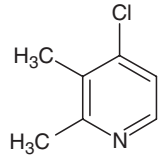
15

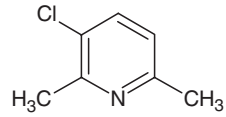
Problems

So there you have it. Our mission to enlighten draws to a close. If you would like to find out if we have been in any way successful, this chapter contains some problems to have a go at. Obviously, real-world problems will normally have other information about them, not just the NMR spectrum. The flow chart in Appendix A.1 gives some indication of useful reminder of the thought processes for real-world problems.

15.1 Ten NMR Problems

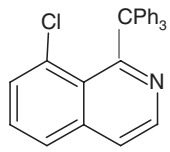
Q1. You are given a sample that is known to be one of the following compounds:

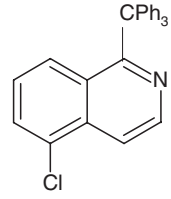




It is not clear whether the compound is a free base, a salt or a partial salt. What would you need to do to be confident beyond reasonable doubt that you could positively identify the compound?

Q2. What key observation might lead you to differentiate the following pair of compounds from nothing more than their proton spectra?





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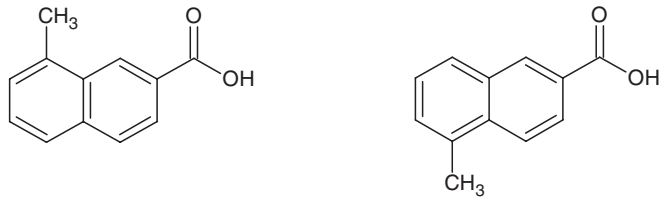
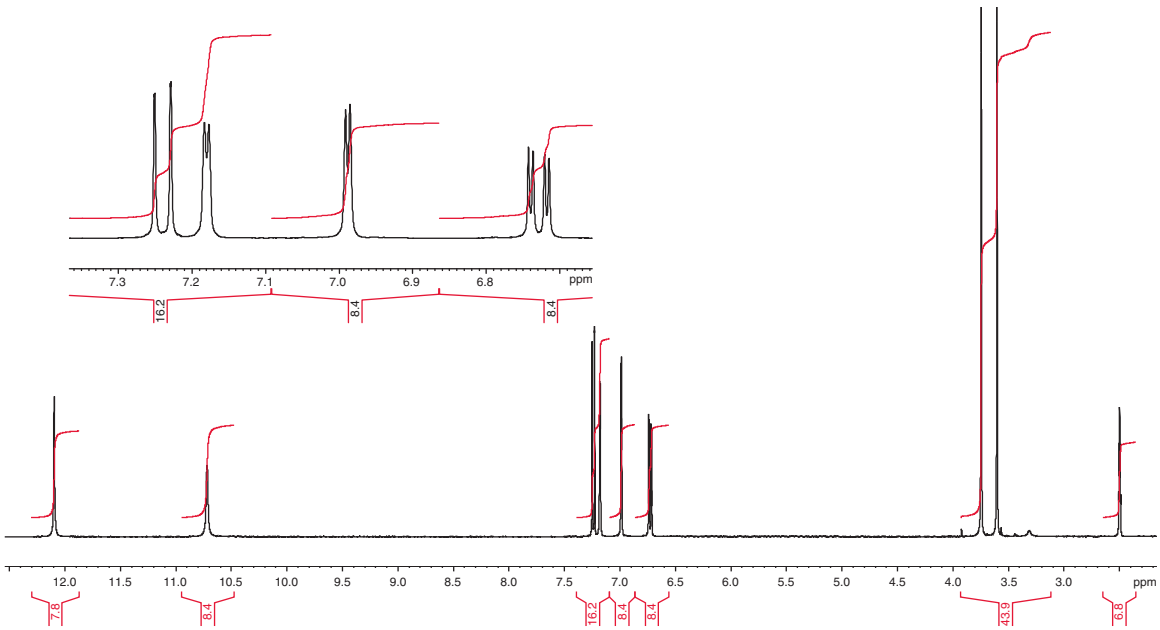
Q3. How would you differentiate this pair of isomers?

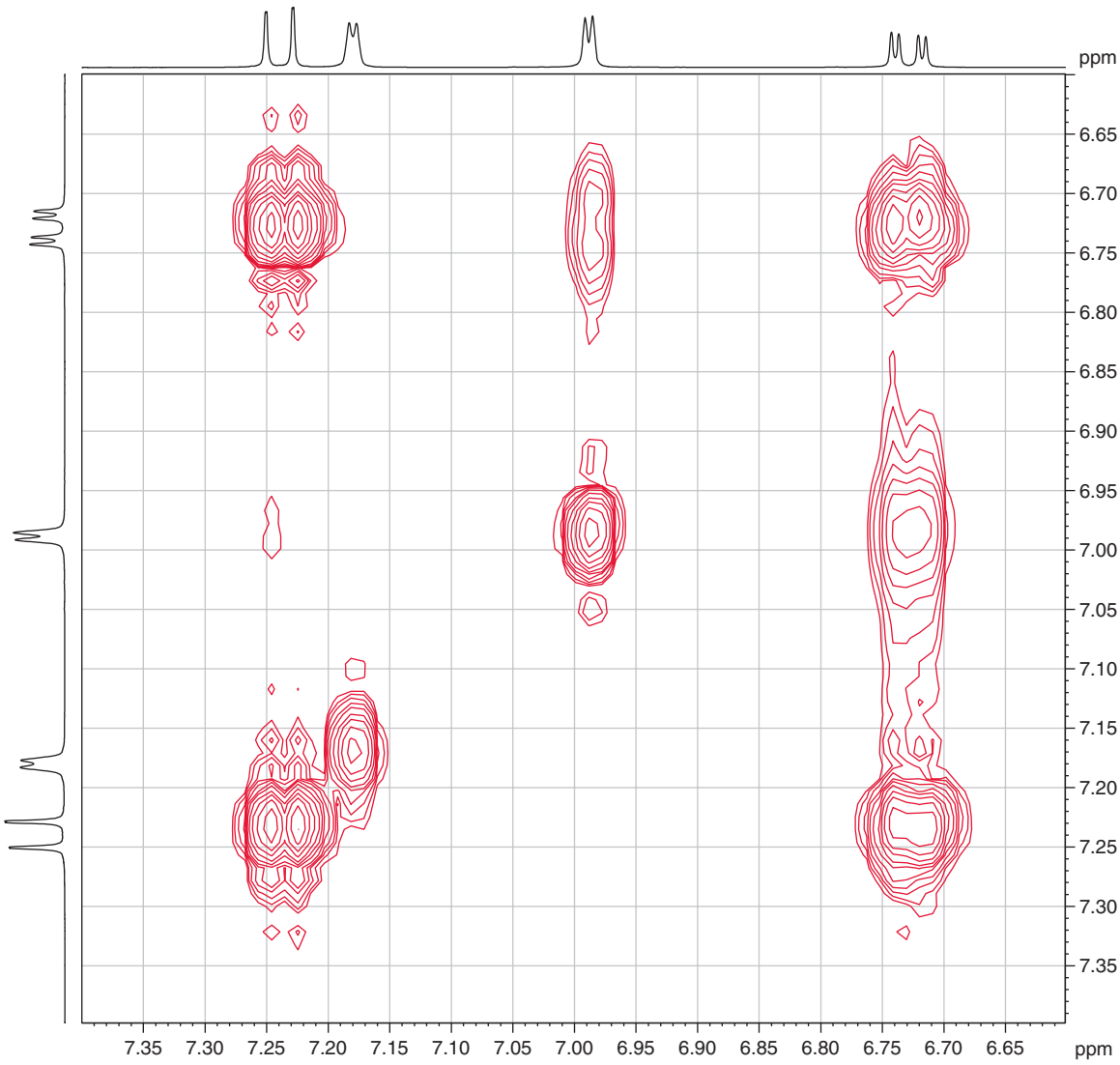


Q4. It's another case of distinguishing two compounds but this time they aren't isomers. (Unfortunately, the mass spec next door is out of action following a sub-optimal 'preventative maintenance' visit from the service engineer and the compounds probably wouldn't ionise anyway!)

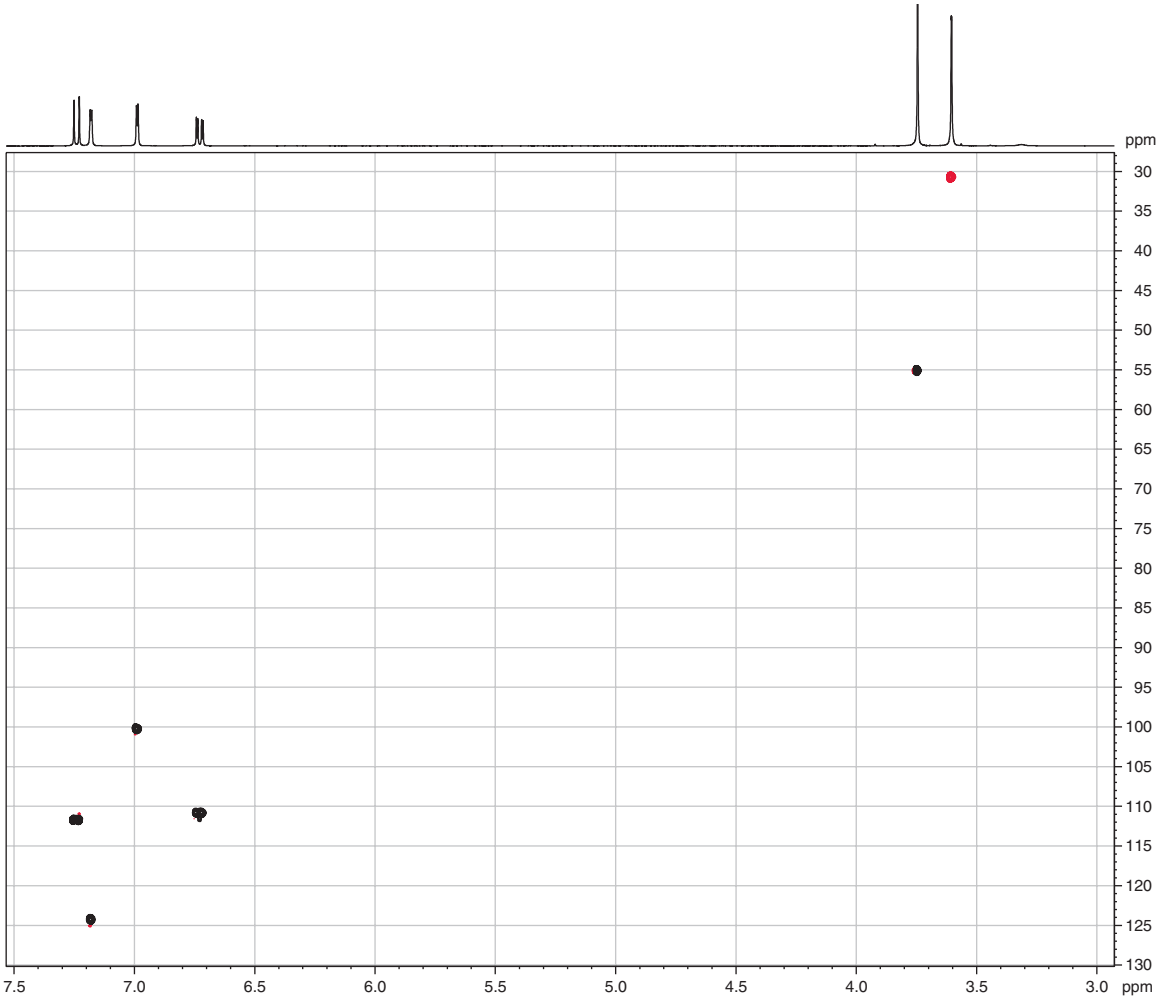
Q5. From Spectra 15.1–15.5, construct a plausible structure for the unknown compound which has a molecular formula: $C_{11}H_{14}NO_3$. (It is known to be an indole and to contain the following fragments: -OCH_3 and -CH_2-COOH.)



