

## 2D experiments

2D experiments show cross-peaks at the intersection of frequencies of nuclei that share a scalar coupling or relax through each other.

2D spectra are made of an array of 1D spectra, in which a time in the pulse sequence,  $d_2$ , is incremented. The pulse sequences are designed to keep in these 1D spectra (frequency dimension that is observed,  $f_2$ ) only the signals that oscillate along the time  $d_2$  at the frequency of the nuclei that couple with, or relax through, the nuclei yielding those signals. A second Fourier transform along time  $d_2$  for each point in the spectra in  $f_2$ , produces the frequencies in the indirect detected dimension,  $f_1$ .

Brief instructions are given below for acquiring homonuclear correlation and indirect detection experiments. For each type of experiment, say HSQC (one-bond heteronuclear correlation), there are better variants which employ more sophisticated hardware, e.g. gHSQC uses gradients, and gHSQCAD uses adiabatic shaped pulses which need a waveform generator. In principle go with the g\*\*\*\*AD experiment. If the instrument doesn't have the appropriate hardware, the experiment will be grayed out.

Take a proton spectrum in experiment 1.

Go to the top menu Edit and drag to Move FID. Move the FID from experiment 1 to experiment 2, and join experiment 2. In the Process tab click the top button Transform to get a copy of the spectrum in experiment 1. Place two cursors around the region of interest and in the Acquire tab click the MoveSW top button. It is a good idea to acquire a spectrum with this new spectral window, and then phase it.

In the top menu Experiments drag to Convert current parameters to do.. and select the experiment you want. From the Homonuclear Correlations menu I recommend Gradient DQF COSY for couplings and ROESYAD for nOEs. From Indirect Heteronuclear Correlations (Basic) I recommend gradient HSQCAD for one-bond corrections and gradient HMBCAD for long-range couplings.

*For H1-H1 correlations:*

In the Acquire – Acquisition tab make the number of points a power of 2 for which the Acquisition time will be around 0.5 s, and the number of increments in t1 half that. For COSY experiments, the digital resolution = Spectral width / Complex points should be of the order of the smallest coupling from which you want to see a cross-peak, since the lines in the cross-peak separated by the active coupling are anti-phase. Click Set LP1 and Weighting, then Acquire.

*For H1-C13 correlations:*

In the defaults tab, set the C13 Spectral Width to the minimum that would cover the region of carbon signals (just the protonated carbons in HSQC). Set the number of scans per increment to a large number, say 16 or 64. One scan will be acquired for all of the increments, then the second and so on, therefore you can stop the experiment when you are satisfied with the signal to noise ratio. Select the H1-C13 multiplicity off if you are not interested in distinguishing the methylene groups from the methines and methyls. In the Acquire – Acquisition tab make the number of points a power of 2 for

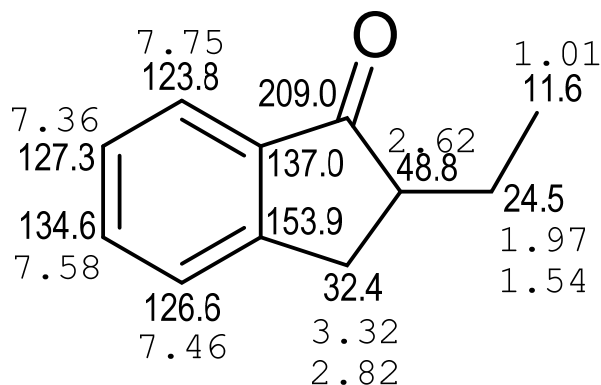
which the Acquisition time will be around 0.5 s, and adjust the number of increments to the desired resolution in *f1*. Click Set LP1 and Weighting, then Acquire.

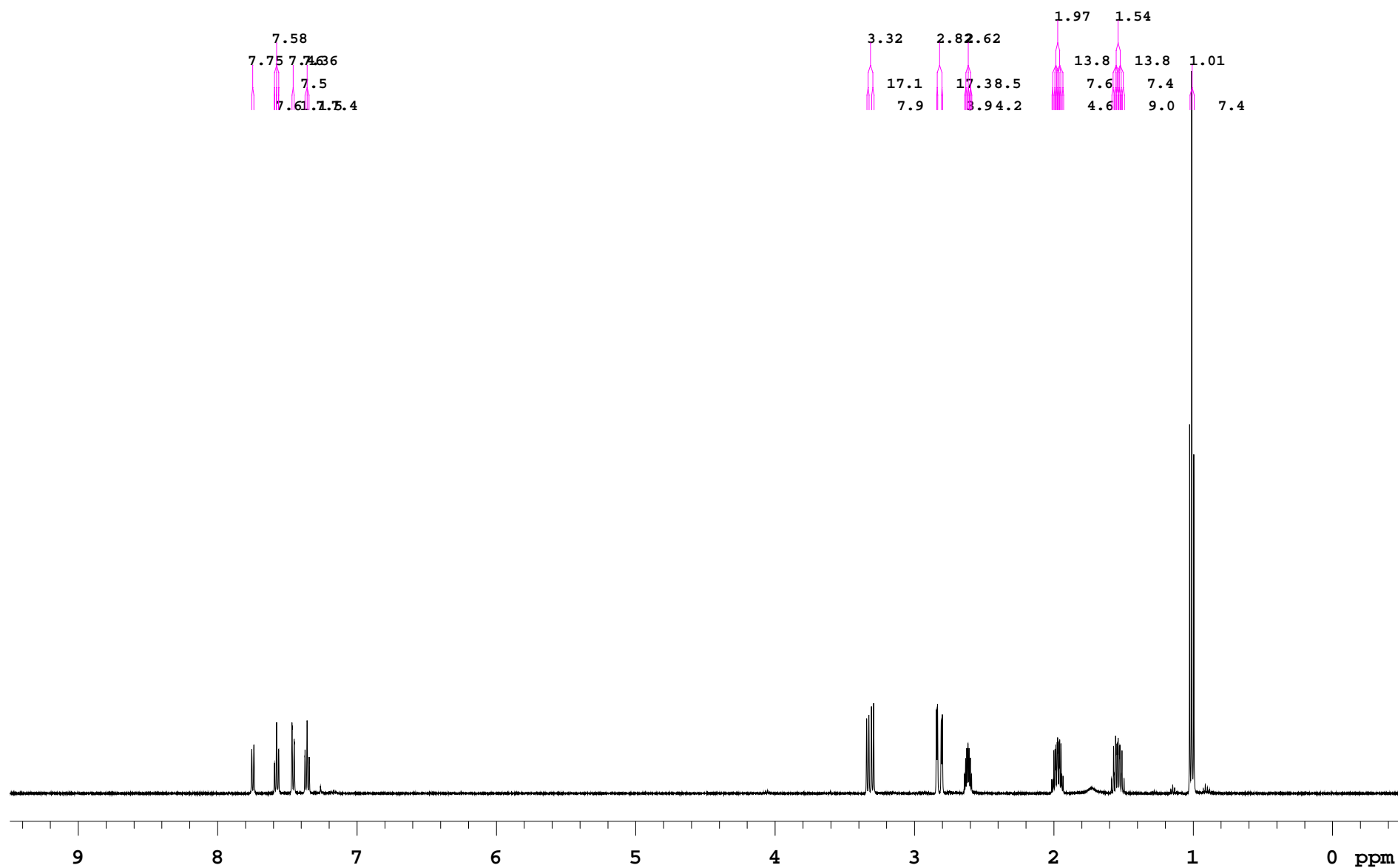
Spectra will be transformed automatically at the end of the acquisition. You can process the spectra at any point, but you may not have the full resolution in *f1* or enough signal to noise ratio. To process the spectra with the default parameters, in the Process tab, click Transform. If you encounter an error, most likely there are not enough increments for linear prediction in *f1*, so go to the Process – more 2D tab and turn it off.

