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Scale-Up of a Chemo-Biocatalytic Route to (2*R*,4*R*)- and (2*S*,4*S*)-Monatin

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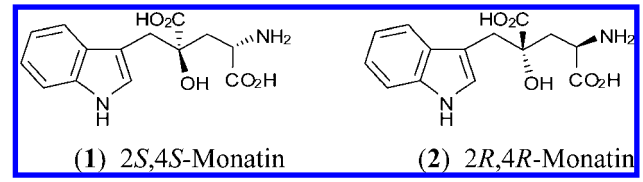
Abstract:

Monatin, a natural sweetener, refers to a collection of four isomers of 2-((1*H*-indol-3-yl)methyl)-4-amino-2-hydroxypentanedioic acid. A chemo-biocatalytic approach to kilogram quantities of enantiopure 2*S*,4*S*-monatin and 2*R*,4*R*-monatin from indole is described. Key steps in the process include a (2 + 3) cycloaddition reaction followed by nickel-catalysed reduction to construct the monatin backbone, and a highly selective enzyme resolution of the 2*S*,4*S*- and 2*R*,4*R*-monatin diastereomeric pair to afford each enantiomer in 99% ee.

Introduction

Monatin is the coined name that describes a collection of four isomers of 2-((1*H*-indol-3-yl)methyl)-4-amino-2-hydroxypentanedioic acid, isolable from *Schlerochitin ilicifolius*, a spiny-leaved hardwood shrub indigenous to South Africa. The isolation of the 2*S*,4*S* isomer of monatin (**1**) from the plant and the elucidation of its structure were reported 20 years ago, together with the identification of this compound as a high-intensity, natural sweetener.[1] Originally, 2*S*,4*S*-monatin was thought to be the only isomer isolated from the extracted roots of *S. ilicifolius*, but it is now known that the plant produces a mixture of all four monatin isomers.[2] Of further interest is the fact that the sweetness potential appears dependent upon the isomer. For example, 2*R*,4*R*-monatin (**2**) and its salts (e.g., Na, NH_3, K) have been shown to elicit the sweetest taste, with a relative sweetness ranging from 1000 to 2700 times that of sucrose.[3,4] By comparison, synthetic sweeteners such as aspartame exhibit relative sweetness of only 180−200 times that of sucrose. As a result, there may be commercial interest in the natural product, monatin, which is present in very low quantities in the bark of the root of *S. ilicifolius* (1.73 g isolated from 160 kg of root).[1a]



A number of synthetic routes to monatin isomers, both stereoselective and racemic, have been reported.[5] However, these processes have been conducted at laboratory scale and are not readily applied to the large-scale production of monatin. In this contribution we describe a chemo-biocatalytic approach for the production of kilogram quantities of enantiopure 2*S*,4*S*- and 2*R*,4*R*-monatin.[6,7]

Results and Discussion

The chemo-biocatalytic approach allows access to all four isomers of monatin by the initial preparation of racemic monatin in three steps from indole (Scheme 1). In this route, the diastereomeric pairs (2*R*,4*R*/2*S*,4*S*-monatin and 2*R*,4*S*/2*S*,4*R*-monatin, hereinafter referred to as *RR*/*SS* monatin and *RS*/*SR* monatin respectively) are separated by crystallization, and each pair may be subjected to enzymatic resolution after esterification and lactonization. This affords, after subsequent deprotection steps, the corresponding individual monatin enantiomers.The route to racemic monatin developed by the CSIR and first described in 1992,[5i] was modified for scale-up to kilogram scale and to exclude reagents such as bromine and sodium/mercury amalgam utilized in the original process. The enzymatic resolution was developed by Altus Biologics,[7a] and here we describe modifications to the process to allow for scale-up to kilogram scale. Although the process described in this paper has not been fully optimized, it represents the first application of a synthetic route to monatin isomers on this scale.

Acrylate derivative (**3**) was prepared from ethyl acrylate by initial Baylis−Hillman[8] reaction with paraformaldehyde to

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(1) (a) Vleggaar, R.; Ackerman, L. G. J.; Steyn, P. S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3095–3098. (b) van Wyk, P. J.; Ackerman, L. G. U.S. Patent 4,975,298, 1990.

(2) Bassoli, A.; Borgonovo, G.; Busnelli, G.; Morini, G.; Drew, M. G. B. *Eur. J. Org. Chem.* **2005**, 1652–1658.

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(4) Amino, Y.; Yuzawa, K.; Mori. K.; Takemoto, T. WO/2003/045914, 2003.

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(7) (a) Buddoo, S. R., Rousseau, A. L., Brady, D., Lalonde, J., Yao, Y., Wang, Y. US Patent Application US20090087888A1, 2009; (b) Brady, D., Steenkamp, L. H., Rousseau, A. L., Buddoo, S. R., Steenkamp, P. A. US Patent Application US20090087829A1, 2009.

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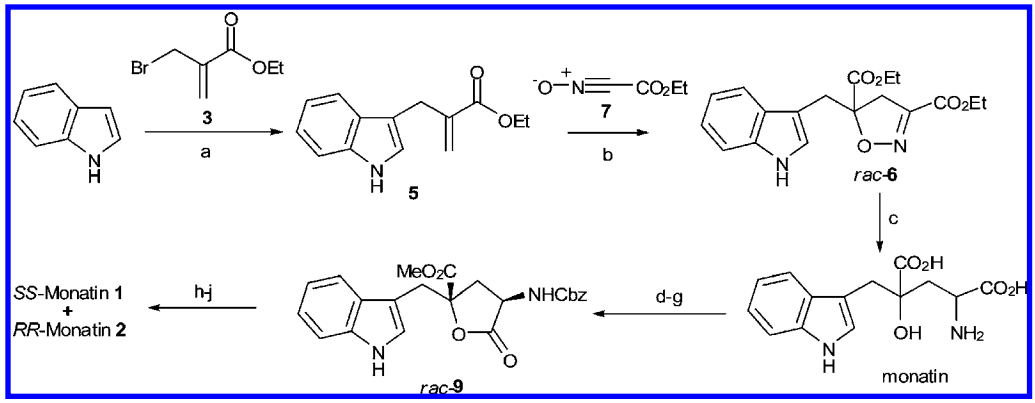
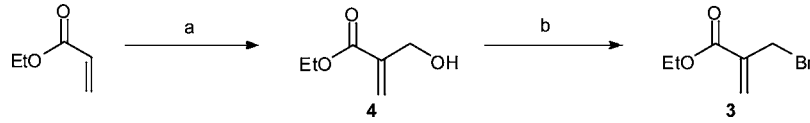
Scheme 1. **Overall schematic approach to monatin enantiomers**[a]



[a] Reagents and conditions: a) Indole, THF, methylmagnesium chloride, 0 °C, 30 min, then (**3**) in THF, 5 °C, 1 h, 49% (11 runs) [Average yields reported over multiple runs, number of runs listed in parentheses after yield given]; b) $CHCl_3$, Et_3N, 30 °C, 2 h, 68% (11 runs); c) EtOH, KOH, sponge Ni (0.5 equiv), H_2, 2 bar, 21 °C, 1.5 h, 98% conversion by HPLC; d) separation of *RR*/*SS* monatin from *RS*/*SR* monatin by crystallization, 76% isolated yield (5 runs); e) methanol, HCl(g), 25 °C, 1.5 h; f) EtOAc, aq $NaHCO_3$, CbzCl, 25 °C, 1 h; g) *p*-TsOH (1%), toluene, 25 °C, 16 h, 60% over three steps from *RR*/*SS* monatin, (5 runs); h) ChiroCLEC-BL, ACN, phosphate buffer (0.3 M, pH 7.5), 23 °C, 15−20 h 98% conversion of *SS*-**9**; i) 10% m/m Pd/C (5%), MeOH, Et_3N, H_2 (3 bar), 40 °C, 1 h; j) aq KOH, 25 °C, 30 min. Yield of *SS*-monatin (**1**) from *SS*-**9**: 52% (5 runs). For *RR*-product, order of deprotection steps i) and j) are reversed. Yield of *RR*-monatin (**2**) from *RR*-**9**: 47% (5 runs).

