

ANALITIČKA KEMIJA II

- uvodno predavanje
- općenito - uzorkovanje; norme i standardi; intelektualno vlasništvo
- STATISTIKA - osnove
- EKSTRAKCIJA, KROMATOGRAFIJA - osnove
- ELEKTROANALITIČKE METODE
- BOLTZMANNOVА RAZDIOBA
- SPEKTROSKOPIJA - osnove; zadaci
- INSTRUMENTACIJA - osnove; zadaci
- ATOMSKA SPEKTROSKOPIJA; zadaci
- MOLEKULSKA SPEKTROSKOPIJA - UV/VIS, fluorescencija
- IR i Ramanova spektroskopija
- NMR - uvod
- **NMR - tehnike**

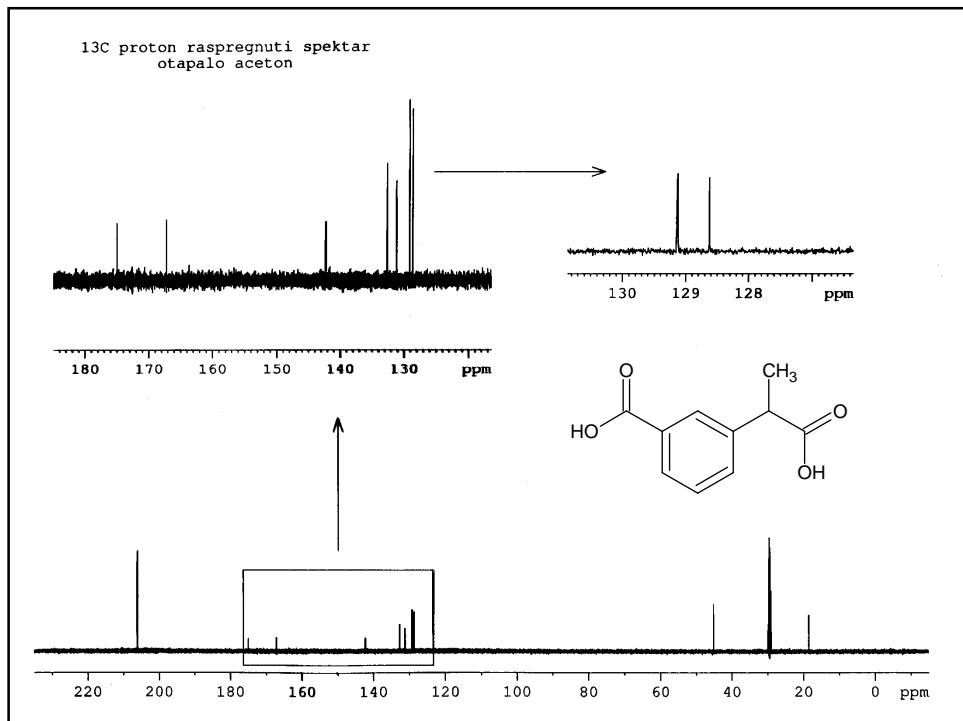
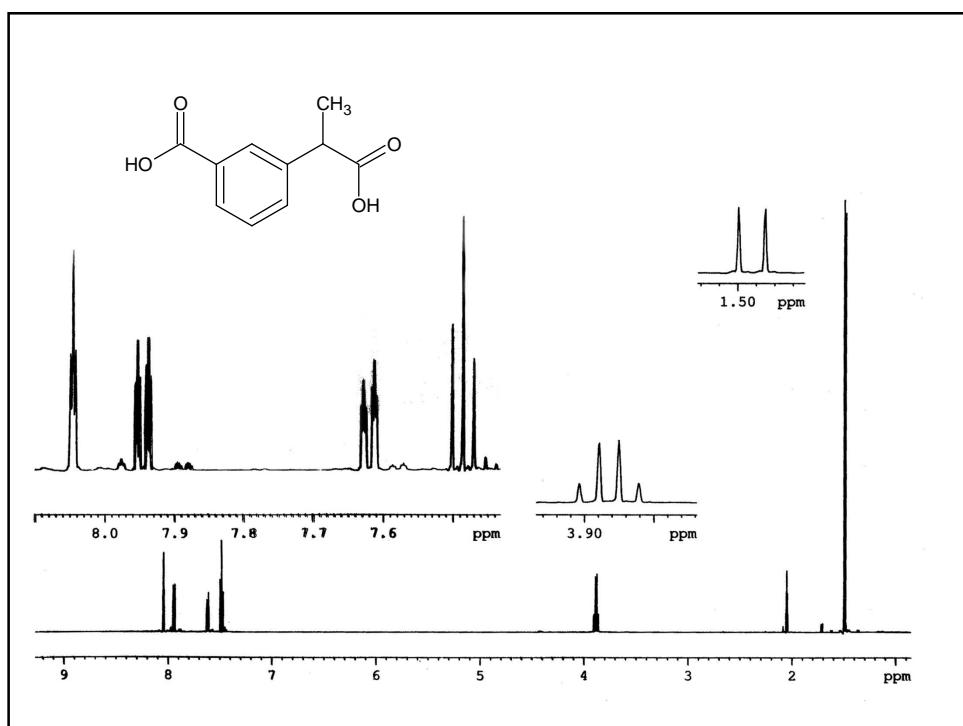
nositelj: prof.dr.sc. P. Novak; šk.g. 2012/13.

Jednodimenzionske tehnike



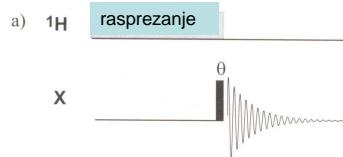
Osnovne višepulsne jednodimenzionske metode

Tehnika	primjena
J-modulirana spinska jeka ili APT (Attached proton test)	Editiranje spektara heterojezgri (^{13}C) prema multiplicitetu
INEPT (Insensitive nuclei enhanced by polarisation transfer)	pojačavanje signala jezgri s malim γ (^{13}C) pomoću prijenosa polarizacije sa jezgri s velikim γ (^1H , ^{19}F). Editiranje spektara heterojezgri prema multiplicitetu. Robusniji od DEPT-a. Nema informacija o kvarternim ^{13}C .
DEPT (Distortionless enhancement by polarisation transfer)	pojačavanje signala jezgri s malim γ (^{13}C) pomoću prijenosa polarizacije sa jezgri s velikim γ . Editiranje spektara heterojezgri prema multiplicitetu. Robusniji od INEPT-a. Nema informacija o kvarternim ^{13}C .
DEPT-Q	Isto kao DEPT ali sadrži kvarterne ^{13}C
PENDANT	pojačavanje signala jezgri s malim γ (^{13}C) pomoću prijenosa polarizacije sa jezgri s velikim γ (^1H , ^{19}F). Editiranje spektara heterojezgri prema multiplicitetu. Sadrži informacije o kvarternim ^{13}C .

