Supporting Information

Lipase-catalyzed acylation of levoglucosan in continuous flow: antibacterial and biosurfactant studies

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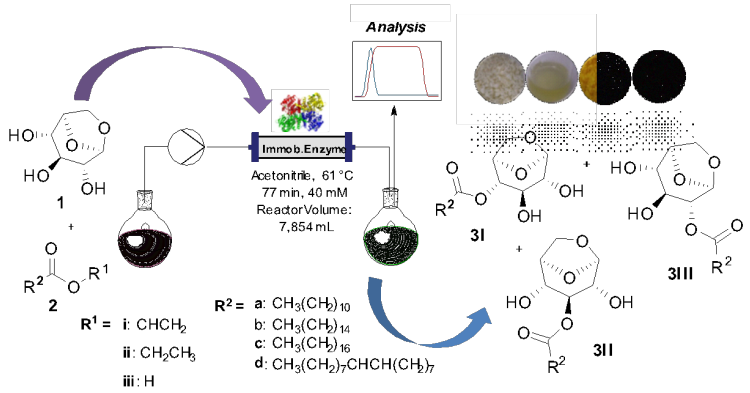
Keywords: Levoglucosan, Lipase, Esters, Design of Experiment, Continuous Flow.

Summary

1. Reaction optimization in continuous flow

1.1. Experimental setup



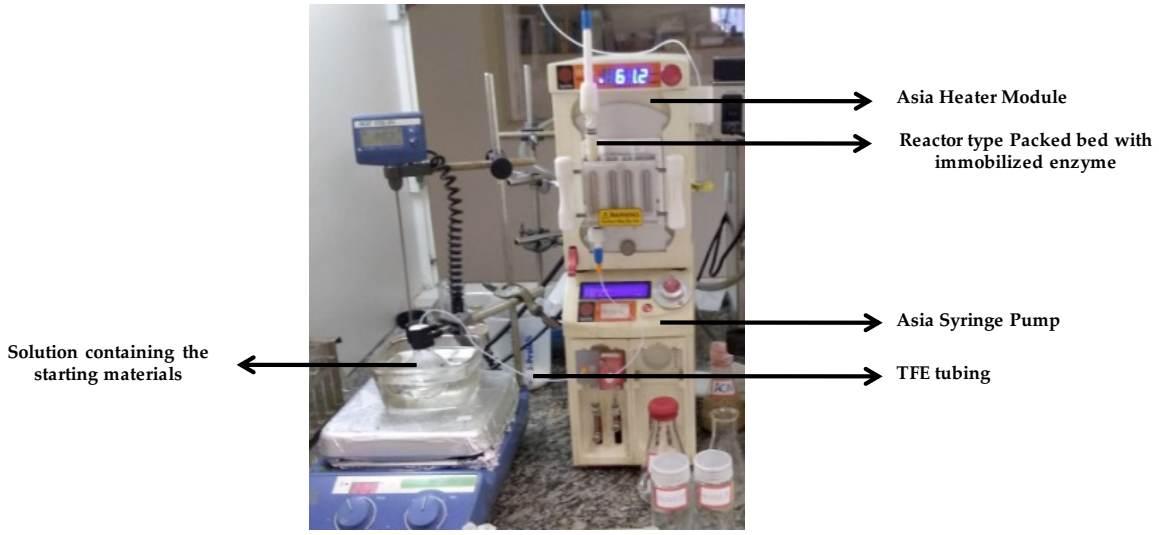


Figure S1: Esterification reaction of levoglucosan with different vinyl esters.*Reactor volume: 7.854mL. **a**: vinyl laurate, **b**: vinyl palmitate, **c**: vinyl stearate and **d**: vinyl oleate. Immobilized Enzyme: **N435** = Novozym 435 (*Candida antarctica lipase* B), immobilized in resin Lewatit VP OC 1600; **CaLB_Epoxy** = *Candida antarctica* lipase B immobilized on epoxy support by our research group.

1.2. CCD planning matrix

Table S1: CCD planning matrix for two variables real and normalized levels and the response obtained.

