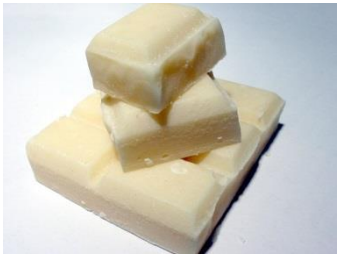
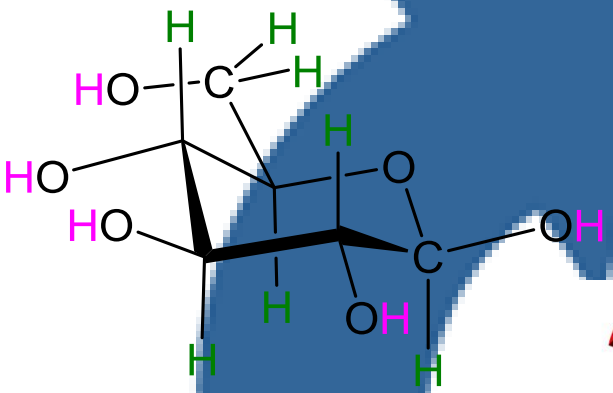
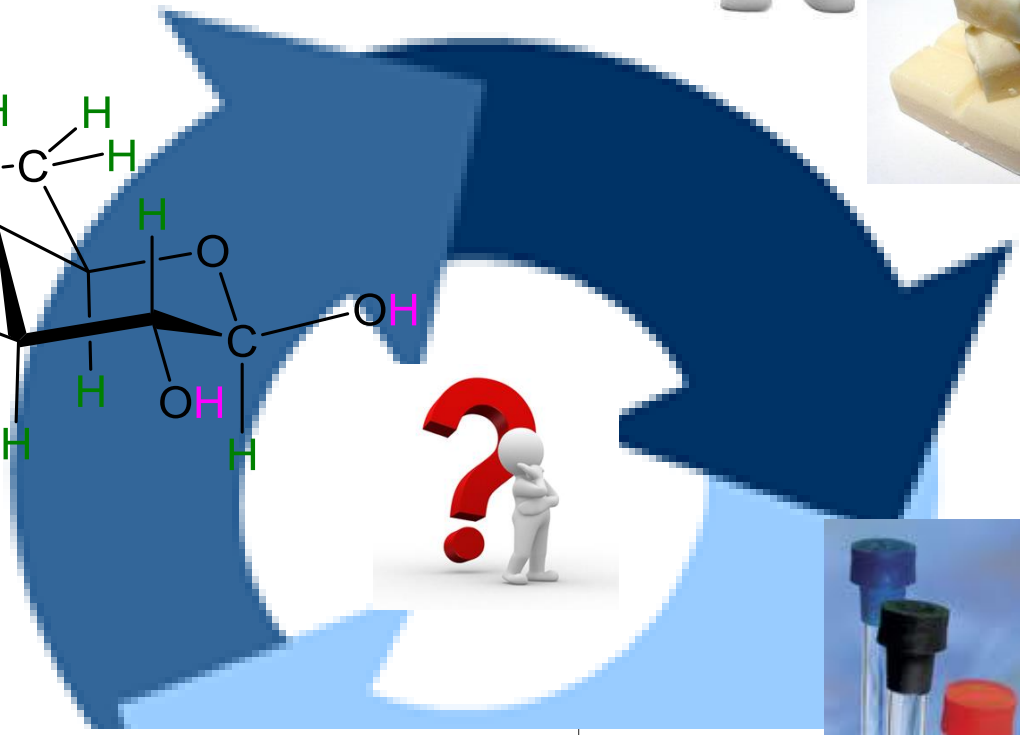
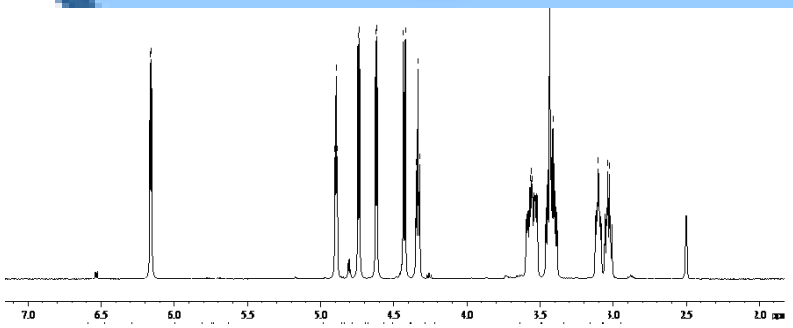
Példák egyszerű szerves vegyületek ^{1}H és ^{13}C jelhozzárendelésére





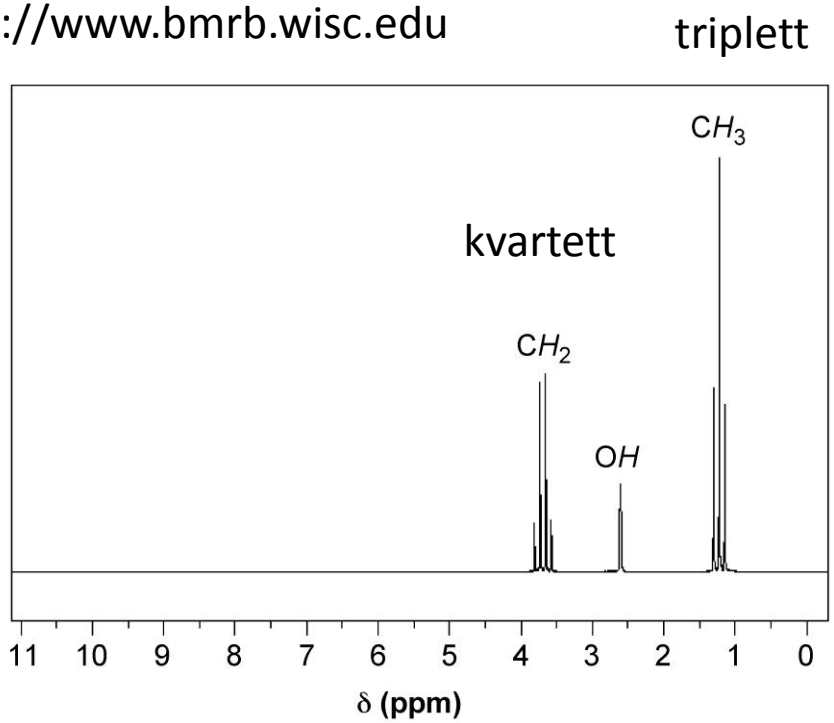




Tartalomjegyzék:

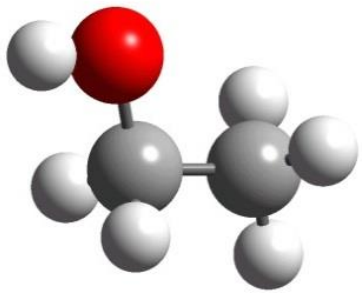
- etanol
- (D)-glükópiranóz

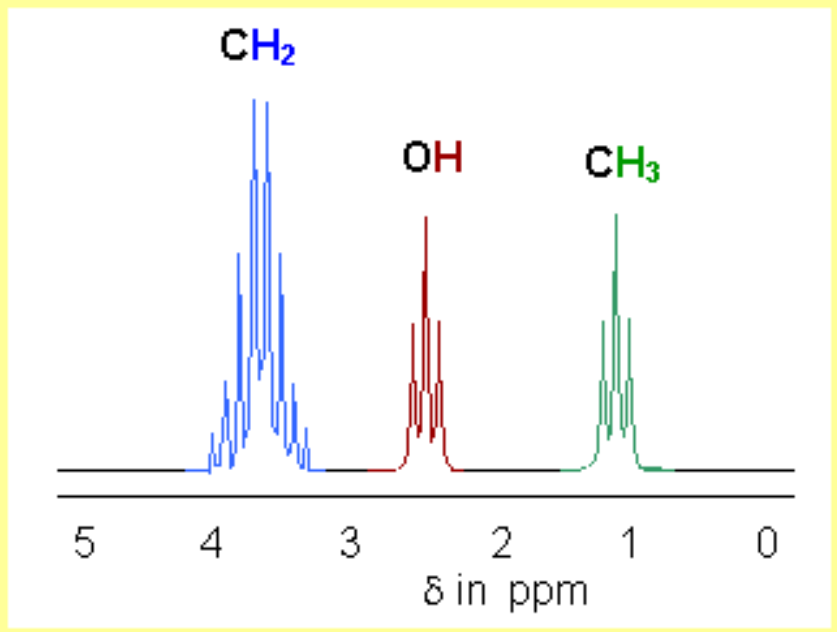
http://www.bmrb.wisc.edu



BMRB
Biological Magnetic Resonance Data Bank

A Repository for Data from NMR Spectroscopy on Proteins, Peptides, Nucleic Acids, and other Biomolecules





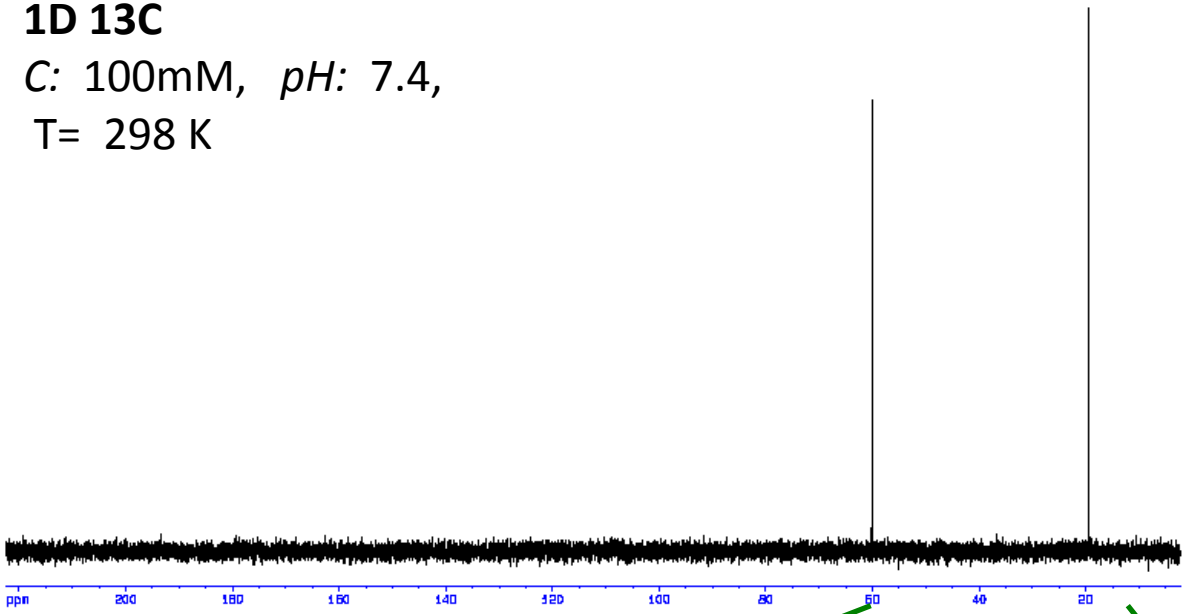
Singlet (s)	1
Doublet (d)	1:1
Triplet (t)	1:2:1
Quartet (q)	1:3:3:1
Quintet	1:4:6:4:1
Sextet	1:5:10:10:5:1
Septet	1:6:15:20:15:6:1

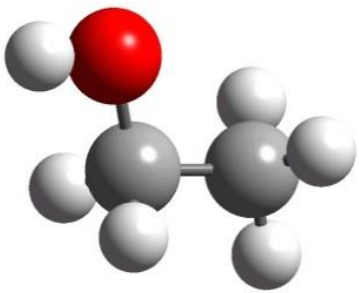
http://en.wikipedia.org/wiki/Nuclear_magnetic_resonance_spectroscopy

