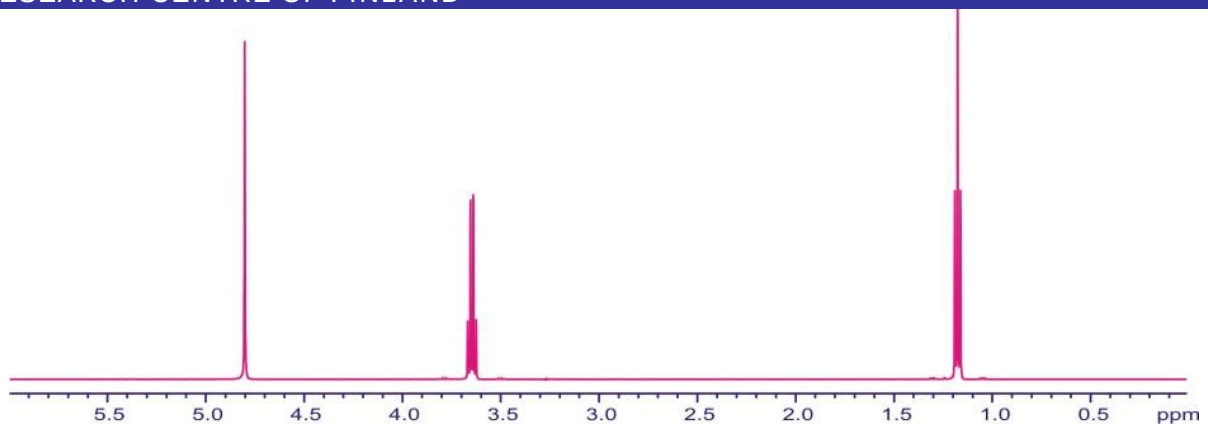
Processing 1D NMR spectra

- weighting funtions
  - the FID is multiplied by a function to weight certain part of it
  - exponential (line broaderning), gaussian or cosine (shifted sine bell) weight the early part => better s/n, broader lines
  - also for forcing the last point of fid to 0
  - sine bell or shifted gaussian to weight the later parts of => enchanced resolution, worse s/n
- Fourier transformation (FT)
  - from time domain (s) to frequency domain (Hz)
- base line correction
  - spline fitting
  - (polynomial) function fitting
- chemical shift reference
  - TMS (tetramethyl silane)
- Integration
  - Determination of peak areas

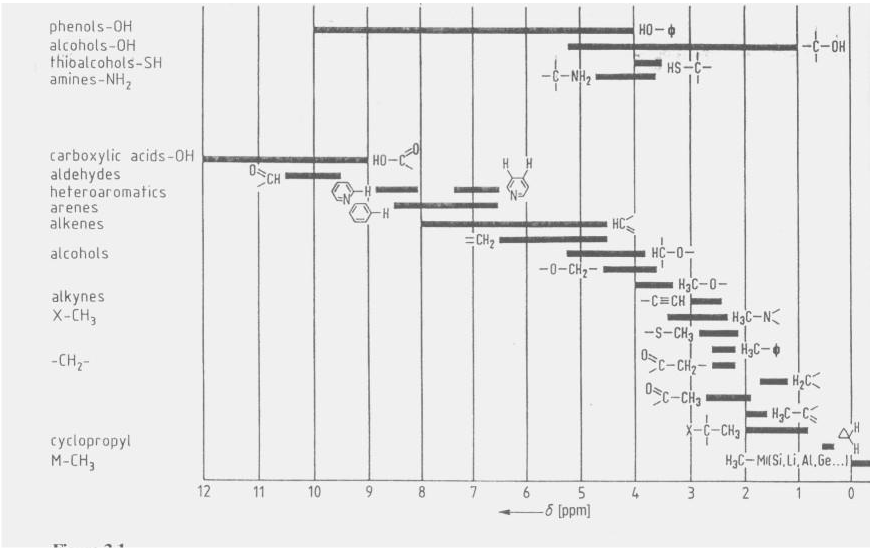

5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

NMR parameters

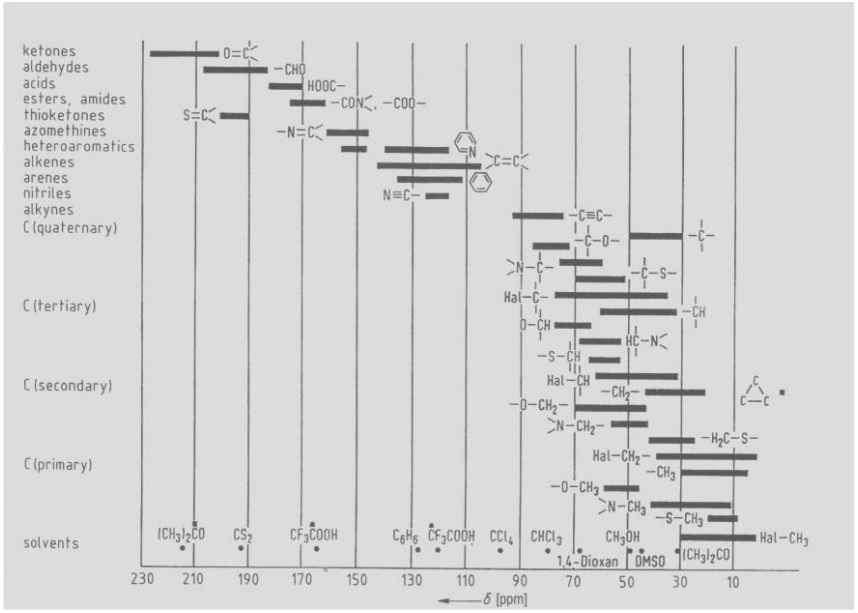
- chemical shift
  - place of the signal
  - local magnetic filed of the nucleus determined by shielding by the electrones (= chemical structure)
  - given as relative value in ppm - independent on external field strength

