NIST
National Institute of Standards and Technology
U.S. Department of Commerce

NMR in Forensic Drug Analysis

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FORENSICS @ NIST

#NISTForensics

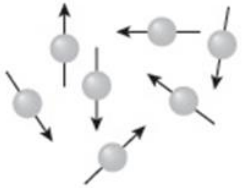
Outline

- Brief Introduction to NMR
- Emerging Synthetic Drugs
- Spin System Modeling of Proton Spectra
- Translating Proton Spectra Across Magnetic Fields
- Benchtop NMR
- Summary and Future Work

A Bit About NMR

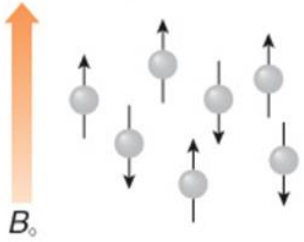
A spinning proton creates a magnetic field.

With no external magnetic field...

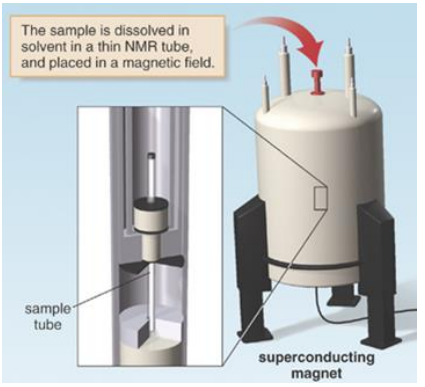


The nuclear magnets are randomly oriented.

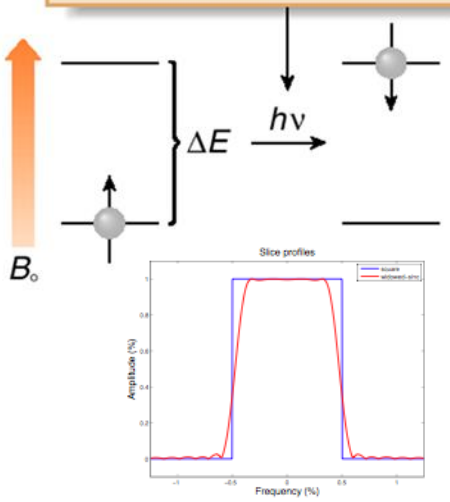
In a magnetic field...



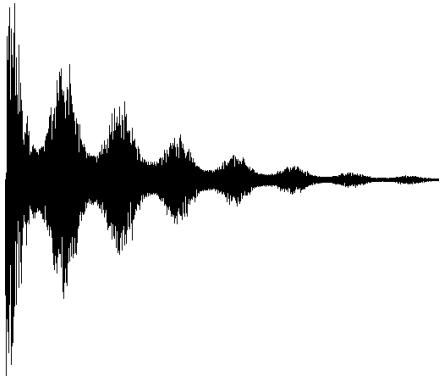
The nuclear magnets are oriented **with or against B_o.**



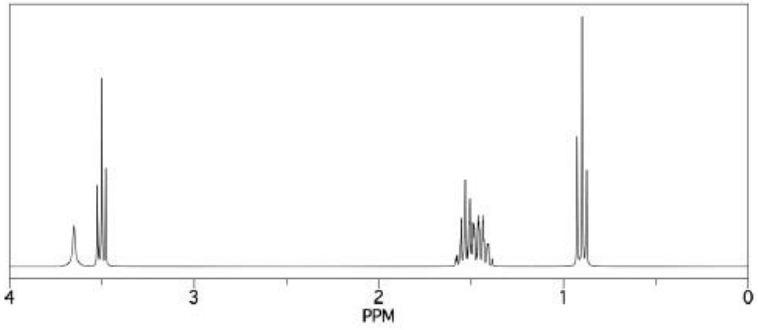
Absorbing RF radiation causes the nucleus to spin flip.



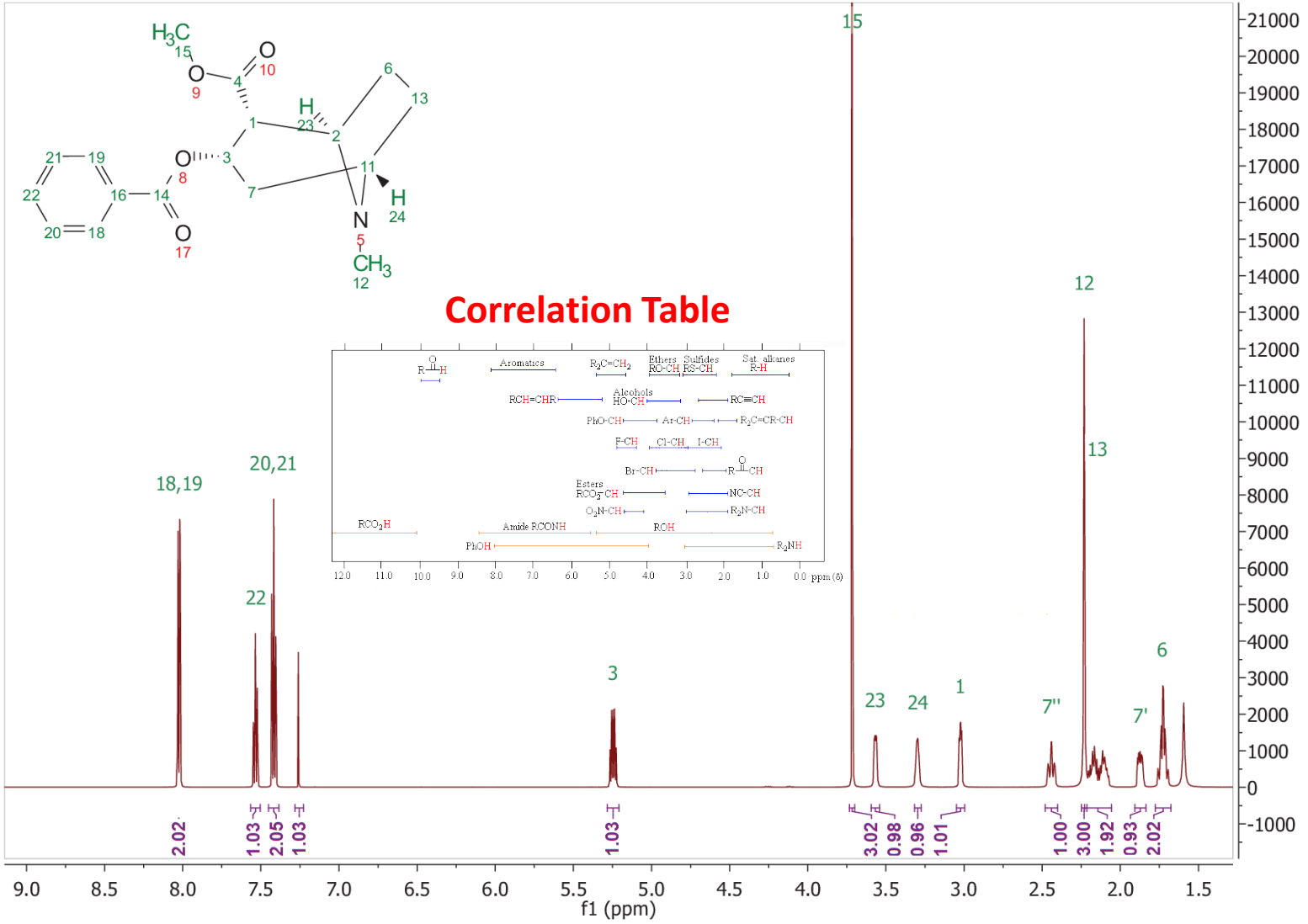
Observed NMR signal is the free induction decay (FID)



Fourier transformation is used to obtain the frequency spectrum from the time domain FID.

