ChemSusChem

Supporting Information

Design and Investigation of a Photocatalytic Setup for Efficient Biotransformations Within Recombinant Cyanobacteria in Continuous Flow

Alessia Valotta,* Lenny Malihan-Yap, Kerstin Hinteregger, Robert Kourist, and Heidrun Gruber-Woelfler*

Contents

1. Design and printing of the tube holders

The tube holders used in this work were designed and 3D printed in house by Alexander Meister, fellow PhD student at IPPE (TU Graz). The 3D model was prepared in SolidWorks and the assembled final product consisted of three parts, which are also marked in Figure S1: the clamps to fix the tubes (with outer diameter 4 mm), the stands equipped with guides for inserting the tube clamps by sliding them in, and finally the top and the bottom plates to fix the stands and keep them in place via standard M3 flat head screws. The tube clamps and stands were printed with a Photon vat photopolymerization printer from Anycubic. For both parts, UV-curable resin supplied by Anycubic was used as 3D printing material. The slicing software provided by Anycubic generated the manufacturing sequence for each slice of the CAD model, which were cured with an exposure time of 10 seconds and set to a layer height of 50 µm. A support structure was added via the slicing software by using its standard settings and a support density of 50%. After printing, the printed parts were cleaned with ethanol and the support structures were removed. The parts were also cured in a UV station from Formlabs, by placing them in the curing station for 15 minutes at room temperature. The bottom and top plates were instead printed in a TEVO Black widow fused deposition modelling 3D printer from polylactic acid (PLA) filament. The .stl file exported from CAD was sliced with the software provided by TEVO, and the layer height for the printing sequence was set to 0.2 mm.

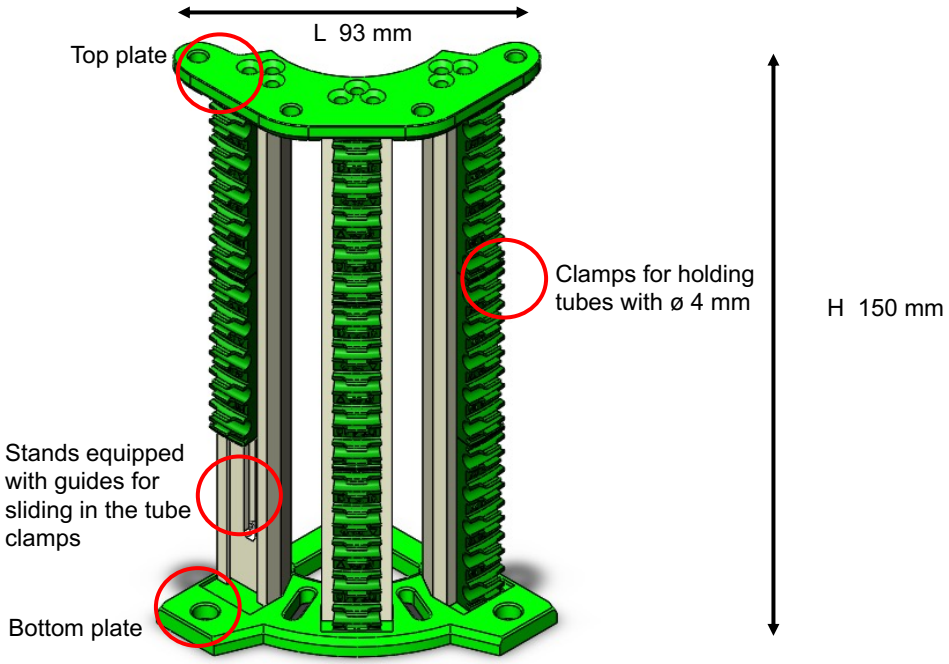


Figure S1. 3D model of the in-house designed and printed tube holders, with the respective main parts marked.

