Supporting Information

Enhanced synthesis of alkyl galactopyranoside by *Thermotoga naphthophila* β-galactosidase catalyzed transglycosylation: A kinetic insight of functionalized ionic liquid-mediated system

Jingwen Yang[a,b], Bianca Pérez[b], Sampson Anankanbil[b], Jingbo Li[b], Renjun Gao[a*], Zheng Guo[b*]

[a]*Key Laboratory for Molecular Enzymology and Engineering, the Ministry of Education, School of Life Science, Jilin University, Changchun 130012, China;*

[b]*Department of Engineering, Aarhus University, Gustav Wieds Vej 10, Aarhus 8000, Denmark;*

[*]*Corresponding Author: Renjun Gao; email: gaorj@jlu.edu.cn; Zheng Guo; email: guo@eng.au.dk.*

Table of Content

Table S1. COSMO-RS predicted results of solubility for lactose and activity coefficient in the biphasic reaction systems at 75 °C.

Ionic liquid	Solubility (g/100 g)	Activity coefficient (γ)		
		Lactose	Water	*n*-octanol
No IL	0.27	2.42	2.52	1.18
BMIM.PF_6	0.42	2.04	2.47	1.20
BMIM.BF_4	0.44	1.92	2.42	1.23
Ecoeng 111P	0.48	1.95	2.33	1.27
Ecoeng 21M	0.42	2.02	2.40	1.24
tOMA.TFA	0.42	2.38	2.47	1.20
Ammoeng 100	0.42	2.20	2.44	1.21
Ammoeng 102	0.47	2.29	2.47	1.20
Ammoeng 111	0.38	2.37	2.49	1.19
Ammoeng 112	0.38	2.33	2.47	1.20
Ammoeng 120	0.45	2.29	2.48	1.19

Solvent: water- *n*-octanol mixture (1:9, v/v).

Table S2. COSMO-RS predicted results of solubility for lactose and activity coefficient in the biphasic reaction systems at 75 °C with different concentrations of ILs.

Ionic liquid	Concentration	Solubility (g/100 g)	Activity coefficient (γ)		
			Lactose	Water	*n*-octanol
No IL		0.27	2.42	2.52	1.18
Ammoeng 111	1.25%	0.33	2.38	2.5	1.19
	2.5%	0.38	2.37	2.49	1.19
	3.75%	0.39	2.29	2.45	1.21
	5%	0.82	2.34	2.45	1.21
Ammoeng 102	1.25%	0.36	2.38	2.50	1.19
	2.5%	0.47	2.29	2.47	1.20
	3.75%	0.48	2.34	2.49	1.19
	5%	0.70	2.43	2.52	1.18
Ecoeng 111P	1.25%	0.38	2.26	2.46	1.21
	2.5%	0.48	1.95	2.42	1.23
	3.75%	0.60	1.93	2.32	1.26
	5%	0.82	1.63	2.19	1.31

Solvent: water- *n*-octanol mixture (1:9, v/v).

Table S3. COSMO-RS predicted results of solubility for lactose and activity coefficient in the biphasic reaction systems at 75 °C with different water contents.

Water content	Without IL				With 2.5% Ammoeng 102			
	Solubility (g/100 g)	Activity coefficient (γ)			Solubility (g/100 g)	Activity coefficient (γ)		
		Lactose	Water	*n*-octanol		Lactose	Water	*n*-octanol
10%	0.27	2.42	2.52	1.18	0.61	2.29	2.47	1.20
15%	0.29	1.57	2.19	1.40	0.62	1.59	2.20	1.39
20%	0.31	1.17	1.98	1.69	0.64	1.12	1.95	1.75
25%	0.32	0.89	1.78	2.20	0.67	0.90	1.79	2.12
30%	0.34	0.75	1.65	2.86	0.69	0.76	1.65	2.84
35%	0.35	0.66	1.52	4.09	0.72	0.68	1.54	3.82
40%	0.37	0.63	1.42	5.75	0.74	0.64	1.42	5.73
45%	0.38	0.63	1.34	8.39	0.75	0.64	1.36	7.75
50%	0.40	0.67	1.27	12.76	0.75	0.67	1.29	11.75
55%	0.41	0.76	1.21	20.31	0.76	0.75	1.22	18.53
60%	0.43	0.91	1.16	33.90	0.76	0.89	1.17	27.89
65%	0.44	1.16	1.12	59.68	0.76	1.06	1.14	47.90
70%	0.45	1.48	1.09	97.82	0.77	1.42	1.10	87.74
75%	0.47	1.99	1.07	166.93	0.77	1.90	1.07	148.94
80%	0.48	3.09	1.04	346.38	0.77	2.92	1.04	306.97

