In search for effective anticancer agents – novel sugar esters based on polyhydroxyalkanoate monomers

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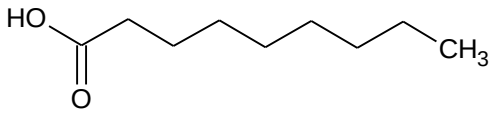
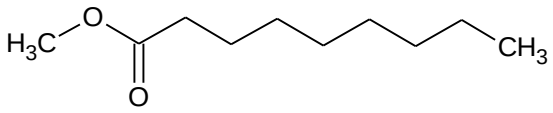
SUPPLEMENTARY

Index of figures and tables:

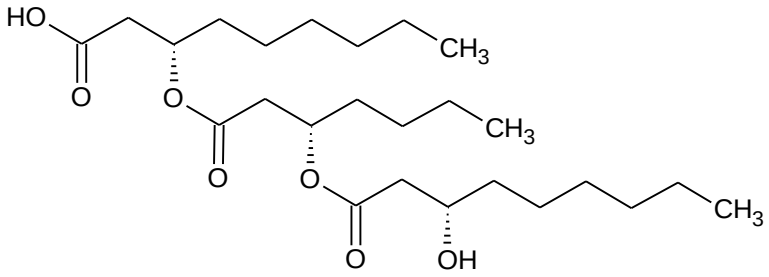
- Fig 13. IR spectra of lactose mono and diesters esters originated from mixture of fluorinated PHN monomers (F-mPHN-lac)
- Fig 14. IR spectra of fluorinated esters set together



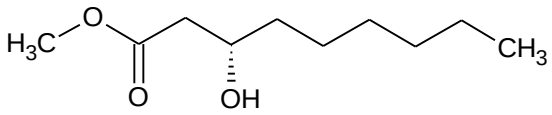
a) **C9**: nonanoic acid



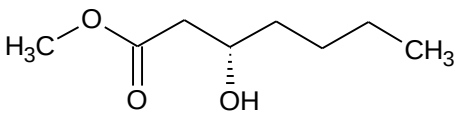
b) **C9-Me**: nonanoic methyl ester



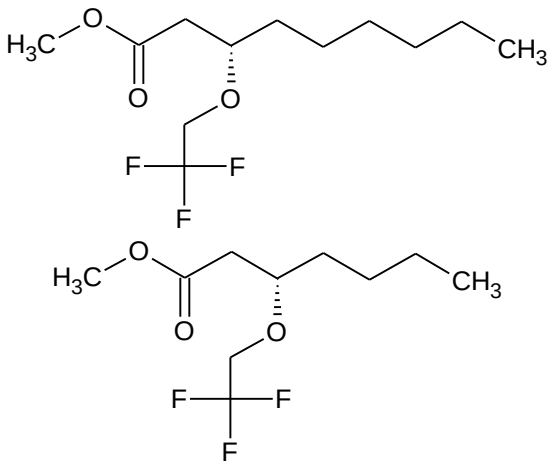
c) **PHN**: poly -(3*R*)-hydroxynonanoate- co- (3*R*)-hydroxyheptanoate



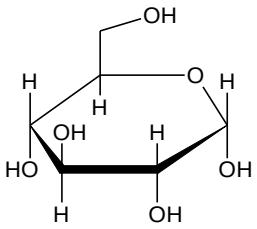
d) **R3OH-C9-Me**: mixture of (3*R*)-hydroxynonanoate methyl esters



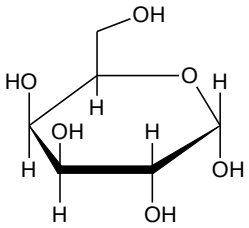
e) **R3OH-C7-Me**: mixture of (3*R*)-hydroxyheptanoate methyl esters



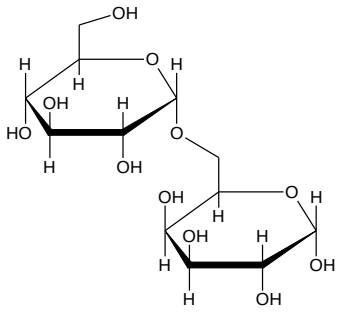
f) **F-mPHN-Me**: mixture of fluorinated PHN monomers: (3*R*)-3-(2,2,2-trifluoroethoxy)nonanoate methyl esters and (3*R*)-3-(2,2,2-trifluoroethoxy)heptanoate methyl esters



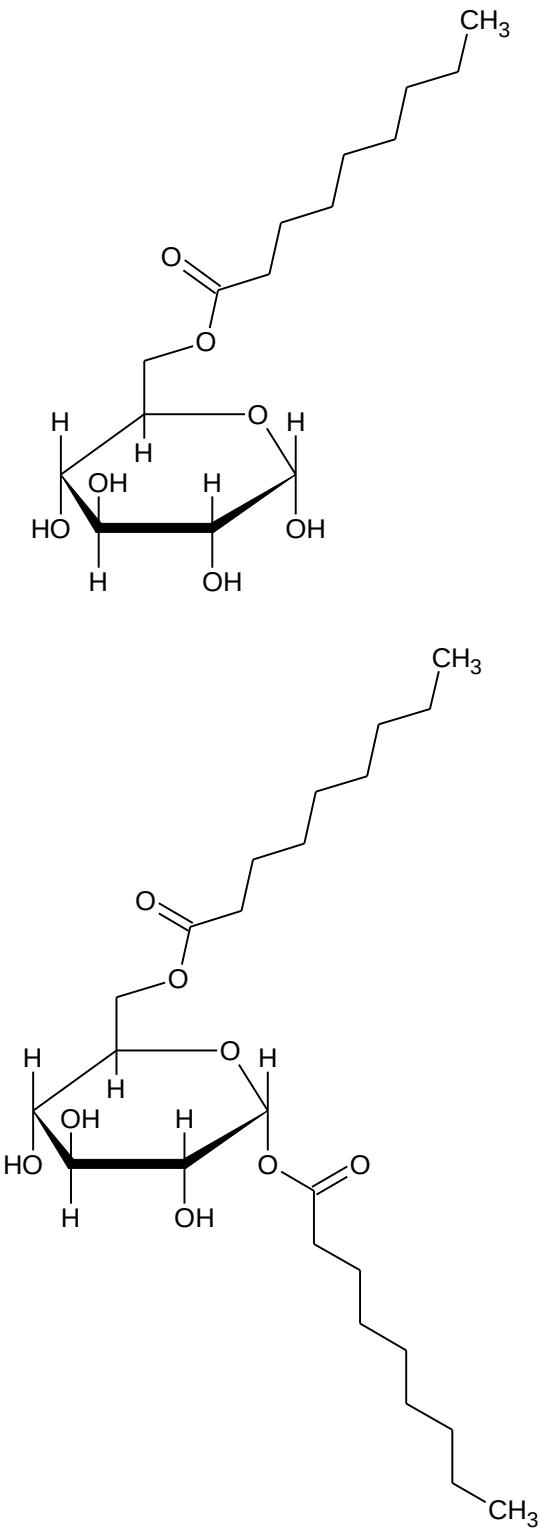
g) **glu**: α-D-glucopiranose



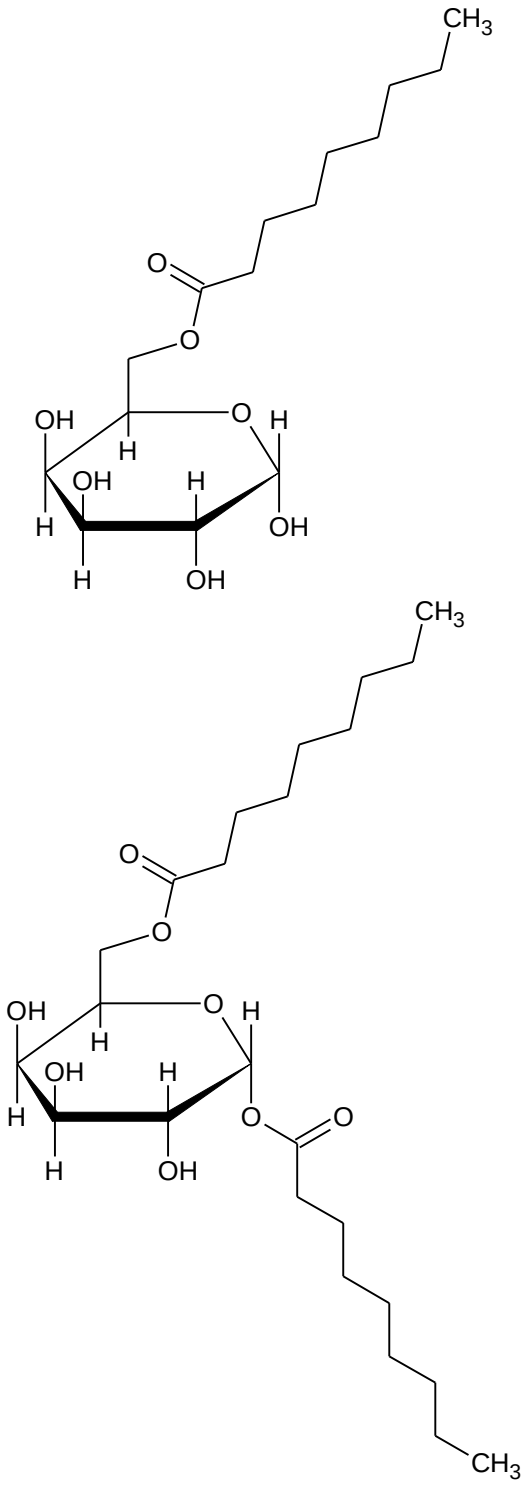
h) **gal**: β-D-galactopyranose



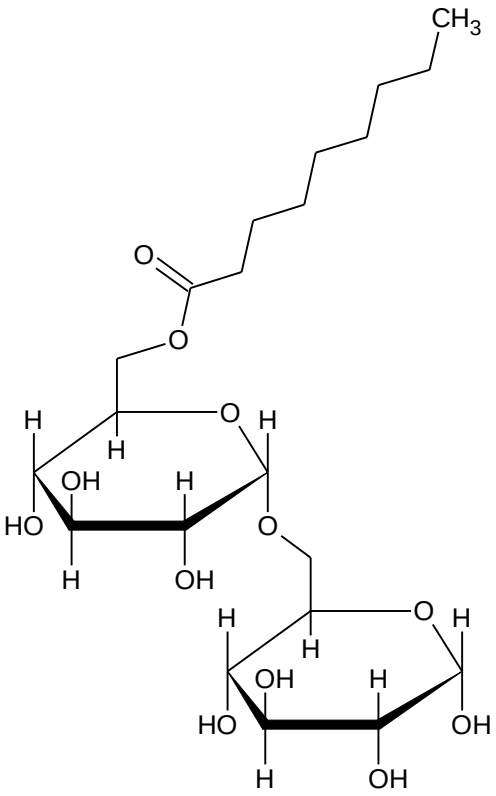
i) **lac**: β-D-Galactopyranosyl-(1→4)-D-glucopiranose



j) **C9-glu**: mixture of glucose nonanoic mono and diesters



k) **C9-gal**: mixture of galactose nonanoic mono and diesters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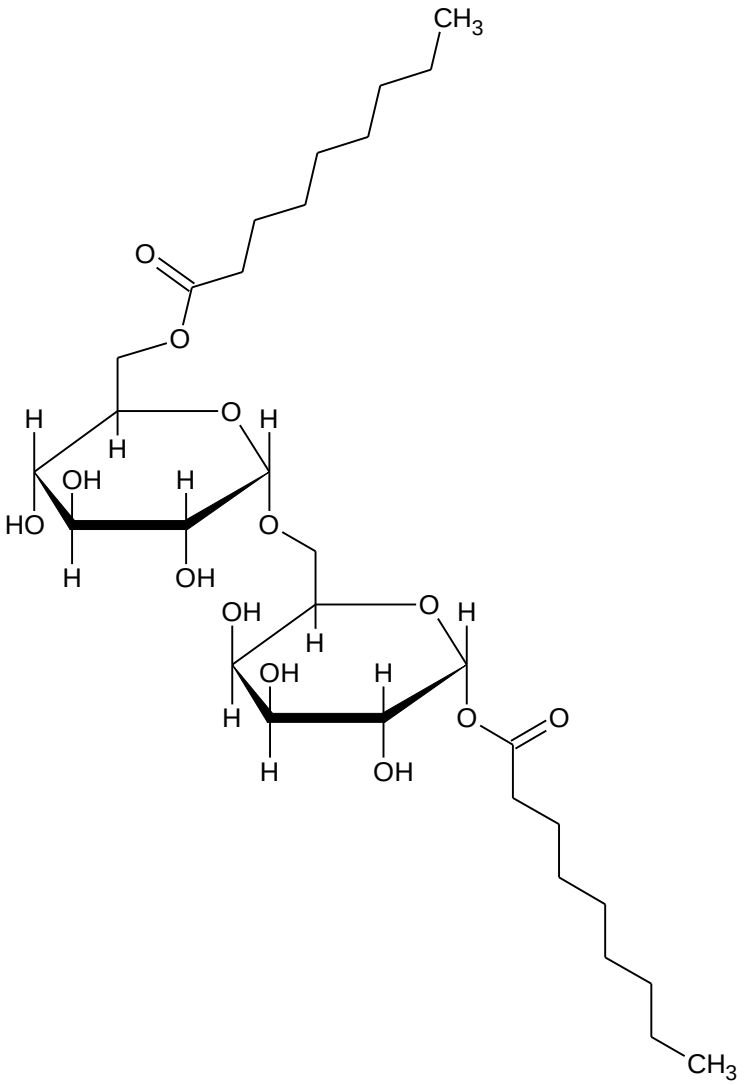
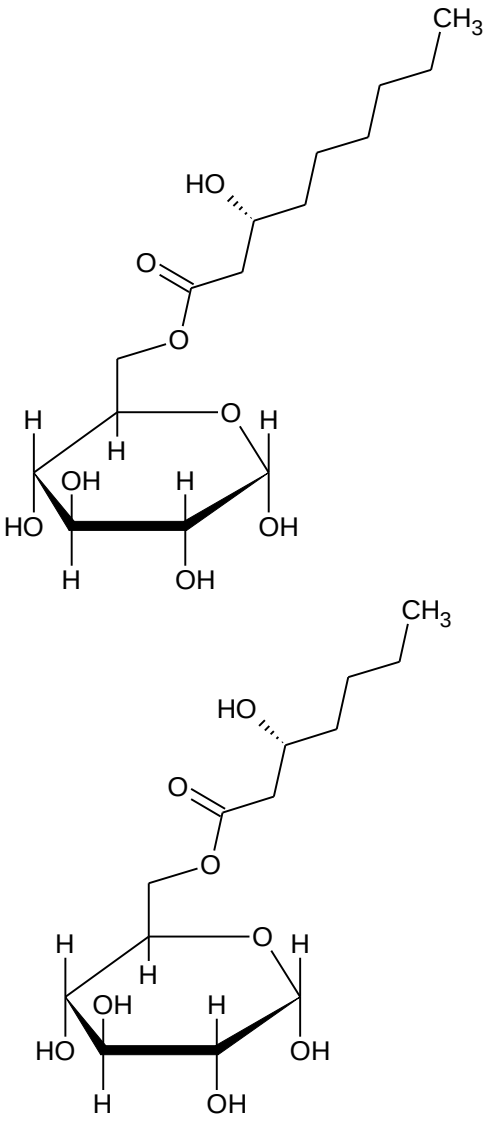


