Improved Procedures for the Synthesis of *N,N*-Diallyltyrosine Intermediates

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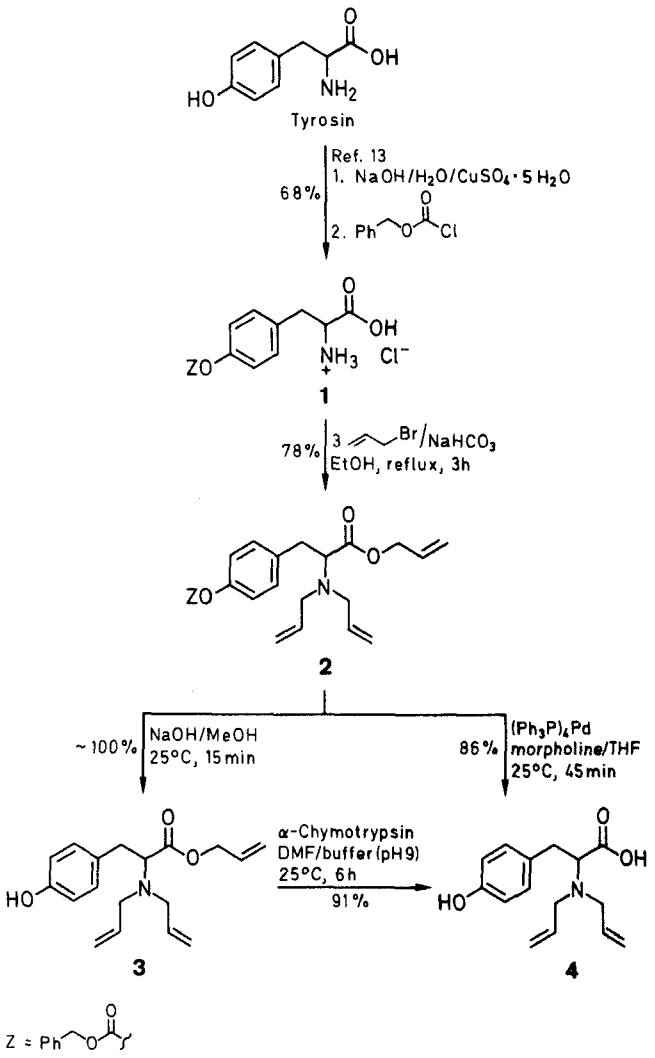
N,N-Diallyltyrosine allyl ester bearing a base-labile phenol protecting group can be obtained from *O*-benzyloxycarbonyltyrosine by a one-step allylation with allyl bromide. This allyl ester is efficiently deprotected with morpholine in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0) or by alkaline cleavage of the *O*-benzyloxycarbonyl group followed by enzymatic hydrolysis using α-chymotrypsin.

The synthesis of *N,N*-diallyltyrosylpeptides has become a general tool for the preparation of new opioid peptides with antagonist properties.[1–9] Refluxing the peptide in the presence of the appropriate alkylating agent and a base has been used to obtain these derivatives; however, such severe conditions do not seem to be generally applicable in peptide chemistry.[5,6,9] As alternatives, rather long and cumbersome reaction pathways have been developed.[9] Thus, the carboxy group of tyrosine is protected, for example, as the methyl or *tert*-butyl ester and the phenolic hydroxy group as the *tert*-butyl ether. Allylation followed by ester cleavage yields *N,N*-diallyl-Tyr(*t*-Bu)-OH. *N,N*-Diallyl-Tyr-OH may be obtained from *N,N*-diallyl-Tyr(*t*-Bu)-OBu-*t* by treatment with trifluoroacetic acid. In any case, five steps are needed. In order to obtain suitable *N*-allyl derivatives of tyrosine by a shorter reaction pathway and in high yield, the use of the allyl ester for intermediate carboxy group protection together with either palladium(0)[10] or enzymatic catalysis[11] for elimination of the ester allyl group is proposed in this work.

Tyrosine was converted into *O*-benzyloxycarbonyl-L-tyrosine hydrochloride (**1**) in high yield by treating the copper(II) complex of tyrosine with benzylcarbonochloridate. Reaction of compound **1** with an excess of allyl bromide produced the fully protected intermediate **2** in a single alkylation step. Finally, the allyl ester was cleaved in good yield by treatment with excess morpholine in tetrahydrofuran in the presence of tetrakis(triphenylphosphine)palladium(0) (allyl group transfer to morpholine). Under the basic conditions employed, the benzyloxycarbonyl group was also eliminated to give *N,N*-diallyltyrosine hydrochloride (**4** · HCl).