Scheme 2. **Preparation of ethyl 2-(bromomethyl)acrylate**[a]



[a] Reagents and conditions: a) paraformaldehyde (30% m/m), H_2O, DABCO (0.1 equiv, 4.5% m/m), NaOH (0.40% m/m), 85 °C, 30 min; b) aq HBr (48%), H_2SO_4 (35% m/m), 15−20 °C, 16 h, 34% over two steps.

afford the intermediate ethyl 2-(hydroxymethyl)acrylate (**4**), followed by bromination to afford the desired 2-(bromomethyl)-acrylate (**3**) (Scheme 2). This process, which was found to be low yielding on laboratory scale (50−100 g scale, 31% average yield over the two steps), proved somewhat problematic on scale-up. However, with modifications of the laboratory procedure, a comparable yield of product was obtained on kilogram scale (34% average yield over the two steps).

On laboratory scale (50−100 g), optimized reactions to prepare (**4**) were carried out under reflux with reactor temperature at 80 °C and in the presence of 10 mol % DABCO catalyst. This gave 60% conversion to the desired product without significant byproduct formation (<10%), as observed by GC analysis. The same results were obtained with 2% DABCO in the presence of triethylamine (7 mol %), which maintains the pH of the reaction mixture between 8 and 9, limiting deactivation of the DABCO catalyst. The product was isolated by extraction into ethyl acetate, followed by evaporation to dryness. The yield of (**4**) in the large-scale Baylis-Hillman reaction under these conditions (80 °C, 2 mol % DABCO, 7 mol % triethylamine) was found to be lower than that obtained on the laboratory scale, with a significantly higher level of polymeric byproduct formed in the process (approximately 30%). The down stream processing of the reaction mixture also proved to be problematic, with poor phase separation during the extraction of (**4**) into ethyl acetate, and loss of product to the aqueous phase occurring as a result of the poor separation. This in turn had a detrimental effect on the subsequent formation of (**3**), produced in an overall yield of only 10% from ethyl acrylate. Upon investigation, triethylamine was shown to facilitate byproduct formation under the prolonged periods of heating associated with the heating and cooling profiles of reaction mixtures on large scale. In order to address this problem, the loading of DABCO catalyst was adjusted to 10 mol %, and triethylamine was excluded from the large-scale reaction. In addition, the reaction was carried out in a closed vessel instead of under reflux conditions to obtain a higher operating temperature of 85 °C and to allow for better utilization of ethyl acrylate (boiling point of 99 °C) and paraformaldehyde. No pressure buildup was observed under these conditions. Using this modified procedure, 70% conversion of ethyl acrylate was observed within 30 min, with minimal byproduct formation on this scale (<10%). The downstream processing of the reaction mixture was modified to facilitate the isolation of (**4**), free of unreacted ethyl acrylate and paraformaldehyde, and polymeric byproduct. This was essential to ensure adequate performance in the subsequent bromination reaction, and to minimise decomposition of the final product (**3**) during distillation. Isolation of (**4**) was therefore achieved by adjusting the pH of the reaction mixture to 4.5 with aqueous sulfuric acid (20% m/m), followed by extraction of ethyl acrylate and polymeric byproduct from the aqueous layer with hexane. The intermediate (**4**) was then isolated from the aqueous phase by extraction with 1,2-dichloroethane.

The bromination reaction to afford (**3**) employed on laboratory scale involved producing a concentrated aqueous hydrobromic acid solution by treatment of 48% aq HBr with hydrogen bromide gas. In the laboratory process reported in 1992,[5i] hydrogen bromide gas was generated from the action of bromine on tetralin. We modified this process to avoid the use of bromine or hydrogen bromide gas on large scale and utilised a method whereby treatment of aqueous hydrobromic acid (48%) with concentrated sulfuric acid (3.25 mol equiv) afforded a concentrated hydrobromic acid solution which performed well in this

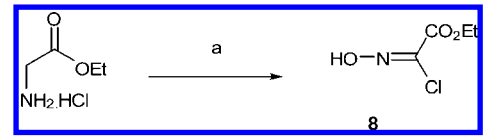
reaction.[9] On laboratory scale, an average overall yield of 31% of (**3**) was achieved from ethyl acrylate over two steps. The bromination of (**4**) to afford (**3**) on large scale was carried out using this modified process. The product was extracted into hexane, washed with aqueous sodium bicarbonate, and purified by distillation (0.8 mmHg, 38−40 °C, reboiler temperature <80 °C) to afford a comparable yield of product on 50 kg scale (34% average isolated yield over the two steps). To avoid decomposition of the final product, the distillation was carried out at <1 mmHg.

Formation of ethyl 2-((1*H*-indol-3-yl)methyl)acrylate (**5**) was achieved by reaction of ethyl 2-(bromomethyl)acrylate (**3**) and indolylmagnesium chloride, which was generated from indole and methylmagnesium chloride in THF. In addition to the formation of the desired product (**5**), other complex indole/acrylate adducts were produced during the reaction and identified as sequential addition products resulting from reaction at the indole nitrogen. A number of screening experiments were therefore conducted on laboratory scale in order to determine the effect of variables such as residual water present in the solvent, order of reagent addition, temperature, stoichiometry, and concentration of reagents on the reaction performance at bench scale (15 L).

At this scale, the reaction performed equally well with solvent that had been predried using sodium metal, and with solvent used directly from the supplier[10] without pretreatment. The order of reagent addition also had no effect on the yield of product and did not result in a decrease in byproduct formation. When acrylate (**3**) was used as the limiting reagent in the reaction, a decrease in byproduct formation was observed as expected; however, this was accompanied by a concomitant decrease in both indole conversion and product yield. Although removal of unreacted indole from the crude reaction mixture could be achieved by steam distillation, the reduction in product yield and prospect of an additional process step prompted us to use conditions which would allow for maximum indole conversion (mole ratio indole/acrylate/Grignard = 1.0:1.08:1.1). The reaction was not adversely affected when carried out at higher concentrations of indole and acrylate (**3**) (31% m/m and 44% m/m, respectively, compared with 18% m/m and 29% m/m respectively), allowing for smaller reaction volumes. The reaction was sensitive to temperature, with increased temperatures resulting in increased levels of byproduct formed.

