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Ketone–Alcohol Hydrogen-Transfer Equilibria: Is the Biooxidation of Halohydrins Blocked?

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Dedicated to the memory of Professor José Manuel Concellón

Abstract: To ensure the quasi-irreversibility of the oxidation of alcohols coupled with the reduction of ketones in a hydrogen-transfer (HT) fashion, stoichiometric amounts of α-halo carbonyl compounds have been employed as hydrogen acceptors. The reason that these substrates lead to quasi-quantitative conversions has been tacitly attributed to both thermodynamic and kinetic effects. To provide a clear rationale for this behavior, we investigate herein the redox equilibrium of a selected series of ketones and 2-propanol by undertaking a study that combines experimental and theoretical approaches. First, the activity of the (*R*)-specific alcohol dehydrogenase from *Lactobacillus brevis* (LBADH) with these substrates was studied. The docking of acetophenone/(*R*)-1-phenyethanol and α-chloroacetophenone/(*S*)-2-chloro-1-phenylethanol in the active site of the enzyme confirms that there seems to be no structural reason for the lack of reactivity of halohydrins. This assumption is confirmed by the fact that the corresponding aluminum-catalyzed Meerwein–Ponndorf–Verley–Oppenauer (MPVO) reactions afford similar conversions to those obtained with LBADH, showing that the observed reactivity is independent of the catalyst employed. While the initial rates of the enzymatic reductions and the IR ν(C=O) values contradict the general belief that electron-withdrawing groups increase the electrophilicity of the carbonyl group, the calculated ΔG values of the isodesmic redox transformations of these series of ketones/alcohols with 2-propanol/acetone support the thermodynamic control of the reaction. As a result, a general method to predict the degree of conversion obtained in the HT-reduction process of a given ketone based on the IR absorption band of the carbonyl group is proposed, and a strategy to achieve the HT oxidation of halohydrins is also shown.

Keywords: computational chemistry · enzyme catalysis · halohydrins · hydrogen transfer · IR spectroscopy

Introduction

Physicists and chemists have unveiled the factors that control and determine the final concentrations of the species involved in a given transformation.[1] The ability to shift a chemical equilibrium is crucial in organic synthesis because it allows the maximization of the yield of the target product(s). Whenever the equilibrium constant does not lead to complete conversion, the tuning of the reaction conditions, such as temperature, pressure, and reagent stoichiometry, as well as medium engineering and product(s) removal from the reaction medium, can be used to drive the reaction toward the desired direction.[2]

The equilibrium constant in redox processes is mainly governed by the difference in oxidation–reduction potentials (ΔE^0) between reagents. In general, oxidizing or reducing species with much higher potentials than the starting materi-

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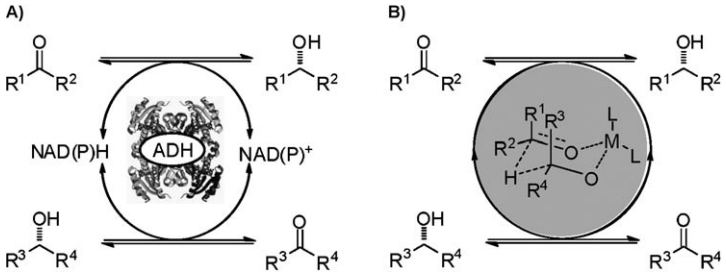
als are used,[3] thus ensuring the completeness of the reaction. However, in some cases this is not possible or not desirable due to incompatibilities with other functional groups in the molecule or because of hazardous conditions. The reduction of ketones coupled with the oxidation of alcohols in a hydrogen-transfer (HT) fashion is an example[4] of a redox process in which the ΔE^0 between the hydrogen donor and the acceptor is not usually large enough to achieve quantitative conversions. Enzymes, such as alcohol dehydrogenases (ADHs; Scheme 1A),[5] and metal complexes (Meerwein–



Scheme 1. Reduction of ketones coupled with the oxidation of alcohols catalyzed by: A) ADHs and B) metal complexes (MPVO).

Ponndorf–Verley reduction/Oppenauer oxidation (MPVO); Scheme 1B)[4] have successfully been employed as catalysts in this kind of transformation. Traditionally, the equilibrium is shifted toward the reduction/oxidation of the ketone/alcohol of interest by sacrificing a small and inexpensive alcohol or ketone, such as 2-propanol or acetone (employed as co-substrate), in a huge molar excess: at least 10 equivalents to afford conversions higher than 90%. However, to maximize the atom efficiency[6] of this type of process, it is highly desirable to find reaction conditions that allow quasi-irreversible HT protocols.[7]