1D 13C

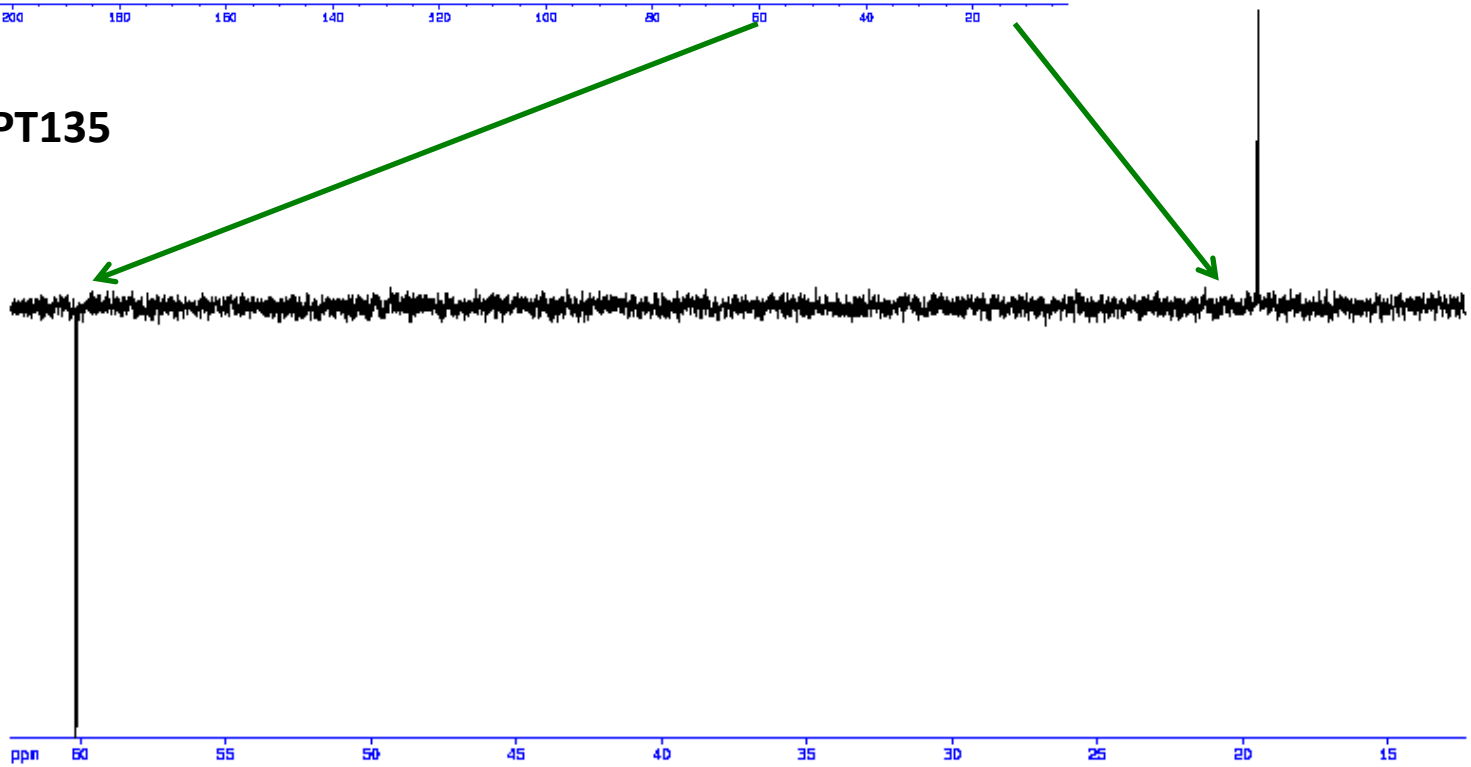
C: 100mM, *pH:* 7.4,

T= 298 K



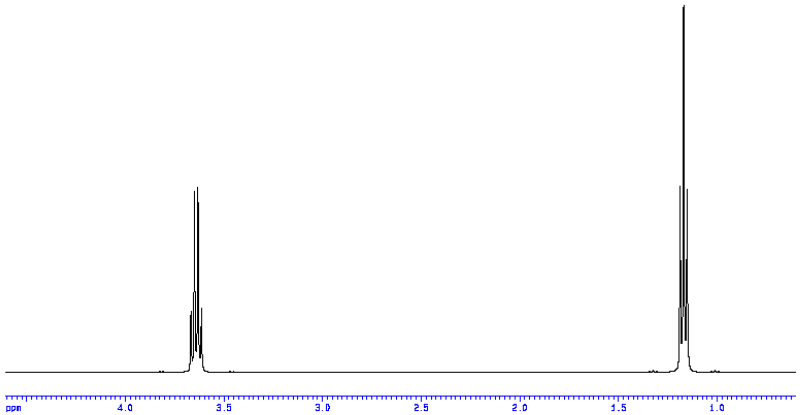


1D DEPT135



2D [1H,1H]-TOCSY

C: 100 mM, *pH:* 7.4, *T:* 298 K



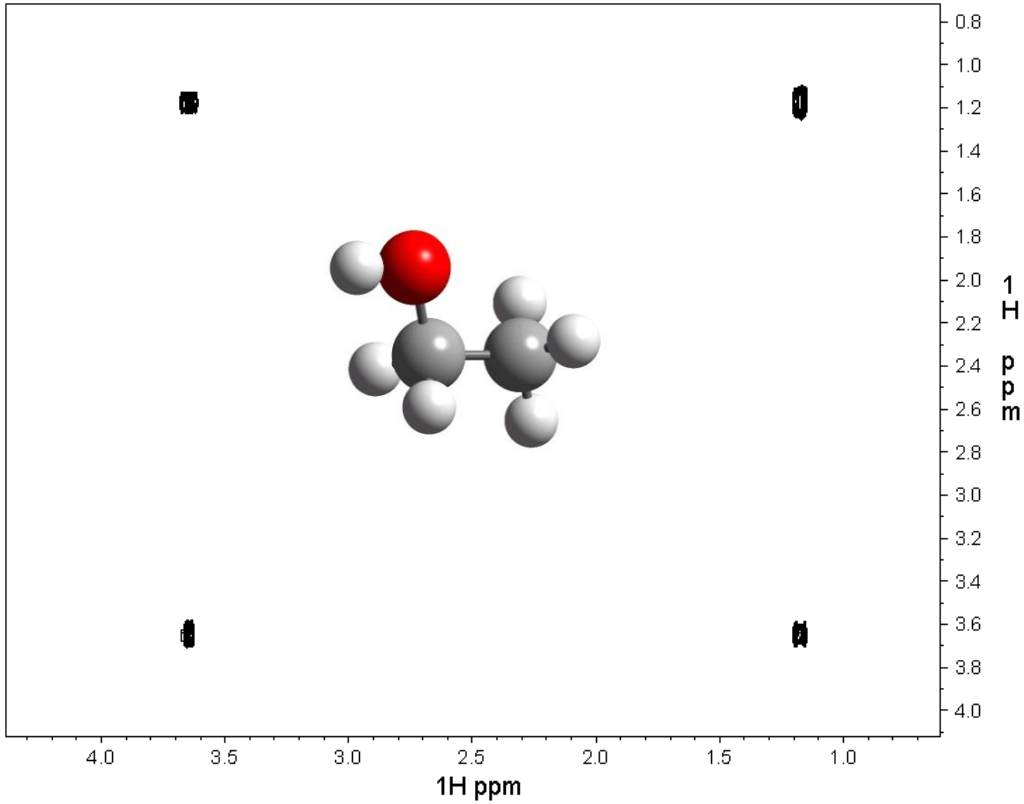
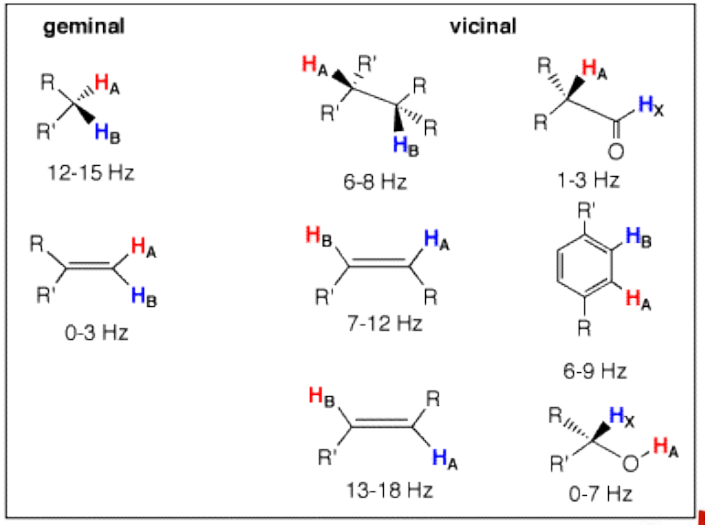
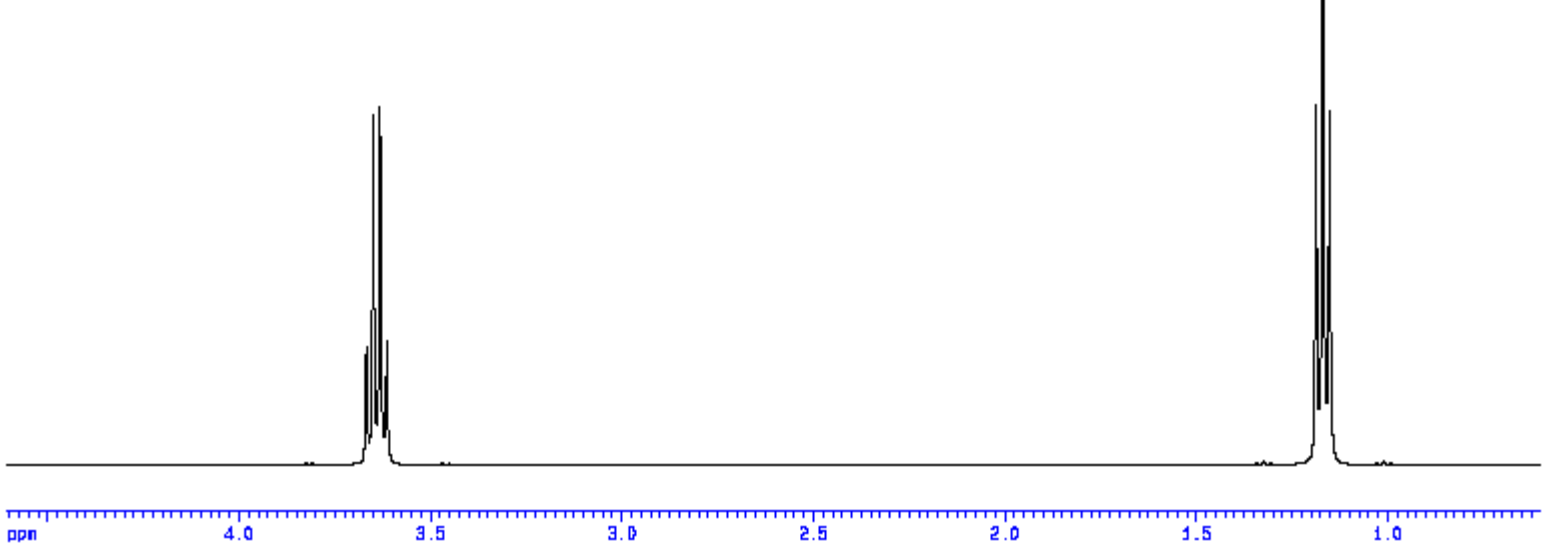
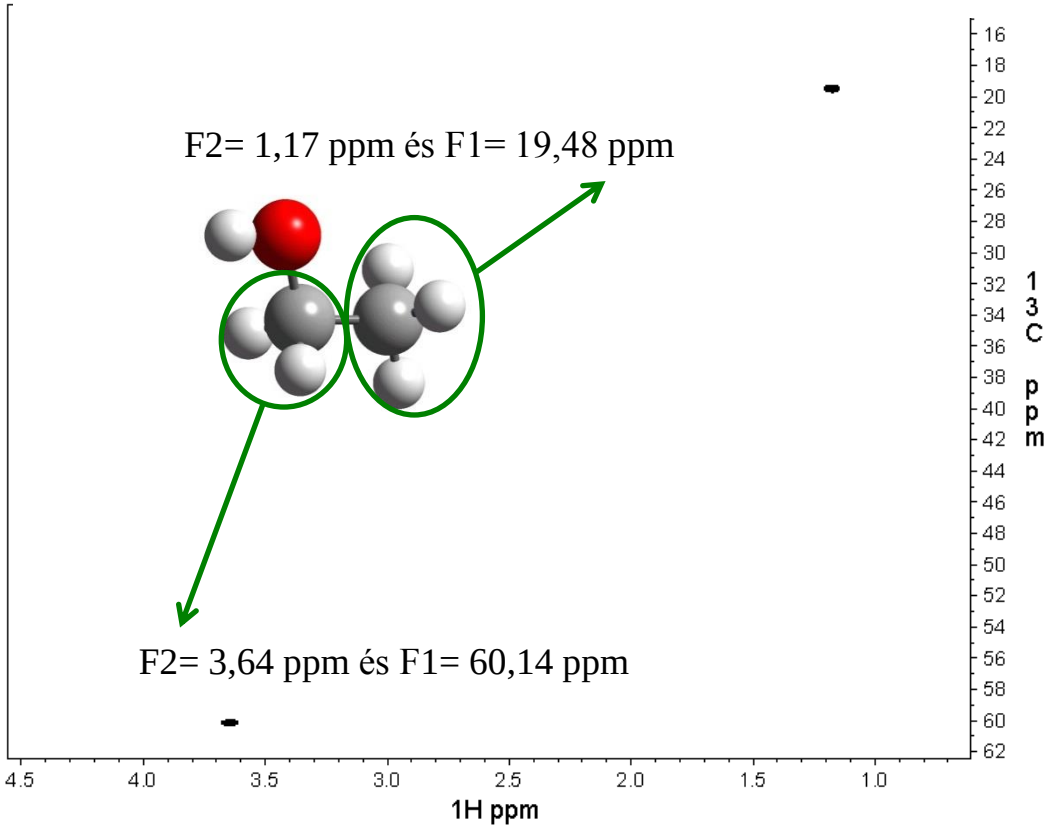


Figure 20. Examples of proton-proton coupling constants (J) as a function of structure.



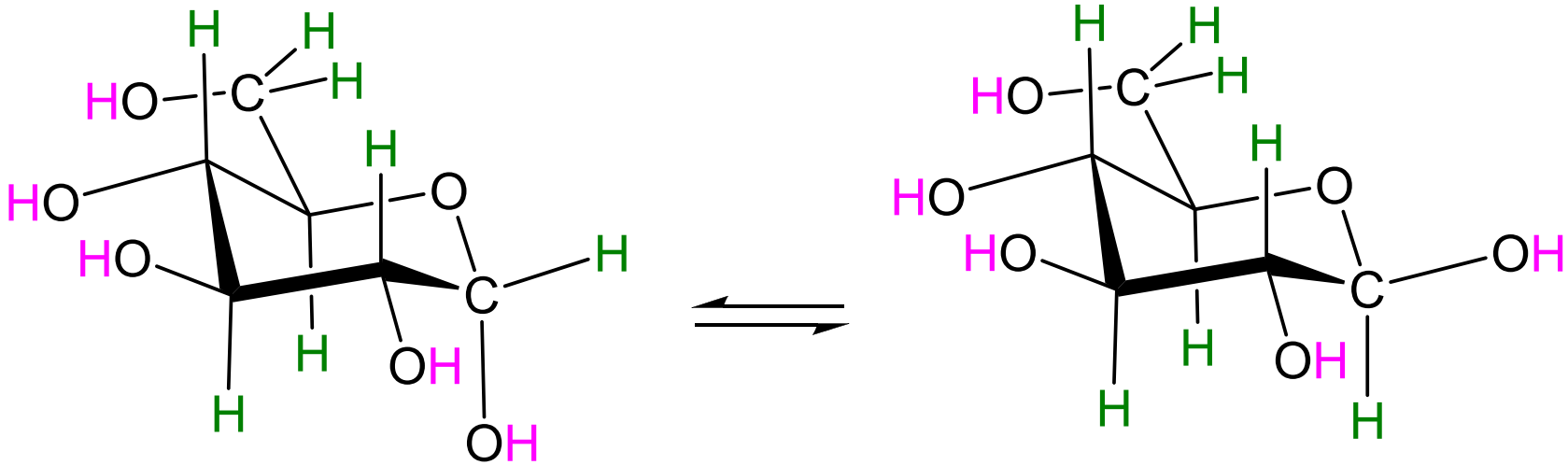




G. Bodenhausen, R. Freeman 1977
Correlation of proton and carbon-13 nmr spectra by heteronuclear two-dimensional spectroscopy

2D [1H,13C]-HSQC
C: 100 mM, *pH:* 7.4, *T:* 298 K

A (D)-Glükópiranóz ^{1}H- és ^{13}C-NMR jeleinek azonosítása



α/β -D-(+)-glükopiranóz: az anomer konfiguráció bár definiált (95/5 : α/β SIGMA) kérdés hogy DMSO-ban megőrződik-e?

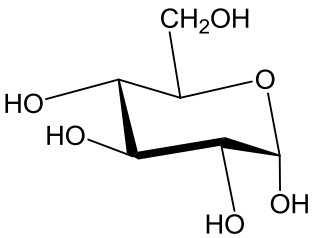
Gyűrű-konformerek: a D-glükóz anomerjeinek és izomerjeinek egyensúlya:

1) Oldatban az α- és β-anomerek illetve a furanóz- és piranóz-gyűrűk egymással **termikus egyensúlyt** tartanak.

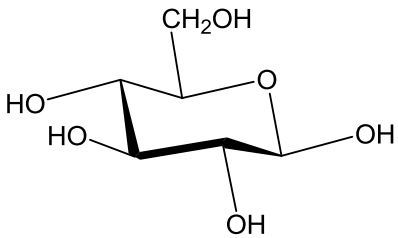
2) A **lineáris forma** fajlagos súlya kicsi, jelenléte mégis fontos egyes jelenségek és reakciók magyarázatához.

