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Stereoselective Monoamine Oxidase-Catalyzed Oxidative Aza-Friedel–Crafts Reactions of *meso*-Pyrrolidines in Aqueous Buffer

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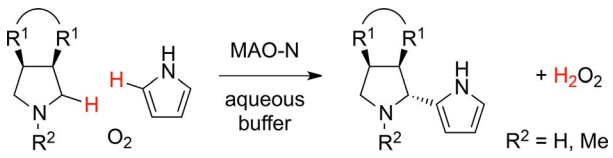
Abstract: We disclose the highly diastereoselective combination of monoamine oxidase-catalyzed oxidation of *meso*-pyrrolidines and aza-Friedel–Crafts reactions in aqueous buffer to give valuable enantioenriched 2-substituted pyrrolidines in a formal double C–H activation process. A range of secondary as well as tertiary amines were shown to be suitable substrates for the biocatalytic oxidation and subsequent addition of a variety of C-nucleophiles.

Keywords: biocatalysis; chemoenzymatic process; oxidation; 2-pyrrolidines; sustainable chemistry

In the transition to sustainable chemistry, the demand for more sophisticated and efficient chemical processes that utilize and generate less hazardous chemicals is ever increasing.[1,2] In order to reduce waste production and energy consumption, the one-pot combination of substrate activation and synthetic transformations in benign media is of great interest. Biocatalysis is particularly useful in this respect, as enzymes are often able to mediate the otherwise difficult activation of building blocks under mild conditions with unrivaled chemo-, regio- and stereoselectivity. The power of chemoenzymatic approaches has been convincingly demonstrated in the synthesis of well-known pharmaceuticals such as sitagliptin,[3] singulair,[4] posaconazole[5] and telaprevir.[6]

In recent years, the direct asymmetric α-functionalization of 2-substituted pyrrolidines has attracted great attention in view of the importance of this structural motif in organocatalysis (e.g., proline derivatives).[7–9] Herein we present a novel one-pot chemoenzymatic oxidative aza-Friedel–Crafts sequence for the synthesis of such chiral 2-substituted pyrrolidines under mild conditions. This approach combines the biocatalytic oxidation of cyclic amines to the corresponding imine derivatives with the subsequent addition of C-nucleophiles in aqueous buffer to yield formally double C–H activation products (Scheme 1).

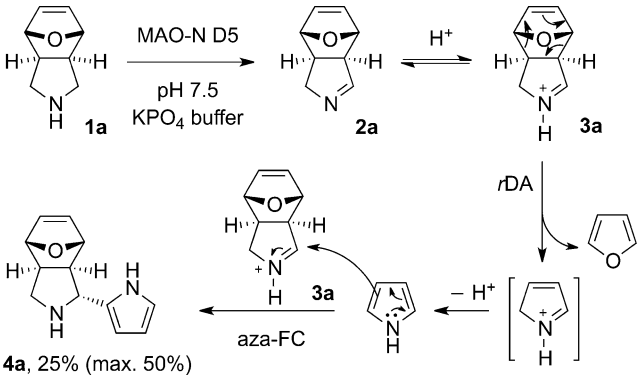
The aza-Friedel–Crafts (aza-FC) reaction[10] can be defined as the 1,2-addition of aromatic and heteroaromatic compounds to imine derivatives. This transformation typically requires organic solvents and strong Lewis or Brønsted acid catalysis, with the exception of a limited number of examples of activator-free phenolic Mannich reactions in aqueous medium.[11] In these cases, the alcohol functionality is proposed to be responsible for both imine activation and regioselectivity control as a result of hydrogen bond formation. Other examples of aqueous aza-FC reactions without an additional catalyst require a highly electrophilic imine.[12,13] Besides the drawback of limited application, these procedures employ imine derivatives that are accessed through additional synthesis and purification steps which are undesirable from a green chemistry perspective.



Scheme 1. One-pot chemoenzymatic oxidative aza-Friedel–Crafts sequence.