We have observed that the biocatalyzed reduction of α-haloketones proceeds quasi-irreversibly when coupled with the oxidation of different secondary alcohols.[8] Thus, although ADHs nicely reduced α-chloroketones into the corresponding 1-chloro-2-alcohols, we did not detect the oxidation of halohydrins like 1-chloro-2-octanol and 2-chloro-1-phenylethanol in a screening of a library of more than 60 commercial ADHs with acetone as a hydrogen acceptor.[9] As a tentative explanation of this reactivity, it was put forward that the redox potential gap between the α-chloroketone/halohydrin pair with respect to the 2-propanol/acetone one is higher than for related non-halogenated pairs.[10] In this regard, it has been shown that halohydrins could additionally be stabilized by the presence of an intramolecular hydrogen bond between the alcohol moiety and the halogen atom,[11] which, on the other hand, could also block the ADH-catalyzed oxidation. Therefore, this behavior seems not to be a particular feature of ADH-catalyzed processes, but rather a general aspect of this type of substrate. Thus, the MPVO-HT (Scheme 1B) of α-halogenated carbonyl compounds also proceeds quasi-irreversibly. In particular, chloral (trichloroacetaldehyde),[12] α-chloroacetophenone,[13] α-bromoacetophenone,[13a] and 1,1,1-trifluoroacetone[14] were quantitatively reduced when employed as stoichiometric hydrogen acceptors in the presence of alcohols like 2-propanol with different Al or Zr complexes as catalysts.[15]

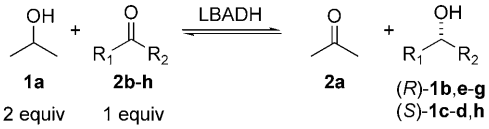
Ketones bearing electron-withdrawing groups (EWGs) are tacitly regarded as electron-deficient ketones and, thus can be more readily attacked by nucleophiles (e.g., hydride) than "non-activated" ketones, which would lead to higher reduction rates. On the other hand, it has also been argued that α-halo substituents increase the stability of the corresponding alcohols by destabilizing the partial positive charge formed during their oxidation,[16] which leads to an increase in the activation energy for the oxidation of halohydrins. These aspects are in favor of both a kinetic blockade of the oxidation of this kind of alcohol and the commonly accepted fact that the Oppenauer oxidation of alcohols is under thermodynamic control, that is, the final composition of the reaction mixture is mostly determined by the reduction potentials of the ketones/aldehydes involved.[4]

In summary, to explain the conversion in this type of process a variety of kinetic and thermodynamic hypotheses have been put forward. To provide an unambiguous rationale for the final conversion of these HT reductions, we have studied the redox equilibrium of a series of selected ketones. The main goals were the correlation of the conversions observed with the catalyst employed and the structure of the substrates, as well as the extent of the influence of kinetics and thermodynamics. To achieve this goal, we undertook a combined experimental and theoretical approach in which several ketone/alcohol complexes bound to the (*R*)-specific NADPH-dependent alcohol dehydrogenase of *Lactobacillus brevis* (LBADH)[17] were first structurally analyzed. Next, the corresponding aluminum-catalyzed MPVO-HT reactions were carried out to observe the influence of the catalyst in these processes. Finally, the enzymatic initial rates and the IR absorption bands of the carbonyl groups were measured, and ab initio calculations of the ketone/alcohol pairs were performed. As a result, a general method to predict the degree of conversion in the HT-reduction of a given ketone was proposed, and a strategy to achieve the HT oxidation of halohydrins was developed.

Results and Discussion

LBADH-catalyzed reduction of ketones under HT conditions: The ketones listed in Table 1 show a wide range of conversions at equilibrium when biocatalytically reduced in the presence of two equivalents of *i*PrOH (**1a**).[8,18] As can be seen, LBADH reduces acetophenone (**2b**; Table 1, entry 1) at approximately 50% conversion. If we use this ketone as a reference substrate, the introduction of electronegative groups at the small substituent leads to much higher conversions. Thus, for 2-chloroacetophenone (**2c**; Table 1, entry 2) the conversion is quantitative and for 2-azido-acetophenone (**2d**; Table 1, entry 3) it is close to com-

Table 1. Equilibrium of the enzymatic reduction of ketones **2b–h** catalyzed by LBADH.



Entry	Compound	R^1	R^2	Conversion [%]
1[a]	**2b**	Ph	Me	49
2[a]	**2c**	Ph	CH_2Cl	>99
3[a]	**2d**	Ph	CH_2N_3	96
4[a]	**2e**	*p*-O_2N-Ph	Me	84
5[a]	**2f**	*p*-MeO-Ph	Me	22
6	**2g**	$MeOCH_2$	Me	89
7[a]	**2h**	MeO_2CCH_2	CH_2Cl	>99

[a] See reference [8].

pletion. The same effect, but to a lesser extent, is observed when the substituents are present at the aromatic ring. Thus, for the electron-withdrawing nitro group (**2e**; Table 1, entry 4) the conversion increases considerably (84%) as compared with acetophenone. Conversely, when an electron-donating group is present at the aromatic ring (**2f**; Table 1, entry 5), the equilibrium is shifted toward the opposite direction.

