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Providing NMR Solutions in Drug Metabolism

To Hyphenate or Not to Hyphenate: Is that the Question?

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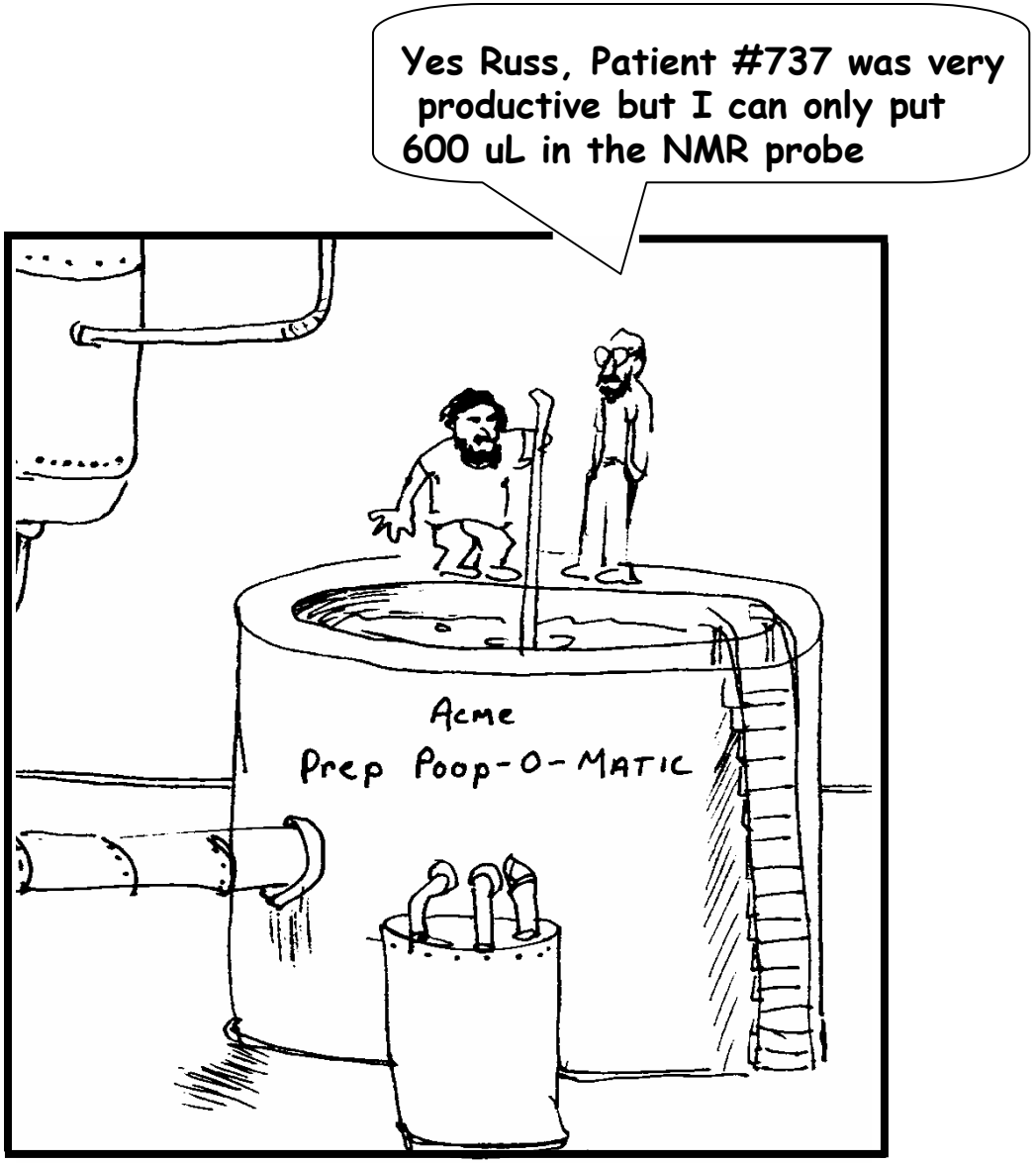
Characterizing Drug Metabolites:

- **Complex Matrices: Bile, Feces, Plasma, and Urine**
  - **Contain a wide array of organic compounds**
  - **Isolation and structural identification are confounded**
- **The concentration of drug metabolites**
  - **Vary with ADME properties**
  - **Ranges: ~µg/mL, down to ≤ 1ng/mL**
- **Circulating drug related components**
  - **Greatest interest**
  - **Limited by concentration and/or volume of plasma**
- **Regulatory requirements are becoming more stringent**
- **Drug potency is increasing**

Example

Dose = 0.5 mg/day,
Primary route of excretion: feces
Collection period = 3 days
10-12 metabolites
No radiolabel
One "productive" patient: 1.5 kg
The math: 1.5 mg/10/1.5 kg
~ 100 ng/g → extraction challenge



Analytical Tools: LC, MS and NMR

- Separations must be a part of the solution
- Many different strategies possible
- Key strategic question: roll of hyphenated techniques
  - LC-MS
  - LC-NMR
  - LC-MS-NMR
  - All of the above with Radiometric Detection

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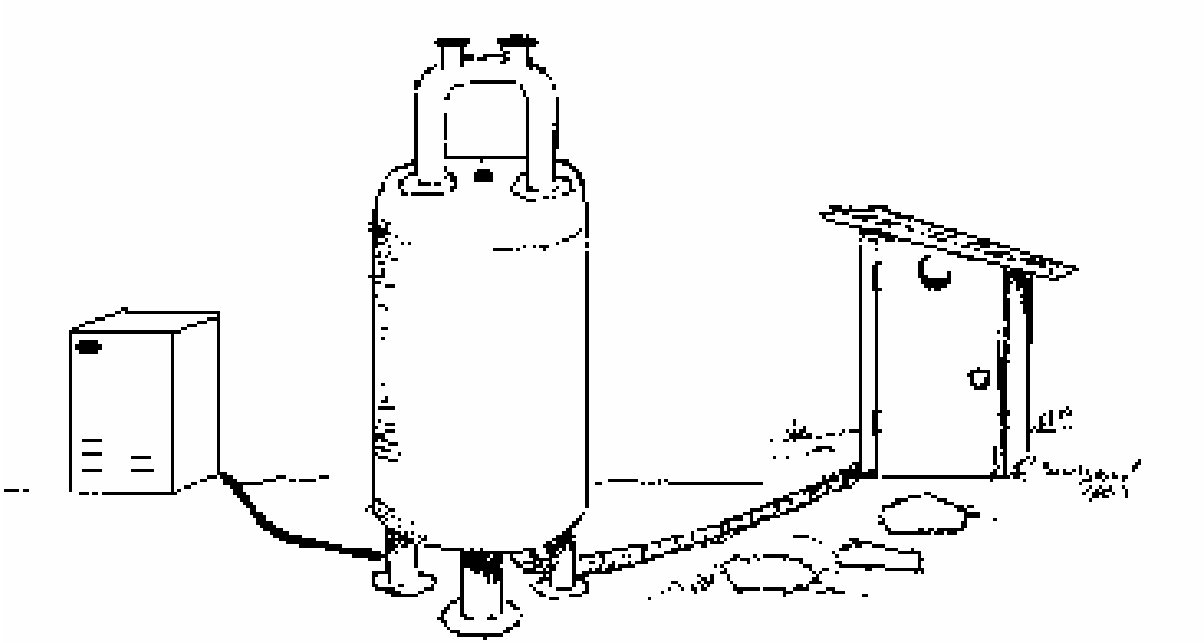
NMR with More Hyphens?

Flush-n-Flow-NMR



LC-MS and LC-NMR in Drug Metabolism

	MS	NMR
Analyze all components in single chromatographic run	±	-
Peak widths limit data capture (on-flow)	±	-
LC method can impact spectroscopy: solvent composition	-	-
Sample manipulation reduced	+	+
Automation	+	-
Conformation vs unknowns	+	-
Efficiency, time saving	-/?	-

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Evolution:

"I got into NMR because there was no heavy lifting or chromatography. However, after seeing the latest in LC-NMR.... I'd be willing to try heavy lifting "

Tim Spitzer, GSK
NMR Spectroscopist

<1997 Low-Field NMR, Tube Only: What's a µg?