3) A furanóz-gyűrű bizonyítéka a D-glükóz **diacetonid** származéka.

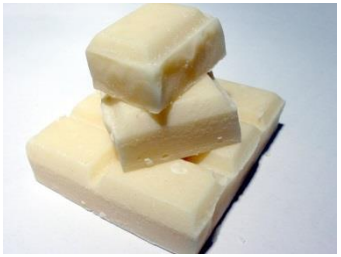
4) sem UV-ban, sem IR-ben a C=O sávok nem azonosíthatók

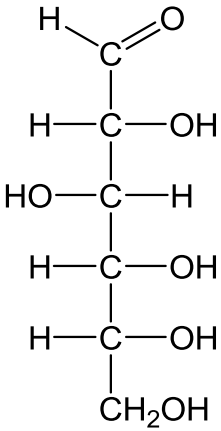


α-D-glükopiranóz
38 %



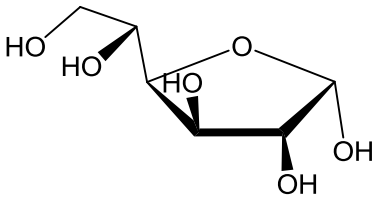
β-D-glükopiranóz
62 %



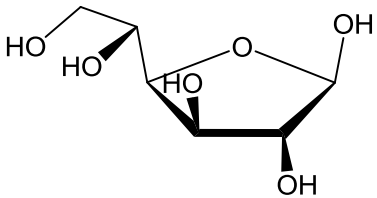


hogy is nézek én ki?

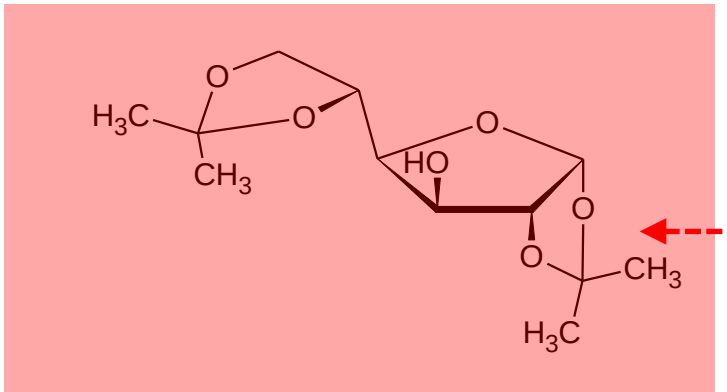
β-D-glükóz
nyílt forma
<0,02%



α-D-glükofuranóz
<0,5 %

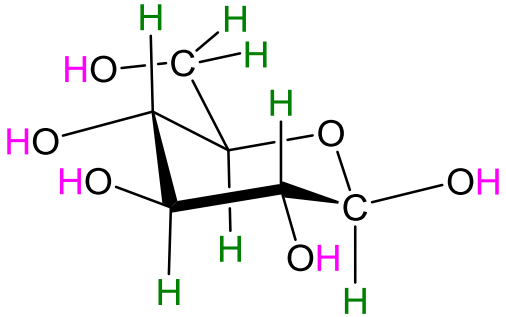


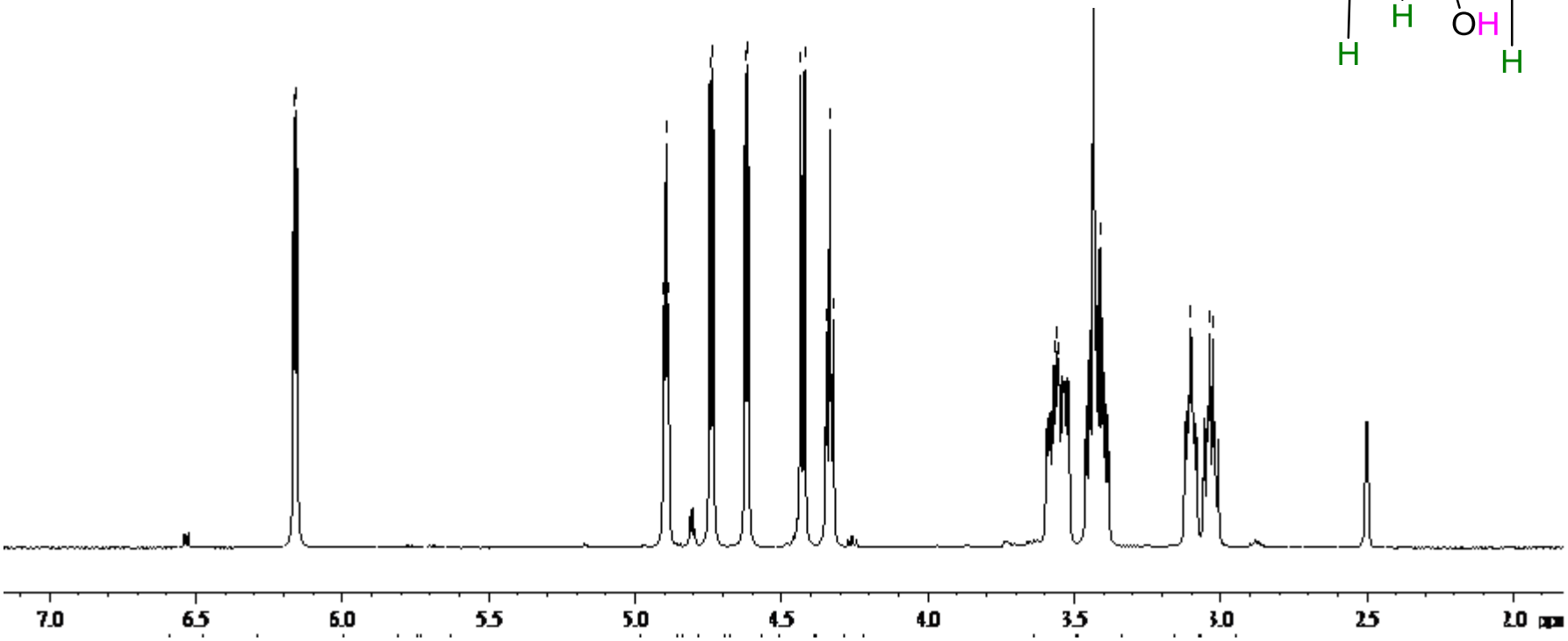
β-D-glükofuranóz
<0,5 %

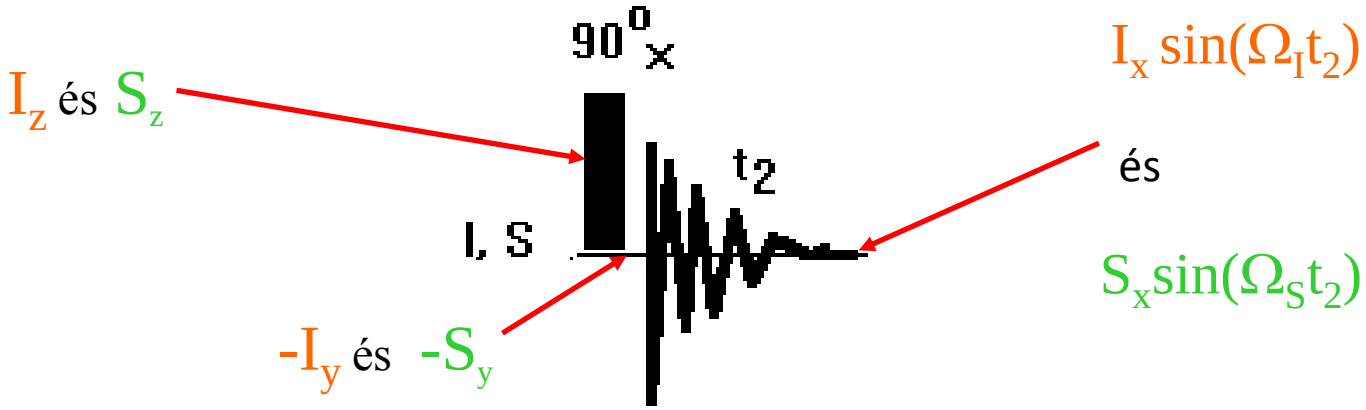


Első tájékozódás (az első 30 perc)

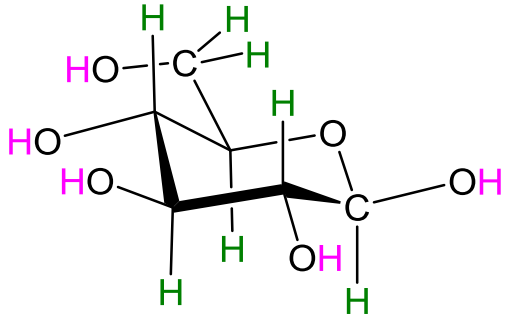
A D-glükózból c~20 mM-os (~ 1,8mg/500ul),
DMSO-s (CD_3-SO-CD_3) oldott készítése

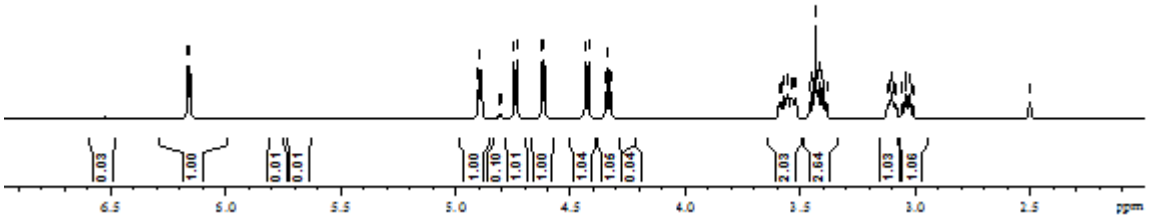


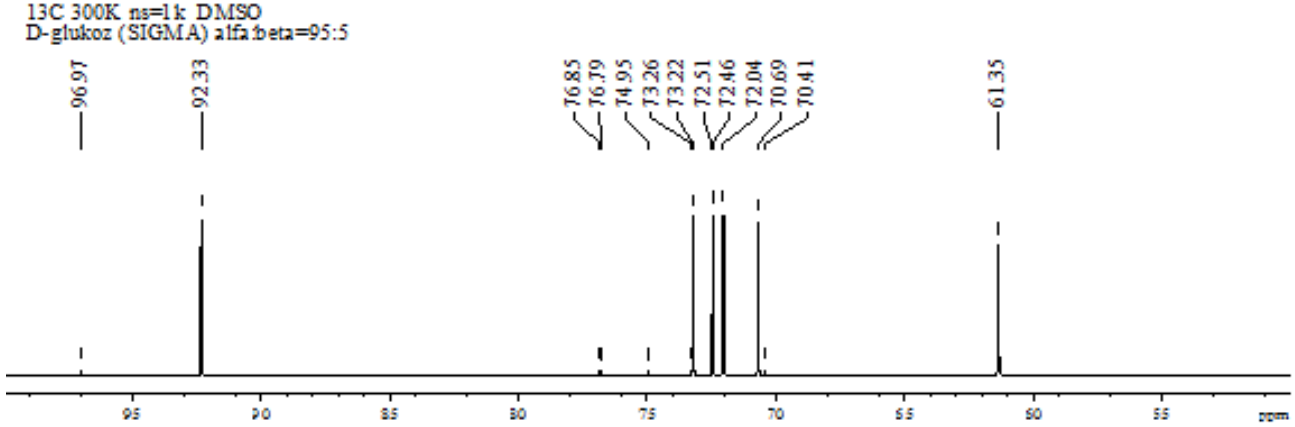




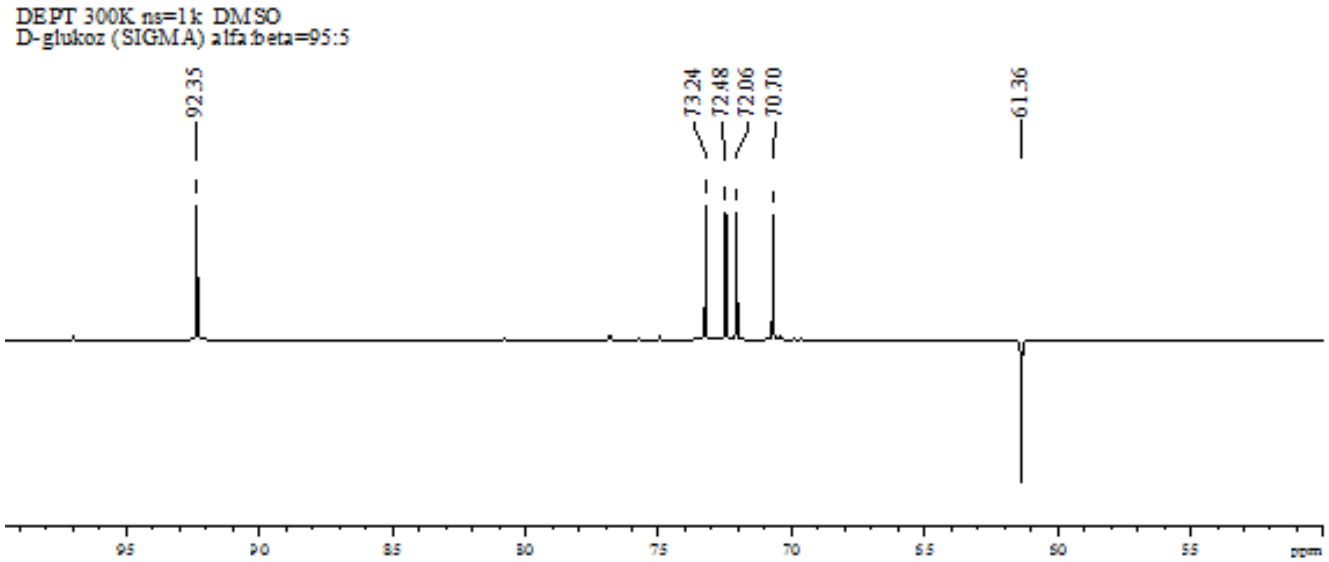
Első tájékozódás (az első 30 perc)





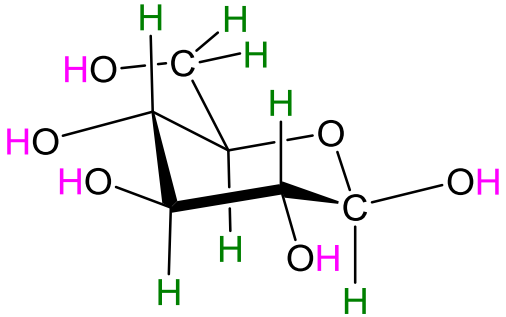


^{13}**C** (zg szén detektált)



^{13}C- DEPT
(szén detektált), amiben
primer (CH_3) és
tercier (CH) **C „felfelé”,**
szekunder szén (CH_2)
„lefelé” áll

