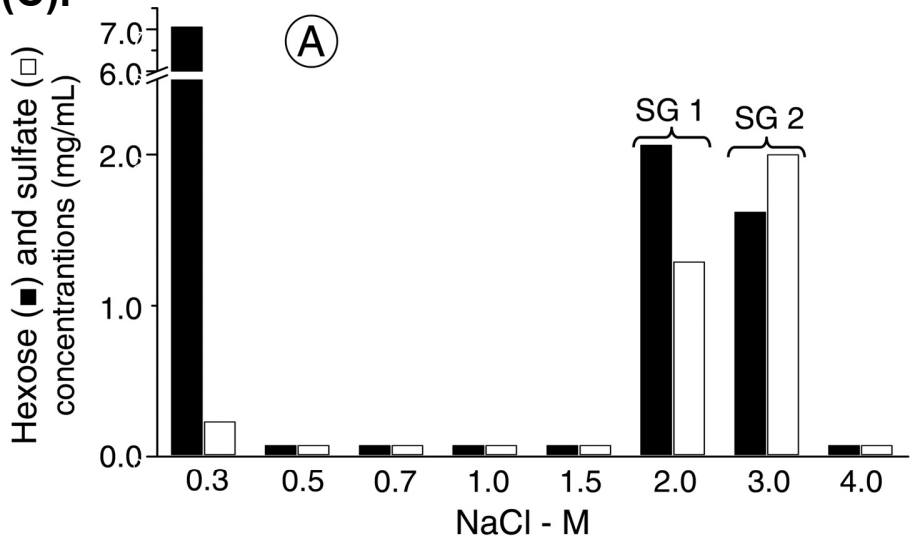
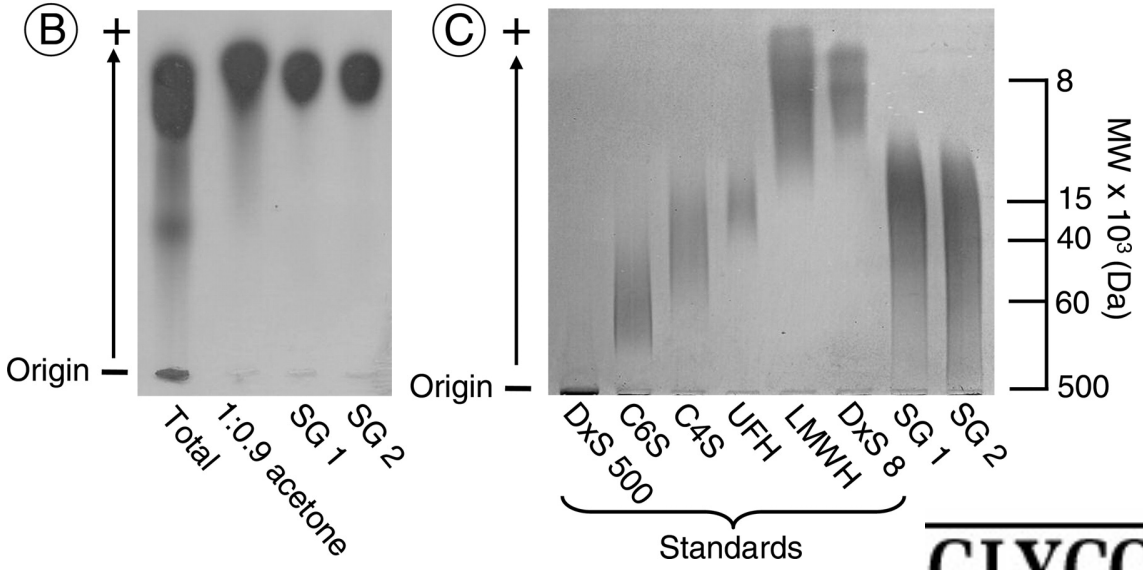
NMR STRUCTURAL DETERMINATION OF A GREEN ALGA SG

Purification of the fractions of sulfated galactan (SG 1 and SG 2) from *C. isthmocladum* by ion-exchange chromatography (A) and electrophoretic analysis by agarose gel (B) and polyacrylamide gel (C).



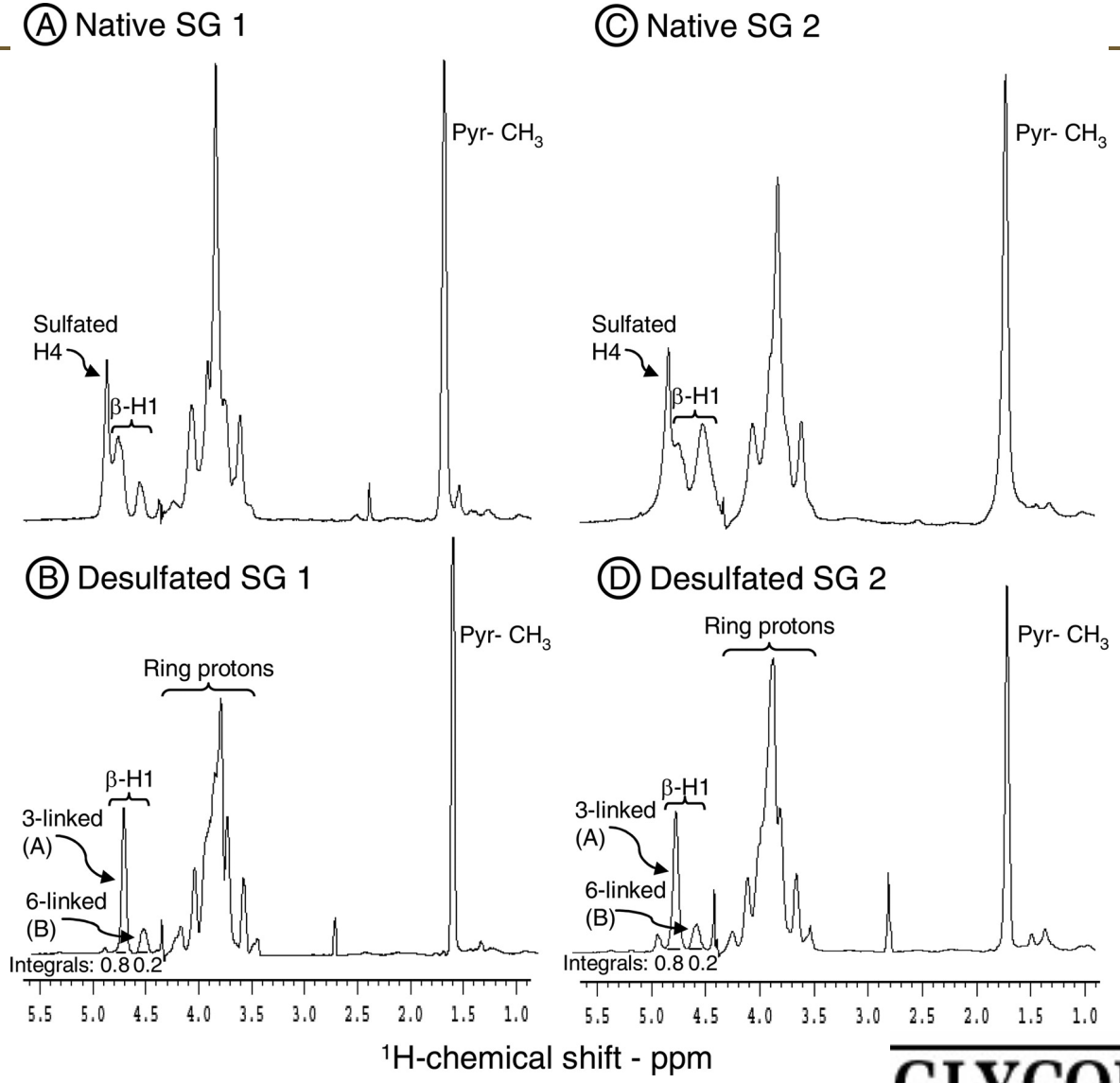


Eduardo H C Farias et al. Glycobiology 2008;18:250-259

GLYCOBIOLOGY

NMR STRUCTURAL DETERMINATION OF A GREEN ALGA SG

1D ^{1}H NMR spectra at 400 MHz of the native SG 1 (A), native SG 2 (C) from *C. isthmocladum*, and the desulfated derivatives of SG 1 (B) and SG 2 (D).

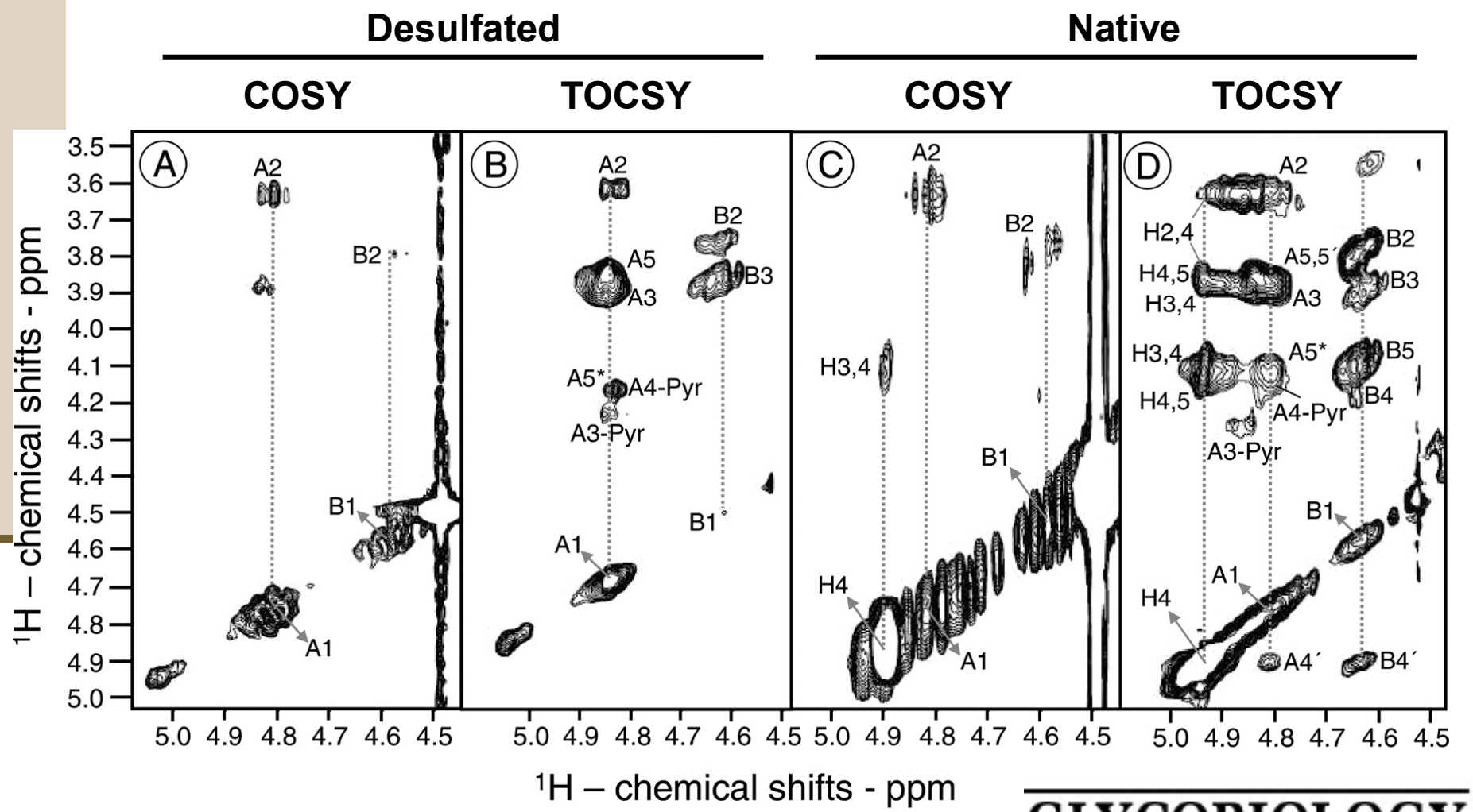


Eduardo H C Farias et al. Glycobiology 2008;18:250-259

GLYCOBIOLOGY

NMR STRUCTURAL DETERMINATION OF A GREEN ALGA SG

Strips of the anomeric regions (expansions from 5.1 to 4.5 ppm) from the COSY (A and C) and TOCSY (B and D) spectra of the desulfated galactan 2 (A and B) and the native SG 2 (C and D) from *C. isthmocladum*.

