Reaction contest: hydrolysis *versus* intramolecular cyclisation reaction in alkyl squaramate esters

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1. Materials and general methods

Chemicals were of commercial origin (Aldrich or Scharlau) and were used as received. ^{1}H, ^{13}C and 2D NMR spectra (at 300 and 600 MHz) and ^{13}C (at 75 and 150 MHz) spectra were recorded on 300 and 600 MHz spectrometers in $CDCl_3$, D_2O or DMSO-d_6 solutions at room temperature. The residual proton signal was used as a reference. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad).

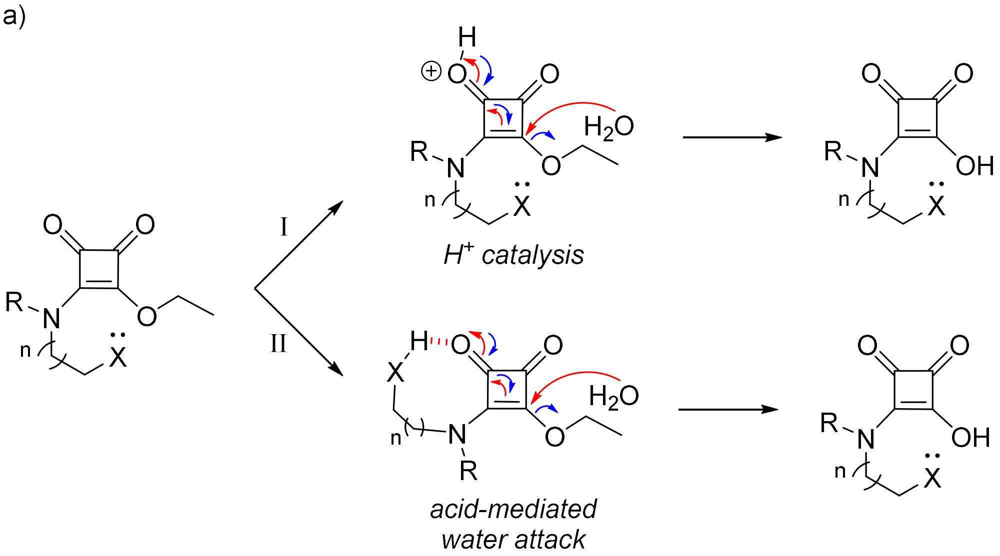
ESI-HRMS mass spectra were recorded on a magnetic sector on an Orbitrap mass spectrometer.

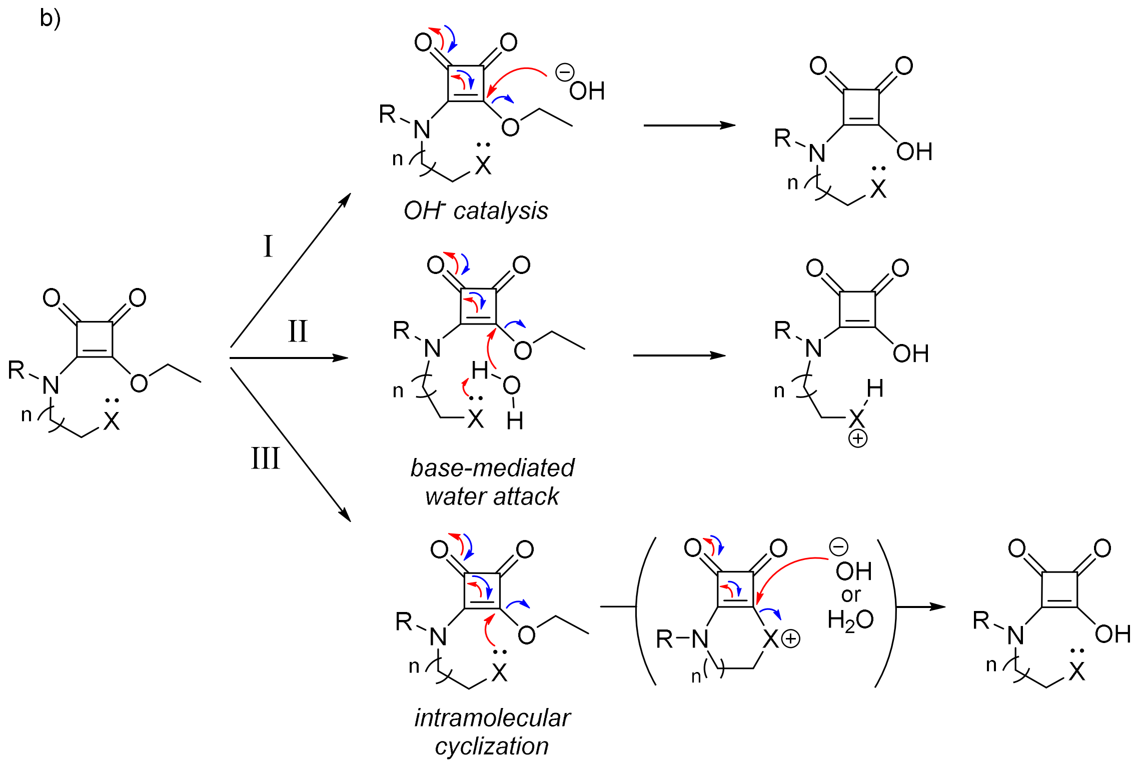
2. Kinetic Experiments

The experiments were carried out on a VARIAN Cary 300 Bio UV-vis spectrophotometer at 37.0 ± 0.1 ºC in water. Squaramate esters were dissolved in 10-2 M buffered solutions (formic acid, acetic acid, cacodylate, PBS and borate) at a final concentration of 30 µM. The ionic strength was 0.15 M NaCl. For the study of squaramate ester **11b**, the parent disulfide **11a** is dissolved in DMSO (30 mM) and reduced *in situ* by addition of 3 equiv of TCEP. The solution is stirred for 10 min to obtain quantitatively **12c**. The resulting solution is diluted to a final concentration of 30 µM with the corresponding buffer for the kinetic studies. The changes in the UV range were analysed with the ReactLabTM Kinetics software (Jplus Consulting Ltd.).[1]

2.1. Proposed hydrolysis mechanisms for squaramate esters

a)





Scheme S1. Proposed mechanisms for the hydrolysis of squaramate esters. a) acidic hydrolysis through acid catalysis by carbonyl protonation (I) and assisted acid-mediated water attack (II). b) alkaline hydrolysis catalysed by direct hydroxide attack (I), assisted base-mediated water attack (II) and assisted intramolecular cyclization and subsequent intermediate-ring opening(III).

2.2. Rate Law for the hydrolysis reaction

$$\mathrm{RX} + \mathrm{H_2O} \rightarrow \mathrm{ROH} + \mathrm{HX}$$

$$-\frac{d(\mathrm{RX})}{dt} = k_\mathrm{h}[\mathrm{RX}] = k_\mathrm{OH}[\mathrm{OH}][\mathrm{RX}] + k_\mathrm{H}[\mathrm{H}][\mathrm{RX}] + k_\mathrm{N}{}'[\mathrm{H_2O}][\mathrm{RX}] \quad (1)$$

Where k_{OH}, k_H and k_N' are the second order rate constants for the base- and acid-catalysed and the neutral processes, respectively.

For processes which are first order respect to the sustrate, and considering the constant value $k_N = k_N'[H_2O]$. Eq. (1) is transformed in Eq (2):

$$k_\mathrm{h} = k_\mathrm{OH}[\mathrm{OH}] + k_\mathrm{H}[\mathrm{H}] + k_\mathrm{N} \quad (2)$$

Considering:

$$[\mathrm{OH}][\mathrm{H}] = K_\mathrm{w} \quad (3)$$

$$k_{\mathrm{h}} = k_{\mathrm{OH}}\frac{K_{\mathrm{w}}}{[\mathrm{H}]} + k_{\mathrm{H}}[\mathrm{H}] + k_{\mathrm{N}} \qquad (4)$$

Therefore, for experiments in alkaline media and pseudo-first order conditions:

$$k_{\mathrm{obs}} = k_{\mathrm{OH}}[\mathrm{OH}] = k_{\mathrm{OH}}\frac{K_{\mathrm{w}}}{[\mathrm{H}]} \qquad (2')$$

Where K_w = 2.04 x 10^{-14} at 37 °C[2]

2.3. Rate Law for the NGP assisted hydrolysis reaction

$$k_{\mathrm{obs}} = k_1[\mathrm{A}] + k_{\mathrm{OH}}[\mathrm{OH}] = k_1\frac{K_{\mathrm{a}}}{K_{\mathrm{a}}+[\mathrm{H}]} + k_{\mathrm{OH}}\frac{K_{\mathrm{w}}}{[\mathrm{H}]} \qquad (5)$$

where $K_a/(K_a+[H])$ is the concentration of squaramate ester in its free unprotonated amino base form ([A]) and k_1 is the rate constant for the assisted hydrolysis.

