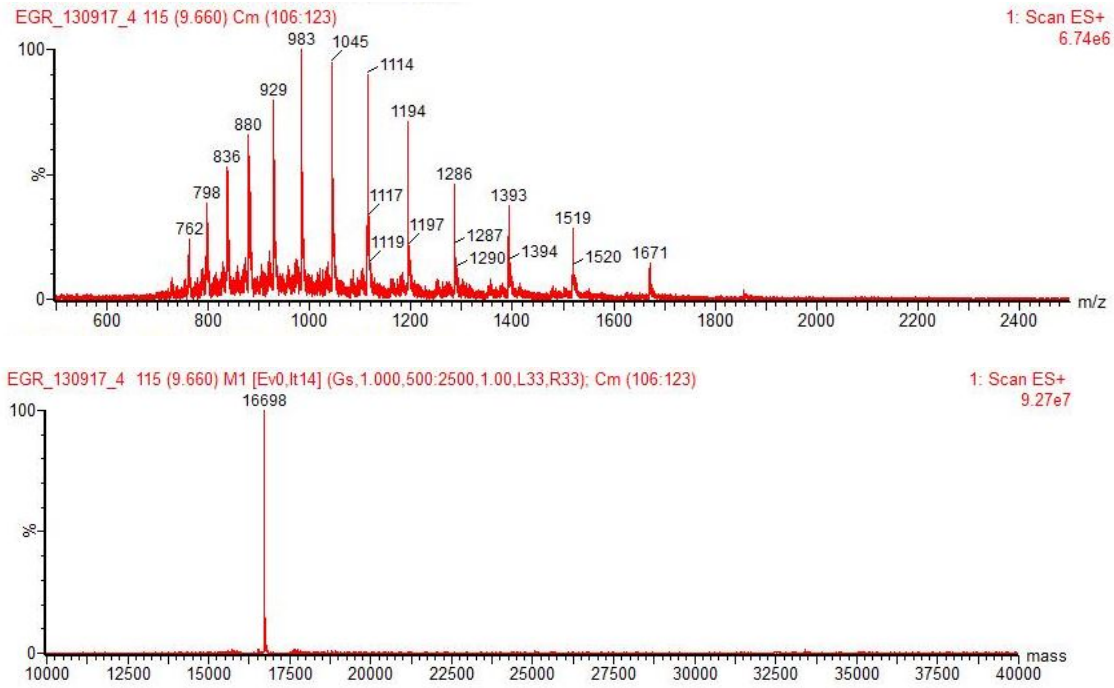


Figure S22. Combined ion series and deconvoluted mass spectrum of the reaction between **C2Am-11** after 30 minutes at room temperature.

6-Methyl-tetrazine-sulfo-cy3 cicloaddition to C2Am-11

A 20 µL aliquot of **C2Am–11** (20 µM) in phosphate Buffer (20 mM, pH 7.0) was thawed. 6-Methyl-tetrazine-sulfo-cy3 (12.5 µL, 5 mM) was added and the resulting mixture was vortexed for 10 seconds. After 24 h of additional mixing at 37 ^{o}C small molecules were removed with an Amicon Ultra-0.5 mL Centrifugal Filter (MWCO 3 KDa). Analysis of the sample by SDS-PAGE according to the general procedure showed complete conversion to the expected product.

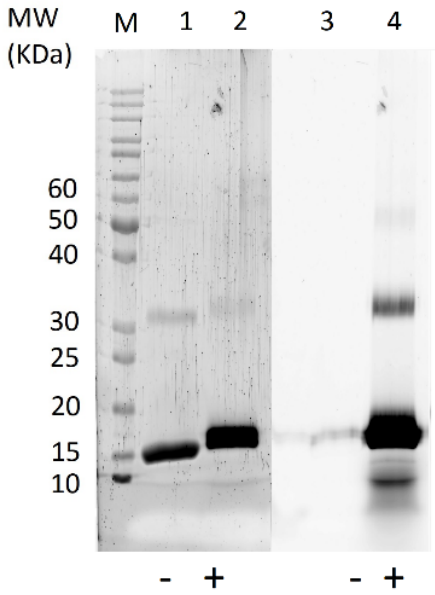


Figure S23. Treatment of **C2Am-11** with 6-methyl-tetrazine-sulfo-cy3 gave a new fluorescent band as detected by SDS-PAGE (lanes 2 and 4) that is consistent with the fluorogenic tetrazine bonding to the azanorbornadiene. Lanes 1 and 2, Spyro Ruby staining and lanes 3 and 4, fluorescence (SDS-PAGE).

12. Binding Streptavidin-Alexa555 to C2Am-11 studies

C2Am-11 was synthetized as previously described, and purified by dialysis overnight at 4 °C. Then, C2Am and **C2Am-11** were subjected to SDS-PAGE according to the general procedure and transferred to a polyvinylidene fluoride (PVDF) membrane (7 x 8.4 cm, pore size 0.2µm, ThermoFisher) by wet transfer at a constant voltage of 20V in NuPAGE™ Transfer buffer for 60 min at room temperature. Then , membrane was blocked for 30 min with 3% (w/v) Bovine serum albumin (BSA)/Tween PBS (TBST) at rt. Membranes were then incubated for 30 minutes with Streptavidin-Alexa555 in TBST buffer (1:1000 dilution, ThermoFisher) and washed 4 times with TBTS buffer. Lastly, membranes were incubated with TBST buffer overnight and visualized by Typhoon TRIO Variable Mode Imaginer at wavelength 555 nm ex and 570 nm em.

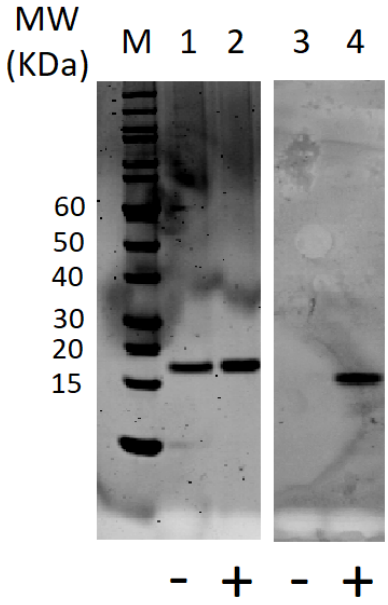


Figure S24. SDS-PAGE (lanes M, 1, 2, 3) and Western Blot (lanes 4 and 3) analysis of the binding between **C2Am–11** and Streptavidin-Alexa555.

13. Cells studies.

Growth and apoptosis protocols.

Cell Studies | HeLa (HeLa ATCC® CCL-2™) cells were grown in a humidified incubator at 37 °C under 5% CO_2 with 90% humidity, and split at approximately 80% confluence using Trypsin-EDTA solution 0.25%. Cells were grown in high glucose DMEM (+ pyruvate) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin and 100 µg/mL streptomycin, 1% non-essential amino acids, 2 mM GlutaMax™ and 10 mM HEPES.

Apoptosis imaging with C2Am-11:Streptavidin-Alexa555

When cells have reached the appropriate density (70% - 80% confluent), the medium was aspirated and cells harvested with 0.25% Trypsin-EDTA. Then, 200 µL of the cell suspension (~50 000 cells) was applied on top of 12 mm glass coverslips pre-coated with poly d-lysine (Corning BioCoat) placed inside a 4-well plate. After 1 h of incubation to allow cells to adhere, 200 µL of additional media was added to flood the wells. Cells were then grown for more 6 - 8 h at 37 °C before apoptosis was induced by treatment for 12 h with 1 µM of actinomycin D in fresh growth media. Untreated cells at the same density were included as a control. After induction of apoptosis the media containing the cytotoxic drug was removed, the cells were washed with D-PBS and then preincubated for 20 min with **C2Am-11** (1.25 µg, 0.075 nmol, 6 µM) in 150 µL of apoptosis binding buffer (Invitrogen™ V13246; 50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4). Cells were then further washed 3 × with PBS before incubating for 10 min at room temperarure °C with the Streptavidin-Alexa555 (2.5 µL, 1 mg/mL, Invitrogen™) diluted in 150 µL of fresh binding buffer. Blocking studies were performed by preincubating apoptotic cells for 10 min with a 10X excess of non-modified C2Am (12.2 µg, 0.75 nmol, 67 µM) before adding **C2Am-11** for 20 min and Streptavidin-Alexa555 for 10 min at room temperature. For fluorescent DNA nuclei staining, Hoechst 33342 (0.8 µg/mL, 1.3 µM, Sigma Aldrich) was incubated with the cells for 20 min at 37 °C (last 20 min of incubation with Streptavidin-Alexa555). After labeling cells were washed in D-PBS 2X and then fixed with D-PBS containing 4% (w/v) formaldehyde for 15 min at room temperature. Finally, cells were further washed 2X with mili-Q water, and mounted on slides with Ibidi mounting medium. Fluorescence microscopy was performed using an inverted epifluorescent microscope (Olympus IX-71) connected to a F-view digital camera (Soft Imaging System). Images were acquired in the Tritc and Hoechst channels and analyzed using the software Cell-F. Same image acquisition settings were used for the control, experimental and blocking data sets. Images were analysed using ImageJ (http://fiji.sc/wiki/index.php).