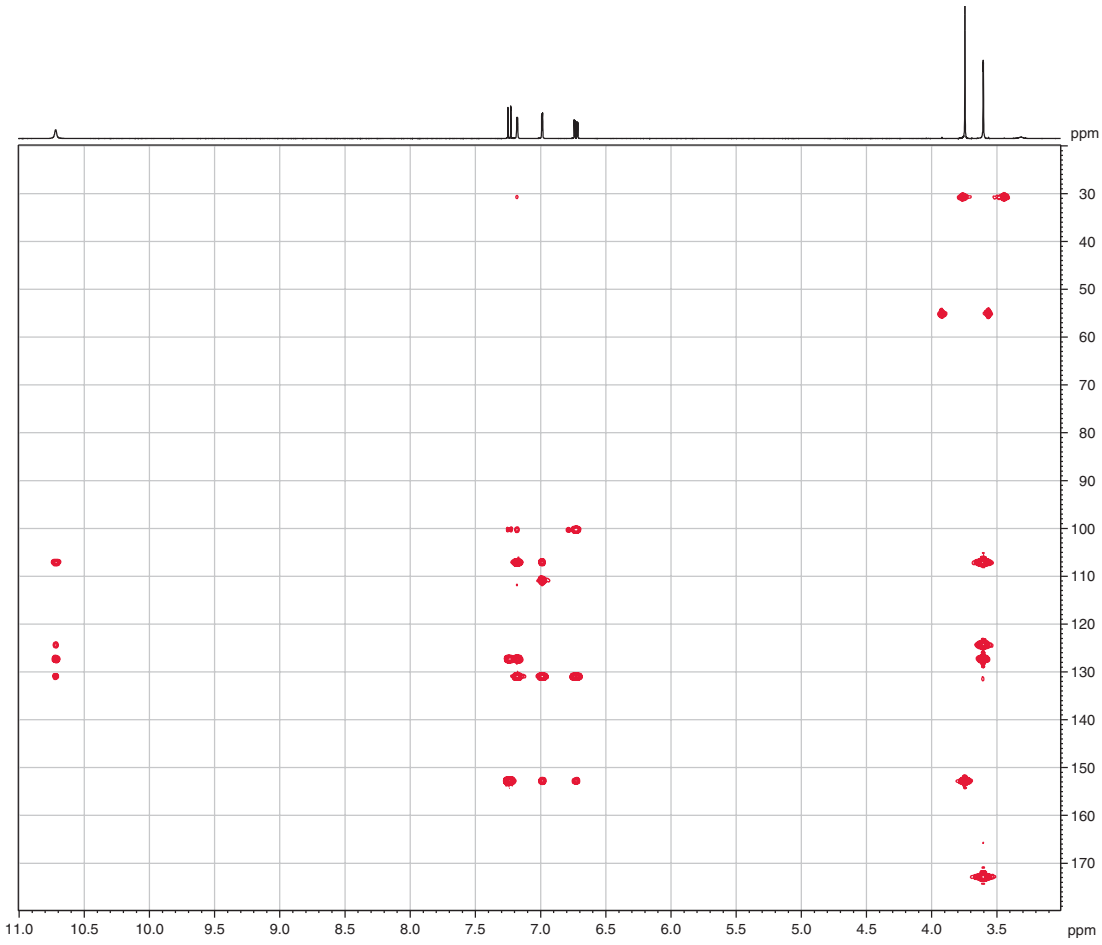
Spectrum 15.1 ^{1}H 1-D.



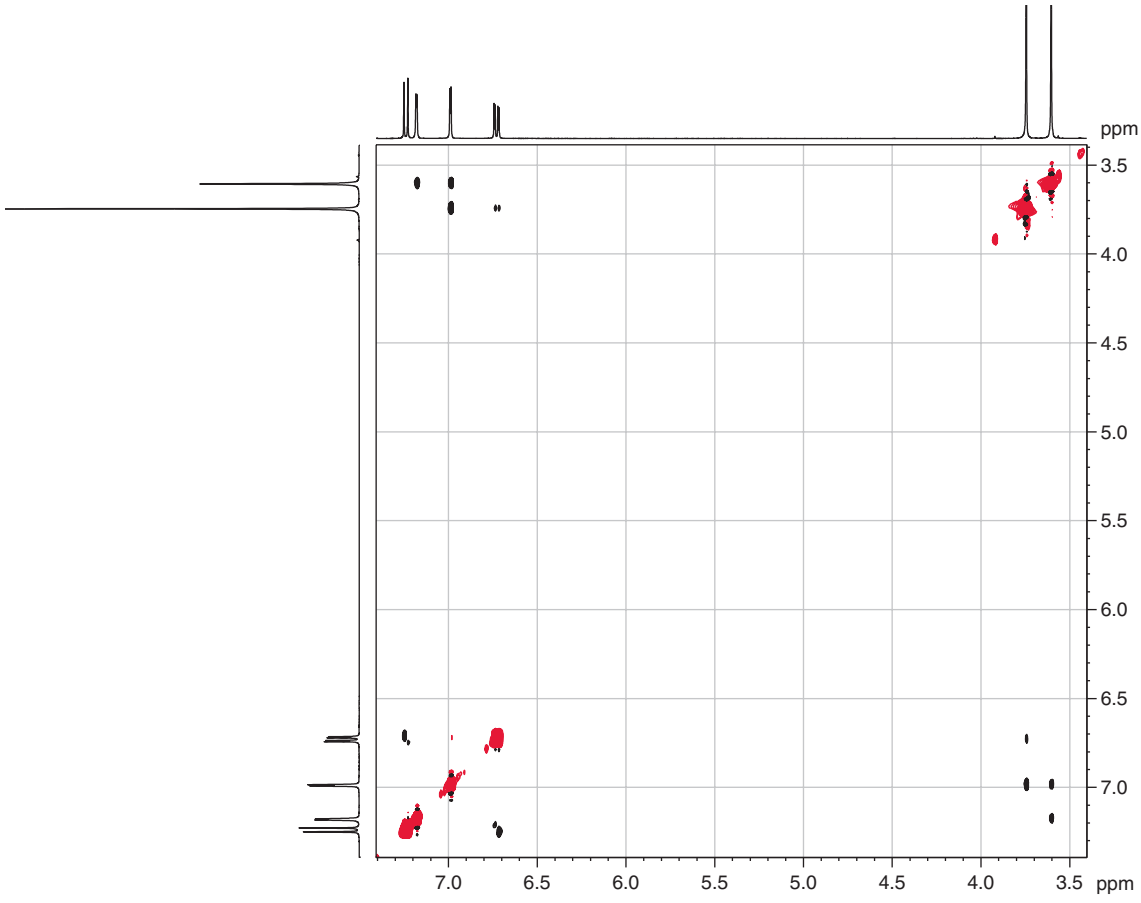
Spectrum 15.2 COSY.



Spectrum 15.3 DEPT-edited HSQC.

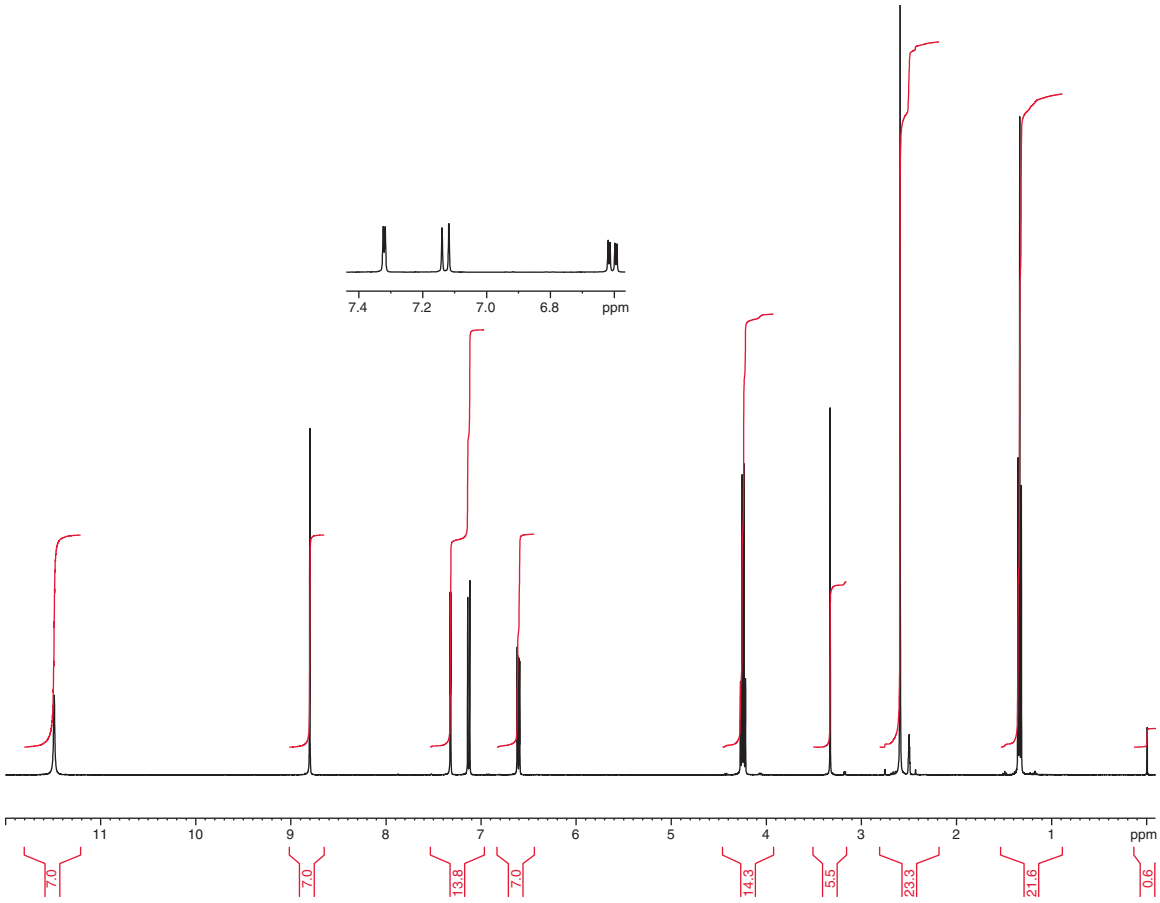


Spectrum 15.4 HMBC.

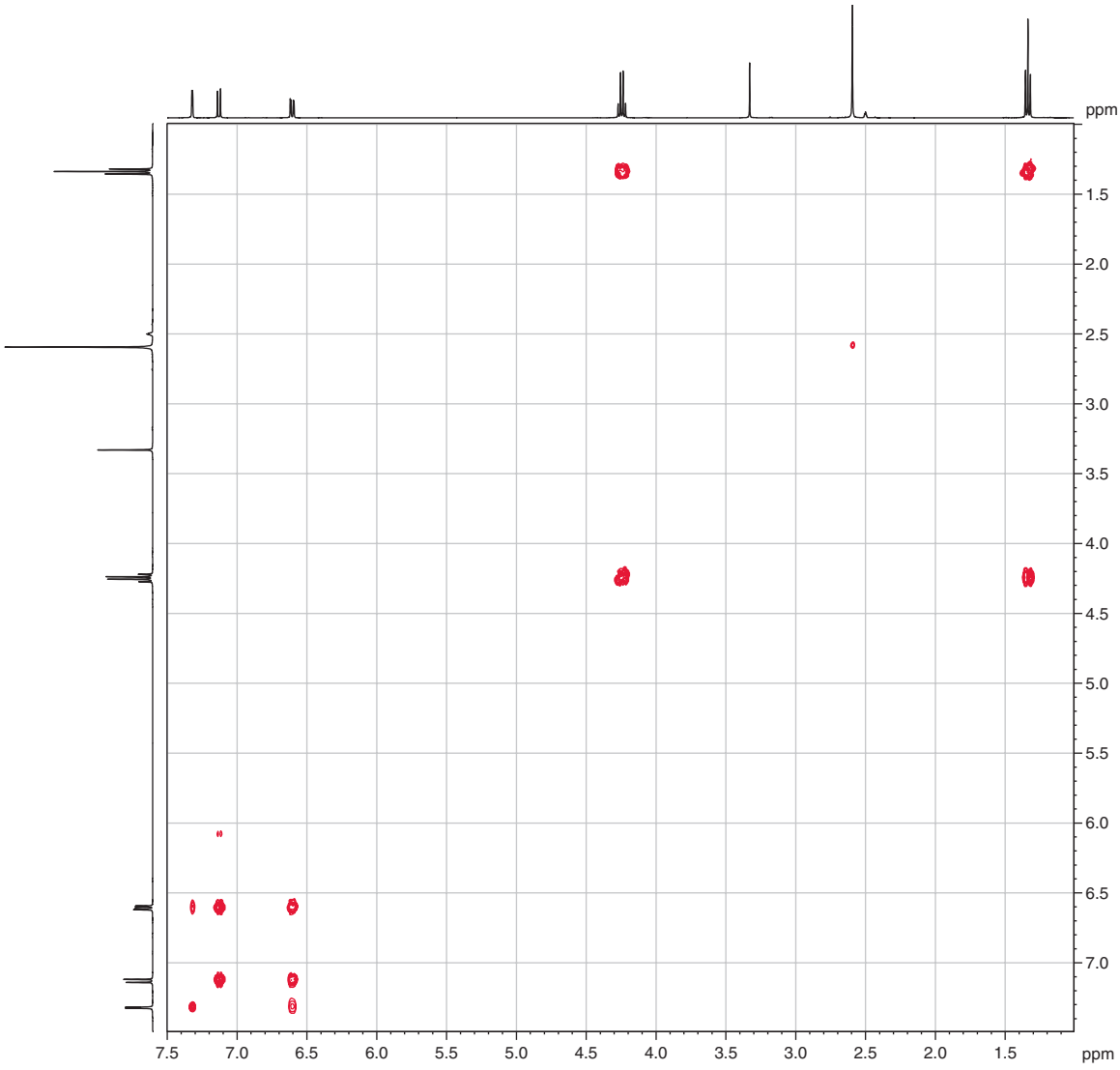


Spectrum 15.5 2-D ROESY.