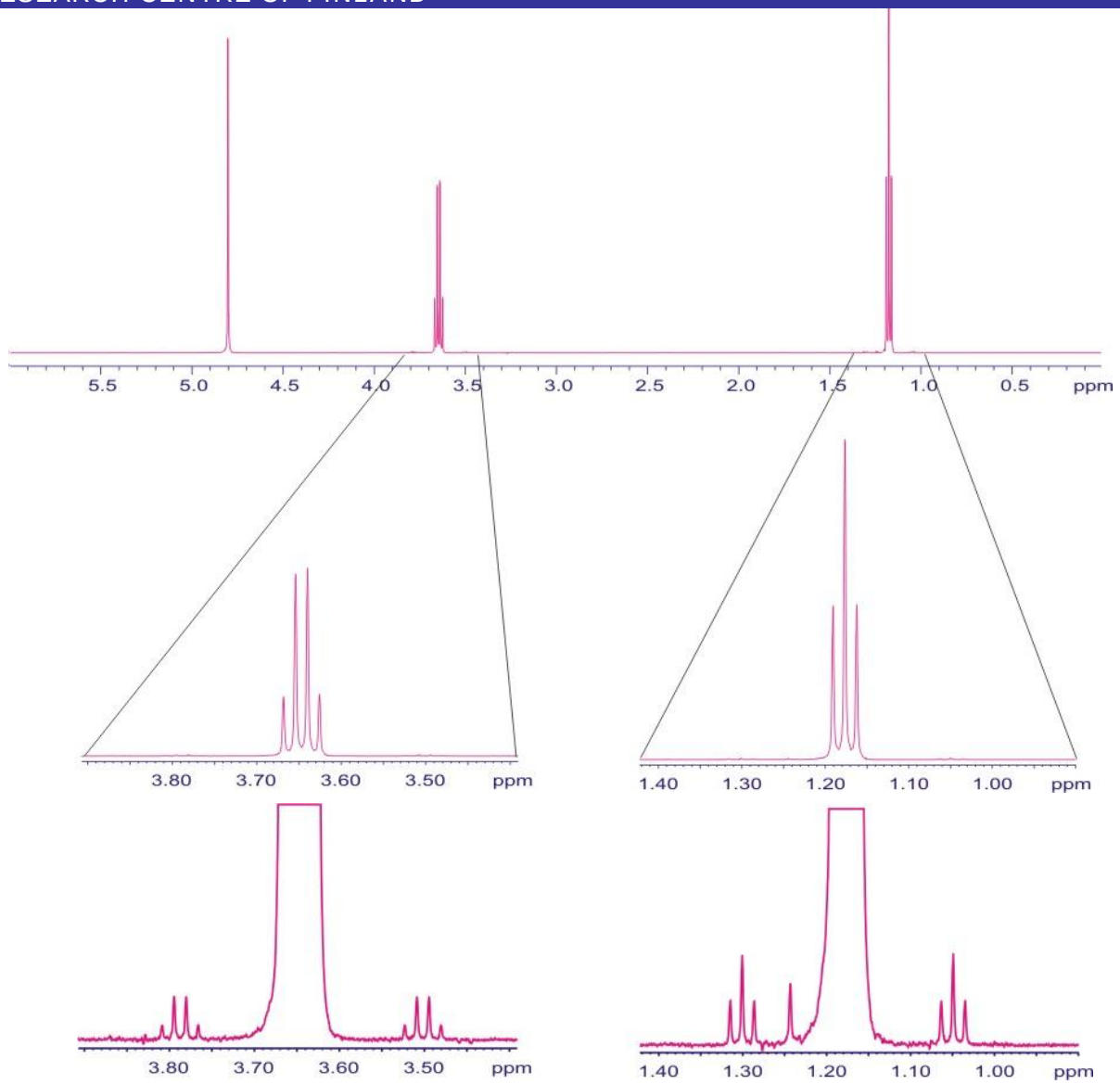
$$\delta_{sample}\,[ppm] = \frac{\nu_{sample} - \nu_{reference}\,[Hz]}{\nu_{reference}\,[MHz]}$$

^{1}H chemical shifts in organic compounds



^{13}C chemical shifts in organic compounds




5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
3.80
3.70
3.60
3.50
ppm
1.40
1.30
1.20
1.10
1.00
ppm
3.80
3.70
3.60
3.50
ppm
1.40
1.30
1.20
1.10
1.00
ppm

- spin - spin coupling
  - the fine structure of NMR signals
  - also between different nuclei (e.g. 13 satelites in 1H spectrum)
  - over 1 - 3 (sometimes 4 or 5) chemical bonds
  - the magnetic field at nucleus is affected by the spin states of the coupled nuclei
  - can be decoupled
- intensitity of the signal
  - propotional to concentration in a quantitative spectrum
  - due to long relaxation times and other factors (e.g. NOE), spectrum not always quantitative
- nuclear Overhauser effect
  - "through space" coupling between to adjacent nuclei
  - also intermolecular
  - can been seen in intensity change by special experiment or in 2D
- relaxation times or line widths
  - data on (intra)molecular motion

Nuclear relaxation

- return to thermal equilibrium (magnetation along +z), T_1
- disappearance of the signal, T_2

T_1 relaxation (spin lattice relaxation)

- energy is transfered from the nuclei to the surroundings
- T_1 relaxation times can vary from seconds to hours
- ^1H T_1 relaxation times usually in the order of seconds
- ^{13}C T_1 varies a lot:
  - data on molecular structure
  - must be taken account in selecting parameters
  - many mechanisms, dipole - dipole interaction with directly bound protons most important -> number of those protons often determines T_1
  - quaternary carbons can have very long T_1 -> weak NMR signal
- fast molecular motion - long T_1

T_2 relaxation

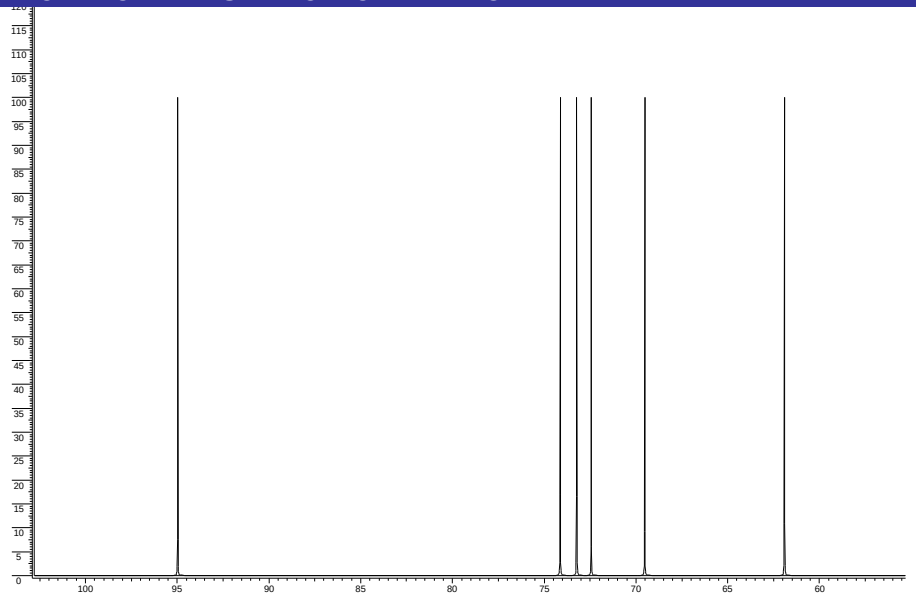
- the decay of the signal
- signal = phase coherence of the nuclear spins
- phase coherence is gradually lost
- mainly because of field inhomogenities
- no energy transfer (no population changes between the spin states)
- T_2 always shorter than T_1

