Comprehensive Signal Assignment of ^{13}C-Labeled Lignocellulose using Multidimensional Solution NMR and ^{13}C Chemical Shift Comparison with Solid-State NMR

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Table S1. Assignment references and notes

	residues	references [reference no.]	assignment note
cello-oligosaccharide	1,4) β-D-Glc*p*	(Kim and Ralph. 2010) 103.1/ 4.39 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-6 were identified in HCCH-COSY
	1,4)β-D-Glc*p* (non reducing end)	cellotetraose, cellotriose, cellobiose	C/H1-6 were identified in ^{13}C-HSQC-TOCSY.
	1,4) β-D-Glc*p* (reducing end)	(Kim and Ralph. 2010) 97.1/ 4.44 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
	1,4) α-D-Glc*p* (reducing end)	(Kim and Ralph. 2010) 92.2/ 5.10 ppm (δ_{13C_1}/ δ_{1H_1}) [1]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
xylans	1,4) β-D-Xyl*p*	(Kim and Ralph. 2010) 101.8/ 4.32 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-5 were identified in HCCH-COSY
	1,4) β-D-Xyl*p* (non reducing end)	Xylooligosaccharide	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
	1,4) β-D-Xyl*p* (reducing end)	(Kim and Ralph. 2010) 97.5/ 4.38 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
	1,4) α-D-Xyl*p* (reducing end)	(Kim and Ralph. 2010) 92.4/ 5.10 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
	3-O-Xyl*p*-substituted 1,4) β-D-Xyl*p*	(Hoije *et al.* 2006) 102.9/4.527 (δ_{13C_1} / δ_{1H_1}), 74.4/3.484 (δ_{13C_2} / δ_{1H_2}), 78.9/3.752 (δ_{13C_3} / δ_{1H_3}), 75.0/3.803 (δ_{13C_4} / δ_{1H_4}), 64.4/4.161 ppm (δ_{13C_5} / δ_{1H_5})*	C/H1-5 were identified in ^{13}C-HSQC-TOCSY and ^{13}C-HSQC-NOESY.
	1,3) α-L-Ara*f*	(Hoije *et al.* 2006) 108.8/5.406 (δ_{13C_1} / δ_{1H_1}), 82.3/4.173 (δ_{13C_2} / δ_{1H_2}), 78.6/3.929 (δ_{13C_3} / δ_{1H_3}), 85.8/4.184 (δ_{13C_4} / δ_{1H_4}), 62.6/3.802, 3.730 ppm (δ_{13C_5} / δ_{1H_5})* [2]	C/H1-5 were identified in HCCH-COSY
	2-O-Xyl*p*-substituted 1,3) α-L-Ara*f*	(Hoije *et al.* 2006) 107.5/5.539 (δ_{13C_1} / δ_{1H_1}), 90.02/4.287 (δ_{13C_2} / δ_{1H_2}), 77.2/4.092 (δ_{13C_3} / δ_{1H_3}), 85.6/4.26 (δ_{13C_4} / δ_{1H_4}), 62.6/3.815, 3.736 ppm (δ_{13C_5} / δ_{1H_5})* [2]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
	1,2) α-L-Ara*f*	(Pitkanen *et al.* 2008) 5.30 ppm (δ_{1H_1})* [3]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
xyloglucans	1,6) α-L-Xyl*p*	(Jia *et al.* 2003) 101.6/4.959 (δ_{13C_1} / δ_{1H_1}), 74.2/3.543 (δ_{13C_2} / δ_{1H_2}), 75.7/3.722 (δ_{13C_3} / δ_{1H_3}), 72.2/3.618 (δ_{13C_4} / δ_{1H_4}), 64.3/3.571, 3.733 ppm (δ_{13C_5} / δ_{1H_5})** [4]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
	1,3) α-L-Ara*f*	(Jia *et al.* 2003) 111.9/5.169 (δ_{13C_1} / δ_{1H_1}), 83.9/4.199 (δ_{13C_2} / δ_{1H_2}), 79.1/3.934 (δ_{13C_3} / δ_{1H_3}), 86.4/4.084 (δ_{13C_4} / δ_{1H_4}), 63.8/3.848, 3.712 ppm (δ_{13C_5} / δ_{1H_5})** [4]	C/H1-5 were identified in ^{13}C-HSQC-TOCSY.
starch	1,4) α-D-Glc*p*	(Kim and Ralph. 2010) 101.8/ 4.32 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-6 were identified in ^{13}C-HSQC-TOCSY.
	1,4) α-D-Glc*p*'	(Kim and Ralph. 2010) 101.8/ 4.32 ppm (δ_{13C_1} / δ_{1H_1}) [1]	C/H1-6 were identified in ^{13}C-HSQC-TOCSY.
lignin	β-O-4(S)*erythro*	(Kim and Ralph. 2010) 71.3/4.87 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$),*** 86.2/4.22 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 60.1/3.73, 3.40 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in HCCH-COSY. C/H2 and 6 were identified in ^{13}C-HSQC-NOESY.
	β-O-4(S)*threo*	(Kim and Ralph. 2010) 71.3/4.87 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$),*** 87.2/4.09 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 60.1/3.73, 3.40 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in HCCH-COSY.
	β-O-4(G)*erythro*	(Kim and Ralph. 2010) 71.3/4.87 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$),*** 83.9/4.45 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 60.1/3.73, 3.40 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in HCCH-COSY.
	β-O-4(G)*threo*	(Kim and Ralph. 2010) 71.3/4.87 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$),*** 84.4/4.39 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 60.1/3.73, 3.40 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in HCCH-COSY.
	β-5	(Kim and Ralph. 2010) 87.1/5.55 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$), 54.3/3.57 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 62.8/3.80 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in ^{13}C-HSQC-TOCSY.
	β-β	(Kim and Ralph. 2010) 85.0/4.67 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$), 53.7/3.06 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 71.0/3.77, 4.14 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in ^{13}C-HSQC-TOCSY.
	cinnamyl alcohol	(Kim and Ralph. 2010) 128.5/6.49 ($\delta_{13C\alpha}$/ $\delta_{1H\alpha}$), 128.7/6.38 ($\delta_{13C\beta}$/ $\delta_{1H\beta}$), 61.7/4.15 ppm ($\delta_{13C\gamma}$/ $\delta_{1H\gamma}$) [1]	C/Hα-γ were identified in ^{13}C-HSQC-TOCSY.

