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Dipeptides Containing D-Serine or D-Isoserine From the Same (*R*)-Aziridine-2-imide by a Simple Reversal of the Synthetic Procedure

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Abstract. The ring expansion of 3-unsubstituted (*R*)-aziridine-2-imide-containing dipeptide under acidic conditions gives rise to oxazoline-5-imide regio and stereoselectively, which can be hydrolysed to the dipeptide containing D-isoserine. On the other hand, the ring opening of 3-unsubstituted (*R*)-aziridine-2-ester dipeptide, easily obtained from the same (*R*)-aziridine, gives the regioisomeric dipeptide containing D-serine. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords. Amino acids; aziridines; oxazolines; rearrangements; regiocontrol.

Introduction

Several natural oligopeptides containing β-hydroxy α-amino acid[1] or α-hydroxy β-amino acid[2] residues possess interesting biological properties and are gaining much interest as potential pharmacological compounds. The introduction of unusual or unnatural amino acid residues in the amino acid sequence could induce massive structural modifications that strongly influence the biological activity. In this paper we describe the synthesis of D-serine and D-isoserine-containing dipeptides through ring opening of aziridine-dipeptides,[3] prepared from the same (*R*)-aziridine, by changing the steps of the same synthetic plan. The asymmetric synthesis of optically pure D-serine[4] or D-isoserine[5] is very lengthy; moreover, hydroxy group protection-deprotection extra steps[6] are often necessary to introduce these residues in a peptidic sequence.

N-Activated aziridines are well known versatile amino acid precursors,[7] as they allow the synthesis of α or β-substituted amino acids by way of regio and stereoselective nucleophilic ring opening reactions in the presence of Lewis acids. Recently we reported that *N*-acyl 3-alkyl aziridine-2-imides are good β-hydroxy α-amino acid precursors[8] and that *N*-(α-amino acyl) 3-methyl aziridines are suitable precursors of threonine-containing dipeptides.[9] These preparations are based on a regioselective ring expansion of the three membered ring to an oxazoline.[10] The reaction spontaneously occurs in the presence of a catalytic amount of Lewis acid, affording the oxazoline with retention of configuration. Concerning the regiochemistry, 3-substituted aziridines afforded in our experiments exclusive attack at C3'.

Following the same strategy, the preparation of serine or isoserine-containing dipeptides starts from the synthesis of 3-unsubstituted aziridine-2-acid derivatives in enantiomerically pure form. Unfortunately, the synthetic procedures reported in the literature[11] often require many steps or, starting from natural serine, they give rise to (2*S*)-aziridines.

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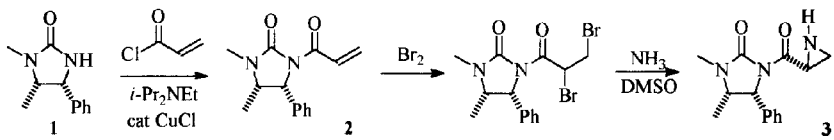
Results and Discussion

Recently we reported the preparation of optically pure 3-unsubstituted (2*R*)-aziridine-2-imide **3**[11f] by way of conjugate addition of *N*-BOC *O*-benzyl hydroxylamine to chiral acryloyl imide **2**. After BOC removal, cyclization gave **3** in good overall yield and 86/14 diastereoselectivity. Including hydroxylamine protection and deprotection, this procedure involved four steps. On the other hand, the straightforward diastereoselective Gabriel-Cromwell[12] reaction from **2** resulted in the synthesis of **3** in a very simple way with 80/20 diastereoselectivity (Scheme 1).

