Note

Concise Synthesis of (2*R*,4*R*)-Monatin

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Monatin, 4-hydroxy-4-(3-indolylmethyl)-glutamic acid, is a naturally occurring sweet amino acid. The (2*R*,4*R*)-monatin isomer has been found to be the sweetest among its four stereoisomers. A concise and efficient synthesis of (2*R*,4*R*)-monatin was accomplished by the alkylation of (4*R*)-*N*-*tert*-butoxycarbonyl (*t*Boc)-4-*tert*-butyldimethylsilyoxy-D-pyroglutamic acid methyl ester with *tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate to give (4*R*)-*N*-*t*Boc-4-*tert*-butyldimethylsilyloxy-4-(*N*-*t*Boc-3-indolylmethyl)-D-pyroglutamic acid methyl ester, *i.e.*, the lactam form of (2*R*,4*R*)-monatin with protecting groups. This was followed by the hydrolysis of the lactam ring and deprotection. The 4-hydroxyl D-pyroglutamic acid derivative was demonstrated to be a suitable precursor for the efficient preparation of (2*R*,4*R*)-monatin in high optical purity because the alkylation proceeded in regioselective and stereoselective manners at C4 to form appropriate asymmetric tetra-substituted carbon center; the resulting alkylated pyroglutamic acid derivative was then easily converted into the linear form of monatin.

Key words monatin; sweet amino acid; pyroglutamic acid; alkylation; tetra-substituted carbon center

Monatin is a naturally occurring amino acid derivative isolated from the bark of *Schlerochiton ilicifolius* roots, a plant native to northwestern Transvaal in South Africa. Monatin has four stereoisomers because of its two asymmetric centers at C2 and C4. Vleggaar *et al.* reported the structure of natural monatin as (2*S*,4*S*)-4-hydroxy-4-(3-indolylmethyl)-glutamic acid and its sweetness as 1200–1400-fold more intense than sucrose.[1] We previously reported that all isomers exhibit a sweet taste; however, (2*S*,4*S*)-monatin was found to be the least sweet isomer, whereas the other three isomers, especially (2*R*,4*R*)-monatin, were found to be intensely sweet (1700 times at 10% sucrose equivalent).[2,3]

A major problem in the synthesis of (2*R*,4*R*)-monatin is the highly diastereoselective formation of a tetra-substituted carbon center at C4. Various chemical synthetic methods have been reported for the formation of the (2*S*,4*S*)-isomer, some of which produce a mixture of stereoisomers,[4–7] whereas others describe stereoselective syntheses.[8–13] In addition, some procedures require several steps after the construction of asymmetric tetra-substituted carbon center to complete the total synthesis.

A few groups have reported the chemoenzymatic synthesis of stereoisomers of monatin, in which the stereogenic center at C4 was introduced by an enantiospecific enzymatic hydrolysis of the chemically synthesized racemic ester derivatives using a protease.[3,14] Since the racemization at C4 of these ester derivatives is impossible, these methods are not efficient for obtaining a single stereoisomer of monatin.

The lactam form of monatin, *i.e.*, the pyroglutamic acid derivative, is an attractive intermediate in the retrosynthetic analysis of monatin. Indeed, Nakamura *et al.* and Tamura *et al.* observed the formation of the lactam form of monatin during their total synthesis.[8,13] A concise method to obtain 4-substituted glutamic acid is the alkylation of the lithium enolate derived from *N*-protected pyroglutamic acid esters, followed by the cleavage of the lactam ring. Ezquerra *et al.* reported that lithium enolates of *N*-*tert*-butoxycarbonyl (*t*Boc)-protected pyroglutamic acid alkyl ester stereospecifically reacted with benzyl bromides, exclusively yielding the *trans* isomer.[15] Bassoli *et al.* also reported the same stereoselectivity in the synthesis of a monatin derivative that lacked the 4-hydroxyl group.[16]

Oliveira and Coelho[12] examined a similar stereospecific synthesis of (2*S*,4*S*)-monatin *via* the oxidation and alkylation of an enolate originating from (*S*)-pyroglutaminol derivative. The alkylation of their derivative proceeded in a regioselective manner because it has a single active hydrogen atom at C4. In addition, the alkylation of their derivative at C4 asymmetric center was proceeded from the less hindered face of the lactam ring opposite from the bulky *tert*-butyldimethylsilyloxymethyl substituent at C2. However, the transformation of the hydroxymethyl group to a carboxylic acid was required in the final stage of their total synthesis.[12]

