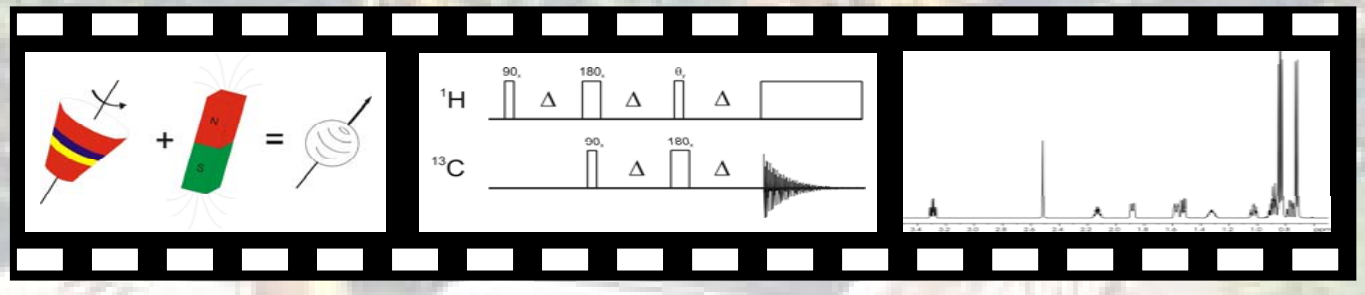
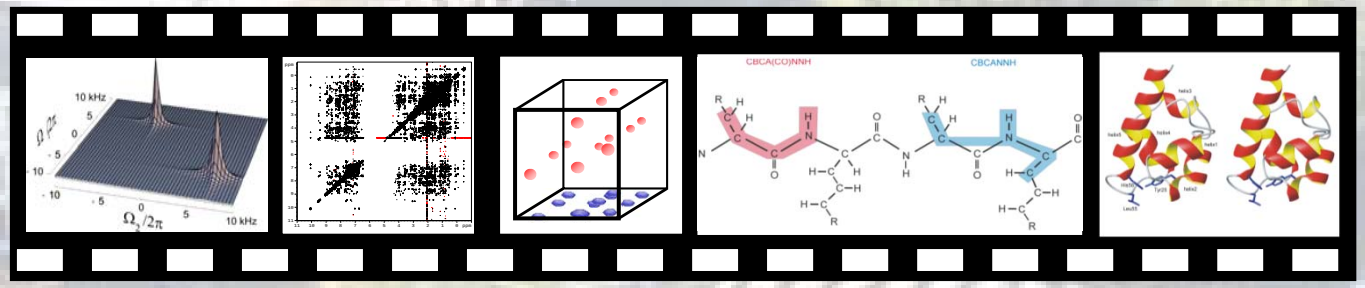


Glycogen phosphorylation

and

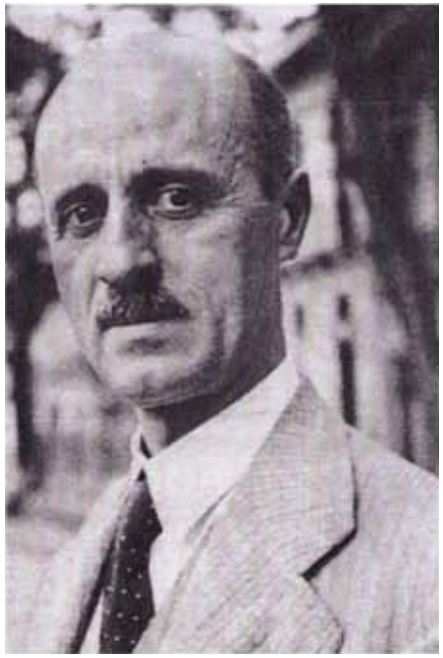
Lafora disease

Gruppenseminar 19.04.2012



In 1911, Dr. Gonzalo Lafora described a myoclonus epilepsy connected with the formation of „amyloid bodies“, it was subsequently called "*Lafora disease*"

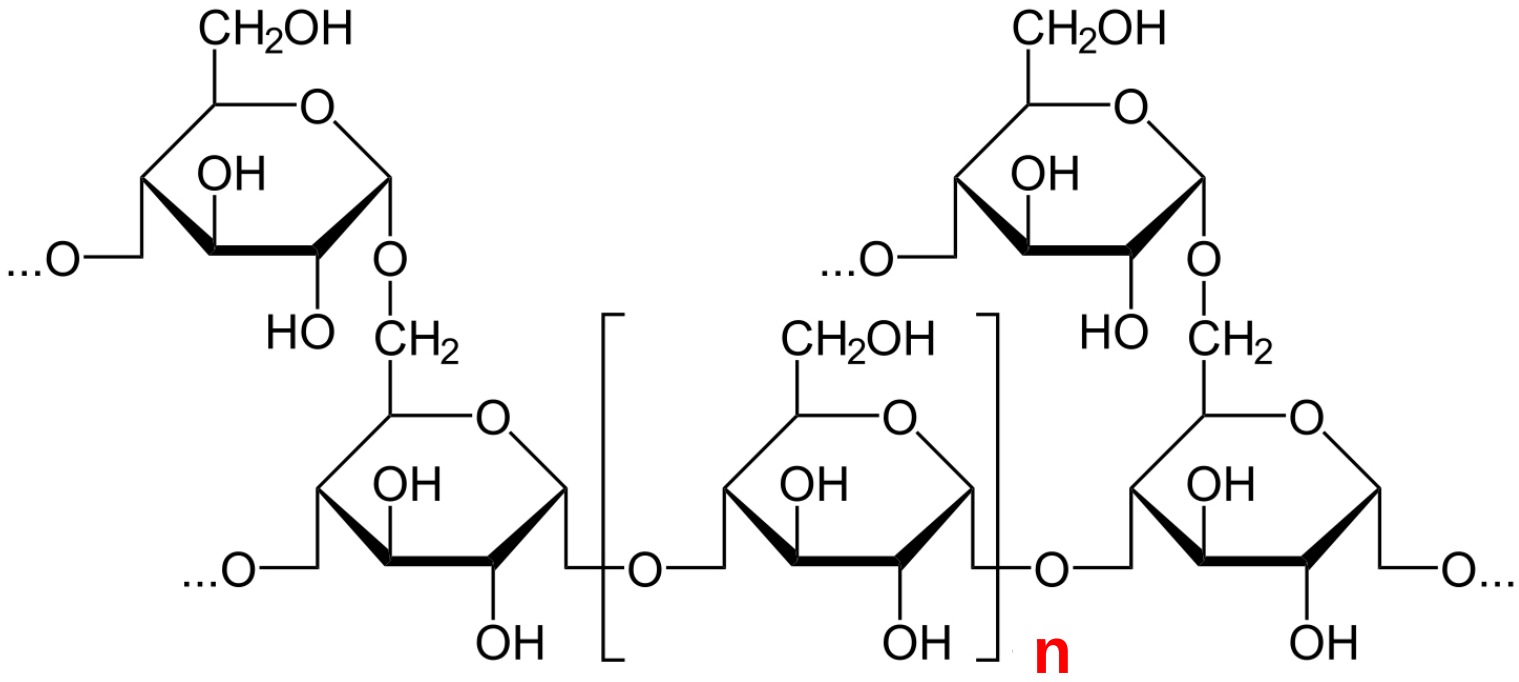
These „Lafora bodies“ turned out to a water-insoluble glycan, a polymer of glucose



The illness usually begins in the second decade of life and leads to death within 10-15 years