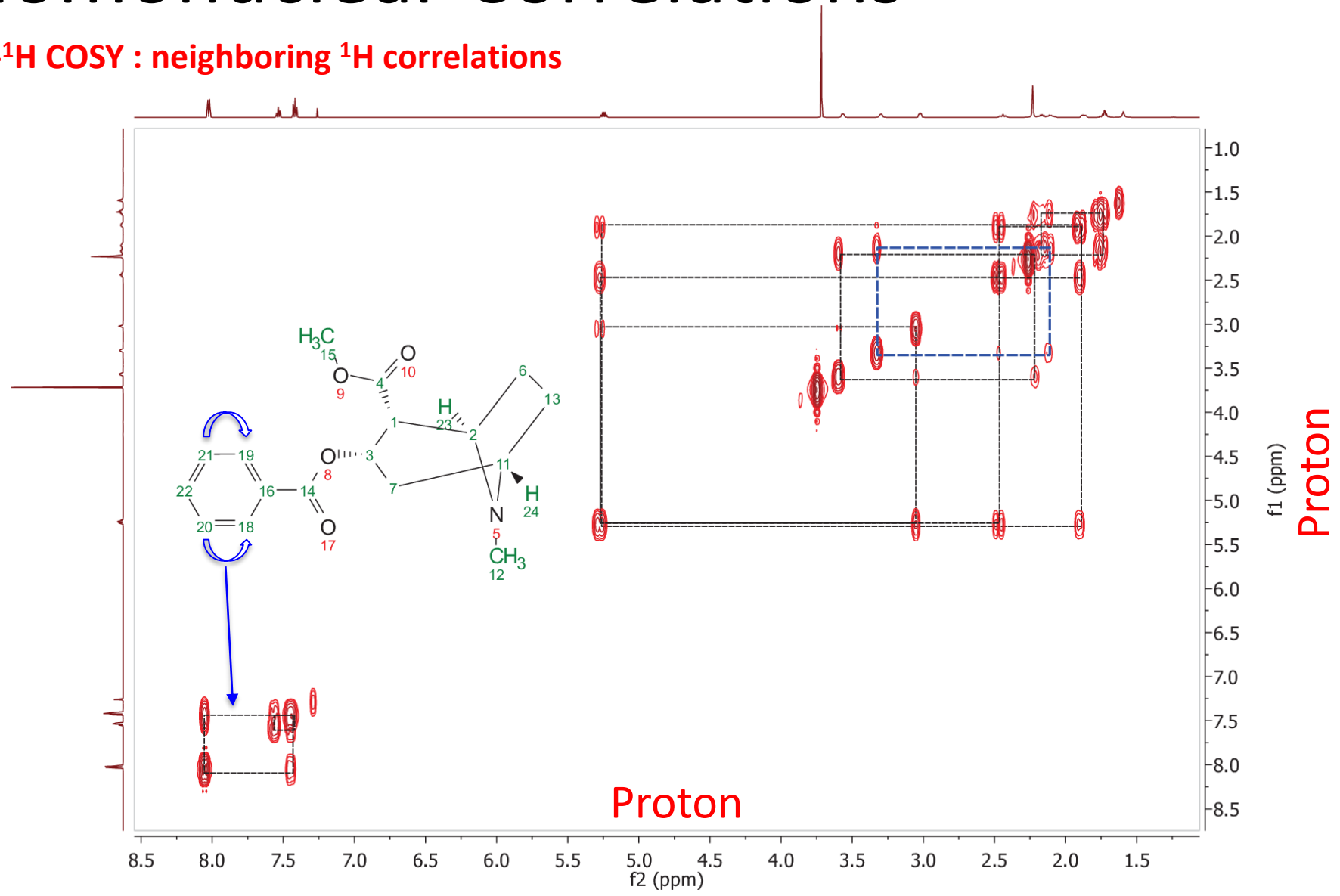


^{1}H NMR of Cocaine



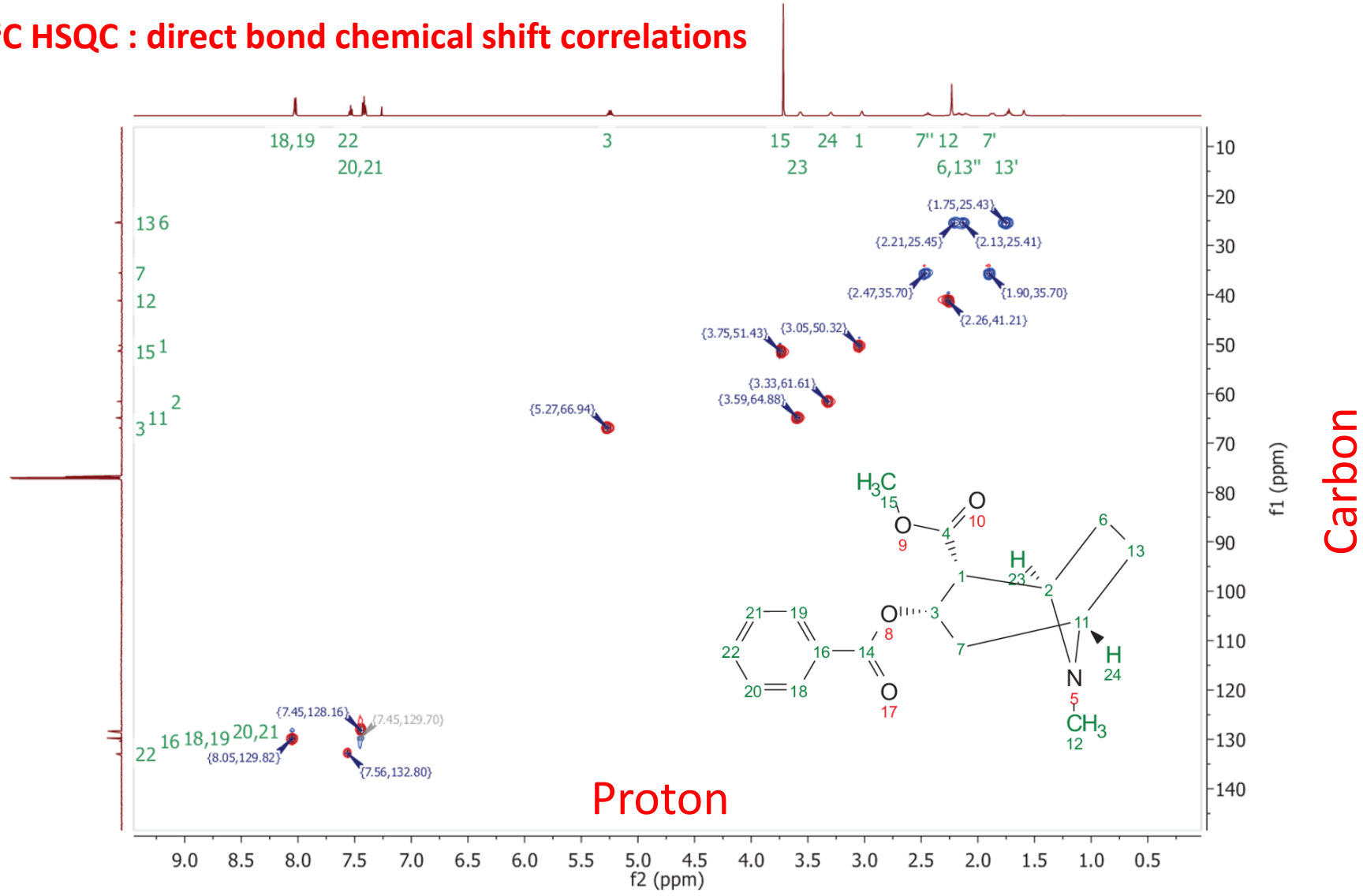
Homonuclear Correlations

^{1}H-^{1}H COSY : neighboring ^{1}H correlations



Heteronuclear Correlations

^{1}H-^{13}C HSQC : direct bond chemical shift correlations



NMR in the Forensic Drug Lab

Essential Structure Elucidation Tool

- NMR Active Nuclei (Spin ½)
  - ^{1}H, ^{13}C, ^{19}F, ^{31}P mainly
- 2D experiments offer a wealth of connectivity information
  - COSY : ^{1}H-^{1}H single bond correlations
  - TOCSY : ^{1}H-^{1}H multi-bond correlations
  - HSQC : ^{1}H-X single-bond single bond connectivity
  - HMBC, HMQC : ^{1}H-X multi-bond single bond connectivity

  There are **MANY** more methods including variants of these and others.

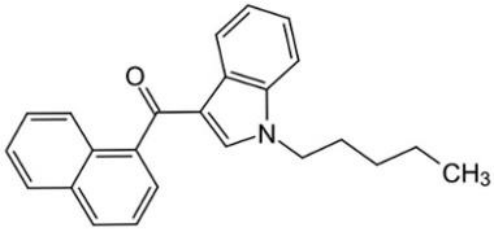
Analytical Tool

- Quantification
  - Absolute purity determinations of reference materials
  - Quantification of many compounds from a single standard
- Powerful screening method for unknowns
  - In most cases, if it's soluble and has a proton you can see it

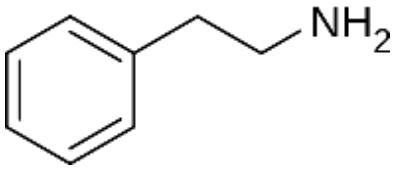
Emerging Synthetic Drugs (ESDs)

- Synthetic drugs mostly in several structurally related families
- Hundreds of new compounds in the last decade

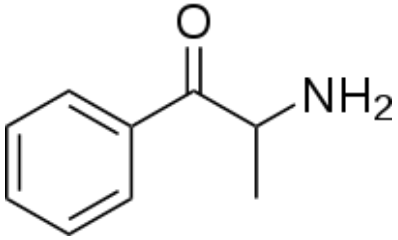
Cannabinoids



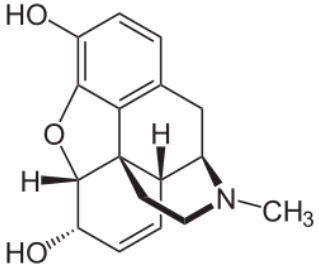
Phenethylamines



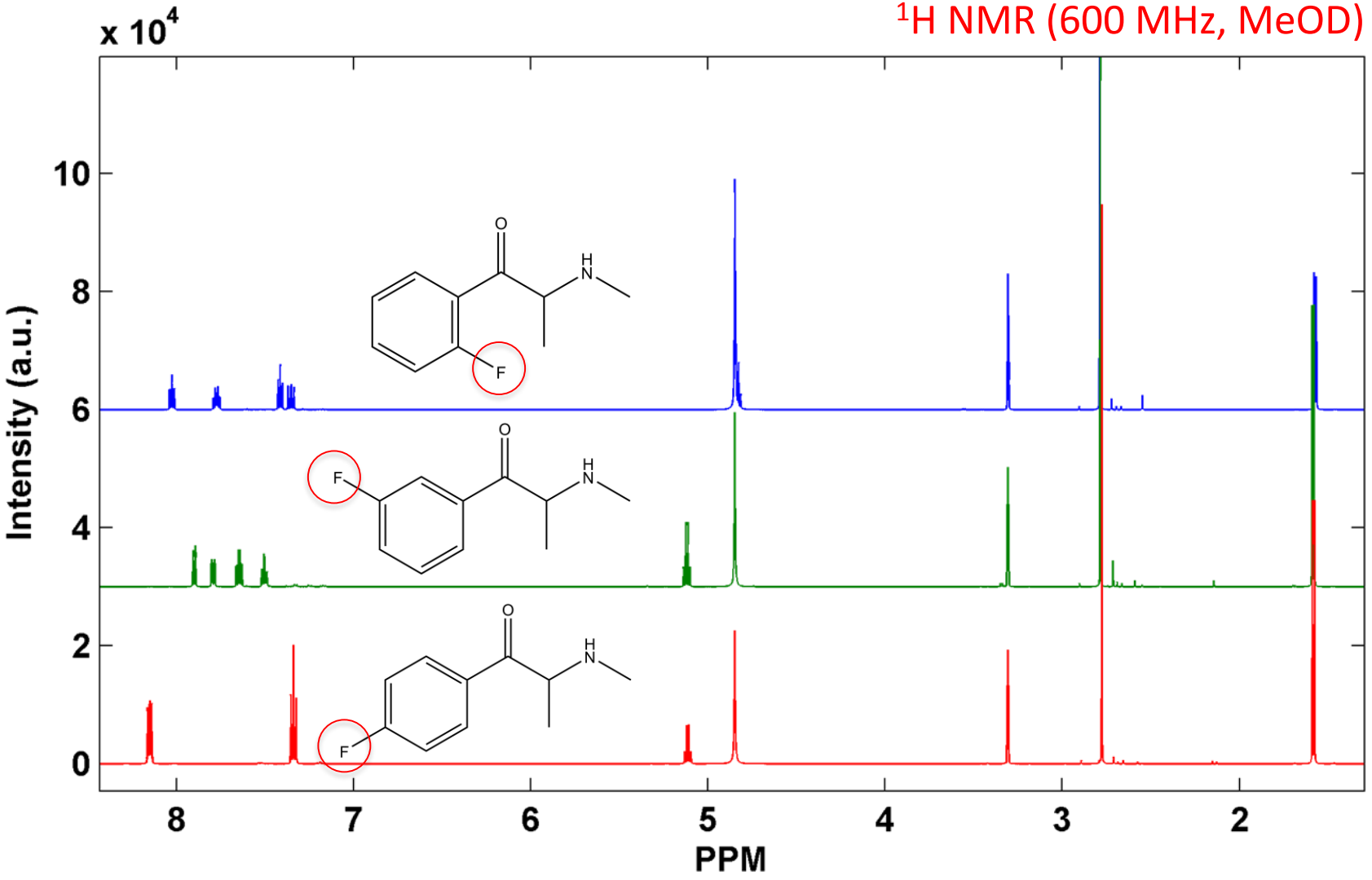
Cathinones



Opioids

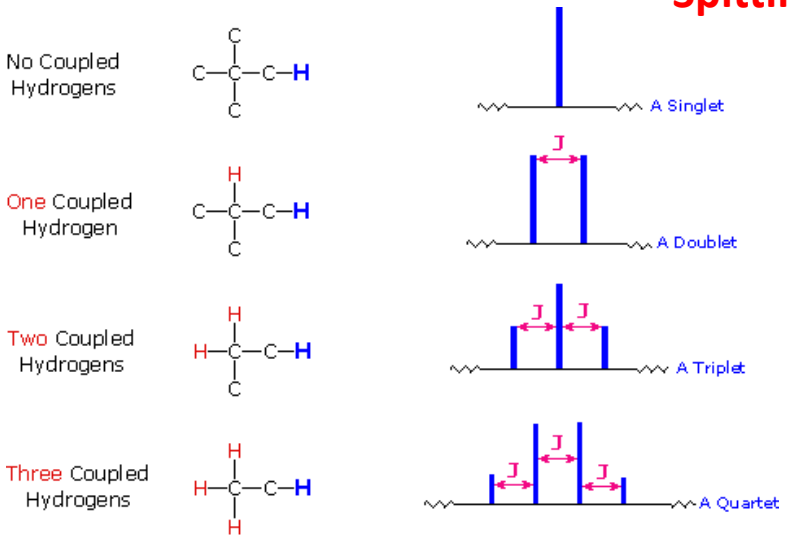


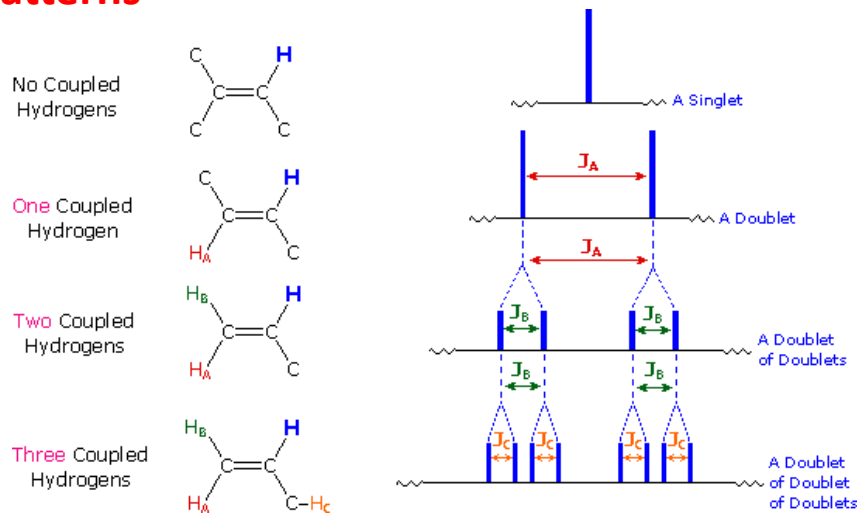
Fluoromethcathinone Regioisomers



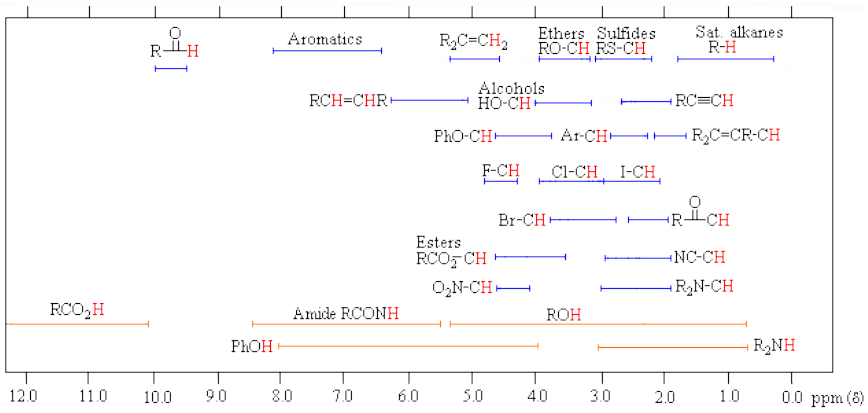
Proton Couplings & Splitting Patterns

Spitting Patterns





^{1}H Correlation Table



www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/nmr/nmr1.htm

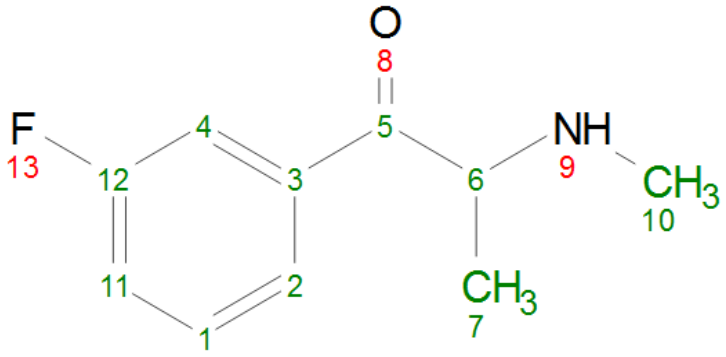
Scalar Coupling Constants

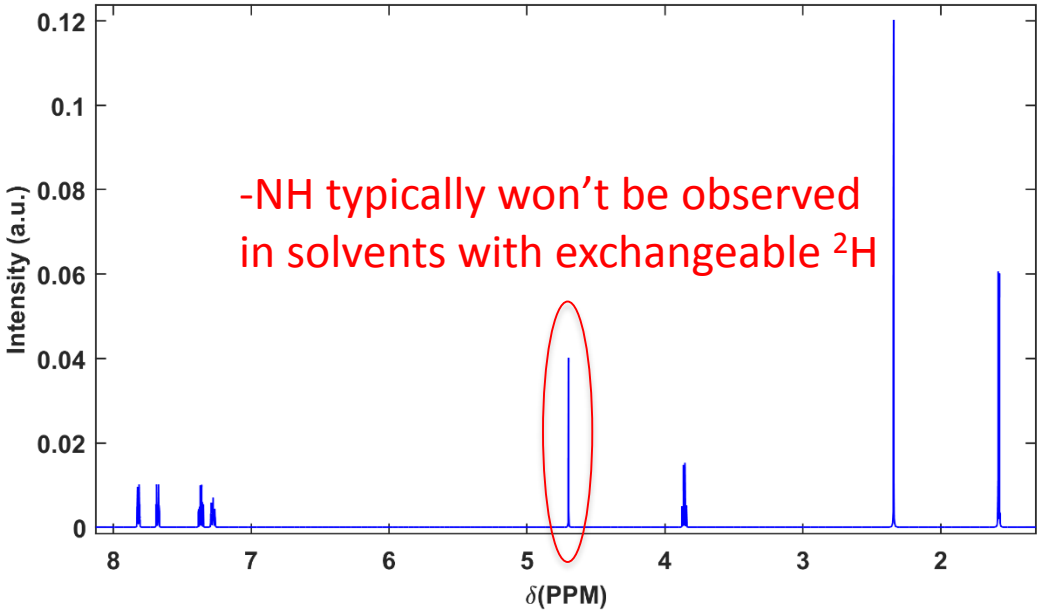
Structural Type	J (Hz)
$H-C-(C)_n-C-H$	0 (unless in a rigid ideal orientation)
H_3C-CH_2-X	6 to 8
$H_3C-CH-X$ (H_3C)	5 to 7
$H-C-C-H$ (X, Y)	2 to 12 (depends on dihedral angle and the nature of X and Y)
$H-C-C=O$	0.5 to 3
$H-C-H$	12 to 15 (must be diastereotopic)

