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The Combination of Hydroformylation and Biocatalysis for the Large-Scale Synthesis of (*S*)-Allysine Ethylene Acetal

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Abstract:

The compound, (*S*)-2-amino-5-[1,3]dioxolan-2-yl-pentanoic acid [(*S*)-allysine ethylene acetal], is a key intermediate in a number of angiotension-I converting enzyme (ACE) and neutral endopeptidase (NEP) inhibitors currently in clinical trials. Through a combination of our hydroformylation and biocatalysis technologies we have developed an efficient five-step synthetic route to this material starting from crotonaldehyde. The development of this process, leading to a large-scale commercial manufacturing campaign, is described in this paper.

Introduction

(*S*)-2-Amino-5-[1,3]dioxolan-2-yl-pentanoic acid [(*S*)-allysine ethylene acetal] is a key intermediate in a number of angiotension-I converting enzyme (ACE) and neutral endopeptidase (NEP) inhibitors currently in clinical trials (Figure 1). The synthesis of racemic allysine ethylene acetal was initially reported *via* a laborious eight-step chemical synthesis from 3,4-dihydro-2*H*-pyran.[1] A wide variety of enzyme classes have been utilised in the preparation of (*S*)-allysine ethylene acetal, including phenylalanine dehydrogenases, amidases, D-amino acid oxidases, and hydantoinases.[2] In the majority of cases, these approaches utilise glutaraldehyde as a key starting material and involve selective monoacetalation of the dialdehyde. Unfortunately, this step is not particularly selective, and isolated yields are typically reported as less than 50%.[2e,3] An alternative bioresolution was sought that could be operated without infringement of existing intellectual property.

The development of an alternative higher-yielding method of producing glutaraldehyde monoacetal from a cheap starting material was key to developing a cost-effective and scalable process to (*S*)-allysine ethylene acetal. We envisaged using crotonaldehyde as a low-cost and readily available starting material for our synthesis (Scheme 1) and transforming it to the desired glutaraldehyde monoacetal (**2**) in two steps: first conversion into the corresponding ethylene acetal (**1**), followed by hydroformylation using a rhodium catalyst. Such a transformation requires the catalysis of two processes: (i) initial isomerisation of (**1**) to give the terminal double bond and (ii) linear selective hydroformylation. The use of hydroformylation for the production of pharmaceutical intermediates and fine chemicals has been a technology area under active development within Chirotech for several years.[4] We have a number of catalytic systems available to us for this transformation and considerable experience in taking such reactions to the manufacturing plant. Having synthesised aldehyde (**2**), this can be readily transformed *via* a Strecker reaction into aminonitrile (**3**). Subsequent conversion into either **4a** or **4b** yields two potential substrates for bioresolution (Scheme 1).

Previous work in our laboratories led to the development of a thermophilic L-acylase from archaeon *Thermoccocus litoralis*,[5] which has been shown to catalyse the bioresolution of a wide variety of *N*-acyl amino acids. Although the L-acylase bioresolution can only generate a maximum 50% yield of (**5**), the process does offer the opportunity to recycle the unwanted enantiomer *via* an intermediate azlactone (Scheme 2).

Results and Discussion

Stage 1: Acetal Formation. Crotonaldehyde ethylene acetal (**1**) was synthesised by following a literature procedure with minor modifications.[6] The literature procedure used benzene

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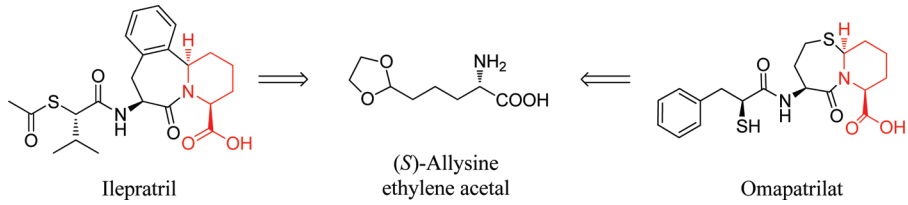
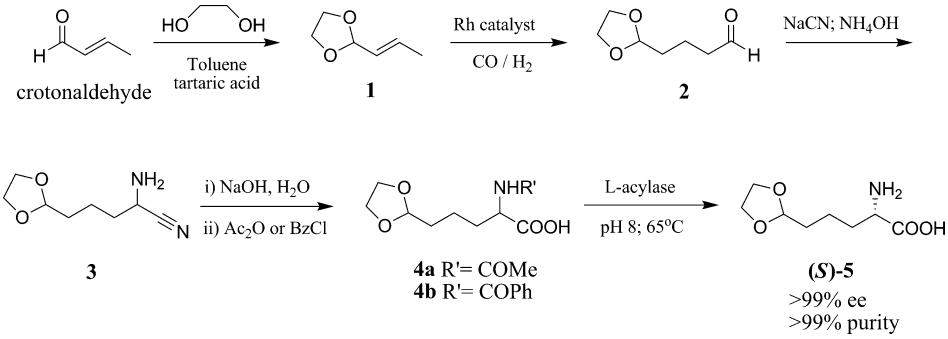
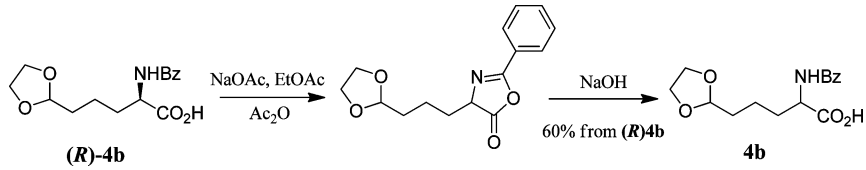


