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A Convenient Synthesis of Protected (R)-α-Phenylproline Derivatives Using the Mitsunobu Reaction

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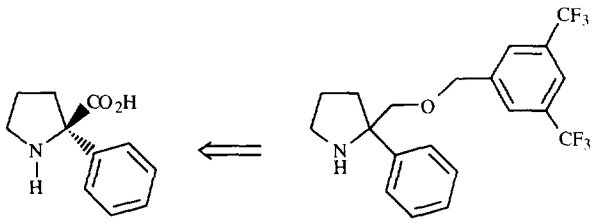
Abstract: Boc-(*R*)-α-phenylproline ethyl ester **1** was prepared in good yield starting from racemic phenylglycine. Condensation of phenylglycine ethyl ester with benzaldehyde furnished *N*-benzylidene phenylglycine ethyl ester which was allylated under phase transfer catalysis conditions. After acidic hydrolysis, the resulting α-allylphenylglycine ethyl ester was enzymatically resolved using PLE. Hydroboration and oxidation of the double bond, Boc-protection and subsequent ring closure using the Mitsunobu reaction protocol gave rise to Boc-(*R*)-α-phenylproline ethyl ester.

INTRODUCTION

Optically pure α-substituted α-amino acids are of considerable interest for the agrochemical and pharmaceutical industry *i.e.* they can be used as building blocks in the preparation of various biologically active molecules. Substituted prolines[1-10] for example have been used as chiral educts in organic synthesis and as tools in peptide research, where they can be used to influence the conformational flexibility of peptidess.[11-13]

We herein report a facile synthesis of protected forms of (*R*)-α-phenylproline **1**, an amino acid that was recently used in the synthesis of the non-peptide substance P antagonist **2**, selective for the NK_1 receptor.[14]



1 **2**

CHEMISTRY

Several methods are known in the literature for the asymmetric synthesis of α-substituted proline derivatives, among them the self-reproduction of chirality method developed by Seebach[15] and the diastereoselective alkylation of a chiral Shiff base by 1,3-diiodopropane, a method investigated by Bajgrowicz.[16]

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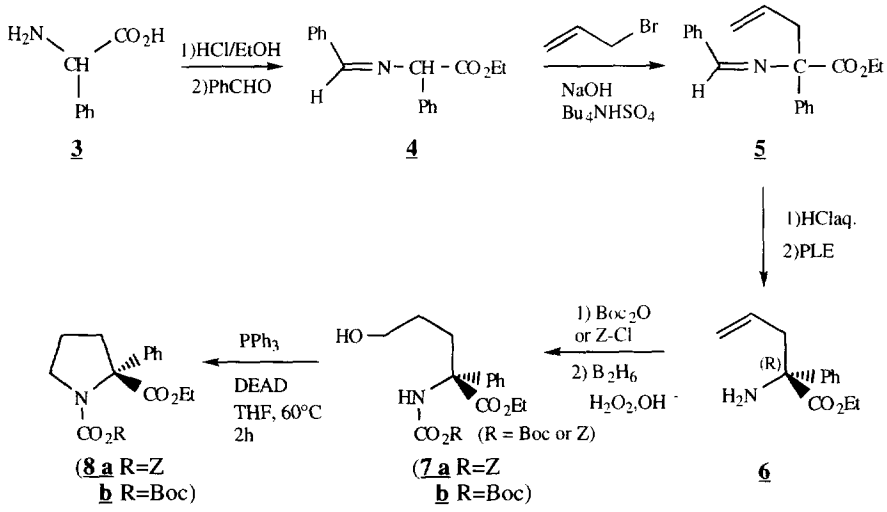
These methods suffer from either a low yield during the introduction of the phenyl group using the expensive and toxic benzene tricarbonylchromium, or from poor diastereoselectivity for the alkylation of the (-) or (+)-2-hydroxypinane-3-one derived Schiff base. A recent publication describes the preparation of α-phenyl proline derivatives via 1,3-dipolar cycloadditions of chiral stabilized azomethine ylids.[17]

We developed a synthetic pathway based on the Mitsunobu ring closure reaction of alcohol **7** (Scheme 1). The Mitsunobu reaction has been successfully used in the preparation of pyrrolidines[17] and proline derivatives such as α-benzylprolinamide.[18] An interesting feature is that a Boc-protected amino acid is obtained, which makes this methodology also very interesting for application in solid phase peptide synthesis (SPPS).

