ÚOCHB AV ČR
IOCB PRAGUE

300 MHz NMR
Bruker Avance II

Aleš Marek| September 23rd 2021

^{3}H characteristics

Isotope	Natural abundance (%)	Nuclear spin	Magnetogyric ratio	Resonance freq MHz at 7T	Relative sensitivity	Half-life	Radiation	E max (MeV)
^{1}H	99.984	½	26.75	300	1.0	-	stable	-
^{2}H (D)	0.156	1	4.11	46	9.65×10^{-3}	-	stable	-
^{3}H (T)	$<10^{-16}$	½	28.53	320	**1.21**	12.32 y	β^-	0.018

Some properties of tritium (^{3}H) and related information	
Mass:	3
Radiation:	Beta (100% abundance)
Decay product (stable):	[^{3}He]helium
Discovered	1934 (Ernest Rutherford, Mark Oliphant, and Paul Harteck)
Biological T½:	10 - 12 days * Large liquid intake (3-4 liters/day) reduces effective T½ by a factor of 2+; 3H is easily flushed from the body
Specific Activity:	2.58 Ci/mL T_2
	29.1 Ci/milliatom
Beta Range:	Air: 6 mm [0.6 cm; 0.25 inches]
	Water: 0.006 mm [0.0006 cm; 3/10,000 inches]
	Solids/Tissue: insignificant [No ^{3}H betas pass through the dead layer of skin]

Lit: Evans E.A. et al., Handbook of Tritium NMR Spectroscopy and Application, **1985**, John Wiley & Sons Ltd.

Why ^{3}H NMR spectra?

APPLICATIONS of ^{3}H NMR spectra

- ✓ To analyze the **patterns of labeling** in tritium labeled compounds
- ✓ Reveal the **position and extend** of tritium labeling
  - to avoid labeling in hydrogen exchangeable positions (e.g., acid hydrogen, alpha carbon in carbonyl compounds, etc.)
- ✓ Determine **specific activity**, purity and identity

ADVANTAGES of ^{3}H nuclei in NMR studies

- ✓ Tritium is an ideal nuclide for high resolution NMR studies – its nucleus, the triton, like the proton has a nuclear **spin of ½** and its high magnetogyric constant gives it a **higher sensitivity** to detection than any other nucleus, including proton by the factor of **1.21**
- ✓ Tritium which have practically zero natural abundance do not suffer from **any background signals or interfering solvent** signals from non-tritiated species.
- ✓ Because of **chemical shifts of the tritium nuclei are the same as those of proton** nuclei -> proton chemical shifts can be applied directly to the interpretation of tritium spectra.

Historical perspectives of ^{3}H NMR:

- The fundamental magnetic properties of tritium (^{3}H) were first reported in **1947** in a series of NMR measurements which demonstrated the triton to be a **spin 1/2** nucleus, with a **positive magnetic moment about 6.66 %** larger than that of the proton.
- **1947** – the ^{3}H nmr signal of **tritiated water** was observed (x100 **Ci** employed, custom build instrument – 30 MHz)

*Multi-Curie quantities in high-speed glass tubes in very expensive nmr spectrometers discourages any rapid development of the technique… only **24 reports of tritium spectra in the first 30 years**, and 14 of those were published in the period 1974-1976.*