Figure 1. **(S)-Allysine ethylene acetal and examples of its reported uses as a chiral intermediate.**

Scheme 1. **Synthetic route to (S)-allysine ethylene acetal from crotonaldehyde**



Scheme 2. **Racemisation of (*R*)-*N*-benzoyl allysine ethylene acetal**



as an azeotroping solvent to remove water. We found that cyclohexane and toluene both worked as well although the relative rates under the same reaction conditions using the different solvents were not compared. The literature procedure reported 76% crude GC yield with complete conversion of crotonaldehyde in one hour. We achieved about 83% conversion of crotonaldehyde in 12 h. After removing the excess ethylene glycol by phase separation, neutralisation with $NaHCO_3$, and evaporation of volatiles under reduced pressure, crude product was obtained as an orange free-flowing oil. Toluene proved to be a slightly beneficial reaction solvent as it helped to remove any unreacted crotonaldehyde prior to purification. Distillation under reduced pressure produced >99% pure product in 54% yield. The lower yield and longer reaction time were most likely due to the much larger scale (about 140 times that described in the literature), which required longer time to remove water by azeotrope.

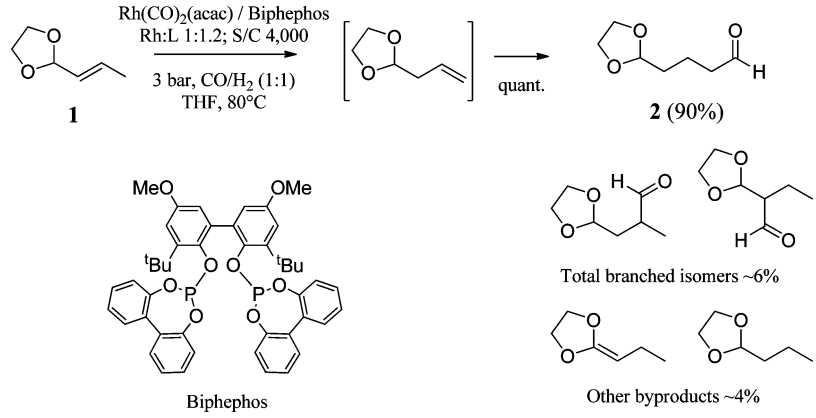
Stage 2: Hydroformylation. After an initial ligand screen (see Supporting Information), Biphephos was chosen as the optimum ligand on the basis of desired product yield, catalyst/product separability, and overall process economics.

Investigation of the process parameters highlighted both higher temperatures and lower pressures to be beneficial for increased reaction rates and increased regioselectivity in favor of the required linear aldehyde.

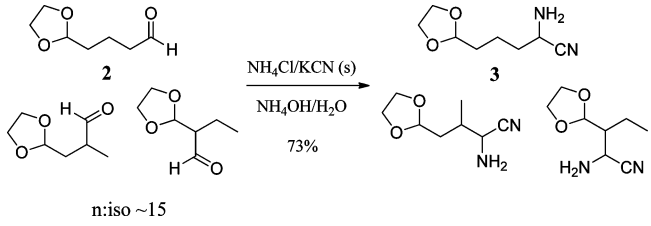
Ultimately, tandem isomerisation/hydroformylation of crotonaldehyde ethylene acetal (**1**) was efficiently achieved in a 50 wt % THF solution at 80 °C, 3 bar Syn gas (CO/H_2 1:1), and molar substrate to catalyst ratio of 4000:1 (0.025 mol %) (Scheme 3). Under these conditions, glutaraldehyde monoethylene acetal (**2**) was obtained with a high degree of selectivity over the corresponding branched regioisomers (~n:iso, 15:1). In addition to the branched aldehydes several other byproducts were observed in a total of up to 4%. These resulted from olefin reduction yielding 2-propyl-1,3-dioxolane and isomerisation of **1** to 2-propylidene-1,3-dioxolane. Formation of the branched aldehydes was of a greater concern due to the fact that the branched aldehydes were more difficult to separate from the linear aldehyde.

Vacuum distillation of the product from the reaction solution was investigated and found to be ineffective due to formation of heavies during the distillation in the presence of the catalyst. Because the next step was to convert the linear aldehyde to aminonitrile in an aqueous solution, on-scale an aqueous extraction process was developed to isolate the aldehydes in water, eliminating the need for vacuum distillation. The phase separation was achieved by first removing the majority of the THF and then adding hexane to the remaining reaction solution. The resulting solution was extracted with an aqueous 0.1 M $NaHCO_3$ solution three times, with the total volume of water used being calculated so as to obtain a solution containing approximately 25 wt % aldehydes. A weak sodium bicarbonate solution was used to ensure continued stability of the acetal product if the solution were to be stored for prolonged periods of time prior to performing the downstream chemistry. Residual THF partitioned mostly in the aqueous phase; however, some THF was extracted into the hexane phase, which enabled the efficient removal of ligand and catalyst into this organic phase. ICP analysis of both phases showed that >98% of the rhodium could be recovered in this manner. This aqueous extraction process also worked very well in isolating and purifying the aldehydes, since any heavies produced stayed with the catalyst

Scheme 3. **Hydroformylation of crotonaldehyde ethylene acetal**



Scheme 4. **Strecker reaction/formation of amino nitrile**



solution and also enhanced the n:iso ratio (up to 24:1) by preferentially extracting some of the branched aldehydes into the organic phase. This process was scaled up with multiple batches being performed in a 300-L pressure vessel that had been appropriately modified for hydroformylation. In each case, the reaction was found to be reproducible with negligible variation in both isolated yield and regioselectivity.

