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Inter- and intramolecular aldol reactions promiscuously catalyzed by a proline-based tautomerase†

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The enzyme 4-oxalocrotonate tautomerase (4-OT), which in nature catalyzes a tautomerization step as part of a catabolic pathway for aromatic hydrocarbons, was found to promiscuously catalyze different types of aldol reactions. These include the self-condensation of propanal, the cross-coupling of propanal and benzaldehyde, the cross-coupling of propanal and pyruvate, and the intramolecular cyclizations of hexanedial and heptanedial. Mutation of the catalytic amino-terminal proline (P1A) greatly reduces 4-OT's aldolase activities, whereas mutation of another active site residue (F50A) strongly enhances 4-OT's aldolase activities, indicating that aldolization is an active site process. This catalytic promiscuity of 4-OT could be exploited as starting point to create tailor-made, artificial aldolases for challenging self- and cross-aldolizations.

Introduction

The aldol reaction is one of the most fundamental carbon–carbon bond-forming reactions in organic chemistry. The reaction is widely used in the production of various chemicals including pharmaceutical compounds.[1–5] Several methodologies have been employed to carry out aldol reactions including acid-, base-, organo-, metal-, and biocatalysis.[4–8] Among them, biocatalysis receives considerable attention due to the fact that biocatalytic aldol reactions[2,3,9] may be performed under environmentally friendly conditions.[6] Aldolases, a specific group of lyases, constitute a class of enzymes that catalyze aldol reactions as their natural activity.[9–20] Besides enzymes with natural aldolase activity (*i.e.*, aldolases), there are few enzymes known that promiscuously catalyze aldol reactions.[3,21–23] Enzyme promiscuity has great promise as a source of synthetically useful catalytic transformations. Hence, further systematic screening of existing enzymes for catalytic promiscuity may prove to be a useful approach to discover new aldolase activities, which could be exploited as starting points to create novel biocatalysts for challenging non-natural aldolizations.

An enzyme that has recently attracted attention concerning its various promiscuous activities is the enzyme 4-oxalocrotonate tautomerase (4-OT).[24–30] It is a member of the tautomerase superfamily, a group of homologous proteins having a β–α–β structural fold and a catalytic amino-terminal proline residue (Pro-1) in common.[31–33] 4-OT takes part in a catabolic pathway for aromatic hydrocarbons in *Pseudomonas putida* mt-2, where it catalyzes the conversion of 2-hydroxyhexa-2,4-dienedioate (**1**, Scheme 1a) into 2-oxohexa-3-enedioate (**2**, Scheme 1a).[34,35] In this tautomerization reaction, Pro-1 acts as a general base (pK_a of Pro-1 ~6.4) abstracting the 2-hydroxyl proton of **1** and transferring it to the C5-position to give **2** (Scheme 1b).[36]

We have recently reported that 4-OT promiscuously catalyzes various C–C bond-forming reactions,[24–29,37,38] including the aldol condensation of acetaldehyde and benzaldehyde to yield cinnamaldehyde.[25,28] In this aldol condensation, 4-OT catalyzes both the initial aldol-coupling step to yield 3-hydroxy-3-phenylpropanal, and the subsequent dehydration step to give cinnamaldehyde.[25,28] 4-OT also catalyzes a retro-aldol reaction with 3-hydroxy-3-phenylpropanal as the substrate.[28] $NaCNBH_3$ trapping, mass spectrometry, and X-ray crystallography[39] experiments strongly suggest a mechanism in which Pro-1 functions as a nucleophile, rather than a base, and reacts with the carbonyl functionality of acetaldehyde to form a covalent enamine intermediate.[25,28] This nucleophilic species reacts with benzaldehyde in an aldol addition. Mechanism-inspired engineering provided an active site