Merino *et al.* reported the synthesis of (4*R*)-*N*-*t*Boc-4-*tert*-butyldimethylsilyoxy-D-pyroglutamic acid methyl ester (**6**), a possible precursor for the synthesis of the lactam form of (2*R*,4*R*)-monatin.[17] Zhang *et al.* also reported the synthesis of (4*S*)-*N*-*t*Boc-4-*tert*-butyldimethylsilyoxy-L-pyroglutamates, an antipode of (**6**).[18] However, the alkylation of the lithium enolate of these compounds has not been examined. As such, we examined the utilization of (**6**) for the synthesis of (2*R*,4*R*)-monatin.[19]

Results and Discussion

The synthetic sequence using commercially available *cis*-4-hydroxy-D-proline (**2**) as a starting material was performed following Zhang *et al.*[18] (Chart 1). The conversion of **2** to (4*R*)-*N*-*t*Boc-*cis*-4-hydroxy-D-proline methyl ester (**4**) (81% yield, two steps) was followed by the protection of the alcohol moiety with *tert*-butyldimethylsilyl (TBDMS) group to give (4*R*)-*N*-*t*Boc-*cis*-4-(*tert*-butyldimethylsilyloxy)-D-proline methyl ester (**5**) in 94% yield.[20,21] The oxidation of **5** using ruthenium oxide and periodate ($NaIO_4$) gave **6** in 88% yield.[22]

A modification of Christiansen *et al.* was followed to generate *tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate (**10**) starting from indole-3-carbaldehyde (**7**)[23] (Chart 2). The usual

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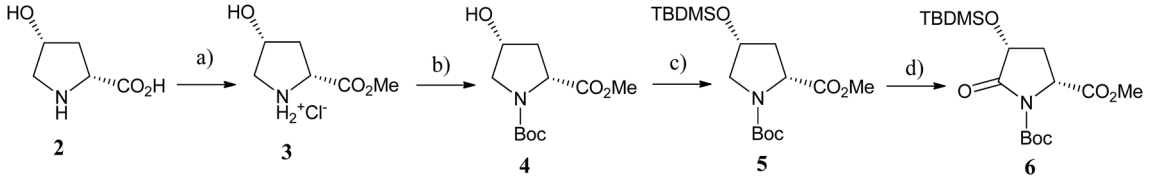
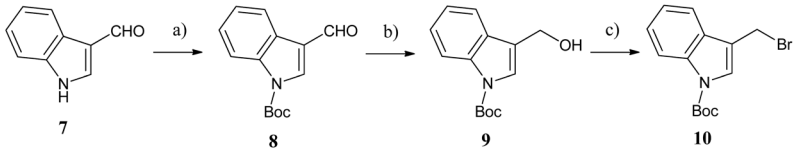


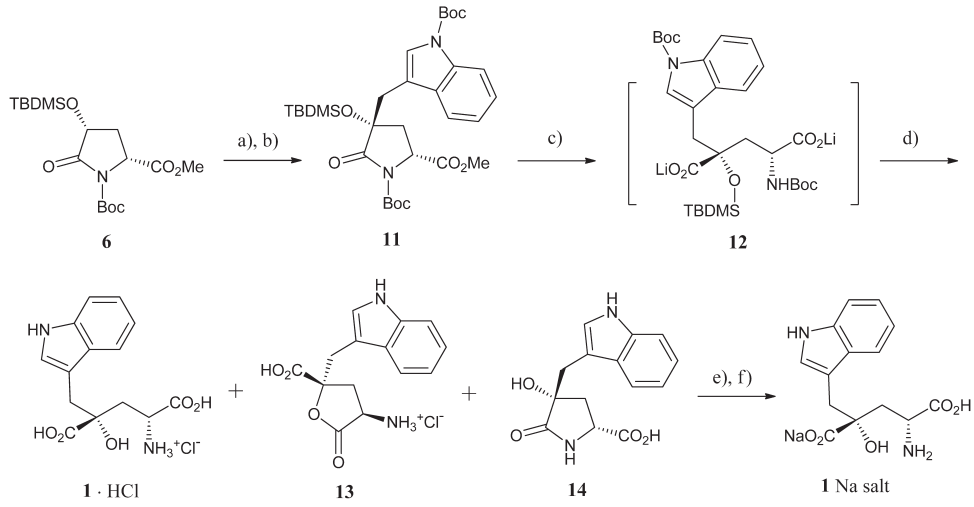
Reagents: (a) HCl–MeOH; (b) $(Boc)_2O$, Et_3N–CH_2Cl_2, 81% for 2 steps; (c) TBDMSCl, imidazole–DMF, 94%; (d) RuO_2–xH_2O, $NaIO_4$–AcOEt, H_2O, 88%.

