III Congreso Latinoamericano
Biorrefinerías
Ideas para un mundo sustentable
19 al 21 de noviembre de 2012, Pucón, Chile

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Lignocellulose deconstruction as shown by 2D-NMR

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IRNAS, CSIC, Sevilla, Spain

Forest-Based Sector Technology Platform

SusChem European Technology Platform For SUSTAINABLE CHEMISTRY

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

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About Lignodeco

LIGNODECO (from LIGNOcellulose DECOnstruction) is the abbreviated name for "Optimized pre-treatment of fast growing woody and nonwoody Brazilian crops by <u>detailed characterization of chemical changes</u> produced in the lignin-carbohydrate matrix", a collaborative research project funded by the EC.

Consortium members

The project is run by a consortium formed by two world-leader companies from Brazil (Suzano) and the EU (Novozymes), four EU research institutes (CIB, CTP, IRNAS and VTT), and one Brazilian University (UFV), responsible for project coordination.

The present study is part of the collaborative work between Lignodeco partners because raw materials from Brazil were treated by IRNAS and Suzanno (including use of Novozymes enzymes), chemically-analyzed by IRNAS and CIB and their bioethanol potential evaluated at VTT

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LIGNODE

RAMEWORK
AMME

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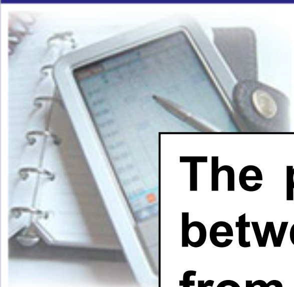

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- [2011/3/5] Second technical meeting
- [2010/8/10] Lignodeco Dissemination
- [2010/7/30] First technical meeting
- [2010/6/1] Selection of woody and nonwoody feedstocks for in-depth characterisation and pre-treatment assays
- [2010/3/8] UFV coordinates project approved by the European Commission
- [2010/1/26] Consortium members kick-off meeting, Brussels, Belgium

Universidade Federal de Viçosa

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LIGNODECO

LIGNOcellulose DECOnstruction

FUNARBE

SEVENTH FRAMEWORK PROGRAMME

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Lignodeco News

- [2012/6/15] **LignoDeco 30th month meeting - Copenhagen on June 11-12, 2012**
- [2011/8/29] **Third technical meeting**
- [2011/8/18] **Lignodeco Dissemination in 3rd Nordic Wood Biorefinery Conference**
- [2011/3/5] **Second technical meeting**
- [2010/8/10] **Lignodeco Dissemination**
- [2010/7/30] **First technical meeting**
- [2010/6/1] **Selection of woody and nonwoody feedstocks for in-depth characterisation and pre-treatment assays**
- [2010/3/8] **UFV coordinates project approved by the European Commission**
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Video

Entrevista

Partners

Pulp and Paper Laboratory

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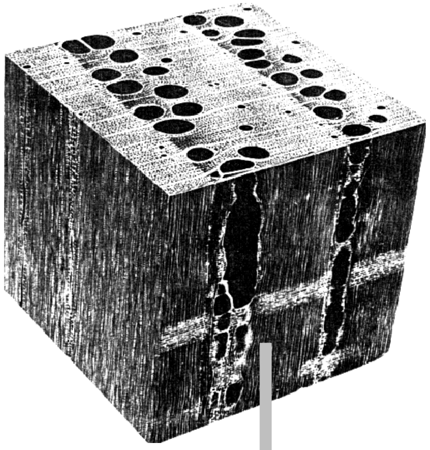
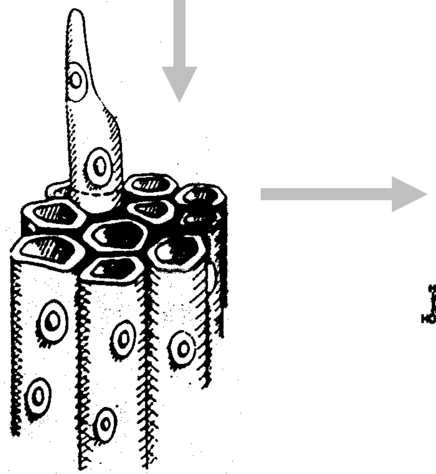
Plant feedstocks for lignocellulose biorefineries (the lignin "barrier")

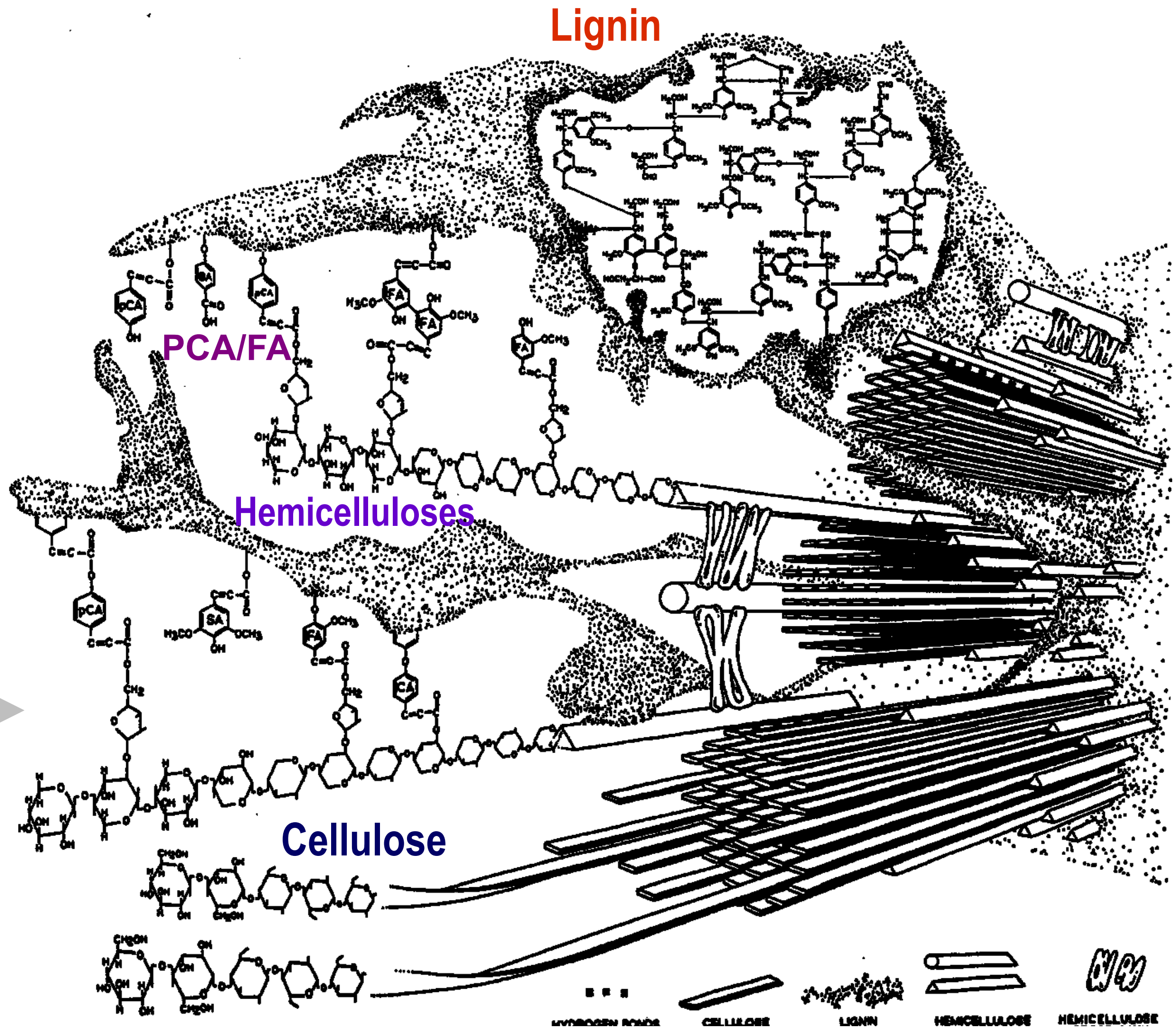
Lignocellulosic crops are a renewable resource that has been well integrated into various **industrial** processes

Eucalyptus clonal propagation

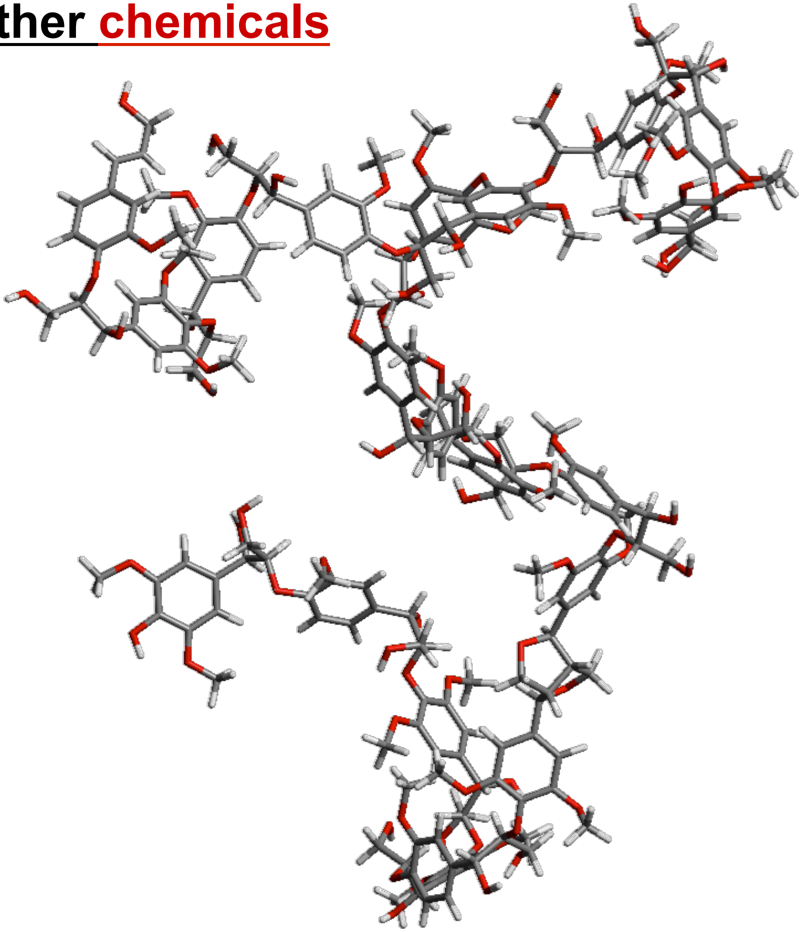
Histological and molecular organization of lignocellulosic materials: Interactions between the structural polymers and other components





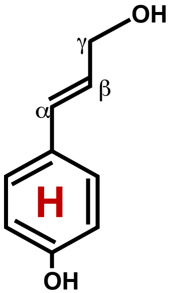


Lignin removal is an central issue for lignocellulose deconstruction in cellulose pulp manufacturing (pulping and bleaching) and a key challenge for its conversion into liquid fuel and other chemicals

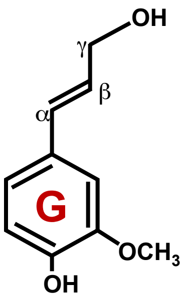


Lignin is a complex polymer formed by random-coupling of radicals from three monolignols giving rise to H, G and S units and different inter-unit linkages →

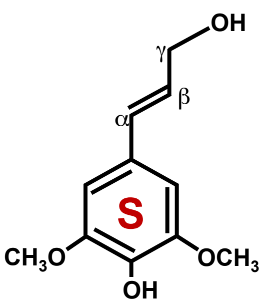
Lignin precursors



p-coumaryl alcohol

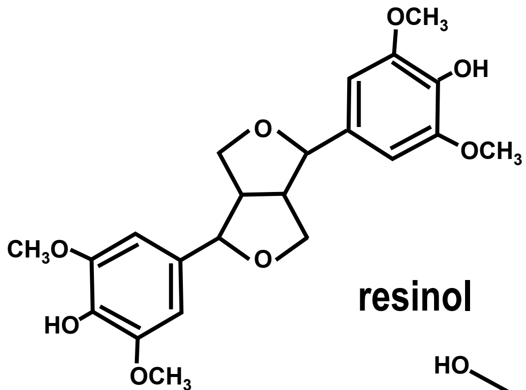


coniferyl alcohol

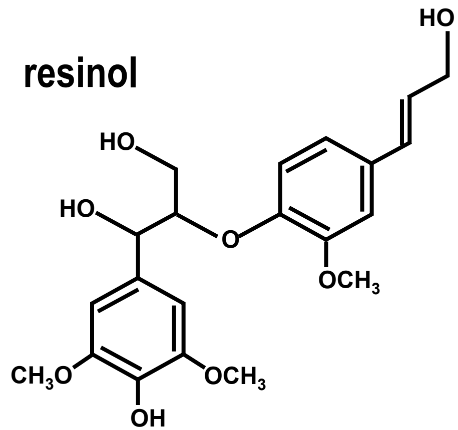


sinapyl alcohol

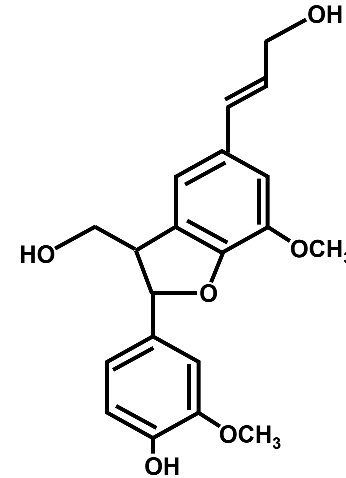
Inter-unit linkages in lignin (lignols)