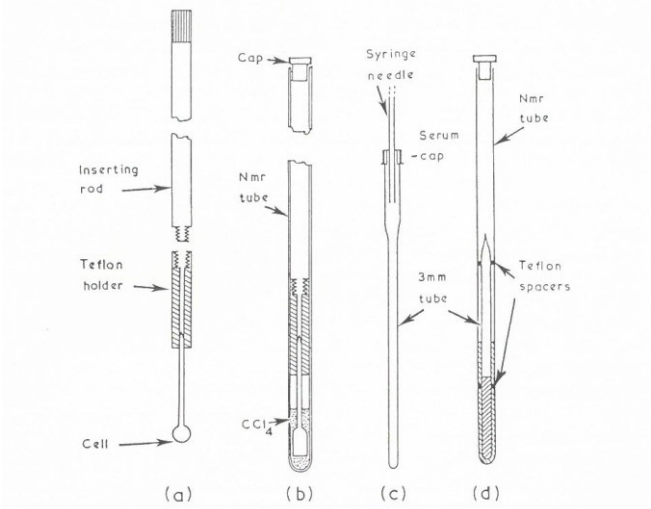
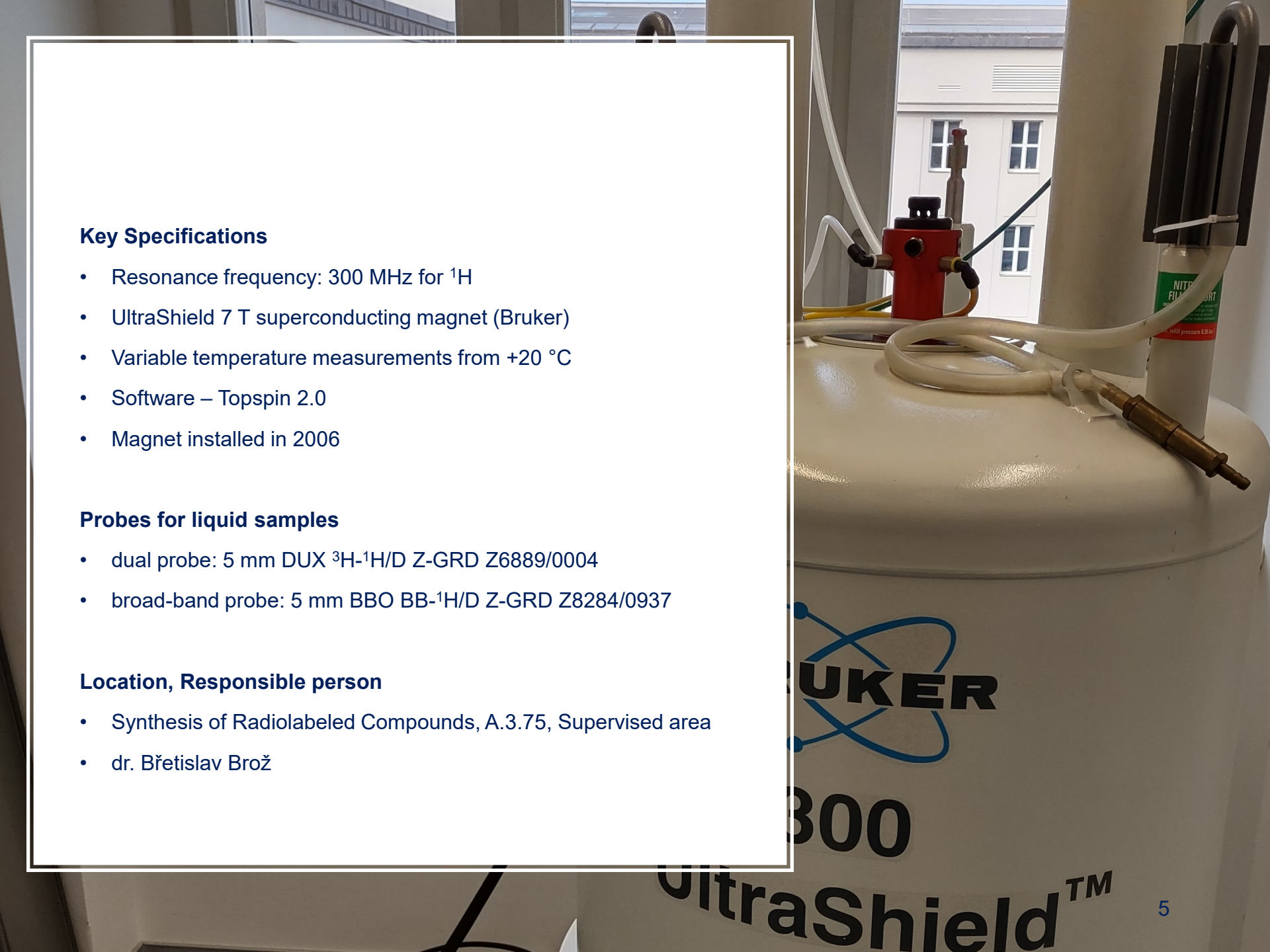


Figure 6. Details concerning microcells for ^{3}H nmr spectroscopy: (a) spherical cell, chuck and insertion tool; (b) nmr tube containing cylindrical microcell; (c) preferred 3 mm tube, as for filling; (d) 3 mm sample tube mounted in 5 mm nmr tube.

- **1964** – first **^{3}H nmr of a tritiated organic compound** – neat sample of [*side-chain*-^{3}H]ethylbenzene (32.1 Ci/mL, Varian A-40, 40 MHz)
- **1968-1971** – practical evaluation into a *safe routine*, it became obvious that only millicurie (**mCi**) amounts of radioactivity were needed. (PerkinElmer R-10, 64 MHz)

Lit: Kubinec M.G, Williams P.G, Tritium NMR, Lawrence Berkley National Laboratory, **1994**, John Wiley and Sons, Ltd., Evans E.A. et al., Handbook of Tritium NMR Spectroscopy and Application, **1985**, John Wiley & Sons Ltd.



