Supporting Information

The mixture of NO_2-LA isomers synthesized was fractionated by preparative and semi-preparative HPLC as described in the main manuscript. A representative HPLC profile is reproduced in this supplement (Figure 1).

The strategy for assignment involved a process of elimination into two groups of isomers and then further eliminating to a single isomer consistent with the spectral data. The first group of isomers was made up of the 9-NO_2- and 13-NO_2-LA. The second group was the 10- and 12-nitrated isomers. 2D COSY, TOCSY and HMBC NMR were used to make the eliminations.

The 2D COSY spectra (Figure 2) of HPLC peak one showed all vinyl protons coupled to the centrally located allylic H11's. This sample must be either the 9- or 13-NO_2-LA since only these isomers have the possibility of this coupling. To discriminate between the 9 and 13 nitrated isomer a combination of 2D experiments were used. The vinyl protons at 5.53 and 5.33 ppm (H9 and H10 of 13-NO_2-LA, respectively) had COSY connectivity to an allylic CH_2 at 2.06 ppm (H8) (Figure 2). This CH_2 at 2.06 ppm was TOCSY coupled to a pair of CH_2's at 1.64 and 2.35 ppm, assigned as H3 and H2, respectively (Figure 3). The assignment of both of these CH_2's was accomplished by HMBC cross peaks to the carbonyl of the carboxylic acid (Figure 4). These results are not consistent with the 9-NO_2 isomer but are consistent with the 13-NO_2-LA. Upon closer inspection of the TOCSY data a weak H9 to H2 and a stronger H9 to H3 connectivity was observed (data not shown). This evidence supports identification of the HPLC peak one isomer as 13-NO_2-LA.

HPLC peak two showed two vinyl protons coupling to the centrally located allylic H11's (Figure 5). This is consistent with either the 12- or 10-NO_2-LA isomers. Using 2D TOCSY NMR, a strong correlation was seen between the vinyl on the nitrated double bond and the terminal methyl (Figure 6). This is not consistent with the 10-NO_2 isomer but is easily observed with the 12-NO_2 isomer; hence, peak two is identified as 12-NO_2-LA.

HPLC peak three was more challenging due to the approximate 50:50 mixture of isomers. However, given the 12- and 13-NO_2 isomers had been assigned with confidence this left peak three to contain a mixture of the 9- and 10-NO_2 isomers. Again, the same strategy employed above was used here. A set of vinyl peaks at 7.03 (H10), 5.32 (H12) and 5.55(H13) all showed COSY connectivity to a central allylic H11 (Figure 7). This coupling was only consistent with the 9-nitro isomer. The other set of vinyl protons were then assigned to the 10-nitro isomer and display only two up-field vinyl protons coupling to the central H11.

Once the initial identity of each HPLC sample was made then assignment of all the proton of carbon NMR spectra (Figure 8) were attempted. Using all the 1D and 2D data a full assignment of the proton spectra were made except for the overlap in mid-chain region from H4 to H6 and H16 and H17 for each isomer. The carbon assignments were not as complete due to the lack of resolution in the 2D HMQC and HMBC experiments. There was also considerable of overlap in the 1D ^{13}C NMR spectra. However, the more critical carbons assignments from C8 to C4 were made with confidence. These assignments are summarized in Tables 1 and 2 of the main manuscript.

The retention of E configuration about both double bonds in all four isomers was established by first noting the downfield chemical shifts of all the protons on the nitrated double bond falls in the rage of 7.02 to 7.09 ppm. Given the deshielding anisotropic effect of the nitro group, this places these protons *cis* to the nitro. The unsubstituted double bond configuration was verified by a series of 1D ^{1}H homonuclear experiments (Figures 9, 10, 11). The central H11 and either the allylic H8 or H14 were decoupled to collapse the vinyl proton to a double triplet. All peaks exhibited the smaller 10 to 11 Hz *cis* coupling.

Figure 1. HPLC profile of NO_2-LA positional isomers

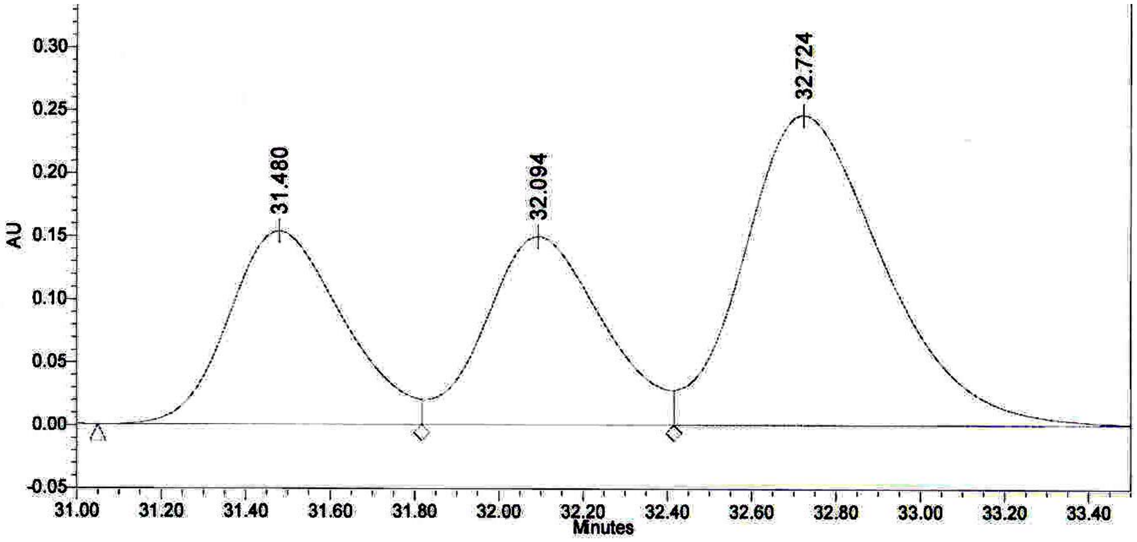


Figure 2. 500 MHz COSY of HPLC peak one (13-NO_2-LA) showing mutual coupling of all vinyl protons to the centrally located H11's. Also shown is the H9 and H10 coupling to H8.

