

Supplementary data for the article:

Gojgic-Cvijovic, G. D.; Jakovljevic, D. M.; Loncarevic, B. D.; Todorovic, N. M.; Pergal, M. V.; Ciric, J.; Loos, K.; Beškoski, V.; Vrvic, M. M. Production of Levan by *Bacillus Licheniformis* NS032 in Sugar Beet Molasses-Based Medium. *International Journal of Biological Macromolecules* **2019**, *121*, 142–151. <https://doi.org/10.1016/j.ijbiomac.2018.10.019>

## Supplementary data

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

G.D. Gojgic-Cvijovic<sup>a\*</sup>, D.M. Jakovljevic<sup>a</sup>, B.D. Loncarevic<sup>a</sup>, N.M. Todorovic<sup>a</sup>, M.V. Pergal<sup>a</sup>, J. Ciric<sup>b</sup>, K. Loos<sup>c</sup>, V.P. Beskoski<sup>d</sup>, M.M. Vrvic<sup>d</sup>

<sup>a</sup> Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoseva 12, Belgrade, Serbia

<sup>b</sup> Charles River Laboratories, Den Bosch, The Netherlands

<sup>c</sup> Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands

<sup>d</sup> Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

#### 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: <sup>13</sup>C NMR spectra of levan LM (a), LS1 (b) and LS2 (c).
5. Fig. S2: <sup>1</sup>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.

---

\* Author for correspondence: [ggojgic@chem.bg.ac.rs](mailto:ggojgic@chem.bg.ac.rs)

Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoseva 12,

P.O. Box 473, 11000 Belgrade, Serbia

Tel: +381 11 2637273, Fax: +381 11 2636061

**Table S1**

Box Behnken experimental design with actual independent variables and observed  
and calculated responses.

Run	Variables			Response	
	X <sub>1</sub> Molasses percentage, %	X <sub>2</sub> Phosphate, g/L	X <sub>3</sub> 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 <sub>1</sub> -Molasses percentage	198.25	1	198.25	10.76	0.0135*	
X <sub>2</sub> -Phosphate	20.66	1	20.19	1.12	0.3247	
X <sub>3</sub> -pH	2084.48	1	2084.48	113.17	< 0.0001*	
X <sub>1</sub> X <sub>2</sub>	352.45	1	352.45	19.14	0.0033*	
X <sub>1</sub> X <sub>3</sub>	41.18	1	41.18	2.24	0.1785	
X <sub>2</sub> X <sub>3</sub>	168.10	1	168.10	9.13	0.0194*	
X <sub>1</sub> <sup>2</sup>	279.41	1	279.41	15.17	0.0059*	
X <sub>2</sub> <sup>2</sup>	82.79	1	82.79	4.49	0.0717	
X <sub>3</sub> <sup>2</sup>	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 <sub>1</sub> + 40.46968X <sub>2</sub> + 452.07393X <sub>3</sub> -0.25032X <sub>1</sub> X <sub>2</sub> - 0.34224X <sub>1</sub> X <sub>3</sub> -2.88122X <sub>2</sub> X <sub>3</sub> -0.052136X <sub>1</sub> <sup>2</sup> -0.49269X <sub>2</sub> <sup>2</sup> -29.02771X <sub>3</sub> <sup>2</sup>					

\* Significant "Prob&gt;F" &lt;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- Glcp
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-Fruf
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-Fruf
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)-Fruf
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)-Fruf
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)-Fruf
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)-Fruf

\*numbers in parentheses indicated abundance.

