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Supporting Information

Synthesis and Reactivity Comparisons of 1-Methyl-3-Substituted Cyclopropene Mini-tags for Tetrazine Bioorthogonal Reactions

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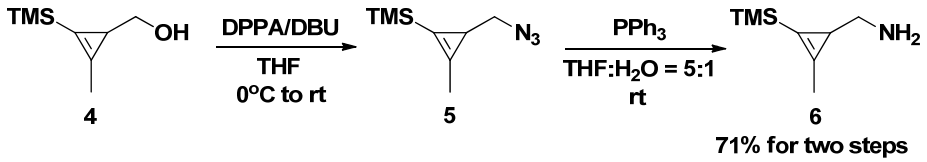
Experimental Section

1. Synthesis of cyclopropene amine **6**

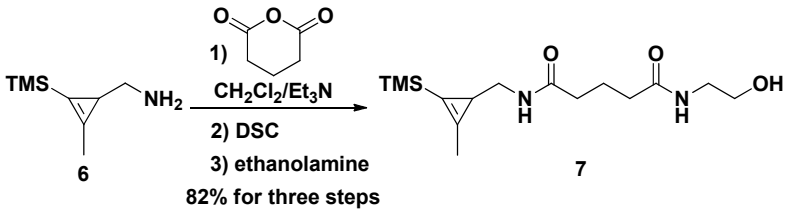


Under the protection of the N_2 gas, to a stirred solution of cyclopropene alcohol **4** (0.50 g, 3.2 mmol) in dry THF (10.0 mL) at 0 °C was added DBU (0.63 g, 4.2 mmol) followed by DPPA (diphenylphosphoryl azide, 1.14 g, 4.2 mmol). The reaction solution was slowly warmed to room temperature and stirred overnight. After TLC indicated that the reaction had completed, most of the THF was evaporated by flushing compressed air and the material was passed through a short silica column using hexanes. The product was collected and the hexanes were evaporated by rotary evaporation at 100 torr, room temperature, affording crude cyclopropene azide **5**. The crude cyclopropene azide **5** was dissolved in 5.0 mL THF and 1.0 mL H_2O, PPh_3 (1.10 g, 4.2 mmol) was added to the solution and stirred at room temperature overnight. After TLC indicated that the reaction was finished, 5.0 mL of 1 M HCl was added to the reaction solution. The THF was evaporated and the aqueous solution was extracted with Et_2O (5 mL x 3). The pH of the water layer was adjusted to pH 9 with sat. $NaHCO_3$ and extracted with CH_2Cl_2 (10 mL x 3). The organic fractions were combined and dried over Na_2SO_4, evaporated at 100 torr, room temperature to afford 355 mg product **6** as yellow oil in 71% yield over two steps.

Cyclopropene azide **5**:
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.17 (s, 9H), 1.57 (m, 1H), 2.22 (s, 3H), 3.01 (dd, J = 15, 5 Hz, 1H), 3.14 (dd, J = 15, 5 Hz, 1H); ^{13}C (125 MHz, $CDCl_3$) δ -1.3, 13.8, 18.7, 59.9, 112.5, 134.9.

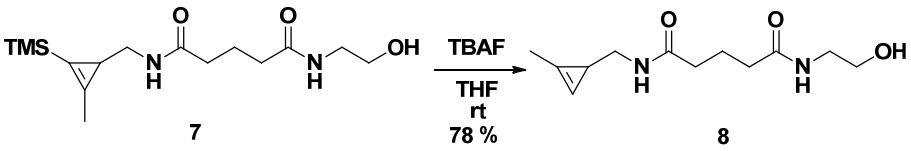
Cyclpropene amine **6**:
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.14 (s, 9H), 1.43 (bs, 1H), 2.18 (s, 3H), 2.57 (bs, 2H); ^{13}C (125 MHz, $CDCl_3$) δ -0.79, 13.7, 23.5, 48.9, 112.7, 136.9; HRMS $[M+H]^+$ m/z calcd. for $[C_8H_{18}NSi]^+$ 156.1203, found 156.1204.

2. Synthesis of compound **7**



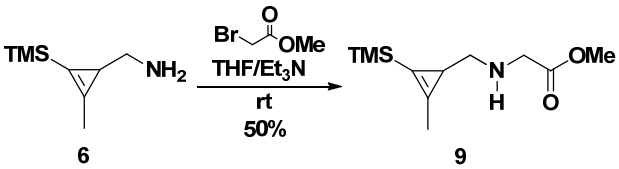
Cyclopropene amine **6** (10.0 mg, 0.065 mmol) was dissolved in CH_2Cl_2 followed by addition of Et_3N (13.0 mg, 0.13 mmol) and glutaric anhydride (11.0 mg, 0.1 mmol). This solution was stirred for 1 hr at room temperature after which *N,N*'-disuccinimidyl carbonate (26.0 mg, 0.1 mmol) was added. The reaction solution was stirred at room temperature for 1 hour after which ethanolamine (6.0 mg, 0.1 mmol) was added. The resulting solution was stirred for an additional hour at room temperature. After evaporating the organic solvent, the residue was purified by preparative TLC (CH_2Cl_2/MeOH=10/1) to afford 16.0 mg of compound **4** as a colorless oil, in 82 % yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.15 (s, 9H), 1.43 (bs, 1H), 1.97 (bs, 2H), 2.17 (s, 3H), 2.27 (m, 4H), 3.04 (bs, 1H), 3.18 (bs, 1H), 3.41 (bs, 2H), 3.71 (bs, 2H), 5.84 (bs, 1H), 6.73 (bs, 1H); ^{13}C (125 MHz, $CDCl_3$) δ -0.86, 13.4, 19.3, 22.3, 29.9, 35.5, 42.8, 46.7, 62.4, 111.9, 135.9, 174.4, 176.7; HRMS $[M+Na]^+$ m/z calcd. for $[C_{15}H_{28}N_2O_3SiNa]^+$ 335.1761, found 335.1762.

3. Synthesis of compound **8**



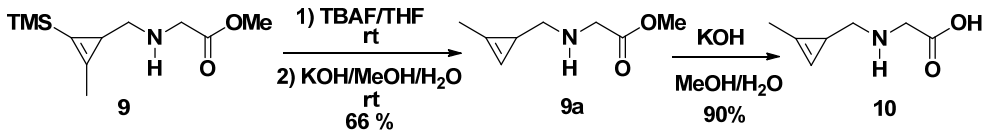
0.05 mL 1.0 M TBAF in THF (0.05 mmol) was added to a stirred solution of compound **7** (15.0 mg, 0.048 mmol) in dry THF (3.0 mL) at room temperature. The reaction solution was stirred at room temperature overnight until the TLC indicated that the starting material was consumed. The organic solvent was evaporated and the residue purified by preparative TLC (CH_2Cl_2/MeOH = 10/1) to afford 9.0 mg compound **8** as colorless oil, in 78% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (m, 1H), 1.94 (m, 2H), 2.11 (s, 3H), 2.24 (m, 4H), 3.11 (m, 1H), 3.22 (m, 1H), 3.40 (m, 2H), 3.71 (m, 2H), 5.86 (bs, 1H), 6.58 (s, 1H), 6.64 (bs, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 11.8, 18.0, 22.3, 29.9, 35.6, 42.7, 45.6, 62.4, 103.0, 121.7, 173.0, 174.0; HRMS $[M+Na]^+$ m/z calcd. for $[C_{12}H_{20}N_2O_3Na]^+$ 263.1366, found 263.1367.

4. Synthesis of compound **9**



Et_3N (101 mg, 1.0 mmol) was added to a stirred solution of compound **6** (130 mg, 0.84 mmol) and methyl bromoacetate (140 mg, 0.84 mmol) in dry THF (3.0 mL) at room temperature. After the reaction solution was stirred at room temperature for 1 hour, LC-MS indicated that there was unreacted starting material **6**, product **9** and a dialkylation product, with **9** as the majority product. Without workup, the residue was directly purified using preparative TLC (EtOAc) to afford 85 mg compound **9**, in 50% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.16 (s, 9H), 1.44 (t, *J* = 5 Hz, 1H), 2.20 (s, 3H), 2.38 (dd, *J* = 10, 5 Hz, 1H), 2.63 (dd, *J* = 10, 5 Hz, 1H), 3.41 (d, J = 24 Hz, 1H), 3.47 (d, *J* = 24 Hz, 1H), 3.72 (s, 3H); ^{13}C (125 MHz, $CDCl_3$) δ -0.83, 13.6, 20.0, 50.5, 52.0, 57.4, 112.4, 136.6, 173.0; HRMS $[M+H]^+$ m/z calcd. for $[C_{11}H_{22}NO_2]^+$ 228.1414, found 228.1415.

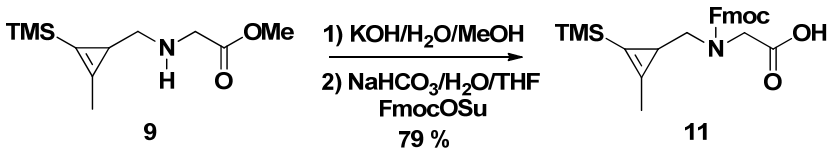
5. Synthesis of compound **10**



TBAF (0.26 mL, 1.0 M in THF) was added to a stirred solution of compound **9** (60.0 mg, 0.26 mmol) in dry THF (0.25 mL) at room temperature. The reaction solution was stirred at room temperature for 8 hours until the TLC indicated that the starting material was consumed. The solvent was evaporated and the residue was purified by preparative TLC (hexane/EtOAc = 2/1) to afford 27.0 mg compound **9a** as colorless oil, in 66 % yield. KOH (15 mg, 0.26 mmol) was added to a stirred solution of 6a (20 mg, 0.13 mmol) in 0.5 mL MeOH/H_2O (4/1) and the resulting solution was stirred overnight. The pH was adjusted to neutral by adding 0.26 mL 1 M HCl and the solution was evaporated to afford the crude product which was purified by preparative TLC (CH_2Cl_2/MeOH/H_2O=10/2/0.2) to afford 16 mg of compound **10** as a white solid, in 90% yield.

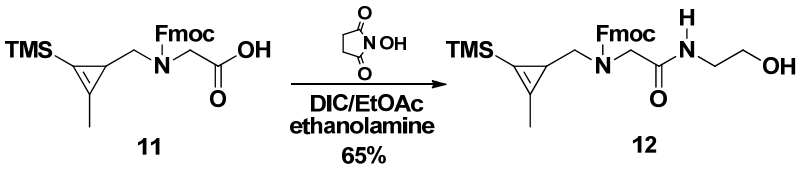
Compound **9a**:
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (m, 1H), 2.13 (s, 3H), 2.50 (dd, *J* = 10, 5 Hz, 1H), 2.59 (dd, *J* = 10, 5 Hz, 1H), 3.42 (s, 2H), 3.71 (s, 3H), 6.64 (s, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 12.1, 18.5, 50.7, 52.0, 56.4, 103.9, 122.3, 173.3.
Compound **10**:
^{1}H NMR (500 MHz, D_2O/DMSO-d_6 = 5/1) δ 1.48 (m, 1H), 2.02 (s, 3H), 2.72 (dd, *J* = 10, 5 Hz, 1H), 2.94 (dd, *J* = 10, 5 Hz, 1H), 3.47 (d, *J* = 15 Hz, 1H), 3.51 (d, *J* = 15 Hz, 1H), 6.65 (s, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 12.1, 15.0, 49.7, 50.3, 56.1, 102.6, 121.4, 172.2.

6. Synthesis of compound **11**



KOH (0.1 mL, 2.0 M KOH/H_2O) was added to a stirred solution of compound **9** (20 mg, 0.088 mmol) in MeOH (3.0 mL) at room temperature. The reaction solution was stirred at room temperature for 6 hours after which TLC indicated that the reaction had completed. The organic solvent was evaporated and the residue was dissolved in 2.0 mL THF. HCl (0.2 mL, 1.0 M HCl/H_2O) was added followed by $NaHCO_3$ (15 mg in 0.1 mL H_2O, 0.18 mmol). FmocOSu (33 mg, 0.1 mmol) was added and the resulting solution was stirred for 3 hours after which TLC indicated that the reaction had finished. The pH was adjusted to 5 by addition of HCl (0.5 mL, 1.0 M HCl/H_2O) and the solution was extracted with EtOAc (10 mL x 2). The organic layers were combined, dried over Na_2SO_4, and evaporated to afford the crude product. The product was purified by preparative TLC (EtOAc) to afford 30 mg of compound **11**, in 79% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.13 (s, 9H), 1.39 (bs, 1H), 2.14 (s, 3H), 2.80-2.89 (m, 1H), 3.61-3.68 (m, 1H), 3.88-4.21 (m, 3H), 4.42 (m, 2H), 7.27-7.73 (m, 8H); ^{13}C (125 MHz, $CDCl_3$) δ -0.85, 13.5, 18.5, 29.9, 47.4, 55.7, 67.7, 111.8, 120.1, 125.1, 127.3, 136.2, 136.3, 141.5, 144.2, 156.0; HRMS $[M+Na]^+$ m/z calcd. for $[C_{25}H_{29}NO_4SiNa]^+$ 458.1758, found 458.1759.

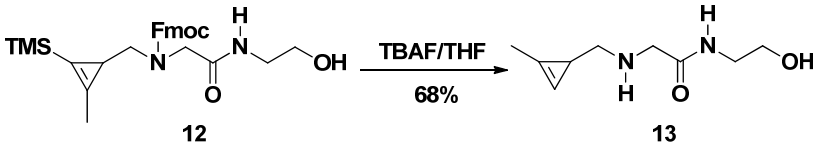
7. Synthesis of compound **12**



DIC (*N,N*-diisopropylcarbodiimide, 10.0 mg, 0.08 mmol) and *N*-Hydroxysuccinimide (9.0 mg, 0.08 mmol) was added to a stirred solution of compound **11** (27.0 mg, 0.06 mmol) in EtOAc (2.0 mL) at room temperature. The reaction solution was stirred at room temperature for 30 minutes after which ethanolamine (5.0 mg, 0.08 mmol) was added. After stirring at room temperature for an additional 30 minutes, the reaction solution was washed with water and the organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by preparative TLC (EtOAc) to afford 20 mg of compound **12** in 65% yield.

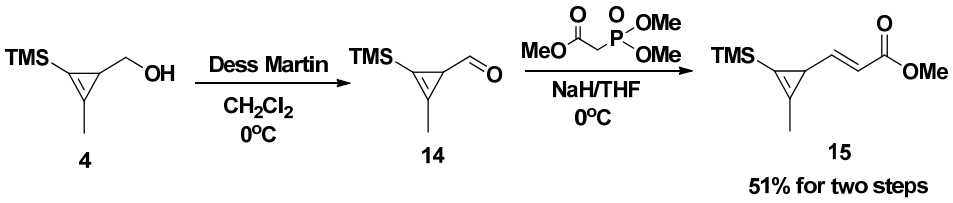
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.14 (s, 9H), 1.34 (bs, 1H), 2.14 (s, 3H), 2.60-2.64 (m, 1H), 3.32-3.39 (m, 2H), 3.63-3.88 (m, 5H), 4.22 (bs, 1H), 4.46-4.52 (m, 2H) 7.30-7.77 (m, 8H); ^{13}C (125 MHz, $CDCl_3$) δ -1.00, 13.3, 18.5, 29.8, 42.5, 47.4, 56.1, 62.2, 67.8, 111.6, 120.1, 124.9, 127.1, 127.9, 136.0, 141.4, 143.9, 157.1, 170.6; HRMS $[M+Na]^+$ m/z calcd. for $[C_{27}H_{34}N_2O_4SiNa]^+$ 501.2180, found 501.2181.

8. Synthesis of compound **13**



0.12 mL 1.0 M TBAF in THF (0.12 mmol) was added to a stirred solution of compound **12** (19.0 mg, 0.04 mmol) in dry THF (1.0 mL) at room temperature. The reaction solution was stirred at room temperature for 16 hours until the TLC indicated an absence of the starting material and intermediate. The organic solvent was evaporated and the residue was purified by preparative TLC (CH_2Cl_2/MeOH/CF_3COOH = 70/10/0.5) to afford 5.0 mg of compound **13** as a white solid, in 68 % yield.
^{1}H NMR (500 MHz, acetone-d_6) δ 1.66 (t, J = 5 Hz, 1H), 2.13 (s, 3H), 2.94 (dd, J = 15, 5 Hz, 1H), 3.09 (dd, J = 15, 5 Hz, 1H), 3.38 (bs, 2H), 3.60 (bs, 2H), 4.00 (bs, 2H), 6.78 (bs, 1H), 8.52 (bs, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 10.4, 13.8, 42.1, 47.6, 54.1, 55.1, 102.0, 120.1; HRMS $[M+Na]^+$ m/z calcd. for $[C_9H_{16}N_2O_2Na]^+$ 207.1104, found 207.1105.

9. Synthesis of cyclopropene aldehyde **14** and compound **15**



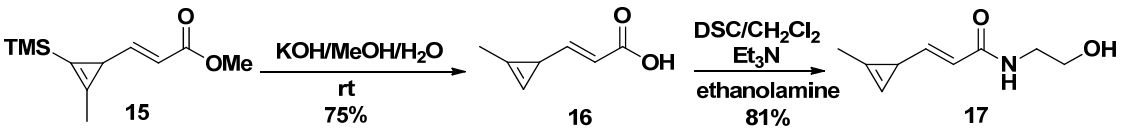
Dess-Martin reagent (750 mg, 1.75 mmol) was added to a stirred solution of compound cyclopropene **4** (250 mg, 1.6 mmol) in CH_2Cl_2 (10.0 mL) at 0°C. The reaction solution was stirred at 0°C for one hour. The reaction was washed with sodium thiosulfate and sodium bicarbonate aqueous solution three times. The organic layer was collected and dried over Na_2SO_4, followed by evaporation at 100 torr. (room

temperature). This afforded the crude aldehyde **14**. Trimethyl phosphonoacetate (296 mg, 1.6 mmol) was dissolved in 10 mL dry THF and cooled to 0°C, NaH (60% in mineral oil, 64 mg, 1.6 mmol) was added and stirred at 0°C for 30 minutes. The crude aldehyde was added to the reaction solution and stirred at 0°C for one hour. The reaction was quenched with water and extracted with EtOAc (20 mL x 3). The organic layers were combined and washed with sat. NaCl solution before drying over Na_2SO_4. After filtration, the solution was evaporated and the residue purified by flash silica column (hexane/EtOAc = 10/1) to afford 172 mg compound of **15**, in 51% yield.

Compound **14**
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.21 (s, 9H), 2.18 (d, *J* = 10 Hz, 1H), 2.27 (s, 3H), 8.69 (d, *J* = 10Hz, 1H); ^{13}C (125 MHz, $CDCl_3$) δ -1.6, 13.3, 35.2, 105.7, 123.9, 206.2.

Compound **15**
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.15 (s, 9H), 2.05 (d, *J* = 10 Hz, 1H), 2.17 (s, 3H), 3.68 (s, 3H), 5.78 (d, *J* = 15 Hz, 1H), 6.64 (dd, *J* = 15, 10 Hz, 1H); ^{13}C (125 MHz, $CDCl_3$) δ -1.3, 12.7, 24.2, 51.2, 110.5, 115.0, 130.3, 160.4, 167.8; HRMS $[M+H]^+$ m/z calcd. for $[C_{11}H_{19}O_2Si]^+$ 211.1149, found 211.1150.

10. Synthesis of compound **17**

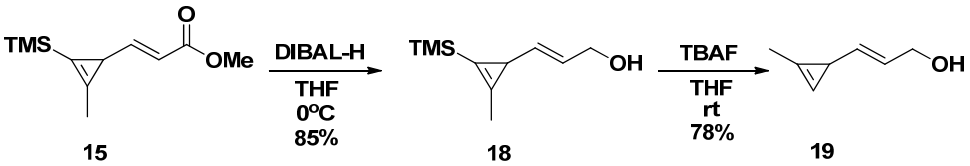


KOH (0.8 mL, 2.0 M KOH/H_2O) was added to a stirred solution of compound **15** (100 mg, 0.48 mmol) in MeOH (4.0 mL) at room temperature. The reaction solution was stirred at room temperature overnight. The organic solvent was evaporated and the residue was dissolved in EtOAc and washed with 1 M HCl. The organic layer was dried over Na_2SO_4 and evaporated to afford crude compound **16**. The crude product was purified by preparative TLC (CH_2Cl_2/MeOH=10/1) to afford 44 mg of compound **16** as a colorless oil, in 75% yield. Compound **16** (10 mg, 0.08 mmol) was dissolved in CH_2Cl_2 and, to this solution, Et_3N (10 mg, 0.1 mmol) and DSC (*N,N'*-disuccinimidyl carbonate, 26 mg, 0.1 mmol) were added. The resulting solution was stirred for 30 min after which ethanolamine (6 mg, 0.1 mmol) was added. The reaction was stirred at room temperature for an additional 1 hour. The organic solvent was evaporated and the residue was purified by preparative TLC (EtOAc) to afford 11 mg of compound **17** as a colorless oil, in 81% yield.

Compound **16**
^{1}H NMR (500 MHz, $CDCl_3$) δ 2.16 (s, 3H), 2.19 (d, *J* = 10Hz, 1H), 5.86 (d, *J* = 15 Hz, 1H), 6.56 (s, 1H), 6.73 (dd, *J* = 15, 10 Hz, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 11.3, 22.5, 100.7, 116.3, 118.5, 161.7, 172.1; HRMS $[M-H]^-$ m/z calcd. for $[C_7H_7O_2]^-$ 123.0452, found 123.0453.

Compound **17**
^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.15 (d, *J* = 8 Hz, 1H), 2.21 (bs, 1H), 3.48 (t, *J* = 6 Hz, 2H), 3.73 (t, *J* = 6 Hz, 2H), 5.85 (d, *J* = 20 Hz, 1H), 5.97 (bs, 1H), 6.53 (dd, *J* = 20, 12 Hz, 1H), 6.56 (s, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 11.3, 22.0, 42.7, 62.9, 102.3, 118.9, 119.1, 154.7, 167.8; HRMS $[M+H]^+$ m/z calcd. for $[C_9H_{13}NO_2Na]^+$ 190.0838, found 190.0839.

11. Synthesis of compound **19**

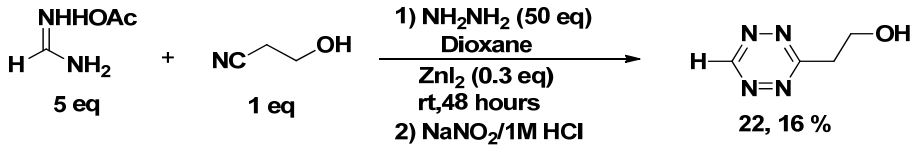


DIBAL-H (1.0M, 0.44 mL) was added to a stirred solution of compound **15** (50 mg, 0.24 mmol) in dry THF (2.0 mL) at 0°C. The reaction was stirred at 0°C for 1 hour and then quenched by addition of 0.1 mL water. The solution was filtered and the organic solvent evaporated to afford the crude product. The residue was purified by preparative TLC (hexane/EtOAc = 5:1) to give 36 mg of compound **18** as a yellow oil, in 85% yield. Compound **18** (20 mg, 0.11 mmol) was dissolved in 0.3 mL THF and TBAF (1.0 M in THF, 0.12 mL) was added. The solution was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by preparative TLC (hexane/EtOAc = 3:1) to afford 9.0 mg of compound **19** as a yellow oil, in 78 % yield.

Compound **18**
^{1}H NMR (500 MHz, $CDCl_3$) δ 0.15 (s, 9H), 1.93 (d, *J* = 10 Hz, 1H), 2.16 (s, 3H), 4.04 (d, *J* = 10 Hz, 2H), 5.26 (dd, *J* = 20, 10 Hz, 1H), 5.64 (dd, *J* = 20, 10 Hz, 1H); ^{13}C (125 MHz, $CDCl_3$) δ -0.91, 12.8, 23.0, 64.3, 111.7, 124.2, 133.1, 143.0.

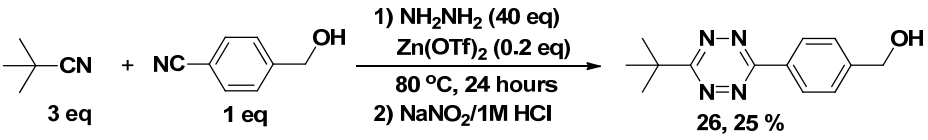
Compound **19**
^{1}H NMR (500 MHz, $CDCl_3$) δ 2.03 (d, *J* = 5 Hz, 1H), 2.12 (s, 3H), 4.07 (d, *J* = 10 Hz, 2H), 5.30 (dd, *J* = 15, 5 Hz, 1H), 5.70 (dd, *J* = 15, 5 Hz, 1H), 6.58 (s, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 11.3, 21.2, 63.9, 102.3, 120.4, 125.5, 141.7.

12. Synthesis of tetrazine **22**



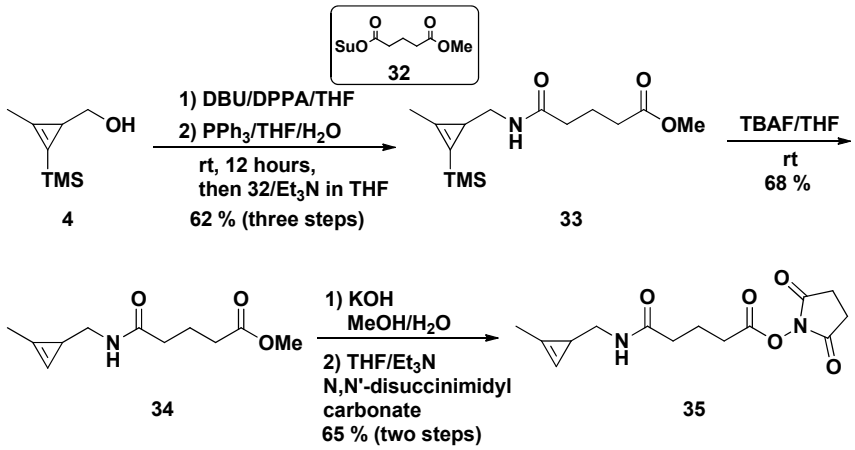
Tetrazine **22** was synthesized by adapting a previously described method (*Angew. Chem. Int. Ed.* **2012**, *51*, 5222–5225). Hydrazine (3.2 g, 100 mmol) was added to a stirring suspension of 3-hydroxy-propionitrile (142 mg, 2 mmol), formamidine acetate (1.01 g, 10 mmol), and ZnI_2 (200 mg, 0.6 mmol) in 1,4-dioxane (3 mL) at room temperature. The reaction was stirred at room temperature for 48 h. Sodium nitrite (20.0 mmol, 1.4 g) in 20 mL of water was slowly added to the solution and followed by slow addition of 1 M HCl during which the solution turned bright red in color and gas evolved. Addition of 1 M HCl continued until gas evolution ceased and the pH value was 3. (**Caution**! This step generates a large amount of toxic nitrogen oxide gasses and should be performed in a well ventilated fume hood). The mixture was extracted with EtOAc and the organic phase dried over sodium sulfate. The EtOAc was removed using rotary evaporation and the residue was purified by preparative TLC (CH_2Cl_2:MeOH = 30:1) to give 41 mg compound **22** as red oil, the yield is 16 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61-3.64 (m, 2H), 4.29 (bs, 2H), 10.26 (s, 1H); ^{13}C (125 MHz, $CDCl_3$) δ 38.2, 60.1, 158.5, 171.6.

13. Synthesis of tetrazine **26**



Tetrazine **26** was synthesized by adapting a previously described method (*Angew. Chem. Int. Ed.* **2012**, *51*, 5222–5225). Hydrazine (1.2 g, 25 mmol) was added to a stirring suspension of trimethylacetonitrile (100 mg, 1.5 mmol), 4-cyanobenzyl alcohol (67 mg, 0.5 mmol), and $Zn(OTf)_2$ (200 mg, 0.6 mmol) at room temperature. The reaction was heated to 80 °C and stirred for 36 h. Sodium nitrite (4 mmol, 280 mg) in 10 mL of water was slowly added to the solution and followed by slow addition of 1 M HCl during which the solution turned bright red in color and gas evolved. Addition of 1 M HCl continued until gas evolution ceased and the pH value is 3. (**Caution**! This step generates a large amount of toxic nitrogen oxide gasses and should be performed in a well ventilated fume hood). The mixture was extracted with EtOAc and the organic phase dried over sodium sulfate. The EtOAc was removed using rotary evaporation and the residue was purified by preparative TLC (hexane/EtOAc = 1:1) to give 35 mg compound **26** as purple solid, the yield is 25 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.62 (s, 9H), 4.03 (s, 2H), 7.58 (d, J = 10 Hz, 2H), 8.58 (d, J = 10 Hz, 2H); ^{13}C (125 MHz, $CDCl_3$) δ 29.3, 38.0, 64.8, 127.5, 128.2, 131.1, 145.7, 163.5, 175.6. HRMS $[M+H]^+$ m/z calcd. for $[C_{13}H_{17}N_4O]^+$ 245.1397, found 245.1395.

