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Research Article

Controlled synthesis of labelled 3-L-chlorotyrosine-[ring-$^{13}C_6$] and of 3,5-L-dichlorotyrosine-[ring-$^{13}C_6$]

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Summary

Pure 3-L-chlorotyrosine-[ring-$^{13}C_6$] is prepared by chlorination of the 5-oxazolidinone of L-tyrosine-[ring-$^{13}C_6$] with SO_2Cl_2 in CH_3COOH-Et_2O and successive one-pot regeneration of the protected aminoacidic functions by BCl_3 in dichloromethane. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: 3-L-chlorotyrosine-[ring-$^{13}C_6$]; 3,5-L-dichlorotyrosine-[ring-$^{13}C_6$]; Sulphuryl chloride; L-tyrosine-[ring-$^{13}C_6$]; Myeloperoxydase

Introduction

Hypochlorous acid, generated by the heme enzyme myeloperoxydase,[1] is a highly reactive species that participates in both oxidation[2,3] and chlorination reactions[4] of various biological targets and converts some residues of L-tyrosine **1a**, present in peptides, to residues of 3-chloro-L-tyrosine **2a**[4,5] and, to a minor extent, of 3,5-dichloro-L-tyrosine **3a** (Fig. 1).[6]

Because of this action of the hypochlorous acid, myeloperoxydase has been implicated in the tissue damage that occurs in numerous diseases that involve inflammatory cells.[7] The chlorinated amino acids 3-chloro-L-tyrosine and 3,5-dichloro-L-tyrosine are found intact in various human fluids and organs and therefore are believed to be important markers for probing the involvement of oxidation by myeloperoxydase in the pathology of particular diseases.[8]

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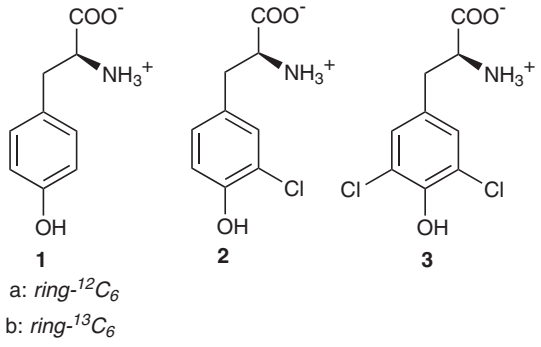


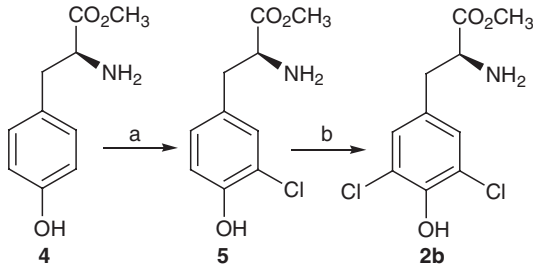
Figure 1. Tyrosine and chlorinated analogues

An accurate quantification of 3-chloro-L-tyrosine and of 3,5-dichloro-L-tyrosine in peptides of various biological substrates requires the use of gas chromatography-mass spectrometry (GC-MS) and dilution of the biological material with isotopically pure 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b** and of 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] **3b** as internal standards. Isotopomers **2b** and **3b** are now commonly obtained by direct halogenation of the free amino acid L-tyrosine-[ring-$^{13}C_6$] **1b** and successive purification by preparative HPLC.[6,9–11] Moreover, despite the great interest in these amino acids no convenient synthetic method is available other than on a very small scale. The reported methods involve reactions in which very small amounts (1–2 mg) of 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b** are prepared and purified by HPLC from the 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] **3b**[9–11] which always accompanies the monochloroderivative. On the other hand, when less than an equivalent of the chlorinating agent is used, some tyrosine accompanies the mono and dichlorinated amino acids and these must be separated by preparative HPLC.[6] This therefore reduces the availability of 3-L-chlorotyrosine-[ring-$^{13}C_6$] for future biological studies. Fortunately, the 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] can be obtained in good yield by exhaustive chlorination, at temperature higher than 40°C, of the free amino acid L-tyrosine-[ring-$^{13}C_6$].[12]

In this paper, we report a convenient method which overcomes the above difficulties and allows us to obtain both 3-chloro- and 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] **2b** and **3b** in a pure state and in sufficient quantities that enable the compounds to be used for both analytical, biological and chemical studies amount suitable not only for analytical purposes but also for other biological or chemical studies.