Table S4. COSMOthermX predicted results of solubility for lactose and activity coefficient in the biphasic reaction systems at different temperatures with 20% and 35% water contents.

Temperature (°C)	Without IL				With 2.5% Ammoeng 102			
	Solubility (g/100 g)	Activity coefficient (γ)			Solubility (g/100 g)	Activity coefficient (γ)		
		Lactose	Water	*n*-octanol		Lactose	Water	*n*-octanol
50	0.11	1.4	2.02	1.7	0.29	0.76	1.55	3.93
55	0.14	1.35	2.01	1.7	0.35	0.74	1.55	3.90
60	0.17	1.30	2.01	1.7	0.42	0.73	1.55	3.90
65	0.21	1.26	2.00	1.69	0.50	0.71	1.54	3.88
70	0.25	1.21	1.99	1.69	0.60	0.70	1.54	3.85
75	0.31	1.17	1.98	1.69	0.72	0.68	1.54	3.82
80	0.37	1.13	1.97	1.69	0.86	0.66	1.53	3.79
85	0.45	1.09	1.97	1.68	1.04	0.65	1.53	3.76
90	0.55	1.05	1.96	1.67	1.26	0.63	1.52	3.72
95	0.66	1.02	1.95	1.66	1.52	0.61	1.52	3.68
100	0.80	0.98	1.93	1.66	1.85	0.60	1.51	3.63

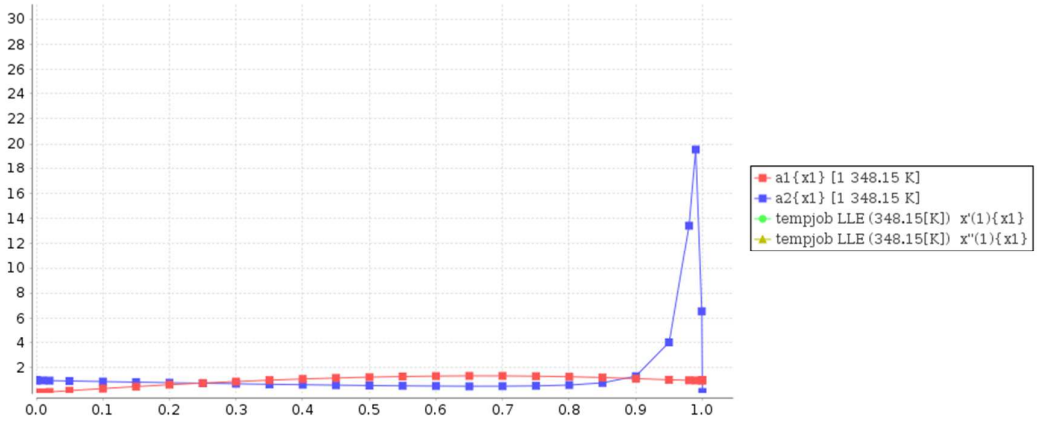
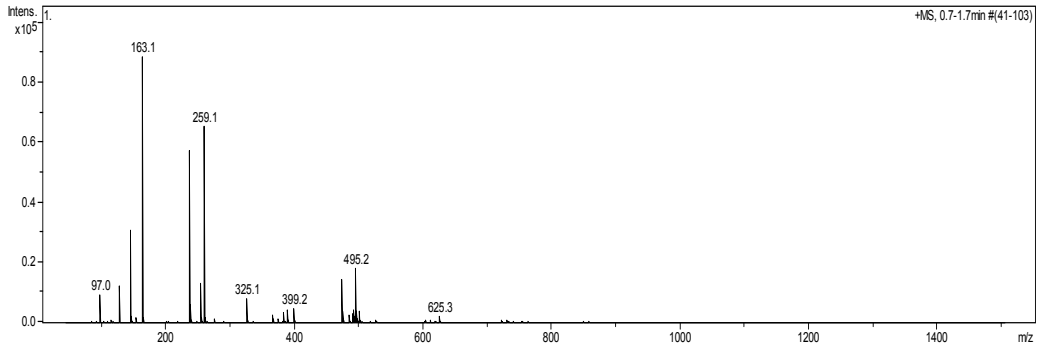