Stage 3: Strecker Reaction. The mixture of linear and branched aldehydes (15:1) isolated from lab-scale hydroformylations were reacted with potassium cyanide in the presence of 35% ammonia solution to generate a 75% yield of predominantly linear amino nitrile product (**3**) (Scheme 4). No significant change in the linear:branched ratio was observed during this step.

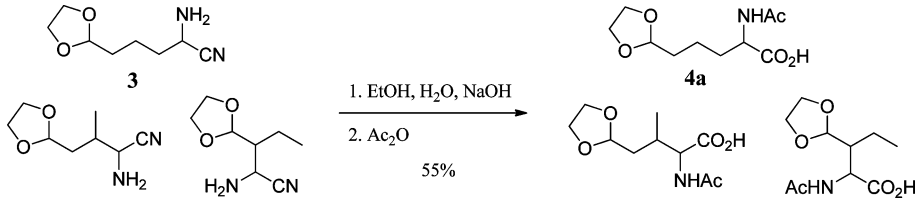
Since the product **2** from stage 2 was isolated as an aqueous solution, it was decided that the process could be streamlined by using this aqueous solution directly in the Strecker reaction. The aldehyde content of the aqueous solution was determined by GC analysis to be between 20 and 30 wt %, and the progress of the reaction was also monitored by GC. Initial Strecker reactions were carried out over 18−20 h with an aldehyde concentration of 6 wt %; however, subsequent development work confirmed that by using an aldehyde concentration of ∼25 wt % the reaction was complete within 5 h. Temperature control of the reaction during addition of **2** was found to be unnecessary. An endotherm resulted from the dissolution of ammonium chloride and potassium cyanide in ammonia solution resulting in a reactor temperature of 13 °C. Slow addition of the aqueous aldehyde solution meant that reaction temperature remained below 30 °C. During laboratory-scale preparation of **3**, potassium cyanide was used as the cyanide source for the reaction; however, during the transfer of the process to the manufacturing plant it was necessary for reasons of cost, safety, and ease of operation to use a 30 wt % aqueous solution of sodium cyanide. The change in cyanide source had no detrimental effect on the process. The Strecker reaction was successfully carried out in our laboratories at multihundred gram scale in 5-L reactors and was subsequently transferred to the manufacturing plant where five batches were successfully carried out in 10,000-L reactors, generating multiton quantities of **3**.

Stage 4: Synthesis of *N*-Acetyl Allysine Ethylene Acetal (4a). Since the key bioresolution step in the synthesis of (*S*)-allysine ethylene acetal (**5**) utilises an L-acylase enzyme, it was initially envisaged that *N*-acetyl allysine ethylene acetal (**4a**) would be an ideal substrate for the bioresolution. Synthesis of **4a** was accomplished by treating **3** with sodium hydroxide in 50% aqueous ethanol and heating the resulting stirred solution at 70 °C for 16 h (Scheme 5). The racemic amino acid product was then acetylated *in situ* with acetic anhydride. Unfortunately, it was discovered that **4a** was highly soluble in water, and consequently, isolation *via* organic extraction only yielded 55−70% product.

Stage 5: Bioresolution of *N*-Acetyl Allysine Ethylene Acetal (4a). Test bioresolutions were carried out using **4a** and a proprietary thermophilic L-acylase from archaeon *Thermoccocus litoralis*. These experiments were carried out at pH 7 and 60 °C with a substrate concentration of 25 g/L (Scheme 6). Within 24 h, conversion had exceeded 40%, and (*S*)-allysine ethylene acetal was produced in greater than 99% ee.

Interestingly, it was noted that branched *N*-acetyl amino acid impurities were not accepted as substrates by the enzyme. Consequently, the bioresolution offered a potential route for removing the branched impurities that had been produced by the hydroformylation step. Unfortunately, these initial results were obtained with an extremely high enzyme loading of 4658 units per gram of substrate. A series of bioresolutions were carried out using isolated **4a** in order to further improve enzyme loading and substrate concentration. The results from these experiments (Table 1) showed that the bioresolution can be successfully carried out at greater than 80 g/L substrate concentration with an enzyme loading of 1 mL (∼2000 units) L-acylase solution per gram of substrate. Bioresolutions reached greater than 40% conversion within 24 h, generating (*S*)-allysine ethylene acetal with ee greater than 99%. Unfortunately, isolation of product from starting material proved to be very difficult as a result of the high water solubility of the *N*-Ac material. An additional concern was the high enzyme loading used in the bioresolution, which raised the question of the

