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Optimized Synthesis of L-*m*-Tyrosine Suitable for Chemical Scale-Up

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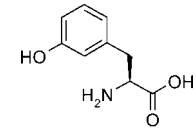
Abstract:

This paper demonstrates how L-*m*-tyrosine 1 can be synthesized on larger-scale via enzyme-catalyzed kinetic resolution of *N*-acyl *m*-tyrosine methyl ester 4. *N*-Acyl *m*-tyrosine methyl ester 4 was prepared by a modification of Erlenmeyer's azalactone synthesis followed by hydrogenation of the resultant dehydroamino acid 12. The optimized four-step synthesis utilizes cheap and readily available starting materials and circumvents difficult purification protocols.

Introduction

α-Amino acids play an important role in drug discovery.[1] The unnatural amino acid L-*m*-tyrosine, **1**, has found broad application in medicinal chemistry and in the study of metabolic pathways in the central nervous system[2,3] since its early synthesis and proof of configuration.[4–6] *m*-Tyrosine and analogues thereof have shown application in the treatment of Parkinson's disease,[7,8] Alzheimer's disease,[9] athritis,[10,11] as agents for pancreatic disorders,[12] in agrichemicals[13] and, in the form of stearyl-tyrosine, as a adjuvant added to antigens or vaccines to increase immune response.[14] The unnatural amino acid was found to be a key motif in three classes of peptidic-nucleoside antibiotics; the mureidomycins,[15–17] the pacidamycins[18] and more recently the napsamycins.[19,20]

L-*m*-Tyrosine **1** is an unnatural amino acid that is both difficult and expensive to obtain in large quantities required for chemical scale-up. The price of L-*m*-tyrosine increases dramatically by a factor of approximately 350 compared to the natural amino acid L-tyrosine.



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Surveying the literature revealed that *N*-α-Cbz-L-*m*-tyrosine **2**[21] and *O*-benzyl-*N*-Boc-L-*m*-tyrosine **3**[22] have been prepared on bench scale by rhodium-catalyzed asymmetric hydrogenation[23] as shown in Scheme 1. The amino acrylic acid methyl esters were prepared by Wadsworth–Emmons reaction between Boc- or Cbz-amino-(dimethoxy-phosphoryl)-acetic acid methyl ester and the corresponding substituted benzaldehyde.

L-*m*-Tyrosine **1** has been also prepared by Williams[24] using an optically active oxazinone as outlined in Scheme 2. This route, although highly selective and resulting in high ee's, would be of considerable expense if scaled up to kilogram scale due to the high cost of the auxiliary.[25]

As the first method of choice, asymmetric hydrogenation was used to prepare L-*m*-tyrosine **1** on large scale in our

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(25) Available in 1 g quantities from Sigma Aldrich at approximately 130 USD/g.

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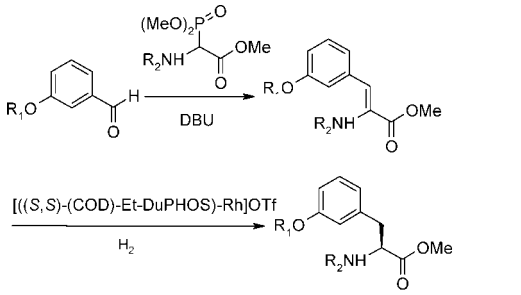
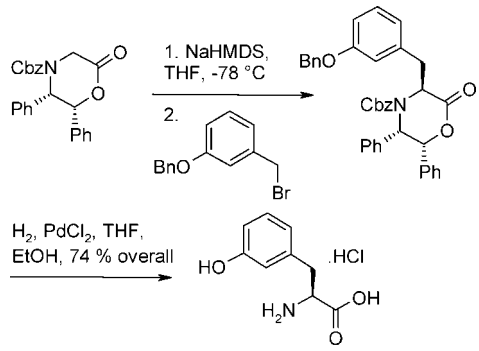
Scheme 1. **Synthesis of L-*m*-tyrosine via rhodium-catalyzed asymmetric hydrogenation of amino acrylic acid methyl esters**



2 R_1 = H, R_2 = Cbz; 81 % (2 steps); 98 % e.e.
3 R_1 = Bn, R_2 = Boc; 92 % (2 steps); high e.e.

Scheme 2. **Synthesis of L-*m*-tyrosine via condensation of *m*-(benzyloxy)benzyl bromide with an optically active oxazinone**



(*S*)-**1**.HCl e.e. > 96 %

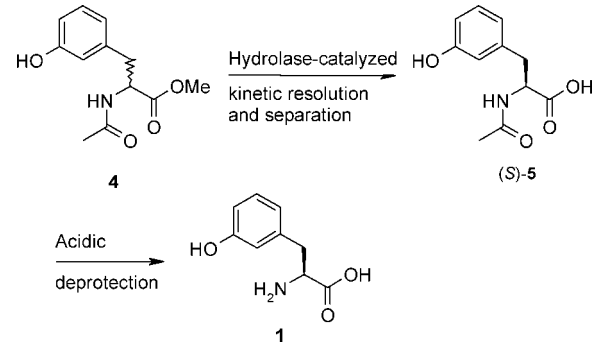
Preparations Laboratories. However, the reaction was not successful. The DuPHOS catalyst required experienced handling due to its high sensitivity to moisture and air. In multiple trials the reaction stalled part of the way through, probably due to catalyst poisoning. Because of time pressure we decided to seek an alternative.

