Supplementary Information

Development and application of a high-throughput method for the purification and analysis of surface carbohydrates from *Klebsiella pneumoniae*

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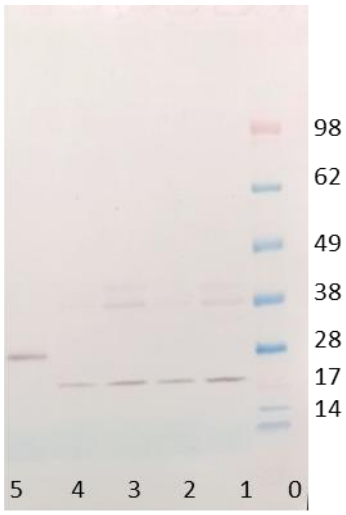
Table S1. Evaluation of OAc level after KAg purification

KAg type	Strain	Extraction method	OAc %
K2	NCTC13810	H_2O	~55%
	NCTC13810	AcOH	~45%
	NCTC11228	AcOH	~98%
	5765B	H_2O	~77%

Table S2: Complete ^{1}H and ^{13}C NMR chemical shifts data of the sugar residues in the polysaccharide produced by strain 4998 (identified as O-acetylated K2) with glycosylation shifts shown in brackets below the carbon chemical shifts; the linkage carbons are underlined.

Residue	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6	OAc
A **→4)-α-D-Glc*p*-(1→**	5.28 99.79 (6.80)	3.61 72.27 (-0.20)	3.86 72.39 (-1.39)	3.66 <u>80.13</u> (9.42)	4.05 71.21 (-1.16)	3.75; 3.79 60.87 (-0.97)	-
B **α-D-Glc*p*A-(1→**	5.17 102.04 (9.08)	3.53 72.65 (0.39)	3.79 74.40 (0.88)	3.51 72.72 (-0.19)	4.10 73.45 (0.98)	- 176.89 (-0.53)	-
C **→3,4)-β-D-Man*p*6Ac-(1→**	4.78 100.86 (6.31)	4.23 71.42 (-0.71)	3.86 <u>81.14</u> (7.11)	4.12 <u>73.21</u> (5.52)	3.80 73.79 (-3.21)	4.66; 4.31 63.51 (1.52)	2.17 21.13 174.61
D **→3)-β-D-Glc*p*-(1→**	4.47 103.20 (6.36)	3.39 73.03 (-2.17)	3.63 <u>83.92</u> (7.16)	3.56 70.91 (0.20)	3.50 77.18 (0.42)	3.75; 3.97 61.76 (-0.08)	-



0: Marker
1: K62 extracted and purified from LB plate
2: K62 extracted and purified from WF plate
3: K64 extracted and purified from LB plate
4: K64 extracted and purified from WF plate
5: Recombinant form of MrkA – MW= 23KDa vs 20kDa of native MrkA monomer

Figure S1. Western Blot analyses shows recognition of K62 and K64 samples by an anti-MrkA antibody confirming the presence of MrkA protein in the purified KAgs.