Chart 1. Synthesis of Compound **6**



Reagents: (a) $(Boc)_2O$, DMAP–CH_3CN, 97%; (b) $NaBH_4$–EtOH, 99%; (c) CBr_4, PPh_3–CH_2Cl_2, 88%.

Chart 2. Synthesis of Compound **10**



Reagents: (a) LiHMDS–THF, −78°C; (b) **10**, 72%; (c) LiOH–iPrOH, THF, H_2O; (d) formic acid, HCl–dioxane; (e) NaOH–H_2O, Δ; (f) Amberlite IR 120B AG, 49% for 4 steps.

Chart 3. Synthesis of (2*R*,4*R*)-Monatin (**1**)

*t*Boc protection method ((*t*Boc)$_2$O, *N*,*N*-dimethylaminopyridine (DMAP), CH_3CN) was applied to **7** to obtain *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (**8**), followed by the reduction of **8** with $NaBH_4$ to give *tert*-butyl 3-(hydroxymethyl)-1*H*-indole-1-carboxylate (**9**) in 96% total yield.[24]

The synthesis of **10** required careful purification at the final stage because of its instability. Thus, the bromination of **9** was performed using carbon tetrabromide (CBr_4) and triphenylphosphine (PPh_3) to generate phosphorus tribromide *in situ* in CH_2Cl_2 at −20°C, followed by the removal of triphenylphosphine oxide by continuous crystallization to give **10** in 88% yield, which was stored in a refrigerator and used as soon as possible.

Next, regioselectivity and stereoselectivity in the alkylation of the lithium enolate of **6** were examined. Reaction sites for the alkylation of **6**, *i.e.*, C2 or C4, as well as the stereoselectivity in the alkylation of the enolate of **6** were of particular interest. Eventually, compound **6** was selectively enolized at C4 using 1.2 eq of lithium hexamethyldisilazide (LHMDS) in tetrahydrofuran (THF) at −78°C, and the enolate form was then reacted with 1.0 eq of **10** to furnish (4*R*)-*N*-*t*Boc-4-*tert*-butyldimethylsilyloxy-4-(*N*-*t*Boc-3-indolylmethyl)-D-pyroglutamic acid methyl ester (**11**) in 72% yield after purification (Chart 3). There was slight improvement in the yield with the use of *N*,*N*′-dimethylpropyleneurea as a co-solvent. As expected from the reaction of the analogue lacking the 4-protected hydroxyl group,[15,16] the alkylation of **6** by **10** proceeded in a stereoselective manner. The stereochemistry at C4 of **11** was confirmed after the transformation of **11** to the linear form of monatin. The relative *trans* configuration at the ring junctions (C2, C4) of **11** obtained by this alkylation coincided with the relative configuration of C2 and C4 of (2*R*,4*R*)-monatin. Therefore, the alkylation at C4 of **6** proceeded in a stereoselective manner from the less hindered face of the pyroglutamic acid ring opposite from the methoxycarbonyl group at C2.[12]

As the electrophile of this reaction, *tert*-butyl 3-(chloromethyl)-1*H*-indole-1-carboxylate,[25] easily obtainable from gramine in two steps without purification, was used instead of **10**; however, no coupling product was obtained, probably because of its low reactivity. *N*-*t*Boc-gramine methiodide was prepared immediately before use from *N*-*t*Boc-gramine and methyl iodide following the preparation of *N*-triisopro-