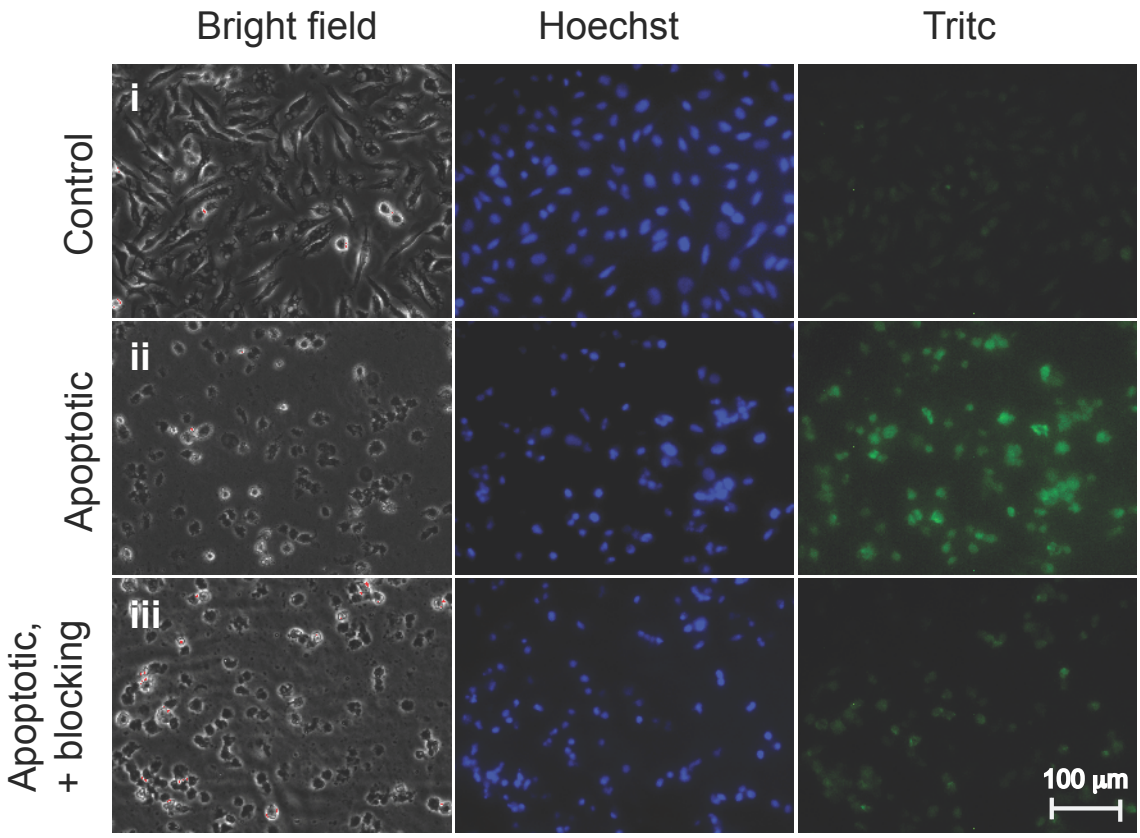


Figure S25. Epifluorescence images of non-apoptotic (i) and apoptotic (ii) HeLa cells after labelling with **C2Am-11**-Streptavidin Alexa 555. Blocking studies (iii) were performed by pre-incubating of apoptotic cells with a 10x excess of non-fluorescent unmodified C2Am before incubation with **C2Am-11-**Streptavidin Alexa 555. Apoptotic cells are shown green, while the nuclei counterstained with Hoechst 33 342 are shown blue.

Apoptosis imaging with C2Am-11:Tetrazine-Cy3

Following a protocol similar to the previously described, healthy and apoptotic cells were preincubated for 20 min with **C2Am-11** (2.48 µg, 0.15 nmol, 13.5 µM) in 150 µL of apoptosis binding buffer. Cells were then washed 2 × before incubating for 1.5 h at 37 °C with the **tetrazine-Cy3** probe (400 eq., 12 µL, 5 mM) diluted in 150 µL of fresh binding buffer. Blocking studies were performed with a 10X excess of non-modified C2Am before adding **C2Am-11**. After labeling cells were fixed as described previously. Confocal images were acquiring on a Leica SP5 confocal microscope equipped with Leica Application Suite Advanced Fluorescence (LAS AF) software and an oil-immersion 63x objective of numerical aperture of 1.4. The Cy3 was imaged upon excitation at 551 nm and detection on a 555 to 575 nm spectral bandwidth, while the excitation and emission wavelengths of Hoechst 33258 were 405 and 425 – 490 nm.

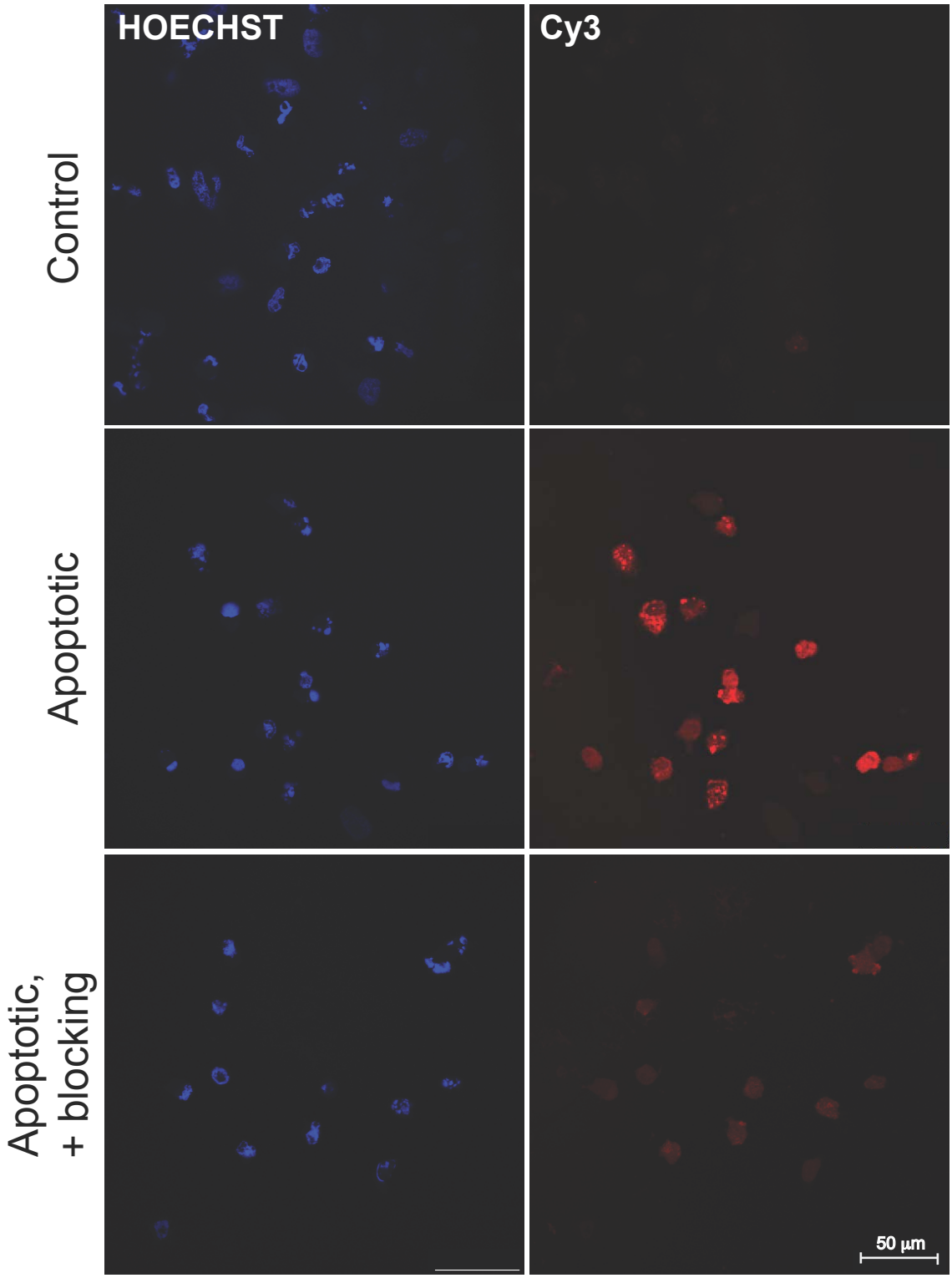


Figure S26. Confocal images of non-apoptotic (control) and apoptotic HeLa cells after labelling first with **C2Am-11** and then with tetrazine-Cy3. Blocking studies were performed by pre-incubating of apoptotic cells with a 10x excess of non-fluorescent C2Am before incubation with **C2Am-11**. Apoptotic cells are shown in green, while the nuclei in blue.