The employment of aliphatic ketones leads to similar results. Thus, the presence of electronegative groups as substituents of the ketone function considerably increases the conversion at equilibrium, that is, the chloromethylene group (**2h**; Table 1, entry 7) is again more efficient than the methoxymethylene group (**2g**; Table 1, entry 6). From these results it can be concluded that, as compared with acetophenone, the oxidation of *i*PrOH is more efficient when ketones bearing EWGs are used. Moreover, this efficiency seems to be independent of the nature of the ketone (aromatic or aliphatic).

Docking of alcohols (*R*)-1b and (*S*)-1c and ketones 2b–c to LBADH: The typical substrates of LBADH are prochiral ketones with a methyl group as the small substituent and, conversely to the majority of ADHs,[19] a more variable (often aromatic) bulky moiety as the large one.[20] The preferred LBADH substrate for in vitro studies is acetophenone (Figure 1A), which is reduced to enantiopure (*R*)-1-phenylethanol (Figure 1B). The stereoselectivity results from an active site that offers two hydrophobic pockets: a small flexible one and a larger open area, which host the small and large substituents of the substrate, respectively. To analyze any possible contribution of substrate–enzyme interactions to the degrees of conversion listed in Table 1, we compared the binding modes obtained for the reference ketone (**2b**) and its corresponding alcohol ((*R*)-**1b**) to those of (*S*)-**1c**/**2c** (see the Experimental Section and Supporting Information for more details), which is the pair with the largest difference in conversion and is structurally most similar to the reference substrates.

For the case of ketone **2c** (Figure 1C), the active site of LBADH remained in the same conformation as in the complex with acetophenone (**2b**, Figure 1A), which indicates that the active site has enough space to accommodate the halogen atom. Thus, the C–Cl bond is oriented in an antiparallel fashion with respect to the plane of the phenolic ring of Tyr189, the amino acid that lies at the entrance of the small pocket of the active site of LBADH. In turn, the binding mode obtained for (*S*)-2-chloro-1-phenylethanol ((*S*)-**1c**; Figure 1D) was qualitatively identical to that of 2-chloroacetophenone (**2c**; Figure 1C). However, a slightly

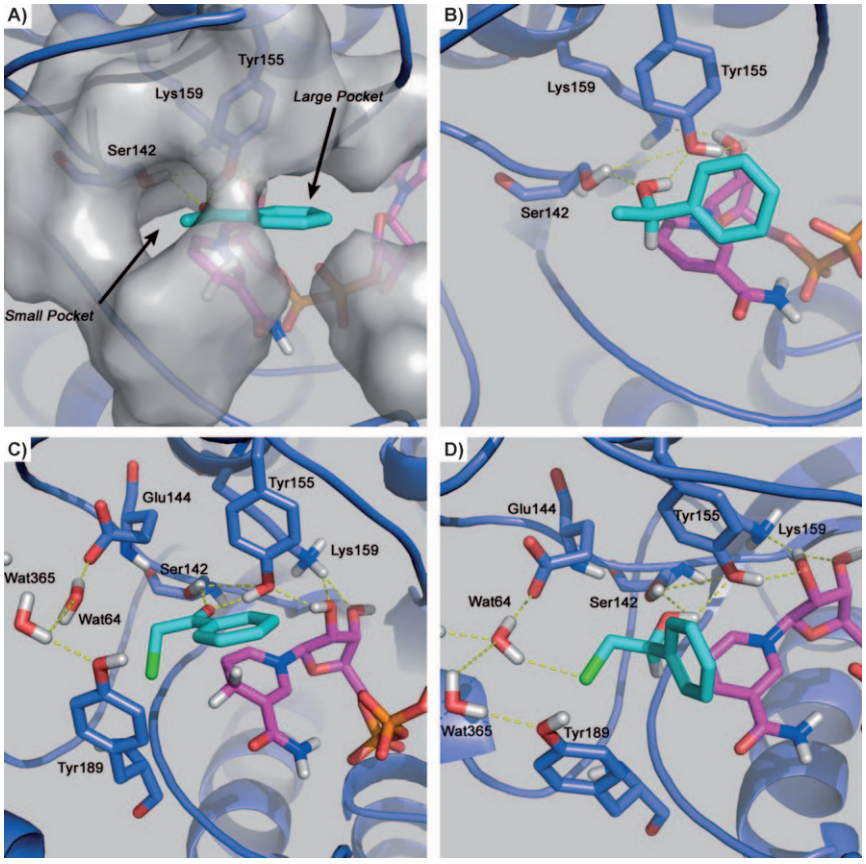
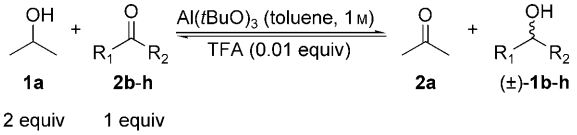


