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Efficient synthesis of pyrenylalanine

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Summary. An efficient synthesis of L-3-(l′-pyrenyl)alanine (Pya), a highly fluorescent amino acid, is described. The amino acid was obtained by the classical asymmetric hydrogenation of chiral 1-acetyl-3-pyrenemethylidene-6-methyl-piperazine-2,5-dione. In the proposed improved procedure mild conditions of the synthesis were applied and the final product – N-tert-butoxycarbonyl-pyrenylalanine – was obtained in good yield. Pyrenylalanine, due to its interesting photophysical properties, can be applied as a fluorescent probe in numerous biochemical and conformational studies.

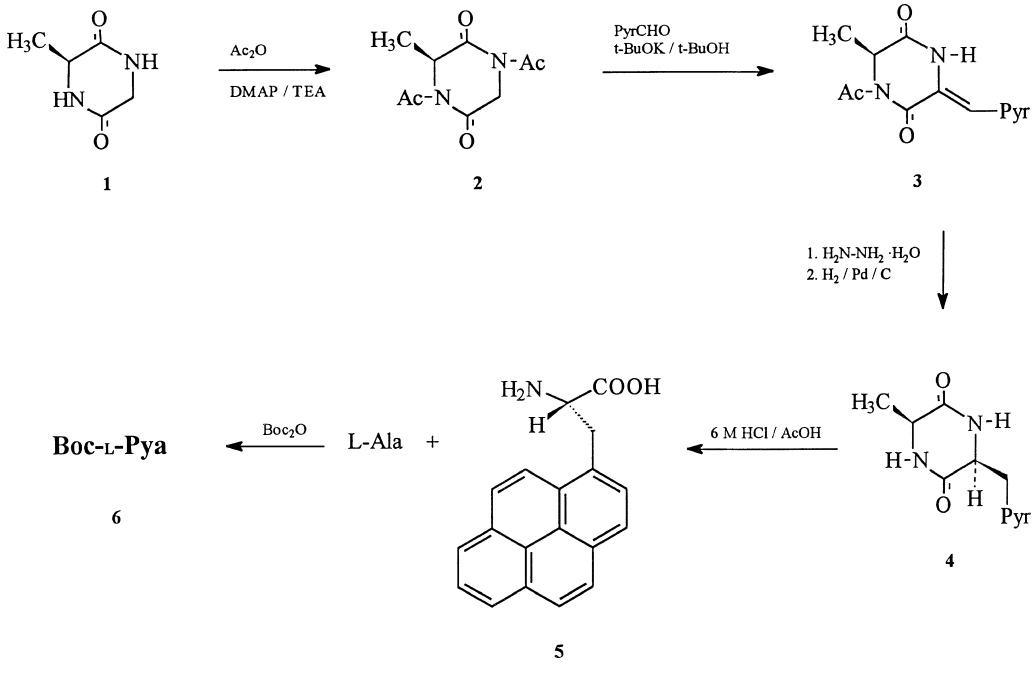
Keywords: Amino acids – Asymmetric synthesis – Pyrenylalanine – Fluorescent compounds

Introduction

Highly fluorescent compounds are very useful tools in conformational studies of biopolymers (Tanaka et al., 1996; Ono et al., 1995; Mihara et al., 1995), investigations of peptide-receptor interaction (Mihara et al., 1985) and measurements of interchromophoric distances (Mekler et al., 1997; Kuragaki et al., 1996). They are also very important for exploring mechanisms of enzyme action and inhibition (Greshkovich et al., 1996), for visualising cancer cells (Graczykowa et al., 1999; Sternberg et al., 1998) and for others applications (Lakowicz, 1999). Introduction of the fluorescent moiety into a peptide chain can be achieved either by a reaction of a fluorescent probe with functional groups present in peptide side chains (carboxylate, amino group, hydroxyl, sulphhydryl) or by direct use of an amino acids bearing fluorescent function at their side chain. Among proteinogenic amino acids only three possess fluorescent properties (Trp, Tyr and Phe), but sometimes native peptides do not contain these amino acid or their photophysical behaviour is complex (Chen et al., 1998). Incorporation of amino acid with photophysical behaviour different from the native fluorescent amino acids into a peptide chain seems to be beneficial. New, synthetic fluorescent amino acid can display shift of absorption and emission spectra to longwave region or can possess higher quantum

yield of fluorescence and longer lifetime of an excited state. Pyrenylalanine (Pya) is one of such new, fluorescent amino acids. There are few synthetic methods, which can be applied for preparation of pyrenylalanine (Egusa et al., 1983; Goedeweeck et al., 1984; Egusa et al., 1985; Mihara et al., 1987). The most efficient are enzymatic resolution of previously obtained racemic mixture of Pya (Mihara et al., 1995; Goedeweeck et al., 1984) and asymmetric catalytic hydrogenation of dehydro-precursor (Mihara et al., 1987). The method based on an alkylation of bornane 10,2-sultam-derived glycine equivalent (Oppolzer et al., 1992) with bromomethylpyrene gave low yield and a lot of side products (Szymańska et al.).