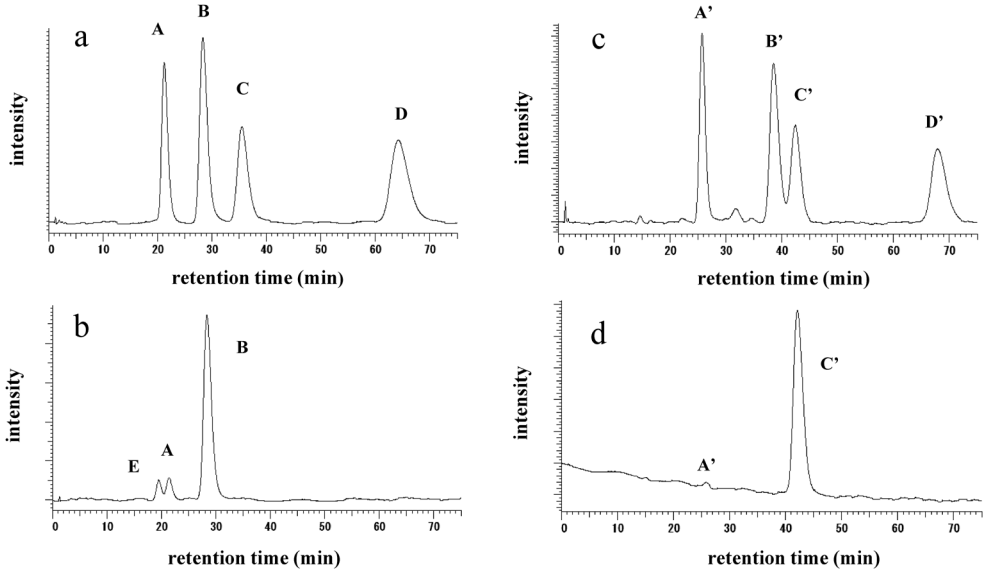
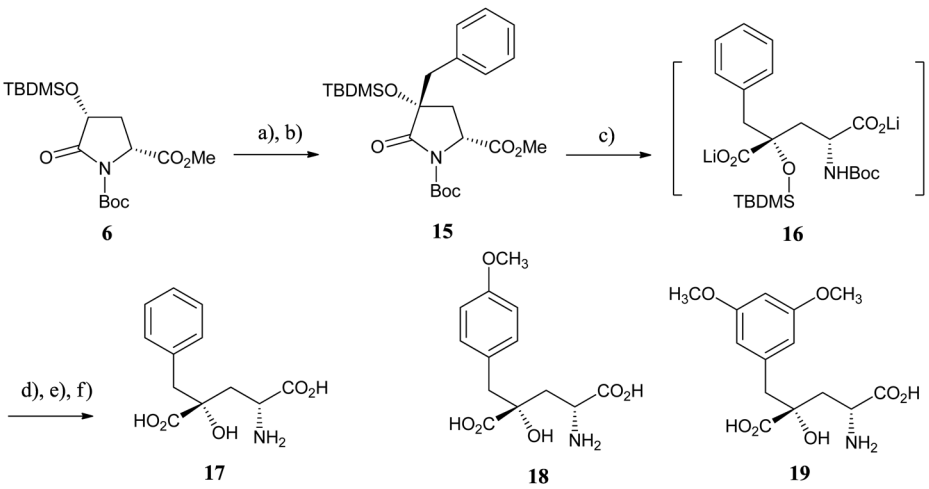


Fig. 1. Chiral HPLC Chromatograms of all Diastereoisomers of (2*R*,4*R*)-Monatin (**1**) (a), the Stereoselective Synthesis of (2*R*,4*R*)-Monatin (**1**) (b), All Diastereoisomers of Compound **17** (c), and the Stereoselective Synthesis of Compound **17** (d)

Chromatogram (a) and (b): A: (2*R*,4*S*)-**1**; B: (2*R*,4*R*)-**1**; C: (2*S*,4*R*)-**1**; D: (2*S*,4*S*)-**1**. Chromatogram (c) and (d): A′: (2*R*,4*S*)-**17**; B′: (2*S*,4*R*)-**17**; C′: (2*R*,4*R*)-**17**; D′: (2*S*,4*S*)-**17**.



Reagents: (a) LiHMDS–THF, −78°C; (b) benzyl bromide, 53%; (c) LiOH–iPrOH, THF, H_2O; (d) formic acid, HCl–dioxane; (e) NaOH–H_2O; (f) Amberlite IR 120B AG, 57% for 4 steps.

Chart 4. Synthesis of Compound **17**

pylsilyl-gramine methiodide reported by Iwao and Motoi[26] The reaction of this compound gave a complex mixture; however, the desired product was obtained in about 10% yield after careful purification by silica gel chromatography.

