Figure

Figure 1. HILIC profiles of maltodextrins (DP2 – DP5) separated on a polyhydroxyethyl A column, using 3.5, 6.5 and 20 mM of ammonium acetate.

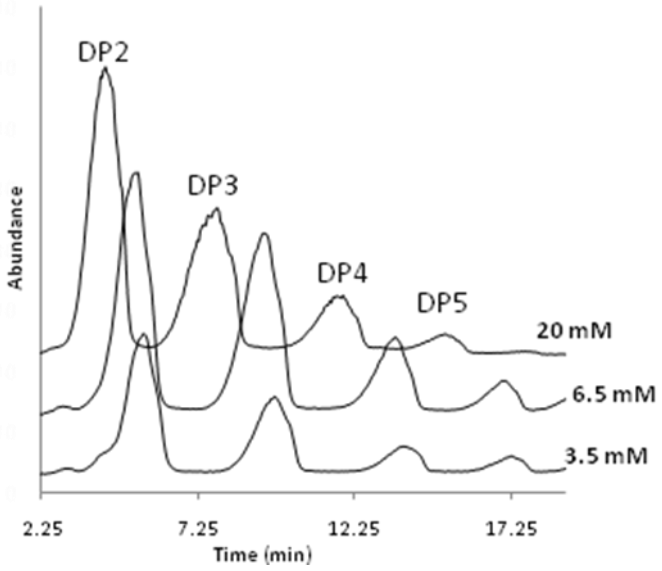
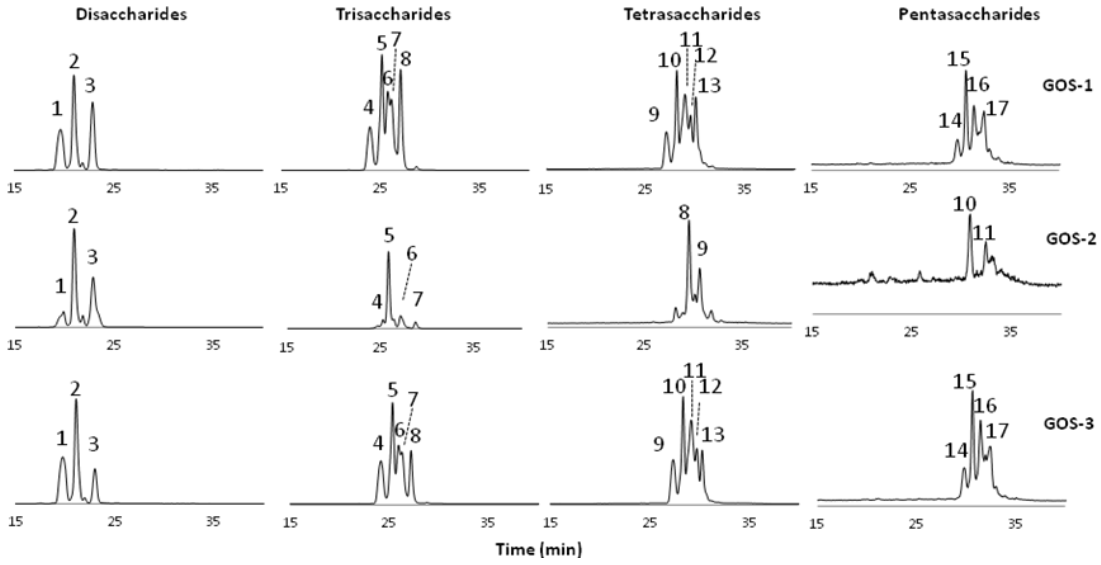


Figure 2. HILIC profiles of commercial GOS separated on an ethylene bridge hybrid with trifunctionally-bonded amide column.



Tables

Table 1. Mobile phases used to optimize the chromatographic methods on a zwitterionic, a polyhydroxyethyl aspartamide and a BEH amide column for the separation of oligosaccharides.

