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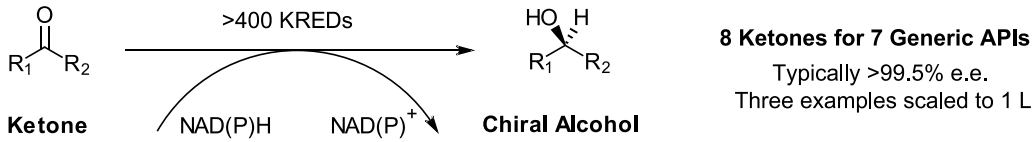

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ABSTRACT: A range of generic active pharmaceutical ingredients were examined for potential chiral alcohol motifs and derivatives within their structures that could be employed as key synthetic intermediates. For seven generic API's, eight precursor ketones were acquired, and then subjected to reduction by >400 commercially available ketone reductases from different suppliers. Positive screening results were achieved for five ketones screened, with multiple ketone reductases available for each successful ketone. Selectivity was typically >99.5% e.e. in most cases, including for the opposite enantiomer. The three best examples were then optimized and quickly scaled up to 1 L scale in high conversion and isolated yield whilst retaining selectivity of >99.5% e.e. for the desired chiral alcohol enantiomer. This work illustrates that where a wide range of enzymes are available, productive enzymes to give either alcohol enantiomer can be readily identified for many

ketones, and rapidly scaled up to produce chiral alcohols. This approach is particularly applicable to generating chiral API intermediates.

KEYWORDS: active pharmaceutical ingredient (API); bio-catalysis; chiral alcohol; ketone; ketone reductase (KRED)

INTRODUCTION

Bio-catalysis has been recognised for some time as a viable technology in the scale-up and manufacture of valuable chiral intermediates in the chemical industry,[1-3] especially pharmaceuticals.[4-5] The benefits of their use have been well-rehearsed, notably their specificity and selectivity under moderate reaction conditions in typically benign solvents. They are not without their drawbacks however, which can include lack of stability under productive reaction conditions, product inhibition, low aqueous solubility for many substrates of interest, and physical challenges such as causing emulsification during isolation. Even so, bio-catalysts can often be modified by a variety of highly successful techniques derived from modern molecular biology, such as directed evolution, random mutagenesis and protein engineering, which can overcome these potential limitations.[6] This provides almost infinite scope for improvement and optimisation of such processes. Consequently, it is thought that for certain transformations, bio-catalysis is likely to provide the most sustainable long-term manufacturing processes for overall efficiency, environmental burden, energy usage etc., especially for higher value products such as active pharmaceutical ingredients (APIs).[3,7,8]

Ketone reductases (KREDs) (EC 1.1.1) are part of the class of aldo-keto reductase enzymes that catalyse reduction-oxidation (redox) reactions between ketones and alcohols (Figure 1). Several examples of KRED reductions of pro-chiral ketones to chiral alcohols have been reported, including a number on industrial scale.[9-11] The synthesis of Montelukast is a well-known example of the highly successful application of a KRED to replace an existing (and expensive) chemical reducing agent (DIP-Cl) in a current manufacturing process,[12] but other potential examples have also been developed (eg. Sulopenem,[13] Atorvastatin,[14] Rosuvastatin,[15] Ticagrelor[16]). Our interest in KREDs was in their potential use as stereo-selective reductants in the synthesis of intermediates for APIs, whether patented or generic. Chiral alcohols can be derived as the obvious targets directly from ketone reductions,[13] but many other chiral intermediates are also readily accessible from chiral alcohols after further functionalisation.

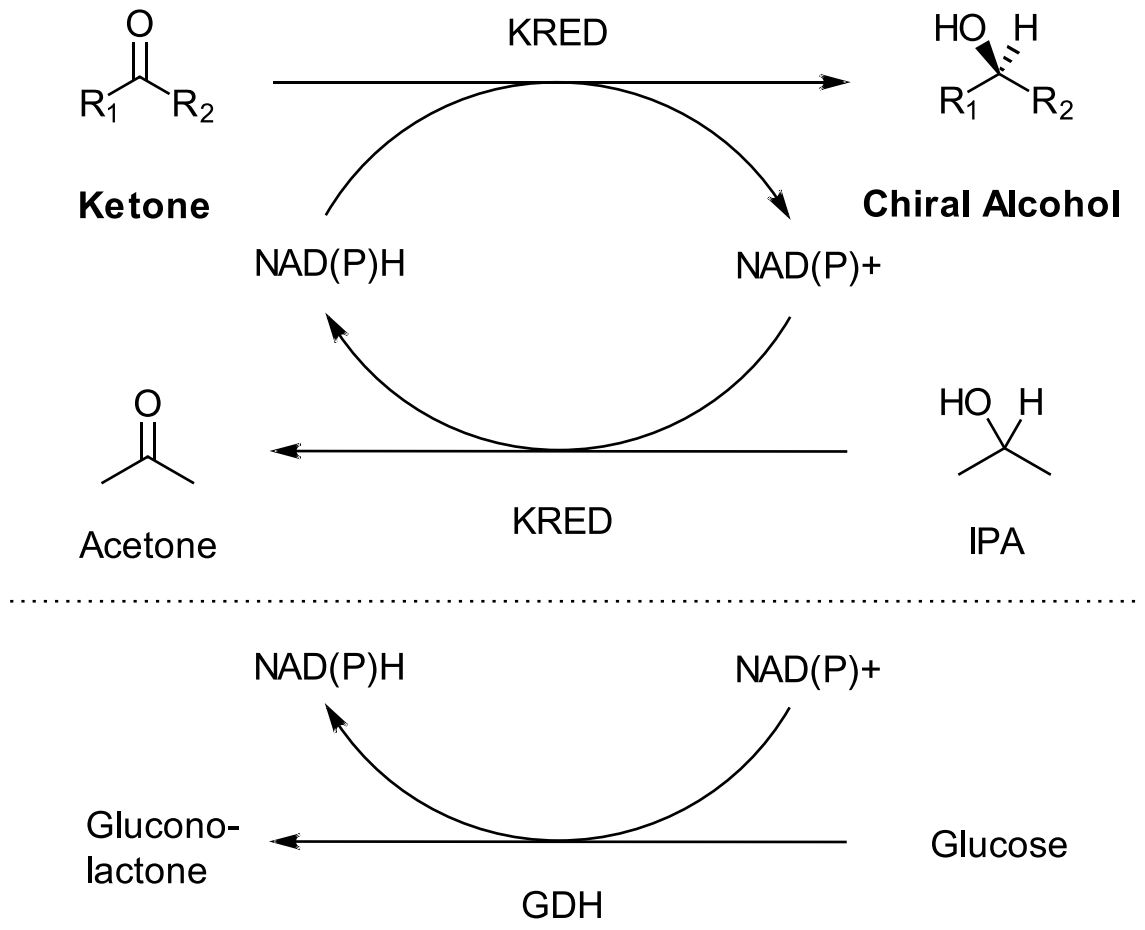


Figure 1. Reaction scheme for a generic KRED reduction of ketone to chiral alcohol with two typical co-factor recycling reactions shown (IPA/KRED and glucose/GDH).

