Amino Acids (2000) 18: 117–127

Amino
Acids

Synthesis of conformationally constrained hydroxy-α-amino acids by intramolecular conjugate addition

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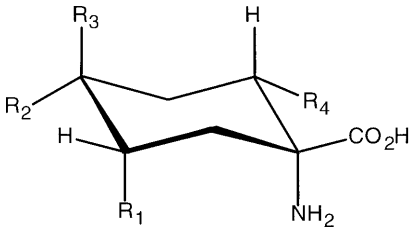
Accepted May 16, 1999

Summary. An efficient and easily applicable method for the synthesis of a variety of hydroxy-α-amino acids analogues of serine and phenylalanine has been established. The method involves the stereoselective intramolecular conjugate addition of the benzamide group to cyclohexenone promoted by Lewis acid. Subsequent transformations of functional groups provide the conformationally constrained 2-hydroxy- and 2,4-dihydroxy-6-phenylcyclohexane-α-amino acids.

Keywords: Amino acids – 5(4H)-Oxazolone – Diels-Alder reaction – Intramolecular conjugate addition – Hydroxy-α-amino acids

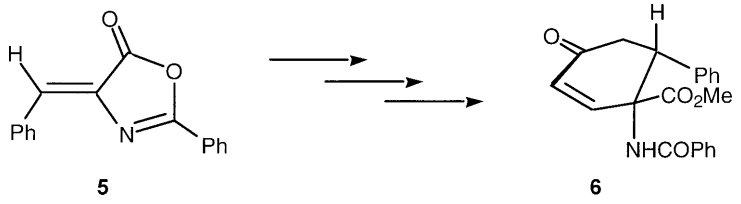
Introduction

Although the synthesis of α-amino acids has received much attention, the synthesis of conformationally constrained α-amino acids has not been developed until their important role in the synthesis of peptides with altered physical properties and biological activity (pseudopeptides, peptidomimetics, …) has been increasingly recognised in the last few years (Barret, 1985; Giannis et al., 1993; Gante, 1994; Liskamp, 1994; Wirth, 1997). Recently, one of us has published a complete and interesting review about the stereoselective synthesis of acyclic quaternary α-amino acids (Cativiela et al., 1998). In this context, and as a part of our research programme on the racemic and asymmetric synthesis of conformationally constrained α-amino acids, we have been interested in the synthesis of hydroxy-α-amino acids, because there are a great number of natural products that contain nonproteinogenic hydroxy-α-amino acid derivatives which display biological activities (Isono et al., 1989; Passerat et al., 1987). Recently, we have reported on the synthesis of several monohydroxycyclohexane-α-amino acids, which are shown in Fig. 1. γ-Hydroxycyclohexane-α-amino acids **1** and **2** were achieved through direct hydroxylation, *via* dihydro-1,3-oxazine intermediates, of the unsaturated



1: $R_1 = OH$, $R_2 = R_3 = R_4 = H$
2: $R_1 = OH$, $R_2 = R_3 = H$, $R_4 = Ph$
3: $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = Ph$
4: $R_1 = R_3 = H$, $R_2 = OH$, $R_4 = Ph$

Fig. 1. Conformationally constrained hydroxy-α-amino acids



5 **6**

Scheme 1. Synthesis of α,β-unsaturated compound **6** from 5(4H)-oxazolone **5**

amino acid derivatives, which can be obtained by Diels-Alder cycloadditions of 1,3-butadiene with methyl and 8-phenylmenthyl 2-acetamido acrylates or (*Z*)-2-phenyl-4-benzylidene-5(4H)-oxazolone (Avenoza et al., 1994a, 1995a, 1996).

On the other hand, δ-hydroxycyclohexane-α-amino acids **3** and **4** were obtained by selective reduction of the carbonyl group present in the enone **6**, which was prepared starting from the Diels-Alder cycloadddition of Danishefsky's diene with (*Z*)-2-phenyl-4-benzylidene-5(4H)-oxazolone **5** and further treatment of the Diels-Alder cycloadducts with DBU-methanol (Avenoza et al., 1995b, 1998) (Scheme 1). In this paper we report the use of compound **6** as starting material to obtain β-hydroxycyclohexane-α-amino acids analogues of serine and phenylalanine.

Material and methods

Solvents were purified according to standard procedures. Analytical TLC was performed by using Polychrom SI F_{254} plates. Column chromatography was performed using Silica gel 60 (230–400 mesh). ^1H and ^{13}C-NMR spectra were recorded on a Bruker ARX-300 spectrometer. ^1H and ^{13}C-NMR spectra were recorded in $CDCl_3$ with TMS as the internal standard and in D_2O-TFA with TMS as the external standard using a coaxial microtube (chemical shifts are reported in ppm on the δ scale, coupling constants in Hz). The assignment of all separate signals in the ^1H-NMR spectra was made on the basis of coupling constants, selective proton-proton homonuclear decoupling experiments, proton-proton COSY experiments and proton-carbon COSY experiments. Melting points were determined on a Büchi SMP-20 melting point apparatus and are uncorrected. Microanalyses were carried out on a CE Instruments EA-1110 analyser and were in good agreement with the calculated values.

