Enantioselective Aldol Addition of Acetaldehyde to Aromatic Aldehydes Catalyzed by Proline-Based Carboligases

Mohammad Saifuddin, Chao Guo, Lieuwe Biewenga, Thangavelu Saravanan, Simon J. Charnock, and Gerrit J. Poelarends*

ABSTRACT: Aromatic β-hydroxyaldehydes, 1,3-diols, and α,β-unsaturated aldehydes are valuable precursors to biologically active natural products and drug molecules. Herein we report the biocatalytic aldol condensation of acetaldehyde with various aromatic aldehydes to give a number of aromatic α,β-unsaturated aldehydes using a previously engineered variant of 4-oxalocrotonate tautomerase [4-OT(M45T/F50A)] as carboligase. Moreover, an efficient one-pot two-step chemoenzymatic route toward chiral aromatic 1,3-diols has been developed. This one-pot chemoenzymatic strategy successfully combined a highly enantioselective aldol addition step catalyzed by a proline-based carboligase [4-OT(M45T/F50A) or TAUT015] with a chemical reduction step to convert enzymatically prepared aromatic β-hydroxyaldehydes into the corresponding 1,3-diols with high optical purity (e.r. up to >99:1) and in good isolated yield (51−92%). These developed (chemo)enzymatic methodologies offer alternative synthetic choices to prepare a variety of important drug precursors.

KEYWORDS: biocatalysis, aldol reaction, aldolases, β-hydroxyaldehydes, carboligases

The aldol reaction is one of the most important methodologies for the straightforward construction of carbon−carbon bonds in synthetic organic chemistry. This reaction allows complex chiral compounds to be rapidly assembled from simpler building blocks. Enzyme catalysis by using native and engineered aldolases offers a powerful strategy to perform this transformation. With the discovery and engineering of novel aldolase activities, an increasing number of applications of aldolases in stereoselective synthesis have been reported. Despite the rapidly expanding biocatalytic toolbox, aldolases that can use various aromatic aldehydes as acceptors for acetaldehyde addition are rare. Such aldolases would provide an attractive synthetic tool for the preparation of chiral precursors to various biologically active compounds and drug molecules.

Our group has previously reported that the enzyme 4-oxalocrotonate tautomerase (4-OT) from Pseudomonas putida mt-2 promiscuously catalyzes the cross-condensation of acetaldehyde (1) with benzaldehyde (2a) to give cinnamaldehyde (4a) (Scheme 1). It has been shown that 4-OT catalyzes both the initial aldol addition to yield 3-hydroxy-3-phenylpropanal (3a) as well as the subsequent rapid dehydration of 3a to give 4a, preventing the accumulation of chiral aldol adduct 3a in the reaction mixture. Rahimi and co-workers applied mutability landscape-guided protein engineering to further improve this promiscuous aldolase activity of 4-OT.

This resulted in the identification of a double mutant, 4-OT M45T/F50A, with a remarkable 3300-fold improved catalytic efficiency (in terms of kcat/Km) over wild-type 4-OT. Although cinnamaldehydes are valuable synthons in their own right, we were especially interested in the enzymatic preparation of chiral aromatic β-hydroxyaldehydes and the corresponding 1,3-diols, which are important precursors to various natural products, bioactive compounds, and drugs (Figure 1). We envisioned that these chiral aldol adducts could be constructed by the enzymatic addition of acetaldehyde to selected aromatic aldehydes, yielding β-
hydroxyaldehydes that are not (or slowly) dehydrated, allowing their accumulation in the reaction mixture. Chemical reduction of the enzymatically prepared aromatic $\beta$-hydroxyaldehydes would provide access to the corresponding aromatic 1,3-diols, which are key intermediates in the synthesis of drugs such as Fluoxetine (Figure 1).5

Herein we report the 4-OT(M45T/F50A)-catalyzed synthesis of various aromatic $\alpha,\beta$-unsaturated aldehydes, starting from acetaldehyde and a number of aromatic aldehydes, in good isolated yields. We also report the one-pot, two-step chemoenzymatic asymmetric synthesis of various aromatic 1,3-diols with high optical purity (e.r. up to >99:1) and in good isolated yield (51−92%). This chemoenzymatic strategy highlights a highly stereoselective aldol addition of acetaldehyde to diverse aromatic aldehydes, catalyzed by either 4-OT(M45T/F50A) or the newly identified carboligase TAUT015, yielding various enantioenriched aromatic $\beta$-hydroxyaldehydes, which are chemically reduced into the

Figure 1. Chiral aromatic $\beta$-hydroxyaldehydes and cinnamaldehydes are valuable precursors to biologically active compounds and drugs.

