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L. Lecointe, Valérie Rolland, L. Pappalardo, M.L. Roumestant, P.H. Viallefont, et al.. Diastereoselective synthesis of non-proteinogenic α -amino acids. *Journal of Peptide Research*, 2008, 55 (4), pp.300-307. <10.1034/j.1399-3011.2000.00681.x>. <hal-04671744>

HAL Id: hal-04671744

<https://hal.science/hal-04671744v1>

Submitted on 28 Sep 2024

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Diastereoselective synthesis of non-proteinogenic α -amino acids

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Key words: asymmetric synthesis; azatyrosine; 2-hydroxypinan-3-one; tribromophenylalanine; trichlorophenylalanine

Abstract: The synthesis of enantiomerically pure azatyrosine, tribromophenylalanine and trichlorophenylalanine is described, using two methods, diastereoselective alkylation and (or) diastereoselective protonation of chiral enolates.

Abbreviations: AIBN, azoisobutyronitrile; EI, electronic impact; Fmoc, 9-fluorenylmethoxycarbonyl; HPLC, high-performance liquid chromatography; NBS, N-bromosuccinimide; THF, tetrahydrofuran; TLC, thin-layer chromatography; FAB, fast atom bombardment; DCM, dichloromethane; LDA, lithium diisopropylamide.

The synthesis of enantiomerically pure non-proteinogenic α -amino acids continues to be the focus of intense research by organic chemists. Analogues of phenylalanine are significant components of a range of biologically active compounds. Here, we report the concise synthesis of three amino acids: azatyrosine **1**, 2,4,6-tribromophenylalanine **2** and 2,4,6-trichlorophenylalanine **3**.

Azatyrosine [$\text{L-}\beta$ -(5-hydroxy-2-pyridyl)-alanine] **1** (Fig. 1) is an antibiotic isolated from *Streptomyces chibaneis* (1,2), which has been shown to promote permanent reversion of ras-dependent transformed cells to the normal phenotype in culture (3) and to inhibit the chemical induction of carcinogenesis in transgenic mice bearing oncogenic human ras (4). These mutations are responsible for kidney, forestomach and skin papilloma. When this study was initiated, only one enantioselective synthesis of **1** had been reported (5). Recently, three other reports (6–8) have been concerned with this compound because of its biological activity. Beside

Dates:

Received 22 June 1999
Revised 9 September 1999
Accepted 4 November 1999

To cite this article:

Lecointe, L., Rolland, V., Pappalardo, L., Roumestant, M.L., Viallefont, Ph. & Martinez, J. Diastereoselective synthesis of non-proteinogenic α -amino acids. *J. Peptide Res.*, 2000, **55**, 300–307.

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ISSN 1397-002X

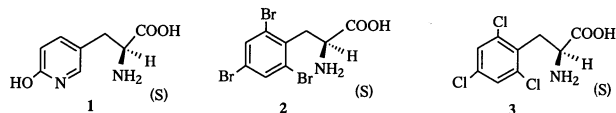


Figure 1.

its own activity, azatyrosine protected on its hydroxyl group by a di-*tert*-butyl phosphono group was incorporated into active peptides using a Fmoc strategy (9) to study structure–activity relationships.

2,4,6-Tribromo phenylalanine **2** and 2,4,6-trichloro phenylalanine **3** (Fig. 1) are analogues of natural phenylalanine and their synthesis has not yet been described. They were prepared in an optically pure form to be incorporated into active peptides and tritio-dehalogenated to serve as radio-active probes. Synthesis of 3',5'-diiodophenylalanine has been described by Escher (10), and this compound has been tritio-dehalogenated following incorporation into angiotensin II and bradykinin.

Experimental Procedures

Materials and Methods

Thin-layer chromatography (TLC) was performed on Merck precoated silica-gel 60F254 plates and spots were visualized using UV light or/and iodine vapour. Column chromatography was performed using Geduran silica gel. Chiral TLC was performed on Macherey-Nagel plates and spots were revealed by 0.2% ninhydrin solution. Chiral high-performance liquid chromatography (HPLC) was performed on a Waters HPLC system with a 486 detector apparatus on a Crown Pack CR+ column. Melting points were obtained on a Büchi 510 apparatus and were not corrected. Optical rotations were recorded using a Perkin–Elmer 141 polarimeter at the sodium D line. Mass spectra were measured on Jeol JMS DX 100 and DX 300 apparatus with fast atom bombardment (FAB) and electronic impact (EI) techniques.

^1H NMR spectra were recorded on a Bruker spectrometer AC 250 or 400 MHz.