To process the spectra manually, go to Process - Weighting. Adjust FT Data size to equal Aqc Pts in *f2* and double or quadruple that in *f1*, then click FT 1D -1<sup>st</sup> increment. You have to do this also when you want to reprocess the spectra, otherwise the processed data is protected.

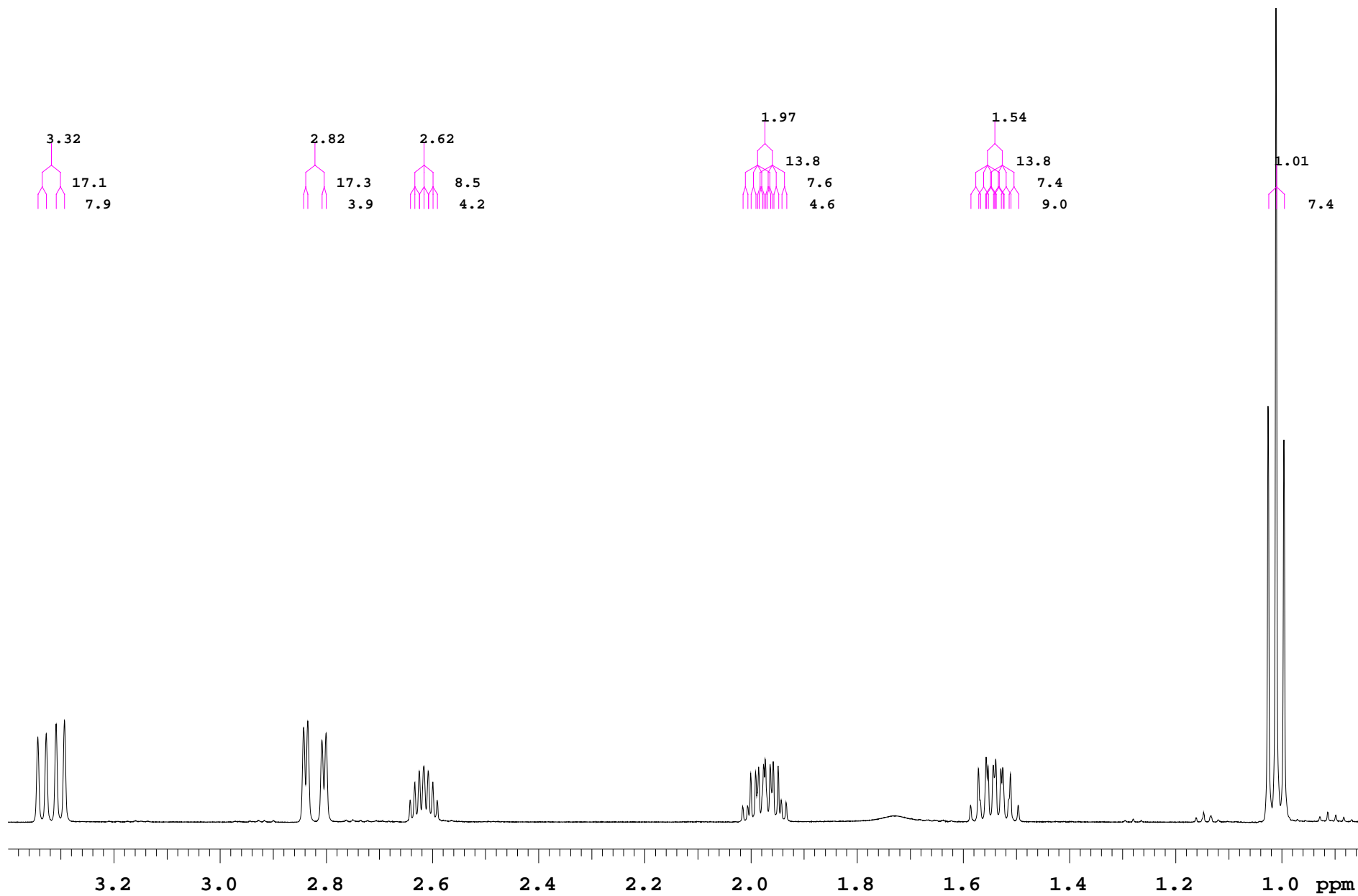
Click interactive weighting, if you want to adjust the weighting in *f2*. Click Transform *f2*, then click on the interferogram with the middle mouse button (MMB) to adjust the vertical scale. Select a signal in *f2* and click Interactive weighting if you want to adjust the weighting in *f1*. Click Full 2D transform. Use the MMB to adjust the vertical scale of the spectrum and the two cursors (controlled by LMB and RMB) together with the zoom and Full Spectrum buttons on the right sidebar to set the displayed region. Go to Process – CS Plot and click Plot all contours to plot the spectrum.