Solvents	Modifiers	Concentration
MeOH : H_2O	Ammonium acetate	5 mM
MeCN : H_2O	Ammonium acetate*	0.1; 3.5; 5; 6.5; 20 mM
	Ammonium hydroxide	0.1%
	Formic acid	0.1%

*Present in aqueous phase with the exception of 5 mM, where the salt was contained in both solvents

Table 2. Retention time (t_R ; min), resolution (R_s) and symmetry of standard carbohydrates analyzed using a zwitterionic, a polyhydroxyethyl aspartamide and a BEH amide column using acetonitrile and water containing 6.5 mM of ammonium acetate.

Column	Maltodextrins	t_R	R_s	Symmetry	Galactose containing oligosaccharides	t_R	R_s	Symmetry
Zwitterionic	Maltose 1	11.1		1.1	Lactulose	10.8		0.7
	Maltose 2	12	0.1	1.1	Galactobiose (α 1-3) 1	11.7	0.2	2.2
	Maltotriose 1	15.1	0.2	1.1	Galactobiose (β 1-4) 1	11.8	0.1	1
	Maltotriose 2	16.1	0	1.1	Lactose 1	12	0	2.1
	Maltotetraose 1	19	0.6	0.9	Lactose 2	12.9	0.6	1.4
	Maltotetraose 2	20	0.3	0.9	Galactobiose (β 1-4) 2	13	0.1	0.8
	Maltopentaose 1	22.5	0.2	1	Melibiose 1	13.4	0.3	1.6
	Maltopentaose 2	23.5	0.5	1.1	Galactobiose (α 1-3) 2	13.7	0.1	0.5
	Maltohexaose 1	25	0.8	1.1	Melibiose 2	14.3	0.3	1
	Maltohexaose 2	26.1	0.5	1.1	Galactobiose (β 1-6) 1	14.5	0.1	1.1
	Maltoheptaose 1	27.3	0.5	1.2	Galactobiose (β 1-6) 2	16	0.5	1.1
	Maltoheptaose 2	28.3	0.4	1.1	Raffinose	16.1	0.1	0.9
					Galactotriose 1	16.2	0	0.9
					Galactotriose 2	17.4	0.3	0.8
					Galactotetraose 1	20.5	0.1	2.7
					Stachyose	22	0.7	0.8
					Galactotetraose 2	22	0	0.8
					Verbascose	26.5	0.3	0.8
Polyhydroxyethyl aspartamide	Maltose	4.3		1.1	Galactobiose (β 1-4)	5.1		0.9
	Maltotriose	7.8	1.6	1.9	Galactobiose (α 1-3)	5.3	0.1	0.6
	Maltotetraose	11.9	1.7	2.1	Lactose	5.9	0.3	1.6
	Maltopentaose	15.1	1.6	1.1	Lactulose	6.5	0.3	1
	Maltohexaose	18.6	2.1	1.3	Galactobiose (β 1-6)	7.6	0.3	2
	Maltoheptaose	20.6	1.4	1.7	Melibiose	7.8	0.3	0.9
					Raffinose	11	0.7	1.3
					Galactotriose	11.5	0.2	1.2
					Stachyose	14.9	1.2	1.2
					Galactotetraose	14.9	0.0	0.8
					Verbascose	20.1	2.8	0.9
BEH amide	Maltose 1	21.4		1.3	Galactobiose (α 1-3) 1	20.8		0.6
	Maltose 2	21.8	0.6	0.6	Galactobiose (β 1-4) 1	21.4	0.5	0.4
	Maltotriose 1	25.7	6.2	2.0	Lactulose	21.5	0.1	1.0
	Maltotriose 2	26	0.5	0.9	Galactobiose (α 1-3) 2	22.0	0.6	0.7
	Maltotetraose 1	29.1	4.7	1.8	Galactobiose (β 1-4) 2	22.3	0.4	1.2
	Maltotetraose 2	29.4	0.4	0.3	Lactose 1	22.5	0.2	0.9
	Maltopentaose 1	31.8	5.3	0.0	Lactose 2	22.5	0.0	1.0
	Maltopentaose 2	31.9	0.2	0.3	Melibiose 1	23.4	1.8	0.9
	Maltohexaose 1	33.8	3.2	10.5	Melibiose 2	23.8	0.6	0.8
	Maltohexaose 2	33.9	0.3	0.6	Galactobiose (β 1-6) 1	24.4	0.7	1.1
					Galactobiose (β 1-6) 2	25.3	0.9	1.3
					Galactotriose 1	25.7	0.4	0.9
					Galactotriose 2	25.8	0.2	0.7