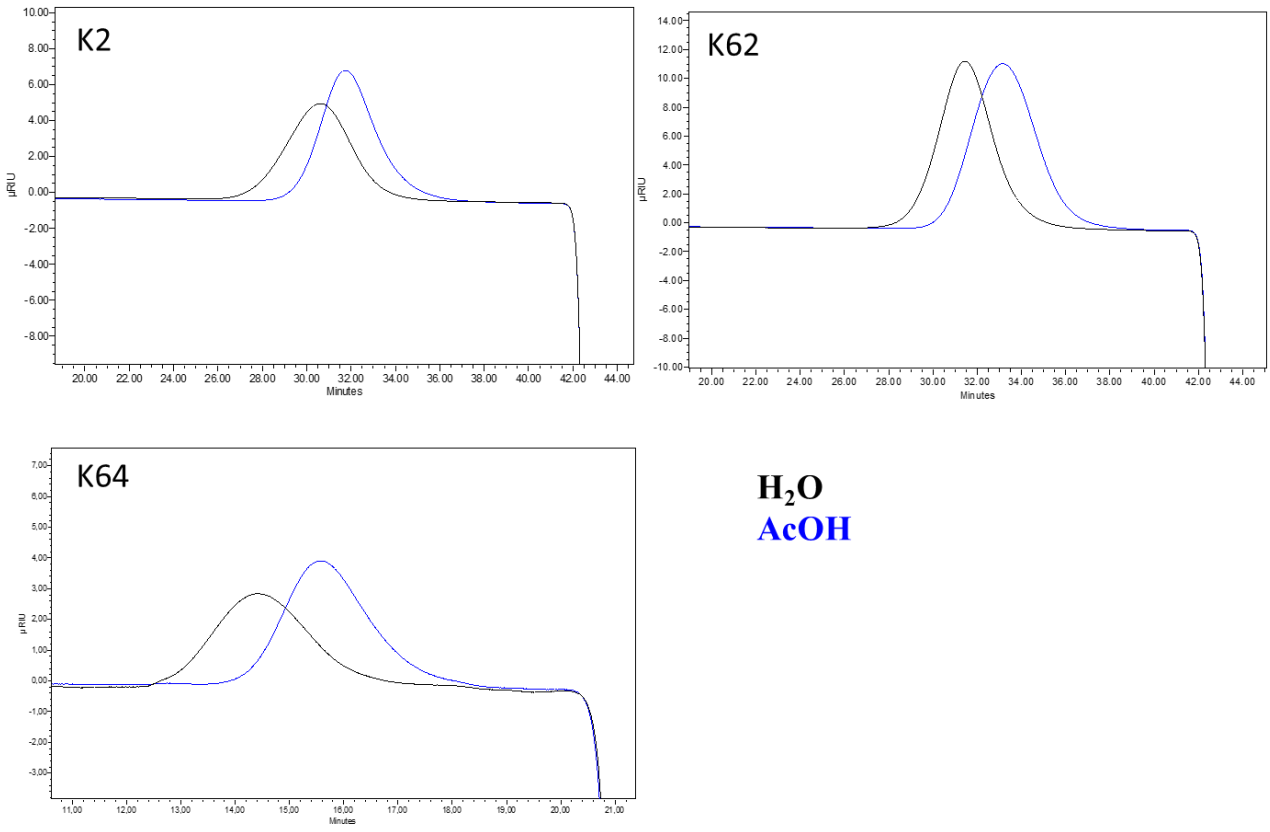


Figure S2. HPLC-SEC profiles highlight differences in the size of purified K-Ag obtained from bacterial extraction with and without AcOH.

