A Stereoselective Synthesis of (2*R*,3*S*)-*N*-Benzoylphenylisoserine Methyl Ester

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β-Amino α-hydroxy acids are important components in a variety of biologically interesting compounds.[1] For instance, it has recently been found that some inhibitors of angiotensin-converting enzyme (ACE), such as microginin, KR1 1314, and KR1 1230,[2] contain β-amino α-hydroxy acids in their structure. These molecules are useful agents in hypertension and congestive heart failure therapy. Other biologically active molecules containing this kind of structure are KN1-272 and KN1-227 tripeptides that are highly potent HIV-1 protease inhibitors and are promising candidates as selective anti-AIDS agents.[3]

The best known molecule containing a β-amino α-hydroxy acid is Taxol (Figure 1), a complex diterpene isolated from the bark of *Taxus brevifolia*. It is considered one of the most active agents in cancer chemotherapy.[4]

Though the role of the (2*R*,3*S*)-phenylisoserine side chain has not yet been fully determined, this part of the molecule seems to be fundamental in the explication of its antitumor activity. In fact, many analogues have recently been synthesized. Unfortunately, however, modifications in the side chain rarely produce advantages; they often lead to either a considerable reduction or a total loss of activity.[5]

We will now report a two-step method for the synthesis of syn β-amino α-hydroxy acids applied to the preparation of (2*R*,3*S*)-phenylisoserine methyl ester moiety[6] based on anti halogenation of the dianion of the methyl (3*R*)-*N*-benzoyl-3-amino-3-phenylpropanoate.

The simplicity of the method and the product's optical purity make it comparable to synthetic procedures previously reported in the literature.

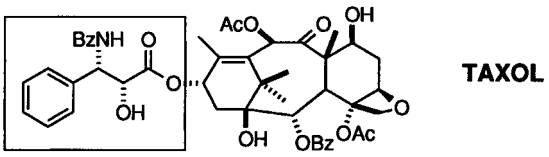

Figure 1.

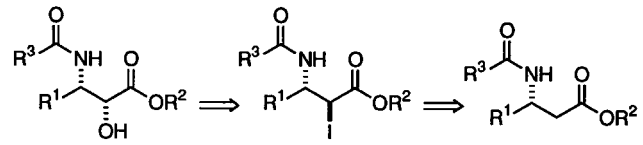

Figure 2.

Seebach and Estermann have previously demonstrated that *N*-acyl-β-amino esters can be alkylated with high stereoselectivity by quenching the lithium dianion with a range of alkylating agents.[7] Further, we performed a similar reaction and observed that the anti product is exclusively formed when the alkylating agent is sufficiently bulky.[8] On the basis of these results, we set up a new strategy that allowed us to transform enantiomerically pure β-amino acids into the corresponding 2,3-anti halo derivatives (successively submitted to S_N2 displacement) to give syn β-amino α-hydroxy derivatives (Figure 2). This synthetic procedure was applied to the preparation of the (2*R*,3*S*)-*N*-benzoylphenylisoserine methyl ester—the Taxol side-chain.

Enantiomerically pure (−)-(*R*)-3-amino-3-phenylpropanoic acid was obtained by enzymatic kinetic resolution of the corresponding *N*-phenylacetyl derivative with penicillin G acylase (PGA) from *Escherichia coli*, immobilized on Eupergit.[9] In fact, it is known that PGA exhibits a high affinity to the phenylacetyl moiety, preferentially hydrolyzing the L configuration of α-, β-, or γ-amino acids at pH 7.[8,10]

The racemic substrate was prepared by reaction of benzaldehyde with malonic acid and ammonium acetate and was isolated as a white precipitate, by filtration, without any further purification.[11] The transformation of the β-amino acid *rac*-**1** into its *N*-phenylacetamide was performed under the Schotten-Baumann conditions, and it afforded the desired product *rac*-**2** in 75% yield (Scheme 1).[10d]

