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PAPER

Candida tenuis xylose reductase catalysed reduction of acetophenones: the effect of ring-substituents on catalytic efficiency†

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The catalytic efficiencies of *Candida tenuis* xylose reductase catalysed reductions of mono-substituted acetophenones are in reasonable correlation with the σ-Hammett coefficients of the substituted phenyl groups. Variations of the substrate transformation rates are hence mainly caused by mesomeric and inductive effects of the substituents, while differences in substrate binding have a secondary relevance. Some substrate ^{1}H NMR chemical shifts and carbonyl IR absorption bands are in reasonable accordance with the catalytic activities and allow the estimation of the transformation rates with good accuracy. The resulting substituted (*S*)-1-phenyl ethanols are generated in very high enantiomeric excess.

Introduction

Since it is known that several oxidoreductases [E.C.1.1.1] act with high transformation rates on natural as well as on non-natural substrates, they have gained a growing significance in synthetic approaches.[1–3] In particular, their asymmetric induction is applied to obtain secondary alcohols of high enantiomeric purity.[3–5] Hence, there is an increase in interest to determine the reaction details about transformations of various ketone bearing molecules. Some oxidoreductases show a quite broad substrate acceptance, which is, however, often limited by the carbonyl bonds' electronic structure and polarisation as well as by size, chirality, and structure of the substrates.[6,7] In particular, the oxidoreductase catalysed transformations of acetophenones are influenced by different substituents of the aromatic ring.[8]

The effects of substituents on electrophilic aromatic substitution (S_NAr) have been studied and quantified since the 1930s.[9,10] Hammett described the electronic influence of substituents on the ionization constant of substituted benzoic acid as the main reason for the reaction velocity in S_NAr reactions.[10] His model was expanded, incorporating steric influences to predict reactions with *ortho*-positioned substituents and furthermore amplified on biochemical reactions.[11,12] Nys and Rekker introduced the log*P*, which describes the substrate solubility in water.[13] The resulting modified equation provides σ-Hammett coefficients for various substrate structures.[14] Furthermore, NMR chemical shifts of the aromatic system have found to be excellent indicators for the electronic influence of the substituents.[15]

In the present study the acetophenone acceptance of xylose reductase [E.C.1.1.1.175] from the yeast *Candida tenuis* (*Ct*XR; AKR2B5) is investigated.[7,16,17] This enzyme reduces its natural substrate xylose to the corresponding alcohol xylitol by utilizing NADPH or NADH,[18] but also accepts a wide variety of non-natural substrates.[7] In particular, some mono-substituted acetophenones are reduced with high catalytic efficiencies.[19] Several others, however, have been found to be accepted very poorly. We now correlate the σ-Hammett coefficients of several acetophenone aromatic moieties with the catalytic efficiencies of the *Ct*XR catalysed transformation. Furthermore, NMR shifts and IR absorption bands of these substrates are compared with the σ-Hammett coefficients and the correspondent *Ct*XR catalytic efficiencies.

Results and discussion

Catalytic efficiency of *Ct*XR catalysed acetophenone reduction

The potential of *Ct*XR to reduce *ortho*-chloro acetophenone (**1**) with high catalytic efficiency has recently been described.[19] Hence, we screened the transformations of 27 mono-substituted acetophenones with respect to the catalytic efficiency (Table 1). The selected substrates carry different substituents in *ortho*-, *meta*-, and *para*-positions, resulting in varying mesomeric and inductive effects. Some bear acidic protons or electron rich moieties, which can act in hydrogen bridge formation during enzyme–substrate binding, as donor or acceptor, respectively.

Acetophenones carrying substituents with (+)-M and (–)-I effects in *ortho*- or *para*-position are reduced with quite reasonable catalytic activities. The best accepted substrate, *ortho*-chloro acetophenone (**1**) turned out to be even faster converted than

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† Electronic supplementary information (ESI) available: values and parameters used for calculation to correlate k_{cat}/K_m of substituted acetophenone reductions with electronic and positional effects of the substituents as shown in Fig. 1. See DOI: 10.1039/c1ob05510k

Table 1 σ-Hammett coefficients[14] of the investigated substrates and determined catalytic efficiency of the according *Ct*XR catalysed transformation

Substrate	σH	catalytic efficiency/ M^{-1} s^{-1}
ortho-chloro acetophenone [**1**]	0.68	340.3
meta-chloro acetophenone [**2**]	0.37	0.8
para-chloro acetophenone [**3**]	0.23	6.6
ortho-bromo acetophenone [**4**]	0.70	260.9
meta-bromo acetophenone [**5**]	0.39	1.3
para-bromo acetophenone [**6**]	0.23	3.2
ortho-iodo acetophenone [**7**]	0.63	60.5
meta-iodo acetophenone [**8**]	0.35	0.3
ortho-fluoro acetophenone [**9**]	0.54	16.5
ortho-cyano acetophenone [**10**]	1.32	134.5
meta-cyano acetophenone [**11**]	0.56	1.4
para-cyano acetophenone [**12**]	0.66	1.8
ortho-nitro acetophenone [**13**]	1.40	17.6
meta-nitro acetophenone [**14**]	0.71	36.7
para-nitro acetophenone [**15**]	0.78	224.4
ortho-hydroxy acetophenone [**16**]	0.04	4.5
meta-hydroxy acetophenone [**17**]	0.12	2.4
para-hydroxy acetophenone [**18**]	–0.37	~ 0.1
ortho-amino acetophenone [**19**]	0.03	~ 0.1
meta-amino acetophenone [**20**]	–0.16	2.2
para-amino acetophenone [**21**]	–0.66	~ 0.1
ortho-methoxy acetophenone [**22**]	0.00	13.9
para-methoxy acetophenone [**23**]	–0.27	~ 0.1
ortho-acetoxy acetophenone [**24**]	–0.37	1.8
para-acetoxy acetophenone [**25**]	0.31	~ 0.1
meta-acetamino acetophenone [**26**]	0.21	~ 0.1
acetophenone [**27**]	0.00	0.5

xylose. However, taking into account that *Ct*XR only reduces the xylose aldehyde form, the natural substrate is still accepted best. Further *ortho*-halo acetophenones with bromo, iodo, and the pseudohalogen cyano substituents as well as *para*-nitro acetophenone (**15**) are also outstandingly well accepted substrates. All other tested acetophenones are much less efficiently reduced by the *Ct*XR.

