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The Synthesis of the High-Potency Sweetener, NC-00637. Part 1: The Synthesis of (*S*)-2-Methylhexanoic Acid

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Abstract:
The synthesis of the high potency sweetener candidate NC-00637 (1) required large quantities of (*S*)-2-methylhexanoic acid (2). This acid was first prepared in small quantities by the use of chiral auxiliaries. For large quantities, resolution by classical means and an enzymatic method were investigated. Asymmetric hydrogenation provided a workable solution.

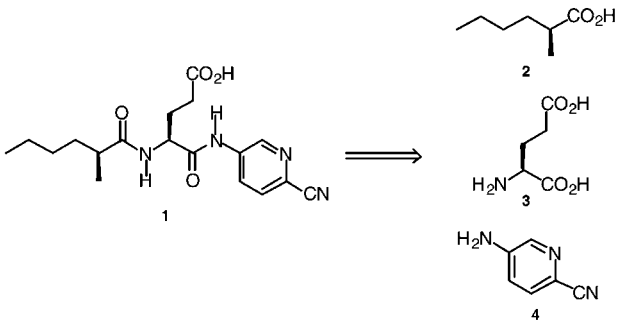
Introduction

Over the past decades, the human desire for sweet, while tempered by the trend for reduced calories, has seen increasing sales of high-potency, low-calorie sweeteners such as saccharin, aspartame, and acesulfam-K.[1] As part of a continuing effort to identify high-potency sweeteners with an excellent taste profile and increased stability compared to aspartame, the molecule NC-00637 (**1**) was identified as a potential candidate.[2,3] Before use as a sweetening agent, any candidate compound must undergo clinical studies to show that it is safe for human use and that there are no adverse biological effects; as a consequence, large quantities of compound are needed for these tests. As food additive studies require extensive animal studies—the only desired effect on humans in the case of NC-00637 (**1**) being sweet taste—a large-scale synthesis has to be "locked in" at a very early stage in development. In this way impurity profiles can be controlled. However, the short time frame for route development means that many potential routes and solutions cannot be investigated.

Inspection of the structure of NC-00637 reveals two obvious disconnections, the two amide bonds (Scheme 1).[4] The molecule can then be assembled in a convergent manner from the three components, (*S*)-2-methylhexanoic acid (**2**), L-glutamic acid (**3**), and the pyridinylamine **4**. Each of these components, or their subsequent coupling reactions, provided a synthetic challenge. This contribution discusses the methods used to prepare the deceptively simple acid **2**.

At the time this work commenced, there were no large-scale methods to access the hexanoic acid **2** by asymmetric synthesis. In addition to an asymmetric hydrogenation, a resolution approach using salt formation and enzymatic resolutions were investigated. However, there was an immediate need for small quantities of the target molecule, and we first investigated the use of chiral auxiliaries for the preparation of the acid **2**. This material was needed to determine which isomer of **2** was in the sweetener candidate as there was some ambiguity.[4]

Scheme 1



Preparation by Alkylation Methods

Although (*S*)-2-methylhexanoic acid (**2**) has a relatively simple constitution, the presence of a single asymmetric centre has made this the key contributor to the synthetic challenge. A number of methods do exist in the literature for the preparation of carboxylic acids with an asymmetric centre at the α-carbon atom (vide infra) including alkylation methods. It was not envisioned that these methods would provide a large-scale economical process, but they would solve the short-term need for a few grams of material. In addition, at this early stage, the enantiomeric excess (ee) required for the 2-methylhexanoic acid (**2**) that would lead to NC-00637 of acceptable diastereoisomeric purity was not known. We, therefore, strived in our initial studies to obtain as high an ee as possible. Subsequent studies showed that an ee of >90% was acceptable as in-process purifications removed the diastereoisomer derived from (*R*)-2-methylhexanoic acid.[4]

Oxazolidinone Approach. The first approach was based on the use of an oxazolidinone as an auxiliary.[5] In this study only the oxazolidinone **5** derived from valine was tried, and the methodology followed that reported by Evans for analogous compounds.[6] Thus, the oxazolidinone **5** was acylated with hexanoyl chloride after deprotonation with

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(1) Ager, D. J.; Pantaleone, D. P.; Henderson, S. A.;. Katritzky, A. R.; Prakash, I.; Walters, D. E. *Angew. Chem.* **1998**, *110*, 1901; *Angew Chem., Int. Ed.* **1998**, *37*, 1803.
(2) DuBois, G. E. Sweeteners, Nonnutritive. In *Encyclopedia of Food Science and Technology*; Hui, Y. H., Ed.; John Wiley: New York, 1991; Vol. 4, pp 2470−2487.
(3) Nofre, C.; Tinti, J.-M. U.S. Patent 5,196,540, 1993.
(4) The chemistry for other components of NC-00637 and the complete synthesis will be published soon.
(5) Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev.* **1996**, *96*, 835.
(6) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127.

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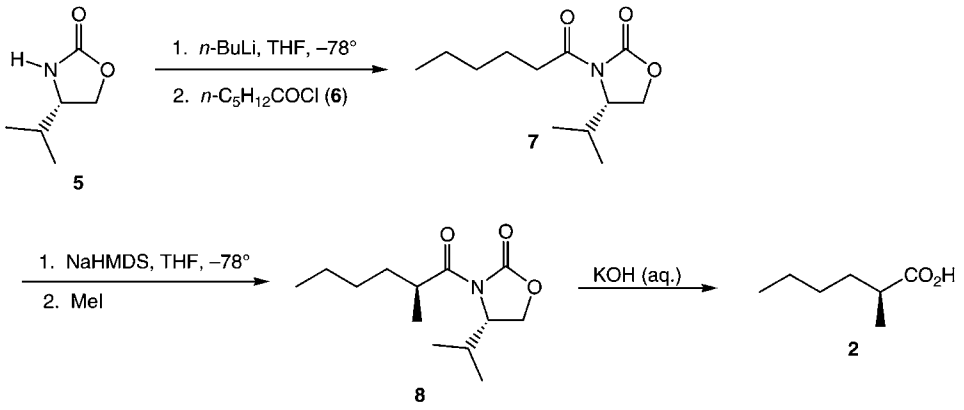
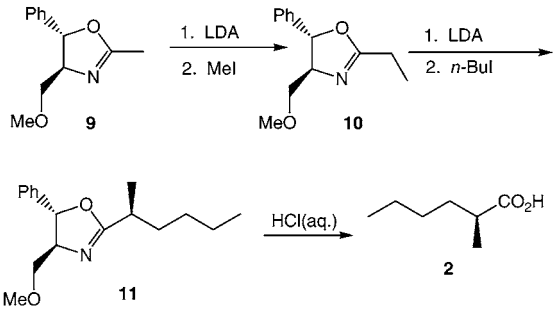
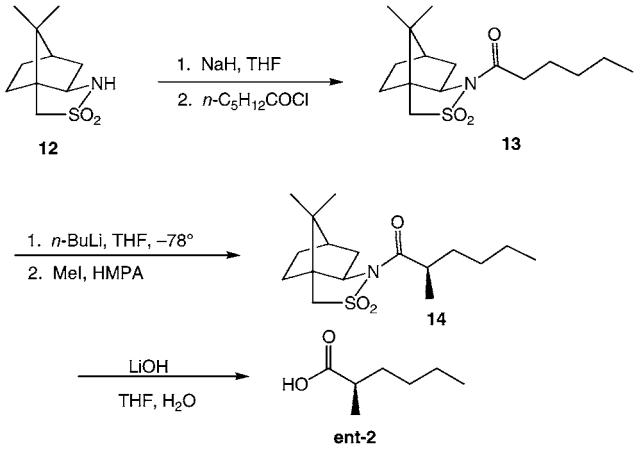
Scheme 2



Scheme 3



Scheme 4



n-butyllithium to give **7** (Scheme 2). The required acid **2** was produced in 80% ee. In this sequence, none of the intermediates was purified extensively, as the goal was to prepare the acid **2**.