1997 500-MHz NMR, LC-NMR : 3mm Tube 50:50

1999 600-MHz NMR, LC-NMR : 3mm Tube 75:25

2001 LC-MS-NMR Option 80:20 LC-NMR

2004 Cold Probe Installed 100% 3mm tube

<2003 LC-MS 100%

2005 nano-ESI: LC-MS 95:5

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Putting the Bits Together

Radio-LC Profiling and Quantification → **Fraction Collection Into 96-Well Plates** → **Reconstructed Radio-Chromatogram**

Isotope Pattern

nanoESI-MS
Accurate mass
MS/MS

nanoESI-MS
Ion trap

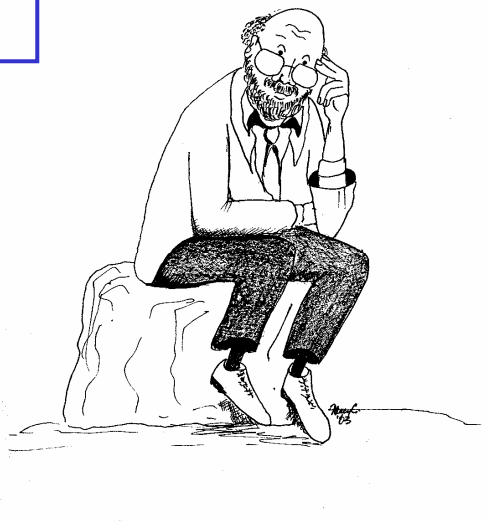
Markush Structure

Isolation Strategy for NMR

NMR 3mm Tube Cold Probe → Metabolite Structure

All data captured on common platform ACD database

Signature: Accurate Mass, Product Ion Spectrum
Note: Single Retention Time



The Best of MS and NMR:

	nanoESI	Cold Probe-NMR
Sensitivity: lot's of it	+	++
Nonselective and Selective detection ie filter out unwanted data	std	add
Discrete Samples	+	+
Extensive Time for Sample Interrogation	++	+
Signal Averaging	+	std
Rich Structural Information	+	+
Complimentary Structural Techniques	std	std

Advantages of nanoESI for metabolite ID

- Increased speed, ≈ 4-5 X increase in experimental work throughput
- Higher quality data - ability to signal average indefinitely
- Reduction in analyte quantity required, ≈ 100 X less material required to get same quality data versus LC/MS
- Rapidly switch between projects (no LC system changeover)
- Rapidly switch between ionization polarities

Advantages of nanoESI for metabolite ID

- Rapidly switch between MS platforms (with same sample)
- Ability to modify analyte and/or spray solvent
- Structures/spectra matched to retention time by default

  (no issues with matching between different LC systems)
- Low intensity product ions now useful for structural ID

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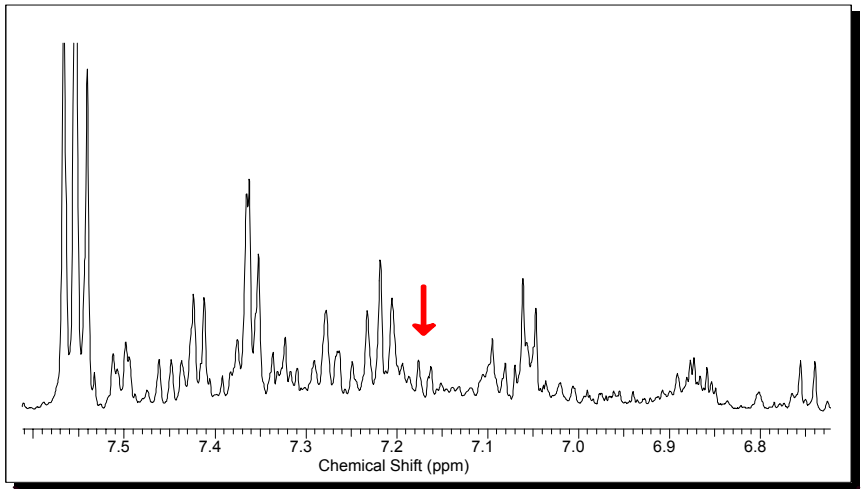
^{1}H Spectrum of Rat Urine

Need Chromatography, Sensitivity, and Selectivity



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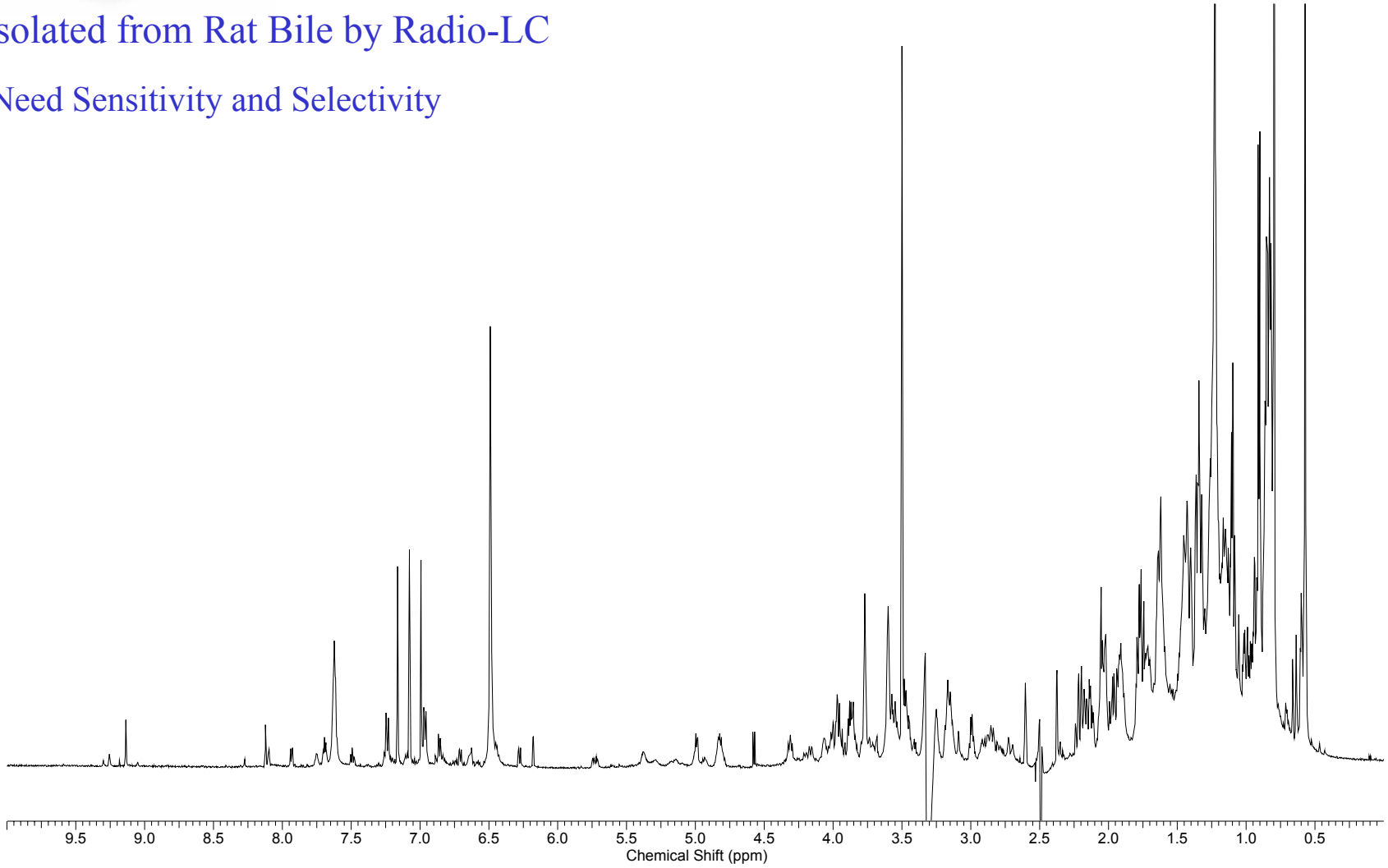
Pure by Radio-LC