Key Specifications

- Resonance frequency: 300 MHz for ^{1}H
- UltraShield 7 T superconducting magnet (Bruker)
- Variable temperature measurements from +20 °C
- Software – Topspin 2.0
- Magnet installed in 2006

Probes for liquid samples

- dual probe: 5 mm DUX ^{3}H-^{1}H/D Z-GRD Z6889/0004
- broad-band probe: 5 mm BBO BB-^{1}H/D Z-GRD Z8284/0937

Location, Responsible person

- Synthesis of Radiolabeled Compounds, A.3.75, Supervised area
- dr. Břetislav Brož

prof. Marek Jindra, CSc.
Biology Center ASCR
České Budějovice

Dr. Aleš Machara
IOCB

Dr. Robert Hanus
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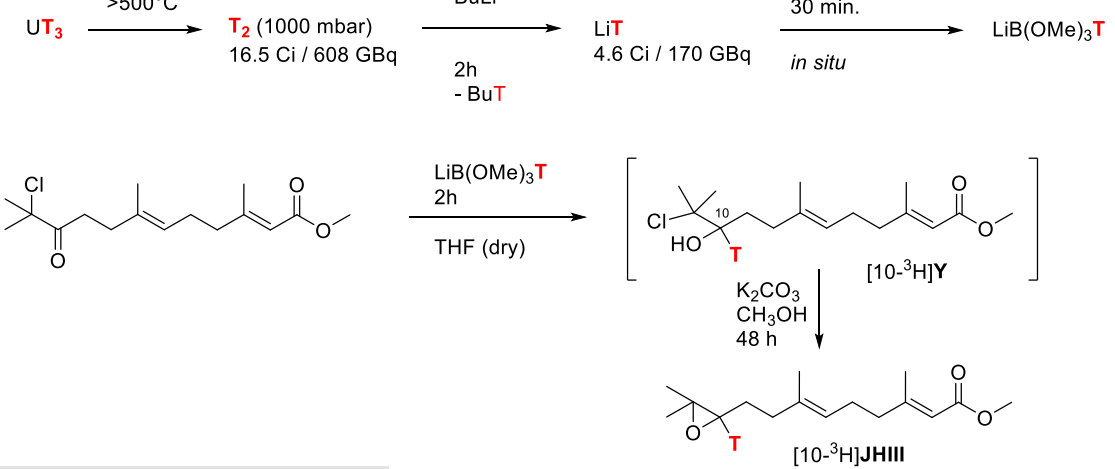
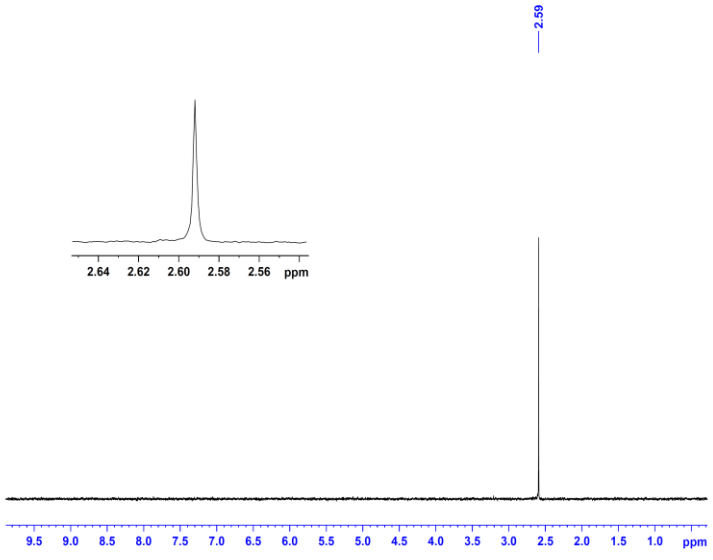
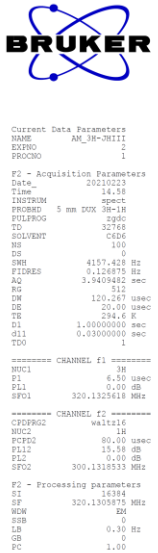
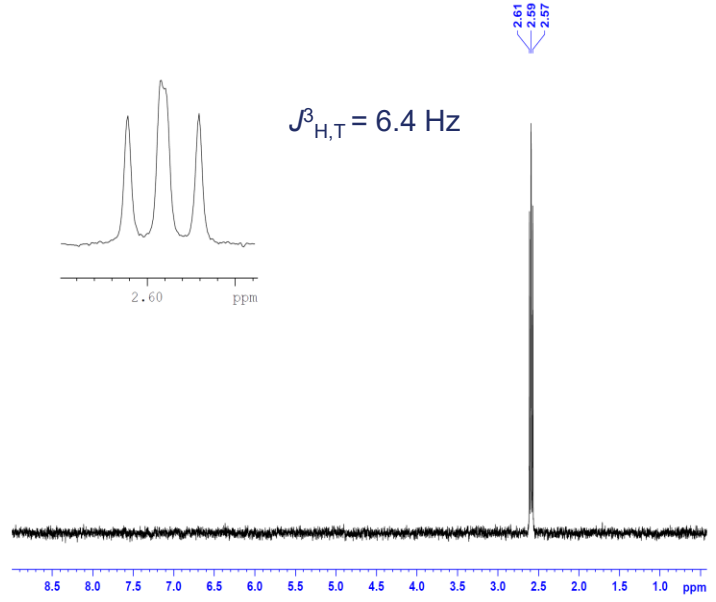