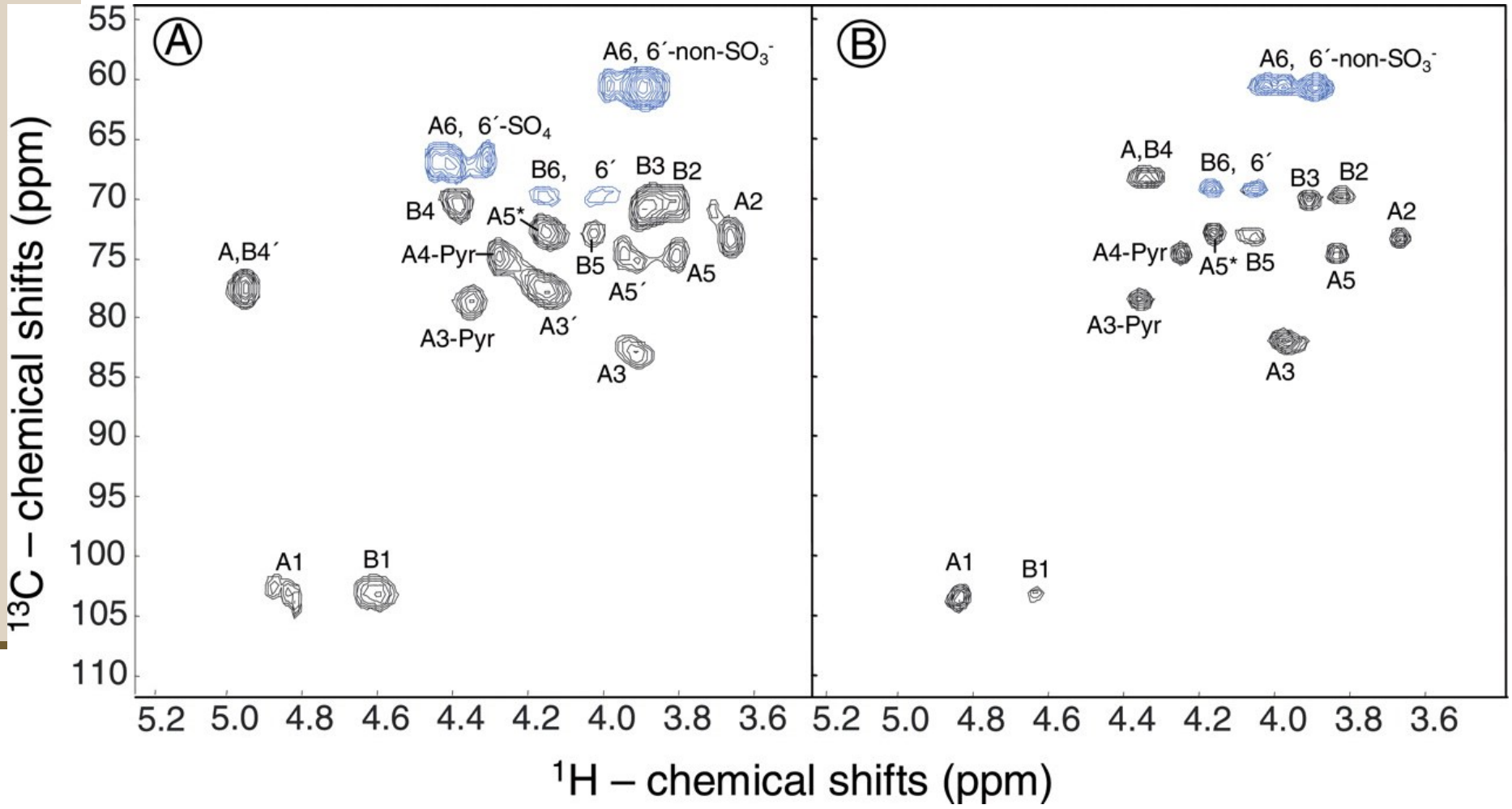


Eduardo H C Farias et al. Glycobiology 2008;18:250-259

GLYCOBIOLOGY

NMR STRUCTURAL DETERMINATION OF A GREEN ALGA SG

$^{1}H/^{13}C$ DEPT-HSQC spectra of the native SG 2 (A) and its desulfated derivative (B).

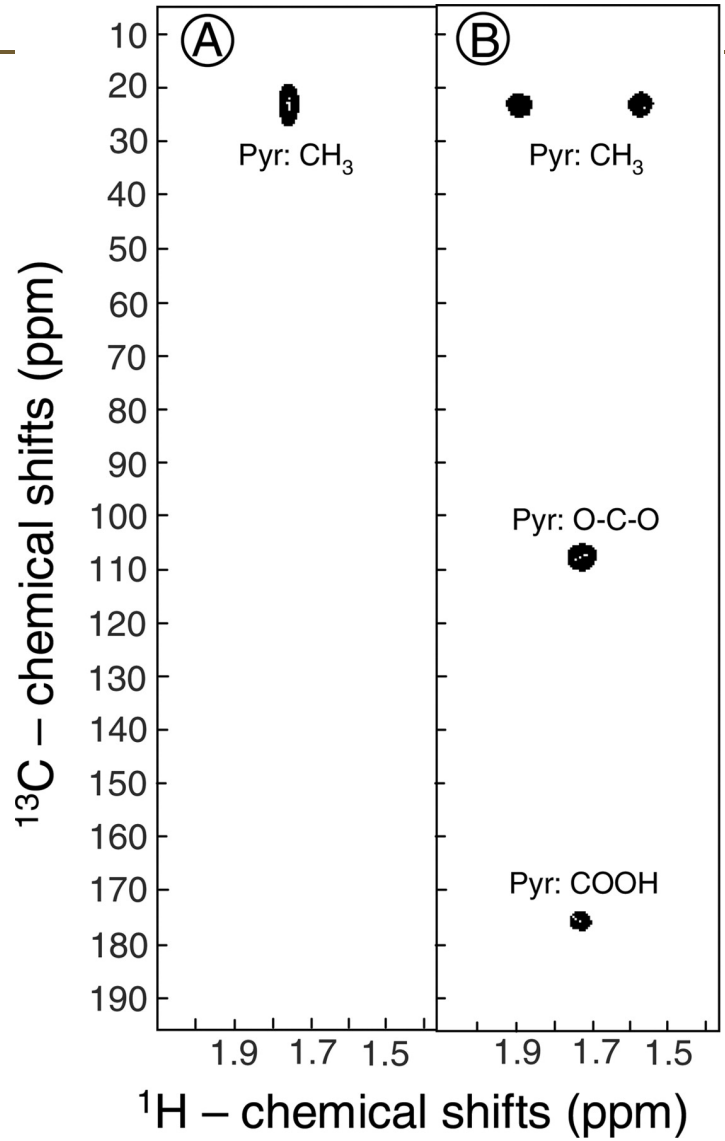


Eduardo H C Farias et al. Glycobiology 2008;18:250-259

GLYCOBIOLOGY

NMR STRUCTURAL DETERMINATION OF A GREEN ALGA SG

$^{1}H/^{13}C$ HSQC (A) and HMBC (B) spectra of the methyl region of the pyruvate group from the native SG 2.

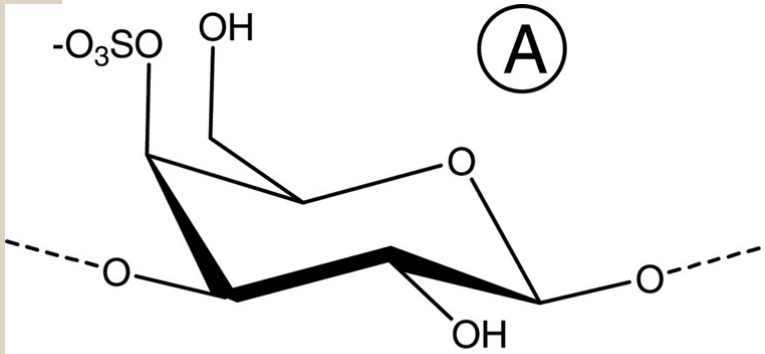


Eduardo H C Farias et al. Glycobiology 2008;18:250-259

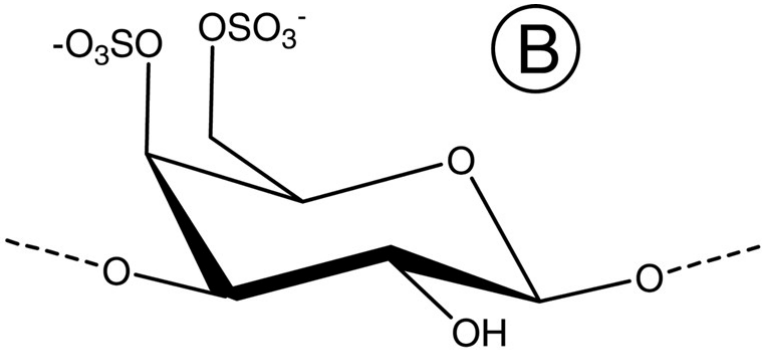
GLYCOBIOLOGY

NMR STRUCTURAL DETERMINATION OF A GREEN ALGA SG

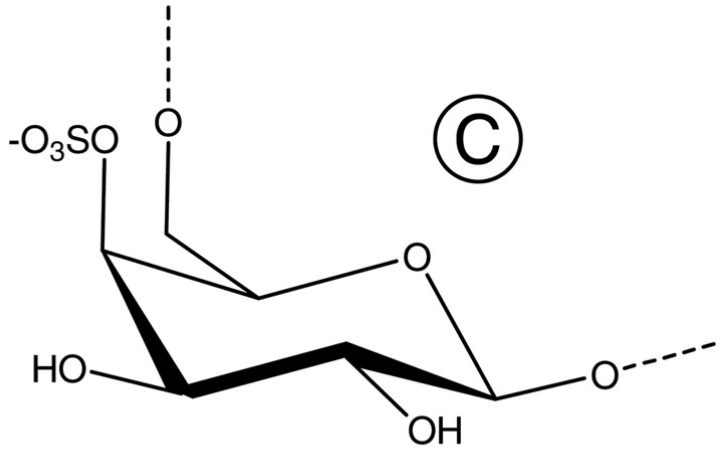
Proposed structures of the components found in the sulfated galactan from the green alga *C. isthmocladum.*



Preponderant



Minor



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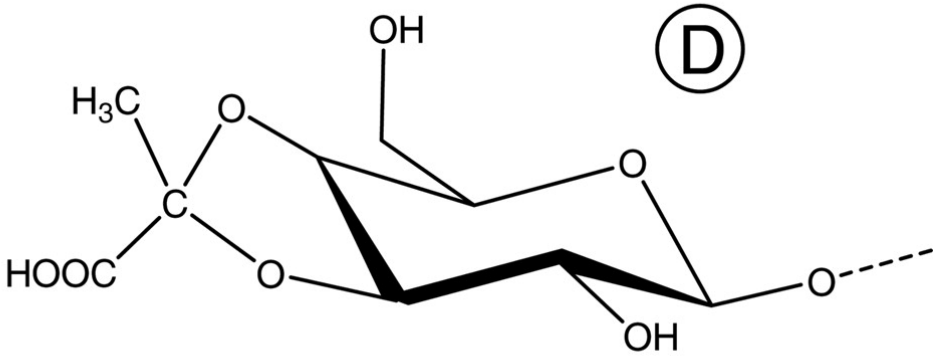