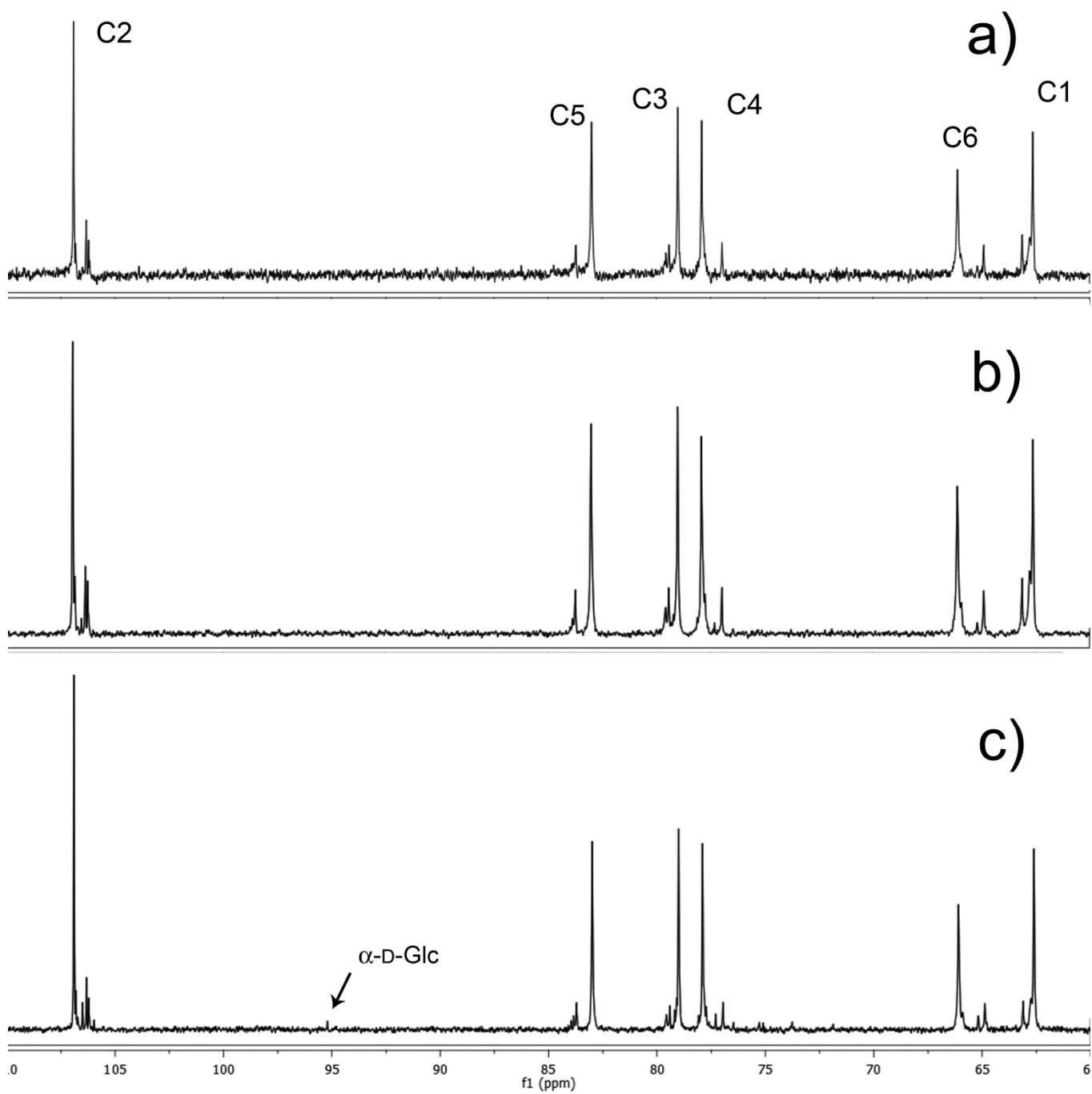


Fig. S1.  $^{13}\text{C}$  NMR spectra of levan LM (a), LS1 (b) and LS2 (c).