Figure 1. Modeled complexes of LBADH with: A) acetophenone (**2b**), B) (*R*)-1-phenylethanol ((*R*)-**1b**), C) 2-chloroacetophenone (**2c**), and D) (*S*)-2-chloro-1-phenylethanol ((*S*)-**1c**). Only polar and reactive hydrogen atoms are shown. The carbon atoms of the ligand, cofactor, and amino acids are shown in cyan, magenta, and blue, respectively. Key amino acids are labeled.

different interaction of the chlorine atom with the aryl ring of Tyr189 is observed (Figure 1D). The C–Cl bond is perpendicular to the plane of the phenolic ring of this amino acid, thus resembling the face-to-edge interaction mode of aryl rings. Apart from this, the binding modes for the **1c**/**2c** pair are identical to those obtained for the **1b**/**2b** one. These results confirm that there is no structural reason that would block the oxidation of (*S*)-**1c** but not the oxidation of alcohol (*R*)-**1b** and, at the same time, would allow the reduction of ketone **2c**. Consequently, if this ketone is reduced, its alcohol should be equally oxidized.

Metal-catalyzed reduction of ketones under HT conditions: The lack of enzyme–substrate interactions that could block the enzymatic oxidation of halohydrin (*S*)-**1c** suggests that the conversions listed in Table 1 are not due to the properties of the enzyme but to the nature of the substrates. To confirm this hypothesis, we tried a different catalyst. In particular, the MPVO-HT of ketones **2b–h** (Table 2) was carried out by using Al(*t*BuO)$_3$ activated in situ with trifluoroacetic acid as the catalyst,[21] and two equivalents of 2-propanol. Methyl 4-chloroacetoacetate (**2h**) was the only substrate that could not be reduced under these conditions because it decomposed. We have attributed this behavior to side aldol condensations, which are known to be potentiated by mixtures of aluminum alkoxides and protic acids, especially if easily enolizable ketones such as **2h** are used.[4b,c] Apart from this substrate, the results obtained were indeed comparable to those afforded by LBADH (Table 1); higher conversions were observed for those ketones with electron-withdrawing groups and the negligible differences between the results of the enzymatic and the metal-catalyzed reactions can be attributed to the different reaction conditions employed.

Table 2. Meerwein–Ponndorf–Verley reduction of ketones **2b–h** catalyzed by Al(*t*BuO)$_3$.



Entry	Compound	R^1	R^2	Conversion [%]
1	**2b**	Ph	Me	52
2	**2c**	Ph	CH_2Cl	95
3	**2d**	Ph	CH_2N_3	84
4	**2e**	*p*-O_2N-Ph	Me	78
5	**2f**	*p*-MeO-Ph	Me	12
6	**2g**	$MeOCH_2$	Me	79
7	**2h**	MeO_2CCH_2	CH_2Cl	n.a.[a]

[a] n.a. = not available.

Initial rates of the LBADH-catalyzed HT of ketones 2b–h: At this point, we were intrigued by the question of why ketones bearing EWGs oxidized *i*PrOH more efficiently. As already mentioned, this behavior has been associated with the higher electrophilicity of the carbonyl carbon in this type of compound,[16] which should be reflected in the kinetics of the reaction. Accordingly, we measured the initial velocities (V_0) of the LBADH-catalyzed reduction of ketones **2c–h** (see the Supporting Information for details) and calculated their ratio with respect to the parent substrate (**2b**; Figure 2). As a general trend, ketones reduced with a higher degree of conversion indeed showed higher initial rates. Thus, 2-chloroacetophenone (**2c**) or *p*-nitroacetophenone (**2e**) were reduced about five times faster than reference substrate **2b**. Also, if the ketone bears two EWGs (**2h**), the enhanced effect on V_0 is even more pronounced. On the contrary, ketone **2f**, which showed lower conversion, also presented a smaller initial rate. However, this correlation was not always true. Thus, the reduction of 2-azidoacetophenone (**2d**) was approximately 20-fold slower than that of the parent substrate (**2b**), but proceeded almost quantitatively. Although the lower V_0 of this ketone could be attributed to the larger size of the azido group as compared with the reference substrate, this is not the case for compound **2g**. This ketone should perfectly fit in the active site of the enzyme, and despite presenting an almost identical V_0 to that of the reference compound, it led to a much higher degree of conversion. Therefore, these data allowed us to draw the following conclusions: ketones with EWGs are, in general, more readily reduced than electron-rich ones, but no general correlation between initial velocities and the degree of conversion can be established, thus ruling out any dominant kinetic contribution to the conversions shown in Table 1. Consequently, the reactions should be, at least mainly, under thermodynamic control.

