



Cite this: *Green Chem.*, 2018, 20, 1754

Received 1st January 2018,  
Accepted 8th March 2018

DOI: 10.1039/c8gc00008e

rsc.li/greenchem

## Pressurized CO<sub>2</sub> as a carboxylating agent for the biocatalytic *ortho*-carboxylation of resorcinol†

Katharina Plasch,<sup>a</sup> Gerhard Hofer,<sup>b</sup> Walter Keller,<sup>b</sup> Sam Hay,<sup>b</sup> Derren J. Heyes,<sup>c</sup> Alexander Dennig,<sup>d</sup> Silvia M. Glueck<sup>b</sup>\*<sup>a,e</sup> and Kurt Faber<sup>\*a</sup>

The utilization of gaseous carbon dioxide instead of bicarbonate would greatly facilitate process development for enzyme catalyzed carboxylations on a large scale. As a proof-of-concept, 1,3-dihydroxybenzene (resorcinol) was carboxylated in the *ortho*-position using pressurized CO<sub>2</sub> (~30–40 bar) catalyzed by *ortho*-benzoic acid decarboxylases with up to 68% conversion. Optimization studies revealed tight pH-control and enzyme stability as the most important determinants.

### Introduction

Great efforts are currently undertaken to utilize the inexpensive, non-toxic and abundantly available waste gas CO<sub>2</sub> as a C<sub>1</sub> carbon source for the syntheses of valuable chemicals, materials or fuels.<sup>1</sup> However, despite the fact that photosynthetic CO<sub>2</sub>-fixation mediated by RuBisCO is one of the most dominant reactions in nature, which binds ~10<sup>11</sup> tons CO<sub>2</sub> p.a.,<sup>2</sup> the chemical activation of CO<sub>2</sub> remains challenging due to the high energy input required for substrate activation.<sup>1</sup> Recently, the catalytic carboxylation of epoxides using salen complexes, zinc salts and double metal cyanide catalysts opened access to poly(ether)-carbonates for the production of polyurethanes<sup>3</sup> and is at the threshold of industrial implementation. Further carboxylation strategies using (transition) metal<sup>4</sup> or organocatalysts<sup>5</sup> have been developed to broaden

the usability of carbon dioxide, but these methods are still in their infancy regarding commercialization.

The harsh reaction conditions (~90 bar, 120–300 °C), varying *ortho/para*-selectivity and incomplete yields are major issues in the large-scale production of salicylic acids *via* the chemical carboxylation of phenolates using pressurized CO<sub>2</sub> gas (Kolbe–Schmitt reaction).<sup>6</sup> Although improved by microwave-heating using a bicarbonate-based ionic liquid, the process still suffers from moderate selectivity and yields.<sup>7</sup>

Biocatalytic methods have been explored as alternatives for the carboxylation of electron-rich (hetero)aromatic compounds to yield the corresponding carboxylic acids.<sup>8</sup> Mild reaction conditions, exquisite regioselectivity and excellent yields (*e.g.* 95% for the bio-carboxylation of resveratrol)<sup>9</sup> emphasize the power of bio-carboxylation processes.

However, in the majority of biocatalytic carboxylation protocols reported so far, bicarbonate is used as a CO<sub>2</sub> source, which needs to be applied at elevated concentrations (~3 M) to shift the equilibrium towards the thermodynamically unfavored carboxylation.<sup>10</sup> In practice, excess bicarbonate is not only wasteful, but also creates problems during work-up (foaming) upon acidification. In contrast, the use of alternative CO<sub>2</sub> sources, such as pressurized or sub/supercritical CO<sub>2</sub> for biocatalytic carboxylation is not well investigated. So far, biocatalytic carboxylations were only successful when additional HCO<sub>3</sub><sup>−</sup> (2–3 M) was applied.<sup>11</sup> In order to develop an operationally simple protocol amenable to scale-up, the use of pressurized CO<sub>2</sub> gas was investigated in the carboxylation of 1,3-dihydroxybenzene (**1**, resorcinol, Fig. 1a) as a test substrate using 2,3-dihydroxybenzoic acid decarboxylase from *Aspergillus oryzae* (2,3-DHBD\_Ao),<sup>12</sup> 2,6-dihydroxybenzoic acid decarboxylase from *Rhizobium* sp. (2,6-DHBD\_Rs)<sup>13</sup> and salicylic acid decarboxylase from *Trichosporon moniliiforme* (SAD\_Tm),<sup>10a</sup> which are highly active in the presence of bicarbonate.<sup>9–11</sup> Special emphasis was devoted to pressure and pH effects on enzyme stability.

