Stereoselective synthesis of α-hydroxy-β-amino acids: the chiral pool approach

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Abstract: A method for the stereoselective homologation of α-amino acids into syn-α-hydroxy-β-amino acids is described, based on the conversion of stereoisomeric cyanohydrins into trans-oxazolines. The synthetic potential of the method is illustrated in the enantioselective formal synthesis of Bestatin.

Keywords: amino acids, cyanohydrins, bestatin, AHPA, oxazoline.

INTRODUCTION

Within a large family of β-amino acids,1 α-hydroxy-β-amino acids occupy an especially important place.2 These compounds are known as constituents of several clinically approved drugs and natural products that show important biological activities: Taxol3 and Taxotere4 (antimitotics), Bestatin (Ubenimex, immunostimulant),5 KRI-1314 (a renin inhibitor),6 Microginin (an ACE inhibitor),7 KNI 272 and R-87366 (HIV-protease inhibitors),8 dideoxykanamycin A (antibacterial),9 to name just a few. Therefore, this class of compounds has attracted considerable interest of organic chemists and numerous methods for their enantioselective synthesis have been devised. However, room still exists for the improvement of the existing methodology.

RESULTS AND DISCUSSION

We sought to develop a semisynthetic approach to α-hydroxy-β-amino acids based on the homologation of α-amino acids. The ready availability and low cost of the optically pure α-amino acids recommend them as a suitable chiral pool for the targeted transformation.10 A one carbon homologation of α-amino acids has

* Dedicated to Professor Živorad Čeković, with the sentiments of gratitude and respect, on the occasion of his 70th birthday.

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been performed via the corresponding amino aldehydes,\textsuperscript{11} using the addition of vinylmetals\textsuperscript{12} or of the acetylide anion\textsuperscript{13} as latent carboxylic groups, followed by subsequent oxidative deprotection. Amino aldehydes have also been homologated by the cyanohydrin reaction,\textsuperscript{14} where trimethylsilyl cyanide,\textsuperscript{15} tributylstannyl cyanide,\textsuperscript{16} with or without added Lewis acids, as well as diethylaluminum cyanide (Nagata’s reagent),\textsuperscript{17} are the most often used reagents for this reaction. The stereoselectivity of the reaction varies and usually a careful choice of reagents and reaction conditions is needed in order to achieve good diastereoselectivity. In our approach, we considered performing the cyanohydrin reaction under operationally simple conditions, with cheap and environmentally the least harmful sodium cyanide. Such a reaction would not be expected to be diastereoselective; therefore, an additional means was needed in order to secure stereocontrolled product formation.

Some time ago, we found that chloroester 1-\textit{syn} can be transformed into \textit{trans}-oxazoline 2 on treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),
in a smooth reaction that formally represents an intramolecular $S_N2$ substitution with the retention of configuration (Scheme 1). No equilibrium was established between the cis- and trans-oxazolines under the reaction conditions, which ruled-out the intermediacy of the former isomer. Performing the reaction in a NMR tube, with spectral monitoring, revealed that the addition of DBU causes rapid epimerization of 1-syn, with the formation of an equimolar mixture of 1-syn and 1-anti; during the course of the reaction, the concentrations of both epimers decrease proportionally to the increase in the concentration of the trans-oxazoline product 2. No other intermediates were detected in the reaction mixture, which indicated the mechanism represented in Scheme 1, where the rapidly equilibrating mixture of isomers is funneled into a single product, through the application of the Curtin-Hammett principle.

Subsequently, it was found by others and us that a similar transformation is also feasible when the more easily available mesylates are substituted for the chlorides. This allowed us to conceive a process for the stereoselective conversion of $\alpha$-amino acids 4 into trans-oxazolines – the synthetic equivalents of $\alpha$-hydroxy-$\beta$-amino acids – as delineated in Scheme 2. After the addition of cyanide to amido aldehyde 5, which is expected to be stereorandom, the mixture of stereoisomeric cyanohydrins 6 would be first converted into the corresponding esters 7 and then mesylated. Treatment of 8 with DBU should bring about the rectification of stereochemistry at C-2 in 9, obviating the need for a tedious chromatographic separation of diastereoisomers. However, this reaction sequence hides a potential pitfall: the easy racemization of the amido aldehyde 5, both under the conditions of

Scheme 2.
oxidation and nucleophilic addition. Indeed, oxidations of α-amino alcohols, as well as nucleophilic additions to α-amino aldehydes, were successfully performed when the amino group was protected as a tertiary amine or carbamate, while the α-amido aldehydes are known to be very sensitive towards racemization.