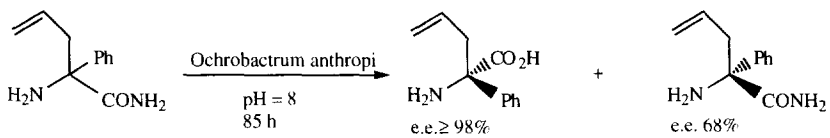
We prepared *N*-benzylidene phenylglycine ethyl ester **4** in 89% yield starting from (*R,S*)-phenylglycine by esterification and condensation of the ethyl ester with benzaldehyde. An allyl group was introduced under phase-transfer catalysis conditions using allylbromide, NaOH and Bu_4NHSO_4 in water/dichloromethane (90% crude yield). *N*-benzylidene-α-allylphenylglycine ethyl ester was then hydrolyzed using aqueous HCl (75% yield) and the resulting α-allylphenylglycine ethyl ester was enzymatically resolved using pig liver esterase[19] (PLE, Amano). Although the enzyme is not completely stereoselective for the hydrolysis of α-allyl-phenylglycine ethyl ester, the (*R*) enantiomer of **6** can be obtained in 96% enantiomeric excess by running the reaction for more than 50%. This (***R*)-6** was converted to the α-phenyl substituted analogue of *L*-proline in a three step procedure. After *N*-protection using Boc_2O or Z-Cl, the double bond was hydroborated, followed by oxidation to α-(3-hydroxypropyl)phenylglycine ethyl ester **7**. A slight difference in yield for the hydroboration/oxidation of the Boc- or Z-protected compound was observed. Using the Boc-group, a yield of 60% of **7a** was obtained, whereas the Z-protected **7b** was obtained in 70% yield.



Scheme 1

Treatment of the alcohol **7b** with 2 eq. triphenylphosphine and 1.5 eq. diethyl azodicarboxylate (DEAD), and refluxing the solution in THF for 2 hours resulted in a ring closure to Boc-α-phenylproline ethyl ester 8b in 55% yield. A higher yield of 63% was obtained for the Z-protected derivative **8a**.

Using PLE, the (*S*)-**6** can not be obtained in sufficent high optical purity (i.e. ≤ 72%[20]) since the enzymatic resolution is run for more than 50%. The α-phenyl substituted *D*-proline derivatives can, however, be prepared from the enantiomerically pure (*S*)-enantiomer of 2-amino-2-phenyl-4-pentenoic acid, which is obtained by resolution of the corresponding amide.[21] Similar to the ethyl ester of 2-amino-2-phenyl-4-pentenoic acid **6**, the racemic amide **9** was prepared in 73% yield by PTC allylation of *N*-benzylidene phenylglycine amide. The resolution of this amide was performed using the amidase of *Ochrobactrum anthropi* NCIMB 40321 (Scheme 2). The (S)-acid **10** was obtained in 38% yield with an e.e. of ≥ 98%. The remaining (R)-amide (49% yield) had an e.e. of 68%. Although this enzymatic resolution is rather sluggish -approximately 40% conversion after 85 h according to ammonia determination- it is fully stereoselective (E ≥ 200). This then gives access to (*S*)-α-phenylproline by the three step procedure described.



9 **10**

Scheme 2

CONCLUSION

The investigated method gives rapid access to both enantiomers of α-phenylproline and it can easily be performed on large scale.

