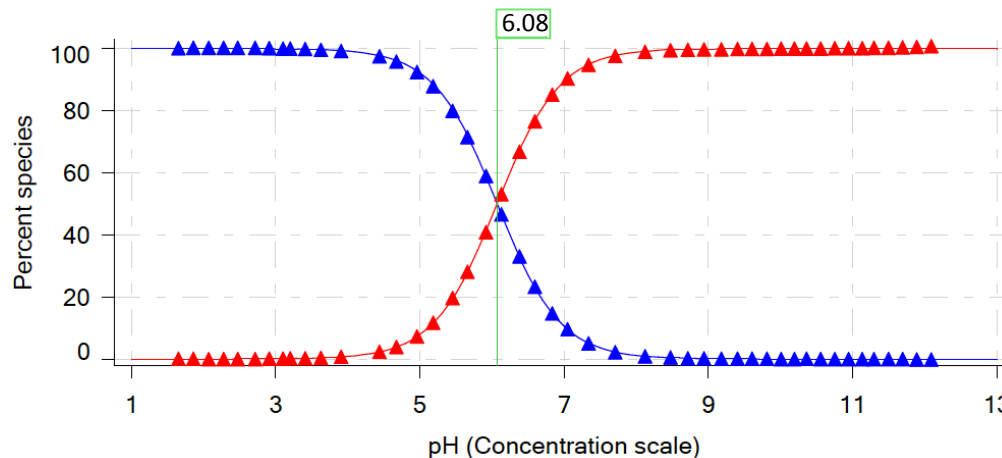


# **NMR Characterization of Microstates of SM07**

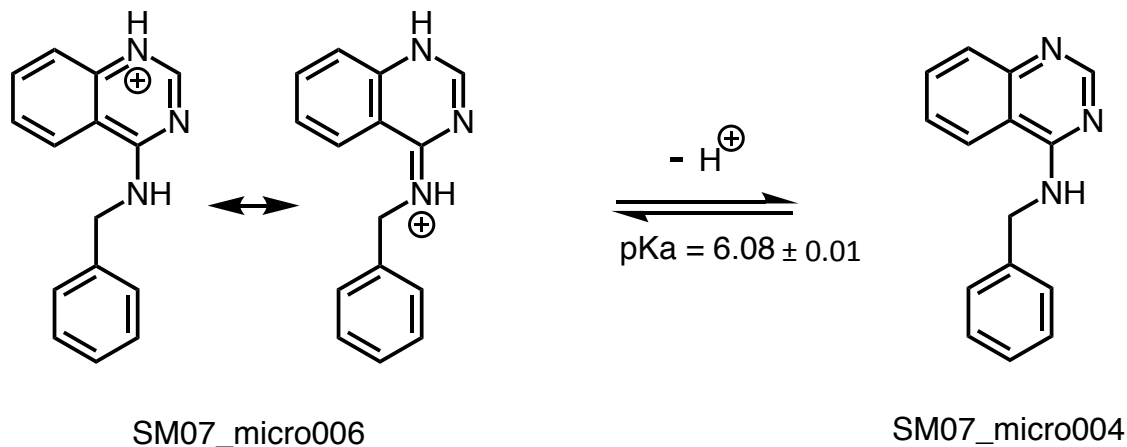
Ikenna Ndukwe  
Xiao Wang  
Mehtap Isik

# NMR Characterization of Microstates of SM07

Distribution of species and pKa value of SM07 was determined with UV-metric pKa measurement with Sirius T3.