Oxazoline Method. As a comparison, the oxazoline auxiliary developed by Meyers was used in a similar alkylation approach (Scheme 3).[7] The oxazoline **9** was methylated to provide the ethyl analogue **10**. A second alkylation then gave the dialkylated derivative **11** that was then hydrolyzed to the product **2**. By this sequence the desired acid was formed with an ee of 62%. A variant employing a camphor derivative has been reported to give higher ee's, but this was not attempted.[8]

Oppolzer's Sultam Auxiliary. The reactions used were standard for alkylations with Oppolzer's sultam as the chiral auxiliary (Scheme 4).[5] The sultam **12** was acylated by reaction with sodium hydride followed by hexanoyl chloride to give the *N*-acyl derivative **13** in 88% yield.[9] Deprotonation with *n*-butyllithium and subsequent reaction of the enolate with methyl iodide in the presence of HMPA gave the alkylated product **14** in about 75% yield. Hydrolysis of **14** gave (*R*)-2-methylhexanoic acid (**ent-2**) with an ee of 90%.[10] The ee of **ent-2** was lower if the acylation was performed with propionyl chloride and the alkylation with *n*-butyl iodide; the yield of the alkylation step was also significantly lower. It has been reported that sodium hexamethyldisilazide can be used as the base in the alkylation step and this avoids the use of HMPA,[9] but this was not attempted.

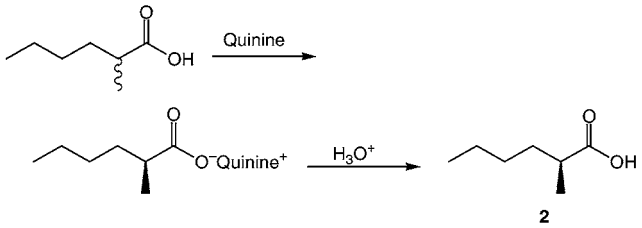
Although this method gave material of acceptable enantiomeric purity that was used to prepare small quantities of **1**, it was not going to be a viable for a large-scale synthesis. At the time this work was performed, we could only find 5 kg of the required sultam (**ent-12**), and the price made its use prohibitive without multiple recycles. Rather than embark on a programme to prepare the sultam, our efforts were concentrated on the resolution and catalytic methods.

Other Approaches. A number of other approaches were considered for the preparation of (*S*)-2-methylhexanoic acid (**2**). In some cases, as the target molecule **2** is relatively simple, there are many variations on a general theme. The use of other auxiliaries in an alkylation approach such as the use of pseudoephedrine[11] was not tried, as the shortcomings outlined above would not be overcome. Other approaches with chiral auxiliaries such as the use of menthone in an S_N2' cuprate displacement on a chiral carbonate[12] were not pursued due to time and resource constraints. The methodology used had to be "tried-and-tested" and familiar to us.

Reduction of 2-methylhexenoic acid (**15**), or an ester of this acid, by hydroboration was investigated. A conjugate

(7) Meyers, A. I. *Acc. Chem. Res.* **1978**, *11*, 3756 and references therein.
(8) Chandrasekhar, S.; Kauser, A. *Tetrahedron: Asymmetry* **2000**, *11*, 2249.
(9) Oppolzer, W.; Moretti, R.; Thomi, S. *Tetrahedron Lett.* **1989**, *30*, 5603.
(10) Oppolzer, W.; Marco-Contelles, J. *Helv. Chim. Acta* **1986**, *69*, 1699.
(11) Myers, A. G.; Yang, B. H.; Chen, H.; McKinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6496; Myers, A. G.; Yang, B. H.; Chen. H.; Gleason, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 9361.
(12) Spino, C.; Beaulieu, C.; Lafreniere, J. *J. Org. Chem.* **2000**, *65*, 7091; compare: Tseng, C. C.; Yen, S. J.; Goering, H. L. *J. Org. Chem.* **1986**, *51*, 2884; Tseng, C. C.; Goering, H. L. *J. Org. Chem.* **1983**, *48*, 3986.

Scheme 5



reduction was required and it was thought that the boron adduct could be reduced to the desired compound. Use of a chiral ligand on boron, as in $IpcBH_2$ could provide the asymmetric induction. All our attempts at hydroboration of **15**, even in the achiral series, were unsuccessful.

To obtain samples of the acid **2** and its enantiomer, and then prepare the intermediates, isomers, and other compounds needed to develop NC-00637 through the clinical phases, a chromatographic separation of racemic 2-methylhexanoic acid was undertaken as an external collaboration. The method was a direct scale-up of the analytical procedure described in the Experimental Section. The racemic 2-methylhexanoic acid was converted to the acid chloride and then condensed with the amino alcohol derived from D-phenylglycine.[13] Chromatographic separation of the diastereoisomers followed by hydrolysis gave material with >98% ee, but the route was tedious as the peak separation was not good. After this, the catalytic hydrogenation method outlined below was used.

Resolution

The resolution of 2-methylhexanoic acid has been reported in the literature by formation of the quinine salt (Scheme 5).[14] The process involved eight recrystallisations of the 2-methylhexanoic acid−quinine salt, and the yield was not reported. The racemate was commercially available (from Lancaster Synthesis) and could be produced at scale. A classical resolution of this type was investigated as it could provide kilogram quantities of material with a relatively cheap resolving agent being employed. It was envisioned that the quinine could be isolated by extraction and recycled if necessary. In addition, the *R*-isomer of the acid could be racemised by a variety of methods and then put back through the process. If the amount of quinine was reduced, the amount of salt that crystallized from the mixture was also reduced. As quinine gave us a cheap, workable method, no in-depth study was performed to see if other bases gave better performance.

In our initial, laboratory studies the first crop of crystals (55% based on the total amount of acid and base used) had an *R:S* content of 26:74. Crystallisation of this mixture resulted in a 75% recovery and an increase in the isomer ratio to 14:86. A further recrystallisation gave 86% recovery and an *R:S* ratio of 9:91. Further crystallizations did increase the *S*-content by 1−2%, but the losses were about 10 wt %. We, therefore, went with two recrystallisations and adjusted the amount of solvent to minimize losses. This also resulted in a slight increase in ee.