Raffinose	26.5	1.0	1.0
Galactotetraose 1	29.3	2.6	1.0
Galactotetraose 2	30.0	0.6	0.8
Stachyose	30.1	0.1	0.7
Verbascose	33.7	3.5	1.3

Table 3. Retention time (t_R ; min), resolution (R_s) and symmetry of standard carbohydrates analyzed with a BEH amide column using acetonitrile: water with 0.1% ammonium hydroxide as mobile phase.

Maltodextrins	**t_R**	**R_s**	**Symmetry**	**Galactose containing oligosaccharides**	**t_R**	**R_s**	**Symmetry**
Maltose	20.3		1	Lactulose	19.8		1.1
Maltotriose	24.7	6.1	1	Galactobiose (β 1-4)	20.6	1.2	1
Maltotetraose	28.2	6.0	1	Galactobiose (α 1-3)	21	0.5	1.1
Maltopentaose	30.9	5.1	0.9	Lactose	21.1	0.1	0.9
Maltohexaose	32.9	3.8	1.1	Melibiose	22.4	2.6	1
Maltoheptaose	34.5	3.3	1.2	Galactobiose (β 1-6)	23.2	1.4	1
				Raffinose	24.7	2.9	0.8
				Galactotriose	24.8	0.2	0.9
				Galactotetraose	28.5	6.5	1
				Stachyose	29.1	1.1	0.9
				Verbascose	32.4	9.5	0.9

Table 4. Relative abundances of characteristic *m/z* ratios of neutral losses from MS^2 of standard disaccharides.

Standard	Glycosidic linkage	Monomeric units	Neutral losses (*m/z* ion)						
			$C_6H_{10}O_5$ (203)	$C_4H_8O_4$ (245)	$C_3H_6O_3$ (275)	$C_2H_4O_2$ (305)	CH_4O_2 (317)	CH_2O (335)	H_2O (347)
α,α,-Trehalose	1→1	Glc, Glc	100.0	-	-	-	-	-	-
Kojibiose	1→2	Glc, Glc	-	93.2	-	-	-	-	-
1,3- Galactobiose	1→3	Gal, Gal	27.0	-	3.1	1.1	-	-	100.0
Nigerose	1→3	Glc, Glc	38.2	-	33.2	-	-	-	100.0
Lactose	1→4	Gal, Glc	27.1	2.2	3.2	100.0	1.7	1.6	72.0
1,4- Galactobiose	1→4	Gal, Gal	42.9	0.8	1.7	100.0	1.1	9.1	46.6
Lactulose	1→4	Gal, Fru	2.3	-	0.1	7.2	14.7	1.3	100.0
1,6- Galactobiose	1→6	Gal, Gal	9.6	11.0	46.2	100.0	-	1.1	17.0
Melibiose	1→6	Gal, Glc	39.7	2.4	16.0	100.0	-	1.4	-

Gal: galactose; Glc: glucose; Fru: fructose

Table 5. Relative abundance for characteristic *m/z* ratios of specific losses from MS^2 of disaccharides from commercial GOS separated in a BEH amide stationary phase.

