Assignment Strategies Using Modern NMR Methods: Quinine in benzene-d6

General methods for performing assignments, and verifying structures using NMR have grown to include now routine two-dimensional NMR experiments.

The following slides show a complete 1H and 13C assignment for quinine; identical methods were used to assign quinidine, which differs only in its stereochemistry from quinine.

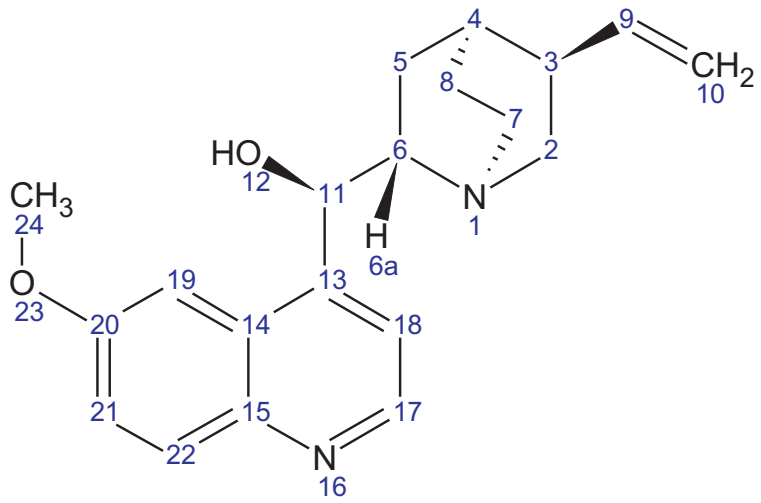
The assignment of quinine and quinidine was once considered a difficult assignment problem; it is reduced by 2D techniques and large magnets to at most a moderately difficult problem.

1H 1d, gDQCOSY, HSQC and gHMBC data are presented. These are four of the most important modern NMR techniques used for chemical assignments, and lack only in NOE data for comprising the major techniques used by chemists for structure assignments.

20 mg of quinine in 0.5 ml benzene was used on our INOVA-600. The sample amount could be reduced to ~1 mg in 80 μl solvent in a 3mm Shigemi tube without increasing experiment times unduly on our 600.

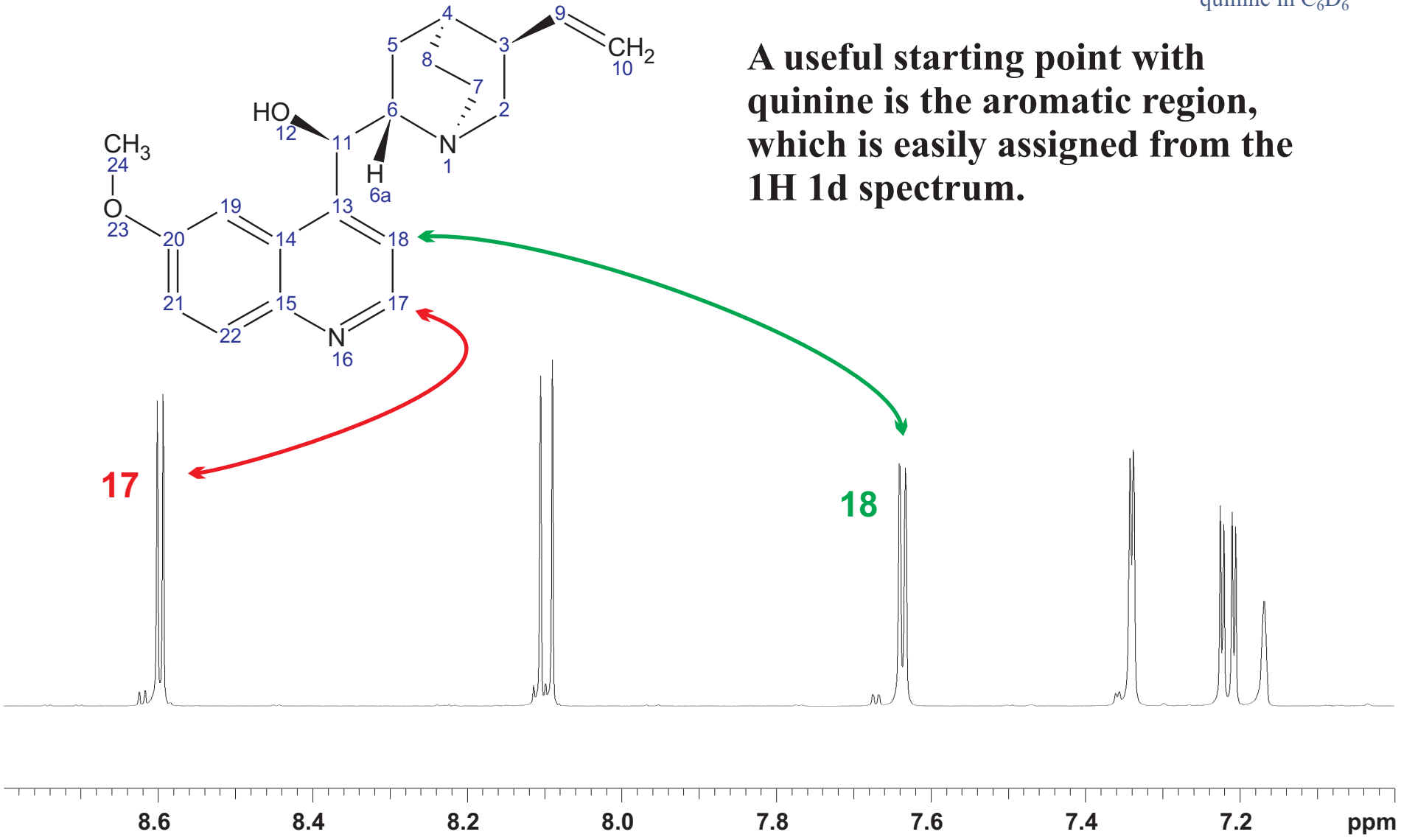
1H 1d spectrum
INOVA-600
quinine in C_6D_6

All assignment strategies should start with an examination of the 1H 1d spectrum.

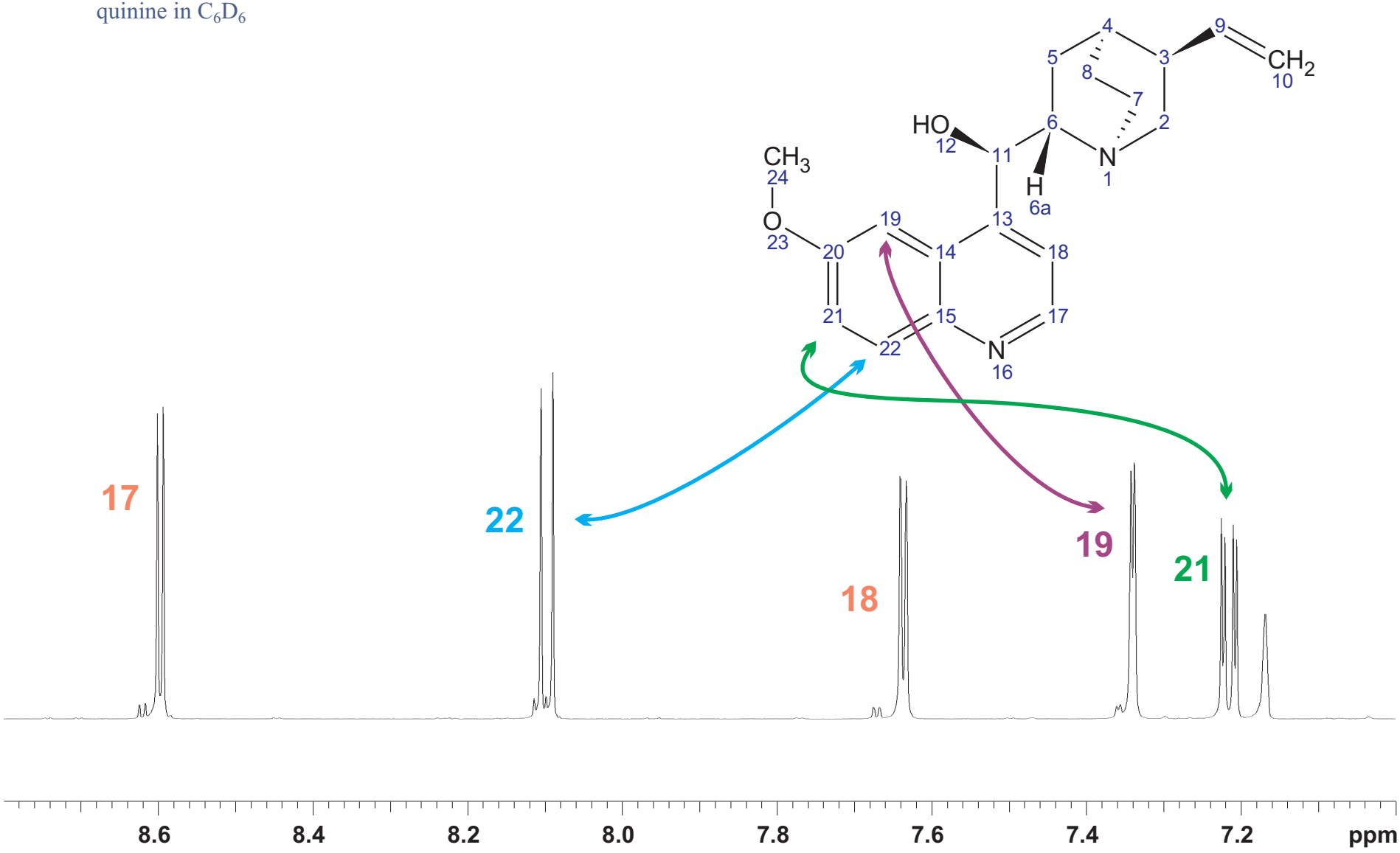


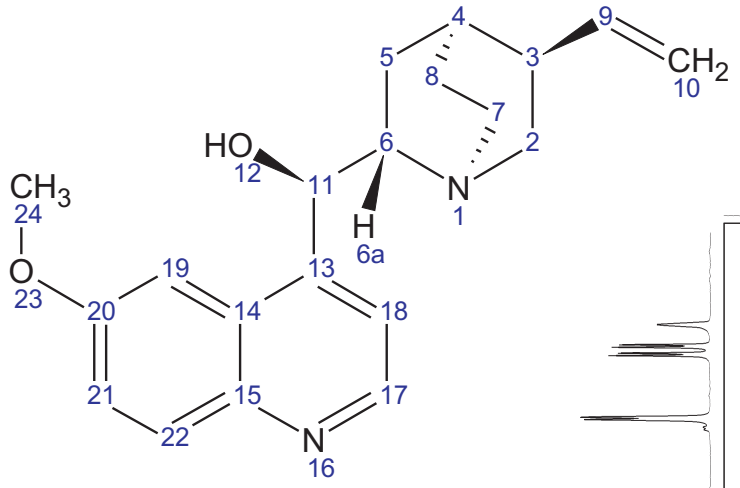
1H 1d spectrum
INOVA-600
quinine in C_6D_6

A useful starting point with quinine is the aromatic region, which is easily assigned from the 1H 1d spectrum.

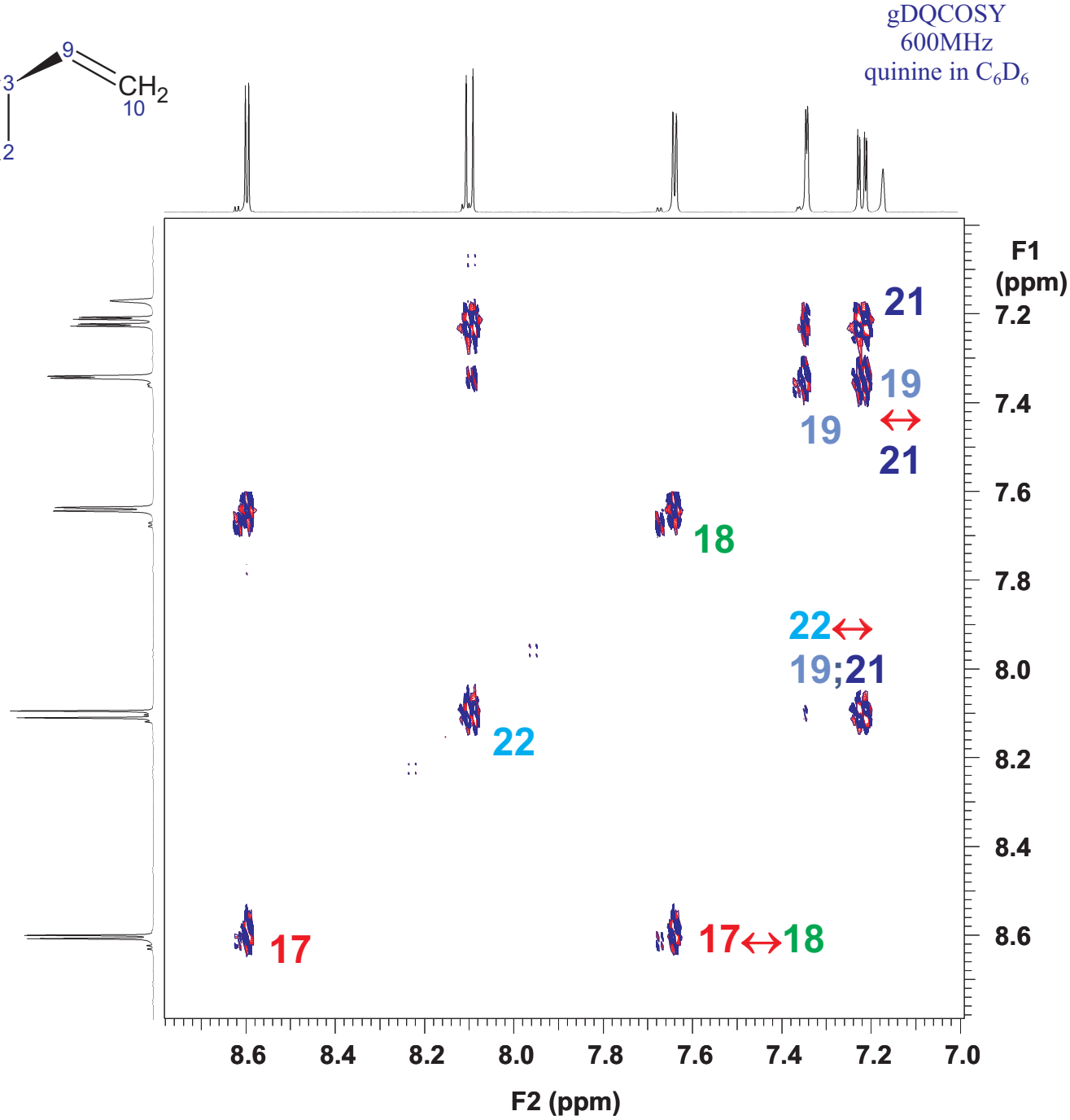


1H 1d spectrum
INOVA-600
quinine in C_6D_6





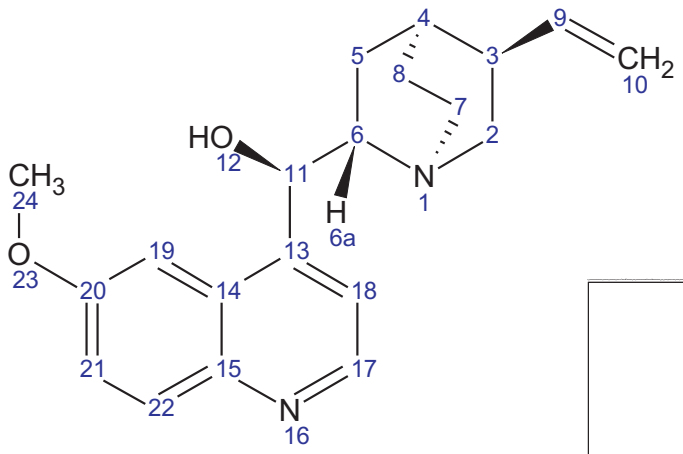
For this portion of the assignments, cosy data (here in the form of a gDQCOSY) do not assist, except to verify what is easily observed in the 1H 1d spectrum.



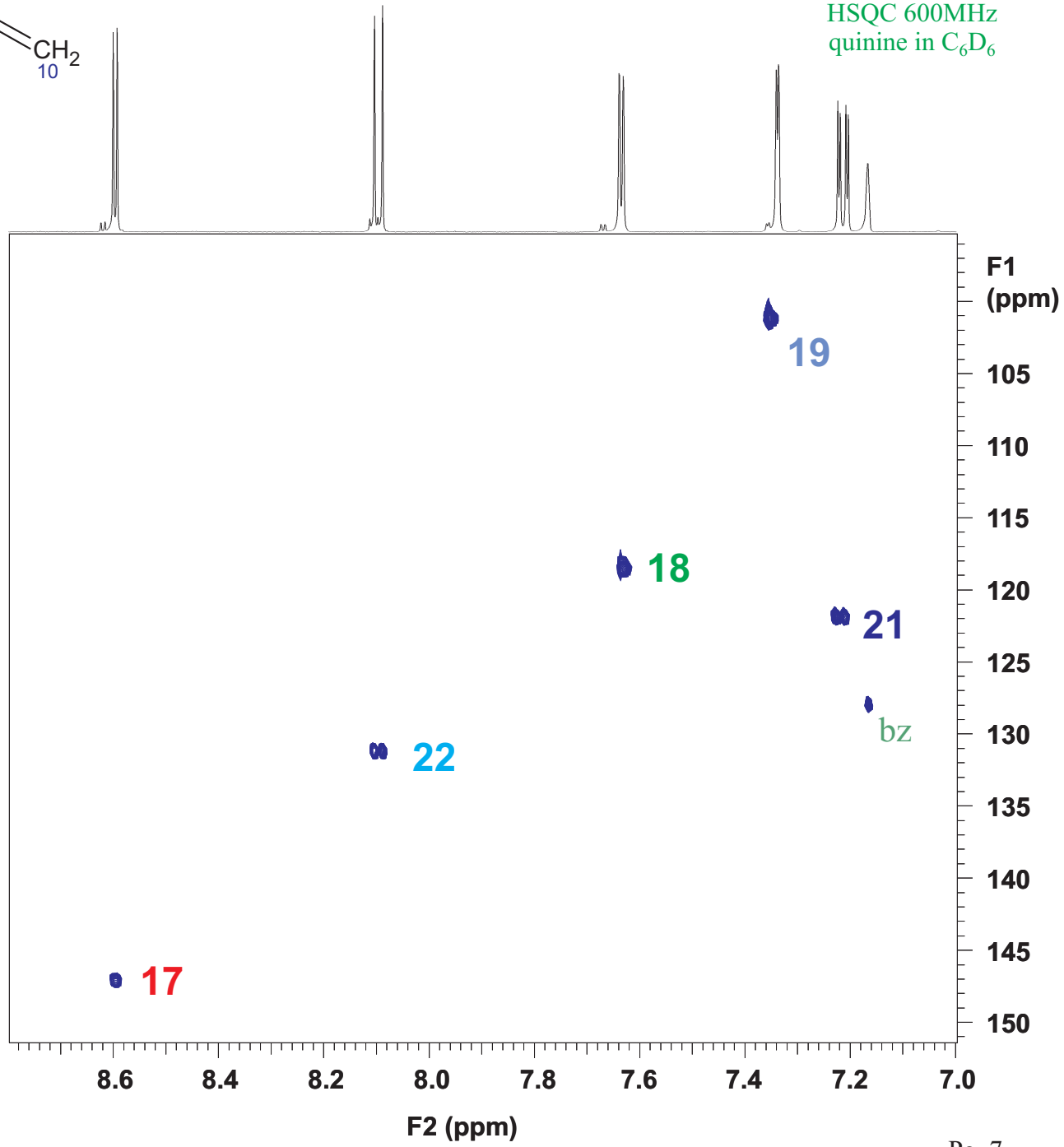
When working with moderately to highly complex data sets, it is imperative to tabularize the data as you work.

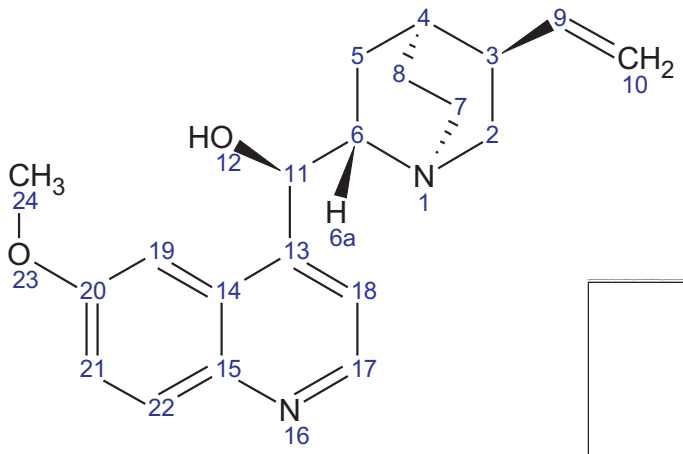
The data must show consistency across all data sets. So far, only the simple consistency between the aromatic assignments of the 1H 1d set and the gDQCOSY data is shown.

Proton	δ (ppm)	# intg	coupling type	J (Hz)		dqcosy connections δ (ppm)	dqcosy connections assign
17	8.60	1	d	4.53		7.64	18(17)
22	8.10	1	d	9.14		7.22	21(22)
18	7.64	1	d	4.55		8.60	17(18)
19	7.34	1	d	2.73		7.22	21(22)
21	7.22	1	dd	9.17		8.10	22(21)
				2.67		7.34	19
			dd(d?)	<1 (.3)			



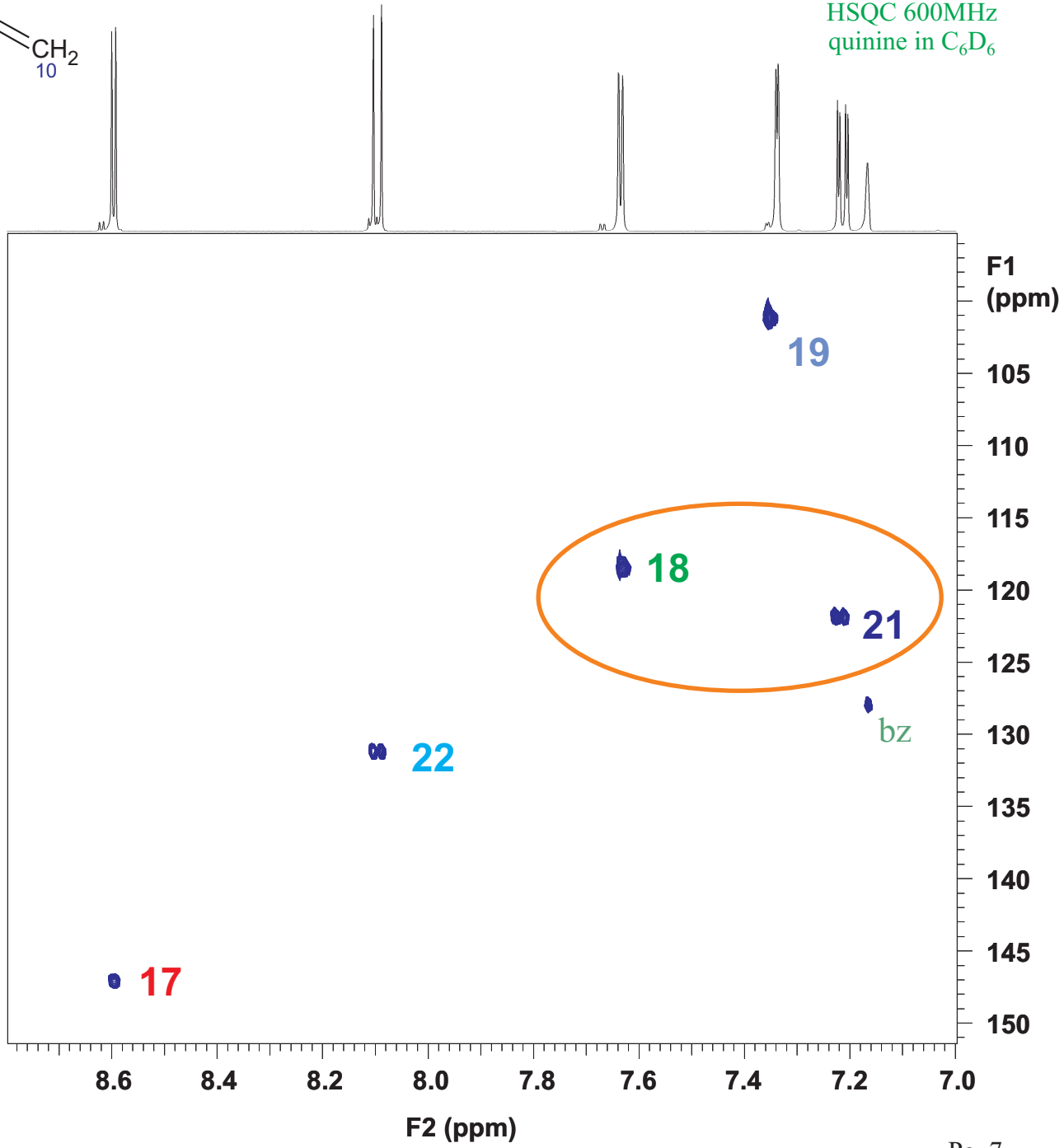
Now 1H-13C HSQC data is used to obtain carbon chemical shifts and assignments. This data has no value at the moment, but will prove useful later.