Structural Type	J (Hz)
$H-C=C$	12 to 18
$H-C=C-H$	7 to 12
$C=C-H$	0.5 to 3
$C=C-C-H$	3 to 11 (depends on dihedral angle)
$C-C\equiv C-H$	2 to 3
C_6H_5-H	o 6 to 9 m 1 to 3 p 0 to 1

Predicting ^{1}H NMR Spectrum

3-Fluoromethcathinone (3-FMC)

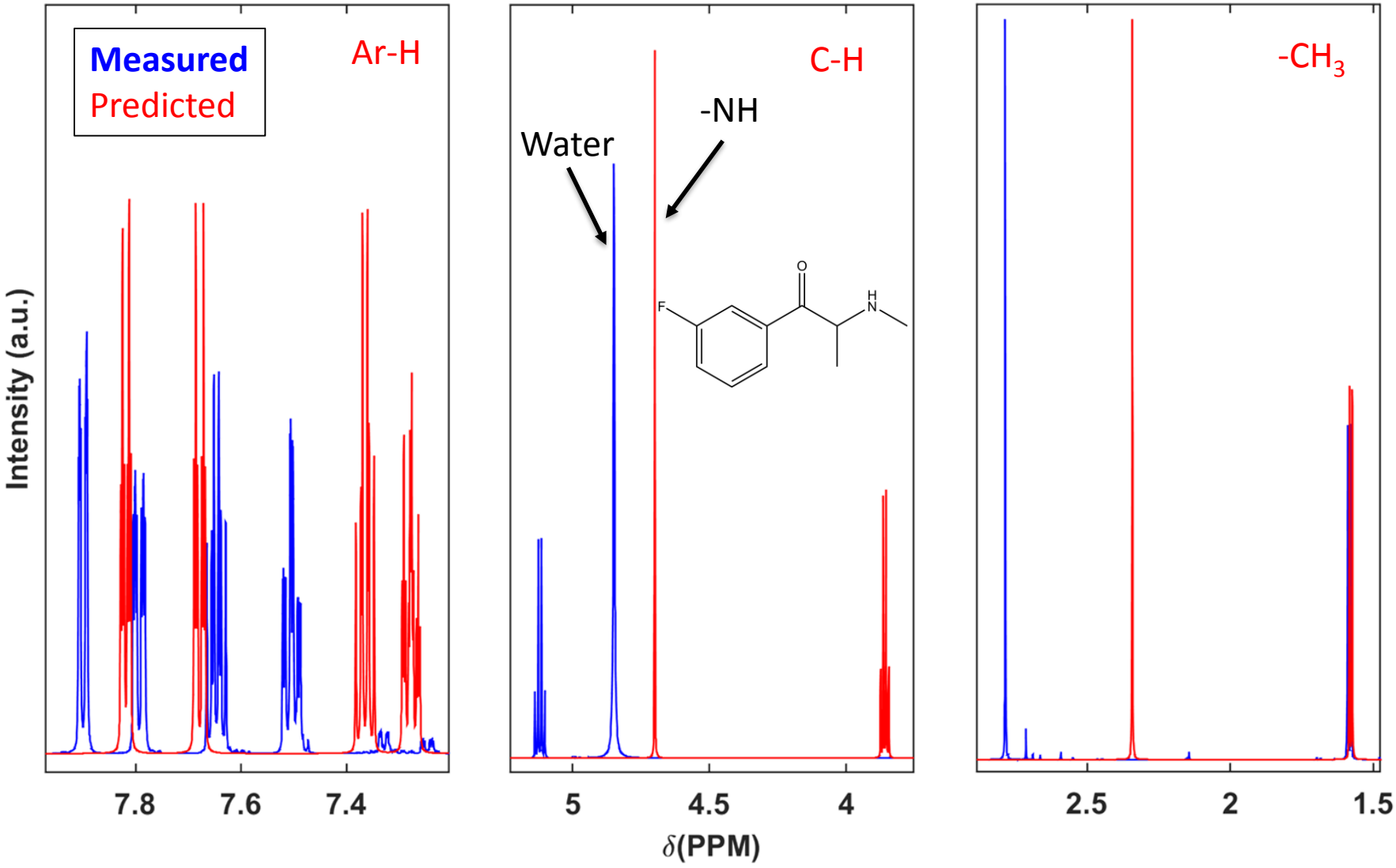




Predicted Chemical Shifts & Coupling Constants

Atom	Shift (ppm)	J (Hz)	
1 CH	7.365	J(1-2)	7.5
		J(1-4)	0.1
		J(1-11)	7.5
		J(1-F13)	5.7
2 CH	7.8147	J(2-1)	7.5
		J(2-4)	2
		J(2-11)	2
		J(2-F13)	0.2
4 CH	7.6787	J(4-1)	0.1
		J(4-2)	2
		J(4-11)	2
		J(4-F13)	8.9
6 CH	3.8598	J(6-7)	6.1
7 CH3	1.5791	J(7-6)	6.1
		J(7)	-12.5
9 NH	4.7		
10 CH3	2.3423	J(10)	-12.5
11 CH	7.2785	J(11-1)	7.5
		J(11-2)	2
		J(11-4)	2
		J(11-F13)	8.9

3-FMC Measured vs Predicted (^{1}H, 600 MHz, MeOD)



Fitting Prediction to Observation

Predicted Chemical Shifts & Coupling Constants

Atom	Shift (ppm)	J (Hz)	
1 CH	7.365	J(1-2)	7.5
		J(1-4)	0.1
		J(1-11)	7.5
		J(1-F13)	5.7
2 CH	7.8147	J(2-1)	7.5
		J(2-4)	2
		J(2-11)	2
		J(2-F13)	0.2
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		J(4-2)	2
		J(4-11)	2
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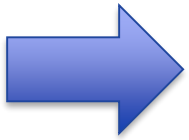
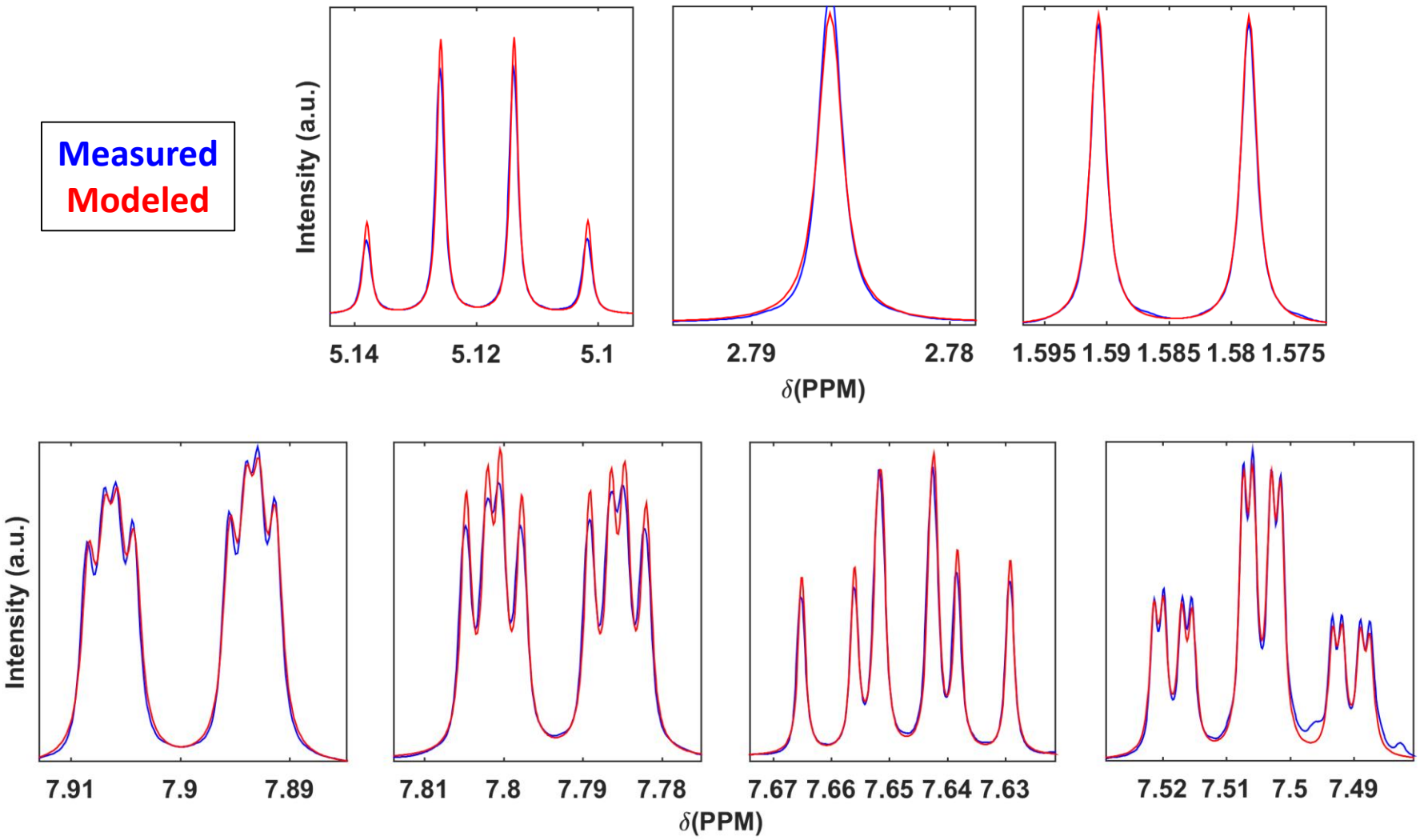
9 NH	4.7		
10 CH3	2.3423	J(10)	-12.5
11 CH	7.2785	J(11-1)	7.5
		J(11-2)	2
		J(11-4)	2
		J(11-F13)	8.9



Fit Chemical Shifts & Coupling Constants

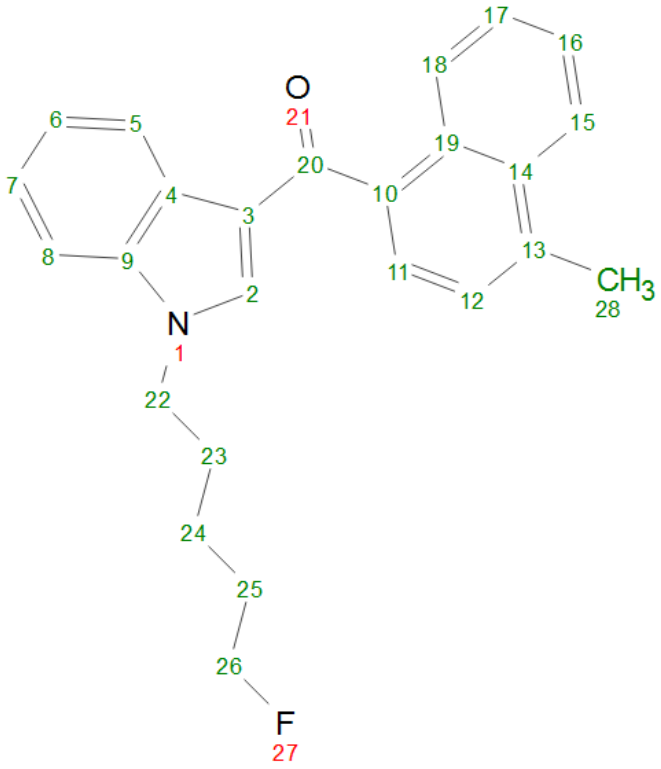
Atom	Shift (ppm)	J (Hz)	
1 CH	7.647	J(1-2)	7.796
		J(1-4)	0
		J(1-11)	8.322
		J(1-F13)	5.484
2 CH	7.8997	J(2-1)	7.796
		J(2-4)	1.615
		J(2-11)	0.908
		J(2-F13)	0
4 CH	7.7934	J(4-1)	0
		J(4-2)	1.615
		J(4-11)	2.647
		J(4-F13)	9.382
6 CH	5.1198	J(6-7)	7.25
7 CH3	1.5846	J(7-6)	7.25
		J(7)	-12.5
9 NH	4.7		
10 CH3	2.786	J(10)	-12.5
11 CH	7.5046	J(11-1)	8.322
		J(11-2)	0.908
		J(11-4)	2.647
		J(11-F13)	8.445

Resulting 3-FMC Model After Fit



Similar results obtained for 2-FMC & 4-FMC

Something a bit more challenging…



(1-(5-fluoropentyl)-1H-indol-3-yl)(4-methyl-1-naphthalenyl)-methanone
aka…..