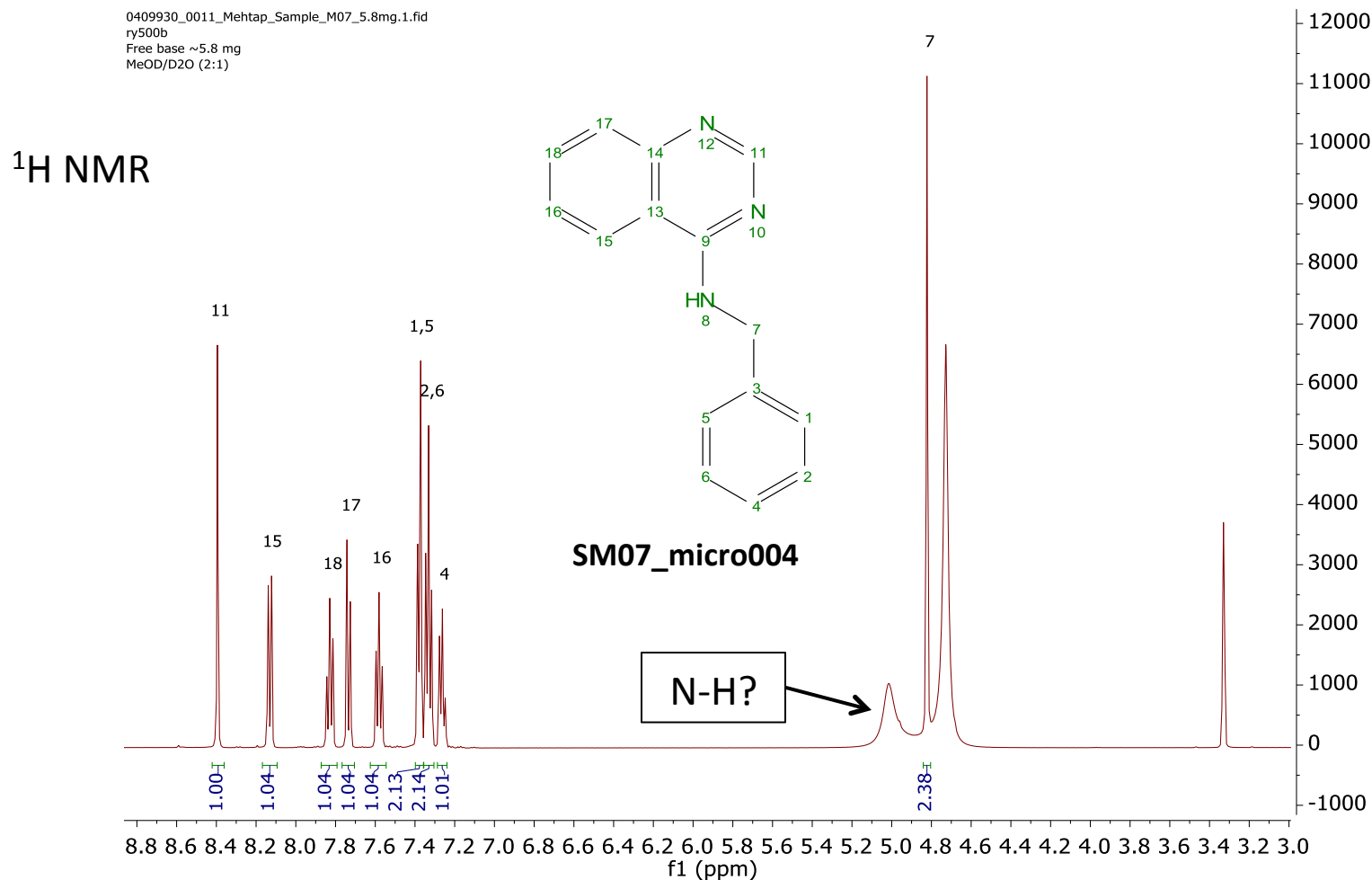


NMR characterization of SM07 showed that  $pK_a 6.08 \pm 0.01$  was related to a microscopic protonation state transition between SM07\_micro006 and SM07\_micro004 microstates.



# NMR Characterization of Microstates of SM07

SM07 structure was assigned with  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY, HSQC, HMBC and  $^{15}\text{N}$  HMBC with 5 – 6 mg of sample in methanol- $d_4$ /D $_2$ O in 2:1 ratio (free-base, pH 5-6\*) – obtaining reference chemical shifts essential for this study.