Alkylating reagents synthesis

2-Bromomethyl-5-methoxypyridine **6**

3-Hydroxy-6-methylpyridine (1 g, 9.15 mmol, 1 eq) dissolved in dimethylformamide (30 mL), was added slowly to sodium hydride (0.44 g, 10 mmol, 1.1 eq washed under argon with petroleum ether and dried). When the addition was complete, methyl iodide (0.69 mL, 10 mmol, 1.1 eq)

was added dropwise and the solution was stirred for 12 h at room temperature. The solution was diluted with water (50 mL) and extracted three times with diethylether (30 mL). The organic phase was washed successively twice with water (15 mL) and with brine (20 mL). After drying over magnesium sulfate, the organic layer was evaporated to dryness under reduced pressure to yield 5-methoxy-2-methylpyridine **5** as a yellow oil (90%) (R_f = 0.7 in diethylether).

To *N*-bromosuccinimide (NBS; 4.05 g, 22.7 mmol, 1.4 eq) and azoisobutyronitrile (AIBN; 400 mg) dissolved in CCl_4 (45 mL) under argon and at 60°C was added 5-methoxy-2-methylpyridine **5** (2 g, 16.25 mmol, 1 eq) dissolved in CCl_4 (30 mL) and the solution was stirred for 4 h at 60°C. After filtration of the insoluble succinimide, the solution was concentrated under vacuum to give a yellow oil in 60% yield (R_f = 0.85 in diethyl ether). This oil was diluted in dry tetrahydrofuran (THF) and used directly for the alkylation.

^1H NMR (CDCl_3) δ (p.p.m.): 3.9 (s, 3H); 4.6 (s, 2H); 7.5 (m, 2H); 8.4 (m, 1H).

2,4,6-Tribromo-1-bromomethylbenzene **7**

N-Bromosuccinimide (0.6 g, 3.36 mmol, 1.4 eq) and a catalytic amount of AIBN (40 mg) were dissolved in CCl_4 (2 mL) under argon at 60°C; tribromotoluene (2 g, 16.25 mmol, 1 eq) dissolved in CCl_4 (30 mL) was added dropwise in 10 min and the solution was stirred for 12 h at 60°C until the starting product disappeared. After filtration of the insoluble succinimide, the solution was concentrated under vacuum to give 2,4,6-tribromo-1-bromomethylbenzene **7** as a yellow powder in 90% yield (R_f = 0.8 in petroleum ether), m.p. = 91°C.

^1H NMR (CDCl_3) δ (p.p.m.): 4.8 (s, 2H); 7.7 (s, 2H). M.S. EI⁺: $[\text{M}]^+$: m/z = 404 with isotopic peaks of Br.

2,4,6-Trichloro-1-bromomethylbenzene **9**

Trichlorobenzoic acid (1 g, 4.4 mmol, 1 eq) was dissolved in freshly distilled THF (22 mL) under argon at –30°C; triethylamine (0.62 mL, 4.4 mmol, 1 eq) was added dropwise. After 5 min stirring, ethyl chloroformate (0.42 mL, 4.4 mmol, 1 eq) was added and after 10 min sodium borohydride (NaBH_4 ; 0.5 g, 13.2 mmol, 3). Anhydrous methanol (44 mL) was added dropwise in 10 min at 0°C. The mixture was stirred for 10 min and neutralized with 1 N HCl solution (4 mL). The solvents were evaporated under reduced pressure and the residue was dissolved in ethyl acetate; the organic phase was washed successively with 1 N HCl, H_2O , NaHCO_3 15%, H_2O , dried on MgSO_4 and evaporated under reduced pressure. 2,4,6-Trichloro-1-hydro-

xymethylbenzene **8** was obtained as white crystals in 54% yield; m.p. = 95°C, R_f = 0.9 in diethylether/petroleum ether (1:1).

^1H NMR (CDCl_3) δ (p.p.m.): 7.4 (s, 2H); 4.9 (s, 2H); 1.6 (s, 1H). M.S. EI^+ : $[\text{M}]^+$: m/z = 209.94 100% with isotopic peaks of Cl (m/z = 211.94, 96.40% and m/z = 212.94, 31.13%).

2,4,6-Trichloro-1-hydroxymethylbenzene **8** (0.3 g, 1.4 mmol, 1.0 eq) was dissolved in anhydrous dichloromethane (DCM; 5 mL). A 1-M PBr_3 solution (1.5 mL, 1.56 mmol, 1.1 eq) was added under argon and the mixture was stirred for 4 h at room temperature; the mixture was extracted with a 15% NaHCO_3 solution, the organic layer dried on MgSO_4 and concentrated under reduced pressure. 2,4,6-Trichloro-1-bromomethylbenzene **9** was obtained as a white solid in 80% yield; m.p. = 40°C, R_f = 0.78 in diethyl ether/petroleum ether (1:5).