Scheme 5. **Hydrolysis and acetylation of amino nitrile**



Scheme 6. **L-Acylase-catalysed bioresolution of *N*-acetyl allysine ethylene acetal**

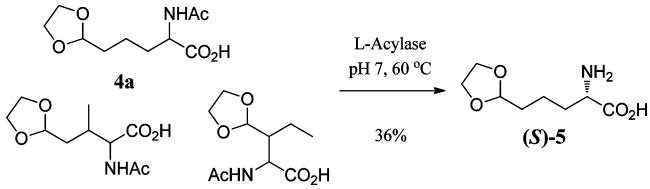
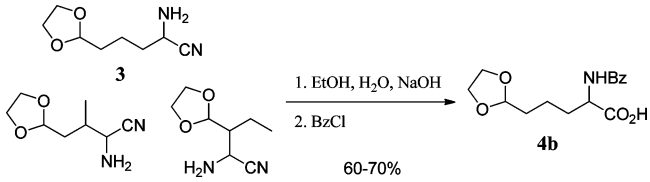


Table 1. **Optimisation of L-acylase catalysed bioresolution of 4a and 4b**

	substrate loading (g/L)	enzyme loading (units/ g substrate)	conversion (%)	ee (*S*)-**5** (%)	reaction time (h)
4a	23	4658	45	>99	24
4a	77	4529	45	>99	19
4a	58	2531	41	>99	19
4a	83	2329	50	>99	19
4b	89.5	657	50	>99	18
4b	261	57	48	>99	18
4b	201	97	49	>99	4
4b	265	97	49	>99	5

Scheme 7. **Hydrolysis and benzoylation of amino nitrile**



economical viability of a process using this resolution. As a result of these concerns, alternative bioresolution substrates were considered. Past experience had shown us that the *Thermococcus litoralis* L-acylase exhibits a preference for *N*-benzoyl substrates over *N*-acetyl substrates, and consequently, the preparation of **4b** was undertaken.

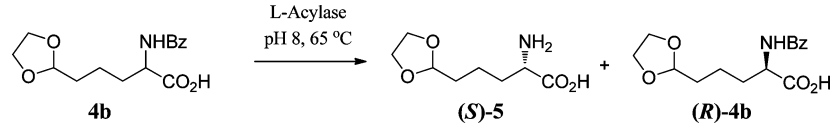
Stage 4: Synthesis of *N*-Benzoyl Allysine Ethylene Acetal (4b). Synthesis of **4b** was accomplished by treatment of **3** with sodium hydroxide in 50% aqueous ethanol and by heating the resulting stirred solution at 70 °C for 16 h, followed by benzoylation of the resulting amino acid under Schotten-Baumann conditions with benzoyl chloride (Scheme 7). Product was isolated by acidification of the aqueous reaction mixture, extraction into ethyl acetate and removal of solvent under reduced pressure to yield **4b** as a white solid.

The high yields and crystalline nature of **4b** offered a good opportunity to streamline the process and carry out stages 3 and 4 of the synthesis in one pot. The crystalline nature of the product meant that any impurities carried through from earlier stages could potentially be removed. Consequently, laboratory experiments were carried out to validate this proposed approach. Aqueous solutions of amino nitrile **3** from the Strecker reaction were successfully hydrolysed; since solutions of **3** were highly alkaline, it was possible to reduce the quantity of sodium hydroxide from 4.2 mol equiv to 1.5 mol equiv. The use of ethanol in the hydrolysis step was rapidly eliminated during development work, and hydrolysis of **3** was found to be complete after heating at 70 °C for 16 h. A key issue identified during development was the need to remove all ammonia from the reaction mix prior to the benzoylation step. Any residual ammonia present was found to react with benzoyl chloride and generate benzamide impurity. The benzoylation was eventually carried out at 10 °C, and the addition of benzoyl chloride was at such a rate as to ensure that the reaction temperature did not exceed 30 °C; the pH could be maintained between 7 and 9 by the addition of 46/48 wt % sodium hydroxide solution. The benzoylation was found to reach completion within 4 h, and product was isolated by acidification of the aqueous reaction mixture, extraction into ethyl acetate, and removal of solvent under reduced pressure to yield **4b** as a white solid. Analysis of the isolated product revealed that it was essentially free of any of the branched impurities that had been produced during the hydroformylation step. Unfortunately, during scale-up it was noted that **4b** exhibited poor solubility in ethyl acetate and had a tendency to precipitate out of solution post extraction. Whilst this issue was not a problem at lab scale, it did present a significant issue at manufacturing scale. To overcome the problem, an alternative isolation procedure was developed, involving acidification of the reaction mixture to pH 4 and recovery of the precipitated product by filtration. The resulting filter cake was washed with ethyl acetate and water in order to remove any potential impurities and dried *in vacuo* to remove traces of solvent. A subsequent production campaign was run in five batches in 10,000-L vessels.

