2014 Workshop on NMR for Glycobiology

Available glycobiology NMR experiments in Academia Sinica NMR core facility

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GRC & HFNMRC
2014.11.04

Part I : Routinely Used Experiments

Step 1: rpar (ex: std* , 1GRC*)
Step 2: getprosol
Step 3: optimize ns, o1 & sw
Step 4: rga
Step 5: zg

Or more carefully /advanced
Step 1: rpar (ex: std*, 1GRC*, 2GRC*)
Step 2: pulsecal (to find 90 deg pulse)
Step 3: getprosol 1H us db (ex: getprosol 1H 10 3.1)
Step 4: optimize ns, o1 & sw
Step 5: rga
Step 6: zg

1 D 1H Experiments

Experiments	Experiment Details	Note
1D_1H-ZG_zg30	1H NMR using 30 deg pulse	Most useful
1D_1H_zggpw5	1H NMR with solvent suppression	Best suppression

1 D 13C Experiments

Experiments	Experiment Details	Note
1D_13C-ZG_zgpg30	13C NMR using 30 deg pulse with 1H decoupled	Most useful
1D_13C_udeft	13C NMR with 1H decoupled	2006 Improved version
1D_13C-ZG-couple_zggd30	13C NMR using 30 deg pulse with 1H coupled	For measuring coupling constant

2D 1H-1H Experiments

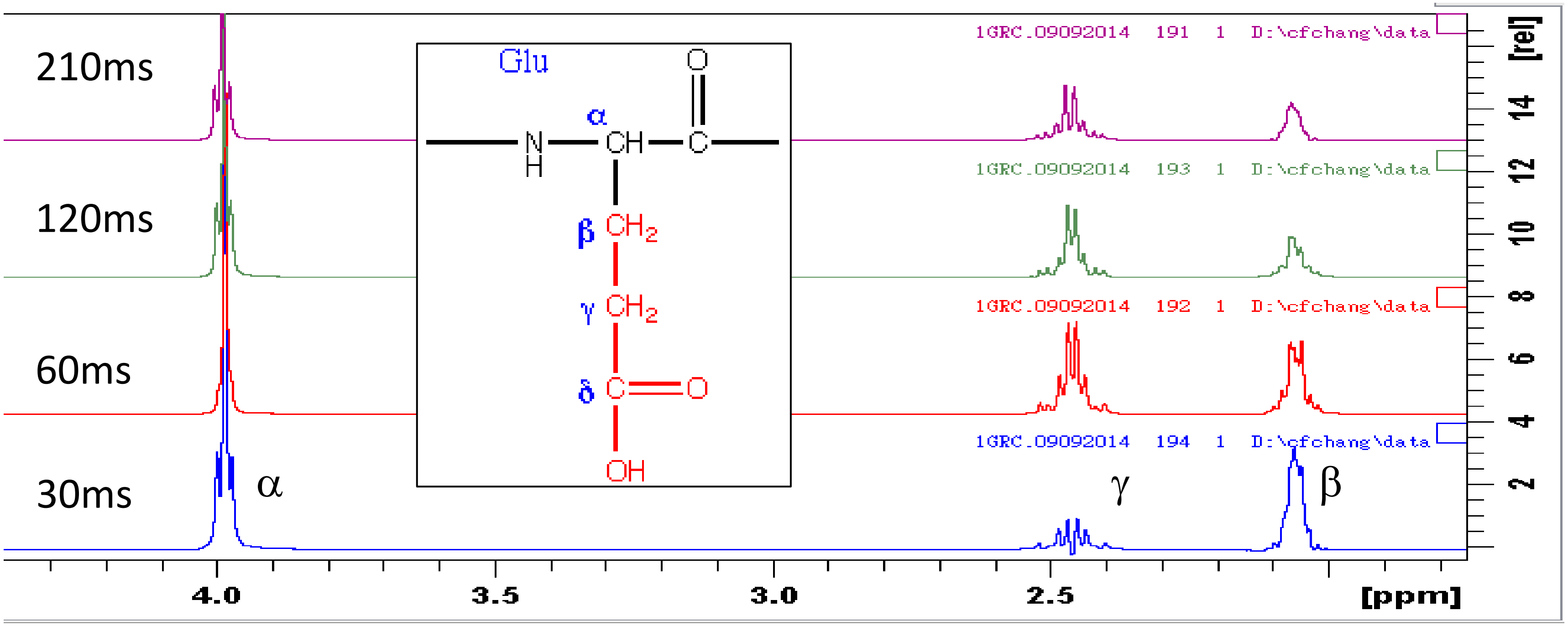
Experiments	Experiment Details	Note
2D_COSY_cosygpppqf	1H-1H COSY	Most useful
2D_TOCSY_dipsi2etgpsi	1H-1H TOCSY	Most useful
2D_NOESY_noesygpphpp	1H-1H NOESY	Most useful
2D_ROESY_roesyetgp	1H-1H ROESY	Most useful

2D 1H-13C Experiments

Experiments	Experiment Details	Note
2D_HSQC_hsqcetgpsisp2.2	1H-13C HSQC	Most useful
2D_HSQC-editing_hsqcedetgpsisp2.2	1H-13C edited HSQC	d21= 1/(2J(YH)): YH, YH3(+), YH2 (-) d21= 1/(4J(YH)): YH2 only
2D_HMBC_hmbcgplpndqf	1H-13C HMBC	Most useful
2D_HMBC_hmbcetgpl3nd	1H-13C HMBC J-filter to suppressed one-bond	Good for "clean" spectrum

Tips on 2D TOCSY & NOESY/ROESY

Experiment	Mixing Time	Note
TOCSY	D9=20-100ms	Longer mixing time, more distant protons can be observed , but… adjacent one might become weaker



Tips on 2D TOCSY & NOESY/ROESY

Experiment	Mixing Time	Note
TOCSY	20-100ms	Longer mixing time, more distant protons can be observed , but… adjacent one might become weaker
NOESY / ROESY	Depends on MW	Need to pay attention on spin diffusion

Molecular Weight	Experiment	Mixing Time	Note
MW<600	NOESY	>500ms (ex:500ms)	Diagonal peaks + Crossed peaks -
600<MW<1500	ROESY	100ms~800ms (ex: 250ms)	All positive
Mw>1500	NOESY (ROESY suffer less spin diffusion but less sensitive too)	50ms~200ms (ex: 120ms)	All positive

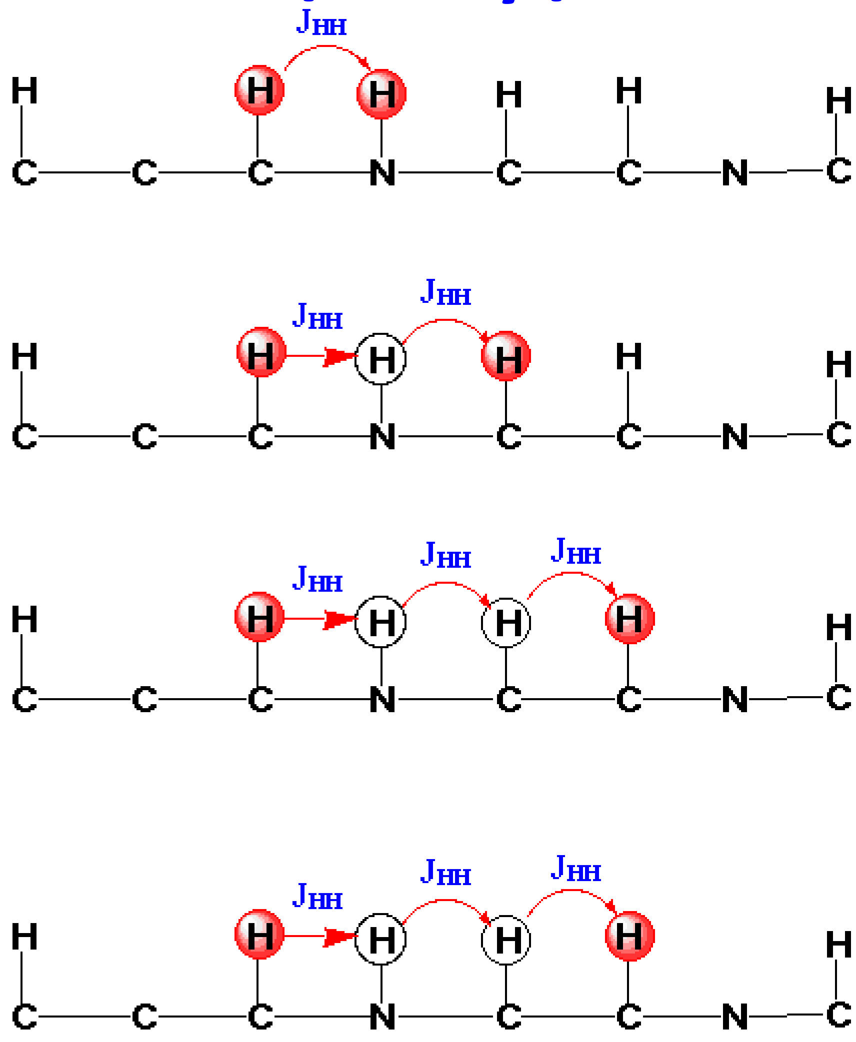
2D COSY /Relay /TOCSY

COSY
(cosyqf)

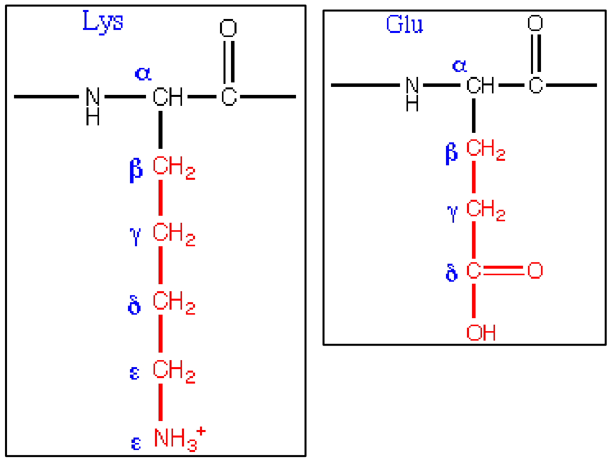
COSY-r1
(cosyqfr1)