To cleave the lactam ring, compound **11** was treated with LiOH in a mixture of isopropyl alcohol (iPrOH), THF, and H_2O to give possible intermediate **12**, the exact structure of which was not confirmed, but a part of the *t*Boc group on the indole ring appeared to be deprotected under basic conditions. To remove the TBDMS group and remaining *t*Boc group, **12**, without purification, was treated with a mixture of 4 N HCl–dioxane and formic acid to give (2*R*,4*R*)-monatin (**1**·HCl); however, HPLC analysis indicated the existence of a significant amount of its lactone (**13**·HCl) and a small amount (5 to 10%) of lactam (**14**). The distinctive structure of monatin is considered to be a cause of the facile formation of five-membered ring compounds under acidic conditions. Indeed, during our previous study on the stability of monatin, we found that monatin and its lactone were in equilibrium in strongly acidic aqueous solutions, which gradually converted to lactam. Therefore, it is suggested that a part of **14** originated from unreacted **11** in the ring-opening step and remaining **14** might be regenerated during deprotection under acidic conditions after ring cleavage by LiOH.

Nakamura *et al.* reported that the lactam form of monatin underwent ring opening to return to the linear form of monatin by hydrolysis with NaOH in refluxing aqueous ethanol for 3 h.[8] Accordingly, a mixture of **1**·HCl, **13**·HCl, and **14** was treated with excess NaOH at 95°C in aqueous ethanol for 3 h to convert to the linear form of (2*R*,4*R*)-monatin sodium salt (**1** sodium salt). The resulting solution was neutralized and desalted, and then, **1** sodium salt was recovered by precipitation

in aqueous ethanol in a 49% yield. In chiral HPLC analysis, (2*R*,4*R*)- and (2*R*,4*S*)-monatin were observed in a ratio of 98 : 2 accompanied by a small amount of lactam (**14**) (Figs. 1a, b). Therefore, product **1** was shown to be of high diastereoisomeric purity at the newly created asymmetric center.

When the order of the original conversion route was reversed, *i.e.*, deprotection under acidic conditions followed by heating at reflux in ethanol with aqueous NaOH for 18 h, the desired linear form of monatin was still obtained; however, compared with the original method, a larger number of impurities were detected by HPLC analysis.

Using a similar procedure, unsubstituted or substituted phenyl analogs of (2*R*,4*R*)-monatin (**17**–**19**) were prepared (Chart 4). Phenyl analog (**17**) was obtained using benzyl bromide as an electrophile in a modest total yield (31% total yield) and good stereoselectivity (>99%, Figs. 1c, d). Because of the low electrophilicity of 4-methoxybenzyl chloride and 3,5-dimethoxybenzyl chloride, the yields of analogs **18** and **19** were unsatisfactory, although excess LHMDS and electrophiles were used. Phenyl analogues **17**–**19** were accordingly faintly sweet because the indole moiety is considered to be indispensable to elicit the strong sweet taste in this series of compounds.

Conclusion

(2*R*,4*R*)-Monatin (**1**) was successfully synthesized with high optical purity, employing the regioselective and stereoselective alkylation of (4*R*)-*N*-*t*Boc-4-*tert*-butyldimethylsilyloxy-D-pyroglutamic acid methyl ester (**6**) with *tert*-butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate (**10**) to give (4*R*)-*N*-*t*Boc-4-*tert*-butyldimethylsilyloxy-4-(*N*-*t*Boc-3-indolylmethyl)-D-pyroglutamic acid methyl ester (**11**). This was followed by the hydrolysis of the lactam ring and deprotection.

Experimental

^{1}H-NMR spectra were obtained using Brucker Avance 400 (400 MHz), and electrospray ionization (ESI)-MS spectra were obtained using Thermo Quest TSQ 700.

Analytical conditions for the determination of the optical purity of monatin stereoisomers: Column: Crownpack CR(+) 4×150 mm. Detection: UV 210 nm. Eluent: **1**–aqueous perchloric acid (pH 1.9)–12% methanol; **17**–aqueous perchloric acid (pH 1.5). Flow rate: 1.2 mL/min. Temperature: 20°C.

(4*R*)-*N*-*t*Boc-4-*tert*-Butyldimethylsilyloxy-D-pyroglutamic acid methyl ester (**6**) was obtained as a colorless oily substance in a total yield of 66% from *cis*-4-hydroxy-D-proline (**2**) in accordance with the method described in the literatures.[18,19–22] *tert*-Butyl 3-(bromomethyl)-1*H*-indole-1-carboxylate (**10**) was obtained as a white solid in a total yield of 85% from indole-3-carbaldehyde (**7**) in accordance with the method described in the literatures.[23,24]

(4*R*)-*N*-*t*Boc-4-*tert*-Butyldimethylsilyloxy-D-pyroglutamic Acid Methyl Ester (6) ^{1}H-NMR ($CDCl_3$) δ: 0.11 (3H, s), 0.16 (3H, s), 0.89 (9H, s), 1.50 (9H, s), 1.99 (1H, dt, J=7.0, 13.0 Hz), 2.57 (1H, dt, J=7.7, 13.0 Hz), 3.76 (3H, s), 4.28 (1H, t, J=7.4 Hz), 4.46 (1H, t, J=7.4 Hz). ESI-MS m/z: 374.61 $(M+H)^+$, 396.59 $(M+Na)^+$.