MAM-2201

Predicted Chemical Shifts & Coupling Constants

Atom	Shift (ppm)	J (Hz)	
2 CH	7.9026	J(2-5)	0.5
5 CH	8.584	J(5-2)	0.5
		J(5-6)	7.5
		J(5-7)	1.5
		J(5-8)	0.5
6 CH	7.3548	J(6-5)	7.5
		J(6-7)	7.5
		J(6-8)	1.5
7 CH	7.22	J(7-5)	1.5
		J(7-6)	7.5
		J(7-8)	7.5
8 CH	7.4558	J(8-5)	0.5
		J(8-6)	1.5
		J(8-7)	7.5
11 CH	7.7937	J(11-12)	7.5
		J(11-18)	0.5
12 CH	7.4556	J(12-11)	7.5
		J(12-15)	0.5
15 CH	7.9936	J(15-12)	0.5
		J(15-16)	7.5
		J(15-17)	1.5
		J(15-18)	0.5
16 CH	7.5243	J(16-15)	7.5
		J(16-17)	7.5
		J(16-18)	1.5
17 CH	7.7431	J(17-15)	1.5
		J(17-16)	7.5
		J(17-18)	7.5

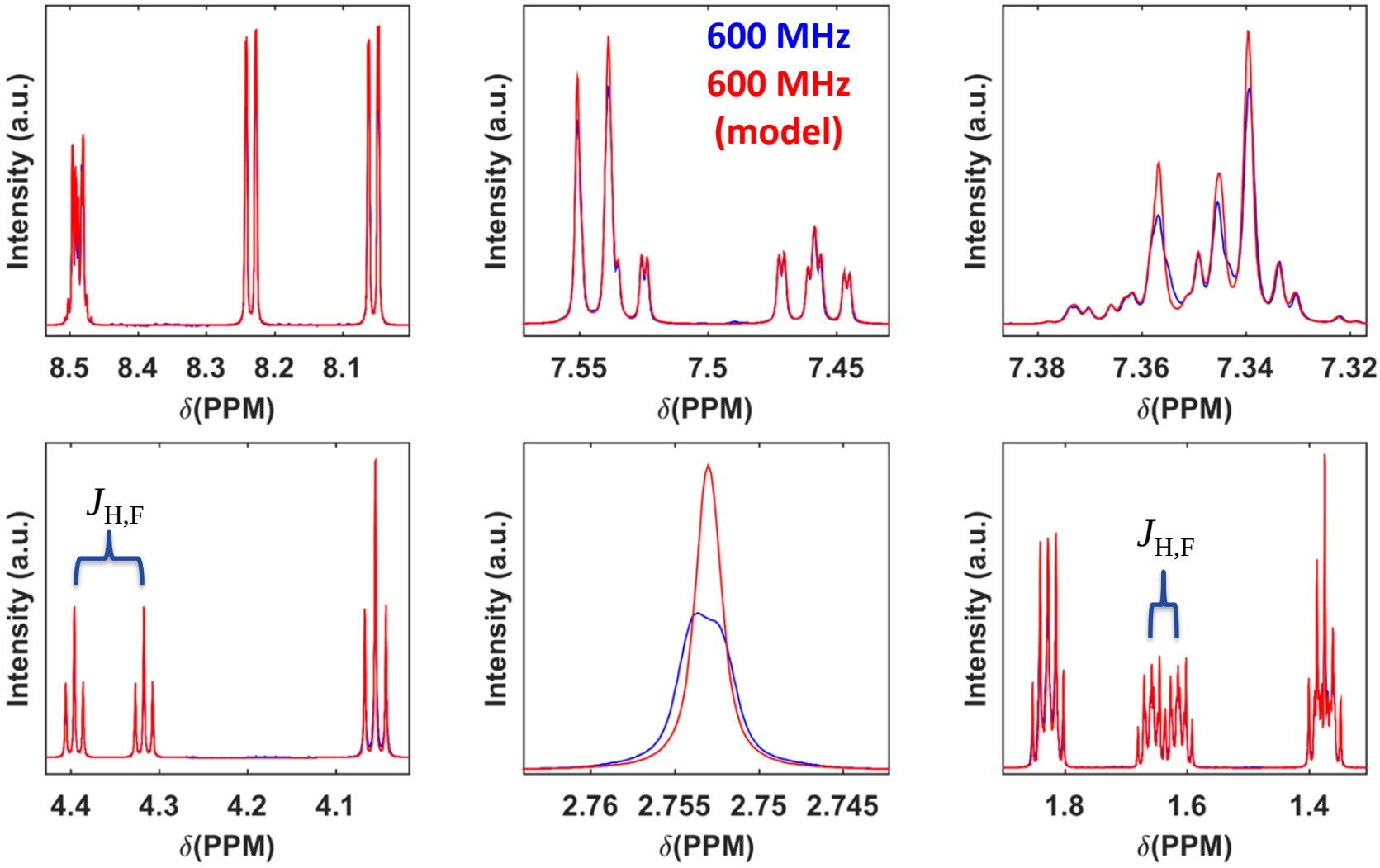
Atom	Shift (ppm)	J (Hz)	
18 CH	8.6891	J(18-11)	0.5
		J(18-15)	0.5
		J(18-16)	1.5
		J(18-17)	7.5
22' CH2	4.32	J(22'-22")	-12.4
		J(22'-23')	7.7
		J(22'-23")	7.7
22" CH2	4.32	J(22"-22')	-12.4
		J(22"-23')	7.7
		J(22"-23")	7.7
23' CH2	1.69	J(23'-22')	7.7
		J(23'-22")	7.7
		J(23'-23")	-12.4
		J(23'-24')	7.9
		J(23'-24")	7.9
23" CH2	1.69	J(23"-22')	7.7
		J(23"-22")	7.7
		J(23"-23')	-12.4
		J(23"-24')	7.9
		J(23"-24")	7.9
24' CH2	1.31	J(24'-23')	7.9
		J(24'-23")	7.9
		J(24'-24")	-12.4
		J(24'-25')	8
		J(24'-25")	8
24" CH2	1.31	J(24"-23')	7.9
		J(24"-23")	7.9
		J(24"-24')	-12.4
		J(24"-25')	8
		J(24"-25")	8

Atom	Shift (ppm)	J (Hz)	
25' CH2	1.47	J(25'-24')	8
		J(25'-24")	8
		J(25'-25")	-12.4
		J(25'-26')	4
		J(25'-26")	4
		J(25'-27)	25.2
25" CH2	1.47	J(25"-24')	8
		J(25"-24")	8
		J(25"-25')	-12.4
		J(25"-26')	4
		J(25"-26")	4
		J(25"-27)	25.2
26' CH2	4.07	J(26'-25')	4
		J(26'-25")	4
		J(26'-26")	-12.4
		J(26'-27)	46.4
26" CH2	4.07	J(26"-25')	4
		J(26"-25")	4
		J(26"-26')	-12.4
		J(26"-27)	46.4
28' CH3	2.667	J(28'-28")	-14.9
		J(28'-28"')	-14.9
28" CH3	2.667	J(28"-28')	-14.9
		J(28"-28"')	-14.9
28"' CH3	2.667	J(28"'-28')	-14.9
		J(28"'-28")	-14.9

Long Story Short…..Almost

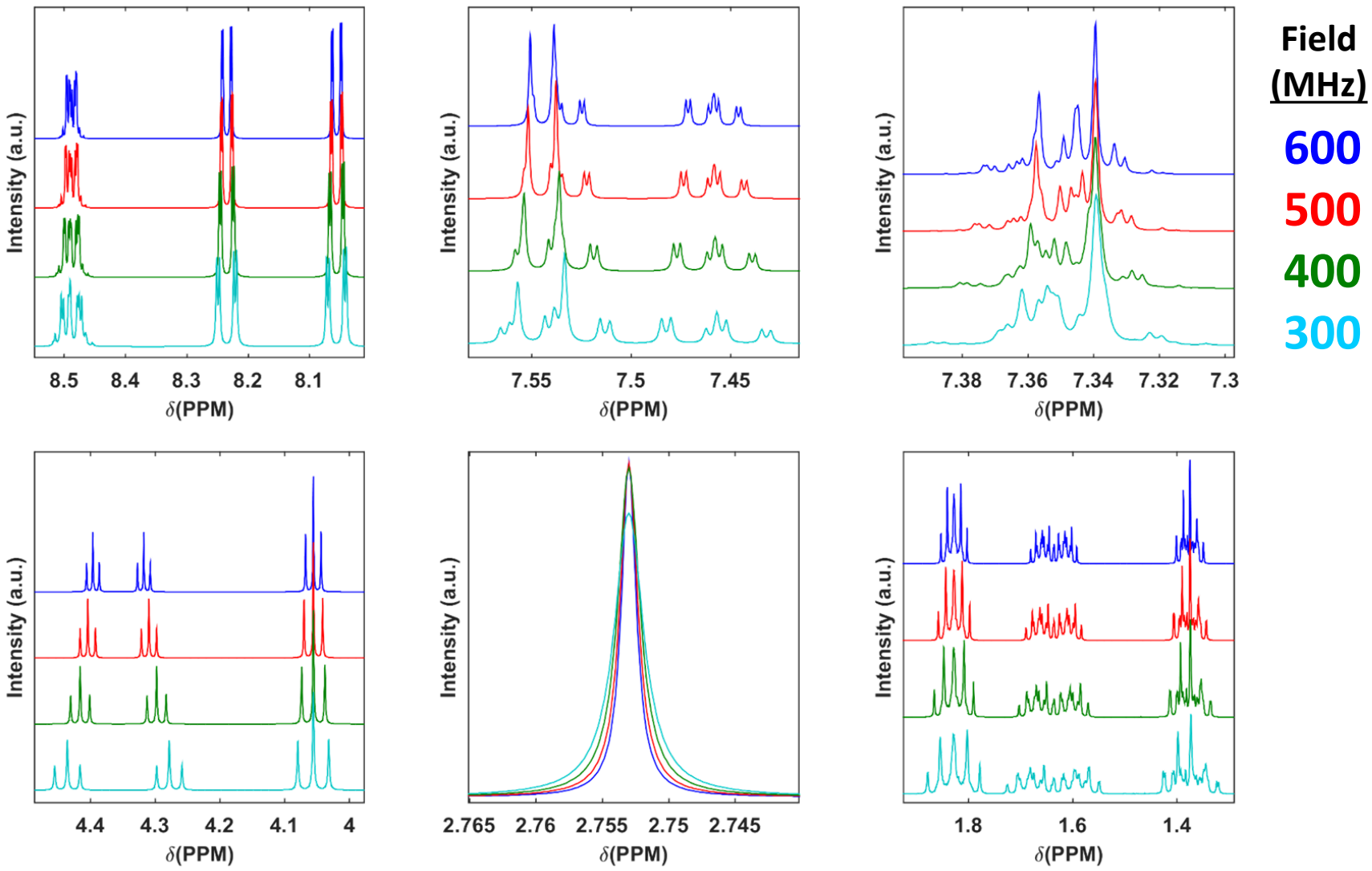
MAM-2201

^{1}H NMR (600 MHz, $CDCl_3$)

