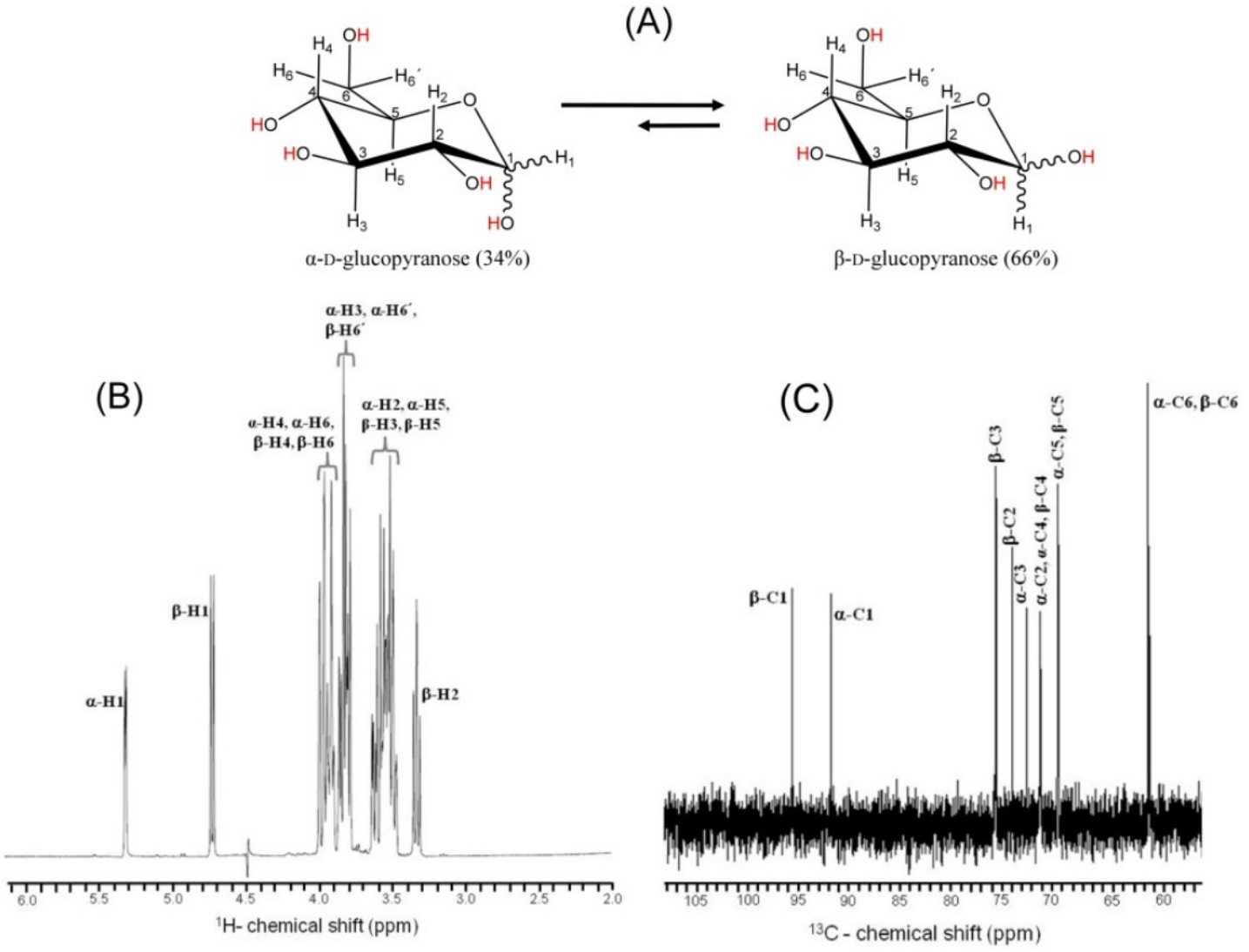
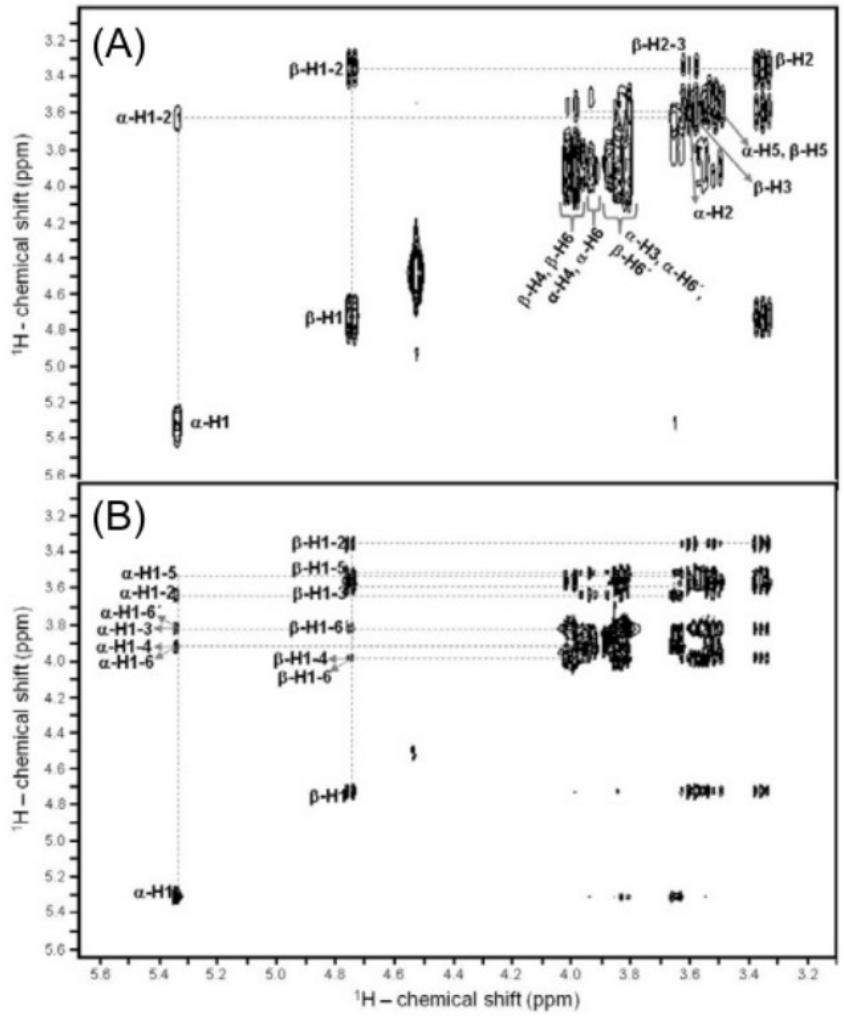
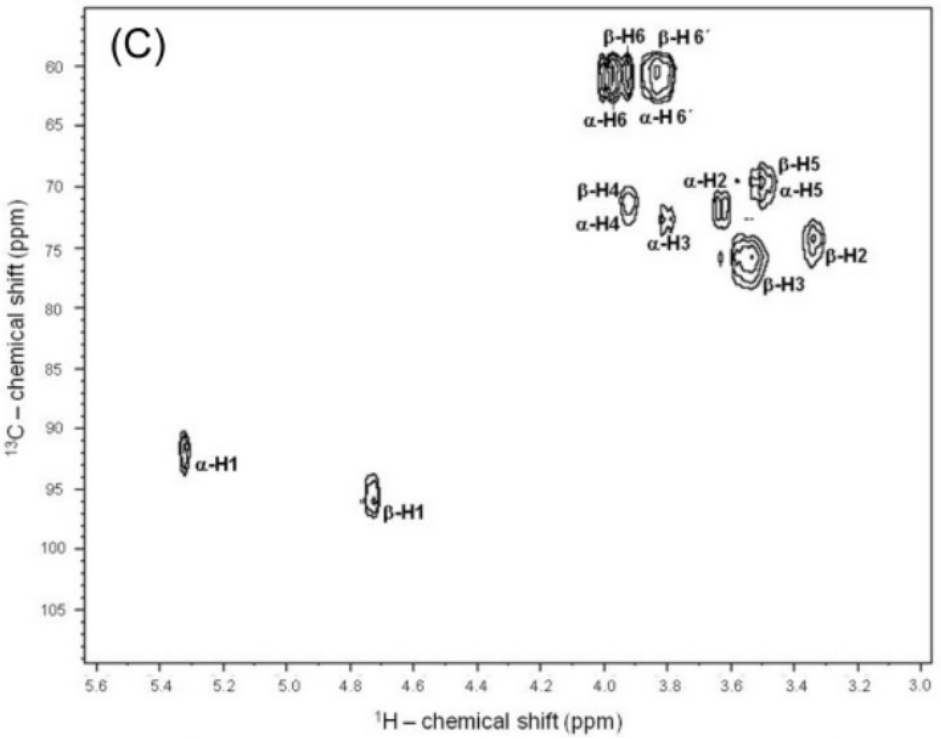
Structure elucidation (1D spectra)



