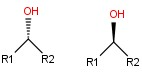
**Dynamic Kinetic Resolution using Enzymes**

In the ideal chemical reaction the product obtained would be completely pure in 100% yield with all the materials of the reaction incorporated. However this can be a challenge to accomplish as starting materials can be racemic along with the products produced. Figure 1 shows an example of a racemic mixture with the enantiomers (denoted as R or S) each with an asymmetric carbon atom (chiral) that are non-superimposable on the other. These compounds are hard to separate due to the similarities in physical and chemical properties, only differing by the spatial arrangement of its atoms. Though this may seem innocuous even a slight difference in the spatial arrangement of the compound can have profound effects in regards to its function.



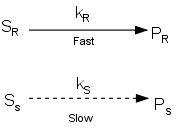
*Figure 1: Example of a racemic mixture*

*Note R2 ≠ R1 if R2 = Cl and R1 = -CH3 this would make 1 the S enantiomer*

Enzymes and receptors are also chiral so can recognise one enantiomer over the other. Here-in lies a significant problem in the pharmaceutical industry. When a racemic medicine is administered the other enantiomer can cause unwanted side effects elsewhere or hinder the effectiveness of the active enantiomer [1]. So enantio-pure drugs are desired and asymmetric catalysis is becoming more important [2]. Natural products used for their various beneficial effects, as drugs or as precursors to synthetic drugs, tend to be chiral and can be in racemic mixtures [3]. As chirality is an important feature to function it can’t be avoided, so a way of separating the enantiomers is required, meaning new pathways to chiral compounds have to be devised. Chiral compounds are not limited in use to pharmaceuticals, and find applications in other areas such as forming chiral polymers with novel properties [4].

Resolution is the separating of enantiomers by chemical or physical means which is usually achieved via conversion to diastereomers, containing additional chiral centres and are not mirror images of one another. Subsequently the diastereomers can be separated due to different chemical properties; from this various techniques have been devised.

Kinetic Resolution



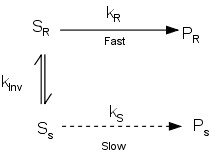
*Figure 2: Overview of classic kinetic resolution*

Kinetic resolution relies on differing reaction rates for the enantiomers. By discriminating, via the use of a chiral catalyst, the desired product is produced while the slower reacting enantiomer is left unreacted. This relies on a large difference in reaction rates in order to achieve a high enantiomeric excess (e.e) and a maximum theoretical yield of only 50%. As the conversion proceeds the slower reacting enantiomer becomes more favourable as the amount of its mirror image depletes, leading to the e.e dropping when the conversion nears 50% (see Figure 3). Another problem is that the unreacted enantiomer and the desired product need to be successfully separated at the end of the kinetic resolution.

*Figure 3: Graph showing changes in e.e during a kinetic resolution*

If the Isolated unreacted enantiomer can undergo racemization, the racemic mixture produced can be subjected to another stage of kinetic resolution. Assuming the theoretical yield of 50% is obtained in each stage by the 5th stage a 97% yield can be achieved however, this increases the time and cost so is impractical after the 2nd stage as the gains significantly decrease. More practical method would be to combine the two processes into a “dynamic” reaction.