Optimum yields of acrylate (**5**) on kilogram scale were therefore obtained when a solution of indolylmagnesium chloride in THF, generated at 0−10 °C over a period of 2 h, was treated with a solution of 2-(bromomethyl)acrylate (**3**) in THF, added over a period of 2 h at 0−5 °C, and left for a further 1 h at this temperature. On a laboratory scale, the product was purified by silica gel column chromatography, and alternative methods of purification were sought for scale-up. Although conventional distillation proved to be successful on a laboratory scale, this led to significant decomposition during extended heating times and loss in yield at bench scale. We assessed an alternative short path distillation of the mixture which was found to minimise the degradation that had been observed using the conventional distillation process. Although this afforded the acrylate (**5**) in lower purity (70−80% m/m by HPLC), losses due to degradation were minimised, and the short path distillation was chosen as the purification method of choice for this intermediate. The acrylate (**5**) was isolated in an average yield of 49% by this process, which was somewhat lower than that obtained on laboratory scale (50 g, 61% yield) where column chromatography was utilized for purification of the product. This may be attributed to losses associated with the short path distillation.

Scheme 3. **Preparation of ethyl 2-chloro-2-(hydroxyimino)acetate**[a]



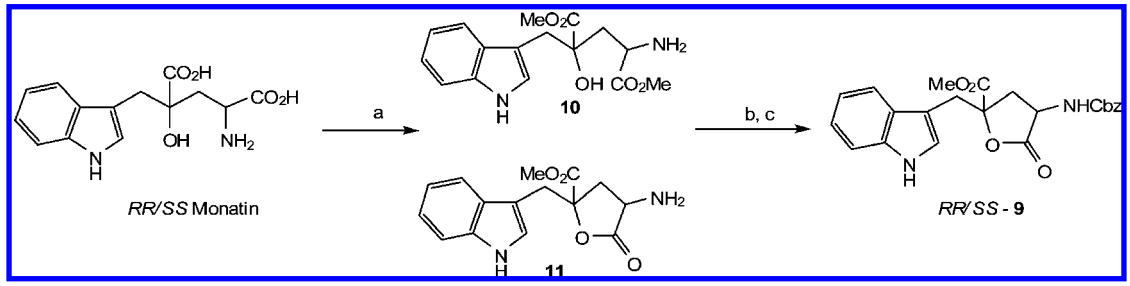
[a] Reagents and conditions: a) 32% aq HCl, HCl(g), $NaNO_2$ (2.0 equiv, in H_2O, 69% m/m), −5−0 °C, 4 h; (40% average yield over 10 runs).

The (2 + 3) cycloaddition step to afford the isoxazoline diester (**6**) from indolyl acrylate (**5**) and carbethoxyformonitrile oxide (**7**) in the presence of triethylamine proved to be robust.[5g,i] The reaction performance was not adversely affected when indolyl acrylate (**5**) of lower purity (70−80%) was utilised as the substrate. Lengthy chromatography of the indolyl acrylate (**5**) was therefore avoided and the product isolated from short path distillation was carried through to the cycloaddition step. On a 2 kg scale, the cycloaddition reaction afforded isoxazoline diester (**6**) in an average yield of 68%. The product was isolated by crystallization, and recrystallized when necessary to achieve the desired level of purity (>98%) for the subsequent hydrogenation step.

The nitrile oxide (**7**) is formed *in situ* from ethyl 2-chloro-2-(hydroxyimino)acetate (**8**) during the cycloaddition reaction. The synthesis of (**8**), starting from glycine ethyl ester hydrochloride, was carried out at 0−5 °C with strict temperature control on 5−10 kg scale (Scheme 3).[11] The product was isolated in an average yield of 40%, which was comparable with the yields obtained on laboratory scale (200 g).[12] The byproduct formed during this reaction, ethyl chloroacetate, is a known sensitizer, and continued exposure to this chemical results in skin rashes and shortness of breath. The symptoms disappear once contact with the chemical is eliminated. The chemical has a low vapour pressure and even though protective equipment is used the risk of exposure is very high. Strict containment measures were put in place in order to minimise exposure to ethyl chloroacetate during the preparation of (**8**). Handling of the material was minimised, and all work was carried out in large-scale fume hoods in a single work area with access control. Effective PPE (including respirators) and a designated wash-up area further reduced the level of exposure to this sensitizer.

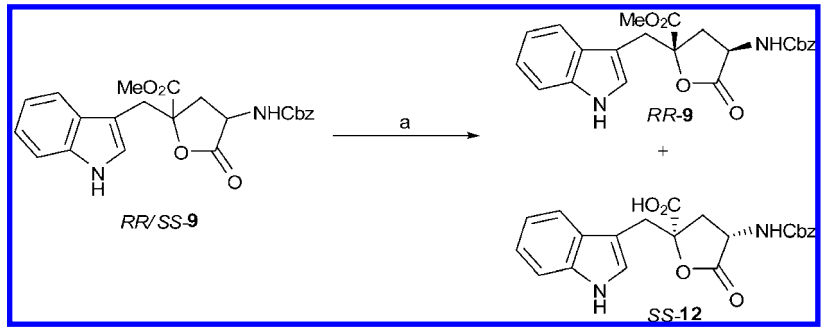
(9) In *Organic Syntheses*, 2nd ed.; Gillman, H., Blatt, A. H., Eds., John Wiley and Sons, Inc.: London, 1958; Collective Vol. 1.
(10) Merck, AR grade, containing 0.03% (m/m) water.
(11) Kozikowski, P.; Adamczyk, M. *J. Org. Chem.* **1983**, *48*, 366–372. Ethyl 2-chloro-2-(hydroxyimino)acetate (**8**) is commercially available in bulk from a number of suppliers; however, costs associated with the purchase and shipping of (**8**) (US $400−700/kg) prompted us to synthesise the material in-house.
(12) The yield of (**8**) on 150 g scale or less was found to be slightly higher, at an average of 56%. However, all reactions carried out at a scale of 200−500 g afforded lower yields of (**8**), with an average yield of 43% obtained on this scale.

Scheme 4. **Preparation of monatin derivatives for enzymatic resolution**[a]



[a] Reagents and conditions: a) methanol, HCl(g), 25 °C, 1.5 h; b) EtOAc, aq $NaHCO_3$, CbzCl, 25 °C, 1 h; c) *p*-TsOH (1%), toluene, 25 °C, 16 h, 60% over three steps from *RR*/*SS* monatin (5 runs).

Scheme 5. **Enzymatic resolution of *RR*/*SS* monatin derivatives**[a]



[a] Reagents and conditions: a) ChiroCLEC-BL, MeCN, phosphate buffer (0.3 M, pH 7.5) 22 °C, 15−20 h, 98% conversion of *SS*-**9**.

Nickel-catalysed hydrogenation of the isoxazoline diester (**6**) was carried out on a 5 kg scale, and the two pairs of monatin diastereomers were separated by crystallization. *RR*/*SS* monatin is only partially water-soluble and was isolated by precipitation from aqueous acetic acid to afford, after subsequent recrystallization, *RR*/*SS* monatin in 76% yield at >98% m/m purity by quantitative HPLC. By comparison, *RS*/*SR* monatin is highly water-soluble, and was isolated by precipitation from ethanol. As our interest lay in the *RR*/*SS* diastereomeric pair, the *RS*/*SR* monatin formed during this process was retained, but not processed further.