Isolated from Rat Bile by Radio-LC

Need Sensitivity and Selectivity

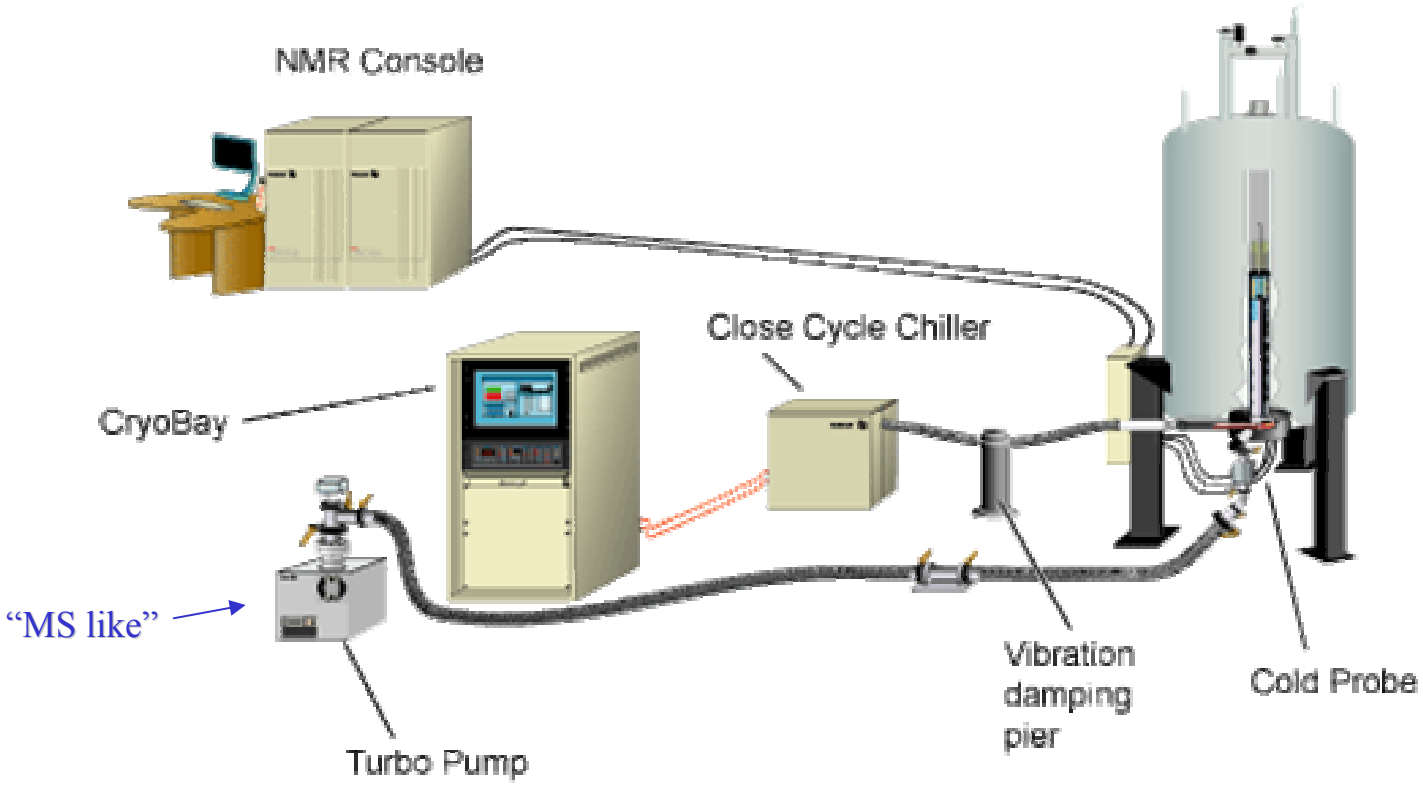


X:\vnmr3\vnmrsys\data\gw328510\scvr02aug00_3

Cold NMR Probes:3-4 Fold Sensitivity

Cryogenically Cooled RF Coil and Preamplifier

Sensitivity increases $\propto \nu^{2/3}$, for example 600-700 ~21% in sensitivity



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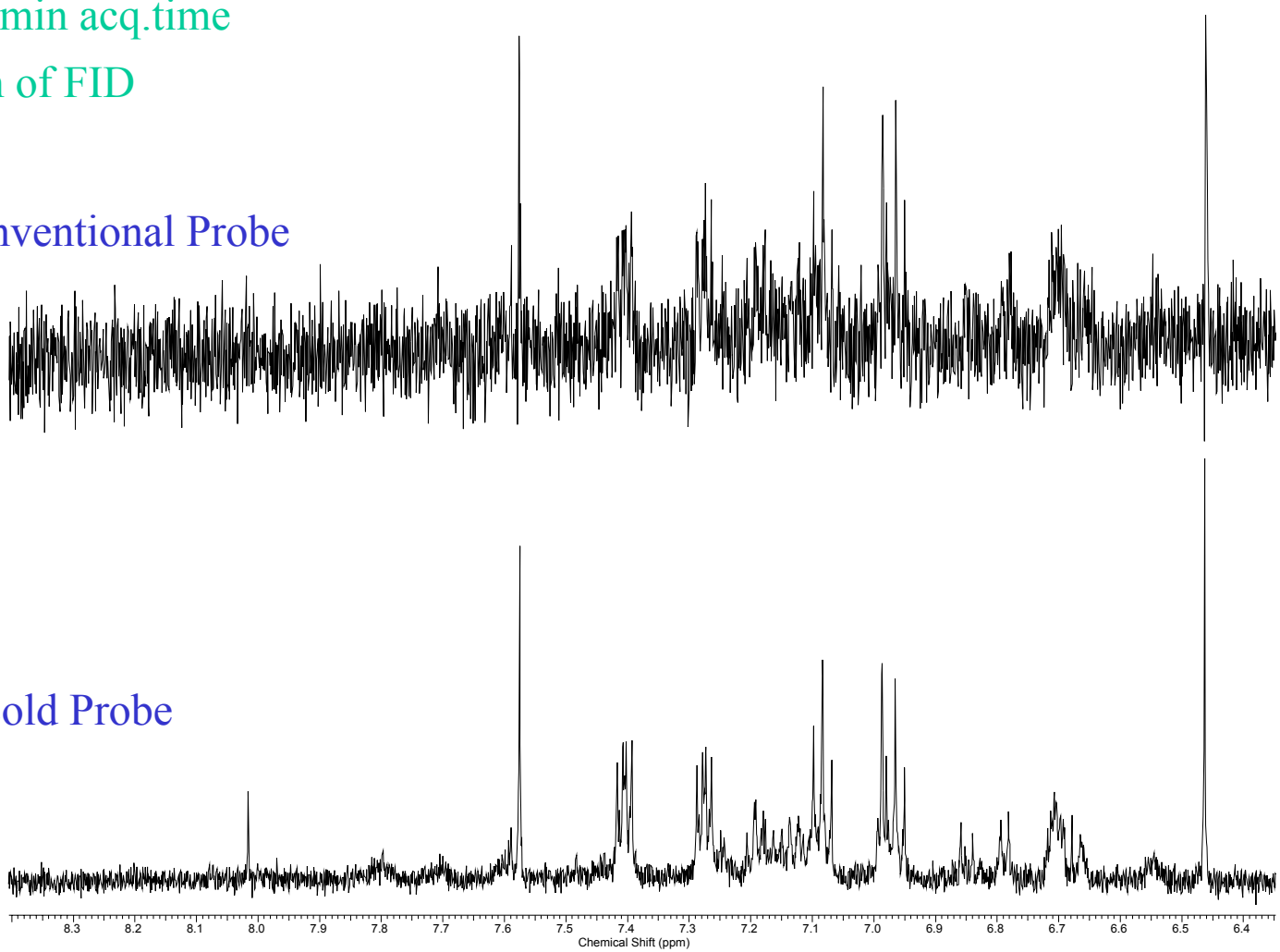
Cold Probe vs Room Temperature Probe