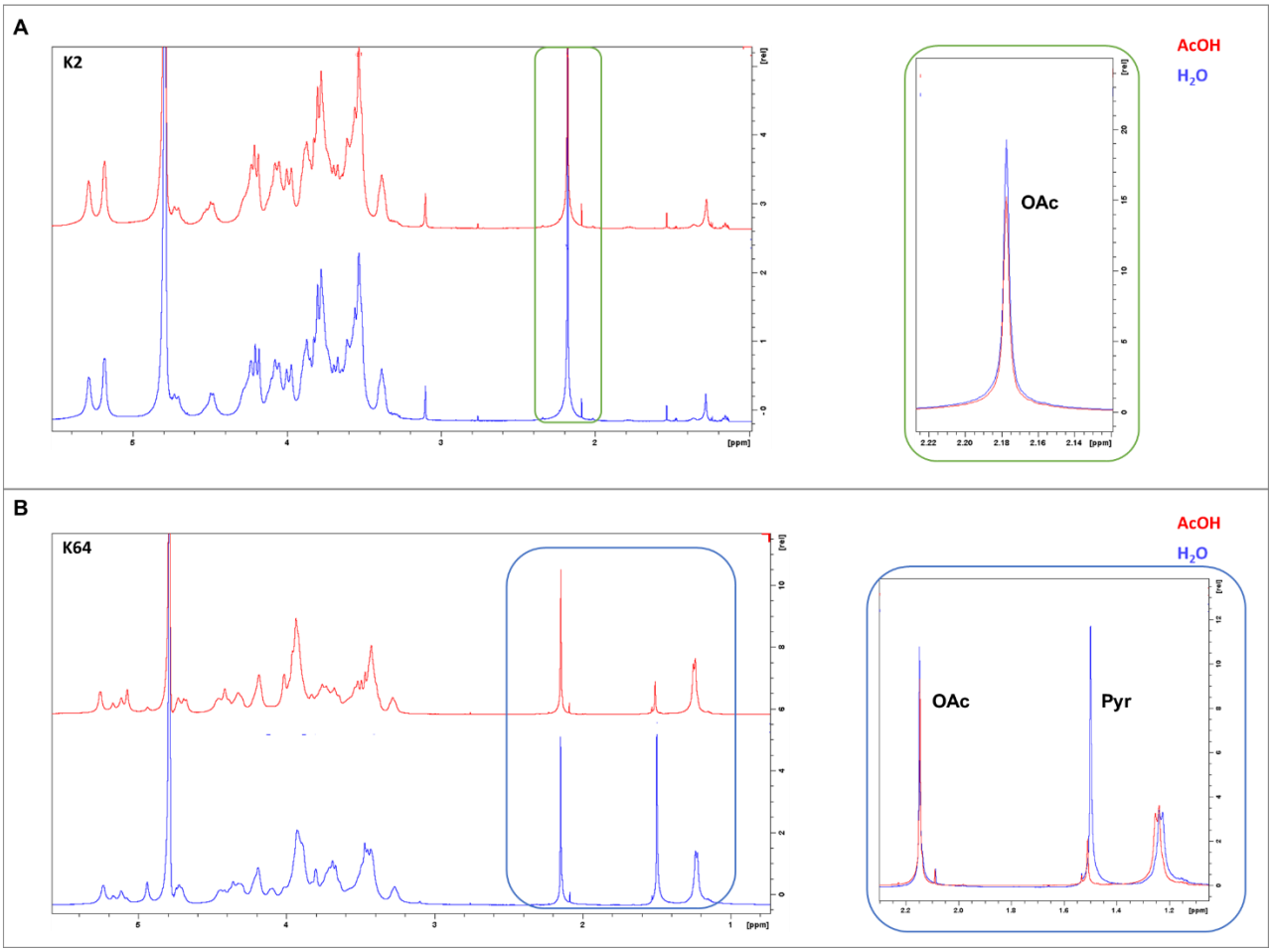


Figure S3. Impact of AcOH on K2 (A) and K64 (B) PS structure integrity by NMR analysis