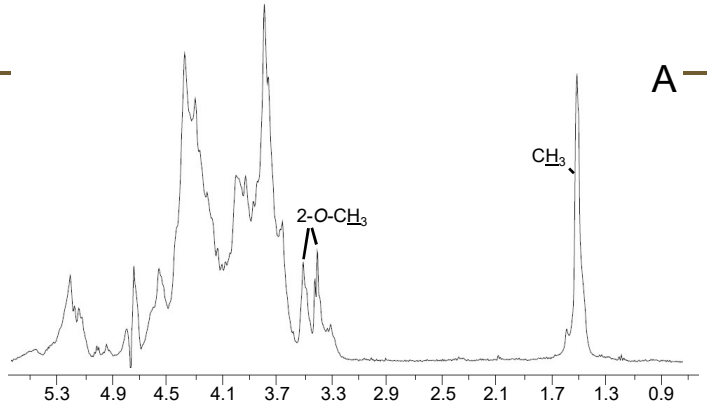
Minor

Eduardo H C Farias et al. Glycobiology 2008;18:250-259

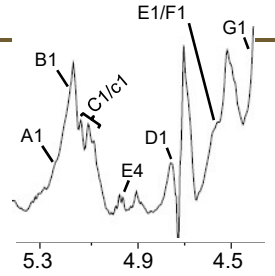
GLYCOBIOLOGY

NMR STRUCTURAL CHARACTERIZATION OF A RED ALGA SG

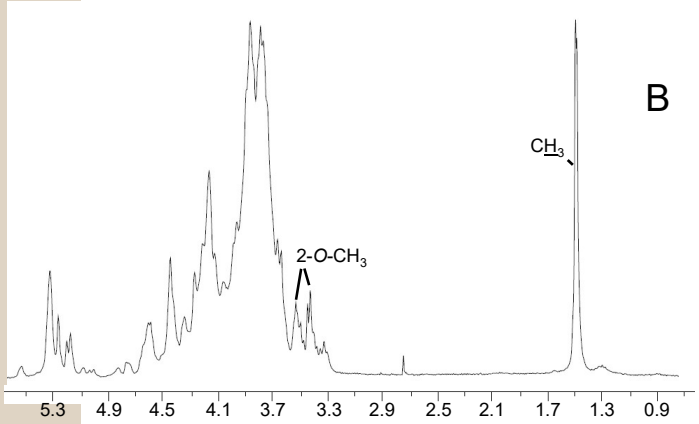
NMR structure characterization of a new red algal SG (*Acanthophora muscoides*)



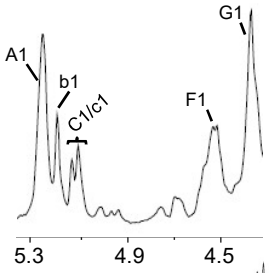
A



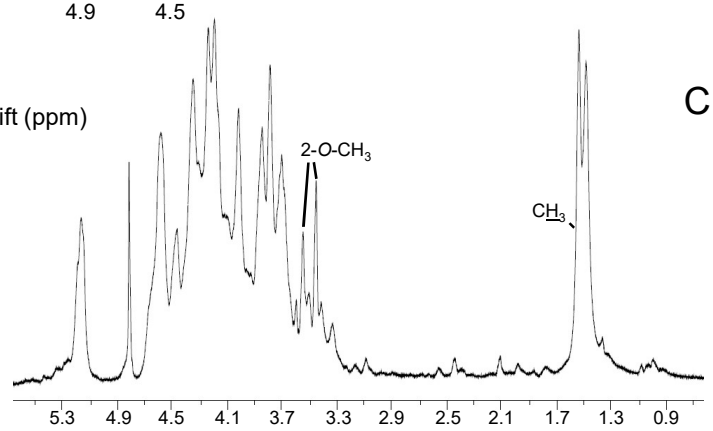
^{1}H-chemical shift (ppm)



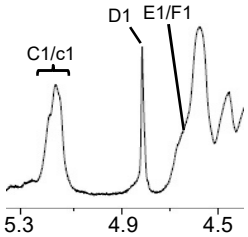
B



^{1}H-chemical shift (ppm)



C



^{1}H-chemical shift (ppm)

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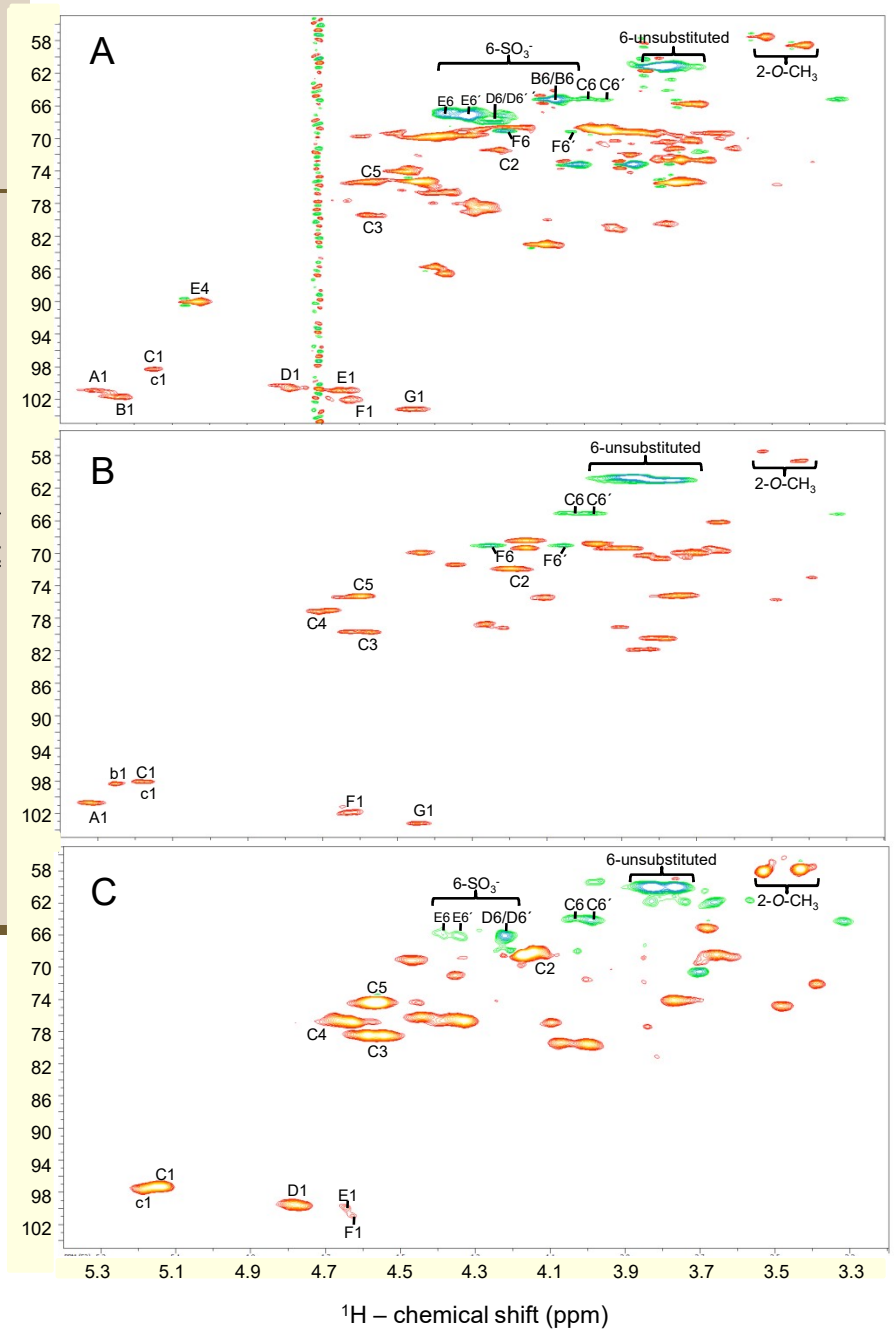
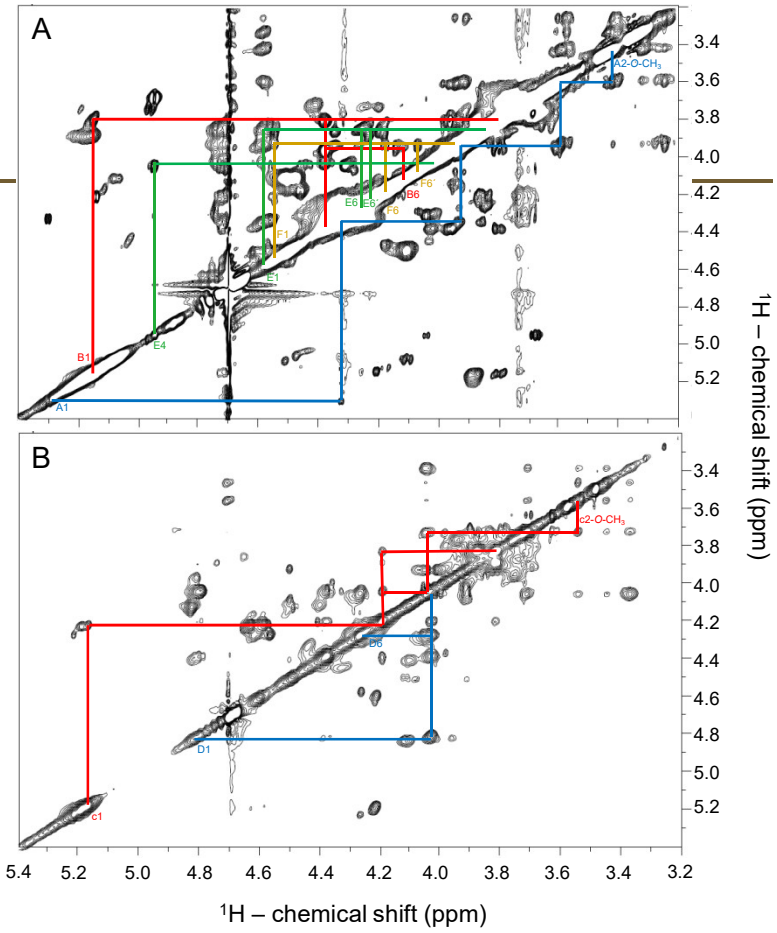
Quinderé ALG et al. J Thromb Haemost 2014;12:43-53

NMR STRUCTURAL DETERMINATION OF A RED ALGA SG





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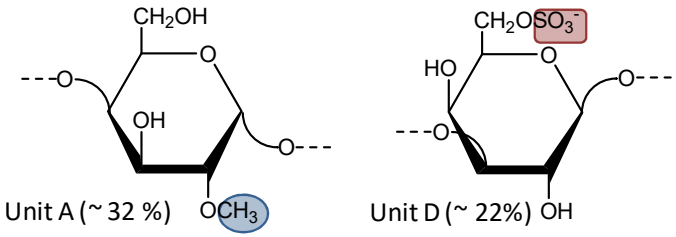
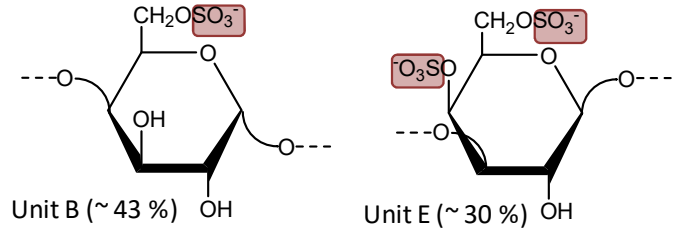
Quinderé ALG et al. J Thromb Haemost 2014;12:43-53

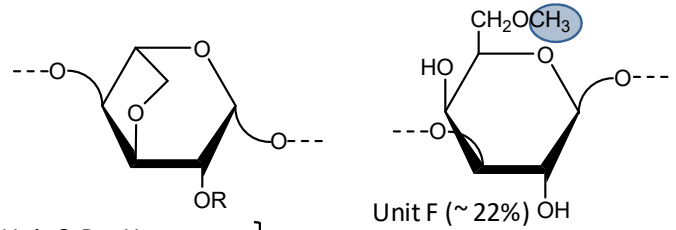
STRUCTURAL COMPARISON OF TWO SGs FROM RED ALGAE

