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Supplementary data

Production of levan by *Bacillus licheniformis* NS032 in sugar beet molasses-based medium

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Content:

1. Table S1: Box Behnken experimental design with actual independent variables and observed and calculated responses.

2. Table S2: Analysis of variance (ANOVA) for response surface quadratic model.

3. Table S3: Fragmentation pattern for the derivatives produced by reductive cleavage.

4. Fig. S1: ^{13}C NMR spectra of levan LM (a), LS1 (b) and LS2 (c).

5. Fig. S2: ^{1}H NMR spectra of levan LM (a), LS1 (b) and LS2 (c).

6. Fig. S3: 2D NMR spectra COSY (a), HSQC (b), HMBC (c) of levan LM.

7. Fig. S4: 2D NMR spectra COSY (a), HSQC (b), HMBC (c) of levan LS2.

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Table S1

Box Behnken experimental design with actual independent variables and observed

and calculated responses.

	Variables			Response	
Run	X_1	X_2	X_3		
	Molasses percentage,%	Phosphate, g/L	Initial pH	Levan observed, g/L	Levan predicted, g/L
1	50	9.0	6.75	51.18	48.39
2	62.5	6.0	6.75	41.98	48.20
3	62.5	3.0	6.0	8.11	6.46
4	62.5	9.0	6.0	16.19	16.17
5	75.0	6.0	6.0	6.92	5.79
6	62.5	6.0	6.75	49.45	48.20
7	75.0	3.0	6.75	38.84	41.62
8	75.0	9.0	6.75	18.52	19.67
9	62.5	6.0	6.75	44.85	48.20
10	62.5	6.0	6.75	52.85	48.20
11	50.0	6.0	6.0	6.53	9.33
12	62.5	6.0	6.75	51.87	48.20
13	62.5	9.0	7.5	33.8	35.60
14	50.0	3.0	6.75	33.95	32.80
15	50.0	6.0	7.5	46.94	48.07
16	62.5	3.0	7.5	51.65	51.67
17	75.0	6.0	7.5	34.5	31.68

Table S2

Analysis of variance (ANOVA) for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4472.56	9	496.95	26.98	0.0001	significant
X_1-Molasses percentage	198.25	1	198.25	10.76	0.0135*	
X_2-Phosphate	20.66	1	20.19	1.12	0.3247	
X_3-pH	2084.48	1	2084.48	113.17	< 0.0001*	
X_1X_2	352.45	1	352.45	19.14	0.0033*	
X_1X_3	41.18	1	41.18	2.24	0.1785	
X_2X_3	168.10	1	168.10	9.13	0.0194*	
X_1^2	279.41	1	279.41	15.17	0.0059*	
X_2^2	82.79	1	82.79	4.49	0.0717	
X_3^2	1122.55	1	1122.55	60.95	0.0001*	
Residual	128.93	7	18.42			
Lack of Fit	42.30	3	14.10	0.65	0.6227	not significant
Pure Error	86.63	4	21.66			
Cor Total	4601.21	16				
R-Squared:	0.9720					
Adj R-Squared:	0.9360					
Pred R-Squared:	0.8235					

Equation:
$Y = -1967.88189 + 9.93075X_1 + 40.46968X_2 + 452.07393X_3 - 0.25032X_1X_2 - 0.34224X_1X_3 - 2.88122X_2X_3 - 0.052136X_1^2 - 0.49269X_2^2 - 29.02771X_3^2$

* Significant "Prob>F" <0.05

Table S3

Fragmentation pattern for the derivatives produced by reductive cleavage.

Peak		Rt	Diagnostic fragments, m/z	Linkage indicated
1	1,5-anhydro-2,3,4,6-tetra-O-methyl-D-glucitol	16.307	101(100)[*],71(73),45(44), 75 (44),88(28)	ter- Glc$p$
2	2,5-anhydro-1,3,4,6-tetra-O-methyl-D-mannitol	16.380	45(100),101(75),71(58),143(53), 89 (39), 99(34), 115 (32)	ter-Fru$f$
3	2,5-anhydro-1,3,4,6-tetra-O-methyl-D-glucitol	16.545	101(100),45(78),71(40), 143(37),89(30)	ter-Fru$f$
4	6-O-acetyl-2,5-anhydro-1,3,4-tri-O-methyl-D-mannitol	18.524	43(100),71(95),101(79),45(76), 111(66), 143 (56),115(41),87(40)	(2→6)-Fru$f$
5	6-O-acetyl-2,5-anhydro-1,3,4-tri-O-methyl-D-glucitol	18.738	101(100),43(81),71(74),117(72), 45(66),111(62),87(45),143(37)	(2→6)-Fru$f$
6	1,6-di-O- acetyl-2,5-anhydro-3,4-di-O-methyl-D-mannitol	20.399	43(100),87(62),71(45),111(31), 117(29),101(23)	(1,2→6)-Fru$f$
7	1,6-di-O- acetyl-2,5-anhydro-3,4-di-O-methyl-D-glucitol	20.699	43(100),87(56),117(46),71(41), 101(29) 111(19)	(1,2→6)-Fru$f$