~525 ng/150 µL (3.5ng/µL)
3 mm tube, 49 min acq.time
No apodization of FID

HFC 3mm Conventional Probe

HCN 5mm Cold Probe



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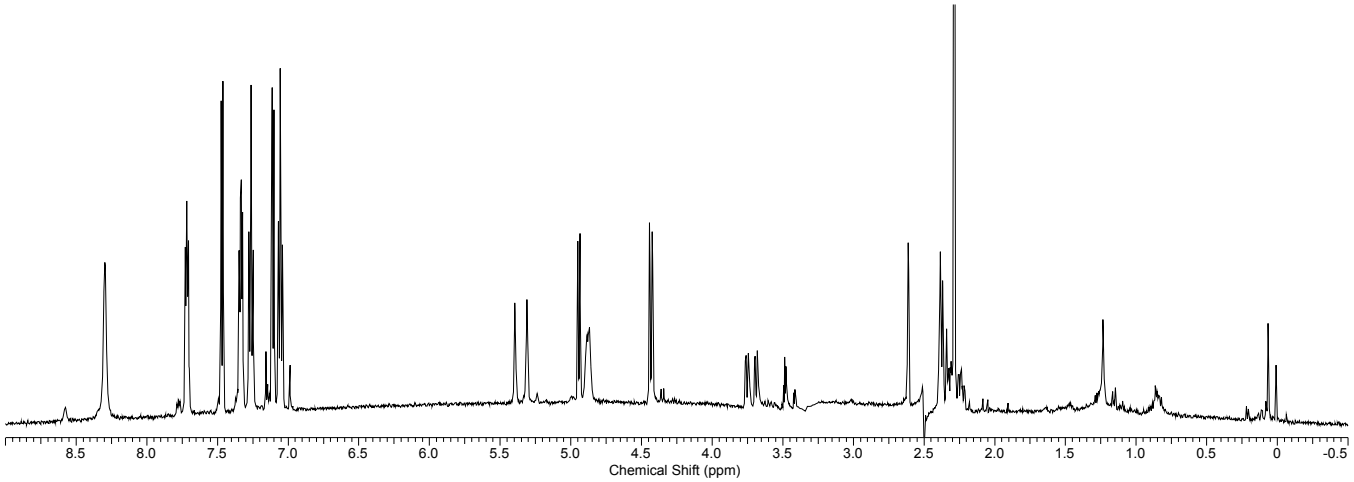
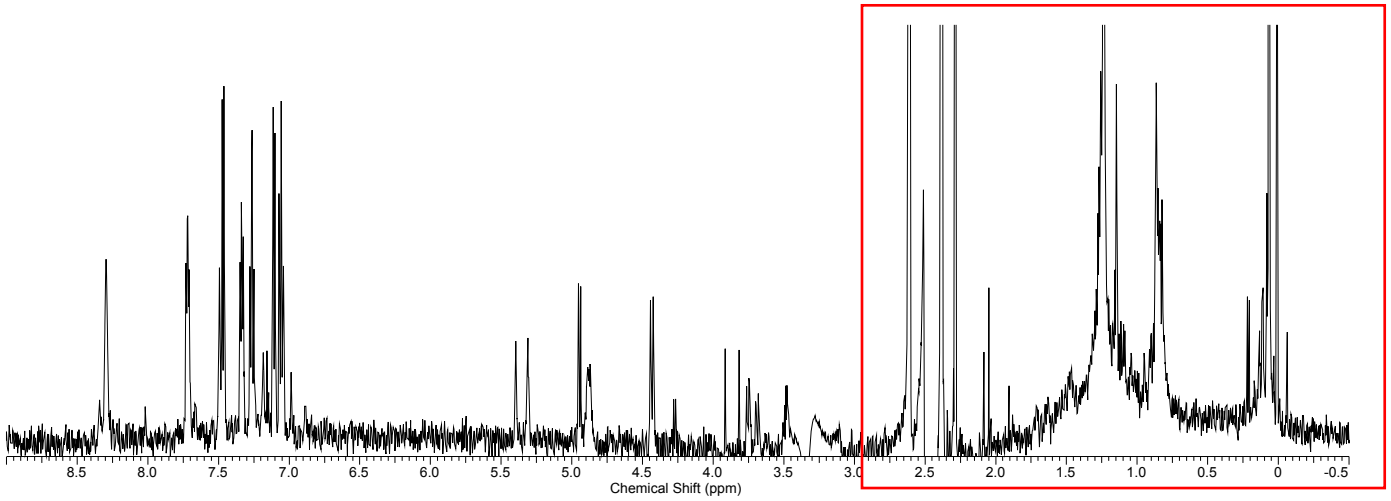
The Background is More of an Issue than Sensitivity