Scheme 2. Enzymatic Aldol Reaction of Acetaldehyde (1) with Various Aromatic Aldehydes (2) Using Promiscuous Proline-Based Tautomeres as Carboligases

Scheme 3. Synthesis of Aromatic $\alpha,\beta$-Unsaturated Aldehydes from Simpler Building Blocks Using the Carboligase 4-OT(M45T/F50A)

$^a$Reaction conditions: reaction mixtures consisted of 100 mM 1, 3 mM 2, and 0.1 mg/mL 4-OT (M45T/F50A) in 20 mM sodium phosphate buffer, 5% DMSO v/v, pH 7.3. The reaction volume was 60 mL. $^b$Reaction time (48 h, 72 h for 2t). $^c$Purified by silica gel column chromatography.
room temperature. The reaction volume was 40 mL. To reduce the aldehyde functionality of the enzymatic products 20 mM sodium phosphate buffer (MOPS for 2k), with 5% DMSO (v/v) as cosolvent, at pH 8.0 (pH 7.3 for 2s to increase its electrophilicity) and room temperature. The reaction volume was 40 mL. To reduce the aldehyde functionality of the enzymatic products 3k–n and 3s, NaBH₄ (30 mM) was added to the reaction mixture followed by incubation for 3 h at room temperature. Reaction time of the enzymatic step. Isolated yield of the aromatic 1,3-diol product. Determined by HPLC analysis on a chiral stationary phase using chemically synthesized racemic standards. The absolute configuration was determined by chiral HPLC using chemically prepared authentic standards with known (R) or (S) configuration. The absolute configuration was assigned based on analogy.

Table 1. Asymmetric Chemoenzymatic Synthesis of Aromatic 1,3-Diols Using 4-OT(M45T/F50A) as Carboligase

| Entry | Aldol acceptor | Product | Reaction time [h] | Isolated yield (%) | e.r. | Abs. Conf. |
|-------|----------------|---------|-------------------|--------------------|------|------------|
| 1     | 2k−n, 2s       | 4a      | 6                 | 92                 | 91.9 | R⁺        |
| 2     | 2i             | 4b      | 7                 | 63                 | 90.10| R⁺        |
| 3     | 2m             | 4c      | 2.5               | 88                 | 90.10| R⁺        |
| 4     | 2n             | 4d      | 5                 | 70                 | 92.8 | R⁻        |
| 5     | 2s             | 4e      | 1                 | 65                 | 99.1 | R⁻        |

“Assay conditions: acetaldehyde (1, 100 mM), aromatic aldehyde (2k–n, 2s, 2 mM), and purified 4-OT(M45T/F50A) (0.125–0.475 mg/mL) in 20 mM sodium phosphate buffer (MOPS for 2k), with 5% DMSO (v/v) as cosolvent, at pH 8.0 (pH 7.3 for 2s to increase its electrophilicity) and room temperature. Reaction volume was 40 mL. To reduce the aldehyde functionality of the enzymatic products 3k–n and 3s, NaBH₄ (30 mM) was added to the reaction mixture followed by incubation for 3 h at room temperature. Reaction time of the enzymatic step. Isolated yield of the aromatic 1,3-diol product. Determined by HPLC analysis on a chiral stationary phase using chemically synthesized racemic standards. The absolute configuration was determined by chiral HPLC using chemically prepared authentic standards with known (R) or (S) configuration. The absolute configuration was assigned based on analogy.

corresponding 1,3-diols in the same pot. The applied carboligases accept a broad range of aromatic aldehydes for acetaldehyde additions, offering alternative synthetic choices to prepare important drug precursors.

We previously reported that an engineered variant of 4-OT (mutant M45T/F50A) can efficiently catalyze the aldol condensation of acetaldehyde (1) with benzaldehyde (2a) to yield cinnamaldehyde (4a). This prompted us to start our investigations by testing a large set of 25 aromatic aldehydes (2b−y and 2ac) as potential aldol acceptor substrates in the 4-OT(M45T/F50A)-catalyzed aldol reaction with donor substrate 1 (Scheme 2). The results demonstrate that the 4-OT(M45T/F50A) enzyme has a remarkably broad substrate scope, accepting 19 aromatic aldehydes with electron withdrawing substituents (2b−t) as aldol acceptor substrates (Scheme 2, Figures S1−S19). On the contrary, aromatic aldehydes with electron-donating substituents (2u−y and 2ac) were not, or very poorly, accepted by 4-OT(M45T/F50A) (Scheme 2, Figures S20−S24, S28). While this may be attributed to unfavorable equilibrium constants for these aromatic aldehydes in the aldol addition with acetaldehyde, the electron donating properties of the substituents on the aromatic ring likely also lead to weak activation of the carbonyl carbon for nucleophilic attack.3,4