In contrast to the halo acetophenones, *para*-nitro acetophenone (**15**) is better accepted by *Ct*XR than the corresponding *ortho*- (**13**) and *meta*-derivatives (**14**). *In situ* ^{1}H-NMR measurements of its transformation show formation of by-products in consecutive or parallel reactions, while in corresponding analyses of *ortho*-halo acetophenone reductions the respective 1-(*ortho*-halophenyl)-ethanols have been exclusively detected as products. The reasons for these differences in chemo- and stereoselectivity are very likely changes in binding. The K_m values of the nitro-derivatives are in the sub-millimolar range [0.05–0.46 mM] and reasonably different from those of all other acetophenones. The limited solubility of acetophenones together with high K_m values prevented saturation of the enzyme with these substrates and the catalytic efficiency was hence obtained from the linear part of the Michaelis–Menten plot.

σ-Hammett coefficients and catalytic efficiency

σ-Hammett coefficients describe electronic and steric effects of substituents to covalently bound structures, incorporating influences from mesomeric and inductive effects. Hence, we carried out a quantitative structure–activity relationship (QSAR) analysis for several acetophenones to predict the influence of the whole aromatic structures on relative reactivity in *Ct*XR catalysed carbonyl transformations.[14] The substituent effects on enzymic rates have been divided into electronic, hydrophobic, and steric characteristics of the substrates. In a first approximation, the dependence of observed parameters on changes in substituent structure was factored in a linear relationship:

$$\log(k_{cat}/K) = \rho\sigma + A\log P + B\log Mol + \Delta S_{eff} \quad (1)$$

Eqn (1) correlates the substituent effects on k_{cat}/K_m with electronic substituent properties, expressed by the σ-Hammett coefficients, as well as the hydrophobic (log*P*) and molecular (log*Mol*) characteristics of acetophenone. log*P* values,[14] and log*Mol* values (logarithm of the molecular volume measured in Å^3) were derived from SciFinder Scholar.[20] The parameter S_{eff} is utilized to take a general positional effect of the substituent into account. It was factored into *meta*-substituted acetophenone (factor 0), *para*-substituted acetophenone (factor 1), and *ortho*-substituted acetophenone (factor 2), as described earlier.[21] In eqn (1), ρ is the σ-Hammett coefficient factor, where A, B and Δ are parameter coefficients. Values of $k_{cat}/K_{subst.acetophenone}$ for *Ct*XR catalysis displayed an 1100-fold variation in the dependence of the substituents. Eqn (1) was fitted to the set of k_{cat}/K_m values excluding the $k_{cat}/K_{NO2\text{-}acetophenone}$. For detailed values used in these calculations see Table S1 in supplementary data.†

Results indicated only electronic and positional factors of the substituents to be important in affecting k_{cat}/K_m. Parameter estimates along with the coefficient of determination (r^2) and the *F*-test value were obtained from two-parameter correlations, as shown in eqn (2). Values of $\log((k_{cat}/K_{subst.acetophenone})/(k_{cat}/K_{acetophenone}))$ are plotted against the σ-Hammett factor and the positional effect giving a reasonable correlation (Fig. 2).

$$\log((k_{cat}/K_{subst.acetophenone})/(k_{cat}/K_{acetophenone})) = (0.93 \pm 0.28)\sigma + (0.67 \pm 0.10)\Delta S_{eff} \quad (2)$$

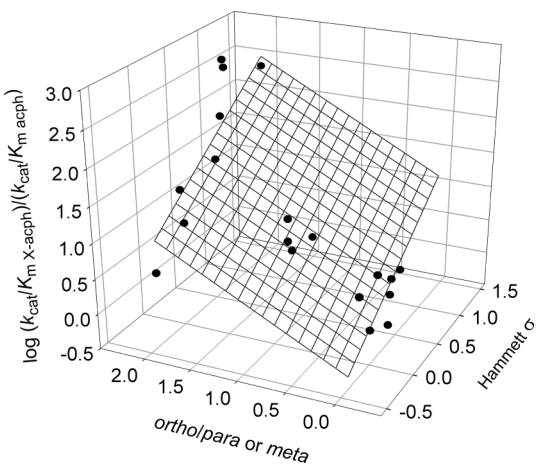


Fig. 1 Correlations of k_{cat}/K_m for substituted acetophenone reductions with electronic (σ) and positional (*ortho*-, *meta*-, and *para*-) effects. The r^2 of 0.74, together with a high *F* value of 0.48, point out a useful fit of the equation to the corresponding data.