* These values were determined using isolated arabinoxylan from barley (*Hordeum vulgare*) in D_2O.

** These values were determined using oligoglycosyl alditols in D_2O. These values are slightly different from their structure.

*** These values do not be distinguished with *erythro* and *threo*.

Table S2. Correspondence of Samples to Hemicelluloses

	order	family	major hemicellulose in primary wall	major hemicellulose in secondary wall
potatoes (*Solanum tuberosum* L.)	Solanaceae	*Solanum*	arabinoxyloglucan	glucronoxylan
chicory (*Cichorium intybus*)	Asterales	*Asteraceae*	xyloglucan	glucronoxylan
corn (*Zea mays*)	Poales	*Poaceae*	β-1,3-1,4-glucan* & glucronoarabinoxylan	glucronoarabinoxylan

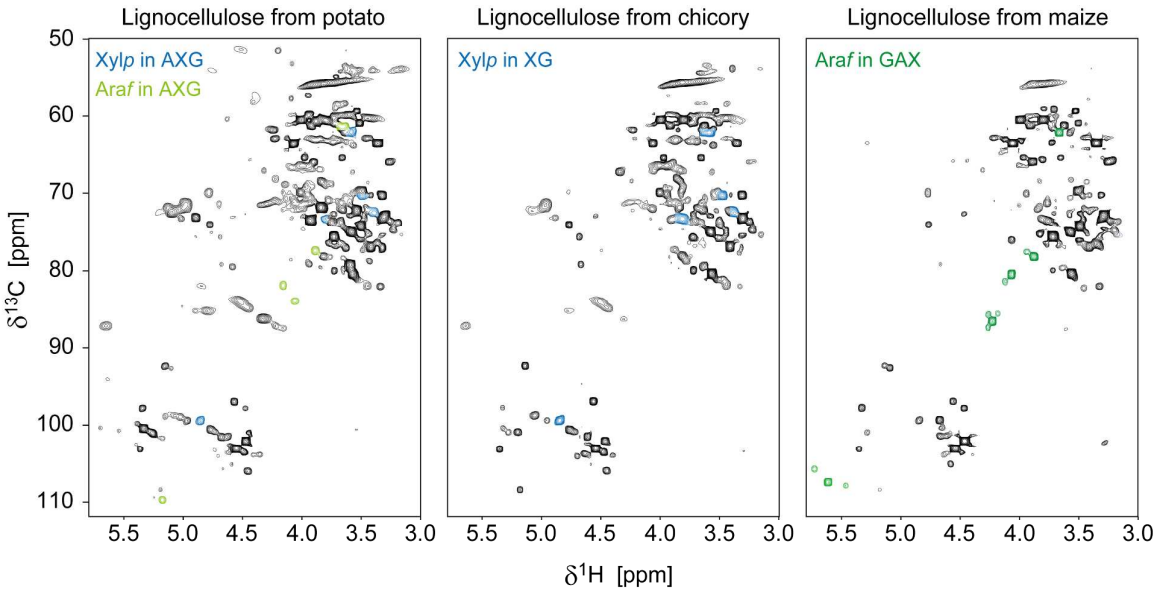

Figure S1. Characteristic hemicellulosic signals on ^{13}C-HSQC spectra

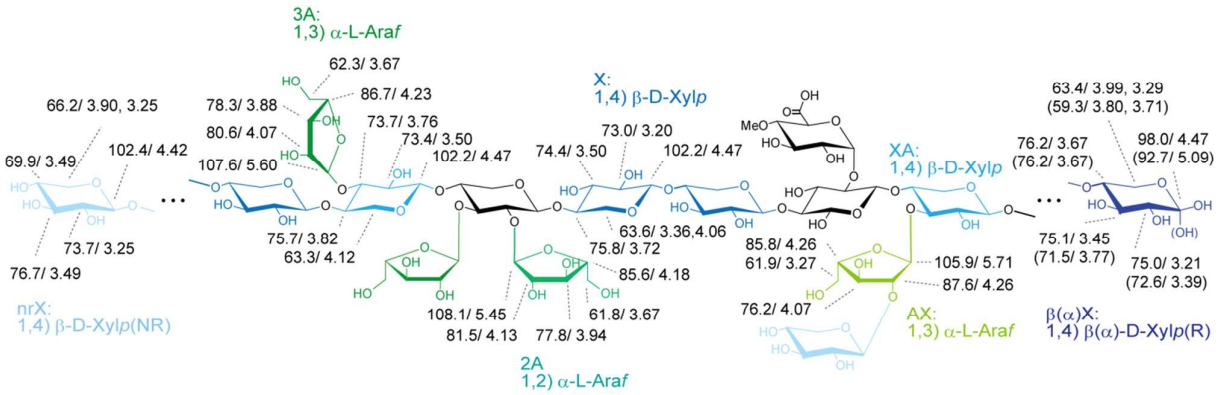

Figure S2. Partial structures of glucuronoarabinoxylan and their chemical shifts.

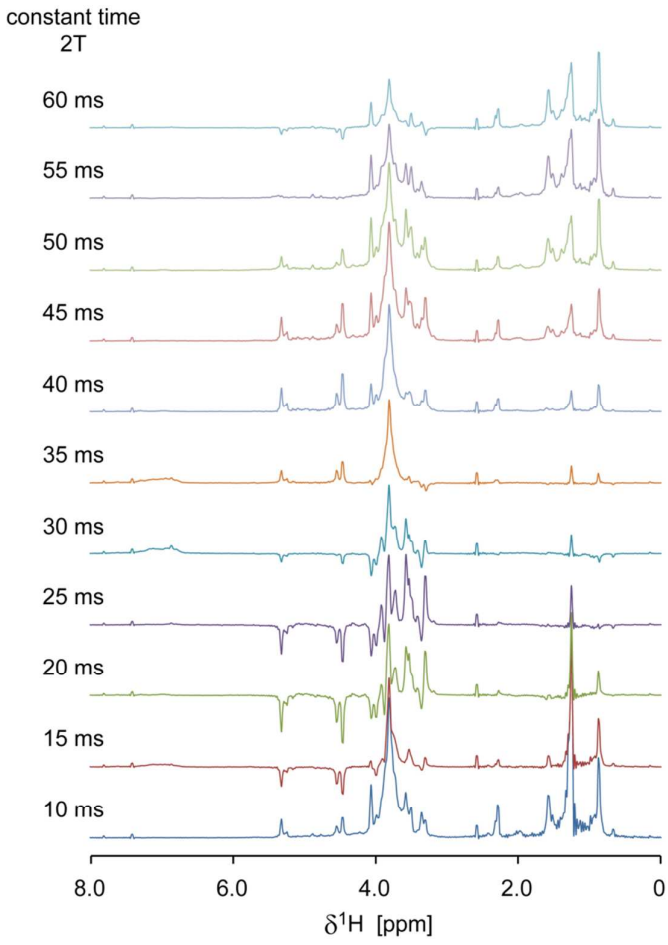

Figure S3. Constant time (2T) series of ^{1}H projections for the ct-HSQC spectra. Signal intensities changed as a function of $\Pi\cos(2T\pi J_{CC})$.