^1H NMR (CDCl_3) δ (p.p.m.): 7.4 (s, 2H); 4.6 (s, 2H). M.S. EI^+ $\text{C}_7\text{H}_4\text{Cl}_3\text{Br}$: $[\text{M}]^+$: m/z = 273.85, 100% with isotopic peaks of Cl (m/z = 271.85, 51.68% and m/z = 275.85, 64.35%).

Schiff's bases

11a, 11b, 11c

Alkyl glycinate hydrochlorides **10a**, **10b**, **10c** (56 mmol, 1 eq) were dissolved in DCM (100 mL) under argon. Benzophenone imine (9.38 mL, 56 mmol, 1 eq) was added and the mixture was stirred overnight at room temperature. After filtration, the solution was evaporated under reduced pressure. The solid was dissolved in diethylether (100 mL), washed with water (200 mL), dried over MgSO_4 and concentrated under reduced pressure. All Schiff's bases were obtained as white powders easily recrystallized in a mixture diethylether/hexane (1:1).

11a: R'' = Me; m.p. = 44–46°C; yield = 80%.

11b: R'' = Et; m.p. = 52–54°C; yield = 70%.

11c: R'' = *tert*-Bu; m.p. = 110–112°C; yield = 74%.

Racemic aminoesters synthesis

Racemic azatyrosine ethylester hydrochloride **15b**

To freshly prepared lithium diisopropylamide (LDA) at -10°C from diisopropylamine (0.78 mL, 5.56 mmol, 1.2 eq) in dry THF (20 mL) under argon and 2.5 N $n\text{BuLi}$ (2.2 mL, 5.53 mmol, 1.2 eq) and cooled at -78°C , was added the cooled Schiff's base **11b** (1.13 g, 4.6 mmol, 1 eq) in THF (10 mL); the mixture was stirred for 30 min to form the enolate. A cooled solution of 2-bromomethyl-5-methoxy-pyridine **6** (1.4 g, 6.9 mmol, 1.5 eq) in THF (10 mL) was added slowly and the mixture was stirred and maintained at

-78°C for 4 h. The solution was stirred overnight, the temperature was allowed to warm to -10°C . The mixture was treated with a NH_4Cl saturated solution. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. The residue was purified on silica gel with diethylether/petroleum ether (1:1) as the eluent. After purification, **12b** was obtained as an oil with 60% yield; R_f = 0.15 in diethylether/petroleum ether (1:1).

^1H NMR (CDCl_3) δ (p.p.m.): 8.1 (s, 1H); 7.5 (m, 2H); 7.25 (m, overlap of 6H); 7.0 (m, 2H); 6.6 (m, overlap of 2H); 4.5 (t, 1H, J = 7.2 Hz); 4.1 (d, 2H, J = 7.1 Hz); 3.75 (s, 3H); 3.4 (d, 2H, J = 7.22 Hz); 1.2 (t, 3H, J = 7.12 Hz).

To the Schiff's base **12b** (0.55 g, 1.42 mmol, 1 eq) dissolved in diethylether (10 mL) at 0°C in an ice bath was added dropwise 1 N HCl (1.7 mL, 1.7 mmol, 1.2 eq) and the mixture was stirred overnight at room temperature. The aqueous phase was evaporated and yielded racemic methoxy-yazatyrosine ethyl ester hydrochloride **15b** as a powder in 70% yield; m.p. = 183–185°C; R_f = 0.5 in EtOH/ NH_4OH (4:1).

^1H NMR (D_2O) δ (p.p.m.): 8.38 (d, 1H, J = 2.39 Hz); 7.87 (dd, 1H, J = 8.83 and 2.46 Hz); 7.72 (d, 1H, J = 8.87 Hz); 4.6 (t, 1H, J = 7.2 Hz); 4.5 (d, 2H, J = 7.1 Hz); 4.0 (s, 3H); 3.55 (d, 2H, J = 7.22 Hz); 1.0 (t, 3H, J = 7.12 Hz).

Racemic 2,4,6-tribromophenylalanine and

2,4,6-trichlorophenylalanine methylester hydrochlorides **16a** and **17a**

To freshly prepared LDA at -78°C was slowly added Schiff's base **11a** followed by 2,4,6-tribromo-1-bromomethylbenzene **7** (4 g, 9.8 mmol, 1.2 eq) in THF (20 mL). The mixture was stirred and maintained at -78°C for 4 h. The solution was then stirred overnight, the temperature being allowed to warm to -10°C . The alkylated Schiff's base was treated with a NH_4Cl saturated solution. The organic phase was dried over MgSO_4 and evaporated under reduced pressure. The product **13a** precipitated in diethylether and was obtained as a yellow powder in 80% yield; m.p. = 141°C; R_f = 0.83 in diethylether/petroleum ether (1:1).