We assumed that the ionization of the squaramidic nitrogen is negligible at the pH interval studied (pK_a ≥ 12)

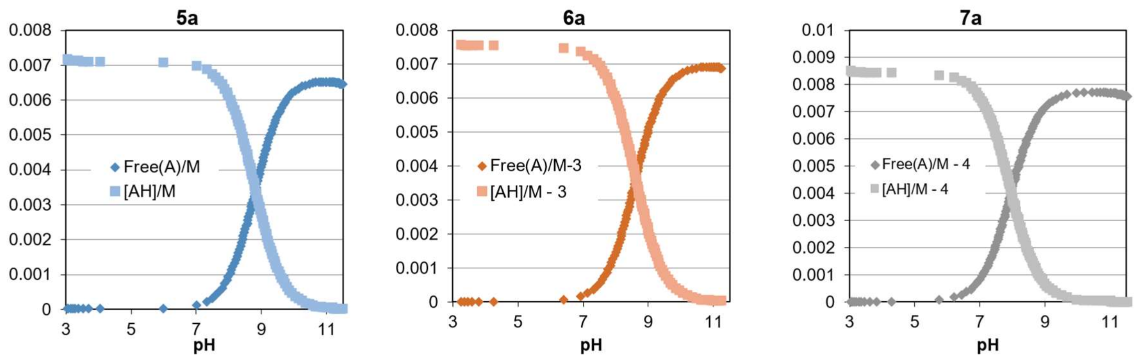


Figure S1. Potentiometric titration for squaramate esters **5a**, **6a** and **7a**. pKa values obtained are 8.66, 8.66 and 7.96, respectively.

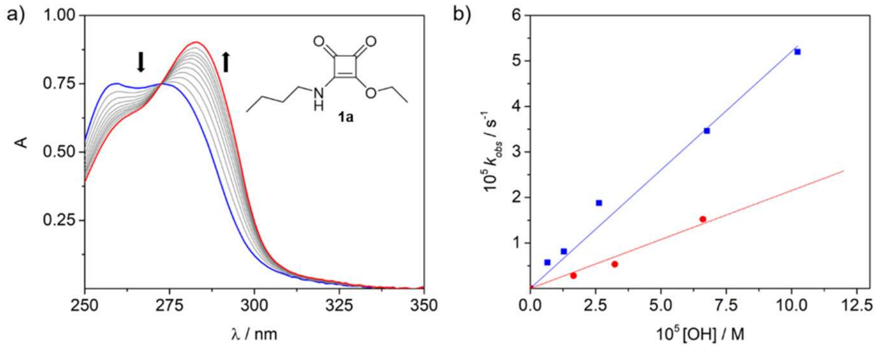


Figure S3. a) Representative example of the changes in the UV spectra observed during the hydrolysis of 30 μM squaramate ester **1a** (10 mM carbonate buffer pH 10, 37 ºC). b) k_{obs} vs [OH(D)] plots for hydrolysis of ester **1a** in H_2O (blue) and D_2O (red).

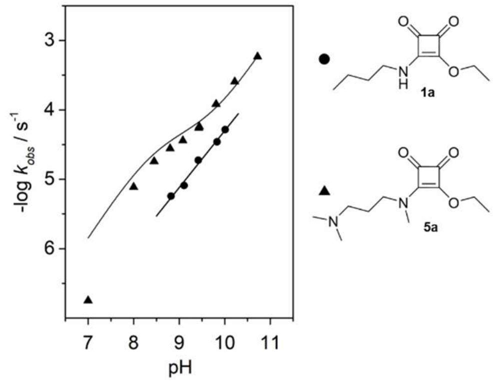


Figure S4. Comparison between hydrolysis rate pH-dependent profiles obtained for the hydrolysis of **1a** (dots) and **5a** (triangles) at 37 ºC.

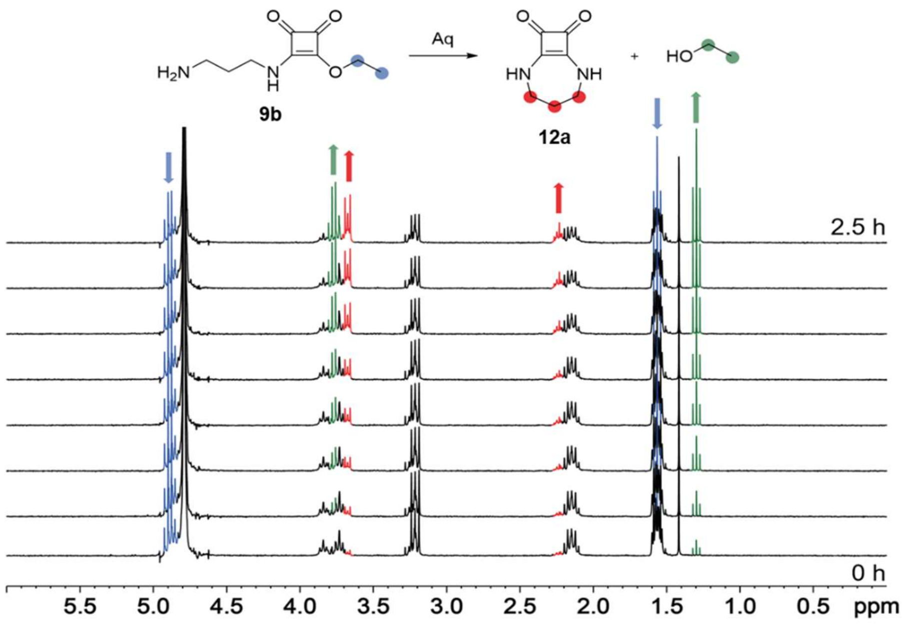
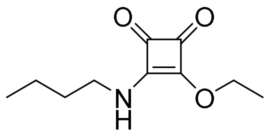


Figure S5.^{1}H-NMR evolution over time of 1 mM squaramate ester **9b** at 0.1 M PBS at pH 7.0 (24 ºC) to give cyclic compound **12a**.

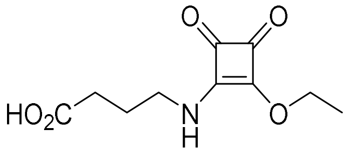
3. Synthetic methods

<u>General procedure for the synthesis of esters:</u> Diethyl squarate (500 mg, 2.94 mmol) in CH_3CN (3 mL) and one equivalent of the corresponding amine were mixed in a round-bottom flask and the mixture was stirred under nitrogen at room temperature for 16h. The crude mixture was concentrated to dryness under reduced pressure and purified by column chromatography (SiO_2) to afford squaramate esters **1a-8a** and **9a** and **9a**. Boc-protected **8a**, **9a** and **9b** were treated with TFA in CH_2Cl_2 to afford the ammonium salts **8b** and **9a and 9b** as trifluoroacetate salts.



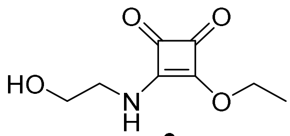
1a

*3-(butylamino)-4-ethoxycyclobut-3-ene-1,2-dione (**1a**).* White solid, 562 mg, 97% yield. Analytical data are in accordance with those reported in literature.[3]



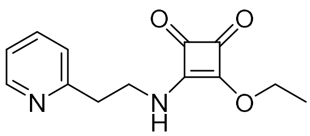
2a

*4-((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)butanoic acid (**2a**).* Yellow solid, 554 mg, 83% yield. Analytical data are in accordance with those reported in literature.[3]



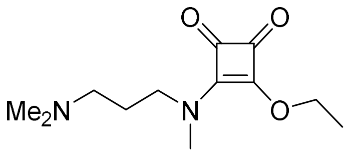
3a

*3-ethoxy-4-((2-hydroxyethyl)amino)cyclobut-3-ene-1,2-dione (**3a**).* White solid, 424 mg, 78% yield. Analytical data are in accordance with those reported in literature.[3]



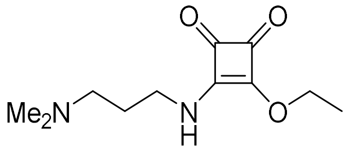
4a

*3-ethoxy-4-((2-(pyridin-2-yl)ethyl)amino)cyclobut-3-ene-1,2-dione (**4a**).* White solid, 51 mg, 41% yield. Analytical data are in accordance with those reported in literature.[4]



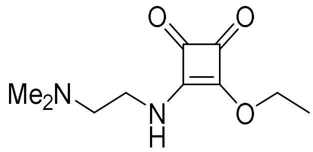
5a

*3-((3-(dimethylamino) propyl) (methyl) amino) - 4 - ethoxycyclobut-3-ene-1,2-dione (**5a**).* Pale yellow oil, 692 mg, 98% yield. Analytical data are in accordance with those reported in literature.[3]



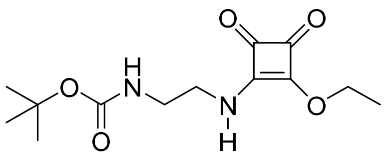
6a

*3 - ((3- (dimethylamino) propyl) amino) - 4-ethoxycyclobut-3-ene-1,2-dione (**6a**).* Pale yellow solid, 605 mg, 91% yield. Analytical data are in accordance with those reported in literature.[3]



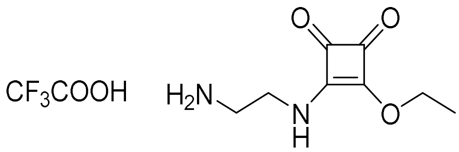
7a

*3-((2-(dimethylamino)ethyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione (**7a**).* Pale yellow solid, 592 mg, 95% yield. Analytical data are in accordance with those reported in literature.[3]



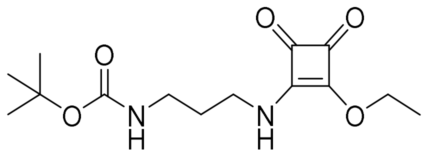
8a

*tert-butyl (2 -((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl) amino) ethyl) carbamate (**8a**).* Silica-gel column chromatography (dichloromethane/tetrahydrofuran gradient = 20:1). Pale oil, 519 mg, 61%. Analytical data are in accordance with those reported in literature[5] ^{1}H-RMN (300 MHz, $CDCl_3$): δ 1.43 (br s, 12H), 3.36 (dd, J = 4.8 Hz, J = 6 Hz, 2H), 3.56 (br s, 1.3H), 3.75 (br s, 0.7H), 4.74 (q, J = 7.2 Hz, 1.3H), 4.86 (br s, 0.7H), 5.93 (br s, 0.5H), 6.37 (br s, 0.5H). ^{13}C-RMN (75 MHz, $CDCl_3$): δ 16.0, 28.4, 40.8, 45.3, 69.9, 79.9, 156.3, 172.9, 177.8, 182.8, 189.8. ESI (+)-HRMS: m/z (%) calcd for $C_{13}H_{21}N_2O_5^+$ $[M+H]^+$ 285,1445; found 285,1446.



