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N-substituted pyrrole carboxylic acid derivatives from 3,4-dihydroxyketons

Tatjana Laudage,[a] Torben Hüsing,[a] Broder Rühmann,[a] Barbara Beer,[a] Luca Schmermund,[a] and Volker Sieber*[a, b, c, d]

Since the chemical industry is largely dependent on petrol-based feedstocks, new sources are required for a sustainable industry. Conversion of biomass to high-value compounds provides an environmentally friendly and sustainable approach, which might be a potential solution to reduce petrol-based starting materials. This also applies for *N*-heterocycles, which are a common structural motif in natural products, pharmaceuticals and functional polymers. The synthesis of pyrroles is a well-studied and established process. Nevertheless, most routes described are not in line with the principles of green and sustainable chemistry and employ harsh reaction conditions and harmful solvents. In this study, 3,4-dihydroxyketons are used as excellent platform chemicals for the production of *N*-substituted pyrrole-2-carboxylic- and pyrrole-2,5-dicarboxylic acids, as they can be prepared from glucose through the intermediate D-glucarate and converted into pyrrolic acid derivatives under mild conditions in water. The scope of this so far unknown reaction was examined using a variety of primary amines and aqueous ammonium chloride leading to pyrrolic acid derivatives with *N*-substituents like alkane-, alkene-, phenyl- and alcohol-groups with yields up to 20%. The combination of both, enzymatic conversion and chemical reaction opens up new possibilities for further process development. Therefore, a continuous chemo-enzymatic system was set up by first employing an immobilized enzyme to catalyze the conversion of D-glucarate to the 3,4-dihydroxyketone, which is further converted to the pyrrolic acid derivatives by a chemical step in continuous flow.

Introduction

In the last decades, the demand for green and sustainable chemistry has increased rapidly as the request for environmentally-friendly and less hazardous processes grows.[1] This demand is accompanied by a rising interest in the bio-based production of chemicals as alternative to conventional fossil-based production pathways.[2] Biocatalytic conversion of renewable resources represents a sustainable approach for the synthesis of platform chemicals.[3] D-Glucarate has been proposed as one such platform molecule, as it can be easily derived from glucose.[4] It is a highly potent starting material for different products, providing access to a variety of derivatives like glucaro-γ-lactones, glucaro-δ-lactones, glucarodilactones and polyhydroxypolyamides.[3b]

In this work, we describe the utilization of D-glucarate for the production of *N*-substituted pyrrolic acid derivatives. Nitrogen-bearing aromatics are important building blocks for the synthesis of functional polymers, active pharmaceutical ingredients[5] and metal-organic frameworks.[6] The conductive nature of pyrrole polymers is utilized in biosensors, which for instance detect copper ions[7] or glucose.[8] They can also serve as anticorrosive coatings.[9] Pyrrole derivatives and their corresponding acids are also of interest in the field of supramolecular chemistry as calixpyrroles.[10] Recently, the utilization of Calix[4]pyrrole-cross-linked porous organic polymers as adsorbent material for water pollutants was investigated.[11]

The chemical synthesis of five-membered heterocyclic compounds was first described by Paal[12] and Knorr[13] mainly focusing on the synthesis of pyrroles and its derivatives (Scheme 1A). Since then, several approaches have been investigated to synthesize the nitrogen bearing compounds. However, traditional processes do not meet the criteria of green chemistry, as they employ transition metal catalysts, hazardous solvents or harsh reaction conditions.[14] Various approaches challenging these issues have been reported in literature. For instance, the improvement of the Paal-Knorr synthesis using ionic liquids,[15] IR radiation in combination with silica gel as catalyst,[16] flow reactors,[17] mechanical activation[18] and even solvent- and catalyst-free conditions have been applied.[19]

[a] *T. Laudage, T. Hüsing, Dr. B. Rühmann, Dr. B. Beer, Dr. L. Schmermund, Prof. Dr. V. Sieber*
Chair of Chemistry of Biogenic Resources,
Campus Straubing for Biotechnology and Sustainability, Technical University of Munich,
Schulgasse 16, 94315 Straubing, Germany.
E-mail: sieber@tum.de

[b] *Prof. Dr. V. Sieber*
Catalytic Research Center, Technical University of Munich,
Ernst-Otto-Fischer-Straße 1, 85748 Garching, Germany

[c] *Prof. Dr. V. Sieber*
SynBiofoundry@TUM, Technical University of Munich,
Schulgasse 22, 94315 Straubing, Germany