In our hands, the salt only had to be recrystallised twice from acetone to provide the acid **2** with an ee of 92%. In our case, GC analysis of the acid **2** by derivatisation was used to monitor the reaction rather than optical rotation that may account for the experimental differences, as our analyses were not susceptible to contamination by small amounts of quinine. The subsequent reactions to NC-00637 involve the formation of diastereoisomers, and the small amount of *R*-isomer of **2** was easily removed.[4]

This resolution method was the first to be successful at scale, and as it was simple, the method was used to make over 100 kg of the chiral acid **2**. It was reproducible at the larger scale and consistently gave material of the same purity. Scale-up was just a multiplication of amounts from the laboratory procedure. The yield of material after all manipulations was about 60% of theoretical. However, the time involved and volumes of solvents coupled with the need to move the process between plants indicated that recycles would be difficult. In addition, some salts were carried over to the distillation step and this made the pot residues very viscous and difficult to clean up. As a result, we continued to look for alternative approaches. However, our findings from this study did allow us to use the method to clean up the optical purity of material obtained by other methods, such as asymmetric hydrogenation.

Enzymatic Approaches. Considerable time and effort was spent looking at enzymatic approaches to **2**. As well as methods to obtain the acid directly, approaches that looked at the use of just one isomer of racemic 2-methylhexanoic acid in the coupling reactions were also investigated.[4] The desired acid **2** has been prepared by enzymatic hydrolysis of ethyl 2-methylhexanoate with PS-30.[15] The conversion was found to be good (45%), but the ee was only 74%. The ee could be increased by lower conversions. Obviously, the undesired isomer is hydrolyzed at a rate that has an affect on the product ee with longer reaction times. This was not considered to be a workable process due to the need for large recycle loops and the problems associated with isolation of the product from dilute aqueous solution.

A number of enzymes were screened with various esters of racemic 2-methylhexanoic acid. PPL, PFL, PLE, and other common lipases and esterases resulted in hydrolysis to provide racemic or up to 30% ee of 2-methylhexanoic acid when small esters were used, such as ethyl or methyl.[16] The *iso*-amyl esters generally gave better selectivity. The Amano PS30 lipase gave an *S*:*R* ratio of 91:9 when the *iso*-amyl ester was hydrolyzed at pH 9. The reaction was slow. A study that varied the reaction conditions resulted in an increase to 85% ee for the formation of **2** from the methyl ester. The enzyme could be recycled through a number of runs without loss of activity. However, the initial cost of the enzyme still did not provide a cost-effective method when compared to that for the quinine resolution or asymmetric hydrogenation. It was found that PS-800 performed much better than PS-30 to give a 94% ee at 55% conversion (of the *S*-isomer)

(13) Compare: Karl, V.; Kaunzinger, A.; Gutser, J.; Steuer, P.; Angles-Angel, J.; Mosandl, A. *Chirality* **1994**, *6*, 420.
(14) Levene, P. A.; Bass, L. *J. Biol. Chem.* **1926**, *70*, 211.
(15) Engel, K.-H. *Tetrahedron: Asymmetry* **1991**, *2*, 165.
(16) Compare: Ozaki, E.; Uragaki, T. Jpn. Patent 09065891; *Chem. Abs.* **1997**, *127*, 276440.

with an 8-fold decrease in the amount of enzyme needed. The reaction still took over 3 days.

As an alternative to hydrolysis of an ester, ester formation was also considered; again this is known in the literature for the esterification of racemic 2-methylhexanoic acid using *Candida cylindracea* lipase (CCL)[11] and by the transesterification of racemic octyl 2-methylhexanoate with oleic acid and CCL.[17] In esterification or transesterification reactions, the conversions were poor (8−15%), but better *R:S* ratios (>10:90) are obtained. For the same reasons as outlined for the hydrolysis reactions, these approaches were not considered further.

In both the hydrolysis and ester formation approaches, a cosolvent such as DMSO had the advantage to ensure contact of reagents with the enzymes. Use of this cosolvent complicated the isolation procedure. In the hydrolysis reactions, it was found that filtration of the enzyme solution prior to the first run removed much of the material that gave rise to emulsions during the isolation steps. Emulsions were still a major problem and with better approaches available, the enzymatic methods were dropped.

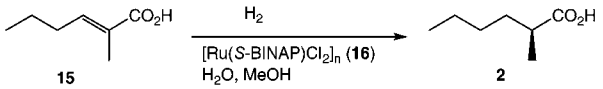
Hydrogenation Approach. At the commencement of this work in the early 1990s, there were relatively few asymmetric hydrogenation catalysts although a number of reports had appeared in the literature describing the reductions of α,β-unsaturated carboxylic acids to the corresponding saturated acids with high enantiomeric excesses.[18] In particular, ruthenium−BINAP complexes have proven expeditious for this transformation.[19]

The two available to us, through Monsanto, were Knowles' catalyst[20] and a ruthenium−BINAP system that was under development for the synthesis of naproxen.[21−23] With our eyes on a robust process, 2-methyl-2-hexenoic acid (**15**) was chosen as the only option for substrate. The other possibility was to use an *exo*-methylene analogue, but this was potentially problematic due to isomerisation of the unsaturation into the more substituted position. In addition, the acid **15** is available as the *E*-isomer as it is used in the flavour industry.

Knowles' catalyst, [Rh(COD)DIPAMP]BF_4, did reduce the α,β-unsaturated acid **15** under a variety of conditions, but in all cases racemic 2-methylhexanoic acid resulted.

The ruthenium catalyst of the type [Ru(*S*-BINAP)XY]$_n$ (where X and Y are either organic or halogen ligands [Our catalyst has X = Y = Cl.]) does provide for useful asymmetric induction to provide **2** (Scheme 6).[22,23] In our case the catalyst was [Ru(*S*-BINAP)Cl_2]$_n$ (**16**). The hydrogenation was carried out in a mixture of methylene chloride, methanol, and water at varying amounts. The catalyst and substrate concentrations were also varied. In all cases, the reaction was asymmetric with ee's of ≥80%.

Scheme 6



Although the ee was not high, as noted above, the selectivity was workable as downstream processing allowed for purification as diastereoisomers are formed.

The reaction was scaled up to >50-kg runs, and at scale, the ability to exclude oxygen was increased. This allowed the amount of catalyst to be decreased significantly. Compared to catalyst usage in the laboratory runs, usage was reduced by about 100-fold at scale. In addition, the amount of solvent could also be reduced so that almost a third of the reactor content was substrate. Degassing of the substrate in solution was relatively straightforward. However, the sequence involved multiple purges, evacuations, and gas-filling steps. As oxygen was present especially at the start of the sequence, the catalyst did not survive if added to the substrate prior to oxygen removal. A method was therefore required to introduce the catalyst after the solution had been purged. There are engineering solutions, but these were not considered to be general so that the methodology can be run in a number of plants and the method can be readily transferred. This eliminated such solutions to the problem as the use of a basket in the reactor. Our solution was to use small cans that are similar to those used for dispensing liquids for high-pressure chromatography or carbonated soda (Figure 1). This approach relies upon the observation that the solid ruthenium catalyst is air-stable in the solid state.

The Ru(BINAP) catalyst (**16**) was placed as a solid in a can linked to the reactor. The headspace could be deoxygenated through the reactor or in a separate sequence. In another can was placed methylene chloride that was also deoxygenated. Once the reactor, lines, catalyst container, and methylene chloride were deoxygenated, the methylene chloride was pushed under nitrogen pressure into the container with the catalyst. The mixture was then agitated—this was usually done by a magnetic stirrer bar that worked through the can even on gallon scale. Laboratory experiments had shown that dissolution was rapid in methylene chloride. After about 0.25 h, the catalyst solution was again pushed by nitrogen pressure into the reactor, which was then put under hydrogen. Because the reaction does not start until being heated, the agitation during the heat-up period was sufficient to allow dispersion and dissolution.