This work aimed to investigate the use of KREDs in the reduction of pro-chiral ketones to generate chiral alcohol intermediates (and by extension their derivatives) for a wide variety of APIs. We were especially interested in generic APIs, and patented APIs which were approaching patent expiry, for which bio-catalytic methods might not have been considered in the original manufacturing routes. Such new synthetic routes using bio-catalysts might well provide more efficient processes, generate potential new intellectual property for commercial advantage, and ultimately be more sustainable.

The APIs specifically chosen were as follows (generic rather than branded trade names have been used in all cases): Aprepitant, an NK1 Substance P antagonist for use as an anti-emetic adjunct with chemotherapy treatments;[17] Atazanavir, a HIV protease inhibitor for use as an anti-retroviral agent;[18] Crizotinib, an ALK and ROS1 inhibitor for use as an anti-cancer agent, predominantly against non-small cell lung cancer (NSCLC);[19] Indacaterol, an ultra-long-acting β2–adrenoreceptor agonist for the treatment of chronic obstructive pulmonary disease (COPD);[20] Montelukast, an LTD_4 leukotriene receptor antagonist for the treatment of asthma;[21] Ticagrelor, a platelet aggregation inhibitor of the P2Y12 receptor for the prevention of thrombosis in patients with acute coronary syndrome;[22] and Vilanterol, another ultra-long-acting β2-adrenoreceptor agonist, also for the treatment of COPD.[23] These details, plus the innovator pharma company, dates and reference for the first patent claim, reference for an early medicinal or process chemistry synthesis route, dates of first major regulatory approval (FDA or EMA) and first major market patent expiry, are listed in Table 1.

Table 1. Background details of APIs investigated in this project

Ketone	API	Therapy Area	Mechanism	Inventor	Patent Date	Patent Reference	Synthesis Reference	Regulatory Approval	Major Patent Expiry
1	Aprepitant	Anti-emetic	NK1 Substance P antagonist	Merck	1995	17a	17b,c	2003	2015
2	Atazanavir	Anti-retroviral	HIV protease inhibitor	BMS	1997	18a	18b	2003	2018
3	Crizotinib	Anti-cancer (NSCLC)	ALK and ROS1 inhibitor	Pfizer	2007	19a	19b,c	2011	2027
4	Indacaterol	COPD	β_2-Adrenoreceptor agonist	Novartis	2002	20a	20b	2009	2020
5	Montelukast	Asthma	LTD_4 receptor antagonist	Merck	1996	21a	21b	1998	2012
6	Montelukast	Asthma	LTD_4 receptor antagonist	Merck	1996	21a	21b	1998	2012
7	Ticagrelor	Thrombosis	$P2Y_{12}$ receptor antagonist	AstraZeneca	2000	22a	22b	2011	2019
8	Vilanterol	COPD	β_2-Adrenoreceptor agonist	GSK	2003	23a	23b	2013	2023

For this programme, we wanted to demonstrate the broad versatility of KREDs to access a range of chiral alcohols. We judged the eight ketones selected to be sufficient to test this general principle based on their degree of structural diversity. It should be stressed that the primary aim of this project was to demonstrate the generality of the application of KREDs in the potential

manufacture of generic APIs, not to fully optimise a method for any specific target. The ketones chosen (**1-8**) are shown in Figure 2.

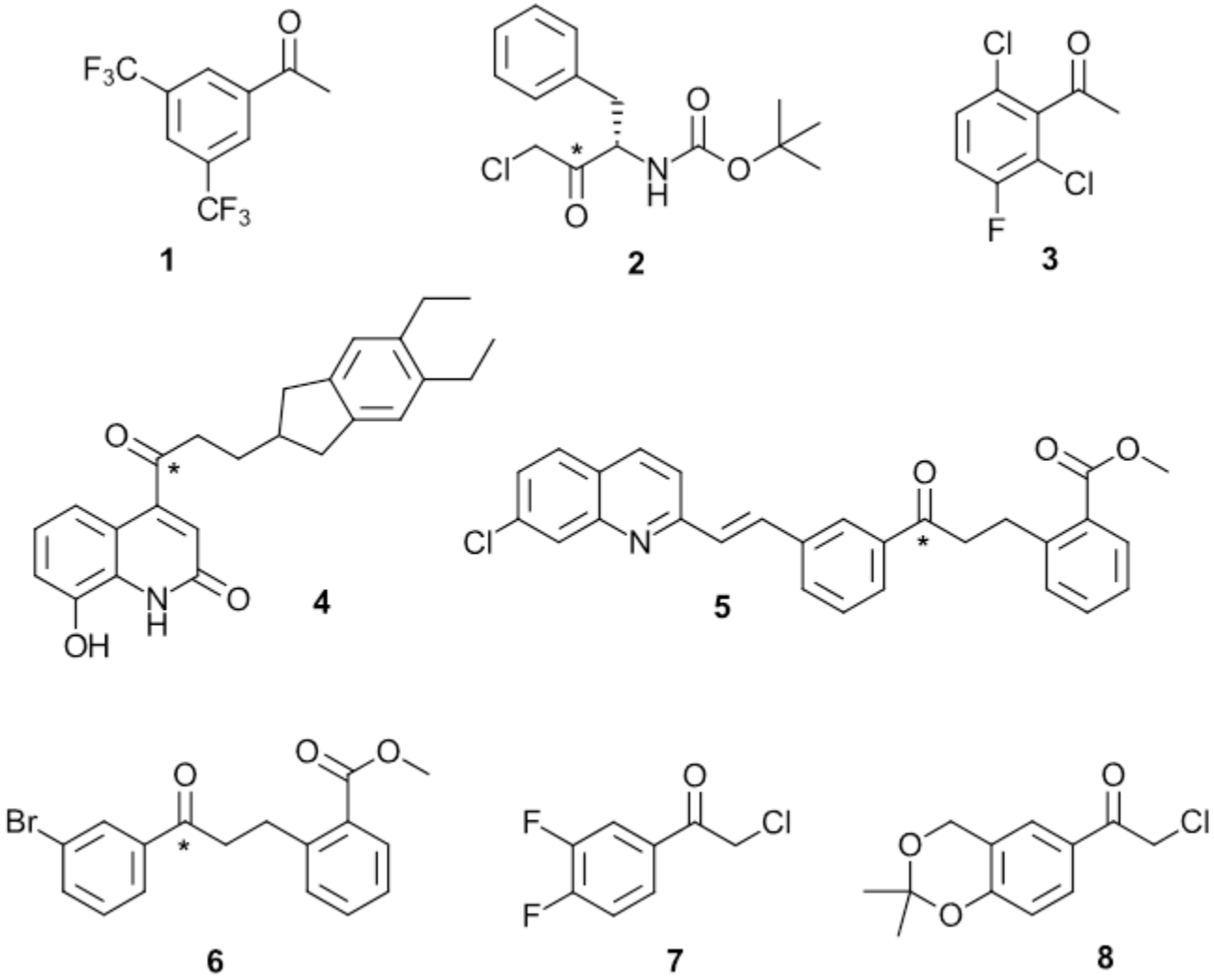


Figure 2. Structures of ketones submitted to KRED reduction in this project (asterisk indicates expected carbonyl group to be reduced where more than one present).