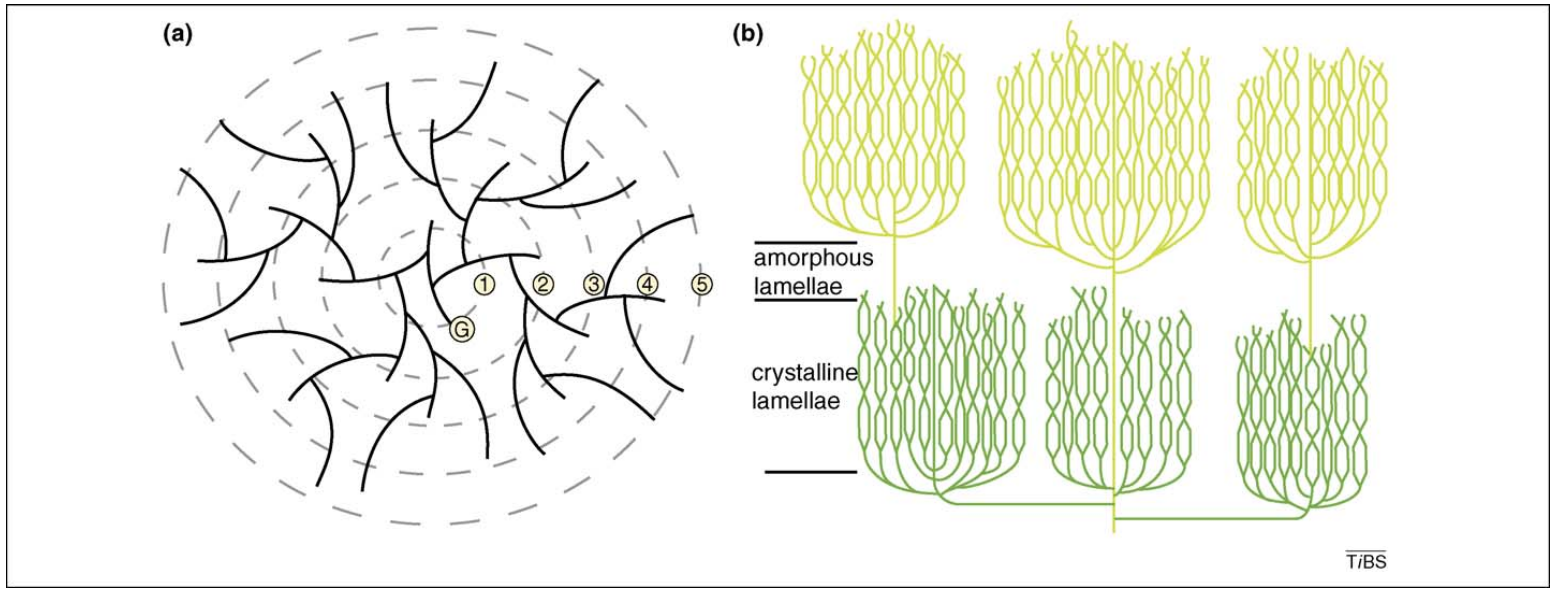
There is *no* cure or medication

Starch and glycogen

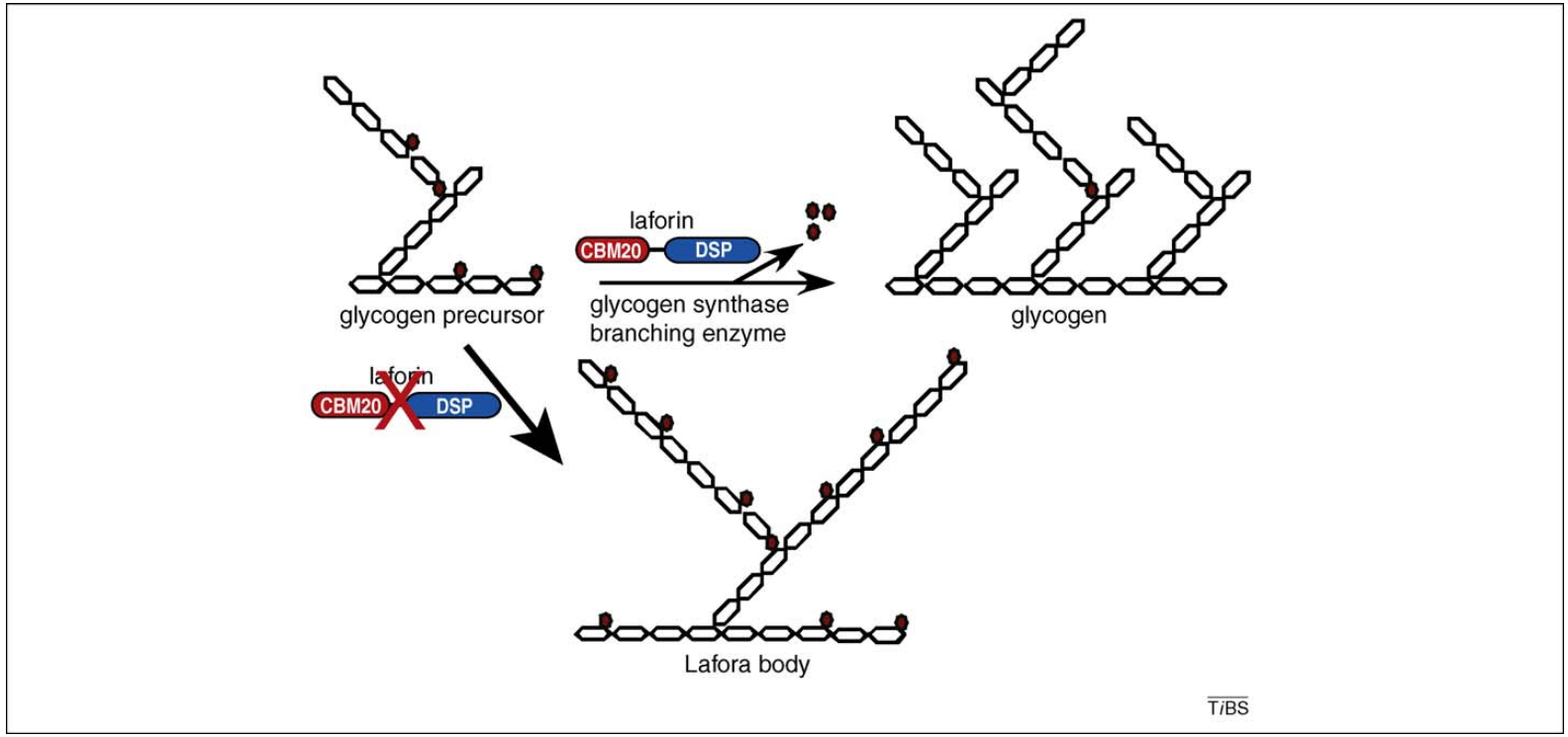


Poly-glucose, chains with 1,4 linkages and branching via 1,6 linkages, n=10 in glycogen (continous), n=20 in starch (discontinous)

Starch and glycogen

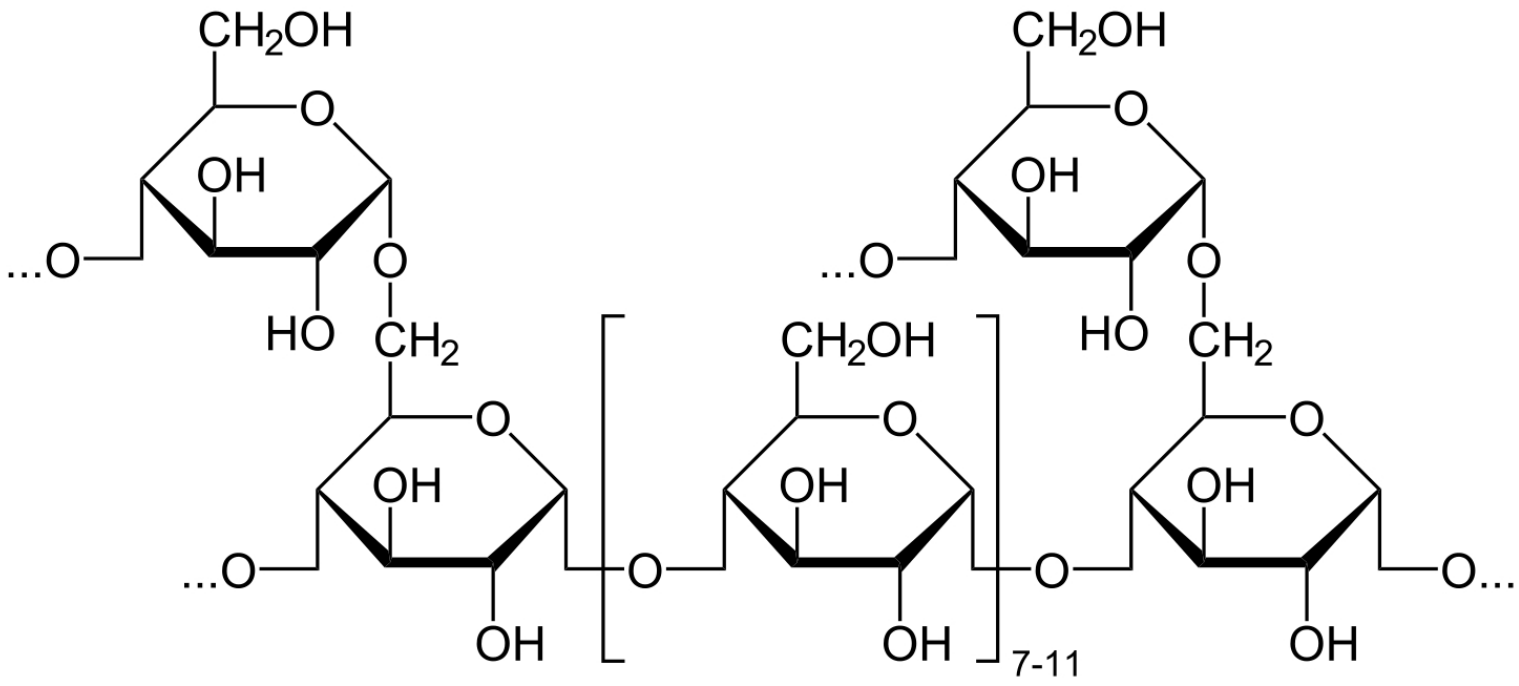


Starch and glycogen are also phosphorylated and the Lafora bodies contain hypophosphorylated material



Laforin is a phosphatase genetically connected with Lafora disease

The question: Where are the phosphates ?



To understand the metabolism one has to know where the phosphates usually are

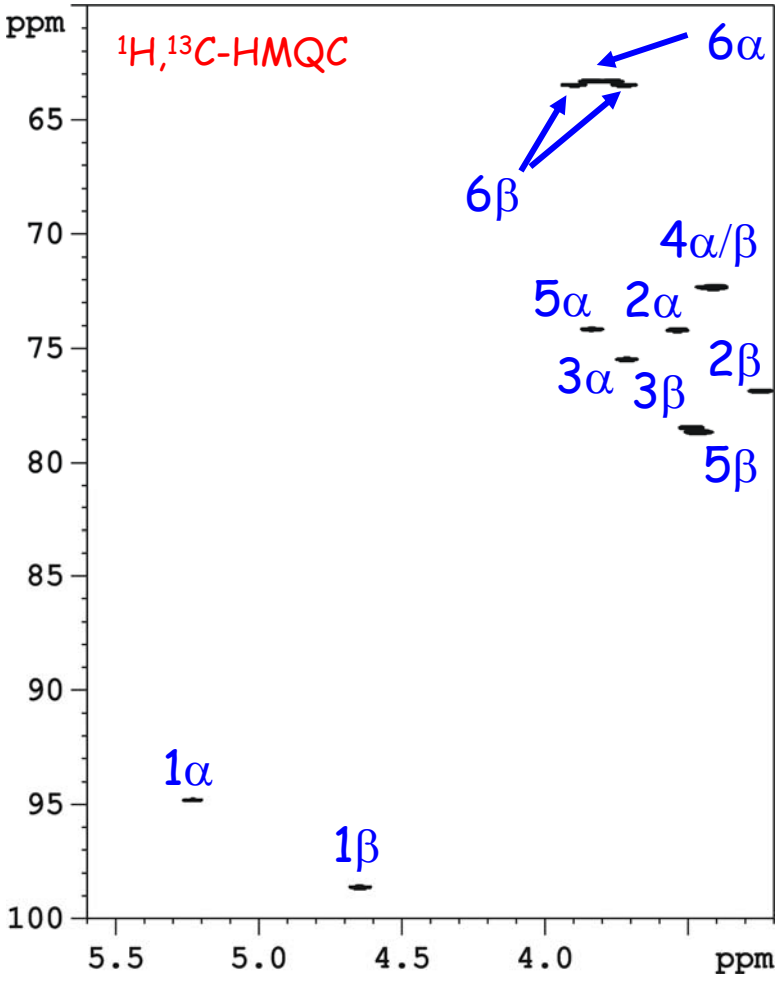
Can we do NMR ?