Some Observations on the Reaction and Mechanism

The 2-methylhexenoic acid (**15**) used for these reductions was exclusively the *E*-isomer (>99%). This material is used in the flavour industry and is prepared by a Wittig reaction. We spent a little time looking at the preparation of **15** and found that a Claisen condensation gave variable isomeric ratios. A Knoevenagel approach gave low yields (<50%) due to self-condensation of the aldehyde. A Perkin reaction

(17) Engel, K.-H. J. *Am. Oil Chem. Soc*. **1992**, *69*, 146.
(18) *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: Orlando, 1985; Vol. 5.
(19) Mashima, K.; Kusano, K.; Ohta, T.; Noyori, R.; Takaya, H. *J. Chem. Soc., Chem. Commun*. **1989**, 1208; Noyori, R.; Takaya, H. *Acc. Chem. Res.* **1990**, *23*, 345; Noyori, R. *Science* **1990**, *248*, 1194; Takaya, H.; Ohta, T.; Mashima, K.; Noyori, R. *Pure Appl*. **1990**, *62*, 1135; Noyori, R. *Chem. Soc. Rev*. **1989**, 187; Uemura, T.; Zhang, X.; Matsumura, K.; Sayo, N.; Kumobayashi, H.; Ohta, T.; Nozaki, K.; Takaya, H. *J. Org. Chem.* **1996**, *61*, 5510; Zhang, X.; Uemura, T.; Matsumura, K.; Sayo, N.; Kumobayashi, H.; Tayaya, H. *Synlett* **1994**, 501.
(20) Knowles, W. S. *Acc. Chem. Res.* **1983**, *16*, 106.
(21) Chan, A. S. C.; Laneman, S. A. U.S. Patent 5,198,561, 1993.
(22) Chan, A. S. C.; Laneman, S. A. U.S. Patent 5,202,473, 1993.
(23) Chan, A. S. C. *CHEMTECH* **1993**, *23*, 46.

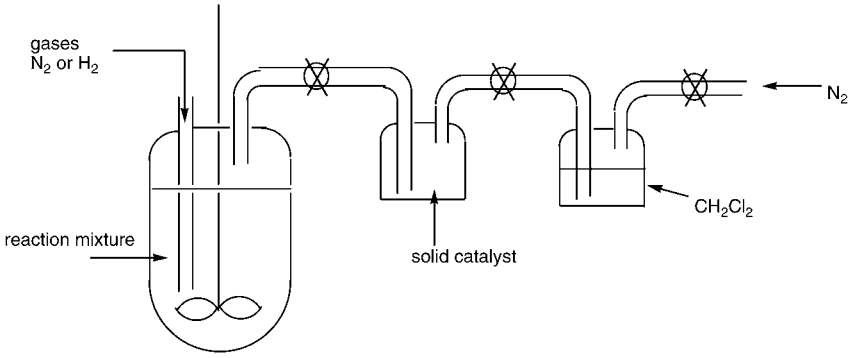


Figure 1. **Reactor configuration for hydrogenations.**

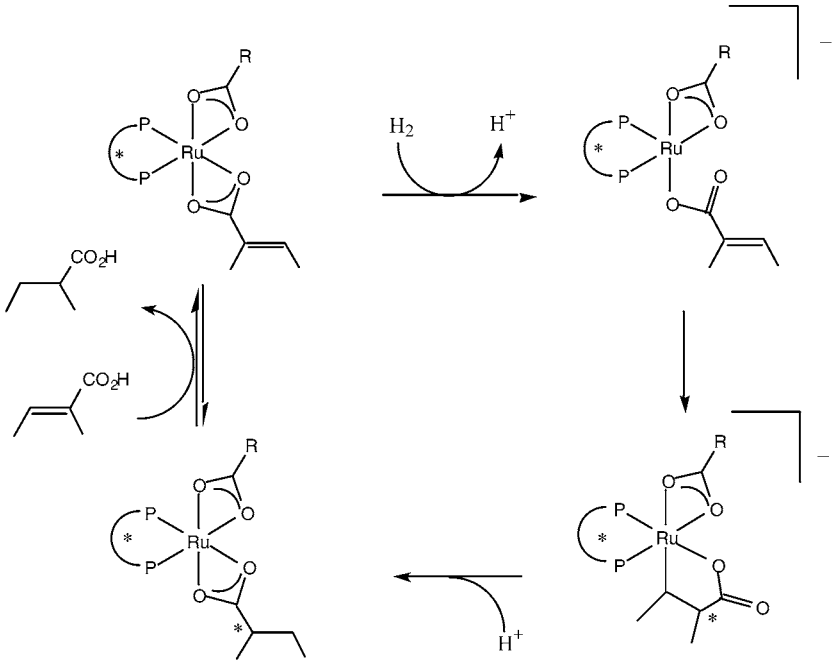


Figure 2. **Mechanistic scheme for the [Ru(BINAP)(O_2CR)$_2$]-catalyzed hydrogenation of tiglic acid.**

between butyraldehyde and propionic anhydride in the presence of pyridine as base gave almost exclusively the desired *E*-isomer of **15**, but the yield (∼60%) was lower than that from a Wittig-type approach, and purification was tedious and required multiple distillations. The Wittig approach was the low-cost route. Our supplier, Bedoukian Research, used this approach.

Studies on the reduction of tiglic acid with Ru(BINAP)-$(OAc)_2$ have shown that the hydrogen addition to the carbon−carbon double bond is not solely derived from a hydrogen molecule; the β-hydrogen comes from gaseous hydrogen while the proton is acquired from the solvent.[26] This was confirmed to be correct in our case by deuterium-labeling experiments with deuterium gas and deuterium oxide.

The rate was reported to be first-order with respect to hydrogen uptake.[27] A mechanism for Ru(BINAP)(O_2CR)$_2$-catalyzed reduction of an enoic acid has been postulated on the basis of the kinetics of these catalytic hydrogenations.[27] Displacement of the ligand on the catalyst by the substrate, in this case, tiglic acid, is followed by reduction of the double bond and the dissociation of the product from the catalyst (Figure 2). A number of assumptions were used to derive the rate law from this catalytic cycle: The carboxylate substitution equilibrium step is rapid, subsequent addition of H_2 is turnover-limiting, and concentration of the catalyst is low compared to that of the substrate.

A study was made of the effects of a number of variables including temperature, concentration, addition of base, and solvents for the hexenoic acid derivative at laboratory scale. There was no observable effect from the addition of base, unlike the case with Noyori-type catalysts or in the synthesis of naproxen.[23] Pressures lower than those needed to obtain high ee with naproxen could be used with the same catalyst system and the enoic acid. The catalyst concentration was not completely optimized but was derived from experiments where the amount was halved—all other conditions were kept constant—until the reaction time became intolerable. These data were used as the starting point for the larger-scale

(24) Chan, A.; Laneman, S. U.S. Patent 5,144,050, 1992.
(25) Chan, A. U.S. Patent 4,994,607, 1991.
(26) Ohta, T.; Takaya, H.; Noyori, R. *Tetrahedron Lett.* **1990**, *31*, 7189.
(27) Ashby, M. T.; Halpern, J. *J. Am. Chem. Soc.* **1991**, *113*, 589.

