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Complete ^{1}H- and ^{13}C-resonance assignments for D-mannooligosaccharides of the β-D-(1 → 2)-linked series released from the phosphopeptidomannan of *Candida albicans* VW.32 (serotype A) *

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ABSTRACT

D-Mannooligosaccharides (dp 1 to > 17) were released by mild acid hydrolysis from the phosphopeptidomannan of a *Candida albicans* strain of A serotype (VW.32). Among these, mannooligosaccharides ranging from bi- to hepta-ose, which were obtained in appreciable amounts, were structurally investigated and found to belong to the β-D-(1 → 2)-linked series. The occurrence of such compounds has already been reported in other *Candida albicans* strains. The complete ^{1}H- and ^{13}C-resonance assignments for *manno*-tri- to *manno*-hepta-ose are reported and general rules applicable for the ^{1}HNMR spectrum analysis of linear mannooligosaccharide of the general structure, β-D-Man*p*-(1 → 2)-[β-D-Man*p*-(1 → 2)]$_n$-β-D-Man*p* are proposed.

INTRODUCTION

Cell wall phosphopeptidomannans from the pathogenic species *Candida albicans* have been shown to contain phosphate diester-linked oligomannosides of the β-(1 → 2)-linked series[1–5]. Although the structural analysis of these acid-labile oligosaccharides was performed by ^{1}H and ^{13}C NMR, the assignment of the chemical shifts of the D-mannose units was not possible owing to the complexity of the NMR patterns[2]. In a preliminary work[6], we assigned the structural reporter

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groups and proposed the rules relative to the β-(1 → 2)-linked D-oligosaccharide series, and we describe herein the complete assignments of the ^1H and ^{13}C NMR spectra of the compounds, obtained by use of homonuclear and heteronuclear COSY experiments.

RESULTS AND DISCUSSION

^1H and ^{13}C NMR spectroscopy of mannooligosaccharides released from the phosphomannan by acid treatment. — The ^1H NMR data obtained at 400 MHz for solutions in D_2O at 27° are given in Table I. The assignments were based on COSY, and relayed and double-relayed COSY experiments. Similarly, the ^{13}C NMR data (100 MHz), based on heteronuclear correlation spectroscopy, are given in Table II.

As previously reported[2,6], it is evident that the series of mannooligosaccharides consists solely of compounds having β-(1 → 2)-linked D-mannose units. Indeed, the coupling constant $J_{1,2}$ is ~ 1.10 Hz for each H-1 proton, except for one, the signal of which is shifted downfield to δ 5.28 and possesses $J_{1,2}$ 1.85 Hz, characteristic of the reducing, terminal α-D-Man residue. Moreover, the heteronuclear COSY spectra show that all the C-2 atom, except one, of the D-Man residues, exibit a downfield shift of ~ 9 ppm, as compared with that of the C-2 resonance of the nonreducing, terminal β-D-mannopyranosyl group. The ^1H- and ^{13}C-chemical shifts of the mannooligosaccharides M-3–M-7 (**1–5**) were assigned by comparison of the homonuclear relayed COSY and ^1H–^{13}C COSY spectra.

Trisaccharide M-3 (**1**). — The basis for the assignment was the identification of the αMan-A H-1 signal owing to $J_{1,2}$ 1.85 Hz, and the βMan-C H-2 signal, shifted upfield to δ 4.165, owing to the nonreducing, terminal position of this β-Man*p* group. Starting from the signal for H-2 of the α form of Man-B and Man-C at δ 4.279 and 4.165, respectively, the signals for H-1 were found from the cross-peak in the normal COSY spectrum at δ 4.856 and 4.865, respectively. The H-1 and H-2 resonances in Man-B and Man-C showed splittings due to the α and β forms of Man-A. The large splitting was attributed to Man-B, and this was confirmed by the ^1H–^{13}C COSY spectrum, which clearly indicated that the H-2 atoms of Man-C(α) and Man-C(β) possess very similar chemical shifts. Man-Aβ H-2 was found to possess a chemical shift identical with that of Man-C(α) H-2 in the heteronuclear COSY spectrum and in the homonuclear COSY spectrum. Starting from these well defined H-2 resonances, the H-3, H-4, and H-5 signals of Man-A, B, and C could be assigned from the double-relayed COSY. The H-6a and H-6b resonances were obtained from the ^1H–^{13}C correlation based on the hypothesis that the anomerization effect affects essentially Man-B and, consequently, enlarges the corresponding ^{13}C resonance (Tables I and II; and Fig. 1).