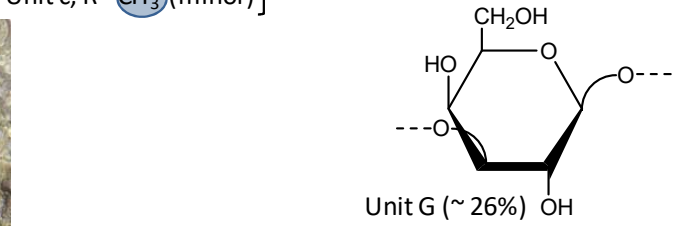
Structural composition of SGs from *Acanthophora muscoides* and *Botriocladia occidentalis*

α-Gal*p* units β-Gal*p* units



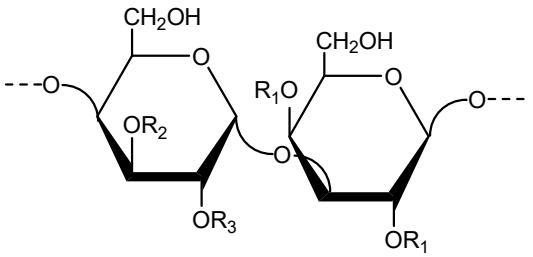






VS

α-Gal*p* units β-Gal*p* units



R_1 = H or SO_3^-

R_2 = H (~ 33%) or SO_3^- (~ 66%)

R_3 = H (~ 66%) or SO_3^- (~ 33%)

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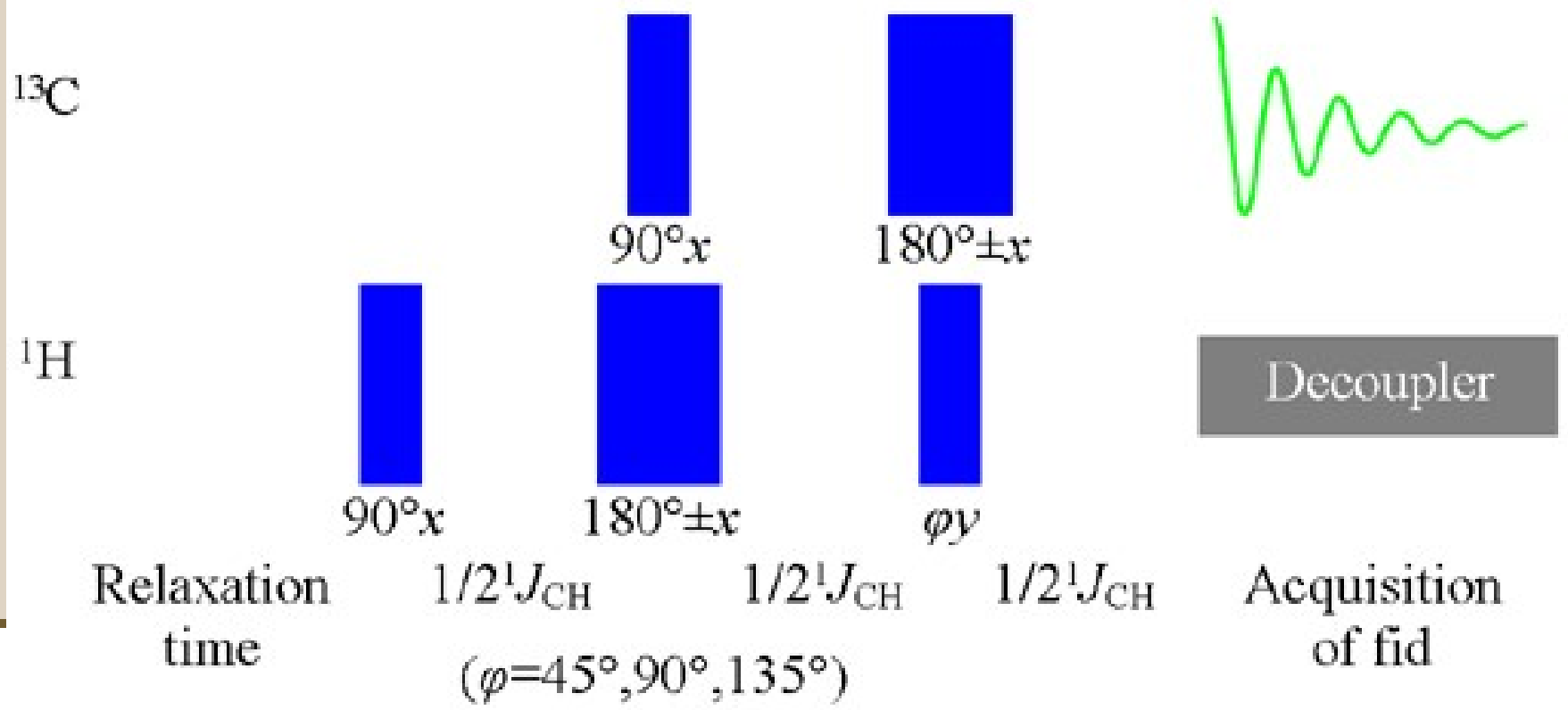
Quinderé ALG et al. J Thromb Haemost 2014;12:43-53

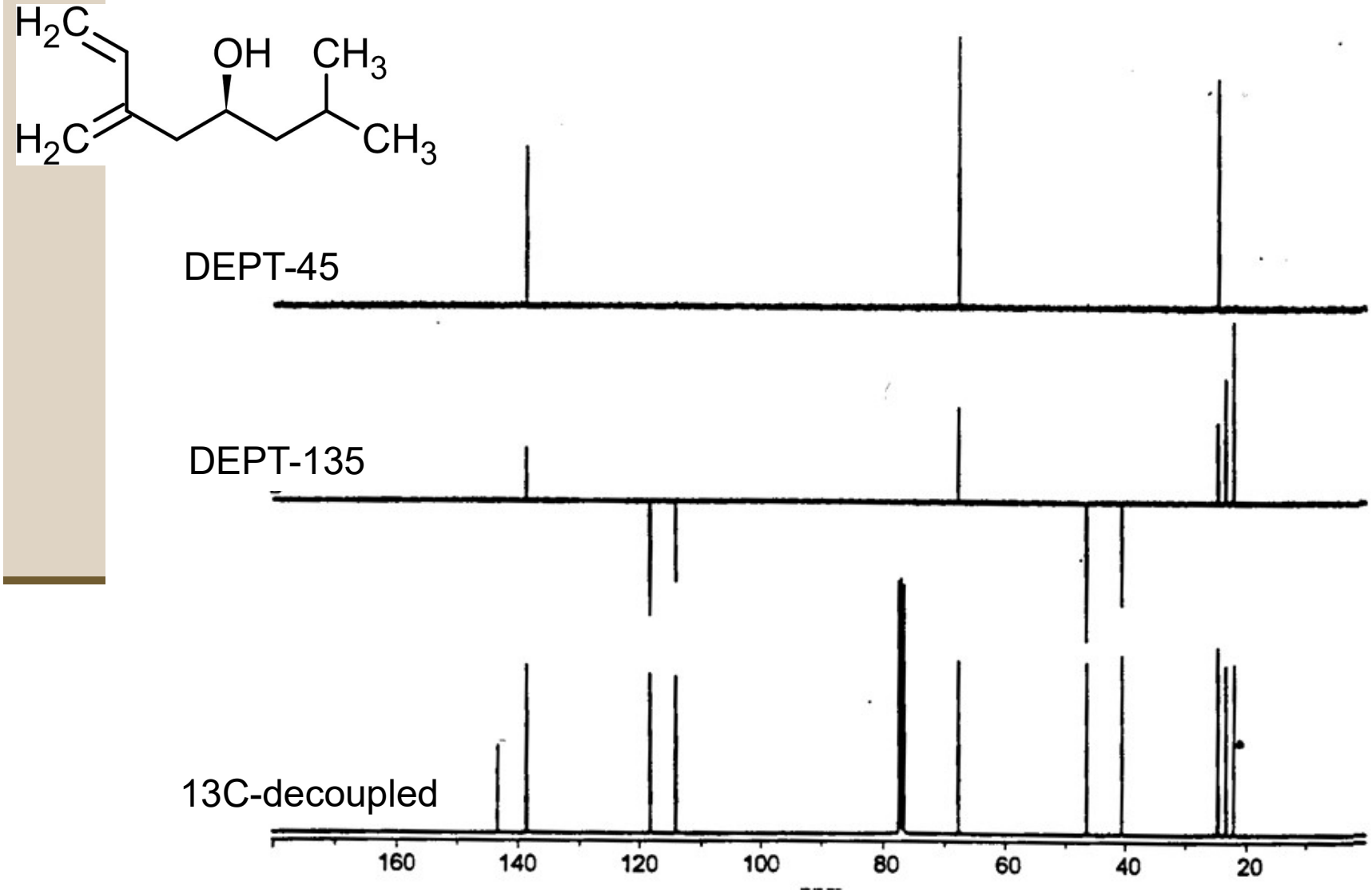
^{13}C DEPT: A way of sorting out the # of Hs attached to a C

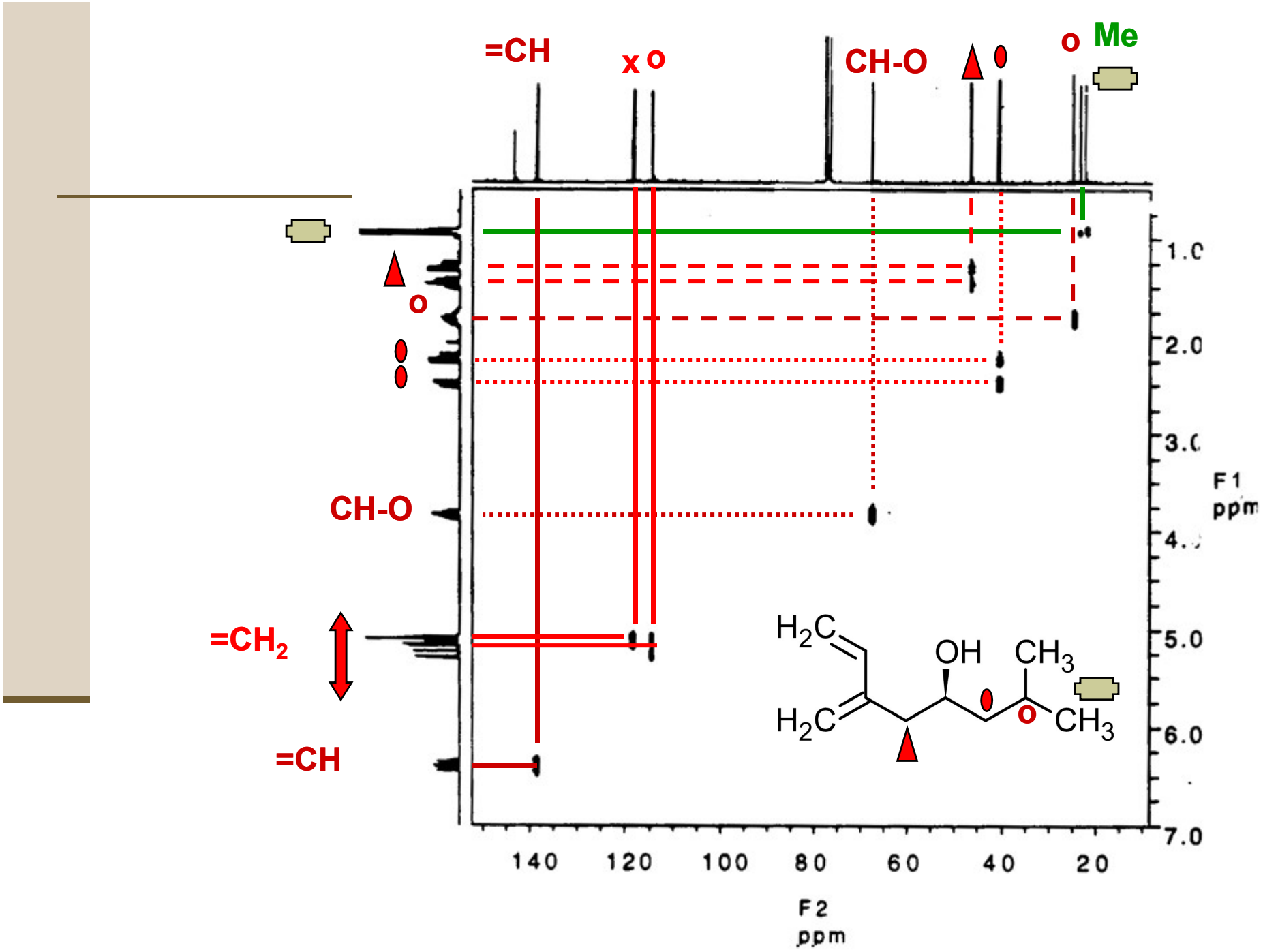
- DEPT is "Distortionless Enhancement by Polarization Transfer" and is used as a means for editing spectra.
- Three DEPT spectra are available for analyses and are termed DEPT-45, DEPT-90 and DEPT-135 (the number indicates the flip angle of the editing proton pulse in the sequence).
- For most practical purposes, **DEPT-135** covers all signals.
- No solvent peaks observed in DEPT.