The *N*-protected monatin lactone (**9**) required for the enzymatic resolution was prepared in three steps as shown in Scheme 4. *RR*/*SS* monatin was treated with methanol saturated with hydrogen chloride gas to afford a mixture of the dimethyl ester of monatin (**10**) as well as the monatin lactone methyl ester (**11**). On laboratory scale, (**10**) and (**11**) were formed in an approximate ratio of 75:25 by HPLC analysis of the reaction mixture, and complete conversion to the lactone methyl ester (**11**) was facilitated by removal of methanol on a rotary evaporator. This process was not feasible on >500 g scale, with all attempts to facilitate complete conversion to (**11**) resulting in either loss in yield or decomposition of the product, and an alternative approach was adopted. The crude mixture of dimethyl ester (**10**) and lactone (**11**) was therefore subjected to *N* protection with benzylchloroformate under basic conditions in a biphasic system. After complete reaction, the product mixture was extracted into ethyl acetate and concentrated under reduced pressure. The *N*-protected lactone/dimethyl ester mixture was treated with catalytic *p*-toluenesulfonic acid (1%) in toluene at room temperature, and the desired product precipitated from the toluene reaction matrix affording pure product in solid form. Using this procedure, *RR*/*SS*-**9** was obtained in an average yield of 60% from *RR*/*SS*-monatin on a 1.5 kg scale.

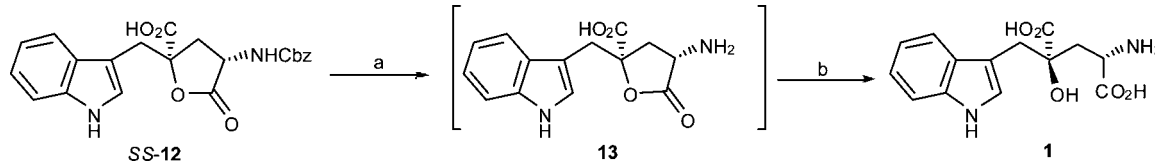
The enzymatic resolution (shown in Scheme 5) was carried out on 1 kg of substrate (**9**) at 22 °C for 19 h and stirrer speeds of 150−200 rpm, and the reaction progress was monitored by HPLC. The enzyme used in this transformation, a protease (subtilisin) from *Bacillus lichenformis*, was immobilized by the CLEC method and designated ChiroCLEC-BL.[13] This biocatalyst had previously demonstrated to be enantioselective for this reaction on a laboratory scale.[14]

The resolution reaction was found to be highly selective over temperatures ranging from 17 to 25 °C, with *RR*-**9** left entirely unreacted with only 2−5% residual *SS*-**9**. A drop in selectivity was observed at higher temperatures (35−40 °C) on laboratory scale with 10−15% of *RR*-**9** consumed in the reaction before 50% conversion of *RR*/*SS*-**9** had been reached. The enzyme was recovered by filtration and could be recycled after washing with a mixture of phosphate buffer and acetonitrile. On laboratory scale, the enzyme was recycled twice with no significant loss in selectivity or activity. After removal of the enzyme by filtration, the unreacted *RR*-**9** was isolated from the resolution reaction mixture by extraction into dichloromethane. The

(13) (a) Margolin, A. L. *Trends Biotechnol.* **1996**, *14*, 223–230. (b) St. Clair, N. L.; Navia, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 7314–7316.

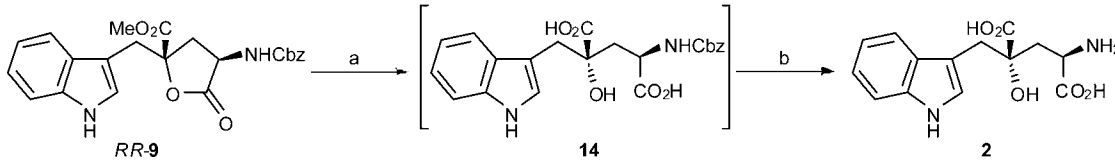
(14) Personal communication with J. Lalonde, Y. Yao, and Y. Wang of Altus Biologics Inc., who selected the enzyme for this resolution, see ref 7a. The ChiroCLEC-BL was supplied by Altus Biologics from a single batch, and the resolution was run five times on this scale. Although ChiroCLEC-BL is no longer commercially available on this scale, the nonimmobilized form of the enzyme, alcalase, which is commercially available, performed equally well in this transformation on laboratory scale. For another example of the use of the ChiroCLEC-BL in synthesis see: Ema, T.; Jittani, M.; Furuie, K.; Utaka, M.; Sakai, T. *J. Org. Chem.* **2002**, *67*, 2144–2151.

Scheme 6. **Deprotection sequence to afford *SS*-monatin (1)[a]**



[a] Reagents and conditions: a) 10% m/m Pd/C (5%), MeOH, Et_3N, H_2 (3 bar), 40 °C, 1 h; b) aq KOH, 25 °C, 30 min, then AcOH.

Scheme 7. **Deprotection sequence to afford *RR*-monatin (2)[a]**



[a] Reagents and conditions: a) *RR*-**9** in DCM, aq KOH, 25 °C, 30 min, then AcOH; b) 10% m/m Pd/C (5%), MeOH, Et_3N, H_2 (3 bar), 40 °C, 1 h.

dichloromethane extract was used without further processing in the deprotection sequence.

Isolation of the resolution product (**12**) was facilitated by addition of dichloromethane to the remaining aqueous phase, followed by acidification to pH 2.0 with 10% aq HCl. This ensured the formation of a well-dispersed precipitate, allowing for recovery and isolation of *SS*-**12** by filtration.

Hydrolysis of the ester groups and removal of the *N*-Cbz protecting group from the products isolated from the enzymatic resolution affords the individual *RR*- and *SS*-monatin enantiomers. In initial studies on laboratory scale, epimerization at the 4-position was observed under the basic conditions required for ester and/or lactone hydrolysis of *RR*-**9** and *SS*-**12**, owing to the acidity of the proton α to the carbonyl group of the lactone. This effect was exacerbated on larger scales (>250 g), where the hydrolysis reaction was much slower and increased epimerization (>15%) was observed.

In an attempt to minimize epimerization, hydrogenation of *SS*-**12** was carried out first, followed by hydrolysis as shown in Scheme 6. Efficient mixing in the hydrogenation reaction is essential to allow for optimal interaction between the gas (hydrogen), solid (catalyst), and liquid phases (substrate in solution). As *SS*-**12** was not sufficiently soluble in methanol, the solvent used for the hydrogenation reaction, the slurry that formed was treated with triethylamine (0.2 equiv) to facilitate dissolution of *SS*-**12**. Palladium supported on carbon was then added and the mixture stirred in a hydrogen atmosphere for 3 h, after which time HPLC analysis indicated complete reaction. The presence of triethylamine at this level was not found to adversely affect the performance of the hydrogenation reaction. The intermediate product (**13**) precipitates out of the reaction matrix; thus, removal of the catalyst by filtration was not feasible. The reaction mixture was therefore treated with aqueous potassium hydroxide and left to stir at room temperature for 1 h before removal of the catalyst by filtration. The filtrate was acidified to pH 4.0 with glacial acetic acid, and the mixture was concentrated to afford a mixture of *SS*-monatin (**1**) and potassium acetate. This mixture was treated with ethanol to dissolve the potassium acetate, and the remaining precipitate was filtered by Nutsche pressure filtration and dried in a vacuum oven. This process was repeated until analysis indicated that the product was free of potassium acetate.[15] This afforded *SS*-monatin isolated as the monopotassium salt in an average yield of 56% from *SS*-**12** at >98% m/m purity (quantitative HPLC), 99.5% de and 99% ee.

