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# α-Chymotrypsin and L-acylase aided synthesis of 5-hydroxypipecolic acid via Jacobsen's hydrolytic kinetic resolution of epoxy amino acids.

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**Suvratha Krishnamurthy,[†*] Jalli Venkataprasad,[†] Tarun Chand Vagvala,[‡] Tetsuji Moriguchi,[†] and Akihiko Tsuge.[†]**

[†]Department of Applied Chemistry, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan.

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

e-mail : suvrathak@gmail.com

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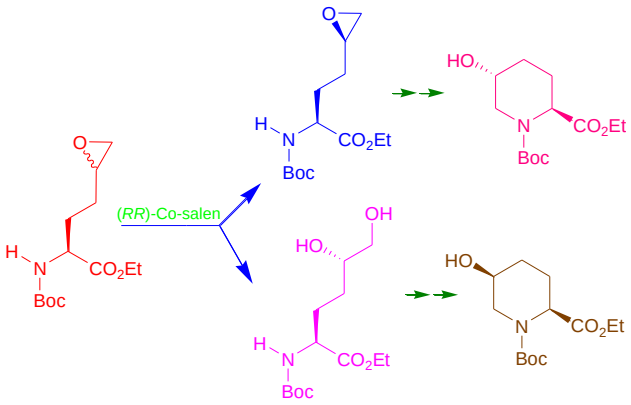



5-hydroxypipecolic acid synthesis from intramolecular reaction of epoxy amino acids; enatiomers separated by hydrolase and diastereomeric epoxide separated by Jacobsen's hydrolytic kinetic resolution.

**ABSTRACT:** Diethyl malonate derivatives were used to synthesize racemic 2-amino-5-hexenoic acid. This racemic 2-amino-5-hexenoic acid (homoallylyglycine) derivatives were efficiently resolved aided by α-chymotrypsin or L-acylase, giving rise to L- and D- enatiomers. These isolated enatiomerically pure amino acids with *tert*-butoxycarbonyl (Boc) protection were oxidised with 3-chloroperbenzoic acid. The oxidation gave rise to an inseparable diastereomeric epoxides due to newly generated chiral center at $C^5$ carbon. The isolation of one of the diastereomeric epoxide was possible by selectively converting the remaining diastereomer into a dihydroxyl compound catalysed by Jacobsen's hydrolytic kinetic resolution (HKR).The isolated epoxide was regioselectively attacked by LiBr to give vicinal halohydrin, with bromide attacking on terminal $C^6$ carbon. Upon (Boc) deprotection of the halohydrin, lead to intramolecular cyclization by attack of free amine at $C^6$ carbon, generating a single isomer of 5-hydroxypipecolic acid which was effortlessly recovered in good yield after re-protection of the amine with (Boc) group. Similarly the dihydroxyl compound isolated earlier was converted to an halohydrin with iodine at the $C^6$ carbon. This was feasible by efficient regioselective mono-tosylation with catalytic ($Bu_2SnO$) followed by iodine substitution. This was utilized to synthesize the 5-hydroxypipecolic acid derivative in the same described sequence consisting of Boc removal by acid treatment, cyclization, reprotection and purification. Finally the same sequence was repeated with D-isomer and two diastereomers were isolated.

## 1. Introduction

5-Hydroxypipecolic acid (5-hydroxypiperidine-2-carboxylic acid) (**1,** Fig.1), a vital non-proteinogenic cyclic δ-hydroxy-α-amino acid found in plants[1,2] and animals[3] occurs as a metabolic by-product. Derivatives of 5-hydroxypipecolic acid are constituents of a tumor necrosis-converting enzyme inhibitor, TNF-α (**3**, Fig.1).[4] Recently *cis*-5-hydroxypipecolic acid (**2**, Fig.1) was utilized as a precursor for the synthesis of *β*-lactamases inhibitor MK-7655[5] (**4**, Fig.1), which combined with Merck's Primaxin® is in phase II clinical trials for the treatment of gram-negative bacterial infections.[6] It is also employed in the synthesis of 5-guanidino pipecolates, as constrained arginine mimetic and exhibited weak to moderate inhibition of nitric oxide synthase (NOS).[7] *cis*-5-hydroxypipecolic acid demonstrates inhibition of *Aspergillus* spp responsible for spoilage of stored grains.[8] It is also reported to display potent inhibitory effects on human platelet aggregation induced by serotonin.[9]

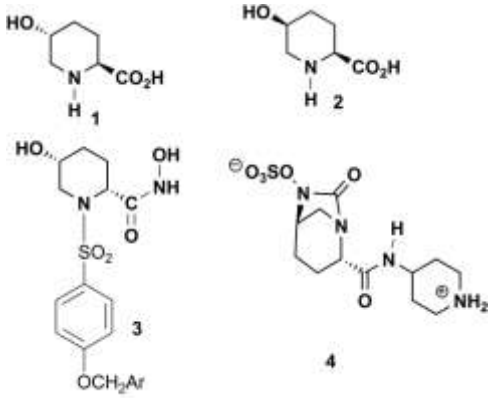


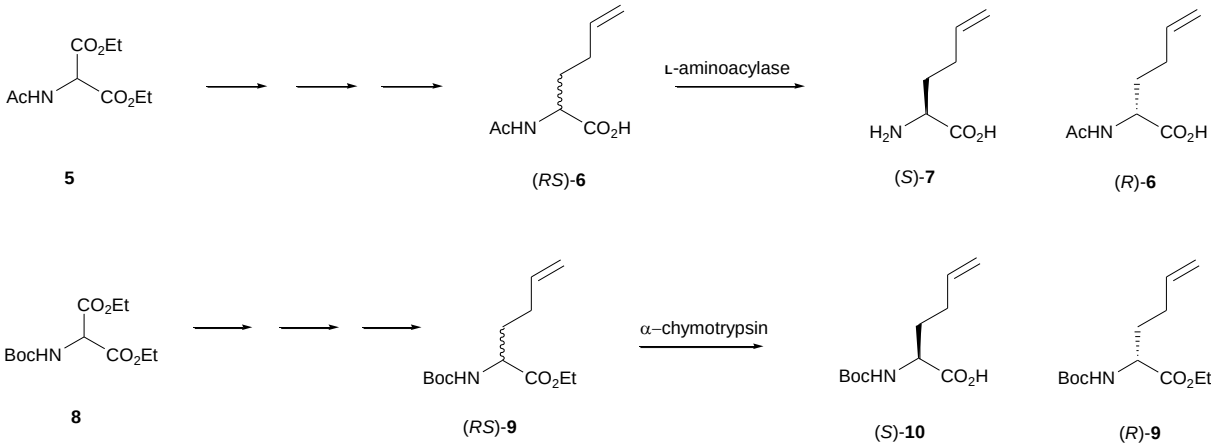

Figure 1.