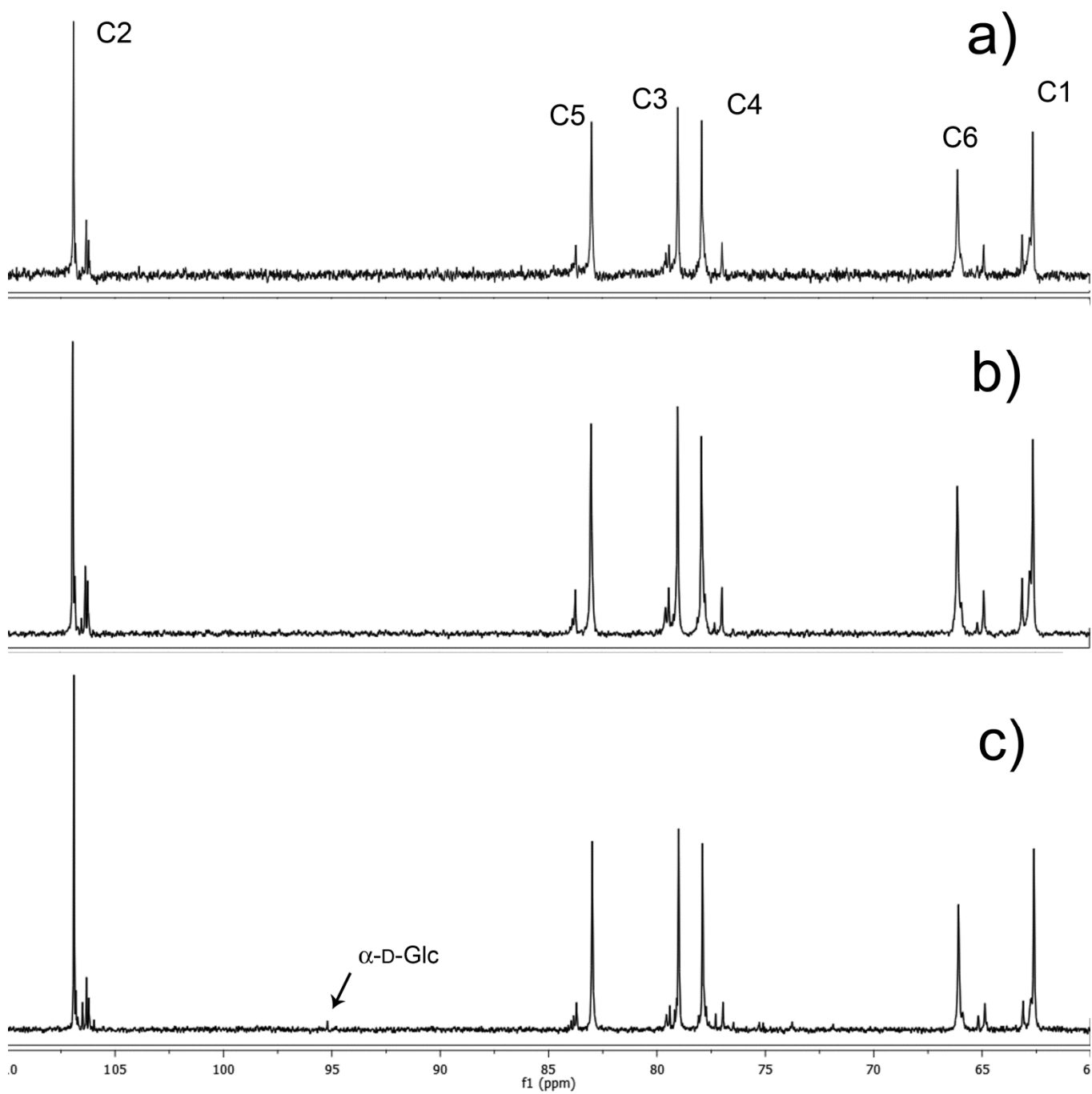*numbers in parentheses indicated abundance.

