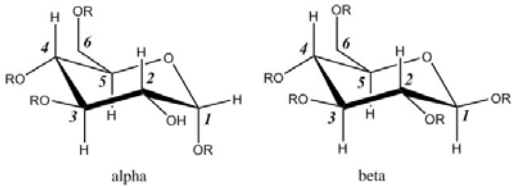
The aim of this workshop is to fully characterise an acetylated disaccharide, **I**. The sample was dissolved in $CDCl_3$ and all data acquired on 800 HMz spectrometer. Our disaccharide has 9 R groups. The R groups will be either the glycosidic linkage or OAc group.

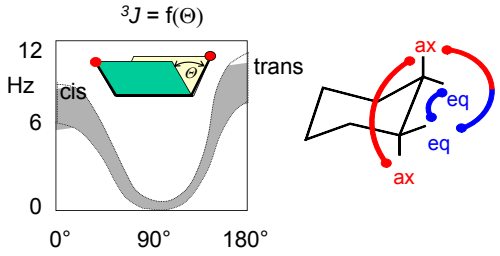
For all sugars, we can have either the α or β anomeric configuration referring to the orientation of the hydrogen at carbon C1, see example below. The glycosidic link will always be from one of the anomeric carbons to the other ring. In our example the linkage has the options to be 1 -> 1, 1 -> 2, 1 -> 3, 1 -> 4 or 1 -> 6.



The coupling constant of the anomeric proton will tell you if the sugar ring is α or β, and the subsequent couplings of the protons around the ring will tell you if adjacent protons are axial or equatorial.

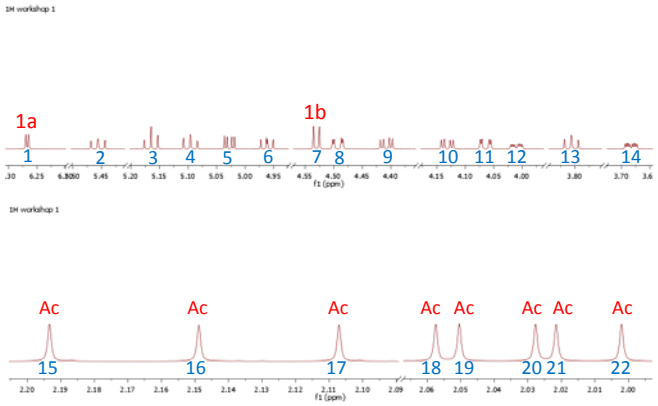
This is determined by the size of the coupling constant that is related to the dihedral angle via Karplus curve (graph 1), for example if protons 1 and 2 are axial-axial, as in the case for β anomeric configuration, there would be a large coupling constant between them compared to the equatorial-axial orientation in the α case.

We can use similar information to determine the orientation of each of our protons.



Orientation	θ	^{3}J [Hz]
ax - ax	180	11.8
ax - eq	60	3.9
eq - eq	60	3.9

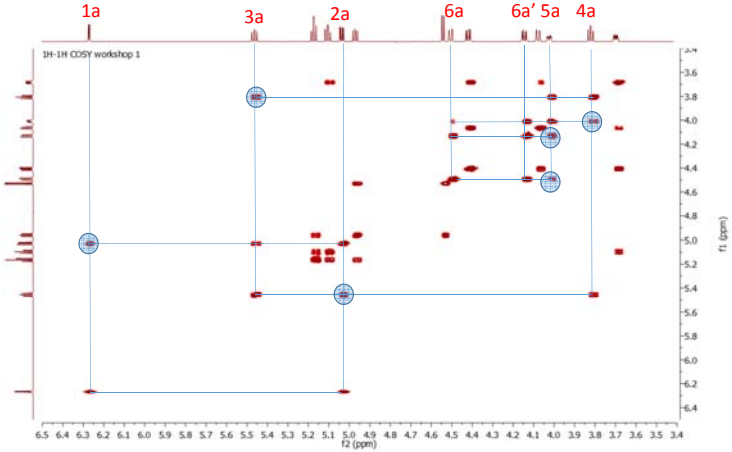
Karplus curve: $^3J_{HH} = A + B\cos\theta + C\cos^2\theta$