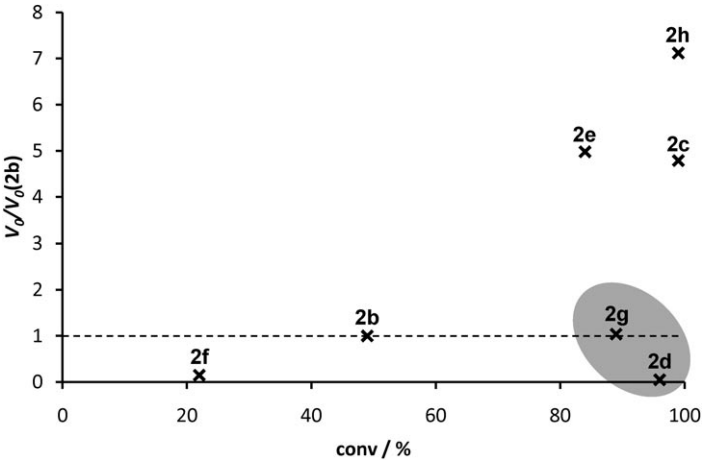


Figure 2. Ratio of the initial rates of the LBADH-catalyzed reduction of ketones **2c–h** with respect to that of ketone **2b**, plotted against the degree of conversion. Outliers are highlighted in grey.

IR carbonyl stretching bands: Neuvonen and Neuvonen have studied the kinetics of the nucleophilic acyl substitutions of different esters and found relationships between different spectroscopic parameters of the carbonyl group and

the rates observed for a series of structurally related compounds.[22] They observed that the IR absorption frequencies of the ester carbonyl group unexpectedly increased with the reaction rate. Therefore, esters bearing EWGs showed higher frequencies (higher double-bond character), which contradicted the tacit assumption of increased electrophilicity of the carbonyl carbon. Alternatively, the authors attributed this rate acceleration to the energetics of the ester substrate: EWGs would decrease the contribution of the dipolar ester resonance form and, therefore, would destabilize the ground-state resonance of the substrate. Inspired by this work and taking into account that a keto function is also constituted by a mixture of two resonance forms (Figure 3),

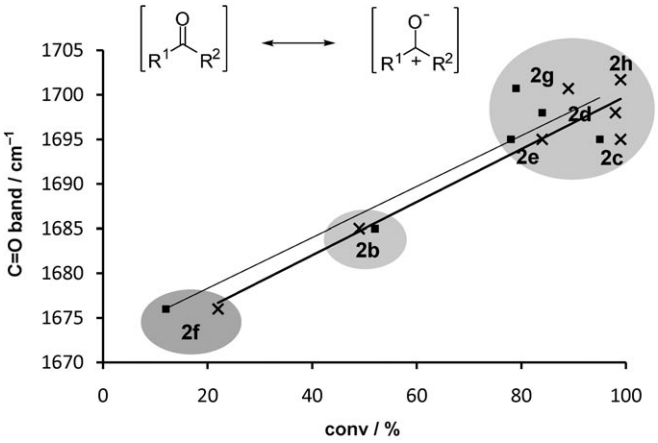
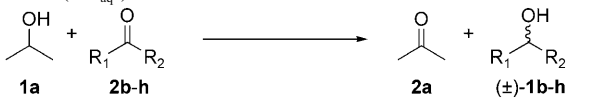


Figure 3. Ketone resonance structures and plot of IR carbonyl stretching bands of substrates **2b**–**h** against the degree of conversion for LBADH- (×; $r^2=0.9144$) and aluminum-catalyzed (■; $r^2=0.8588$) HT reactions.

we measured the frequencies of the IR carbonyl absorption bands of ketones **2b**–**h** (see the Supporting Information, Table S4) and plotted them against the degree of conversion at equilibrium shown in Tables 1 and 2 (Figure 3).[23] As can be seen, the presence of EWGs also leads to higher frequencies,[24] and a good correlation between them and the conversions was observed: higher values of the carbonyl stretching band (right-hand zone) correspond to ketones reduced with higher degrees of conversion and vice versa (left-hand zone). Similarly to the case of esters, this tendency can also be attributed to the fact that ketones with EWGs should be more unstable because the dipolar form is now disfavored, which destabilizes their ground-state resonance form. If the transition state is not affected by the substitution, this would make them more reactive but, more importantly, should affect the energy difference of the alcohol/ketone pairs and thus the equilibrium constant. This hypothesis goes in hand with a thermodynamic control of the HT reactions and shows that a preliminary IR spectrum can be safely used as a reliable method to qualitatively predict whether a ketone will be a good hydrogen acceptor under HT conditions, even for structurally different ketones.[23]

Ab initio calculations: The results obtained so far indicate that neither the catalyst nor the kinetics are responsible for the different conversions observed in the HT transformations, and suggest that this behavior has to be mainly attributed to the thermodynamics of the reaction (i.e., the ΔE^0 differences between the 2-propanol/acetone and the alcohol/ketone counterpart). To corroborate this, the Gibbs free energies for the isodesmic transformations shown in Table 3 have been calculated in both solution and gas phases.