The positive value of $\rho\sigma$ implies that a higher partial positive charge on the reactive carbonyl carbon leads to a more rapid enzymatic reaction. Since *ortho*-substituted acetophenones were the preferred substrates of *Ct*XR, followed by *para*-substituted

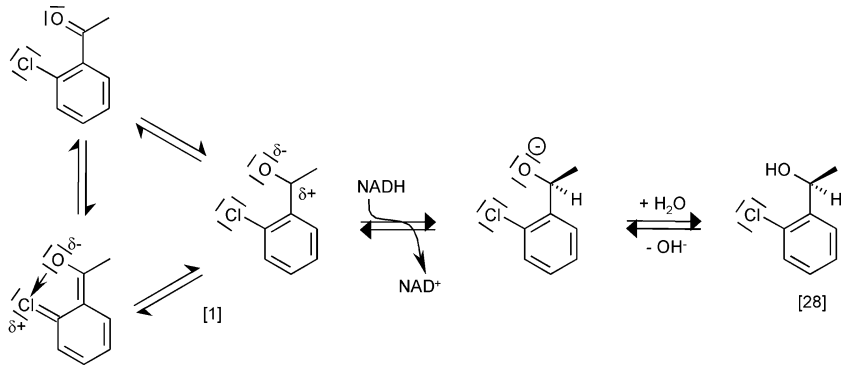


Fig. 2 Polarisation of the *ortho*-chloro acetophenone (**1**) visualised by the mesomeric structures including orbital overlap from negatively charged oxygen to positively charged chlorine. Hydride transfer from NADH attacks the carbonyl carbon leading to the (*S*)-1-(*ortho*-chloro phenyl) ethanolate, which is directly protonated to gain product **28**.

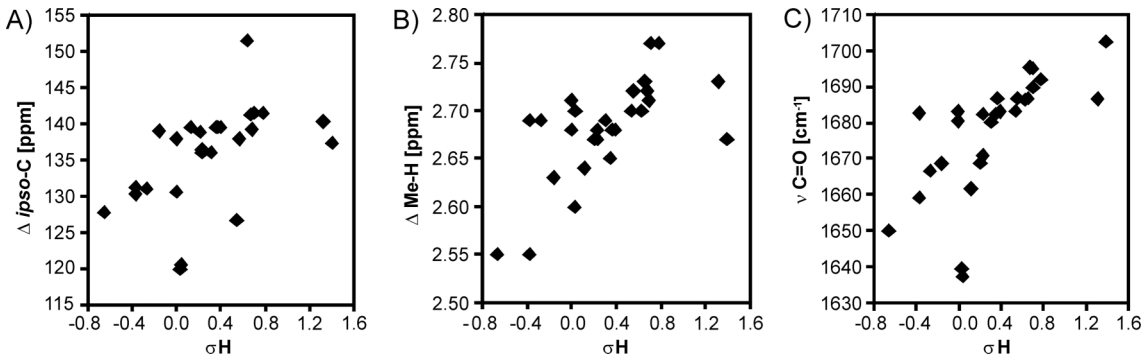


Fig. 3 Shown are the correlations between σ-Hammett coefficients of the substituted aromatic moieties and some spectroscopic data of the substrates. Panel (A) indicates the ^{13}C NMR shift of the *ipso*-carbon neighboured to the carbonyl group, (B) shows the ^{1}H NMR shifts of the methyl groups, and (C) demonstrates the IR wave numbers [ν, C═O] of the carbonyl groups. In all three cases a reasonable relationship between the spectroscopic data and the σ-Hammett coefficients can be seen for most substrates. Outliers belong to compounds carrying acidic protons forming intramolecular hydrogen bridges, which entirely influence the NMR shifts and the IR bands.

acetophenones, Δ had a positive value, which could be mainly correlated with the electronic parameters. The main reasons for the preference of *ortho*-halo acetophenones are hence positive mesomeric and negative inductive effects of the halogens, leading to a polarisation of the carbonyl group. A concomitant orbital overlap of occupied and partially negative charged oxygen p-orbitals with empty orbitals of the spatial neighboured halogen atoms can further stabilise the polarisation of the carbonyl bond. The hydride transfer from NADH to the carbonyl carbon is hence favoured (Fig. 2). The size of the halogen atoms lead to differences in orbital overlap and consequently to slight variations of catalytic activities.

Independence of *Ct*XR activity on substrate size and hydrophobicity has been previously shown for other substrates.[16,21] This behaviour can be explained by the ability of *Ct*XR to accommodate a wide variety of substrates. Hence, the different acetophenone moieties have a subordinated influence to bind *Ct*XR. Even their ability to form hydrogen bridges does not lead to changes of substrate fitting in the active site nor to a modification of the reaction velocity. The only exception is the nitro group leading to a lower K_m value. *Ct*XR binds all further tested compounds without larger influences of the substituents regarding formation of additional non covalent binding and of required freedom in the active site.

NMR shifts and IR absorption bands

Based on the relation between σ-Hammett coefficients and catalytic activity, the NMR chemical shifts and IR absorption bands of the substrates have been taken into account. Such correlations should allow to predict *Ct*XR catalytic activity from the spectral data of the acetophenones, as shown earlier for other molecules.[22] In particular, the ^{13}C NMR shifts of the *ipso*-, carbonyl-, and methyl carbons as well as the ^{1}H NMR shift of the methyl protons and the IR bands of the carbonyl groups are of interest, as they are present in all substrates.

The NMR shifts of carbonyl carbons which are attacked by the hydride as well as the methyl carbon signals do not show any significant changes within the tested compounds. However, the *ipso*-carbon ^{13}C NMR signals (Fig. 3a) and the methyl proton ^{1}H NMR signals (Fig. 3b) show reasonable shift differences. In both cases NMR chemical shifts increase by trend with growing σ-Hammett coefficients. IR bands of the carbonyl groups also show a good direct proportional correlation between the wave numbers and the σ-Hammett coefficients (Fig. 3c). However, spectral data of substrates, which can provide protons for hydrogen bridges like *ortho*-hydroxy acetophenone (**16**) and *ortho*-amino acetophenone (**19**) are affected by these further intramolecular influences and possess larger deviations.

The comparison of catalytic activity with the NMR shifts and IR absorption bands has only been performed when a sensible correlation between spectroscopic data and the σ-Hammett coefficients have been determined. Correlating ^{13}C NMR shifts of *ipso*-carbons with the catalytic activity leads to large statistical spreading and does not cause a sufficient relation. However, in the case of the methyl group ^{1}H NMR shifts, a general correlation between increasing chemical shifts and growing catalytic efficiency can be observed, as shown in Fig. 4a. These ^{1}H NMR shifts can hence be used to estimate the catalytic efficiency of the *Ct*XR catalysed transformation and allow a pre-selection of possibly accepted acetophenones without the necessity to take calculated σ-Hammett coefficients into account.