Expansions of the ^{1}H NMR spectrum of **I**

Chemical Formula: $C_{28}H_{38}O_{19}$

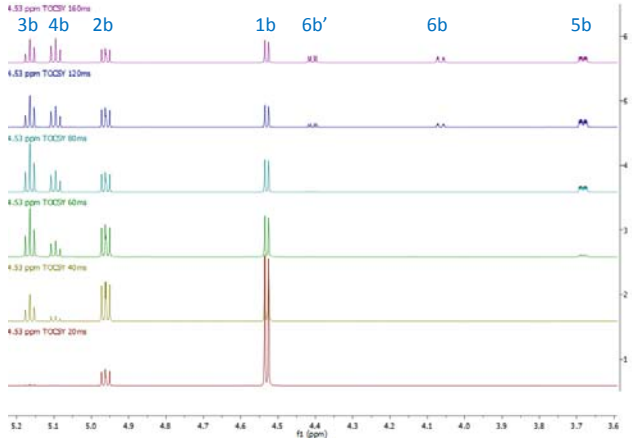
1. Comparing the chemical formula and the proton spectrum, determine how many proton environments disaccharide **I** has and how many OAc groups are present. *22 environments, 8 acetyl groups.*
2. Identify the two anomeric protons, label them 1a and 1b (starting from the more deshielded one)



2D COSY spectrum of **I**

3. Starting at the anomeric proton 1a, establish the spin system for ring a. Label protons of this ring sequentially, 1a -> 2a -> 3a etc. Write down the chemical shifts of ring a into the Table provided.

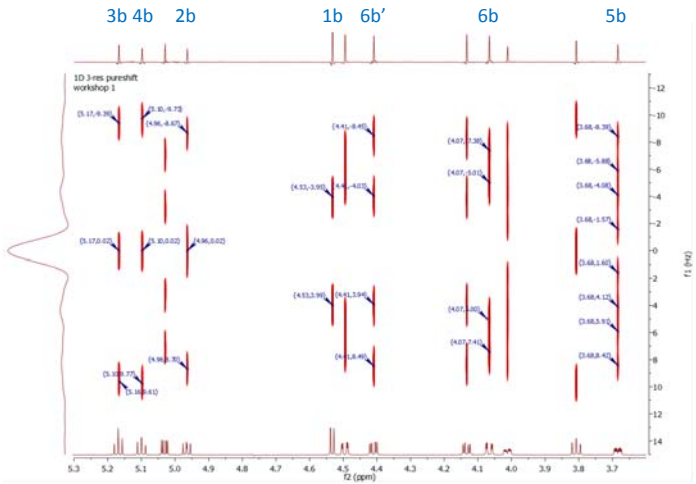
#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d			3.92			
2a	5.03	dd			3.92, 10.40			
3a	5.46	dd			10.40, 9.00			
4a	3.81	dd			9.00, 10.38			
5a	4.01	ddd			10.38, 2.36, 4.21			
6a	4.49	dd			2.36, 12.52			
6a'	4.13	dd			12.52, 4.21			
1b								
2b								
3b								
4b								
5b								
6b								
6b'								



Series of 1D TOCSY spectra of **I** with selective excitation of proton 1b and gradually increasing mixing times