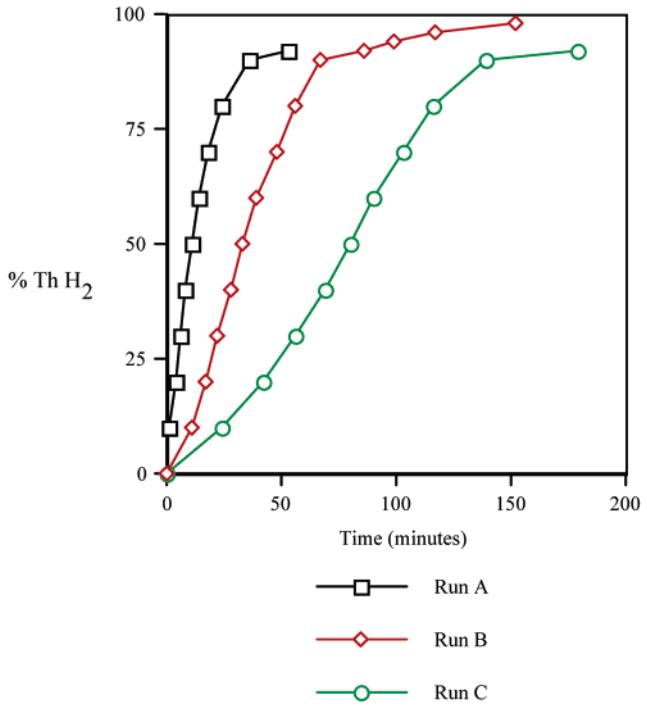


Figure 3. **% Theoretical hydrogenation uptake vs time for the hydrogenation of 2-methyl-2-hexenoic acid with [Ru(*S*-BINAP)Cl_2]$_n$.**

reductions, and as stated above, the amount of catalyst could be significantly decreased. Again, this was achieved by halving the amount of catalyst used in a run until hydrogen uptake did not occur after a few minutes at 80 °C. If this failure occurred, addition of more catalyst then allowed the reduction to proceed.

The theoretical percentage of hydrogen uptake was monitored over numerous runs and the curve is quite unusual. It has an inflection point at ~50% theoretical hydrogen (Figure 3). All of the asymmetric hydrogenations show a similar phenomenon, complete with inflection point. The variability was often due to deoxygenation or other variables that could not be quantified, as under identical conditions, significant variations were observed in laboratory runs. Figure 3 shows three runs under identical experimental conditions including the same lots of catalyst, substrate and solvents. At larger scale, this variation was considerably less noticeable. The inflection point does not differ significantly from 50% even when the catalyst loading is varied. The hydrogen uptake begins immediately upon addition of catalyst in Halpern's tiglic acid case.[27] In our system, there is a slight lag time even after the reaction solution reaches the 80 °C reaction temperature. Figure 4 shows a computer printout of a monitored hydrogenation.

Our results do not fit Halpern's proposed rate equation, which is first-order in alkene and hydrogen. Figure 5 shows representative plots of experimental hydrogen uptake derived from Figure 3 with an additional run, again, under identical conditions. From this a plot of hydrogen concentration, as $\ln[(H_2\ \text{uptake})_\infty - (H_2\ \text{uptake})_t]$ vs time should give a straight line if Halpern's hypothesis holds for our system.[27]

Reactions were stopped at various stages, and the mixtures were analyzed by GC. The hydrogen uptake was found to

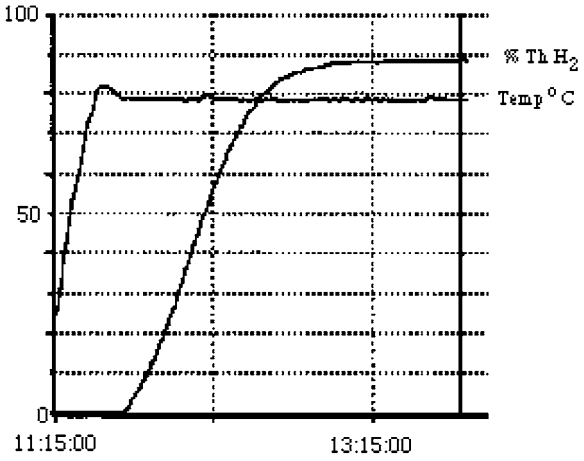


Figure 4. **Computer printout of % theoretical hydrogenation uptake.**

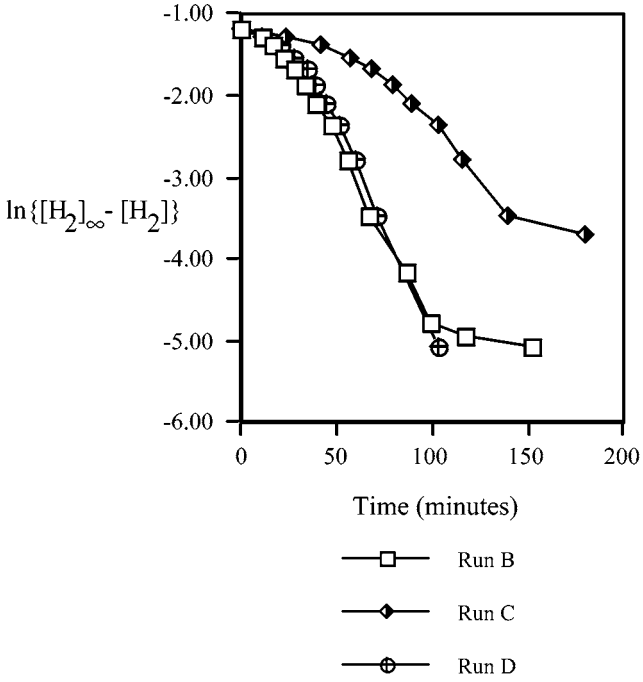


Figure 5. **Plot of H_2 uptake vs time for the Ru(BINAP)Cl_2-catalyzed hydrogenation of 2-methyl-2-hexenoic acid (15).**

correspond to the amount the reaction had proceeded when compared to the amounts of **15**, **2**, and **ent-2** present. In addition, the amount of asymmetric induction was found to be constant throughout the reaction.

The hydrogenation was also monitored by FT infrared spectroscopy in a modified Fischer−Porter apparatus. Thus, the hydrogen uptake could also be monitored during the reaction. The IR spectra of the substrate and the product are sufficiently different in the carbonyl region and at 1200−1100 cm^{-1} to allow the reaction to be followed. The plot of percent theoretical hydrogen uptake showed the usual inflection point at about 50% of theory. The superimposed spectra (Figure 6) in the carbonyl region show that the substrate disappears during the reaction without any significant changes in rate, while the product builds from the zero time point throughout the reaction.