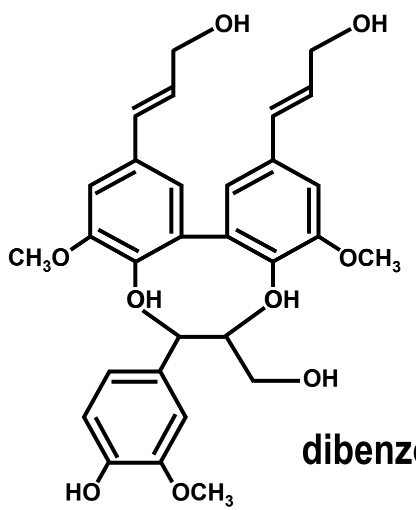
resinol



β-O-4 ether

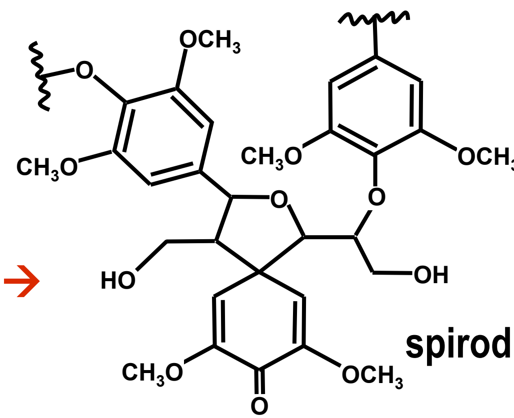


phenylcoumaran



dibenzodioxocin

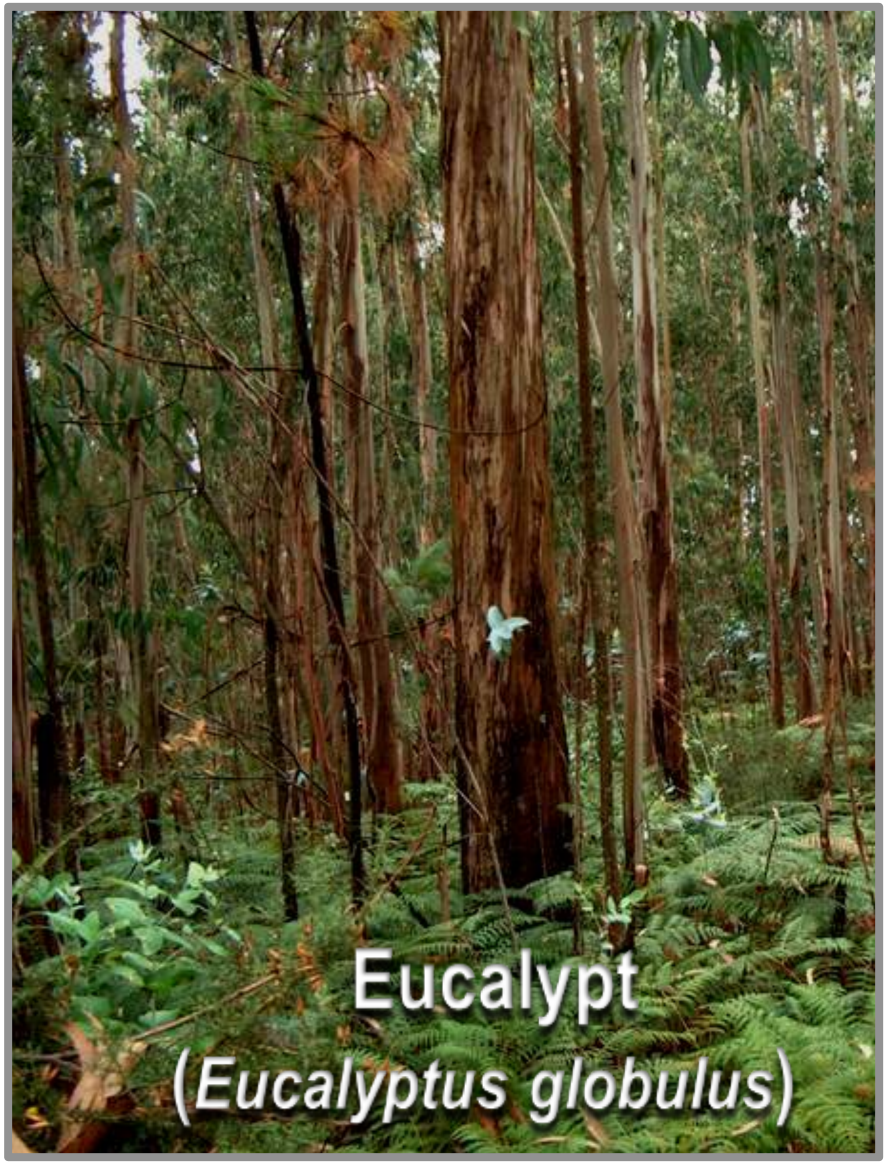
← discovered by 2D NMR! →



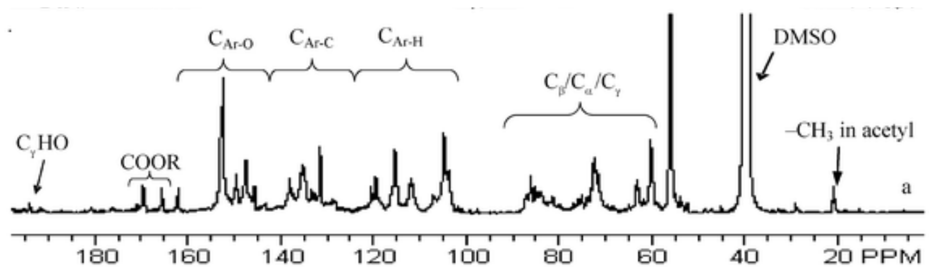
spirodienone

Several approaches including physical, chemical and biological pretreatments, are being studied in LIGNODECO for deconstructing different lignocellulosic feedstocks and removing lignin

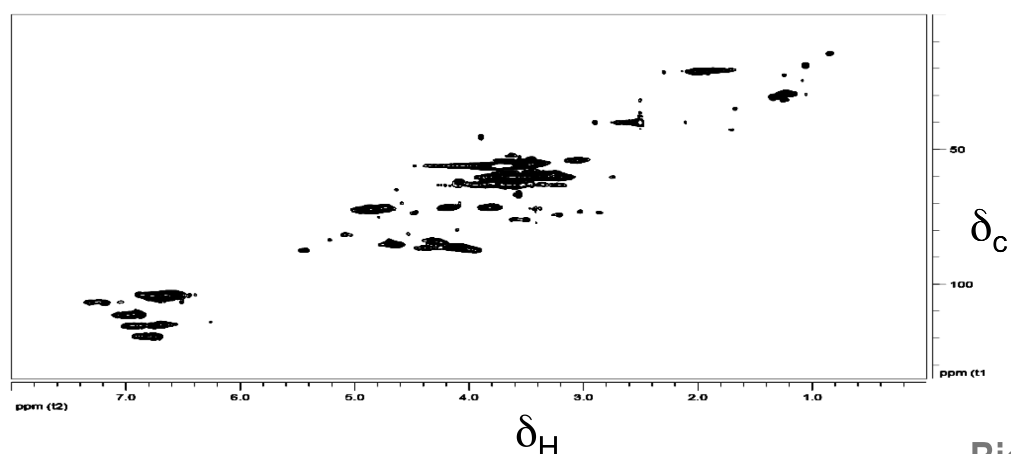
Two nonwoody and woody plant feedstocks (with ~22% lignin) were compared in LIGNODECO:



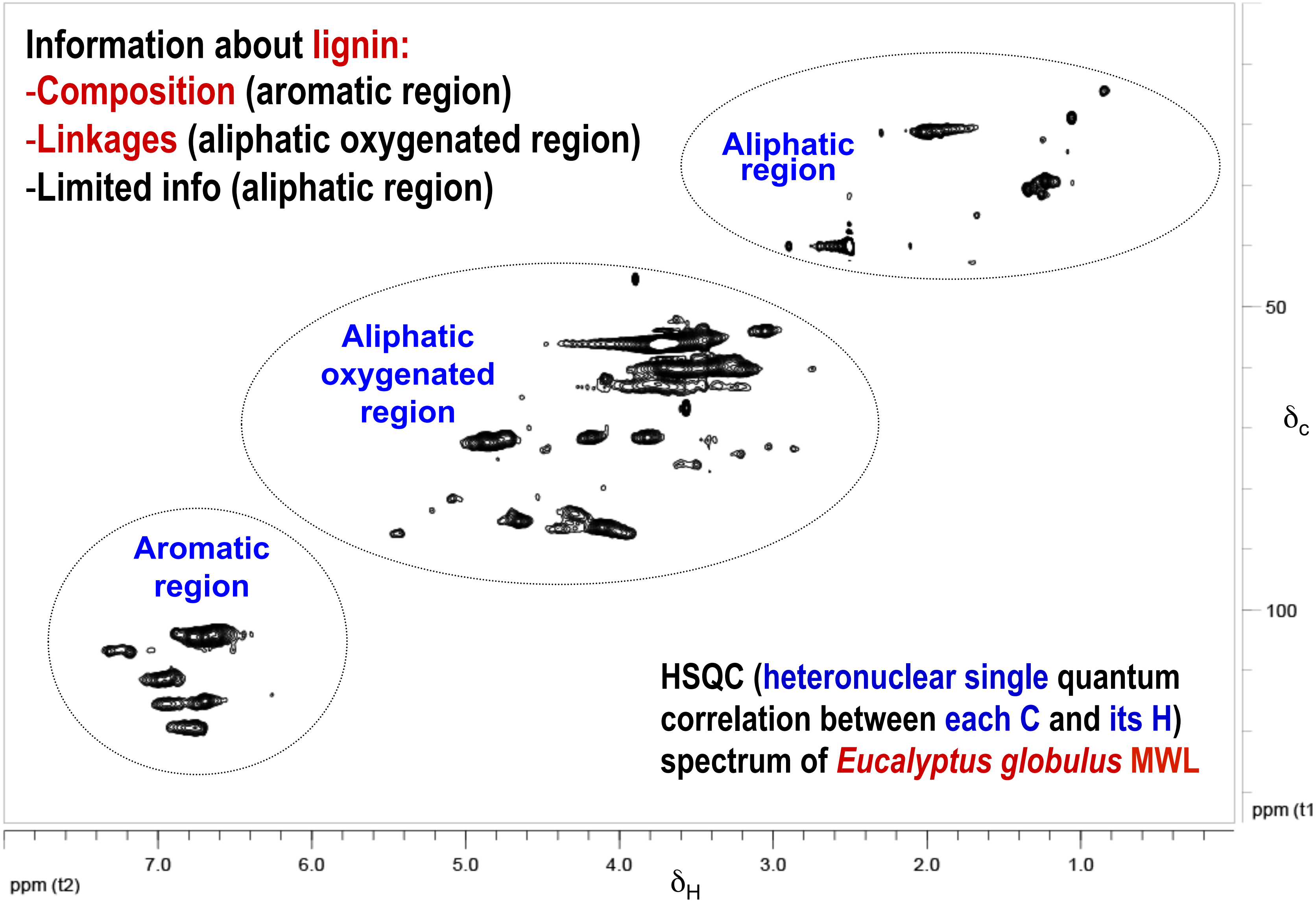
NMR has been classically used to analyze lignin but signal overlapping was a major problem in 1D NMR.



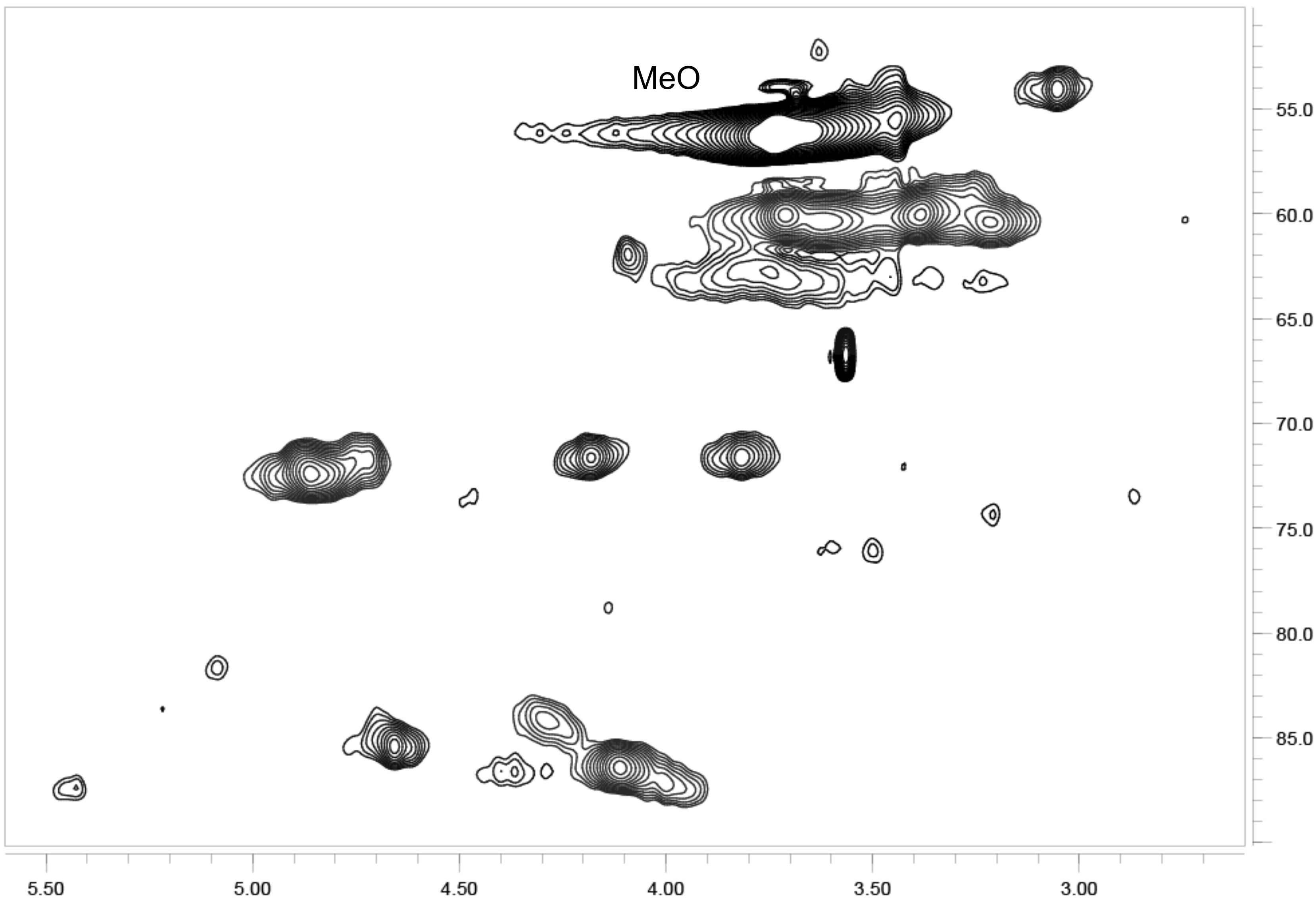
2D-NMR solved the above problem, and provided an invaluable tool for better understanding the complex structure of lignin.