EXP	X_1	X_2	Conversion %
1	(-1)	(-1)	31
2	(1)	(-1)	38
3	(-1)	(1)	50
4	(1)	(1)	54
5	(-1,42)	(0)	26
6	(1,42)	(0)	36
7	(0)	(-1,42)	28
8	(0)	(1,42)	54
9	(0)	(0)	54
10	(0)	(0)	54
11	(0)	(0)	56

1.3. Hydrolytic Activity Assay

The activities of free and immobilized lipases were determined as follows: 1 mL of extract or 10 mg of each supported enzyme were added to 19 mL of an emulsion prepared with olive oil (5 % v/v) and arabic gum (10 % v/v) in sodium phosphate buffer (100 mM, pH 7.0). The reactions were carried out under stirring (200 rpm) at 35 °C for 30 min. The reactions were then stopped by the addition of 20 mL of acetone-ethanol mixture (1:1 v/v) and the fatty acids produced were extracted under agitation (200 rpm) for 10 min and titrated until end-point (pH 11.0) with NaOH solution (0.04 N). The blank assays were performed by adding the extract just after the addition of the acetone-ethanol solution to the flask. One unit of lipase activity (U) was defined as the amount of enzyme which catalyzes the release of 1 µmol of fatty acids per minute, under the assay conditions.[1,2]

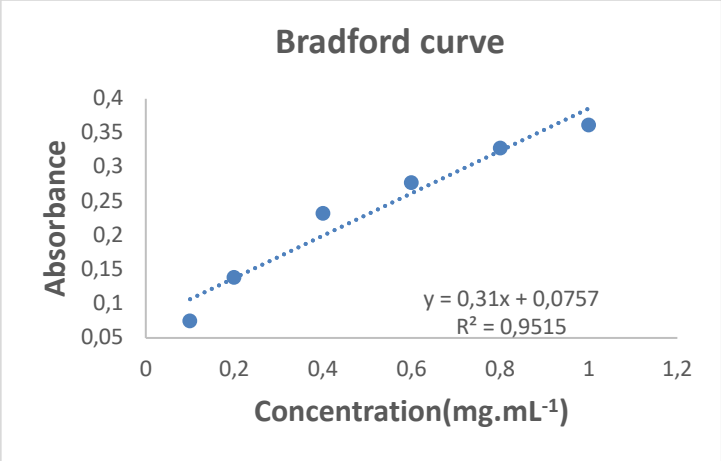


Figure S2: Bradford curve for protein quantification.

Table S2: Immobilization efficiency of e new biocatalysts compared to that of N435

Biocatalilyst Immobilized	Amount of protein (mg/g of support)	IPC (%)[a]	H_A ($U.g^{-1}$ of support)[b]	S_A ($U.mg^{-1}$ of protein)[c]
N435[2]	15.0	-	248.0	16.53
CalB_Epoxy	9.0	>99	372.0	41.33

[a]IPC: immobilized protein concentration; [b] Hydrolytic activity; [c] Specific activity

[a] IPC (%): $\left(\frac{P_0 - P_f}{P_0}\right) * 100$

P_0 and P_f are the initial and final time protein concentrations in the supernatant ($mg.mL^{-1}$), respectively.

[b] Hydrolytic activity (H_A): $\frac{(V - V_b)}{t * V_a} * M * 1000$

Where, V and V_b are the volume (mL) of NaOH solution used for sample and blank titration, respectively; t is the reaction time (min); M is the molarity ($mmol.mL^{-1}$) and V_a is the sample volume (mL).

[c] Specific activity (S_A): $\frac{U}{milligrams\ of\ protein}$

1.4. Solubility tests of levoglucosan and acyl donors in acetonitrile

Levoglucosan and acyl donors vinyl laurate, ethyl laurate, lauric acid, vinyl stearate, vinyl oleate and vinyl palmitate were subjected to solubility tests. 40 and 100 mM solutions were prepared in a total volume of 500 mmL of acetonitrile. The temperatures tested were 45, 50 and 55 °C and shaken at 200 rpm in an

orbital shaker incubator for 30 min. The numbers below have been assigned in relation to the solubility of the reagents:

1 – Insoluble: turbid solution and background body;

2 – Partially soluble: turbid solution;

3 – Completely soluble: Clear solution

Table S3: Solubility tests of levoglucosan and acyl donors in acetonitrile

Reagent	Temperature (°C)					
	45		50		55	
	40 mM	100 mM	40 mM	100 mM	40 mM	100 mM
Levoglucosan	1	1	1	1	3	3
Lauric acid	3	3	3	3	3	3
Ethyl laurate	2	2	2	2	2	2
Vinyl laurate	2	2	3	3	3	3
Vinyl stearate	2	2	3	3	3	3
Vinyl palmitate	3	3	3	3	3	3
Vinyl oleate	3	3	3	3	3	3

2. Analytical Methods

2.1. Gas Chromatography Analysis

The reactions were monitored by thin layer chromatography (TLC) and, subsequently, 10 µL of each sample were collected and derivatized with 10 µL of BSTFA in an orbital shaker incubator at 200 rpm, 60 °C and 90 min. After that, they were swelled with ethyl acetate to 0.5 mL and analyzed in a gas chromatograph equipped with a mass spectrometry detector (CG-MS).[3,4] Helium was applied as carrier gas in all analyses.