The material is treated with a-amylase and amyloglucosidase to yield short fragments

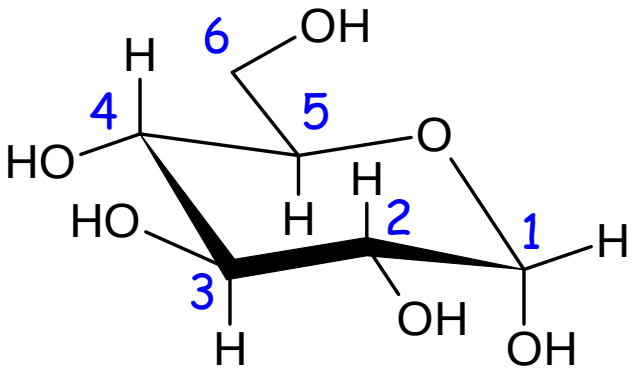
Ultracentrifugation and anion exchange chromatography yield samples enriched with phosphoglucans

The material is heterogenous with respect to chain length but homogenous with respect to chemical composition

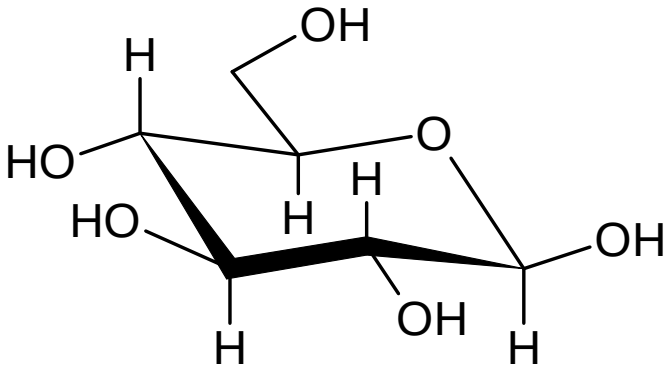
Glucose

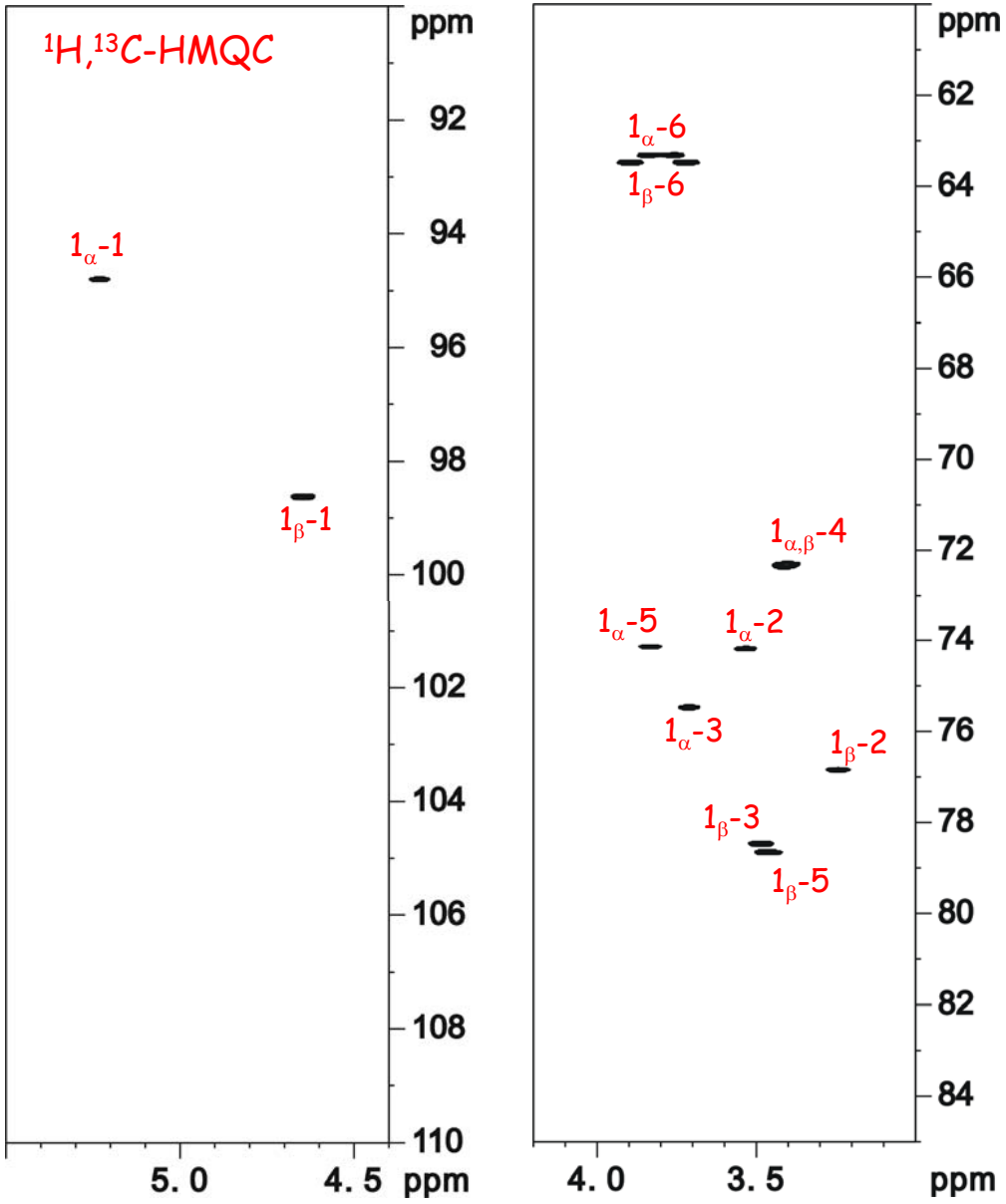

ppm
$^{1}H,^{13}C$-HMQC
6α
6β
4α/β
5α
2α
3α
3β
2β
5β
1α
1β
65
70
75
80
85
90
95
100
5.5
5.0
4.5
4.0
ppm

α


H
6
OH
4
5
HO
O
H
HO
H
2
1
H
3
OH
H
OH

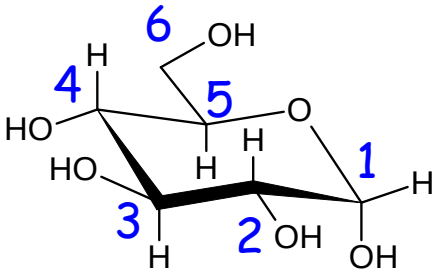
β


OH
H
HO
O
H
HO
H
OH
OH
H
H

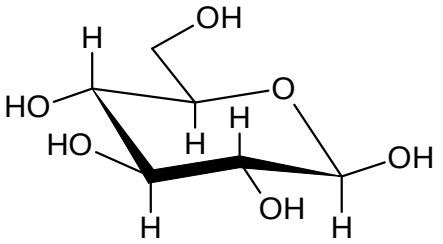

$^1H,^{13}C$-HMQC
ppm
92
94
96
98
100
102
104
106
108
110
5.0
4.5
ppm
1_α-1
1_β-1
ppm
62
64
66
68
70
72
74
76
78
80
82
84
4.0
3.5
ppm
1_α-6
1_β-6
$1_{\alpha,\beta}$-4
1_α-5
1_α-2
1_α-3
1_β-2
1_β-3
1_β-5