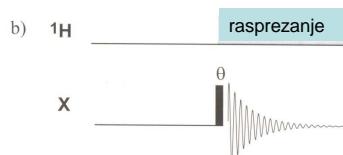


Tehnike dvostrukih rezonancija

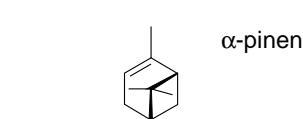
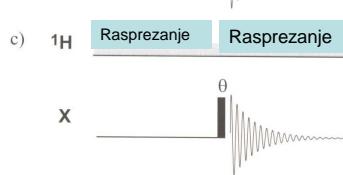
a) Spregnuti spektar s NOE



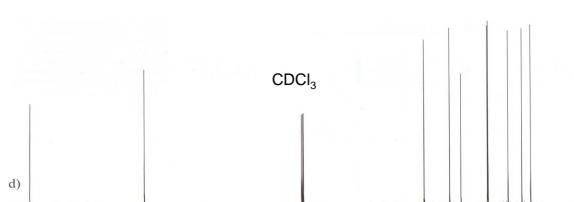
b) Raspregnuti spektar bez NOE



c) Raspregnuti spektar s NOE (rf polje primjenjeno s dvije različite snage-power gated)



d) Raspregnuti spektar s NOE-power gated



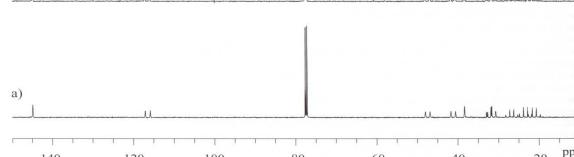
c) Raspregnuti spektar bez NOE

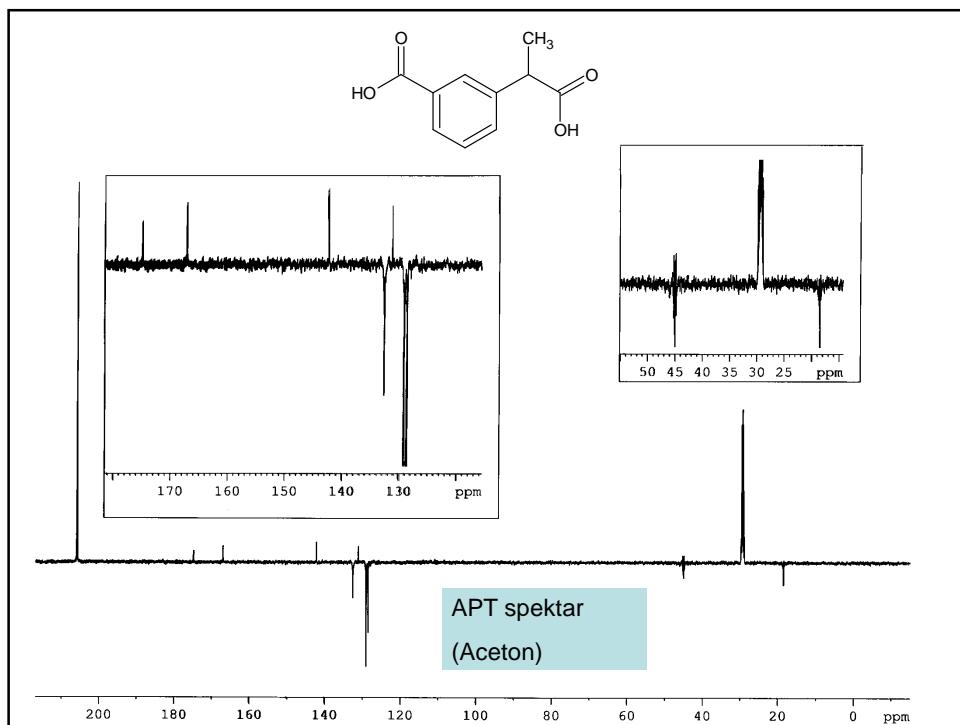
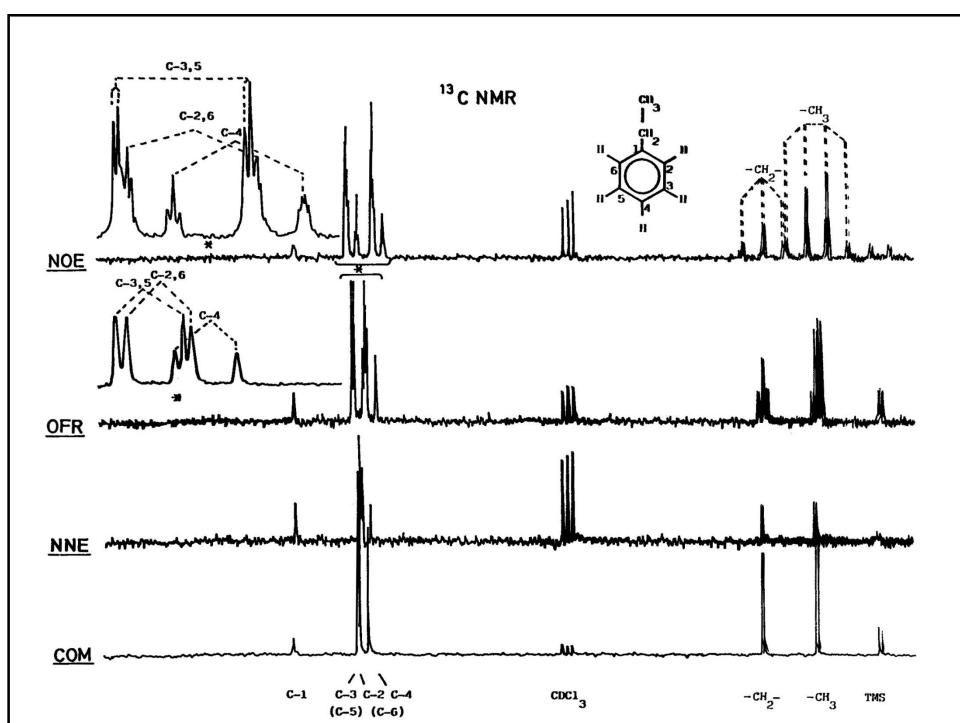


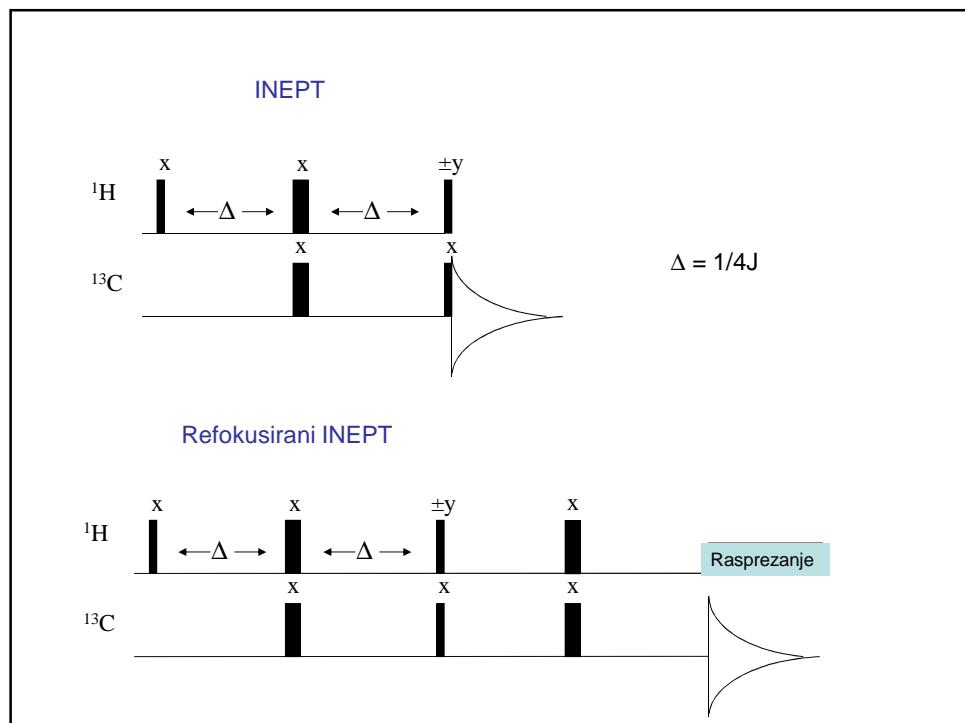
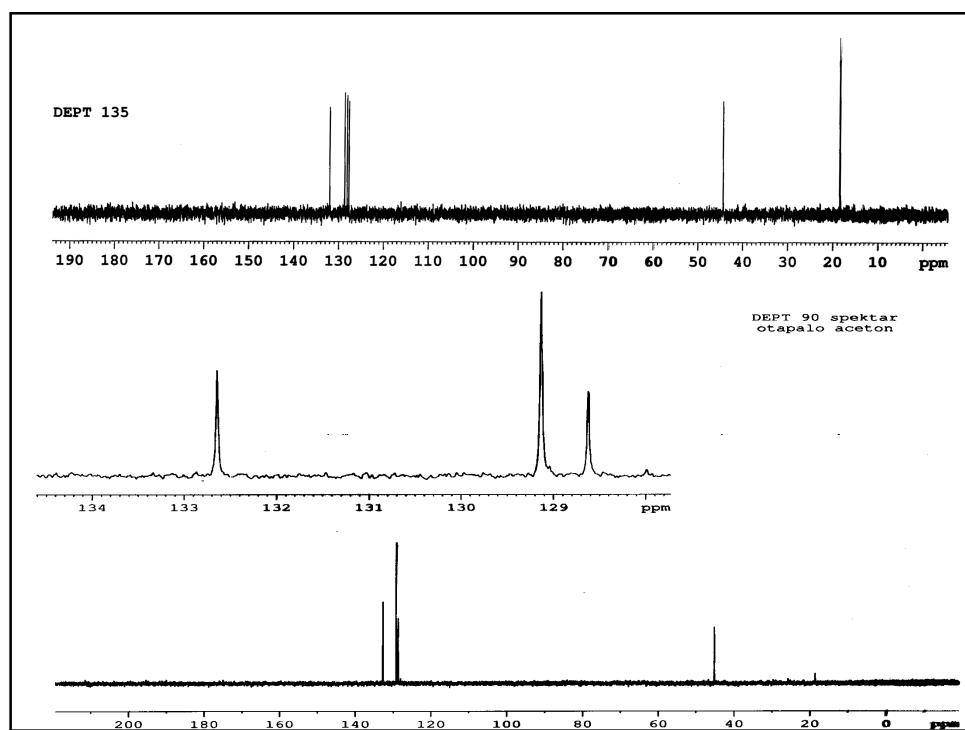
b) Spregnuti spektar s NOE