The exposure of enzymes to scCO<sub>2</sub>‡ pressure has an impact on activity, stability or selectivity,<sup>14</sup> which is either due to con-

<sup>a</sup>Department of Chemistry, Organic & Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria. E-mail: S.Glueck@Uni-Graz.at, Kurt.Faber@Uni-Graz.at

<sup>b</sup>Institute of Molecular Biosciences, University of Graz, Humboldtstrasse 50, 8010 Graz, Austria

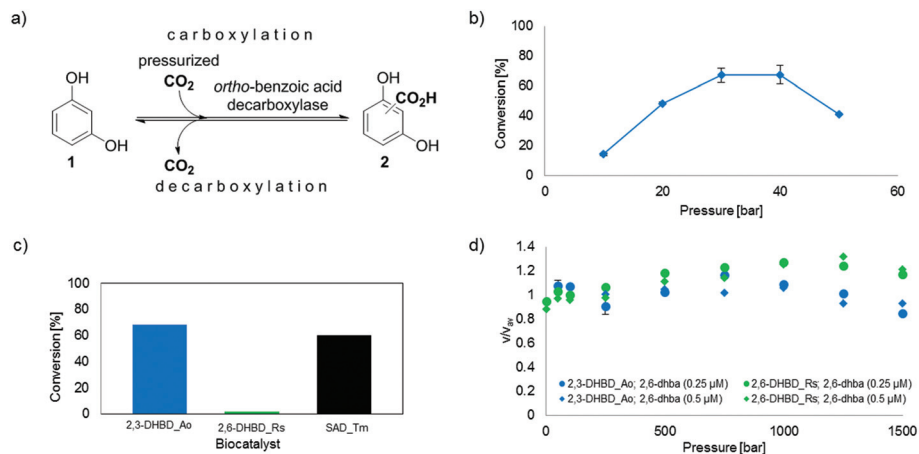
<sup>c</sup>Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK

<sup>d</sup>Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Petersgasse 12, 8010 Graz, Austria

<sup>e</sup>Austrian Centre of Industrial Biotechnology (ACIB), Petersgasse 14, 8010 Graz, Austria

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c8gc00008e





**Fig. 1** (a) Enzyme-catalyzed de/carboxylation of resorcinol (**1**). Carboxylated product **2** is a mixture of regio-isomeric 2,6- (2,6-dhba, **2a**) and 2,4-dihydroxybenzoic acid (2,4-dhba, **2b**) with a ratio of 3 : 4;<sup>10b</sup> (b) CO<sub>2</sub> pressure dependence of the carboxylation of **1** using 2,3-DHBD\_Ao; (c) carboxylation activity of decarboxylases with CO<sub>2</sub> (30 bar) using **1** as a substrate; (d) stopped-flow measurements of the decarboxylation of 2,6-dhba (**2a**) with pressure-pretreated ( $\leq 1.5$  kbar) 2,3-DHBD\_Ao and 2,6-DHBD\_Rs.

formational changes in their secondary and tertiary structure<sup>15</sup> or due to the chemical modification of basic amino acid residues (e.g. Lys, Arg, His) by *N*-carboxylation forming carbamates.<sup>16</sup> The most prominent is the carboxylation of lysine residues (e.g. in RuBisCO,<sup>17</sup> urease<sup>18</sup>), which is required for structural reasons (e.g. ligand for binding of metal ions in RuBisCO)<sup>17</sup> or even mandatory for catalysis (e.g.  $\beta$ -lactamase OXA-10 from *Pseudomonas aeruginosa*,<sup>19</sup> biotin-dependent enzymes<sup>20</sup>). In contrast to these rare beneficial effects, the scCO<sub>2</sub> treatment of enzymes was reported to cause a decrease or complete loss of enzyme activity due to enforced conformational changes (e.g. horseradish peroxidase,<sup>15b</sup> lipase,<sup>15a</sup> tyrosinase<sup>15d</sup>).