Unfortunately, this deprotection sequence could not be applied to *RR*-**9** as lactam formation occurs readily during the removal of the Cbz protecting group. The dichloromethane extract from the resolution reaction containing *RR*-**9** was therefore treated with aqueous potassium hydroxide to facilitate hydrolysis. The biphasic system limited epimerization at the 4-position during ester hydrolysis (Scheme 7).

The aqueous phase containing the intermediate *RR*-diacid (**14**) was then treated with methanol and acetic acid to afford a homogeneous solution, followed by catalytic reduction with Pd/C in a hydrogen atmosphere to facilitate complete removal of the Cbz protecting group. The reaction mixture was filtered from catalyst and the filtrate concentrated to afford a mixture of *RR*-monatin and potassium acetate. This mixture was treated as described for the *SS*-product to afford *RR*-monatin as the monopotassium salt in an average yield of 60% from *RR*-**9** at >99% m/m purity (quantitative HPLC), 97% de and 99% ee.

Conclusions

We have prepared kilogram quantities of both 2*S*,4*S*-monatin and 2*R*,4*R*-monatin in 99% ee using a chemo-biocatalytic approach. No other synthetic route published has afforded monatin enantiomers on this scale, and isolation of kilogram quantities of monatin from the plant source is not feasible owing to the low concentrations present in *S. ilicifolius* and the scarceness of the plant. Key steps in the synthesis which proved to be robust on scale-up were the (2 + 3) cycloaddition reaction and the highly selective enzymatic resolution. This route potentially allows access to all four monatin isomers in sizable quantities.

(15) ^{1}H NMR spectroscopy was used to monitor approximate levels of potassium acetate with each subsequent ethanol wash by the presence of a peak at δ 2.1 ppm. As the products (**1**) and (**2**) are isolated as the monopotassium salt, the accurate potassium level (% m/m) was determined by atomic absorption once the peak in the ^{1}H NMR spectrum was no longer present.

Table 1

time (min)	% eluent A	% eluent B
0.00	90	10
30.0	10	90
35.0	90	10
45.0	90	10

Eluent A: 3.5 g of phosphoric acid diluted with 900 mL of nano pure water, pH adjusted to 3 by addition of triethylamine. Volume made up to 1000 mL with nano pure water, mobile phase then degassed and filtered. **Eluent B**: 100% acetonitrile. The flow rate was set at 1.0 mL/min and UV detection at 280 nm.

Experimental Section

General. NMR spectra were run on either a Varian 200 MHz Gemini 2000 instrument, or on a 400 MHz Varian INOVA instrument. A 20 L Buchi rotary evaporator was used to carry out solvent recovery at bench scale, while solvent recovery at larger scale was carried out using a 40 L glass batch distillation apparatus. In all cases the term "reduced pressure" refers to a pressure of 2 kPa.

GC Method A. DB-Wax 5 column. Program: initial temperature 80 °C, initial time 2 min, rate 20 °C/min, final time 0 min, final temperature 240 °C.

HPLC Method A. Luna 5 μ C18 (2), 150 mm × 4.6 mm I.D, at 25 °C. A linear gradient elution system was set up as shown in Table 1.

HPLC Method B. Chiracel OD, 250 mm × 4.6 mm I.D at 25 °C, eluting with hexane/EtOH/TFA (70:30:0.1, prepared by mixing 700 mL of hexane, 300 mL of ethanol, and 1 mL of trifluoroacetic acid in a 1000 mL volumetric flack, degassing, and filtering). Flow rate set at 1 mL/min, 220 nm UV detection.

HPLC Method C. Crownpak CR (+) AD, 15 cm × 4.0 mm at 35 °C, eluting with a 15% methanol in aq $HClO_4$ solution at pH1.5 (prepared by diluting 16.3 g of commercially available 70% perchloric acid with nano pure water to 1000 mL to give a perchloric acid solution of pH 1. Further dilution of pH 1 perchloric acid solution (316 mL) with 1000 mL of nano pure water affords a perchloric acid solution of pH 1.5. Eluent prepared by mixing 850 mL of perchloric acid solution (pH 1.5) with 150 mL of methanol in a 1000 mL volumetric, degassing, and filtering), flow rate set at 1 mL/min, 220 nm UV detection.

Ethyl 2-(Bromomethyl)acrylate (3). Paraformaldehyde (18.8 kg, 625 mol) dissolved in water (62.5 kg) was treated with a 10% aqueous sodium hydroxide solution (250 g, 6.50 mol) and heated to 80 °C in a 100 L stainless steel reaction vessel (with a pressure rating of 6 bar) equipped with a Rushton turbine (150 rpm). To this preheated mixture were added DABCO (0.10 equiv, 2.8 kg, 25 mol) and ethyl acrylate (25.0 kg, 250 mol). The contents were heated to 85 °C with stirring, and the reaction progress was monitored by GC analysis (retention time of product, GC method A, 3.93 min). After complete conversion of starting material, the reaction mixture was cooled to 25 °C and treated with aqueous sulfuric acid (4.4 kg of a 20% solution) to adjust the pH of the mixture to 4.5. The reaction mixture was extracted with hexane (2 × 16.5 kg) to facilitate removal of ethyl acrylate and some byproduct. The remaining aqueous phase was then extracted with 1,2-dichloroethane (2 × 20 kg). The combined organic extracts were analysed for residual paraformaldehyde[16] by adding a 2 g sample of the extract to 100 mL of a 125 g/L solution of sodium sulfite. Three drops of phenolphthalein were added and the mixture titrated to a colourless end point with 0.5 N sulfuric acid. If no paraformaldehyde was present, the solvent was removed at 40−60 °C under reduced pressure. If paraformaldehyde was present, the organic extract was washed again with water, and the titration repeated. The resultant residue containing ethyl 2-(hydroxymethyl)acrylate (27.0 kg, contaminated with DCE) was analysed for moisture content.

Ethyl 2-(hydroxymethyl)acrylate (27.0 kg, 207 mol) was charged to a 400 L glass lined reactor (ex Pfaudler), equipped with a propeller type impeller (100 rpm). Aqueous HBr (48%, 3.25 equiv, 112 kg, 672 mol) was added, and the mixture was cooled to 12 °C. Sulfuric acid (2.0 equiv, 39.0 kg, 398 mol) was added at such a rate that the internal temperature did not exceed 15 °C to avoid the formation of byproduct resulting from polymerisation. After complete addition of the sulfuric acid, the mixture was left to stir at room temperature (recorded as varying between 22−26 °C) overnight. The reaction mixture was extracted with hexane (3 × 33 kg), and the combined hexane extracts were washed twice with a 5% aqueous sodium bicarbonate solution (2 × 33 kg). The washed hexane extract was then transferred to the distillation column and the solvent removed at 40 °C under reduced pressure. The temperature was increased to 50 °C to remove any water that might be present. The residue containing ethyl 2-(bromomethyl)acrylate **3** was then distilled (0.8 mmHg, 38−40 °C, reboiler temperature <80 °C to avoid decomposition of the final product) to afford the desired product (16.4 kg, 34% yield) in >95% purity by GC (retention time, GC method A, 4.62 min). ^{1}H NMR (200 MHz; $CDCl_3$) δ 1.33 (t, J = 7.2 Hz, 3H), 4.19 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 5.95 (s, 1H), and 6.33 (s, 1H).