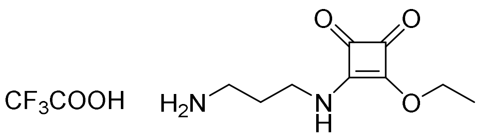
8b

*2- ((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl) amino) ethan - 1 - aminium - trifluoroacetate (**8b**).* Boc-protected ester **8a** (500 mg, 1.76 mmol) was dissolved in DCM (20 ml) followed by 700 µL of TFA (8.80 mmol). The reaction was stirred at room temperature for 8h and the solvent was removed under rotary evaporation. The remaining TFA was removed by suspending the resulting oil in hexane (× 3) yielding **8b** as a white amorphous solid, 514 mg, yield 98%. Analytical data are in accordance with those reported in literature.[6] ^{1}H-RMN (300 MHz, DMSO-d_6): δ = 1.37 (t, J = 6.9 Hz, 3H), 3.00 (m, J = 6 Hz, 2H), 3.52 (br, 0.9H), 3.70 (br, 1.1H), 4.66 (q, J = 6.9 Hz, 2H), 7.864 (br s, 3H), 8.576 (br s, 0.5H), 8.72 (br s, 0.5H). ^{13}C-RMN (75 MHz, CDCl$_3$): δ =15.6, 41.1, 41.5, 69.0, 172.1, 176.9, 182.4, 188.2. ESI (+)-HRMS: m/z (%) calcd for $C_8H_{13}N_2O_3^+$ [M+H]$^+$ 185,0921; found 185,0921.



9a

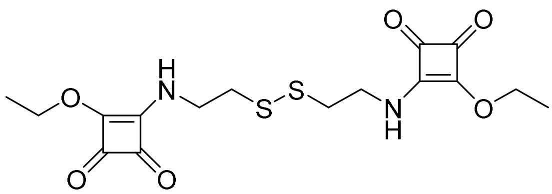
*tert-butyl (3-((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)propyl)carbamate (**9a**).* Silica-gel column chromatography (dichloromethane/tetrahydrofuran gradient = 20:1). Pale oil, 479 mg, 91% yield. %. Analytical data are in accordance with those reported in literature[¡Error! Marcador no definido.] ^{1}H-RMN (300 MHz, CDCl$_3$): δ 1.44 (s, 9H), 1.46 (t, J = 6.9 Hz, 3H), 1.72 (m, 2H), 3.23 (br s, 2H), 3.47 (br s, 1.1H), 3.69 (br, 0.9H), 4.77 (q, J = 6.9 Hz, 2H), 6.68 (br, 1H), 6.93 (br s 1H). ^{13}C-RMN (75 MHz, CDCl$_3$): δ 16.0, 28.5, 30. 5, 31.5, 32.2, 36.5, 36.9, 41.0, 41.7, 69.8, 80.0, 156.8, 157.5, 172.8, 177.2, 177.8, 183.2, 183.4, 189.1, 189.4. ESI (+)-HRMS: m/z (%) calcd for $C_{14}H_{22}N_2NaO_5^+$ [M+Na]$^+$ 321.1421; found 321.1420.



9b

*3- ((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl) amino) propan-1-aminium-trifluoroacetate (**9b**).* Boc-protected ester **9a** (120 mg, 0.40 mmol) was dissolved in DCM (10 ml) followed by 157 µL of TFA (2.00 mmol). The reaction was stirred at room temperature for 8h and the solvent was removed under rotary evaporation. The remaining TFA was removed by suspending the resulting oil in hexane (× 3) yielding a white solid, 122 mg, 97%. Analytical data are in accordance with those reported in literature[6] ^{1}H-RMN (300 MHz, DMSO-d_6): δ 1.38 (t, J = 7.0 Hz, 3H), 1.79 (m, J = 7.5

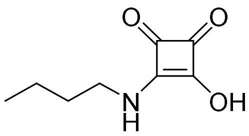
Hz, 2H), 2.83 (m, J = 6.5 Hz, 2H), 3.35 (q, J = 6.9 Hz, 1H), 3.53 (q, J = 7.2 Hz, 1H), 4.66 (q, J = 7.1 Hz, 2H), 7.68 (br s, 3H), 8.62 (br s, 0.5H), 8.79 (br s, 0.5H). ^{13}C-RMN (75 MHz, DMSO-d_6): δ 16.2, 29.2, 36.9, 41.1, 69.9, 172.1, 174.8, 186.0, 189.5. ESI (+)-HRMS: m/z (%) calcd for $C_9H_{15}N_2O_3^+$ $[M+H]^+$ 199.1077; found 199.1077.



11a

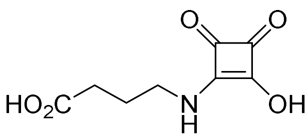
*4,4' - ((disulfanediylbis(ethane-2,1-diyl)) bis (azanediyl)) bis(3-ethoxycyclobut-3-ene-1,2-dione) (**11a**).* Cystamine dihydrochloride (330 mg, 1.47 mmol) was suspended in CH_3CN (20 mL). After the addition of DIPEA (1.5 mL, 17.64 mmol) the mixture was stirred at room temperature for 10 minutes and then, diethyl squarate (500 mg, 2.94 mmol) was added at once. The reaction was stirred at 50 ºC for 6h and the solvent was removed under rotary evaporation. The resulting crude mixture was purified by silica-gel column chromatography (CH_2Cl_2/CH_3CN 1:1 v/v) to afford **11a** as an amorphous white solid, 383 mg (yield 76%). ^{1}H-RMN (300 MHz, DMSO-d_6): δ 1.37 (t, J = 7.2 Hz, 6H), 2.91 (t, J = 6.0 Hz, 4H), 3.59 (q, J = 6.0 Hz, 2.1H), 3.77 (q, J = 6.0 Hz, 1.9H), 4.65 (q, J =7.2 Hz, 4H), 8.69 (br s, 0.9H), 8.88 (br s, 1.1H). ^{13}C-RMN (75 MHz, DMSO-d_6): δ = 15.7, 37.8, 38.2, 42.6, 69.0, 172.4, 172.8, 176.9, 177.1, 182.2, 182.4, 189.1, 189.2. ESI (+)-HRMS: m/z (%) calcd for $C_{16}H_{20}N_2NaO_6S_2^+$ $[M+Na]^+$ 423.0660; found 423.0663.

<u>*General procedure for the synthesis of acids* **1c**-**8c**:</u> Squaramate acids **1c**-**8c** were obtained by treatment of the corresponding esters with H_2O at 60 ºC for 16h. Removal of the solvent by rotary evaporation afforded the final product.



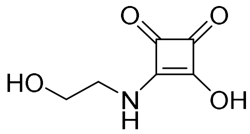
1c

*3-(butylamino)-4-hydroxycyclobut-3-ene-1,2-dione (**1c**).* White solid, 38 mg, 89% yield. Analytical data are in accordance with those reported in literature.3



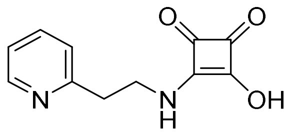
2c

*4-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl)amino)butanoic acid (**2c**).* White solid, 40 mg, 88% yield. Analytical data are in accordance with those reported in literature.[3]



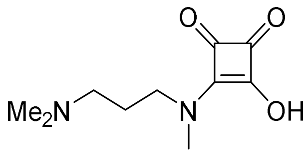
3c

*3-hydroxy-4-((2-hydroxyethyl)amino)cyclobut-3-ene-1,2-dione (**3c**).* White solid, 26 mg, yield 99%. ^{1}H-RMN (300 MHz, D_2O): δ = 3.56 (t, J = 5.4 Hz, 2H), 3.61 (t, J = 5.4 Hz, 2H).. ^{13}C-RMN (75 MHz, DMSO-d_6): δ = 45.8, 60.8, 167.9, 183.5, 184.5, 185.2. ESI (-)-HRMS: m/z (%) calcd for $C_6H_6NO_4^-$ $[M]^-$ 156.0302; found 156.0299.[3]



