Supplementary Information

Development of regioselective deacylation of peracetylated β-D-monosaccharides using lipase from *Pseudomonas stutzeri* under sustainable conditions.

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1. General information

Peracetylated monosaccharides and disaccharides, solvents (ethanol, tetrahydrofuran THF- and 2-methyltetrahydrofuran –2-MeTHF-) were purchased from Sigma Aldrich. Solvent **S1**, was a gift from COGNIS IP Management GmbH now part of BASF. Solvents **S2**, **S3**, **S4**, **S5**, **S6** and **S7** were a generous gift from Prof. Ignacio García,[26] (Universidad de Navarra, Spain; solvents features are shown in Table S2 see supplementary information) Commercially available lipases from *Pseudomonas stutzeri* (PSL) and *Alcaligenes sp*. (QLC) were purchased from Meyto Sangyo (Japan). CAL-B was purchased from Sigma Aldrich. HPLC Jasco with evaporative light scattering detector (ELSD) using NH_2P50-4E amino column (Asahipak, Japan) eluted at 0.8 mL/min, 80% acetonitrile and 20% water was used. NMR spectra were recorded in $CDCl_3$ (δ=ppm) on a Bruker AV. 250 MHz and a Bruker AV. 400 MHz Spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to $CDCl_3$ (^{1}H: δ 7.27 ppm) and $CDCl_3$ (^{13}C: δ

77.0 ppm). Structures assignment were performed by means of 2D-COSY (COrrelation SpectroscopY), HSQC (Heteronuclear Single Quantum Correlation) experiments and HMBC (Heteronuclear Multiple Bond Correlation).

2. Typical procedure for the hydrolysis of peracetylated sugars

15.0 mg of the peracetylated sugar were solved in 500 μL of solvent containing 50 μL of distilled water. The reaction was started by the addition of the lipase (see Table S1) and the reaction progress was monitorized by HPLC. Products were purified by column chromatography using dichloromethane:methanol (95:5) as eluent. Solvent was dried and purified products were analysed by ^{1}H-NMR and ^{13}C-NMR.

Table S1. Deacylation of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose catalized by several lipases under different solvents conditions.

Assay	Enzyme	Solvent	Yield 1a (%)
1	CAL-B	ethanol	8
2	CAL-B	**S1**	3
3	CAL-B	**S2**	3
4	CAL-B	**S3**	27
5	CAL-B	**S4**	1
6	CAL-B	THF	3
7	CAL-B	MeTHF	7
8	PSL	ethanol	58
9	PSL	**S1**	96
10	PSL	**S2**	76
11	PSL	**S3**	62
12	PSL	**S4**	2
13	PSL	THF	15
14	PSL	MeTHF	25
15	QLC	ethanol	1
16	QLC	S1	1
17	QLC	S2	1
18	QLC	S3	2
19	QLC	S4	3
20	QLC	THF	5
21	QLC	MeTHF	4

3. Physicochemical features of the solvents used in this study

Table S2. Physicochemical features of the solvents used in this study.[S1-S3]

Solvent	log P[a]	Density
S1	1.42	0.905
S2	1.42	1.359
S3	0.14	0.942
S4	1.71	1.270
S5	–0.60	1.068
S6	0.27	0.912
S7	1.14	1.121

[a] Values obtained from literature [S4-S6]

4. Hydrolysis of peracetylated substrates using *P. stuzeri* lipase

15.0 mg of the peracetylated sugar were solved in 500 μL of solvent containing 50 μL of distilled water. The reaction was started by the addition of 10.0 mg of PSL. The mixture was shaked at 30 ºC and the reaction progress was monitorized by HPLC. Products were purified by column chromatography using dichloromethane:methanol (95:5) as eluent. Solvent was dried and purified products were analysed by ^{1}H-NMR and ^{13}C-NMR.[S7, S8]

2,3,4,6-Tetra-*O*-acetyl-α/β-D-galactopyranose (1a): colorless oil, 90 % yield. **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 5.52 (bd, *J* = 3.4 Hz, 1H, H-1α-anomer), 5.48 (dd, 1H, *J* = 1.3 Hz), 5.41 (dd, 1H, *J* = 3.5 Hz), 5.18 (dd, 1H, *J* = 3.4 Hz), 4.48 (t, 1H, *J* = 6.7 Hz), 4.11-4.09 (m, 2H), 2.10 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm):** 171.5, 171.3, 171.2, 171.0, 96.9 (C-1 β-anomer), 91.0 (C-1 α-anomer), 71.9, 71.3, 69.2, 68.1, 62.7, 21.9, 21.7, 21.6, 21.5. Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.28 %; H, 5.79 %; found: C, 48.09; H, 5.80. $[\alpha]^{20}_{D}$: +54.6 (c 1.98, $CHCl_3$).

