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Introducing Complex NMR Mixtures at the Undergraduate Level: Isomerization, Separation and Analysis of the Diels-Alder Adducts from the Reaction of Methylcyclopentadiene and Maleic Anhydride (Part II)

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Abstract This paper introduces undergraduate students to advanced NMR techniques to help elucidate the structures of *endo* and *exo* norbornene isomers formed during the Diels-Alder reaction between cyclopentadiene and methylcyclopentadiene with maleic anhydride. The microwave assisted isomerization of the norbornene derivatives resulted in a mixture of *endo* and *exo* isomers that were separated and analyzed using advanced NMR spectroscopy techniques, with a focus on the spectral interpretation and unequivocal assignment of the *endo* and *exo* stereoisomerism when possible.

Keywords Diels-Alder Reaction, Methylcyclopentadiene, *Endo/Exo* Isomerism, Complex Mixtures, Nuclear Magnetic Resonance Analysis, Nuclear Overhauser Effect (NOE)

1. Introduction

The Diels-Alder reaction between cyclopentadiene (Cp) and maleic anhydride (MA) is well described in laboratory manuals and textbooks at the undergraduate level and represents a simple and effective teaching tool in organic chemistry. [1–3] However, at the undergraduate level, this reaction suffers from a lack of diversity, as too often this reaction generates only a single isomer, the *endo* isomer **1*en***, due to the symmetry of the staring materials as well as the reaction conditions (**Figure 1**).

Figure 1. Diels-Alder adducts resulting from the reaction between cyclopentadiene and maleic anhydride

In part I of this work, we showed that methylcyclopentadiene (MeCp) can generate a complex mixture of isomers during the Diels-Alder reaction with maleic anhydride, yielding two pairs of enantiomers **2*en*** and **3*en*** (**Figure 2**), thus exposing the students to a more challenging spectral interpretation training. [4]

There, we used that case study to introduce undergraduate students to the analysis of complex and inseparable mixtures and taught them how to elucidate each structure using advanced NMR techniques and the separation of isomers using chemical modifications. The complete structure elucidation of the Diels-Alder reaction adducts and their L-alanine derivatives was accomplished using ^{1}H, ^{13}C, APT, HSQC and HMBC NMR spectroscopy experiments in all cases. However, we assumed the stereochemistry of all adducts to be *endo* as the Diels-Alder reaction was done at low temperature (Kinetic control).

In the present paper we will discuss the assignment of the *endo* and *exo* configuration of the Diels Alder adducts, and introduce the students to tools to help answer to following question: *How would you confirm the exo and endo configuration of these Diels-Alder products?*

Since we have previously shown that students could readily acquire the *exo* and *endo* *cis*-norbornene-5,6-dicarboxylic anhydride isomers using microwave irradiation, followed by their separation using chromatography on silica gel, [5] in this study we were able to use a similar approach to yield all the major isomers of 1- and 5-methyl-5-norbornene-2,3-dicarboxylic anhydride.

To start this investigation, the students will begin with the synthesis and full characterization of all the Diels Alder adducts resulting from the reaction of Cp and MeCp with MA, followed by a familiarization with 1D and 2D NOESY experiments using simple norbornene systems before moving to the more complex derivatives.

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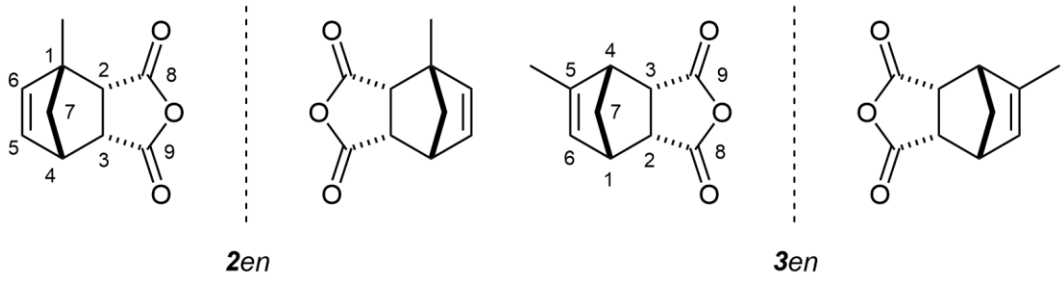


Figure 2. Kinetic Diels-Alder adducts **2*en*** and **3*en*** resulting from the reaction between methylcyclopentadiene isomers and maleic anhydride

2. Experimental

General. All reagents and solvents were purchased from Sigma-Aldrich and used without further purification. All NMR spectra (^{1}H, ^{13}C, APT, COSY, HSQC, HMBC) were recorded on a Bruker Avance III 400 MHz or a Bruker Avance III 500 MHz spectrometer and were referenced to the residual chloroform ($CDCl_3$) resonance at 7.26 ppm for proton and 77.16 ppm for carbon. All 1D and 2D NOESY spectra were recorded on a Bruker Avance II 600 MHz spectrometer. All microwave irradiations were performed on a 6 mL scale using toluene as a solvent in an Anton Parr Monowave 50 at 200℃ in a 10 mL capped vial fitted with a magnetic stir bar. All chromatography separations were performed on a Teledyne Combiflash system using ethyl acetate/hexanes mixtures as eluent.

Safety Precautions. Before performing the experiments, students should familiarize themselves with the hazards of the reagents used by consulting their respective SDS. Care should be taken during the microwave irradiation of the norbornene derivatives, and the pressure of the reaction carefully monitored. The microwave, CombiFlash apparatus and the rotary evaporator should be placed in a well ventilated area, preferably in a fume hood or under a secondary air extractor to minimize contacts with chemical fumes.

2.1. Synthetic Procedures

All synthetic procedures were optimized to allow the students to perform each reaction and purification either within a single four hour laboratory period, or within two laboratory periods (eight hours) when an overnight reaction was required.

2.1.1. *Cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride (**1*en***) and *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (**1*ex***) were purchased from Sigma-Aldrich and used as is.

2.1.2. The mixture of *endo*-1-methyl-5-norbornene -2,3-dicarboxylic anhydride (**2*en***) and *endo*-5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride (**3*en***) was prepared according to the procedure previously described. [4]

2.1.3. The L-alanine derivatives of **4*en***, **5*en***, **4*ex*** and **5*ex*** were prepared according to the procedure previously described. [5]

2.2. Microwave Irradiation Procedure

A mixture of **2*en*** and **3*en*** was dissolved in toluene (6 mL) and the solution was irradiated in an Anton Parr Monowave 50 at 200℃ for 15 minutes with constant stirring. After irradiation, the toluene was removed under vacuum and the reaction mixture was purified using a Teledyne Combiflash system using ethyl acetate/hexanes mixture as eluent to yield two fractions containing compounds **2*en*** and **3*en*** and compounds **2*ex*** and **3*ex***, respectively *(See supplementary information)*.

2.3. NOESY Spectra Acquisition

Proton 1D and 2D NOESY spectra were recorded on a Bruker Avance II HD 600 MHz spectrometer equipped with a quadruple nucleus cryoprobe. All spectra were acquired at 298K. 1D selective NOESY spectra were acquired with the Bruker pulse sequence selnogpzs which significantly suppresses the zero quantum coherence signals resulting from *J*-coupling between the selectively irradiated proton and the *J*-coupled proton. [6] All mixing times were set to 0.5 sec. A total of 32 scans were collected for each 1D NOESY spectrum. Similarly, the 2D NOESY spectra were acquired with the suppression of zero quantum coherence, using the sequence noesygpphzs. Eight scans were collected for each of the 256 increments in each 2D NOESY experiment.

2.4. Spectral Processing and Deconvolution

The Mnova software package was used to process spectra. 1D NMRs were phased and baselines were corrected by use of the Whittaker smoother algorithm. Spectra were referenced to $CDCl_3$ 7.26ppm (singlet) for ^{1}H and 77.16 ppm (triplet) for ^{13}C. Exponential apodization was used to increase signal to noise ratio [7] and no zero filling was used. Global spectral deconvolution (GSD) was used to filter off solvent and impurities signals with a refinement level of 2 cycles. [8] 2D spectra were phased and sin-bell apodization (0°) was used for COSY and HMBC, sin-square apodization (90°) was used for HSQC. Baselines were corrected by use of the Whittaker smoother algorithm in both axes for all 2D spectra.

3. Results and Discussion

3.1. 5-norbornene-2,3-dicarboxylic Anhydride Derivatives

One of the easiest ways to tackle the *endo* and *exo* isomerism assignment for students is to start with the ubiquitous spectra of 5-norbornene-2,3-dicarboxylic anhydride isomers 1*en* and 1*ex*. From commercially available 1*en* and 1*ex*, students are able to compile all the necessary NMR spectra (^{1}H, ^{13}C, APT, COSY, HSQC, HMBC) and assign all the relevant peaks in each of the ^{1}H and ^{13}C-NMR spectra.

As shown in the ^{1}H-NMR spectra of 1*en* and 1*ex* displayed in **Figure 3**, the apparent difference between the two isomers in the ^{1}H-NMR spectrum consists of the chemical shift of protons H_3 /H_9 as well as H_{8a} and H_{8b}. For the student, note that H_{8a} and H_{8b} have different chemical shifts because they are in different environments. In later courses, they will learn that they are diastereotopic. The *endo* isomer signals tend to be more deshielded compared to the *exo* isomer. While these spectra can confirm the difference between the *endo* and *exo* isomers, they are however not enough to allow the students to unequivocally assign the stereochemistry of each isomer.

Therefore, to clearly determine the configuration of the two isomers we introduced the students to nuclear Overhauser effect spectroscopy (NOESY), an important tool often used to identify stereochemistry. Could 1D and 2D NOESY experiments allow the unequivocal structure interpretation using the correlation between NOE enhancements and their $1/r^6$ dependence of internuclear distances between protons, [9] a question the students will attempt to answer in the following investigation.

3.1.1. *Cis*-5-norbornene-*endo*-2,3-dicarboxylic Anhydride (1*en*)

The 2D NOESY spectrum shows diagonal peaks and cross peaks; with cross peaks connecting resonances from nuclei that are spatially close rather than those that are coupled to each other through-bonds (**Figure 4**).

The 2D NOESY spectrum of 5-norbornene-*endo*-2,3-dicarboxylic anhydride **1*en*** displayed in **Figure 4** shows several correlations between protons. As an example, both H_8 protons show correlations with H_4 and H_7 while only one of them shows a correlation with H_3, H_9. Such observed correlations not only allow the students to differentiate between H_{8a} and H_{8b} but also hints at the possible *endo* configuration for isomer **1*en***. Other important correlations are observed between H_4, H_7 and H_5, H_6 as well as between the diastereotopic protons H_{8a} and H_{8b}.

Theoretically, more correlations could be observed but do not appear. As an example, the correlation between H_{8a}, H_6 and H_5 should be expected. Nevertheless, due to the relative longer distance compared with that between H_{8a} and H_4, the signal for this correlation is too weak to be observed under the experimental conditions (e.g. limited number of scans). This is a limitation of the 2D NOESY experiment that students should be made aware of.

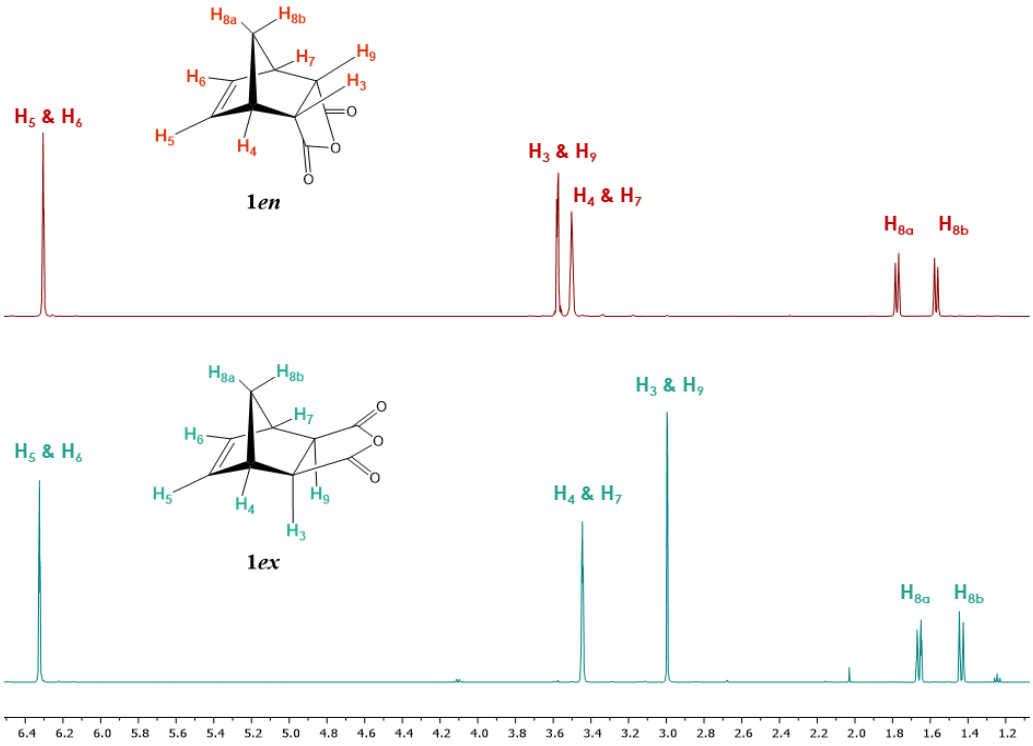


Figure 3. Stacked plot of the ^{1}H NMR spectra of **1*ex*** and **1*en*** isomers of *cis*-5-norbornene-2,3-dicarboxylic anhydride (Recorded in $CDCl_3$, 1.2 to 7.0 ppm region of the spectra shown only for clarity purpose)

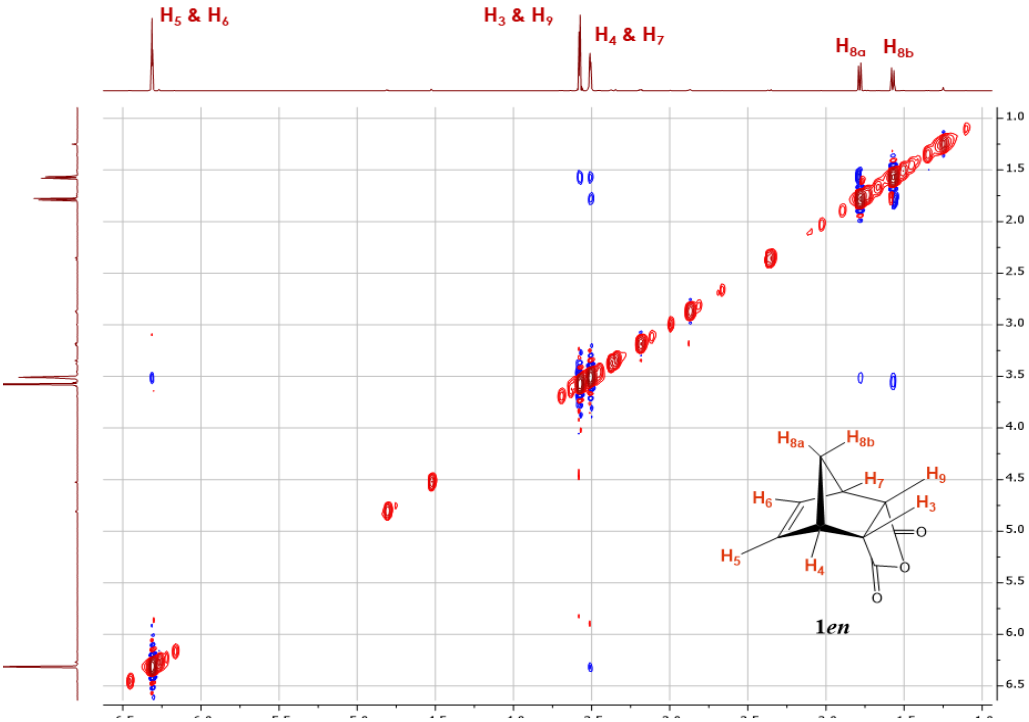


Figure 4. 2D NOESY spectrum of 5-norbornene-*endo*-2,3-dicarboxilate anhydride **1*en*** (Recorded in $CDCl_3$, 1.0 to 7.0 ppm region of the spectra shown for clarity of purpose)

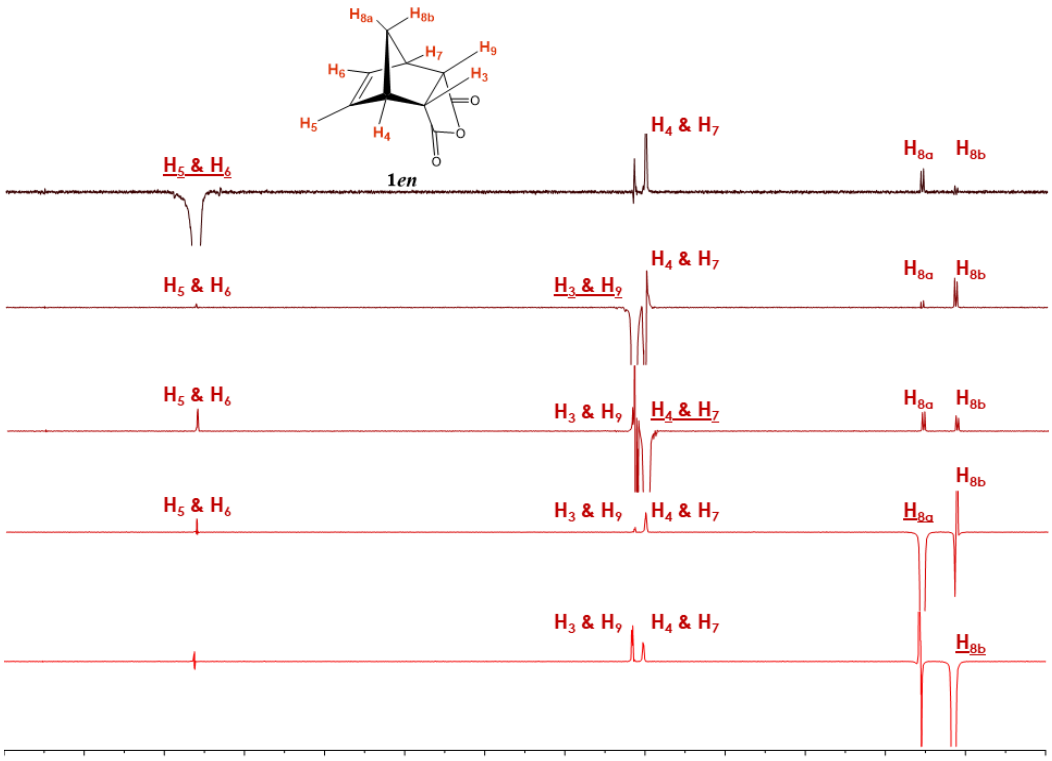


Figure 5. Stacked plot for the 1.0-7.0 ppm region of **1*en*** 1D NOESY spectra with marked irradiated protons (Underlined). Irradiated signals are shown in negative phase with all positive phase signal considered true NOE, while false NOE signals show both positive and negative phases

To further confirm the observed correlations, we introduced the students to the 1D NOESY experiments (**Figure 5**). The 1D NOE spectrum shows a negative or positive change of NMR signal intensity when another resonance signal is saturated by irradiation with a radiofrequency field. The change in resonance intensity is a consequence of cross relaxation between the irradiated nucleus and its neighbors in space. [6]

As an example, when protons H_5 and H_6 were irradiated, a positive nuclear Overhauser effect was observed for H_{8a}; H_3

and H_9 as well as H_4 and H_7 for isomer **1*en*** (**Figure 5**). The irradiation of H_{8b} as predicted showed a positive nuclear Overhauser effect for H_3 and H_9 as well as for H_4 and H_7. For the most part the correlations seen in the 2D NOESY were observed in the 1D NOESY spectra with the addition of a new system in which protons H_{8a}, H_5 and H_6 were found to be spatially close.

Interestingly, protons H_5 and H_6 and H_3, H_9 also showed nuclear Overhauser effects, although the NOE signals are much weaker, which suggests a relatively longer distance between these nuclei or possibly relayed effects through other protons which are close. Therefore, particular attention has to be given to the NOE intensities and the phase of the peaks. Most NOE experiments are efficient at determining the relative proximity between protons in space (typically 5 Å) based on the strength of the observed NOE intensity, making them appropriate for most structural studies. [10]

To further attempt to explain these observed correlations, students can be provided with the crystal structure of **1*en***, and asked to investigate the atom proximity in the structure. With the help of **Figure 6**, student can easily correlate the NOE intensity with the distance between the atoms. Indeed, the shortest distance between H_5 and H_4 (2.6 Å) resulted in a strong NOE, while a longer distance with H_{8a} (3.5 Å) and H_3 (4.1 Å) resulted in a weaker NOE, and even weaker NOE with H_{8b} distant by 4.3 Å. *(Students should be cautioned about using a crystal structure to explain a phenomenon observed in solution, however, due to the rigidity of the system studied the distances deduced from the crystal structure, will still follow the trend seen in solution.)*

To undergraduate students, processing 1D NOESY experiments may appear to be demanding, so they should be encouraged to organize the observed correlations in a table (**Table 1**) as well as to focus their initial efforts on the strong NOEs.

3.1.2. *Cis*-5-norbornene-*exo*-2,3-dicarboxylic Anhydride (1*ex*)

A similar method can be used to characterize the 5-norbornene-*exo*-2,3-dicarboxylic anhydride isomer **1*ex***. After recording all the usual NMR spectra (^{1}H, ^{13}C, APT, COSY, HSQC and HMBC), the students can establish the correlations between the protons in the 2D NOESY spectrum (**Figure 7**).

Table 1. 1D and 2D NOE observed correlations for 5-norbornene-*endo*-2,3-dicarboxylic anhydride **1*en***. *(w stands for weak observed NOE)*

Irradiated proton	1D NOE correlation				2D NOE correlation			
H_{8a}	H_5 & H_6	H_4 & H_7			H_4 & H_7			
H_{8b}	H_4 & H_7	H_3 & $H_{9\,w}$			H_4 & H_7			
H_3 & H_9	H_5 & H_6	H_4 & H_7	$H_{8b\,w}$		H_4 & H_7	H_5 & H_6		
H_4 & H_7	H_5 & H_6	H_3 & H_9	H_{8a}	H_{8b}	H_{8a}	H_{8b}	H_3 & H_9	H_5 & H_6
H_5 & H_6	H_4 & H_7	H_3 & H_9	H_{8a}	$H_{8b\,w}$	H_4 & H_7	H_3 & H_9		

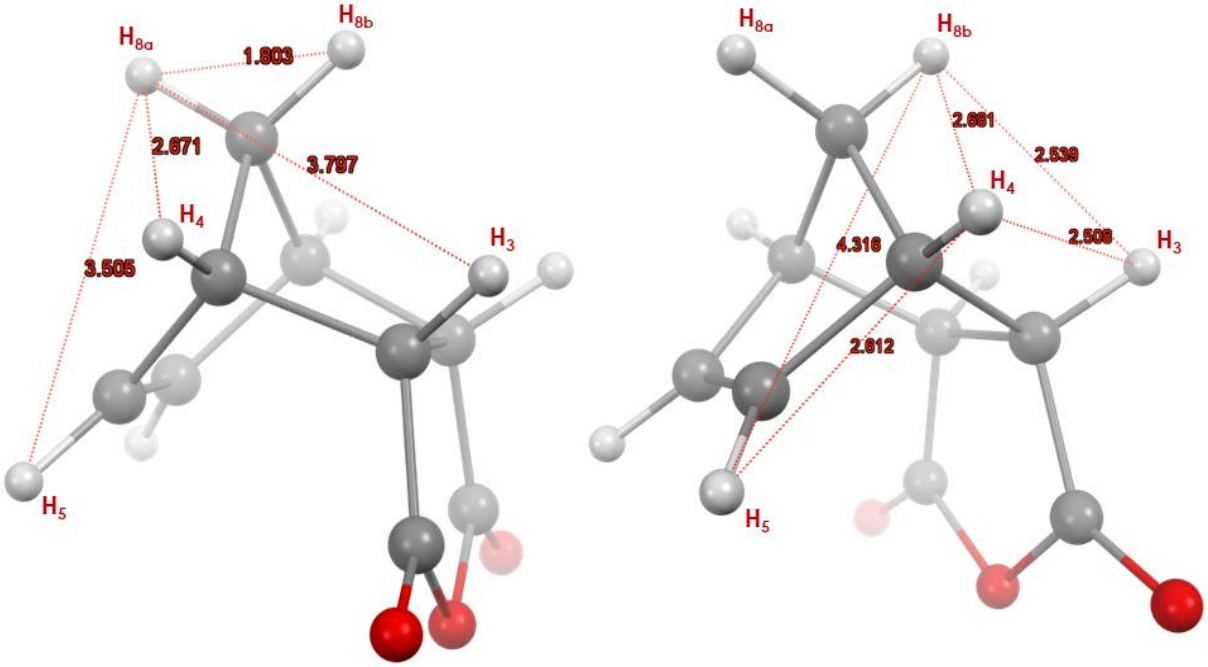


Figure 6. Crystal structure of **1*en*,** left structure showing distances between H_{8a} to H_5, H_4, H_3, H_{8b} and right structure showing distances between H_{8b} to H_5, H_4, H_3, and between H_5, H_4 and H_4, H_3 in Angstroms. (CCDC Database Identifier: NBORAN12, Deposit Number: 1508074)

Figure 7 shows that both H_{8a} and H_{8b} protons have only a correlation with H_4 and H_7 unlike the *endo* isomer which showed a correlation between H_{8b} with H_3 and H_9. This suggests the presence of the *exo* isomer configuration, due to the protons H_3 and H_9 being on the opposite face in the *exo* isomer compared to the *endo* isomer. Additionally, protons H_5 and H_6 show a correlation with protons H_3 and H_9 and H_4 and H_7. Interestingly, for compound **1*ex***, the discrimination between H_{8a} and H_{8b} is not possible using the 2D NOESY spectrum as both protons only show a correlation with H_4 and H_7. Hence, it was important to run several 1D NOESY experiments.

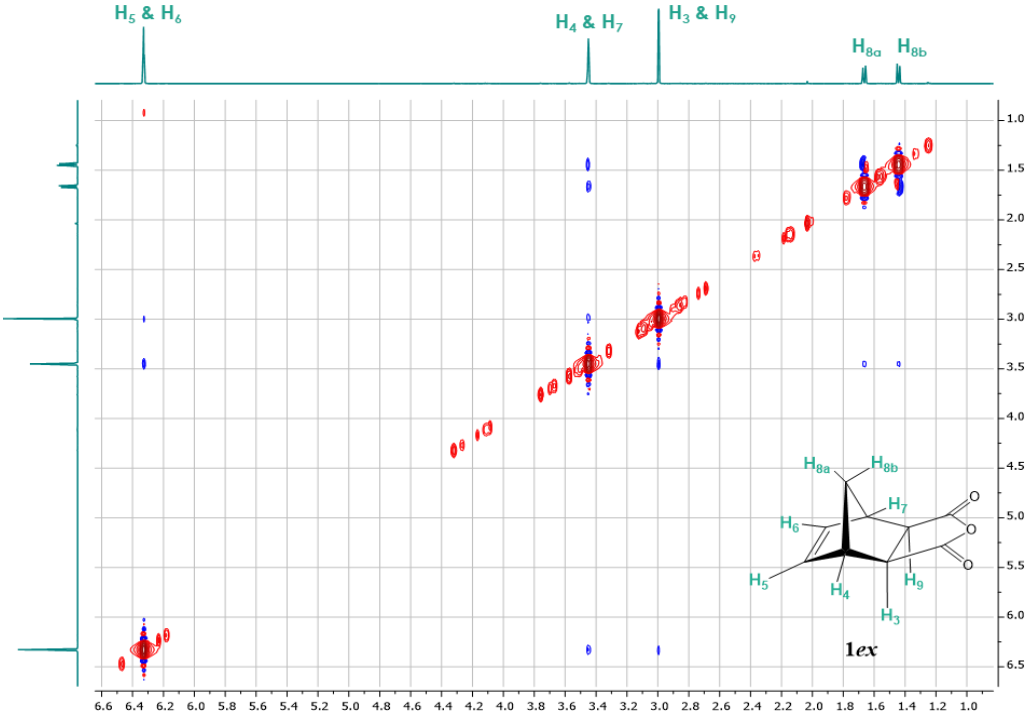


Figure 7. 2D NOESY spectrum of 5-norbornene-*exo*-2,3-dicarboxylic anhydride **1*ex*** (Recorded in $CDCl_3$, 1.0 to 7.0 ppm region of the spectrum shown for clarity of purpose)

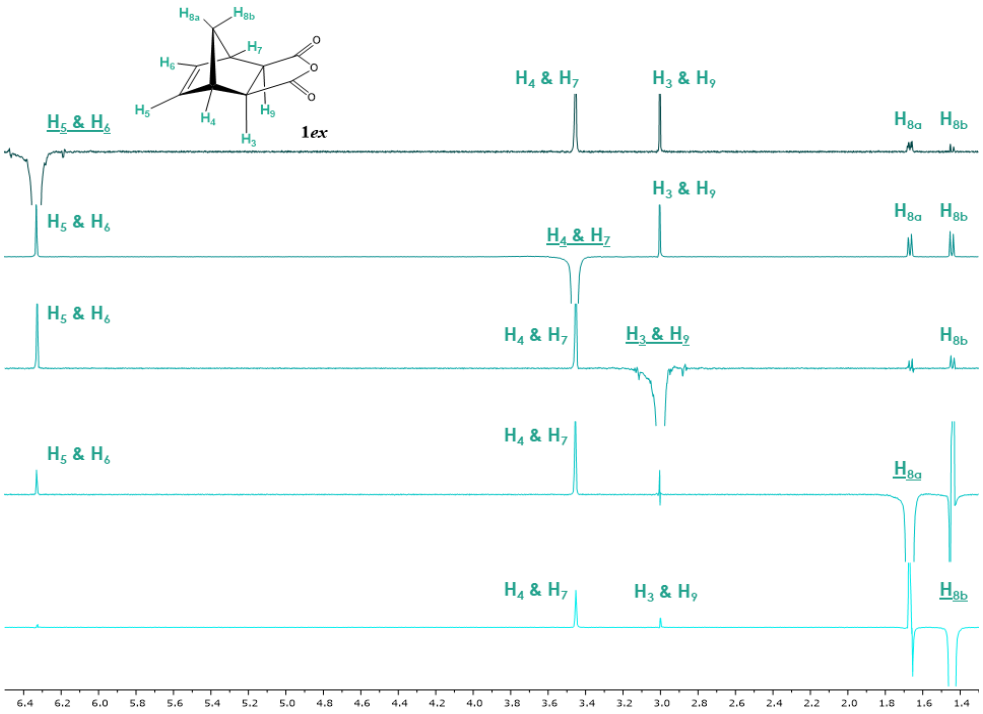


Figure 8. Stacked 1D NOESY spectra of 5-norbornene-exo-2,3-dicarboxylic anhydride with irradiated protons H_5 & H_6, H_4 & H_7, H_3 & H_9, H_{8a} and H_{8b}. Irradiated protons are underlined. Spectra are processed having irradiated signal in negative phase. Positive phase signals are considered true NOE, while false NOE signals show both positive and negative phases

Table 2. 1D and 2D NOE correlations summary in 5-norbornene-*exo*-2,3-dicarboxylic anhydride **1*ex*.** *(w stands for weak observed NOE)*

Irradiated proton	1D NOE correlation			2D NOE correlation		
H_{8a}	H_5 & H_6	H_3 & $H_{9\,w}$	H_4 & H_7	H_4 & H_7		
H_{8b}	H_3 & H_9	H_4 & H_7		H_4 & H_7	H_3 & H_9	
H_4 & H_7	H_5 & H_6	H_{8a}	H_{8b}	H_{8a}	H_{8b}	H_5 & H_6
H_3 & H_9	H_5 & $H_{6\,w}$	$H_{8a\,w}$	H_{8b}		H_{8b}	
H_5 & H_6	H_4 & H_7	H_{8a}	H_{8b}	H_4 & H_7		

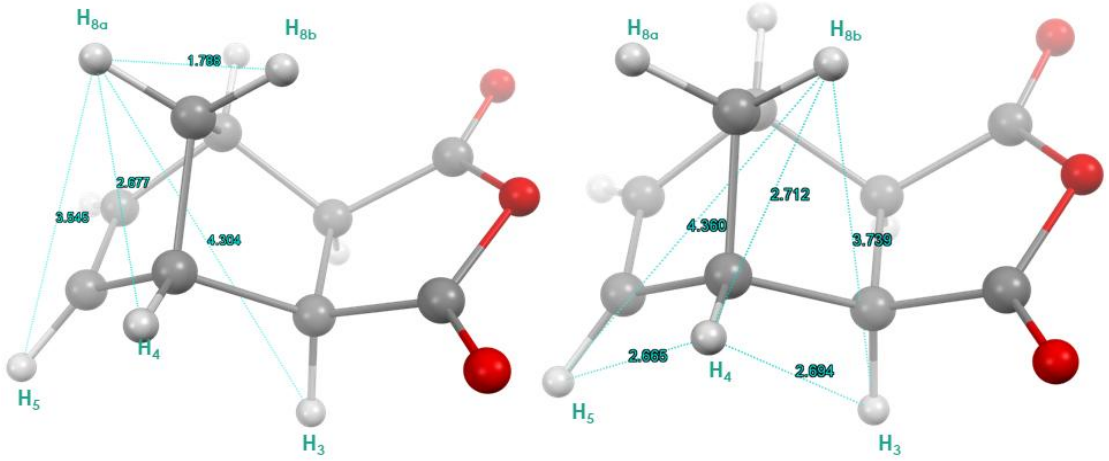


Figure 9. Crystal structure of **1*ex*,** left structure showing distances between H_{8a} and H_5, H_4, H_3, H_{8b}. Right structure showing distance between H_{8b} and H_5, H_4, H_3 and between H_5, H_4 and H_4, H_3 in Angstroms. (CCDC Database Identifier: NBONAN01, Deposit Number: 1217845)

The 1D NOESY experiments for **1*ex*** are shown in **Figure 8**. The irradiation of proton H_{8a} showed NOEs to protons H_5, H_6 which are close to each other in space and reside on the same face of the cyclopentene moiety. Interestingly, irradiation of H_5 and H_6 not only confirms the NOE with H_{8a} but also shows a minor Overhauser effect with H_{8b}. The magnetisation transfer between H_{8b} and H_5, H_6 is known **[**11**]** and probably occurring due to the orbital overlap between the double bond and H_{8b}. The greater signal intensity of H_{8a} compared to H_{8b} indicates that H_{8b} is further away from H_5, H_6 compared to H_{8a}.