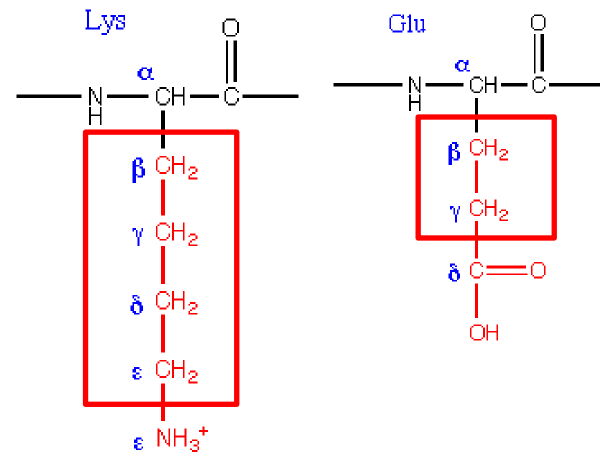
COSY-r2
(cosyqfr2)

TOCSY

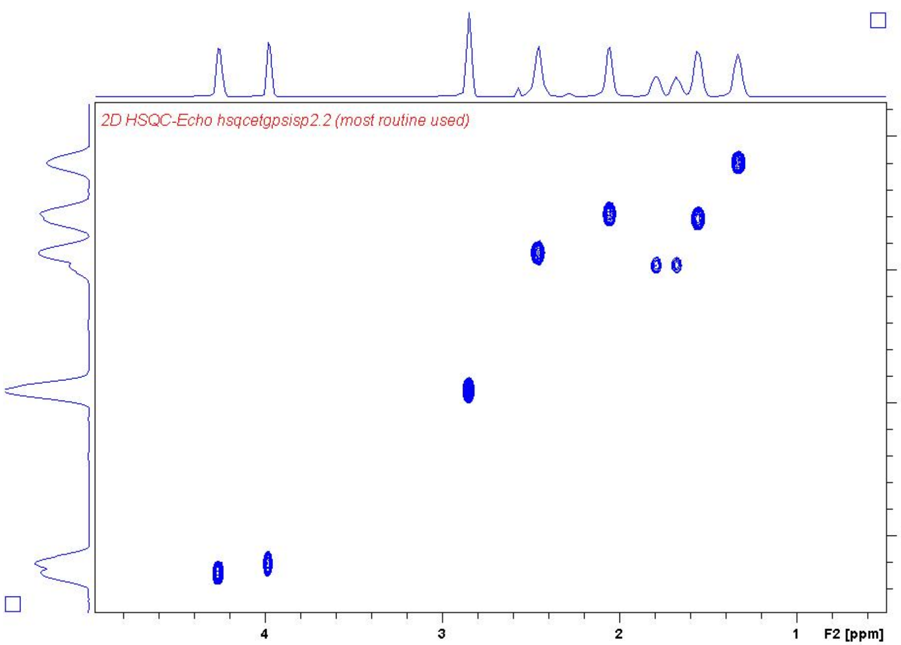


Example on different version HSQC

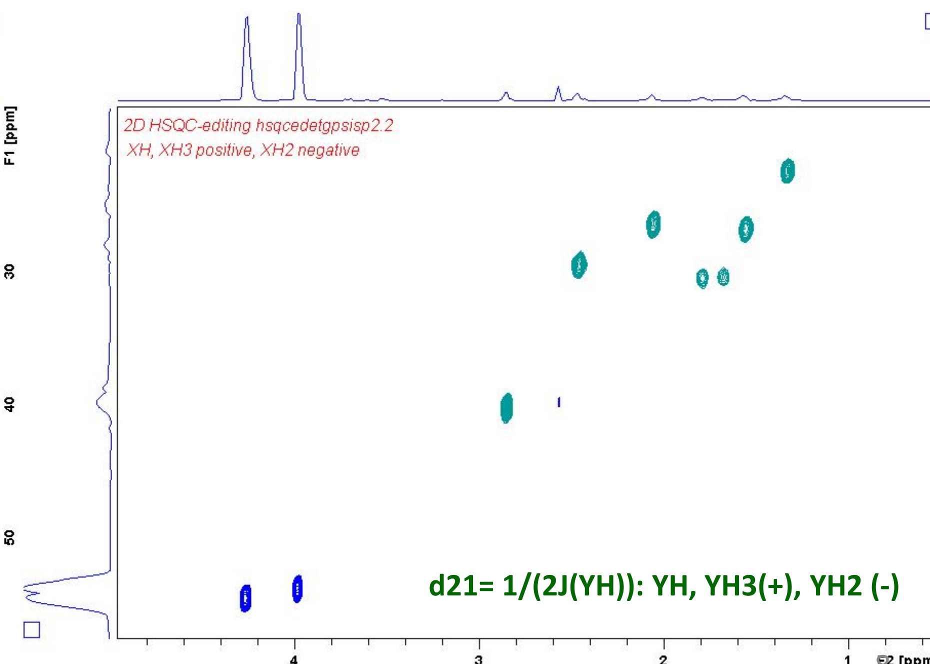




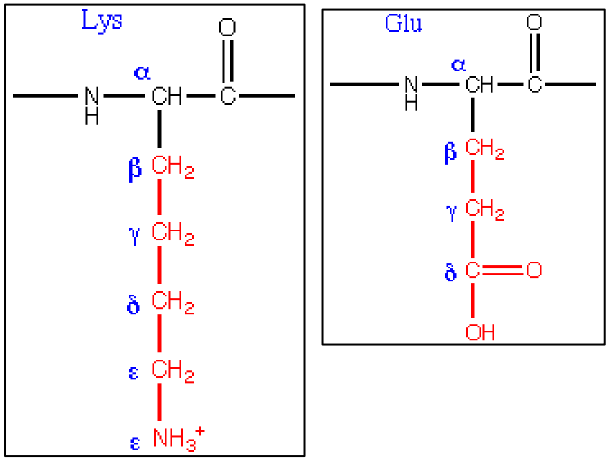
2D_HSQC_hsqcetgpsisp2.2

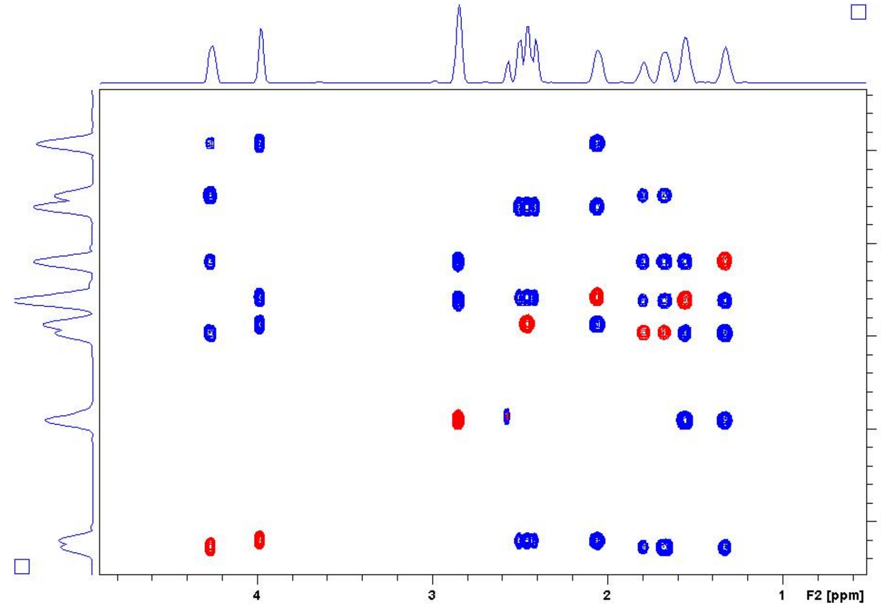


2D_HSQC-editing_hsqcedetgpsisp2.2

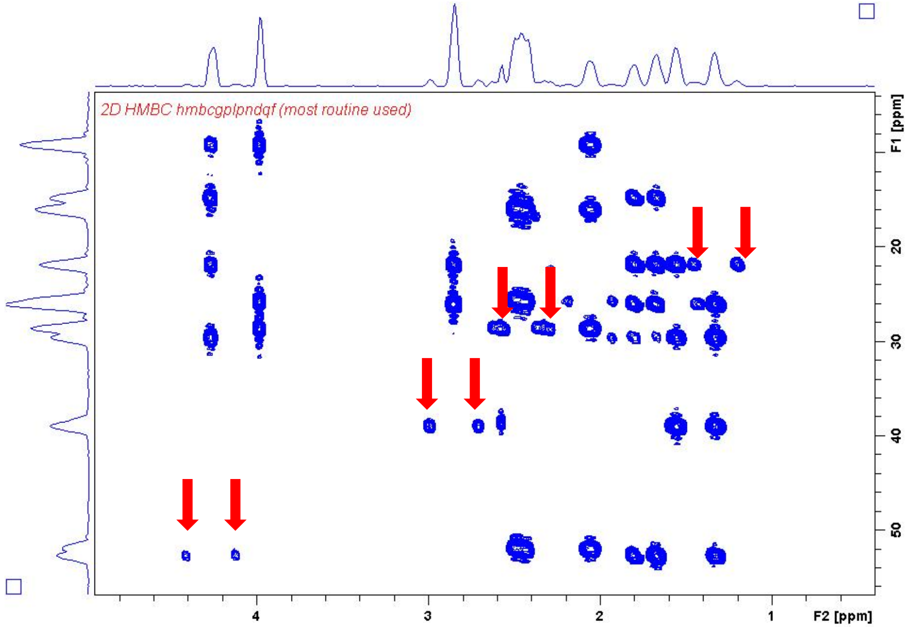


Example on different version HMBC

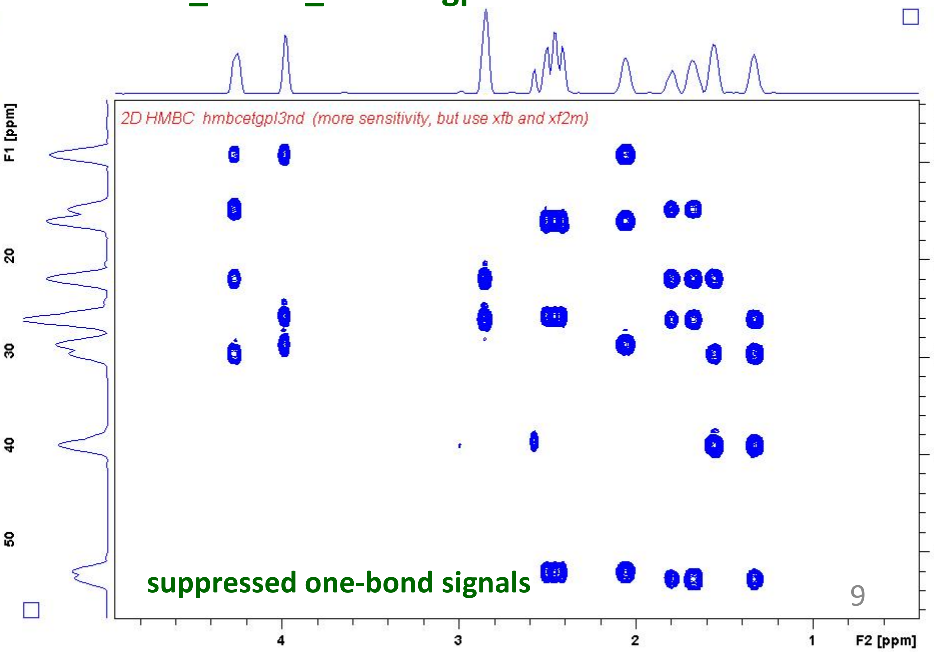




2D_HMBC_hmbcgplpndqf

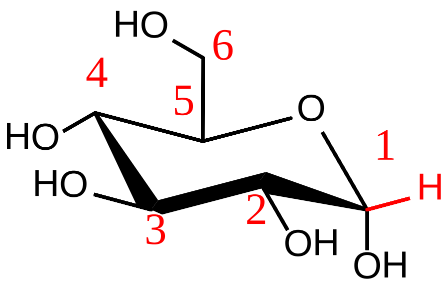


2D_HMBC_hmbcetgpl3nd

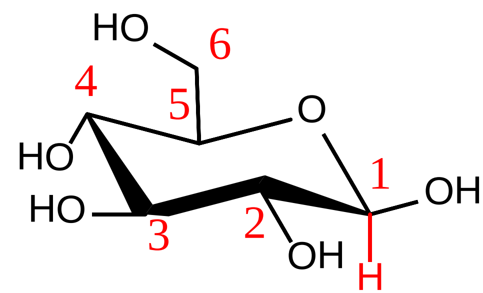


Part II: Experiments to help identify "anomer"

α or β ?

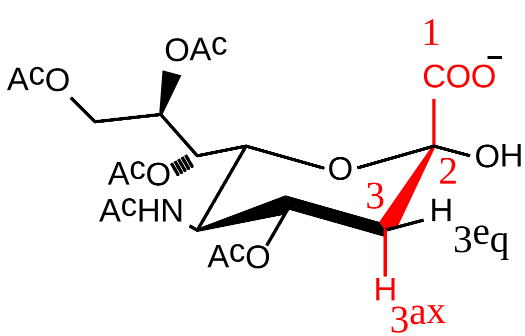


saccharides

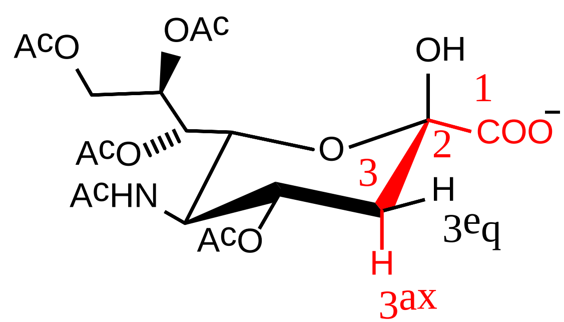


α-anomer

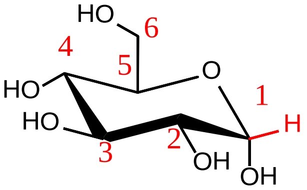
β-anomer



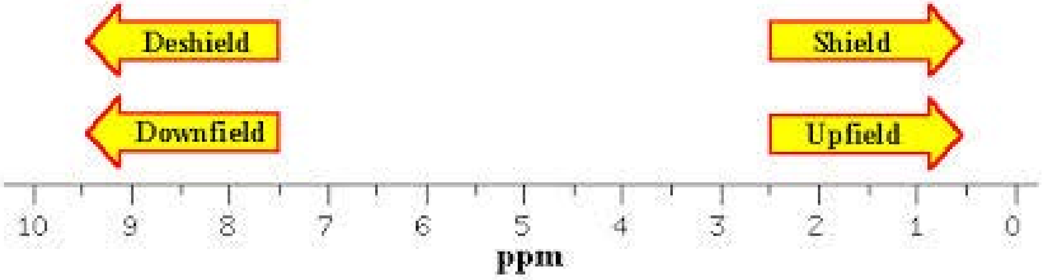
sialic acid



For saccharides



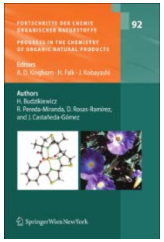
- In D-pyranoses in $^{4}C1$ conformation, the 1H for α-anomer resonance appears Downfield in comparison with the β-anomer



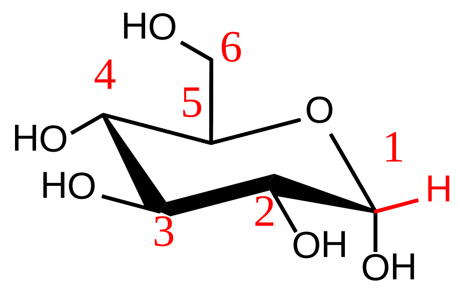
- *The vicinal coupling constant between the anomeric* H-1 and H-2 protons indicates their relative orientation, i.e., a large coupling constant value (^{3}J = 7-8 Hz) for an axial orientation and smaller values for the axial-equatorial (^{3}J = 4 Hz) or equatorial-equatorial (^{3}J = <2 Hz) ones.

- The 13C-1H ($^{1}J_{CH}$) coupling constant is a more reliable criterion to determine conclusively the anomeric configuration in pyranoses. For D-sugars in the $^{4}C1$ conformation, the α-anomeric configuration has a 1JCH value of 170 Hz, which is 10 Hz higher than that observed (1JCH = 160 Hz) for the β-anomer . This difference is reversed for L-sugars.