^{1}H relatív „intenzitások” **integrálok** arányosak az adott rezonancia frekvenciához (kémia eltolódáshoz) rendelt hidrogének számával



memo: DMSO-ban nincs csere az oldószer és az **OH protonok** között

6 db ^{1}H jel mindegyik integrálja egységnyi

2*2 db ^{1}H jel mindegyik integrálja 2 és 2

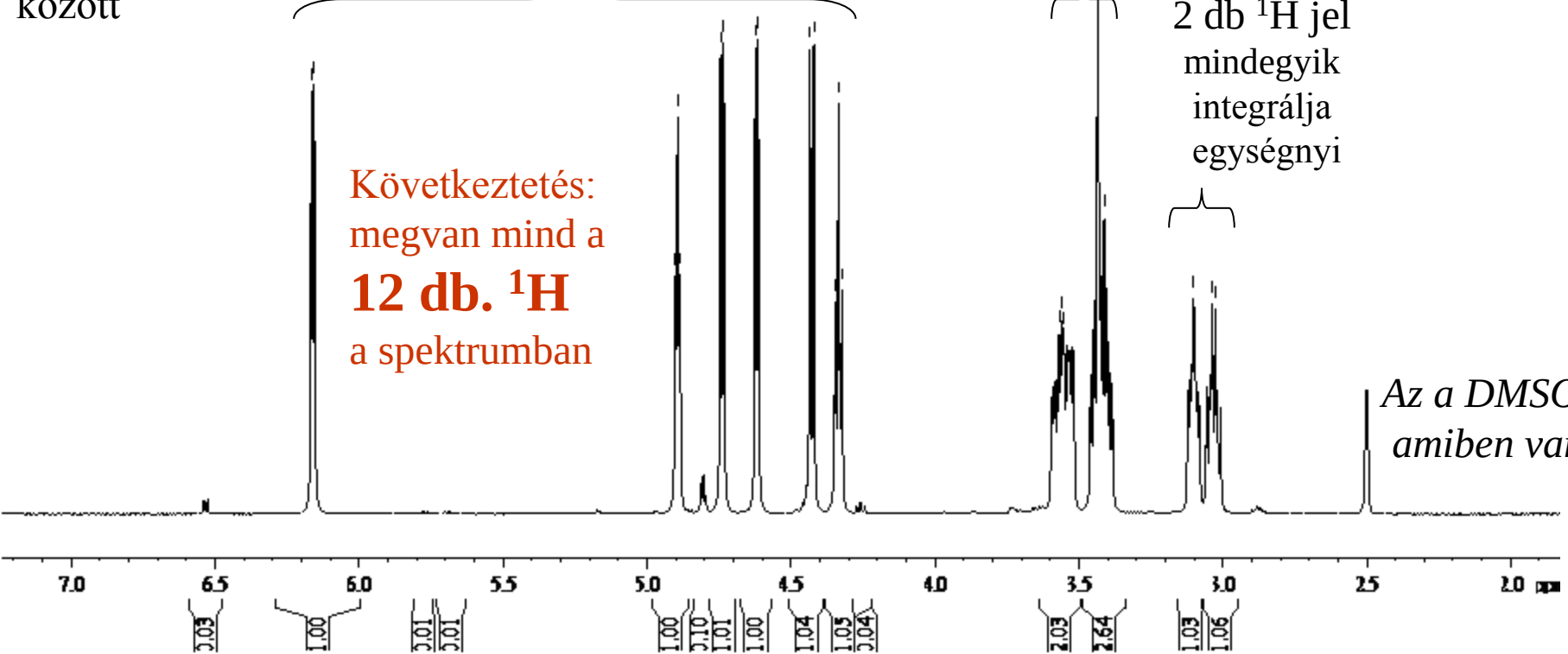
memo: DMSO-ban itt van a H_2O is!

12 db H atom van a glükózban 5 +7

2 db ^{1}H jel mindegyik integrálja egységnyi

Következtetés: megvan mind a **12 db. ^{1}H** a spektrumban

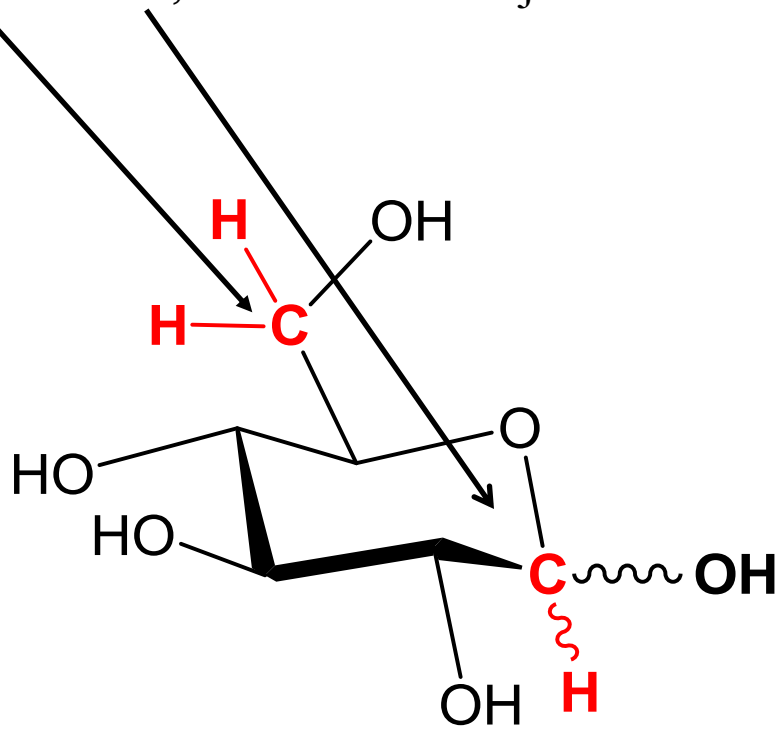
Az a DMSO amiben van ^{1}H



kérdés: hol és hogyan kezdjük az asszignációt, az NMR jel(ek) és atom(ok) összekapcsolást?

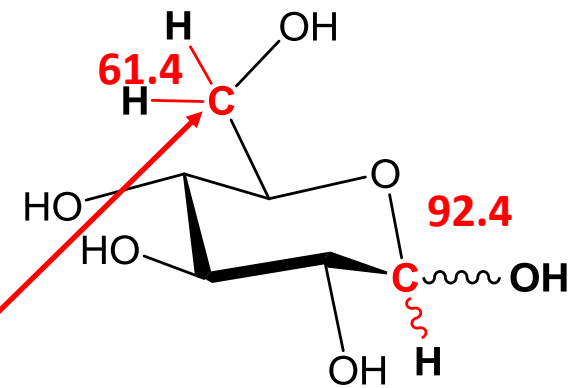
megoldás: használjunk adatbázist, s azt találhatjuk, hogy

a karakterisztikus kémiai eltolódásaik alapján a legkönnyebben azonosítható ^{1}H és ^{13}C atom a C6 és a C1, valamint a hozzájuk tartozó H atomok: H1, H61 és H62.

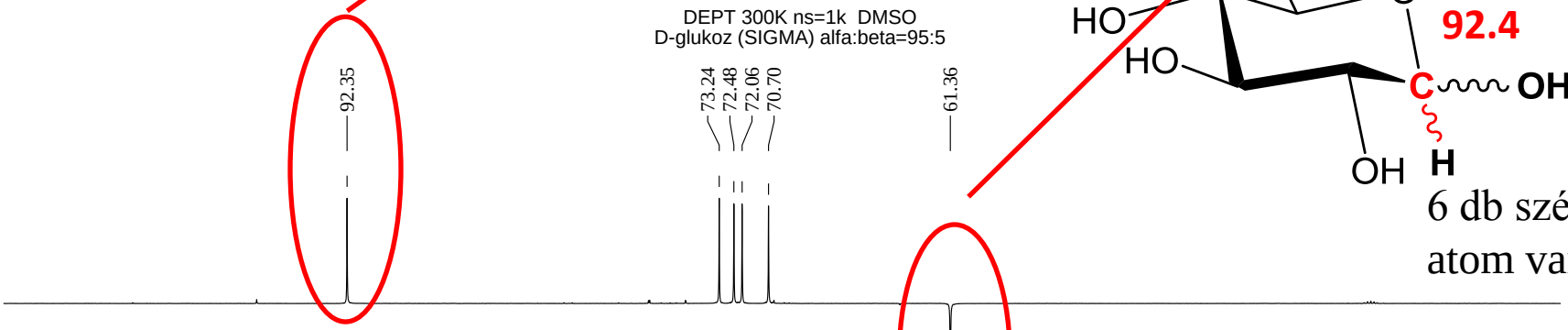


memo: noha az anomer konfiguráció nem definiált, ezért lehet akár α– akár β- annak térállása

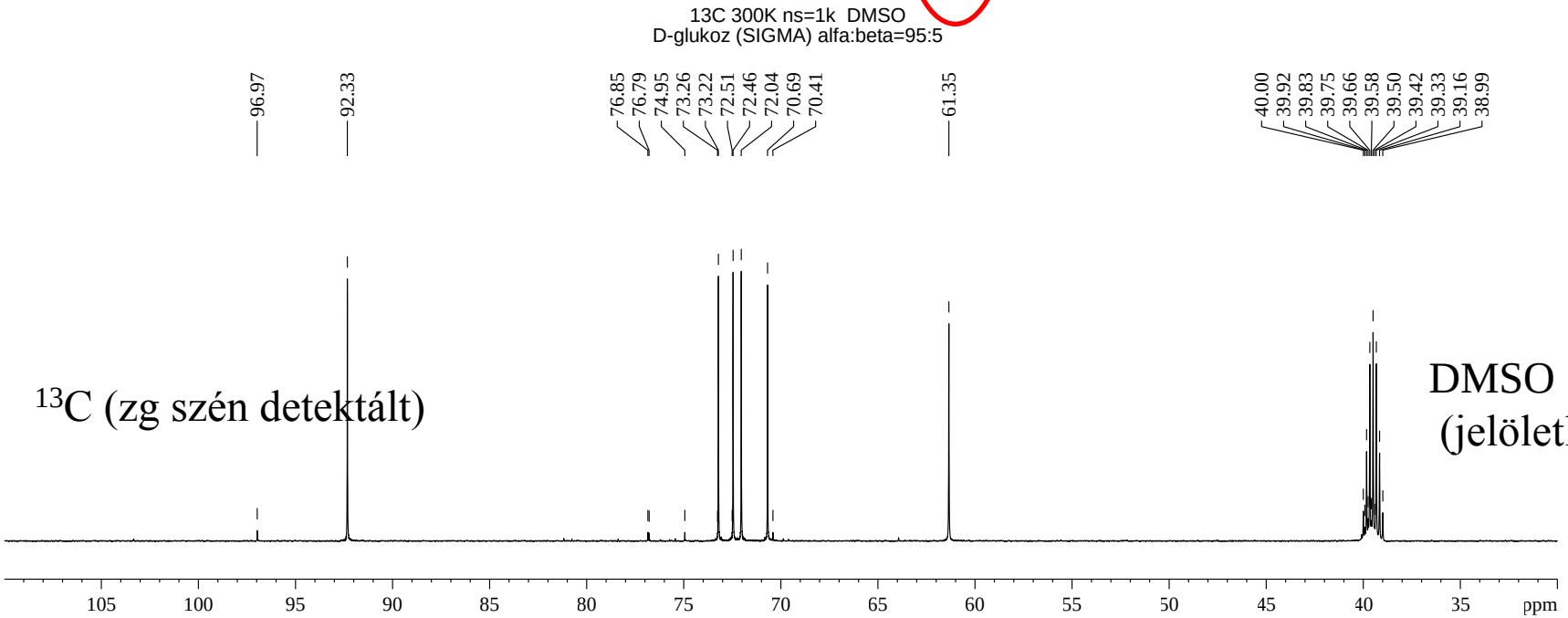
geminális helyzetű 2 O
közötti szén jellegzetes
eltolódása: 92,4 ppm



6 db szén
atom van benne



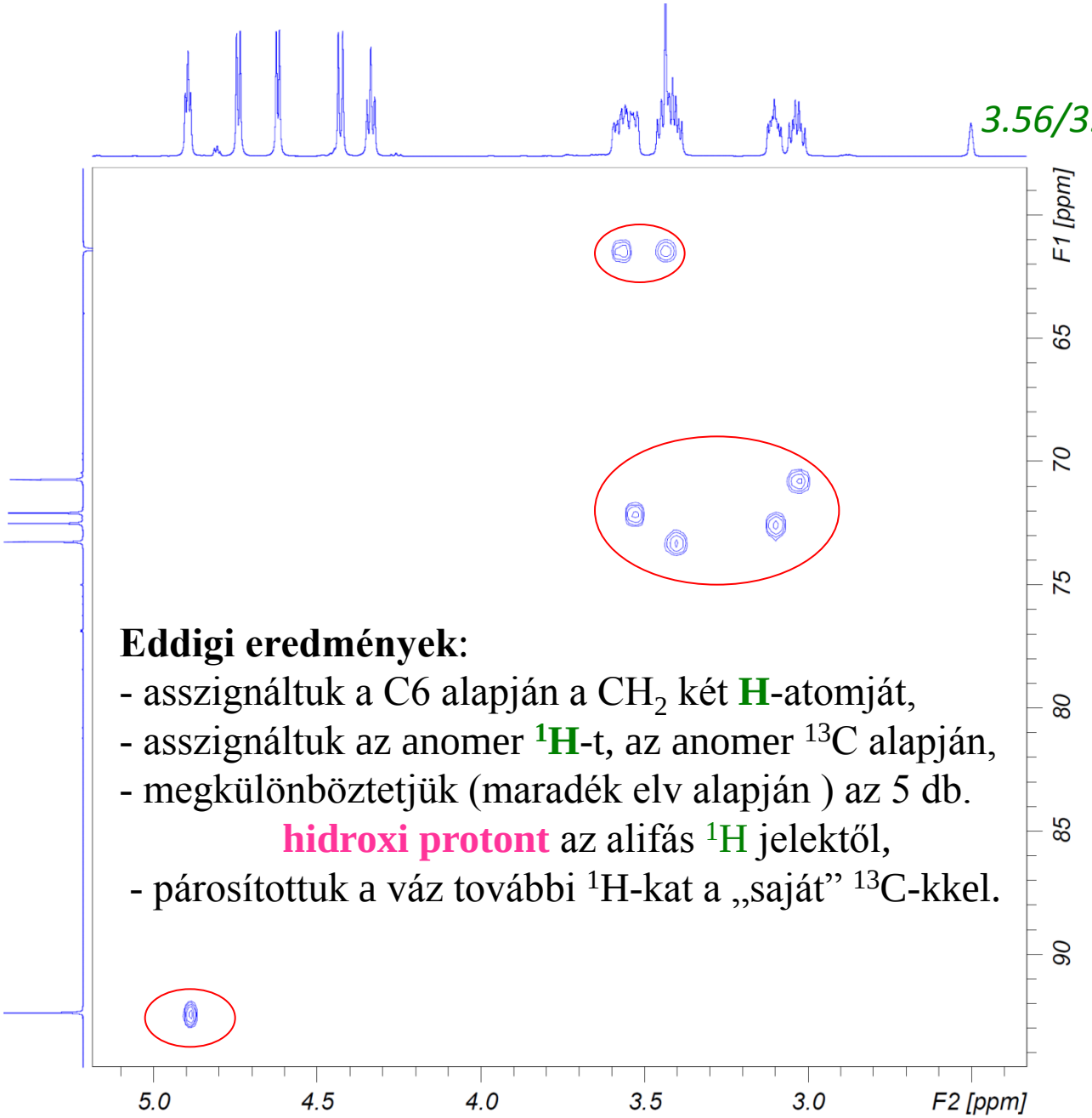
^{13}C (DEPT) alapján a CH_2 szén atom („lefelé” áll) egyértelműen azonosítható