Analyses were performed in a GC-MS (Shimadzu CG2010 –SLB-5MS capillary column 30 m, 0.25 mm ID, 0.25 um film thickness). Samples were prepared by diluting 20 µL of the final product in 980 µL of ethyl acetate. The injector and detector temperatures were 250 °C, and the oven was maintained at 80 °C for 4 min, increased to 200 °C at a rate of 6 °C/min for 10 min, increased to 310 °C at a rate of 8 °C/min, when it was held constant for 4 min, totaling 51.75 min.

In this section, we will show the illustrative chromatograms obtained in the steps of higher product conversions, in the esterification reactions with vinyl laurate using CalB_Epoxy and N435 for the synthesis of CFAEs.

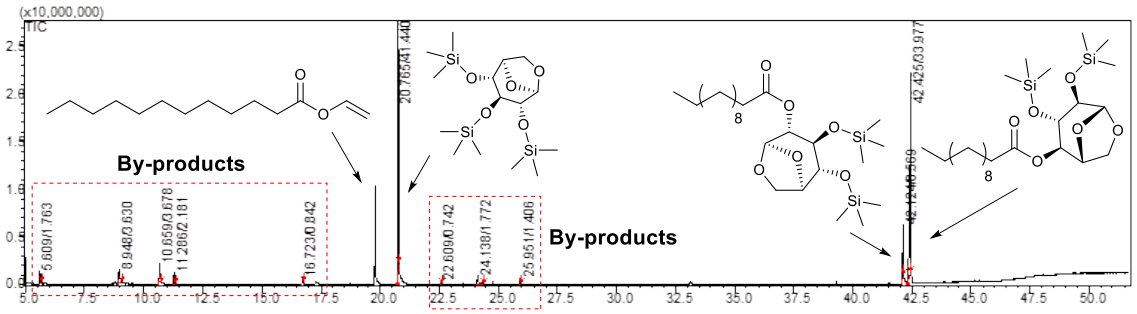


Figure S3: Chromatogram of levoglucosan reaction with vinyl laurate using CalB_Epoxy (derivatized) 61 °C.

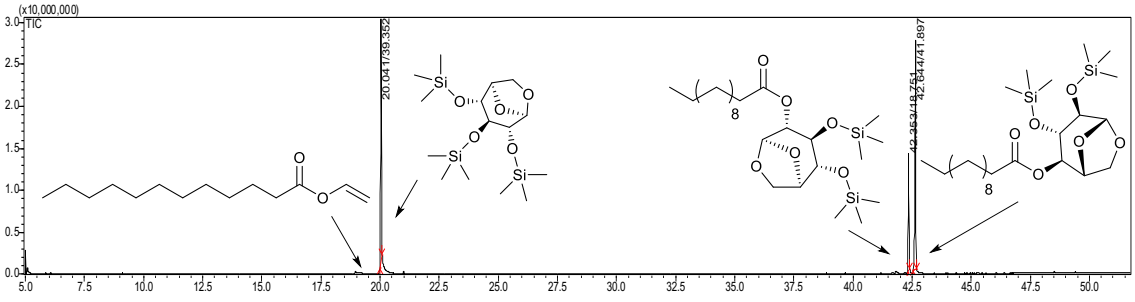


Figure S4: Chromatogram of levoglucosan reaction with vinyl laurate using N435 (derivatized) 61 °C

In this section, we will show the illustrative chromatograms obtained of monoesters levoglucosan that were separated from unreacted reagents by flash chromatography

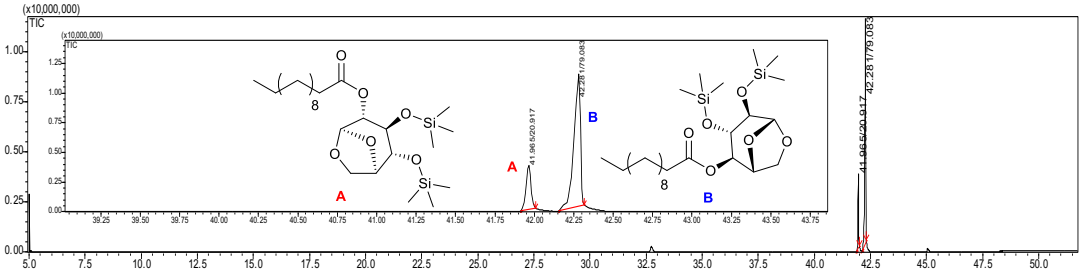


Figure S5: Chromatogram of MONLAU. Mixture of 4- and 2-: O-Lauryl-1,6-anhydroglucopyranose.