## Experimental

### General

Resorcinol (**1**) and 2,6-dihydroxybenzoic acid (**2a**) were purchased from Sigma Aldrich and 2,4-dihydroxybenzoic acid (**2b**) was obtained from Fluka Analytical. SYPRO orange was purchased from Invitrogen. The pressure reactor system (DigiCAT-system from the HEL Group, volume 16 mL) was equipped with a gas inlet and a magnetic stirrer. A HisTrapFF column with a Ni-NTA Matrix and a PD10 desalting column were obtained from GE Healthcare and Vivaspin 20 (30 kDa) was obtained from Sartorius AG. CO<sub>2</sub> gas (3.0 = 99.9% purity) was obtained from the Linde Group. High pressure stopped-flow measurements were performed with a Hi-Tech Scientific HPSF-56 high pressure stopped-flow spectrophotometer from TgK Scientific. A thermal cycler and a CFX real time system for fluorescence measurements were from Bio Rad and a WebQC calculator was used for pH-calculations.<sup>21</sup> 2,3-Dihydroxybenzoic acid decarboxylase from *Aspergillus oryzae* (2,3-DHBD\_Ao), 2,6-dihydroxybenzoic acid decarboxylase from

*Rhizobium* sp. (2,6-DHBD\_Rs) and salicylic acid decarboxylase from *Trichosporon moniliiforme* (SAD\_Tm) were cloned and overexpressed as previously described.<sup>22</sup>

### General procedure for biotransformation under pressurized CO<sub>2</sub> gas

Lyophilized whole cells (90 mg *E. coli* host cells containing the corresponding overexpressed enzyme with an activity of  $5.7 \pm 0.7$  U mg<sup>-1</sup> 2,3-DHBD\_Ao and  $38.1 \pm 0.8$  U mg<sup>-1</sup> 2,6-DHBD\_Rs, respectively) were rehydrated in TRIS-HCl buffer (2850  $\mu$ L, pH 9.0, 100 mM) for 30 min. The substrate **1** [10 mM final concentration, dissolved in 150  $\mu$ L MeOH (5% v/v)] was added to the enzyme solution (3 mL final volume) which was transferred into a pressure reactor. After CO<sub>2</sub> gas (30 bar) was applied *via* an additional gas inlet for  $\sim 1$  h, the reaction mixture was stirred at 50 rpm for 24 h at 30 °C in the tightly sealed pressure reactor. After 24 h the reaction was stopped by taking 100  $\mu$ L of the reaction mixture and diluting it in 900  $\mu$ L of H<sub>2</sub>O/MeCN/trifluoroacetic acid (TFA, 50 : 50 : 3) to precipitate the enzyme, which was removed by centrifugation (10 min, 14 000 rpm). The supernatant was directly used for measurements on a reversed-phase HPLC system.

For CO<sub>2</sub> pressure studies 10, 20, 30, 40 and 50 bar CO<sub>2</sub> gas was applied.

For buffer concentration studies 100, 250 and 500 mM TRIS-HCl buffer was applied.

The CO<sub>2</sub> pressure pretreatment experiments with 2,6-DHBD\_Rs were performed under the same conditions as described above at 10, 40 and 50 bar CO<sub>2</sub> gas (30 mg whole cells, 950  $\mu$ L TRIS-HCl buffer, pH 9.0, 100 mM), however, without the addition of the substrate. The pressure pretreated enzyme was then used for the decarboxylation of 2,6-dhba **2a** [final concentration 10 mM, dissolved in 50  $\mu$ L MeOH (5% v/v)] in a glass vial (1 mL final volume). The vials were tightly



sealed with screw caps and samples were shaken for 24 h, 120 rpm at 30 °C.

For the determination of the enzymatic activity, the lyophilized whole cells of 2,3-DHBD\_Ao and 2,6-DHBD\_Rs (10 mg mL<sup>-1</sup>) were rehydrated in TRIS-HCl buffer (950 μL, pH 9.0, 100 mM) for 30 min. The substrate **2a** (10 mM final concentration) was added to the enzyme solution (1 mL final volume). The vials were shaken at 30 °C with 120 rpm for 0, 1, 2, 4, 6, 8, 10, 12, 15 and 20 min.