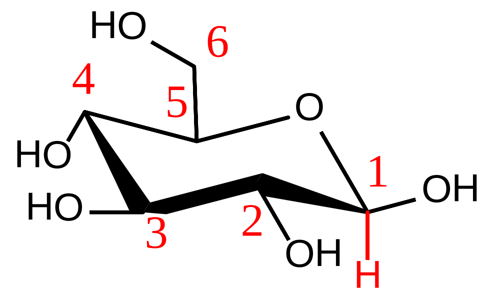
Reference:
Fortschritte der Chemie organischer Naturstoffe
Progress in the Chemistry of Organic Natural Products
Volume 92, page 126



For saccharides



α-anomer

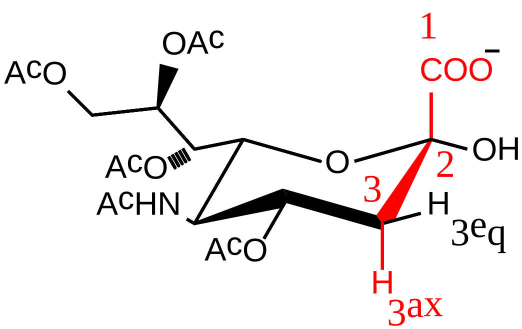


β-anomer

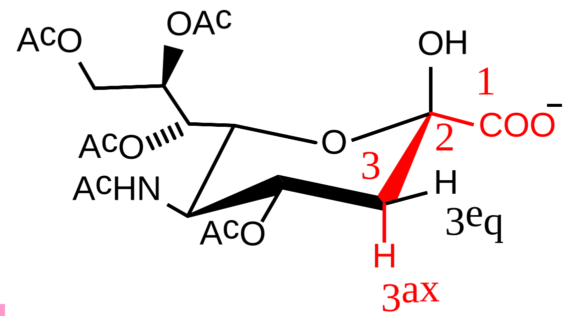
Saccharide Anomer	α	β
1H chemical shift	Downfield (larger)	Upfield (smaller)
$^{3}J_{1H\text{-}2H}$	< 4Hz	~7-8 Hz
$^{1}J_{1H\text{-}1C}$	~170Hz	~160Hz
13C Chemical Shift	Upfield (smaller)	Downfield (larger)

For Sialic Acid

α-anomer



β-anomer

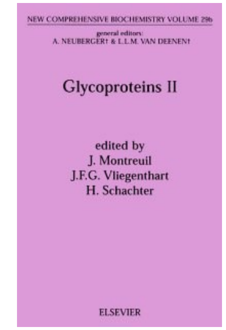


Saccharide Anomer	α	β
1H chemical shift		
$^{3}J_{1H\text{-}2H}$		
$^{1}J_{1H\text{-}1C}$		
13C Chemical Shift		

Cannot be applied!!

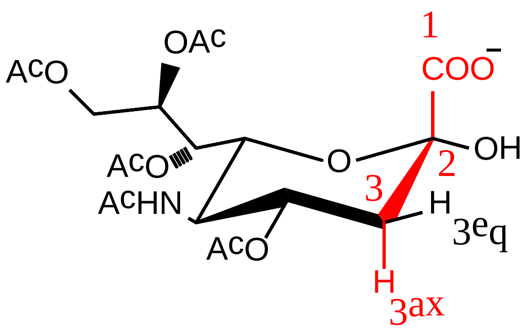
For Sialic Acid

In more biophysical studies, several aspects of sialic acids have been investigated by NMR spectroscopy. Although so far mainly α-forms of bound sialic acid have been detected, good differentiation systems for α- and β-forms are essential, and a number of empirical rules have been reported [336]. In a heteronuclear 2D-approach it could be demonstrated that the determination of the geminal C,H coupling constant ^2J(C2,H3a) offers a unique criterion for the anomeric assignment in sialic acid glycosides (α, –8 Hz; β, –3 to –4 Hz) [337]. Also the values of the vicinal C,H coupling constants ^3J(C1,H3a) can be applied for this differentiation (α, ~6 Hz; β, ~1 Hz) [55,336,338]. More details with respect to anomeric determinations have been reviewed in ref. [339].