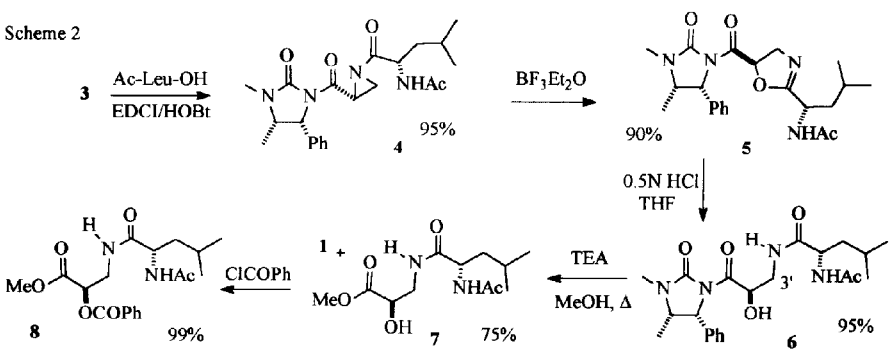
Scheme 1



The stereochemistry of the reaction is controlled by the (-)-(4*R*,5*S*)-1,5-dimethyl-4-phenylimidazolidin-2-one **1**[13] chiral auxiliary, which is also available in the opposite configuration. The acryloyl imide **2**[11f] was treated with bromine and the dibromo derivative so obtained was converted into an aziridine by bubbling ammonia in DMSO. The reaction gave a mixture of diastereoisomers in 80/20 ratio and the major *R* isomer **3**[11f] was isolated by flash cromatography.

The aziridine **3** was coupled with *N*-Ac leucine using EDCI/HOBt at 0°C in dichloromethane and DMF, giving in quantitative yield the aziridine-dipeptide **4** (Scheme 2). This compound fastly rearranged to oxazoline **5** upon treatment with boron trifluoride-Et_2O complex giving a single regio and stereoisomer. The final hydrolysis of **5** under acidic conditions afforded the leucine-isoserine dipeptide **6**.

Scheme 2



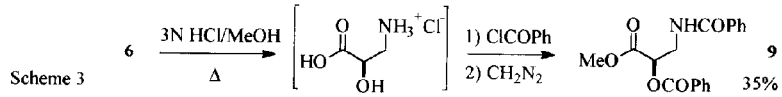
The regiochemistry of the ring expansion was determined by ^1H NMR decoupling experiments performed on **6**; indeed, it was observed that the HN peptidic proton couples with the two H3' protons. To remove and recover the chiral auxiliary, **6** was treated with lithium hydroxide or lithium hydroperoxide under the conditions

described by Evans for oxazolidin-2-ones,[14] but yields of free dipeptide were less than 20%. In an attempt to obtain the corresponding dipeptide methyl ester **7**, compound **6** was treated with methanol in the presence of boron trifluoride-Et_2O complex, giving **7** in poor yield. On the contrary, on refluxing **6** for 3h in methanol in the presence of an excess of triethylamine,[15] ester **7** was obtained in 75% yield, as revealed by ^1H NMR of the crude mixture. Extended reaction times gave an increasing amount of undetermined by-products arising from decomposition of **7**. The crude mixture containing **7**, unreacted **6** and **1** was treated with benzoyl chloride and triethylamine and the resulting **8** was purified by flash chromatography.

To definitively confirm the regiochemistry of **5**, **6**, **7**, and **8**, commercially available *rac*-isoserine methyl ester was coupled with *N*-Ac leucine under the above reported conditions, and the diastereomeric mixture so obtained showed a ^1H NMR which contained the ^1H NMR spectra of **7**.

The determination of the absolute stereochemistry of the isoserine moiety was obtained by refluxing **6** in 3N HCl/MeOH for 2h. These drastic conditions caused the complete cleavage of the chiral auxiliary and the formation of a mixture of dipeptide acid Ac-Leu-Isoser-OH, free isoserine and *N*-Ac leucine. After derivatization of the mixture by treatment with benzoyl chloride and diazomethane (Scheme 3), *N*, *O*-dibenzoyl isoserine methyl ester **9** was isolated and its absolute stereochemistry assumed (*R*) by comparison with a sample obtained by derivatization of optically pure (*S*)-isoserine.[16]