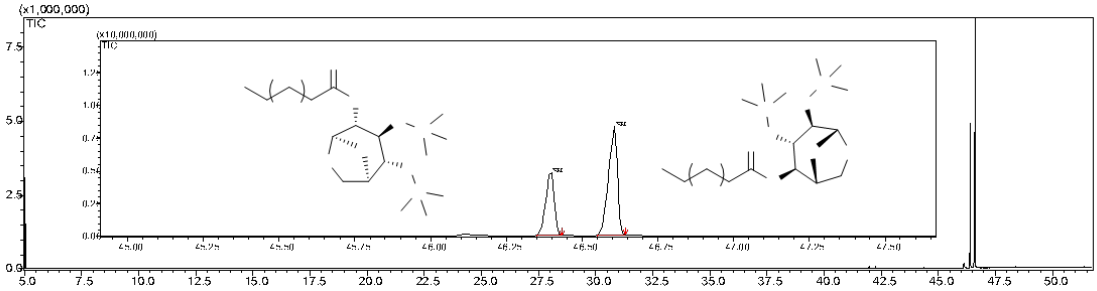


Figure S6: Chromatogram of MONPAL. Mixture of 4- and 2-: O-Palmitoyl-1,6-anhydroglucopyranose.

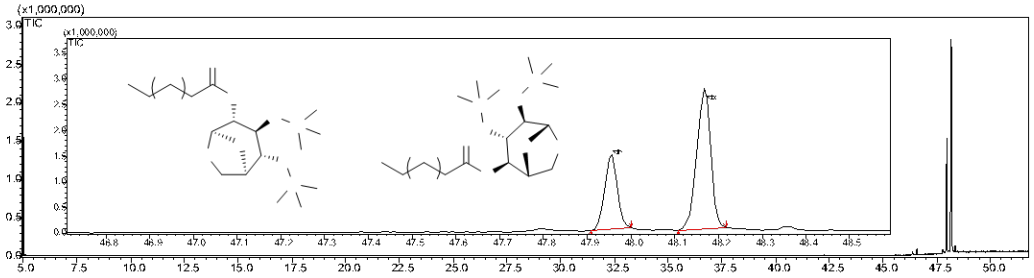


Figure S7: Chromatogram of MONEST. Mixture of 4- and 2-: O-Estearyl-1,6-anhydroglucopyranose.

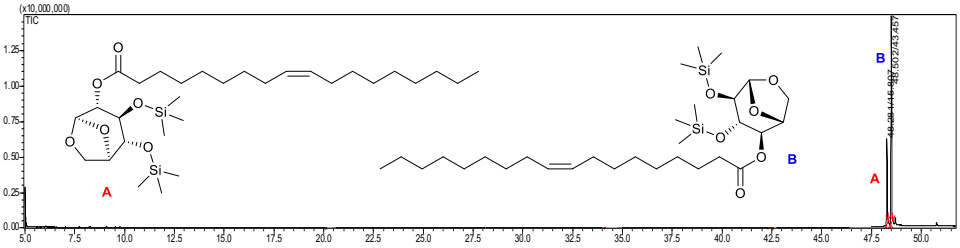


Figure S8: Chromatogram of MONOLE. Mixture of 4- and 2-: O-Oleoyl-1,6-anhydroglucopyranose.

2.2. Determination of surface activity

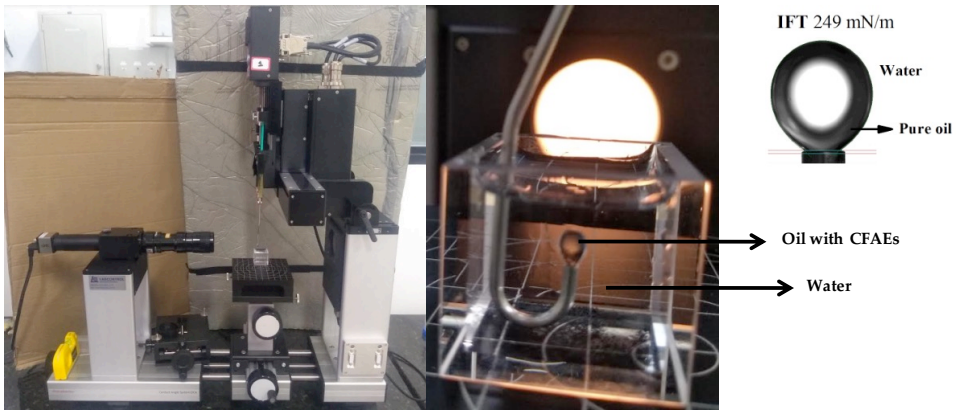


Figure S9: Evaluation of pendant drop (PD) method using Goniometer OCA25 (DataPhysic Instruments, Germany)