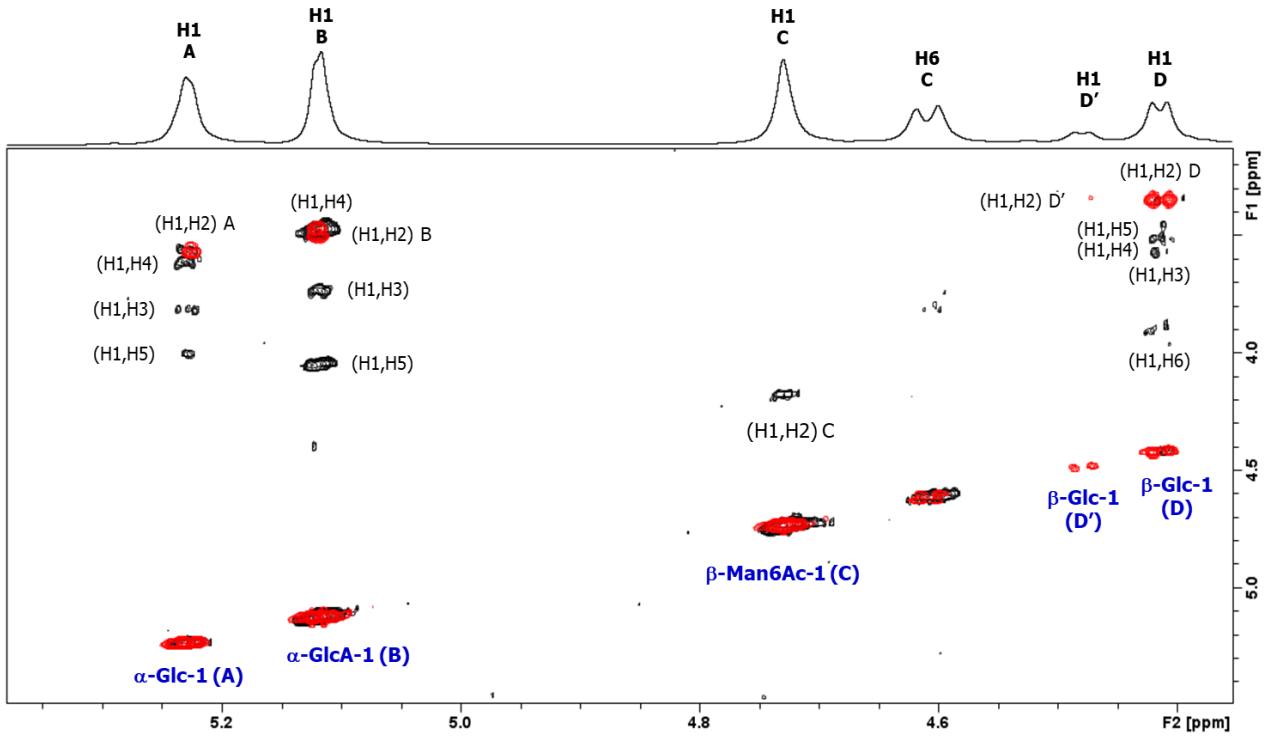


Figure S4. ^{1}H-^{1}H NMR COSY (red)/TOCSY (black) overlay of the anomeric region of K-Ag from strain 4998 recorded at 600 MHz and 343 K. The crosspeaks for the O-acetylated tetrasaccharide RU are labeled (A = α-Glc, B = α-GlcA, C = β-Man6Ac and D = β-Glc).

