# NMR Characterization of Microstates of SM14

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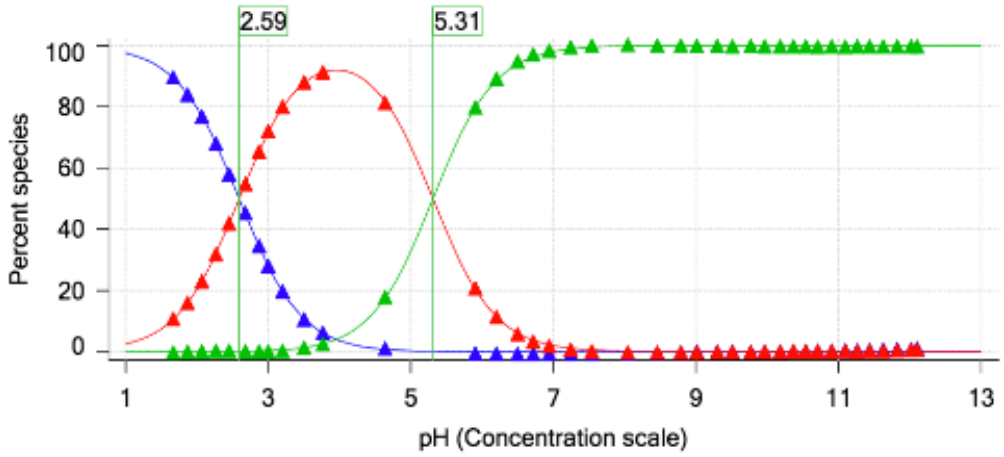
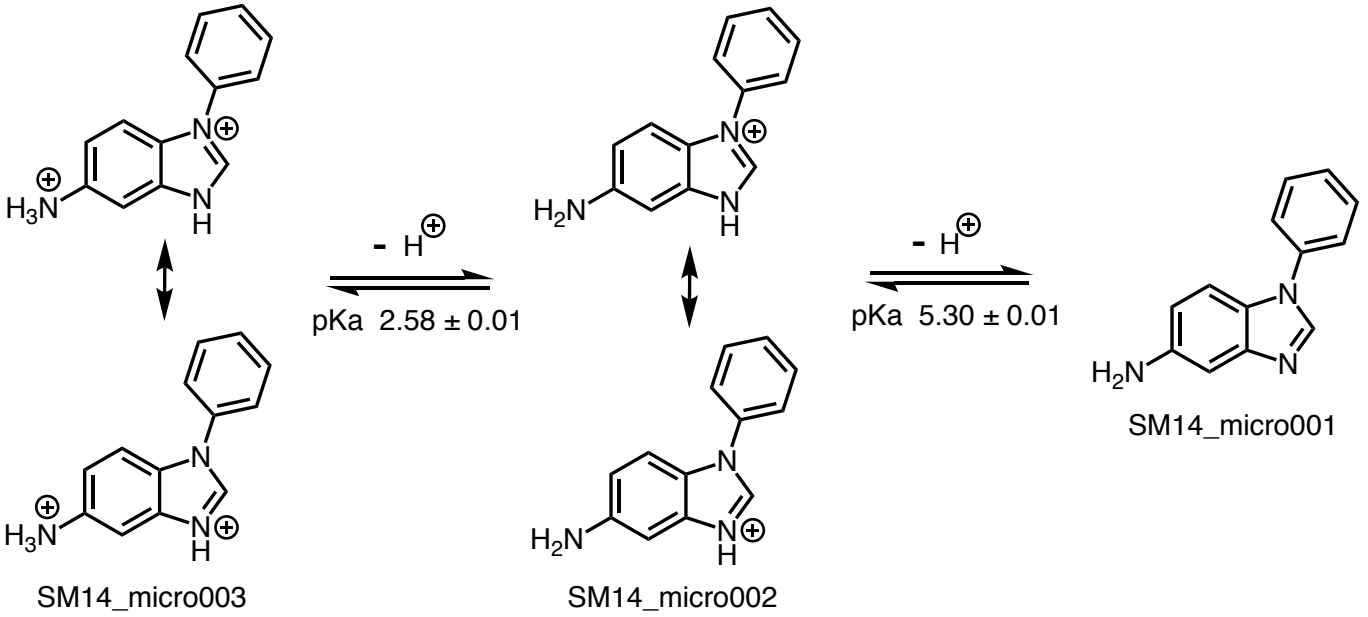
**UV-metric pKa measurement with Sirius T3**
**Distribution of Species**