Table S4: Variation of the interface tension with the concentration of CFAEs: MONLAU, MONPAL, MONEST and MONOLE in oil at 25 °C

Compound	CONCENTRATION (mM)											
	0	5	10	20	30	40	50	60	75	100	120	150
	IFT[mN/m]											
MONLAU	249,39	176,6	166,9333	137,1033	115,3817	111,8117	96,39	88,71833	90,55667	90,55667	90,49333	89,97667
MONPAL	249,39	184,85	155,966	133,252	121,818	105,58	88,214	88,53	86,576	89,48	88,356	86,346
MONEST	249,39	226,435	222,73	212,1933	200,3917	178,6117	177,4317	171,5917	141,16	135,56	140,1933	139,655
MONOLE	249,39	237,5083	198,1483	188,79	158,9	148,5333	134,6317	102,4817	105,3367	104,0533	103,1367	105,6033

Table S5: Variation of the interface tension with the concentration of MONLAU in oil at 25 °C

Compound	CONCENTRATION (mM)										
	5	10	20	30	40	50	60	75	100	120	150
	IFT[mN/m]										
MONLAU	175,6	168,13	136,85	114,99	111,95	96,87	89,31	90,58	91,3	90,78	91,14
	176,47	167,52	136,77	115,16	111,98	96,78	89,14	90,8	91,17	90,52	89,14
	177,91	166,94	136,75	115,21	111,8	95,49	88,73	91,14	91,17	89,91	88,73
	177,24	166,34	136,7	115,9	111,4	96,77	88,53	90,02	90,58	90,42	90,52
	176,31	166,12	137,69	115,36	111,83	95,9	88,43	90,78	89	90,91	89,91
	176,07	166,55	137,86	115,67	111,91	96,53	88,17	90,02	90,12	90,42	90,42
Average	176,6	166,9333	137,1033	115,3817	111,8117	96,39	88,71833	90,55667	90,55667	90,49333	89,97667
Sd	0,837568	0,766385	0,525268	0,341843	0,213112	0,565084	0,435266	0,452975	0,885362	0,348578	0,905951

Sd: Standard deviation

Table S6: Variation of the interface tension with the concentration of MONPAL in oil at 25 °C

Compound	CONCENTRATION (mM)										
	5	10	20	30	40	50	60	75	100	120	150
	IFT[mN/m]										
MONPAL	185,71	154,56	134,59	123,12	106,58	89,39	88,34	87,11	89,76	88,9	86,17
	184,15	155,57	134,41	121,98	106,01	88,95	87,91	86,17	89,63	88,52	86,06
	184,4	155,95	132,51	121,66	105,55	88,88	87,87	86,06	89,48	88,11	86,06
	184,28	156,79	131,9	121,7	105,28	87,05	89,36	86,05	89,36	88,34	86,05
	185,71	156,96	132,85	120,63	104,48	86,8	89,17	87,49	89,17	87,91	87,39
Average	184,85	155,966	133,252	121,818	105,58	88,214	88,53	86,576	89,48	88,356	86,346
Sd	0,790032	0,974797	1,190722	0,890461	0,788321	1,196089	0,699035	0,676077	0,229891	0,381615	0,585688

Sd: Standard deviation

Table S7: Variation of the interface tension with the concentration of MONEST in oil at 25 °C

Compound	CONCENTRATION (mM)										
	5	10	20	30	40	50	60	75	100	120	150
	IFT[mN/m]										
MONEST	225,68	221,57	211,57	200,04	177,94	178,01	171,7	142,56	136,51	137,8	141,25
	227,78	221,45	210,91	200,71	177,92	177,36	171,37	142,25	136,27	139,01	141,36
	226,61	223,17	213,17	199,72	181,39	176,78	170,87	138,66	133,18	142,56	140,88
	225,87	222,59	212,59	200,66	176,82	177,31	171,9	141,25	136,14	142,25	137,09
	227,44	225,4	211,75	200,58	179,85	178,43	171,47	141,36	135,42	138,66	135,47
	225,23	222,2	213,17	200,64	177,75	176,7	172,24	140,88	135,84	140,88	141,88
Average	226,435	222,73	212,1933	200,3917	178,6117	177,4317	171,5917	141,16	135,56	140,1933	139,655
Sd	1,018916	1,456503	0,927053	0,411165	1,68135	0,679865	0,471568	1,380188	1,225186	1,989067	2,683123