10 µg/150 µL



1 µg/150 µL



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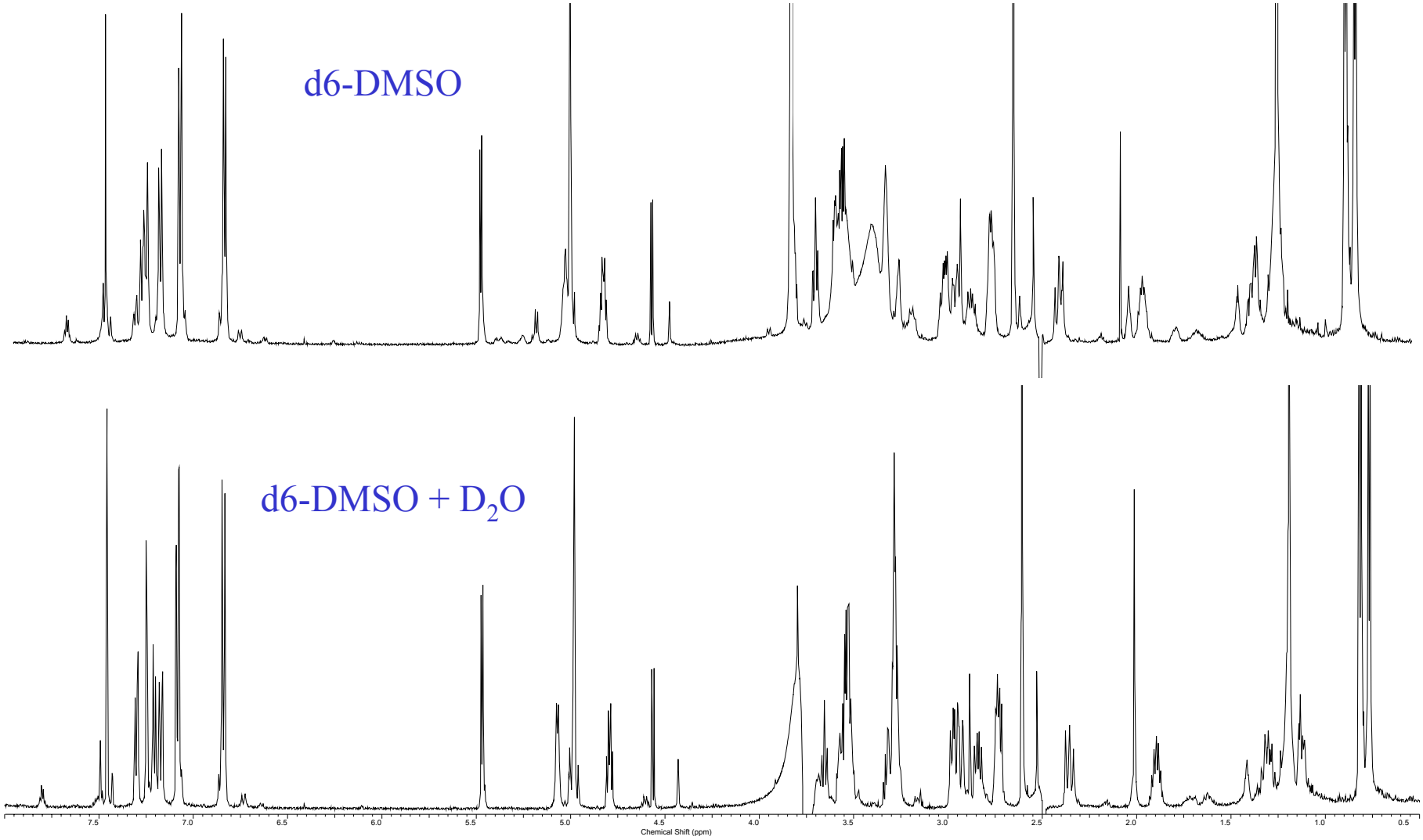
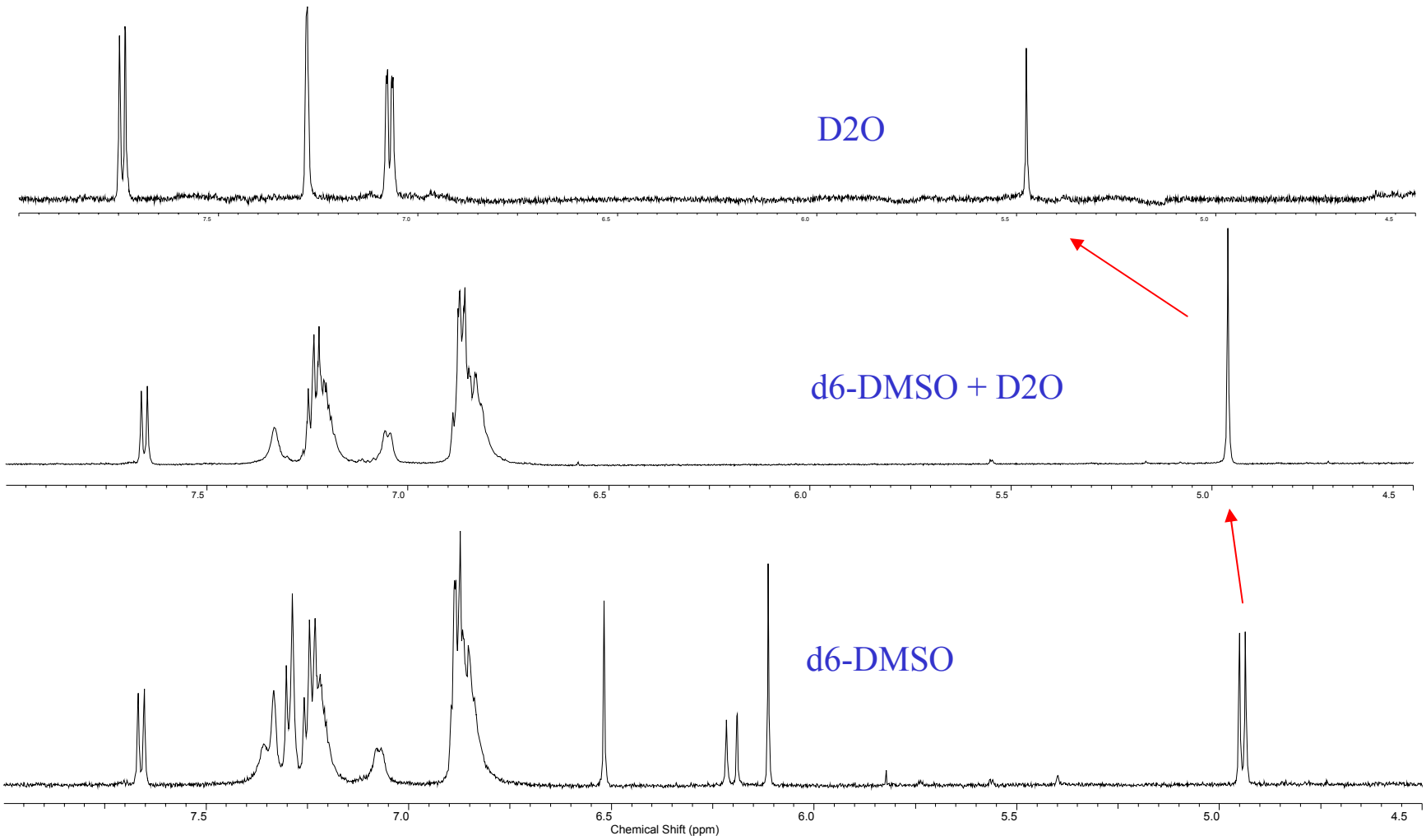
Solvent Effects: An "Old School" Tool



Solvent Effects and Solubility

Metabolite isolated from 10g of rat feces



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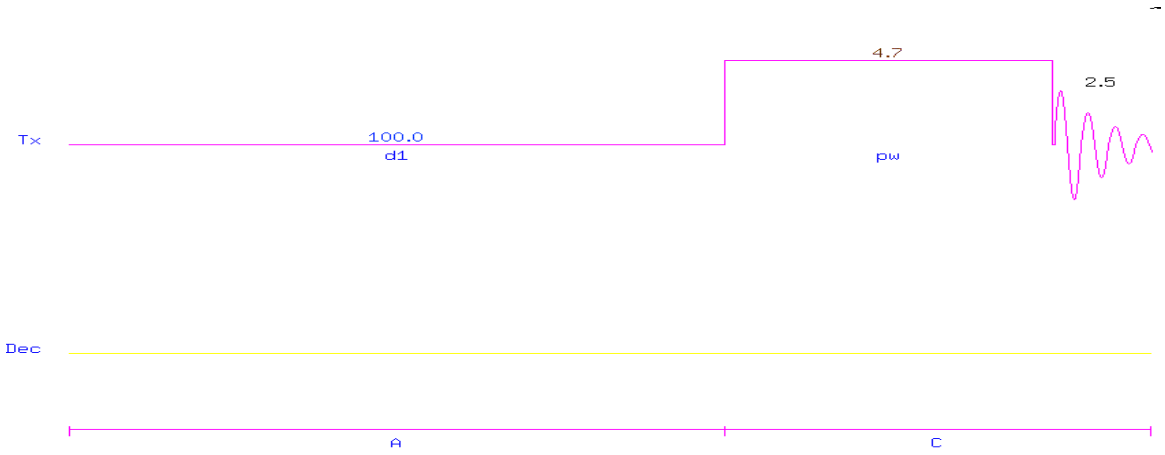
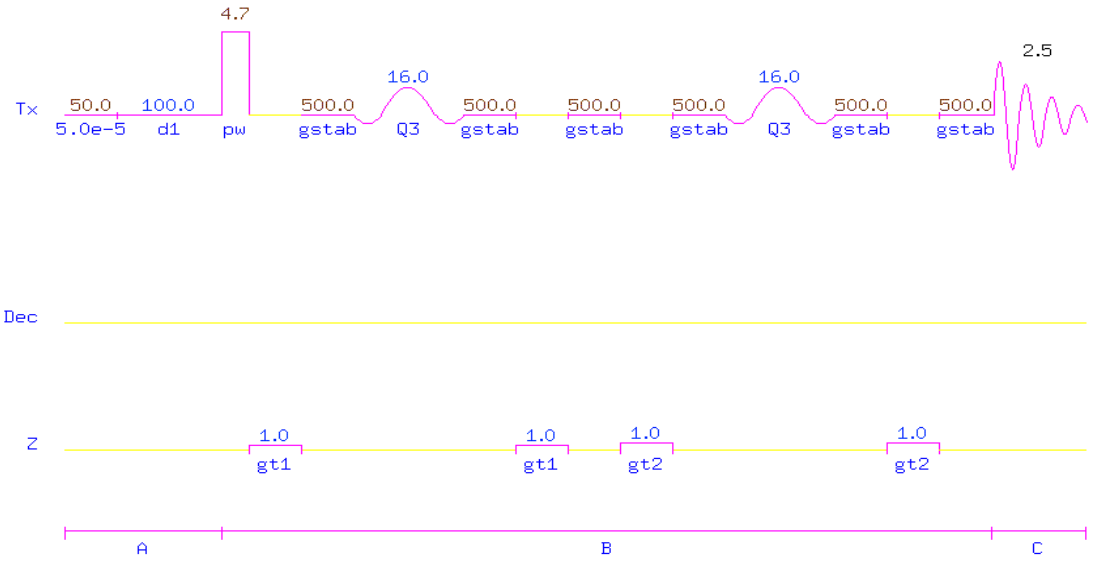
Adding Selectivity to a Non-Selective Method