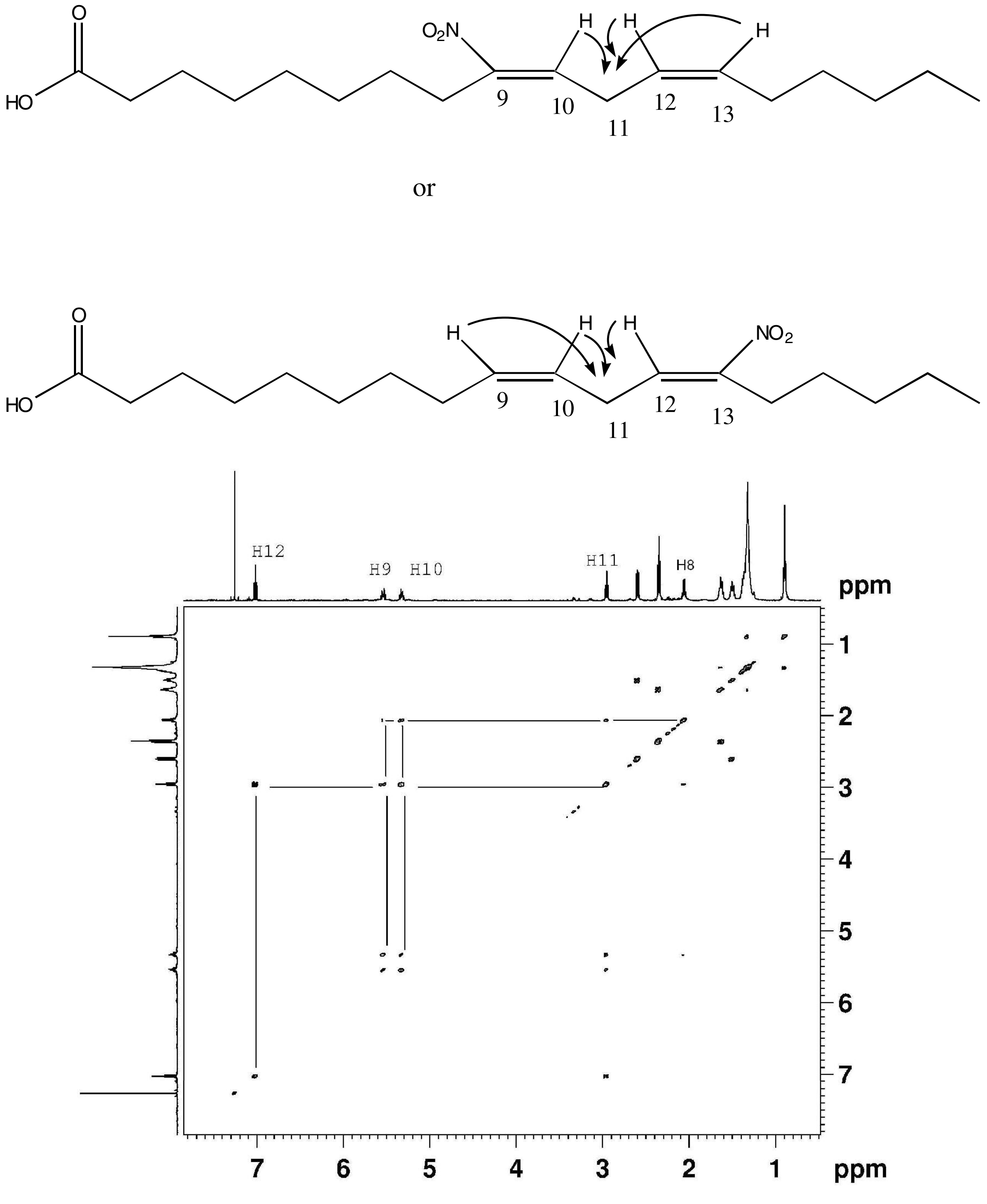


Figure 3. TOCSY of peak one (13-NO_2-LA) showing allylic H8 coupling to methylenes H2 and H3 which are α and β to the carbonyl (see HMBC in Figure 4)