NMR line width

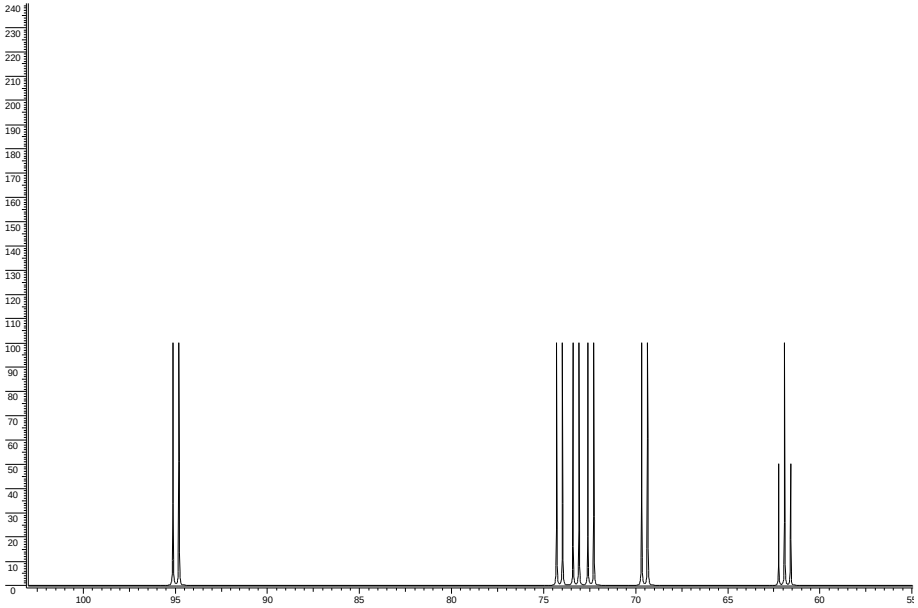
- determined as half-height width of the NMR signal
- depends on relaxation
- in non-viscous solution state for small molecules T_1 and T_2 on the same order of magnitude and both contribute
- in slowly moving molecules (large molecules, viscous solution) T_1 very long, T_2 very short -> broad lines
- to record a quantitative spectrum relaxation delay (between repetition of the pulse sequence) must be at least 5 x T_1
  - for ^{13}C often impossible

^{13}C NMR spectrum

- much less sensitive than ^{1}H
  - natural abundance only 1.1%
  - gyromagnetic ratios about 4 times smaller
- ^{1}H spin - spin coulings splitt the signals => usually recorded with ^{1}H broadband decoupling



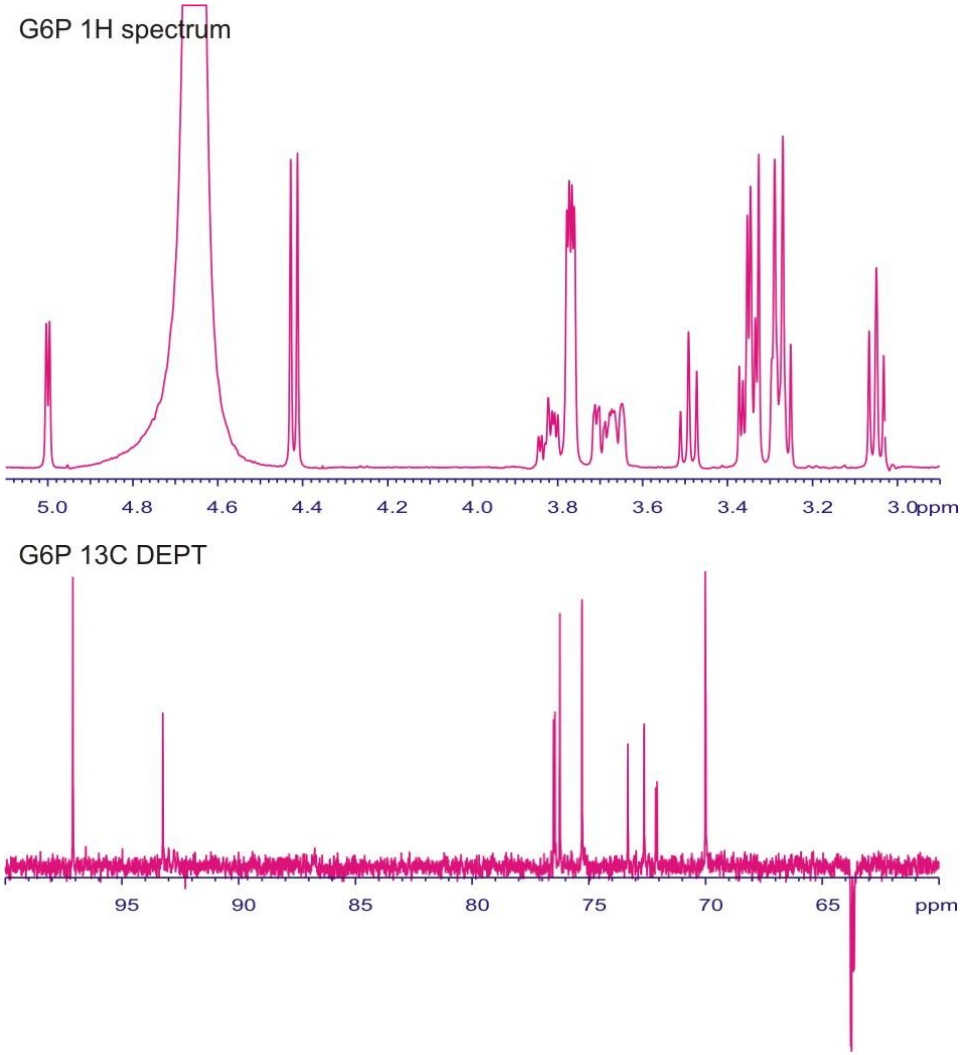
Predicted ^{13}C spectrum
of α-glucose
with ^{1}H BB decoupling