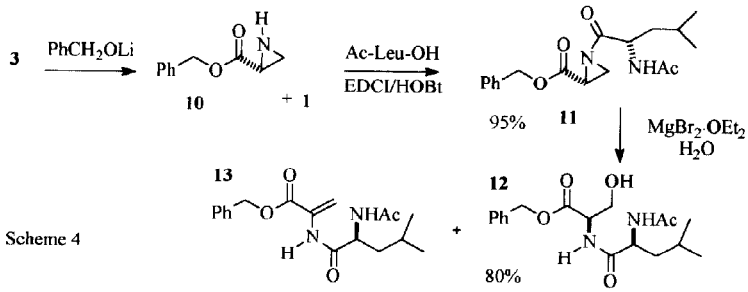
Scheme 3



In order to also prepare a dipeptide containing a D-serine residue, we decided to remove the chiral auxiliary in an earlier stage of the procedure, and to transform the aziridine-2-imide **3** into an aziridine-2-ester. Indeed, in our experience *N*-acyl aziridine-2-esters or amides undergo a slow ring expansion to oxazolines compared to *N*-acyl aziridine-2-imides.[8] For this reason, we anticipated that by treatment of an aziridine-2-ester dipeptide with 1 equivalent of water in the presence of a Lewis acid, a fast ring opening of aziridine in the less hindered C3 position could likely occur.[7c]

Aziridine **3** was transformed into benzyl ester **10**[11f] with lithium benzyloxide in THF, according to a procedure reported in the literature by us (Scheme 4). On the other hand, treatment of **3** with triethylamine in refluxing methanol[15] gave the corresponding methyl aziridine-2-carboxylic acid in 40% yield only.

Scheme 4



We performed the coupling of **10** and *N*-Ac leucine with EDCI/HOBt, and this reaction gave (*R*)-aziridine-2-ester dipeptide **11** in essentially quantitative yield. We unsuccessfully attempted to react **11** with traces of water in the presence of boron trifluoride-Et_2O complex. Finally, treatment of **11** with $MgBr_2{\cdot}Et_2O$ and 1.1 equivalents of water in THF gave D-serine-containing dipeptide **12**. The presence of a larger amount of water should be avoided, for we observed that under these conditions the Lewis acid promoted dehydration to benzyl 2-leucylamino acrylic acid **13**.

The regiochemistry was determined again with ^1H NMR decoupling experiments, which demonstrated that in this case the H2 proton couples with the HN proton. Concerning the stereochemistry of the serine residue, it is sufficient to mention that the ring opening of **11** occurs by cleavage of the C3-N bond, and that no epimerization is possible at this position.

Conclusion

Starting from the same optically pure 3'-unsubstituted aziridine imide **3** it is possible to obtain both a serine and an isoserine dipeptide with retention of the (*R*) configuration by varying the synthetic sequence. Indeed, the ring expansion and hydrolysis of (*R*)-aziridine-containing dipeptide **4** carrying the chiral auxiliary **1** gives a D-isoserine-containing dipeptide which can be transformed into the dipeptide Ac-Leu-D-Isoser-OMe by final removal of **1**. On the other hand, the removal of **1** at the beginning gives access to (*R*)-aziridine-2-ester-containing dipeptide **11** which is hydrolysed to the dipeptide Ac-Leu-D-Ser-OBzl

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Experimental Section

General methods. ^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz respectively in $CDCl_3$ with a Varian Gemini apparatus, and chemical shift are reported in ppm relative to the solvent peak. IR spectra were recorded with a FT-IR 210 Nicolet spectrometer. Optical rotations were determined with a Perkin-Elmer 241 polarimeter at room temperature. EI MS was obtained with a VG 7070 at 70 eV. TLC were performed on Merck fluoresent silica gel plates. Flash cromatography was performed at medium pressure with Merck silica gel 60 (230-400 mesh). THF was distilled from sodium benzophenone ketyl. CH_2Cl_2 was distilled from P_2O_5. DMSO and DMF were distilled from activated molecular sieves. Solvents for flash-chromatography were simply distilled. Reagents were purchased from Aldrich and used without purification.