Stage 5: Bioresolution of *N*-Benzoyl Allysine Ethylene Acetal (4b). A trial bioresolution was carried out on 500-mg scale at a substrate concentration of 89 g/L of *N*-benzoyl allysine ethylene acetal and an enzyme loading of 657 units per gram. The bioresolution proved to be successful, yielding (*S*)-allysine ethylene acetal with an ee > 99%, at a conversion of approximately 50% within a 19 h reaction time (Scheme 8). Initial trial experiments also demonstrated that the bioresolution of **4b** could be accomplished at significantly lower enzyme loadings than for the bioresolution of **4a** (Table 1). Furthermore, through the use of **4b** we observed a cleaner and more effective separation of product from starting material, allowing for the potential recycle and racemisation of (*R*)-**4b** *via* an intermediate azlactone (Scheme 2).

Significant optimisation work on the bioresolution step was undertaken, resulting in an improvement in substrate concentration from 89 g/L to over 260 g/L and a reduction in enzyme loading from 657 units per gram of substrate to 57 units per

Scheme 8. **L-Acylase-catalysed bioresolution of *N*-benzoyl allysine ethylene acetal**



gram of substrate. The bioresolution was successfully carried out at multihundred gram scale in a 5-L reactor using these optimum conditions, leading to complete resolution within 18 h. However, during transfer of the process to the manufacturing plant, it was decided to carry out the reaction at the higher enzyme loading of 97 units per gram in order to benefit from the quicker reaction time (5 h cf. 18 h). Isolation of amino acids from aqueous solutions is frequently problematic and often results in poor recoveries with the product contaminated with inorganic salts. Initial attempts to recover (*S*)-allysine ethylene acetal from the bioresolution mixture involved filtration through a plug of Celite to remove any precipitated protein material followed by concentration of the aqueous solution to approximately one-third volume. Addition of two volumes of ethanol led to precipitation, and after cooling to 10 °C, the product was recovered by filtration, washed with further cold ethanol, and dried *in vacuo* to give a 40% isolated yield. Reproducing this yield proved difficult, and unacceptably high salt content resulted in the evaluation of alternative antisolvents to aid product recovery. Isopropanol was briefly looked at, but the recovered precipitate contained significant contamination with *N*-benzoyl allysine ethylene acetal and benzoic acid. A switch to 1,2-dimethoxyethane (DME) was the key breakthrough and marked a switch to isolating material *via* recrystallisation rather than a precipitation approach. The aqueous bioresolution solution was partially concentrated under reduced pressure and stirred at 60 °C. DME (2 volumes) was slowly added taking care to ensure that no product precipitation occurred during the addition. The resulting solution was gradually cooled to 10 °C and left stirring for 12 h in order to allow product to crystallise. (*S*)-allysine ethylene acetal was subsequently isolated as a white crystalline solid in good yield (>35%) and excellent chemical and optical purity (>99%).

Conclusions

A robust five-step process for the production of (*S*)-allysine ethylene acetal has been developed using a combination of two catalytic technologies, namely rhodium-catalysed tandem isomerisation/hydroformylation and bioresolution. Starting from crotonaldehyde, the synthesis involved acetal formation with ethylene glycol followed by linear selective isomerisation/hydroformylation to yield predominantly glutaraldehyde monoethylene acetal. After an aqueous extraction to remove and recover the transition metal catalyst, a Strecker reaction followed by hydrolysis and benzoylation afforded the appropriate *N*-substituted racemic amino acid. Bioresolution of this racemate using a thermophilic L-acylase from archaeon *Thermoccocus litoralis* not only enabled separation of the linear product from the corresponding branched isomers but ultimately provided material with >99% ee in >99% purity. The development of this commercial process led to a large-scale manufacturing campaign that produced multihundred kilograms of this valuable chiral intermediate.

Experimental Section

Materials and Methods. All the materials were purchased from commercial suppliers and used without further purification. L-Acylase enzyme solution was produced via a generic fed-batch fermentation and subsequent standard downstream processing.[5] Optical rotations were measured on a Perkin-Elmer 341 polarimeter, and data are reported as $[\alpha]^{25}_{D}$ (concentration in g/100 mL, solvent). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chemical shifts are reported in ppm relative to the residual deuterated solvent. GC analysis was performed on a Perkin-Elmer Autosystem XL equipped with a FID detector. HPLC analysis was carried out on an Agilent 1100 system equipped with a UV detector.

Crotonaldehyde Ethylene Acetal (1). A 3-L three-neck flask was fitted with a Dean−Stark trap, an overhead stirrer, a temperature probe, and a gas inlet on the condenser. The flask was then placed under an atmosphere of nitrogen by slowly purging for 30 min. Tartaric acid (6.41 g, 42.7 mmol) was then charged to the flask. Crotonaldehyde (599.1 g, 8.55 mol) and toluene (700 mL) were then transferred into the flask followed by ethylene glycol (636.7 g, 10.26 mol). No exotherm was observed. The solution was heated to reflux using an oil bath at 120 °C (internal temperature of bulk = 102 °C) until collection of the aqueous phase slowed significantly (approximately 12 h). A total of 165 g of aqueous phase was collected (GC analysis showed this to contain small quantities of crotonaldehyde, toluene, and ethylene glycol). Stirring was stopped to allow the solution to settle into two phases, a colourless top phase containing the product and a yellow bottom phase containing most of the excess ethylene glycol and tartaric acid. After removal of the yellow bottom phase and back extraction with toluene (2 × 100 mL) to extract any residual product, the toluene washes were combined with the top organic phase. Analysis of this solution by GC prior to distillation showed crotonaldehyde conversion to be approximately 83%. Solid $NaHCO_3$ (10.1 g, 120 mmol) was added to ensure neutralisation of any residual acid prior to distillation. (NB: This is essential to prevent the appearance of crotonaldehyde *via* the back reaction during distillation.) The above procedure was repeated twice, and the batches were combined prior to fractional distillation. Initial volatiles (unreacted crotonaldehyde and toluene, together with approx 10% w/w of the desired acetal) were removed on a rotary evaporator to leave a crude orange-coloured solution. Distillation was performed under reduced pressure in a 3-L round-bottomed flask using a glass column packed with stainless steel Knitmesh (diameter = 30 mm, height = 325 mm). Distillation of 1308 g of the crude reaction mixture gave an initial fraction consisting of mainly toluene with a trace amount of crotonaldehyde and the desired