RESULTS AND DISCUSSION

Initial enzyme screening studies

Initial qualitative screening was based on 96-well plate format and visualised using a standard colorimetric NAD(P)H sensitive formazan assay; a positive response was indicated by the development of a red colour from the coupled bio-catalytic reaction with formazan.[24,25] Plates were pre-loaded with KREDs, buffer, co-solvent and co-factor, to which the diluted test substrate was added. Where chiral alcohols were available, the reverse reaction (i.e. oxidation) was screened first, since a positive response in such cases proved that the enzyme was competent to produce the desired alcohol enantiomer. Where chiral alcohols were not available, screening of the racemic alcohols proved that at least one enantiomer could be obtained by KRED reduction. Where alcohols were not commercially available, chiral and racemic samples were prepared by chemical reduction methods as noted (see Experimental Section and Supplementary Information). This also provided marker samples necessary to support analytical method development. This initial screening assay yielded qualitative results in which the response (intensity of the red colour) was indicative of some degree of reaction conversion. In the few cases for which alcohols could not easily be prepared, initial screening had to be conducted on the available ketones in test-tubes (3 mL scale), as described further below.

All the positive responses from both sets of screening reactions (reduction or oxidation) were then screened for the desired (reduction) reaction on a 3 mL test-tube scale to confirm results. This quantitative screening round eradicated any false positive results, and also enabled weak responses against particular substrate/KRED combinations to be evaluated for further study or eliminated from further screening rounds. Over the course of the project, each ketone was initially screened against over 400 KREDs from all commercial sources.[26-28]

Confirmed positive results from the initial qualitative screening rounds were obtained for ketones **1**, **2**, and **6-8** from a range of KREDs, with no positive results for ketones, **3**, **4** or **5**, as

summarised in Table 2. Ticagrelor ketone (**7**) was unusual in having a high number of positive responses (36) for both enantiomers in very high e.e., although most published results favour (S)-**7**. A successful KRED reduction has also been achieved on a slightly larger ketone relevant to the synthesis of Ticagrelor.[29] Positive results were also obtained for ketones **1**, **2**, **6**, and **8** in high e.e. for the desired enantiomer, with one or two examples for the opposite enantiomer in each case. For example, the opposite enantiomer to that required for Aprepitant ((R)-**1**) is a key structural motif in at least two other related pharmaceuticals, Casopitant[30] and Vestipitant,[31] the latter of which is still in development (Figure 3). Although the same final (R)-stereochemistry is required to that of Aprepitant, substitution by the nitrogen in Casopitant and Vestipitant requires the opposite (S)-enantiomer as the starting material to achieve this. In summary, positive results were obtained for five out of eight ketones screened on 3 mL reaction scale (Table 2).

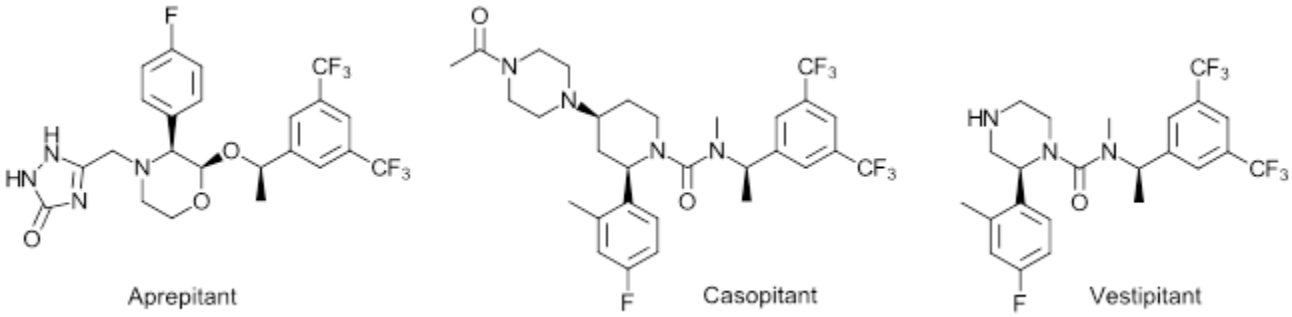


Figure 3. Structures of Aprepitant, Casopitant and Vestipitant.

Table 2. Screening results by KRED source with enantiomer distribution and highest e.e. for each API

	Desired Enantiomer		Other Enantiomer		Overall
Series	*Total*	*Highest e.e*	*Total*	*Highest e.e*	**Total**
1	24	99.5%	2	99%	26
2	9	99.5%	1	50%	10
3	0	-	0	-	0
4	0	-	0	-	0
5	0	-	0	-	0
6	5	95.4%	1	71%	6
7	20	99.5%	14	99%	36
8	6	99.8%	2	99.4%	8

Table 2 illustrates the following key points. Firstly, it was possible to rapidly obtain multiple positive results in five out of eight cases using KREDs on a range of pharmaceutically representative ketones. Additionally, the opposite enantiomer was also obtained in five cases and with high e.e. in three cases, albeit by fewer enzymes. This again shows the power and versatility of bio-catalysis in that positive results could be obtained in very high e.e.s for both enantiomers.

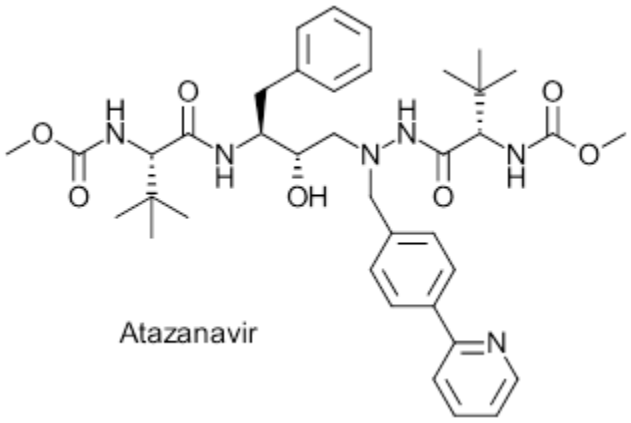
In the cases where no positive results were obtained, it is suggested that the ketones **4** and **5** were either too bulky to fit in the enzyme active site, or their solubility in the reaction medium was too low, since no conversion was detected. For ketone **3**, a benzophenone very similar in structure to the successfully reduced ketones **1** and **7**, it is suggested that the two *ortho*-chlorine substituents effectively prevented reaction at the ketone with the KREDs tested, since this was the differentiating factor between **3** and the other two (**1** and **7**). Two *ortho* substituents are known to be problematic, although it must be acknowledged that Codexis have achieved KRED reduction of **3** in excellent enantioselectivity (>99% e.e.) and high yield (94%), albeit with a mutant KRED derived from an alcohol dehydrogenase, and after extensive optimisation.[32] This does however illustrate well the previously stated advantage of bio-catalysis that excellent solutions to chemical transformations can often be improved despite initial poor or absence of reactivity.