Now 1H-13C HSQC data is used to obtain carbon chemical shifts and assignments. This data has no value at the moment, but will prove useful later.

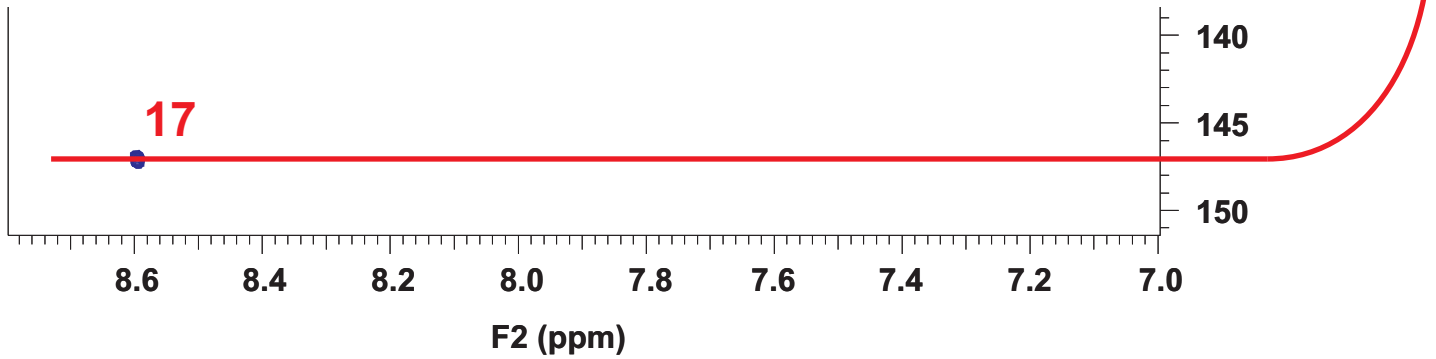
Note how simple it is to assign the carbons using the 1H correlations.



Carbon assignments and chemical shifts can now be added to the table from the HSQC.

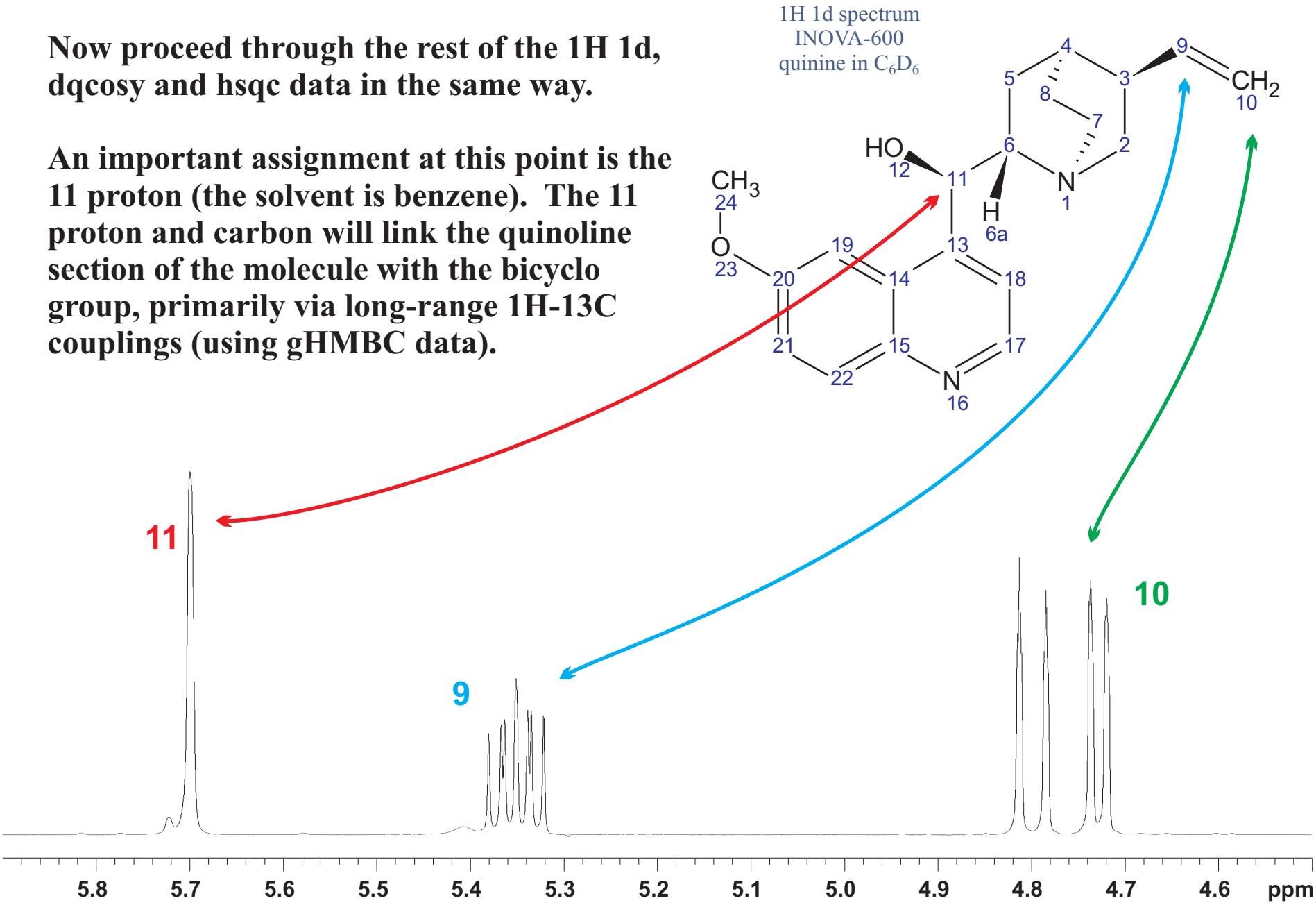
Aside: We need $(\gamma_H/\gamma_C)^{3/2}$ =8 times less concentration for an HSQC than for a direct 13C 1d experiment (or can perform the experiment at least 64× faster at the same concentration!). And we obtain *more* information with the HSQC because of the 1H-13C correlations.

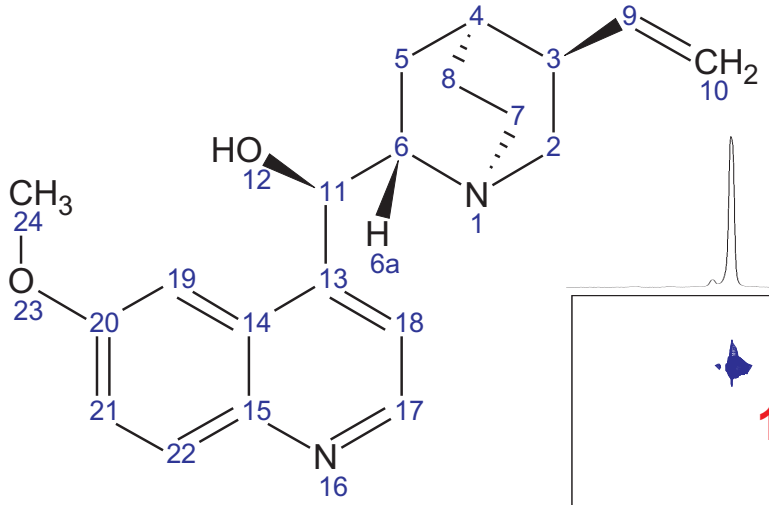
Proton	δ (ppm)	# intg	coupling type	J (Hz)	dqcosy connections δ (ppm)	dqcosy connections assign	gHSQC δ_C(ppm)
17	8.60	1	d	4.53	7.64	18(17)	147.2
22	8.10	1	d	9.14	7.22	21(22)	132.2
18	7.64	1	d	4.55	8.60	17(18)	119.4
19	7.34	1	d	2.73	7.22	21(22)	102.1
21	7.22	1	dd	9.17	8.10	22(21)	122.7
				2.67	7.34	19	
			dd(d?)	<1 (.3)			



Now proceed through the rest of the 1H 1d, dqcosy and hsqc data in the same way.

An important assignment at this point is the 11 proton (the solvent is benzene). The 11 proton and carbon will link the quinoline section of the molecule with the bicyclo group, primarily via long-range 1H-13C couplings (using gHMBC data).

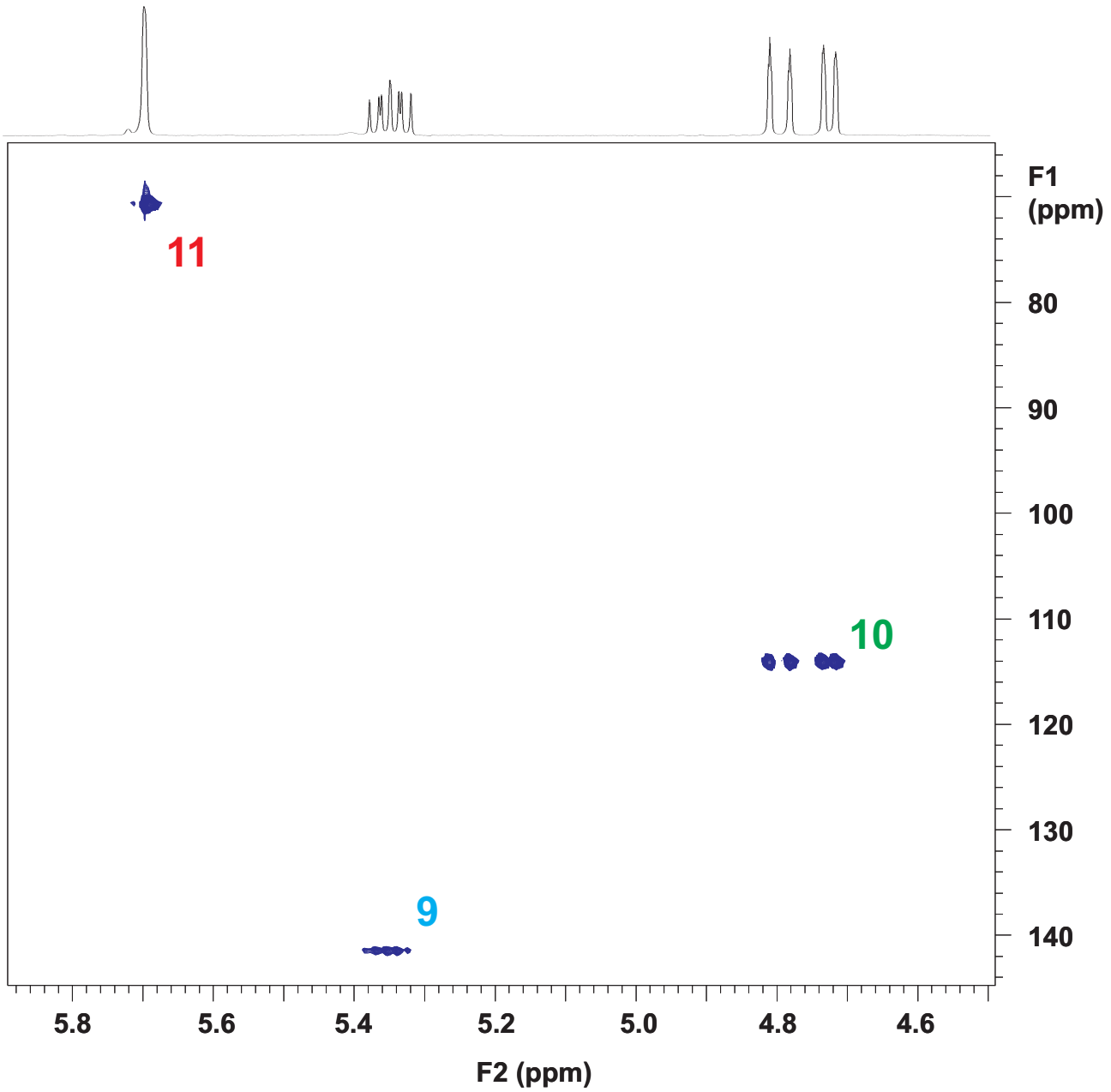


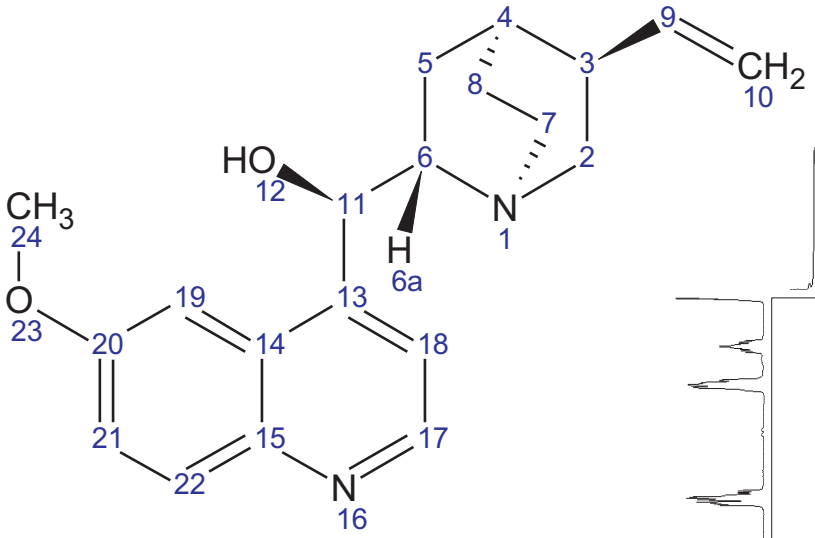


HSQC 600MHz
quinine in C_6D_6

Again, the HSQC provides simple 13C assignments from the 1H correlations.

In this case, it also provides a simple confirmation (via 13C chemical shift) of the 11 proton assignment.



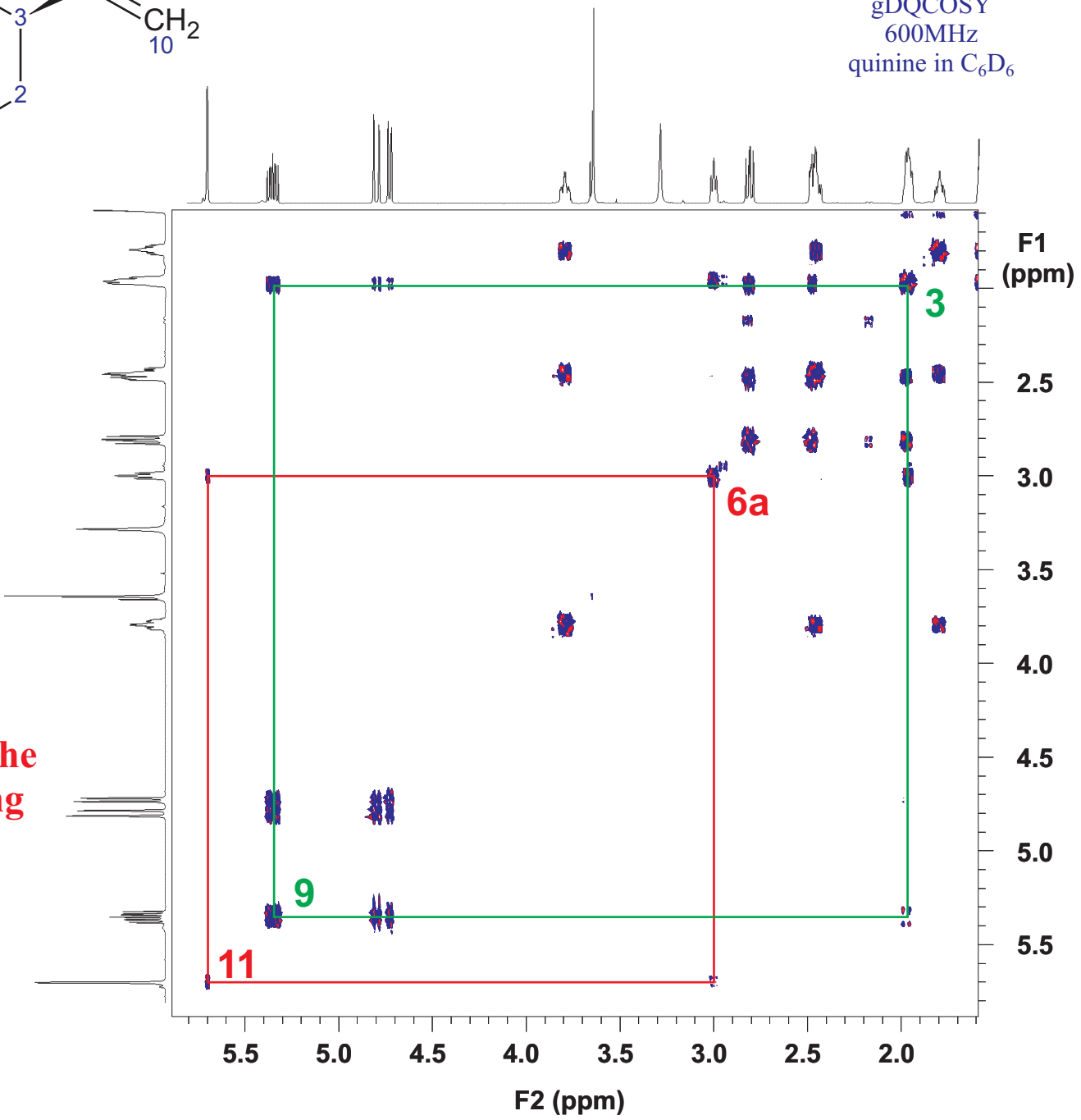


gDQCOSY
600MHz
quinine in C_6D_6

The dqcosy provides easy entries into the aliphatic assignments.

The 11 proton correlates with the 6a proton at 3.00 ppm. Note the small coupling—11 appears in the 1D as a singlet—suggesting the torsion angle is ~90°.

The 9 proton correlates with the 3 proton at 1.97 ppm.

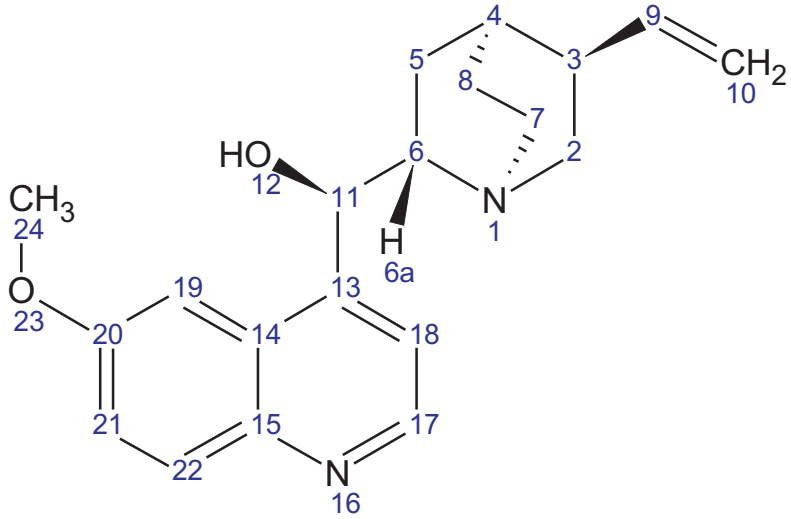


It is vital to continue to tabulate the data. Question marks can be inserted to show uncertain assignments. It is important to indicate weak correlations in the dqcosy and hsqc in the table.

Proton	δ (ppm)	# intg	coupling type	J (Hz)	dqcosy connections δ (ppm)	dqcosy connections assign	gHSQC δ_C(ppm)
17	8.60	1	d	4.53	7.64	18(17)	147.2
22	8.10	1	d	9.14	7.22	21(22)	132.2
18	7.64	1	d	4.55	8.60	17(18)	119.4
19	7.34	1	d	2.73	7.22	21(22)	102.1
21	7.22	1	dd	9.17	8.10	22(21)	122.7
				2.67	7.34	19	
			dd(d?)	<1 (.3)			
11?	5.70	1	s		3.0(wk)	6a?	71.8
			s(d?)	~1			
9a	5.35	1	ddd	16.99	4.80	10b	142.4
				10.26	4.73	10a	
				7.89	1.97	3?	
10b	4.80	1	dt	17.07	5.35	9a	115
				1.28	4.73	10a	
				1.28	1.97	3?	
10a	4.73	1	dt	10.30	5.35	9a	115
				0.80	4.80	10b	
				0.80	1.97	3?	

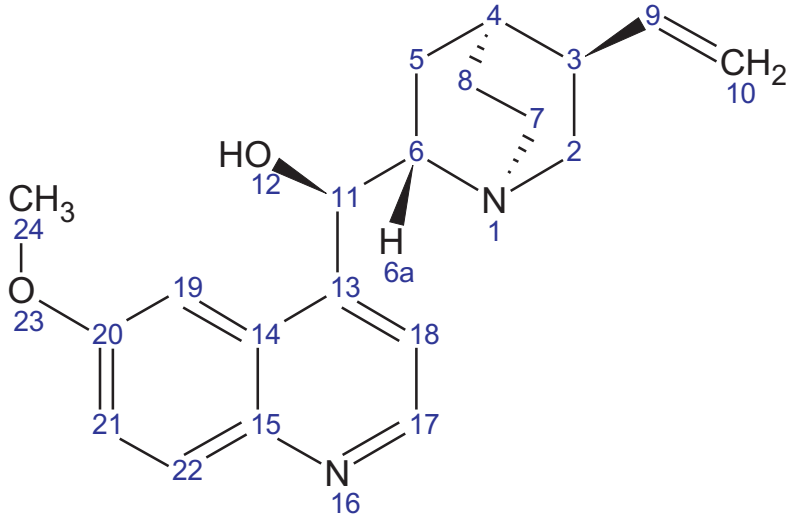
Standard practices such as counting protons via integrals must not be ignored in the "glitz" of using 2D techniques.