^1H NMR (CDCl_3) δ (p.p.m.): 3.48 (dd, 1H, J = 3.74 and 10.07 Hz); 3.75 (dd, 1H, J = 10.22 and 10.09 Hz); 3.8 (s, 3H); 4.73 (dd, 1H, J = 3.72 and 10.17 Hz); 6.7 (m, 2H); 7.3 (m, overlap of 6H); 7.5 (s, 2H); 7.7 (m, 2H).

13b: R'' = Et; yield = 65%; orange powder; m.p. = 112°C.

13c: R'' = *tert*-Bu; yield = 90%; orange powder; m.p. = 131°C.

Using the same protocol, the Schiff's base of trichlorophenylalanine methyl ester **14a** was obtained as crystals in 95% yield; R_f = 0.8 in diethylether/petroleum ether (1:1).

^1H NMR (CDCl_3) δ (p.p.m.): 3.43 (dd, 1H, $J = 4.04$ and 13.68 Hz); 3.68 (dd, 1H, $J = 10.04$ and 13.66 Hz); 3.8 (s, 3H); 4.65 (dd, 1H, $J = 3.99$ and 10.0 Hz); 6.7 (m, 2H); 7.3 (m, 6H); 7.45 (s, 2H); 7.7 (m, 2H).

14c: $R'' = \text{tert-Bu}$: yield = 45%; white crystals; m.p. = 132°C .

The Schiff's base of tribromophenylalanine methylester **13a** (2.5 g, 4.2 mmol, 1 eq) was dissolved in DCM (20 mL) at 0°C in an ice bath. 1 N HCl (6 mL, 6 mmol, 1.4 eq) was added dropwise and the mixture was stirred overnight at room temperature. The aqueous phase was evaporated, dissolved in a minimum of methanol and precipitated in diethylether to afford the racemic tribromophenylalanine methyl ester hydrochloride **16a** as a powder in 80% yield; $R_f = 0.5$ in EtOH/ NH_4OH (4:1).

^1H NMR (D_2O) δ (p.p.m.): 3.48 (dd, 1H, $J = 10.2$ and 10.07 Hz); 3.75 (dd, 1H, $J = 10.22$ and 10.09 Hz); 3.8 (s, 3H); 4.73 (dd, 1H, $J = 10.2$ and 10.17 Hz); 7.76 (s, 2H).

16b: $R'' = \text{Et}$: yield = 85%.

16c: $R'' = \text{tert-Bu}$: yield = 90%.

The Schiff's base of trichlorophenylalanine methylester **14a** was hydrolysed as described for **13a** to afford trichlorophenylalanine methyl ester hydrochloride **17a** as a powder in 71% yield; $R_f = 0.5$ in EtOH/ NH_4OH (4:1).

^1H NMR (D_2O) δ (p.p.m.): 3.53 (dd, 1H, $J = 7.32$ and 14.16 Hz); 3.66 (dd, 1H, $J = 8.66$ and 14.18 Hz); 3.88 (s, 3H); 4.46 (dd, 1H, $J = 7.4$ and 8.7 Hz); 7.6 (s, 2H).

17c: $R'' = \text{tert-Bu}$: yield = 70%.

Diastereoselective protonation of chiral Schiff's bases

Schiff's bases **18b**, **19a**, **20a** were prepared from racemic amino esters arising from hydrochlorides **15b**, **16a**, **17a**. Starting from **15b** the standard procedure in toluene using BF_3 , Et_2O as catalyst (11) was used and afforded the Schiff's base **18b** in 60% yield. The two diastereomers were detected R_f : 0.14 and 0.3 (Et_2O).

Starting from **16a** and **17a**

To the amino ester from **16a** (3 mmol, 1.5 eq) in dichloromethane (50 mL) was added 2-hydroxy pinan-3-one (2 mmol, 1 eq), BF_3 , Et_2O (4 drops) and 4-Å molecular sieves (10 g). The mixture was treated for 3 days at 40°C , filtered on celite and the filtrate evaporated under reduced pressure to yield the Schiff's base **19a** in 45% yield after purification on silica gel column chromatography [two diastereomers R_f : 0.75 and 0.85 (Et_2O)]. **17a** gave the Schiff's base **20a** in 50% yield [two diastereomers R_f : 0.67 and 0.35, ether/petroleum ether (1:2)]

To *tert*-BuOK (10.45 mmol, 2.1 eq) dissolved in anhydrous THF (20 mL) and cooled at -78°C under argon was added dropwise the cooled Schiff's base **18b** or **19a** or **20a** (5 mmol, 1 eq) dissolved in THF (20 mL). The mixture was stirred for 30 min at -78°C and hydrolysed with a saturated solution of NH_4Cl . The aqueous phase was extracted three times with Et_2O , the organic phase was dried (MgSO_4) and concentrated under reduced pressure. Diastereomeric excesses were evaluated by ^1H NMR spectroscopy and HPLC analysis on a C18 inverse phase column at 254 nm with acetonitrile/water (40:60) as the eluent.

