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A convenient two step transformation of tyrosine into the antihypertensive amino acid (*S*)-4-hydroxy-3-hydroxymethylphenylalanine

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Abstract—Treatment of tyrosine with paraformaldehyde and catalytic amounts of *p*-toluenesulfonic acid, at reflux in toluene, directly generates benzyl (*S*)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate, which on treatment with boron trichloride in dichloromethane, affords (*S*)-4-hydroxy-3-hydroxymethylphenylalanine.
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1. Introduction

The amino acid (*S*)-4-hydroxy-3-hydroxymethylphenyl-alanine **1**, a homologue of tyrosine **2**, isolated from seeds of *Caesalpinia tinctoria*,[1] is considered important in medicine for its antihypertensive properties, for its ability to chelate heavy metal ions and for its utility in studies concerning the treatment of Parkinson's disease.[2] Due to these properties, (*S*)-4-hydroxy-3-hydroxymethylphenylalanine **1** has been the subject of some chemical[2-4] and chemoenzymatic[5] syntheses, affording racemic mixtures[2a,3] or the enantiomeric pure compound (*S*)-**1** (Fig. 1).[2b,4,5]
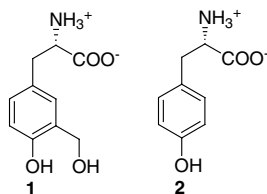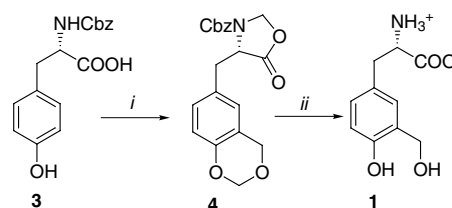

Figure 1.

Herein we report an alternative, short and efficient synthesis of the unnatural amino acid **1**, starting from commercial *N*-benzyloxycarbonyltyrosine **3** and using two simple but unexpected cascade reactions (Scheme 1).
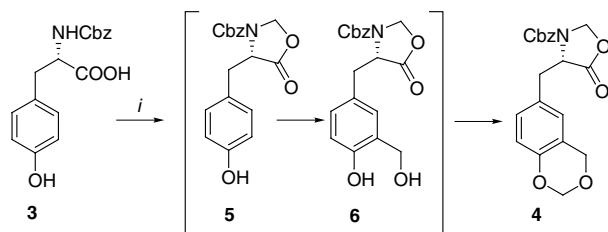

Scheme 1. Reagents and conditions: (i) paraformaldehyde (8 M equiv on **3**), *p*-TsOH, toluene, reflux, 1 h; (ii) BCl₃ (5 M equiv on **4**), CH₂Cl₂, 25 °C, 0.5 h.

2. Results and discussion

The first step is the treatment of the benzyloxycarbonyltyrosine **3** with excess of paraformaldehyde and catalytic amounts of *p*-toluenesulfonic acid at reflux in toluene to afford, via azeotropic removal of water, the benzyl (*S*)-4-(4H-1,3-benzodioxin-6-yl-methyl)-5-oxo-1,3-oxazolane-3-carboxylate **4** in high yields (≥80%). The second reaction (Scheme 1) causes the regeneration of all the protected functions of **4**, by treatment at 25 °C of a solution of **4** in dichloromethane with boron trichloride, a reagent previously used only for the scission of the aromatic methoxyl or methyl-endioxy groups.[6]

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The one-pot formation of **4** clearly involves three successive transformations (Scheme 2): the formation of the oxazolidinone **5**, the formylation of its aromatic ring and the acetalization of the formed 3-hydroxymethyl derivative **6** with formaldehyde.


Scheme 2. Reagents and conditions: (i) paraformaldehyde (8 M equiv on **3**), *p*-TsOH, toluene, reflux, 1 h.

This cascade of three reactions was in part unexpected on the basis of the previous report[7] that the reaction of benzyloxycarbonyltyrosine **3** in benzene, with paraformaldehyde and catalytic amounts of a sulfonic acid, via azeotropic removal of water, affords the 5-oxazolidinone **5**. In our work, we have seen that the 5-oxazolidinone **4** is the main product of the reaction and it is obtained in high yields ($\geqslant 80\%$) by simply performing the same reaction in refluxing toluene, in place of benzene, and continuing the reflux until the disappearance of **5**, the first formed compound. Moreover we observed that variable amounts of the 5-oxazolidinone **4** constantly accompany the formation of the 5-oxazolidinone **5** even in the case of reactions performed in refluxing benzene or in toluene at 25 °C for 36 h. The 5-oxazolidinone **4** forms in high yields also by treatment of pure **5** with paraformaldehyde. This could also explain the low yields of **5** (i.e., 37%) reported[7] in its preparation from benzyloxycarbonyl tyrosine **3**, and those just a little higher obtained by us at 25 °C (55%).