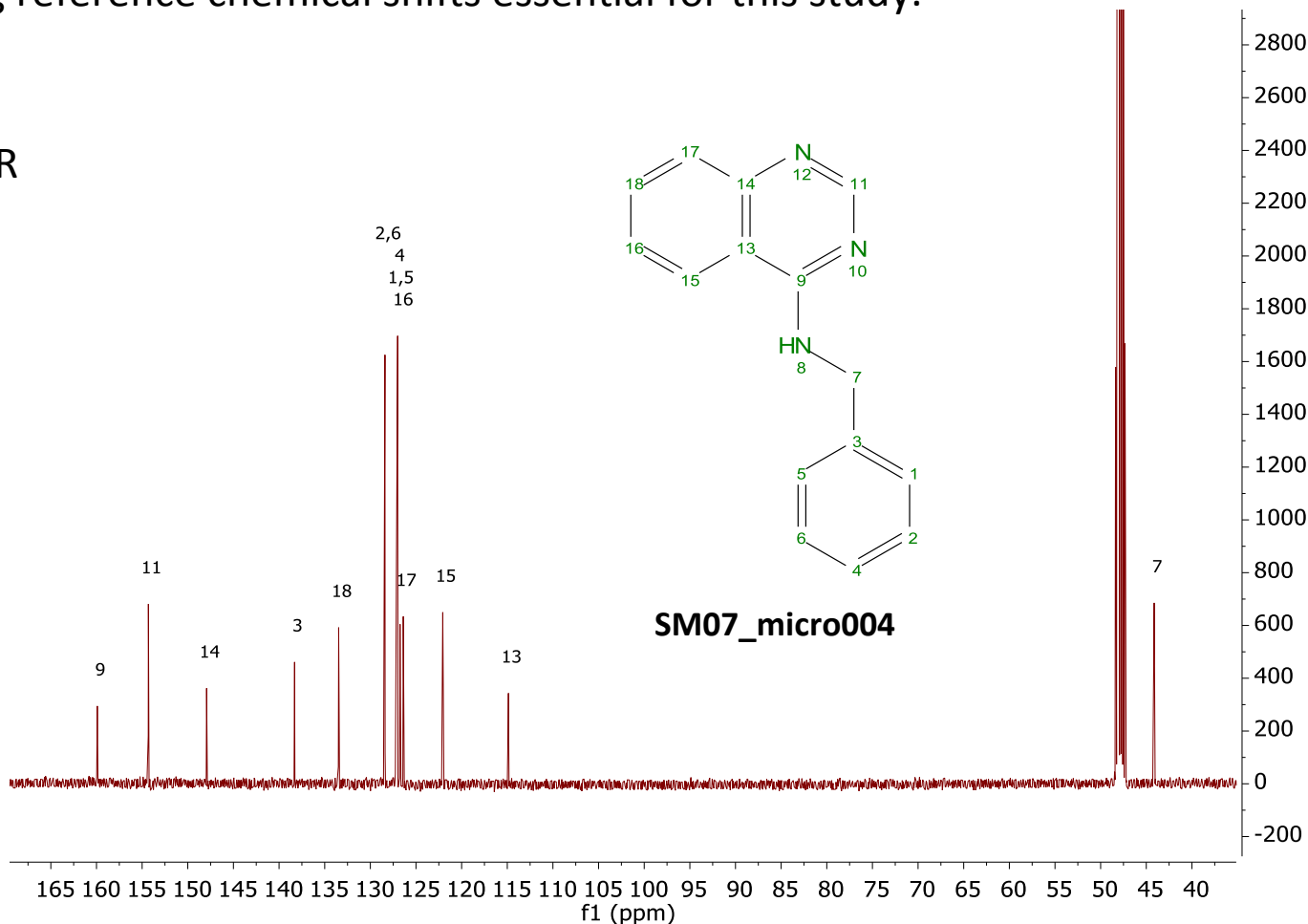


\* Measured with pH paper.

# NMR Characterization of Microstates of SM07

SM07 structure was assigned with  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY, HSQC, HMBC and  $^{15}\text{N}$  HMBC with 5 – 6 mg of sample in methanol- $\text{d}_4/\text{D}_2\text{O}$  in 2:1 ratio (free-base, pH 5-6\*) – obtaining reference chemical shifts essential for this study.