Schiff's bases of protected azatyrosine **21b**, of phenylalanine derivatives **22a** and **23a** were obtained as oils in 95% yield.

R_f (**21b**) = 0.3 (diethylether).

^1H NMR (C_6D_6) δ (p.p.m.): 0.2 (s, 3H); 1.18 (s, 3H); 1.28 (s, 3H); 2.53–1.5 (m, 6H); 3.18 (s, 3H); 3.33 (s, 3H); 3.57 (dd, 1H, $J_1 = 13.45$ Hz and $J_2 = 4.24$ Hz); 5.2 (dd, 1H, $J_1 = 9.44$ Hz and $J_2 = 4.26$ Hz); 6.61 (dd, 1H, $J_1 = 8.49$ Hz and $J_2 = 2.97$ Hz); 6.82 (d, 1H, $J = 8.46$ Hz); 8.24 (d, 1H, $J = 2.91$ Hz).

R_f (**22a**) = 0.75 (diethylether).

^1H NMR (CDCl_3) δ (p.p.m.): 0.15 (s, 3H); 0.98 (s, 3H); 1.4 (s, 3H); 1.5–2.5 (m, overlap of 6H); 3.38 (s, 3H); 3.45 (dd, 1H, $J_1 = 4.52$ Hz and $J_2 = 13.05$ Hz); 3.9 (dd, 1H, $J_1 = 13.14$ Hz and $J_2 = 10.54$ Hz); 4.85 (dd, 1H, $J_1 = 4.45$ Hz and $J_2 = 10.51$ Hz); 7.3 (s, 2H).

R_f (**23a**) = 0.35 [diethylether/petroleum ether (1:2)].

^1H NMR (CDCl_3) δ (p.p.m.): 0.70 (s, 3H); 1.20 (s, 3H); 1.4 (s, 3H); 1.6–2.6 (m, overlap of 6H); 3.45 (dd, 1H, $J_1 = 13.60$ Hz and $J_2 = 5.02$ Hz); 3.55 (dd, 1H, $J_1 = 13.61$ Hz and $J_2 = 9.44$ Hz); 3.7 (s, 3H); 7.3 (s, 2H).

Optically pure amino acids

(S)Azatyrosine 1

The Schiff's base **21b** (1.8 g, 5 mmol) was hydrolysed in a mixture of 1 M boric acid (31 mL) in THF (2.4 mL) and phosphate buffer pH 6.8 (5 mL). The mixture was stirred for 30 min at room temperature (pH 6.2–6.5). After concentration of THF, 1 N HCl was added (pH 2) and the aqueous phase was extracted three times with diethylether (10 mL). The aqueous phase was neutralized with 15% NaHCO_3 and extracted three times with dichloromethane (15 mL). The organic phase was dried (MgSO_4) and concentrated under reduced pressure. The optically pure aminoester was obtained in 70% yield as an oil [$R_f = 0.5$; EtOH/ NH_4OH (4:1)].

The amino ester (1 g, 4.7 mmol, 1 eq) was stirred for 3 days at room temperature in 1 N HCl (19 mL). The solution was concentrated under reduced pressure and the residue purified on DOWEX 50WX8 (60–170 mesh) using a mixture of H₂O/NH₄OH to give 5-methoxy azatyrosine in 80% yield; m.p. = 150–152°C.

¹H NMR (D₂O) δ : 3.3 (m, 2H); 3.95 (s, 3H); 4.1 (m, 1H); 7.4 (d, 1H, J = 8.6 Hz); 7.5 (dd, 1H, J = 8.5 Hz and J_2 = 2.75 Hz); 8.25 (d, 1H, J = 2.7 Hz). MS FAB⁺ [MH⁺] = 197.

To 5-methoxy azatyrosine (0.07 g, 0.36 mmol, 1 eq) dissolved in dichloromethane (25 mL) under argon at –78°C was slowly added 1 M BBr₃ (1.4 mL, 4 eq) and DCM (10 mL). The mixture was stirred for 30 min at –78°C and the temperature was allowed to increase to room temperature and was then maintained for 12 h at this temperature. The solution was cooled to 0°C, water was added and the mixture evaporated under reduced pressure. The residue was purified on DOWEX 50WX8 (60–170 mesh) using a mixture of H₂O/NH₄OH; m.p. = 260–262°C.

$[\alpha]_D^{25} = +53.8$ (c = 1, 1 N HCl) [lit⁴] + 55 c = 1, 1 N (HCl).