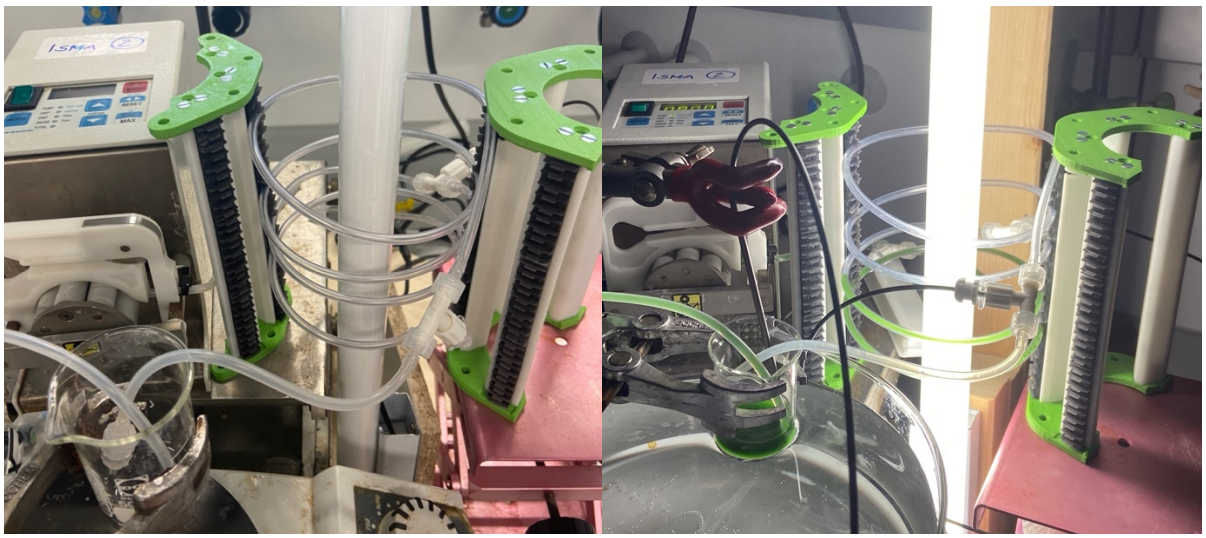


Figure S2. Real life image of the used experimental setup. To the left, a picture of the setup after cleaning, including the peristaltic pump, light source, tube holders, coil reactor and magnetic stirrer. To the right, a picture of the setup during operation, in this case during the experiments where the reaction solution was kept at 30°C by using a temperature controlled water bath.

2. Synthesis of 1a and isolation of 1b

The substrate **1a** was synthesized according to literature procedure[1]. 80 mL of acetic acid are dosed into a 250mL-flask and 34 g of ammonium acetate is added. 16 mL of citraconic anhydride are dropwise added to the solution. The solution was mixed and heated under reflux for 2.5 h. The obtained solution is an orange-brown oil. After cooling the mixture to room temperature, the acetic acid is removed in vacuo and the remaining solution is mixed with 100 mL of 0.9% saline solution and extracted 8 times per 50 mL ethyl

acetate with a separating funnel. The organic phase is dried over sodium sulphate and then filtrated. Ethyl acetate is removed from the solution per vacuo.
The crude product is purified by column chromatography. Therefore, 246.06 g of silica gel is used as a stationary phase in a 500mL-column to purify 5.22g of the crude product. The mobile phase is made out of petroleum ether and ethyl acetate in the ratio of 1:1. After all the fractions containing the product are collected, the solvent is removed per vacuo. The dried product is an off-white solid.
For the isolation of **1b**, the reaction mixture is extracted after the reaction. Therefore, the reaction solution is extracted three times in a separating funnel with ethyl acetate in a ratio of 1:1.33 (v:v). The organic phase is dried with sodium sulphate and the solvent removed per vacuo. The dried product is a white solid.
The NMR-spectrum of **1a** and **1b** were measured using a Bruker Avance III 300 MHz spectrometer by dissolving 20 mg of each substance in $CDCl_3$. After evaluating the NMR spectrum of **1a**, as it can be seen in Figure S3, a purity of >99% is calculated. Figure S4 shows the NMR-spectrum of **1b**, whereby a purity of 95.7% is determined.