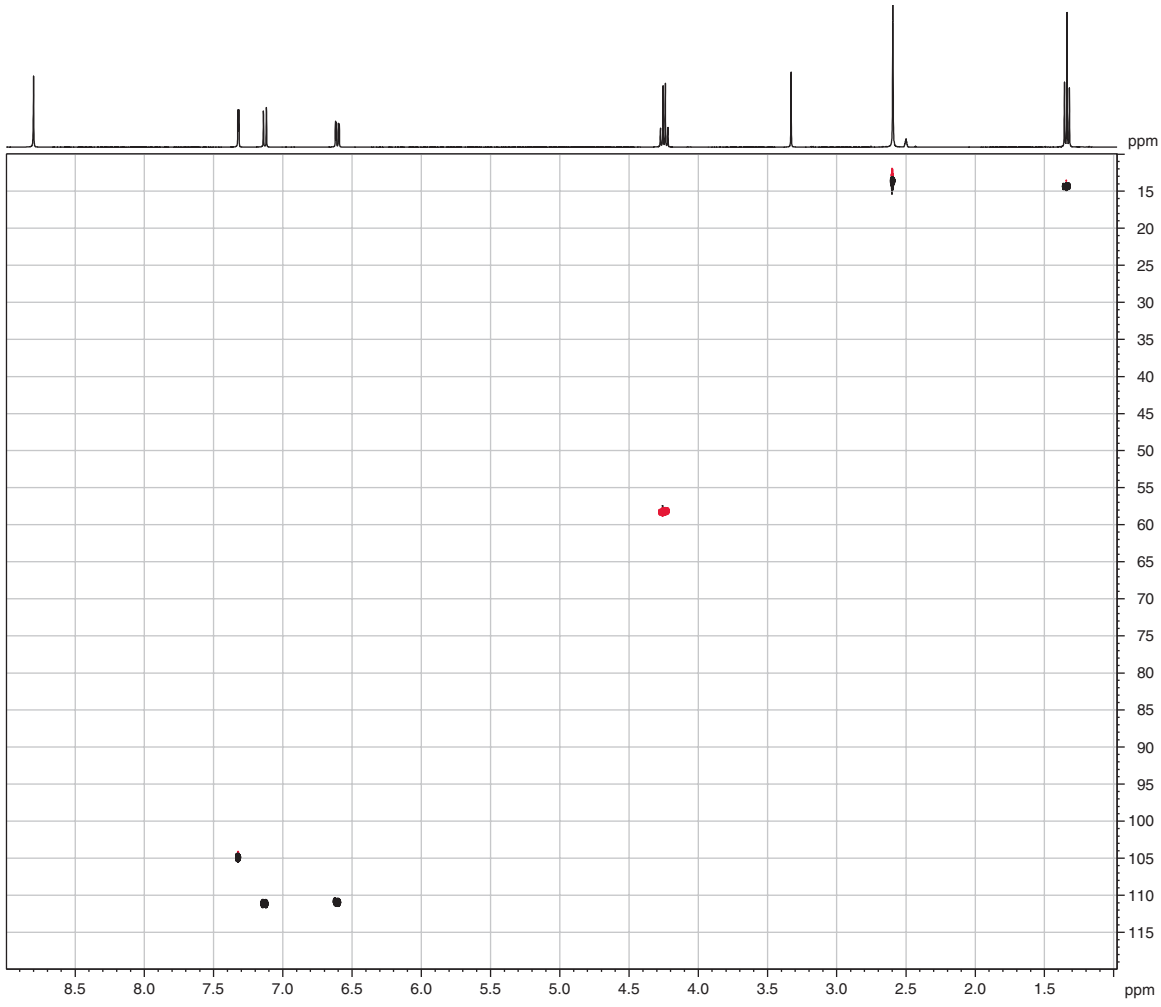
Q6. It's another indole problem but maybe a bit more difficult. It has a formula of $C_{12}H_{13}NO_3$. The spectra are given in Spectra 15.6–15.9. Enjoy!



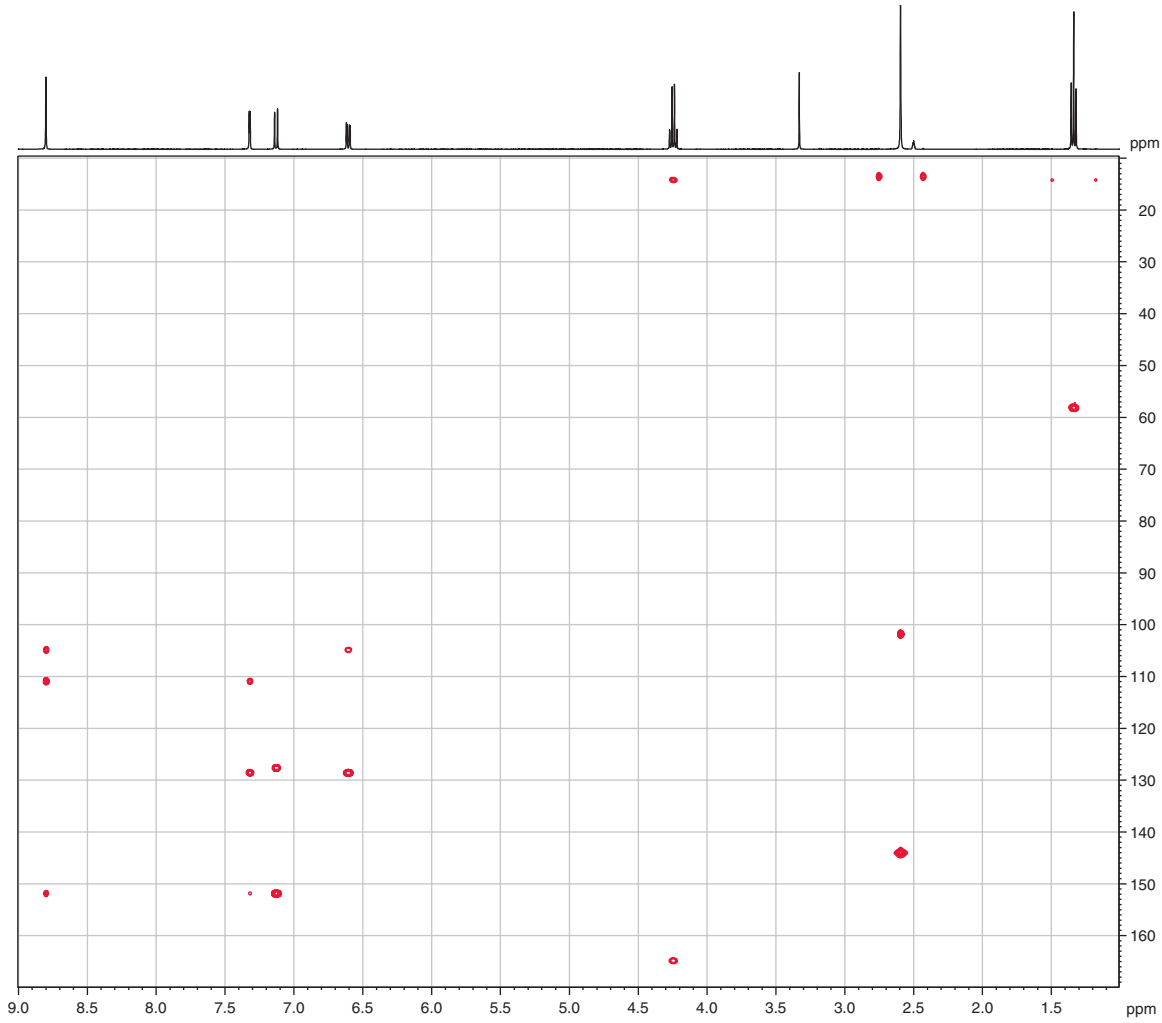
Spectrum 15.6 ^{1}H 1-D.



Spectrum 15.7 COSY.

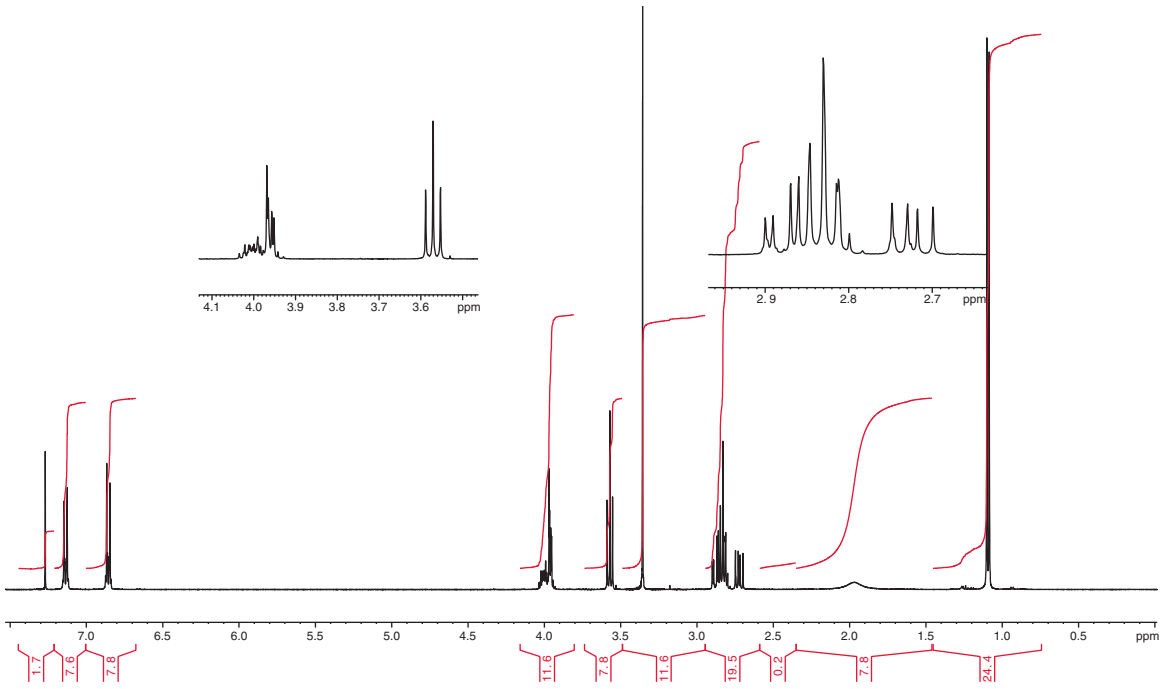


Spectrum 15.8 DEPT-edited HSQC.

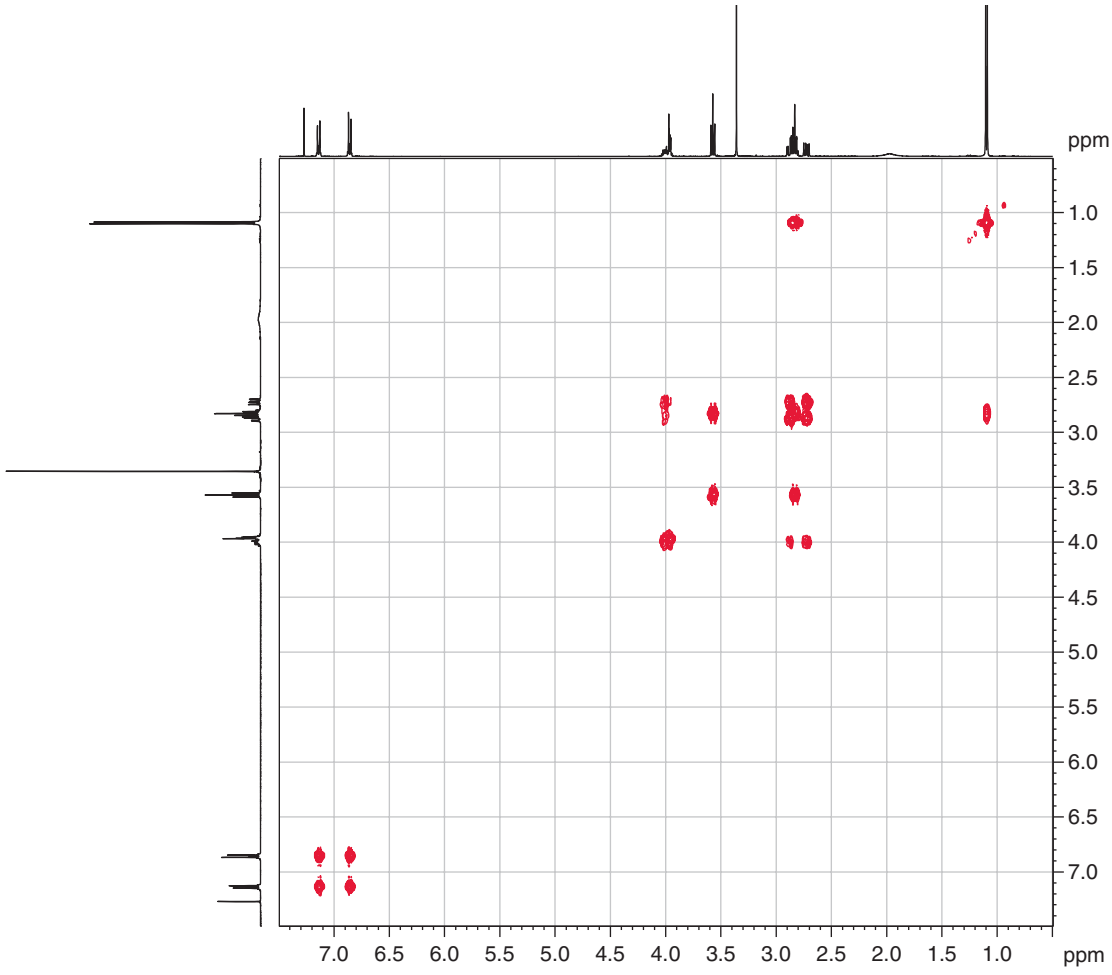


Spectrum 15.9 HMBC.