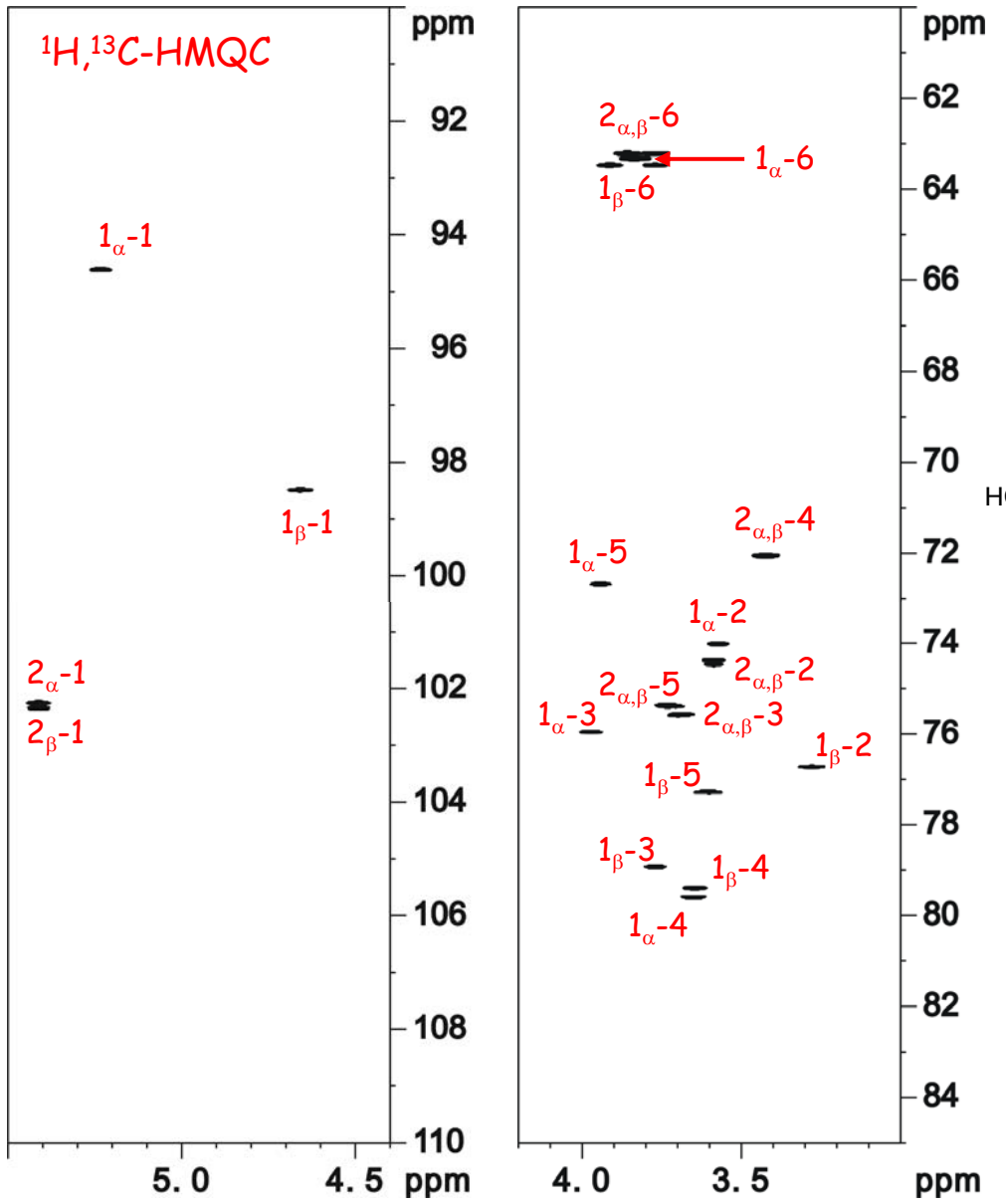
Glucose

α


6
OH
H
4
5
HO
O
H
HO
H
1
H
3
2
OH
H
OH

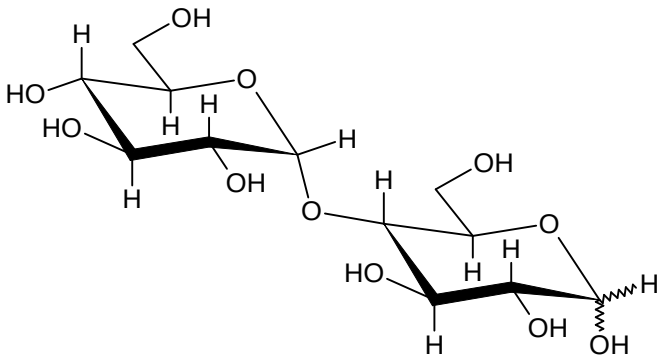
β


OH
H
HO
O
H
HO
H
OH
H
OH
H

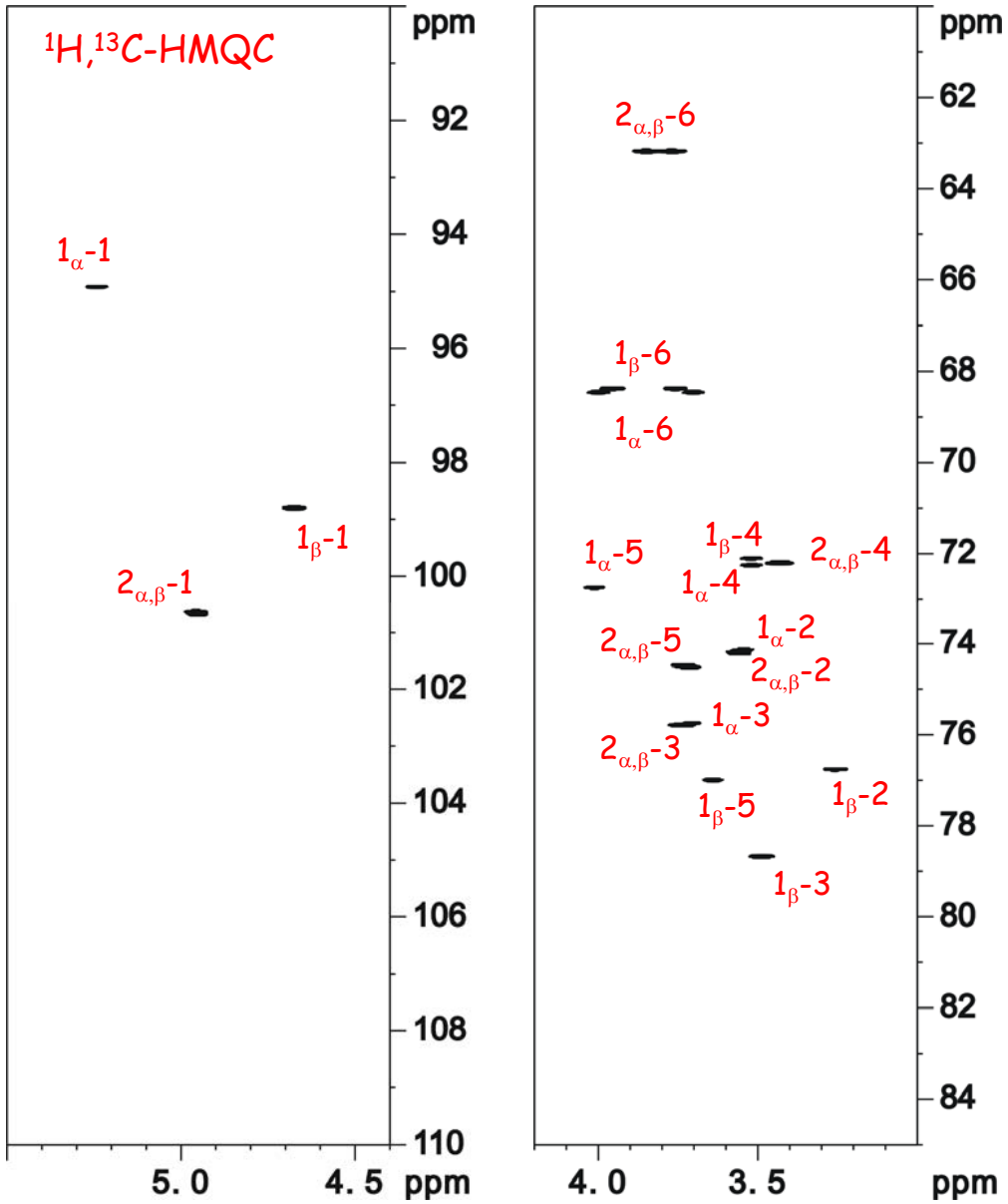


^{1}H,^{13}C-HMQC
ppm
92
94
96
98
100
102
104
106
108
110
5.0
4.5
ppm
1_α-1
1_β-1
2_α-1
2_β-1
ppm
62
64
66
68
70
72
74
76
78
80
82
84
4.0
3.5
ppm
$2_{\alpha,\beta}$-6
1_α-6
1_β-6
$2_{\alpha,\beta}$-4
1_α-5
1_α-2
$2_{\alpha,\beta}$-2
$2_{\alpha,\beta}$-5
1_α-3
$2_{\alpha,\beta}$-3
1_β-2
1_β-5
1_β-3
1_β-4
1_α-4

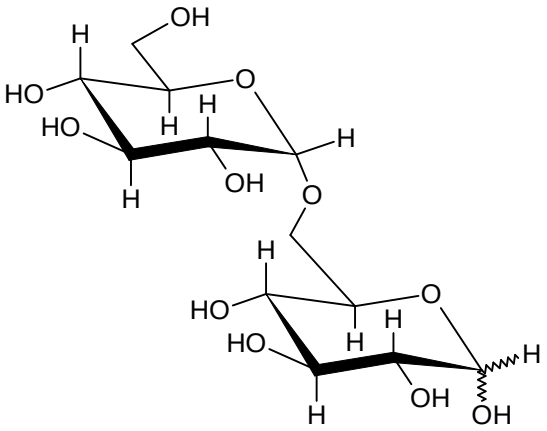
Maltose

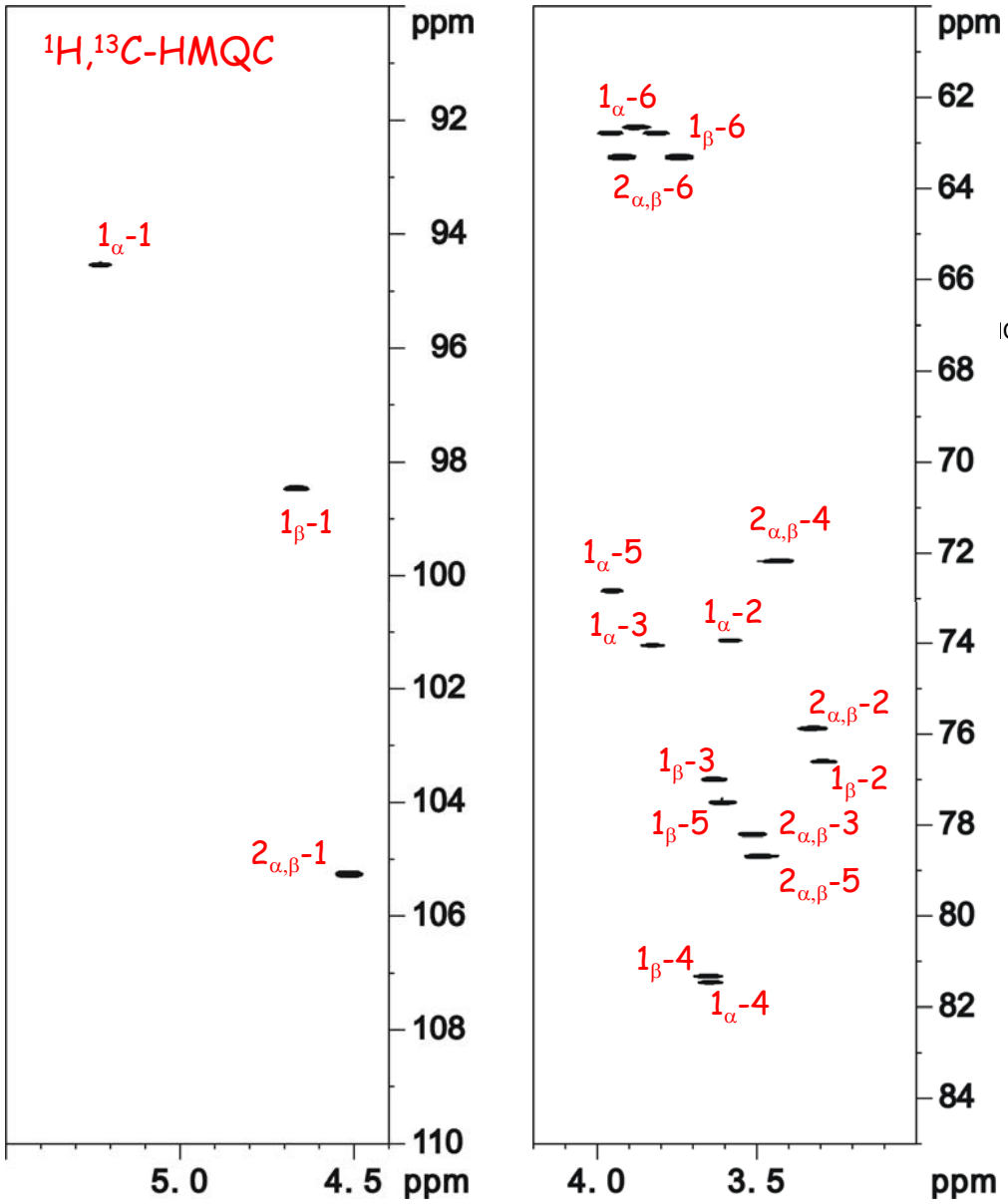


OH
H
HO
O
H
H
HO
H
H
OH
H
OH
O
O
H
HO
H
H
H
OH
OH

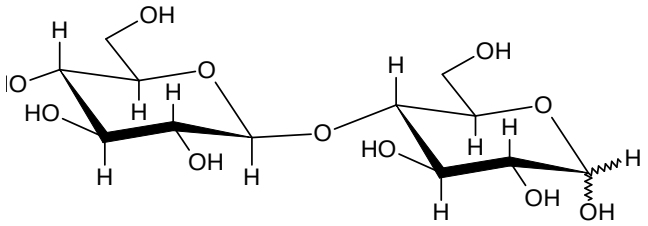

$^{1}H,^{13}C$-HMQC
ppm
92
94
96
98
100
102
104
106
108
110
5.0
4.5
ppm
1_{α}-1
1_{β}-1
$2_{\alpha,\beta}$-1
ppm
62
64
66
68
70
72
74
76
78
80
82
84
4.0
3.5
ppm
$2_{\alpha,\beta}$-6
1_{β}-6
1_{α}-6
1_{α}-5
1_{β}-4
$2_{\alpha,\beta}$-4
1_{α}-4
$2_{\alpha,\beta}$-5
1_{α}-2
$2_{\alpha,\beta}$-2
1_{α}-3
$2_{\alpha,\beta}$-3
1_{β}-5
1_{β}-2
1_{β}-3