***tert*-Butyl 3-(Bromomethyl)-1*H*-indole-1-carboxylate (10)** ^{1}H-NMR ($CDCl_3$) δ: 1.66 (9H, s), 4.69 (2H, s), 7.29–7.38 (2H, m), 7.44–7.50 (1H, m), 7.64–7.69 (2H, m), 8.13 (1H, d, J=7.8 Hz).

(4*R*)-*N*-*t*Boc-4-*tert*-Butyldimethylsilyloxy-4-(*N*-*t*Boc-3-indolylmethyl)-D-pyroglutamic Acid Methyl Ester (11) LHMDS was added (1.7 mol/L in THF, 29.10 mL, 50.34 mmol) to a stirred solution of compound **6** (15.67 g, 41.95 mmol) in anhydrous THF (60 mL) under an argon atmosphere at −78°C. The resulting solution was stirred at −78°C for 1 h. A solution of compound **10** (13.01 g, 41.95 mmol) in anhydrous THF (20 mL) was added portionwise into the reaction solution; the solution was stirred at −78°C for 25 min and then warmed to room temperature (r.t.) and allowed to stir for 2 h. The reaction was quenched with aqueous NH_4Cl (50 mL) and extracted with AcOEt (100 mL, thrice). Combined organic layers were washed with water (50 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel 300 g, *n*-hexane–AcOEt=20 : 1–4 : 1) to give **11** as a pale yellow foam (18.20 g, 30.19 mmol). The ^{1}H-NMR spectrum showed a peak marked by a very small amount (several percent) of impurity beside the isomers of the compound.

^{1}H-NMR ($CDCl_3$) δ: 0.14 (3H, s), 0.30 (3H, s), 0.87 (9H, s), 1.45 (9H, s), 1.66 (9H), 2.09 (1H, dd, J=4.5, 13.6 Hz), 2.42 (1H, dd, J=9.2, 13.6 Hz), 3.02 (1H, d, J=14.6 Hz), 3.19 (1H, d, J=14.6 Hz), 3.71 (3H, s), 4.16 (1H, dd, J=4.5, 9.2 Hz), 7.22–7.25 (1H, m), 7.31 (1H, t, J=7.1 Hz), 7.49–7.52 (2H, m), 8.17 (1H, br d, J=7.9 Hz). ESI-MS m/z: 626.07 $(M+Na)^+$.

(2*R*,4*R*)-Monatin (1) Sodium Salt LiOH–H_2O (20.14 g, 480 mmol) was added to a solution of **11** (18.0 g, 29.86 mmol) in a mixed solvent of isopropyl alcohol (50 mL), THF (50 mL), and water (100 mL) cooled to 0°C. The solution was stirred at 0°C for 10 min, warmed to r.t., and stirred for 16 h. The solvent was evaporated *in vacuo*, and the residue was suspended in water (50 mL). The pH of the solution was then adjusted to approximately 3 with aqueous HCl (2 N). The aqueous phase was extracted with AcOEt (80 mL, thrice). Combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a colorless foam (**12**, 15.3 g).

A solution of HCl (4 N in dioxane, 40 mL) was added portionwise to a solution of the abovementioned residue in formic acid (40 mL) cooled to 0°C. The mixture was stirred at 0°C for 5 min, warmed to r.t., and stirred for 30 min. The mixture was concentrated *in vacuo*, and the residue was triturated and washed by Et_2O (30 mL) and then with AcOEt (30 mL) to give a yellow powder (**1**·HCl, **13**·HCl, **14**, 13.22 g).

A solution of the abovementioned residue in a mixed solvent of EtOH (160 mL) and aqueous NaOH (2 N, 40 mL) was heated at 95°C for 3 h, and the insoluble material was removed by filtration after cooling to r.t. The filtrate was concentrated *in vacuo*. The residue was dissolved in water (100 mL), and the solution was successively washed with AcOEt (50 mL) and Et_2O (50 mL). The solution was neutralized by the addition of Amberlite IR 120B AG (H^+), filtered, and concentrated *in vacuo*. The residue was suspended in EtOH (50 mL), and the insoluble material (NaCl) was removed by filtration. The filtrate was concentrated *in vacuo*, and the residue was crystallized with aqueous EtOH (95%) at r.t. to give **1** sodium salt as a white crystal (4.64 g, 14.71 mmol). Analysis was conducted by HPLC using a chiral column and revealed the (2*R*,4*R*) and (2*R*,4*S*) forms of isomers having an integrated peak ratio of 98 : 2.