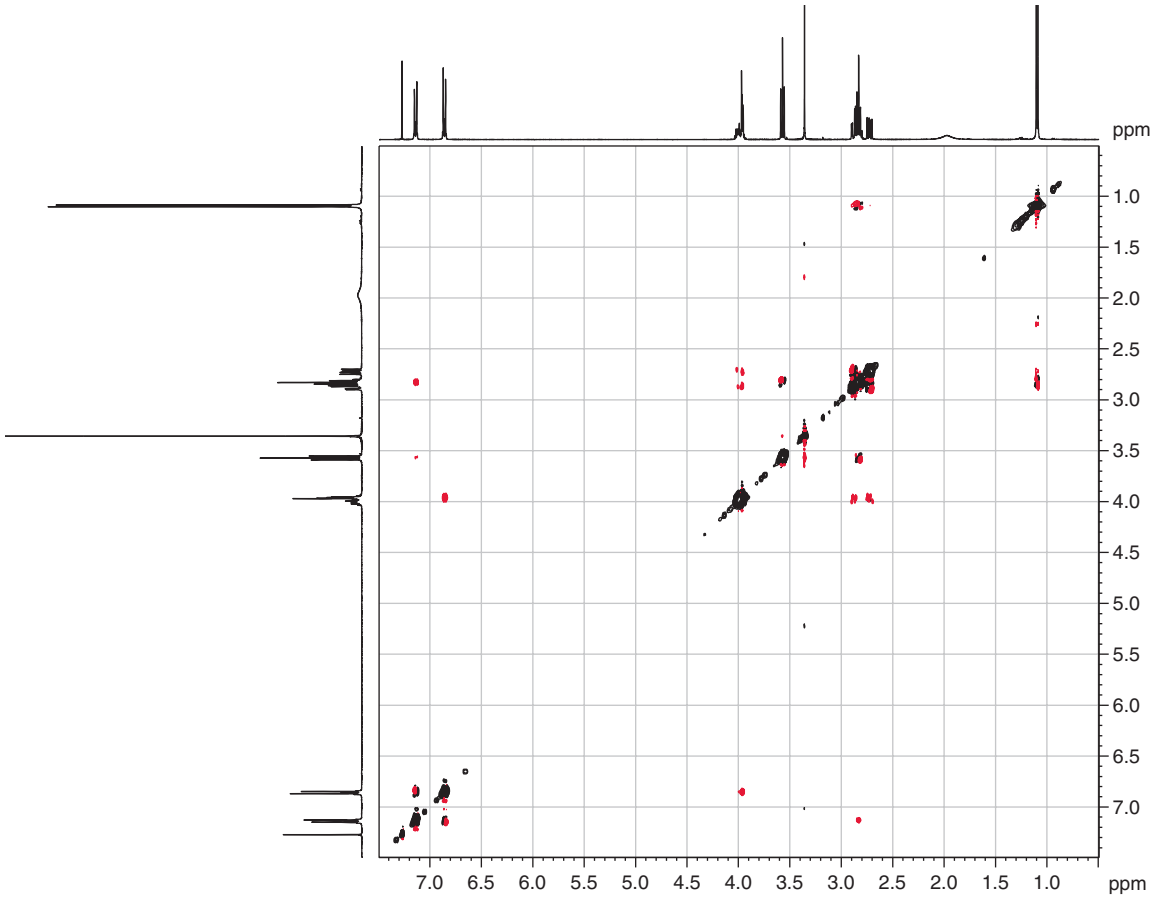
Q7. Propose a structure from Spectra 15.10–15.13. The compound is known to be a free base and is composed of only carbon, hydrogen, oxygen and nitrogen. It has a molecular weight of 267. To further complicate matters, the compound was extracted into $CDCl_3$ solution from D_2O/sodium carbonate so that no exchangeable protons can be observed.



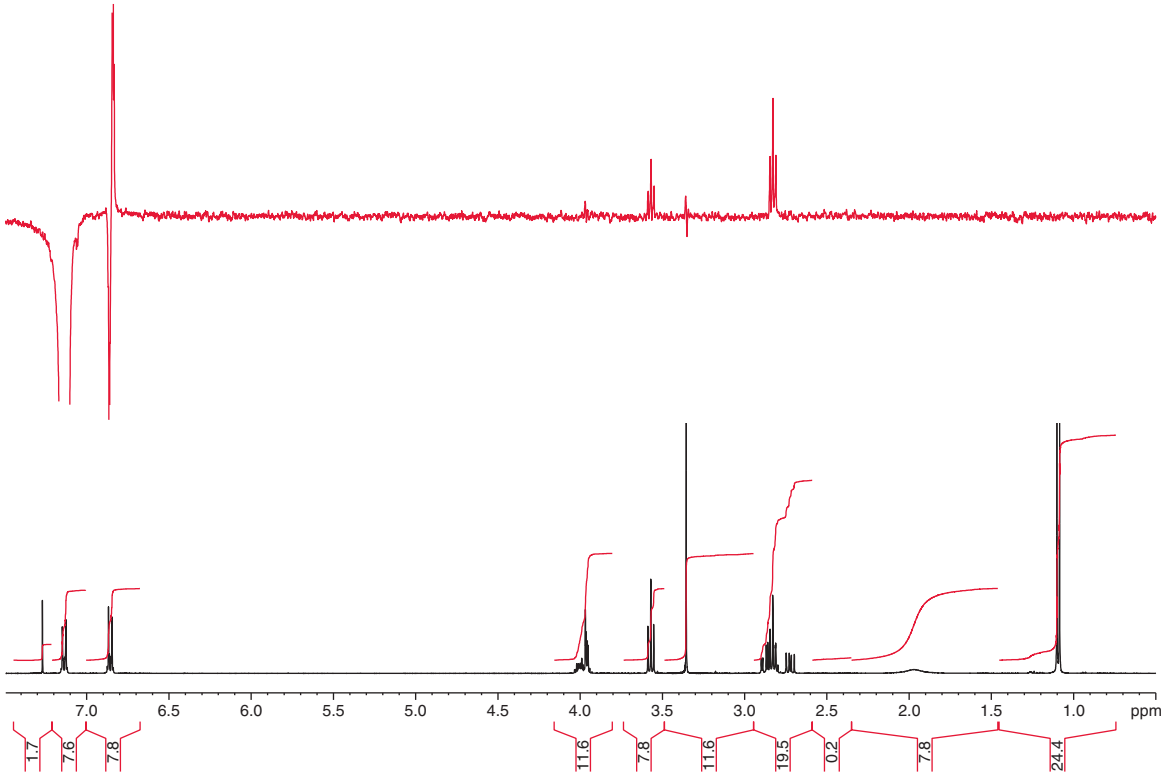
Spectrum 15.10 ^{1}H 1-D.



Spectrum 15.11 COSY.

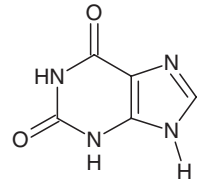


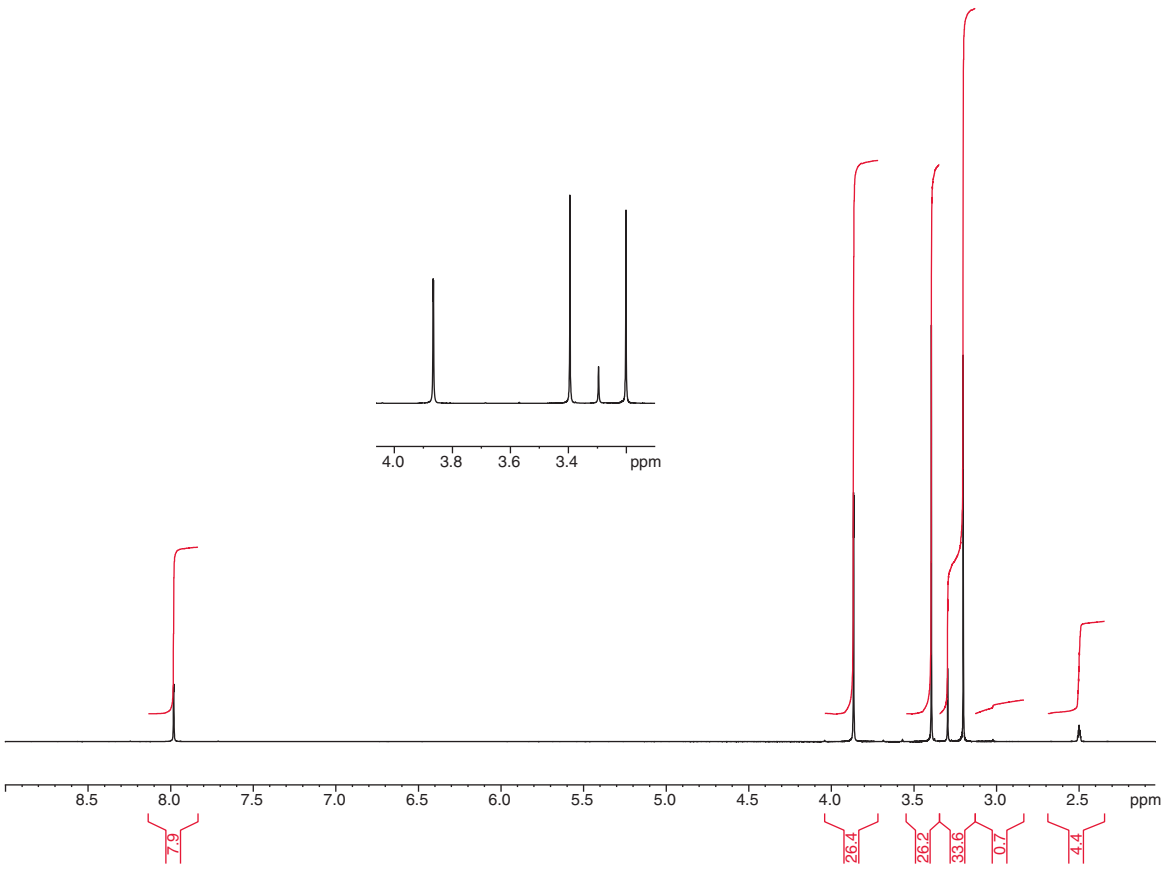
Spectrum 15.12 2-D ROESY.



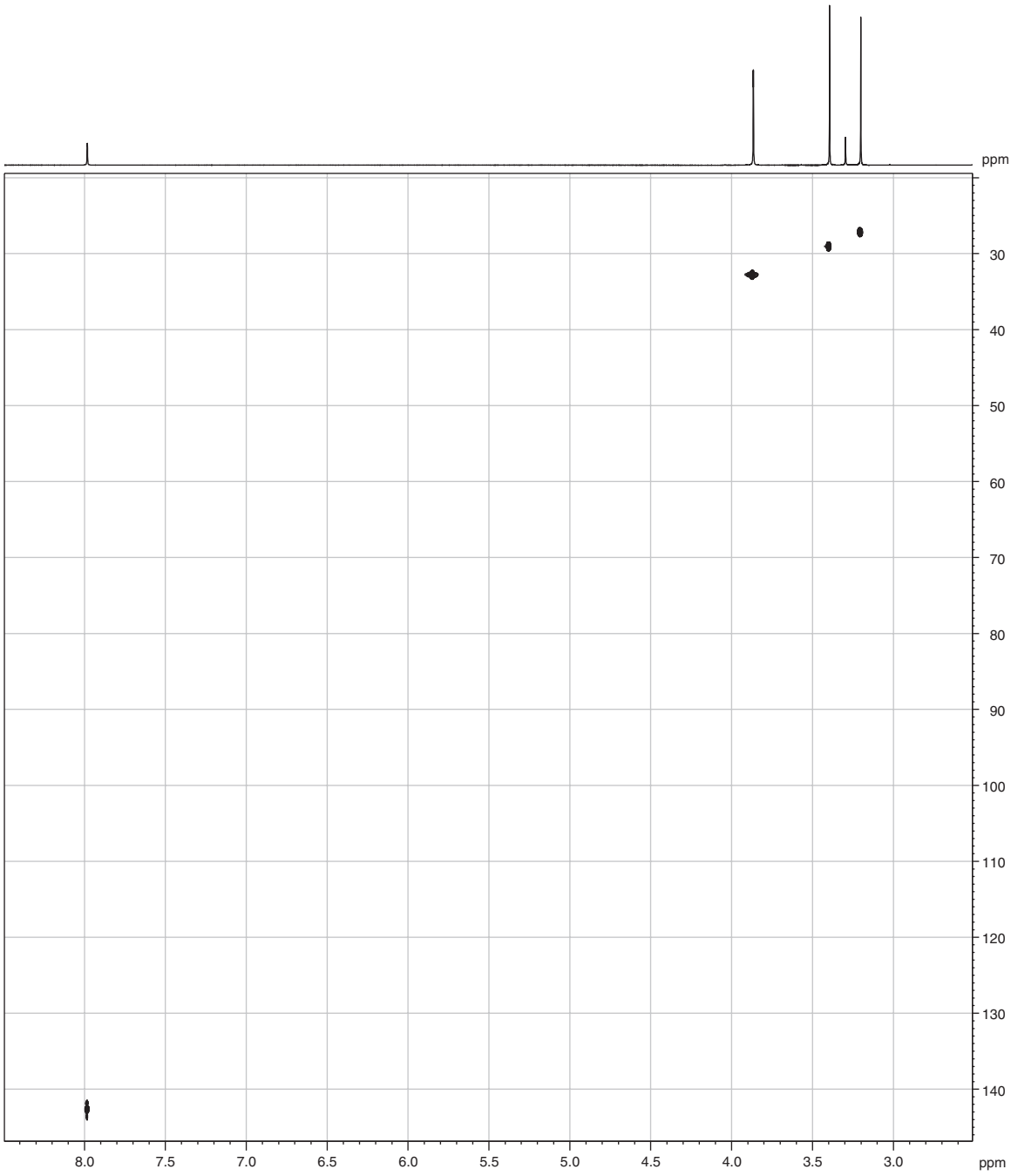
Spectrum 15.13 1-D ROESY (irradiation at 7.15 ppm).

