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Asymmetric synthesis of L-proline regio- and stereoselectively labelled with deuterium

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Abstract

A synthesis of L-proline where all of the ring methylenes are stereoselectively labelled with deuterium is described. A catalytic deuteration of protected 3,4-dehydro-L-proline using transition metal catalyst followed by RuO_4-oxidation gave a [3,4-D_2]pyroglutamic acid derivative. A *syn*-selective deuteration of the aminal derived from the pyroglutamate with Et_3SiD–$BF_3 \cdot OEt_2$ furnished (2*S*,3*S*,4*R*,5*S*)-[3,4,5-D_3]proline. The present procedure is also applied to the synthesis of the corresponding (2*S*,3*S*,4*R*,5*R*)-isomer.

1. Introduction

The availability of amino acids regio- and stereoselectively labelled with stable isotopes, such as D, ^{13}C and ^{15}N, is valuable both for stereochemical studies on the enzymatic transformations[1] and for the determination of the three-dimensional structure of the proteins by NMR spectroscopy.[2] In particular, stereoselective deuterium-labelling of the diastereotopic hydrogen and the methyl group is the most important and essential technique for these investigations. In the light of the importance of these amino acids, we have recently developed methods for regio- and stereoselective deuterium-labelling of amino acid side chains.[3]

Proline is the sole cyclic amino acid in mammalian proteins and is usually located in bend and loop structures where it provides important conformational constraints in globular proteins.[4] Furthermore, proline and its metabolites, mainly hydroxyproline, have also figured as components of biologically-active compounds such as antibiotics.[5] Therefore, specimens of L-proline stereospecifically labelled with deuterium(s) in the ring methylene(s) are required for understanding its unusual conformational preference and the stereochemical course of its metabolic reactions.

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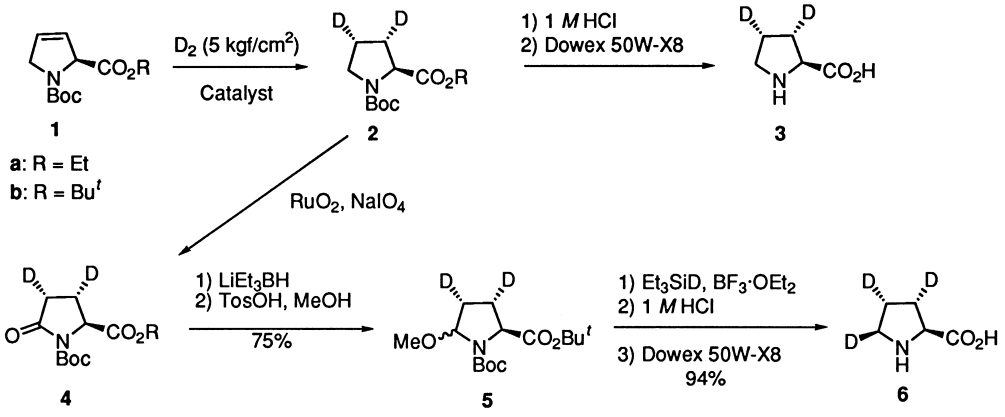
In connection with stereochemical studies on proline hydroxylase, stereoselective labelling at the γ-carbon was achieved by reduction of tosylates derived from 4-hydroxyproline with $LiAlD_4$ or $LiAlT_4$[6] with an improved protocol being recently developed by Baldwin et al.[7] The samples of [3,4-T_2]proline prepared by catalytic tritiation of the dehydroproline were also used in the above study.[8] For stereoselective labelling at the β-[9] or δ-position,[10] methods based on a combination of chemical and enzymatic reactions were explored. In this paper we wish to report on the first synthesis of [3,4,5-D_3]proline in which all of the prochiral methylenes are stereoselectively labelled with deuterium.[11]

2. Results and discussion

In order to incorporate deuterium atoms stereoselectively into the 3- and 4-positions of L-proline, a catalytic deuteration of the corresponding dehydro-L-proline seems to be the most efficient and convenient procedure. We therefore chose both ethyl and *t*-butyl 3,4-dehydro-L-prolinate **1a**[12] and **1b** derived from *trans*-4-hydroxy-L-proline as starting materials (Scheme 1).



Scheme 1.