In this paper we present an improved procedure of the classical asymmetric hydrogenation of previously prepared chiral 1-acetyl-3-pyrenemethylidene-6-methyl-piperazine-2,5-dione (Mihara et al., 1987). In the described procedure milder conditions were applied for preparation of the precursor and the final amino acid was obtained in better yield (see Scheme 1). Pyrenylalanine was isolated and purified as N-tert-butoxycarbonyl derivative, which additionally allowed to improve yield.



Scheme 1. Synthesis of N-tert-butoxycarbonyl-pyrenylalanine (Boc-L-Pya)

Discussion

Pyrenylalanine (Pya) in an α-amino acid bearing fluorescent moiety in its side chain. Its photophysical properties (high fluorescence quantum yield {QY = 0,56 for Boc-L-Pya using 0,1 N quinine sulphate solution in sulphuric acid as a standard (QY = 0,55) (Szymańska et al.)}, long lifetime of an excited state

{τ = 147 ns for Boc-L-Pya} (Szymańska et al.)) are making the compound very suitable as a fluorescent probe in conformational studies of peptides by means of fluorescence and fluorescence resonance energy transfer (FRET) (Tanaka et al., 1996; Ono et al., 1995; Mihara et al., 1995), and in investigations of the interaction between peptides and membranes (Lee et al., 1989; Xu et al., 1992). More comprehensive applications of Pya require more efficient method of its synthesis. In our approach we improved the procedure delivered by H. Mihara and co-workers (Mihara et al., 1987). First of all we used milder conditions for acetylation of cyclic dipeptide c(Gly-L-Ala) **1**. Instead of acetylation by refluxing of c(Gly-L-Ala) with acetic anhydride for several hours (Kanamera et al., 1979), we applied procedure with dimethylaminopyridine (DMAP) as the acylation catalyst (Ragnarsson et al., 1998). This procedure gave better yield at milder conditions (reaction was carried out at room temperature) and less amount of side products was observed. Additionally, our procedure is more convenient from safety point of view as it avoids heating of the substrate with acetic anhydride. A higher yield for the condensation reaction of 1,4-diacetyl-3-methyl-piperazine-2,5-dione **2** with pyrene aldehyde was obtained, mostly because of application of tetrahydrofuran (THF) which improved solubility of the substrates and the product. Finally, we decided to change isolation method of pyrenylalanine from the mixture of amino acids (Ala and Pya). Instead of ion chromatography for separation of the free amino acids, we transformed them into N-tert-butoxycarbonyl derivatives and applied column chromatography on silica gel. This modification gave us simple and efficient method of amino acids separation. Moreover, we obtained derivative of pyrenylalanine ready to use in peptide synthesis – N-tert-butoxycarbonyl protected amino acid.

Experimental

Synthesis of cyclic dipeptide c(Gly-L-Ala) **1** was performed as described previously (Kanamera et al., 1979).

*Synthesis of 1,4-diacetyl-3-methyl-piperazine-2,5-dione **2***

To a stirred solution of 4.35 g (34 mmoles) of c(Gly-L-Ala) **1** in CH_3CN (200 ml) 6.42 ml (68 mmoles) of acetic anhydride, 8.20 ml (68 mmoles) of triethylamine and 0.83 g (6.8 mmoles) of dimethylaminopyridine were added. The stirring was continued until all substrates were consumed (TLC inspection). Then the volatile solvents were stripped off under reduced pressure and an oily residue was dissolved in ethyl acetate (150 ml). The organic phase was washed subsequently with water (3 × 20 ml), brine (2 × 20 ml) and dried ($MgSO_4$). Filtration of drying agent followed by evaporation of ethyl acetate gave brownish oil, which was purified by flash chromatography (Still et al., 1978) on silica gel (Kieselgel 60, 0,05–0,2 mm, Machery Nagel) using diethyl ether/petroleum ether (1:1, v/v) mixture as a mobile phase. Removal of the solvents from appropriate fractions gave the final product with 85% yield

(6.1 g); mp = 70–72°C (mp = 69–71°C (Kanamera et al., 1979)); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1,53 (3H, *d*, *J = 7,5 Hz*, $^{\beta}CH_3$ -Ala), 2,60 (6H, *s*, CH_3CO), 3,93 (1H, *br. m*, CH_2-Gly), 4,15 (1H, *br. m*, CH_2-Gly), 5,25 (1H, *q*, *J = 7,5 Hz*, $^{\alpha}$CH-Ala); IR (KBr) ν_{max} (cm^{-1}): 1713 (*br.* C=O).