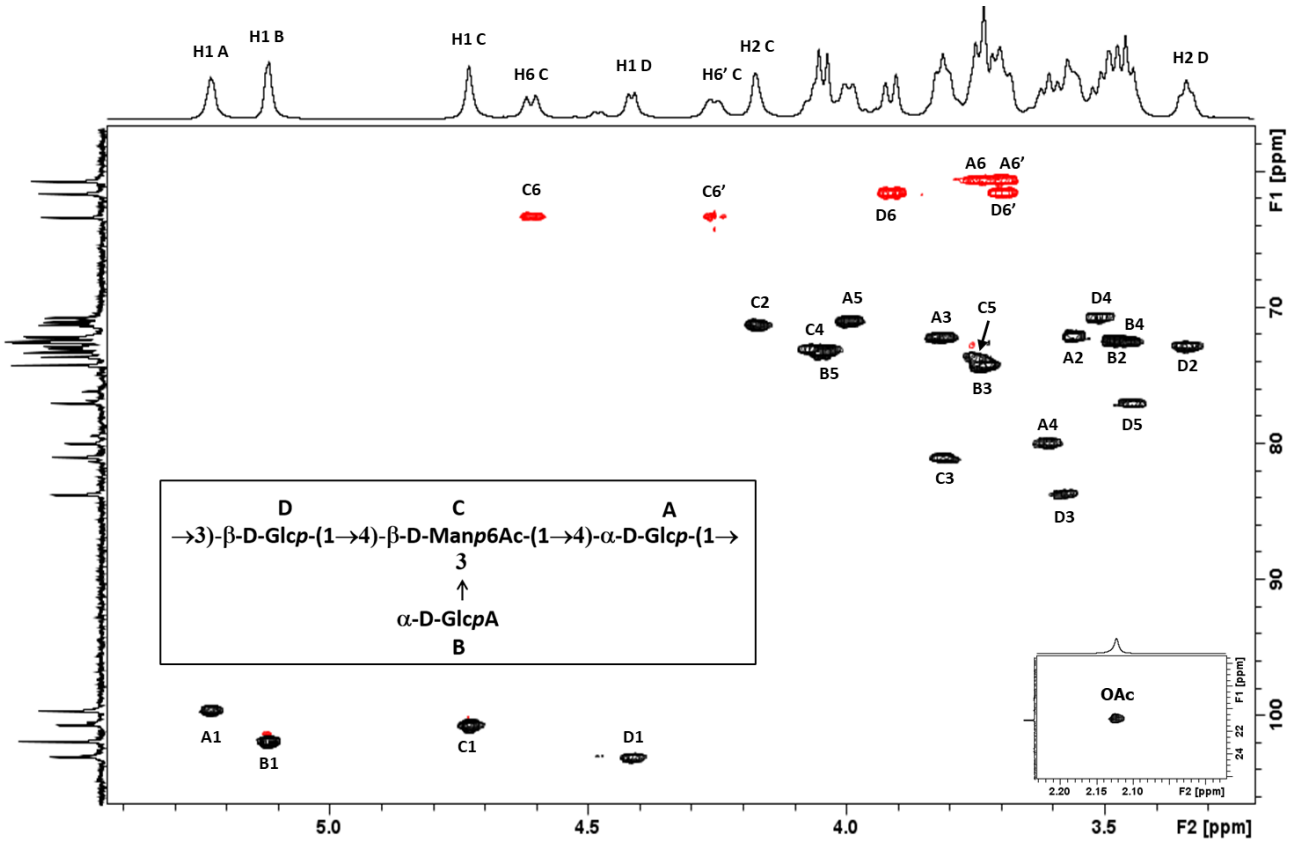


Figure S5. The HSQC-DEPT spectrum of K-Ag from strain 4998 recorded at 600 MHz and 343 K, methylene signals are inverted (in red), the inset shows the methyl region. All the O-acetylated tetrasaccharide repeat unit proton/carbon crosspeaks have been labeled according to the carbon atom of the corresponding residue (A = α-Glc, B = α-GlcA, C = β-Man6Ac and D = β-Glc).

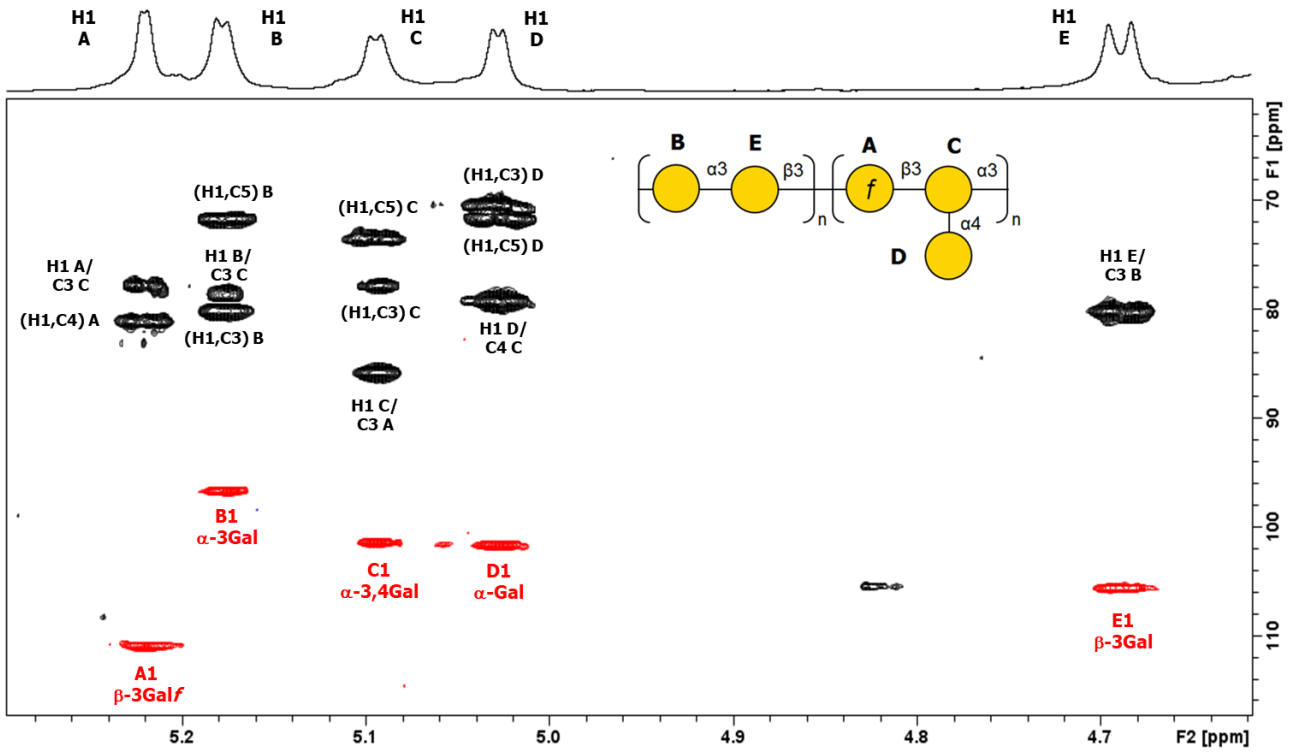


Figure S6. ^{1}H-^{1}H NMR COSY (red)/TOCSY (black) overlay of the anomeric region of O-Ag from strain 7008B recorded at 600 MHz and 323 K. The crosspeaks for the pentasaccharide repeat unit have been labeled (A = β-3Gal*f*, B = α-3Gal*p*, C = α-3,4Gal*p*, D= α-Gal*p* and E = β-3Gal*p*).