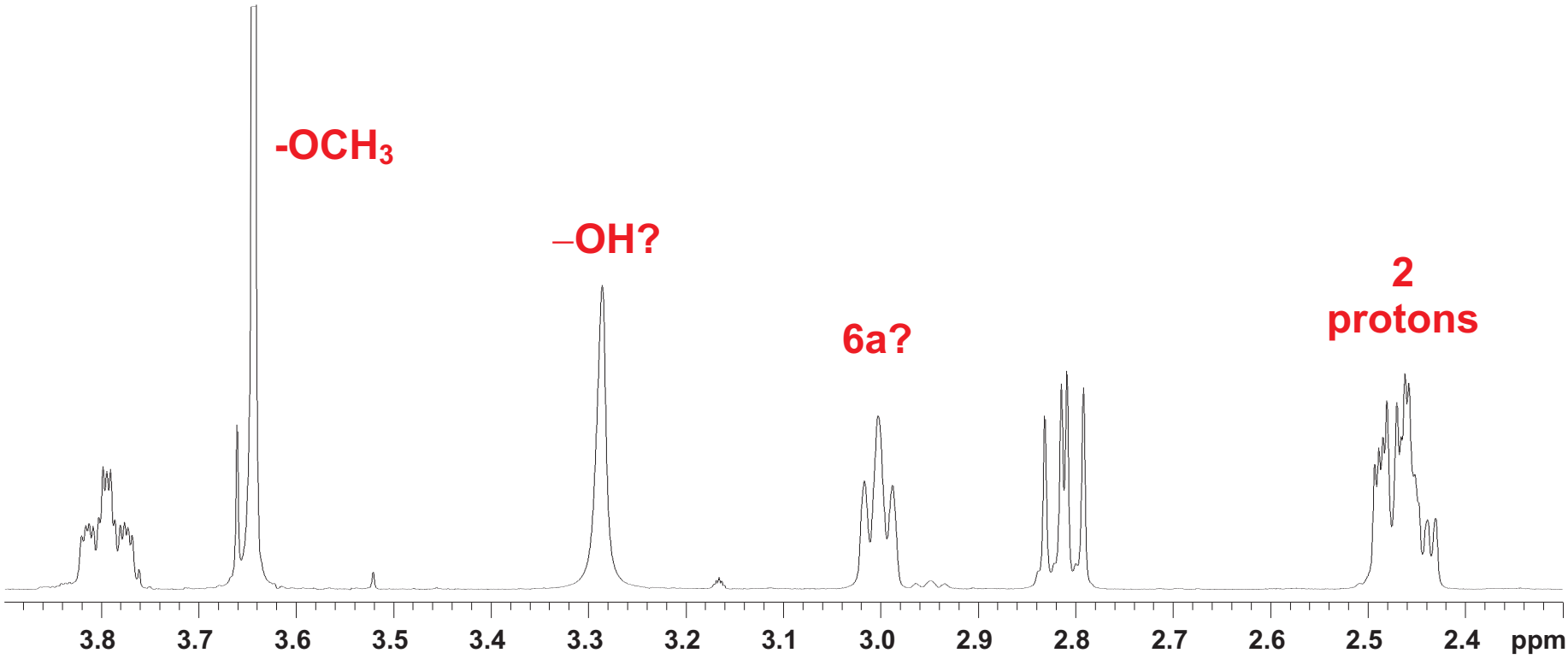
Nine protons have already been assigned. All protons should be observed, with the possible exception of the –OH group. 15 more are integrated, exactly the number expected *including* the –OH proton. This proton is therefore present (likely at 3.29ppm).



Closer inspection of the aliphatic region of the 1H 1d spectrum shows that most of the protons are resolved at 600MHz, but regions such as 2.4 to 2.5 ppm may be troublesome.

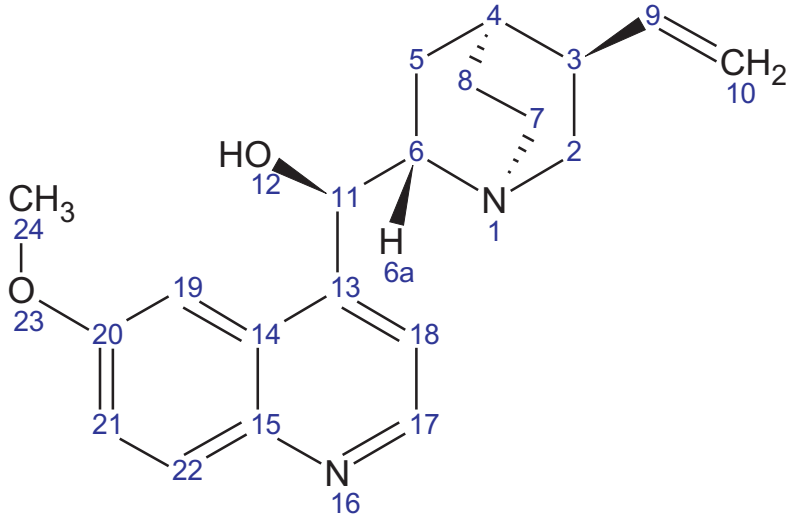
The multiplet at 3.0 ppm has already been tentatively assigned to the 6a proton via the coupling to 11 from the dqcosy spectrum.

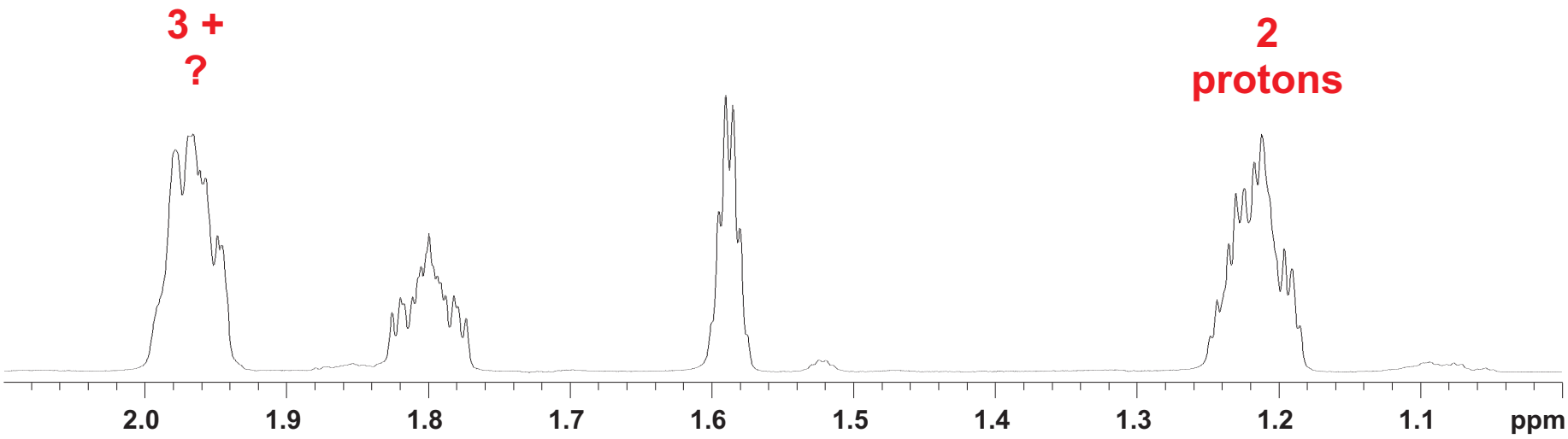


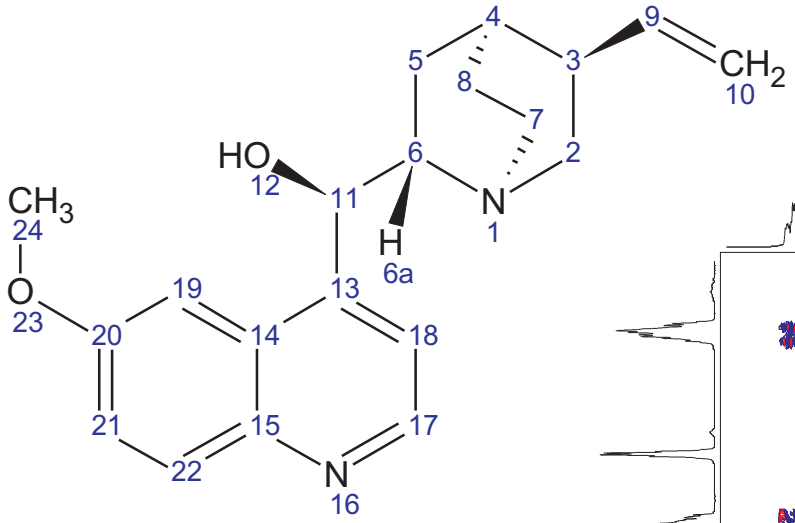


One of the protons at 1.97 ppm (it has an integral of 2) is already tentatively assigned to the 3 proton, from coupling to the 9 proton in the dqcosy spectrum. The 1.25 to 1.18 ppm region also has two overlapping protons.

dqcosy should provide most of the assignments, if we keep in mind that protons closer to the 1 nitrogen will be pushed to higher frequencies. Thus, the 5, 4 and 8 protons are likely to be in this low frequency (upfield) region.

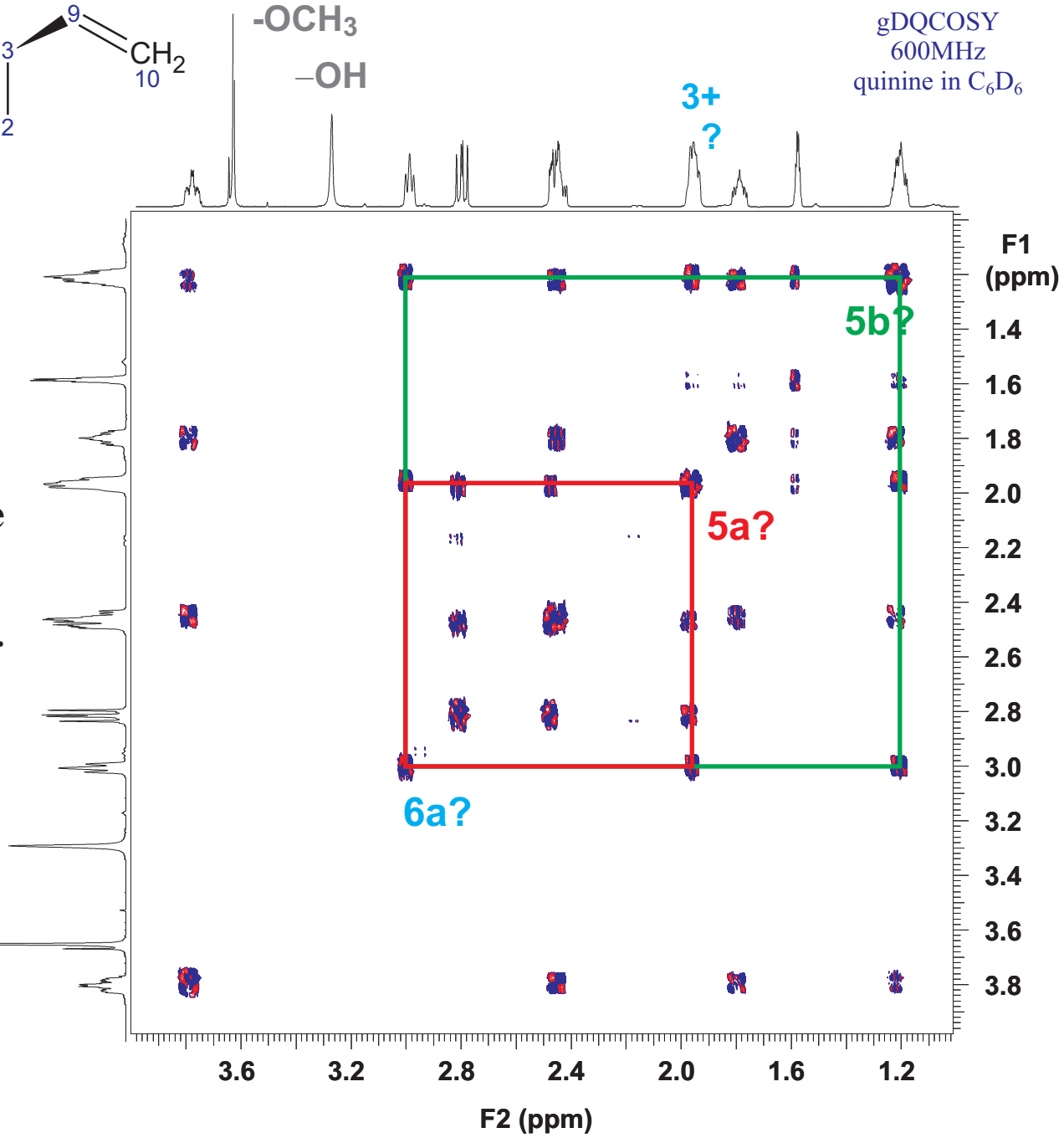






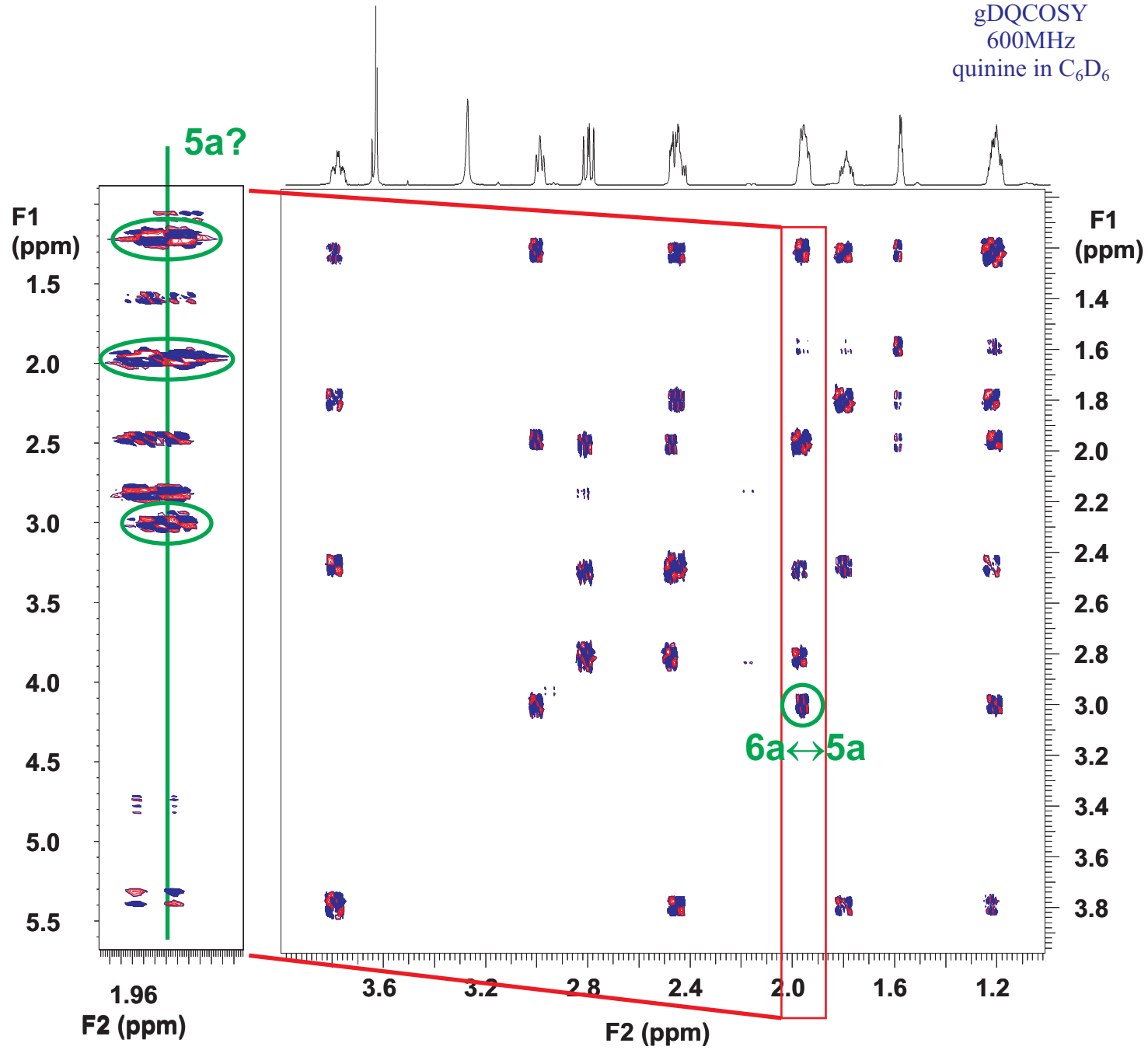
A key question is where to start (entry points). The obvious answer here is the 6a and 3 protons. The 3 proton is messy, as another unknown proton is overlapping, so start with 6a at 3.00ppm.

Other than 11, 6a will strongly couple only to the 5a and 5b protons. One of these is overlapping with the 3 proton.



An expansion of the 3,5a region at 1.96 ppm shows that 5a is on the low-freq side of the multiplet.

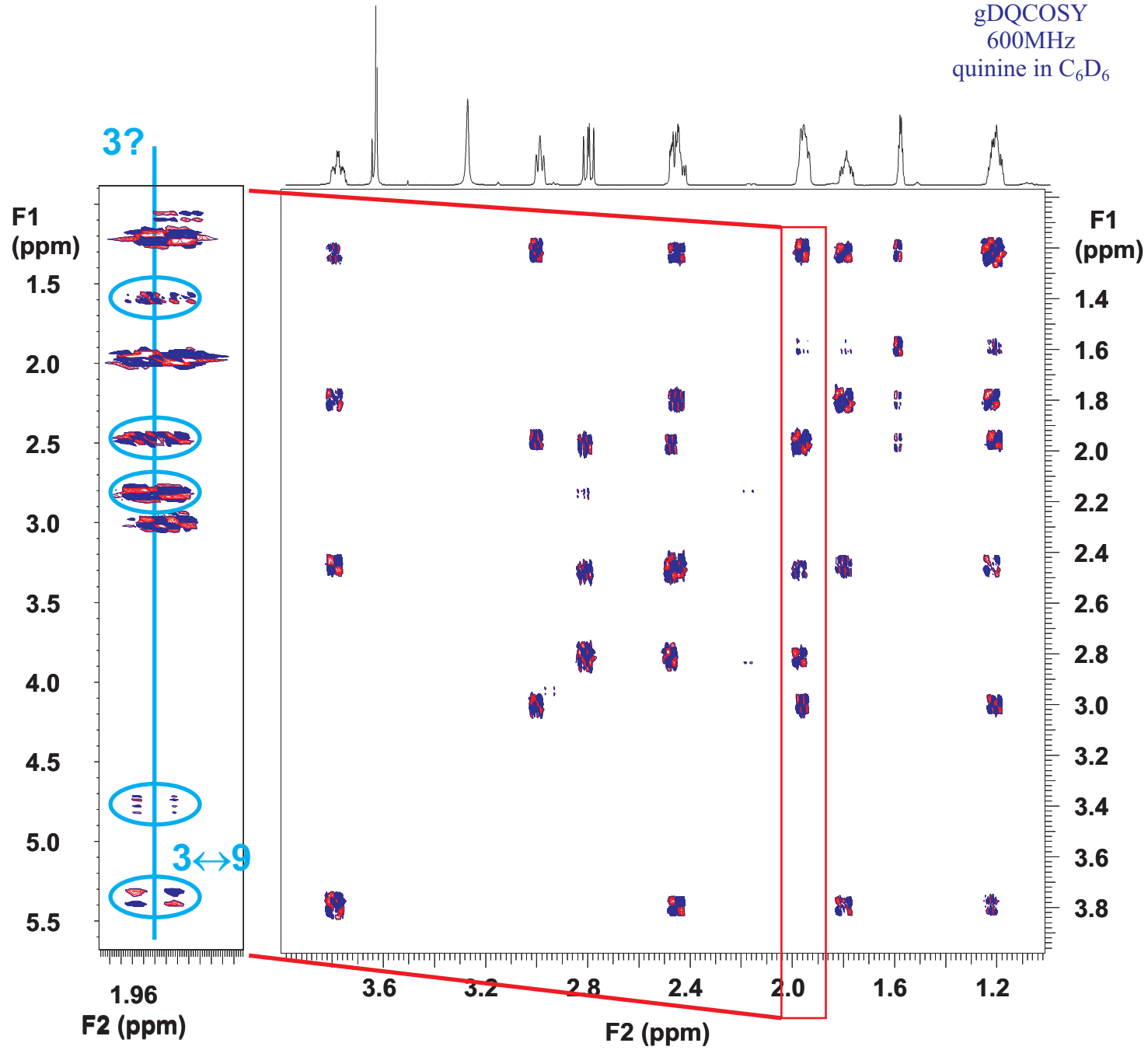
In the table, 3 can be assigned to 1.960 ppm, and 5a to 1.957 ppm (keeping in mind that δ can be improved later).



A number of protons can be assigned with the 3 dqcosy correlations:

1.59 ppm (wk)
2.47 ppm
2.81 ppm
4.73 ppm (wk)
4.80 ppm (wk)

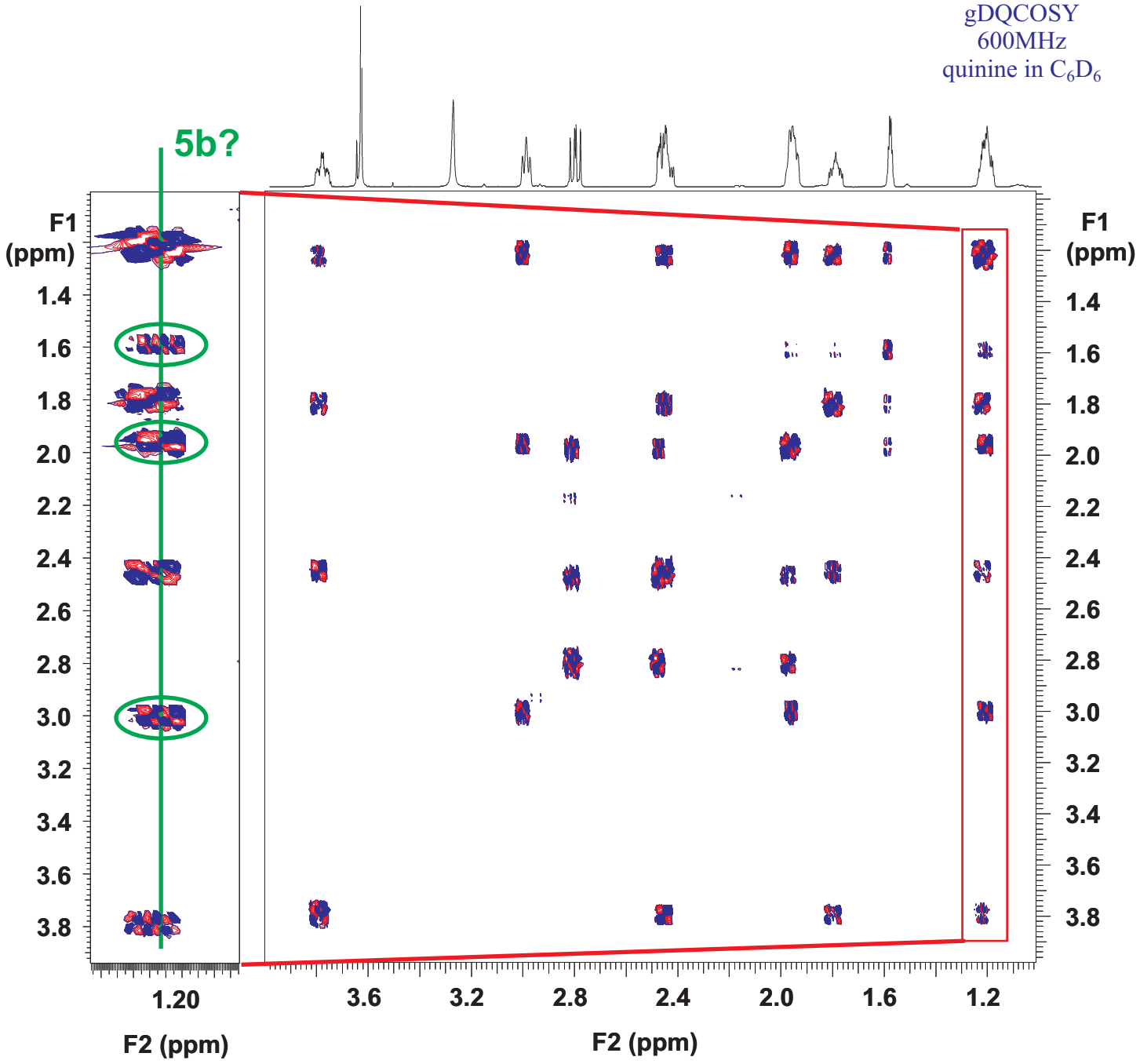
Although some of these may seem suspect, they can be included if verification is made with other data at a later time.