14. Copies of ^{1}H-NMR and ^{13}C-NMR spectra for the new compounds.

The heating of the sample favours the retro-Diels-Alder transformation of the compound. Thus, the sample could not be heated, what complicated the assignment of the ^{1}H and ^{13}C-NMR spectra (broad and duplicate signals) due to the presence of *N*-Boc rotamers and also due to the existence of two diasteroisomers.

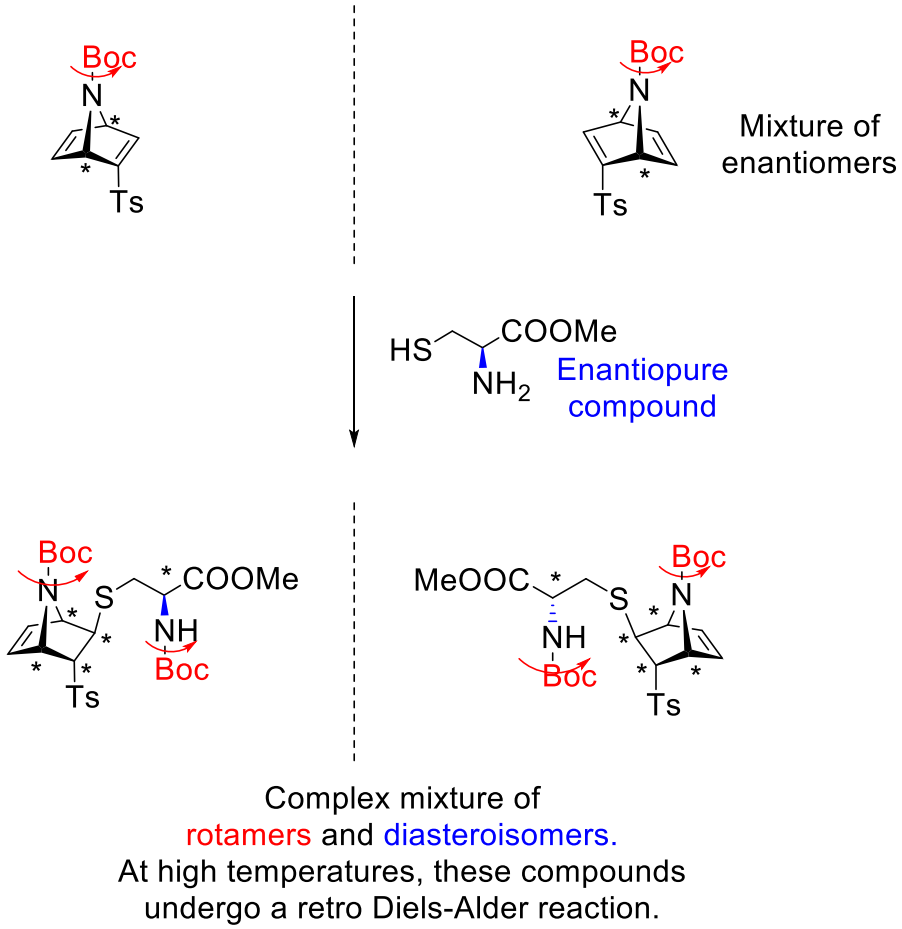


Figure S27. Difficulty in the NMR signals appearance.