*Follow the cross-peaks in the 2D spectra of 2-ethylindanone:*

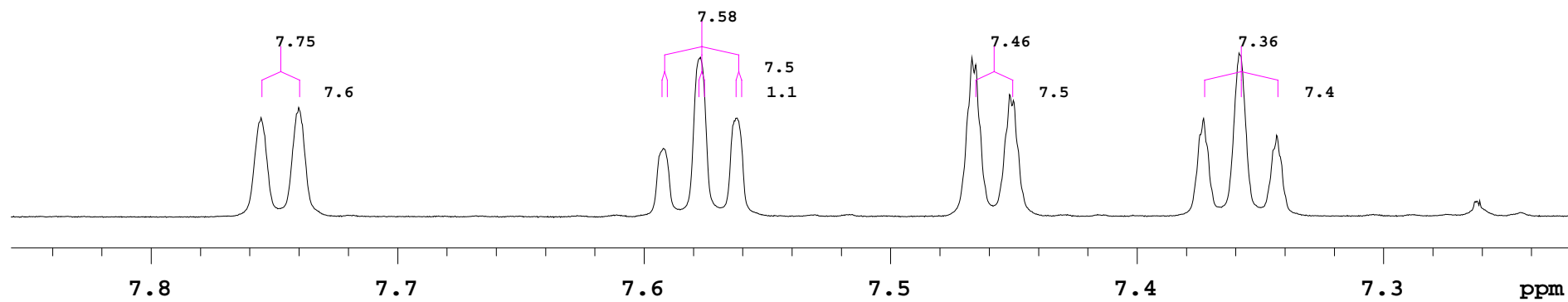




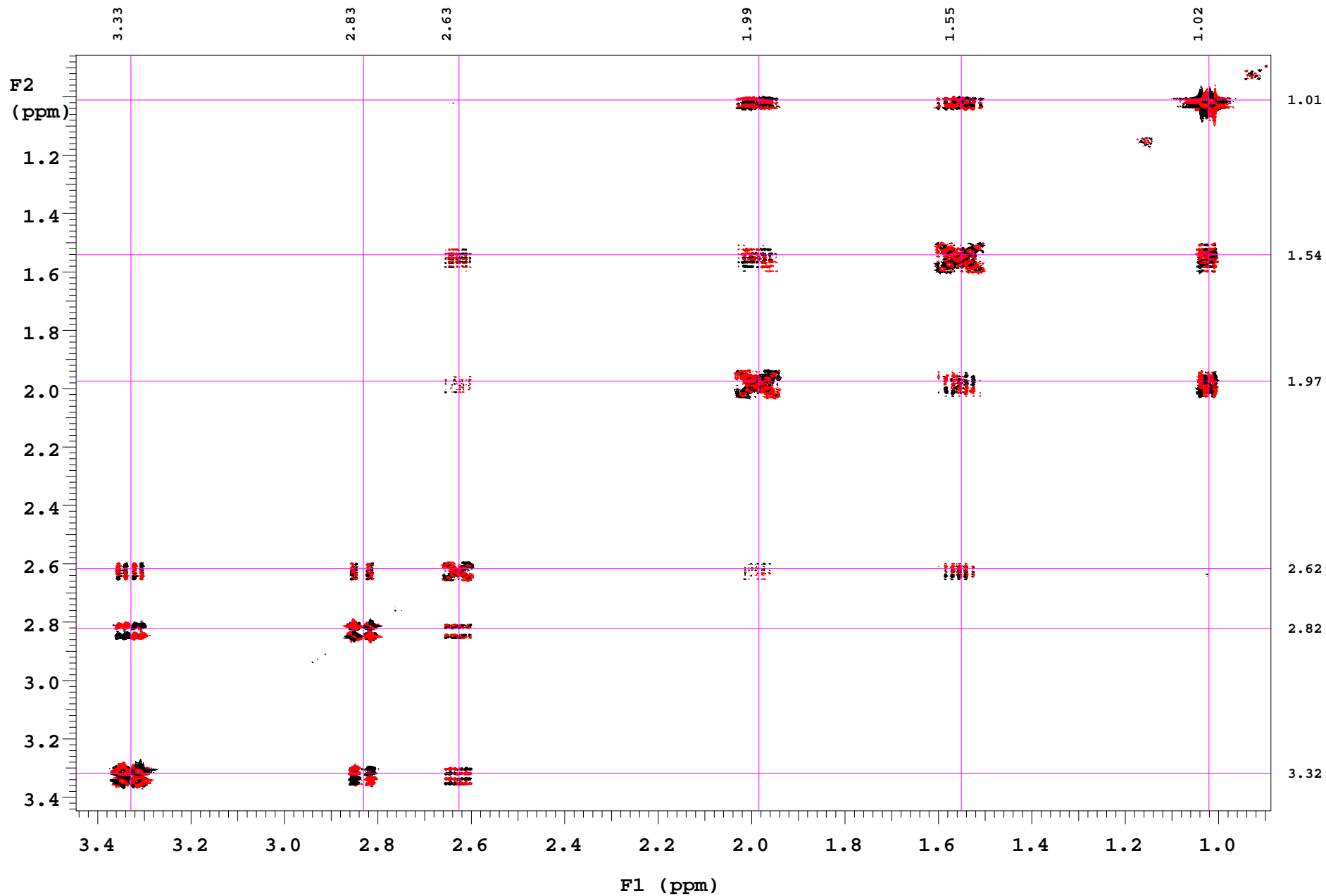
<sup>1</sup>H spectrum of 2-ethylindanone.