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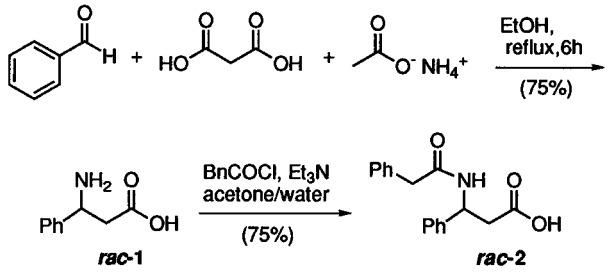
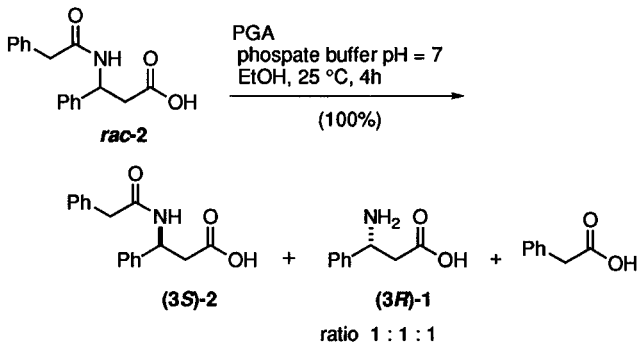
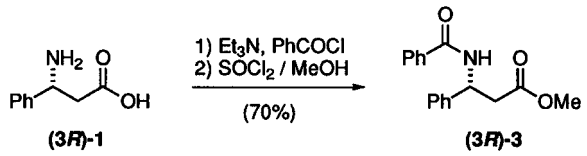
Scheme 1



Scheme 2



Scheme 3

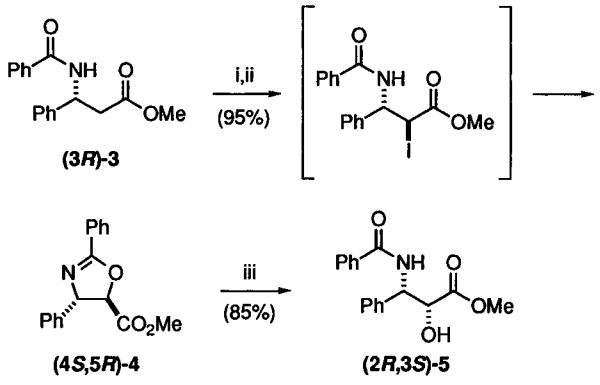


The enzymatic resolution of *N*-(phenylacetyl)-3-amino-3-phenylpropanoic acid (**2**) was performed by dissolving the compound in a pH 7 solution of ethanol and phosphate buffer and by adding PGA to the mixture. The (*R*)-*N*-(phenylacetyl)amide was completely hydrolyzed after 4 h at room temperature, while the unreacted *S* enantiomer was recovered by extraction with ethyl acetate as a 1:1 mixture with phenylacetic acid. This ratio was determined by comparing the methylene peak of (3*S*)-**2** with phenylacetic acid in the ^{1}H NMR spectrum[8] (Scheme 2).

(3*R*)-3-Amino-3-phenylpropanoic acid (**1**) was obtained enantiomerically pure. The enantiomeric excess was established by converting the β-amino acid into the corresponding *N*-benzoyl methyl ester, (3*R*)-**3**, which was analyzed by chiral HPLC chromatography, in comparison with a mixture of *rac*-**3** (estimated error is about ±1%). (3*R*)-**3** was obtained from (3*R*)-**1** by reaction with Et_3N and benzoyl chloride under the Schotten−Baumann conditions and was successively transformed into the corresponding methyl ester by adding it to a solution of $SOCl_2$ and methanol (Scheme 3).