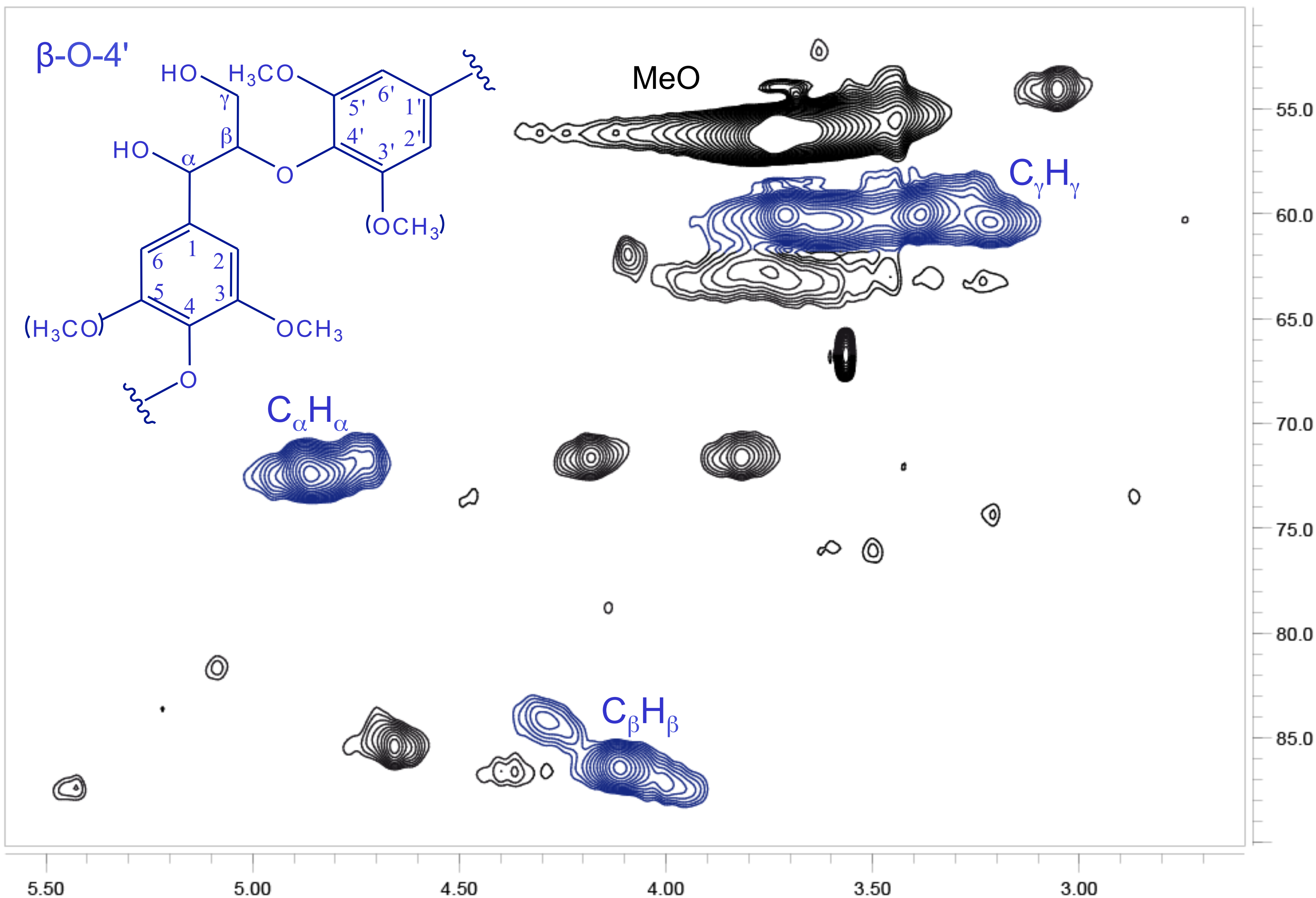
Lignin 2D-NMR: Whole HSQC spectrum



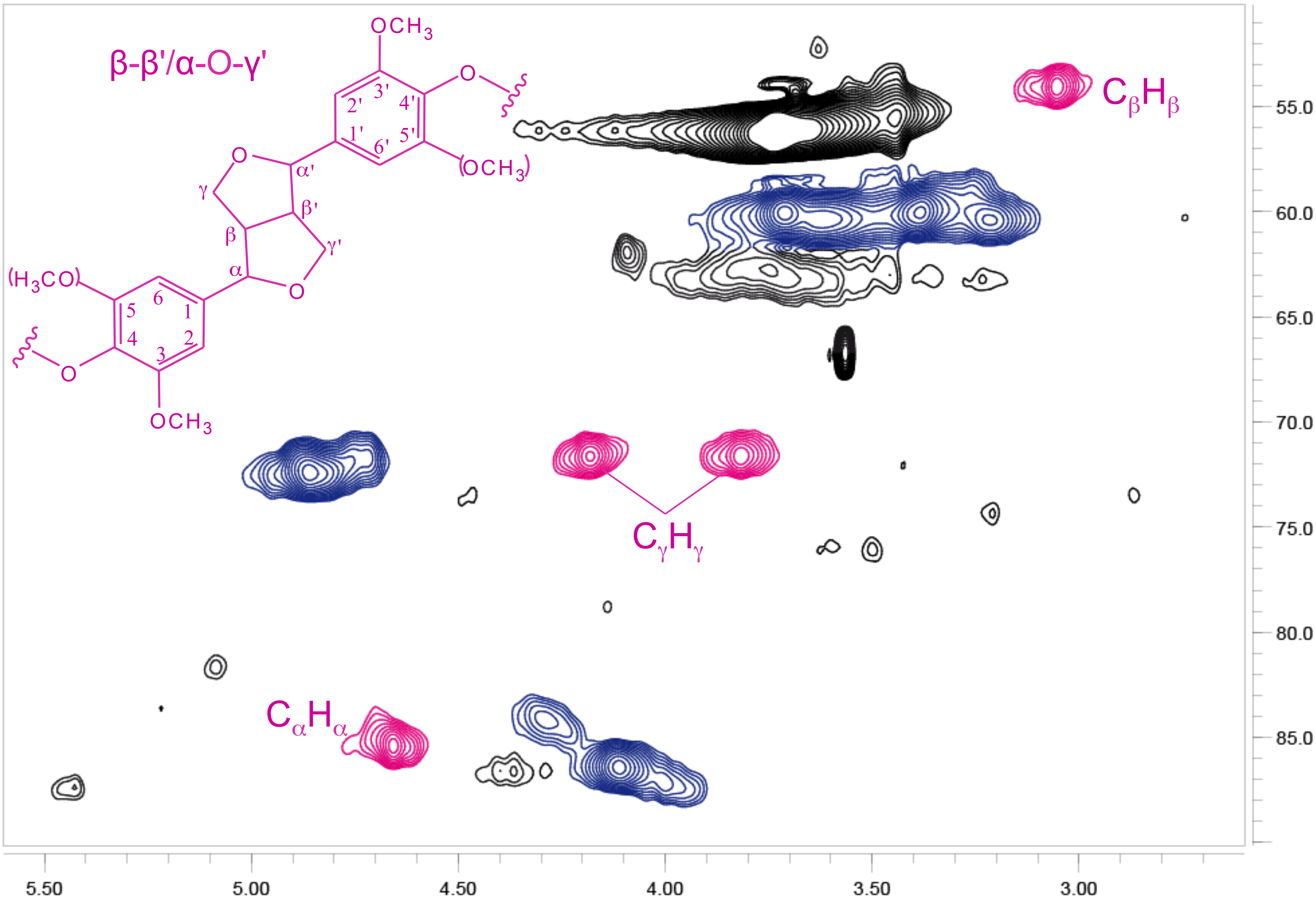
Lignin 2D-NMR: Aliphatic oxygenated region



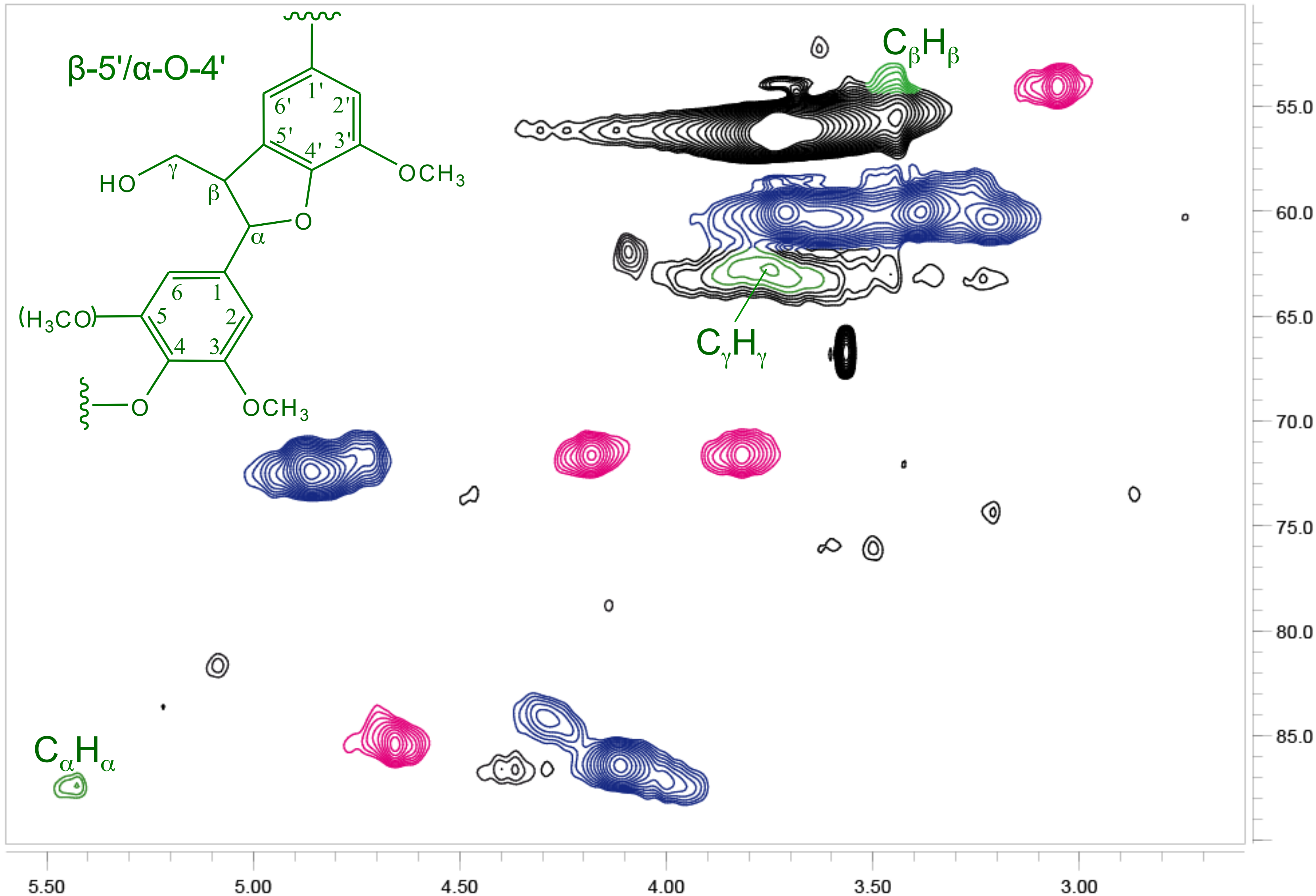
Lignin 2D-NMR: Aliphatic oxygenated region (β-O-4' linkages)



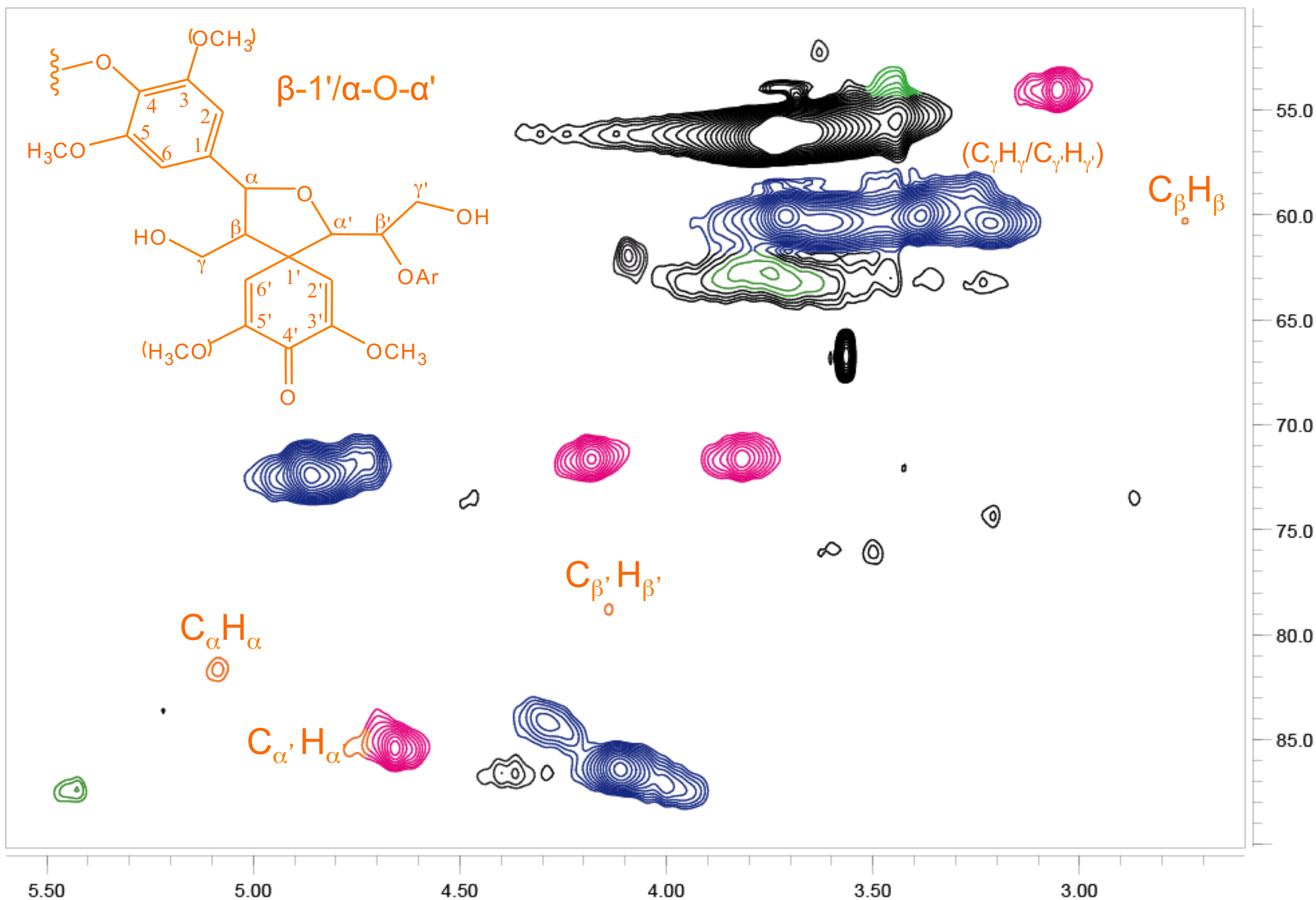
Lignin 2D-NMR: Aliphatic oxygenated region (resinols)