14. Synthesis of methylcyclopropene amide tag **35**



Under the protection of N_2, to a stirred solution of cyclopropene alcohol **4** (90 mg, 0.58 mmol) in dry THF (3.0 mL) at 0 °C was added DBU (131 mg, 0.87 mmol) followed by DPPA (diphenylphosphoryl azide, 263 mg, 0.87 mmol). The reaction solution was slowly warmed to room temperature and stirred overnight. After TLC indicated that the reaction had completed, most of the THF was evaporated by flushing compressed air and the material was passed through a short silica column using hexanes. The product was collected and the hexanes were evaporated by rotary evaporation at 100 torr, room temperature, affording crude cyclopropene azide **5**. The crude cyclopropene azide **5** was dissolved in 4.0 mL THF and 0.6 mL H_2O, PPh_3 (227 mg, 0.87 mmol) was added to the solution and stirred at room temperature overnight. After TLC indicated that the reaction was finished, compound **32** (140 mg, 0.58 mmol) and Et_3N (60 mg, 0.58 mmol) in 2 mL THF was added and stirred at room temperature for 20 minutes. The THF was evaporated and 10 mL water was added, the aqueous solution was extracted with EtOAc (10 mL ×3). The organic fractions were combined and dried over Na_2SO_4, evaporated to afford crude product. The crude product was purified by silica column (Hexane:Acetone = 3:1) to afford 120 mg product **33** as yellow oil in 62 % yield over three steps.^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (t, J = 10 Hz, 1H), 1.91-1.97 (m, 2H), 2.15 (s, 3H), 2.16-2.19 (m, 2H), 2.36 (t, J = 8 Hz, 2H), 3.03-3.08 (m, 1H), 3.14-3.19 (m, 1H), 3.64 (s, 3H), 5.41

(bs, 1H); ^{13}C (125 MHz, $CDCl_3$) δ -0.9, 13.4, 19.4, 21.2, 33.3, 35.8, 46.3, 51.8, 111.8, 136.1, 171.9, 173.9. HRMS $[M+H]^+$ m/z calcd. for $[C_{14}H_{26}NO_3Si]^+$ 284.1674, found 284.1675.

TBAF (0.35 mL, 1.0 M in THF) was added to a stirred solution of compound **33** (100.0 mg, 0.35 mmol) in dry THF (2.0 mL) at room temperature. The reaction solution was stirred at room temperature for 30 minutes until the TLC indicated that the starting material was consumed. The solvent was evaporated and the residue was purified by silica column (hexane/acetone = 2/1) to afford 50 mg compound **34** as colorless oil, in 68 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49-1.51 (m, 1H), 1.89 (m, 2H), 2.08 (s, 3H), 2.17 (t, J = 8 Hz, 2H), 2.34 (t, J = 8 Hz, 2H), 3.07-3.13 (m, 1H), 3.18 (m, 1H), 3.63 (s, 3H), 5.62 (bs, 1H), 6.54 (s, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 11.8, 18.1, 21.2, 33.3, 35.8, 45.3, 51.8, 103.0, 121.7, 172.2, 174.0. HRMS $[M+H]^+$ m/z calcd. for $[C_{11}H_{18}NO_3]^+$ 212.1281, found 212.1280.

KOH (18 mg, 0.32 mmol) was added to a stirred solution of **34** (45 mg, 0.21 mmol) in 2.0 mL MeOH/H_2O (4/1) and the resulting solution was stirred overnight. The pH was adjusted to neutral by adding 0.32 mL 1 M HCl and the solution was evaporated to afford carboxylic acid intermediate. The crude intermediate was dissolved in THF (5 mL) and followed by addition of Et_3N (32 mg, 0.32 mmol) and N, N'-disuccinimidyl carbonate (54 mg, 0.21 mmol). The solution was stirred at room temperature for 5 hours and worked up by addition of brine (20 mL) and extracted by EtOAc (20 mL × 3). The combined organic layer was washed by brine (60 mL) and dried over Na_2SO_4, evaporated in vacuum to afford crude product. The crude product was purified by silica column (EtOAc) to afford 40 mg compound **35** as colorless oil, yield is 65 % for two steps. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.49-1.51 (m, 1H), 2.06-2.08 (m, 2H), 2.08 (s, 3H), 2.25 (t, J = 7.5 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 2.82 (bs, 4H), 3.09-3.22 (m, 4H), 5.86 (bs, 1H), 6.54 (s, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 11.7, 17.9, 21.0, 25.7, 30.0, 34.7, 45.3, 102.9, 121.6, 168.5, 169.4, 171.5. HRMS $[M+H]^+$ m/z calcd. for $[C_{14}H_{19}N_2O_5]^+$ 295.1292, found 295.1291.

15. Tetrazine stability

Tetrazine compounds **24**, **25**, and **26** at 1 mM concentration were incubated at room temperature in 50% DMF, 50% PBS pH 7.4 with and without 1 mM cysteine. Samples with 1 mM cysteine were incubated for 3 hours (Figure S1a), samples in DMF/PBS were left at room temperature for 48 hours (Figure S1b). The decomposition of tetrazine was determined by monitoring the disappearance of the characteristic tetrazine absorption maximum between 527-529 nm.

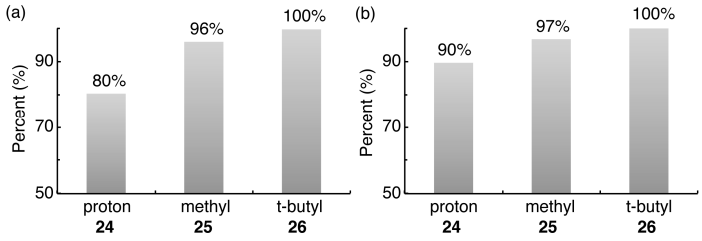
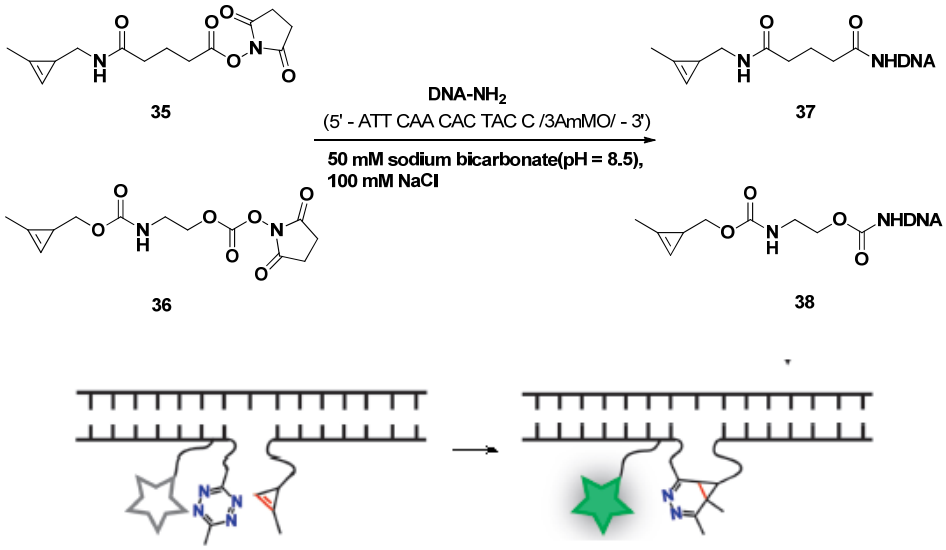


Figure S1. Tetrazine series stability over time. Tetrazines **24** (proton), **25** (methyl), and **26** (t-butyl) stability in 50% DMF, 50% PBS pH 7.4. (a) Tetrazine samples at 1 mM were incubated with 1 mM cysteine for 3 hours at room temperature. (b) Tetrazine samples at 1 mM were incubated at room temperature for 48 hours.

16. Modified oligonucleotide synthesis, characterization, and stability



Amine-modified 13-nucleotide DNA sequence was purchased from Integrated DNA Technologies, Inc, as 5′ - ATT CAA CAC TAC C /3AmMO/ - 3′. Cyclopropene carbamate NHS ester (N-hydroxysuccinimide) **36** was reacted in excess with the terminal amine of the oligonucleotide in order to afford the conjugated cyclopropene probe **38**, as described in reference (*Nucleic Acids Res.* **2013***, 41,* e148). Cyclopropene **35** was conjugated in a similar manner. Resulting DNA probes were purified by HPLC, lyophilized, and resuspended in ddi H_2O for storage. Probe molecular weights were confirmed by ionization spray mass spectrometry. For cyclopropene **37** calculated MW was 4252.9, observed m/2 1419.3, m/3 1068.5 (Figure S2a). For cyclopropene carbamate **38** calculated MW was 4270.9, observed m/2 1422.4, m/3 1067.0. Cyclopropene carbamate **38** was further confirmed by MALDI MS, shown in reference (*Nucleic Acids Res.* **2013***, 41,* e148).

Each DNA cyclopropene probe at 1 μM in 50 mM MOPS pH 7.5, 250 mM NaCl was incubated with 1 μM DNA template that contains a fully complementary region for cyclopropene probe hybridization, as well as a neighboring region that allows for a tetrazine-**25**-modified 13 nucleotide fluoresce in DNA probe as described in reference (*Nucleic Acids Res.* **2013***, 41,* e148). Resultant tetrazine-cyclopropene click reaction was studied as a function of the unquenching of the fluorescein emission (Figure 3). The product of the reaction between cyclopropene carbamate **38** and tetrazine **25** modified DNA probes was confirmed by MALDI MS, shown in reference (*Nucleic Acids Res.* **2013***, 41,* e148). The corresponding product, obtained using cyclopropene amide **37** DNA probe was confirmed by the ionization spray MS, shown in Figure S2b.

DNA probe stability was tested after multiple freeze/thaw cycles (5-7 times frozen at -80°C, defrosted to room temperature for experiments, and frozen again). HPLC traces of freshly purified samples are compared with the final traces in Figure 3.

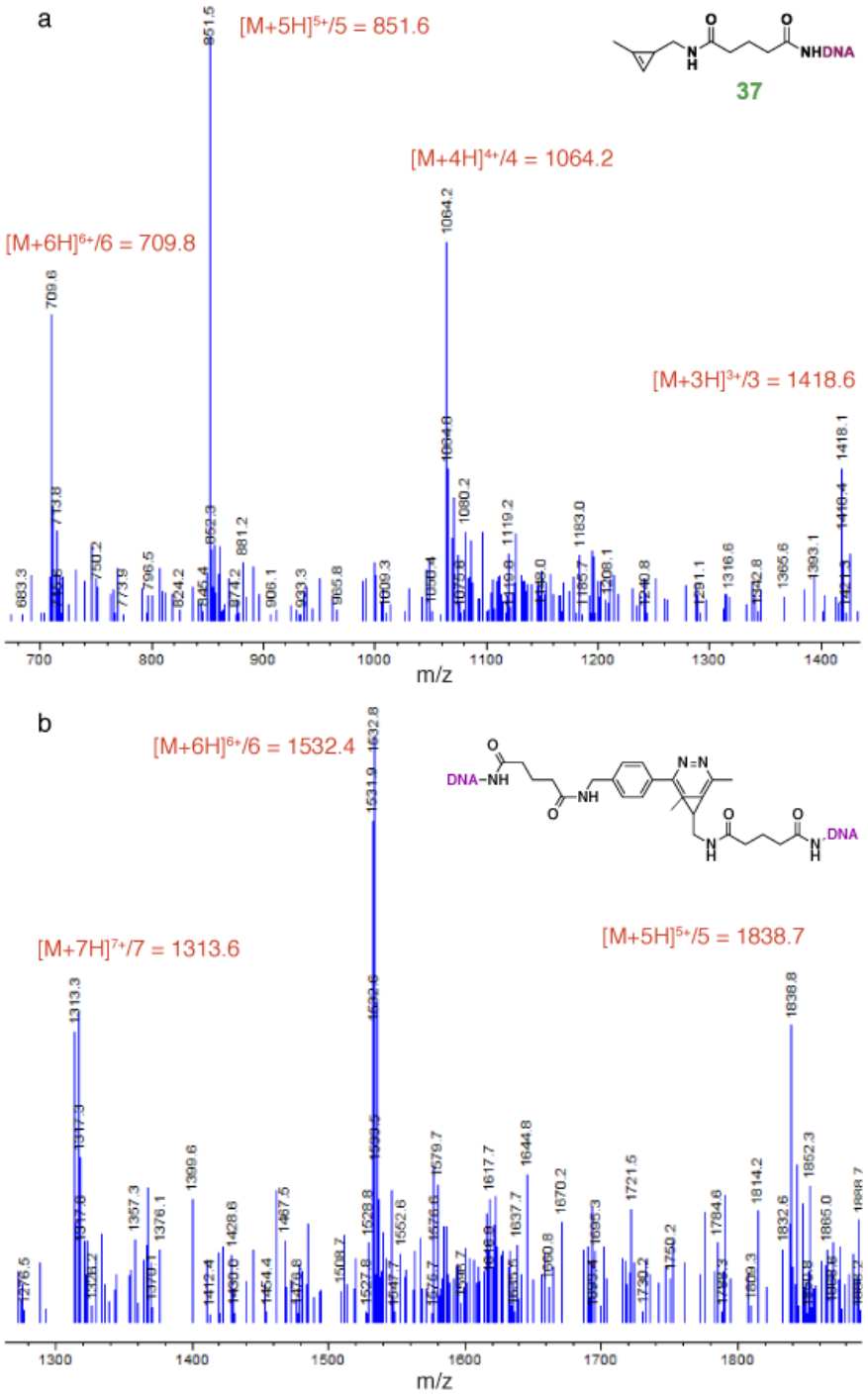
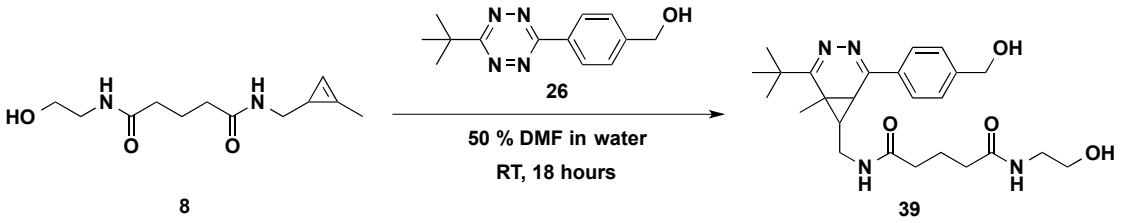


Figure S2. Molecular weight confirmation of the cyclopropene amide **37** DNA probe reaction by ionization spray MS. (a) Reactant cyclopropene amide **37** modified 13 nt DNA probe with a calculated MS of 4252.9, with the corresponding peaks indicated. (b) Product of the templated click reaction of a 13 nt fluorescently-labeled tetrazine **25** and a 13 nt cyclopropene **37** modified DNA probes along a matching template strand. Calculated product MS is 9188.5, with the corresponding peaks indicated.

17. Characterization of the reaction between tetrazine **26** and methylcyclopropene amide **8**



Tetrazine **26** (100 mM in dry DMF, 5 µL) and methylcyclopropene amide **8** (250 mM in DMF, 2.2 µL) were combined in 250 µL of H_2O and 243 µL DMF at a final concentration of 1 mM for tetrazine **26** and 1.1 mM for cyclopropene **8**. The reaction solution was agitated for 18 hours at room temperature then analyzed by LC-MS. Only one isomer of product **39** is depicted. The LC-MS trace of **26** and **39** are shown in Figure 4. The mass spectrum of **39** is included on page 58.

Computational Section

1. Computational details and references

All calculations were performed with the Gaussian 09.[1] The geometry optimization of all the minima and transition states involved was carried out at the M06-2X level of theory[2] with the 6-31G(d) basis set.[3] The vibrational frequencies were computed at the same level to check whether each optimized structure is an energy minimum or a transition state and to evaluate its zero-point vibrational energy (ZPVE) and thermal corrections at 298 K. A quasiharmonic correction was applied during the entropy calculation by setting all positive frequencies that are less than 100 cm^{-1} to 100 cm^{-1}.[4] Solvent effects in water were computed at the M06-2X/6-311+G(d,p) level using the gas-phase optimized structures at the M06-2X/6-31G(d) level. Solvation energies were evaluated by a self-consistent reaction field (SCRF) using the CPCM model,[5] where UFF radii were used. Fragment distortion energies were computed at the M06-2X/6-311+G(d,p) level using the M06-2X/6-31G(d) geometries. The frontier molecular orbitals and their energies were computed at the HF/6-311+G(d,p) level using the M06-2X/6-31G(d) geometries and were shown in Figure S3. DFT calculations indicated that the electron rich substituents, such as methoxy and dimethylamino groups, would greatly decrease the tetrazine reactivity in the inverse-electron-demand Diels-Alder cycloaddition (Figure S4).

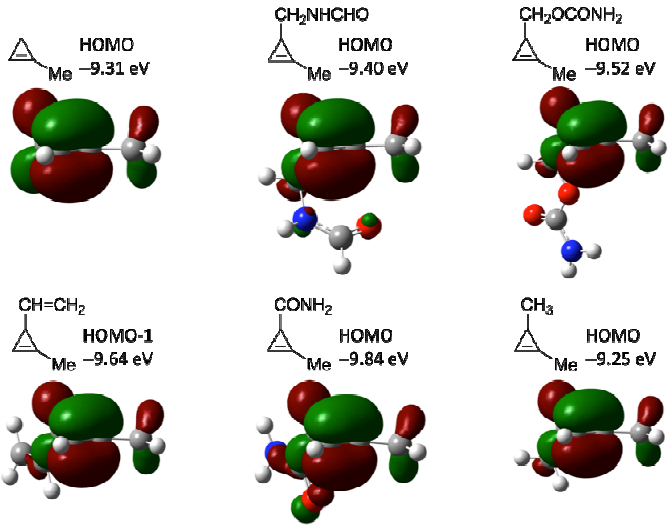


Figure S3. Frontier molecular orbitals and energies of cyclopropenes.

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[1] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, *Gaussian 09*, Revision C.01, Gaussian Inc.: Wallingford CT, **2010**.
[2] (a) Y. Zhao, D. G. Truhlar, *Theor. Chem. Acc.* **2008**, *120*, 215. (b) Y. Zhao, D. G. Truhlar, *Acc. Chem. Res.* **2008**, *41*, 157.
[3] W. J. Hehre, L. Radom, P. v. R. Schleyer, J. A. Pople, *Ab Initio Molecular Orbital Theory*, Wiley: New York, **1986**.
[4] (a) Y. Zhao, D. G. Truhlar, *Phys. Chem. Chem. Phys.* **2008**, *10*, 2813. (b) R. F. Ribeiro, A. V. Marenich, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem. B* **2011**, *115*, 14556.
[5] (a) V. Barone, M. Cossi, *J. Phys. Chem. A* **1998**, *102*, 1995. (b) M. Cossi, N. Rega, G. Scalmani, V. Barone, *J. Comput. Chem.* **2003**, *24*, 669.

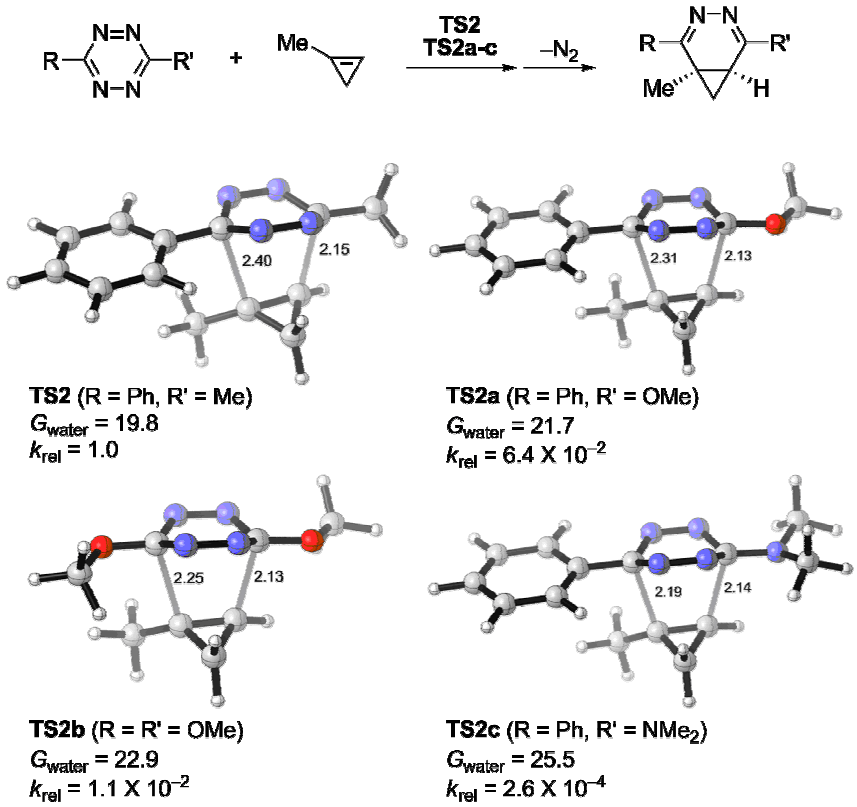
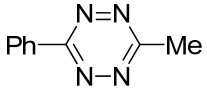


Figure S4. M06-2X/6-31G(d)-optimized transition-state structures for the Diels-Alder reactions of 3-methyl-6-phenyltetrazine and three electron-rich tetrazines with methylcyclopropene (distances in Å) and M06-2X/6-311+G(d,p)//6-31G(d)-computed activation free energies in water (G_{water}, in kcal mol^{-1}) and relative rate constants (k_{rel}).

2. Coordinates and energies of stationary points



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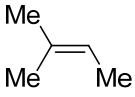
C	2.914514	0.002002	-0.004776
N	2.286373	1.185045	-0.013603
N	0.978371	1.184179	-0.014694
N	2.285782	-1.182613	-0.010781
N	0.979590	-1.182536	-0.011796
C	-1.132554	0.000373	-0.002316
C	-1.832432	-1.210453	-0.000541
C	-1.833907	1.210369	0.001671
C	-3.222108	-1.207236	0.005216
H	-1.278576	-2.142601	-0.003913
C	-3.223528	1.205487	0.007459
H	-1.281016	2.143117	-0.000140
C	-3.919740	-0.001315	0.009189
H	-3.762260	-2.148716	0.006646
H	-3.764837	2.146294	0.010386

H	-5.005486	-0.001963	0.013630
C	4.409585	-0.001802	0.025091
H	4.762236	-0.135256	1.052977
H	4.794207	-0.830688	-0.571667
H	4.791714	0.947790	-0.350841



G_{water} = -196.378261 Hartree

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C	0.731532	-0.675726	-0.000007
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C	2.107239	-0.076288	0.000005
H	2.096599	1.015200	-0.000096
H	2.672697	-0.404630	0.879758
H	2.672878	-0.404869	-0.879548
C	-0.623413	1.452372	0.000003
H	0.319449	2.000814	0.000110
H	-1.198406	1.767176	-0.879510
H	-1.198634	1.767121	0.879384
C	-1.739209	-0.816056	0.000018
H	-2.344365	-0.564342	0.879873
H	-2.344269	-0.564417	-0.879924
H	-1.566694	-1.895371	0.000066

G_{water} = -155.851322 Hartree

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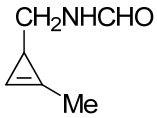
C	0.154946	0.090503	-0.000350
C	-0.920209	0.809908	0.000056
H	-1.471896	-1.176296	0.913313
H	-1.472177	-1.176350	-0.913211
H	-1.404386	1.774021	-0.000113
C	1.625469	-0.067329	0.000149
H	2.132201	0.900741	0.000045
H	1.944834	-0.633462	0.881439
H	1.945561	-0.634037	-0.880502



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C	-1.344857	-1.009359	0.883984
C	-0.770228	-1.154024	-0.490421
H	-1.538762	-1.497708	1.827249
H	-1.310601	-1.741463	-1.242235
C	0.740413	-1.180168	-0.749007
H	1.074921	-2.209770	-0.913010

H	0.977355	-0.605066	-1.648923
C	-1.886296	1.419371	-0.155945
H	-2.574111	1.432637	-1.007944
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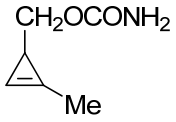
H	2.201986	0.995789	1.405052



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H	-2.131475	-0.535413	2.240679
C	0.062086	-1.177135	-0.308638
H	0.539951	-2.010779	0.217916
H	0.269937	-1.302805	-1.377421
O	0.637711	0.050638	0.139259
C	1.974764	0.141012	-0.018915
O	2.668768	-0.713420	-0.519492
N	2.429227	1.320799	0.498171
H	3.369185	1.583352	0.247576
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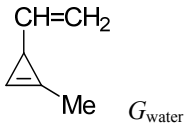

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H	-0.944533	2.358118	-0.472022
C	1.378935	-0.104750	-0.399705
H	0.507593	0.480388	1.496220
C	-2.190483	-0.940884	-0.015496
H	-3.134720	-0.576405	-0.426762
H	-1.828548	-1.767688	-0.635101
H	-2.372008	-1.343254	0.986389
H	1.173834	-0.179645	-1.468931
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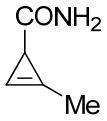
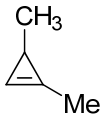


G_{water} = -324.543723 Hartree

C	0.975991	1.202168	0.701333
C	1.386902	0.244054	-0.055696
C	-0.004719	0.744609	-0.338145
H	1.095188	1.869805	1.539035
C	-1.140863	-0.145445	0.088432
O	-1.024220	-0.978451	0.969515
H	-0.197928	1.362323	-1.221699
C	2.328514	-0.812502	-0.475622
H	3.251457	-0.777297	0.106900
H	1.851562	-1.786687	-0.329116
H	2.570763	-0.714204	-1.538148
N	-2.299646	0.019231	-0.618297
H	-3.115370	-0.481838	-0.297555
H	-2.439342	0.823584	-1.209265



G_{water} = -195.131186 Hartree

C	-0.685716	0.206784	0.034439
C	0.003444	1.281046	-0.188019
C	0.743755	0.170246	0.498557
H	0.071276	2.282536	-0.585351
C	-1.930745	-0.588044	-0.047853
H	-2.268156	-0.869273	0.955190
H	-2.729430	-0.032687	-0.545229
H	-1.751504	-1.516576	-0.599893
C	1.793558	-0.656829	-0.223730
H	2.785056	-0.196606	-0.147229
H	1.865357	-1.666757	0.195570
H	1.544061	-0.746137	-1.286163
H	0.937560	0.266279	1.572748

TS1 G_{water} = -762.836149 Hartree

C	-2.320800	-0.950760	-0.191911
N	-1.732108	-0.778216	-1.432929
N	-0.468603	-0.603112	-1.427019
C	0.162458	-0.669554	-0.207803
N	-0.349760	-1.511796	0.752186
N	-1.613940	-1.668975	0.758928
C	-2.121523	0.966562	0.532850
C	-0.756956	1.232346	0.701672
H	-2.650439	0.680228	1.441309
C	-3.803554	-1.204893	-0.203443
H	-4.265638	-0.705322	-1.055584

H	-3.977614	-2.280541	-0.294270
H	-4.269381	-0.865341	0.725198
C	-0.169470	1.099307	2.084413
H	-0.655320	0.303040	2.654313
H	0.907762	0.913040	2.054214
H	-0.322741	2.045400	2.620378
C	1.624471	-0.440024	-0.196102
C	2.392829	-0.822779	0.908946
C	2.250518	0.164726	-1.291964
C	3.764299	-0.589767	0.921280
H	1.905740	-1.317015	1.743165
C	3.621321	0.395008	-1.273873
H	1.653235	0.438135	-2.155622
C	4.381489	0.022047	-0.166953
H	4.352688	-0.891579	1.782267
H	4.098988	0.863839	-2.128656
H	5.451885	0.203370	-0.155313
C	-2.934729	1.757621	-0.469905
H	-2.562092	1.627388	-1.489370
H	-3.986117	1.465245	-0.449800
H	-2.882611	2.826050	-0.227638
C	-0.077184	2.228355	-0.203992
H	-0.314820	2.058457	-1.258002
H	-0.419844	3.239420	0.057317
H	1.008174	2.201702	-0.081364

TS2 G_{water} = -722.320778 Hartree

C	2.546162	-0.711711	0.071931
N	1.960672	-0.768964	1.304528
N	0.674236	-0.723308	1.328436
C	0.034891	-0.663029	0.128375
N	0.613984	-1.211670	-0.983647
N	1.893681	-1.252107	-1.012201
C	2.105366	1.336950	-0.420822
C	0.784248	1.544758	-0.438072
C	1.520072	2.072813	0.747979
H	2.952403	1.415552	-1.087416
H	1.661604	3.152270	0.840635
H	1.454951	1.547928	1.702553
C	4.045656	-0.722359	0.041472
H	4.439120	0.043979	0.712265
H	4.412811	-1.696532	0.377240
H	4.402012	-0.549570	-0.975795
C	-0.331096	1.949452	-1.332996
H	-0.382770	1.300501	-2.211469
H	-1.295148	1.936112	-0.820002
H	-0.138516	2.975185	-1.670081
C	-1.432674	-0.492855	0.139878
C	-2.196912	-0.843092	-0.976824
C	-2.054164	0.067479	1.259754
C	-3.572009	-0.635028	-0.969088
H	-1.703364	-1.279562	-1.838749
C	-3.428709	0.275101	1.260067
H	-1.449615	0.335293	2.120442

C	-4.190536	-0.074816	0.146329
H	-4.162459	-0.912359	-1.836817
H	-3.907055	0.712195	2.131078
H	-5.263754	0.089369	0.148407