product (168.1 g, boiling point: 38 °C at 72 mbar, oil bath temp = 60 °C, internal bulk temperature = 43 °C). After discarding a small forerun (~5 g), the final product was isolated as the next fraction (820 g, boiling point: 66−74 °C at 70 mbar, oil bath temp = 95 °C, internal bulk temperature = 82 °C), analysed by GC and ^{1}H NMR, and found to contain the desired acetal as a mixture of *cis*- and *trans*-isomers. The distillation was stopped with 309.9 g of an orange residue remaining that GC showed to contain undistilled product and unidentified heavies. ^{1}H NMR for the major *trans*-isomer (400 MHz, $CDCl_3$) δ 5.83 (dq, J_{H-H} = 15.5, 6.6 Hz, 1H). 5.38 (ddq, J_{H-H} = 15.5, 8.7, 1.6 Hz, 1H), 5.05 (d, J_{H-H} = 8.7, 1H); 3.71−3.92 (m, 4H,), 1.63 (dd, J_{H-H} = 6.6, 1.6 Hz. 3H). GC sample preparation: Dilute 2 μL to 1 mL with acetonitrile. DB5 30 m × 0.25 mm ×0.25 μm, oven program 60 °C for 8 min, then 10 °C/min to 250 °C (hold 3 min). Retention times (relative response factors): crotonaldehyde 2 min (1.0), ethylene glycol 2.2 min (0.4), toluene 2.9 min (1.7), *trans*-acetal 5.4 min (0.9), *cis*-acetal 5.5 min (0.9).

4-[1.3]-Dioxolan-2-yl Butanal (2). Crotonaldehyde ethylene acetal (**1**) (533 g, 4.67 mol) was charged to a 2-L Parr pressure vessel fitted with a glass liner, double impeller, injection port, bursting disk, pressure relief valve, and pressure gauge. THF (533 mL) was added and the vessel assembled. The reaction solution was deoxygenated by pressurising with nitrogen (6 bar), stirring vigorously (1000 rpm), and venting. This procedure was repeated five times. In the meantime, $Rh(CO)_2(acac)$ (301 mg, 1.17 mmol) and Biphephos (1.10 g, 1.40 mmol) were charged to a glass liner of a 50 mL Parr vessel and the vessel assembled. This was purged 6 times by pressurising to 4 bar with Syn gas (CO/H_2 1:1) and venting. Degassed THF (10 mL) was added through a septum and the vessel charged to 10 bar with Syn gas. This was heated to 40 °C for 30 min with rapid stirring and then the activated catalyst solution removed into a syringe and transferred to the 2-L Parr vessel. The 2-L Parr vessel was then charged to 3 bar with Syn gas, heated to 80 °C, and stirred vigorously (1000 rpm), maintaining the desired pressure with constant addition of Syn gas until gas uptake ceased. The vessel was cooled to room temperature, purged with nitrogen, and sampled. ^{1}H NMR spectroscopy and GC analysis showed the reaction to be complete (<1% starting material), yielding a mixture of the desired linear aldehyde product (approximately 90%), the corresponding branched regioisomers (6%), 2-propyl-1,3-dioxolane and 2-propylidene-1,3-dioxolane (~4% in total). The solution was concentrated on a rotary evaporator at 45 °C to give a clear, mobile, yellow oil. ^{1}H NMR for the linear product (400 MHz, $CDCl_3$) δ 9.71 (s, 1H), 4.80 (d, J_{H-H} = 3.3 Hz. 1H), 3.76−3.94 (m, 4H), 2.45 (m, 2H), 1.51−1.74 (m, 4H). ^{13}C NMR for the linear product (100 MHz, $CDCl_3$) δ 202.0, 103.9, 64.5, 43.4, 32.8, 16.3. GC sample preparation: Dilute 2 μL to 1 mL with acetonitrile. DB5 30 m × 0.25 mm ×0.25 μm, Oven program 60 °C for 8 min, then 10 °C/min to 250 °C (hold 3 min). Retention times: THF 1.9 min, *trans*-acetal 5.4 min, *cis* acetal 5.5 min, branched aldehydes 13.1 and 13.2 min, linear aldehyde (**2**) 14.4 min.