The irradiation of H_{8b} shows a NOE with H_4, H_7 and a minor NOE with H_3, and H_9. The intensity of the H_3, H_9 peak is lower compared to H_4, H_7 and therefore indicates that H_3, H_9 are spatially further from H_{8b}. In turn, irradiation of H_3, H_9 also show a NOE with H_{8b}. If the students recall the 1D NOE for 5-norbornene-*endo*-2,3-dicarboxylate anhydride (**Figure 5**) they can readily observe that upon irradiation of H_{8b} the intensities of H_3, H_9 and H_4, H_7 signals are inverted relative to the *exo* isomer which shows that in the *exo* isomer H_3, H_9 are at a greater distance from H_{8b} and that in the *endo* isomer H_3, H_9 are much closer to H_{8b}.

Overall, 1D NOEs are consistent with the structure of the *exo* isomer and are summarized in **Table 2**. This is confirmed by the two important groups of proton correlations, the first one between H_{8a} and H_5, H_6 and second one between H_{8b} and H_4, H_7 and H_3, H_9.

These observed correlations, can also be associated with the crystal structure of the **1*ex***, and the atoms spatial proximity in the structure. With the help of **Figure 9**, students can correlate the NOE intensity with the distance between the atoms. Indeed, the shortest distance between H_5, H_6 and H_{8a} (3.5 Å) resulted in a medium to strong NOE, while a longer distance with H_{8b} (4.4 Å) resulted in a weak NOE.

Finally, with the analysis of the collected 2D and 1D NOESY data students can have a series of tools to help differentiate between the *endo* and *exo* isomers of 5-norbornene-2,3-dicarboxylic anhydride. These NMR experiments can unambiguously answer the question that may rise on how to distinguish between the *exo* and *endo* Diels-Alder products with the help of advanced NMR techniques. To make sure these techniques are well understood, the students can now be exposed to a more challenging assignment using the Diels-Alder reaction between methylcyclopentadiene and maleic anhydride and asked to assign the configuration of the resulting adducts. [4]

3.2. *Endo*-5-Methyl-5-norbornene-2,3-dicarboxylic Anhydride (3*en*) and *Exo*-5-methyl-5-norbornene-2,3-dicarboxylic Acid Anhydride (3*ex*)

The analysis of the Diels-Alder reaction products between the methylcyclopentadiene isomers and maleic anhydride followed the same process as in case of *exo* and *endo* isomers of 5-norbornene-2,3-dicarboxylic anhydride. The *endo* isomers **2*en*** and **3*en*** can readily be obtained according to the reported procedure [4], while the exo isomers **2*ex*** and **3*ex*** can be generated using microwave irradiation of the endo isomers in toluene at 200℃ for 15 min (**Scheme 1**).

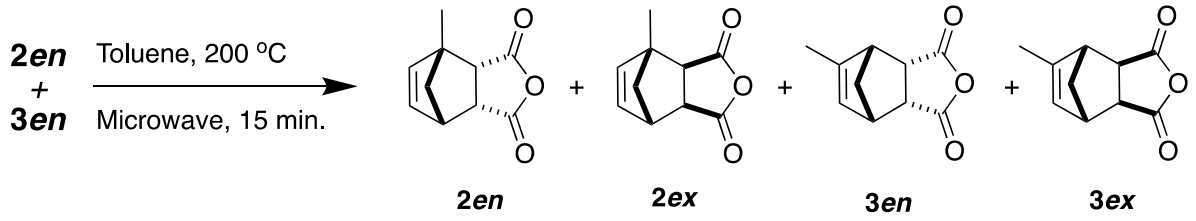
Interestingly, during the isomerization the ratio of isomer **2** to **3** decreased dramatically from 43/57 to 20/80 after 15 minutes of irradiation, and subsequently to 14/86 after 90 minutes of irradiation. Such a dramatic change in ratios permitted an easy separation of isomers **3*en*** *and* **3*ex*** in good purity.

With the NMR structure elucidation of *endo* isomer 3*en* previously described,[4] the *exo* isomer 3*ex* was submitted to the similar procedure and its structure was solved using ^{1}H, ^{13}C, APT, COSY, HSQC, HMBC experiments.

Figure 10 shows the ^{1}H-NMR spectra of both isomers for comparison. Just as in the case of isomers **1*en*** and **1*ex***, the biggest difference between the isomers consists of more deshielded H_5 and H_6 signals in the *endo* as opposed to the *exo* isomer, while the chemical shifts of H_4, H_1 and H_{7a}, H_{7b} show the same tendency and are shifted to lower field in the *endo* isomer. [11]

The 1D and 2D NOESY spectra were subsequently collected to help assign the *exo* and *endo* configuration.

Figure 11 displays the 2D NOESY spectrum for **3*en***. The cross peaks between H_{7b} and H_5, H_6 indicate the close spatial proximity of these protons, while the presence of additional cross peaks in the spectrum indicates the close proximity of H_{7a} and H_3 protons. These observations suggest the presence of the *endo* configuration. However, additional validation using 1D NOESY is required due to T_1 noise that can lead to ambiguous cross peak readings in the 2D spectrum.



Scheme 1. Microwave assisted isomerization and rearrangement of the Diels-Alder Adducts **2*en*** and **3*en*** to **2*ex*** and **3*ex***

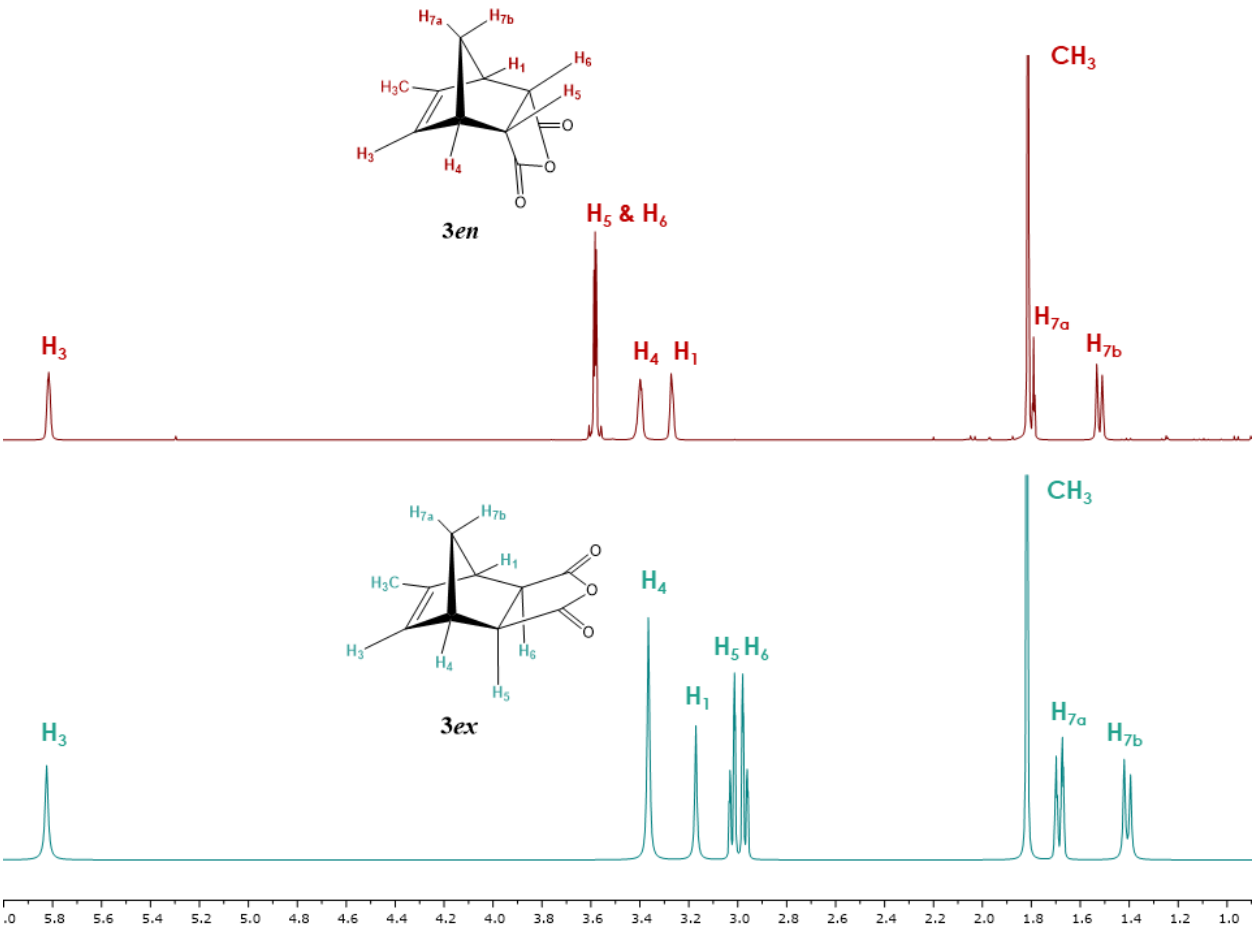


Figure 10. ^{1}H NMR spectra of exo and endo isomers of 5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride **3ex** and **3en**. (Minor impurities were removed by deconvolution for clarity purposes, see supplementary information for the non- deconvoluted spectra.)

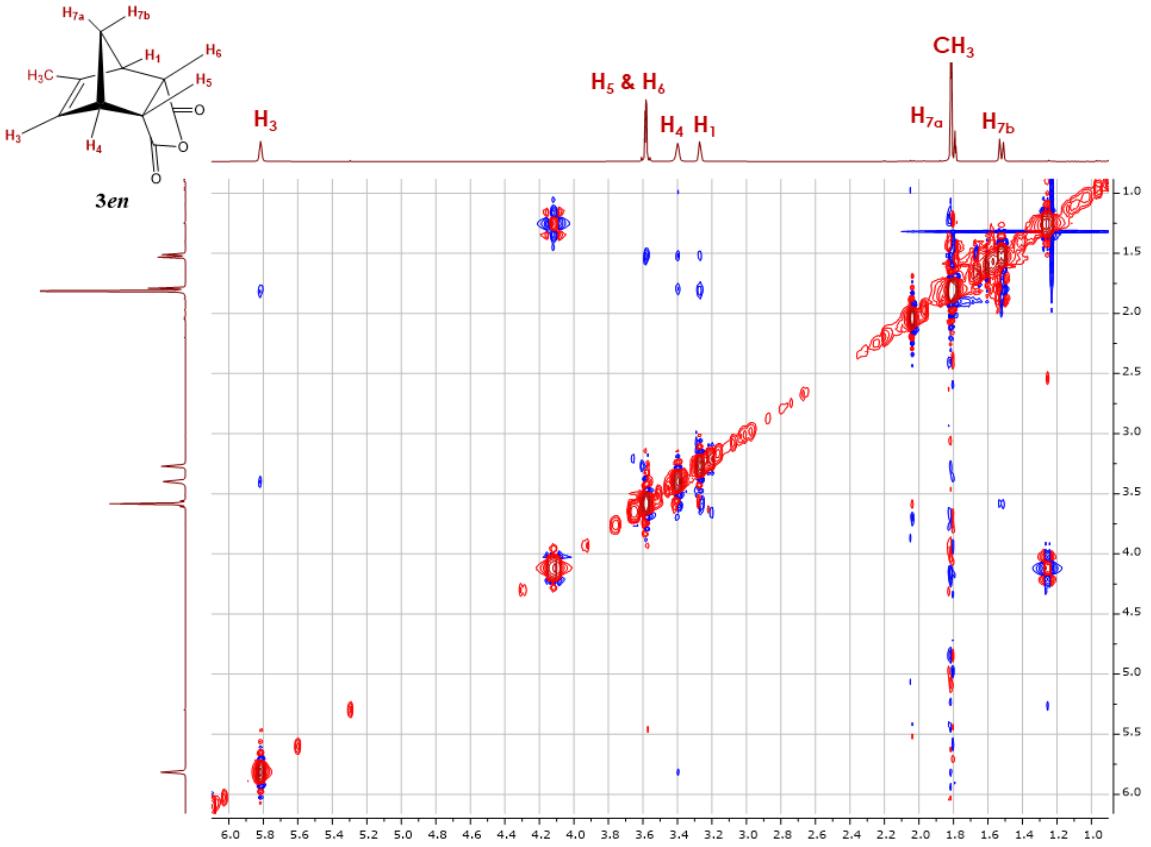


Figure 11. 2D NOESY of *endo*-5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride **3*en***

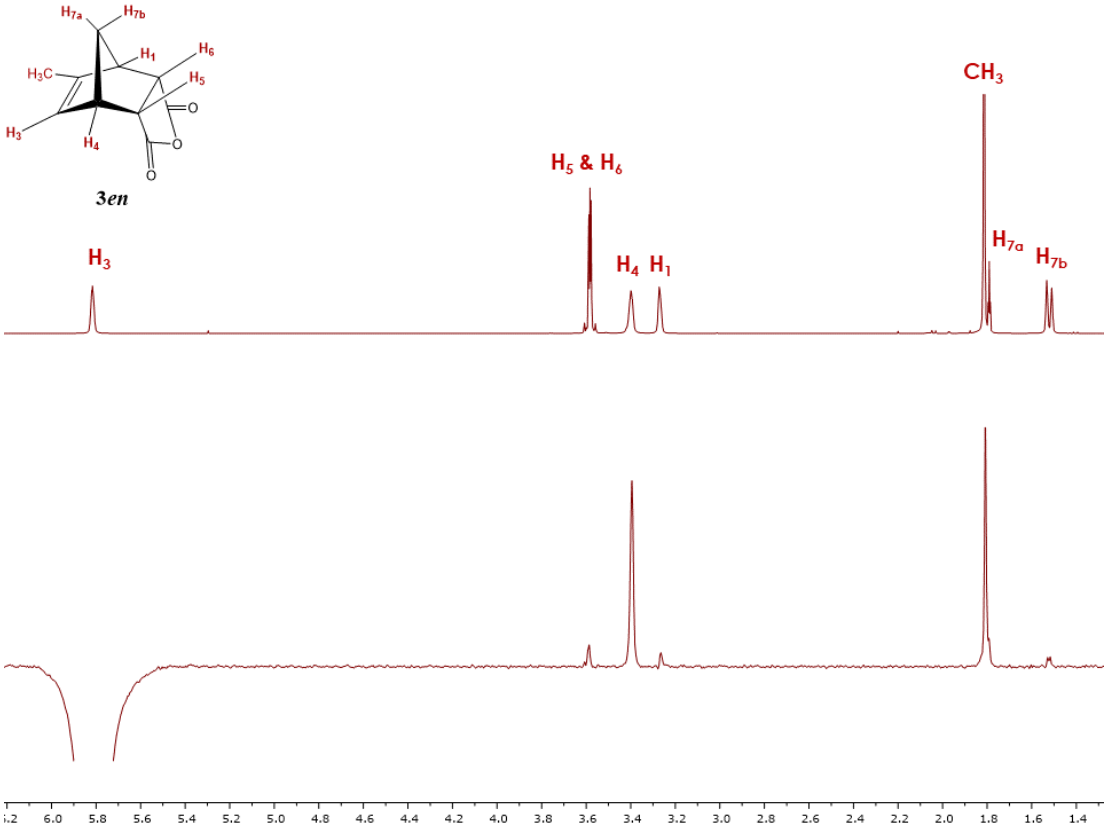


Figure 12. Stacked ^{1}H-NMR and 1D NOESY spectra of endo-5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride **3*en*** with H_3 proton irradiated

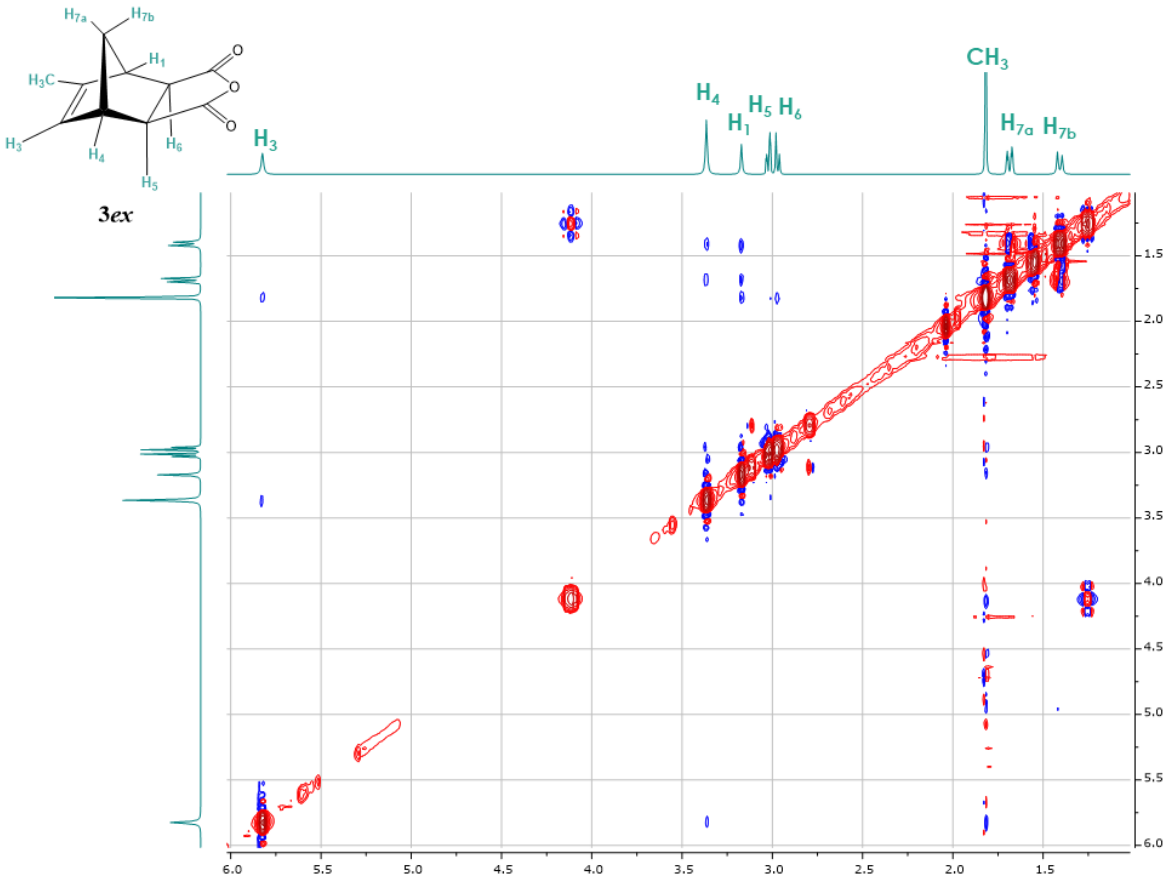


Figure 13. 2D NOESY spectrum of endo-5-Methyl-5-norbornene-2,3-dicarboxylic acid anhydride **3*ex***

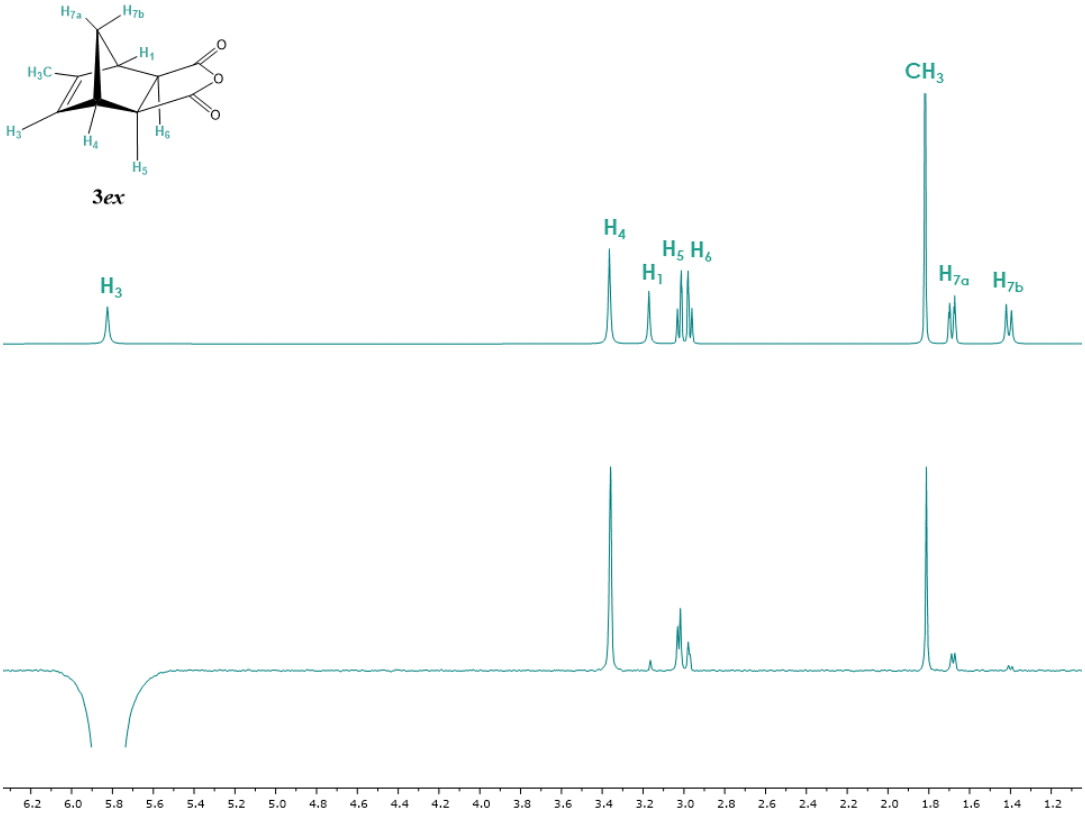


Figure 14. Stacked ^{1}H-NMR and 1D NOESY spectra of exo-5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride **3*ex*** with H_3 proton irradiated

The zero-quantum suppression 1D NOESY for **3*en*** shown in **Figure 12** allows the students to "filter off" the *J*-coupling interactions and further confirm the spatial configuration of the endo isomer.

After the comprehensive analysis of **1*en*** and **1*ex*** isomers, students can now limit the number of 1D NOE acquisitions by simply irradiating the readily accessible alkene proton H_3. Indeed, the irradiation of H_3 provides immediate information on the adduct **3** configuration. Strong correlations are observed for neighbouring protons H_4, methyl group and H_{7a}, and weak correlations between protons H_1, H_3, H_{7b} and H_5 & H_6. It is now important to compare intensities of cross peaks between protons H_3 and H_5 & H_6 for both 1D NOESY spectra of ***3en*** and ***3ex*** to clearly confirm the structure of adducts. The intensity of cross peak $H_3 - H_5$ & H_6 is smaller in the spectrum displayed on **Figure 12** compared to **Figure 14** (discussed below) and therefore suggests that **Figure 12** depicts the endo isomer ***3en***. Irradiation of other protons in the *endo* adduct also reaffirm the suggested configuration *(See supplementary information for all spectra)*.

The two dimensional NOESY spectrum in **Figure 13** for the *exo* isomer **3*ex*** shows no correlation between protons H_5, H_6 and the bridge diastereotopic protons H_{7a} and H_{7b}, indicating that they are not in close proximity. Additionally, due to a large amount of t_1 noise and the close chemical shifts of H_5, H_6 protons, cross peak interpretation is not really informative. However, a series of 1D NOE spectra allowed for the unequivocal *exo* assignment. While all the protons for ***3ex*** were irradiated the most informative spectrum resulted from the irradiation of the alkene proton H_3. As seen in **Figure 14**, H_3 shows a strong correlation with protons H_4 and H_5 & H_6. The intensity of cross peak $H_3 - H_5$ & H_6 is also greater compared to that in the ***3en*** isomer in **Figure 12**, which is a clear indication of their spatial proximity, and a good validation of the *exo* configuration.

Since most of the studies on 3*en* and 3*ex* adducts were performed on samples containing small impurities, a quick way to generate clean analytical samples was to react adducts 3*en* and 3*ex* with L-alanine methyl ester under basic conditions and separate the resulting diastereomers. [12] These two new diastereomers 4*en* and 4*ex* can easily be separated by chromatography on silica gel and submitted to NMR spectroscopic investigation to further assign and validate their configuration.

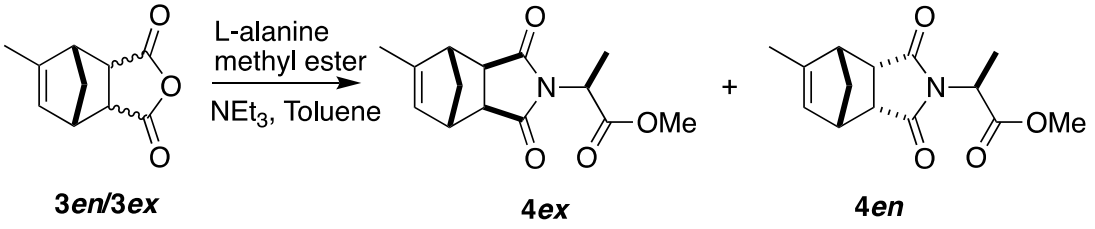
3.3. L-Alanine Methyl Ester Derivatives of 5-Methyl-5-norbornene-2,3-dicarboximide

The L-alanine modification of 5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride derivatives was previously described to separate the mixtures of the diastereomers **2*en*** and **3*en***. [4] This modification can serve as an alternative confirmation in the structure elucidation of the *exo* and *endo* isomers. The L-alanine methyl ester *endo* and *exo* isomers **4*en*** and **4*ex*** were obtained by microwave isomerization of Diels Alder products **3*en*/3*ex*** followed by derivatization of the reaction mixture with L-alanine methyl ester under basic conditions (**Scheme 2**). [5,12]

Since the structure analysis of the endo isomer 4*en* was previously completed using ^1H, ^{13}C, COSY, HSQC, HMBC experiments [4], the same NMR experiments can be used to assign the structure of the *exo* isomer 4*ex (See supplementary information)*. Additionally, 1D and 2D NOESY experiments were used to differentiate between the *exo* and *endo* configuration. As in the case of 5-Methyl-5-norbornene-2,3-dicarboxylic acid anhydride, **Figure 15** shows a similar trend for the ^1H NMR chemical shifts between *exo* and endo isomers, with several signals being more deshielded for the *exo* isomer. The most drastic differences are observed for the chemical shifts of protons H_5 and H_6 which occur at 2.8-2.6 ppm in case of the *exo* isomer and 3.2-3.3 ppm in case of the *endo* isomer.