Similarly, an expansion of the 5b,? region shows two sets of correlations that can be tentatively assigned. The low-freq cross-peaks are assigned to 5b:

1.59 ppm
1.96 ppm
3.00 ppm

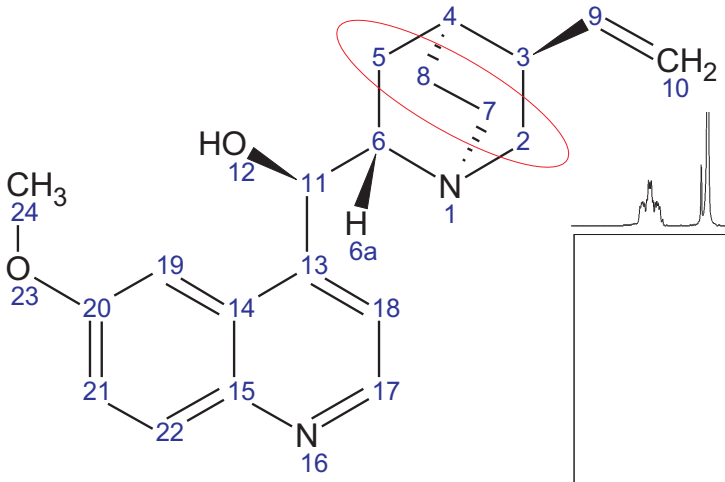
leaving 1.80, 2.44 and 3.79 ppm peaks correlating to the other proton.



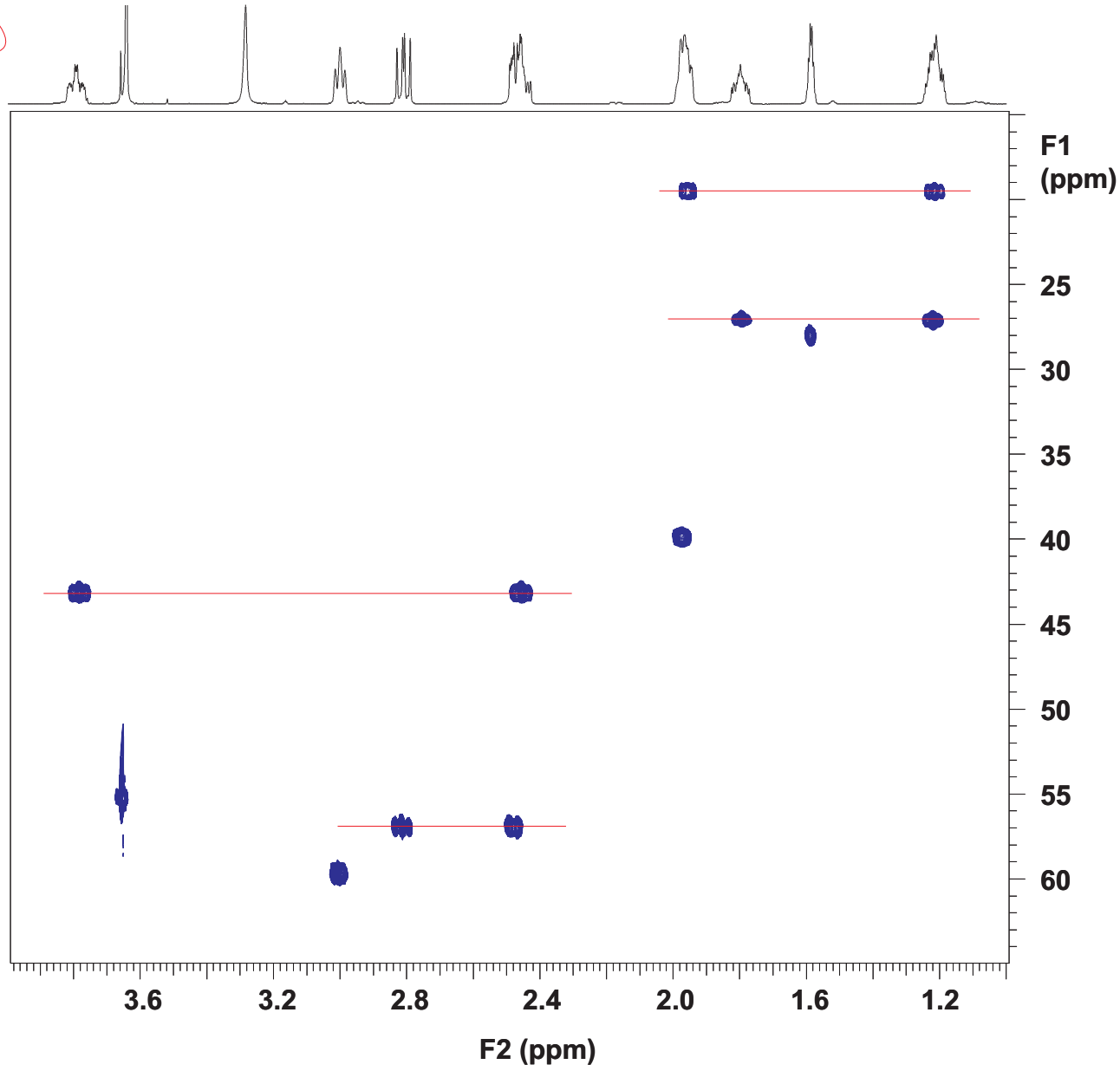
The table now contains a lot of data, including carbon assignments from the HSQC on the next page.

Many of the assignments are tentative at this point; they need more data for confirmation.

Proton	δ (ppm)	# intg	coupling type	J (Hz)	dqcosy connections δ (ppm)	dqcosy connections assign	gHSQC δ_C(ppm)
11?	5.70	1	s s(d?)	 ~1	3.0(wk)	6a?	71.8
9a	5.35	1	ddd	16.99 10.26 7.89	4.80 4.73 1.97	10b 10a 3?	142.4
10b	4.80	1	dt	17.07 1.28 1.28	5.35 4.73 1.97	9a 10a 3?	115
10a	4.73	1	dt	10.30 0.80 0.80	5.35 4.80 1.97	9a 10b 3?	115
24	3.64	3	s s(d?)	 0.48	none		56.1
-OH?	3.28	1	s		none		
6a?	3.00	1	t	8.78	5.7(wk) 1.97 1.22	11? 5a? 5b?	60.7
3?	1.97	1	m	2.40	2.476 2.81 5.35 4.80(wk) 4.73(wk) 1.59(wk)	2b? 2a? 9a 10b 10a 4?	40.7
5a?	1.96(hsqc)	1		~7	3.00 1.22	6a? 5b?	20.5
5b?	1.22u	1	m	3.00	1.59 1.96 3.00	4? 5a? 6a	20.5



HSQC 600MHz
quinine in C_6D_6



HSQC provides a simple method of identifying –CH2– groups. Four groups are expected in the aliphatic region:

5, 8, 7, and 2

with only 10 outside this region.

This plot shows an HSQC data set obtained with the parameter:

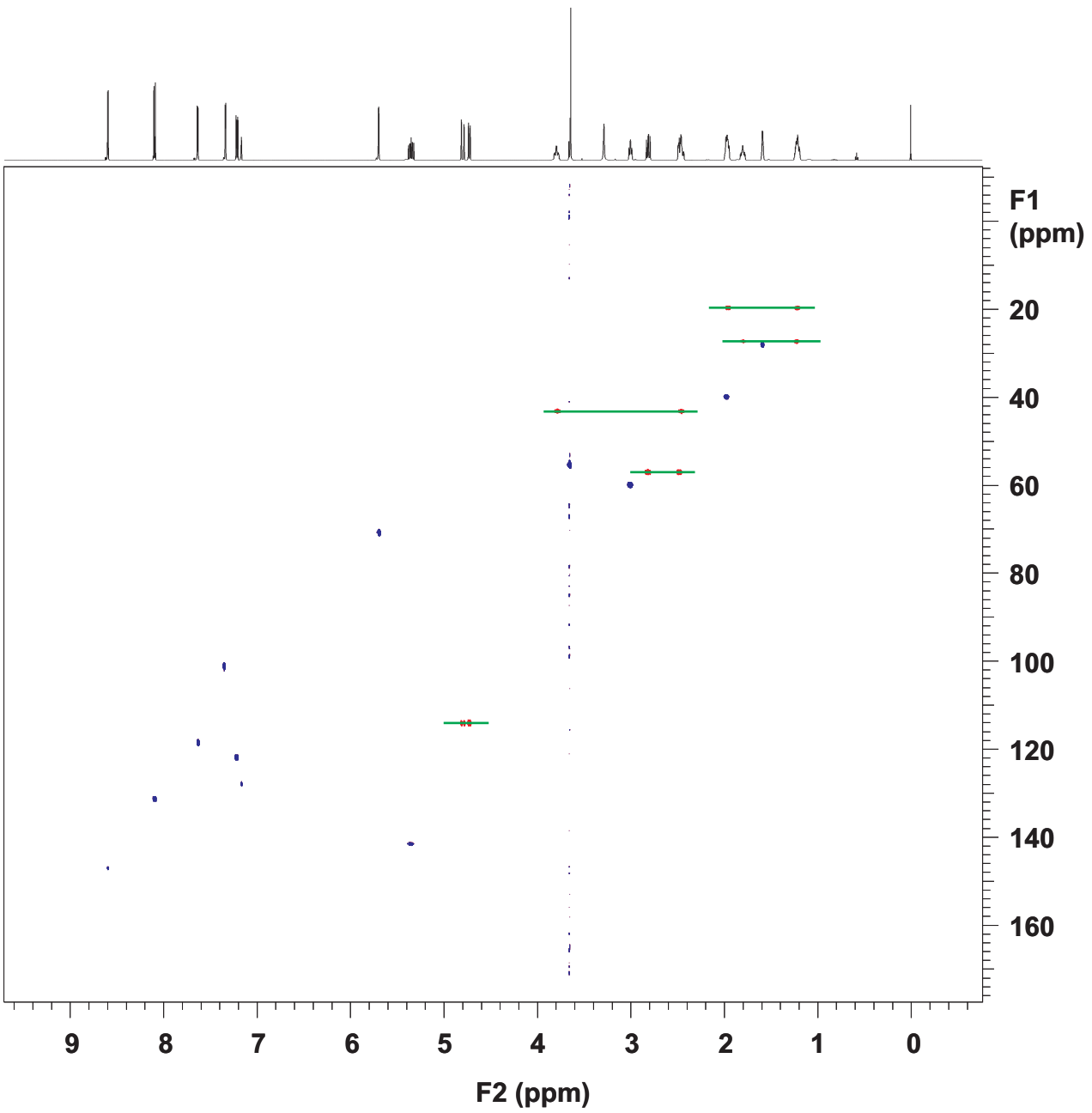
mult=2

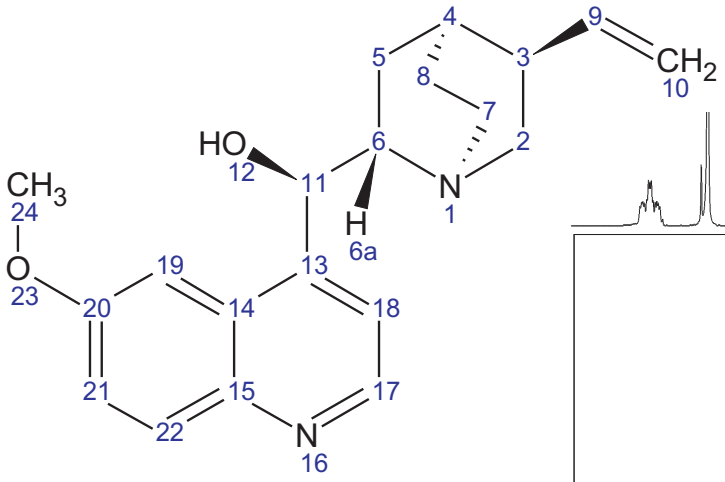
giving a DEPT-135 appearance to the data. The four aliphatic CH2 groups plus the vinyl methylene are observed as inverted in intensity (red) compared to the other correlations (blue).

For quinine, mult=2 data is not needed; it involves loss in sensitivity, and should not be run, unless required and the conc is high.

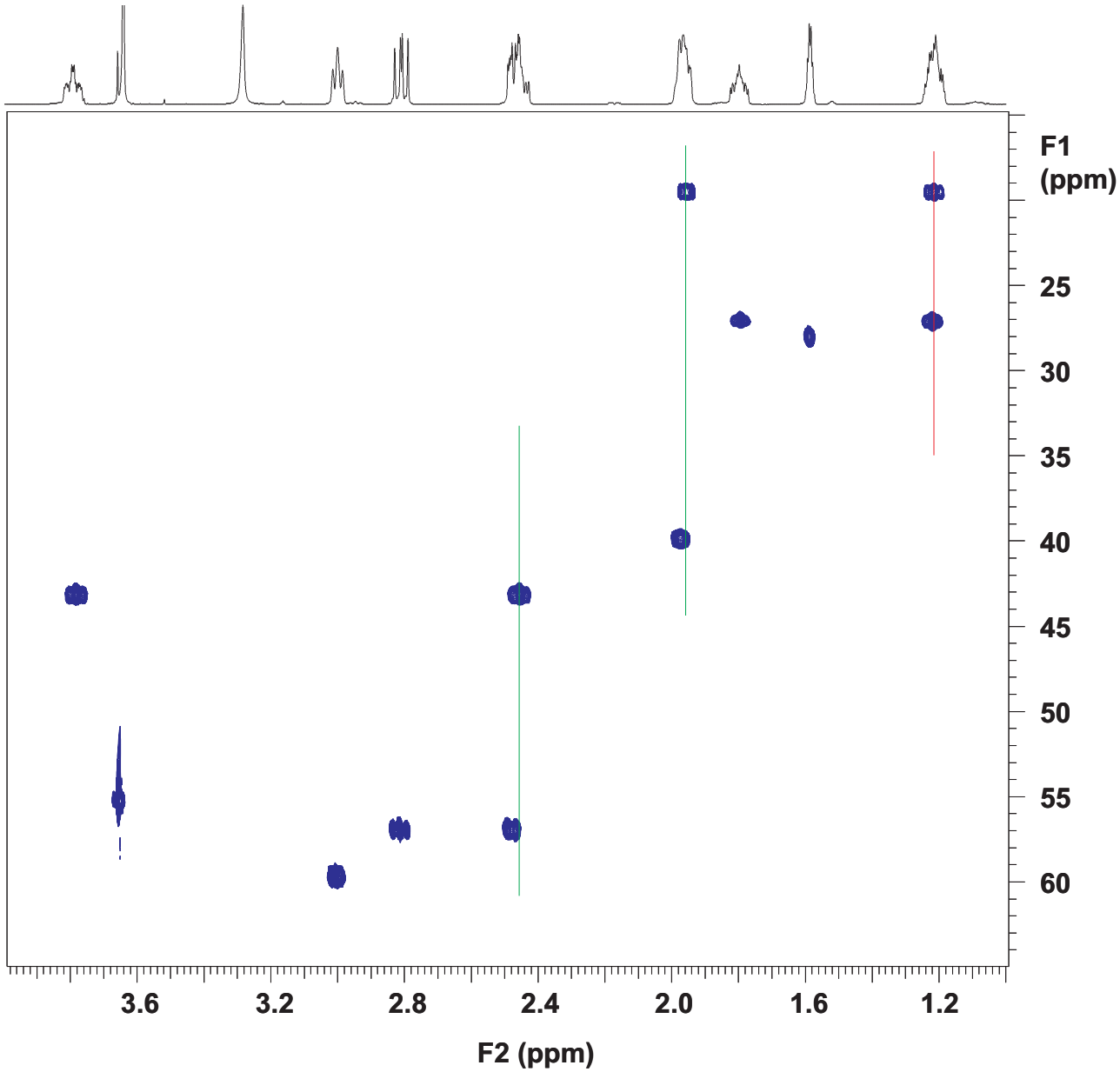
HSQCAD is a new experiment that greatly improves data quality in mult=2 spectra.

HSQC 600MHz
quinine in C_6D_6



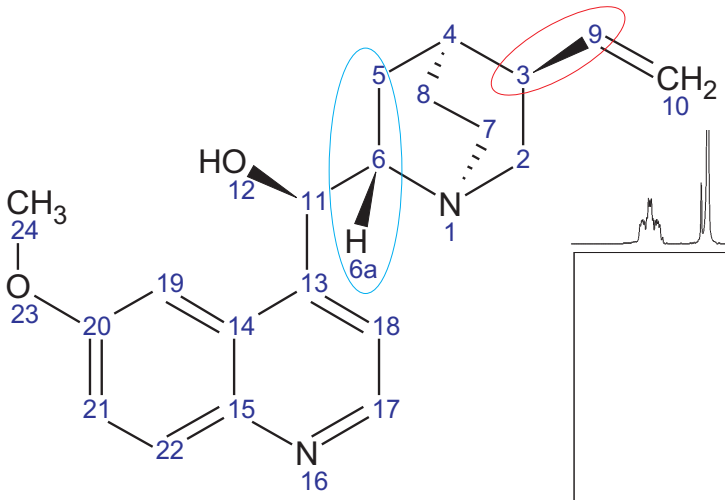


HSQC 600MHz
quinine in C_6D_6

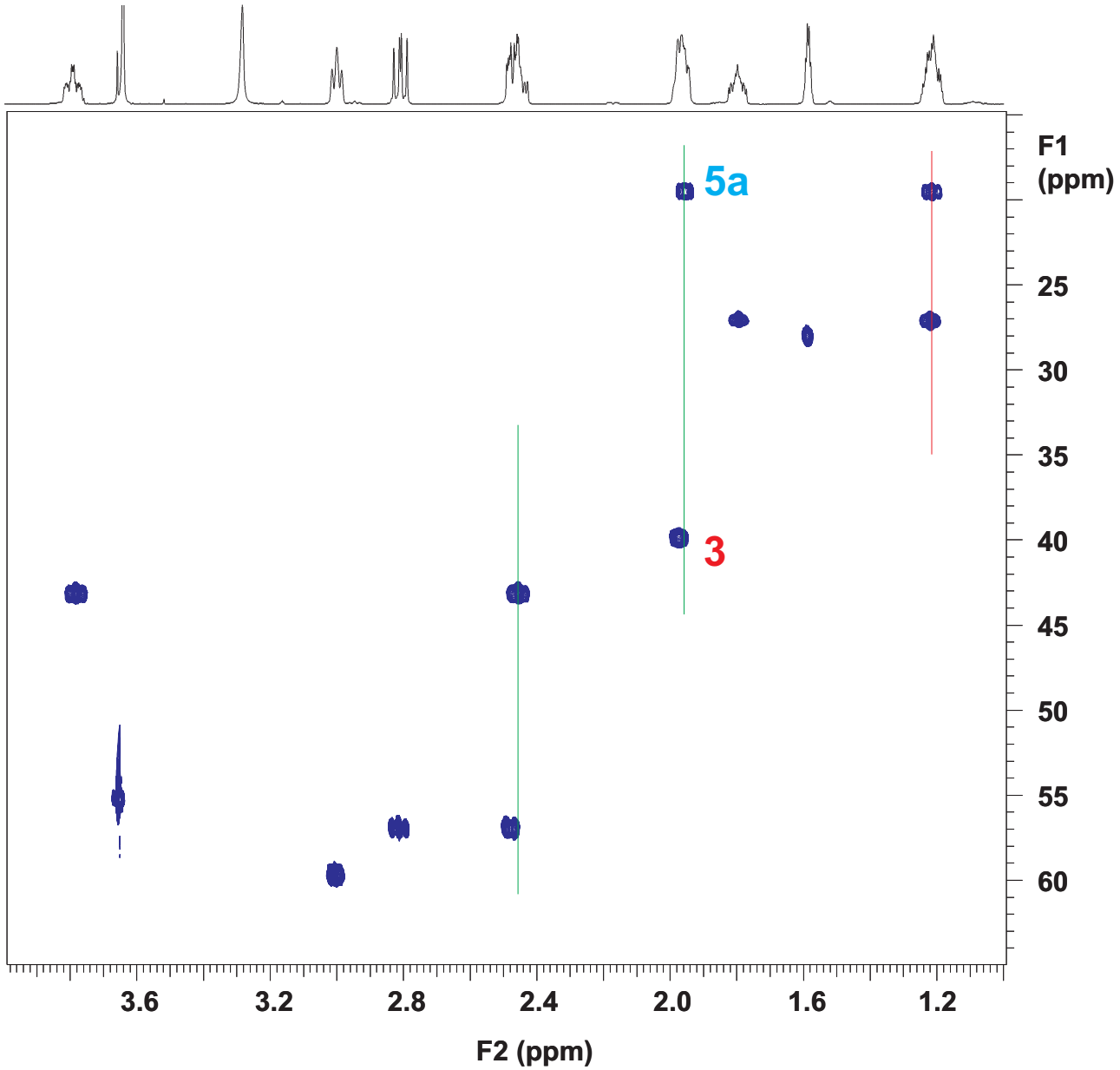


HSQC also displays the resolution in proton chemical shifts observed in the dqcosy spectra, *which is not obtained in the 1H 1d spectra*.

The δ differences are clear at ~2.42ppm and ~1.97ppm, but not so at ~1.22ppm. More points (np) in the direct F2 dimension may have provided sufficient resolution at 1.22ppm.



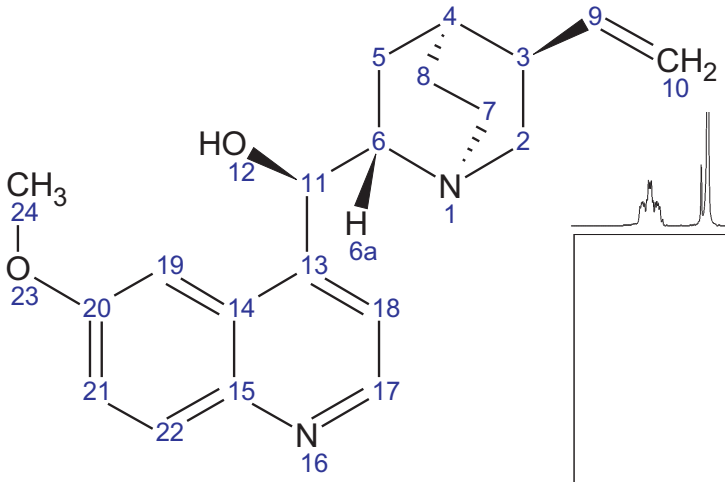
HSQC 600MHz
quinine in C_6D_6



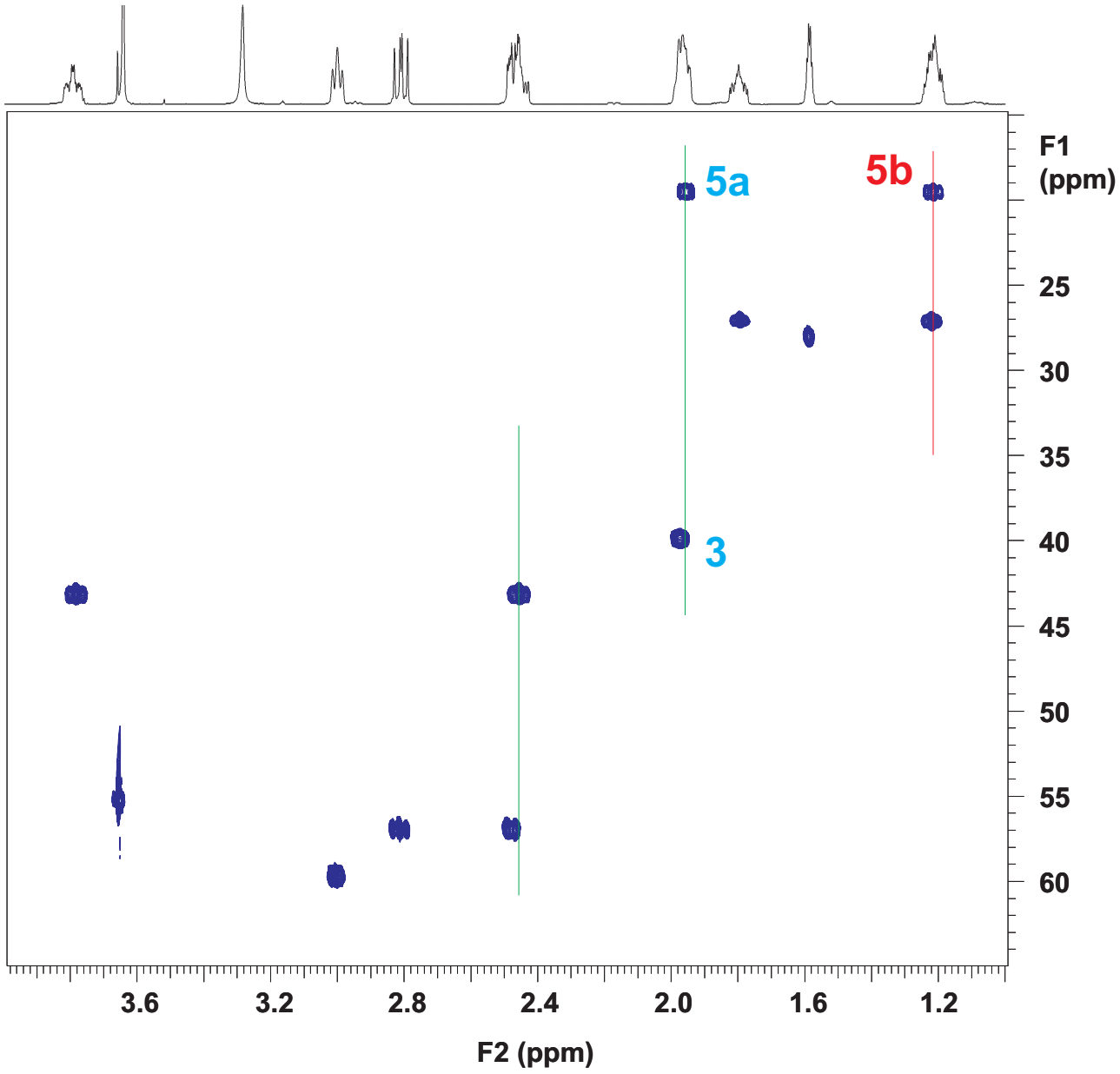
The 3 and 5a assignments are now confirmed via 13C δ.