	Disaccharides								
	MS^2								
	Neutral loss	**$C_6H_{10}O_5$**	**$C_4H_8O_4$**	**$C_3H_6O_3$**	**$C_2H_4O_2$**	**CH_4O_2**	**CH_2O**	**H_2O**	***Tentative identification***
Sample	**Peak / *m/z***	**203**	**245**	**275**	**305**	**317**	**335**	**347**	
GOS-1	1	6.8	0.3	45.1	0.3	-	-	100.0	Gal-(1→3)-Glc
	2	67.4	42.8	0.9	100.0	-	4.1	50.6	Gal-(1→4)-Glc and Gal-(1→2)-Glc
	3	3.4	6.1	25.3	100.0	0.6	1.9	3.7	Gal-(1→6)-Glc
GOS-2	1	4.0	-	12.8	3.8	4.3	-	100.0	Gal-(1→3)- Glc + lactulose
	2	16.7	7.4	0.4	100.0	2.7	5.9	52.3	Gal-(1→4)-
	3	2.1	6.7	28.0	100.0	0.2	1.9	4.7	Gal-(1→6)-Glc
GOS-3	1	7.7	-	38.9	0.8	1.6	0.4	100.0	Gal-(1→3)-Glc
	2	51.7	30.8	0.6	100.0	-	4.2	43.6	Gal-(1→4)- and Gal-(1→2)- Glc
	3	2.1	5.7	21.5	100.0	0.2	1.8	5.3	Gal-(1→6)-Glc

Table 6. Relative abundance for characteristic *m/z* ratios of specific losses from MS^2 and MS^3 of trisaccharides from commercial GOS separated on a BEH amide stationary phase.

Trisaccharides									
MS^2									
	Neutral loss	**$C_6H_{12}O_6$**	**$C_6H_{10}O_5$**	**$C_4H_8O_4$**	**$C_3H_6O_3$**	**$C_2H_4O_2$**	**CH_2O**	**H_2O**	*Identification*
Sample	**Peak / *m/z***	**347**	**365**	**407**	**437**	**467**	**497**	**509**	
GOS-1	4	18.2	100.0	-	70.4	4.0	-	99.7	-Gal-(1→3)-Glc
	5a	3.2	100.0	66.2	5.3	3.2	-	5.3	-Gal-(1→2)-Glc
	5b	12.5	86.4	7.2	5.9	100.0	0.4	43.4	-Gal-(1→4)-Glc
	6	46.0	96.0	7.4	1.0	100.0	1.9	30.7	-Gal-(1→4)-Glc
	7	1.7	60.4	6.1	23.8	100.0	1.3	17.8	-Gal-(1→6)-Glc
	8	2.9	76.0	13.6	5.0	100.0	2.5	14.1	-Gal-(1→4)-Glc +-Gal-(1→2)-Glc
GOS-2	4	9.6	89.9	3.8	12.5	100	-	88.3	-Gal-(1→6)-x
	5	32.1	22.7	4.0	1.7	100	3.0	45.7	-Gal-(1→4)-x
	6	10.1	33.6	2.6	12.2	100	0.5	7.8	-Gal-(1→6)-x
	7	0.4	4.7	2.4	9.8	100	-	1.0	-Gal-(1→6)-x
GOS-3	4	12.8	77.7	-	62.9	5.1	0.6	100.0	-Gal-(1→3)-Glc
	5a	6.0	90.8	100.0	0.8	1.9	-	8.8	-Gal-(1→2)-Glc
	5b	8.8	99.1	6.0	5.0	100.0	2.2	37.3	-Gal-(1→4)-Glc
	6	34.8	100.0	2.2	2.2	58.9	-	35.3	-Gal-(1→4)-Glc + -Gal-(1→6)-Glc
	7	3.4	62.4	5.9	27.2	100.0	1.2	25.2	-Gal-(1→6)-Glc
	8	2.0	72.4	5.8	7.4	100.0	2.6	21.6	-Gal-(1→4)-Glc +-Gal-(1→6)-Glc
MS^3									
	Neutral loss	**$C_6H_{10}O_5$**	**$C_4H_8O_4$**	**$C_3H_6O_3$**	**$C_2H_4O_2$**	**CH_4O_2**	**CH_2O**	**H_2O**	***Identification***
Sample	**Peak / *m/z***	**203**	**245**	**275**	**305**	**317**	**335**	**347**	
GOS-1	4	4.5	-	100.0	-	-	-	88.1	Gal-(1→3)-
	5a	50.5	88.3	-	100.0	-	24.6	-	Gal-(1→4)- + x*-(1→2)-
	5b	17.2	-	-	100.0	1.0	9.4	26.0	Gal-(1→4)-
	6	-	14.6	15.2	100.0	-	6.5	22.6	Gal-(1→4)- + Gal-(1→6)-
	7	-	3.8	31.2	100.0	-	2.4	18.1	Gal-(1→6)-
	8	-	5.7	21.4	100.0	-	1.3	5.9	Gal-(1→6)-