TS3 G_{water} = -930.268989 Hartree

C	-1.157638	1.092987	0.003500
N	-0.727152	1.643495	1.171472
N	0.346595	2.352124	1.125959
C	0.962284	2.437031	-0.092092
N	0.196206	2.411343	-1.235545
N	-0.873797	1.709944	-1.185333
C	0.672813	-0.444554	-0.239224
C	1.678960	0.439971	-0.267953
C	1.496890	-0.341492	1.000205
H	2.495440	0.710442	-0.923916
H	1.086803	0.198744	1.856756
C	-2.315374	0.177768	0.060811
C	-2.644268	-0.445007	1.268531
C	-4.108351	-1.011407	-1.033133
C	-3.700637	-1.346844	1.320241
H	-2.065733	-0.212929	2.156864
C	-4.434270	-1.632912	0.170128
H	-4.678913	-1.230766	-1.930240
H	-3.951916	-1.828947	2.259971
H	-5.258013	-2.338938	0.212525
C	2.462118	-1.476234	1.367328
H	2.709252	-1.423173	2.430472
H	2.001167	-2.449892	1.178242
C	0.003234	-1.500720	-1.040823
H	-0.850763	-1.932448	-0.513558
H	-0.333056	-1.101462	-2.001600
H	0.746786	-2.285407	-1.225440
C	3.837165	-2.106324	-0.568380
O	2.986878	-2.815061	-1.070005
C	-3.051478	-0.109460	-1.091972
H	-2.787229	0.380072	-2.023363
C	2.231918	3.234762	-0.144225
H	2.692514	3.141449	-1.129570
H	2.011110	4.290319	0.038222
H	2.923101	2.889355	0.627424
N	3.701373	-1.449418	0.610444
H	4.435381	-0.816776	0.894524
H	4.826275	-1.946955	-1.033904

TS4 G_{water} = -1005.50615 Hartree

C	-1.770820	0.834387	0.067553
N	-1.475889	1.421628	1.259592
N	-0.708358	2.455221	1.229245
C	-0.240878	2.828114	0.000953
N	-1.017374	2.607712	-1.112845
N	-1.784471	1.582376	-1.078185

C	0.443290	0.025141	-0.367314
C	1.105776	1.189579	-0.366623
C	1.266248	0.318095	0.840910
H	1.731688	1.767158	-1.032535
H	0.762689	0.635135	1.758472
C	-2.554372	-0.417947	0.091375
C	-2.576383	-1.201504	1.249364
C	-3.937145	-2.048210	-1.030249
C	-3.275849	-2.402818	1.261882
H	-2.043797	-0.858647	2.130766
C	-3.957049	-2.829348	0.123193
H	-4.470799	-2.374306	-1.917662
H	-3.289458	-3.008188	2.162872
H	-4.502965	-3.767717	0.135640
C	2.548217	-0.438243	1.112290
H	3.234926	0.156035	1.724224
H	2.353581	-1.374676	1.645705
C	0.109227	-1.131046	-1.239520
H	-0.519012	-1.863972	-0.728248
H	-0.397861	-0.798872	-2.149593
H	1.052303	-1.615861	-1.516522
O	3.164908	-0.725552	-0.144846
C	4.327936	-1.414061	-0.056048
O	4.818732	-1.789723	0.982043
N	4.866168	-1.586782	-1.295911
H	5.631180	-2.238448	-1.368169
H	4.299125	-1.414875	-2.111096
C	-3.237235	-0.846842	-1.050156
H	-3.215994	-0.229352	-1.941752
C	0.685669	4.006794	-0.034691
H	1.097089	4.131201	-1.037955
H	0.134869	4.913980	0.230166
H	1.492994	3.869981	0.687306

TS5 G_{water} = -799.674464 Hartree

C	-2.005089	-1.656075	-0.260938
N	-1.533624	-1.179125	-1.452447
N	-0.302189	-0.805242	-1.470773
C	0.409728	-0.962036	-0.320451
N	0.077886	-1.958141	0.557250
N	-1.148591	-2.326749	0.582087
C	-2.011337	0.159244	0.873283
C	-0.785837	0.690507	0.928253
C	-1.755303	1.361124	-0.001660
H	-2.780564	-0.186946	1.550695
H	-1.658818	1.174779	-1.074511
C	-3.450846	-2.052654	-0.222037
H	-4.074833	-1.231195	-0.579617
H	-3.611773	-2.917900	-0.871603
H	-3.736056	-2.324467	0.795987
C	0.283671	1.091342	1.880829
H	0.590643	0.244171	2.500042
H	1.158453	1.495736	1.366846
H	-0.117765	1.872812	2.536996

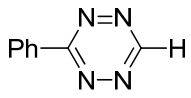
C	1.788922	-0.433876	-0.286773
C	2.709157	-0.917606	0.647495
C	2.159762	0.591295	-1.161666
C	3.990024	-0.379009	0.702283
H	2.409850	-1.715387	1.319003
C	3.440893	1.127500	-1.099686
H	1.435277	0.959869	-1.880990
C	4.358257	0.644745	-0.167459
H	4.703240	-0.760784	1.426213
H	3.724314	1.925744	-1.778673
H	5.357524	1.066598	-0.119073
C	-2.314528	2.691312	0.344381
H	-2.447735	2.899088	1.406898
C	-2.653010	3.608992	-0.558882
H	-2.531189	3.422385	-1.622880
H	-3.061727	4.571472	-0.269987

TS6 G_{water} = -891.008115 Hartree

C	-0.695002	-1.094885	-0.258881
N	-0.026659	-1.152963	-1.444152
N	1.148975	-1.677185	-1.428251
C	1.613606	-2.087931	-0.210831
N	0.721292	-2.552344	0.727920
N	-0.449039	-2.033147	0.707429
C	0.745740	0.499695	0.777821
C	1.898906	-0.163370	0.708610
C	1.712411	0.958288	-0.270714
H	2.692813	-0.499712	1.359815
H	1.531273	0.707438	-1.318574
C	-1.988464	-0.382301	-0.234199
C	-2.909687	-0.611216	0.791530
C	-2.268249	0.567775	-1.220822
C	-4.101348	0.105405	0.824925
H	-2.682923	-1.351666	1.551484
C	-3.459884	1.281769	-1.180745
H	-1.544105	0.736726	-2.011162
C	-4.378389	1.053356	-0.157657
H	-4.816367	-0.076889	1.621176
H	-3.671555	2.020076	-1.947935
H	-5.307519	1.614045	-0.125979
C	-0.194207	1.137355	1.734061
H	-1.046920	1.595298	1.228603
H	-0.556279	0.409205	2.464571
H	0.371924	1.918252	2.254041
C	2.439072	2.244591	0.013210
O	2.718890	2.583274	1.151206
C	3.000133	-2.657644	-0.178845
H	3.299325	-2.856562	0.851784
H	3.022253	-3.598328	-0.736523
H	3.702531	-1.963812	-0.645169
N	2.742711	2.993294	-1.079674
H	3.252871	3.853854	-0.946500
H	2.552771	2.676971	-2.016976

TS7 G_{water} = -761.601449 Hartree

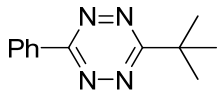
C	-0.170511	-0.866579	-0.231187
N	0.489282	-0.785399	-1.418536
N	1.763011	-0.973149	-1.394571
C	2.320291	-1.194889	-0.167483
N	1.587744	-1.839191	0.801339
N	0.319773	-1.655801	0.772184
C	0.794471	1.123568	0.716399
C	2.086206	0.773933	0.683115
C	1.627366	1.775810	-0.338389
H	2.907472	0.636936	1.373920
H	1.532759	1.406590	-1.362826
C	-1.609878	-0.534692	-0.219804
C	-2.434746	-0.986990	0.813629
C	-2.138859	0.280120	-1.225089
C	-3.777763	-0.626357	0.837801
H	-2.013759	-1.621093	1.586724
C	-3.481551	0.639040	-1.193852
H	-1.487458	0.624494	-2.022144
C	-4.303645	0.187852	-0.162591
H	-4.416017	-0.982596	1.640507
H	-3.887834	1.273899	-1.975209
H	-5.351624	0.470881	-0.139356
C	1.991998	3.244308	-0.233858
H	2.943030	3.444334	-0.737562
H	1.227320	3.871785	-0.702904
H	2.091097	3.553927	0.811804
C	-0.296498	1.487850	1.658894
H	-1.243164	1.666958	1.144042
H	-0.440125	0.707237	2.410899
H	-0.007895	2.412788	2.173021
C	3.808949	-1.372483	-0.129130
H	4.080779	-2.300626	-0.640354
H	4.299407	-0.541302	-0.639473
H	4.152348	-1.432382	0.905259



G_{water} = -527.213293 Hartree

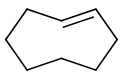
C	0.795954	-0.000001	-0.000312
C	3.344786	0.000000	0.000332
N	2.735472	-1.186514	0.000292
N	1.426931	-1.186701	-0.000008
N	2.735472	1.186514	-0.000108
N	1.426931	1.186700	-0.000451
C	-0.681194	-0.000001	-0.000145
C	-1.381274	1.210967	0.000099
C	-1.381274	-1.210966	-0.000212
C	-2.770752	1.206739	0.000257
H	-0.828010	2.143429	0.000139
C	-2.770754	-1.206738	-0.000063
H	-0.828012	-2.143429	-0.000401

C	-3.467186	0.000000	0.000177
H	-3.311732	2.147647	0.000449
H	-3.311730	-2.147649	-0.000139
H	-4.552928	0.000003	0.000302
H	4.428939	0.000000	0.000779



G_{water} = -684.332497 Hartree

C	0.710003	0.020206	0.000062
C	-1.864249	0.037408	-0.000053
N	-1.226883	1.210762	0.000391
N	0.085538	1.202508	0.000532
N	-1.234581	-1.151974	-0.000099
N	0.066927	-1.163009	-0.000107
C	2.188326	0.006553	0.000018
C	2.878443	-1.209729	0.000217
C	2.899456	1.210908	-0.000246
C	4.268223	-1.217679	0.000145
H	2.317422	-2.137528	0.000406
C	4.288922	1.194914	-0.000315
H	2.353428	2.147758	-0.000385
C	4.975517	-0.017485	-0.000120
H	4.800774	-2.163503	0.000310
H	4.837839	2.131309	-0.000537
H	6.061236	-0.026840	-0.000180
C	-3.379904	0.004828	-0.000105
C	-3.843689	-0.753787	-1.254410
H	-3.425984	-1.764041	-1.274616
H	-4.936169	-0.826338	-1.256182
H	-3.535294	-0.229924	-2.165345
C	-3.843721	-0.751825	1.255337
H	-3.535524	-0.226427	2.165457
H	-4.936190	-0.824555	1.257119
H	-3.425849	-1.761977	1.277251
C	-3.957663	1.420698	-0.001186
H	-3.640204	1.983006	0.881168
H	-3.639784	1.981799	-0.884155
H	-5.050681	1.359178	-0.001392



G_{water} = -312.998444 Hartree

C	-0.413966	-0.522065	-1.358397
H	-1.490184	-0.336794	-1.334889
C	0.413966	0.522065	-1.358397
H	1.490184	0.336794	-1.334889
C	0.034981	-1.871510	-0.901221
H	-0.510280	-2.704358	-1.358222
H	1.102540	-2.008137	-1.112700
C	-0.034981	1.871510	-0.901221
H	-1.102540	2.008137	-1.112700

H	0.510280	2.704358	-1.358222
C	0.183001	1.877834	0.635533
H	1.260794	1.924503	0.841500
H	-0.248046	2.798155	1.048278
C	-0.413966	0.660993	1.376189
H	-0.560669	0.965017	2.418884
H	-1.420918	0.465721	0.984525
C	-0.183001	-1.877834	0.635533
H	0.248046	-2.798155	1.048278
H	-1.260794	-1.924503	0.841500
C	0.413966	-0.660993	1.376189
H	0.560669	-0.965017	2.418884
H	1.420918	-0.465721	0.984525

TS8 G_{water} = -890.982386 Hartree

C	-1.193301	1.157782	-0.010780
N	-0.828642	1.775517	1.145385
N	0.144777	2.619915	1.077533
C	0.713370	2.756650	-0.150999
N	-0.038098	2.620096	-1.290524
N	-1.006042	1.784272	-1.214283
C	0.856982	-0.117239	-0.278408
C	1.737657	0.892764	-0.298733
C	1.645486	0.095016	0.968680
H	2.512854	1.271302	-0.950622
H	1.155515	0.578016	1.817359
C	-2.204588	0.086170	0.070210
C	-2.401739	-0.586889	1.279538
C	-3.846393	-1.335419	-0.982432
C	-3.316552	-1.630727	1.352376
H	-1.832159	-0.283424	2.152139
C	-4.040102	-2.007700	0.222111
H	-4.410993	-1.623993	-1.863528
H	-3.465355	-2.151800	2.292988
H	-4.753268	-2.824214	0.281025
C	2.746532	-0.898329	1.361479
H	3.008499	-0.758707	2.413344
H	2.396942	-1.927116	1.235671
C	0.321860	-1.239694	-1.090284
H	-0.469086	-1.780781	-0.566028
H	-0.064678	-0.874220	-2.045581
H	1.154742	-1.926440	-1.282601
C	4.159585	-1.490815	-0.560092
O	3.392551	-2.326766	-0.996101
C	-2.929930	-0.292520	-1.063003
H	-2.768292	0.236811	-1.996011
N	3.957065	-0.769869	0.570489
H	4.624409	-0.050512	0.808707
H	5.119368	-1.251900	-1.052359
H	1.554469	3.437312	-0.213873

TS9 G_{water} = -1048.100333 Hartree

C	1.177712	-1.121575	0.032165
N	0.571238	-1.425089	1.212138
N	-0.693505	-1.657583	1.176785
C	-1.309493	-1.538733	-0.039417
N	-0.593100	-1.839162	-1.174409
N	0.666495	-1.607362	-1.139229
C	0.118438	1.012854	-0.250426
C	-1.156565	0.608395	-0.254820
C	-0.662748	1.268207	0.996883
H	-2.018124	0.681575	-0.902281
H	-0.492136	0.621687	1.861027
C	2.602761	-0.735454	0.068255
C	3.156394	-0.245595	1.254808
C	4.714125	-0.392910	-1.050176
C	4.482807	0.168928	1.283798
H	2.537332	-0.195399	2.144799
C	5.264388	0.097637	0.131840
H	5.320215	-0.451032	-1.948993
H	4.907661	0.550317	2.207174
H	6.299523	0.424338	0.155712
C	-1.099014	2.696189	1.346576
H	-1.287150	2.774720	2.420055
H	-0.314054	3.413744	1.090304
C	1.139986	1.697450	-1.083323
H	2.100548	1.778748	-0.569443
H	1.282432	1.169319	-2.030028
H	0.756850	2.702324	-1.297194
C	-2.241418	3.712028	-0.574317
O	-1.214842	3.985324	-1.164750
C	3.387299	-0.807752	-1.086155
H	2.947318	-1.190296	-2.001045
C	-2.801741	-1.858236	-0.054216
N	-2.298563	3.120082	0.644416
H	-3.202092	2.862299	1.014500
H	-3.241231	3.941897	-0.984615
C	-2.922970	-3.357446	0.280908
H	-2.493606	-3.566050	1.264769
H	-3.977239	-3.654365	0.285556
H	-2.396401	-3.960269	-0.466046
C	-3.534038	-1.043798	1.017840
H	-3.517405	0.027013	0.787505
H	-4.580048	-1.363731	1.068165
H	-3.076025	-1.191911	1.999521
C	-3.421362	-1.614353	-1.433457
H	-4.467876	-1.935879	-1.415673
H	-3.410396	-0.556534	-1.716726
H	-2.894684	-2.176330	-2.207545

TS10 G_{water} = -840.187486 Hartree

C	-1.012127	1.217003	-0.071701
C	1.047417	2.567439	-0.371057
C	1.880567	0.575300	-0.219291
H	2.301894	0.644720	-1.224137
C	0.787513	-0.247208	-0.067038

H	0.499175	-0.515263	0.950951
N	0.469568	2.641470	0.863788
N	-0.589427	1.933004	1.017422
N	0.277100	2.409408	-1.487025
N	-0.784494	1.704997	-1.329095
C	0.441437	-1.242532	-1.131181
H	-0.624575	-1.491756	-1.144768
H	0.707005	-0.834113	-2.114236
C	2.864215	0.727700	0.908849
H	3.406566	1.679197	0.869204
H	2.327453	0.698150	1.864578
C	1.260192	-2.525979	-0.858926
H	1.094223	-3.215599	-1.694761
H	0.853951	-3.021074	0.032823
C	3.870637	-0.440944	0.833545
H	4.513833	-0.391042	1.719825
H	4.529660	-0.289653	-0.031835
C	3.242632	-1.844236	0.740987
H	3.993536	-2.555743	1.100807
H	2.411428	-1.915586	1.455529
C	2.773122	-2.313278	-0.667130
H	3.263218	-3.268048	-0.885587
H	3.139397	-1.617001	-1.433589
C	-2.143947	0.285067	0.121294
C	-2.909736	-0.135884	-0.970243
C	-2.436058	-0.196976	1.400312
C	-3.958825	-1.028911	-0.779003
H	-2.677083	0.249475	-1.957508
C	-3.484752	-1.090684	1.584596
H	-1.843294	0.144976	2.243113
C	-4.248079	-1.508801	0.496289
H	-4.553973	-1.347923	-1.629055
H	-3.708217	-1.460641	2.580480
H	-5.066011	-2.207653	0.642263
H	1.977325	3.109964	-0.501540

TS11 G_{water} = -879.472359 Hartree

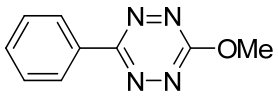
C	0.944301	-1.151631	-0.016487
C	-1.287102	-2.265699	-0.235917
C	-1.782092	-0.143807	-0.105920
H	-2.252106	-0.196518	-1.089916
C	-0.568227	0.509800	-0.042213
H	-0.213515	0.809895	0.945544
N	-0.670776	-2.371051	0.985434
N	0.466230	-1.792312	1.098238
N	-0.511144	-2.216933	-1.369759
N	0.627284	-1.644617	-1.256858
C	-0.131536	1.379511	-1.183558
H	0.957454	1.469618	-1.249382
H	-0.492116	0.950419	-2.126644
C	-2.725815	-0.059240	1.062191
H	-3.411093	-0.911008	1.116533
H	-2.146704	-0.043087	1.993136
C	-0.741975	2.784785	-0.983127

H	-0.506905	3.381451	-1.872200
H	-0.234243	3.275423	-0.142342
C	-3.552626	1.238371	0.933357
H	-4.156450	1.342565	1.842577
H	-4.265203	1.124828	0.105229
C	-2.740042	2.527494	0.714983
H	-3.372119	3.362339	1.035849
H	-1.881042	2.534724	1.399326
C	-2.261171	2.818240	-0.736045
H	-2.610405	3.818669	-1.013145
H	-2.757018	2.133447	-1.437153
C	2.216289	-0.406141	0.128625
C	3.004607	-0.120579	-0.990266
C	2.622084	0.034717	1.391515
C	4.186250	0.598553	-0.843138
H	2.683594	-0.473465	-1.964726
C	3.804099	0.752902	1.532302
H	2.011465	-0.204943	2.256378
C	4.588207	1.037531	0.416227
H	4.795724	0.815400	-1.715019
H	4.115761	1.089669	2.516182
H	5.511236	1.598306	0.528015
C	-2.624774	-2.930997	-0.383966
H	-2.487887	-3.908545	-0.854482
H	-3.081058	-3.074257	0.596134
H	-3.291379	-2.343400	-1.020659

TS12 G_{water} = -997.297872 Hartree

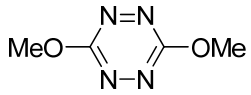
C	-1.610947	-1.255314	-0.042317
C	0.900778	-1.130544	0.063174
C	0.287539	0.938639	-0.046628
H	0.794974	1.133345	0.899747
C	-1.098144	0.912277	-0.015921
H	-1.600322	1.039541	-0.974433
N	0.355232	-1.499269	-1.147237
N	-0.916881	-1.548658	-1.200347
N	0.250098	-1.487861	1.220478
N	-1.025952	-1.555566	1.162712
C	-1.802388	1.501261	1.175987
H	-2.805583	1.095784	1.316857
H	-1.227773	1.278444	2.083555
C	0.986120	1.458851	-1.272725
H	1.981439	1.022427	-1.402503
H	0.392611	1.206325	-2.159916
C	-1.927053	3.028719	0.986134
H	-2.351091	3.443447	1.908250
H	-2.664591	3.225979	0.196004
C	1.123513	2.990652	-1.154668
H	1.540437	3.363144	-2.097625
H	1.866135	3.221394	-0.379315
C	-0.177315	3.749090	-0.841651
H	-0.035973	4.783999	-1.171446
H	-0.987500	3.352812	-1.469083
C	-0.626290	3.775491	0.646090

H	-0.772606	4.821231	0.937135
H	0.184070	3.406533	1.288961
C	2.379274	-1.030797	0.147392
C	2.979385	-0.711184	1.368123
C	3.176482	-1.242098	-0.980847
C	4.362196	-0.602738	1.458891
H	2.352535	-0.565207	2.242653
C	4.560374	-1.133845	-0.884045
H	2.701164	-1.498488	-1.921922
C	5.155983	-0.813135	0.333175
H	4.822152	-0.357101	2.411168
H	5.174860	-1.305352	-1.762594
H	6.236087	-0.729052	0.405596
C	-3.127089	-1.464647	-0.141822
C	-3.790396	-1.482786	1.240966
H	-4.874280	-1.572241	1.111088
H	-3.438242	-2.334197	1.827615
H	-3.590417	-0.584003	1.826621
C	-3.780842	-0.418709	-1.052910
H	-3.253626	-0.357448	-2.011026
H	-4.816707	-0.709848	-1.255594
H	-3.800734	0.576553	-0.596763
C	-3.345661	-2.855439	-0.774731
H	-2.830670	-3.627484	-0.193396
H	-4.416349	-3.086654	-0.779236
H	-2.970603	-2.886854	-1.799641



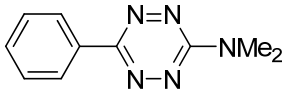
G_{water} = -641.707742 Hartree

C	-0.046608	0.097476	-0.000015
C	2.499247	0.288006	0.000521
N	1.978456	-0.935789	0.000420
N	0.667509	-1.028509	0.000217
N	1.797924	1.437922	0.000050
N	0.505909	1.330904	-0.000120
O	3.814594	0.447260	0.000021
C	4.606811	-0.740448	-0.000093
H	4.399766	-1.340293	-0.889417
H	5.638439	-0.393529	-0.000504
H	4.400372	-1.340019	0.889554
C	-1.522131	0.000243	-0.000086
C	-2.303451	1.160149	-0.000415
C	-2.140965	-1.253793	0.000182
C	-3.690025	1.062286	-0.000477
H	-1.815904	2.128761	-0.000622
C	-3.527405	-1.343629	0.000113
H	-1.527605	-2.147923	0.000430
C	-4.305045	-0.187308	-0.000215
H	-4.291970	1.965496	-0.000731
H	-4.002705	-2.319593	0.000324
H	-5.388291	-0.260561	-0.000263



G_{water} = -525.252980 Hartree

C	-1.226238	-0.316848	-0.000071
C	1.226239	0.316851	0.000098
N	0.923084	-0.985302	0.000549
N	-0.341297	-1.318279	0.000390
N	0.341299	1.318281	0.000359
N	-0.923084	0.985303	0.000227
O	2.501393	0.694018	-0.000051
O	-2.501392	-0.694020	-0.000305
C	-3.478384	0.344442	-0.000300
H	-3.377296	0.972036	-0.889325
H	-4.438373	-0.169142	-0.000949
H	-3.378137	0.971362	0.889291
C	3.478382	-0.344443	-0.000548
H	3.376445	-0.972588	-0.889078
H	4.438372	0.169135	-0.002378
H	3.378982	-0.970815	0.889534



G_{water} = -661.106338 Hartree

C	-0.403972	-0.000350	-0.000005
C	2.166323	-0.000250	0.000049
N	1.534110	-1.197238	0.000111
N	0.233121	-1.181317	0.000043
N	1.533768	1.197142	0.000127
N	0.233259	1.181155	0.000104
C	4.219351	-1.272088	0.000071
H	3.962579	-1.864161	-0.883357
H	5.290367	-1.070395	-0.000930
H	3.964050	-1.863294	0.884536
C	-1.883254	-0.000097	-0.000099
C	-2.586534	1.208676	-0.000321
C	-2.586774	-1.208722	0.000044
C	-3.976515	1.205033	-0.000394
H	-2.034024	2.141724	-0.000424
C	-3.976777	-1.204824	-0.000033
H	-2.034429	-2.141869	0.000214
C	-4.675573	0.000174	-0.000250
H	-4.515998	2.147188	-0.000566
H	-4.516420	-2.146882	0.000085
H	-5.761325	0.000317	-0.000306
N	3.513885	0.000097	-0.000068
C	4.219066	1.272384	0.000422
H	3.963098	1.864306	-0.883352
H	3.962950	1.863754	0.884526
H	5.290102	1.070818	0.000453

TS2a G_{water} = -797.524453 Hartree

C	-0.205200	-0.504610	-0.307717
N	0.354771	-0.269896	-1.537768
N	1.632365	-0.258120	-1.612351
C	2.288666	-0.437625	-0.423176
N	1.750583	-1.254181	0.536945
N	0.470101	-1.269255	0.601755
C	0.426061	1.471241	0.699011
C	1.752372	1.403877	0.512007
C	0.990714	2.335242	-0.380667
H	2.661044	1.428714	1.096489
H	0.851600	2.056402	-1.425708
C	-0.595242	1.570689	1.776405
H	-0.533745	0.715254	2.454814
H	-0.391795	2.482937	2.349635
H	-1.608569	1.637765	1.375379
C	4.368374	-0.854370	0.581920
H	4.242575	-1.935917	0.668635
H	5.405923	-0.603239	0.366050
H	4.055872	-0.390980	1.524368
O	3.621215	-0.343122	-0.515429
H	1.048450	3.412727	-0.210809
C	-1.681217	-0.454353	-0.220248
C	-2.357870	-1.135632	0.795072
C	-2.398966	0.322488	-1.133725
C	-3.742239	-1.042059	0.890143
H	-1.790169	-1.735874	1.498171
C	-3.782424	0.414847	-1.031056
H	-1.862816	0.846481	-1.918571
C	-4.457186	-0.266375	-0.019542
H	-4.264212	-1.576899	1.677587
H	-4.336128	1.019904	-1.742316
H	-5.537552	-0.192285	0.059092

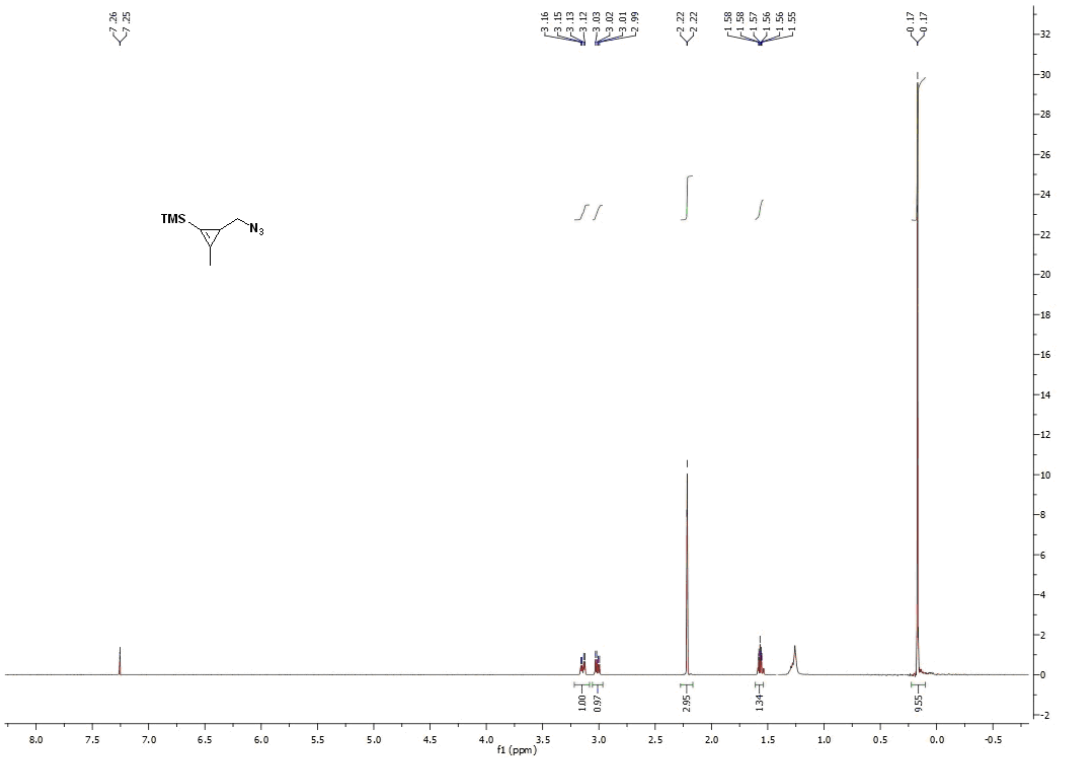
TS2b G_{water} = -681.067857 Hartree

C	0.966651	-0.793633	-0.292842
N	0.561228	-1.167747	0.951613
N	-0.683008	-1.006073	1.226376
C	-1.431662	-0.448762	0.228847
N	-1.126028	-0.710944	-1.083532
N	0.113255	-0.883333	-1.356412
C	0.709869	1.433306	-0.048472
C	-0.595754	1.508536	0.248408
C	0.358656	1.729636	1.378165
H	-1.502244	1.962688	-0.125716
H	0.459901	0.950210	2.133718
C	1.701571	1.882690	-1.063769
H	1.389839	1.584933	-2.067749
H	1.763687	2.976204	-1.020552
H	2.697279	1.477362	-0.874633
C	-3.613709	-0.003290	-0.516292
H	-3.700523	-0.888169	-1.150959
H	-4.569493	0.229535	-0.048494