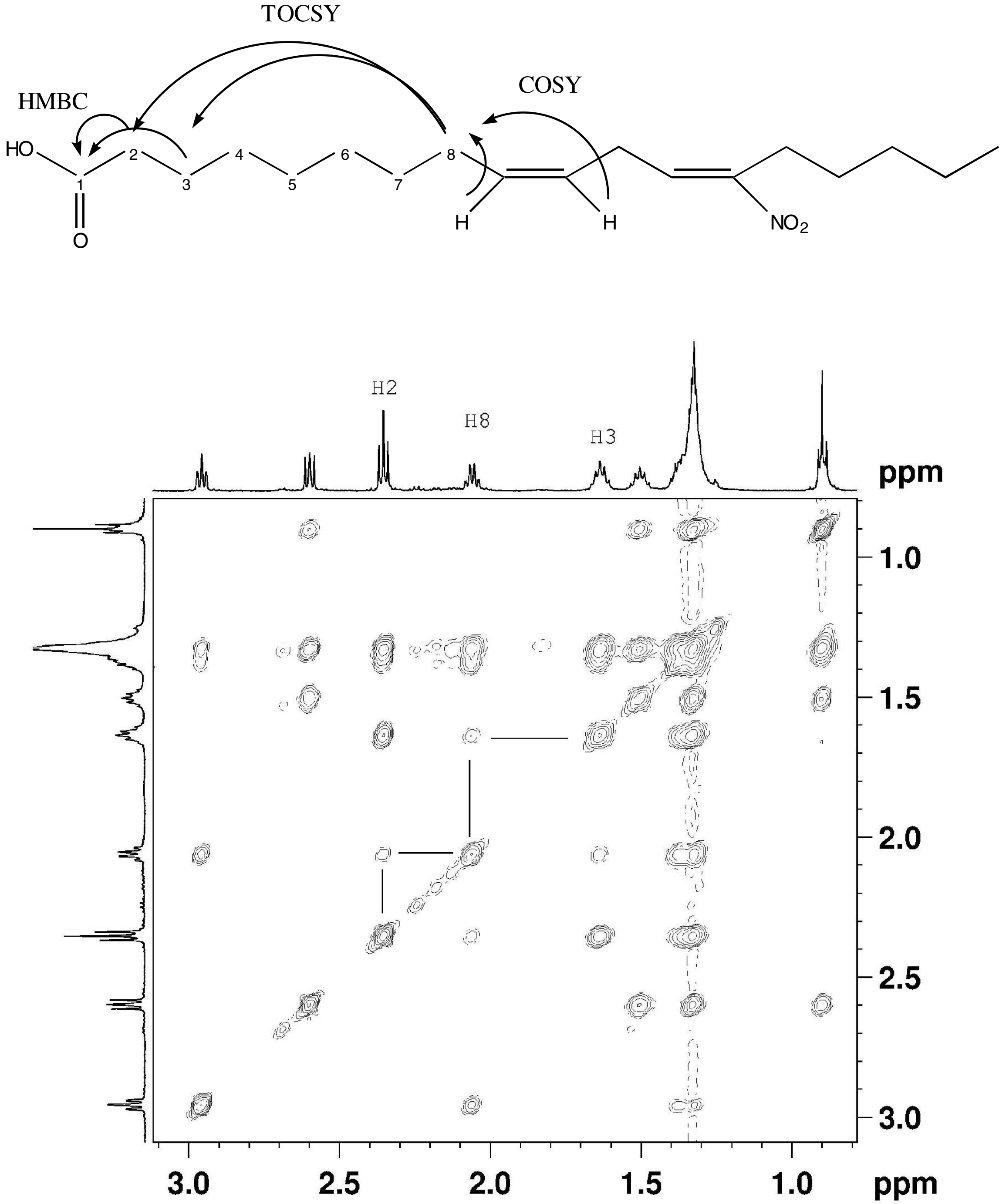


Figure 4. HMBC of peak one showing connectivity between the carbonyl carbon C1 and H2 and H3. This establishes H2 and H3's identity with confidence.