Lastly, note that during the screening phase (up to 3 mL scale), suitable control reactions were included in all reaction screens to check for potentially reduced e.e. arising from achiral background reduction from some other component or contaminant, and for inhibition of the enzyme. Specifically, glucose dehydrogenase (GDH) (EC 1.1.99.10) (the most likely source of background reduction in the reaction), was shown to have no activity against any of the substrate targets. KRED-free control reactions were conducted in duplicate on 3 mL scale with all other components present to determine the presence of any background reduction, and also with each individual component missing (organic solvent, substrate, or buffer).

Results from all these control studies concluded that as expected there was no background reduction within the limit of detection, and that e.e.s determined by analytical LC methods were therefore reliable. It was also confirmed that sampling methods did not lead to contamination between reactions or variability in the results.

Reaction optimisation studies

For further development, optimisation and scale-up studies, we chose to focus on the ketone examples of Aprepitant (**1**), Atazanavir (**2**) and Ticagrelor (**7**) (Figures 3 and 4), since these gave good conversions in high e.e.s and both enantiomers were produced (Table 3).



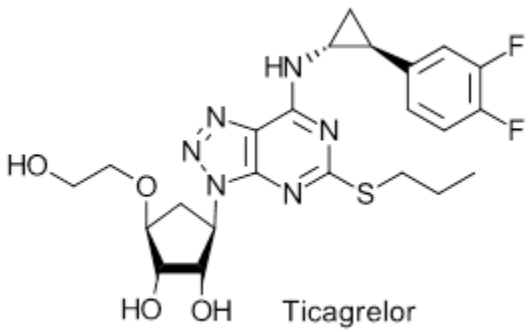


Figure 4. Structures of Atazanavir and Ticagrelor.

Despite having found multiple active KREDs for most of the ketones, a single KRED showed the greatest activity whilst retaining high selectivity for all three ketones chosen for further investigation. This greatly simplified further optimisation of the reaction conditions and aided finding a largely common set of conditions.

Table 3. Conversion, stereochemical product, enantiomeric excess, and LC methods used for selected ketones 1, 2 and 7 after initial screening studies

Series	Conversion at 24 hrs	Achiral LC Method	Stereochemical Product	e.e. (%)	Chiral LC Method	Compared to:
1	>99%	A	R	>99.5%	C	Purchased marker
2	>88%	A	2S, 3S (a)	>99.5%	A (b)	Supplied marker
7	>99%	B	S	>99.5%	D	Synthesis

(a) 2-position already set as (S), 3-position reduced by KRED
(b) d.e. determined from achiral LC method since diastereomers result in this case

Following these initial screening studies, the key factors to investigate further were the co-factor recycling system and solvent composition, along with continuous parameters such as pH and temperature. Substrate concentration and KRED loading were optimised during the scale-up studies.

The first key factor to finalise was the co-factor recycling system, which was also linked to the solvent composition. For a stoichiometric oxidation or reduction reaction it is only the recycling of the co-factor that is essentially catalytic. Recycling of the expensive co-factor, NAD(P)H as used here, is also essential on cost grounds. Iso-propanol (IPA) can be used as the co-substrate in many cases, with KREDs and alcohol dehydrogenase producing acetone as the co-product from the reverse reaction (cf. Figure 1). Acetone is easily lost by evaporation from the

reaction medium, which helps to drive the coupled forward reaction, promoting the reduction of the target substrates (see screening procedure P).

Another commonly used co-factor recycling system is the NAD(P)H / glucose dehydrogenase (GDH) (EC 1.1.99.10) / glucose combination, which was also trialled in this study (see screening procedure G). However, this combination requires a second enzyme (GDH) and pH adjustment to compensate for production of the acidic co-product (gluconolactone/gluconic acid). A direct comparison of the IPA/KRED and glucose/GDH co-factor recycling systems for these substrates concluded that the glucose/GDH system was very marginally faster; however, the simpler IPA/KRED system was retained at this stage since it also greatly aided the solvent selection.

The identification of a single KRED as the preferred enzyme for all three substrates greatly simplified further optimisation of the reaction conditions and aided finding a largely common set of conditions. However, none of the ketones or alcohols were water soluble so an organic solvent was required to help solubilise them. The choice of solvent depended strongly on the physical properties of the substrates, and the tolerance of the enzyme towards the organic component. In order to overcome solubility issues, particularly those posed by ketone **2**, a range of solvents were examined. The solvents trialled were: acetone, DMSO, hexane, iso-propanol (IPA), methyl *tert*-butyl ether (MTBE), THF and toluene; and in ratios of 1:9, 3:7 and 1:1 with water. As expected, more lipophilic solvents resulted in phase separation with water. Water/IPA mixtures were found to be best for ketones **1** and **7**. IPA was an ideal co-solvent since it was required as the co-substrate in screening procedure P to enable regeneration of the NADPH co-factor. Investigations into fine-tuning the solvent systems then largely focused on reaction tolerance towards increasing IPA concentration (to aid substrate and product solubility). Reactions of **1** and **7** showed that high

conversion to the desired product (>99%) could be achieved within 4 h until the concentration of IPA had reached 20%; above this level of IPA, enzyme activity began to decline markedly, as Figure 5 shows for ketone **7**. A 4:1 ratio of water:IPA was also found to be the most that could be tolerated for reduction of ketone **2** without reducing conversion below the maximum observed of ~85-90%.

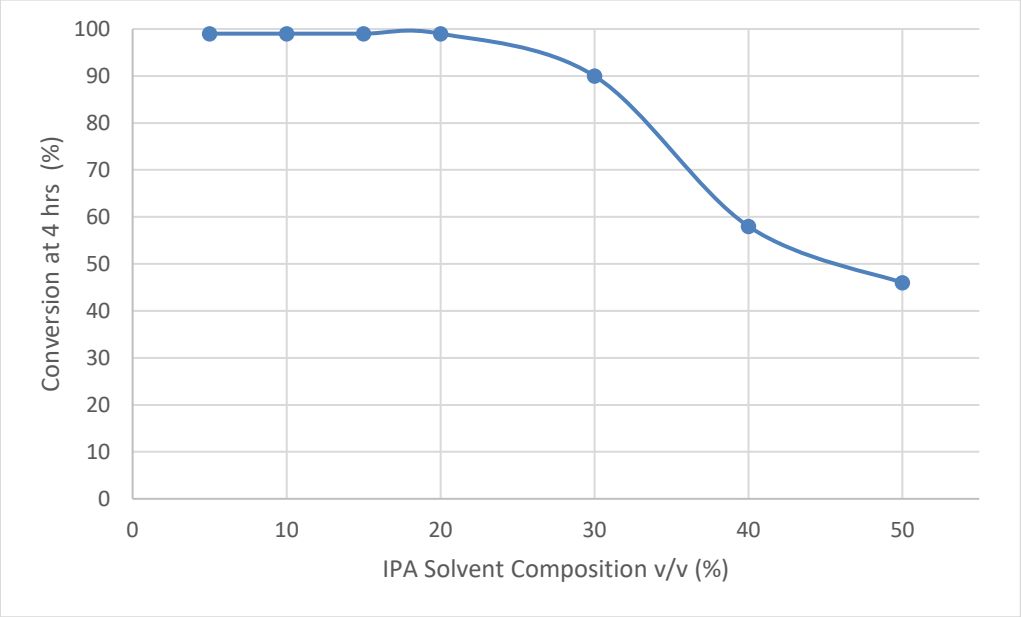


Figure 5. Conversion at 4 hour time points determined on ketone **7** showing reducing tolerance to increasing IPA concentration in water.