The data from the IR study and hydrogen uptake showed the same kinetics. This gave us the confidence that either

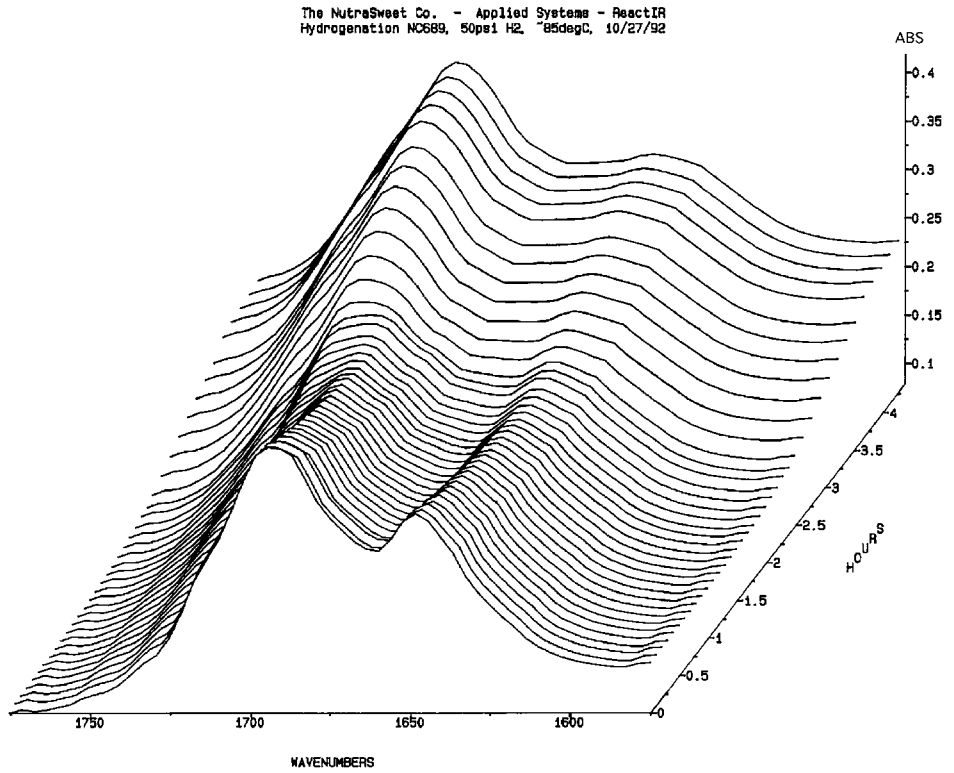


Figure 6. **Superimposed infrared spectra.**

method could be used to monitor the reaction. Why our system that uses the catalyst **16** differs from the findings of Halpern with tiglic acid could be due to a number of reasons and factors: In the tiglic acid system, reduction was observed as soon as hydrogen pressure was applied, whereas our system required heating to 80 °C. The catalyst precursor **16** is polymeric in nature, unlike that used in Halpern's studies, and some dissociation to an active species may have to occur. Thus, as the reaction progresses, more and more catalyst is entering the catalytic cycle. For the reduction of the hexenoic acid **15**, the concentration of substrate is much higher than for the tiglic acid study, and thus, some factors that were not important for tiglic acid may become noticeable. Certainly oxygen inhibition seems to play an important role as the amount of catalyst needed at larger scale was 2 orders of magnitude less than that for laboratory-scale runs. In addition, it was found that 25 purges were more than sufficient to ensure removal of oxygen.

Obviously, more studies could be performed with the $[Ru(BINAP)Cl_2]_n$ catalyst system to determine why there is run-to-run variability as well as to unravel the mechanism, but time did not allow this.

As studies were continuing towards the target molecule, NC-00637, while these reactions were being performed, it was found that a 90% ee for **2** was acceptable because the diastereoisomers resulting from reaction of **ent-2** were simple to remove in subsequent steps.[4]

It was found that the addition of quinine to the acid **2** prior to the final distillation did not result in an increase in ee of the final product, but if quinidine (~10 mol %) were added, then ee's of 95−98% could be achieved. Presumably this is because the quinidine is a pseudoenantiomer of quinoline and forms a salt with the *R*-isomer **ent-2**. However, this method was not used at scale due to the problems with the residue as seen during the quinine resolution approach (vide supra).

One interesting observation was that in one 22-kg run, after distillation of **2**, it turned blue on exposure to air. Although metal ions could not be detected in the material, redistillation gave a colourless, air-stable product.

Summary

A large-scale method was required to obtain (*S*)-2-methylhexanoic acid (**2**), a component of the sweetener candidate, NC-00637 (**1**). The use of chiral auxiliaries provided the small quantities necessary for preliminary biological testing. For larger amounts of the acid **2**, a classical resolution approach using quinine was found to be workable. Although a large number of methods were explored for enzymatic resolution, none were found to be cost-effective compared to alternatives. Asymmetric hydrogenation of the enoic acid **15** did provide a direct asymmetric approach to **2** although only 90% ee could be attained. This was not a problem as subsequent reactions to **1** removed the undesired isomer.

Experimental Section

The melting points are uncorrected. IR spectra (Nujol) were recorded on a Nicolet FT-IR spectrometer. ^1H NMR spectra were recorded on a GE 300 spectrometer in $CDCl_3$ using TMS as internal standard. Optical rotation was measured on a Perkin-Elmer 241 Polarimeter.

Laboratory hydrogenations were performed in mechanically stirred Parr reactors with external heating. In some

cases, Fischer−Porter bottles fitted with appropriate inlet and outlet tubes and valves were used for screenings; external heat was applied through an oil batch, and stirring was magnetic. The systems included pressure sensors that were computer-monitored to allow for the reactions to be followed by hydrogen uptake.

Racemic 2-methylhexanoic acid was purchased from Lancaster Synthesis. The 2-methyl-2-hexenoic acid was purchased from Bedoukian Research, Danbury, CT.

Analytical Methods. Unless otherwise noted, the method used to determine ee's was an automated, precolumn derivatisation procedure using 1-3(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP), and *R*-phenylglycinol and a Hewlett-Packard 1090 HPLC system. A Supelco LC-8, 3 μ, 25 mm × 46 mm i.d. column was used at 40 °C. The mobile phase was Millipore water (solvent A) and acetonitrile (solvent B) with detection at 210 nm. The column was equilibrated with 77% A and 23% B. After sample injection (10 μL), the system was held at these conditions for 15 min followed by 18 min with a gradient change to 0% A and 100% B. These conditions were held for another 4 min followed by equilibration with 77% A and 23% B. Retention times were 13.4 and 15.6 min for the *R*- and *S*-isomers, respectively.

An achiral method was achieved by diluting a sample with water or mobile phase. This was then injected (15 mL) onto a Supelcosil LC-18 5 mm, 25 cm × 4.6 mm column eluting at 1 mL/min with a mobile phase comprising buffer (800 mL of 0.01 M potassium phosphate, monobasic and 0.001 M tetrabutylammonium phosphate) and acetonitrile (200 mL). Detection was at 200 nm. 2-Methylhexanoic acid had a retention time of 5.2 min, and the 2-methyl-2-hexenoic acid, 6.8 min.