Ethyl 2-((1*H*-Indol-3-yl)methyl)acrylate (5). A solution of indole (2.00 kg, 17.1 mol) in THF (3.88 kg) was charged to a GR-15 glass reactor (ex Buchi) which was equipped with a propeller-type impeller (100−300 rpm) and cooled to 0 °C under an atmosphere of nitrogen. A solution of methylmagnesium chloride (1.1 equiv, 3.0 M in THF, 6.63 kg, 18.8 mol) was added over 105 min, and with the slight exotherm, the reactor temperature was maintained between 5 and 10 °C, and then stirred at 150 rpm for a further 30 min under these conditions. A solution of (**3**) (1.08 equiv, 3.54 kg, 18.4 mol) in THF (3.57 kg) was then added over 2 h, while maintaining the reaction temperature below 5 °C, and then stirred for an additional 1 h at this temperature. The reaction mixture was then quenched with water (7.5 kg) and heated to 20 °C in order to dissolve salts. The aqueous and organic phases were separated, and the aqueous phase was extracted twice with ethyl acetate (10 kg). The ethyl acetate/THF phases were combined and concentrated under reduced pressure at 40−90 °C. The organic residue was passed through a short path distillation unit (KD-5 SPD ex UIC) to facilitate removal of high-boiling components at a feed temperature of 90 °C, and vacuum of 0.02 mmHg. The column temperature was maintained at 170−190 °C, and a flow rate of 140−300 mL/h ensured

(16) Borgstrom, P.; Horsch, W. G. *J. Am. Chem. Soc.* **1923**, *45*, 1493–1497.

recovery of product at >70% m/m purity (boiling point of (**5**) 47−52 °C at 0.02 mmHg). The distillate (2.38 kg, 76.8% m/m) contained 1.83 kg of (**5**) (47% yield) as determined by quantitative HPLC (HPLC Method A, retention time 17.06 min). A pure sample of (**5**), obtained by silica gel column chromatography (20:80 ethyl acetate/hexane), was analysed by ^{1}H and ^{13}C NMR spectroscopy. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (t, J = 7.2 Hz, 3H), 3.85 (s, 2H), 4.29 (q, J = 7.2 Hz, 2H), 5.55 (s, 1H), 6.27 (s, 1H), 7.03 (d, J = 2.6 Hz, 1H), 7.03−7.29 (m, 2H), 7.38 (dt, J = 8.0 and 1.1 Hz, 1H), 7.59−7.63 (m, 1H), and 8.16 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.15, 27.56, 60.64, 111.09, 112.72, 118.98, 119.21, 121.82, 122.78, 125.16, 127.25, 136.30, 139.78, and 167.37.

Ethyl 2-Chloro-2-(hydroxyimino)acetate (8). Glycine ethyl ester hydrochloride (6.30 kg, 45.0 mol) was dissolved in hydrochloric acid (32% m/v, 5.13 kg) in a glass-lined CR-26 reactor (ex Buchi), equipped with a retreat blade impeller (100−300 rpm). The solution was cooled to 0 °C and hydrogen chloride gas was bubbled into the reactor *via* a sparging tube for 1 h while maintaining the temperature below 5 °C. The reaction mixture was then cooled to −5 °C, and a solution of sodium nitrite (1.0 equiv, 3.11 kg, 45.0 mol) in water (4.5 kg) was dosed into the reactor using a diaphragm pump, while maintaining the temperature between −5 and 0 °C. This operation was complete within an hour. A second portion of gaseous HCl was sparged into the reactor over 1 h at 0−5 °C. This was followed by a second portion of sodium nitrite (1.0 equiv, 3.11 kg, 45.0 mol) in water (4.5 kg) which was added over one hour while maintaining the temperature between −5 and 0 °C. The reaction mixture was stirred for an additional 1 h and was then extracted with chloroform (3 × 2 kg) at ambient temperature. Isolation of the product from the reaction mixture by crystallisation resulted in significant losses in yield on this scale. The organic extracts were therefore combined, dried ($MgSO_4$), and evaporated *in vacuo* (40 °C). An oily residue remained which crystallised upon cooling to ambient temperature. The crystals were filtered, washed with hexane (2 kg) at 5 °C, and dried under vacuum to afford the product (**8**) (2.53 kg, 36.9% yield) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (t, J = 7.2 Hz, 3H), 4.36 (q, J = 7.2 Hz, 2H), and 10.06 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.89, 63.85, 132.77, and 158.67.

Diethyl 5-((1*H*-Indol-3-yl)methyl)-4,5-dihydroisoxazole-3,5-dicarboxylate (6). Ethyl 2-((1*H*-indol-3-yl)methyl)acrylate **5** from short path distillation (2.38 kg, 76.8% m/m, containing 1.83 kg, 7.98 mol of **5**) was dissolved in chloroform (4.3 kg), and triethylamine (2.0 equiv, 1.62 kg, 16.0 mol) was added to this solution with stirring in a glass-lined CR-26 reactor (ex Buchi), equipped with a retreat blade impeller (100−300 rpm). A solution of 2-chloro-2-(hydroxyimino)acetate (**8**) (1.8 equiv, 2.18 kg, 14.4 mol) in chloroform (8.7 kg) was added slowly to the reaction mixture over a period of 45−70 min, with the reaction temperature maintained below 30 °C. The reaction progress was monitored by HPLC. After complete conversion of starting material, the reaction mixture was quenched with water (13 kg), and the phases were separated. The organic phase was washed twice more with water (2 × 13 kg) and concentrated under vacuum, and the crude residue was treated with ethanol/water (3.2 kg, 80:20 v/v) to facilitate precipitation of the product out of solution. The precipitate was filtered to afford 1.80 kg (67% yield) of desired product (**6**) at >98% m/m purity by HPLC (HPLC Method A, retention time 14.90 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, J = 7.4 Hz, 3H), 1.30, (t, J = 7.2 Hz, 3H), 3.20 (d, J = 18.4 Hz, 1H), 3.43 (d, J = 15.6 Hz, 1H), 3.51 (q, J = 15.6 Hz, 1H), 3.61 (d, J = 18.4 Hz, 1H), 4.19−4.29 (m, 4H), 7.12−7.20 (m, 3H), 7.36 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), and 8.20 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.95, 31.22, 40.36, 62.13, 62.33, 91.87, 107.86, 111.15, 118.61, 119.82, 122.15, 124.17, 127.79, 135.72, 151.55, 159.97, and 170.83.