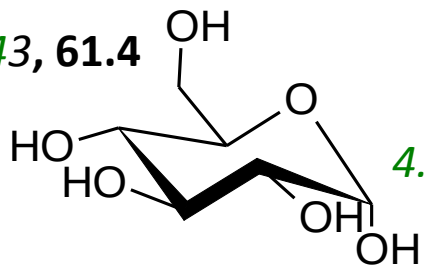
^{13}C (zg szén detektált)

DMSO
(jelöletlen)

Használjunk hetero korrelációs spektrumot (**^{1}H,^{13}C-HSQC**) a C1 és a C6 **H**-jainak azonosításához



3.56/3.43, **61.4**



4.89, **92.4**

CH:

3.03, **70.8**
3.11, **72.5**
3.41, **73.2**
3.54, **72.1**

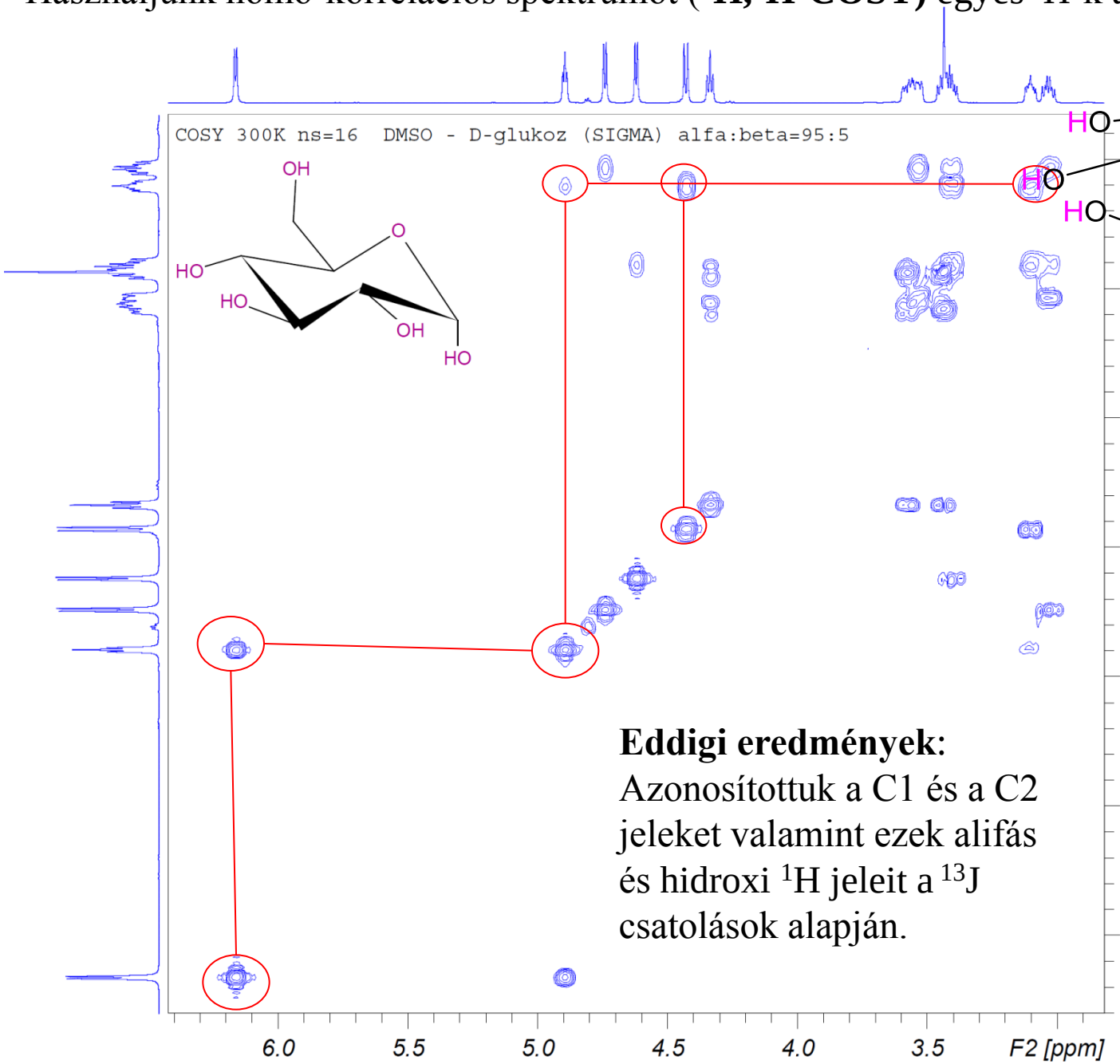
OH:

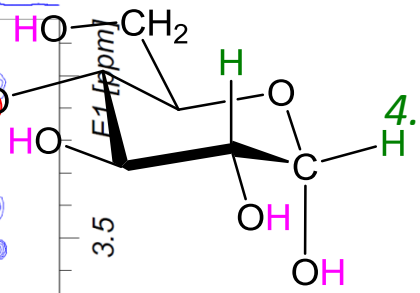
4.33
4.42
4.61
4.74
6.16

Eddigi eredmények:

- asszináltuk a C6 alapján a CH_2 két **H**-atomját,
- asszináltuk az anomer **^{1}H**-t, az anomer ^{13}C alapján,
- megkülönböztetjük (maradék elv alapján) az 5 db. **hidroxi protont** az alifás ^{1}H jelektől,
- párosítottuk a váz további ^{1}H-kat a „saját” ^{13}C-kkel.

Használjunk homo-korrelációs spektrumot (**^{1}H,^{1}H-COSY)** egyes H-k azonosításához.





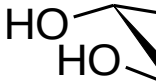
4.89, **92.4**

CH:

3.03, **70.8**
3.11, **72.5**
3.41, **73.2**
3.54, **72.1**

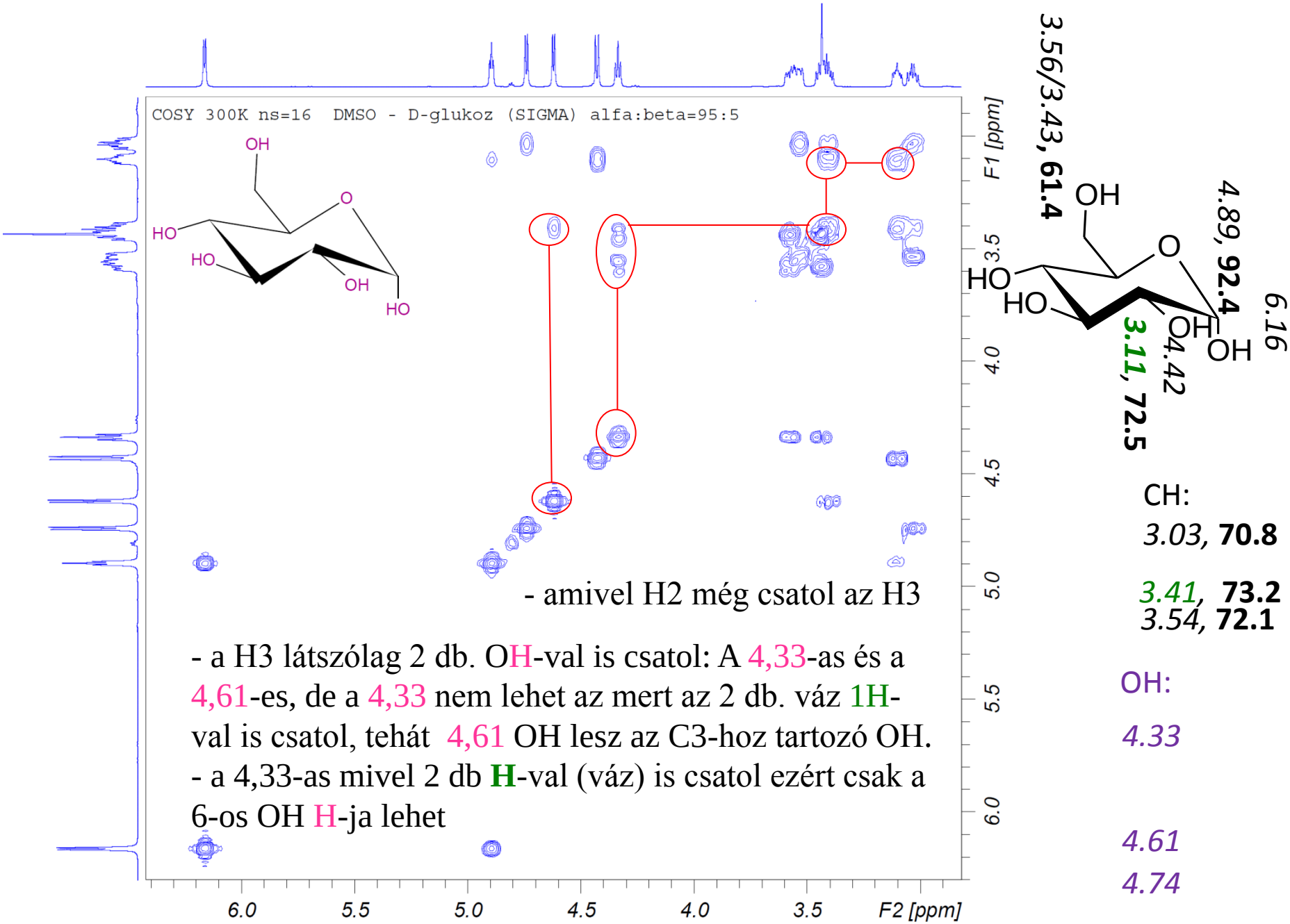
OH:

4.33
4.42
4.61
4.74
6.16



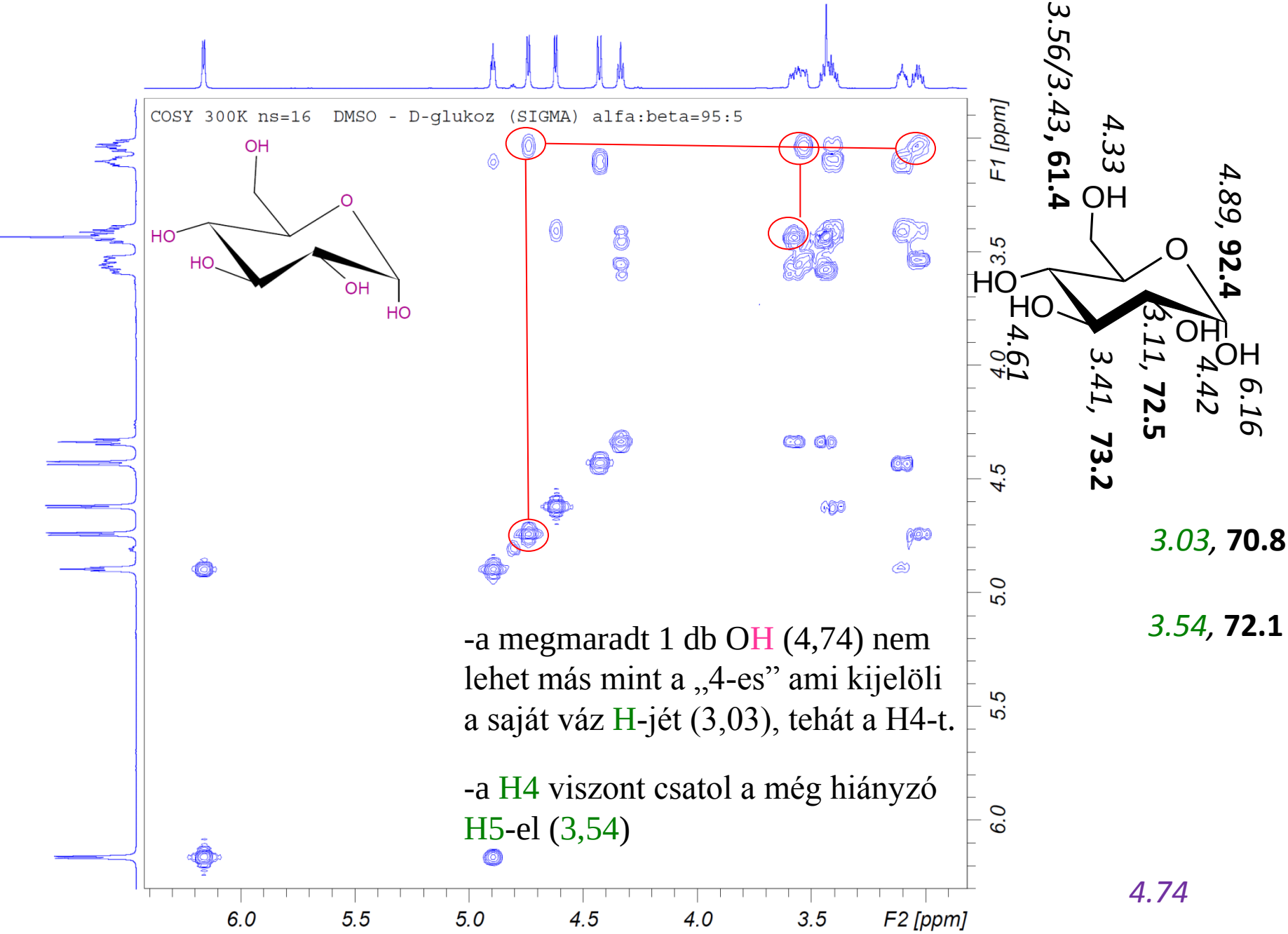
Eddigi eredmények:
Azonosítottuk a C1 és a C2 jeleket valamint ezek alifás és hidroxi ^{1}H jeleit a ^{13}J csatolások alapján.

Használjunk homo-korrelációs spektrumot (**^{1}H,^{1}H-COSY)** egyes H-k azonosításához.