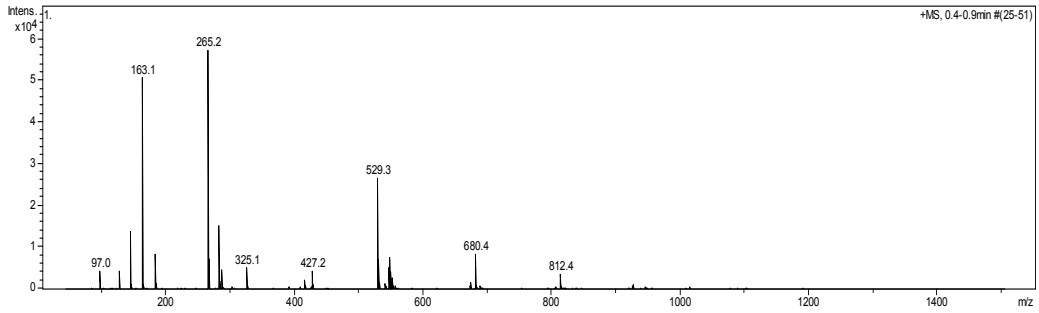
Figure S1. Liquid-liquid phase diagram of water and *n*-octanol at 75 °C. (Red: water; blue: *n*-octanol; x axis: water content)

MS spectra of alkyl galactopyranoside

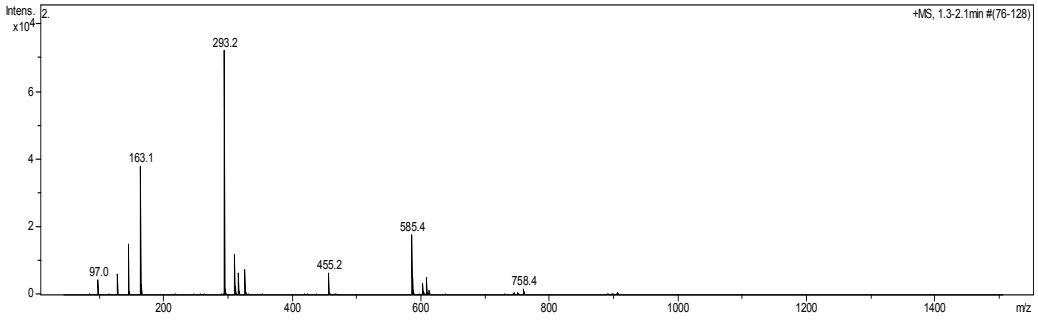
Butyl galactopyranoside.



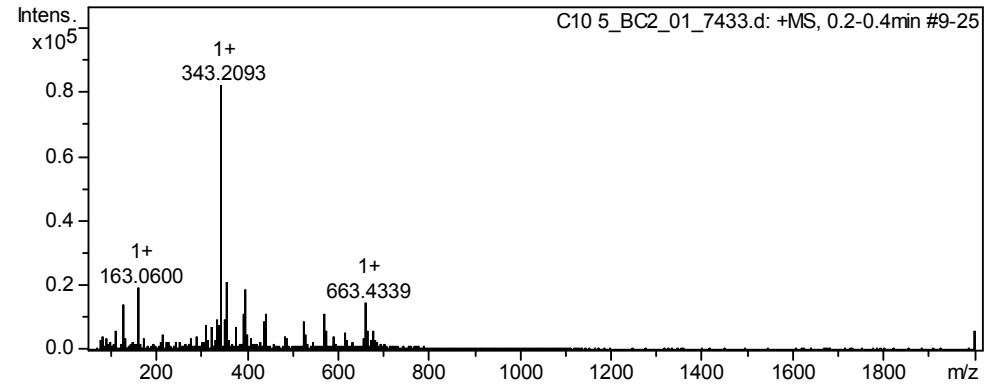
Hexyl galactopyranoside.