Results and discussion

Our initial procedure for the preparation of 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b** (Scheme 1) exploits the observation reported by Yu *et al.*[12] and by Masilamani



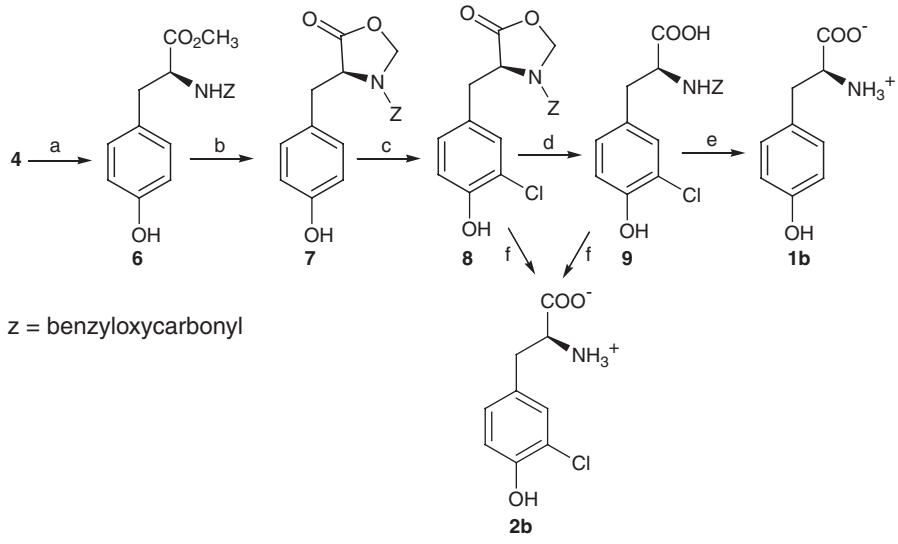
Scheme 1. a: SO_2Cl_2, AcOH-Et_2O, 0°C, 1 h; b: K_2CO_3, MeOH, 25°C, 12 h

and Rogic,[13] that sulphuryl chloride (SO_2Cl_2), at low temperature, provides a route to the efficient aromatic monochlorination of phenolic amino esters or phenols.

In fact, treatment of the methyl ester[14] **4** of the commercially available L-tyrosine-[ring-$^{13}C_6$] with one equivalent of SO_2Cl_2, in acetic acid and diethyl ether, at 0°C, afforded the 3-chloro-L-tyrosine ester **5** in nearly pure form. However, in agreement with the literature,[12] variable amounts of the dichlorinated compound were also formed. As reported by Yu *et al.*,[12] the amount of dichlorinated product decreases if the reaction is performed below 25°C and with a slow addition of the chlorinating agent. In order to ensure this condition, we dissolved SO_2Cl_2 in diethyl ether and added the solution previously cooled at −10°C to the amino acid cooled to 0–5°C. Under these conditions, the monochloroderivative **5** was obtained in constantly high yields (75%) but accompanied by some dichlorinated compound (4–5% by HPLC). The monochloroderivative **5** was then treated with potassium carbonate in aqueous methanol to afford the 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b**, which was isolated by HPLC.[†]

To obtain pure 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b**, and simplify the purification process, we decided to set up a second procedure based on the chlorination of a completely protected tyrosine. For this, our recent work on the chemistry of benzyloxycarbonyl-5-oxazolidininones was of immediate utility.[15–18] In our work,[16] under controlled conditions,[17] the tyrosine methyl ester **4** (Scheme 2) could be transformed into the 5-oxazolidinone-[ring-$^{13}C_6$] **7**, having both the aminoacidic functions suitably protected. This was obtained by transforming **4** into the benzyloxycarbonyl ester **6** and reacting it with paraformaldehyde in toluene, in the presence of *p*-toluenesulphonic acid.[16,17] The obtained 5-oxazolidinone **7** was then chlorinated, using sulphuryl chloride