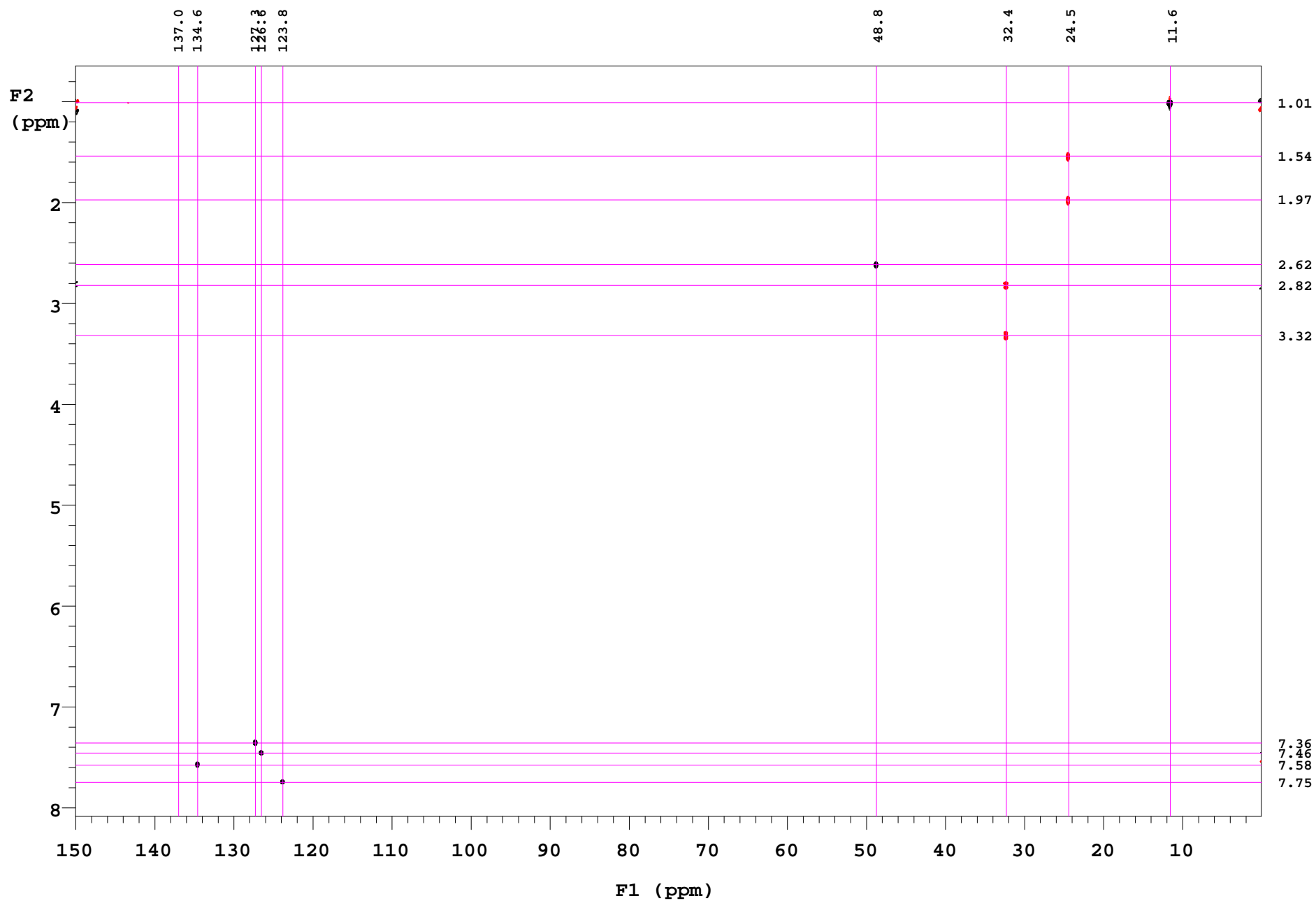
$^1\text{H}$  spectrum of 2-ethylindanone, expansion of the aliphatic region. The protons can be assigned based on their multiplicity.