Enzymatic reactions are widely used in our unit as this expertise is available in-house. This led us to explore the preparation of L-*m*-tyrosine via enzyme-catalyzed kinetic resolution of racemic *N*-acyl *m*-tyrosine methyl ester **4** using Alcalase based on work by Roper and co-workers[26,27] and previous knowledge gained in-house.[28] The advantages of such a route include the use of cheap and readily available enzymes, in reactions which are easily scaled up and show very high selectivity. This route would also provide us with both enantiomers of the resultant *m*-tyrosine which may be of interest during the drug discovery and early development phases.

Results and Discussion

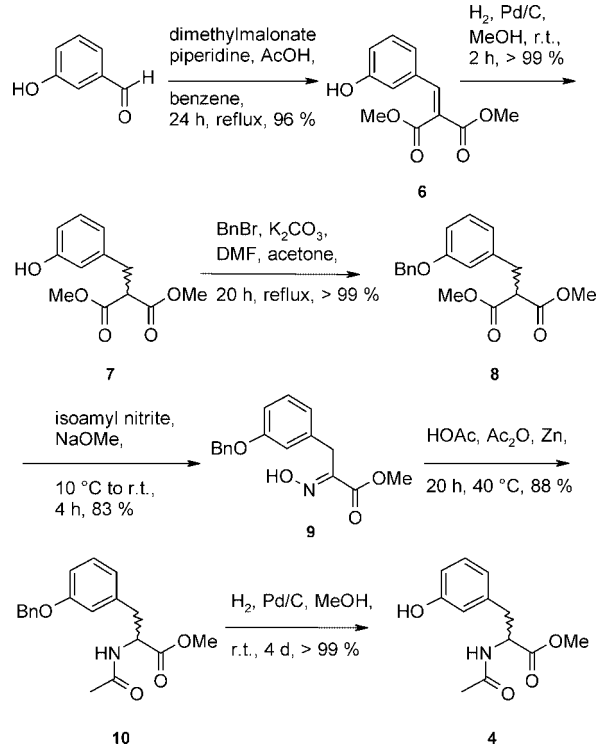
We envisaged the production of L-*m*-tyrosine **1** by acetyl-deprotection of the product of kinetic resolution, as detailed in Scheme 3. Previous experience told us that the *N*-acetyl protected amino acid methyl ester would be a good substrate for hydrolase-catalyzed kinetic resolution. In order to make this process valuable, we needed to find a large-scale synthesis of

Scheme 3. **Proposed hydrolase-catalyzed kinetic resolution of *N*-acyl *m*-tyrosine methyl ester 4**



4 (*S*)-**5** **1**

Scheme 4. **Synthesis of racemic *N*-acyl *m*-tyrosine methyl ester 4 via Knoevenagel condensation**



6 **7** **8** **9** **10** **4**

racemic *N*-acyl *m*-tyrosine methyl ester **4** utilizing cheap and readily available starting materials and with the fewest possible chemical steps.

***N*-Acyl *m*-Tyrosine Ethyl Ester via Knoevenagel Condensation.** Scheme 4 details the initial synthesis route used to prepare 3 kg of racemic *N*-acyl *m*-tyrosine methyl ester **4** via Knoevenagel condensation of 3-hydroxybenzaldehyde with dimethylmalonate. This route to unnatural amino acids was chosen due to considerable previous experience with this robust and reliable chemistry.[28] After subsequent hydrogenation of the double bond and benzyl protection diester **8** was converted to oxime **9** by reaction with isoamyl nitrite. Isoamyl nitrite may constitute a safety risk as it decomposes exothermically at elevated temperatures. Differential scanning calorimetry (DSC) measurements showed the onset of a large exotherm at 160 °C of 1857 J g^{-1}. Oxime hydrolysis and removal of the benzyl protecting group gave *N*-acyl *m*-tyrosine methyl ester **4** in 70% overall yield. Careful purification of benzyl-protected amino acid **10** was required before hydrogenation. Large amounts of water