GOS-2	4	-	10.7	-	100.0	11.8	5.8	20	Gal-(1→4)- and Gal-(1→2)-
	5	6.8	2.6	23.8	100.0	2.2	15.9	33.9	Gal-(1→6)-
	6	27.3	43.1	53.6	100.0	-	16.0	8.2	Gal-(1→6)-
	7	-	-	96.9	-	-	-	-	Gal-(1→3)-
GOS-3	4	-	-	100.0	7.4	-	-	50.4	Gal-(1→3)-
	5a	56.2	59.2	-	100.0	-	4.5	17.4	Gal-(1→4)- + x*-(1→2)-
	5b	5.4	9.0	-	100.0	1.2	5.5	29.7	Gal-(1→4)-
	6	23.1	30.9	6.8	100.0	14.1	-	18.5	Gal-(1→4)- + Gal-(1→2)-
	7	-	-	53.1	100.0	-	6.7	21.5	Gal-(1→6)-
	8	12.4	8.5	7.6	100.0	-	6.3	23.2	Gal-(1→6)-

* x: correspond to an unknown monosaccharide unit not previously described in the literature.

Table 7. Relative abundance for characteristic *m/z* ratios of specific losses from MS^2, and MS^3 of tetrasaccharides from commercial GOS separated on a BEH amide stationary phase.