The subsequent 2D NOESY spectrum for the *endo* adduct **4*en*** shows similar cross peaks between bridge diastereotopic proton H_{7b} and H_5, H_6 as was previously observed for *endo*-5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride (**Figure 16**) as well as a cross peak between H_{7a} and H_3.

The 1D NOESY spectrum, however, was more informative when focused on the H_3 proton, avoiding the ambiguous cross peaks in the 2D NOESY spectrum caused by the t_1 noise and the *J*-couplings. As in adducts **2** and **3**, the H_3 proton signal does not overlap with any other signal and is easily detected in the ^1H-NMR alkene region (5 to 6 ppm). The 1D NOESY spectrum (**Figure 17**) with irradiated protons H_3 shows several correlations with H_{7a}, the methyl groups CH_3 (13) and CH_3 (15) as well of what appears to be a strong correlation with H_4. However, since H_4, H_5 and H_6 overlap in the adduct **4*en***, the analysis of the adduct **4*ex*** is required to lift any doubt on the **4*en*** configuration.



Scheme 2. L-alanine functionalization of the 5-methyl-5-norbornene-2,3-dicarboxylic acid anhydride isomers mixture

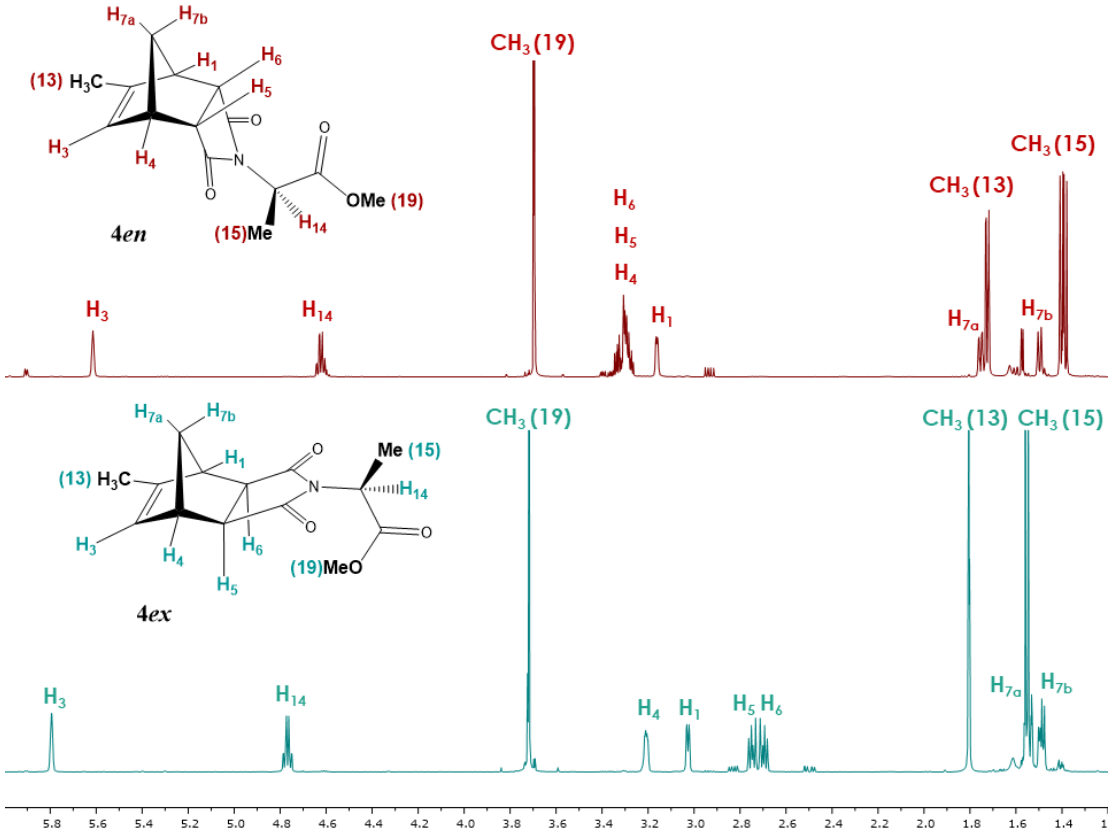


Figure 15. ^{1}H NMR spectra of isomers **4*ex*** and **4*en***

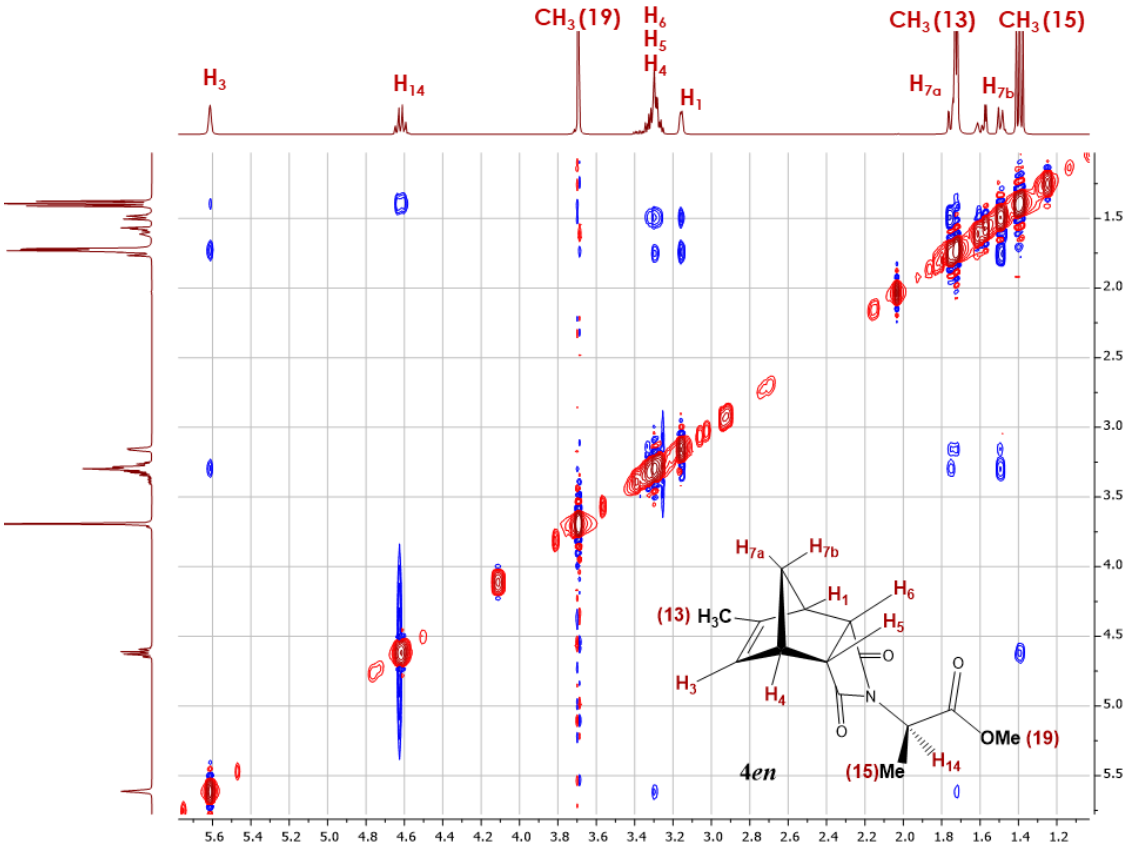


Figure 16. 2D NOESY spectrum of adduct **4*en***

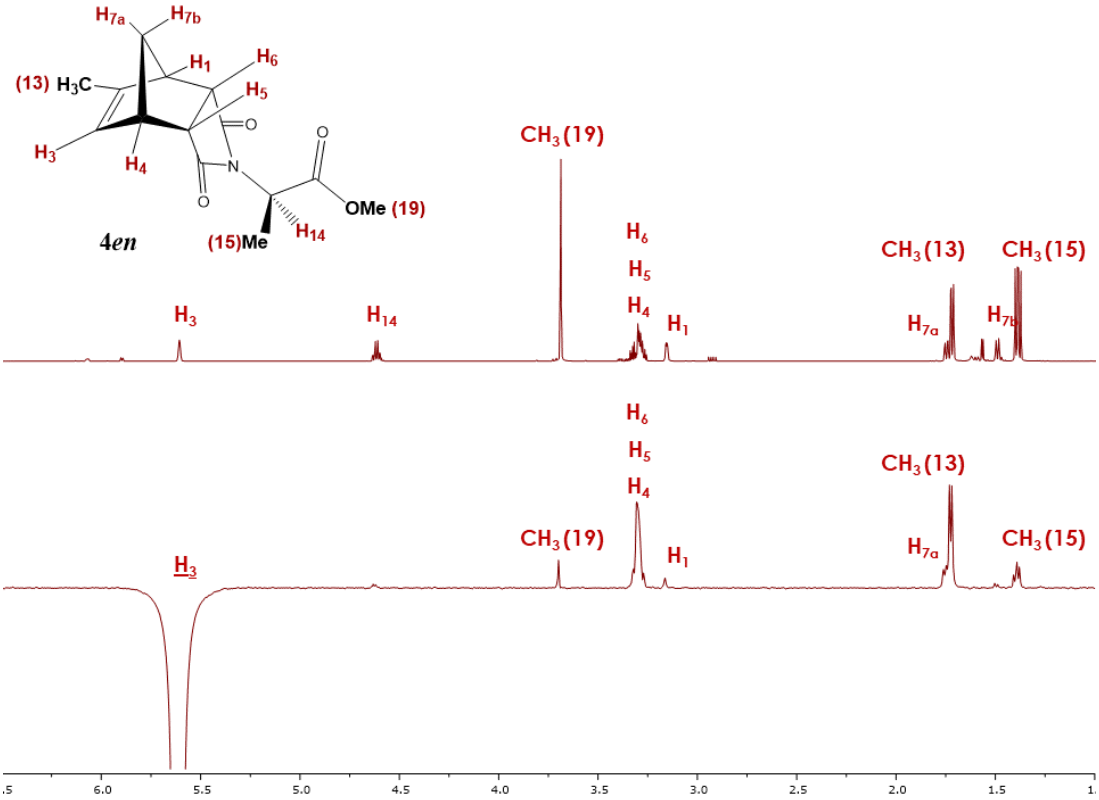


Figure 17. Stacked ^{1}H-NMR and 1D NOESY spectra of adduct ***4en*** with irradiated H_3 proton

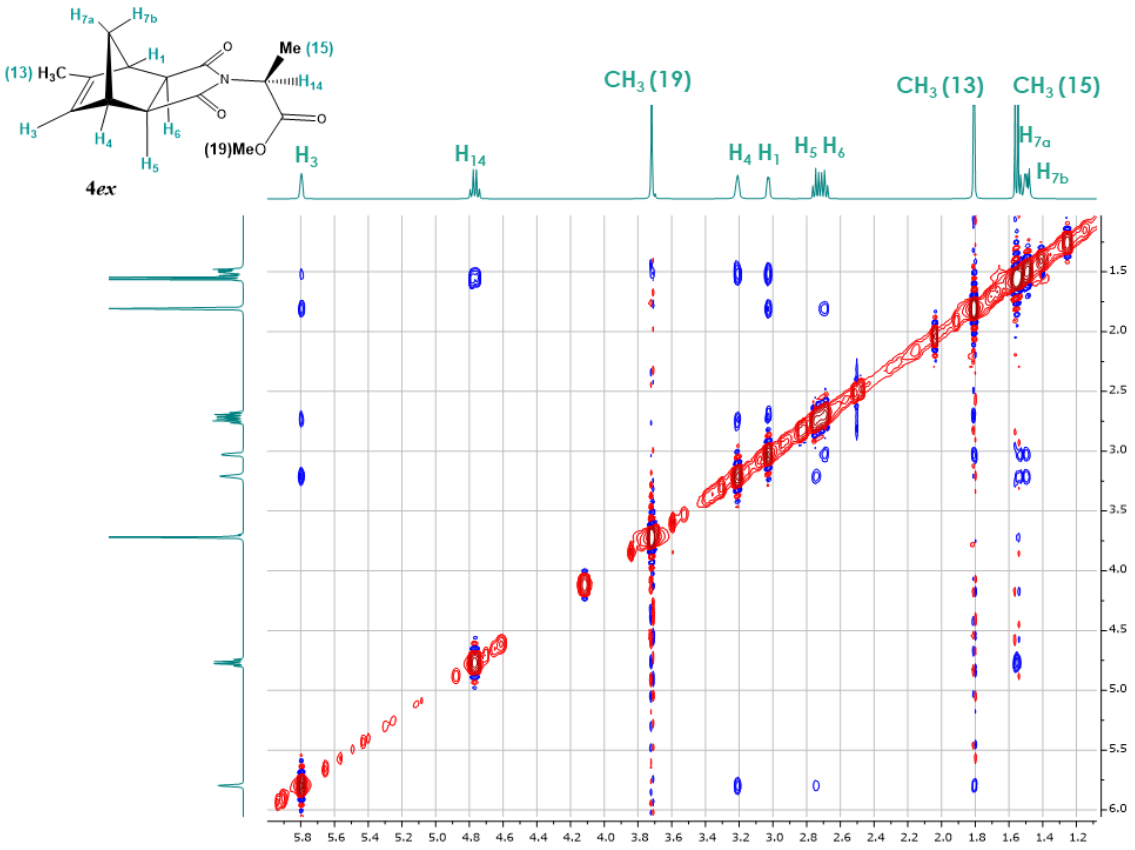


Figure 18. 2D NOESY spectrum of adduct ***4ex***

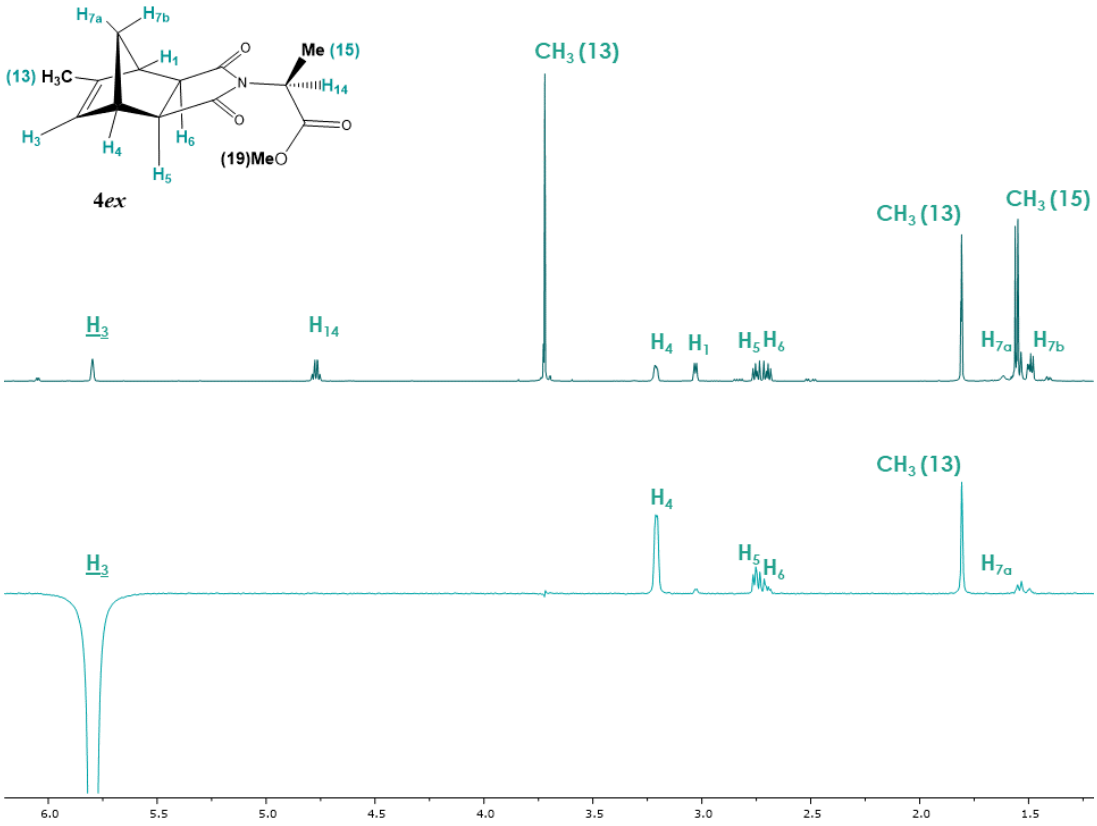


Figure 19. Stacked ^{1}H-NMR and 1D NOESY spectra of adduct ***4ex*** with H_3 proton irradiated

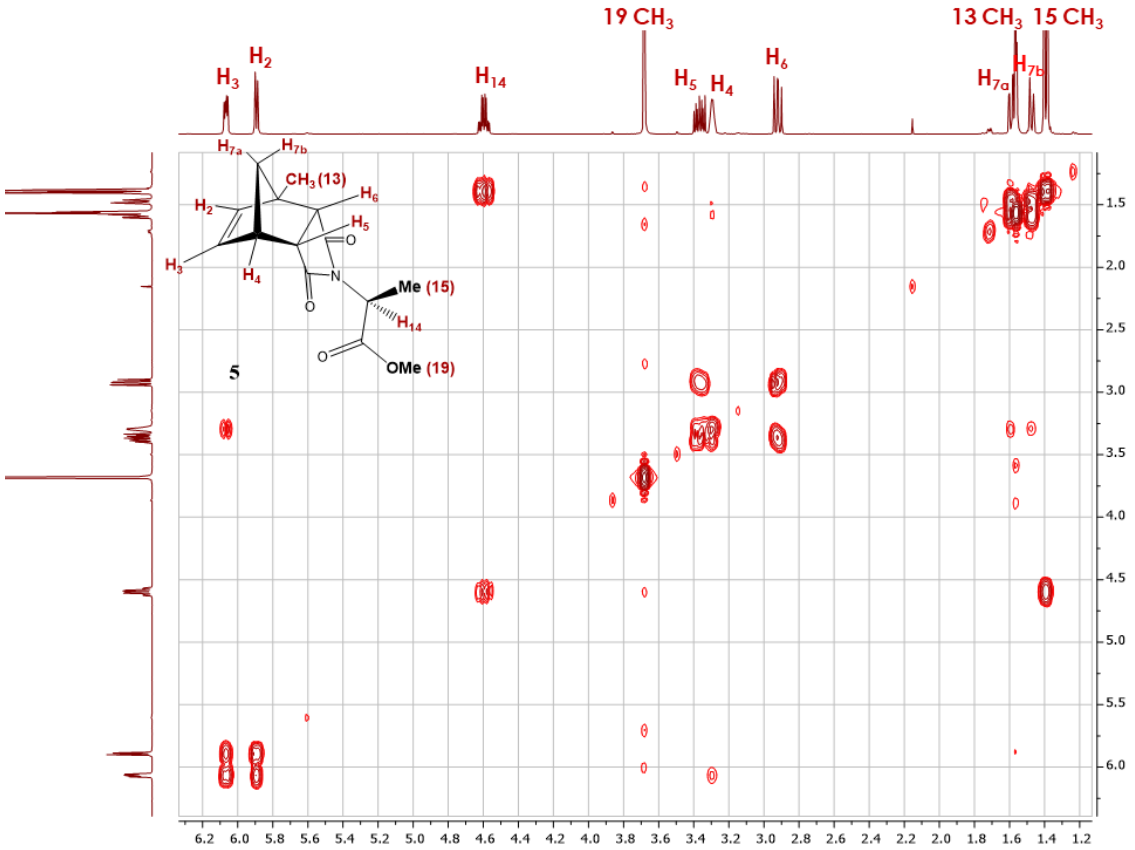


Figure 20. 2D NOESY spectrum of adduct **5**

The 2D NOESY spectrum for the *exo* isomer **4*ex*** showed no correlation between bridge protons H_{7a}, H_{7b} and H_5, H_6 as expected (**Figure 18**). Instead H_5, H_6 show correlations with H_1, H_3, H_4 protons. Additionally, correlations between H_3 and H_{7a}, CH_3 (13) protons are observed, providing further evidence in favor of the *exo* configuration for adduct **4*ex***. The 1D NOESY spectrum (**Figure 19**) confirms that upon irradiation of H_3 magnetization transfers to protons H_4, H_5, H_6, methyl group CH_3 (13) as well as to the diastereotopic bridge proton H_{7a}. Students should be encouraged to compare the *exo* and *endo* isomers spectra since that is the key strategy for configuration determination.

3.4. Determining the Configuration of 1-methyl-5-norbornene-2,3-dicarboximide L-alanine Derivative (5)

At this point and to summarize the student's learnings, it is important to provide them with a derivative of unknown configuration and ask them to assign its structure as well as confirm its configuration. From previous and current work, several derivatives of **2*en*** and **2*ex*** can be isolated, purified and used as unknowns for this particular exercise.

When an L-alanine methyl ester derivatives of 1-methyl-5-norbornene-2,3-dicarboximide (**5**) [4] was assigned to the students, a full NMR package was recorded and the proton signals in the molecule assigned when possible. Subsequently, the 2D NOESY spectrum was recorded and the spectrum analyzed (**Figure 20**). The clear correlations between the alkene protons H_2 and H_3 with H_4 were to be expected, however the absence of correlation with protons H_{7b} and H_5 and H_6 needed to be further investigated using 1D NOE experiments.

As seen in **Figure 21**, upon selective irradiation of proton H_2 and H_3, correlations with proton H_4 was confirmed while additional information was acquired. Indeed, these experiments confirmed the assignment of H_2 and H_3, with H_2 showing a correlation to the methyl group Me (13). With no correlation observed with any of the H_5 and H_6 protons the students could definitely assign the configuration of compound **5** as *endo*.

4. Conclusions

Using the ubiquitous Diels Alder reaction between readily available undergraduate laboratory reagents, the students were able to synthesize methyl substituted norbornene-2,3-dicarboxylic anhydride adducts. Upon microwave irradiation of these adducts a mixture of the *endo* and e*xo* conformers were formed and were separated using standard chromatography techniques. Such mixtures of conformers proved to be the ideal targets to introduce the students to 2D and 1D Nuclear Overhauser Effect (NOE) NMR spectroscopy experiments.

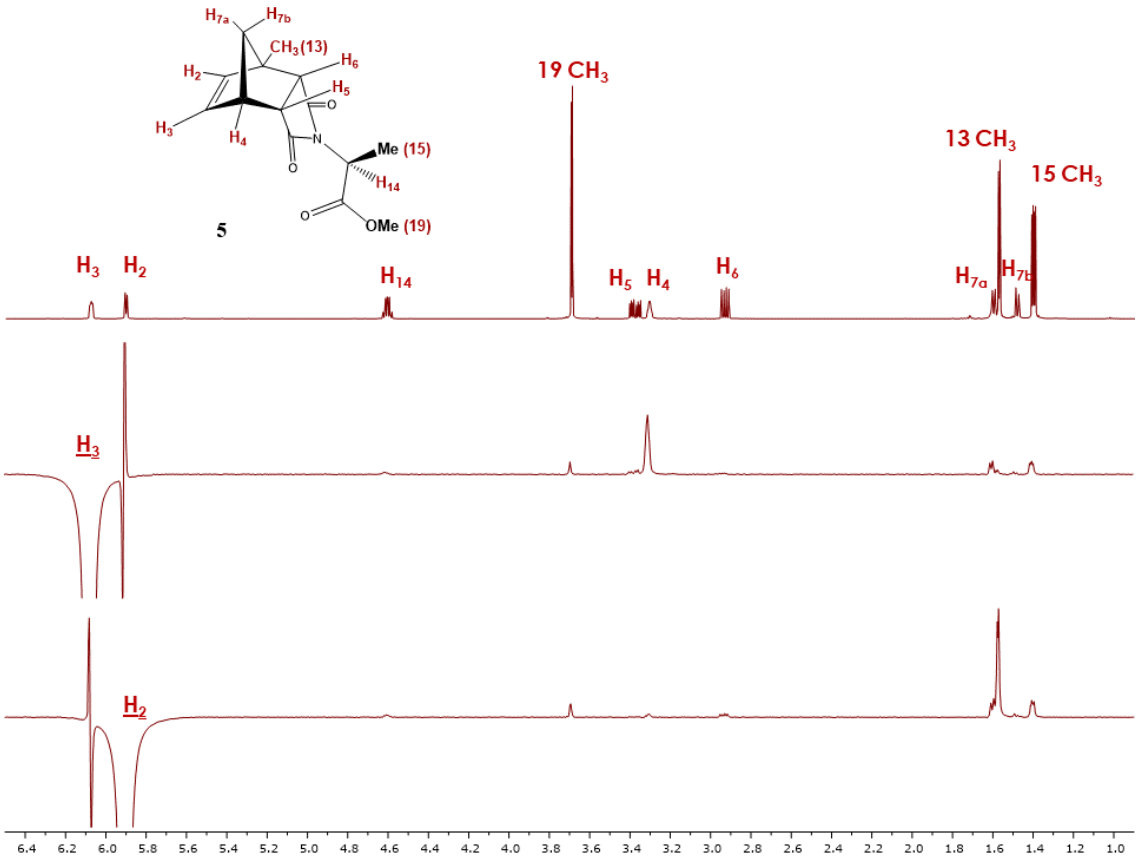


Figure 21. Stacked ^{1}H-NMR and 1D NOESY spectra of adduct **5** with irradiated H_2 and H_3 proton

ACKNOWLEDGEMENTS

The authors thank the Simon Fraser University (SFU) Dean of Science Office for financial support and the SFU Chemistry Department for logistical support.

Supporting Information

All raw "full package" NMRs as well as labelled relevant NMR spectra and correlation tables are available as supplementary information.

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Supporting Information

Chromatographic purification of 3en, 3ex.

The crude reaction mixture extract (1.4 g) was purified using flash liquid chromatography on a Teledyne CombiFlash Rf system equipped with a 2x24 g normal-phase RediSep Rf Gold silica column. The elution was done with an ACS grade hexanes/ethyl acetate mixture. The sample was dry packed using a minimal amount of Fisher 230-400 mesh normal-phase silica gel. The separation was done using the following parameters. Flow Rate –35 ml/min; Equilibration Volume –5.0 CV (Column Volume); Run length –20 CV; Fraction volume –7 mL; UV wavelength –254 nm & 280 nm. Gradient: 25% ethyl acetate over 2 CV; 25% – 33% over 4 CV; 33% – 66% ethyl acetate over 4 CV; 66% ethyl acetate over 4 CV; 100% ethyl acetate over 8 CV. Diastereomer ***3ex*** was eluted first in fractions 6-10 (480 mg) followed by ***3en*** fractions. 12-17 (900 mg).

Chromatographic purification of 4en, 4ex.

The crude reaction mixture extract (1.1 g) was purified using flash liquid chromatography on a Teledyne CombiFlash Rf system equipped with a 2x24 g normal-phase RediSep Rf Gold silica column. The elution was done with an ACS grade hexanes/ethyl acetate mixture. The sample was dry packed using a minimal amount of Fisher 230-400 mesh normal-phase silica gel. The separation was done using the following parameters: Flow Rate –35 ml/min; Equilibration Volume –5.0 CV (Column Volume); Run length –15 CV; Fraction volume –7 mL; UV wavelength –254 nm & 280 nm. Gradient: 33% ethyl acetate – 33 % over 2 CV; 33% – 42% ethyl acetate over 4 CV; 42% – 45% ethyl acetate over 3 CV; 100% ethyl acetate over 6 CV. Diastereomer ***4ex*** was eluted first in fractions 8-14 (78 mg) followed by ***4en*** fractions. 15-21 (1000 mg).