Nonselective Excitation

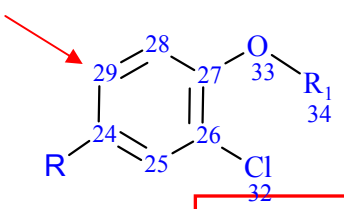


Selective Excitation



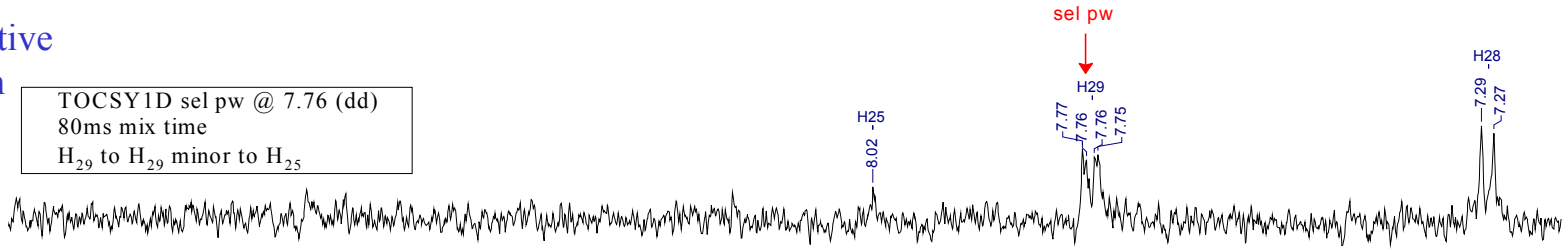
Selective TOCSY Exp

Rat Fecal Metabolite

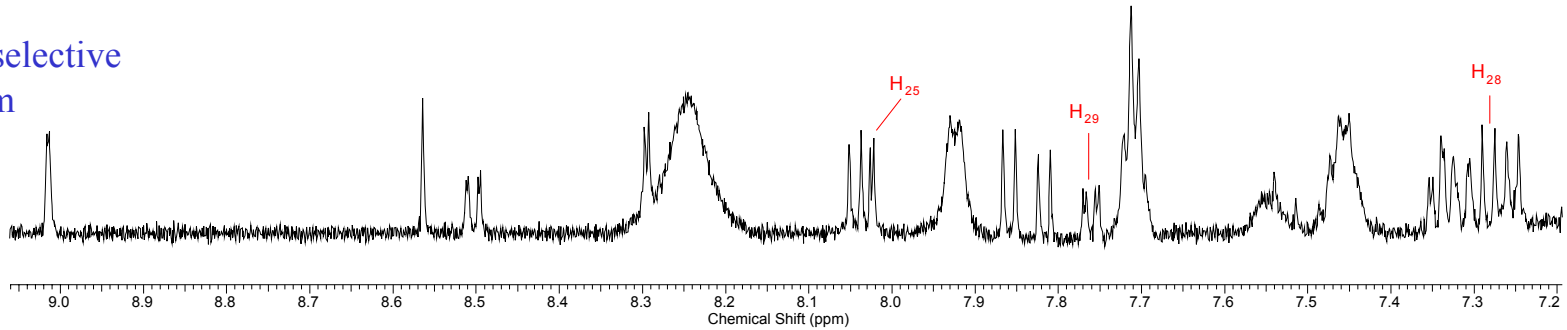


Selective Zoom

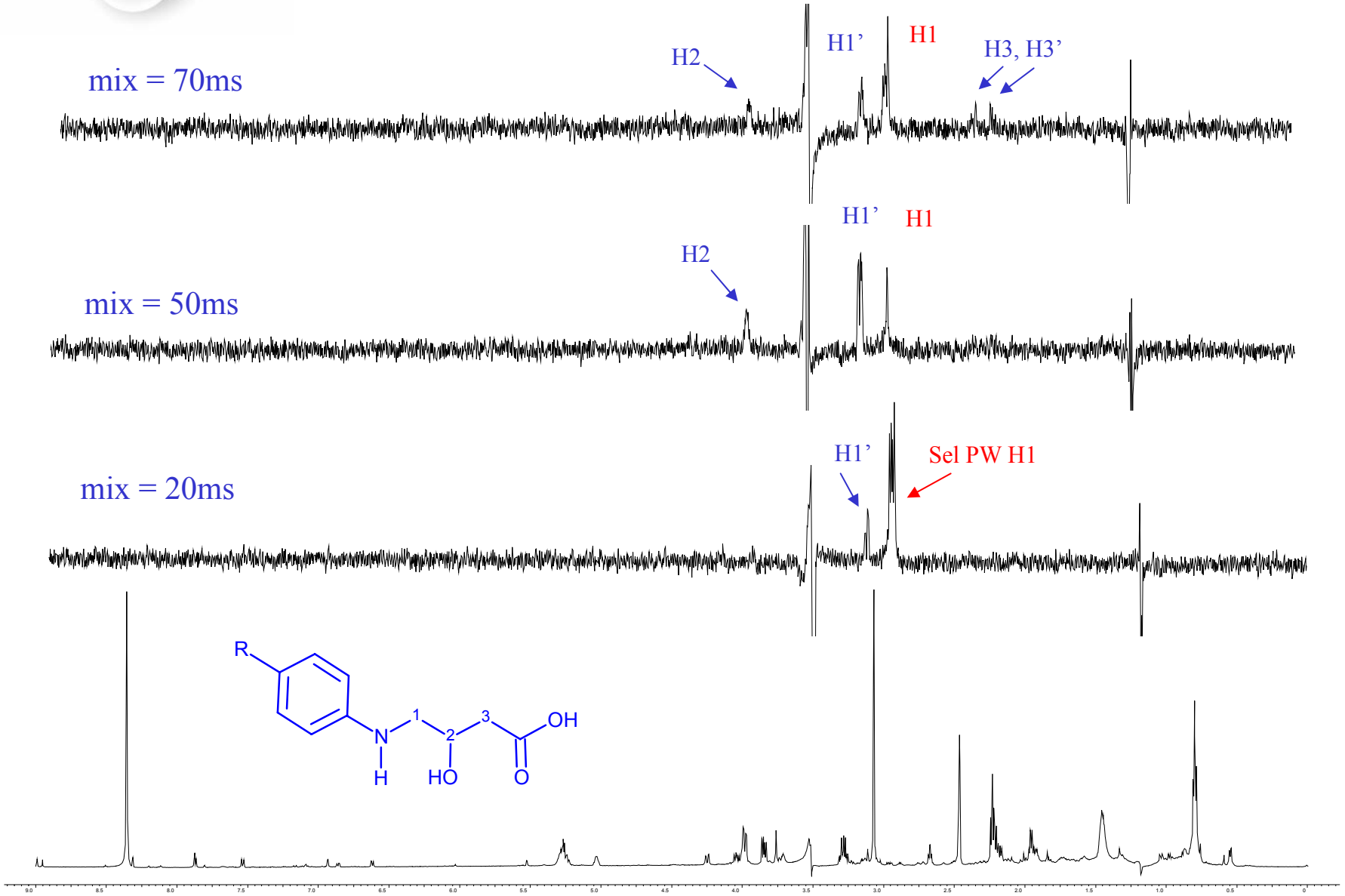
TOCSY1D sel pw @ 7.76 (dd)
80ms mix time
H_{29} to H_{29} minor to H_{25}