Due to its above mentioned biological relevance and ability to induce rigidity owing to its cyclic structure, several groups have attempted the synthesis of 5-hydroxypipecolic acid.[7,10-14] In this context glycine amino acid residues functionalized with epoxide side chains are synthetically valuable precursors for generation of cyclic amino acids. We recently synthesized 4-hydroxyproline isomers from epoxide derived from 2-amino-4-pentenoic acid (allyl glycine), by selective intramolecular reaction of amine on $C^5$ carbon.[15] Similarly the synthesis of 5-hydroxypipecolic acid can be achieved by selective attack of amine group on $C^6$ carbon of 2-amino-5-hexenoic acid epoxide (homoallyl glycine). Several reports exists on the synthesis of 5-hydroxypipecolic acid derivatives via intramolecular reactions of epoxy amino acid.[1,16] However these synthetic procedures yield an unresolved diastereomeric mixture of desired *cis*- and *trans*-5-hydroxypipecolic acids, [1,16,17] along with the undesirable 5-hydroxymethylprolines.[1,17] A straightforward method generating 5-hydroxypipecolic acid derivatives bypassing the laborious need to isolate the *cis*- and *trans*- diastereomers in the final stages combined with elimination of the formation of regioisomeric proline is desired. In this paper we would like to present the synthetic scheme circumventing the drawbacks imposed by the established synthetic procedures involving generation of 5-hydroxypipecolic acid by intramolecular reaction of epoxy amino acid. To the best of our knowledge this is the first work reporting the synthesis of 5-hydroxy pipecolic acid wherein the 2*S* (L-) and 2*R* (D-) isomers were efficiently separated by enzymatic kinetic resolution. Whereas the epoxide precursors that give rise to *cis*- and *trans*- isomers of 5-hydroxypipecolic acid were separated via Jacobsen's Co catalyzed HKR.

## 2. Results and Discussion

### L-acylase and α-Chymotrypsin catalyzed enzymatic resolution of 2-amino-5-hexenoic acid derivatives.

Enatiomerically pure 2-amino-5-hexenoic acid derivatives were synthesized by enzymatic resolution of acetyl amino acid and *tert*-Boc amino acid ester, with L-aminoacylase and α-chymotrypsin respectively (Scheme 1).




**Scheme 1**. Enzymatic resolution of 2-amino-5-hexenoic acid derivatives.

Diethyl acetamidomalonate (**5**) was reacted with excess of 4-bromo-1-butene in the presence of sodium ethoxide in absolute ethanol to obtain the alkylated diester. The diester was half saponified and decarboxylated simultaneously, by treating with solid NaOH in EtOH:$H_2O$ mixture (1:1, v/v) and refluxing for 24 h. This gave the desired racemic acetyl amino acid (*RS*)-**6**, (47 % overall yield based on **5**) suitable for the L-acylase catalyzed enzymatic resolution.

An aqueous solution of (*RS*)-**6**, maintained at 38 °C, with pH 7.5 adjusted with LiOH (aq) was treated with L-aminoacylase from *Aspergillus* genus.[18] After 24 h, the solution was concentrated and acidified with 1M HCl to pH 3 and extracted with EtOAc to remove the unreacted (*R*)-**6**. The remaining acidic solution was passed through ion exchange resin (DOWEX® 50WX8) to separate (*S*)-**7**, which was selectively eluted with $NH_4OH$ (1M). Due to high water solubility of the acetyl amino acid the yields were less, but pure D- and L-2-amino-5-hexenoic acid were obtained with good chemical and enatiomeric purity ascertained by $^1H$ NMR and chiral HPLC.

Subsequently diethyl (2-Boc-amino)malonate (**8**), was treated with excess 4-bromo-1-butene in presence of sodium ethoxide to obtain alkylated diester. The later was selectively saponified with solid LiOH to obtain the half acid half ester, which was successfully decarboxylated in refluxing toluene to obtain (*RS*)-**9** (59 % overall yield with respect to **8**).

Enzymatic resolution was carried by suspending the racemic (*RS*)-**9** in phosphate buffer (pH 8, 0.1 mmol) at 38 °C and treating with α-chymotrypsin. To avoid slight deviance of pH caused by formation of (*S*)-**10**, minimal amount of $NH_4OH$ (1M) was added and maintained at pH 8. After 24 h, the mixture was cooled to room temperature and alkalinity was enhanced by addition of (4% $NaHCO_3$) followed by extraction with EtOAc to recover unreacted (*R*)-**9** (51 %). The remaining alkaline aqueous solution was subsequently acidified with solid citric acid to pH 3, extracted with EtOAc to obtain (*S*)-**10** (46%).