Tetrasaccharide M-4 (**2**). — As for trisaccharide M-3, the signals for H-1 and H-2 in αMan-A and Man-D, which are the terminal-reducing and nonreducing units, respectively, were deduced from the 1D ^1H NMR spectrum. The signals for

TABLE I

^{1}H NMR chemical shifts

Compounds, units, and anomers	Chemical shifts (δ)						
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
M-3 (**1**)							
C (α)	4.865	4.165	3.626	3.562	3.34	3.935	3.717
(β)	4.954	4.147	3.626	3.56	3.37	[a]	[a]
B (α)	4.856	4.279	3.668	3.606	3.394	3.942	3.751
(β)	4.914	4.415	3.668	3.606	3.397	[a]	[a]
A α	5.275	4.110	3.900	3.626	3.785	3.867	3.744
β	4.982	4.165	3.674	3.497	3.36	3.90	3.72
M-4 (**2**)							
D	4.930	4.153	3.620	3.568	3.384	3.92	3.75
C (α)	4.941	4.418	3.640	3.585	3.363	3.94	3.73
(β)	5.041	4.382	3.647	3.592	3.40	[a]	[a]
B (α)	4.843	4.256	3.698	3.517	3.391	3.94	3.75
(β)	4.883	4.385	3.698	3.517	3.40	[a]	[a]
A α	5.280	4.120	3.903	3.909	3.801	3.86	3.74
β	4.990	4.177	3.681	3.483	3.384	n.d.	n.d.
M-5 (**3**)							
E	4.953	4.156	3.626	3.568	3.397	~ 3.92	~ 3.75
D	4.929	4.406	3.668	3.503	3.356	~ 3.92	~ 3.75
C	5.031	4.388	3.637	3.589	3.40	~ 3.92	~ 3.75
B (α)	4.847	4.259	3.702	3.507	3.391	~ 3.92	~ 3.75
(β)	4.892	4.388	3.702	3.507	3.391	[a]	[a]
A α	5.280	4.119	3.907	3.613	3.797	~ 3.92	~ 3.92
β	4.990	4.176	3.685	3.486	[a]	[a]	[a]
M-6 (**4**)							
F	4.959	4.156	3.627	3.569	3.404	~ 3.92	~ 3.75
E	4.935	4.415	3.674	3.504	3.360	~ 3.92	~ 3.75
D	5.011	4.389	3.657	3.589	3.401	~ 3.92	~ 3.75
C	5.011	4.372	3.668	3.507	3.384	~ 3.92	~ 3.75
B (α)	4.847	4.266	3.709	3.510	3.394	~ 3.92	~ 3.75
(β)	4.893	4.389	3.705	[a]	[a]	[a]	[a]
A α	5.281	4.119	3.907	3.606	3.798	~ 3.82	~ 3.72
β	4.990	4.176	3.685	3.480	3.398	[a]	[a]
M-7 (**5**)							
G	4.959	4.156	3.630	3.568	3.406	3.92	3.70 ~ 3.75
F	4.934	4.416	3.678	3.502	3.361	3.92	3.70 ~ 3.75
E	5.058	4.387	3.652	3.589	3.39	3.92	3.70 ~ 3.75
D	5.033	4.371	3.678	3.502	3.39	3.92	2.70 ~ 3.75
C	5.018	4.379	3.673	3.590	3.39	3.92	3.70 ~ 3.75
B (α)	4.847	4.260	3.709	3.502	3.393	3.92	3.70 ~ 3.75
(β)	4.894	4.389	[a]	[a]	[a]	[a]	[a]
A α	5.280	4.119	3.906	3.608	3.801	3.82	[a]
β	4.990	4.177	3.682	3.480	3.99	3.90	3.70

[a] Not determined.