2*S*,4*S*/2*R*,4*R*-Monatin Diastereomeric Pair. Potassium hydroxide (4.0 equiv, 3.01 kg, 53.6 mol) was dissolved in ethanol (37 kg) in a 75-L glass-lined reactor (ex Pfaudler), equipped with a propeller-type impeller (100 rpm), and cooled to 21 °C. To this was added isoxazoline diester (**6**) (4.60 kg, 13.4 mol) with stirring. Upon complete dissolution of the substrate, sponge nickel catalyst (4.60 kg, A-7063 from AMC, approximately 50% wet) was added to the reactor with stirring (215 rpm) to minimise settling. The reactor was sealed and purged with nitrogen before hydrogen was fed to the reactor to a total pressure of 2 bar (g). The reaction was run at this pressure for 30 min with the hydrogen supply line open. The conversion after 30 min was 72% by HPLC analysis. Hydrogen was then used to further pressurise the reactor to 3 bar (g) and the system maintained for 25 min, after which 97% conversion of starting material was obtained. The system was then pressurised to 5 bar (g) and run for 30 min to ensure complete conversion of the isoxazoline diester. The incremental increase in pressure was implemented to contain the exotherm of the hydrogenation reaction, which resulted in temperatures >25 °C. After complete reaction, the reactor was vented and flushed with nitrogen (1 bar (g)) for 5 min, prior to being pressurised to 1.5 bar (g) with nitrogen. The material was filtered through a Nutsche filter with nitrogen at this pressure. The catalyst bed was not filtered to dryness owing to the pyrophoric nature of the catalyst. The filtrate (46.1 kg) was then concentrated in a 40 L vessel equipped with a steam coil, condenser, and receiving vessel. The final mass of the concentrate was 10.8 kg. The concentrate was treated with water in a 16 L glass-lined, stirred reactor at 20−25 °C and acidified to a pH of 4.5 with glacial acetic acid. The *RR*/*SS* diastereomeric pair of monatin isomers precipitated from this medium over a period of 12−15 h at 20 °C. The slurry was drained and centrifuged to isolate the precipitate, which is a 90:10 mixture of *RR*/*SS*-monatin and *RS*/*SR*-monatin. To enrich this ratio, the mixture was dissolved in aqueous ammonium hydroxide solution at pH 8.5 and then acidified with glacial acetic acid to pH 4.5 to facilitate precipitation. The reactor was again cooled to approximately 20 °C and maintained for 2 h, after which the slurry was drained and centrifuged. The procedure for the second precipitation was repeated twice to obtain 1.54 kg of *RR*/*SS* monatin (79% of theoretical yield) of purity >98% m/m by quantitative HPLC, and >96% de. (HPLC Method A, retention time of *RR*/*SS* diastereomeric pair, 5.24 min). ^{1}H NMR (400 MHz, D_2O + NaOD) δ 1.51 (dd, J = 14.4 and 10.8 Hz, 1H), 2.15 (dd, J = 14.4 and 2.0 Hz, 1H), 2.81 (d, J = 14.6 Hz, 1H), 2.99 (d, J = 14.6 Hz, 1H), 3.07 (dd,

J = 10.8 and 2.0 Hz, 1H), 6.88−6.98 (m, 3H), 7.23 (d, J = 8.0 Hz, 1H) and 7.48 (d, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, D_2O + NaOD) δ 35.25, 42.69, 54.68, 80.71, 109.49, 111.36, 118.78, 119.06, 121.28, 124.49, 127.78, 135.52, 181.38, and 182.30. *RS/SR* monatin can be isolated from the mother liquor of the first precipitation by first concentrating this mixture under reduced pressure, followed by addition of ethanol to the concentrate. *RS/SR* monatin precipitates from this matrix, and can be recrystallized from ethanol/water to afford *RS/SR* monatin in >99% de (HPLC Method A, retention time of *RS/SR* diastereomeric pair, 4.96 min). ^{1}H NMR (400 MHz, D_2O) δ 1.99 (dd, J = 15.0 and 9.7 Hz, 1H), 2.27 (d, J = 15.0 Hz, 1H), 3.02 (s, 2H), 3.78 (d, J = 9.7 Hz, 1H), 6.92−7.03 (m, 3H), 7.28 (d, J = 8.0 Hz, 1H) and 7.53 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 33.58, 38.65, 51.78, 78.01, 109.03, 111.42, 118.86, 119.03, 121.40, 124.56, 127.62, 135.58, 174.56, and 180.82.

Methyl 2-((1*H*-Indol-3-yl)methyl)-4-(benzyloxycarbonylamino)-5-oxotetrahydrofuran-2-carboxylate (9). A slurry of *RR*/*SS*-monatin (1.47 kg, 5.03 mol) in MeOH (13 L) in a glass lined CR-26 reactor (ex Buchi), equipped with a retreat blade impeller (100−300 rpm) was saturated with HCl gas while maintaining the temperature between 35 °C - 45 °C. The reaction mixture was then stirred at room temperature for 2 h after which HPLC analysis showed complete consumption of starting material and the formation of two products: dimethyl ester (**10**) and lactone methyl ester (**11**) (75:25 by HPLC analysis). Solvent was removed under reduced pressure at 35 °C - 40 °C to afford an oil that was used without further purification. The crude mixture of monatin esters (**10**) and (**11**) was dissolved in EtOAc (5 L) and added to a saturated aq $NaHCO_3$ solution (7 L). The reaction mixture was cooled to 5 °C - 10 °C, and benzyl chloroformate (approximately 1.0 equiv, 720 mL) was added to the reactor with stirring over a period of 30 min. The reaction mixture was stirred for an additional 1 h, after which HPLC analysis showed complete consumption of starting material. The phases were separated and the aqueous phase was extracted with EtOAc (5 L). The combined organic phases were washed with water and dried ($MgSO_4$). Removal of solvent (8 L) under reduced pressure afforded a brown oil which was dissolved in toluene (6 L), treated with *p*-TsOH (1 mol %, 9.6 g, 0.050 mol) and the reaction mixture stirred at room temperature for 15 h. The white precipitate that formed was filtered and dried to afford methyl 2-((1*H*-indol-3-yl)-methyl)-4-(benzyloxycarbonylamino)-5-oxotetrahydrofuran-2-carboxylate (**9**) (1.28 kg, 60% yield, 98% m/m purity by HPLC). (HPLC Method B, retention time of *RR*-CBz lactone 11.47 min, retention time of *SS*-CBz lactone 8.88 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (dd, J = 13.2 and 10.0 Hz, 1H), 2.84 (dd, J = 13.2 and 10.0 Hz, 1H), 3.37 (d, J = 15.0 Hz, 1H), 3.46 (d, J = 15.0 Hz, 1H), 3.48−3.51 (m, 1H), 3.83 (s, 3H), 5.02 (s, 2H), 5.08 (br s, 1H), 7.14−7.38 (m, 9H), 7.64 (d, J = 7.2 Hz, 1H) and 8.27 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.60, 36.37, 50.41, 53.21, 67.30, 84.42 110.05, 111.44, 118.61, 120.32, 122.64, 124.61, 127.39, 128.11, 128.31, 128.55, 135.74, 135.88, 155.52, 171.49, and 173.46.