Structure elucidation (2D spectra)





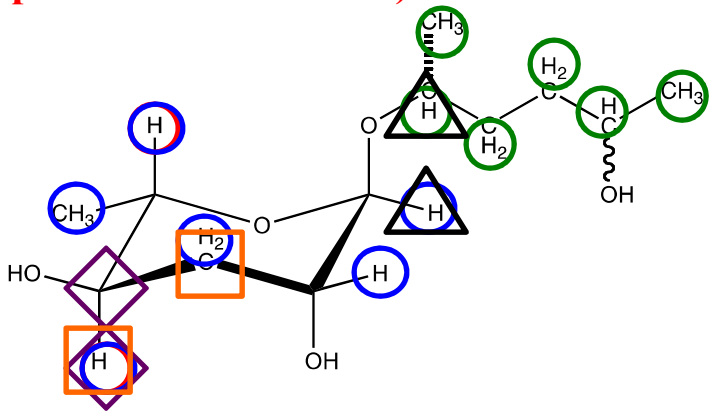
α-D-Glc*p*	β-D-Glc*p*
α-H1 – 5.32	β-H1 – 4.74
α-H2 – 3.63	β-H2 – 3.37
α-H3 – 3.83	β-H3 – 3.60
α-H4 – 3.92	β-H4 – 3.92
α-H5 – 3.50	β-H5 – 3.5
H6/6´ - 3.91/3.82	H6/6´ - 3.91/3.82
α-C1 – 91.4	β-C1 – 95.9
α-C2 – 71.8	β-C2 – 74.1
α-C3 – 72.4	β-C3 – 75.8
α-C4 – 71.2	β-C4 – 71.2
α-C5 – 69.8	β-C5 – 69.8
α-C6 – 60.3	β-C6 – 60.3

Information Content of Common 2D NMR Experiments

COSY (Correlation Spectroscopy): J coupling (generally up to 3 covalent bonds)

TOCSY (Total Correlation Spectroscopy):
J coupling along coupled networks

Blue is one TOCSY network, green is another



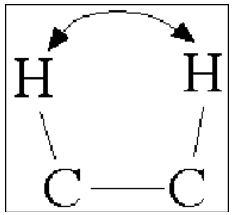
HSQC (Heteronuclear Single Quantum Correlation):
Directly bonded ^{13}C-^{1}H or ^{15}N-^{1}H

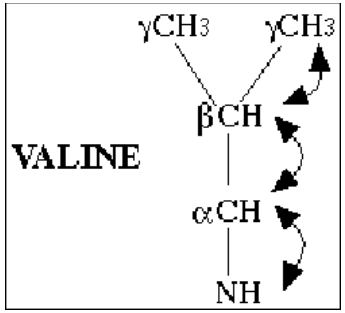
HMBC (Heteronuclear Multiple Bond Correlation):
2 or 3 bond ^{13}C---^{1}H or ^{15}N----^{1}H

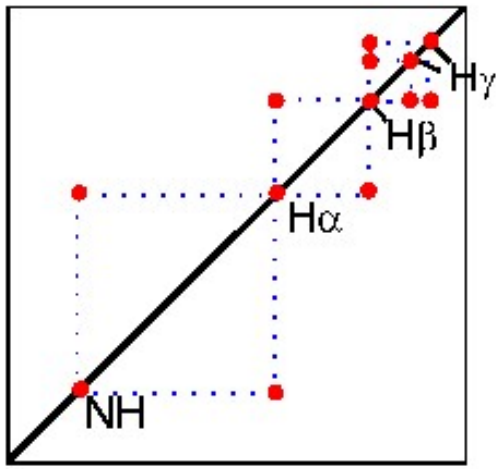
NOESY (Nuclear Overhauser Effect Spectroscopy)
Or ROESY (Rotating Frame Overhauser Effect Spectroscopy):
^{1}H to ^{1}H distances up to 5-6 Å

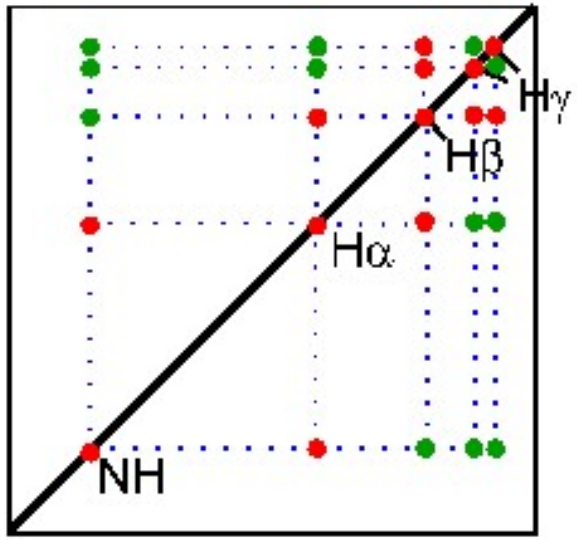
COSY & TOCSY

Correlation	Principal Technique(s)	Comments
	^1H–^1H COSY	Proton J coupling typically over 2 or 3 bonds.
	^1H–^1H TOCSY	Relayed proton J couplings within a coupled spin system. Remote protons may be correlated provided there is a continuous coupling network in between them.

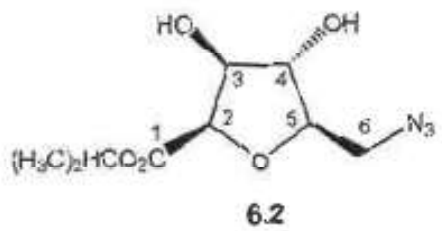




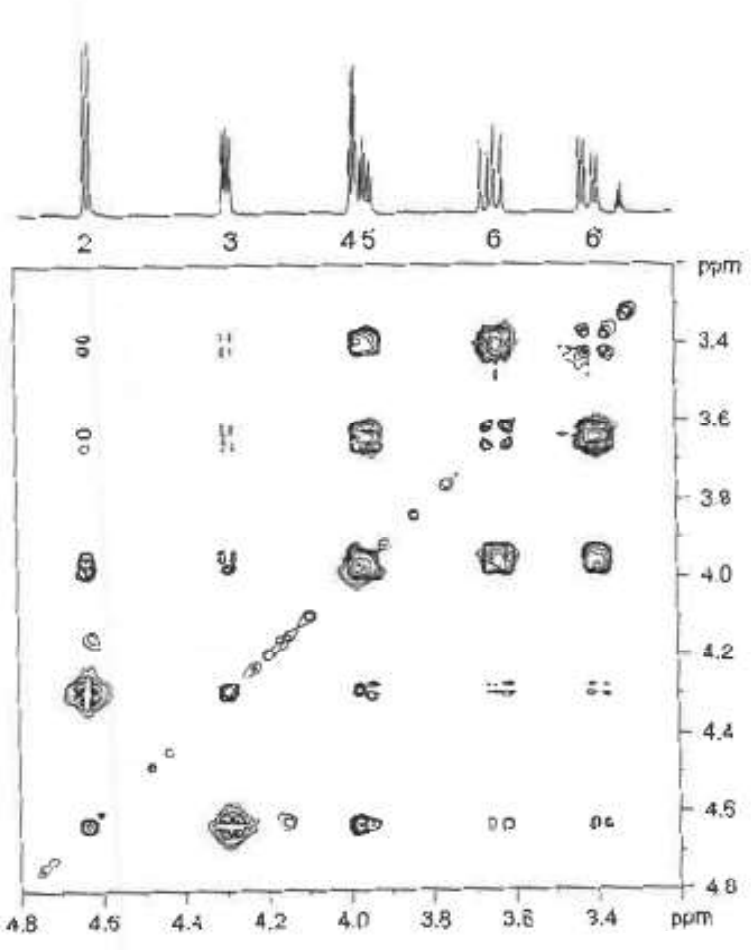




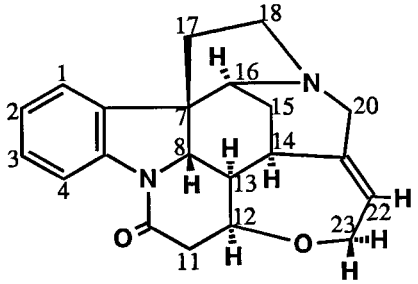
TOCSY: assessment of spins in a ring with multiple HC-CH