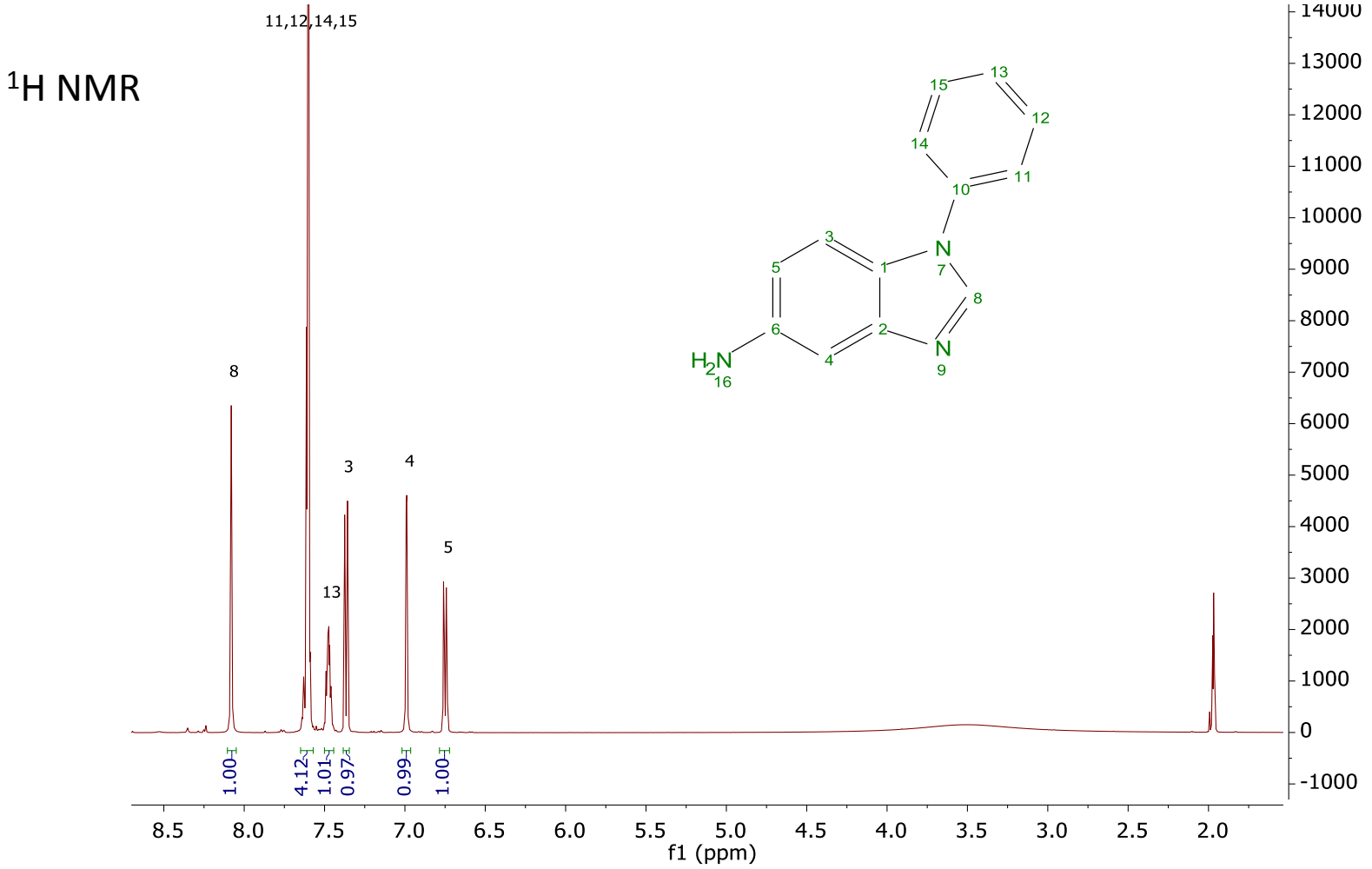



The NMR characterized protonation pathway indicates that the UV-metric pKas of SM14, correspond to transitions between the following microstates:



# NMR Characterization of Microstates of SM14

SM14 structure was assigned with $^{1}H$ NMR, $^{13}C$ NMR, COSY, HSQC, $^{13}C$ and $^{15}N$ HMBC experiments.