(2*R*,4*R*)-Methyl 2-((1*H*-Indol-3-yl)methyl)-4-(benzyloxycarbonylamino)-5-oxotetrahydrofuran-2-carboxylate *RR*-9 and (2*S*,4*S*)-2-((1*H*-Indol-3-yl)methyl)-4-(benzyloxycarbonylamino)-5-oxotetrahydrofuran-2-carboxylic Acid *SS*-12. *RR*/*SS*-Lactone (**9**) (1.0 kg, 2.34 mol) was dissolved in MeCN (6.0 kg) and added to phosphate buffer (0.3 M, pH 7.5, 24 L) in a glass-lined reactor (CR-26 ex Buchi) fitted with an anchor stirrer. A suspension of the enzyme (706 mL, ChiroCLEC-BL, batch lot BLSD004[14]) was added to the reactor, and the resulting mixture stirred at 22 °C for 19 h, with a stirrer speed of 155 rpm. After this time, approximately 98% of the *SS*-lactone had been consumed as observed by HPLC analysis. DCM (6.1 kg) was added to allow for ease of filtration (Whatman No.1, Nutsche pressure filter pressurised with nitrogen) to recover the enzyme, and the phases were separated. The aqueous phase was extracted twice with DCM (2 × 5 kg), and the combined organic phases, containing *RR*-**9** (0.48 kg by quantitative HPLC analysis, 96% of theoretical yield, containing 2% residual *SS*-**9**) were concentrated to a volume of approximately 2.5 L under reduced pressure. This concentrate was used without further processing in the subsequent deprotection sequence to afford *RR*-monatin (**2**). The aqueous phase from the resolution reaction was treated with DCM (5.3 kg) and a 10% aq HCl solution (2.88 kg) to adjust the pH to 2.0. *SS*-**12** precipitates from this mixture, and was isolated by filtration, which gave, after drying, 0.5 kg of the desired product as a white solid in 99% theoretical yield, 100% m/m *SS*-**12** by HPLC (HPLC Method B, retention time of *RR*-**9** 11.47 min, retention time of *SS*-**12** 6.45 min).^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (dd, J = 13.2 and 9.6 Hz, 1H), 2.76 (dd, J = 13.2 and 9.6 Hz, 1H), 3.37 (d, J = 14.8 Hz, 1H), 3.45 (d, J = 18.8 Hz, 1H), 3.61 (dt, J = 9.6 and 8.0 Hz, 1H), 5.02 (s, 2H), 6.25 (d, J = 8.0 Hz, 1H), 7.08−7.17 (m, 3H), 7.27−7.39 (m, 7H), 7.64 (d, J = 7.6 Hz, 1H), and 9.67 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.59, 35.93, 50.37, 66.78, 84.10, 107.10, 111.57, 118.59, 119.58, 121.85, 124.89, 127.61, 127.95, 128.04, 128.41, 136.09, 136.19, 155.82, 172.97, and 174.04.

2*S*,4*S*-Monatin (1). *SS*-**12** (440 g, 1.08 mol) was treated with methanol (4.2 kg) in an 8 L Parr reactor, and triethylamine (6.7 g, 0.067 mol) was added to facilitate dissolution, with warming to 40 °C. The solution was added to a pressure vessel and treated with 10% Pd/C (44 g of 50% wet paste). The pressure vessel was sealed, evacuated, and purged with nitrogen twice. The reactor was then pressurised to 3 bar with hydrogen, and the resulting suspension was stirred (500 rpm) at 40 °C. The reaction progress was monitored by HPLC. After 1 h, complete consumption of starting material had been achieved; the reactor was depressurised, purged with nitrogen gas, and cooled to 25 °C. A solution of KOH (4.0 equiv, 242 g, 4.31 mol) in water (2.2 kg) was added to the reaction mixture with stirring while maintaining the temperature at 25 °C. The reaction progress was monitored by HPLC, and after 1 h the reaction was complete. The catalyst was removed by filtration and the reaction mixture acidified to pH 6.5 with glacial acetic acid. The reaction mixture was concentrated under reduced pressure to a volume of 600 mL, and the product (**1**) precipitated from this solution. The precipitate isolated was washed with ethanol (3 × 1 L) until free of potassium acetate and dried to afford

193 g of 2*S*,4*S*-monatin (**1**); 54% yield, 98% m/m purity by HPLC, 99.5% de and 99% ee (HPLC Method C, retention time of **1** 26.44 min).^{1}H NMR (400 MHz, D_2O) δ 1.81 (dd, J = 15.2 and 12.0 Hz, 1H), 2.43 (dd, J = 15.2 and 1.6 Hz, 1H), 2.84 (d, J = 14.4 Hz, 1H), 3.05 (d, J = 14.4 Hz, 1H), 3.38 (dd, J = 12.0 and 1.6 Hz, 1H), 6.89−6.99 (m, 3H), 7.24 (d, J = 8.0 Hz, 1H), and 7.48 (d, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 35.21, 38.04, 53.60, 80.14, 108.99, 111.57, 119.04, 119.20, 121.54, 124.86, 127.84, 135.77, 174.13, and 179.93.

2*R*,4*R*-Monatin (2). The solution of *RR*-**9** in dichloromethane (approx 2.5 L from the enzyme resolution extraction containing 0.48 kg, 1.14 mol *RR*-**9** by quantitative HPLC analysis) was treated with a solution of KOH (4.0 equiv, 264 g, 4.72 mol) in water (2.4 kg) with stirring, while maintaining the temperature at 25 °C. The reaction progress was monitored by HPLC, and after 1 h the reaction was complete. After this time the phases were separated, and the pH of the aqueous phase was adjusted to 7.0 using glacial acetic acid (approximately 2 equiv, 140 g). Methanol (2.5 kg) was then added to the solution and the resulting mixture transferred to an 8 L Parr reactor. The mixture was treated with 10% Pd/C (48 g of 50% wet paste), and the pressure vessel sealed, evacuated, and purged with nitrogen twice, and then pressurised to 3 bar with hydrogen. The resulting suspension was stirred at 25 °C and reaction progress monitored by HPLC at half-hourly intervals. After 1 h, all of the starting material had been converted to product, and the reactor was depressurised and purged with nitrogen gas. The catalyst was removed by filtration and the pH of the solution adjusted to 6.5 with glacial acetic acid. The resulting mixture was concentrated to a volume of approximately 600 mL and the product allowed to precipitate from this mixture. The precipitate isolated was washed with ethanol (3 × 1 L) to facilitate removal of potassium acetate and dried to afford 240 g of 2*R*,4*R*-monatin (**2**); 64% yield, 99% m/m purity by HPLC, 97% de and 99% ee (HPLC Method C, retention time of **2** 13.32 min).^{1}H NMR (400 MHz, D_2O) δ 1.85 (dd, J = 15.2 and 12.0 Hz, 1H), 2.47 (dd, J = 15.2 and 2.0 Hz, 1H), 2.87 (d, J = 14.4 Hz, 1H), 3.08 (d, J = 14.4 Hz, 1H), 3.42 (dd, J = 12.0 and 2.0 Hz, 1H), 6.92−7.04 (m, 3H), 7.28 (d, J = 8.4 Hz, 1H), and 7.52 (d, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 35.12, 37.95, 53.47, 80.06, 108.80, 111.47, 118.95, 119.08, 121.45, 124.76, 127.70, 135.65, 174.05, and 179.82.

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