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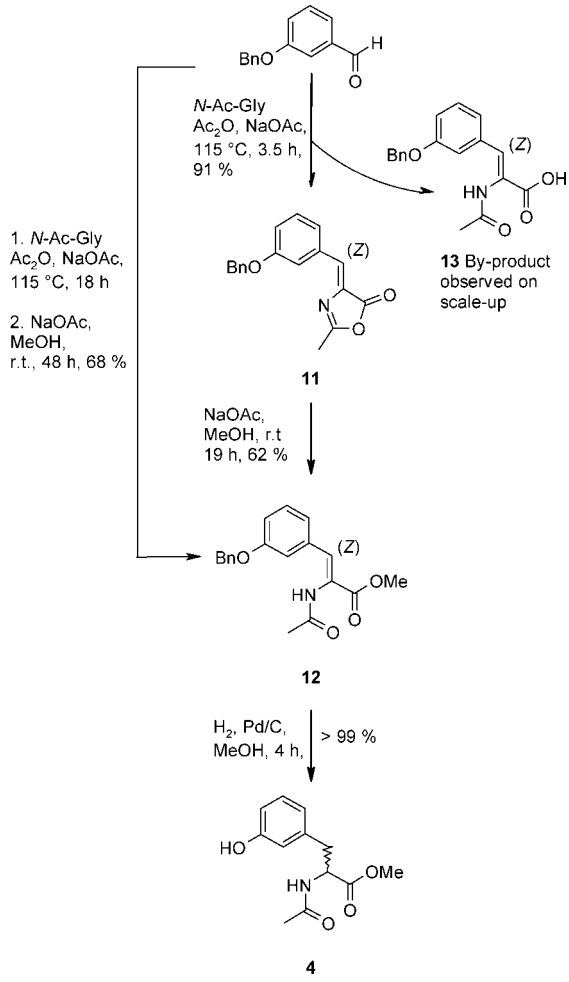
Scheme 5. **Synthesis of racemic *N*-acyl *m*-tyrosine methyl ester 4 via Erlenmeyer amino acid synthesis**



were required for reaction quenching, otherwise any residual zinc poisoned the palladium catalyst used in hydrogenation and stalled the reaction.

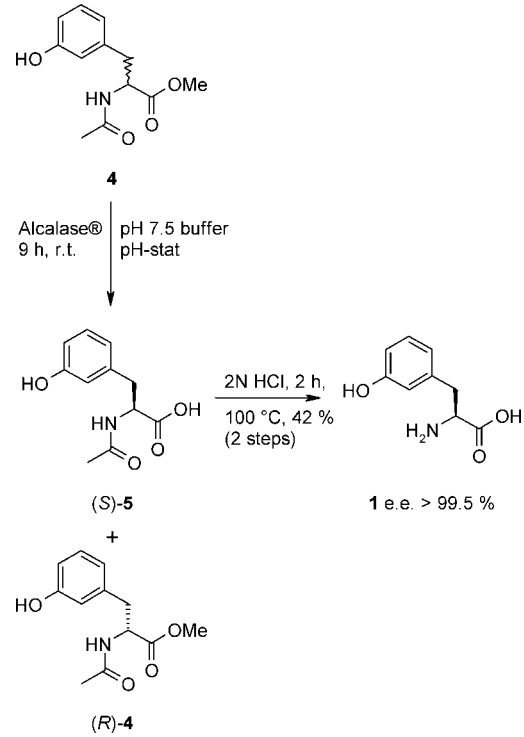
The synthetic route was high yielding but constituted six chemical steps. It became obvious that a shorter route would not only save considerable time but might prove to be more economically favorable.

***N*-Acyl *m*-Tyrosine Methyl Ester via Erlenmeyer Azlactone Synthesis.** A route to *N*-acyl *m*-tyrosine methyl ester **4**, based upon the Erlenmeyer synthesis of amino acids via oxazolone formation, is outlined in Scheme 5 (main branch).[29–31]

In a trial scale reaction, *m*-benzyloxybenzaldehyde was reacted with *N*-acetylglycine to yield 2-methyl-oxazol-5-one **11** in high yield as a bright yellow solid. The presence of a single (*Z*)-geometric isomer for this oxazolone was confirmed by X-ray analysis.

The ring was opened using sodium acetate in methanol to yield the acrylic acid methyl ester in moderate yield. Purification of the solid formed by trituration with 50% EtOAc in hexanes gave the product as a pale yellow crystalline solid. Simultaneous benzyl deprotection and hydrogenation of dehydroamino acid

Scheme 6. **Alcalase-catalyzed kinetic resolution of *N*-acyl *m*-tyrosine methyl ester 4**



12 occurred in a single reaction to give *N*-acyl *m*-tyrosine methyl ester **4** in quantitative yield (overall yield 56%).

When this reaction was scaled to 100 g we encountered a number of problems with the isolation of azalactone **11**. When the reaction mixture was poured into a mixture of ethanol/water (1:2) the major product isolated was acid **13** formed by hydrolysis of the required product. In order to circumvent this problem, the reaction mixture from the first step was poured directly into methanol containing sodium acetate, thus reducing the two-step procedure to a one-pot procedure (see Scheme 5, left hand side- branch). This approach showed a yield of 68%, more than 12% higher in yield than the two steps performed individually.

Enzymatic Kinetic Resolution. Finally, racemic *N*-acyl *m*-tyrosine methyl ester **4** was converted to (*S*)-*N*-acyl *m*-tyrosine **5** via Alcalase (protease from *Bacillus licheniformis*)-catalyzed kinetic resolution, followed by acetyl-deprotection to give L-*m*-tyrosine **1** in 42% yield over two steps (Scheme 6). The enzyme was highly selective for the (*S*)-enantiomer, thus giving the required L-*m*-tyrosine **1** in an excellent ee of >99.5%.

The Alcalase shows a remarkably broad substrate tolerance and high enantioselectivity against a range of nonproteinogenic racemic amino acid derivatives. It has been previously shown to hydrolyze *N*-acetyl-protected amino acid esters of mono-, di- or trisubstituted phenylanilines,[28] and even *tert*-leucine,[32] with high enantioselectivity.