(4*R*,5*S*,2'*R*)-1,5-Dimethyl-3-[(2'-aziridinyl)carbonyl]-4-phenylimidazolidin-2-one (3). *Gabriel-Cromwell method.* Bromine (0.32 mL, 6.2 mmol) is added to a solution of **2** (1.0 g, 4.1 mmol) in CH_2Cl_2 (20 mL) at 0°C with exclusion of light. After 6 h the reaction is quenched with 1M Na_2SO_3 (10 mL) and extracted three times with CH_2Cl_2. The collected organic layers are dried over Na_2SO_4 and the solvent is evaporated at reduced pressure, giving an oily residue. This dibromo derivative is used without further purification. It is dissolved in DMSO (5 mL) and ammonia is bubbled at 0°C until solvent saturation. After 1 h the solution is diluted with

EtOAc (50 mL), the organic layer is washed three times with small portions of water, and it is dried over Na_2SO_4. After evaporation of the solvent at reduced pressure, the residue is purified by flash chromatography (EtOAc:Cyclohexane 80:20) giving pure (2'*R*)-**3**[11f] (0.72 g, 68%) and its (2'*S*) diastereoisomer (0.18 g, 17%).

(4*R*,5*S*,2'*R*,2''*S*)-1,5-Dimethyl-3-{[1'-(2''-acetamido-4''-methylpentanoyl)-2'-aziridinyl]carbonyl}-4-phenylimidazolidin-2-one (4). A mixture of aziridine-2-imide **3** (0.36 g, 1.4 mmol), *N*-acetyl leucine (0.29 g, 1.7 mmol), EDCI (0.32 g, 1.7 mmol), triethylamine (0.39 mL, 2.8 mmol), and HOBt (0.23 g, 1.7 mmol) in CH_2Cl_2 (20 mL) and DMF (2 mL) is stirred under nitrogen at 0°C. After 6 h the reaction is diluted with Et_2O (40 mL) and washed with: sat. $NaHCO_3$ (5 mL); 1M HCl (5 mL); brine (5 mL). The organic layer is dried over Na_2SO_4, then solvent is removed at reduced pressure. The residue is purified by flash chromatography (EtOAc:Cyclohexane 50:50) giving **4** (0.55 g, 95%) as a waxy solid. IR (neat) ν 3200, 1743, 1680 cm^{-1}; ^1H NMR δ 0.76 (d, J = 6.2 Hz, 6H, CH_3), 0.82 (d, J = 6.6 Hz, 3H, CH_3), 1.38-1.70 (m, 2H, C*H*$_2$C*H*), 1.93 (s, 3H, $COCH_3$), 2.50 (dd, J = 1.6, 3.1 Hz, 1H, C*H*$_2$N), 2.63 (dd, J = 1.6, 6.0 Hz, 1H, C*H*$_2$N), 2.87 (s, 3H, NCH_3), 3.94 (dq, 1H, J = 6.6, 8.6 Hz, CH_3C*H*CHPh), 4.54 (dt, J = 4.8, 7.9 Hz, 1H, C*H*NAc), 4.94 (dd, J = 3.1, 6.0 Hz, 1H, COC*H*CH$_2$), 5.29 (d, J = 8.6 Hz, 1H, CH_3CHC*H*Ph), 6.06 (d, J = 7.9 Hz, 1H, NH), 7.05-7.39 (m, 5H, Ph); ^{13}C NMR δ 14.8, 21.8, 22.7, 24.6, 28.1, 30.9, 35.0, 41.4, 52.6, 52.8, 54.3, 59.5, 127.0, 128.1, 128.3, 128.4, 135.8, 155.2, 166.5, 169 8, 184.0; $[\alpha]^{20}_D$ = -27 (*c* 2.4 MeOH). Anal. Calcd for $C_{21}H_{30}N_4O_4$: H 7.51, C 62.67, N 13.9; found: H 7.50, C 62.69, N 13.8.