l) **C9-lac**: mixture of lactose nonanoic mono and diesters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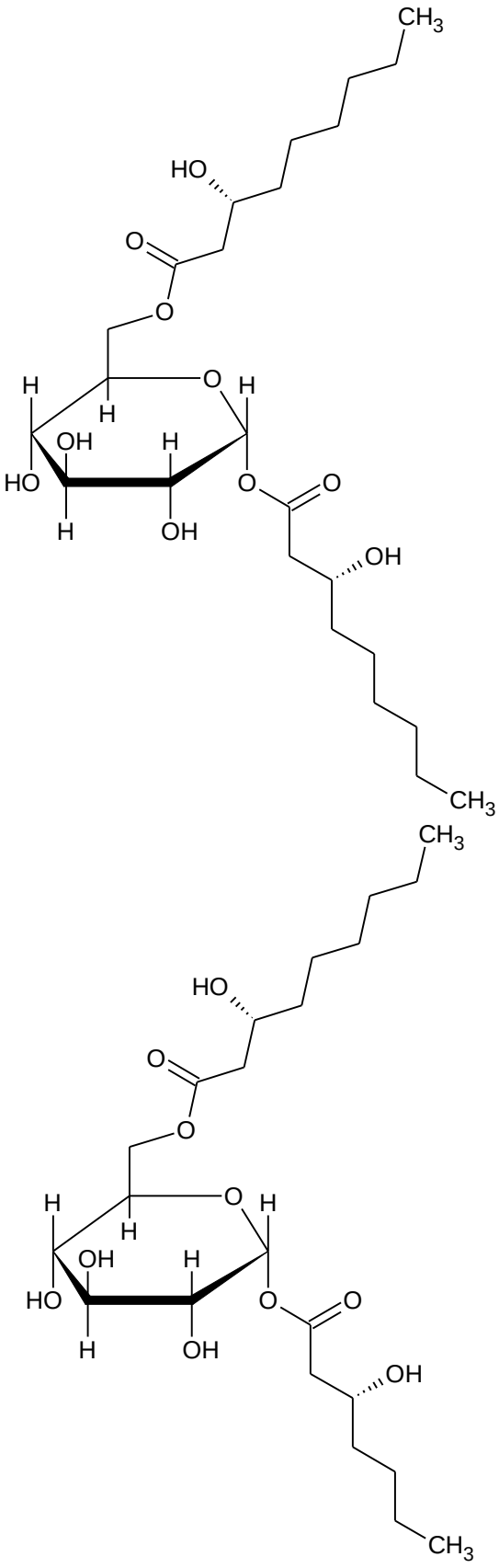
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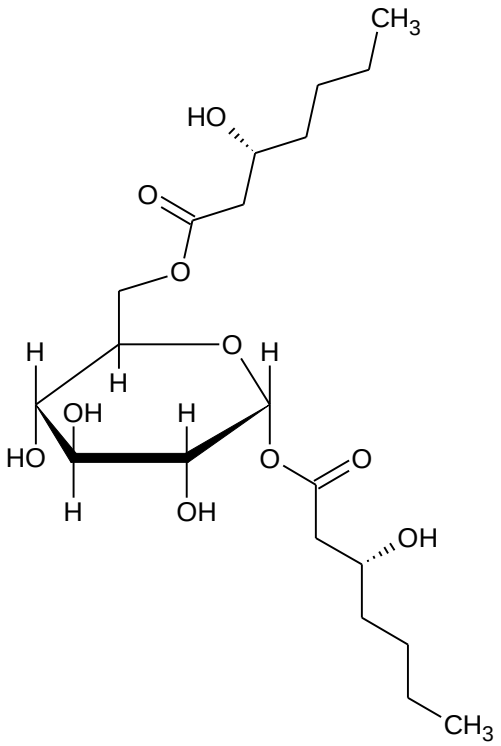
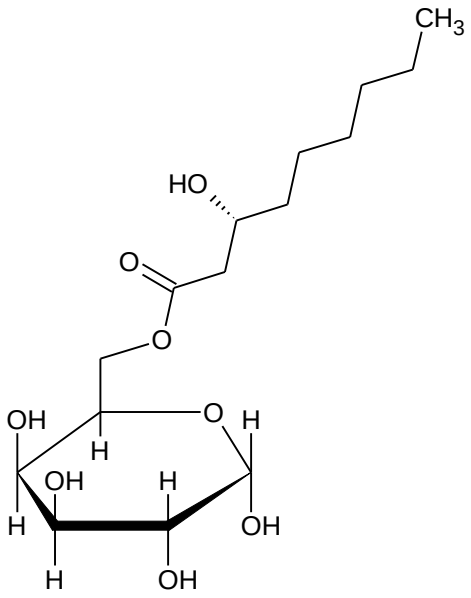
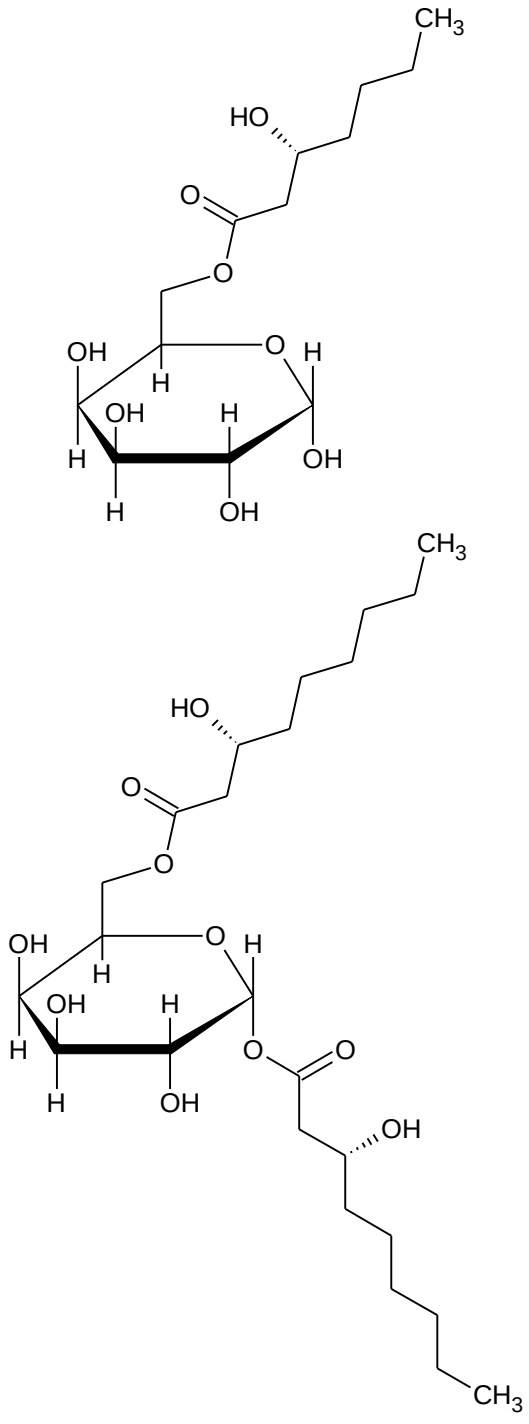


m) **mPHN-glu**: mixture of (3*R*)-hydroxynonanoate glucose esters and (3*R*)-hydroxyheptanoate glucose mono and diesters

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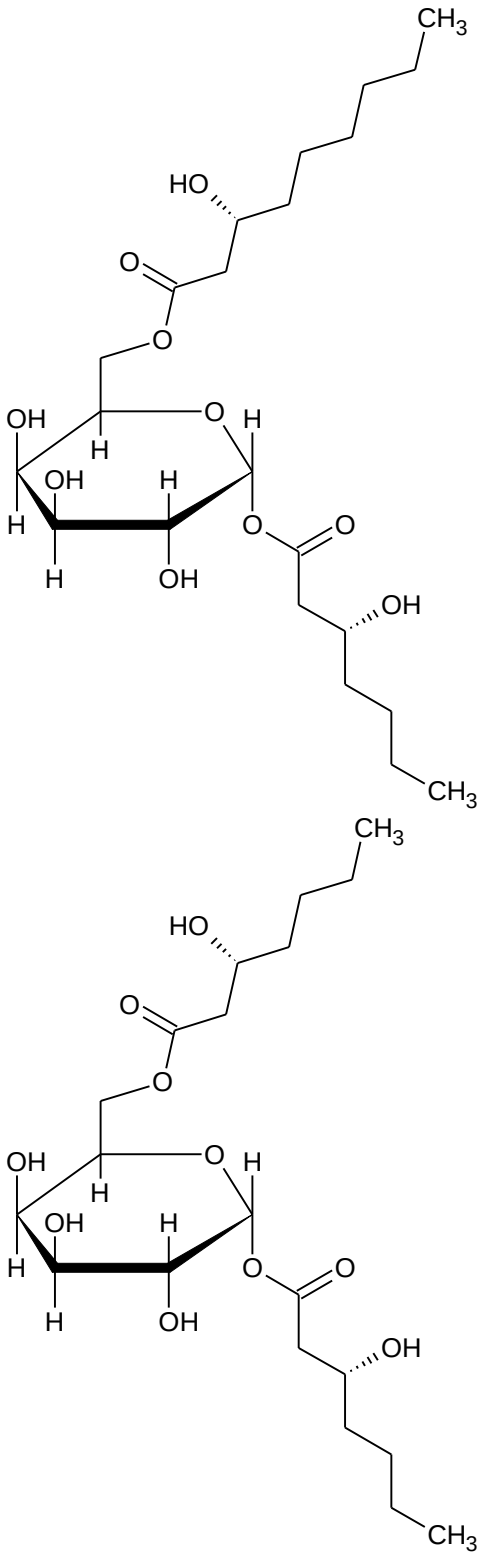
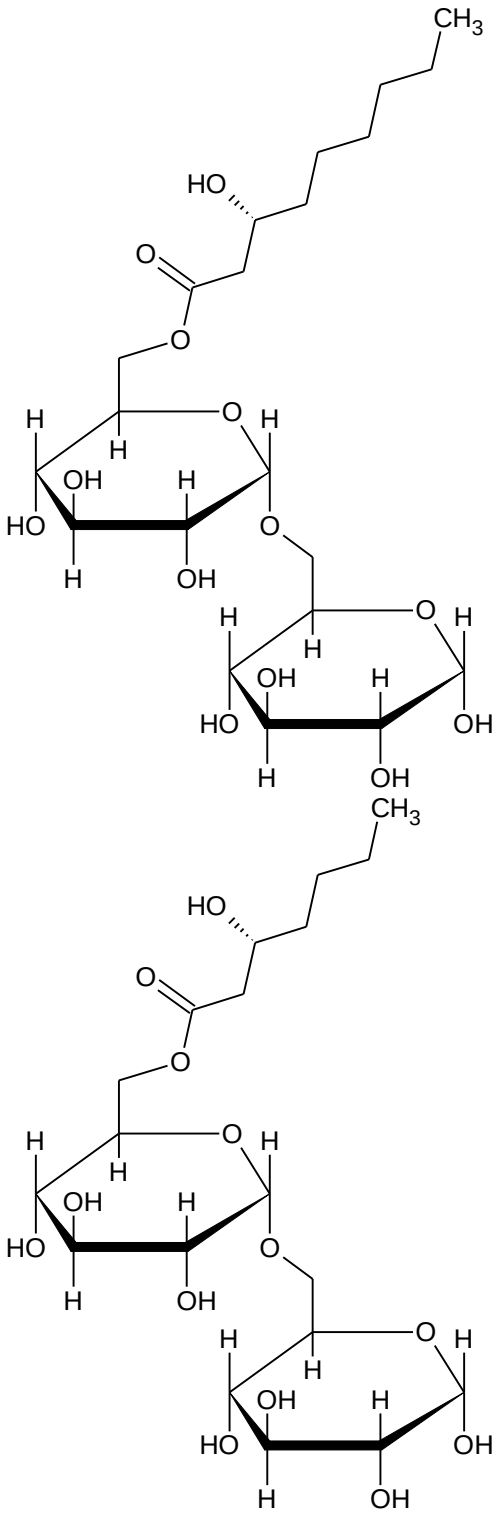


n) **mPHN-gal**: mixture of (3*R*)-hydroxynonanoate galactose esters and (3*R*)-hydroxyheptanoate galactose mono and diesters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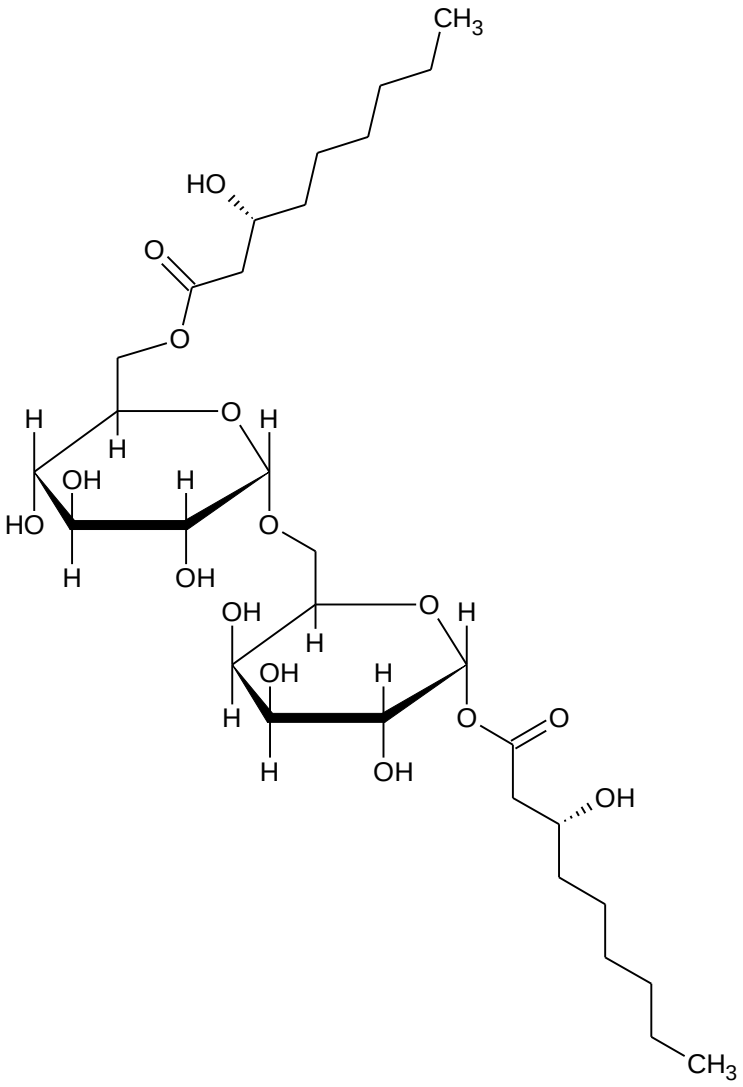
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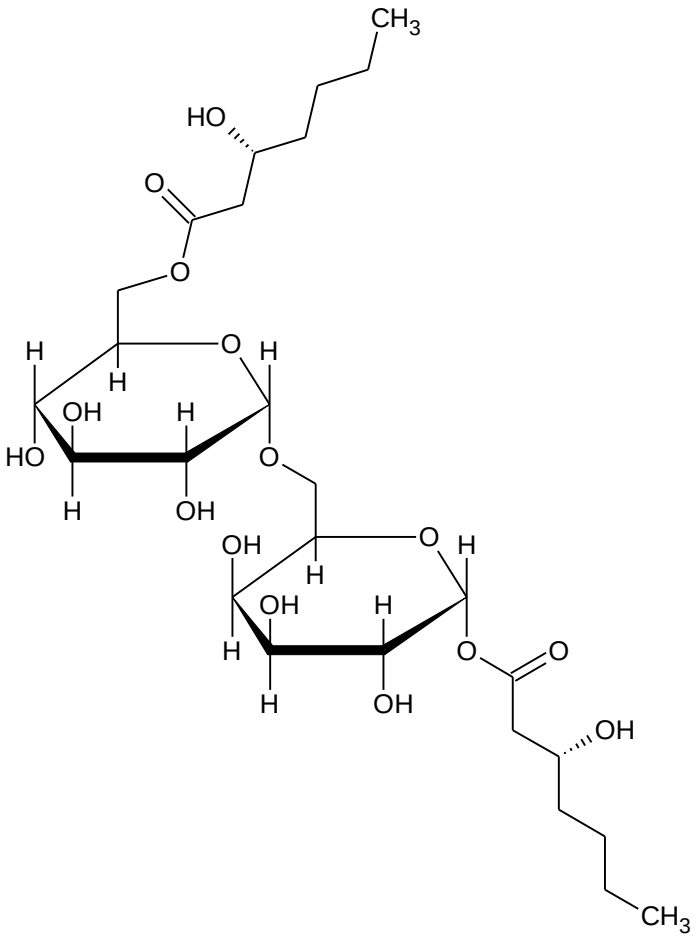
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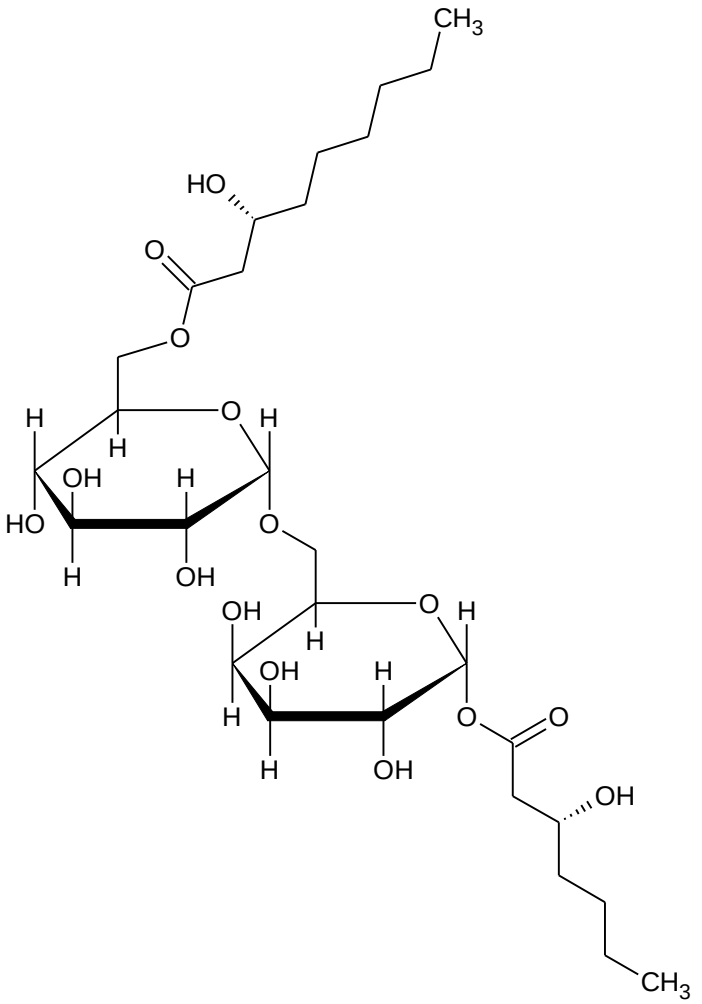
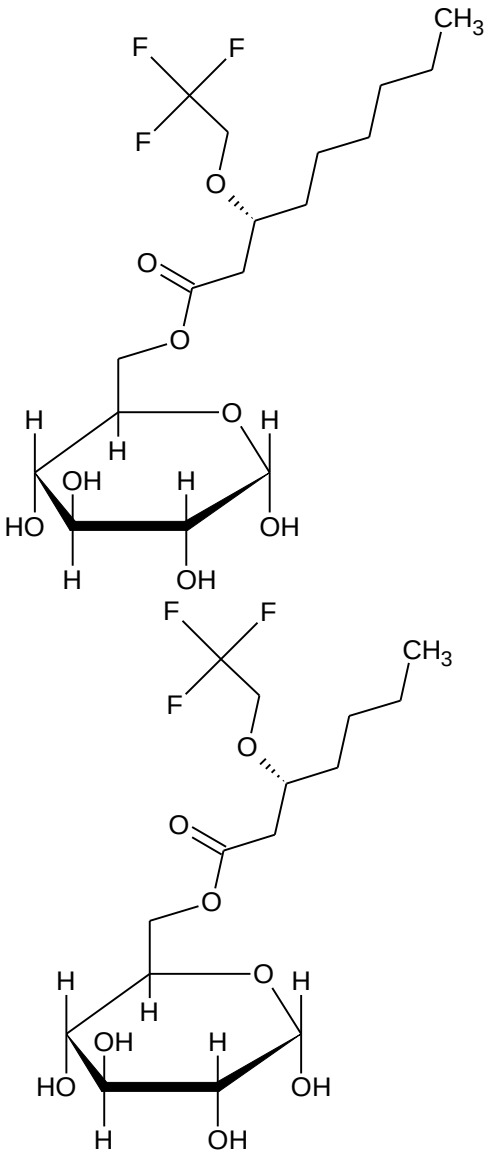