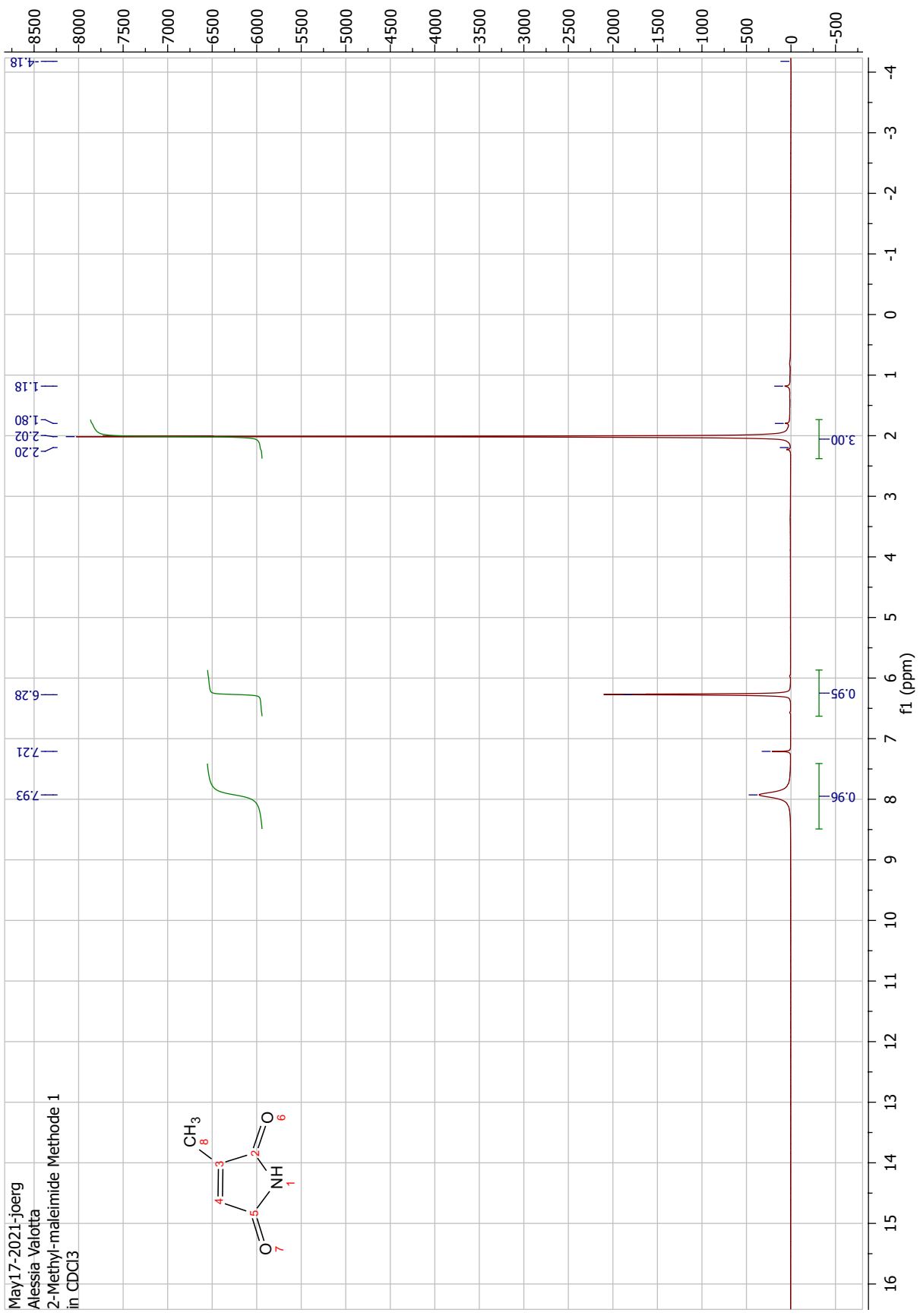


Figure S3. H-NMR spectrum of substrate **1a**.