(5*R*,4'*R*,5'*S*,1''*S*)-4,5-dihydro-2-(1''-acetamido-3''-methyl-1''-butyl)-5-[(1',5'-dimethyl-4'-phenyl imidazolidin-2'-on-3'-yl)carbonyl]oxazole (5). A solution of **4** (0.55 g, 1.3 mmol) and boron trifluoride-Et_2O complex (0.17 mL, 1.3 mmol) is stirred in CH_2Cl_2 (10 mL) at r.t. under nitrogen for 2 h. Then sat. $NaHCO_3$ (5 mL) is added and the reaction is extracted three times with CH_2Cl_2. The organic layer is dried over Na_2SO_4 and solvent is removed at reduced pressure. After flash chromatography (EtOAc:Cyclohexane 30:70), **5** (0.49 g, 90%) is obtained as an oil. IR (neat) ν 3287, 3050, 1733, 1690, 1394, 1260 cm^{-1}; ^1H NMR δ 0.82 (d, J = 6.7 Hz, 3H, CH_3), 0.92 (d, J = 6.0 Hz, 6H, CH_3), 1.44-1.76 (m, 3H, C*H*$_2$C*H*), 1.98 (s, 3H, $COCH_3$), 2.84 (s, 3H, NCH_3), 3.84 (dd, J = 6.2, 14.6 Hz, 1H, C*H*$_2$N), 3.98 (dq, J = 6.7 Hz, 8.6 Hz, 1H, CH_3C*H*CHPh), 4.32 (dd, J = 10.8, 14.6 Hz, 1H, C*H*$_2$N), 4.81 (dt, J = 4.6, 9.0 Hz, 1H, CHNAc), 5.24 (d, J = 8.6 Hz, 1H, CH_3CHC*H*Ph), 6.02 (dd, J = 6.2, 10.8 Hz, 1H, C*H*CH$_2$N), 6.19 (d, J = 9.0 Hz, 1H, NH), 7.10-7.41 (m, 5H, Ph); ^{13}C NMR δ 14.8, 22.3, 22.6, 23.0, 24.7, 28.1, 41.8, 51.5, 54.5, 59.2, 59.8, 77.2, 127.1, 128.0, 128.3, 135.5, 154.5, 155.3, 165.4, 177.1; MS *m/z* 224 (19), 190 (80), 181 (3), 143 (25), 99 (30), 56 (100); $[\alpha]^{20}_D$ = -60 (*c* 1 MeOH). Anal. Calcd for $C_{21}H_{30}N_4O_4$: H 7.51, C 62.67, N 13.9; found: H 7.51, C 62.64, N 13.8.

(4*R*,5*S*,2'*R*,2''*S*)-1,5-Dimethyl-3-[3'-(2''-acetamido-4''-methylpentanoyl)amino-2'-hydroxypropionyl]-4-phenylimidazolidin-2-one (6). To a stirred solution of **5** (0.49 g, 1.2 mmol) in THF (10 mL), 0.5 M HCl (3 mL) is added at 0°C. After 1 h THF is evaporated at reduced pressure and $NaHCO_3$ is added until neutrality. The mixture is extracted three times with CH_2Cl_2, and the collected organic layers are dried over Na_2SO_4. Solvent is removed at reduced pressure affording **6** (0.47 g, 95%), without further purification. IR (neat) ν 3300, 3050, 1720, 1650, 1550, 1490 cm^{-1}; ^1H NMR δ 0.82 (d, J = 6.6 Hz, 3H, CH_3), 0.92 (d, J = 5.2 Hz, 6H, CH_3), 1.40-1.62 (m, 3H, C*H*$_2$CH), 1.96 (s, 3H, $COCH_3$), 2.86 (s, 3H, NCH_3), 3.47 (dt, J = 4.8, 9.5 Hz, 1H, NC*H*$_2$CH),