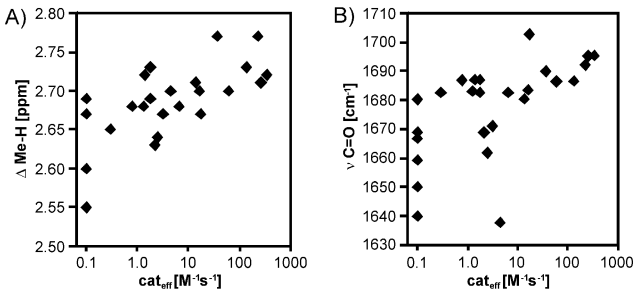


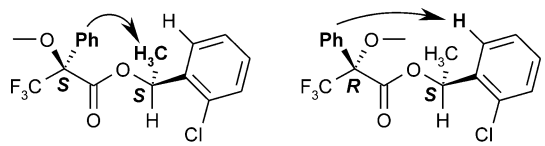
Fig. 4 Relationship between the catalytic efficiencies of *Ct*XR catalysed transformations and the ^{1}H NMR shifts of the methyl groups (A) as well as the IR bands [ν, C═O] of the carbonyl groups (B). The logarithmic tendency of these correlations is obvious for most substrates, except for those forming intramolecular hydrogen bridges. The transformation rates and catalytic efficiencies of the poorly accepted substrates could not exactly be determined for $cat_{eff} < 0.2\ M^{-1}\ s^{-1}$.

The IR absorption bands of the carbonyl group also have a reasonable correlation with the catalytic efficiency and are therefore a quite well suited parameter to predict the catalytic efficiency (Fig. 4b). Substrates forming intramolecular hydrogen bridges by providing an acidic proton again show a larger deviance. These spectral data can hence be used to roughly estimate the catalytic efficiency of the *Ct*XR catalysed transformation and allow a pre-selection of possibly accepted acetophenones without the necessity to take calculated σ-Hammett coefficients into account. The results reveal that acetophenones whose C═O ν is below 1683 cm^{-1} are poorly active towards *Ct*XR catalysed reduction. This wavelength therefore represents a "Break Even Point" for sensible activity tests.

Previous IR studies of pyruvate reduction by lactate dehydrogenase have shown a shift in wave number ($\Delta\nu = 35\ cm^{-1}$) upon pyruvate binding to the enzyme. Active site residues are responsible for the carbonyl moiety polarization ($^{+}C–O^{-}$) and the frequency shift. Half of the rate enhancement by lactate dehydrogenase is due to such enzyme-induced carbonyl activation.[23] Catalytic efficiencies of acetophenone reductions by *Ct*XR, however, follow the intrinsic reactivities of substrates reflected by IR shifts. The enzyme adopts the inherent carbonyl activity and reacts accordingly. The k_{cat}/K_m value variation of *Ct*XR for acetophenone reductions is therefore ascribed to electronic effects of the substituent in a type of substrate-assisted catalysis.[8]

Absolute configuration of the resulting products

Oxidoreductase catalysed reduction of different acetophenones leads to corresponding chiral secondary alcohols with a varying enantiomeric excess (ee).[8] Low ee-values can either be explained by chemical epimerisation or by variation of substrate binding positions in the active site, as is possible for the investigated acetophenones. Hence, we checked the enantiomeric excess and the absolute configuration of the two most efficiently generated products 1-(*ortho*-chloro)-phenyl ethanol (**28**) and 1-(*ortho*-bromo)-phenyl ethanol (**29**). Optical rotation and Mosher's ^{1}H NMR shift method[24] have been applied for this purpose. In particular, the generated Mosher esters **30**, **31**, **32**, and **33** indicated both products to be formed in the (*S*)-configuration with ≥99% ee (Fig. 5). Due to such high enantiomeric purity, large catalytic activity, and good substrate access, the resulting chiral (*S*)-1-phenyl ethanols are interesting enantiomerically pure intermediates for synthetic approaches.



(*S*)-MTPA ester [30] (*R*)-MTPA ester [31]

Fig. 5 Influence of the phenyl ring onto chemical shifts of the methyl group and *ortho*-chlorophenyl group in the (*S*)-1-(*ortho*-chlorophenyl)-ethanol side chains in the two diastereomeric (*S*)-1-(*ortho*-chlorophenyl) ethyl-(*R*)-α-methoxy-α-trifluoromethylphenyl acetates (MTPA esters, **30** (*S*,*S*) and **31** (*S*,*R*)). Different proton shifts of the diastereomers are used to determine the absolute configuration of the (*ortho*-chlorophenyl)-ethanol according to Dale and Mosher.[24]

Conclusions

*Ct*XR catalysed reductions of mono-substituted acetophenones show a large variety of different catalytic efficiencies. These variations are mainly caused by mesomeric and inductive effects of the substituents, which influence the polarisation of the carbonyl group and affect the hydride transfer from NADH to the substrate. As σ-Hammett coefficients of the substituted phenyl groups are in reasonably good correlation with the reaction velocities, enzyme–substrate binding obviously has a subordinated influence on the *Ct*XR catalytic efficiencies. This unusual relation has to be further investigated with respect to high enantiomeric purity of the products, which point a definite binding of the well accepted substrates in the active site. ^{1}H NMR chemical shift of the methyl proton and the IR absorption band of the carbonyl group provide a "reactivity scale" to predict the catalytic efficiency of a certain substrate transformation, because these spectroscopic indicators are influenced by the substituent effects comparable to the catalytic efficiencies. The well accepted substrates are transformed with high enantiomeric excess to products, which can act as key intermediates in synthesis of natural products and pharmacological active compounds.[19] Reliable reactivity scales for reductases on one hand diminish labour input in the screening of substrates libraries for the production of pharmaceutical

key intermediates. On the other hand, they facilitate substrate engineering to activate poor substrates.