H-1 and H-2 in Man-B, which possesses a similar environment in M-3 and M-4, were assigned to δ 4.843 and 4.256, respectively. This was confirmed by the C-1 resonance at δ 100.51 in the C–H correlation experiment. The other ^1H chemical shifts were assigned in the double-relayed COSY spectrum, and the ^{13}C-chemical shifts obtained from the heteronuclear COSY spectrum (Tables I and II; and Fig. 2).

Oligosaccharides M-5–M-7 (**3–5**). — As for oligosaccharides M-3 and M-4, the signals relative to the terminal reducing and nonreducing units could be directly assigned from the 1D ^1H NMR spectra. The signals for H-1 and H-2 in Man-B of oligosaccharides Man-5 (**3**), -6(**4**), -7(**5**) exhibited remarkably stable chemical shift values (δ_{H-1} 4.847 and δ_{H-2} 4.259–4.266) due to the strong influence of the reducing unit. For Man-C, -D and -E of **5**, which possess the same environment and consequently should give superposable NMR parameters, the H-2 resonance was assigned to the bulk observed at δ 4.37–4.38. Moreover, a comparison of the spectra of **3**, **4**, and **5** highlighted the distinctive H-1 and H-2 signals at δ 4.93 Nd 4.40–4.41, respectively, which were assigned to the H-1 and H-2 resonances of the penultimate Man unit. Likewise, the H-4 signal found at δ 3.589 could be considered as being characteristic either of Man-C, or of the antepenultimate Man residue. This second hypothesis was retained on the basis that the corresponding anomeric proton assigned from the double-relayed COSY spectrum (Man-D in **4**, Man-E in **5**) possesses a similar chemical shift, which is different from that of Man-C of **3**. Finally, the complete ^1H and ^{13}C resonance assignment for **3** and **5** was achieved by use of ^1H–^1H and ^1H–^{13}C COSY experiments. However, it was not possible to perform a C–H correlation experiment for **5** owing to the small amount of pure compound available. Therefore, the ^{13}C resonances were assigned on the basis of the 1D-spectrum by comparison with the data for **1–4**.

EXPERIMENTAL

Fractionation of the oligosaccharides. — Phosphopeptidomannans were extracted according to the method described by Kocourek and Ballou[7], slightly modified since only a single extraction in citrate buffer was made. The acid-labile, phosphate-bound oligomannosides were obtained by mild hydrolysis in 10 mM HCl[8]. The short oligosaccharides (M-2 to M-7) were separated by paper chromatography (Whatman No. 3) with 5:5:1:3 pyridine–EtOAc–acetic acid–water as solvent.

NMR spectroscopy. — The 400-MHz ^1H NMR experiments were performed with a Bruker AM-400 WB spectrometer equipped with a 5-mm ^1H–^{13}C mixed probe-head, operating in the pulse FT mode and controlled by an Aspect 3000 computer. After three exchanges with ^2H_2O (99.95 atoms Aldrich) and intermediate lyophilizations, the products (concn ~ 50 mg/0.5 mL ^2H_2O) were analyzed with a spectral width of 3000 Hz for 16 K-frequency domain points and time-domain data points giving a final digital resolution of 0.365 Hz/point. The 100-MHz