EXPERIMENTAL SECTION

General Procedures

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AC-250 spectrometer, using residual isotopic solvent as internal reference. Chemical shifts are given in δ(ppm). Mass spectra were recorded on a AEI 902S spectrometer (FAB ionisation) or on a VG Quattro II spectrometer (electrospray ionisation, ESP) as indicated. Analytical thin layer chromatographies were performed using precoated glass-backed plates (Merck Kieselgel 60 F_{254}) and visualised with ultraviolet light or iodine. Column chromatographies were performed using Merck Kieselgel 60 (230-400mesh). Ethanol was dried by distillation from magnesium. THF was dried by distillation over sodium/benzophenone.

Phenylglycine ethyl ester hydrochloric acid salt

A solution of D,L-phenylglycine **3** (50g, 31mmol) in 150mL of EtOH was saturated using gaseous HCl, cooled to 0°C and further saturated with HCl. The solution was stirred overnight and evaporated. The resulting white solid was washed with ether and dried.

Yield: 71.3g (331 mmol) =100 %

^1H NMR (D_2O): 7.65 (m, 10H, H_{arom}), 5.5(s, 1H, H_α), 4.4 (q, 2H, $COO\underline{CH_2}CH_3$), 1.34 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS (m/z, %RA) : 180(M^++1, 100)

Mp: 199°C

TLC: R_f = 0.75 (CH_3CN(4)/MeOH(1)/H_2O(1))

N-Benzylidene-α-phenylglycine ethyl ester (4)

To a suspension of phenylglycine ethyl ester hydrochloride (64.6g, 300mmol) in 400mL CH_2Cl_2 was added 44g Na_2SO_4 and 32mL benzaldehyde (315 mmol). NEt_3 (44mL) was added to this suspension over a period of 30 minutes. The slurry was gently warmed for 30 minutes and the reaction was then allowed to proceed for 15 hours at ambient temperature. The precipitate was filtered and washed with a chloroform/diethyl ether (1:1) solution. The filtrate was then washed twice with 400mL water. The organic solutions were evaporated and the resulting oil was recrystallized overnight by addition of 30 mL acetone.

Yield: 71g (267 mmol) = 89% , colourless crystals

TLC: R_f = 0.55 ($CHCl_3$(3)/EtOAc(1))

^1H NMR ($CDCl_3$): 8.41 (s, 1H, H_{imine}), 7.88-7.2 (m, 10H, H_{arom}), 5.2(s, 1H, Hα), 4.15 (q, 2H, $COO\underline{CH_2}CH_3$), 1.23 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS (m/z, %RA) : 268(M^++1, 100), 194(80), 135(50), 91(100)

Mp: 68-70°C

N-Benzylidene-α-allyl α-phenylglycine ethyl ester (5)

N-Benzylidene-α-phenylglycine ethyl ester **4** (53.4g, 200mmol), 46g allyl bromide (0.38mol. 1.8 eq.) and 7.2g Bu_4NHSO_4 in 500mL CH_2Cl_2 were added to 350mL of a 10N NaOH solution. This solution was vigorously stirred during 4 hours. Dichloromethane (500mL) was added to the mixture and the organic phase was separated from the aqueous layer. After evaporation of the organic phase, the resulting oil was used in the following step without further purification.

Yield: 90% (crude product)

^1H NMR (250 MHz, $CDCl_3$): 8.2(s,1H,H_{imine}), 7.9-7.2 (m, 10H, $H_{arom.}$), 5.75 (m, 1H, H_γ), 5.1-4.9 (m, 2H, H_δ, $H_{\delta'}$), 4.2 (q, 2H, $COO\underline{CH_2}CH_3$), 3.2-2.6 (m, 2H, H_β, $H_{\beta'}$), 1.2 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS :(m/z, %RA) : 308(M^++1, 100), 266(18), 234(20), 186(40)

Hydrolysis to α-allyl-α-phenylglycine ethyl ester (6)

To a solution of *N*-benzylidene-α-allyl-phenylglycine ethyl ester **5** (47g, 153mmol) in 400mL diethyl ether was added 195mL 1N HCl and the reaction mixture was stirred overnight. The organic phase was separated from the water layer. The water layer was made alkaline (pH 9) by adding 3N NaOH solution. After several extractions with CH_2Cl_2, evaporation gave rise to a colourless oil which was purified by Kugelrohr distillation (110°C, 0.05mm Hg).