The lithium dianion of (3*R*)-**3** was formed by reaction with LiHMDS (2.2 equiv) in THF at 0 °C and was treated with iodine (2.4 equiv) at −60 °C for 1h. The reaction was then quenched with NH_4Cl, and ethyl acetate was added. The mixture was washed with a saturated solution of $Na_2S_2O_3$. After the usual workup, the oxazoline **4** was obtained in a 95% yield with a 98:2 diastereomeric ratio. If the reaction is performed at −30 °C, the competitive formation of the corresponding aziridine will be observed, whereas keeping the temperature below −60 °C will result in an incomplete conversion of the

Scheme 4[a]



[a] Reagents and conditions: (i) LiHMDS (1 M solution in THF, 2.2 equiv), 0 °C, 1 h; (ii) I_2 (2,4 equiv), −60 °C, 1 h; (iii) 1 M HCl/methanol 1:3, reflux, 5 h.

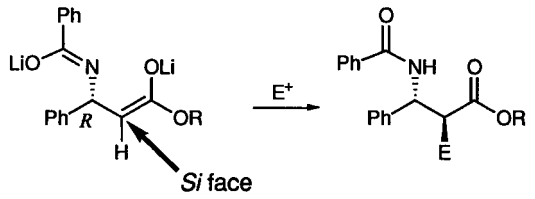


Figure 3.

iodointermediate to oxazoline. The trans configuration of compound **4** was assigned on the basis of H_4 and H_5 ($J_{H4,H5}$ = 6.4 Hz) value of the ^{1}H NMR coupling constant, compared with known compounds.[6d] The oxazoline **4** was then dissolved in 1 N HCl/MeOH, and the mixture was refluxed for 5 h to obtain (2*R*,3*S*)-*N*-benzoylphenylisoserine methyl ester **5** in 85% yield (Scheme 4).

Though the nature of diastereoselectivity in the reaction of dianion with an electrophile varies, the high diastereoselectivity of syn or anti α-hydroxy β-amino acids can be preferentially obtained by monitoring the reaction conditions.

Direct hydroxylation of the enolate dianion has been reported by two research groups. Davis and Reddy[12] reported that the reaction of the lithium dianion generated in the presence of LDA and LiCl with (+)-(camphorsulfonyl)oxaziridine afforded **5** in a 86:14 syn/anti diastereomeric ratio. Similar results have been obtained by Hanessian[6c] using KHMDS as metalling agent with either complex MoO_5−pyridine−HMPT (MoOPH) or (±)-*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine as electrophile. By contrast, if LiHMDS is used as metallating agent, the reaction provides a mixture of syn and anti α-hydroxy β-amino acids in a 10:90 diastereomeric ratio. To rationalize the predominant syn selectivity, the authors proposed an intramolecular eight-membered chelate dianion that does not account for the results obtained when the LiHMDS was used. In our case, the working model is the one proposed by Seebach,[13] whose main product originates from the addition of the I^+ to the *Si* face enolate (Figure 3).

In conclusion, we have shown a short and facile synthesis of (2*R*,3*S*)-*N*-benzoylphenylisoserine methyl

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ester. The key step is the iodination reaction of the lithium dianion of methyl (3*R*)-*N*-benzoyl-3-amino-3-phenylpropanoate, which directly affords the corresponding trans oxazoline with high yield and diastereoselectivity. The enantiomerically pure *β*-amino acid was obtained by enzymatic kinetic resolution of the *N*-(phenylacetyl)amide of the racemate with PGA.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 300 or 200 MHz. ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts are reported in ppm relative to the solvent peak of $CHCl_3$, defined to be δ 7.27 ppm. Infrared spectra were recorded with an FT-IR spectrometer. Melting points were determined in open capillaries and are uncorrected. HPLC analyses were performed utilizing a CHIRALCEL OD-H column (cellulose derivative coated on 5 μm silica gel as packing material, column size 0.46 cm i.d. × 25 cm, flow rate 0.5 mL/min, maximum column pressure 50 kg/cm^2, operating temperature 0−40 °C). Flash chromatography was performed with Merck silica gel 60 (230−400 mesh). THF was distilled from sodium benzophenone ketyl.