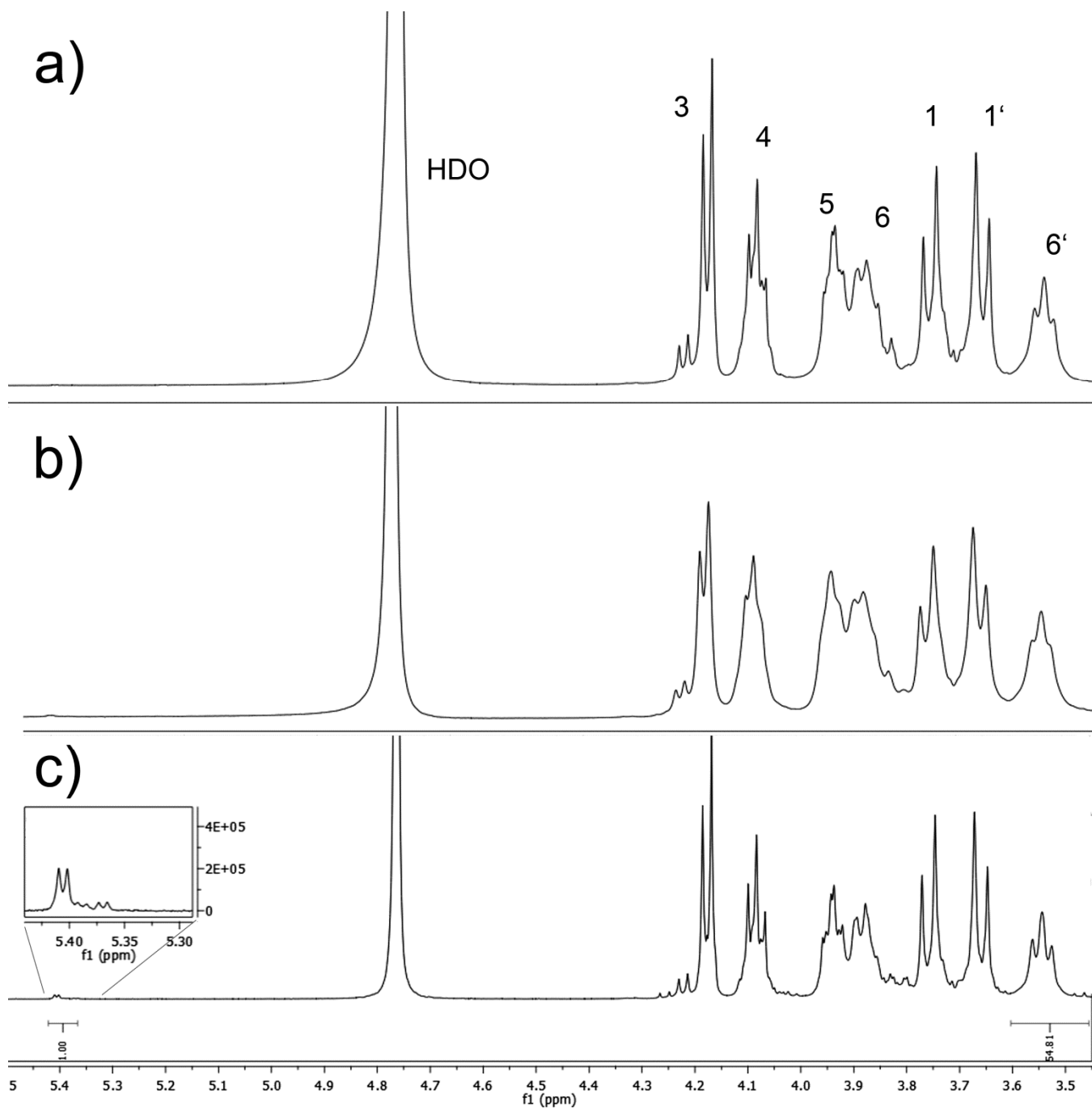


Fig. S2.  $^1\text{H}$  NMR spectra of levan LM (a), LS1 (b) and LS2 (c).