2,3,4,6-Tetra-*O*-acetyl-α/β-D-glucopyranose (2a): colorless oil, 93 % yield. **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 5.54 (t, *J* = 9.7 Hz, 1H), 5.46 (d, 1H, *J* = 3.5 Hz , H-1 α-anomer), 5.09 (t, 1H, *J* = 9.8 Hz), 4.91 (q, 1H, *J* = 3.0 Hz), 4.74 (d, 1H, *J* = 8.5 Hz , β-anomer), 4.29-4.12 (m, 3H), 2.10 (s, 3H), 2.09

(s, 3H), 2.04 (s, 3H), 2.02 (s, 3H).. **^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm):** 171.8, 171.7, 171.1, 170.6, 96.4 (C-1 β-anomer), 91.0 (C-1 α-anomer), 72.0, 70.8, 69.4, 68.1, 62.9, 21.6, 21.5. Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.28 %; H, 5.79 %; found: C, 48.12; H, 5.81. $[\alpha]^{20}_D$: +46.9 (c 2.49, $CHCl_3$).

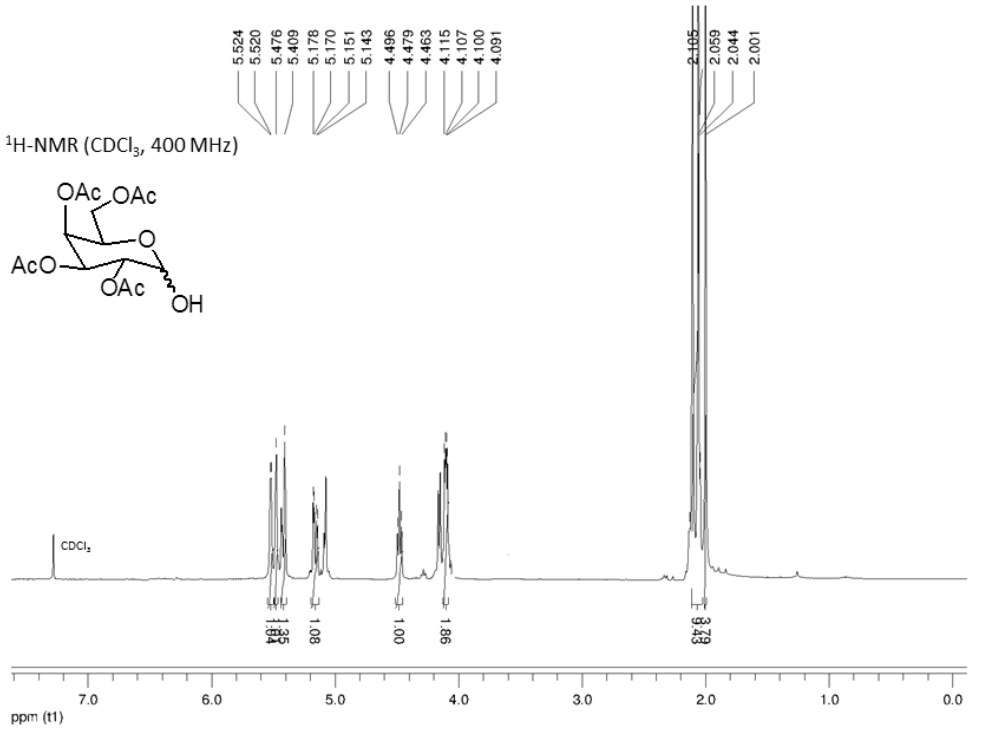
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α/β-D-glucopyranose (4a): colorless oil, 65 % yield. **^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):** 6.00 (d, *J* = 9.5 Hz, 1H, NH), 5.33-5.26 (m, 2H), 5.14 (t, 1H, *J* = 9.4 Hz), 4.65 (d, 1H, *J* = 8.5 Hz, H-1 β-anomer), 4.32-4.27 (m, 1H), 4.23-4.20 (m, 2H), 4.16-4.10 (m, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm):** 172.42, 171.93, 171.47, 170.39, 92.48, 71.89, 69.18, 68.46, 63.04, 53.26, 24.09, 21.07, 21.58 . Anal. Calcd. for $C_{14}H_{21}NO_9$: C, 48.41 %; H, 6.09 %; N, 4.03; found: C, 48.29; H, 6.10; N, 4.04.