Isomaltose

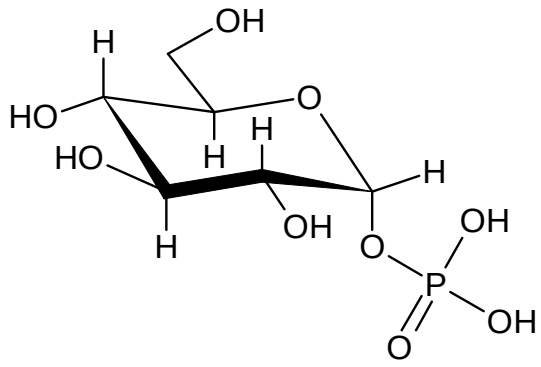

H
OH
HO
O
HO
H
H
H
H
OH
O
H
HO
O
HO
H
H
OH
H
OH


$^{1}H,^{13}C$-HMQC
ppm
92
94
96
98
100
102
104
106
108
110
5.0
4.5
ppm
1_{α}-1
1_{β}-1
$2_{\alpha,\beta}$-1
ppm
62
64
66
68
70
72
74
76
78
80
82
84
4.0
3.5
ppm
1_{α}-6
1_{β}-6
$2_{\alpha,\beta}$-6
$2_{\alpha,\beta}$-4
1_{α}-5
1_{α}-3
1_{α}-2
$2_{\alpha,\beta}$-2
1_{β}-3
1_{β}-2
1_{β}-5
$2_{\alpha,\beta}$-3
$2_{\alpha,\beta}$-5
1_{β}-4
1_{α}-4