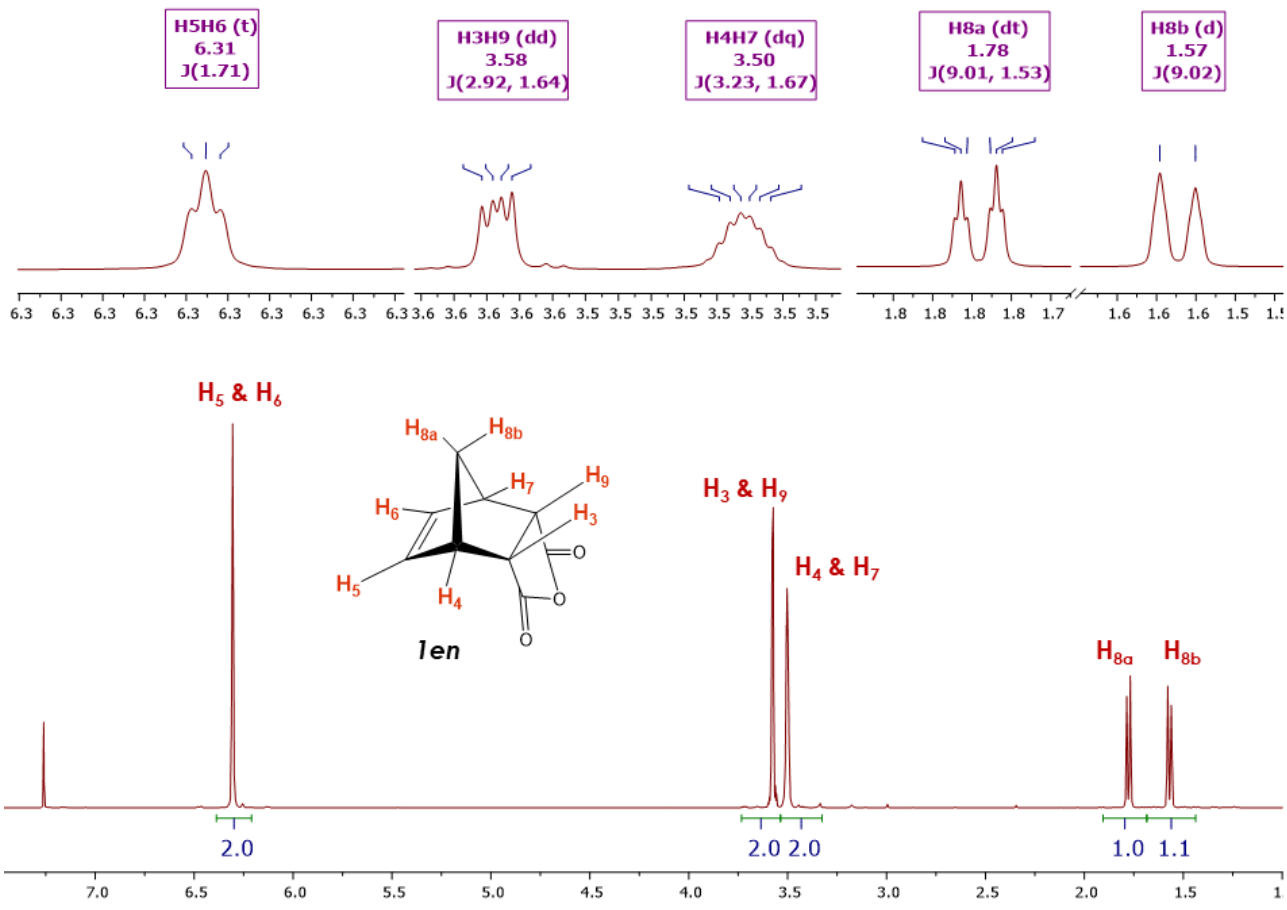

Figure S.1. ^{1}H NMR of ***1en***

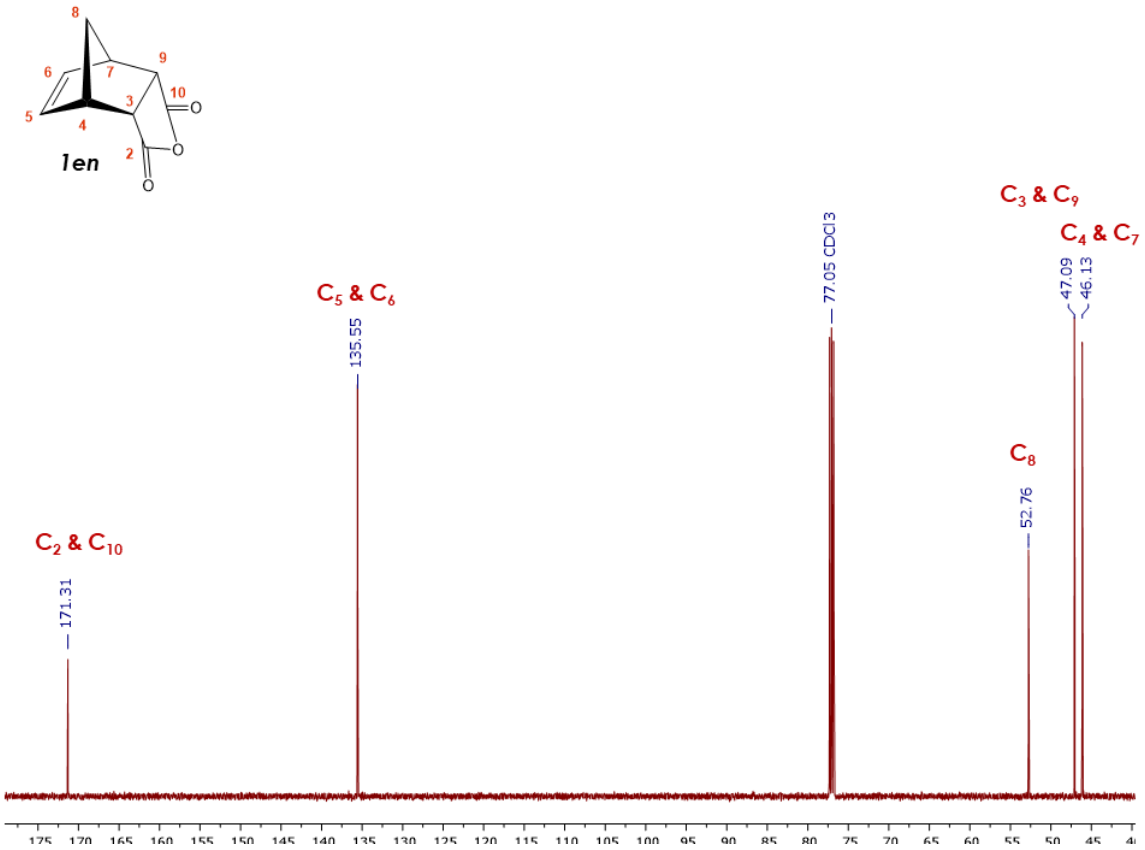

Figure S.2. ^{13}C NMR of ***1en***

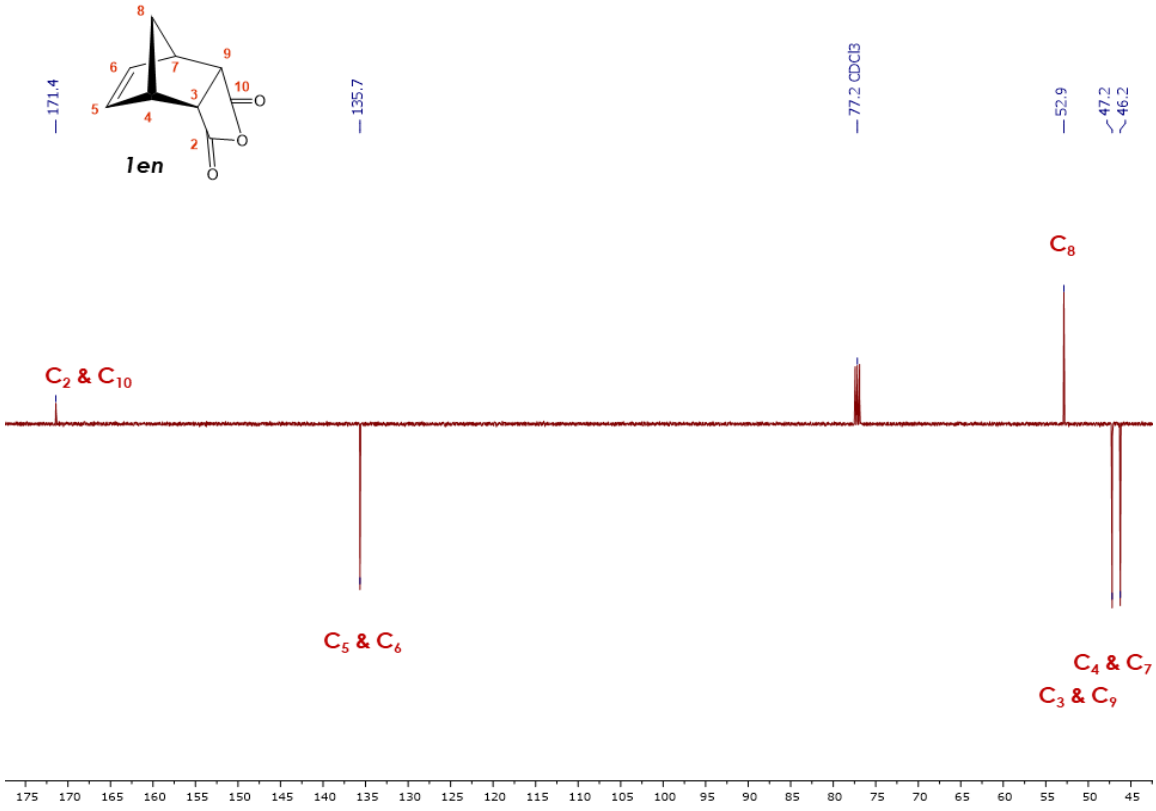

Figure S.3. APT of ***1en***

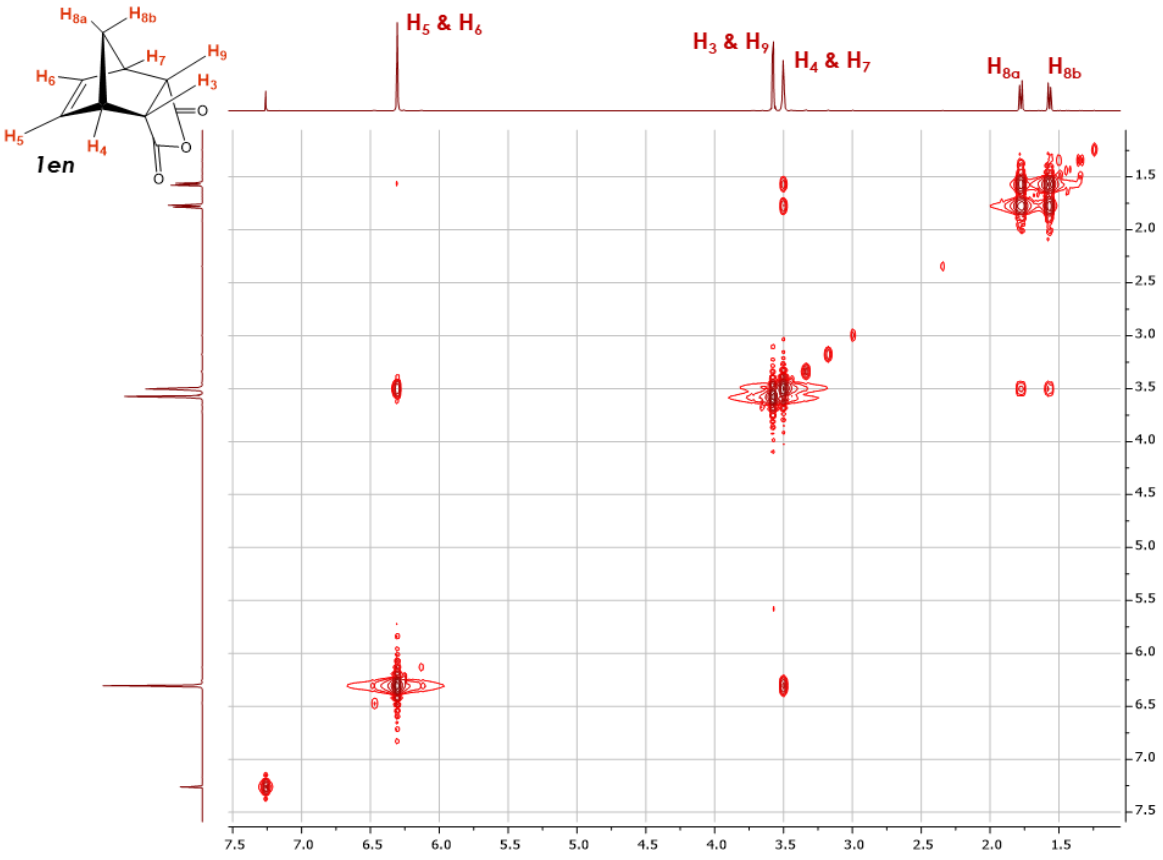

Figure S.4. COSY of ***1en***

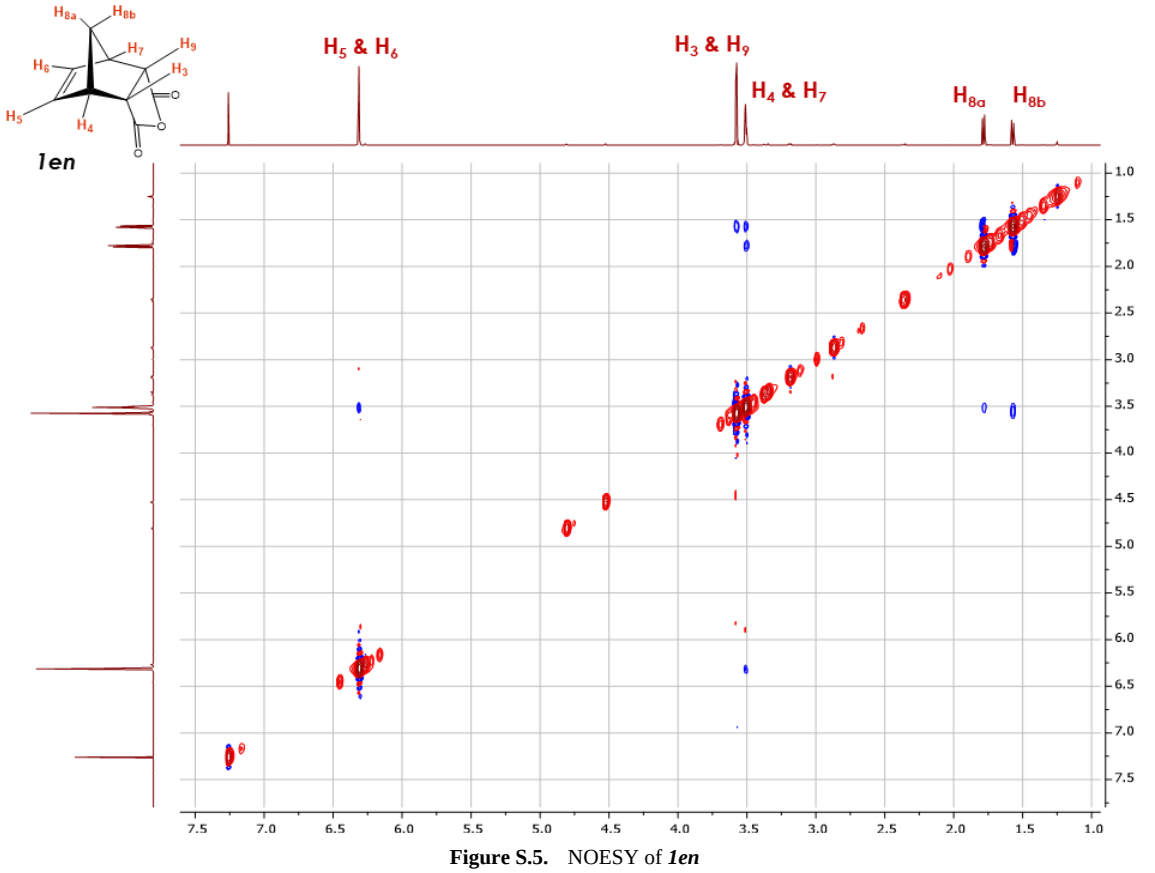

Figure S.5. NOESY of ***1en***

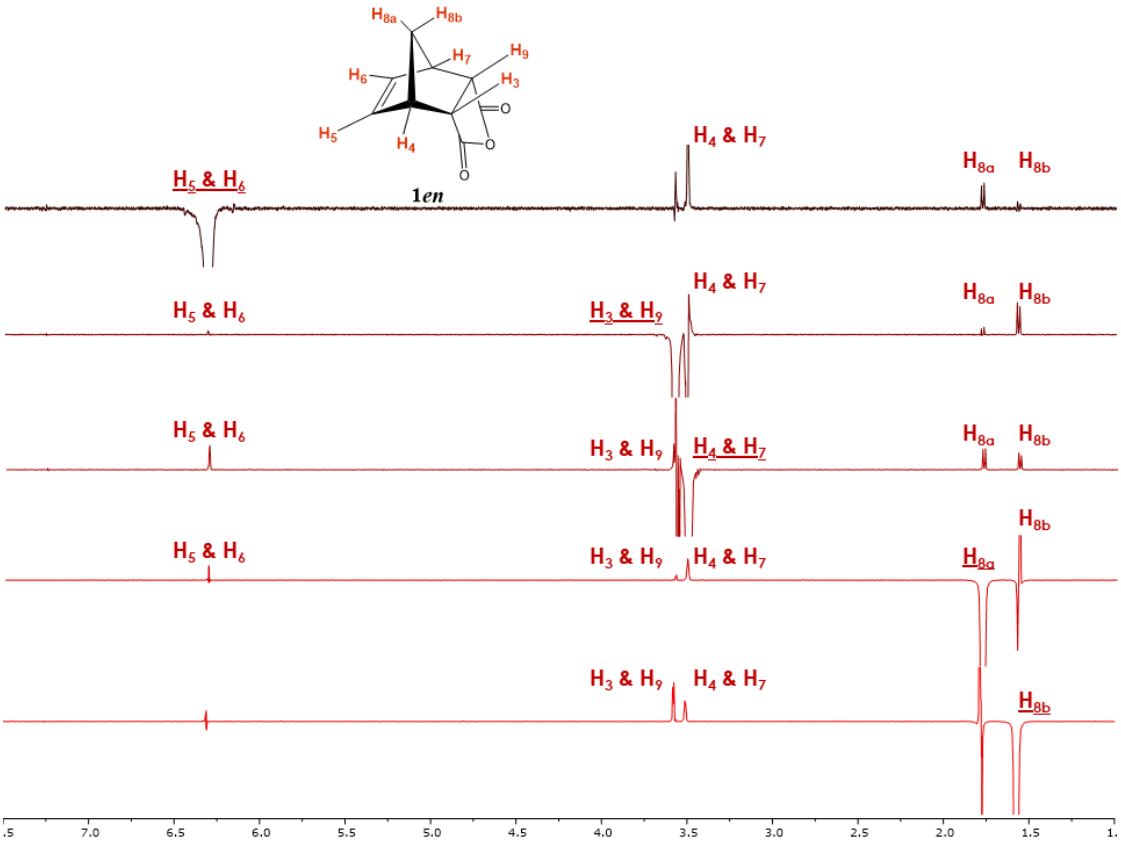

Figure S.6. 1D NOEs of ***1en***

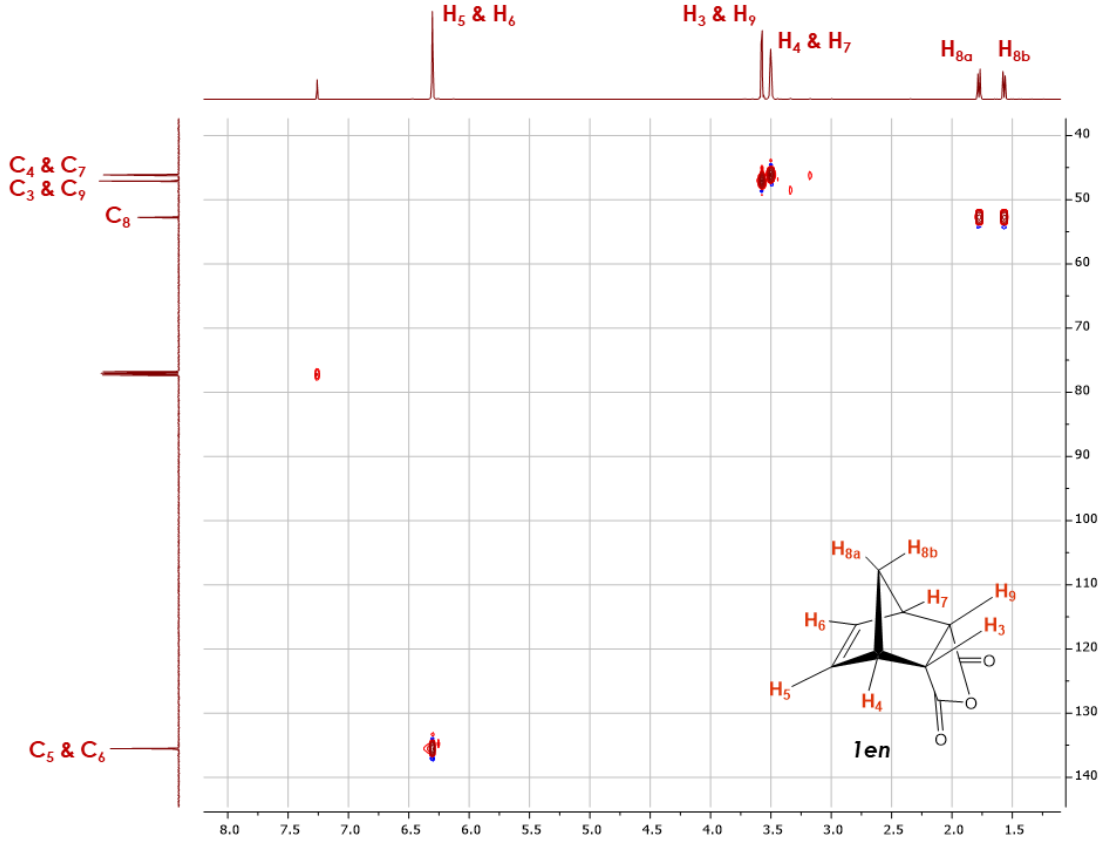

Figure S.7. HSQC of ***1en***

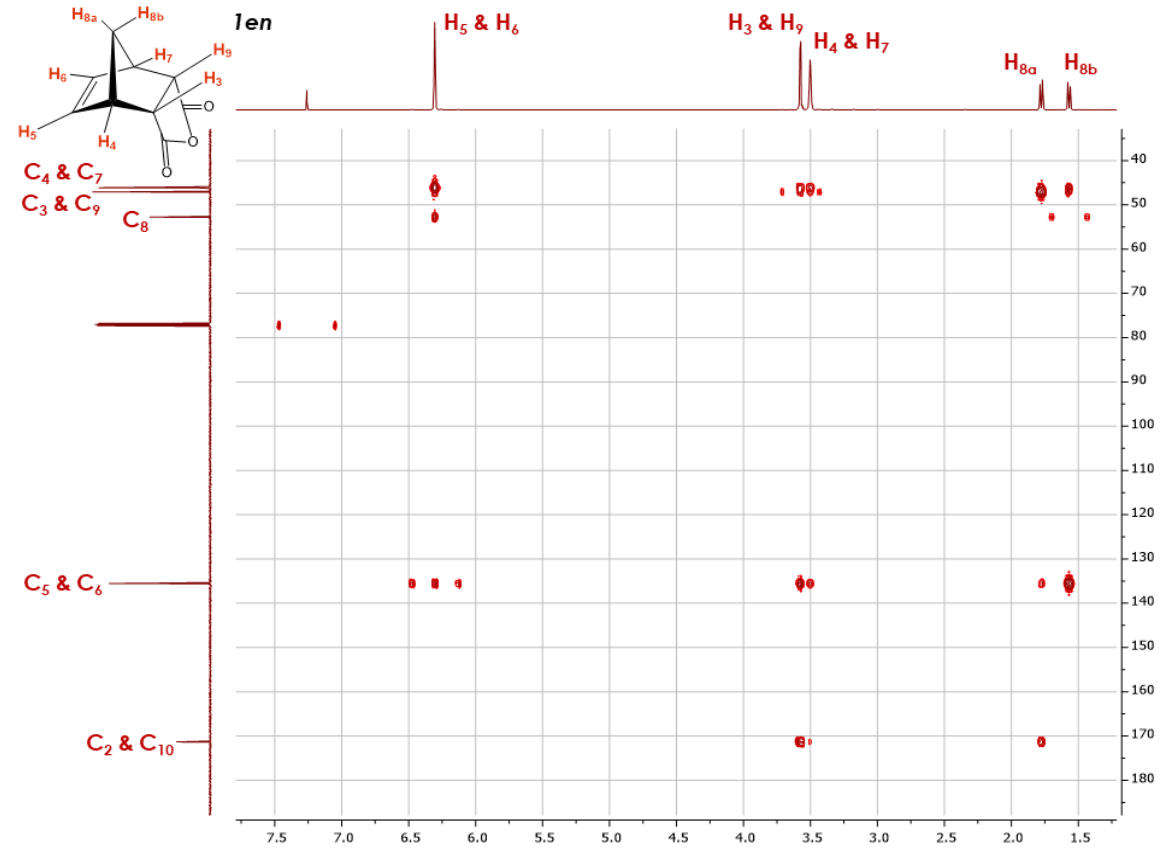

Figure S.8. HMBC of ***1en***

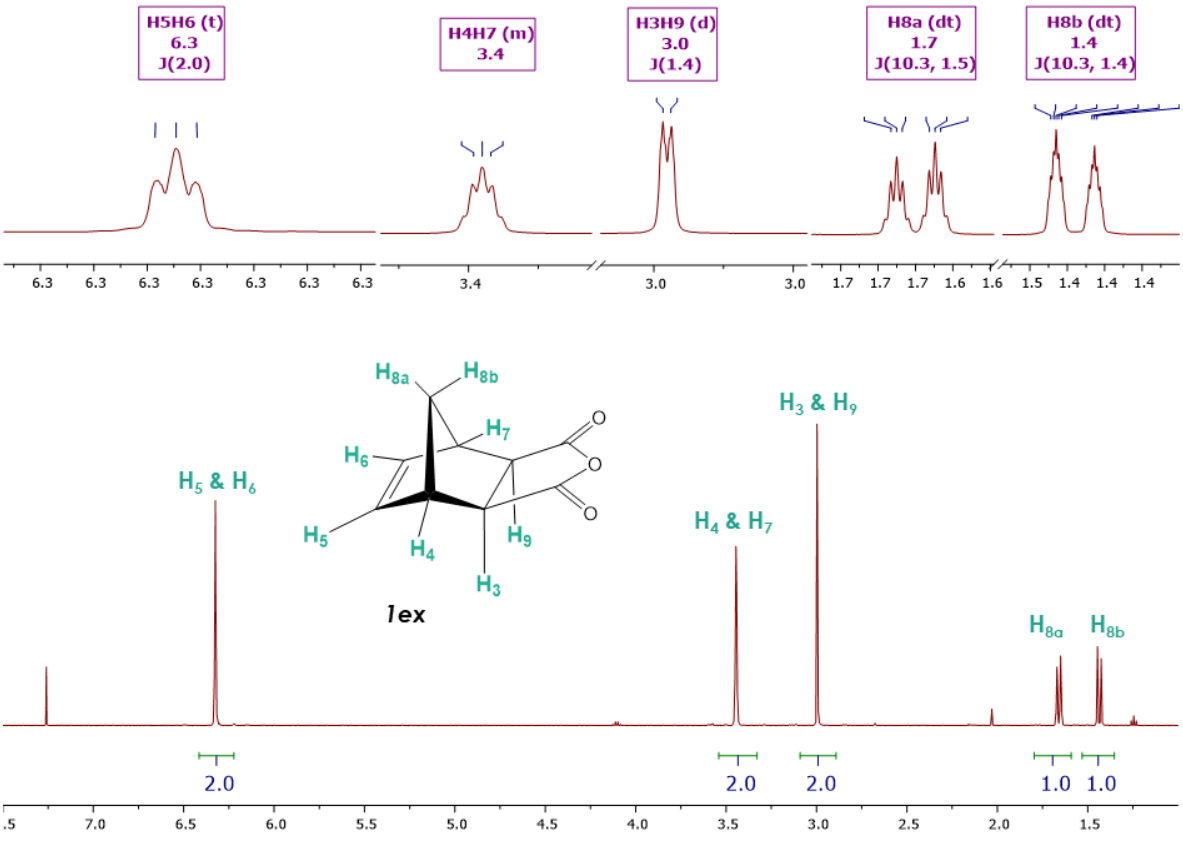

Figure S.9. ^{1}H NMR of ***1ex***

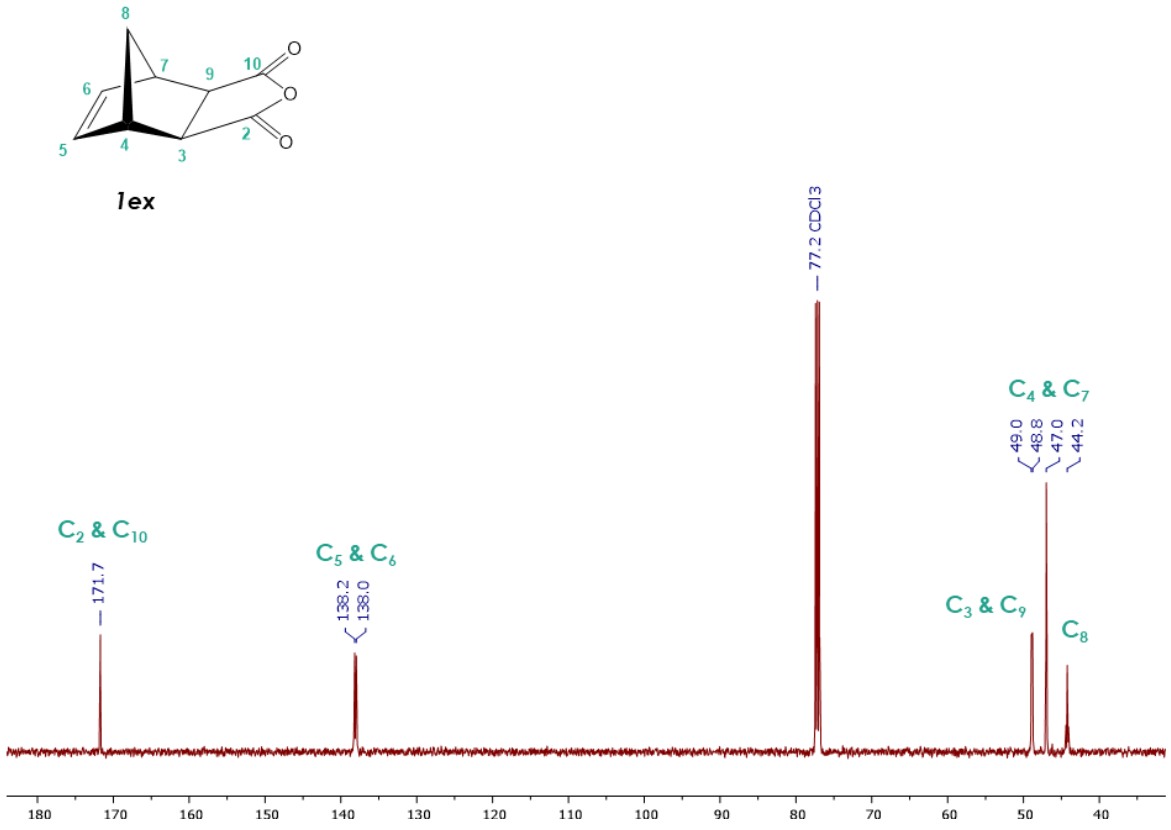

Figure S.10. ^{13}C NMR of ***1ex***