Yield: 25g (115 mmol, 75%)

^1H NMR ($CDCl_3$): 7.8-6.9 (m, 5H, H_{arom}), 5.65 (m, 1H, H_γ), 5.1-5.0 (m, 2H, H_δ, $H_{\delta'}$), 4.15 (q, 2H, $COO\underline{CH_2}CH_3$), 2.95 (m, 1H, H_β), 2.65 (m, 1H, $H_{\beta'}$), 1.9 (br s, 2H, NH_2), 1.2 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS :(m/z, %RA) : 220 (M^++1, 100), 203(30), 146(100), 104(55), 131(100), 91(100)

TLC: R_f=0.43 (s-BuOH(75)/MeOH(15)/H_2O(10))

Enzymatic resolution of (R,S)-α-allyl-α-phenylglycine ethyl ester

The ethyl ester **6** (12.8g, 59 mmol) was added to 100mL 0.05M KH_2PO_4 buffer (pH 8). The pH was adjusted to 8 by slow additon of a sulfuric acid solution. 500 mg pig liver esterase (PLE, AMANO) was added to this solution. The enzymatic hydrolysis was continued at 28°C and was monitored by TLC. During the course of the reaction, the pH was kept at 8 by automatic addition of a 0.5N NaOH solution with an auto-titrator.

The hydrolysis was allowed to proceed for 24 hours. A total amount of 8.2 mL 0.5 N NaOH was added at that time. The enzyme was filtered over celite and the ethyl ester was recuperated by extractions with ethyl acetate.

A total amount of 4.2g ethyl ester **6** (*R*-isomer) was recovered after enzymatic hydrolysis. The (*S*)-isomer was not recovered. The enantiomeric excess of the (*R*)-isomer was determined by HPLC *via* chiral precolumn derivatisation with o-phthalaldehyde/N-acetyl cysteine.[22,23] e.e.=96%.

Yield: 4.2g (65% of total amount of (R)-isomer in racemic mixture)

TLC : R_f = 0.16 (Et_2O(1)/pentane(2))

Z-Protection of (R)-α-allyl-α-phenylglycine ethyl ester

(*R*)-α-Allyl-α-phenylglycine ethyl ester **6** (2g, 9mmol) was dissolved in 30 mL dioxane(3)/water(1). One equivalent of K_2CO_3 (1.26g) was added, followed by 1.1 eq. of benzyloxycarbonyl chloride (10mmol, 1.56g). The reaction was continued for 2 hours. Water (20 mL) was added to the reaction mixture and this solution was extracted with ethyl acetate. The organic layer was washed with a $KHSO_4$ solution (10%), $NaHCO_3$ solution (6%) and brine. After drying of the organic layer over $MgSO_4$, evaporation yielded a colourless oil.

Yield: 2.76g (7.83mmol, 86%)

TLC: R_f = 0.7 (petroleumether(3)/ethyl acetate(1))

^1H NMR ($CDCl_3$): 7.6-7.2 (m, 10H, H_{arom}), 6.4 (s, 1H, NH_{amide}), 5.7 (m, 1H, H_γ), 5.2-4.9 (m, 4H, H_δ, $H_{\delta'}$, OCH_2Ph), 4.15 (q, 2H, $COO\underline{CH_2}CH_3$), 3.6-3.1 (m, 2H, H_β, $H_{\beta'}$), 2.15 (s,1H,OH), 1.15(t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS (m/z, % RA) : 354(M^++1, 100), 91(100)

Boc-Protection of (R)-α-allyl-α-phenylglycine ethyl ester

(*R*)-α-Allyl-α-phenylglycine ethyl ester **6** (2g, 9mmol) was dissolved in 30mL dioxane(3)/water(1). One equivalent of K_2CO_3 was added, followed by 1.1 eq. of Boc_2O. The reaction was continued for 3 hours. Water (20mL) was added to the reaction mixture and this solution was extracted with ethyl acetate. The organic layer was washed with 10%$KHSO_4$ solution, 6%$NaHCO_3$ solution and brine. After drying of the organic layer over $MgSO_4$, evaporation yielded a colourless oil.