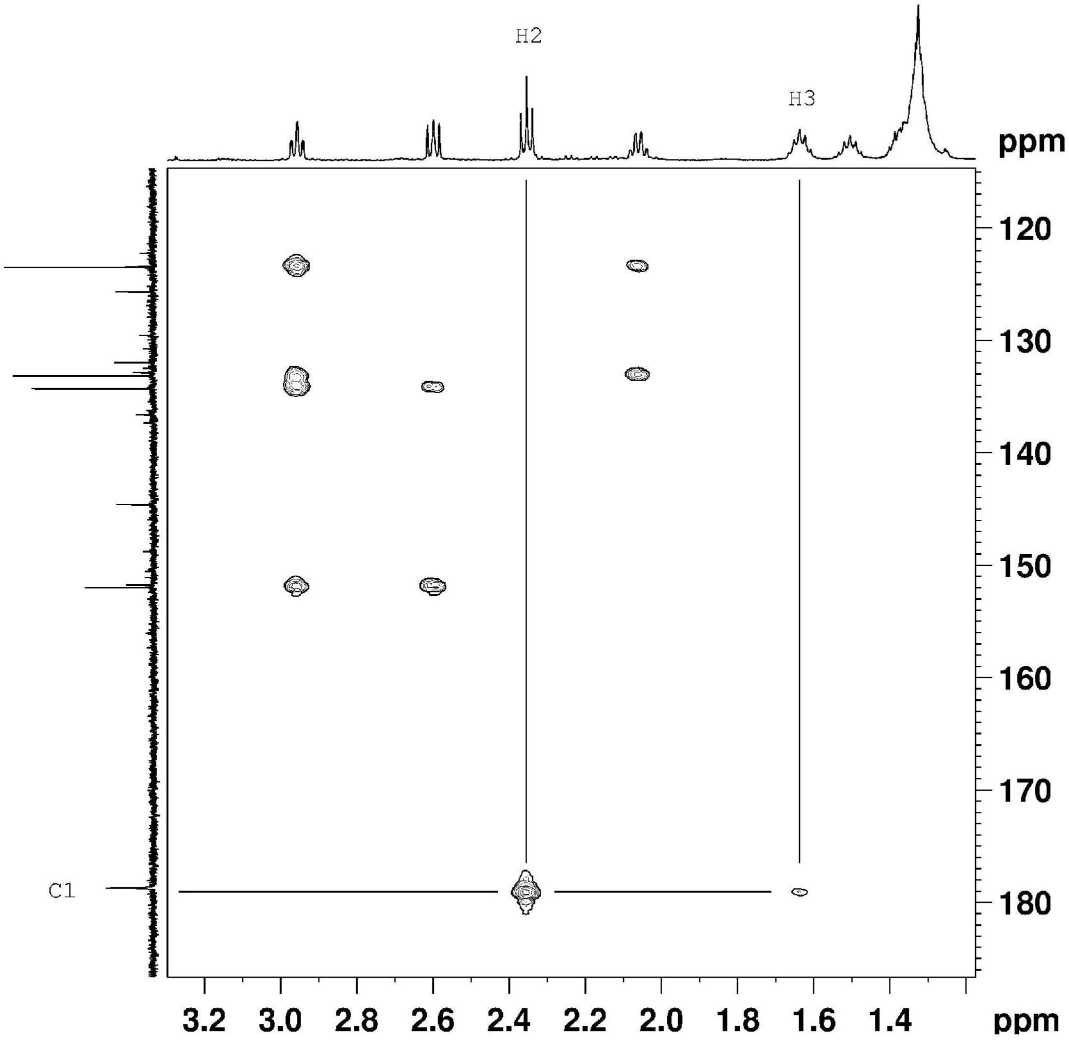
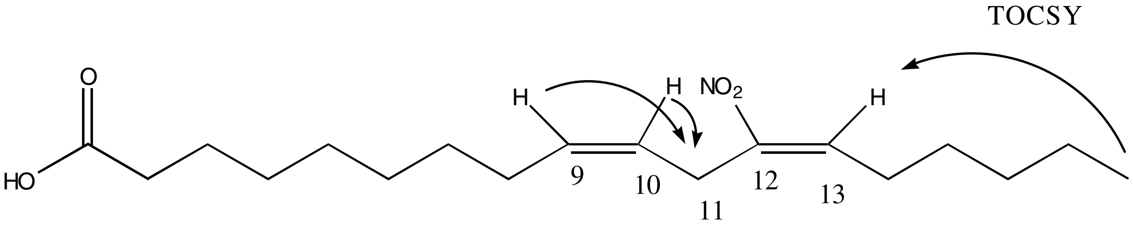
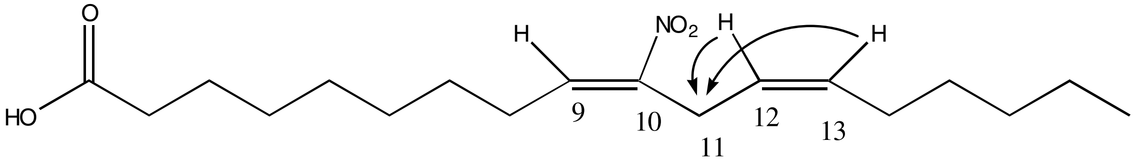


Figure 5. 2D COSY of HPLC peak two showing only two vinyl protons coupling to the central H11.