Methyl cis-endo-*3,6-diphenyl-8-oxo-2-oxa-4-azabicyclo[4.3.0]non-3-ene-5-carboxylate.* ***7***

Enone **6** (500 mg, 1.40 mmol) was dissolved in dry dichloromethane (20 mL) under an inert atmosphere and trimetylsilyl triflate (933 mg, 4.30 mmol) was then slowly added. After 1 h stirring at room temperature, the reaction was quenched by addition of a saturated NH_4Cl solution (2 mL). The residue was extracted with ethyl acetate (3 × 30 mL) and the organic layer dried over anhydrous $MgSO_4$, filtered and evaporated to give 487 mg of a mixture of compounds **7** and **6** in a ratio of 90:10, which was used in the next step without purification. Nevertheless an analytical sample of compound **7** could be obtained after flash silica gel column chromatography eluting with hexane-ethyl acetate (70:30).

Found C: 72.23, H: 5.52, N: 3.88
Anal. Calc. for $C_{21}H_{19}NO_4$ C: 72.18, H: 5.48, N: 4.01
^{1}H-NMR($CDCl_3$): δ = 2.53(dd, 1H, J_{7x-7n} = 18.6, J_{7x-6x} = 3.6, H_{7x}); 2.68(dd, 1H, J_{7n-7x} = 18.6, J_{7n-6x} = 13.1, H_{7n}); 3.05–3.10(m, 2H, H_{9x} + H_{9n}); 3.66(s, 3H, COOMe); 3.90(dd, 1H, J_{6x-7n} = 13.1, J_{6x-7x} = 3.6, H_{6x}); 5.24('t', 1H, J_{1x-9n} ~ J_{1x-9x} = 3.0, H_{1x}); 7.18–7.62(m, 8H, Arom.); 7.98–8.03(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 40.8, 41.5, 45.4, 52.7(C_6, C_7, C_9, COOMe); 79.8, 81.1(C_1, C_5); 126.2, 127.8, 128.4, 128.5, 128.8, 128.9, 132.3, 137.2 (Arom.); 165.1(C=N); 173.9(COOMe); 207.4(CO).

Methyl cis-endo-*3,6-diphenyl*-endo-*8-hydroxy-2-oxa-4-azabicyclo [4.3.0]non-3-ene-5-carboxylate.* ***8***

A solution of compound **7** (300 mg, 0.85 mmol) in dry tetrahydrofuran (15 mL) was added to a suspension of sodium borohydride (321 mg, 8.50 mmol) in dry tetrahydrofuran (5 mL) kept at −78°C under an inert atmosphere. The reaction mixture was allowed to stand at the same temperature for 1 h. After that, water (2 mL) was added and the excess of sodium borohydride was destroyed by the dropwise addition of a 10% HCl solution. The resulting mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography eluting with hexane-ethyl acetate (70:30) to give 281 mg of compound **8** (94%) as an oil.

Found C: 71.83, H: 6.09, N: 3.80
Anal. Calc. for $C_{21}H_{21}NO_4$ C: 71.78, H: 6.02, N: 3.99
^{1}H-NMR($CDCl_3$): δ = 1.79(m, 1H, H_{7n}); 2.20–2.30(m, 2H, H_{7x} + H_{9n}); 2.42(ddd, 1H, J_{9x-9n} = 15.6, J_{9x-8x} = 6.0, J_{9x-1x} = 3.9, H_{9x}); 3.31(dd, 1H, J_{6x-7n} = 13.8, J_{6x-7x} = 1.8, H_{6x}); 3.61(s, 3H, COOMe); 4.16–4.26(m, 1H, H_{8x}); 5.01('t', 1H, J_{1x-9n} ~ J_{1x-9x} = 3.9, H_{1x}); 7.20–7.58(m, 8H, Arom.); 8.02–8.09(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 33.4, 34.5, 44.7, 52.6(C_6, C_7, C_9, COOMe); 65.6(C_8), 79.6(C_5) 82.1(C_1) 127.3, 128.2, 128.4, 128.6, 128.9, 129.6, 132.1, 139.3(Arom.); 164.7(C=N); 174.5(COOMe).

Methyl 1-amino-t-*2-benzoyloxy*-t-*4-hydroxy*-t-*6-phenylcyclohexane*-r-*1-carboxylate trifluoroacetate.* ***9***

Method A (Starting from **8***)*: Trifluoroacetic acid (650 mg, 5.70 mmol) was added to a solution of 1,3-oxazoline **8** (200 mg, 0.57 mmol) in 4:1 tetrahydrofuran-water (20 mL). After 5 h stirring at 80°C the solvent was evaporated and, in order to remove most of the trifluoroacetic acid, the solvent was removed under reduced pressure. The oily residue was then dissolved in diethyl ether and the solvent and the residual trifluoroacetic acid were distilled off *in vacuo*. This operation was repeated to ensure complete removal of the trifluoroacetic acid. In this way 223 mg (81%) of compound **9** were obtained as a white solid.