Q8. The following heterocycle is known to have been methylated at three positions. How would you determine which they are? *Note*: Spectra 15.14–15.16 acquired in DMSO.

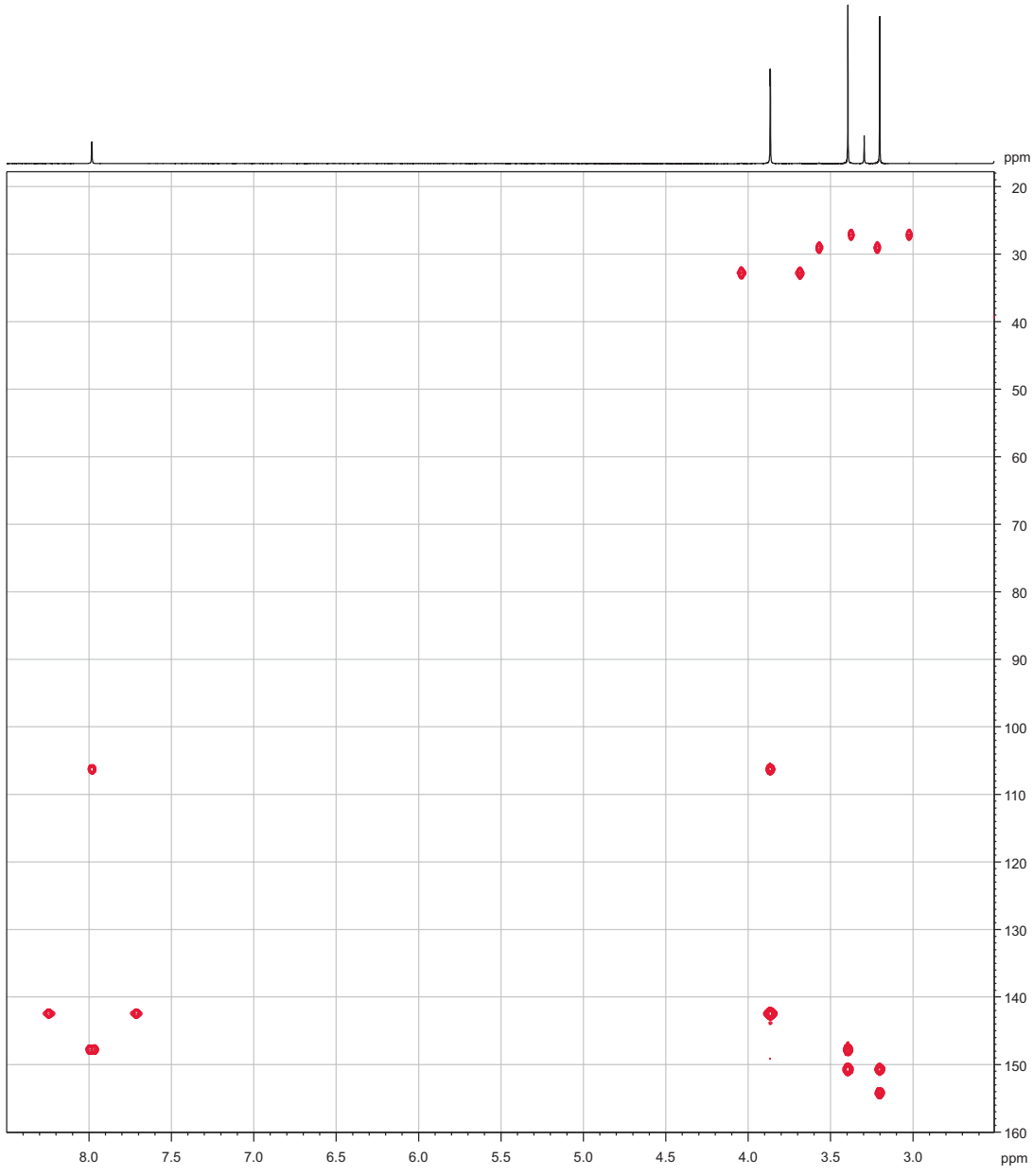




Spectrum 15.14 1H 1-D.



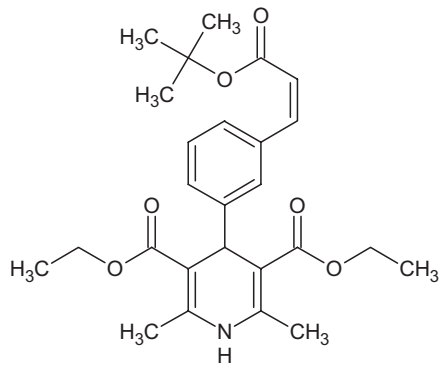
Spectrum 15.15 DEPT-edited HSQC.

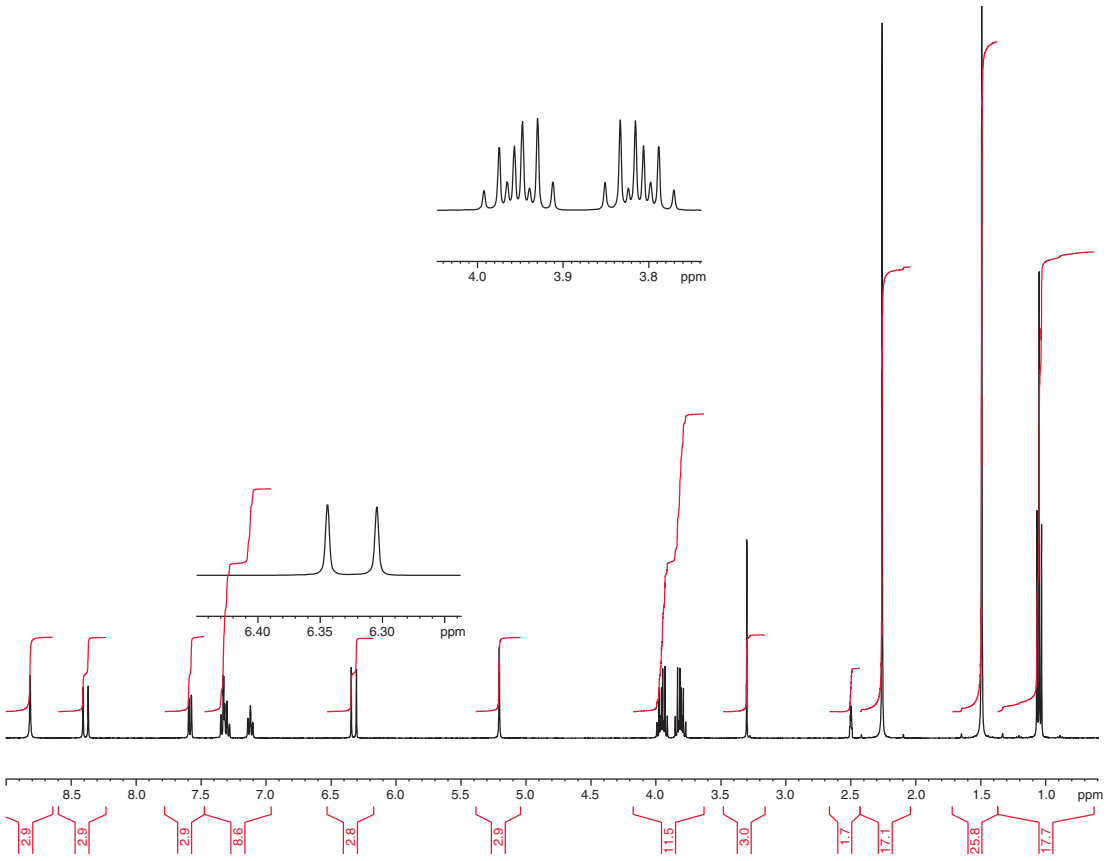


Spectrum 15.16 HMBC.

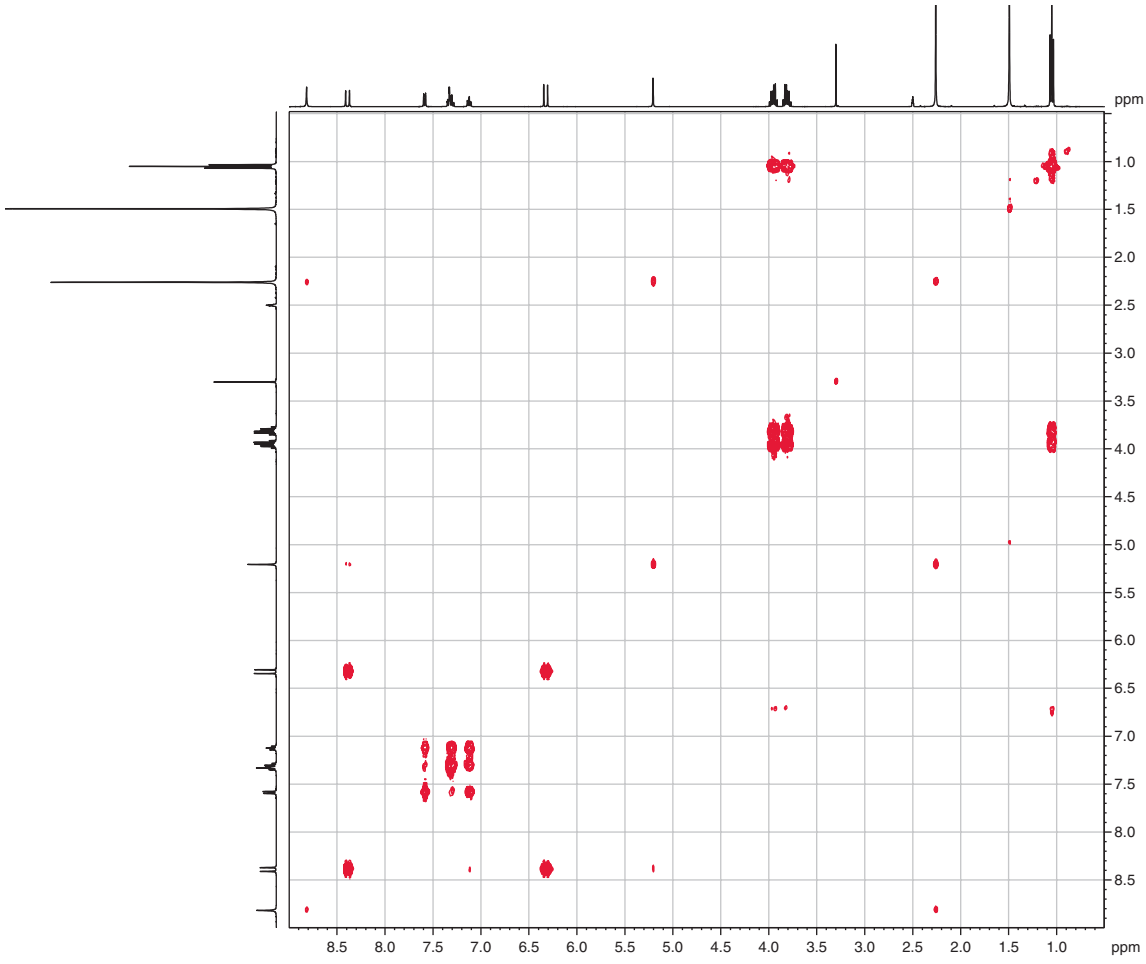
Q9. A compound comes to you for a routine check (Spectra 15.17–15.21). The alleged structure is shown below but previous experience of compounds from this source makes you naturally suspicious! Examine the proton spectrum (Spectrum 15.17) to see if these suspicions are justified and if so, can you suggest an alternative structure that better fits the data? What extra level of

reassurance would you like to see before you would be totally happy with the compound and what technique(s) would you employ to achieve this?