(±)-3-Amino-3-phenylpropanoic Acid (1). Benzaldehyde (20 mmol, 2 mL), malonic acid (20 mmol, 2.08 g), and ammonium acetate (40 mmol, 3.08 g) were refluxed in ethanol (30 mL) for 6 h. The reaction mixture was decanted overnight. The amino acid **1** precipitated from the reaction mixture as a white solid and was isolated by filtration in 75% yield: mp 216−218 °C dec (lit.[10d] mp 218−219 °C dec); ^{1}H NMR (D_2O) δ 2.70 (ABX, 2H, J = 6.7, 7.9, 16.2 Hz), 4.47 (dd, 1H, J = 6.7, 7.9 Hz), 7.28 (m, 5H); ^{13}C NMR (D_2O) δ 30.8, 52.2, 126.5, 128.8, 128.9, 135.1, 176.5. Anal. Calcd for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.39; H, 6.84; N, 8.46.

(±)-*N*-(Phenylacetyl)-3-amino-3-phenylpropanoic Acid (2). Phenylacetyl chloride (13 mmol, 1.72 mL) in acetone (5 mL) was added dropwise to a stirred solution of (±)-3-amino-3-phenylpropanoic acid (**1**) (10 mmol, 1.65 g) and triethylamine (24 mmol, 3.35 mL) in water (15 mL) and acetone (5 mL) at −5 °C. The mixture was stirred for 2 h at −5 °C and then for 3 h at room temperature. After the precipitate of triethylammonium chloride was filtered off, the acetone was removed under reduced pressure and the residue was extracted twice with ethyl acetate. Then 2 M HCl was added to the aqueous layer until pH 2 was reached, and the solution was extracted with ethyl acetate, dried over Na_2SO_4, and concentrated to give 2.1 g of compound **2** (75% yield): mp = 138−140 °C (lit.[10d] mp 134−140 °C); IR (Nujol) 3278, 1702, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.83 (ABX, 2H, J = 5.8, 6.0, 16.1 Hz), 3.61 (s, 2H), 5.41 (m, 1H), 6.53 (d, 1H, J = 8.5 Hz), 7.30 (m, 10H), 9.20−9.70 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 39.4, 43.8, 49.3, 126.1, 127.5, 127.7, 128.8, 129.0, 129.4, 133.7, 139.8, 170.4, 176.0; HRMS calcd for (M^+) 283.120 843 6, found 283.120 339 3. Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94. Found: C, 72.12; H, 6.01; N, 4.97.

Enzymatic Hydrolysis of (±)-*N*-(Phenylacetyl)-3-amino-3-phenylpropanoic Acid (2). PGA immobilized on Eupergit (200 mg) was added to a pH 7 solution of the amide acid **2** (7.5 mmol, 2.12 g) in 1 M phospate buffer (50 mL) and ethanol (7 mL). The reaction mixture was allowed to stir at room temperature for 4 h. Then, after the immobilized enzyme was filtered off, the ethanol was removed under reduced pressure and 2 M HCl was added to the aqueous solution until pH 3. The mixture was extracted twice with ethyl acetate, dried over Na_2SO_4, and concentrated to recover phenylacetic acid and the unhydrolyzed amide (1.51 g, 100% yield, 3.75 mmol of (3*R*)-**2** and 3.75 mmol of phenylacetic acid). The aqueous layer concentrated gave (*S*)-3-amino-3-phenylpropanoic acid as hydrochloride. The amino acid hydrochloride was used in the following step without further purification.