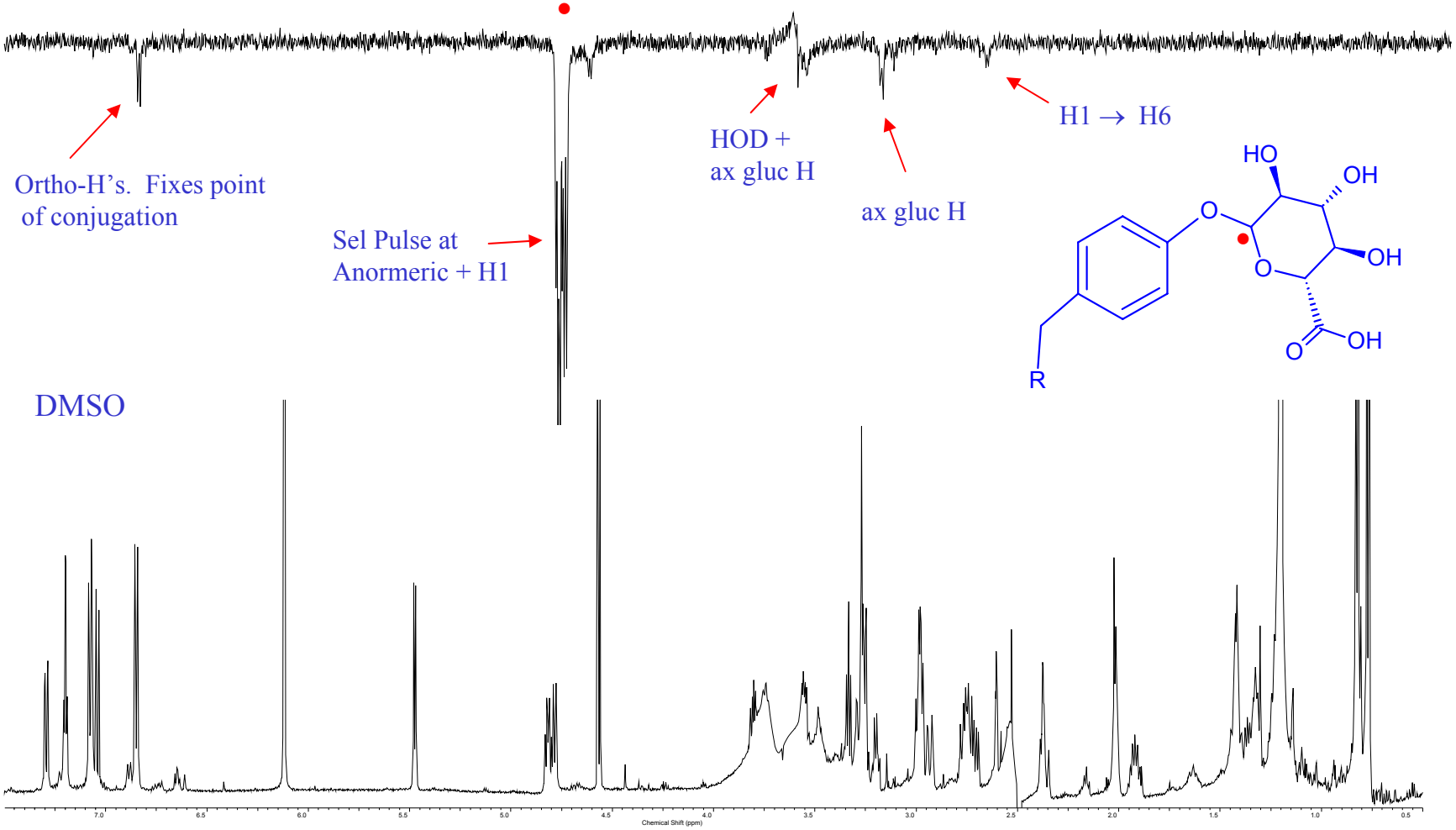
Nonselective Zoom



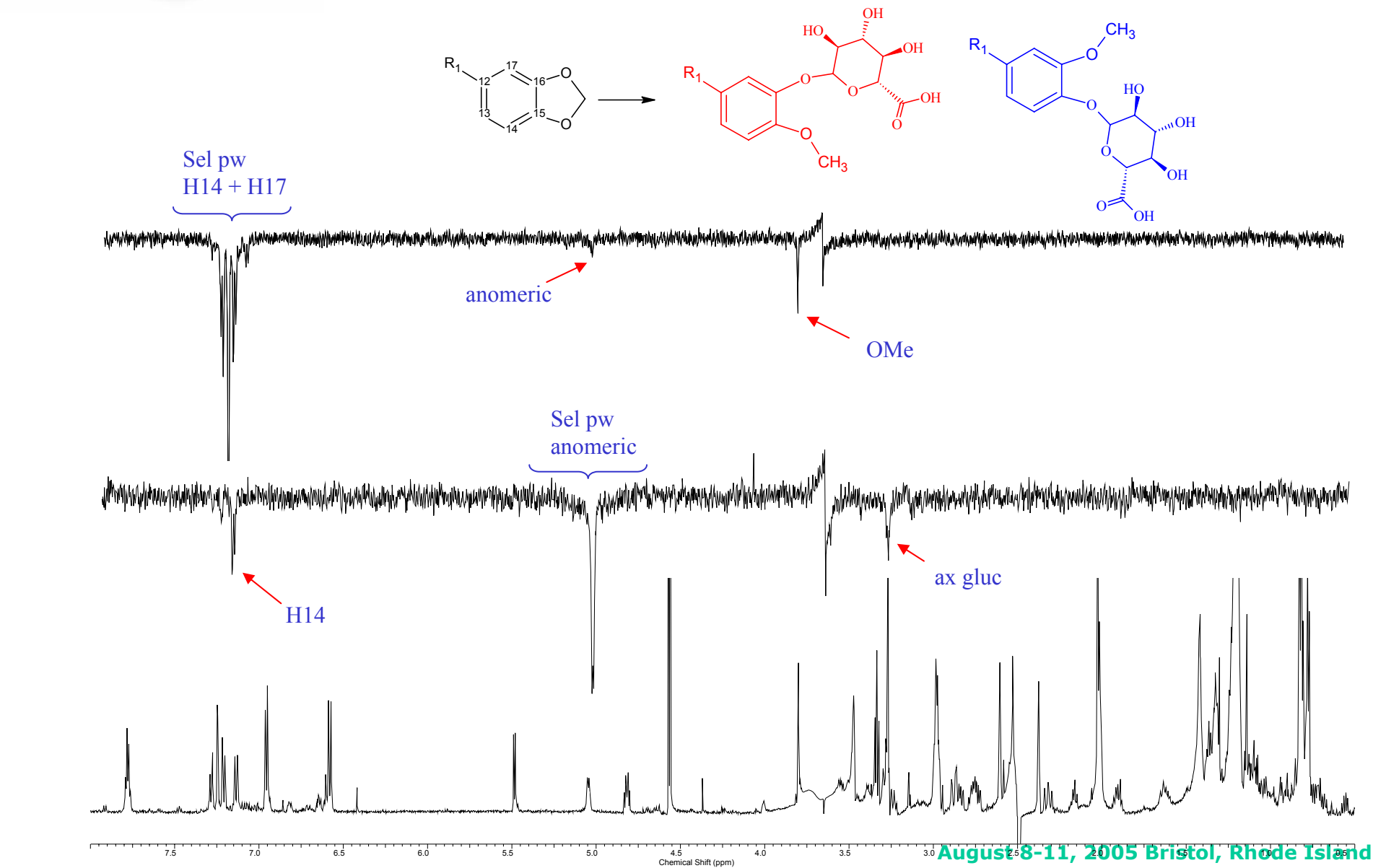
TOCSY with Varied Mixing Times



Selective NOE Exp: Through Space Correlations



Selective NOESY



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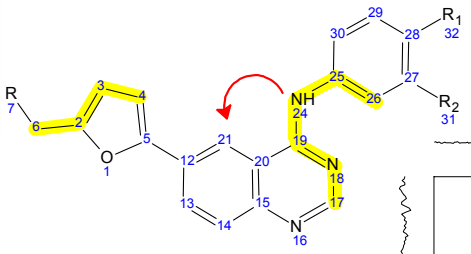
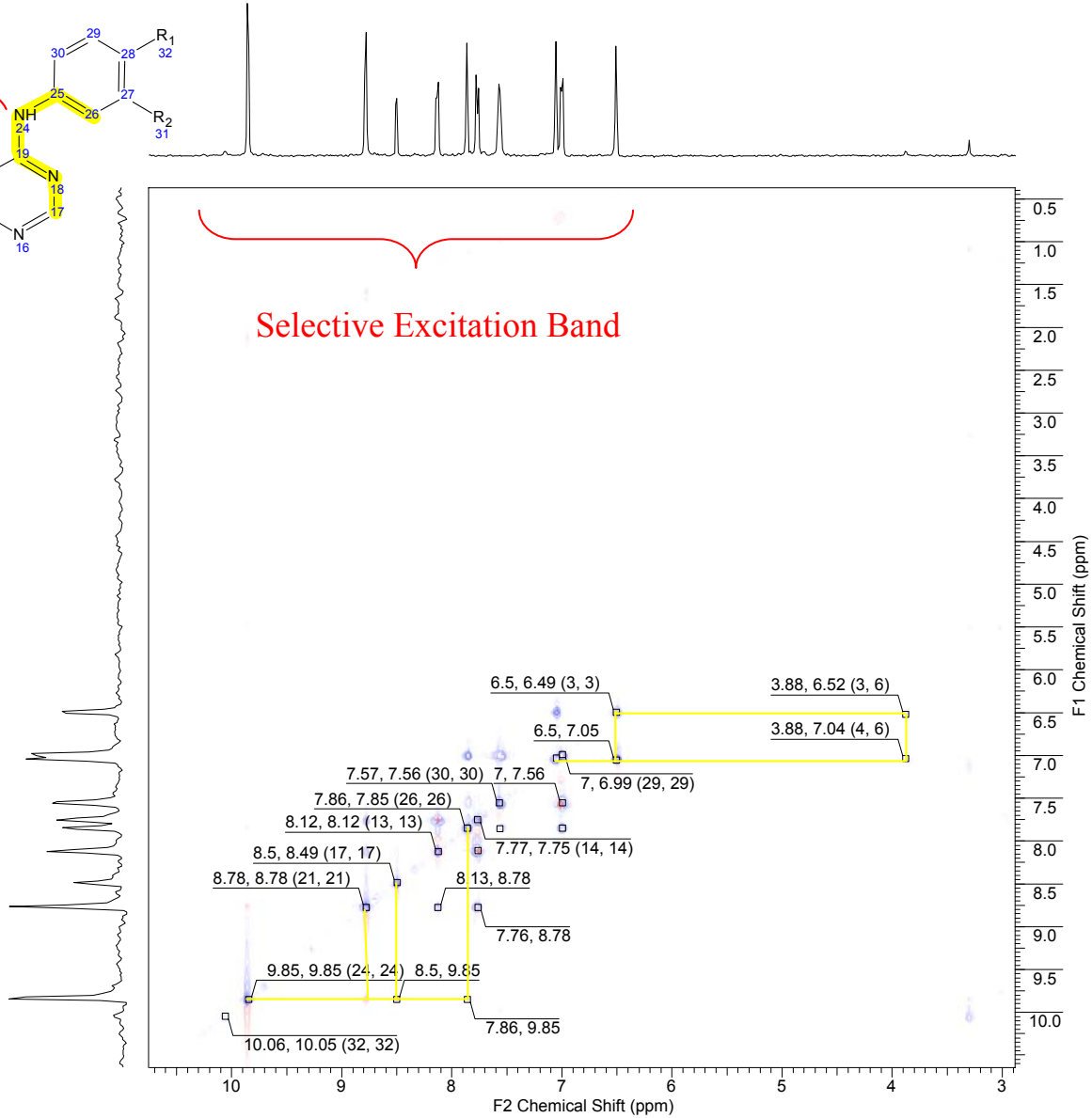


Band-Selective 2D-TOCSY

2D Correlations
With reduced overlap



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Conclusions

There are many different analytical approaches to characterizing drug metabolites

Complimentary use of MS and NMR