Tetrasaccharides								
				MS^2				
	Neutral loss	**$C_6H_{12}O_6$**	**$C_6H_{10}O_5$**	**$C_4H_8O_4$**	**$C_3H_6O_3$**	**$C_2H_4O_2$**	**H_2O**	***Identification***
Sample	**Peak / *m/z***	**509**	**527**	**569**	**599**	**629**	**671**	
GOS-1	9	13.6	100.0	-	61.7	1.2	17.8	-(1→3)-
	10a	9.0	100.0	78.3	3.4	11.8	8.1	-(1→2)- + -(1→6)-
	10b	5.4	100.0	14.2	4.0	49.2	19.2	-(1→6)- + -(1→2)-
	11	5.1	100.0	3.7	6.2	20.5	6.2	-(1→6)-
	12	10.0	100.0	12.3	7.0	94.8	8.5	-(1→4)- + unkown
	13	6.4	84.8	4.1	-	100.0	12.3	-(1→4)-
GOS-2	8	70.5	42.9	2.7	3.5	100.0	24.3	-(1→4)- + -(1→6)-
	9	19.8	23.0	4.3	3.6	100.0	26.9	-(1→4)- + -(1→6)- +-(1→2)-
GOS-3	9	9.3	100.0	1.2	71.1	5.2	26.1	-(1→3)-
	10a	3.2	100.0	-	1.0	9.4	8.6	-(1→6)-
	10b	6.5	100.0	-	2.2	53.2	19.6	-(1→6)-
	11	6.8	100.0	-	-	38.0	6.0	-(1→4)-
	12	5.6	100.0	7.8	3.3	92.6	4.9	-(1→4)- + unkown
	13	3.0	98.1	3.3	1.9	100.0	13.7	-(1→4)- + unkown
				MS^3				
	Neutral loss	**$C_6H_{12}O_6$**	**$C_6H_{10}O_5$**	**$C_4H_8O_4$**	**$C_3H_6O_3$**	**$C_2H_4O_2$**	**H_2O**	***Identification***
Sample	**Peak / *m/z***	**347**	**365**	**407**	**437**	**467**	**509**	
GOS-1	9	4.5	35.9	-	100.0	10.0	31.0	-(1→3)-
	10a	-	100.0	37.3	-	45.3	-	-(1→4)- + -(1→2)-
	10b	8.2	77.4	11.0	4.1	100.0	19.0	-(1→6)- + -(1→4)-
	11	22.4	100.0	16.0	-	52.3	44.8	-(1→4)- + -(1→2)-
	12	5.4	48.5	-	17.0	100.0	10.0	-(1→4)- or -(1→6)-
	13	6.8	32.1	19.5	9.9	100.0	27.3	-(1→6)- + unknown
GOS-2	8	-	62.2	-	-	100.0	-	-(1→4)-
	9	-	20.0	-	-	100.0	-	-(1→4)-
GOS-3	9	6.7	50.2	-	100.0	1.2	26.0	-(1→3)-

10a	-	100.0	30.3	-	75.8	18.0	unknown
10b	9.6	91.2	18.0	7.4	100.0	21.4	-(1→6)- + -(1→4)- +-(1→2)-
11	-	100.0	4.4	15.9	63.0	32.5	-(1→4)- + -(1→6)-
12	-	24.9	12.0	-	100.0	13.9	-(1→4)-
13	-	100.0	14.2	-	11.0	11.7	unknown

Table 8. Relative percentages of quantified and identified oligosaccharides using a BEH amide stationary phase in commercial GOS.