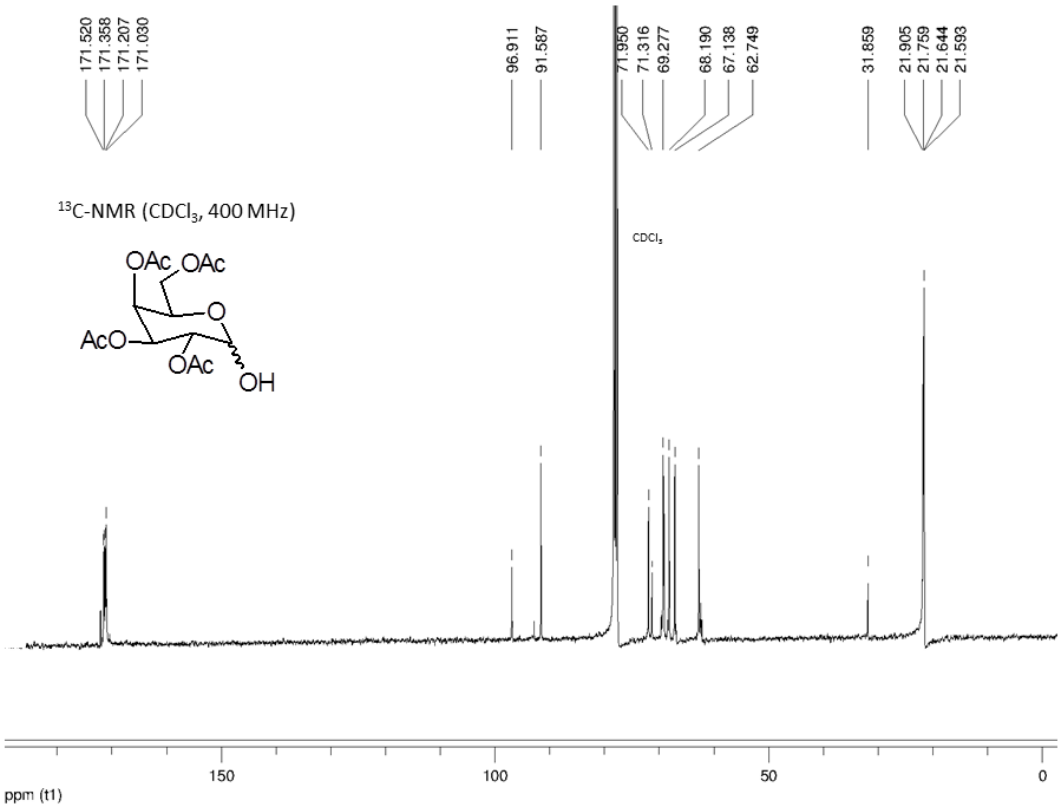
5. Alcoholysis of peracetylated substrates using *P. stuzeri* lipase

15.0 mg of substrate (1β or 1α) were dissolved in 500 µL of dry alcohol (1-butanol, S2, S3, S5, S6 and S7). 10.0 mg of PSL and molecular sieves (50.0 mg) were added to the mixture and the reactions were shaked at 30 °C during 48 h. Alcoholysis reactions were monitorized by HPLC. After complete reaction, products were purified by column chromatography using dichloromethane:methanol (95:5) as eluent. Solvent was evaporated and purified products were analysed by ^{1}H-NMR and ^{13}C-NMR, NMR spectra were similar to those reported above and were concordant with literature.[S7, S8]