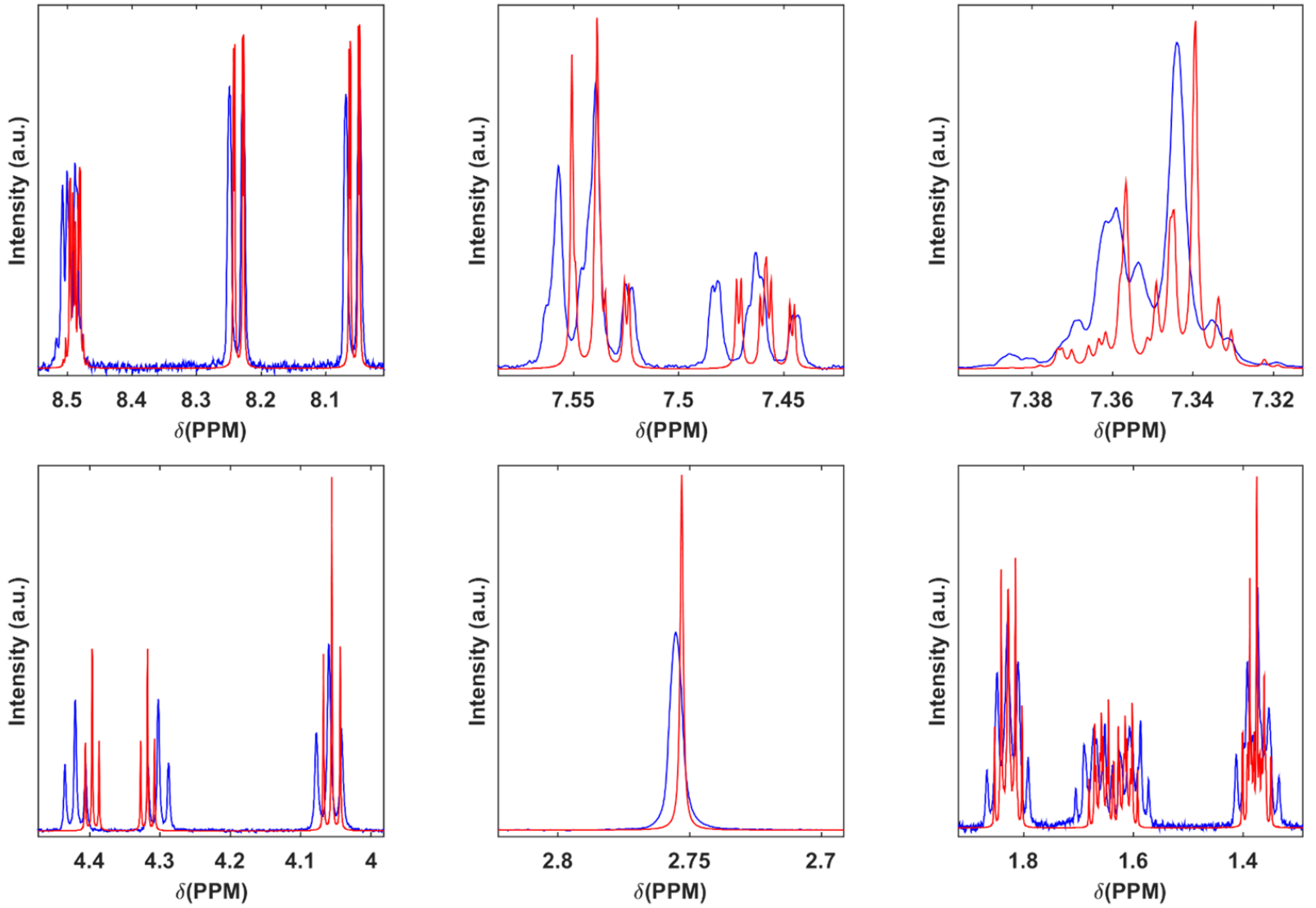


Side Note: A better fit has since been obtained

Translating Model to Different Field Strengths

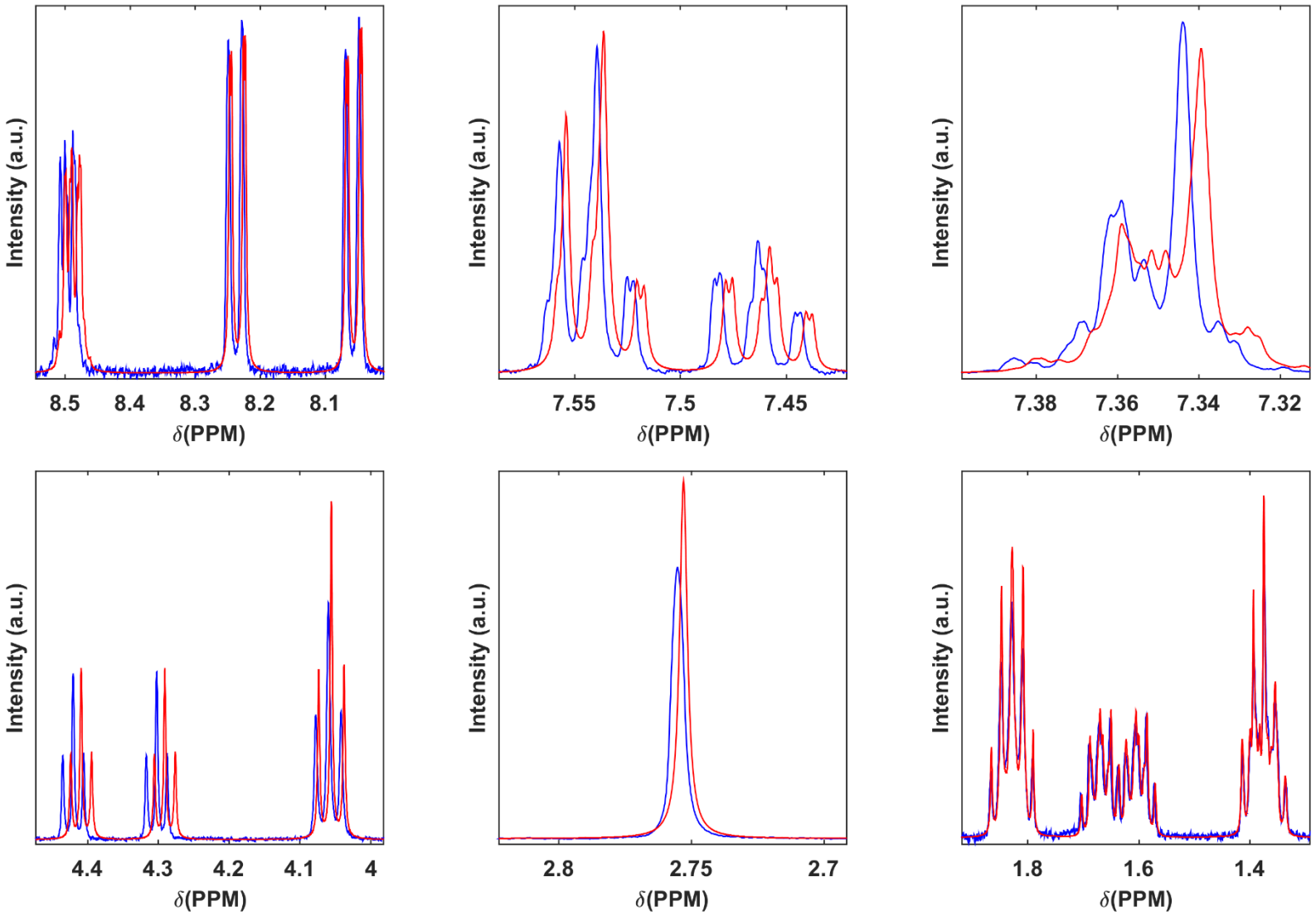


^{1}H NMR Spectrum of MAM-2201 @ 400 MHz



400 MHz
600 MHz (model)

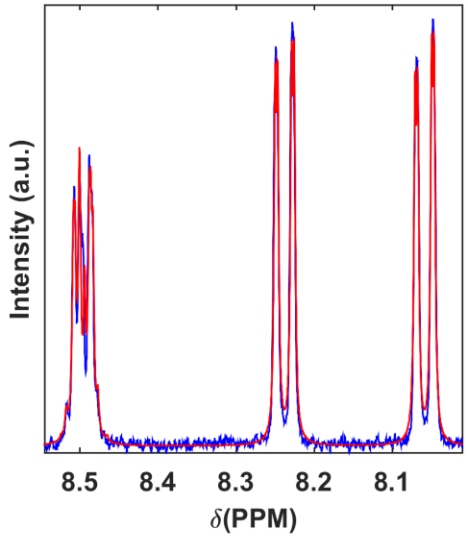
Transform 600 MHz Model to 400 MHz

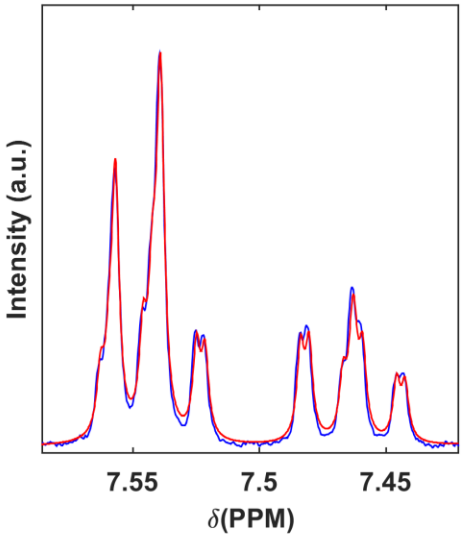


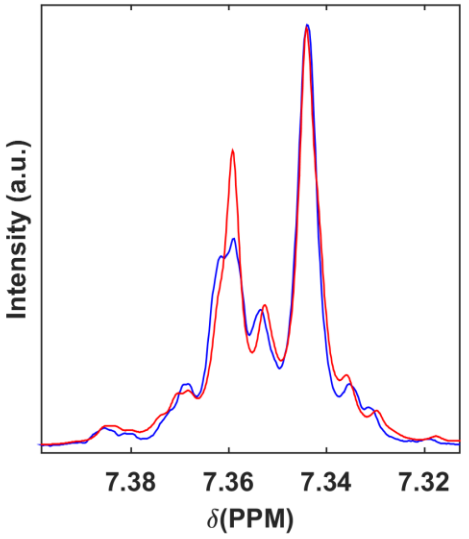
400 MHz (20°C)