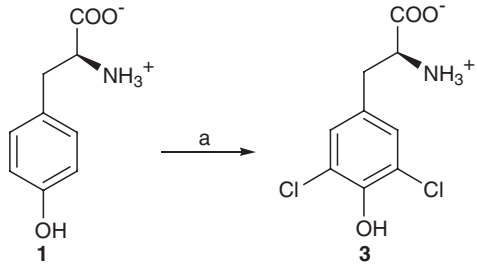
[†] For the determination of the optical purity, each amino acid (3 mg) was converted into the corresponding ester hydrochloride salt by treatment with methanolic HCl (0.2 ml; 25°C, 12 h). The mixture was then evaporated and the residue was then treated with a solution of trifluoracetic anhydride and trifluoracetic acid (0.2 ml, 1:1, v:v), and analysed by GLC on a chiral column (see 'General' section).



Scheme 2. a: $PhCH_2O$ COCl, aqueous $NaHCO_3$, 25°C, 5 h; b: paraformaldehyde, *p*-TsOH, toluene, 80°C, 30 min; c: SO_2Cl_2, AcOH-Et_2O, 0°C, 1 h; d: $NaHCO_3$, MeOH–H_2O, reflux, 10 min; e: H_2, Pd/C, EtOH; f: BCl_3, dichloromethane, 25°C, 1 h

and the controlled conditions used above for the methyl ester **4**.[12] From the reaction the monochloroderivative-[ring-$^{13}C_6$] **8** was obtained accompanied by trace amounts of the corresponding less polar 3,5-dichloroderivative, from which it can be separated by rapid chromatography on silica. Starting from the oxazolidinone **8**, we first attempted to obtain **2b**, by regeneration of the aminoacidic functions in two steps, using a mild hydrolytic procedure to open the 5-oxazolidinone ring by treatment with aqueous $NaHCO_3$[17,18] and subjecting the obtained carbobenzyloxy acid **9** to hydrogenolysis cleavage of the carbobenzyloxy group. Unfortunately, while the opening of the 5-oxazolidinone **8** to the acid **9** occurs with satisfactory yields, the hydrogenolysis of **9** causes, under all conditions used (different solvents and catalyst), the loss of the chlorine[19] substituent with the formation of the starting L-tyrosine-[ring-$^{13}C_6$] **1b**. A better and more satisfactory result was, however, obtained, by performing the regeneration of the amino group by means of BCl_3 in dichloromethane, according to our recent protocol.[18] Thus we performed in *one-pot* the simultaneous cleavage of the 5-oxazolidinone ring and of the carbobenzyloxy group and obtained the desired 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b** uncontaminated by the 3,5-dichloro homologous.

The second desired compound, the 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] **3b** was obtained by simple exhaustive chlorination of the tyrosine **1b** with SO_2Cl_2 (3 molar equivalents) at 25°C (Scheme 3).



Scheme 3. a: SO_2Cl_2, sulpholane-Et_2O, 25°C, 24 h

In conclusion we have devised a simple route to prepare the 3-chloro-L-tyrosine-[ring-$^{13}C_6$] **2b** and the 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] **3b** in pure form and in sufficient quantity to be used as internal standards in the quantification of the isotopomers formed by *in vivo* action of the heme enzyme myeloperoxydase.

Experimental

General

Nuclear magnetic resonance spectra were recorded at 298 K on a Bruker AM-500 spectrometer, operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ units) relative to $CHCl_3$, fixed at 7.24 ppm, or to HDO, fixed at 4.54 ppm, for the ^{1}H spectra and relative to dioxane, fixed at 67.60 ppm, for the ^{13}C spectra.[20] ^{1}H-NMR and ^{13}C-NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; bs, broad singlet; m, multiplet), coupling constant(s) in Hz, assignment of proton(s).