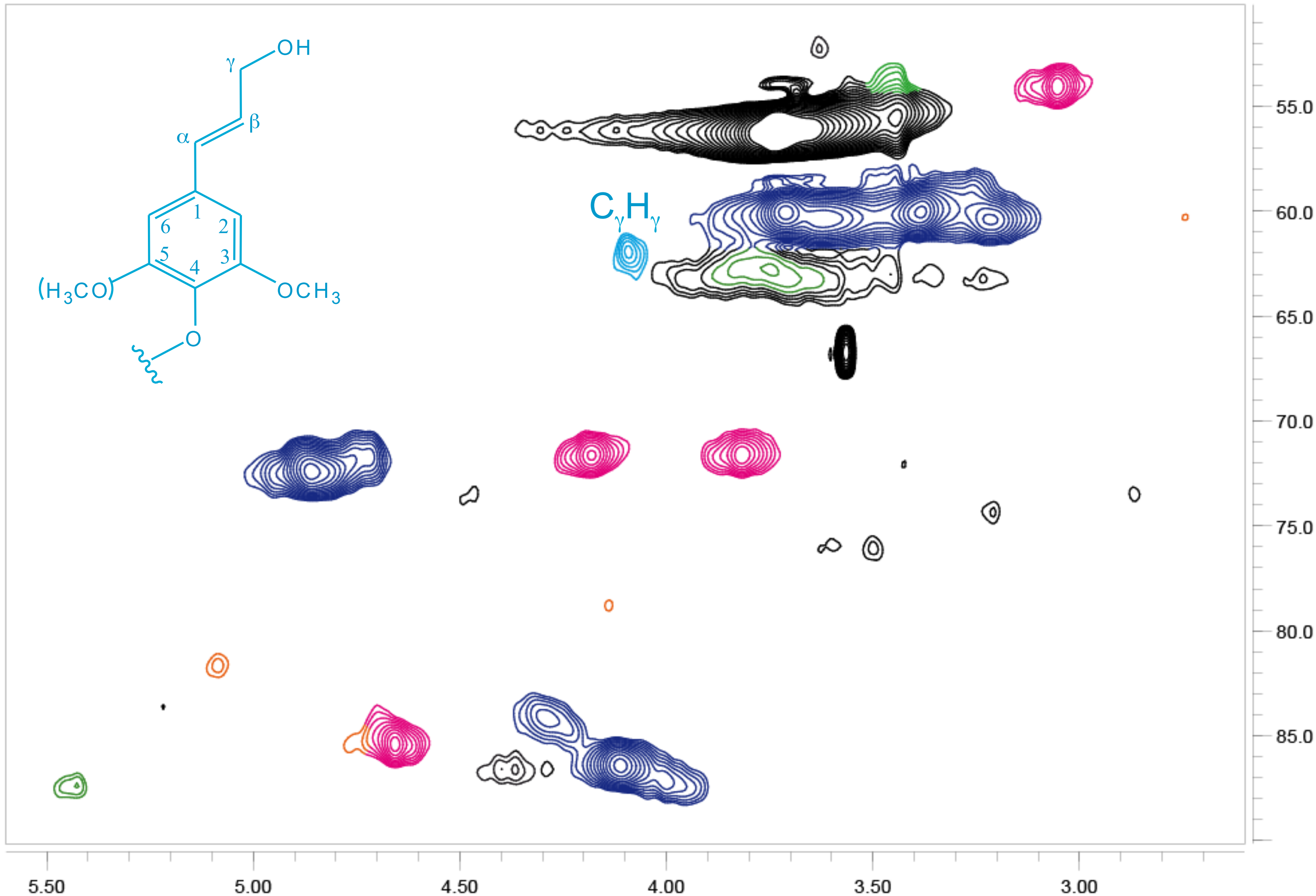
Lignin 2D-NMR: Aliphatic oxygenated region (phenylcoumarans)



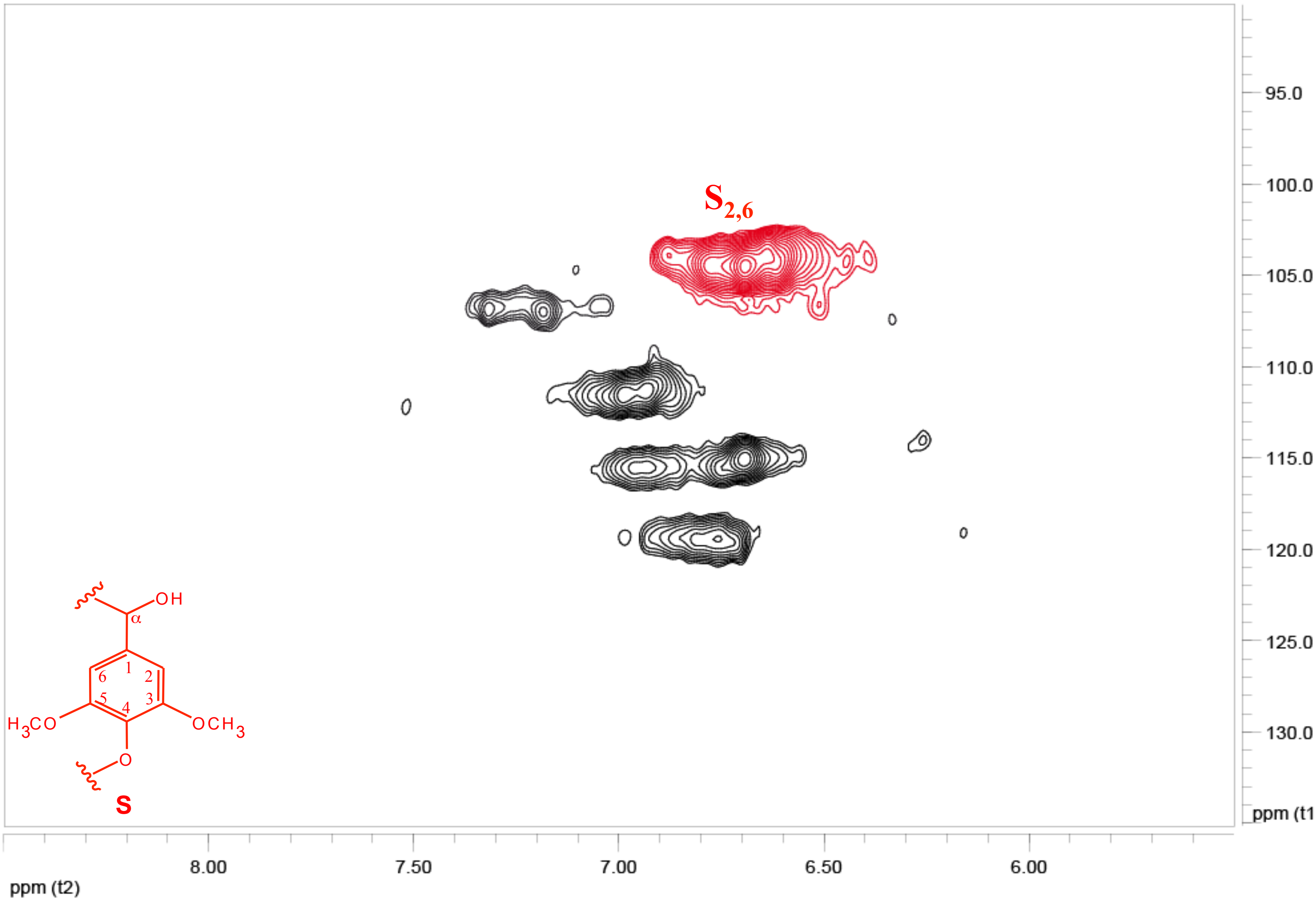
Lignin 2D-NMR: Aliphatic oxygenated region (spirodienones)



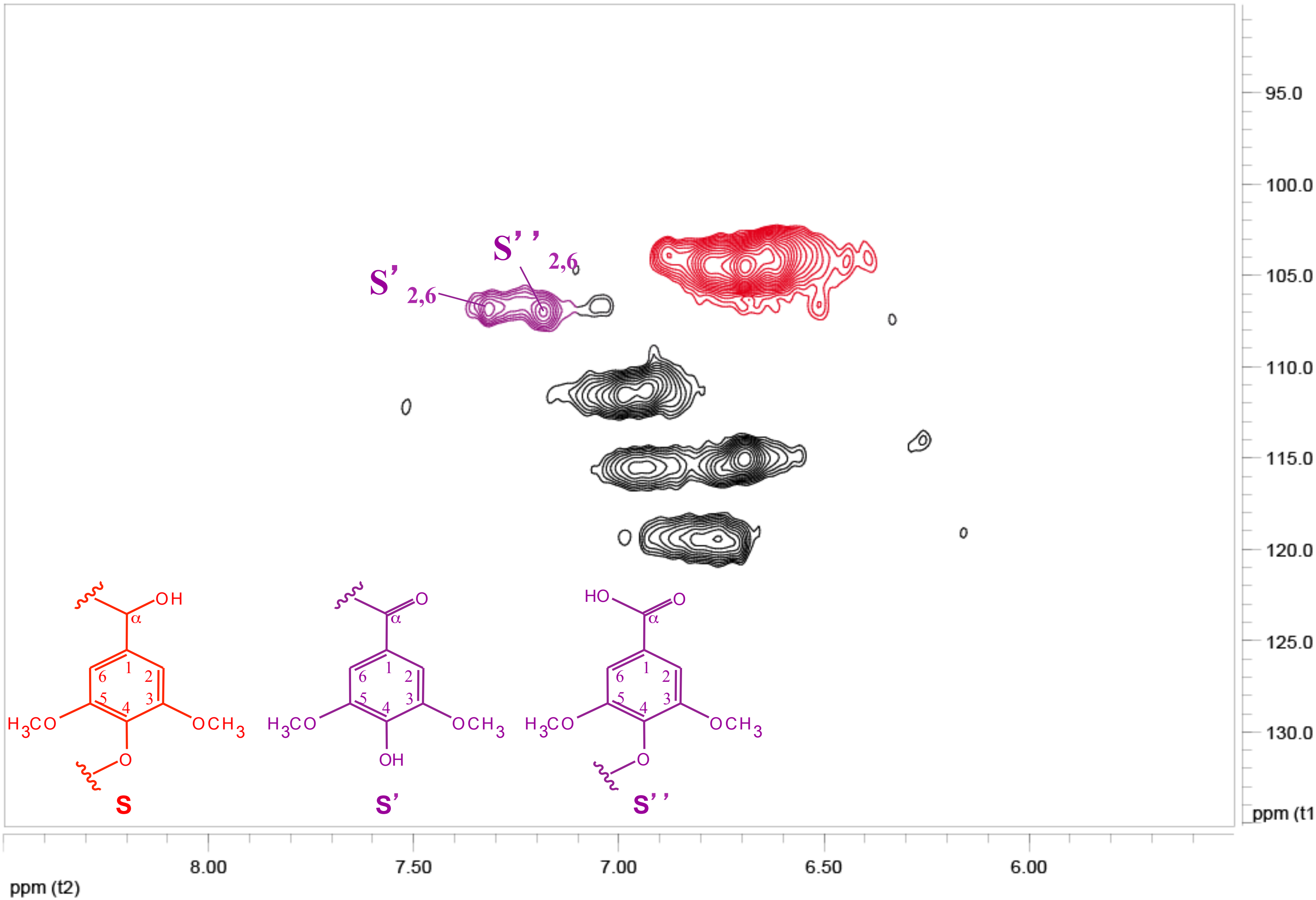
Lignin 2D-NMR: Aliphatic oxygenated region (cinnamyl ends)