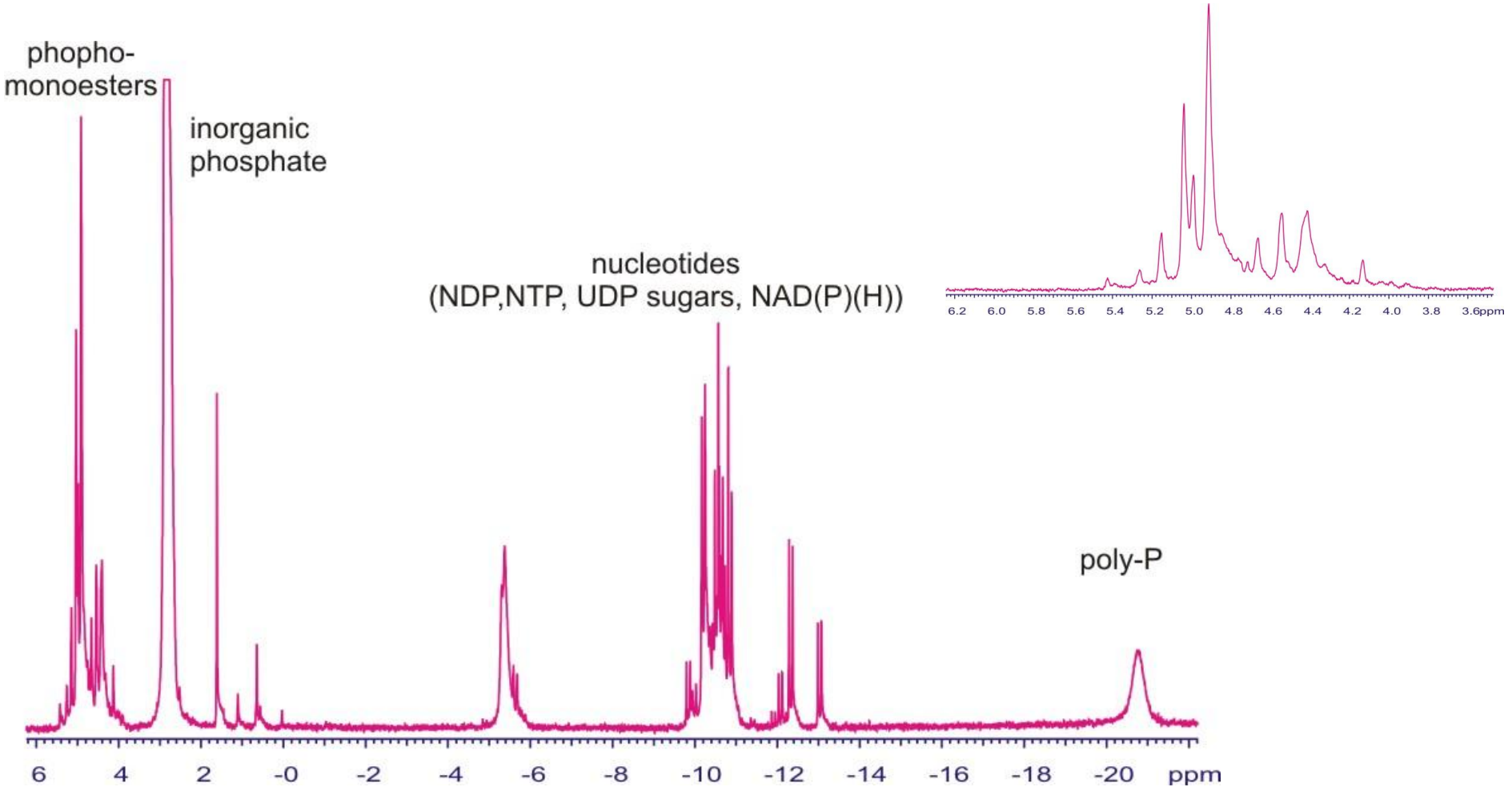
Predicted ^{13}C spectrum
of α-glucose
without ^{1}H BB decoupling

^{13}C NMR spectrum

- much less sensitive than ^{1}H
  - natural abundance only 1.1%
  - gyromagnetic ratios about 4 times smaller
- ^{1}H spin - spin couplings splitt the signals => usually recorded with ^{1}H broadband decoupling
  - without decoupling signals at least 50% lower
- BB decoupling also enhances the signals due to heteronuclear NOE => not quantitative, intensity depends on number of directly bound protons


G6P 1H spectrum
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0ppm
G6P 13C DEPT
95
90
85
80
75
70
65
ppm

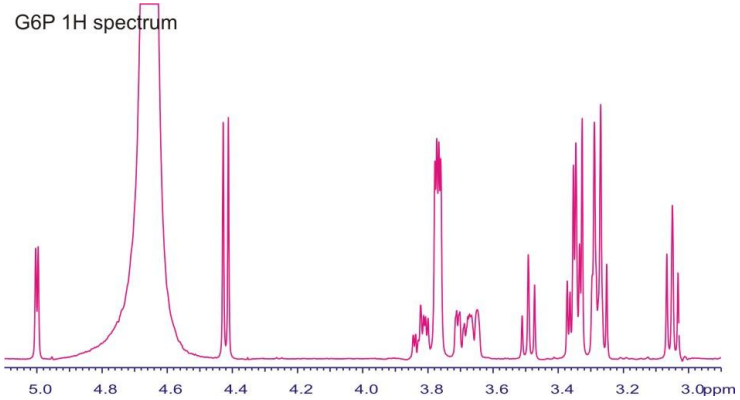
31P spectrum of yeast extract

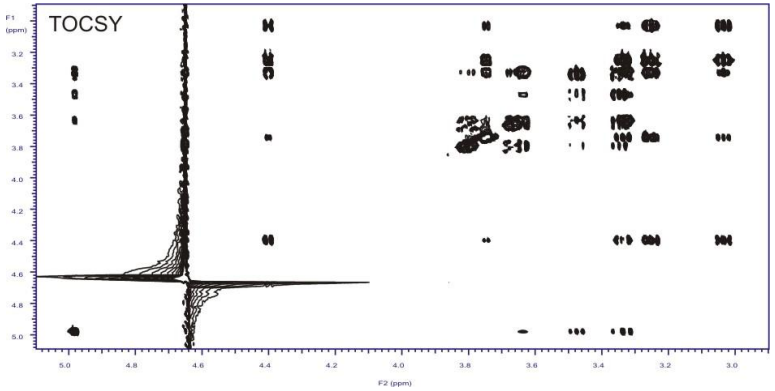


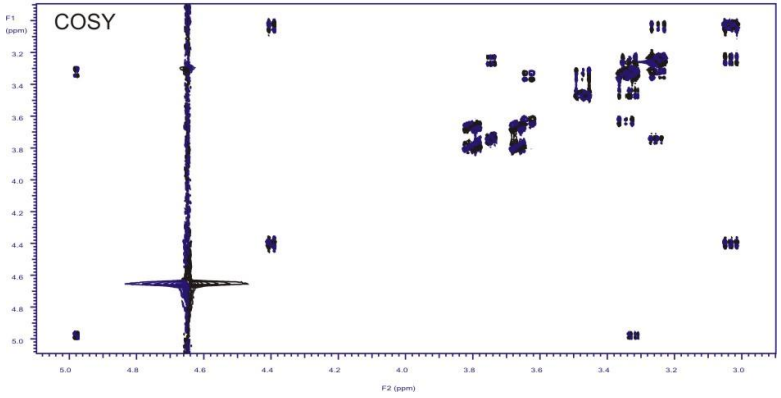
2D NMR

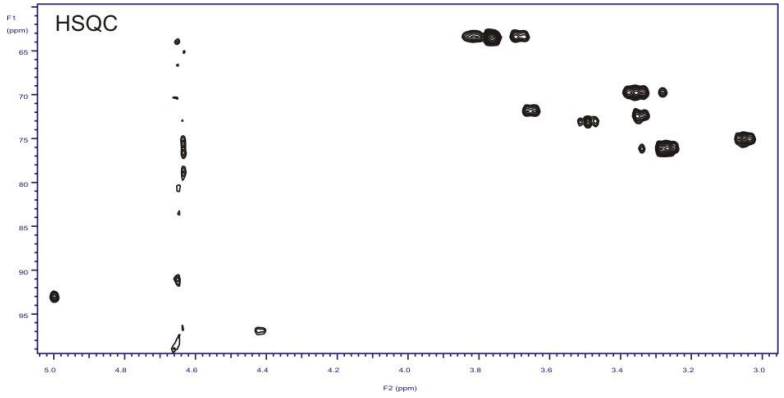
Preparation - evolution -mixing -detection