^{13}C DEPT pulse sequence

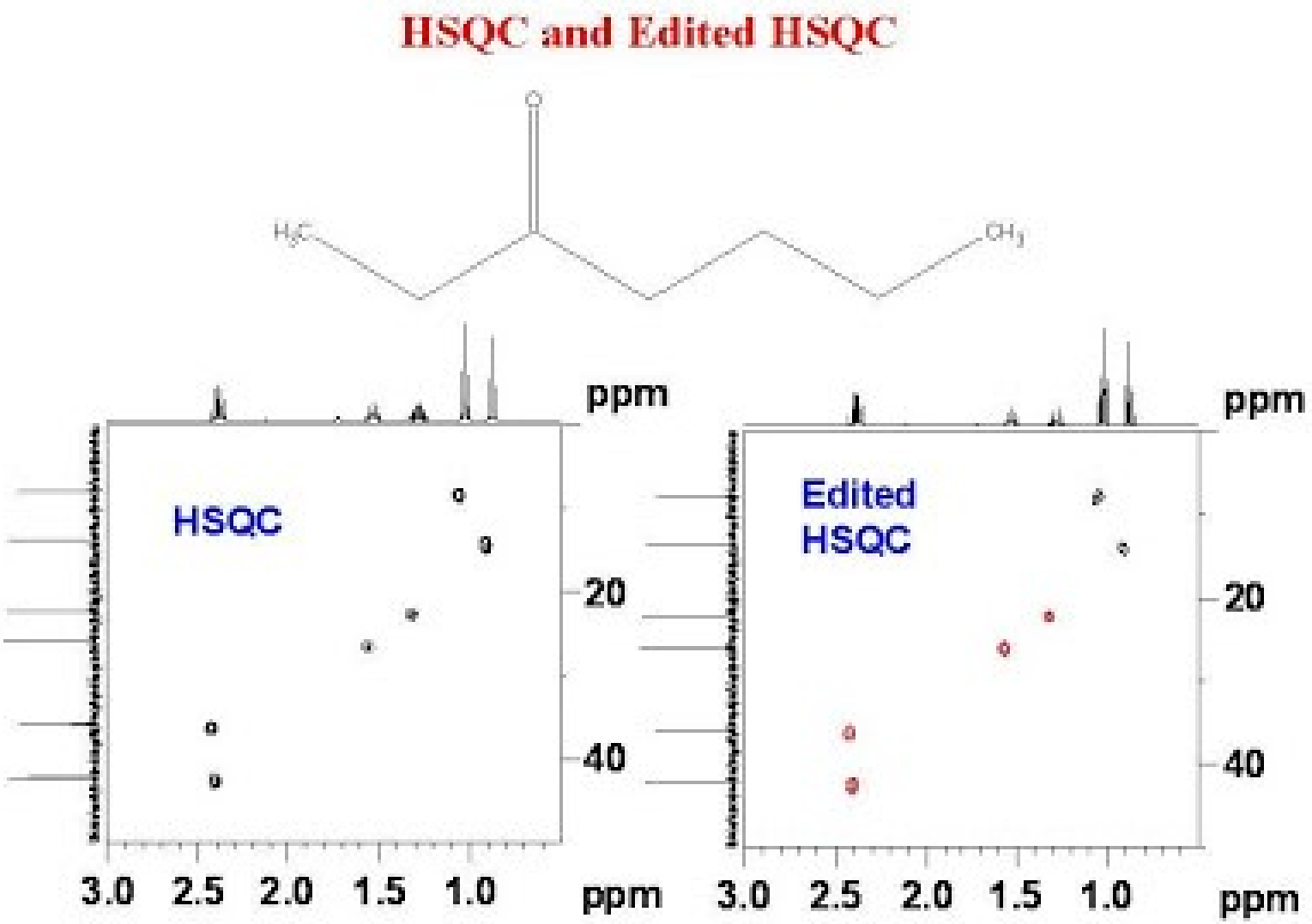


Spectral assignments for ipsenol



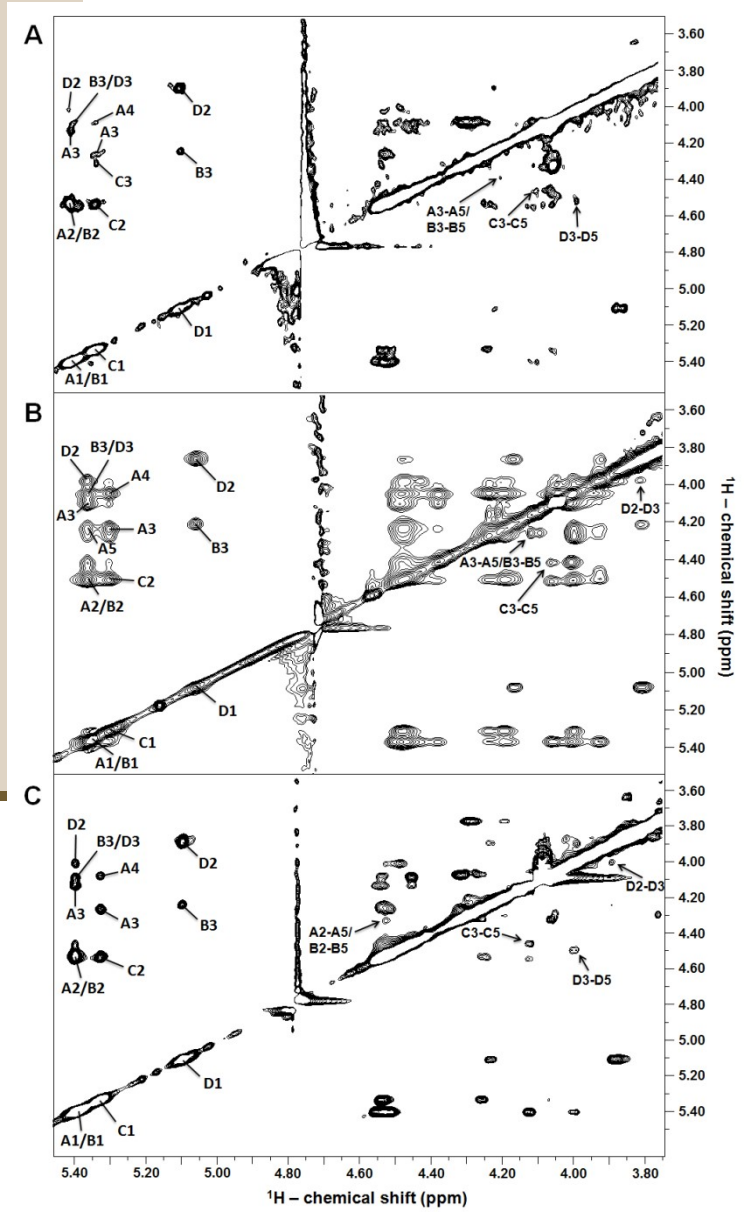

=CH
x o
CH-O
o
Me
o
CH-O
=CH2
=CH
H2C
OH
CH3
H2C
o
CH3
1.0
2.0
3.0
4.0
5.0
6.0
7.0
F1
ppm
140
120
100
80
60
40
20
F2
ppm

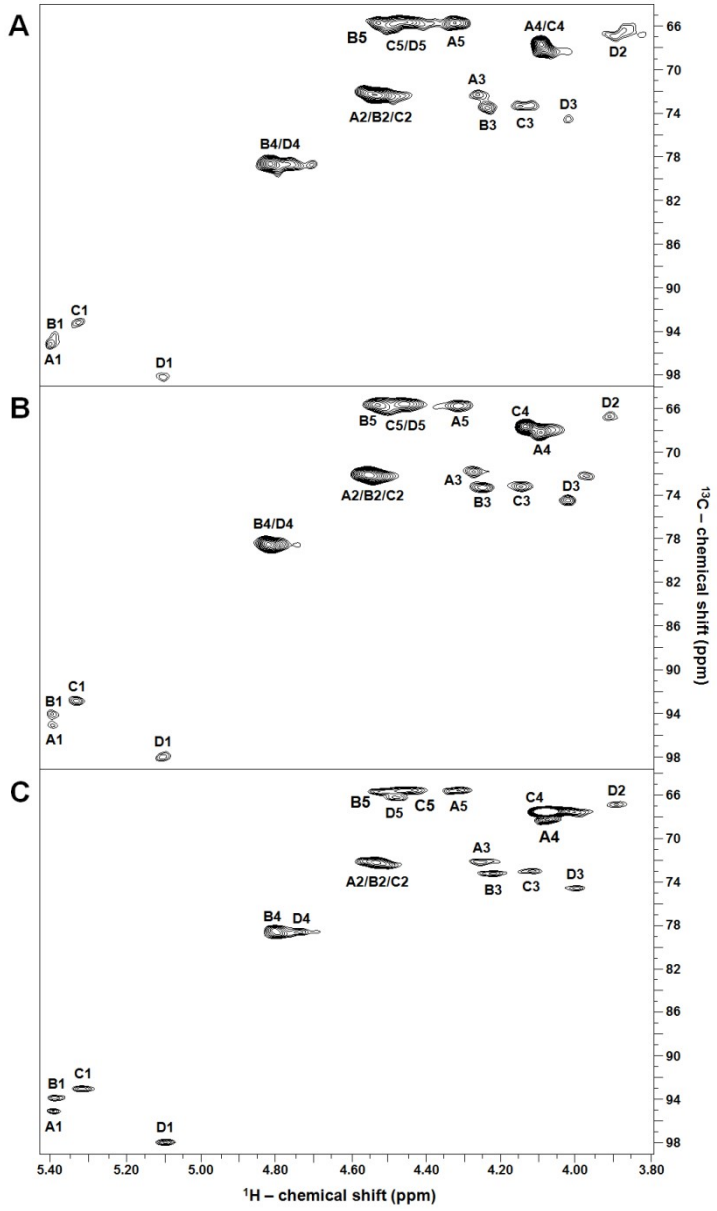
HSQC vs edited HSQC spectra



Ring and chain conformations and dynamics of native SF, SG and derived oligosaccharides