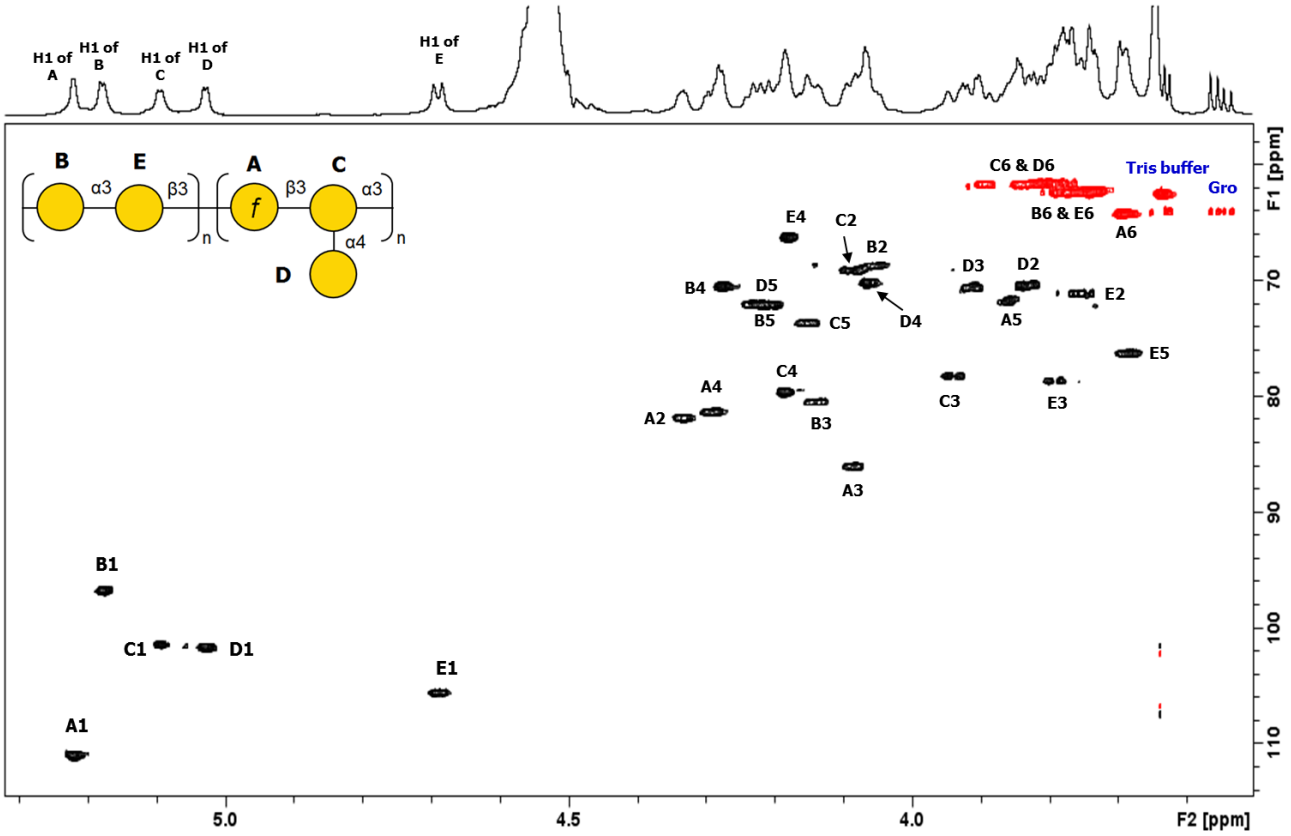


Figure S7. The HSQC-DEPT spectrum of O-Ag from strain 7008B recorded at 600 MHz and 323 K, methylene signals are inverted (in red). All the pentasaccharide repeat unit proton/carbon crosspeaks have been labeled according to the carbon atom of the corresponding residue in the RU (A = β-3Gal*f*, B = α-3Gal*p*, C = α-3,4Gal*p*, D= α-Gal*p* and E = β-3Gal*p*). Additional peaks are due to buffer and glycerol (Gro).