We previously reported a biocatalytic oxidation of *meso*-pyrrolidines with an engineered monoamine oxidase (MAO-N D5) to give 1-pyrrolines with high enantioselectivity.[14] In continuation of this research, we investigated the biocatalytic oxidation of *exo*-configured *meso*-pyrrolidine **1a** in aqueous buffer.[15] Intriguingly, 2-pyrrolylpyrrolidine **4a** was obtained as the reaction product rather than the expected imine **2a**. To unravel which chemoenzymatic reaction cascade is responsible for the formation of **4a**, we investigated this reaction in greater detail.

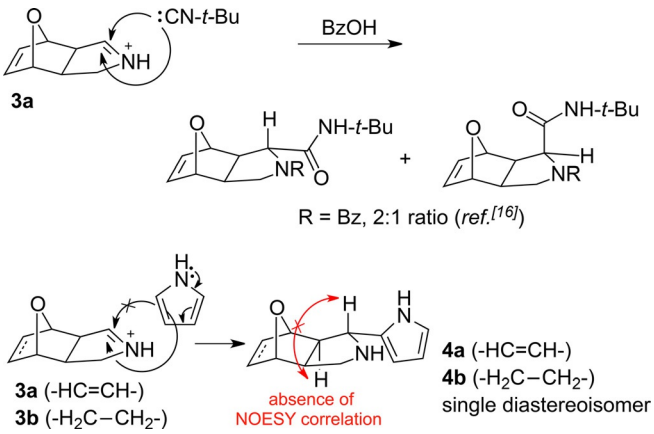
The mechanism for the formation of **4a** likely starts with the expected biocatalytic oxidation of **1a** to give imine **2a** (Scheme 2). However, **2a** is apparently unstable under the reaction conditions and undergoes



Scheme 2. Proposed mechanism for the formation of **4a**.

a *retro*-Diels–Alder (*r*DA) reaction to give furan and pyrrole. Since *rac*-**2a** can readily be prepared with a different method,[16] we hypothesize that the *r*DA reaction proceeds only from protonated **2a** under aqueous conditions. Based on additional experiments (see the Supporting Information),[17] we believe that the aqueous buffer is a sufficiently good proton and/or hydrogen bond donor to activate **2a** toward both *r*DA reaction and aza-FC reaction. Thus, the formed pyrrole adds to unconverted (protonated) **2a** *via* an aza-FC reaction to afford the 2-pyrrolylpyrrolidine **4a** (25%) in a maximum theoretical yield of 50%. This one-stage sequence consisting of biocatalytic oxidation, *r*DA and aza-FC requires only a single purification step, which is a major advantage.

In terms of stereochemistry, we were delighted to obtain 2-pyrrolylpyrrolidine **4a** as a single diastereoisomer, albeit with 70% *ee*.[14] The excellent diastereoselectivity of this aza-FC reaction is a highly interesting feature, since the corresponding Ugi-type three-component reaction (Ugi-type 3CR) with *tert*-butyl isocyanide and benzoic acid proceeds with only moderate diastereoselectivity (Scheme 3).[16] Plausibly, the steric properties of the C-nucleophile are responsible

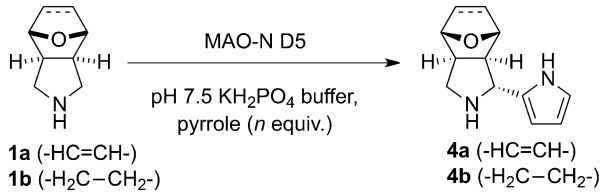


Scheme 3. Representation of diastereoselectivity for the aza-Friedel–Crafts reaction of *exo*-configured **3a** and **3b** with pyrrole, compared to the Ugi-type 3CR with *tert*-butyl isocyanide.

for the high degree of stereoinduction during this transformation.[18]