Method B (Starting from **14***)*: Tributyltin hydride (0.16 mL, 0.24 mmol) was added to a solution of iodo derivative **14** (100 mg, 0.20 mmol) in dry dichloromethane (20 mL) kept under an inert atmosphere. After 3 h stirring at room temperature the solvent was evaporated and, in order to remove the organotin compounds, the residue was chromatographed on silica gel eluting with hexane-ethyl acetate (1:1) to afford 70 mg (94%) of methyl 1-amino-*t*-2-benzoyloxy-*t*-4-hydroxy-*t*-6-phenylcyclohexane-*r*-1-carboxylate which was dissolved in trifluoroacetic acid (3 ml) to obtain this compound as a trifluoroacetate derivate (compound **9**). Then, the excess of trifluoroacetic acid was eliminated in the same way above described in method A. Isolated yield, 90 mg (98%).

Found C: 57.03, H: 5.12, N: 2.96
Anal. Calc. for $C_{23}H_{24}NO_7F_3$ C: 57.14, H: 5.00, N: 2.89
^{1}H-NMR($CDCl_3$): δ = 2.19(m, 1H); 2.45–2.59(m, 2H); 2.94(m, 1H); 3.41(m, 1H, H_{6a}); 3.52(s, 3H, COO<u>Me</u>); 3.94–4.12(m, 1H, H_{4a}); 5.65–5.75(m, 1H, H_{2a}); 7.10–7.49(m, 8H, Arom.); 8.25–8.31(m, 2H, Arom).
^{13}C-NMR($CDCl_3$): δ = 34.0, 34.5, 44.7, 53.8(C_3, C_5, C_6, COO<u>Me</u>); 66.4, 68.2, 72.3(C_1, C_2, C_4); 128.1, 128.4, 128.6, 128.7, 129.5, 130.7, 133.7, 134.7(Arom.); 164.9(OCOPh); 168.6(<u>C</u>OOMe).

Methyl 1-amino-t-*2,*t-*4-dihydroxy*-t-*6-phenylcyclohexane*-r-*1-carboxylate hydrochloride.* ***10***

Compound **9** (100 mg, 0.29 mmol) was dissolved in 2N HCl (30 mL) and heated under reflux for 48 h. The solvent was evaporated, the residue of amino ester hydrochloride was dissolved in water (20 mL) and washed with diethyl ether (3 × 10 mL). The aqueous solution was evaporated *in vacuo* to give 76 mg (93%) amino ester hydrochloride **10** as a white solid.

Found C: 55.65, H: 6.54, N: 4.73
Anal. Calc. for $C_{14}H_{20}NO_4Cl$ C: 55.72, H: 6.68, N: 4.64
^{1}H-NMR(D_2O): δ = 1.50(m, 1H); 1.98–2.13(m, 2H); 2.34(m, 1H); 3.60(dd, 1H, J_{6a-5a} = 11.4, J_{6a-5e} = 6.0, H_{6a}); 3.84(s, 3H, COO<u>Me</u>); 3.94–4.12(m, 1H, H_{4a}); 4.54(dd, 1H, J_{2a-3a} = 12.3, J_{2a-3e} = 4.2, H_{2a}); 7.20–7.47(m, 5H, Arom.).
^{13}C-NMR(D_2O): δ = 24.5, 28.2, 34.2, 45.6, 57.1, 60.6, 60.7(C_1, C_2, C_3, C_4, C_5, C_6, COO<u>Me</u>); 119.8, 120.5, 121.1, 127.1(Arom.); 162.4(<u>C</u>OOMe).

1-amino-t-*2,*t-*4-dihydroxy*-t-*6-phenylcyclohexane*-r-*1-carboxylate acid.* ***11***

Compound **10** was dissolved in 10N HCl (30 mL) and heated under reflux for 48 h. The solvent was evaporated, the residue of aminoacid hydrochloride was dissolved in EtOH (6 mL) and propylene oxide (2 mL) was added. The mixture was heated under reflux for 1 h and partially precipitated. After removal of the EtOH, the residue was dissolved in distilled water (2 mL) and eluted through a C_{18} reverse-phase Sep-pak cartridge which, after removal of water, gave 51 mg (77%) of α-amino acid **11** as a white solid.