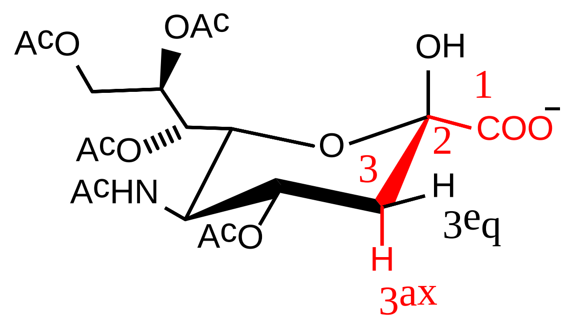


Reference:
Glycoprotein II, page 289

α-anomer



β-anomer



Sialic Acid Anomer	α	β
$^3J_{C1\text{-}H3ax}$	**~6 Hz**	**~1Hz**
$^2J_{C2\text{-}H3a}$	~-8Hz	~-3, -4Hz

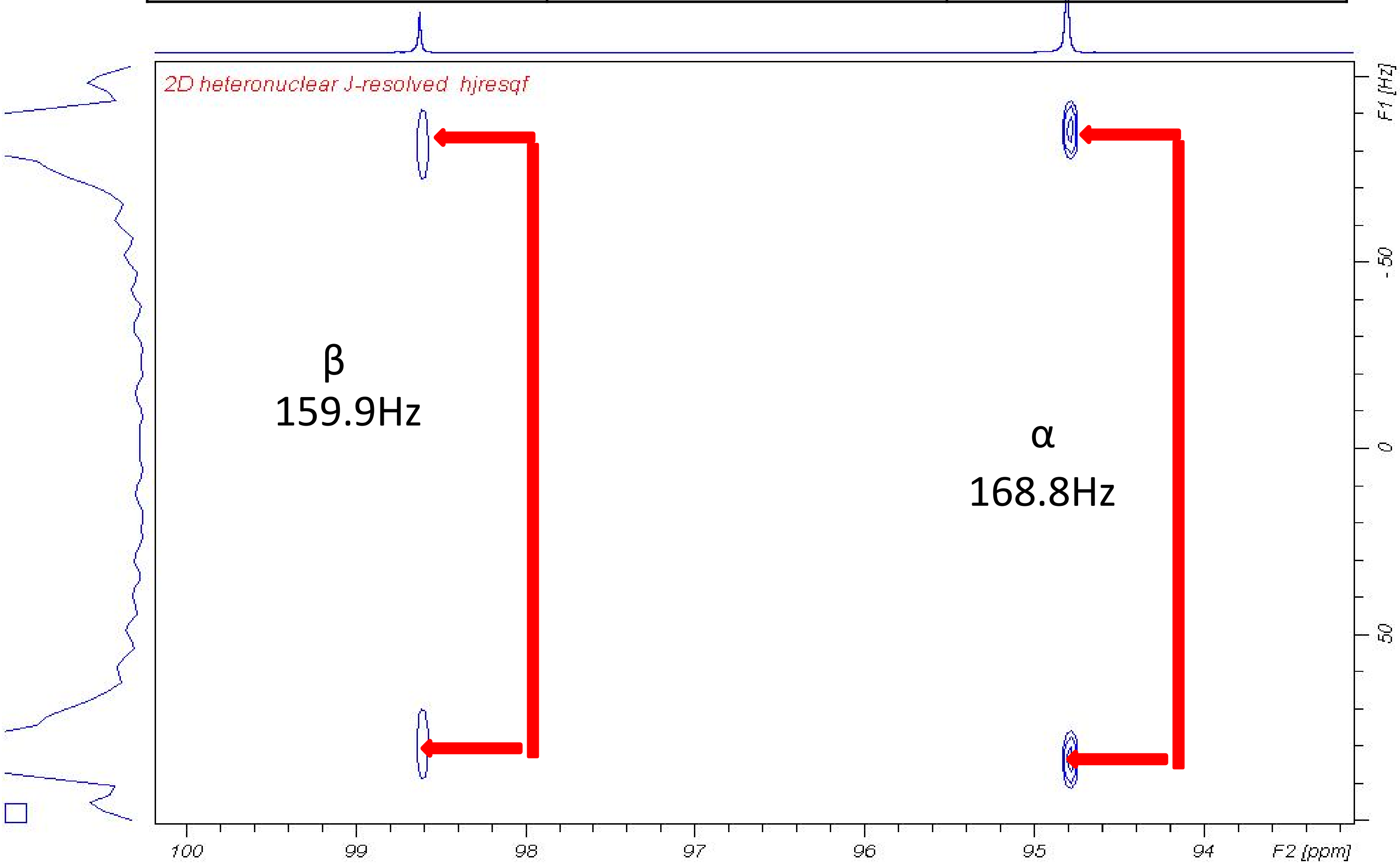
Experiments to help identify "anomer"

2D Experiments for Coupling Constant

Experiments	Experiment Details	Note
2D_HomoJres_jresqf	For determination of HH coupling	1H-1H
2D_HeterJres_hjresqf	For determination of CH coupling	13C detection
2D_HSQC-nodec_hsqcetgpsisp.cf	For determination of XH one bond coupling	1H-13C Most useful
2D_HeterJres-sel_hjresqf_inept.cl	For determination of XH long range coupling	13C detection Good for long range CH
2D_CPMG_HSQMBC_hsqcetgpjclrndxy	For determination of XH long range coupling	1H-13C

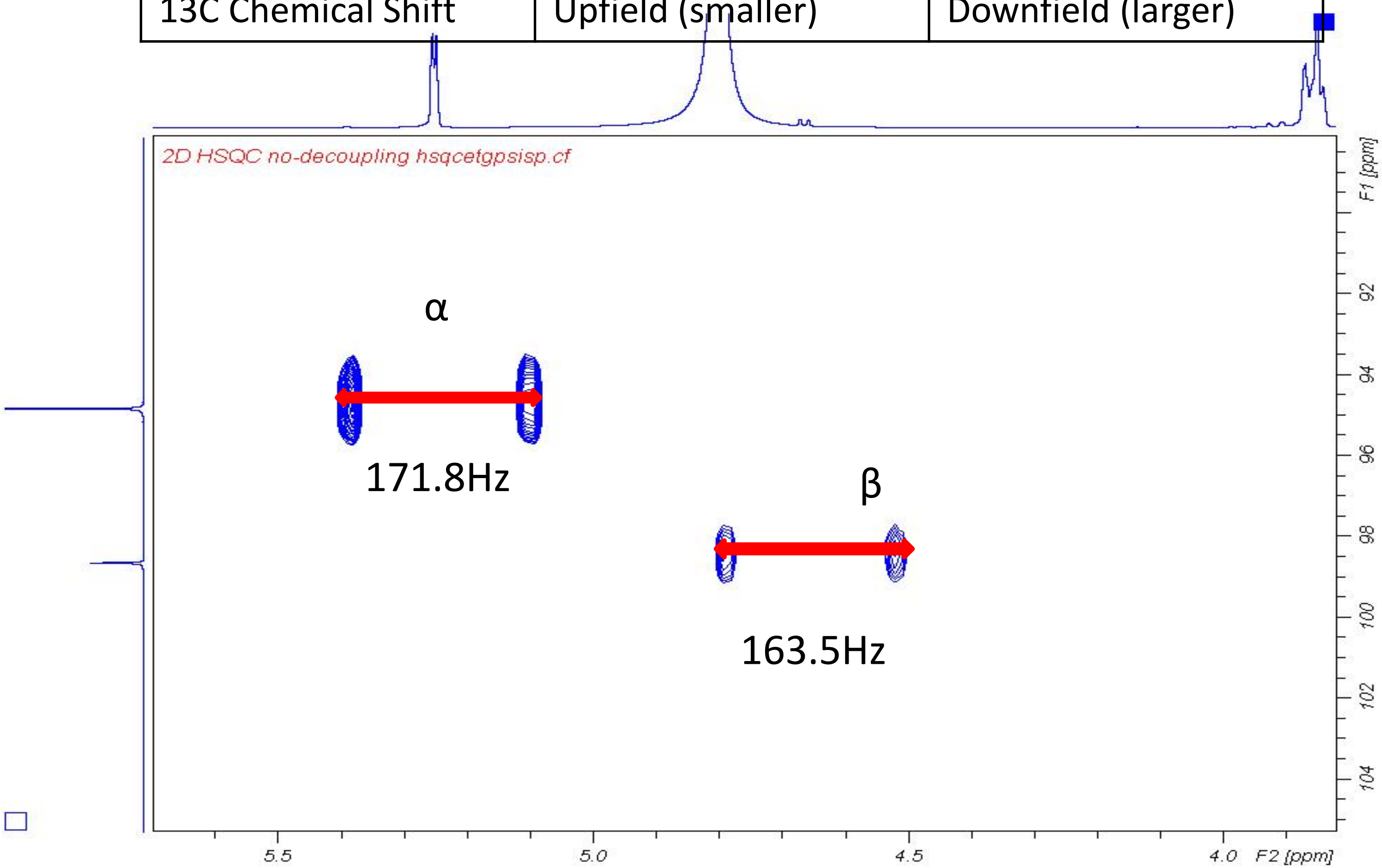
2D_HeterJres_hjresqf

Saccharide Anomer	α	β
1H chemical shift	Downfield (larger)	Upfield (smaller)
$^{3}J_{1H\text{-}2H}$	7-8 Hz	< 4Hz
$^{1}J_{1H\text{-}1C}$	**~170Hz**	**~160Hz**
13C Chemical Shift	Upfield (smaller)	Downfield (larger)