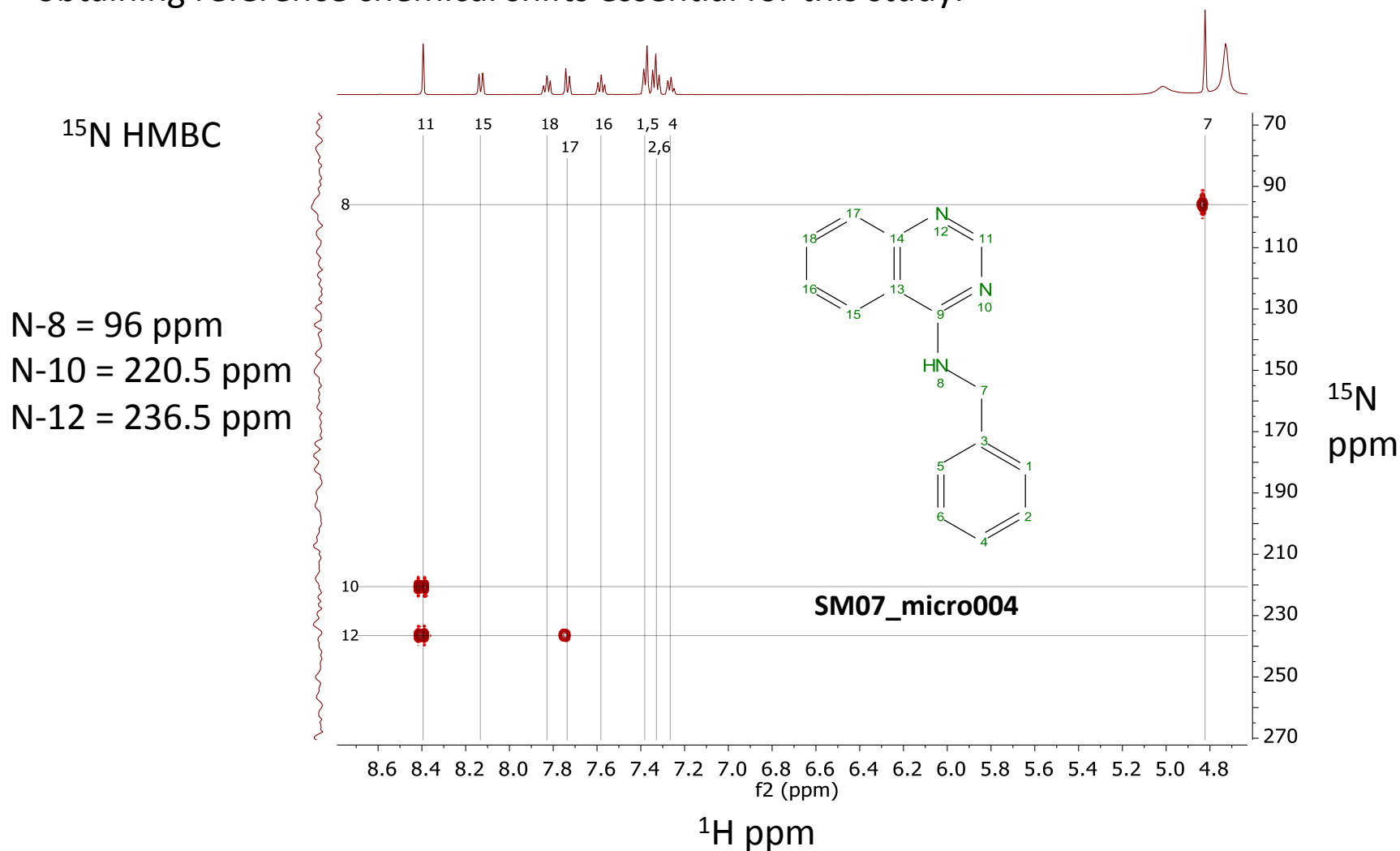
$^{13}\text{C}$  NMR



\* Measured with pH paper.

# NMR Characterization of Microstates of SM07

SM07 structure was assigned with  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY, HSQC, HMBC and  $^{15}\text{N}$  HMBC with 5 – 6 mg of sample in methanol- $d_4$ / $\text{D}_2\text{O}$  in 2:1 ratio (free-base, pH 5-6\*) – obtaining reference chemical shifts essential for this study.