Fig. S1. ^{13}C NMR spectra of levan LM (a), LS1 (b) and LS2 (c).

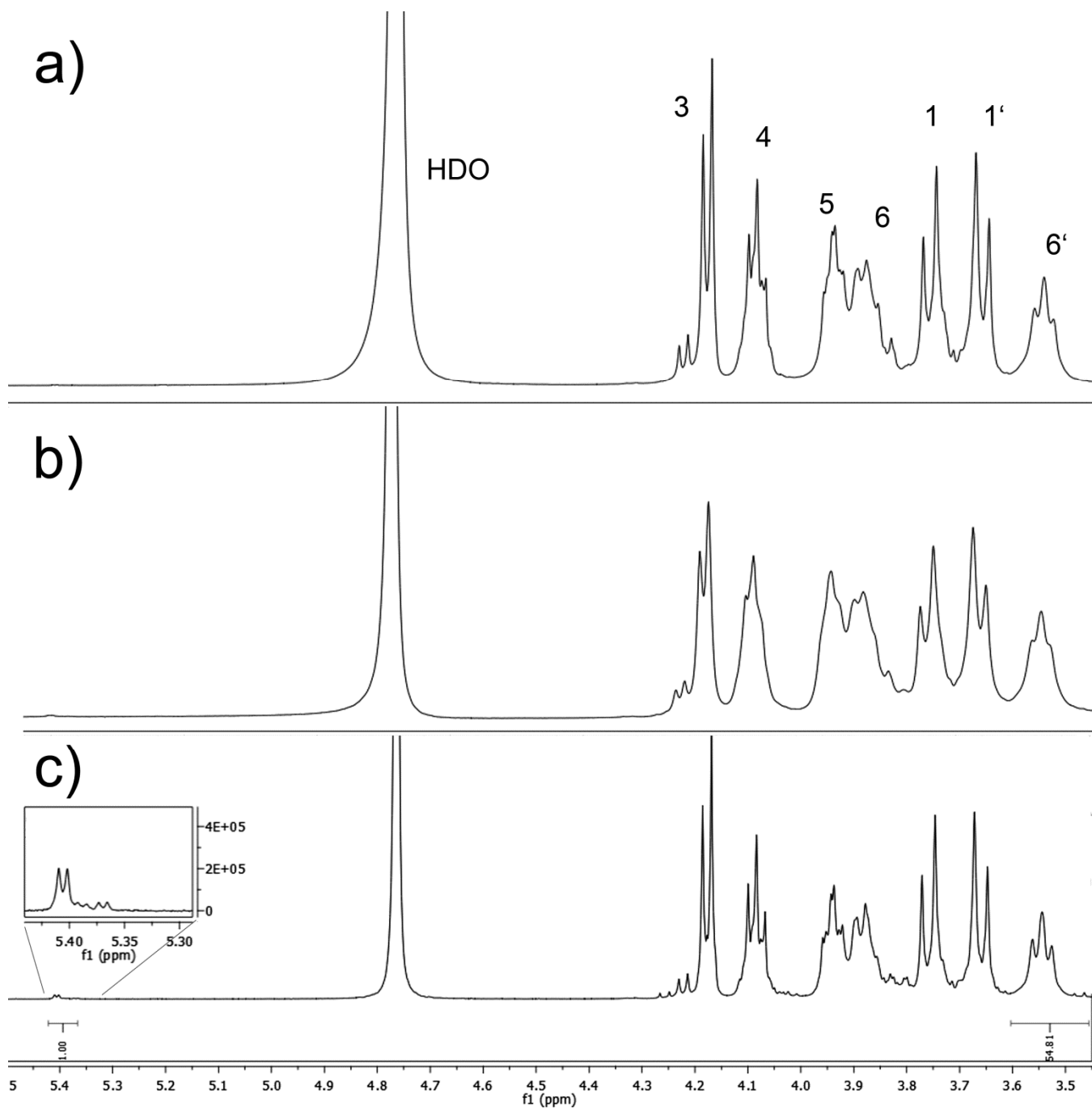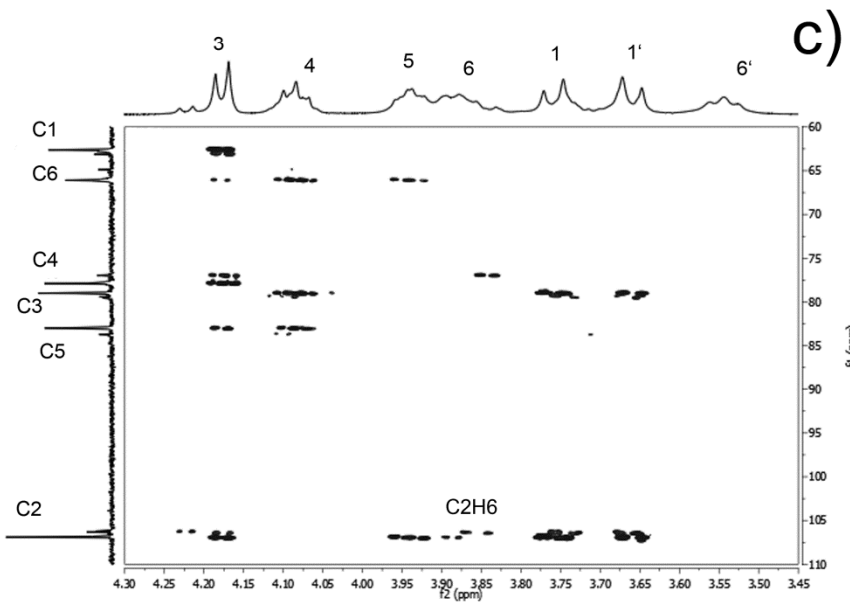**Fig. S2.