Given these interesting observations, we proceeded to optimize the oxidative aza-FC reaction of *meso*-pyrrolidine **1a**. Our initial oxidation of **1a** with the biocatalyst afforded 2-pyrrolylpyrrolidine **4a** in 25% isolated yield (Table 1, entry 1). We slightly increased the efficiency by including pyrrole (2 equiv.) in the reaction mixture (entry 2), but the *r*DA reaction of protonated **2a** impeded further improvements. To circumvent this issue, we opted to employ a similar *meso*-pyrrolidine (**1b**) that is unable to undergo the *r*DA reaction. To our delight, product **4b** was obtained with considerably increased enantioselectivity and yield compared to **4a** (entries 2 and 3) while maintaining the diastereoselectivity. However, a side product was formed which is plausibly the addition product of **4b** to iminium ion **3b** (double aza-FC).[19] In order to further optimize the reaction conditions, we performed the oxidative aza-FC reaction of **1b** with varying equivalents of pyrrole (entries 3–6). A significant decrease in conversion was observed with increasing pyrrole stoichiometry, which is probably caused by competitive enzyme inhibition by the excess of pyrrole. To our surprise, the enantioselectivity was also found to decrease with increasing pyrrole stoichiometry. Plausibly, binding of pyrrole in one or more hydrophobic pockets in the enzyme alters its conformation, thereby affecting the enantioselectivity. In order to maximize both yield and enantioselectivity, we performed the oxidative aza-FC reaction as a one-pot, two-stage protocol, in which pyrrole (2 equiv.) is added after the oxidation is complete (17 h). Using this procedure, **4b** was isolated in improved yield and with high enantioselectivity without the formation of any side product (entry 7). We further explored the reaction conditions in terms of molarity, pH and type

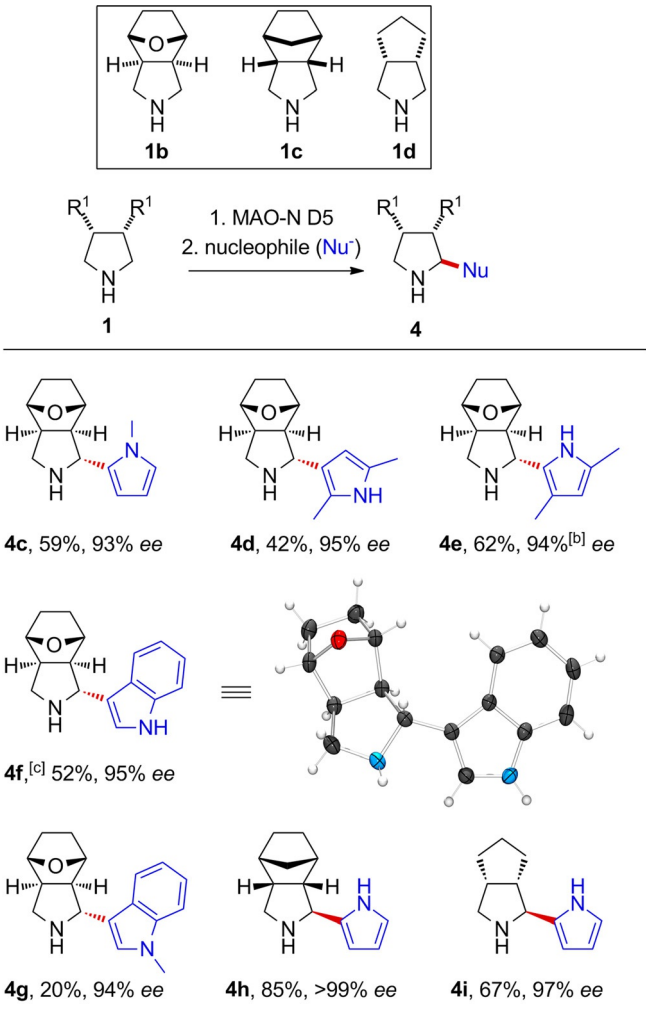
Table 1. Optimization of reaction conditions.[a]



Entry	Substrate	Product	*n*	Yield[b] [%]	*ee*[c]
1	**1a**	**4a**	0	25	70%
2	**1a**	**4a**	2	36	70%
3	**1b**	**4b**	1	55 [12][d,e]	98%
4	**1b**	**4b**	2	56 [6][d,e]	92%
5	**1b**	**4b**	5	23[d]	89%
6	**1b**	**4b**	10	trace[d]	75%
7[f]	**1b**	**4b**	2	67	95%