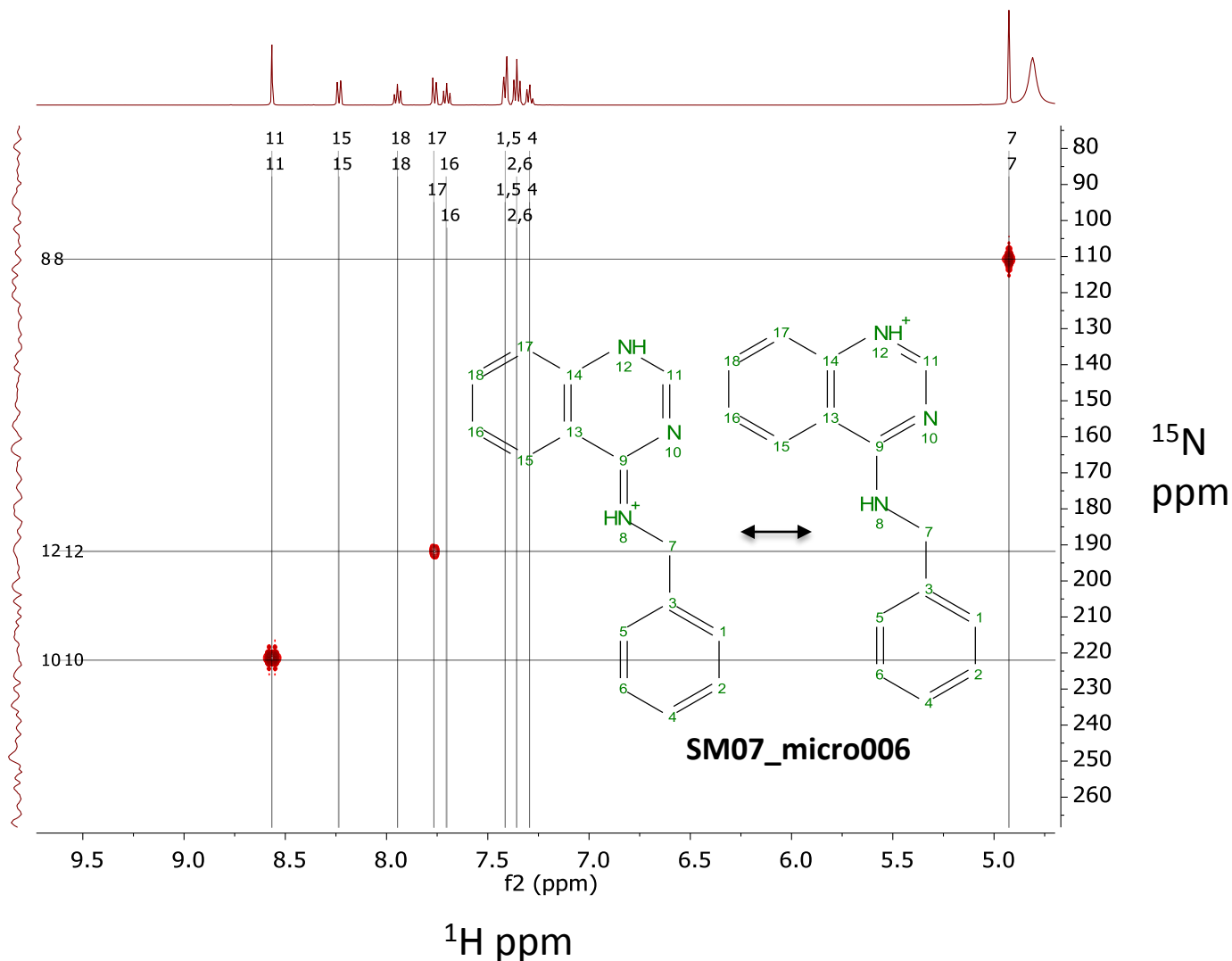


\* Measured with pH paper.

# NMR Characterization of Microstates of SM07

$^{15}\text{N}$  HMBC after addition of about 0.5 equivalent of trifluoroacetic acid (TFA) to the solution (pH 4.5 – 5.5\*).

