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Introduction

NMR spectroscopy has emerged as the most important tool for the structure determination of oligo- and polysaccharides. Whilst component and linkage analysis are still performed by chemical methods the determination of anomeric configuration and sequence is now routinely performed by NMR.

Since the NMR spectra of most carbohydrates are unique, it is possible to determine their structure from a single NMR spectrum. The computer program CASPER generates trial structures from information of component and linkage analyses and calculates their NMR spectra. The trial structures are then ranked according to their agreement with the experimental spectrum.

Results

The five structures with the smallest deviation between calculated and experimental spectra are shown together with the complete assignments of all chemical shifts.

Output from a sequence determination

Best fitting structures

- Structure 1, error=3.75 (0.12)
->3)[aDGlc-(1->6)]bDGlcNAc-(1->2)aLRha-(1->2)aLRha-(1->3)aLRha-(1->3)
- Structure 2, error=5.45 (0.17)
->3)[aDGlc-(1->6)]bDGlcNAc-(1->2)aLRha-(1->3)aLRha-(1->2)aLRha-(1->2)
- Structure 3, error=7.79 (0.24)
->3)[aDGlc-(1->2)aLRha-(1->6)]bDGlcNAc-(1->2)aLRha-(1->3)aLRha-(1->3)
- Structure 4, error=7.97 (0.25)
->6)[aDGlc-(1->2)aLRha-(1->3)]bDGlcNAc-(1->2)aLRha-(1->3)aLRha-(1->3)
- Structure 5, error=8.11 (0.25)
->3)[aDGlc-(1->6)]bDGlcNAc-(1->3)aLRha-(1->2)aLRha-(1->2)aLRha-(1->2)

Total and per-resonance deviations

HTML-form with the input for CASPER

Title:

Source:

Residue	Linkage position	1	2	3	4	5	6
L-Rhap	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
L-Rhap	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
L-Rhap	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
D-GlcpNAc	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
D-Glcp	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
none	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Chemical shifts: C H

Source of chemical shifts: text area file

101.85 79.61 70.83 73.31 70.04 17.48
 101.58 79.05 70.94 73.20 69.95 17.56
 101.85 71.47 78.28 72.45 69.88 17.36
 103.03 56.39 82.24 69.40 75.21 66.97
 23.20 174.88
 99.07 72.45 74.11 70.66 72.84 61.65

Load shifts from: Bläddra...

Correct by subtracting: ppm

Number of shifts - Required: Actual: Clear text area

Minimum number of coupling constants of different magnitudes

	small	medium	large
$^1J_{CH}$	<input type="text" value="0"/> (<2 Hz)	<input type="text" value="0"/> (2-7 Hz)	<input type="text" value="0"/> (>7 Hz)
$^1J_{CH}$	<input type="text" value="0"/> (<169 Hz)		<input type="text" value="0"/> (>169 Hz)

Save form Start simulation

11 glycosyl residues to choose from

Chemical shifts are entered here

A filled form can be saved locally

Linkage positions are checked for consistency

Coupling constants may be used as restrictions

Assignment of resonances

Experimental	Simulated	Exp-Sim	Assignment
174.88	174.97	-0.09	bDGlcNAc - CO
103.03	102.76	0.27	bDGlcNAc - 1
101.85	102.05	-0.20	aLRha - 1

Structure 1

->3)[aDGlc-(1->6)]bDGlcNAc-(1->2)aLRha-(1->2)aLRha-(1->3)aLRha-(1->3)

Simulated structure

->2)aLRha-(1->	101.81	79.49	70.83	73.52	69.98	17.46		
	5.13	4.12	3.87	3.35	3.74	1.26		
->2)aLRha-(1->	101.79	79.26	70.92	73.00	70.03	17.55		
	5.13	4.07	3.94	3.49	3.79	1.30		
->3)aLRha-(1->	102.05	71.39	78.76	72.17	70.02	17.37		
	4.85	3.87	3.78	3.54	4.00	1.25		
->3,6)bDGlcNAc-(1->	102.76	56.32	82.48	69.58	75.24	66.74	23.15	174.97
	4.75	3.83	3.64	3.61	3.62	3.97	3.83	2.06
aDGlc-(1->	98.85	72.43	74.11	70.67	72.77	61.65		
	4.96	3.57	3.74	3.44	3.73	3.77	3.86	

Experimental structure

->2)aLRha-(1->	101.85	79.61	70.83	73.31	69.88	17.48		
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
->2)aLRha-(1->	101.58	79.05	70.94	73.20	70.04	17.56		
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
->3)aLRha-(1->	101.85	71.47	78.28	72.45	69.95	17.36		
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
->3,6)bDGlcNAc-(1->	103.03	56.39	82.24	69.40	75.21	66.97	23.20	174.88
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
aDGlc-(1->	99.07	72.45	74.11	70.66	72.84	61.65		
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	

Error=3.75 pp

The main drawback of CASPER has been the interface and the fact that the program and databases have been in a state of flux. Using an interface based on HTML-forms and server-side scripts allows us to retain creative control over the program whilst making it available to the widest possible audience.

How well CASPER succeeds in picking the correct structure depends mainly on the number of possible structures. Trials with known structures show that most ¹³C-NMR spectra are simulated with a deviation of <0.3 ppm/resonance. In the case of structures with three residues in the repeating unit this is sufficient to determine the correct structure.