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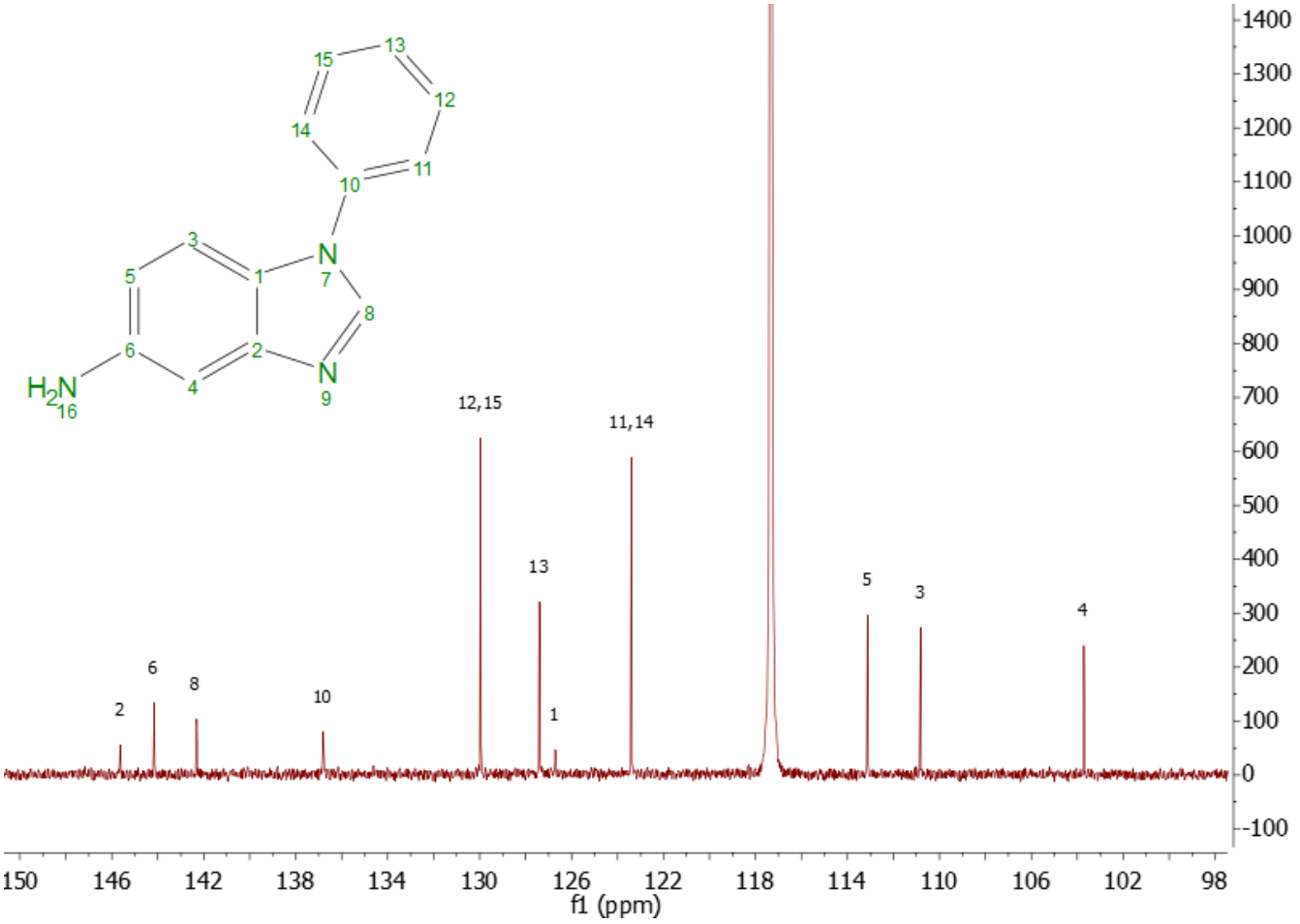
$^{13}C$ NMR