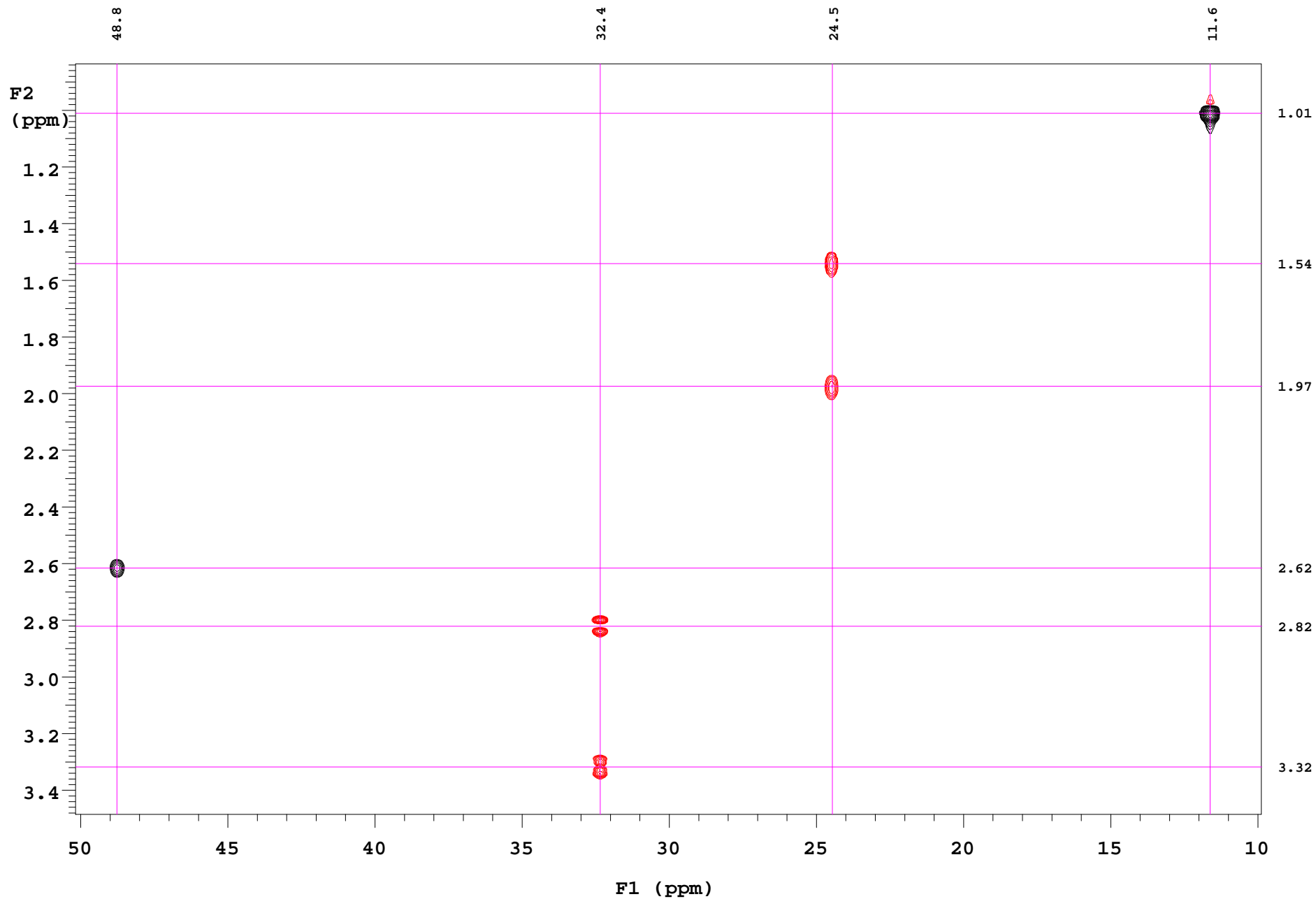
<sup>1</sup>H spectrum of 2-ethylindanone, expansion of the aromatic region.



$^1\text{H}$ - $^1\text{H}$  gDQCOSY spectrum of 2-ethylindanone, on the aliphatic region.

