

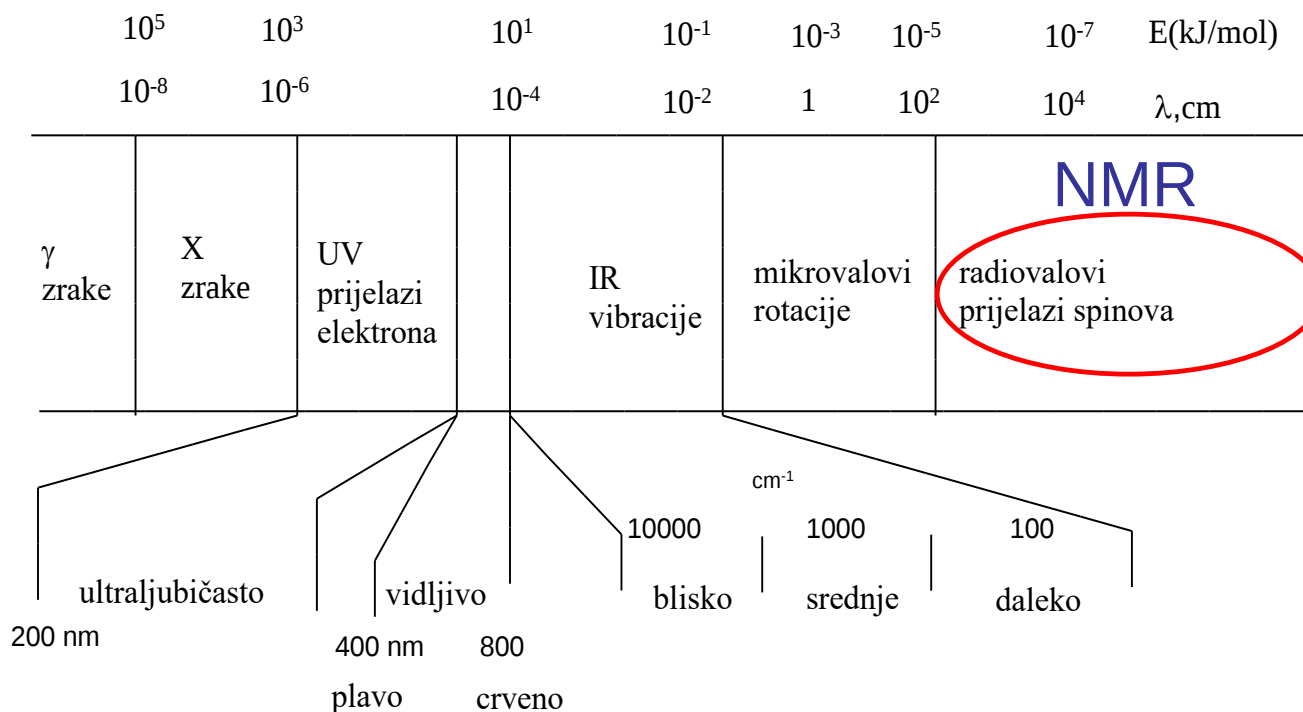
Nuklearna Magnetna Rezonancija NMR

POVIJESNI RAZVOJ NMR-a

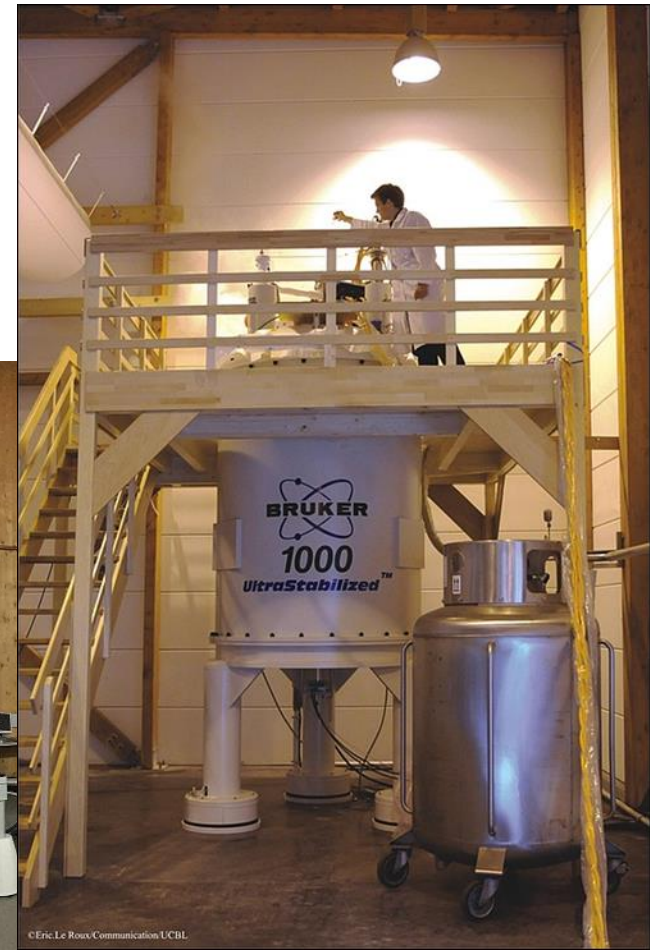
- 1924. W. Pauli - teorijski temelji NMR
- 1939. Rabi i sur. - dokaz o postojanju nuklearnog spina (molekularni snop LiCl)
- 1944. Nobelova nagrada iz fizike Rabiju
- 1946. Bloch (Stanford) i Purcell (Harvard)- prvi uspješni eksperimenti efekta NMR
- 1952. Nobelova nagrada iz fizike za NMR Blochu i Purcellu
- 1953. Prvi komercijalni NMR spektrometar (Varian 30 MHz)
- 1960. Spektrometri od 100 MHz
- 1970. Pulsni spektrometri s Fourierovom transformacijom
- 1980. Spektrometri od 400 MHz
- 1970.-1980. Dvodimenzijske metode i tehnike (2D NMR)

- 1970. Oslikavanje magnetnom rezonancijom (magnetic resonance imaging, MRI)
- 1991. Nobelova nagrada iz kemije za NMR, R. R. Ernst
- 1992. Magnet od 17.61 T (750) MHz
- 1995. Magnet od 21.14 T (900 MHz)
- 1999. Prvi komercijalni 900 MHz spektrometar
- 2002. Nobelova nagrada iz kemije za NMR, K. Wütrich
- 2003. Nobelova nagrada iz medicine za MRI, P. Lauterbur i P. Mansfield (fizičari)
- 2005. Magnet od 22.31 T (950 MHz)
- 2009. Magnet od 1000 MHz (1 GHz)
- 2015. Magnet od 1200 MHz (1.2 GHz)
- 2023. Magnet od 1300 MHz (1.3 GHz)

Spektar elektromagnetnoga zračenja



NMR spektrometar 1.2 GHz 24,6 T



ICE NMR



Integrirani
NMR sustav-
INCA



BEST NMR



900 MHz



950 MHz



950 MHz





„benchtop” 80 MHz spektrometar NMR s dodatkom za automatsku izmjenu uzorka

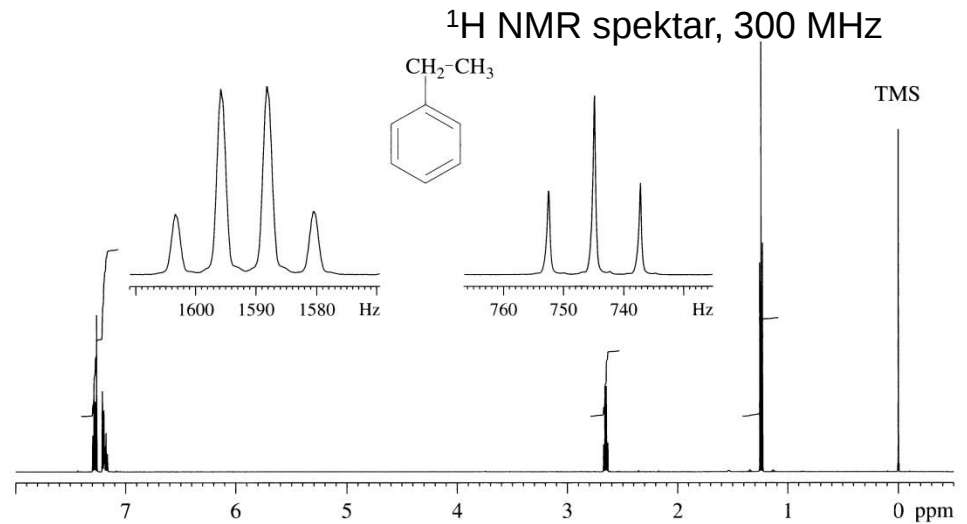
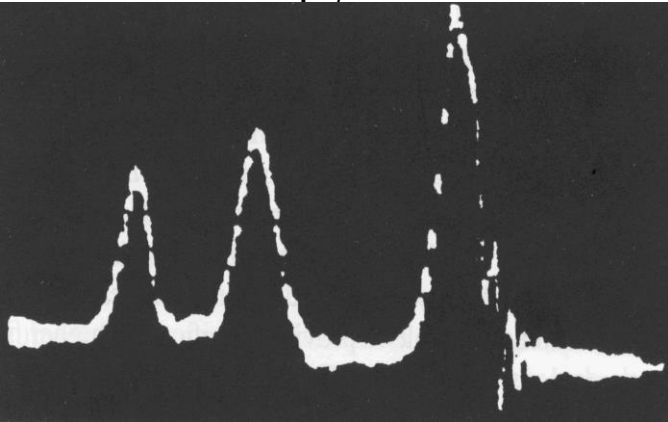


LC-SPE-krio/NMR sustav

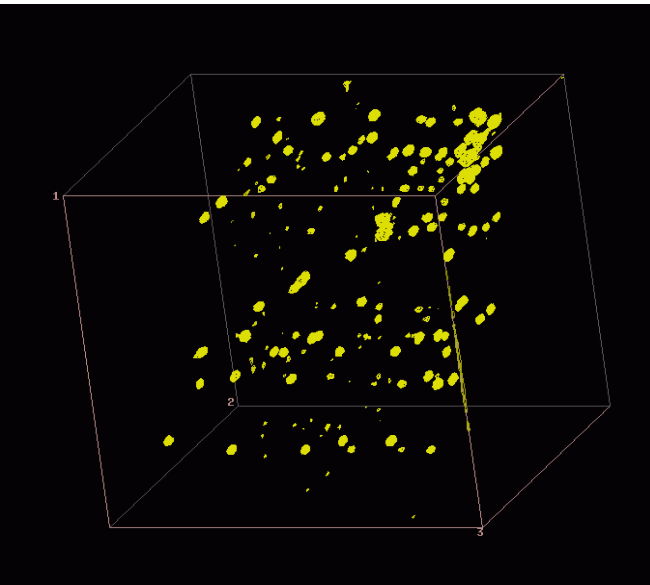


NMR visoke rezolucije

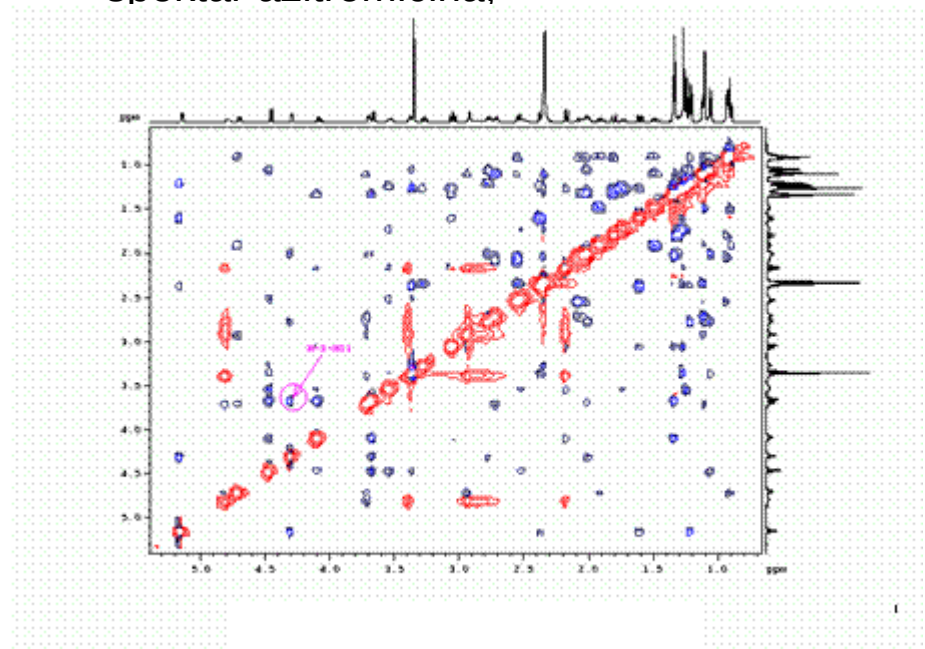
Prvi publicirani NMR spektar visoke rezolucije, 30 MHz