H	-3.282745	0.837746	-1.135941
C	3.167999	-0.911346	0.486993
H	3.074670	-1.832292	1.066204
H	4.161290	-0.835241	0.045537
H	2.976126	-0.055133	1.143656
O	-2.714690	-0.235909	0.560446
O	2.262377	-0.908675	-0.612349
H	0.510866	2.742687	1.757616

TS2c G_{water} = -816.916982 Hartree

C	-0.442523	-0.395387	-0.275026
N	0.205650	0.016563	-1.414406
N	1.483135	0.024723	-1.387417
C	2.073183	-0.352712	-0.196910
N	1.442291	-1.331603	0.559737
N	0.169453	-1.338140	0.520227
C	0.036154	1.282635	1.052503
C	1.380541	1.297323	0.972445
C	0.646474	2.357085	0.211986
H	2.236753	1.243775	1.628277
H	0.590479	2.280307	-0.874999
C	-1.036178	1.195879	2.084337
H	-0.993075	0.237823	2.610022
H	-0.871917	1.998134	2.812887
H	-2.031247	1.320926	1.652822
N	3.442007	-0.275680	-0.110617
H	0.640570	3.382708	0.588034
C	-1.924456	-0.390771	-0.317045
C	-2.664456	-1.256545	0.492131
C	-2.586561	0.526330	-1.137181
C	-4.054393	-1.204870	0.475928
H	-2.141173	-1.965664	1.124794
C	-3.976191	0.574693	-1.147829
H	-2.001099	1.193877	-1.761540
C	-4.713792	-0.289599	-0.340861
H	-4.625133	-1.882800	1.103276
H	-4.484980	1.290029	-1.786827
H	-5.798731	-0.249179	-0.349284
C	4.151485	-1.283043	0.657689
H	5.170280	-0.927026	0.828364
H	3.662187	-1.435499	1.620168
H	4.193146	-2.252136	0.140339
C	4.150957	0.356163	-1.207437
H	3.651848	1.288256	-1.479101
H	5.167356	0.580894	-0.875175
H	4.195530	-0.278542	-2.103102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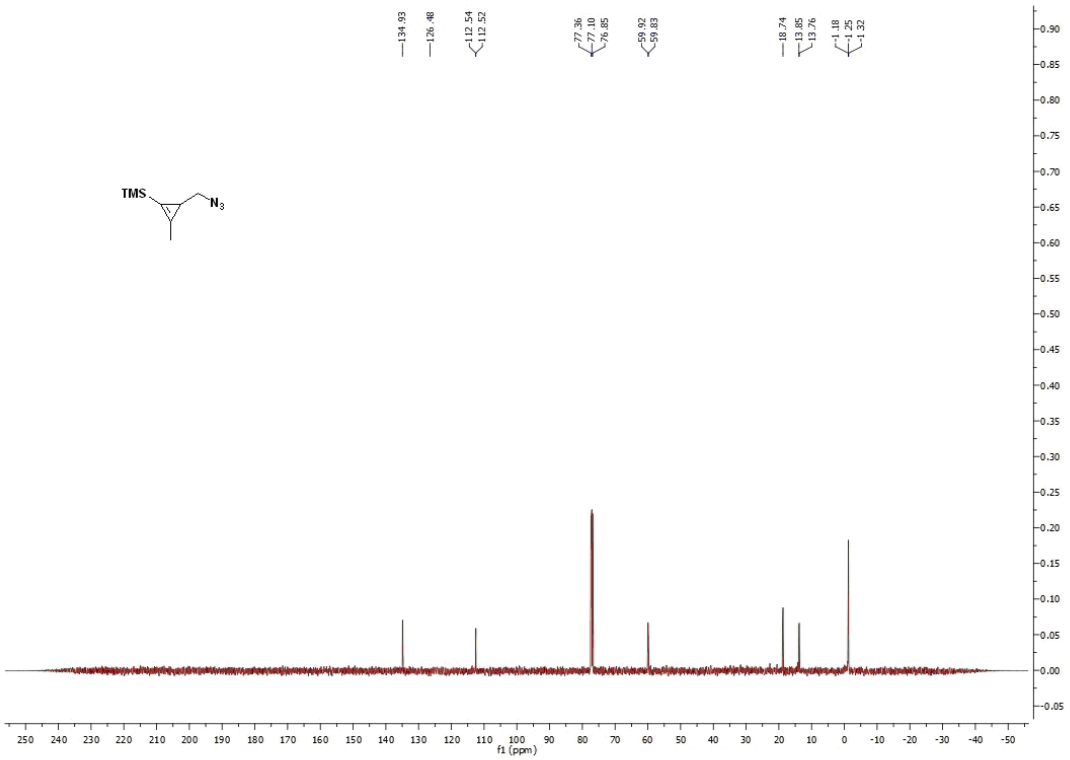
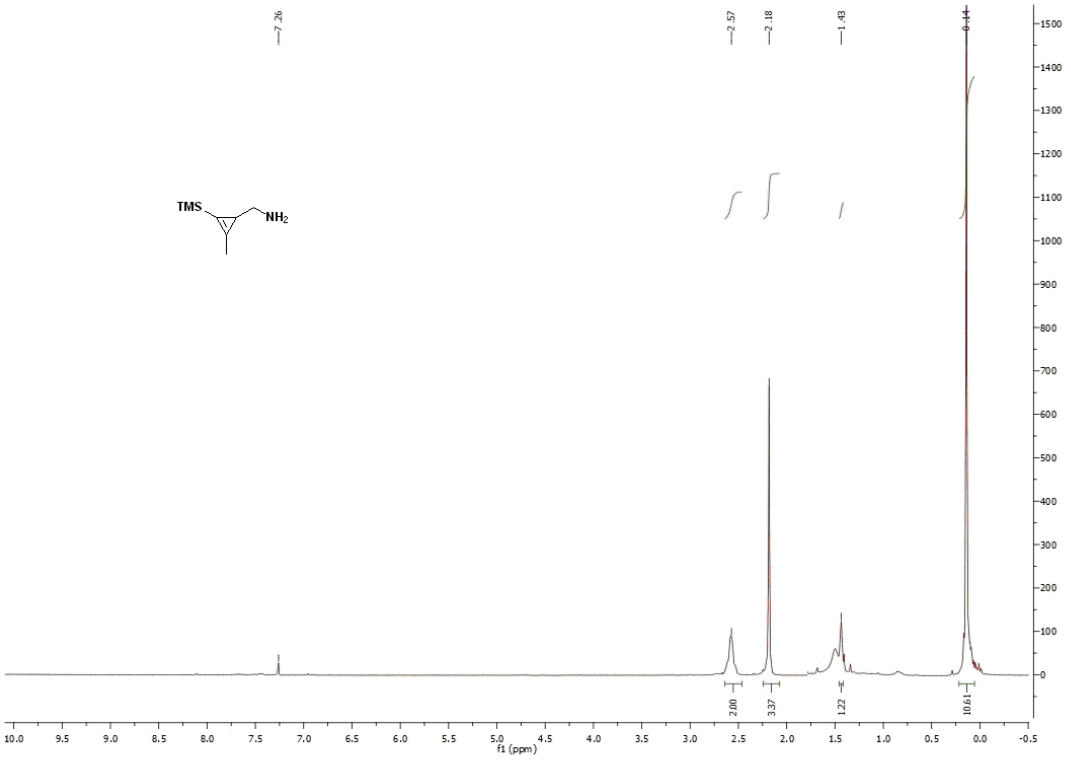
7.25
3.16
3.15
3.13
3.12
3.03
3.02
3.01
2.99
2.22
2.22
1.58
1.58
1.57
1.56
1.56
1.55
0.17
0.17
TMS
N3
1.00
0.97
2.95
1.34
9.55
f1 (ppm)


134.93
126.48
112.54
112.52
77.36
77.10
76.85
59.92
59.83
18.74
13.85
13.76
1.18
1.25
1.32
TMS
N3
f1 (ppm)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NH2
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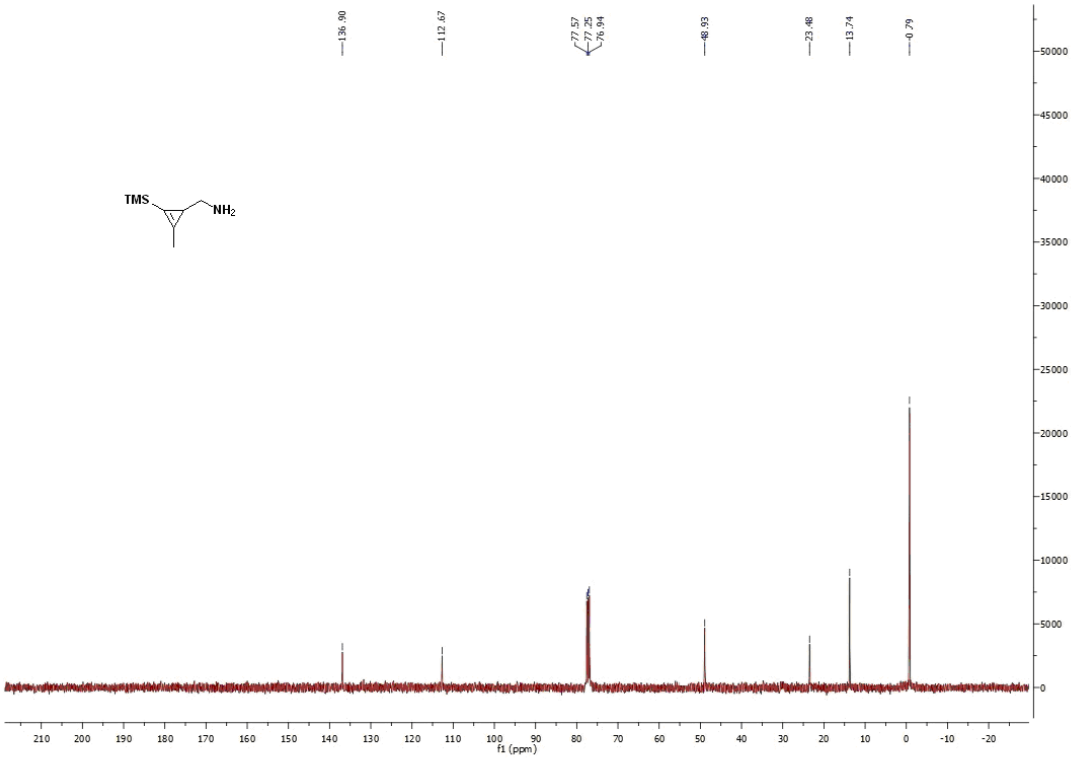
2.57
2.18
1.48
0.14
2.00
3.37
1.22
10.61
f1 (ppm)


TMS
NH2
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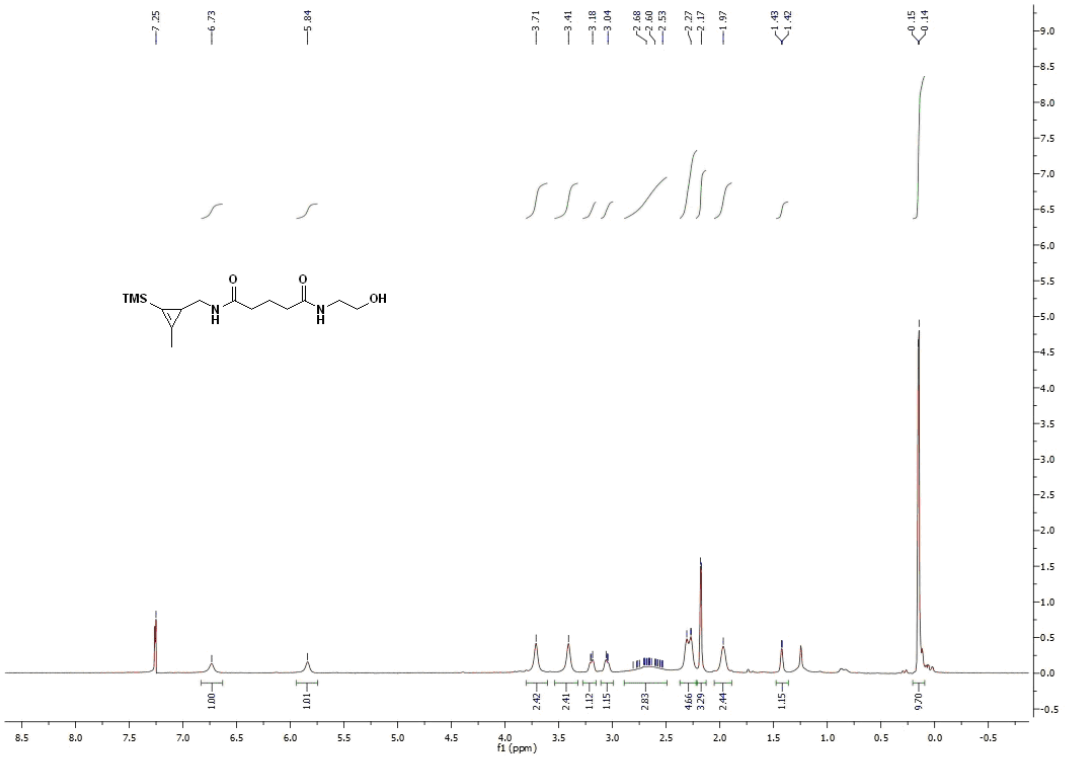
77.25
76.94
48.93
23.48
13.74
0.79
f1 (ppm)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6.73
5.84
3.71
3.41
3.18
3.04
2.68
2.60
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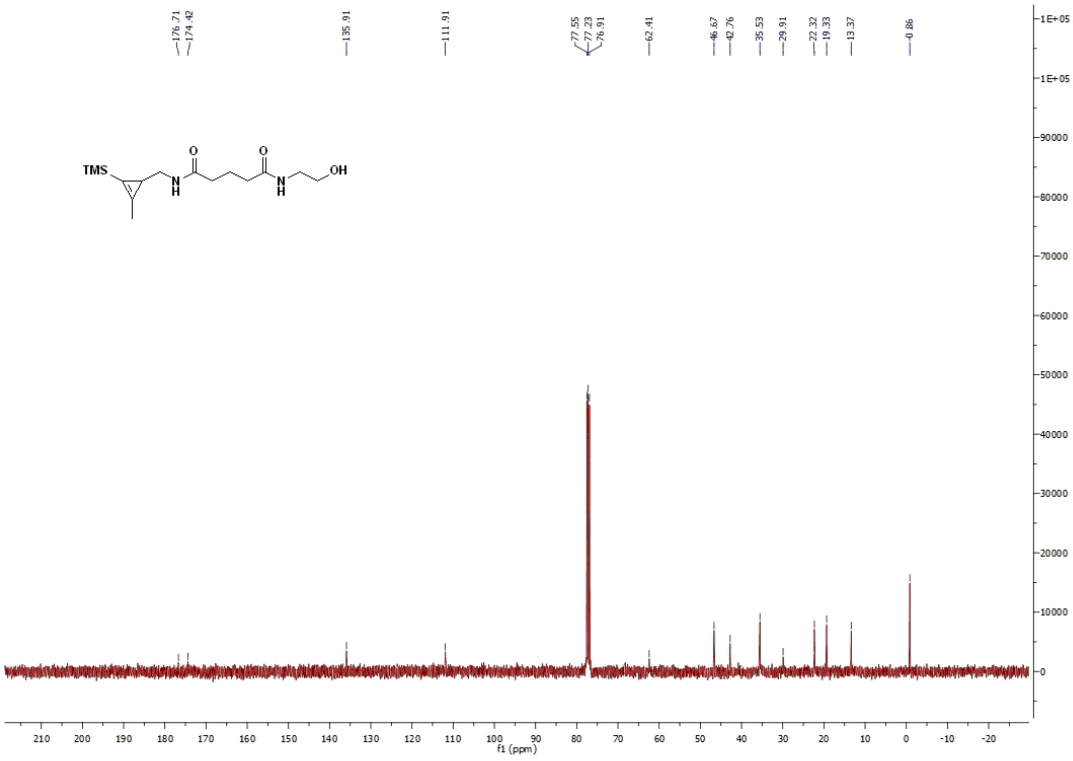
2.27
2.17
1.97
1.43
1.42
0.15
0.14
TMS
OH
f1 (ppm)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174.42
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77.55
77.23
76.91
62.41
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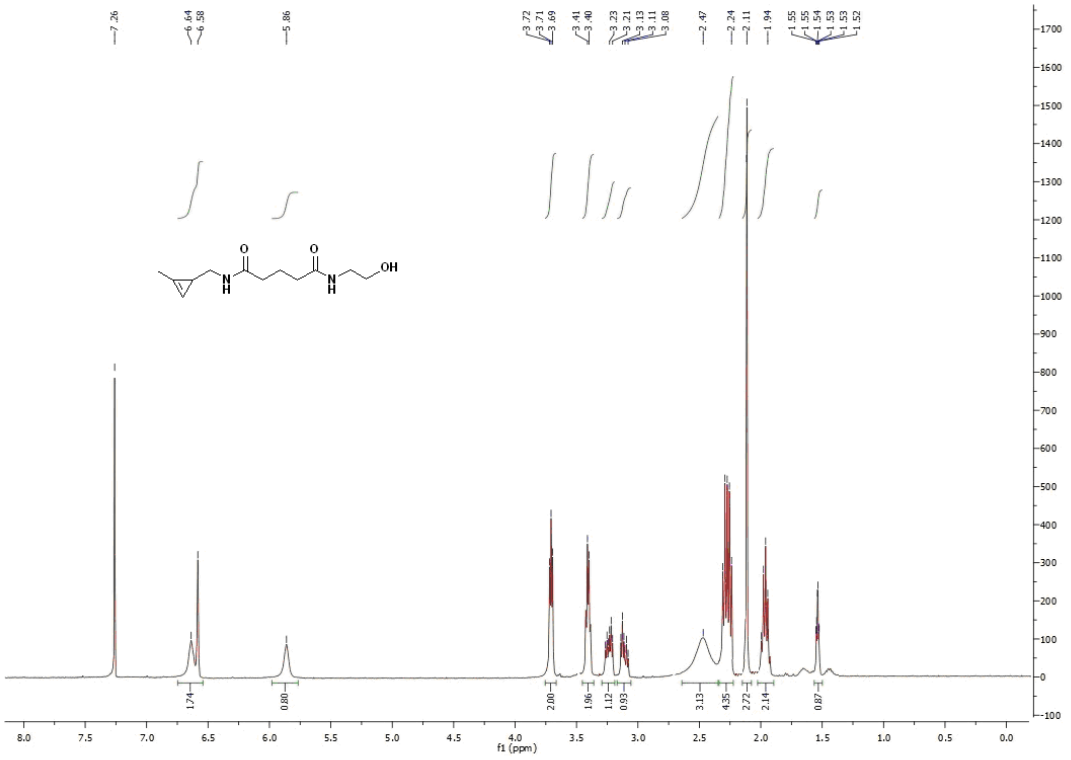
42.76
35.53
29.91
22.32
19.33
13.37
0.86
TMS
OH
f1 (ppm)