o) **mPHN-lac**: mixture of (3*R*)-hydroxynonanoate lactose and (3*R*)-hydroxyheptanoate lactose mono and diesters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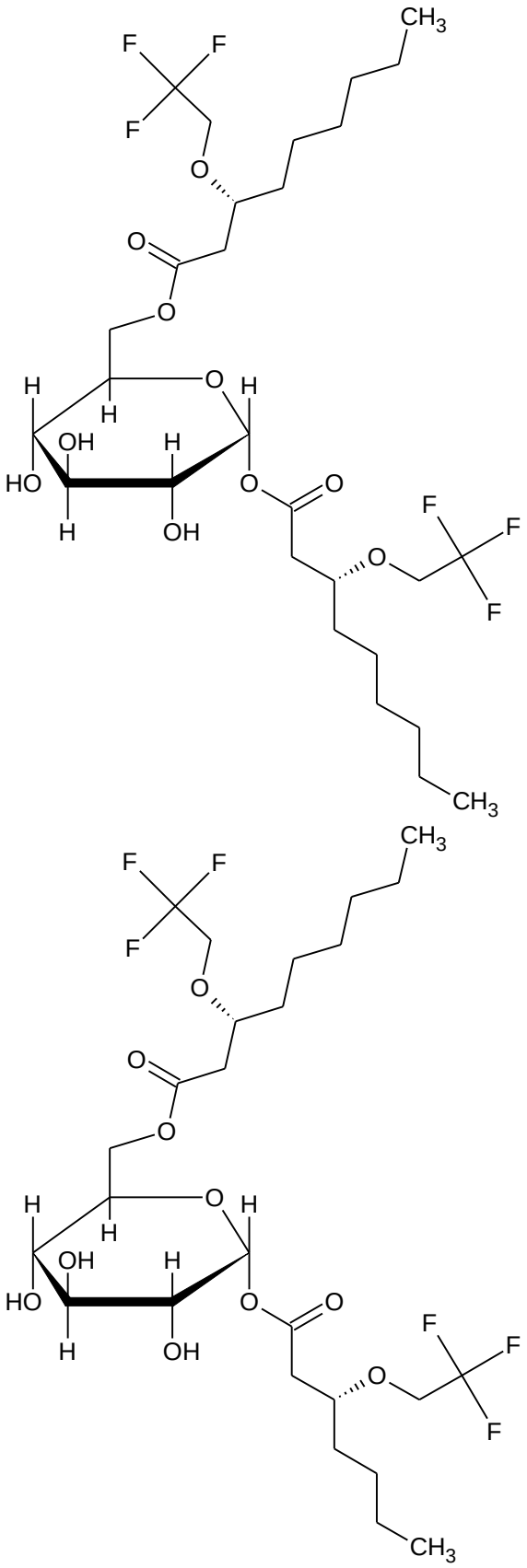
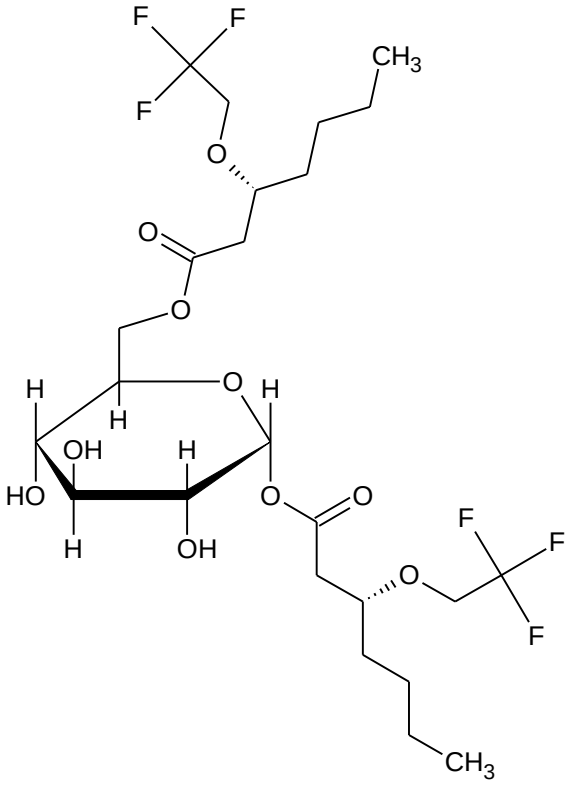
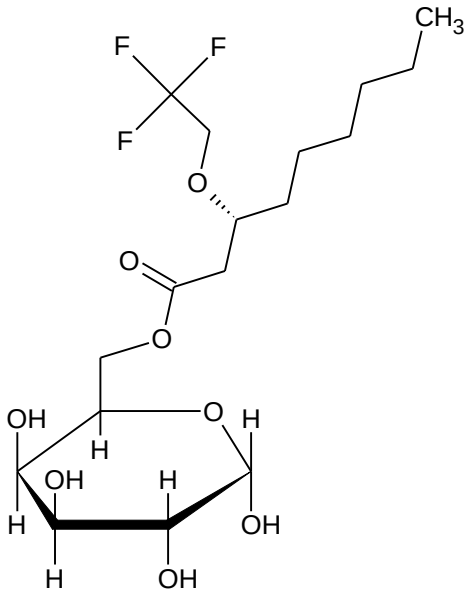
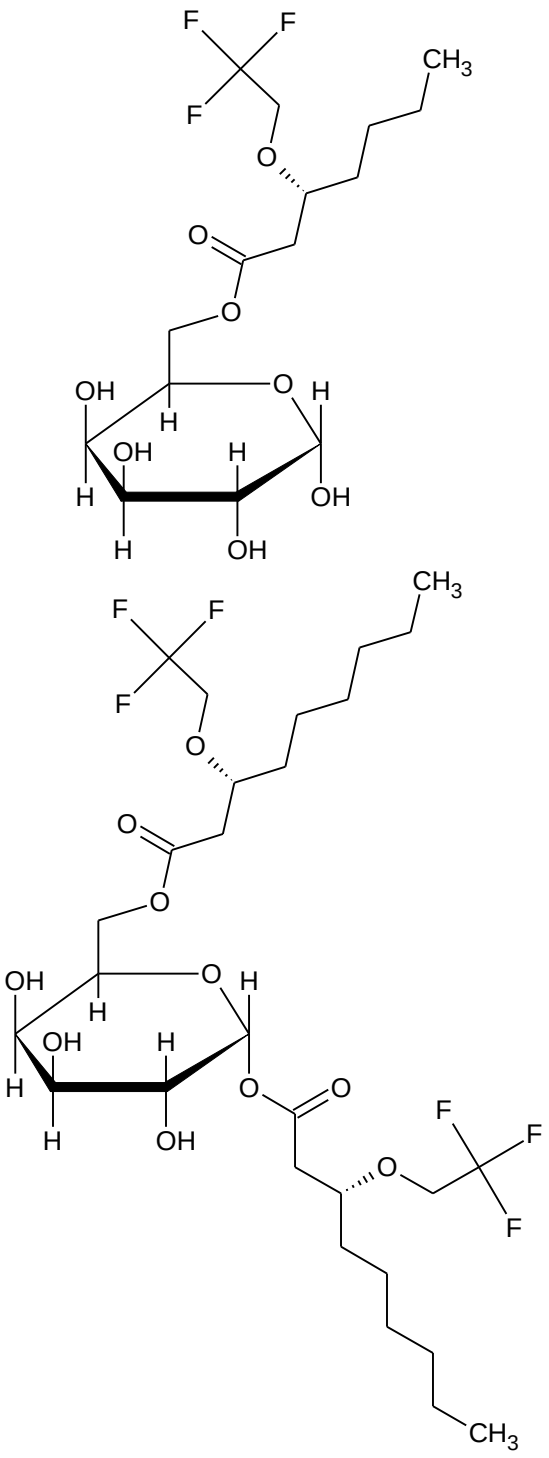




p) **F-mPHN-glu**: mixture of (3*R*)-3-(2,2,2-trifluoroethoxy)nonanoate glucose and (3*R*)-3-(2,2,2-trifluoroethoxy)heptanoate glucose mono andesters

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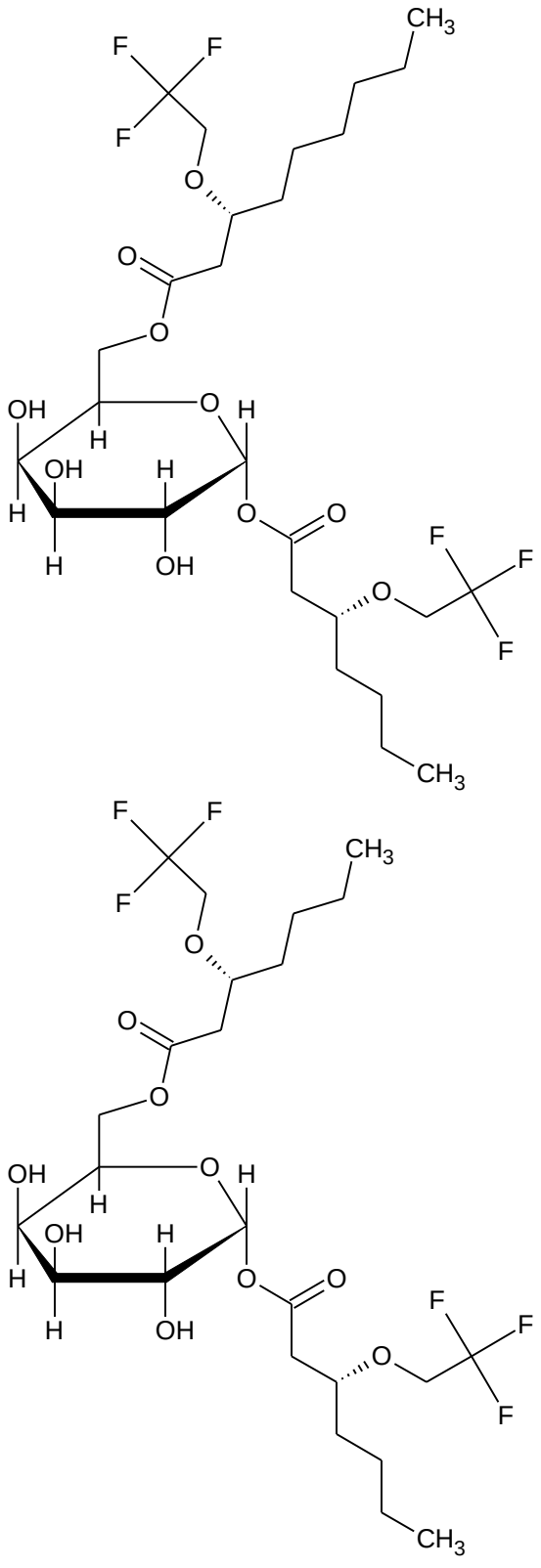
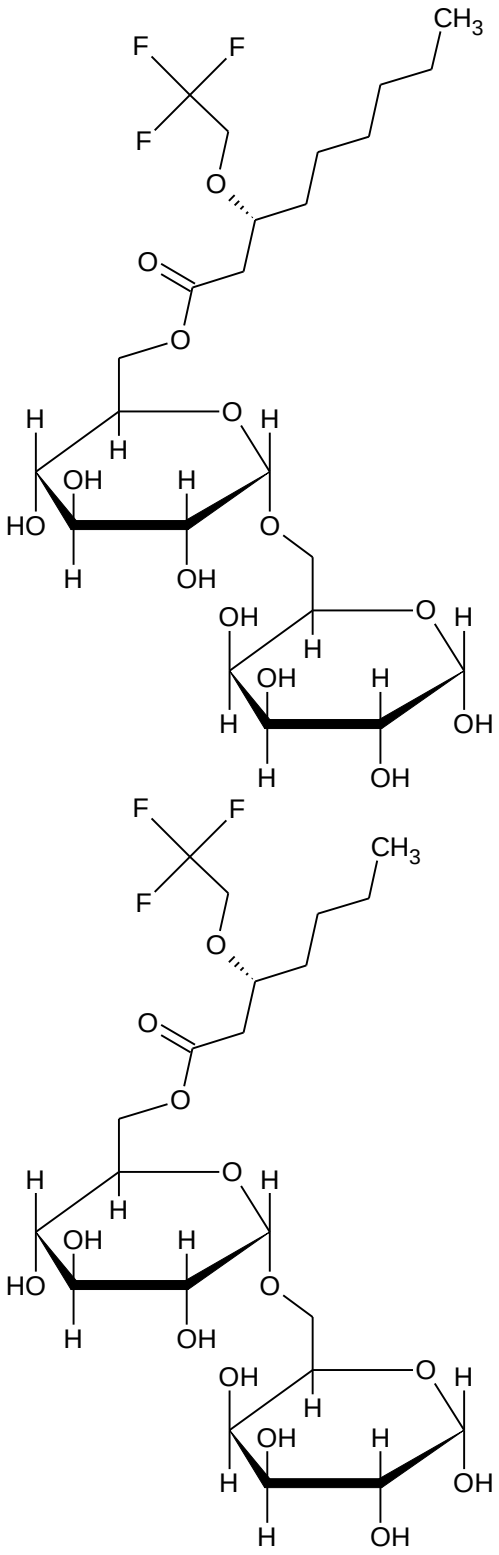


