RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2014, **4**, 2482

Epoxy amino acids produced from allylglycines intramolecularly cyclised to yield four stereoisomers of 4-hydroxyproline derivatives†

Suvratha Krishnamurthy,[a] Toru Arai,*[a] Kanae Nakanishi[b] and Norikazu Nishino[b]

Received 17th September 2013
Accepted 12th November 2013

DOI: 10.1039/c3ra45184d

www.rsc.org/advances

Derivatives of 2-amino-4-pentenoic acid (allylglycine) were efficiently resolved using Subtilisin or acylase. The side-chain unsaturated bond of the enantiomerically pure amino acid with *tert*-butoxycarbonyl (Boc) protection was smoothly epoxidized with *m*-chloroperbenzoic acid. When the Boc protection of the amino group was removed, the amino group intramolecularly attacked the side-chain epoxide, generating compounds with five-membered rings: the 4-hydroxyproline derivatives. Two diastereomeric products were formed through the cyclisation reaction, for example, (2*S*,4*S*)-4-hydroxyproline benzyl ester (*cis*-**8**) and (2*S*,4*R*)-4-hydroxyproline benzyl ester (*trans*-**8**) were formed from (2*S*)-amino acid with a side-chain epoxide. Compound (2*S*,4*S*)-4-hydroxyproline benzyl ester (*cis*-**8**) was transformed to a lactone (*cis*-hydroxyproline lactone, **10**) with the removal of benzyl alcohol. The *cis*-conformation was essential for the intramolecular ester exchange reaction; in fact, no lactone formation was observed for the *trans* isomer (*trans*-**8**). The separation of *cis*-hydroxyproline lactone and the *trans*-isomeric hydroxyproline benzyl ester was facile and clear, in contrast to the difficult separation of *cis*- and *trans*-hydroxyproline derivatives. Thus, two diastereomers of hydroxyproline derivatives for L-hydroxyproline and also for D-hydroxyproline were obtained, *i.e.*, four diastereomers of hydroxyproline derivatives.

Introduction

Compound (2*S*,4*R*)-4-hydroxyproline (*trans*-L-hydroxyproline, L-Hyp) is the most abundant non-coded amino acid in nature (Fig. 1). L-Hyp residues are generated by post-translational modification and are essential to stabilize the triple-helical supercoil of collagen.[1–4] Reactions of proline hydroxylase using ascorbic acid and molecular oxygen are highly relevant for molecular biology but still not well understood. Recent structure-determination of proline hydroxyalse will afford several mechanistic and kinetic studies. L-Hyp is also found in biochemicals such as echinocandins, which are important antifungal peptides.[5] Although prolyl 4-hydroxylase selectively forms *trans*-hydroxylated prolines, (2*S*,4*S*)-4-hydroxyproline (*cis*-L-hydroxyproline, L-*cis*Hyp) exists in an immunosuppressive natural product, microcolin A.[6] Recently L-*cis*Hyp derivatives have been tested as potential anticancer drugs because L-*cis*Hyp inhibits collagen biosynthesis by preventing procollagen folding.[7,8] The conformation of 4-hydroxyproline's five-membered ring has been thoroughly elucidated recently.[9] Furthermore, non-natural hydroxyprolines have been incorporated into a synthetic antagonist of a histamine H_3 receptor[10] and into a diverse artificial polypeptide library.[11]

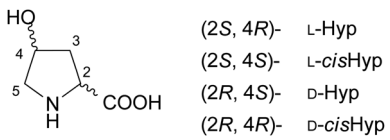


Fig. 1 Stereoisomers of 4-hydroxyprolines.

Various methods have been proposed for the synthesis of hydroxyprolines. Inversion of the 4-hydroxyl group of natural L-Hyp under Mitsunobu conditions[6,12–15] and other reactions[10,16,17] gave L-*cis*Hyp. Hydroxyproline derivatives have been elaborated by using chiral compounds as starting materials.[18,19] Biological methods have also been applied to obtain the diastereomers of hydroxyprolines.[8,20,21] Here, the proposed methods provides four isomers of hydroxyprolines combining the enzymatic and organic reactions with moderate reaction yields and facile separation procedures.[22]

Results and discussion

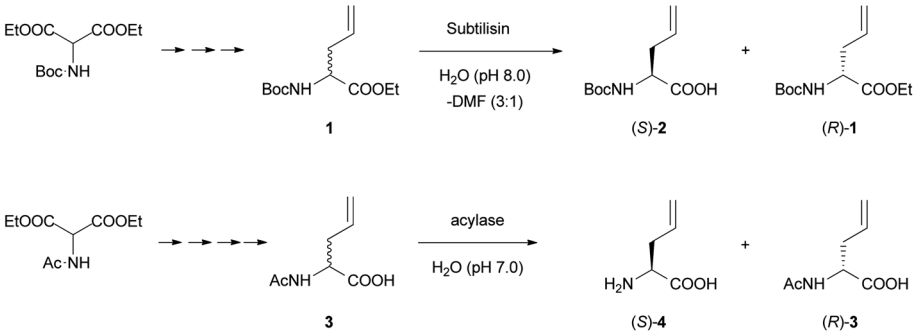
Enzymatic resolutions of 2-amino-4-pentenoic acid (allylglycine) derivatives as starting materials

The racemic 2-amino-4-pentenoic acid (DL-allylglycine) derivatives were synthesized from malonate derivatives and resolved

[a]*Department of Applied Chemistry, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan. E-mail: arai@che.kyutech.ac.jp*

[b]*Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan*

† Electronic supplementary information (ESI) available: NMR spectra for all novel compounds and the selected compounds. See DOI: 10.1039/c3ra45184d



Scheme 1 Enzymatic synthesis of 2-amino-4-pentenoic acid derivatives.

enzymatically. Subtilisin resolution of amino acid esters and acylase resolution of acetyl amino acids were tested (Scheme 1).

Racemic ethyl 2-Boc-amino-4-pentenoate (**1**, Boc = *tert*-butoxycarbonyl) was synthesized from diethyl (2-Boc-amino) malonate. Reaction of this malonate with excess allyl bromide in refluxing EtOH using NaOEt produced the allyl-substituted malonate, which was half saponified and then refluxed in toluene for the decarboxylation to generate racemic **1** (91% yield in three steps based on diethyl (2-Boc-amino)malonate).

In H_2O–DMF (3 : 1, v/v), **1** was enzymatically hydrolyzed using Subtilisin while the pH was maintained at 8.0 (aqueous NH_3) at 40 °C. After 3 h of enzymatic treatment, unreacted (*R*)-**1** was initially recovered *via* diethyl ether extraction from the aqueous reaction mixture. The aqueous reaction mixture was subsequently acidified to pH 3 and then (*S*)-2-Boc-amino-4-pentenoic acid ((*S*)-**2**) was extracted with EtOAc. Both yields of recovered (*R*)-**1** and selectively hydrolyzed (*S*)-**2** were approximately 48% based on racemic **1**. The chemical and enantiomeric purities of (*S*)-**2** and (*R*)-**1** (after deprotection) were ascertained by ^{1}H NMR, chiral HPLC (eluting with dilute Cu^{2+} solution) and the specific rotation measurements, in which no impurities were found in ^{1}H NMR and in chiral HPLC.[23–26] Thus, (*S*)-**2** and (*R*)-**1** were successfully obtained from racemic **1** by kinetic enzymatic (Subtilisin) resolution.