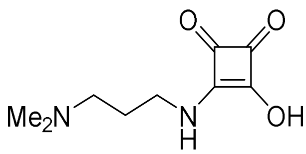
4c

*3-hydroxy-4-((2-(pyridin-2-yl)ethyl)amino)cyclobut-3-ene-1,2-dione (**4c**).* White solid, 16 mg, 95% yield. Analytical data are in accordance with those reported in literature.[7]



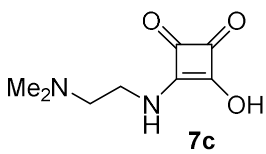
5c

*3-((3-(dimethylamino)propyl)(methyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione (**5c**).* White solid, 23 mg, 97% yield. Analytical data are in accordance with those reported in literature.[3]



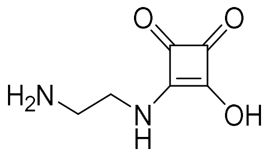
6c

*3-((3-(dimethylamino)propyl)amino)-4-ethoxycyclobut-3-ene-1,2-dione (**6c**).* Pale ochre amorphous solid, 18 mg, 94% yield. Analytical data are in accordance with those reported in literature.[3]



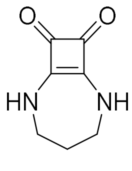
7c

*3-((2-(dimethylamino)ethyl)amino)-4-hydroxycyclobut-3-ene-1,2-dione (**7c**).* White solid, 22 mg, yield 85%. Analytical data are in accordance with those reported in literature.[8]



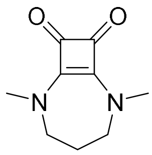
8c

*3-((2-aminoethyl)amino)-4-hydroxycyclobut-3-ene-1,2-dione (**22**).* White solid, 39 mg, yield 92%. Analytical data are in accordance with those reported in literature.[9] ^{1}H-RMN (300 MHz, D_2O): δ 2.67 (t, J = 6.0 Hz, 2H), 3.43 (t, J = 6.0 Hz, 2H). ^{13}C-RMN (75 MHz, DMSO-d_6): δ 25.7, 43.0, 183.1, 184.5, 185.2. ESI (-)-HRMS: m/z (%) calcd for $C_6H_7N_2O_3^-$ $[M]^-$ 155.0462; found 155.0456.



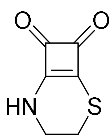
12a

*2,6-diazabicyclo[5.2.0]non-1(7)-ene-8,9-dione (**12a**).* White prisms, 760 mg, yield 84%. Prepared as reported in the literature.[10]



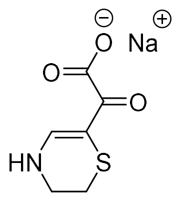
12b

*2,6-dimethyl-2,6-diazabicyclo[5.2.0]non-1(7)-ene-8,9-dione (**12b**).* White needles. 0.71 g, yield 67%. Prepared as reported in the literature.[10]



12c

*2-thia-5-azabicyclo[4.2.0]oct-1(6)-ene-7,8-dione (**11c**).* To 453 mg (5.88 mmol) of cysteamine dissolved in 21 mL of H_2O were added 1 g (5.88 mmol) of ethyl squarate dissolved in 30 mL of EtOH. The crude reaction was stirred at room temperature for 1h and the resulting precipitate was filtered out and washed with H_2O (3 × 5 mL) and Et_2O (3 × 5 mL). Pale yellow solid, 411 mg, yield 45%. ^{1}H-RMN (300 MHz, DMSO-d_6, D_2O: 20%): δ = 3.22 (m, J = 4.8 Hz, 2H), 3.81 (m, J = 4.8 Hz, 2H). ^{13}C-RMN (75 MHz, DMSO-d_6): δ = 25.2, 46.2, 161.2, 178.4, 186.2, 187.0. ESI (+)-HRMS: m/z (%) calcd for $C_6H_6NO_2S^+$ $[M+H]^+$ 156.0114; found 156.0113.



13

*Sodium 2-(3,4-dihydro-2H-1,4-thiazin-6-yl)-2-oxoacetate (**13**).* 100 mg of cyclosquaramide **12c** were suspended in 75 mL of H_2O. Then, the pH was adjusted to 8 with NaOH 1M. The solution was heated up to 50 ºC for 5h adjusting the pH between 7.5 and 8.5. After the reaction time, the solvent was removed by rotary evaporation resulting a pale yellow solid. 120 mg, yield 95%. ^{1}H-RMN (600 MHz, DMSO-d_6): δ 2.70 (t, J = 4.8 Hz, 2H), 3.41 (m, 2H), 7.25 (br s, 1H), 7.72 (d, J = 6.6 Hz, 1H). ^{13}C-RMN (150 MHz, DMSO-d_6): δ 22.5, 41.9, 97.5, 144.6, 169.5, 192.0. ESI (-)-HRMS: m/z (%) calcd for $C_6H_6NO_3S^-$ $[M]^-$ 172.0074; found 172.0074.

4. NMR Spectra of Selected Compounds

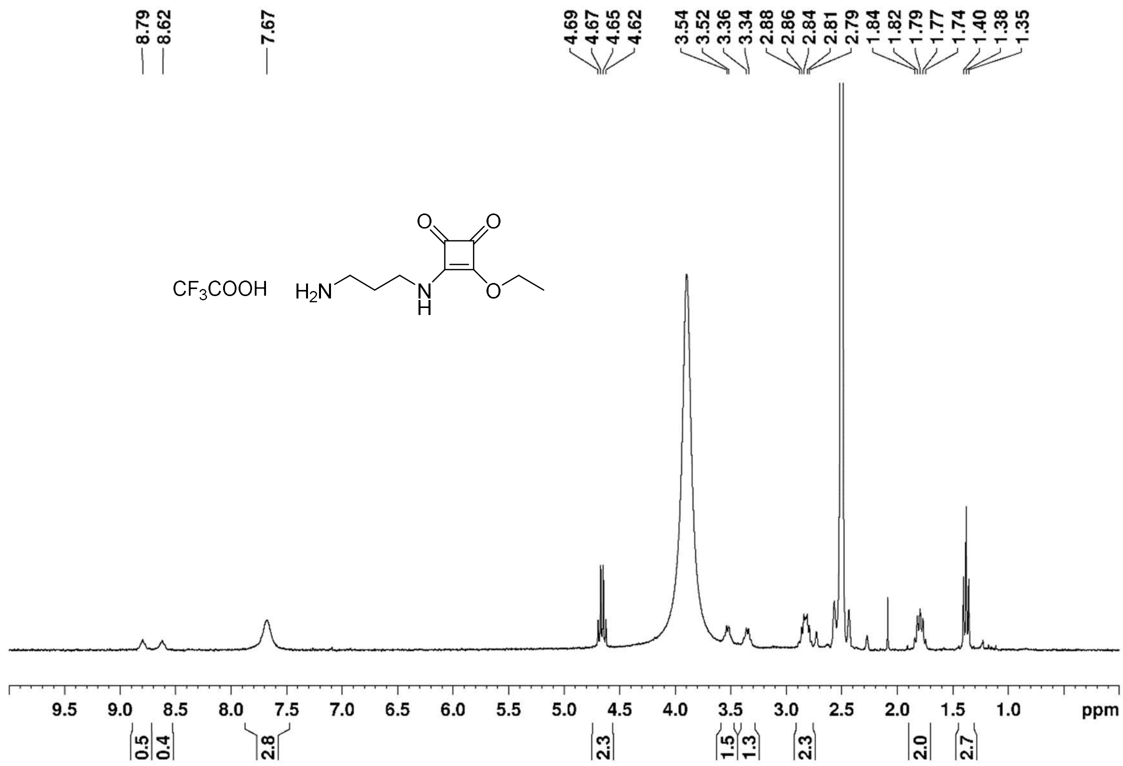


Figure S6. ^{1}H NMR spectrum of **9b** in DMSO-d_6, 300 MHz, 298 K.