Synthesis of 1-acetyl-3-pyrenemethylidene-6-methyl-piperazine-2,5-dione ***3***

Reaction was carried out under argon atmosphere

To a chilled to 0°C solution of 1.5 g (7 mmoles) of 1,4-diacetyl-3-methyl-piperazine-2,5-dione **2** in THF (20 ml) a solution of 0.78 g (7 mmoles) t-BuOK in t-BuOH (15 ml) was added dropwise during 1 h, followed by 2.4 g (10.5 mmoles) of pyrene aldehyde (portionwise). The stirring was continued at 0°C for 2 h and then at room temperature for 20 h. The solvents were removed under reduced pressure and the residue was dissolved in ethyl acetate (50 ml). The organic phase was washed with water (2 × 10 ml) and dried ($MgSO_4$). Filtration of drying agent followed by evaporation of the solvent gave a yellow solid, which was purified by column chromatography on silica gel (Kieselgel 60, 0,05–0,2 mm, Merck) using diethyl ether/petroleum ether (3 : 1, v/v) mixture as a mobile phase. Removal of the solvents from appropriate fractions gave the final product with 65% yield (1.73 g). The product was immediately used in a next step i.e. removal of the acetyl group and hydrogenation.

Synthesis of cyclic dipeptide c(L-Pya-L-Ala) ***4***

To a solution of 1-acetyl-3-pyrenemethylidene-6-methyl-piperazine-2,5-dione **3** (1.73 g, 4.53 mmoles) in dimethylformamide (20 ml) hydrazine hydrate (0.66 ml, 13.5 mmoles) was added. The resulting mixture was stirred at room temperature until TLC indicated consumption of the substrate (3–4 h). The volatile solvents were evaporated and the obtained residue (yellow solid) was dissolved in dimethylformamide (75 ml). To the resulting solution 10% Pd/C (0.1 g) was added and hydrogenation was continued until TLC inspection showed disappearance of the substrate (16 h). The catalyst was filtered off and the solvent was stripped off under reduced pressure. Crystallisation of the residue from dimethylformamide/diethyl ether yielded the product – cyclic dipeptide c(L-Pya-L-Ala) **4**: m = 0.79 g (51% yield); mp = 270–274°C (decomp.) (mp = 273–275°C (Mihara et al., 1987)). The dipeptide was used immediately in the next step of the synthesis (hydrolysis).

Hydrolysis of cyclic dipeptide ***4***

The cyclic dipeptide c(L-Pya-L-Ala) **4** (0.79 g, 2.33 mmoles) was suspended in 100 ml of 6 M HCl and AcOH mixture (1 : 1, v/v) and heated at 110°C for 20 h. Solvents were evaporated and the residue (greenish solid) was used directly in the next step – preparation of N-tert-butoxycarbonyl derivatives of amino acids.

Synthesis of tert-butoxycarbonyl-L-pyrenylalanine (Boc-L-Pya) ***6***

The mixture of amino acids obtained in the previous step (L-Ala and L-Pya) was dissolved in 2M NaOH (2.5ml) and t-BuOH (5ml). Then di-tert-butyldicarbonate (1.36g, 6.25mmoles) was added portionwise (0.5h) and pH was maintained at 8–9. The resulting mixture was stirred for 16h at room temperature. Then t-BuOH was stripped off under reduced pressure and the residue was diluted with water. The aqueous phase was washed with pentane (2×10ml), acidified with 1M $KHSO_4$ and extracted with ethyl acetate (3×20ml). The organic fractions were collected, washed with water (1×10ml), brine (1×10ml) and dried ($MgSO_4$). Filtration of drying agent, followed by removal of the solvent under reduced pressure yielded the mixture of Boc-protected pyrenylalanine and alanine which was separated by means of column chromatography on silica gel (Kieselgel 60, 0,05–0,2mm, Machery Nagel, mobile phase: methylene chloride/methanol = 9:1, v/v). Removal of the solvents from appropriate fractions gave the final product (Boc-L-Pya) with 51.3% yield (0.46g) for the last two steps; mp = 136–139°C (mp = 133–135°C (Mihara et al., 1987)); $[\alpha]_D^{23} = -72.5^0$ (c = 0.3, DMF), ($[\alpha]_D^{23} = -72.9^0$, c = 0.3, DMF (Mihara et al., 1987), $[\alpha]_D^{23} = -82.0^0$, c = 0.3, DMF (Mihara et al., 1995)); ^{1}H-NMR (DMSO-d_6) δ (ppm): 1,24 (9H, *s*, $(CH_3)_3$C-), 3,49–3,55 (1H, *br. m*, $^{\beta}$CH), 3,85–3,89 (1H, *br. m*, $^{\beta}$CH), 4,32–4,37 (1H, *br. m*, $^{\alpha}$CH), 7,99–8,39 (9H, *m*, aromatic); IR (KBr) ν_{max} (cm^{-1}): 3323 (N-H), 1749 (C=O, acid), 1663 (C=O, urethane).

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