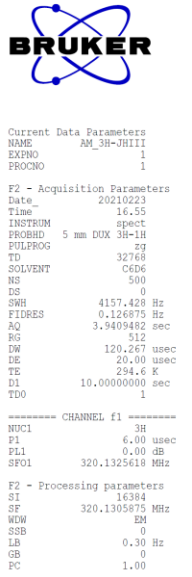
SA = 25.7 Ci/mmol (0.88 T)
320 mCi

Regiospecificity of ^{3}H-labeling





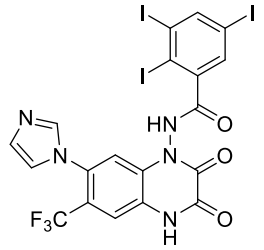




^{3}H{^{1}H} NMR of [10-^{3}H]JHIII (320 MHz, C_6D_6, 5 mCi)

^{3}H-^{1}H NMR of [10-^{3}H]JHIII (320 MHz, C_6D_6, 5 mCi)

Dr. Darryl S. Pickering, assoc. prof.
Dept. of Drug Design and Pharmacology
Faculty of Health and Medical Sciences
University of Copenhagen

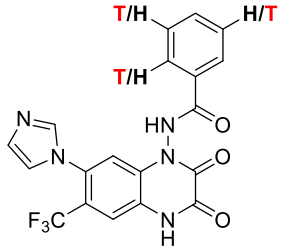


Chemical Formula: $C_{19}H_9F_3I_3N_5O_3$
Molecular Weight: 793.02

1.7 mg

Pd/C [10%]
T_2 (912 mbar, 6.2 Ci)
DIPEA
2h

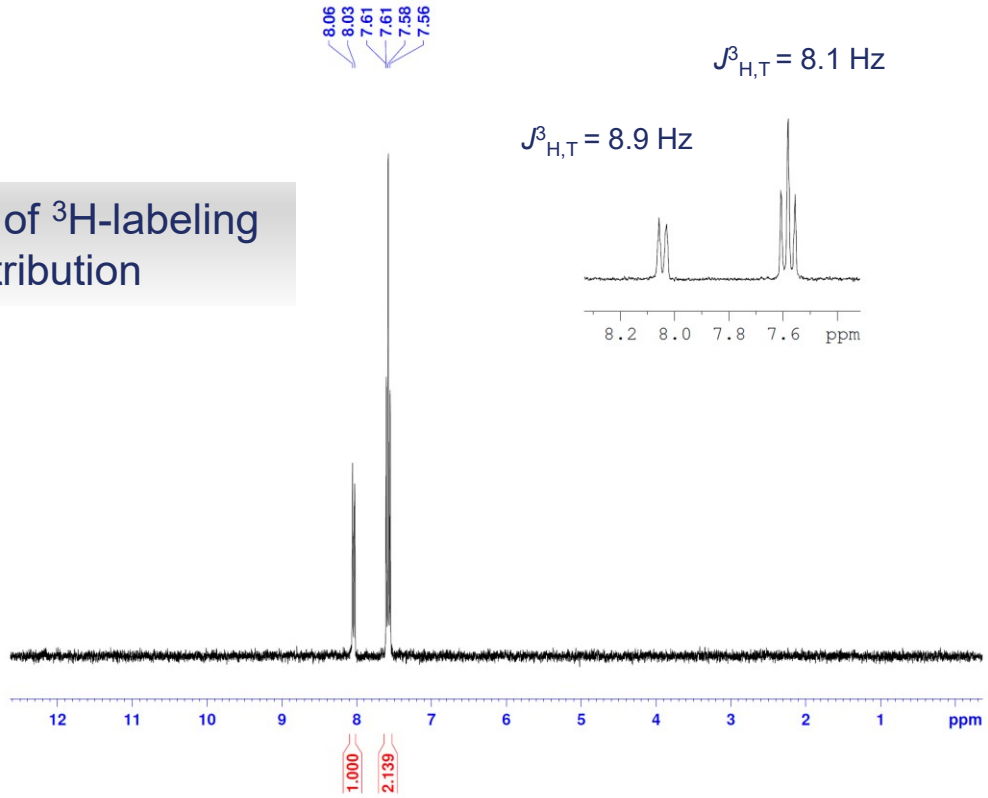
DMF (dry)