[a] *Conditions (one stage):* pyrrolidine **1** (1 equiv.), pyrrole (n equiv.), MAO-N D5 freeze-dried whole cells (500 mg per mmol of **1**), 200 mM potassium phosphate buffer (pH 7.5, 50 mL per mmol of **1**), 37 °C, 400 rpm shaking, 17 h.
[b] Isolated yield, unless stated otherwise.
[c] Determined with chiral HPLC by comparison with the racemic mixture.
[d] Yield determined by NMR spectroscopy with 2,5-dimethylfuran (0.5 equiv.) as an internal standard.
[e] Yield of proposed side product[19] in square brackets as determined by NMR spectroscopy with 2,5-dimethylfuran (0.5 equiv.) as an internal standard.
[f] *Conditions (two stages):* pyrrolidine **1** (1 equiv.), MAO-N D5 freeze-dried whole cells (500 mg per mmol of **1**), 200 mM potassium phosphate buffer (pH 7.5, 50 mL per mmol of **1**), 37 °C, 400 rpm shaking, 17 h; then pyrrole (2 equiv.), 37 °C, 400 rpm shaking, 24 h.

Table 2. Substrate scope.[a]



[a] *Conditions (two stages):* 1) pyrrolidine **1** (1 equiv.), MAO-N D5 freeze-dried whole cells (500 mg per mmol of **1**), 200 mM potassium phosphate buffer (pH 7.5, 50 mL per mmol of **1**,), 37 °C, 400 rpm shaking, 17 h; 2) pyrrole (2 equiv.) and DMSO (5% v/v, only for **4f** and **4g**), 37 °C, 400 rpm shaking, 24 h. Single diastereoisomers were observed by NMR analysis of the crude product.
[b] Approximate value as a result of overlap with small impurities in the HPLC chromatogram (see the Supporting Information).
[c] X-ray structure of **4f** with displacement ellipsoids drawn at 50% probability level.[20]

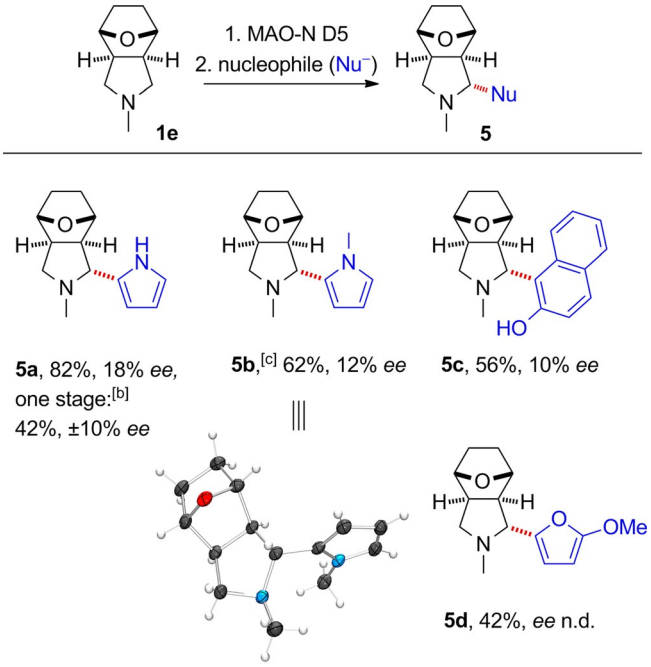
of buffer, but these factors did not significantly affect the reaction outcome (see the Supporting Information).

With the optimized conditions in hand, the scope of the nucleophile was explored with a range of pyrroles and indoles. To our delight, the desired products **4c–i** were obtained in moderate to good yields with high enantioselectivity (Table 2). In all cases, clean conversion to single diastereoisomers of the products was observed. In order to determine the relative and absolute configuration of these adducts, 2-(3-indolyl)pyrrolidine **4f** was crystallized and its structure was confirmed by X-ray crystallography.[20] The analogous products **4a–e** and **4g** were assumed to have the same relative configuration,[21] which is supported by the absence of a NOESY correlation between the protons at the 2- and 3-positions of the pyrrolidine (as indicated in Scheme 3). Other heteroaromatic nucleophiles, including 3-methylindole, 2-methylfuran, 2-methoxyfuran and *N*-phenylpyrrole, were found to be unreactive towards **2b**[22] under these conditions. Further investigation showed that a range of other C-nucleophiles [*N*,*N*-dimethylaniline, 1,3-dimethoxybenzene, *p*-cresol, 1-phenyl-1-trimethylsiloxyethylene, ethyl vinyl ether and 4-(cyclohex-1-en-1-yl)-morpholine] did not show significant reactivity either. To further evaluate the reaction scope, we tested different *meso*-pyrrolidines. Pleasingly, the examined substrates **1c** and **1d** underwent clean conversion to the desired 2-pyrrolylpyrrolidines **4h** and **4i** as single diastereoisomers. Their relative and absolute configurations were