Cellobiose

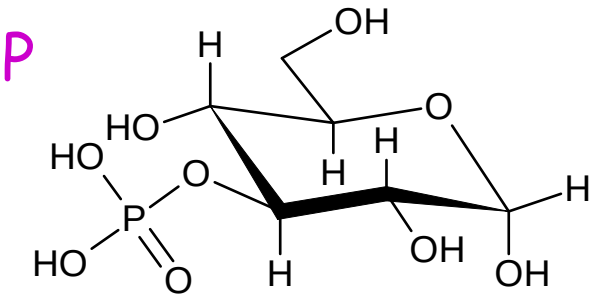

H
OH
O
HO
H
H
OH
H
O
H
OH
O
HO
H
H
H
OH
OH
H

G1P

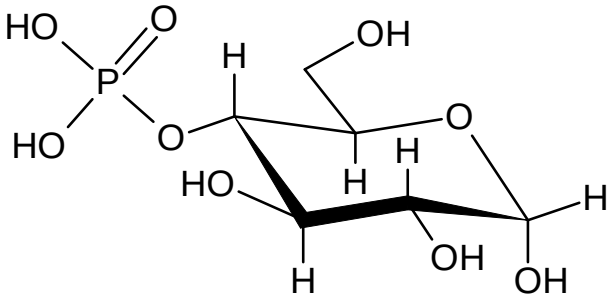


G2P is not easy to come by

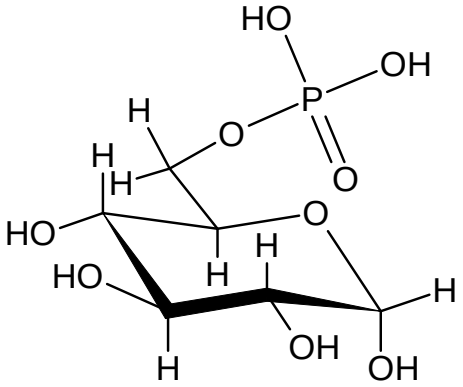
G3P



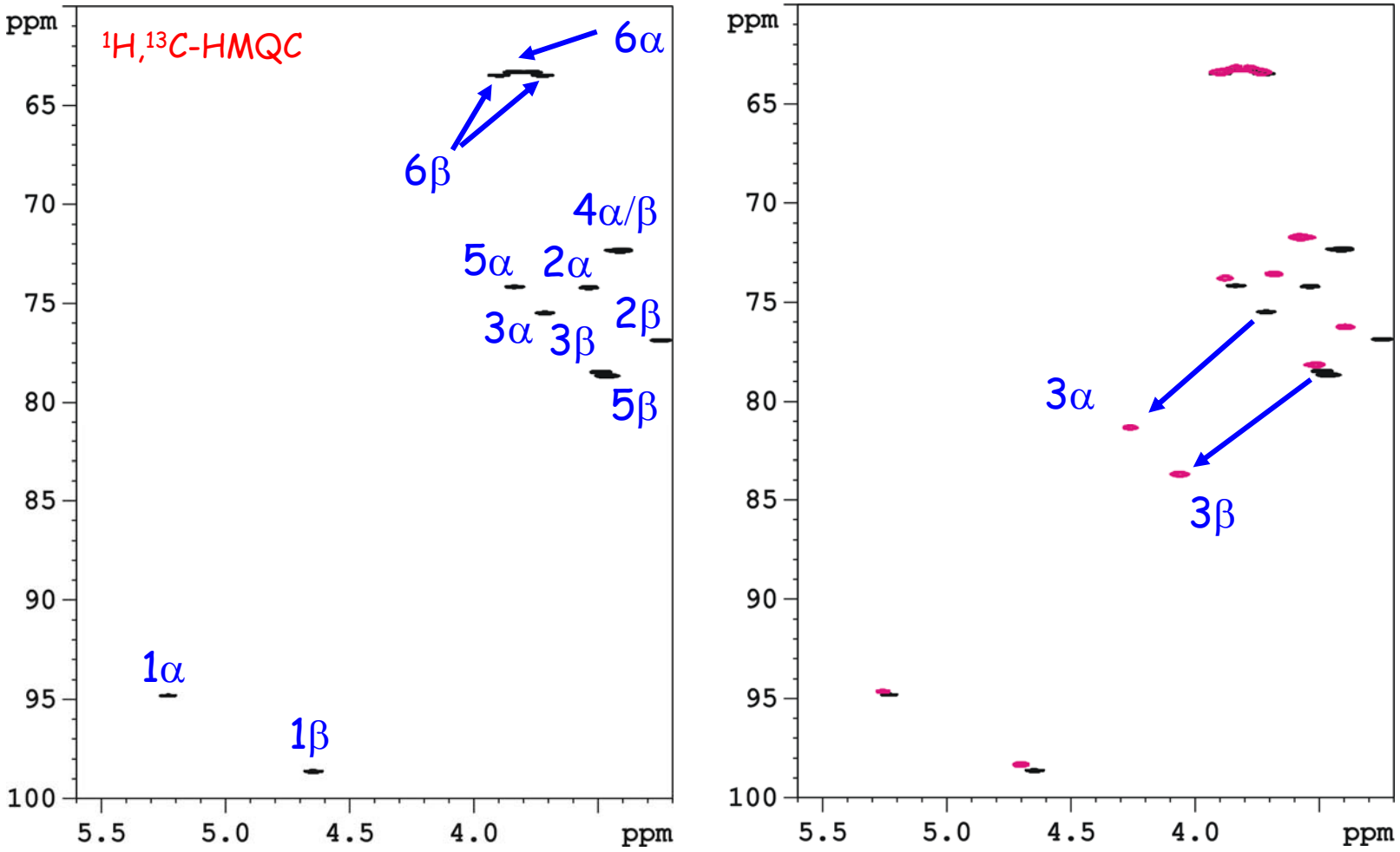
G4P

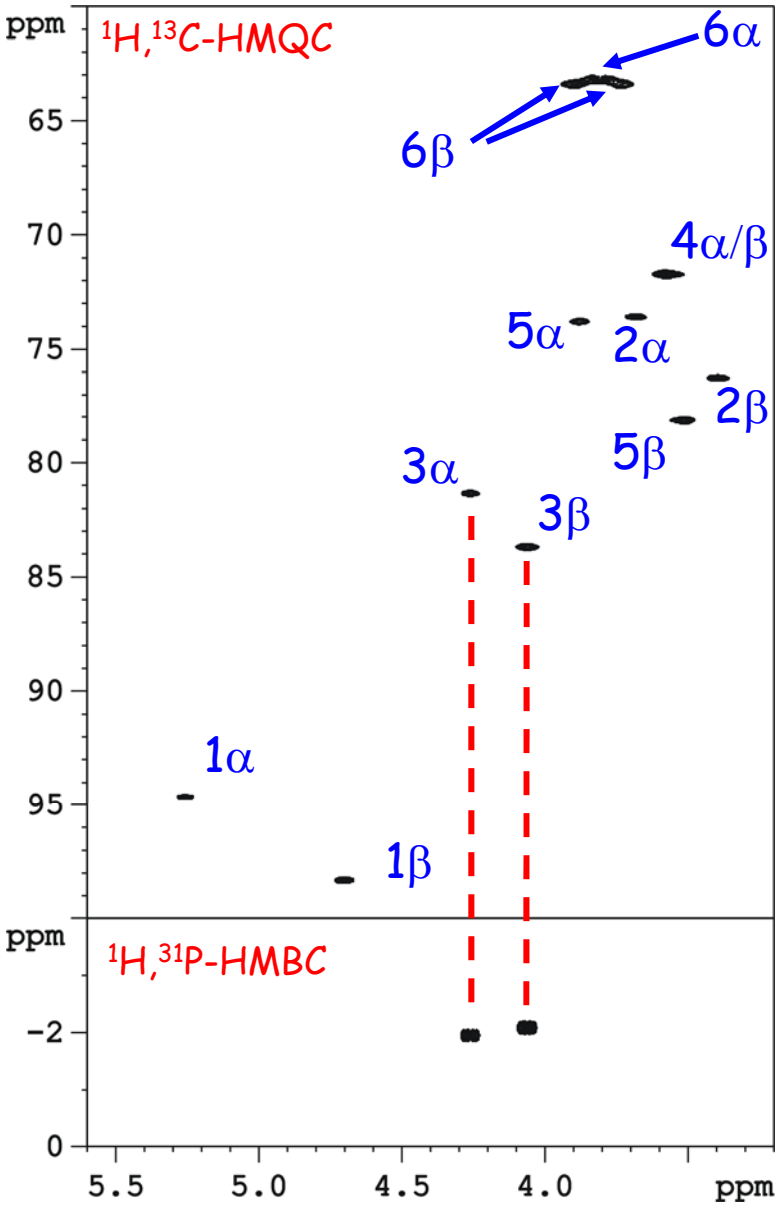


G6P

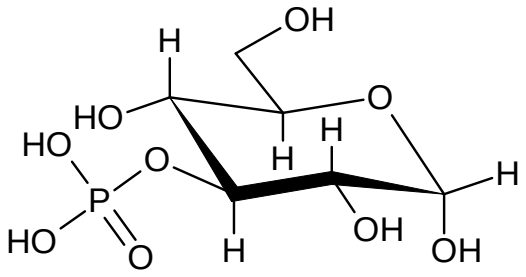


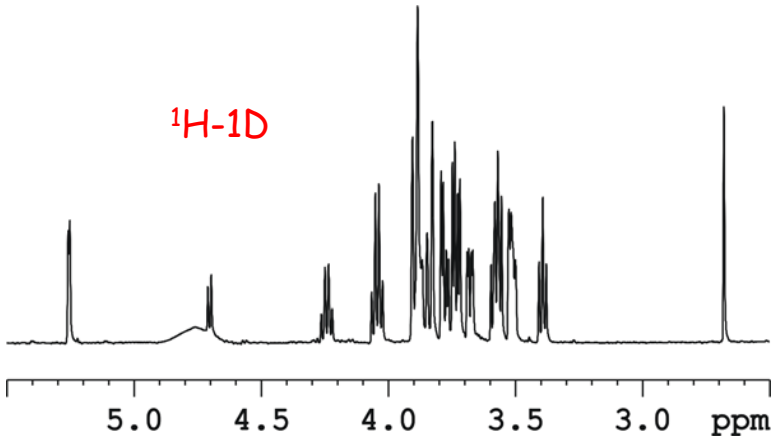
Glucose/G3P

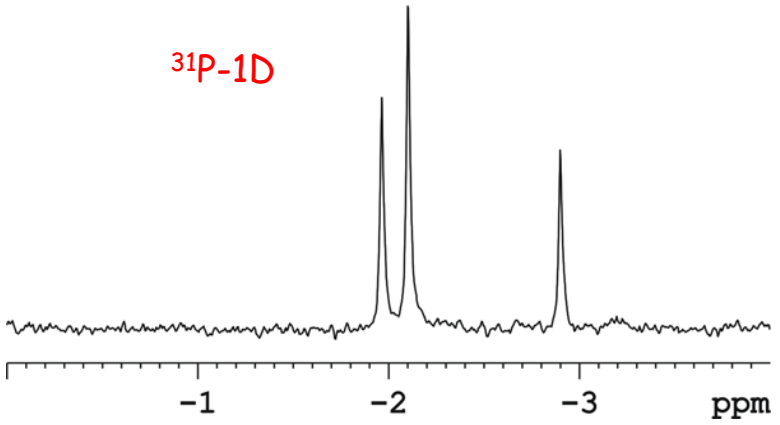

$^{1}H,^{13}C$-HMQC
ppm
65
70
75
80
85
90
95
100
5.5
5.0
4.5
4.0
ppm
6α
6β
4α/β
5α
2α
3α
3β
2β
5β
1α
1β
ppm
65
70
75
80
85
90
95
100
5.5
5.0
4.5
4.0
ppm
3α
3β

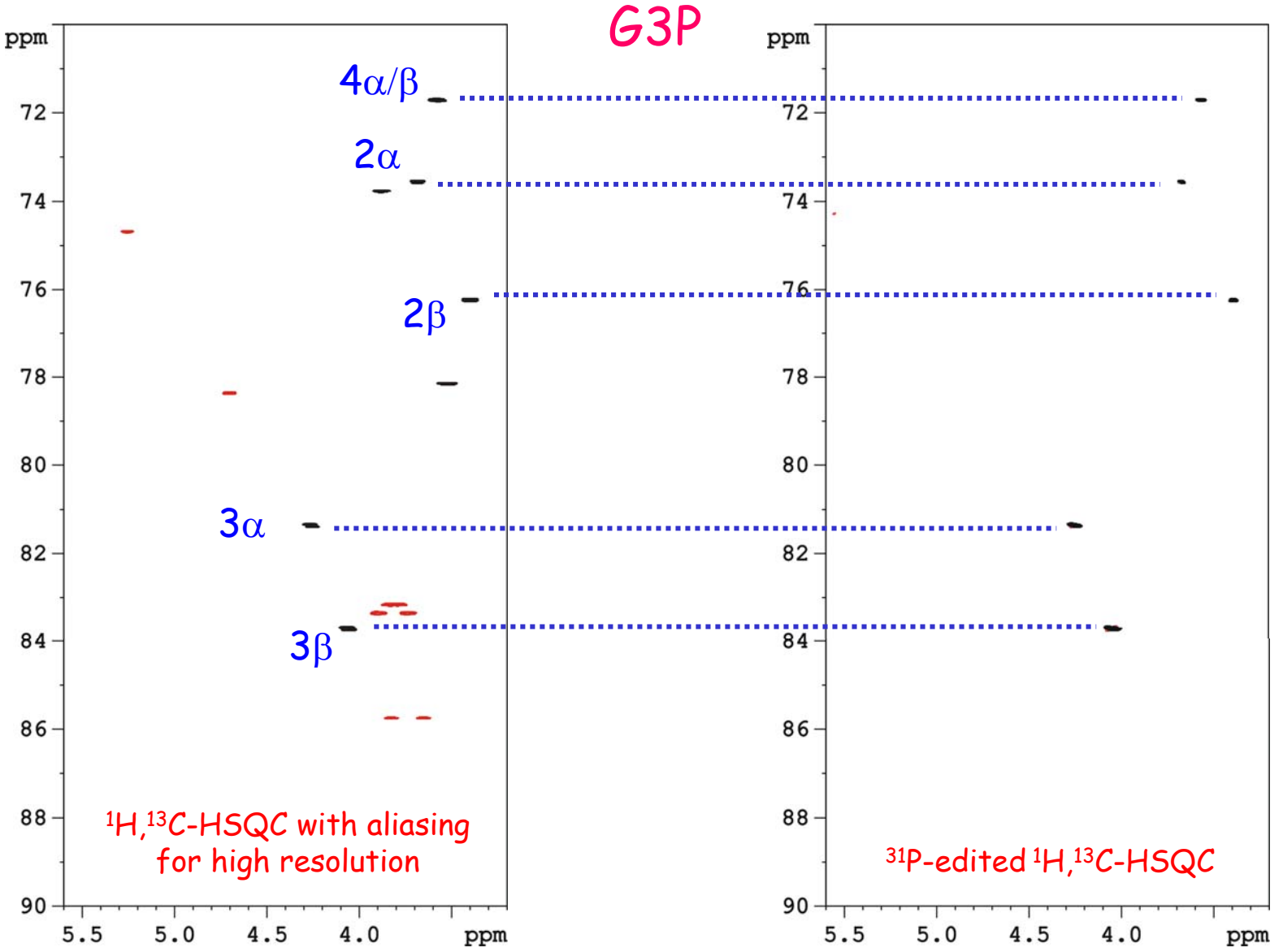

ppm
^{1}H,^{13}C-HMQC
6α
6β
4α/β
5α
2α
2β
5β
3α
3β
1α
1β
65
70
75
80
85
90
95
ppm
^{1}H,^{31}P-HMBC
−2
0
5.5
5.0
4.5
4.0
ppm

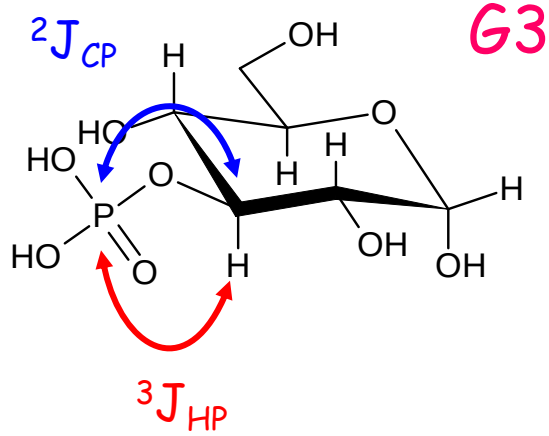
G3P


H
OH
HO
O
HO
H
H
O
P
H
HO
O
H
OH
OH


^{1}H-1D
5.0
4.5
4.0
3.5
3.0
ppm

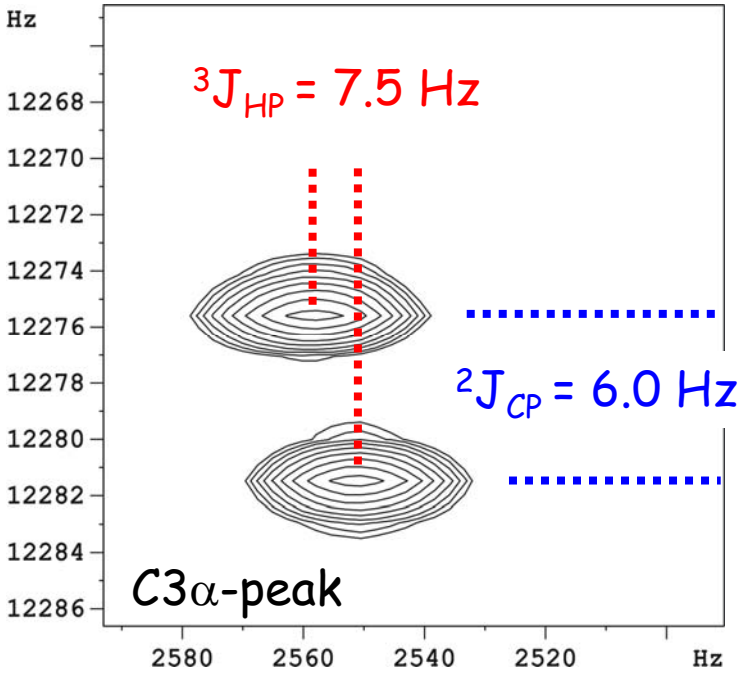

^{31}P-1D
−1
−2
−3
ppm

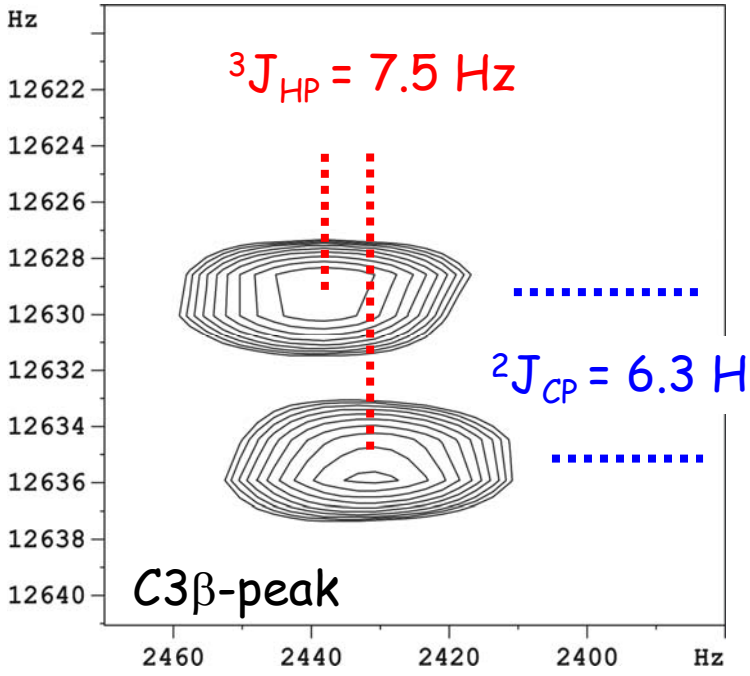

G3P
ppm
72
74
76
78
80
82
84
86
88
90
5.5
5.0
4.5
4.0
ppm
4α/β
2α
2β
3α
3β
$^{1}H,^{13}C$-HSQC with aliasing for high resolution
^{31}P-edited $^{1}H,^{13}C$-HSQC