[d] *Prof. Dr. V. Sieber*
School of Chemistry and Molecular Biosciences, The University of Queensland,
68 Copper Road, St. Lucia 4072, Australia

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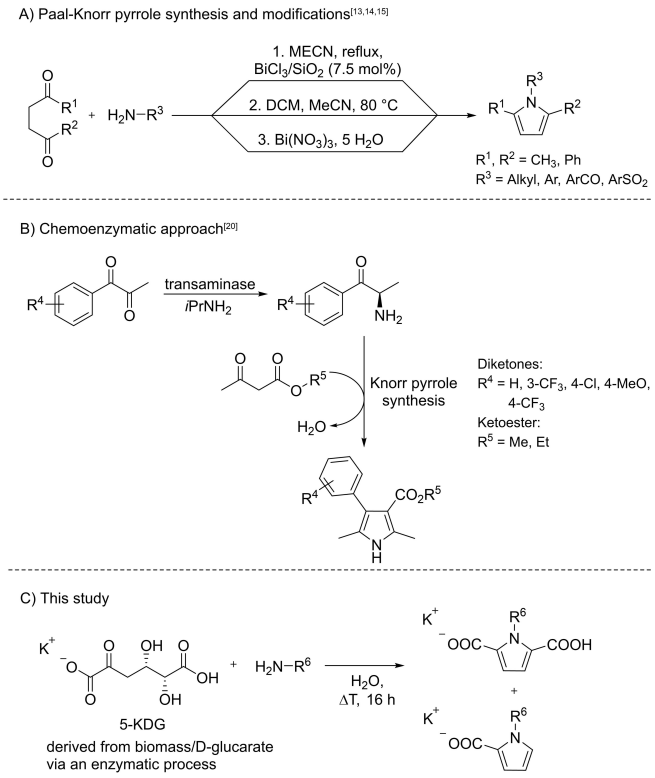


Scheme 1. Comparison of different strategies to synthesize substituted pyrroles. A) Classical synthesis of pyrroles via slightly modified Paal-Knorr procedures; B) Chemoenzymatic synthesis of substituted pyrroles combining a selective enzymatic amination of diketons with a classic Knorr pyrrole synthesis; C) Synthesis of *N*-substituted pyrrole-2-carboxylic and pyrrole-2,5-dicarboxylic acids starting from 3,4-dihydroxyketones derived enzymatically from D-glucarate.

Furthermore, a chemo-enzymatic approach to synthesize pyrroles was published combining the selective amination of α-diketons by a transaminase with a classical Knorr synthesis using β-keto esters in the second step (Scheme 1B).[20] Apart from that, a chemo-enzymatic pathway for the synthesis of pyrroles in a two phase system was established combining olefin metathesis with a monoamine oxidase used for aromatization.[21]

Recently, attention shifted towards biomass-derived starting materials, directly from C_6-carbohydrates, or via platform chemicals such as 5-hydroxymethylfurfural (HMF).[22] In this study, a previously undescribed reaction using 3,4-dihydroxyketons as platform chemicals for the preparation of *N*-substituted pyrrole-2-carboxylic and pyrrole-2,5-dicarboxylic acids (Scheme 1C) was conducted. The C_6-substrate was prepared based on a previously published enzymatic reaction cascade for the production of α-ketoglutarate from D-glucarate.[23] In this study, a glucarate dehydratase (GlucD) from *Actinobacillus succinogenes* 130Z was used to convert D-glucarate to 5-keto-4-deoxy-D-glucarate (5-Kdg, **1**). This enzyme was now incorporated into a chemo-enzymatic synthesis of *N*-substituted pyrrolic acid derivatives (Scheme 1C). The combination of biocatalysis and chemical processes for the utilization of biomass gives additional access to sustainable production of chemicals.[24] However, this poses a challenge, since chemical and enzymatic reactions often require different conditions and compatibility problems may arise.[21,24a] Two phase systems using whole cells in combination with chemical catalysts[21] or the compartmented combination of both, catalysis via immobilization of the enzyme,[24a] avoid these problems and lead to suitable work flows.

Here, we show the production of both, previously described and novel, *N*-substituted pyrrolic acid derivatives deriving from the renewable D-glucarate in aqueous medium without the application of chemical catalysts.