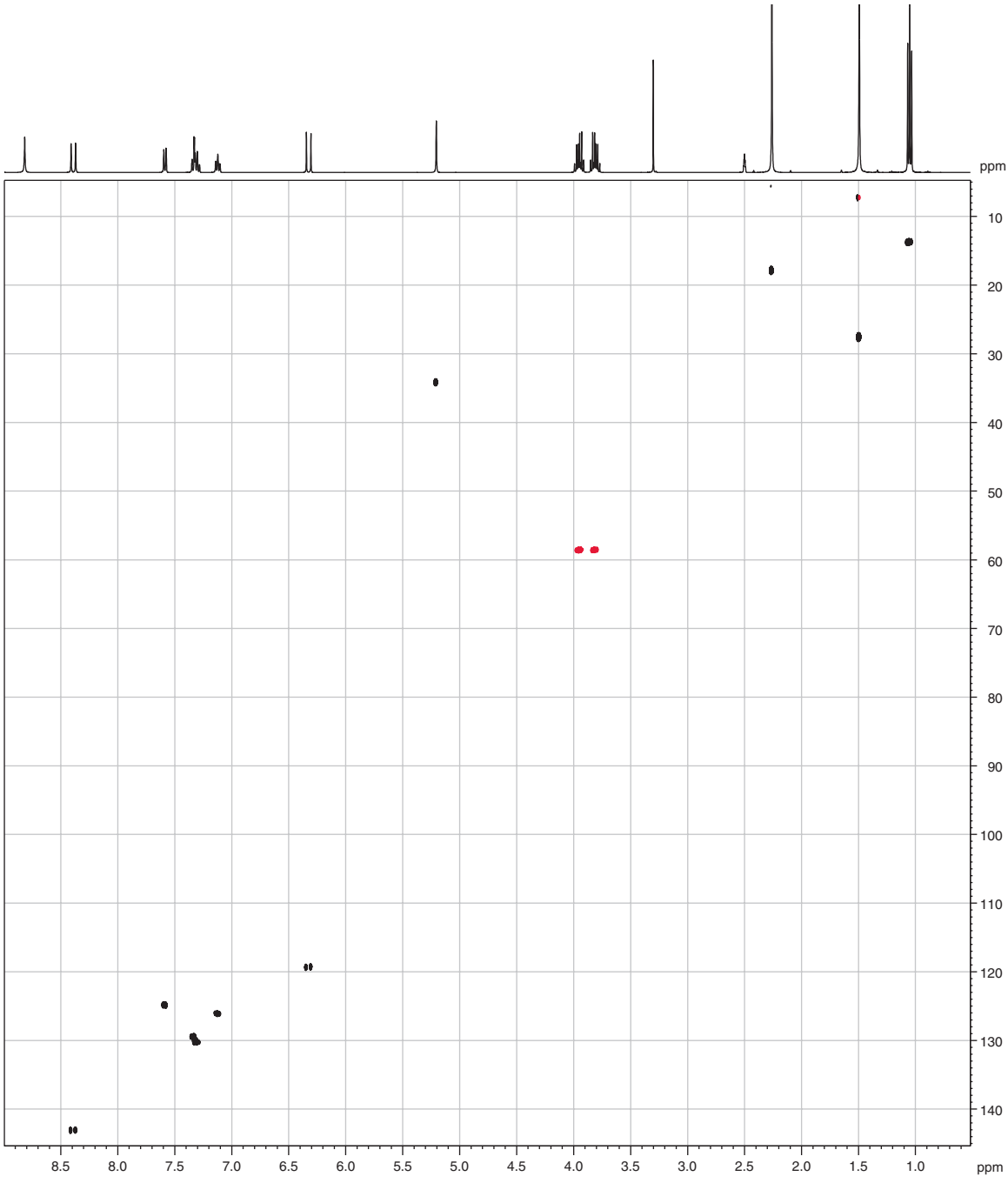




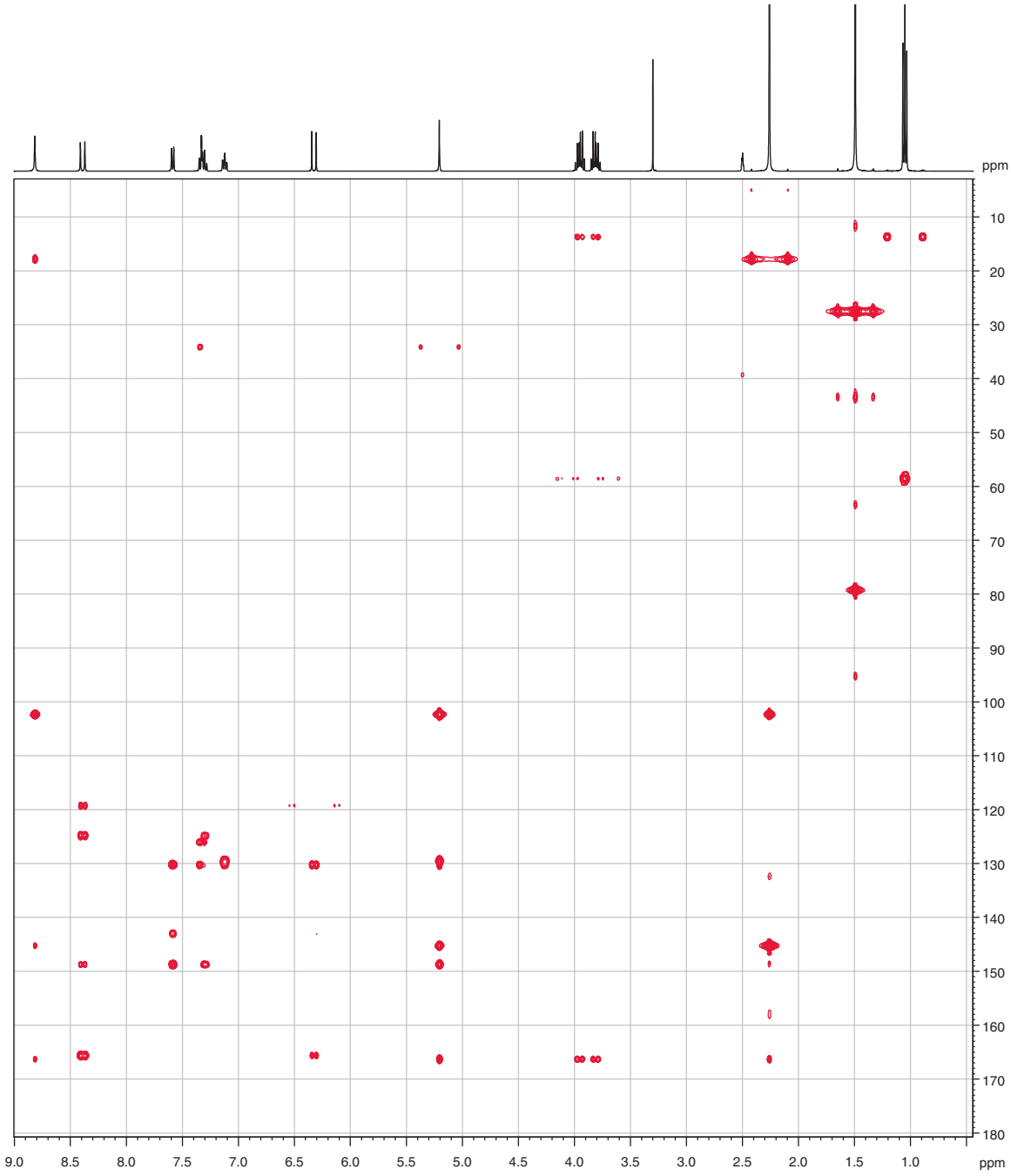
Spectrum 15.17 ^{1}H 1-D.



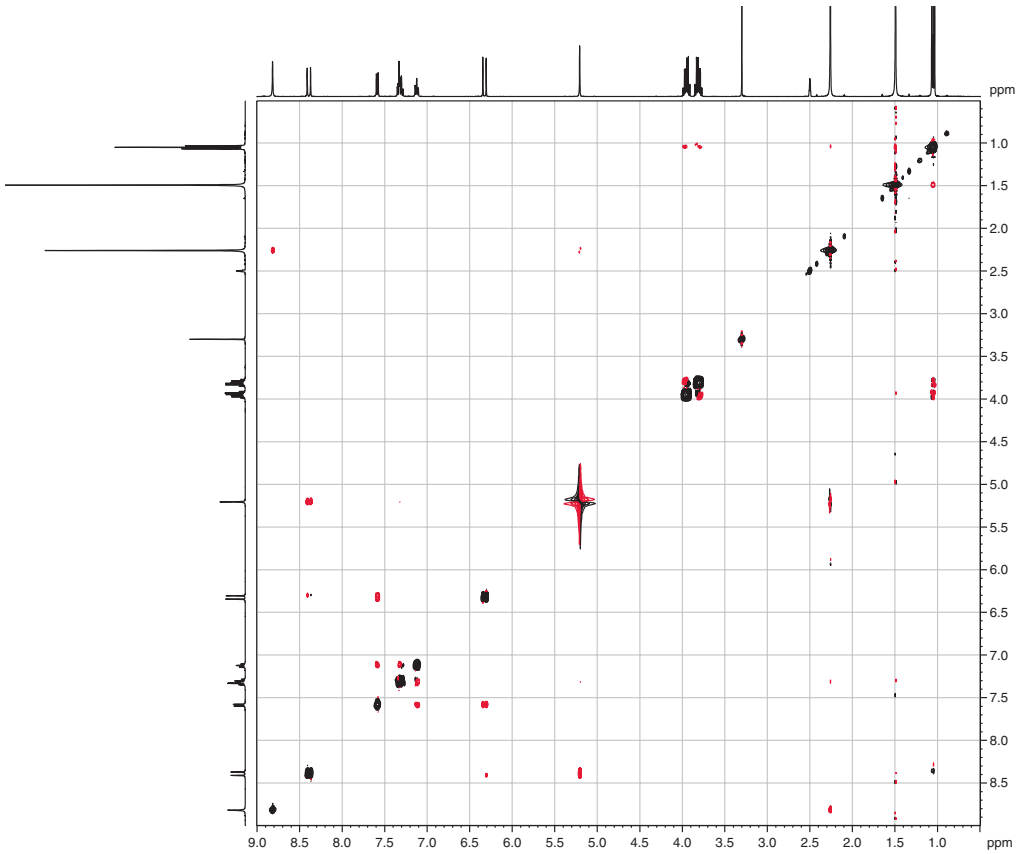
Spectrum 15.18 COSY.



Spectrum 15.19 DEPT-edited HSQC.



Spectrum 15.20 HMBC.



Spectrum 15.21 2-D ROSEY.

Q10. You have secured the post of 'Head of Structural Verification' in a small pharmaceutical company (by the strategic deployment of some particularly interesting pictures of the chairman at last year's Xmas party) and you have a capital budget of £ 350 000 for the year. What do you spend it on?

15.2 Hints

Q1. Run the proton spectrum in a suitable solvent. It's always the best way to begin! Stop and think about it. The answer may be right there in front of you. Maybe there is no need for any further experiments. As the state of protonation of the pyridine nitrogen is unknown so chemical shift information may be unreliable but the spin coupling should be relatively unaffected by this.

Q2. Think about through-space interactions.

Q3. This pair of isomers would indeed give similar proton spectra. There would be no obvious feature available from the basic proton spectrum to facilitate differentiating them. You need a further technique that can be used to exploit the different spatial relationships between the protons in both compounds.

Q4. Two very simple little molecules but they have no structural difference between them. It looks like it will have to be a question of discrimination on the basis of some predictable and *significant* chemical shift differences.

Q5. Check out the proton spectrum first! Extract as much information as possible before considering any of the other spectra. Given that the proton spectrum serves to eliminate most of the potential isomers, select the spectrum that yields the information required most directly and unambiguously.

Q6. Once again, wring all the information available from the proton spectrum first. The proton spectrum is your friend! What can you deduce about the number, nature and likely positions of the substituent(s)? What do you need to 'firm up?' What further techniques are needed?

Q7. This is a tricky one! Take your time and see if you can identify any 'special feature' in the 1-D proton spectrum which will help give you an important lead. Think about the consequences of protons having similar chemical shifts – both when they are coupled to each other and when they are not.

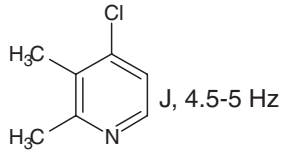
Q8. Take a look at the proton spectrum. Which site has not been methylated? So far so good. Can proton techniques help any further? If not, it could be a good idea to acquire some data for another nucleus.

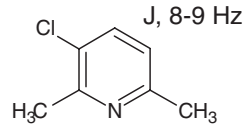
Q9. Check out the aromatic region. Are you happy with the splitting pattern? Now do the same with the alkene.

Q10. Hmm! Tricky one. But there can only be one answer.

15.3 Answers

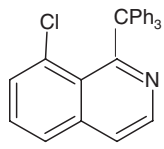
Q1. Measure the coupling between the two pyridine protons accurately. Now check the value against data quoted in Table 5.5 and all should become clear. . .