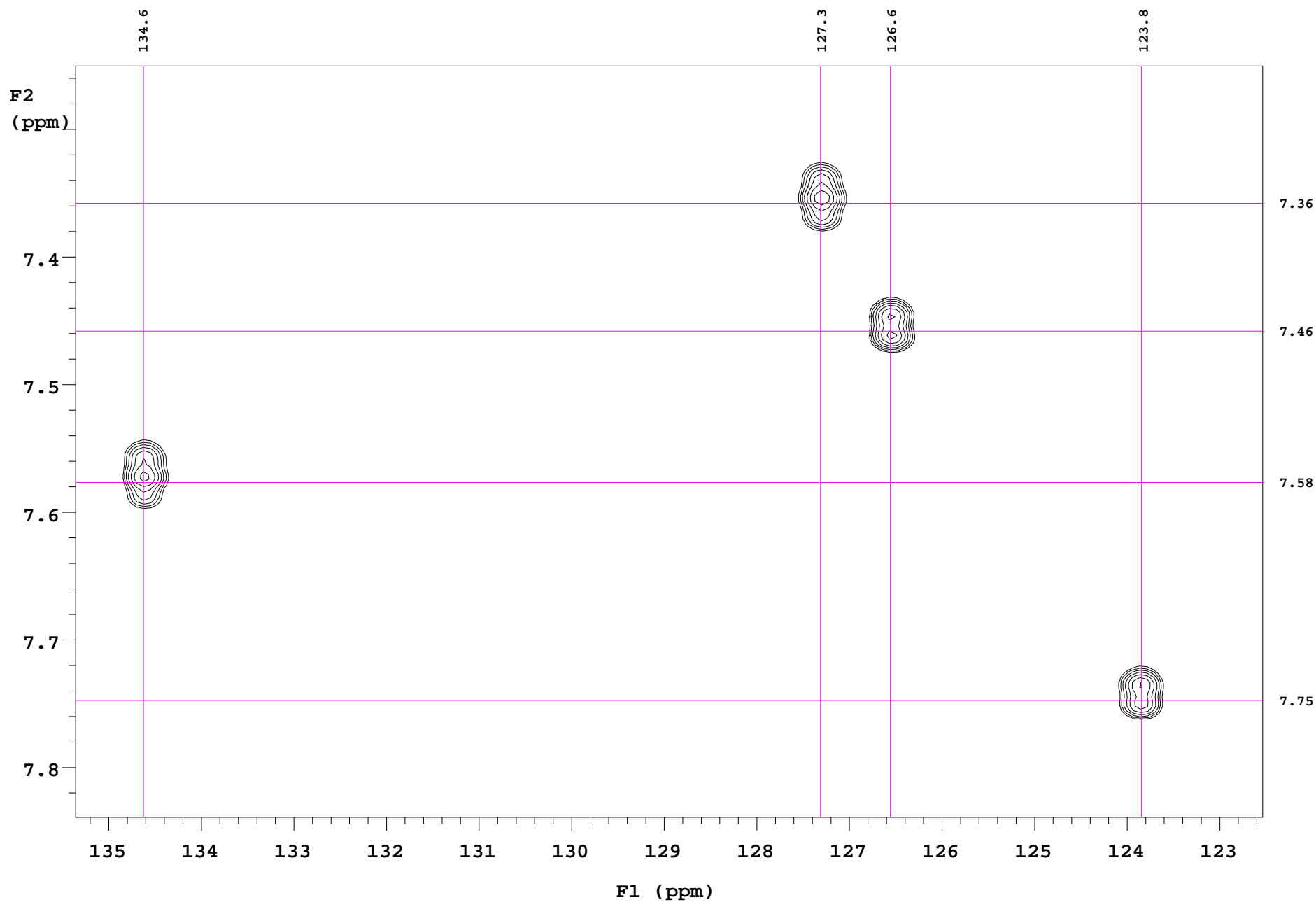


$^1\text{H}$ - $^{13}\text{C}$  gHSQC spectrum of 2-ethylindanone, multiplicity edited.

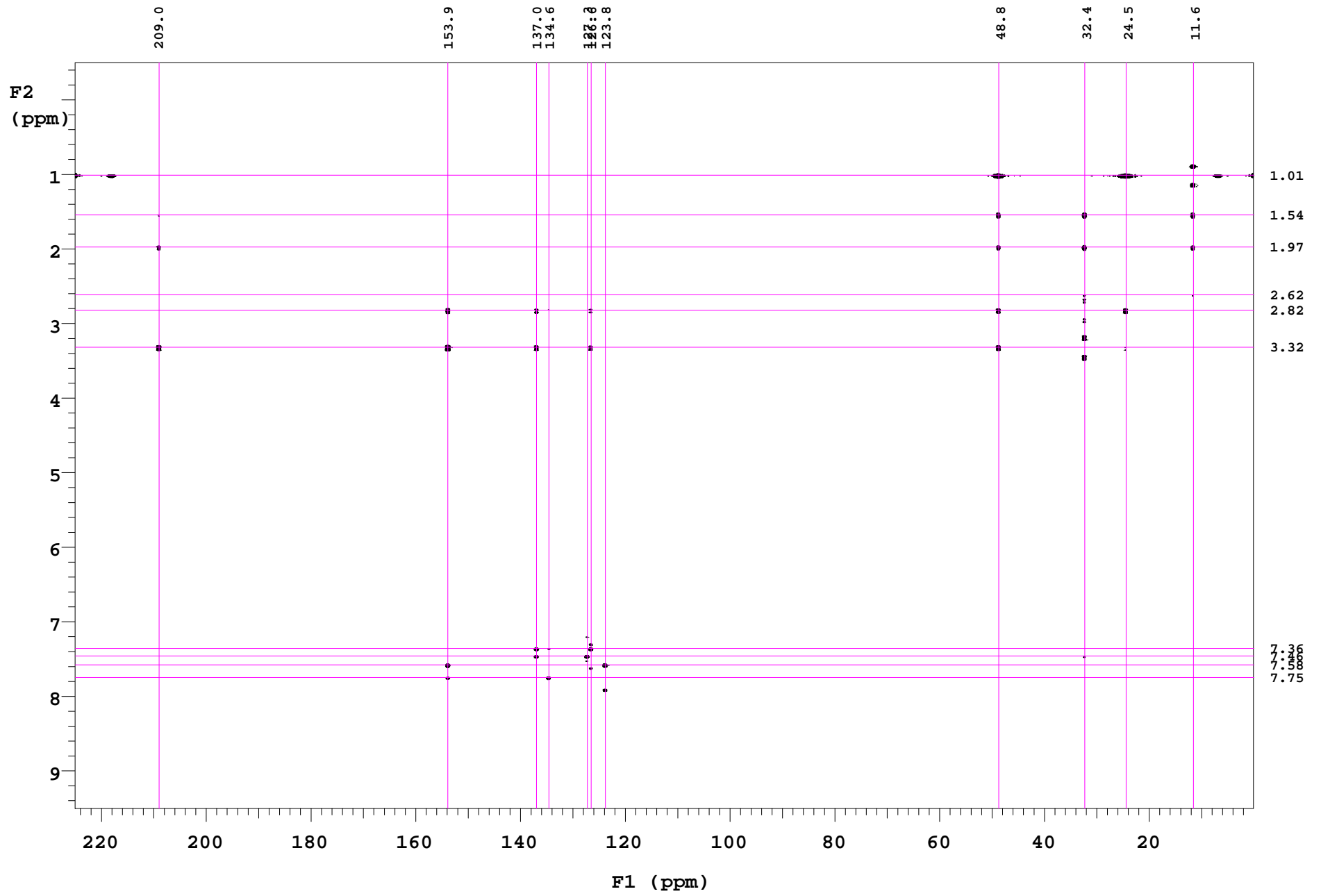


$^1\text{H}$ - $^{13}\text{C}$  gHSQC spectrum of 2-ethylindanone, aliphatic region. The methylene peaks have opposite phase to the methyl and methine ones.