TABLE II

^{13}C NMR chemical shifts

Compounds, units, and anomers	Chemical shifts (δ)					
	C-1	C-2	C-3	C-4	C-5	C-6
M-3 (1)						
C (α)	102.23	71.70	74.21	68.60	77.63	62.42
(β)	102.12	71.78	74.26	68.57	77.63	[a]
B (α)	100.28	79.85	73.44	68.24	77.63	62.00
(β)	102.32	79.41	73.37	68.28	77.46	[a]
A α	93.27	79.82	70.54	68.06	73.77	61.78
β	94.91	80.94	73.48	68.07	77.56	
M-4 (2)						
D (α)	102.29	71.71	74.28	68.11	77.51	62.14
(β)	102.26	71.71	74.28	68.14	[a]	[a]
C (α)	102.52	79.68	73.53	68.45	77.59	62.50
(β)	102.44	79.63	73.55	68.49	[a]	[a]
B (α)	100.51	80.71	73.19	68.30	77.42	61.91
(β)	102.46	80.31	73.23	68.30	[a]	[a]
A α	93.41	80.09	70.41	68.77	73.71	61.74
β	94.82	81.34	73.25	68.77	77.48	[a]
M-5 (3)						
E	102.26	71.73	74.24	68.09	77.48	62.19
D	102.70	79.77	73.28	68.54	77.44	62.47
C	102.42	80.29	73.61	68.35	77.44	61.96
B	100.52	80.90	73.09	68.40	77.38	61.83
A α	93.40	80.09	70.44	68.77	73.73	61.75
β	94.84	81.36	[a]	68.77	[a]	[a]
M-6 (4)						
F	102.28	71.74	74.26	68.10	77.45	62.19
E	102.70	80.52	73.20	68.54	77.45	62.48
D	102.42	79.81	73.37	68.54	77.45	62.08
C	100.62	80.40	73.57	68.54	77.45	62.08
B	100.53	80.92	73.11	68.36	77.30	61.90
A α	93.40	80.09	70.44	68.83	73.74	61.79
β	94.86	81.37	[a]	68.83	[a]	[a]
M-7 (5)						
C	102.32	71.77	74.28	68.13	77.48	62.22
F	102.77	80.64	73.23	68.52	77.48	62.51
E	102.65	80.55	73.30	68.66	77.48	62.07
D	102.48	79.84	73.36	68.62	77.48	62.01
C	100.65	80.47	73.60	68.55	77.48	61.93
B	100.57	80.93	73.13	68.40	77.28	61.89
A α	93.44	80.12	70.47	68.85	73.76	61.82
β	94.90	81.40	[a]	[a]	[a]	[a]

[a] Not determined.

C B A
β-D-Man*p*-(1 → 2)-[β-D-Man*p*-(1 → 2)]$_n$-D-Man

1 (M-3) $n = 1$ C → B → A

2 (M-4) $n = 2$ D → C → B → A

3 (M-5) $n = 3$ E → D → C → B → A

4 (M-6) $n = 4$ F → E → D → C → B → A

5 (M-7) $n = 5$ G → F → E → D → C → B → A

^{13}C NMR experiments were performed with the standard Bruker pulse-program POWGATE with ^{1}H broad-band, composite-pulse decoupling. The spectral width was 23000 Hz for 32 K frequency-domain points and time-domain data giving a final digital resolution of 1.387 Hz/point. A 90° pulse (6μs) and a 1-s recycle delay were used. The chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured relative to the methyl signal of internal acetone (δ 2.225 for ^{1}H and δ 31.55 for ^{13}C) for a solution in $^{2}H_2O$ at 300 K.

The 2D homonuclear COSY 45 experiments were performed with the standard Bruker pulse-program COSY. In these experiments, the spectral width was 1800