N-8 = 110.7 ppm  
N-10 = 222.0 ppm  
N-12 = 191.7 ppm

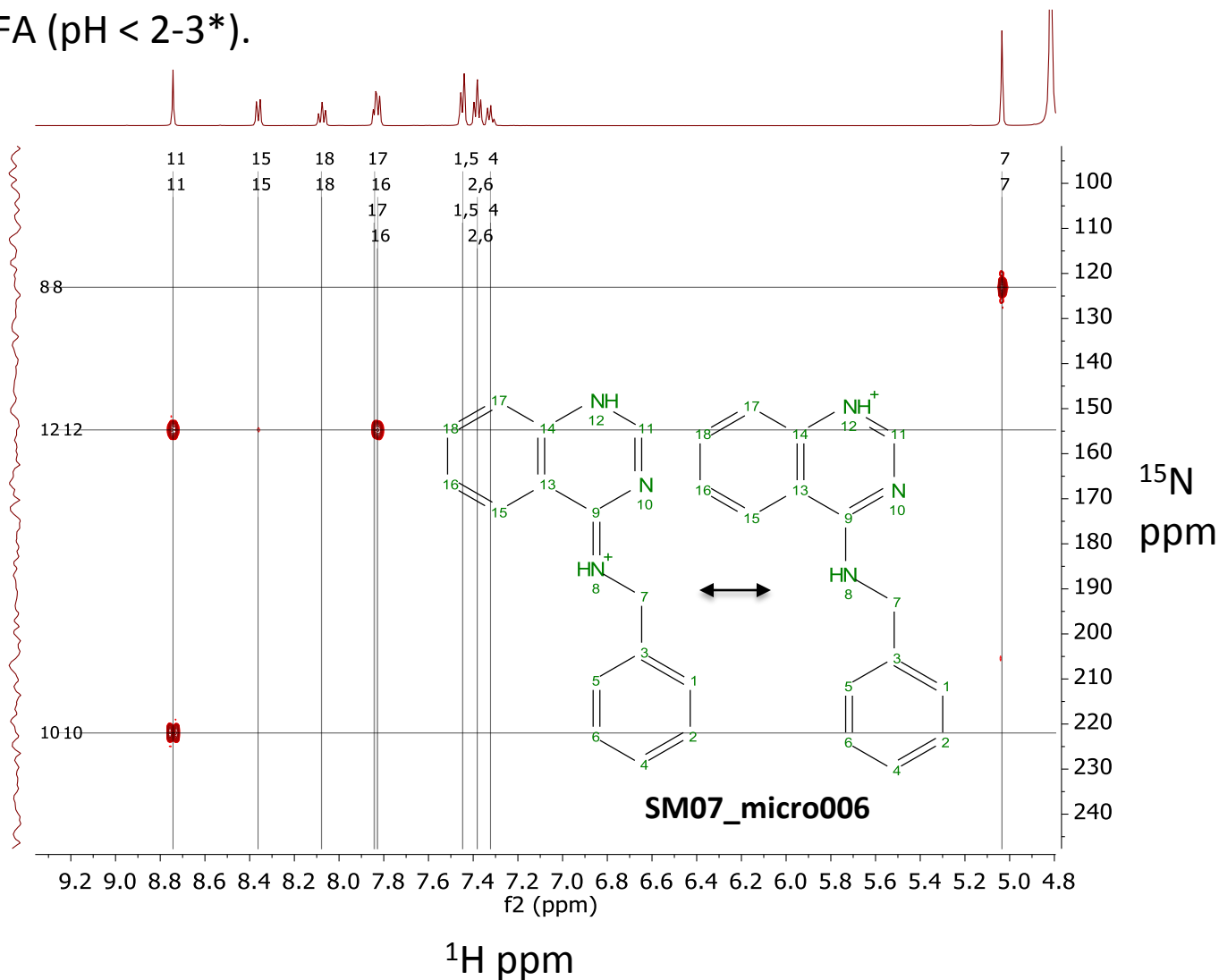


\* Measured with pH paper.

# NMR Characterization of Microstates of SM07

$^{15}\text{N}$  HMBC after addition of about 1 equivalent (less ~5%) of trifluoroacetic acid (TFA) to the solution – note that no further change was observed even after addition of ~ 5 equivalents of TFA (pH < 2-3\*).

N-8 = 123.1 ppm  
N-10 = 222.0 ppm  
N-12 = 154.7 ppm



\* Measured with pH paper.