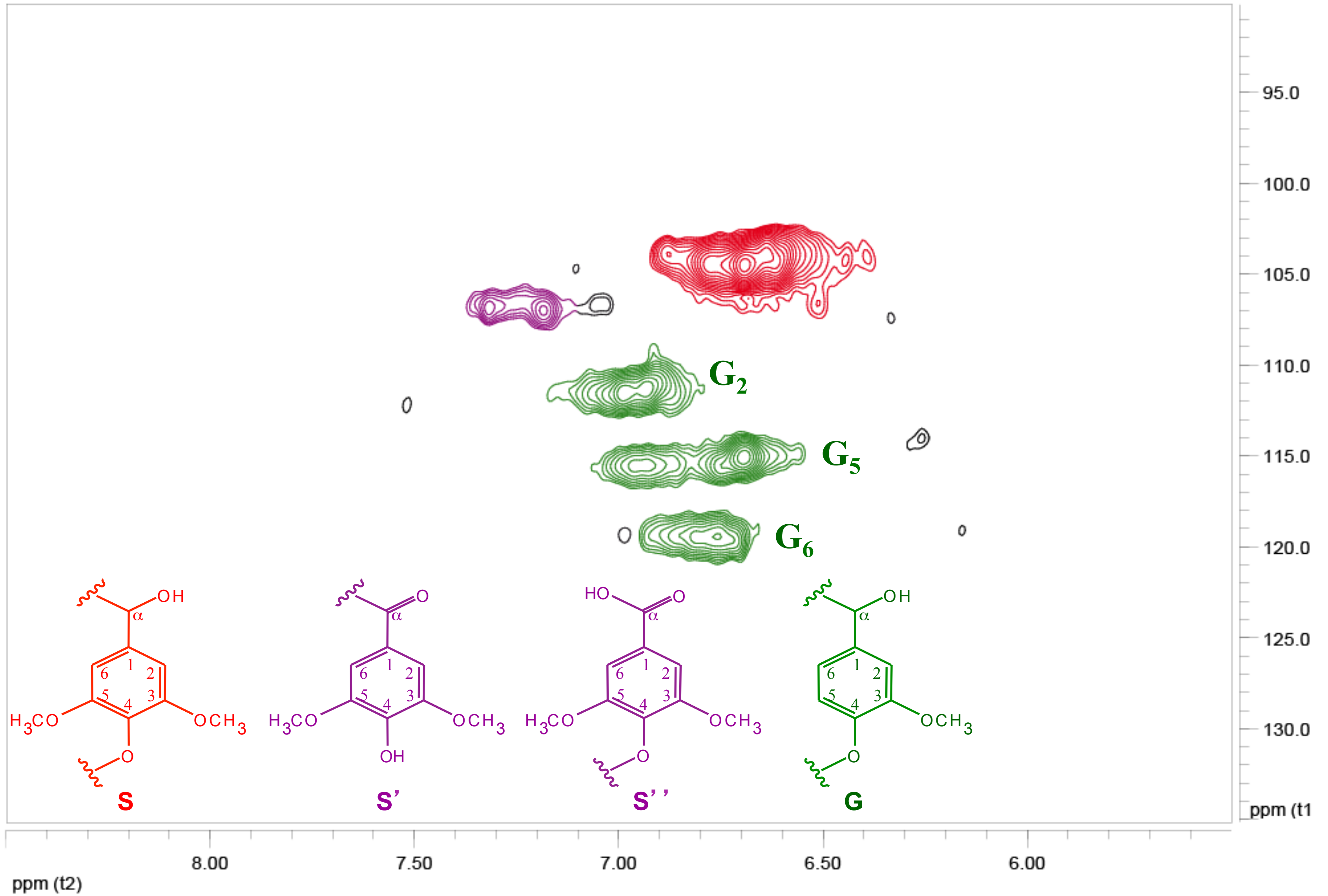
Lignin 2D-NMR: Aromatic region (S units)



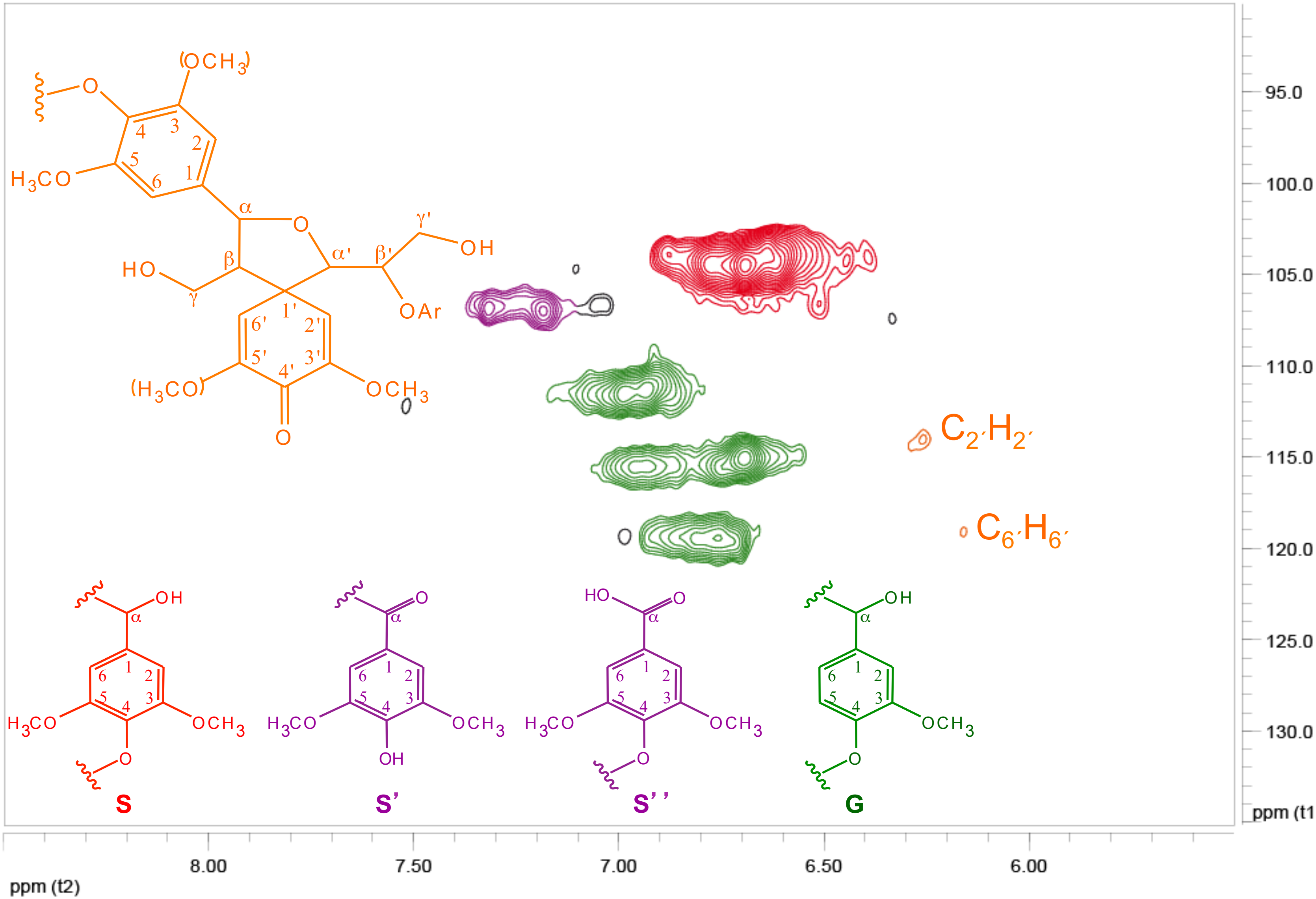
Lignin **2D-NMR**: Aromatic region (C$_\alpha$-oxidized S units)



Lignin 2D-NMR: Aromatic region (G units)



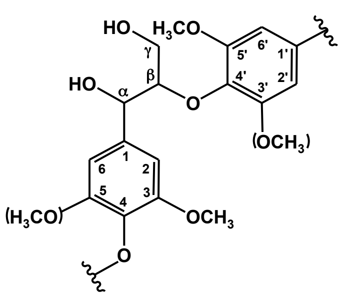
Lignin 2D-NMR: Aromatic region (spirodienone aromatic signals)



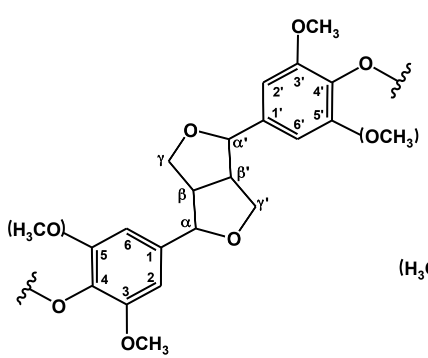
An example of lignin 2D-NMR analysis: Five eucalypt species

S/G ratio, inter-unit linkages and end units (percentage of side-chains)

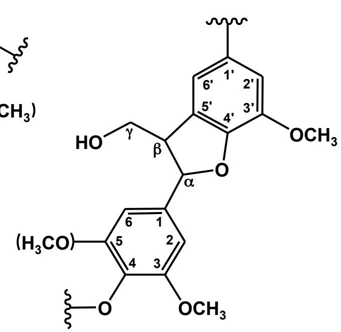
	E. globulus	*E. nitens*	*E. maidenii*	*E. grandis*	*E. dunnii*
β-O-4´ aryl ether	69.3	71.7	69.7	66.9	65.9
Resinol	18.2	16.1	16.4	16.5	19.0
Phenylcoumaran	2.9	4.0	3.6	6.8	4.0
Spirodienone	2.8	1.3	3.6	2.9	4.2
β-O-4´-Cα=O	2.0	1.3	1.7	1.7	1.9
Cinnamyl end-groups	4.7	5.7	4.9	5.3	4.9
S/G ratio	2.9	2.7	2.4	1.7	2.7



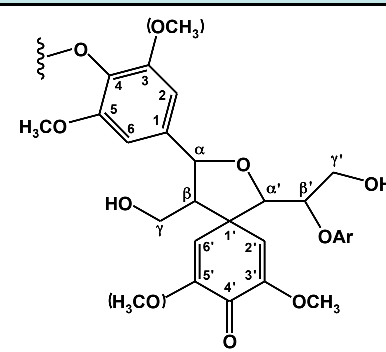
β-*O*-4´



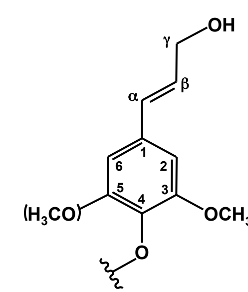
Resinol



Phenylcoumaran



Spirodienone



Cinnamyl ends

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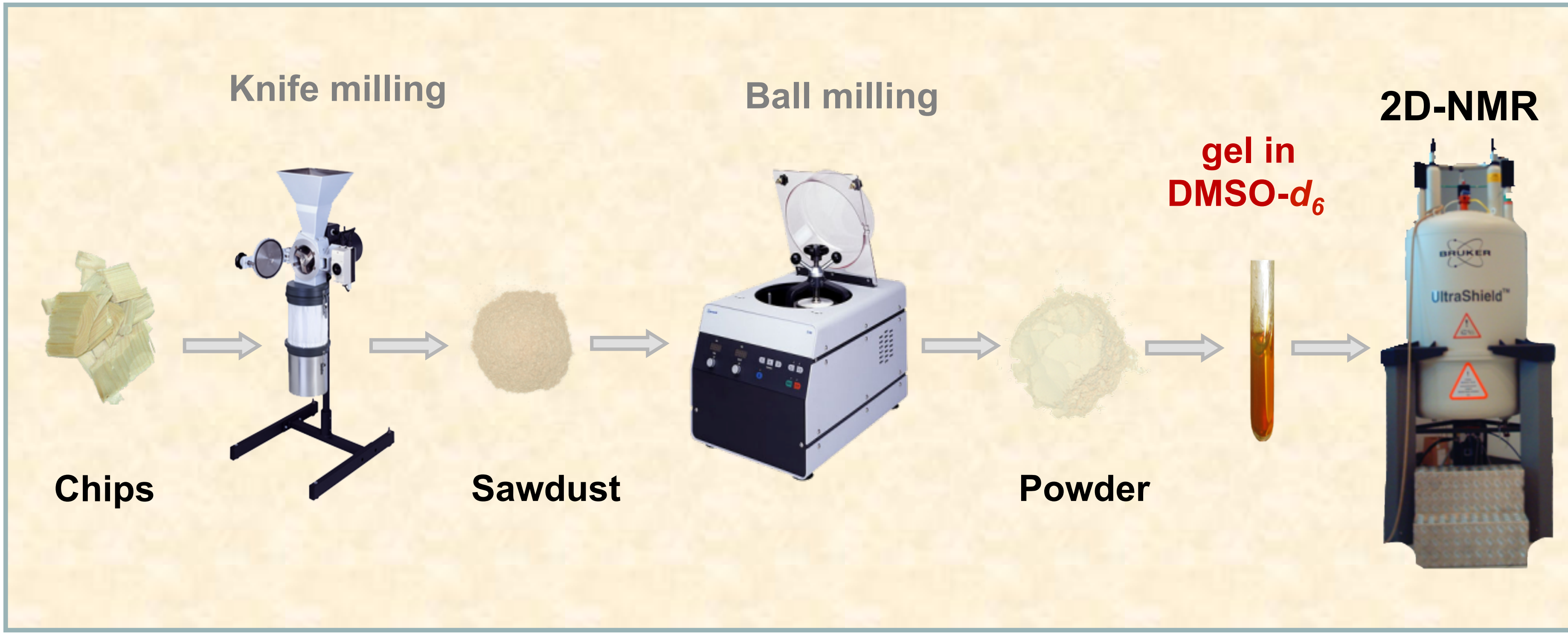
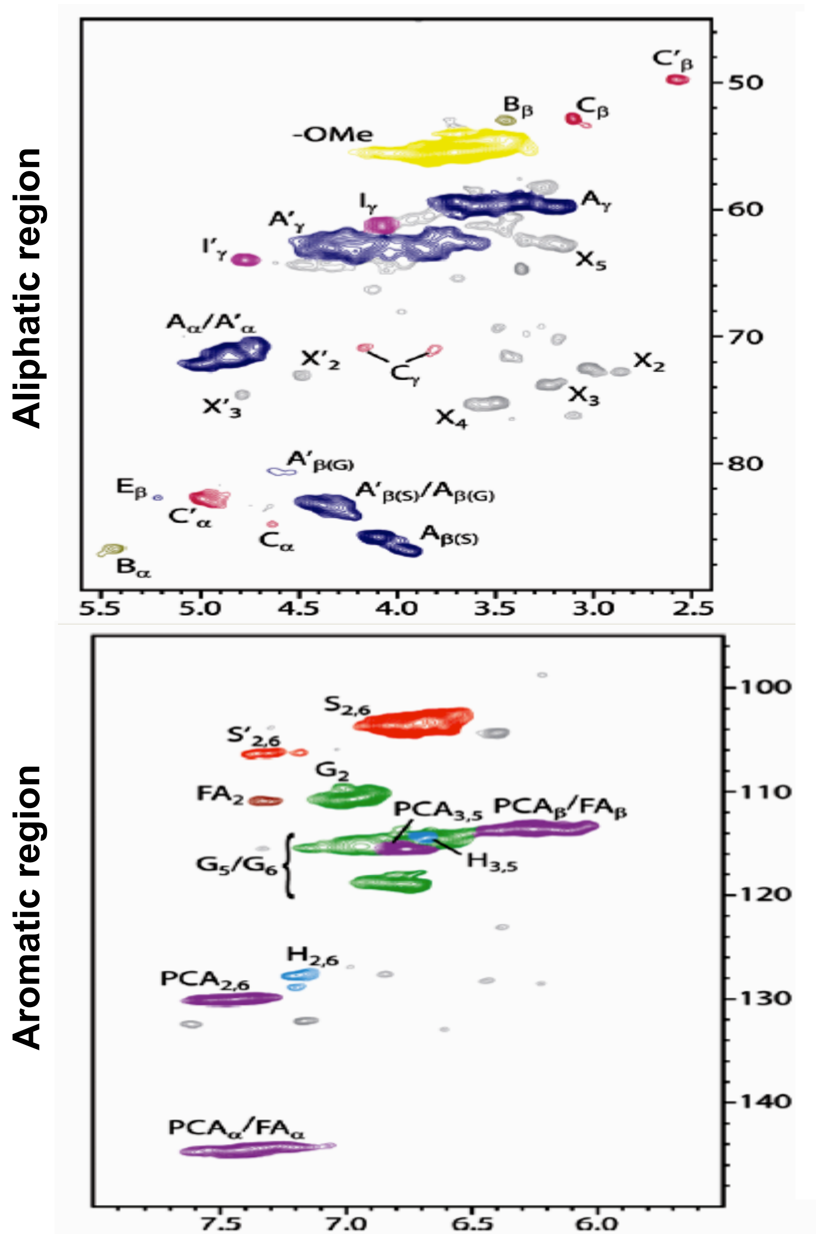
Moreover it has been recently shown that lignocellulose lignin (and polysaccharides) can be "in situ" analyzed by 2D-NMR without their prior isolation (!)