Figure S28. ^{1}H-NMR (DMSO-D_6, 300 MHz) of **9.**

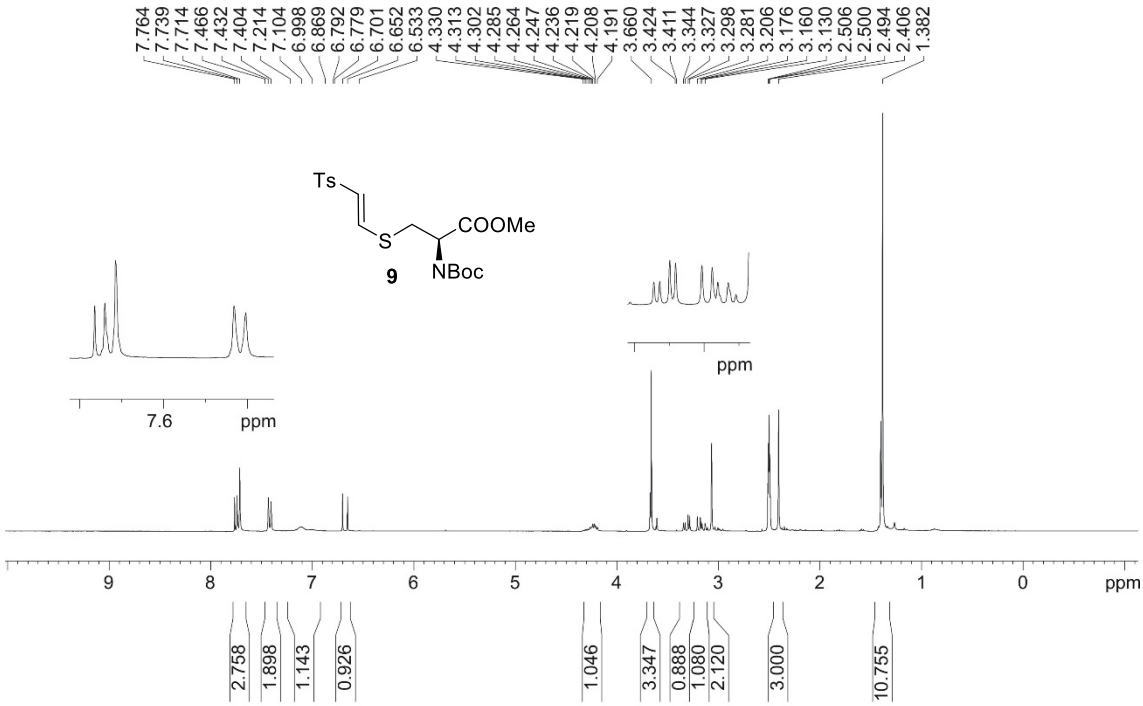


Figure S29. ^{13}C-NMR (DMSO-D_6, 75 MHz) of **9.**

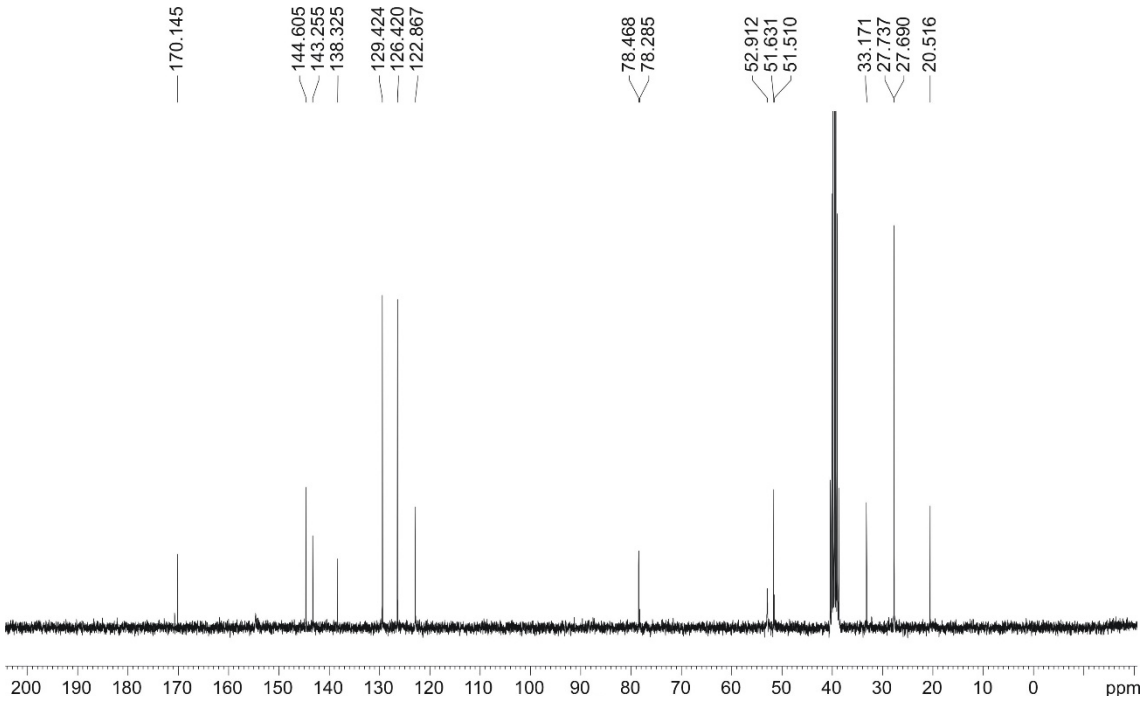


Figure S30. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **5.**

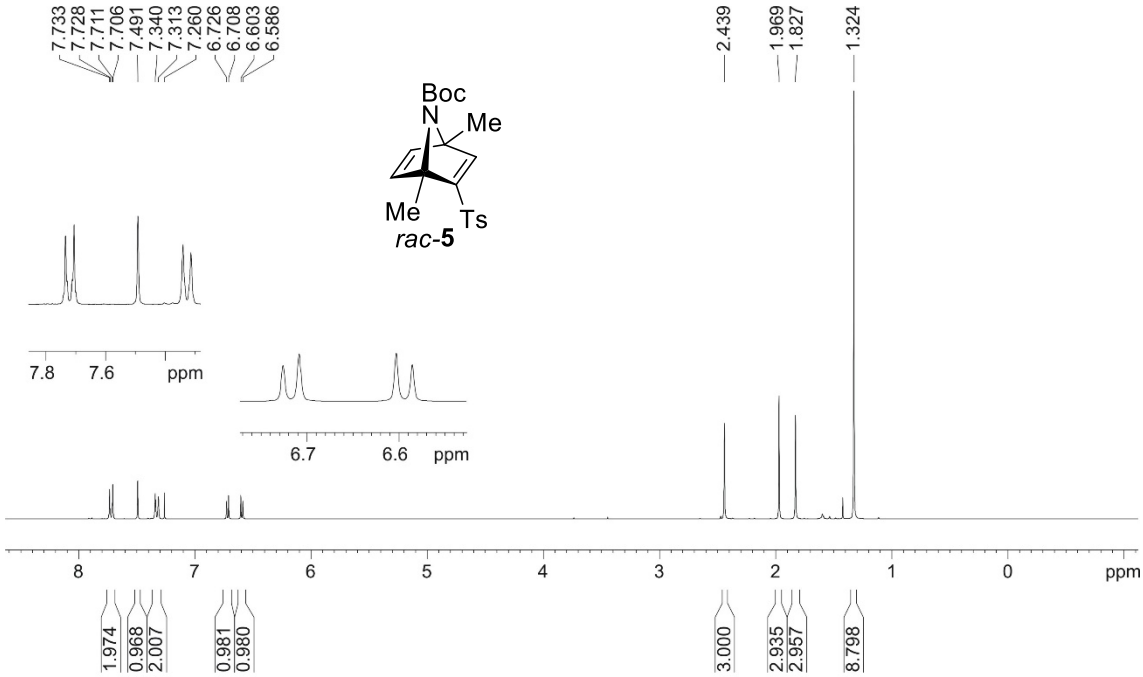


Figure S31. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **5.**

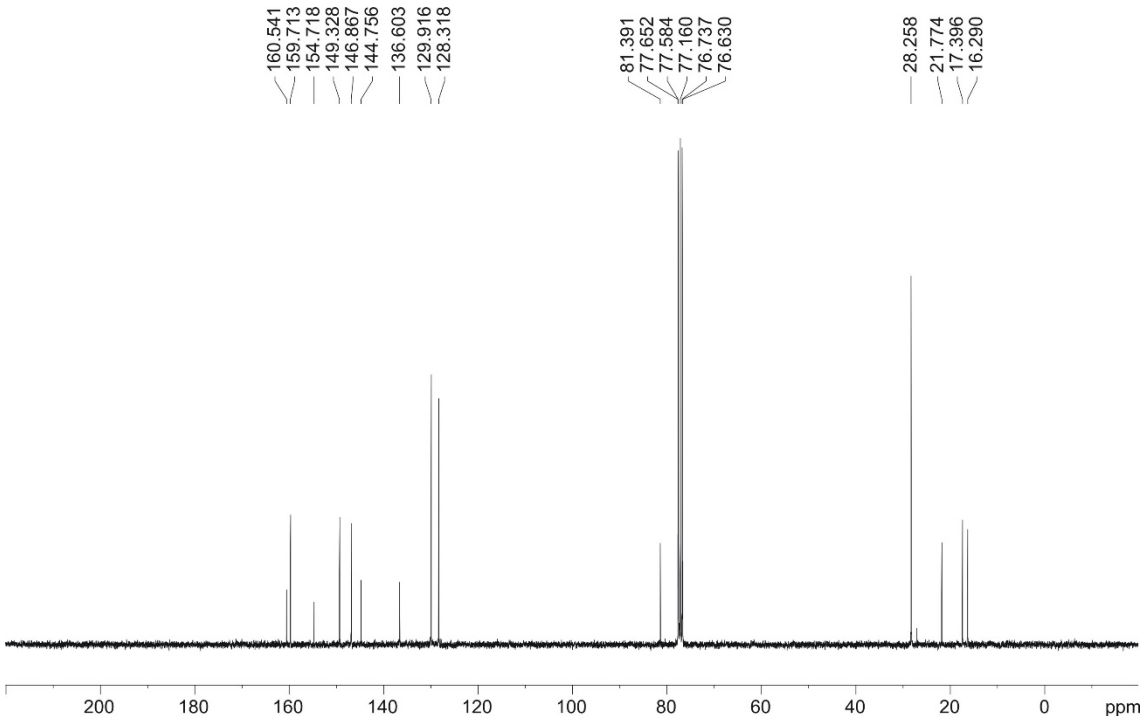


Figure S32. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **2.**