(3*S*)-Methyl *N*-Benzoyl-3-amino-3-phenylpropanoate (3). Benzoyl chloride (4.88 mmol, 0.57 mL) in acetone (5 mL) was added dropwise to a stirred solution of the recovered (*S*)-3-amino-3-phenylpropanoic acid hydrochloride (**1**) (3.75 mmol, 0.62 g) and triethylamine (13 mmol, 1.81 mL) in water (15 mL) and acetone (5 mL) at −5 °C. The mixture was stirred for 2 h at −5 °C and for 3 h at room temperature. After the precipitate of triethylammonium chloride was filtered off, the acetone was removed under reduced pressure and the residue was extracted twice with ethyl acetate. Then 2 M HCl was added to the aqueous layer until pH 2 was reached, and the solution was extracted with ethyl acetate, dried over Na_2SO_4, and concentrated to give 0.75 g of *N*-benzoyl derivative. A solution of $SOCl_2$ (5.6 mmol, 0.4 mL) in methanol (50 mL) was stirred for 2 h at −15 °C, and then the previously obtained *N*-benzoyl amide was added in one portion. The mixture was gradually warmed to room temperature overnight. The solution was concentrated under reduced pressure, and compound **3** was obtained in 67% overall yield (0.71 g, 2.5 mmol) after flash chromatography (cyclohexane/ethyl acetate 6:4 as eluant): mp = 108−112 °C; IR (Nujol) 3358, 1720, 1649 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.00 (ABX, 2H, J = 5.5, 5.7, 15.9 Hz), 3.63 (s, 3H), 5.65 (ddd, 1H, J = 5.5, 5.7, 8.4 Hz), 7.25 (m, 10H), 7.82 (d, 1H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 39.6, 49.7, 51.8, 126.2, 127.0, 127.6, 128.5 128.6, 131.5, 134.1, 140.5, 166.5, 172.0; $[\alpha]_D$ = −17.9 (*c* 4.52, $CHCl_3$); HRMS calcd for (M^+) 283.120 843 6, found 283.121 449 2.

(4*S*,5*R*)-2,4-Diphenyl-5-(methoxycarbonyl)-2-oxazoline (4). To a stirred solution of ester **3** (2.5 mmol, 0.7 g) in dry THF (10 mL) was added LiHMDS (5.5 mmol, 5.5 mL, 1 M solution in THF) in one portion under argon at 0 °C. After 1 h, the mixture was cooled to −60 °C, and a solution of iodine (6 mmol, 1.52 g) in THF (5 mL) was added dropwise. The reaction was quenched after 1 h with an aqueous saturated solution of NH_4Cl (10 mL), and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed twice with a solution of $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated. Compound **4** was obtained as an oil in 93% yield (0.65 g, 2.32 mmol) after flash chromatography on silica gel (cyclohexane/ethyl acetate 9:1 as eluant): IR (film) 1760, 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H), 4.93 (d, 1H, J = 6.5 Hz), 5.47 (d, 1H, J = 6.5 Hz), 7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 52.6, 74.5, 83.0, 126.3, 126.4, 126.6, 128.3, 128.6, 128.7, 131.8, 141.0, 163.8, 170.4; $[\alpha]_D$ = +14.0 (*c* 0.8, $CHCl_3$) (lit.[6d] $[\alpha]_D$ = +13.0 (c 1, $CHCl_3$)); HRMS calcd for (M^+) 281.105 193 5, found 281.106 003 3.

(2*S*,3*R*)-*N*-Benzoyl-3-phenylisoserine Methyl Ester (5). A solution of compound **4** (2.3 mmol, 0.65 g) in methanol (15 mL) and 1 N HCl (5 mL) was refluxed for 5 h. After the solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 and washed twice with water. The organic layer was dried over Na_2SO_4 and concentrated. Compound **5** was obtained pure in 85% yield (0.65 g) after flash chromatography on silica gel (cyclohexane/ethyl acetate 8:2 as eluant): mp = 182−184 °C (lit.[6e] mp = 184−185 °C); IR (Nujol) 1733, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.40 (bs, 1H), 3.84 (s, 3H), 4.64 (d, 1H, J = 2.0 Hz), 5.75 (dd, 1H, J = 2.0, 9.1 Hz), 7.03 (d, 1H, J = 9.1 Hz), 7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 53.3, 54.8, 73.2, 126.9, 127.1, 127.9, 128.5, 128.6, 131.8, 138.7, 166.3, 173.4; $[\alpha]_D$ = −47.6 (*c* 0.6, CH_3OH) (lit.[6e] $[\alpha]_D$ = −48 (*c* 0.92, CH_3OH)). Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68. Found: C, 68.30; H, 5.69; N, 4.74.

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