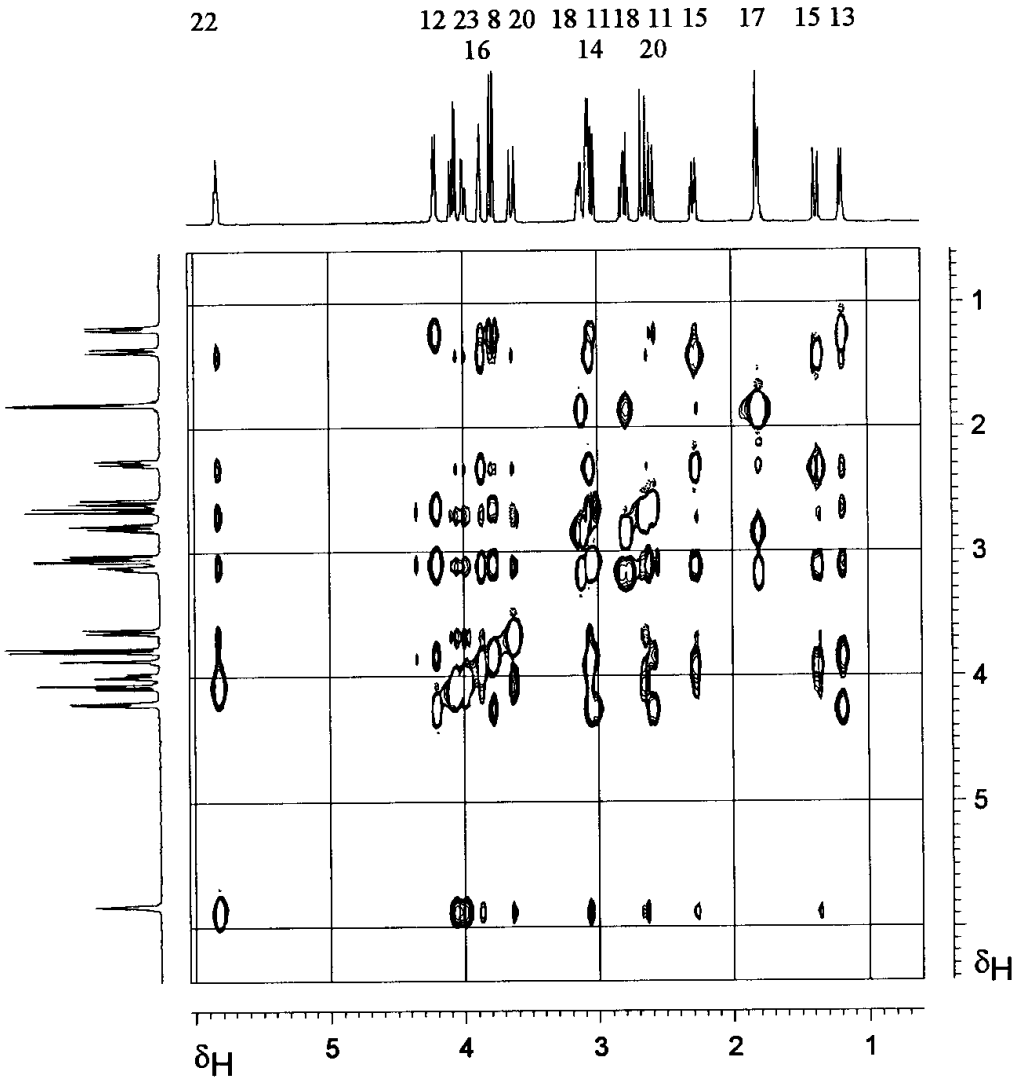


6.2

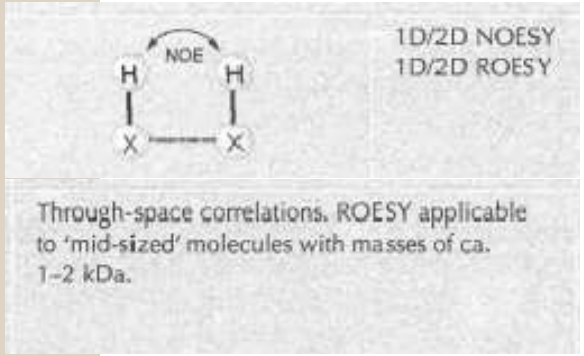


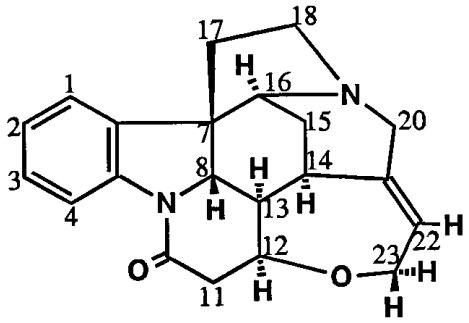
TOCSY of strychnine

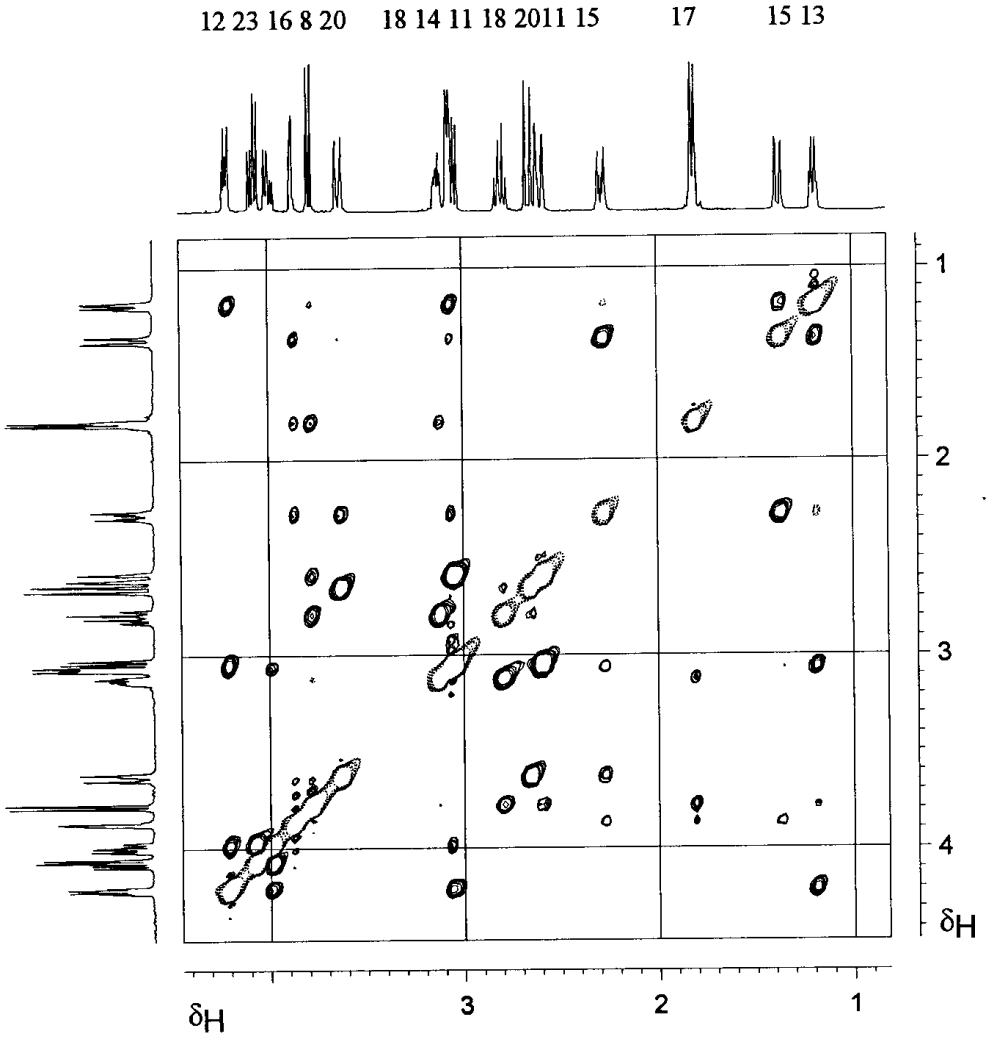




NOESY of strychnine

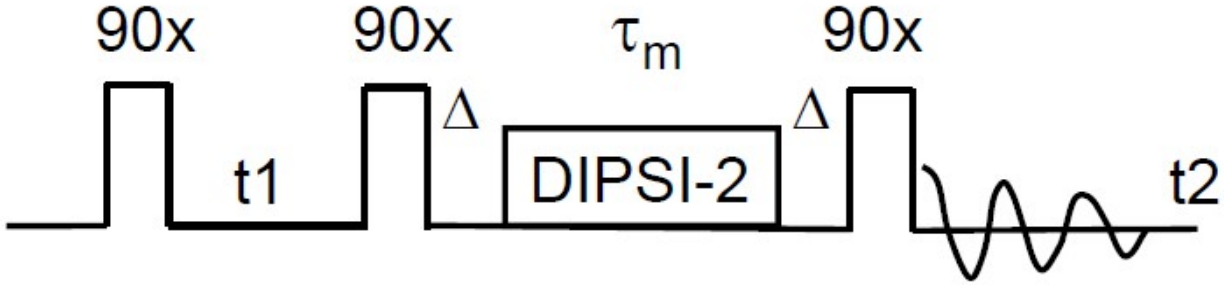






TOtal Correlation SpectroscopY (TOCSY)

- Cross-peaks for all members of spin system



- Δ is a short delay to change transmitter power
- DIPSI-2 is an isotropic mixing sequence –
- τ_m is mixing time

The general **TOCSY** block consists of an **spin-lock** period during which an isotropic mixing sequence applied along an specific axis (WALTZ, **DIPSI** or MLEV) is applied.

TOtal Correlation SpectroscopY (TOCSY)

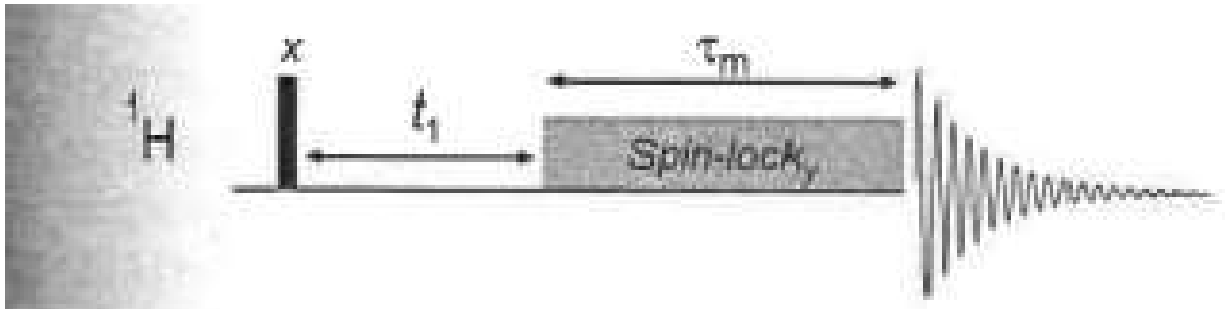


FIGURE 6.28 **The TOCSY sequence.** The spin-lock mixing time, τ_m, replaces the single mixing pulse of the basic COSY experiment.