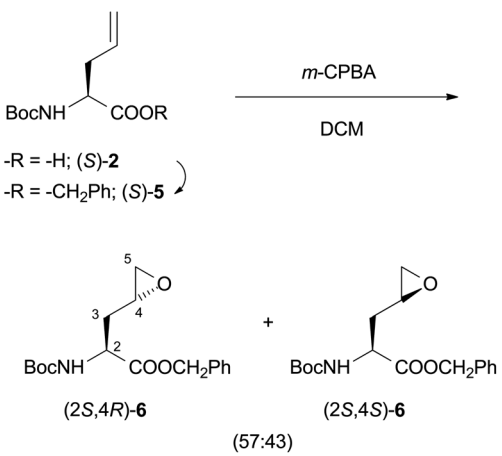
Next, diethyl acetamidomalonate, which is less expensive than diethyl (2-Boc-amino)malonate, was selected as a starting material. Similar to diethyl (2-Boc-amino)malonate, diethyl acetamidomalonate was reacted with allyl bromide, half saponified, decarboxylated in refluxing toluene and then subjected to second saponification affording racemic 2-acetamido-4-pentenoic acid (**3**, Fig. S1 in ESI,† 37% yield in four steps based on diethyl acetamidomalonate).

This racemic acetyl amino acid **3** was treated with acylase under pH 7.0 (aqueous LiOH) conditions with Co^{2+} at 37 °C.[27–29] After 24 h, the solution was acidified and extracted with EtOAc to afford unreacted (*R*)-**3**, which was purified by silica gel column chromatography to yield a brown semi-solid compound. The remaining acidic solution was subjected to the ion-exchange resin (–SO_3H type) and then product (*S*)-2-amino-4-pentenoic acid ((*S*)-**4**) eluted when the resin was washed with aqueous NH_3, which became a white solid after evaporation. In these procedures, the isolations of products **3** and **4** were not easy because these compounds were highly water soluble. The isolated amounts of (*R*)-**3** and (*S*)-**4** were not high (28% and 22%, respectively, based on diethyl acetamidomalonate). However, these procedures (*i.e.*, kinetic enzymatic resolution using acylase) also produced the chemically and enantiomerically pure (*S*)-**4** and (*R*)-**4** (obtained from (*R*)-**3**),[30] which were analyzed by ^{1}H NMR and chiral HPLC.

Epoxidation of amino acid derivatives with unsaturated side chains

Kinetically resolved Boc-amino acid (*S*)-**2** was esterified with benzyl bromide using Et_3N to afford benzyl (*S*)-2-Boc-amino-4-pentenoate ((*S*)-**5**) in 64% yield. This Boc-amino acid ester with an unsaturated side chain was epoxidized with excess *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2.[31–33] The reaction was nearly completed after 24 h at room temperature (TLC), then the excess *m*-CPBA was subsequently reduced with Na_2SO_3. After being washed and chromatographed over silica gel (hexane–15% EtOAc), Boc-amino acid benzyl esters with side-chain epoxy group, benzyl (2*S*)-2-Boc-amino-3-(2-oxiranyl)propionate isomers ((2*S*)-**6**) were obtained in 69% yield (Scheme 2).[34,35]

Compound (2*S*)-**6** was obtained as a diastereomeric mixture owing to the newly generated chiral center at C^4, though these isomers were not separated under our TLC conditions. ^{1}H NMR spectra of (2*S*)-**6** in $CDCl_3$ (Fig. 2 and S2†) showed two sets of



Scheme 2 Epoxidation of side chain C=C bond of benzyl (*S*)-2-Boc-amino-4-pentenoate.

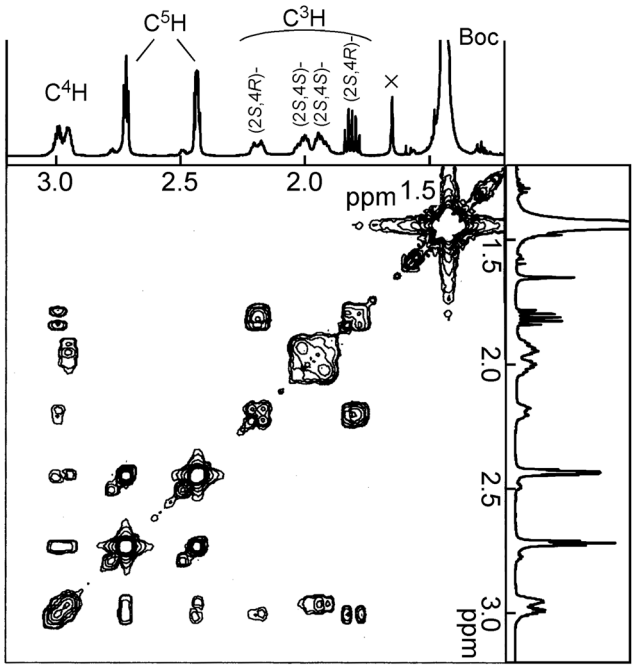


Fig. 2 ^{1}H NMR (1D and 2D-COSY) spectra of (2*S*)-**6** in $CDCl_3$ (high-field region). See Scheme 2 for C^3H, C^4H and C^5H.

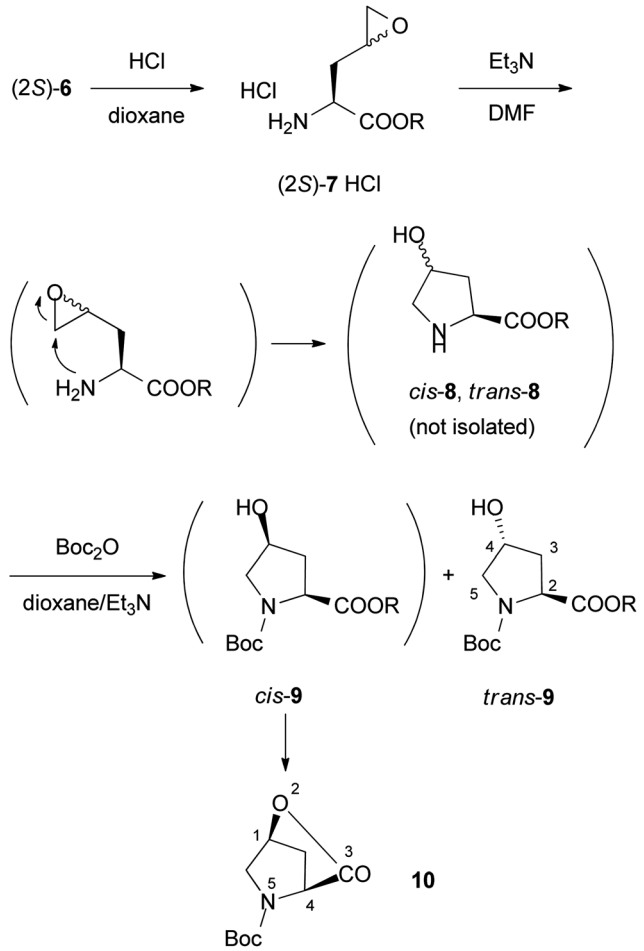
C^3H protons, δ 2.19 and 1.81 ppm, 0.43H, respectively, for (2*S*,4*R*)-**6** and δ 2.00 and 1.95 ppm, 0.57H, respectively, for (2*S*,4*S*)-**6** (see subsequent discussion for assignments). The geminal couplings between the C^3H protons of (2*S*,4*R*)-**6** and also between the C^3H protons of (2*S*,4*S*)-**6** were clearly observed in the 2D ^{1}H–^{1}H COSY spectrum (Fig. 2 and S3†). C^4H signals ($\sim\delta$ 2.99 and 2.96) and also some ^{13}C NMR signals (Fig. S4†) appeared as two sets. These diastereomers were assigned with reference to the pure methyl (2*S*,4*S*)-2-Boc-amino-3-(2-oxiranyl) propionate reported earlier.[35]