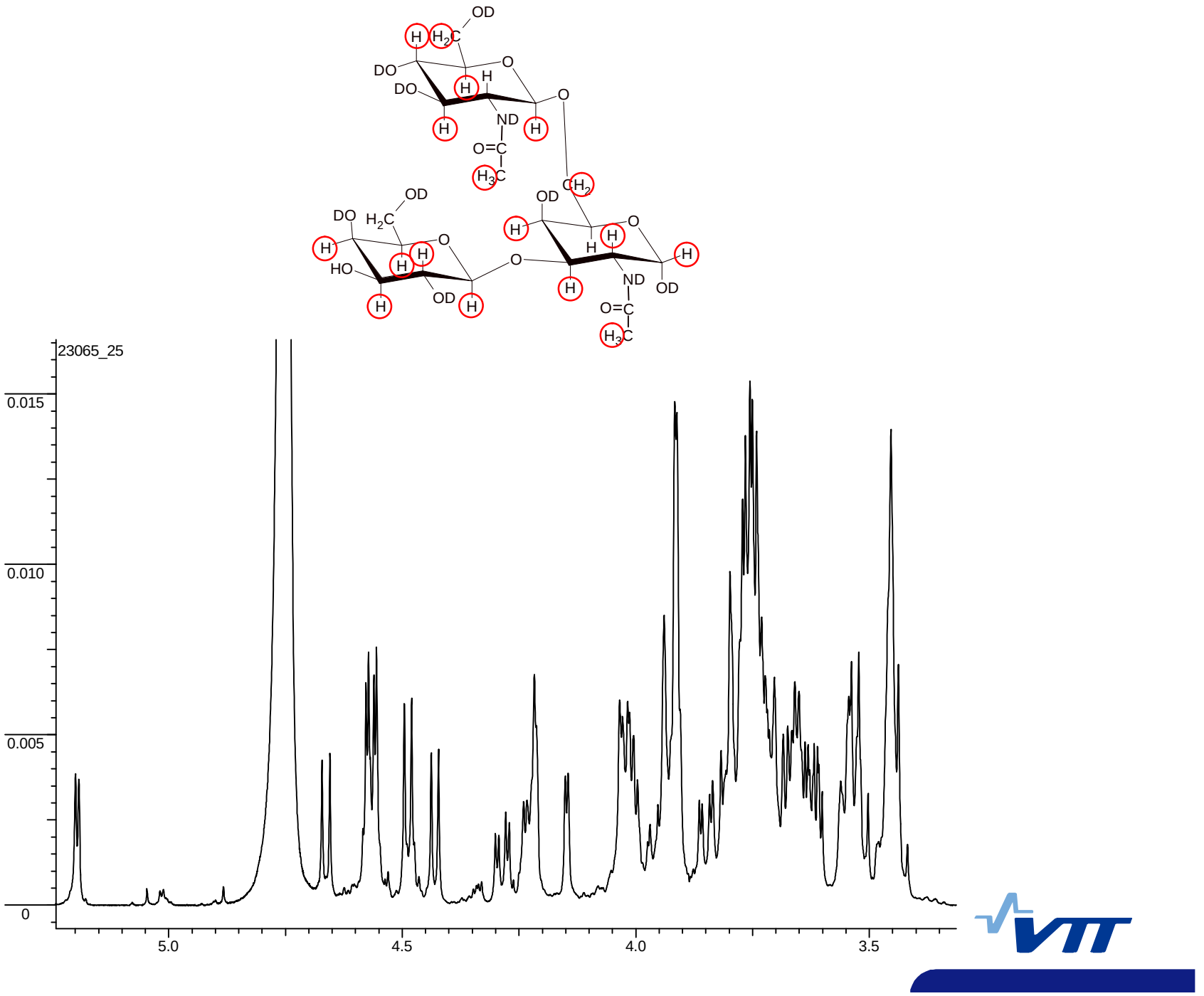
2D NMR

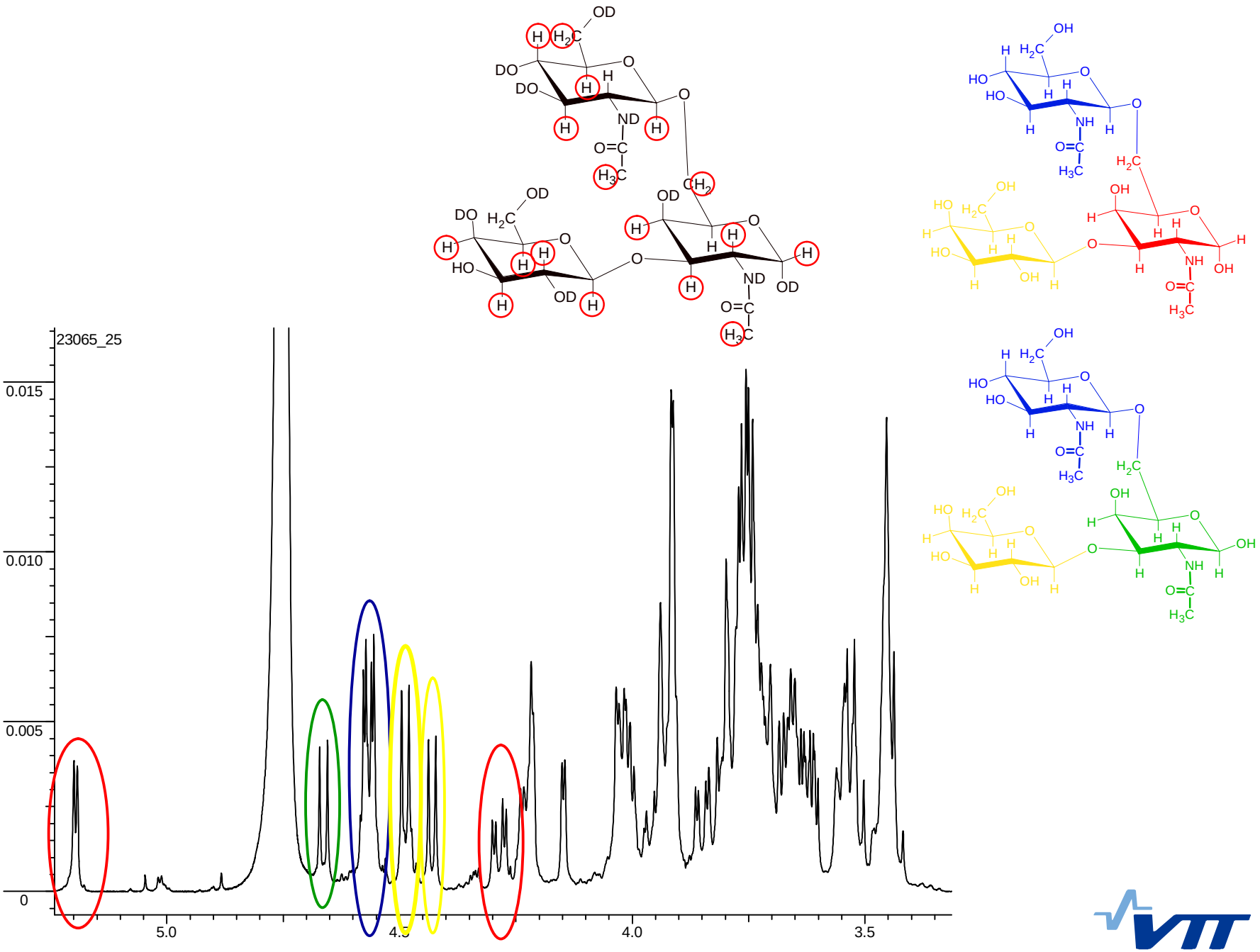

G6P 1H spectrum
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