Accurate mass measurement: Molecular Formula eliminates NMR "blind spots" to insensitive or non-active nuclei eg O, S, N

Without hyphenation each technique can be optimized without compromise

MS Key: exact mass measurements and automated nanoflow esi

NMR Key: Cryogenically cooled probes selective excitation in homonuclear experiments

NMR limitations: purity > amount

Next innovation: Separations?

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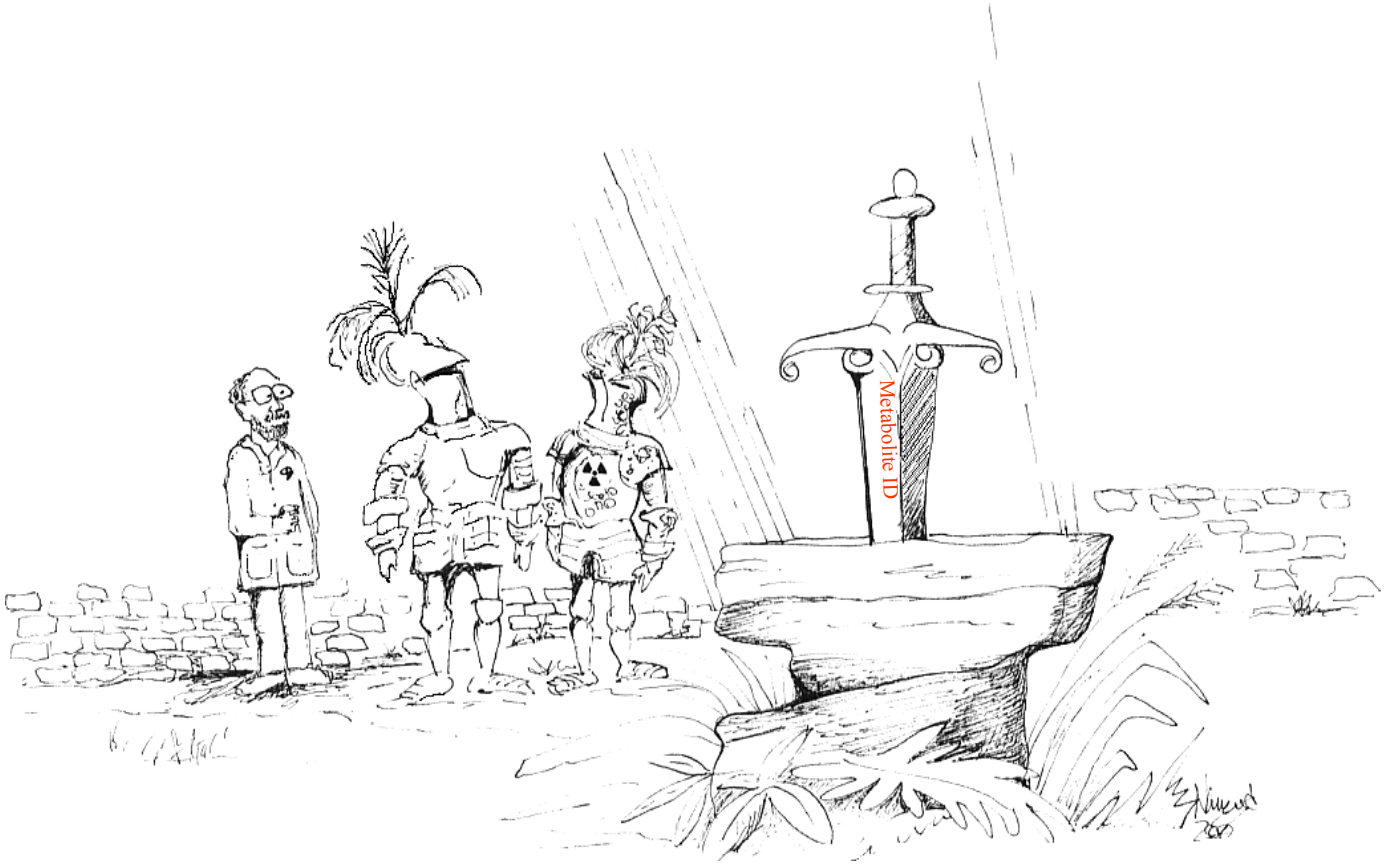

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"Get along Lil' Doggie"
"Happy Trails to You"
"Home on the Range"
"Back in the Saddle Again"