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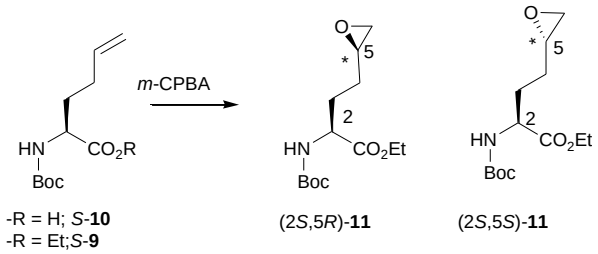
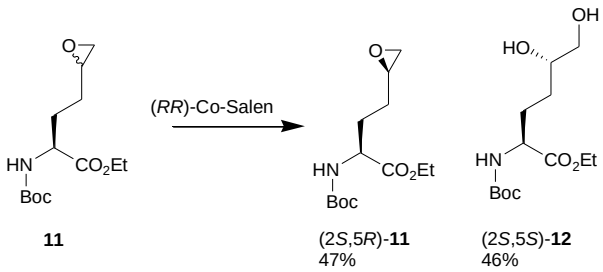



**Scheme 2.** *m*-CPBA oxidation of the alkene side chain of (*S*)-**9**.

***m*-CPBA oxidation.**(Scheme 2)

Kinetically resolved Boc-amino acid (*S*-**10**) was esterified with ethyl bromide in the presence of $Et_3N$ to obtain (*S*)-**9** in 84% yield. This fully protected Boc-amino acid ester with unsaturated side chain was epoxidized with excess 3-chloroperbenzoic acid (*m*-CPBA) by treating the solution of (***S***)-**9** in DCM with excess *m*-CPBA at 0 °C, followed by 24 h stirring at room temperature. Subsequently excess *m*-CPBA was cautiously reduced with 10% $Na_2SO_3$ at 0 °C. The DCM layer was separated and washed (4% $NaHCO_3$ and brine), concentrated and chromatographed ($SiO_2$) to obtain **11** (90%, yield).

The resulting epoxide was a diastereomeric mixture (2*S*,5*R*)-**11** and (2*S*,5*S*)-**11** due to the newly generated chiral centre at $C^5$ carbon. However the diastereomers moved as a single spot with same $R_f$ value on TLC and could not be separated.$^1$H NMR analysis in ($CDCl_3$) did not show any set of distinguishable peaks for the individual diastereomers. In this context *m*-CPBA oxidation of similar epoxides is believed to produce 1:1 mixture of both diastereomers, nonetheless it was reported that these diastereomers were inseparable.[16,19]




**Scheme 3.** Jacobsen's hydrolytic kinetic resolution of (2*S*,5*S*)-11 and (2*S*,5*R*)-11

**Hydrolytic kinetic resolution (HKR) of epoxy amino acids.**[20,21] (Scheme 3)

To aid the separation of the diastereomeric epoxide, **11** was treated with (0.55 eq) of $H_2O$ in the presence of (0.5 mol%) of (*RR*)-Co-Salen and AcOH, at room temperature in THF. This produced the chiral diol (2*S*,5*S*)-**12** and chiral epoxide (2*S*,5*R*)-**11** which had a considerable $R_f$ difference. In fact the chiral epoxide (2*S*,5*R*)-**11** and chiral diol (2*S*,5*S*)-**12**, were easily separated by silica gel column chromatography in good yield, 47% and 46%, respectively.

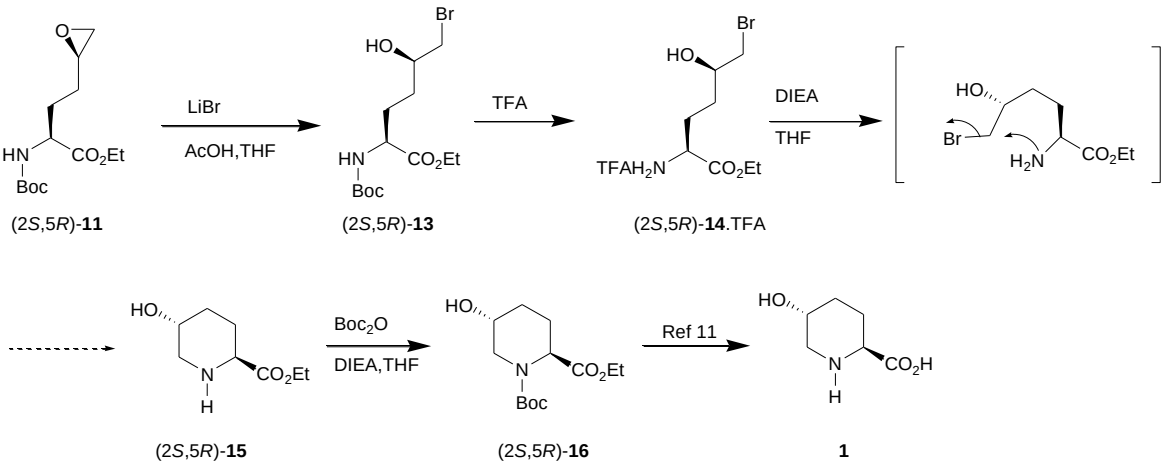
***tert*-butoxycarbonyl (Boc) removal and intramolecular cyclization.**(Scheme 4)

Next, the chiral epoxide (2*S*,5*R*)-**11** was treated with LiBr in the presence of AcOH in THF, which attacked the $C^6$ carbon selectively giving rise to bromohydrin **13**.[22] This bromo amino acid was isolated in good yield (92%) after ($SiO_2$) purification, which was suitable for synthesis of 5-hydroxypipecolic acid. To aid the intramolecular cyclization, Boc group of **13** was removed by treating it with TFA. After

1h TFA was removed to obtain, **14.**TFA in quantitative yield which was not purified owing to its hydrophilic character, instead it was used for next step directly.

**14**.TFA, was dissolved in THF at room temperature and treated with DIEA (2 eq) and stirred. DIEA treatment leads to the neutralization of TFA and simultaneously gives rise to highly nucleophilic and free amine. This amine will attack intramolecularly the $C^6$ carbon, thereby producing the 5-hydroxypipecolic acid. Due to the formation of HBr after intramolecular cyclization, another equivalent of DIEA was required to neutralize this and complete the cyclization. TLC analysis at the end of 4 h, (butanol/pyridine/acetic acid/$H_2O$, 4:1:1:2, v/v/v/v) by staining with ninhydrin showed the absence of the reactant and appearance of a yellow spot, which is characteristic of the cyclic amino acids. This confirmed that the nitrogen intramolecularly attacked the side chain to produce the piperidine ring, *i.e.*, (2*S*,5*R*)-**15**. To aid the purification process the nitrogen of the piperidine ring was again reprotected with Boc group by treatment with $Boc_2O$ in presence of DIEA to generate the Boc-5-hydroxypipecolic acid derivatives. 5-hydroxypipecolic acid, (2*S*,5*R*)-**16** was separated as an oil (80%) by silica gel column chromatography (50% EtOAc in hexane).