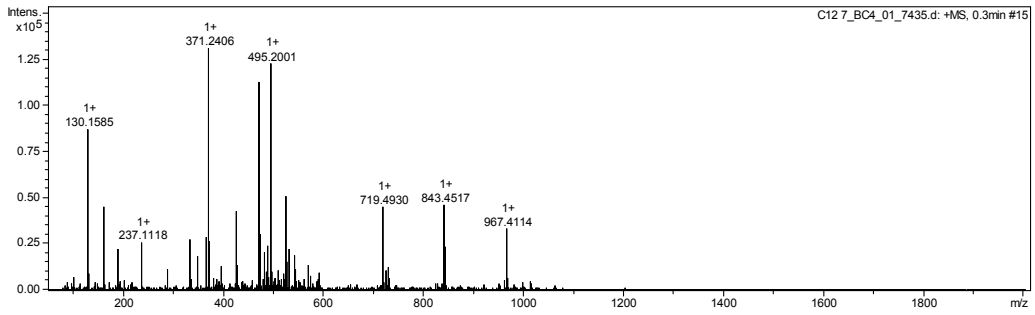
Octyl galactopyranoside.



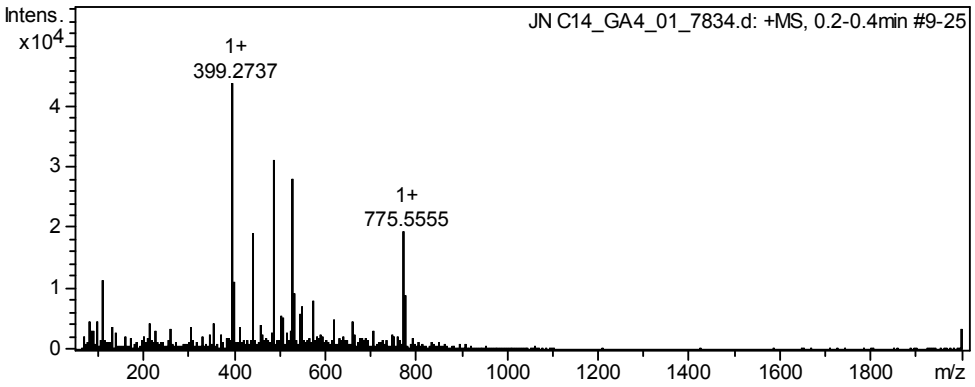
Decyl galactopyranoside



Dodecyl galactopyranoside

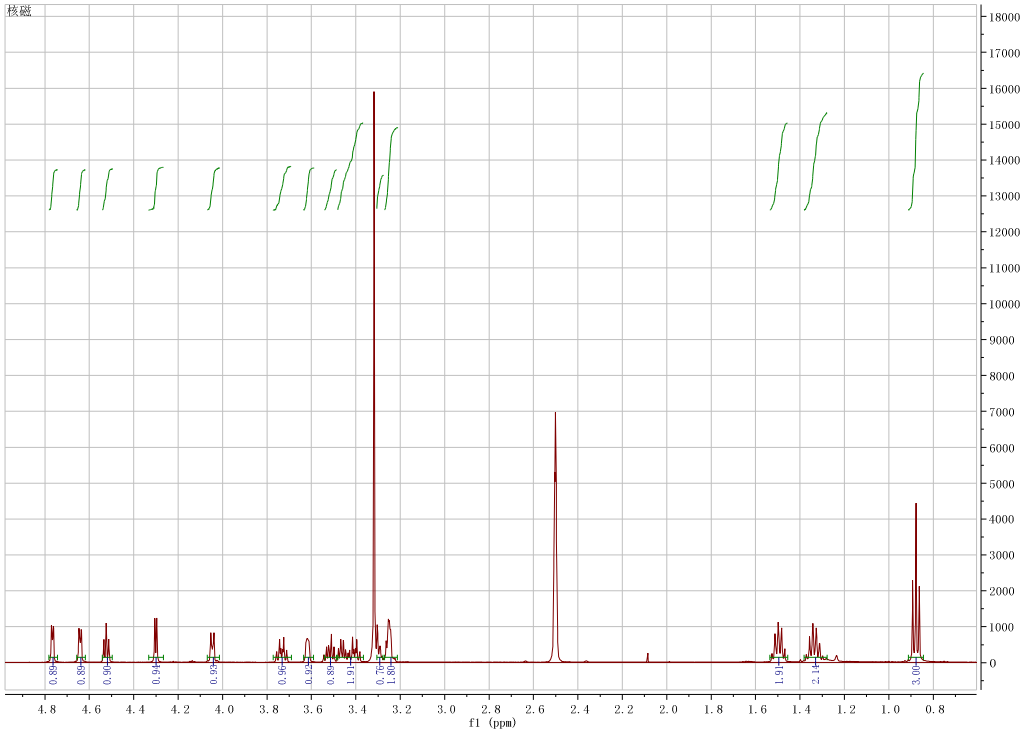


Tetradecyl galactopyranoside.



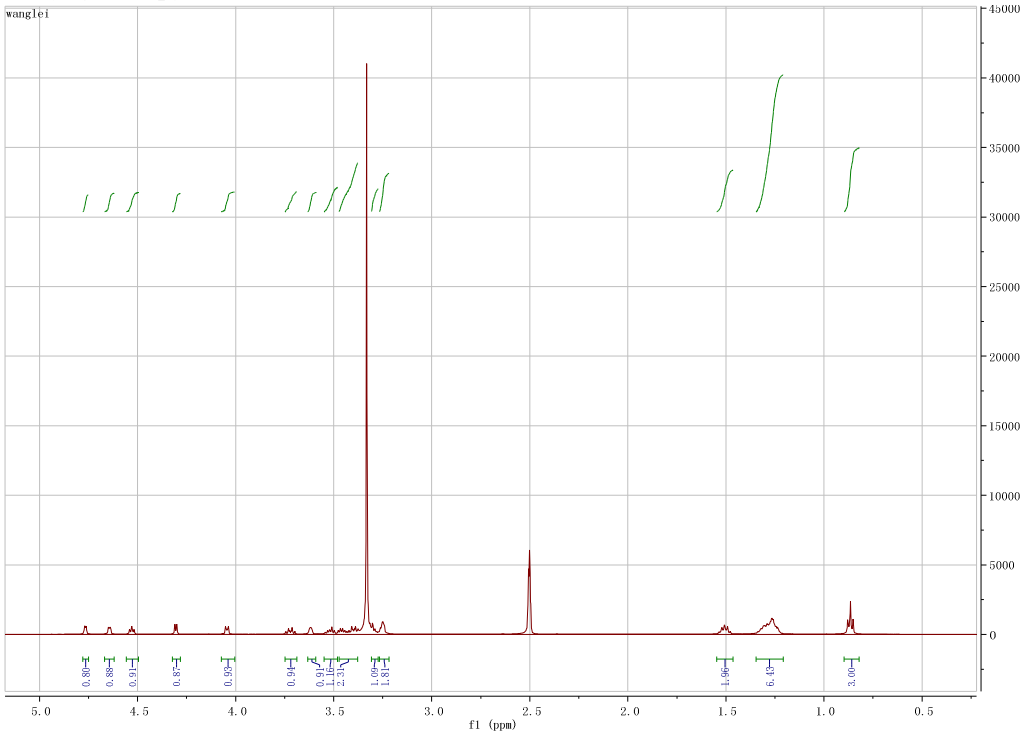
1H-NMR of alkyl galactopyranoside

Butyl galactopyranoside.



Butyl galactopyranoside. Rf (ethyl acetate-methanol-water (17:2:1, v/v)): 0.40. ^{1}H NMR (500 MHz, DMSO-d6) δ/ppm 0.82–0.91(m, 3H), 1.28–1.38 (m, 2H), 1.46–1.54 (m, 2H), 3.21–3.27 (m, 2H), 3.28–3.30 (m, 1H), 3.36–3.49 (m, 2H), 3.49–3.57 (m, 1H), 3.59–3.66 (s, 1H), 3.68–3.79 (dt, 1H), 4.00–4.08 (m, 1H), 4.27–4.33 (d, 1H), 4.48–4.56 (t, 1H), 4.62–4.68 (d, 1H), 4.75–4.81 (t, 1H); HPLC-MS: exact mass calculated for $C_{10}H_{20}O_6Na$ [M+Na$^+$]: 259.1 m/z, found 259.1 m/z.