This epoxidation of allylglycine derivative **5** (with a $-CH_2-CH{=}CH_2$ side chain) slightly favored (2*S*,4*S*)-**6** diastereomer than (2*S*,4*R*)-**6** diastereomer at a ratio of 57 : 43 (within 1% error in three independent experiments). In this context, (*R*)-vinylglycine (with a $-CH{=}CH_2$ side chain) was epoxidized in an 80 : 20 ratio for (2*R*,3*R*)- and (2*R*,3*S*)-diastereomers.[31] However no stereoselectivity has been noted for the epoxidation of butenylglycine (with a $-CH_2-CH_2-CH{=}CH_2$ side chain).[32,33]

Intramolecular cyclisation to generate *cis*-hydroxyproline lactone and *trans*-hydroxyproline ester

Next, intramolecular attack of the epoxy ring by the amino group of the amino acid was performed (Scheme 3). First, the Boc protection of (2*S*)-**6** was removed by treating with 4 mol L^{-1} HCl in dioxane for 2.5 h.[36] TLC analysis showed the complete deprotection; the spot of (2*S*)-**6** disappeared and a ninhydrin-positive spot appeared. The HCl salt of benzyl (2*S*)-2-amino-3-(2-oxiranyl)propionate ((2*S*)-**7**·HCl, not isolated) was quantitatively produced as a white solid upon evaporation.

After (2*S*)-**7**·HCl was dissolved in DMF (4.6 mmol in 30 mL DMF), two equivalents of Et_3N were added to neutralize HCl.



Scheme 3 Formation of *N*-Boc-L-*cis*-hydroxyproline lactone (**10**) and benzyl *N*-Boc-L-*trans*-hydroxyproline (*trans*-**9**) (–R = $-CH_2Ph$).

The free amino group of (2*S*)-**7** slowly attacks the epoxy ring intramolecularly. After 24 h, TLC analysis (butanol/pyridine/acetic acid/H_2O, 4/1/1/2 by volume) of the reaction mixture showed that (2*S*)-**7** still remained (R_f = 0.34, purple color with ninhydrin) and also showed two ninhydrin-active yellow spots at R_f = 0.52 and 0.79 (yellow spotting with ninhydrin is a characteristic of proline-like molecule). After 72 h, TLC results showed the disappearance of the reactant. ^{1}H NMR analysis of the evaporated reaction mixture showed the absence of characteristic epoxide signals at δ 2.72 and 2.44 (C^5H signals). These facts strongly suggest that the nucleophilic ring opening of the epoxide occurred intramolecularly, possibly to produce five-membered cyclic imino acids (secondary amino acids), *cis*-L-hydroxyproline benzyl ester (*cis*-**8**) and *trans*-L-hydroxyproline benzyl ester (*trans*-**8**).

However, these products (a mixture of L-hydroxyproline benzyl esters *cis*-**8** and *trans*-**8**) were not isolated, because their isolation required polar conditions for the silica gel column chromatography. Instead, this reaction mixture was re-dissolved in dioxane and reacted with a small excess of Boc_2O using Et_3N to re-protect the secondary amino groups to give *N*-Boc-*cis*-L-hydroxyproline benzyl ester (*cis*-**9**) and *N*-Boc-*trans*-L-hydroxyproline benzyl ester (*trans*-**9**). After 24 h, evaporation

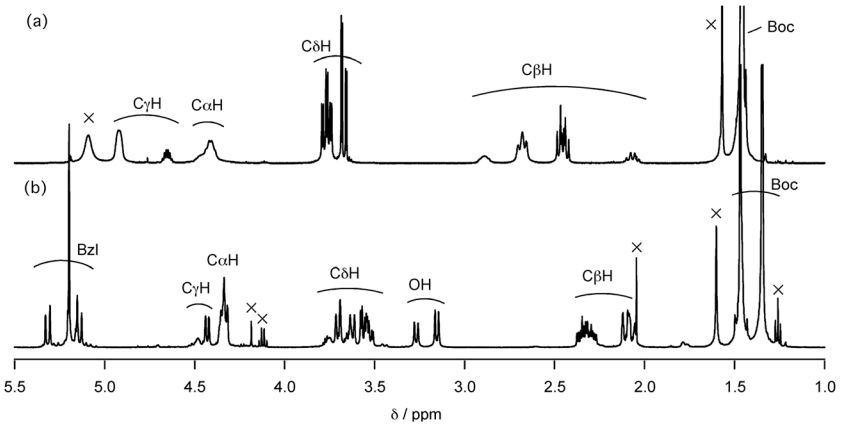


Fig. 3 ^{1}H NMR spectra of (a) **10** and (b) *trans*-**9** in $CDCl_3$ (high-field region). X denotes solvents and known impurities. See Fig. 4 for $C_\alpha H$, $C_\beta H$, $C_\gamma H$ and $C_\delta H$.

and washing with brine afforded the Boc-protected products as yellow oil. Two spots were observed on TLC, which were ninhydrin negative and $H_3(PMo_{12}O_{40})$ positive. ^{1}H NMR analysis was difficult at this stage because the spectrum exhibited many overlapping peaks; however, two products were successfully isolated by silica gel column chromatography using hexane–50% EtOAc (v/v) as the eluent.

The first eluted product was a white solid and was identified as (1*S*,4*S*)-*tert*-butyl-3-oxo-2-oxa-5-azabicyclo[2.2.1]heptane-5-carboxylate (L-*cis*-hydroxyproline lactone, **10**),[12,13,37,38] not the expected *N*-Boc-*cis*-L-hydroxyproline benzyl ester, *cis*-**9**. Compound **10** was obtained as a white solid in 29–42% yield based on (2*S*)-**6**. The ^{1}H NMR spectrum of **10** showed no signals of a benzyl group and –OH group (Fig. 3a and S5†). This spectrum is slightly complex because Boc-proline (hydroxyproline) derivative usually exists as a two-rotamers mixture based on the secondary amino *N*-carbamate C bond (Fig. 4a).[11,39] In fact, two sets of signals for a major isomer and a minor isomer were observed in the spectrum of **10**: $C_\gamma H$ δ 4.92 (0.79H, major), 4.65 (0.21H, minor); $C_\alpha H$ 4.47 (minor), 4.41 (major); $C_\beta H$ 2.89 (0.21H, minor), 2.68 (0.79H, major), 2.45 (0.79H, major), 2.06 (0.21H, minor). The geminal couplings between the βCH protons of major isomers and also between those of minor isomers were clearly observed in the 2D ^{1}H–^{1}H COSY spectrum (Fig. S6†). The spin–spin coupling between $C_\beta H$ and $C_\gamma H$ was observed only for one set of $C_\beta H$ protons, probably because of their dihedral angles. The 2D NOESY spectrum (Fig. S7†) indicated a NOE signal between $C_\beta H$ (δ 2.68 and 2.06) and $C_\gamma H$ (Fig. 4b). This NOE signal is the direct evidence for the bicyclic structure of **10** because monocyclic *trans*-**9** showed no NOE signals under the same conditions (see below). The most intense peak appeared at m/z = 272 (and 274) of $[M + H + Na^{35}Cl]^+$ (and $[M + H + Na^{37}Cl]^+$) in the ESI-mass spectrum (Fig. S8†), where M (213) indicated the intramolecular lactone formation, with no signals derived from *cis*-**9**.