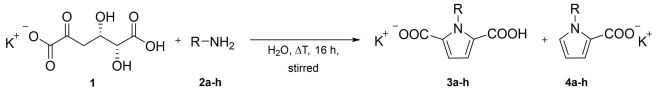
Results and Discussion

We recently demonstrated the enzymatic production of 5-Kdg (**1**) from D-glucarate.[23,25] Here, we scaled the reaction up to concentrations of 1.0 M for subsequent experiments. 5-Kdg (**1**) was then used for a novel synthesis route to pyrrolic acid derivatives in presence of primary amines or ammonium salts without the addition of any catalyst and no other solvent than water. This is in contrast to the common Paal-Knorr synthesis, which is only known for diketones and to the best of our knowledge has never been described for 3,4-dihydroxyketons. Consequently, the focus of this study was set on the investigation of the reaction scope of the formation of pyrrolic acid derivatives from 5-Kdg (**1**) and the combination of the chemical and biochemical reactions.

Scope of the reaction. To investigate the scope of the reaction 5-Kdg (**1**) was reacted with a variety of primary amines (Table 1) to obtain the *N*-substituted pyrrole carboxylic acid derivatives (Scheme 2).

At 70 °C, formation of pyrrolic acid derivatives was observed for all primary amines tested. Within 16 h, the starting material was converted, with pyrrolic acid yields of 1 to 20%, as confirmed by qNMR studies (Table 1). All reactions were accompanied by strong browning, possibly indicating the formation of humins.[26] In most cases, the addition of 1.0 M sulfuric acid led to precipitation (Table S1). In order to obtain more pure products at a small batch size (1 mmol), products were extracted after precipitation with EtOAc. In larger scales, products may be separated by filtration after precipitation by acid.[10]

The highest yield of targeted dicarboxylic acid was observed with *n*-hexylamine (**2c**) (20%, Entry 3) accompanied by slight formation of monocarboxylic acid. Using unsaturated aliphatic 3-buten-1-amine (**2e**) as reactant the formation of the monocarboxylic acid (**4e**) was likewise observed. The dicarboxylic acid (**3e**) reached 10% yield (Entry 5). Similar yields for the dicarboxylic acid have resulted from the conversions with



Scheme 2. Synthesis of different pyrrole acid derivatives (**3**/**4**) using 5-Kdg (**1**) and different primary amines (**2**).

Table 1. qNMR yields of the reaction of 5-Kdg (**1**) with a variety of primary amines at 70 °C. The reactions were stirred for 16 h; dicarboxylic acid (DCA), carboxylic acid (CA).

Entry	Amine	Pyrrole derivatives	qNMR yields/ [%]
1	$NH_4^+Cl^-$ **2a**	DCA (**3a**) CA (**4a**)	13 12
2	HO, NH_2 **2b**	DCA (**3b**) CA (**4b**)	9 9
3	NH_2 **2c**	DCA (**3c**) CA (**4c**)	20 2
4	NH_2 **2d**	DCA (**3d**) CA (**4d**)	1 3
5	NH_2 **2e**	DCA (**3e**) CA (**4e**)	10 1
6	NH_2 **2f**	DCA (**3f**) CA (**4f**)	11 4
7	NH_2 **2g**	DCA (**3g**) CA (**4g**)	10 5
8	NH_2 **2h**	DCA (**3h**) CA (**4h**)	9 8

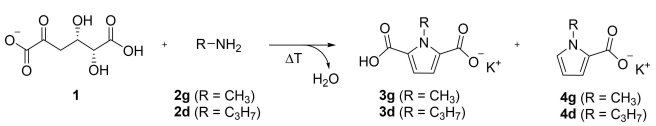
cyclopropylamine (**2f**) and methylamine (**2g**). However, corresponding decarboxylated pyrrolic acid could be determined with 4 to 5% yield (Entry 6, 7). Reactions of 5-Kdg (**1**) with ammonium chloride (**2a**), ethanolamine (**2b**) and aniline (**2h**) led to formation of the corresponding dicarboxylic acid (**3a,b,h**) and monocarboxylic acid (**4a,b,h**) in approximately one to one ratio (Entry 1, 2, 8). The conversion with ammonium chloride (**2a**) gave yields of 12 to 13%. Ethanolamine (**2b**) and aniline (**2h**) led to yields around 9%. Using isopropylamine (**2d**), 1% yield of the corresponding dicarboxylic acid (**3d**) and 3% of the monocarboxylic acid (**4d**) were achieved.

Isolated yields ranged from 2 to 11%. Differences in isolated and qNMR yield may result from different temperatures used and loss of product during purification. In some cases, it was not possible to isolate all products detected in the qNMR. More sophisticated purification procedures were not developed, since this was not the scope of this work, but will be targeted in future studies. Nevertheless, these results show a wide range of the reaction and demonstrate that indeed many different functional groups can be introduced into pyrrolic acid via this new pathway.