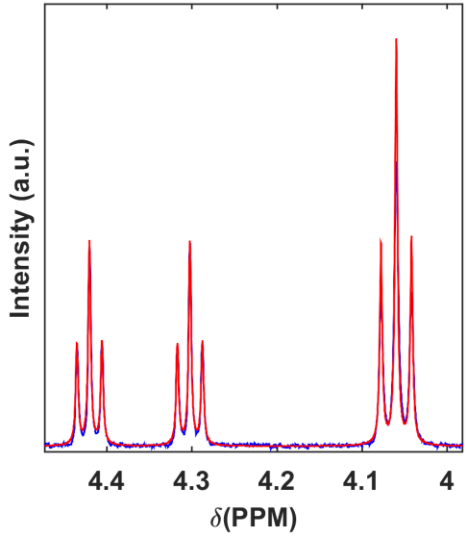
600 MHz (25°C) Evaluated @ 400 MHz

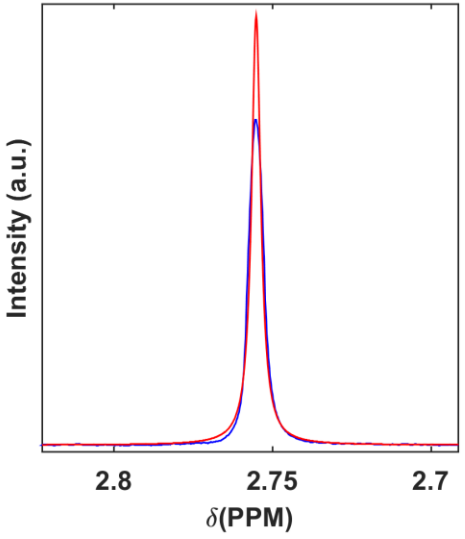
Chemical Shifts of Model Adjusted

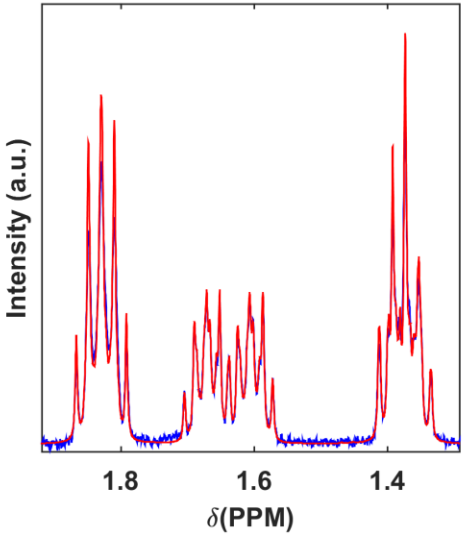






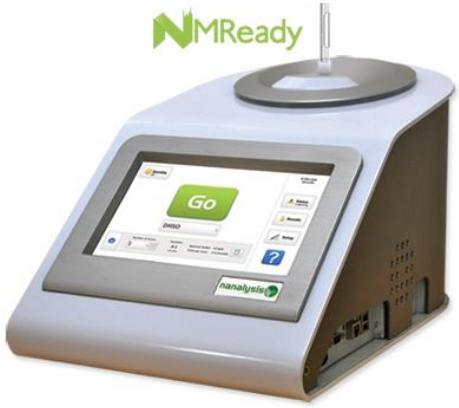




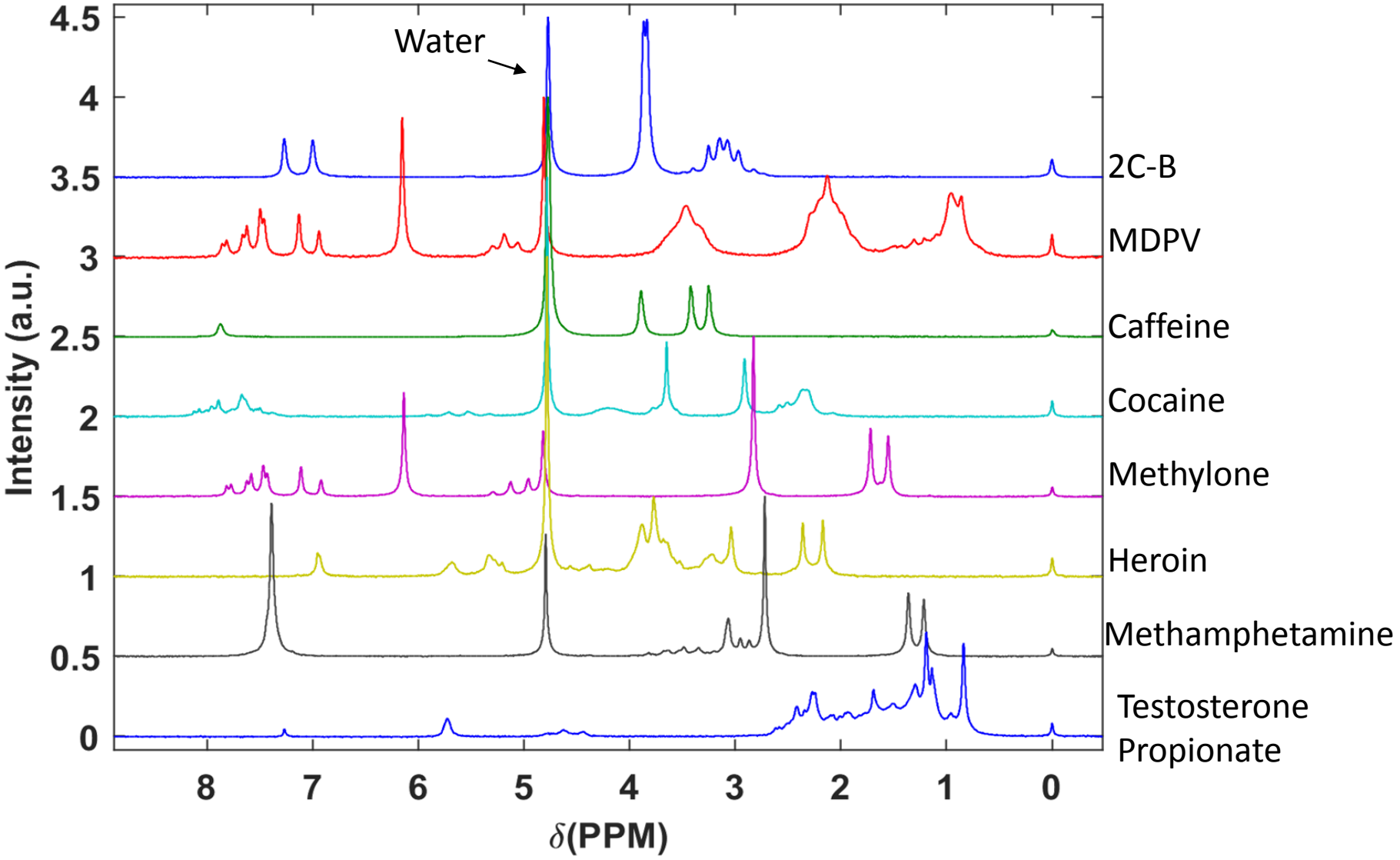


Benchtop NMR

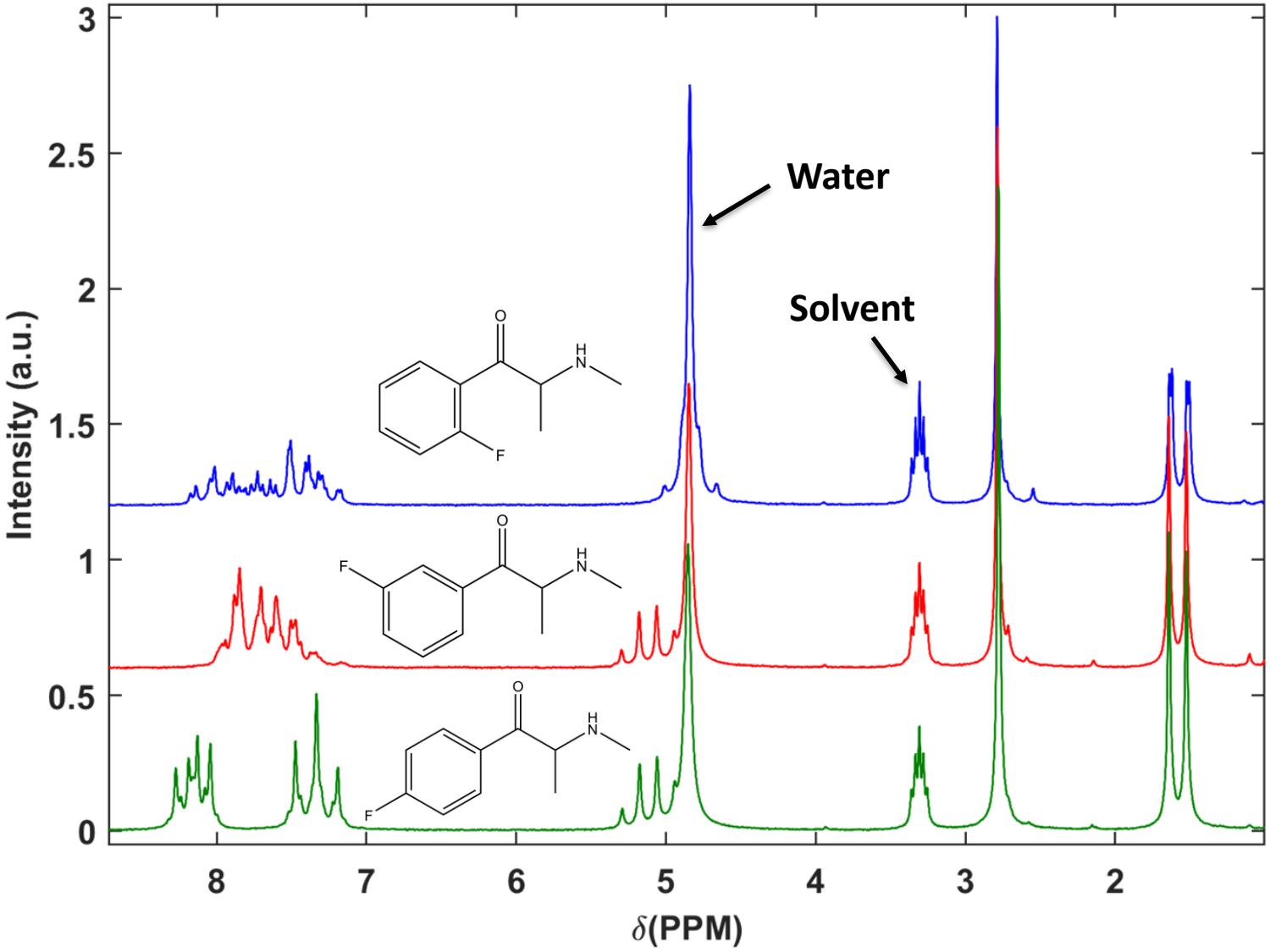
- 40 – 90 MHz Permanent Magnet Systems
- Range from ~ $40K - $100K
- No cryogens, little maintenance
- Easy to Use
- Portable to varying extents
- Some 2D spectral capabilities



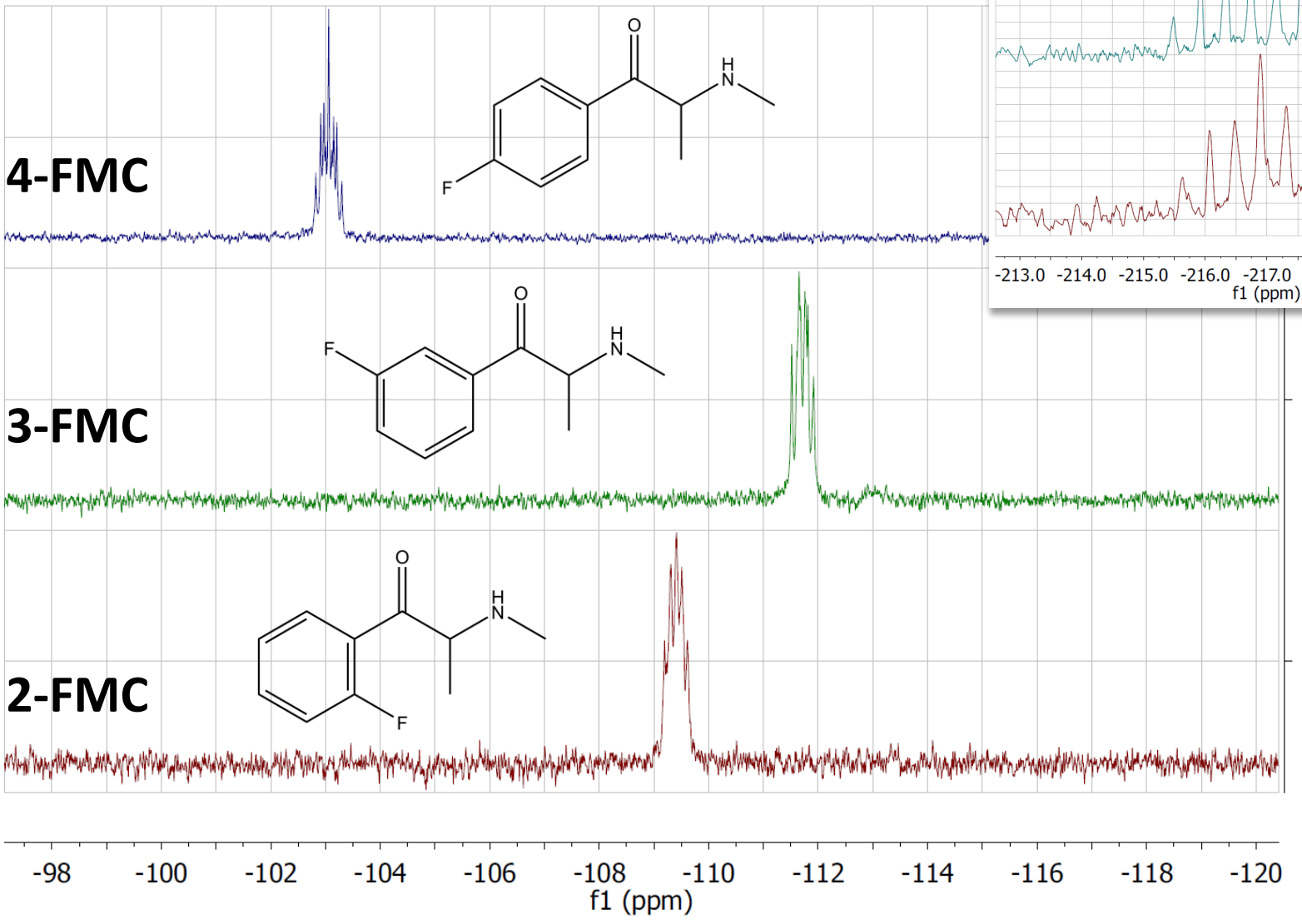
^{1}H Spectrum Survey (42 MHz)

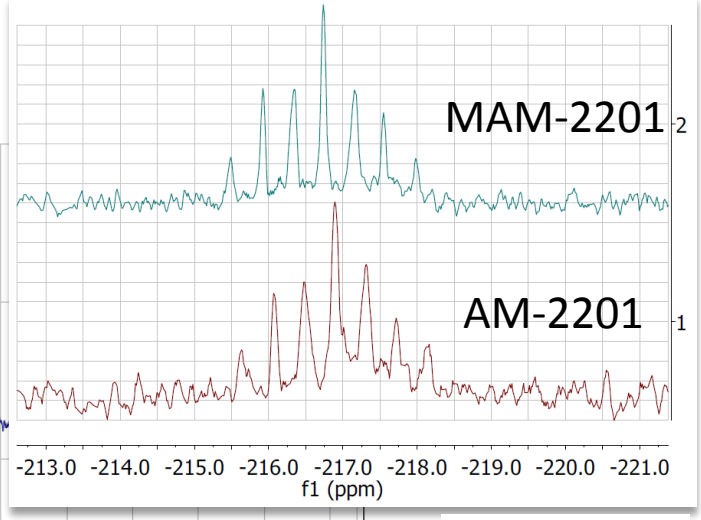


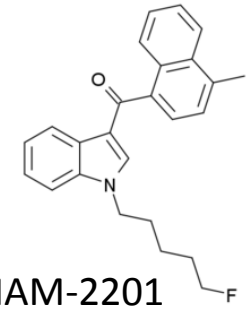
Fluoromethcathinones (^{1}H, 62 MHz, MeOD)



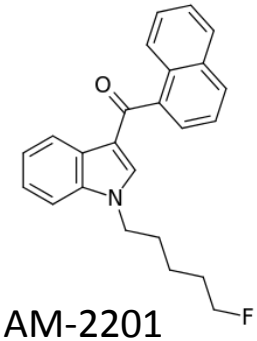
^{19}F NMR Spectra (~58 MHz)