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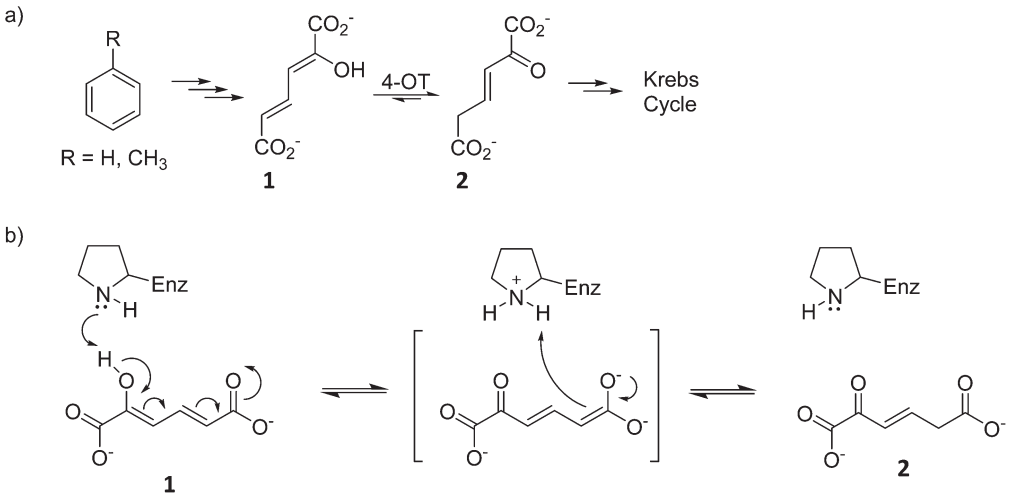
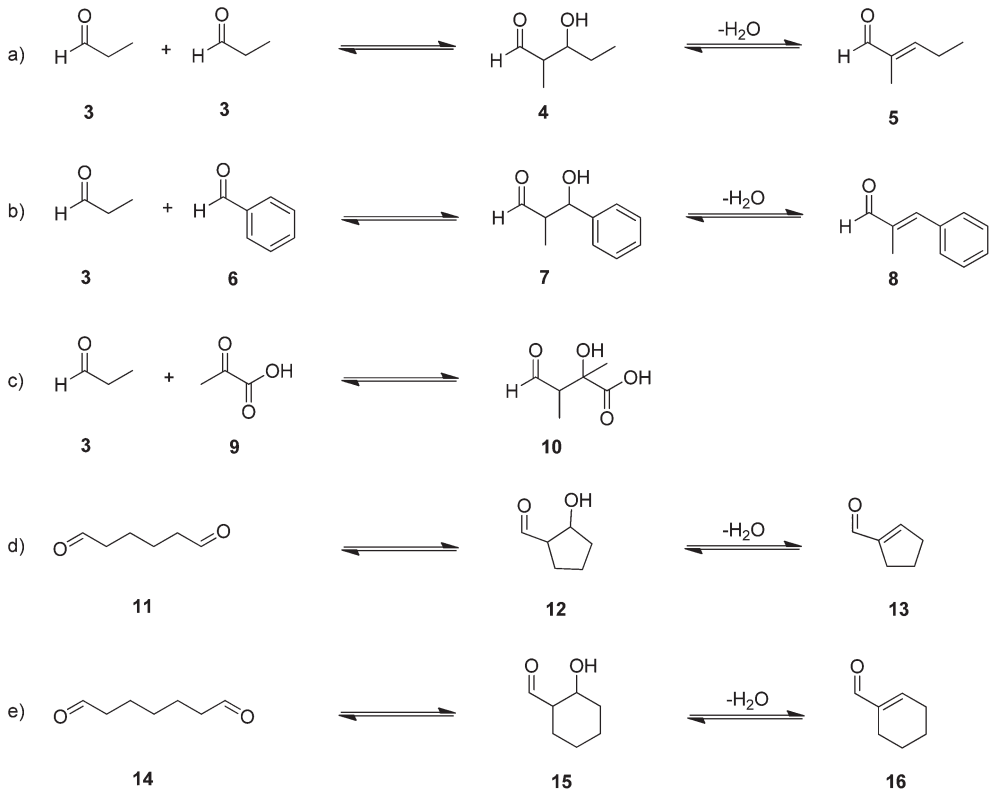


Scheme 1 (a) Tautomerization of 2-hydroxyhexa-2,4-dienedioate (**1**) to yield 2-oxohex-3-enedioate (**2**) naturally catalyzed by 4-OT as part of a degradative pathway for aromatic compounds; (b) the catalytic mechanism for 4-OT with the role of the key residue Pro-1 indicated.

mutant (F50A) with strongly enhanced aldol condensation activity (600-fold in terms of k_{cat}/K_m),[28] while having greatly reduced tautomerase activity (~2000-fold in terms of k_{cat}/K_m).[40] In the present study, we show that WT 4-OT and the 4-OT F50A mutant accept various carbonyl compounds as substrates for both inter- and intramolecular aldol reactions (Scheme 2).