deduced from our previous studies on diastereoselective α-functionalizations of **1c** and **1d**.[8c]

Next, we investigated the application of *N*-methylpyrrolidine **1e** in the oxidative aza-FC reaction with different nucleophiles. To our delight, a wider variety of aromatic and heteroaromatic nucleophiles was applicable for this substrate, presumably as a result of the increased electrophilicity of the iminium ion. The desired 2-substituted pyrrolidines **5a–d** were obtained in reasonable to good yields and as single diastereoisomers (Table 3). In order to determine the relative stereochemistry, 2-pyrrolylpyrrolidine **5b** was crystallized and its structure was corroborated by X-ray crystallography.[20] Disappointingly, products **5a–c** were isolated with low optical purity (≤16% *ee*). Possibly, the biocatalyst has low stereoselectivity in the oxidation of *meso*-pyrrolidine **1e**. However, in this case one would expect to find identical *ee* values for **5a–d**. Another plausible explanation is a post-oxidation racemization by an internal redox-neutral hydride transfer mechanism, as commonly observed for cyclic iminium ions.[23] This racemization has been described to proceed through an azomethine ylide intermediate as depicted in Scheme 4. The low optical purity of **5a–c** may be caused by a combination of low stereoselectivity during the biocatalytic oxidation of **1e** and subsequent partial racemization.[24,25] Despite the decreased enantioselectivity for **1e**, our method is significantly more benign than many other direct α-functionalizations of tertiary amines.[7] Moreover, the synthesis of *rac*-**5a–c** (as racemic standards for *ee* determination) required a laborious three-step protocol involving oxidation, Brønsted acid-mediated addition and *N*-methylation.[26] Thus, our one-pot, two-stage oxidative aza-FC reaction has many advantages over the conventional chemical synthesis of 2-substituted pyrrolidines.

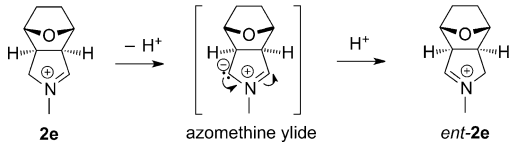
Table 3. Substrate scope.[a]



[a] *Conditions (two stages):* 1) pyrrolidine **1e** (1 equiv.), MAO-N D5 *freeze-dried* whole cells (500 mg per mmol of **1e**), 200 mM potassium phosphate buffer (pH 7.5, 50 mL per mmol of **1e**), 37 °C, 400 rpm shaking, 17 h; 2) pyrrole (2 equiv.) and DMSO (10% v/v, only for **5c**), 37 °C, 400 rpm shaking, 24 h. Single diastereoisomers were observed on NMR spectroscopy of the crude mixture.

[b] *Conditions (one stage):* pyrrolidine **1e** (1 equiv.), pyrrole (2 equiv.), MAO-N D5 freeze-dried whole cells (500 mg per mmol of **1e**), 200 mM potassium phosphate buffer (pH 7.5, 50 mL per mmol of **1e**), 37 °C, 400 rpm shaking, 17 h. Compound **5a** was isolated as an inseparable mixture with **6** (6%) and the yields were calculated based on the molar ratio as determined by NMR analysis.[24,25]

[c] X-ray structure of **5b** with displacement ellipsoids drawn at 50% probability level.[20]



Scheme 4. Proposed mechanism for racemization of **2e**.