Out of the 19 well-accepted aromatic aldehydes, 14 substrates undergo an aldol condensation reaction with formation of the corresponding α,β-unsaturated aldehydes (Figures S1−S19). To demonstrate the synthetic usefulness of 4-OT(M45T/F50A) for the preparation of α,β-unsaturated aldehydes, nine aromatic aldehydes (2b−d, 2f−j, and 2t) that could be efficiently converted by 4-OT(M45T/F50A) were selected and used in semipreparative scale synthesis (Scheme 3). The corresponding α,β-unsaturated aldehydes (4b−d, 4f−j, and 4t) could be isolated in moderate to good yields (Scheme 3, Figures S30−S47). Notably, ortho-substituted benzaldehydes (e.g., 2b, 2f, 2h, and 2i) were better accepted by 4-OT(M45T/F50A) than para-substituted benzaldehydes (2p, 2r, 2o, and 2q). These results underscore the potential of 4-OT(M45T/F50A) for the synthesis of versatile aromatic α,β-unsaturated aldehydes, which are important precursors to various abundantly prescribed drugs (Figure 1).}

Interestingly, for the 4-OT(M45T/F50A)-catalyzed aldol addition of 1 to 2k−n and 2s, depletion of the starting substrates was observed without significant accumulation of the corresponding α,β-unsaturated aldehydes (Figures S10−S13, S18). These results led us to hypothesize that in these instances the initially formed aldol adducts, β-hydroxylaldehydes 3k−n and 3s, were not (or slowly) dehydrated and accumulated in the reaction mixture. To confirm this hypothesis, an analytical scale experiment was performed using 4-OT(M45T/F50A) in NaPi buffer (0.3 mL, pH 7.3, 5% DMSO), containing 1 (50 mM) and 2k (2 mM). Reaction progress was monitored by following the depletion of 2k by UV−vis spectrophotometry, and NaBH₄ was added to the reaction mixture (after 1 h) to convert the aldol adduct into
the more stable 1,3-diol $5k$. Analysis of chemoenzymatic product $5k$ by HPLC on a chiral stationary phase, using authentic standards with known absolute configuration, revealed high enantiococontrol at the site of addition with formation of the $R$ enantiomer (e.r. = 94:6). These results demonstrate that the enzyme 4-OT(M45T/F50A) can indeed catalyze enantioselective aldol additions.

Having established that 4-OT(M45T/F50A) has potential for the asymmetric synthesis of enantioenriched aromatic $\beta$-hydroxyaldehydes, we first optimized the reaction conditions by varying the pH and the concentration of acetaldehyde, buffer, and enzyme (Figure S88). Using the optimized conditions, the one-pot two-step chemoenzymatic cascade synthesis of aromatic 1,3-diols 3k−n and 5s, via enzymatically prepared $\beta$-hydroxyaldehydes 3k−n and 3s, was achieved with high enantioselectivity (e.r. up to 99:1) and in good isolated yield (63−92%) (Table 1, Figures S48−S62).

To expand the number of enzymatically accessible $\beta$-hydroxyaldehyde products (e.g., 3b−d, 3f, and 3g) in principle two different approaches can be used: (i) the use of protein engineering to create new variants of 4-OT(M45T/F50A) that possess the desired aldol addition activity but lack dehydration activity. To selectively eliminate the dehydration activity of 4-OT(M45T/F50A), a structure-based approach is favored, which awaits the determination of the enzyme crystal structure complexed with one of the $\beta$-hydroxyaldehyde products (work in progress). In this study, we therefore have chosen to screen a panel of 50 commercially available 4-OT homologues for enzymes with the desired aldol addition activity to yield enantioenriched $\beta$-hydroxyaldehydes. For initial activity testing, we used donor substrate 1 and aldol acceptor substrate 2b. Interestingly, out of the 50 tested 4-OT homologues, three enzymes (TAUT015, TAUT021, and TAUT028) were found to show significant activity toward the aldol addition of 1 to 2b to give the desired product 3b without promoting significant dehydration leading to formation of 4b (Figure S29). Notably, the same reaction catalyzed by 4-OT(M45T/F50A) resulted in the formation of $\alpha,\beta$-unsaturated aldehyde 4b as the sole product, providing additional support that the $\beta$-hydroxyaldehyde dehydration step is enzyme catalyzed.