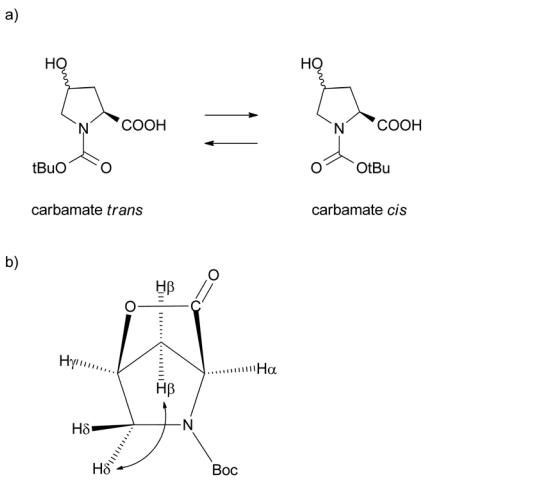


Fig. 4 (a) Two conformers of Boc-hydroxyproline. (b) NOE signals observed for **10**.

The second eluted product from the column was yellowish oil and was identified as *N*-Boc-L-*trans*-hydroxyproline benzyl ester (*trans*-**9**). Compound *trans*-**9** was obtained as yellowish oil in 18–38% yield based on (2*S*)-**6**. The ^{1}H NMR of *trans*-**9** (Fig. 3b) also indicated a mixture of rotamers but clearly showed the signals of a benzyl group at δ 7.35 (5H) and ~5.2 (2H). No NOE signals were observed under the same measurement conditions used for **10**; however, an –OH signal was detected at ~3.2 as an exchanging proton by phase-sensitive NOESY. The ^{1}H NMR and MS spectra of this sample agreed with those of the authentic sample of *trans*-**9** sample, which was separately synthesized from commercial *N*-Boc-L-*trans*-hydroxyproline and benzyl bromide.[40] The mass spectrum of *trans*-**9** showed peaks at m/z = 322 for $[M + H]^+$ and fragment peaks derived from $[M + H]^+$, where M (321) supported the structure of *trans*-**9**.

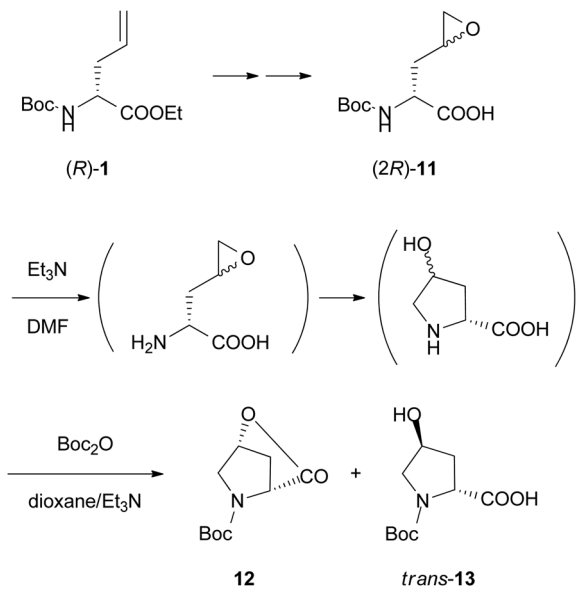
The ^{1}H NMR spectra of *trans*-**9** and **10** shows significant differences in the positions of $C_\gamma H$ signals (δ 4.92, 4.65 for **10** and δ ~4.4 for *trans*-**9**) and $C_\beta H$ signals (δ 2.89, 2.68, 2.45, 2.06 for **10** and δ 2.31, 2.07 for *trans*-**9**). The two $C_\beta H$ protons of **10** (δ 2.89 and 2.68) were high-field shifted compared to **9** and the other two $C_\beta H$ protons of **10** (δ 2.45 and 2.06) were similar to **9**. Interestingly, the high-field-shifted $C_\beta H$ protons of **10** (δ 2.89 and 2.68) showed NOE signals with $C_\delta H$ signals.

The –OH and ester (–$COOCH_2Ph$) groups are closely located in *cis*-**9**; therefore, lactone molecule **10** may be intramolecularly formed in the work-up or purification process. Fortunately, lactone **10** is less polar than *cis*-**9** and *trans*-**9**; therefore, separation between **10** and *trans*-**9** was easy. Thus, practical syntheses of both **10** (L-*cis*-hydroxyproline derivative) and *trans*-**9** (L-*trans*-hydroxyproline derivative) were successfully performed.

Hydroxyproline derivatives with (2*R*)-stereoconfiguration and *O*-benzyl derivatives for peptide synthesis

To prepare the D-hydroxyproline derivatives with (2*R*)-stereoconfigurations, (*R*)-**1** obtained by the Subtilisin resolution was epoxidized by *m*-CPBA and saponified to obtain (2*R*)-2-Boc-amino-3-(2-oxiranyl)propionic acid ((2*R*)-**11**) (Scheme 4 and Fig. S9†). This Boc-amino acid with a side-chain epoxide group was deprotected with HCl/dioxane, neutralized with Et_3N and then (after completion of the nucleophilic opening of the epoxide, 72 h) re-protected by Boc_2O similar to the procedure used to prepare the (2*S*)-isomeric series (Scheme 3). Silica gel chromatography afforded (1*R*,4*R*)-*tert*-butyl-3-oxo-2-oxa-5-aza-bicyclo[2.2.1]heptane-5-carboxylate (**12**)[10] and *N*-Boc-D-*trans*-hydroxyproline benzyl ester (*trans*-**13**) in 43% and 32% yields, respectively. Products **12** and *trans*-**13** are the mirror images of **10** and commercial *N*-Boc-L-*trans*-hydroxyproline, respectively. The protection of the amino acid –COOH group was not necessary in intramolecular cyclisation.

For practical use of *cis*-L-hydroxyproline derivatives in peptide synthesis, *O*-benzyl *N*-Boc-*cis*-L-hydroxyproline ((2*S*,4*S*)-*N*-Boc-4-benzyloxyproline, **14**) was synthesized. Compound *cis*-L-hydroxyproline **10** was cleaved with aqueous NaOH (slight excess). The *N*-Boc-*cis*-L-hydroxyproline Na salt was obtained after evaporation, to which NaH and benzyl bromide were added to produce **14**. Contrary to commercially available *O*-benzyl *N*-Boc-*trans*-L-hydroxyproline ((2*S*,4*R*)-*N*-Boc-4-benzyl-oxyproline), **14** was less soluble in $CDCl_3$, therefore, the ^1H NMR spectra of **14** and *O*-benzyl *N*-Boc-*trans*-L-hydroxyproline were acquired in CD_3OD (Fig. S10 and S11†). The $C_\beta H$ protons showed difference; $C_\beta H$ protons appeared at ~2.34 (m, 2H) in the *cis*-isomer (**14**) and appeared at 2.44 (m, 1H) and 2.07 (m, 1H) in the *trans*-isomer. Utilization of **14** for the synthesis of biologically active peptides by the introduction of *cis*-L-hydroxyproline residues is underway.



Scheme 4 Formation of *N*-Boc-D-*cis*-hydroxyproline lactone (**12**) and benzyl *N*-Boc-*trans*-D-hydroxyproline (*trans*-**13**).

Conclusions

In summary, (2*S*,4*R*)-, (2*S*,4*S*)-, (2*R*,4*S*)- and (2*R*,4*R*)-hydroxyproline derivatives were synthesized. The configuration of the chiral center at C^2 position was determined by the enzymatic resolution of the racemic amino acid ester (Subtilisin) or an acetyl amino acid (acylase). The L- and D-amino acids derivatives were obtained in enantiomerically pure forms. After the epoxidation of the side chain by treatment with *m*-CPBA, the amino acids with side-chain epoxide groups were subjected to the nucleophilic attack of the amino acid H_2N- group to the epoxide ring. Intramolecular cyclisation generated 4-hydroxyproline with five-membered rings.