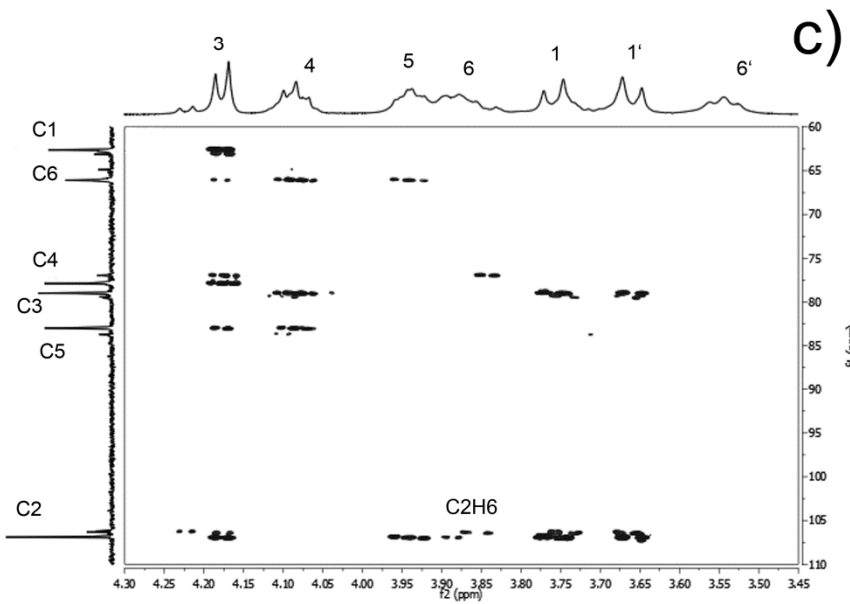
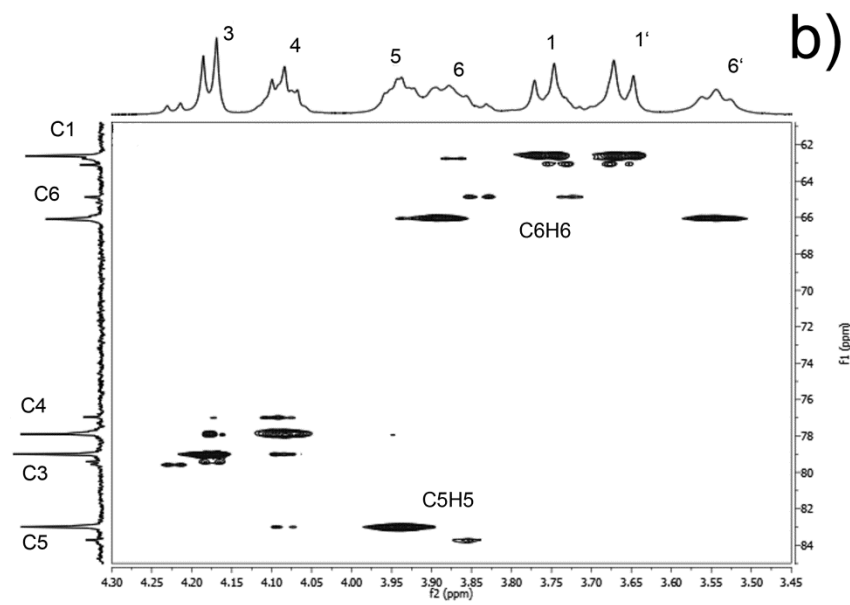
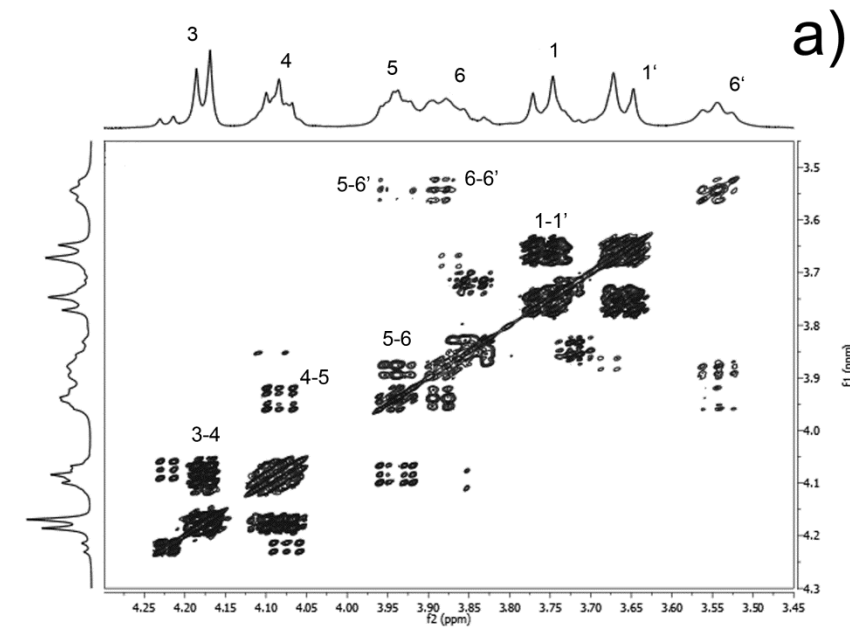


Fig. S3. 2D NMR spectra COSY (a), HSQC (b), HMBC (c) of levan LM.