r) **F-mPHN-gal**: mixture of (3*R*)-3-(2,2,2-trifluoroethoxy)nonanoate galactose and (3*R*)-3-(2,2,2-trifluoroethoxy)heptanoate galactose mono and diesters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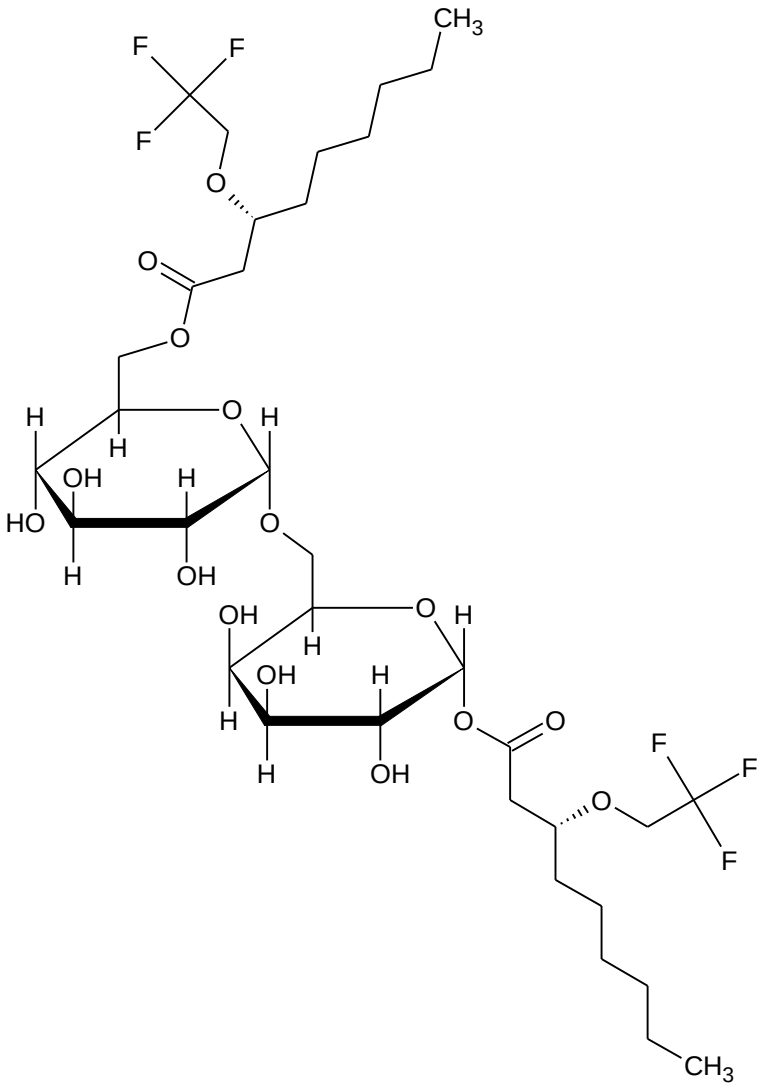
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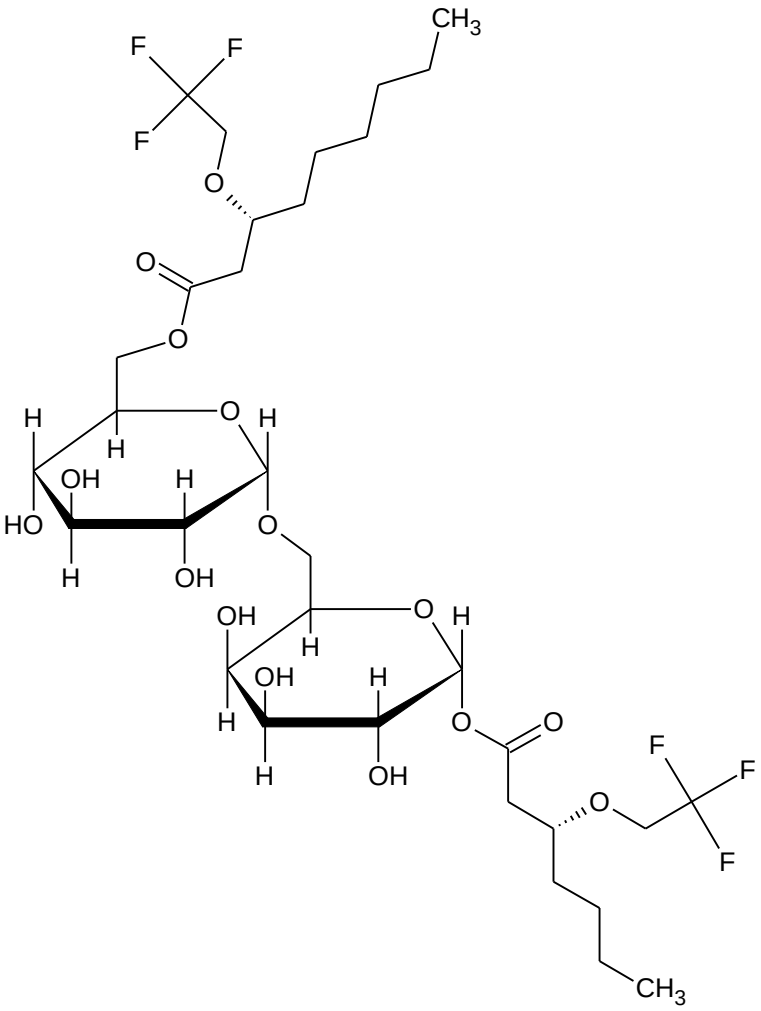
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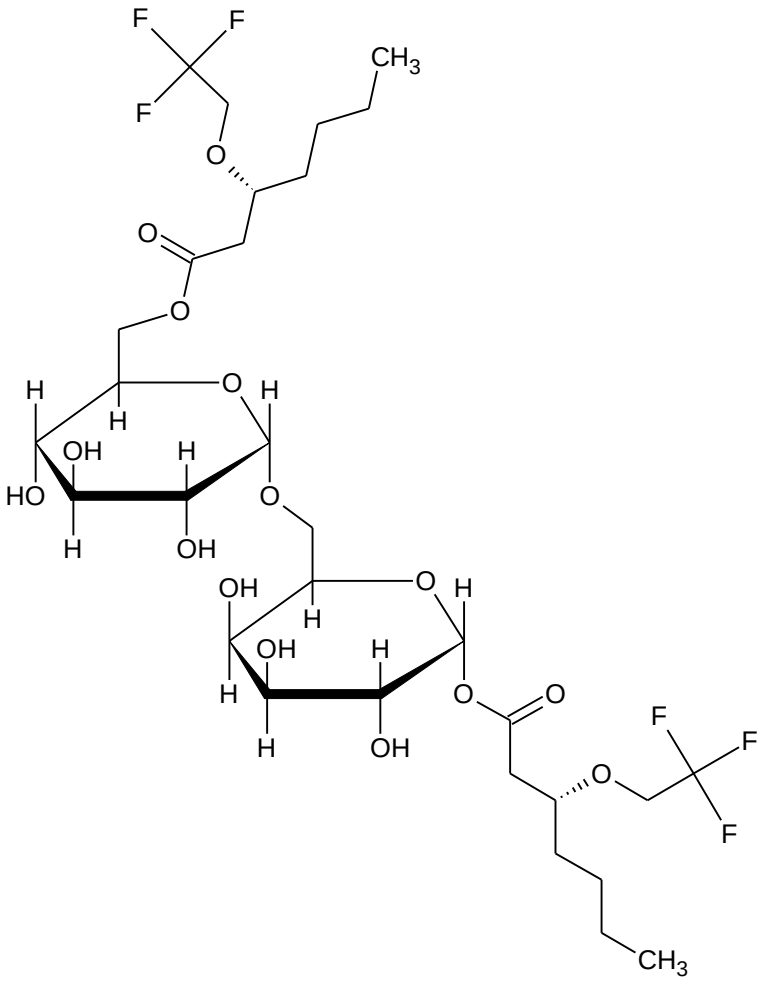


s) **F-mPHN-lac**: mixture of (3*R*)-3-(2,2,2-trifluoroethoxy)nonanoate lactose and (3*R*)-3-(2,2,2-trifluoroethoxy)heptanoate lactose mono and diesters

Fig 1. **List of compound names, shortcuts and structures generated in ChemSketch**

Table 1. Precursor and product ions of sugar esters obtained in MRM LC-MS QQQ analysis:

Compound:		MRM: Precursor ion -> product ion	Fragmentor:	Collision energy:
C9-gluc	C9 monoester	[M+K]-: 359.14 -> 341.3	93	6
		[M+K]-: 359.14 ->193.3		18
		[M+K]-: 359.14 -> 41.1		74
		[M+K]-: 359.14 -> 202.5		26
	C9 C9 diester	[M+Na]+: 483.69 -> 69	142	22
C9-gal:	C9 monoester	[M+K]-: 359.14 -> 341.3	98	6
		[M+K]-: 359.14 -> 43.1		54
		[M+K]-: 359.14 -> 202.4		18
	C9 C9 diester	[M+Na]+: 483.69 -> 69	142	22
C9-lac:	C9 monoester	[M+H_2O+H]-: 517.2 -> 157.1	136	22
		[M+H_2O+H]-: 517.2 -> 282.1		46
	C9 C9 diester	[M+Na]+: 657.3 -> 157.1	131	30
mPHN- glu:	C9 monoester	[M+Cl]-: 371,15 -> 35	98	14
	C7 monoester	[M+K]+: 429.11 -> 73.1		
		[M+MeOH]+: 340.15 -> 295.7	132	6
		[M+MeOH]+: 340.15 -> 180.4		26
	C9 C9 diester	[M+Cl]-: 527,26 -> 35	137	30
	C7 C7 diester	-	-	-
	C9 C7 diester	[M+Cl]-: 499.23 -> 35	137	18
mPHN- gal:	C9 monoester	[M+Cl]-: 371.15 -> 35	98	14
	C7 monoester	[M+K]+: 429.11 -> 73.1		
	C9 C9 diester	[M+H]+: 493.3 -> 59.1	113	18
		[M+H]+: 493.3 -> 42.1		126
	C7 C7 diester	[M+H]+: 437.24 -> 23.1	181	30
mPHN -lac:	C9 monoester	[M+H2O+H]-: 517.2 -> 157.1	116	26
		517.2 -> 282.1	116	38
	C9 C9 diester	[M+Na]+: 657.3 -> 157.1	111	26
F-mPHN-gluc:	C9 monoester	[M+K]+: 457.14 -> 23	172	34
	C7 monoester	[M+K]:+429.11 -> 73.1	245	46
		[M+K]:+429.11 -> 45		94
		[M+K]:+429.11 -> 43.1		178
		[M+K]:+429.11 -> 218.7		22
	C9 C9 diester wth two –CF_3	[M+MeOH]+: 688.3 -> 73.1	147	70
		[M+MeOH]+: 688.3 -> 355.1		26
	C9 C7 diester with one –CF_3	[M+Na]+: 651.26 -> 73	235	78
		[M+Na]+: 651.26 -> 45.1		166
	C9 C7 diester with one –CF_3	[M-H]-: 627.26 -> 325	191	38
F-mPHN-gal:	C9 C7 diester with two –CF_3	[M+H]+: 629.28 -> 324.4	113	14
	C7 C7 diester with one –CF_3 group	[M+H]+: 457.14 -> 23.1	167	38
F-mPHN-lac:	C9 C9 diester with two –CF_3 groups	[M+MeOH]+: 851.36 -> 73.1	162	114
		[M+MeOH]+: 851.36 -> 436.4		50
	C9 C9 diester with one –CF_3 group	[M+Na]+: 759.34 -> 73.1	127	78
		[M+Na]+: 759.34 -> 45		190
	C7C7diester with one -CF_3	[M+Na]+: 703.28 -> 355.1	118	30
	C7C7 with two –CF_3 groups	[M+Na]+: 833.28 -> 73.1	181	122
		[M+Na]+: 833.28 -> 45		198

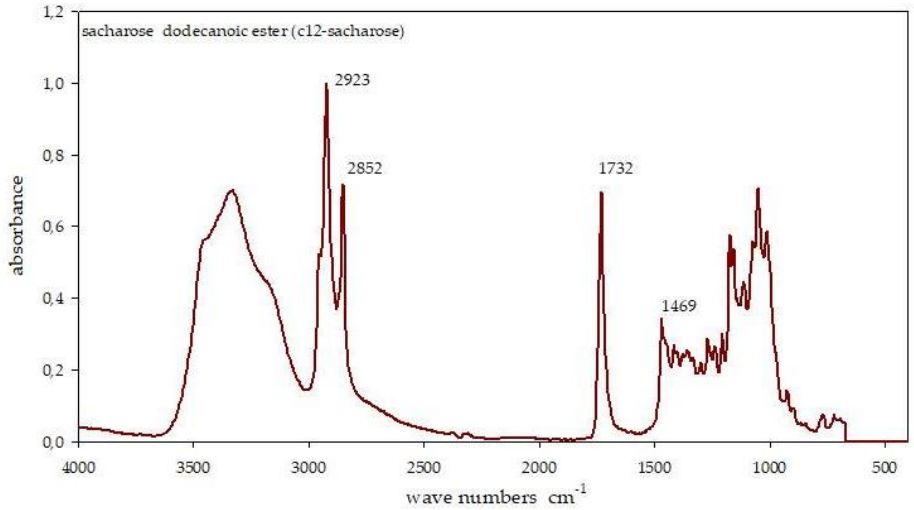


Fig 2. IR spectrum of C12- (sucrose monolaurate) as a referring compound

Following peaks inform about:

- Stretching vibrations: 3000- 3500 cm^{-1} indicate presence -OH group
- Stretching vibrations: 2900 cm^{-1} come from -CH_2 aliphatic residues
- Stretching vibrations around 1725 cm^{-1} responsible for ester bonds