Optical rotations were taken at 25°C on a Perkin-Elmer 241 polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2/g^1. Chiral GCL analyses were carried out on a Hewlett-Packard 5890 gas chromatograph equipped with a octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex E)[21] capillary column (25 m, 0.25 mm ID, purchased from Macherey-Nagel). The HPLC system was equipped with a RP-18 column (LiChroCART, 125 mm, 4 mm ID, 5 µm purchased from Merck) eluted with H_2O/MeOH/AcOH 95:5:1 v/v at 1 ml/min; the detection was performed at 276 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 HPLC and/or by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light, 50% sulphuric acid or 0.2% ninhydrin in ethanol and heated as developing agent. E. Merck 230-400 mesh silica gel was used for flash column chromatography.[22]

*Preparation of the (2S)-2-amino-3-(4-hydroxyphenyl-[ring-$^{13}C_6$])-propionic acid methyl ester (**4**) hydrochloride*

Thionyl chloride (80 mg; 0.66 mmol) was added to methanol (2 ml) cooled at −15°C and the solution was kept for 1 h at this temperature. Then, crystalline L-tyrosine-[ring-$^{13}C_6$] (100 mg; 5.4 mmol) was added and the suspension was stirred at room temperature for 24 h, when a clear solution formed. The solvent was evaporated, the residue was tritured with methanol and the solvent removed again under reduced pressure. The crude product was dried under high vacuum and crystallized from methanol-diisopropyl ether to afford, after filtration, the title ester **4** as hydrochloride in quantitative yields (122 mg, 96.6%): mp 189–192°C; $[\alpha]_D^{22} = +71$ (*c* 1, py), [(lit.[14] mp 190–191°C); $[\alpha]_D^{19} = +70.9$ (*c* 1, py)]; ESI/MS (positive) *m/z*: 202.3 ($M+H^+$); ^{1}H-NMR (D_2O): δ 7.16 (2H, d, $J = 158.0$, 2′-H_2), 6.91 (2H, d, $J = 160.7$, 3′-H_2), 4.38 (1H, m, 2-H), 3.84 (3H, s, OCH_3), 3.26 (1H, m, 3-Ha), 3.17 (1H, m, 3-Hb); ^{13}C-NMR (D_2O): δ 170.8 (s, CO_2CH_3), 155.8 (dt, $J = 64.8$ and 7.6, 4′-C), 131.4 (ddd, $J = 57.2$, 57.2 and 6.2, 2′-C and 6′-C), 126.0 (dt, $J = 57.2$ and 8.6, 1′-C), 116.5 (ddd, $J = 64.8$, 57.2 and 6.7, 3′-C and 5′-C), 54.8 (s, OCH_3), 54.1 (s, 2-C), 35.3 (d, $J = 44.8$, 3-C). Analytically calculated for $^{12}C_4\,^{13}C_6H_{14}ClNO_3$: C 53.05, H 5.94, Cl 14.92, N 5.89. Found: C 52.99, H 6.09, Cl 15.18, N 5.75.

*Preparation of the 3-chloro-L-tyrosine-[ring-$^{13}C_6$] (**2b**), via the 3-chloro-L-tyrosine-[ring-$^{13}C_6$] methyl ester (**5**) hydrochloride*

L-tyrosine-[ring-$^{13}C_6$] methyl ester **4** hydrochloride (80 mg; 0.34 mmol) was suspended in a mixture of AcOH-Et_2O (1–0.5 ml) and cooled at 0–5°C. A solution of SO_2Cl_2 (27.3 μl; 46 mg; 0.34 mmol) in Et_2O (0.5 ml), cooled at −10°C, was then slowly added. The reaction mixture was stirred for 1 h at 0°C, then the solvent was evaporated under a steam of nitrogen and the residue was crystallized from methanol-diisopropyl ether to afford the 3-chloro-L-tyrosine-[ring-$^{13}C_6$] methyl ester **5** (as hydrochloride), a white solid (69 mg, 75%) containing some 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] (4–5%, HPLC). The product gave: mp 168–169°C; ESI/MS (positive) *m/z* (relative intensity): 238.1 (31, [^{37}Cl]$M+H^+$), 236.1 (100, [^{35}Cl]$M+H^+$); ^{1}H-NMR (D_2O): δ 7.33 (1H, d, $J = 164.2$, 2′-H), 7.12 (1H, dd, $J = 158.4$ and 7.9, 6′-H), 7.04 (1H, dd, $J = 160.5$ and 7.9, 5′-H), 4.42 (1H, m, 2-H), 3.88 (3H, s, OCH_3), 3.29 (1H, dd, $J = 14.9$ and 5.0, 3-Ha), 3.19 (1H, dd, $J = 14.9$ and 7.7, 3-Hb). The obtained product was then dissolved in a saturated methanolic solution of potassium carbonate (2.5 ml) and stirred at room temperature for 12 h. Then the solvent was evaporated and the residue recovered with water and applied to a Dowex 50 × 8-200 cation-exchange resin column, activated with 2 M HCl and washed with water. After washing with water, the amino acid was recovered by eluting