0ppm

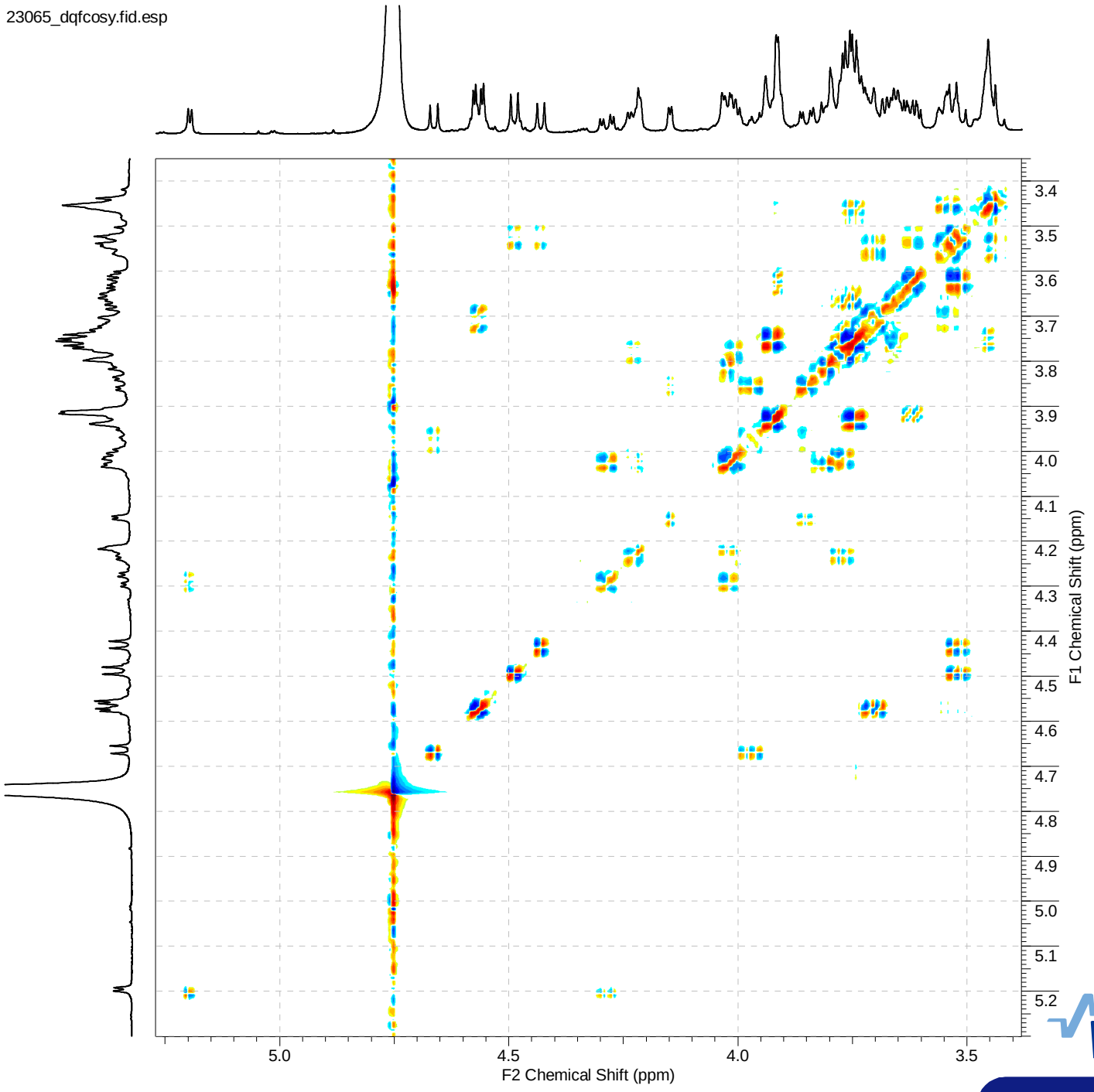

TOCSY
F2 (ppm)

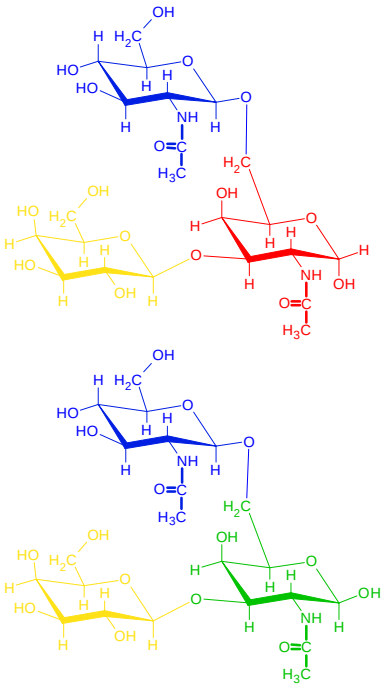

COSY
F2 (ppm)