Chemical Formula:
$C_{19}H_{12}T_3F_3N_5O_3$
Molecular Weight: 424.38

SA = 40.1 Ci/mmol (1.37T)
28 mCi

Regiospecificity of ^{3}H-labeling
Quantitative distribution



BRUKER

```
Current Data Parameters
NAME         AM_EM-17_HOT
EXPNO                   3
PROCNO                  1

F2 - Acquisition Paramete
Date_            20190604
Time                4.22
INSTRUM             spect
PROBHD   5 mm DUX 3H-1H
PULPROG                zg
TD                  32768
SOLVENT              MeOD
NS                   3072
DS                      0
SWH             4157.428 H
FIDRES          0.126875 H
AQ              3.9409482 s
RG                    512
DW                120.267 u
DE                 20.00 u
TE                 294.5 K
D1            10.00000000 s
TD0                     1

======== CHANNEL f1 =====
NUC1                   3H
P1                  6.00 u
PL1                 0.00 d
SFO1         320.1325618 M

F2 - Processing parameter
SI                  16384
SF           320.1305980 M
WDW                    EM
SSB                     0
LB                  0.30 H
GB                      0
PC                   1.00
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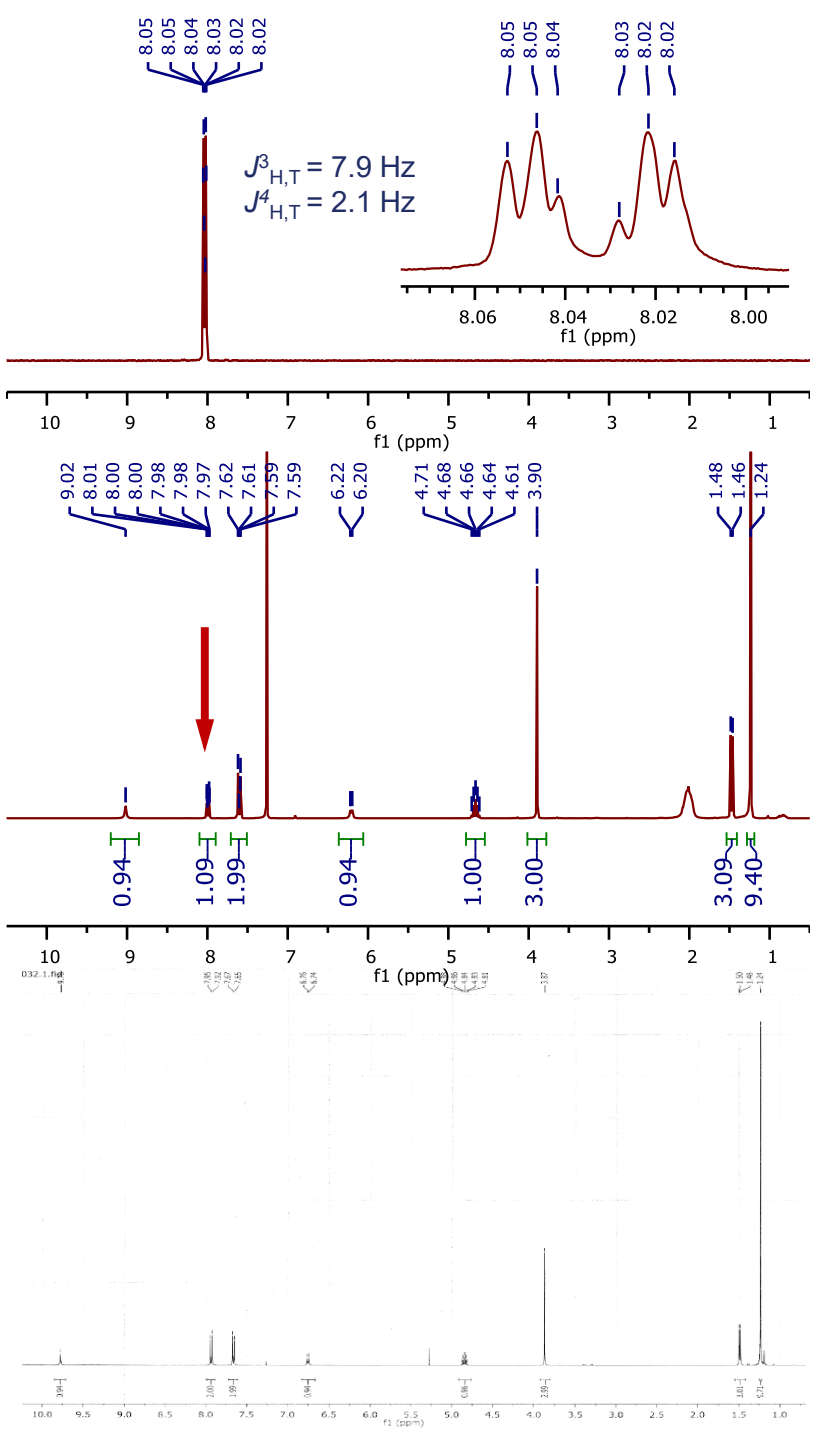
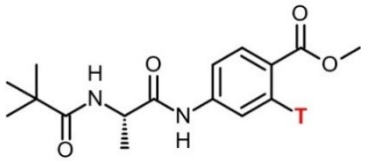
^{3}H-^{1}H NMR of [^{3}H]EM17 (10 mCi, CD_3OD)





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Dr. Břetislav Brož
IOCB

^{3}H-^{1}H NMR spectrum (320.1 MHz, $CDCl_3$)
the **hot** [^{3}H]-AN-1-099.

^{1}H NMR spectrum (300.1 MHz, $CDCl_3$)
the **hot** [^{3}H]-AN-1-099.

⇒ **Specific activity** = SA_{NMR}

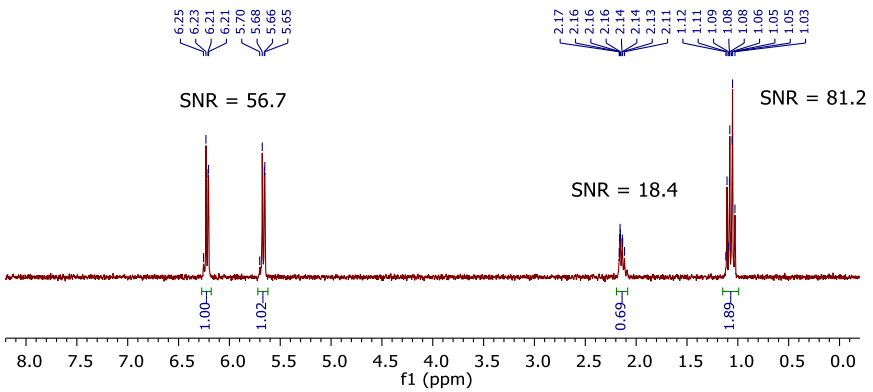
⇒ (2.00-1.09)×29.1 = <u>26.5 Ci/mmol (0.91 T)</u>

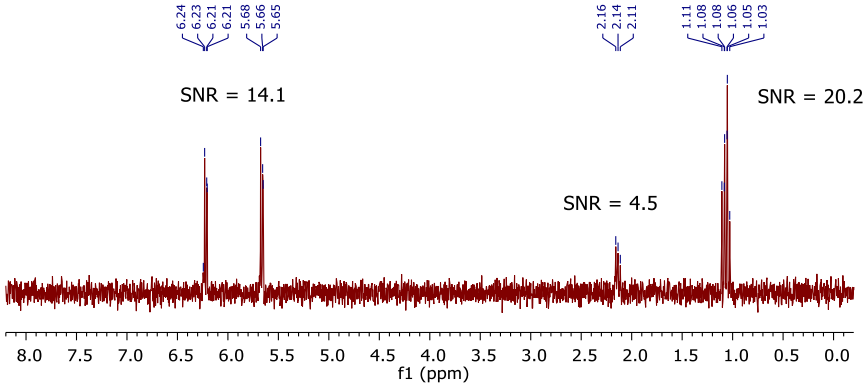
(SA_{MS} = 26.1 Ci/mmol)

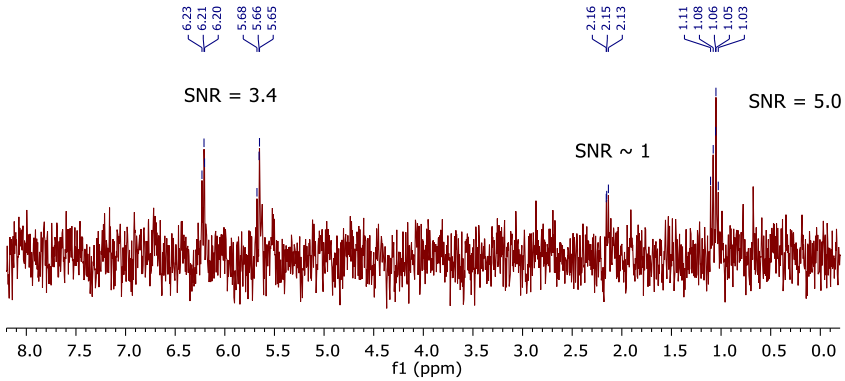
^{1}H NMR spectrum (400.1 MHz, $CDCl_3$)
the **unlabeled** [^{1}H]-AN-1-099.

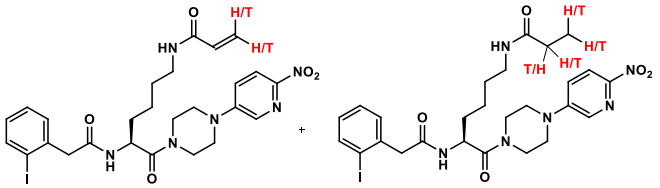
Sensitivity of ^{3}H-nuclei in NMR

$^{3}H\{^{1}H\}$ NMR spectra, Bruker Avance II 300 MHz, **320.13 MHz, 25 °C**









15 mCi, approx. **200 µg**
500 µL MeOD, NS = 64, LB = 0.5;
time of the experiment: 5 min 20 s