In summary, we have developed a one-pot chemoenzymatic oxidative aza-Friedel–Crafts reaction under benign conditions for the direct α-functionalization of a range of pyrrolidines including both secondary and tertiary amines. These typically unreactive substrates were activated by means of biocatalytic oxidation in aqueous buffer, followed by the addition of a variety of C-nucleophiles without the requirement of an additional catalyst. The desired 2-substituted pyrrolidines were obtained in reasonable to good yields and as single diastereoisomers with generally high enantioselectivity. This contribution demonstrates the high potential of one-pot chemoenzymatic transformations to produce functionalized chiral small molecules in a sustainable manner.

Experimental Section

General Procedure for the Oxidative aza-Friedel–Crafts Reaction

To a suspension of rehydrated freeze-dried MAO-N cells (500 mg per mmol of **1**, 30 min, 37 °C, 400 rpm) in aqueous phosphate buffer (50 mL per mmol, 200 mM, pH 7.5) was added pyrrolidine **1** (1.0 equiv.). The reaction mixture was shaken for 17 h (400 rpm, 37 °C) after which the nucleophile (2.0 equiv.) was added, followed by continued shaking for 24 h. The suspension was centrifuged (1 h, 4000 rpm) and the supernatant was collected, adjusted to pH 12–13 with

2 M aqueous NaOH, extracted with CH_2Cl_2 (3×100 mL per mmol of **1**), dried (Na_2SO_4) and concentrated under vacuum. Purification was achieved with flash chromatography (SiO_2) with an eluent gradient of MeOH (1–5%) in CH_2Cl_2.

Acknowledgements

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[7] For selected reviews, see: a) S. A. Girard, T. Knauber, C. J. Li, *Angew. Chem.* **2014**, *126*, 76–103; *Angew. Chem. Int. Ed.* **2014**, *53*, 74–100; b) E. A. Mitchell, A. Peschiulli, N. Lefevre, L. Meerpoel, B. U. W. Maes, *Chem. Eur. J.* **2012**, *18*, 10092–10142; c) C. J. Li, *Acc. Chem. Res.* **2009**, *42*, 335–344.

[8] For oxidative α-functionalizations developed in our group, see: a) C. de Graaff, L. Bensch, S. J. Boersma, R. C. Cioc, M. J. van Lint, E. Janssen, N. J. Turner, R. V. A. Orru, E. Ruijter, *Angew. Chem.* **2015**, *127*, 14339–14342; *Angew. Chem. Int. Ed.* **2015**, *54*, 14133–14136; b) A. Znabet, S. Blanken, E. Janssen, F. J. J. de Kanter, M. Helliwell, N. J. Turner, E. Ruijter, R. V. A. Orru, *Org. Biomol. Chem.* **2012**, *10*, 941–944; c) A. Znabet, E. Ruijter, F. J. J. De Kanter, V. Köhler, M. Helliwell, N. J. Turner, R. V. A. Orru, *Angew. Chem.* **2010**, *122*, 5417–5420; *Angew. Chem. Int. Ed.* **2010**, *49*, 5289–5292.

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[10] For an overview of catalytic asymmetric Friedel–Crafts alkylations, see: J.-R. Chen, W.-J. Xiao, S.-L. You, in: *Catalytic Asymmetric Friedel–Crafts Alkylations*, (Eds.: M. Bandini, A. Umani-Ronchi), Wiley-VCH, Weinheim, **2009**, pp 101–144.

[11] These reactions also meet the definition of an aza-Friedel–Crafts reaction, see: a) T. B. Nguyen, O. Lozach, G. Surpateanu, Q. Wang, P. Retailleau, B. I. Iorga, L. Meijer, F. Guéritte, *J. Med. Chem.* **2012**, *55*, 2811–2819; b) T. B. Nguyen, Q. Wang, F. Guéritte, *Eur. J. Org. Chem.* **2011**, 7076–7079; c) P. D. MacLeod, Z. Li, C.-J. Li, *Tetrahedron* **2010**, *66*, 1045–1050.

[12] As a result of the instability of acyclic imines in water, the scope of the aqueous aza-Friedel–Crafts reaction is limited in general.