Found C: 62.08, H: 6.70, N: 5.50
Anal. Calc. for $C_{13}H_{17}NO_4$ C: 62.12, H: 6.82, N: 5.57
^{1}H-NMR(D_2O/TFA): δ = 1.51(m, 1H); 1.95–2.15(m, 2H); 2.32(m, 1H); 3.50(m, 1H, H_{6a}); 3.92(m, 1H, H_{4a}); 4.42(m, 1H, H_{2a}); 7.10–7.50(m, 5H, Arom).
^{13}C-NMR(D_2O/TFA): δ = 33.1, 36.9, 42.4, 65.8, 68.8, 69.2(C_1, C_2, C_3, C_4, C_5, C_6); 128.3, 128.9, 129.5, 135.7(Arom.); 172.2(<u>C</u>OOH).

Methyl 1-benzamido-t-*4-hydroxy*-t-*6-phenyl-2-cyclohexene*-r-*1-carboxylate.* ***12***

Allylic alcohol **12** was obtained as an oil in a similar way to that described for compound **8**, starting from enone **6** (300 mg , 0.84 mmol). Isolated yield, 280 mg (94%).

Found C: 71.63, H: 6.11, N: 3.89
Anal. Calc. for $C_{21}H_{21}NO_4$ C: 71.78, H: 6.03, N: 3.98
^{1}H-NMR($CDCl_3$): δ = 2.28(ddd, 1H, J_{5e-5a} = 12.0, J_{5e-4a} = 6.0, J_{5e-6a} = 3.0, H_{5e}); 2.45('t'd, 1H, $J_{5a-5e} \sim J_{5a-6a}$ = 12.0, J_{5a-4a} = 9.0, H_{5a}); 3.55(dd, 1H, J_{6a-5a} = 12.0, J_{6a-5e} = 3.0, H_{6a}); 3.76(s, 3H, COOMe); 4.50–4.59(m, 1H, H_{4a}); 6.15(d, 1H, J_{2-3} = 12.0, H_2); 6.27(dd, 1H, J_{3-2} = 12.0, J_{3-4a} = 3.0, H_3); 6.61(brs, 1H, NH) 7.11–7.58(m, 10H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 33.9, 46.4, 53.1, 62.4, 67.6 (C_1, C_4, C_5, C_6, COOMe); 126.7, 126.8, 128.0, 128.1, 128.6, 128.8, 131.7, 131.8, 134.4, 138.8(Arom, C_2, C_3); 167.4(NHCOPh); 173.0(COOMe).

Methyl cis-endo-*3,6-diphenyl*-exo-*9-iodo*-endo-*8-hydroxy-2-oxa-4-azabicyclo[4.3.0]non-3-ene-5-carboxylate.* ***13***

A mixture of compound **12** (250 mg, 0.71 mmol) and iodine (718 mg, 2.89 mmol) in 50 mL of dioxane was stirred at room temperature. After 2 h, the reaction was diluted with ethyl ether, washed with 10% $Na_2S_2O_3$, dried over anhydrous $MgSO_4$, filtered and evaporated to give the iodo-1,3-oxazoline **13**, which was then purified by silica gel column chromatography using hexane-ethyl acetate (70:30) as an eluent. Isolated yield, 302 mg of a white solid (88%). Mp: 67–70°C

Found C: 52.93, H: 4.35, N: 2.90
Anal. Calc. for $C_{21}H_{20}NO_4I$ C: 52.84, H: 4.22, N: 2.93
^{1}H-NMR($CDCl_3$): δ = 1.95('t'd, 1H, $J_{7n-7x} \sim J_{7n-6x}$ = 13.5, J_{7n-8x} = 10.8, H_{7n}); 2.16(d't', 1H, J_{7x-7n} = 13.5, $J_{7x-6x} \sim J_{7x-8x}$ = 3.6, H_{7x}); 3.51(s, 3H, COOMe); 3.62(dd, 1H, J_{6x-7n} = 13.5, J_{6x-7x} = 3.6, H_{6x}); 3.81(dd, 1H, J_{9n-8x} = 10.8, J_{9n-1x} = 8.4, H_{9n}); 3.97('t'd, 1H, $J_{8x-9n} \sim J_{8x-7n}$ = 10.8, J_{8x-7x} = 3.6, H_{8x}); 5.45(d, 1H, J_{1x-9n} = 8.4, H_{1x}); 7.26–7.56(m, 8H, Arom.); 8.03–8.10(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 35.0, 41.8, 45.1, 53.0(C_5, C_6, C_7, COOMe); 71.1, 80.5, 90.0(C_1, C_8, C_9) 127.1, 127.6, 128.3, 128.4, 128.8, 129.1, 132.3, 138.7(Arom.); 166.0(C=N); 173.1(COOMe).

Methyl 1-amino-t-*2-benzoyloxy*-c-*3-iodo*-t-*4-hydroxy*-t-*6-phenylcyclohexane*-r-*1-carboxylate trifluoroacetate.* ***14***

Amino ester **14** was obtained as a white solid in a similar way to that described for compound **9** (method A), starting from iodo-1,3-oxazoline **13** (250 mg, 0.52 mmol). Isolated yield, 255 mg (82%). Mp: 149–152°C.