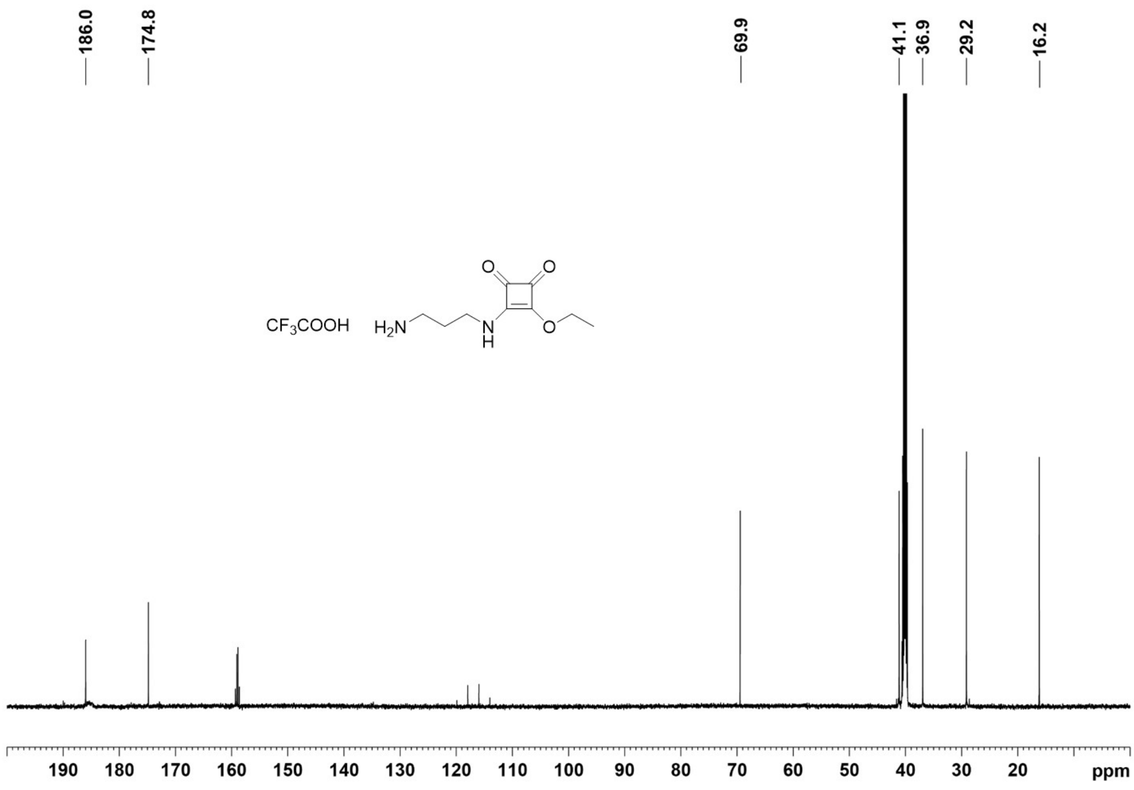


Figure S7. ^{13}C NMR spectrum of **9b** in DMSO-d_6, 300 MHz, 298 K.

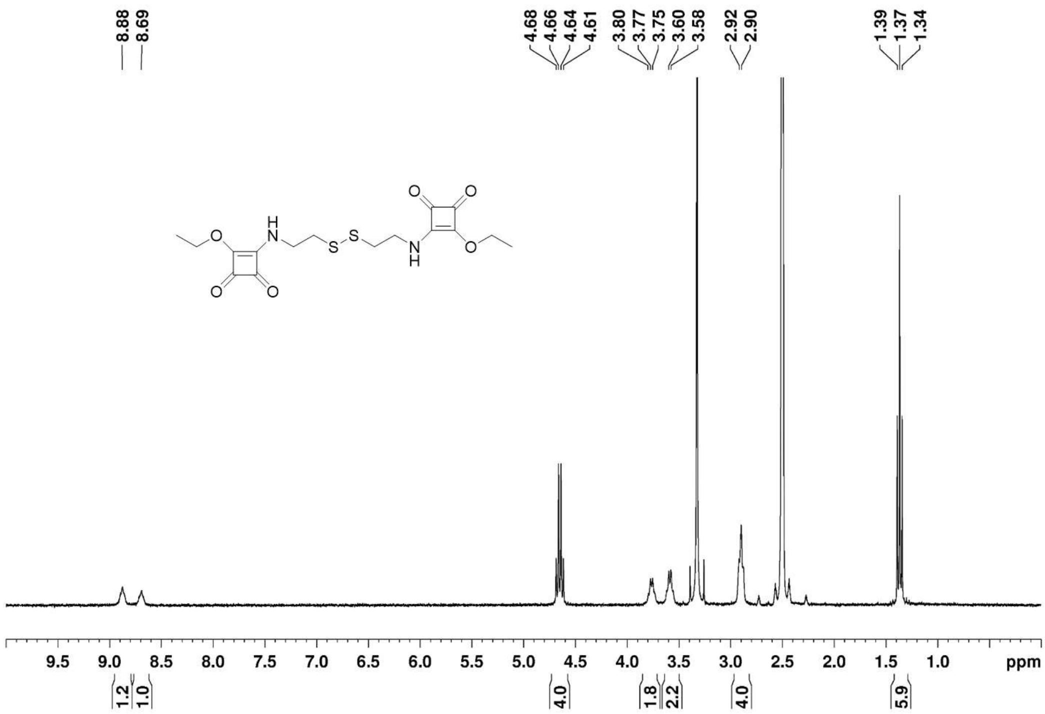


Figure S8. ^{1}H NMR spectrum of **11a** in DMSO-d_6, 300 MHz, 298 K.

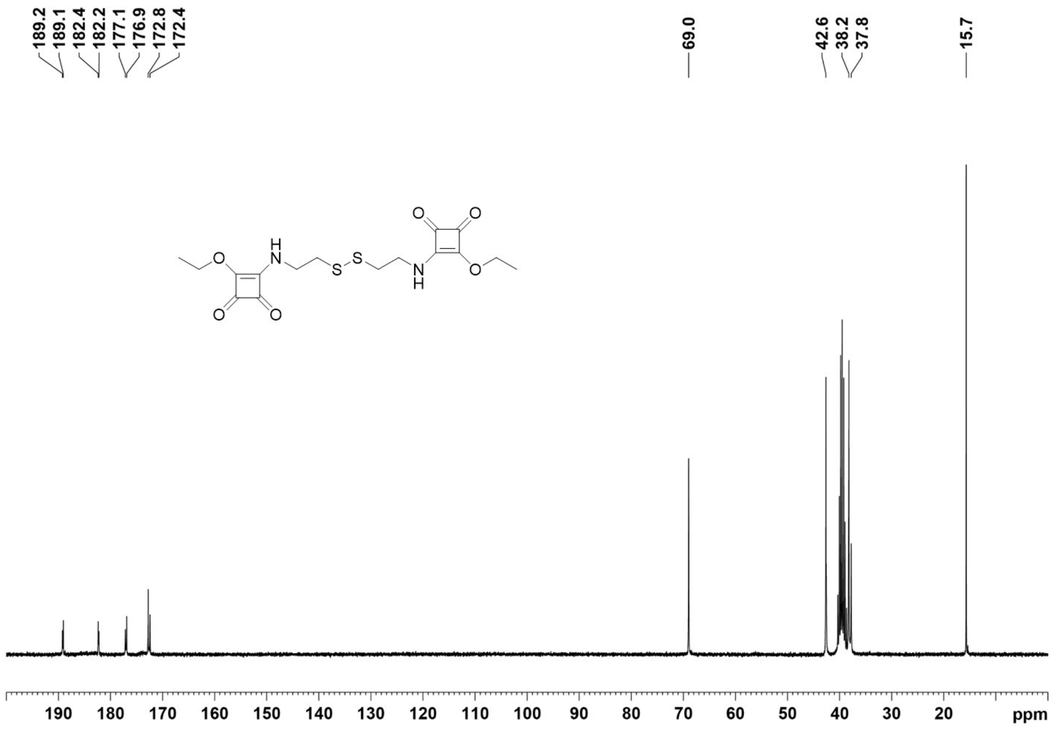


Figure S9. ^{13}C NMR spectrum of **11a** in DMSO-d_6, 75 MHz, 298 K.

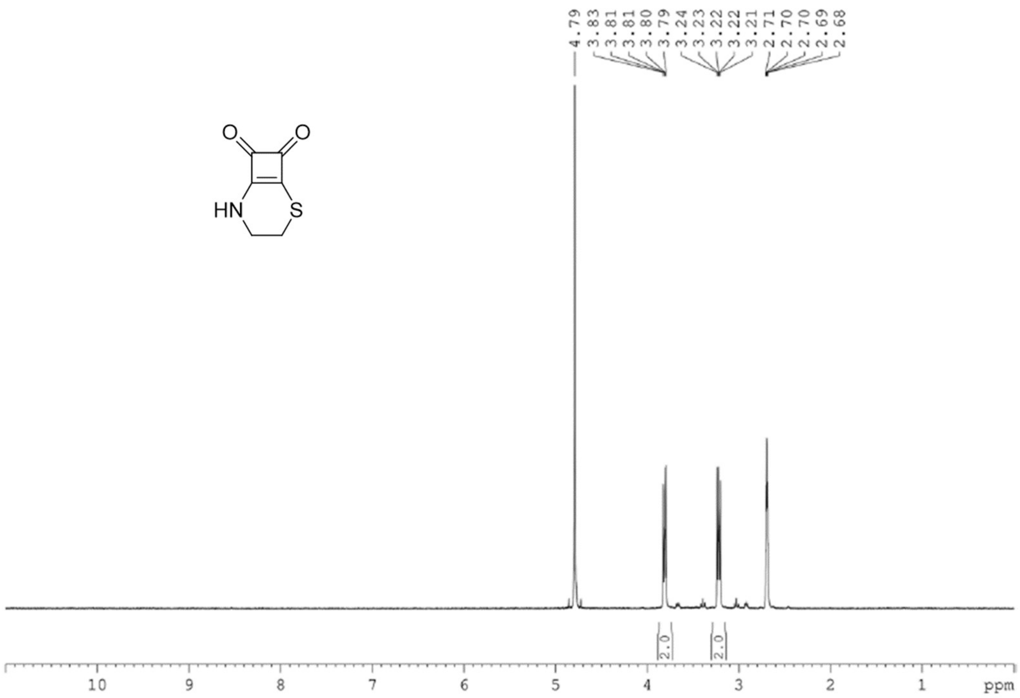


Figure S10. ^{1}H NMR spectrum of **12c** in DMSO-d_6, D_2O: 20%, 300 MHz, 298 K.