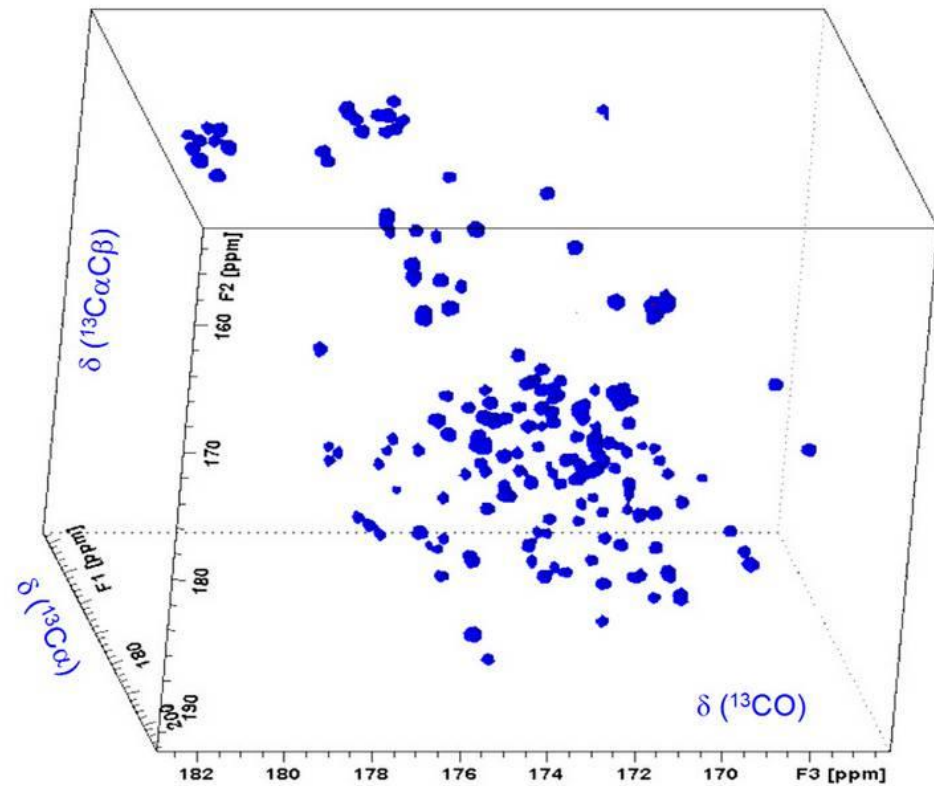
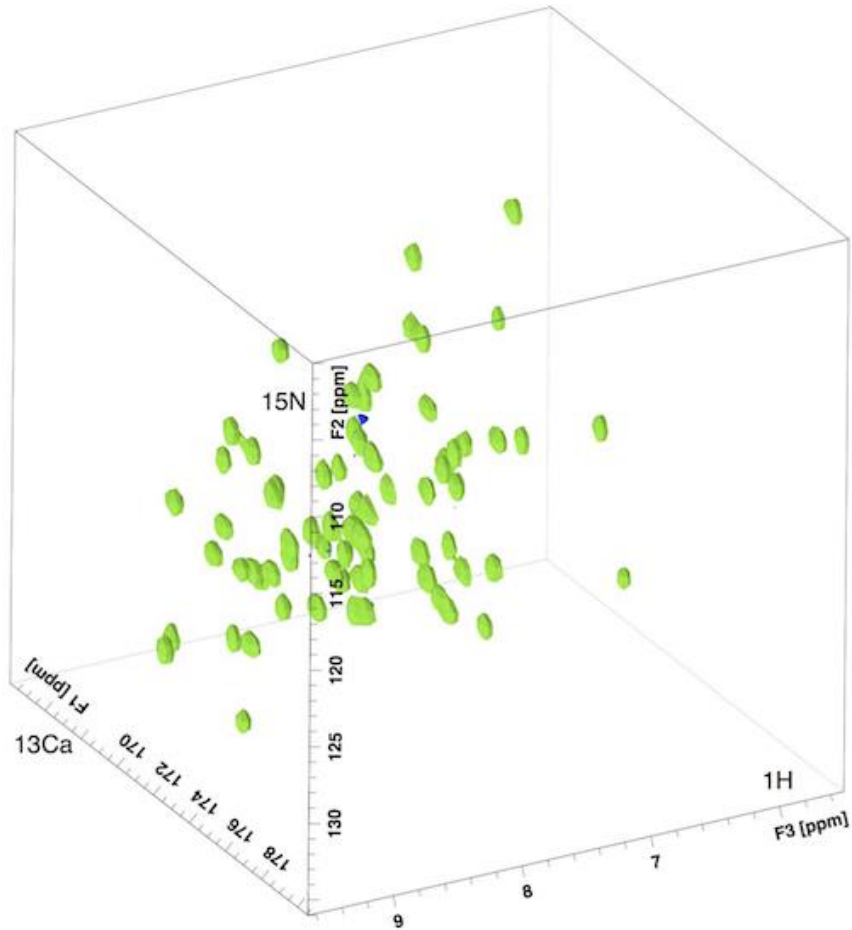
3D HMQC-TOCSY NMR spektar azitromicina 500 MHz



2D TPPI-NOESY
spektar azitromicina,



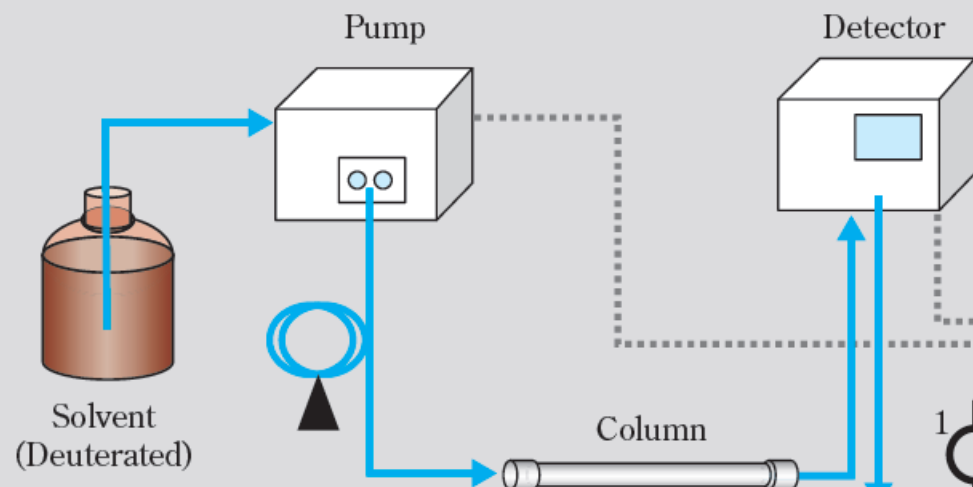
3D NMR spektri proteina



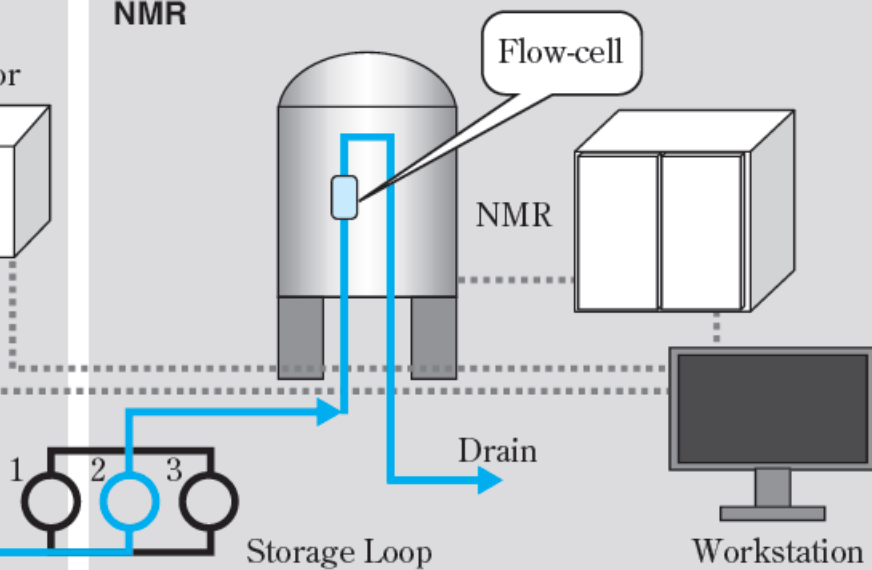
Sprega tekućinske kromatografije i NMR-a

LC-NMR

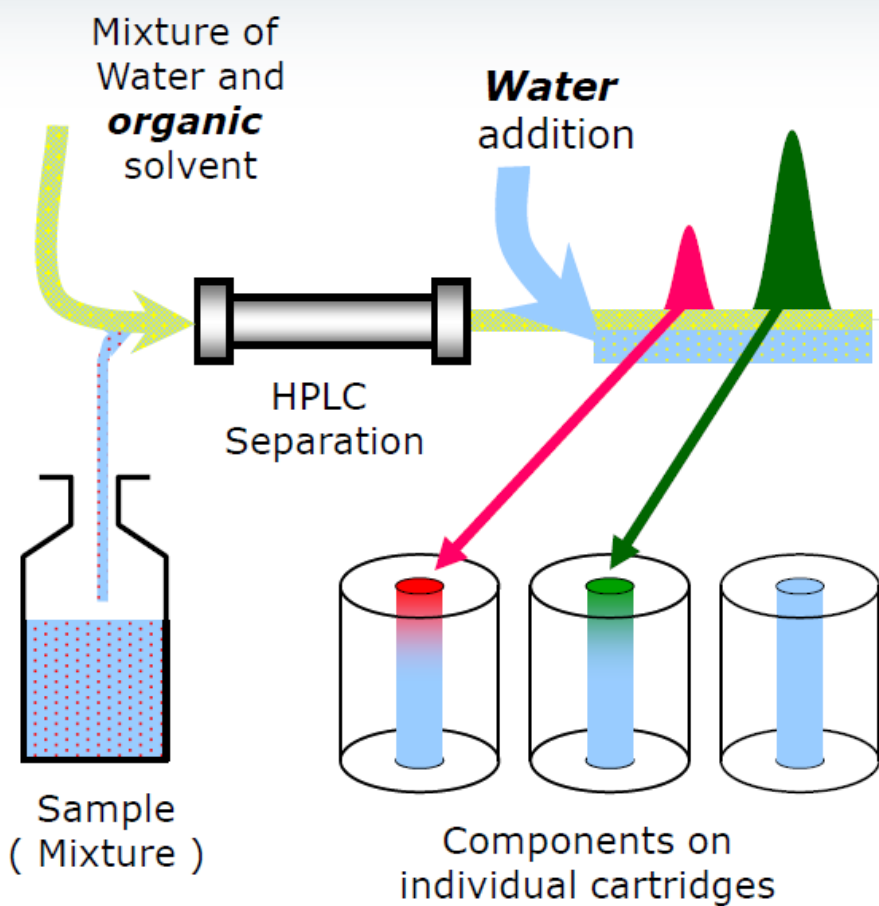
HPLC



NMR

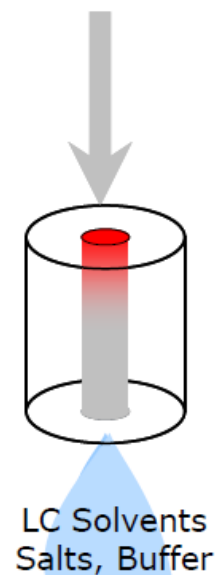


Ekstrakcija na čvrstoj fazi

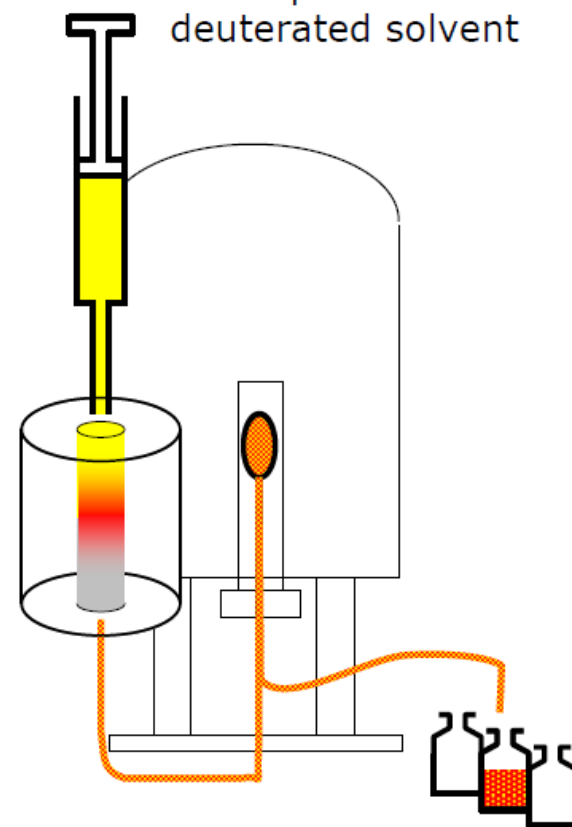


1. Wash
 H_2O

2. Dry
with N_2



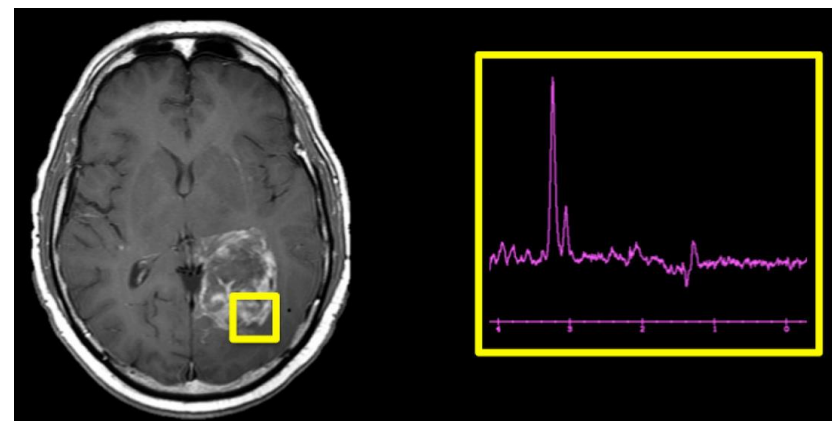
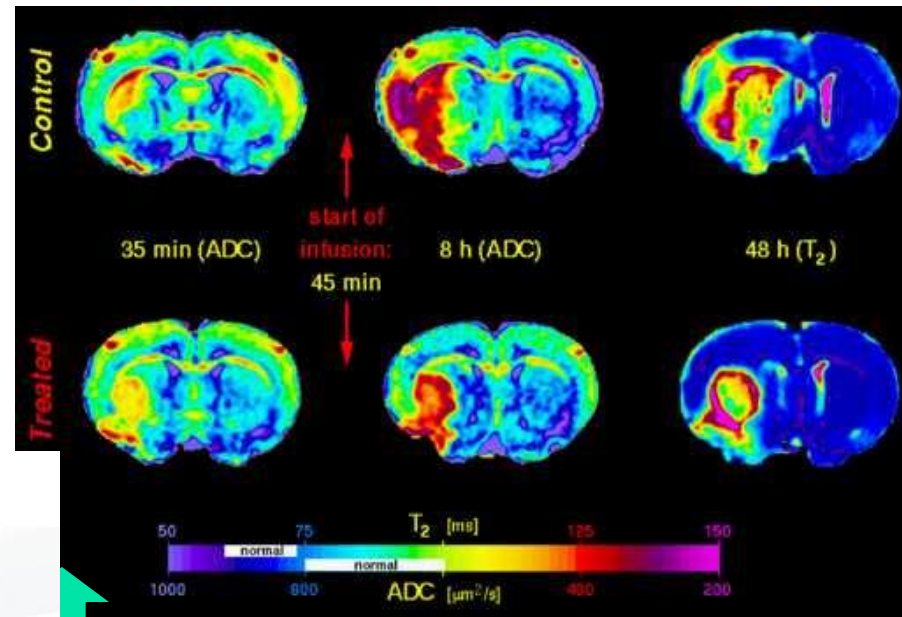
Transfer directly into
flow probe with
deuterated solvent