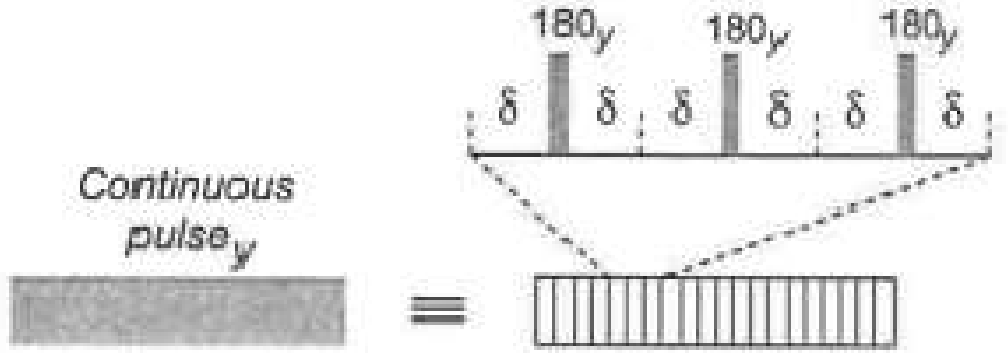
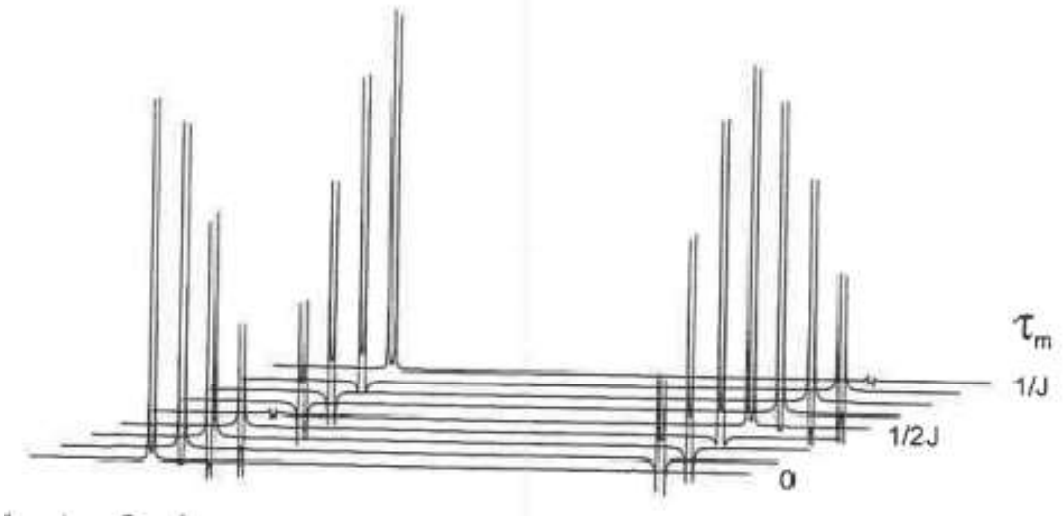


FIGURE 6.29 **The spin lock in its simplest form is a single, long, low-power pulse.** This can be viewed as a continuous sequence of closely spaced 180 degree pulses bracketed by infinitely small periods δ.

TOCSY: interferogram and spin-system



Practical Considerations for TOCSY

- Both cross-peaks and auto-peaks are in-phase and can be phased absorptive
- Cross-peaks do not necessarily indicate direct coupling – but show virtual coupling
- The magnitude of cross-peaks depends on the topology of the spin system, magnitudes of all couplings involved, efficiency of mixing sequence, relaxation during τ_m.
- Chose τ_m 75-100 ms for long transfer, 30-50 ms for one to two couplings.