^{1}H-NMR (D_2O) δ: (sodium salt of (2*R*,4*R*)-monatin) 2.06 (1H, dd, J=11.6, 15.2 Hz), 2.68 (1H, dd, J=2.0, 15.2 Hz),

3.10 (1H, d, J=14.8 Hz), 3.30 (1H, d, J=14.8 Hz), 3.64 (1H, dd, J=2.0, 11.6 Hz), 7.16 (1H, brt, J=8.0 Hz), 7.23 (1H, brt, J=8.0 Hz), 7.24 (1H, s), 7.50 (1H, d, J=8.0 Hz), 7.74 (1H, d, J=8.0 Hz). ^{13}C-NMR (D_2O, 100 MHz) δ: 38.1, 41.0, 56.5, 83.1, 111.9, 114.5, 121.9, 122.1, 124.5, 127.8, 130.7, 138.7, 177.1, 182.9. ESI-MS m/z: 291 $(M-H)^-$. FAB-MS m/z: 315.0963 (M+Na) (Calcd for $C_{14}H_{16}N_2NaO_5$: 315.0957).

(2*R*,4*R*)-2-Amino-2,3-dideoxy-4-*C*-(1*H*-indol-3-ylmethyl)pentaric Acid 1,4-Lactone (13, Monatin Lactone) ^{1}H-NMR (D_2O) δ: 2.38 (1H, dd, J=10.7, 13.6 Hz), 2.92 (1H, dd, J=9.8, 13.6 Hz), 3.17 (1H, dd, J=9.8, 10.7 Hz), 3.37 (2H, d, J=2.6 Hz), 7.16 (1H, t, J=6.9 Hz), 7.18 (1H, t, J=8.2 Hz), 7.27 (1H, s), 7.46 (1H, d, J=8.0 Hz), 7.68 (1H, d, J=8.0 Hz). ESI-MS m/z: 275.51 $(M+H)^+$.

(2*R*,4*R*)-4-Hydroxy-4-(1*H*-indol-3-ylmethyl)-5-oxo-proline (14, Monatin Lactam) ^{1}H-NMR (DMSO-d_6) δ: 1.80 (1H, dd, J=6.8, 13.0 Hz), 2.41 (1H, dd, J=8.0, 13.0 Hz), 2.89 (1H, d, J=14.1 Hz), 2.97 (1H, d, J=14.1 Hz), 3.45 (1H, dd, J=6.8, 8.0 Hz), 5.45 (1H, brs), 6.93–6.98 (1H, m), 7.04 (1H, t, J=7.0 Hz), 7.16 (1H, d, J=2.3 Hz), 7.32 (1H, d, J=8.0 Hz), 7.62 (1H, d, J=8.0 Hz), 7.97 (1H, s), 10.88 (1H, brs). ESI-MS m/z: 275.06 $(M+H)^+$.

***N*-*t*Boc-(4*R*)-4-*tert*-Butyldimethylsilyloxy-4-benzyl-D-pyroglutamic Acid Methyl Ester (15)** To a stirred solution of **6** (1.09 g, 3.0 mmol) in anhydrous THF (10 mL) under an argon atmosphere at −78°C was added LHMDS (1.7 mol/L in THF, 2.1 mL, 3.6 mmol). The resulting solution was stirred at −78°C for 1 h. Benzyl bromide (0.38 mL, 3.15 mmol) was added dropwise into the reaction solution, the solution was stirred at −78°C for 25 min then warmed to r.t. and allowed to stir for 1.5 h. The reaction was quenched with aqueous NH_4Cl (10 mL) and extracted with AcOEt (50 mL, twice). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by preparative thin layer chromatography (PTLC) to give **15** as a white solid (741 mg, 1.60 mmol). The ^{1}H-NMR spectrum showed a single stereoisomer.