Sd: Standard deviation

Table S8: Variation of the interface tension with the concentration of MONOLE in oil at 25 °C

Compound	CONCENTRATION (mM)										
	5	10	20	30	40	50	60	75	100	120	150
	IFT[mN/m]										
MONOLE	238,53	198	187,84	158,84	152,43	134,95	102,67	107,41	102,94	103,4	105,85
	237,96	198,18	188,69	157,61	147,96	134,53	103,88	107,12	103,66	102,67	107,41
	237,79	197,36	187,67	161,79	148,21	135,48	102,21	104,61	104,33	103,88	107,12
	236,89	199,73	190,55	160,04	147,95	134,67	101,69	104,97	104,02	102,21	104,61
	236,67	197,66	191,98	158,04	147,34	134,29	101,77	102,94	103,4	101,69	104,97
	237,21	197,96	186,01	157,08	147,31	133,87	102,67	104,97	105,97	104,97	103,66
Average	237,5083	198,1483	188,79	158,9	148,5333	134,6317	102,4817	105,3367	104,0533	103,1367	105,6033
Sd	0,707288	0,827053	2,152626	1,753362	1,943097	0,553115	0,80415	1,674511	1,055683	1,196021	1,468982

Sd: Standard deviation

2.3. Antibacterial Assays

Table S9: Strains used in the antibacterial assays

Bacterial species	Code	Isolation source	Susceptibility profile
Staphylococcus aureus subsp. *aureus* Rosenbach	ATCC 29213	Injury	Sensitive to methicillin
	ATCC 33591	Clinical isolate	Resistant to methicillin
	ATCC 25933	Clinical isolate	Sensitive to methicillin
Staphylococcus aureus	517	Blood	Resistant to methicillin