** ¹H NMR spectra of levan LM (a), LS1 (b) and LS2 (c).
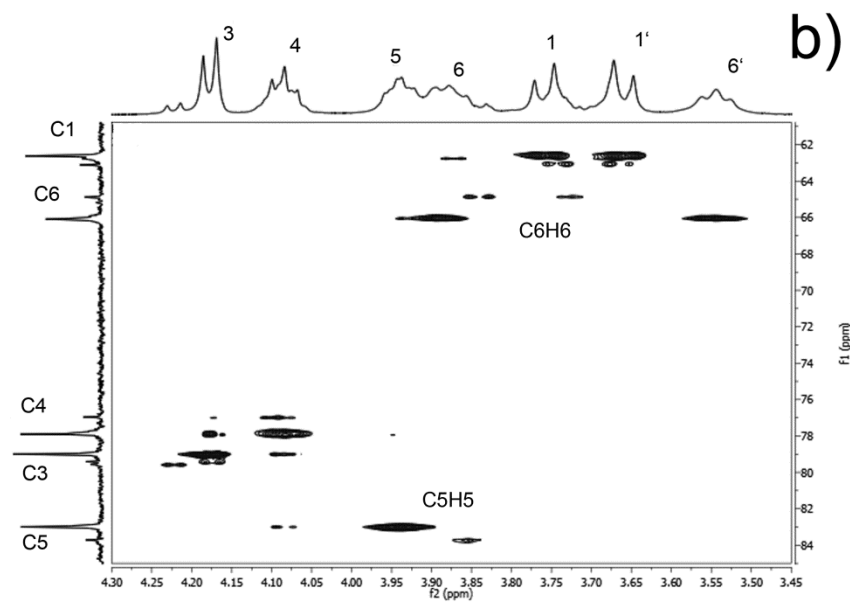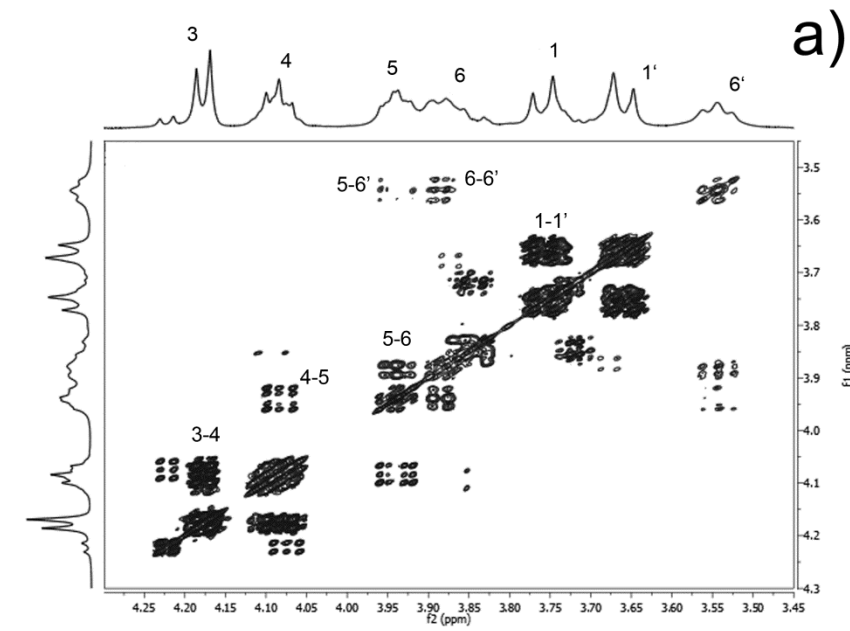