Experimental

General

NMR spectra were measured on a Bruker Avance DRX-400 or a DRX-600 (Bruker) operating at 400.13 MHz and 600.13 MHz, respectively. Measurements were performed at a temperature of 300.20 K using the Bruker Topspin 1.3 software. The acetophenones were dissolved in a 750 μL D_2O : CD_3OD mixture (9 : 1 v : v) and transferred into 5 mm high-precision NMR sample tubes. All spectra were referenced to solvent signals of CH_3OD at 3.34 ppm (^1H) or $CHCl_3$ at 7.26 ppm (^1H) and CD_3OD at 49.50 ppm (^{13}C) or $CDCl_3$ at 77.16 ppm (^{13}C). For *in situ* NMR on DRX-600 all enzymatic transformations were performed directly in the NMR sample tube. Samples were prepared by adding acetophenones (15–58 mg) dissolved in 75 μL CD_3OD to a solution of a corresponding amount of NADH in 0.675 mL K_3PO_4 buffer (50 mM) in D_2O (pD 6.60).[25,26] The *Ct*XR was finally added in 1–2 μL amounts from an aq. stock solution. Measurements were performed at regular intervals over a total time of 3–6 h.

IR-spectra were recorded with a FT-IR spectrometer Spectrum System 2000 (Perkin Elmer). The catalytic efficiency was performed at a Beckman DU-800 spectrometer (Beckman Coulter) observing the decrease of the NADH signal at 340 nm. Optical rotation was measured at the sodium D line using a 100 mm path length cell on a Perkin Elmer Automatic Polarimeter 341.

Materials

If not otherwise indicated, all reagents were obtained from commercial suppliers (Sigma–Aldrich–Fluka or Acros Organics) and used without further purification or drying. TLC was performed with Merck silica gel 60 F_{254} pre-coated plates. Silica gel column chromatography was carried out on silica gel 60 M (40–63 μm, Macherey & Nagel).

Recombinant *Candida tenuis* xylose reductase was produced in *Escherichia coli* and purified to apparent homogeneity as described earlier.[27] Initial rate measurements were carried out using reported protocols.[16] All experiments were performed at 25 °C in 50 mM potassium phosphate buffer, pH 7.0. 5% ethanol was added to the buffer to enhance the solubility of the acetophenones. Initial rates were obtained under conditions in which the substrate concentration was varied and the NADH concentration was constant and saturating (230 μM NADH). The enzyme concentration in the assays was in the range of 0.1–9 μM, depending on the activity towards the respective substrate. Appropriate controls containing enzyme and coenzyme, or the substrate and coenzyme were determined under conditions otherwise exactly identical to the enzymic assay. Data processing and statistical analysis were carried out as reported elsewhere.[28] The limited solubility of most acetophenones prevented saturation of the enzyme. The catalytic efficiency was obtained from the part of the Michaelis–Menten plot where under conditions of [substrate] « K_m, the reaction rate is linearly dependent on substrate concentrations with a slope that equals (k_{cat}/K_m) divided by the molar concentration of the enzyme.

***ortho*-Bromo acetophenone (4)[29–31].** Anthranilic acid (0.2 g, 1.5 mmol) was dissolved in 2 mL H_2SO_4 (3.0 N) and mixed with a hot solution of $CuSO_4$ (0.25 g, 1 mmol), KBr (0.36 g, 3 mmol), 0.2 mL H_2SO_4, and copper swarfs (0.2 g) in 2 mL H_2O and refluxed for 2 h. Then a solution of $NaNO_2$ (0.1 g, 1.5 mmol) in 1.0 mL H_2O was added drop wise and the reaction was stirred at 80–90 °C until the gas evolution stopped. The mixture was extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated. The residue was dissolved in 5 mL Et_2O, mixed with $SOCl_2$, refluxed for 2 h, and concentrated. The concentrated residue was mixed under argon with Fe(III)-acetylacetonate (8.5 mg, 0.8 mmol) in 15 mL abs. Et_2O and cooled to –78 °C. A solution of CH_3MgI (0.124 g, 1.0 mmol) in 1.5 mL abs. Et_2O was added. After the colour changed from red/orange to brown/green the reaction was quenched with saturated NH_4Cl solution and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, concentrated, and purified by flash chromatography with a EtOAc : CH_2Cl_2 mixture (2 : 3 v : v) to afford 0.06 g (38%) **4**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.78 (1H, d, J = 7.92 Hz), 7.68 (1H, d, J = 7.52 Hz), 7.50 (1H, m), 7.48 (1H, m), 2.71 (3H, s); δ_C 208.1, 141.5, 135.4, 134.2, 130.6, 129.2, 119.3, 31.2; ν_{max}/cm^{-1} 1695.0 (C═O).

***meta*-Bromo acetophenone (5)[29].** *meta*-Amino acetophenone (**20**) (2.0 g, 15 mmol) was dissolved in 20 mL H_2SO_4 (3.0 N) and mixed with a hot solution of $CuSO_4$ (2.5 g, 10 mmol), KI (3.6 g, 30 mmol), 2 mL H_2SO_4, and copper swarfs (2.0 g) in 20 mL H_2O and refluxed for 2 h. Then a solution of $NaNO_2$ (1.0 g, 15 mmol) in 10 mL H_2O was added dropwise and the reaction was stirred at 80–90 °C until the gas evolution stopped. The mixture was extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated to give 1.6 g (52%) **5**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.20 (1H, m), 7.99 (1H, d, J = 8.21 Hz), 7.87 (1H, d, J = 8.20 Hz), 7.50 (1H, m), 2.68 (3H, s); δ_C 203.4, 139.5, 138.0, 132.7, 132.0, 128.8, 27.7, one ^{13}C n.d.;[32] ν_{max}/cm^{-1} 1683.1 (C═O).