2D_HSQC_nodec_hsqcetgpsisp.cf

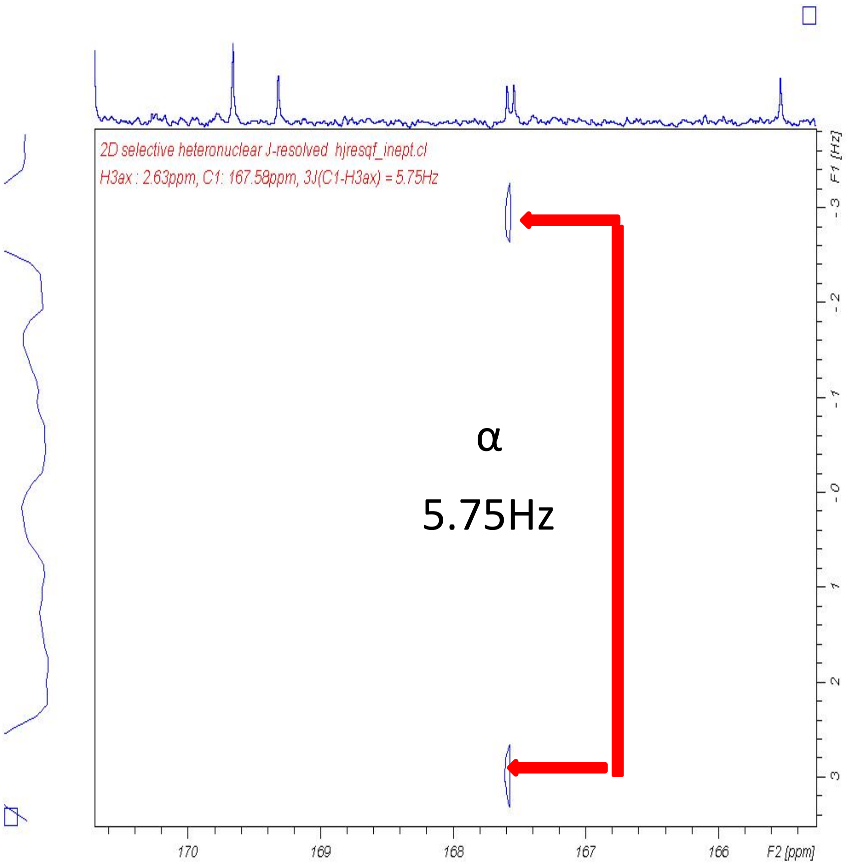
Saccharide Anomer	α	β
1H chemical shift	Downfield (larger)	Upfield (smaller)
$^{3}J_{1H\text{-}2H}$	7-8 Hz	< 4Hz
$^{1}J_{1H\text{-}1C}$	**~170Hz**	**~160Hz**
13C Chemical Shift	Upfield (smaller)	Downfield (larger)

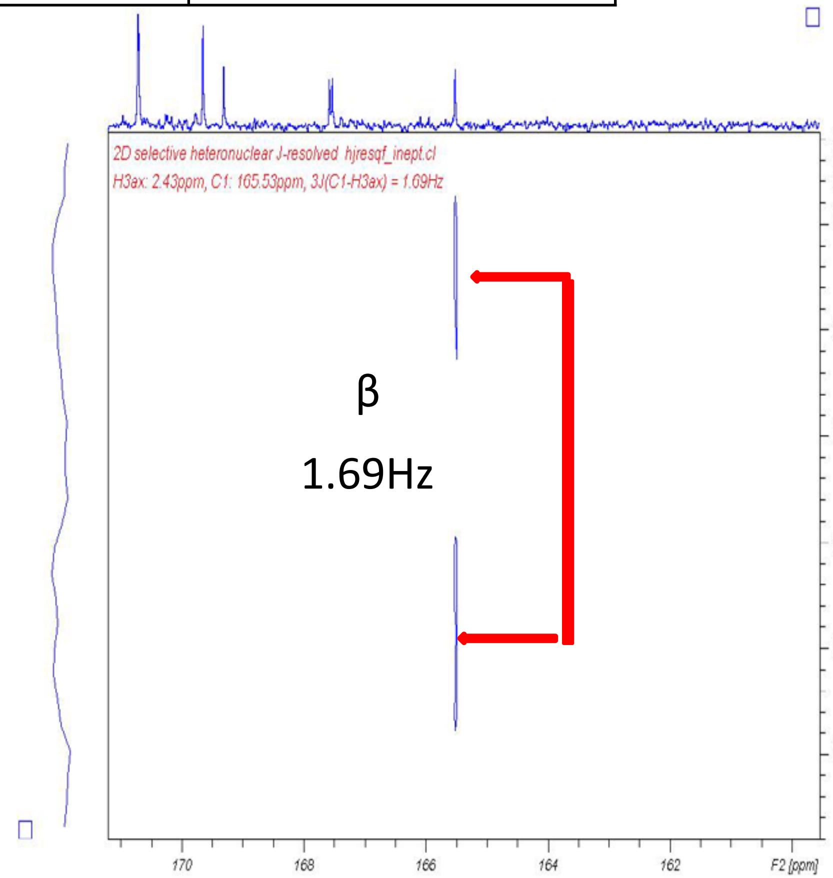


2D_HeterJres-sel_hjresqf_inept.cl

But 13C-dectection, so better with 13C-cryorpobe

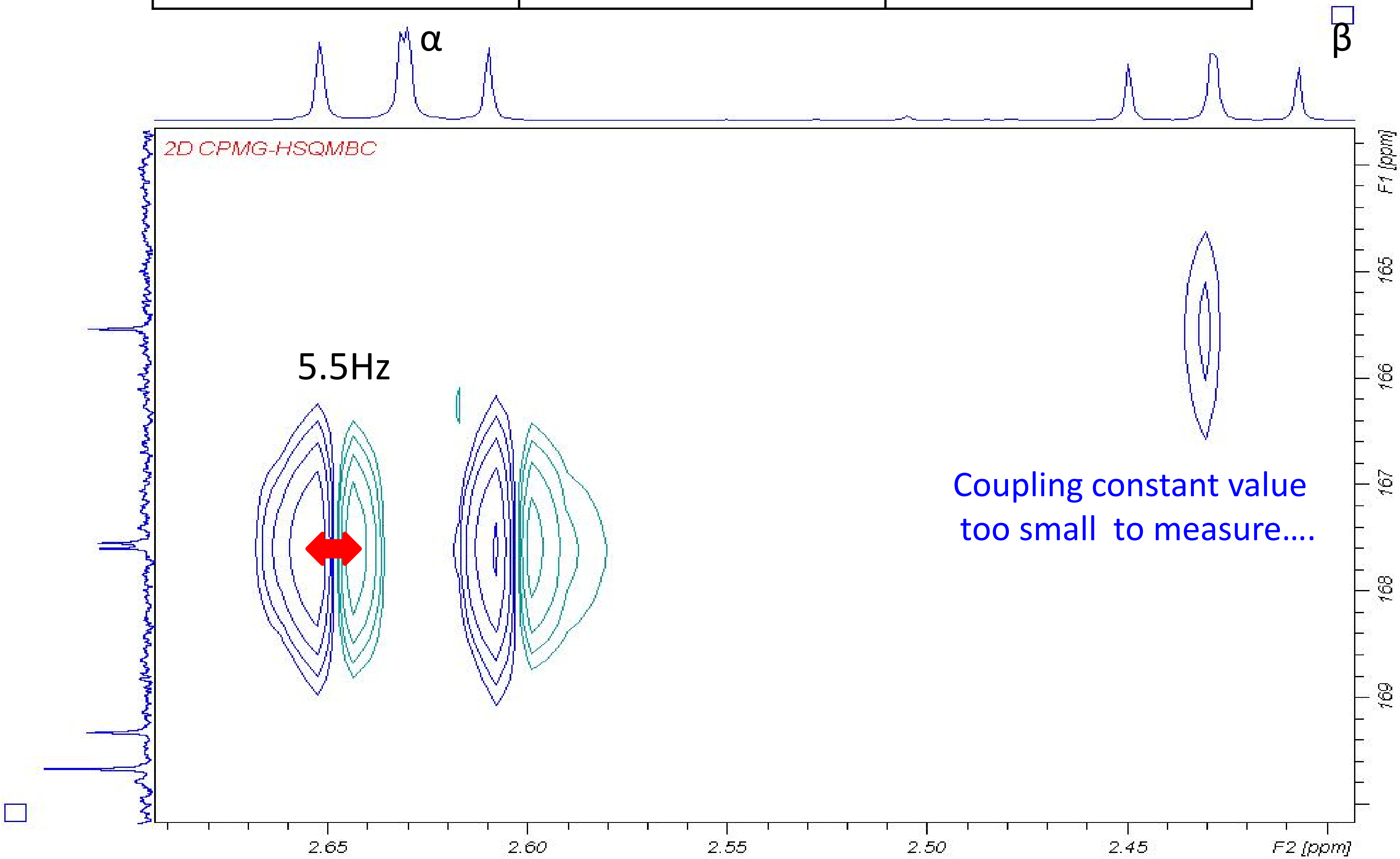
Sialic Acid Anomer	α	β
$^{3}J_{C1\text{-}H3ax}$	~6 Hz	~1Hz
$^{2}J_{C2\text{-}H3ax}$	~-8Hz	~-3, -4Hz





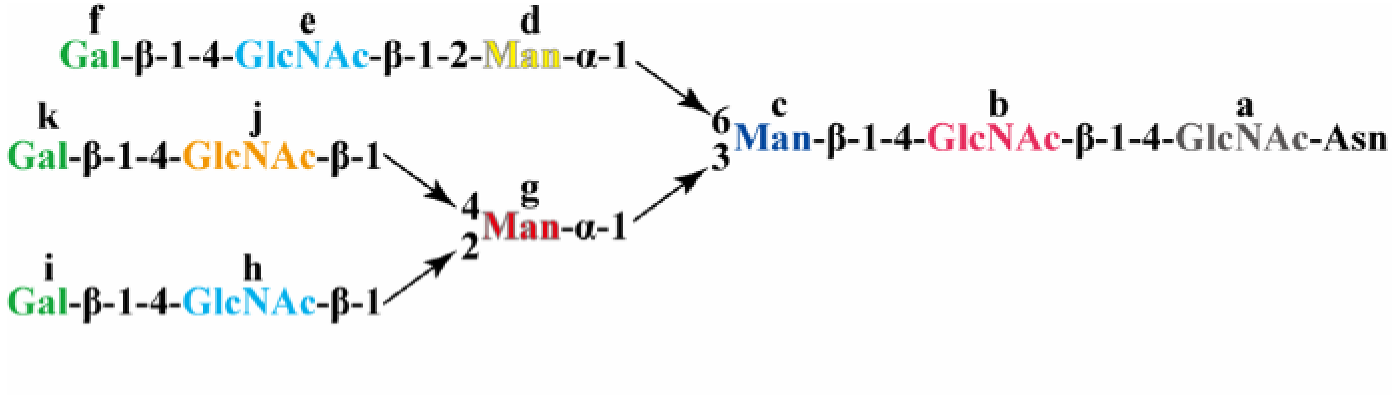
2D_CPMG_HSQMBC_hsqcetgpjclrndxy

Sialic Acid Anomer	α	β
$^{3}J_{C1\text{-}H3ax}$	~6 Hz	~1Hz
$^{2}J_{C2\text{-}H3ax}$	~-8Hz	~-3, -4Hz