We next investigated the substrate scope of the best performing enzyme, TAUT015, which shows 28% overall sequence identity to 4-OT (Figure S89), in additions using 1 as donor substrate and a number of selected benzaldehyde derivatives as potential aldol acceptor substrates. The results show that, like 4-OT(M45T/F50A), TAUT015 does not accept benzaldehydes with electron donating substituents (2u, 2v, 2a, and 2ab) on the aromatic ring (Figures S25−S27). However, several benzaldehydes with electron withdrawing substituents (2b−d, 2f, and 2g) were well accepted by TAUT015. Encouraged by these findings, semipreparative scale reactions were carried out under optimized reaction conditions. The reaction progress (1 with 2b−d, 2f, and 2g)
was monitored by UV–vis spectrophotometry, and the enzymatically prepared β-hydroxylaldehydes 3b−d, 3f, and 3g were reduced to the corresponding aromatic 1,3-diols (3b−d, 3f, and 3g) using NaBH₄. These one-pot, two-step chemoenzymatically prepared compounds 3b−d, 3f, and 3g were obtained in good isolated yields (up to 72%) and with high e.r. values (e.r. up to >99:1) (Table 2, Figures S63−S87). Note that enzymatic access to these useful chiral synths requires the use of TAUT015 as carboligase and is not possible with 4-OT(M45T/F50A). Hence, the enzyme TAUT015 nicely supplements the toolbox of biocatalysts for the production of important chiral aromatic β-hydroxylaldehydes and corresponding 1,3-diols.

In conclusion, our results indicate that members of the 4-OT family of enzymes, which possess an uncommon catalytic amino-terminal proline, can function as novel carboligases, promoting the cross-aldol reaction of acetaldehyde with diverse aromatic aldehydes to give access to a range of important α,β-unsaturated aldehydes as well as enantioenriched β-hydroxylaldehydes. It is feasible that their promiscuous aldol addition activities, which are beyond the synthetic scope of currently known aldolases, can further be optimized by directed evolution or computational redesign. It is important to emphasize that the controlled cross-aldol addition of acetaldehyde is a particularly challenging synthetic aim because acetaldehyde is a relatively reactive and difficult to tame chemical.15,16 Remarkably, the promiscuous 4-OT enzymes accept acetaldehyde as a nucleophile in aldol additions, which is unparalleled among the aldolases, with the notable exception of 2-deoxy-D-ribose-5-phosphate aldolase (DERA) and D-fructose-6-phosphate aldolase (FSA), which are also able to use acetaldehyde as a nucleophilic substrate.2g,kl As such, these proline-based 4-OT enzymes nicely complement the toolbox of biocatalysts for (asymmetric) C–C bond formation and open up new opportunities to develop practical enzymatic processes for the more sustainable and step-economic synthesis of valuable drug precursors starting from simple building blocks. Further screening of related proline-based enzymes might prove to be a valuable approach to discover new aldolase activities that could be exploited to develop synthetically useful biocatalysts for carbon–carbon bond formation.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscatal.0c00039.

Additional experimental procedures and characterizations of compounds (PDF)

**AUTHOR INFORMATION**

* Corresponding Author

Gerrit J. Poelarends — Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, 9713 AV Groningen, The Netherlands; orcid.org/0000-0002-6917-6368; Phone: +31503633354; Email: g.j.poelarends@rug.nl; http://www.rug.nl/staff/g.j.poelarends/

**Authors**

Mohammad Saitfuddin — Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, 9713 AV Groningen, The Netherlands

Chao Guo — Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, 9713 AV Groningen, The Netherlands

Lieve Biewenga — Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, 9713 AV Groningen, The Netherlands

Thangavelu Saravanan — Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, 9713 AV Groningen, The Netherlands

Simon J. Charnock — Procomix Ltd., Haltwhistle, Northumberland NE49 9HN, U.K.

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acscatal.0c00039

**Author Contributions**

M.S. and C.G. contributed equally.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We acknowledge financial support from The Netherlands Organization of Scientific Research (VICI grant 724.016.002), the European Research Council (PoC grant 713483), and the European Union’s Horizon 2020 Research and Innovation Programme under grant agreement No 635595 (CarbaZymes).