The chiral center at the C^4 position was generated with low stereoselectivity; however, 4-hydroxyproline derivatives with *cis* orientations, *i.e.*, (2*S*,4*S*)- and (2*R*,4*R*)-hydroxyproline derivatives, formed bicyclic lactone molecules during the work-up process. Intramolecular ester exchange reactions occurred, *i.e.*, the benzyl ester moieties was attacked by the 4-OH group. The lactone molecules were more hydrophobic than hydroxyprolines; therefore, the silica gel column chromatographic separation of (2*S*,4*R*)-isomer (L-hydroxyproline) and (2*S*,4*S*)-isomer (L-*cis*-hydroxyproline lactone) was straightforward. Thus, stereoisomers of hydroxyproline derivatives were prepared successfully.

Experimental section

General

Reagents and solvents including Subtilisin (Carslberg, from *Bacillus lichenifirmis*) and L-aminoacylase (from *Aspergillus genus*, 3×10^4 unit per g) were from commercial sources. DCM and DMF were used after routine drying. DOWEX 50WX8 (50–100) was used as an ion exchange resin, after thoroughly washing with aqueous HCl. TLC was visualized by UV (F_{254}), I_2, $H_3(PMo_{12}O_{40})$ and/or ninhydrin. Daicel CHIRALPAK WH column (250 × 4.6 mm) was used for chiral HPLC of the amino acids eluting with 0.25 mmol L^{-1} $CuSO_4$ (1.0 mL min^{-1}), 50 °C and 220 nm detection. ^1H (1D, 2D-COSY, 2D-NOESY) and ^{13}C NMR spectra were measured in $CDCl_3$ or CD_3OD at 500 or 600 MHz. Normal- and high resolution-FAB mass and ESI TOF mass spectra were measured routinely. Optical rotations were measured in a 100 mm cell at 26 °C.

Kinetic resolutions of 2-amino-4-pentenoic acid derivative by Subtilisin, racemic ethyl 2-Boc-amino-4-pentenoate (1)[23]

Diethyl (2-Boc-amino)malonate (25.4 mL, 100 mmol) and NaOEt (100 mmol) in 100 mL EtOH was refluxed for 30 min. Allyl

bromide (11.2 mL, 130 mmol) was added to the above cooled mixture and further refluxed for 5 h. AcOH (5.7 mL) was added after cooling, then the mixture was evaporated, dissolved in EtOAc, washed (brine) and again evaporated to obtain diethyl 2-allyl-(2-Boc-amino)malonate (28.7 g). To the EtOH (60 mL) solution of diethyl 2-allyl-(2-Boc-amino)malonate (16.3 g, 51.7 mmol), 60 mL of aqueous NaOH (1.0 mol L^{-1}) was added at 0 °C. After 3 h, the mixture was concentrated, acidified (pH 3, citric acid) and extracted with EtOAc. After evaporation, crude ethyl 2-Boc-amino-2-ethoxycarbonyl-4-pentenoate was obtained (15.2 g). This ester was not purified but dissolved in 100 mL toluene and refluxed for 6 h. Racemic **1** was obtained after evaporation as a viscous liquid (12.5 g, 51.4 mmol, 91% based on diethyl (2-Boc-amino)malonate). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.70 (m, 1H, C^4H), 5.13 (m, 2H, C^5H), 5.06 (d, $J = 7$ Hz, 1H, NH), 4.36 (q, $J = 7$ Hz, 1H, C^1H), 4.20 (m, 2H, OCH_2), 2.55 and 2.50 (m, total 2H, C^2H), 1.44 (s, 9H, Boc), 1.28 (t, $J = 7$ Hz, 3H, OCH_2CH_3), which was similar to the literature data.[26]

(*S*)-2-Boc-amino-4-pentenoic acid ((*S*)-2)

The solution of racemic **1** (17.4 g, 71.5 mmol) in 72 mL DMF was diluted with 200 mL H_2O, then 70 mg (~840 units) of Subtilisin was added. The mixture was stirred at 40 °C for 3 h, while maintaining its pH at 8.0 (aqueous NH_3). The mixture was extracted by diethyl ether, then the remaining aqueous solution was acidified (pH 3, citric acid). (*S*)-**2** was extracted with EtOAc, washed (brine) and evaporated to yield 7.53 g (35.0 mmol, 49% based on racemic **1**) as an oil. HPLC (after removal of Boc with 4 mol L^{-1} HCl in dioxane): Only (*S*)-isomer 2-amino-4-pentenoic acid was detected at retention time 23.18 min. $[\alpha]_D^{26} = +9.65°$ ($c = 1.3$, MeOH). ^{1}H NMR was similar to the literature data.[25]

Ethyl (*R*)-2-Boc-amino-4-pentenoate ((*R*)-1)

The diethyl ether extract of the Subtilisin reaction mixture was evaporated to afford (*R*)-**1** (8.54 g, 35.1 mmol, 49% based on racemic **1**). HPLC (after removal of -OEt with 1 mol L^{-1} aqueous NaOH, 2 h, then Boc removal): Only (*R*)-isomer 2-amino-4-pentenoic acid was detected at retention time 19.38 min.

Kinetic resolutions of 2-amino-4-pentenoic acid derivative by acylase, racemic 2-acetamido-4-pentenoic acid (3)

Diethyl acetamidomalonate (10.0 g, 46.0 mmol) and NaOEt (50.7 mmol) was refluxed in 40 mL EtOH for 5 h, then allyl bromide (5.0 mL, 59 mmol) was added to the cooled reaction mixture and further refluxed for 15 h. After cooling, neutralized (2 mL AcOH) mixture was evaporated, taken up in EtOAc, washed (brine) and again evaporated to obtain an oily diethyl 2-acetylamido-2-allylmalonate quantitatively (11.9 g). This crude malonate (11.9 g, supposed to be 46.0 mmol) dissolved in 45 mL EtOH was mixed with 56 mL aqueous NaOH (1.0 mol L^{-1}) at 0 °C. The reaction was complete after 3 h (TLC), then EtOH was evaporated, the remaining aqueous solution was washed (diethyl ether), acidified (pH 2, aqueous HCl) and extracted with EtOAc. Evaporation of the organic phase afforded white solid of ethyl 2-acetamido-2-(ethoxycarbonyl)-4-pentenoate (10.8 g). A part of this ethyl 2-acetamido-2-(ethoxycarbonyl)-4-pentenote (9.30 g, 39.6 mmol) was refluxed in 90 mL toluene for 24 h, which afforded crude ethyl 2-(acetamido)-4-pentenoate (5.92 g) after evaporation. This crude ethyl ester was hydrolyzed with excess NaOH (with two batches) in EtOH–H_2O (1.3 : 1, v/v) at 0 °C for 3 h. After evaporation of EtOH, the remaining aqueous solution was washed (diethyl ether), acidified (aqueous HCl) and extracted with EtOAc. Evaporation of the organic phase afforded racemic **3** as a creamy oil (total 2.29 g, 14.6 mmol, 37% from diethyl acetamidomalonate). ^{1}H NMR ($CDCl_3$, 500 MHz, Fig. S1†): δ 6.00 (d, $J = 7$ Hz, 1H, NH), 5.74 (m, 1H, C^4H), 5.19 (m, 2H, C^5H), 4.65 (m, 1H, C^2H), 2.64 and 2.59 (m, 2H, C^3H), 2.06 (s, 3H, Ac).