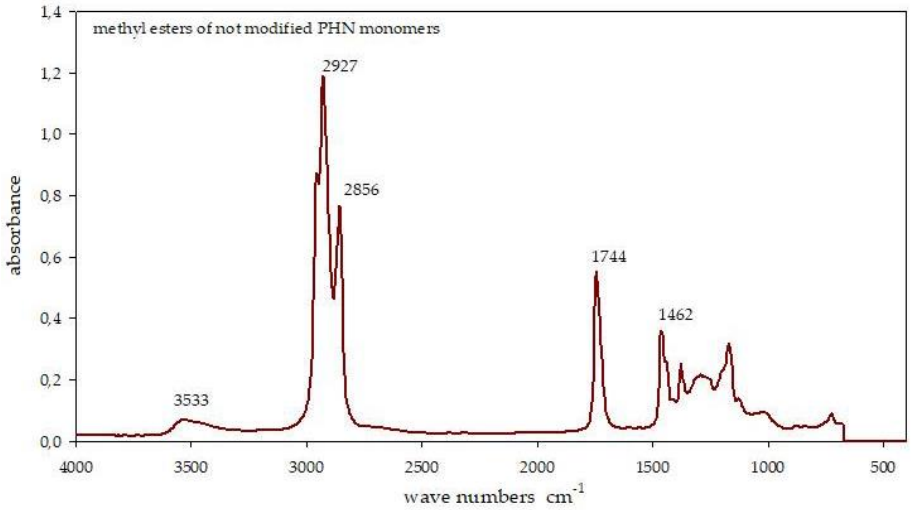
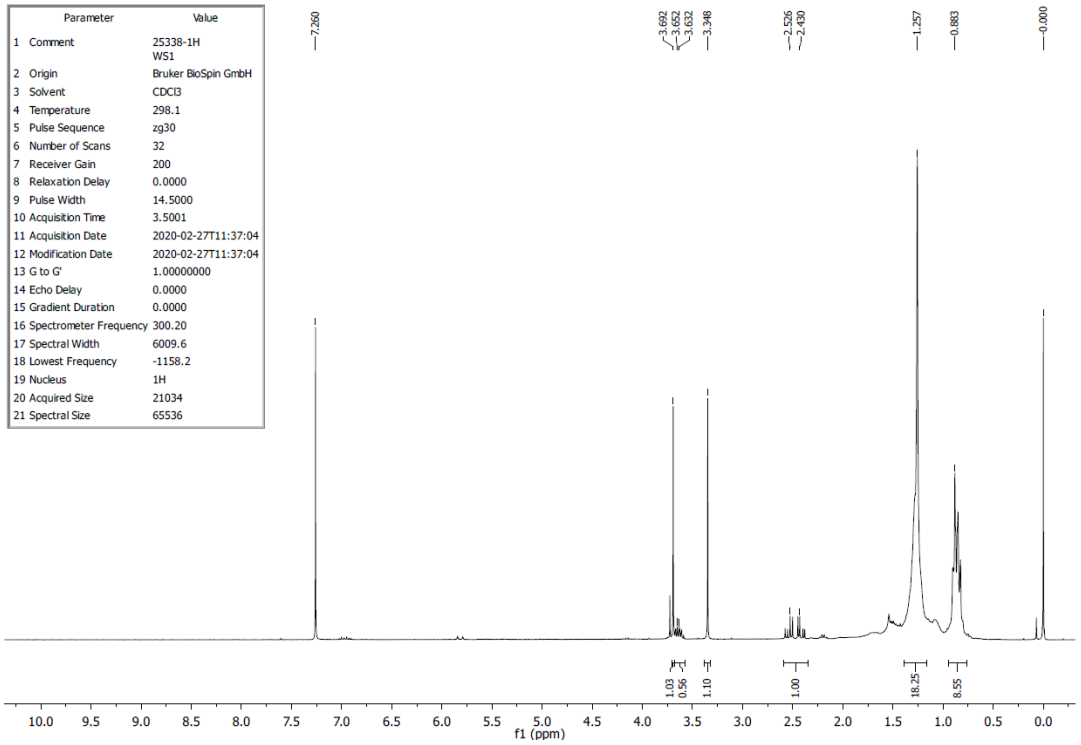
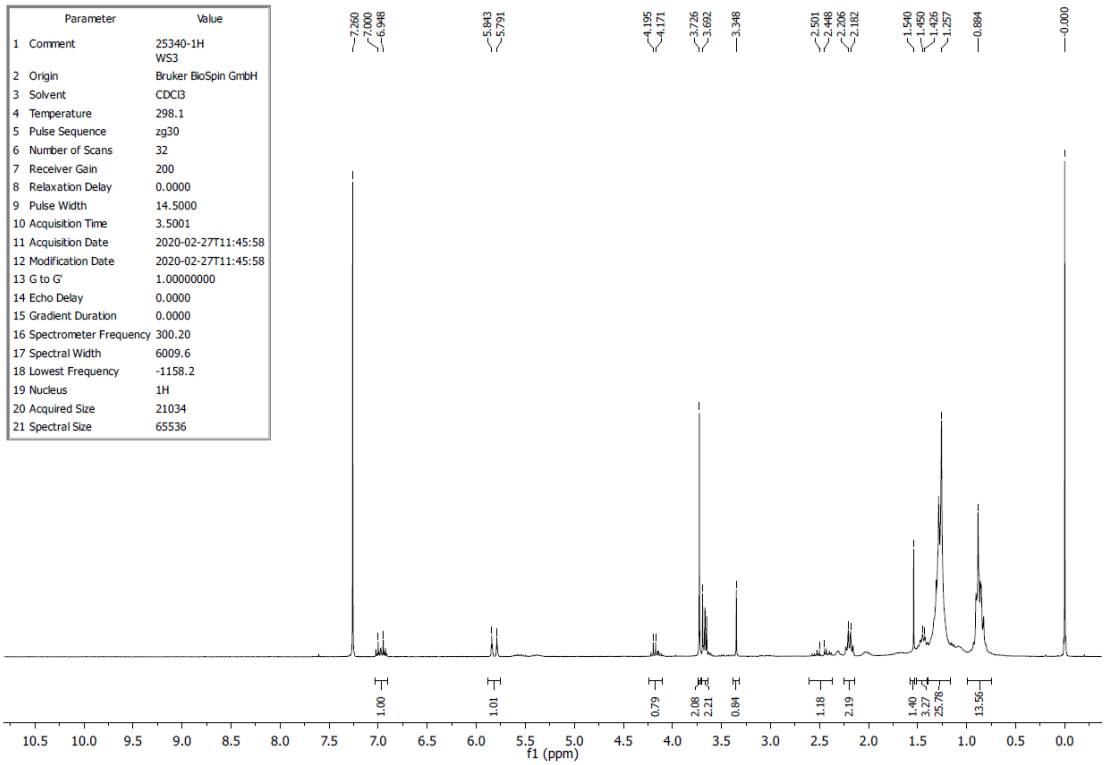


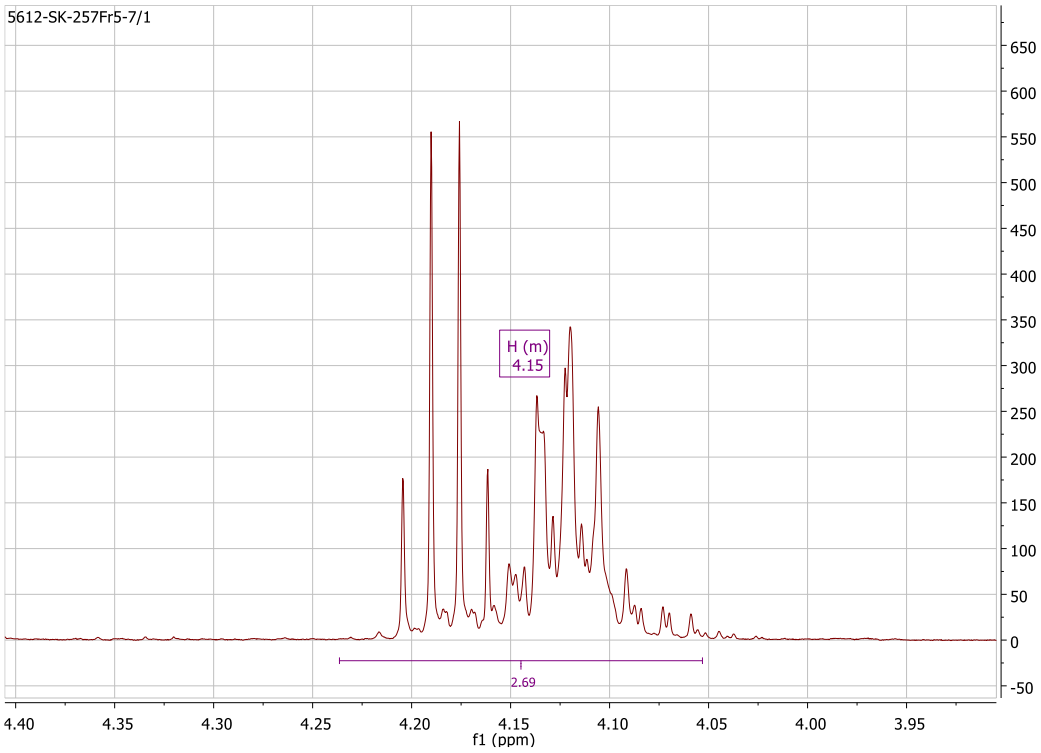
Fig 3. IR spectra of methyl esters of not modified PHN monomers (mixture of 3-(*R*)- hydroxynonanoic and 3-(*R*)- hydroxyheptanoic acids)

	Parameter	Value
1	Comment	25338-1H WS1
2	Origin	Bruker BioSpin GmbH
3	Solvent	CDCl3
4	Temperature	298.1
5	Pulse Sequence	zg30
6	Number of Scans	32
7	Receiver Gain	200
8	Relaxation Delay	0.0000
9	Pulse Width	14.5000
10	Acquisition Time	3.5001
11	Acquisition Date	2020-02-27T11:37:04
12	Modification Date	2020-02-27T11:37:04
13	G to G'	1.00000000
14	Echo Delay	0.0000
15	Gradient Duration	0.0000
16	Spectrometer Frequency	300.20
17	Spectral Width	6009.6
18	Lowest Frequency	-1158.2
19	Nucleus	1H
20	Acquired Size	21034
21	Spectral Size	65536

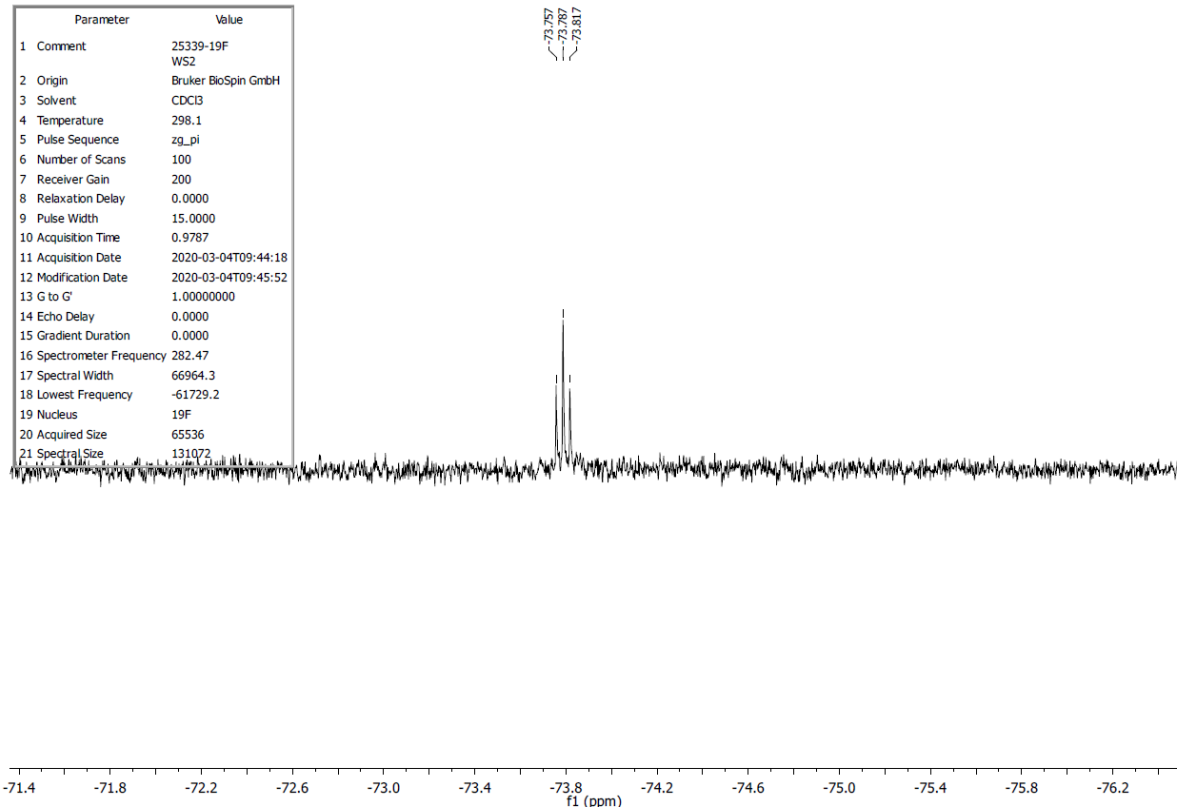


	Parameter	Value
1	Comment	25340-1H WS3
2	Origin	Bruker BioSpin GmbH
3	Solvent	CDCl3
4	Temperature	298.1
5	Pulse Sequence	zg30
6	Number of Scans	32
7	Receiver Gain	200
8	Relaxation Delay	0.0000
9	Pulse Width	14.5000
10	Acquisition Time	3.5001
11	Acquisition Date	2020-02-27T11:45:58
12	Modification Date	2020-02-27T11:45:58
13	G to G'	1.00000000
14	Echo Delay	0.0000
15	Gradient Duration	0.0000
16	Spectrometer Frequency	300.20
17	Spectral Width	6009.6
18	Lowest Frequency	-1158.2
19	Nucleus	1H
20	Acquired Size	21034
21	Spectral Size	65536

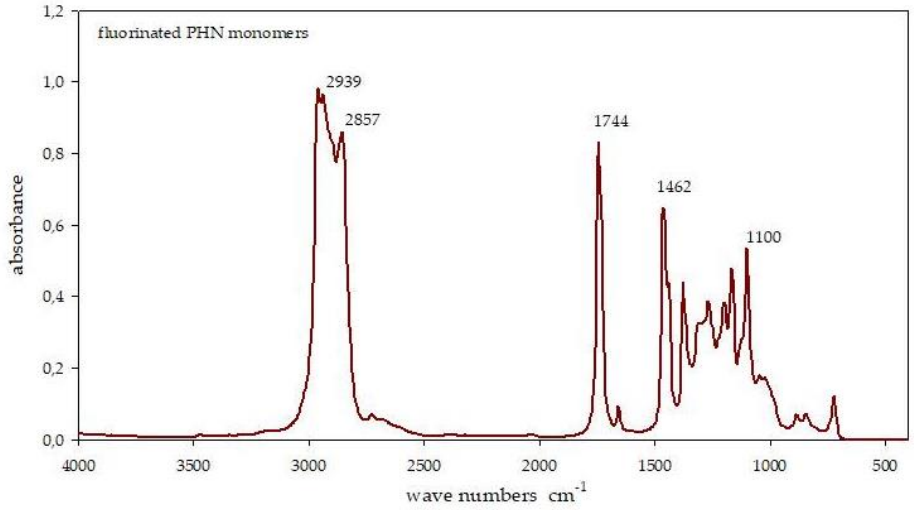




a) [1]HNMR spectra



b) [19]F NMR spectra of the modified PHN monomers confirmed a presence of fluoric moieties



c) IR spectra of the purified PHN mixture after modification

Fig 4. Spectra analysis of methyl esters of methyl esters of 3-(*R*)- (2 -2′- 2′′- trlifluoroethyl)-nonanoic and heptanoic acids (fluorinated PHN monomers or mPHN-F)

IR Spectra description:

Stretching vibrations between ~ 1360-1090 cm^{-1} suggests presence of -CF_3 groups

Stretching vibrations between ~ 1360-1000 cm^{-1} and 1110-1000 cm^{-1} suggests presence of –CF bonds

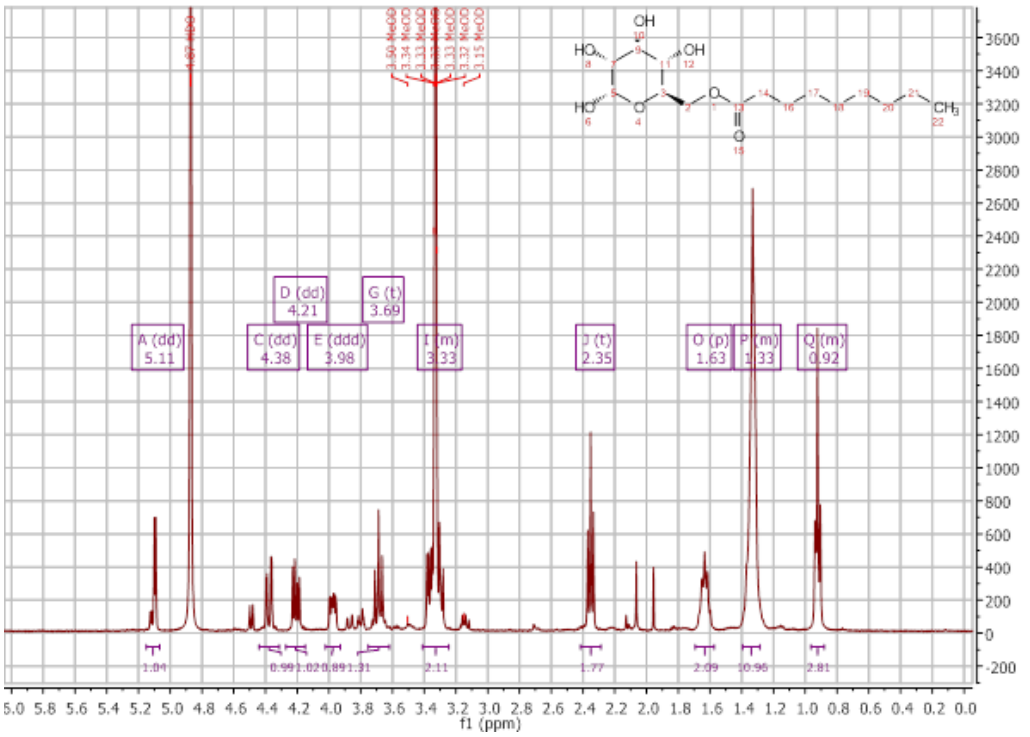
And 2859 cm^{-1} for -C-O-C etheric bonds