All screening experiments were carried out at least in triplicates and all reactor experiments at least in duplicates.

### High pressure stopped-flow system

High pressure stopped-flow measurements were performed under a pressure of 1 bar–1.5 kbar using a purified enzyme (0.5 μM 2,3-DHBD\_Ao and 2,6-DHBD\_Rs, respectively) in TRIS-HCl buffer (pH 9.0, 100 mM) with two different 2,6-dhba concentrations (0.25 and 0.5 μM **2a**, dissolved in 5% v/v MeOH) over 1 min at 30 °C. Spectral changes of the reaction were monitored at 320 nm. All screening experiments were carried out at least in triplicates.

### Thermostability experiments

For differential scanning fluorimetry, protein solution [10 μL, 0.2 g L<sup>-1</sup> in 5 mM MES (pH 6), 150 mM NaCl], SYPRO orange (10 μL, 1:500 diluted in sterile ultrapure water) and multi-component buffer (pH 4 to 9) (10 μL, 1:2:2 molar ratio of L-malic acid, MES and TRIS; 1 M total concentration)<sup>23</sup> were mixed in 96 well plates. Using a C1000 thermal cycler, the solution was heated at 1.2 °C per minute, from 25 °C to 95 °C. Fluorescence was measured every 0.3 °C, using channel 2 of a CFX real time system. For the smaller step size experiment between pH 4 and 5, sodium citrate buffer (100 mM) was used and the temperature range extended from 10 °C to 95 °C. The melting temperature  $T_m$  was calculated as the minimum of the first derivative of the fluorescence *vs.* the temperature. All experiments were carried out in triplicates.

### Analytics

**HPLC analysis.** HPLC/UV experiments were performed on a HPLC Agilent 1260 Infinity system with a diode array detector and a reversed phase Phenomenex Luna column C18 (100 Å, 250 × 4.6 mm, 5 μm, column temperature 24 °C). Conversions were determined by comparison with calibration curves for products and substrates prepared with an authentic reference material. All compounds were spectrophotometrically detected at 263 nm. The method was run over 22 min with H<sub>2</sub>O/TFA (0.1%) as the mobile phase (flow rate 1 mL min<sup>-1</sup>) and a MeCN/TFA (0.1%) gradient (0–2 min 5%, 2–15 min 5–100%, 15–17 min 100%, 17–22 min 100–5%).

**HR-MS analysis.** HR-MS analysis was performed on a nanoHPLC (Ultimate 3000 RSLCnano system – Dionex) system coupled with q-TOF Maxis II-ETD with an ESI-ionisation in positive mode.

## Results and discussion

In spite of the previous reports that carboxylation of phenols requires significant concentrations of bicarbonate (~0.5 M minimum, levelling off at ~1 M to reach a flat plateau at ~3 M) to achieve appreciable conversion levels,<sup>11</sup> but fails with CO<sub>2</sub> (gas) alone,<sup>11b</sup> we tested the use of commercial sparkling water as a medium for the carboxylation of resorcinol (**1**) using 2,3-DHBD\_Ao. Surprisingly, conversions of 23% and 7% were measured in water samples containing only 55 mM or 4 mM HCO<sub>3</sub><sup>-</sup>, respectively, which favorably compares to 22% obtained under standard conditions (3 M bicarbonate) (for details see the ESI†).

In order to evaluate the usability of CO<sub>2</sub> (gas) for carboxylation, the influence of various levels of CO<sub>2</sub> pressure (10–50 bar) on the conversion of resorcinol (**1**, 10 mM, TRIS-HCl buffer 100 mM, pH 9.0) using 2,3-DHBD\_Ao<sup>12</sup> was determined (Fig. 1b). A bell-shaped curve of the CO<sub>2</sub> pressure with an optimum between 30 and 40 bar was found corresponding to a maximum conversion of 68% of carboxylated product (**2**). The conversion was very low below ≤10 bar and dropped significantly at 50 bar. A time study proved that under these conditions equilibrium was reached at ~24 h (see ESI, Fig. S3†).