Part III: Experiments to help "building unit" assignment

Ex: Polysaccharides

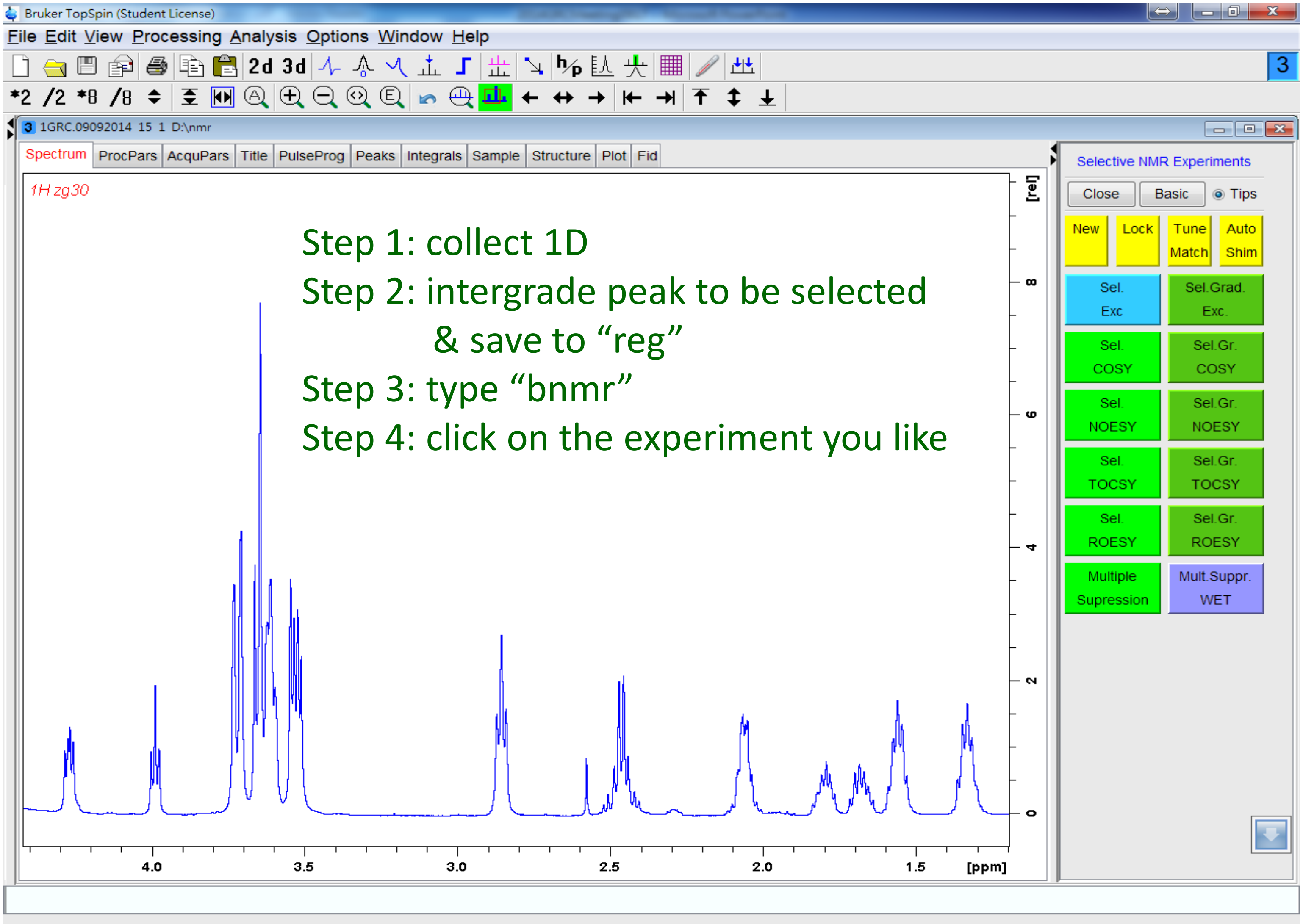


or

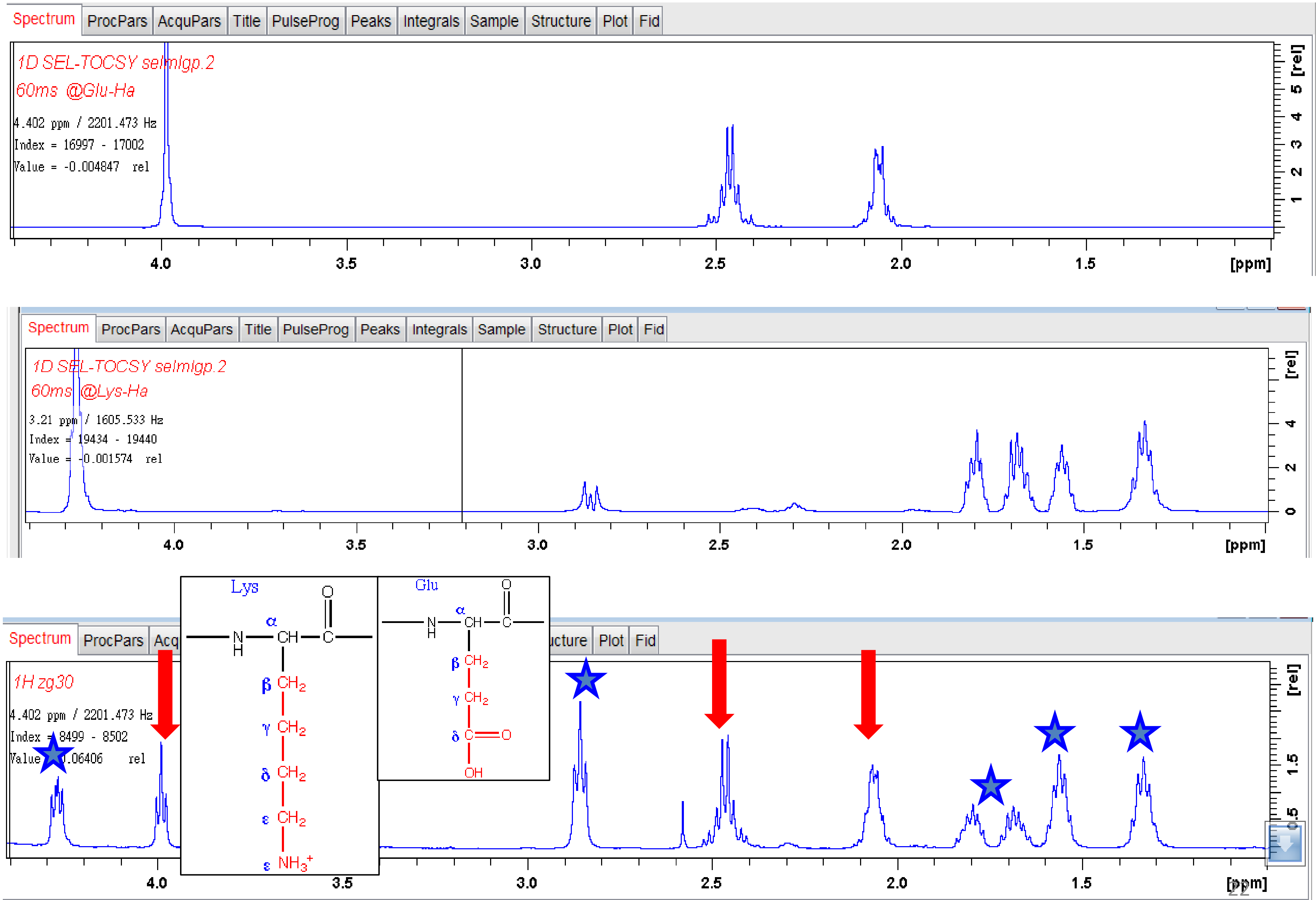
Peptides

1D Selected Excitation Experiments

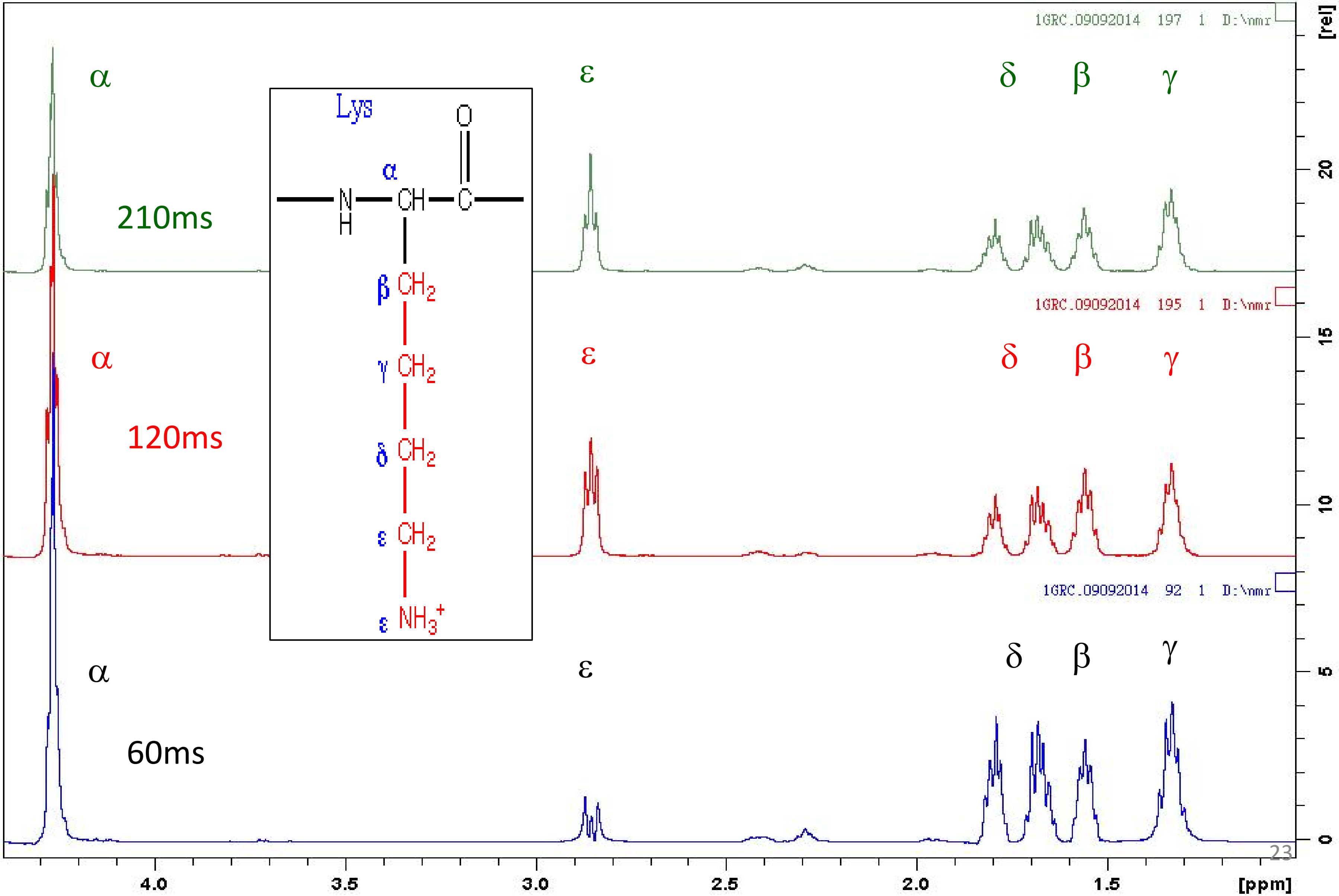
Use button NMR



1D Selected TOCSY selmlgp.2



Tips on selected TOCSY : optimize mixing time



2D Selected Excitation Experiments

Selected TOCSY based

Available online at www.sciencedirect.com

ScienceDirect

Carbohydrate Research 343 (2008) 1333–1345

Carbohydrate RESEARCH

2D Selective-TOCSY–DQFCOSY and HSQC–TOCSY NMR experiments for assignment of a homogeneous asparagine-linked triantennary complex type undecasaccharide

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[b] *Research and Technical Center, Otsuka Chemical Co. Ltd, 463 Kagasuno Kawauchi, Tokushima 771-0193, Japan*