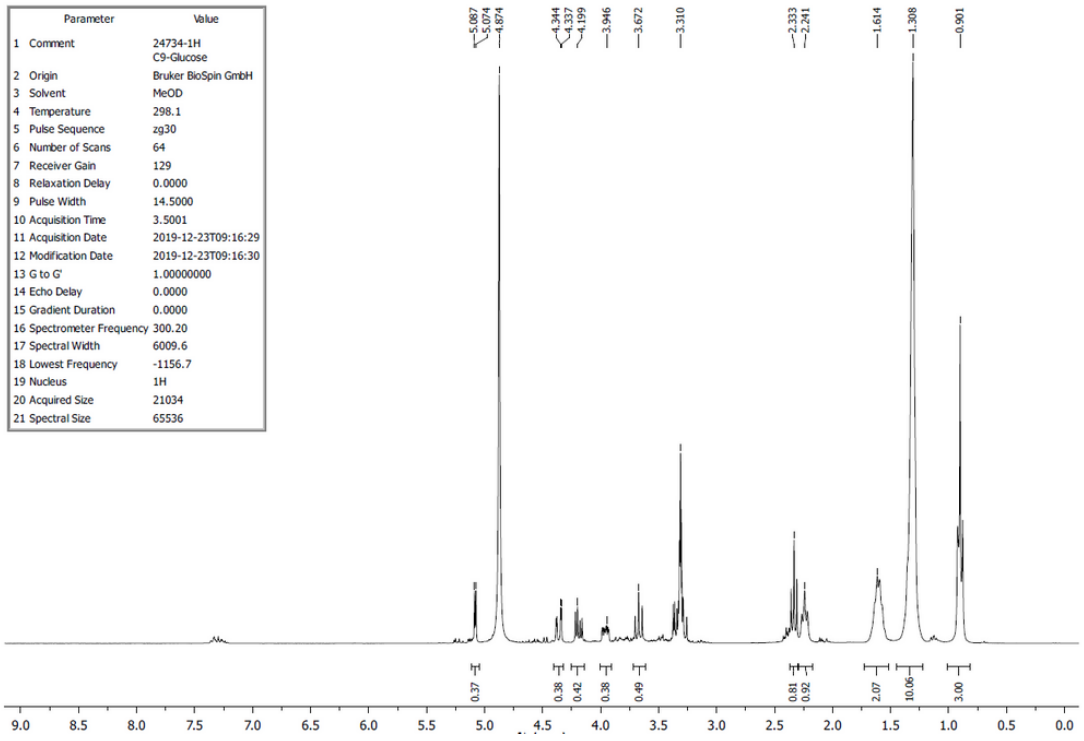
1734 cm^{-1} stretching vibrations inform about presence of ester bonds between methyl groups and carboxyl groups of PHN monomers

Spectrum profile refers to inverted IR spectra of 2,2,2-Trifluoroethyl Methyl Ether taken from*:*

"Low-Resolution Microwave, Infrared, and Raman Spectra, Conformational Stability, and Vibrational Assignment of 2,2,2-Trifluoroethyl Methyl Ether"; J. Phys. Chem. 1987, 91, 1334-1344

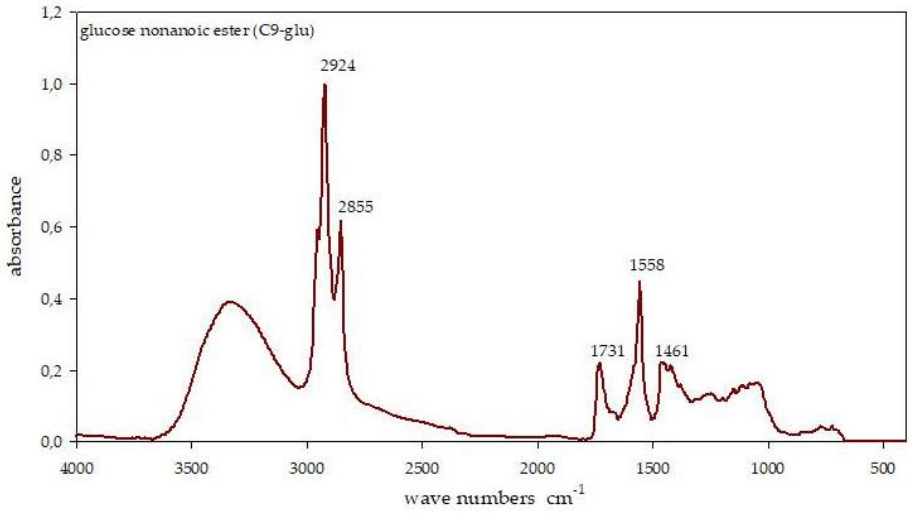
Additionally, lack of large peak between 3000-3500 cm^{-1} suggests –OH groups are not present in a structure of the analyzed compound which confirms that reaction of protecting –OH group occurred





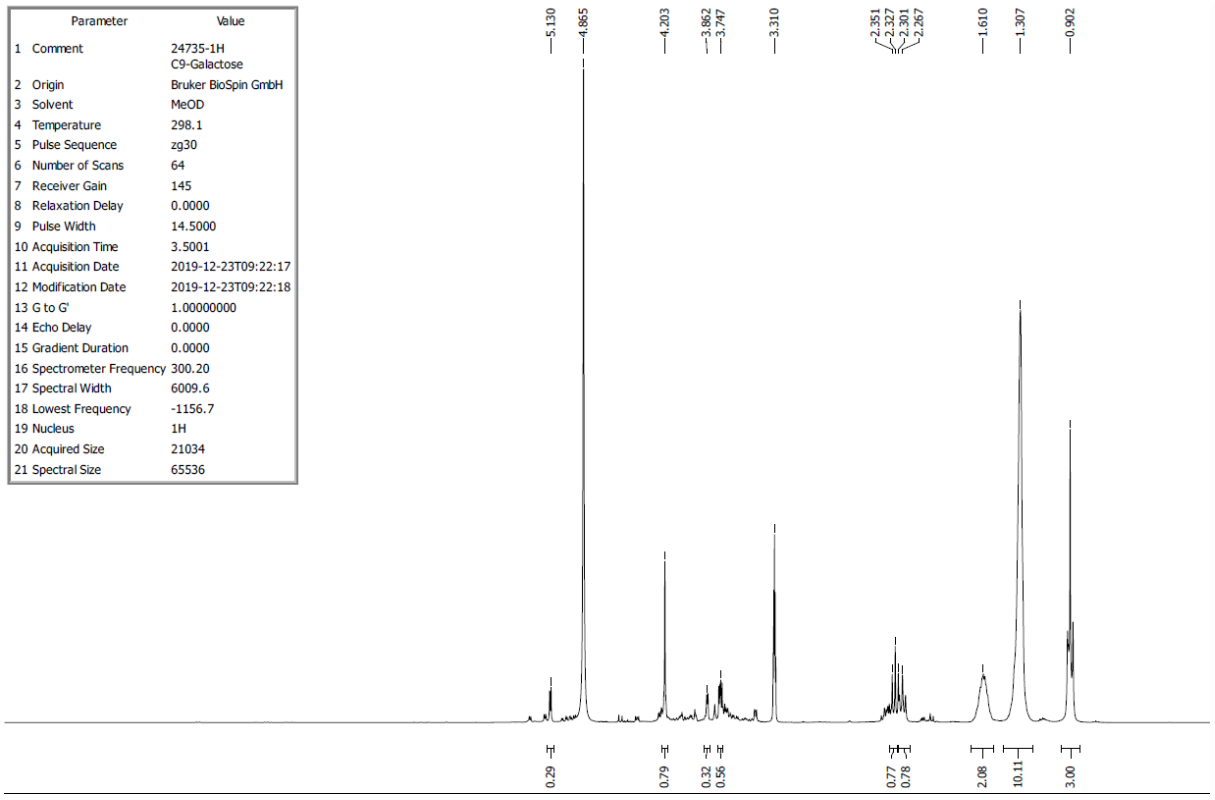
	Parameter	Value
1	Comment	24734-1H C9-Glucose
2	Origin	Bruker BioSpin GmbH
3	Solvent	MeOD
4	Temperature	298.1
5	Pulse Sequence	zg30
6	Number of Scans	64
7	Receiver Gain	129
8	Relaxation Delay	0.0000
9	Pulse Width	14.5000
10	Acquisition Time	3.5001
11	Acquisition Date	2019-12-23T09:16:29
12	Modification Date	2019-12-23T09:16:30
13	G to G'	1.00000000
14	Echo Delay	0.0000
15	Gradient Duration	0.0000
16	Spectrometer Frequency	300.20
17	Spectral Width	6009.6
18	Lowest Frequency	-1156.7
19	Nucleus	1H
20	Acquired Size	21034
21	Spectral Size	65536

a) [1]HNMR spectra

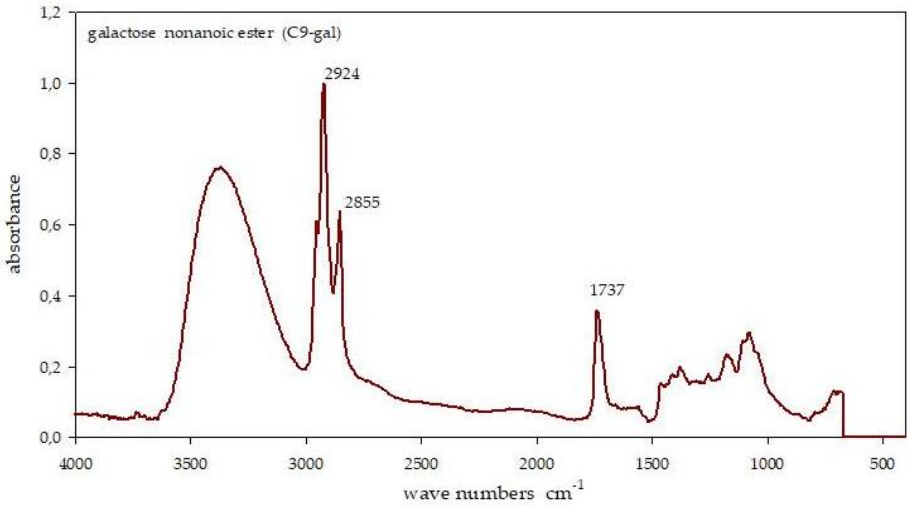


b) IR spectra

Fig 5. Spectral analysis of glucose nonanoate (C9-glu)



a) ^{1}HNMR spectra



a) IR spectra

Fig 6. Spectral analysis galactose nonanoate (C9-gal)

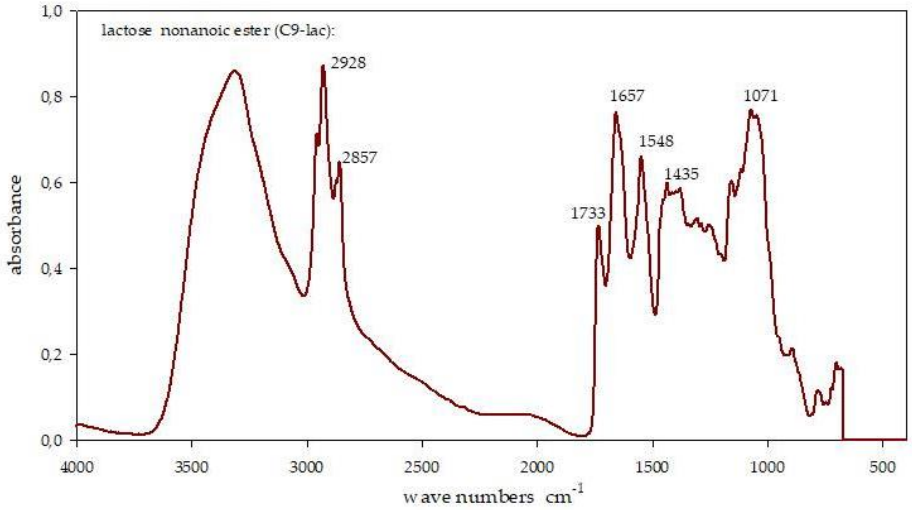
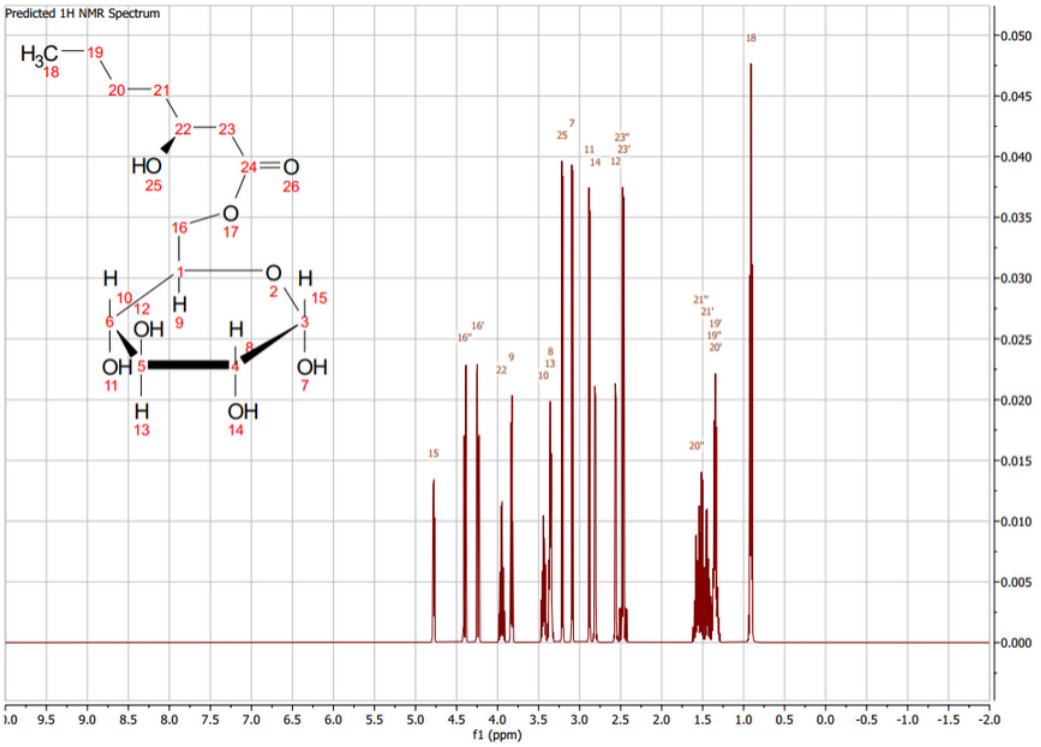
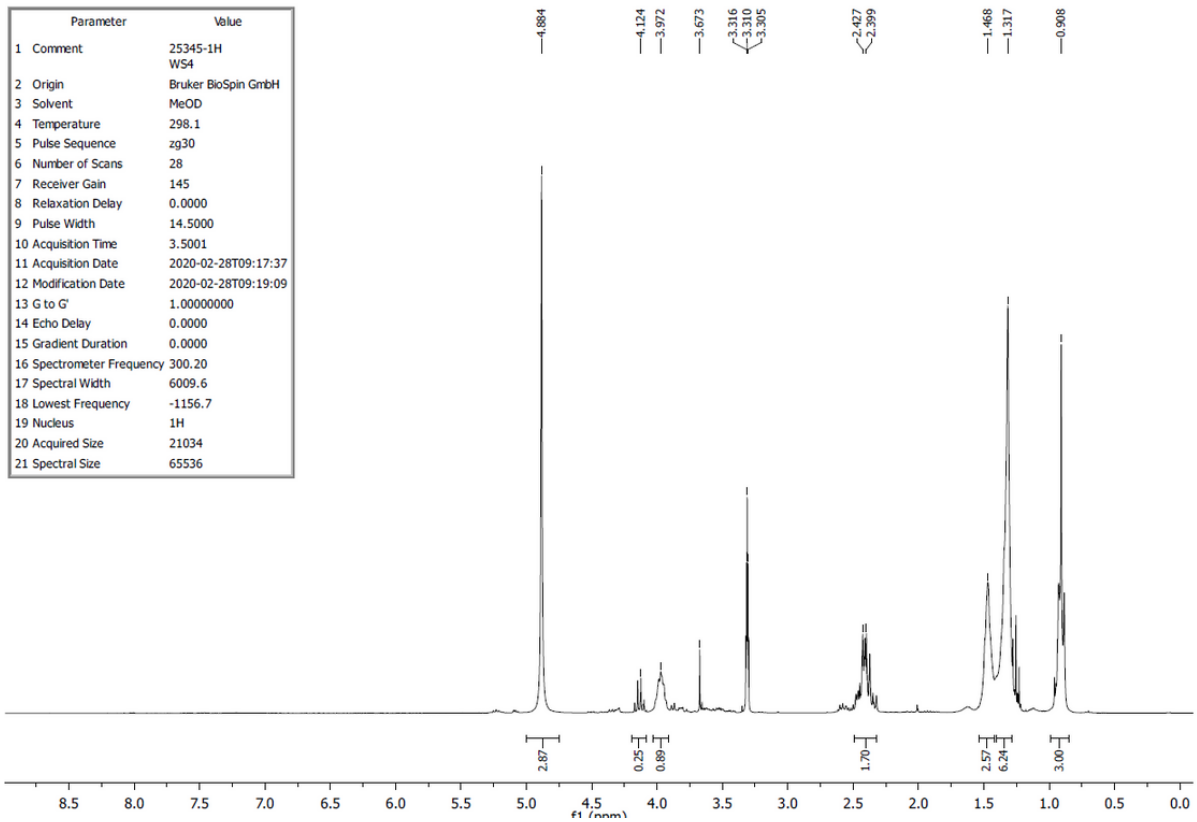