3 was initially assigned via dqcosy correlation with the 9 proton.

5a was initially assigned via dqcosy correlation with the 6a proton.

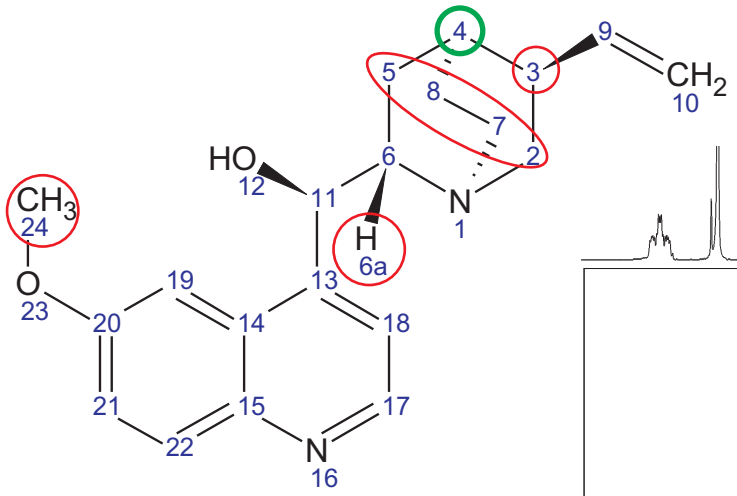


HSQC 600MHz
quinine in C_6D_6

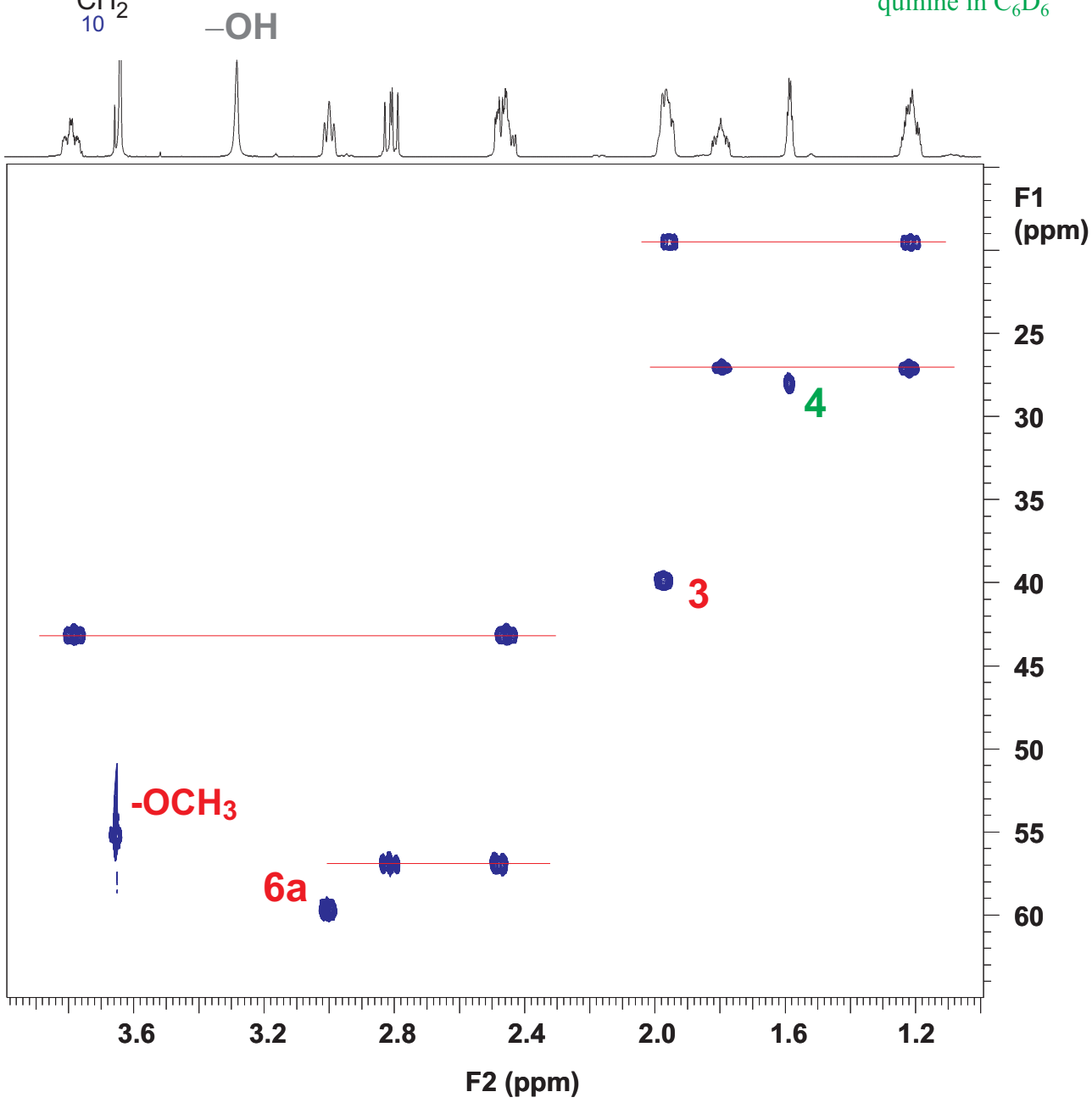


5b is confirmed by the carbon chemical shift (being identical to 5a).

The assignment of 5b being on the low-frequency side of the 1.22ppm multiplet is still tentative.

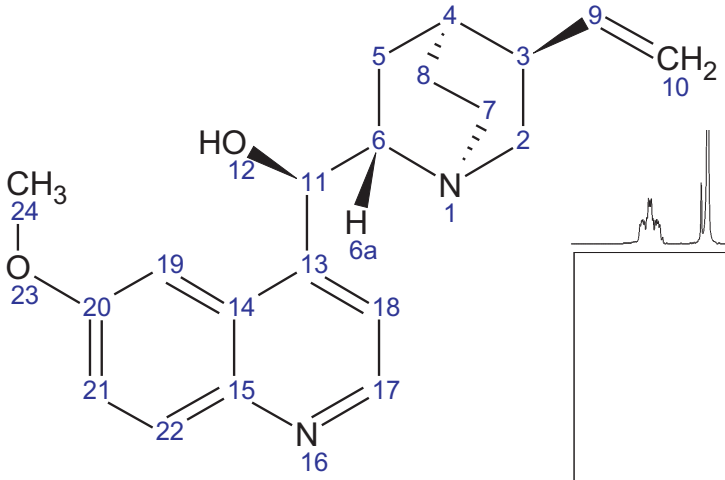


HSQC 600MHz
quinine in C_6D_6



6a was assigned via the dqcosy correlation with 11. The last >CH– proton left is 4, which can now be unambiguously assigned to the 1.59ppm multiplet.

This multiplet is narrow, esp. for a proton that has many 3-bond neighbors: the dqcosy shows three weak correlations to 3, 5b and 8a. The torsion angles are all close to 90°.



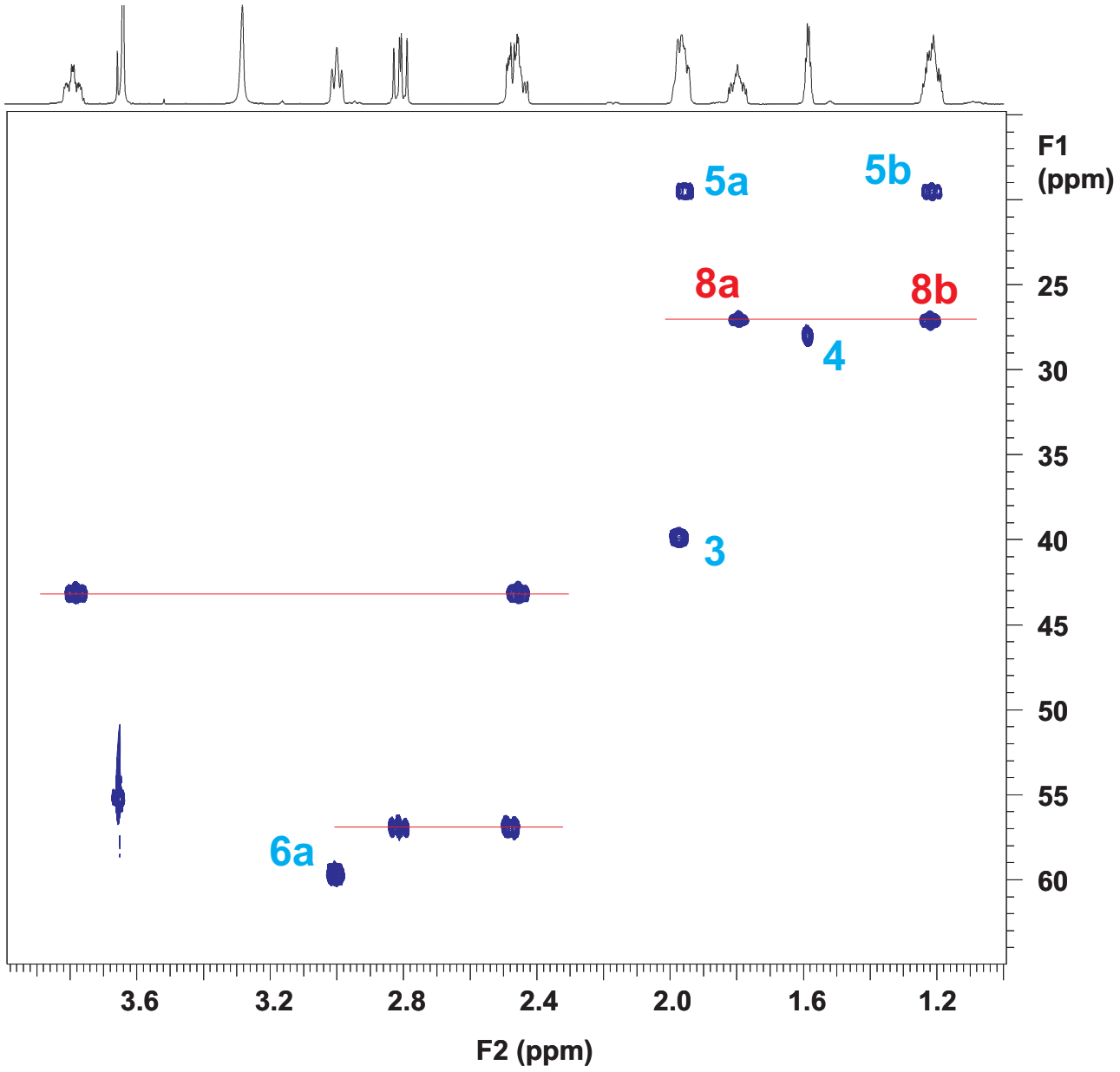
HSQC 600MHz
quinine in C_6D_6

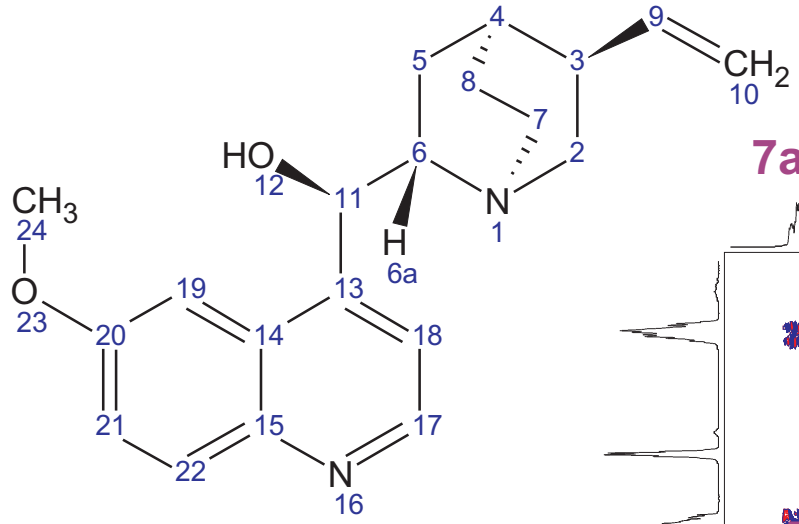
These assignments leave three CH2 groups unassigned. The CH2 at

13C → 27ppm and
1H → 1.80,1.22ppm

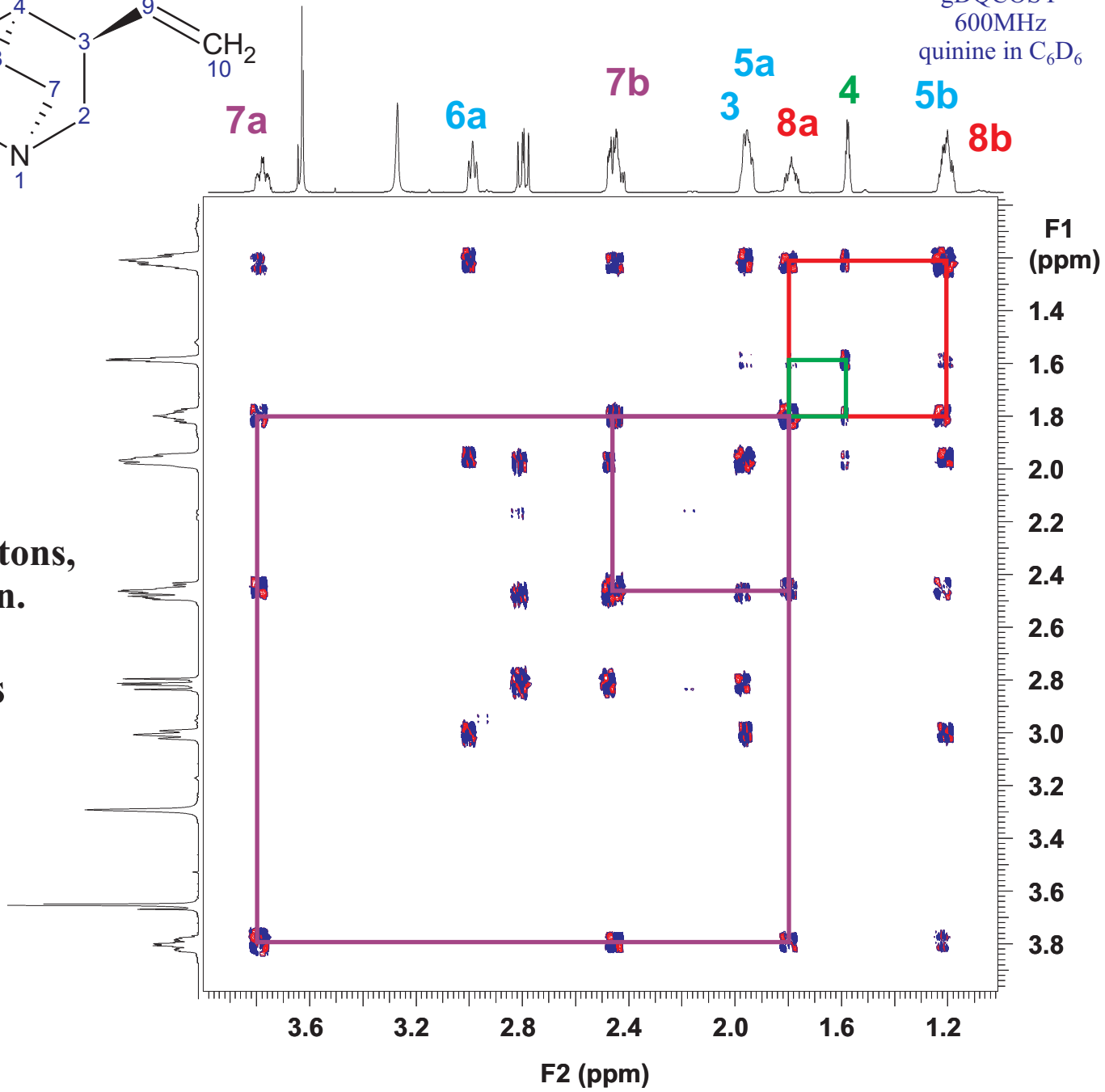
can be safely assigned to the 8 carbon and 8a,8b protons from chemical shift considerations.

This assignment can be confirmed, and the other two CH2's assigned using the dqcosy data.





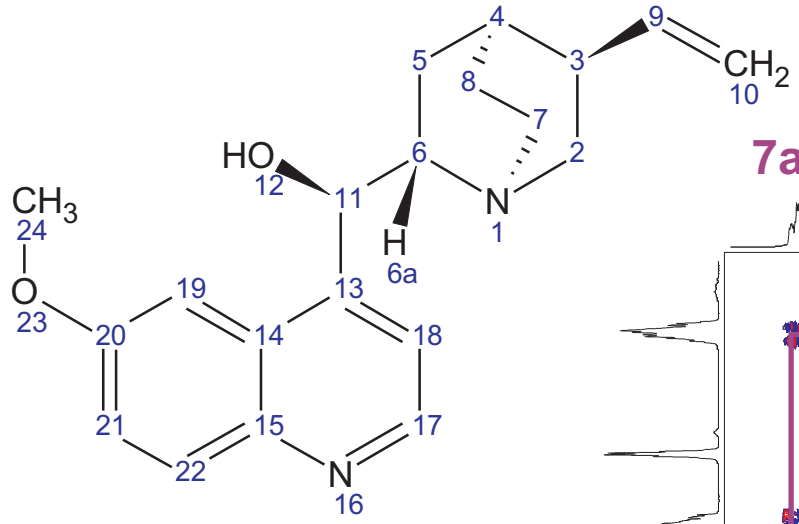
gDQCOSY
600MHz
quinine in C_6D_6



The 8 protons should correlate to the 7 protons, as well as the 4 proton.

The 8a correlations are shown:

to 8b
to 4
to 7b
to 7a



gDQCOSY
600MHz
quinine in C_6D_6

The 8b correlations are shown:

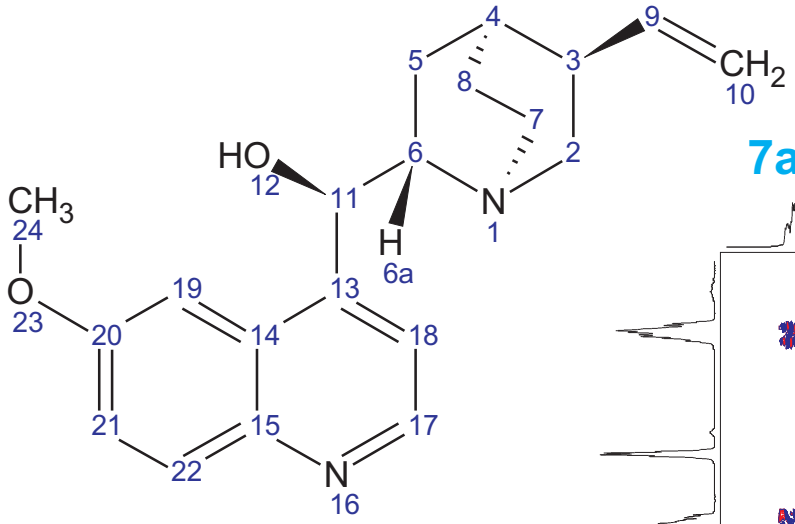
to 8a

to 4 (+ 5b correl.)

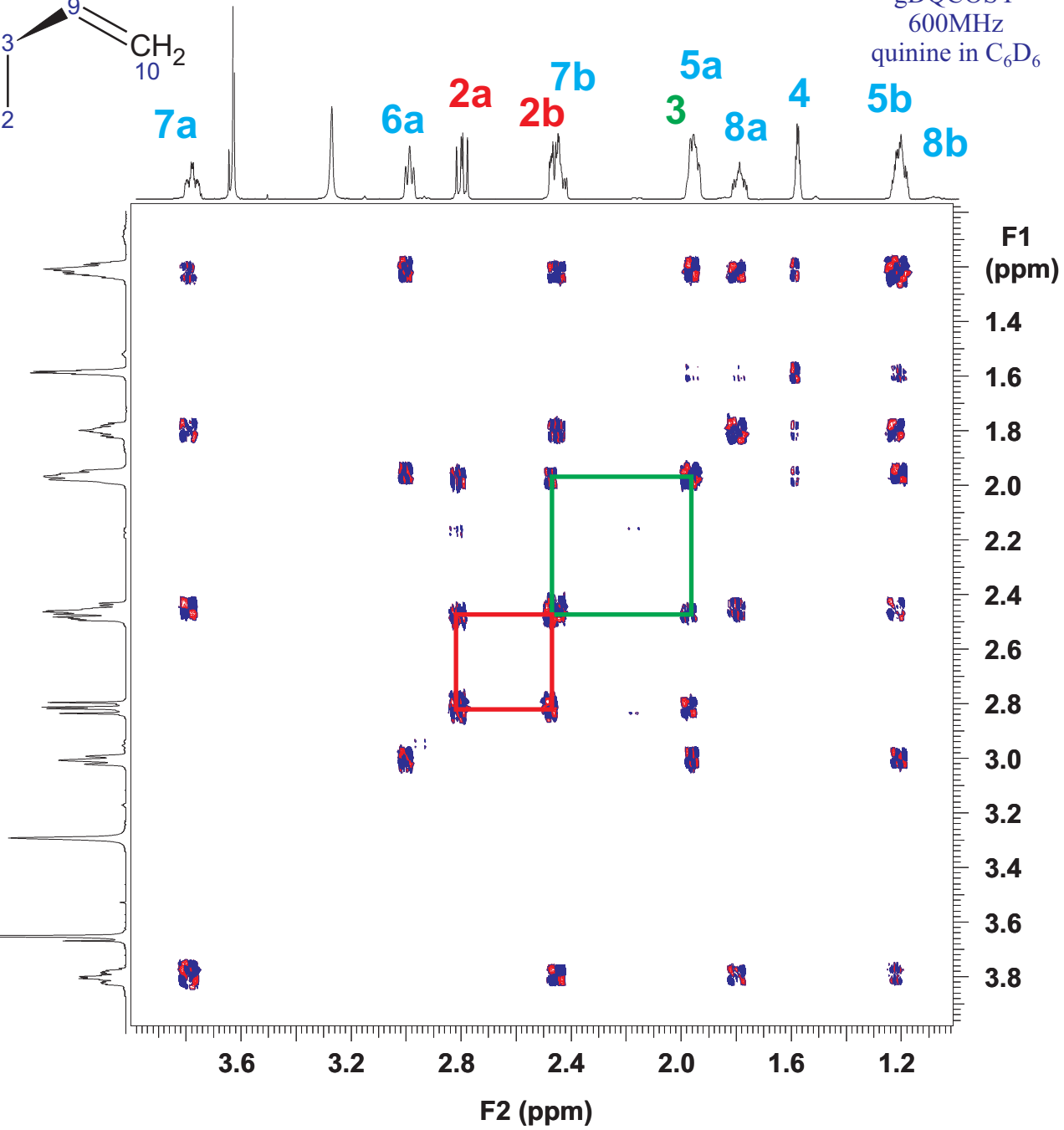
to 7b

to 7a

The 5b correlations are shown in light gray.