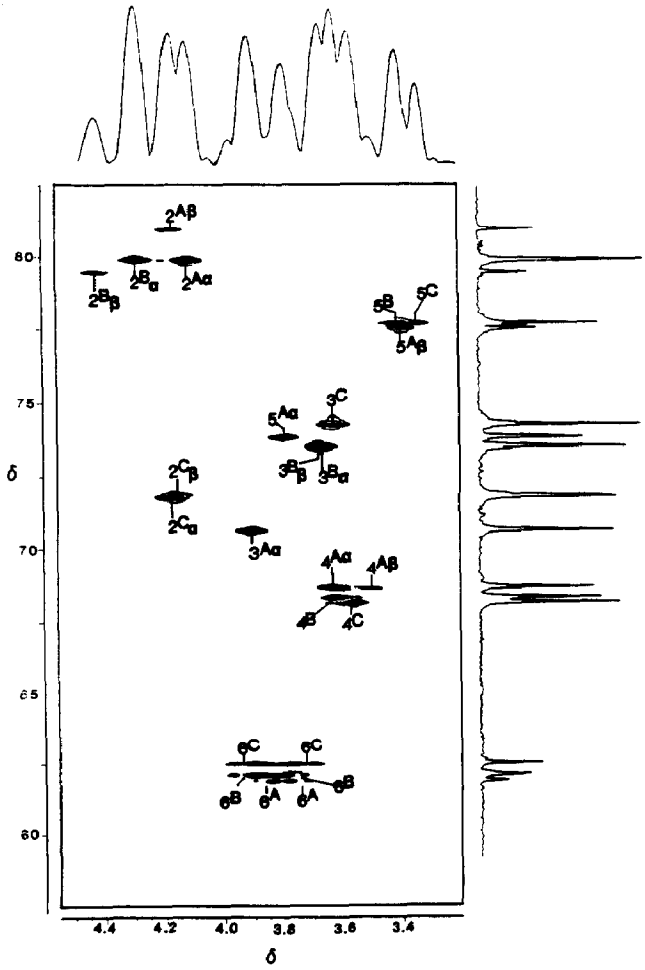

Fig. 1. ^{1}H–^{13}C COSY spectra of oligosaccharide M-3 (1).

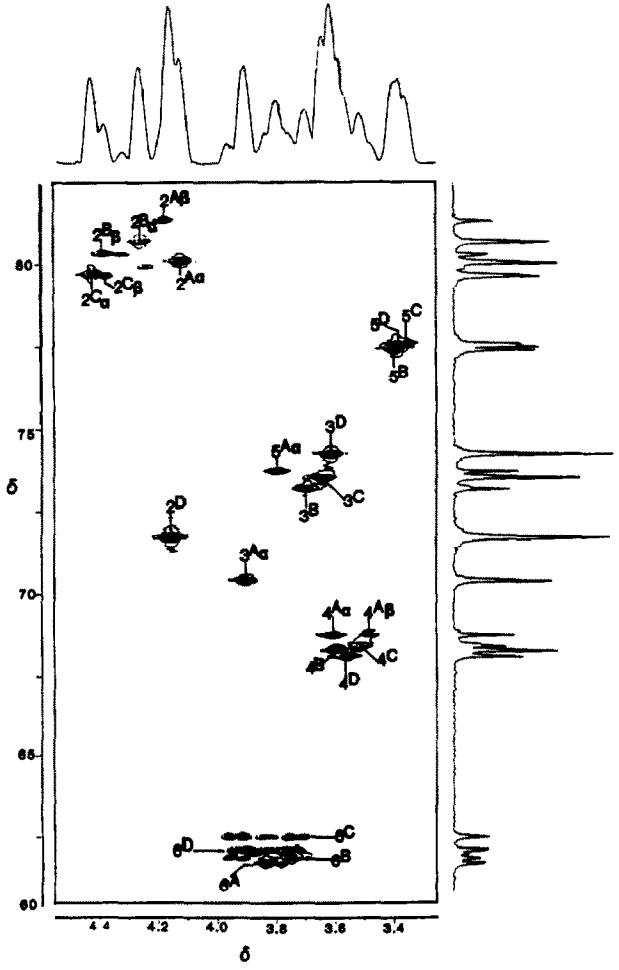

Fig. 2. $^{1}H-^{13}C$ COSY spectra of oligosaccharide M-4 (2).

Hz. The ^{1}H-90° pulse was 10.6 μs. 256 W × 2 K data matrices were acquired which were zero-filled prior to FT to obtain a 1 K × 2 K spectral data matrix; a sine-bell squared function was used in both dimensions.

The 2D homonuclear COSY with simple- and double-relay transfers was performed with the standard Bruker pulse-program COSYRCT and the pulse-program COSYDR[9]. For example, the COSYDR experiment was performed with the sequence, D_1-90-D_ϕ-90-D_2-180-D_2-90-D_3-180-D_3-90-fid, where $D_1 = 2$ s, 90,180 = 90°, 180° 1H pulse (90° = 10.6 μs), D_ϕ = incremental delay (initial = 3 μs), and $D_2 = D_3 = 35$ ms. In all experiments for a spectral width of 1800 Hz, 256 W × 2 K data matrices were obtained, which were zero-filled to 1 K × 2 K prior to FT; a sine-bell squared function was used in both dimensions.