or



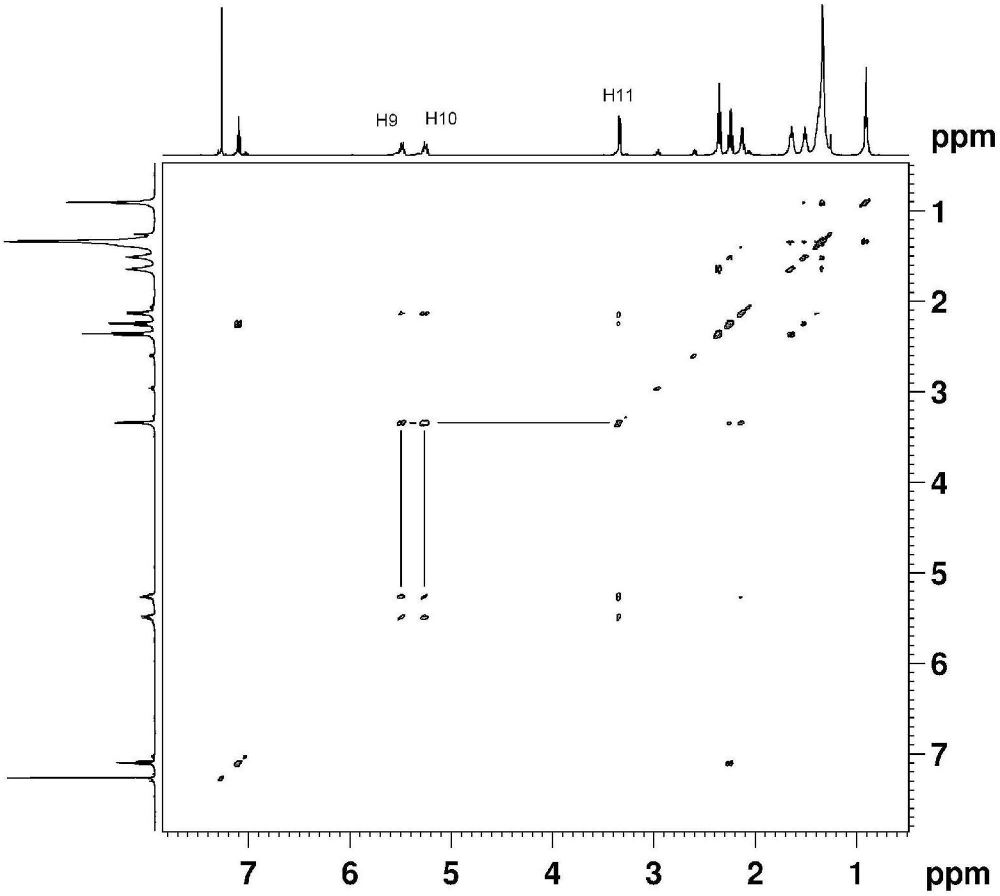


Figure 6. 2D TOCSY of peak two showing coupling from the terminal methyl H18 to the vinyl proton H13.

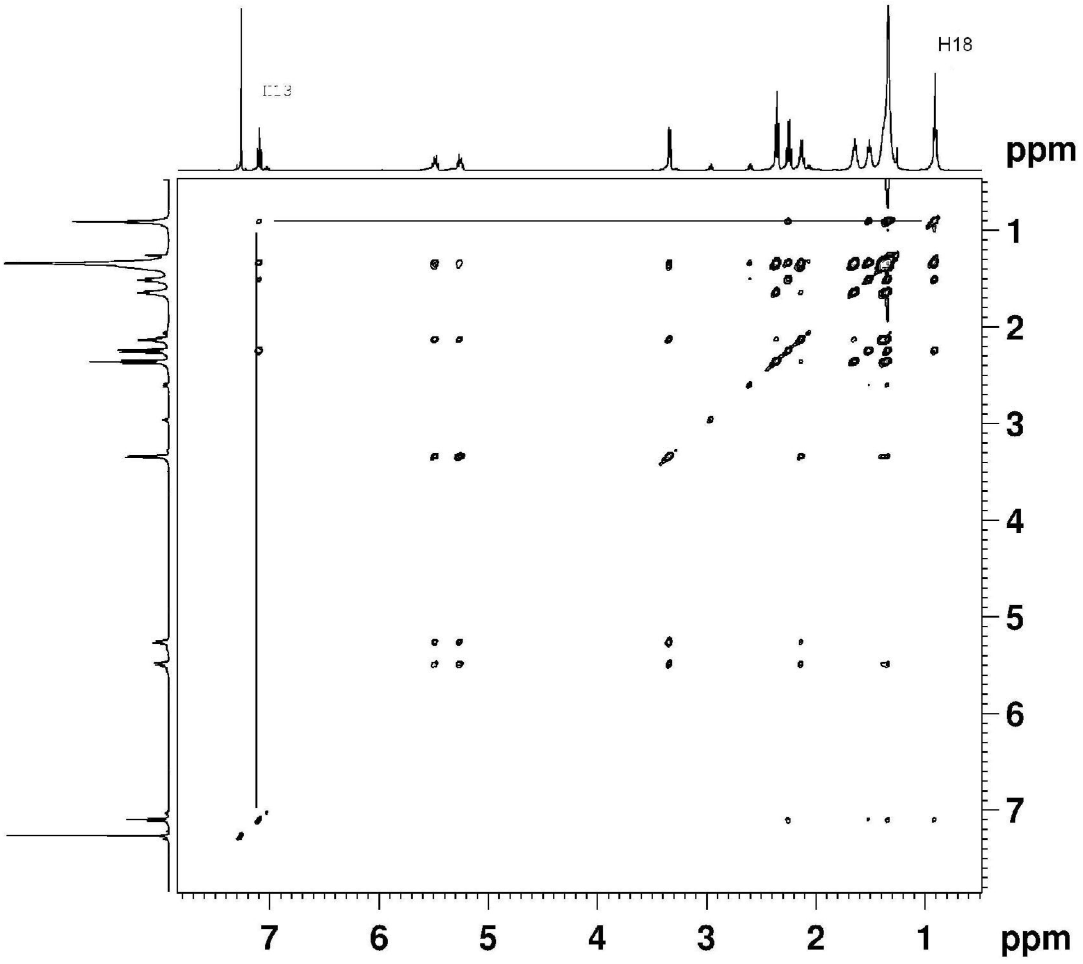


Figure 7. 2D COSY HPLC peak three showing the 9-NO_2 isomer's vinyls all coupling to the central allylic H11. Also shown are only two of the 10-NO_2 isomer's vinyls (H12 and H13) coupling to the central H11.