Although a few papers concerning a catalytic deuteration[13] or tritiation[8] of racemic 3,4-dehydroproline itself have been published, no papers concerning the protected derivatives were found in the literature. A catalytic deuteration of compound **1a** was carried out using 10% palladium on carbon in MeOD for 24 h under medium pressure of deuterium gas (5 kgf/cm^2). The regio- and stereoselectivities of the deuteration were checked by ^{1}H NMR spectroscopy after being deprotected because the ^{1}H NMR spectrum of the deuterated ethyl prolinate **2a** was complicated by the *s-cis*/*s-trans* isomerism often found in the *N*-acylproline derivatives.[14] The ^{1}H NMR spectrum of the deuterated proline **3** so obtained revealed that a considerable extent of H–D scrambling occurred. Screening for a transition metal catalyst such as PdO, PtO_2, Rh/C, Ru/C, $RhCl(PPh_3)_3$ and $RuCl_2(PPh_3)_3$ was carried out and the selected examples which resulted in relatively clean formation of the [3,4-D_2]proline **3** are listed in Table 1.

When $RuCl_2(Ph_3)_3$ was used as a catalyst, the selectivity of deuteration was the best, giving the [3,4-D_2]proline **3** in 77% yield after deprotection of the ethyl [3,4-D_2]prolinate **2a** with 1 M HCl followed by Dowex 50W-X8 (entry 2). The enantiomeric excess was checked by HPLC analysis using a chiral stationary phase column, and was found to be 98% ee (L-form). The ^{1}H NMR spectrum of the [3,4-D_2]proline **3** was shown in Fig. 1A. Although the deuterium content of the 4*R*-proton cannot be estimated due to the signal overlapping, the signal intensity of the 3*S*-proton corresponds only to the 0.03 proton, indicating a stereoselective formation of (2*S*,3*S*,4*R*)-isomer. This stereochemical outcome

Table 1
Stereoselective deuteration of protected dehydroprolinate **1a** and **1b**

entry	dehydroproline	catalyst	deuterated proline **3**		
			%[a]	% D[b]	%ee[c]
1	**1a**	$RhCl(PPh_3)_3$	62	82	97
2	**1a**	$RuCl_2(PPh_3)_3$	77	97	98
3	**1a**	Ru/C	92	93	99
4	**1b**	$RhCl(PPh_3)_3$	60	78	99
5	**1b**	$RuCl_2(PPh_3)_3$	64	87	99
6	**1b**	Ru/C	97	90	99

[a] Based on the starting olefin. [b] Deuterium content of the *3S*-proton determined by ^1H NMR spectroscopy. [c] Determined by HPLC analysis. The value refers to α-position.

can be attributed to a predominant deuterium addition to the olefin **1a** *anti* to the resident ester group. The corresponding *t*-butyl ester **1b** was also subjected to the above deuteration and Ru on carbon proved to be the most efficient catalyst in this case (entry 6, vide infra).

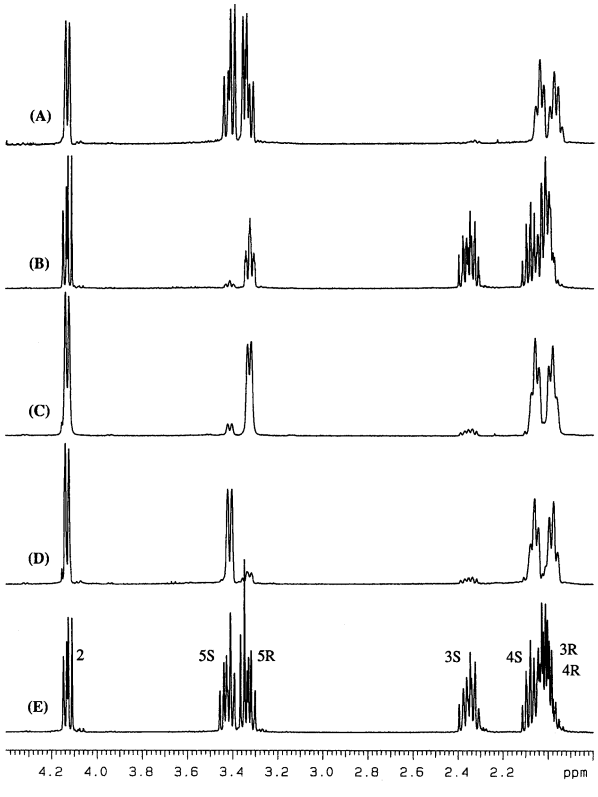
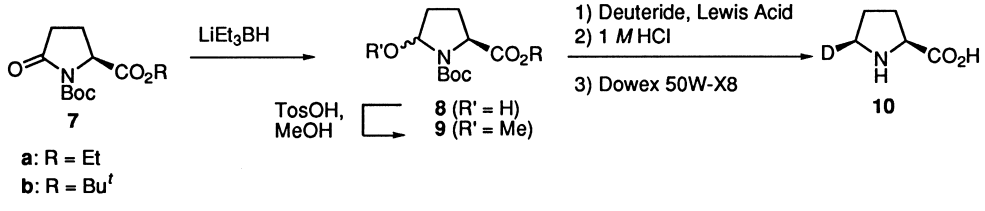