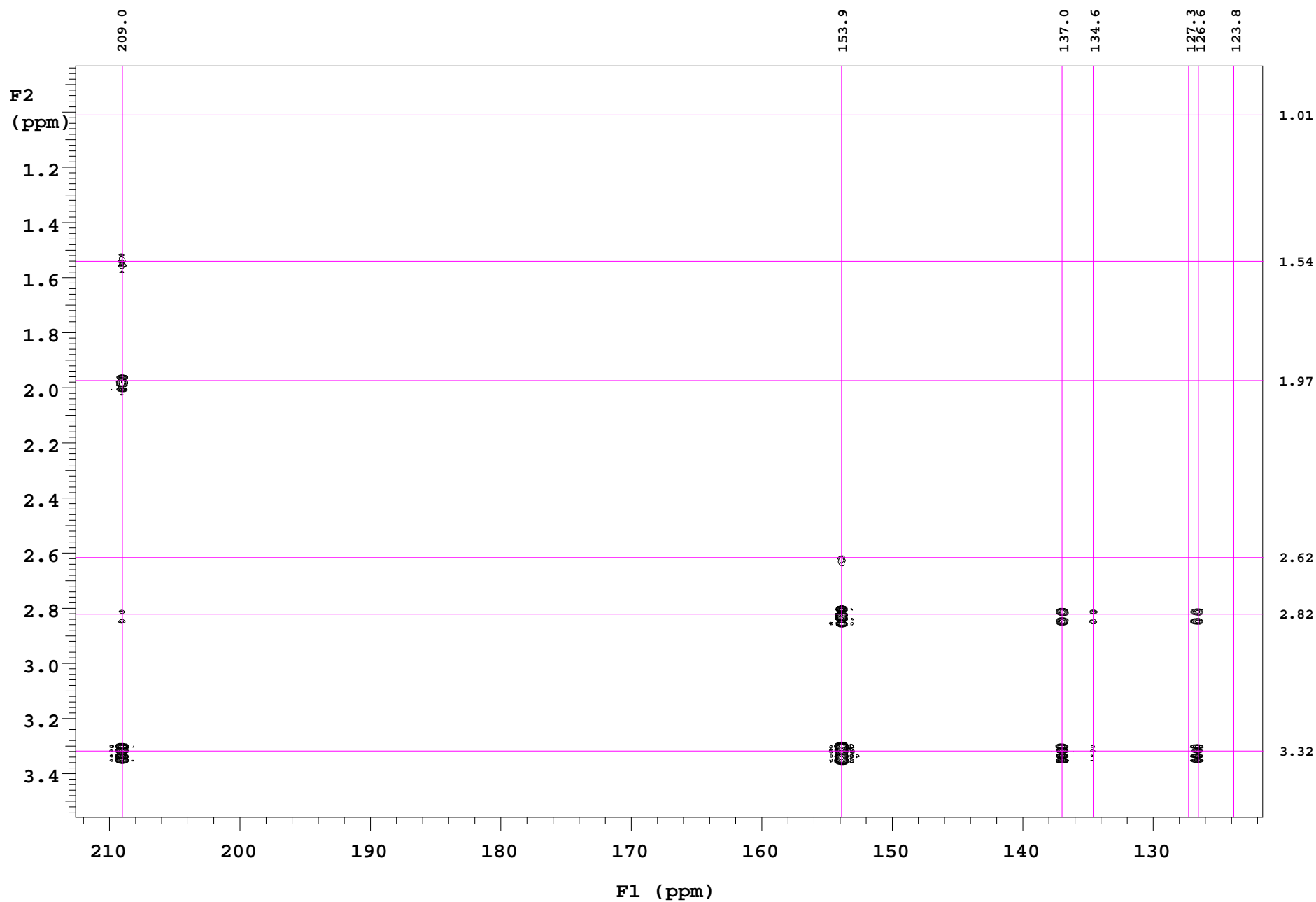




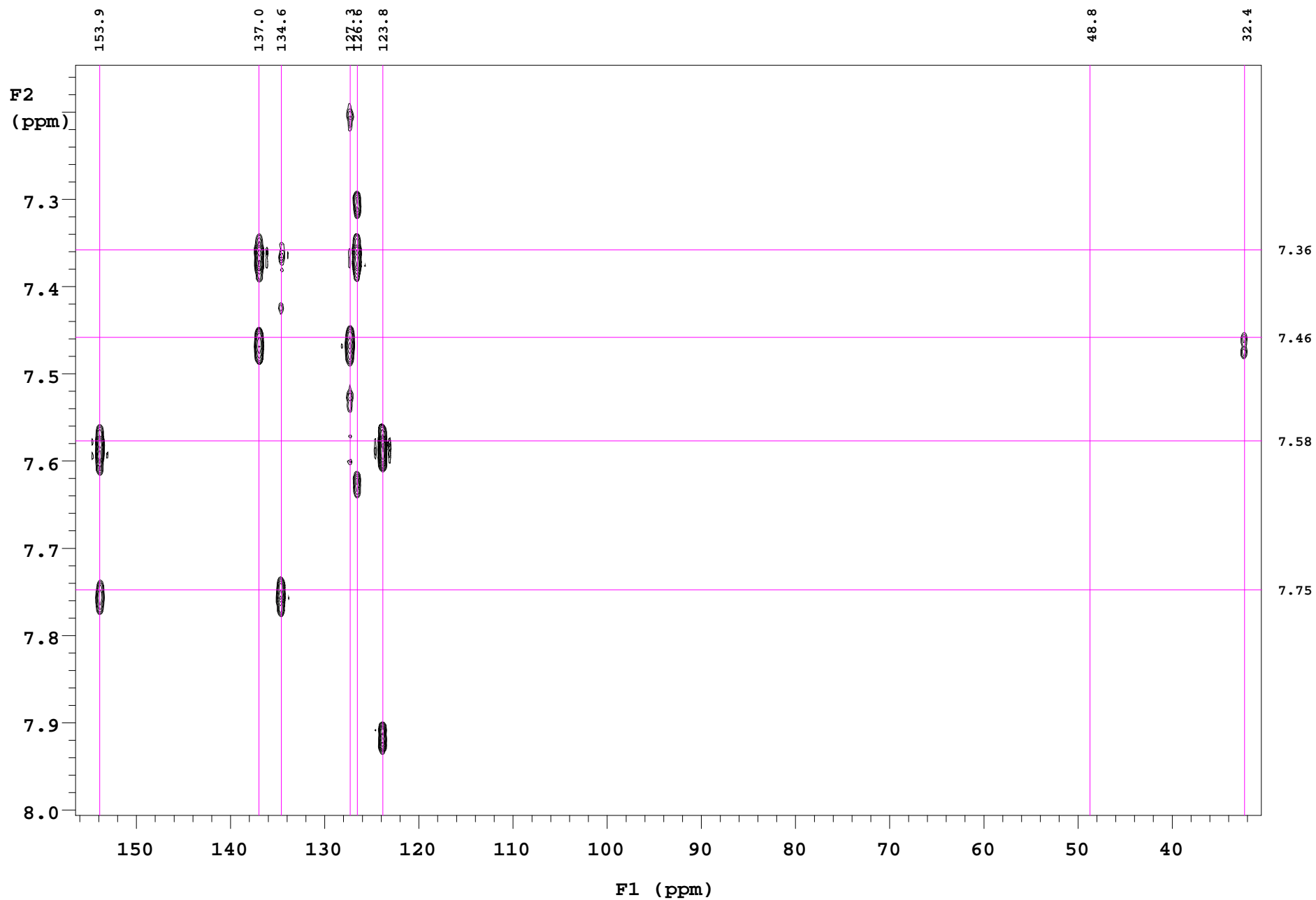
$^1\text{H}$ - $^{13}\text{C}$  gHSQC spectrum of 2-ethylindanone, aromatic region.