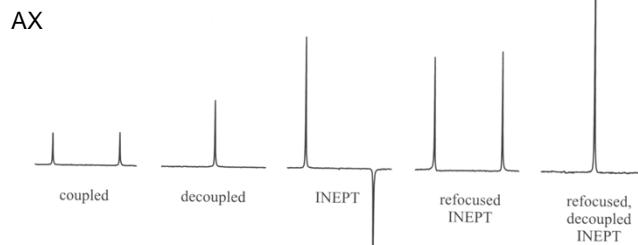


a) Spregnuti spektar bez NOE









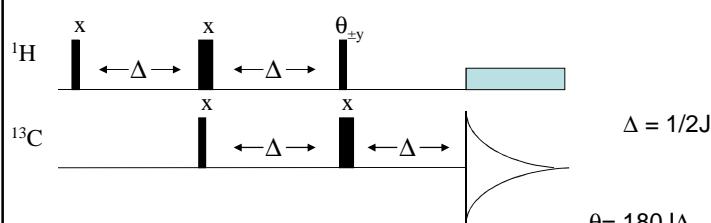
Povećanje osjetljivosti!

$$I_{\text{INEPT}} = I_0 (\gamma_H / \gamma_X)$$

$$I_{\text{NOE}} = I_0 (1 + \gamma_H / 2\gamma_X)$$

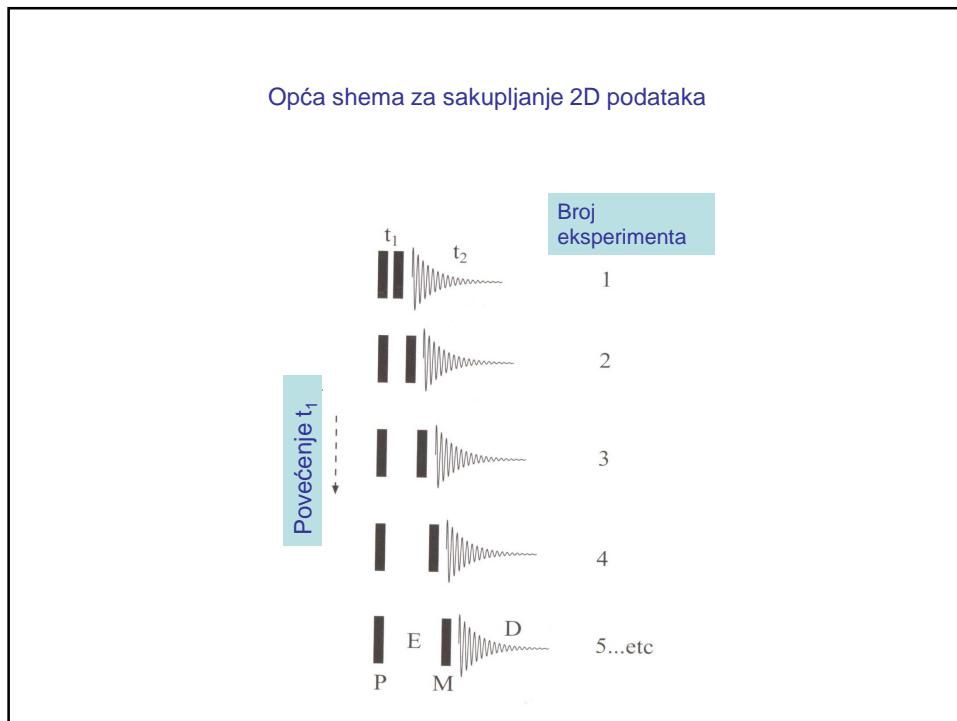
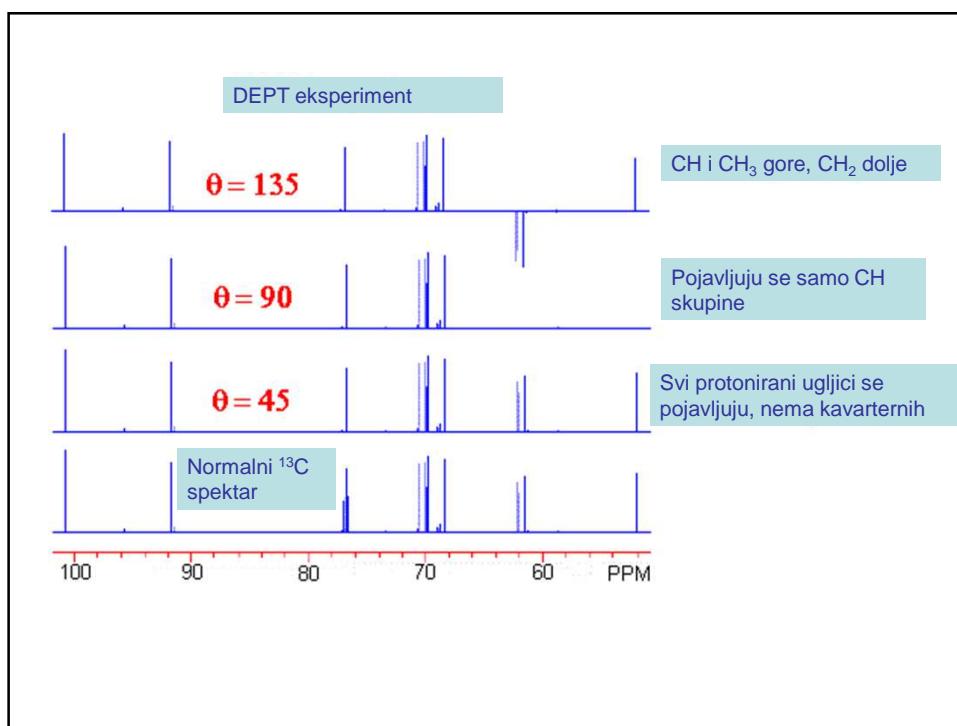
X	¹³ C	¹⁵ N	²⁹ Si	³¹ P
I_{INEPT}	3.98	9.87	5.03	2.47
I_{NOE}	2.99	-3.94	-1.52	2.24