7.26
6.64
6.58
5.86
3.72
3.71
3.69
3.41
3.40
3.23
3.21
3.13
3.11
3.08
2.47
2.24
2.11
1.94
1.55
1.55
1.54
1.53
1.53
1.52
1.74
0.80
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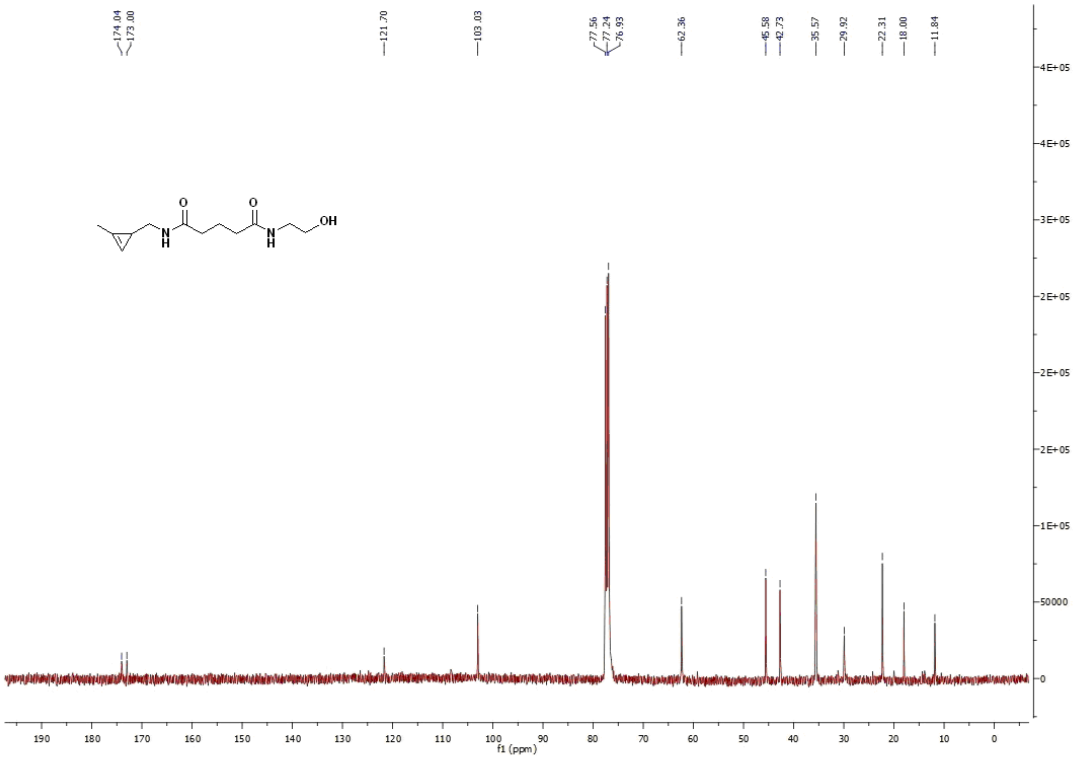
1.96
1.12
0.93
3.13
4.35
2.72
2.14
0.87
f1 (ppm)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173.00
121.70
103.03
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77.24
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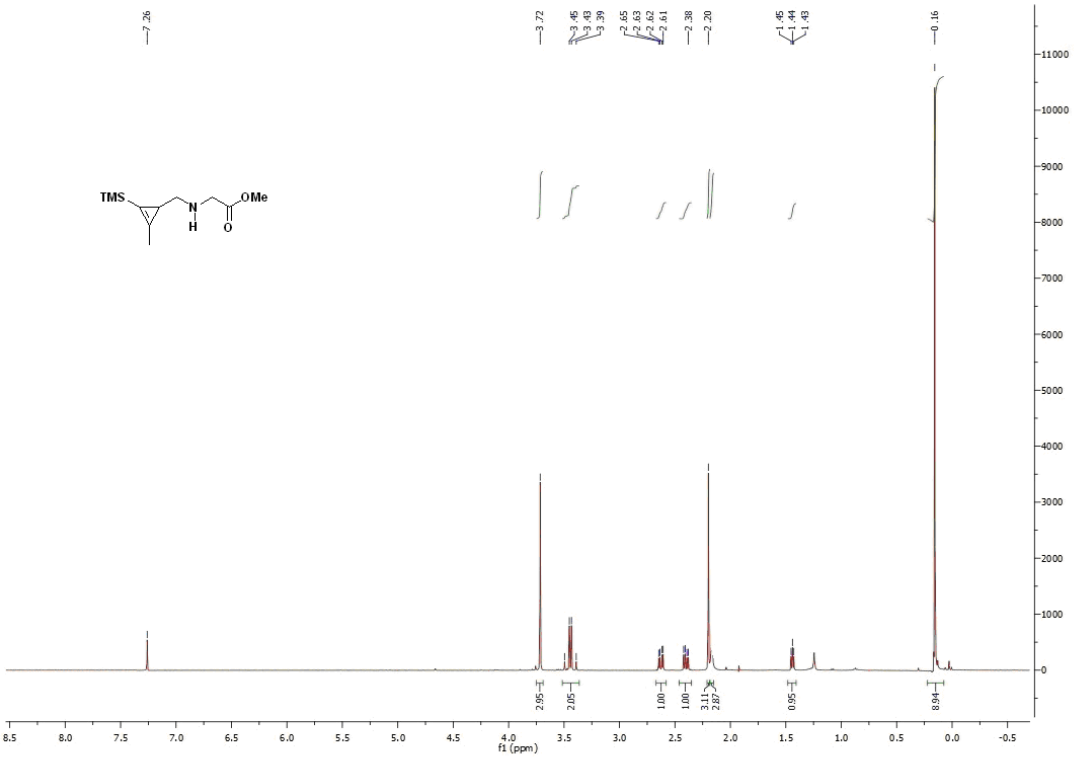
62.36
45.58
42.73
35.57
29.92
22.31
18.00
11.84
f1 (ppm)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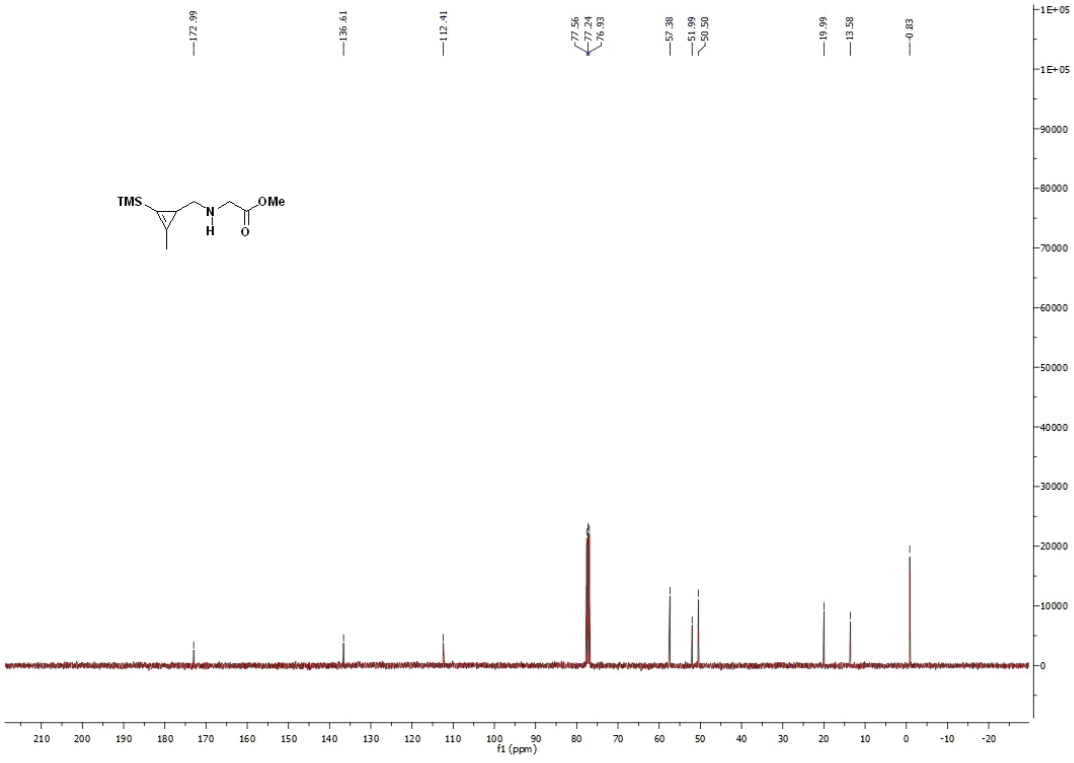
3.72
3.46
3.43
3.39
2.65
2.63
2.62
2.61
2.38
2.20
1.45
1.44
1.43
0.16
TMS
N
H
OMe
O
2.95
2.05
1.00
1.00
3.11
2.87
0.95
8.94
f1 (ppm)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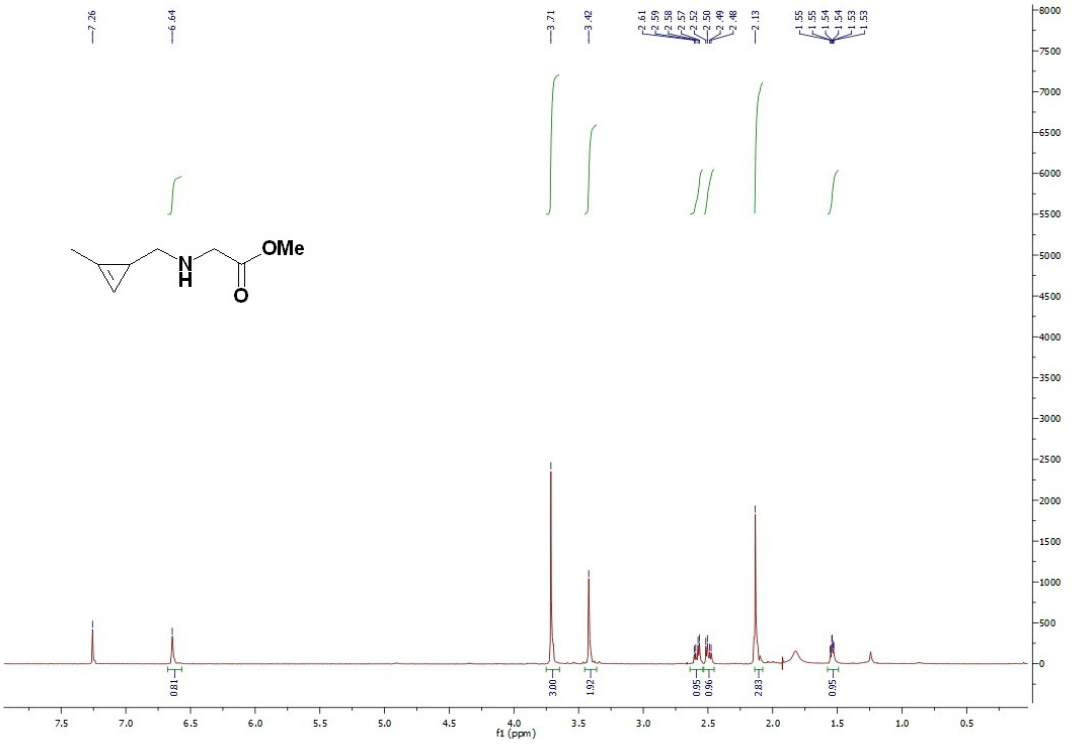
136.61
112.41
77.56
77.24
76.93
57.38
51.99
50.50
19.99
13.58
0.83
TMS
N
H
OMe
O
f1 (ppm)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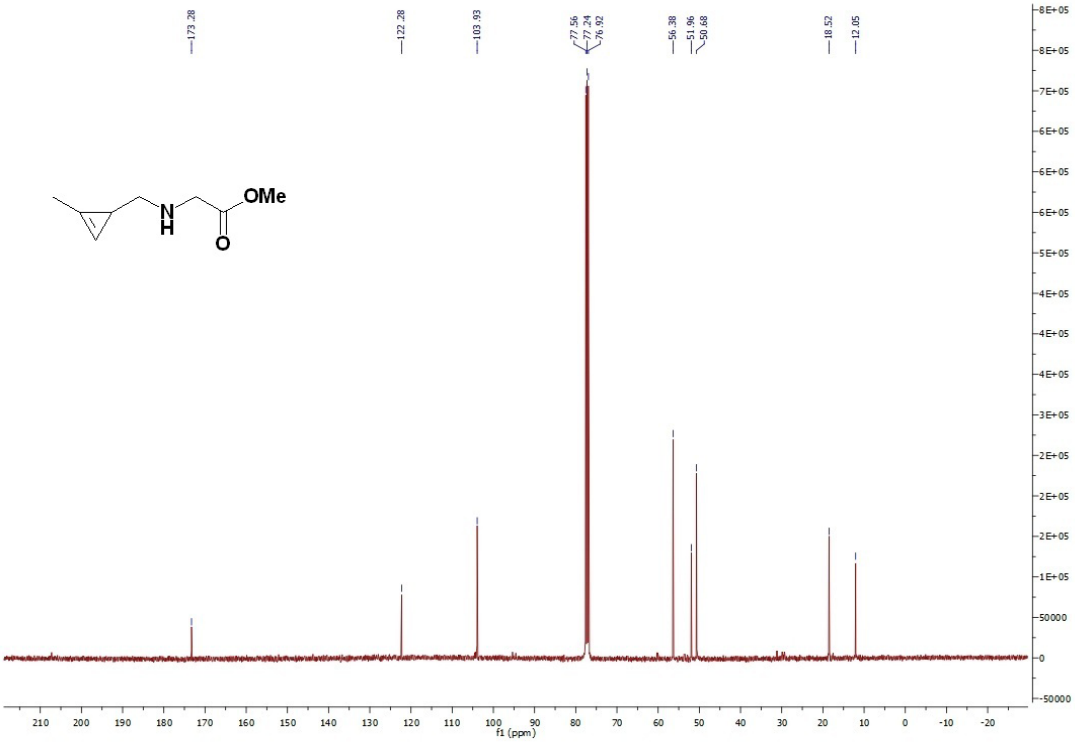
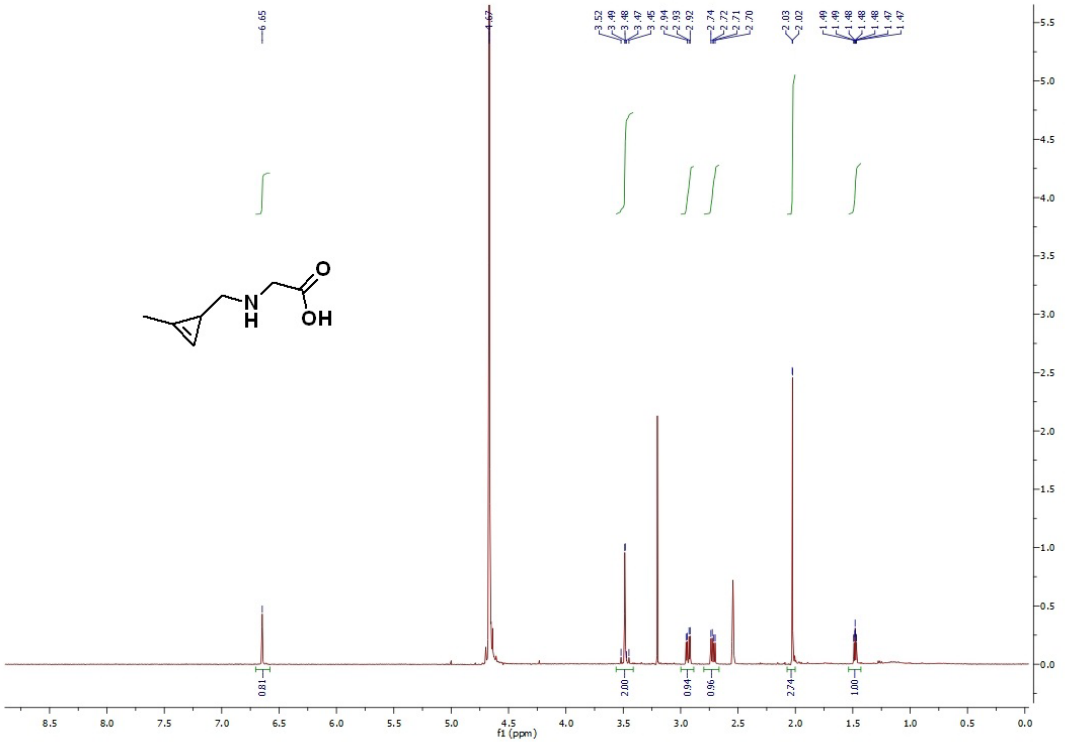
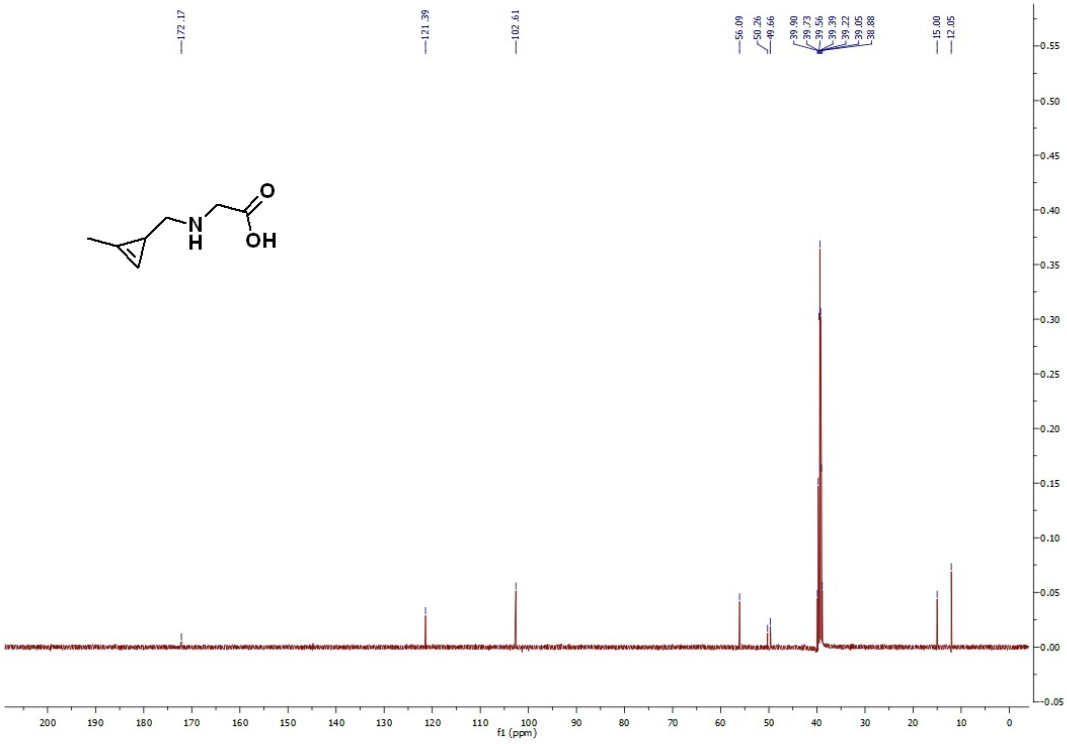
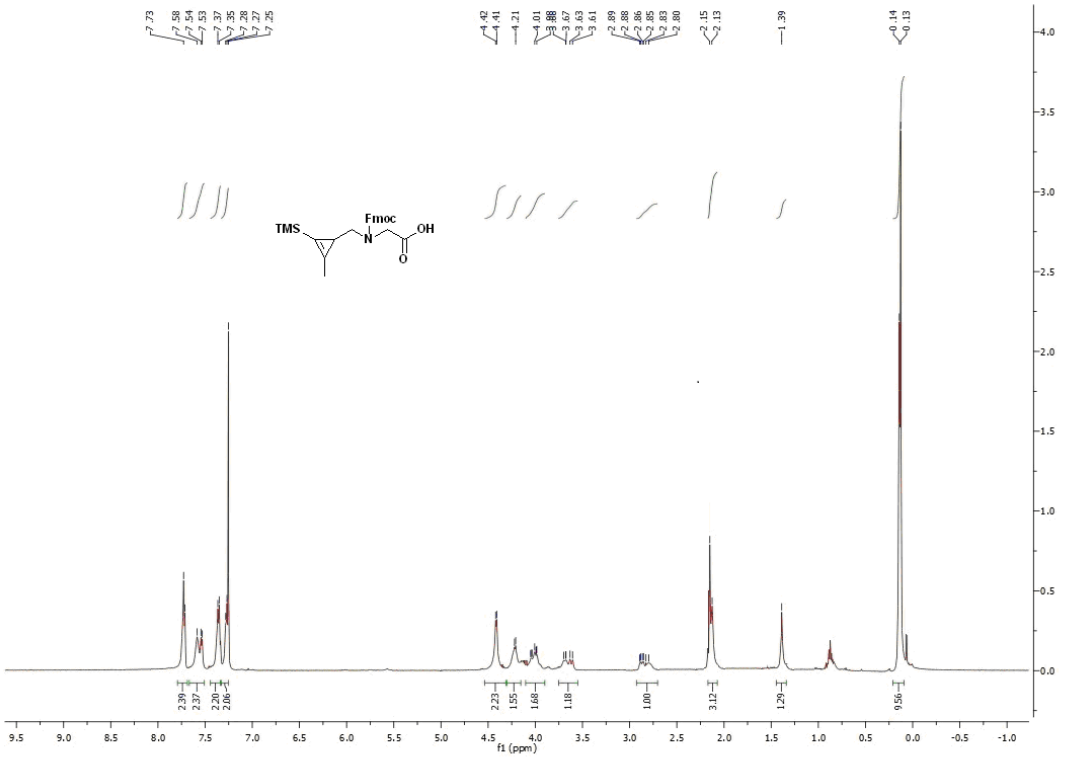
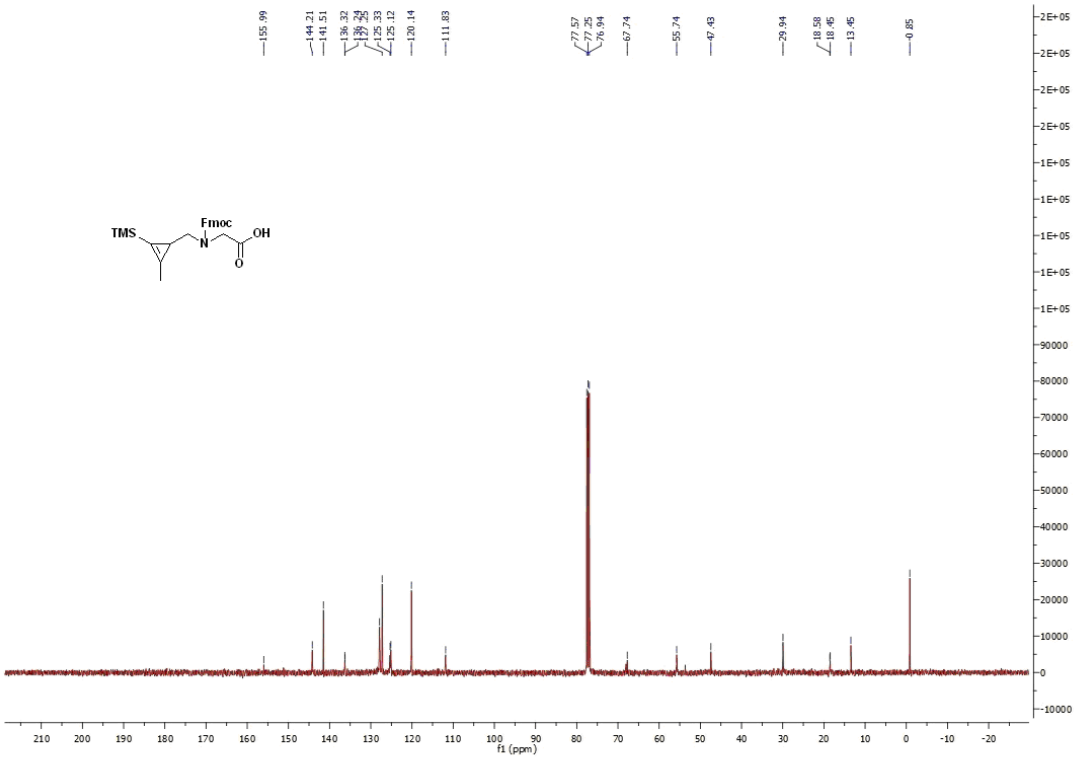
6.64
3.71
3.42
2.61
2.59
2.58
2.57
2.52
2.50
2.49
2.48
2.13
1.55
1.55
1.54
1.54
1.53
1.53
OMe
N
H
O
0.81
3.00
1.92
0.95
0.96
2.83
0.95
f1 (ppm)

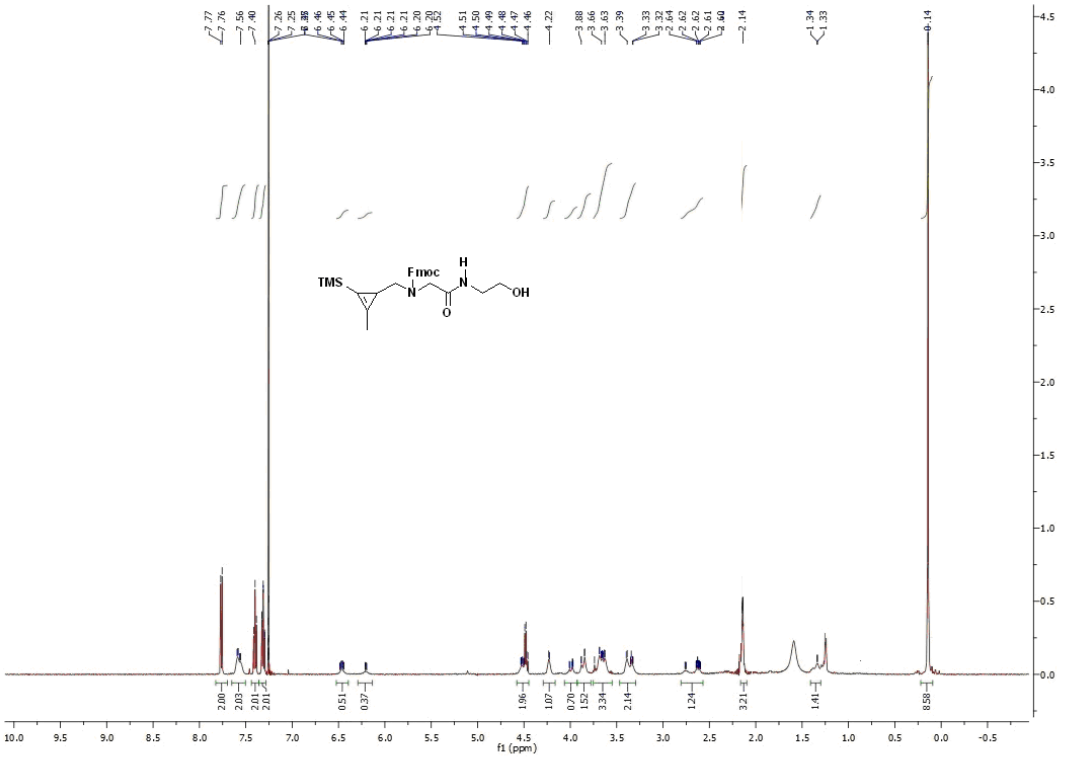

173.28
122.28
103.93
77.56
77.24
76.92
56.38
51.96
50.68
18.52
12.05
OMe
N
H
O
f1 (ppm)

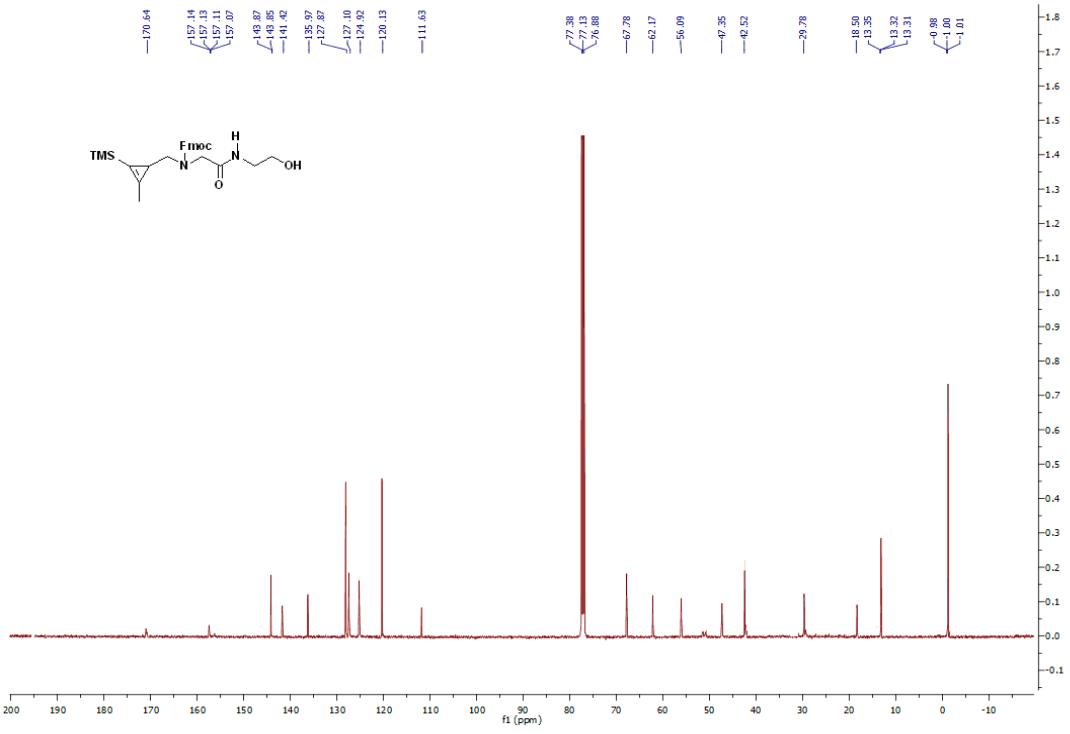

6.65
4.67
3.52
3.49
3.48
3.47
3.45
2.94
2.93
2.92
2.74
2.72
2.71
2.70
2.03
2.02
1.49
1.49
1.48
1.48
1.48
1.47
1.47
0.81
2.00
0.94
0.96
2.74
1.00
f1 (ppm)

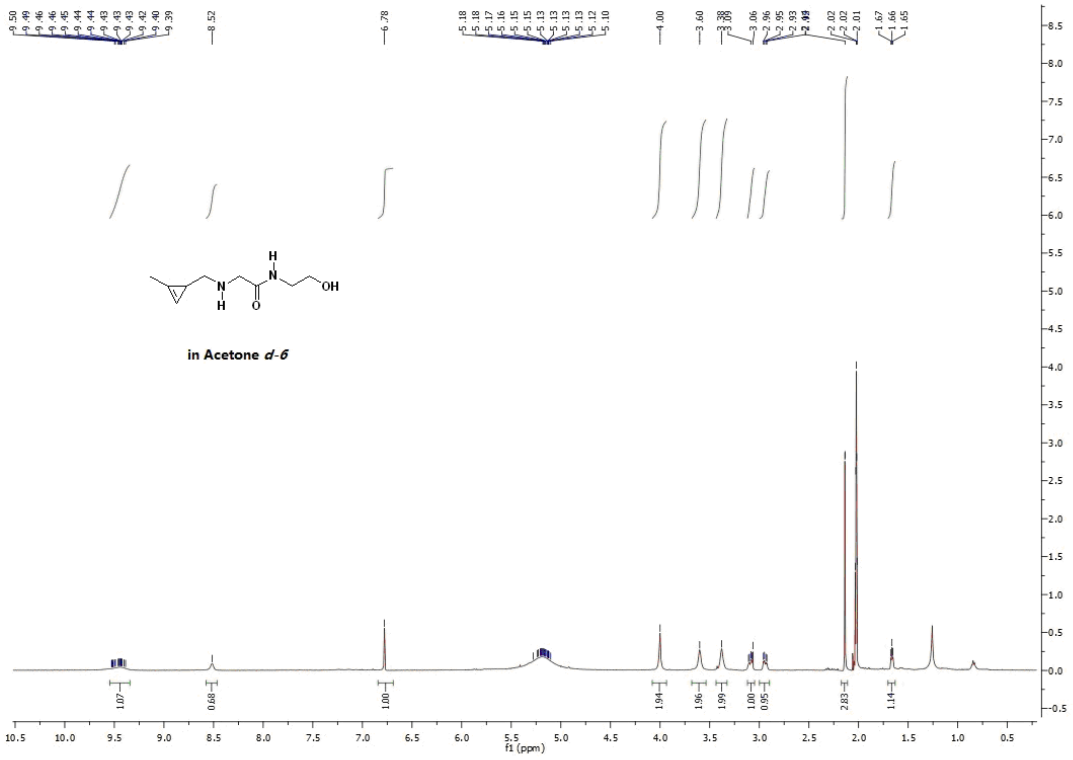

172.17
121.39
102.61
56.09
50.26
49.66
39.90
39.73
39.56
39.39
39.22
39.05
38.88
15.00
12.05
f1 (ppm)

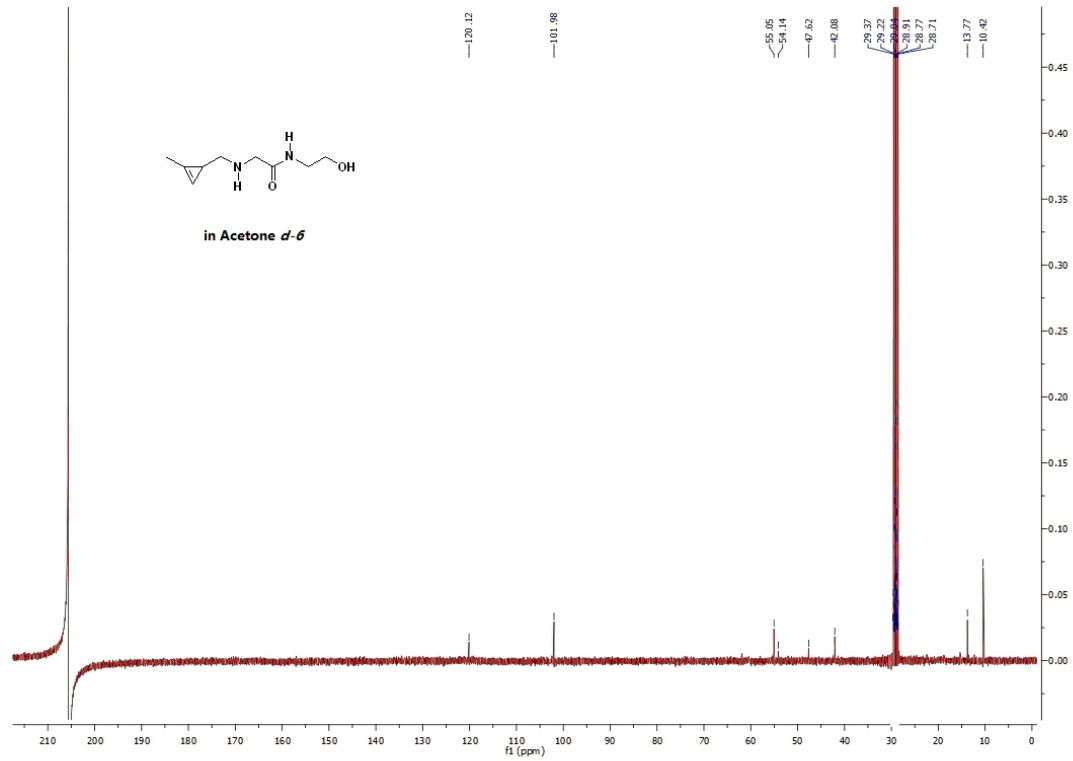

TMS
Fmoc
N
OH
O
f1 (ppm)

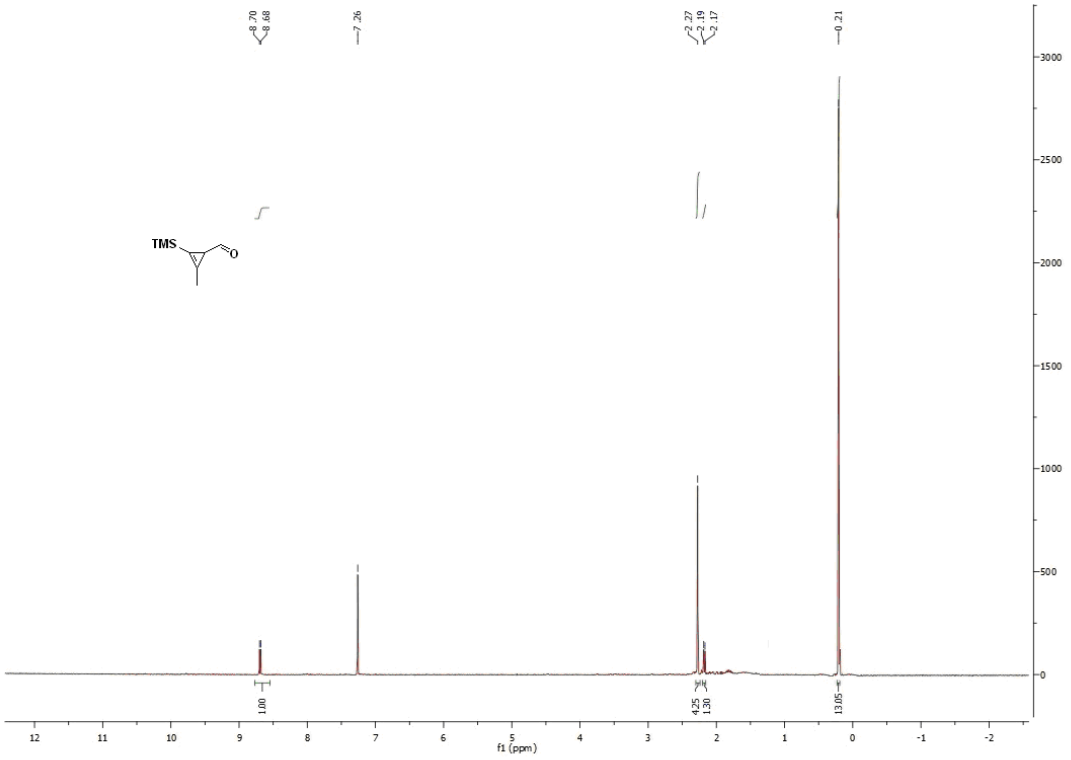

TMS
Fmoc
N
OH
O
f1 (ppm)