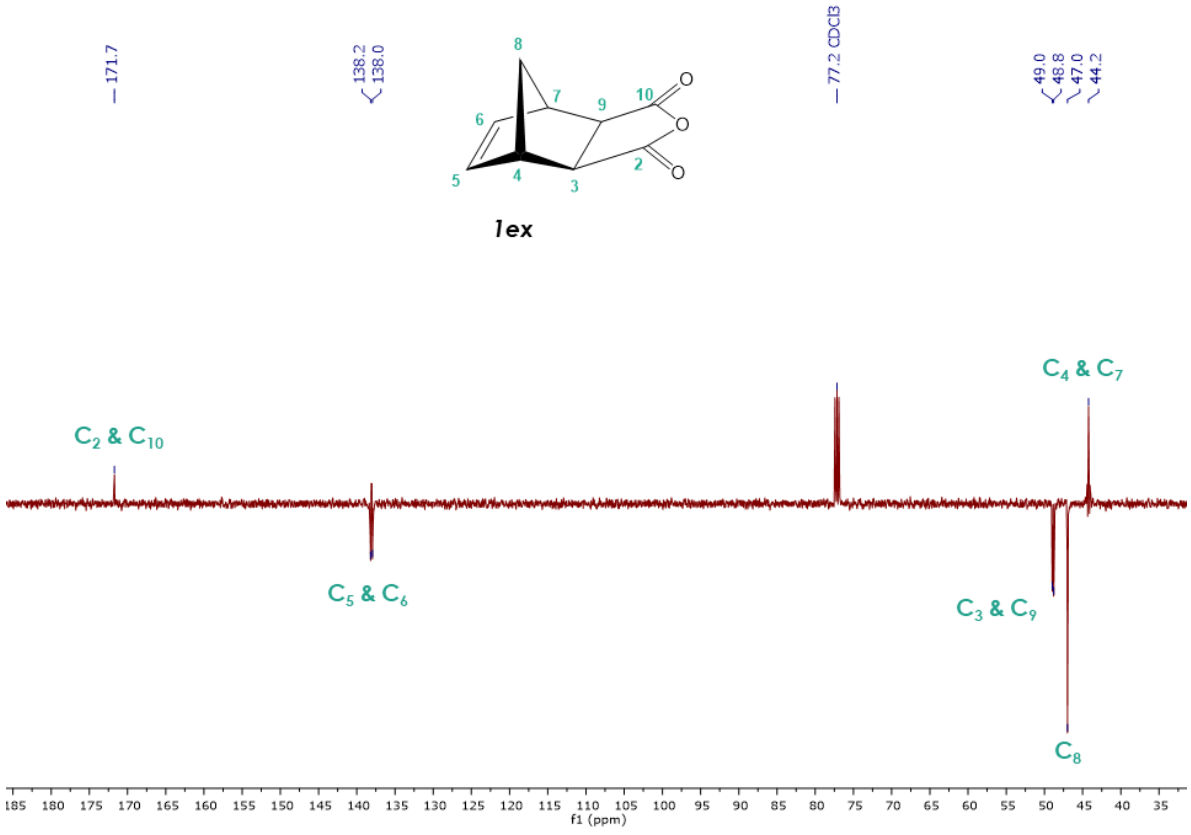

Figure S.11. APT of ***1ex***

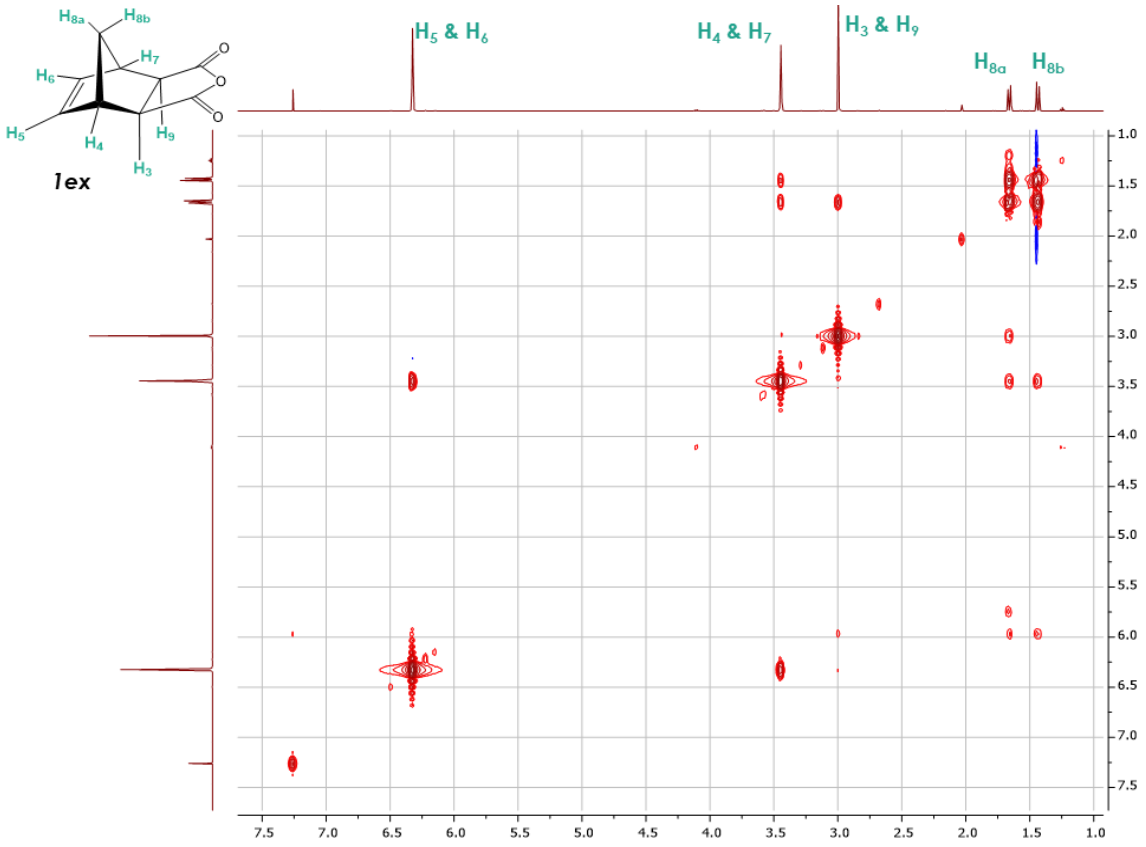

Figure S.12. COSY of ***1ex***

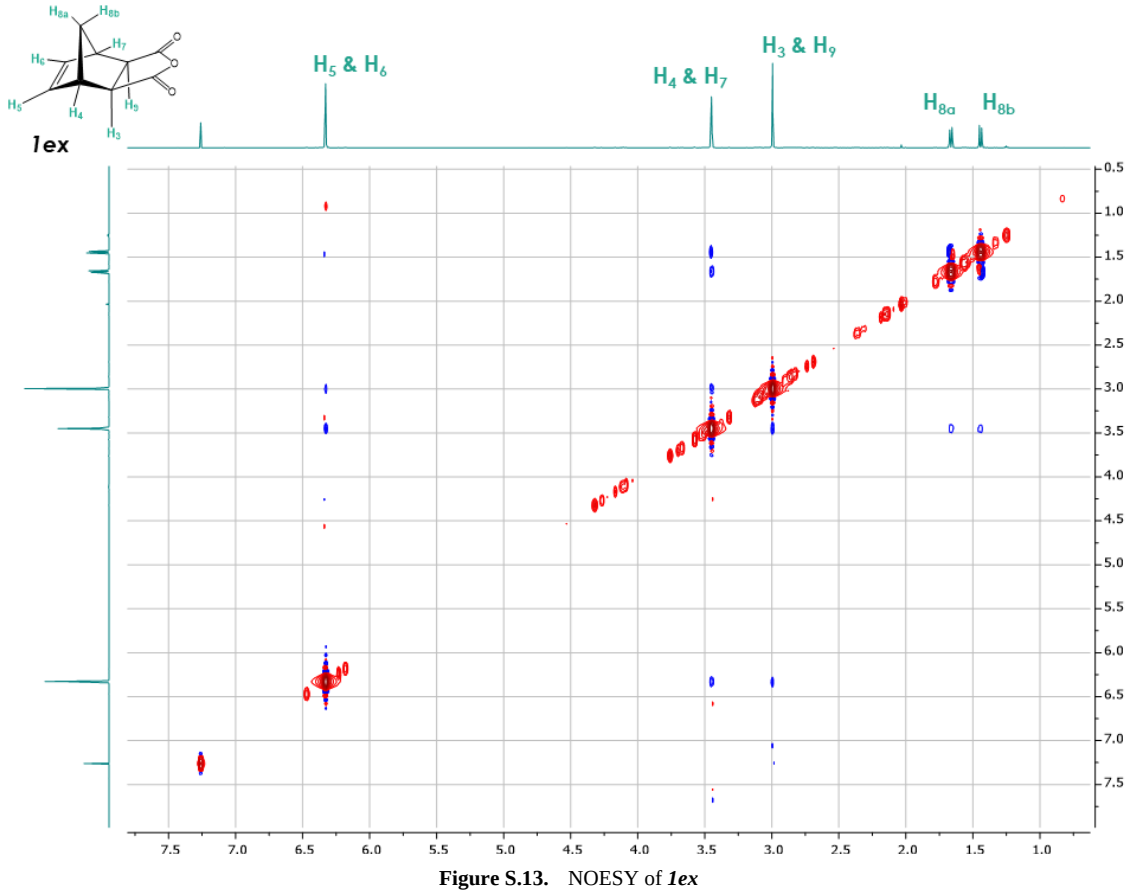

Figure S.13. NOESY of ***1ex***

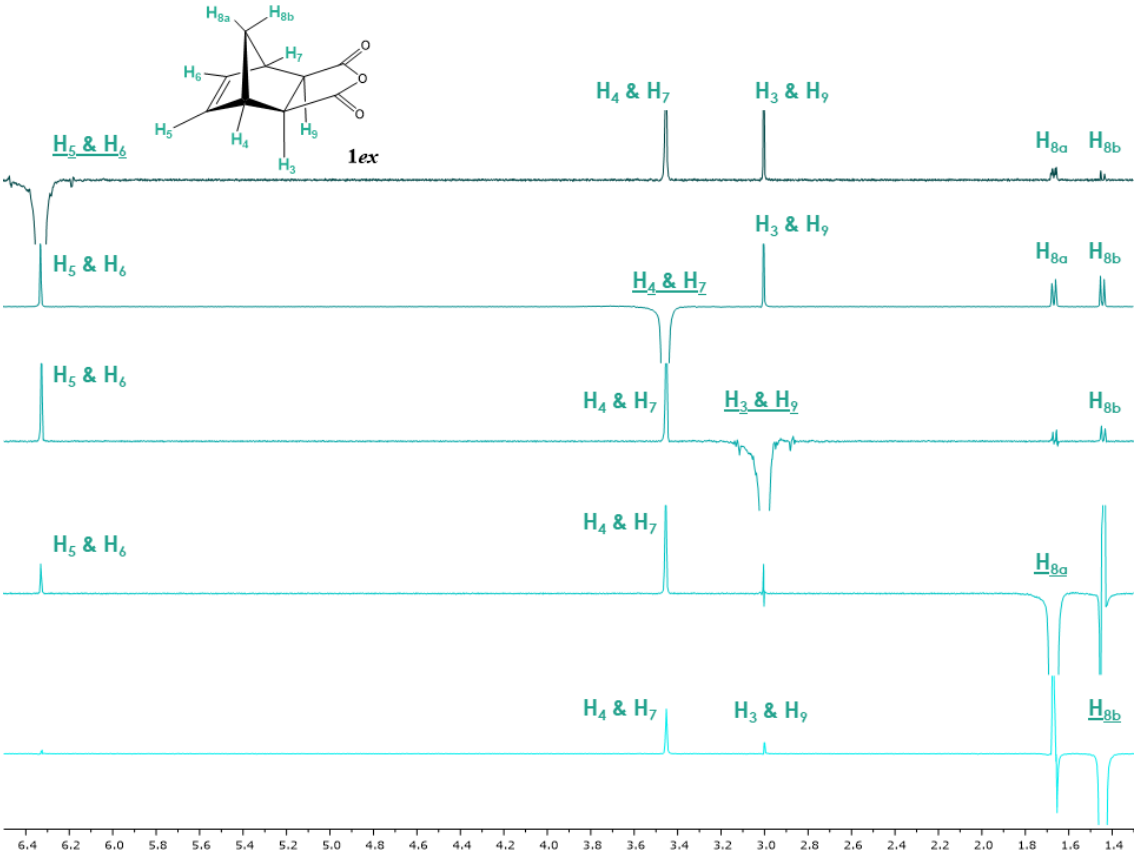

Figure S.14. 1D NOEs of ***1ex***

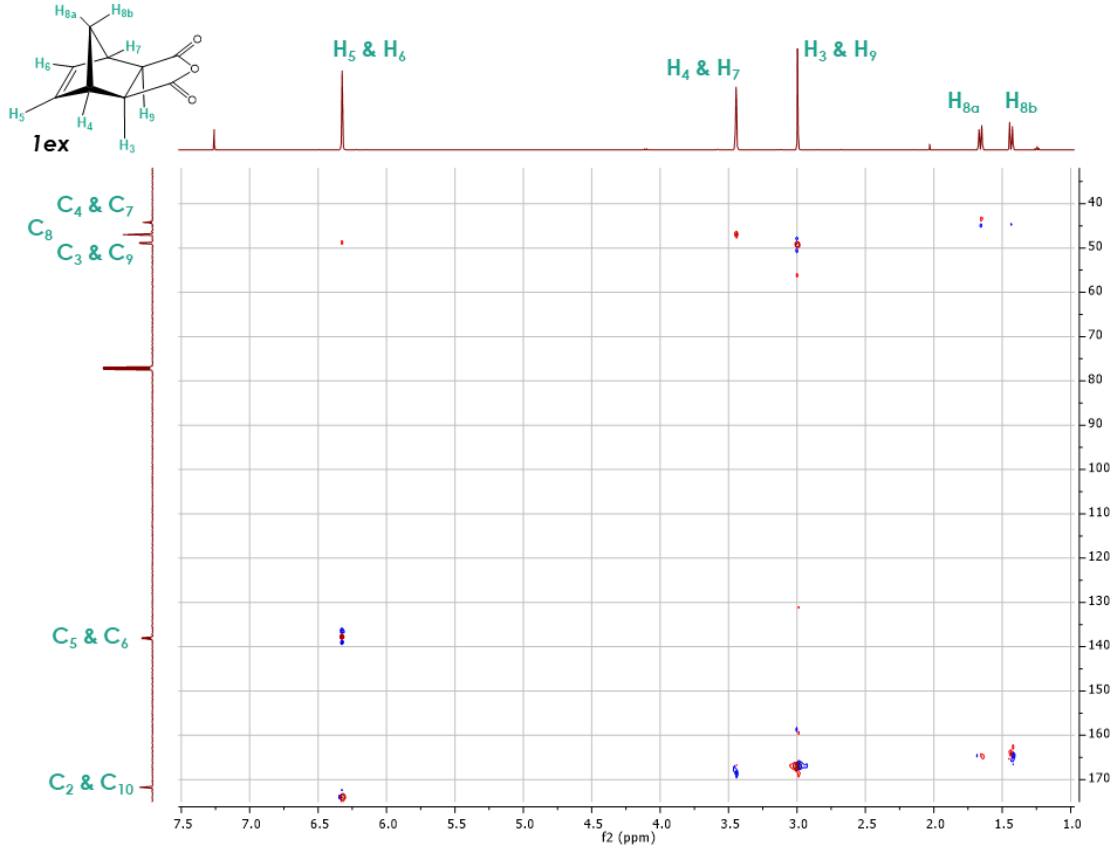

Figure S.15. HSQC of ***1ex***

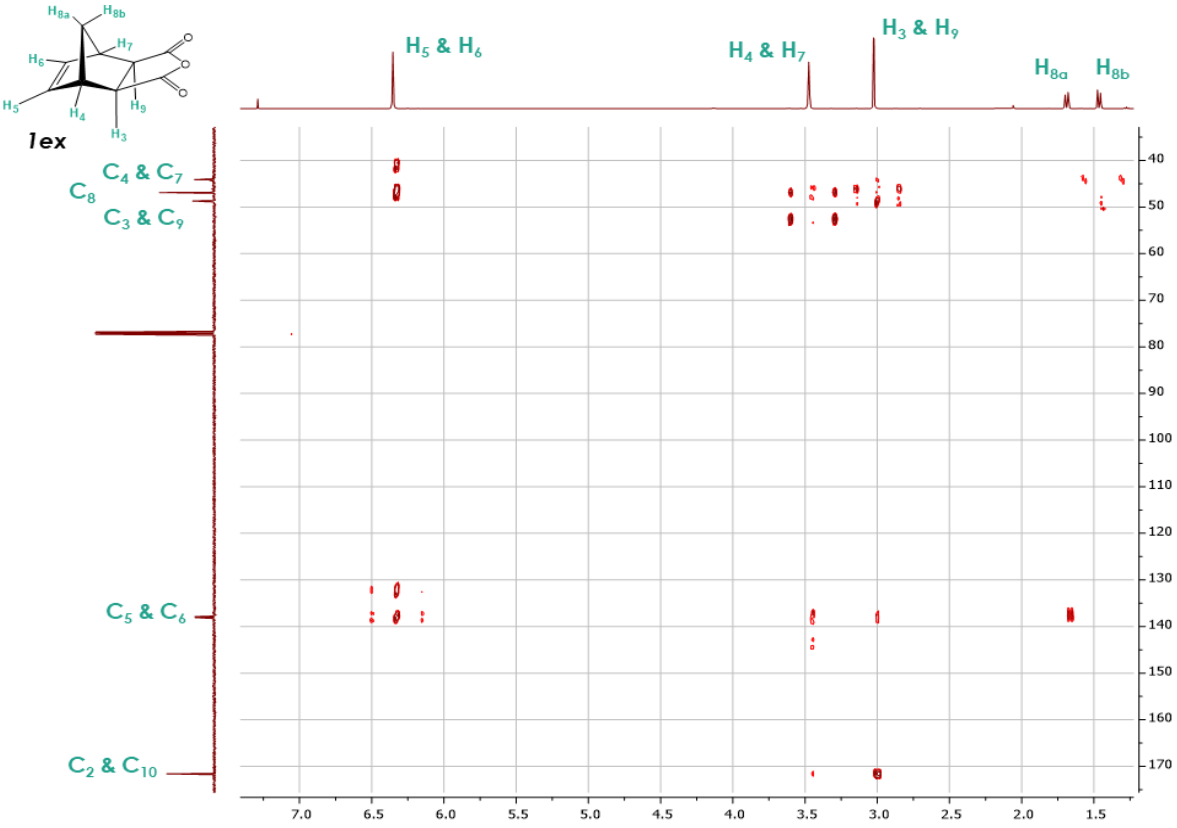

Figure S.16. HMBC of ***1ex***

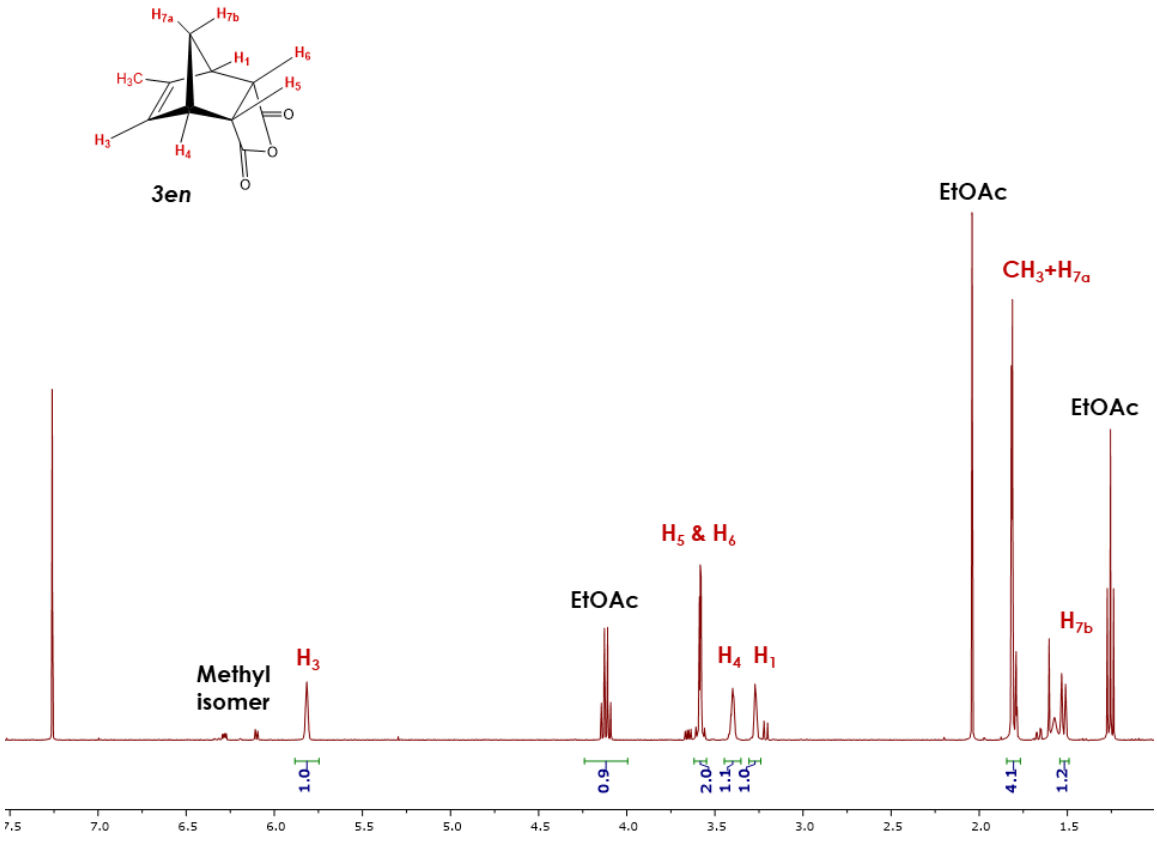

Figure S.17. ^{1}H NMR of ***3en***

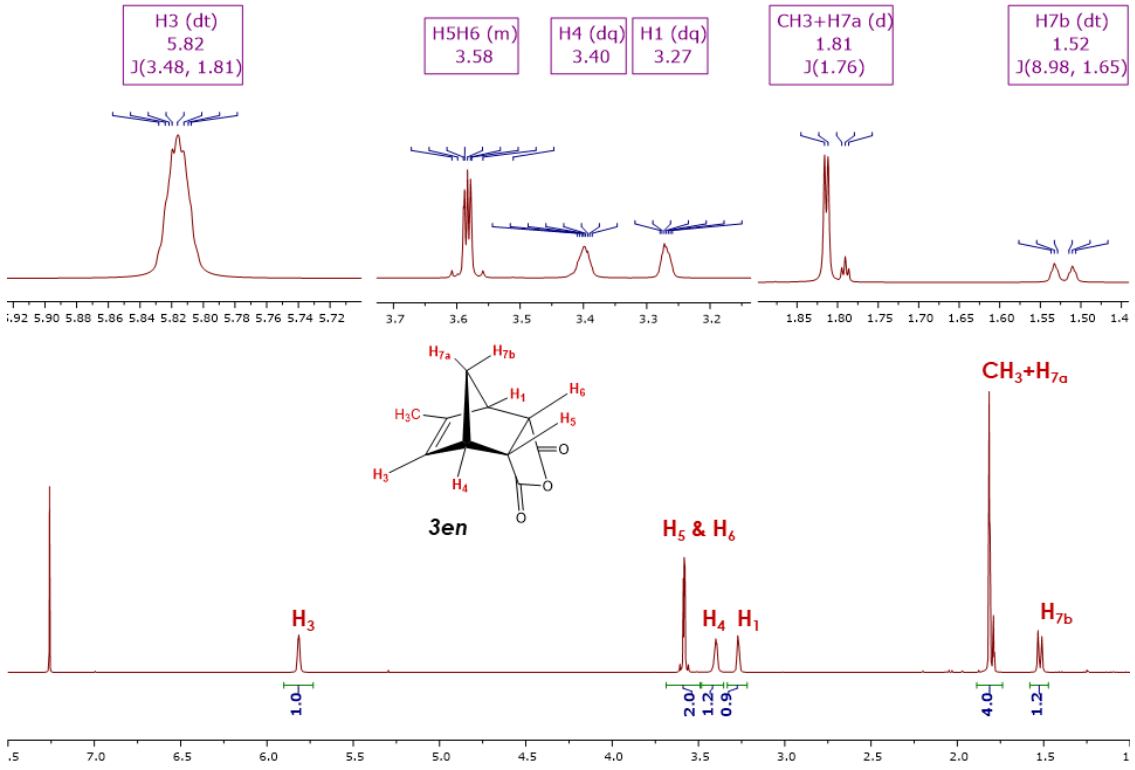

Figure S.18. Deconvoluted 1H NMR of ***3en***

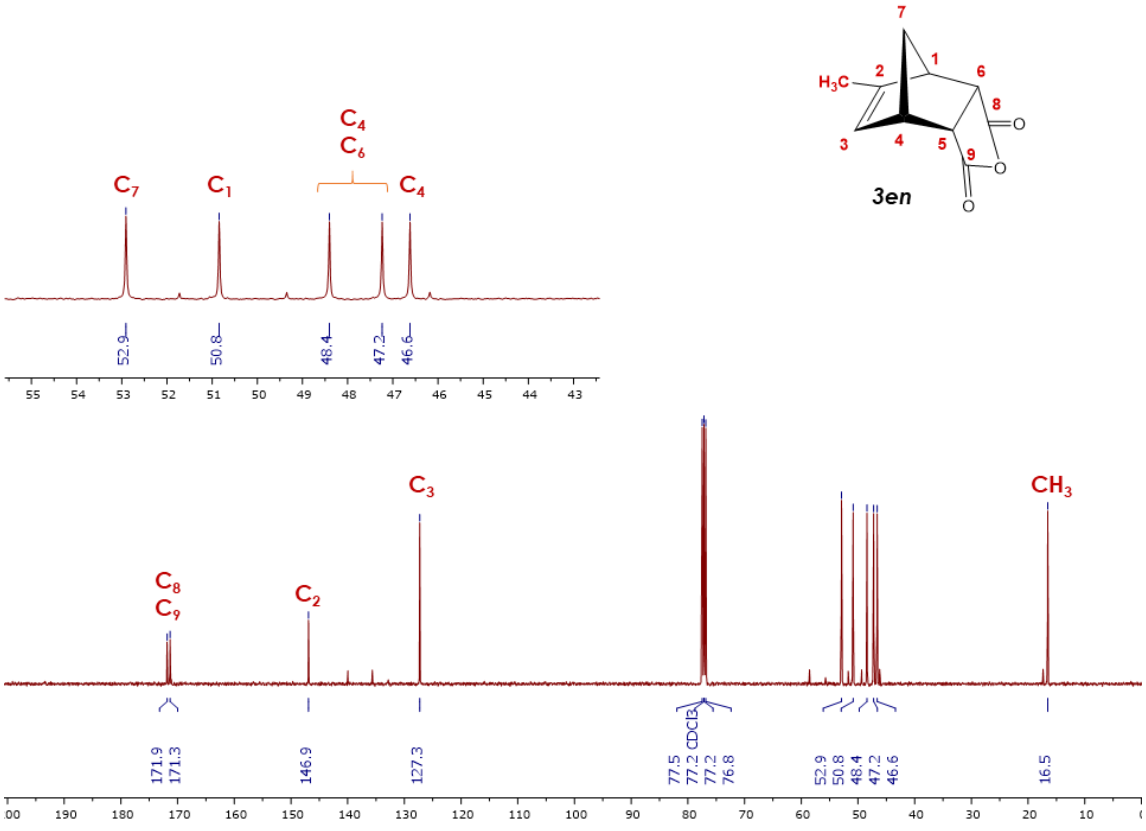

Figure S.19. ^{13}C NMR of ***3en***

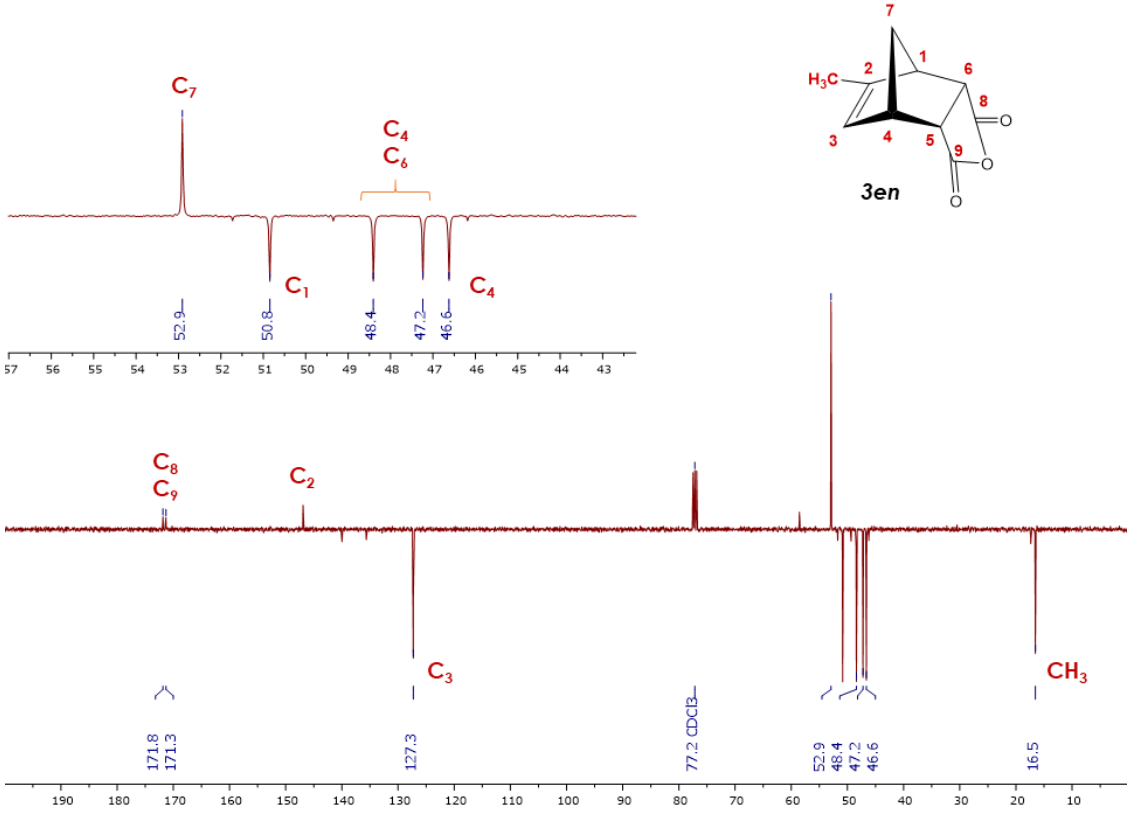

Figure S.20. APT of ***3en***