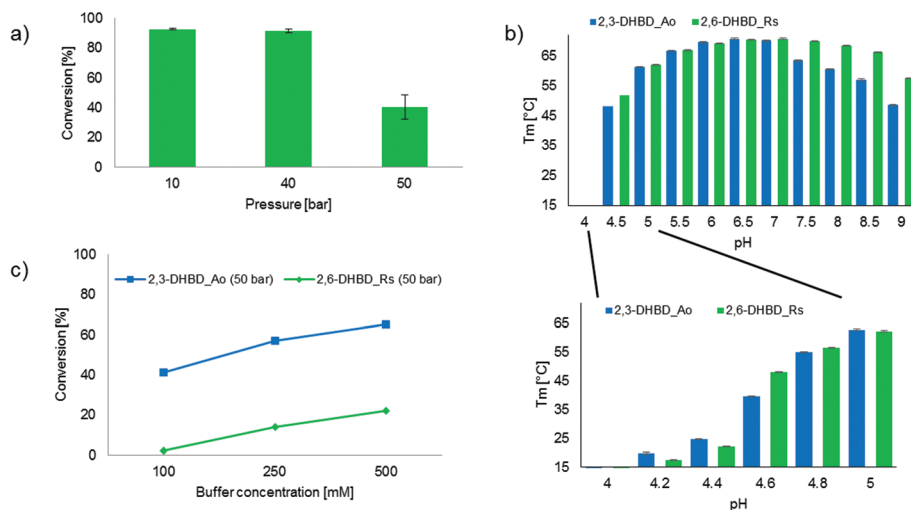
In order to examine whether pressurized CO<sub>2</sub> gas (30 bar) is also accepted by other decarboxylases, 2,6-DHBD\_Rs<sup>13</sup> and SAD\_Tm<sup>10a</sup> were tested (Fig. 1c). While SAD\_Tm yielded similar results obtained with 2,3-DHBD\_Ao (66% and 60% conv., respectively), 2,6-DHBD\_Rs did not lead to an appreciable amount of carboxylated product (**2**, conv. <2%). This result corroborates a previous observation, that 2,6-DHBD\_Rs is inactive under 50–80 bar of CO<sub>2</sub>.<sup>11b</sup>

To answer the question whether pressure *per se* (a physical consequence) or pressurized carbon dioxide (a chemical effect) is responsible for the inactivation of 2,6-DHBD\_Rs, high pressure stopped-flow experiments were performed. For reasons of simplicity, the activity of (hydrostatic) pressure-pretreated 2,6-DHBD\_Rs was determined in the (energetically favored) decarboxylation direction with **2a** as a substrate (Fig. 1d). The fairly constant velocity ( $v/v_{av} = 0.8–1.3$ ) of substrate consumption (monitored by a decrease of absorbance at 320 nm) of both enzymes pretreated with up to 1.5 kbar reveals their general pressure stability (Fig. 1d, see also ESI, Fig. S5–S7†). Consequently, the inactivation of 2,6-DHBD\_Rs can be explicitly assigned to the action of pressurized CO<sub>2</sub>.

In order to determine whether the CO<sub>2</sub> dependent inactivation of 2,6-DHBD\_Rs is reversible, the biocatalyst was pretreated with CO<sub>2</sub> pressure (10, 40 and 50 bar, respectively) before measuring its decarboxylation activity (Fig. 2a). The sharp drop in conversion between pretreatments with 40 and 50 bar CO<sub>2</sub> (92% *versus* 40% conv.) clearly indicates that 2,6-DHBD\_Rs is irreversibly deactivated beyond ~40 bar CO<sub>2</sub>.

Since carbamate formation *via* the carboxylation of lysine residues is a prime suspect for enzyme deactivation, HR-MS measurements were performed. However, no difference in mass between the native and CO<sub>2</sub> pressure (50 bar) treated 2,6-DHBD\_Rs was detected, thus inactivation is most likely not





**Fig. 2** (a) Decarboxylation activity of CO<sub>2</sub> pressure pretreated (10, 40 and 50 bar) 2,6-DHBD\_Rs using 2a as a substrate; (b) DSF comparison of the pH dependent melting temperature of 2,3-DHBD\_Ao and 2,6-DHBD\_Rs in multicompound buffer (pH 4 to 9) and citrate buffer (pH 4 to 5); (c) Influence of TRIS-HCl buffer concentration on the carboxylation of 1 using 2,6-DHBD\_Rs and 2,3-DHBD\_Ao under 50 bar CO<sub>2</sub> pressure.

caused by the carboxylation of basic amino acid residues (see ESI, Fig. S4†).