500 µCi, approx. **7 µg**
450 µL MeOD, NS = 8192, LB = 0.5;
time of the experiment: 11 h 23 min

100 µCi, approx. **1.5 µg**
450 µL MeOD, NS = 12288, LB = 1.0;
time of the experiment: 17 h 5 min

selected NMR parameters: recycle delay length (d1) = 1.00 s, 90° RF pulse length (p1) = 6.5 µs, acquisition time (aq) = 3.941 s

Doc. RNDr. Jiří Novotný, DSc.
Department of Physiology
Charles university

Dr. Břetislav Brož
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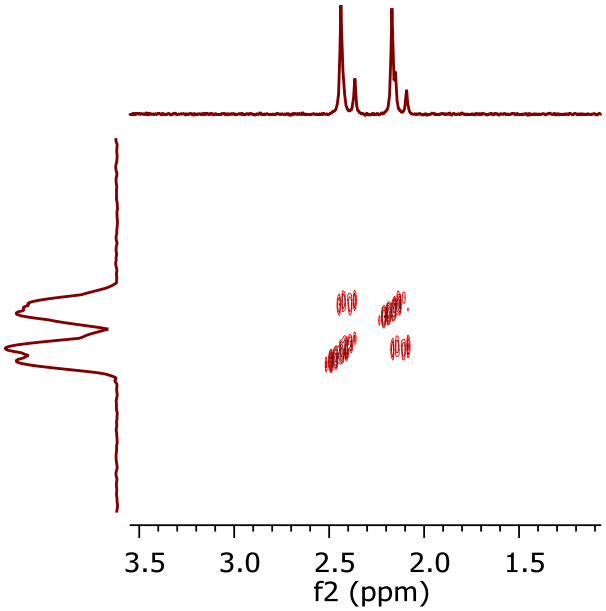
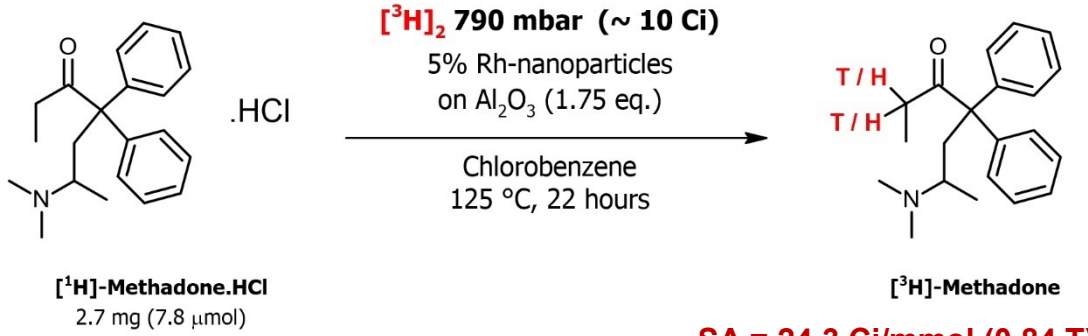
^{3}H{1H} NMR
(320 MHz, CD_2Cl_2)



^{3}H-^{3}H COSY NMR (320 MHz, CD_2Cl_2)

^{3}H-Methadone

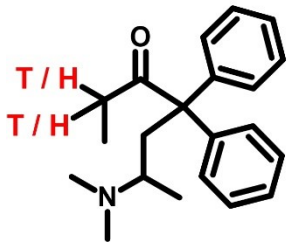
late-stage-labeling by catalyzed Hydrogen Isotope Exchange (HIE)



[^{1}H]-Methadone.HCl
2.7 mg (7.8 μmol)

[^{3}H]-Methadone

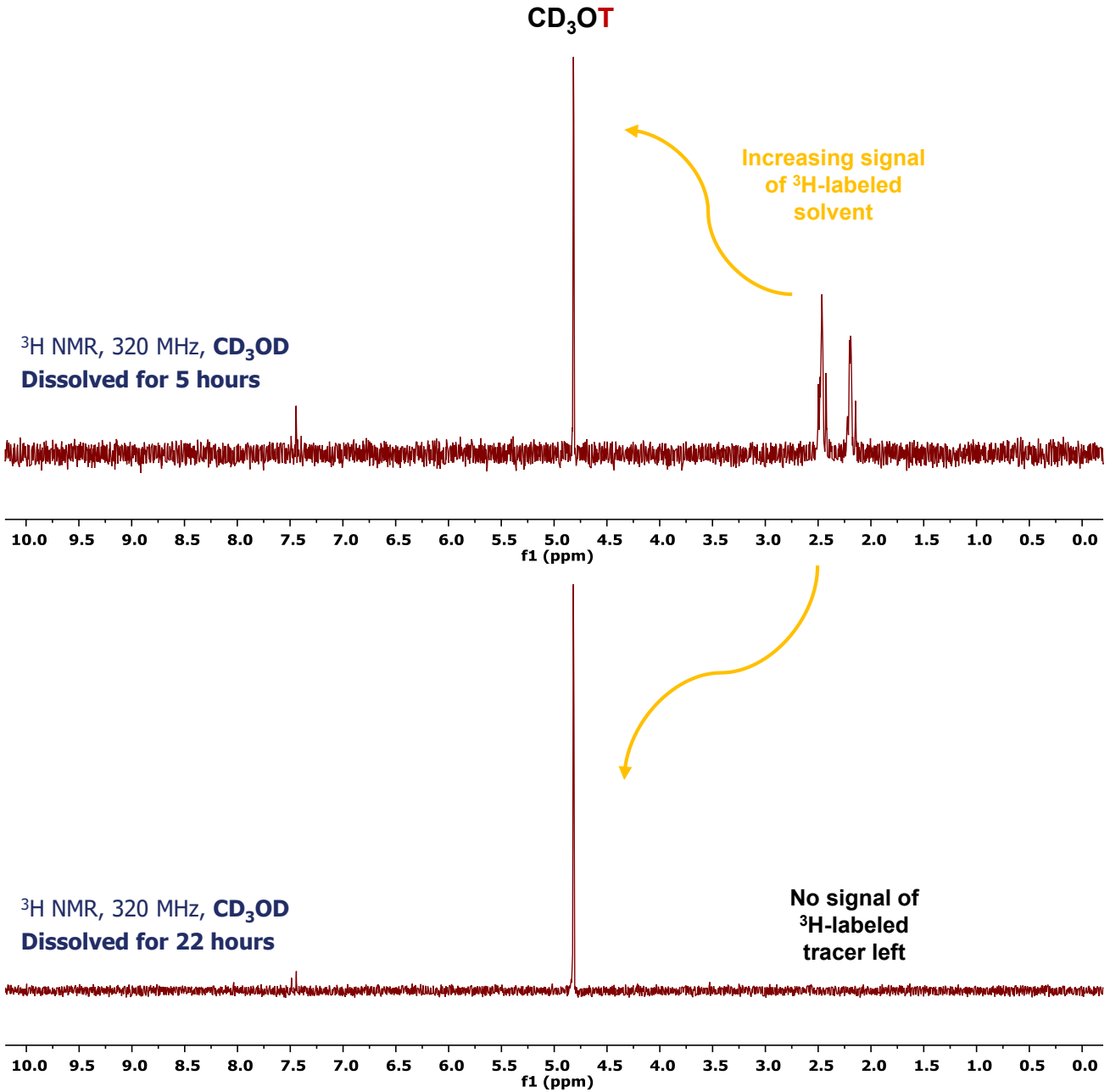
SA = 24.3 Ci/mmol (0.84 T)



SA = 24.3 Ci/mmol
0.84 T per molecule

Example of **undesirable** ^{3}H-labeling of hydrogen **exchangeable** position.
=> Loss of label in CD_3OD ☹