The importance of mixing time in TOCSY

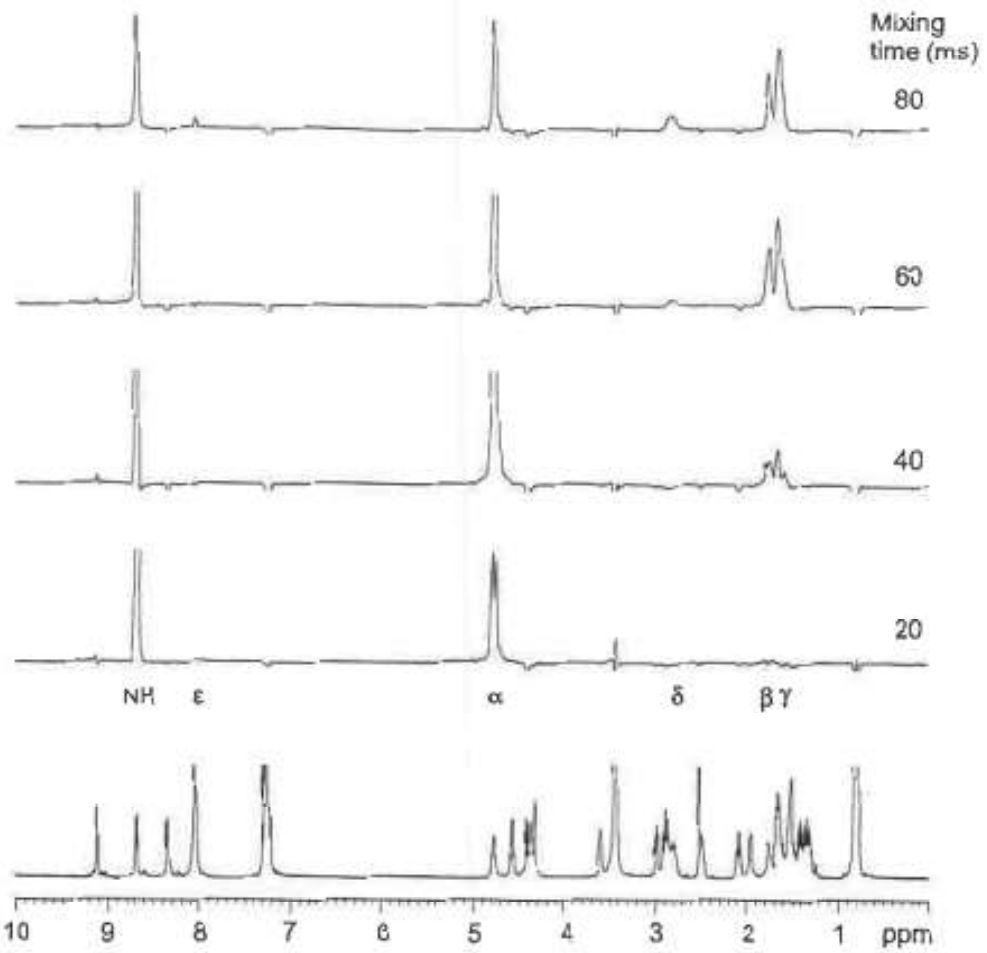
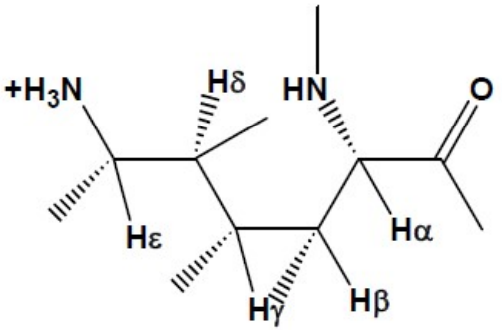
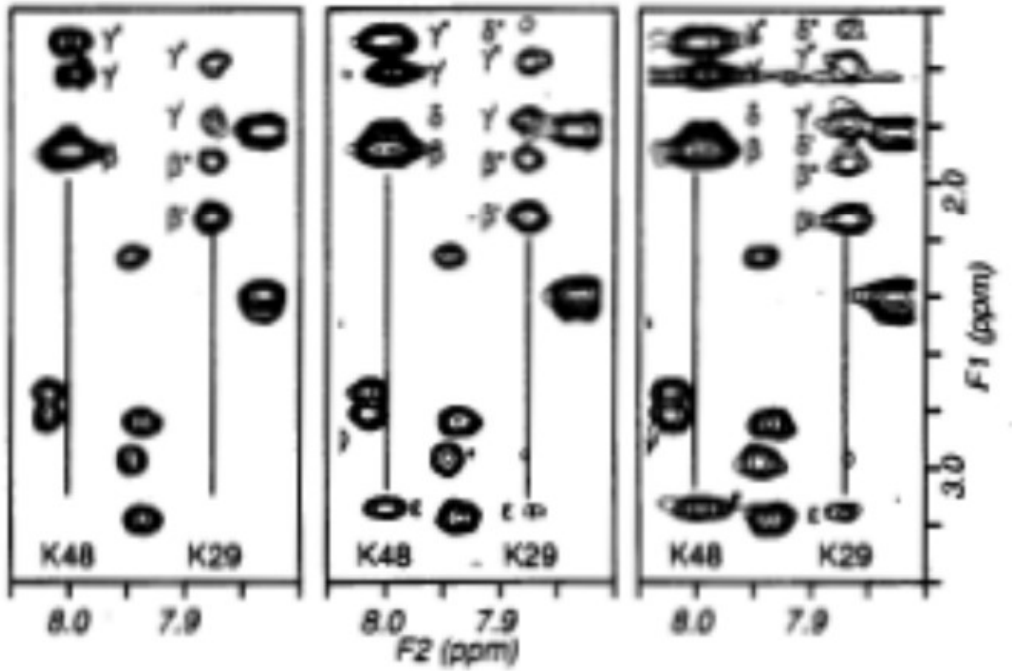


FIGURE 6.36 **The time-depeadent propagation of magnetisation along a proton spin system.** This is illustrated for the ornithine residue of gramicidin-S 6.8 where are extracted from 2D TOCSY spectra at the amide NH proton shift and show progressive transfer along the sidechain as the mixing time increases.