In preparation of the aqueous solution, hexane was added (171 g) to the concentrated reaction mixture (438 g) together with a 0.1 M aqueous solution of $NaHCO_3$ (370 g). The phases were mixed, allowed to separate into a triphasic mixture consisting of a top hexane phase, a middle organic phase, and a lower aqueous phase. The lower aqueous phase was separated off. The remaining top two organic phases were then extracted twice more with further quantities of 0.1 M $NaHCO_{3\ (aq)}$ (2 × 370 g), and the aqueous phases were combined. GC analysis of this aqueous phase showed this to contain 25 wt % aldehyde content.

2-Amino-5-[1,3]dioxolan-2-yl-pentanenitrile (3). Into a 10-L jacketed vessel was placed ammonium chloride (92.86 g, 1.74 mol) and potassium cyanide (113.05 g, 1.74 mol) in water (2.25 L) and 35% aqueous ammonia (2 L). The solution was stirred under a nitrogen atmosphere and cooled to below 0 °C. To the cooled solution was slowly added the crude reaction mixture of 4-[1.3]-dioxolan-2-yl butanal (**2**) (250 g, 1.74 mol) over a period of 60 min, taking care that the temperature remained below 0 °C. After the addition was complete, the reaction mixture was allowed to slowly warm up to room temperature and was left stirring overnight. After overnight stirring the reaction was halted and extracted with ethyl acetate (3 × 2.5 L). The combined organics were then dried over magnesium sulphate and concentrated under reduced pressure to yield **3** as a yellow oil (216 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.88 (t, J = 4.4 Hz, 1H), 4.00−3.92 (m, 2H), 3.91−3.81 (m, 2H), 3.69 (t, J = 6.8 Hz, 1H), 1.85−1.76 (m, 2H), 1.76−1.54 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.41, 104.34, 65.32, 43.70, 35.15, 33.33, 20.26.

***N*-Acetyl Allysine Ethylene Acetal (4a).** Into a 50-mL round-bottom flask was placed **3** (725 mg, 4.3 mmol) in 50% aqueous ethanol (20 mL), together with sodium hydroxide (720 mg, 18 mmol, 4.2 equiv). The reaction mixture was stirred continuously and heated under refluxing conditions for 4 h. After this time the mixture was cooled to below 5 °C by means of a salt−ice bath, and acetic anhydride (490 μL, 5.11 mmol, 1.2 equiv) was added to the stirred solution. The reaction mixture was allowed to warm up to room temperature and was left stirring overnight. The mixture was then acidified to pH 3 with 6 M hydrochloric acid solution and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over magnesium sulphate and concentrated under reduced pressure to yield 530 mg (55%) of the *N*-acetyl allysine ethylene acetal (**4a**) as an orange oil. ^{1}H NMR (400 MHz, CD_3OD) δ 4.84 (t, J = 4.6 Hz; 1H), 4.37 (dd, J = 5.2, 9.2 Hz, 1H), 4.00−3.89 (m, 2H), 3.89−3.79 (m, 2H), 2.01 (s, 3H), 1.95−1.83 (m, 1H), 1.81−1.59 (m, 3H), 1.59−1.43 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.94, 173.78, 105.74, 66.26, 54.08, 34.72, 32.91, 22.71, 21.80.

(*S*)-Allysine Ethylene Acetal (5) *via* 4a. Into a 50-mL jacketed vessel was placed **4a** (500 mg, 2.16 mmol) dissolved in distilled water (20 mL). The solution was continuously stirred and heated to 60 °C; the pH of the solution was adjusted to 7 by dropwise addition of 1 M sodium hydroxide solution. L-Acylase enzyme solution (1 mL, 2329 units) was then added to the reaction, and the mixture was left stirring for 24 h at 60 °C. The reaction mixture was then acidified to pH 3 with 6 M hydrochloric acid solution and extracted with ethyl acetate (3 × 25 mL) in order to remove residual *N*-acetyl allysine ethylene acetal. The pH of the aqueous phase was readjusted to 7.3 using 5 M sodium hydroxide solution, was concentrated to one-quarter

volume and diluted with isopropanol (20 mL). The resulting white precipitate was recovered by filtration and dried overnight in a vacuum oven, yielding 150 mg (36%) of (*S*)-allysine ethylene acetal. The product was analysed by HPLC on chiral stationary phase (Chirex (D)-penicillamine 50 mm × 4.6 mm; 5 μm; 30 °C; 0.5 mL/min methanol/2 mM copper sulphate pentahydrate in water 5:95; 254 nm). Retention times: (*S*)-allysine ethylene acetal = 14 min and (*R*)-allysine ethylene acetal = 25 min. (*S*)-allysine ethylene acetal ee = 99%. ^{1}H NMR (400 MHz, D_2O) δ 4.84 (t, J = 4.8 Hz, 1H), 4.06−3.97 (m, 2H), 3.97−3.83 (m, 2H), 3.63 (t, J = 6.2 Hz; 1H), 1.99−1.80 (m, 2H), 1.78−1.63 (m, 2H), 1.59−1.34 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 175.00, 104.42, 64.85, 54.86, 32.46, 30.52, 19.32.