Results and discussion

In separate experiments, WT 4-OT, 4-OT F50A, and 4-OT P1A (0.15 mM each) were incubated with propanal **3** (50 mM) in NaH_2PO_4 buffer (20 mM, pH 7.3) and reaction progress was monitored by UV spectroscopy. The UV spectra of the reaction



Scheme 2 Promiscuous aldol reactions catalyzed by 4-OT; (a) self-condensation of propanal (**3**); (b) cross-coupling of propanal (**3**) and benzaldehyde (**6**); (c) cross-coupling of propanal (**3**) and pyruvate (**9**); (d) intramolecular cyclization of hexanedial (**11**) and (e) intramolecular cyclization of heptanedial (**14**).

mixture incubated with the 4-OT F50A mutant (Fig. 1A) showed an increase in absorbance at 234 nm in course of time, which corresponds to the formation of product **5** ($\lambda_{max,5}$ = 234 nm). A slight increase in absorbance at 234 nm was observed during the assay with WT 4-OT (Fig. 1B). During the assay with the 4-OT P1A mutant, a negligible increase in absorbance at 234 nm was observed after 20 h (Fig. 1C) while no change in absorbance was observed for the reaction mixture without enzyme (Fig. 1D). These data indicate that WT 4-OT catalyzes the self-condensation of **3**, though its activity is relatively low, and that the active-site mutation of Phe-50 to Ala strongly increases the activity of 4-OT for self-condensation of **3**.

The self-condensation of **3** catalyzed by WT 4-OT, 4-OT F50A or 4-OT P1A was also monitored by ^{1}H NMR spectroscopy to verify that the product of the reaction is **5**. In separate experiments, these enzymes (1.0 mol% relative to **3**), which were redissolved in NaD_2PO_4 buffer (20 mM, pD 7.6), were incubated with **3** (30 mM) in NaD_2PO_4 (20 mM, pD 7.6). A control experiment (reaction mixture without enzyme) containing **3** (30 mM) in NaD_2PO_4 buffer (20 mM, pD 7.6) was also performed. During all experiments, an equilibrium between the hydrated (63%) and unhydrated (37%) form of **3** was reached in the time between mixing all sample components and recording the first ^{1}H NMR spectrum (≤5 min) (Fig. 2B and Scheme S2†). Therefore, we could not distinguish whether the enzymes accelerate reaching this equilibrium or not.

Interestingly, the acidic protons of **3**, which are located at the C2-position (marked with g in Fig. 2B), were exchanged with deuterium in the reaction mixtures incubated with WT 4-OT or 4-OT F50A (Fig. 2D and E). The exchange most likely only takes place at C2 of the unhydrated form of **3** (*i.e.*, propanal) and not at C2 of the hydrated form (*i.e.*, propane-1,1-diol-d_2) since the protons at C2 of the latter are not acidic (Scheme S2†). However, since the rate for reaching equilibrium between unhydrated and hydrated form is relatively high compared to the rate of H–D exchange, the vanishing of signals g (protons at C2 of unhydrated form) and j (protons at C2 of hydrated form) was witnessed in equal proportion (Fig. 2D and E and Scheme S2†). The ^{1}H NMR data showed 90% H–D exchange (in 24 h) in the presence of WT 4-OT and 65% H–D exchange (in 24 h) in the presence of 4-OT F50A. A relatively low rate of H–D exchange was found for the control sample without enzyme (6%, 24 h) and for the sample with 4-OT P1A (5%, 24 h), indicating that the H–D exchange is enzyme-catalyzed and that the Pro-1 residue is involved in catalysis (Fig. 2C and F). Two possible mechanisms of proton–deuterium exchange during which Pro-1 either acts as a base or nucleophile are visualized in Scheme S3.† The mechanism shown in Scheme S3B† is sup-