The 2D heteronuclear-correlated experiments were performed with simultaneous ^{1}H broad-band decoupling using the standard Bruker pulse-program XH-

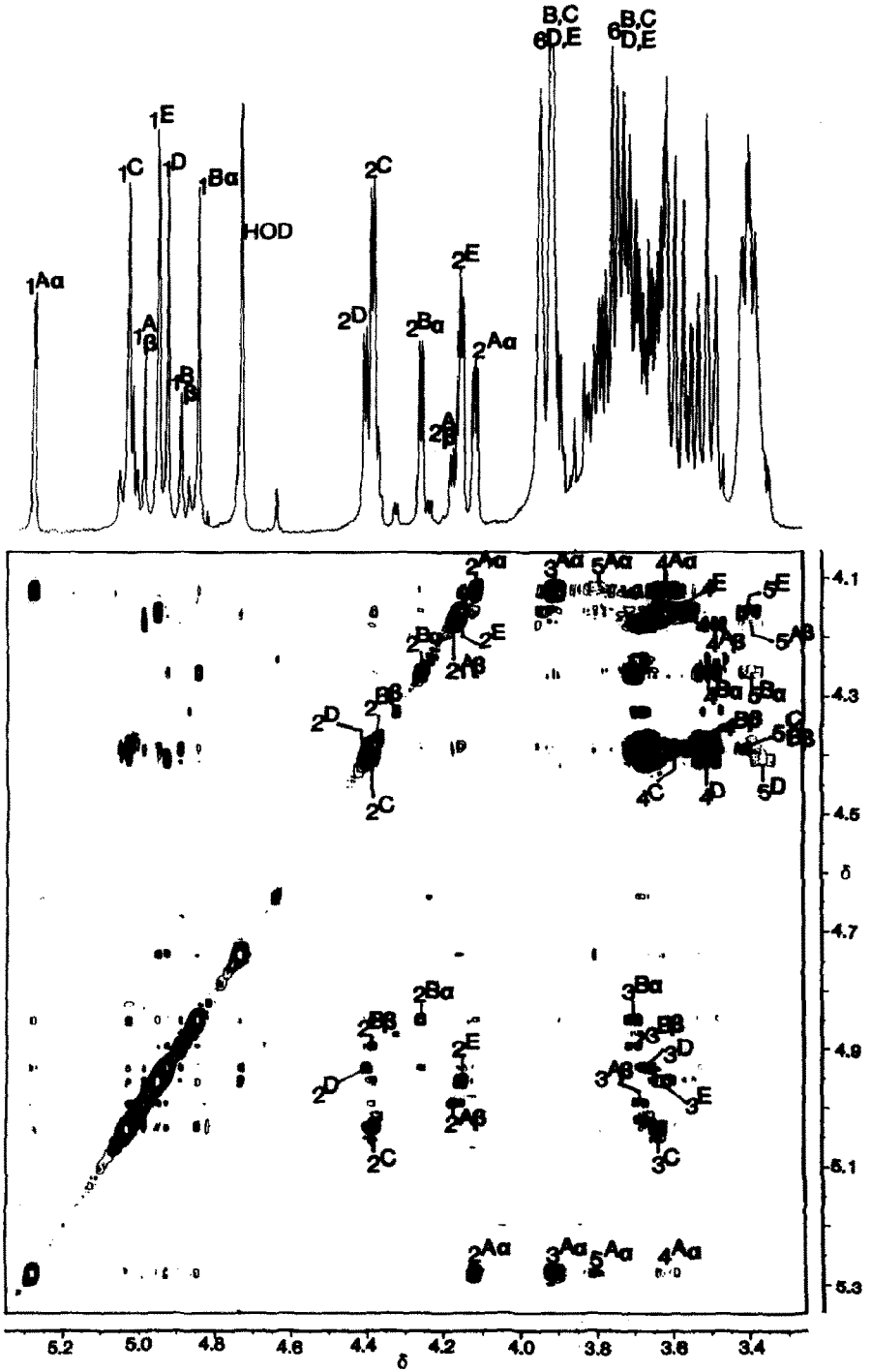

Fig. 3. Double-relayed COSY spectrum of oligosaccharide M-5 (3).