- amivel H2 még csatol az H3

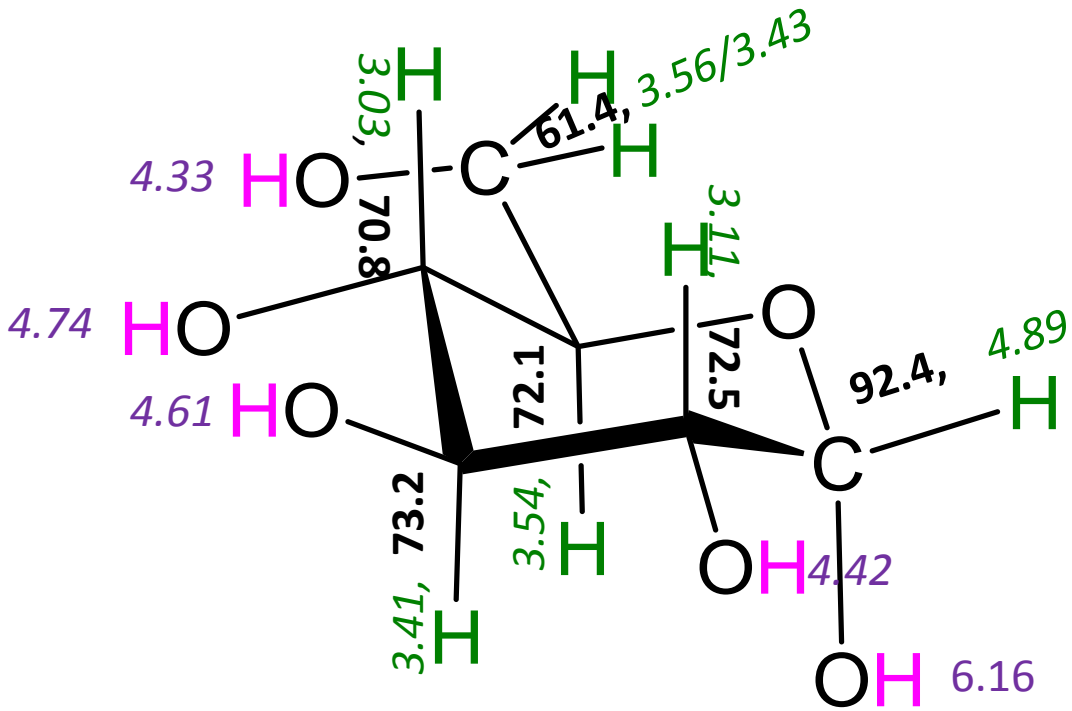
- a H3 látszólag 2 db. OH-val is csatol: A 4,33-as és a 4,61-es, de a 4,33 nem lehet az mert az 2 db. váz 1H-val is csatol, tehát 4,61 OH lesz az C3-hoz tartozó OH.
- a 4,33-as mivel 2 db **H**-val (váz) is csatol ezért csak a 6-os OH H-ja lehet



-a megmaradt 1 db OH (4,74) nem lehet más mint a „4-es” ami kijelöli a saját váz H-jét (3,03), tehát a H4-t.

-a H4 viszont csatol a még hiányzó H5-el (3,54)

A (D)-Glükópiranóz összes ^{1}H- és ^{13}C- NMR jeleit azonosítottuk, s hozzárendeltük a megfelelő atomokhoz:



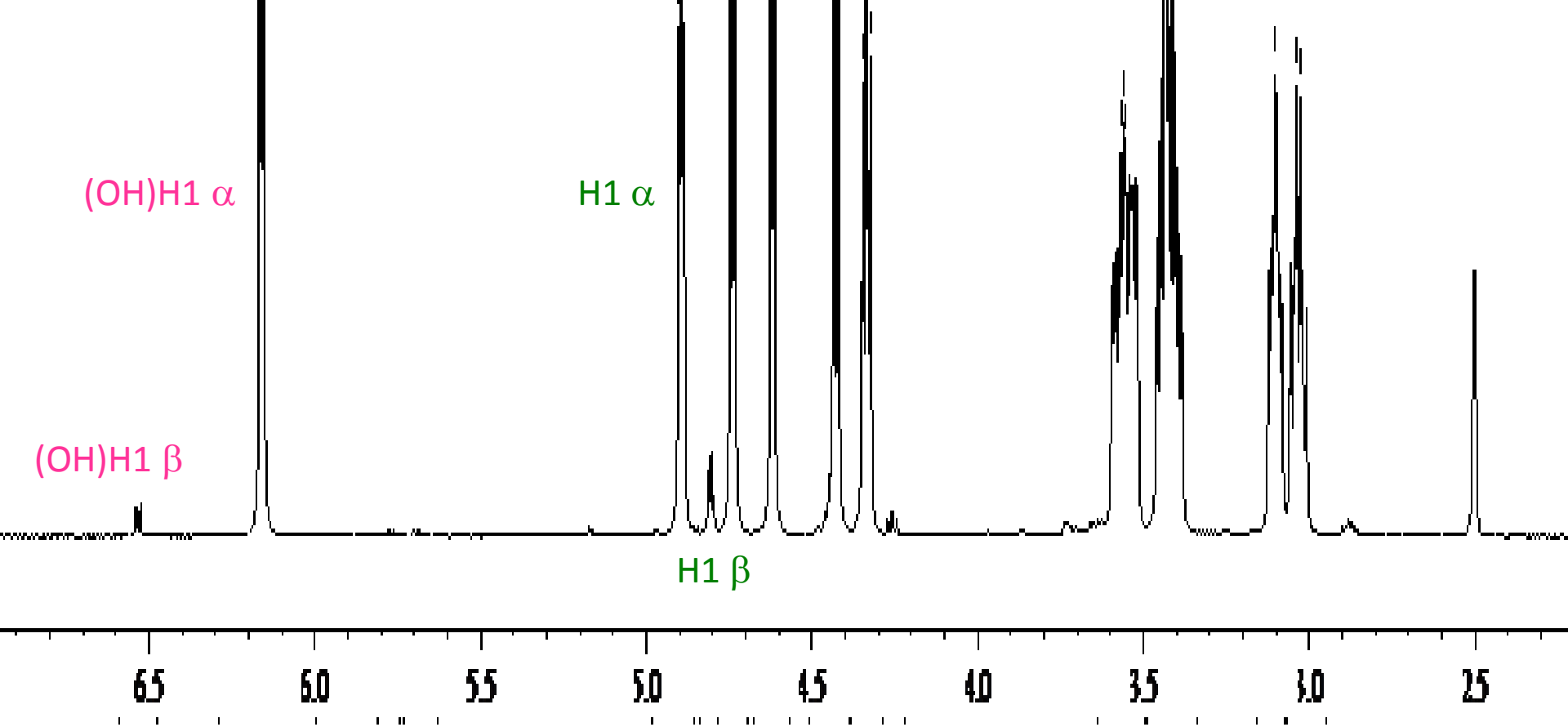
kérdés: α vagy β anomert határoztuk meg?

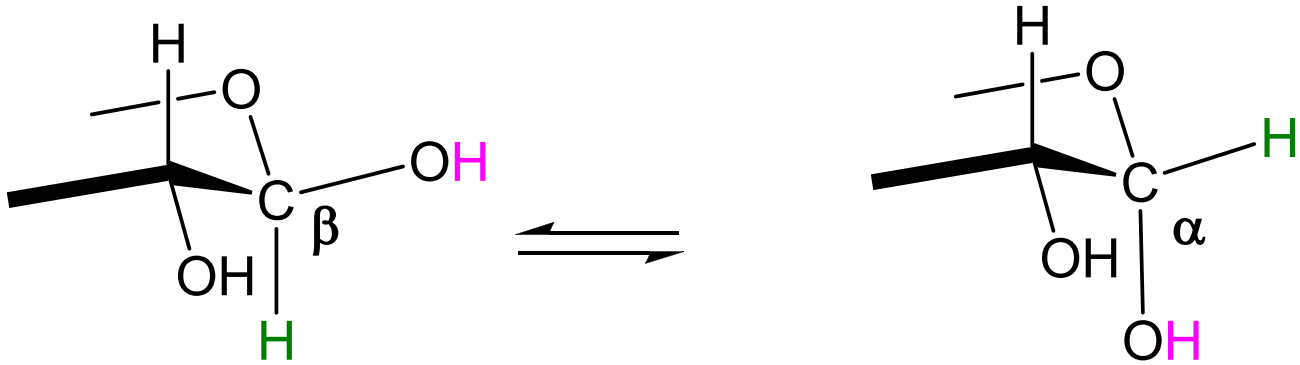
Adatok D_2O és T=303K mérve

	C1	C2	C3	C4	C5	C6
α	92.77	72.15	73.43	70.32	72.10	61.27
β	96.59	74.81	76.43	70.27	76.61	61.42

válasz: az α–anomert.

memo: mutrotációhoz savanyú proton kell, s elvben abs. DMSO-ban ilyen nincs.


(OH)H1 α
H1 α
(OH)H1 β
H1 β
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5


H
O
OH
C
β
OH
H
H
O
H
C
α
OH
OH

The Mutarotation of Glucose in Dimethyl Sulphoxide (DMSO)

Laura Lilley

8/29/2011

Glucose is the most important energy source for many organisms, existing as two isomers, α-glucose and β-glucose. When dissolved in water, these isomers react until an equilibrium mixture forms. This process is called mutarotation and can be observed by polarimetry. The objective of this research was to study the effect of various concentration of dimethyl sulfoxide (DMSO) on the equilibrium constant (K eq) and rate constant (k obs) of the mutarotation of glucose. Three sets of polarimetry experiments were performed. The first experiment was to develop an experimental method by observing the mutarotation of glucose in distilled water. The K eq was found to be 1.6 and the k obs was found to be 0.033 min -1 , both agree with the literature. The second experiment was to measure the mutarotation of glucose with varying concentrations of hydrochloric acid (HCl). The purpose of this experiment was to determine the optimal concentration of HCl such that the reaction occurred in a reasonable amount of time. The optimal concentration of HCl was found to be 0.010 M. The mutarotation reaction required between one and three hours to reach equilibrium. The third experiment involved the effect of varying concentrations of DMSO (X DMSO = 0.027, 0.20, and .83) with a constant concentration of HCl (0.010 M). The results of these reactions showed a general trend that as the concentration of DMSO increased the K eq increased; however, the values were not significantly different. The rate constant results showed that there was a significant difference for X DMSO = 0.027 (k obs 0.034) and X DMSO = 0.200 (k obs 0.011).

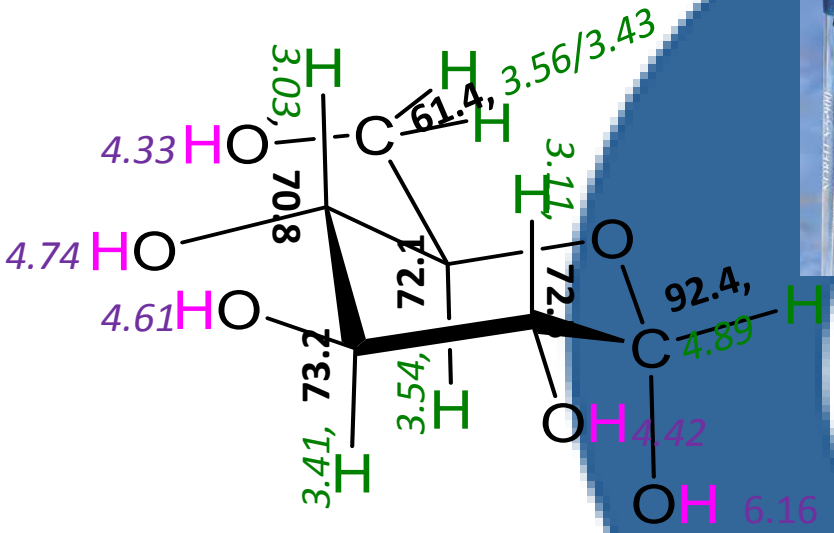
Two main conclusions can be drawn from these data:

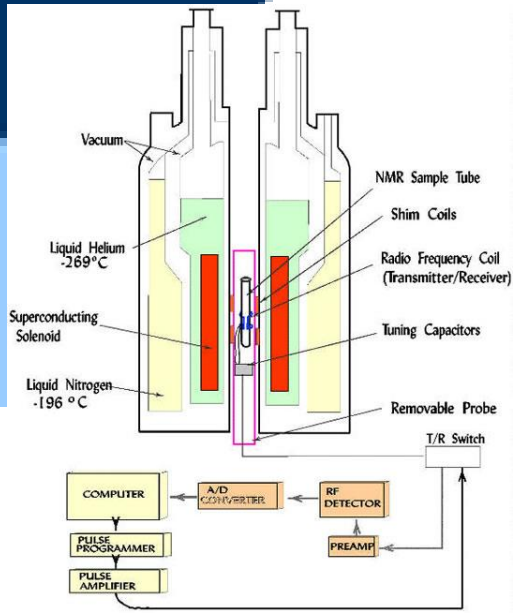
(1) **DMSO slows** the mutarotation of glucose and

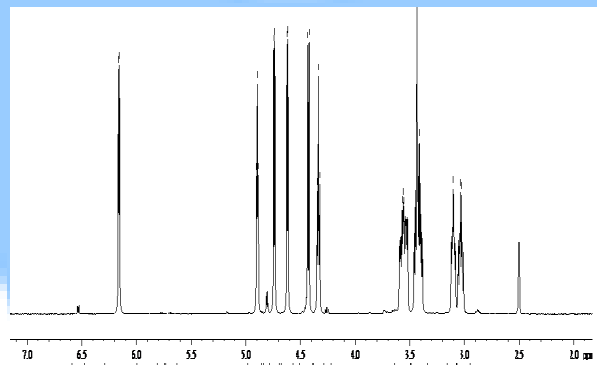
(2) **water is necessary** to stabilize the transition state of the mutarotation of glucose.

kérdés: mire jó az NMR- és az asszignált spektrum?

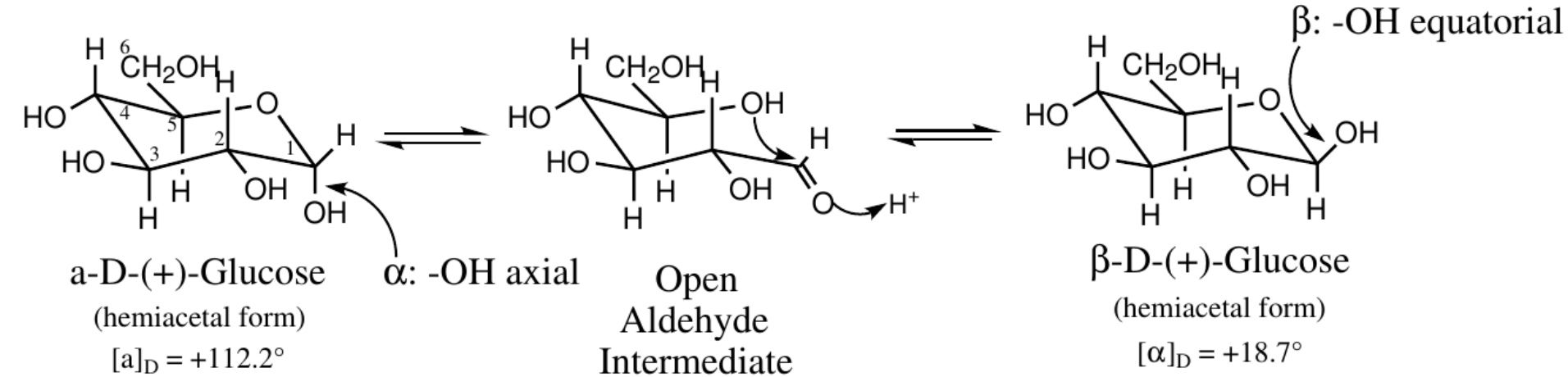
válasz : hogy „belelássunk” a molekulába: (atomi felbontás, szerkezeti információ, stb.