Hexyl galactopyranoside.

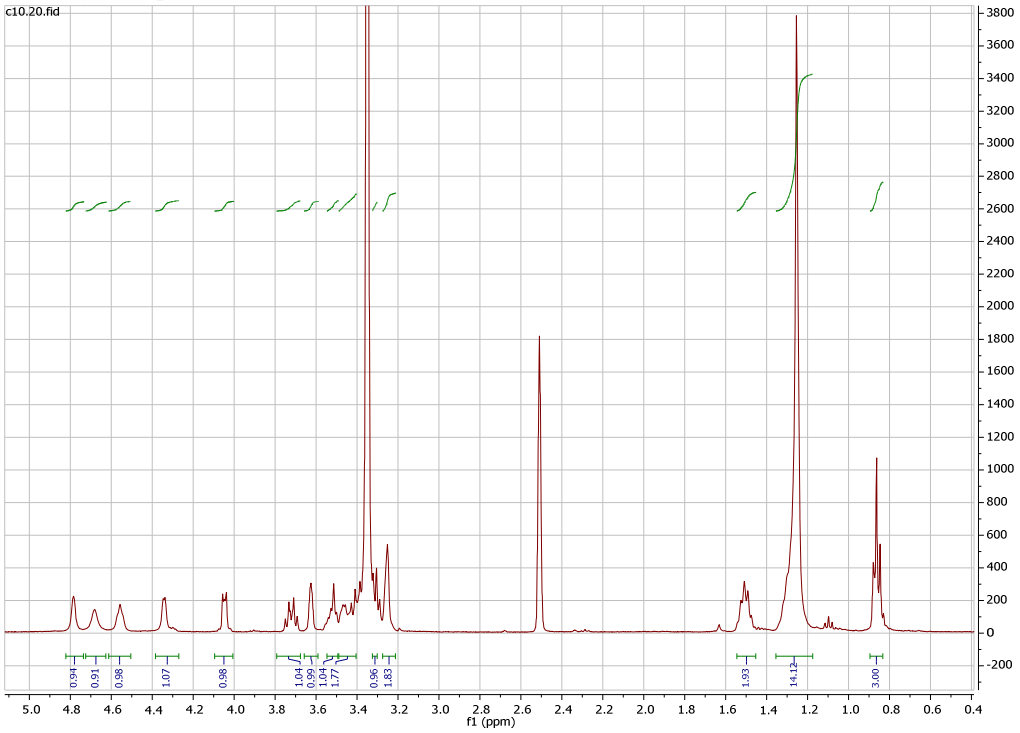


Hexyl galactopyranoside. Rf (ethyl acetate-methanol-water (17:2:1, v/v)): 0.42. ^{1}H NMR (500 MHz, DMSO-d6) δ/ppm 0.81–0.89(t, 3H), 1.21–1.34 (tdd, 6H), 1.46–1.53 (m, 2H), 3.22–3.26 (d, 2H), 3.26–3.30 (m, 1H), 3.38–3.48 (m, 2H), 3.48–3.54 (dd, 1H), 3.59–3.65 (s, 1H), 3.68–3.74 (m, 1H), 4.02–4.07 (d, 1H), 4.28–4.34 (d, 1H), 4.49–4.54 (t, 1H), 4.61–4.67 (d, 1H), 4.75–4.80 (d, 1H); HPLC-MS: exact mass calculated for $C_{12}H_{25}O_6$ [M+H$^+$]: 265.2 m/z, found 265.2 m/z.

Octyl galactopyranoside.