The important factor of pH was readily screened in the range 5.0 to 8.5 against ketone **1** under standard conditions. Conversion was measured at an intermediate 2 hour time point to allow for some differentiation in results. Figure 6 clearly shows that the KRED is not sensitive to pH changes within the range pH 6.0 and pH 8.0. Consequently, all subsequent reactions in the study were performed at pH 7.0, which allowed a tolerance of one pH log unit on either side of this

value. The stereoselectivity of the enzyme between pHs 5.0 and 8.5 was constant and all reactions gave an enantiomeric excess of >99.5%.

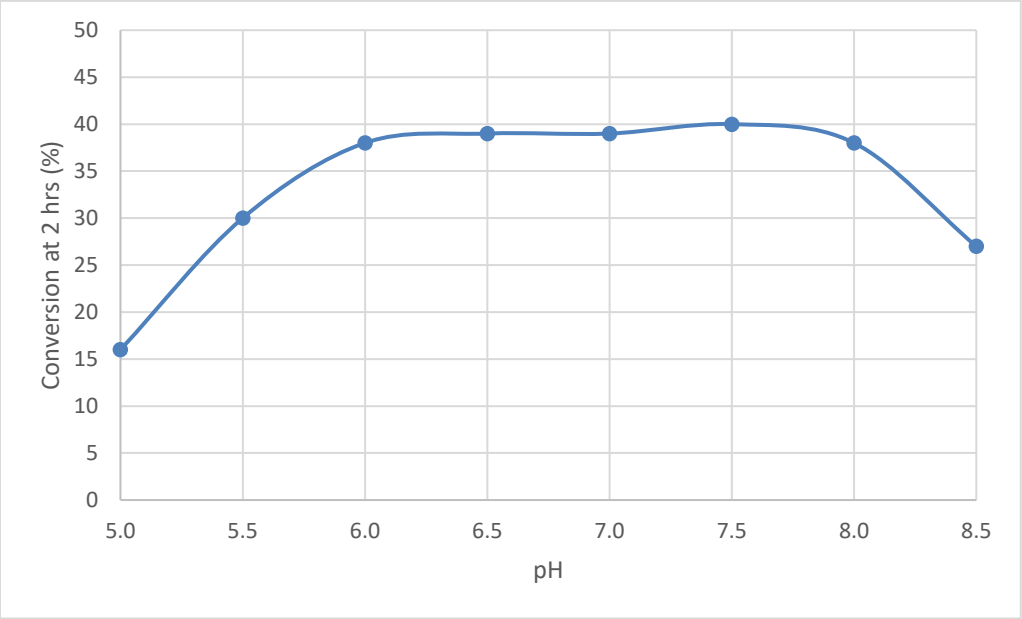


Figure 6. Conversion at 2 hour time points determined on ketone **1** showing pH tolerance range.

The optimum temperature was also easily determined in a similar manner, again determined on ketone **1**. As Figure 7 shows, the highest levels of conversion were at approximately 40 °C, with the reaction reaching completion in <1 hour under standard screening conditions (i.e. high enzyme loading, low substrate loading). Significant reaction progress was also made at 50 °C (and slightly less so at 30 °C), with complete conversion within 2 hours. The ability of this KRED to withstand higher temperatures was potentially advantageous when addressing the solubility issues which arose in the scale up, especially for ketone **2**.

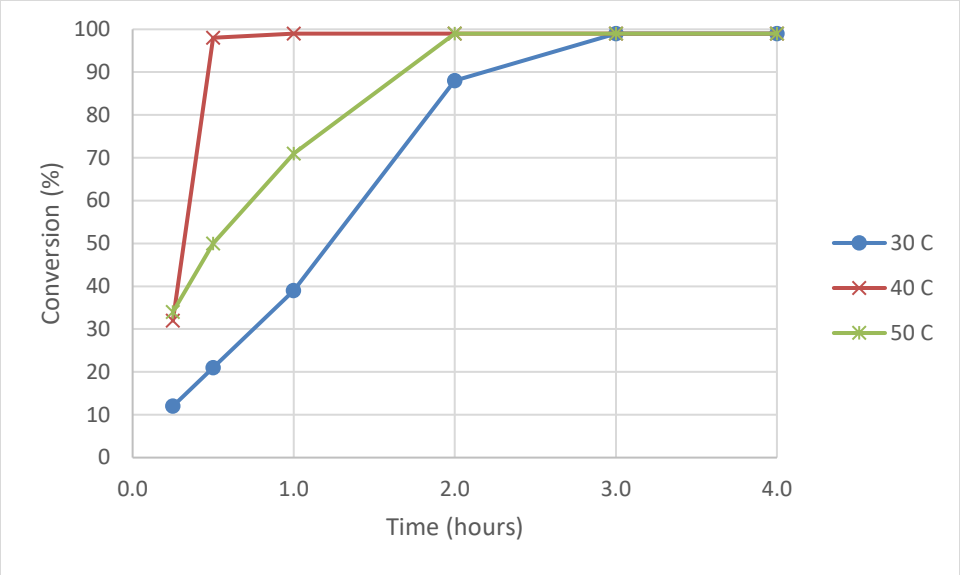


Figure 7. Conversion against time determined on ketone **1** at different temperatures.

Scale-up studies

Screening and optimisation studies had been conducted at high substrate dilutions, typically 10 mg/mL ketone. Enzyme loadings were thus relatively high at this scale (up to 3 mL). With a view to scale-up however, substrate concentrations need to be much higher for manufacturing efficiency (ideally >100 mg/mL), and enzyme loadings much lower, to enable greater cost reduction.[33] Consequently, initial scale-up studies were conducted to determine what concentration of ketone could be achieved in the reaction. For ketones **1** and **7**, a solubility of 100 mg/mL (10 relative volumes) was readily achievable under the conditions of screening procedure P at 5.0 or 8.0 wt% KRED loading with no difficulties concerning substrate solubility or loss of enzymatic efficiency. For the less soluble, more challenging substrate ketone **2** however, it was immediately apparent that a 100 mg/mL solution was far too ambitious; even screening reactions which had been conducted at 10 mg/mL became thick slurries. It was speculated that this could be

caused by the alcohol product **2** precipitating from the reaction mixture. However, on this scale (3 mL) mixing was still sufficient and the reaction mixture did not solidify.