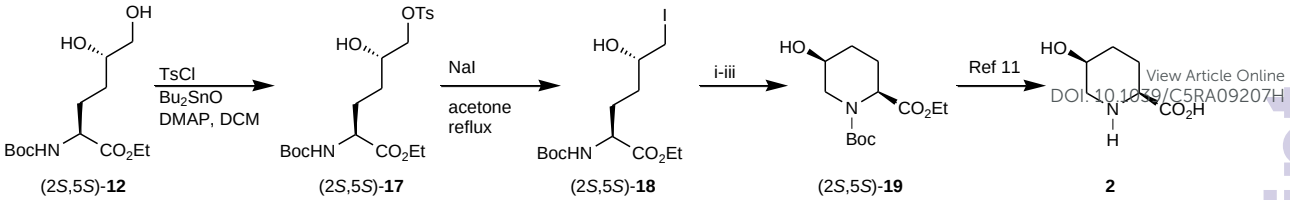
Subsequently the oil (2*S*,5*R*)-**16** was converted to **1.HCl**. The spectral data, melting point and specific rotation of **1.HCl**, were in good agreement with the reported values.[23]




**Scheme 4.** Formation of (2*S*,5*R*)-hydroxypipecolic acid by intramolecular cyclization

**Regioselective halogenation, *N*-Boc removal and intramolecular cyclization.**

In order to transform the chiral diol **12** into 5-hydroxypipecolic acid, it was necessary to transform **12** into amino acid with a good leaving group at $C^6$ carbon similar to **13**. This was achieved by selective tosylation of diol by treating with TsCl in the presence of catalytic $Bu_2SnO$ (30 mol%), DMAP (cat.) and triethylamine at -10 °C, yielding the mono tosylated product in almost quantitative yield.[24-26] The temperature was maintained between -10 °C to 0 °C in order to retain the acid labile Boc group. The tosyl group in **17** was replaced with iodo group by refluxing it in the presence of NaI (6 eq) in acetone. After 8 h, acetone was replaced with EtOAc washed with (sat.$Na_2S_2O_3$), brine and chromatographed on ($SiO_2$) resulting (2*S*,5*S*)-**18** (95%) used for synthesis of 5-hydroxypipecolic acid (Scheme 5).



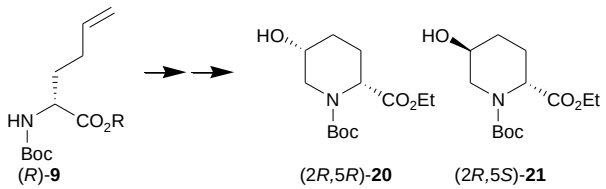



**Scheme 5. Formation of (2S,5S)-hydroxypipecolic acid by intramolecular cyclization i.TFA; ii. DIEA, THF; iii. $Boc_2O$, DIEA,THF.**

To aid the intramolecular cyclization Boc group of **18** was removed by treating with TFA at room temperature. After 1h the solution was concentrated to obtain Boc- deprotected amino acid salt of TFA in quantitative yield, which was used for subsequent step directly.

After dissolving in THF at room temperature and treating it with DIEA (2 eq) free amine was generated. The free amine attacked the $C^6$ carbon intramolecularly generating 5-hydroxypipecolic acid. After 3 h, to aid the purification process the nitrogen of the amino acid was again reprotected with Boc group by treating with $Boc_2O$ in presence of DIEA to generate the Boc-5-hydroxypipecolic acid derivatives. The fully protected 5-hydroxypipecolic acid (2*S*,5*S*)-**19** was easily separated as an oil by silica gel column chromatography (50% EtOAc in hexane) in 76 % yield. Subsequently the oil (2*S*,5*S*)-**19** was converted to **2.HCl**. The spectral data, melting point and specific rotation of **2.HCl**, were in good agreement with the reported values.[27,28]

**Synthesis of (2*R*,5*R*)-20 and (2*R*,5*S*)-21.** Finally the synthesis of the (2*R*,5*R*) and (2*R*,5*S*)- isomers of the 5-hydroxypiecolic acid was easily achieved starting from (2*R*)-**9** (Scheme 6), [ESI-S2].




**Scheme 6**. Formation of (2*R*,5*S*) and(2*R*,5*R*) isomers by intramolecular cyclization

## 3. Experimental section

**General:** Reagents, dry solvents, and enzymes were from commercial sources. DOWEX 50WX8 (50–100) was used as an ion exchange resin, after washing with 1M HCl. (*R*,*R*)-(−)-N,N′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) was purchased from Sigma-Aldrich®. TLC was visualized by one of the following, UV (F254), $I_2$, $H_3(PMo_{12}O_4)$ or ninhydrin. Chiral HPLC analysis were performed on *Column I:* CHIRALPAK IA column (4.6 × 250 mm, Diacel Chemical Industries, Ltd.), n-hexane:2-propanol:TFA (97:3:0.1, v/v/v) with a flow rate of 1mL/min, detection at 220 nm; *Column II:* CHIRALCEL OD column (4.6 × 250 mm, Diacel Chemical Industries, Ltd.), n-hexane:2-propanol:TFA (90:10:0.1) with a flow rate of 1mL/min, detection at 220 nm. $^1H$ were measured in $CDCl_3$, $CD_3OD$ or $D_2O$ at 500 or 400 MHz and $^{13}C$ NMR were measured in $CDCl_3$, $CD_3OD$ or $D_2O$ at 125 MHz. Normal- and high resolution-FAB mass were measured routinely. Optical rotations were measured using Jasco Polarimeter (P-1010). Melting point (m.p.) were recorded by As One melting point instrument (Model Number ATM-01).