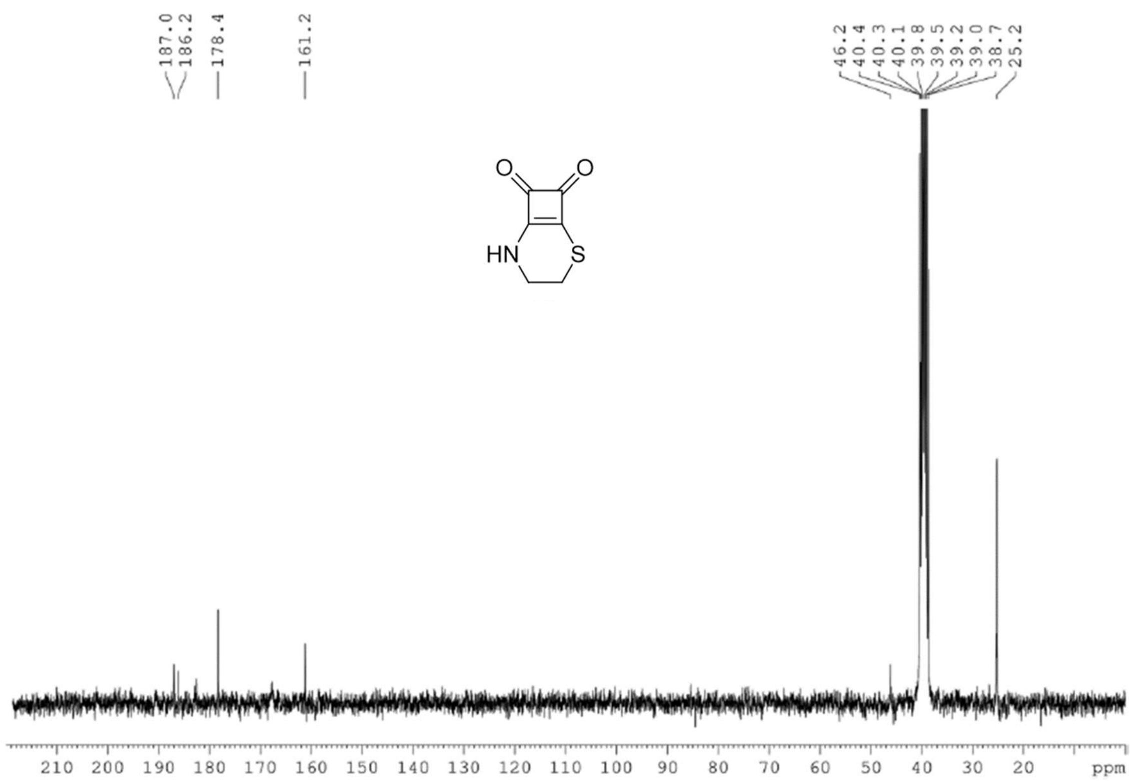


Figure S11. ^{13}C NMR spectrum of **12c** in DMSO-d_6, D_2O: 20%, 75 MHz

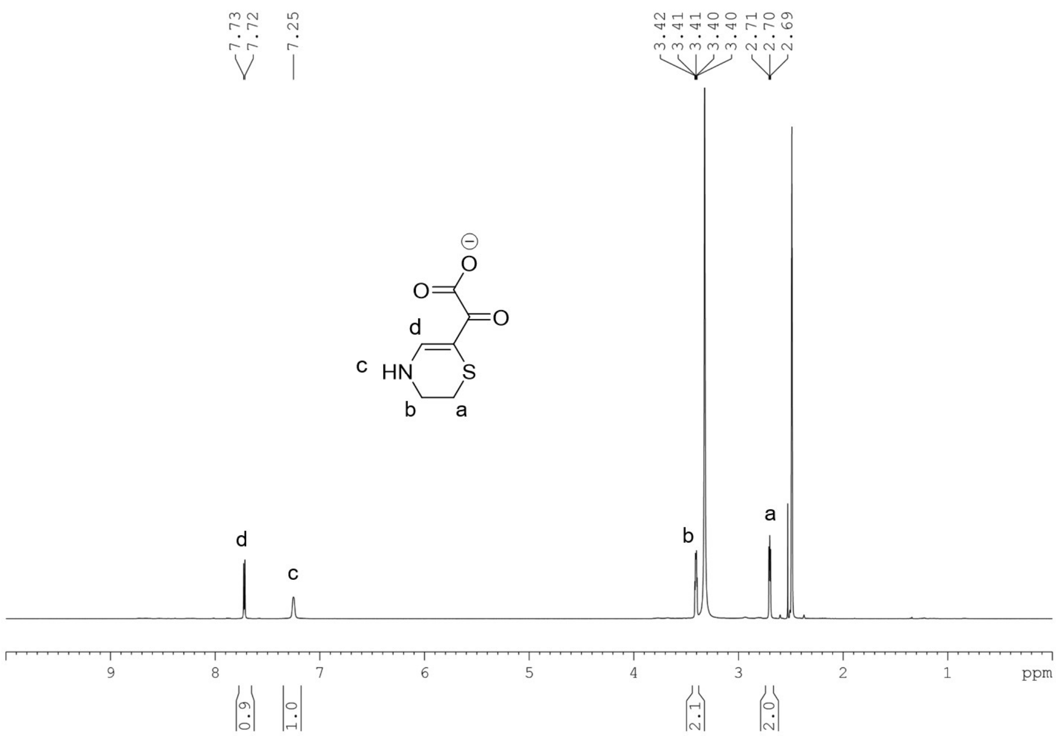


Figure S12. ^{1}H-NMR of **13** (DMSO-d_6, 298K) and the corresponding signal assignation.

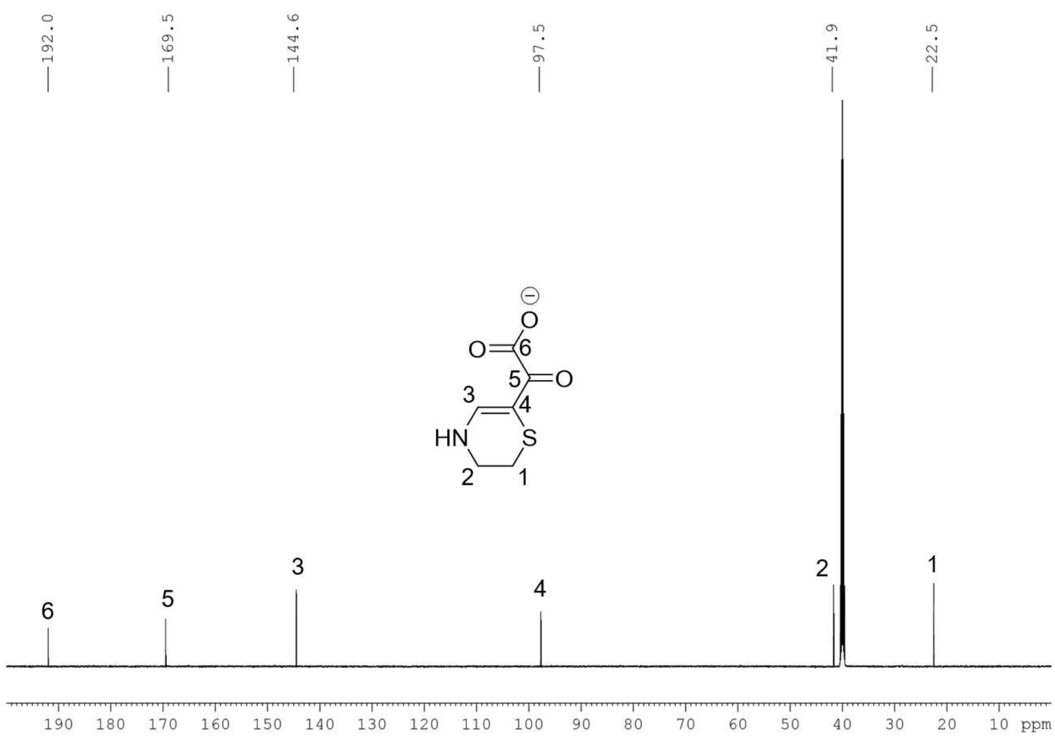


Figure S13. ^{13}C-NMR of **13** (DMSO-d_6, 298K) and the corresponding signal assignation.

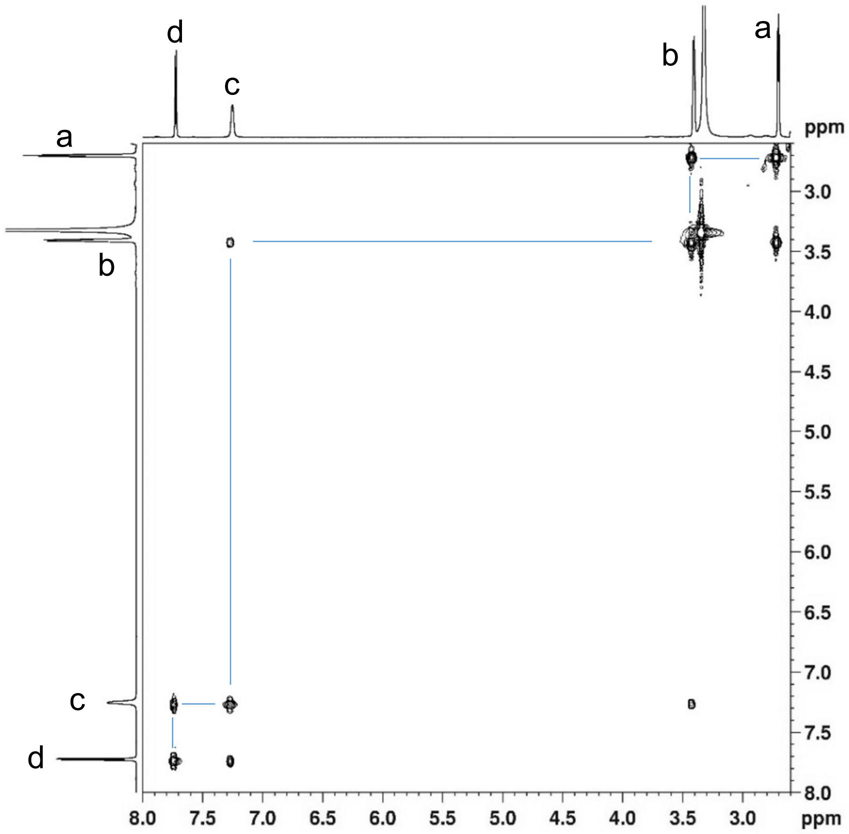


Figure S14. ^{1}H-^{1}H-COSY experiment of **13** (DMSO-d_6, 298K) and the corresponding signal correlations.