A screening study for the reduction of ketone **2** under conditions of screening procedure P at 40°C was then conducted on larger 10 mL scale so that physical effects could more easily be observed. Ketone concentrations of 17, 20, 25 and 33 mg/mL were trialled and found to give similar conversions of ~75-80% after 2 hours, and ~85-90% at extended reaction times. However, the most concentrated reaction mixture had begun to solidify within 1 hour. At the other concentrations, the mixture was relatively mobile and stirring was sufficient, despite some thickness developing. Interestingly, this study showed that the concentration of the ketone in solution, and hence the reaction mixture consistency, had neither a beneficial nor adverse effect on the conversion to the desired alcohol. Despite this, a compromise between the reaction concentration and the consistency of the reaction mixture needed to be reached, since 25 mg/mL was still deemed dilute (40 relative volumes). Physical problems due to agitation tend to increase on scale-up,[33,34] despite often improved stirring efficiencies when using larger (jacketed) vessels equipped with mechanical stirrers. For the purposes of this study, further optimisation studies on ketone **2** were performed at 25 mg/mL.

As noted, ketone **2** was much more soluble in IPA at 50 °C than at 40 °C, and this factor was combined with portion wise addition in 0.1 equivalent doses to take advantage of improved solubility. However, consistency worsened as the reaction progressed until the reaction mixture eventually had the same consistency as previous reactions at 40 °C added in a single initial portion. As a result, neither change was implemented.

Each ketone reduction was then sequentially scaled up from a 3 mL test-tube through 10, 50 and 100 mL round-bottomed flasks with magnetic stirring, to 250, 500 and 1000 mL in heated jacketed reactors with mechanical stirring. The purpose of 10 mL and 50 mL reactions was to ensure that the optimised conditions would prove to be robust as the reaction volume increased. The stepwise increase in reaction volume was primarily to understand the potential effects of vessel size, vessel type and agitation, which can cause unexpected problems.[33,34] Reactions for ketones **1** and **7** scaled up with no demanding issues, although conversions dropped slightly to ~85% and ~95% respectively from ≥99% at the 3 mL scale with a 5 wt% loading of KRED. To retain the high level of conversion previously achieved in screening studies, the loading of KRED was increased from 5.0 wt.% to 8.0 wt.% as noted above.

Reactions for ketone **2** gave a thick reaction mixture up to ~100 mL. Scaling this to larger volumes seemed doubtful since the reaction mixtures had become thicker with increasing volume. However, conducting a 100 mL reaction in a 250 mL jacketed vessel with a more efficient mechanical (turbine) stirrer improved the consistency of the reaction mixture. A relatively low rate of stirring at 130 rpm was used to avoid the formation of emulsions or stable foams. A conversion of 85% was achieved after 4 hours, dropping only marginally to 79% when scaled to 250 mL reaction volume in the same vessel.

Reactions were also conducted on 250 mL for ketones **1** and **7**, and on 500 mL for all three ketones. However, as the charge of KRED increased, the enzyme tended to form a solid mass in the reaction when added directly to the reactor to the ketone/IPA/buffer mix. Although no reduction in reaction rate was observed at this scale, the formation of a solid mass of enzyme could result in an uneven distribution in the reaction mixture and likely problems on further scaling. This was readily avoided by pre-mixing the KRED in the buffered aqueous reaction medium to give a

homogeneous mixture and adding to the 1000 mL reactor with stirring at 40 °C. The ketone (**1** or **7**) was dissolved in IPA and added to the reaction mixture which avoided the enzyme aggregating into a solid mass. This reverse addition was not necessary with ketone **2** because lower KRED loading and more dilute conditions were used. Indeed, when added last in smaller scale reactions, ketone **2** tended to form a solid layer above the solvent. This had to be prevented since contact between the ketone and enzyme would be reduced, potentially limiting the reduction reaction. KRED was therefore mixed in buffered aqueous medium to a homogeneous mixture and then added to the ketone/IPA slurry in the reactor. This ensured that the enzyme was well dissolved and did not aggregate in the reaction.

Table 4. Summary of reaction conditions and results for reduction of ketones 1, 2 and 7 by KRED on 1000 mL scale

Ketone	**1**	**2**	**7**
Conditions			
KRED loading (wt%)	8.0	5.0	8.0
Concentration (mg/mL)	100	25	100
pH	7.0	7.0	7.0
Temperature (°C)	40	40	40
Agitation (rpm)	230	130	230
Results			
Conversion at 4 hrs (%)	77%	72%	77%
Conversion at 24 hrs (%)	90%	86%	96%
Mass recovery (%)	91%	70%	94%
e.e. (%)	>99.5%	>99.5%	>99.5%

The reactions were scaled up for all three ketones in a 1000 mL jacketed reactor with mechanical stirring using the optimised conditions developed so far. The finalised conditions in all aspects, along with comparative results on 1000 mL scale, are summarised in Table 4. Mass recoveries were high and gave product alcohols of quality reflecting the conversions achieved.

Throughout the project, the preferred KRED gave exceptionally high e.e.s, and this was maintained at 1000 mL scale. This was pleasing and a good indication that this key parameter would be maintained on further scale-up.

CONCLUSIONS

In conclusion, KRED reductions have been identified for five out of eight short-listed ketone precursors present in the generic APIs chosen for investigation. In five cases the desired chiral alcohols were obtained with high selectivity (>99.5% e.e.) in good conversion and isolated yield. After further focused reaction screening and some preliminary optimisation, three of the best examples were then scaled to 1 L scale.

Therefore, we conclude that KRED reductions can be applied as a general strategy in the synthesis of key chiral alcohols and their derivatives present in generic APIs and other high value materials. The successful scale-up to 1 L with only minimal optimisation so far demonstrates that these reductions have the potential for successful application on manufacturing scale, as has been shown elsewhere.[9-12] There is clearly scope to apply this technology to many further examples.

Lastly, in addition, successful results were also obtained in typically high selectivity (>99.5% e.e.) for the opposite enantiomer in five examples. This further shows the versatility of bio-catalysis in general, and KREDs in particular, to produce opposite enantiomers from the same prochiral precursors. It should be noted that all KREDS were obtained from commercial sources, which would not delay initial scale-up, and provided both enantiomers without modification. This powerfully demonstrates the utility of commercially available KREDS in the potential manufacture of many other high value materials.

EXPERIMENTAL SECTION

General Procedures. Standard materials and reagents for the synthesis of several ketones and their respective alcohols were purchased variously from Sigma Aldrich (Gillingham, UK), Fisher Scientific (Loughborough, UK), Fluorochem (Hadfield, UK) and TCI (Zwijndrecht, Belgium). Solvents for reactions and HPLC grade solvents for analysis were purchased from Fisher Scientific. All reagents and solvents were purchased at standard grades and used as supplied except where noted otherwise. Specifically, ketones **1**, **3** and **7** were purchased from Fluorochem; ketones **2**, **4**, and **5** were supplied by industrial partners; and ketones **6** and **8** were prepared by synthesis (see Supplementary Information). Marker samples of racemic alcohols were prepared by standard reduction of the relevant ketone with sodium borohydride or related reagents – see Table 5, representative example below and Supplementary Information.