**REFERENCES**

1. (a) Gröger, H.; Vogl, E. M.; Shibasaki, M. New Catalytic Concepts for the Asymmetric Aldol Reaction. *Chem. - Eur. J.* 1998, 4, 1137−1141. (b) Palomo, C.; Oiarbide, M.; García, J. M. Current Progress in the Asymmetric Aldol Addition Reaction. *Chem. Soc. Rev.* 2004, 33, 65−75. (c) Yamashita, Y.; Yasukawa, T.; Yoo, W. J.; Kitano, T.; Kobayashi, S. Catalytic Enantioselective Aldol Reactions. *Chem. Soc. Rev.* 2018, 47, 4388−4480.

2. (a) Wong, C. H.; Junceda, E. G.; Chen, L.; Blanco, O.; Gijzen, H. J. M.; Steenma, D. H. Recombinant 2-Deoxyribose-5-phosphate Aldolase in Organic Synthesis: Use of Sequential Two-Substrate and Three-Substrate Aldol Reactions. *J. Am. Chem. Soc.* 1995, 117, 3333−3339. (b) Fessner, W. D. Enzyme Mediated C-C Bond Formation. *Curr. Opin. Chem. Biol.* 1998, 2, 85−97. (c) Machajewski, T. D.; Wong, C. H. The Catalytic Asymmetric Aldol Reaction. *Angew. Chem., Int. Ed.* 2000, 39, 1352−1374. (d) DeSantis, G.; Liu, J.; Clark, D. P.; Heine, A.; Wilson, I. A.; Wong, C. H. Structure-Based Mutagenesis Approaches Toward Expanding the Substrate Specificity of D-2-Deoxyribose-5-phosphate Aldolase. *Bioorg. Med. Chem.* 2003, 11, 43−52. (e) Clapés, P.; Garrabou, X. Current Trends in Asymmetric Synthesis with Aldolases. *Adv. Synth. Catal.* 2011, 353, 2263−2283. (f) Fesko, K.; Khadjawi, M. G. Biocatalytic Methods for C-C Bond Formation. *ChemCatChem* 2013, 5, 1248−1272. (g) Clapés, P.; Fessner, W. D.; Sprenger, G. A.; Samland, A. K. Recent Progress in Stereoselective Synthesis with Aldolases. *Curr. Opin. Chem. Biol.* 2010, 14, 154−167. (h) Chen, Q.; Chen, X.; Cui, Y.; Ren, J.; Lu, W.; Feng, J.; Wu, Q.; Zhu, D. A New D-Threonine Aldolase as a Promising Biocatalyst for Highly Stereoselective Preparation of Chiral Aromatic β-Hydroxy-a-Amino acids. *Chem. Sci. Technol.* 2017, 7, 5964−5973. (i) Schmidt, N. G.; Eger, E.; Krouth, W. Building Bridges: Biocatalytic C-C Bond Formation toward Multifunctional Products. *ACS Catal.* 2016, 6, 4286−4311. (j) Obexer, R.; Godina, A.; Garrabou, X.; Mittl, P. R. E.; Baker, D.; Griffith, A. D.; Hilvert, D. Emergence of a Catalytic Tetrad during Evolution of a Highly Active Artificial Aldolase. *Nat. Chem.* 2017, 9,
dron Lett. Biocatalytic Asymmetric Aldol Reaction in Buffer Solution. ACS Catal. 2018, 8, 8804–8809. (l) Xie, Z. B.; Wang, N.; Jiang, G. F.; Yu, X. Q. Biocatalytic Asymmetric Aldol Reaction in Buffer Solution. Tetrahedron Lett. 2013, 54, 945–948. (m) Rolldán, R.; Hernández, K.; Joglar, J.; Bujons, J.; Parella, T.; Fessner, W. D.; Clapés, P. Aldolase-Catalyzed Asymmetric Synthesis of N-Heterocycles by Addition of Simple Aliphatic Nucleophiles to Hydroxyaldehydes. ACS Catal. 2017, 7, 5005–5009.

50–56. (k) Rolldán, R.; Hernandez, K.; Joglar, J.; Bujons, J.; Parella, T.; Sánchez-Moreno, I.; Hélaine, V.; Lemaire, M.; Guérard-Hélaine, C.; Fessner, W. D.; Clapés, P. Biocatalytic Aldol Addition of Simple Aliphatic Nucleophiles to Hydroxyaldehydes. ACS Catal. 2018, 8, 8804–8809. (l) Xie, Z. B.; Wang, N.; Jiang, G. F.; Yu, X. Q. Biocatalytic Asymmetric Aldol Reaction in Buffer Solution. Tetrahedron Lett. 2013, 54, 945–948. (m) Rolldán, R.; Hernández, K.; Joglar, J.; Bujons, J.; Parella, T.; Fessner, W. D.; Clapés, P. Aldolase-Catalyzed Asymmetric Synthesis of N-Heterocycles by Addition of Simple Aliphatic Nucleophiles to Aminoaldehydes. Chem. - Eur. J. 2017, 23, 5005–5009.