The importance of mixing time in TOCSY

Example TOCSY for Lysine

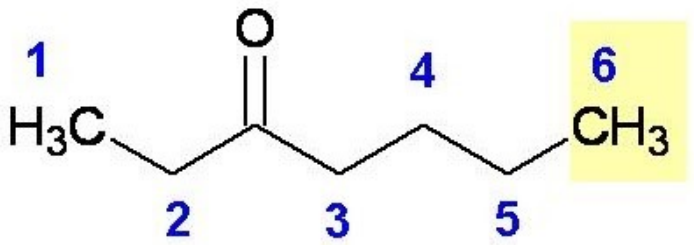


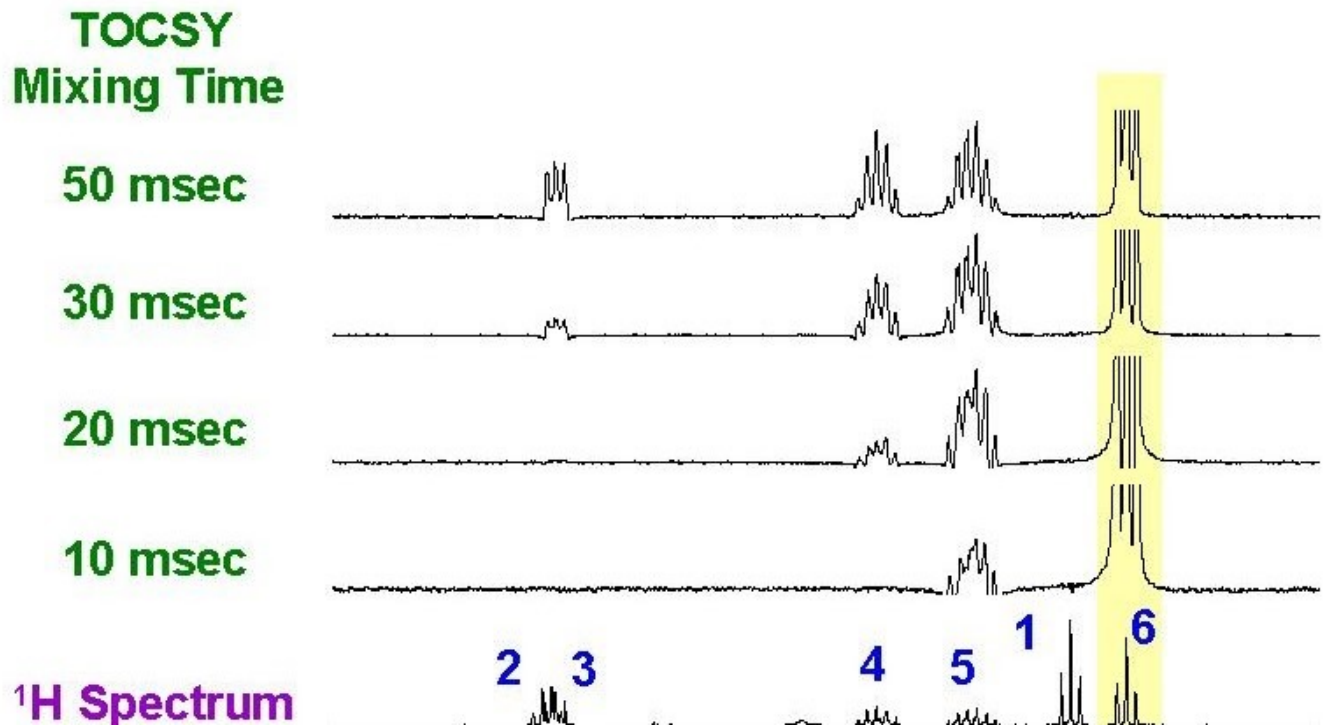


- Mixing times of 48, 83, and 102 ms
- From Cavanagh, Fairbrother, Palmer and Skelton

Practical Considerations for TOCSY

1D Selective Gradient TOCSY as a Function of Mixing Time





TOCSY: different pulse sequences

MLEV-17 vs DIPSI-2

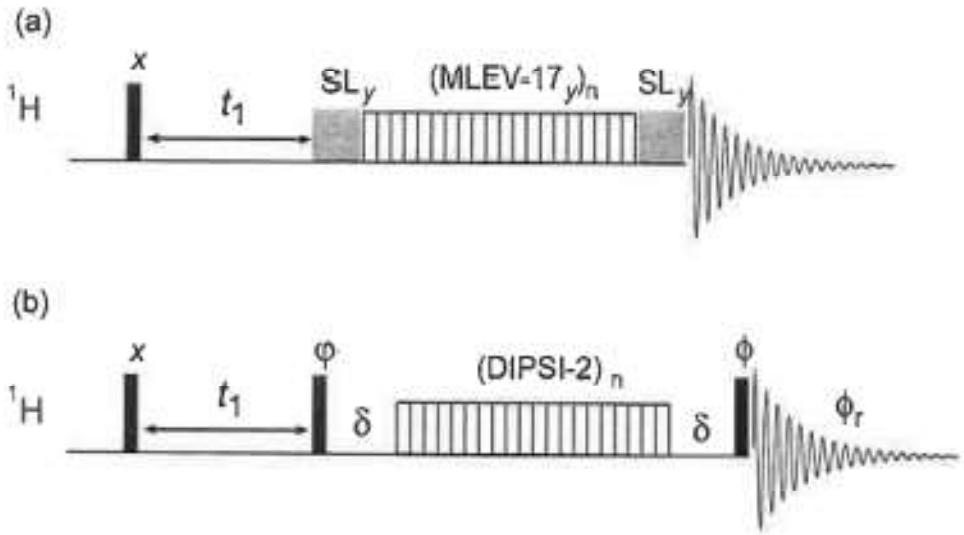


FIGURE 6.37 **Practical schemes for implementing TOCSY.** These are based on (a) the MLEV-17 mixing scheme and (b) the DIPSI-2 isotropic mixing scheme. The MLEV sequence is bracketed by short, continuous wave, spin lock trim pulses (SL) to provide pure-phase data. In scheme (b) this can be achieved by phase-cycling the 90 degree *z*-filter pulses that surround the mixing scheme. This demands the independent inversion of each bracketing 90 degree pulse with coincident receiver inversion, thus $\varphi = x, -x, x, -x$; $\phi = x, x, -x, -x$ and $\phi_r = x, -x, -x, x$. The δ periods allow for the necessary power switching.

TOCSY: different pulse sequences

MLEV-16 vs MLEV-17 with gradients

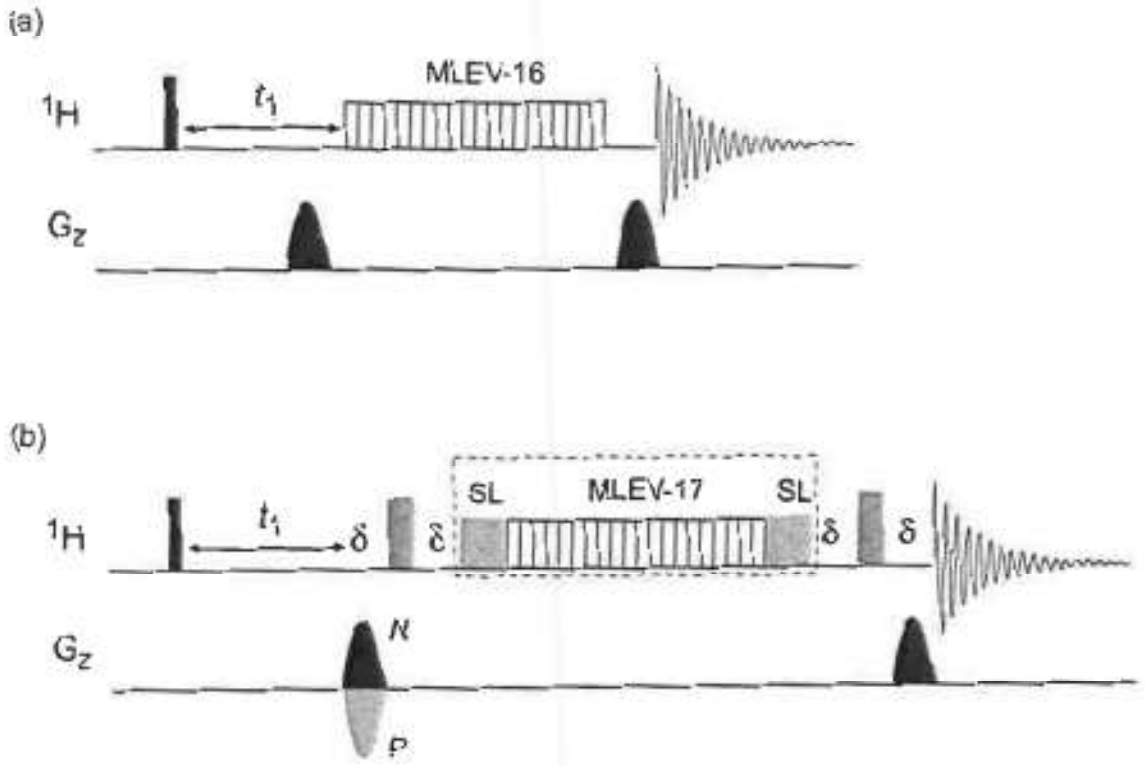


FIGURE 6.38 **Gradient-selected TOCSY.** Sequence (a) is suitable for absolute value presentations with a 1:1 gradient combination selecting the N-type spectrum. Sequence (b) provides phase-sensitive data sets via the echo–antiecho method for which separate P- and N-type data are collected through inversion of the first gradient. The boxed section may contain any suitable mixing element including the 90 degree–DIPSI2–90 scheme described above.

1D selective TOCSY with or without gradient

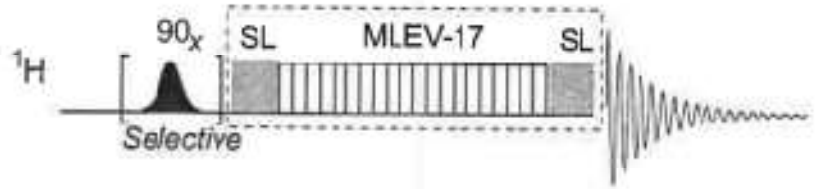


FIGURE 6.39 **The general sequence for 1D selective TOCSY.** Any suitable selective 90 degree pulse scheme (see Section 12.4) can be used to selectively excite the target resonance from which transfer is initiated. The boxed section can contain any suitable mixing element.