We decided to test the feasibility of this reaction sequence by applying it in the synthesis of (2S,3R)-3-amino-2-hydroxy-4-phenylbutanoic acid (also known as allophenylnorstatine; abbreviated as (2S,3R)-AHPA, (2S,3R)-AHPBA, or Apns). This important acid is a constituent of several aminopeptidase inhibitors: Bestatin, Phebestin and Probestin (Fig. 1). The suitable starting compound for the synthesis would be D-phenylalanine.

The synthesis, represented in Scheme 3, started with the reduction of D-phenylalanine with lithium aluminum hydride, followed by in situ benzoylation to give the known α-amido alcohol. The oxidation/cyanide addition sequence was tried with several reagents, the efficiencies of which were compared in terms of the product yield and optical purity. To determine the optical purity of the product cyanhydrin, this compound was reduced with sodium borohydride back into the starting alcohol, the optical rotation of which was compared to the original values. The combination of the Swern oxidation and cyanide addition using potassium cyanide in the presence of benzyltriethylammonium chloride (TEBA) afforded cyanohydrin in 66 % yield. Quenching the reaction mixture with benzoyl chloride afforded a somewhat higher yield of the corresponding benzoate (74 %), but this compound turned out to be less suitable for the subsequent transformations. 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) mediated oxidation, followed by potassium cyanide/TEBA treatment, gave in 77 % yield. The attractive feature of this method is its operational simplicity. However, the optical purity of was unsat-
is satisfactory (58 % ee). With the presumption that partial racemization may have occurred in the second step, under the influence of the relatively basic “naked” cyanide anion, the experimental procedure was modified in that the cyanohydrin reaction was accomplished with in situ generated hydrogen cyanide. Gratifyingly, 14 was obtained in almost quantitative yield, with an optical purity exceeding 93 %. Methanolysis of 14 produced a mixture of the desired ester 15 and the corresponding acid, which was converted into 15 with diazomethane (86 % from 14). The mesylation of 15 and the oxazoline formation were envisaged to be accomplished as a one pot transformation. Treatment of 15 with mesyl chloride, in the presence of triethylamine, led to almost instantaneous formation of mesylate 16. Addition of DBU to the reaction mixture then promoted the isomerization/cyclization sequence which yielded the trans-oxazoline 17 in 70 % yield (accompanied with 1.7 % of the cis-oxazoline). Hydrolysis of 17 was accomplished with refluxing hydrochloric acid, to give (2S,3R)-AHPA in 88 % yield. The optical rotation of the product ([α]_D = +29.3°) corresponded well with the literature value for the optically pure compound ([α]_D = +27.4°). Thus, starting from D-phenylalanine, the synthesis of (2S,3R)-AHPA was accomplished in 7 steps and in 48 % overall yield.

The successful accomplishment of the above synthesis demonstrated that at least some α-amido aldehydes can survive the oxidation/addition sequence without isomerization. However, the proclivity of these compounds towards racemization is known to vary and to be structure dependent. To test the generality of

![Scheme 3.](image-url)
the procedure, several other α-amido alcohols were prepared from the corresponding α-amino acids, and then submitted to the oxidation/cyanide addition sequence. The optical purity of the α-amido aldehydes 20 and cyanohydrins 21 was determined by their reduction with sodium borohydride into the starting alcohols 19 and comparison of the optical rotation values of the original sample and the reduction product. The results of these experiments are represented in Scheme 4. In the case of valinol 19a, the oxidation with TEMPO/sodium hypochlorite, followed by the addition of a cyanide (potassium cyanide/TEBA), afforded the optically pure cyanohydrin 21a. With leucinol 19b the situation was more complicated. TEMPO-mediated oxidation of 19b afforded the optically enriched aldehyde 20b with 68 % ee. Recently, it was shown that sensitive α-amido aldehydes can racemize under the reaction conditions employed for the TEMPO-mediated oxidation. It was suggested that the highest level of optical purity of the α-amido aldehyde products can be attained when Dess-Martin periodinane (DMP) is used as the oxidant, under modified experimental conditions. Indeed, when the oxidation of 19b was performed according to this procedure, the optical purity of 20b was 96 % ee! However, the cyanohydrin 21b was obtained with a low degree of optical purity (64 %), indicating that 20b isomerized under the experimental conditions, even though the cyanohydrin reaction was effected with hydrogen cyanide — the mildest reagent for this type of transformation. Thus, the high proclivity of the leucine derived α-amidoaldehyde 20b towards isomerization prevents its use as an intermediate in the homologation protocol. Similarly, when the same sequence of reactions — oxidation/addition — was applied to the phenylglycinol derivative 19c, the cyanohydrin 21c was obtained with an optical purity not exceeding 75 % ee.