*Yield:*2.67g (8.4 mmol, 92%)

TLC: R_f = 0.65 (petroleum ether(3)/ethyl acetate(1)

^1H NMR ($CDCl_3$): 7.6-7.2 (m, 5H, H_{arom}), 5.76-5.60 (m, 1H, H_γ), 5.20-5.0(m, 2H, H_δ, $H_{\delta'}$), 4.15 (q, 2H, $COO\underline{CH_2}CH_3$), 2.9 (m, 1H, H_β), 2.7 (m, 1H, $H_{\beta'}$), 2.13 (s, 1H, OH), 1.4 (s, 9H, t-butyl), 1.2 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS (m/z, %RA) :320(20), 220(100), 147(100)

N-Z-α-(3-Hydroxypropyl)-α-phenylglycine ethyl ester (7a)

Z-α-Allyl-α-phenylglycine ethyl ester (2g, 5.66mmol) was dissolved in 40 mL dry THF. $BH_3.Me_2S$ (17mmol, 850µL 10 M solution in THF) was added to this solution at 0°C. The solution was stirred for 1 hour. After slow addition of 3 eq. NaOH (5.67mL 3N solution) at 0°C, 3 eq. H_2O_2 (1.9mL 30% solution) were added. The temperature was kept at 0°C during these additions. The solution was allowed to reach room temperature and was then further stirred for one hour. After addition of 20mL of water, the reaction mixture was evaporated (removal of THF) and extracted with ethyl acetate to yield a colourless oil which was purified by column chromatography using petroleumether(3)/ethyl acetate(1) as eluent.

Yield: 1.47g (3.96mmol, 70%)

TLC: R_f = 0.26 (petroleum ether(3)/ethyl acetate(1))

^1H NMR ($CDCl_3$): 7.29 (m, 10H, H_{arom}), 6.40 (s, 1H, NH_{amide}), 5.0 (s, 2H, OCH_2Ph), 4.16 (q, 2H, $COO\underline{CH_2}CH_3$), 3.67 (s, 1H, OH), 2.9-1.3 (m, 6H, H_β, $H_{\beta'}$, H_γ, $H_{\gamma'}$, H_δ, $H_{\delta'}$), 1.15 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS :(m/z, %RA) : 372 (M^++1,50), 330 (20), 237 (40), 161 (50), 147 (100), 91 (100)

N-Boc-α-(3-Hydroxypropyl)-α-phenylglycine ethyl ester (7b)

N-Boc-α-(3-hydroxypropyl)-phenylglycine ethyl ester was prepared starting from 2g (6.27 mmol) *N*-Boc-α-allyl-α-phenylglycine ethyl ester following the above described experimental conditions.

Yield: 1.27g (3.67 mmol, 60%), colourless oil.

TLC: R_f = 0.3 (petroleum ether(3)/Ethyl acetate(1))

^1H NMR ($CDCl_3$): 7.44-7.2 (m, 5H, H_{arom}), 6.1 (br s, 1H, NH_{amide}), 4.1 (q, 2H, $COOCH_2CH_3$), 3.65 (m, 2H, H_δ, $H_{\delta'}$), 2.7-2.5 (m, 2H, H_β, $H_{\beta'}$), 1.7-1.5 (m, 2H, H_γ, $H_{\gamma'}$), 1.4(s, 9H, $C(CH_3)_3$), 1.1(t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS (m/z, % RA) : 338(M^++1, 50), 238(100), 147(100), 91(60)

(R)-Z-α-phenylproline ester (8a)

N-Z-α-(3-Hydroxypropyl)-α-phenylglycine ethyl ester (500mg, 1.35 mmol) was dissolved in 10 mL of dry THF. To this solution was added 707mg PPh_3 (2.7 mmol, 2 eq.) and 315μL (2mmol, 1.5eq.) diethyl azodicarboxylate (DEAD). The solution was then refluxed for 2 hours. After evaporation of the remaining slurry 20 mL hexane was added and the solution was refluxed. Acetonitrile was added until all material had dissolved. After overnight storage in the refrigerator, the triphenylphosphine oxide crystals were filtered off and washed with hexane. The combined organic fractions were evaporated and the remaining oil was purified on by column chromatography using petroleum ether(3)/ethyl acetate(1) as eluent.