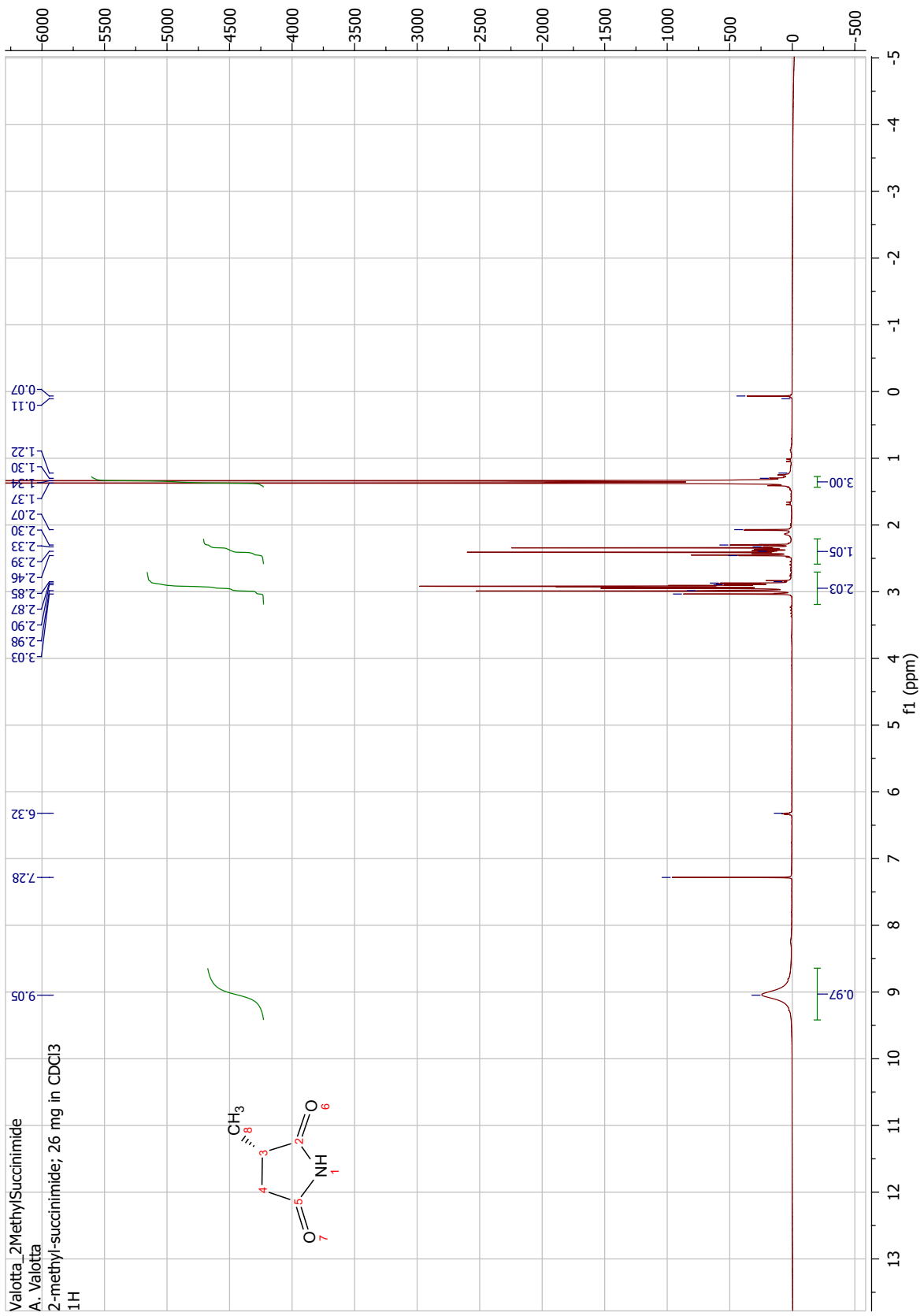


Figure S4. H-NMR spectrum of product **1b**.

3. Setup used for the CSTR experiment

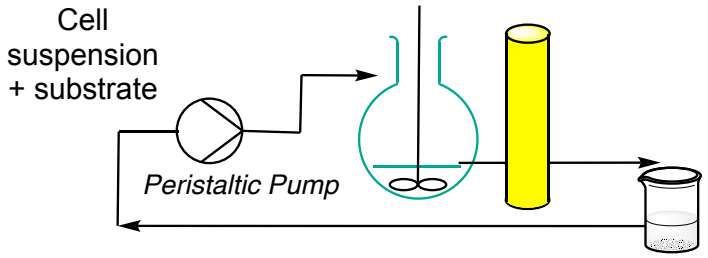


Figure S6. Graphical scheme of the CSTR experiment. The CSTR experiment was carried out by circulating the reaction solution (15 mL as in the coil experiment) with 0.8 mL min^{-1} through a stirred tank filled up to the internal volume of 4.71 mL, same as the internal volume of the coil, and kept at a distance of 4 cm from the light source.

4. Calculation of the E factor

We hereby report in Table S1 the parameters for the evaluation of the E factor. For this calculation, only the multiple feeding reaction is considered, and further process steps (*e.g.* cell cultivation) have not been taken into account, as the simple E factor was chosen to only assess the sustainability of the presented setup.

Table S1. Parameters for the E factor evaluated for both coil and BCR reactors under optimal operating conditions during a double feeding experiment. The values for the BCR are taken from literature.[2]

Parameter	Coil	BCR
Reaction volume [mL]	15	200
Operating time [h]	6	
Conversion [%]	100	50
BG-11 ingredients [g]	0.061	0.82
Water [g]	14.95	199.5
Cells [g_{CDW}]	0.054	0.48
1a concentration [mM]	40	
1b concentration [mM]	40	20
MW 1a [g/mol]	111.11	
MW 1b [g/mol]	111.13	
1a fed [g]	0.066	0.88
1b formed [g]	0.065	0.44
E without water/cells	0.92	2.8
E with cells	1.74	3.93

5. Emission spectrum of the used light source

All experiments were carried out using a fluorescent tube lamp from OSRAM, equipped with G13 socket attachment and delivering cold visible light (4000 K) with 18 W power and providing 1350 lumen. The lamp was mounted on an in-house-constructed lamp holder in order to simplify the buildup of the tubular reactor around the lamp. The light emission spectrum of the lamp is reported in Figure S5.