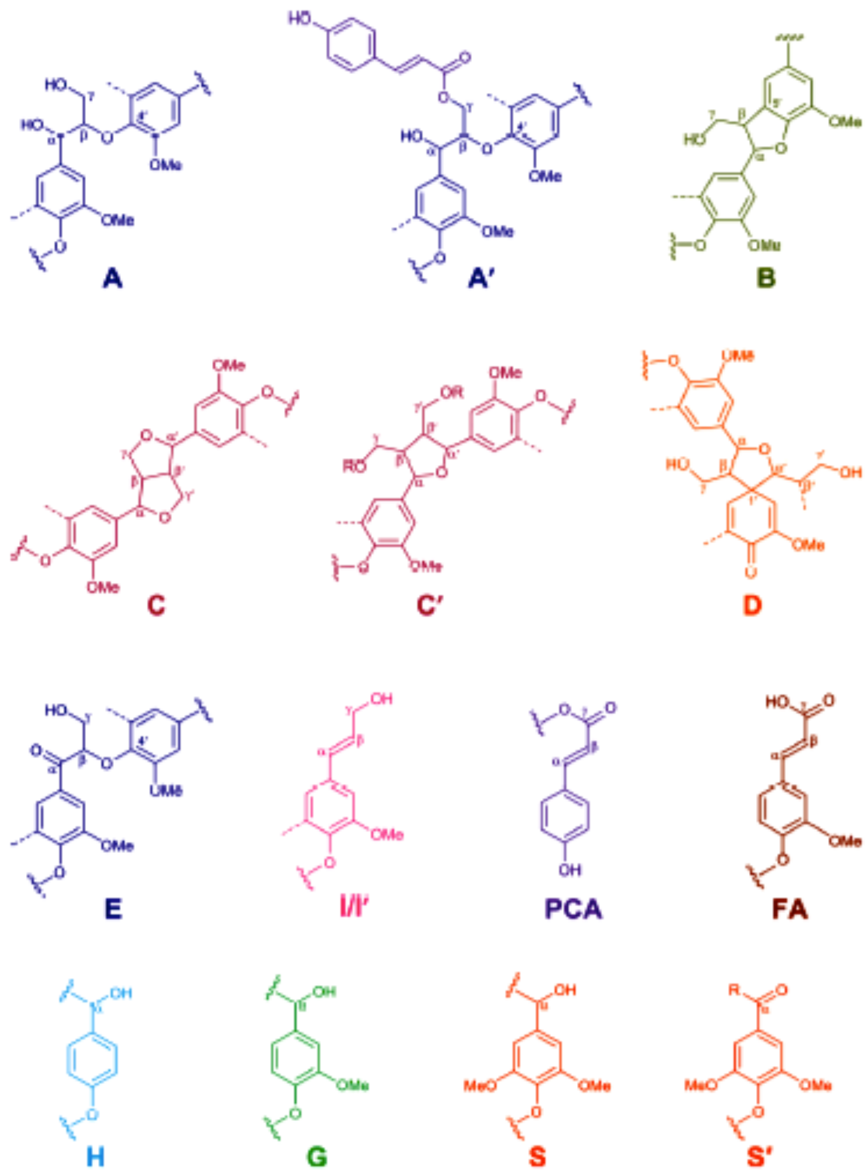


This technique has been used to investigate changes during lignocellulose pretreatment

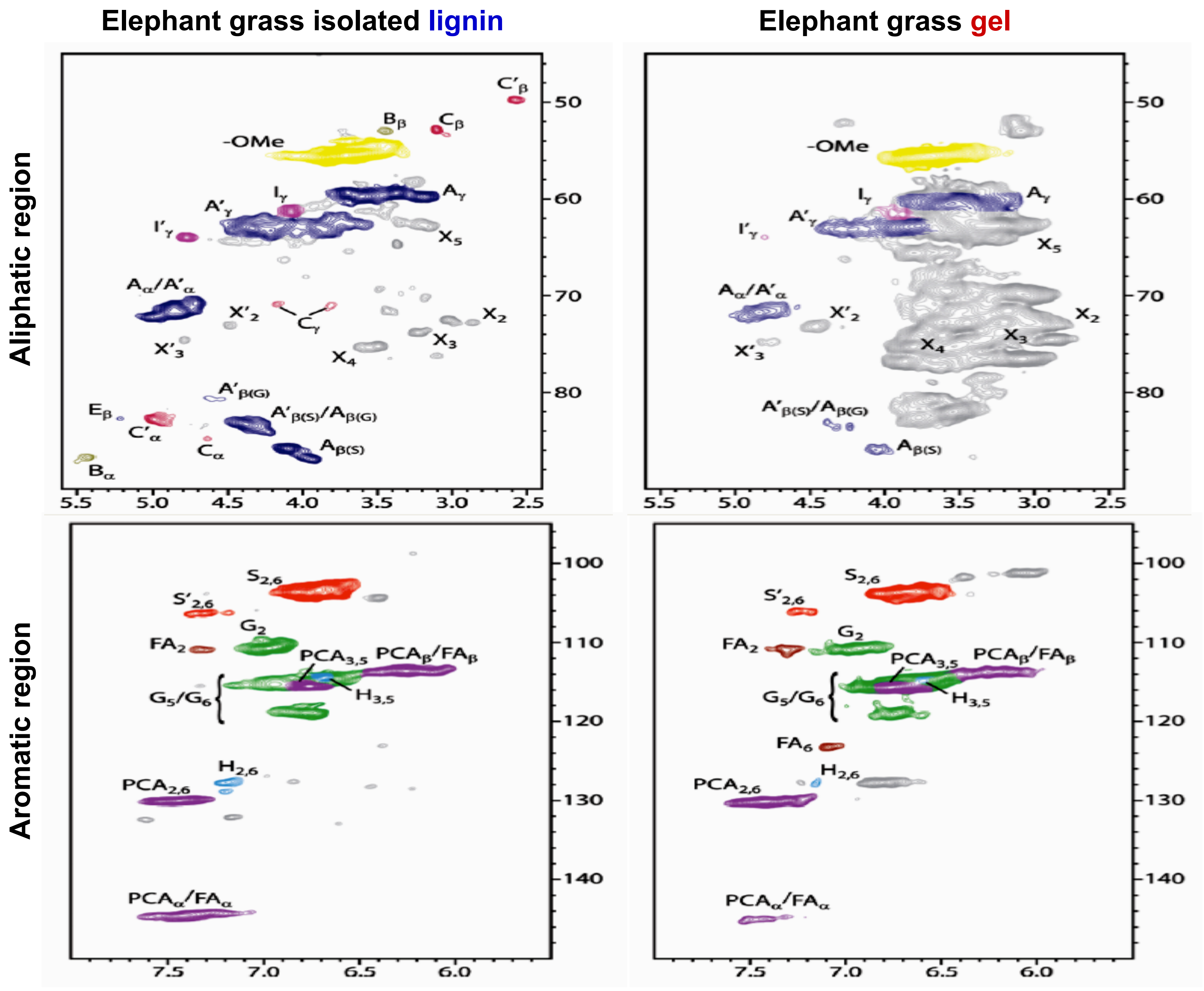
"In situ" analysis (lignocellulose gel) *vs* isolated lignin analysis

Elephant grass isolated lignin

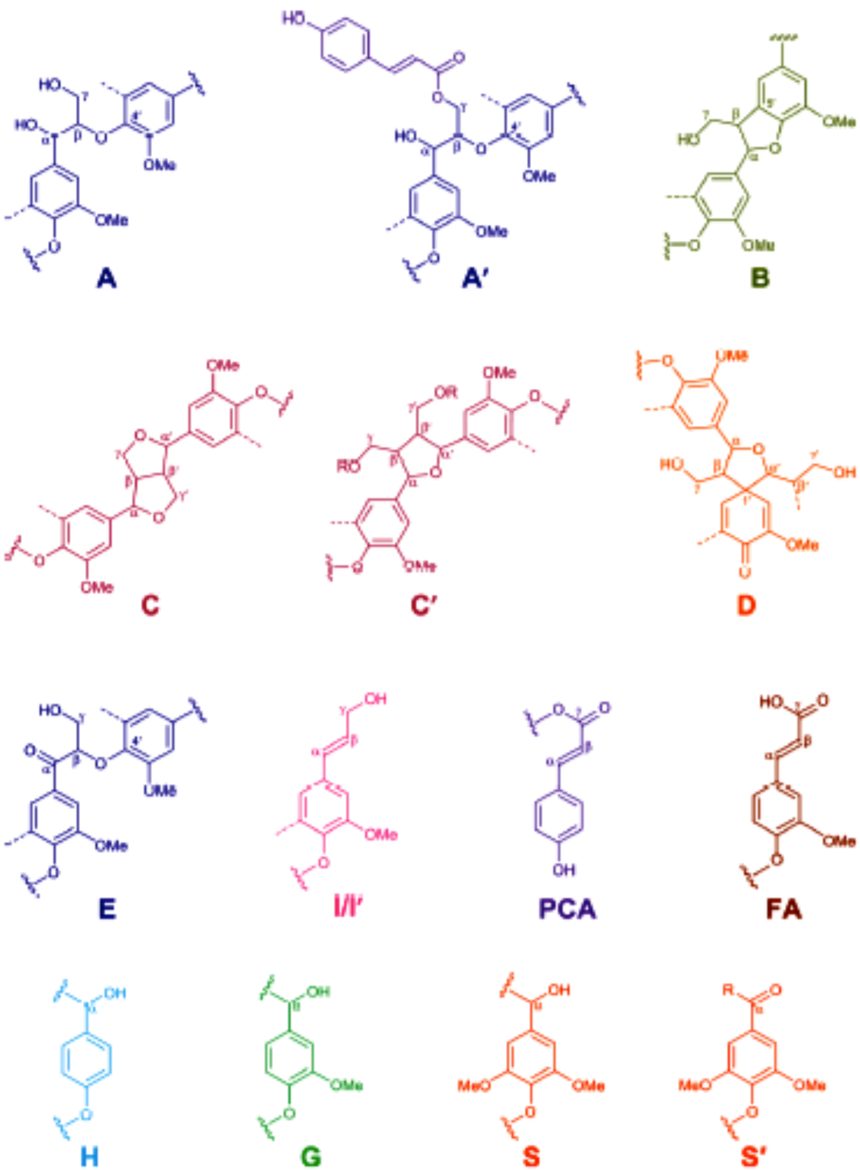




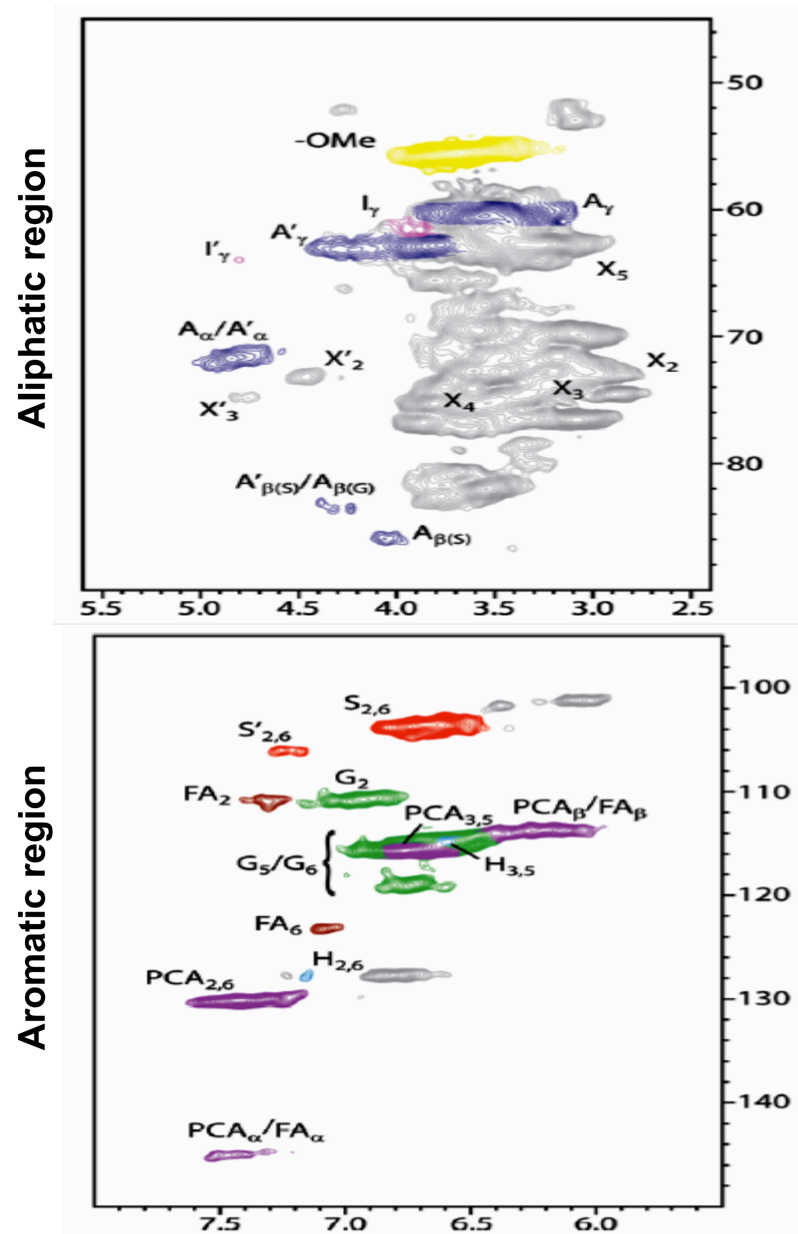
"In situ" analysis (lignocellulose gel) *vs* isolated lignin analysis