**Kinetic resolutions by L-acylase from *Aspergillus* genus.**

**(*RS*)-2-(acetamido)-5-hexenoic acid (*RS*)-6**. Sodium metal (5.10 g, 222.0 mmol) was added to a flask containing 195 mL of absolute ethanol maintained at 0 °C. After complete dissolution of sodium, **5** (43.5 g, 200 mmol) was added in one portion and the resulting solution was refluxed. After 3h to this refluxing solution 4-bromo-1-butene (25.0 g, 185 mmol) was added and continued to reflux. After 24 h the mixture was concentrated and the residue dissolved in EtOAc (600 mL) and washed with 10% citric acid, 4% $NaHCO_3$, brine, dried ($MgSO_4$), filtered and evaporated to get the alkylated diester.

This diester was dissolved in 1:1 (v/v), mixture of ethanol and water (230 mL) at 0 °C, and to this solution solid NaOH (7.0 g, 175 mmol) was added, this solution was then refluxed. After 24h the ethanol was removed and the resulting solution was dissolved in 4% $NaHCO_3$ and extracted with diethyl ether to remove the unreacted starting material. Then the remaining alkaline aqueous solution was acidified to pH 3 with 1M HCl and extracted with EtOAc (20 mL × 20). The combined organic layer was washed with brine, dried ($MgSO_4$), filtered and evaporated to give (*RS*)-**6** as a white powder (15 g, 47 % w.r.t. **5**). mp 119-121 °C [lit.[29] 106-108 °C]; $^1$H NMR (400 MHz, $CD_3OD$) 5.89-5.79 (m, 1H), 5.10-5.00 (m, 2H), 4.37 (m, 1H), 2.14 (m, 2H), 1.99 (s, 3H), 1.96-1.71 (m, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$) 175.6, 173.5, 138.5, 116.2, 53.3, 32.1, 31.2, 22.5; LRMS-FAB (*m/z*) 343 (67), 194 (58), 172 (100), 126 (68), 83 (68); HRMS-FAB (*m/z*) calcd for $C_8H_{14}N_1O_3$ ($[M+H]^+$)172.0974 found 172.0909.

**(*S*)-2-amino-5-hexenoic acid (*S*)-7 and (*R*)-2-(acetamido)-5-hexenoic acid (*R*)-6**. (*RS*)-**6** (3.00 g, 17.5 mmol) and $CoCl_2$•$6H_2O$ (14 mg, 50 μmol) was dissolved in 60 mL of $H_2O$ at 38 °C, while maintaining pH at 7.5 with aqueous LiOH. L-aminoacylase from *Aspergillus* genus (0.8 g) was then added and stirred. The pH was monitored at 7.5 with small amount of $NH_4OH$ (1M). After 24 h, the reaction mixture was concentrated, acidified to pH 2-3 with 1M HCl. The unreacted (*R*)-**6** was extracted with EtOAc, washed with brine, dried ($MgSO_4$), filtered and evaporated to give (*R*)-**6** (1.23 g, 41%). (*Column II*. ee 88%).

The remaining acidic aqueous phase was applied to a column of ion exchange resin (DOWEX® 50WX8). After sample charging and elution with 1M HCl, followed by $H_2O$, finally the compound (*S*)-**7** was eluted with 1M $NH_4OH$. Evaporation of this eluant afforded (*S*)-**7** as white solid (0.72g, 32 %). (after *N* acetylation, *Column II*. ee > 99%) $^1$H NMR ($D_2O$, 500 MHz) 5.84-5.76 (m, 1H), 5.12-5.03 (m, 2H), 3.72-3.69 (m, 1H), 2.15-2.11 (m, 2H), 1.99-1.85 (m, 2H); $^{13}$C NMR ($D_2O$, 125 MHz) 174.7, 137.0, 115.9, 54.3, 29.7, 28.6; LRMS-FAB (*m/z*) 130 (100).

**Kinetic resolutions by α-chymotrypsin.**

**(*RS*)-ethyl 2-*N*-(*tert*-butoxycarbonyl)-2-amino-5-hexenoate (*RS*)-9**. Metallic sodium (0.92 g, 39.9 mmol) was added to EtOH (17.0 mL) at 0 °C and stirred until complete dissolution of sodium. **8** (10.0 g, 36.3 mmol) was then added at the same temperature and stirred for 30 min. Finally 4-bromo-1-butene (5.6 mL, 54.8 mmol) was added in one portion and the resulting yellow solution was refluxed for 24 h. After 24 h, the solution was partitioned between $H_2O$ and EtOAc, extracting the desired product into EtOAc. The EtOAc was then washed with 10% citric acid, brine, dried ($MgSO_4$), filtered and evaporated to obtain an oil (9.51 g), which was suspended in EtOH:$H_2O$ (18:8, v/v) and cooled to 0 °C. This solution was treated with solid LiOH.$H_2O$ (1.33 g, 31.8 mmol) and stirred overnight at 0 °C. The solution was then mixed with 4% $NaHCO_3$ to make the solution alkaline and extracted with diethyl ether to remove any unreacted starting material. The remaining alkaline solution was then acidified to pH 3 with solid citric acid and extracted with EtOAc. The EtOAc was then washed with brine, dried ($MgSO_4$), filtered and evaporated to give the mono ester mono acid (8.30 g). This was dissolved in

toluene (25 mL) and refluxed. After 18 h the solvent was evaporated at 40-50 °C to give a crude mixture which directly purified by silica gel column chromatography (hexane:EtOAc, 80:20, v/v) to give (*RS*)-**9** (5.5 g, 59%, after 3 steps). $^1$H NMR (500 MHz, $CDCl_3$) 5.84-5.76 (m, 1H), 5.07-5.00 (m, 2H), 4.31-4.30 (m, 1H), 4.21 (m, 2H), 2.15-2.09 (m, 2H), 1.95-1.88 (m, 1H), 1,75- 1.68 (m,1H), 1.45 (s, 9H) 1.28 (m, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) 172.8, 155.3, 137.1, 115.6, 79.8, 61.3, 53.1, 32.1, 29.5, 28.3, 14.2; LRMS-FAB (*m/z*) 258 (50), 230 (21), 202 (100), 158 (93) and 128(18); HRMS-FAB (*m/z*) calcd for $C_{13}H_{24}N_1O_4$ ([M+H]$^+$) 258.1705, found 258.1707.