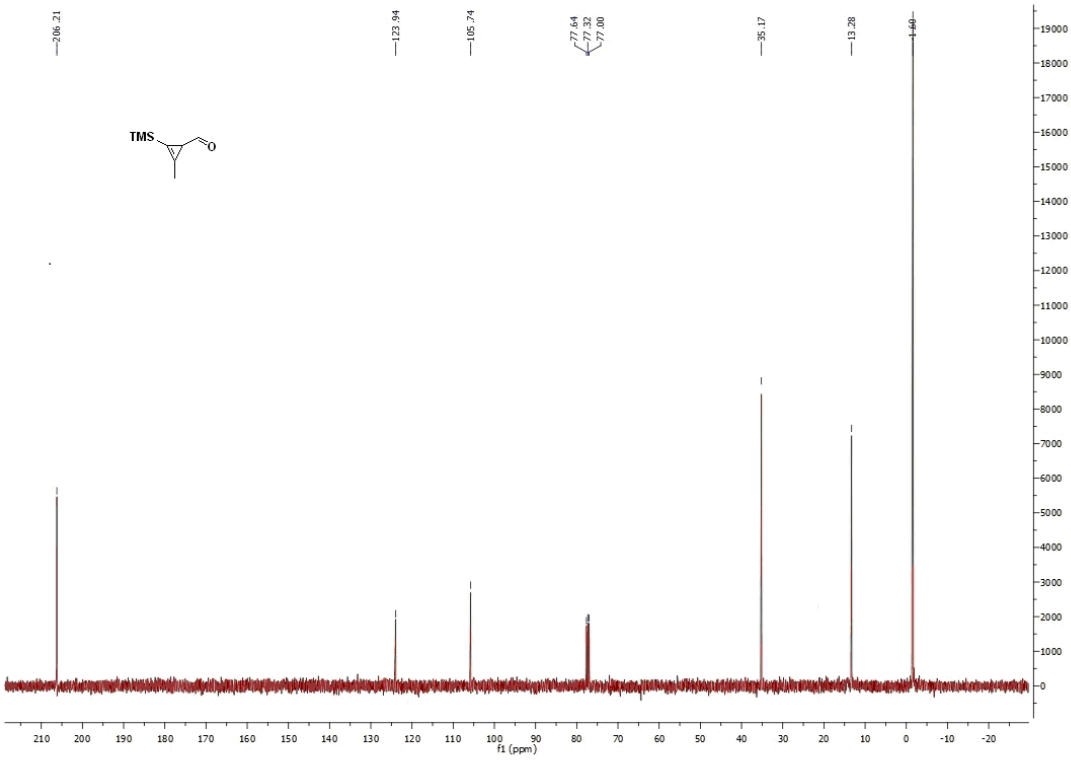

TMS
Fmoc
N
H
N
O
OH
f1 (ppm)

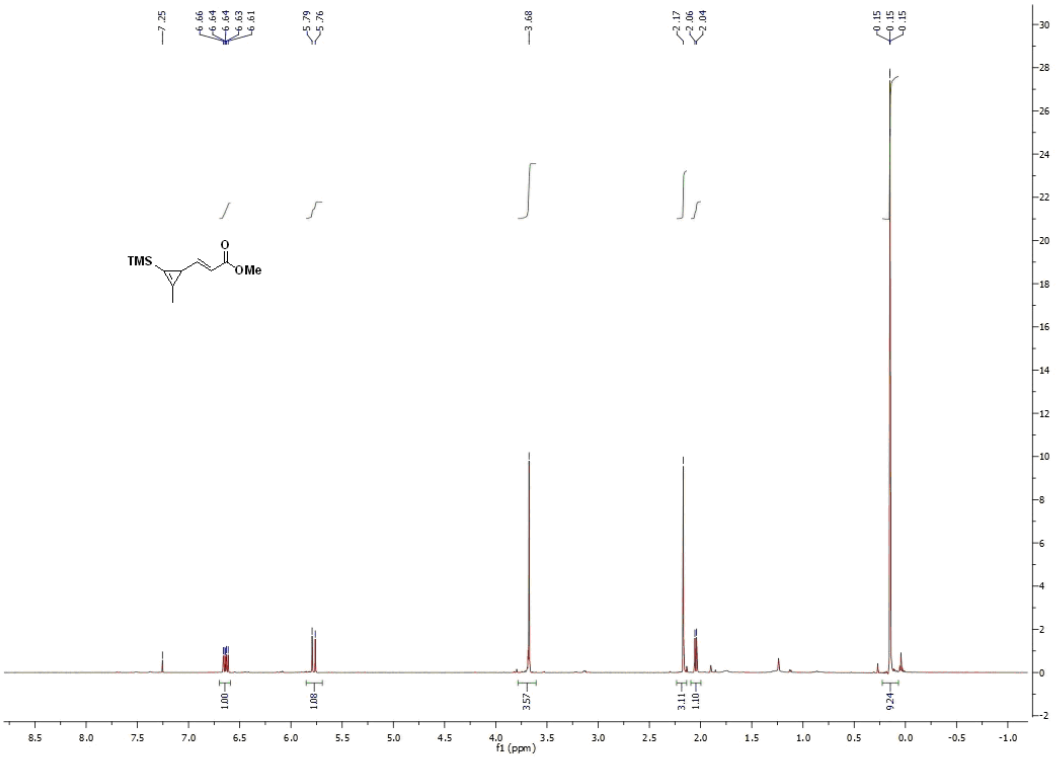

TMS
Fmoc
N
H
N
O
OH
f1 (ppm)

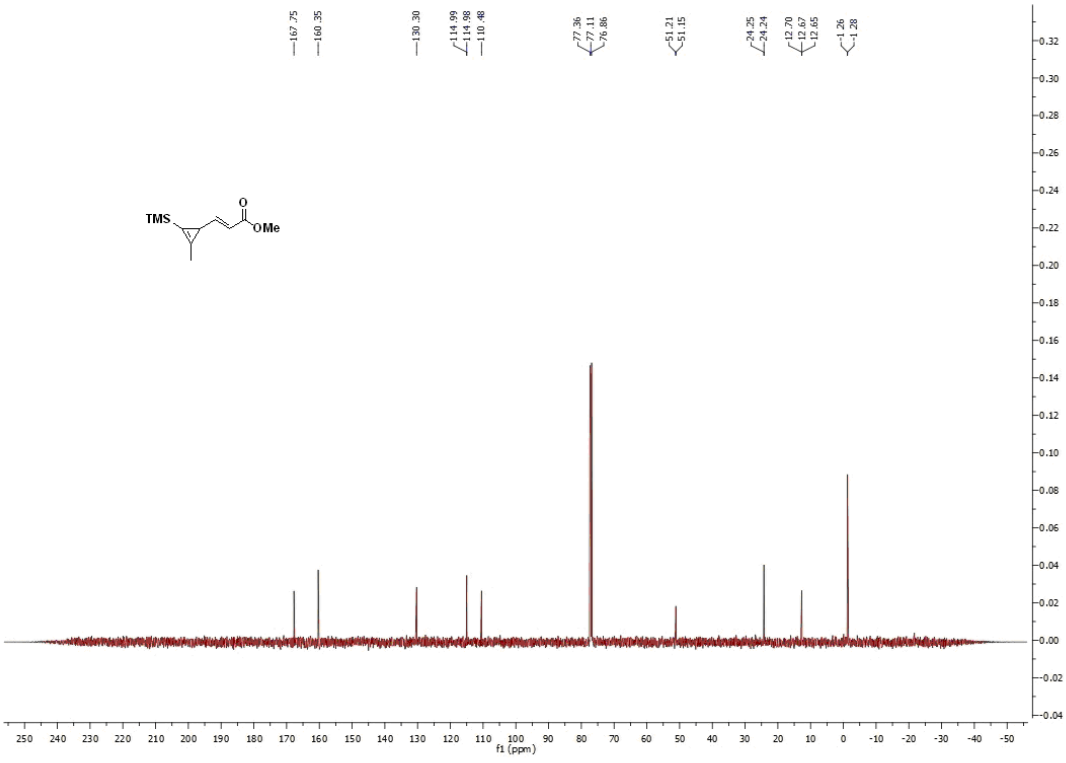

in Acetone d-6
f1 (ppm)

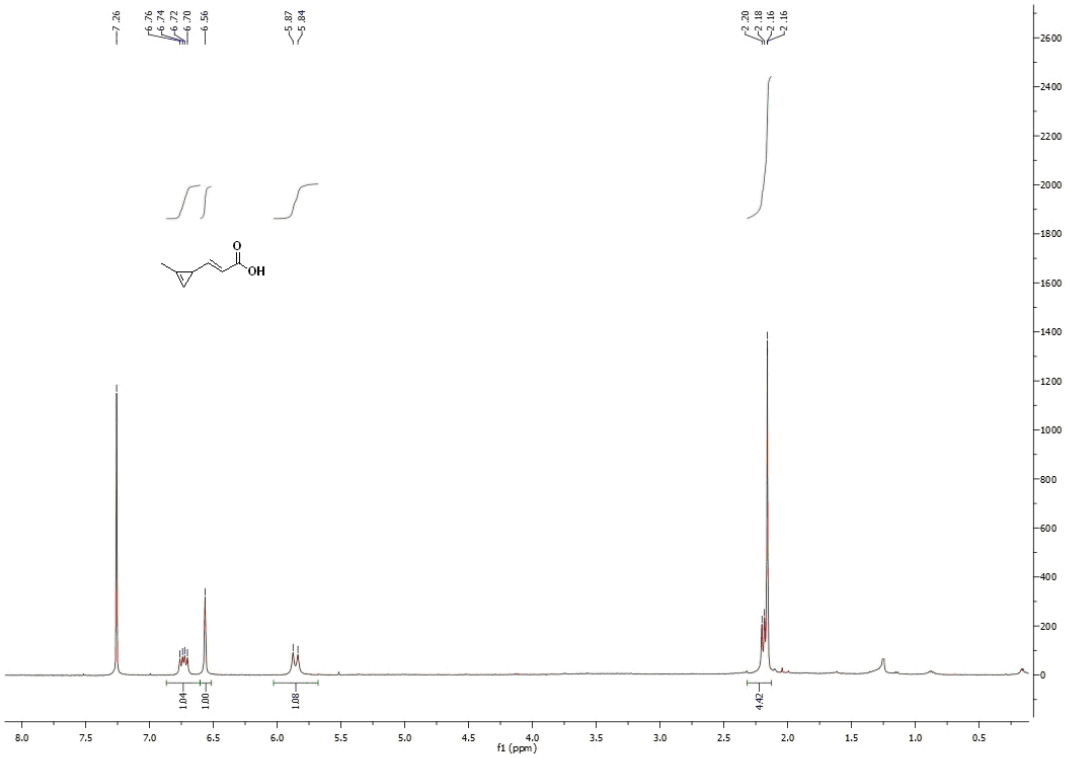

in Acetone d-6
f1 (ppm)

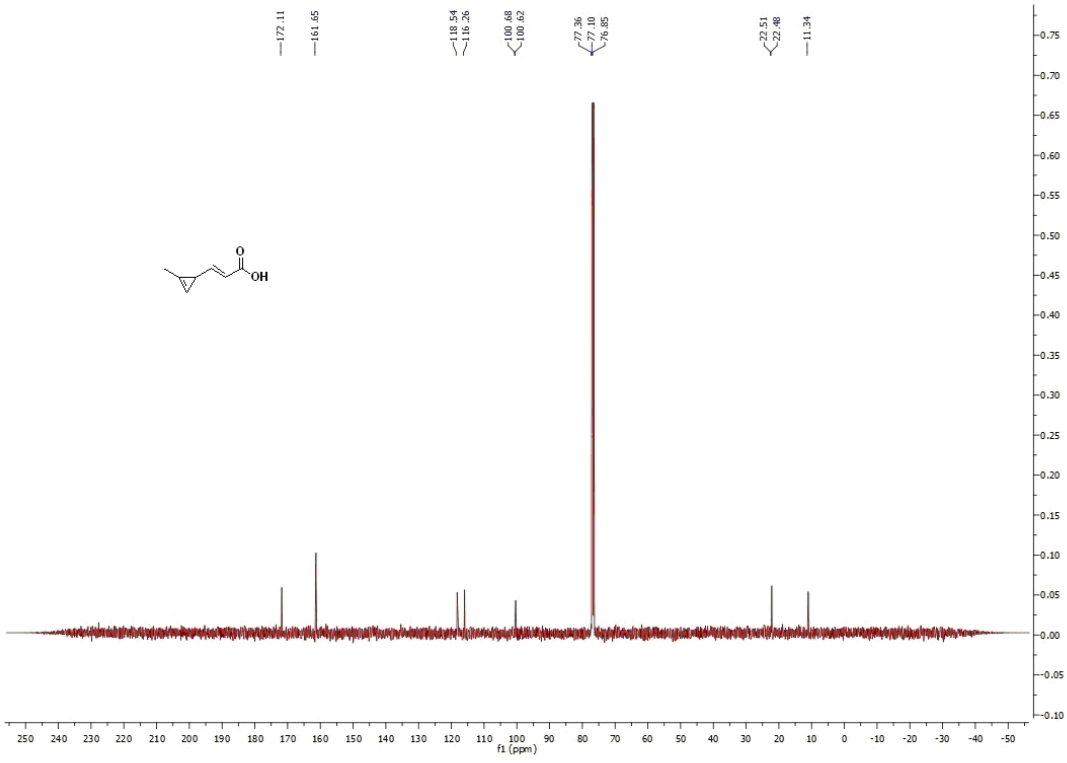

8.70
8.68
7.26
2.27
2.19
2.17
0.21
TMS
O
1.00
4.25
1.30
13.05
f1 (ppm)


206.21
123.94
105.74
77.64
77.32
77.00
35.17
13.28
1.60
TMS
O
f1 (ppm)


7.25
6.66
6.64
6.64
6.63
6.61
5.79
5.76
3.68
2.17
2.06
2.04
0.15
0.15
0.15
TMS
O
OMe
1.00
1.08
3.57
3.11
1.10
9.24
f1 (ppm)


167.75
160.35
130.30
114.99
114.98
110.48
77.36
77.11
76.86
51.21
51.15
24.25
24.24
12.70
12.67
12.65
1.26
1.28
TMS
O
OMe
f1 (ppm)


7.26
6.76
6.74
6.72
6.70
6.56
5.87
5.84
2.20
2.18
2.16
2.16
O
OH
1.04
1.00
1.08
4.42
f1 (ppm)


172.11
161.65
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100.62
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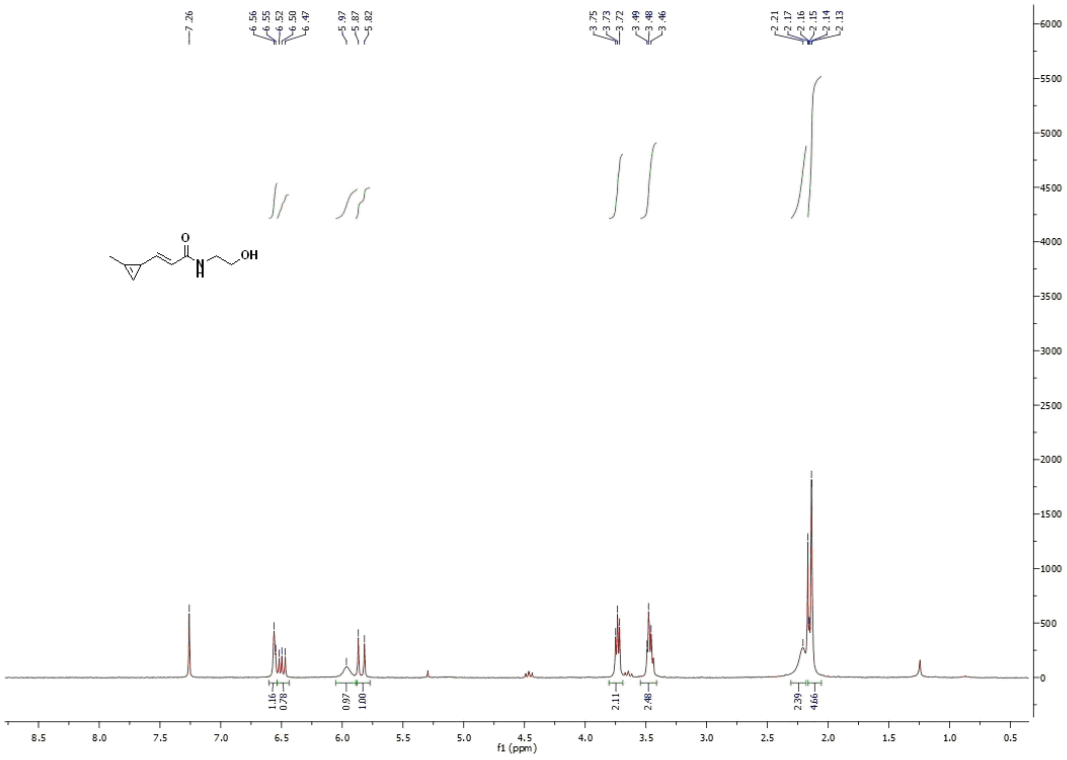
77.10
76.85
22.51
22.48
11.34
O
OH
f1 (ppm)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6.55
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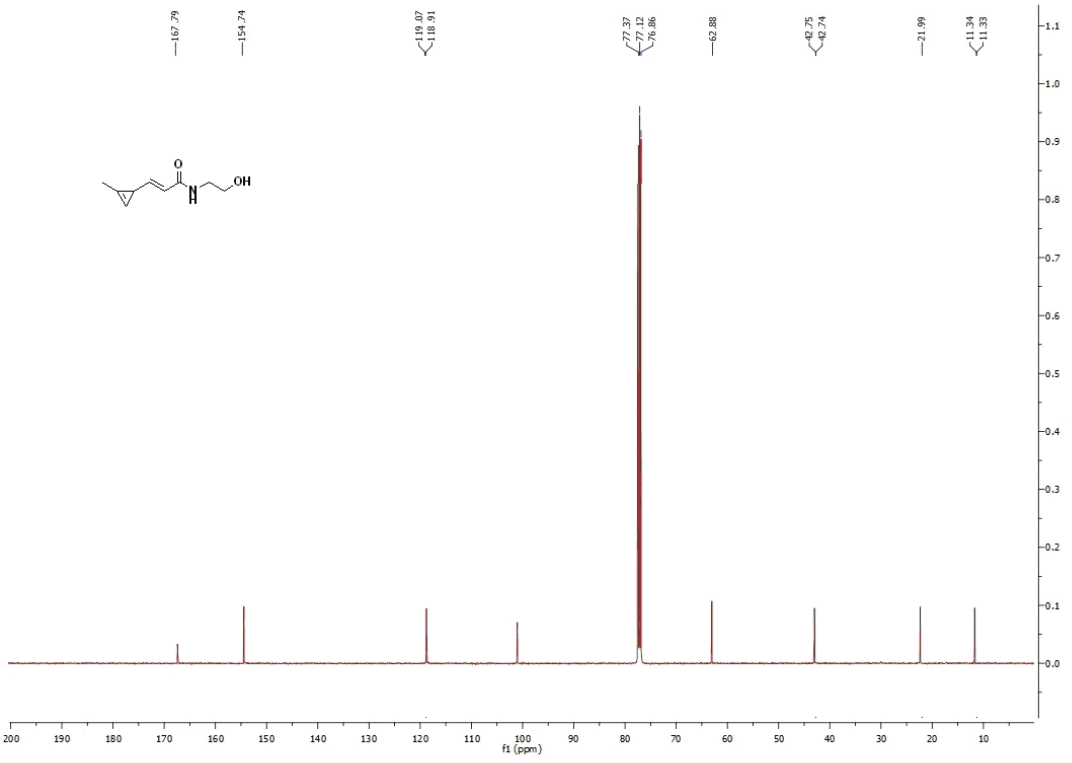
6.50
6.47
5.97
5.87
5.82
3.75
3.73
3.72
3.49
3.48
3.46
2.21
2.17
2.16
2.15
2.14
2.13
O
N
H
OH
1.16
0.78
0.97
1.00
2.11
2.48
2.39
4.66
f1 (ppm)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154.74
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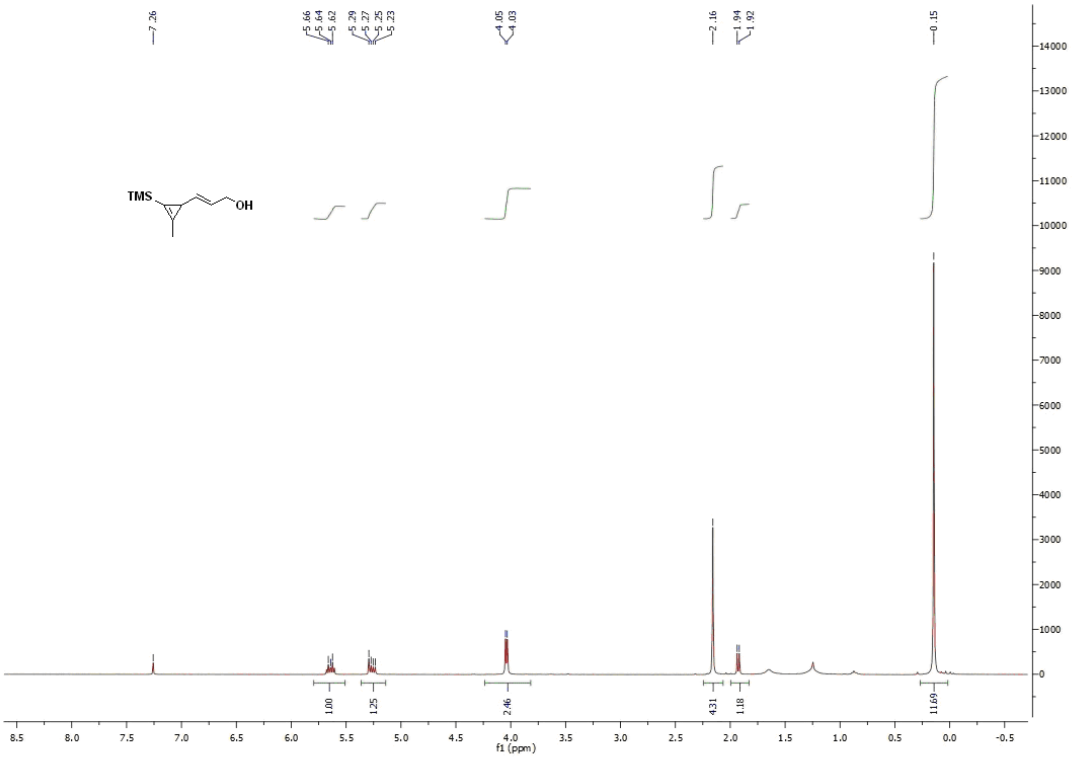
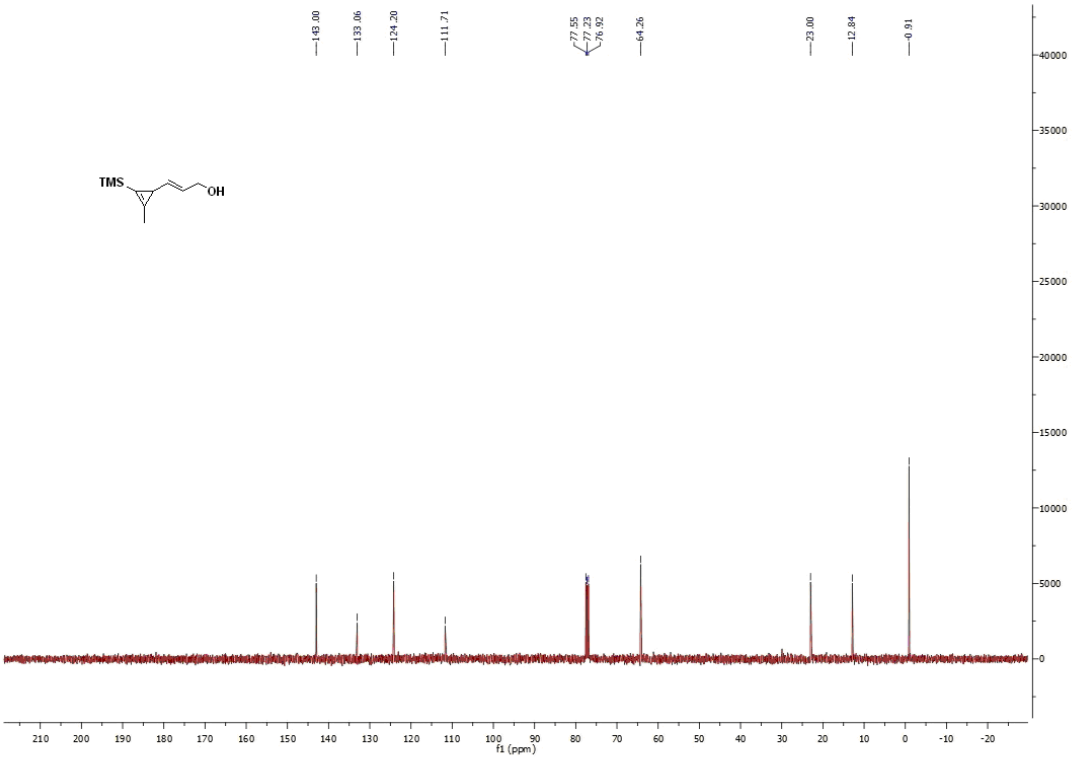
62.88
42.75
42.74
21.99
11.34
11.33
O
N
H
OH
f1 (ppm)