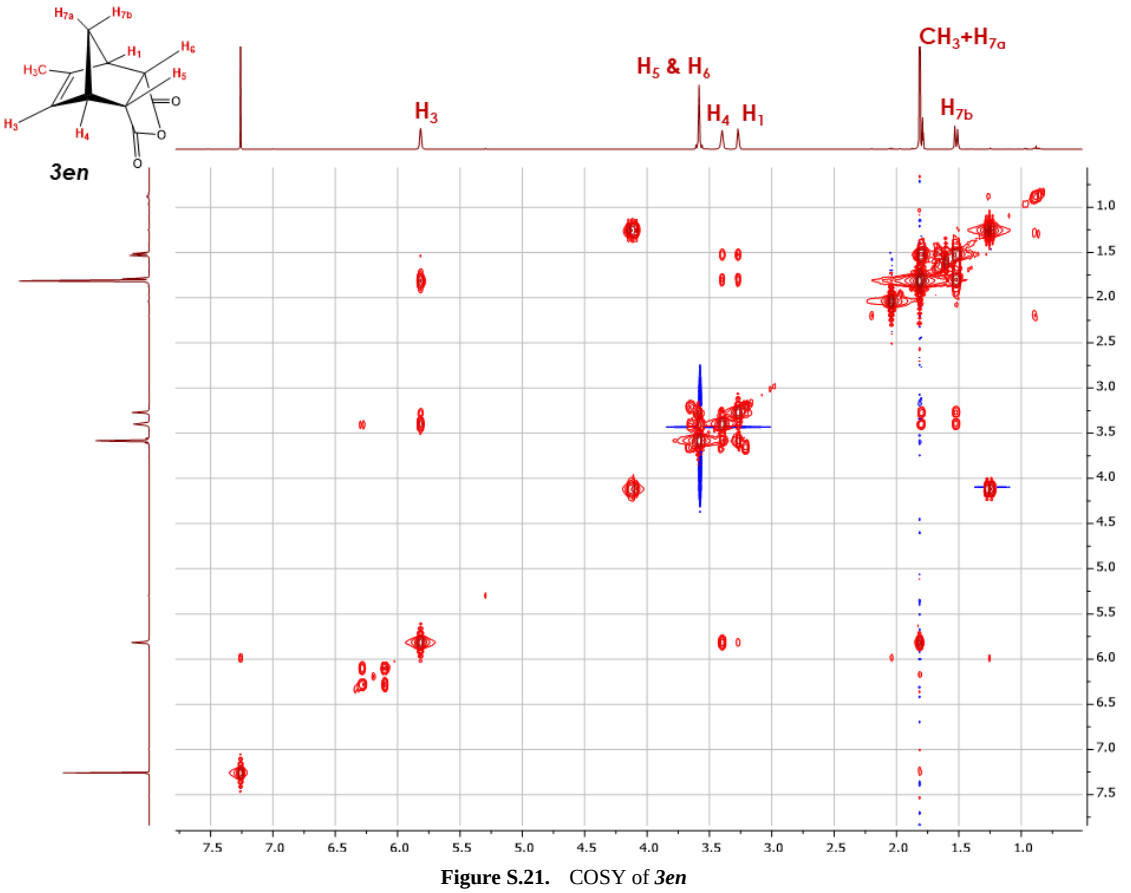

Figure S.21. COSY of ***3en***

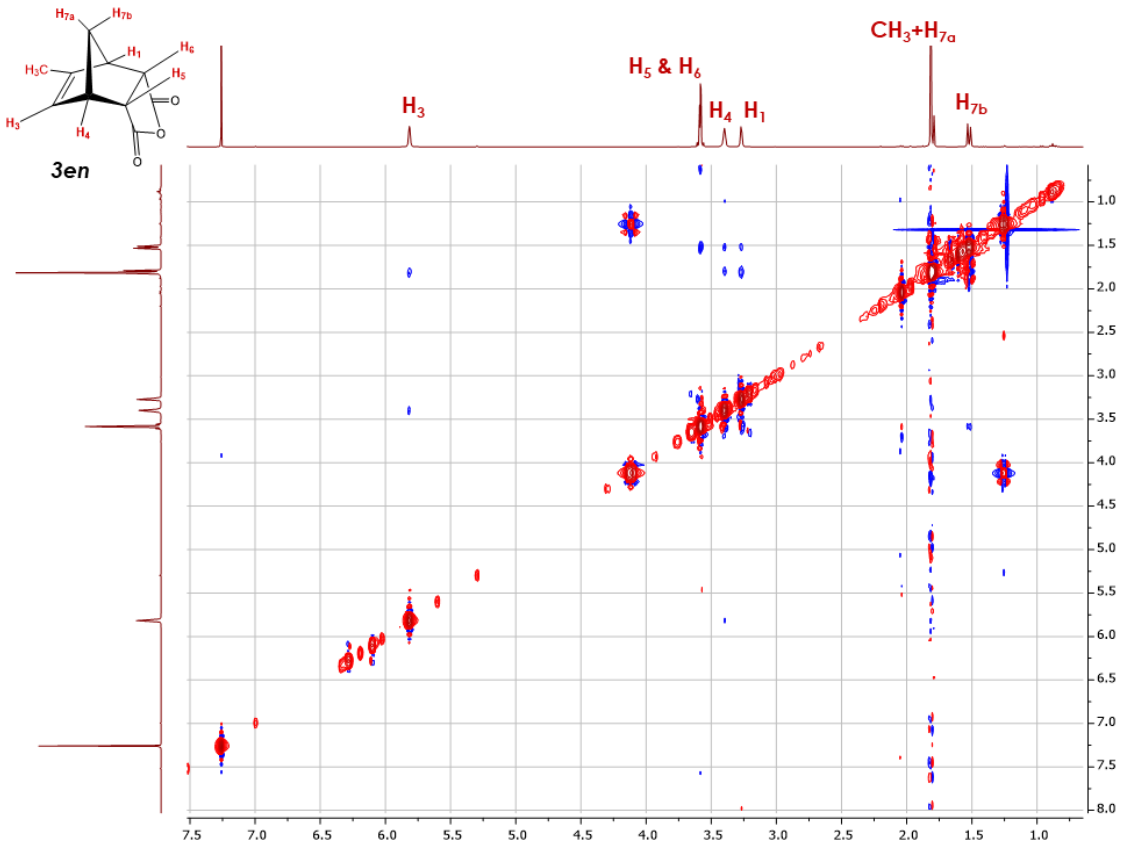

Figure S.22. NOESY of ***3en***

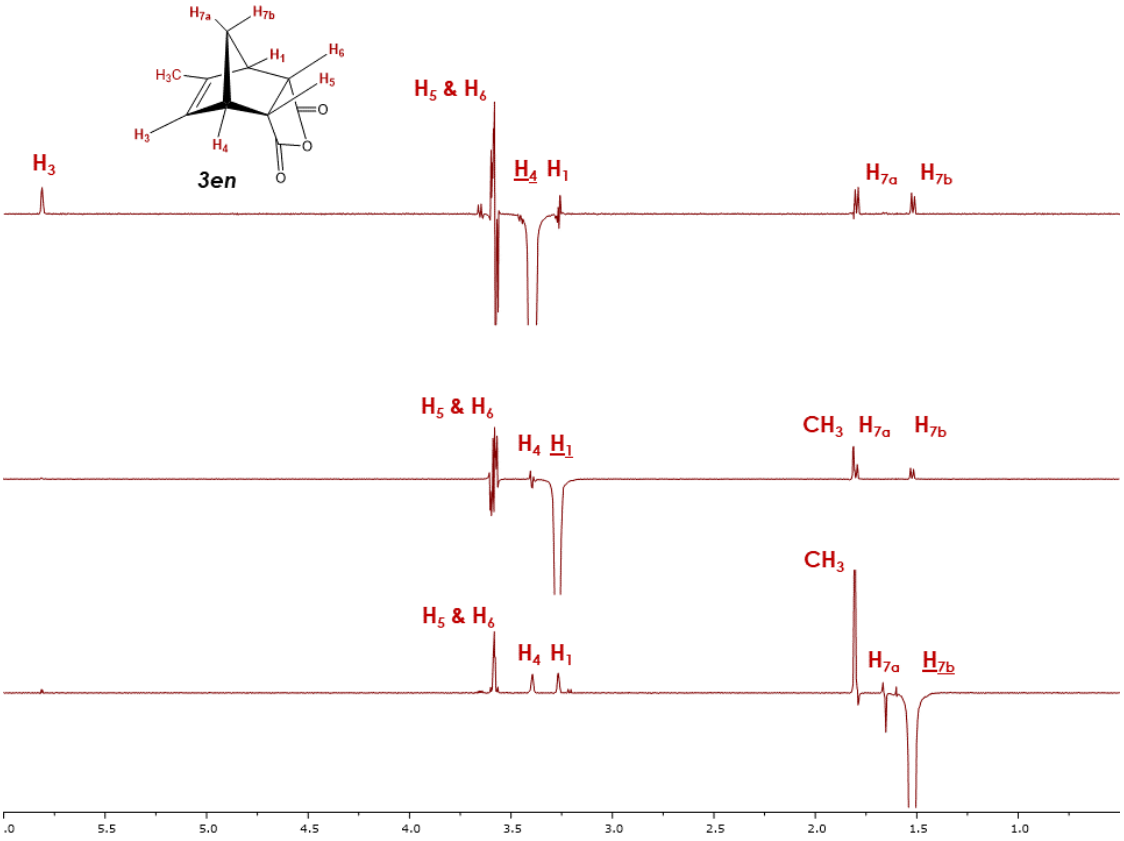

Figure S.23. 1D NOE of ***3en***

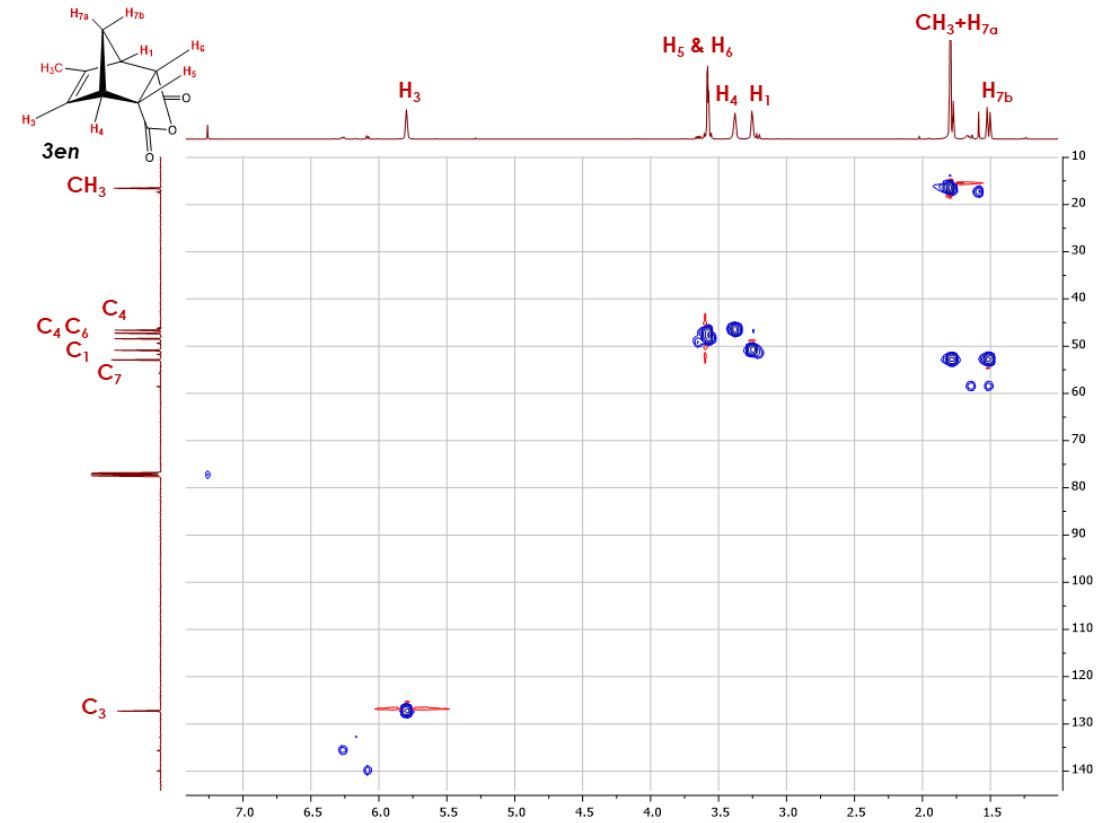

Figure S.24. HSQC of ***3en***

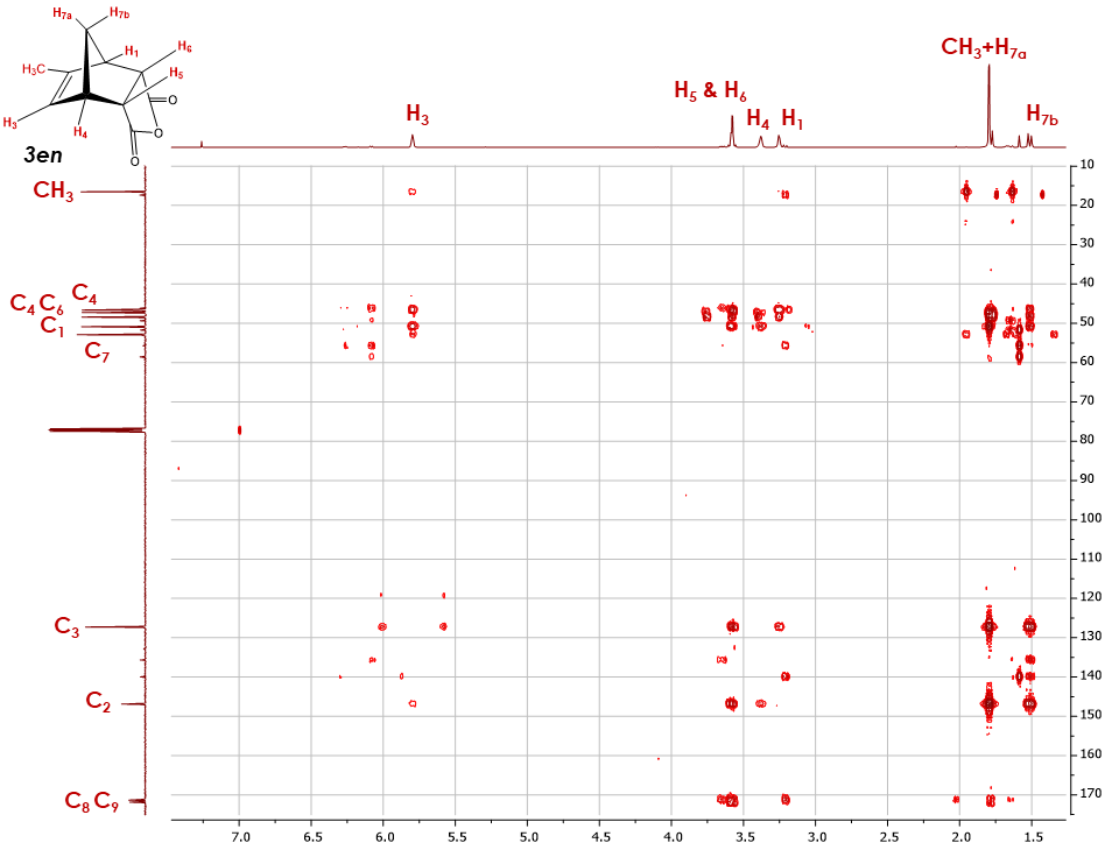

Figure S.25. HMBC of ***3en***

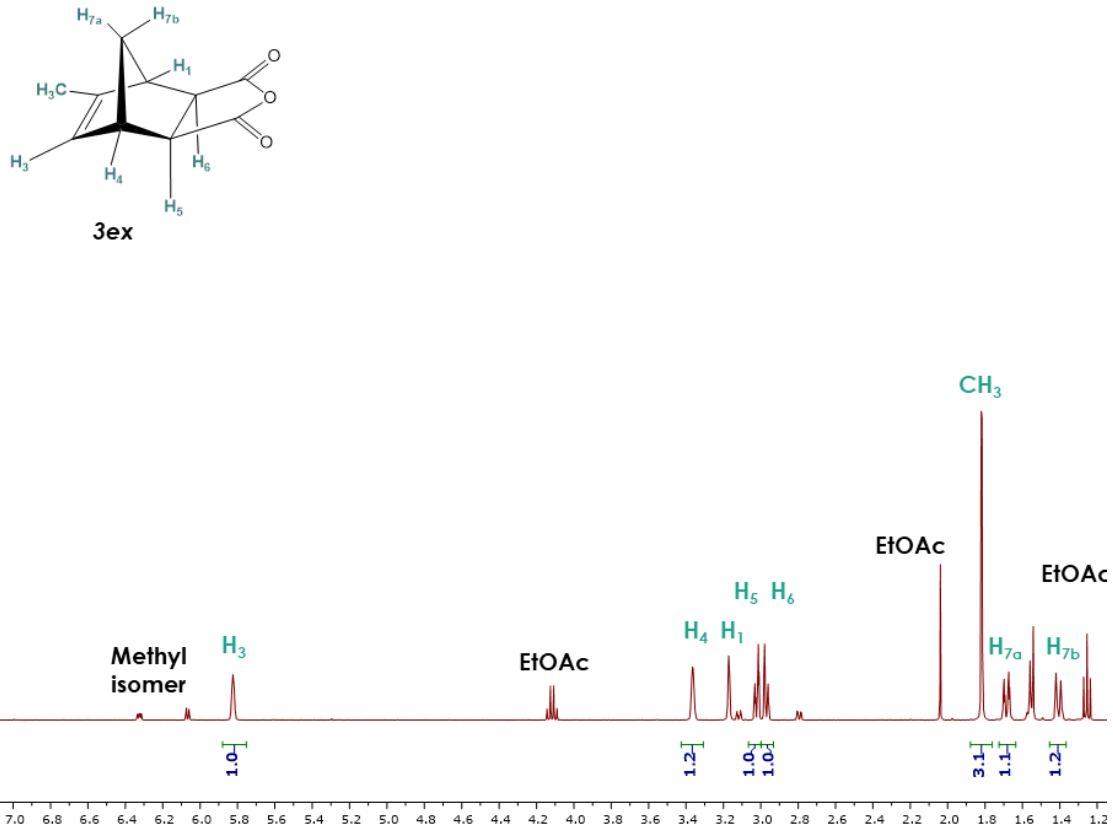

Figure S.26. ^{1}H NMR of ***3ex***

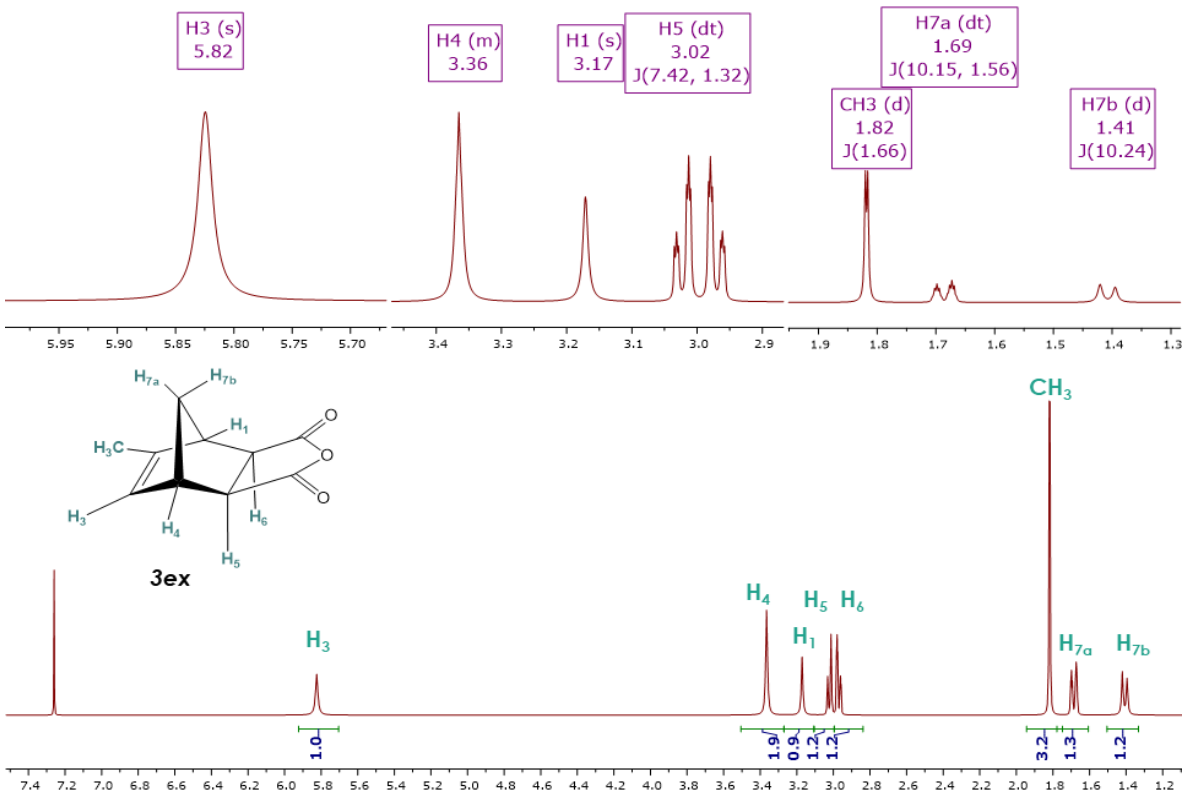

Figure S.27. Deconvoluted 1H NMR of ***3ex***

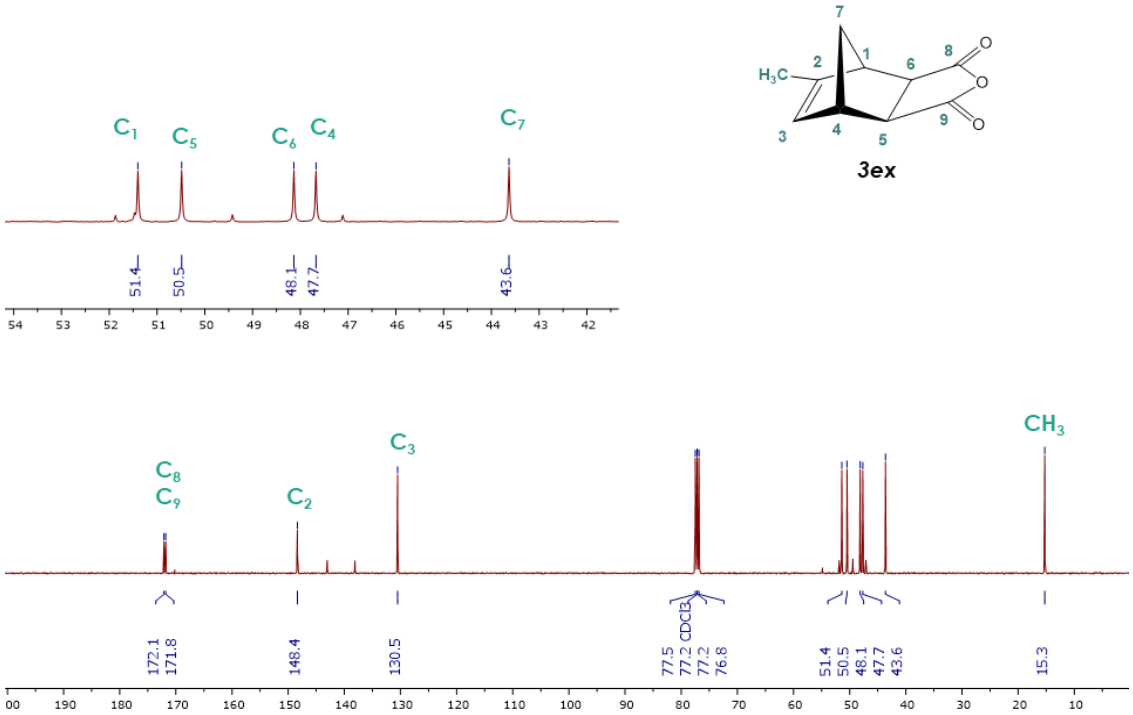

Figure S.28. ^{13}C NMR of ***3ex***

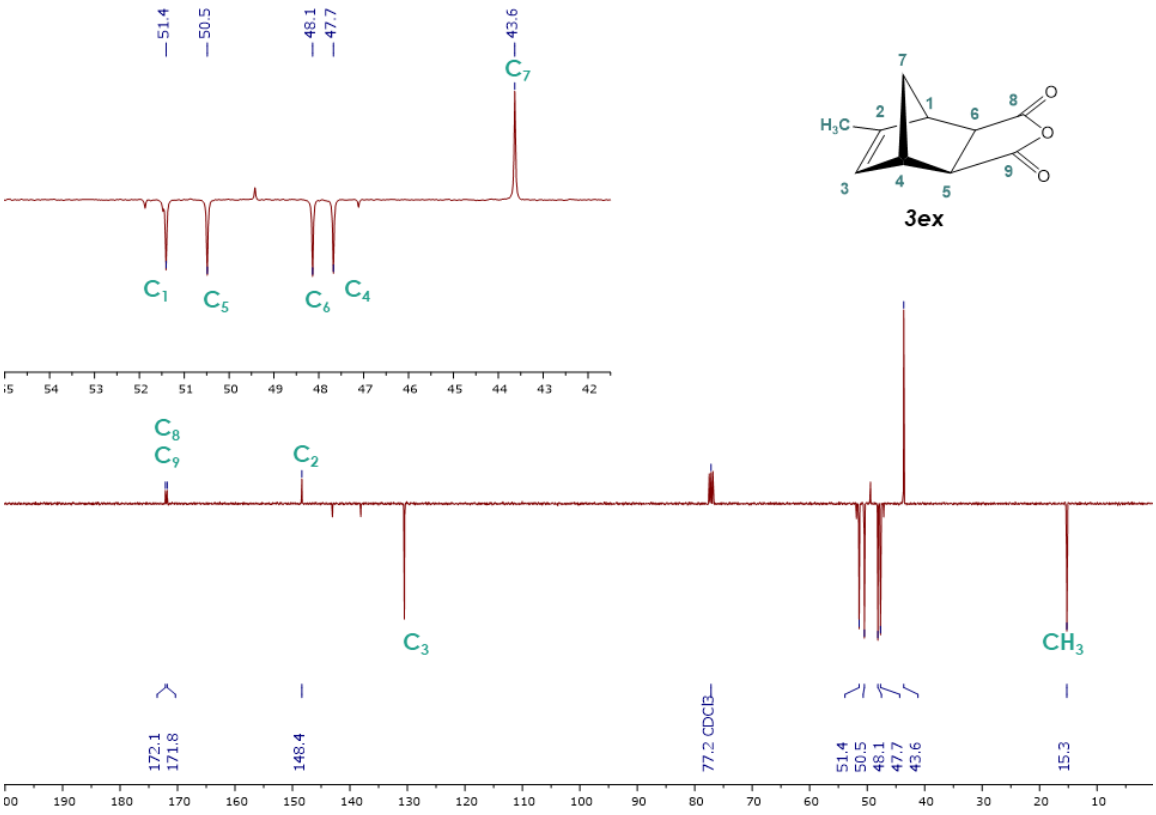

Figure S.29. APT of ***3ex***

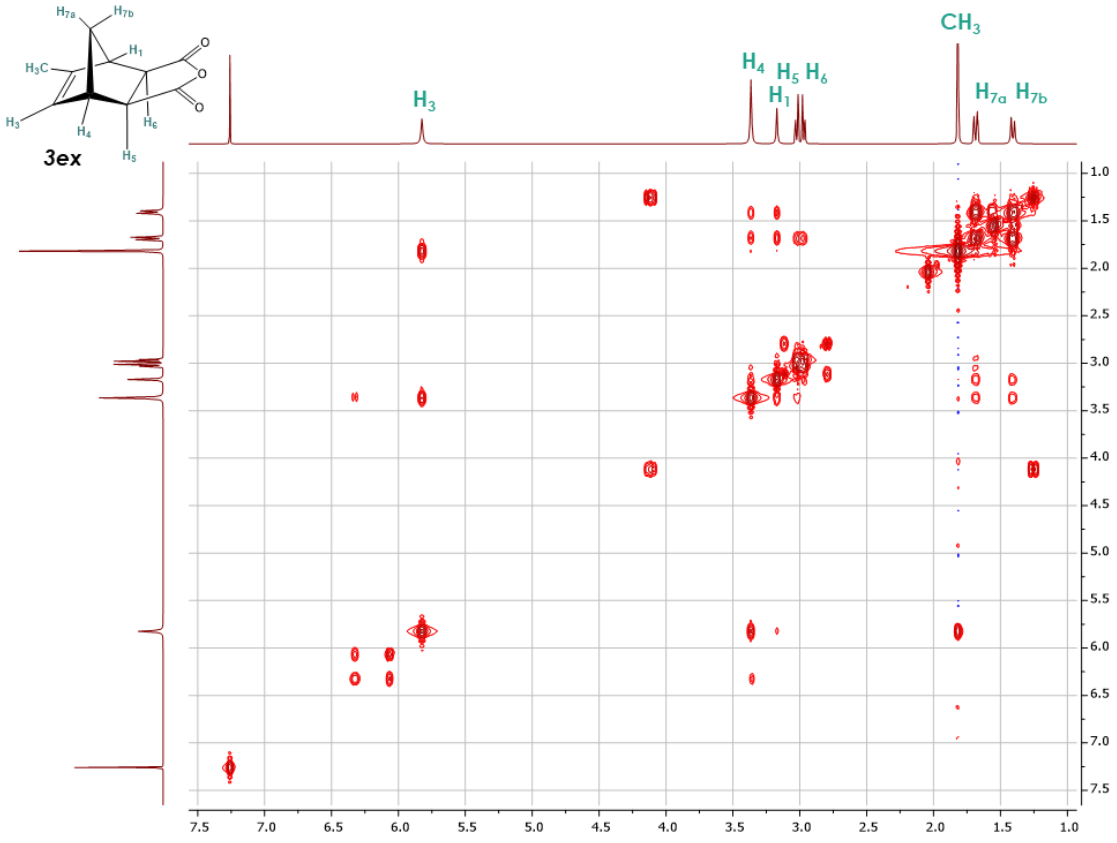

Figure S.30. COSY of ***3ex***