A GC method was used for final and in process analyses. Column was a Supelco permethylated cyclodextrin polysiloxane fused silica capillary (60 m × 0.25 mm i.d., 0.25 micrometer film) at 130° (isothermal). Detection was by flame ionization. Samples were injected at ∼1 mg/mL in acetonitrile. Septum purge was 2 mL/min, column flow 0.6 mL/min, split vent 25 mL/min, aux gas 30 mL/min, air 330 mL/min, H_2 30 mL/min. Injector temperature was 250 °C and the column head pressure ∼21 psi. Retention times were *S*-2-methylhexanoic acid 17.99 min, *R*-2-methlyhexanoic acid 18.65 min, *Z*-2-methyl-2-hexenoic acid 21.94, min and *E*-2-methyl-2-hexenoic acid, 23.57 min.

Oxazolidinone Route. To a solution of the oxazolidinone **5** (2.5 g, 19.4 mmol) in dry THF (65 mL) was added 1.6 M *n*-butyllithium in hexane (13.3 mL, 21.3 mmol) at −78 °C. After stirring for 30 min, hexanoyl chloride (**6**) (2.87 g, 21.3 mmol) was added neat. The mixture was warmed to room temperature and stirred overnight. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate. The combined organic phase was washed with brine and dried ($MgSO_4$). After removing solvents, 4.36 g of crude product **7** was obtained (99%).

The *N*-acyl compound **7** from the previous step was dissolved in dry THF (75 mL) and cooled to −78 °C when 1 M $NaN(SiMe_3)_2$ in THF (21.1 mL, 21.1 mmol) was added slowly. After 30 min, MeI (13.6 g, 96 mmol) was added, and the solution was warmed to −20 °C. At this time, saturated aqueous NH_4Cl was added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The extracts were washed with brine and dried ($MgSO_4$). Evaporation of the solvents under vacuum gave 4.05 g (91%) of crude product **8**.

The compound **8** from the previous step was dissolved in MeOH (34.5 mL) and stirred at 0 °C. Then 2 N KOH (34.5 mL)was added and the mixture stirred for 45 min. The mixture was then extracted with ethyl acetate (2 × 25 mL). The aqueous phase was acidified to pH 2 and extracted with ethyl acetate (3 × 30 mL). The combined extracts were dried (Na_2SO_4) and evaporated to give the product **2** liquid with an ee of 80%.

Oxazoline Route. To a solution of diisopropylamine (2.9 mL, 21 mmol) in dry THF (54 mL) at −78 °C was added 1.6 M *n*-butyllithium in hexane (13.1 mL, 21 mmol). After 20 min, a solution of the oxazoline **9** (4.1 g, 20 mmol) in THF (19 mL) was added portionwise over 15 min. After stirring for 45 min, MeI (1.5 mL, 24 mmol) in THF (10 mL) was added slowly. After an additional 2 h, the mixture was warmed to −50 °C and poured into ice−water (250 mL). The mixture was extracted with ether (3 × 40 mL). The combined extracts were washed with brine and dried ($MgSO_4$). After removal of the solvents under reduced pressure 4.04 g of product **10** was obtained (92%).

To a solution of diisopropylamine (2.7 mL, 19.2 mmol) in THF (20 mL) at 0 °C was added 1.6 M *n*-butyllithium (12 mL, 19.2 mmol). After 10 min, this solution was added dropwise to a solution of **10** in THF (50 mL) at −78 °C. After 30 min, *n*-butyl iodide (2.5 mL, 21.9 mmol) was added dropwise and stirred for 2 h. After warming to room temperature, the mixture was poured into ice−water (100 mL), which was extracted with ether (2 × 120 mL). The combined extracts were dried ($MgSO_4$). The crude product was purified by chromatography (SiO_2 eluting with 20% EtOAc/hexanes). About 2.7 g of product **11** was obtained (67%) along with 0.78 g of starting material **10**.

To 4.5 N HCl (36 mL) was added 1.8 g of **11**, and the resultant mixture was heated under reflux for 3.5 h. After cooling to room temperature, the mixture was extracted with ether (2 × 80 mL) and dried ($MgSO_4$). After removal of the solvents in vacuo, the acid **2** was purified by distillation (bulb-to-bulb). The *S/R* isomer ratio was 81/19 and gave 1.0 g.

Sultam Route. Sodium hydride (0.48 g of a 50% suspension in oil, 10 mmol) was washed with dry THF (2 × 5 mL) under nitrogen. The washings were removed by decantation. Dry THF (25 mL) was then added to the solid and the mixture cooled in an ice bath. A solution of the sultam **12** (2.15 g, 10 mmol) in THF (50 mL) was added dropwise over 30 min and then stirred for an addition 1 h. Saturated aqueous NH_4Cl solution (100 mL) was added and the mixture extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with 1 M NaOH (2 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered, and

evaporated to give the crude acyl derivative **13** (2.76 g, 88%) which was used without further purification.

To a solution of the sultam **13** (2.50 g, 7.98 mmol) in THF (35 mL) that had been cooled to −78° was added 1.6 M *n*-butyllithium in hexane (5 mL, 8 mmol). The mixture was stirred for 0.5 h when a solution of methyl iodide (0.5 mL, 5 mmol) in HMPA (3 mL) was added. The reaction was allowed to come to ambient temperature over 2 h. Saturated aqueous NH_4Cl solution (100 mL) was added and the mixture extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with water (2 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered, and evaporated to give the crude alkylated acyl derivative **14** (1.96 g, 75%) which was used without further purification.

The alkylated product **14** (1.96 g, 5.99 mmol) was dissolved in a solution of lithium hydroxide (0.143 g, 12 mmol) in 1:1 aqueous THF (50 mL). The reaction was monitored by TLC (SiO_2, 20% EtOAc/hexanes). After stirring at ambient temperature for 3h, the solution was acidified with 2 M hydrochloric acid to pH 2. The mixture was extracted with ethyl acetate (3 × 30 mL), dried (Na_2SO_4), filtered, evaporated under reduced pressure, and then distilled (bulb-to-bulb) to give the product (0.43 g, 60%) which had an *R:S* isomer ratio of 95:5.

Resolution with Quinine.[14] To a solution of racemic 2-methylhexanoic acid (78 g, 0.6 mol) in acetone (600 mL) was added quinine (194.4 g, 0.6 mol) and the mixture heated to reflux. The clear solution was cooled to room temperature, and the crystallized solid was collected by filtration, washed with acetone (60 mL), and dried in air. This solid was recrystallized two more times with acetone (2 × 360 mL). The resulting solid was added to hydrochloric acid (1 N, 400 mL). The clear solution, thus obtained, was extracted with ethyl acetate (2 × 90 mL), washed with water (100 mL), and concentrated on a rotary evaporator to get crude *S*-2-methylhexanoic acid (31.4 g, 80%). Distillation of the crude product at 48−49 °C/0.12 mm gave pure *S*-2-methylhexanoic acid as a colorless oil (23.48 g, 60%, *R:S* ratio 4:96).