**(*R*)-ethyl 2-*N*-(*tert*-butoxycarbonyl)-2-amino-5-hexenoate (*R*)-9 and (*S*) 2-*N*-(*tert*-butoxycarbonyl)-2-amino-5-hexenoic acid (*S*)-10.** (*RS*)-**9** (4.80 g, 18.6 mmol) was suspended in phosphate buffer ( 360 mL, 0.1 M; pH 8.00), warmed to 38 °C and α-chymotrypsin (28 mg, 40 units/mg) was added in one portion. The resulting mixture was stirred at 38 °C for 24 h while monitoring the pH 8 with little amount of (1M $NH_4OH$). After 24 h the above solution was made more alkaline by addition of 4% $NaHCO_3$ and was extracted with EtOAc (20 ml × 10). The combined EtOAc layer (200 mL) was washed with brine, dried ($MgSO_4$), filtered and evaporated to give (*R*)-**9** ( 2.42 g, 51 %). (*Column I.* ee > 64%); $[\alpha]_D^{25}$ = - 9.30° (*c* = 2.44, $CH_2Cl_2$). The remaining alkaline solution was then acidified to pH 3, with solid citric acid and extracted with EtOAc (30 ml × 10). The combined EtOAc layer (300 mL) was washed with brine, dried ($MgSO_4$), filtered and evaporated to give **(*S*)-10** (1.95 g, 46 %). (*Column I.* ee > 99%); $[\alpha]_D^{20}$ = - 1.18° (*c* = 1.30, $CH_3OH$). {lit.$^{30}$ $[\alpha]_D^{20}$ = - 1.1° (*c* = 1.30, $CH_3OH$)}; $^1$H NMR (500 MHz, $CD_3OD$) 5.81-5.73 (m, 1H), 5.01-4.98 (m, 3H), 4.04-4.02 (m, 1H), 2.18-2.08 (m, 2H), 1.91-1.85 (m, 1H), 1.75-1.68 (m, 1H), 1.44 (s, 9H); $^{13}$C NMR (125 MHz, $CD_3OD$) 175.0, 156.7, 137.1, 114.6, 79.1, 52.9, 30.8, 29.7, 27.3; LRMS-FAB (*m/z*) 275 (35), 252 (16), 230 (17), 174 (100) 130 (65); HRMS-FAB (*m/z*) calcd for $C_{11}H_{20}N_1O_4$ ([M+H]$^+$) 230.1392, found 230.1336.

***m*-CPBA oxidation.**

**(*S*)-ethyl 2-*N*-(*tert*-butoxycarbonyl)-2-amino-5-hexenoate (*S*)-9.** (*S*)-**10** (1.31 g, 5.72 mmol) was dissolved in DMF (15 mL) and cooled with an ice-water bath. This solution was treated with triethylamine (1.20 mL, 8.58 mmol) and finally ethyl bromide (2.6 mL, 35 mmol). The resulting solution was then allowed to attain room temperature and continued to stir. After 24 h the solution was concentrated to get a crude mixture as an oil. This crude mixture was purified by silica gel column chromatography (hexane:EtOAc, 80:20, v/v) to give (*S*)-**9** (1.24 g, 84 %). $[\alpha]_D^{25}$ = + 8.53° (*c* = 2.46, $CH_2Cl_2$). [$^1$H NMR same as (*RS*)-**9**].

**ethyl 2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)butanoate, (2*S*,5*R*)-11 and (2*S*,5*S*)-11.** (*S*)-**9** (1.2 g, 4.67 mmol) was dissolved in DCM 20.0 mL and cooled to 0 °C with ice-water bath. To this solution *m*-CPBA (1.57 g, 7.0 mmol) was added in one portion and stirred. The ice-water bath was removed once the *m*-CPBA completely dissolved and allowed to stir at room temperature. After 24 h the crude reaction mixture was again cooled with in an ice-water bath, treated with 10% aqueous $Na_2SO_3$ (30 mL), then the solution was stirred vigorously to reduce the excess *m*-CPBA. After 2h the organic phase was separated and washed with 4% $NaHCO_3$, brine, dried ($MgSO_4$), filtered and evaporated to get a clear oil. This was purified by silica gel column chromatography (hexane:EtOAc, 70:30, v/v) to get pure **11** as a clear oil (1.15 g, 90 %). $^1$H NMR (500 MHz, $CDCl_3$) 5.10-5.05 ( m, 1H), 4.32 (m, 1H) 4.20 (q, 2H), 2.93 (m, 1H), 2.76 (m, 1H), 2.49 (m, 1H,) 1.99 (m, 1H) 1.8 (m, 1H), 1.68 (m, 1H) 1.51 (m, 1H), 1.45 (s, 9H), 1.29 (t, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) 172.5 (172.4), 155.4, 79.9, 61.5 (61.4), 53.2 (53.0), 51.6 (51.5), 47.1 (47.0), 29.2, 28.4, 28.3, 14.2(14.1); LRMS-FAB (*m/z*) 274 (42), 218 (100), 174 (49); HRMS-FAB (*m/z*) calcd for $C_{13}H_{24}N_1O_5$ ([M+H]$^+$) 274.1654, found 274.2659.