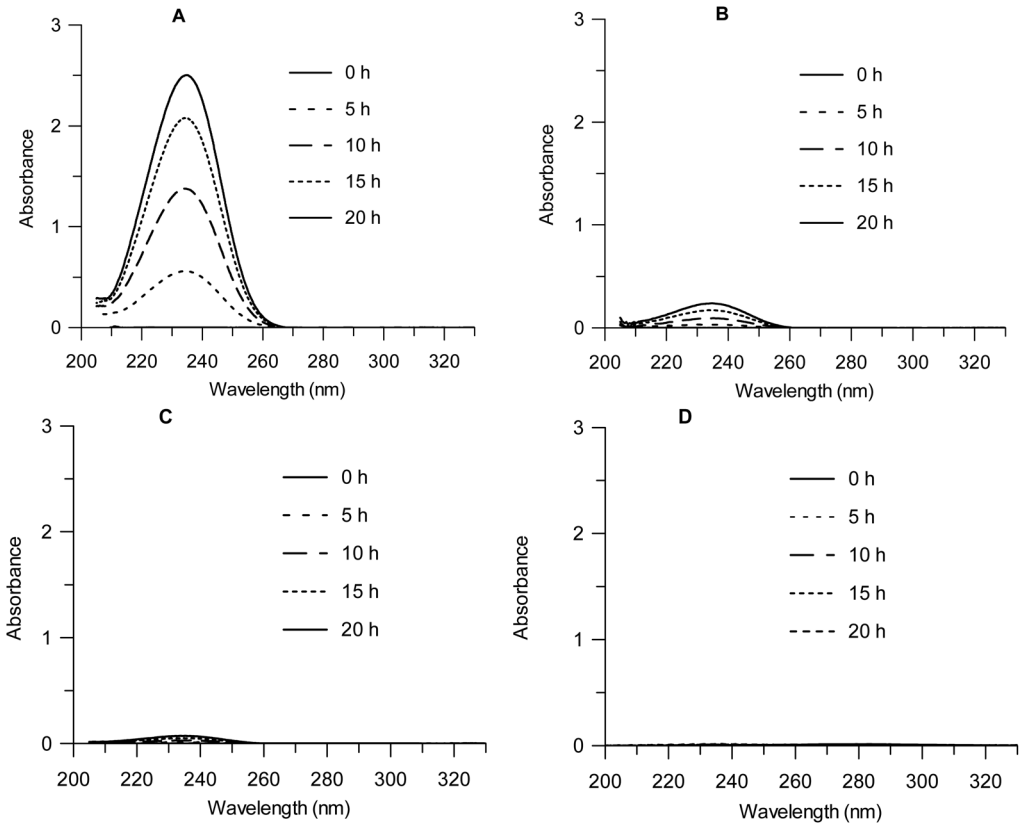


Fig. 1 UV spectra showing the self-condensation of propanal (**3**) and the formation of 2-methyl-2-pentenal (**5**, $\lambda_{max,5}$ = 234 nm). Propanal (**3**, 50 mM) in 20 mM NaH_2PO_4 buffer (pH 7.3) was incubated with (A) 4-OT F50A mutant, (B) WT 4-OT, (C) 4-OT P1A mutant, (D) no enzyme (control sample).