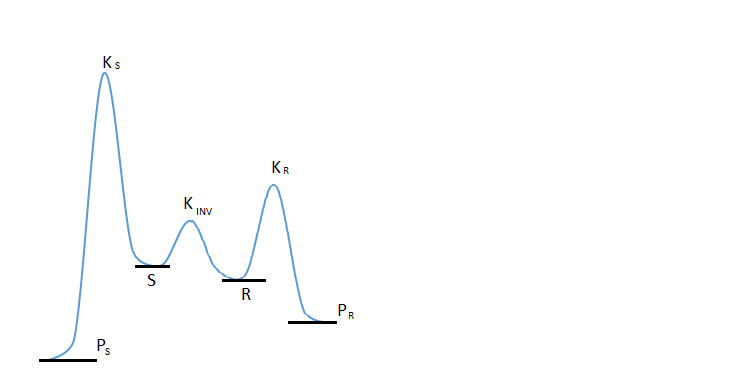
Dynamic Kinetic Resolution



*Figure 4: Overview of Dynamic Kinetic Resolution (DKR)*

Dynamic kinetic resolution (DKR) is a kinetic resolution with an additional feature: the enantiomers are being inverted in-situ and thus are in an equilibrium with each other. The fast conversion of one enantiomer into the desired product causes a depletion, which shifts the inversion equilibrium in favour of producing more of the faster reacting enantiomer. This results in a skewed reaction making higher yields achievable; theoretically 100%. Also importantly this technique achieves high e.e as there is always a racemic mixture present so the asymmetric catalyst isn’t in direct contact with only the undesired enantiomer.

In essence there are two processes going on at once: racemization of the enantiomers and the conversion of one enantiomer into the desired product, which needs to be fine-tuned as there compatibility is vital. There are ideal conditions that have to be met for a successful DKR including those for a kinetic resolution and avoiding side or spontaneous reactions [5]. The resolution reaction should be irreversible thereby preventing a drop in e.e. and maintaining the shift in equilibrium. The rate of inversion (kInv) needs to be greater than or equal to the rate of the asymmetric conversion (kR) avoiding the supply of the wanted enantiomer depleting thus making the unwanted enantiomer more favourable. High selectivity in the catalyst is desired otherwise the rate of inversion needs to be significantly higher than the rate of conversion to achieve high yields.



*Figure 5: energy profile of a DKR. Note that DKR is an example of the Curtin-Hammett Principle.*

Racemization

Racemization is arguably the most important aspect of a DKR as its optimization is essential for a DKR to run efficiently. Unless racemization is spontaneous certain conditions have to be met to induce it, which can lead to compatibility problems with the rest of the DKR as the asymmetric catalyst has its own favourable conditions. Examples of this are specific pH and temperature requirements. Therefore this step needs to be tuned to give a balance that maintains an effective inversion but doesn’t hinder the conversion to the desired product; the use of a catalyst in this step keeps the conditions in an acceptable range. Racemization can be accomplished by various methods such as acid or base catalysis [6], but this role can also be accomplished by enzymes such as dehydrogenase.

Biocatalyic Asymmetric Conversion- Why Enzymes for DKR?

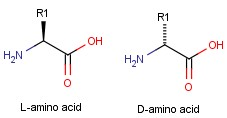
Chiral asymmetric catalysts based upon transition metals are commonly used to in both the racemization and conversion steps of a DKR but have to be synthesised and usually require a rare metal at its core, most commonly ruthenium is used [7]. Enzymes can offer a more attractive alternative.

Enzymes are becoming more viable as tools in organic synthesis especially as the direction of chemistry is moving towards being more environmentally responsible [8] and enzymes can provide alternative pathways to desired compounds in much greener reactions. Enzymes are more environmentally friendly than metal based catalysts as they are biodegradable. In terms of efficiency enzymes can increase reaction rates magnitudes higher [9] than there non-bio counterparts and be used in lower quantities.

Enzymes typically act under milder conditions such as low temperature (~ 30⁰C) and near neutral pH. This meansthat reactions can be untaken in ambient conditions avoiding the need for specialized machinery needed to incorporate extreme conditions. Another benefit of this is that it avoids side reactions which would be able to take place at higher temperatures and pressures such as rearrangements, isomerism and racemization. Though this does limit the potential for speeding up a slow reaction.

Enzymes were commonly dismissed as only working in non-aqueous media however this is not the case and can even work to temperatures around 70⁰C and beyond in organic solvents [10]. Thus allowing for organic substrates that would not dissolve successfully in water to interact with the enzyme. Enzymes can perform in the presence of others [11], if the conditions required are similar for both, leading to one pot reactions where multiple enzyme-catalysed reactions are feasible. This of course would not be true with protease enzymes. Enzymes have been shown to be able to carry out reactions on synthesised substances opening their usefulness as they are not limited to natural substrates and can perform a wide variety of transformations.