We also prepared *N,N*-diallyltyrosine (**4**) by an alternative procedure. As previously described,[11] allyl esters of α-amino acids can be cleaved under mild conditions by enzymatic hydrolysis. Taking advantage of the selectivity of certain hydrolyzing enzymes for the ester group, we have developed a selective deprotection sequence for **2** based on our findings[12] on the reactivity of aspartic acid allyl esters. Thus, compound **4** may also be obtained from **2** by an enzymatic approach: The *O*-benzyloxycarbonyl group protecting the phenolic OH of tyrosine derivative **2** is cleaved by treatment with an equimolecular amount of sodium hydroxide in methanol at room temperature. The resultant intermediate **3** is isolated by extraction and treated with bovine pancreatic α-chymotrypsin to achieve allyl ester hydrolysis with formation of *N,N*-diallyltyrosine (**4**) in a clean and simple step in good yield.



Bovine pancreatic α-chymotrypsin (E.C. 3.4.21.1; 350 U/mg) was purchased from Merck, Darmstadt. Purification of some intermediates was carried out by flash chromatography[14] using silica gel (40–63 μm) in 5 × 15 cm columns, and elution with an appropriate solvent system at a linear flow rate of 5 cm/min. MPLC purifications were achieved with reversed-phase C_{18} (40–63 μm), 2.5 × 31 cm column with gradient elution from A (0.1% aq TFA) to B (0.1% TFA in MeCN/0.1% aq TFA, 7:3) over 40 min at a flow rate of 5 mL/min. HPLC analyses were performed on a reversed-phase C_{18}, 0.4 × 25 cm column with isocratic elution using 0.1% aq TFA/0.1% TFA in MeCN in proportions indicated for each compound and UV detection at 215 nm. TLC analyses were performed on silica gel plates, 230–400 mesh, 0.25 mm (Merck, Darmstadt).

Melting points were determined in a Kofler apparatus and are uncorrected. Microanalyses were performed at the "Servei de Microanàlisi del C.I.D. (C.S.I.C.)" in Barcelona using a C,H,N microanalyzer model 1106 from Carlo Erba. ^{1}H-NMR spectra were recorded on a 80 MHz Bruker instrument.

O-Benzyloxycarbonyl-L-tyrosine Hydrochloride (1):

This derivative is prepared from L-tyrosine according to the procedure of Lit.[13] and recrystallized from MeOH/H_2O; yield: 68%; colorless solid, mp 218 °C (Lit.[13] mp 215 °C).

TLC: R_f 0.49 (BuOH/AcOH/H_2O, 4:1:1).

$C_{17}H_{18}ClNO_5$ (351.8) calc. C 50.04 H 5.15 N 3.98; found 49.78 5.36 4.10

^{1}H-NMR (DMSO-d_6/TMS): δ = 3.15 (d, 2H, β-Tyr), 4.23 (t, 1H, α-Tyr), 5.25 (s, 2H, Z), 6.7–7.1 (4H_{arom}, Tyr), 7.45 (s, 5H_{arom}, Z).

N,N-Diallyl-O-benzyloxycarbonyl-L-tyrosine Allyl Ester (2):

To a stirred solution of **1** (2.5 g, 7.1 mmol) in absolute EtOH (100 mL) is added anhydrous $NaHCO_3$ (5.97 g) and stirring is continued for a few minutes at 25°C. Then, allyl bromide (8.6 g, 71 mmol) is added dropwise and the mixture is refluxed for 3 h. The precipitated NaBr is then filtered off. The organic solution is evaporated to dryness and the oily residue is purified by flash chromatography using petroleum ether/EtOAc (7:3) as eluent; yield: 2.39 g (79%).

TLC: R_f 0.67 (petroleum ether/EtOAc, 1:1).

$C_{26}H_{29}NO_5$ (435.5) calc. C 71.70 H 6.71 N 3.21; found 71.59 6.39 3.30

^{1}H-NMR ($CDCl_3$/TMS): δ = 2.9–3.6 (m, 4H, 2CH_2N), 3.3 (d, 2H, β-Tyr), 3.8 (t, 1H, α-Tyr), 4.6 (d, 2H, OCH_2–C), 4.9–5.55 [m, 8H, CH_2(Z), 3C=CH_2], 5.65–6.1 (m, 3H, 3CH=C), 6.7–7.40 (9H_{arom}, Tyr and Z).