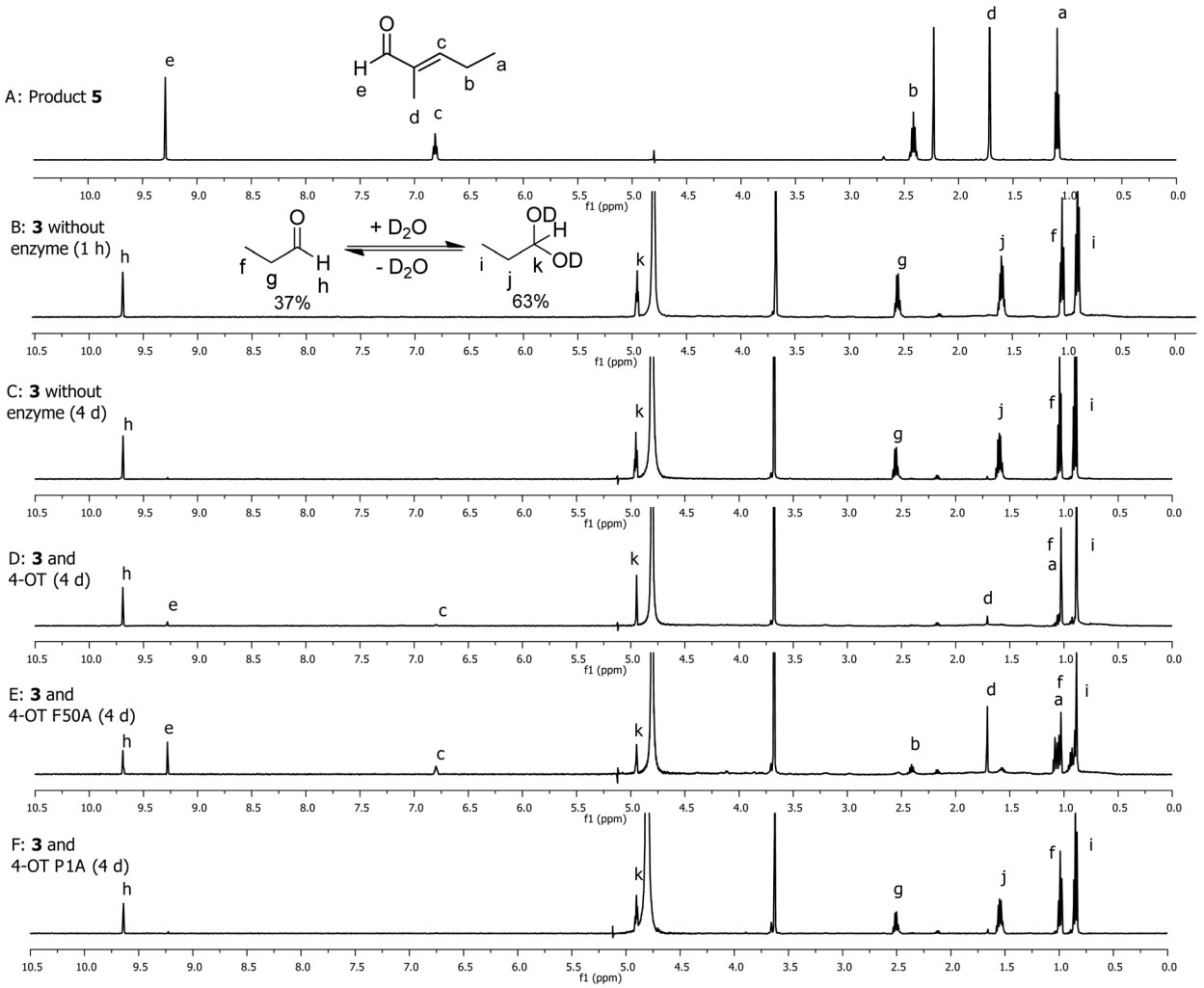


Fig. 2 Stack plot of ^{1}H NMR spectra. (A) ^{1}H NMR spectrum of an authentic sample of **5** (2-methyl-2-pentenal). Signals of **5** are marked with a, b, c, d, and e; (B) propanal (**3**) incubated in 20 mM NaD_2PO_4 buffer at pD 7.6 for 1 h. Equilibrium is reached between unhydrated and hydrated forms of **3** and signals are marked with f, g, h and i, j, k, respectively; (C) propanal (**3**) incubated in 20 mM NaD_2PO_4 buffer at pD 7.6 for 4 d. Signals of unhydrated and hydrated forms of **3** are marked with f, g, h and i, j, k, respectively; (D) propanal (**3**) incubated in 20 mM NaD_2PO_4 buffer at pD 7.6 with WT 4-OT for 4 d. Acidic protons of **3** (marked with g and j in spectrum B) are completely exchanged with deuterium. As a result, methyl signals f and i have changed from triplets into singlets (compare with spectrum B). Signals of **5** are marked with a, c, d, and e; (E) propanal (**3**) incubated in 20 mM NaD_2PO_4 buffer at pD 7.6 with 4-OT F50A for 4 d. Signals of **5** are marked by a, b, c, d, and e. (F) Propanal (**3**) incubated in 20 mM NaD_2PO_4 buffer at pD 7.6 with 4-OT P1A for 4 d.

ported by previous $NaCNBH_3$ trapping and mass spectrometry experiments, demonstrating that Pro-1 reacts with the carbonyl functionality of **3** to form an enamine intermediate.[25]

Signals corresponding to product **5** were identified in ^{1}H NMR spectra of the reaction mixtures incubated with WT 4-OT and 4-OT F50A for 4 d (Fig. 2D and E). Notably, the majority of acidic protons of **3** (at C2) were exchanged with deuterium (relatively fast reaction) before **3** underwent self-condensation (relatively slow reaction) to yield product **5** (Scheme S2†). During conversion, the C2 atoms of the two molecules of **3** become C2 and C4 of **5**, respectively. As a result, we noticed that signal b (protons at C4) of **5** was missing in the ^{1}H NMR spectra of the reaction mixture incubated with WT 4-OT and this signal had lower integration area than expected in the ^{1}H NMR spectra of the reaction mixture incubated with 4-OT F50A (compare Fig. 2A, D, and E). The yield[41] of **5** in the reaction mixtures incubated with WT 4-OT and 4-OT F50A, reached to ~5% and ~27% after 4 d, respectively (Fig. 2D and E). Only 1 and 2% yield of **5** was detected in the control and 4-OT P1A sample, respectively, after 4 d (Fig. 2C and F). Incubation of **3** with synthetic WT 4-OT (prepared by total chemical synthesis)[42] also led to the formation of **5** (see also Fig. S3D†). This experiment confirms that 4-OT is responsible for catalysis and rules out the possibility that catalysis was effected by a contaminating enzyme co-purified from the expression strain. In conclusion, the ^{1}H NMR data verified that WT 4-OT and 4-OT F50A catalyze the self-condensation of **3** and confirmed the essential role of Pro-1 in catalysis.[43]

Importantly, no ^{1}H NMR signals corresponding to the presumed aldol product **4**, or its hydrated form, were detected over the entire course of the reaction. The question whether the dehydration of **4** to yield **5** is enzyme-catalyzed or proceeds *via* a chemical process thus remained. To address this question, compound **4** was chemically synthesized and tested as a potential substrate for WT 4-OT and 4-OT F50A. The conversion of **4** to **5** was monitored by UV spectroscopy. The sets of spectra recorded from the reaction mixtures incubated with WT 4-OT or 4-OT F50A revealed an increase in absorbance at 234 nm, which corresponds to the formation of **5** ($\lambda_{max,5}$ = 234 nm) (Fig. S1B and S1C†). A control experiment in which **4** was incubated without enzyme showed a significantly lower rate of formation of **5** (Fig. S1A†). These results indicate that both WT 4-OT and 4-OT F50A facilitate the dehydration of **4** to give **5**; hence, they catalyze both the aldol coupling and aldol dehydration steps.