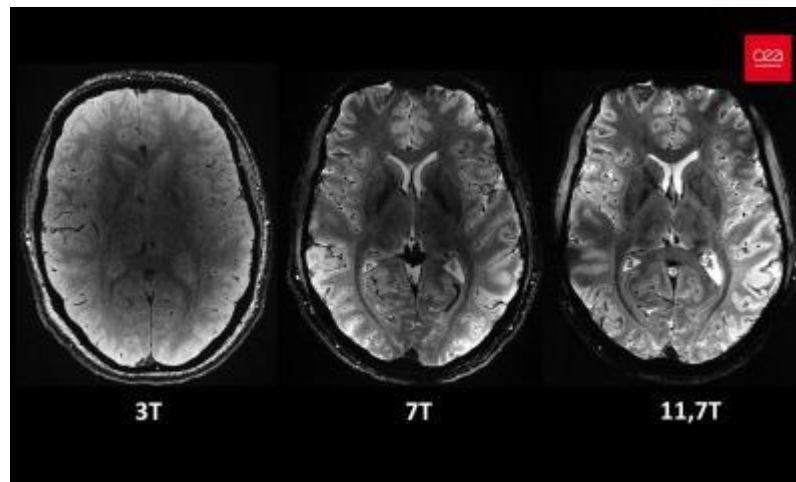
LC-SPE-NMR-MS sustav



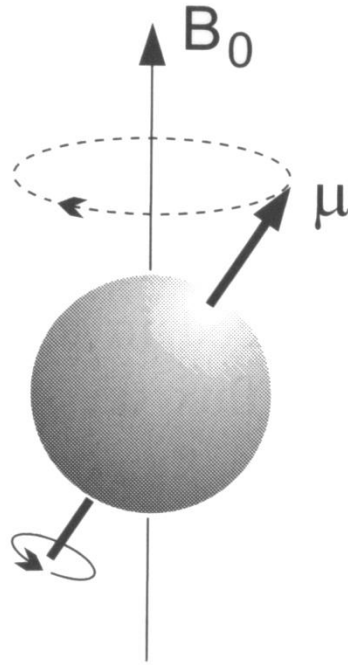
MRI/MRS sustav



Najjači uređaj MRI od 11,7 T



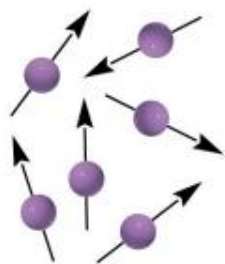
Spin jezgre i rezonancija



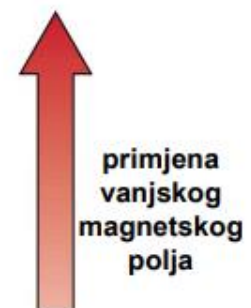
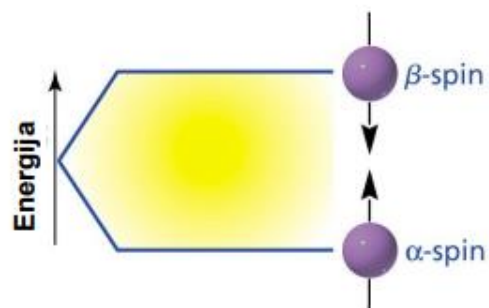
$$\mu = \gamma P$$

$$P = I \hbar / 2\pi$$

$$I = (0, 1/2, 1, 3/2, \dots)$$

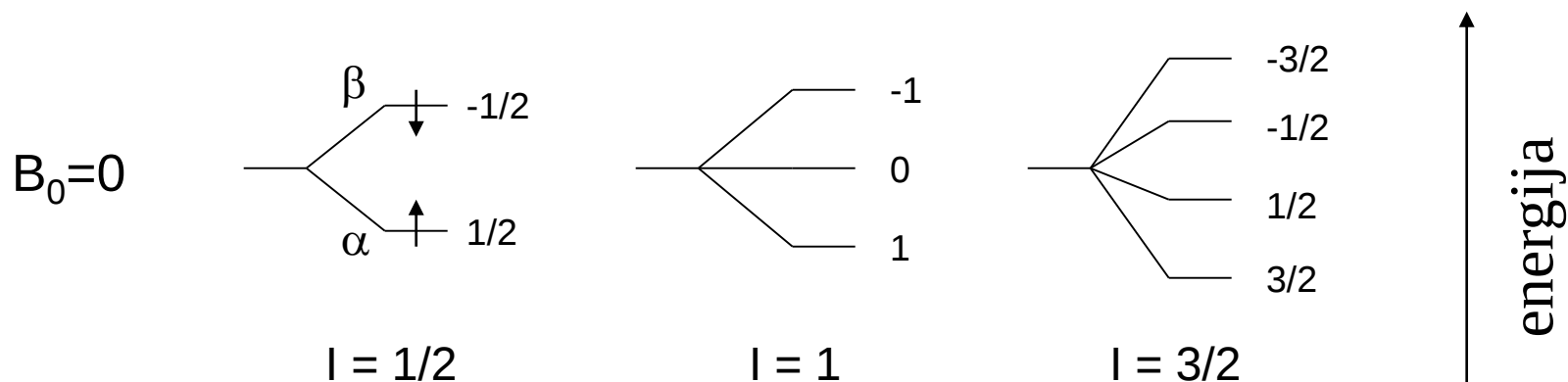


bez vanjskog
magnetskog
polja



primjena
vanjskog
magnetskog
polja

Jezgra s kvantnim brojem I može imati $2I_n + 1$ orijentaciju



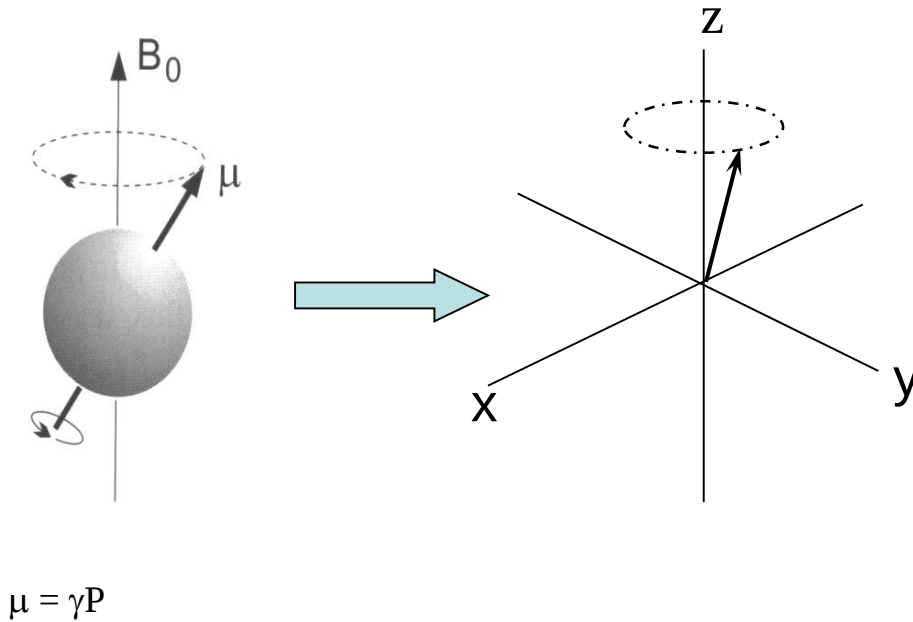
Svojstva nekih jezgri sa spinom $1/2$

izotop	Prorodna zastupljenost (%)	NMR frekvencija (MHz) $B_0=11.7$ T	Relativna osjetljivost
^1H	99.98	400.0	1.0
^3H	0	426.7	1.2^a
^{13}C	1.11	100.6	1.76×10^{-4}
^{15}N	0.37	40.5	3.85×10^{-6}
^{19}F	100	376.3	0.83
^{29}Si	4.7	79.5	3.69×10^{-4}
^{31}P	100	161.9	6.63×10^{-2}

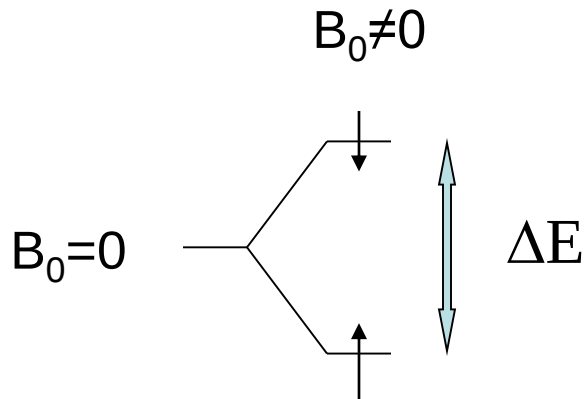
^aako ima 100% ^3H

Dogovorom je prihvaćeno da je smjer primjenjenog magnetnog polja smjer osi z u Cartesijevom koordinatnom sustavu

Frekvencija precesije
ili Larmorova frekvencija



Rezonancija



$$\mu = \gamma P$$

$$P = I\hbar/2\pi$$

$$E = -\mu_0 B_0 = -\gamma\hbar I B_0/2\pi$$

$$\Delta E = \gamma\hbar B_0/2\pi$$

$$\Delta E = h\nu$$

$$\nu = \gamma B_0/2\pi$$

Osnovni uvjet rezonancije

$$\nu_1 = \nu_0$$

Boltzmanova raspodijela

$$N_{\alpha} / N_{\beta} = e^{\Delta E/kT}$$

N- broj jezgri

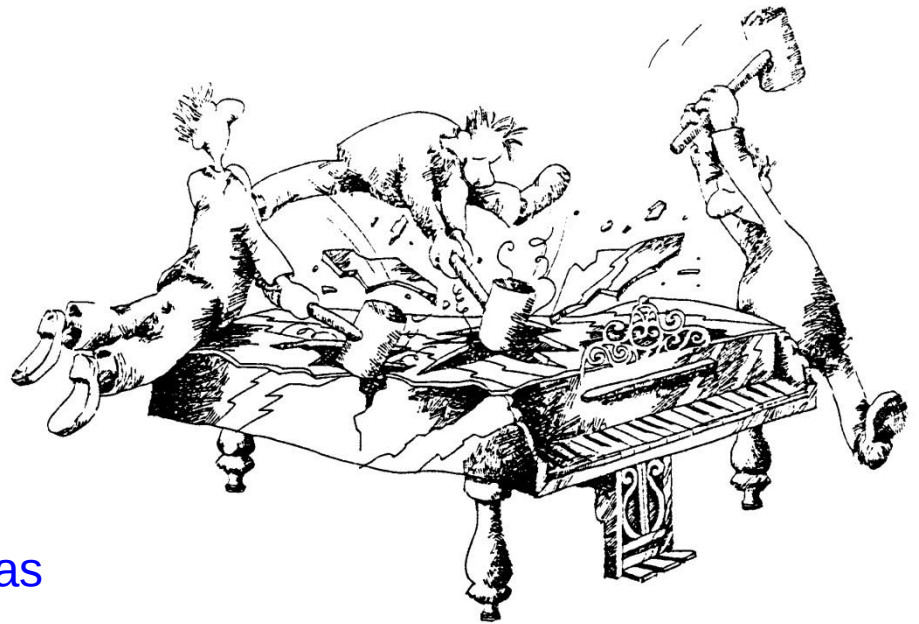
k- Boltzmanova konstanta ($1.38066 \times 10^{-23} \text{ JK}^{-1}$)

T- temperatura

Samo 1 jezgra na milijun više u osnovnom energijskom stanju !!



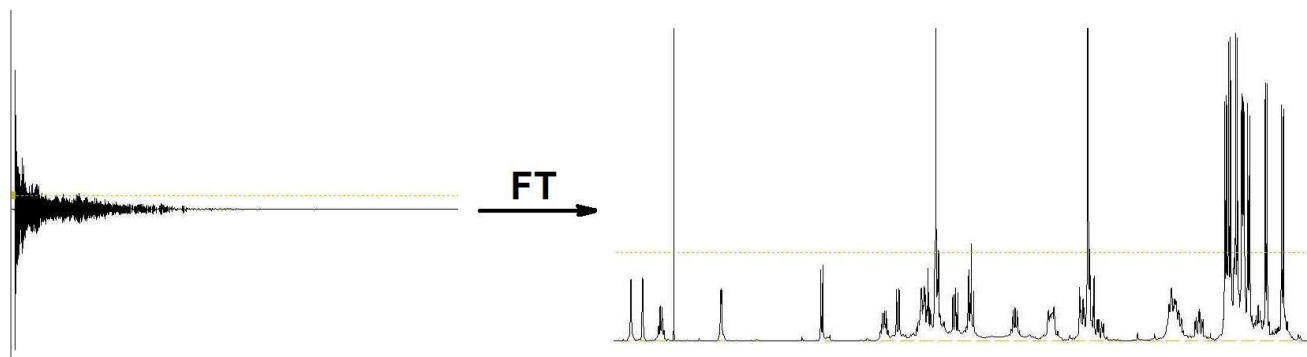
nekad



danas

vremenska domena

frekvencijska domena



$$F(\omega) = \int_{-\infty}^{+\infty} f(t) e^{i\omega t} dt$$

$$e^{i\omega t} = \cos \omega t + i \sin \omega t$$

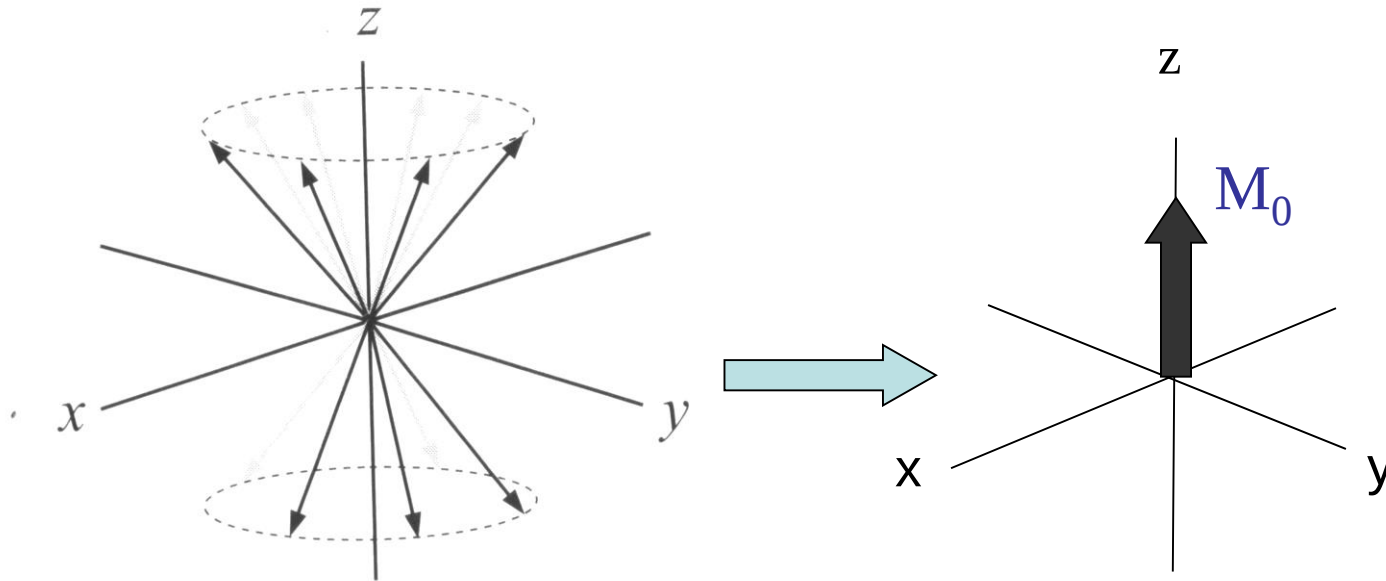
realno

imaginarno

Apsorpcijski
signal

Disperzijski
signal

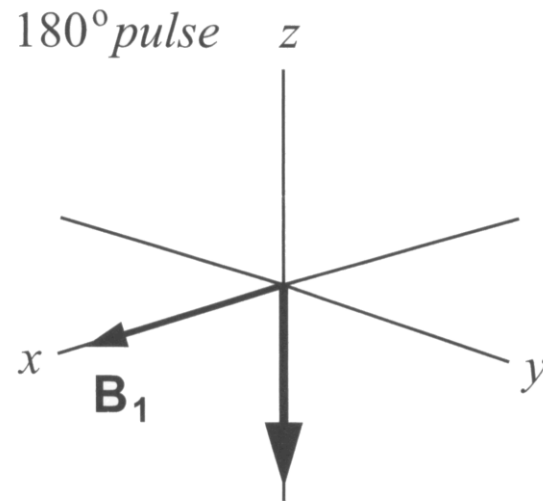
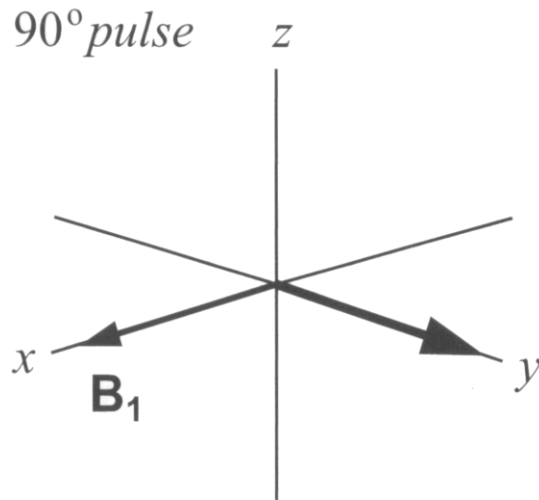
Vektorski model