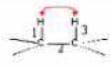
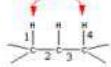
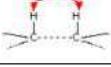
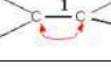
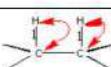
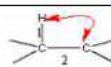
DEPT (Distortionless Enhancement by Polarisation Transfer)

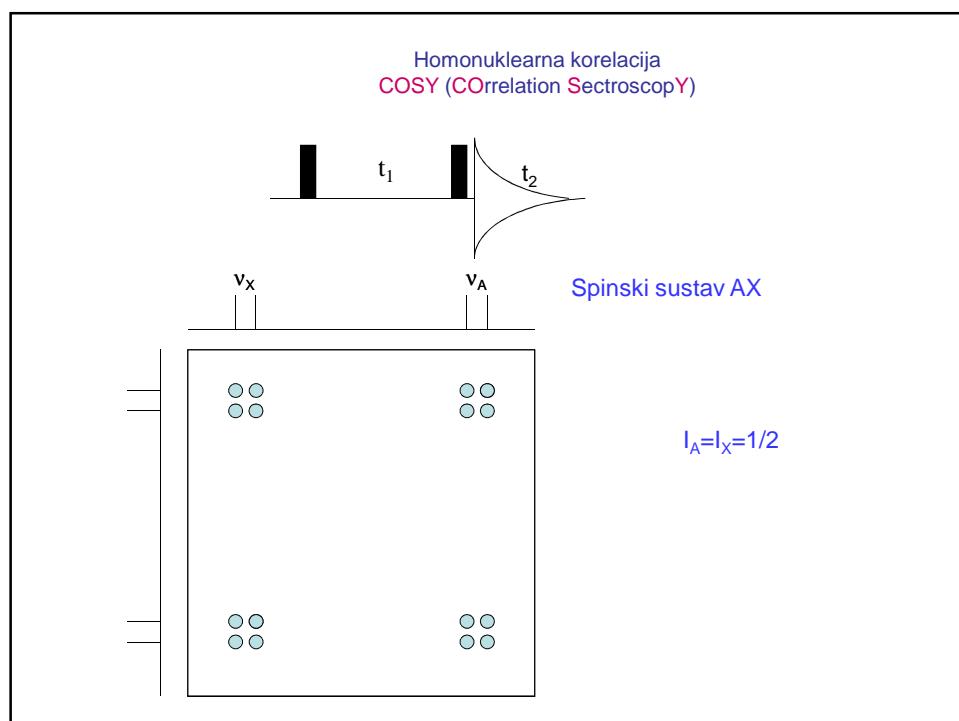


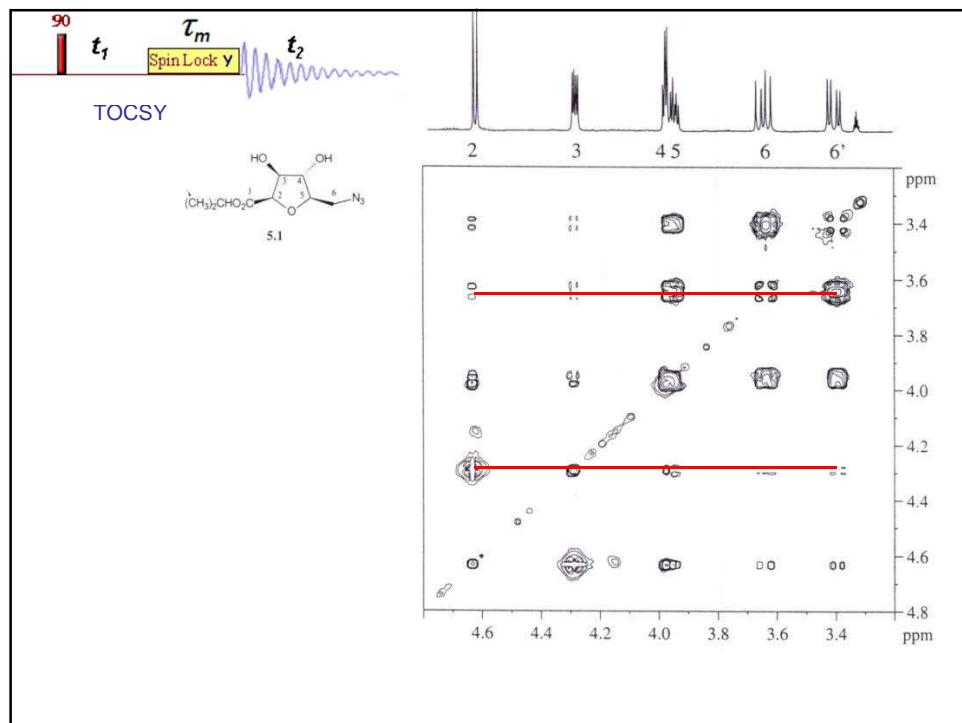
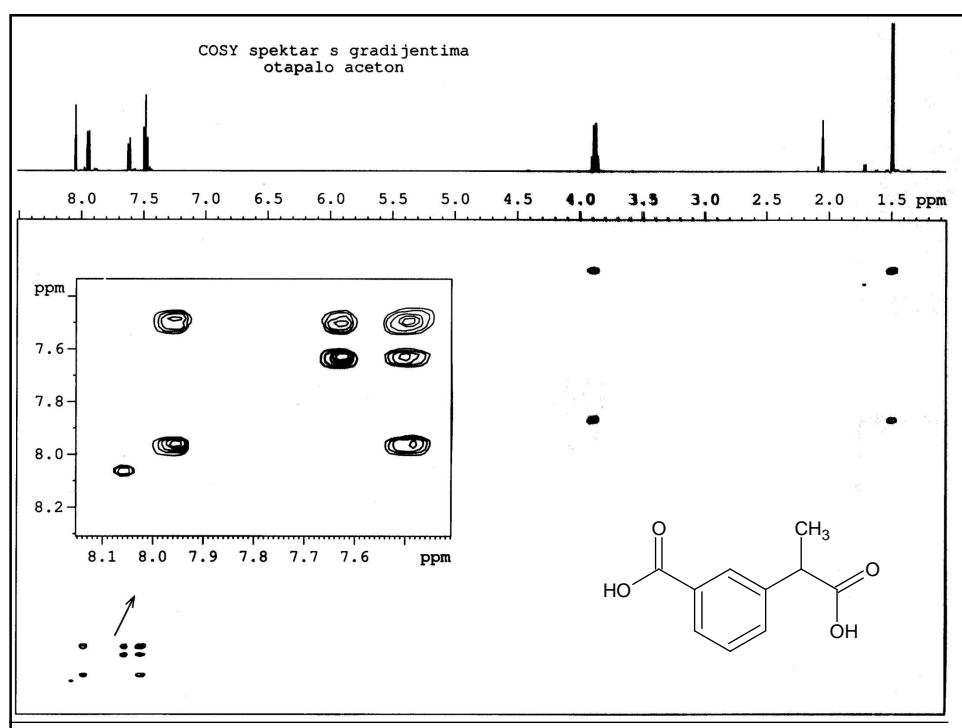
- Faza i intenzitet signalata ugljikovih atoma ovisi o kutu θ
- Slično kao i kod INEPT-a, no s tom razlikom što se kod DEPT-a editiranje postiže različitim kutem θ , a ne vrijednostima Δ
- Stoga je i učinkovitost editiranja veća za DEPT