Next, we investigated the activity of WT 4-OT and 4-OT F50A for the cross-coupling of **3** and **6** (Scheme 2b). In separate experiments, WT 4-OT, 4-OT F50A, and 4-OT P1A (0.15 mM each) were incubated with **3** (50 mM) and **6** (2 mM) in NaH_2PO_4 buffer (20 mM, pH 7.3), and the reactions were monitored by UV spectroscopy (Fig. S2†). The UV spectra of the reaction mixture incubated with 4-OT F50A showed a decrease in absorbance at 250 nm over a period of 5 h indicating the depletion of **6** ($\lambda_{max,6}$ = 250 nm). However, after 5 h the absorbance at 250 nm increased again and a new peak with a maximum at 241 nm appeared, which may indicate the formation of **5** ($\lambda_{max,5}$ = 234 nm) by self-condensation of **3** (Fig. S2A†). Neither formation of dehydrated aldol product **8** ($\lambda_{max,8}$ = 288 nm) was observed under these conditions, nor formation of aldol coupling product **7** as it most likely does not show significant absorbance above 220 nm.[44] During the experiments with WT 4-OT and the 4-OT P1A mutant a negligible decrease at 250 nm was observed (Fig. S2B and S2C†). We did not detect any alteration in the UV spectra of the reaction mixture without enzyme (Fig. S2D†).

^{1}H NMR experiments were performed to detect the products and clarify the reactions taking place in the presence of WT 4-OT and 4-OT F50A (Fig. S3 and Scheme S4†). In separate experiments, WT 4-OT, 4-OT F50A, and 4-OT P1A (2.0 mol% compared to **6**) were incubated with **3** (30 mM) and **6** (15 mM) in NaD_2PO_4 buffer (20 mM, pD 7.6). Analysis of the ^{1}H NMR spectra of the 4-OT F50A-catalyzed reaction revealed accumulation of aldol coupling product **7** to ~36% after 1 d, after which it decreased to 20% after 14 d (Fig. S4†).[41] The yield of **8** steadily increased in time to 15% after 14 d. These observations suggest a relatively fast aldol addition of **3** to **6** to yield **7** and a relatively slow subsequent dehydration of **7** to **8**, which results in initial accumulation of **7**. Characteristic ^{1}H NMR spectroscopic signals of **7** (at 5.23 and 9.74 ppm),[45] the hydrate of **7** (*i.e.* **7′**, at 4.94 ppm. See Scheme S4† for structure of **7′**), and **8** (at 2.03 and 9.49 ppm)[46,47] present in the reaction mixture incubated with 4-OT F50A are shown in Fig. S3.† The ^{1}H NMR data also indicated activity of WT 4-OT for the cross-coupling of **3** and **6** albeit lower than that of 4-OT F50A (Fig. S3†). For the reaction mixture without enzyme and the reaction mixture incubated with 4-OT P1A, only trace amounts of **7**, **7′**, and **8** were detected after 14 d.

In contrast to the UV experiments during which no absorbance corresponding to **8** was observed, the presence of signals for product **8** in the ^{1}H NMR spectra of the reaction mixture incubated by 4-OT F50A provided evidence that **7** was slowly converted to **8**. This anomaly may be explained by the different molar ratios of compounds **3** to **6** applied during the UV (**3**/**6** = 25 : 1) and ^{1}H NMR (**3**/**6** = 2 : 1) experiments and by the fact that the ^{1}H NMR signals corresponding to **8** were detected after 4 d (Fig. S3†) while the UV experiments were followed for a shorter time period of 20 h. Whether the dehydration reaction (**7** to **8**) is enzyme-catalyzed or buffer-catalyzed requires further investigation. Also, compound **5** ($\lambda_{max,5}$ = 234 nm) was detected, by ^{1}H NMR spectroscopy, in the reaction mixtures incubated with WT 4-OT and the 4-OT F50A mutant, which indeed explains the appearance of the absorbance at ~241 nm witnessed during the UV experiments (Fig. S2A†). This observation means that self-condensation of **3** into **5** and cross-coupling of **3** and **6** take place simultaneously and are in competition with each other. In conclusion, the ^{1}H NMR data revealed that WT 4-OT and the 4-OT F50A mutant catalyze the cross-coupling of **3** and **6**.