Table 3. Total number of conformers (N_{conf}) of the computationally examined alcohol/ketone pairs and their Boltzmann-averaged free energies [kcal mol^{-1}] in both solution ($\Delta\bar{G}_{aq}$) and the gas phase ($\Delta\bar{G}_{gas}$) for the redox equilibrium with respect to 2-propanol/acetone. The corresponding changes in the free energy as evaluated over the most stable conformers in solution (ΔG^{min}_{aq}) are also indicated.



	R^1	R^2	N_{conf}	$\Delta\bar{G}_{aq}$	$\Delta\bar{G}_{gas}$	ΔG^{min}_{aq}
2a	Me	Me	1:1	–	–	–
2b	Ph	Me	2:1	−0.61	−0.20	−0.36
2c	Ph	CH_2Cl	6:2	−4.86	−4.98	−4.29
2d	Ph	CH_2N_3	12:2	−5.10	−4.86	−4.17
2e	*p*-O_2N-Ph	Me	3:1	−1.65	−1.16	−0.92
2f	*p*-MeO-Ph	Me	4:2	0.56	0.71	1.17
2g	$MeOCH_2$	Me	16:3	−3.55	−3.85	−3.07
2h	MeO_2CCH_2	CH_2Cl	57:9	−7.04	−7.15	−5.82

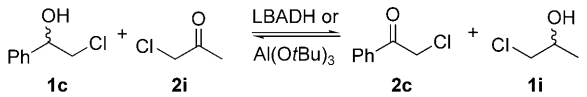
Analysis of the $\Delta\bar{G}_{aq}$ values showed that the quantum chemical results agreed quite well with the experimental data and consequently the same trends were observed. In other words, reactions in which either aromatic or aliphatic ketones contain electronegative groups in the α-position (**2c**, **2d**, **2g**, and **2h**) had lower $\Delta\bar{G}_{aq}$ values than ketones containing deactivating substituents in the aromatic ring (**2e**). Acetophenone (**2b**) showed a value close to 0 kcal mol^{-1}, and expectedly, the reduction of ketone **2f** was disfavored. Solvation effects were clearly not determining because the differences between $\Delta\bar{G}_{aq}$ and $\Delta\bar{G}_{gas}$ were systematically below 0.5 kcal mol^{-1} and the exact same trends were predicted in both environments.

The influence of the conformational flexibility of each reactant and product was also examined by comparing $\Delta\bar{G}_{aq}$ and ΔG^{min}_{aq}, in which the former was obtained as an average over the most stable conformers of each particular structure according to a Maxwell–Boltzmann distribution and the latter was calculated considering only the most stable conformer for each substrate. Even when ΔG^{min}_{aq} energies were slightly higher than $\Delta\bar{G}_{aq}$, the information obtained from both sets of energies was practically equivalent. From this assessment it may seem that performing a conformational analysis was irrelevant for the calculation of free energies of reaction. However, although restricting the calculation of the Gibbs free energies of reaction to the most stable conformer had no significant effect on $\Delta\bar{G}_{aq}$ from a quantitative point of view, in terms of finding true reasons for explaining

the shift in the redox equilibrium, skipping the conformational complexity of a certain system may result in misleading conclusions. To illustrate this point, we have focused on substrate **1c**. In principle, it would not be misconceived to think that oxidation of 2-chloro-1-phenylethanol to 2-chloroacetophenone can be hampered by the fact that an OH···Cl intramolecular hydrogen bond can be formed for the former.[11] Such an intramolecular hydrogen bond occurred in two of the six existing conformers in the conformational landscape, specifically in the most stable conformer and in the fourth local minima (according to Gibbs free energies in solution). However, it was not present in the four remaining conformers, which were very close in energy to the previous ones (all together the six conformers lay within an interval of free energy of 1 kcal mol^{-1}). In other words, the co-existence of the six conformers in solution is incompatible with the shift in the redox reaction towards 2-chloro-1-phenylethanol due to a formation of an intramolecular hydrogen bond in this alcohol. This is in agreement with the fact that for 2-azido-1-phenylethanol (in which such an intramolecular hydrogen bond is not possible), the degree of conversion and the $\Delta\bar{G}_{aq}$ value were very similar to those obtained for 2-chloroacetophenone. Therefore, we conclude that none of the above-mentioned factors (solvation effects and conformational flexibility) have a large impact in the Gibbs free energies of reaction, which means that the constant of the redox equilibrium mostly depends on the intrinsic stability of the alcohol/ketone pairs considered.