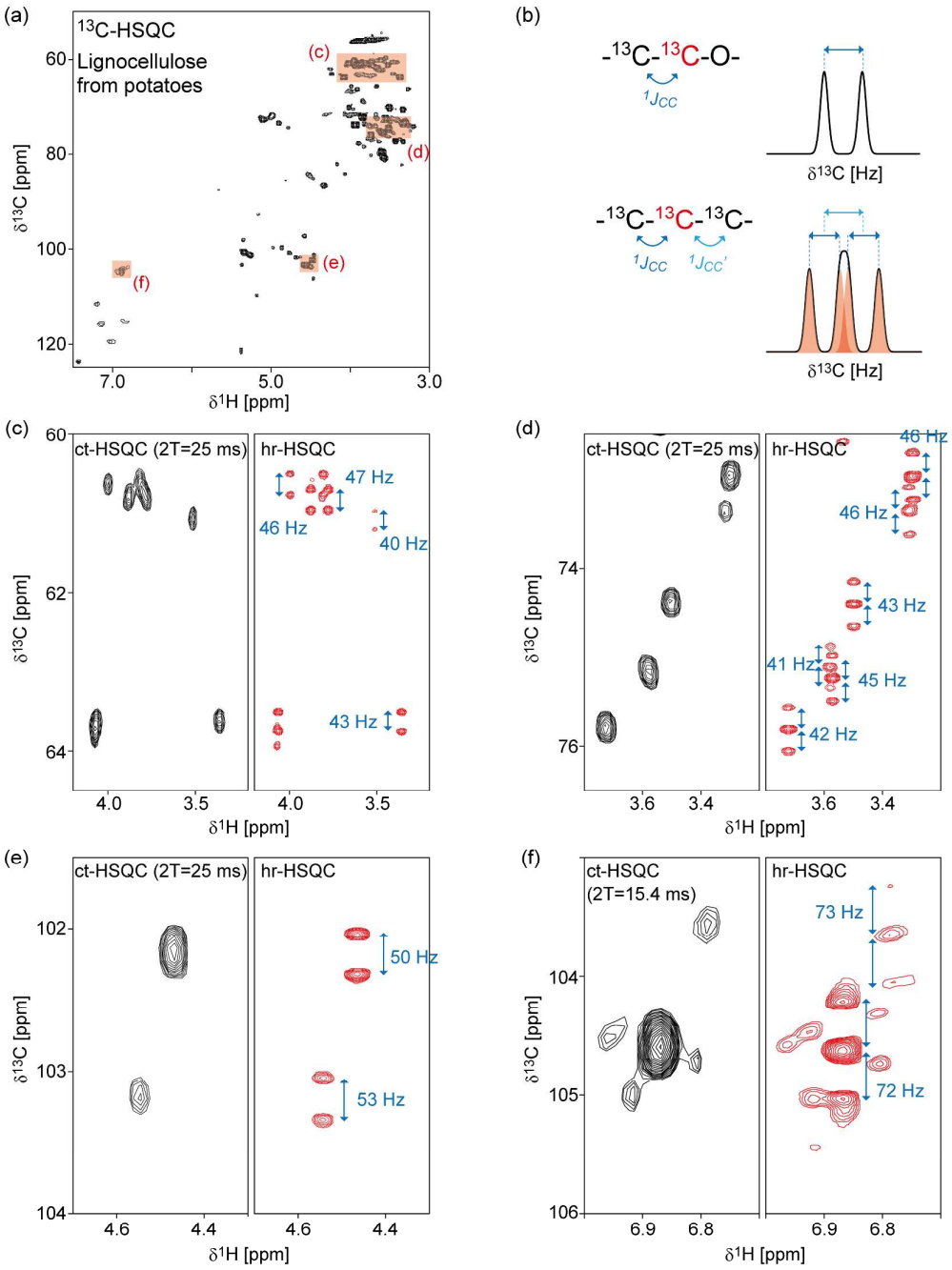

Figure S4. The evaluation of $^{1}J_{CC}$ values using high-resolution ^{13}C-HSQC to optimize values of 2T in ct-HSQC. (a) ^{13}C-HSQC spectrum of ^{13}C-labeled lignocellulose from potatoes. (b) ^{13}C-^{13}C splitting pattern of uniformly ^{13}C-labeled compounds. The evaluation of $^{1}J_{CC}$ values in aliphatic (c), (d), anomeric (e), and aromatic region (f). Left and right spectra are ct-HSQC and high-resolution ^{13}C-HSQC spectra respectively. In high-resolution ^{13}C-HSQC experiments, 2048 complex f1 (^{13}C) points were recorded and the spectral widths of the f2 dimensions were 60 ppm.