Next, we examined whether 4-OT catalyzes the cross-coupling of **3** and **9** to give **10** (Scheme 2c). In separate experiments, WT 4-OT, 4-OT F50A, and 4-OT P1A (2.0 mol% compared to **9**) were incubated with **3** (30 mM) and **9** (15 mM) in NaD_2PO_4 buffer (20 mM, pD 7.6), and reactions were monitored by ^{1}H NMR spectroscopy (Fig. S5 and Scheme S5†). The protons of the methyl group of compound **9** are acidic and we noticed that the methyl signal of **9** was missing in the ^{1}H NMR spectra recorded of the reaction mixtures incubated with WT 4-OT and 4-OT F50A mutant for 1 h as a result of >99% H–D exchange. No H–D exchange of the acidic protons of **9** was observed in the control sample without enzyme after 1 h, whereas only negligible H–D exchange was detected in the reaction mixture incubated with 4-OT P1A after 1 h (7%).

We analyzed the ^{1}H NMR data to obtain the yield[41] of **10** in the reaction mixtures incubated with WT 4-OT and 4-OT F50A (Fig. S5 and S6†). The yield of **10** reached steady levels of 38 and 44% (based on substrate **9**) after 4 d in the reaction mixtures incubated with WT 4-OT and 4-OT F50A, respectively. Prolonged incubation of up to 8 d did not increase yields in both experiments. Signals corresponding to both diastereoisomers of **10** were detected in the ^{1}H NMR spectra of both the WT 4-OT and the 4-OT F50A sample (Fig. S5 and S7†). No signals corresponding to **10** were detected in the reaction mixture without enzyme and the 4-OT P1A sample after 4 d. The ^{1}H NMR data indicated that WT 4-OT and 4-OT F50A both catalyze the cross-coupling of **3** and **9** and that the Pro-1 residue is crucial for catalysis (Scheme S5†). Furthermore, when synthetic WT 4-OT was used instead of recombinant WT 4-OT, under otherwise identical reaction conditions, a similar yield of **10** was obtained (Fig. S7†).

In addition to those of **10**, signals corresponding to **5** were observed as well in the ^{1}H NMR spectra of the reaction mixture

incubated with WT 4-OT and the 4-OT F50A mutant (Fig. S5†). The yield of **5** reached to 25 and ~30% (based on substrate **3**) after 4 and 8 d, respectively, in the sample containing the 4-OT F50A mutant. This means that the cross-coupling of **3** and **9** into **10** and the self-condensation of **3** into **5** are two competing conversions taking place simultaneously, having a reducing effect on the yield of **10**. No ^{1}H NMR signals corresponding to the dehydration product of **10**, nor the hydrated form of **10** (conversion of carbonyl moiety to diol), were detected during the entire course of the reaction. To the best of our knowledge, **10** has not been reported in the literature before. We have chemically synthesized **10** (Scheme S1†) and found that its spectroscopic data (exact mass, ^{1}H and ^{13}C NMR spectroscopy) are identical to those of enzymatically obtained **10** (Fig. S7†).

To test whether WT 4-OT and 4-OT F50A are able to catalyze a retro-aldol reaction of **10**, these enzymes were incubated with chemically synthesized **10** and the reactions were monitored by ^{1}H NMR spectroscopy. The spectroscopic analysis showed the formation of product **9** in reaction mixtures incubated with WT 4-OT or 4-OT F50A (Fig. S8†). ^{1}H NMR signals for product **3** were not observed during the course of the reaction, most likely because of evaporation of **3** (which is highly volatile) from the reaction mixture. The control experiment in which **10** was incubated without enzyme did not show formation of **9**. These data indicate that WT 4-OT and 4-OT F50A are capable of catalyzing a retro-aldol reaction using **10** as the substrate.