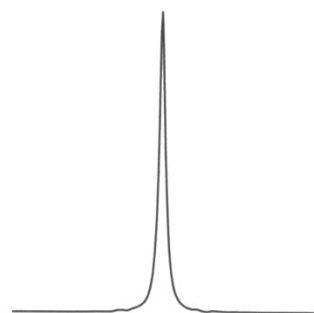
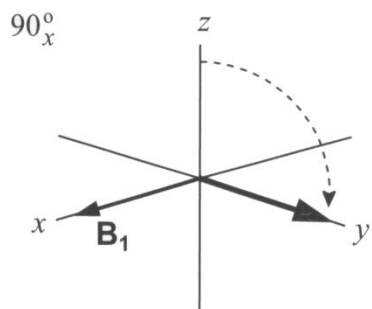


S obzirom da ima više istovrsnih spinova u stanju α vektor ukupne magnetizacije ima smjer $+z$

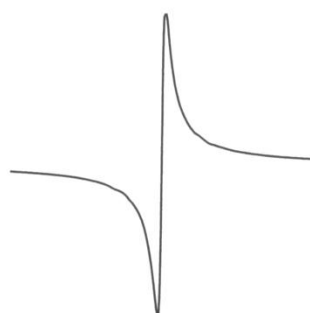
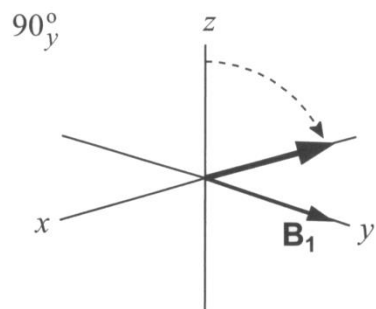
Radiofrekvencijski puls

Pulsni kut $\Phi = 360\gamma B_1 t_p / 2\pi$

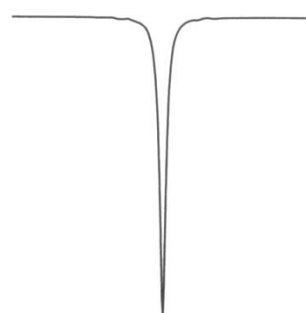
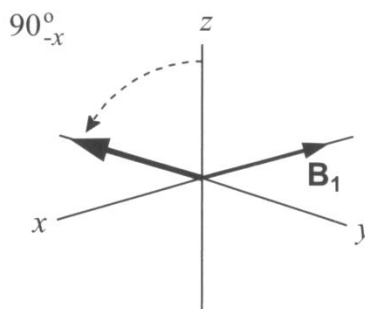




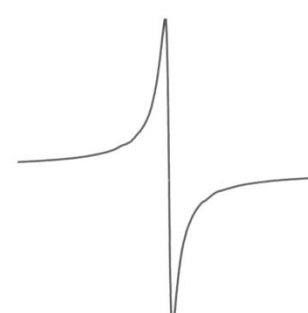
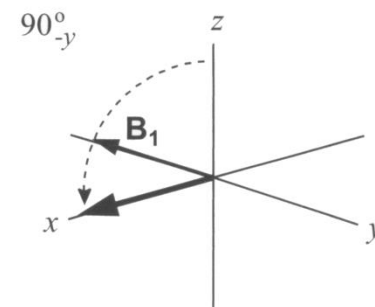
Pozitivna
apsorpcija



Pozitivna
disperzija

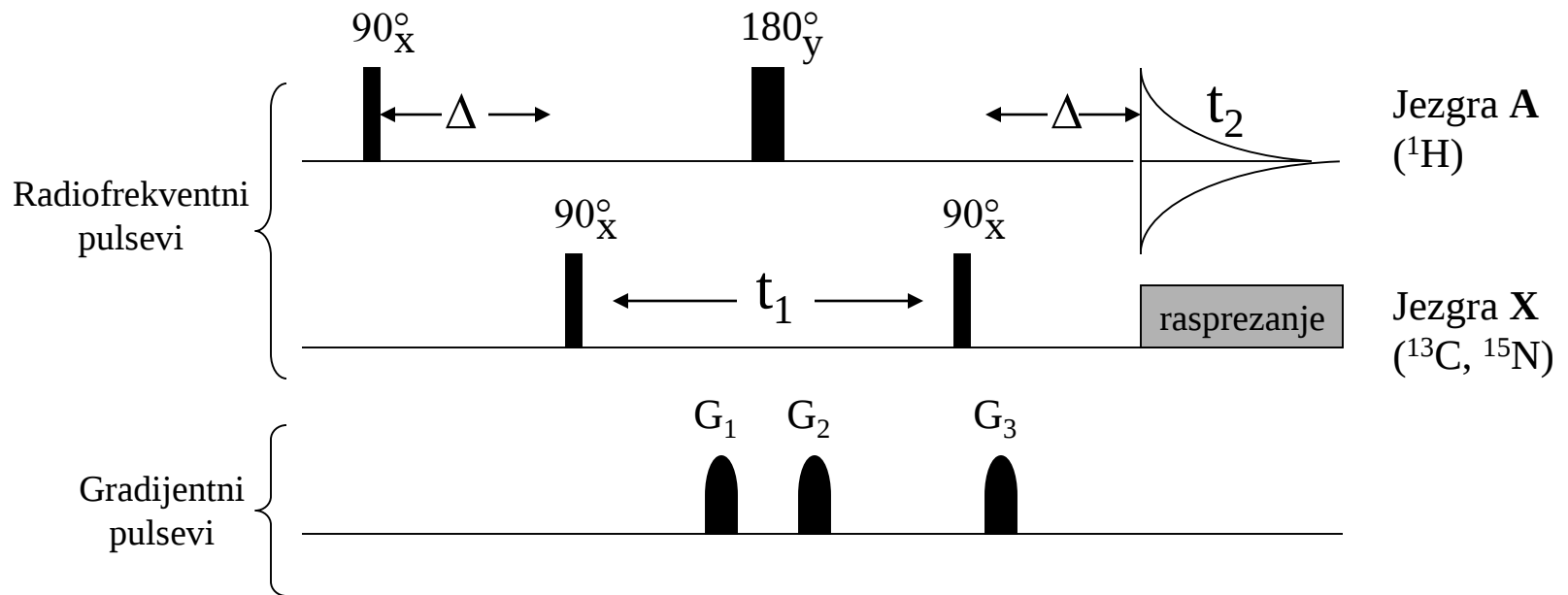


negativna
apsorpcija



Negativna
disperzija

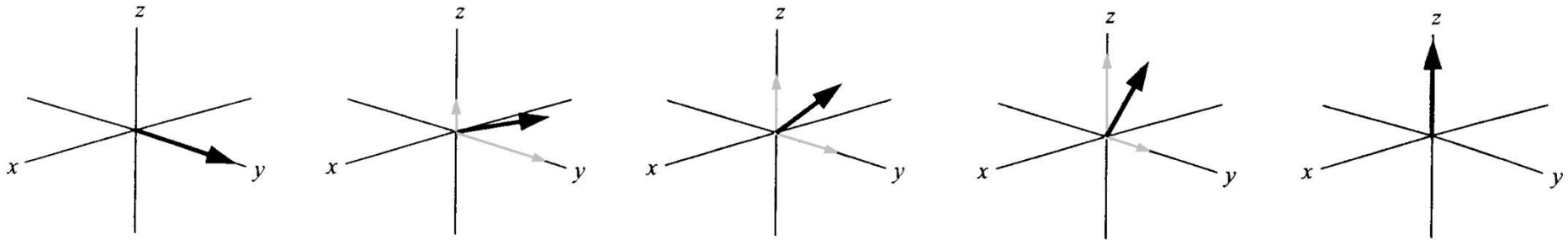
Pulsni slijed



NMR parametri

a) Vremena opuštanja ili relaksacije

- vrijeme opuštanja spin-rešetka (longitudinalno), T_1
 - $dM_z/dt = (M_0 - M_z)/T_1$ Blochova jednačina
 - Intenzitet NMR signala

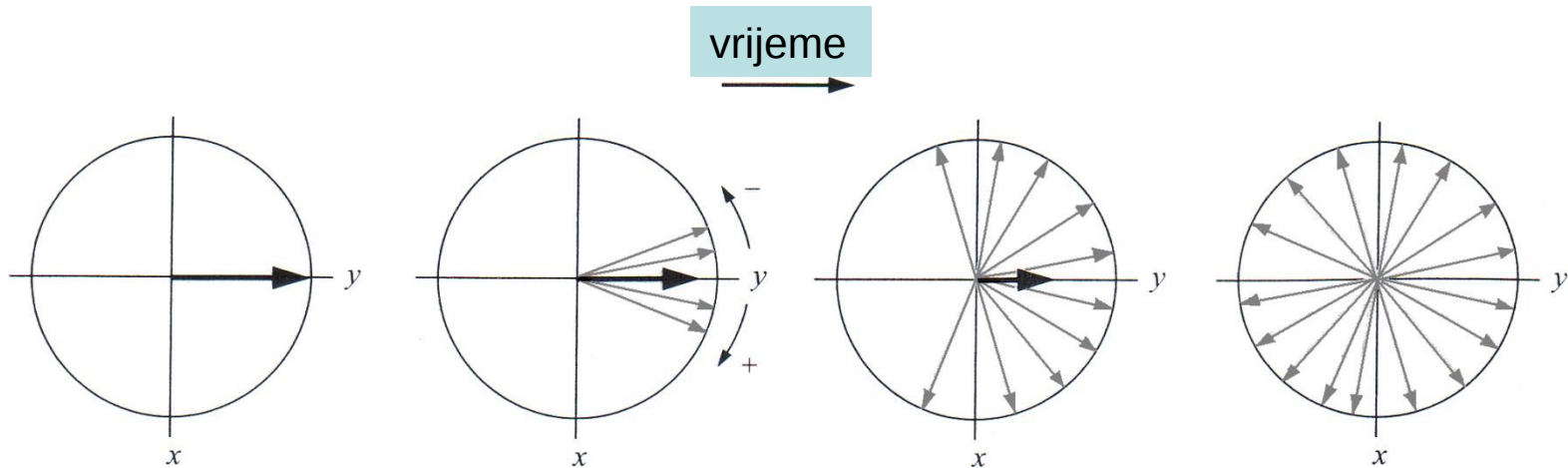


➤) vrijeme opuštanja spin-spin (transverzalno), T_2

➤ $dM_x/dt = (M_x)/T_2$

➤ $dM_y/dt = (M_y)/T_2$

➤ Širina NMR linija $\Delta\nu_{1/2} = 1/T_2$



Mehanizmi opuštanja spin-rešetka

➤ $1/T_1^{\text{uk}} = 1/T_1^{\text{DD}} + 1/T_1^{\text{CSA}} + 1/T_1^{\text{SR}} + 1/T_1^{\text{SC}}$

DD- dipol-dipol (najučinkovitiji)

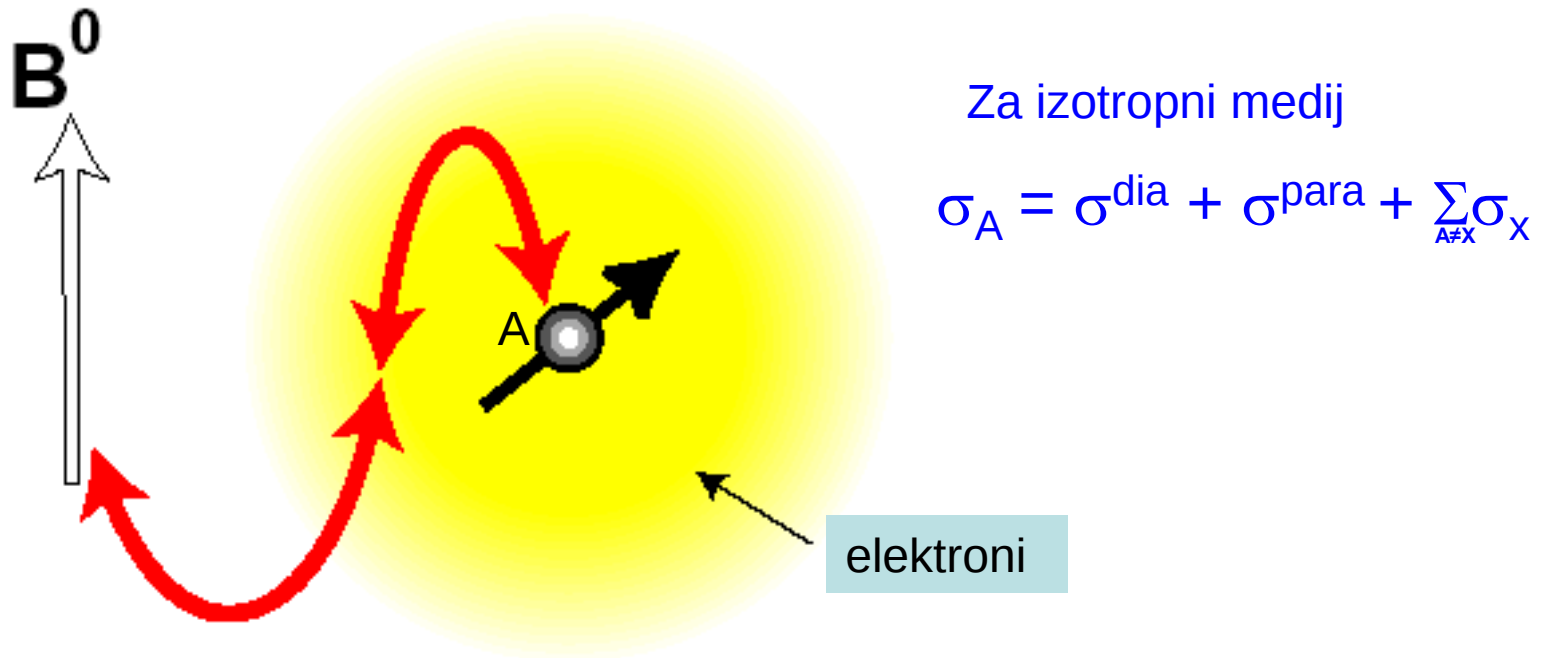
CSA- anizotropija kemijskog pomaka

SR- spinska rotacija (rotori npr. CH₃)