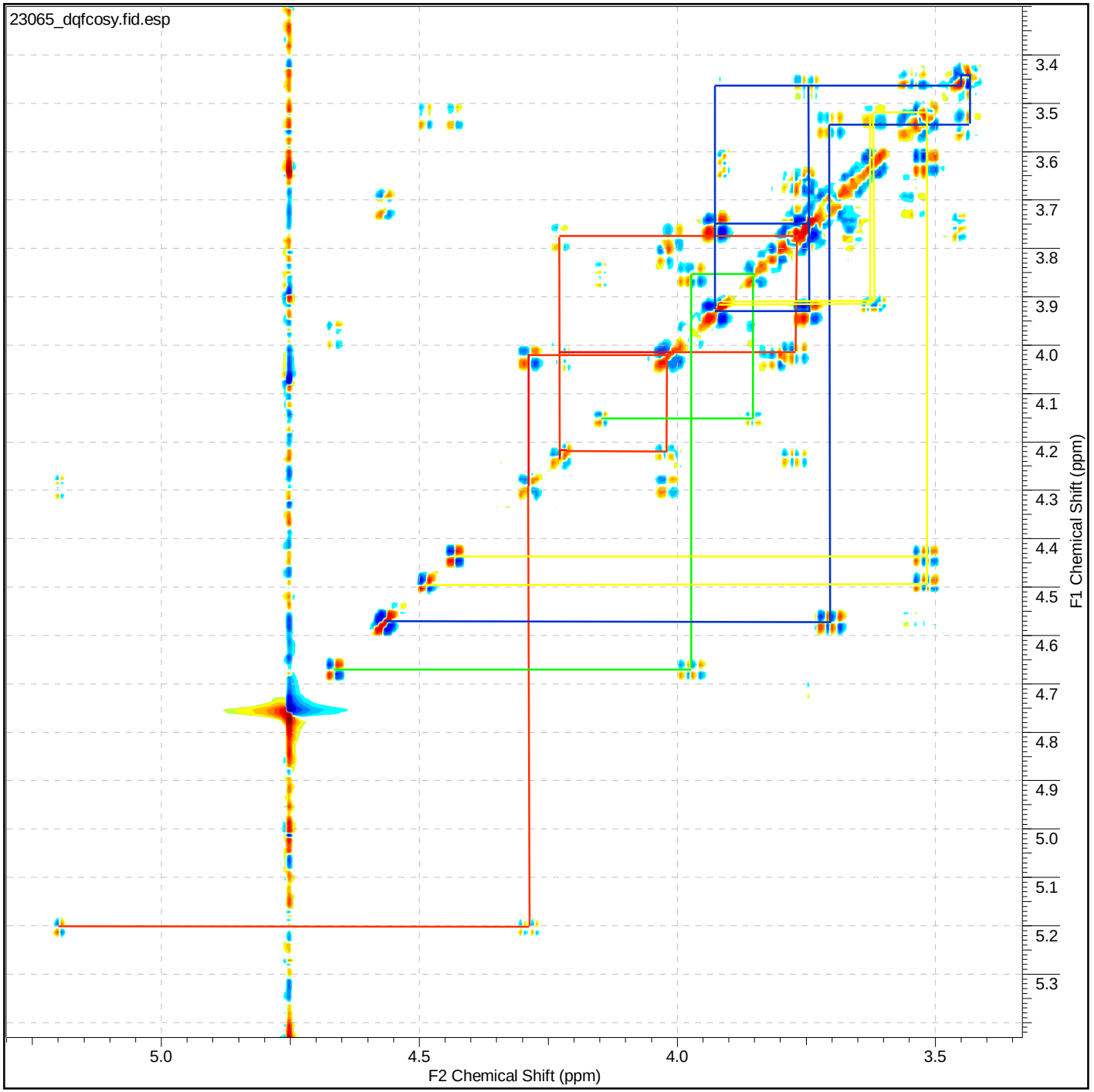

HSQC
F2 (ppm)