3.75-3.85 (m, 1H, NCH_2CH), 4.05 (dq, J = 6.6, 8.5 Hz, 1H, CH_3CHCHPh), 4.40 (dt, J = 5.7, 6.1 Hz, 1H, CHNAc), 5.17 (d, J = 8.5 Hz, 1H, CH_3CHCHPh), 5.16-5.23 (m, 1H, CHOH), 6.10 (d, J = 6.1 Hz, 1H, NHAc), 6.51-6.62 (m, 1H, NHCH_2), 7.10-7.41 (m, 5H, Ph); ^{13}C NMR δ 14.2, 22.0, 22.1, 22.4, 24.0, 28.3, 41.0, 51.3, 52.1, 54.0, 59.5, 59.9, 127.1, 128.4, 128.6, 136.0, 155.3, 167.0, 170.1, 170.2; $[\alpha]^{20}_D$ = -1.7 (*c* 1.6 $CHCl_3$). Anal. Calcd for $C_{21}H_{32}N_4O_5$: H 7.67, C 59.98, N 13.32; found: H 7.67, C 60.00, N 13.31.

Methyl (2*R*,2'*S*)-3-(2'-acetamido-4'-methylpentanoyl)amino-2-benzoyloxypropanoate (8). A mixture of **6** (0.24 g, 0.57 mmol), and triethylamine (0.31 mL, 2.3 mmol) in methanol (5 mL) is refluxed under nitrogen. After 3 h solvent is removed at reduced pressure. The residue, which contains **1**, **7** and unreacted **6**, is diluted with CH_2Cl_2 (5 mL), and stirred with ClCOPh (0.10 mL, 0.11 mmol) and cat. DMAP at 0°C for 2 h. The reaction is quenched with water (5 mL) and extracted three times with CH_2Cl_2. The organic layers are collected and dried over Na_2SO_4. Solvent is evaporated at reduced pressure and the residue is purified by flash chromatography (EtOAc:Cyclohexane 50:50, to EtOAc) giving sequentially **8** (0.12 g, 74%), reagent **6** (0.048 g, 20%) and **1** (0.088 g, 80%), $[\alpha]^{20}_D$ = -43.5 (*c* 1.5, MeOH); lit.[13] $[\alpha]^{20}_D$ = -44.5 (*c* 2, MeOH). **8**: IR (neat) ν 3310, 3064, 1742, 1719, 1645, 1550 cm^{-1}; ^{1}H NMR δ 0.81 (d, J = 6.0 Hz, 3H, CH_3), 0.85 (d, J = 6.0 Hz, 3H, CH_3), 1.40-1.63 (m, 3H, CH_2CH), 1.89 (s, 3H, $COCH_3$), 3.66 (dt, J = 4.5, 14.2 Hz, 1H, CHCH_2N), 3.74 (s, 3H, $COOCH_3$), 3.98 (dt, J = 6.9, 14.2 Hz, 1H, CHCH_2N), 4.50 (dt, J = 6.3, 8.4 Hz, 1H, CHNAc), 5.36 (dd, J = 4.5, 6.6 Hz, 1H, CHOCOPh), 6.63 (d, J = 8.4 Hz, 1H, AcNH), 7.38-7.44 (m, 3H, Ph); 7.57 (t, J = 7.3 Hz, 1H, NH), 8.06 (d, J = 7.0 Hz, 2H, Ph); ^{13}C NMR δ 22.3, 22.6, 22.9, 24.7, 39.9, 41.0, 51.6, 52.6, 71.2, 128.3, 128.8, 128.9, 133.4, 165.5, 168.5, 170.3, 172.7; $[\alpha]^{20}_D$ = -41.9 (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{19}H_{26}N_2O_6$: H 6.93, C 60.30, N 7.40; found: H 6.91, C 60.27, N 7.39.