Having established that WT 4-OT and 4-OT F50A catalyze various intermolecular aldolizations, we next investigated whether these enzymes can catalyze the intramolecular aldol-cyclizations of hexanedial (**11**) and heptanedial (**14**) (Scheme 2d and e). Dials **11** and **14** were synthesized *in situ* from *trans*-1,2-cyclohexanediol (**17**) and *trans*-1,2-cycloheptanediol (**18**) based on a modified literature procedure (Scheme S6†).[48] First, the conversion of **11** to the cyclic aldehyde **12**, which may dehydrate to α,β-unsaturated aldehyde **13**, was monitored by UV spectroscopy. The sets of spectra recorded from the experiments with WT 4-OT and the 4-OT F50A mutant both show an increase of absorbance at 245 nm in course of time, which presumably corresponds to the formation of **13** ($\lambda_{max,\mathbf{13}}$ = 236 nm, 99.5% EtOH) (Fig. S9†).[49–51] To ascertain this finding, the enzymes WT 4-OT and 4-OT F50A (1.0 mol% compared to **11**) were incubated separately with **11** (15.4 mM in 20 mM NaD_2PO_4, pD = 7.6) and reaction progress was followed by ^{1}H NMR spectroscopy (Fig. S10†). A control reaction was also carried out using the same reaction mixture but without enzyme. We calculated the yield[41] of compounds **12** and **13** in course of time from their characteristic ^{1}H NMR peaks (Fig. S10†). Since **11** and **12** are present in equilibrium with their corresponding hydrated form (monohydrate **11′**, dihydrate **11″** and hydrate **12′**), their presence is determined by the sum of the unhydrated and hydrated forms (*i.e.* presence of **11** = **11** + **11′** + **11″**). The hydrated form of **13** was not observed. As illustrated in Fig. S11,† the yield of aldol coupling product **12** was ~67% in the reaction mixture incubated with 4-OT F50A after 24 h while the yield of dehydrated product **13** was ~23% after 24 h. These observations imply the accumulation of pharmaceutically important compound **12**[50] in this period of time as a result of a slower dehydration step (*i.e.*, **12** to **13**) compared to the aldol cyclization step (*i.e.*, **11** to **12**). The yields of **12** and **13** were 40 and 10%, respectively, in the reaction mixture incubated with WT 4-OT after 24 h. The yields of **12** and **13** only reached to 10% and 1%, respectively, in the control sample without enzyme after 24 h.

The cyclization of **14** was monitored using the same methods and conditions as described for **11**. UV spectroscopic assays showed an increase of absorbance at 236 nm for reaction mixtures incubated with WT 4-OT and 4-OT F50A (Fig. S13†) within 12 h. Increase in the absorbance at 236 nm presumably corresponds to the formation of **16** ($\lambda_{max,\mathbf{16}}$ = 230 nm)[52] *via* dehydration of **15**. Incubation of **14** in the absence of enzyme or in the presence of 4-OT P1A resulted in a minor increase of absorbance at 236 nm. The ^{1}H NMR spectroscopic assays indicated that the yield of **15** was ~81% in the reaction mixture incubated with 4-OT F50A after 21 h [Fig. S14 and S15,† yield of **15** = **15** + **15′** (monohydrate)]. Only a small amount of compound **16** (11%) was observed after 21 h (Fig. S14 and S15†) indicating that the relatively fast aldol-cyclization step was followed by relatively slow dehydration (the hydrate of **16** was neither observed in the sample containing WT 4-OT nor in the presence of the 4-OT F50A mutant). The yields of **15** were ~42% and 6% in the WT 4-OT and control samples after 21 h, respectively. The yield of **16** reached to ~8% (21 h) in the reaction mixture containing WT 4-OT. A negligible amount of compound **16** (1.5%, 21 h) was detected in the control sample.

To eliminate the possible role of any contaminating enzyme from *E. coli* in catalysis of the intramolecular cyclization of **11** and **14**, we performed separate experiments in which recombinant and synthetic WT 4-OT were incubated, under identical conditions, with either compound **11** or **14**, and the reactions were monitored by UV spectroscopy. The increases in absorbance at 245 nm (in the presence of **11**) and 236 nm (in the presence of **14**) were similar for both recombinant and synthetic WT 4-OT (Fig. S12 and S16†). Taken together, the data clearly demonstrate that both WT 4-OT and 4-OT F50A catalyze the intramolecular cyclization of **11** and **14**. Clarification on whether these enzymes catalyze the dehydration step requires further investigation. Note that all of the four reaction products **12**, **13**, **15** and **16** are valuable building blocks in organic synthesis, especially for the preparation of pharmaceuticals.[53–61]

Conclusion

In summary, we have demonstrated that 4-OT promiscuously catalyzes different types of aldol reactions, namely intermolecular self-condensation, intermolecular cross-coupling and intramolecular cyclization. This work provides the basis for further development of synthetically useful aldolases through activity-enhancing mutations and extensions of the

substrate scope. Work to engineer 4-OT into a more efficient aldolase for the self-condensation of propanal, yielding an α,β-unsaturated aldehyde as valuable building block for organic synthesis, is under way in our laboratory and the results will be reported in due course. Such a newly engineered biocatalyst may have preparative utility and complement existing chemical catalysts.

Experimental

For experimental procedures and characterization of compounds, see ESI.†

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