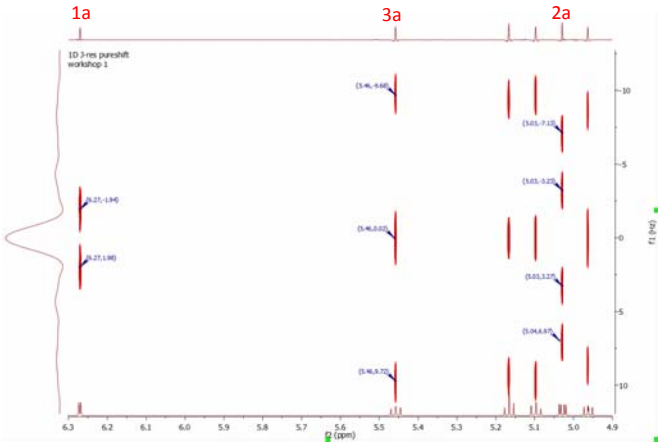
4. Starting at anomeric proton 1b, assign the protons of the second ring system. Label protons of this ring sequentially, 1b -> 2b -> 3b etc. Write down the chemical shifts of ring **b** into the Table provided.

#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d			3.92			
2a	5.03	dd			3.92, 10.40			
3a	5.46	dd			10.40, 9.00			
4a	3.81	dd			9.00, 10.38			
5a	4.01	ddd			10.38, 2.36, 4.21			
6a	4.49	dd			2.36, 12.52			
6a'	4.13	dd			12.52, 4.21			
1b	4.53	d						
2b	4.96	dd						
3b	5.16	dd						
4b	5.10	dd						
5b	3.68	ddd						
6b	4.41	dd						
6b'	4.06	dd						



Pure-shift 2D *J*-resolved spectrum of **I**

5. Determine the coupling constants for protons of ring **b**. Write the values into the Table provided.
6. By inspecting *J* couplings of both rings, determine the relative configuration of all chiral carbons and hence the type of monosaccharides **a** and **b**.

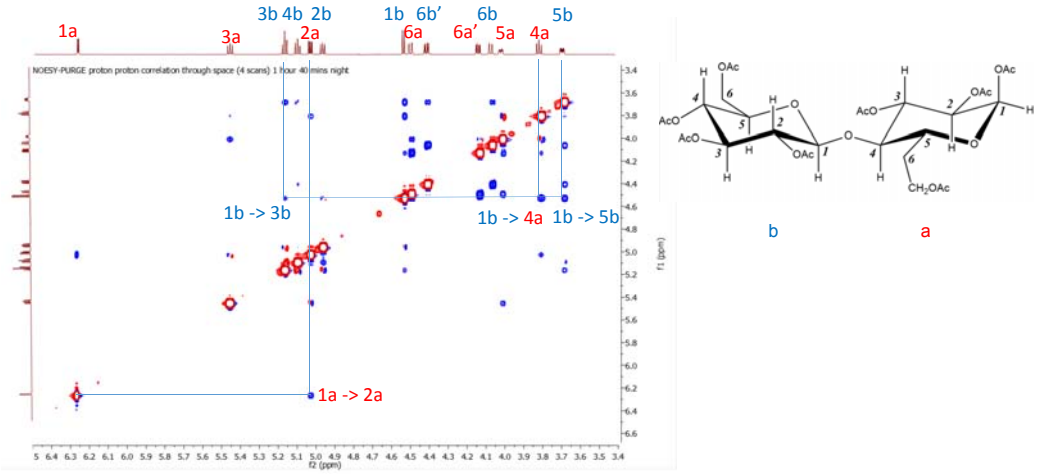


Pure-shift 2D *J*-resolved spectrum of **I**

Example looking at spin system a:
1a d: 1.94 + 1.98 = 3.92 Hz
2a dd: 7.13 - 3.23 = 3.9 Hz, 7.13 + 3.27 = 10.40 Hz
3a dd: 9.68 + 9.72 = 19.40 – 10.40 (known from 2a) = 9.00 Hz