[c] *International Graduate School of Arts and Sciences, Yokohama City University, 22-2 Seto Kanazawa-ku, Yokohama 236-0027, Japan*

Received 18 October 2007; received in revised form 22 February 2008; accepted 3 March 2008
Available online 18 March 2008

2D Selected-TOCSY type experiment

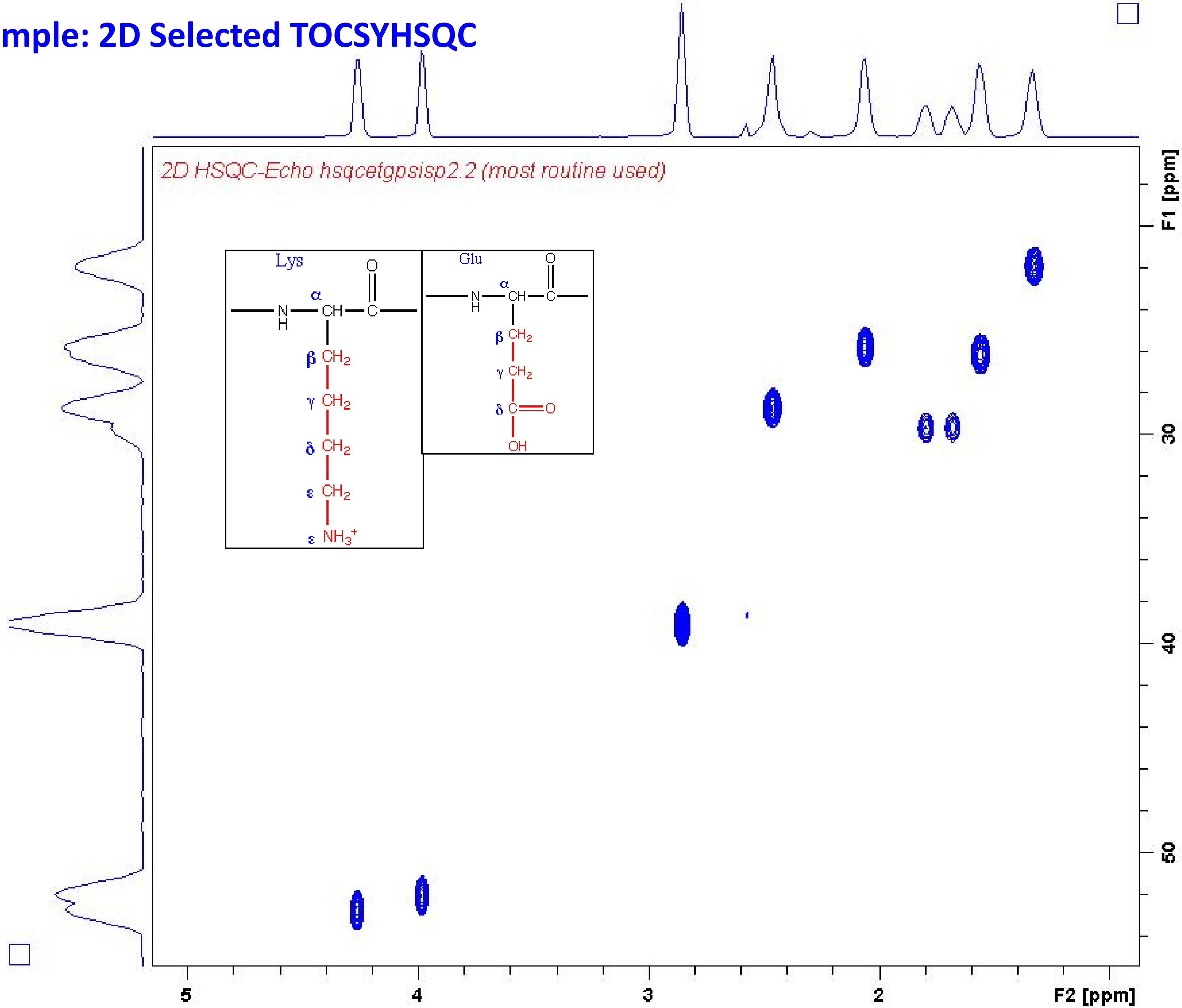
GRC Parameter Set	Experiment Details	Note
2D_SEL_TOCSYHSQC_ sel-tocsyhsqc.cf	Selected "building unit" HSQC	Need to adjust tocsy mixing time
2D_SEL_TOCSYDQFCOSY_ sel-tocsycosy.cf	Selected "building unit" DQFCOSY	Need to adjust tocsy mixing time
2D_SEL_TOCSYNOESY_ sel-tocsynoesy.cf	Selected "building unit" NOESY	Need to adjust tocsy mixing time & noesy mixing time

Step 1: set up cnst21=O1 (center of the spectrum) in ppm
Step 2: set up const22= The peak you like to select in ppm
Step 3: optimize d9 (ms) (ex: 60-210ms)

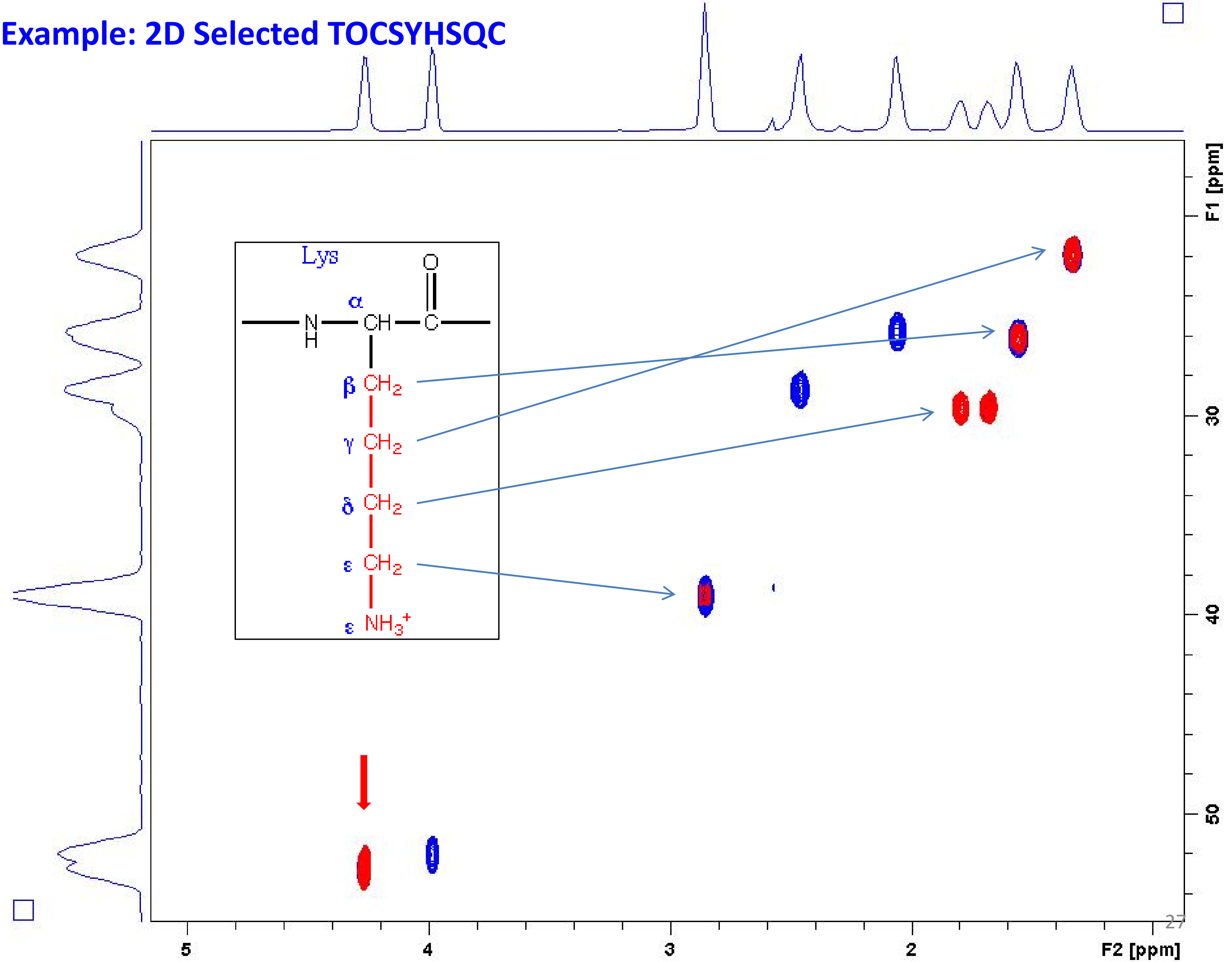
For selected-TOCSY-NOESY
Step 4: optimize d8 (ms) (ex: 300ms)