¹H NMR (D₂O) δ : 3.29 (m, 2H); 4.2 (m, 1H); 7.4 (d, 1H, J = 8.6 Hz); 7.55 (dd, 1H, J = 8.5 Hz and J_2 = 2.8 Hz); 8.25 (d, 1H, J = 2.7 Hz).

(S) 2,4,6-Tribromophenylalanine **2** and trichlorophenylalanine **3** Schiff's base **22a** (1.0 g, 1.65 mmol), or Schiff's base **23a**, was dissolved in THF (4 mL) and an aqueous solution of 15% citric acid (8.3 mL, 6.5 mmol, 4 eq) was added dropwise. The mixture was stirred at room temperature for 2 days and the aminoester precipitated. It was filtered and washed with diethylether. The amino ester (0.37 g, 0.8 mmol) was treated with 5 M HCl (1.7 mL, 10) in ether (5 mL) for 3 days at room temperature. A precipitate formed and was filtered (80% yield for **2** and **3**) and purified by sublimation. Because of their insolubility the $[\alpha]_D^{25}$ of **2** and **3** were not evaluated.

¹H NMR (**2** and **3**) were carried out in 2 M NaOH at 60°C.

¹³C NMR (**2** and **3**) were carried out in 2 M NaOH at 80°C.

R_f (**2**) = 0.75 in EtOH/NH₄OH (4:1).

m.p. = 210–212°C.

¹H NMR (**2**): δ (p.p.m.): 2.8 (dd, 1H, J_1 = 8.66 Hz and J_2 = 13.62 Hz); 3.0 (dd, 1H, J_1 = 6.87 Hz and J_2 = 13.67 Hz); 3.4 (dd, 1H, J_1 = 8.58 Hz and J_2 = 6.96 Hz); 4.8 (H₂O); 7.6 (s, 2H).

¹³C NMR (**2**): δ (p.p.m.): 42.1, 56.6, 121.1, 126.8, 135.5, 138.1, 182.5.

M.S. EI [M]⁺: m/z = 402 and [M-COOH]⁺: m/z = 356 with isotopic peaks of Br.

Anal. calcd for C₉H₈O₂NBr₃: C, 26.89; H, 2.00; N, 3.48. Found: C, 26.58; H, 1.98; N, 3.30.

R_f (**3**) = 0.72 in EtOH/NH₄OH (4:1).

¹H NMR (**3**): δ (p.p.m.): 2.92 (dd, 1H, J_1 = 8.7 Hz and J_2 = 13.7 Hz); 3.2 (dd, 1H, J_1 = 6.85 Hz and J_2 = 13.65 Hz); 3.4 (dd, 1H, J_1 = 8.7 Hz and J_2 = 7.02 Hz); 4.7 (H₂O); 7.76 (s, 2H).

¹³C NMR (**3**): δ (p.p.m.): 44.0, 57.8, 126.2, 128.8, 137.5, 139.1, 180.4.

3: M.S. FAB + [MH⁺] = 268.

Diastereoselective alkylation

21c by diastereoselective alkylation with CH₃MgBr

The Schiff's base of *tert*-butyl glycinate was prepared using the standard procedure from (2R, 3R, 5R)-2-hydroxypinan-3-one in toluene with BF₃·Et₂O as the catalyst (11) in 85% yield. After purification by chromatography on a silica-gel column, the Schiff's base (1 eq) dissolved in THF was cooled at –15°C under argon. A 3 M solution of CH₃MgBr (1.1 eq) was then added slowly, the mixture was stirred for 30 min, added to freshly prepared LDA at –10°C (1.1 eq), cooled at –78°C and stirred for 30 min to form the enolate. A cooled solution of 2-bromomethyl-5-methoxypyridine **6** (1.5 eq) in THF was then added slowly and the mixture was stirred and maintained at –78°C for 4 h. The solution was then stirred overnight, the temperature increased to –10°C, and was treated with a NH₄Cl saturated solution. The organic phase was dried (MgSO₄) and evaporated under reduced pressure. The residue was purified on a silica-gel column with diethylether/petroleum ether (1:1) as the eluent to yield **21c** as an oil (yield, 60%).