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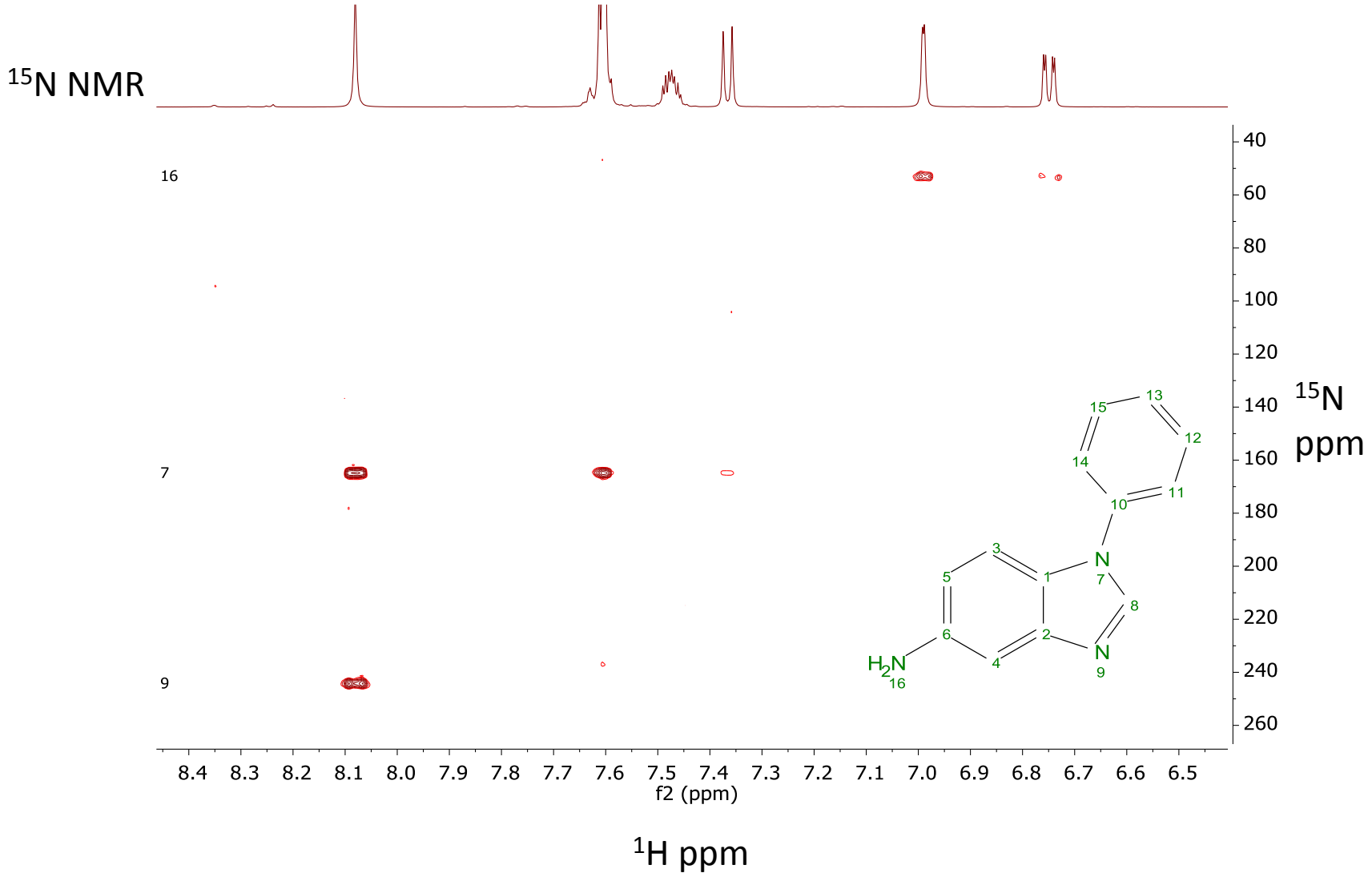