Yield: 300 mg (0.85 mmol, 63%)

TLC: R_f=0.65 (petroleum ether(3)/ethyl acetate(1))

^1H NMR ($CDCl_3$): 7.4-7.2 (m, 10H, H_{arom}), 5.1 (s, 2H, OCH_2Ph), 4.15 (q, 2H, $COO\underline{CH_2}CH_3$), 3.5 (m, 2H, H_δ, $H_{\delta'}$), 2.2-1.5 (m, 4H, H_γ, $H_{\gamma'}$, H_β, $H_{\beta'}$), 1.2 (t, 3H, $COOCH_2\underline{CH_3}$)

EI/MS (m/z, % RA) : 354(M^++1, 100), 282(80)

(R)-N-Boc-α-phenylproline ethyl ester (8b)

N-Boc-α-phenylproline ethyl ester was prepared using the same method described above, starting from 500mg (1.48mmol) **7b**.

Yield: 260mg (0.82mmol, 55%)

TLC: R_f = 0.6 (petroleum ether(3)/ethyl acetate(1))

^1H NMR ($CDCl_3$): 7.25 (m, 5H, H_{arom}), 4.15 (q, 2H, $COO\underline{CH_2}CH_3$), 3.5 (m, 2H, H_δ, $H_{\delta'}$), 2.2-1.5 (m, 4H, H_γ, $H_{\gamma'}$, H_β, $H_{\beta'}$), 1.4 (s, 9H, t-butyl), 1.2 (t, 3H, $COOCH_2\underline{CH_3}$)

FAB/MS (m/z, % RA) : 320(M^++1, 30), 220(70), 147(100)

(R,S) 2-amino-2-phenyl-4-pentenoic amide (9)

This compound was prepared according to known literature procedures in 73% chemical yield.[21]

^1H NMR ($CDCl_3$): 7.2-7.6 (m, 6H, H_{arom} + amide), 5.6-5.8 (m, 2H, amide + -CH=), 5.12 (t, 2H, $=CH_2$), 3.20 (d, 1H, Hβ), 2.65 (d, 1Hβ').

Mp : 116-116.5°C

Resolution of (R,S)-2-amino-2-phenyl-4-pentenoic amide using Ochrobactrum anthropi

A suspension of 10.07g (53mmol) of racemic 2-amino-2-phenyl-4-pentenoic amide **9** in 90mL of water was neutralized with diluted acetic acid to pH 7.8. *Ochrobactrum anthropi* NCIMB 40321 (6.2g) was added and the mixture was incubated at 40°C in an orbital shaker (170 rpm). After 22h (NH_3 determination: conversion 25%) an additional amount of 10.8g of *Ochrobactrum anthropi* NCIMB 40321 was added. After 85 h (conversion approximately 40%) the reaction was stopped by acidification with 4N HCl solution to pH 2. The cells were removed by centrifugation (25 min, 10000 rpm) followed by filtration of the supernatant over Celite. The solution was brought to pH 9 with 4N NaOH solution and extracted with $CHCl_3$ (3 x 100mL). After drying of the organic layer 4.9 g (25.8mmol, 49%) of (*R*)-amide was isolated as a slightly yellowish oil, which slowly solidified on standing. E.e. 68%; $[\alpha]^{20}_D$ -21.2 (c 1.5, 1N HCl). The aqueous layer was neutralized to pH 7.0 with 4N HCl solution and concentrated at a rotary evaporator to approximately 20 mL. After cooling of the remaining suspension and filtration 3.8 g (9.9mmol, 38 %) of (*S*)-acid was obtained as white solid after drying. M.p. > 250°C; E.e. ≥ 98%, $[a]^{20}_D$ +21.0 (c 1, 1N HCl).

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