7.26
5.66
5.64
5.62
5.29
5.27
5.25
5.23
4.05
4.03
2.16
1.94
1.92
0.15
TMS
OH
1.00
1.25
2.46
4.31
1.18
11.69
f1 (ppm)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133.06
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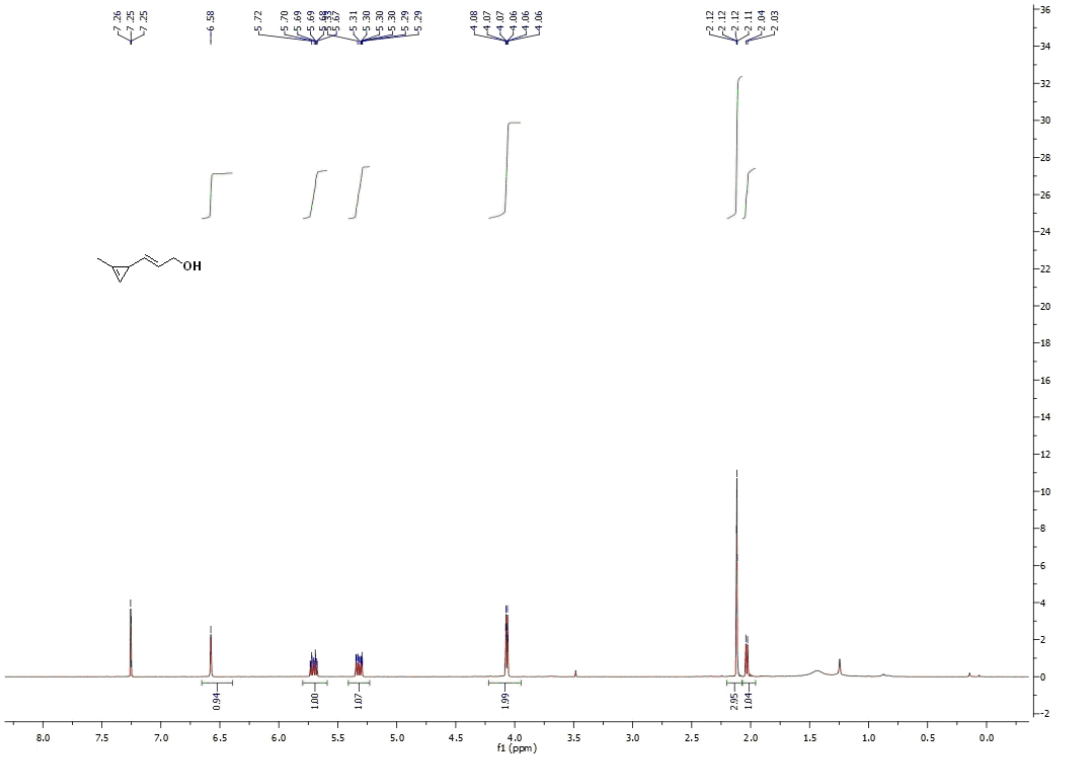
111.71
77.55
77.23
76.92
64.26
23.00
12.84
0.91
TMS
OH
f1 (ppm)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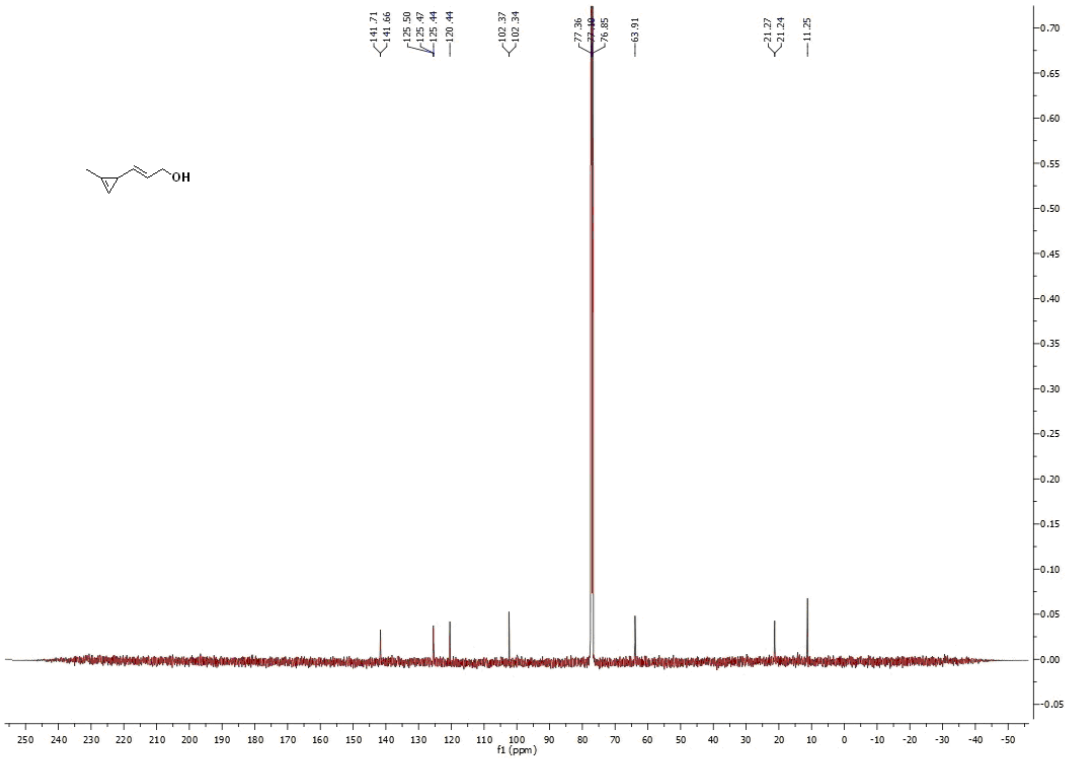
7.25
7.25
6.58
5.72
5.70
5.69
5.69
5.68
5.67
5.31
5.30
5.30
5.29
5.29
4.08
4.07
4.07
4.06
4.06
4.06
2.12
2.12
2.12
2.11
2.04
2.03
OH
0.94
1.00
1.07
1.99
2.95
1.04
f1 (ppm)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102.34
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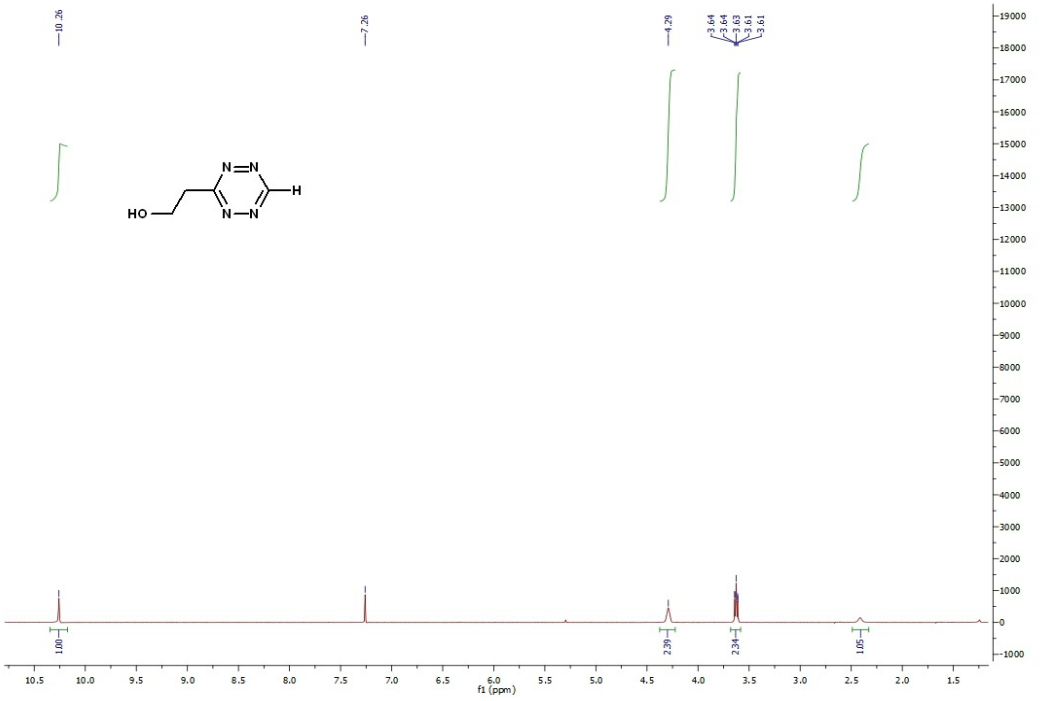
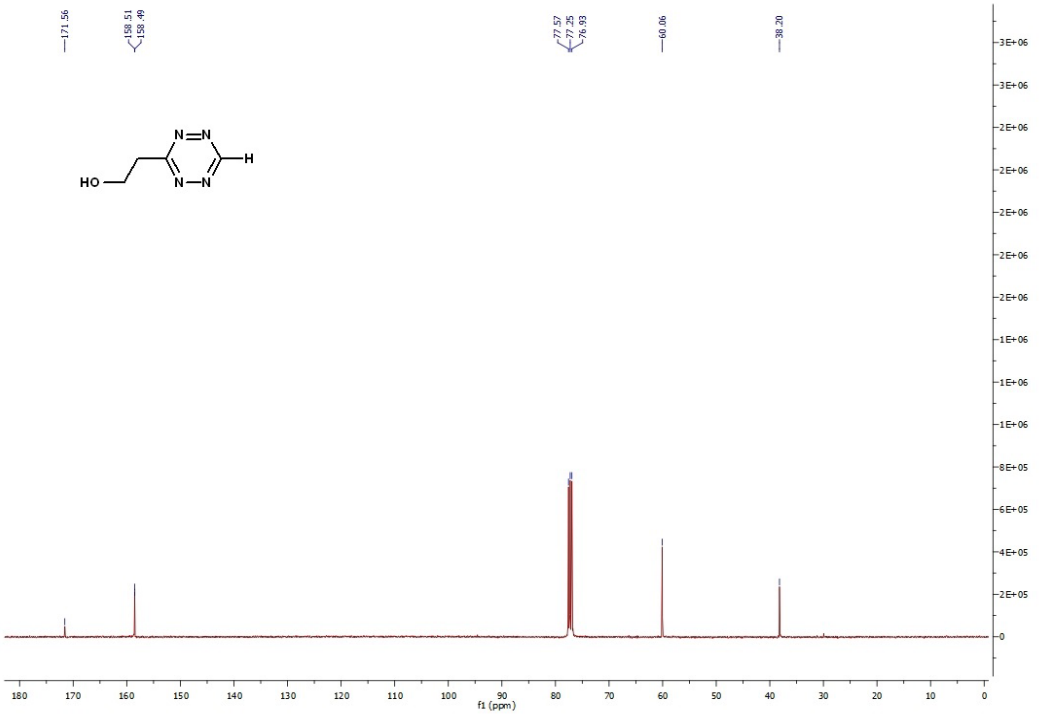
77.10
76.85
63.91
21.27
21.24
11.25
OH
f1 (ppm)


10.26
7.26
4.29
3.64
3.64
3.63
3.61
3.61
HO
N
N
N
N
H
1.00
2.39
2.34
1.05
f1 (ppm)


171.56
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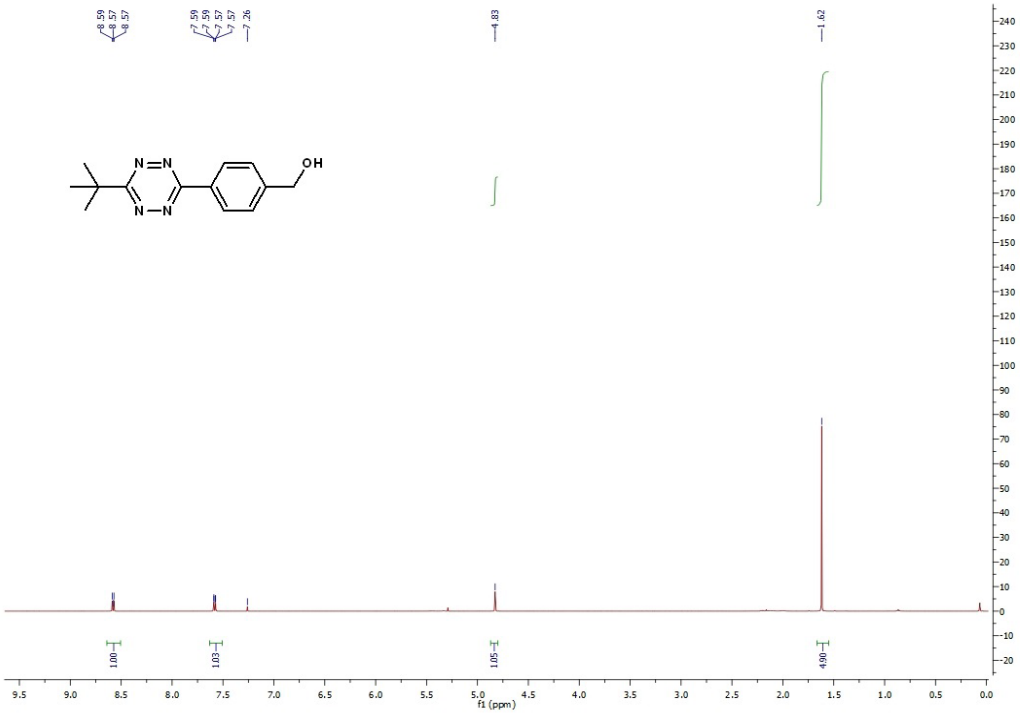
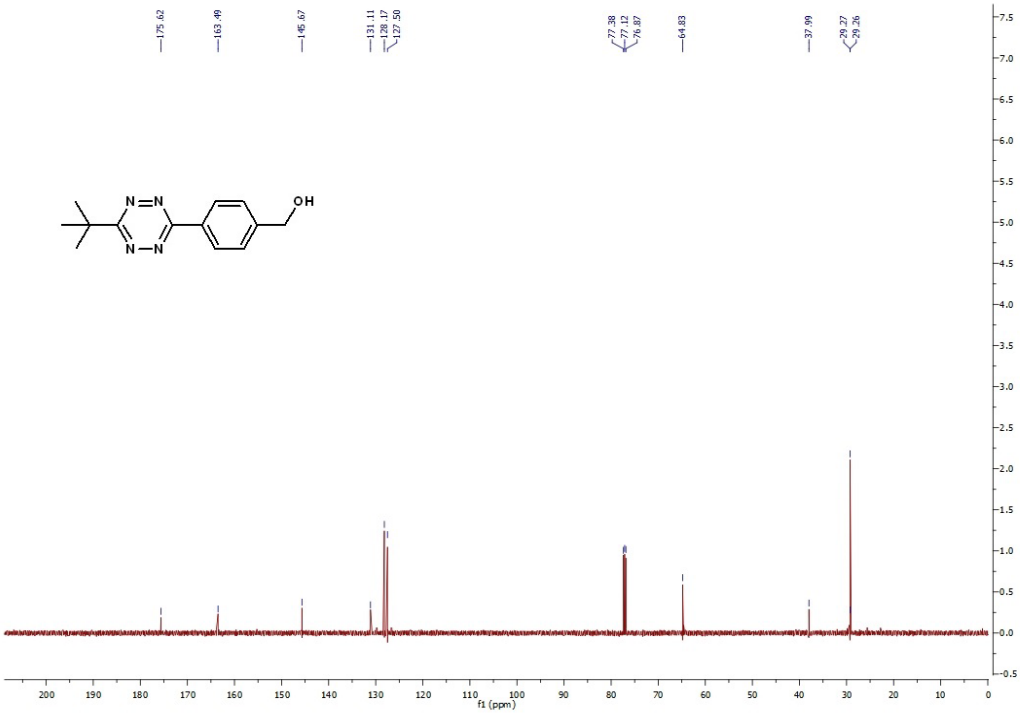
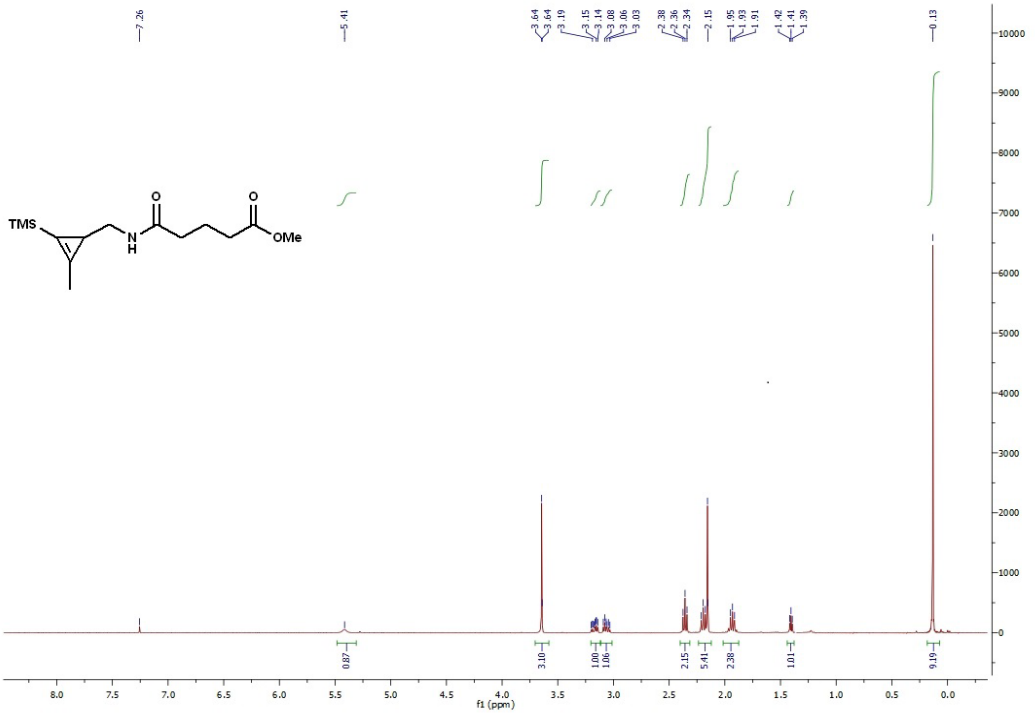
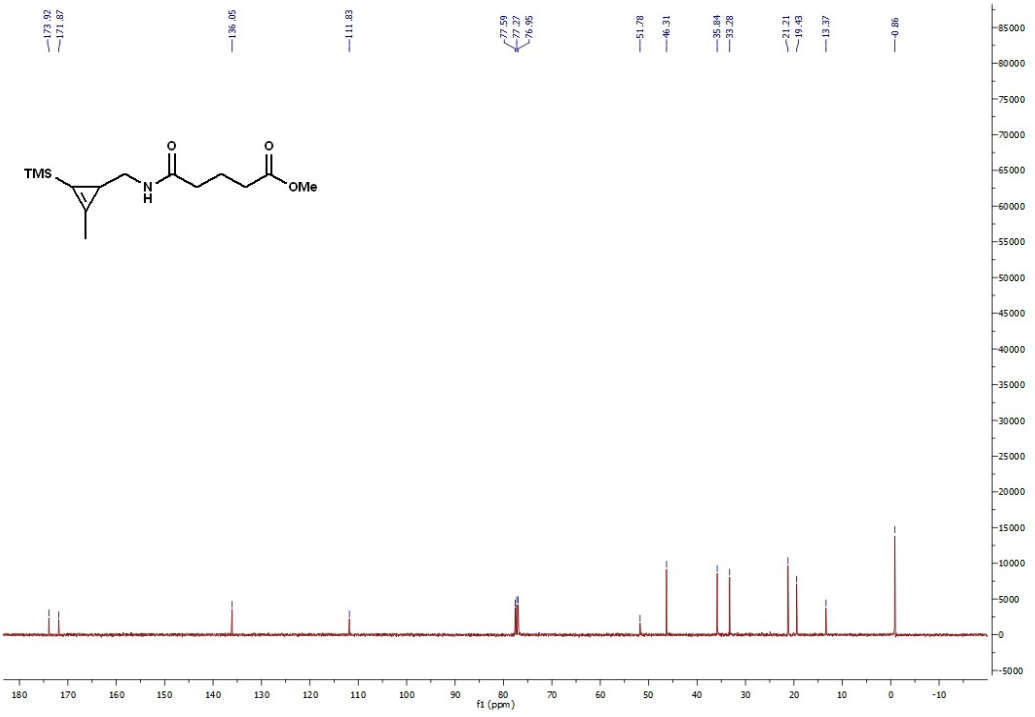
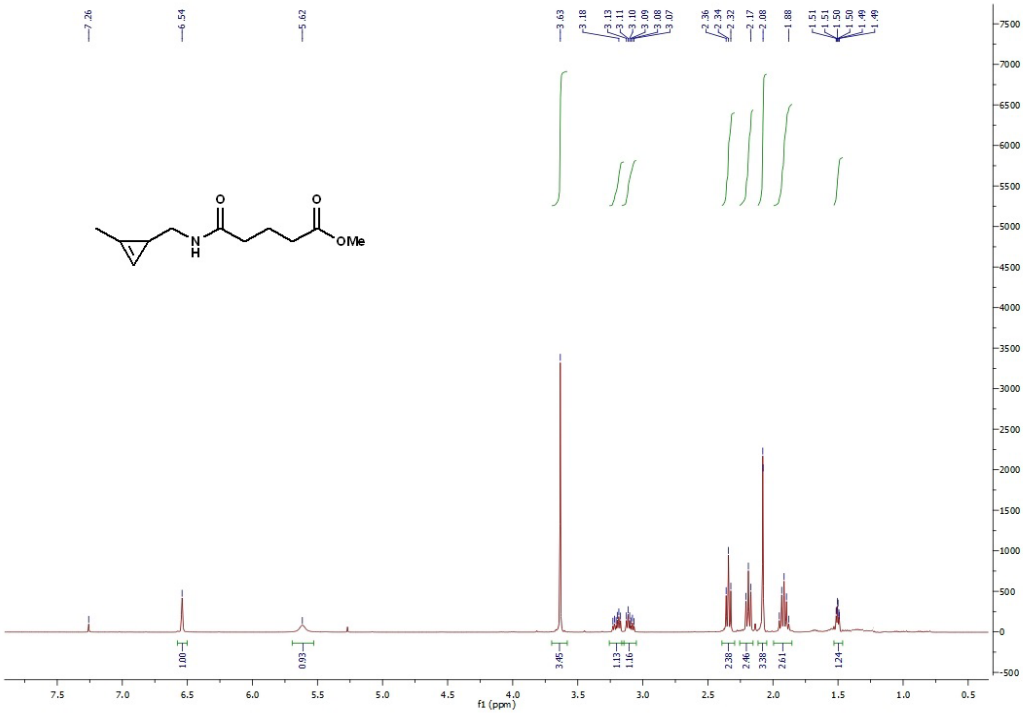
158.49
77.57
77.25
76.93
60.06
38.20
HO
N
N
N
N
H
f1 (ppm)

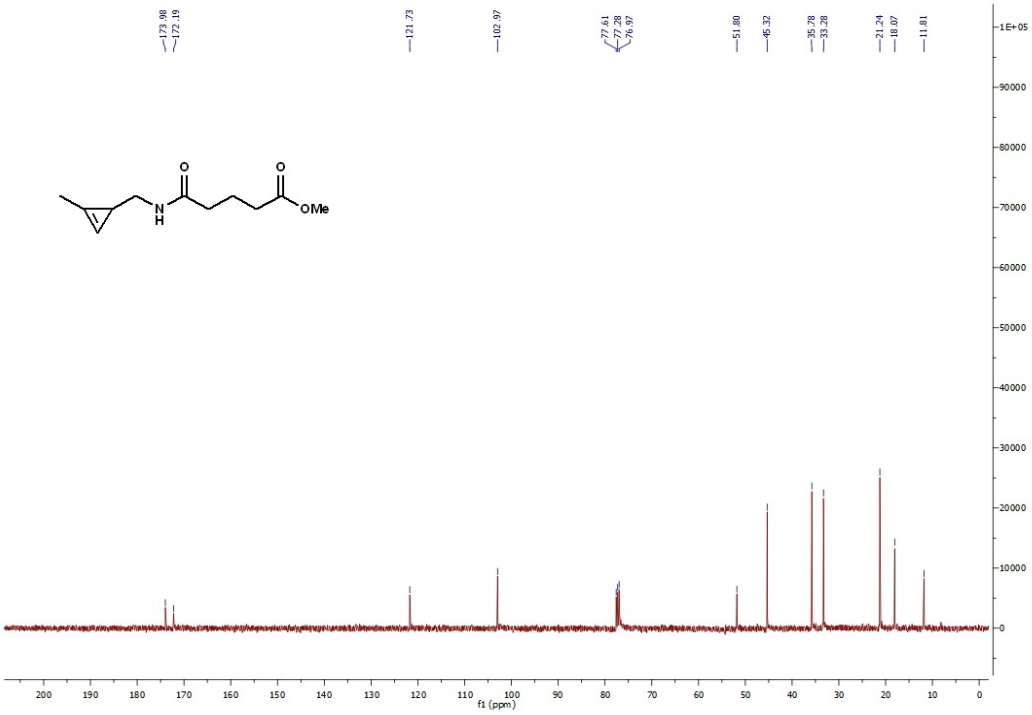

8.59
8.57
8.57
7.59
7.59
7.57
7.57
7.26
4.83
1.62
OH
1.00
1.03
1.05
4.90
f1 (ppm)

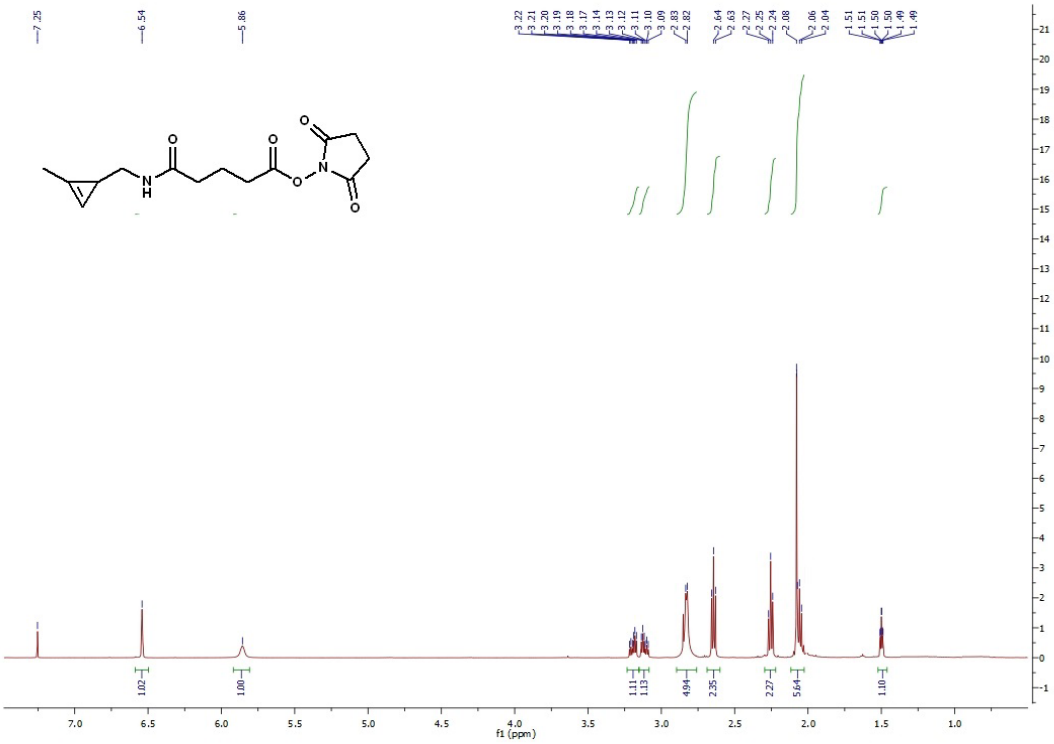

175.62
163.49
145.67
131.11
128.17
127.50
77.38
77.12
76.87
64.83
37.99
29.27
29.26
OH
f1 (ppm)

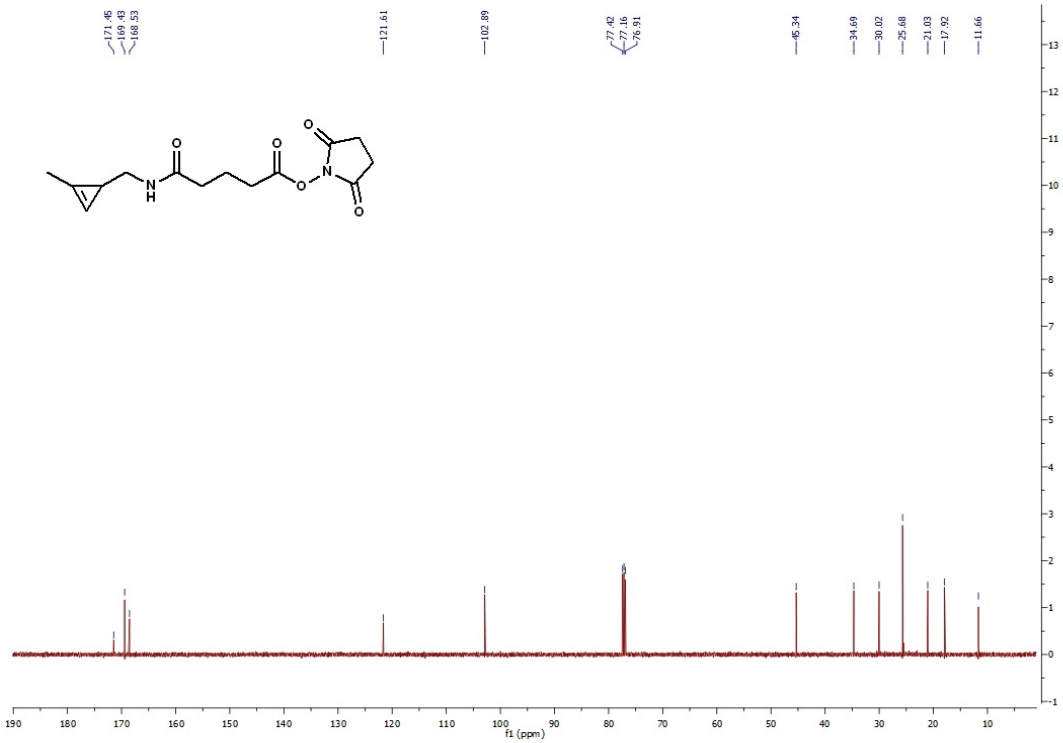

TMS
O
N
H
O
OMe
7.26
5.41
3.64
3.64
3.19
3.15
3.14
3.08
3.06
3.03
2.38
2.36
2.34
2.15
1.95
1.93
1.91
1.42
1.41
1.39
0.13
0.87
3.10
1.00
1.06
2.15
5.41
2.38
1.01
9.19
f1 (ppm)

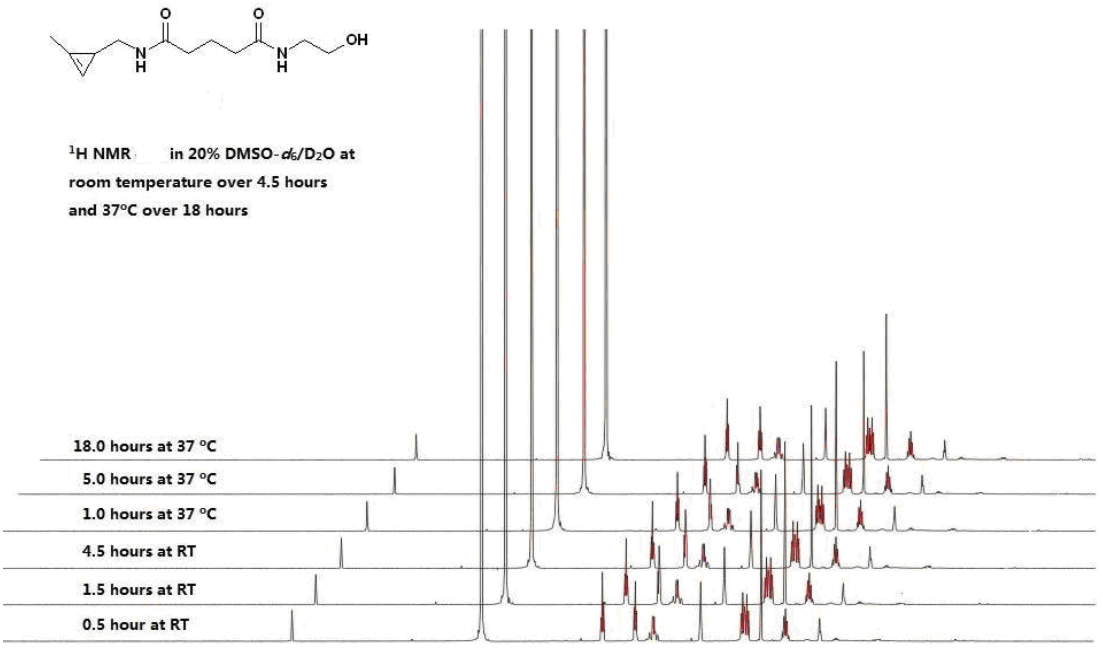

TMS
O
N
H
O
OMe
173.92
171.87
136.05
111.83
77.59
77.27
76.95
51.78
46.31
35.84
33.28
21.21
19.48
13.37
0.86
f1 (ppm)