with 0.5 M ammonia solution.[23] The eluates containing the amino acids were collected to afford **2b** (45.2 mg, 80%) that showed a major peak in HPLC ($t_r = 8.8$ min; 95%) accompanied by a minor peak ($t_r = 22.3$ min; 5%) corresponding to the dichloroderivative **3b**. Purification of a sample by preparative HPLC gave pure **2b** : $[\alpha]_D^{22} = -23.57$ (*c* 1, H_2O); ESI/MS (negative) *m/z* (relative intensity): 441.0 (60, [^{35}Cl]M + [^{37}Cl]M-H$^+$), 439.0 (100, [^{35}Cl]M + [^{35}Cl]M-H$^+$), 222.0 (27, [^{37}Cl]M-H$^+$), 220.3 (85, [^{35}Cl]M-H$^+$); ^{1}H-NMR (D_2O): δ 7.23–6.49 (3H, m, 2′-H, 5′-H and 6′-H), 4.26 (1H, dd, $J = 7.7$ and 5.6, 2-H), 3.21 (1H, dd, $J = 14.9$ and 5.6, 3-Ha), 3.08 (1H, dd, $J = 14.9$ and 7.7, 3-Hb); ^{13}C-NMR (D_2O): δ 171.9 (s, 1-C), 152.4 (dd, $J = 68.7$ and 64.8, 4′-C), 131.4 (dd, $J = 64.8$ and 57.2, 2′-C), 129.9 (ddd, $J = 57.2$, 57.2 and 7.6, 6′-C), 128.2 (ddd, $J = 57.2$, 57.2 and 7.6, 1′-C), 121.4 (ddd, $J = 74.4$, 67.7 and 6.7, 3′-C), 118.1 (ddd, $J = 63.9$, 58.2 and 5.7, 5′-C), 56.7 (s, 2-H), 36.0 (d, $J = 43.9$, 3-H).

*Preparation of the 3-chloro-L-tyrosine-ring-[ring-$^{13}C_6$] (**2b**), via the chlorination of benzyl 4-(4-hydroxybenzyl-[ring-$^{13}C_6$])-5-oxo-1,3-oxazolidine-3-carboxylate (**7**)*