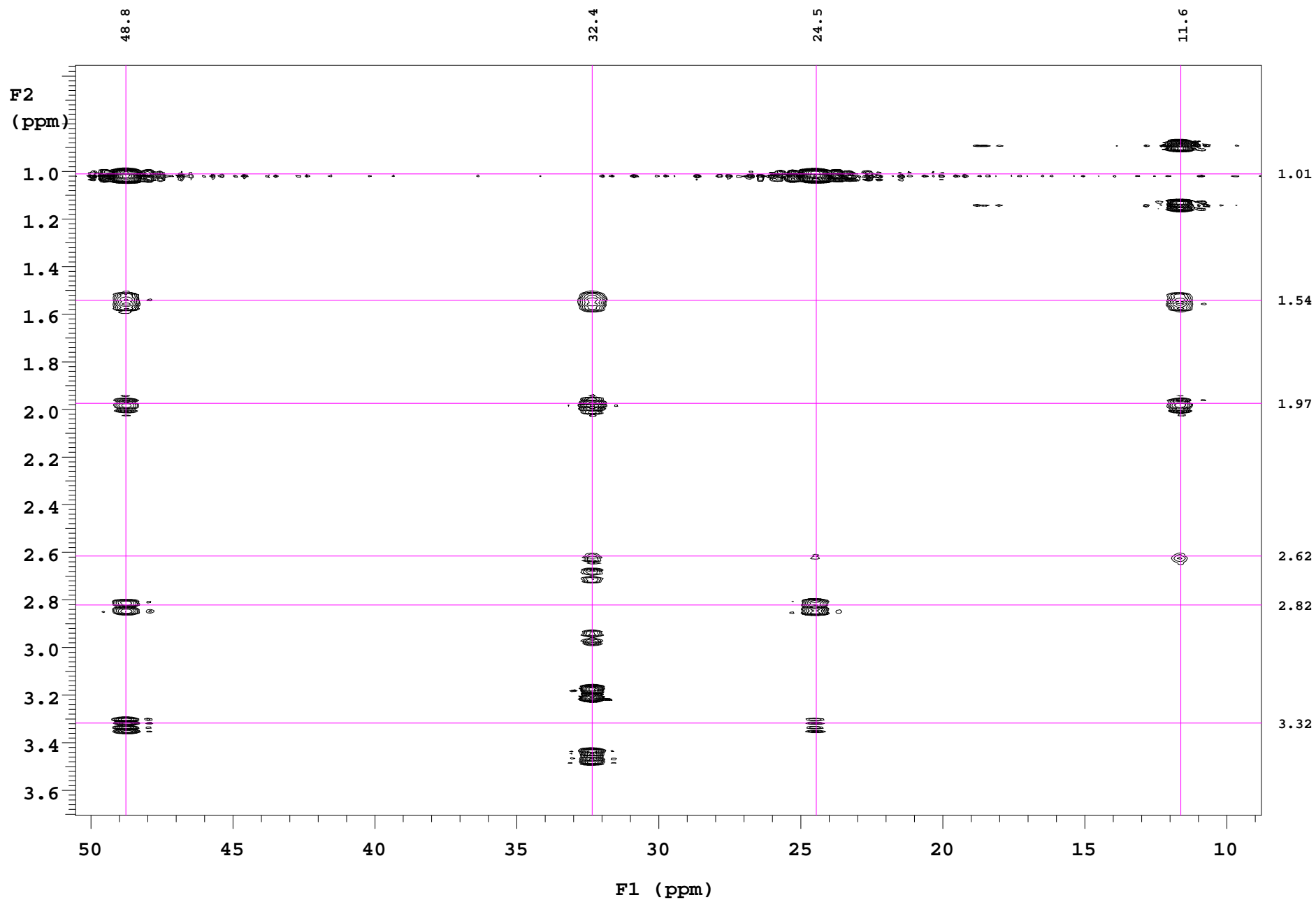
$^1\text{H}$ - $^{13}\text{C}$  gHMBC spectrum of 2-ethylindanone.



$^1\text{H}$ - $^{13}\text{C}$  gHMBC spectrum of 2-ethylindanone, expansion. Not all the carbons and protons separated by 2 or 3 bonds show cross-peaks, since the coupling constant may be close to 0.



<sup>1</sup>H-<sup>13</sup>C gHMBC spectrum of 2-ethylindanone, expansion. The cross-peaks of 7.46 with 32.4 allows for the assignment of this proton. The cross-peaks of 7.46 with 127.3 and 137.0 allows for the assignment of these carbons.



$^1\text{H}$ - $^{13}\text{C}$  gHMBC spectrum of 2-ethylindanone. The experiment is optimized for the long-range coupling constant of 8 Hz. The one-bond cross-peaks may show (3.32, 2.82, 1.01) or may not (2.62, 1.97, 1.54), but are easily recognized by the much larger coupling constant.