This is not to say there are not drawbacks to the use of enzymes which need to be considered. The biggest being that enzymes created by natural evolution are usually of one enantiomeric form so therefore are selective to one substrate enantiomer. With synthetic asymmetric catalysts it is a case of adapting the synthesis to create its mirror image meaning there is access to both enantiomeric products. Enzymes can’t be modified as easily by swapping the L-amino acid for its mirror image the D-amino acid (Figure 6). Site directed mutagenesis and directed evolution are techniques that can be employed to try and create an enzymes mirror image, though is often trial and error and be a long process [12] though there has been discoveries of enzymes that are equivalent to mirror images (opposite selectivity) so form complementary enantiomer pairs [13,18]



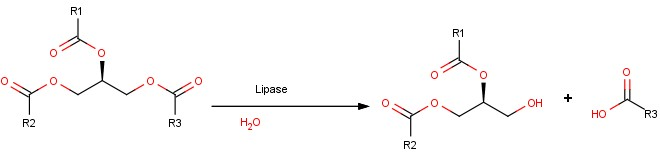
*Figure 6: amino acid enantiomers*

Enzymes can be used in non-aqueous media so can be utilized in organic synthesis, though this usually leads to significant loss of reactivity [10] as water is the preferred medium. The nature of how enzymes work to intrinsic detail is still not sufficiently discovered and some enzymes can be limited in regards to the need for co-factors (e.g. ATP, NADH) to function and substitutes can’t be used resulting in more expenditure being required and the co-factor having to be recycled often by use of a sacrificial compound.

Another drawback is the fact enzymes can be inhibited and this can be initiated by a whole array of different components such as high substrate concentrations, products produced and can be sensitive to non-enzymatic catalysts used in the racemization step. Through more research these drawbacks can be overcome as techniques such as directed evolution can lead to more efficient enzymes being produced.

Lipases in Dynamic Kinetic Resolution

One of the most commonly used enzymes in DKR are lipases which can be produced in quantities that are practical for use on an industrial scale [14] and used in organic solvents [10ii]. They don’t require co-factors to operate and can be used in conjunction with metal catalysts. They can be immobilised [15] enabling them to be recycled and protected from chemical, thermo, and solvation degradation. An immobilised enzyme can be used with other immobilised enzymes without direct interaction meaning that its shelf life is dramatically increased bring costs down.



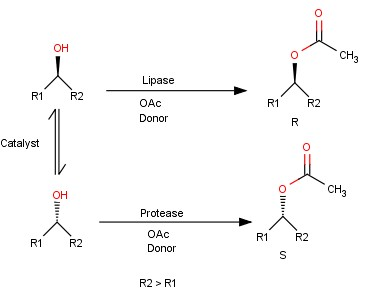
*Figure 7: Hydrolysis by lipase*

In nature Lipases catalyse the hydrolysis and synthesis of lipids (Figure 7) but these transformations can be applied to manmade synthetic compounds can show high selectivity and versatility with the range of reactions they can assist.

Dynamic kinetic resolution of Secondary Alcohols via Esterification

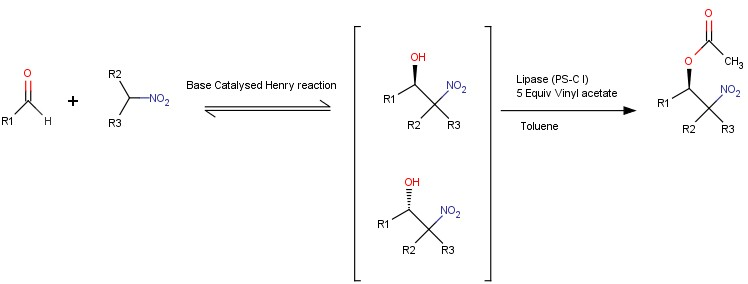
Secondary alcohols can be resolved by lipases [16] which show selectivity towards the R enantiomer and perform esterification by attaching an acetyl moiety. Meanwhile the S enantiomer is constantly being inverted, most commonly by a Ru based catalyst. This esterification requires an acetyl source Lipases offer an efficient pathway to various versatile chiral building blocks such as β-hydroxy nitriles [17]