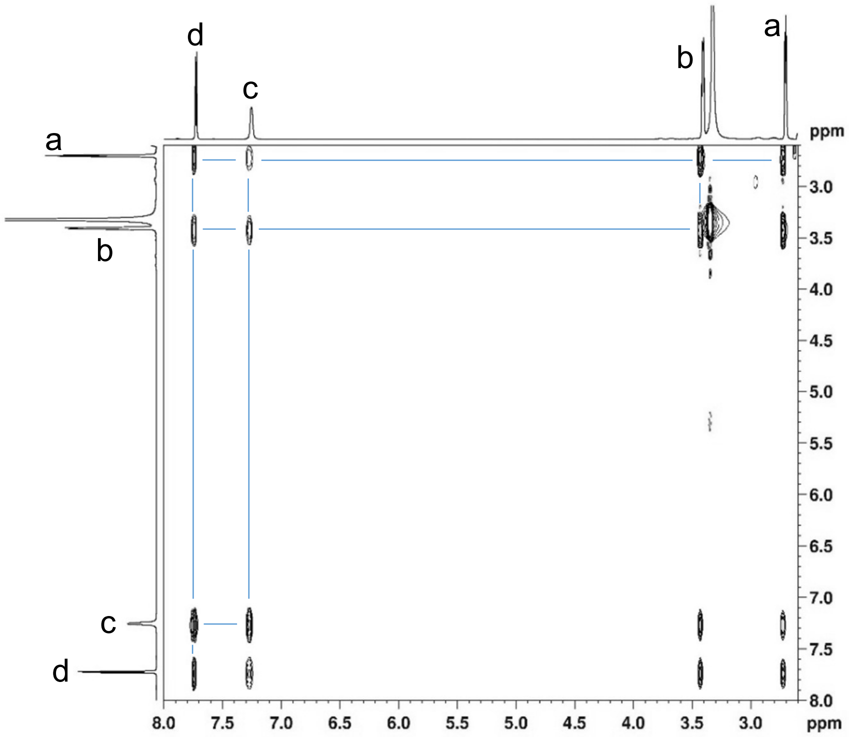


Figure S15. ^{1}H-^{1}H-TOCSY experiment of **13** (DMSO-d_6, 298K) with correlation of coupled protons.

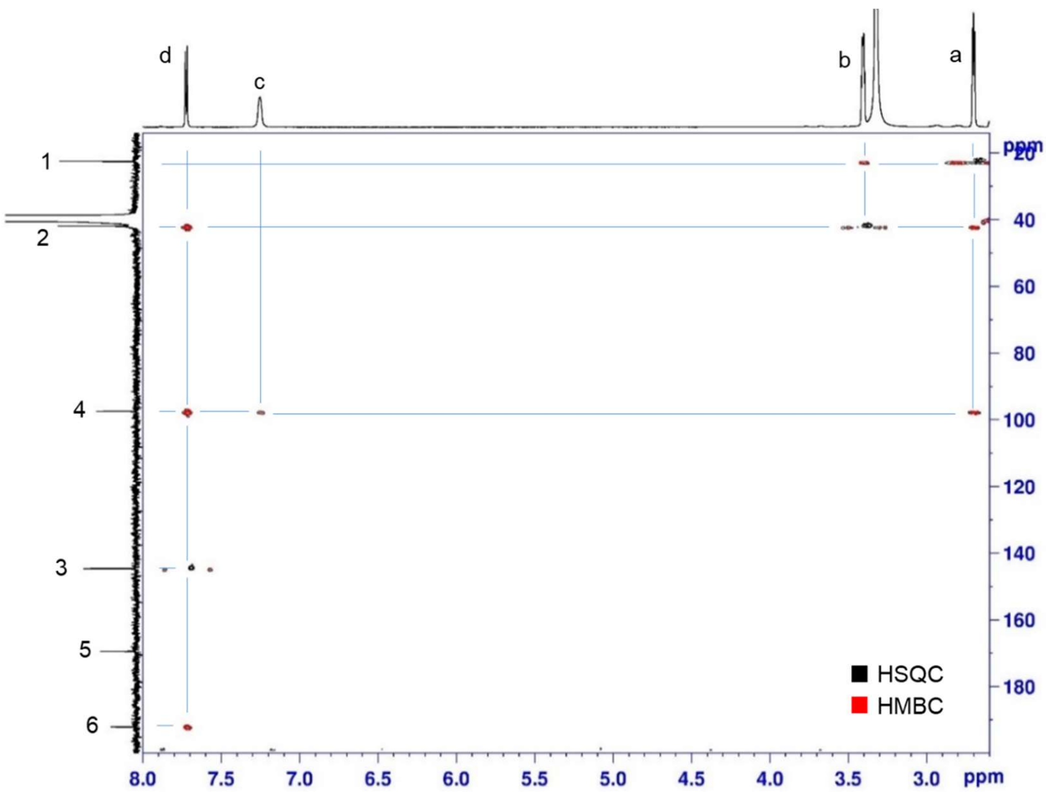


Figure S16. ^{1}H-^{13}C-HSQC and HMBC (black and red respectively) experiments of **13** (DMSO-d_6, 298K) with the corresponding correlations.

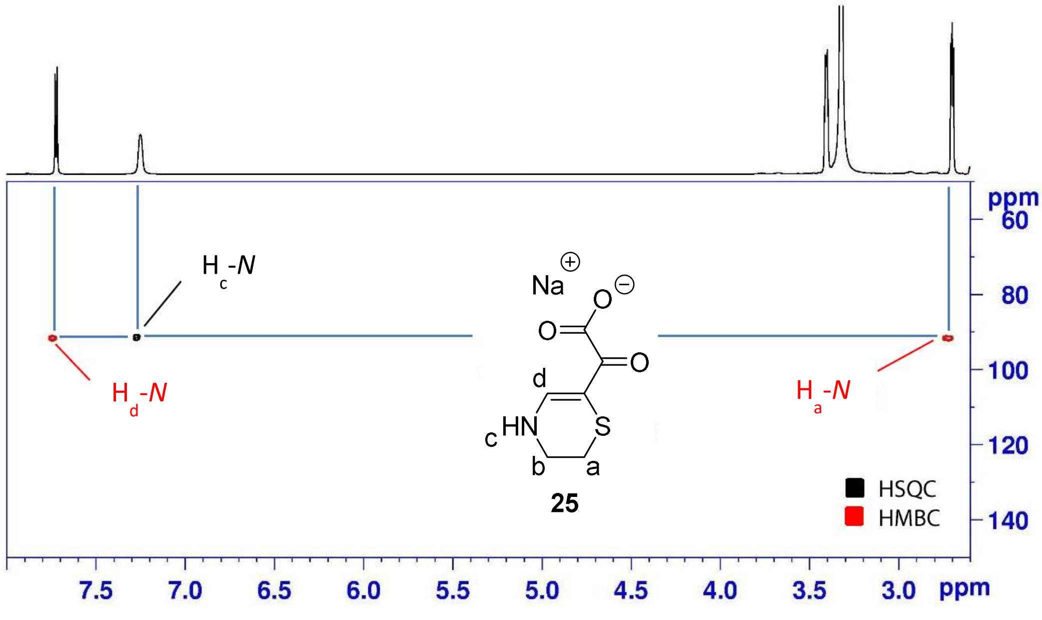


Figure S17. ^{1}H-^{15}N-HSQC and HMBC (black and red respectively) experiments of **13** (DMSO-d_6, 298K) with the corresponding assignation.

5. Computational Studies

All the geometric and energy calculations have been performed using ORCA 5.0 program[11] by means of the PBE0[12]-D4[13] method in combination with the def2-TZVP[14] basis set. The minimum or transition state nature of the compounds have been determined by frequency calculations. All minima have zero imaginary frequencies and the transition state have only one imaginary frequency that connects the starting and final products. The transition states have been computed using the NEB-TS (Nudged Elastic Band with TS optimization)[15] methodology as implemented in ORCA 5.0 program.

Computed structures

Cartesian coordinates (in Å) for all computed species. Geometries are computed at the PBE0-D4/def2-TZVP level of theory.