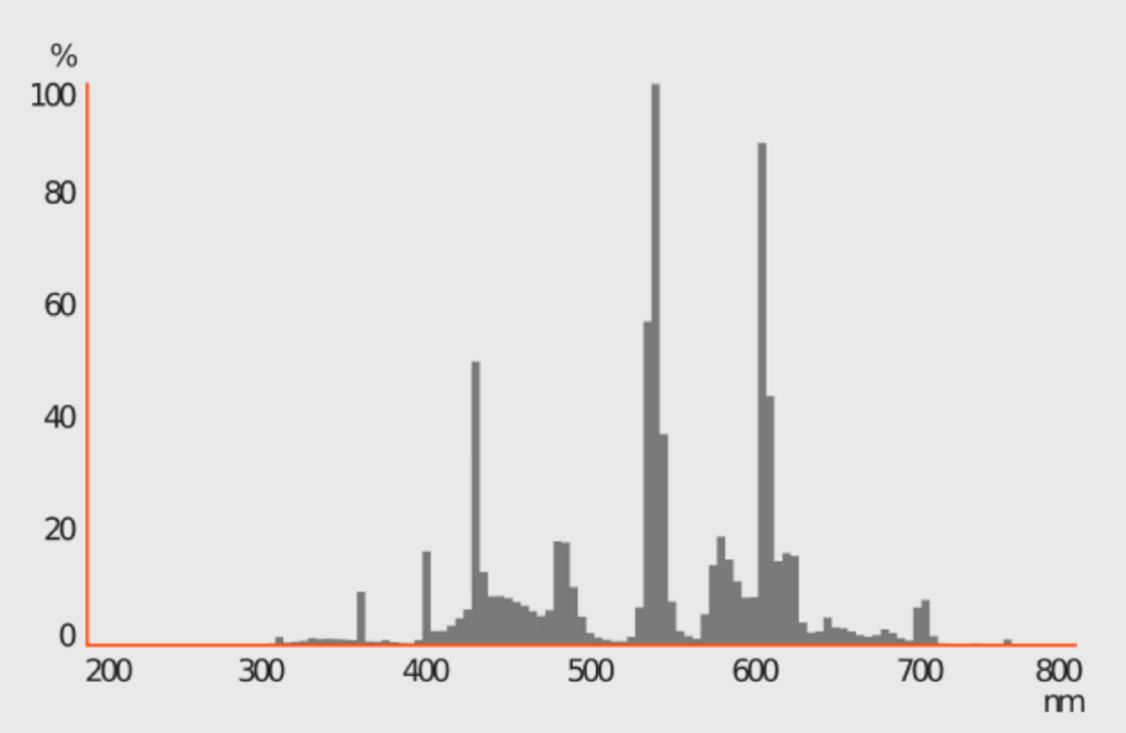


Figure S5. Light spectrum of the used OSRAM fluorescent tube lamp. Available at the supplier[3].

6. GC methods

Table S2. Gas Chromatography Flame Ionization Detector Method. Adapted from[2,4].

<table>
<tr><th colspan="2"></th><th>1 a-b</th><th>2a-b-c
3a-b</th></tr>
<tr><td rowspan="5">Column parameters</td><td>Column</td><td colspan="2">Optima 5-MS</td></tr>
<tr><td>Film thickness</td><td colspan="2">0.25 µm</td></tr>
<tr><td>Length</td><td colspan="2">30 m</td></tr>
<tr><td>Inner diameter</td><td colspan="2">320 µm</td></tr>
<tr><td>Stationary phase</td><td colspan="2">5 % Diphenyl – 95 % Dimethylpolysiloxane</td></tr>
<tr><td rowspan="6">Autosampler and injection port</td><td>Injection volume</td><td>4 µL</td><td>1 µL</td></tr>
<tr><td>Injection temperature</td><td colspan="2">230 °C</td></tr>
<tr><td>Carrier gas</td><td colspan="2">N_2</td></tr>
<tr><td>Total flow</td><td>16.8 mL/min</td><td>21 mL/min</td></tr>
<tr><td>Column flow</td><td>0.8 mL/min</td><td>1 mL/min</td></tr>
<tr><td>Split ratio</td><td colspan="2">20</td></tr>
<tr><td>Temperature program</td><td></td><td><table><tr><th>Rate [°C/min]</th><th>Temp. [°C]</th><th>Hold [min]</th></tr><tr><td>---</td><td>100</td><td>3</td></tr><tr><td>30</td><td>310</td><td>4</td></tr></table></td><td><table><tr><th>Rate [°C/min]</th><th>Temp. [°C]</th><th>Hold [min]</th></tr><tr><td>---</td><td>60</td><td>5</td></tr><tr><td>10</td><td>200</td><td>3</td></tr><tr><td>25</td><td>300</td><td>3</td></tr></table></td></tr>
<tr><td rowspan="4">FID detector</td><td>Temperature</td><td colspan="2">320 °C</td></tr>
<tr><td>Sampling rate</td><td>6.25 pts/s</td><td>12.5 pts/s</td></tr>
<tr><td>H_2 flow</td><td colspan="2">40 mL/min</td></tr>
<tr><td>Air flow</td><td colspan="2">400 mL/min</td></tr>
<tr><td rowspan="3">Retention time</td><td>Substrate</td><td>1a: 5.45 min</td><td>2a: 8.29 min
3a: 6.01 min</td></tr>
<tr><td>Product</td><td>1b: 5.98 min</td><td>2b: 7.45 min
3b: 5.1 min</td></tr>
<tr><td>Internal standard</td><td>6.99 min</td><td>14.52 in</td></tr>
</table>