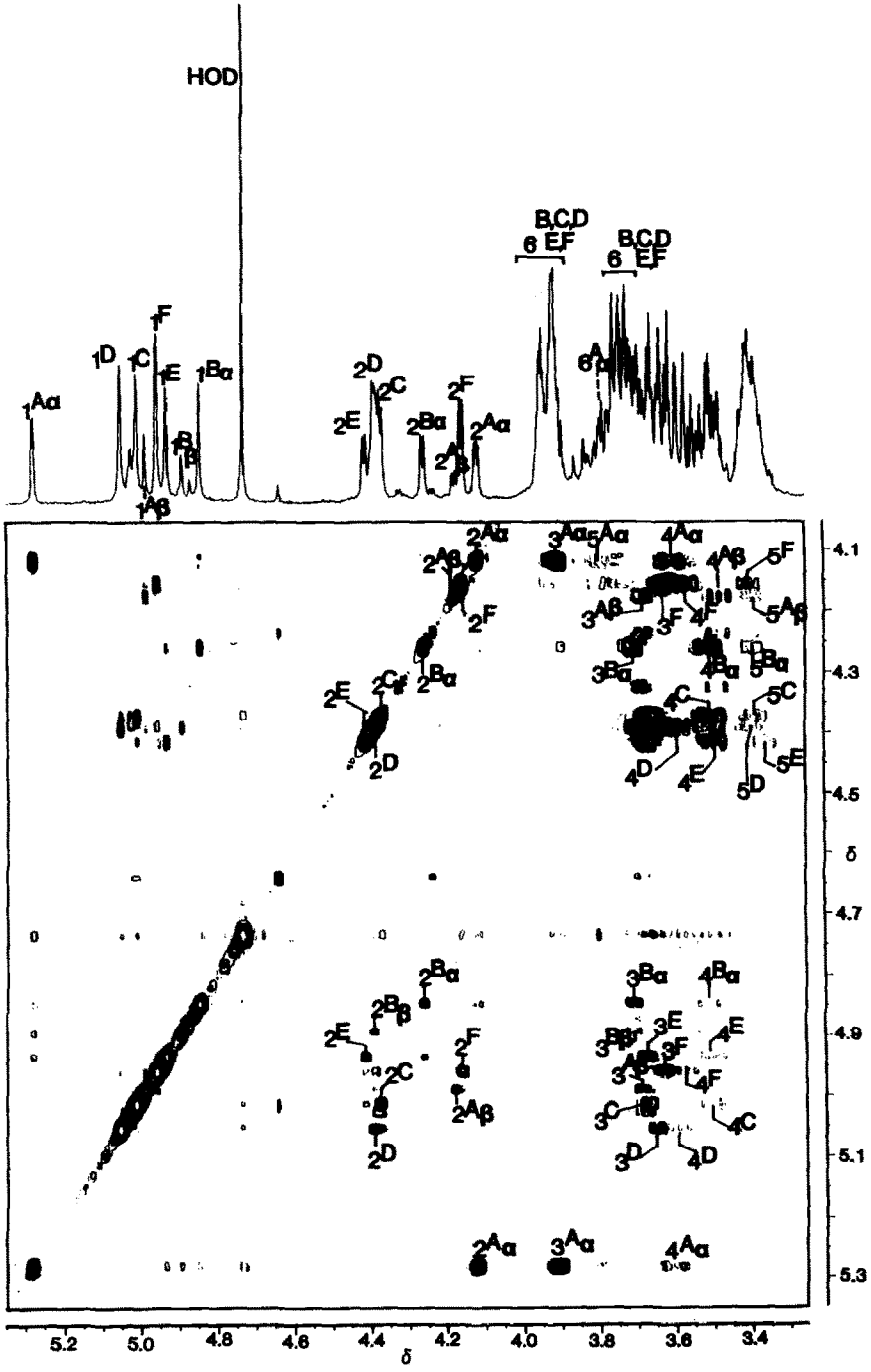

Fig. 4. Double-relayed COSY spectrum of oligosaccharide M-6 (4).