Conclusions

In summary, we have developed a new enzymatic method to prepare L-*m*-tyrosine, which is complementary to asymmetric

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hydrogenation. Such a method might be considered whenever both enantiomers of the unnatural amino acid are required. Additionally, the synthesis of key intermediate *N*-acyl *m*-tyrosine methyl ester **4** has been successfully reduced from an six-step to a two-step procedure, starting from cheap and readily available starting materials, requiring minimal purification, and unlike the longer synthesis in which isoamyl nitrite was utilized, it does not include problematic reagents. The overall yield for the optimized two-step synthesis is comparable to the six-step synthesis at 68%; however, the shortened procedure developed herein has the following four advantages: (a) the time needed for the two-step synthesis is far less than performing a six-step synthesis; (b) the space/time yield is higher compared to the longer six-step synthesis; (c) the two-step synthesis developed is not only ecological but economical more sound, and finally, (d) this synthesis could be easily adapted to amino acids containing different aryl-substitution patterns.

Experimental Section

All reagents were obtained commercially and used as received unless otherwise noted. The Alcalase used was Novo Nordisk, Alcalase 2.5 L, type DX, PMN04666, 2.67 AU/g proteolytic activity. TLC was performed on Merck silica gel plates 60 F-254, Art. no. 5729, with detection by UV (254 nm) or molybdate dip (3 g $KMnO_4$; 20 g K_2CO_2; 5 mL 5% NaOH; 300 mL H_2O).

Reverse-phase HPLC analyses were performed on an Agilent-1100 machine using a Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD column, acetonitrile and water both containing 0.05% TFA, a column temperature of 35 °C, with a flow rate of 1.0 mL/min and measuring at 216 nm. The standard gradient used was 5–100% MeCN over 6 min, 100% MeCN for 1.5 min followed by 100–5% MeCN over 0.5 min.

Normal-phase chiral separations were performed using either a Chiralpak AD-H 250 × 4.0 mm column in the case of **4**, a hexane/ethanol mixture at 80:20, a column temperature of 20 °C, with a flow rate of 1.0 mL/min and measuring at 210 nm; or a Chirobiotic TAG 250 × 4.6 mm column in the case of **1**, a water/methanol mixture at 40:60, a column temperature of 20 °C, with a flow rate of 1.5 mL/min and measuring at 215 nm.

NMR was performed using a 400 MHz Varian machine, AS 400 Oxford. ^{1}H shifts were referenced to DMSO-d_6 at 2.49 ppm and $CDCl_3$ at 7.25 ppm with tetramethylsilane as internal standard for ^{1}H NMR. MS was measured using VG Platform (Fisons Instruments), Spectraflow 783 Detector, HP 1100 Series HPLC. Melting points were measured using a Büchi, B-545 machine.

N*-Acyl *m*-Tyrosine Ethyl Ester via Knoevenagel Condensation (according to Scheme 4).** *2-(3-Hydroxy-benzylidene)-malonic Acid Dimethyl Ester* ***6. 3-Hydroxybenzaldehyde (2705 g, 22.2 mol) was added to a 25-L reactor containing dimethylmalonate (2538 mL, 22.2 mol) dissolved in toluene (10 L), acetic acid (250 mL, 4.37 mol) and pyridine (25 mL, 0.43 mmol) were added to give a light yellow cloudy solution. The mixture was heated to 110 °C (internal temperature) and stirred (using an overhead stirrer) at reflux for 24 h under nitrogen with a Dean–Stark trap to collect the water formed. The reaction was monitored by HPLC using the conditions shown below. After 24 h, the reaction mixture was cooled to room temperature, and the toluene removed by distillation. The residue was dissolved in dichloromethane (10 L) and washed with brine (5 L) and twice with saturated sodium bicarbonate (2 × 5 L). The combined aqueous phases were extracted with dichloromethane (2 × 5 L), and the combined organic phases were dried over anhydrous sodium sulfate and concentrated in vacuo to give a clear red colored oil. The oil was stirred under hexane (5 L) until pink crystals formed, which were removed by filtration (5106 g, 97%). Mp 73.6–73.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.76 (3H, s), 3.79 (3H, s), 6.85 (1H, s), 6.86 (1H, d, J = 7.8 Hz), 6.91 (1H, d, J = 7.8 Hz), 7.24 (1H, t, J = 7.8 Hz), 7.64 (1H, s), 9.78 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 53.4 (t), 115.8 (s), 119.0 (s), 121.6 (s), 125.4 (q), 130.8 (s), 134.0 (q), 142.7 (s), 158.3 (q), 164.6 (q), 167.1 (q). HRMS (FAB) calcd for $C_{12}H_{12}O_5$ + Na 259.0577, found 259.0576. Anal. Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12; N, 0. Found: C, 61.04; H, 5.08; N, < 0.3. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 20–100% MeCN (6′) 100% MeCN (1.5′) 100–20% MeCN (0.5′)) $R_{f\,(\text{aldehyde})}$ = 1.28 min; $R_{f\,(\text{product})}$ = 2.72 min; purity 78%.