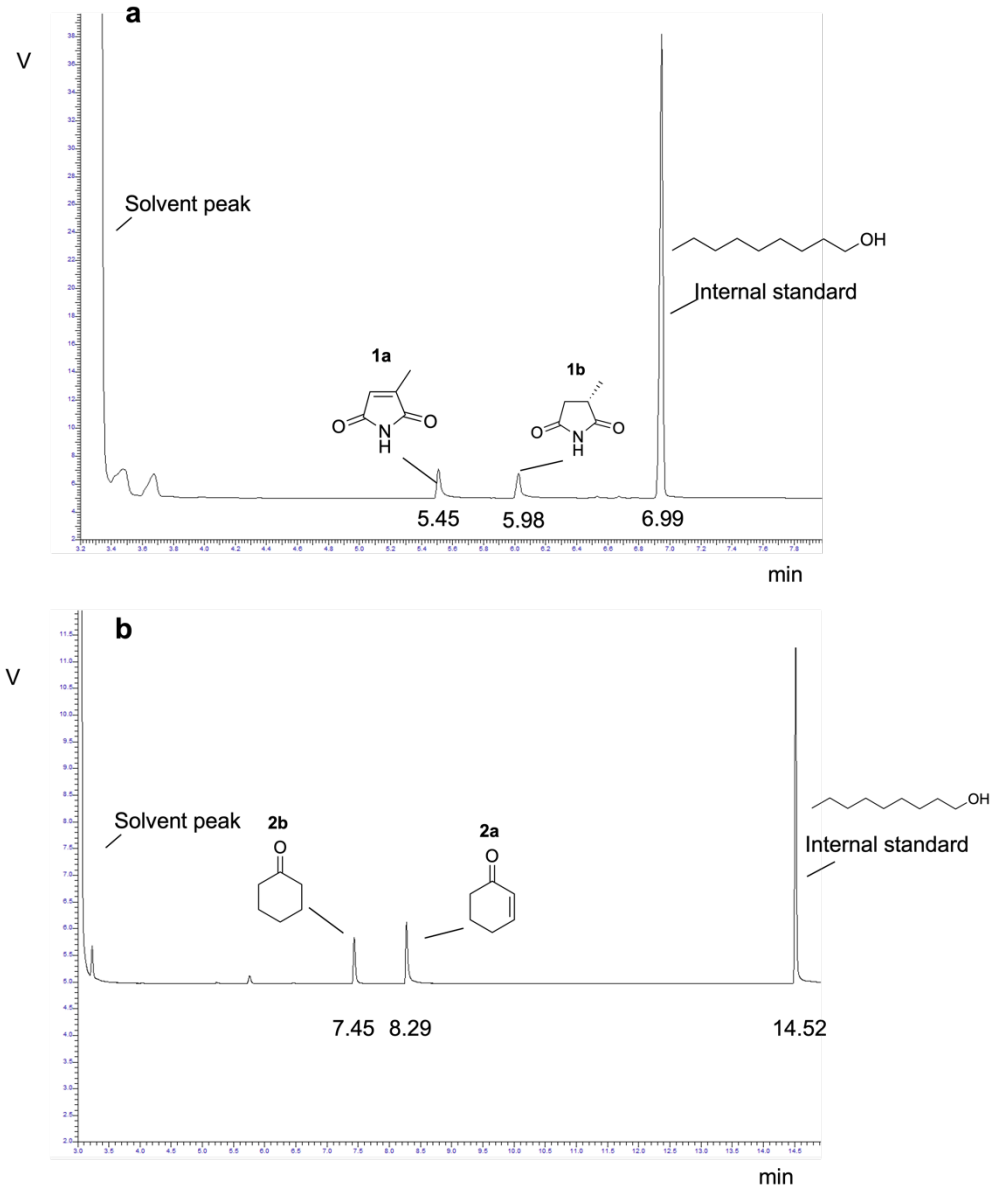
Table S3. Chiral GC method. Adapted from[2].