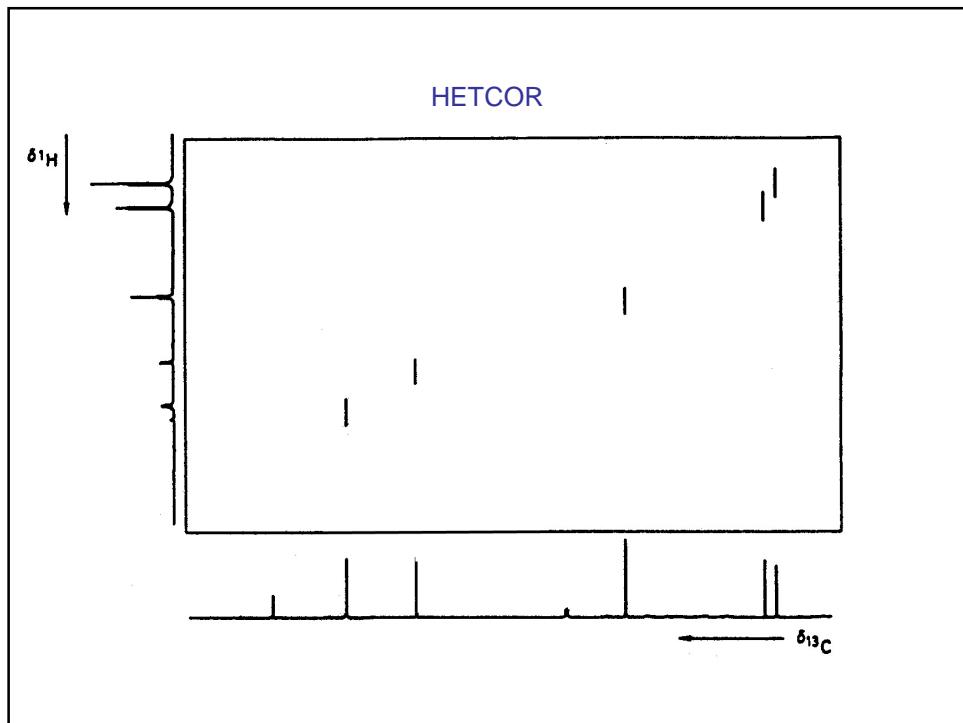
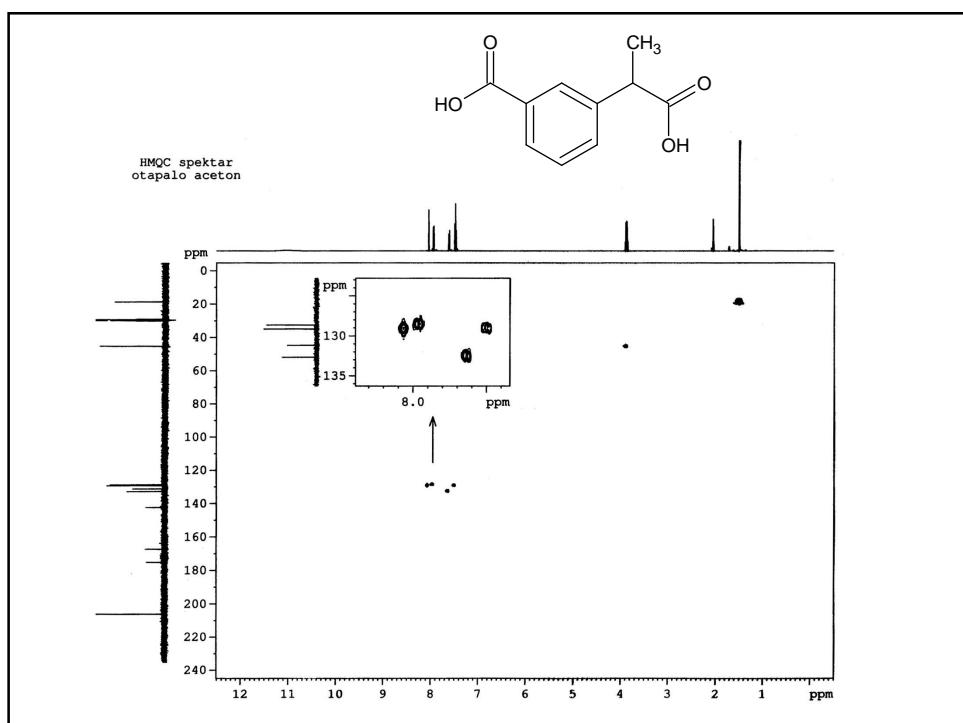
	DEPT-45	DEPT-90	DEPT-135
XH	+	+	+
XH ₂	+	0	-
XH ₃	+	0	+

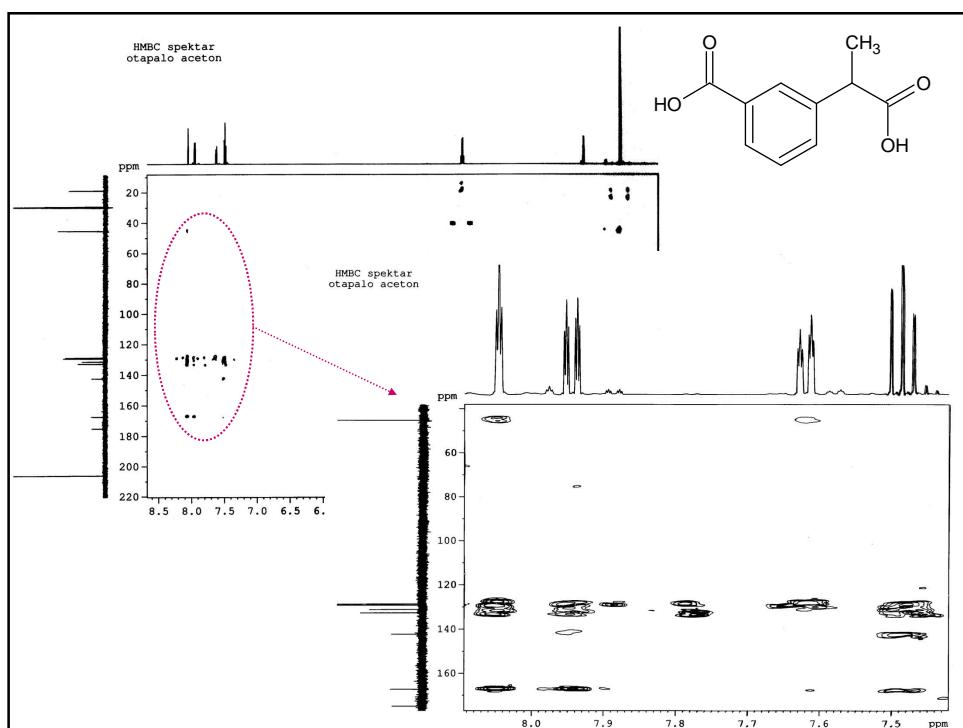


EKSPERIMENT	F2 DIMENZIJA	F1 DIMENZIJA	INFORMACIJE
Homonuklearne korelacije			
COSY TOCSY	δ_H, J_{HH}	δ_H, J_{HH}	 $n_{J_{HH}}$ ($n \leq 3$)
COSY LR TOCSY	δ_H, J_{HH}	δ_H, J_{HH}	 $n_{J_{HH}}$ ($n > 3$)
Relayed COSY	δ_H, J_{HH}	δ_H, J_{HH}	 $n_{J_{HH}}$ ($n > 3$)
NOESY ROESY	δ_H, J_{HH}	δ_H, J_{HH}	 N.O.E.
2D INADEQUATE	δ_C, J_{CC}	δ_C, J_{CC}	 $^1J_{CC}$
Heteronuklearne korelacije			
HETCOR HSQC (inverzni)	δ_C	δ_H, J_{HH}	 $^1J_{CH}$
COLOC HMBC (inverzni)	δ_C	δ_H, J_{HH}	 $n_{J_{CH}}$ ($n > 1$)