*(a) Benzyloxycarbonylation of the (2S)-2-amino-3-(4-hydroxyphenyl-[ring-$^{13}C_6$])-propionic acid methyl ester (**4**) hydrochloride.* The methyl ester hydrochloride **4** (60 mg; 0.25 mmol) was dissolved in an aqueous solution of $NaHCO_3$ (2 ml, 0.3 M) and the solution cooled to 0°C. Then, benzyl chloroformate (45 µl, 0.32 mmol) was added under stirring and the solution kept at room temperature for 5 h. After extraction with ethyl acetate, the organic layers were then washed with water and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a crude oil which, after column chromatography (eluting with hexane/ethyl acetate, 60/40, v/v), gave the pure methyl (2S)-2-benzyloxycarbonylamino-3-(4-hydroxyphenyl-[ring-$^{13}C_6$])propanoate **6** (40.1 mg, 48.0%): mp 90.5–91.5°C, [(lit.[24] mp 91–92°C)]; $[\alpha]_D^{22} = +37.8$ (*c* 1, $CHCl_3$); ESI/MS (negative) *m/z* (relative intensity): 669.1 (100, M + M-H$^+$), 334.3 (72, M-H$^+$); ^{1}H-NMR ($CDCl_3$): δ 7.36–7.29 (5H, m, aromatics), 6.92 (2H, dd, $J = 155.6$ and 7.5, 2′-H and 6′-H), 6.69 (2H, dd, $J = 161.7$ and 6.0, 3′-H and 5′-H), 5.37 (1H, d, $J = 8.2$, NH), 5.11 (1H, d, $J = 12.3$, A part of a AB system, $NHCO_2CHHC_6H_5$), 5.07 (1H, d, $J = 12.3$, B part of a AB system, $NHCO_2CHHC_6H_5$), 4.63 (1H, ddd, $J = 8.2$, 6.1 and 5.4, 2-H), 3.71 (3H, s, OCH_3), 3.06 (1H, dd, $J = 13.9$ and 5.4, 3-Ha), 2.98 (1H, dd, $J = 13.9$ and 6.1, 3-Hb); ^{13}C-NMR ($CDCl_3$): δ 172.43 (s, $COOCH_3$), 156.0 (dt, $J =$ 64.8 and 9.5, 4′-C), 131.1 (ddd, $J = 57.2$, 57.2 and 5.7, 2′-C and 6′-C), 127.7 (dt, $J = 57.2$ and 9.5, 1′-C), 116.2 (ddd, $J = 64.8$, 64.8 and 5.7, 3′-C and 5′-C), 67.1 (s, OCH_2Ph), 54.9 (s, OCH_3), 52.4 (s, 2-C), 37.5 (s, 3-C). Analytically calculated for $^{12}C_{12}$ $^{13}C_6H_{19}NO_5$: C 65.64, H 5.81, N 4.25. Found: C 65.42, H 5.72, N 4.54.

(b) Preparation of benzyl 4-(4-hydroxybenzyl-[ring-$^{13}C_6$])-5-oxo-1,3-oxazolidine-3-carboxylate (7). A mixture of methyl ester **6** (80 mg; 0.24 mmol), *p*-toluenesulfonic acid (22.8 mg; 0.12 mmol) and toluene (3 ml) was placed in a flask. Then paraformaldehyde (18 mg; 0.6 mmol) was added to the solution, which was heated to 80°C. After 30 min the reaction was stopped, the solution was washed with aqueous $NaHCO_3$ and extracted with AcOEt; the organic layer was dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure, a crude oil was obtained that, after column chromatography (eluting with dichloromethane/acetone, 100/0.5, v/v), gave the pure **7** (44.8 mg, 56.0%); mp 111°C; $[\alpha]_D{}^{22} = +208.0$ (*c* 1, $CHCl_3$) [(lit.[25] mp 110–112°C; $[\alpha]_D{}^{22} = +206.0$ (*c* 1, $CHCl_3$)]; ESI/MS (positive) *m/z* (relative intensity): 753.6 (100, $M + M + 2CH_3OH + Na^+$), 388.3 (72, $M + CH_3OH + Na^+$); ^{1}H-NMR (DMSO-d_6): δ 7.41–7.36 (5H, m, aromatics), 6.78 (2H, dd, $J = 158.5$ and 7.6, aromatic), 6.62 (2H, dd, $J = 161.0$ and 7.6, aromatic), 5.26 (1H, d, $J = 3.7$, OC*H*HN), 5.20 (2H, m, OCH_2Ph), 4.54 (1H, bs, COCHN), 4.45 (1H, bs, OCH*H*N), 3.09 (1H, m, CHC*H*HAr), 2.91 (1H, dd, $J = 13.9$ and 2.5, CHCH*H*Ar). Analytically calculated for $^{12}C_{12}\,^{13}C_6H_{17}NO_5$: C 66.05, H 5.23, N 4.28. Found: C 65.88, H 5.33, N 4.11.