Diastereoselective alkylation – general procedure

To freshly prepared LDA (2.1 eq) was added the cooled Schiff's base (1 eq) and the mixture was stirred for 30 min to form the enolate. A cooled solution of 2,4,6-tribromo-1-bromomethylbenzene **7** (or 2,4,6-trichloro-1-bromomethylbenzene **9**) (1.5 eq) in THF was then added slowly

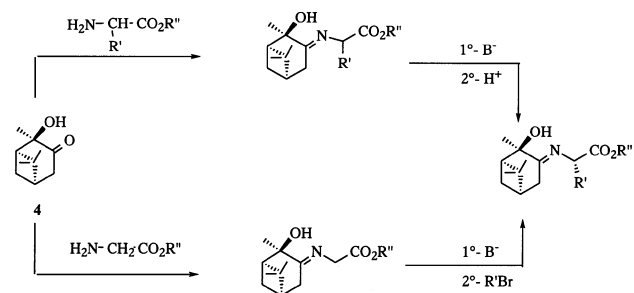


Figure 2.

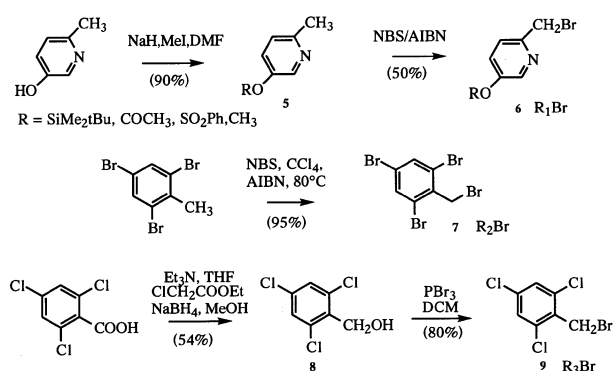


Figure 3.

and the mixture was stirred and maintained at -78°C for 4 h, and the temperature was allowed to increase to -10°C .

Treatment and purification were as described previously.

Results and Discussion

Two efficient methods of diastereoselective synthesis for these optically pure amino acids are described: diastereoselective protonation (11) and diastereoselective alkylation (12) of enolates of chiral Schiff's bases prepared from 2-hydroxypinan-3-one **4** (13) and α -amino esters (Fig. 2); the first step was the preparation of the alkylating agents (Fig. 3).

Commercially available 2-methyl 5-hydroxy pyridine was easily transformed into 2-bromomethyl-5-methoxy pyridine **6** (R₁Br), which is necessary for the synthesis of azatyrosine **1**, after protection of the hydroxyl group and bromination using NBS in the presence of AIBN, in 45 % overall yield. Of several protecting groups tested (SiMe₂tBu, COCH₃, SO₂Ph, CH₃), the methyl group gave the best results in the following steps.

For the preparation of tribromophenylalanine **2**, the electrophile 2,4,6-tribromo-1-bromomethyl benzene **7**

Figure 5.

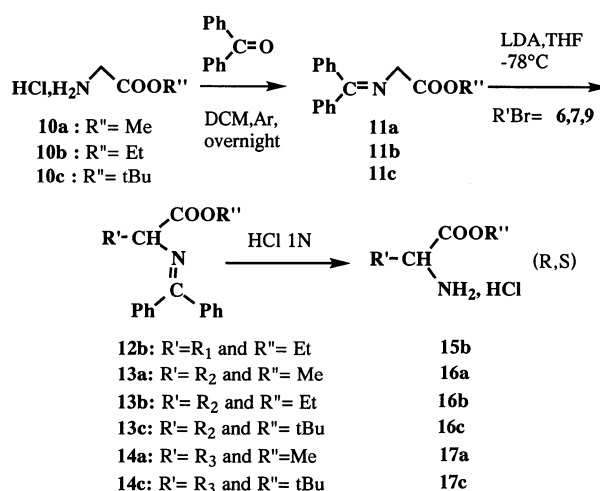
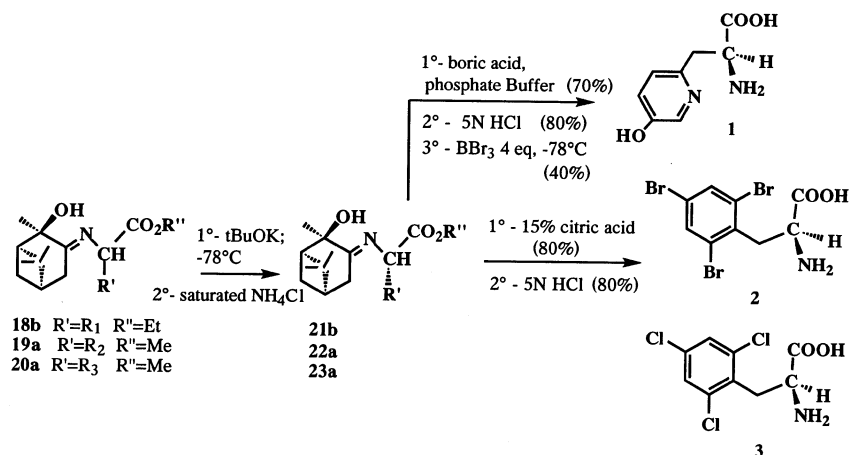


Figure 4.