Also the second step of the procedure, the direct transformation of the 5-oxazolidinone **4** into the (*S*)-4-hydroxy-3-hydroxymethylphenylalanine **1**, by the action of BCl₃ in dichloromethane (Scheme 1), is unexpected on the basis of the known literature, which reports that 5-oxazolidinones are stable to the action of acids[8,9] and that benzyloxycarbonyl protecting groups are stable to BCl₃.[10] However, on studying this reaction, we have shown that using a large excess of BCl₃ (5 M equiv), both 5-oxazolidinone and benzyloxycarbonyl groups can be cleaved.

Following the reaction by HPLC and TLC, we were also able to monitor the intermediary formation of the *N*-benzyloxycarbonylmethanol **7** and of the benzyloxycarbonyl derivative **8** (HPLC and MS evidence). Thus, at the same time, we obtained evidence that the benzyloxycarbonyl group of **4** is the protecting group cleaved last. The intermediate formed **7** is obtained as the main compound of the reaction using a lower excess of BCl₃ (3 M equiv). It is quantitatively transformed on treatment with NaHCO₃ in aqueous methanol for a few minutes, the conditions we reported for the opening of

the 5-oxazolidinone nucleus.[11] On the contrary, all attempts to isolate in satisfactory yields the benzyloxycarbonyl **8** from the direct treatment of **4** with BCl₃ were unsuccessful (Scheme 3).
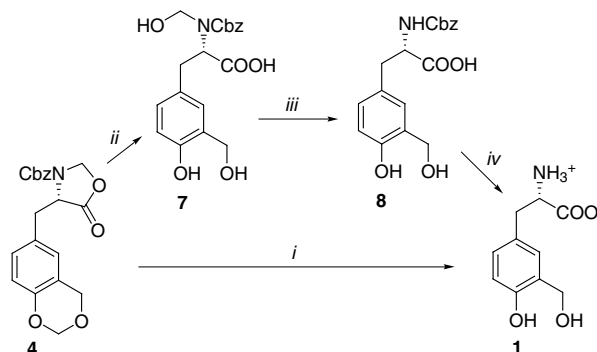
Scheme 3. Reagents and conditions: (i) BCl₃ (5 M equiv on **4**), CH₂Cl₂, 25 °C, 0.5 h; (ii) BCl₃ (3 M equiv on **4**), CH₂Cl₂, 0 °C, 3 h; (iii) NaHCO₃, H₂O–MeOH (1:1), reflux, 10 min.; (iv) BCl₃ (3 M equiv on **8**), CH₂Cl₂, 25 °C, 1 h.

3. Conclusion

In conclusion we report herein a simple but nonobvious preparation of (*S*)-4-hydroxy-3-hydroxymethylphenylalanine **1** using two reactions each, unexpected on the base of the literature reports. Moreover the result appears of a more broad and general interest since it expands the knowledge on the reactivity of both 5-oxazolidinone[11,12] and benzyloxycarbonyl groups, two widely used[13] protecting groups in amino acid chemistry.

4. Experimental

4.1. General

Nuclear magnetic resonance spectra were recorded at 298 K on Bruker AM-500 spectrometer operating at 500.13 MHz for ¹H and 125.76 MHz for ¹³C. Chemical shifts are reported in parts per million (ppm, δ units) relative to solvent signal (residual proton signal for proton spectra or carbon signal for carbon spectra).[14] ¹H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; br s, broad singlet; m, multiplet), coupling constant(s) in hertz, assignment of proton(s). Optical rotations were taken at 24 °C on a Perkin–Elmer 241 polarimeter and $[\alpha]_D$ values are given in $10^{-1} \deg \text{cm}^2 \text{g}^{-1}$. HPLC analyses were carried out on a RP-18 column (LiChroCART, 125 mm, 4 mm ID, 5 μm purchased from Merck); the mobile phase was MeOH/H₂O/TFA 50:50:0.5 v/v/v; the flow rate was 1 mL/min and the detection was performed at 275 nm. Mass spectra were obtained using a Finnigan LCQdeca (ThermoQuest) ion trap mass spectrometer fitted with an electrospray source (ESI). All reactions

were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F254) using UV light, 50% sulfuric acid or 0.2% ninhydrin solution in ethanol and heat as developing agent. E. Merck 230–400 mesh silica gel was used for flash column chromatography.[15]