SC- skalarna sprega (bliska Larmorova frekvencija ¹³C-
³⁷Br)

b) Kemijski pomak, nuklearno zasjenjenje

Levitt



Elektroni koji okružuju jezgru A stvaraju magnetno polje (zasjenjenje) koje utječe na ukupno magnetno polje koje osjećaju jezgre

- $B_{\text{ef}} = B_0 - B_{\text{ind}}$

$$B_{\text{ind}} = \sigma_{\text{ef}} B_0$$

- $B_{\text{ef}} = B_0 - (1 - \sigma_{\text{ef}}) B_0$

$$\nu = \gamma B_0 / 2\pi$$

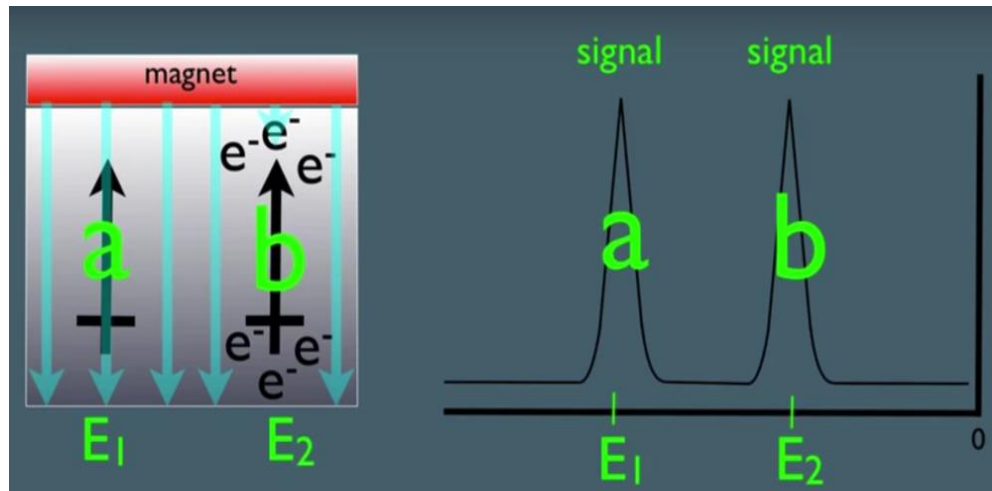
Uvjet rezonancije

$$\nu = \gamma (1 - \sigma_{\text{ef}}) B_0 / 2\pi$$

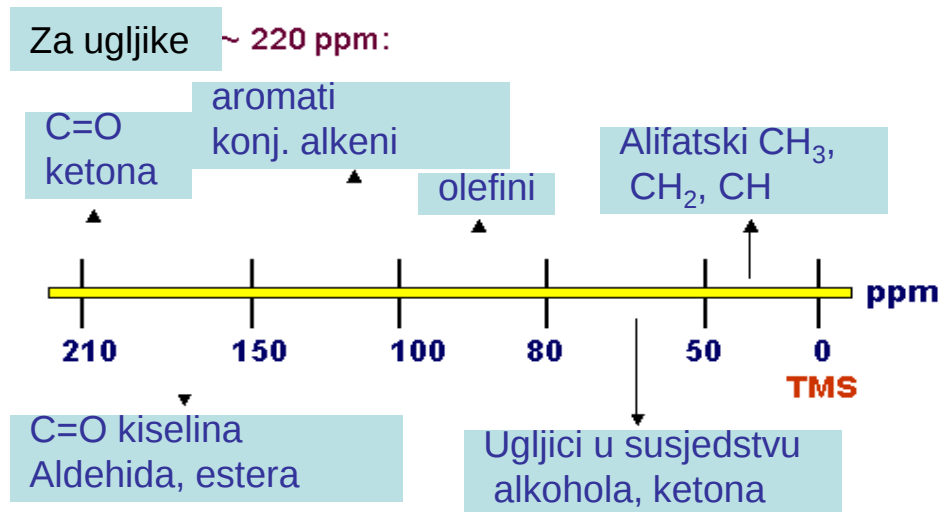
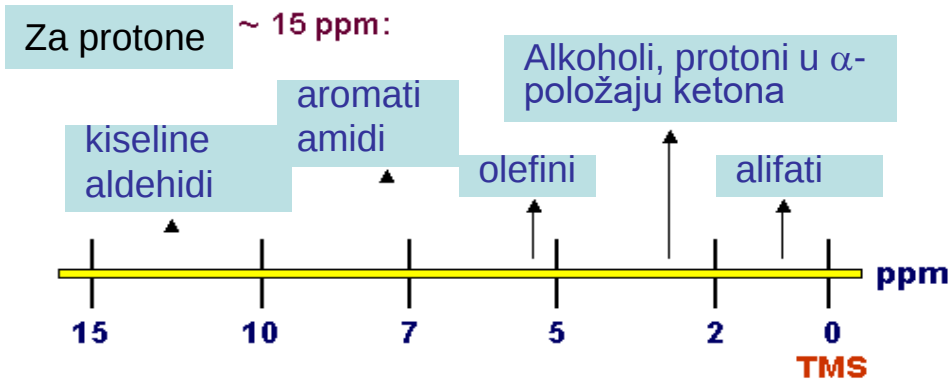
Referentna δ Skala

$$\begin{aligned} \delta_{\text{uzorak}} &= \nu_{\text{uzorak}} - \nu_{\text{referentno}} / \nu_{\text{referentno}} \\ &= \Delta\nu \text{ (Hz)} / \nu_{\text{referentno}} \text{ (MHz)} \end{aligned}$$

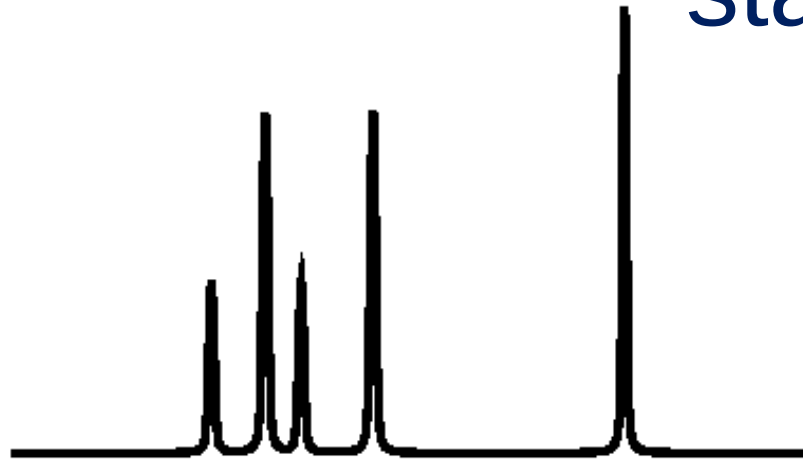
Skala u ppm!



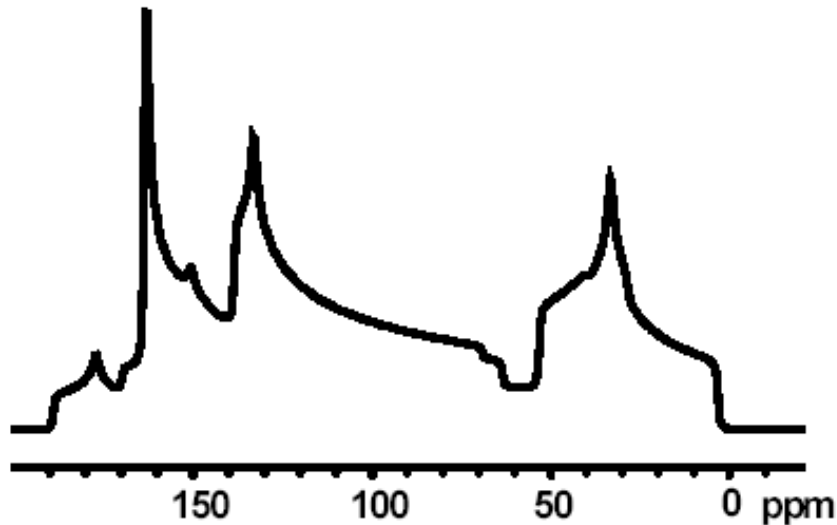
Skala za različite jezgre



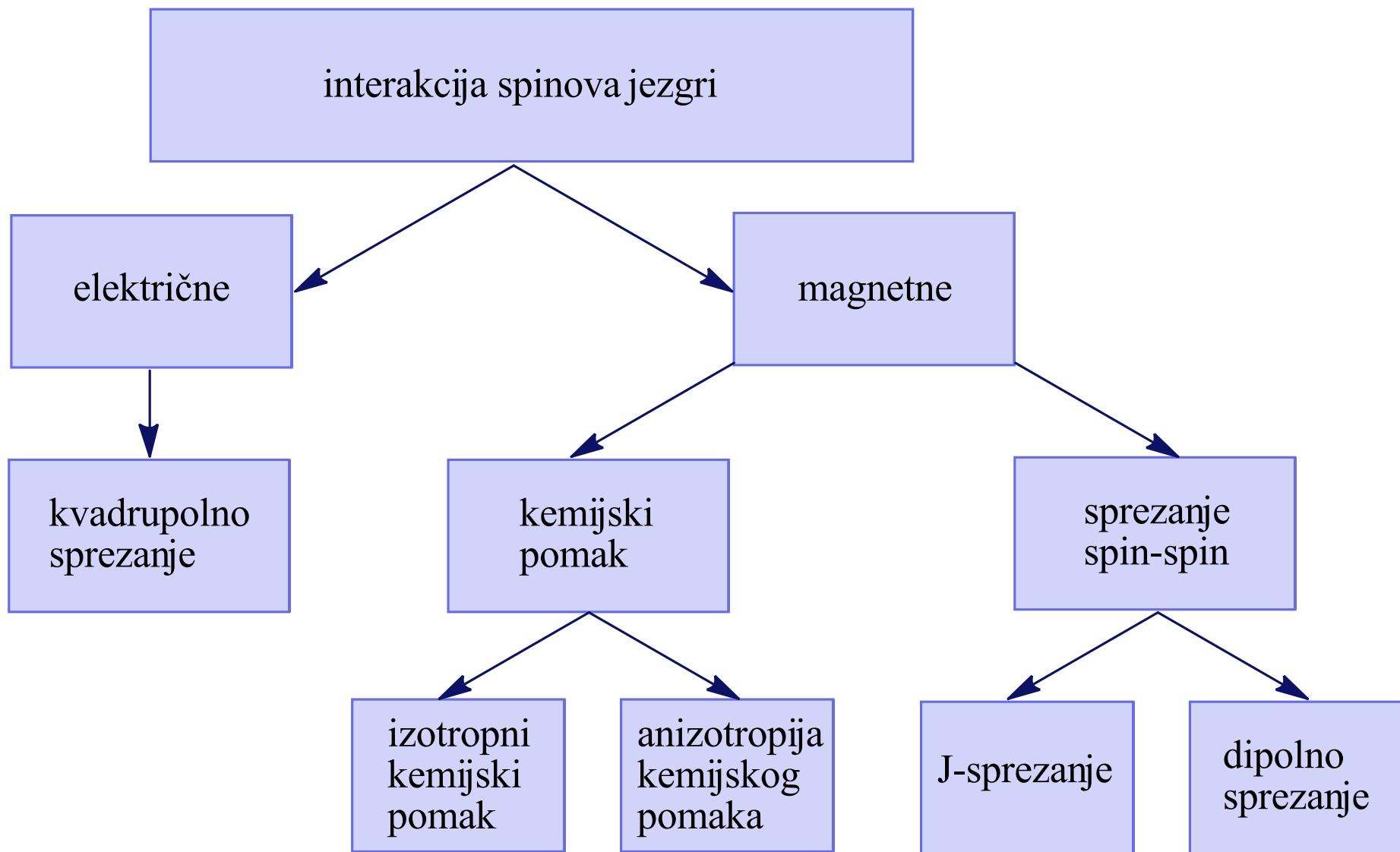
NMR spektroskopija u čvrstom stanju



- Spektar ^{13}C NMR u otopini

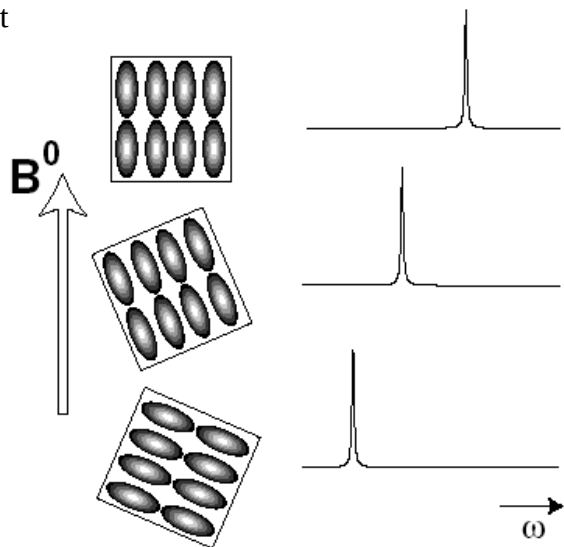


- spektar ^{13}C NMR u čvrstom stanju



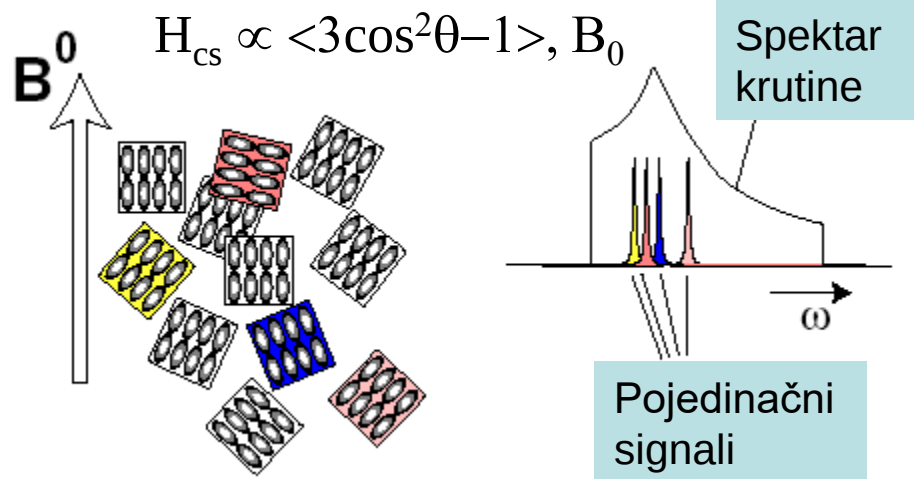
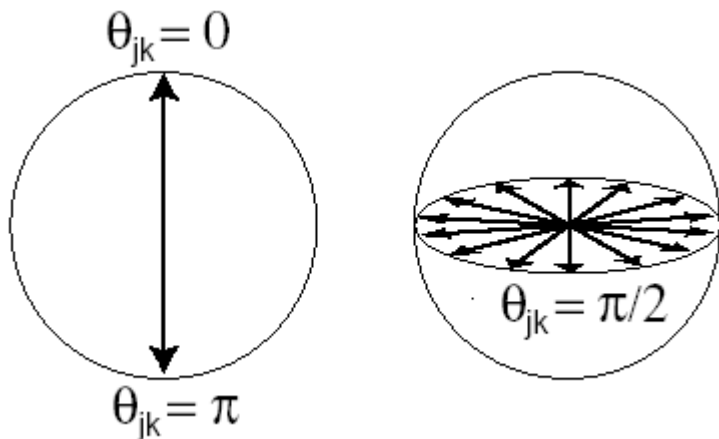
Anizotropija kemijskog pomaka (CSA)

Levitt

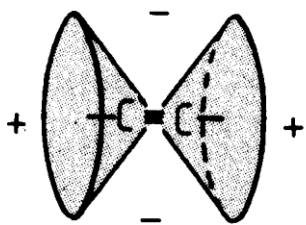


U NMR-u tekućina gibanje (tumbanje) molekula uzrok je simetričnosti vremenski uprosječenog elektronskog zasjenjenja. U NMR-u čvrstog stanja, molekule se ne gibaju izotropno pa kemijski pomak ne ovisi samo o kemijskom identitetu atoma nego i o prostornom odnosu između molekule i vanjskog magnetnog polja.