Figure 1. 400 MHz ^1H NMR spectra of (A) (2*S*,3*S*,4*R*)-[3,4-D_2]proline **3**, (B) (2*S*,5*S*)-[5-D]proline **10**, (C) (2*S*,3*S*,4*R*,5*S*)-[3,4,5-D_3]proline **6**, (D) (2*S*,3*S*,4*R*,5*R*)-[3,4,5-D_3]proline **12**, and (E) unlabelled proline in D_2O

We next examined the selective deuterium-labelling of the diastereotopic hydrogen at the δ-position. Since a stereoselective reduction of the amido carbonyl moiety was considered to be the most convenient approach for this purpose, a preliminary examination was performed using unlabelled ethyl and *t*-butyl pyroglutamate **7a** and **7b** as starting materials. Among several methods for conversion of the pyroglutamic acid into proline,[15] we adopted a procedure of Pedregal et al.[16] which involves a stepwise process for reducing the carbonyl moiety. An additional advantage of this method for our purpose was the availability of the corresponding deuterated-reducing agents. As shown in Scheme 2, a reduction of the protected pyroglutamate **7** with lithium triethylborohydride was carried out at −78°C to give 5-hydroxyprolinate **8** and the resulting amino alcohol **8** was directly, or after conversion to the corresponding methyl ether **9**, subjected to a reductive deuteration using the deuteriosilane–Lewis acid system. The results are summarised in Table 2. For example, *t*-butyl 5-methoxyprolinate **9b** was treated with Et_3SiD in the presence of $BF_3{\cdot}OEt_2$ at −78°C for 2 h and subsequent deprotection procedure gave [5-D]proline **10** in 78% yield based on the 5-methoxyprolinate **9b** (entry 9). The enantiomeric excess at the C-2 position was checked by HPLC analysis to be 99% ee (L-form) and the stereoselectivity of the deuteration was determined by ^1H NMR integration of the δ-proton signals by comparison with the reported data.[10,13] As shown in Fig. 1B, formation of (2*S*,5*S*)-[5-D]proline **10**, the *syn*-isomer, was predominant over the (2*S*,5*R*)-isomer in the ratio of 90:10. Similar *syn*-selective addition in the acyliminium intermediate which seemed to be inconsistent with the steric interaction was also observed in a few Lewis acid catalysed amido alkylation reactions.[17] According to Danishefsky's hypothesis,[18] favorable orbital interaction over steric interaction, so-called Cieplak-type stereoelectronic effect,[19] was responsible for the observed *syn*-selectivity. In our case the emerging σ* orbital at the δ carbon may be more stabilised by interacting with the σ C–H bond rather than σ C-ester bond at the α carbon.



Scheme 2.

Other silanes such as tris(trimethylsilyl)deuteriosilane (TMS_3SiD), Ph_3SiD or Ph_2SiD_2, were also tested as a reducing agent and a significant decrease in the selectivity was observed, probably due to