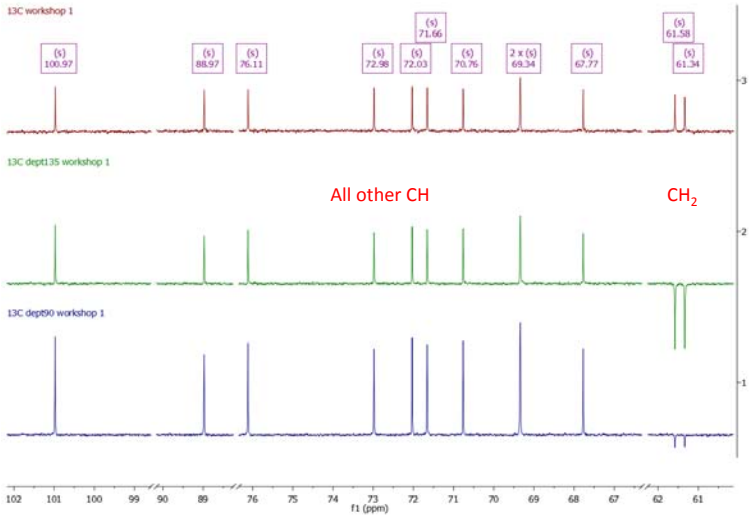
All coupling constants between H1-H5 protons in both rings are large, with an exception of *J*(H1a,H2a). This indicated that proton H1a is equatorial and both monosaccharide are glucoses in β (ring b) and α (ring a) configuration, respectively.

#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d		e	3.92			
2a	5.03	dd		a	3.92, 10.40			
3a	5.46	dd		a	10.40, 9.00			
4a	3.81	dd		a	9.00, 10.38			
5a	4.01	ddd		a	10.38, 2.36, 4.21			
6a	4.49	dd		n/a	2.36, 12.52			
6a'	4.13	dd		n/a	12.52, 4.21			
1b	4.53	d		a	7.94			
2b	4.96	dd		a	7.94, 9.43			
3b	5.16	dd		a	9.43, 9.57			
4b	5.10	dd		a	9.57, 9.93			
5b	3.68	ddd		a	9.93, 2.51, 4.31			
6b	4.41	dd		n/a	4.31, 12.39			
6b'	4.06	dd		n/a	12.39, 2.37			