3 (a) Zandvoort, E.; Geertsema, E. M.; Quax, W. J.; Poelarends, G. J. Enhancement of the Promiscuous Aldolase and Dehydration Activities of 4-Oxalocrotonate Tautomerase by Protein Engineering. ChemBioChem 2012, 13, 1274–1277. (b) Rahimi, M.; van der Meer, J. Y.; Geertsema, E. M.; Poddar, H.; Baas, B. J.; Poelarends, G. J. Mutations Closer to the Active Site Improve the Promiscuous Aldolase Activity of 4-Oxalocrotonate Tautomerase More Effectively than Distant Mutations. ChemBioChem 2016, 17, 1225–1228. (c) Rahimi, M.; van der Meer, J. Y.; Geertsema, E. M.; Poelarends, G. J. Engineering a Promiscuous Tautomerase into a More Efficient Aldolase for Self-Condensations of Linear Aliphatic Aldehydes. ChemBioChem 2017, 18, 1435–1441. (d) Rahimi, M.; Geertsema, E. M.; Miao, Y.; van der Meer, J. Y.; van den Bosch, T.; de Haan, P.; Zandvoort, E.; Poelarends, G. J. Inter- and Intramolecular Aldol Reactions Promiscuously Catalyzed by a Proline-based Tautomerase. Org. Biomol. Chem. 2017, 15, 2809–2816.

4 (a) Rej, R. K.; Das, T.; Hazra, S.; Nanda, S. Chemoenzymatic Asymmetric Synthesis of Fluoxetine, Atomoxetine, Nisoxetine, and Duloxetine. Tetrahedron: Asymmetry 2013, 24, 913–918. (b) Wenthur, C. J.; Bennett, M. R.; Lindsley, C. W. Classics in Chemical Neuroscience: Fluoxetine (Prozac). ACS Chem. Neurosci. 2014, 5, 14–23. (c) Fátima, Â d.; Lapis, A. A. M; Pilli, R. A. A Concise Total Synthesis of (R)-Fluoxetine, a Potent and Selective Serotonin Reuptake Inhibitor. J. Braz. Chem. Soc. 2005, 16, 495–499. (d) Roenes, S.; Agarwal, V. K. Enantioselective synthesis of (R)-tolterodine using lithiation/borylation—protodeboronation methodology. Can. J. Chem. 2012, 90, 965–974. (e) Sabitha, G.; Padmaja, P.; Yadav, J. S. A Concise Total Synthesis of Diospongins A and B. Helv. Chim. Acta 2008, 91, 2235–2239. (f) Guo, C.; Saffuddin, M.; Saravanam, T.; Shariff, M.; Poelarends, G. J. Biocatalytic Asymmetric Michael Additions of Nitromethane to α,β-Unsaturated Aldehydes via Enzyme-bound Iminium Ion Intermediates. ACS Catal. 2019, 9, 4369–4373. (g) Fan, X.; Rodriguez-Escrich, C.; Wang, S.; Sayalero, S.; Pericas, M. A. Highly Enantioselective Cross-Aldol Reactions of Acetaldehyde Mediated by a Dual Catalytic System Operating under Site Isolation. Chem. - Eur. J. 2014, 20, 13089–13093. (S) Hayashi, Y.; Itoh, T.; Aratake, S.; Ishikawa, H. A Diarylprolinol in an Asymmetric, Catalytic, and Direct Crossed- Aldol Reaction of Acetaldehyde. Angew. Chem., Int. Ed. 2008, 47, 2082–2084. (6) Martinez, A.; Van Gemmeren, M.; List, B. Unexpected Beneficial Effect of ortho-Substituents on the (S)-Proline-Catalyzed Asymmetric Aldol Reaction of Acetone with Aromatic Aldehydes. Synlett 2014, 25, 961–964. (7) Yang, J. W.; Chandler, C.; Stadler, M.; Kampen, D.; List, B. Proline-Catalysed Mannich Reactions of Acetaldehyde. Nature 2008, 452, 453–455.