(R₂Br) was obtained in 95 % yield after bromination of 2,4,6-tribromotoluene using NBS/AIBN. Finally 2,4,6-trichlorobenzoic acid afforded 1-bromomethyl-2,4,6-trichlorobenzene **9** (R₃Br) (useful for the synthesis of trichlorophenylalanine **3**) in 43 % overall yield after reduction of the acid function (14) and bromination of the alcohol using PBr₃.

In the first strategy, diastereoselective protonation, the racemic amino esters azatyrosine, tribromo and trichlorophenyl alanine alkyl esters (*tert*-butyl, methyl, ethyl) were easily prepared by alkylation of alkyl *N*-diphenylmethylene glycinate in THF using the bromides described previously and LDA as the base followed by hydrolysis using 1 N HCl (Fig. 4).

Chiral Schiff's bases were prepared from 2-hydroxypinan-3-one **4** and amino esters using the standard method: reflux in benzene or toluene in the presence of BF₃, Et₂O; **18b** was obtained in 60 % yield using this method. When **16a** and **17a** were used as starting materials, poor yields were obtained; the amino esters and (or) their imines were not stable under the experimental conditions used and were degraded.

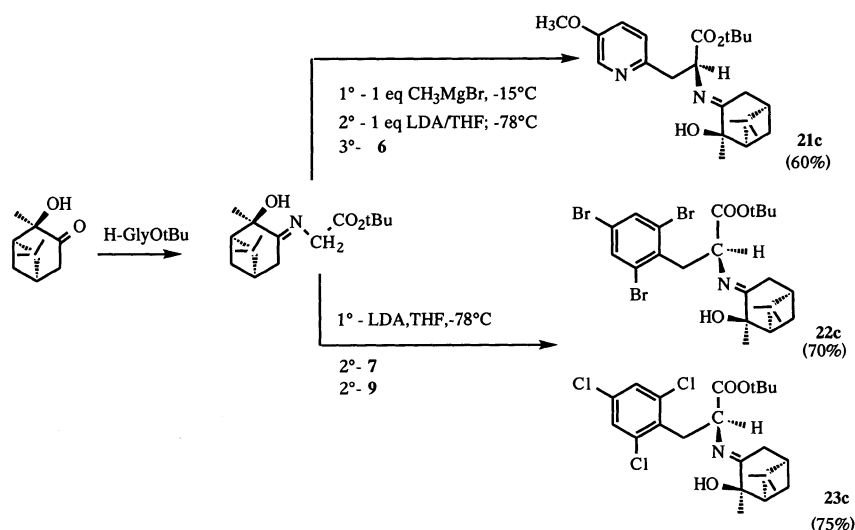


Figure 6.

Schiff's bases **19a** and **20a** were prepared in 50% yield in dichloromethane at 40°C in the presence of BF_3 , Et_2O and molecular sieves.

Two bases were used for enolate formation: KHMDs and tert-BuOK at -78°C in anhydrous THF; protonation was carried out using a saturated solution of NH_4Cl . Under these conditions azatyrosine, tribromo and trichloro phenylalanine were obtained in good yields and excellent enantioselectivity after hydrolysis of the imine (boric and or citric acid) and ester functions (5 N HCl). In the case of azatyrosine, the limiting step was the deprotection of the hydroxyl function, which was performed using boron tribromide (40% yield) (Fig. 5).

The second strategy examined was the diastereoselective alkylation by the bromides **6**, **7** and **9** of the enolate of the chiral Schiff's base prepared from 2-hydroxypinan-3-one and tert-butyl glycinate at -78°C (Fig. 6). Two methods were used for enolate formation. In method A, the chiral Schiff's base was treated with two equivalents of LDA in anhydrous THF under argon at -78°C ; in method B, the same chiral Schiff's base was treated with 1 eq CH_3MgBr followed by

1 eq LDA in anhydrous THF under argon at -78°C . For the three bromides **6**, **7** and **9** the chemical yields were the same (70–75%) using the two methods, the diastereoselectivity was excellent using method A or B for tribromo and trichloro phenylalanine; however, for azatyrosine method B yielded the best results (d.e. $> 98\%$) in the absence of CH_3MgBr , 95% diastereoselectivity was detected (^1H NMR and HPLC).

Conclusion

The three amino acids azatyrosine **1**, tribromophenylalanine **2** and trichlorophenylalanine **3** were obtained in an enantiomerically pure form using the two methods diastereoselective alkylation and diastereoselective protonation. The former, diastereoselective alkylation, afforded target compounds in better yields than diastereoselective protonation; for this latter method the modest yield arises essentially from the difficulty of synthesis of the chiral Schiff's bases from racemic amino esters.

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