Fig 7. IR spectra of lactose nonanoate (C9-lac)

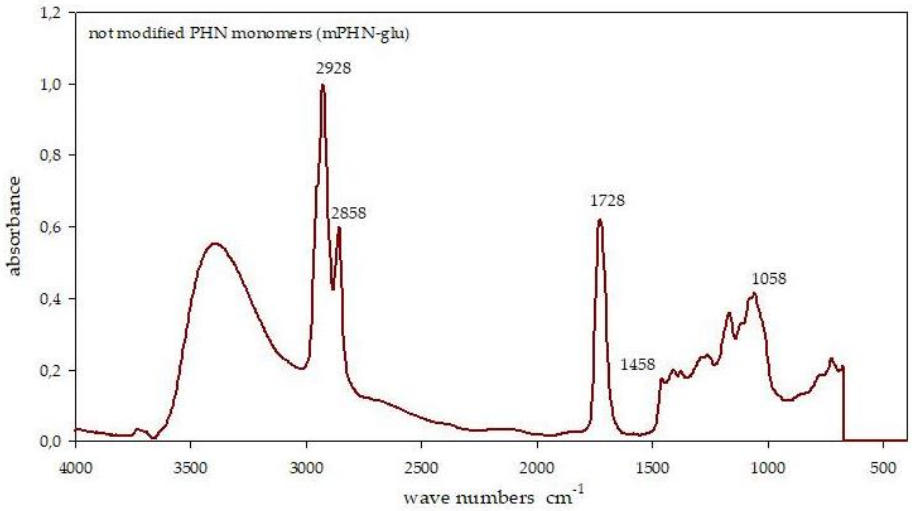


a) [1]HNMR spectra prediction

	Parameter	Value
1	Comment	25345-1H WS4
2	Origin	Bruker BioSpin GmbH
3	Solvent	MeOD
4	Temperature	298.1
5	Pulse Sequence	zg30
6	Number of Scans	28
7	Receiver Gain	145
8	Relaxation Delay	0.0000
9	Pulse Width	14.5000
10	Acquisition Time	3.5001
11	Acquisition Date	2020-02-28T09:17:37
12	Modification Date	2020-02-28T09:19:09
13	G to G'	1.00000000
14	Echo Delay	0.0000
15	Gradient Duration	0.0000
16	Spectrometer Frequency	300.20
17	Spectral Width	6009.6
18	Lowest Frequency	-1156.7
19	Nucleus	1H
20	Acquired Size	21034
21	Spectral Size	65536

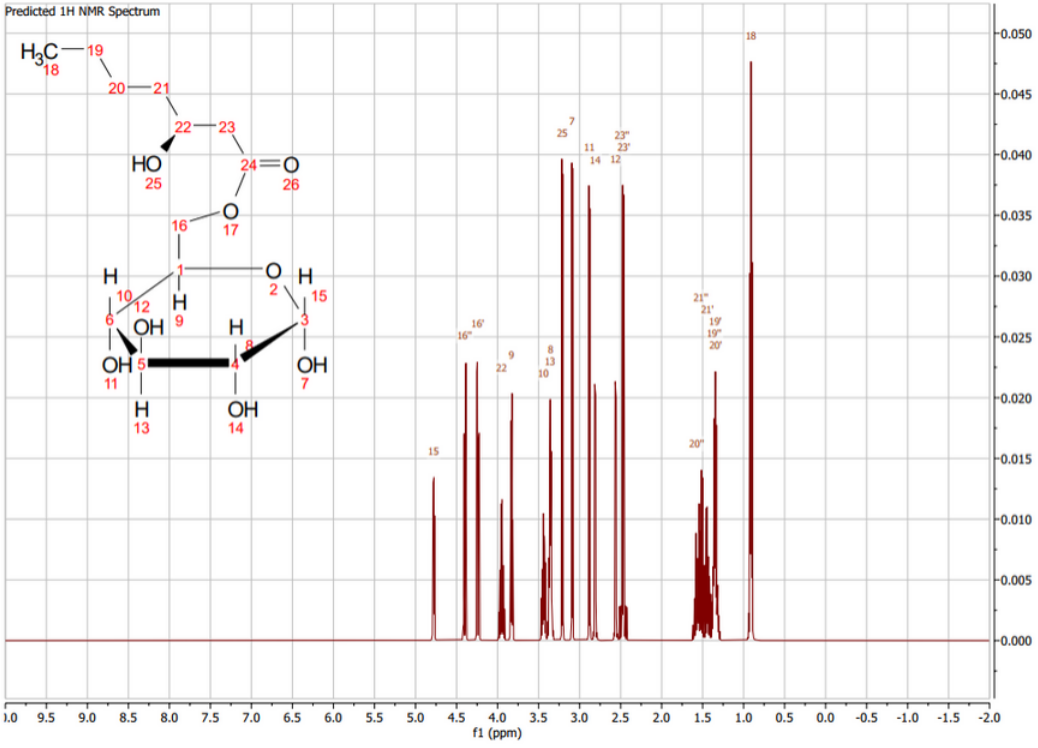


b) [1]HNMR spectra: [1]H NMR (300 MHz, Methanol-d4) δ 4.93 (s, 3H), 4.10 – 3.89 (m, 1H), 3.33 (td, *J* = 3.7, 2.1 Hz, 1H), 2.55 – 2.29 (m, 2H), 1.62 – 1.21 (m, 12H), 1.02 – 0.85 (m, 3H)

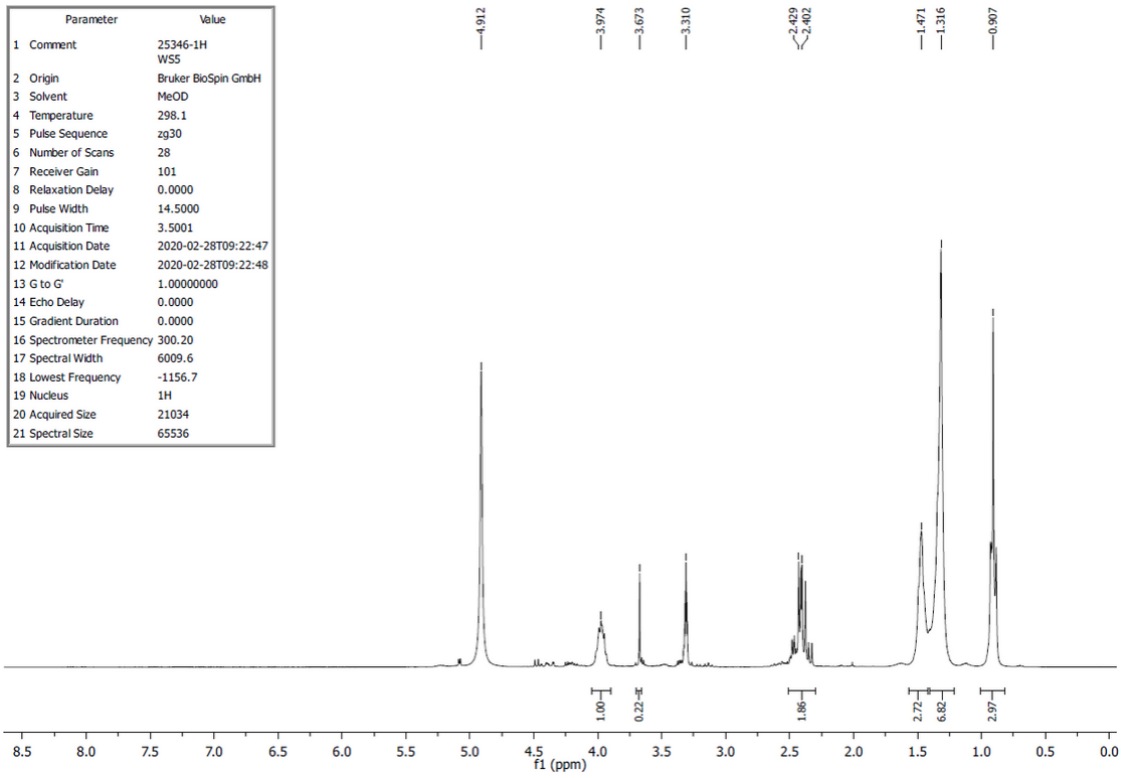


c) IR spectra

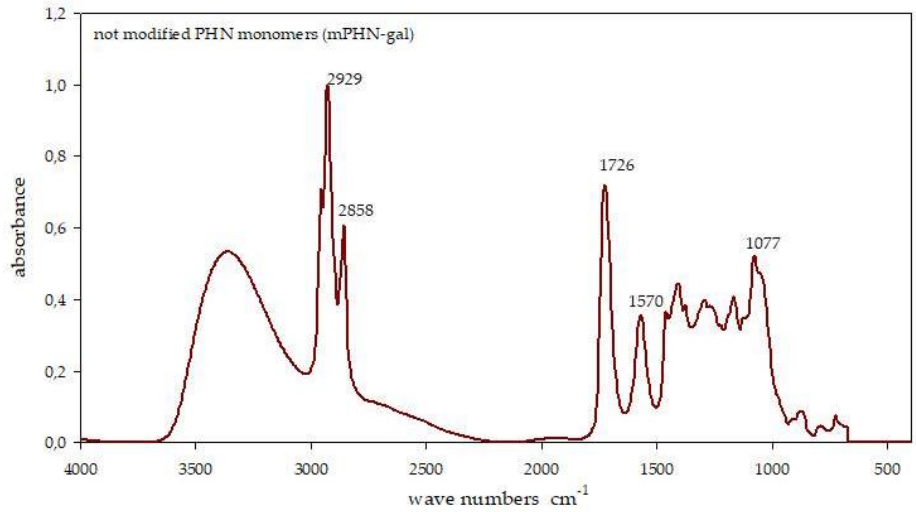
Fig 8. Spectral analysis of glucose mono and diesters esters originated from mixture of not modified PHN monomers (mPHN-glu)



a) [1]HNMR spectra prediction

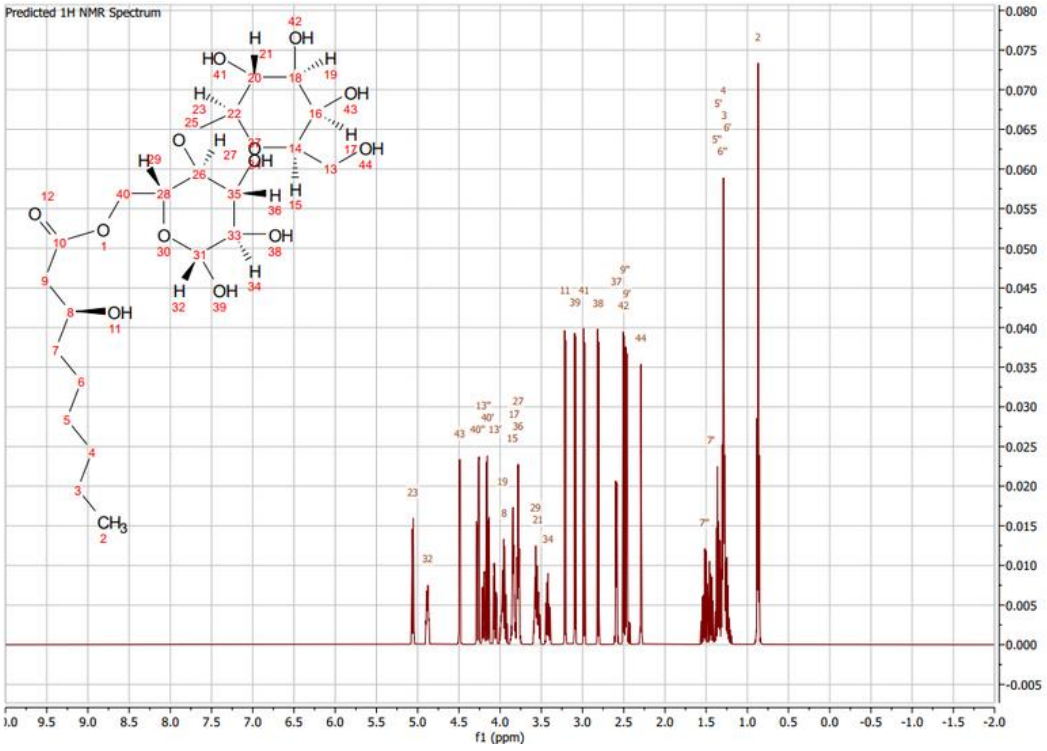


b) [1]HNMR spectra: [1]H NMR (300 MHz, Methanol-d4) δ 4.94 (s, 3H), 3.99 (dtd, *J* = 9.5, 7.0, 3.4 Hz, 1H), 3.33 (p, *J* = 1.7 Hz, 1H), 2.54 – 2.28 (m, 2H), 1.63 – 1.22 (m, 12H), 1.03 – 0.84 (m, 3H).