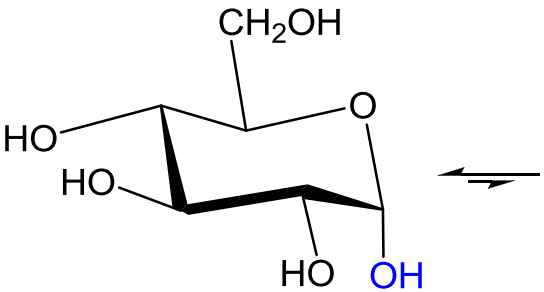




Az α-D-glükóz és a β-D-(+)-glükóz anomerek egyensúlyának nyomonkövetése 1H – és 13C NMR spektroszkópia segítségével:



Conformational Analysis of Sugars by 1H NMR Spectroscopy and Polarimetry
Adapted by R. E. Krull, M. Cocco, A. Freyer, and R. Minard from "Application of NMR to Biochemical Kinetics" by E. N. Drake and C. E. Brown, J. Chem. Ed., 54 (1977), 124-7. Revised 10/19/00



Adatok D_2O és T=303K mérve

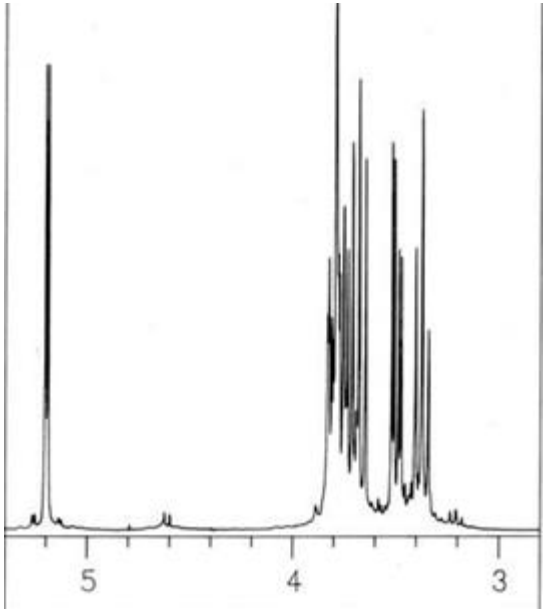
	C1	C2	C3	C4	C5	C6
α	92.77	72.15	73.43	70.32	72.10	61.27
β	96.59	74.81	76.43	70.27	76.61	61.42

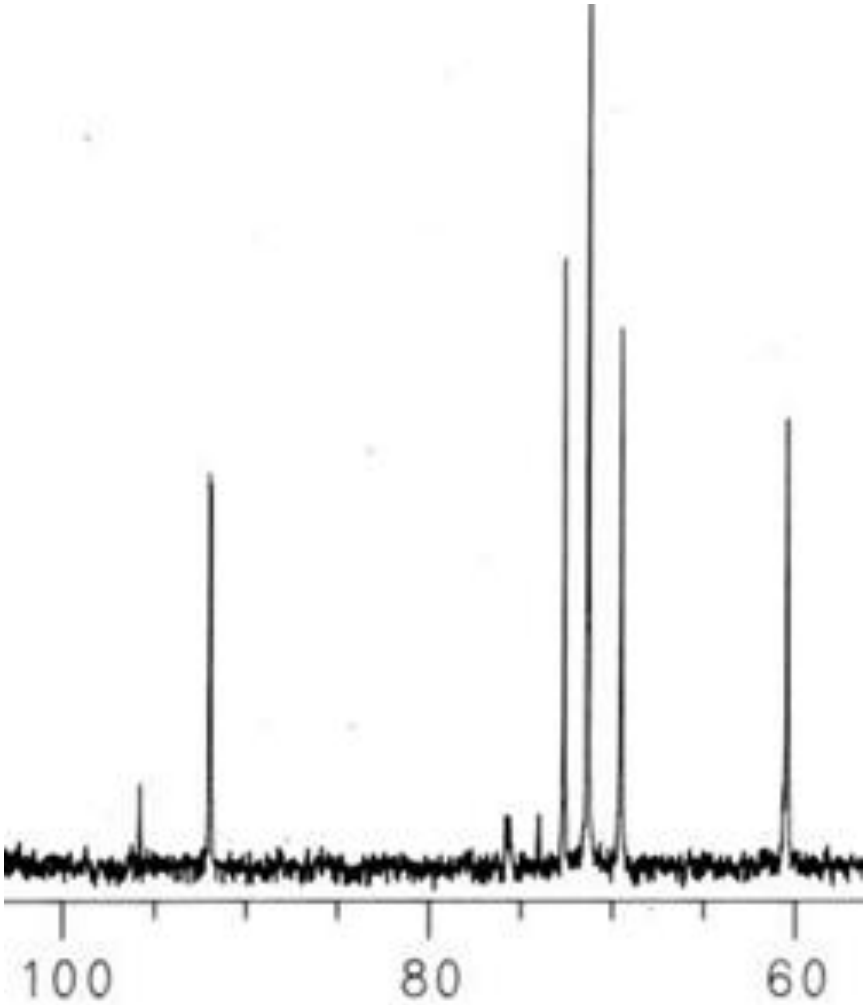
EtOH vagy H_2O-ból kristályosítva

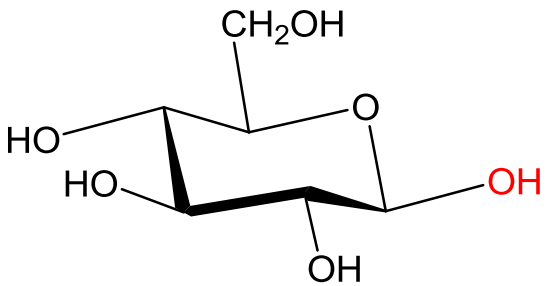
α-D-(+)-glükopiranóz

op. 146°C, $[\alpha]_D^{25}$ = **+112°**

egyensúlyi oldatban: 38 %







Adatok D_2O és T=303K mérve

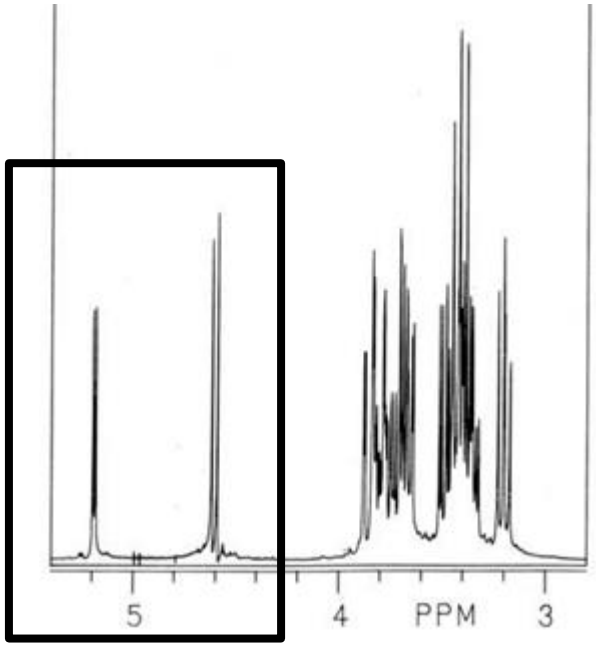
	C1	C2	C3	C4	C5	C6
α	92.77	72.15	73.43	70.32	72.10	61.27
β	96.59	74.81	76.43	70.27	76.61	61.42

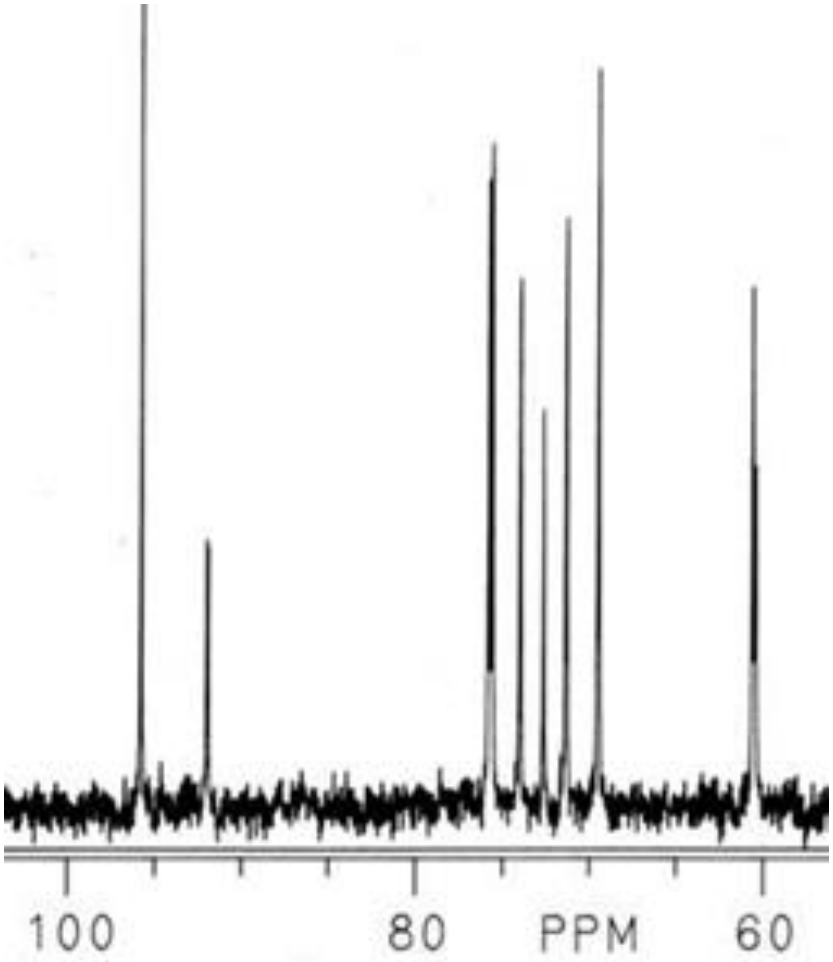
vizes AcOH-ból vagy piridin kristályosítva

β-D-(+)-glükopiranóz

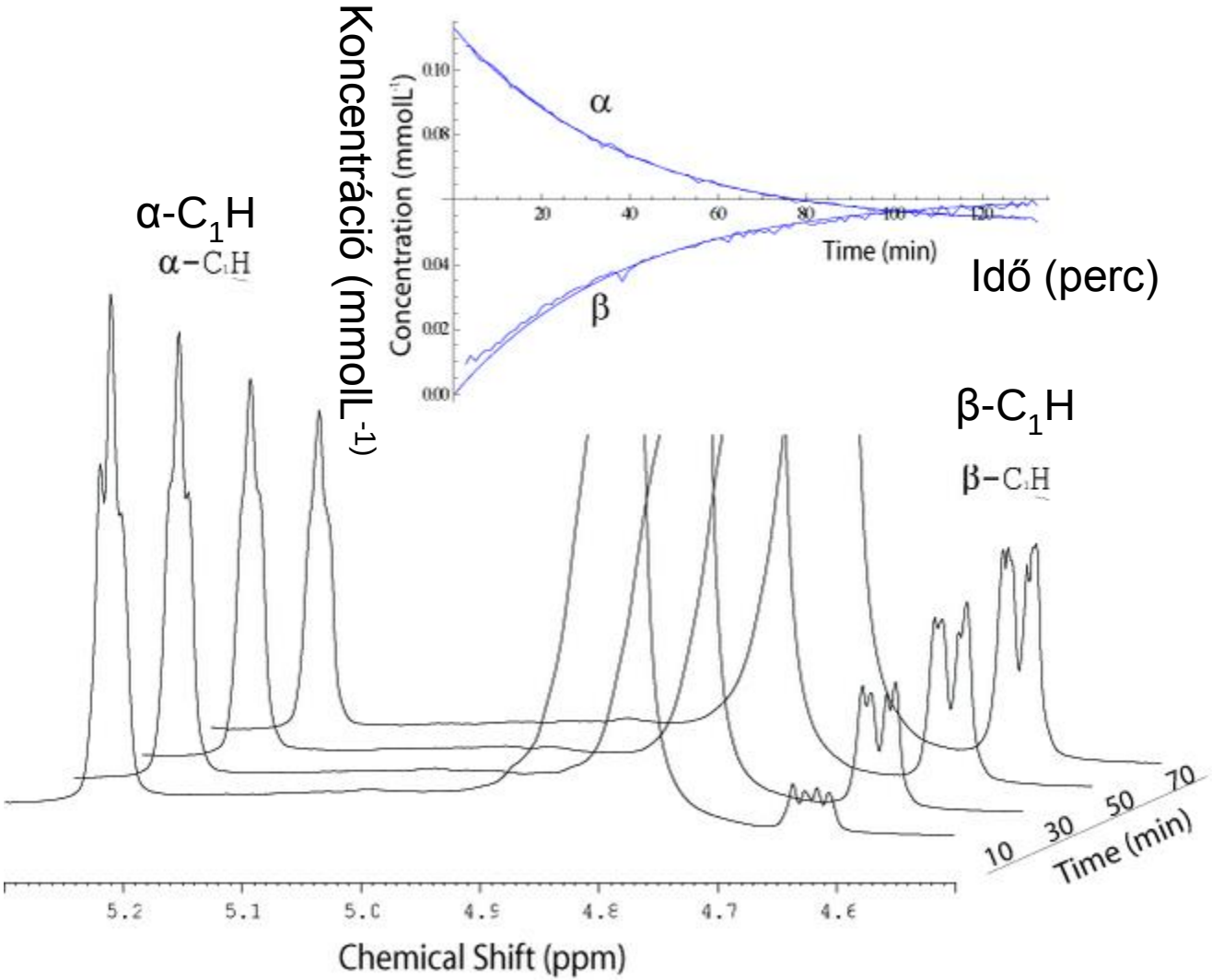
op. 150°C, $[\alpha]_D^{25}$ = **+18,7°**