(c) Chlorination of the benzyl 4-(4-hydroxybenzyl-[ring-$^{13}C_6$])-5-oxo-1,3-oxazolidine-3-carboxylate (7). The ester **7** (54 mg; 0.162 mmol) was suspended in a mixture of AcOH-Et_2O (240 µl–1.1 ml) and the whole cooled at 0–5°C. To this mixture, SO_2Cl_2 (13.8 µl; 0.180 mmol) dissolved into Et_2O (0.60 ml), and cooled to −10°C, was then slowly added. The formed solution was then stirred at 0°C for 1 h and then diluted with an aqueous solution of $NaHCO_3$. The mixture was then extracted with AcOEt, washed with water and dried over Na_2SO_4. Evaporation of the solvent, under reduced pressure, afforded a crude residue that, after column chromatography (eluting with hexane/ethyl acetate, 75/25, v/v), gave pure benzyl 4-(3-chloro-4-hydroxybenzyl-ring-$^{13}C_6$)-5-oxo-1,3-oxazolidine-3-carboxylate **8** (40.5 mg, 68.0%): mp 81–82°C; $[\alpha]_D{}^{22} = +167.7$ (*c* 1, $CHCl_3$); ESI/MS (positive) *m/z* (relative intensity): 424.1 (35, [^{37}Cl]$M + CH_3OH + Na^+$), 422.1 (100, [^{35}Cl]$M + CH_3OH + Na^+$); ^{1}H-NMR (DMSO-d_6): δ 7.39–7.32 (5H, m, aromatics), 6.95 (1H, d, $J = 163.2$, aromatic), 6.82 (1H, dd, $J = 163.5$ and 8.2, aromatic), 6.75 (1H, ddd, $J = 158.9$, 8.1 and 1.3, aromatic), 5.29 (1H, d, $J = 4.0$, OC*H*HN), 5.16 (2H, m, OCH_2Ph), 4.63 (1H, bs, COCHN), 4.55 (1H, m, OCH*H*N), 3.08 (1H, m, CHC*H*HAr), 2.92 (1H, dd, $J = 14.1$ and 3.5, CHCH*H*Ar). Analytically calculated for $^{12}C_{12}\,^{13}C_6H_{16}ClNO_5$: C 59.76, H 4.46, Cl 9.80, N 3.87. Found: C 60.01, H 4.22, Cl 9.86, N 3.91.

*(d) Regeneration of the aminoacidic function of **8**, preparation of 3-chloro-L-tyrosine-[ring-$^{13}C_6$] (**2b**)*. The oxazolidinone **8** (80 mg; 0.22 mmol) was dissolved in dichloromethane (8 ml) and treated at 25°C with BCl_3 (0.6 ml of

a 1 M solution in dichloromethane; 0.64 mmol). After 1 h, the solution was diluted with H_2O, and applied on a Dowex 50×8-200 cation-exchange resin column and processed as described above in the saponification of the methyl ester **5**. The eluates containing the amino acid were collected to afford pure **2b** (24.4 mg, 50.1%) that showed a single peak in HPLC ($t_r = 8.8$ min) and all physicochemical properties reported above.

(e) Preparation of (2S)-2-benzyloxycarbonylamino-3-(3-chloro-4-hydroxypheny-[ring-$^{13}C_6$])-propanoic acid **9**. The oxazolidinone **8** (30 mg; 0.081 mmol) was dissolved in a saturated solution of $NaHCO_3$ in MeOH-H_2O (1:1 v/v) (2 ml) and refluxed for 10 min.[15] Then the solution was acidified, extracted with AcOEt and the organic layers were washed with water and dried over Na_2SO_4. Evaporation of the solvent, under reduced pressure, afforded the acid **9** (24.5 mg, 85.0%): mp 140–141°C; $[\alpha]_D^{22} = +33.0$ (*c* 1, $CHCl_3$); ESI/MS (negative) *m/z* (relative intensity): 712.8 (6, [^{37}Cl]M + [^{37}Cl]M-H$^+$), 710.8 (31, [^{37}Cl]M + [^{35}Cl]M-H$^+$), 708.8 (51, [^{35}Cl]M + [^{35}Cl]M-H$^+$), 355.8 (30, [^{37}Cl]M-H$^+$), 353.8 (100, [^{35}Cl]M-H$^+$); ^{1}H-NMR ($CDCl_3$): δ 7.34–7.26 (5H, m, aromatics), 7.08 (1H, d, $J = 158.0$, 6′-H), 6.90 (2H, dd, $J = 165.9$ and 8.6, 2′-H and 5′-H), 5.28 (1H, d, $J = 1.23$, NH), 5.10 (1H, d, $J = 11.93$, A part of a AB system, $NHCO_2CHHC_6H_5$), 5.04 (1H, d, $J = 11.93$, B part of a AB system, $NHCO_2CHHC_6H_5$), 4.59 (1H, m, 2-H), 3.08 (1H, m, CHC*H*HAr), 2.96 (1H, m, CHCH*H*Ar); ^{13}C-NMR ($CDCl_3$): δ 150.6 (ddd, $J = 71.5$, 70.5 and 6.7, 4′-C), 119.9 (ddd, $J = 71.5$, 67.7 and 5.7, 3′-C), 116.3 (ddd, $J = 58.2$, 10.5 and 5.7, 5′-C), 130.3-128.0 (m, overlapping, 1′-C, 2′-C and 6′C). Analytically calculated for $^{12}C_{11}\,^{13}C_6H_{13}ClNO_5$: C 58.38, H 4.61, Cl 10.14, N 4.00. Found: C 58.63, H 4.73, Cl 10.05, N 3.83.