Stability of 5-Kdg and the pyrrolic acid derivatives. Decarboxylation occurred in all cases. Therefore, product stability was ensured by incubation of unsubstituted pyrrole-2,5-dicarboxylic acid (PDCA, **3a**) in aqueous solution at 95 °C for 24 h at pH 6. In addition, reaction conditions described in literature indicate the stability of PDCA, (**3a**) at elevated temperatures (up to 100 °C) in basic environment and up to 75 °C in acidic environment over several hours.[10] A conceivable reason for decarboxylation is therefore the decomposition of the substrate 5-Kdg (**1**) or of any reaction intermediate. Preliminary tests showed the decomposition of 5-Kdg (**1**) at 95 °C with a half-life of approximately 2 h (Figure S1).

According to literature, the decarboxylation of α-keto acids can be catalyzed by adding primary amines and heating to 50 to 170 °C.[27] Therefore, we hypothesized that this could be the case for 5-Kdg (**1**) as well. It is proposed that the formed Schiff base leads to a nitrogen ylide. The more basic character of nitrogen compared to oxygen implicated that probably more nitrogen ylide is formed than one containing oxygen, leading to the catalytic effect of the amine (Scheme 3).[27a] Furthermore, phenyl pyrrole (**5h**) could be isolated after conversion of 5-Kdg (**1**) with aniline (**2h**) (Entry 8), indicating a double decarboxylation. Acid catalyzed decarboxylation of pyrrole-2-carboxylic acid (PCA) (**4a**) has been well studied.[28] Literature proposed the addition of water to the carboxyl group of PCA (**4a**) followed by a release of protonated carbonic acid.[28b] In qNMR analysis no double decarboxylated products were observed in the crude reaction mixture indicating the loss of the second carboxylic group during the acidic workup. Further, it was conceivable that the addition of the phenyl ring has an impact on the reactivity of the compound, as it increases the electron density, facilitating the protonation of the carboxyl group and thus also the decarboxylation.

Although we presume that substituents at the amine should have an influence on solubility, reactivity as well as specificity, no general trend was identified. Nevertheless, the described reactions proceeded successfully using amines ranging from simple ammonia chloride to aliphatic, unsaturated, aromatic amines and amino alcohols. This enabled the synthesis of previously undescribed compounds such as 1-hexyl-1*H*-pyrrole-2,5-dicarboxylic acid (**3c**, (Entry 3), 1-isopropyl-1*H*-pyrrole-2,5-dicarboxylicacid (**3d**, Entry 4), 1-(but-3-en-1-yl)-1*H*-pyrrole-2,5-dicarboxylic acid (**3e**, Entry 5), 1-cyclopropyl-1*H*-pyrrole-2,5-dicarboxylic acid (**3f**, Entry 6) and 1-(2-hydroxyethyl)-1*H*-pyrrole-2,5-dicarboxylic acid (**3b**, Entry 2). This compounds open up potential new applications in the field of material science and others. To the best of our knowledge, polymers consisting of PDCA (**3a**) are not described in literature. Due to structural similarity to furan-2,5-dicarboxylic acid, of which the biopolymer polyethylene furanoate consists,[29] it is possible that PDCA (**3a**) may also serve as building block or additive of polymers. The possibility of modification at the nitrogen residue opens up further ways of varying the properties of the resulting copolymers by introducing modifications like crystallization



Scheme 3. Conversion from 5-Kdg (**1**) to 1-methyl-1*H*-pyrrole-2,5-dicarboxylic acid (**3g**) and 1-isopropyl-1*H*-pyrrole-2,5-dicarboxylic acid (**3d**).

inhibitors (e.g. through aliphatic residues) and new functionalities like increased fire resistance through e.g. aromatic residues or halogenated residues. This will be determined in further studies.

Investigation of reaction temperature. After having shown that the reaction can be carried out with different primary amines, further studies were set on the optimization of the reaction temperature as decarboxylation may occur due to thermal activation. The simple methylamine (**2 g**) and the more complex, branched isopropylamine (**2 d**) were used as nitrogen sources to find overall suitable conditions for the reaction process in water (Scheme 3). Following the principle of green chemistry to utilize co-substrates in stoichiometric amounts, both reactants were used in equimolar ratios in order to avoid an excess of amines. After a reaction time of 16 h the samples were acidified using H_2SO_4 (1.0 M). The products were extracted with EtOAc, followed by evaporation of the solvent. Afterwards, NMR measurements of the extracted substances were carried out. In order to investigate the influence of temperature on the reaction process, the reaction of 5-Kdg (**1**) with methylamine (**2 g**) and isopropylamine (**2 d**) were carried out at room temperature, 50 °C, 70 °C, 80 °C and 95 °C. Temperatures below 70 °C led to significantly lower crude product yields than at higher temperatures (Figure 1). Whereas, at 70 °C, 80 °C and 95 °C there were no major differences between the crude yields with around 20 % 1-methyl-1*H*-pyrrole-2,5-(di)carboxylic acid (**3 g**,**4 g**) and around 28 % for 1-isopropyl-1*H*-pyrrole-2,5-(di)carboxylic acid (**3 d**,**4 d**).