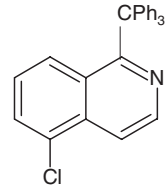




This data is solid. There are numerous other confirmatory experiments that could be performed of course but they would not be really necessary.

Q2.

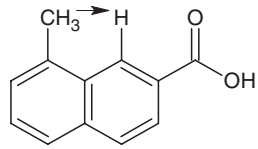


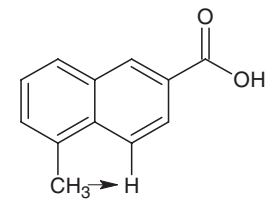


The compound on the left should give a relatively unremarkable proton spectrum as none of its protons are in a position to get anywhere near the highly anisotropic trityl (-CPh_3) group.

The proton para to the chlorine in the compound on the right however, would certainly be held very close to the trityl moiety and be likely to exhibit an unexpected chemical shift and would probably be shielded by a whole 1 ppm and maybe more.

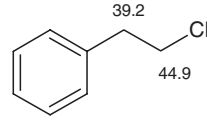
Q3.

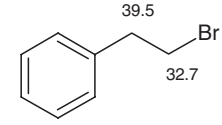




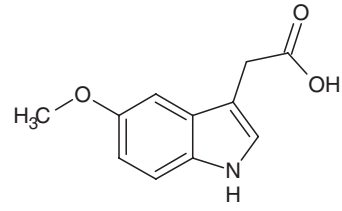
Whilst these two isomers could undoubtedly be differentiated by using HMBC, this is a problem that would be best solved by using an NOE technique. The aryl methyl would provide an ideal target for irradiation. Such an experiment would be expected to give strong enhancements in either case as shown above. As couplings of any enhanced signals are maintained in Overhauser experiments, the distinction between the two would be immediately obvious.

Q4. The proton spectra of these two compounds are very similar and so it would be unwise to try to discriminate between them in this way. The ^{13}C spectra however, would show differences in the alkyl chain which would be both significant and predictable.





Q5. The correct structure is shown below:

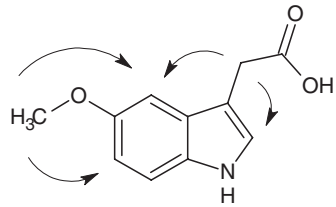


From the proton spectrum, it can be seen that there is one substituent on the 6-membered indole ring and because one of the signals on this ring (the doublet of doublets) has a chemical shift of 6.7 ppm, then it is a requirement for this substituent to be shielding in character. Check the shifts of indole itself in the relevant table! Given the choice of the two substituents, then it must be the -OCH_3 that is located on the six-membered ring. Given the observed coupling pattern for this ring, two positions for this substituent would be possible: the 5 and 6 positions. This will be defined later by reference to some further spectroscopic method.

The -CH_2-COOH substituent must therefore reside on the five-membered ring and it defines its position by the chemical shift of the proton that remains on this ring. This substituent has relatively little influence on the chemical shifts of protons ortho to it and so a shift of 7.18 ppm is immediately indicative of a proton in the 2 position and therefore the substituent in the 3

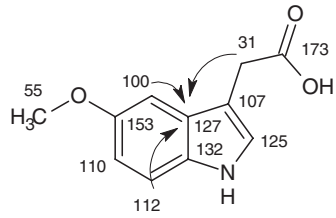
position. Note that all groups of the type -CH_2-X tend to be fairly neutral in terms of their shielding/deshielding characteristics on aromatic systems, with the exception of -CH_2-NR_3^+ which is moderately deshielding. (With the group in the 2 position and a proton in the 3 position, we would be looking for a chemical shift of about 6.7 ppm for this proton).

The final piece of the puzzle must be to define the position of the methoxy group and this is best performed in this case by use of an NOE-based experiment. The ROESY experiment shows clear enhancements, as indicated below:



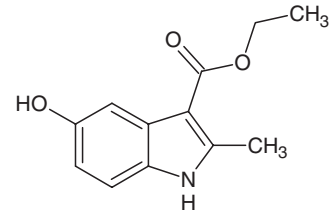
The common enhancements from both the substituents to the 4 proton is pivotal in defining the methoxy group as a 5 rather than a 6 substituent. Note the small coupling between the indole NH and the indole 2 proton that can be seen in the COSY spectrum. Observing this coupling is *not* proof that this proton is in the 2 rather than the 3 position as the 'zigzag' path between the indole NH and an indole 3 proton facilitates a similar sized, four-bond coupling between them! This is typical in indoles and analogous heterocyclic compounds.

So, full marks if you opted for the NOE-based approach to solving this problem. This of course, in no way implies that solving by the HMBC approach is wrong! A full carbon assignment with key correlations is shown below:



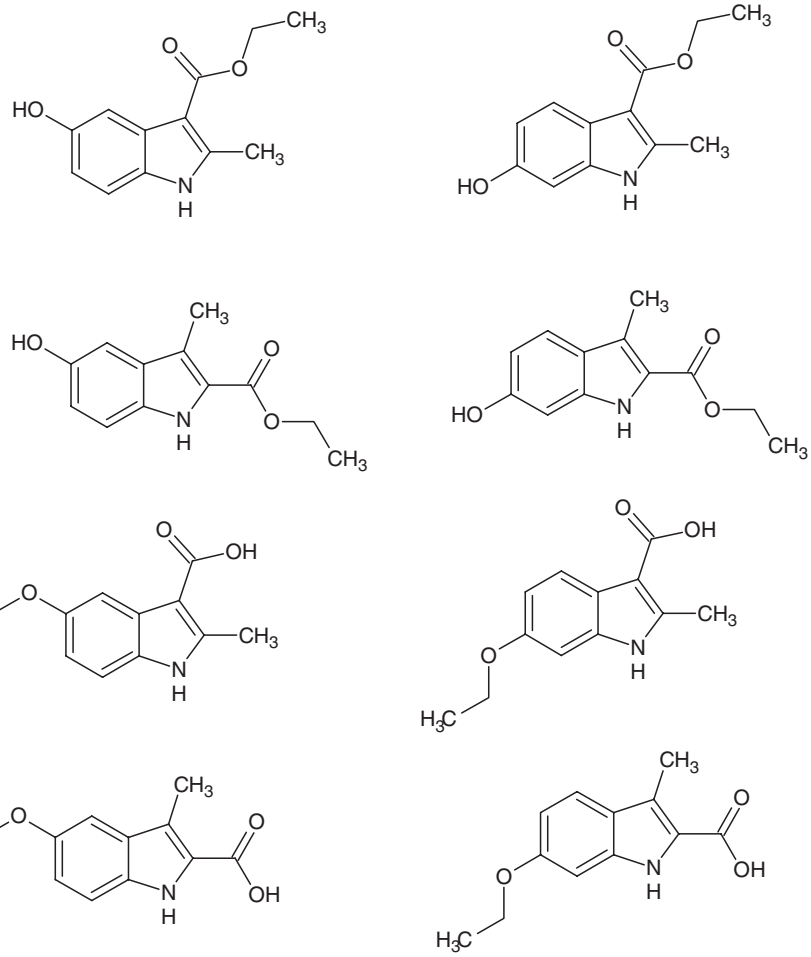
Use of the HSQC and HMBC spectra to assign the compound and establish key connectivities in this way, unambiguously establishes the substitution positions of both groups but is a lot more labour-intensive than the NOE-based approach.

Q6. The correct structure is shown below:



Once again, the proton spectrum reveals a shielding group attached to the six-membered ring of the indole in either the 5 or the 6 position and, given the molecular formula, this has to be oxygen-based. It is also clear that there is an ethyl group present and the shift of the -CH_2 of this group shows that it is either part of an aryl-ethyl ether, or that it is part of an ethyl ester (note that both systems would give similar shifts for the respective -CH_2s!). We also have evidence of a methyl singlet with a shift typical for a methyl attached to an aromatic moiety. Given that there are clearly three protons on the six-membered indole ring (and therefore only one substituent) and that no other heterocyclic protons are visible, it is logical to conclude that there must be two substituents on the five-membered ring, one of which being a methyl group and the other either a carboxylic acid or an ethyl ester, depending on whether the substituent on the six-membered ring is a phenol or an ethyl ether.

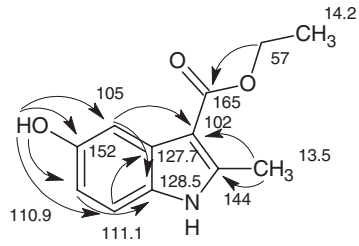
Given the molecular formula, this gives eight possible isomers which would all give perfectly plausible structures to fit the observed proton spectrum:



Note that any attempt to differentiate carboxylic acids from phenols on the basis of how broad their respective signals are is to be discouraged in the strongest possible terms! Whilst carboxylic acids *tend* to be broader than phenols, it is by no means guaranteed that this is always the case. Steric and electronic factors and hydrogen bonding can reverse this in certain situations.

Conclusive validation of the correct structure in this case takes a little thought. NOE-based experiments will tend to be less useful in these circumstances because in isomers where the methyl group is in the 2 position, no useful NOEs may be observed – note that an ethyl ester is inherently flexible and NOEs between the ethyl ester protons and any aromatic protons would be unlikely and unreliable. Furthermore, relying on exchangeable protons for the purpose of gathering NOE data is not recommended and is often unfeasible if such signals are broad. Attempting to gather NOE data from compounds which contain more than one exchangeable is even more ill advised. In this case, the possibility exists for bogus relayed NOEs from the phenol via the indolic NH!

HSQC/HMBC is the way to nail this problem. The full assignment with key correlations is shown below:

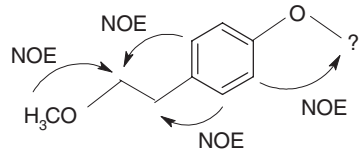


The first obvious deduction is that the compound must be an ethyl ester rather than an ethyl ether as the -CH_2 correlates to a carbonyl carbon at 165 ppm. No other protons correlate to this carbon so even without any ^{13}C prediction, the ethyl ester is confirmed. The relative positions of the ester and methyl substituents are confirmed as if they were reversed, a common correlation from both the methyl protons and the indole 7 proton to one of the ring junction carbons (127.7 ppm in this compound) would be expected. Finally, the position of the -OH is confirmed by comparison with ^{13}C prediction data for both the 5 and the 6 isomer and by the weak but significant correlation from the 4 proton to the 3 carbon at 102 ppm. Note that correlations from the -OH in this case are a bonus. Exchangeable signals are often too broad to give useful correlations.

Q7. Casting an eye over the proton spectrum, the AB part of an ABX system immediately presents itself at 2.88 and 2.73 ppm. This means the molecule contains a chiral centre!

Working methodically from left to right, it is clear from the proton spectrum that the compound has a single aromatic ring and that it is 1,4 disubstituted. One of the substituents is fairly neutral as one half of the aromatic AA′BB′ system has a chemical shift of about 7.14 ppm whilst the other is quite strongly shielding as the other half of the AA′BB′ is at about 6.8 ppm. Since the compound is known to contain both oxygen and nitrogen, it is quite reasonable to deduce that the shielding entity must be one of these two atoms. Further investigation will be required to determine which it is in due course.

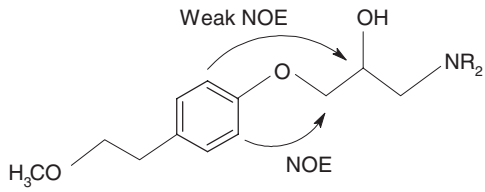
Now consider the ROESY experiment. It is clear that the aromatic protons at 7.14 ppm show an enhancement to the multiplet, or rather, one element of the multiplet, at around 2.83 ppm. This would be a typical shift for an alkyl substituent. Similarly, the aromatic protons at 6.8 ppm enhance protons between 4.1 and 3.9 ppm. This would be a typical shift for an aromatic O alkyl substituent and is important information. Remember that the compound has been base extracted from D_2O/sodium carbonate and for this reason, no exchangeable protons will be visible. The fact that both sets of aromatic protons show enhancements to different alkyl protons means that there must be alkyl substituents on both ends of the ring (as opposed to an -OH at one end and everything else attached at the other). The aromatic ring therefore conveniently splits the molecule into two segments that can be dealt with separately. The 2-D ROESY has certainly proven to be very useful so far but the severely overlapped nature of the alkyl protons makes it difficult to see *exactly* what is being enhanced. For this reason, specific 1-D ROESY experiments hold a big advantage as the enhanced multiplet is always reconstructed complete with all couplings. Two signals show enhancement from the aromatic protons at 7.15 ppm and they have the appearance of a pair of coupled triplets. By inspecting the ordinary 1-D proton spectrum, it becomes clear that this must be a $-CH_2-CH_2-$ system with the triplet at 2.83 ppm more intense in appearance than its coupled partner at 3.57 ppm. This shift looks good for another oxygen and in fact, a $-OCH_3$ as another 1-D ROESY irradiating the singlet at 3.35 ppm establishes the connection between this singlet and the triplet $-CH_2$ at 3.57 ppm. So piecing together what we have so far, we're looking at something like this:



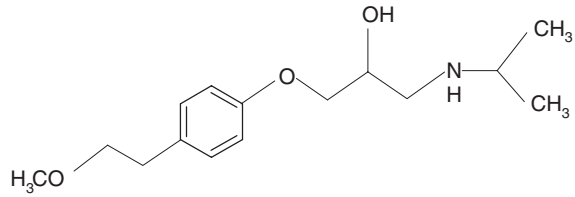
Concentrating now on the right hand side of the molecule and re-examining the signals which show enhancement from the aromatic protons at 6.8 ppm, it would seem that the entire multiplet (4.05–3.93 ppm) which integrates for three protons is part of a close-coupled non-first order spin system. The coupling between these protons is not at all clear from the COSY spectrum because the chemical shifts of the protons are so close. The coupling is more apparent from close scrutiny of the 1-D proton spectrum. The right hand side of this multiplet (3.98–3.93 ppm) consists of heavily roofed eight-line system which is characteristic of the AB part of an ABX system where the shifts of 'A' and 'B' are extremely close. The A-X and B-X couplings are not obvious from the COSY because the 'X' is extremely close to 'A' and 'B' and in fact is the left hand side of the multiplet (4.05–3.98 ppm)!