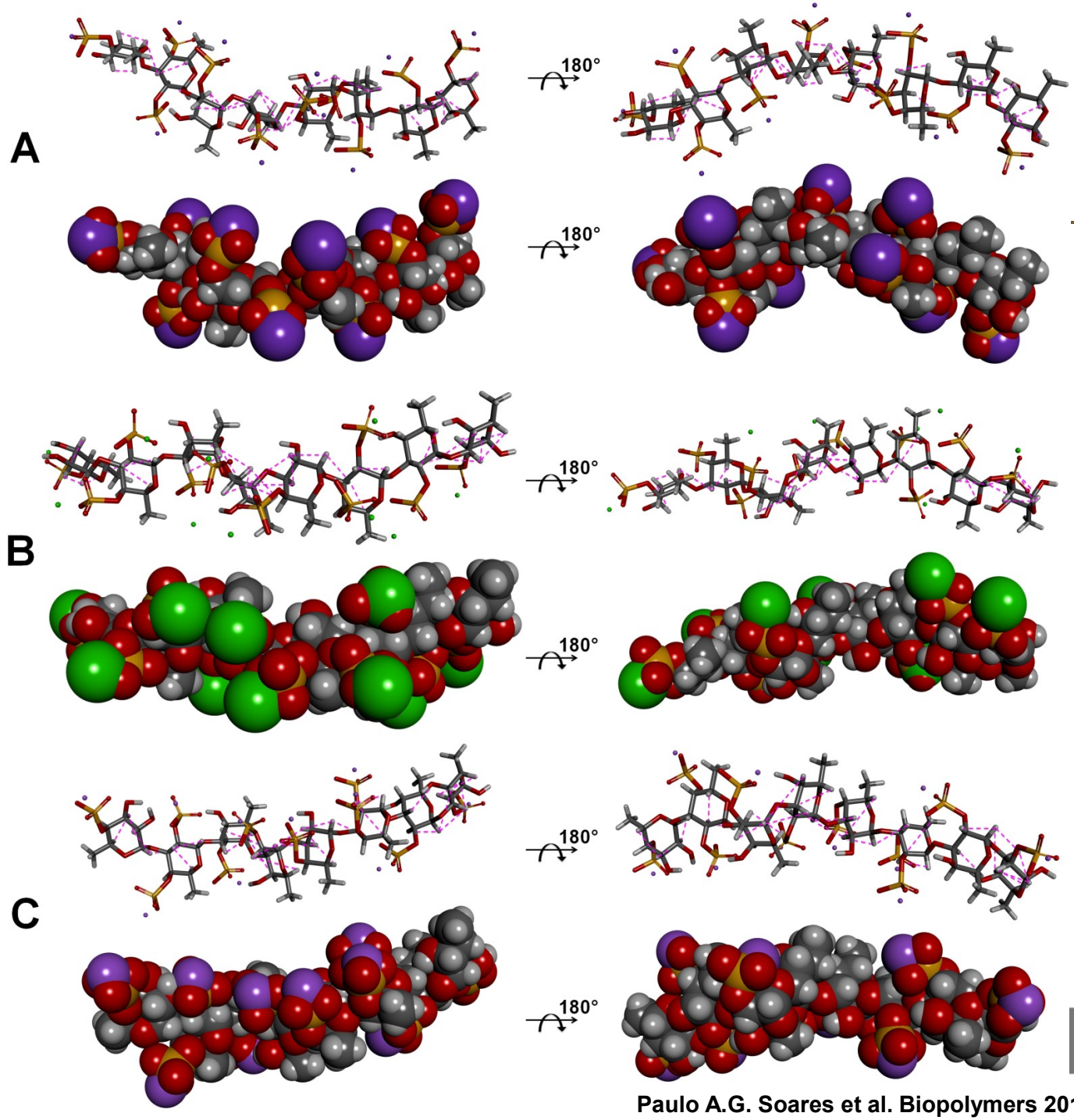
Counterion effects (Na^+, Ca^{2+}, Li^+)





Biopolymers

Paulo A.G. Soares et al. Biopolymers 2016;105:840-851

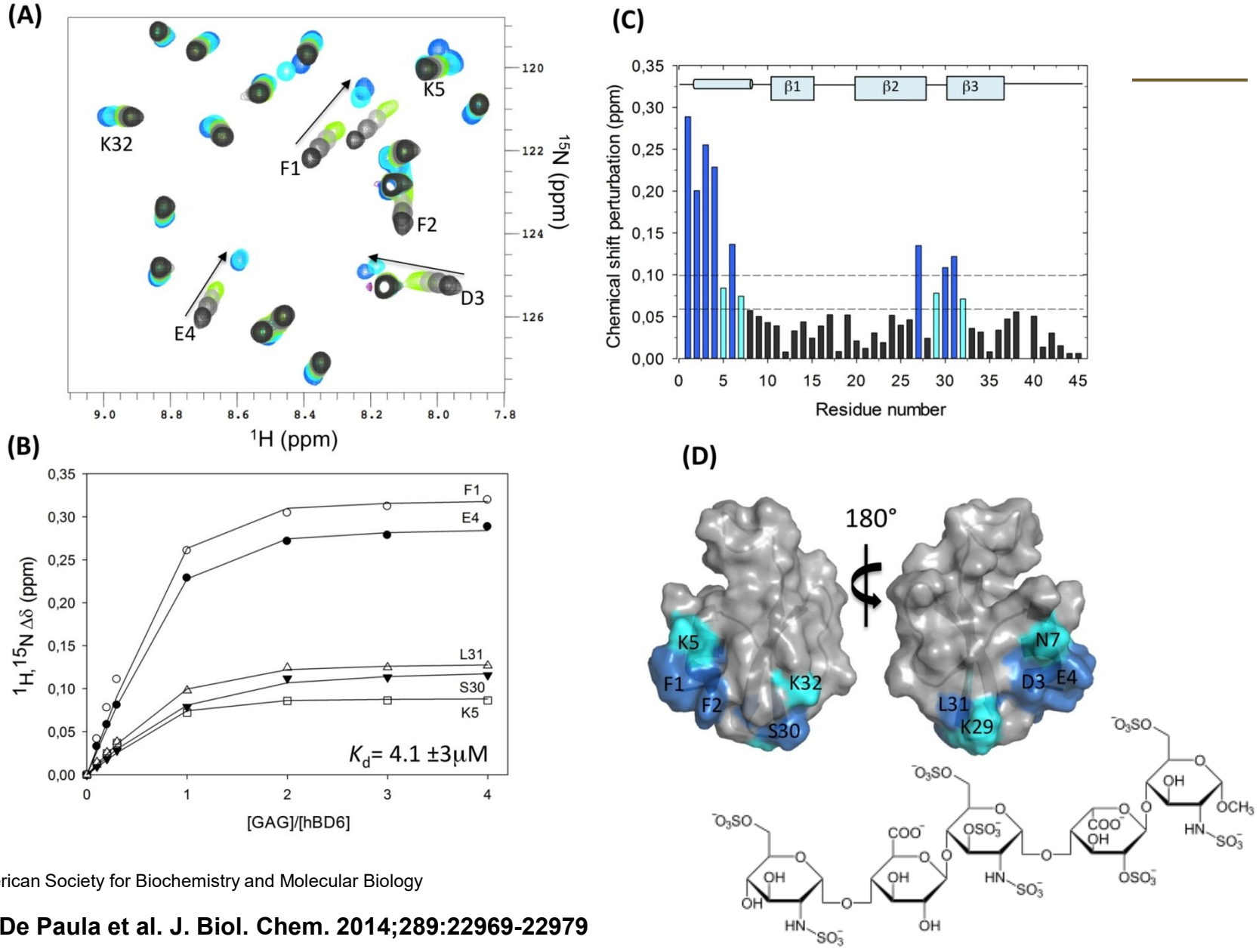


Biopolymers

Paulo A.G. Soares et al. Biopolymers 2016;105:840-851

Protein-sulfated glycan interactions

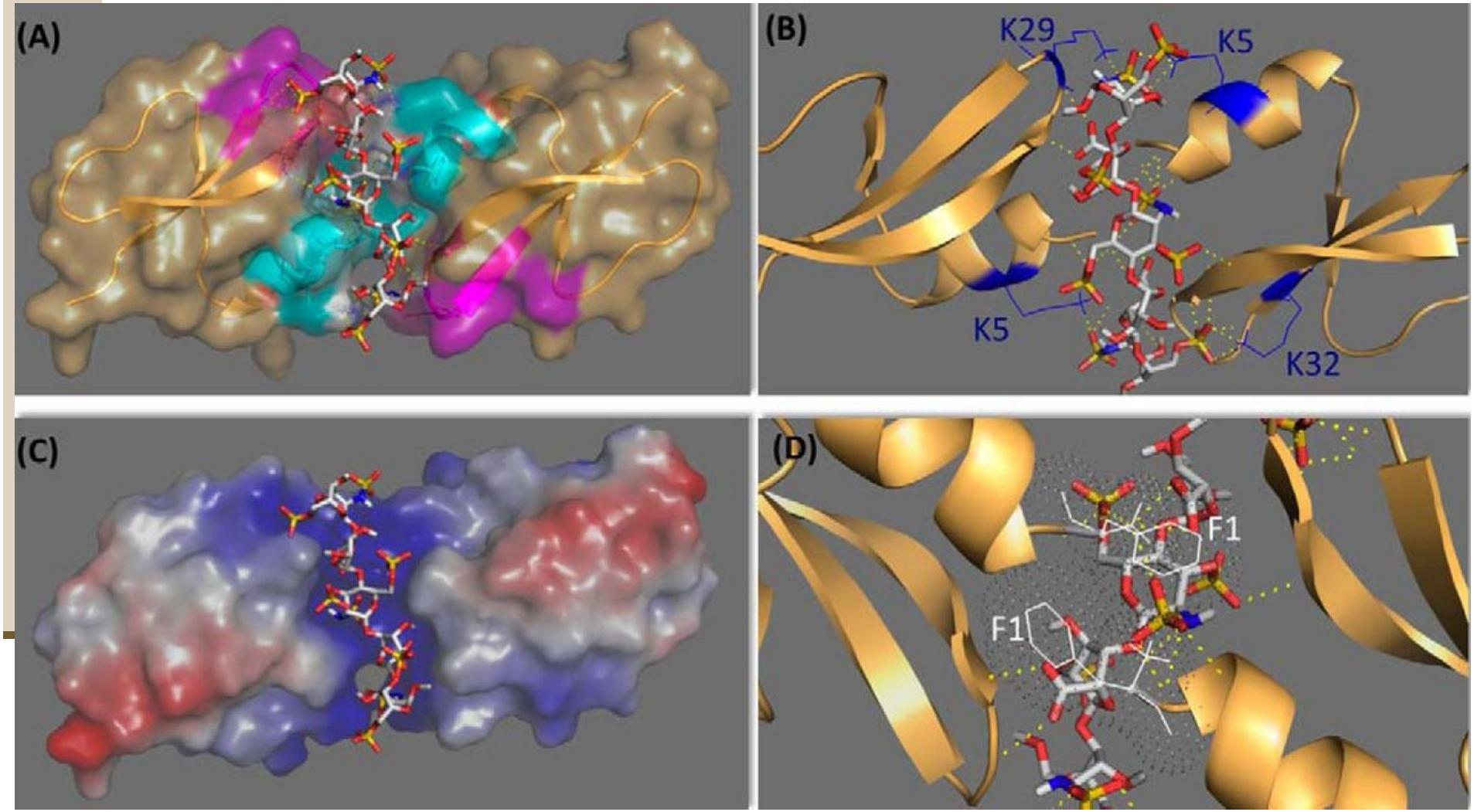
Chemical shift perturbation of fondaparinux on ^{15}N-labeled hBD6



Viviane S. De Paula et al. J. Biol. Chem. 2014;289:22969-22979

Protein-sulfated glycan interactions

NMR-restrained HADDOCK models for the ternary complex ^{15}N-hBD6:fondaparinux



Viviane S. De Paula et al. J. Biol. Chem. 2014;289:22969-22979

jbc

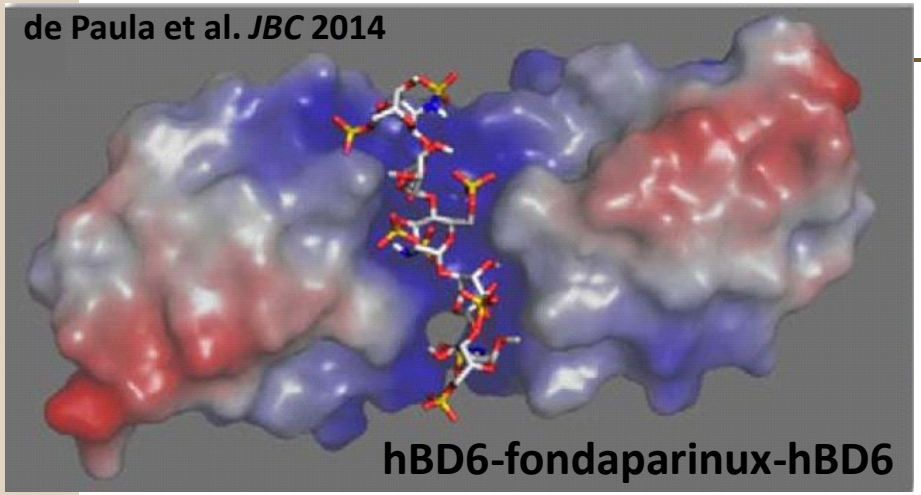
Glycobiology. 2014 Nov;24(11):991-1003. doi: 10.1093/glycob/cwu065. Epub 2014 Jun 27.