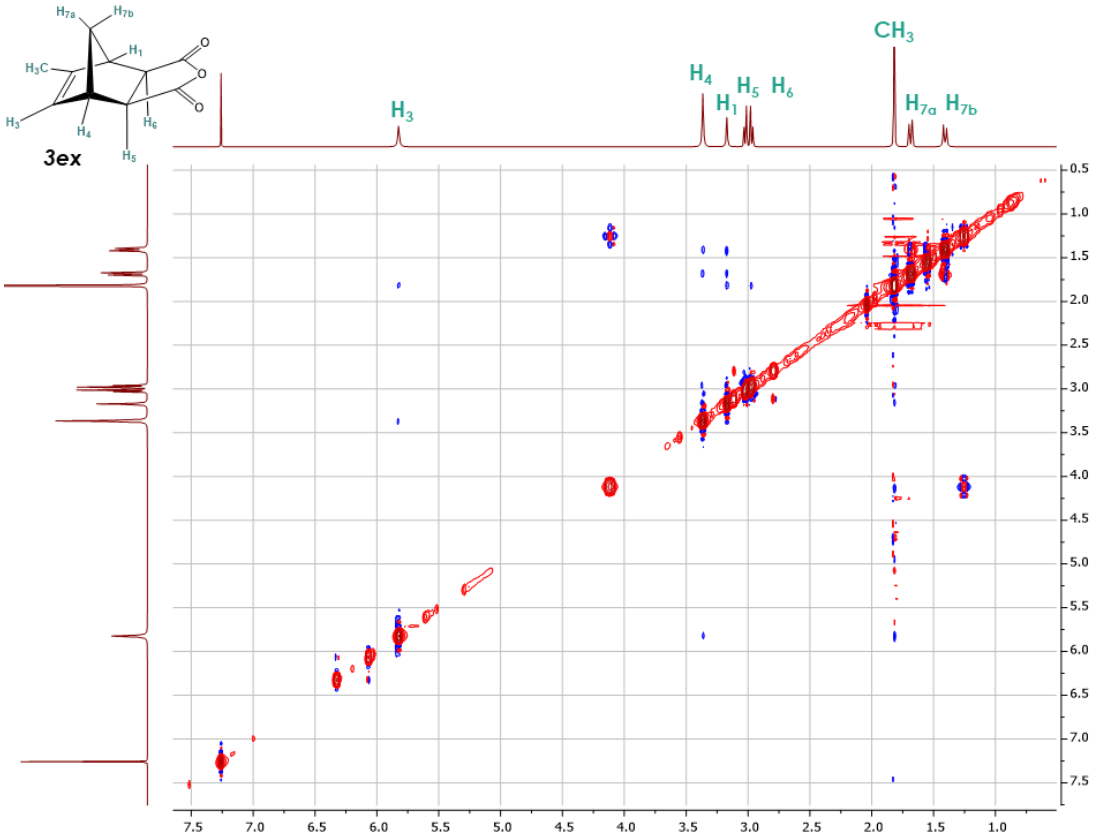

Figure S.31. NOESY of ***3ex***

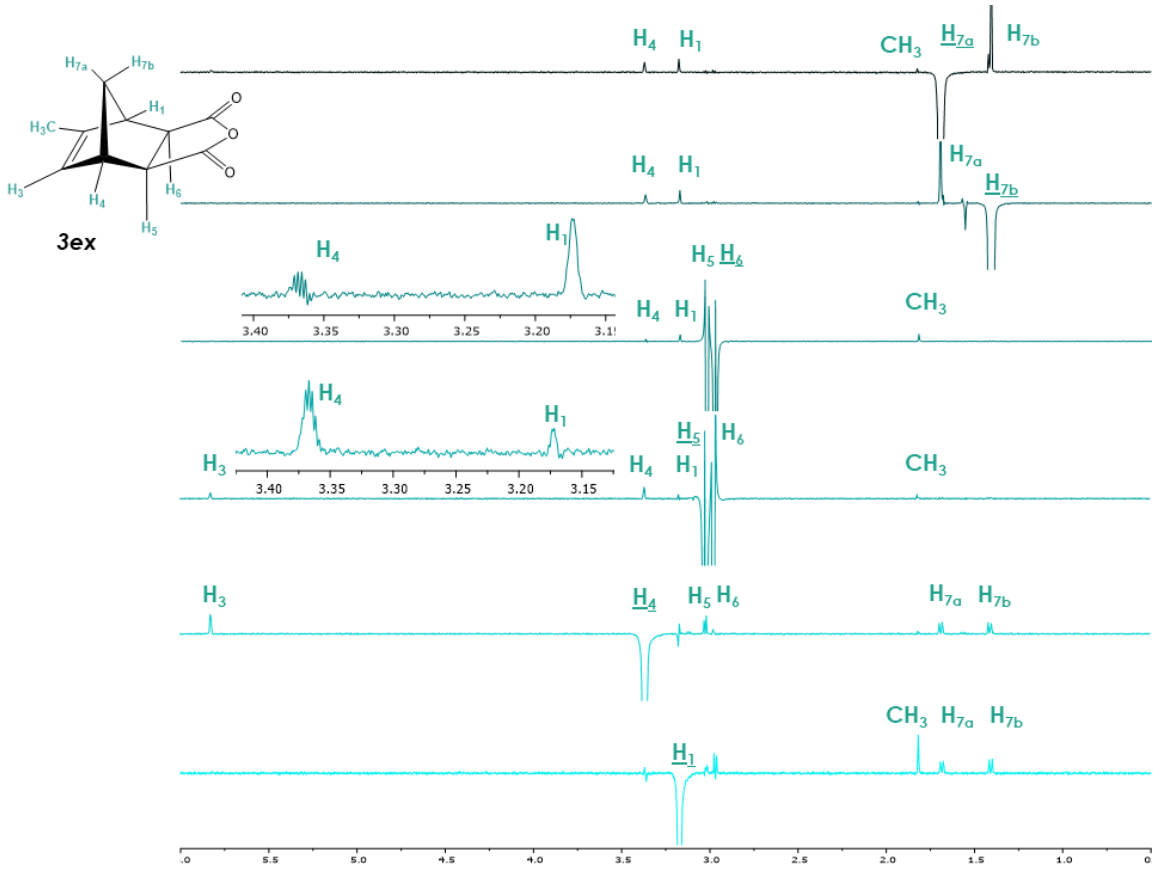

Figure S.32. 1D NOE of ***3ex***

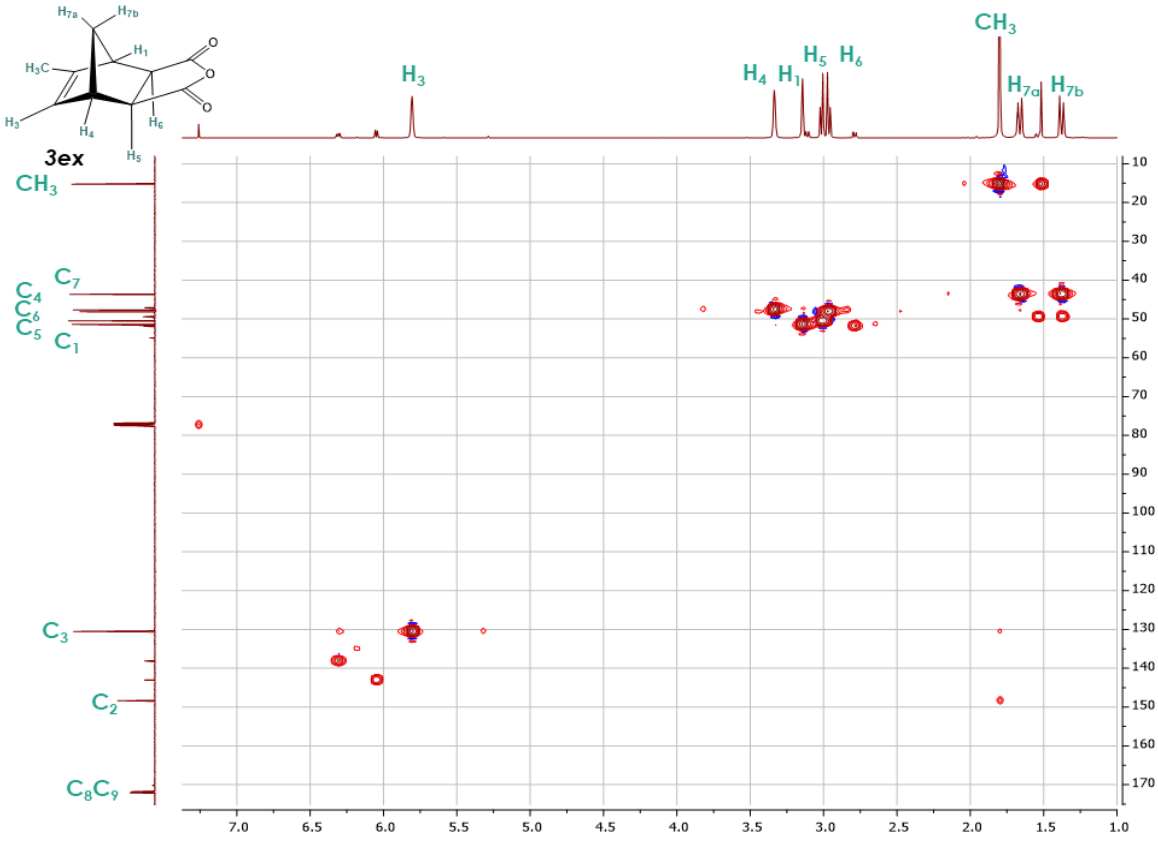

Figure S.33. HSQC of ***3ex***

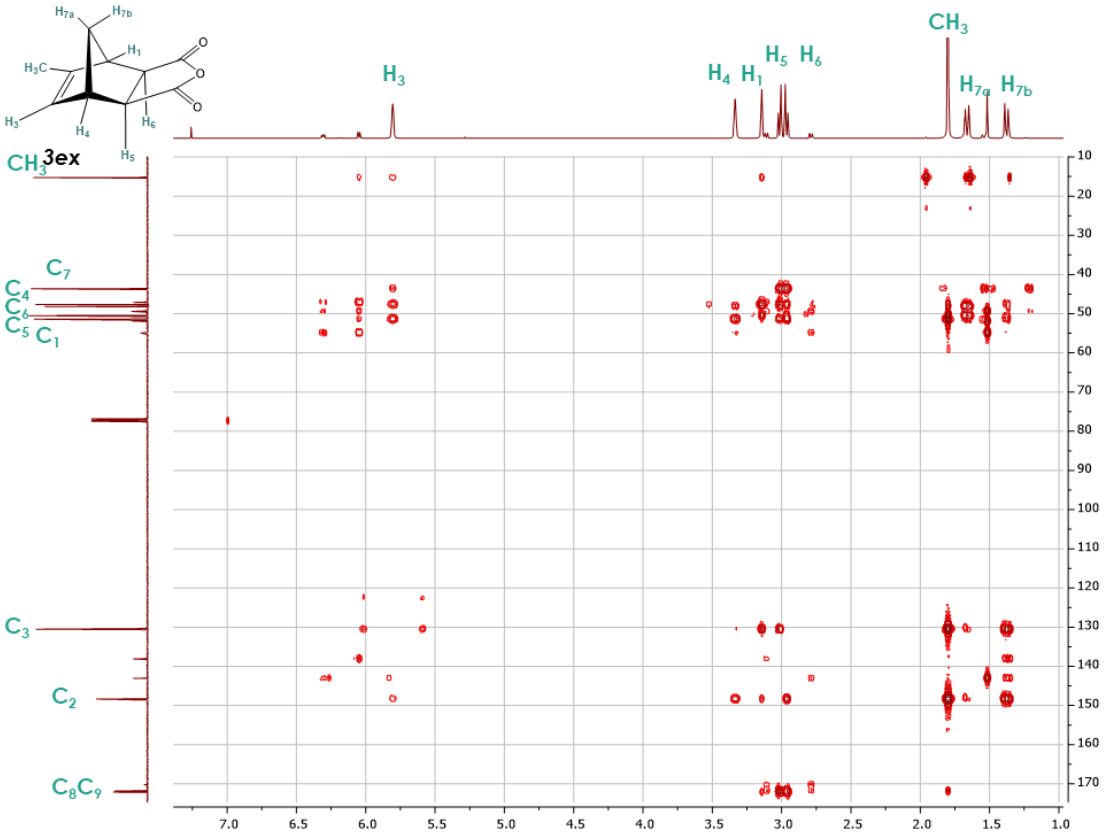

Figure S.34. HSQC of ***3ex***

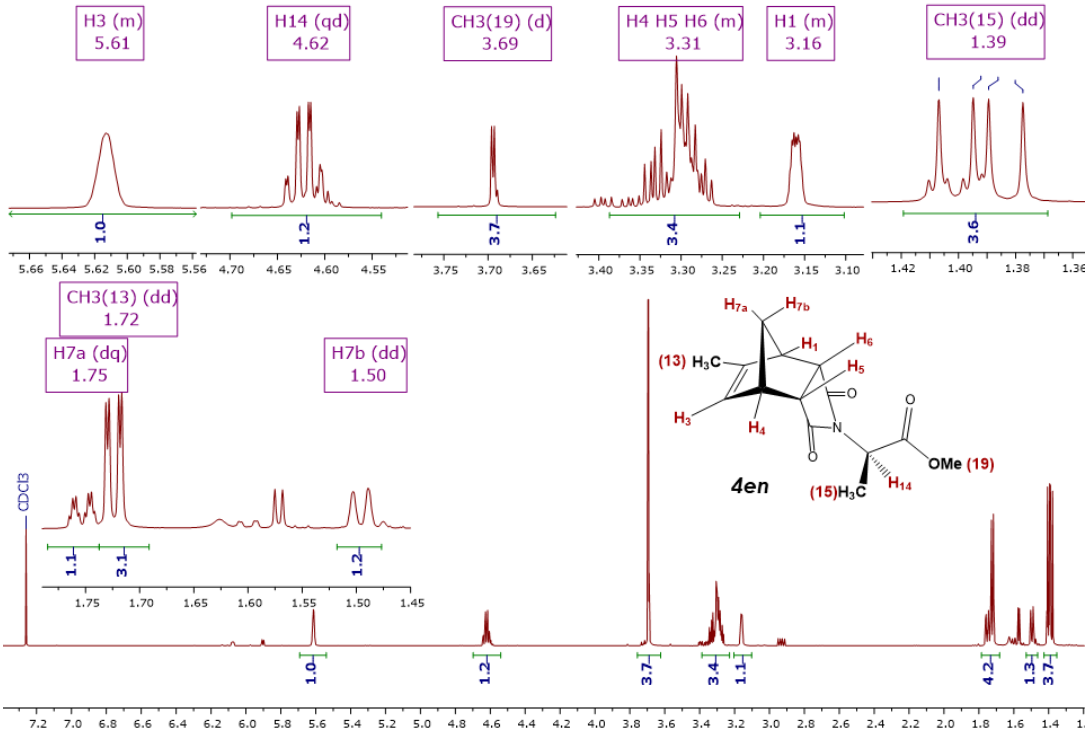

Figure S.35. ^{1}H NMR of ***4en***

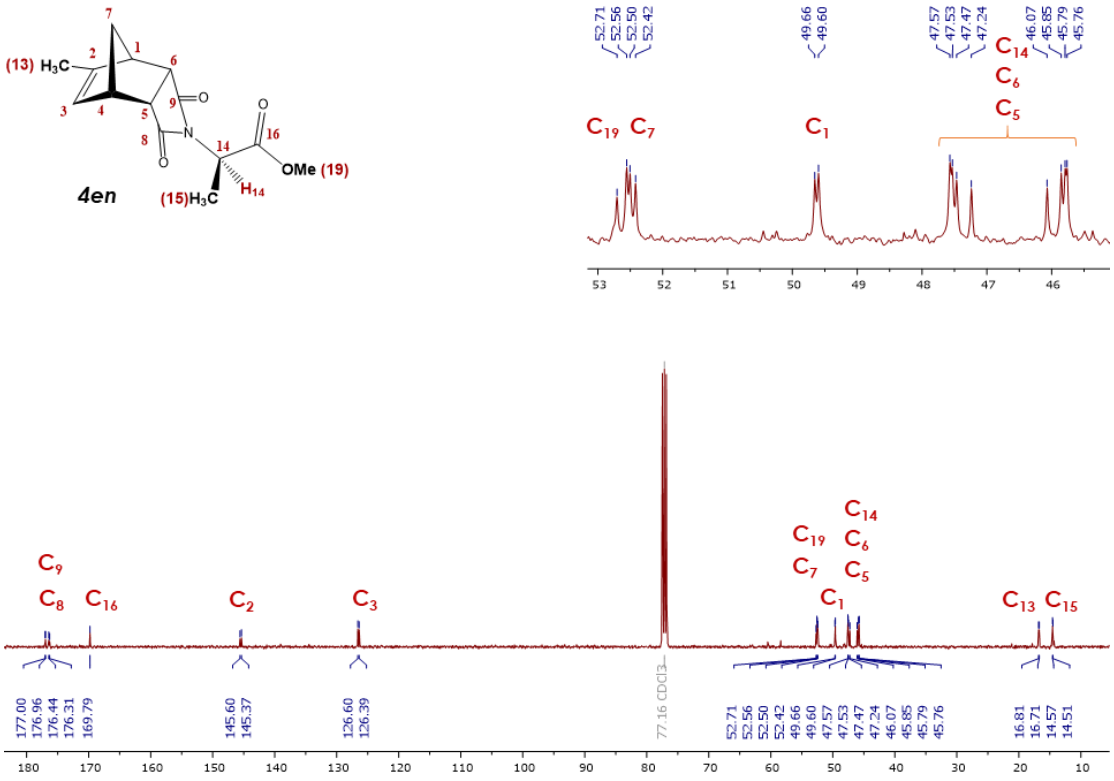

Figure S.36. ^{13}C NMR of ***4en***

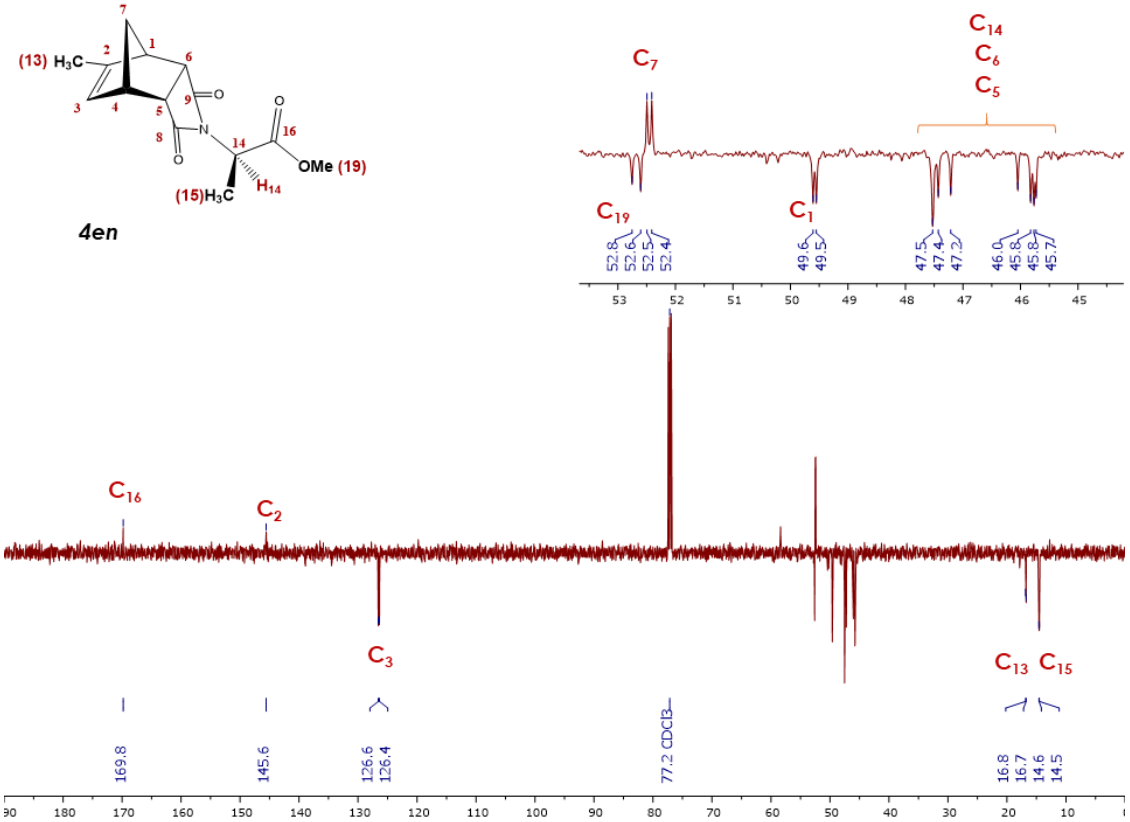

Figure S.37. APT of ***4en***

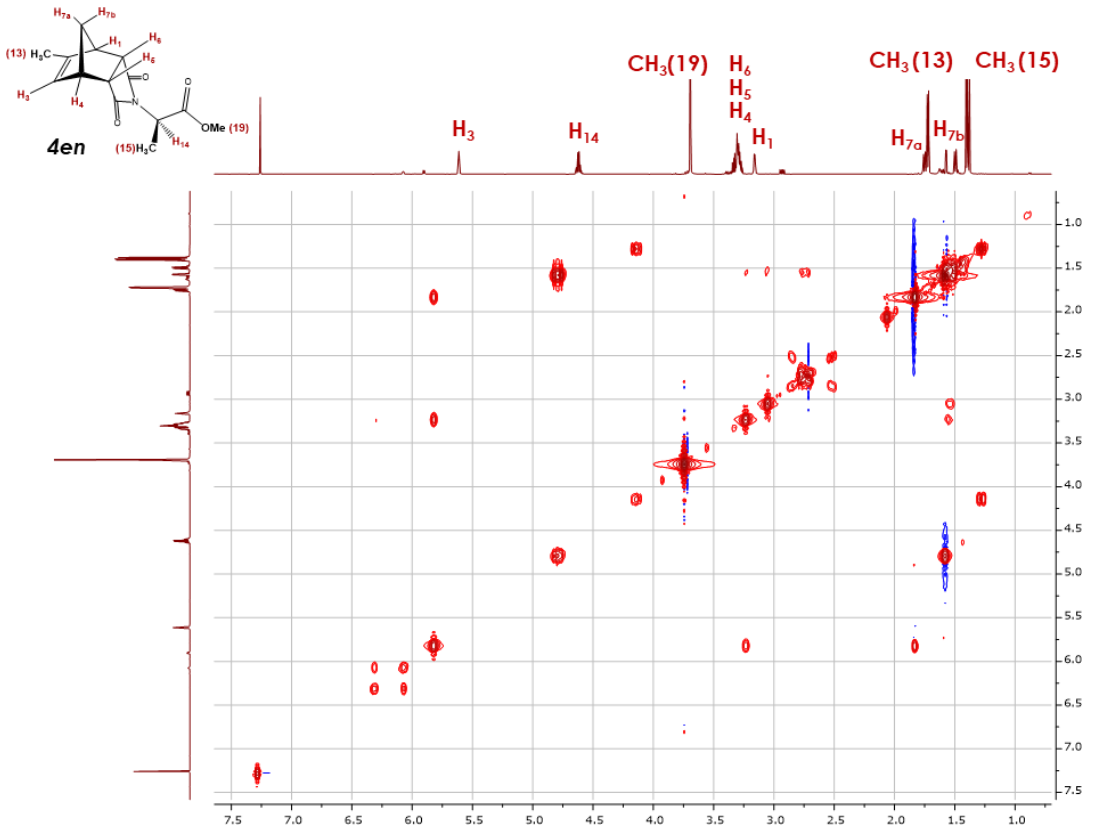

Figure S.38. COSY of ***4en***

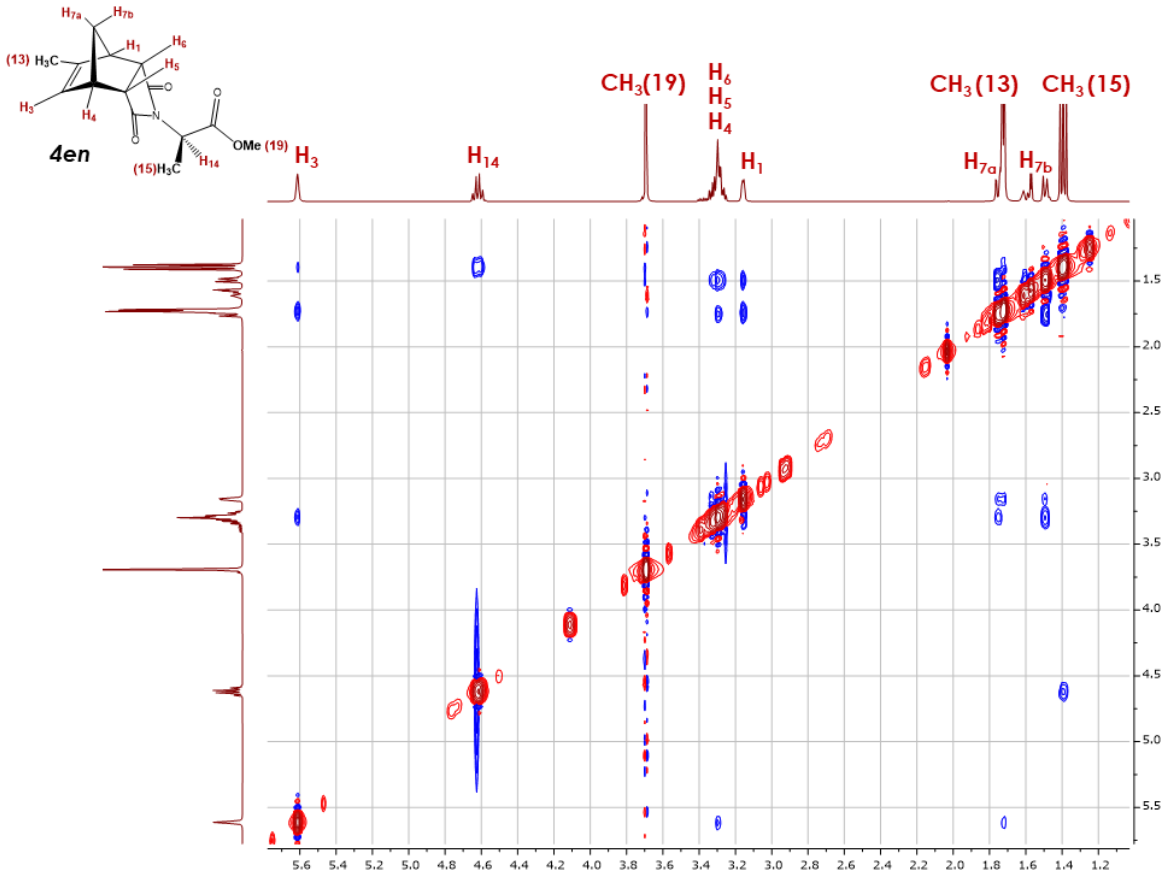

Figure S.39. NOESY of ***4en***

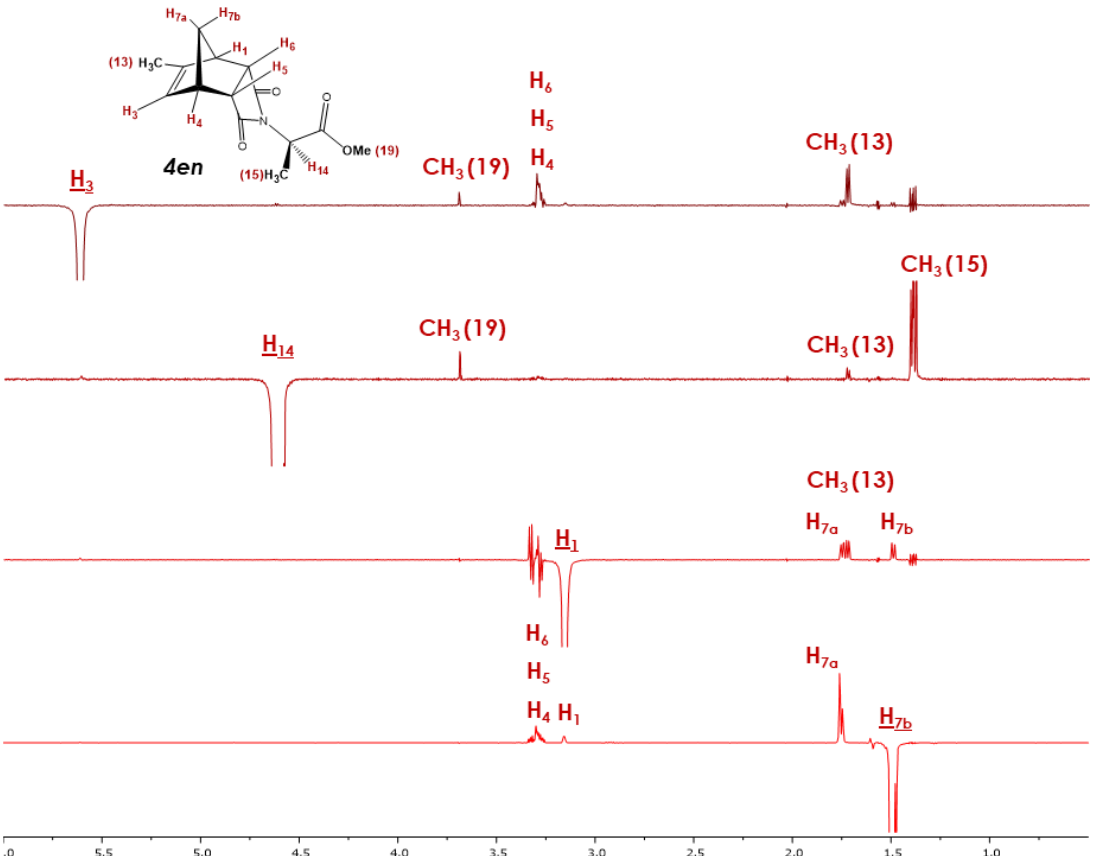

Figure S.40. 1D NOEs of ***4en***