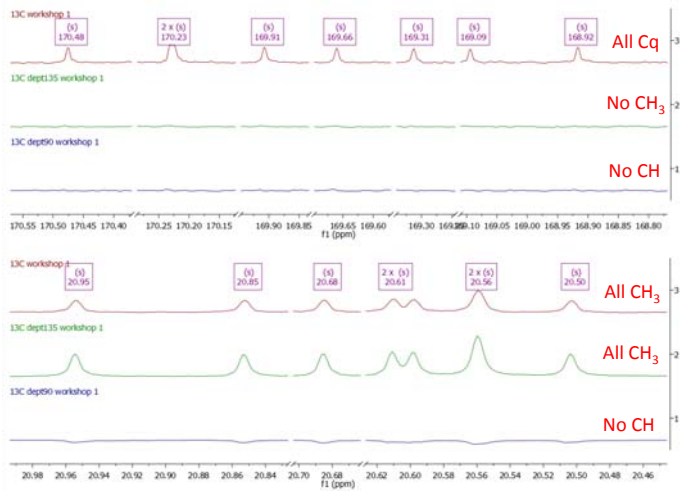


2D NOESY spectrum of **I**

7. By assigning the cross peaks of protons 1a and 1b determine how the two monosaccharides are linked. *1b -> 4a*



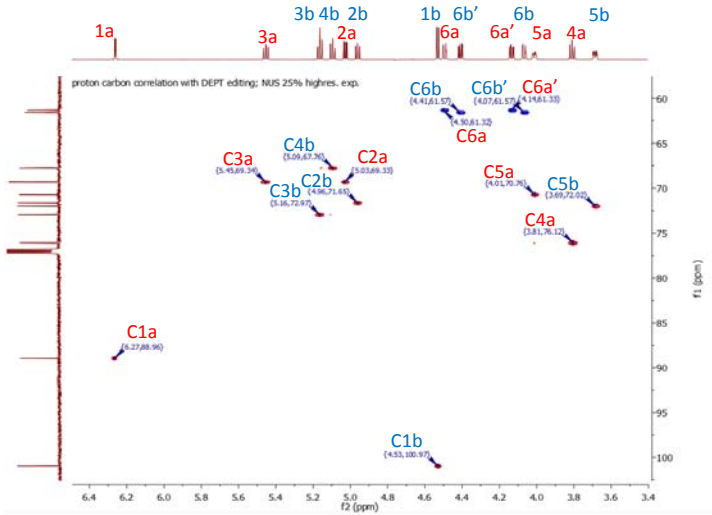
Expansions of ^{13}C 1D and DEPT spectra of **I**



Expansions of ^{13}C 1D and DEPT spectra of **I**

Chemical Formula: $C_{28}H_{38}O_{19}$

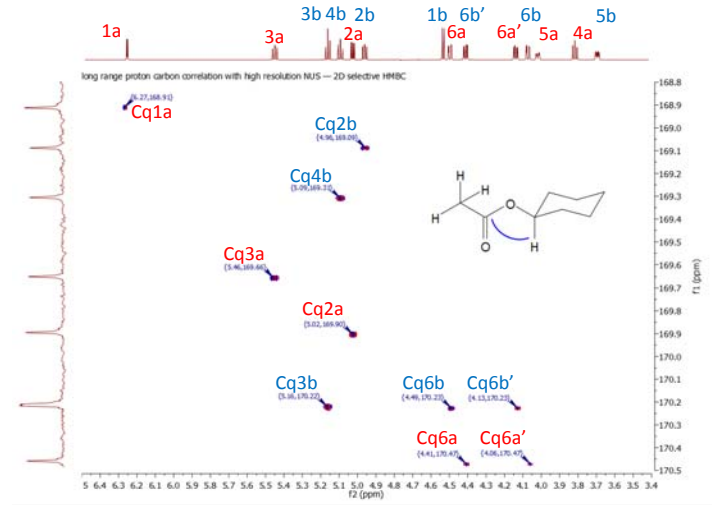
8. Determine the number of Cq, CH, CH_2 and CH_3. Compare to the chemical formula *8 x Cq, 10 x CH, 2 x CH_2, 8 x CH_3*



Partial 2D ^{1}H, ^{13}C HSQC spectrum of **I**

9. Using the assigned proton resonances from Table **I**, assign the ring carbons of **I**. Write the assigned ^{13}C chemical shifts into Table **I**

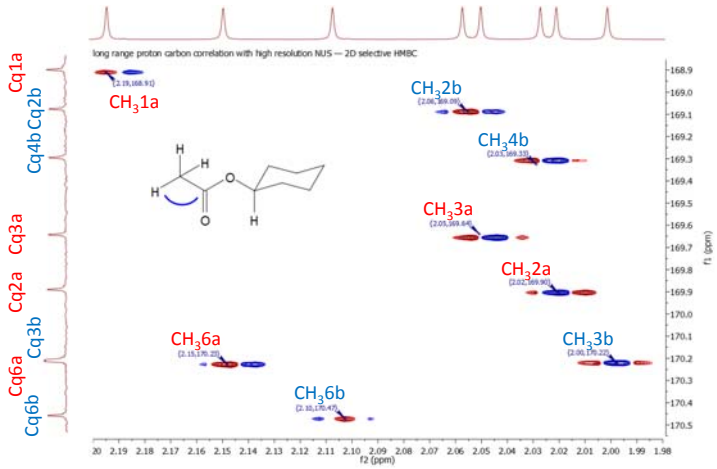
#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d		e	3.92	88.96		
2a	5.03	dd		a	3.92, 10.40	69.33		
3a	5.46	dd		a	10.40, 9.00	39.34		
4a	3.81	dd		a	9.00, 10.38	76.12		
5a	4.01	ddd		a	10.38, 2.36, 4.21	70.76		
6a	4.49	dd		n/a	2.36, 12.52	61.32		
6a'	4.13	dd		n/a	12.52, 4.21	61.32		
1b	4.53	d		a	7.94	100.97		
2b	4.96	dd		a	7.94, 9.43	71.65		
3b	5.16	dd		a	9.43, 9.57	72.97		
4b	5.10	dd		a	9.57, 9.93	67.76		
5b	3.68	ddd		a	9.93, 2.51, 4.31	72.02		
6b	4.41	dd		n/a	4.31, 12.39	61.57		
6b'	4.06	dd		n/a	12.39, 2.37	61.57		