If the S enantiomer is desired the protease subtilisin (Figure 8) [18] can be used however it is not as practical as lipase with lower activity, selectivity and stability in non-aqueous media. However DKR with an aminocyclopentadienyl ruthenium complex catalysing the inversion of the enantiomers was shown to be able to transform secondary alcohols to acyl derivatives in THF at 25⁰C in yields ranging from 77-94% with high e.e values of 95-99%. The authors also demonstrated how complementary subtilisin is by using m-butanoyloxyphenyl-1-ethanol as the substrate and comparing a DKR with a lipase in toluene with subtilisin in THF under the same conditions (25⁰C 3d) with similarly high yields (94% S enantiomer: 95% R enantiomer) and an e.e >99.5%

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*Figure 8: General DKR scheme for secondary alcohol resolution by lipase or its complementary enzyme pair protease*

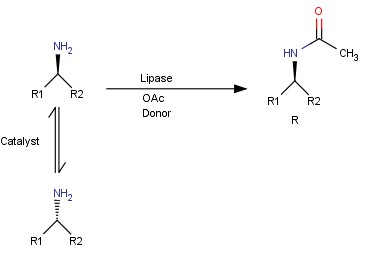
An interesting application of this chemistry is the combination of it with classical organic synthesis reactions. A good example is carbon-carbon bond formation via a henry reaction (Figure 9) [19] which also causes the required racemisation of the enantiomers, via base catalysis, resulting in a one pot synthesis of β-nitroalkanol derivatives. The authors engineered a common industrial process which used enzyme kinetic resolution resulting in yields of only 50% converting it into a more efficient DKR affording high yields (≤ 90%) as well as high e.e (92-99%).



*Figure 9: Henry reaction (aka Nitro-Aldol) combined with secondary alcohol DKR R2 and R3= CH3*

*When R1 = 4-O2N-C6H4 a 90% yield was obtained with an e.e of 99%* [18]

Synthesis of Amides from DKR of amines.

**

*Figure 10: General DKR of amide synthesis*

Lipases versatility means it can also form (R)-amides from primary amines in conjunction with an acyl donor. A number of functionalized primary (R)-amides have been successfully synthesised using Novozyme 435 (lipase from *Candida Antarctica*) at 90⁰C in toluene with isopropyl acetate as the acyl group donor [20] the authors developed a new route to norsertraline from the DKR of 1,2,3,4-tetrahydro-1-naphthylamine ( Yield 70 % e.e 99%).

Like with esterification there are examples of (S)-amides being formed, examples in literature include from the DKR of amino acid thioesters with the protease subtilsin [21] and (S)-amides from aliphatic amines with alkaline protease [22]. The DKR with alkaline protease produced (S)-amides in moderate yields (65-73%) using irradiation at 350nm in the presence of azobisisobutyronitrile (ALBN) toperform racemisation.

Synthesis of Acids (hydrolysis) from DKR

As shown in figure 7 lipases can also perform hydrolysis resulting in the formation of a carboxylic moiety [23] and this can be used in a DKR. Figure 11 shows and example involving ibuprofen [24] which the (S) - enantiomer is the active agent and the (R) - enantiomer causes side effects and due to the ability of conversion in-vivo to the (S)-enantiomer it is sold as a racemic mixture. However using acid catalysis and a lipase it is possible to obtain the (S)-enantiomer in 86% yield with a e.e of 99.4%

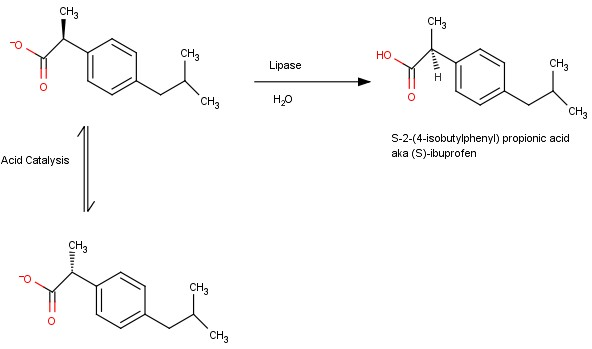


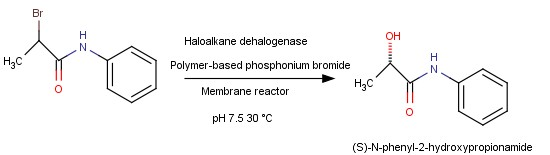
Figure 11 DKR using lipase to resolve racemic ibuprofen the S enantiomer is the most potent [24]