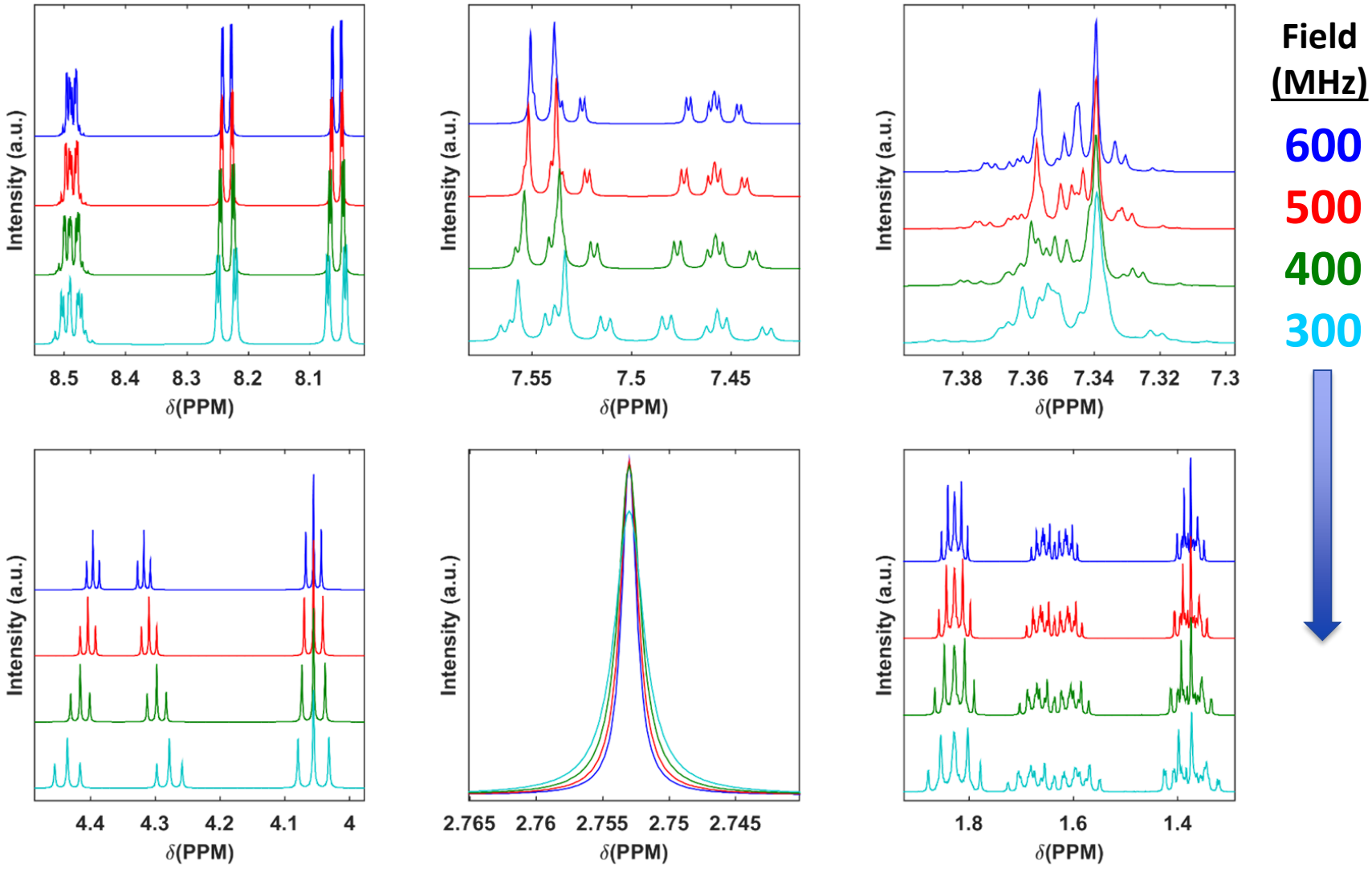


MAM-2201



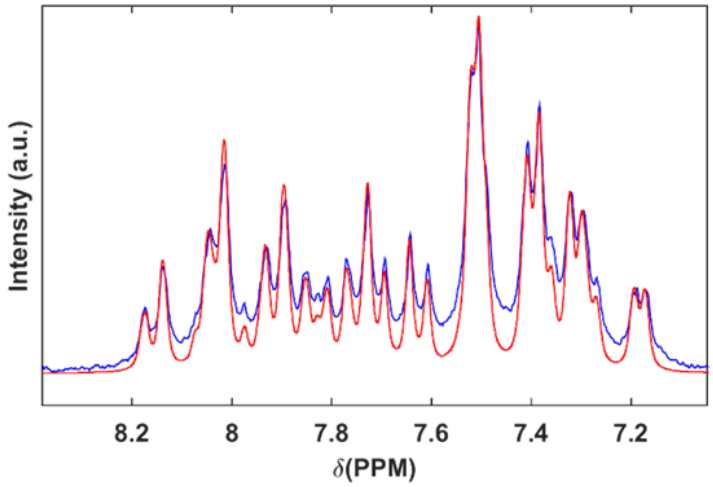
AM-2201

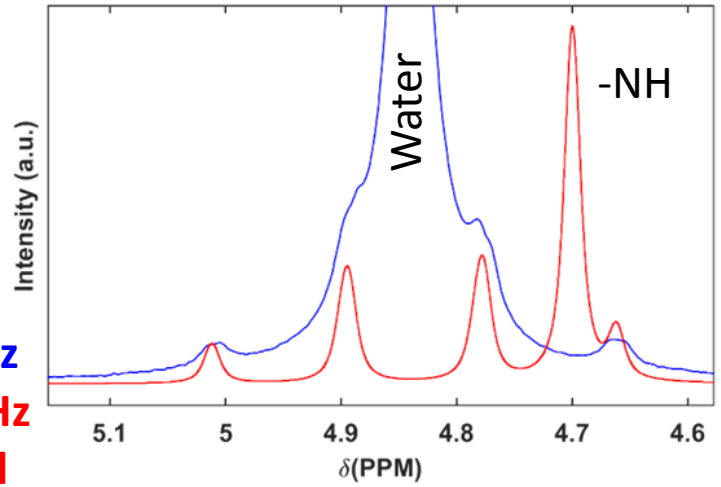
Field Translation….. How Low Can You Go?



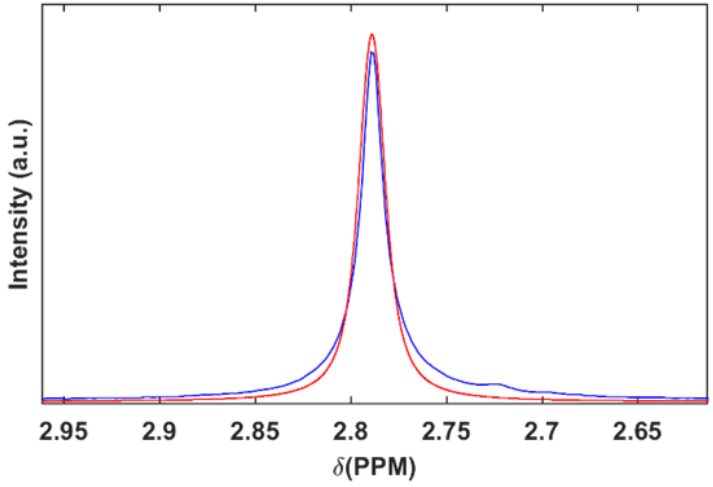
600 MHz Model Transformed to 62 MHz

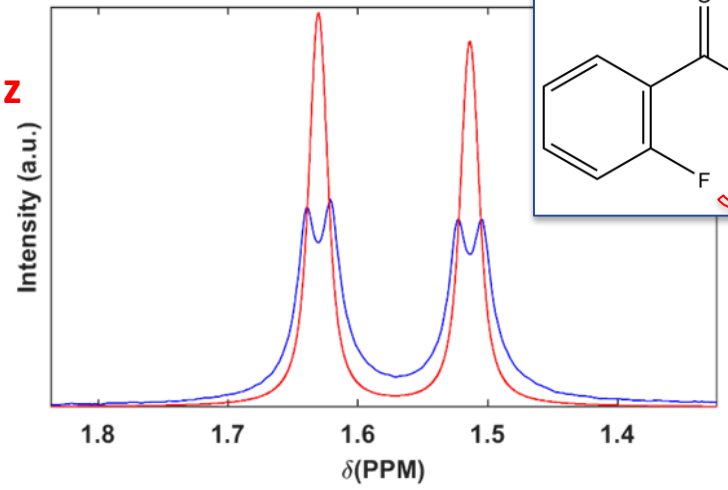
2-Fluoromethcathinone



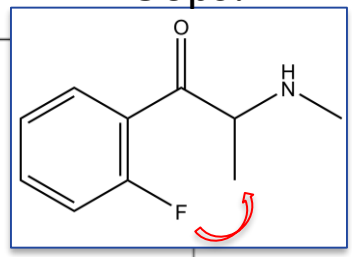


62 MHz
600 MHz Model Evaluated @ 62 MHz



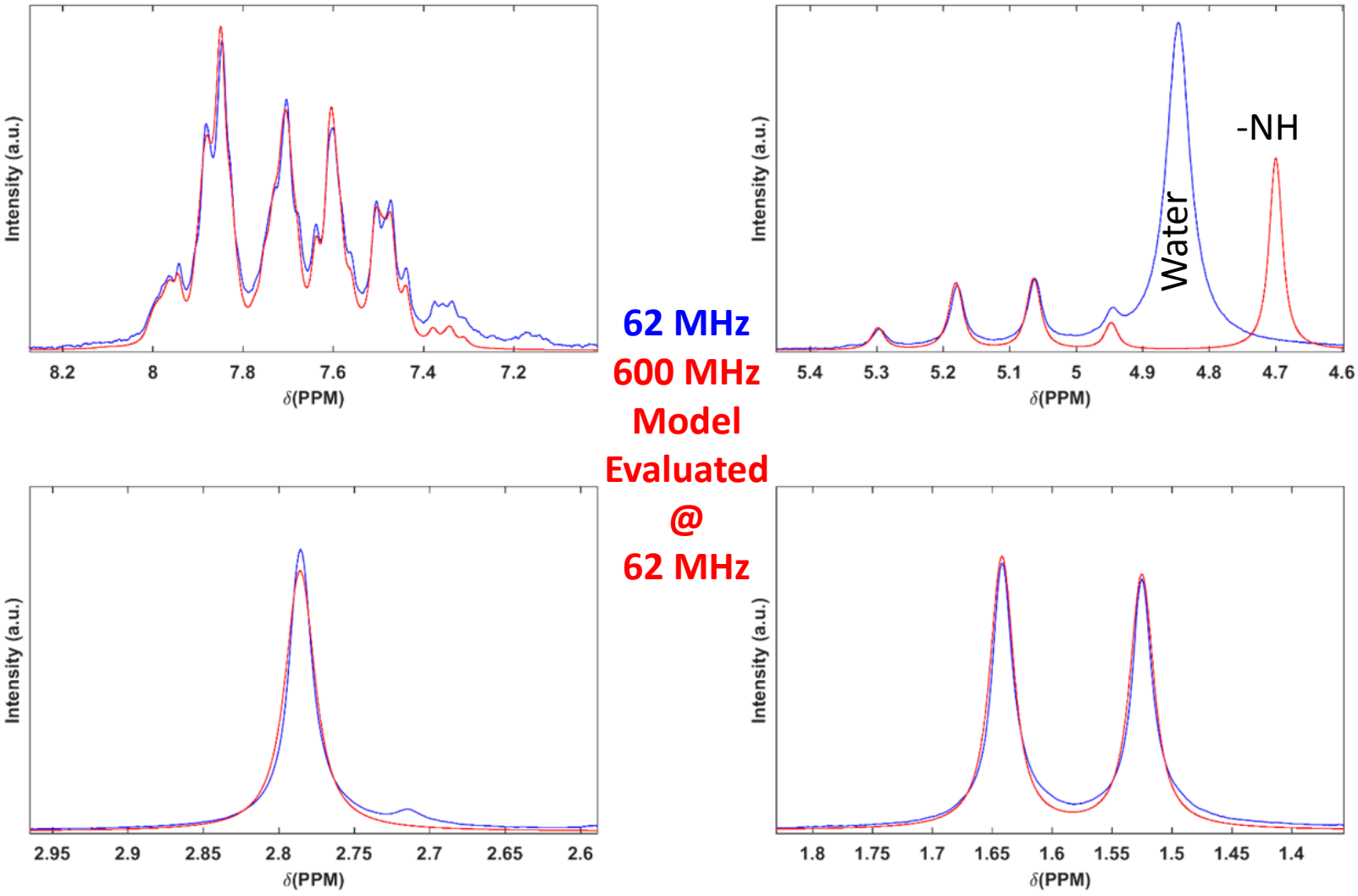


Oops!