Taking into account the good correlation obtained between the IR absorption bands of the carbonyl group and the experimental conversions, we examined several theoretical descriptors of the reactive C=O bond to confirm the hypothesis on the resonance (de)stabilization of ketones upon substitution (Table S6 in the Supporting Information). Thus, either the bond distance of the C=O bond (Figure S2A in the Supporting Information) or its charge density at the corresponding bond-critical point (BCP; Figure S2B in the Supporting Information) correlate reasonably well with the average free energy in solution ($\Delta\bar{G}_{aq}$) for the reduction of **2b**–**g** by 2-propanol: the shorter the C=O bond distance (or the larger the accumulation of charge density at the BCP), the more favorable the reduction of the keto group. We also examined the variation in the local energy density at the C=O BCPs ($H(r_c)$) because this quantity is a stable property with regard to the ab initio level of theory (Figure S2C in the Supporting Information). The value of $H(r_c)$ is negative for all the C=O bonds, which indicates a predominant covalent nature. $H(r_c)$ showed a good correlation with the relative free energies, thus showing that the more ionic or polarized the C=O bond is (i.e., the lower $H(r_c)$ in absolute value), the less likely it is to be reduced. This observation reinforces the hypothesis of the destabilization of the ketones based on the experimental IR absorption bands (Figure 3).

Overcoming HT limitations by combining halohydrins with α-haloketones: At this point, we were interested in oxidizing thermodynamically impeded alcohols (i.e., halohydrins) under standard HT conditions. Because the combination of an activated ketone (e.g., 2-chloroacetophenone) with a nonactivated alcohol (e.g., 2-propanol) led to complete oxidation of the alcohol due to the high ΔE^0 difference between both pairs, employing a pair of substrates both containing activated ketones should lead to an equilibrium far away from quasi-quantitative conversions. To prove this, we performed the reduction of ketone **2c** by using 1-chloro-2-propanol (**1i**) as a hydrogen acceptor,[25] and the opposite transformation (alcohol **1c** with chloroacetone **2i**) by using both LBADH and Al(*t*BuO)$_3$ (Scheme 2).



Scheme 2. Reduction of α-haloketones under HT conditions by using halohydrins as hydrogen acceptors.

By using LBADH and different ratios of the hydrogen acceptor and donor (from 1:2 to 4:1), we observed enzymatic conversions in the range of 3–12% in both directions (starting materials **1c**+**2i** or **2c**+**1i**) after 24 h. Leaving these reactions for longer (7 d) and adding fresh biocatalyst and cofactor every day[26] led to higher conversions (>30%). When the aluminum catalyst was employed with two equivalents of the halohydrin and one equivalent of the α-chloroketone, conversions of around 20% were achieved after 24 h. Therefore, these results clearly demonstrate that the oxidation of halohydrins is not blocked and that they can be oxidized under HT conditions by selecting an acceptor with a suitable redox potential. On the other hand, the different concentrations of products measured for the forward and reverse reactions clearly show that thermodynamic equilibrium was not reached after 24 h. Therefore, the oxidation of halohydrins is also under some kinetic control, which is further supported by the fact that the higher temperature of the metal-catalyzed HT reaction as compared with the enzymatic one allowed higher conversions.

Conclusion

We have studied the redox equilibrium of a series of ketones to understand the extent of their HT reduction. As a general feature, higher conversions are obtained with substrates bearing EWGs, regardless of the catalyst employed. This suggests that the observed conversions are due to the intrinsic nature of the substrates. In the same sense, the docking of α-haloketone **2c** and its corresponding alcohol (**1c**) in the active site of LBADH suggests that the quasi-quantitative conversion attained is not likely to be due to any enzyme–substrate interaction that could block the oxidation of the halohydrin.

The higher reactivity of ketones bearing EWGs has traditionally been attributed to a higher electrophilicity of the carbonyl carbon because of the inductive effect of these substituents. However, the initial rates of reaction do not always correlate with the conversions and the normalized IR frequencies of the carbonyl groups not only suggest the opposite but show a good correlation with the experimental conversions, thus affording a straightforward and easy predictive method for determining the position of the equilibrium that will be attained for a given ketone. Therefore, it seems that the extent of the reduction of these ketones is not under kinetic control and, thus, it should be under thermodynamic control. This is in agreement with the good correlation observed for the conversions and the calculated Gibbs free energy ketone/alcohol differences against 2-propanol/acetone. This behavior has been explained on the basis that the presence of EWGs would destabilize the ground resonance state of the ketones by decreasing the contribution of the dipolar resonant form. This hypothesis explains the general higher reactivity of these substrates and suggests that the equilibrium constants of these reactions are mainly driven by the different energies of the ketones. Moreover, the higher contribution of the resonant double-bond form leads to higher frequencies in the absorption band, which was further confirmed by the calculated values of different suitable descriptors of the C=O bond.

Once the lack of reactivity of halohydrins was clarified, it became evident that the oxidation of halohydrins and related alcohols should be feasible by using another ketone bearing an EWG as a hydrogen acceptor. Such reactions did indeed proceed to a relatively high extent but they did not reach equilibrium in the time frames used. Therefore, in the case of alcohols bearing EWGs, the oxidation is slowed down as compared with other hydrogen donors, and longer reaction times or higher temperatures are required to reach the equilibrium concentrations.