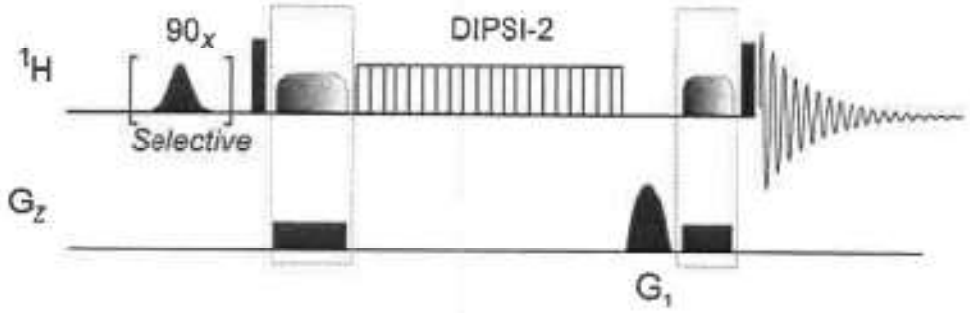


FIGURE 6.43 **The zero-quantum dephasing scheme applied to TOCSY.** The boxed regions contain the dephasing elements in which the gradients are applied during the swept inversion pulse; the two elements are of different durations to avoid accidental refocusing. G_1 represents a purge gradient.

1D selective TOCSY in structural assignment

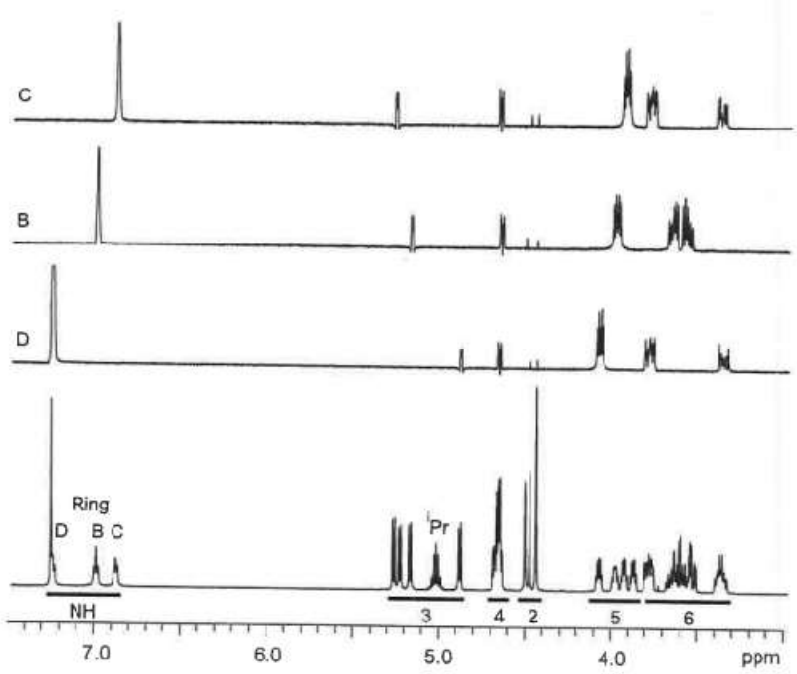
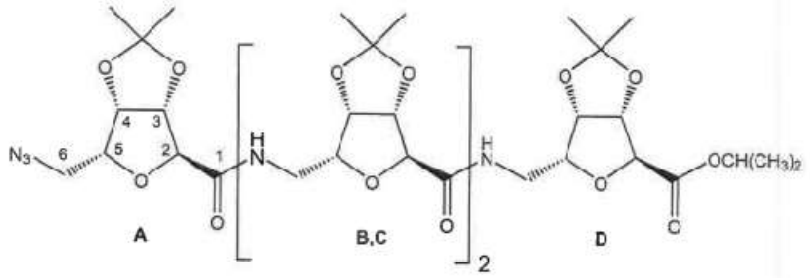


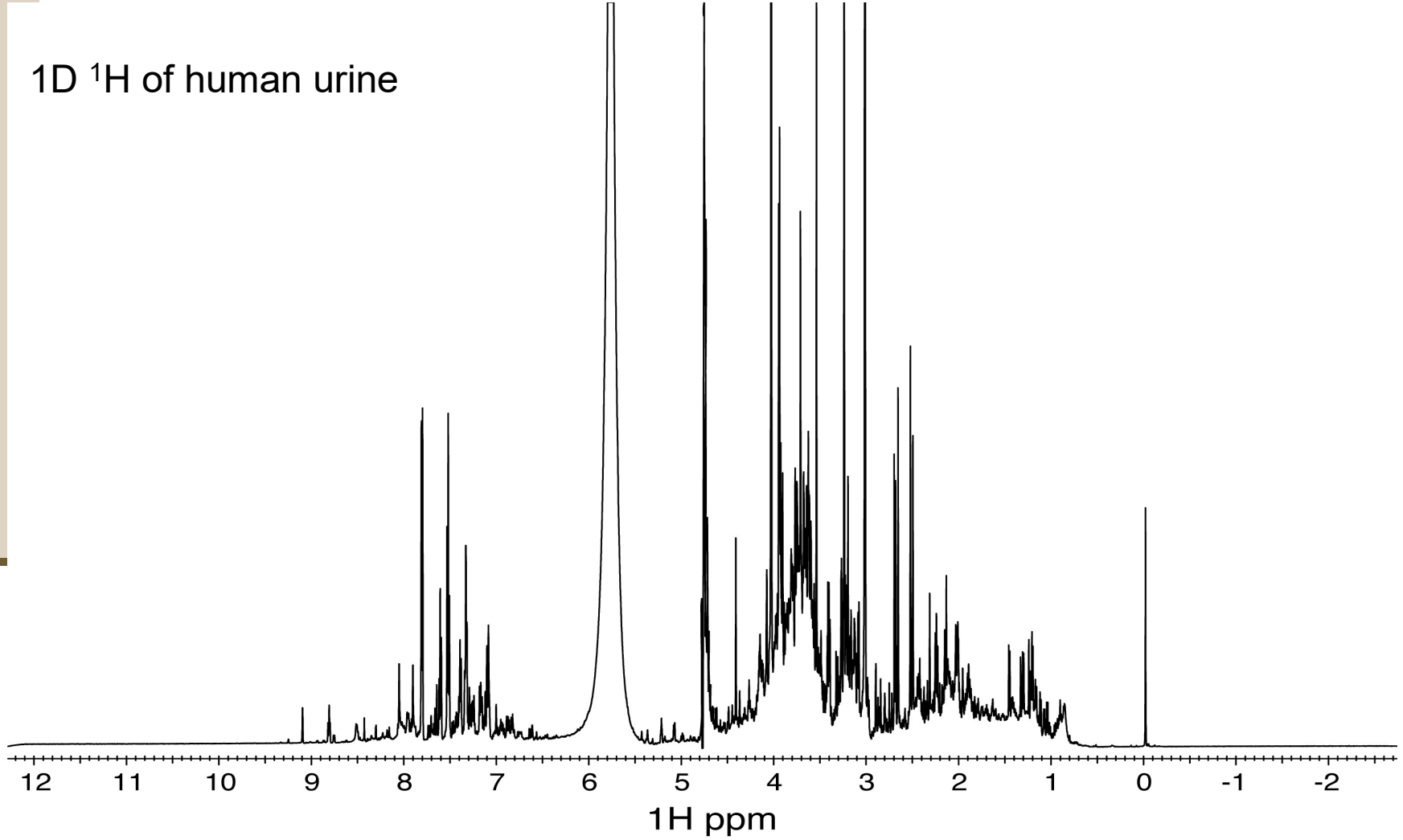
FIGURE 6.40 1D TOCSY spectra of the tetrameric carbopeptoid 6.10 in $CDCl_3$. Each amide proton was selectively excited and used as the starting point for coherence transfer. Selective excitation was achieved with the excitation sculpting method of Section 12.4 and mixing used a 97-ms spin lock.