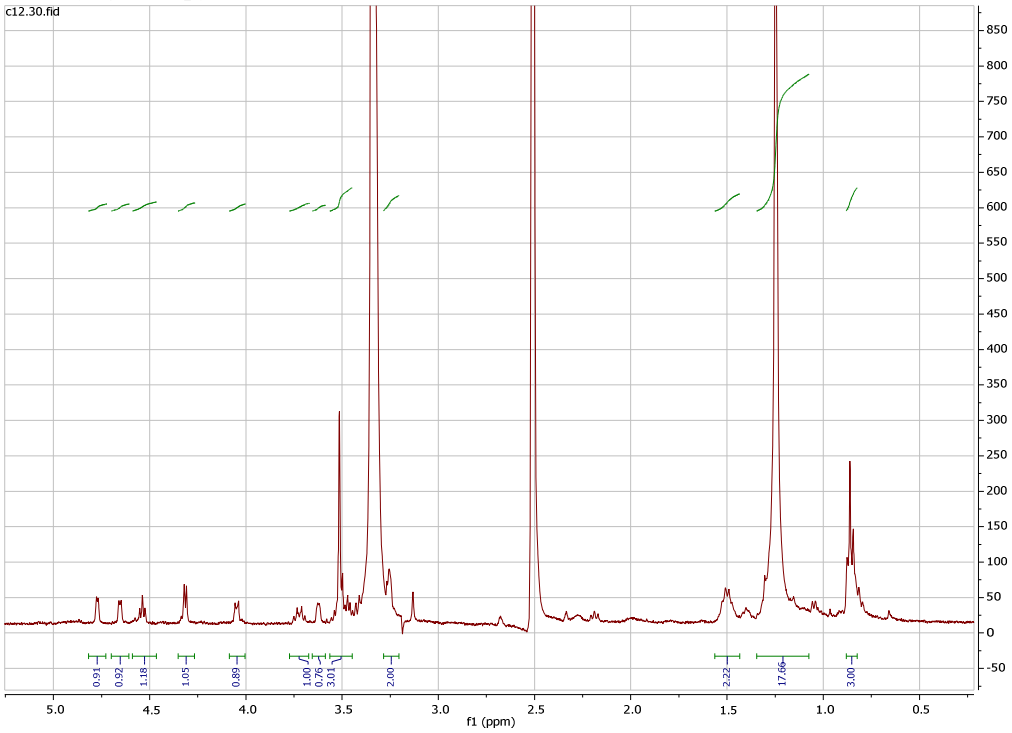
Octyl galactopyranoside. Rf (ethyl acetate-methanol-water (17:2:1, v/v)): 0.45. ^{1}H NMR (500 MHz, DMSO-d6) δ/ppm 0.82–0.91(t, 3H), 1.20–1.34 (m, 10H), 1.46–1.53 (m, 2H), 3.23–3.26 (m, 2H), 3.27–3.30 (dd, 1H), 3.37–3.48 (m, 2H), 3.49–3.54 (m, 1H), 3.60–3.64 (s, 1H), 3.68–3.74 (dt, 1H), 4.01–4.06 (t, 1H), 4.28–4.33 (d, 1H), 4.50–4.55 (t, 1H), 4.62–4.68 (d, 1H), 4.74–4.80 (d, 1H); HPLC-MS: exact mass calculated for $C_{14}H_{29}O_6$ [M+H$^+$]: 293.2 m/z, found 293.2 m/z.