Experimental Section

General experimental methods: Alcohol dehydrogenases and ketones **1a–i** were purchased from commercial sources. Racemic alcohols (±)-**2a–i** were either synthesized by conventional reduction of the corresponding ketones ($NaBH_4$, MeOH, RT) or purchased from commercial sources. All other reagents, including catalysts and solvents, were of the highest quality available. Flash chromatography was performed by using silica gel 60 (230–400 mesh). ^1H and ^{13}C NMR and DEPT spectra were obtained by using a DPX-300 spectrometer (^1H: 300.13 MHz, ^{13}C: 75.5 MHz) for routine experiments. Gas chromatography (GC) analyses were performed by using a standard gas chromatograph with nitrogen as the carrier. UV and IR spectra were performed by using standard UV/Vis and IR spectrophotometers, respectively.

Enzymatic HT protocol by using LBADH: Generally, LBADH (3 U) was added to Tris-HCl buffer (600 μL, 50 mM, pH 7.5; 1 mM $MgCl_2$, 1 mM NADPH) in an Eppendorf vial (1.5 mL). Then, the corresponding ketone (25 mM) and 2-propanol (50 mM, 1.8 μL, 2 equiv) were added to the mixture. Reactions were shaken at 30 °C and 150 rpm for 24 h. The reactions were stopped by extraction with ethyl acetate (2×0.6 mL). The organic layer was separated by centrifugation (1.5 min, 13000 rpm) and dried over Na_2SO_4. Conversions were determined by GC analysis.

HT protocol by using Al(*t*BuO)$_3$: In a sealed Schlenk tube, Al(*t*BuO)$_3$ (100 μL of a 0.1 M solution in dry toluene), the corresponding ketone (0.5 mmol), 2-propanol (1 mmol, 77 μL), and trifluoroacetic acid (100 μL of a 0.1 M solution in dry toluene) were added under N_2 to dry CH_2Cl_2 (4 mL). The sealed tube was then stirred at 60 °C for 24 h. To determine conversions, aliquots (500 μL) were withdrawn, the catalyst was quenched with one drop of water, and Na_2SO_4 was added. Conversions were determined by GC analysis.

General protein–ligand computational methods: All molecular mechanics computations were performed by using the molecular modeling package Molecular Operating Environment (MOE) 2007.09 (Chemical Computing Group).[27] In all cases the Amber99 force field,[28] the corresponding dictionary charges as implemented in MOE, and the Generalized Born solvation model[29] with relative dielectric constant values of 2 and 80 for the protein matrix[30] and the solvent, respectively, were selected. A nonbonded cut-off of 8 Å with a smoothing function of between 8 and 10 Å were used. In all molecular dynamics simulations, the NVT ensemble and the Nosé–Poincaré–Anderson equations were selected. The initial and simulation temperatures were set to 0 and 300 K, respectively, a temperature relaxation time of 10 fs was selected, and the length of the heating and simulation periods was 1 ps. No constraints were imposed on any bond and a step size of 1 fs was used. The convergence criterion of the energy minimizations (EM) was set to a gradient value of 0.01 or 0.001 kcal mol^{-1} if only parts of the protein or the whole enzyme–substrate–cofactor complex, respectively, were minimized. The rest of parameters were set to their default values. High-quality pictures of representative structures were generated with PyMOL 0.99.[31]

Ab initio calculations: Gibbs free energies of reactants and products corresponding to the reaction described in Table 1 were calculated according to the following Equation (1):

$$\Delta\bar{G}_{aq} = \Delta\bar{G}^{HF}_{aq} + \bar{E}_{MP2/CBS} + \Delta\bar{G}^{COSMOS}_{solv} - TS_{conf} \quad (1)$$

in which $\Delta\bar{G}^{HF}_{aq}$ represents the thermal contributions (translational, rotational and vibrational) to Gibbs free energy, $\bar{E}_{MP2/CBS}$ is the MP2 electronic energy evaluated at the complete basis set (CBS) limit, $\Delta\bar{G}^{COSMOS}_{solv}$ is the solvation free energy and S_{conf} is the conformational entropy. In this expression, the energy components (except S_{conf}) are averaged over all the existing conformers of each particular structure according to a Maxwell–Boltzmann distribution.

For the isodesmic transformations, energies were averaged over the most stable conformers (N_{conf}) of each particular structure according to a Maxwell–Boltzmann distribution. For the sake of completeness, relative free energies of reaction were calculated in solution ($\Delta\bar{G}_{aq}$) and in the gas phase ($\Delta\bar{G}_{gas}$). Relative free energies (ΔG^{min}_{aq}) evaluated over the most stable conformers in solution are also given. Further details of the ab initio calculations are provided in the Supporting Information.

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