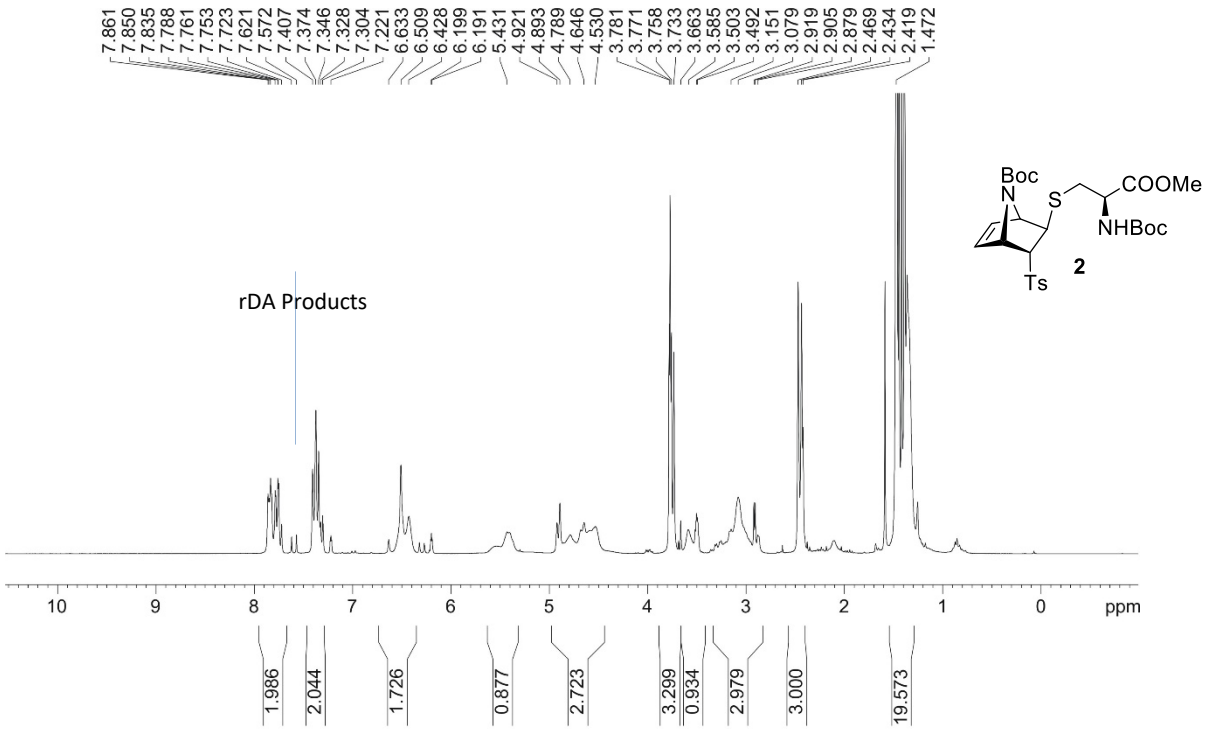
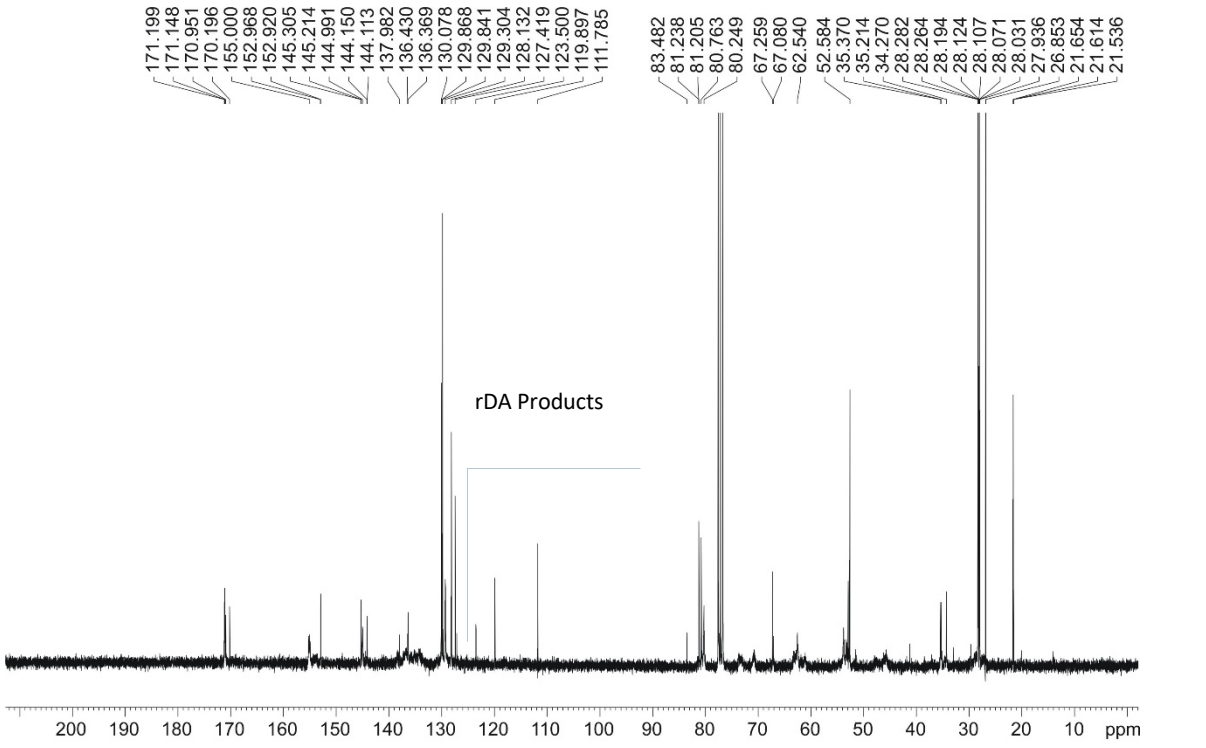


Figure S33. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **2.**



*Note: The heating of the sample favours the retro-Diels-Alder transformation of the compound. Thus, the sample could not be heated, what complicated the assignment of the ^{1}H and ^{13}C-NMR spectra (broad and duplicate signals) due to the presence of N-Boc rotamers.