Other Enzymes used in DKR

Although lipases are the most utilised enzyme for dynamic kinetic resolutions, due to their ease of use, other groups of enzymes have also been researched. Different enzyme families can facilitate reactions that can’t be performed by lipases. As such they can occupy a niche if they can be successfully optimised in a DKR. Hereafter are some recent examples.

*Haloalcohol dehalogenase*

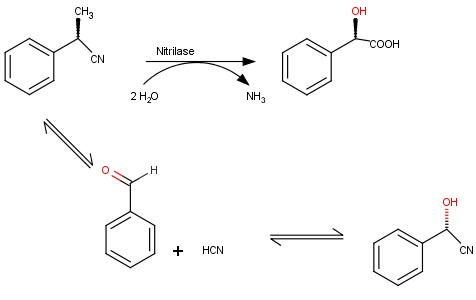
These enzymes convert a halogen on an alkane into a hydroxyl group and recently a DKR has been successfully developed to obtain (S)-α-hydroxyamides from racemic α-bromoamides. [25]. Figure 12 shows this reaction which resulted in a 63% yield and 95% e.e. The purpose of this reaction was to synthesize α-hydroxyamide “scaffolds” which the authors noted is present in bioactive compounds such as vitamin B5.



*Figure 12: DKR using haloalkane dehalogenase*

*Nitrilase*

Nitrilases perform hydrolysis on nitrile functional groups converting them to carboxylic acids and ammonia in one step. This provides a more attractive alternative to using a nitrile hydratase and then an amidase to do the same. To do this reaction chemically requires harsher conditions which is not ideal in a synthetic strategy when a sensitive chiral compound is desired. Side products are also produced when this conversion is performed chemically and nitrilase does not suffer the yield losses. Figure 5 shows a DKR using nitrilase [26]



*Figure 13: DKR leading to formation of (R)-(-)-mandelic acid*

*Baeyer-Villiger Monooxygenase*

Baeyer-Villiger oxidation is the formation of an ester by oxidation of a ketone with a peroxyacid. The reason why this enzyme shares the name is it can perform this reaction as shown in figure 14 [27]. (R)- 3-Methyl-3,4-dihydroisocoumarin (R1= methyl) was obtained in 84% yield and 82% e.e. The enzyme requires a co-factor which in itself needs to be replenished by a surficial substrate. This is not ideal as it introduces unused waste products.

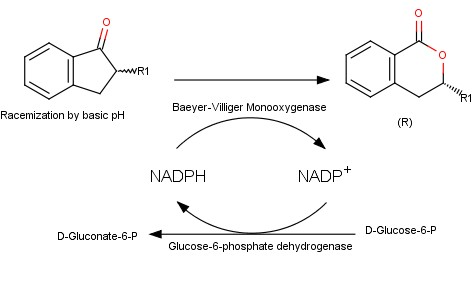
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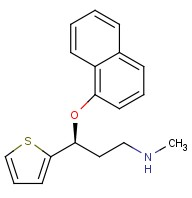
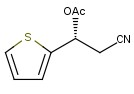
Figure 14: DKR using Baeyer-Villiger Monooxygenase 3,4-Dihydroisocoumarins show beneficial medicinal properties such as antifungal uses[27].

DKR in synthesis of chiral Drugs

1. S-Profens

Although an example of this has been covered by lipases (see figure 11) [24] an interesting alternative to this is present in literature [28]. This uses alcohol dehydrogenase from *sulfolobus solfataricus* which is a hyper thermophile, meaning it is an example of an extremophiles which are organisms which thrive in extreme conditions. Therefore its enzymes have evolved to perform in these environments and can be exploited for bio-catalytic reactions. DKR was successfully performed at pH 9 (base catalysed racemisation of enantiomers) at 80⁰C affording (S)-precursors to non-steoridal anti-inflammatory drugs such as naproxen, ibuprofen and ketoprofen in high yields (≤98%) and high e.e (≤99%).