Large Scale. In a 30-gal reactor, racemic 2-methylhexanoic acid (7.8 kg) and quinine (19.4 kg) in acetone (60 L) were heated under reflux for 1 h. The mixture was cooled to ambient temperature overnight. The solid was collected on a Nutsche filter, rinsed with acetone (6 L), and dried in a nitrogen stream. This solid was then added to acetone (36 L) and heated under reflux for 1 h after which time visible inspection showed that all solids had dissolved. The mixture was cooled to ambient temperature over 4 h. The solid was collected on a Nutsche filter, rinsed with acetone (5 L) and dried under a nitrogen stream. This last recrystallisation was then repeated. The resultant solid was added to 1 N HCl (40 L) and extracted with EtOAc (2 × 90 L). The combined extracts were washed with water (10 L). The EtOAc was removed by vacuum distillation to give the crude acid **2** (∼3.0 kg). Redistillation was performed as described for asymmetric hydrogenations in a thermosyphon.

Enzymatic Methods. Enzymatic screening studies were carried out in 2-mL HPLC vials containing several small glass beads to aid mixing. Racemic 2-methylhexanoic acid ester (0.5 mmol) was added to 0.1 M potassium phosphate buffer (pH 7.0) in order to make a final volume of 1.0 mL. Enzymes to be screened were first prepared by dissolving 50 mg in 10 mM phosphate buffer (5 mL) at pH 7.0. After dissolution, the reaction was initiated by the addition of 50 μL of enzyme solution. The vials were sealed with Teflon caps and placed in a rotary shaker (300 rpm) overnight at ambient temperature. At the end of the incubation period, 20 μL of 6 N HCl was added followed by ether (1.0 mL). After mixing, the ether was removed and the extraction repeated. The combined organic extracts were evaporated to dryness and dissolved in acetonitrile (0.1 mL) for HPLC analysis.

pH Stat Reaction. Racemic ethyl 2-methylhexanoate (1.37 g, 8.7 mmol) was mixed with potassium phosphate buffer (9.0 mL of 5.0 mM solution, pH 8.0). The mixture was vigorously stirred at room temperature and the reaction initiated by the addition of solid PS-30 lipase (Amano). The reaction pH was maintained by the addition of 1 N NaOH using a Radiometer pH stat system. After the rate of base addition leveled off, the reaction was terminated by acidification to pH 2.0 with 6 N HCl. Extraction in a manner similar to that described in the screening experiments provided material for analysis.

To investigate isolation procedures this reaction was also performed with 70 g (0.44 mol) of the ester in 5.0 mM buffer (0.93 L, pH 7.0). The enzyme was added as a solid (3.5 g).

Asymmetric Hydrogenation. Ruthenium−(*S*-BINAP) catalyst, $[Ru(S\text{-BINAP})Cl_2]_n$ (0.135 g) was placed in a 450-mL pressure vessel. Methylene chloride (27 mL) was placed in a second 450-mL pressure vessel. Then methanol (225 mL), water (27 mL), and 2-methyl-2-hexenoic acid (40.5 g) were placed in a third 450-mL pressure vessel. Each vessel was purged 100 times with nitrogen. The methylene chloride was then pushed over to the catalyst by a positive nitrogen pressure. The resultant mixture was stirred until the catalyst dissolved. The water, methanol, and substrate mixture was then added to the catalyst solution by use of a positive nitrogen pressure. The reaction mixture was purged four times with hydrogen. The reaction vessel was charged to 50 psig with hydrogen and heated to 80 °C. The reaction was run until the theoretical amount of hydrogen had been taken up. The reaction vessel was purged with nitrogen. The reaction mixture was concentrated by distillation under vacuum (up to 40 °C/25 mmHg). The crude product was then purified by vacuum distillation (bp 65−70 °C/0.2 mmHg). The isolated yield of the product **2** was typically 85%. The product was analyzed by derivatisation; the acid chloride was prepared by reaction with thionyl chloride, followed by reaction with (*S*)-α-phenylethylamine. The diastereoisomeric ratio was determined by HPLC or GC. The ratio of *S*:*R* varied from 86:14 to 92:8, a typical run giving around 90:10. $[\alpha]_D = +17.4°$ (neat): NMR ($CDCl_3$) δ 0.9 (3H, t) 1.18 (3H, d), 1.3−1.8 (6H, m), 2.47 (1H, sex), and 11 (1H, br).

Large Scale. At larger scale, the catalyst was dissolved in CH_2Cl_2 and this solution was then added to the substrate

in aqueous methanol (Figure 1). In one 2-gal canister was placed [Ru(*S*-BINAP)Cl_2]$_n$ (**16**) (22.68 g) and a magnetic stirrer bar. Methylene chloride (5.2 L) was placed in the second canister. These tanks were purged 25 times with N_2 up to 30 psig, releasing the pressure at the end of the sequence. In a 20-gal autoclave fitted with a dip tube for gas introduction, was charged the enoic acid **15** (6.8 kg), methanol (38 L), and DI water (4.5 L). The reactor was purged three times with N_2 to 10 psig through the dip tube. When all purges were complete, the CH_2Cl_2 was pushed into the catalyst canister by N_2. The mixture was stirred for 15 min. Again by use of N_2 pressure, the catalyst solution was added to the reactor. The reactor was purged by pressurizing to 100 psig for 2 min through three cycles to ensure that there were no gas leaks. The system was then purged with H_2 through four cycles. Hydrogen pressure was adjusted to 50 psig. The reaction was heated to 80 °C. The hydrogen pressure was monitored by a pressure transducer and the reactor filled up from a reservoir as needed to keep the pressure at 50 psig. When hydrogen uptake ceased, the hydrogen was vented, and the system was purged three times with N_2 (20 psig). The contents of the reactor were transferred to 50-gal drums, rinsing with methanol (10 L).

For one reaction a 200-gal autoclave was used. In this case, the amount of catalyst was kept the same so that the 2-gal canisters could be used. Thus, the catalyst **16** (22.68 g) was dissolved in CH_2Cl_2 (5.2 L) as above. This was added to a purged solution of the enoic acid **15** (51 kg) in methanol (180 L) and water (30 L). All other operations were performed as described for the 20-gal series.

Workup of these reactions was performed at a different site. The contents of two drums were charged to a 100-gal reactor system, rinsing with MeOH (2 L). This was then put under vacuum (~25 in. Hg) and warmed to 30 °C to remove most of the methanol. When ~70 kg remained, the contents of the reactor were transferred to a 30-gal reactor system. The volume was reduced again under vacuum, heating to 30 °C. When the contents of the reactor were about 45 kg, the vacuum was released and the mixture allowed to stand for 0.5 h. The lower layer was removed to waste. The upper layer was placed in a 50-L thermosyphon, rinsing in with methanol (5 kg). Silicone oil (15 kg) was added as chaser. If the volume was too large, the crude **2** was added as methanol was removed. The last amounts of methanol were removed under vacuum with gentle heating. The product was collected >100 °C at 28−29 in. Hg. Overall yields were cumulative and ~75%.

Kinetic Experiments. The hydrogenations were run in both Fischer−Porter bottles and Parr reactors. In both cases pressure transducers were used to monitor the hydrogen pressure. All solvents and substrates were degassed with nitrogen prior to use. In a typical run, 2-methyl-2-hexenoic acid (36.7 g, 316 mmol), catalyst (135 mg), water (27 mL), and methanol (225 mL) were combined in the reactor in a glovebox. The vessel was then sealed and transferred from the glovebox. After 10 cycles of hydrogen, the vessel was charged to 50 psig and heated to 80 °C. The hydrogen uptake was monitored via a computerized hydrogenation system.

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