***para*-Bromo acetophenone (6)[29].** Compound **6** was prepared from *para*-amino acetophenone (**21**, 2.0 g, 15 mmol) and KBr (3.6 g, 30 mmol), following the procedure for compound **5** to give 1.7 g (55%) **6**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.92 (2H, d, J = 8.54 Hz), 7.76 (2H, d, J = 8.54 Hz), 2.67 (3H, s); δ_C 203.6, 136.0, 133.4 (2C), 131.6 (2C), 27.6, one ^{13}C n.d.;[32] ν_{max}/cm^{-1} 1670.9 (C═O).

***ortho*-Iodo acetophenone (7)[29–31].** Compound **7** was prepared from anthranilic acid (0.2 g, 1.5 mmol) and KI (0.36 g, 2 mmol), following the procedure for compound **4** to give 0.12 g (61%) **7**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.08 (1H, d, J = 7.96 Hz), 7.68 (1H, d, J = 7.68 Hz), 7.56 (1H, m), 7.29 (1H, m), 2.70 (3H, s); δ_C 216.8, 151.6, 142.3, 134.1, 130.1, 129.8, 30.7, one ^{13}C n.d.;[32] ν_{max}/cm^{-1} 1686.2 (C═O).

***meta*-Iodo acetophenone (8)[29].** Compound **8** was prepared from *meta*-amino acetophenone (**20**, 2.0 g, 15 mmol) and KI (3.6 g, 22 mmol), following the procedure for compound **5** to give 2.3 g (63%) **8**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.38 (1H, m), 8.07 (1H, d, J = 7.83 Hz), 8.02 (1H, d, J = 7.87 Hz), 7.34 (1H, m), 2.65 (3H, s); δ_C 203.6, 141.6, 139.5, 136.9, 130.8, 130.5, 27.9, one ^{13}C n.d.;[32] ν_{max}/cm^{-1} 1682.4 (C═O).

***ortho*-Cyano acetophenone (10)[29].** Compound **10** was prepared from *ortho*-amino acetophenone (**19**, 1.0 g, 7 mmol) and KCN

(1.8 g, 23 mmol), following the procedure for compound **5** to give 0.21 g (20%) **19**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.18 (1H, d, J = 7.96 Hz), 7.95 (1H, d, J = 7.32 Hz), 7.87 (1H, m), 7.80 (1H, m), 2.73 (3H, s); δ_C 202.1, 140.3, 137.8, 137.1, 134.9, 132.5, 120.8, 110.5, 28.4; ν_{max}/cm^{-1} 1686.5 (C═O).

***meta*-Cyano acetophenone (11)[29].** Compound **11** was prepared from *meta*-amino acetophenone (**20**, 1.0 g, 7 mmol) and KCN (1.8 g, 23 mmol), following the procedure for compound **5** to give 0.35 g (24%) **11**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.41 (1H, m), 8.30 (1H, d, J = 7.27 Hz), 8.05 (1H, d, J = 7.78 Hz), 7.75 (1H, m), 2.72 (3H, s); δ_C 202.8, 138.4, 137.9, 134.5, 133.9, 131.8, 120.4, 112.7, 26.3; ν_{max}/cm^{-1} 1686.8 (C═O).

***ortho*-Nitro acetophenone (13)[33].** A solution of *ortho*-amino acetophenone (**19**, 1.0 g, 7.4 mmol) in 20 mL $CHCl_3$ was added to a refluxing solution of *meta*-chloro perbenzoic acid (5.1 g, 30 mmol) in $CHCl_3$ and refluxed for 2 h. The mixture was filtrated, washed with 20 mL NaOH solution (1 N), dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography on silica gel with CH_2Cl_2 to afford 0.42 g (43%) **13**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.21 (1H, m), 7.88 (1H, m), 7.76 (1H, m), 7.65 (1H, m), 2.67 (3H, s); δ_C 207.1, 146.5, 137.1, 136.4, 133.0, 128.9, 126.0, 31.1; ν_{max}/cm^{-1} 1702.5 (C═O).

***meta*-Nitro acetophenone (14)[33].** Compound **14** was prepared from *meta*-amino acetophenone (**20**, 1.0 g, 7.4 mmol), following the procedure for compound **13** to give 0.66 g (55%) **14**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.83 (1H, s), 8.53 (1H, d, J = 8.76 Hz), 8.41 (1H, d, J = 7.76 Hz), 7.82 (1H, m), 2.77 (3H, s); δ_C 202.1, 138.2, 135.6, 131.2, 128.9, 124.2, 27.3, one ^{13}C n.d.;[32] ν_{max}/cm^{-1} 1689.9 (C═O).

***para*-Nitro acetophenone (15)[33].** Compound **15** was prepared from *para*-amino acetophenone (**21**, 1.0 g, 7.4 mmol), following the procedure for compound **13** to give 0.68 g (56%) **15**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.40 (2H, d, J = 8.90 Hz), 8.22 (2H, d, J = 8.90 Hz), 2,77 (3H, s); δ_C 196.5, 150.5, 141.5, 129.9 (2C), 124.2 (2C), 27.0; ν_{max}/cm^{-1} 1692.0 (C═O).

***ortho*-Methoxy acetophenone (22)[34].** MeI (0.8 mL, 12.5 mmol) was added to a solution of *ortho*-hydroxy acetophenone (**16**, 0.2 g, 15 mmol) and K_2CO_3 (1.0 g, 75 mmol) in 150 mL acetone and refluxed for 12 h. After filtration of the precipitated salt the mixture was concentrated. The residue was purified by flash chromatography on silica gel with CH_2Cl_2 to afford 0.2 g (98%) **22**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.78 (1H, d, J = 6.90 Hz), 7.70 (1H, m), 7.26 (1H, d, J = 7.77 Hz), 7.17 (1H, m), 3.99 (3H, s), 2.71 (3H, s); δ_C 205.7, 158.9, 135.3, 130.6, 127.3, 120.9, 112.9, 55.9, 30.6; ν_{max}/cm^{-1} 1680.4 (C═O).