***For Best Result , you may use shape tool to optimize shape pulse power level !!**

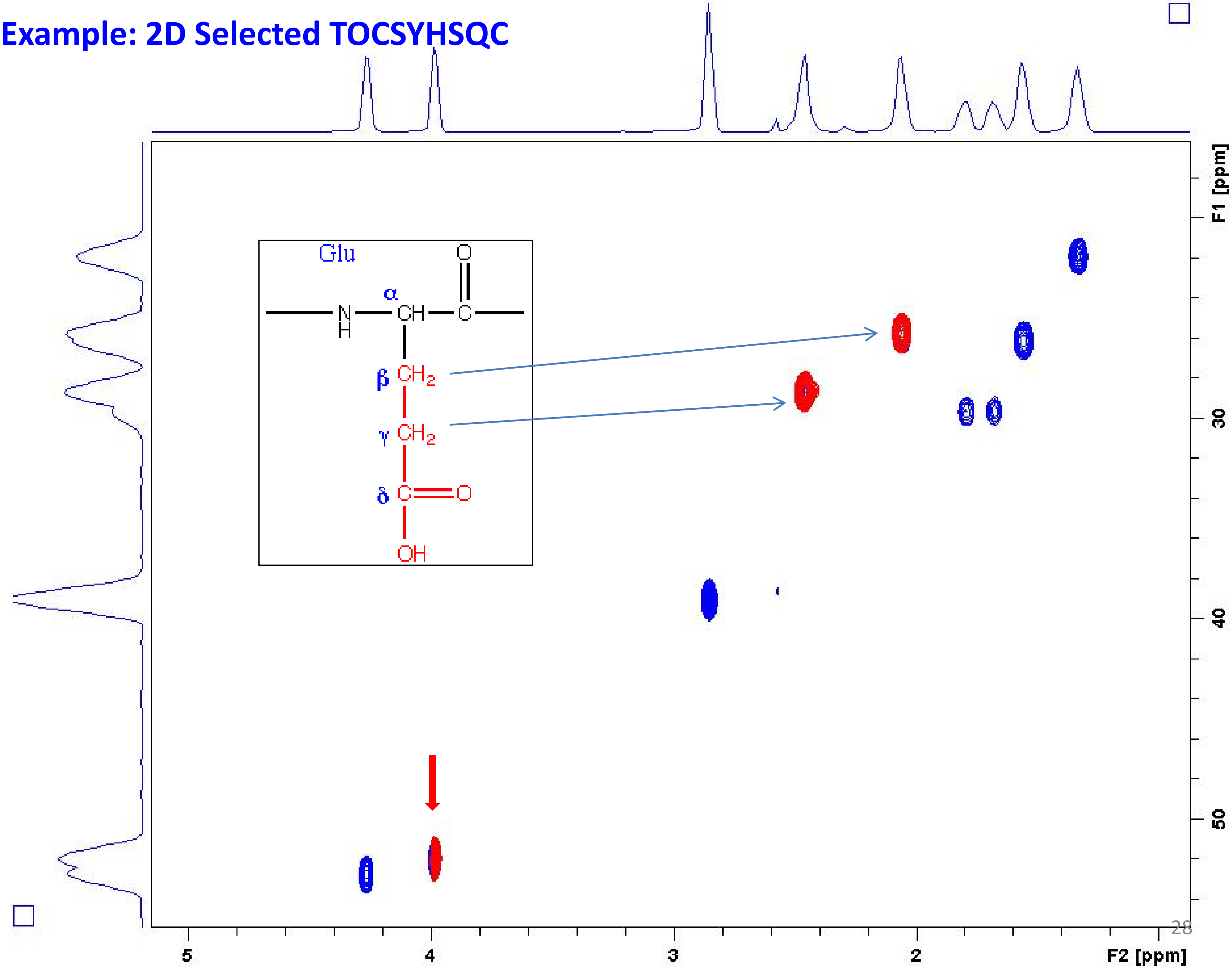
Example: 2D Selected TOCSYHSQC

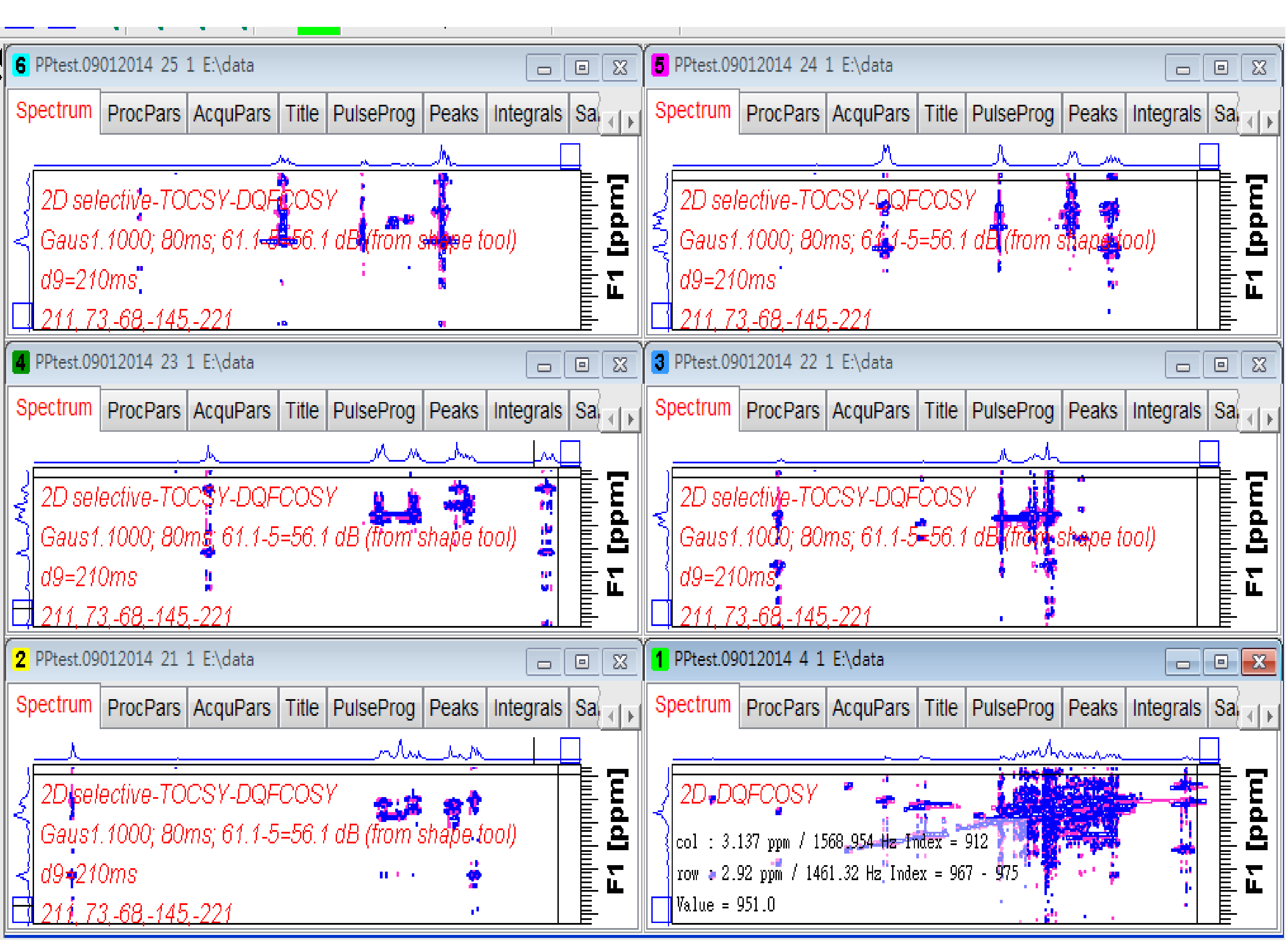


Example: 2D Selected TOCSYHSQC



Example: 2D Selected TOCSYHSQC





6 PPtest.09012014 25 1 E:\data
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
2D selective-TOCSY-DQFCOSY
Gaus1.1000; 80ms; 61.1-5=56.1 dB (from shape tool)
d9=210ms
211, 73,-68,-145,-221
F1 [ppm]
5 PPtest.09012014 24 1 E:\data
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
2D selective-TOCSY-DQFCOSY
Gaus1.1000; 80ms; 61.1-5=56.1 dB (from shape tool)
d9=210ms
211, 73,-68,-145,-221
F1 [ppm]
4 PPtest.09012014 23 1 E:\data
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
2D selective-TOCSY-DQFCOSY
Gaus1.1000; 80ms; 61.1-5=56.1 dB (from shape tool)
d9=210ms
211, 73,-68,-145,-221
F1 [ppm]
3 PPtest.09012014 22 1 E:\data
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
2D selective-TOCSY-DQFCOSY
Gaus1.1000; 80ms; 61.1-5=56.1 dB (from shape tool)
d9=210ms
211, 73,-68,-145,-221
F1 [ppm]
2 PPtest.09012014 21 1 E:\data
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
2D selective-TOCSY-DQFCOSY
Gaus1.1000; 80ms; 61.1-5=56.1 dB (from shape tool)
d9=210ms
211, 73,-68,-145,-221
F1 [ppm]
1 PPtest.09012014 4 1 E:\data
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
2D DQFCOSY
col : 3.137 ppm / 1568.954 Hz Index = 912
row : 2.92 ppm / 1461.32 Hz Index = 967 - 975
Value = 951.0
F1 [ppm]

Available glycobiology NMR experiments in Academia Sinica NMR core facility