Found C: 44.30, H: 3.63, N: 2.24
Anal. Calc. for $C_{22}H_{21}NO_7IF_3$ C: 44.37, H: 3.56, N: 2.35
^{1}H-NMR($CDCl_3$): δ = 2.13(m, 1H, H_{5e}); 2.58(m, 1H, H_{5a}); 3.62(dd, 1H, J_{6a-5a} = 14.1, J_{6a-5e} = 3.6, H_{6a}); 3.69(s, 3H, COOMe); 4.10(m, 1H, H_{4a}); 4.70('t', 1H, $J_{3a-2a} \sim J_{3a-4a}$ = 11.4, H_{3a}); 6.02(d, 1H, J_{2a-3a} = 11.4, H_{2a}), 7.17–7.63(m, 8H, Arom.); 8.02–8.09(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 32.9, 38.1, 43.9, 53.4(C_1, C_5, C_6, COOMe); 66.6, 73.1, 76.6(C_2, C_3, C_4); 128.1, 128.3, 128.5, 128.5, 129.2, 130.2, 133.9, 135.7(Arom.);
164.3(OCOPh); 171.1(COOMe).

Methyl cis-endo-*3,6-diphenyl-2-oxa-4-azabicyclo[4.3.0]non-3-ene-5-carboxylate.* ***15***

Under an inert atmosphere, a solution of compound **8** (200 mg, 0.57 mmol) in dry acetonitrile (20 mL) was added to a suspension of 4-(dimethylamino)pyridine

(188 mg, 1.54 mmol) in dry acetonitrile (5 mL) and the mixture was treated with phenoxythiocarbonyl chloride (189 mg, 0.62 mmol). After 12 h stirring at room temperature, the acetonitrile was removed by evaporation, the residue was dissolved in dichloromethane (30 mL) and the solution was washed with water (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered and evaporated to give 220 mg (80%) of methyl *cis-endo*-3,6-diphenyl-*endo*-8-phenoxythiocarbonyloxy-2-oxa-4-azabicyclo[4.3.0]non-3-ene-5-carboxylate, which was used in the next step without purification.
^{1}H-NMR($CDCl_3$): δ = 2.09(m, 1H); 2.42–2.55(m, 2H); 2.66(m, 1H); 3.39(dd, 1H, J_{6x-7n} = 14.1, J_{6x-7x} = 3.0, H_{6x}); 3.60(s, 3H, COO<u>Me</u>); 5.07('t', 1H, $J_{1x-9n} \sim J_{1x-9x}$ = 3.6, H_{1x}); 5.70(m, 1H, H_{8x}); 6.88–7.53(m, 13H, Arom.); 8.05–8.12(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 29.6, 29.6, 44.1, 52.7, 77.8, 79.6, 80.8(C_1, C_5, C_6, C_7, C_8, C_9, COO<u>Me</u>); 115.4, 121.9, 126.5, 127.5, 128.3, 128.3 128.8, 129.0, 129.5, 132.0, 138.8, 153.2(Arom.); 165.4(C=N); 174.4(<u>C</u>OOMe); 194.3 (OCOSPh).

To a solution of this compound (168 mg, 0.35 mmol) in dry toluene (20 mL), tributyltin hydride (0.28 mL, 1.06 mmol) and 2,2′-azobisisobutyronitrile (20 mg, 0.12 mmol) were added. The reaction mixture was heated at 100°C for 5 h under an inert atmosphere. The solvent was evaporated and the residue was purified by column chromatography on silica gel using a mixture of hexane-ethyl acetate (70:30) as eluent, to give 110 mg (91%) of compound **15** as an oil.

Found C: 75.36, H: 6.25, N: 4.23
Anal. Calc. for $C_{21}H_{21}NO_3$ C: 75.20, H: 6.31, N: 4.17
^{1}H-NMR(CD_3Cl): δ = 1.26(m, 1H); 1.70–1.88(m, 3H); 2.04–2.14(m, 2H); 3.40(dd, 1H, J_{6x-7n} = 13.2, J_{6x-7x} = 3.9, H_{6x}); 3.53(s, 3H, COO<u>Me</u>); 5.03('t', 1H, $J_{1x-9n} \sim J_{1x-9x}$ = 3.0, H_{1x}); 7.16–7.57(m, 8H, Arom.); 8.03–8.11(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 23.2, 23.6, 28.7, 44.1, 52.3, 80.0, 82.2(C_1, C_5, C_6, C_7, C_8, C_9, COO<u>Me</u>); 127.0, 128.1, 128.3, 128.7, 129.1, 129.6, 131.7, 140.9(Arom.); 164.9(C=N); 174.9(<u>C</u>OOMe).