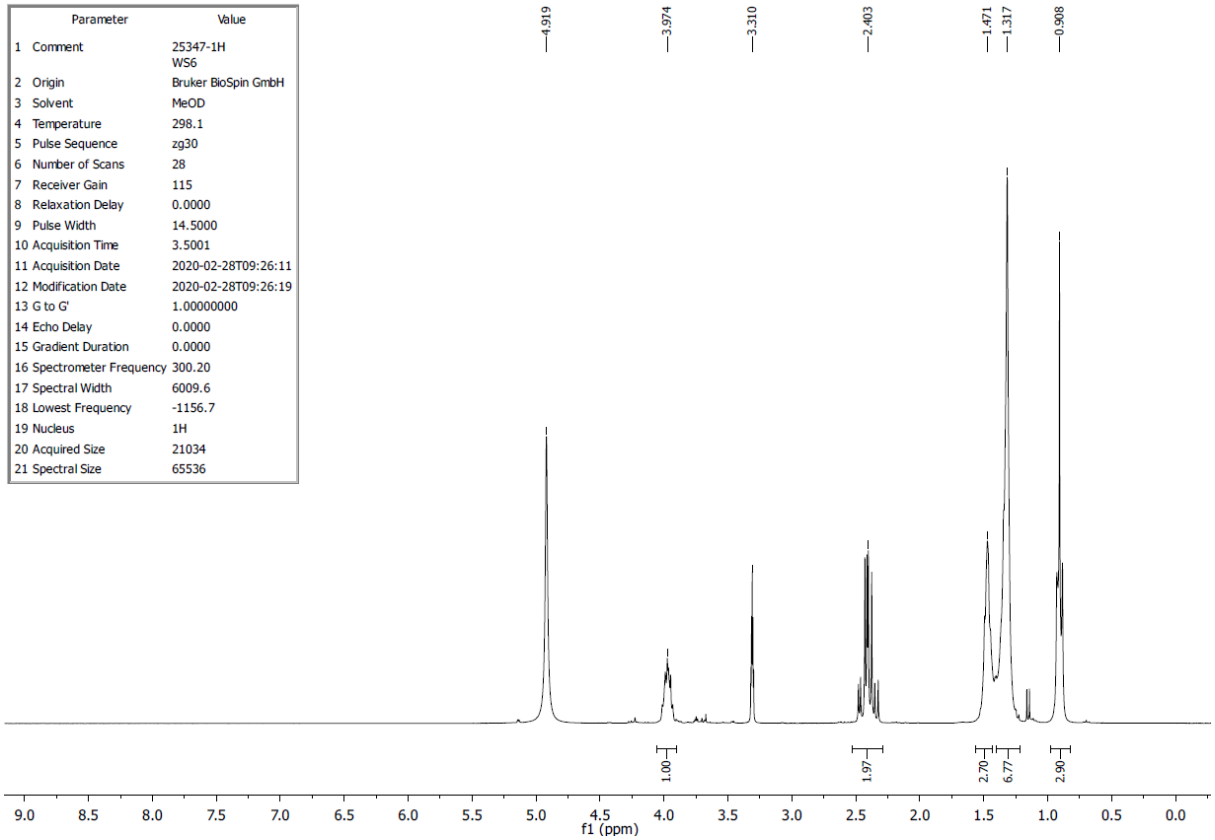


c) IR spectra

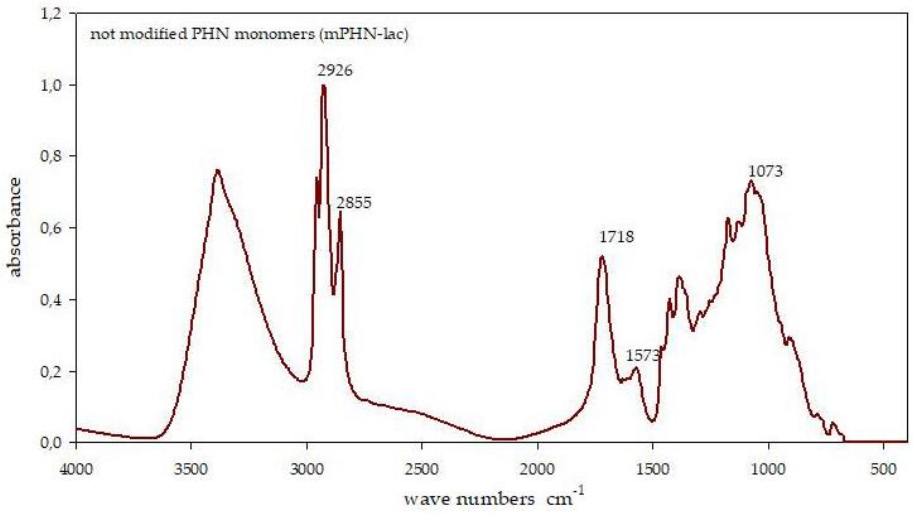
Fig 9. Spectral analysis of galactose mono and diesters esters originated from mixture of not modified PHN monomers (mPHN-gal)



a) [1]HNMR spectra prediction



b) ^{1}HNMR spectra: (300 MHz, Methanol-d4) δ 4.90 (s, 3H), 4.16 (q, *J* = 7.1 Hz, 1H), 4.07 – 3.91 (m, 1H), 3.33 (p, *J* = 1.6 Hz, 1H), 2.55 – 2.31 (m, 2H), 1.58 – 1.23 (m, 11H), 1.03 – 0.82 (m, 3H)



c) IR spectra

Fig 10. Spectral analysis of lactose mono and diesters esters originated from mixture of not modified PHN monomers (mPHN-lac)

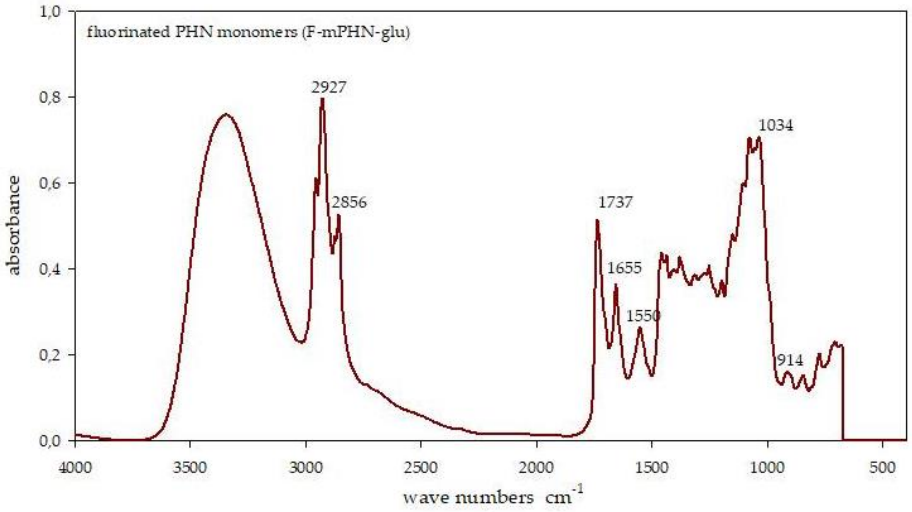


Fig 11. IR spectra of glucose mono and diesters esters originated from mixture of fluorinated PHN monomers (F-mPHN-glu)

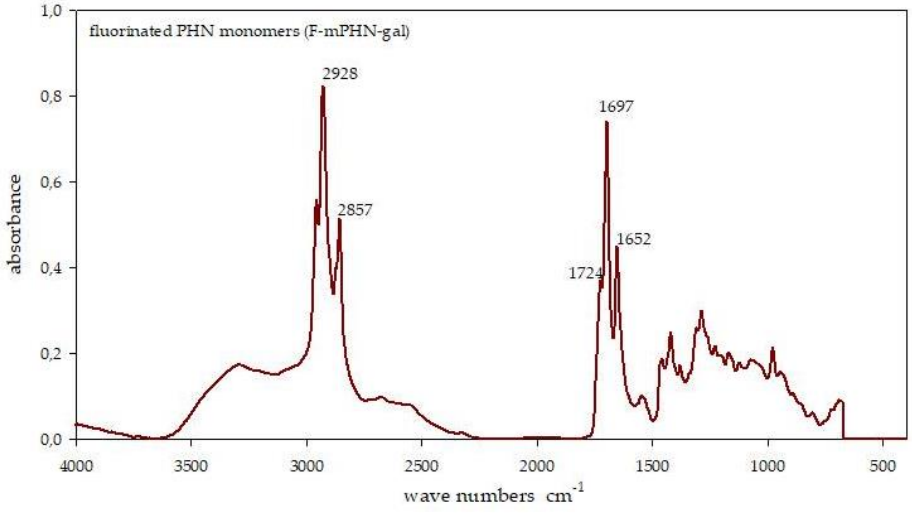


Fig 12. IR spectra of galactose mono and diesters esters originated from mixture of fluorinated PHN monomers (F-mPHN-gal)

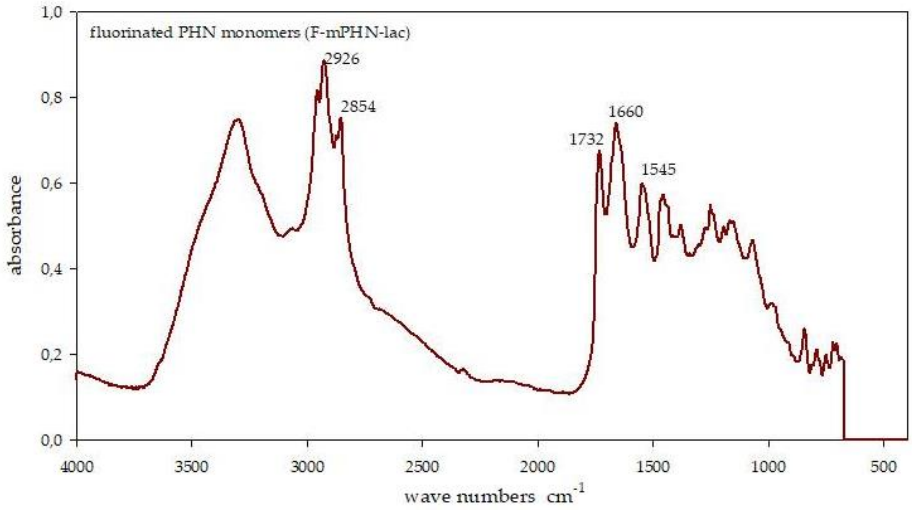


Fig 13. IR spectra of lactose mono and diesters esters originated from mixture of fluorinated PHN monomers (F-mPHN-lac)

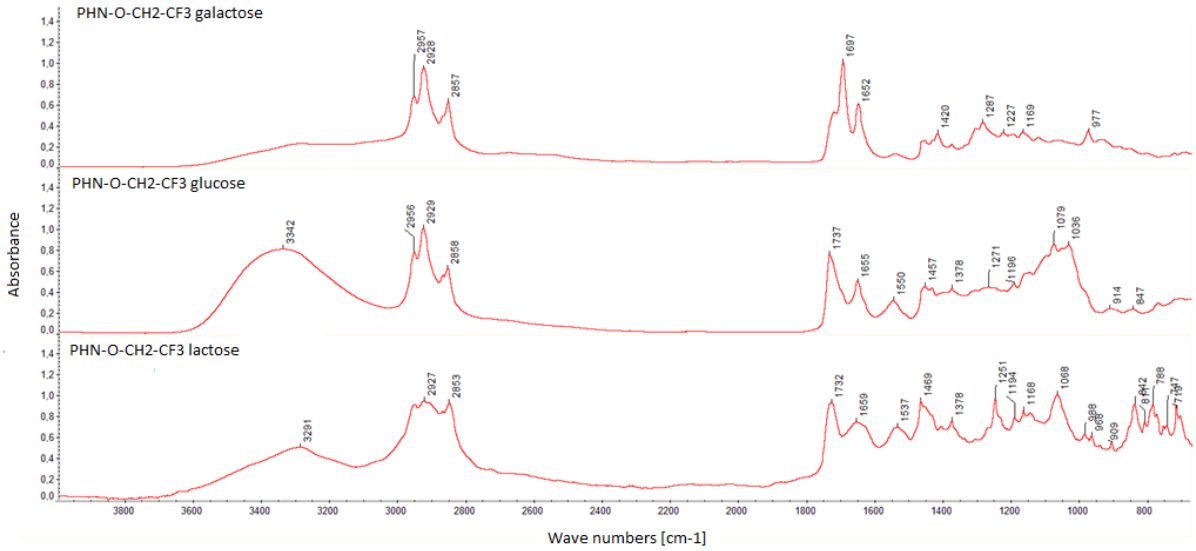


Fig 14. IR spectra of fluorinated esters set together

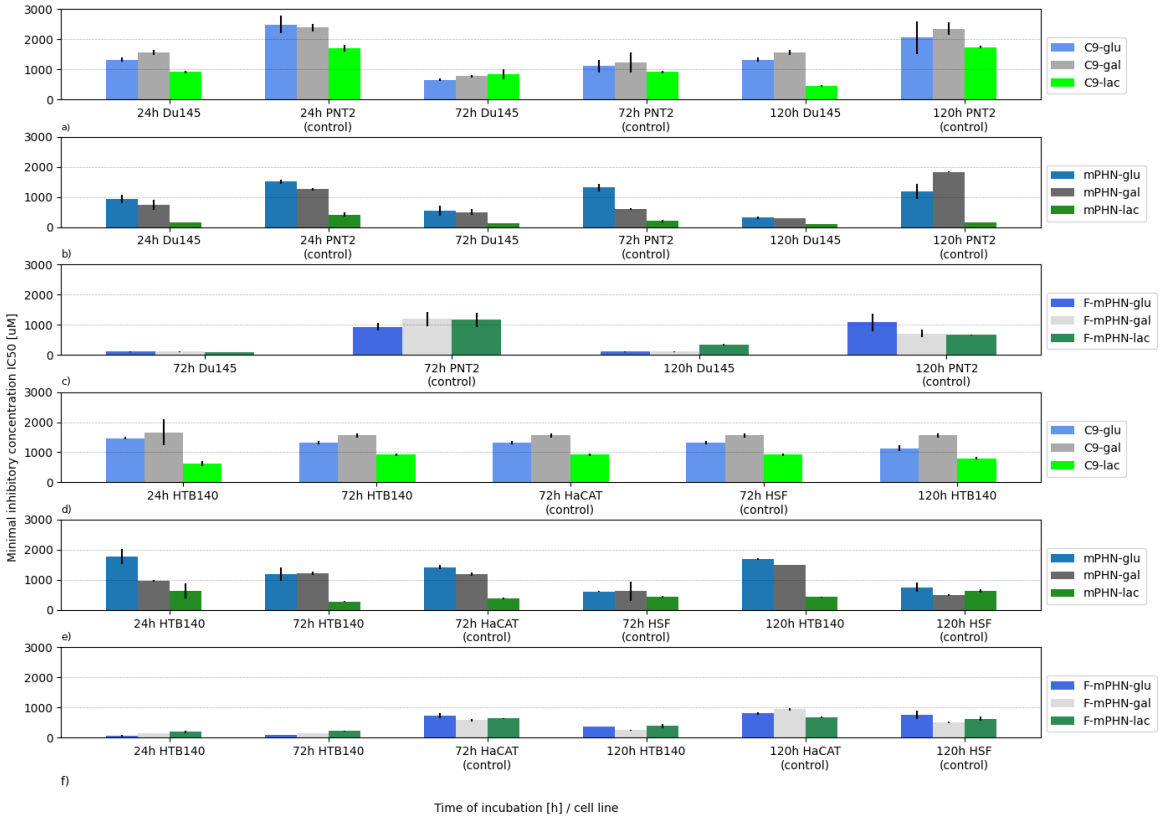


Fig 15. Cytotoxicity of SFAE determined by MTT assay, where IC_{50} is a concentration [μM] of a particular compound that causes reduction of a tested cell culture to 50% :

a) nonanoic acid originated SFAE tested on DU145 / PNT2 (control)

b) mPHN originated SFAE tested on DU145 / PNT2 (control)

c) F-mPHN originated SFAE tested on DU145 / PNT2 (control)

d) nonanoic acid originated SFAE tested on HTB40 /HaCat/ HSF (control)

e) mPHN originated SFAE tested on HTB40 /HaCat/ HSF (control)

f) F-mPHN originated SFAE tested on HTB40 /HaCat/ HSF (control)

Table 2. Cytotoxicity of SFAE determined by MTT assay: – not determined; d – standard deviation. Red fillings mark the most lowest concentrations, green- the highest.

	Time [h]	C9-glu	C9-gal	C9-lac	PHN-glu	PHN-gal	PHN-lac	F-mPHN-glu	F-mPHN-gal	F-mPHN-lac
DU145 (prostate cancer)	24	1317.1	1560.5	919.5	932.1	750.9	161.2	-	-	-
	d	66	78	46	146	169	3	-	-	-
	72	658.6	780.3	836.9	541.1	500.0	131.4	100.0	100.0	83.8
	d	33	39	169	159	100	5	5	5	4
	120	1317.1	1560.8	459.8	315.7	304.6	91.3	100.0	100.0	335.0
	d	66	78	23	41	2	3	5	5	17
PNT2 (control)	24	2491.5	2390.9	1707.2	1507.6	1256.8	421.0	-	-	-
	d	285	120	110	80	32	79	-	-	-
	72	1110.5	1233.3	919.5	1312.2	612.5	199.5	926.8	1183.5	1156.4
	d	207	327	46	115	31	42	127	238	234
	120	2053.1	2355.3	1743.7	1180.2	1838.4	165.2	1075.5	704.9	662.5
	d	538	220	33	247	20	5	296	117	8
HTB 140 (melanoma)	24	1468.8	1659.0	621.6	1767.6	967.3	640.0	63.3	155.9	189.0
	d	46	434	77	253	26	250	24	3	49
	72	1317.1	1560.5	919.5	1195.7	1224.9	283.4	89.3	155.9	216.9
	d	66	78	46	232	61	8	2	3	21
	120	1133.9	1560.5	805.3	1689.4	1496.4	435.0	372.4	247.9	394.2
	d	92	78	51	35	2	10	3	11	70
HaCAT (human keratinocytes-control)	72	1317.1	1560.5	919.5	1427.3	1200.0	383.9	733.7	580.0	644.1
	d	66	78	46	71	60	25	81	37	17
	120	-	-	-	-	-	-	800.8	945.9	670.0
	d	-	-	-	-	-	-	40	47	33
HSF (human skin fibroblasts-control	72	1317.1	1560.5	919.5	613.4	624.2	445.8	-	-	-
	d	66	78	46	12	322	24	-	-	-
	120	-	-	-	761.2	499.6	631.1	761.2	499.6	631.1
	d	-	-	-	143	25	64	143	25	64