^{1}H-NMR ($CDCl_3$) δ: 0.13 (s, 3H), 0.29 (s, 3H), 0.86 (s, 9H), 1.46 (s, 9H), 1.96 (1H, dd, J=5.9, 13.5 Hz), 2.45 (1H, dd, J=8.8, 13.5 Hz), 2.88 (1H, d, J=13.3 Hz), 3.16 (1H, d, J=13.3 Hz), 3.71 (3H, s), 3.80 (1H, dd, J=5.9, 8.8 Hz), 7.20–7.31 (5H, m). ESI-MS m/z: 464.83 $(M+H)^+$, 486.81 $(M+Na)^+$.

(2*R*,4*R*)-4-Hydroxy-4-benzylglutamic Acid (17) Sodium Salt To a solution of **15** (379 mg, 0.82 mmol) in the mixed solvent of isopropyl alcohol (6 mL) and water (5 mL) cooled to 0°C was added LiOH–H_2O (550 mg, 13.12 mmol). The solution was stirred at 0°C for 10 min, warmed to r.t., then stirred for 5 h. The solvent was evaporated *in vacuo*, the residue was suspended in water (20 mL), and the pH of the solution was adjusted to approximately 3 with aqueous HCl (2 N). The aqueous phase was extracted with AcOEt (50 mL, twice). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a colorless foam (**16**, 495 mg).

To a solution of the above residue in formic acid (4 mL) cooled to 0°C was added a solution of HCl (4 N in dioxane, 4 mL) dropwise. The mixture was stirred at 0°C for 5 min, warmed to r.t., then stirred for 30 min. The mixture was concentrated under reduced pressure then the residue was dissolved in water (15 mL) and washed with Et_2O (20 mL) and AcOEt (20 mL). The aqueous layer was neutralized with aqueous NaOH (2 N) and Amberlite IR 120B AG (H^+) then filtered. The filtrate was concentrated *in vacuo* to about one fifth of volume and 20 mL of ethanol was added. The resulting crystals were collected by filtration and dried under reduced pressure to give 130 mg (0.47 mmol) of **17** sodium salt. Analysis was conducted by HPLC using a chiral column and revealed only the (2*R*,4*R*) and (2*R*,4*S*) forms of isomers having an integrated peak ratio of 99 : 1 or higher.

^{1}H-NMR (D_2O) δ: (sodium salt of (2*R*,4*R*)-4-hydroxy-4-benzylglutamic acid) 1.95 (1H, dd, J=11.7, 15.3 Hz), 2.56 (1H, d, J=15.3 Hz), 2.81 (1H, d, J=13.5 Hz), 3.07 (1H, d, J=13.5 Hz), 3.55 (1H, d, J=11.7 Hz), 7.19–7.31 (m, 5H). ESI-MS m/z: 252.0 $(M-H)^-$. FAB-MS m/z: 252.0862 (M−H) (Calcd for $C_{12}H_{14}NO_5$: 252.0872).

(2*R*,4*R*)-4-Hydroxy-4-(4-methoxybenzyl)glutamic Acid (18) Sodium Salt The same operation described for **17** was performed using 4-methoxybenzyl chloride, and **18** was obtained in an overall yield of 13.5% as a white solid.

^{1}H-NMR (D_2O) δ: 1.53 (1H, dd, J=10.6, 14.3 Hz), 2.18 (1H, dd, J=2.5, 14.3 Hz), 2.66 (1H, d, J=13.8 Hz), 2.90 (1H, d, J=13.8 Hz), 3.14 (1H, dd, J=2.5, 10.6 Hz), 3.68 (3H, s), 6.79 (2H, m), 7.06 (2H, m). ESI-MS: 305.9 $(M+Na)^+$, 281.8 $(M-H)^-$.

(2*R*,4*R*)-4-Hydroxy-4-(3,5-dimethoxybenzyl)glutamic Acid (19) Sodium Salt The same operation described for **17** was performed using 3,5-dimethoxybenzyl chloride and **19** was obtained in an overall yield of 18.5% as a white solid.

^{1}H-NMR (D_2O) δ: 1.59 (1H, dd, J=10.7, 14.3 Hz), 2.23 (1H, dd, J=2.4, 14.3 Hz), 2.72 (1H, d, J=13.5 Hz), 2.98 (1H, d, J=13.5 Hz), 3.21 (1H, dd, J=2.4, 10.7 Hz), 3.72 (6H, s), 6.38 (1H, m), 6.43 (2H, m). ESI-MS: 336.2 $(M+Na)^+$, 311.8 $(M-H)^-$.

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Conflict of Interest The author is an employee of Ajinomoto Co., Inc. and has no further conflicts of interest to declare.

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