***para*-Methoxy acetophenone (23)[34].** Compound **23** was prepared from *para*-hydroxy acetophenone (**18**, 0.2 g, 15 mmol) following the procedure for compound **22** to give 0.2 g (98%) product **23**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.01 (2H, d, J = 8.96 Hz), 7.09 (2H, d, J = 8.96 Hz), 3.90 (3H, s); 2.69 (3H, s); δ_C 204.0, 165.2, 132.6 (2C), 131.0, 115.5 (2C), 56.0, 31.7; ν_{max}/cm^{-1} 1666.6 (C═O).

***ortho*-Acetoxy acetophenone (24)[35].** A solution of *ortho*-hydroxy acetophenone (**16**, 0.2 g, 15 mmol) and acetic anhydride (0.94 g, 92 mmol) in 14 mL pyridine was heated for 30 min and then ice water with HCl was added. The mixture was extracted with CH_2Cl_2, dried over $MgSO_4$, and concentrated to give 0.25 g (94%) **24**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.99 (1H, m), 7.70 (1H, m), 7.48 (1H, m), 7.25 (1H, m), 2.63 (3H, s), 2.38 (3H, s); δ_C 204.4, 174.6, 149.4, 136.1, 132.4, 131.3, 128.4, 125.0, 30.0, 21.8; ν_{max}/cm^{-1} 1682.6 (C═O).

***para*-Acetoxy acetophenone (25)[35].** Compound **25** was prepared from *para*-hydroxy acetophenone (**18**, 0.5 g, 38 mmol), following the procedure for compound **24** to give 0.62 g (92%) **25**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.09 (2H, d, J = 8.80 Hz), 7.31 (2H, d, J = 8.80 Hz), 2.69 (3H, s), 2.39 (3H, s); δ_C 204.0, 174.1, 155.8, 136.0, 131.9 (2C), 123.5 (2C), 27.7, 21.9; ν_{max}/cm^{-1} 1680.1 (C═O).

***meta*-Acetamino acetophenone (26)[35].** Compound **26** was prepared from *meta*-amino acetophenone (**20**) (1.0 g, 7 mmol), following the procedure for compound **24** to give 1.14 g (92%) **26**. δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.02 (1H, s), 7.83 (1H, d, J = 7.80 Hz), 7.69 (1H, d, J = 9.04 Hz), 7.56 (1H, m), 2.67 (3H, s), 2.22 (3H, s); δ_C 204.6, 174.5, 138.9, 138.6, 131.0, 128.4, 126.9, 122.7, 27.7, 24.3; ν_{max}/cm^{-1} 1668.8 (C═O).

***ortho*-Chloro acetophenone (1).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.72 (1H, d, J = 6.96 Hz), 7.56 (2H, m), 7.48 (1H, m), 2.72 (3H, s); δ_C 207.7, 139.2, 134.3, 132.2, 131.8, 130.9, 128.7, 31.4; ν_{max}/cm^{-1} 1695.2 (C═O).

***meta*-Chloro acetophenone (2).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.03 (1H, s), 7.94 (1H, m), 7.72 (1H, m), 7.55 (1H, m), 2.68 (3H, s); δ_C 203.9, 139.5, 135.8, 135.1, 131.7, 128.7, 128.4, 27.8; ν_{max}/cm^{-1} 1686.8 (C═O).

***para*-Chloro acetophenone (3).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.99 (2H, d, J = 8.56 Hz), 7.59 (2H, d, J = 8.56 Hz), 2.68 (3H, s); δ_C 204.16, 141.2, 136.4, 131.6 (2C), 130.3 (2C), 27.6; ν_{max}/cm^{-1} 1682.4 (C═O).

***ortho*-Fluoro acetophenone (9).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.86 (1H, m), 7.69 (1H, m), 7.35 (1H, m), 7.29 (1H, m), 2.70 (3H, d, J = 3.68 Hz); δ_C 203.2, 163.2, 137.2, 131.8, 126.6, 126.0, 118.3, 31.6; ν_{max}/cm^{-1} 1683.3 (C═O).

***para*-Cyano acetophenone (12).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.13 (2H, d, J = 8.44 Hz), 7.94 (2H, d, J = 8.44 Hz), 2.73 (3H, s); δ_C 203.5, 141.3, 134.3 (2C), 130.2 (2C), 120.2, 117.1, 27.92; ν_{max}/cm^{-1} 1686.8 (C═O).

***ortho*-Hydroxy acetophenone (16).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.97 (1H, d, J = 8.04 Hz), 7.62 (1H, m), 7.08 (1H, m), 7.02 (1H, d, J = 8.56 Hz), 2.70 (3H, s); δ_C 208.8, 161.1, 138.5, 133.3, 121.3, 120.4, 118.9, 27.7; ν_{max}/cm^{-1} 1637.5 (C═O).

***meta*-Hydroxy acetophenone (17).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.57 (1H, d, J = 7.76 Hz), 7.44 (1H, m), 7.10 (1H, s), 7.18 (1H, m), 2.64 (3H, s); δ_C 205.0, 157.3, 139.5, 133.7, 122.5, 122.4, 116.0, 27.7; ν_{max}/cm^{-1} 1661.6 (C═O).

***para*-Hydroxy acetophenone (18).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.87 (2H, d, J = 8.82 Hz), 6.91 (2H, d, J = 8.82 Hz), 2.55 (3H, s); δ_C 203.8, 163.0, 132.9 (2C), 130.3, 116.8 (2C), 27.1; ν_{max}/cm^{-1} 1659.1 (C═O).