Chiral GC-FID (GC-2030 Plus, Shimadzu, Japan)

Column	Phase	β-6TBDAc (Column ID: 23254-3)
	Dimension	Length = 50 m; Film Thickness = 0.25 µm; Inner Diameter = 0.25 mm
	Temperature	180 °C
FID Detector	Temperature	250 °C
	Air flowrate	200 mL min^{-1}
	H_2 flowrate	32 mL min^{-1}
Injection port	Volume	1.0 µL
	Carrier Gas	N_2 (24.0 mL min^{-1})
	Temperature	230 °C
	Split Ratio	20
Temperature Program		1) 180 °C (2 min); 2) 220 °C (5 °C min^{-1} for 3 min)
Retention time	**1a**	7.06 min
	1b	10.8 (*R*) 10.3 (*S*) min
	Internal standard	5.1 min

7. GC chromatograms and calibration curves

A calibration curve was determined for all compounds, with 5 points (between 2 and 20 mM) and $R^2 > 0.98$. Calibration slopes [GC area/mM]: **1a** 1131.03, **1b** 765.24, **2a** 916.6, **2b** 731.16, 3a 245.17, **3b** 540.63. The concentrations for each compound in the samples were calculated from the peak areas and the calibration slopes and were normalized with the internal reference area (*n*-decanol was used as internal standard).
Typical chromatograms are reported in Figure S6. The chromatograms from the chiral GC-FID measurement carried out to determine the optical purity of **1b** are reported in Figure S7.


a
V
Solvent peak
1a
1b
OH
Internal standard
5.45
5.98
6.99
min
b
V
Solvent peak
2b
2a
OH
Internal standard
7.45
8.29
14.52
min

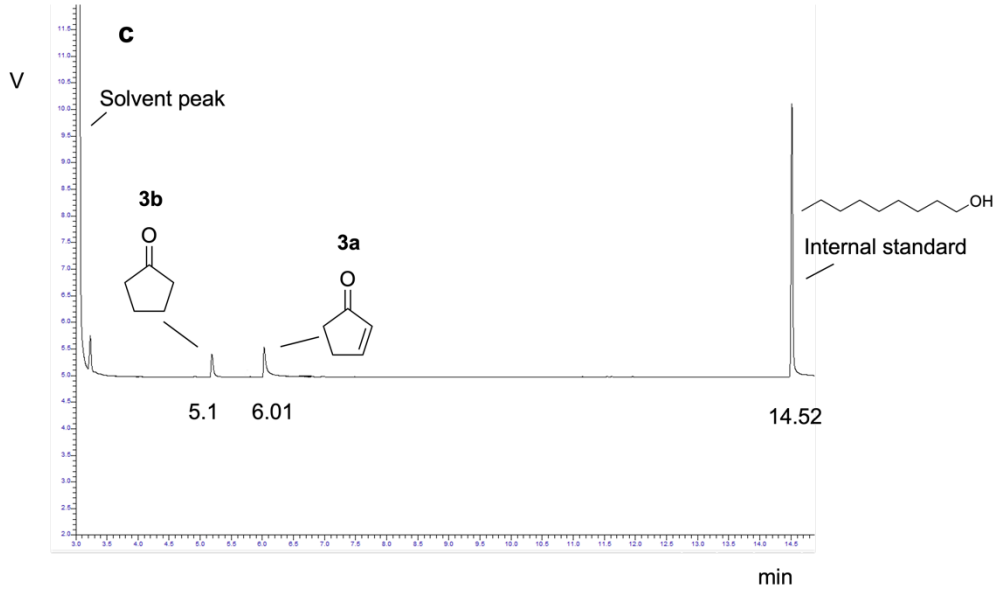


Figure S6. Example of GC chromatograms for all the substrates screened in this work (**1a-3a**) and the obtained reduced products (**1b-3b**), measured in the non-chiral GC column. *n*-decanol has been used as internal reference.