*(f) Regeneration of 3-chloro-L-tyrosine-[ring-$^{13}C_6$] (***2b***) from (2S)-2-benzyloxycarbonylamino-3-(3-chloro-4-hydroxypheny-ring-$^{13}C_6$)-propanoic acid (***9***)*. The 5-oxazolidinone **9** (31 mg; 0.087 mmol) was dissolved in dichloromethane (2 ml) and treated at 25°C with BCl_3 (440 µl of a 1 M solution in dichloromethane; 0.44 mmol). After 1 h the solution was diluted with H_2O, and applied on a Dowex 50×8-200 cation-exchange resin column to be recovered as described above. The eluates containing the amino acid were collected to afford pure **2b** (9.6 mg, 50.0%) with all physicochemical properties reported above for **2b**, purified by HPLC.

*Preparation of the 3,5-dichloro-L-tyrosine-[ring-$^{13}C_6$] (***3b***)*

L-tyrosine-[ring-$^{13}C_6$] **1b** (50 mg; 0.27 mmol) was suspended in sulpholane (1 ml) and the mixture was stirred at 25°C under argon. Then SO_2Cl_2 (0.1 ml; 1.23 mmol) dissolved in diethyl ether (1.0 ml) was added dropwise. After addition, a solution gradually formed which was stirred for 24 h at 23°C. Then,

anhydrous dichloromethane was added until precipitation of a white solid was complete; then it was filtered and dried under vacuum to give the title compound **3b** as the hydrochloride (69.5 mg, 88.0%). The compound gave a single spot in TLC, eluting with a solution of $CHCl_3$/MeOH/NH_3 (6/4/1; v/v/v), Rf = 0.26, and shows a single peak in HPLC (t_r = 22.3 min): mp 205–206°C, (from AcOH); $[\alpha]_D^{22} = +2.9$ (*c* 1, H_2O); ESI/MS (negative) *m*/*z* (relative intensity): 258.2 (10, [$^{37}Cl_2$]M-H^+), 256.1 (69, [^{35}Cl,^{37}Cl]M-H^+), 254.2 (100, [$^{35}Cl_2$]M-H^+); ^{1}H-NMR (D_2O): δ 7.27 (2 H, d, *J* = 162.8, 2′-H and 6′-H), 4.24 (1 H, dd, *J* = 7.5 and 5.7, 2-H), 3.24 (1 H, dd, *J* = 14.9 and 5.7, 3-Ha), 3.12 (1 H, dd, *J* = 14.9 and 7.5, 3-Hb); ^{13}C-NMR (D_2O): δ 171.9 (s, 1-C), 148.2 (t, *J* = 72.5, 4′-C), 130.0 (ddd, *J* = 62.9, 59.1 and 9.5, 2′-C and 6′-C), 128.1 (dt, *J* = 59.1 and 3.8, 1′-C), 122.8 (ddd, *J* = 3′-C and 5′-C), 54.6 (s, 2-C), 35.1 (d, *J* = 42.9, 3-C). Analytically calculated for $^{12}C_3\,^{13}C_6H_{10}Cl_3NO_3$: C 38.99, H 3.45, Cl 36.36, N 4.79. Found: C 39.10, H 3.61, Cl 36.45, N 4.70.

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