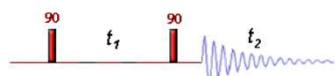






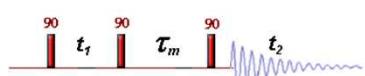
Interakcije kroz prostor- križna relaksacija (NOESY, ROESY)

COSY

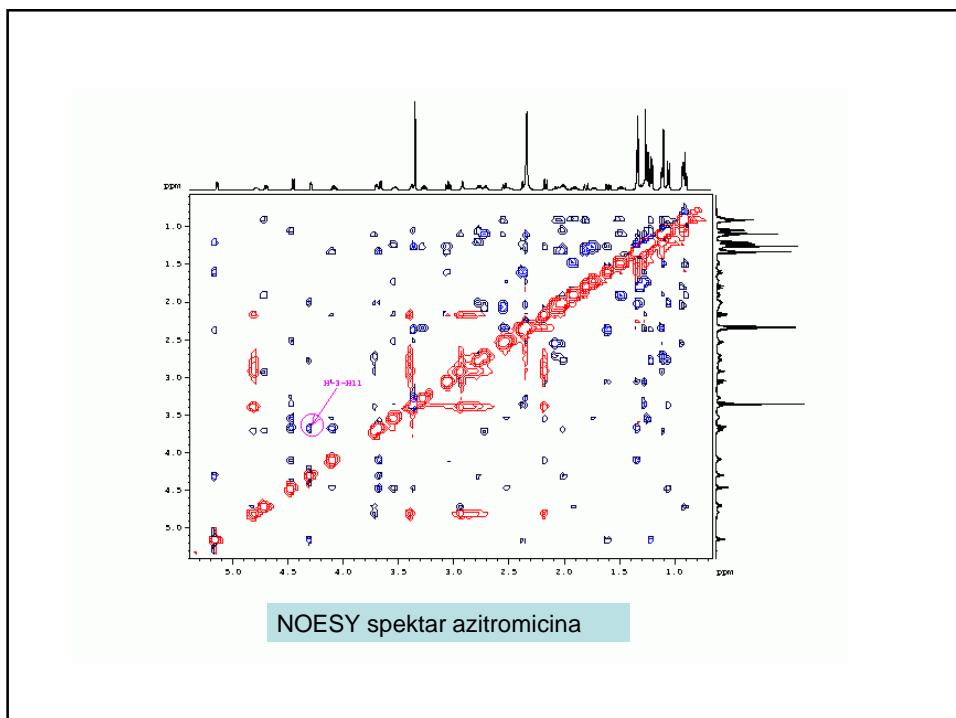


1. Skalarne interakcije

NOESY



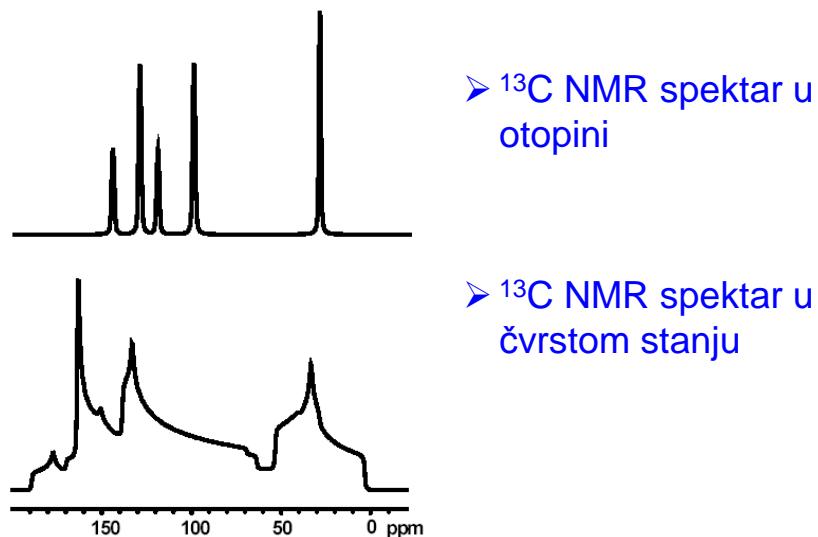
1. Skalarne interakcije, dipolarne interakcije, kemijska izmjena
2. Vrijeme miješanja,
3. Prijenos magnetizacije kroz prostor



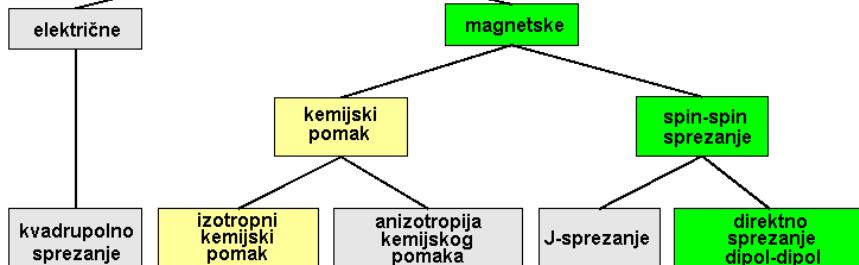
Protokol za određivanje ili potvrdu strukture molekula

postupak	tehnika	informacija
^1H spektar	1D	kemijski pomaci, konstante sprega, integrali
^1H - ^1H korelacija	COSY	povezanost protona preko sprega (asignacija)
^{13}C spektar s editiranjem	DEPT, APT, PENDANT	broj atoma C i multipletnost (C, CH, CH_2 , CH_3) često nepotrebno ako se koriste inverzne 2D tehnike s editiranjem
spektri hetero-jezgri (^{31}P , ^{19}F ..)	1D	kemijski pomaci i homo-/hetero-nuklearne konstante sprega
^1H - ^{13}C korelacija kroz jednu kemijsku vezu	HMQC, HSQC	asignacija atoma C i H, multipletnost iz HSQC spektra s editiranjem
^1H - ^{13}C korelacija kroz više kemijskih veza	HMBC	povezanost atoma C i H kroz više veza, povezanost preko hetero-atoma, identifikacija strukturnih fragmenata
korelacija kroz prostor preko NOE efekta	1D ili 2D NOESY, ROESY	stereokemijska struktura: konfiguracija i konformacija

NMR spektroskopija u čvrstom stanju



interne interakcije spinova jezgara



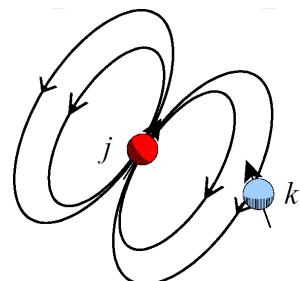
➤ Interakcije ovisne o položaju u prostoru

$$\delta = \delta_{iso} + \frac{1}{2} \delta_{CSA} (3 \cos^2 \theta - 1 + \eta \sin^2 \theta \cos 2\phi)$$