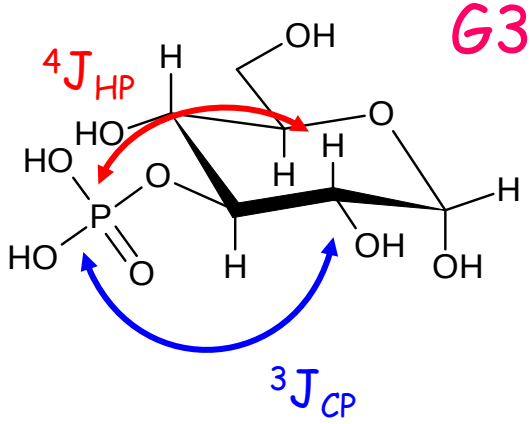
G3P

E.COSY pattern in the $^1H,^{13}C$-HSQC

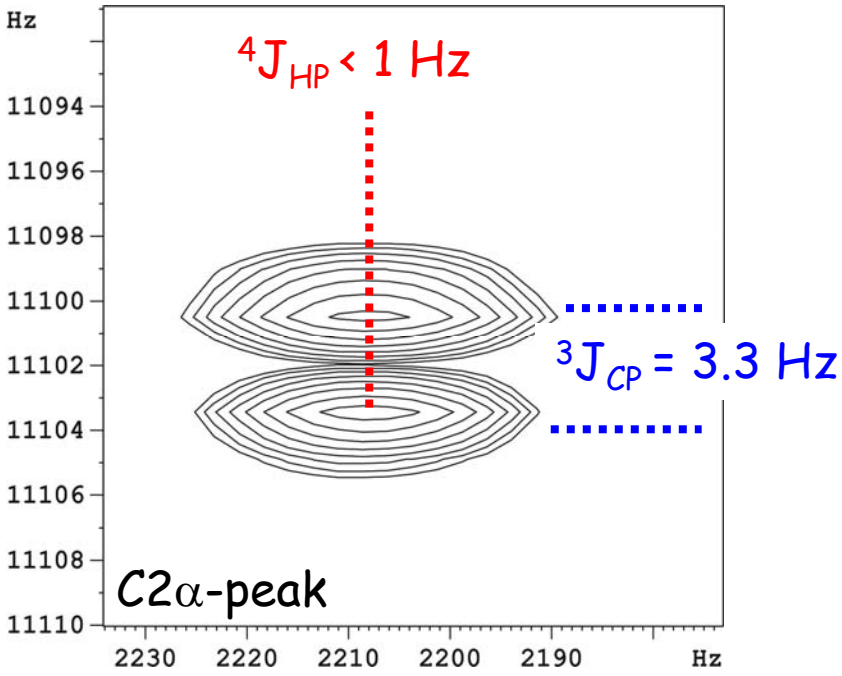


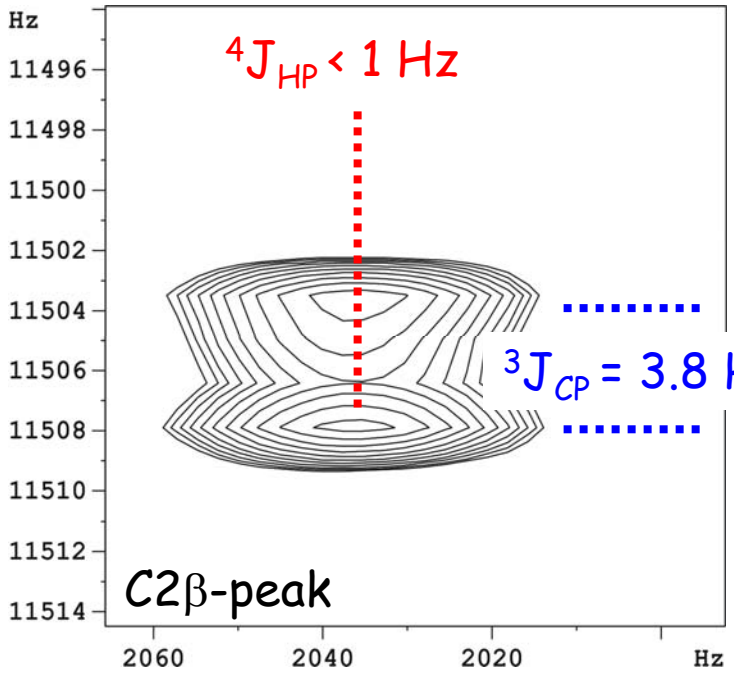


G3P



E.COSY pattern in the $^1H,^{13}C$-HSQC



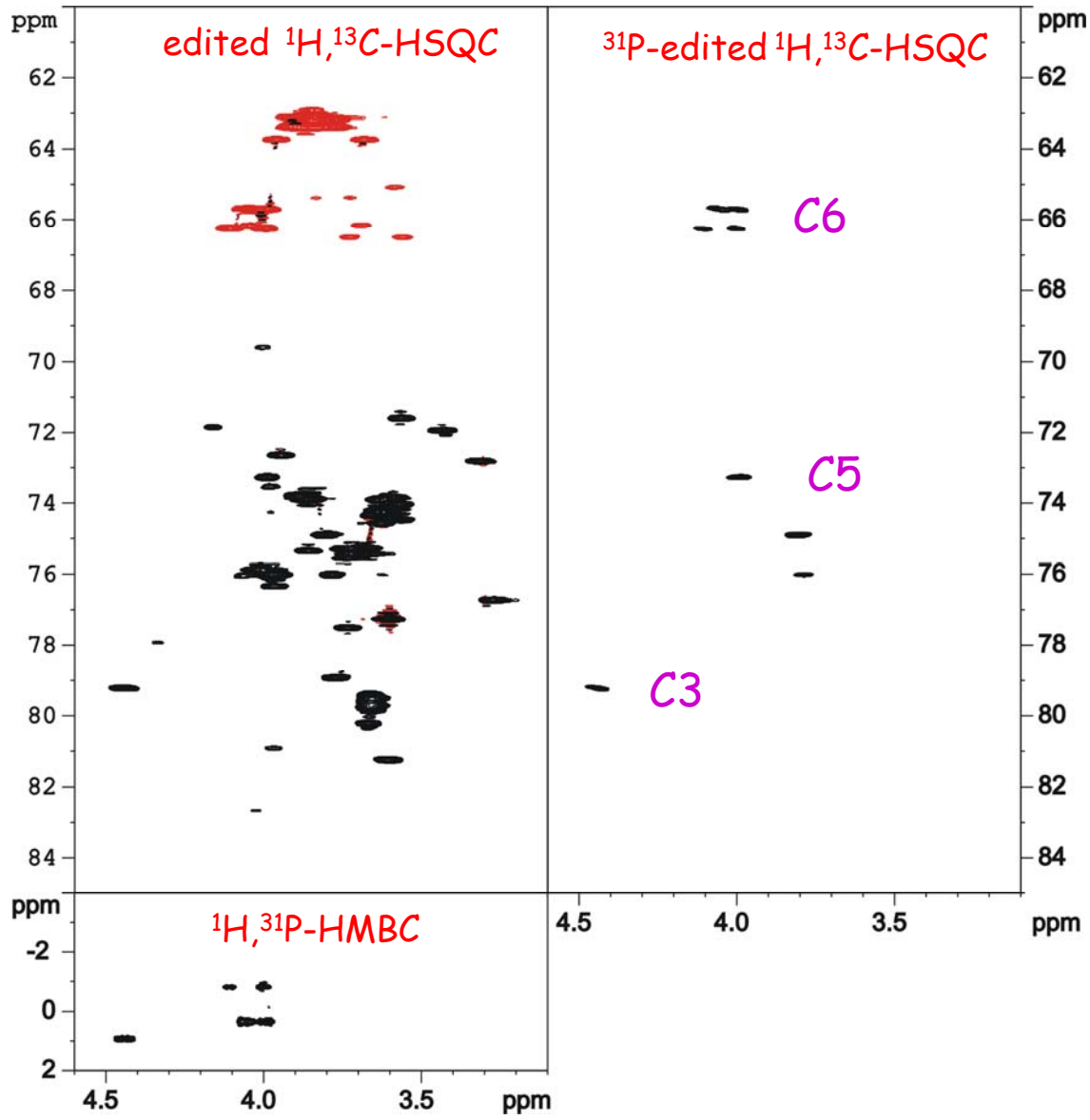


Then the real thing !