Additionally, as decarboxylation products have been observed before, the relative ratios between dicarboxylic acid and monocarboxylic acid at different temperatures were estimated based on the NMR spectra of extracted crude products (Figure 2).

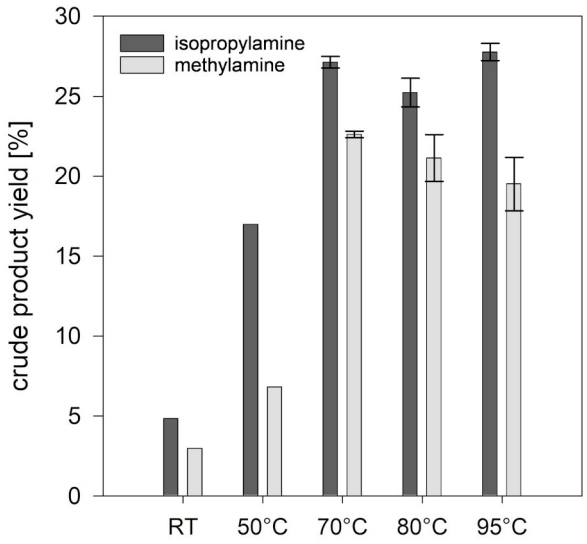


Figure 1. Crude product yields determined by weight analysis, of the reaction of 5-Kdg (**1**) with isopropylamine (**2 d**) and methylamine (**2 g**) at different temperatures for 16 h. Results at 70 °C, 80 °C and 95 °C were determined in duplicates.

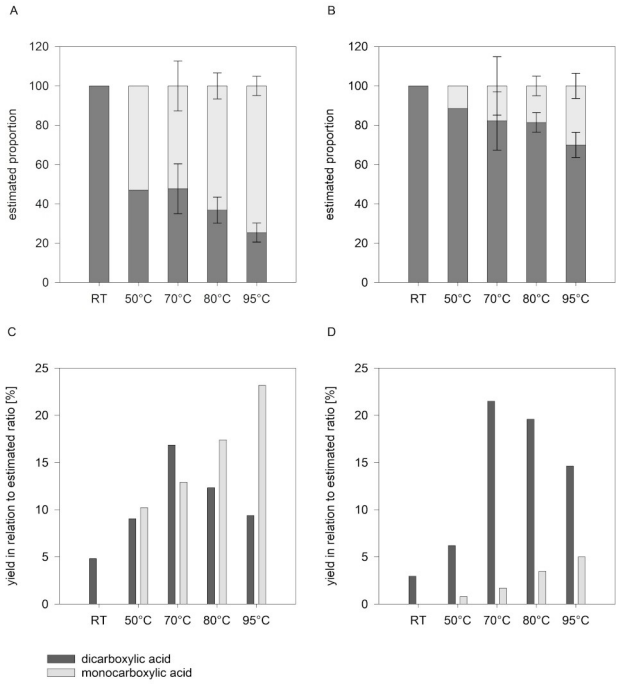
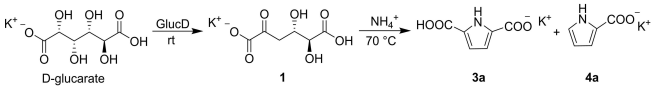


Figure 2. NMR-based analysis of relative ratios between dicarboxylic acids and monocarboxylic acids formed in the reaction of 5-Kdg (**1**) with (A,C) isopropylamine (**2 d**) and (B,D) methylamine (**2 g**); (A,B) NMR-based estimated ratios of monocarboxylic acid and dicarboxylic acid; Reactions at 70 °C, 80 °C and 95 °C were determined in duplicates; (C,D) estimated yields in relation to relative ratios of mono- and dicarboxylic acid.