egyensúlyi oldatban: 62 %

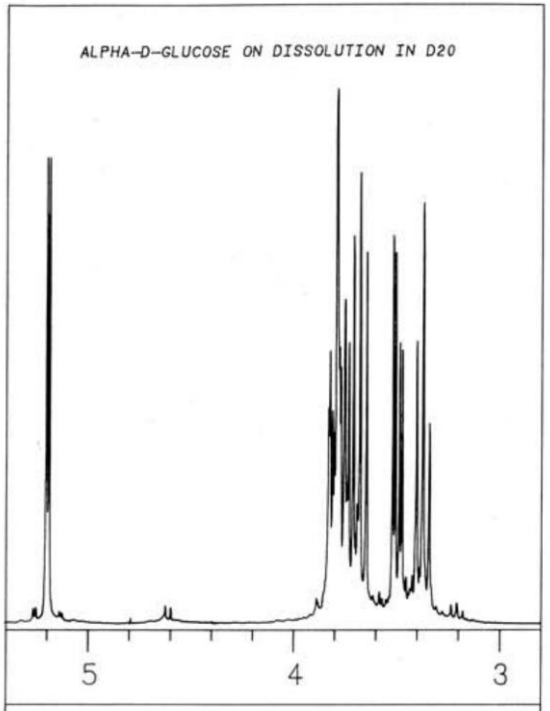




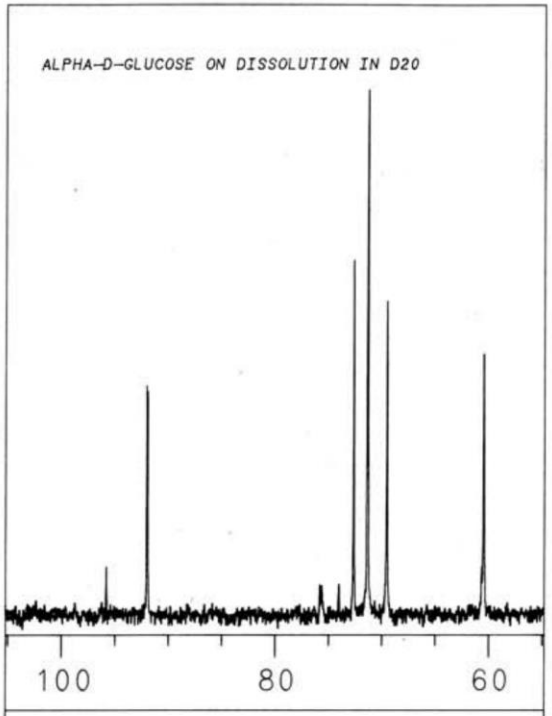
^{1}H NMR (400.13MHz) idő függő spektrumok, α-D-glükóz és β-D-glükóz mutarotációját mutatják be. T=25°C, acetát puffer pH 5.2.



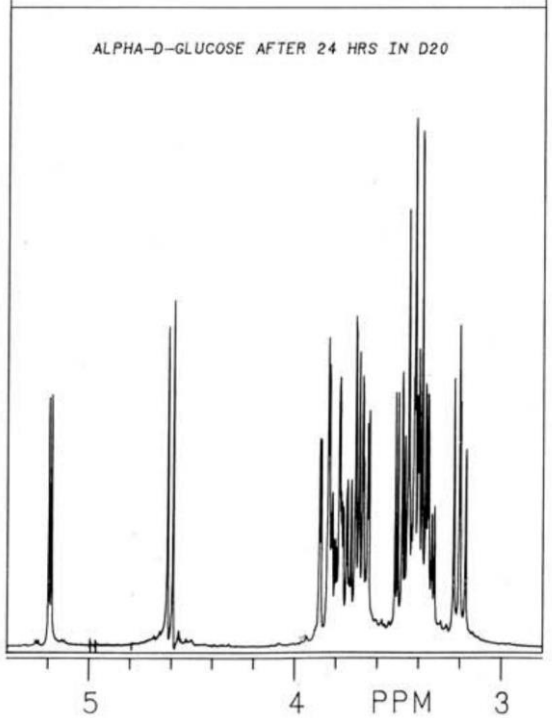
http://aups.org.au/Proceedings/38/77P/



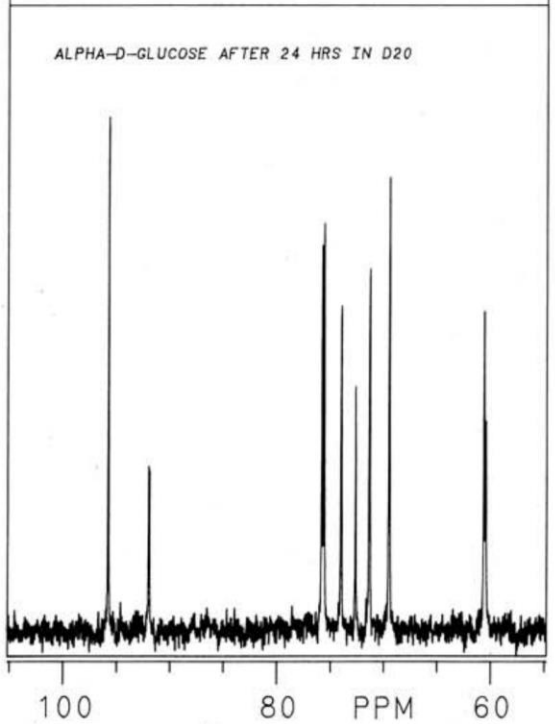
NMR and the Structure of D-Glucose,
Journal of Chemical Education, Volume 68 Number 12 December 1991, 1003-1004



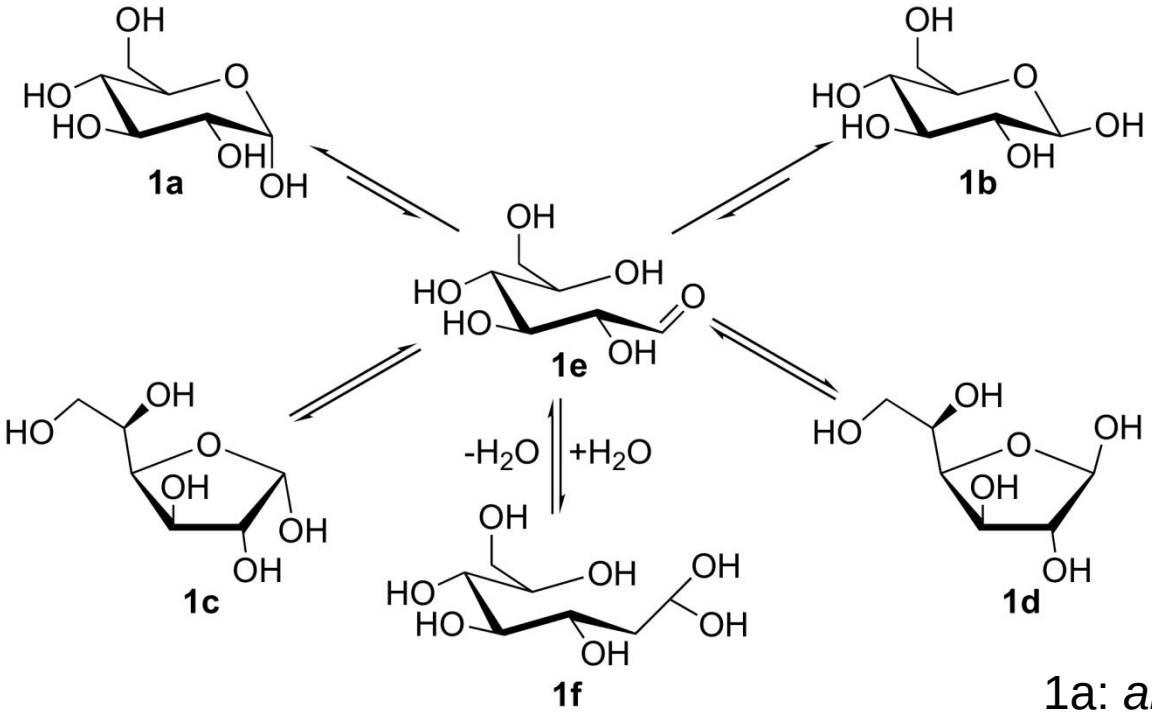
Az ^{1}H NMR és ^{13}C spektrum az α-D-(+)-glükóz esetében közvetlenül oldás után. D_2O-ban.



Az 1H NMR és 13C spektrum az α-D-(+)-glükóz esetében D_2O-ben. 24 órával az oldás után.



D-glükóz vizes oldatának jellemzése. ^{13}C NMR méréssel. (T=30°)

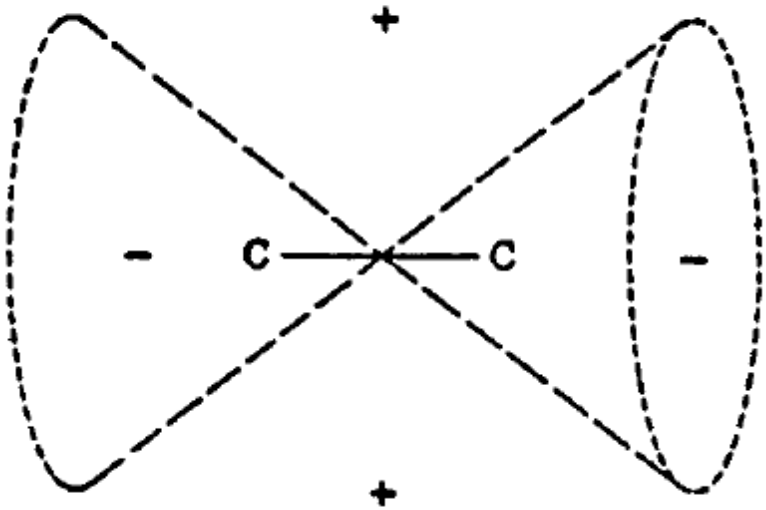


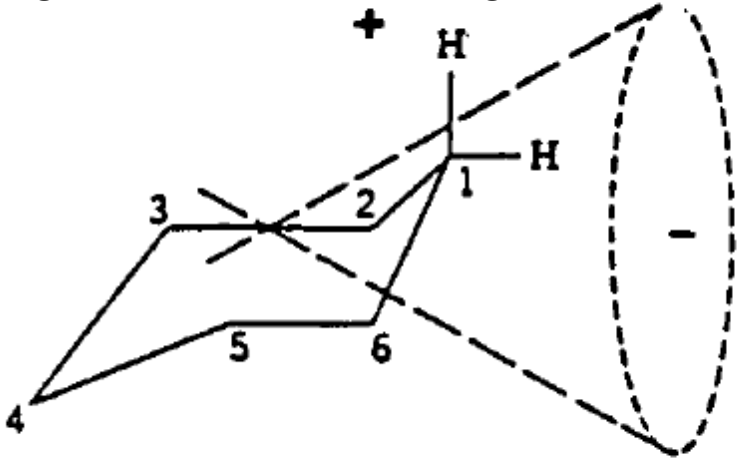
Monoszaharidok vizes oldatban ciklizációval stabilizálódnak, mind a 6 lehetséges tautomer kimutatható $^{2}H_2O$-ban ^{13}C NMR-el.

1a: *alpha*-D-glucopyranose (37.63%)
1b: beta-D-glucopyranose (61.96%)
1c:alpha-glucofuranose (0,108%)
1d: beta-glucofuranose (0,28%)
1e: D-glucose aldehyde (0,004%)
1f: D-glucose hydrate form (0,0059%)

Zhu, Y.; Zajicek, J.; Serianni, A. S. J. Org. Chem. 2001,
66, 6244–6251.

Deshielding of equatorial proton of a rigid six-membered ring:





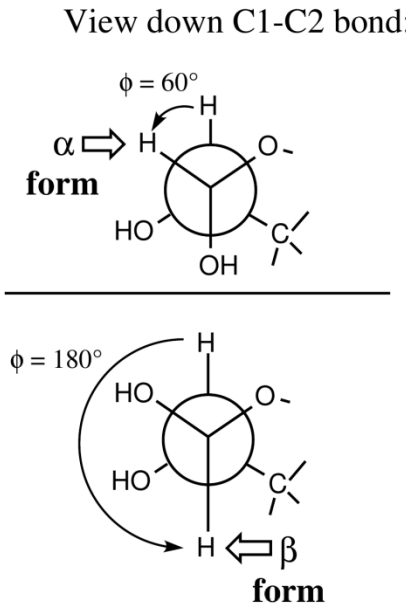
Deshielding of equatorial six-membered ring.

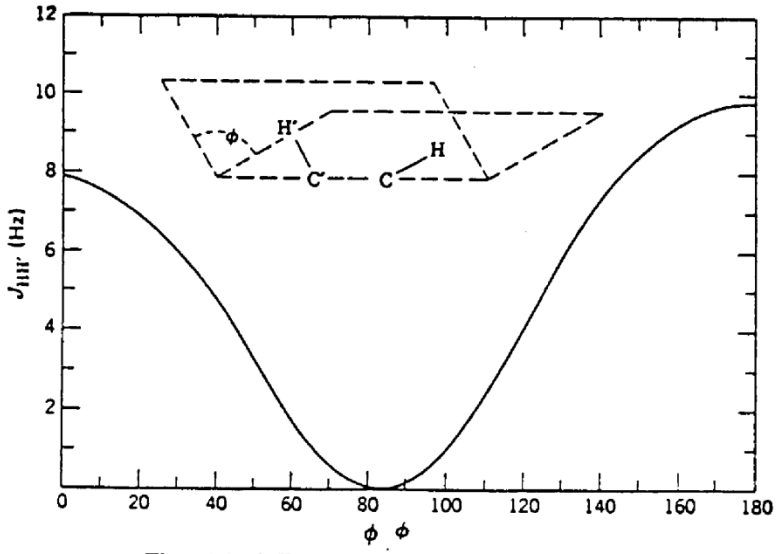
Elektromos árnyékolás:
C-C kötés mentén + és – árnyékolási zónák találhatóak.
(+) esetben “upfield shift” tapasztalható - ppm skálán negatív irányú jeleltolódást eredményez.
(-) zónába esik “downfield shift”, ppm skálán pozitív irányú eltolódás lesz.

Elektromos árnyékolás alapján eldönthető a hidrogének axiális/ekvatoriális állása.
Elektronnegatív atomoknál szintén “downfield shift” tapasztalható.
Ezzel magyarázható a glükóz α és β hidrogénjeinek sokkal magasabb eltolódása.

Csatolási állandók:
Csatolás n+1 számúra hasítja fel az NMR jelet. (n- szomszédos H-ek száma) Az így kapott csúcsok távolsága a pontos térbeli elhelyezkedéstől függ.
Ezt a Karplus egyenlet írja le:

Csatolási állandók változása a térszög függvényében:





The vicinal Karplus correlation. Relationship between dihedral angle and coupling constant for vicinal protons.

Coupling	Angle, ϕ	Calc. J (Hz)	Observed J
ax/ax	180	9 Hz	8 to 14 Hz
ax/eq	60	1.8 Hz	1 to 7 Hz
eq/eq	60	1.8 Hz	1 to 7 Hz