The complexity of this spectrum does not end there however as two key features of this spectrum must now be addressed. First, the 'X' part of the ABX system we have just discussed consists of far more than the normal four lines; and second, the four-line multiplets centred at 2.88 and 2.73 ppm are clearly 'A' and 'B' parts of a second ABX system! These features are linked in that the COSY spectrum clearly shows that the complex 'X' part (4.05–3.98 ppm) is in fact coupled to both the 'A' and 'B' parts of the second ABX system. Therefore, we can deduce that

the 'X' part is common to *both* ABX systems. Chemical shifts indicate that a likely arrangement of hetero atoms would give a right hand side for the molecule looking like this:



Almost home and dry now! Back to the COSY. The six-proton doublet at 1.1 ppm shows a coupling to something at 2.83 ppm. We know that the triplet at 2.83 ppm is part of the closed spin system on the left hand side of the molecule and therefore cannot in any way be responsible for this correlation. Measuring the integral from 2.91–2.78 ppm reveals the presence of *four* protons. One of them has already been assigned as part of the second ABX system and the triplet at 2.83 ppm accounts for two protons. Then, the implication must be that one proton is almost completely hidden from view beneath these two signals. In terms of chemical shifts, an isopropyl group attached to the nitrogen would fit perfectly. So fitting it all together, we have:



(This is the drug Metoprolol, a beta-blocker). Obviously, a great many deductions have to be made to arrive at a structure from scratch in this way and whilst each one in this example is valid in its own right and they all fit together perfectly well with no obvious conflicts, structural verification via the HMQC/HMBC route would be advisable!

Q8. A quick inspection of the proton spectrum for this compound confirms that a heterocyclic proton is present at 8.0 ppm so C-methylation cannot have taken place. Furthermore, the proton spectrum confirms the presence of three methyl signals at approximately 3.9, 3.4 and 3.2 ppm. There is little more to be gleaned from the proton spectrum except for the fact that the methyl at 3.9 ppm is slightly broader than the other two. This is indicative of a small long-range coupling to the heterocyclic proton though this information is only of limited value. It is clear that another nucleus must be examined.

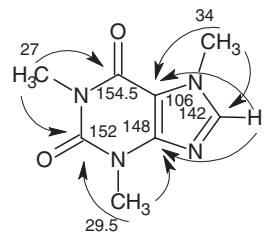
As the parent compound contains four nitrogen atoms, it might be tempting to opt for proton–nitrogen HMBC but the technique would be of limited value in this case. ^{13}C spectroscopy offers by far the most comprehensive solution. The HSQC spectrum shows that the chemical shifts for the methyls are approximately 33, 29 and 27 ppm. It is immediately clear that the methyl groups must therefore all be attached to the nitrogen atoms and not to any of the oxygens (which would give shifts in the 55–65 ppm range).

The information required to solve this problem will come from the HMBC experiment. After first discounting the one-bond couplings that have come through (either by reference to the HSQC experiment or just by observation) it can be seen that the heterocyclic CH shows two, three-bond correlations to carbons at 148 and 106 ppm. Since the carbon shifts of the methyl groups indicates that O-methylation is not an option, it is safe to assume that the oxygen atoms will still be in the form of conjugated amidic or urea carbonyl functions. The chemical shift of such carbonyls will always be in the 150–160 ppm range. We know the shift of the carbon bearing the solitary heterocyclic proton (142 ppm) and of the two remaining quaternary carbons, the one flanked by two nitrogens is likely to be far more de-shielded than the other so even without using ^{13}C prediction software, this problem should be relatively straightforward.

The salient features of the HMBC could be summarised as follows:

1. There is a common correlation from the methyl protons at 3.9 ppm and the heterocyclic proton (8.0 ppm) to a quaternary carbon at 106 ppm.
2. This proton also correlates to another quaternary carbon at 148 ppm.
3. The methyl protons at 3.2 ppm correlate to two quaternary (carbonyl) signals at 154.5 and 152.0 ppm.
4. The methyl protons at 3.4 ppm correlate to one of the carbonyls at 152 ppm and also to the quaternary carbon at 148 ppm (see item 2, above).

Putting all this information together we have: caffeine.



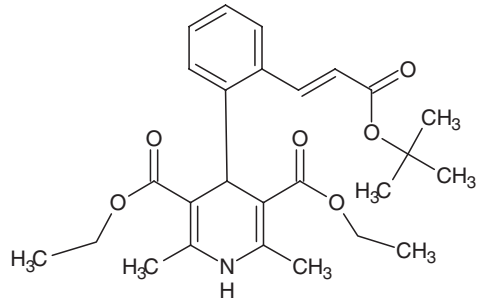
This summarizes the proton–carbon correlations and shows all the ^{13}C chemical shifts. Note that no other arrangement of the methyl groups would satisfy the observations made. For example, had one of the methyl groups been attached to the other nitrogen in the five-membered ring, then the correlation to a carbon anywhere near 106 ppm would have been replaced by one to a carbon nearer to 150 ppm.

Note also that though the methyl protons at 3.9 ppm correlate to the carbon at 142 ppm, there is no guarantee that the corresponding proton at 8.0 ppm will show a correlation to the carbon of this methyl group (34 ppm). In fact this correlation does exist but it is a lot weaker than the others and does not show up in the plot without turning up the gain to the point where the rest of the spectrum becomes difficult to understand. The apparent intensities of the observed correlations reflect the size of the proton–carbon couplings concerned. The (methyl) proton–heterocyclic carbon coupling must be significantly different from the C**H**-methyl (carbon) coupling.

Q9. At first glance, the proton spectrum for this compound looks excellent. The protons are, with the exception of two aromatic protons, well separated and this is always a bonus! The alkene protons draw immediate attention as they sit on either side of the aromatic protons and the doublet at about 8.4 ppm is definitely the alkene closest to the aromatic ring. Its coupling partner, closest to

the t-butyl ester is the doublet at approximately 6.32 ppm. The coupling between these two alkene protons looks large and measurement indicates that it is in fact 16 Hz. This is too large to support the proposed *cis* alkene and is far more in keeping with *trans* geometry! As an interesting footnote to this question of alkene configuration, a trans alkene on an aromatic ring will generally show NOEs between *both* alkene protons and the aromatic proton(s) ortho to the point of substitution, whilst the corresponding *cis* alkene can only show an NOE from *one* of the alkene protons and the ortho protons on the aromatic ring. This could provide useful back up information if the observed coupling was in any way doubtful.

Furthermore, scrutiny of the aromatic region shows coupling patterns that are not consistent with 1,3 substitution. Given that the aromatic protons are relatively well spread out – and this is an important point as little or nothing could be deduced about the substitution pattern if the substituents were such that all the aromatic protons were heavily overlapped – we should be looking to see two doublet of doublets, one with two small (meta) couplings and one with two larger (ortho) couplings. What we do observe is a pair of broad triplet structures, a broad doublet with one ortho coupling and a doublet of doublets dominated by an ortho coupling. This pattern can only occur in 1,2 disubstituted aromatic rings. Thus a far more plausible structure would be:



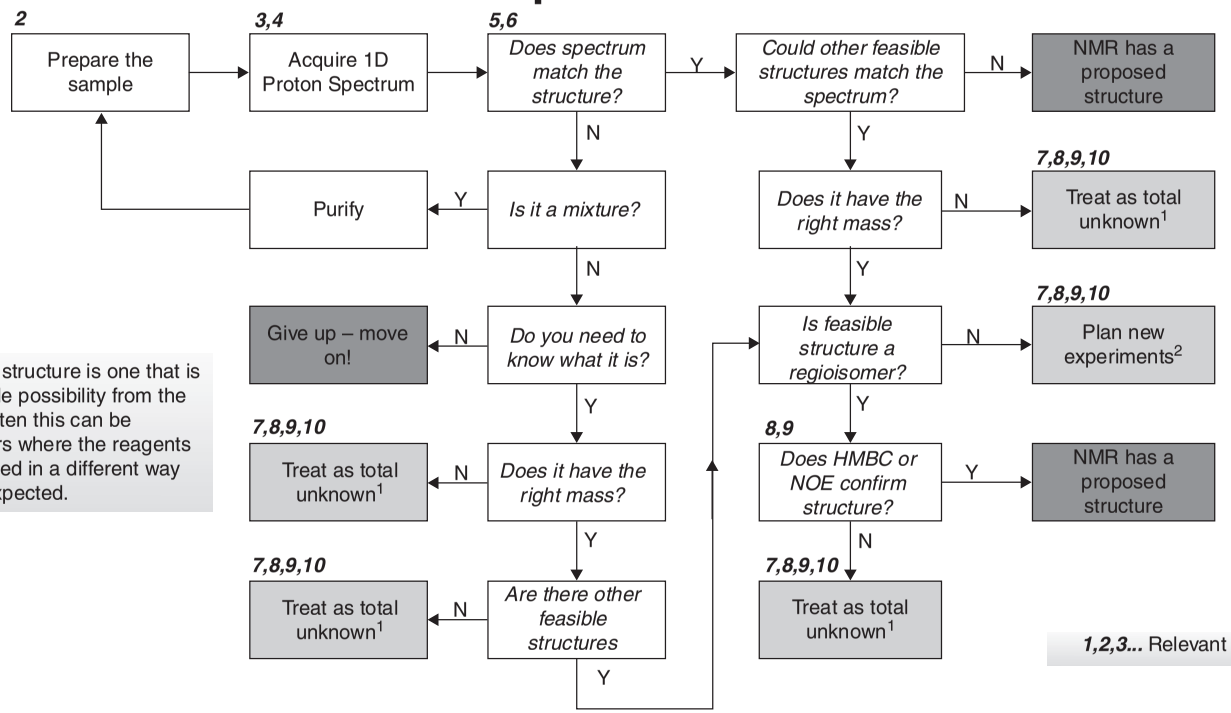
The ethyl ester protons are worthy of note in this molecule. Though there is no chiral centre present, these are non-equivalent by virtue of being diastereotopic (remember the 'Z test?').

In order to be as fully confident as possible with this compound, given the two errors already apparent, it would be advisable to check it out thoroughly with HSQC, HMBC and a ROESY. This would establish the relative positions of the ethyl ester and methyl groups. A mass spectrum might be a good idea as well!

Q10. Flippancy aside, there is at least a semiserious aspect to this tongue in cheek question. Without wishing to cause offence to any mass spectroscopist or devotee of any other form of spectroscopy, we hope that we've demonstrated (to some extent at least) the unrivalled power and flexibility of the NMR technique for elucidating chemical structures. The quality and depth of the information available is remarkable and the range of associated techniques gives the method huge versatility. If an organic compound can be dissolved then it *will* give NMR signals – no question about it. NMR may be used in a quantitative as well as qualitative manner and given the right hardware, can be applied to several key nuclei.

Spend the money wisely – on the best NMR system you can get your hands on – and don't forget to keep your camera handy at next year's office party – you might fancy an upgrade.

NMR Interpretation Flow Chart



A "feasible" structure is one that is a reasonable possibility from the reaction. Often this can be regioisomers where the reagents have attached in a different way from that expected.

[1] Treat as unknown
In the case of a total unknown it is a case of the more data, the better. Solving this sort of problem is like doing a jigsaw puzzle. You piece together information from a variety of sources to come up with a feasible structure. You then test that structure with more experiments to ensure you get a consistent answer. As a minimum you should consider COSY, HSQC, HMBC, 1D 13C. Don't forget – NMR is not the only technique so look at mass spectrometry (accurate mass in particular) and IR to help.

[2] Plan new experiments
If you have two or more possible structures that fit the data, you will need to look for differences that can be identified by NMR. Often this is HMBC and/or NOE (to identify key connectivities). Every problem is different so you need to use all your skills to look for tools that can help distinguish the putative structures. As in the case of total unknowns, don't use NMR to the exclusion of other techniques as they may be able to make the choice much easier.

Don't forget! NMR on its own cannot prove a structure.

Appendix A.1 Useful thought processes for tackling NMR problems.