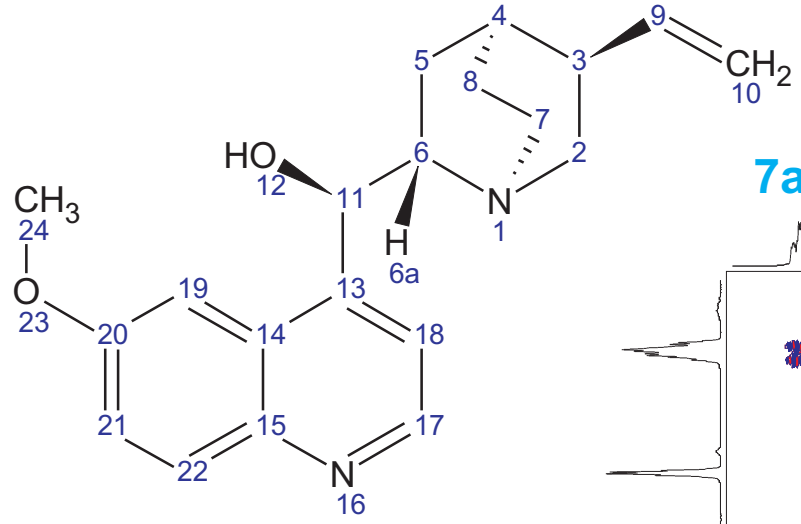
gDQCOSY
600MHz
quinine in C_6D_6



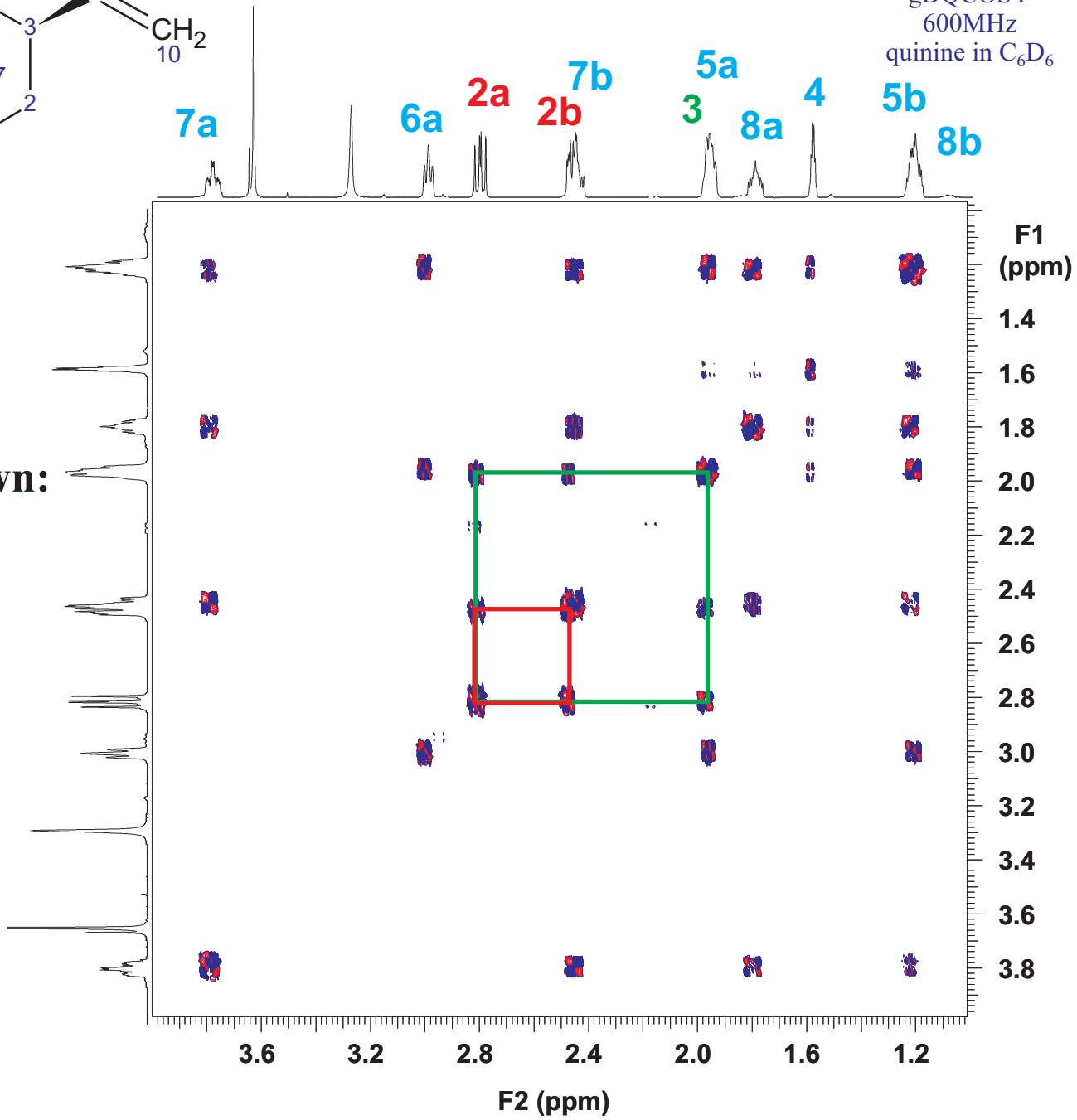
The protons 2a and 2b should correlate only with each other and proton 3.

Proton 2a is a dd; we could have used this multiplet as a starting (entry) point in the assignments.

2b correlations are shown:
to 2a
to 3



gDQCOSY
600MHz
quinine in C_6D_6

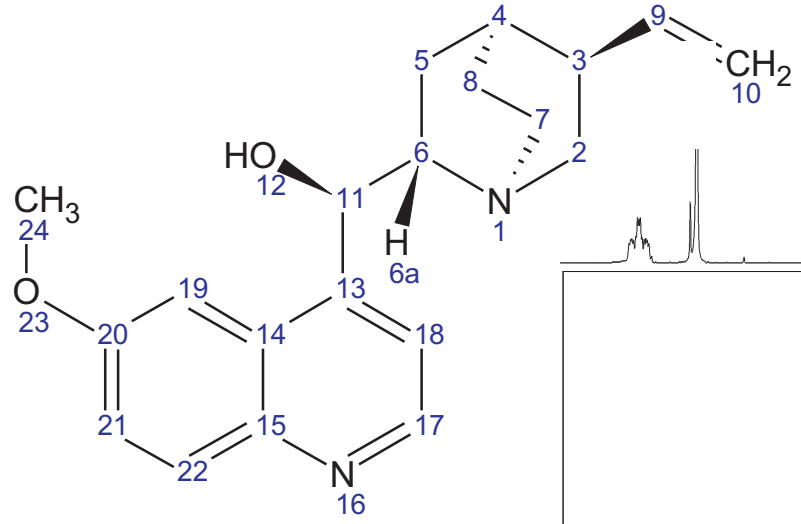


2a correlations are shown:

to 2b and to 3

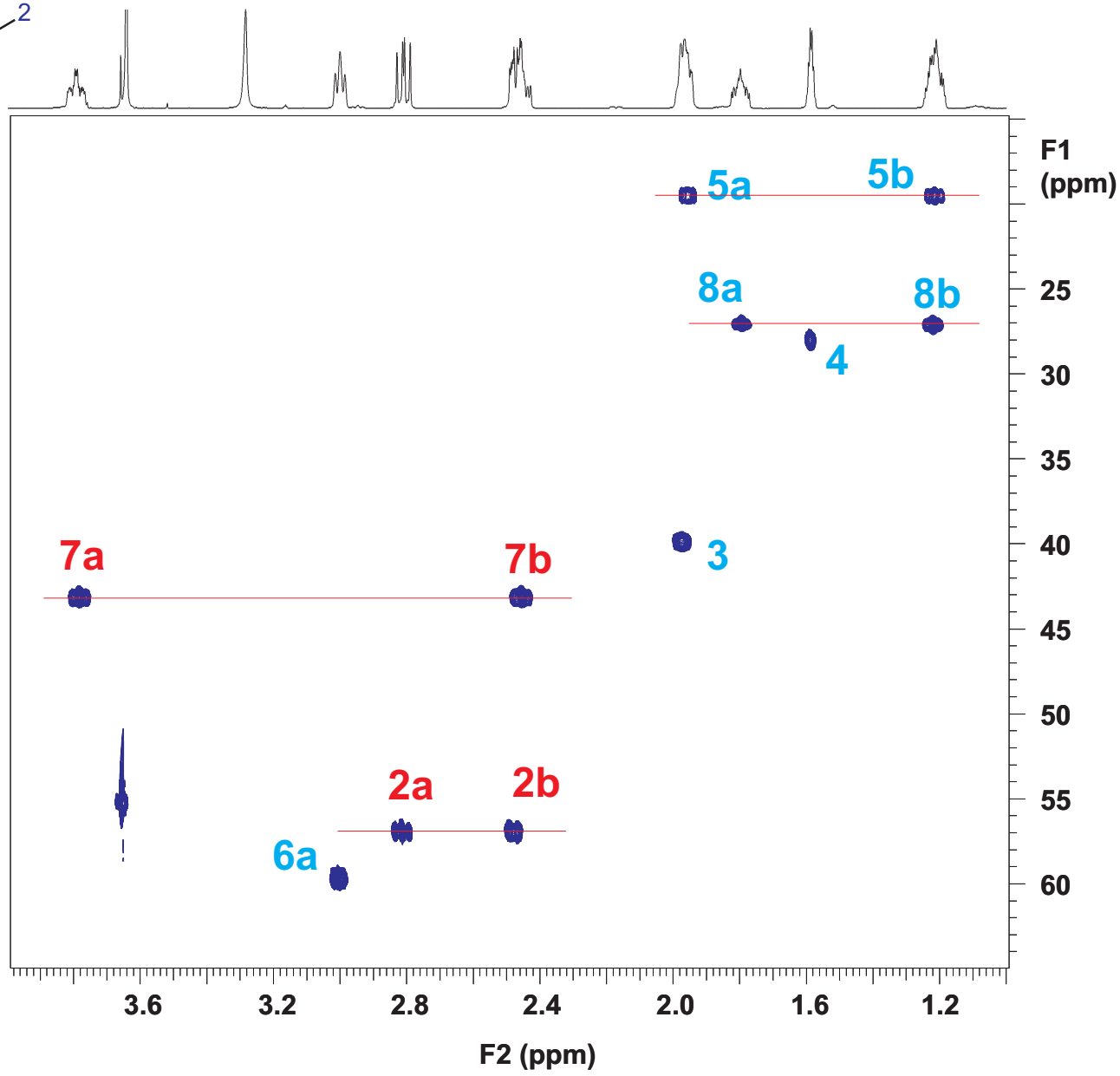
finishing all proton assignments.

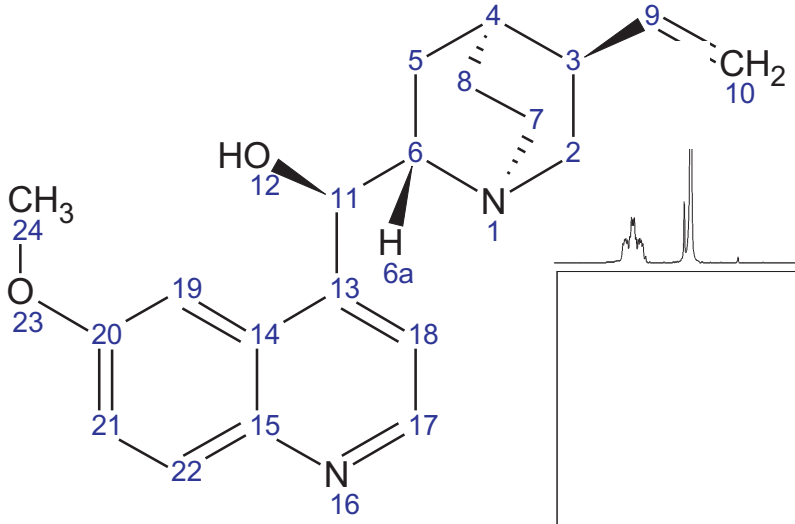
The keys to this procedure were careful tabularization of the data and the use of multiple sets of data.



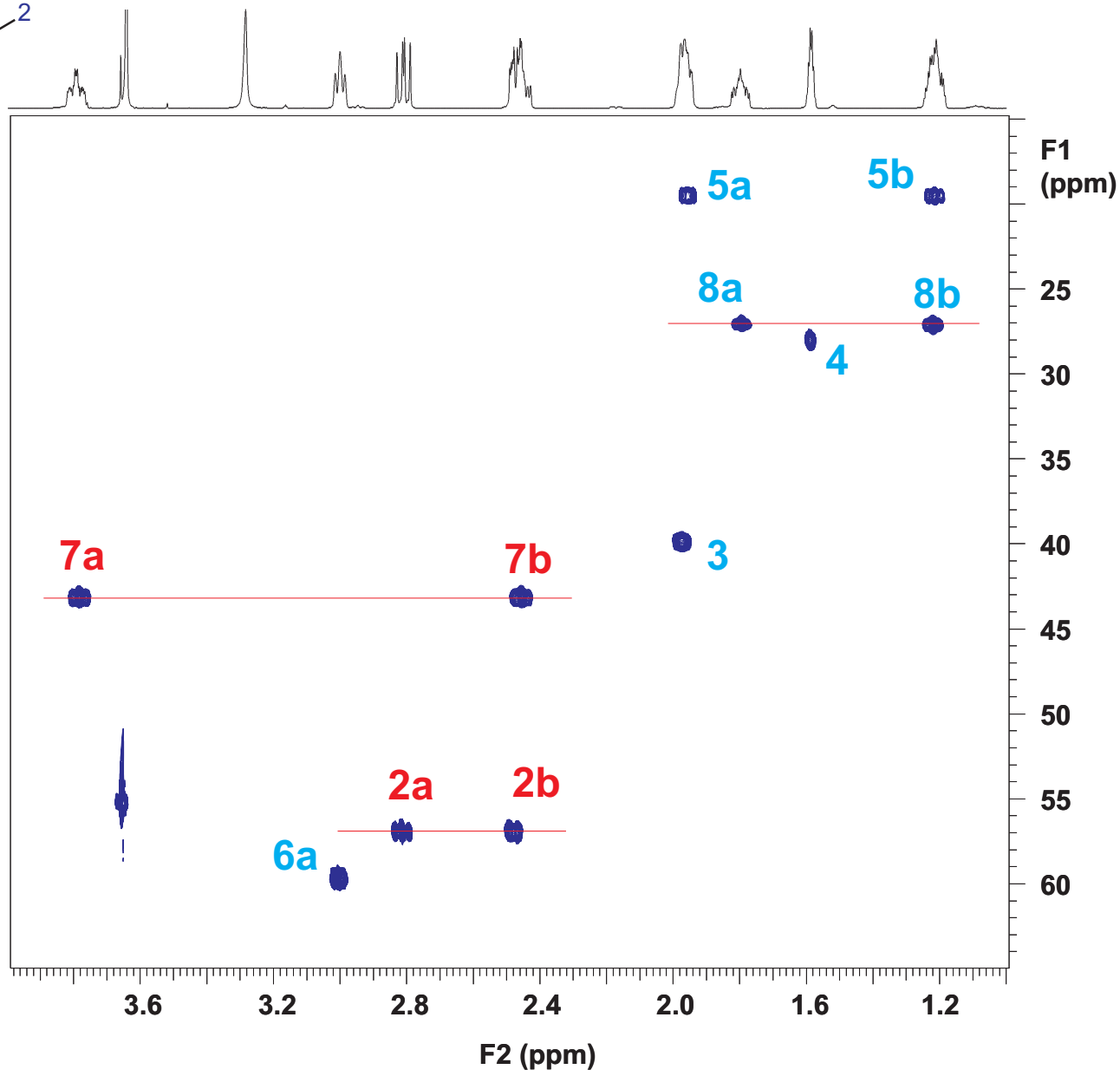
gDQCOSY
600MHz
quinine in C_6D_6

The HSQC data finishes all protonated-carbon assignments. The 7 and 3 carbons could have been safely assigned based on chemical shift.



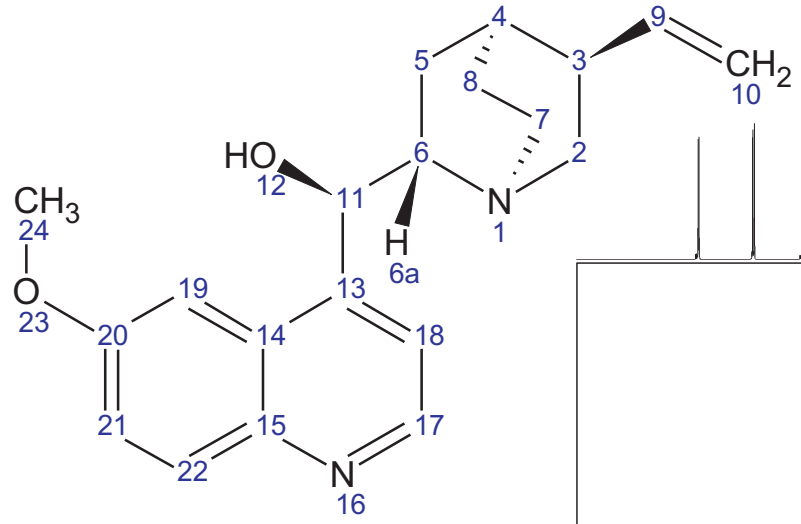


gDQCOSY
600MHz
quinine in C_6D_6



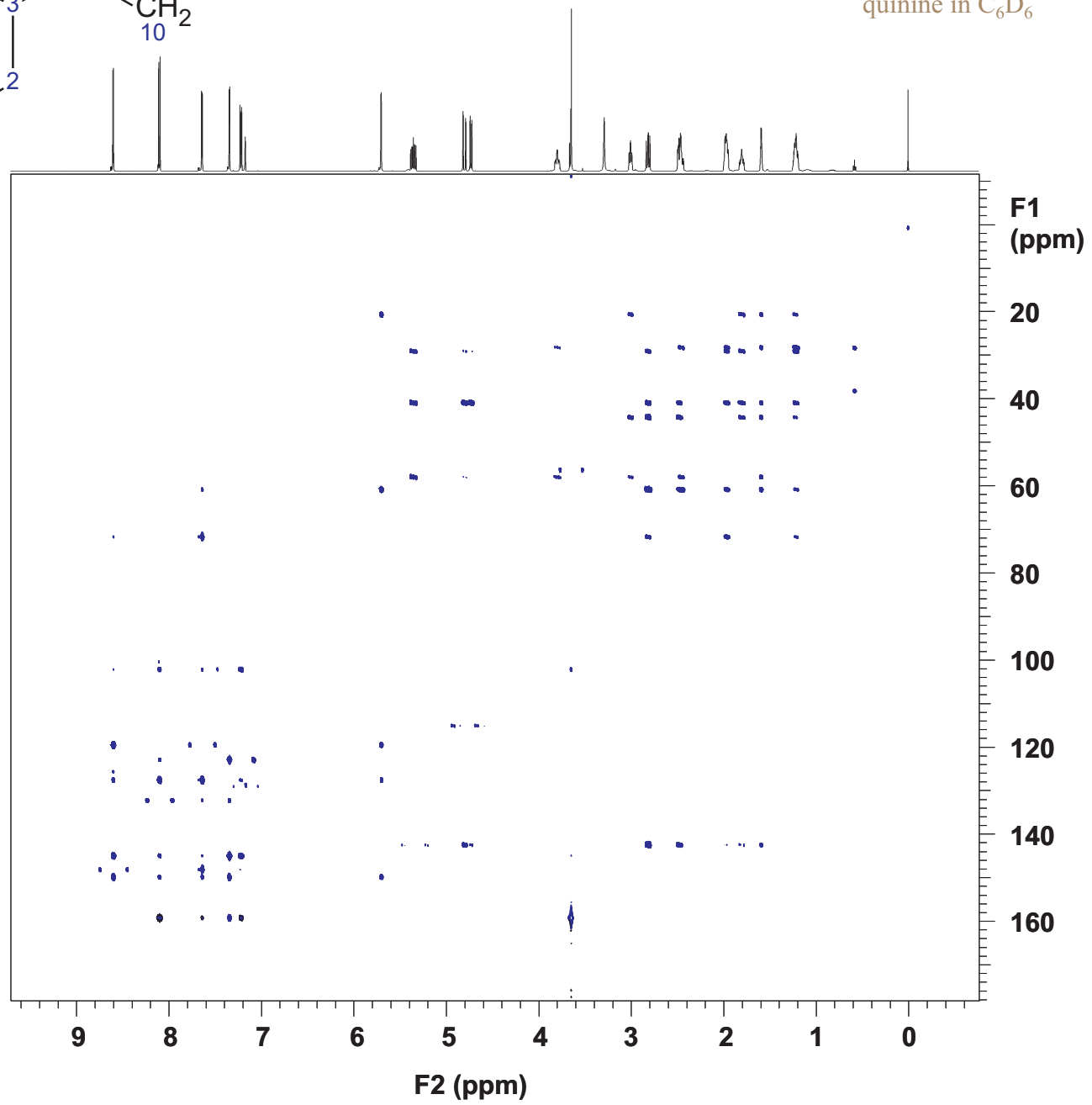
gHMBC is not needed for 1H quinine assignments, but

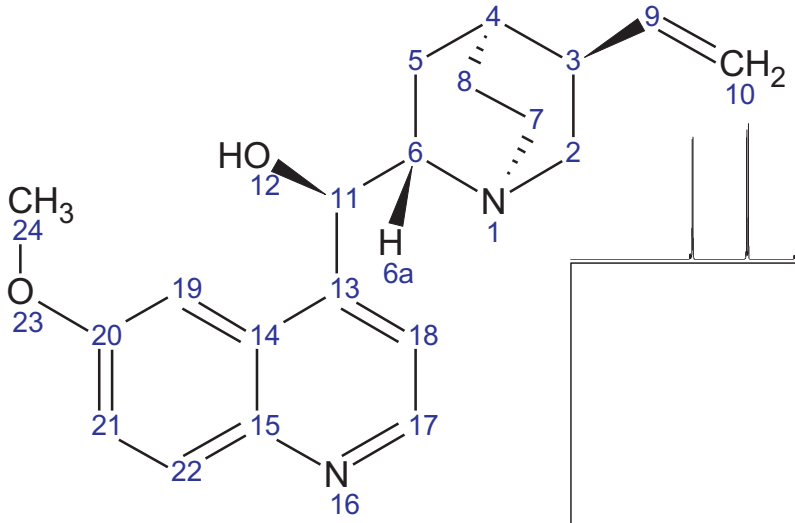
gHMBC provides the only method for quaternary 13C assignments of low conc samples.



gHMBC 600MHz
quinine in C_6D_6

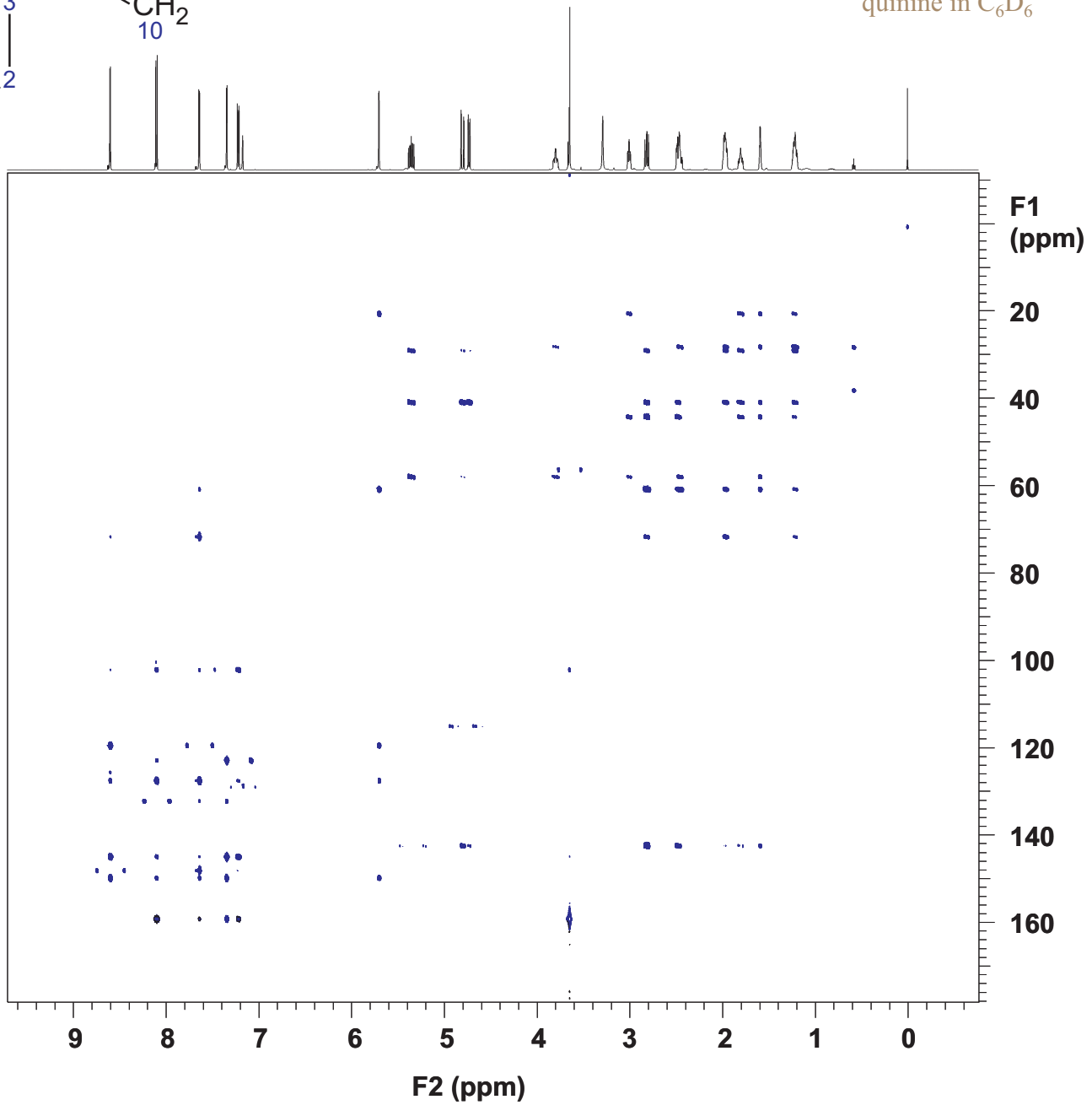
gHMBC produces a seeming maze of data, but with careful inspection and tabularization of the data, this can be the key data set for difficult assignments.