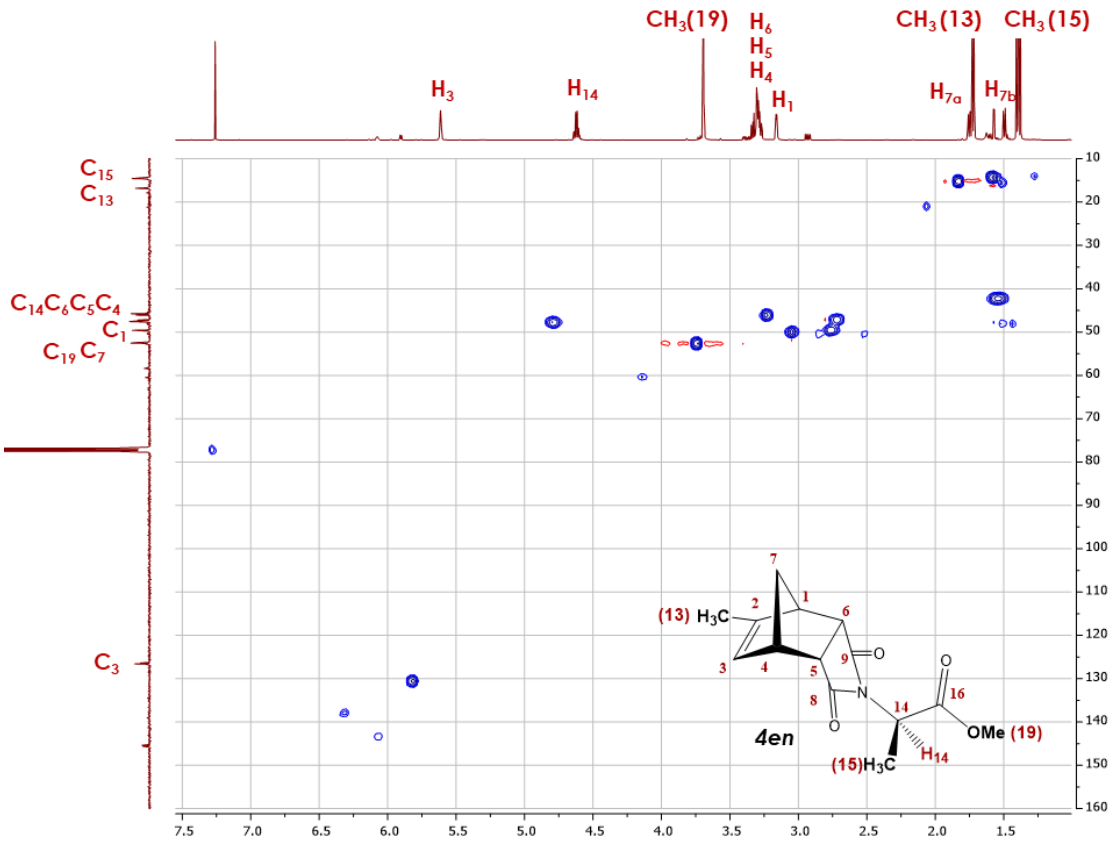

Figure S.41. HSQC of ***4en***

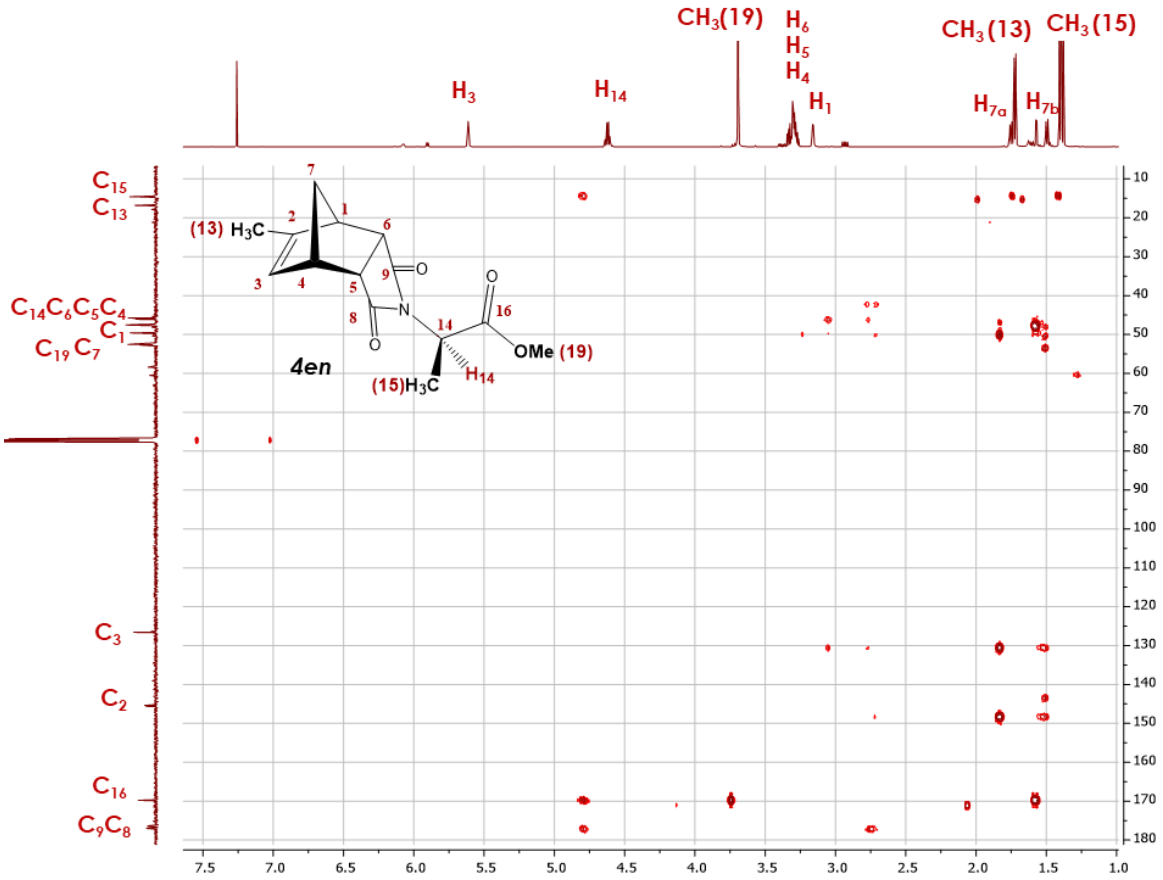

Figure S.42. HMBC of ***4en***

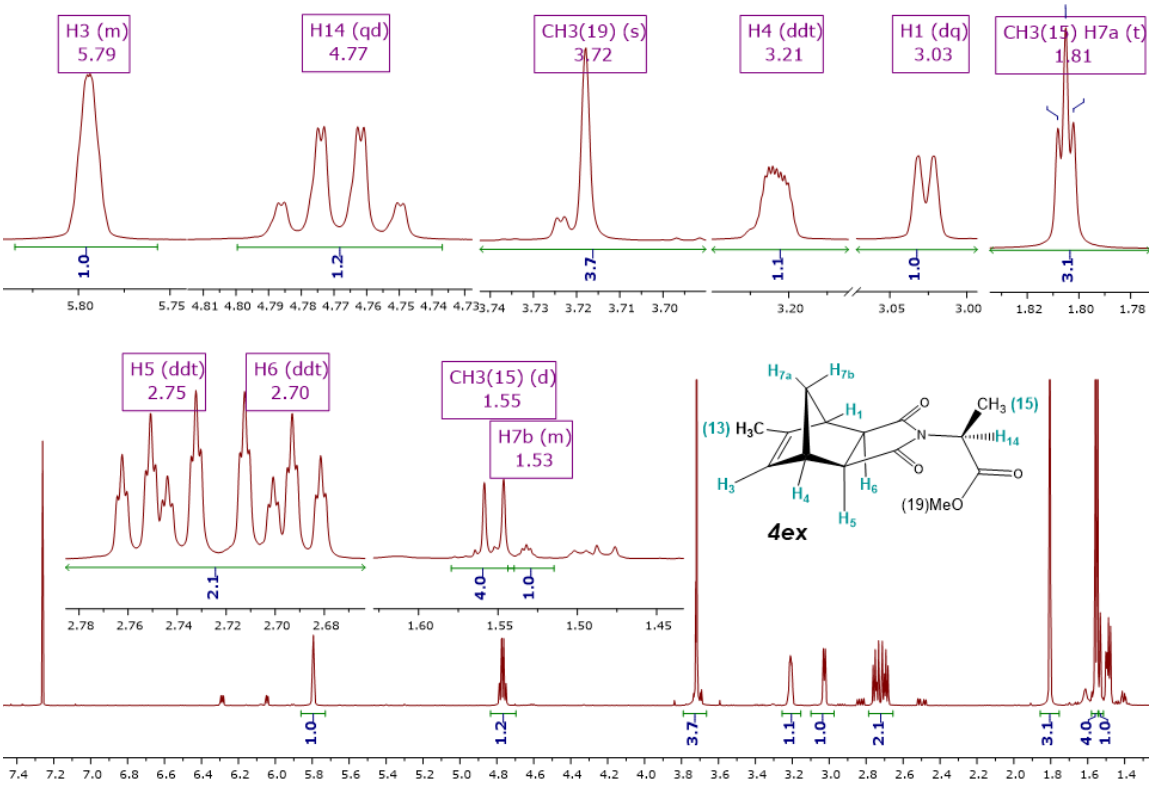

Figure S.43. ^{1}H NMR of ***4ex***

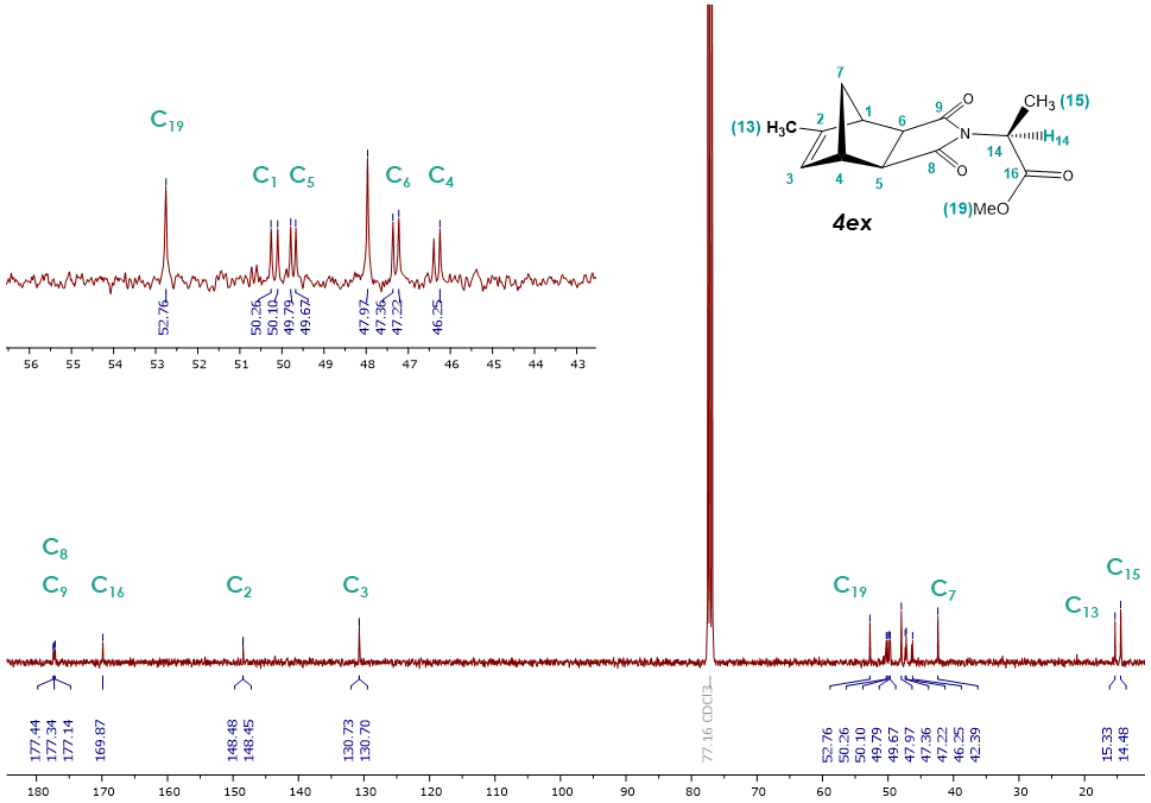

Figure S.44. ^{13}C NMR of ***4ex***

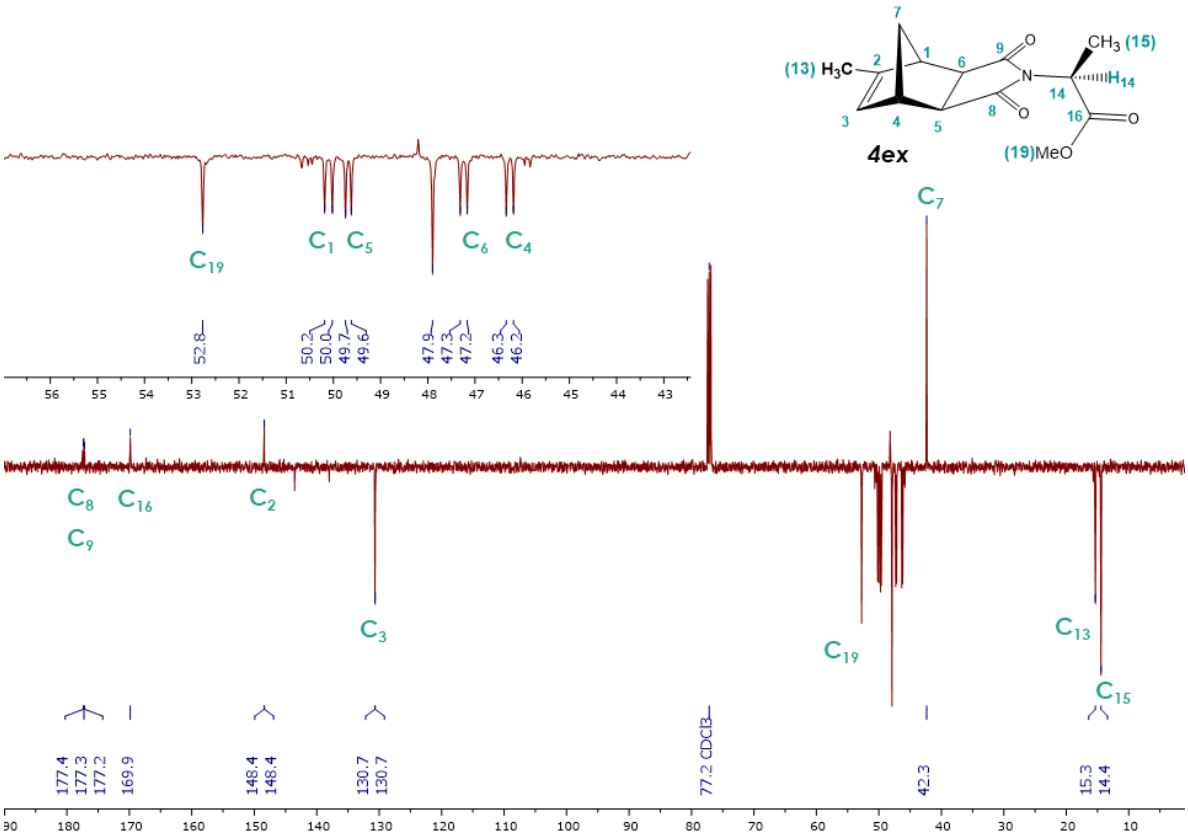

Figure S.45. APT of ***4ex***

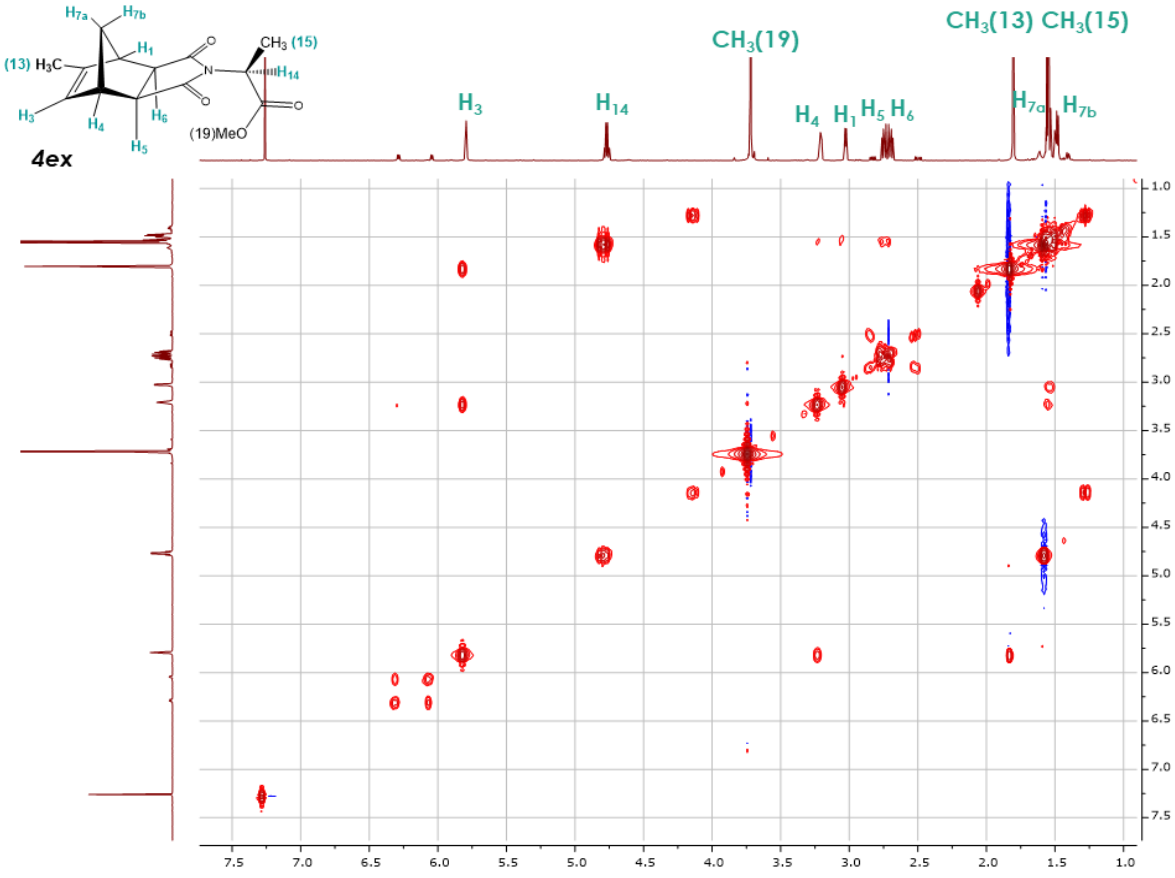

Figure S.46. COSY of ***4ex***

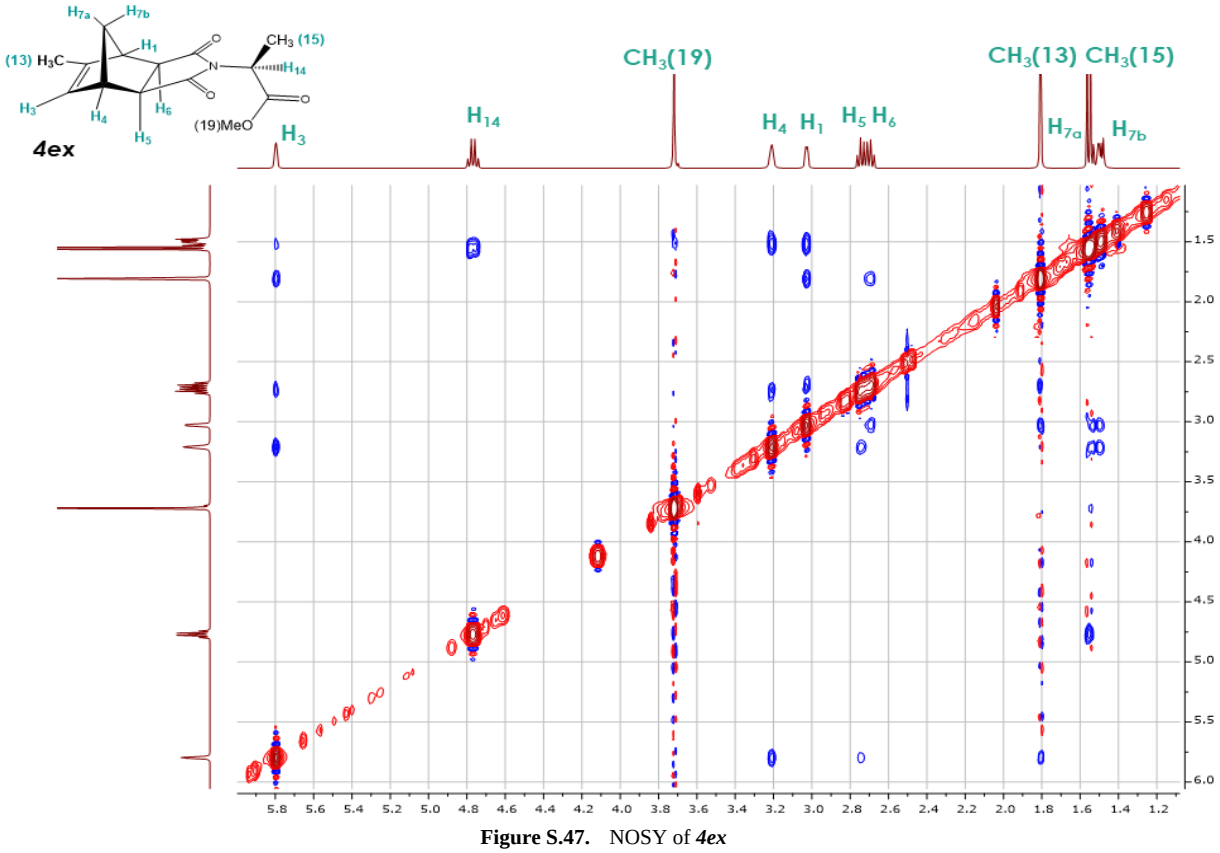

Figure S.47. NOSY of ***4ex***

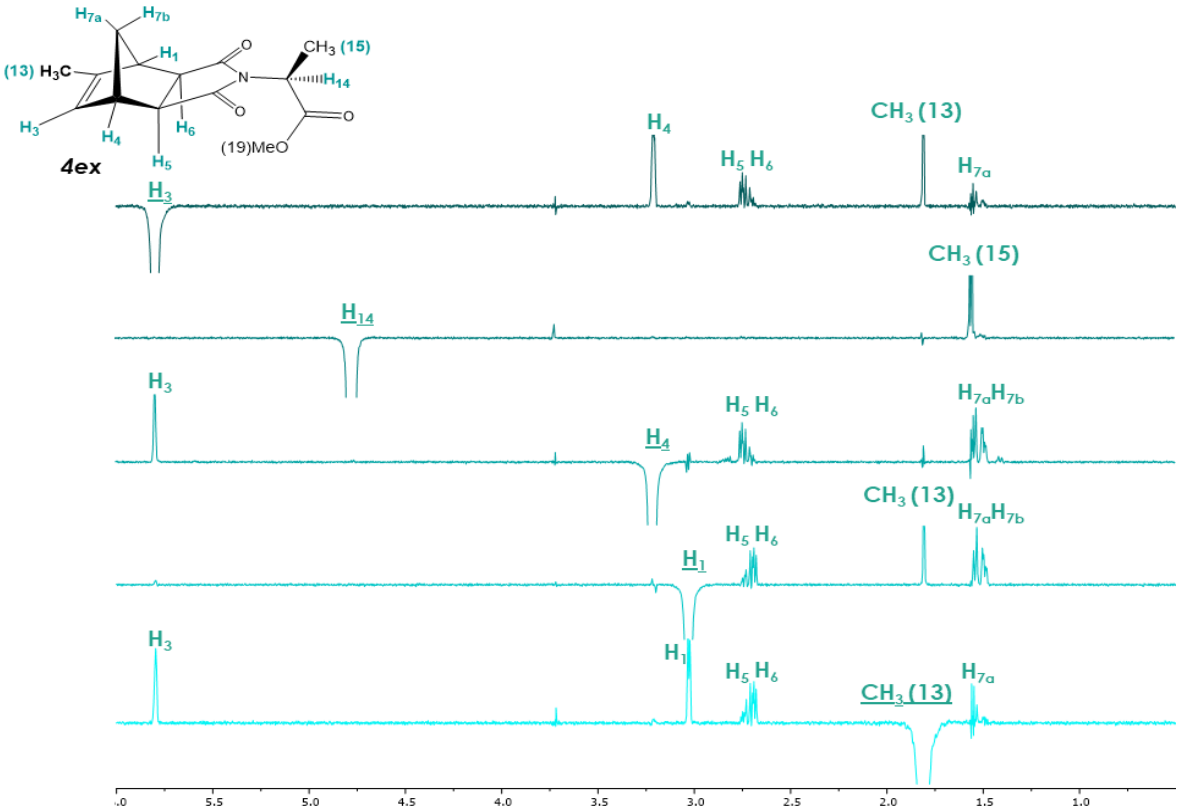

Figure S.48. 1D NOEs of ***4ex***

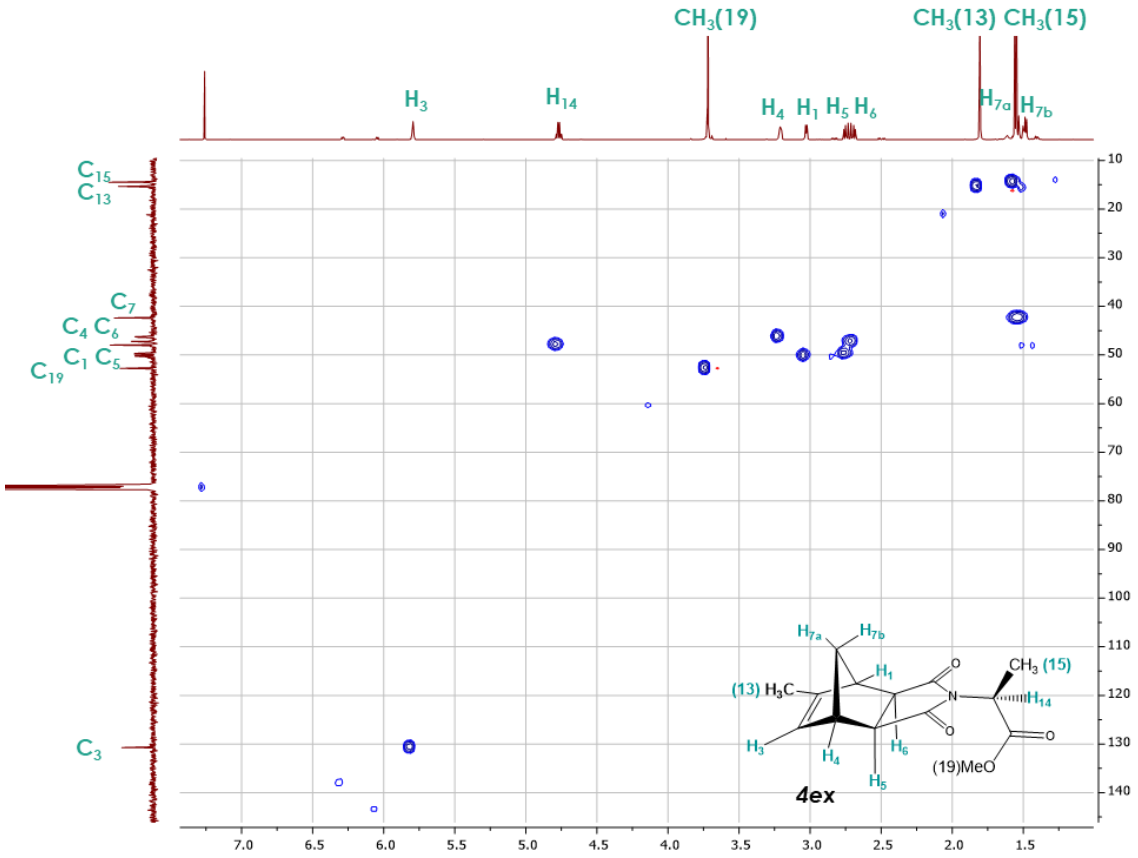

Figure S.49. HSQC of ***4ex***

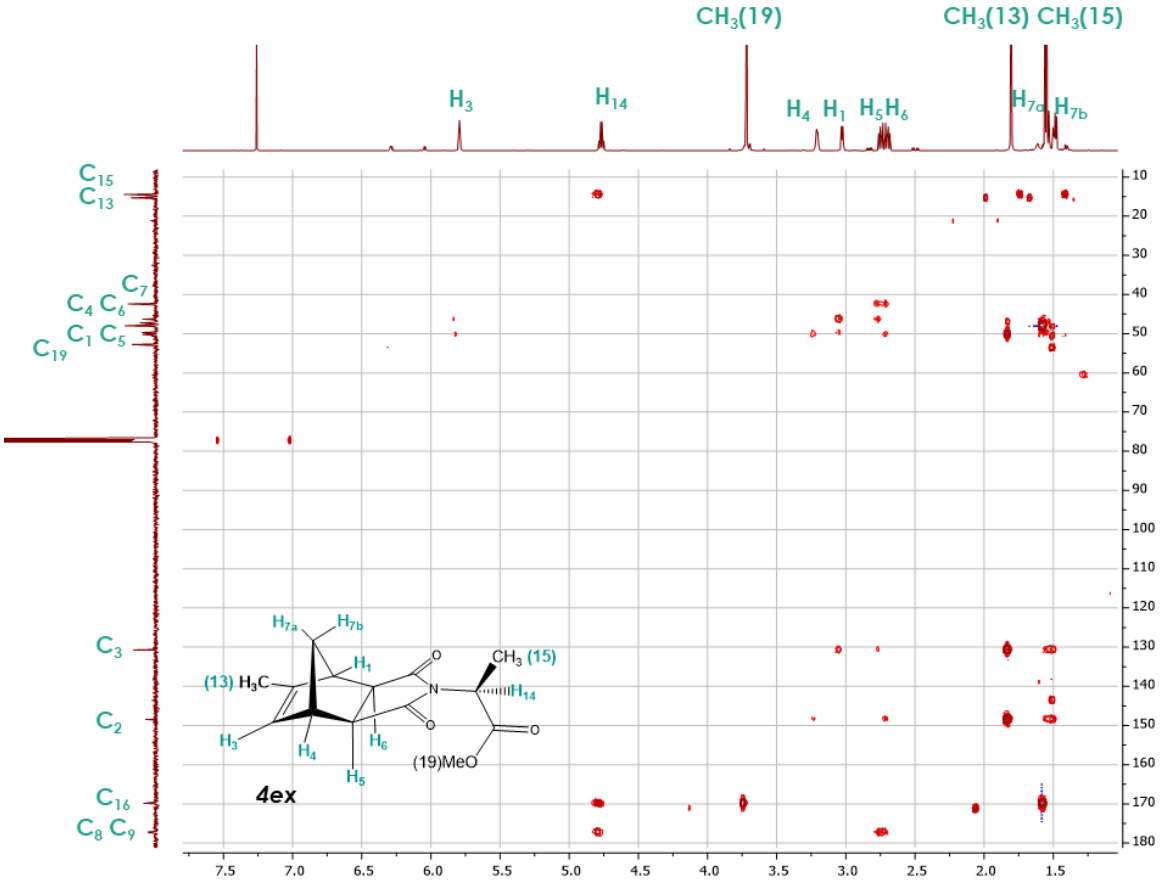

Figure S.50. HMBC of ***4ex***