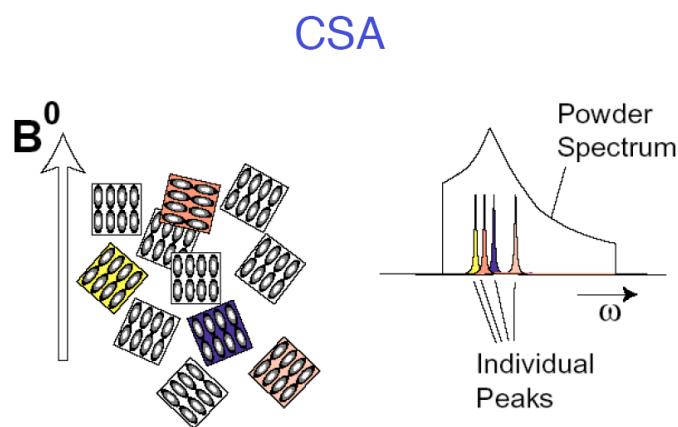
➤ Dipolarne interakcije

^1H , $^1\text{H} \approx 40 \text{ kHz} - 120 \text{ kHz}$

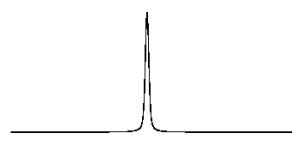
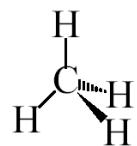
^{13}C , $^1\text{H} \approx 3 \text{ kHz} - 30 \text{ kHz}$



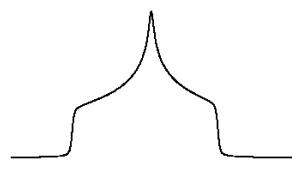
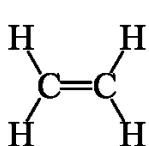
Utjecaj anizotropije kemijskog pomaka (CSA) na NMR spektre u čvrstom stanju



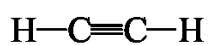
➤ Anizotropija kemijskog pomaka



➤ Sferna simetrija



➤ Zrcalna simetrija

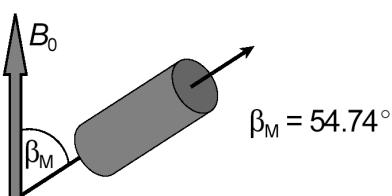


➤ Osovinska simetrija

Čarobni trik...

$$(3\cos^2\theta - 1) = 0 \quad \text{kada je } \theta = 54.74^\circ$$

➤ beskonačno dugačak cilindar



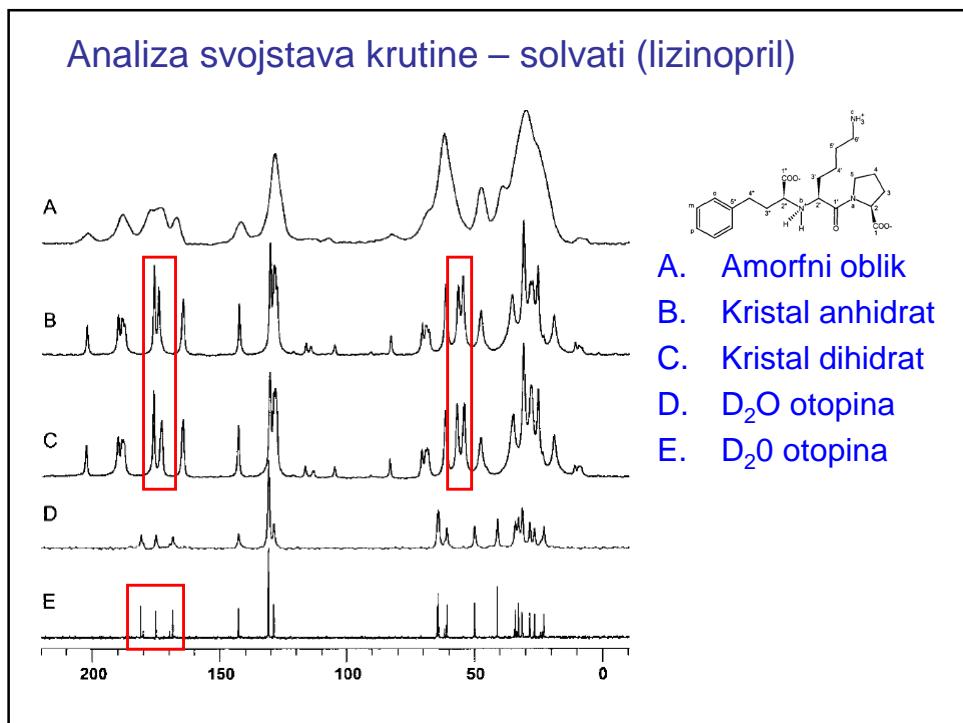
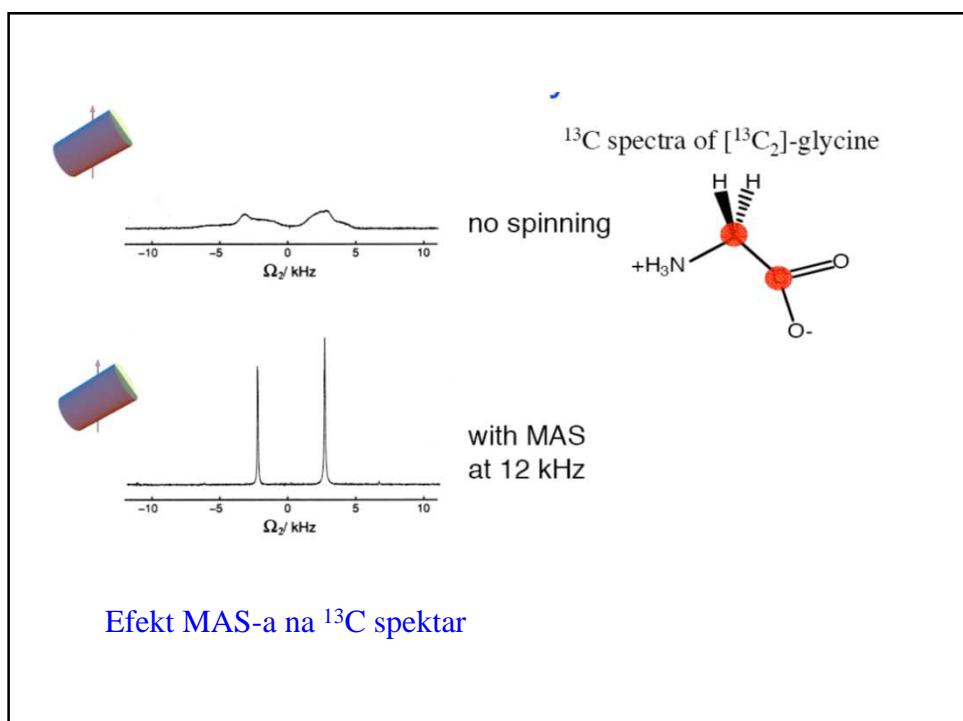
➤ E. R. Andrew i I. J. Lowe

Magic Angle Spinning

MAS

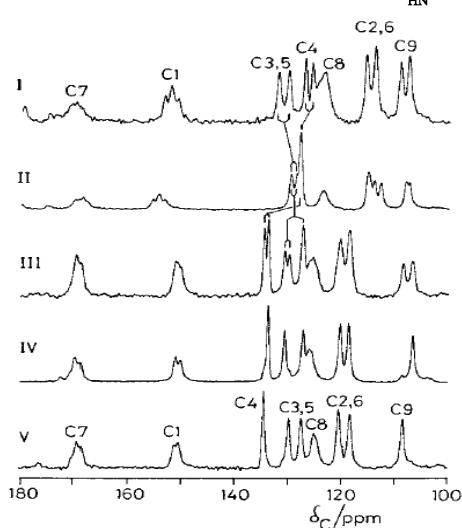
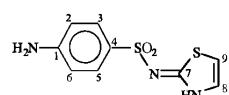
CP-MAS

- dipolarne interakcije
- $^1\text{H}, ^1\text{H} \approx 40 \text{ kHz} - 120 \text{ kHz}$
- $^{13}\text{C}, ^1\text{H} \approx 3 \text{ kHz} - 30 \text{ kHz}$