In both cases, no monocarboxylic acid was detectable in the NMR after incubation for 16 h at RT. With increasing temperature, the proportion of pyrrole monocarboxylic acid also increases. This confirms the previously postulated decarboxylation at elevated temperatures. The different *N*-substituents also had an impact on the ratio of mono- and dicarboxylic acid. Decarboxylation was more pronounced in the reaction with isopropylamine (**2 d**), where only 50 to 25 % dicarboxylic acid remained at 70 °C, 80 °C, and 95 °C, respectively (Figure 2A). In case of the reaction with methylamine (**2 g**), the dicarboxylic acid decreases with increased temperature from 100 % to 70 % (Figure 2B). This leads to the assumption that the extended alkyl chain destabilizes a carboxyl group during the reaction process and promotes its elimination. As the crude product yields are in a similar range between 70 °C and 95 °C and the decarboxylation is promoted by elevated temperatures, the highest yield of the dicarboxylic acids was achieved at 70 °C (Figure 2C, D).

Coupled continuous chemo-enzymatic synthesis. Subsequent to our experiments, a continuous chemo-enzymatic approach to synthesize pyrrolic acids starting from D-glucarate was intended (Scheme 4).

Therefore, the conversion profile of 5-Kdg (**1**) with different ammonium salts at 70 °C was investigated (Figure 3). The reaction of 5-Kdg with NH_4Cl and $(NH_4)_2SO_4$ proceeds quite similar. 5-Kdg (**1**) was consumed after 56 h and PDCA (**3 a**) and PCA (**4 a**) were formed in almost equal quantities of approximately 25 % (Figure 3A, B). The highest yield of PDCA (**3 a**) of



Scheme 4. Reaction scheme of the coupled continuous chemo-enzymatic approach.

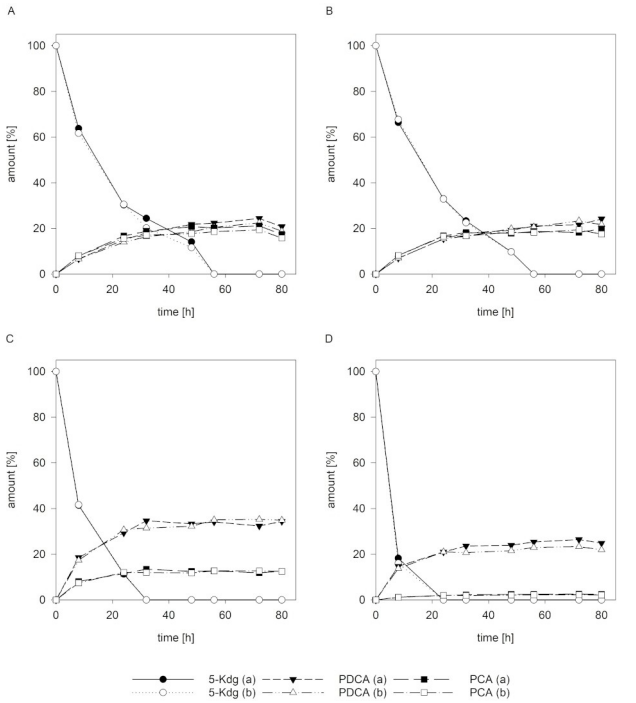


Figure 3. Investigation of the reaction course of 5-Kdg (**1**) with different ammonium salts over 80 h at 70 °C in duplicates; (A) Incubation of 5-Kdg (**1**) with NH_4Cl at pH 8; (B) 5-Kdg (**1**) with $(NH_4)_2SO_4$ at pH 8; (C) 5-Kdg (**1**) with NH_4CO_3 at pH 9; (D) 5-Kdg (**1**) with NH_4OH at pH 10.

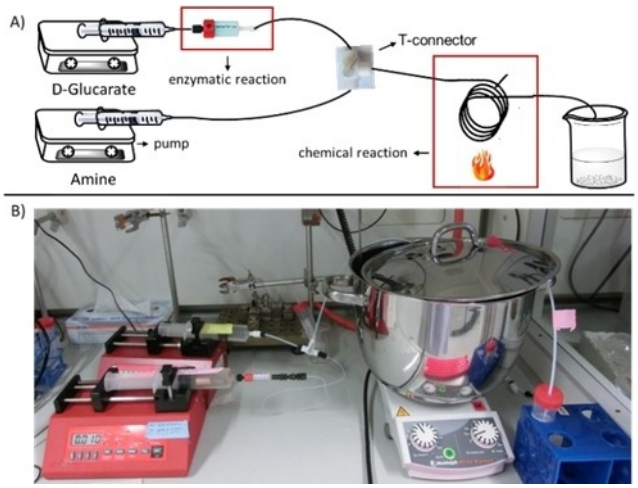


Figure 4. The setup for the coupled continuous chemo-enzymatic synthesis of pyrrolic acids from D-glucarate is shown; A) planned set up, B) final laboratory setup.