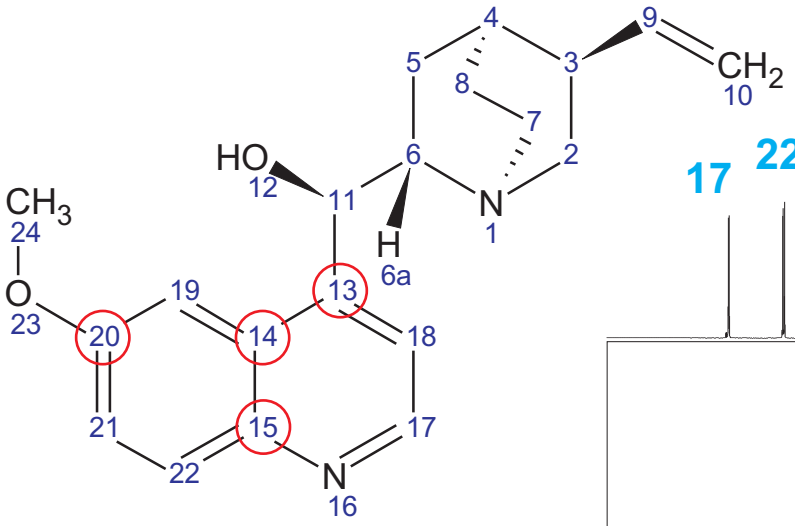




gHMBC 600MHz
quinine in C_6D_6

gHMBC provides the best method for making quaternary carbons assignments (but relying on the presence of long-range 1H-13C J-couplings).





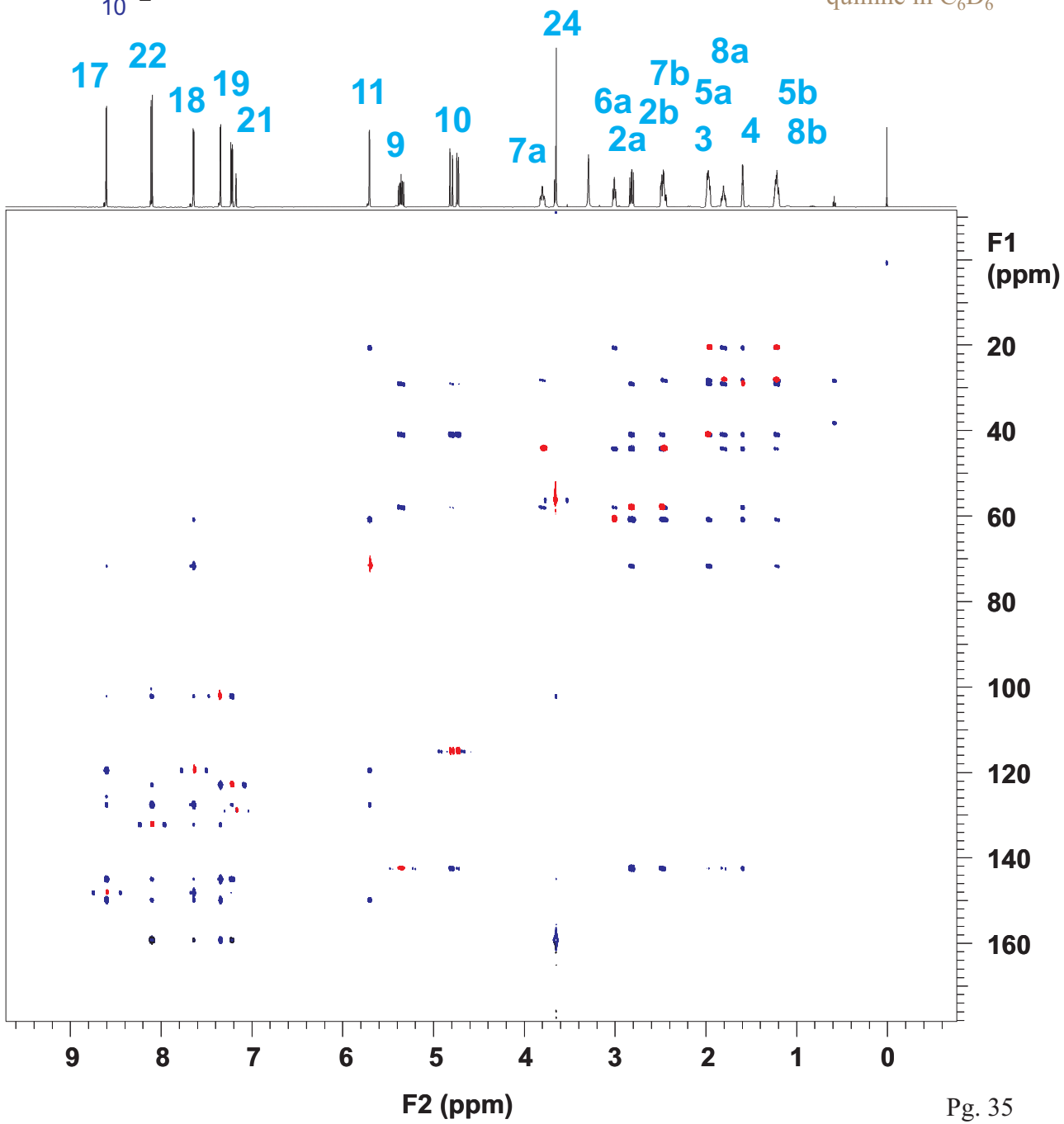
gHMBC 600MHz
quinine in C_6D_6

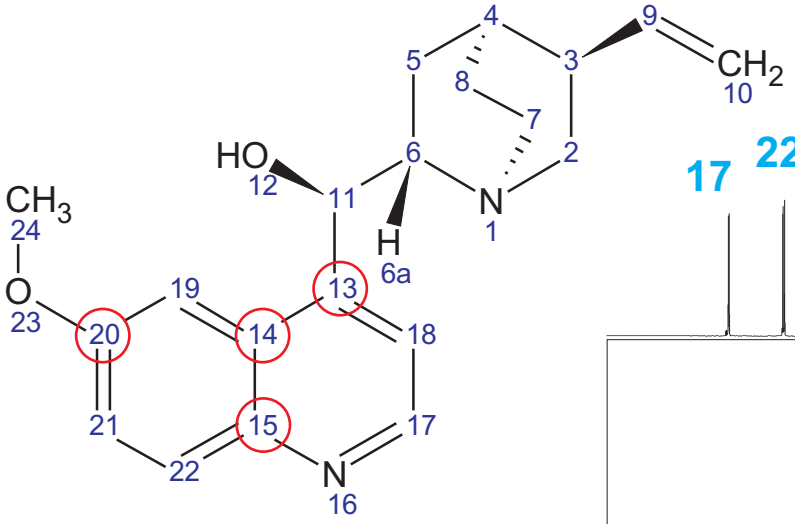
Quaternary 13C assignments:

Look for carbon chemical shifts missing an HSQC correlation.

The hsqc data are shown in red.

Only four quaternary carbons exist in quinine.



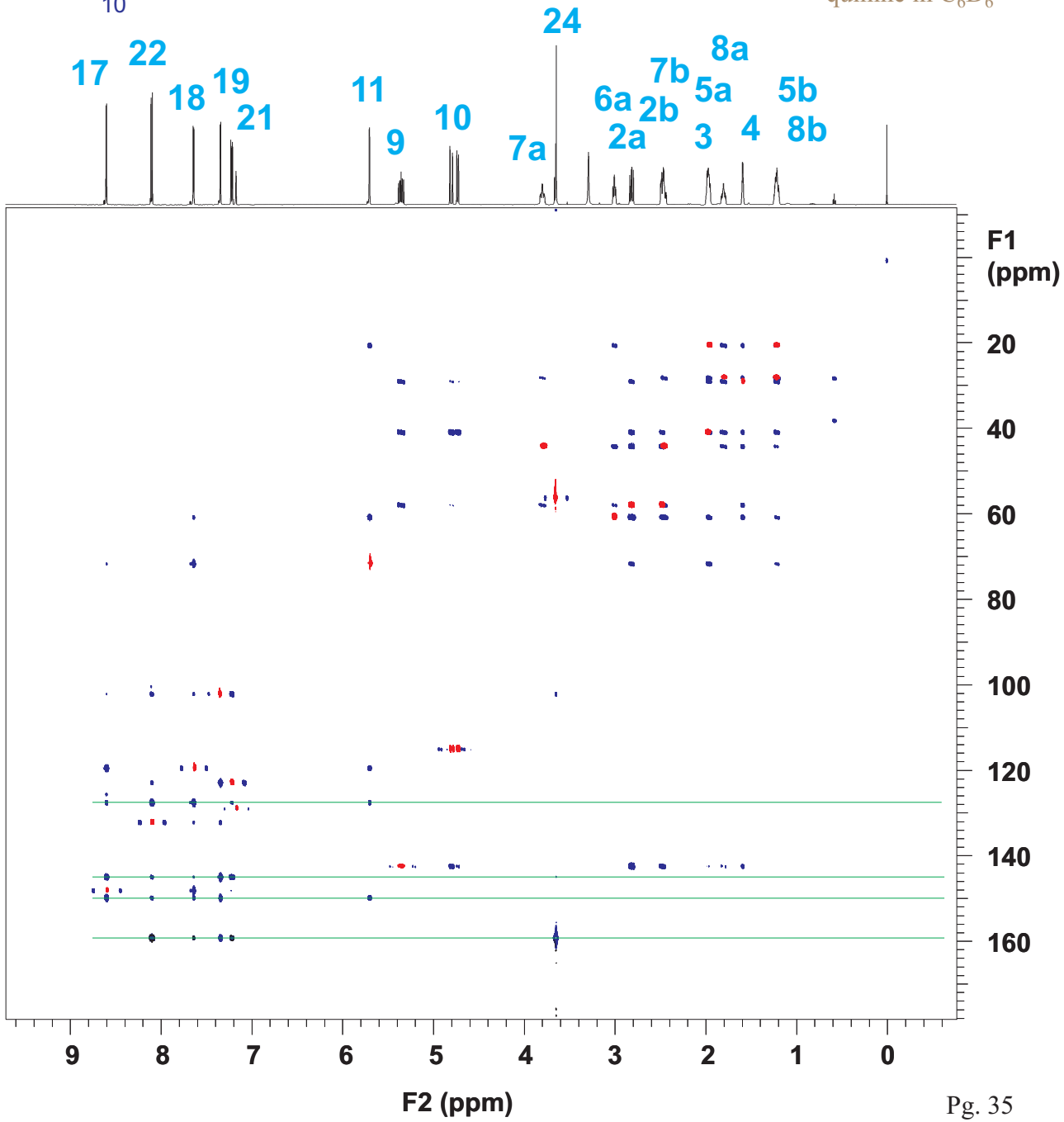


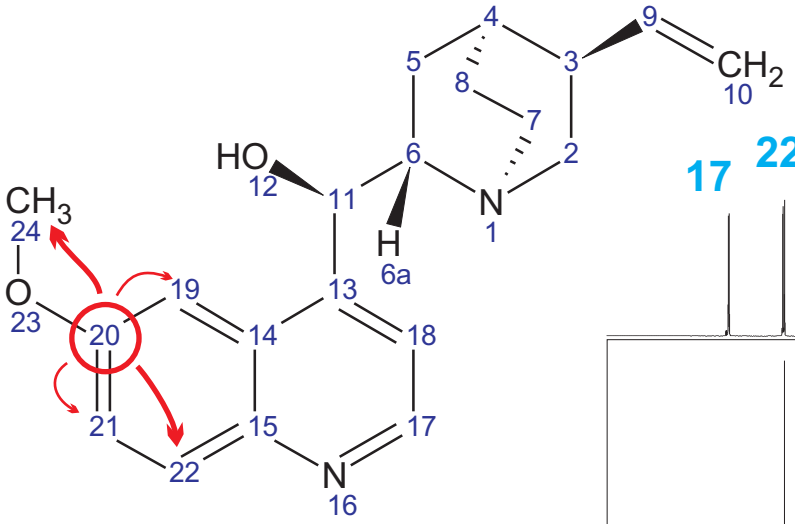
Quaternary 13C assignments:

The four quaternary carbons are found at:

127.5 ppm
144.9 ppm
149.8 ppm
159.1 ppm

gHMBC 600MHz
quinine in C_6D_6

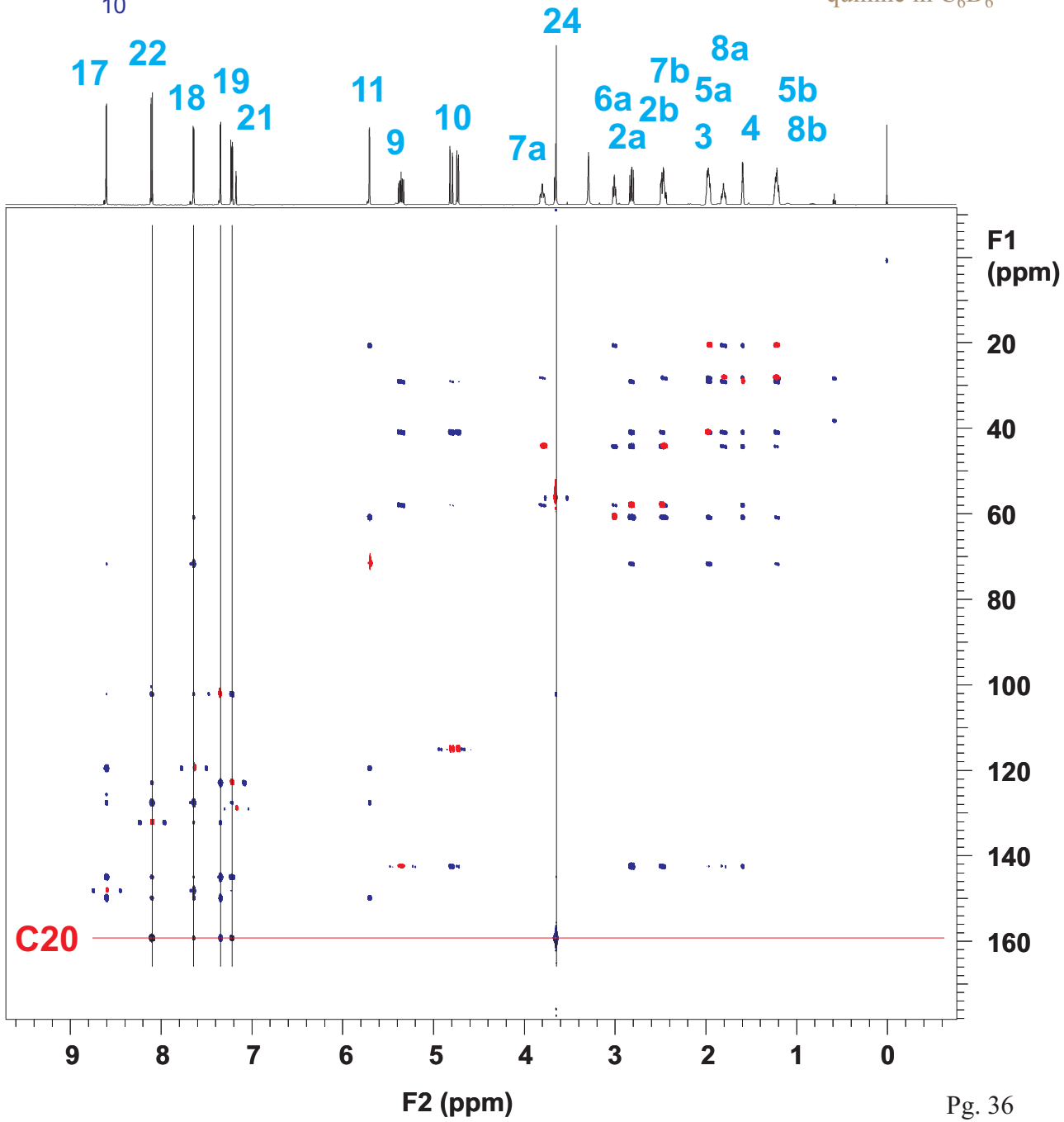


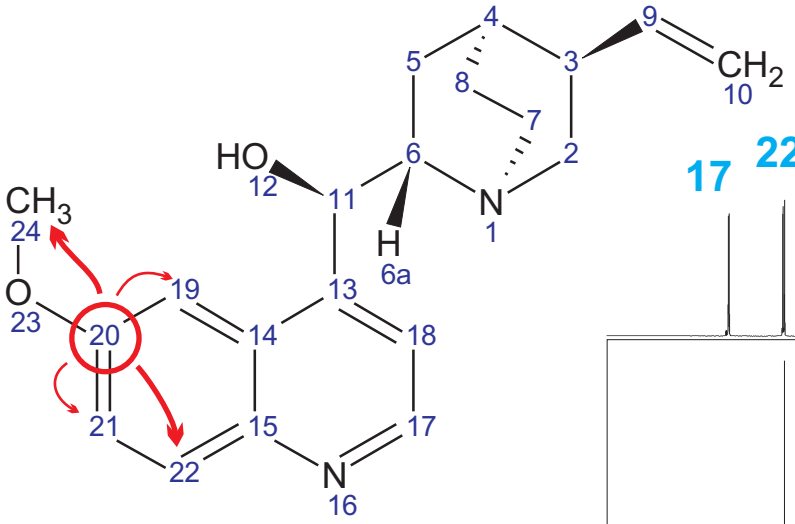


gHMBC 600MHz
quinine in C_6D_6

The quaternary carbon at 159.1 ppm has a correlation to the 24 methyl protons; this carbon must be the 20 carbon.

The strongest other correlation is to proton 22, a 3-bond J_{CH}. Correlations to protons 19 and 21 are moderately strong; the weak apparent coupling to proton 18 cannot be trusted (5-bond).





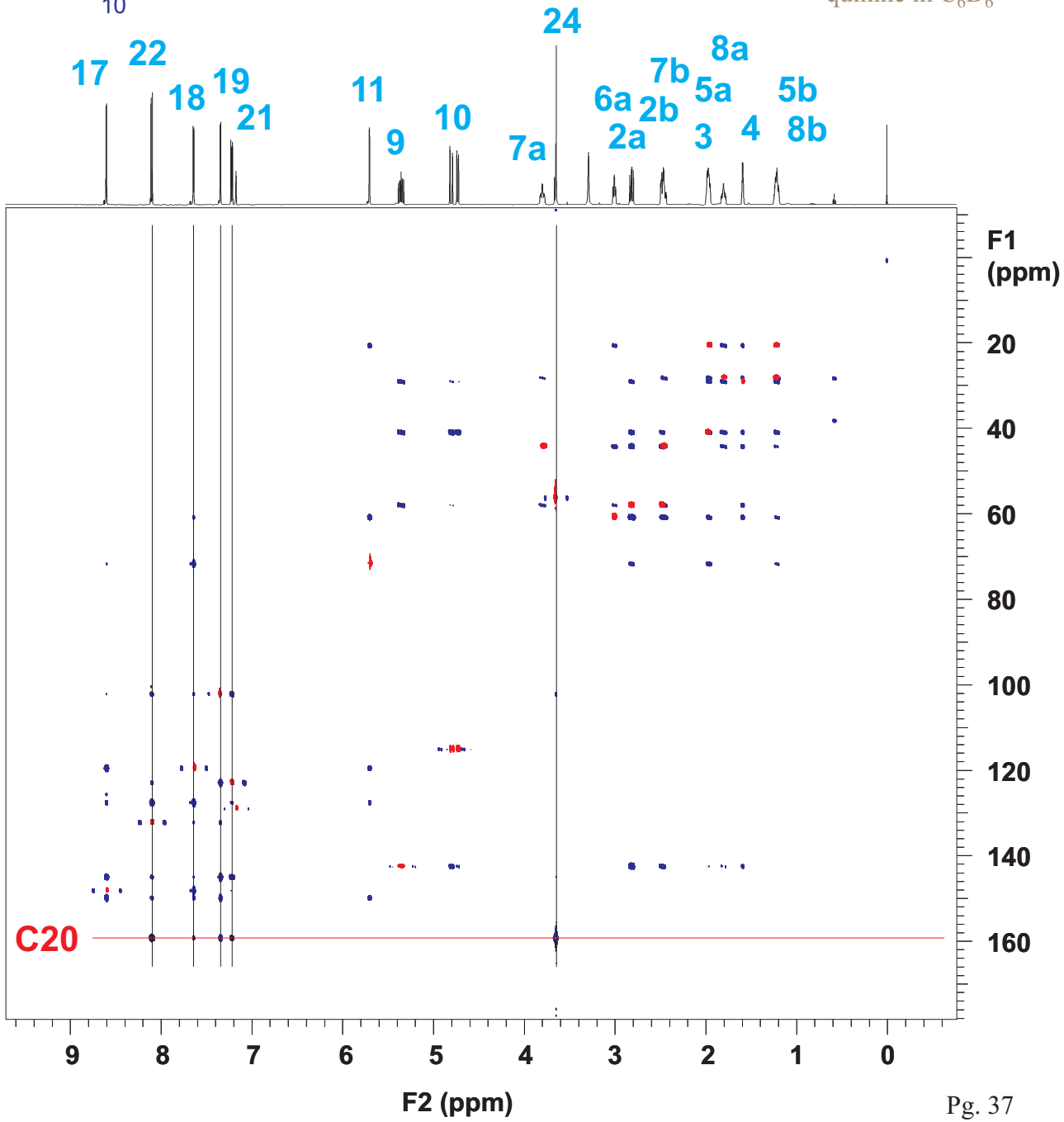
The C20 assignment displays a general and very useful aspect of aromatic J_{CH} values:

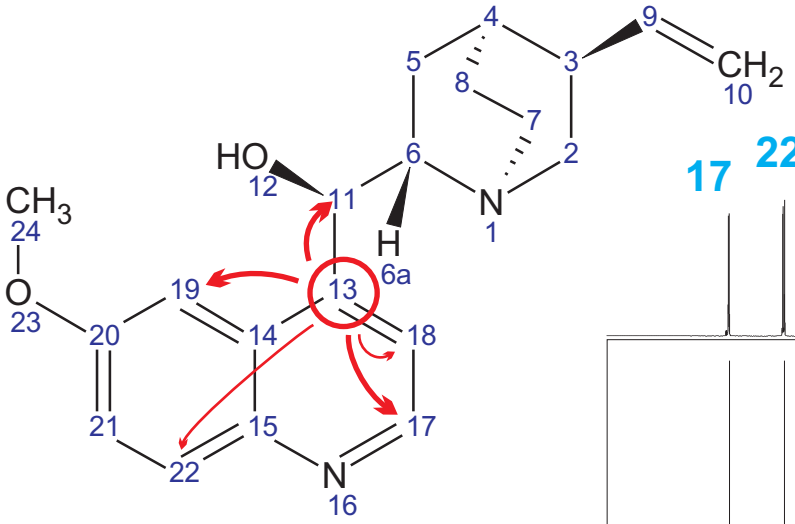
$^{2}J_{CH}$ range 1 to 4 Hz

$^{3}J_{CH}$ range 7 to 10 Hz.