Methyl 1-amino-t-*2-benzoyloxy*-t-*6-phenylcyclohexane*-r-*1-carboxylate trifluoroacetate.* ***16***

Amino ester **16** was obtained as a white solid in a similar way to that described for compound **9** (method A), starting from 1,3-oxazoline **15** (100 mg, 0.30 mmol). Isolated yield, 114 mg (83%). Mp: 138–140°C.

Found C: 59.18, H: 5.27, N: 3.12
Anal. Calc. for $C_{23}H_{24}NO_6F_3$ C: 59.08, H: 5.18, N: 3.00
^{1}H-NMR($CDCl_3$): δ = 1.35(m, 1H); 1.69(m, 1H); 1.86–1.98(m, 2H); 2.26–2.48(m, 2H); 3.50(dd, 1H, J_{6a-5a} = 13.5, J_{6a-5e} = 3.6, H_{6a}); 3.67(s, 3H, COO<u>Me</u>); 5.74(dd, 1H, J_{2a-3a} = 11.8, J_{2a-3e} = 4.6, H_{2a}); 7.08–7.33(m, 8H, Arom.); 7.96–8.02(m, 2H, Arom.).
^{13}C-NMR($CDCl_3$): δ = 21.4, 23.2, 24.3(C_3, C_4, C_5); 46.5, 52.8, 67.5, 72.8(C_1, C_2, C_6 COO<u>Me</u>); 126.8, 127.1, 127.4, 127.9, 128.5, 129.0, 132.8, 134.2(Arom.); 164.8(OCOPh); 168.6(<u>C</u>OOMe).

1-amino-t-*2-hydroxy*-t-*6-phenylcyclohexane*-r-*1-carboxylic acid.* ***17***

In a similar way to that described above for dihydroxy-α-amino acid **11**, starting from amino ester **16** (90 mg, 0.25 mmol), β-hydroxy-α-amino acid **17** was obtained as a white solid in 76% yield.

Found C: 66.42, H: 7.35, N: 6.02
Anal. Calc. for $C_{13}H_{17}NO_3$ C: 66.36, H: 7.28, N: 5.95
^{1}H-NMR(D_2O/TFA): δ = 1.48–2.09(m, 6H); 3.55(m, 1H, H_{6a}); 4.40(m, 1H, H_{2a}); 7.15–7.50(m, 5H, Arom.).

^{13}C-NMR(D_2O/TFA): δ = 21.9, 24.2, 28.0, 45.5, 69.6, 70.8(C_1, C_2, C_3, C_4, C_5, C_6); 128.2, 128.5, 129.3, 136.8(Arom.); 172.3(COOH).

Results and discussion

Due to the availability of product **6** (Avenoza et al., 1994b) in this paper we would like to report a strategy of hydroxy-functionalisation on this enone **6** in order to obtain different β-hydroxycyclohexane-α-amino acids using an intramolecular conjugate addition of the benzamide group to vinyl ketone moiety of cyclohexenone ring. Although the intramolecular conjugate addition of heteronucleophiles is a well known process in the synthesis of heterocyclic compounds (Asao et al., 1994; Perlmutter, 1992) there are few examples in the literature in which the amide group behaves as an oxygen nucleophile. Moreover, when the double bond attacked is in a constrained ring, the cyclisation occurs with quite predictable stereochemical and regiochemical control (Trost, 1991). In our case, the reaction took place with a very good yield when a Lewis acid was added, allowing the direct hydroxylation in a syn relationship to the amide group through the 1,3-oxazoline intermediate **7** (Scheme 2).

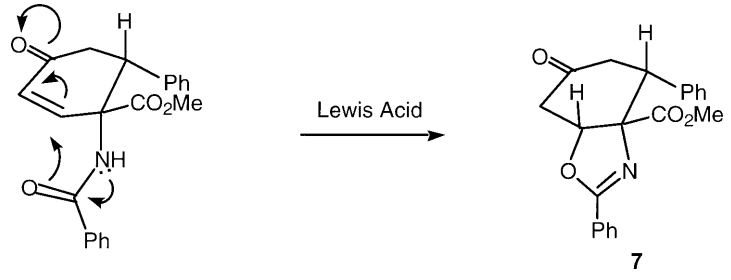
The reaction was carried out in an inert atmosphere using dry CH_2Cl_2 as a solvent at room temperature and, in order to improve the yield, several Lewis acids ($AlCl_3$, $AlCl_2Et$, $AlMe_3$, $TiCl_4$ and TMSOTf) were tested under various conditions. Under the optimum conditions, treatment of enone **6** with 3.0 equivalents of trimethylsilyl triflate at room temperature over 1 h afforded the desired cyclisation product **7** in 90% yield as a single diastereoisomer.

The ratio of the **6** and **7** products was determined by the integration of the signals corresponding to the H_{6a} proton of enone **6** and H_{1x} proton of oxazoline **7** in the ^{1}H-NMR spectrum of the mixture **6**, **7**.