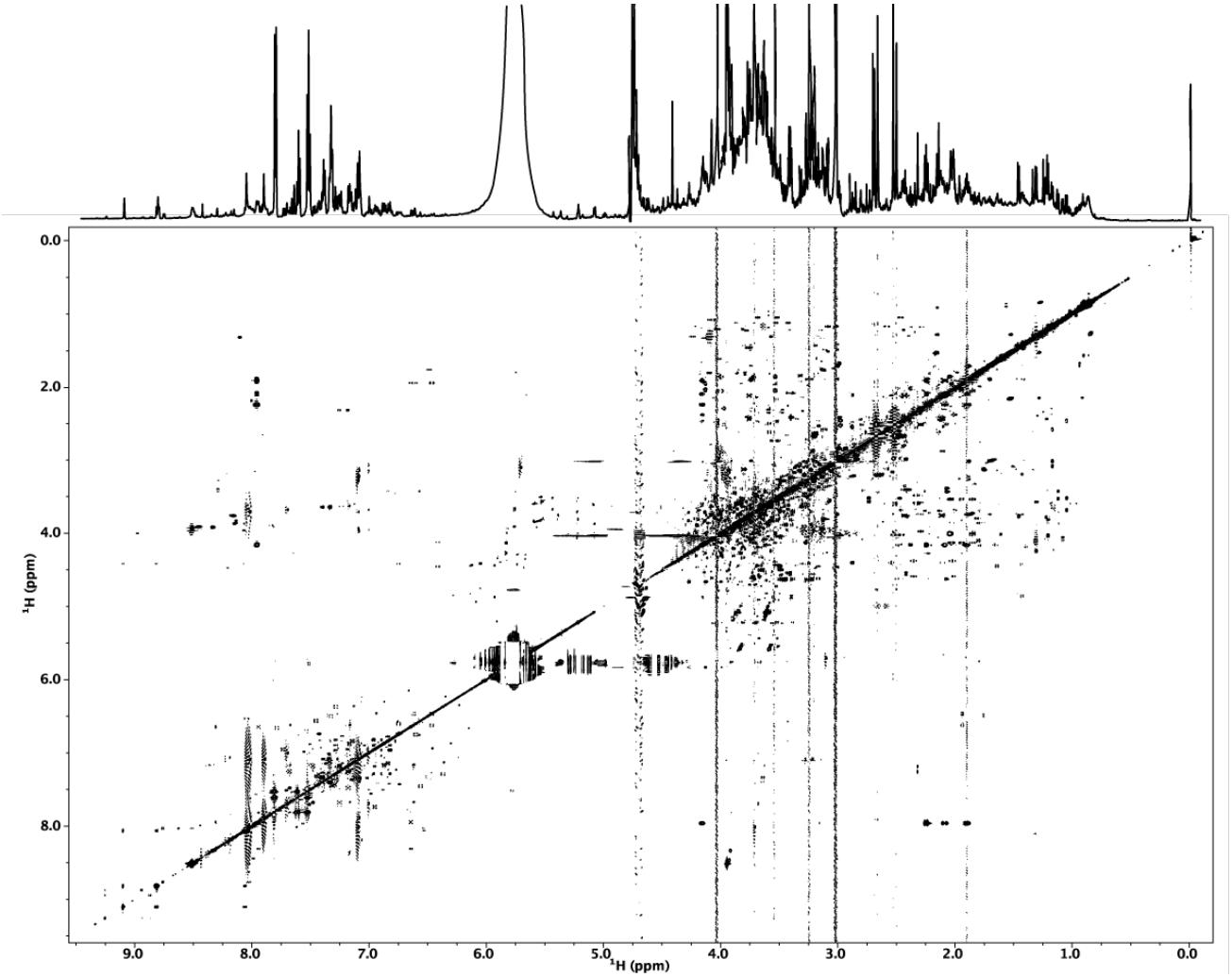
6.10

NMR in metabolomics – not possible through 1D!

1D ^{1}H of human urine

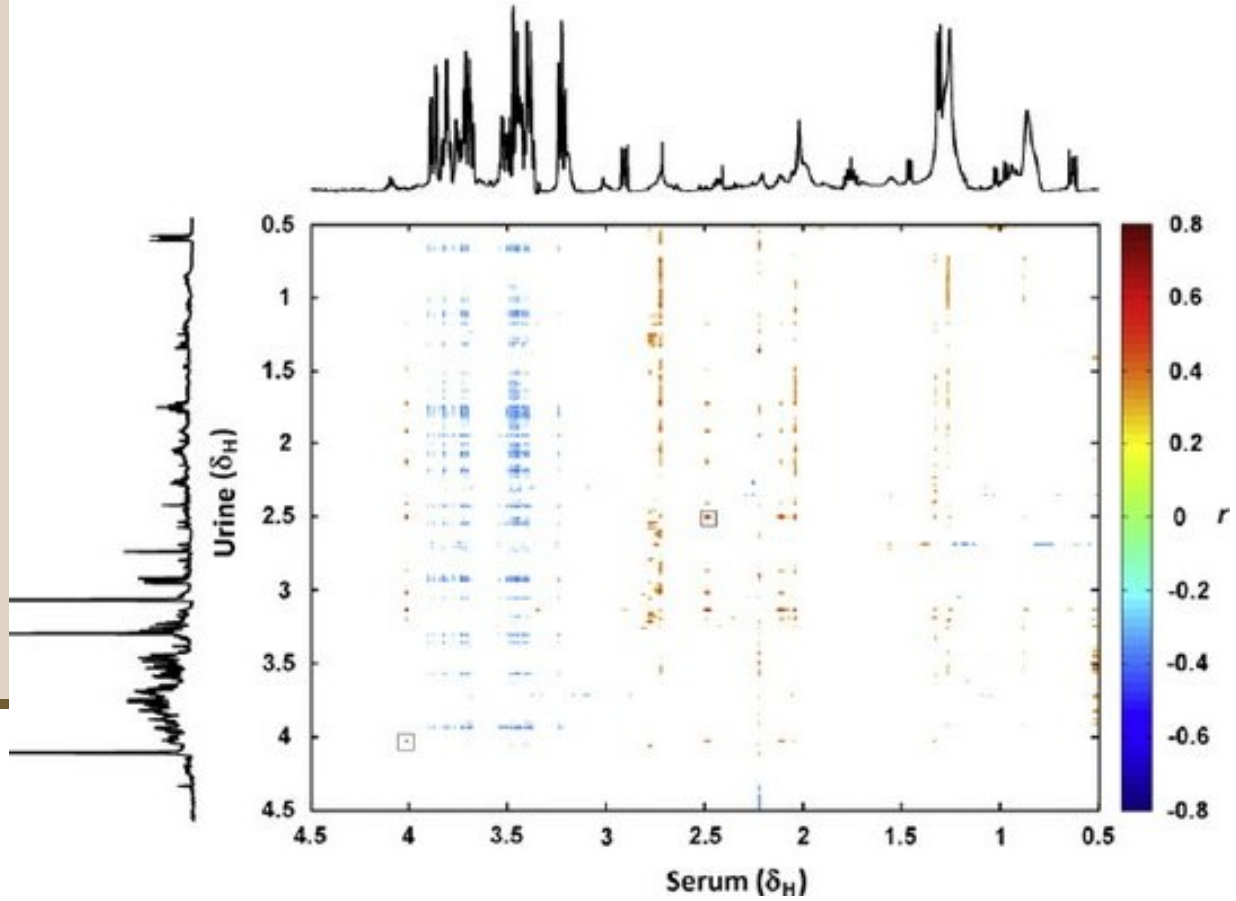


TOCSY in metabolomics - molecular screening of organic fluids such as urine, plasma, saliva



2D ^{1}H-^{1}H TOCSY spectrum of human urine

Statistical TOCSY (sTOCSY) in metabolomics



Two-dimensional statistical urine-serum correlation spectroscopy of the aliphatic region of NMR spectra. The diagonal peaks at δ 4.08 and 2.45 indicated with small rectangles are from creatinine and succinate, respectively.