Table 5. Summary of material sources, alcohols and analytical methods

	Ketone	rac-Alcohol	Chiral Alcohol	Analytical Methods			
#	Source	Reductions	Source	LC (A)	GC (B)	Chiral LC (C)	Chiral LC (D)
1	Purchased	NaBH4	Purchased (R)	Yes	-	Yes	-
2	Supplied	NaBH4	Supplied (2S,3S)	Yes	-	(a)	(a)
3	Purchased	NaBH4	n/a	Yes	-	-	-
4	Supplied	n/a	n/a	Yes	-	-	-
5	Supplied	Red-Al	n/a	Yes	-	-	-
6	Synthesised	NaBH4	n/a	Yes	-	Yes	-
7	Purchased	NaBH4	Synthesised (S)	-	Yes	-	Yes
8	Synthesised	NaBH4	n/a	Yes	-	-	-
	n/a not attempted						
	(a) Achiral LC method A also served to determine d.e. for series **2** compounds						

KREDs (EC 1.1.1.1 and EC 1.1.1.2, alcohol dehydrogenases with NAD^+ and $NADP^+$ acceptors respectively) were obtained from Codexis (Redwood City, CA),[26] Johnson Matthey (Royston, UK)[27] and Prozomix Ltd (Haltwhistle, UK).[28] Glucose dehydrogenase (GDH) (EC 1.1.99.10) and co-factors NAD^+ and $NADP^+$ were also supplied by Prozomix.

Screening Procedures

Typical tube-scale screening with GDH/glucose recycle (screening procedure G). The aqueous solvent mixture was prepared using 250 mM potassium phosphate, 2.0 mM magnesium sulphate, 1.1 mM $NADP^+$, 1.1 mM NAD^+, 80 mM D-glucose and10 units/mL GDH adjusted to pH 7.0.[26] Ketone (0.15mmol) and the relevant KRED (10 wt%) were added to a 9:1 aqueous:iso-propanol reaction mixture (3.0 mL) in a test-tube and heated to 30 °C with magnetic stirring. Reactions were sampled at intervals by taking aliquots (20 µL) diluted into acetonitrile (800 µL) for LC-MS (method A) or methanol (800 µL) for GC-MS (method B). Variations in screening procedures included the stoichiometry of ketone and KRED, choice of organic solvent, aqueous:organic solvent ratio, concentration, pH and temperature.

Typical tube scale screening with IPA/co-factor recycle (screening procedure P). The aqueous solvent mixture was prepared using 125 mM potassium phosphate, 1.25 mM magnesium sulphate and 1.0 mM $NADP^+$ adjusted to pH 7.0.[26] Ketone (0.15mmol) and the relevant KRED (10 wt%) were added to an 9:1 aqueous:iso-propanol reaction mixture (3.0 mL) in a test-tube and heated to 30 °C with magnetic stirring. Reactions were sampled at intervals by taking aliquots (20 µL) diluted into acetonitrile (800 µL) for LC-MS (method A) or methanol (800 µL) for GC-MS (method B). Variations in screening procedures included the stoichiometry of ketone and KRED, choice of organic solvent, aqueous:organic solvent ratio, concentration, pH and temperature.

Scale-up Preparations

*Reduction of ketone **1** to chiral alcohol (R)-**1** on 1 L scale*. Aqueous buffer solution (800 mL) was prepared as described in screening procedure P and added to a suitably serviced 1000 mL jacketed reactor. KRED (8.0 g, 8 wt.%) was added and the contents mechanically stirred at 230

rpm whilst heated at 40 °C until a colourless solution was achieved. Iso-propanol (200 mL) and ketone **1** (100 g, 390 mols) were added and stirring maintained at 230 rpm. Reaction progress was determined by LC (method A). Once the reaction had reached completion, agitation and heating were stopped. The aqueous reaction mixture was extracted three times with dichloromethane (400 mL each). The aqueous phase was then filtered through Celite and extracted twice more with dichloromethane (200 mL each). The dichloromethane extracts were combined and filtered through Celite, which was rinsed with further dichloromethane (200 mL). The combined dichloromethane filtrates were dried over magnesium sulfate, filtered under gravity and the solvent evaporated under vacuum to yield the desired chiral alcohol product (R)-**1** as a yellow solid (91 g, 91%). Achiral HPLC (Method A, Rt 3.76 mins, conversion 90%); chiral HPLC (Method C, Rt 7.53 mins, (R)-**1**, >99.5% e.e.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (2H, s), 7.79 (1H, s), 5.01-5.07 (1H, m), 2.01 (1H, d, J = 3.5 Hz), 1.55 (3H, d, J = 6.5 Hz).

*Reduction of ketone **7** to chiral alcohol (S)-**7** with KRED on 1 L scale*. The same procedure was used as for the large scale reduction of ketone **1** to chiral alcohol (R)-**1** on the same scale (100 g, 525 mmol) and volume (1000 mL), with all other factors being identical, except as noted. Reaction progress was determined by GC (method B). Evaporation of the solvent extracts yielded the desired chiral alcohol product (S)-**7** as a dark yellow oil (94 g, 93%). Achiral GC (Method B, Rt 6.64 mins, conversion 96%); chiral HPLC (Method D, Rt 12.28 mins, (S)-**7**, >99.5% e.e.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07-7.28 (3H, m), 4.83-4.91 (1H, m), 3.71 (1H, dd, J = 3.5 and 11.5 Hz), 3.59 (1H, dd, J = 8.5 and 11.5 Hz), 2.89 (1H, br s).

*Reduction of ketone (2S)-**2** to chiral alcohol (2S,3S)-**2** with KRED on 1 L scale*. Iso-propanol (200 mL) and ketone (2S)-**2** (25.0 g, 84 mmols) were added to a suitably serviced 1000 mL jacketed reactor and stirred at 130 rpm. Aqueous buffer solution (800 mL) was prepared as

described in screening procedure P and KRED (1.25 g, 5 wt.%) added and the resulting mixture stirred to achieve a homogeneous solution. This was added to the reaction vessel and heated to 40 °C whilst stirring was maintained at 130 rpm. Reaction progress was determined by LC (method A). Once reaction had reached completion, agitation was increased to 250 rpm for 15 minutes, then agitation and heating were stopped. Work-up and extractions were as for large-scale reduction of ketone **1** above. Evaporation of the solvent extracts yielded the crude desired chiral alcohol product (2S,3S)-**2** as a yellow oil (17.6 g, 70%). Achiral LC (Method A, conversion 86%, Rt 2.55 mins, (2S,3S)-**2**, >99.5% e.e.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.34 (2H, m), 7.21-7.26 (3H, m), 4.58 (1H, br s), 3.79-3.97 (2H, m), 3.64-3.72 (1H, m), 3.56-3.63 (1H, m), 3.16 (1H, br s), 2.88-3.04 (2H, m), 1.38 (9H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) 155.9, 137.3, 129.5, 128.6, 126.6, 80.0, 73.5, 54.4, 47.6, 35.8, 28.2.