4.2. Synthesis of (S)-4-hydroxy-3-hydroxymethylphenylalanine 1

4.2.1. Synthesis of benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate 4. A mixture of the (S)-N-benzyloxycarbonyltyrosine **3** (3.00 g; 9.5 mmol), p-toluenesulfonic acid (0.180 g; 0.95 mmol) and toluene (160 mL) were placed in a flask set for reflux and azeotropic separation of water. Then paraformaldehyde 2.28 g (76 mmol) was added to the refluxing solution in five portions, allowing the solution to clear before each subsequent addition. After 1 h the reaction was stopped, the solution was washed with aqueous NaHCO$_3$ and dried over anhydrous Na$_2$SO$_4$. After evaporation of the solvent under reduced pressure, the residue was chromatographed (eluting with hexane/ethyl acetate 60:40, v/v) to afford, the benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate **4** (2.81 g; Y = 80%): mp 97–98 °C, (from dichloromethane, diisopropyl ether: sinterizes at 88 °C); [α]$_D$ = +193.4 (c 1, CHCl$_3$); IR ν_{max} (KBr) 1796, 1665 cm^{-1}; ^{1}H NMR (DMSO-d_6): 7.45–7.25 (5H, overlapping, H-aromatics), 6.80 (1H, d, J = 8.1, H-aromatic), 6.72 (1H, d, J = 8.1, H-aromatic), 6.66 (1H, br s, H-aromatic), 5.29 (1H, d, J = 4.0), 5.25–5.16 (4H, overlapping), 4.77–4.70 (2H, AB system), 4.63 (1H, d, J = 4.0), 4.57 (1H, m), 3.12 (1H, dd, J = 14.1 and 3.4), 2.95 (1H, dd, J = 14.1 and 4.5). ^{13}C NMR (CDCl$_3$): δ 171.8, 152.5, 135.5, 129.4, 128.8, 128.4, 127.2, 125.1, 126.2, 121.5, 117.1, 91.2, 78.2, 78.8, 67.9, 67.7, 66.0, 56.4, 35.5, 34.3. Anal. Calcd for C$_{20}$H$_{19}$NO$_6$: C, 65.03; H, 5.18; N, 3.79. Found: C, 65.21; H, 5.06; N, 3.83.

4.2.2. Regeneration of the protected functions of 4. The benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate **4** (0.300 g; 0.81 mmol) was dissolved in dichloromethane (45 mL) and treated at 25 °C with BCl$_3$ (4.05 mL of a 1 M solution in dichloromethane; 4.05 mmol). After 30 min, the solvent was concentrated under reduced pressure and the reaction mixture was poured into ice cold water. The aqueous layer was applied on a Dowex 50X 8-200 cation-exchange resin column, activated with 2 M HCl and washed with water. After washing with water, the amino acid **1** was recovered eluting with 0.5 M ammonia solution.[16] The eluates containing the amino acid were collected and lyophilized to afford pure **1** (0.137 g, Y = 80%) as a white powder: mp > 300 °C; HPLC: R_t = 1.31 min; [α]$_D$ = −10.3 (c 0.5, 0.1 M HCl), [lit.[4] = −9.5 (c 0.5, 0.1 M HCl), lit.[2b] = −30.49]; ^{1}H NMR (DMSO-d_6): δ 7.16 (1H, d, J = 1.3, 2-H aromatic), 6.92 (1H, dd, J = 8.1 and 1.3, 5-H aromatic), 6.68 (1H, d, J = 8.1, 4-H aromatic), 4.45 (2H, s, CH$_2$OH), 3.34 (1H, dd, J = 8.5 and 4.0, 2-H), 3.04 (1H, dd, J = 14.1 and

4.0, 3-Ha), 2.73 (1H, dd, J = 14.1 and 8.5, 3-Hb). Anal. Calcd for C$_{10}$H$_{13}$NO$_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.74; H, 6.10; N, 6.78.

4.3. Synthesis of benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate 4 from benzyl (S)-4-(4-hydroxybenzyl)-5-oxo-1,3-oxazolane-3-carboxylate 5

A mixture of the oxazolidinone **5**[7] (0.350 g; 1.1 mmol), p-toluenesulfonic acid (0.020 g; 0.10 mmol) and toluene (30 mL) were placed in a flask set for reflux and azeotropic separation of water. Then paraformaldehyde (0.264 g; 8.8 mmol) was added to the refluxing solution in two portions, allowing the solution to clear before the second addition. After 1 h, the reaction was stopped, the solution was washed with aqueous NaHCO$_3$ and dried over anhydrous sodium sulfate. The solvent was then evaporated under reduced pressure and the residue was chromatographed (eluting with hexane/ethyl acetate 60:40, v/v) to afford the benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate **4** (0.308 g; Y = 78%): mp 97–98 °C (from dichloromethane, diisopropyl ether: sinterizes at 88 °C); [α]$_D$ = +194.5 (c 1, CHCl$_3$). All physicochemical properties were identical to those reported above.