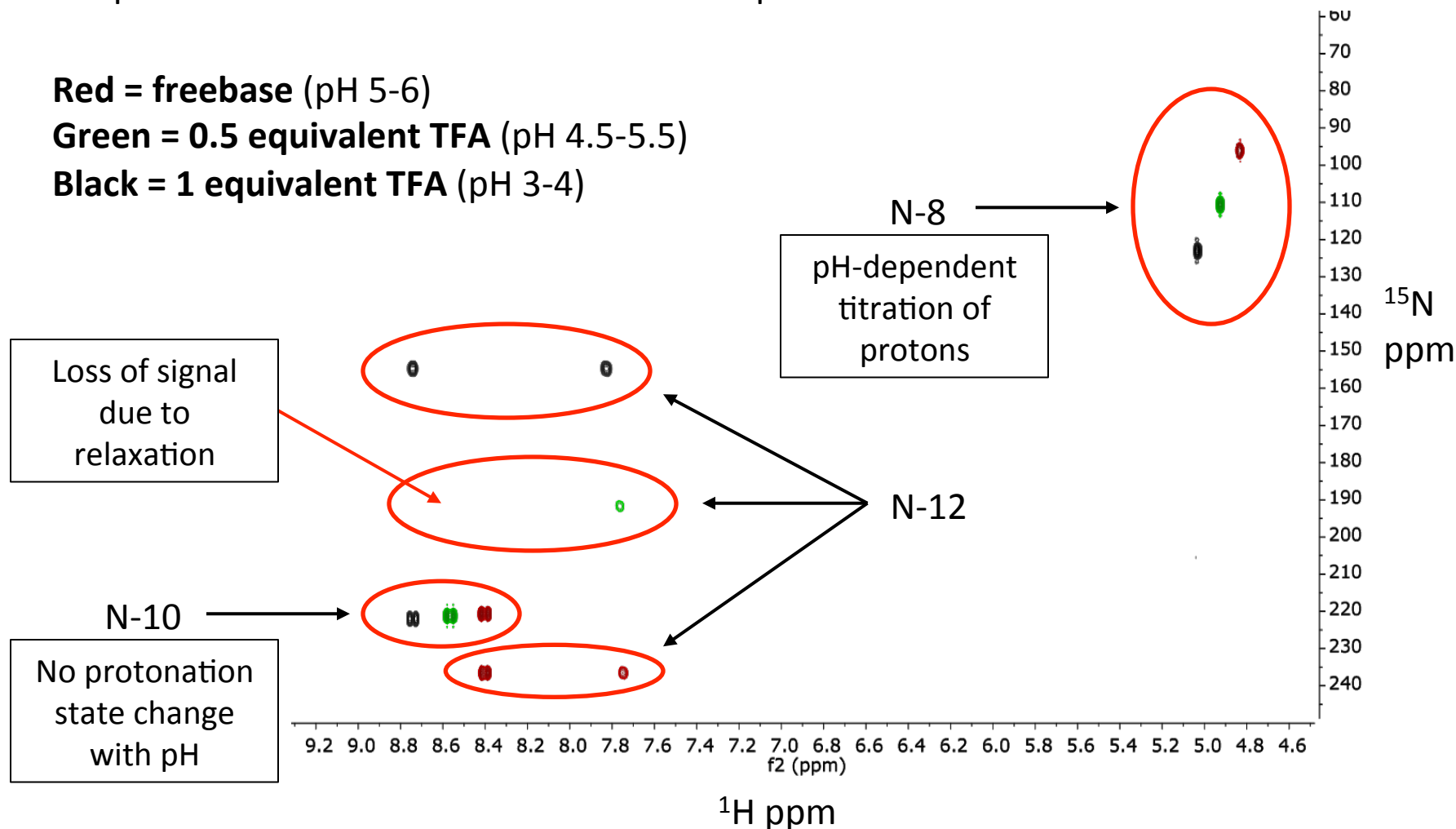
# NMR Characterization of Microstates of SM07

Overlay of  $^{15}\text{N}$  HMBC at free-base, 0.5 equivalent and 1 equivalent of TFA. Notice that the chemical shifts of N-8 and N-12 in the 0.5 TFA equivalent solution are at the midpoint between the freebase and the 1 equivalent solution.

**Red = freebase** (pH 5-6)

**Green = 0.5 equivalent TFA** (pH 4.5-5.5)

**Black = 1 equivalent TFA** (pH 3-4)



\* Solution pH values were measured with pH paper.