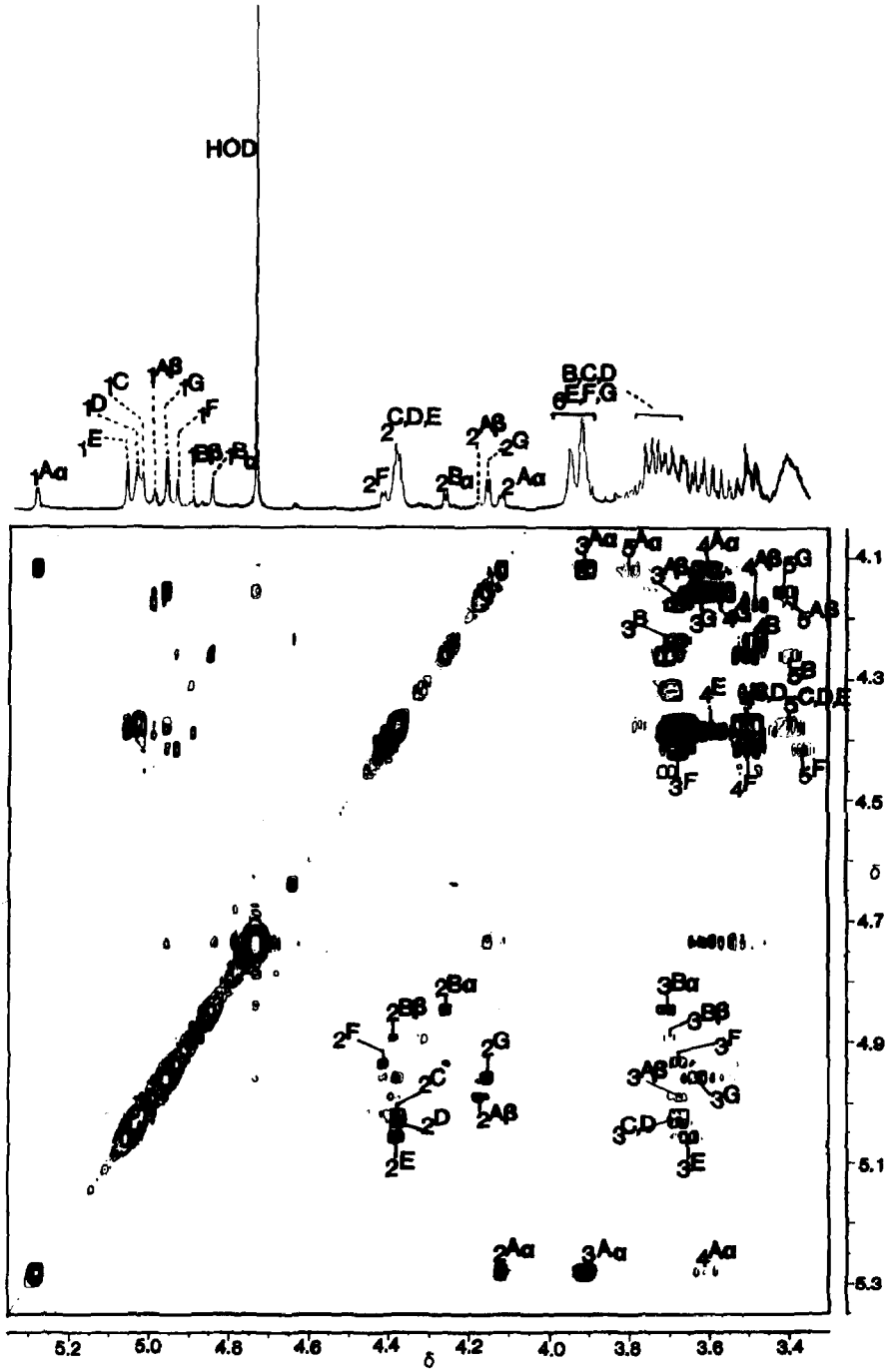

Fig. 5. Double-relayed COSY spectrum of oligosaccharide M-7 (5).

CORRD. Refocusing delays were adjusted to an average $J_{C,H}$ 142 Hz. Spectral windows of 10000 Hz with 4096 data points for ^{13}C, and 900 Hz with 128 data points for ^{1}H were employed. The ^{1}H- and ^{13}C-90° pulse width was 10.6 and 6 μs, respectively. A 128 W × 4 K data matrix was acquired which was zero-filled prior to FT to obtain a 512 W × 4 K spectral data matrix. The F1 domain was multplied by a sine-bell function and the F2 domain by a line-broadening function (LB = 1 Hz) prior to processing.

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