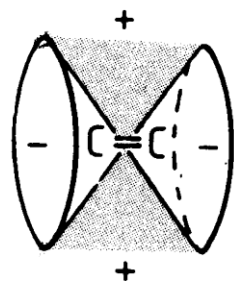
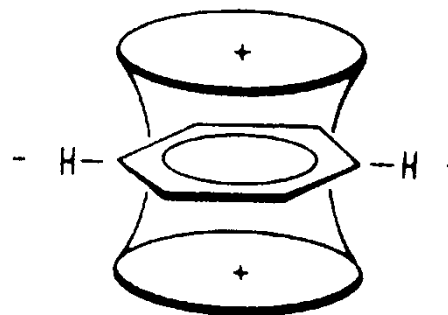
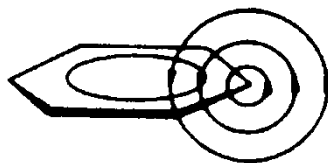
Vjerojatnost orijentacije



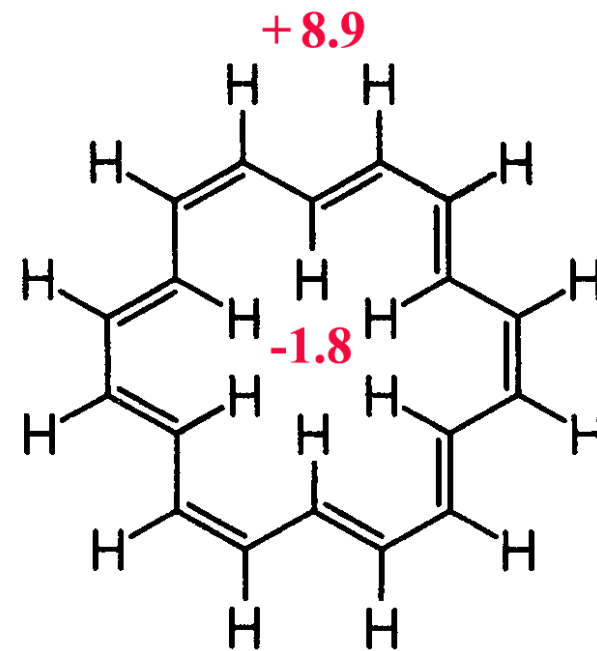
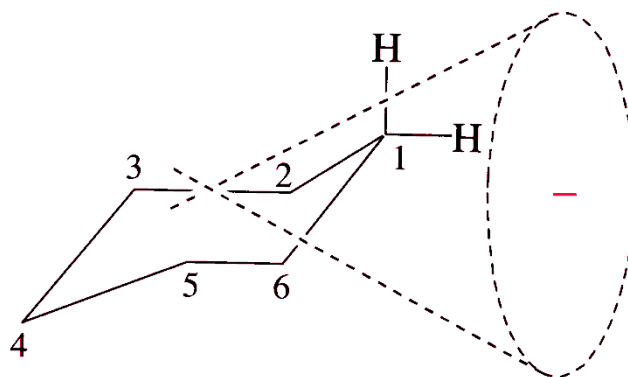
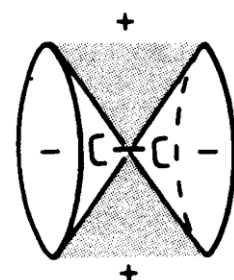
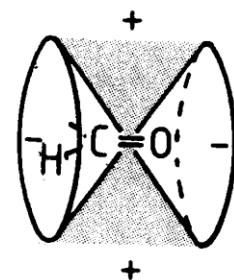
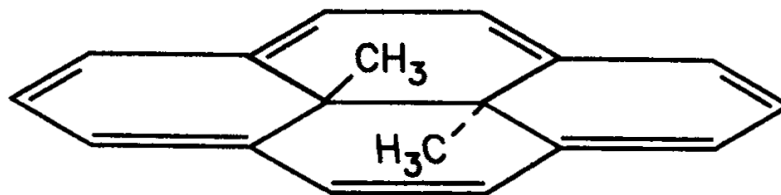
Anizotropija kemijskog pomaka i prstenaste struje



B_0

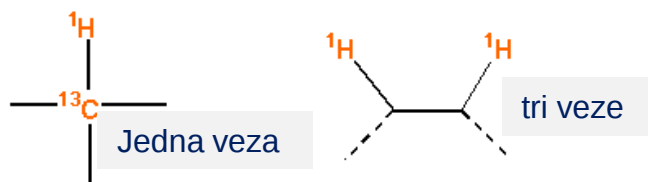


$$\delta(\text{CH}_3) = -4.25$$

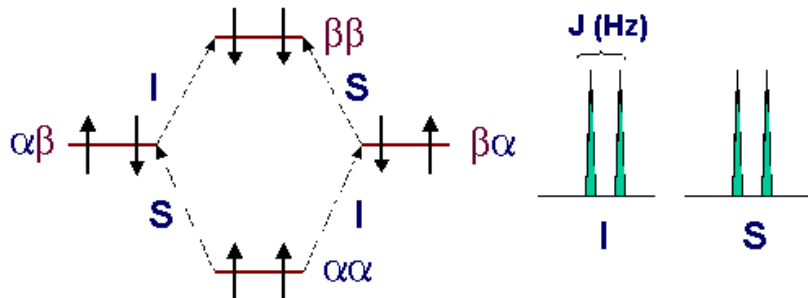


c) Konstanta sprege spin-spin

stanje spina susjedne jezgre može utjecati na energetske razine promatrane jezgre. Za takve jezgre kažemo da su međusobno spregnute preko jedne ili više kemijskih veza



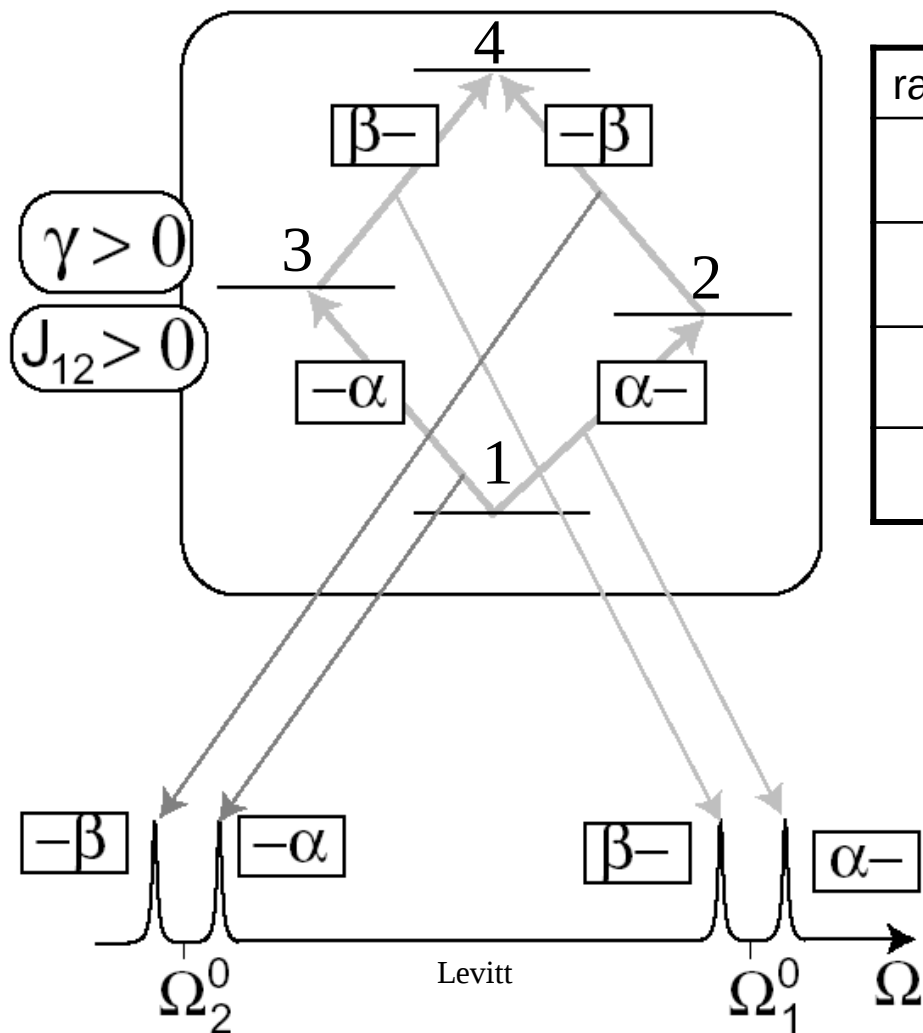
Energetski dijagram. Svaki spin sada ima dvije podrazine ovisno o stanju spina s kojim je u sprezi



Razlika između dvije linije dubleta se zove konstanta sprege J i ima jedinicu Hz

Način sprezanja bitan je za identifikaciju spinskog sustava u molekuli i za određivanje njene strukture

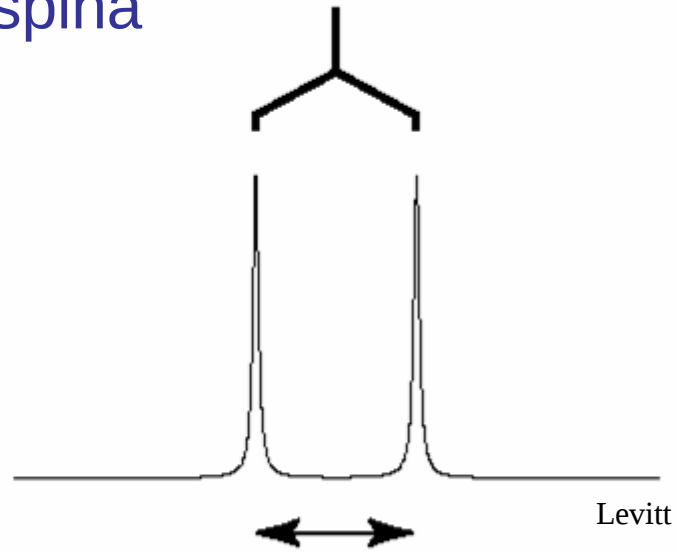
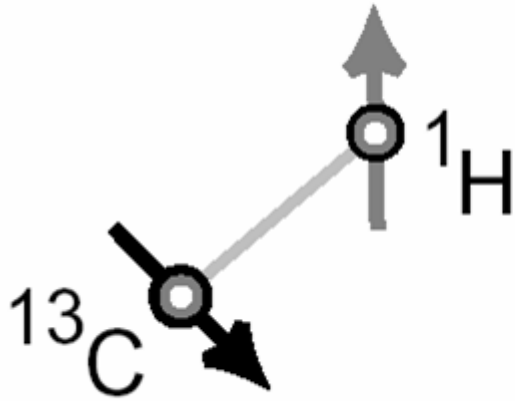
Homonuklearna sprega J : 2 spina



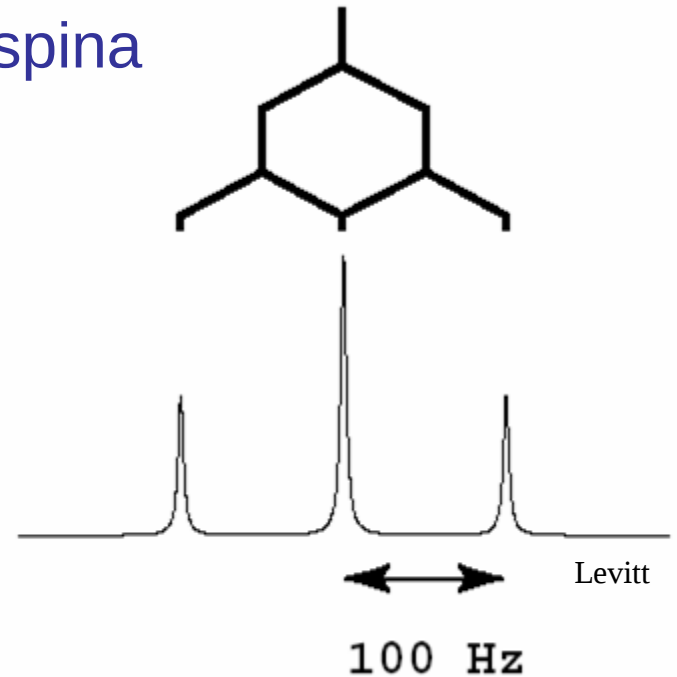
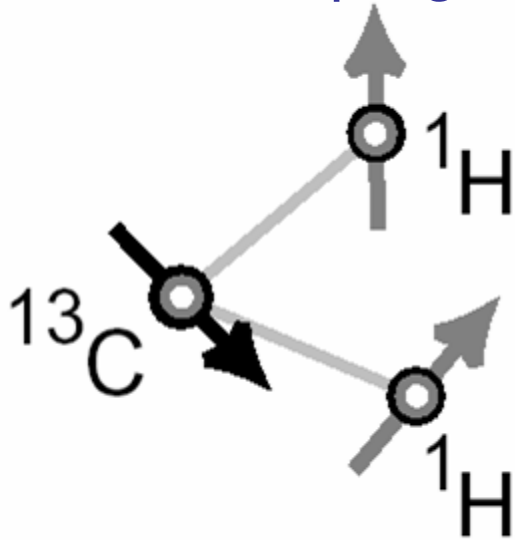
razina	Spinsko stanje	energija
1	$\alpha\alpha$	$-\frac{1}{2}\nu_0^1 - \frac{1}{2}\nu_0^2 + \frac{1}{4}J_{12}$
2	$\alpha\beta$	$+\frac{1}{2}\nu_0^1 - \frac{1}{2}\nu_0^2 - \frac{1}{4}J_{12}$
3	$\beta\alpha$	$-\frac{1}{2}\nu_0^1 + \frac{1}{2}\nu_0^2 - \frac{1}{4}J_{12}$
4	$\beta\beta$	$+\frac{1}{2}\nu_0^1 + \frac{1}{2}\nu_0^2 + \frac{1}{4}J_{12}$

prijelaz	energija
$1 \rightarrow 2$	$\nu_0^1 - \frac{1}{2}J_{12}$
$3 \rightarrow 4$	$\nu_0^1 + \frac{1}{2}J_{12}$
$1 \rightarrow 3$	$\nu_0^2 - \frac{1}{2}J_{12}$
$2 \rightarrow 4$	$\nu_0^2 + \frac{1}{2}J_{12}$