To conclude, stereoselective conversion of a stereoisomeric mixture of cyano-
hydrin mesylates into trans-oxazoline allows for a stereoselective synthesis of 
\( \alpha \)-hydroxy-\( \beta \)-amino acids starting from \( \alpha \)-amino acids. In all cases the transformation is diastereoselective and gives syn-products. However, the enantioselectivity of the reaction sequence depends on the substrate structure. While phenylalanine and valine derived \( \alpha \)-hydroxy-\( \beta \)-amino acids are obtained with excellent optical purity, with phenylglycine and leucine partial racemization occurs. Further investigations are needed in order to establish the scope and limitations of this reaction sequence.

**EXPERIMENTAL**

**General experimental**

All reactions were performed in dried glassware under an inert (Ar) atmosphere. Petroleum ether refers to the fraction with the distillation range 70–90 °C. All chromatographic separations\(^{29}\) were performed on Silica, 10–18, 60A, ICN Biomedicals. Standard techniques were used for the purification of reagents and solvents.\(^{29}\) NMR Spectra were recorded on a Varian Gemini 200 instrument, \(^{1}H\)-NMR at 200 MHz, \(^{13}C\)-NMR at 50 MHz, for samples in deuterated chloroform. Chemical shifts are expressed in ppm using tetramethylsilane as the internal standard, coupling constants (\(J\)) are in Hz. IR Spectra were recorded on a Perkin-Elmer 457 grating FT instrument, and are expressed in cm\(^{-1}\). Mass spectra were obtained on a Finnigan ITD S 700 instrument. Microanalyses were performed at the Vario EL III instrument CHNOS Elemental Analyzer, Elemental Analysensysteme GmbH, Hanau-Germany. Melting points were determined on a Koehler hot-stage apparatus and are uncorrected.

\((R)\)-(+)\(-N\)-benzoylphenylalaninol (12). This compound was obtained according to the previously described procedure for the preparation of its enantiomer,\(^{12}\) in 91 % yield. White crystals, mp 176 °C (lit.\(^{24}\) mp 169–170.5 °C, lit.\(^{12}\) mp 179–180 °C); Anal. calcd. for \(C_{16}H_{17}NO_{2}\): C 75.27, H 6.71; N 5.49, found: C 75.21, H 6.66, N 5.59, \([\alpha]_{D} = +82.4^\circ\) (c 0.5, MeOH), \([\alpha]_{D} = +93.4^\circ\), c 0.97, MeOH, lit.\(^{12}\) for the \(\text{ent-12} \ [\alpha]_{D} = –18^\circ\) (c 1.5, MeOH); \(IR\)\(_{KBr}\): 3308, 1640; \(^{1}H\)-NMR (DMSO-\(d_{6}\)): 2.88 (dd, \(J = 13.4, J = 9.3, 1H\)), 3.03 (dd, \(J = 13.4, J = 5.2, 1H\)), 3.59 (m, \(J = 9.3, J = 5.2, J = 2.5, 1H\)), 3.90 (d, \(J = 2.5, 1H\)), 7.31 (m, 5H), \(^{13}C\)-NMR (DMSO-\(d_{6}\)): 35.6 (CH\(_2\)); 54.1, 67.6, 127.7, 129.3, 129.9 (CH), 136.4, 173.2 (C).

\((R)-(+)\(-N\)-benzoylphenyalaninal cyanohydrin (14). To a cold (0 °C), rapidly stirred two-phase mixture of 12 (1 g, 3.92 mmol), sodium bromide (8.1 mg, 78 \(\mu\)mol), TEMPO free radical (12.3 mg, 78 \(\mu\)mol), THF (12 mL), dichloromethane (12 mL) and water (2 mL), was added a solution of sodium hypochlorite 2.9 mL of 11 % solution, 4.3 mmol) containing sodium bicarbonate (1.68 g, 20 mmol), over a period of 15 min and stirred for an additional 10 min. The layers were separated, the aqueous layer was extracted with dichloromethane, the combined organic extract was washed with a solution of sodium iodide (0.58 g) in 10 % aqueous sodium hydrogensulfate, then with 10 % aqueous sodium thiosulfate and finally with 7 % aqueous sodium bicarbonate. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure. The crude aldehyde 13 was dissolved in dichloromethane (13 mL), cooled (0 °C), and a solution of hydrogen cyanide in methanol (prepared by the addition of acetic acid (640 mg, 10.7 mmol) to a solution of potassium cyanide (644 mg, 9.9 mmol) in methanol (5.25 mL)) was added in one portion under vigorous stirring. After 5 min, the reaction was quenched by the addition of diethyl ether (26 mL) and a mixture of brine (20 mL) and saturated aqueous sodium bicarbonate (5 mL). The organic phase was separated, the aqueous phase was extracted with diethyl ether, the combined organic extract was washed with brine/sodium bicarbonate mixture, dried over anhy. sodium sulfate and the solvent was removed under reduced pressure to give 1.08 g (98 %) of the crude title compound 14, as a mixture of stereoisomers in the ratio 1:1.23.
The optical purity of 14 was determined in the following manner: to a solution of 14 (60 mg, 0.214 mmol) in ethanol (1 mL) was added sodium borohydride (40 mg, 1.06 mmol), at r.t., with stirring. After completion of the reaction the mixture was acidified with 1.5 M hydrochloric acid, diluted with water and extracted with ethyl acetate. The combined organic extract was washed with saturated aqueous sodium bicarbonate, water, brine, dried over anhy. magnesium sulfate, the solvent was evaporated under the reduced pressure and the residue was purified by dry-flash chromatography (eluent: petroleum ether/aceticone = 4/1). The optical rotation value for the product was $[\alpha]_D = +77.2^\circ$ (c 0.5, MeOH), which correspond to 93.4 % ee.