4.4. Synthesis of benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate 4 from (S)-N-benzyloxycarbonyl-4-hydroxy-3-hydroxymethylphenylalanine 8

A mixture of the benzyloxycarbonyl derivative **8**[4,17] (0.200 g; 0.58 mmol), p-toluenesulfonic acid (0.11 g; 0.06 mmol) and toluene (20 mL) was placed in a flask set for reflux and azeotropic separation of water. Then paraformaldehyde (0.138 g; 4.6 mmol) was added to the refluxing solution in two portions, allowing to the solution to clear before the second addition. After 1 h the reaction is stopped, the solution was washed with NaHCO$_3$ and dried over anhydrous sodium sulfate. The solvent was then evaporated under reduced pressure and the residue was chromatographed (eluting with hexane/ethyl acetate 60:40, v/v) to afford, benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate **4** (0.192 g; Y = 90%): mp 97–98 °C (from dichloromethane, diisopropyl ether: sinterizes at 88 °C); [α]$_D$ = +192.5 (c 1, CHCl$_3$). All physicochemical properties were identical to those reported above.

4.5. Synthesis of (S)-N-benzyloxycarbonyl-4-hydroxy-3-hydroxymethylphenylalanine 8 from benzyl (S)-4-(4H-1,3-benzodioxin-6-ylmethyl)-5-oxo-1,3-oxazolane-3-carboxylate 4

The oxazolidinone **4** (0.300 g; 0.81 mmol) was dissolved in dichloromethane (45 mL) and treated at 0 °C with BCl$_3$ (2.43 mL, of a 1 M solution in dichloromethane; 2.43 mmol). After 3 h, the reaction mixture was poured into a ice cold saturated solution of NaCl and the

organic layer was separated. After drying over anhydrous sodium sulfate, the solvent was evaporated and the crude product **7** [0.273 g; HPLC: R_t = 2.39 min, ESI-MS(positive) m/z: 398 (M+Na)$^+$, 420 (M+2×Na−H)$^+$] was then directly used into the successive reaction. For this, the crude **7** was dissolved into a solution of aqueous methanol (10 mL, 50%), saturated with NaHCO$_3$ and refluxed for 10 min. At this time, the reaction was stopped, the solvent was concentrated, and the residue acidified with aqueous 1 M HCl and extracted with ethyl acetate to afford the (S)-N-benzyloxycarbonyl-4-hydroxy-3-hydroxymethylphenylalanine **8**[4,17] (0.238 g; Y = 85%): mp 132–133 °C (from dichloromethane-diisopropyl ether); HPLC: R_t = 3.44 min, ESI-MS(positive) m/z: 368 (M+Na)$^+$, 713 (2×M+Na)$^+$; ESI-MS(negative) m/z: 344 (M−H)$^-$, 689 (2×M−H)$^-$; $[\alpha]_D$ = +10.2 (c 0.92, CH$_3$COOH), [lit.[4] = +10.4 (c 0.75, CH$_3$COOH)]; ^{1}H NMR (DMSO-d_6): δ 9.19 (1H, br s, COO*H*), 7.54 (1H, d, J = 8.05, aromatic), 7.34–7.26 (4H, overlapping, aromatics), 7.16 (1H, br s, aromatic), 6.91 (1H, d, J = 8.1, aromatic), 6.64 (1H, d, J = 8.1, aromatic), 4.98–4.93 (2H, AB system, OC*H*$_2$Ph), 4.43 (2H, br s, C*H*$_2$OH), 4.08 (1H, m, 2-H), 2.93 (1H, dd, J = 14.1 and 3.4, 3-Ha), 2.71 (1H, dd, J = 14.1 and 10.7, 3-Hb). Anal. Calcd for C$_{18}$H$_{19}$NO$_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 62.65; H, 5.62; N, 4.17. All other physicochemical characteristics were identical with those reported.[4,17]

4.6. Synthesis of (S)-4-hydroxy-3-hydroxymethylphenylalanine 1 from (S)-N-benzyloxycarbonyl-4-hydroxy-3-hydroxymethylphenylalanine 8

The (S)-N-benzyloxycarbonyl-4-hydroxy-3-hydroxymethylphenylalanine **8**[4,17] (0.250 g; 0.72 mmol) was dissolved in dichloromethane (35 mL) and treated at 25 °C with BCl$_3$ (2.16 mL of a 1 M solution in dichloromethane; 2.16 mmol). After 1 h, the solvent was concentrated under reduced pressure and the reaction mixture was poured into ice cold water. The aqueous layer was applied on a Dowex 50X 8-200 cation-exchange resin column, activated with 2 M HCl and washed with water. After washing with water, the amino acid **1** was recovered eluting with 0.5 M ammonia solution.[16] The eluates containing the amino acid were concentrated and dried to afford pure **1** (0.118 g, Y = 77%): $[\alpha]_D^{24}$ = −9.9 (c 0.5,

0.1 M HCl). All other physicochemical characteristics were identical with those reported.[2b,4,5]

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