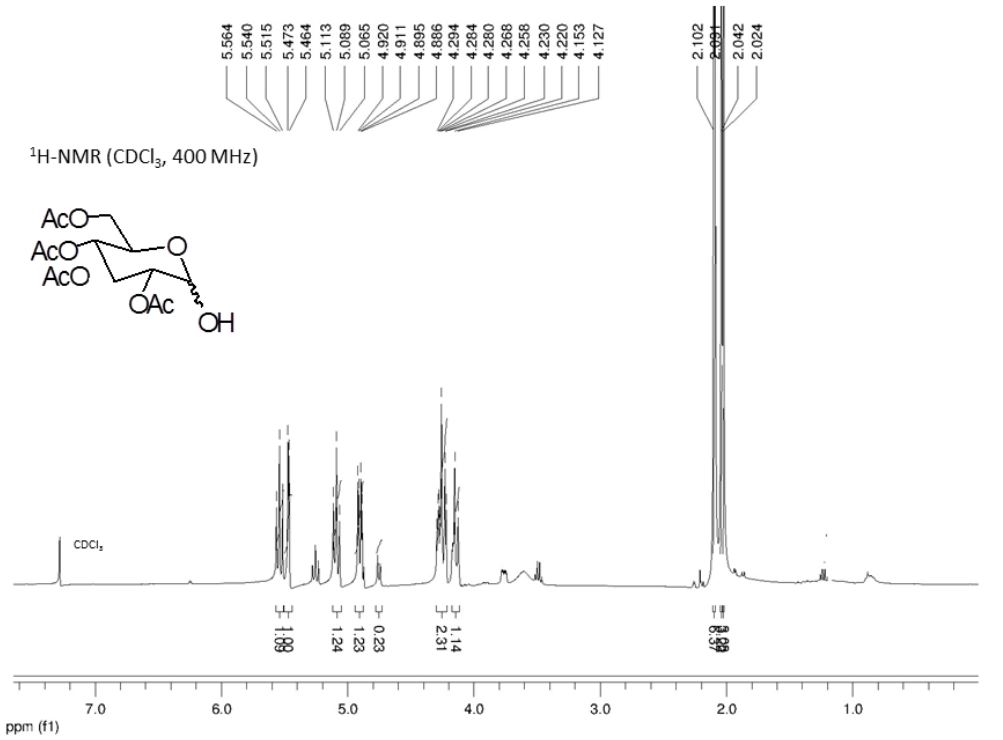
6. NMR spectra of the obtained compounds

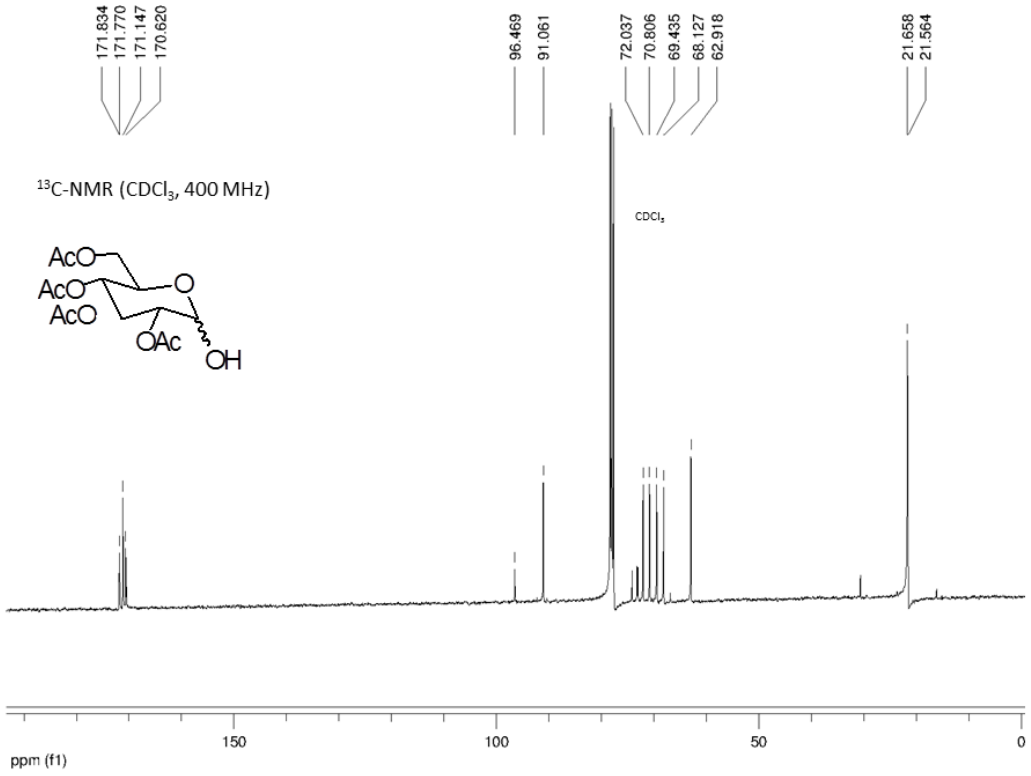
2,3,4,6-Tetra-*O*-acetyl-α/β-D-galactopyranose (1a):