2.4. Nuclear Magnetic Resonance

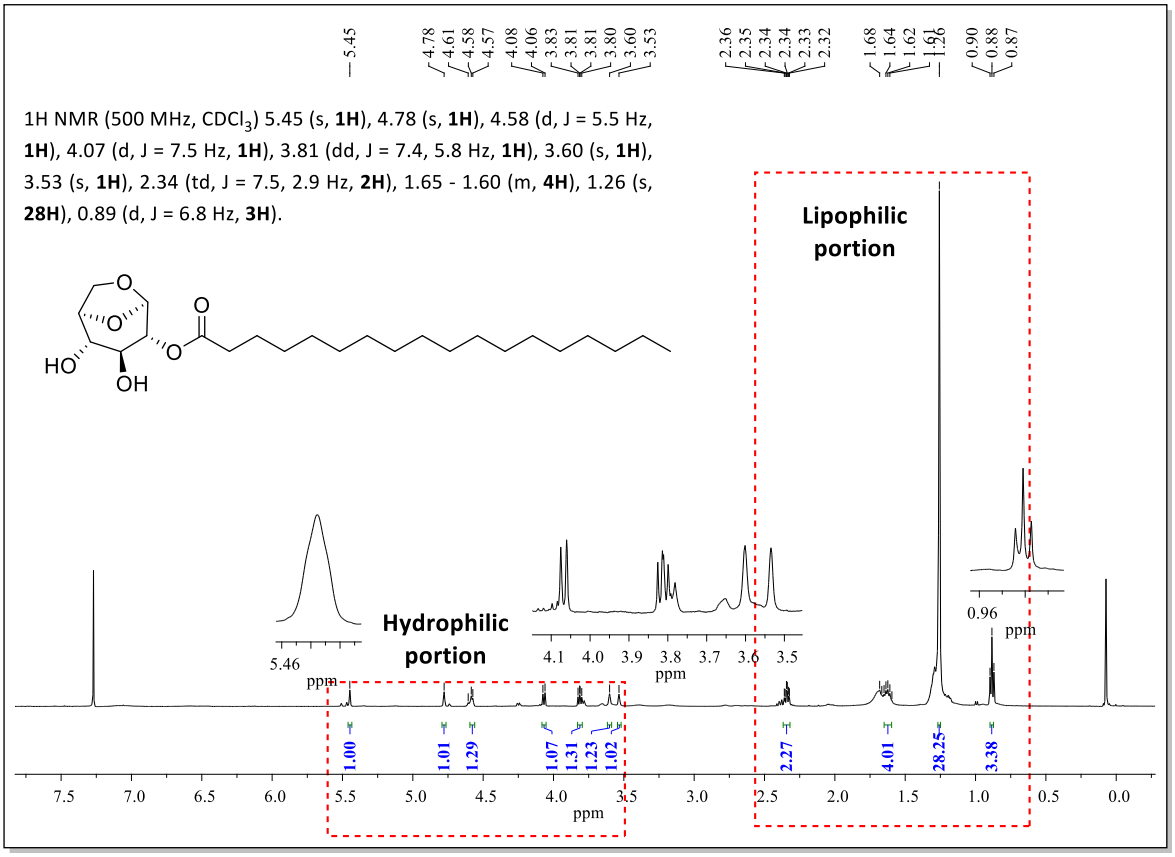


Figure S10: 1H-NMR Spectrum for 2-O-Estearyl-1,6-anhydroglucopyranose ($CDCl_3$).

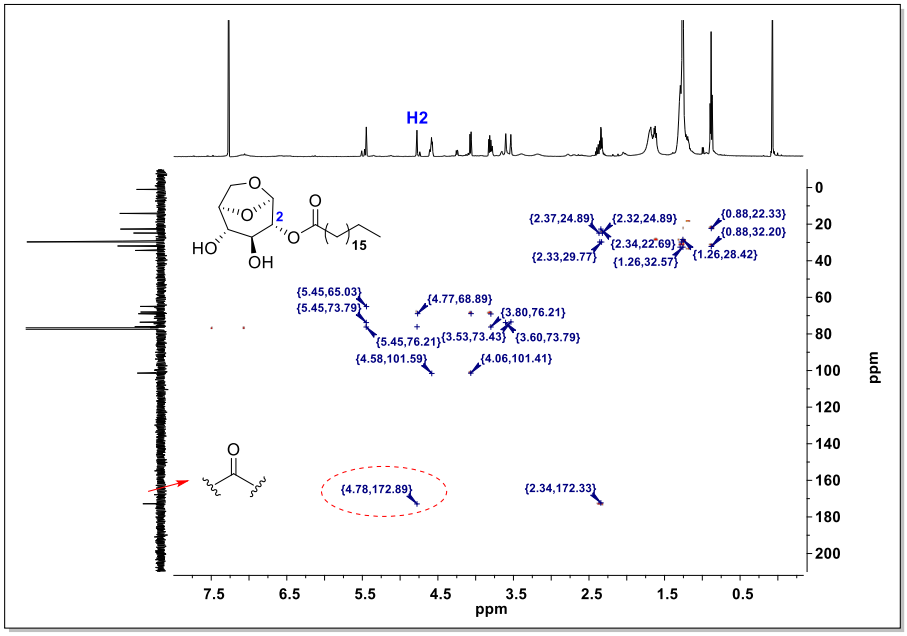


Figure S11: HMBC for 2-O-Estearyl-1,6-anhydroglucopyranose (CDCl3).