***N*-Benzoyl Allysine Ethylene Acetal (4b).** Into a 5-L jacketed vessel was placed 35% aqueous ammonia solution (860 mL). To the stirred solution was added ammonium chloride (159.84 g, 2.99 mol) and potassium cyanide (194.6 g, 2.99 mol). The resulting suspension was left stirring for 10 min at 15 °C. An aqueous solution of **2** (1760 mL containing 430 g or 2.99 mol aldehyde) was then added dropwise to the reaction mixture over a period of 45 min. The temperature rose to 30 °C, and the reaction mixture became homogeneous. The reaction was left stirring at ambient temperature for 4 h. GC and NMR analyses confirmed that all starting material had been consumed. The reaction mixture was transferred into a three-neck, 5-L round-bottom flask, and sodium hydroxide (220 g, 5.5 mol, 1.8 equiv) was added. The reaction mixture was heated under refluxing conditions (70 °C pot temperature) for 15 h. NMR analysis confirmed the formation of amino acid and the absence of any amino nitrile. Excess ammonia was then removed under reduced pressure. A total of 1000 mL of ammonia/water was removed, and no odor of ammonia was detected in the reaction mix. The concentrated reaction mixture was transferred into a 5-L jacketed vessel and diluted with 500 mL of deionised water, and the pH was adjusted to 11.5 by addition of concentrated hydrochloric acid (230 mL). After cooling to below 15 °C, benzoyl chloride (248 mL, 2.13 mol, 0.71 equiv) was added dropwise to the vigorously stirred reaction mixture over a period of 20 min. The pH was maintained between 7 and 9 by multiple additions of 46−48% sodium hydroxide solution (105 mL). The reaction temperature rose to 29 °C. The mixture was washed with 500 mL of ethyl acetate; this wash was discarded. The aqueous phase was acidified to pH 3.5 with concentrated HCl (150 mL) and extracted with a total of 2.5 L of ethyl acetate.A total of 410 g of *N*-benzoyl allysine ethylene acetal was isolated. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (br s, 1H), 8.59 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 6.8 Hz, 2H), 7.59−7.42 (m, 3H), 4.76 (t, J = 4.6 Hz, 1H), 4.41−4.30 (m, 1H), 3.90−3.79 (m, 2H), 3.79−3.67 (m, 2H), 1.92−1.71 (m, 2H), 1.67−1.33 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.07, 166.85, 134.33, 131.69, 128.58, 127.79, 103.84, 64.51, 52.90, 33.25, 30.80, 21.03.

(*S*)-Allysine Ethylene Acetal (5) *via* 4b. Into a 5-L jacketed vessel heated to 65 °C was placed *N*-benzoyl allysine ethylene acetal (**4b**) (457.3 g, 1.56 mol) dissolved in a mixture of water (600 mL) and 2 M sodium hydroxide solution (725 mL) giving a pH of 7.5. The reaction mixture was continuously stirred, and L-acylase enzyme solution (19 mL, 44,251 units) was added. Stirring was continued at 65 °C for 5 h after which time NMR analysis indicated that the reaction had reached ~50% conversion. Concentration under reduced pressure removed water (600 mL). The remaining aqueous phase was returned to the 5-L vessel and heated to 60 °C, and DME (1.5 L) was then added via a dropping funnel to the reaction mix at 60 °C. Precipitation started to occur, and the mixture was stirred at this temperature for 40 min. The stirred solution was cooled to 10 °C and was left stirring overnight at this temperature. The resulting precipitated amino acid was recovered by filtration, washed with further DME (400 mL), and dried *in vacuo* to yield 105.2 g (36%) of white crystalline solid. Mp >200 °C dec; $[\alpha]^{25}_D$ +3.7° (*c* 1, H_2O); >99% ee; ^{1}H NMR (400 MHz, D_2O) δ 4.84 (t, J = 4.8 Hz, 1H), 4.06−3.97 (m, 2H), 3.97−3.83 (m, 2H), 3.63 (t, J = 6.2 Hz; 1H), 1.99−1.80 (m, 2H), 1.78−1.63 (m, 2H), 1.59−1.34 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 175.00, 104.42, 64.85, 54.86, 32.46, 30.52, 19.32.

Racemisation of (*R*)-*N*-Benzoyl Allysine Ethylene Acetal (4b). Into a two-neck 100-mL round-bottom flask was placed (*R*)-*N*-benzoyl allysine ethylene acetal (5 g, 17 mmol) and sodium acetate (200 mg, 2.4 mmol) in ethyl acetate (25 mL). The solution was stirred at 80 °C, and acetic anhydride (2.5 mL, 26.5 mmol, 1.5 equiv) was slowly introduced into the reaction vessel. The reaction mixture was then continuously stirred at 80 °C for a further 3 h. After this time the vessel was allowed to cool to room temperature, and the contents were extracted with saturated sodium bicarbonate solution (20 mL). To the organic phase was then added 5 M sodium hydroxide solution (40 mL). The resulting mixture was vigorously stirred for 1 h, after which time the phases were separated, and the aqueous phase was acidified to pH 3 with 6 M hydrochloric acid solution. The acidified solution was then extracted with of ethyl acetate (2 × 40 mL), combined extracts were dried over magnesium sulphate, and concentration under reduced pressure yielded 3.3 g of an off-white solid. The material was then slurried in MTBE to remove benzoic acid impurities, filtered, and dried *in vacuo* to yield 2.74 g (60%) of racemic *N*-benzoyl allysine ethylene acetal.

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Supporting Information Available

Hydroformylation ligand screening data, ^{1}H and ^{13}C NMR spectra of key products and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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