2-(3-Hydroxy-benzyl)-malonic Acid Dimethyl Ester ***7.*** Malonic acid dimethyl ester **6** (5106 g, 21.6 mol) was dissolved in methanol (20 L) containing Pd/C (5%) (120 g) and shaken under 1.2 bar H_2 for 4 h. After 100% H_2 consumption the reaction was stopped, and the reaction mixture was filtered through a plug of Hyflo to remove the carbon and any palladium impurities. The methanolic solution obtained was concentrated in vacuo to yield a clear yellow oil (5164 g, > 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.98 (2H, d, J = 8.2 Hz), 3.59 (6H, s), 3.77 (1H, t, J = 7.8 Hz), 6.57 (1H, s), 6.59 (1H, s), 7.03 (1H, t, J = 7.0 Hz), 9.31 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 34.8 (d), 53.0 (t), 53.4 (s), 114.3 (s), 116.2 (s), 119.8 (s), 130.0 (s), 139.6 (q), 158.0 (q), 169.5 (q). HRMS (FAB) calcd for $C_{12}H_{14}O_5$ + Na 261.0733, found 261.0733; calcd for $C_{12}H_{14}O_5$ + K 277.0473, found 277.0472. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 20–100% MeCN (6′) 100% MeCN (1.5′) 100–20% MeCN (0.5′)) R_f = 2.49 min; purity 91%.

2-(3-Benzyloxy-benzyl)-malonic Acid Dimethyl Ester ***8.*** Dimethyl ester **7** (5164 g, 21.7 mol) was dissolved in acetone (15 L) in a 25-L reactor containing potassium carbonate (3600 g, 26.1 mol) and dimethylformamide (90 mL). Benzylbromide (3 L, 25.3 mol) was added slowly via a dropping funnel to give a yellow suspension. The reaction mixture was heated to 68 °C (internal temperature) and heated at reflux for 20 h. After this time a white solid had formed, which was removed by filtration. The filtrate was concentrated in vacuo to give the product as a yellow oil (7471 g, > 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.04 (1H, d, J = 8.2 Hz), 3.07 (1H, d, J = 7.0 Hz), 3.58 (6H, s), 3.87 (1H, t, J = 7.8 Hz), 5.04 (1H, s), 6.75 (1H, m), 6.83–6.87 (2H, m), 7.17 (3H, t, J = 7.8 Hz), 7.29–7.43 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 34.8 (d), 53.0 (t), 53.2 (s), 69.7 (d), 113.6 (s), 115.9 (s), 121.8 (s), 128.4 (s), 128.5 (s), 129.1 (s), 130.1 (s), 137.7 (q), 139.8 (q), 159.0 (q), 169.5 (q). HRMS (FAB) calcd for $C_{19}H_{20}O_5$ + Na 351.1203, found 351.1202; calcd for $C_{19}H_{20}O_5$ + K 367.0942, found

367.0942. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 20–100% MeCN (6′) 100% MeCN (1.5′) 100–20% MeCN (0.5′)) R_f = 4.55 min; material taken on crude to next step.

3-(3-Benzyloxy-phenyl)-2-[hydroxyimino]-propionic Acid Methyl Ester ***9.*** Dimethyl ester **8** (3328 g, 10.1 mol) was added to a 25-L reactor containing methanol (15 L) to give a clear yellow solution. Sodium methoxide (2160 mL, ~5.4 M in MeOH, 11.8 mol) was added dropwise, resulting in a small exotherm. The reaction was not allowed to heat over 40 °C, and once addition was finished, the reaction was cooled to 10 °C (internal temperature). Isoamyl nitrite (1500 mL, 11.1 mol) was added to the cooled mixture over 30 min with care not to increase the temperature over 15 °C. Note: the DSC of isoamyl nitrite showed a large exotherm starting at 160 °C. The reaction mixture was stirred at 10 °C for 2 h, and after this time the solution was allowed to warm to room temperature. 2 N HCl (5 L) was added to pH 7, and the mixture was allowed to stir for 30 min before concentrating to remove the methanol. The thick beige suspension was diluted with methanol (1 L) and filtered to remove the solid. The solid was placed in a large rotation flask and azeotroped with toluene (2 × 2.5 L) to remove residual water. The beige solid formed was resuspended in hexane (5 L) and stirred at 0 °C for 1–2 h. The solid was removed by filtration and dried under vacuum at 40 °C overnight (2527 g, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.69 (3H, s), 3.80 (2H, s), 5.02 (2H, s), 6.75 (1H, d, J = 7.8 Hz), 6.82–6.84 (2H, m), 7.17 (1H, t, J = 7.8 Hz), 7.30–7.43 (5H, m), 12.50 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 30.7 (d), 52.9 (t), 69.7 (d), 112.9 (s), 116.0 (s), 121.7 (s), 128.4 (s), 128.5 (s), 129.1 (s), 130.1 (s), 137.7 (q), 138.6 (q), 150.0 (q), 159.1 (q), 164.8 (q). HRMS (FAB) calcd for $C_{17}H_{17}NO_4$ + Na 322.1050, found 322.1049. Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68. Found: C, 68.01; H, 5.68; N, 4.56. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 20–100% MeCN (6′) 100% MeCN (1.5′) 100–20% MeCN (0.5′)) R_f = 4.03 min; purity 86%.