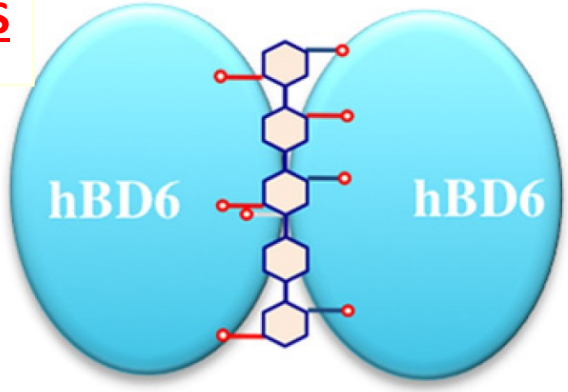
Biological findings from the recent NMR-based studies of glycosaminoglycan-protein interactions.

Pomin VH[1].

GLYCOBIOLOGY

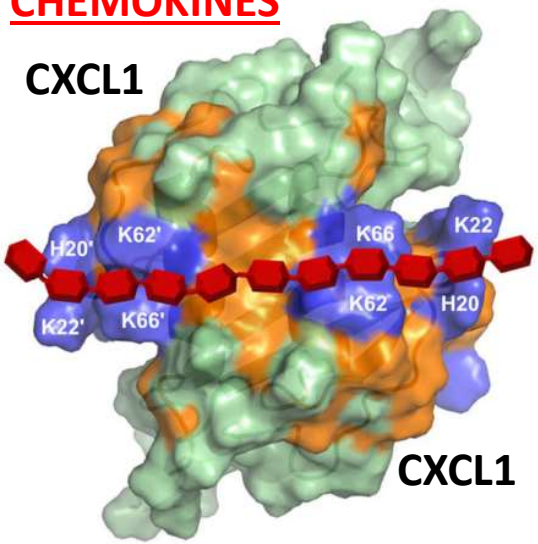
DEFENSINS



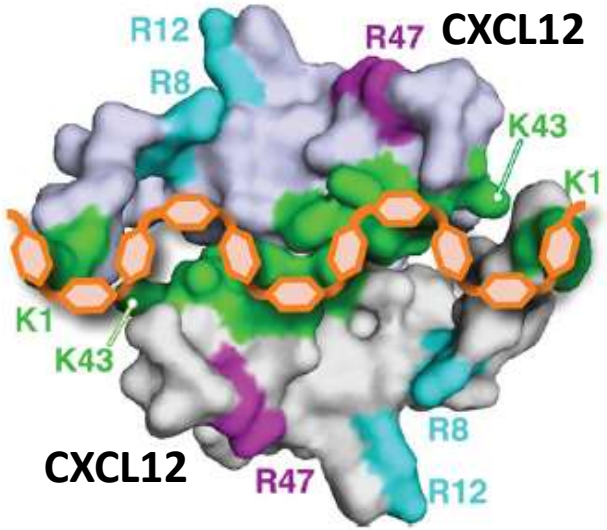


Sandwich-like dimerization

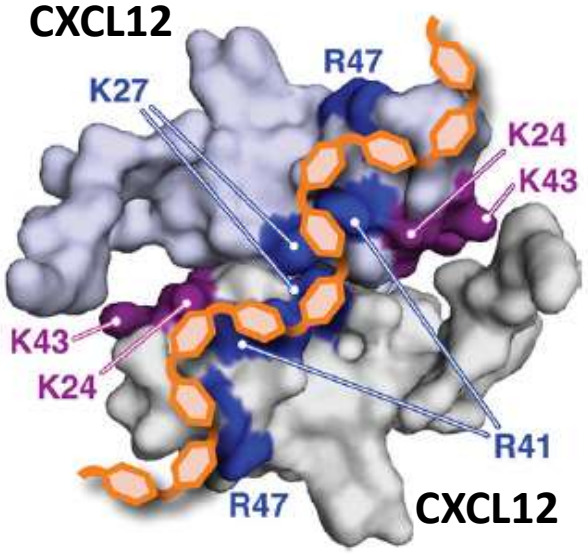
CHEMOKINES



Poluri et al. *JBC* 2013

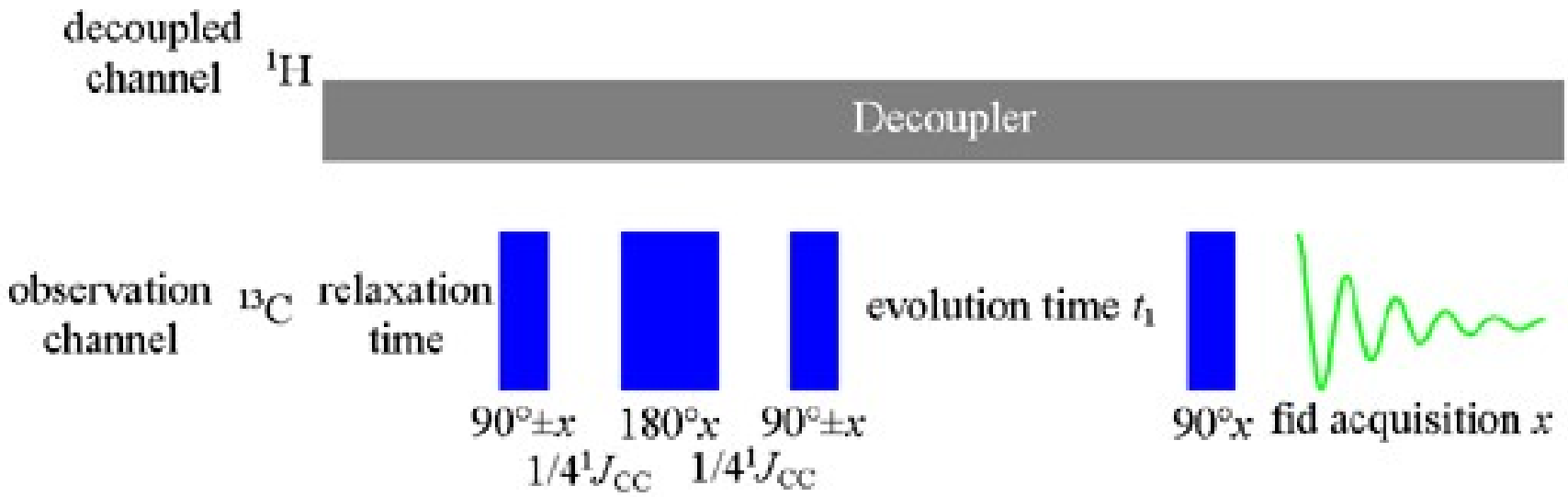


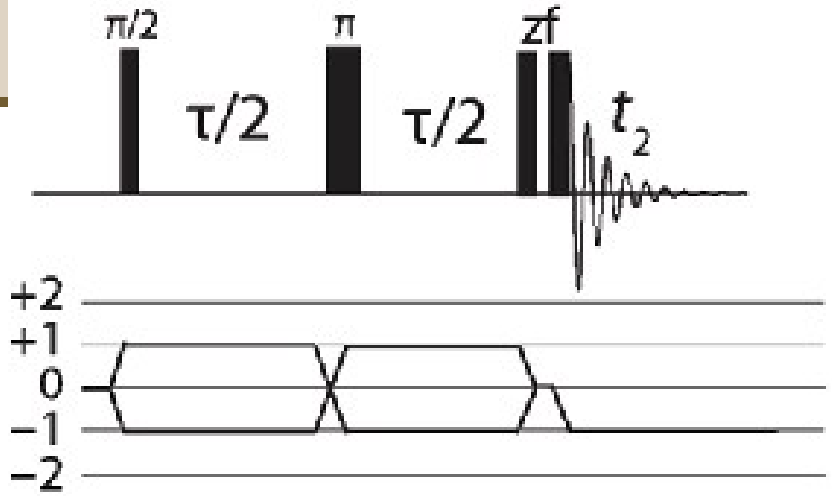
Laguri et al. *JACS* 2011



Ziarek et al. *JBC* 2013

2D ^{13}C-^{13}C INADEQUATE (Incredible Natural Abundance DoublE QUAntum Transfer Experiment)





2D ^{13}C-^{13}C INADEQUATE (Incredible Natural Abundance DoublE QUAntum Transfer Experiment)

- C-C correlation experiment.
- Relies on two neighboring ^{13}C.
- Chance of ^{13}C-^{13}C = 1/10.000.
- Double quantum dimension (f2) VS Single Quantum Dimension (f1).
- For proper acquisition, it needs signal/noise of 25/1 with 1 transient ^{13}C NMR experiment to get spectrum in 24 h.
- Assuming a compound of 150 Da, needs 700 mg in 0.7 mL solvent (~ 6M).

Structural characterization of a hyaluronidase inhibitor derived from marine *Streptomyces*

Mar. Drugs **2014**, *12*, 491-507; doi:10.3390/md12010491

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marine drugs

ISSN 1660-3397

www.mdpi.com/journal/marinedrugs

Article

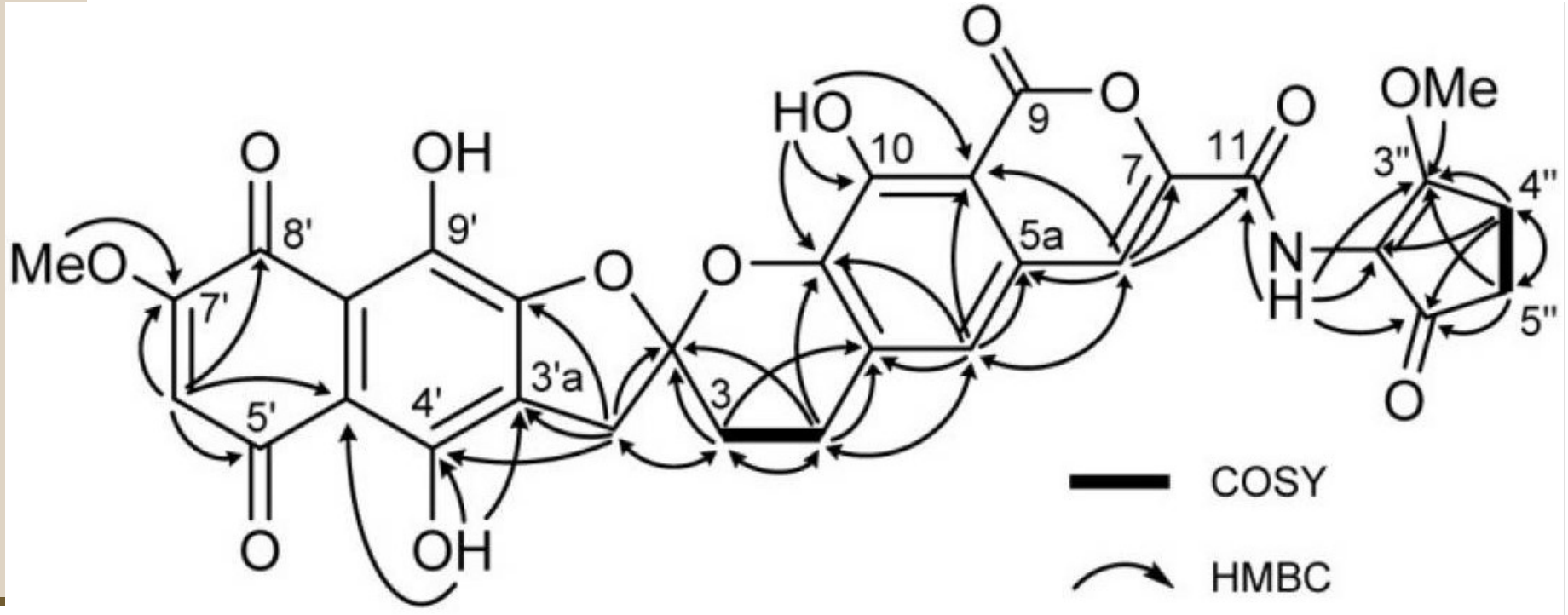
Hyaluromycin, a New Hyaluronidase Inhibitor of Polyketide Origin from Marine *Streptomyces* sp.