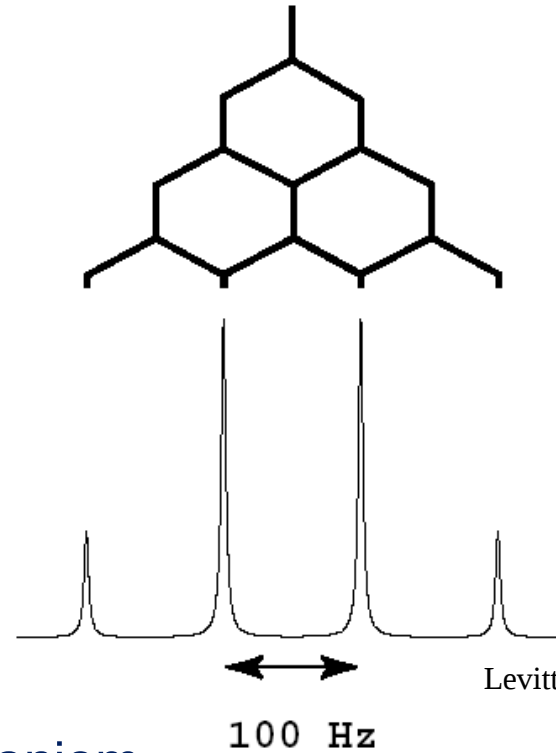
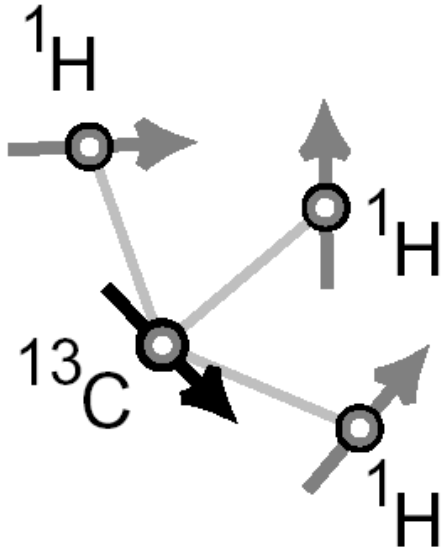
Heteronuklearna sprega J : 2 spina



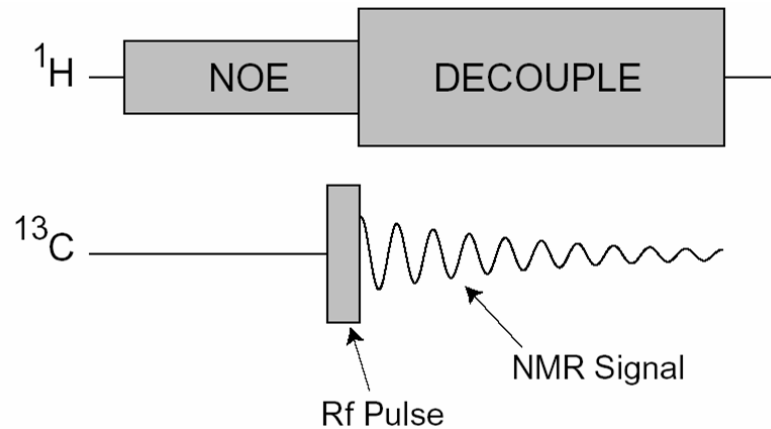
Heteronuklearna sprega J : 3 spina

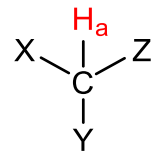
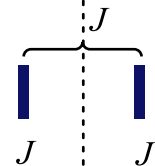
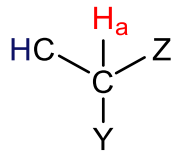
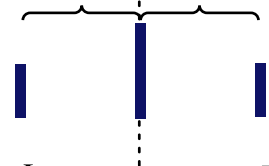
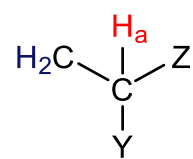
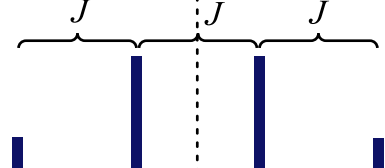
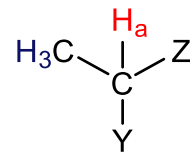
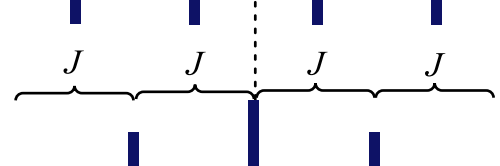
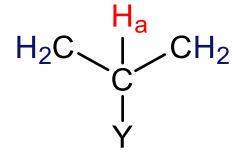


Heteronuklearna sprega J : 4 spina



Spektri se mogu pojednostavniti rasprezanjem



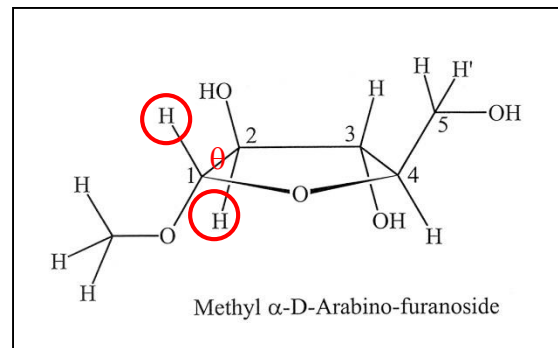
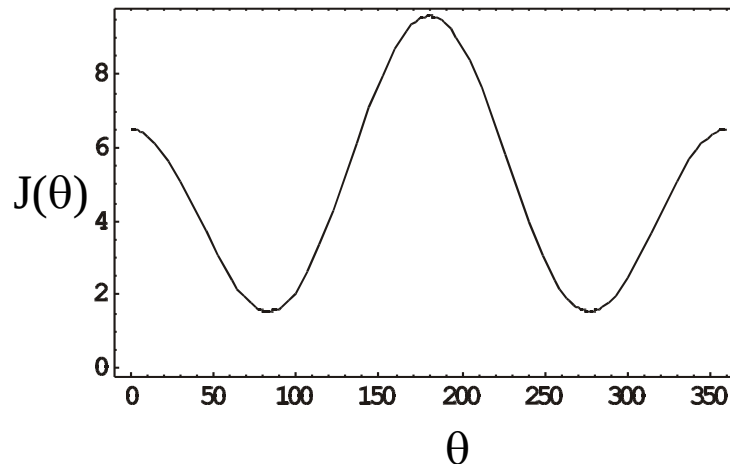
Multipletnost	Relativni intenzitet	Izgled signala	Primjer
<i>Singlet</i> (s)	1		
<i>Dublet</i> (d)	1 1		
<i>Triplet</i> (t)	1 2 1		
<i>Kvartet</i> (q)	1 3 3 1		
<i>Kvintet</i>	1 4 6 4 1		

Spin-spin sprege kroz tri veze

Martin Karplus je pokazao da vicinalna sprega između ^1H atoma ovisi o diedarskom kutu između njih. Ova relacija se može iskazati pomoću **Karplusove jednadžbe**:

$$J(\theta) = A\cos^2(\theta) + B\cos(\theta) + C$$

A , B , i C su empirijski određeni parametri.



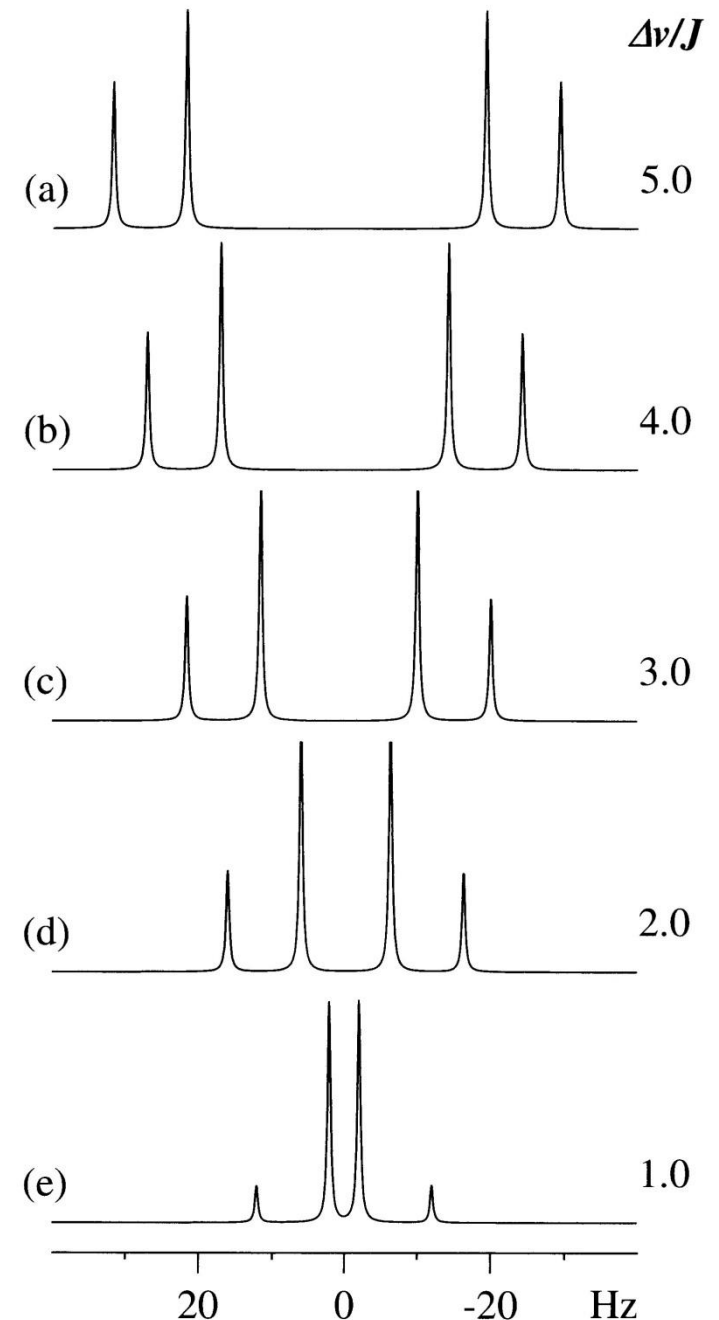
Sprega J omogućuje procjenu molekulske konformacije!

Spinski sustavi

Pople-ova notacija: slova u abecedi označavaju kemijske pomake

Prvi red: $\Delta\nu/J \geq 10$
oznake A i X

Viši red: $\Delta\nu/J \leq 10$
oznake A, B, C



$\text{ClCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{Cl}$
(CDCl_3)

60 MHz

(a)

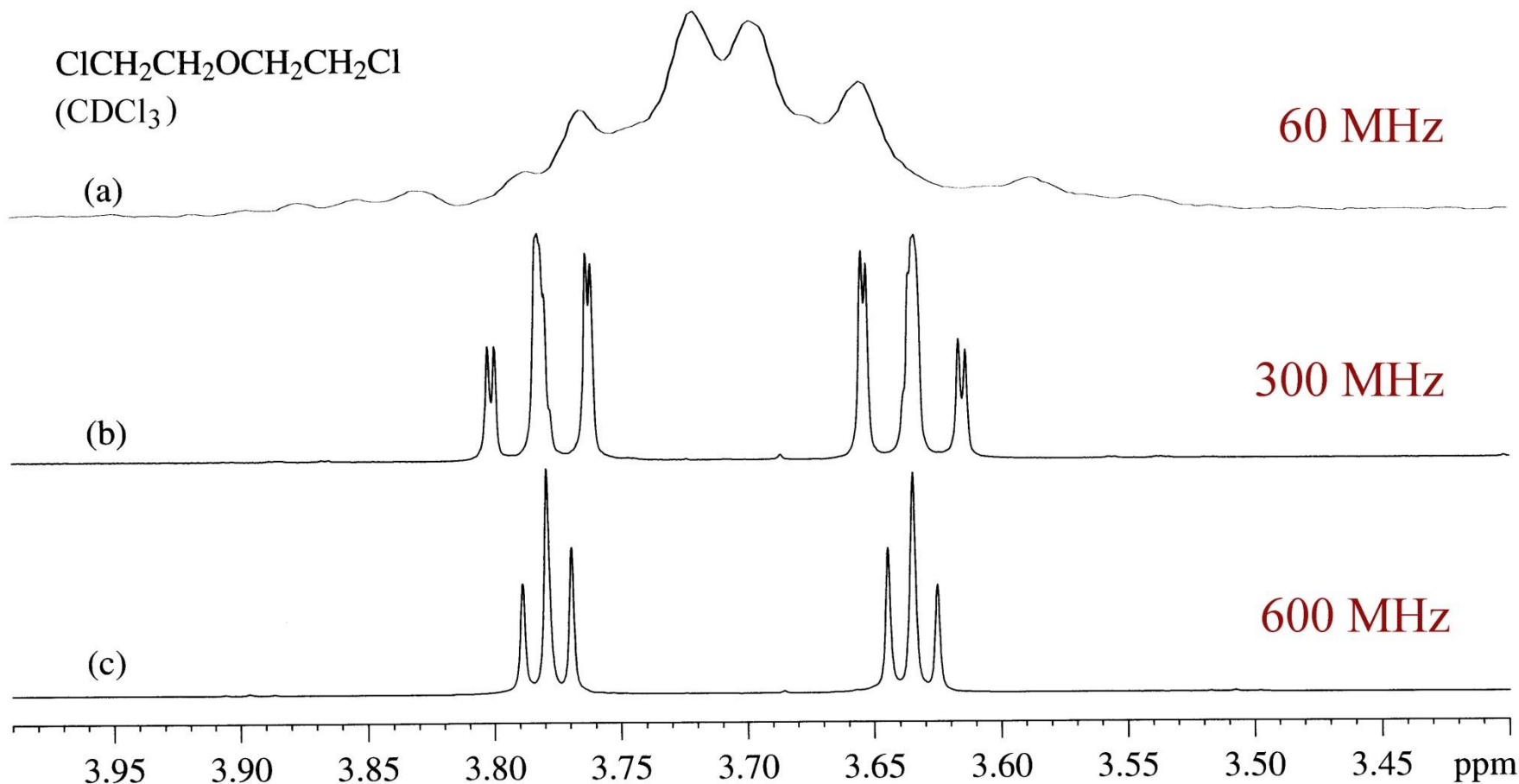
300 MHz

(b)