Figure S34. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **7.**

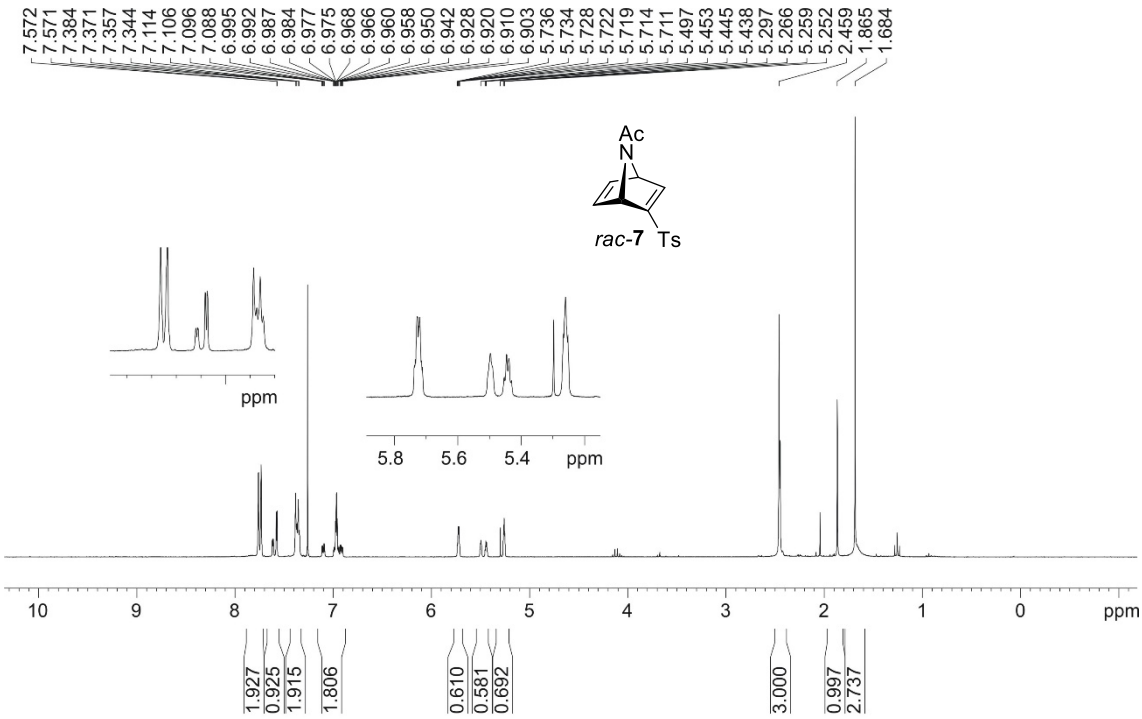


Figure S35. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **7.**

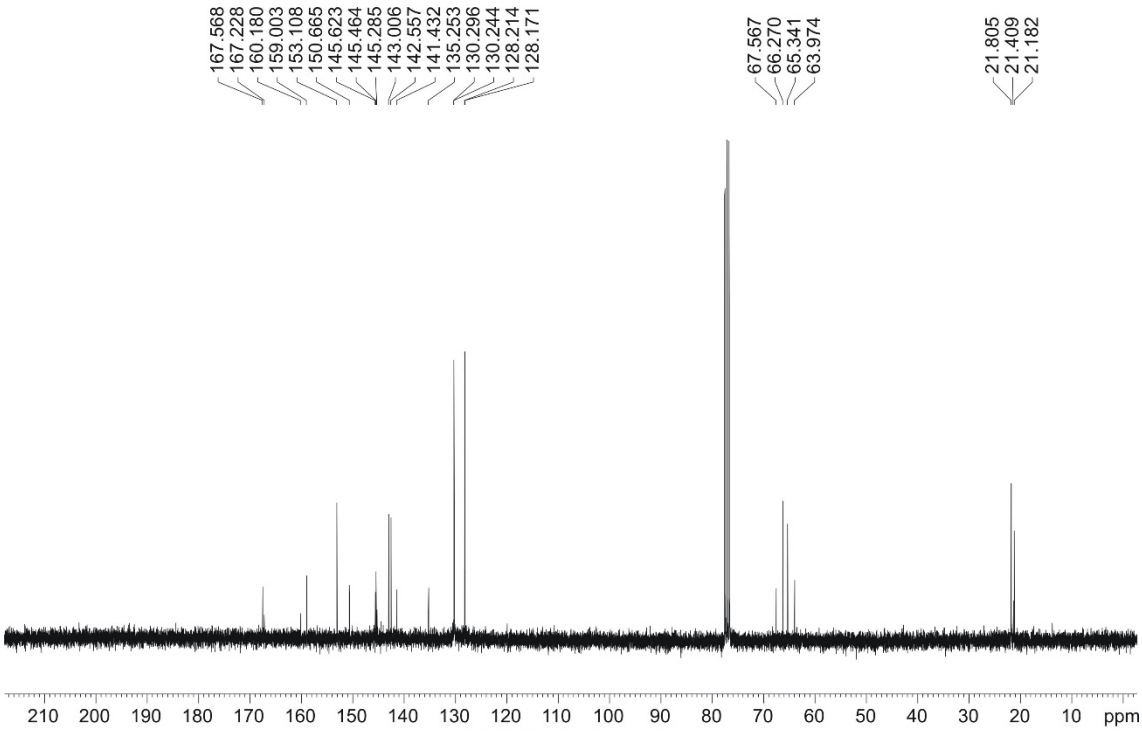


Figure S36. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **8.**

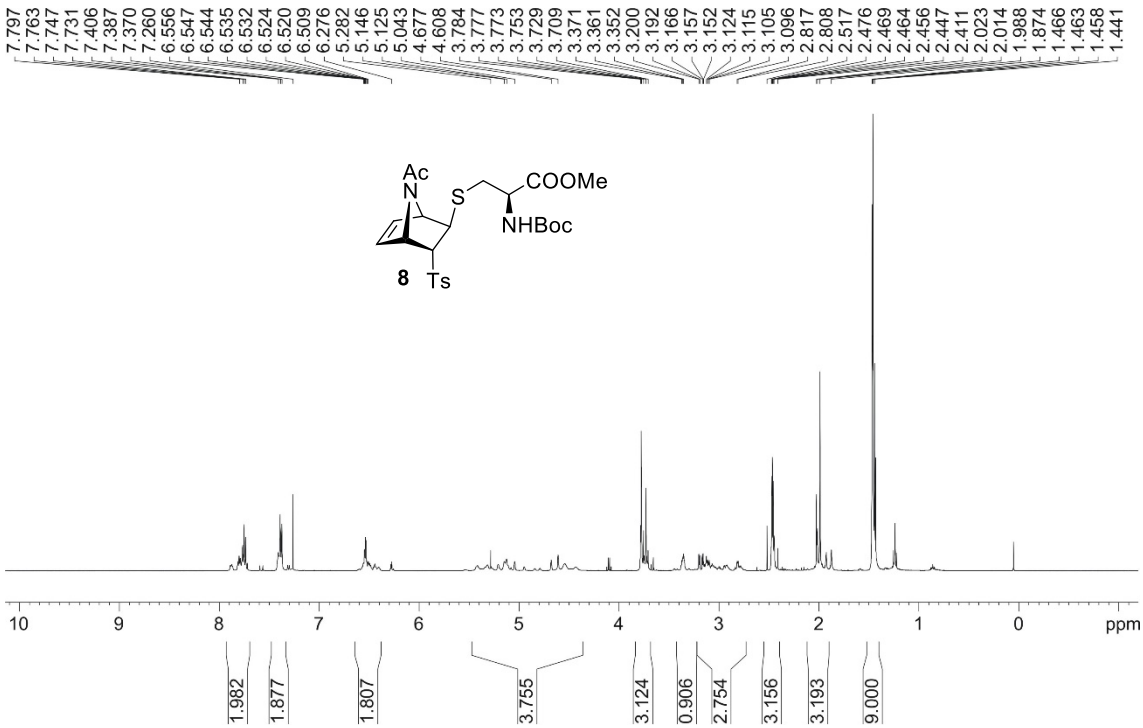
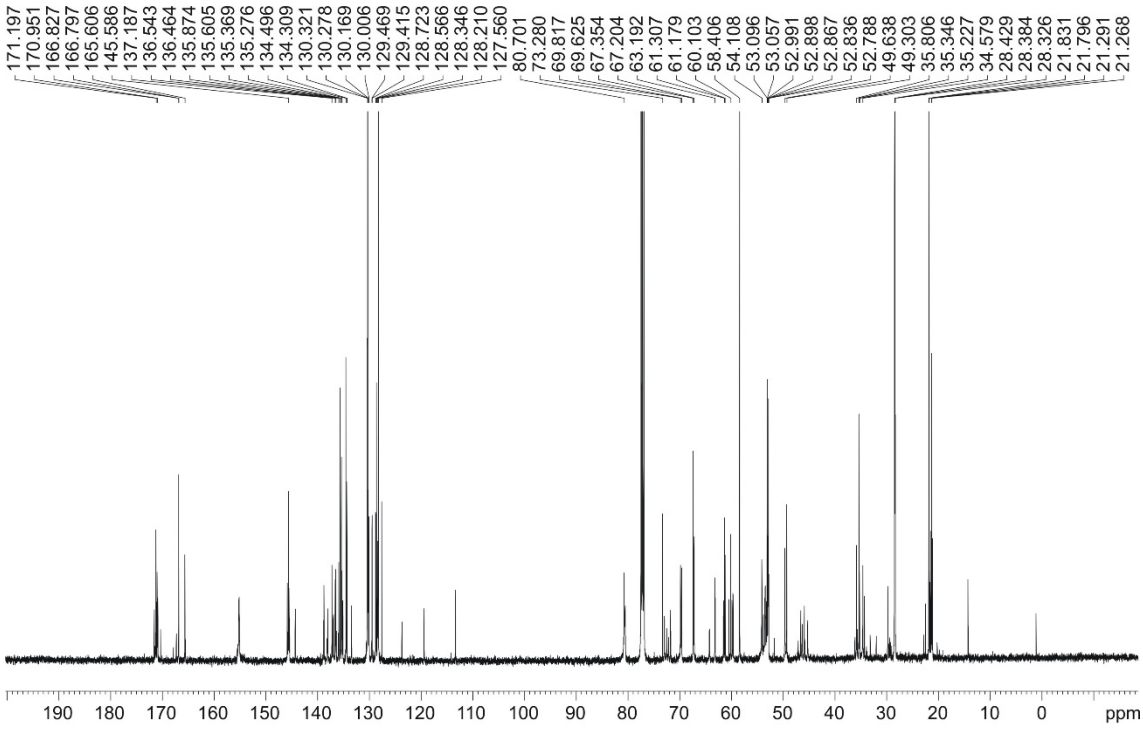
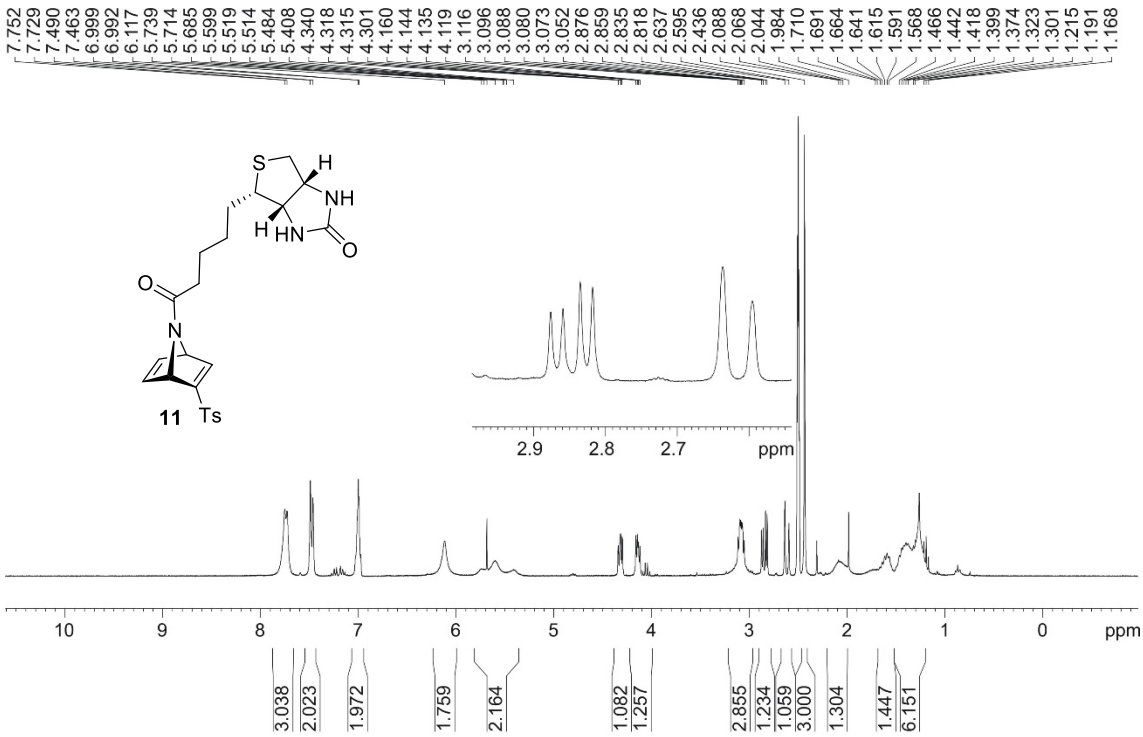


Figure S37. ^{13}C-NMR ($CDCl_3$, 75 MHz) of **8.**



*Note: The heating of the sample favours the retro-Diels-Alder transformation of the compound. Thus, the sample could not be heated, what complicated the assignment of the ^{1}H and ^{13}C-NMR spectra (broad and duplicate signals) due to the presence of N-Boc and N-Ac rotamers.

Figure S38. ^{1}H-NMR (DMSO-D_6, 300 MHz) of **11.**



*Note: broad signals in ^{1}H-NMR spectrum due to the presence of N-Boc rotamers (spectrum registered at 80 °C).