(*S*)-2-Amino-4-pentenoic acid ((*S*)-4)

Racemic **3** (1.60 g, 10.2 mmol) and $CoCl_2 \cdot 6H_2O$ (11 mg, 50 μmol) was dissolved in 16 mL H_2O at 37 °C, while maintaining its pH at 7.0 with aqueous LiOH (*ca.* 14 mL). L-Aminoacylase (0.46 g, 13 800 units) in 4.0 mL H_2O was added. After 24 h, while adjusting the pH at 7.0 with small amount of aqueous NH_3, the reaction mixture was concentrated, acidified to pH 1.5 (aqueous HCl) and the unreacted (*R*)-**3** was extracted with EtOAc.

The acidic aqueous phase was applied to a column of ion exchange resin (DOWEX 50WX8, 150 × 20 mm). After sample charging and washing with 1.0 mol L^{-1} aqueous HCl, (*S*)-**4** eluted with 1.0 mol L^{-1} aqueous NH_3. Evaporation of the eluent afforded (*S*)-**4** as white solids (330 mg, 2.87 mmol, 28% based on racemic **3**). Only (*S*)-isomer of 2-amino-4-pentenoic acid was detected at retention time 23.60 min. ^{1}H NMR (CD_3OD, 500 MHz): δ 5.81 (m, 1H, C^4H), 5.22 (m, 2H, C^5H), 3.58 (m, 1H, C^2H), 2.66 and 2.57 (m, 2H, C^3H), which was identical to the commercial sample.

(*R*)-2-Acetamido-4-pentenoic acid ((*R*)-3)

The EtOAc extract of the acidified acylase reaction mixture was evaporated to give (*R*)-**3** as a brown oil (2.53 g). A part (1.75 g) of this sample was chromatographed over silica gel ($CHCl_3$–MeOH–AcOH = 90/10/2, by volume) to afford pure (*R*)-**3** as a semi-solid (249 mg, 1.58 mmol, 22% based on racemic **3**). HPLC (after removal of Ac by 1 mol L^{-1} aqueous HCl, reflux):[30] Only (*R*)-isomer of 2-amino-4-pentenoic acid was detected at retention time 19.53 min.

Epoxidation in the side chain of (*S*)-2, benzyl (*S*)-2-Boc-amino-4-pentenoate ((*S*)-5)

To the solution of (*S*)-**2** (6.93 g, 32.2 mmol) in 100 mL DMF, benzyl bromide (5.13 g, 45.0 mmol) and Et_3N (2.43 g, 36 mmol) was added at 0 °C. After 5 h, the mixture was evaporated, taken up in EtOAc, washed (aqueous citric acid/aqueous $NaHCO_3$) and again evaporated. The residue was chromatographed over silica gel ($CHCl_3$–1% MeOH, v/v) to obtain pure (*S*)-**5** (6.32 g, 20.7 mmol, 64%) as an oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.36 (m, 5H, Ph), 5.65 (m, 1H, C^4H), 5.17 (m, 2H, CH_2Ph), 5.1 (m, 3H, overlap of NH and C^5H), 4.43 (q, $J = 7$ Hz, 1H, C^2H), 2.52 (m, 2H, C^3H), 1.43 (s, 9H, Boc), which was similar to the literature data.[41]

Benzyl (2*S*)-2-Boc-amino-3-(2-oxiranyl)propionate ((2*S*)-6)

To the ice-cooled solution of (*S*)-**5** (8.00 g, 26.2 mmol) in 100 mL DCM, *m*-CPBA (9.59 g, 40.0 mmol) in 50 mL DCM was added in 5 portions and warmed to room temperature. After 24 h, the solution was concentrated to *ca.* 1/4 volume and was mixed with Na_2SO_3 solution (3.0 g in 25 mL H_2O). The biphasic mixture was stirred vigorously for 2 h, then the organic phase was washed (aqueous $NaHCO_3$) and evaporated to obtain the crude (2*S*)-**6** (9.26 g) as a white syrup, which was purified by silica gel column chromatography (hexane–15% EtOAc, v/v) to obtain pure (2*S*)-**6** (5.79 g, 18.0 mmol, 69%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz, Fig. 2, S2 and S3†): δ 7.36 (m, 5H, Ph), 5.31 (d, 1H, $J = 8$ Hz, NH), 5.19 (m, 2H, CH_2Ph), 4.52 (m, 1H, C^2H), 2.99 (m) and 2.96 (m) (C^4H of (2*S*,4*R*)-**6** and C^4H of (2*S*,4*S*)-**6**, respectively, total 1H), 2.72 (t, 1H, $J = 5$ Hz, C^5H), 2.44 (m, 1H, C^5H), 2.19 (m, 0.43H, C^3H of (2*S*,4*R*)-**6**), 2.00 (m, 0.57H, C^3H of (2*S*,4*S*)-**6**), 1.95 (m, 0.57H, C^3H of (2*S*,4*S*)-**6**), 1.81 (m, 0.43H, C^3H of (2*S*,4*R*)-**6**), 1.45 (s, 9H, Boc). ^{13}C NMR ($CDCl_3$, 125 MHz, Fig. S4†): δ 171.9 and 171.8, 155.3, 135.3, 128.6, 128.5, 128.4, 80.1, 67.4, 52.1 and 52.0, 49.1 and 49.0, 46.7 and 46.5, 35.6 and 35.5, 28.3. FAB-MS: *m*/*z* 344 ($[M + Na]^+$, 7), 322 ($[M + H]^+$, 14), 266 ($[M + H–C_4H_8]^+$, 100), 222 ($[M + H–C_4H_8–CO_2]^+$, 38); HR-FAB-MS $[C_{17}H_{24}NO_5]^+$ ($[M + H]^+$): calculated = 322.1655, found = 322.1639.

Intramolecular epoxide opening to generate *cis*-hydroxyproline lactone and *trans*-hydroxyproline ester, *tert*-butyl (1*S*,4*S*)-3-oxo-2-oxa-5-azabicyclo[2.2.1]heptane-5-carboxylate (10) and *N*-Boc-(2*S*,4*R*)-4-hydroxyproline benzyl ester (*trans*-9)

(2*S*)-**6** (1.49 g, 4.64 mmol) in 3.0 mL of dioxane was added to the ice-cooled solution of HCl in dioxane (4 mol L^{-1}, 40.0 mL) within 5 min and warmed to room temperature. After 2.5 h, the solution with white precipitate was evaporated and kept *in vacuo* overnight. HCl salt of benzyl 2-amino-3-(2-oxiranyl)propionate ((2*S*)-**7**·HCl) was obtained quantitatively (1.20 g) as a white oily solid, which was not characterized.