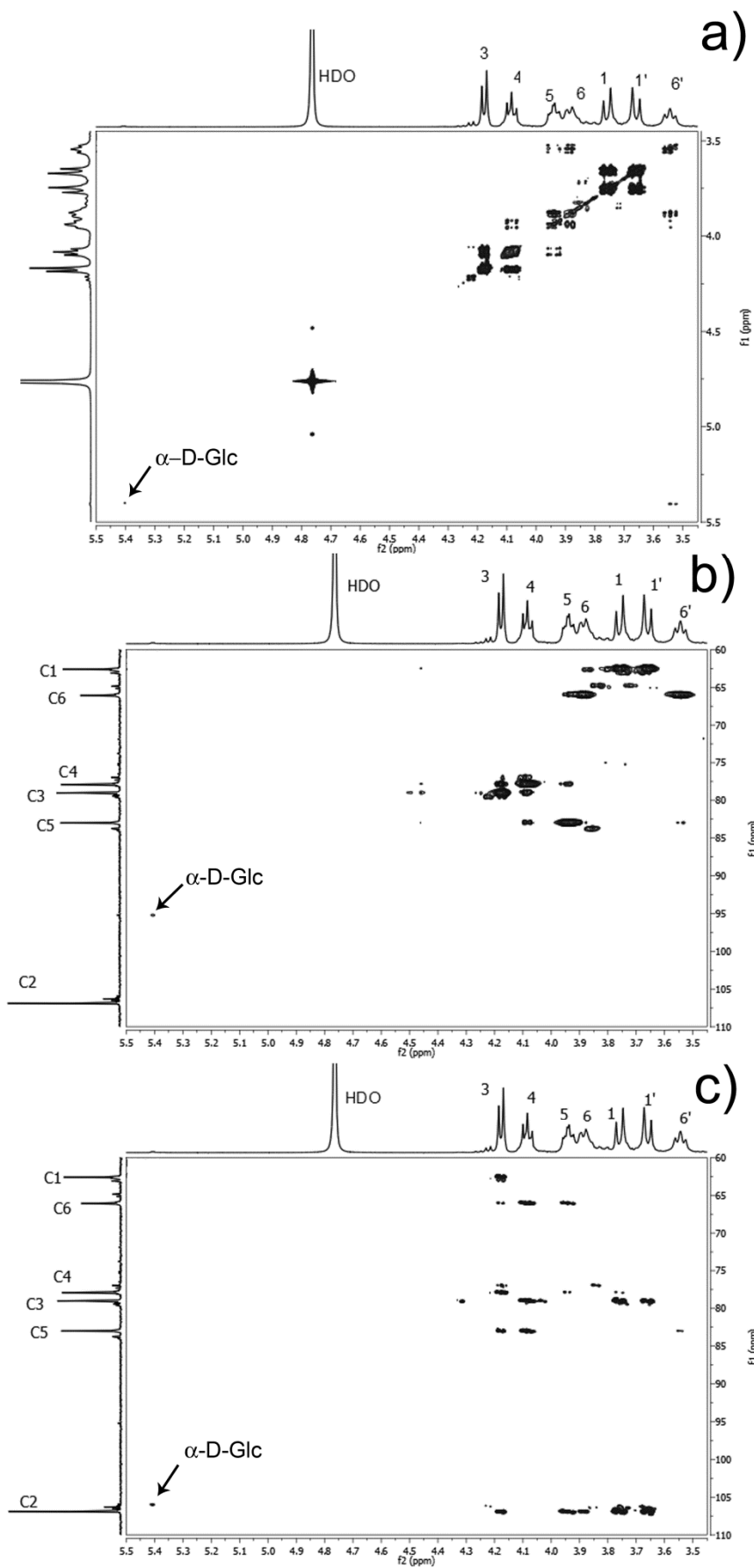**Fig. S3. 2D NMR spectra COSY (a), HSQC (b), HMBC (c) of levan LM.**

Fig. S4. 2D NMR spectra COSY (a), HSQC (b), HMBC (c) of levan LS2.