Enjuro Harunari [1,*], Chiaki Imada [1], Yasuhiro Igarashi [2], Takao Fukuda [2], Takeshi Terahara [1] and Takeshi Kobayashi [1]

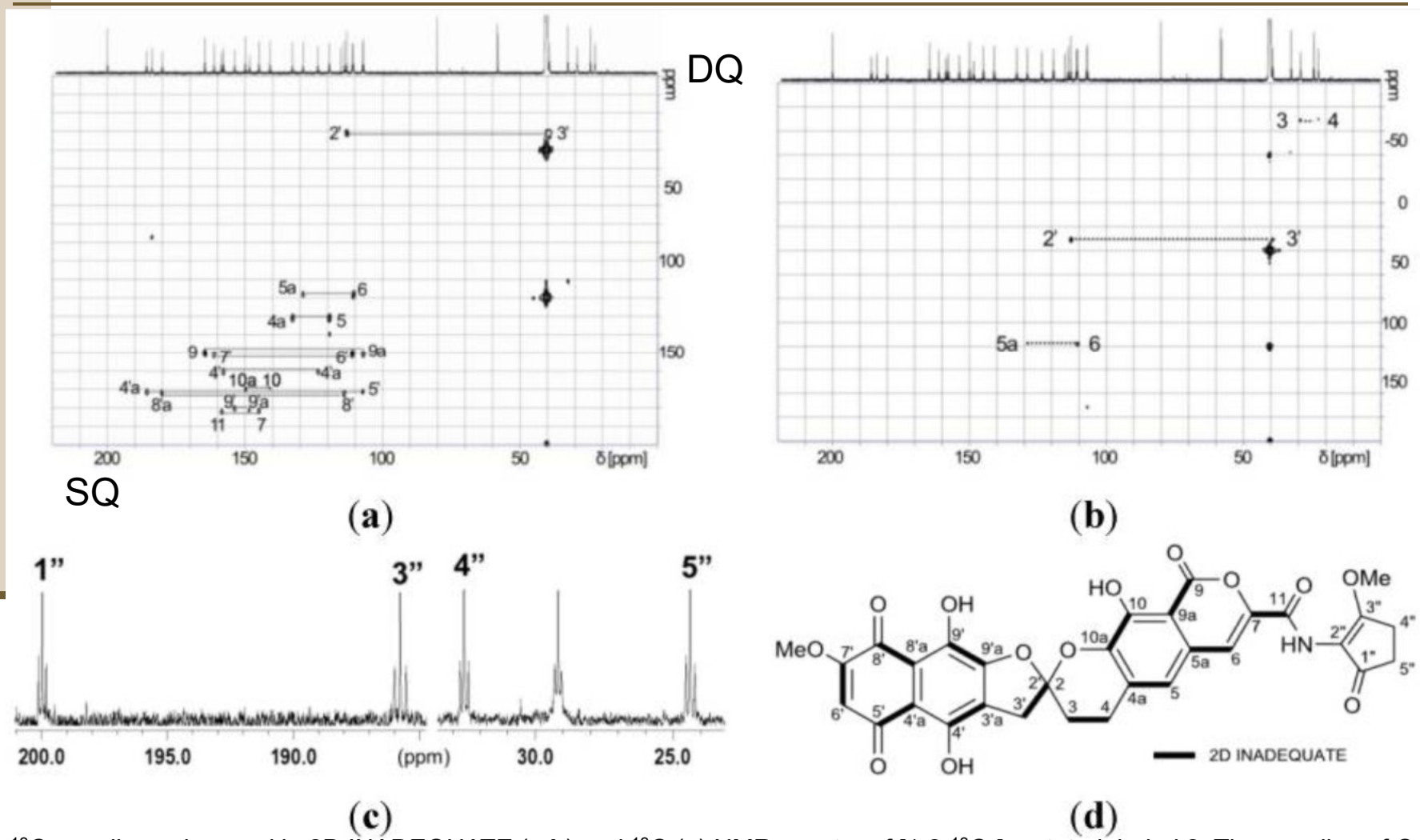
[1] Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan;
E-Mails: imada@kaiyodai.ac.jp (C.I.); terahara@kaiyodai.ac.jp (T.T.);
takeshik@kaiyodai.ac.jp (T.K.)

[2] Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan; E-Mails: yas@pu-toyama.ac.jp (Y.I.); z02113@st.pu-toyama.ac.jp (T.F.)

Structural characterization of a hyaluronidase inhibitor derived from marine *Streptomyces*

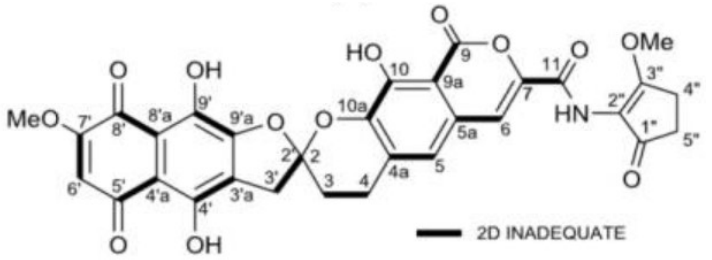


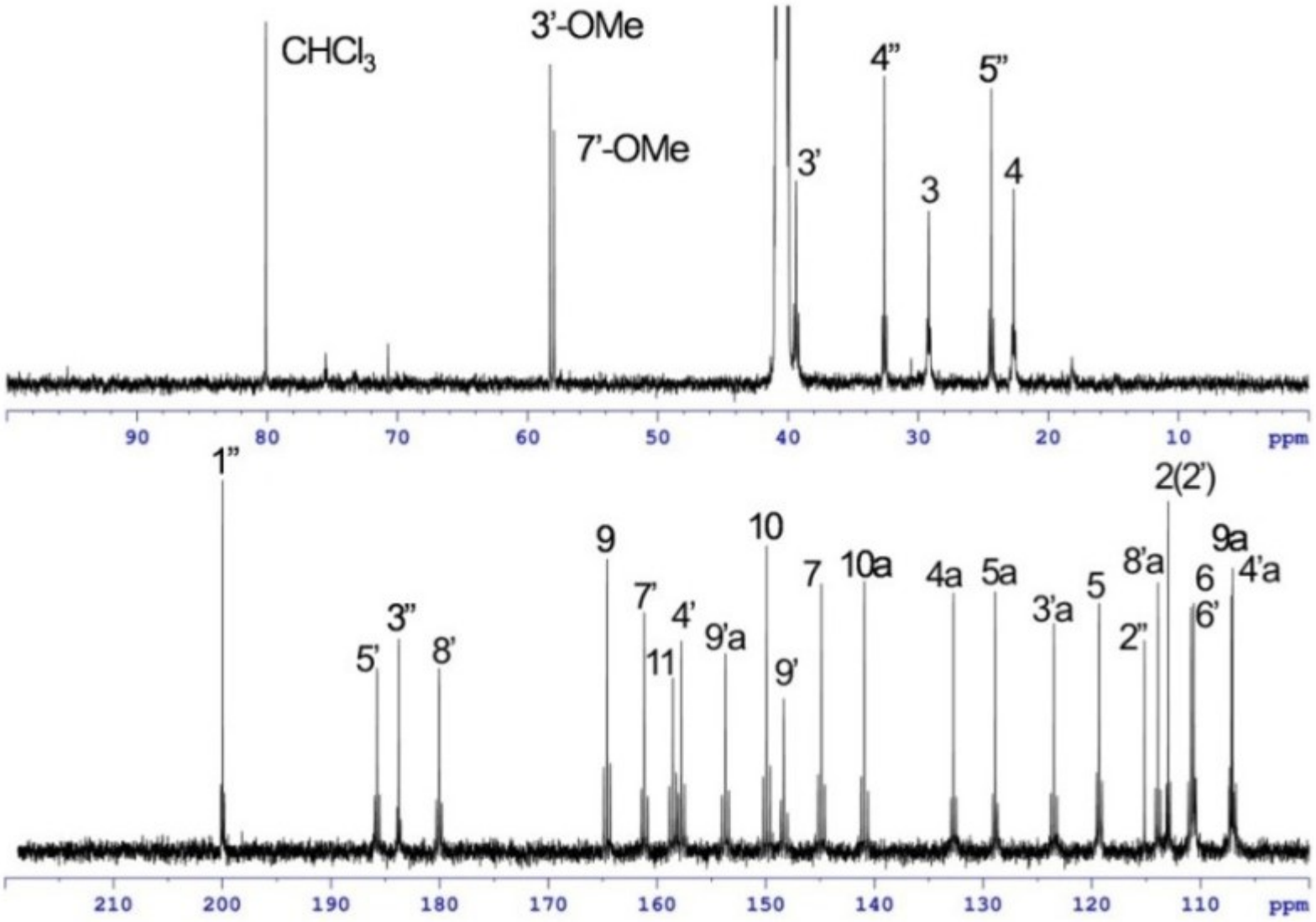
Structural characterization of a hyaluronidase inhibitor derived from marine *Streptomyces*



^{13}C-^{13}C couplings observed in 2D INADEQUATE (**a**,**b**) and ^{13}C (**c**) NMR spectra of [1,2-$^{13}C_2$]acetate-labeled **2**. The coupling of C-1″/C-5″ and C-3″/C-4″ were only observed in the ^{13}C NMR spectrum (**c**). (**a**) Optimized for $^{1}J_{cc}$ = 50 Hz; (**b**) Optimized for $^{1}J_{cc}$ = 35 Hz; (**c**) ^{13}C NMR spectra; (**d**) Observed in 2D INADEQUATE.

^{13}C assignments of hyaluronidase *inhibitor*





Sensitivity in NMR

Table-13.1: Quantities of material (of M.Wt. approx. 400) required on a 400 MHz instrument over 12 hours of measuring time.

	Experiment	Amount required (in mg)
A)	1D Experiments	
1.	1D ^{1}H-NMR	0.004
2.	Broadband decoupled ^{13}C-NMR	4
3.	DEPT	5
4.	1D INADEQUATE	75
B)	2D Experiments (^{1}H / ^{13}C)	
1.	COSY	
2.	Delayed COSY	4
3.	Phase-sensitive COSY	4
4.	Heteronuclear 2D shift correlation (one-bond)	10
5.	Long range heteronuclear 2D shift correlation	20
6.	Heteronuclear multiple bond connectivity (HMBC)	2
7.	NOESY	8
8.	Homonuclear relayed coherence transfer	10
9.	Heteronuclear relayed coherence transfer	30
10.	Homonuclear 2D J-resolved	1
11.	Heteronuclear 2D J-resolved	40
12.	2D INADEQUATE	300

If more sample is available, the measuring time will be correspondingly reduced.