(2*R*)-Methyl 3-benzoylamino-2-benzoyloxypropanoate (9). A mixture of **6** (0.24 g, 0.57 mmol) and 3N HCl (2 mL) in MeOH (5 mL) is refluxed for 2h. After evaporation of THF at reduced pressure, the mixture is extracted twice with EtOAc. The aqueous layer is neutralized with 2N NaOH. Acetone (5 mL) is added and the mixture is cooled at 0°C. Then K_2CO_3 (0.23 g, 1.7 mmol) and ClCOPh (0.20 mL, 1.7 mmol) are added and the reaction is stirred for 2 h. The organic solvent is evaporated at reduced pressure, pH is adjusted to 4 with 0.5 M HCl, and the mixture is extracted twice with EtOAc. The collected organic layers are dried over Na_2SO_4, solvent is evaporated at reduced pressure and the residue is treated with an excess of diazomethane solution in Et_2O. Solvent is evaporated at reduced pressure and the residue is purified by flash chromatography (EtOAc:Cyclohexane 30:70) affording isolated **9** (0.065 g, 35%) as an oil. IR (neat) ν 3330, 3064, 1757, 1719, 1645, 1539, 1272, 1117 cm^{-1}; ^{1}H NMR δ 3.81 (s, 3H, $COOCH_3$), 4.05-4.15 (m, 2H, CH_2N), 5.48 (t, J = 5.4 Hz, 1H, CHOCOPh), 6.65 (t, J = 5.7 Hz, 1H, NH), 7.30-7.60 (m, 6H, Ph), 7.75 (d, J = 8.0 Hz, 2H, Ph), 8.10 (d, J = 8.0 Hz, 2H, Ph); ^{13}C NMR δ 40.5, 52.7, 71.5, 127.0, 128.4, 128.5, 128.6, 128.9, 129.9, 131.7, 133.6, 165.7, 167.7, 169.0; $[\alpha]^{20}_D$ = -10.9 (*c* 0.5, $CHCl_3$); authentic sample of (*S*)-*N*, *O*-dibenzoyl isoserine methyl ester, from (*S*)-isoserine:[16] $[\alpha]^{20}_D$ = +9.4 (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{18}H_{17}NO_5$: H 5.28, C 66.05, N 4.28; found: H 5.27, C 66.02, N 4.28.

Benzyl (2*R*,2'*S*)-1-(2'-acetamido-4'-methylpentanoyl)aziridine-2-carboxylate (11). Under the same conditions reported for the synthesis of **4**, a mixture of **10** (0.18 g, 1.0 mmol), *N*-acetyl leucine (0.21 g, 1.2

mmol), EDCI (0.23 g, 1.2 mmol), triethylamine (0.28 mL, 2.0 mmol), and HOBt (0.16 g, 1.2 mmol) in CH_2Cl_2 (10 mL) and DMF (1 mL) is stirred for 6 h. After the above reported work up, the mixture is purified by flash chromatography (Et_2O:Cyclohexane 50:50) giving **11** (0.32 g, 95%) as an oil. IR (neat) 3190, 1743, 1690, 1670 cm^{-1}; ^1H NMR δ 0.90 (d, J = 6.0 Hz, 3H, CH_3), 0.91 (d, J = 6.0 Hz, 3H, CH_3), 1.60-1.80 (m, 3H, CH_2CH), 1.97 (s, 3H, $COCH_3$), 2.58 (d, J = 2.7 Hz, 1H, CH_2N), 2.63 (d, J = 5.7 Hz, 1H, CH_2N), 3.51 (dd, J = 2.7, 5.7 Hz, 1H, $CHCH_2N$), 4.51 (m, 1H, CHNAc), 5.22 (s, 2H, CH_2Ph), 5.89 (bs, 1H, NH), 7.33-7.41 (m, 5H, Ph); ^{13}C NMR δ 22.0, 22.7, 22.9, 24.8, 30.5, 34.6, 41.7, 52.8, 67.5, 128.4, 128.5, 128.6, 135.0, 167.8, 170.0, 183.6; MS *m/z* 241 (3), 177 (22), 156 (19), 128 (49), 91 (75), 86 (100); $[\alpha]^{20}_D$ = +35.0 (*c* 1.6, $CHCl_3$). Anal. Calcd for $C_{18}H_{24}N_2O_4$: H 7.28, C 65.04, N 8.43; found: H 7.29, C 65.02, N 8.41.