Figure S39. ^{13}C-NMR (DMSO-D_6, 75 MHz) of **11.**

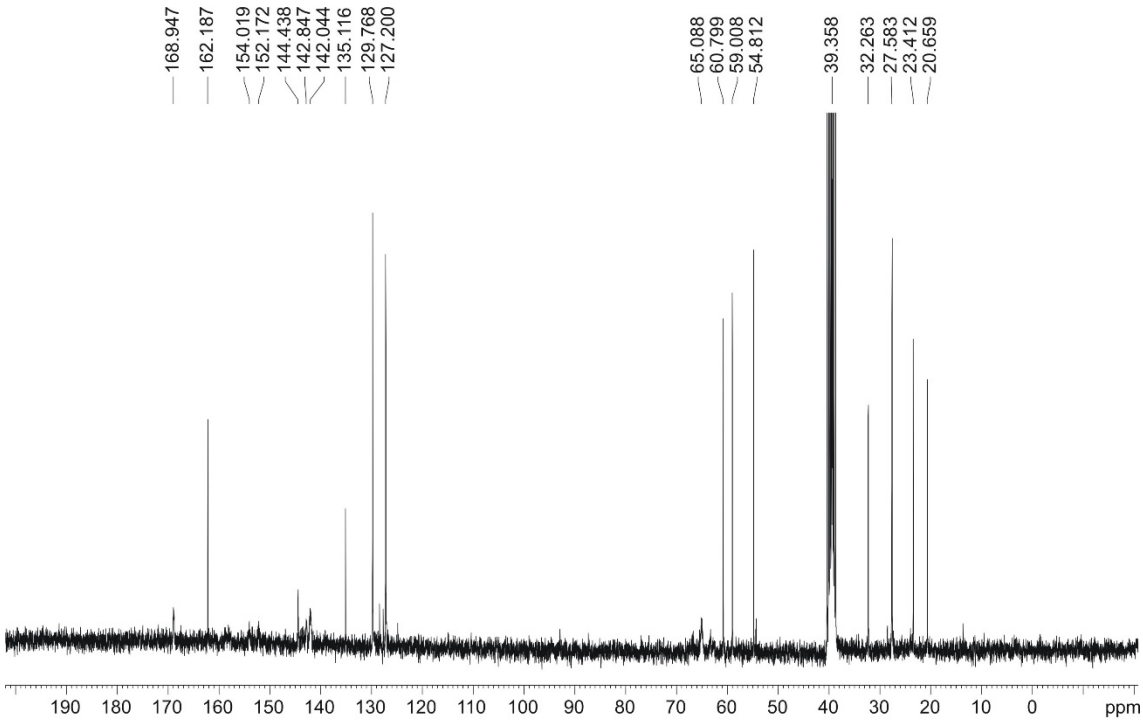
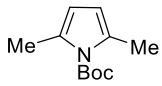