(2*S*)-**7**·HCl thus obtained was dissolved in 30 mL of DMF, then Et_3N (1.3 mL, 9.3 mmol) was added and the resultant yellowish mixture was stirred for 72 h. After evaporation, a white solid was yielded (1.51 g). A mixture of (2*S*,4*S*)-4-hydroxyproline benzyl ester ((2*S*,4*S*)-**8**) and (2*S*,4*R*)-4-hydroxyproline benzyl ester ((2*S*,4*R*)-**8**) were obtained quantitatively, which were analyzed by TLC and ^{1}H NMR, but these benzyl esters were not isolated. Instead, some of this crude benzyl esters (1.40 g, 4.30 mmol) was mixed with 30 mL of dioxane, then Et_3N (0.59 mL, 4.23 mmol) and Boc_2O (1.13 g, 5.18 mmol) in 3.0 mL dioxane was added. After 18 h, the mixture was concentrated, dissolved in EtOAc, washed (brine) and evaporated to afford yellow oil (1.12 g). 0.49 g (calculated to be 1.88 mmol) of this crude product was purified by silica gel column chromatography (hexane–50% EtOAc, v/v) to obtain pure **10** (yellowish solid after crystallization from EtOAc, 115 mg, 539 μmol, 29%) and *trans*-**9** (brownish oil, 106 mg, 330 μmol, 18%).

tert-Butyl (1*S*,4*S*)-3-oxo-2-oxa-5-azabicyclo[2.2.1]heptane-5-carboxylate (10)

^{1}H NMR ($CDCl_3$, 500 MHz, Fig. 3, S5, S6 and S7†): δ 4.92 (br s, 0.79H, $C_\gamma H$ of major isomer), 4.65 (sextet, $J = 5$ Hz, 0.21H, $C_\gamma H$ of minor isomer), 4.47 and 4.41 (both m, total 1H, $C_\alpha H$ of minor and major isomers, respectively), 3.66–3.79 (m, 2H, $C_\delta H$), 2.89 (m, 0.21H, $C_\beta H$ of minor isomer), 2.68 (t, $J = 20$ Hz, 0.79H, $C_\beta H$ of major isomer), 2.45 (dt, $J = 10$ and 13 Hz, 0.79H, $C_\beta H$ of major isomer), 2.06 (q, $J = 11$ Hz, 0.21H, $C_\beta H$ of minor isomer), 1.46 (s, 9H, Boc), which were similar to the literature data.[37] ESI-MS (Fig. S8†): *m*/*z* 272 ($[M + H + Na^{35}Cl]^+$), 274 ($[M + H + Na^{37}Cl]^+$), 216 ($[272–C_4H_8]^+$).

N-Boc-(2*S*,4*R*)-4-hydroxyproline benzyl ester (*trans*-9)

^{1}H NMR ($CDCl_3$, 500 MHz, Fig. 3): δ 7.35 (m. 5H, Ph), 5.07–5.27 (m, 2H, CH_2Ph), 4.44–4.48 (m, 2H, $C_\alpha H$, $C_\gamma H$), 3.65 (minor isomer) and 3.63 (major isomer) (both d, $J = 4$ Hz, total 1H, $C_\delta H$), 3.56 (major isomer) and 3.45 (minor isomer) (both d, $J = 12$ Hz, total 1H, $C_{\delta'}H$), 3.27–3.16 (m, 1H, OH), 2.31 (m, 1H, $C_\beta H$), 2.07 (m, 1H, $C_\beta H$), 1.46 (s, 3.2H, minor isomer, Boc), 1.35 (s, 5.8H, major isomer, Boc). FAB-MS: *m*/*z* 322 ($[M + H]^+$, 26), 266 ($[M + H–C_4H_8]^+$, 76), 222 ($[M + H–C_4H_8–CO_2]^+$, 100).

cis-Hydroxyproline lactone and *trans*-hydroxyproline of (2*R*)-isomers, (2*R*)-2-Boc-amino-3-(2-oxiranyl)propionic acid ((2*R*)-11)

The reaction of (*R*)-**1** (7.30 g, 30.0 mmol) with *m*-CPBA (1.5 equivalent) in DCM, in a similar manner to the synthesis of (2*S*)-**6**, yielded ethyl (2*R*)-2-Boc-amino-3-(2-oxiranyl)propionate (5.98 g, 23.1 mol, 77%). Without purification, 3.63 g of this ethyl ester (14.9 mmol) in 50 mL EtOH was mixed with 17 mL aqueous NaOH (1.0 mol L^{-1}) at 0 °C. After 2 h, AcOH (1.2 mL) was added and EtOH was evaporated. The remaining aqueous solution was washed (diethyl ether/aqueous $NaHCO_3$), acidified and extracted with EtOAc. Evaporation of the organic phase afforded (2*R*)-**11** as white solid (2.31 g, 10.0 mmol, 55% from (*R*)-**1** in two steps). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.63 (d, 1H, C^2H), 4.49 (m, 1H, C^4H), 3.77 (m, 1H, C^5H), 3.61 (m, 1H, C^5H), 2.46, 2.34, 2.02 (m, total 2H, C^3H), 1.44 (s, 9H, Boc). ^{13}C NMR (CD_3OD, 125 MHz, Fig. S9†): δ 178.3 and 177.3, 157.7, 80.9 and 79.7, 79.5, 64.7 and 64.1, 52.0 and 51.1, 31.4 and 31.2, 28.7. FAB-MS: *m*/*z* = 254 ($[M + Na]^+$, 29), 232 ($[M + H]^+$, 29), 176 ($[M + H–C_4H_8]^+$, 100); HR-FAB-MS $[C_{10}H_{18}NO_5]^+$ ($[M + H]^+$): calculated = 232.1185, found = 232.1193.

tert-Butyl (1*R*,4*R*)-3-oxo-2-oxa-5-azabicyclo[2.2.1]heptane-5-carboxylate (12) and *N*-Boc-(2*R*,4*S*)-4-hydroxyproline ethyl ester (*trans*-13)

(2*R*)-**11** (2.71 g, 11.7 mmol) was added to the HCl solution in dioxane (4 mol L^{-1}, 25 mL, 0 °C) and warmed to room temperature. After 1 h, the mixture was evaporated to obtain (2*R*)-2-amino-3-(2-oxiranyl)propionic acid quantitatively. This (2*R*)-2-amino-3-(2-oxiranyl)propionic acid was dissolved in 40 mL DMF, then Et_3N (2.00 mL, 14.4 mmol) was added and the resultant mixture was stirred for 72 h. Boc_2O (2.55 g, 11.7 mmol) and Et_3N (1.63 mL, 11.7 mmol) were further added. After 8 h, the mixture was concentrated, dissolved in EtOAc, washed

(aqueous citric acid) and evaporated to obtain yellow oil (1.45 g). This crude product was purified by silica gel column chromatography (hexane–50% EtOAc, v/v) to obtain **12** (ref. 10) (1.07 g, 5.03 mmol, 43%) and *trans*-**13** (866 mg, 3.74 mmol, 32%). The ^{1}H NMR spectra of these compounds were similar to the data of their enantiomers, **10** and Boc-Hyp, respectively.

4-Benzyl derivatives of (2*S*)-hydroxyprolines, (2*S*,4*S*)-*N*-Boc-4-benzyloxyproline (14)

The EtOH (3.0 mL) solution of **10** (213 mg, 1.00 mmol) was mixed with 1.2 mL aqueous NaOH (1.0 mol L^{-1}) for 1 h, then the mixture was evaporated *in vacuo*. The residue was dissolved in 3.0 mL THF, 55% NaH (52.4 mg, 1.2 mmol) and benzyl bromide (205 mg, 1.2 mmol) was added and stirred for 4 h. After evaporation, the residue taken up in EtOAc was washed and again evaporated to yield **14** as a white solid (267 mg, 83.1 μmol, 83%). ^{1}H NMR (CD_3OD, 500 MHz, Fig. S10†): δ 7.31 (m, 5H, Ph), 4.49 (m, 2H, CH_2Ph), 4.34 and 4.28 (m, total 1H, $C_\alpha H$), 4.16 (m. 1H, $C_\gamma H$), 3.61 (m, 1H, $C_\gamma H$), 3.48 (m, 1H, $C_\gamma H$), 2.34 (m, 2H, $C_\beta H$), 1.46 and 1.43 (s, total 9H, Boc). ^{13}C NMR (CD_3OD, 125 MHz, Fig. S11†): δ 175.9 and 175.6, 156.3 and 156.1, 139.5, 129.3, 129.0, 128.7, 81.5, 78.4 and 77.4, 73.2 and 72.0, 59.2 and 58.8, 53.4 and 52.7, 36.8 and 36.0, 28.6. ESI-MS: *m*/*z* 344 ($[M + Na]^+$), 288 ($[M + Na–C_4H_8]^+$), 244 ($[M + Na–C_4H_8–CO_2]^+$); HR-ESI-MS $[C_{17}H_{23}N_1Na_1O_5]^+$ ($[M + Na]^+$): calculated = 344.1474, found = 344.1460.