H_{6a} (**6**): 4.02(dd, 1H, J_{6a-5a} = 10.8, J_{6a-5e} = 4.8).

H_{1x} (**7**): 5.24('t', 1H, $J_{1x-9n} \sim J_{1x-9x}$ = 3.0).

The oxazoline product **7** could not be purified from the starting enone (10%) by column chromatography because of the retroreaction caused by the action of silica gel. Nevertheless, an analytical sample was chromatographed on silica gel in order to characterize this labile compound **7**. The relative stereochemistry of this compound was assigned on the basis of ^{1}H-NMR spectrum, decoupling and nOe experiments (Severn et al., 1993), in which relevant nOe enhancements were observed in the H_6 and H_1 protons when H_{7x} proton was presaturated. (Fig. 2)



Scheme 2. Intramolecular conjugate addition on compound **6**

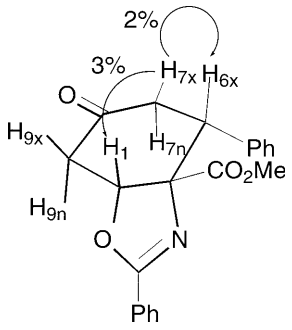


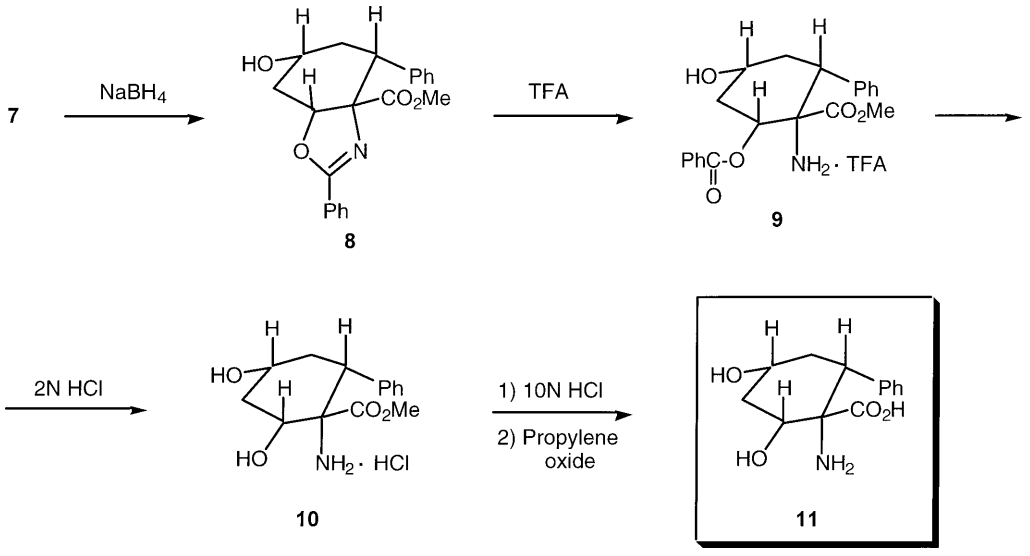
Fig. 2. Nuclear Overhauser experiment (nOe) in 1,3-oxazoline intermediate **7**

As the result of this simple reaction, a stereogenic centre was created with excellent stereocontrol, demonstrating the synthetic potential of this intramolecular strategy. In this way, the oxazoline **7** was used as a starting material to synthesize different β-hydroxycyclohexane-α-amino acids.

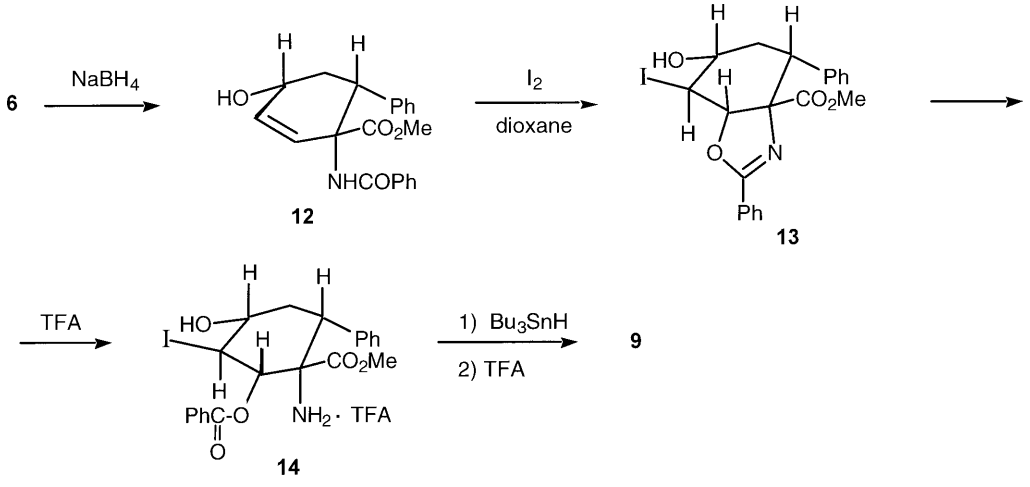
1,3-Oxazoline **7** is a direct precursor of a dihydroxycyclohexane-α-amino acid, indeed, the carbonyl group was reduced, using sodium borohydride in tetrahydrofuran at −78°C, to give exclusively the equatorial alcohol **8**, which was treated with trifluoroacetic acid in tetrahydrofuran-water at 80°C in order to open the 1,3-oxazoline ring, allowing the synthesis of amino ester derivative **9** in quantitative yield. This compound was partially hydrolysed in 2N HCl under reflux to give the dihydroxy-α-amino methyl ester derivative **10** with 69% yield. To obtain the desired 2,4-dihydroxy-6-phenylcyclohexane-α-amino acid **11** by hydrolysis of the methyl ester group, it was necessary to use 10N HCl under reflux over 3 days. In this way the amino acid was obtained as a hydrochloride derivative and to achieve the free amino acid **11** the compound **10** was treated with propylene oxide in ethanol under reflux (Scheme 3).