Curcuma starch as a plant model

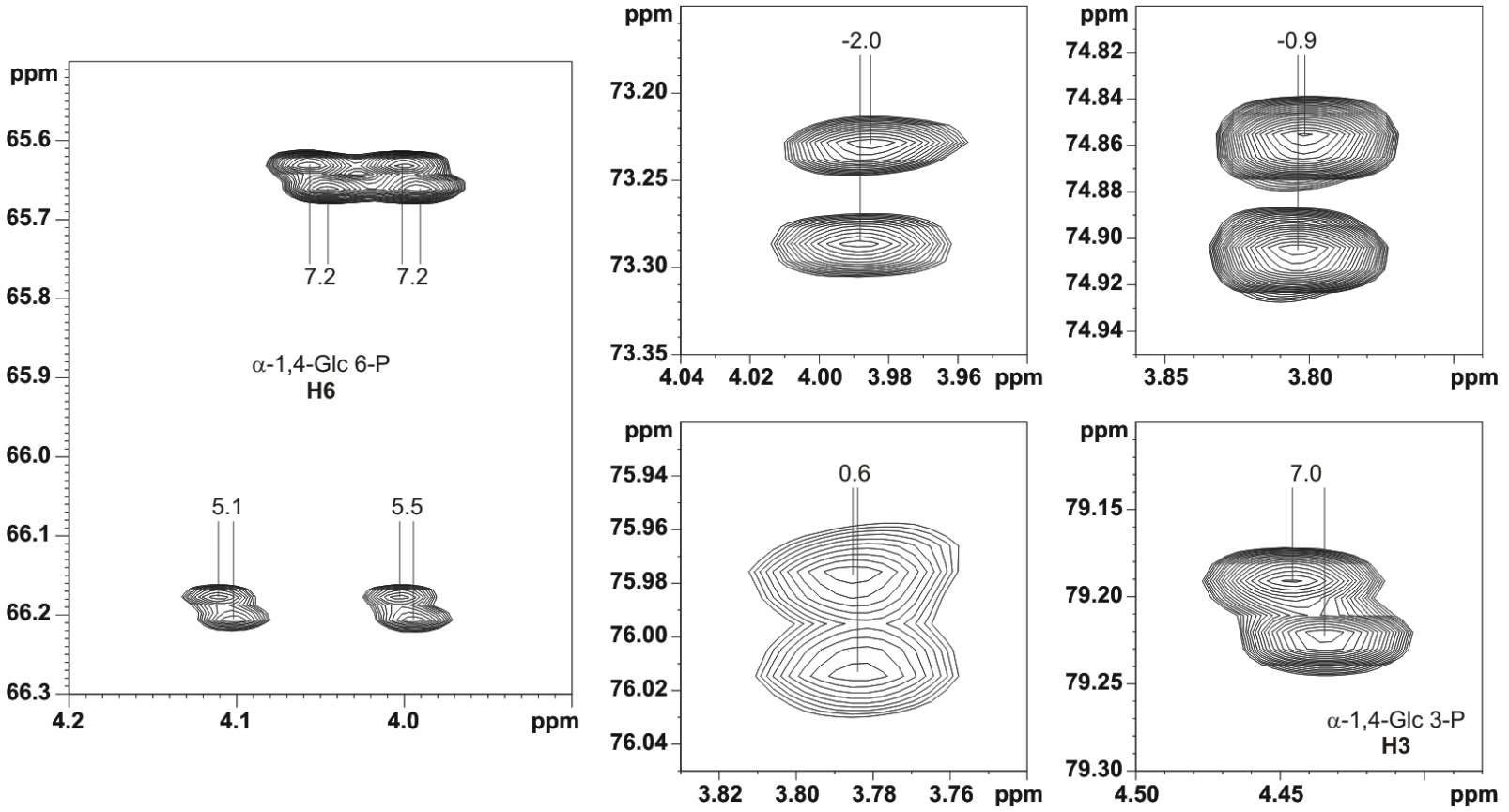
Glycogen from mice liver of

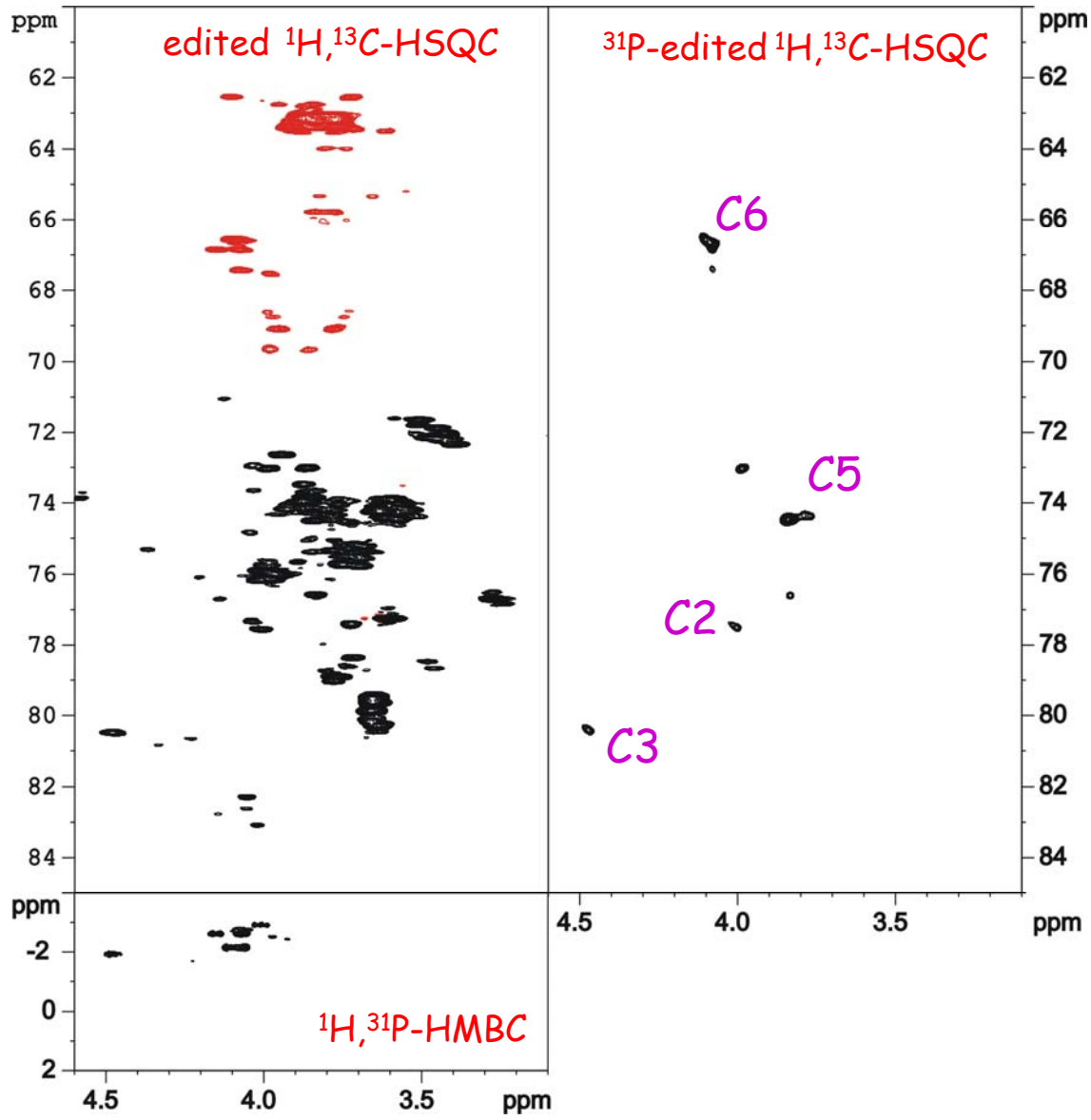
mice which lack laforin



Curcuma starch

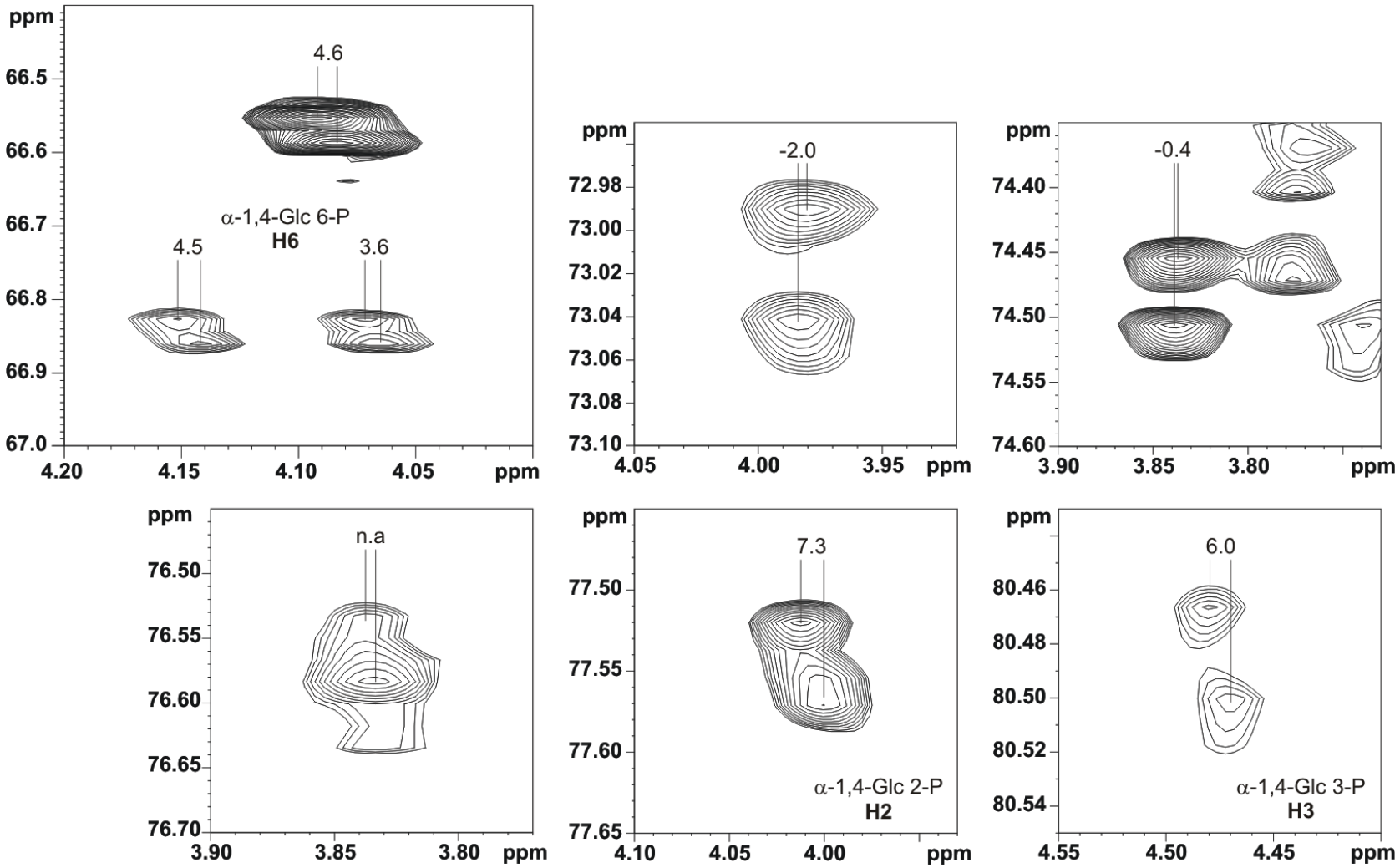
Curcuma starch




edited $^{1}H,^{13}C$-HSQC
^{31}P-edited $^{1}H,^{13}C$-HSQC
$^{1}H,^{31}P$-HMBC
ppm
62
64
66
68
70
72
74
76
78
80
82
84
-2
0
2
4.5
4.0
3.5
C6
C5
C2
C3

Glycogen

Glycogen


ppm
66.5
66.6
66.7
66.8
66.9
67.0
4.20
4.15
4.10
4.05
ppm
4.6
α-1,4-Glc 6-P
H6
4.5
3.6
ppm
72.98
73.00
73.02
73.04
73.06
73.08
73.10
4.05
4.00
3.95
ppm
-2.0
ppm
74.40
74.45
74.50
74.55
74.60
3.90
3.85
3.80
ppm
-0.4
ppm
76.50
76.55
76.60
76.65
76.70
3.90
3.85
3.80
ppm
n.a
ppm
77.50
77.55
77.60
77.65
4.10
4.05
4.00
ppm
7.3
α-1,4-Glc 2-P
H2
ppm
80.46
80.48
80.50
80.52
80.54
4.55
4.50
4.45
ppm
6.0
α-1,4-Glc 3-P
H3

Summary

Glucan-phosphorylation can be detected using NMR

Starch is phosphorylated at positions 3 and 6

Glycogen is phosphorylated at positions 2,3 and 6

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