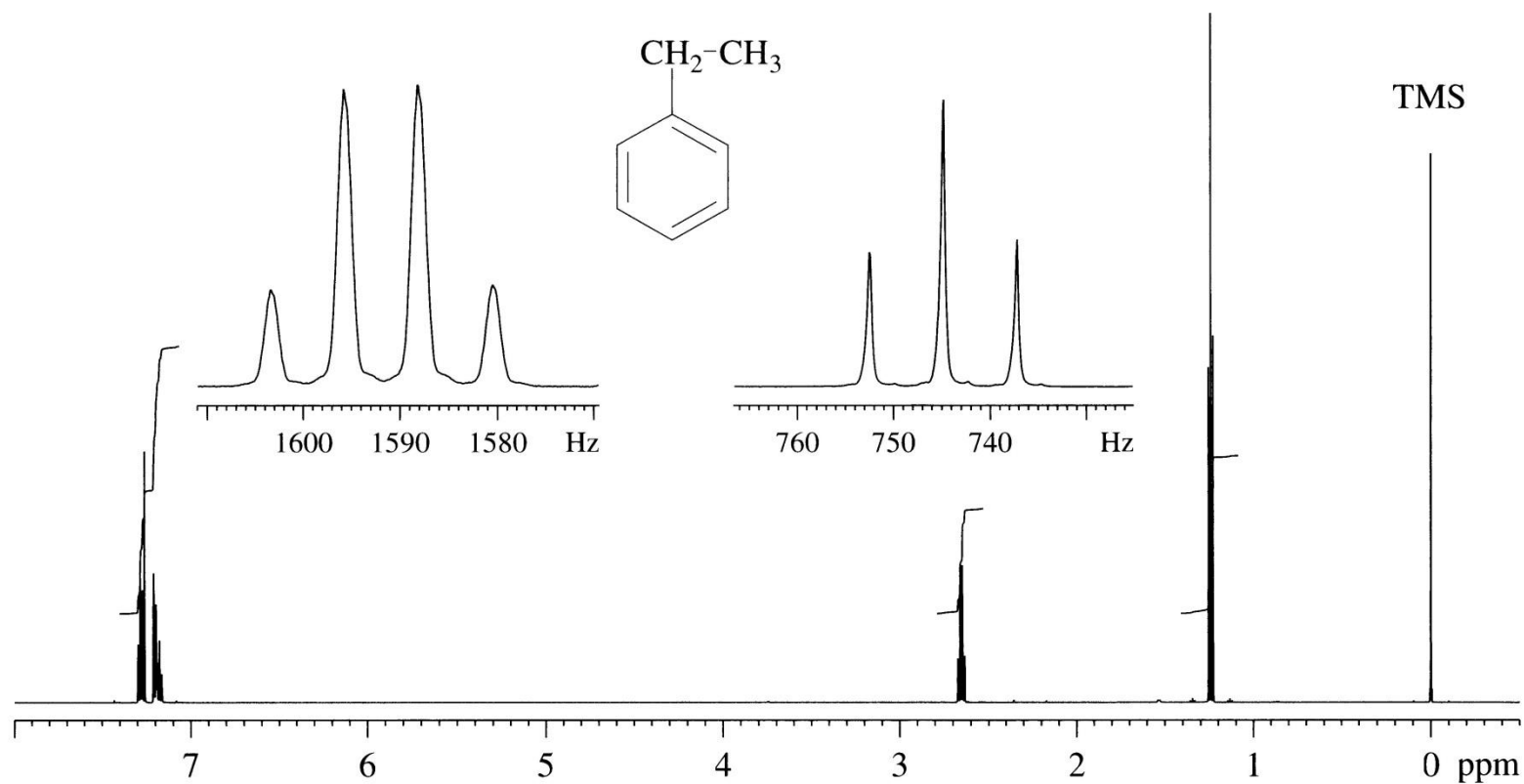
600 MHz

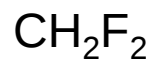
(c)

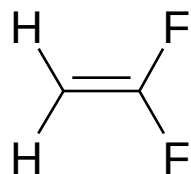
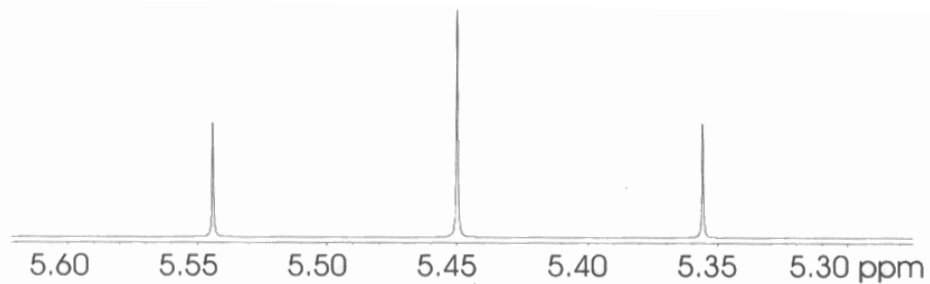
3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 ppm

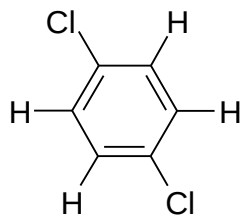
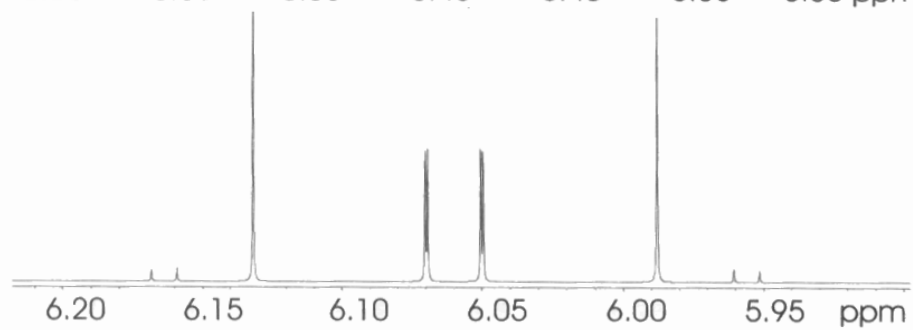


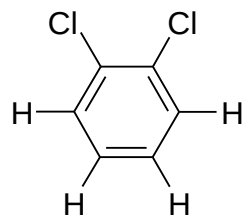
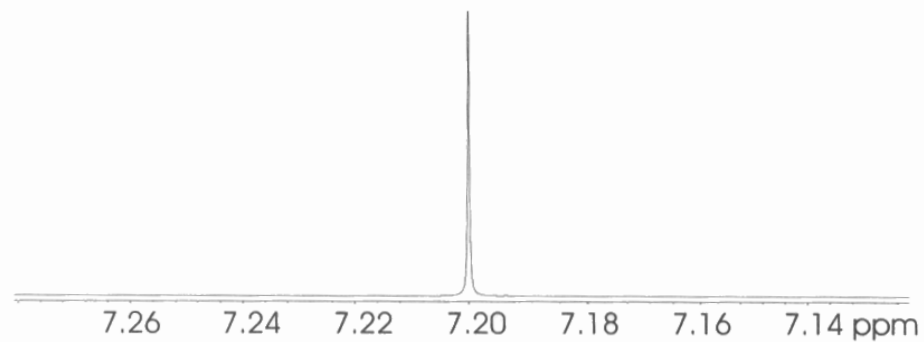
Više spinskih sustava unutar jedne molekule

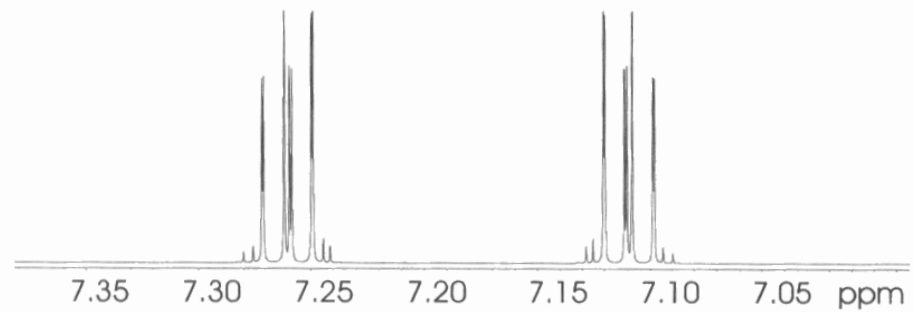




$$\text{A}_2\text{X}_2$$


$$\text{AA'XX'}$$


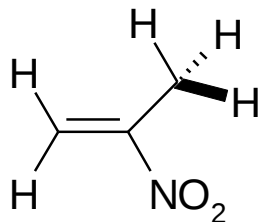
$$\text{A}_4$$


$$\text{AA'BB'}$$


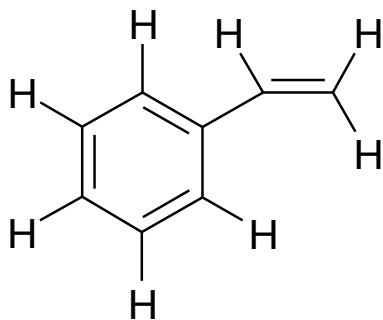
Cijepanja prvoga reda za neke spinske sustave

Struktura	Multiplet za Ha	Struktura	Multiplet za Ha		
$J_{ab} = J_{ac}$					
1			7	 $\nu_b \neq \nu_{b'}$ $J_{ab} > J_{ab'}$	
2			8	 $\Delta\nu_{ab} > 0; \nu_b = \nu_{b'}$ $J_{ab} = J_{ab'}$	
3			9	 $J_{ac} > J_{ab}$	
4			10	 $J_{ab} = J_{ab'} > J_{ac}$	
5			11	 $J_{ab} > J_{ab'} > J_{ac}$	
6			12	 $J_{ab} > J_{ac}$	

Odredite spinski sustav za slijedeće molekule



A_3MX



AMX

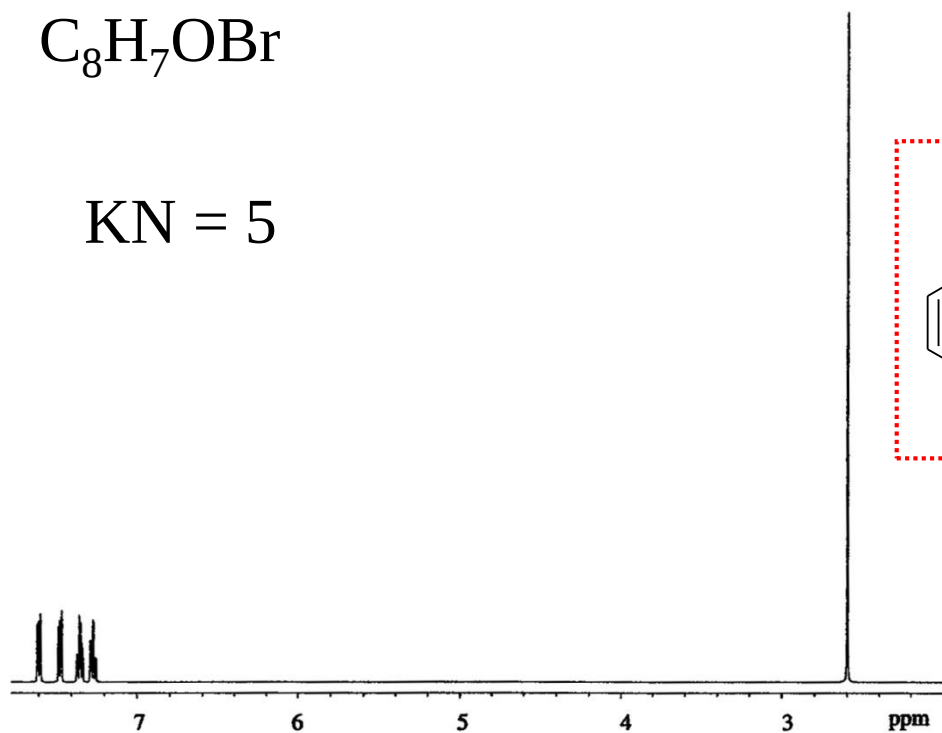
+

A_2B_2C

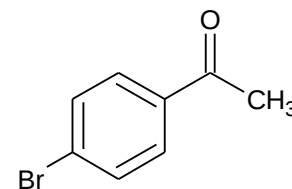
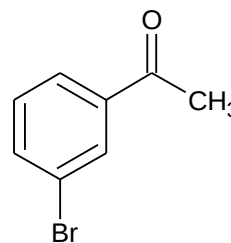
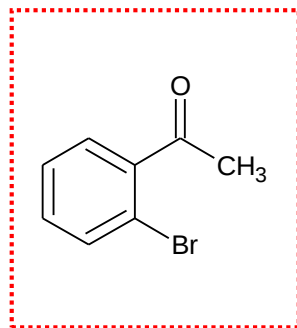
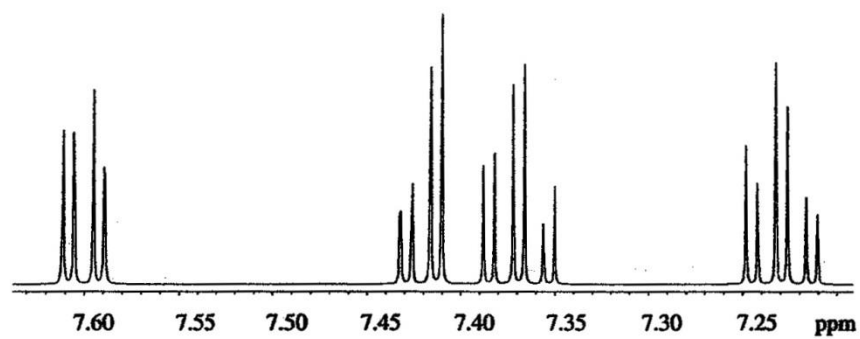
(AA'BB'C)

$\text{C}_8\text{H}_7\text{OBr}$

$\text{KN} = 5$



Expansion
2.1 Hz/mm



Odredite položaj supstitucije metilnih skupina u dimetilpiridinu