"In situ" analysis (lignocellulose gel) *vs* isolated lignin analysis



Elephant grass gel



2D-NMR "in situ" analysis of lignocellulose during biological pretreatment: Enzymatic treatment using a laccase-mediator/alkaline extraction sequence

Plant feedstocks were treated with laccase-mediator without a prior chemical treatment

Conditions for the enzymatic treatments LEp

Doses laccase	10 – 50 U/g
Doses HBT	2.5 %

Elephant grass

Trametes villosa **laccase**
(and HBT as mediator)

4 Cycles LEp	Lignin content (%)
Control	21.1
Laccase (10 U g^{-1})-HBT	18.8
Laccase (25 U g^{-1})-HBT	16.4
Laccase (50 U g^{-1})-HBT	14.3
Laccase (50 U g^{-1})	20.7

ControlEp - LEp treatment: △ **6.8 % KL**

About 32% lignin reduction
(with respect to the laccase-less control)

Plant feedstocks were treated with laccase-mediator without a prior chemical treatment

Conditions for the enzymatic treatments LEp

Doses laccase	10 – 50 U/g
Doses HBT	2.5 %

Eucalypt wood

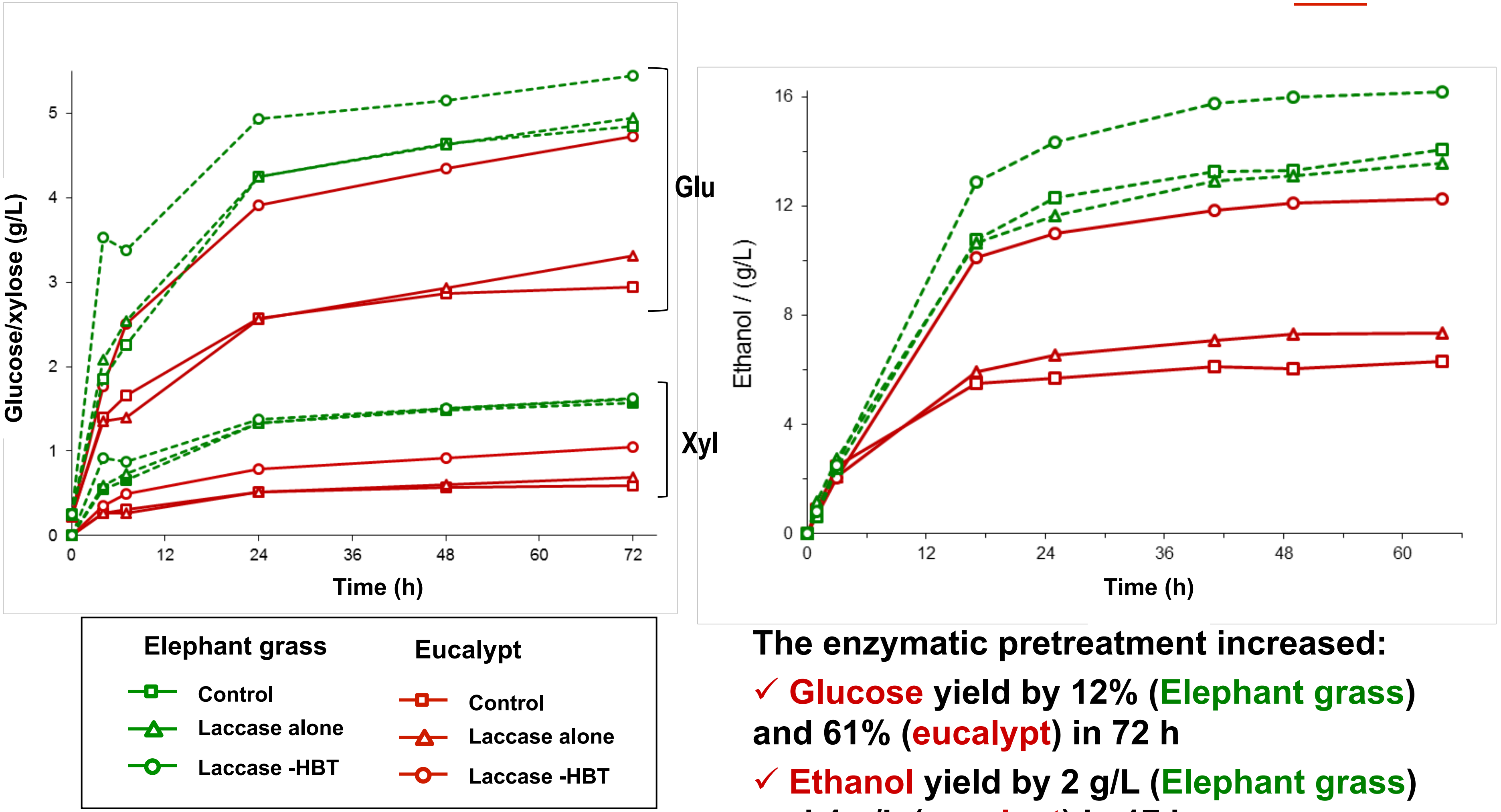
***Trametes villosa* laccase**
(and HBT as mediator)

4 Cycles LEp	Lignin content (%)
Control	18.0
Laccase (10 U g^{-1})-HBT	12.2
Laccase (25 U g^{-1})-HBT	11.9
Laccase (50 U g^{-1})-HBT	9.4
Laccase (50 U g^{-1})	17.5

ControlEp - LEp treatment: △ **8.6 % KL**

Nearly 50% lignin reduction!

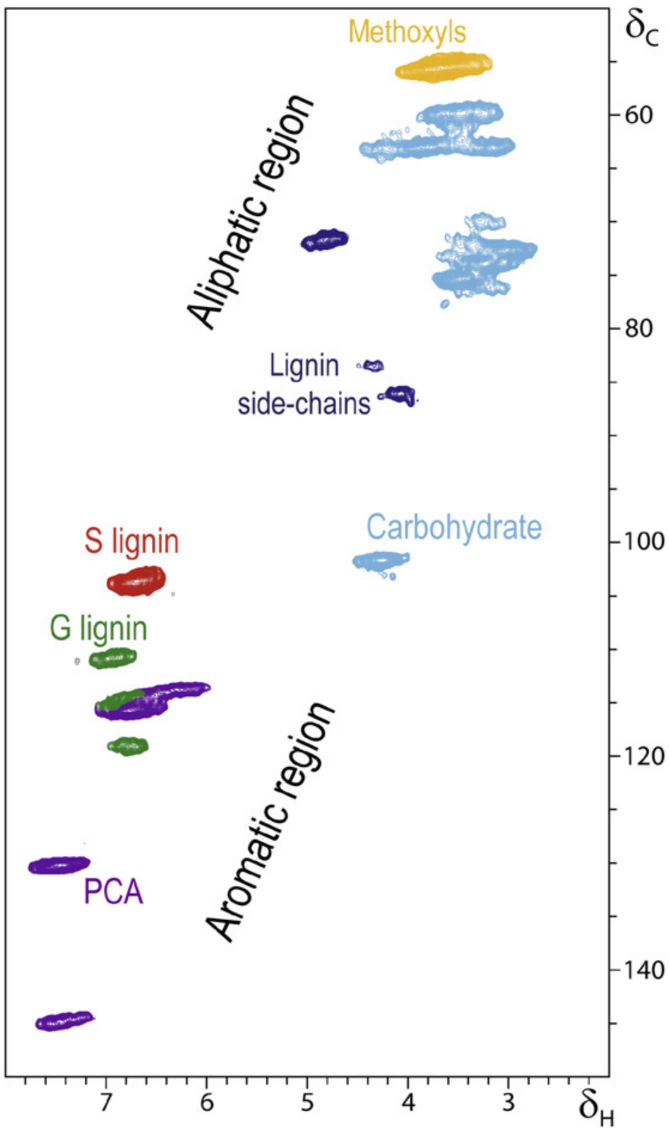
Elephant grass and eucalypt samples pretreated with laccase (25 U/g) and HBT were further evaluated for saccharification and fermentation at VTT



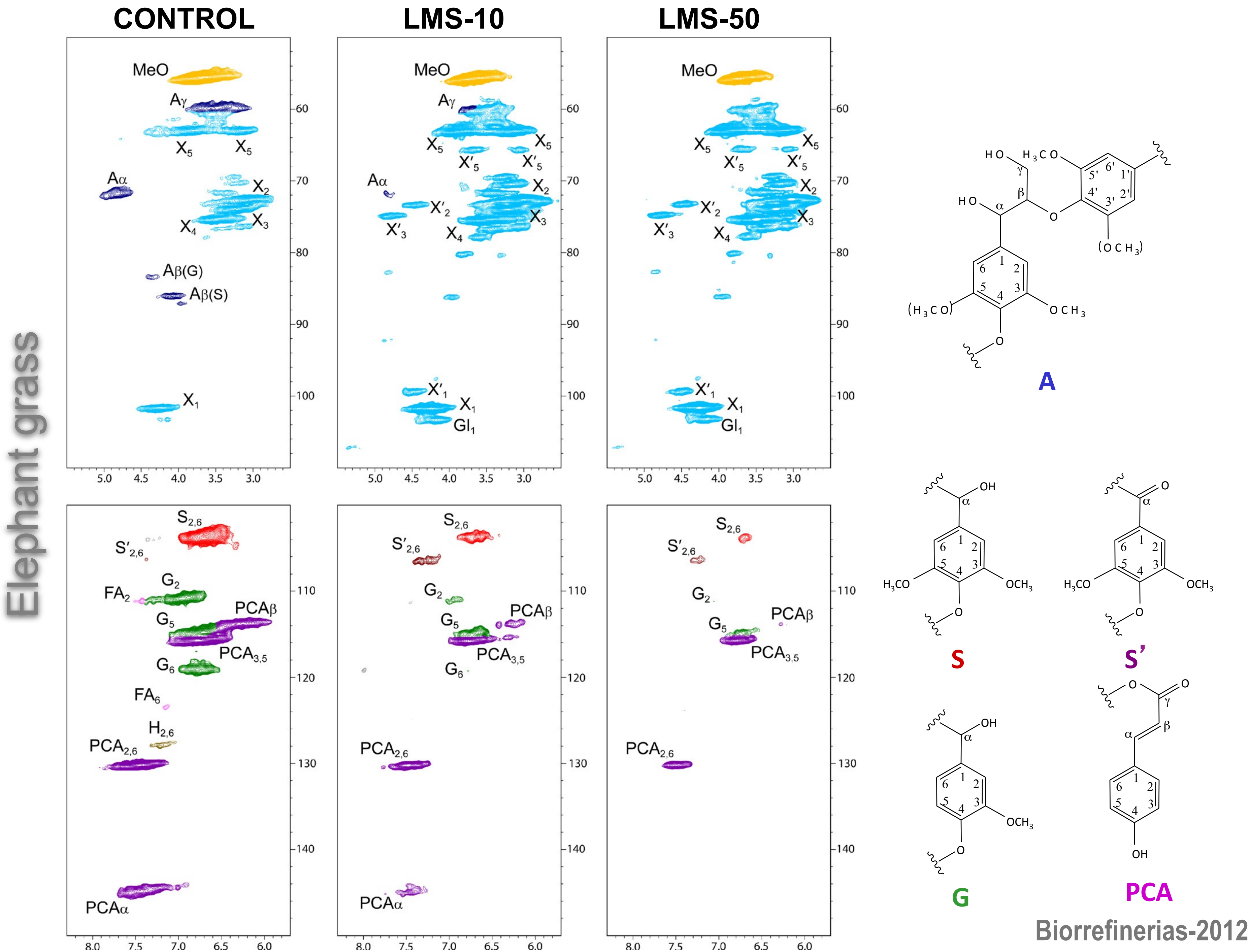
The enzymatic pretreatment increased:

- ✓ Glucose yield by 12% (Elephant grass) and 61% (eucalypt) in 72 h
- ✓ Ethanol yield by 2 g/L (Elephant grass) and 4 g/L (eucalypt) in 17 h

...and lignin modifications were "in situ" analyzed by 2D-NMR →



HSQC NMR spectrum of whole plant biomass (Elephant grass) swollen in dimethylsulfoxide-d_6