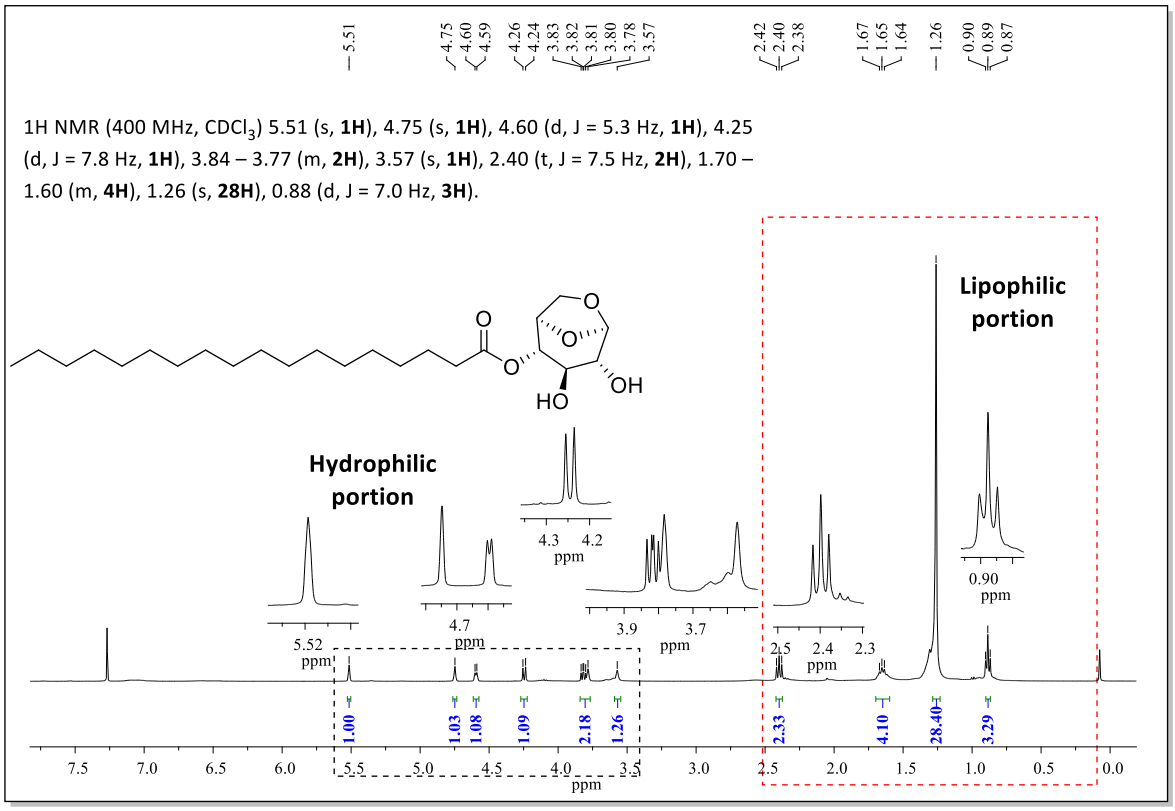


Figure S12:1H-NMR Spectrum for 4-O-Estearyl-1,6-anhydroglucopyranose ($CDCl_3$).

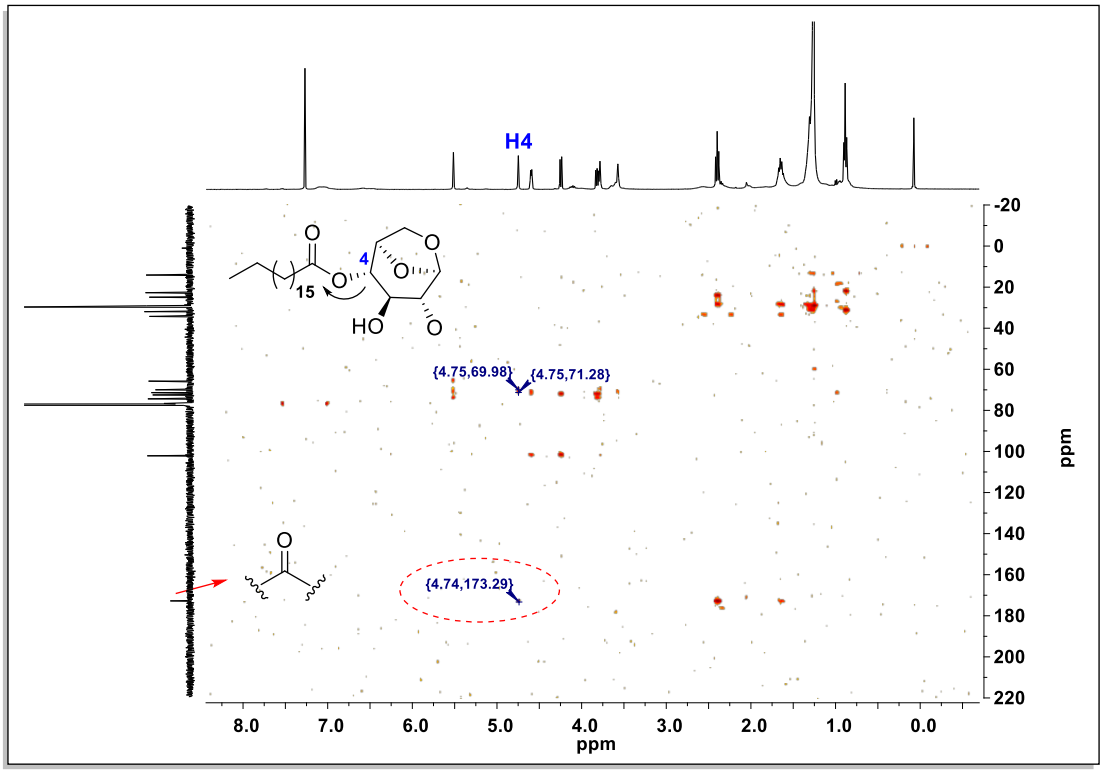


Figure S13: HMBC for 4-O-Estearyl-1,6-anhydroglucopyranose (CDCl3).

Reference

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