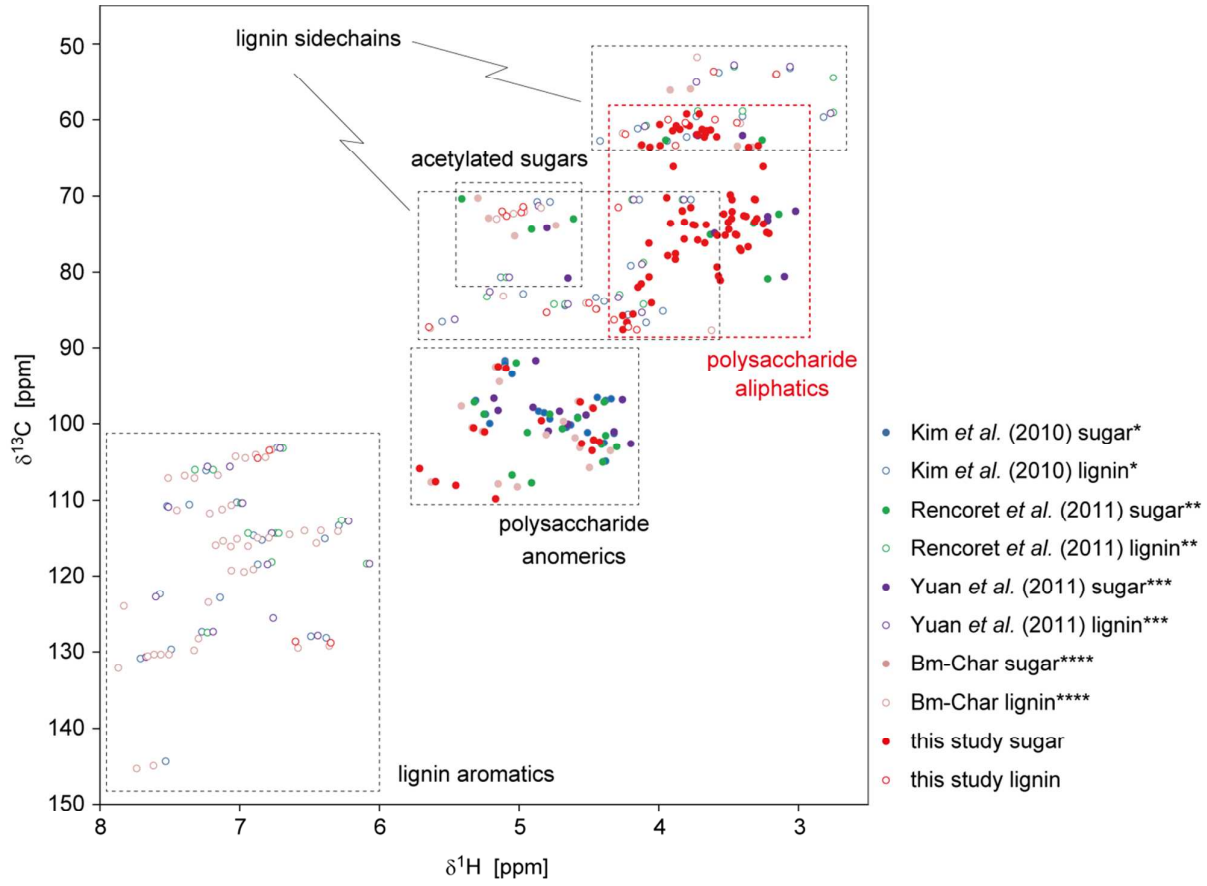

Figure S5. Comparison of signal assignments between this study and previous reports. Previous reports focused on lignin and the well-resolved anomeric signals of the polysaccharide. The use of ^{13}C labeled samples in the current study combined with multidimensional NMR delivered significant advances for signal assignments, especially for the aliphatic region of polysaccharides. Note that the plots assigned in this study include only fully assigned components, whereas those from other studies included only partly assigned signals. *Reference number1 ** Reference number5 *** Reference number6 **** This data was obtained from Bm-Char which is a web tool used to characterize the chemical structure of lignocellulosic biomass using NMR data.[7,8] (https://database.riken.jp/ecomics/index.html)

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