Table 2
Transformation of prolinate **8** or **9** into [5-D]proline **10**

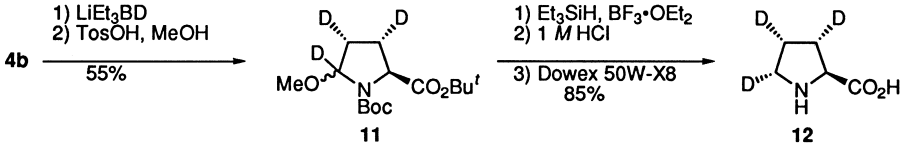
entry	prolinate	Lewis acid (2 equiv.)	deuteride (2 equiv.)	**10**	
				%	*5S* / *5R*[a]
1	**8a**	$BF_3{\cdot}Et_2O$	Et_3SiD	64	87 / 13
2	**9a**	$BF_3{\cdot}Et_2O$	Et_3SiD	64	89 / 11
3	**8a**	$BF_3{\cdot}Et_2O$	TMS_3SiD	74	74 / 26
4	**8a**	$BF_3{\cdot}Et_2O$	Ph_3SiD	55	55 / 45
5	**8a**	$BF_3{\cdot}Et_2O$	Ph_2SiD_2	87	59 / 41
6	**8a**	TMSOTf	Et_3SiD	87	87 / 13
7	**8a**	Ph_3CClO_4	Et_3SiD	52	88 / 12
8	**8b**	$BF_3{\cdot}Et_2O$	Et_3SiD	96	89 / 11
9	**9b**	$BF_3{\cdot}Et_2O$	Et_3SiD	78	90 / 10

[a] Determined by 400 MHz ^1H NMR.

an additional steric repulsion between the bulky silane and the ester moiety (entries 3–5). We previously reported that the deuteriogermane and deuteriostannane were slightly more effective than deuteriosilane for the *syn*-selective deuteration;[11] however, here we employed only deuteriosilanes from ecological and economical perspectives. Utilisation of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and trityl perchlorate (Ph_3CClO_4) as a Lewis acid resulted in the same degree of selectivity (entries 6 and 7). Focusing on the difference in the ester moiety, the *t*-butyl ester proved to be slightly advantageous (entries 8 and 9).

Since a preparation of [3,4,5-D_3]proline from ethyl [3,4-D_2]prolinate **2a** was minutely described in our previous communication, we here report a synthesis of (2*S*,3*S*,4*R*,5*S*)-[3,4,5-D_3]proline from *t*-butyl [3,4-D_2]prolinate **2b** obtained using the reaction conditions shown in entry 6 (Table 1). An oxidation of the prolinate **2b** was carried out using RuO_4, prepared in situ from RuO_2 and $NaIO_4$,[20] to afford *t*-butyl [3,4-D_2]pyroglutamate **4b** in a quantitative yield. After conversion of the pyroglutamate **4b** into *t*-butyl 5-methoxy[3,4-D_2]prolinate **5b** in 75% yield, the 5-methoxyprolinate **5b** was subjected to the above deuteration procedure (Table 2, entry 9) to afford [3,4,5-D_3]proline **6** in 94% yield. The enantiomeric excess at the α-position was determined by HPLC analysis to be 99% ee (L-form) and the relative configuration was confirmed by ^{1}H NMR spectrum. As shown in Fig. 1C, the signal intensity of the residual 3*S*-proton of compound **6** corresponded to 0.08 proton and the ratio of 5*S*- and 5*R*-proton signals was 10:90, indicating a selective formation of (2*S*,3*S*,4*R*,5*S*)-isomer.

Using the present protocol, it was logically possible to obtain the corresponding (5*R*)-isomer simply by changing the deuterium source. However, attempts to reduce the amido carbonyl moiety of the ethyl [3,4-D_2]pyroglutamate **4a** with lithium triethylborodeuteride resulted in failure due to insufficient deuterium incorporation. The origin of the hydrogen atom incorporated in the δ-position is still not clear. Therefore, we attempted to prepare the (5*R*)-isomer from the *t*-butyl [3,4-D_2]pyroglutamate **4b** as shown in Scheme 3. Thus, the pyroglutamate **4b** was initially reduced with lithium triethylborodeuteride and the deuterated amino alcohol so formed was converted to methyl ether **11** in 55% yield. Finally, the 5-methoxy[3,4-D_2]prolinate **11** was subjected to Et_3SiH–$BF_3\cdot OEt_2$ system followed by deprotection to give (2*S*,3*S*,4*R*,5*R*)-[3,4,5-D_3]proline **12** in 85% yield with 99% ee (L-form). The relative configuration was also confirmed by ^{1}H NMR spectrum (Fig. 1D) and it was found that the ratio of (5*S*)- and (5*R*)-isomer was 13:87.



Scheme 3.