36 % was obtained with ammonium bicarbonate. Full consumption of 5-Kdg (**1**) under these conditions was reached after 32 h (Figure 3C). Almost no formation of PCA (**4 a**) could be observed with NH_4OH. PDCA (**3 a**) yields of 25 % were obtained and 5-Kdg (**1**) was no longer detectable after 24 h (Figure 3D). All these observations indicate the stability of the products, but also suggest an influence of the pH during the reaction. This has to be determined in future studies. Nevertheless, due to highest PDCA (**3 a**) yield, ammonium bicarbonate was chosen for the subsequent continuous chemo-enzymatic process.

Due to the temperature needed for the chemical step, we aimed for a compartmentalized setup. For this purpose, the enzyme GlucD was immobilized on a 1 mL IMAC affinity column via its His_6-tag. The substrates, D-glucarate and ammonium bicarbonate, were supplied by two syringe pumps at a flow rate of 0.01 mL/min. The syringe containing D-glucarate was connected to the loaded IMAC affinity column, allowing the conversion to 5-Kdg (**1**). Both flows were combined via a T-connector and the reaction mixture was led through a tube heated to 70 °C with a final flow rate of 0.02 mL/min. The setup is shown in Figure 4. This setup achieved 4 % PCA (**4 a**) and 12 % PDCA (**3 a**). Unfortunately, the same yields as in the batch approach could not be achieved, but this experiment demonstrates that pyrrole acids can be produced in a chemo-enzymatic continuous system.

Different approaches for chemo-enzymatic synthesis of pyrroles are described in literature. For example, the chemo-enzymatic synthesis of pyrroles from diallylamines/-anilines was enabling compartmentalization by using a suitable choice of reaction media and solvents.[21] Thereby, whole cell biocatalysis was combined with a Grubbs-catalyst allowing a one pot cascade metathesis. However, phase separation must be performed with an organic solvent. This is avoided with the route described here, where the reaction is carried out exclusively in water. In both cases, the purification steps were implemented via silica chromatography. In some cases, precipitation during the acidification of the reaction mixture was observed. It is described that pyrrole dicarboxylic acids can be precipitated and filtered,[10,30] similar precipitation was also detected during this study. Therefore, in larger scale approaches, it might be possible to obtain the products by precipitation.

Furthermore, the compartmented one pot reaction described in literature takes place at 37 °C and leads to isolated yields in the range of 5 to 84 % depending on the *N*-substituent.[21] Apart from this, other chemo-enzymatic approaches were conducted without any compartmentalization because of the compatibility and properties of the individual partial reactions.[20,31] However, a one-pot reaction is not feasible at the temperatures required for the formation of pyrrolic acid derivatives from D-glucarate, since the enzyme would be inactivated.

As mentioned above, compatibility problems can be avoided by separating the different steps of the route.[24a] The continuous chemo-enzymatic synthesis offers the possibility to produce different *N*-substituted pyrrolic acid derivatives from platform chemicals using compartmentalization. The immobilization of the enzymes is advantageous, as it allows a

continuous process. In addition, the lysate can be used directly, which makes prior purification of the enzymes unnecessary. By compartmentalizing the reaction steps, the isolation of the intermediate product 5-Kdg (**1**) is avoided, which saves time and costs. In addition, the set up offers the possibility to obtain different products quickly, by using exchangeable compartments. In summary, we provide the possibility to convert renewable biomass into *N*-substituted heterocycles with carboxylic acid functional groups. Various functional groups at the *N*-substituent will give rise to a wide variety of application areas.

Conclusions

While common synthetic routes to pyrroles do not meet the standards of green und sustainable chemistry, this study presents a simple, biobased and environmentally benign approach starting from the platform chemical D-glucarate. The conversion of the 3,4-dihydroxyketone, 5-Kdg (**1**), with aqueous ammonium sources and a variety of primary amines takes place under mild conditions and leads to the aimed products, while H_2O is the only by product. It was possible to introduce different *N*-substituents comprising alkane-, alkene-, phenol- and alcohol groups, demonstrating the broad scope of the reaction. Furthermore, the introduction of these various functional groups provides the opportunity to synthesize novel pyrrolic acid derivatives, which are interesting building blocks for polymers or pharmaceutical compounds. We assume that higher yields can be reached by further reaction and process optimization. The combination of the enzymatic preparation of the 3,4-dihydroxyketone and a synthetic step yields a simple route towards different *N*-substituted pyrrole acids starting from platform chemicals. A continuous chemo-enzymatic process starting from D-glucarate was implemented by immobilizing GlucD.