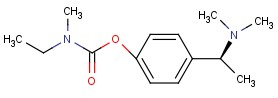
ii) Duloxetine [29]



*Figure 15: (R)-Precusor (left) and (S)-Duloxetine (right)*

(S)-Duloxetine is a serotonin-norepinephrine reuptake inhibitor and DKR has been shown to be viable as a step in its synthesis using lipase (*Candida antarctica* Lipase B) to produce (S)-Duloxetine from a (R)-precursor formed from esterification of (R)- β-hydroylnitrile

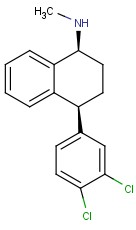
iii) Rivastigmine [30]



*Figure 16: Rivastigmine*

Rivastigmine is an acetylcholinesterase inhibitor which is used as treatment for Alzheimer’s disease and lipase (*Candida antarctica* Lipase B) was used to synthesis a precursor by esterification of a secondary alcohol. The authors also recycled the enzymes used and found that the lipase maintained its activity in 5 runs (4th run afforded a lower conversion due to insufficient potassium carbonate), which when replenished for the 5th run still achieved high yields (>97%).

# iv) Sertraline [31]



*Figure 17: Sertraline*

Sertraline is a prescribed antidepressant and the authors used DKR utilizing lipase resolving a diol to the (R,R)-diacetyl enantiomer (98% yield, 99.9 e.e) which can then subsequently be converted to sertraline through additional steps.

Conclusions and outlook

Enzymatic dynamic kinetic resolution offers a viable alternative to classical chemical reactions in regards to being more environmentally friendly and being able to perform delicate chemical modifications on complex and chiral compounds. They can be incorporated into a synthetic strategy and be used in non-aqueous media thus becoming more attractive as more research is performed. This is a growing area of chemistry with significant interest. Though the mirror image of a synthetic chiral asymmetric catalyst can be easily obtained, complementary pairs of enzymes are being discovered and utilized. As shown enzymatic dynamic kinetic resolution can be used to produce intermediates in the synthesis of compounds with therapeutic effects.

References

1. i) Höglund, P.; Eriksson, T.; Björkman, S. *Journal of pharmacokinetics and biopharmaceutics* **1998**, *26*, 363–83.

ii) Mannschreck, A.; Kiesswetter, R.; von Angerer, E. *Journal of Chemical Education* **2007**, *84*, 2012.

2. Busacca, C. a.; Fandrick, D. R.; Song, J. J.; Senanayake, C. H. *Advanced Synthesis & Catalysis* **2011**, *353*, 1825–1864.

3. Finefield, J. M.; Sherman, D. H.; Kreitman, M.; Williams, R. M. *Angewandte Chemie (International ed. in English)* **2012**, *51*, 4802–36.

4. Hilker, I.; Rabani, G.; Verzijl, G. K. M.; Palmans, A. R. a; Heise, A. *Angewandte Chemie (International ed. in English)* **2006**, *45*, 2130–2.

5. Strauss, U. .; Felfer, U.; Faber, K. *Tetrahedron: Asymmetry* **1999**, *10*, 107–117.

6. Ebbers, E.; Ariaans, G.; Houbiers, J. P. M.; Bruggink, A.; Zwanenburg, B. *Tetrahedron,* **1997**, *53*, 9417–9476.

7. Pellissier, H. *Tetrahedron* **2011**, *67*, 3769–3802.

8. Anastas, P.; Bartlett, L.; Kirchhoff, M.; Williamson, T. *Catalysis Today* **2000**, *55*, 11–22.

9. Menger, F. M. *Accounts of Chemical Research* **1993**, *26*, 206–212.

10. i) Klibanov, A. M. *Accounts of Chemical Research* **1990**, *23*, 114–120.