In conclusion, we have completed a stereoselective synthesis of (2*S*,3*S*,4*S*,5*S*)-[3,4,5-D_3]proline **6** based on the catalytic deuteration of the dehydro-L-prolinate **2** and the *syn*-selective reduction of aminal **5** derived from the [3,4-D_2]pyroglutamate **4** with Et_3SiD–$BF_3\cdot OEt_2$ system. During the course of these investigations, [3,4-D_2]proline **3** and [5-D]proline **10** were also obtained. In addition, the present procedure was applied to the synthesis of the corresponding (2*S*,3*S*,4*R*,5*R*)-isomer **12** simply by changing the deuterium source. These amino acids are considered to be useful for unambiguous assignments of ^{1}H NMR signals of the ring methylenes, especially the (2*S*,3*S*,4*R*,5*S*)-isomer which is a promising probe for monitoring the ring dynamics through the vicinal spin–spin coupling constants.

3. Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O on a Varian UNITY-400 spectrometer. All chemical shifts are reported as δ values (ppm) relative to residual chloroform (7.26 ppm) or sodium 3-(trimethylsilyl)[2,2,3,3-D_4]propionate (0 ppm). High resolution mass spectra (EI) were obtained on a JEOL JMS-AX-500 spectrometer with DA7000 data system using perfluorokerosene as an internal standard. For ion-exchange chromatography, Dowex 50W-X8 activated with 1 M HCl was used. Enantiomeric excess was determined on a Senshu SSC-3100 high-pressure liquid chromatography system equipped with chiral MCIGEL CRS10W column from Mitsubishi Kasei Co. and 2 mM $CuSO_4$ solution as an eluent. Catalytic hydrogenation was performed in an Ishii CHA-S medium-pressure catalytic hydrogenator. All other reagents were of commercial grade and used as supplied.

3.1. t-*Butyl (*2S*)*-N-t-*butoxycarbonyl-3,4-dehydroprolinate* ***1b***

The *t*-butyl 3,4-dehydroprolinate **1b** was obtained as a colorless oil in 51% yield (5 steps) from *trans*-4-hydroxy-L-proline according to the reported procedure[12] described for the preparation of the corresponding ethyl ester **1a**. ^{1}H NMR ($CDCl_3$) δ 1.440, 1.443, 1.45 and 1.47 (4 s, 18H), 4.10 and 4.27 (2 m, 2H), 4.81 and 4.88 (2 m, 1H), 5.69 and 5.73 (2 m, 1H), 5.90 and 5.94 (2 m, 1H). HRMS *m/z* 269.1586 (M^+, calcd for $C_{14}H_{23}NO_4$: 269.1627).

3.2. *(2*S*,3*S*,4*R*)-[3,4-D_2]Proline* ***3***

To a solution of ethyl (2*S*)-*N*-*t*-butoxycarbonyl-3,4-dehydroprolinate (**1a**, 1.21 g, 5.00 mmol) in MeOD (25 ml) was added a solution of $RuCl_2(PPh_3)_3$ (23.9 mg, 0.025 mmol) in benzene (2 ml), and the resultant solution was stirred under medium pressure (5 kgf/cm^2) using deuterium gas at room temperature overnight. After removal of the solvent, the residue was submitted to a short chromatography on silica gel to remove the catalyst, giving ethyl *N*-*t*-butoxycarbonyl[3,4-D_2]prolinate (**2a**, 1.23 g) as an oil in a quantitative yield. ^{1}H NMR ($CDCl_3$) δ 1.24 and 1.26 (2 t, *J*=7 Hz, 3H), 1.40, 1.45 (2 s, 9H), 1.92 (m, 2H), 3.33–3.56 (m, 2H), 4.10–4.28 (m, 3H). HRMS *m/z* 246.1698 [$(M+H)^+$, calcd for $C_{12}H_{20}D_2NO_4$: 246.1674].

Deprotection of the [3,4-D_2]prolinate **2a** (0.363 g, 1.50 mmol) was carried out in 1 M HCl (15 ml) at 110°C for 3 h followed by a treatment with Dowex 50W-X8 to give [3,4-D_2]proline (**3**, 135 mg, 77%) as a colorless solid, mp 214–217°C (dec.). ^{1}H NMR (D_2O) δ 1.97 (ddd, *J*=7, 7 and 7 Hz, 1H), 2.05 (dd, *J*=7 and 7 Hz, 1H), 2.33 (dd, *J*=7 and 7 Hz, 0.03H), 3.33 (dd, *J*=12 and 7 Hz, 1H), 3.41 (dd, *J*=12 and 7 Hz, 1H), 4.13 (d, *J*=7 Hz, 1H). HRMS *m/z* 117.0762 (M^+, calcd for $C_5H_7D_2NO_2$: 117.0759).