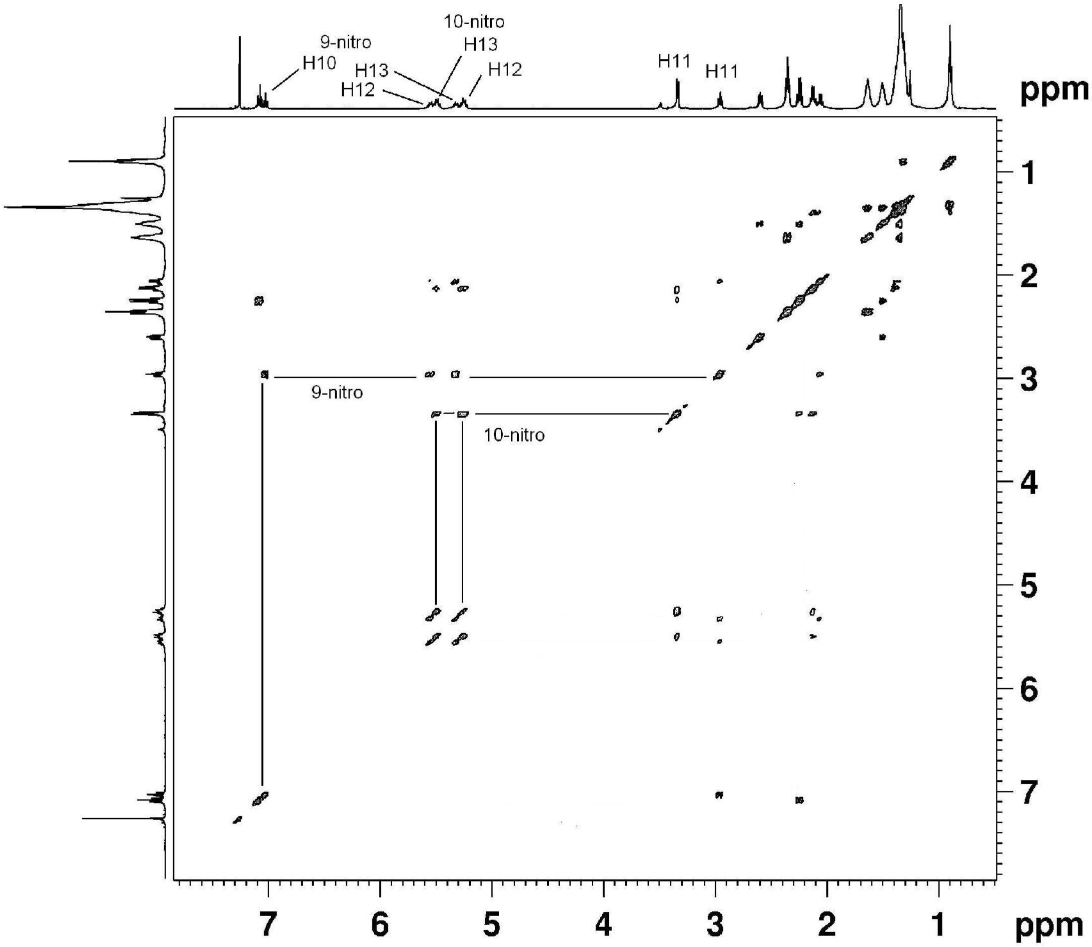
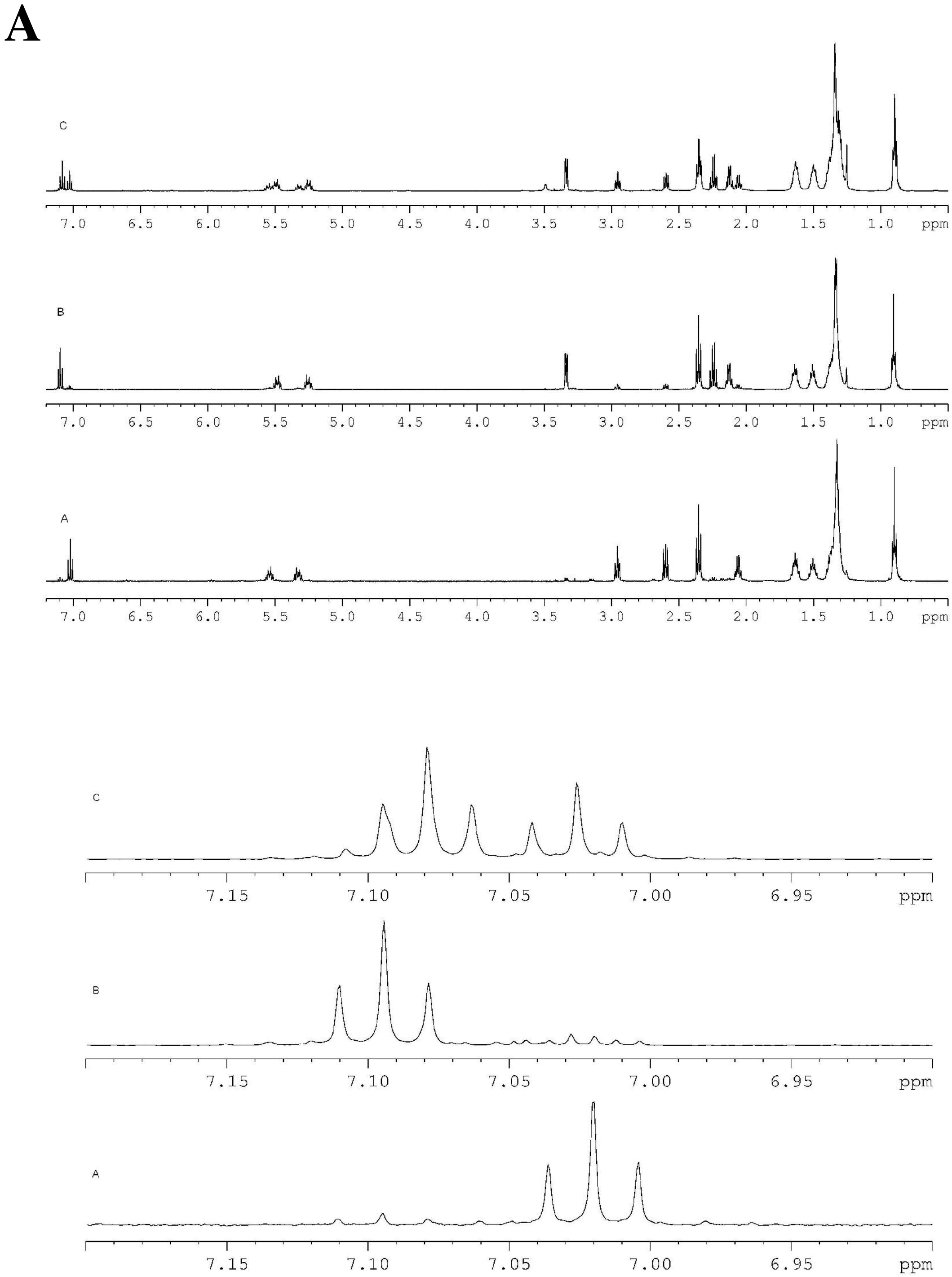
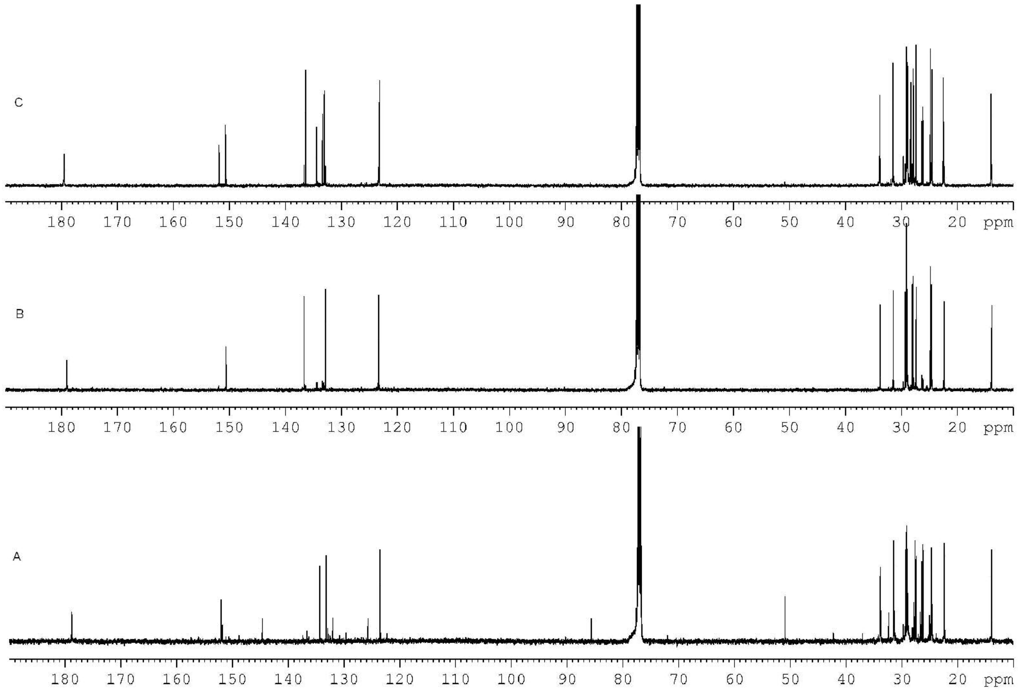