Carbon dioxide is readily dissolved in the aqueous reaction medium leading to a drop in pH due to the dissociation of H<sub>2</sub>CO<sub>3</sub>.<sup>24</sup> This effect was applied by Hofland *et al.*<sup>25</sup> using CO<sub>2</sub> gas as a 'volatile acid' within a range of pH 4–9 to precipitate proteins. To evaluate whether differences in pH-dependent structural stability between 2,3-DHBD\_Ao and 2,6-DHBD\_Rs could explain their disparate activity, differential scanning fluorimetry (DSF) experiments were performed with both proteins. A first experiment using a multi-component buffer system (L-malic acid, MES, TRIS)<sup>23</sup> shows a broad pH-window from pH 4 to 9, while a second run using citrate buffer and smaller increments reveals details within the pH range of 4 to 5. Overall, 2,3-DHBD\_Ao and 2,6-DHBD\_Rs behave similarly over the whole pH range. Both are thermally most stable between pH 6–7 and show a continuous decrease in denaturation temperature upon higher or lower pH levels (Fig. 2b). Both enzymes are unstable already at room temperature when the pH of the medium reaches below 4.6.

Given that this pH is likely reached in water in a CO<sub>2</sub> pressurized system (30 bar CO<sub>2</sub> in 0.1 M TRIS-buffer corresponds to a calculated pH of 4.6),<sup>21</sup> the influence of the buffer capacity was investigated. An increase of buffer concentration/capacity (TRIS-HCl buffer, 100, 250 and 500 mM) to compensate for acidification due to H<sub>2</sub>CO<sub>3</sub> dissociation and product formation considerably improved the conversion of the carboxylation of 1 with both enzymes (2,3-DHBD\_Ao ~1.5-fold increase; 2,6-DHBD\_Rs ~10-fold increase) (Fig. 2c). These results as well as the DSF analysis clearly indicate that the pH value in the pressure chamber is at the edge of the operational pH-window for both enzymes, with 2,3-DHBD\_Ao performing slightly better.

**Table 1** Comparison of the atom economy of various carboxylation methods of resorcinol (1)

Method	Yield [%]	Atom economy [%]	Ref.
Biocatalytic (CO <sub>2</sub> gas)	68	100	This work
Biocatalytic (HCO <sub>3</sub> <sup>-</sup> )	22	73	22
Chemical (Kolbe-Schmitt)	56	52	6b
Chemical (Kolbe-Schmitt)	47	61	26
Chemical (microwave-assisted Kolbe-Schmitt)	62	55	7

Since the economic usage of resources constitutes an important parameter, the atom economy of various *o*-carboxylation systems was compared (Table 1, see the ESI†). An excellent atom efficiency of 100% combined with a good yield (68%) verifies the benefit of the biocatalytic approach using CO<sub>2</sub> (gas). By way of comparison, the biocatalytic alternative using high amounts of bicarbonate shows a significant drop in atom efficiency (73%), which further drops in the case of traditional chemical (52% and 61%, respectively) or microwave-assisted methods (55%).

## Conclusion

In summary, we have demonstrated that pressurized carbon dioxide can be used directly as a carboxylating agent in the enzyme catalyzed *o*-carboxylation of a phenol as an alternative to the high concentration of bicarbonate. Two enzyme candidates (2,3-DHBD\_Ao and SAD\_Tm) readily accepted the alternative CO<sub>2</sub> source for the carboxylation of the model substrate resorcinol. In contrast, 2,6-DHBD\_Rs was inapplicable under





CO<sub>2</sub> pressure due to irreversible inactivation, which was correlated to a decrease in pH caused by the dissociation of H<sub>2</sub>CO<sub>3</sub>.