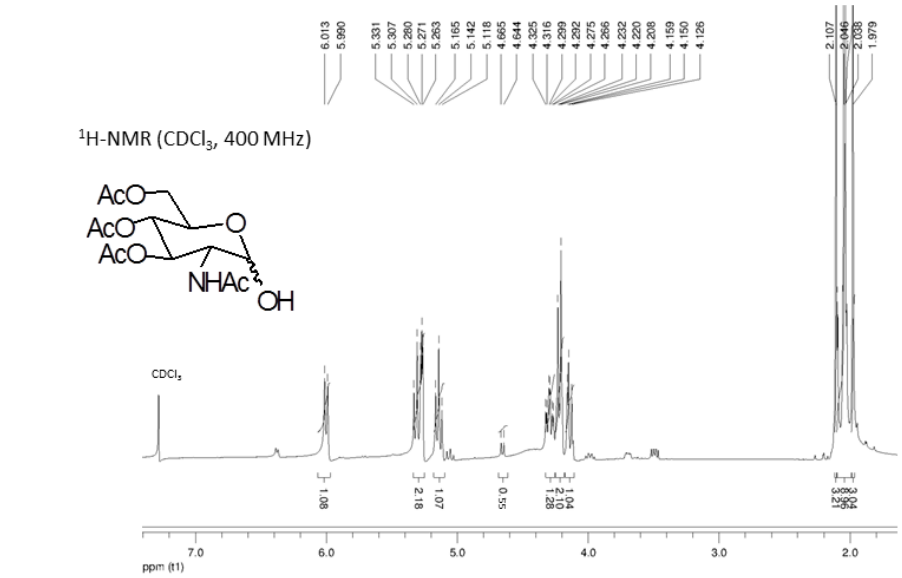


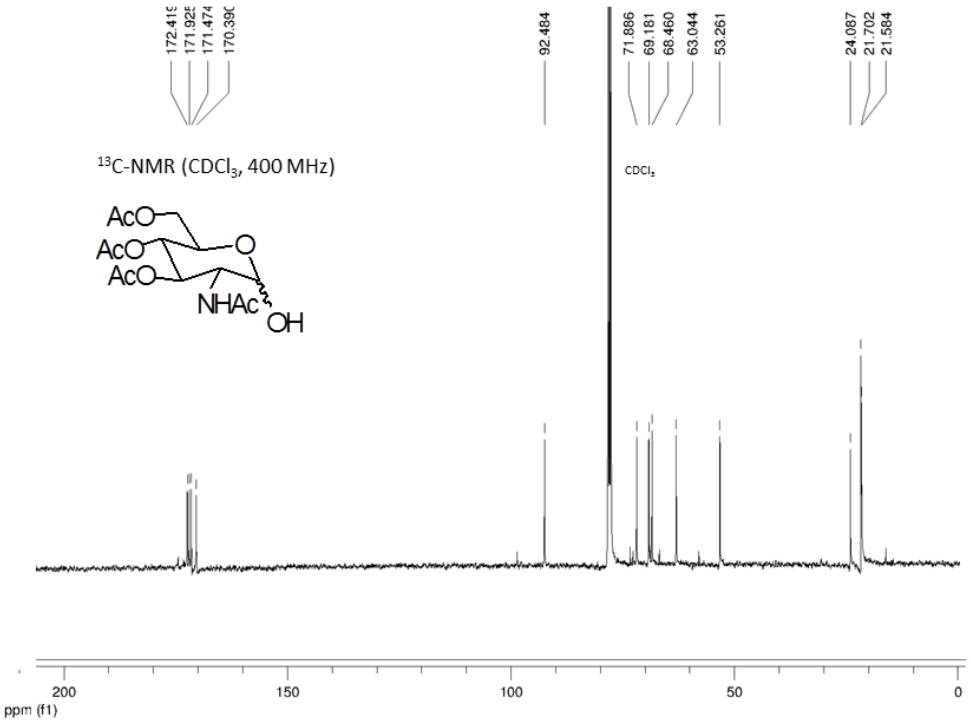
2,3,4,6-Tetra-*O*-acetyl-α/β-D-glucopyranose (2a):





2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α/β-D-glucopyranose (4a):





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