Decyl galactopyranoside



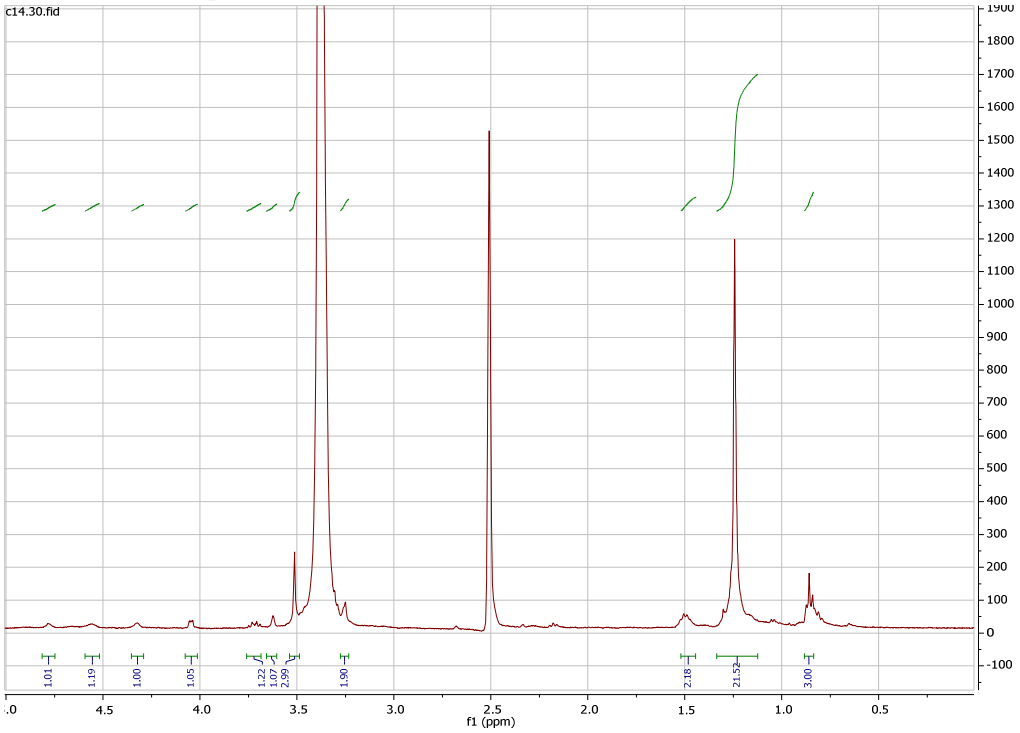
Decyl galactopyranoside. Rf (ethyl acetate-methanol-water (17:2:1, v/v)): 0.48. ^{1}H-NMR (400MHz, DMSO-d6) δ/ppm 0.83-0.90 (m, 3H), 1.18-1.35 (s, 14H), 1.45-1.55 (m, 2H) 3.21-3.28 (s, 2H), 3.30-3.32 (m, 1H), 3.41-3.49 (m, 2H), 3.49-3.55 (m, 1H), 3.59-3.66 (s, 1H), 3.68-3.79 (dt, 1H), 4.01-4.10 (m, 1H), 4.27-4.39 (s, 1H), 4.51-4.61 (s, 1H), 4.63-4.73 (s, 1H), 4.74-4.82 (s, 1H). HR-MS (ESI-IT): exact mass calculated for $C_{16}H_{32}O_6Na$ [M+Na^+]: 343.2091 m/z, found 343.2093 m/z.

Dodecyl galactopyranoside



Dodecyl galactopyranoside. Rf (ethyl acetate-methanol-water (17:2:1, v/v)): 0.51. ^{1}H-NMR (400MHz, DMSO-d6) δ/ppm 0.82-0.88 (m, 3H), 1.07-1.35 (m, 18H), 1.43-1.56 (m, 2H), 3.20-3.29 (s, 2H), 3.45-3.56 (m, 3H), 3.59-3.65 (d, 1H), 3.67-3.77 (dt, 1H), 4.01-4.09 (m, 2H), 4.27-4.35 (m, 1H), 4.47-4.59 (m 1H), 4.61-4.70 (m, 1H), 4.73-4.82 (m, 1H). HR-MS (ESI-IT): exact mass calculated for $C_{18}H_{36}O_6Na$ [M+Na^+]: 371.2404 m/z, found 371.2406 m/z.

Tetradecyl galactopyranoside.



Tetradecyl galactopyranoside. Rf (ethyl acetate-methanol-water (17:2:1, v/v)): 0.53. ^{1}H-NMR (400MHz, DMSO-d6) δ/ppm 0.84-0.88 (m, 3H), 1.12-1.34 (m, 22H), 1.44-1.52 (m, 2H), 3.23-3.28 (s, 2H), 3.49-3.54 (m, 3H), 3.61-3.66 (s, 1H), 3.69-3.76 (dt, 1H), 4,01-4.08 (m, 1H), 4.29-4.35 (s, 1H), 4.52-4.59 (s, 1H), 4.75-4.81 (s, 1H). HR-MS (ESI-IT): exact mass calculated for $C_{20}H_{40}O_6Na$ [M+Na^+]: 399.2717 m/z, found 399.2737 m/z.