Reference Marker Preparations

Typical preparation of racemic alcohols with sodium borohydride ($NaBH_4$). A solution of ketone **7** (100 mg, 0.53 mmol) dissolved in methanol (4.0 mL) was cooled in an ice bath to 0 °C and sodium borohydride (12 mg, 0.32 mmol) added in one portion. The reaction mixture was stirred at 0 °C for 40 minutes, then at room temperature for 1 hour, after which the reaction mixture was quenched with saturated aq. NH_4Cl solution. The methanol was removed by vacuum distillation and the residual aqueous phase was extracted three times with ethyl acetate (5 mL each). The combined organic extracts were dried ($MgSO_4$) and concentrated to dryness. The crude product was purified by flash silica gel chromatography eluting with 10% ethyl acetate in petroleum ether to yield the desired racemic alcohol **7** as a colourless oil (62 mg, 61%).[35] TLC R_f 0.20 (20% EtOAc-petrol). HPLC (Method B, Rt 6.64 mins, 93% purity); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23-7.28 (1H, m), 7.13-7.20 (1H, m), 7.08-7.12 (1H, m), 4.88 (1H, dt, J = 3.5 and 8.5

Hz), 3.72 (1H, dd, *J* = 3.5 and 11.5 Hz), 3.59 (1H, dd, *J* = 8.5 and 11.5 Hz), 2.71 (1H, d, *J* = 3.5 Hz); m/z (EI^+) 141 (M^+).

Reduction of other ketones to racemic alcohols using this and related methods are noted in Table 5 and the Supplementary Information.

Determination of absolute stereochemistry for scale-up examples. Reference marker alcohols of known stereochemistry were purchased for (R) and (S)-**1** (Fluorochem) and supplied for (2S,3S)-**2**, all of which could be compared to the results of KRED reductions for ketones **1** and **2** using the appropriate chiral LC method. Neither enantiomer of alcohol **7** could be purchased, so ketone **7** was reduced by a KRED using general screening method 1 to give **7** in >99% e.e. This was in turn converted to its acetate ester and the sign of optical rotation ($[\alpha]^{20}_D$ = + 39.8 (c 0.7, $CHCl_3$)) compared to the known literature value ($[\alpha]^{20}_D$ = + 47.4 (c 0.7, $CHCl_3$)),[35] which identified this sample as having been derived from (S)-**7**. Absolute stereochemistry was not formally determined on other compounds which were not taken into the scale-up studies – only racemic alcohol marker samples were needed in these cases to determine if any stereoselectivity had been achieved.

Chromatographic methods

Reaction monitoring and analysis of isolated samples. KRED reduction reactions were monitored by HPLC-MS (method A) or GC-MS (method B) as appropriate. Synthetic chemistry reaction monitoring (for synthesis of ketones and alcohols) used the same or slightly modified methods. E.e.s were determined by either of two chiral HPLC methods (C and D). Isolated samples were analysed for achiral purity (LC or GC) and enantiomeric purity (LC only) using the same methods (A to D). Identification and purity by ^{1}H NMR spectroscopy used standard methods on

Bruker or Varian 300, 400 or 500 MHz spectrometers in solvents as noted. Identification of isolated samples by mass spectrometry used methods A or B.

Reaction monitoring by HPLC-MS (method A). Instruments: Agilent 1100 with DAD, and Waters ZQ (ESCI) MS; column: Ace Ultra Core 2.5, Super C18, 75 mm x 3.0 mm i.d.). Samples were prepared by taking 20 μL dissolved in 800 μL of acetonitrile and centrifuged at 3000 rpm for 4 mins before analysis. Injection volume was 2.0 μL, column temperature 40 °C, and detection at 220-300 nm. Eluent A was 10% acetonitrile in water with 0.05% formic acid, eluent B was 100% acetonitrile with 0.05% formic acid. Run time was 5 mins with a flow rate of 1.25 mL/min. Starting eluent at 0.00 mins was 80% eluent A with 20% eluent B rising linearly to 99% eluent B at 4.00 mins, then decreasing linearly to 20% eluent B at 5.00 mins, with a 1.00 min post-time. Mass spectrometry ionisation by ESI^+ used 20 eV and by ESI^- used 50 eV, both with *m/z* range of 100-1000 amu. Retention times: ketone **1** (3.95 min); alcohol **1** (3.76 min); ketone **2** (2.96 min); (S)-alcohol (2S, 3S)-**2** (2.56 min); (R)-alcohol (2S, 3R)-**2** (2.80 min).

Reaction monitoring by GC-MS (method B). Instrument: Varian Saturn 2000 GC-MS; column: J&W Scientific HP-1MS, 30 m x 2.5 mm with 0.25 μm film). Samples were prepared by taking 20 μL dissolved in 800 μL of methanol and centrifuged at 3000 rpm for 4 mins before analysis. Injection volume was 1.0 μL, inlet temperature 300 °C, split ratio 50:1, and the carrier gas helium. Total run time was 17.55 mins with a constant flow rate of 1.2 mL/min and heating profile as follows: 60 °C at 0.00 mins, hold for 0.55 mins, ramp at +15.0 K/min for 4.00 minutes to 120 °C then ramp at +20.0 K/min 11.00 mins to a final temperature of 300 °C, hold for 2.00 mins at 300 °C, then cool back to 60 °C. Mass spectrometry ionisation was by EI^+ with *m/z* range of 50-650 amu. Retention times: ketone **7** (6.80 min); racemic alcohol **7** (6.64 min).

Chiral HPLC method C. Instrument: Agilent 1100; column: Diacel OD-H, 250 mm x 4.6 mm i.d., particle size 5.0 μm). Samples were prepared by taking 20 μL dissolved in 1.0 mL of heptane and centrifuged at 3000 rpm for 4 mins before analysis. Injection volume was 10.0 μL, column temperature 30 °C, and detection at 220 nm. Run time was 11.0 mins with a flow rate of 1.20 mL/min using an isocratic eluent of 2% ethanol in 98% heptane. Retention times: ketone **1** (3.69 min); alcohol (S)-**1** (6.72 min); alcohol (R)-**1** (8.01 min).

Chiral HPLC method D. Instrument: Agilent 1100; column: Phenomenex A2 Lux, 250 mm x 4.6 mm i.d., particle size 5.0 μm). Samples were prepared by taking 20 μL dissolved in 1.0 mL of diluent (30% ethanol in 70% heptane) and centrifuged at 3000 rpm for 4 mins before analysis. Injection volume was 2.0 μL, column temperature 30 °C, and detection at 220 nm. Run time was 20.0 mins with a flow rate of 1.20 mL/min using an isocratic eluent of 2% ethanol in 98% heptane. Retention times: ketone **7** (9.16 min); alcohol (S)-**7** (10.42 min); alcohol (R)-**7** (11.09 min).

ASSOCIATED CONTENT

The Supporting Information is available free of charge on at: [**ACS to insert details**]
Chiral LC and NMR spectra of alcohols **1**, **2** and **7**; synthesis of ketones **6** and **8**; preparation of additional racemic alcohol marker samples (pdf).

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Notes

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