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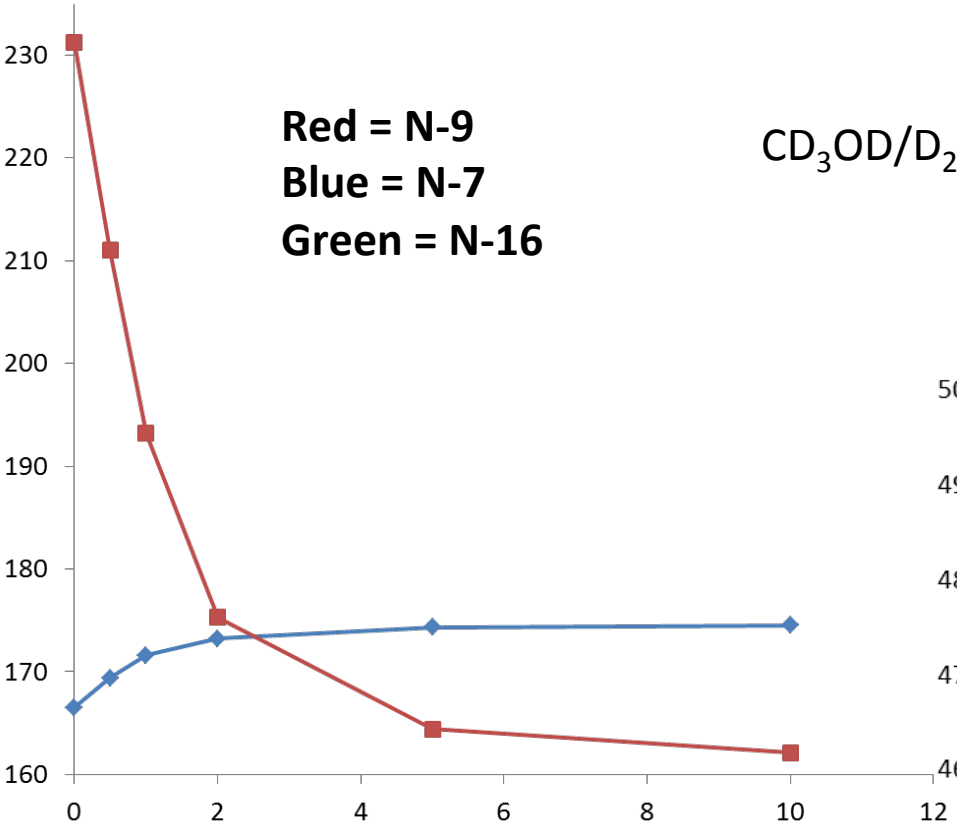
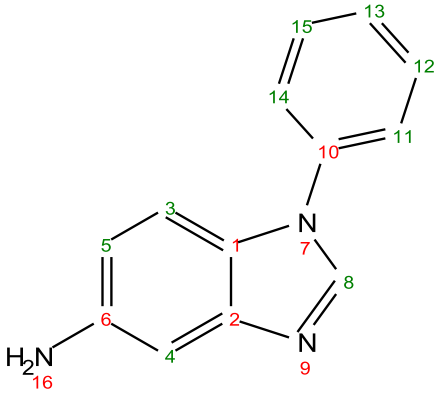
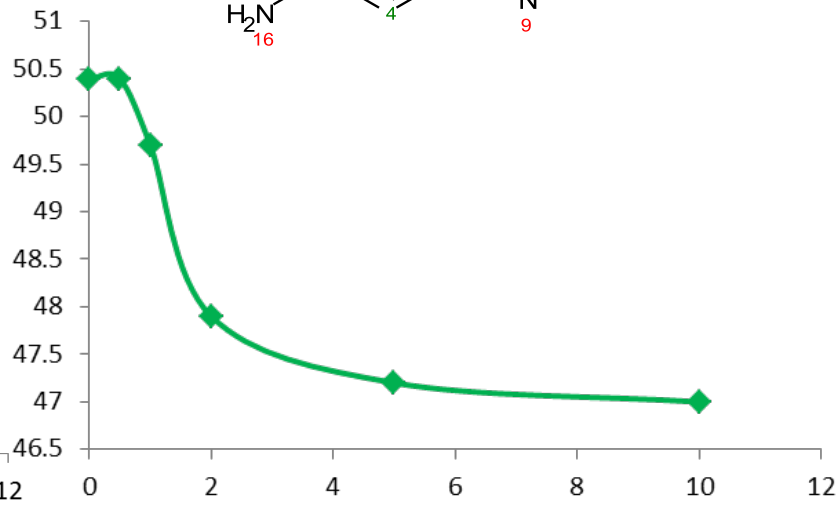
Determining the protonation sites for SM14 was more challenging – due to the possibility of double protonation. Water/methanol strong solvent effects, coupled with multiple protonation states, complicated the data interpretation. For instance, titration of SM14 in methanol/water (plots below) showed continued protonation of N-9 even after 5 equivalents of TFA was added. Using aprotic solvent (acetonitrile-$d_3$) was necessary for unambiguous interpretation of the results.