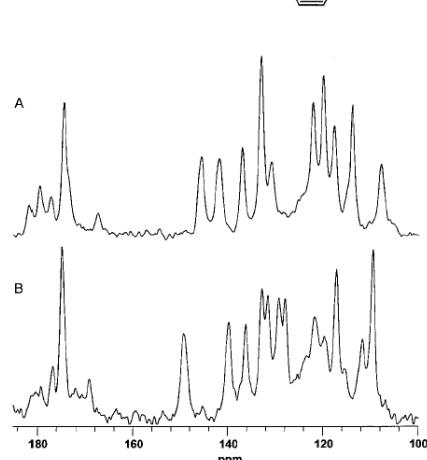
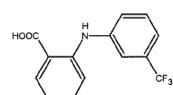


Analiza svojstava krutine – polimorfi

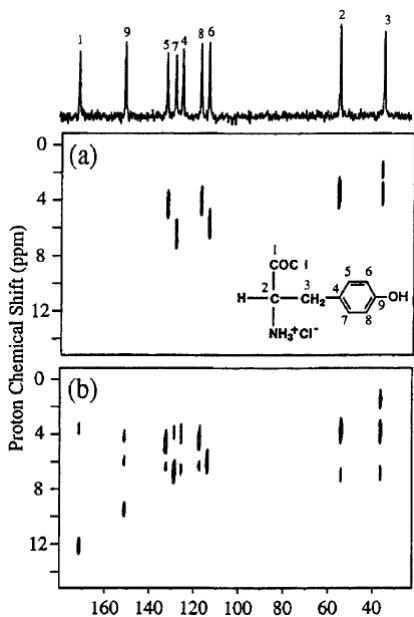
sulfatiazol



flufenamska kiselina



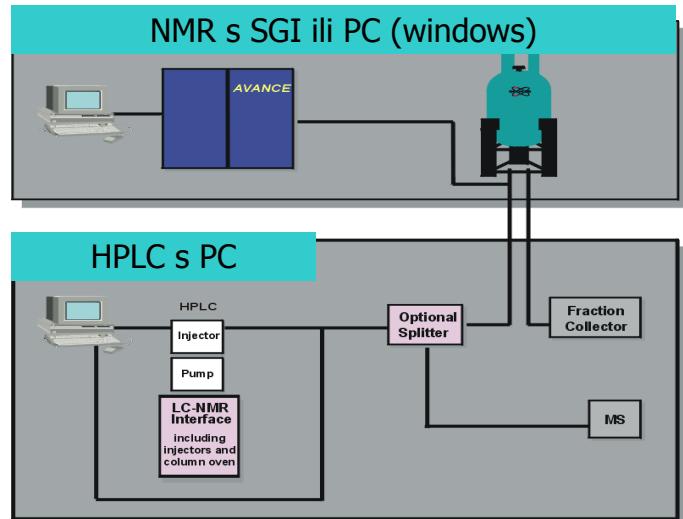
Strukturna analiza – L-tirozin hidroklorid



a) MAS-J-HMQC

b) dipolarni HETCOR

Sprega tekućinske kromatografije i NMR-a



Načini izvedbe LC-NMR-a

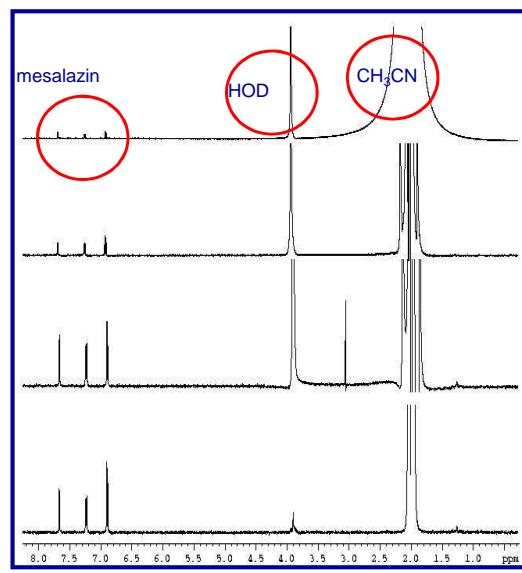
- Kontinuirani protok (on-flow)
 - Izvedivo za ^1H i ^{19}F (osjetljivost)
 - Za uzorke nepoznatih kromatografskih svojstava
 - Labilni spojevi (razgradnja, izomerizacija)
- Zaustavljeni protok u vremenskim intervalima (time-slice)
 - Loše odvojive komponente u smjesi
 - Slaba ili nikakva UV kromofora
 - Slabo definirana retencijska vremena
- Zaustavljeni protok (stopped-flow)
 - Poznata retencijska vremena komponenata u smjesi
 - Poznate UV kromofore
- Skladištenje u kapilarnim petljama (loop transfer)
 - LC pikovi se skladište u kapilarne petlje
- LC-SPE-NMR
 - LC pikovi se skladište u SPE (solid phase extraction) patronama (cartridge)

Tehnike supresije signala otapala

- Prezasićenje (NOESY tip, “shaped” pulsevi)
 - Jedno-, dvo-, i višestruka supresija signala
- WET (water suppression enhanced trough T_1 effects)
- WATERGATE (water suppression by gradient-tailored excitation)
- Excitation sculpting (dvostruka pfg spinska-jeka)

Protonski spektri mesalazina u 71% CH_3CN i 29% D_2O

a) običan spektar



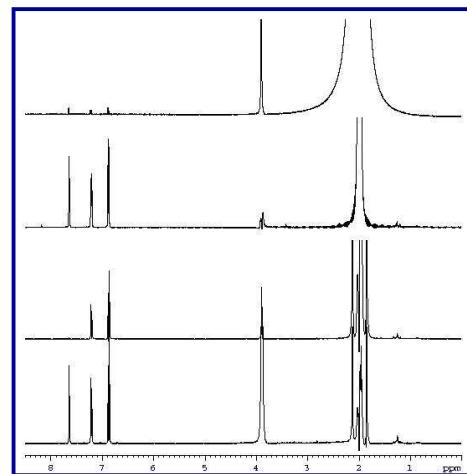
b) prezasićenje CH_3CN

c) dvostruko prezasićenje CH_3CN i HDO

d) Dvostruko prezasićenje CH_3CN i HDO uz ^{13}C rasprezanje

Protonski spektri mesalazina u 71% CH_3CN i 29% D_2O

a) Običan spektar



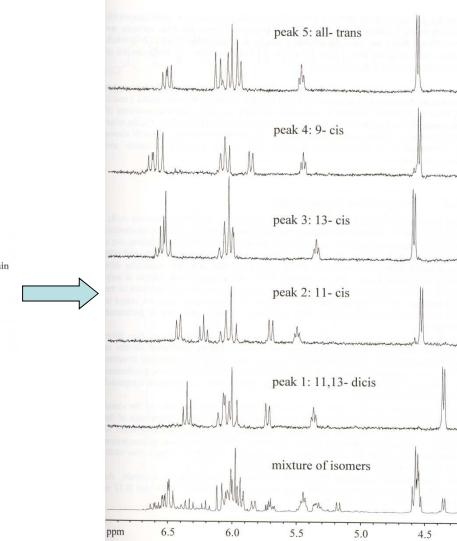
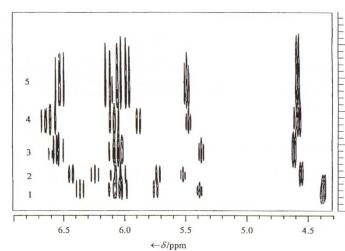
b) WET višestruka supresija
otapala

c) 3-9-19 WATERGATE (W3)

d) 3-9-19 WATERGATE (W3)

Tipični LC-NMR spektri kontinuiranoga protoka

^1H LC-NMR spektri kontinuiranog
protoka



Odvajanje izomera vitamina A