***ortho*-Amino acetophenone (19).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.87 (1H, m), 7.40 (1H, m), 6.85 (1H, m), 6.79 (1H, m), 2.60 (3H, s); δ_C 205.8, 151.5, 136.7, 134.0, 119.9, 119.3, 118.2, 28.7; ν_{max}/cm^{-1} 1639.7 (C═O).

***meta*-Amino acetophenone (20).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.46 (1H, d, J = 7.80 Hz), 7.38 (1H, m), 7.37 (1H, m), 7.12 (1H, d, J = 7.88 Hz), 2.63 (3H, s); δ_C 205.7, 148.2, 139.0, 131.2, 123.2, 121.2, 116.8, 27.7; ν_{max}/cm^{-1} 1668.6 (C═O).

***para*-Amino acetophenone (21).** δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.85 (2H, d, J = 8.74 Hz), 6.82 (2H, d, J = 8.74 Hz), 2.55 (3H, s); δ_C 203.5, 154.8, 132.9 (2C), 127.8, 115.8 (2C), 26.8; ν_{max}/cm^{-1} 1650.0 (C═O).

Acetophenone (27). δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 8.02 (2H, d, J = 7.60 Hz), 7.58 (2H, m), 7.40 (1H, m), 2.68 (3H, s); δ_C 205.6, 137.9, 135.6, 130.3 (2C), 130.0 (2C), 27.7; ν_{max}/cm^{-1} 1683.0 (C═O).[36]

1-(*ortho*-Chloro-phenyl) ethanol (28). The transformation is performed with the whole cell transformation technique described by Kratzer *et al.*[19,37] δ_H (400 MHz, D_2O/CD_3OD, CH_3OH) 7.57 (1H, m), 7.30 (1H, d, J = 7.76 Hz), 7.28 (1H, m), 7.19 (1H, m), 5.27 (1H, m), 1.47 (3H, d, J = 6.47); δ_C 143.0, 131.6, 129.4, 128.4, 127.2, 126.4, 67.0, 23.5; $[\alpha]_D^{20}$ −58 (c 0.01 in $CHCl_3$).[38]

1-(*ortho*-Bromo-phenyl) ethanol (29). The transformation is proceeded with the whole cell transformation technique described by Kratzer *et al.*[19,37] δ_H (400 MHz, $CDCl_3$, $CHCl_3$) 7.57 (1H, m), 7.49 (1H, m), 7.32 (1H, m), 7.10 (1H, m), 5.23 (1H, m), 1.47 (3H, d, J = 6.40 Hz); δ_C 144.6, 132.7, 128.8, 127.8, 126.6, 121.7, 69.2, 23.6.

(*S*)-1-(*ortho*-Chlorophenyl) ethyl-(*S*)-α-methoxy-α-trifluoromethylphenylacetate (30)[24]. 1-(*ortho*-chloro)-phenyl ethanol (**28**, 0.01 g, 0.06 mmol) was esterified with enantiomerically pure (*R*)-Mosher acid chloride (0.02 g, 0.08 mmol, 99% ee) in the presence of triethylamine (0.08 g, 0.08 mmol), and DMAP (0.12 mg, 0.001 mmol) dissolved in CH_2Cl_2 resulting in the (*S*)-1-(*ortho*-chlorophenylethyl)-(*S*)-α-methoxy-α-trifluoromethylphenyl acetate (**30**). Selected δ_H (400 MHz, $CDCl_3$, $CHCl_3$) δ 7.39 (1H, m), 1.54 (3H, d, J = 6.61 Hz).

(*S*)-1-(*ortho*-Chlorophenyl) ethyl-(*R*)-α-methoxy-α-trifluoromethylphenylacetate (31)[24]. Compound **31** was prepared from 1-(*ortho*-chloro)-phenyl ethanol (0.01 g, 0.06 mmol) and enantiomerically pure (*S*)-Mosher acid chloride (0.02 g, 0.08 mmol, 99% ee), following the procedure for compound **30** and resulting in the (*S*)-1-(*ortho*-chlorophenylethyl)-(*R*)-α-methoxy-α-trifluoromethylphenyl acetate (**31**). Selected δ_H (400 MHz, $CDCl_3$, $CHCl_3$) 7.14 (1H, m), 1.59 (3H, d, J = 6.60 Hz).

(*S*)-1-(*ortho*-Bromophenyl) ethyl-(*S*)-α-methoxy-α-trifluoromethylphenylacetate (32)[24]. Compound **32** was prepared from 1-(*ortho*-bromo)-phenyl ethanol (**29**, 0.012 g, 0.06 mmol) and enantiomerically pure (*R*)-Mosher acid chloride (0.02 g, 0.08 mmol, 99% ee), following the procedure for compound **30** and resulting in the (*S*)-1-(*ortho*-bromophenylethyl)-(*S*)-α-methoxy-α-trifluoromethylphenyl acetate (**32**). Selected δ_H (400 MHz, $CDCl_3$, $CHCl_3$) 7.37 (1H, m), 1.54 (3H, d, J = 6.61 Hz).

(*S*)-1-(*ortho*-Bromophenyl) ethyl-(*R*)-α-methoxy-α-trifluoromethylphenylacetate (33)[24]. Compound **33** was prepared from 1-(*ortho*-bromo)-phenyl ethanol (**29**, 0.012 g, 0.06 mmol) and enantiomerically pure (*S*)-Mosher acid chloride (0.02 g, 0.08 mmol, 99% ee), following the procedure for compound **30** and resulting in the (*S*)-1-(*ortho*-bromophenylethyl)-(*R*)-α-methoxy-α-trifluoromethylphenyl acetate (**33**). Selected δ_H (400 MHz, $CDCl_3$, $CHCl_3$) 7.13 (1H, m), 1.59 (3H, d, J = 6.61 Hz).

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