Experimental Section

Heterologous expression and purification of GlucD. Expression of *glucD* was performed using *Escherichia coli* (*E. coli*) BL21(DE3) containing the plasmid of interest in of autoinduction medium (1 L) containing kanamycin (100 μg/mL). The preculture was incubated in LB medium (100 mL) with kanamycin (100 μg/mL) at 37 °C overnight on a rotary shaker (180 rpm). Expression cultures were inoculated to an initial OD_{600} of 0.1 and incubated for 3 h at 37 °C, followed by 21 h at 16 °C. The purification was performed as described in literature.[23] Aliquots of the protein were frozen in liquid nitrogen and stored at −80 °C.

Immobilization of GlucD. For immobilization, 6 mL of GlucD (7.5 mg/mL) was applied to an IMAC affinity column (1 mL HisTrap™ FF), which was preliminary equilibrated with potassium phosphate (50 mM, pH 8.0) using a peristaltic pump (LA-102, Landgraf Laborsysteme HLL GmbH). After application of the protein the column was washed with 10 cv 50 mM potassium phosphate buffer. Equilibration was conducted with two column volumes (cv) D-glucarate (1.0 M, pH 7.5, titrated with KOH) containing $MgSO_4$ (5 mM). Complete conversion of the substrate was verified by HPLC.

Preparation of 5-Kdg. GlucD was used to prepare 5-Kdg (**1**) enzymatically according to Beer *et al.*.[23] D-glucarate (1.0 M, pH 6.5, titrated with KOH) was converted by 5 U of GlucD in a total volume of 12 mL containing 8.3 mM potassium phosphate from the enzyme preparation and 5 mM $MgSO_4$. The reaction mixture was incubated at room temperature until complete conversion of the substrate. It was stopped by ultrafiltration with a VivaSpin column (10 kDa cut-off, GE Healthcare). Complete conversion was confirmed by HPLC analysis. The flow through was stored at −20 °C. The aqueous solution of 5-Kdg (**1**) was used for subsequent reactions.

Production of different pyrrolic acid derivatives starting from 5-Kdg. General procedure: Each amine and 5-Kdg (**1**) were mixed in an equimolar ratio and stirred in a suitable reaction vial or round bottom flask at 95 °C. The reaction was monitored by thin-layer chromatography determining the decrease of 5-Kdg (**1**, $R_f = 0.65$). As mobile phase methanol, water and ethyl acetate in 3 : 1 : 1 ratio was used. Thin-layer chromatography was analyzed via UV-light or staining solution. The reaction mixture was cooled to room temperature and the pH adjusted to 1 using H_2SO_4 (1.0 M).the mixture was extracted with EtOAc, the organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The reaction mixtures were purified with individual procedures (SI).

Investigation of the reaction course with different ammonium salts. For investigation of the reaction course with different ammonium salts, 5-Kdg (**1**, 1.0 M) was mixed with NH_4Cl (**2 a**), $(NH_4)_2SO_4$, NH_4CO_3 or NH_4OH in an equimolar ratio and heated to 70 °C for 80 h. Time-point samples were analyzed for pyrrole-2,5-dicarboxylic acid (PDCA, **3 a**), pyrrole-2-carboxylic acid (PCA, **4 a**) and 5-Kdg (**1**) via HPLC.

Continuous chemo-enzymatic synthesis of pyrrolic acids. For chemo-enzymatic production of pyrrolic dicarboxylic acids the IMAC affinity resin column, on which the enzyme was immobilized, was equilibrated with D-glucarate (1.0 M, pH 7.5, titrated with KOH) containing 5 mM $MgSO_4$. Syringe pumps at a flow rate of 0.01 mL/min were used, containing syringes with ammonium bicarbonate (1.0 M) and D-glucarate (1.0 M, pH 7.5, titrated with KOH) containing 5 mM $MgSO_4$. The syringe with D-glucarate was connected to the IMAC affinity resin column with immobilized enzyme. Both streams of the pumps were combined in a T-piece. The length of the tubing with the merged streams was chosen so that at the flow rate of 0.02 mL/min, the reaction time reached at least 32 h. The tubing passed through a water bath at 70 °C temperature. The reaction mixture was collected in a 50 mL falcon tube. After 65 h samples were taken and analyzed. Determination and quantification of the products was done by HPLC. The experimental setup is shown in Figure 4.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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