Two other components were also identified:

Allyl-Tyr(Z)-O-allyl; R_f 0.45 (TLC; petroleum ether/EtOAc 1:1).

^{1}H-NMR ($CDCl_3$/TMS): δ = 2.9–3.5 (m, 2H, CH_2N), 4.55 (d, 2H, OCH_2–C), 4.9–5.4 (m, 4H, 2C=CH_2), 5.25 [dd, 2H, CH_2(Z)], 5.6–6.1 (m, 2H, 2CH=C).

(Allyl)$_2$-Tyr(Z)-OH; R_f 0.05 (TLC; petroleum ether/EtOAc 1:1).

^{1}H-NMR ($CDCl_3$/TMS): δ = 2.9–3.5 (m, 2H, CH_2N), 4.9–5.4 (m, 4H, 2C=CH_2), 5.25 [dd, 2H, CH_2(Z)], 5.6–6.1 (m, 2H, 2CH=C).

N,N-Diallyltyrosine Allyl Ester (3):

Compound (0.5 g) is stirred with a 1 N solution (25 mL) of NaOH in MeOH for 15 min. The mixture is then evaporated and extracted with EtOAc (3 × 20 mL). Drying ($MgSO_4$) and evaporation of the extract affords **3**; yield: 331 mg g (~ 100%).

TLC: R_f 0.48 ($CHCl_3$/MeOH/AcOH, 95:5:3); 0.92 (MeCN/H_2O/AcOH, 17:2:1); 0.58 (petroleum ether/EtOAc 5:8).

HPLC: t_R = 4.7 min (0.1% aq TFA/0.1% TFA in MeCN, 2:3)

$C_{18}H_{23}NO_3$ (301.4) calc. C 71.72 H 7.69 N 4.64; found 71.41 7.88 4.98

^{1}H-NMR ($CDCl_3$/TMS): δ = 2.9–3.6 (d, 4H, 2CH_2N), 3.3 (dd, 2H, β-Tyr), 3.8 (t, 1H, α-Tyr), 4.6 (d, 2H, OCH_2–C), 5.1–5.4 (m, 6H, 3C=CH_2), 5.7–6.2 (m, 3H, 3CH=C), 6.7, 7.0 (4H_{arom}, Tyr).

N,N-Diallyl-L-tyrosine Hydrochloride (4 · HCl):

Method A, from **2** by Ester Cleavage with Morpholine/Pd(0): To a stirred solution of ester **2** (0.376 g, 0.84 mmol) in anhydrous THF (90 mL) kept in the dark and under argon, $(Ph_3P)_4Pd$ (100 mg) and morpholine (3 mL) are added and the mixture is stirred for 45 min at 25°C. The solvent is then evaporated, the residue is dissolved in 0.1 N aq HCl (1.5 mL), and this solution is filtered. The solid product thus isolated is purified by MPLC as described in the general experimental section. The final product is dissolved in 0.1 N aq HCl (3 mL) an lyophilized to give **4** as a white amorphous solid; yield: 213 mg (86%).

TLC: R_f 0.46 (MeCN/H_2O/AcOH 17:2:1).

HPLC: t_R = 3.1 min (0.1% aq TFA-0.1% TFA in CH_3CN, 1:1)

$C_{15}H_{20}ClNO_3$ (297.8) calc. C 60.50 H 6.77 N 4.70; found 60.83 6.92 4.56

^{1}H-NMR (D_2O/TMS_{ext}): δ = 3.15 (dd, 2H, β-Tyr), 3.55 (d, 4H, 2CH_2N), 5.1–5.4 (m, 4H, 2C=CH_2), 5.7–6.2 (m, 2H, 2CH=C), 6.75, 7.15 (4H_{arom}, Tyr).

Method B, from **3** by Enzymatic Ester Cleavage: To a stirred solution of compound **3** (0.280 g, 0.93 mmol) in DMF (45 mL), a mixture of α-chymotrypsin (100 µm) in $NaHCO_3$/Na_2CO_3 buffer (pH 9.0; 55 mL) is slowly added at 25°C. After 6 h, the mixture is lyophilized and the solid product purified by MPLC as described in the general experimental section. It is then dissolved in 1 N aqueous HCl (3 mL) and lyophilized to give **4** as an amorphous solid; yield: 257 mg (91%).

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