Sample	DP	Peak number	%	Identification
GOS-1	DP2	1	15.11 (0.07)[§]	Gal-(1→3)-Glc
		2	22.20 (0.18)	Gal-(1→4)-Glc + Gal-(1→2)-Glc
		3	17.07 (0.16)	Gal-(1→6)-Glc
	DP3	4	3.86 (0.02)	Gal-(1→3)-Gal-(1→3)-Glc
		5a+5b	8.55 (0.07)	Gal-(1→4)-Gal-(1→2)-Glc + x*-(1→2)-Gal-(1→2)-Glc + Gal-(1→4)-Gal-(1→4)-Glc
		6	5.05 (0.12)	Gal-(1→4)-Gal-(1→4)-Glc + Gal-(1→6)-Gal-(1→4)-Glc
		7	5.00 (0.11)	Gal-(1→6)-Gal-(1→6)-Glc
		8	6.38 (0.04)	Gal-(1→6)-Gal-(1→4)-Glc + Gal-(1→6)-Gal-(1→2)-Glc
	DP4	9	1.25 (0.02)	x-(1→3)-x-(1→3)-x-(1→3)-x
		10a+10b	2.58 (0.04)	x-(1→6)-x-(1→4)-x-(1→y)-x + x-(1→6)-x-(1→2)-x-(1→y)-x + x-(1→6)-x-(1→6)-x-(1→4)-x + x-(1→6)-x-(1→4)-x-(1→4)-x
		11	3.66 (0.04)	x-(1→6)-x-(1→4)-x-(1→y)-x + x-(1→6)-x-(1→2)-x-(1→y)-x
		12	1.44 (0.03)	x-(1→4)-x-(1→4)-x-(1→6)-x or x-(1→y)-x-(1→6)-x-(1→6)-x
		13	2.24 (0.07)	x-(1→4)-x-(1→6)-x-(1→y)-x + x-(1→4)-x-(1→y)-x-(1→y)-x
	DP5	14	1.13 (0.02)	Unknown
		15	1.82 (0.04)	Unknown
		16	1.11 (0.03)	Unknown
		17	1.56 (0.02)	Unknown
GOS-2	DP2	1	9.26 (0.18)	Gal-(1→3)- Glc + lactulose
		2	37.89 (1.65)	Gal-(1→4)-Glc
		3	29.17 (0.39)	Gal-(1→6)-Glc
	DP3	4	1.53 (0.35)	Gal-(1→4)-Gal-(1→6)-x + Gal-(1→2)-Gal-(1→6)-x
		5	17.62 (0.24)	Gal-(1→6)-Gal-(1→4)-x
		6	2.99 (0.11)	Gal-(1→6)-Gal-(1→6)-x
		7	0.34 (0.18)	Gal-(1→3)-Gal-(1→6)-x
	DP4	8	1.02 (0.06)	x-(1→4)-x-(1→4)-x-(1→y*)-x + x-(1→6)-x-(1→4)-x-(1→y*)-x
		9	0.50 (0.02)	x-(1→4)-x-(1→4)-x-(1→y*)-x + x-(1→6)-x-(1→4)-x-(1→y*)-x + x-(1→6)-x-(1→4)-x-(1→y*)-x
	DP5	10	tr	Unknown
		11	tr	Unknown
GOS-3	DP2	1	18.88 (0.25)	Gal-(1→3)-Glc
		2	26.11 (0.48)	Gal-(1→4)- + Gal-(1→2)- Glc
		3	8.34 (0.04)	Gal-(1→6)-Glc
	DP3	4	5.61 (0.05)	Gal-(1→3)-Gal-(1→3)-Glc
		5a + 5b	10.20 (0.08)	Gal-(1→4)-Gal-(1→2)-Glc + x*-(1→2)-Gal-(1→2)-Glc + Gal-(1→4)-Gal-(1→4)-Glc
		6	4.96 (0.15)	Gal-(1→4)-Gal-(1→4)-Glc + Gal-(1→4)-Gal-(1→2)-Glc + Gal-(1→4) -Gal-(1→6)-Glc + Gal-(1→2) -Gal-(1→6)-Glc

		7	4.30 (0.09)	Gal-(1→6)-Gal-(1→6)-Glc
		8	3.80 (0.05)	Gal-(1→6)-Gal-(1→4)-Glc + Gal-(1→6)-Gal-(1→6)-Glc
	DP4	9	1.66 (0.09)	x-(1→3)-x-(1→3)-x-(1→4)-x
		10a + 10b	3.09 (0.19)	x-(1→6)-x-(1→y)-x-(1→y)-x + x-(1→6)-x-(1→6)-x-(1→y)-x + x-(1→6)-x-(1→4)-x-(1→y)-x + x-(1→6)-x-(1→2)-x-(1→y)-x
		11	4.18 (0.17)	x-(1→4)-x-(1→4)-x-(1→6)-x + x-(1→4)-x-(1→6)-x-(1→6)-x
		12	1.34 (0.12)	x-(1→4)-x-(1→4)-x-(1→y)-x + x-(1→y)-x-(1→4)-x-(1→y)-x
		13	1.38 (0.12)	x-(1→4)-x-(1→y)-x-(1→6)-x + x-(1→y)-x-(1→y)-x-(1→6)-x + x-(1→4)-x-(1→y)-x-(1→3)-x + x-(1→y)-x-(1→y)-x-(1→3)-x
	DP5	14	1.13 (0.04)	Unknown
		15	1.83 (0.09)	Unknown
		16	1.73 (0.06)	Unknown
		17	1.45 (0.02)	Unknown

*x: unknown monosaccharide unit.

*y: an unknown bond

[§]Standard deviation (n = 3)

tr: traces