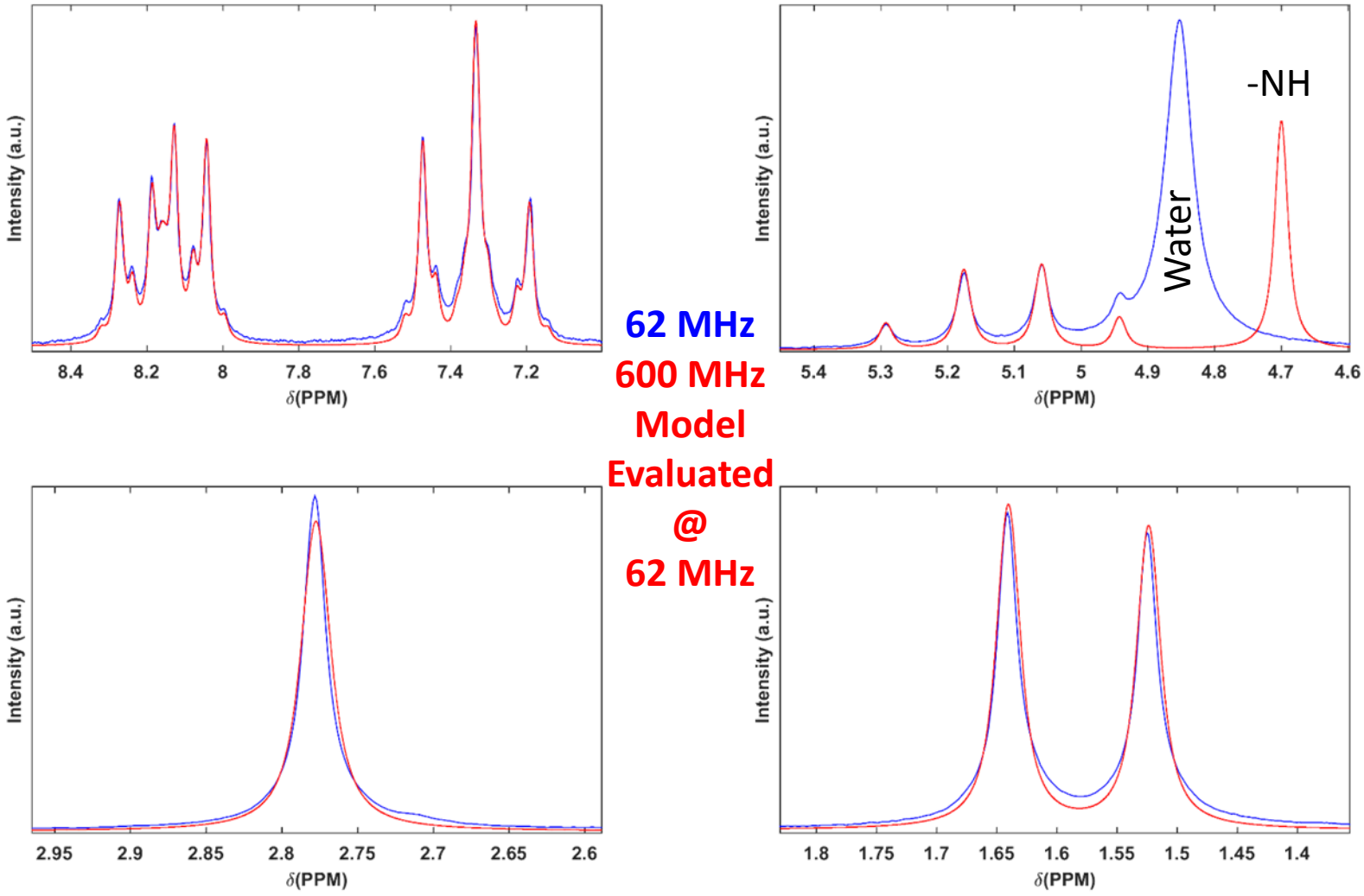
600 MHz Model Transformed to 62 MHz

3-Fluoromethcathinone



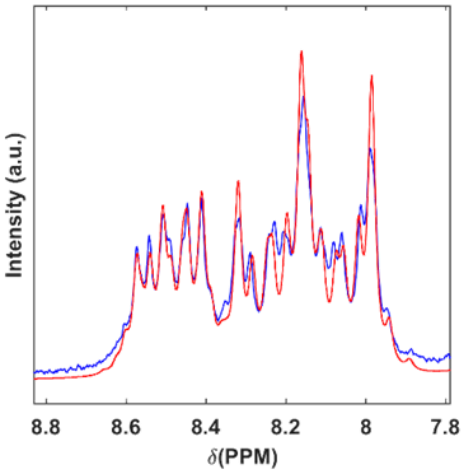
600 MHz Model Transformed to 62 MHz

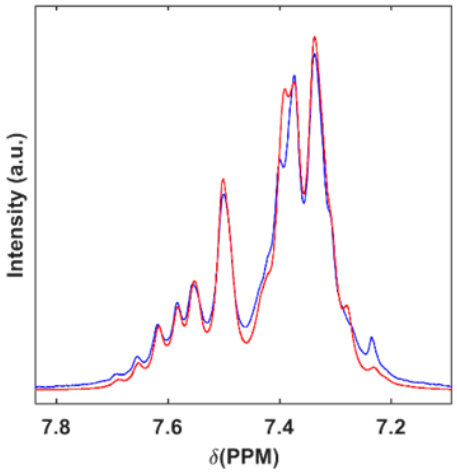
4-Fluoromethcathinone

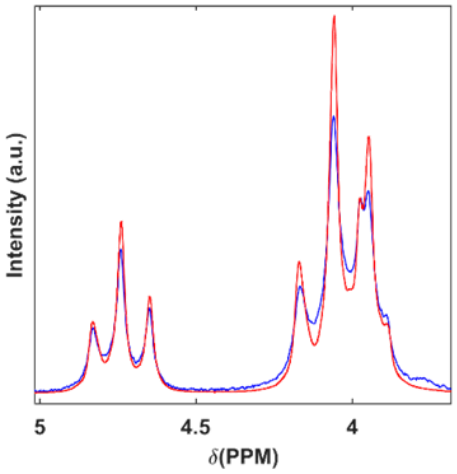


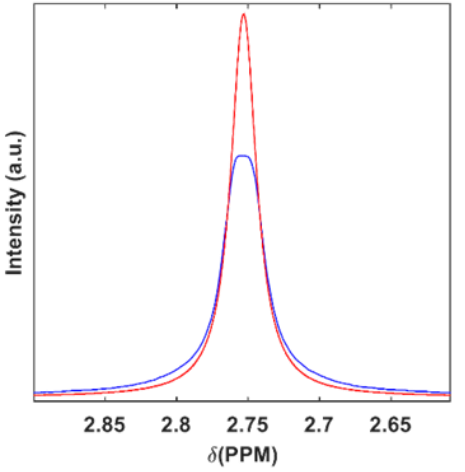
600 MHz Model Transformed to 62 MHz

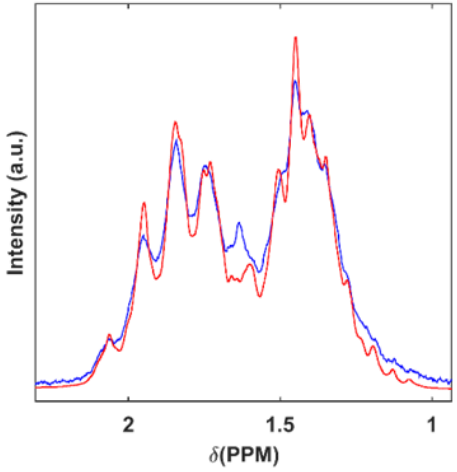
MAM-2201

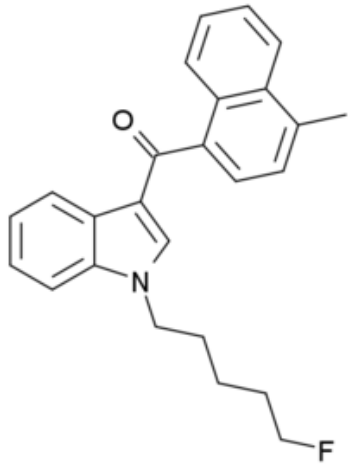












Summary & Future Work

- Briefly discussed the (well-known) utility of NMR as a powerful tool in structure elucidation
- Demonstrated the utility of spin system modeling of proton NMR spectra
  - Facilitate data comparison between instruments/fields
  - Potential for rapid confirmation of chemical ID
- Going Forward
  - Build up library of modeled spectra
  - Explore mixture analysis at high and low field

Acknowledgements

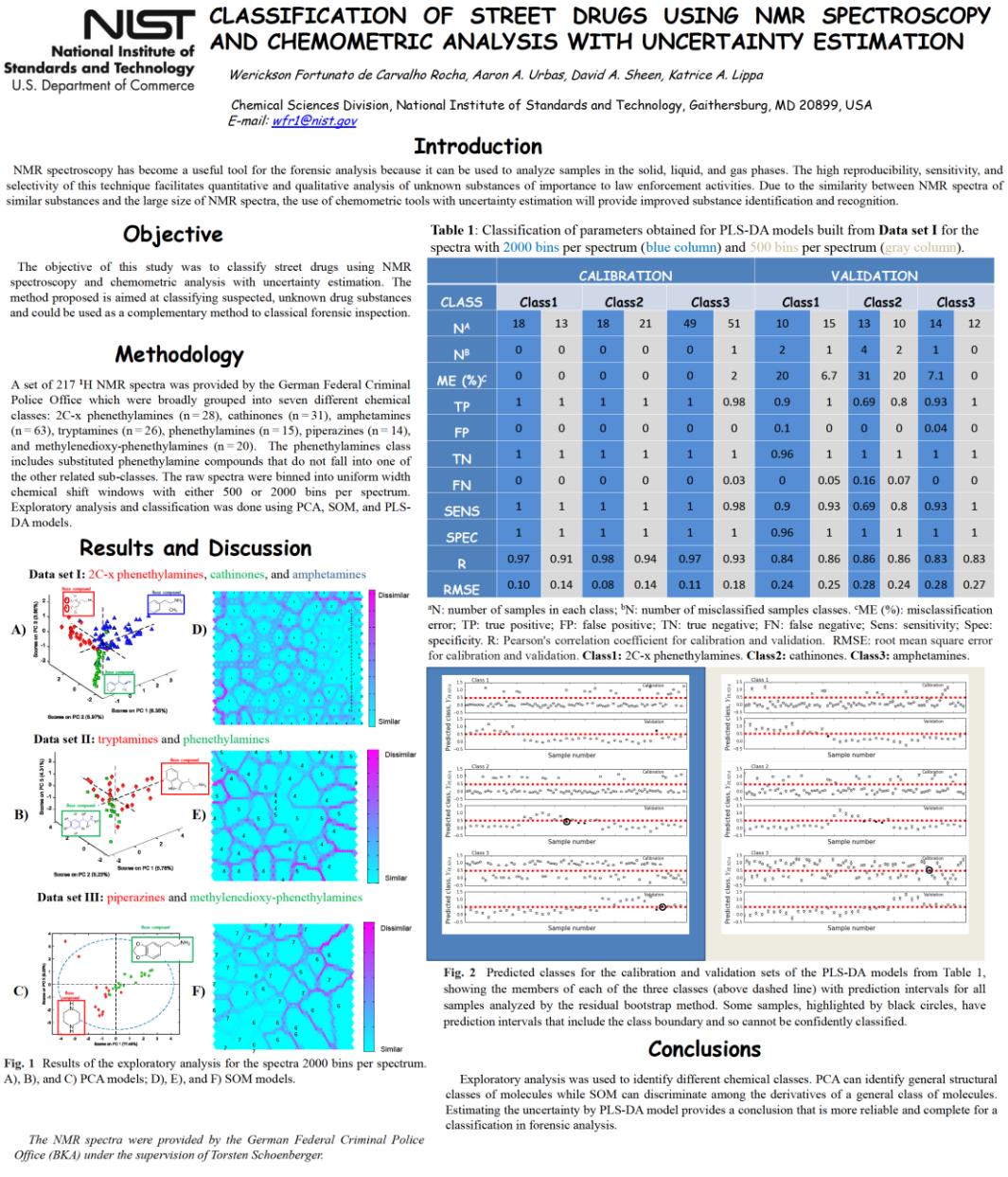
- Support from the NIST Special Programs Office
- Samples from the DEA Special Testing and Research Laboratory

Disclaimer

Commercial equipment identified in this presentation is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose."

Thanks!

Check out our poster on drug classification of NMR spectra.



NIST National Institute of Standards and Technology U.S. Department of Commerce

CLASSIFICATION OF STREET DRUGS USING NMR SPECTROSCOPY AND CHEMOMETRIC ANALYSIS WITH UNCERTAINTY ESTIMATION

Werickson Fortunato de Carvalho Rocha, Aaron A. Urbas, David A. Sheen, Katrice A. Lippa

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Introduction

NMR spectroscopy has become a useful tool for the forensic analysis because it can be used to analyze samples in the solid, liquid, and gas phases. The high reproducibility, sensitivity, and selectivity of this technique facilitates quantitative and qualitative analysis of unknown substances of importance to law enforcement activities. Due to the similarity between NMR spectra of similar substances and the large size of NMR spectra, the use of chemometric tools with uncertainty estimation will provide improved substance identification and recognition.

Objective

The objective of this study was to classify street drugs using NMR spectroscopy and chemometric analysis with uncertainty estimation. The method proposed is aimed at classifying suspected, unknown drug substances and could be used as a complementary method to classical forensic inspection.

Methodology

A set of 217 ^{1}H NMR spectra was provided by the German Federal Criminal Police Office which were broadly grouped into seven different chemical classes: 2C-x phenethylamines (n = 28), cathinones (n = 31), amphetamines (n = 63), tryptamines (n = 26), phenethylamines (n = 15), piperazines (n = 14), and methylenedioxy-phenethylamines (n = 20). The phenethylamines class includes substituted phenethylamine compounds that do not fall into one of the other related sub-classes. The raw spectra were binned into uniform width chemical shift windows with either 500 or 2000 bins per spectrum. Exploratory analysis and classification was done using PCA, SOM, and PLS-DA models.

Results and Discussion

Data set I: 2C-x phenethylamines, cathinones, and amphetamines

Data set II: tryptamines and phenethylamines

Data set III: piperazines and methylenedioxy-phenethylamines

Fig. 1 Results of the exploratory analysis for the spectra 2000 bins per spectrum. A), B), and C) PCA models; D), E), and F) SOM models.

The NMR spectra were provided by the German Federal Criminal Police Office (BKA) under the supervision of Torsten Schoenberger.

Table 1: Classification of parameters obtained for PLS-DA models built from **Data set I** for the spectra with 2000 bins per spectrum (blue column) and 500 bins per spectrum (gray column).

	CALIBRATION						VALIDATION					
CLASS	Class1		Class2		Class3		Class1		Class2		Class3	
N[A]	18	13	18	21	49	51	10	15	13	10	14	12
N[B]	0	0	0	0	0	1	2	1	4	2	1	0
ME (%)[C]	0	0	0	0	0	2	20	6.7	31	20	7.1	0
TP	1	1	1	1	1	0.98	0.9	1	0.69	0.8	0.93	1
FP	0	0	0	0	0	0	0.1	0	0	0	0.04	0
TN	1	1	1	1	1	1	0.96	1	1	1	1	1
FN	0	0	0	0	0	0.03	0	0.05	0.16	0.07	0	0
SENS	1	1	1	1	1	0.98	0.9	0.93	0.69	0.8	0.93	1
SPEC	1	1	1	1	1	1	0.96	1	1	1	1	1
R	0.97	0.91	0.98	0.94	0.97	0.93	0.84	0.86	0.86	0.86	0.83	0.83
RMSE	0.10	0.14	0.08	0.14	0.11	0.18	0.24	0.25	0.28	0.24	0.28	0.27

[A]N: number of samples in each class; [B]N: number of misclassified samples classes. [C]ME (%): misclassification error; TP: true positive; FP: false positive; TN: true negative; FN: false negative; Sens: sensitivity; Spec: specificity. R: Pearson's correlation coefficient for calibration and validation. RMSE: root mean square error for calibration and validation. **Class1:** 2C-x phenethylamines. **Class2:** cathinones. **Class3:** amphetamines.

Fig. 2 Predicted classes for the calibration and validation sets of the PLS-DA models from Table 1, showing the members of each of the three classes (above dashed line) with prediction intervals for all samples analyzed by the residual bootstrap method. Some samples, highlighted by black circles, have prediction intervals that include the class boundary and so cannot be confidently classified.

Conclusions

Exploratory analysis was used to identify different chemical classes. PCA can identify general structural classes of molecules while SOM can discriminate among the derivatives of a general class of molecules. Estimating the uncertainty by PLS-DA model provides a conclusion that is more reliable and complete for a classification in forensic analysis.