**Red = N-9**
**Blue = N-7**
**Green = N-16**

$CD_3OD/D_2O$



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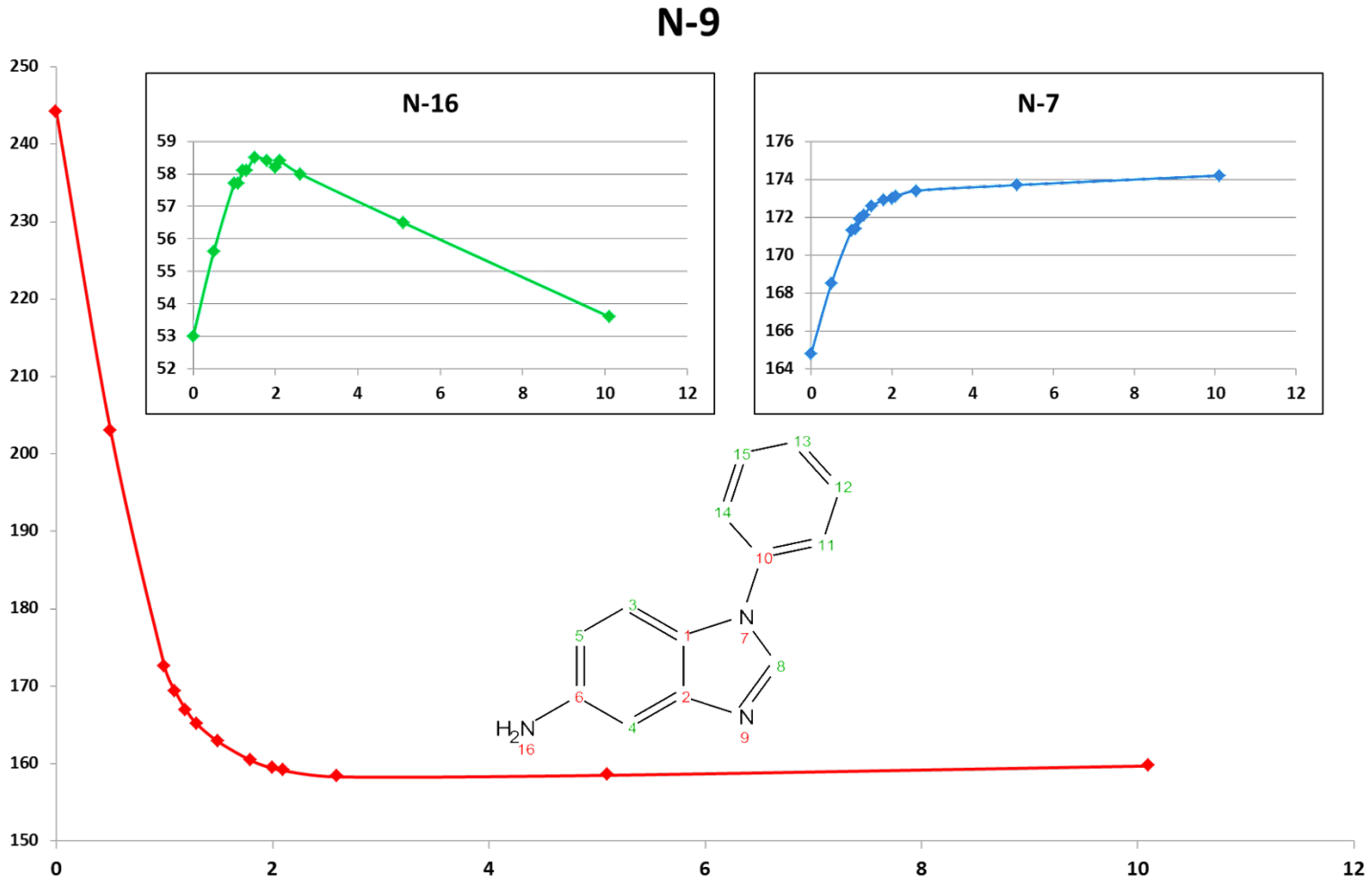
We therefore chose an aprotic solvent, acetonitrile-$d_3$, for the investigation of SM14. Titration of SM14 in acetonitrile provided a much clearer picture of its protonation states. N-9, with a larger chemical shift change ~ 72 ppm at 1 equivalent of TFA, clearly is the site of first protonation. At this point, the changes in chemical shifts observed for N-7 ($\Delta\delta \approx 6.5$) and N-16 ($\Delta\delta \approx 5$) are due to electronic effects. At roughly 2.5 equivalents of TFA, complete protonation of N-9 was attained. The second protonation site, N-16, shows the expected pseudo-linear trend for the protonation of the “anilino” nitrogen with further addition of TFA.



# NMR Characterization of Microstates of SM14

The protonation pathway is shown below; resonance structures are in a fast equilibrium.