For a more recent example using lipases successfully in non-aqueous media:

ii) Romero, C. M.; Pera, L. M.; Loto, F.; Baigori, M. D. *Catalysis Letters* **2012**, *142*, 1361–1368.

11. Gihani, M. T.; Williams, J. M. *Current opinion in chemical biology* **1999**, *3*, 11–5.

12. Nair, N.; Denard, C.; Zhao, H. *Current Organic Chemistry* **2010**, *14*, 1870–1882.

13. Mugford, P. F.; Wagner, U. G.; Jiang, Y.; Faber, K.; Kazlauskas, R. J. *Angewandte Chemie (International ed. in English)* **2008**, *47*, 8782–93.

14. Jaeger, K.-E.; Eggert, T. *Current opinion in biotechnology* **2002**, *13*, 390–7.

15. Sheldon, R. A. *Advanced Synthesis & Catalysis* **2007**, *349*, 1289–1307.

16. Runmo, A.; Pàmies, O.; Faber, K.; Bäckvall, J. *Tetrahedron letters* **2002**, *43*, 2983–2986.

17. Pàmies, O.; Bäckvall, J.-E. *Advanced Synthesis & Catalysis* **2002**, *344*, 947–952.

18. Kim, M.-J.; Chung, Y. Il; Choi, Y. K.; Lee, H. K.; Kim, D.; Park, J. *Journal of the American Chemical Society* **2003**, *125*, 11 494–5.

19. Vongvilai, P.; Larsson, R.; Ramström, O. *Advanced Synthesis & Catalysis* **2008**, *350*, 448–452.

20. i) Thalén, L. K.; Zhao, D.; Sortais, J.-B.; Paetzold, J.; Hoben, C.; Bäckvall, J.-E. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2009**, *15*, 3403–10.

ii) Shakeri, M.; Engström, K.; Sandström, A. G.; Bäckvall, J.-E. *ChemCatChem* **2010**, *2*, 534–538.

21. Arosio, D.; Caligiuri, A.; D’Arrigo, P.; Pedrocchi-Fantoni, G.; Rossi, C.; Saraceno, C.; Servi, S.; Tessaro, D. *Advanced Synthesis & Catalysis* **2007**, *349*, 1345–1348.

22. El Blidi, L.; Vanthuyne, N.; Siri, D.; Gastaldi, S.; Bertrand, M. P.; Gil, G. *Organic & biomolecular chemistry* **2010**, *8*, 4165–8.

23. Wen, W.; Ng, I.; Tsai, S. *Journal of Chemical Technology and Biotechnology* **2006**, *1721*, 1715–1721.

24. Fazlena, H.; Kamaruddin, a H.; Zulkali, M. M. D. *Bioprocess and biosystems engineering* **2006**, *28*, 227–33.

25. Westerbeek, Alja, et al.. *ACS Catalysis* **2011**. *1 (12)* 1654-1660.

26. i) Banerjee, A.; Kaul, P.; Banerjee, U. C. *Archives of microbiology* **2006**, *184*, 407–18.

ii) Xue, Y.-P.; Xu, S.-Z.; Liu, Z.-Q.; Zheng, Y.-G.; Shen, Y.-C. *Journal of industrial microbiology & biotechnology* **2011**, *38*, 337–45.

27. Rioz-Martínez, A.; de Gonzalo, G.; Torres Pazmiño, D. E.; Fraaije, M. W.; Gotor, V.  *J. org chem* **2010**, *75*, 2073–6.

28. Friest, J.; Maezato, Y.; Broussy, S. *Journal of the …* **2010**, 5930–5931.

29. Träff, A.; Lihammar, R.; Bäckvall, J.-E. *The Journal of organic chemistry* **2011**, *76*, 3917–21.

30. Han, K.; Kim, C.; Park, J.; Kim, M.-J. *The Journal of organic chemistry* **2010**, *75*, 3105–8.

31. Krumlinde, P.; Bogár, K.; Bäckvall, J.-E. *Chemistry (Weinheim an der Bergstrasse, Germany)* **2010**, *16*, 4031–6.