Benzyl (2*R*,2'*S*)-2-(2'-acetamido-4'-methylpentanoyl)amino-3-hydroxypropionate (12). A mixture of **11** (0.32 g, 0.95 mmol), $MgBr_2$-Et_2O complex (0.049 g, 0.19 mmol), and water (0.018 mL, 0.95 mmol) in THF (10 mL) was stirred for 4 h at 0°C. Solvent was evaporated at reduced pressure, the residue was diluted in CCl_4 (5 mL), and the suspension was filtered on celite. Compound **12** (0.26 g, 80%) was precipitated from the filtrate by adding cold pentane. The mother liquor was evaporated at reduced pressure and the residue was analyzed by ^1H NMR, revealing a 1:1 mixture of **12** and **13**. An alternative purification by flash chromatography (Et_2O:Cyclohexane 40:60) was also attempted, but **13** (0.30 g, 94%) was obtained as the unique product.

12: IR (neat) 3320 br, 3050, 1735, 1680, 1660 cm^{-1}; ^1H NMR δ 0.95 (d, J = 6.0 Hz, 6H, CH_3), 1.55-1.80 (m, 3H, CH_2CH), 1.98 (s, 3H, $COCH_3$), 3.74 (dd, J = 3.6, 10.8 Hz, 1H, CH_2OH), 3.81 (dd, J = 3.6, 10.8 Hz, 1H, CH_2OH), 4.50-4.60 (m, 1H, CHNAc), 4.99 (dt, J = 3.6, 7.5 Hz, 1H, CHN), 5.17 (d, J = 12.3 Hz, 1H, CH_2Ph), 5.24 (d, J = 12.3 Hz, 1H, CH_2Ph), 6.00 (bs, 1H, NHAc), 7.20 (d, J = 7.5 Hz, 1H, NH), 7.35-7.50 (m, 5H, Ph); ^{13}C NMR δ 21.8, 22.0, 22.8, 24.2, 32.3, 40.4, 51.2, 52.5, 67.7, 127.8, 128.0, 128.1, 134.0, 168.0, 171.1, 174.1; MS *m/z* 332 (4), 177 (20), 128 (38) 91 (90), 86 (100); $[\alpha]^{20}_D$ = -47.3 (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{18}H_{26}N_2O_5$: H 7.48, C 61.70, N 7.99; found: H 7.47, C 61.73, N 8.00.

13: IR (neat) 3050, 1730, 1695, 1660 cm^{-1}; ^1H NMR δ 0.96 (d, J = 6.0 Hz, 3H, CH_3), 0.98 (d, J = 6.0 Hz, 3H, CH_3), 1.42-1.61 (m, 3H, CH_2CH), 2.02 (s, 3H, $COCH_3$), 4.54 (dt, J = 5.4, 8.1 Hz, 1H, CHNAc), 5.28 (s, 2H, CH_2Ph), 5.91 (d, J = 8.1 Hz, 1H, NHAc), 5.92 (s, 1H, =CH), 6.59 (s, 1H, =CH), 7.25-7.45 (m, 5H, Ph), 8.35 (bs, 1H, NH); ^{13}C NMR δ 21.7, 22.5, 22.6, 24.5, 40.5, 52.2, 67.4, 109.4, 127.8, 128.1, 130.5, 134.6, 163.1, 170.0, 170.2; MS *m/z* 332 (M^+, 1), 276 (4), 241 (5), 177 (16), 156 (13), 128 (46) 91 (79), 86 (100); $[\alpha]^{20}_D$ = -43.9 (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{18}H_{24}N_2O_4$: H 7.28, C 65.04, N 8.43; found: H 7.28, C 65.01, N 8.42.

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