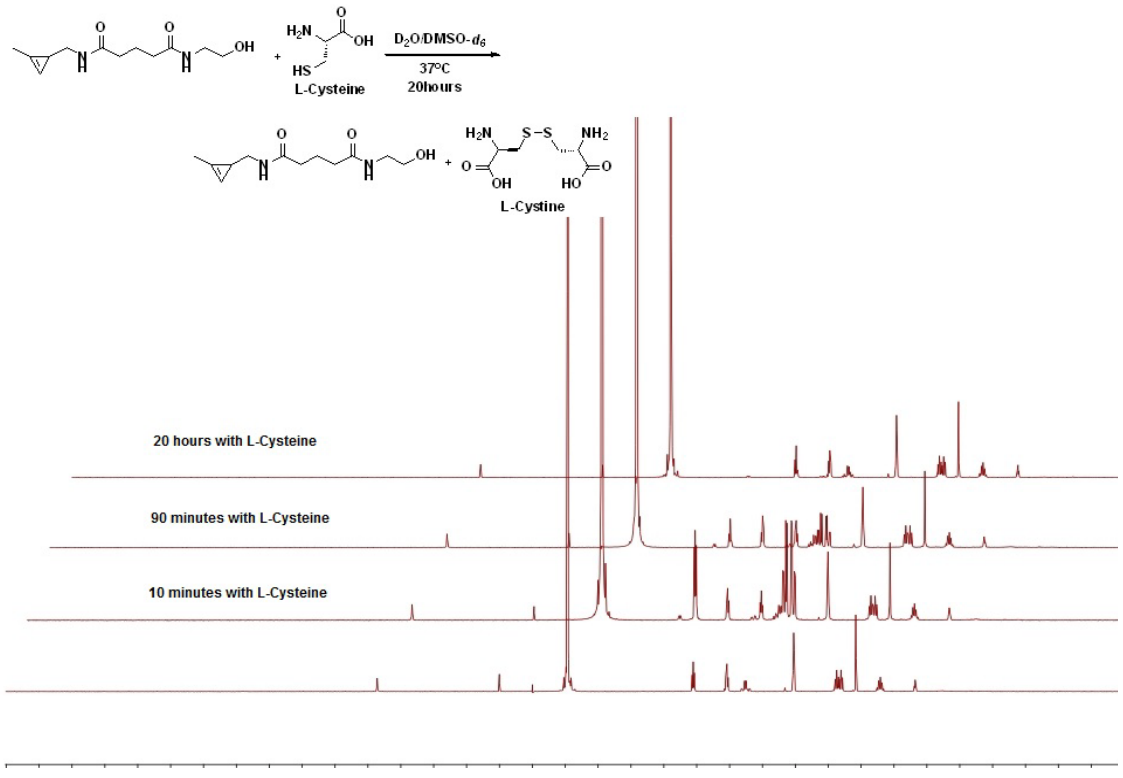

7.26
6.54
5.62
3.63
3.18
3.13
3.11
3.10
3.09
3.08
3.07
2.36
2.34
2.32
2.17
2.08
1.88
1.51
1.51
1.50
1.50
1.49
1.49
O
O
N
H
OMe
1.00
0.93
3.45
1.13
1.16
2.38
2.46
3.38
2.61
1.24
f1 (ppm)

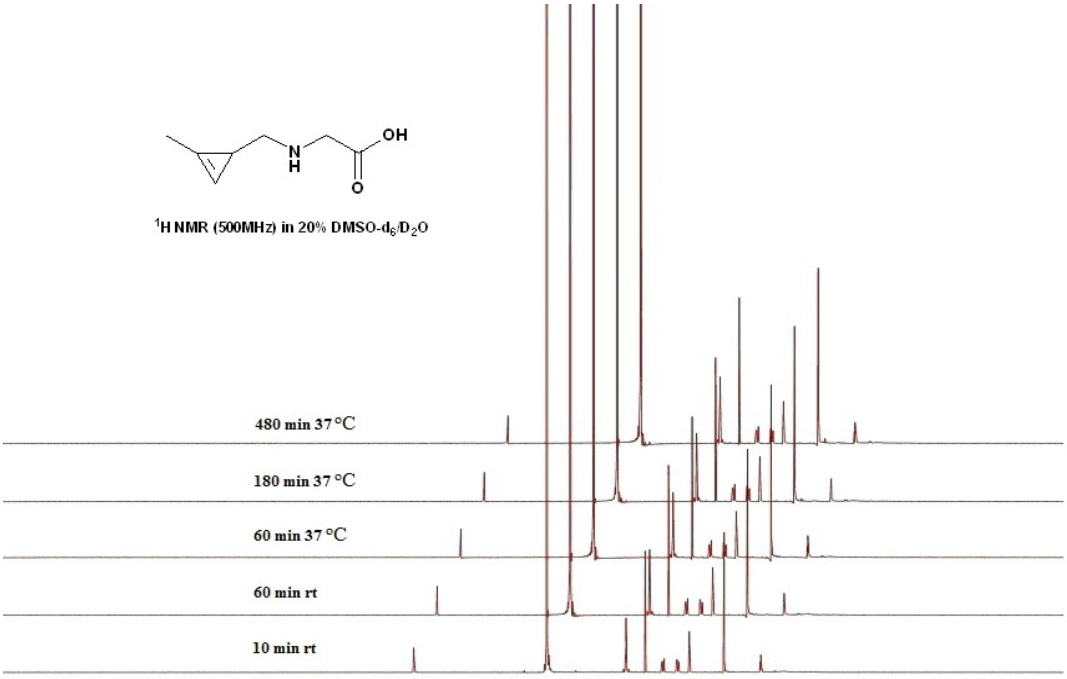

173.98
172.19
121.73
102.97
77.61
77.28
76.97
51.80
46.32
35.78
33.28
21.24
18.07
11.81
O
O
N
H
OMe
f1 (ppm)

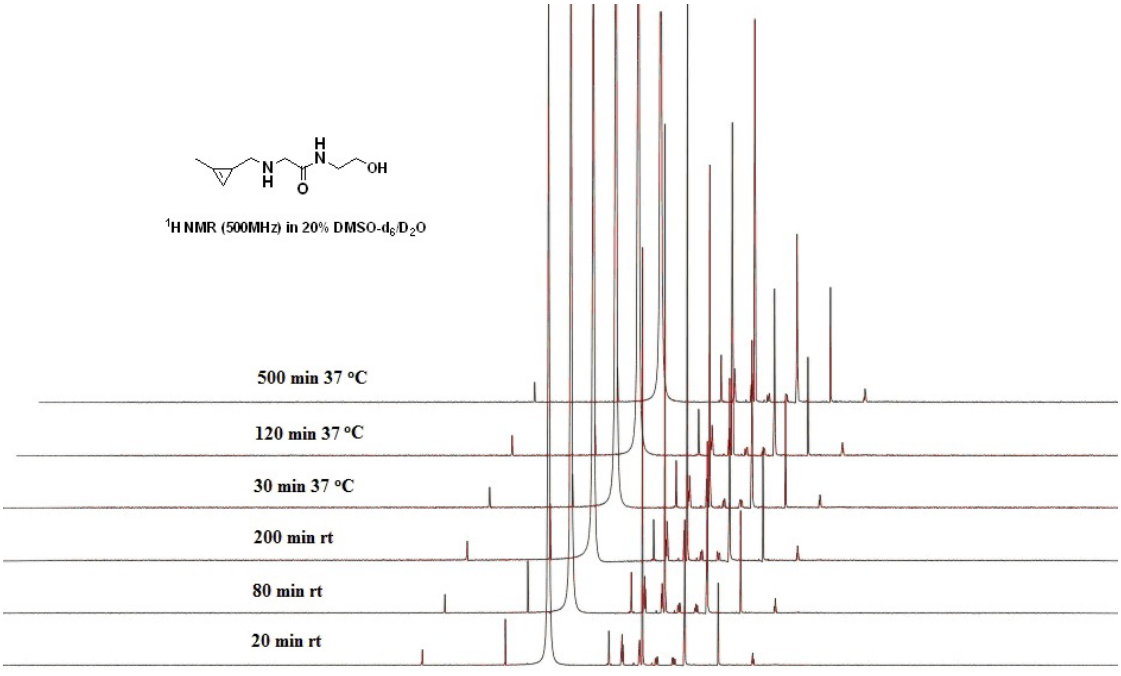

7.25
6.54
5.86
3.22
3.21
3.20
3.19
3.18
3.17
3.14
3.13
3.12
3.11
3.10
3.09
2.83
2.82
2.64
2.63
2.27
2.25
2.24
2.08
2.06
2.04
1.51
1.51
1.50
1.50
1.49
1.49
1.02
1.00
1.11
1.13
4.94
2.35
2.27
5.64
1.10
f1 (ppm)

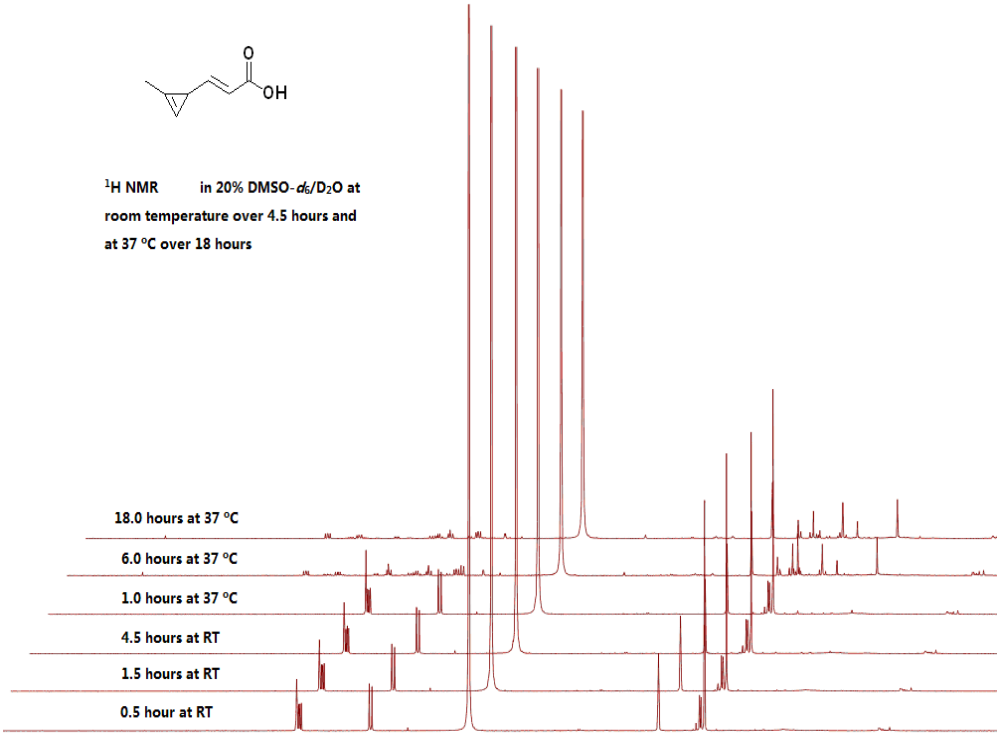

171.45
169.43
168.53
121.61
102.89
77.42
77.16
76.91
45.34
34.69
30.02
25.68
21.03
17.92
11.66
f1 (ppm)

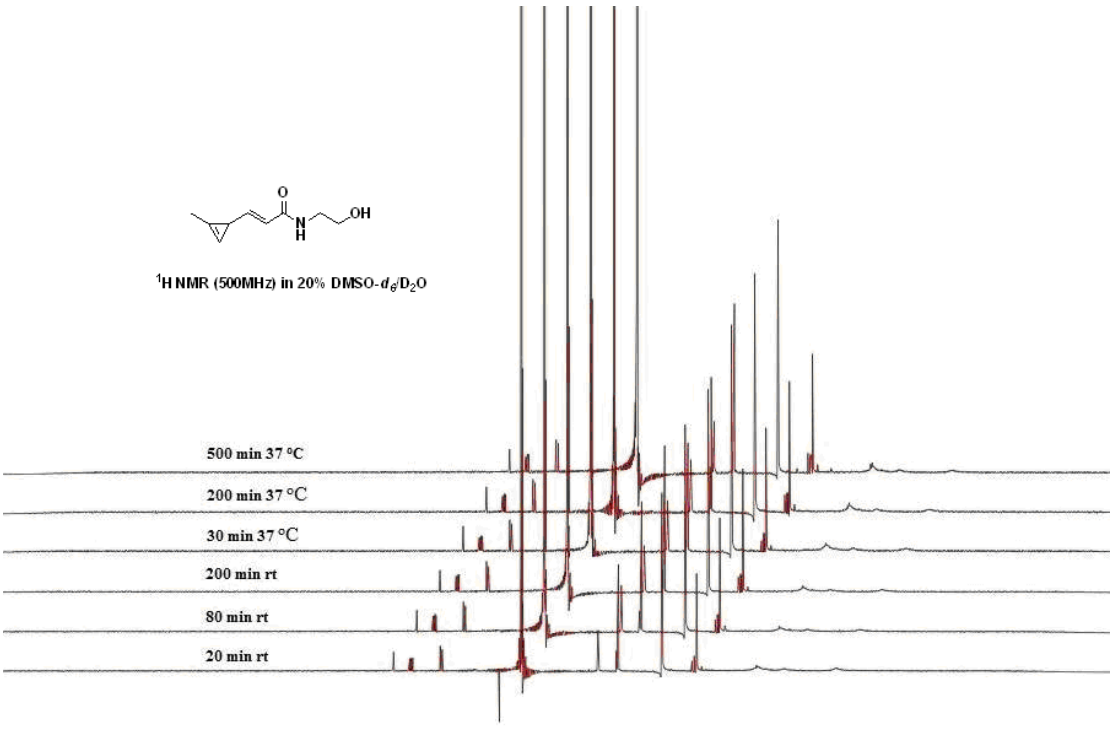

^{1}H NMR in 20% DMSO-d_6/D_2O at
room temperature over 4.5 hours
and 37°C over 18 hours
18.0 hours at 37 °C
5.0 hours at 37 °C
1.0 hours at 37 °C
4.5 hours at RT
1.5 hours at RT
0.5 hour at RT

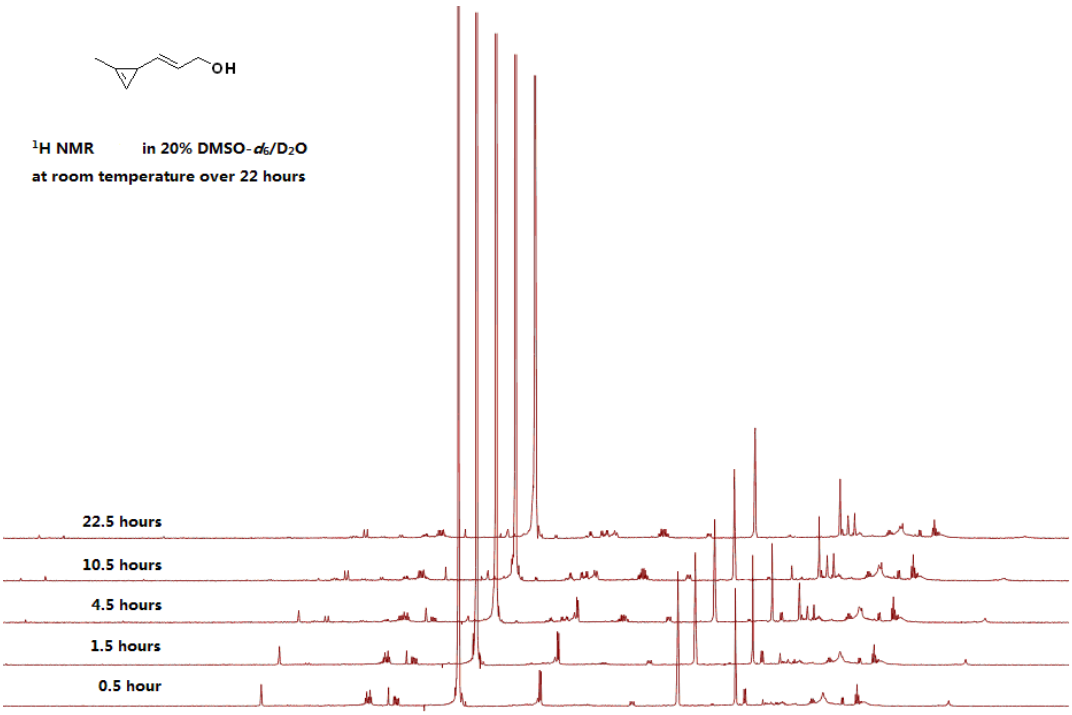

L-Cysteine
D_2O/DMSO-d_6
37°C
20hours
L-Cystine
20 hours with L-Cysteine
90 minutes with L-Cysteine
10 minutes with L-Cysteine

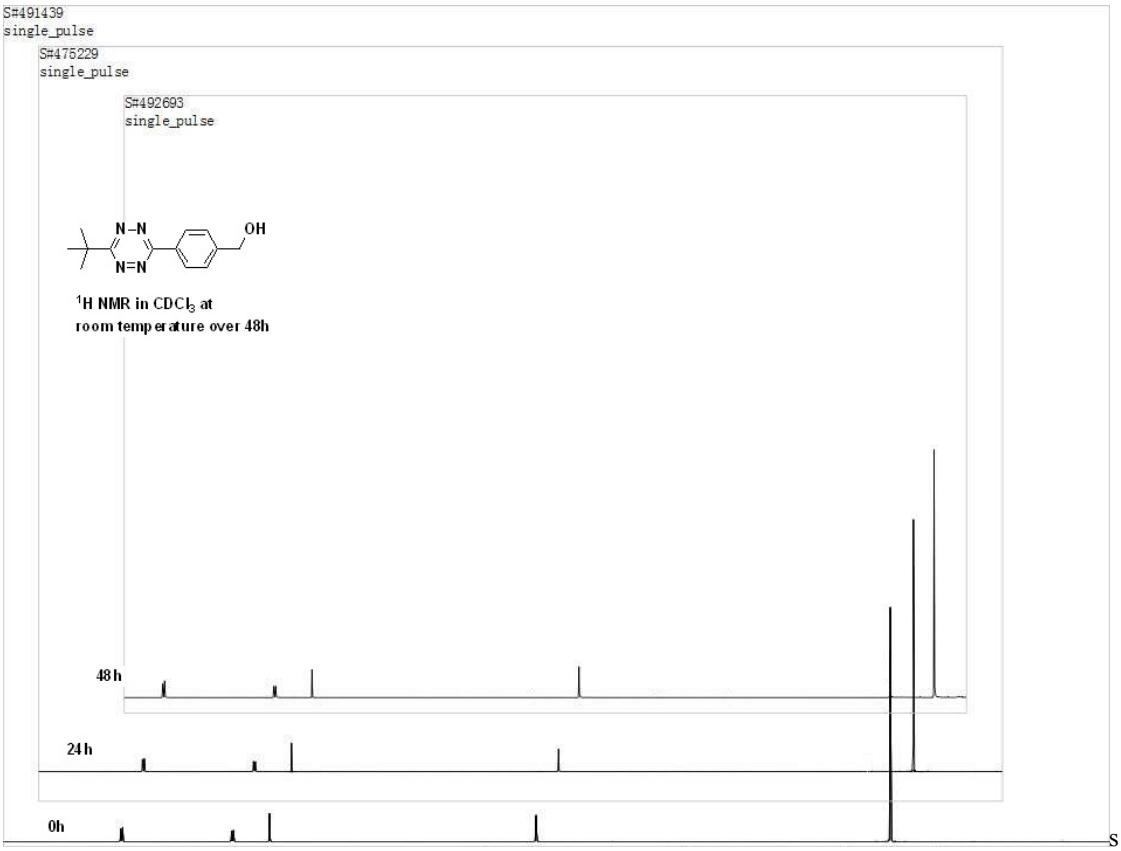

OH
N
H
O
^{1}H NMR (500MHz) in 20% DMSO-d_6/D_2O
480 min 37 °C
180 min 37 °C
60 min 37 °C
60 min rt
10 min rt


H
N
N
H
O
OH
^{1}H NMR (500MHz) in 20% DMSO-d_6/D_2O
500 min 37 °C
120 min 37 °C
30 min 37 °C
200 min rt
80 min rt
20 min rt

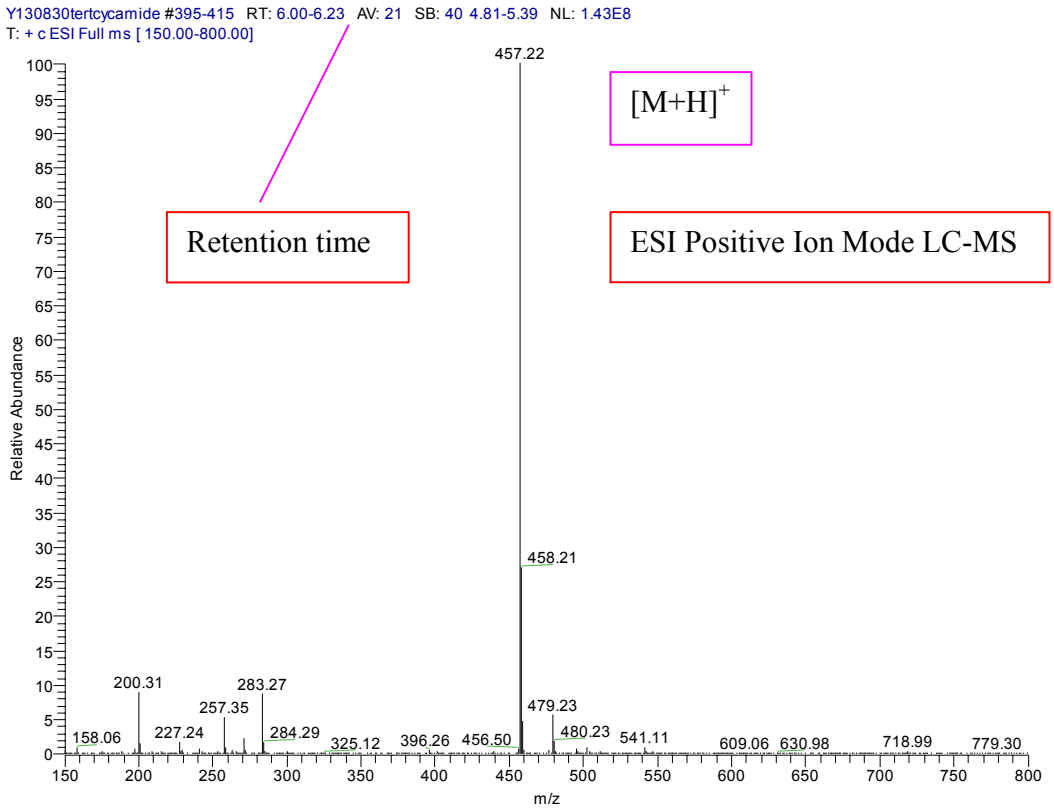

O
OH
^{1}H NMR in 20% DMSO-d_6/D_2O at
room temperature over 4.5 hours and
at 37 °C over 18 hours
18.0 hours at 37 °C
6.0 hours at 37 °C
1.0 hours at 37 °C
4.5 hours at RT
1.5 hours at RT
0.5 hour at RT

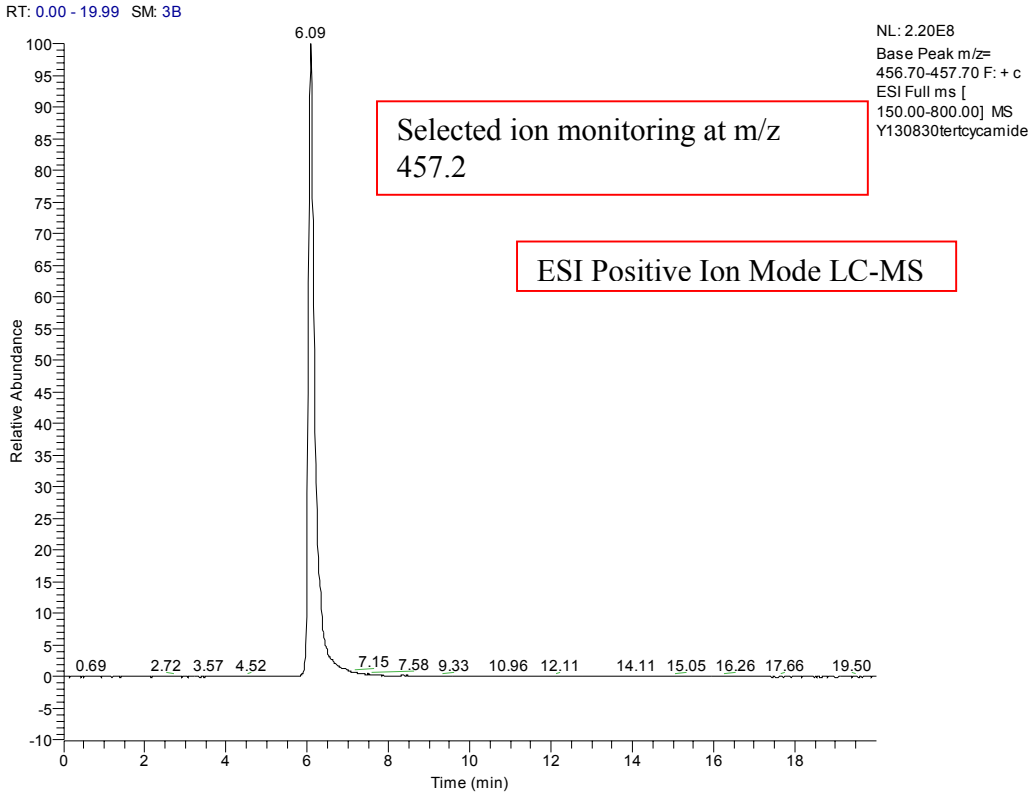

O
N
H
OH
^{1}H NMR (500MHz) in 20% DMSO-d_6/D_2O
500 min 37 °C
200 min 37 °C
30 min 37 °C
200 min rt
80 min rt
20 min rt

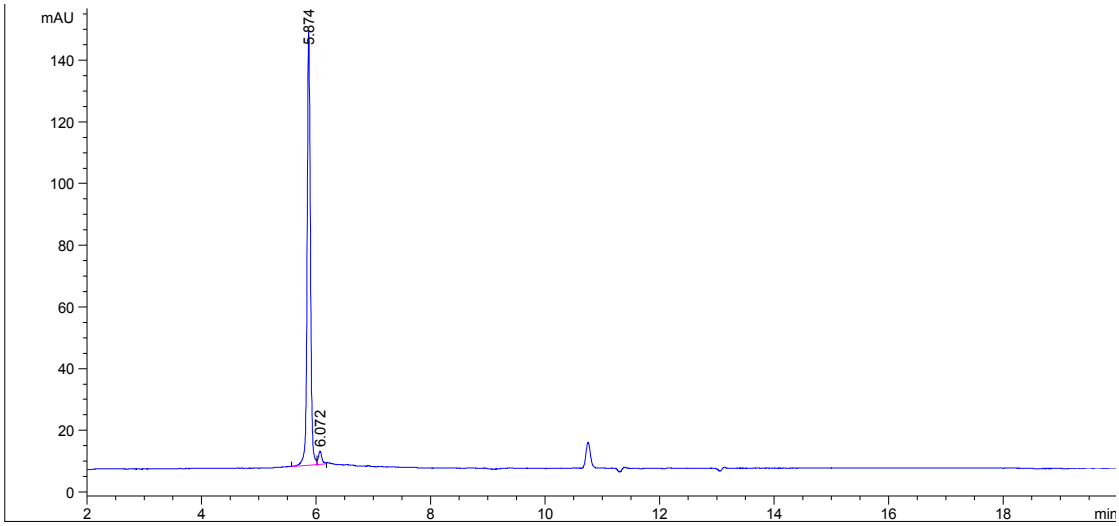

OH
^{1}H NMR in 20% DMSO-d_6/D_2O
at room temperature over 22 hours
22.5 hours
10.5 hours
4.5 hours
1.5 hours
0.5 hour



ample was kept in ambient lighting during stability experiment


Y130830tertcycamide #395-415 RT: 6.00-6.23 AV: 21 SB: 40 4.81-5.39 NL: 1.43E8
T: + c ESI Full ms [150.00-800.00]
457.22
[M+H]+
Retention time
ESI Positive Ion Mode LC-MS
458.21
200.31
283.27
257.35
479.23
158.06
227.24
284.29
325.12
396.26
456.50
480.23
541.11
609.06
630.98
718.99
779.30
Relative Abundance
m/z


RT: 0.00 - 19.99 SM: 3B
NL: 2.20E8
Base Peak m/z= 456.70-457.70 F: + c ESI Full ms [150.00-800.00] MS Y130830tertcycamide
6.09
Selected ion monitoring at m/z 457.2
ESI Positive Ion Mode LC-MS
0.69
2.72
3.57
4.52
7.15
7.58
9.33
10.96
12.11
14.11
15.05
16.26
17.66
19.50
Relative Abundance
Time (min)




```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MWD1 B, Sig=280,6 Ref=600,20
 Signal has been modified after loading from rawdata file!

Peak RetTime Type  Width     Area      Height     Area
  #    [min]         [min]    [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   5.874 BV    0.0604  561.36523  141.14607  96.7316
   2   6.072 VB    0.0644   18.96776    4.30873   3.2684
```