Partial 2D ^{1}H, ^{13}C HMBC spectrum of **I**. The carbonyl carbons/ring protons region.

10. Using the assigned proton resonances from Table **I**, assign the carbonyl carbons of **I**. Write the assigned ^{13}C chemical shifts into Table **I**

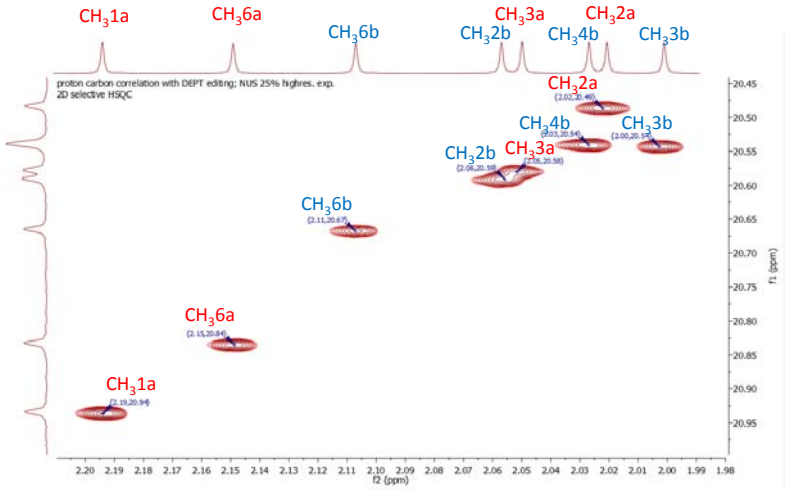
#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d		e	3.92	88.96	168.91	
2a	5.03	dd		a	3.92, 10.40	69.33	169.09	
3a	5.46	dd		a	10.40, 9.00	39.34	169.66	
4a	3.81	dd		a	9.00, 10.38	76.12	-	
5a	4.01	ddd		a	10.38, 2.36, 4.21	70.76	-	
6a	4.49	dd		n/a	2.36, 12.52	61.32	170.23	
6a'	4.13	dd		n/a	12.52, 4.21	61.32	170.23	
1b	4.53	d		a	7.94	100.97	-	
2b	4.96	dd		a	7.94, 9.43	71.65	169.90	
3b	5.16	dd		a	9.43, 9.57	72.97	170.22	
4b	5.10	dd		a	9.57, 9.93	67.76	169.31	
5b	3.68	ddd		a	9.93, 2.51, 4.31	72.02	-	
6b	4.41	dd		n/a	4.31, 12.39	61.57	170.47	
6b'	4.06	dd		n/a	12.39, 2.37	61.57	170.47	



Partial 2D ^{1}H, ^{13}C HMBC spectrum of **I.** The carbonyl carbons/methyl protons region.