3-bond C-H gHMBC correlations in aromatic systems will always be stronger than 2-bond correlations (for jnxh=8).

gHMBC 600MHz
quinine in C_6D_6



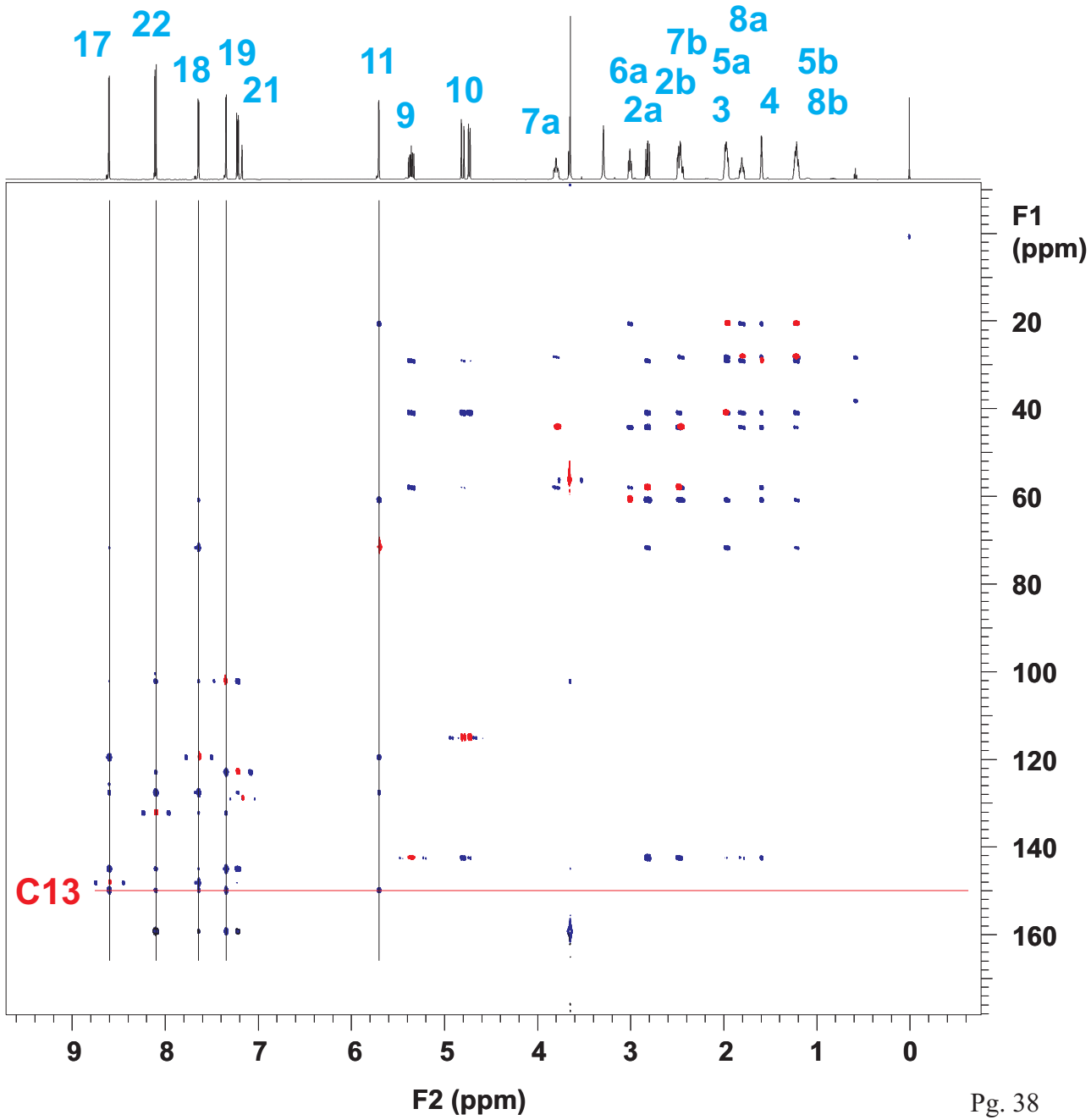


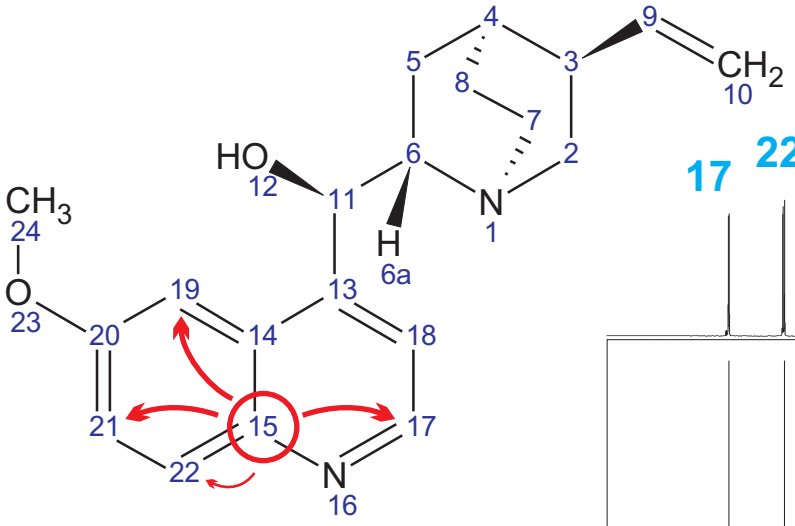
The next quaternary is at 149.8 ppm, with correlations to the:

17 proton
22 proton (wk)
18 proton (wk)
19 proton
11 proton

This must be C13;
C14 would not correlate (strongly anyway) to H17, and the correlation C14 to H22 would be strong.

gHMBC 600MHz
quinine in C_6D_6



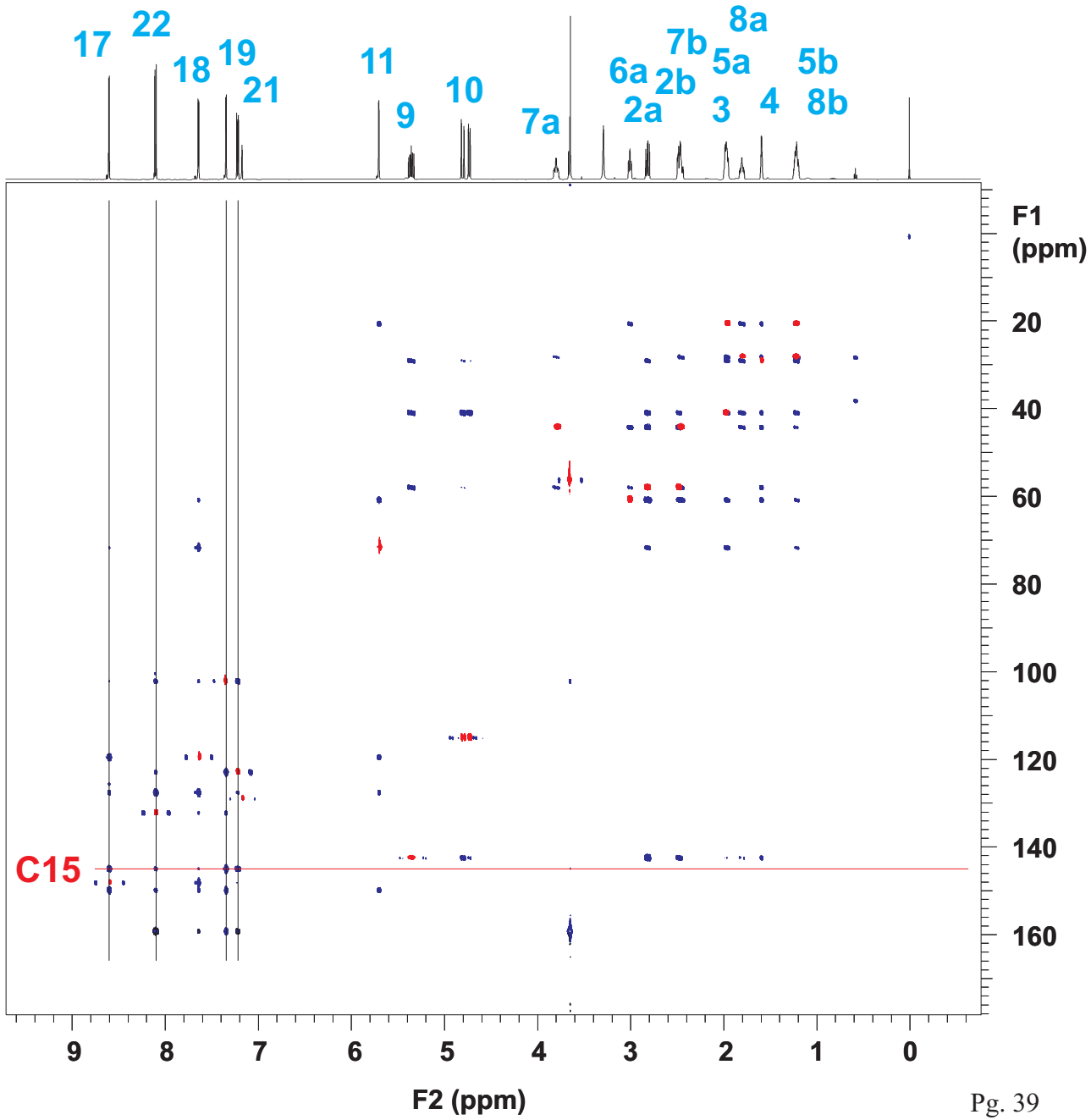


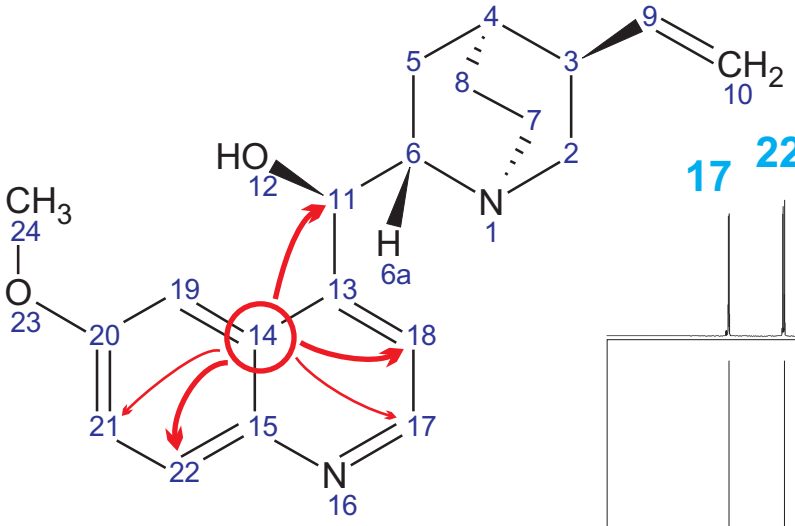
gHMBC 600MHz
quinine in C_6D_6

The next quaternary is at 144.9 ppm, with correlations to the:

17 proton
22 proton (wk)
19 proton
21 proton

This must be C15, because of the strong 3-bond aromatic correlations.

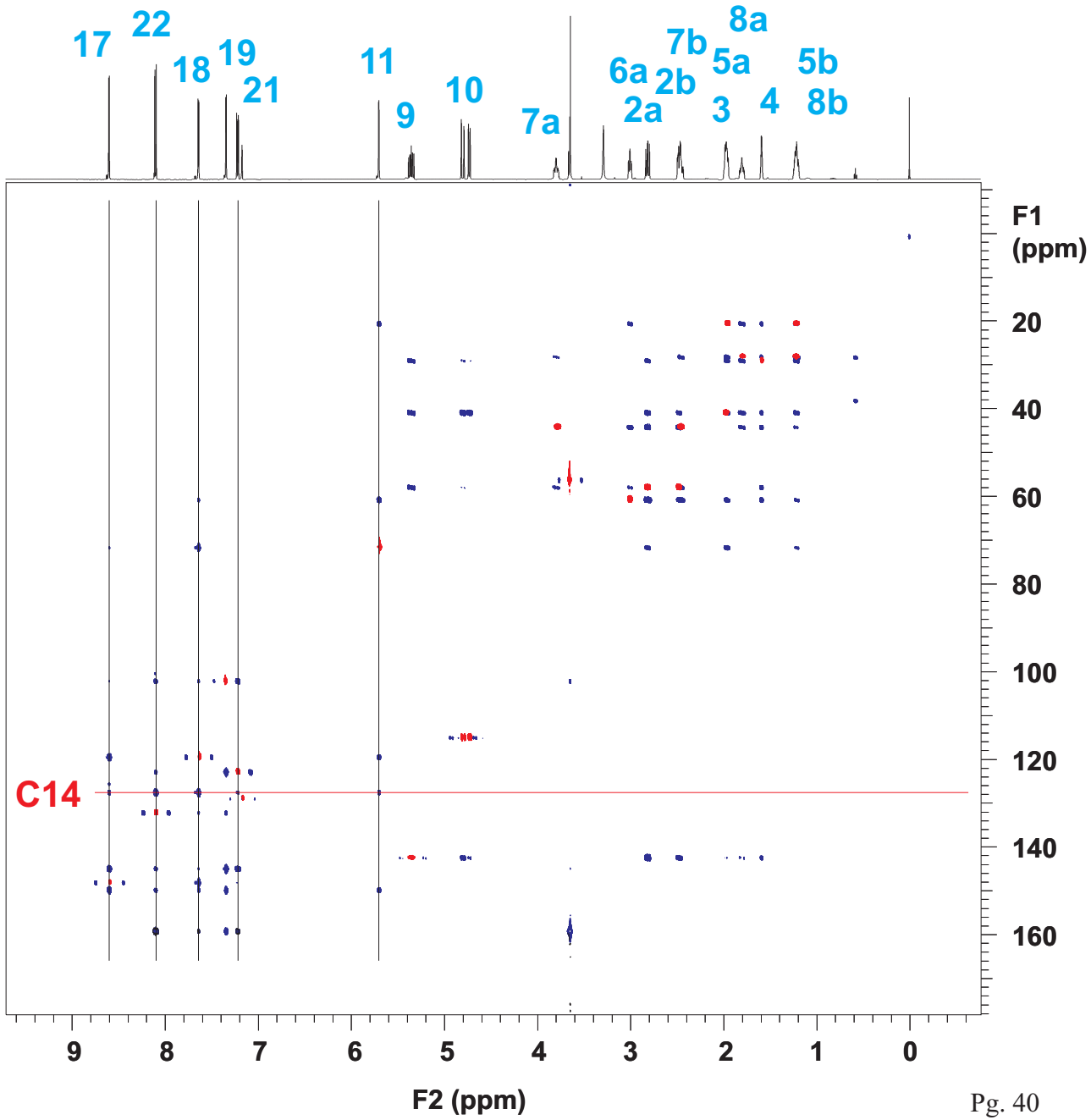


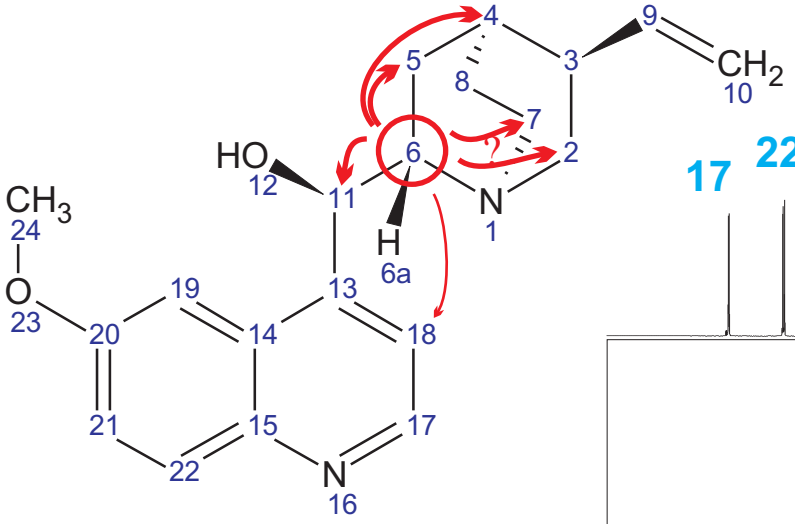


gHMBC 600MHz
quinine in C_6D_6

Last is C14; we should expect H22 and H18 to give strong (3-bond) correlations. H11 should show a correlation, but of unpredictable strength.

17 is weak
22 strong
18 strong
21 is weak
11 is moderate

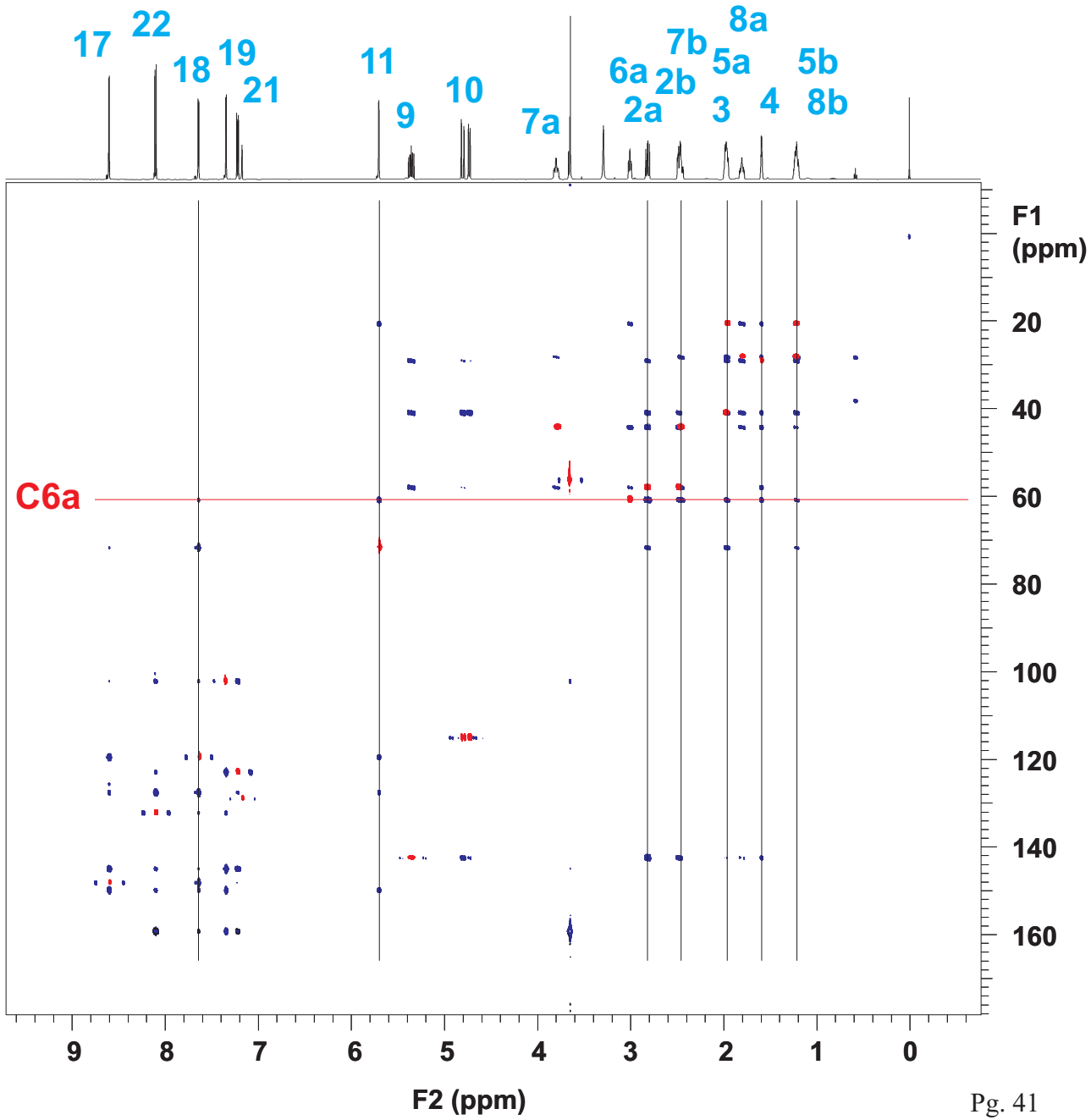


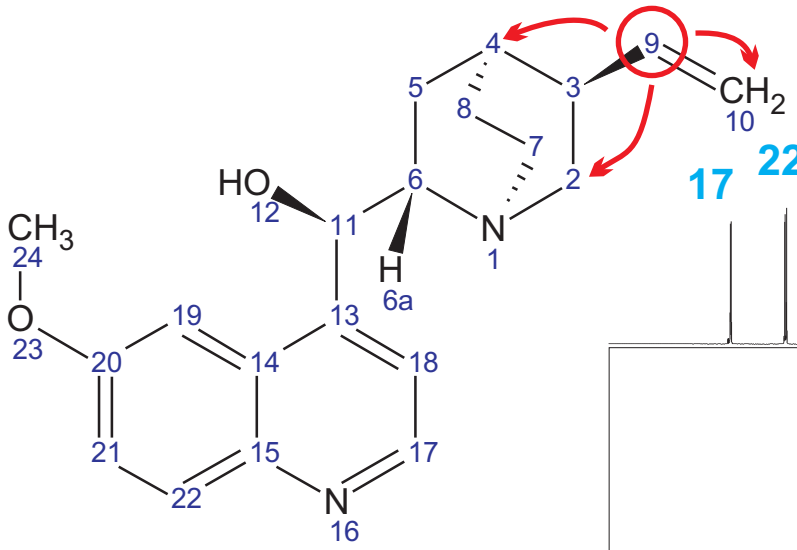


gHMBC 600MHz
quinine in C_6D_6

A look at the 6 carbon shows correlations to:

18 proton (wk)
11 proton
2a proton
2b proton (7b?)
5a proton (low-freq side)
4 proton
5b proton





gHMBC 600MHz
quinine in C_6D_6

The 9 carbon shows correlations to:

10 protons
2a proton
2b proton (high-freq side)
4 proton (wk)
3 proton (wk)
5a and/or 8a (wk)