2-Acetylamino-3-(3-benzyloxy-phenyl)-propionic Acid Methyl Ester ***10.*** Oxime **9** (3000 g, 10.0 mol) was added to a 30-L reactor containing acetic acid (14 L) and acetic anhydride (5.6 L, 59.3 mol) to give a cloudy yellow solution. The reaction mixture was warmed in a warm water bath to 40 °C (internal temperature) and zinc powder (1700 g, 26 mol) was added in portions. The internal temperature was kept between 40 and 45 °C throughout the zinc addition. After complete addition, the grey mixture was stirred at 40 °C for a further 20 h. The mixture was quenched (in two portions due to limited reactor size) with water (12.5 L, Note: a large amount of water was used to remove any zinc that could poison the Pd-catalyst in the next step), then the mixture was filtered and the solid was washed with ethyl acetate and water. The filtrate was concentrated to remove the acetic acid, and the residue was dissolved in water (10 L) and ethyl acetate (5 L). The aqueous phase was extracted three more times with ethyl acetate (3 × 5 L), and the combined organic phases were washed three times more with water (5 L). Thorough washing is important to remove all zinc salts that may disturb catalysis in the next step. The organic phase was dried over anhydrous sodium sulfate and concentrated in vacuo to give a brown oil (2888 g, 88%), which partially crystallized on standing. Mp 78.3–78.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.78 (3H, s), 2.82 (1H, dd, J = 9.4 and 13.7 Hz), 2.96 (1H, dd, J = 5.5 and 13.7 Hz), 3.57 (3H, s), 4.43 (1H, m), 5.05 (2H, s), 6.78 (1H, d, J = 7.8 Hz), 6.84–6.88 (2H, m), 7.18 (1H, t, J = 7.8 Hz), 7.31–7.43 (5H, m), 8.33 (1H, d, J = 7.8 Hz). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 22.9 (t), 37.4 (d), 52.5 (t), 54.2 (s), 69.7 (d), 113.5 (s), 116.3 (s), 122.1 (s), 128.4 (s), 128.5 (s), 129.1 (s), 129.9 (s), 137.8 (q), 139.5 (q), 158.9 (q), 170.0 (q), 172.9 (q). HRMS (FAB) calcd for $C_{19}H_{21}NO_4$ + H 328.1543, found 328.1543; calcd for $C_{19}H_{21}NO_4$ + Na 350.1363, found 350.1362. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.26; H, 6.36; N, 3.95. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 20–100% MeCN (6′) 100% MeCN (1.5′) 100–20% MeCN (0.5′)) R_f = 3.74 min; purity >99%.

2-Acetylamino-3-(3-hydroxy-phenyl)-propionic Acid Methyl Ester ***4.*** Benzyl-protected *m*-tyrosine **10** (1800 g, 5.5 mol) was dissolved in methanol (18 L) containing Pd/C (10%) (180 g) and shaken under 1.2 bar H_2 for 4 days. After 100% H_2 consumption the reaction was stopped, and the reaction mixture was filtered through a plug of Hyflo to remove the carbon and any palladium impurities. The methanolic solution obtained was concentrated in vacuo to yield a viscous brown oil (1323 g, >99%). The product could be crystallized according to the procedure used in the next synthesis. Mp 112.6–112.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.78 (3H, s), 2.76 (1H, dd, J = 9.4 and 13.7 Hz), 2.89 (1H, dd, J = 5.5 and 13.7 Hz), 3.57 (3H, s), 4.38 (1H, m), 6.58–6.60 (3H, m), 7.03 (1H, t, J = 8.0 Hz), 8.31 (1H, d, J = 7.4 Hz), 9.29 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 22.9 (t), 37.4 (d), 52.5 (t), 54.3 (s), 114.2 (s), 116.5 (s), 120.2 (s), 129.8 (s), 139.2 (q), 157.9 (q), 170.0 (q), 172.9 (q). HRMS (FAB) calcd for $C_{12}H_{15}NO_4$ + H 238.10738, found 238.10733; calcd for $C_{12}H_{15}NO_4$ + Na 260.08933, found 260.08922. Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.33; H, 6.23; N, 5.84. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 20–100% MeCN (6′) 100% MeCN (1.5′) 100–20% MeCN (0.5′)) R_f = 2.80 min; purity >99%.

N*-Acyl *m*-Tyrosine Methyl Ester via Erlenmeyer Azlactone Synthesis (according to Scheme 5).** *4-[1-(3-Benzyloxy-phenyl)-meth-(Z)-ylidene]-2-methyl-4H-oxazol-5-one* ***11. *m*-Benzyloxybenzaldehyde (47.5 g, 224 mmol) was added to a 350 -mL flask containing *N*-acetylglycine (28.8 g, 246 mmol) and sodium acetate (20.6 g, 251 mmol), and acetic anhydride (112 mL, 5.27 mmol) was added. The mixture was stirred (using an overhead stirrer) at 115 °C for 3.5 h under nitrogen to give a dark brown solution. The starting materials took 30 min to completely dissolve. The reaction was monitored by TLC in 10% EtOAc in hexanes ($R_{f(SM)}$ = 0.25, $R_{f(P)}$ = 0.18). The reaction mixture was cooled in an ice bath to 20 °C and then poured into water (2 L) diluted with ethanol (1 L). The yellow/