Fortunately,
^{3}H-label turned out to be non-exchangeable in **H_2O**/AcCN for weeks…! ☺
Formulated in DMSO.



Better safe than sorry: handling significant amount of radioactivity…

~ 1974

Safety first!

PRECAUTIONS IN TRITIUM HANDLING

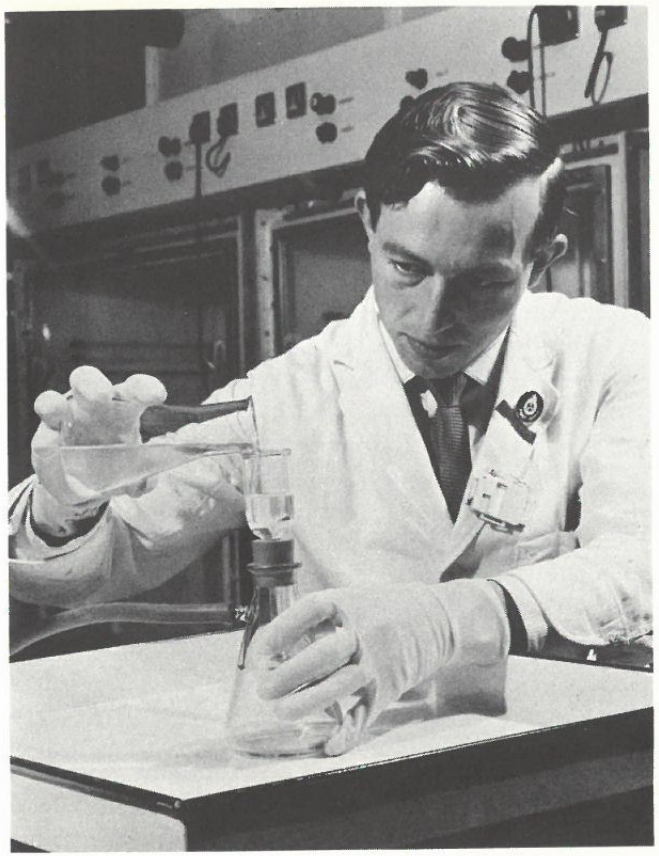


Figure 3.5. Filtration of a tritiated compound. Note the operation over a spill tray

In IOCB about these days…
☺

Chapter 3: PRECAUTIONS IN TRITIUM HANDLING

Evans E. A., Tritium and its Compounds, **1974**,
Butterworth & Co (Publishers) Ltd

Summary

- ✓ **high sensitivity** of detection of the ^{3}H nucleus - only reasonably **small amount of radioactivity** (0.1 mCi to 10 mCi per site, 3.7 to 370 MBq) to give a well-defined spectrum
- ✓ proton chemical shifts can be applied directly to the interpretation of tritium spectra
- ✓ pattern of ^{3}H-labeling
- ✓ quantitative distribution

- ✓ specific activity (SA)
- ✓ purity and identity of synthesized ^{3}H-tracer

Aleš Marek
23rd 2021
—
Synthesis of radiolabeled compounds

Thank you for your attention.