Acknowledgements

^{1}H NMR NOESY spectra and ESI mass spectra were measured at Institute for Materials Chemistry and Engineering, Kyushu University.

Notes and references

1 M. Pekkala, R. Hieta, U. Bergmann, K. I. Kivirikko, R. K. Wierenga and J. Myllyharju, *J. Biol. Chem.*, 2004, **279**, 52255–52261.
2 J. Myllyharju, *Top. Curr. Chem.*, 2005, **247**, 115–147.
3 K. L. Gorres and R. T. Raines, *Crit. Rev. Biochem. Mol. Biol.*, 2010, **45**, 106–124.
4 N. R. Rose, M. A. McDonough, O. N. F. King, A. Kawamura and C. J. Schofield, *Chem. Soc. Rev.*, 2011, **40**, 4364–4397.
5 R. A. Cacho, W. Jiang, Y.-H. Chooi, C. T. Walsh and Y. Tang, *J. Am. Chem. Soc.*, 2012, **134**, 16781–16790.
6 A. M. Mandal, J. Hines, K. Kuramochi and C. M. Crews, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4043–4047.
7 P. M. Simon, J. Pachence, B. Belinka, G. J. Poiani, S.-E. Lu, C. A. Tozzi and D. J. Riley, *Pulm. Pharmacol. Ther.*, 2006, **19**, 242–250.
8 B. T. M. Hoa, T. Hibi, R. Nasuno, G. Matsuo, Y. Sasano and H. Takagi, *J. Biosci. Bioeng.*, 2012, **114**, 160–165.
9 M. D. Shoulders, F. W. Kotch, A. Choudhary, I. A. Guzei and R. T. Raines, *J. Am. Chem. Soc.*, 2010, **132**, 10857–10865.
10 D. J. Pippel, L. K. Young, M. A. Letavic, K. S. Ly, B. Naderi, A. Soyode-Johnson, E. M. Stocking, N. I. Carruthers and N. S. Mani, *J. Org. Chem.*, 2010, **75**, 4463–4471.
11 A. K. Pandey, D. Naduthambi, K. M. Thomas and N. J. Zondlo, *J. Am. Chem. Soc.*, 2013, **135**, 4333–4363.
12 D. Papaioannou, G. Stavropoulos, K. Karagiannis, G. W. Francis, T. Brekke and D. W. Aksnes, *Acta Chem. Scand.*, 1990, **44**, 243–251.
13 J. A. Gómez-Vidal and R. B. Silverman, *Org. Lett.*, 2001, **3**, 2481–2484.
14 N. K. Yee, V. Farina, I. N. Houpis, N. Haddad, R. P. Frutos, F. Gallou, X.-j. Wang, X. Wei, R. D. Simpson, X. Feng, V. Fuchs, Y. Xu, J. Tan, L. Zhang, J. Xu, L. L. Smith-Keenan, J. Vitous, M. D. Ridges, E. M. Spinelli, M. Johnson, K. Donsbach, T. Nicola, M. Brenner, E. Winter, P. Kreye and W. Samstag, *J. Org. Chem.*, 2006, **71**, 7133–7145.
15 J. A. Smith, G. P. Savage and O. E. Hutt, *Aust. J. Chem.*, 2011, **64**, 1509–1514.
16 M. Seki and K. Matsumoto, *Biosci., Biotechnol., Biochem.*, 1995, **59**, 1161–1162.
17 P. D. Croce and C. La Rosa, *Tetrahedron: Asymmetry*, 2002, **13**, 197–201.
18 E.-S. H. El-Ashry and A. El Nemr, *Carbohydr. Res.*, 2003, **338**, 2265–2290.
19 H. B. Mereyala, G. Pathuri and L. Nagarapu, *Synth. Commun.*, 2012, **42**, 1278–1287.
20 R. Hara and K. Kino, *Biochem. Biophys. Res. Commun.*, 2009, **379**, 882–886.
21 C. Klein and W. Hüttel, *Adv. Synth. Catal.*, 2011, **353**, 1375–1383.
22 K. Nakanishi, M. I. Nurul, L. A. Watanabe and N. Nishino, in *Peptide Science 2010*, ed. N. Fujii and Y. Kiso, The Japanese Peptide Society, 2011, p. 197.
23 B. S. Schricker, K. Thirring and H. Berner, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 387–390.
24 B. Mohar, A. Štimac and J. Kobe, *Tetrahedron: Asymmetry*, 1994, **5**, 863–878.
25 A. G. Myers and J. L. Gleason, *Org. Synth.*, 1999, **76**, 57–66.
26 L. Sanière, L. Leman, J.-J. Bourguignon, P. Dauben and R. H. Dodd, *Tetrahedron*, 2004, **60**, 5889–5897.
27 H. K. Chenault, J. Dahmer and G. M. Whitesides, *J. Am. Chem. Soc.*, 1989, **111**, 6354–6364.
28 N. Nishino, T. Arai, Y. Ueno and M. Ohba, *Chem. Pharm. Bull.*, 1996, **44**, 212–214.
29 L. A. Watanabe, S. Haranaka, B. Jose, M. Yoshida, T. Kato, M. Moriguchi, K. Soda and N. Nishino, *Tetrahedron: Asymmetry*, 2005, **16**, 903–908.
30 B. Layek and J. Singh, *Biomacromolecules*, 2013, **14**, 485–494.
31 K. J. Shaw, J. R. Luly and H. Rapoport, *J. Org. Chem.*, 1985, **50**, 4515–4523.
32 S. Hoarau, J. L. Fauchère, L. Pappalardo, M. L. Roumestant and P. Viallefont, *Tetrahedron: Asymmetry*, 1996, **7**, 2585–2593.
33 P. Allevi, M. Galligani and M. Anastasia, *Tetrahedron: Asymmetry*, 2002, **13**, 1901–1910.
34 N. Kurokawa and Y. Ohfune, *J. Am. Chem. Soc.*, 1986, **108**, 6041–6043.
35 D. Guillerm, K. Lavrador and G. Guillerm, *Synth. Commun.*, 1995, **25**, 877–882.

36 G. Han, M. Tamaki and V. J. Hruby, *J. Pept. Res.*, 2001, **58**, 338–341.
37 M. M. Bowers-Nemia and M. M. Joullié, *Heterocycles*, 1983, **20**, 817–828.
38 M.-C. Lechner, E. Aubert, G. Guichard and C. Didierjean, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2008, **64**, 2039/1–2039/6.
39 M. Kuemin, Y. A. Nagel, S. Schweizer, F. W. Monnard, C. Ochsenfeld and H. Wennemers, *Angew. Chem., Int. Ed.*, 2010, **49**, 6324–6327.
40 S.-p. Zhang, X.-k. Fu and S.-d. Fu, *Tetrahedron Lett.*, 2009, **50**, 1173–1176.
41 S. J. Ryan, Y. Zhang and A. J. Kennan, *Org. Lett.*, 2005, **7**, 4765–4767.