Spectral data for the mixture of isomers: $IR_{KB}$: 3314, 1647, 1631, 1530, 1491, 1449, 1082; $^1\text{H}-\text{NMR}$: 2.29–3.22 (m), 3.28 (dd, $J_1 = 14.0, J_2 = 6.2, 82.97–83.22+83.28=2\text{Hz}$), 4.29 (m), 4.51–4.75 (m, $84.29+64.51=4.75\text{Hz}$), 5.57 (d, $J = 5.7$), 6.12 (d, $J = 7.4, 85.57+86.12=1\text{Hz}$), 6.47 (br. d), 6.61 (d, $J = 5.0, 86.47+76.61=1\text{Hz}$), 7.20–7.65 (m, 10H); $^{13}\text{C}-\text{NMR}$ (CDCl$_3$ + CD$_2$OD): 35.13 (CH$_2$), 35.53 (CH$_2$), 54.39 (CH), 54.57 (CH), 61.95 (CH), 63.91 (CH), 118.43 (CN), 118.87 (CN), 126.89 (CH), 127.04 (CH), 127.09 (CH), 128.50 (CH), 128.69 (CH), 128.78 (CH), 129.00 (CH), 129.02 (CH), 131.90 (CH), 132.00 (CH), 133.30 (C), 133.43 (C), 136.11 (C), 136.68 (C), 168.60 (C), 169.35 (C).

(RR,N)-4-benzyl-3-amino-2-hydroxy-4-phenylbutanoic acid methyl ester, mixture of (2R)- and (2S)-isomers (15). A cold (0 °C) solution of 14 (1.02 g, 3.64 mmol) in a mixture of diethyl ether/methanol (51 mL of the Et$_2$O/MeOH = 3/1) was saturated with gaseous hydrogen chloride. The reaction mixture was stirred for 24 h at a temperature below 5 °C, then water (11 mL) was added and stirring was continued for 24 h at the same temperature, followed by 24 h at r.t. The reaction mixture was evaporated to dryness and the residue was taken up in tetrahydrofuran (20 mL). Diazomethane solution in diethyl ether was added dropwise, until the yellow colour persisted. After 15 min the solvent was evaporated and the crude product was purified by dry-flash chromatography (eluent: petroleum ether/aceticone = 4/1), to give 0.98 g (86.4 %) of the title compound 15 as a mixture of isomers in 1:1.1 ratio.

$IR_{KB}$ (for the mixture of isomers): 3420, 1741, 1639, 1535, 1326, 1112; $^1\text{H}-\text{NMR}$ (less polar isomers): 3.0 (dd, $J_1 = 13.4, J_2 = 9.0, 1\text{H}$), 3.10 (dd, $J_1 = 13.4, J_2 = 6.8, 1\text{H}$), 3.60 (s, 3H), 4.20 (d, $J = 1.9, 1\text{H}$), 4.80 (m, 1H), 6.53 (d, $J = 9.3, 1\text{H}$), 7.20–7.53 (m, 8H), 7.65–7.69 (m, 2H); $^{13}\text{C}-\text{NMR}$ (less polar isomer): 37.63 (CH$_2$), 52.67 (CH or CH$_3$), 53.40 (CH$_3$ or CH), 70.16 (CH), 126.64 (CH), 126.91 (CH), 128.40 (CH), 128.52 (CH), 129.32 (CH), 131.48 (CH), 134.11 (C), 137.26 (C), 167.39 (C), 174.07 (C); $^1\text{H}-\text{NMR}$ (more polar isomer): 2.90 (dd, $J_1 = 14.3, J_2 = 6.8, 1\text{H}$), 3.01 (dd, $J_1 = 14.3, J_2 = 7.6, 1\text{H}$), 3.59 (s, 3H), 4.44 (d, $J = 3.6, 1\text{H}$), 4.86 (m, 1H), 6.50 (d, $J = 8.4$), 7.10–7.57 (m, 8H), 7.62–7.70 (m, 2H); $^{13}\text{C}-\text{NMR}$ (more polar isomer): 35.34 (CH$_2$), 52.63 (CH or CH$_3$) 53.34 (CH$_3$ or CH), 72.14 (CH), 126.81 (CH), 126.94 (CH), 128.56 (CH), 128.67 (CH), 129.42 (CH), 131.67 (CH), 134.08 (C), 136.84 (C), 167.47 (C), 173.05 (C).