In order to corroborate this synthetic route, we have synthesised compound **9** starting from enone **6** using a different strategy which includes the reduction of the carbonyl group of enone **6** with sodium borohydride at −78°C to give the equatorial allylic alcohol **12** and a further electrophile-initiated cyclofunctionalization reaction involving an oxygen nucleophile. The treatment of alcohol **12** with iodine in dioxane at room temperature then produced the direct hydroxylation in a syn relationship to the amide group through the iodo 1,3-oxazoline intermediate **13**, which was efficiently hydrolysed with trifluoroacetic acid in tetrahydrofuran-water at 80°C to afford the amino ester derivative **14**. Subsequent deiodination reaction using tributyltin hydride in dichloromethane at room temperature and further treatment with trifluoroacetic acid gave the required compound **9** (Scheme 4).

Because we have previously obtained the δ- and γ-hydroxy-α-amino acids **2**, **3** and **4** with phenylcyclohexane skeletons, we have decided to increase this family of compounds synthesizing the β-hydroxy-α-amino acid **17** by means of the above described synthetic route, which is based on the intramolecular conjugate addition.

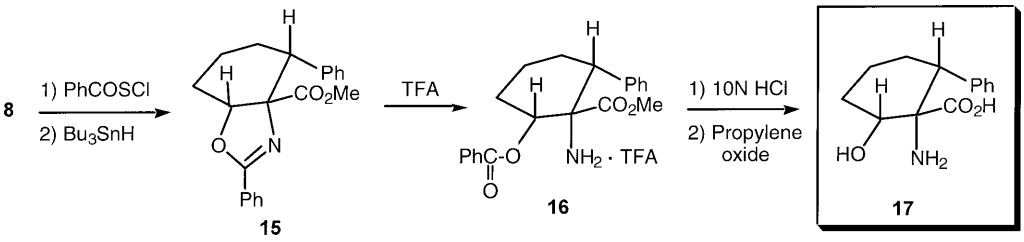


Scheme 3. Synthesis of 2,4-dihydroxy-α-amino acid **11** from 1,3-oxazoline intermediate **7**



Scheme 4. Synthesis of compound **9** from α,β-unsaturated compound **6**

Compound **8** was treated with phenoxythiocarbonyl chloride in the presence of 2,4-dimethylaminopyridine (DMAP) to produce the corresponding phenoxythiocarbonyl ester. This compound was then deoxygenated by treatment with tributyltin hydride and a catalytic amount of 2,2′-azobisisobutyronitrile (AIBN) (Khazauchi et al., 1993). The product, after purification by flash chromatography, gave a 70% yield of deoxygenated 1,3-oxazoline derivative **15**. The conversion of this compound into the desired conformationally constrained 2-hydroxy-6-phenylcyclohexane-α-amino acid was then easily achieved by opening of the 1,3-oxazoline heterocycle of intermediate **15** by the action of trifluoroacetic acid in tetrahydrofuran-water at 80°C, allowing the synthesis of the compound **16** in quantitative yield. Further



Scheme 5. Synthesis of 2-hydroxy-α-amino acid **17** from compound **8**

hydrolysis with 10N HCl under reflux over 3 days produced the required hydroxy-α-amino acid **17** with a good yield (Scheme 5).

In summary, we have developed a new strategy, using an intramolecular conjugate addition of the benzamide group to cyclohexenone, that allows the stereoselective synthesis of two conformationally constrained hydroxy-α-amino acids analogues of serine and phenylalanine.

Acknowledgements

We are indebted to the *Dirección General de Investigación Científica y Técnica* (PB97-0998-C02-02) and to the *Universidad de La Rioja* for theirs generous support. J. H. B. thanks the *Ministerio de Educación y Ciencia* for a doctoral fellowship.

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Received February 5, 1999