brown solid formed upon stirring for 30 min at room temperature was collected by filtration and dried at room temperature under a filter paper over the weekend to give a yellow/brown lumpy solid. The solid was crushed to give a bright yellow solid (59.7 g, 91%) and taken on crude for the next synthesis step. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.38 (3H, s), 5.13 (2H, s), 7.14 (1H, dd, J = 2.6 and 8.0 Hz), 7.17 (1H, s), 7.32–7.47 (6H, m), 7.73 (1H, d, J = 7.8 Hz), 7.89 (1H, s). The sample contained 18% acidic by-product **13** by ^{1}H NMR. ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 16.0 (t), 70.0 (d), 118.2 (s), 118.4 (s), 125.5 (s), 128.6 (s), 128.6 (s), 129.1 (s), 130.3 (s), 130.6 (s), 133.5 (q), 135.0 (q), 137.4 (q), 159.0 (q), 167.5 (q), 168.0 (q). HRMS (FAB) calcd for $C_{18}H_{15}NO_3$ + H 294.11247, found 294.11238; calcd for $C_{18}H_{15}NO_3$ + Na 316.09441, found 316.09436. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 5–100% MeCN (6′) 100% MeCN (1.5′) 100–5% MeCN (0.5′)) $R_{f\,(\text{aldehyde})}$ = 5.57 min; $R_{f\,(\text{product})}$ = 6.11 min.

*(Z)-2-Acetylamino-3-(3-benzyloxy-phenyl)-acrylic Acid **13** as Isolated By-Product.* Isolated as a the major product from 100 g scale-up of the previous reaction. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.95 (3H, s), 5.11 (2H, s), 7.34–7.54 (9H, m), 9.48 (1H, s), 12.68 (1H, br s). MS (ES^+) calcd for $C_{18}H_{17}NO_4$ + H 312, found 312; (ES^-) calcd for $C_{18}H_{17}NO_4$ – H 310, found 310. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 5–100% MeCN (6′) 100% MeCN (1.5′) 100–5% MeCN (0.5′)) R_f = 4.55 min.

*(Z)-2-Acetylamino-3-(3-benzyloxy-phenyl)-acrylic Acid Methyl Ester **12** (one-pot procedure).* *m*-Benzyloxybenzaldehyde (100 g, 471 mmol) was added to a 1-L flask containing *N*-acetylglycine (100 g, 466 mmol) and sodium acetate (42.9 g, 522 mmol), and acetic anhydride (232 mL, 2450 mmol) was added. The mixture was stirred (using an overhead stirrer) at 115 °C for 18 h under nitrogen to give a dark brown solution. The reaction was monitored by TLC in 10% EtOAc in hexanes ($R_{f\,(\text{SM})}$ = 0.25, $R_{f\,(\text{P})}$ = 0.18) and by HPLC until the aldehyde concentration reached a minimum. The reaction mixture was cooled in an ice bath to 20 °C then poured into methanol (2.5 L) containing sodium acetate (76 g, 926 mmol), and the clear brown solution was stirred at room temperature for 48 h, after which time TLC in 50% EtOAc in hexanes indicated near complete conversion ($R_{f\,(\text{SM})}$ = 0.57, $R_{f\,(\text{P})}$ = 0.13). The reaction was concentrated in vacuo to remove the methanol, then diluted with EtOAc (1 L) and washed with water (2 L). The aqueous layer was washed twice more with EtOAc (2 × 500 mL), and the combined EtOAc layers were washed once more with brine (1 L). The organic layer was dried over anhydrous sodium sulfate containing charcoal (50 g) before being concentrated in vacuo to yield a brown crystalline solid. The crude material was triturated with 40% methylene chloride in hexane (300 mL) followed by the addition of 100 mL of hexane (now 30% methylene chloride in hexane), and the pale yellow solid obtained was washed with heptane (100 mL) before being dried in the vacuum oven at 40 °C for 2 h (104 g, 68%). Mp 113.8–115.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.97 (3H, s), 3.69 (3H, s), 5.11 (2H, s), 7.02 (1H, dd, J = 2.1 and 8.0 Hz), 7.11 (1H, s), 7.21 (1H, d, J = 7.8 Hz), 7.30–7.44 (7H, m), 9.67 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 23.1 (t), 52.9 (t), 69.9 (d), 116.5 (s), 116.7 (s), 123.2 (s), 127.6 (q), 128.4 (s), 128.6 (s), 129.2 (s), 130.4 (s), 131.4 (s), 135.4 (q), 137.6 (q), 159.0 (q), 166.3 (q), 170.1 (q). HRMS (FAB) calcd for $C_{19}H_{19}NO_4$ + H 326.13854, found 326.13869; calcd for $C_{19}H_{19}NO_4$ + Na 348.12042, found 348.12063. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 69.97; H, 5.78; N, 4.45. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 5–100% MeCN (6′) 100% MeCN (1.5′) 100–5% MeCN (0.5′)) R_f = 4.97 min; > 92% purity.