Figure S40. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **S3.**



S3

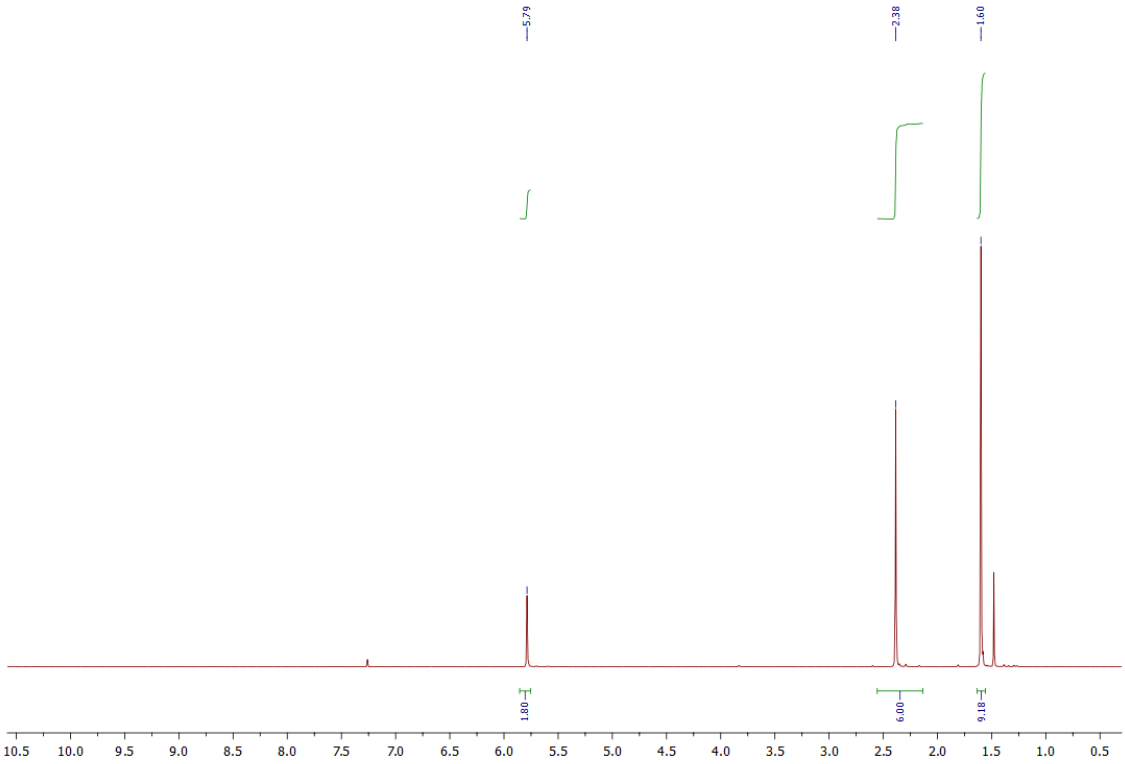


Figure S41. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **12.**

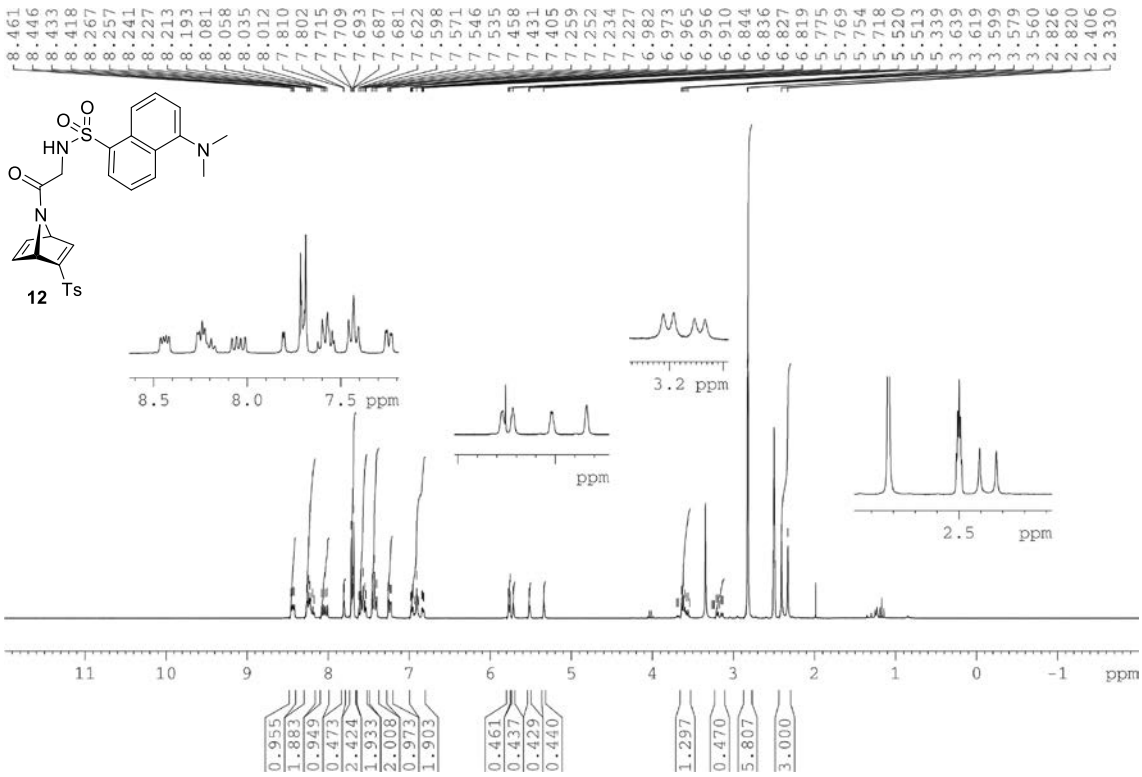


Figure S42. ^{13}C-NMR (DMSO-D_6, 75 MHz) of **12.**

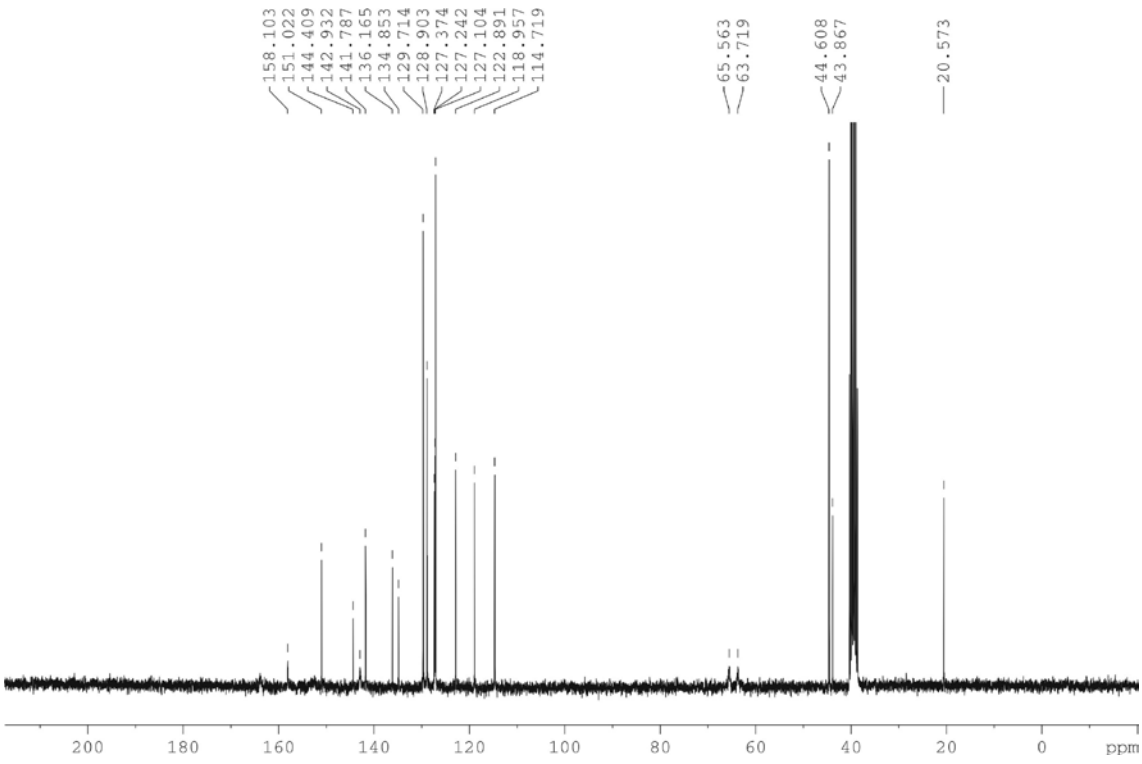


Figure S43. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **S1**

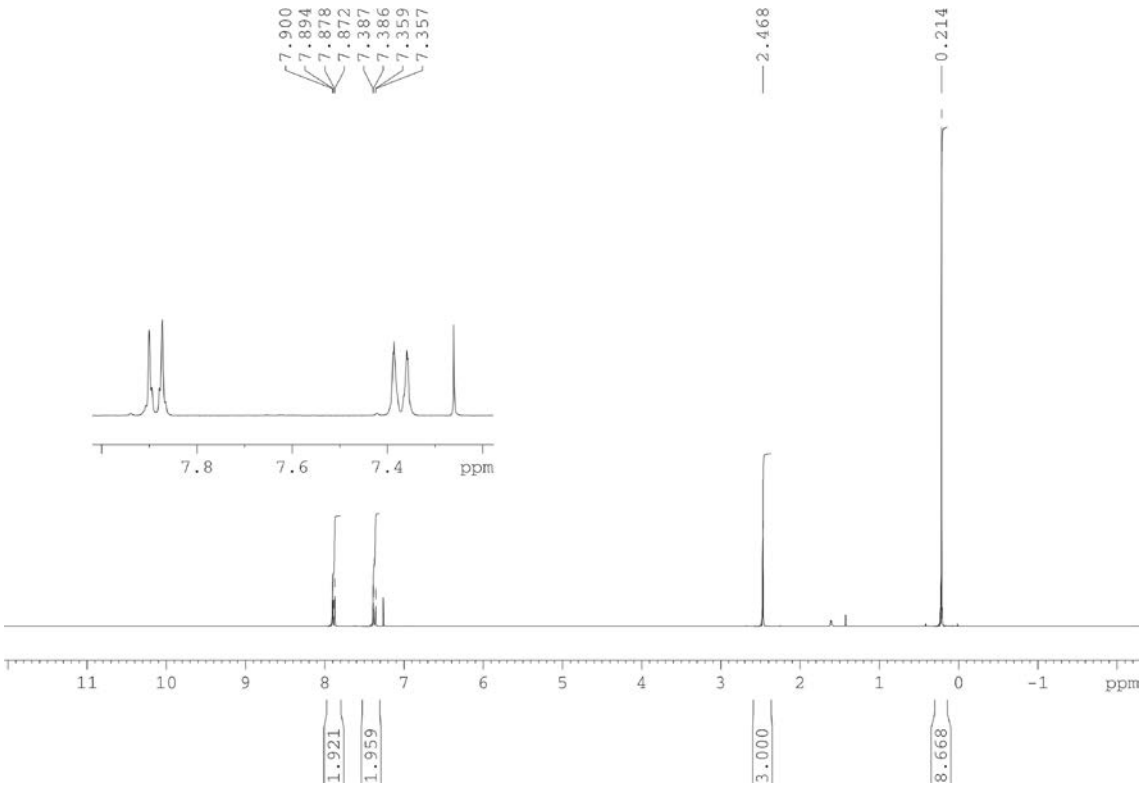


Figure S44. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **S2**

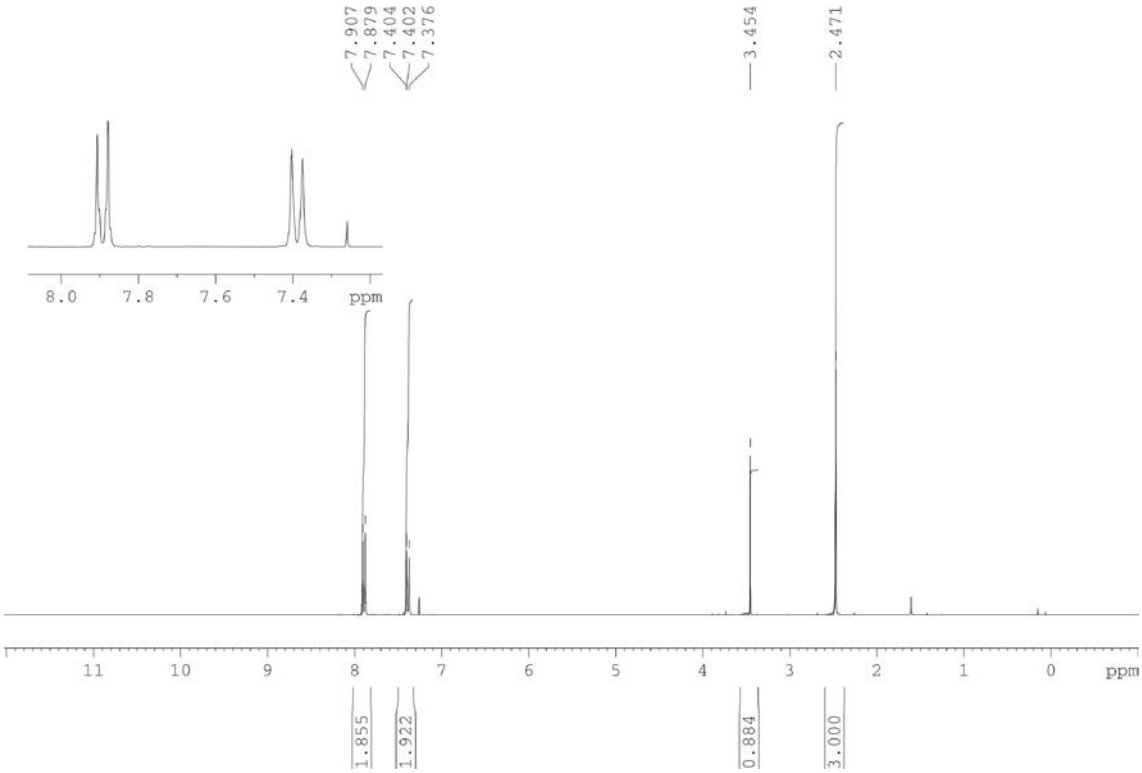


Figure S45. ^{1}H-NMR ($CDCl_3$, 300 MHz) of **1**