11. Using the assigned carbonyl carbon resonances from Table **I**, assign the methyl protons of **I**. Write the assigned ^{1}H chemical shifts into Table **I**

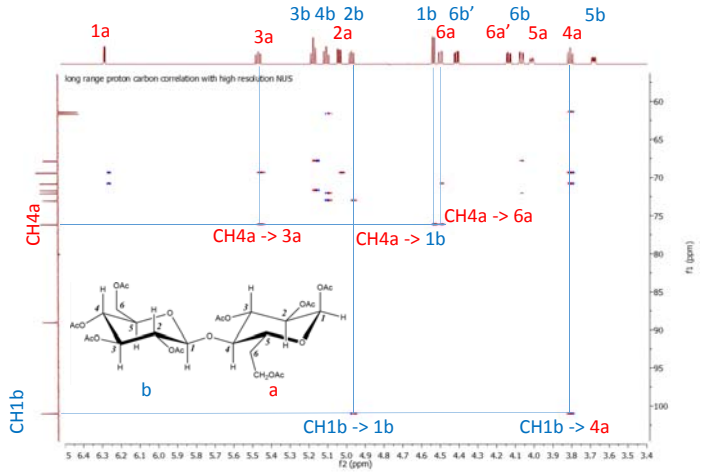
#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d	2.19	e	3.92	88.96	168.91	
2a	5.03	dd	2.02	a	3.92, 10.40	69.33	169.09	
3a	5.46	dd	2.05	a	10.40, 9.00	39.34	169.66	
4a	3.81	dd	--	a	9.00, 10.38	76.12	-	
5a	4.01	ddd		a	10.38, 2.36, 4.21	70.76	-	
6a	4.49	dd	2.15	n/a	2.36, 12.52	61.32	170.23	
6a'	4.13	dd	2.15	n/a	12.52, 4.21	61.32	170.23	
1b	4.53	d	-	a	7.94	100.97	-	
2b	4.96	dd	2.06	a	7.94, 9.43	71.65	169.90	
3b	5.16	dd	2.00	a	9.43, 9.57	72.97	170.22	
4b	5.10	dd	2.03	a	9.57, 9.93	67.76	169.31	
5b	3.68	ddd	-	a	9.93, 2.51, 4.31	72.02	-	
6b	4.41	dd	2.10	n/a	4.31, 12.39	61.57	170.47	
6b'	4.06	dd	2.10	n/a	12.39, 2.37	61.57	170.47	



Partial 2D ^{1}H, ^{13}C HSQC spectrum of **I.** The methyl carbons/methyl protons region.

12. Using the assigned methyl proton resonances from Table **I**, assign the methyl carbons of **I**. Write the assigned ^{13}C chemical shifts into Table **I**

#	δH(CH)	m	δH(CH_3)	axial/equatorial	J	δC(H)	δC(quaternary)	δC(CH_3)
1a	6.27	d	2.19	e	3.92	88.96	168.91	20.94
2a	5.03	dd	2.02	a	3.92, 10.40	69.33	169.09	20.49
3a	5.46	dd	2.05	a	10.40, 9.00	39.34	169.66	20.54
4a	3.81	dd	--	a	9.00, 10.38	76.12	-	-
5a	4.01	ddd		a	10.38, 2.36, 4.21	70.76	-	-
6a	4.49	dd	2.15	n/a	2.36, 12.52	61.32	170.23	20.84
6a'	4.13	dd	2.15	n/a	12.52, 4.21	61.32	170.23	20.84
1b	4.53	d	-	a	7.94	100.97	-	-
2b	4.96	dd	2.06	a	7.94, 9.43	71.65	169.90	20.59
3b	5.16	dd	2.00	a	9.43, 9.57	72.97	170.22	20.54
4b	5.10	dd	2.03	a	9.57, 9.93	67.76	169.31	20.54
5b	3.68	ddd	-	a	9.93, 2.51, 4.31	72.02	-	-
6b	4.41	dd	2.10	n/a	4.31, 12.39	61.57	170.47	20.67
6b'	4.06	dd	2.10	n/a	12.39, 2.37	61.57	170.47	20.67



Partial 2D ^{1}H, ^{13}C HMBC spectrum of **I.** The ring carbons/ring protons region.

13. Focusing on the long-range correlations of the anomeric carbons and protons, identify the glycosidic linkage between monosaccharides **a** and **b**. Is this result consistent with the structure determined by homonuclear techniques?