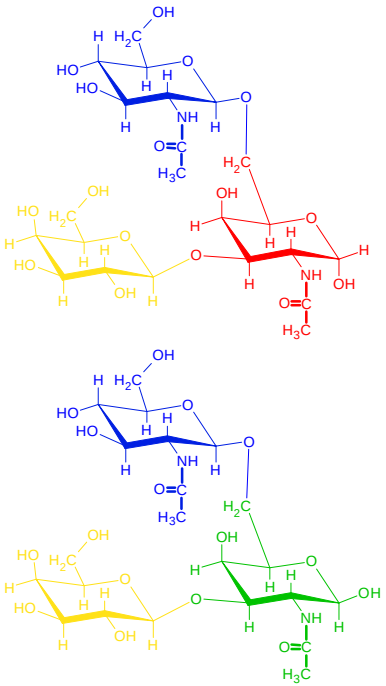

23065_25
0.015
0.010
0.005
0
5.0
4.5
4.0
3.5

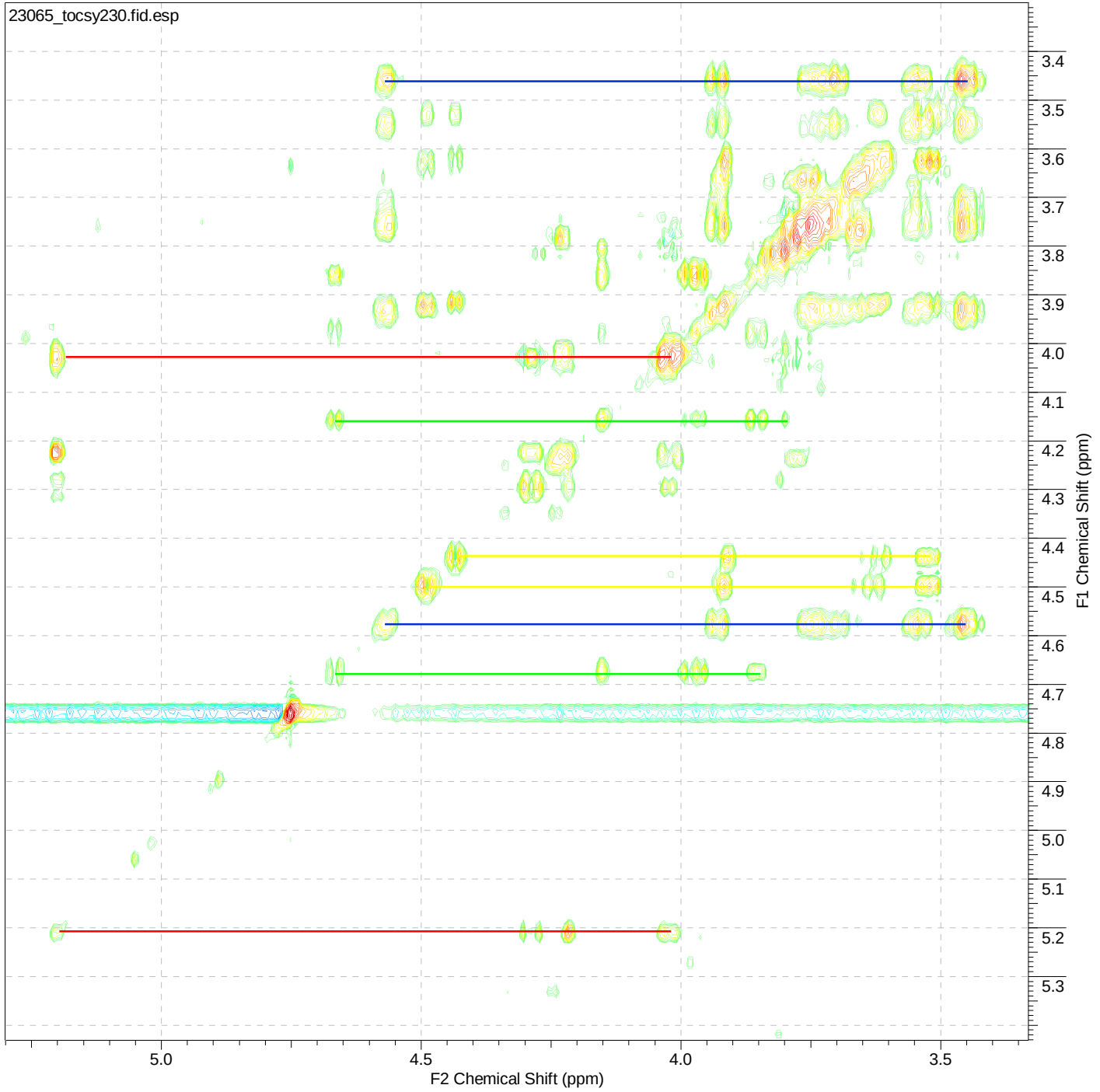

23065_25
0.015
0.010
0.005
0
5.0
4.5
4.0
3.5

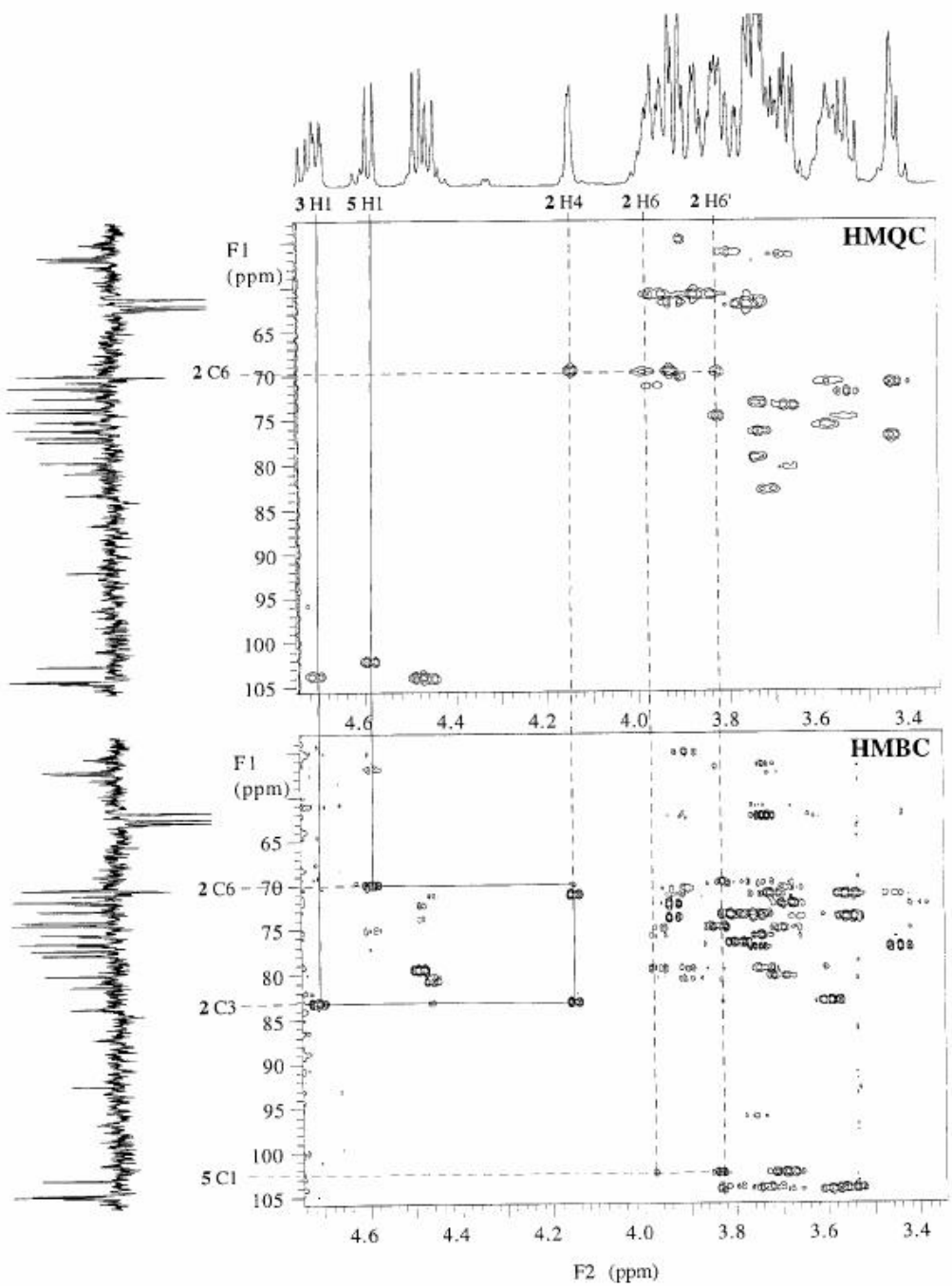

23065_dqfcosy.fid.esp
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
F1 Chemical Shift (ppm)
5.0
4.5
4.0
3.5
F2 Chemical Shift (ppm)




23065_dqfcosy.fid.esp
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)
5.0
4.5
4.0
3.5
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3




23065_tocsy230.fid.esp
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
F1 Chemical Shift (ppm)
5.0
4.5
4.0
3.5
F2 Chemical Shift (ppm)


3 H1
5 H1
2 H4
2 H6
2 H6'
HMQC
F1
(ppm)
2 C6
65
70
75
80
85
90
95
100
105
4.6
4.4
4.2
4.0
3.8
3.6
3.4
HMBC
F1
(ppm)
2 C6
2 C3
5 C1
F2 (ppm)

Common 2D NMR experiments

- COSY, correlated spectroscopy
  - between two protons over 2-3 chemical bonds
- TOCSY, total correlated spectroscopy (HOHAHA)
  - between protons of the whole spin system
- NOESY (ROESY), nuclear Overhauser effect spectroscopy
  - between two protons close in space (< 5Å)
  - inversely proportional to sixth power of the distance
- HSQC and HMQC, heteronuclear single/multiple quantum coherence
  - between a proton and a heteroatom (e.g. ^{13}C, ^{15}C) over one bond
- HMBC, heteronuclear multiple bond correlation
  - between a proton and a heteroatom over 2-3 bonds
- DOSY, diffusion ordered spectroscopy
  - separates molecules according to their diffusion rate

Pulse sequence of the ^{1}H-^{31}P HSQC-AD-TOCSY experiment