12a

```
C   -1.17081948692606      3.67526442674609     -0.16833248076613
C   -2.74193782674321      3.94273683768036     -0.28393301345172
C   -1.43925162931313      2.26281997154961      0.14059321265998
C   -2.78672737099286      2.41327553359818      0.03444610183954
C   -1.15964836416653     -0.14836500243787      0.20429838862624
C   -3.70956007286923      0.17240396975576     -0.07545898196942
N   -3.85498404390013      1.59475887673387      0.09967182564127
H   -1.17818962914670     -0.38705145397189     -0.87164389734255
H   -4.65085853148694     -0.30481780947649      0.22187634746085
H   -4.67855192232910      2.00919888541622     -0.31848129539354
H   -0.47099557813845     -0.86322884404748      0.66596200783561
H   -3.53075749757556     -0.09352766351353     -1.13428627513185
O   -0.15293992233170      4.31679696836815     -0.31688780302808
O   -3.19245873002859      4.66033059436112      0.91661596751284
H   -3.73703047922516      5.32654124377091      0.48163373253907
O   -3.30527613641461      4.37873945634902     -1.35343155237372
C   -2.57150982933483     -0.41511199848143      0.74577467415148
H   -2.65575199992573     -0.06880184550992      1.78219531828961
H   -2.70816902782221     -1.50421325107781      0.74676798682807
N   -0.59972656732032      1.16603970132035      0.37798413393566
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12a-TS

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C   -1.68089791119688      2.38936912998725      0.23767603018826
C   -3.00534660744992      2.52074202701743     -0.04479348768754
C   -1.21048549936356      0.01779965682806      0.41413305808330
C   -3.72138172739170      0.16105071917205     -0.08726484333145
N   -3.98070622960284      1.58009056092519      0.02219193280166
H   -1.12793597506821     -0.19467261651470     -0.67173545352632
H   -4.65572197328373     -0.36899627443820      0.13659815901318
H   -4.85397114704933      1.86450579159731     -0.39520532051510
H   -0.50681262489640     -0.64760858595742      0.92580328022993
H   -3.42868911048309     -0.11358500215538     -1.11828310586090
O   -0.04521906528806      4.05548445348216     -0.35358203025215
O   -3.08279687452405      5.16506966069460      0.37857539016007
H   -2.93296706873320      4.69499682879257      1.20473692942649
O   -2.81399110714301      4.65606327840679     -1.79276136686536
C   -2.62871722984249     -0.34052554397775      0.84226052464001
H   -2.80842828635393      0.03322878118663      1.85629578586006
H   -2.70396668411700     -1.43378965294162      0.86861542111618
N   -0.86023697722003      1.37402803739990      0.75092406083708
H   0.10210088121686      1.59141207013961      0.51121267929068
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Oa

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C	-3.24297882515035	2.50028354850233	0.04343716301421
C	-1.22849987911261	0.03834865686922	0.33721813380117
C	-3.75003121817364	0.01619109809410	-0.00073439240793
N	-4.06296426790464	1.42293989801482	0.18141579543579
H	-1.15542077650649	-0.10560875889664	-0.76261279059268
H	-4.64630010269348	-0.55491598655288	0.27564672931997
H	-5.01360246098153	1.61642624497571	-0.09159793606800
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H	-3.55001214887642	-0.20194272968590	-1.06733503481330
O	0.02213593034132	3.82565261210136	0.00757667702447
O	-2.58039876628777	5.49398222679759	0.61025824605021
H	-3.03911983465144	6.24220766088962	0.20386117961486
O	-2.19787829881073	5.23088435395072	-1.56880842095495
C	-2.57007337258376	-0.50914795430111	0.78908234547372
H	-2.70344225857247	-0.28447634047321	1.85385771345344
H	-2.56047648435559	-1.59970542539467	0.66114264388579
N	-1.03270315132484	1.39427843621993	0.75704833419923
H	-0.07760657123862	1.70868335581147	0.60770267437237

12c

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C	-3.18444254470842	3.73037202221028	0.14826748096792
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C	-2.91507027593280	2.19938587080327	-0.00131175444255
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C	-3.09056995003030	-0.20315925916568	-0.23354687806721
S	-0.53146365391703	0.94091638754557	-0.50176892147257
N	-3.67222233767031	1.10053254784678	-0.01773685455498
H	-1.23582378470189	-1.25964264128885	0.04259201933697
H	-3.67794587777701	-0.94554179430688	0.32013270512449
H	-4.64491424292634	1.18724052364968	0.21675207224766
H	-1.62944525790890	-0.11781924767128	1.34720122145338
H	-3.12275922713858	-0.48500171454422	-1.29374609620035
O	-0.72408426644369	4.63614354604917	-0.07675836072031
O	-3.51192390459584	4.00362245843132	1.54636578136059
H	-4.18098861154474	4.68371397782986	1.40552983792315
O	-3.97647045245619	4.30216185371909	-0.67452051559487

12c-TS

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C	0.84937117220068	0.54293928487379	-0.45302162850789
C	-0.48731761950807	0.38813541446930	-0.23573743132254
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C	-0.43063150874905	-2.07883889710175	-0.34290289277877
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H	-0.45642547110421	-2.46946674025168	-1.36764070579467
O	1.99658917000980	2.66470417492783	-0.09756192864305
O	-0.79086181791327	2.47093438436873	1.40949552410712
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O	-1.12600799590429	3.02546031640747	-0.74563874768301

Oc

C	-0.97932653186479	3.52902365915384	0.24533481004234
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C	-3.02887633042278	-0.28340084942690	0.35497820343051
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O	0.21965240134414	3.81740259731670	0.24496474087063
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H	-2.89904861285826	5.88462408759518	-0.79722675006392
O	-2.25685471685104	5.33319981869635	1.25042693220882

12d

C	-1.21815531144670	3.76660154716416	0.07682890799615
C	-2.79747011283518	3.99835823531063	-0.11444970082774
C	-1.41734201690566	2.32903686507849	-0.20669930084779
C	-2.76700740160683	2.45889927990812	-0.33388679934439
C	-1.19924187535045	-0.36615019935181	0.54021034510599
C	-3.59263034106217	0.18080387236803	-0.29292560887669
S	-0.37328356390744	0.94378603067919	-0.42899284144124
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H	-0.64241290118511	-0.53506284967636	1.46703844189369
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O	-3.41957446450771	4.20776723354185	1.20465966620375
H	-3.89881448432661	5.02162023893321	1.01116086202166
O	-3.27513104899339	4.75863808606967	-1.02317766981732
C	-2.66396502949059	-0.12680932408025	0.88448724695867
H	-2.74185676978683	0.69769162719394	1.59801138909069
H	-3.02648081148969	-1.02409933141642	1.40097785943099

12d-TS

C	-1.20535723180306	3.71795666360875	0.01400911866876
C	-2.59755964965438	4.43120946374110	0.01676843876464
C	-1.62634533122967	2.37635890510709	-0.29011935424462
C	-2.98753473243897	2.51130361203496	-0.33772125501867
C	-1.20172220068899	-0.21003097086610	0.61517056937883
C	-3.58560522643918	0.10898207196271	-0.35856025429606
S	-0.60039042088079	0.98021504480340	-0.60955845347717
N	-3.88467085929321	1.53190745929885	-0.49463131568383
H	-0.89379766574955	-1.18524581899294	0.22254221909512
H	-4.54206352206523	-0.41449768973540	-0.26061991504200
H	-4.85245754104676	1.78488479355893	-0.39391644530533
H	-0.68396204353407	-0.06253077772383	1.56773041818514
H	-3.10167595166814	-0.26663177693967	-1.26577738370442
O	-0.12110040366171	4.22543555636875	0.22248973733481
O	-3.03826749796740	4.64160691701771	1.33929778542559
H	-3.64464191110978	5.38294616931499	1.21771556733133
O	-2.96061358139142	5.25294513044738	-0.82949483721458
C	-2.71323381868711	-0.18330416689365	0.86215529738265
H	-2.93569254217732	0.58219767387338	1.60896005810516
H	-3.00385472851323	-1.14516225998640	1.30384886431468

Od

C	-1.18602852895556	3.82474660562959	-0.15793583502014
C	-2.23056070501379	4.88589369175435	-0.53240382190487
C	-1.75746636118844	2.55330222816264	0.14669009836471
C	-3.13025136517860	2.55231256022188	0.06209503597106
C	-1.17016059656466	-0.21421995445621	0.17259799058452
C	-3.70056901418517	0.06878707263696	-0.02088219062871
N	-3.93474330010925	1.48130739176338	0.24625795773266
H	-1.15277160958494	-0.25036731565903	-0.92483265617970
H	-4.62355527547911	-0.45340673157504	0.26219143788350
H	-4.90316842437023	1.70184418616124	0.07341169895171
H	-0.42976086233120	-0.93477652775609	0.53464153349754
H	-3.56808524816630	-0.09204056128675	-1.10590267348743
O	-0.00941063301639	4.17246342367314	-0.18238571256906
O	-2.67400007064401	5.57433294892608	0.54738268982625
H	-3.22804757692474	6.27246343780577	0.17243690813807
O	-2.47295794459364	5.26798771765074	-1.65286794327604
C	-2.54309036343848	-0.60884998636118	0.68148034882396
H	-2.59824714686164	-0.42568795781132	1.75994869591799
H	-2.67178601009463	-1.68847574499286	0.50872854194219
S	-0.55742781329922	1.36468074551271	0.76046942543179

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