[13] For examples, see: a) J. Iwanejko, E. Wojaczyńska, J. Wojaczyński, J. Bąkowicz, *Tetrahedron Lett.* **2014**, *55*, 6619–6622; b) E. Wojaczyńska, J. Bąkowicz, M. Dorsz, J. Skarżewski, *J. Org. Chem.* **2013**, *78*, 2808–2811; c) M. Minakawa, H.-M. Guo, F. Tanaka, *J. Org. Chem.* **2008**, *73*, 8669–8672.

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[15] The choice of substrate is based on the envisioned use of furan as a chiral auxiliary that can be removed by *retro*-Diels–Alder reaction after diastereoselective α-functionalization.

[16] C. de Graaff, L. Bensch, M. J. van Lint, E. Ruijter, R. V. A. Orru, *Org. Biomol. Chem.* **2015**, *13*, 10108–10112.

[17] Test experiments showed no reaction between *rac*-**2a** and pyrrole in organic solvents such as dichloromethane, methanol and acetonitrile, while **4a** was isolated as a single product for the identical reaction in aqueous buffer (see the Supporting Information).

[18] The fact that a reaction between *rac*-**2a** and pyrrole in the presence of a Brønsted acid (TFA) is completely diastereoselective as well (see the Supporting Information), implies that the C-nucleophile is responsible for the degree of stereoinduction rather that the reaction medium. Considering the steric properties of the nucleophile, *tert*-butyl isocyanide is rather linear compared to pyrrole with an *sp*-hybridized nitrogen separating the *sp*-hybridized nucleophilic carbon from the steric bulk. Therefore, the excellent diastereoselectivity towards **4a** presumably results from a significantly larger stereodifferentiation between the diastereotopic faces

of **3a** and **3b** by pyrrole compared to *tert*-butyl isocyanide.

[19] This hypothesis is based on similarities in the ^{1}H NMR spectrum when compared to side product **6**/**6′** (ref.[25]) that was obtained using a one-stage protocol with substrate **1e**. In correspondence with our expectation, the proposed side product (using substrate **1b**) was formed to a higher extent with a lower concentration of pyrrole (Table 1, entries 3 and 4) and was not observed at higher concentration (Table 1, entries 5 and 6).

[20] CCDC 1434414 (**4f**) and CCDC 1434415 (**5b**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

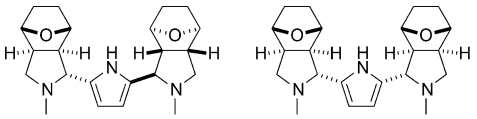
[21] The absolute stereochemistry is determined during the biotransformation and should thus be the same for all products derived from **1b**.

[22] Imine (−)-**2b** was isolated as a stable hemiaminal by the biocatalytic oxidation of *meso*-pyrrolidine **1b** with MAO-N. Imine *rac*-**2b** was isolated as a stable hemiaminal by a two-step protocol of N-halogenation of *meso*-pyrrolidine **1b** followed by base-mediated elimination (see the Supporting Information) or in dehydrated form by IBX-mediated oxidation as in ref.[16]

[23] D. Seidel, *Acc. Chem. Res.* **2015**, *48*, 317–328.

[24] In an attempt to trap the iminium ion before racemization, we performed the reaction with a one-stage protocol having pyrrole present during the biocatalytic oxidation. However, the *ee* decreased even further in this case. The negative influence of pyrrole on the enantioselectivity of the biocatalyst as observed earlier (see Table 1, entries 3–6) presumably cancels out the *ee* gain of reduced racemization.

[25] Using a one-stage protocol, a side product (**6** or **6′**) was formed that we isolated as a mixture with the desired product **5a**. The proposed structures are based on 1D- and 2D-NMR and MS analysis of the product mixture (see the Supporting Information). Due to difficulties in isolation of the side product, we were unable to differentiate between *meso*-**6** and the C_2-symmetric **6′**. The mechanism to its formation is proposed to proceed through addition of the pyrrole moiety of **5a** to iminium ion **2e**. We speculate that this reaction occurs in a hydrophobic pocket of the enzyme, since it was only observed with the one-stage procedure. The maximum theoretical yield for **6**/**6′** is 50%.



[26] TFA-mediated addition of the nucleophile to imine **2b** was not achieved in the synthesis of *rac*-**5d**.