(4R,5S)-methyl 4-benzyl-4,5-dihydro-2-phenylxazol-5-carboxylate (17). To a cold (0 °C) solution of 15 (526 mg, 1.68 mmol) and triethylamine (0.35 mL, 2.52 mmol) in dichloromethane (11 mL) was added mesyl chloride (0.16 mL, 2.02 mmol), with vigorous stirring, under an argon atmosphere. After 10 min DBU (0.75 mL, 5.04 mmol) was added and the reaction mixture was stirred at r.t. for 6 h. The solution was washed with 1 M hydrochloric acid, aqueous sodium bicarbonate and water, dried over anhy. sodium sulfate and the solvent was removed under reduced pressure. Purification by dry-flash chromatography (eluent: petroleum ether/aceticone = 95/5) afforded 350 mg (71 %) of the trans-oxazoline 17 as a viscous oil, followed by 8.5 mg (1.7 %) of the cis-oxazoline. Anala. calcd. for C$_9$H$_{12}$N$_2$O$_2$: C 73.20 %, H 5.80 %, N, 4.74 %, found: C 73.63, H 5.73 %, N 4.35 %; $[\alpha]_D = -31.4^\circ$ (c 1, CH$_2$Cl$_2$); $IR_{\text{film}}$: 3030, 2955, 2920, 1757, 1657, 1495, 1452, 1438, 1288, 1213, 1082, 1059; $^1\text{H}-\text{NMR}$: 2.96 (dd, $J_1 = 6.6, 1\text{H}$), 3.18 (dd, $J_1 = 14.0, J_2 = 5.8, 1\text{H}$), 3.69 (s, 3H), 4.64 (dd, $J_1 = 11.8, J_2 = 6.1, 1\text{H}$), 4.74 (d, $J = 6.1, 1\text{H}$), 7.16–7.58 (m, 8H), 7.95–8.03 (m, 2H); $^{13}\text{C}-\text{NMR}$: 41.32 (CH$_3$), 52.47 (CH$_2$), 72.67 (CH), 79.50 (CH), 126.75 (CH), 128.37 (CH), 128.47 (CH), 128.50 (CH), 129.67 (CH), 131.69 (CH), 136.62 (C), 163.24 (C), 170.88 (C).

(2S,3R)-AHPA HCl. A solution of 17 (330.6 mg, 1.12 mmol) in 10 % hydrochloric acid (15 mL) was refluxed (110 °C) for 8 h. The reaction mixture was evaporated to dryness, dry ethanol was
added (2 × 5 mL) and evaporated to dryness again. Diethyl ether (7 mL) was added to the solid residue, heated to reflux for 10 min, and the hot solvent was decanted (this procedure was repeated two more times). The crude product was recrystallized from isopropanol/diethyl ether, to give 229.6 mg (88.5 %) of (2S,3R)-AHPA hydrochloride.

White crystals, mp 185–186 ºC; Anal. calcd. for C 10H14ClNO3: C 51.84 %, H 6.09 %, N 6.05 %; found: C 51.54 %, H 6.31 %, N 6.19 %; $\delta^1H-NMR$ (DMSO-d$_6$): 2.88 (dd, $J_1 = 13.4$, $J_2 = 9.3$, 1H), 3.03 (dd, $J_1 = 13.4$, $J_2 = 5.2$, 1H), 3.59 (m, 1H), 3.90 (d, $J = 2.5$, 1H), 7.31 (m, 5H); $\delta^{13}C-NMR$ (DMSO-d$_6$): 35.62 (CH$_2$), 54.06 (CH), 67.58 (CH), 127.68 (CH), 129.32 (CH), 129.91 (CH), 136.45 (C), 173.18 (C).

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