**Jacobsen's Hydrolytic Kinetic Resolution (HKR).**

**ethyl 2-[(*tert*-butoxycarbonyl) amino]-4-(2-oxiranyl)butanoate (2*S*,5*R*)-11 and ethyl 2-[(*tert*-butoxycarbonyl) amino]-5,6-dihydroxyhexanoate, (2*S*,5*S*)-12. 11** (1 g, 3.65 mmol) was taken in a vial equipped with a stir bar, to this was added (*RR*)-Co-Salen (0.5 mol %, 12 mg), followed by acetic acid (30 µL) and THF (0.3 mL). Finally $H_2O$ (2 mmol, 36 µL) was added in one portion at room temperature and stirred. After 48 h, the solution was directly purified by silica gel column chromatography, eluting the column by (EtOAc:hexane, 30:70, v/v) gave (2*S*,5*R*)-**11** (0.47 g, 47 %) as a clear oil. Further elution with (EtOAc:haxane:methanol, 60:40:2, v/v/v) gave (2*S*,5*S*)-**12** (0.49 g, 46 % ). $^1$H (500 MHz, $CDCl_3$) 5.25-5.14 (m, 0.66H), 4.36 (m, 0.73H), 4.21 (q, 2H), 3.75 (br s, 0.91H), 3.64 (br s, 0.96H), 3.46 (m, 0.98H), 2.03-1.93(m, 1.90H), 1.84-1.68 (m, 0.82H), 1.60-1.48 (m, 5H), 1.45, 1.44 (both s, total 9H) 1.29 (t, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) 173.0 (172.8), 155.8, 80.0 (79.9), 71.7 (71.5), 66.6, (66.5), 61.4, 53.4, 29.0, 28.7, 28.3, 14.1; LRMS-FAB (*m/z*) 356 (33), 354 (35), 300 (53), 298 (55), 256 (98), 254 (100); HRMS-FAB (*m/z*) calcd for $C_{13}H_{26}N_1O_6$ ($[M+H]^+$) 292.1760, found 292.1767.

**Synthesis of (2*S*,5*R*)-5-hydroxypipecolic acid derivatives.** (Scheme 4)

**ethyl 2-[(*tert*-butoxycarbonyl) amino]-5-hydroxy-6-bromohexanoate (2*S*,5*R*)-13. 11** (0.46 g, 1.71 mmol) was dissolved in THF (15 mL) and cooled to 0 °C. To this solution AcOH (0.30 mL, 5.25 mmol) was added followed by LiBr (0.24 g, 2.76 mmol). The solution was then warmed to room temperature and stirred. After 16h, when the TLC analysis showed the complete disappearance of the epoxide, the solution was concentrated in vacuum and the residue taken in EtOAc. Washed with 4% $NaHCO_3$, brine, dried( $MgSO_4$), filtered and evaporated to get crude product which was purified by silica gel column chromatography eluting with (EtOAc:hexane, 40:60 v/v) to get **13** (0.55 g, 92 %). $^1$H NMR (500 MHz, $CDCl_3$) 5.21 (bd, 1H), 4.37 (m, 1H), 4.21 (m, 2H), 3.82 (br s, 1H), 3.50-3.38 (m, 2H), 2.87 (bd, 1H), 2.05-1.91 (m, 1H), 1.86-1.54 (m, 3H), 1.45 (s, 9H), 1.29 (t, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$), 172.5, 155.7, 80.2, 70.8, 61.5, 53.1, 40.1, 30.6, 29.7, 28.3, 14.2; LRMS-FAB (*m/z*) 356 (33), 354 (35), 300 (53), 298 (55), 256 (98), 254 (100); HRMS-FAB (*m/z*) calcd for $C_{13}H_{25}N_1O_5Br_1$ ($[M+H]^+$) 354.0916, found 354.0881.

**1-*tert*-butyl 2-ethyl 5-hydroxypiperidine-1,2-dicarboxylate (2*S*,5*R*)-16**. **13** (0.26 g, 0.73 mmol) was dissolved in TFA (5 ml) and allowed to stand at room temperature. After 1 h when the reaction was complete (TLC) the excess TFA was evaporated. The crude product was redissolved in diethyl ether and evaporated to remove excess TFA, to get **14**.TFA as a white sticky oil.

**14**.TFA was dissolved in THF (5 mL) and to this DIEA (0.25 mL, 1.46 mmol) was added, and the resulting solution was stirred at room temperature. After 4h the solution was treated with additional DIEA (0.13 mL, 0.73 mmol) and $Boc_2O$ (0.32 g, 1.46 mmol). The resulting solution stirred at room temperature. After 12 h the solution was concentrated and the residue was dissolved in ethyl acetate and washed with 4% $NaHCO_3$, 10% citric acid, brine, dried ($MgSO_4$), filtered and evaporated to get an oil purified by silica gel column chromatography (EtOAc:hexane, 50:50, v/v) to get **16** as an oil (0.16 g, 80%). $^1$H (500 MHz, $CDCl_3$) 4.91, 4.74 (both br s, 1H), 4.20 (m, 2H), 4.08-3.95 (m, 2H), 3.24-3.10 (m, 1H), 2.17-2.00 (m, 2H), 1.89-1.76 (m, 2H), 1.47, 1.45 ( both s, total 9H); $^{13}$C (125 MHz, $CDCl_3$) 171.7 (171.5), 156.5 (156.2), 80.4, 61.1, 54.7 (53.5), 48.1 (47.1), 28.2, 27.2 (26.9), 20.3, 14.3; LRMS-FAB (*m/z*) 274 (57), 218 (100), 174 (100), 144 (50); HRMS-FAB (*m/z*) calcd. for $C_{13}H_{24}N_1O_5$ ($[M+H]^+$) 274.1654, found 274.1656.