Overall, the use of pressurized CO<sub>2</sub> gas significantly improves the efficiency of biocatalytic carboxylations and facilitates downstream-processing of this benign and sustainable approach in using CO<sub>2</sub> as a carbon feedstock for the synthesis of organic acids.

## Conflicts of interest

There are no conflicts of interest to declare.

## Acknowledgements

Funding by the Austrian Science Fund (FWF, projects I 1637-N19 and P 26863-N19) is gratefully acknowledged. This work has been supported by the Austrian BMWFJ, BMVIT, SFG, Standortagentur Tirol and ZIT through the Austrian FFG-COMET-Funding Program. Joerg H. Schrittwieser (University of Graz) is cordially thanked for his support regarding the atom efficiency experiments. Special thanks to Stefan E. Payer and Haifeng Liu (University of Graz) for their helpful ideas and fruitful discussions. Ruth Birner-Gruenberger and Laura Liesinger from the Institute of Pathology (Medical University of Graz) are cordially thanked for their support in HR-MS analysis.

## References

‡scCO<sub>2</sub> = supercritical carbon dioxide.

- (a) Q. Liu, L. Wu, R. Jackstell and M. Beller, *Nat. Commun.*, 2015, **6**, 5933–5948; (b) M. Aresta, A. Dibenedetto and A. Angelini, *Chem. Rev.*, 2014, **114**, 1709–1742.
- G. Schneider, Y. Lindqvist and C.-I. Bränden, *Annu. Rev. Biophys. Biomol. Struct.*, 1992, **21**, 119–143.
- (a) S. Elmas, M. A. Subhani, W. Leitner and T. E. Müller, *Beilstein J. Org. Chem.*, 2015, **11**, 42–49; (b) J. Langanke, A. Wolf, J. Hofmann, K. Böhm, M. A. Subhani, T. E. Müller, W. Leitner and C. Gürtler, *Green Chem.*, 2014, **16**, 1865–1870.
- (a) T. Fujihara and Y. Tsuji, *J. Jpn. Pet. Inst.*, 2016, **59**, 84–92; (b) K. Huang, C.-L. Sun and Z.-J. Shi, *Chem. Soc. Rev.*, 2011, **40**, 2435–2452.
- (a) V. Saptal, D. B. Shinde, R. Banerjee and B. M. Bhanage, *Catal. Sci. Technol.*, 2016, **6**, 6152–6158; (b) B. A. Vara, T. J. Struble, W. Wang, M. C. Dobish and J. N. Johnston, *J. Am. Chem. Soc.*, 2015, **137**, 7302–7305; (c) C. J. Whiteoak, A. Nova, F. Maseras and A. W. Kleij, *ChemSusChem*, 2012, **5**, 2032–2038.
- (a) A. S. Lindsey and H. Feskey, *Chem. Rev.*, 1957, **57**, 583–614; (b) S. S. Mathur, A. Vijayan, V. H. Nougare, S. R. Bhosale, M. K. Dapake, S. D. Parkar, P. D. Damania, N. S. Jagtap, A. Kalirajan, R. H. Khamkar, N. J. Jain, M. M. More, S. B. Aher and S. S. Padwal, *Processes for the preparation of bispyribac sodium and intermediates thereof*, WO 2014128719A2; CAN, 2014, **161**, 390016.
- A. Stark, S. Huebschmann, M. Sellin, D. Kralisch, R. Trotzki and B. Ondruschka, *Chem. Eng. Technol.*, 2009, **32**, 1730–1738.
- (a) R. Lewin, M. L. Thompson and J. Micklefield, *Enzyme Carboxylation and Decarboxylation*, in *Science of Synthesis: Biocatalysis*, ed. K. Faber, W.-D. Fessner and N. J. Turner, Thieme, Stuttgart, New York, 2015, vol. 2, pp. 133–157; (b) A. Alissandratos and C. J. Easton, *Beilstein J. Org. Chem.*, 2015, **11**, 2370–2387; (c) S. M. Glueck, S. Gümüs, W. M. F. Fabian and K. Faber, *Chem. Soc. Rev.*, 2010, **39**, 313–328.