Figure 8. 1D 1H's (Bold A) and 13C's (Bold B). A) HPLC peak one. B) HPLC peak two. C) HPLC peak three.



B


C
B
A
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

C

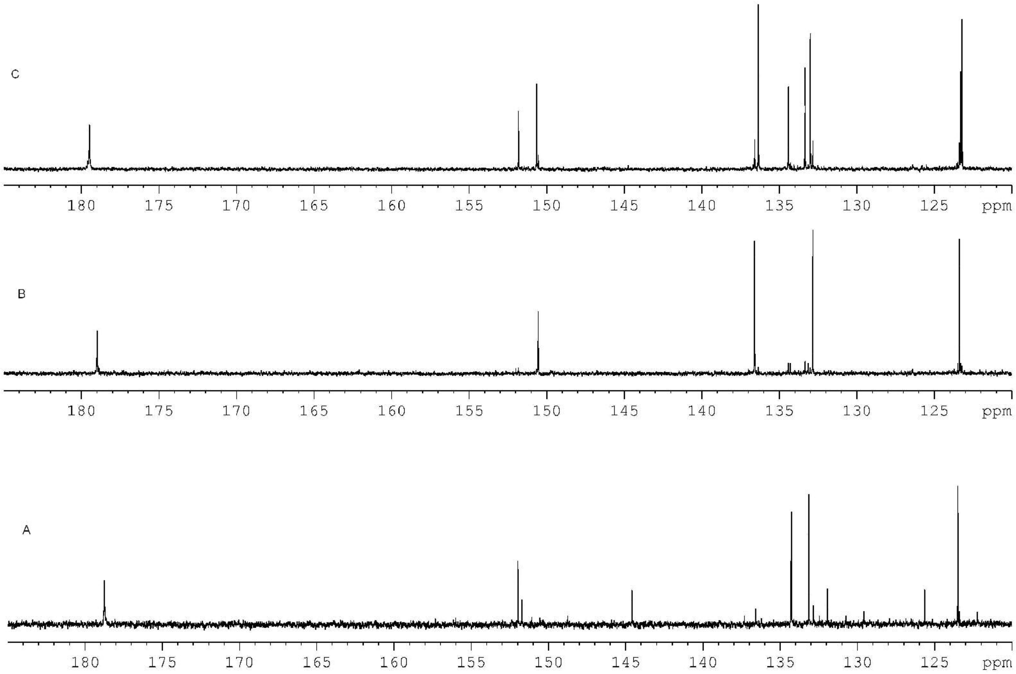

C
B
A
180 175 170 165 160 155 150 145 140 135 130 125 ppm

Figure 9. HPLC peak one homonuclear decoupling. A) Reference spectrum. B) H11 decoupled showing the cis 10.76 Hz coupling between H9 and H10. C) H8 decoupled to reveal cis coupling.