CONTROL
LMS-10
LMS-50
Elephant grass
MeO
Aγ
X5
X'5
Aα
X2
X'2
X3
X'3
X4
Aβ(G)
Aβ(S)
X1
X'1
Gl1
S2,6
S'2,6
G2
FA2
PCAβ
G5
PCA3,5
G6
FA6
H2,6
PCA2,6
PCAα
A
S
S'
G
PCA
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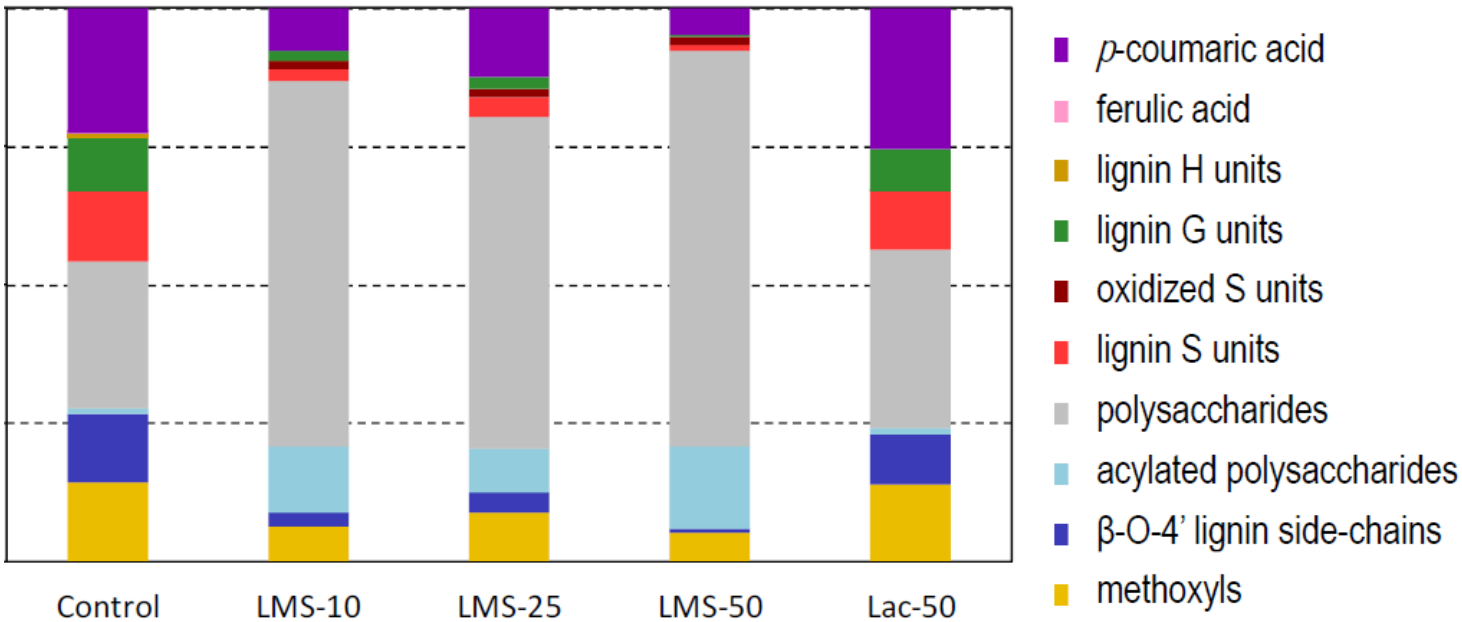
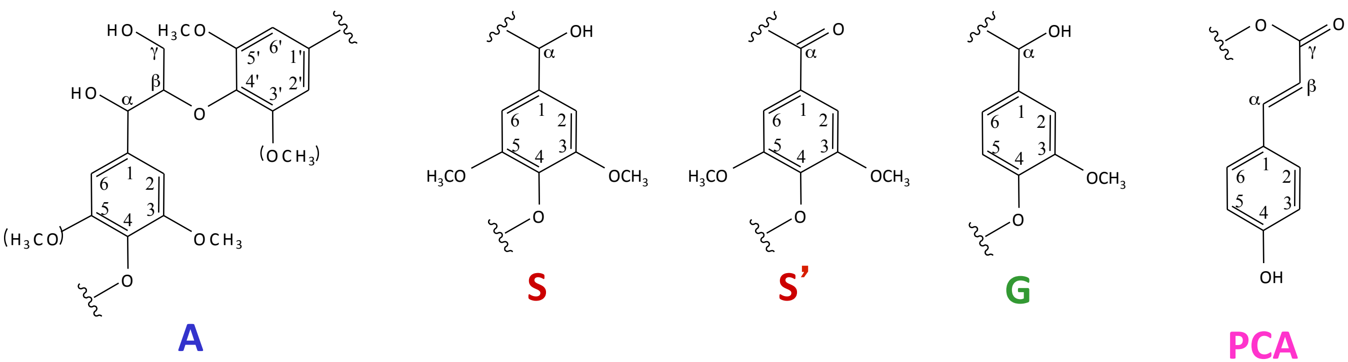
A relative decrease in lignin carbon and an increase in polysaccharide carbon is observed at increasing enzyme doses

Elephant grass





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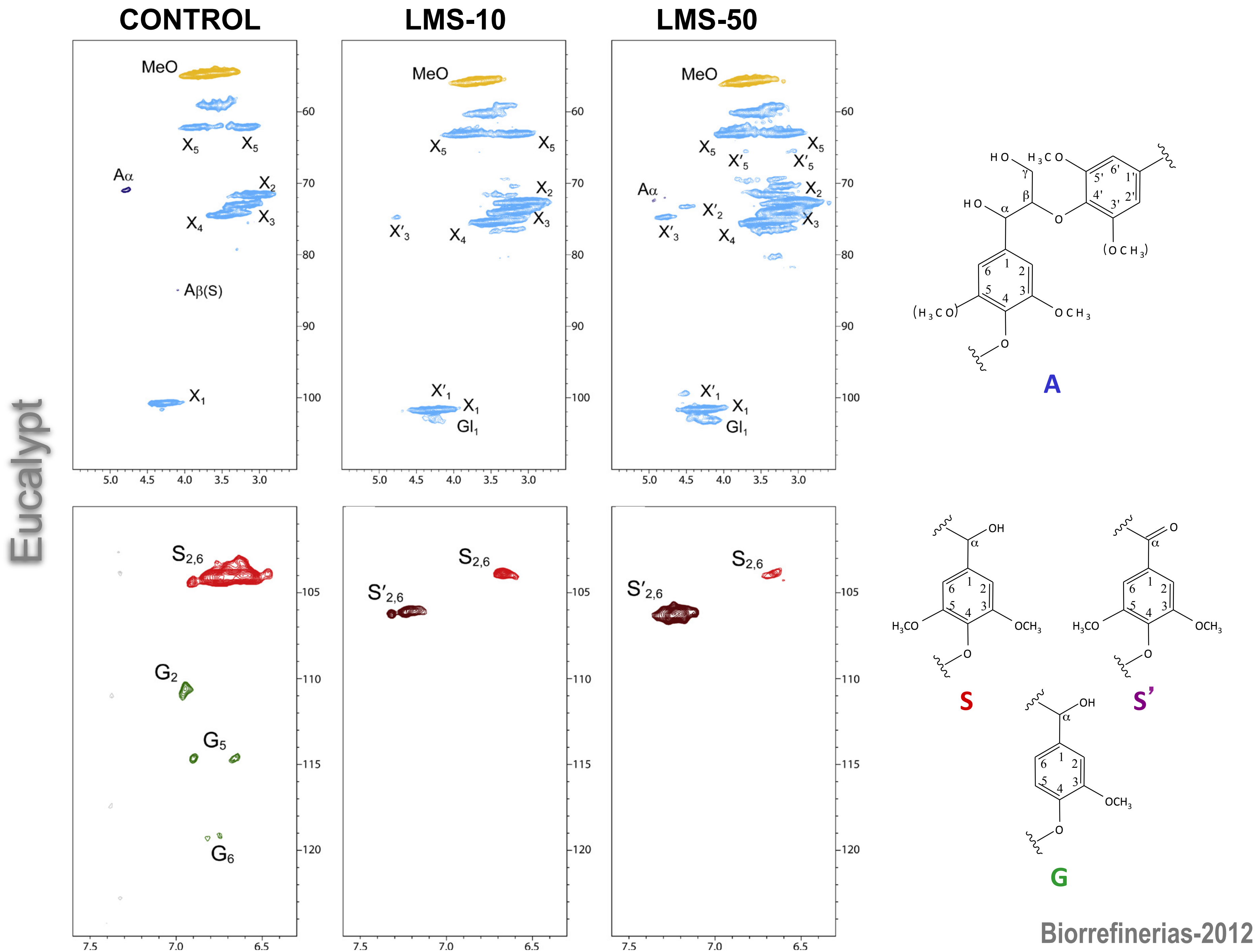

CONTROL
LMS-10
LMS-50
Eucalypt
MeO
X5
X'5
Aα
X2
X3
X4
X'2
X'3
Aβ(S)
X1
X'1
Gl1
S2,6
S'2,6
G2
G5
G6
A
S
S'
G
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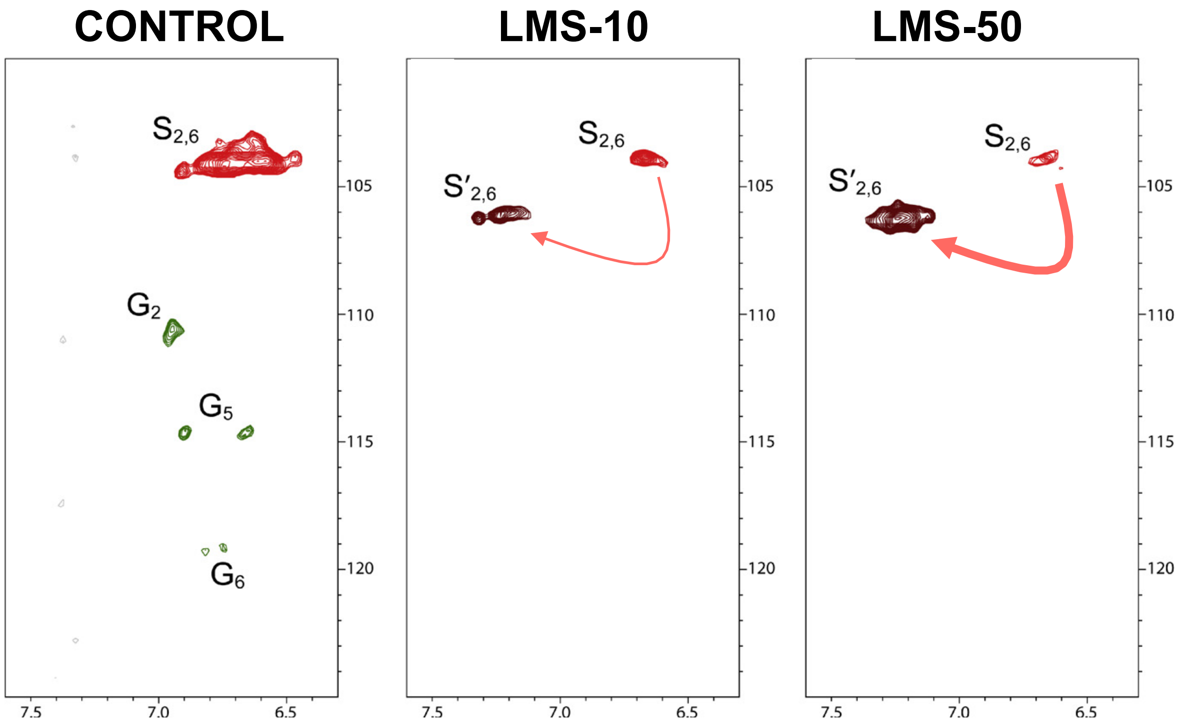
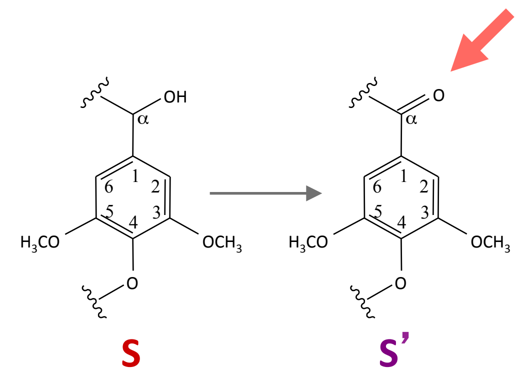
Eucalypt



Side-chain oxidation



(S' >60% lignin units!)

- ✓ S' structures after pulp treatment with laccase-HBT have been identified by HMBC as aromatic acids and ketones (Ibarra et al *Holzforschung* 61:634, 2007)
- ✓This agrees with results from dimer degradation by laccase-HBT (Kawai et al *EMT* 30:482, 2002)
- ✓We had reported oxidative degradation of lignin side-chains (resulting in lignin-linked aromatic acids and aldehydes) during fungal decay of wheat straw (Camarero et al *RCMS* 11:331, 1997)

✓ We show that plant biomass can be delignified by enzymes (30-50% removal) by applying a sequence including laccase-mediator and alkaline extraction stages (such treatment results in improved cellulose hydrolysis and higher ethanol production)

✓ The HSQC NMR spectra of the whole samples show a decrease of both aromatic and aliphatic lignin signals and provide evidence for a Cα-oxidation degradation mechanism with oxidized S units representing over 60% of all lignin units in the treated eucalypt wood

(subsequent assays with low-cost commercial laccase and a phenolic mediator are yielding promising results)

Other examples of 2D-NMR analysis during lignocellulose deconstruction are available from the LIGNODECO project:

2D-NMR "in situ" analysis of lignocellulose during physical pretreatment: Solvent fractionation

2D-NMR "in situ" analysis of lignocellulose during chemical pretreatment: Alkaline cooking under different optimized conditions

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- Funding Projects

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Development of optimized enzymatic pretreatments for the deconstruction of lignocellulosic materials (LINOCELL, AGL2011-25379)

Optimized pre-treatment of fast growing woody and nonwoody Bralizian crops by detailed characterization of chemical changes produced in the lignin-carbohydrate matrix (LIGNODECO, www.lignodeco.com.br)

- All the Collaborators in these Projects

Lignin removal is an central issue for lignocellulose deconstruction in cellulose pulp manufacturing (pulping and bleaching)...

Lignin removal is an central issue for lignocellulose deconstruction in cellulose pulp manufacturing (pulping and bleaching)... and a key challenge for its conversion into liquid fuel and other chemicals