**Synthesis of (2*S*,5*S*) 5-hydroxypipecolic acid derivatives.** (Scheme 5)

**ethyl 2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-tosylhexanoate (2*S*,5*S*)-17. 12** ( 0.45 g, 1.53 mmol) was dissolved in DCM (25.0 mL) and cooled to -10 °C, to this stirred solution triethylamine (0.25 mL, 1.8 mmol) followed by $Bu_2SnO$ (0.11 g, 0.46 mmol, 30 mol%) and DMAP (10 mg, cat.) were added. The solution was then stirred for 15 min at -10 °C finally TsCl (0.32 g, 1.68 mmol) was added portion wise over a period of 1 h. The resulting solution was stirred for an additional 1 h. At the end of 2h, the reaction was quenched with 4% $NaHCO_3$ and DCM was replaced with EtOAc. The EtOAc layer was washed with brine, dried($MgSO_4$), filtered and evaporated to give a crude mixture purified by silica gel column chromatography (EtOAc:hexane, 30:70 to 50:50, v/v) to give **17** (0.67 g, 97 %). $^1$H (500 MHz, $CDCl_3$) 7.80 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 5.10 (br d, 1H), 4.27 (m, 1H), 4.19 (m, 2H), 4.12 (m, 1H), 4.00 (m, 1H), 3.88 (m, 2H), 2.46 (s, 3H), 1.91-1.77 (m, 2H), 1.55-1.46 (m, 2H), 1.43 (s, 9H); $^{13}$C (125 MHz, $CDCl_3$) 172.5, 155.5, 145.1, 132.6, 130.0, 128.0, 80.0, 73.7, 68.8, 61.5, 53.0, 28.7, 28.3, 21.7, 14.2; LRMS-FAB (*m/z*) 446 (15), 390 (42), 347 (37), 346 (100); HRMS-FAB (*m/z*) calcd for $C_{20}H_{32}N_1O_8S_1$ ($[M+H]^+$) 446.1849, found 446.1864.

**ethyl 2-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-6-iodohexanoate (2*S*,5*S*)-18. 17** (0.55 g, 1.23 mmol) was dissloved in acetone (30 mL) and to this solution NaI (1 g, 6.67 mmol) was added and the resulting solution was refluxed for 8 h. After 8 h, the acetone was replaced with EtOAc and washed with sat.$Na_2S_2O_3$, brine, dried ($MgSO_4$), filtered and evaporated to give an oil purified by silica gel chromatography (EtOAc:hexane, 50:50, v/v) to give **18** (0.47 g, 95 %). $^1$H (500 MHz, $CDCl_3$) 5.11 (bd, 1H), 4.32 (m, 1H), 4.21 (m, 2H), 3.58 (m, 1H), 3.36 (m, 1H), 3.22 (m, 1H), 2.16 (bd, 1H), 1.93-1.92 (m, 1H), 1.82-1.80 (m, 1H), 1.70-1.66 (m,1H), 1.60-1.53 (m, 1H), 1.45 (s, 9H), 1.28 (m, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) 172.5, 155.5, 80.1, 70.3, 61.5, 53.1, 32.1, 29,1, 28.3, 15.8, 14.2; LRMS-FAB (*m/z*) 402 (35), 346 (69), 302 (100), 284 (37); HRMS-FAB (*m/z*) calcd for $C_{13}H_{25}N_1O_5I_1$ ($[M+H]^+$) 402.0777, found 402.0794.

**1-*tert*-butyl 2-ethyl 5-hydroxypiperidine-1,2-dicarboxylate (2*S*,5*S*)-19**. **18** (0.262 g, 0.65 mmol) was dissolved in TFA (4 ml) and allowed to stand at room temperature. After 1 h when the reaction was complete (TLC) the excess TFA was evaporated, the crude product was redissolved in diethyl ether and evaporated to remove excess TFA, to obtain Boc deprotected product as a salt of TFA, which was dissolved in THF (5 mL) and to this DIEA (0.23 mL, 1.30 mmol) was added, and the resulting solution was stirred at room temperature. After 4h when the reaction was complete which was confirmed by TLC, the solution was treated with additional DIEA (0.12 mL, 0.65 mmol) and $Boc_2O$ (0.28 g, 1.30 mmol) and the resulting solution stirred at room temperature. After 12 h the solution was concentrated and the residue was dissolved in ethyl acetate and washed with 4% $NaHCO_3$, 10% citric acid, brine, dried ($MgSO_4$), filtered and evaporated to get an oil purified by silica gel column chromatography (EtOAc:hexane, 50:50, v/v) to get **19** as an oil (0.13 g, 76 %). $^1$H NMR (500 MHz, $CDCl_3$) 4.87-4.58 (m, 1H), 4.28-4.04 (m, 3H), 3.71-3.58 (m, 1H), 2.82-2.64 (m, 1H), 2.35-2.19 (m, 1H) 2.04-1.94 (m, 1H), 1.78-1.67 (m, 1H), 1.62-1.53 (m, 1H), 1.47, 1.44 (both s, total 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) 171.5 (171.4), 155.5 (155.2), 80.5, 66.8 (66.7), 61.3, 54.0 (52.8), 48.5 (47.6), 30.5 (30.0), 28.3, 25.0 (24.8), 14.2; LRMS-FAB (*m/z*) 274 (70), 218 (60), 174 (100), 144 (45); HRMS-FAB (*m/z*) calcd for $C_{13}H_{24}N_1O_5$ ($[M+H]^+$) 274.1654, found 274.1658.

## 4. Conclusion

Starting from a common intermediate all the four isomers of 5-hydroxypipecolic acid (2*S*,5*R*)-, (2*S*,5*S*)-, (2*R*,5*S*)- and (2*R*,5*R*) were synthesized from commercially available malonate derivatives. Enatiomerically pure 2-amino-5-hexenoic acids were obtained by enzymatic resolution of the racemic

amino acid ester (α-chymotrypsin) or acetyl aminoacid (L-acylase). Oxidation of the unsaturated side chain of the enatiomerically pure L-2-amino-5-hexenoic acid with *m*-CPBA, generated the diastereomeric, inseparable epoxides. Co catalysed hydrolytic kinetic resolution converted the diastereomeric epoxide into separable components, each of them were correspondingly transformed into $C^6$ halo amino acids. Followed by, intramolecular nucleophilic attack of the amino -$NH_2$ group at $C^6$ carbon generating the respective 5-hydroxypipecolic acid diastereomers. Similarly starting from D-2-amino-5-hexenoic acid, two diastereomers of 5-hydroxypipecolic acid were synthesized. Ultimately synthesis of a single diastereomer was successfully achieved surpassing the difficulty of isolation of the *cis*- and *trans*- diastereomers, in a facile fashion. Application of these compound to synthesize biologically important cyclic tetra peptides are being investigated which will be reported in future.

## ■ Associated content

**Supporting Information**

Synthesis of (2*R*) isomers, $^1$H NMR and $^{13}$C NMR spectra.

## ■ Author information

**Corresponding Author**

*suvrathak@gmail.com

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