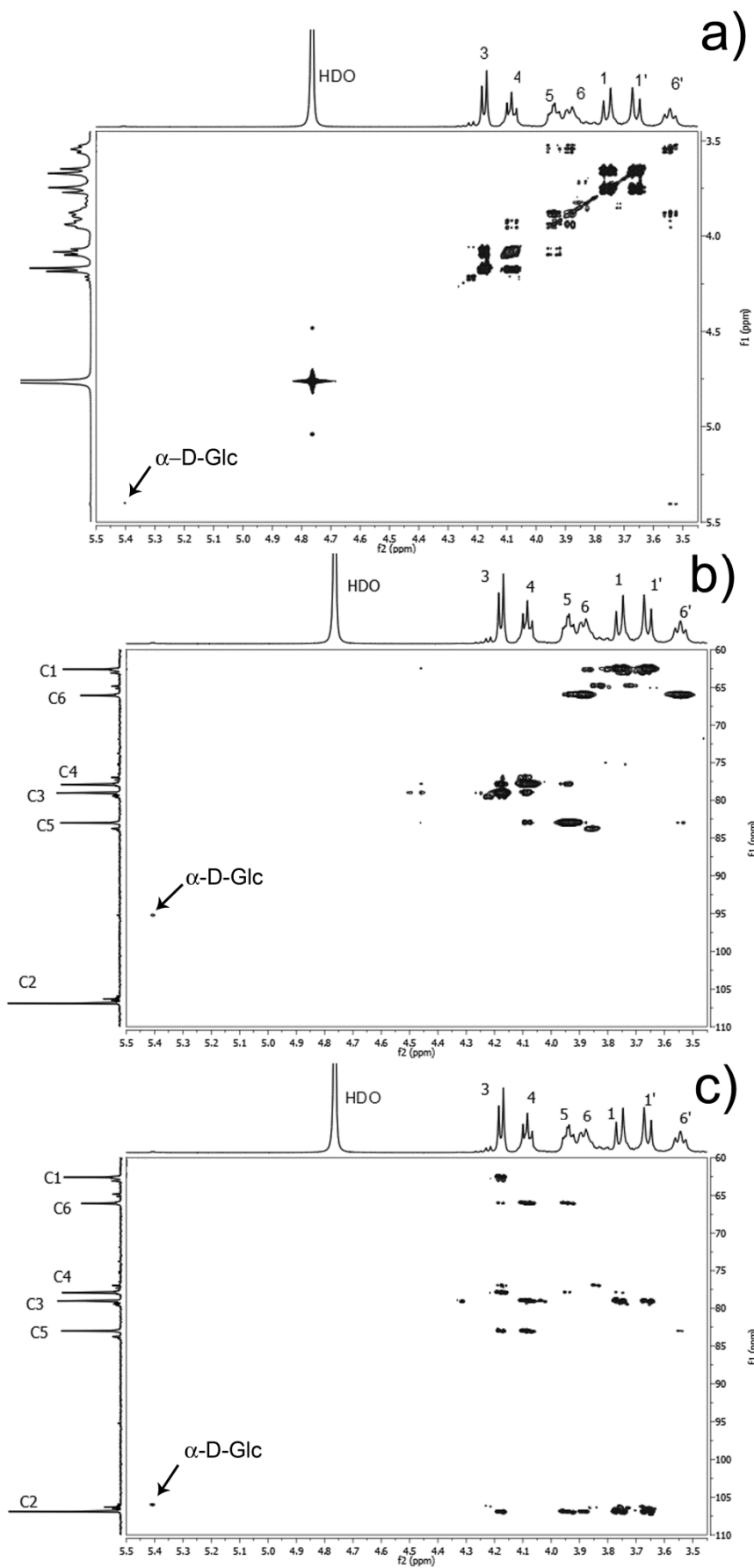


Fig. S4. 2D NMR spectra COSY (a), HSQC (b), HMBC (c) of levans LS2.