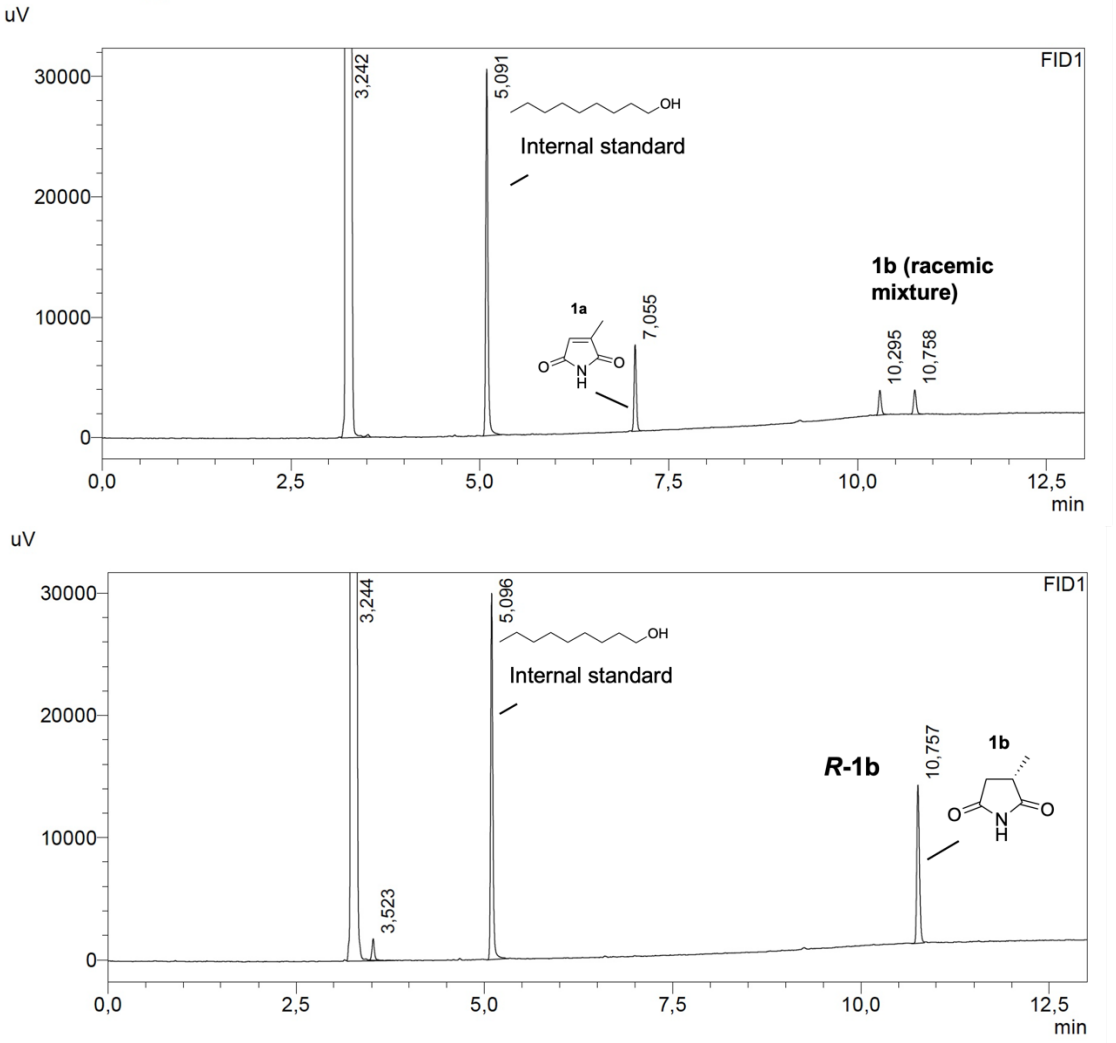


Figure S7. Sample GC chromatograms for the determination of the optical purity of **1b.** Top picture: sample containing 5 mM **1a**, 5 mM of a racemic mixture of **1b**, and internal standard *n*-decanol. Bottom picture: sample containing **1b** isolated after the double feeding experiment. The sample comprised 10 mM of **1b** dissolved in ethyl acetate containing the internal standard.

References

[1] K. Köninger, Á. Gómez Baraibar, C. Mügge, C. E. Paul, F. Hollmann, M. M. Nowaczyk, R. Kourist, Á. Gómez Baraibar, C. Mügge, C. E. Paul, F. Hollmann, M. M. Nowaczyk, R. Kourist, *Angew. Chemie Int. Ed.* **2016**, *55*, 5582–5585.

[2] M. Hobisch, J. Spasic, L. Malihan-Yap, G. D. Barone, K. Castiglione, P. Tamagnini, S. Kara, R. Kourist, *ChemSusChem* **2021**, *14*, 3219–3225.

[3] OSRAM, "Produktdatenblatt," can be found under https://images.obi.at/PROD/DE/document/204/204311_specification_1.pdf, **n.d.**

[4] L. Assil-Companioni, H. C. Büchsenschütz, D. Solymosi, N. G. Dyczmons-Nowaczyk, K. K. F. Bauer, S. Wallner, P. Macheroux, Y. Allahverdiyeva, M. M. Nowaczyk, R. Kourist, *ACS Catal.* **2020**, *10*, 11864–11877.