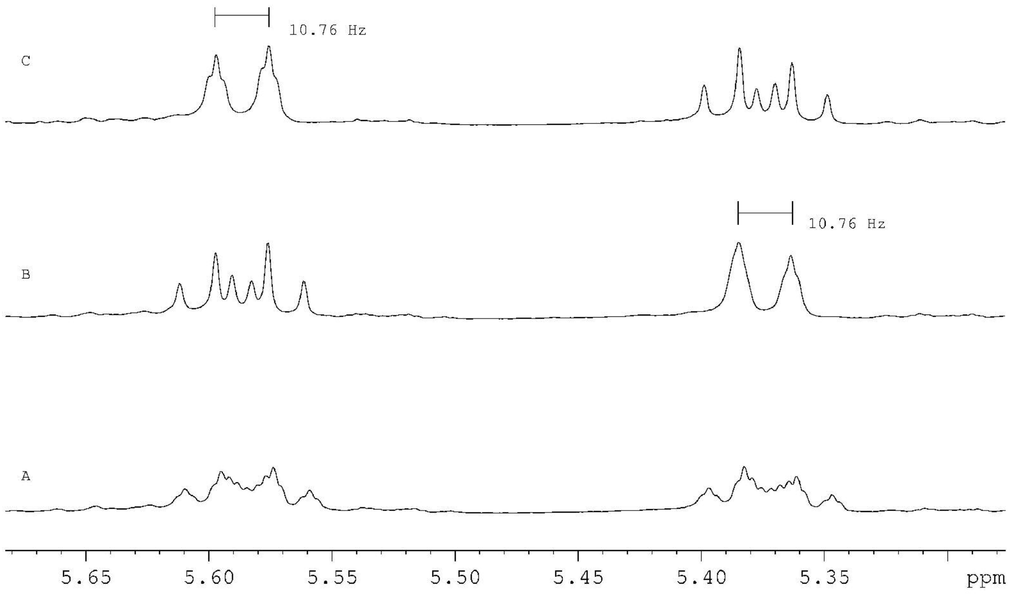


Figure 10. HPLC peak two homonuclear decoupling. A) Reference spectrum. B) H11 decoupled showing the cis 11.00Hz coupling between H9 and H10. C) H8 decoupled to reveal cis coupling.

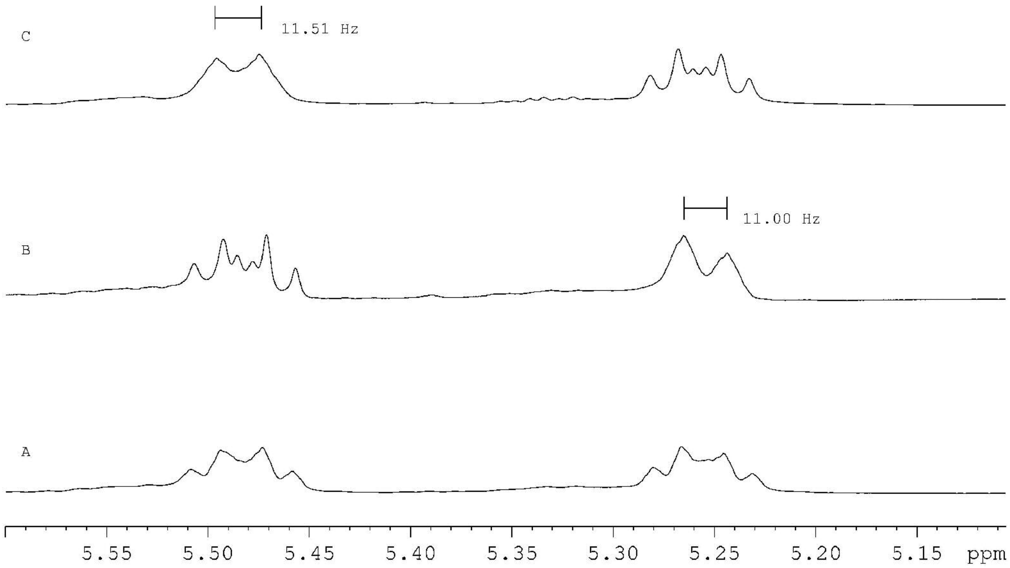


Figure 11. HPLC peak three homonuclear decoupling. A) Reference spectrum. B) 10-NO_2 isomer's H11 decoupled showing the cis 11.00 Hz coupling between H12 and H13. C) 10-NO_2 isomer's H14 decoupled to reveal cis coupling. D) 9-NO_2 isomer's H11 decoupled showing the cis 10.51 Hz coupling between H12 and H13. E) 9-NO_2 isomer's H14 decoupled showing the same cis 10.51 Hz coupling.