*2-Acetylamino-3-(3-hydroxy-phenyl)-propionic Acid Methyl Ester **4**.* Acrylic acid methyl ester **11** (40.8 g, 124 mmol) was dissolved in methanol (627 mL) containing Pd/C (10%) (4.23 g) and shaken under 1.1 bar H_2 for 4 h. After 100% H_2 consumption the reaction was stopped, and the reaction mixture was filtered through a 3 cm plug of Hyflo to remove the carbon and any palladium impurities. The methanolic solution obtained was concentrated in vacuo to yield a clear pale brown gummy/foamy solid (30.4 g, > 99%). If necessary the solid was crystallized from 10% dichloromethane in hexanes to give a cream colored solid. Mp 105.1–105.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.78 (3H, s), 2.75 (1H, dd, J = 9.0 and 13.7 Hz), 2.88 (1H, dd, J = 5.9 and 13.7 Hz), 3.57 (3H, s), 4.37 (1H, dd, J = 5.7 and 8.0 Hz), 6.57–6.60 (3H, m), 7.03 (1H, t, J = 8.0 Hz), 8.31 (1H, d, J = 7.8 Hz), 9.30 (1H, br s). ^{13}C NMR (101 MHz, DMSO-d_6, DEPT) δ 22.9 (t), 37.4 (d), 52.5 (t), 54.3 (s), 114.2 (s), 116.5 (s), 120.2 (s), 129.8 (s), 139.2 (q), 157.9 (q), 170.0 (q), 172.9 (q). HRMS (FAB) calcd for $C_{12}H_{15}NO_4$ + H 238.10738, found 238.10739; calcd for $C_{12}H_{15}NO_4$ + Na 260.08933, found 260.08923; calcd for 2($C_{12}H_{15}NO_4$) + Na 497.18944, found 497.18949. Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 59.94; H, 6.09; N, 5.97. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 5–100% MeCN (6′) 100% MeCN (1.5′) 100–5% MeCN (0.5′)) R_f = 3.17 min.

*(S)-2-Acetylamino-3-(3-hydroxy-phenyl)-propionic Acid **5**.* In a typical experiment, racemic *N*-acyl *m*-tyrosine methyl ester **4** (34.1 g, 144 mmol) was suspended in phosphate buffer at pH 7.5 (300 mL) and 100 μg of Alcalase was added. The mixture was stirred at room temperature, and the pH was kept constant using a pH-stat by adding a 1 N NaOH solution. After about 9 h, the conversion reached 50% (consumption of 72 mL of the NaOH solution). The aqueous phase was extracted twice with dichloromethane (2 × 250 mL), and then the combined organic phases were washed with water, dried over anhydrous sodium sulfate and concentrated in vacuo to give (*R*)-*N*-acyl *m*-tyrosine methyl ester (*R*)-**4** (16.6 g, 70 mmol, 49%) as a colorless resin. The spectroscopic data were identical to those of racemic *N*-acyl *m*-tyrosine methyl ester (±)-**4**. $[\alpha]^{20}_{589}$ = −76.7 (*c* 1.16, $CHCl_3$); HPLC (Chiralpak AD-H 250 × 4.0 mm hexanes/EtOH 80:20 flow 1 mL/min) ee 99.2%. The crude aqueous phase containing (*S*)-*N*-acyl *m*-tyrosine (*S*)-**5** was used for the next step without purification.

*L-m-Tyrosine **1**.* Concentrated HCl solution (82 mL) was added to the crude aqueous phase containing (*S*)-*N*-acyl *m*-tyrosine (*S*)-**5** (409 mL), and the mixture was heated for 2 h

at 100 °C followed by complete evaporation of the liquid. The residue was redissolved in water (100 mL), and the pH was adjusted to 6 by adding 4 N NaOH solution. The precipitate formed was filtered and dried in vacuo to yield L-*m*-tyrosine **1** (11.1 g, 42%) as colorless crystals. Mp 278.0–278.4 °C. $[\alpha]^{20}_{589} = -7.9$ (*c* 1, 1 N HCl) [lit.: $[\alpha]^{25}_{D} = -7.9$ (*c* 2, 1 N HCl)];[4,5] HPLC (Chirobiotic TAG 250 × 4.6 mm H_2O/MeOH 40:60 flow 1.5 mL/min) ee >99.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.88 (1H, dd, J = 8.2 and 14.5 Hz), 3.05 (1H, dd, J = 5.1 and 14.4 Hz), 3.79 (1H, dd, J = 5.1 and 8.2 Hz), 6.63–6.70 (3H, m), 7.12 (1H, t, J = 8.5 Hz). ^{1}H NMR (126 MHz, D_2O, DEPT) δ 36.6 (d), 56.0 (s), 114.7 (s), 116.2 (s), 121.5 (s), 130.6 (s), 137.0 (q), 156.0 (q), 174.1 (q). HRMS (FAB) calcd for $C_9H_{11}NO_3$ + H 182.08117, found 182.08121; calcd for $C_9H_{11}NO_3$ + Na 204.06311, found 204.06308. HPLC (Macherey-Nagel CC 70/4 Nucleosil 100-3 C18 HD, 5–100% MeCN (6′) 100% MeCN (1.5′) 100–5% MeCN (0.5′)) R_f = 1.527 min; > 99% purity.

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