- K. Plasch, V. Resch, J. Hitce, J. Popłoński, K. Faber and S. M. Glueck, *Adv. Synth. Catal.*, 2017, **359**, 959–965.
- (a) K. Kirimura, H. Gunji, R. Wakayama, T. Hattori and Y. Ishii, *Biochem. Biophys. Res. Commun.*, 2010, **394**, 279–284; (b) C. Wuensch, J. Gross, G. Steinkellner, A. Lyskowski, K. Gruber, S. M. Glueck and K. Faber, *RSC Adv.*, 2014, **4**, 9673–9679.
- (a) T. Matsui, T. Yoshida, T. Yoshimura and T. Nagasawa, *Appl. Microbiol. Biotechnol.*, 2006, **73**, 95–102; (b) L. Pesci, S. M. Glueck, P. Gurkov, I. Smirnova, K. Faber and A. Liese, *FEBS J.*, 2015, **282**, 1334–1345; (c) T. Matsuda, Y. Ohashi, T. Harada, R. Yanagihara, T. Nagasawa and K. Nakamura, *Chem. Commun.*, 2001, 2194–2195.
- R. Santha, N. A. Rao and C. S. Vaidyanathan, *Biochim. Biophys. Acta*, 1996, **1293**, 191–200.
- (a) M. Yoshida, N. Fukuhara and T. Oikawa, *J. Bacteriol.*, 2004, **186**, 6855–6863; (b) M. Goto, H. Hayashi, I. Miyahara, K. Hirotsu, M. Yoshida and T. Oikawa, *J. Biol. Chem.*, 2006, **281**, 34365–34373.
- (a) S. Cantone, U. Hanefeld and A. Basso, *Green Chem.*, 2007, **9**, 954–971; (b) S. Garcia, N. M. T. Lourenço, D. Lousa, A. F. Sequeira, P. Mimoso, J. M. S. Cabral, C. A. M. Afonso and S. Barreiros, *Green Chem.*, 2004, **6**, 466–470.
- (a) D. Chen, H. Zhang, J. Xu and Y. Yan, *Enzyme Microb. Technol.*, 2013, **53**, 110–117; (b) F. Gui, F. Chen, J. Wu, Z. Wang, X. Liao and X. Hu, *Food Chem.*, 2006, **97**, 480–489; (c) X. Liao, Y. Zhang, J. Bei, X. Hu and J. Wu, *Innovative Food Sci. Emerging Technol.*, 2009, **10**, 47–53; (d) W. Hu, Y. Zhang, Y. Wang, L. Zhou, X. Leng, X. Liao and X. Hu, *Langmuir*, 2010, **27**, 909–916.
- M. L. Drummond, A. K. Wilson and T. R. Cundari, *J. Phys. Chem. B*, 2012, **116**, 11578–11593.
- M. Oliva, V. S. Safont and J. Andres, *J. Phys. Chem. A*, 1999, **103**, 8725–8732.
- E. Jabri, M. Carr, R. Hausinger and P. Karplus, *Science*, 1995, **268**, 998–1004.
- J. Li, J. B. Cross, T. Vreven, S. O. Meroueh, S. Mobashery and H. B. Schlegel, *Proteins: Struct., Funct., Bioinf.*, 2005, **61**, 246–257.
- P. V. Attwood and J. C. Wallace, *Acc. Chem. Res.*, 2002, **35**, 113–120.



- 21 pH Calculator – Calculates pH of a Solution, <https://www.webqc.org/pHsolver.php>, accessed 02/22/2018.
- 22 C. Wuensch, S. M. Glueck, J. Gross, D. Koszelewski, M. Schober and K. Faber, *Org. Lett.*, 2012, **14**, 1974–1977.
- 23 J. Newman, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2004, **60**, 610–612.
- 24 (a) Z. Duan and R. Sun, *Chem. Geol.*, 2003, **193**, 257–271; (b) A. C. Pierre, *ISRN Chem. Eng.*, 2012, 753687.
- 25 G. W. Hofland, A. de Rijke, R. Thiering, L. A. M. van der Wielen and G.-J. Witkamp, *J. Chromatogr. B: Biomed. Sci. Appl.*, 2000, **743**, 357–368.
- 26 D. K. Hale, A. R. Hawdon, J. I. Jones and D. I. Packham, *J. Chem. Soc.*, 1952, 3503–3509.