3.3. t-*Butyl (2*S*,3*S*,4*R*)*-N-t-*butoxycarbonyl[3,4-D_2]prolinate* ***2b***

A mixture of *t*-butyl-*N*-*t*-butoxycarbonyl-3,4-dehydroprolinate (**1b**, 1.03 g, 4.00 mmol) and Ru/C (0.1 g) in MeOD (40 ml) was stirred at room temperature overnight under the medium pressure (5 kgf/cm^2) of deuterium gas. After removal of the catalyst using a Celite pad, evaporation of the solvent gave [3,4-D_2]prolinate **2b** (1.63 g, 97%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.43, 1.44 and 1.45 (3 s, 18H), 1.89 (m, 1.8H), 2.00–2.18 (m, 0.2H), 3.32–3.54 (m, 2H), 4.09 and 4.17 (2 br s, 1H). HRMS *m/z* 273.1874 (M^+, calcd for $C_{14}H_{23}D_2NO_4$: 273.1909).

3.4. t-Butyl (2S,3S,4R)-N-t-butoxycarbonyl[3,4-D_2]pyroglutamate **4b**

To an aqueous solution of 10% sodium metaperiodate (120 ml) was added RuO_2 (219 mg, 1.65 mmol), and the black suspension turned into a yellow solution. Then to this solution was added a solution of [3,4-D_2]prolinate **2b** (3.00 g, 11.1 mmol) in ethyl acetate (45 ml). The resulting two-phase mixture was vigorously stirred at room temperature and monitored to completion by TLC. The layers were separated and to the organic phase was added 2-propanol (2 ml) and stirred for 1 h. After removal of the precipitated RuO_2 using a Celite pad, the filtrate was dried over $MgSO_4$, concentrated, and chromatographed on silica gel to afford [3,4-D_2]pyroglutamate **4b** (3.18 g, 100%) as an oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.49 (s, 9H), 1.96 (dd, *J*=10 and 2 Hz, 0.9H), 2.25 (dd, *J*=10 and 10 Hz, 0.1H), 2.43 (d, *J*=10 Hz, 0.1H), 2.57 (d, *J*=10 Hz, 0.9H), 4.46 (d, *J*=2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 21.19 (t, *J*=21 Hz), 27.85 (6 C), 30.64 (t, *J*=21 Hz), 59.47, 82.10, 83.09, 149.37, 170.32, 173.11. HRMS *m/z* 288.1778 [$(M+H)^+$, calcd for $C_{14}H_{22}D_2NO_5$: 288.1780).

3.5. (2S,3S,4R,5S)-[3,4,5-D_3]Proline **6**

To a solution of *t*-butyl [3,4-D_2]pyroglutamate **4b** (578 mg, 2.00 mmol) in THF (20 ml) was added a solution of 1 M $LiEt_3BH$ in THF (2.4 ml, 2.40 mmol) at −78°C under Ar atmosphere and the reaction mixture was stirred for 0.5 h. Then the reaction was quenched with saturated aqueous $NaHCO_3$ (6 ml) at −78°C and the mixture was warmed up to 0°C. After addition of 30% H_2O_2 (1 ml), the mixture was stirred for a further 20 min and concentrated. The residue was extracted with ether, washed with H_2O and dried over $MgSO_4$. After removal of the solvent, the crude *t*-butyl 5-hydroxyprolinate was directly treated with MeOH (13 ml) in the presence of *p*-toluenesulfonic acid (30 mg, 0.174 mmol) for 16 h. The mixture was diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (7:3) afforded oily *t*-butyl (2*S*,3*S*,4*R*,5*RS*)-*N*-*t*-butoxycarbonyl-5-methoxy[3,4-D_2]prolinate (**5**, 457 mg, 75%) as unseparable diastereoisomers. ^{1}H NMR ($CDCl_3$) δ 1.437, 1.440, 1.45, 1.46, 1.48 and 1.49 (6 s, 18H), 1.84–2.11 (m, 2H), 3.34, 3.39, 3.40 and 3.43 (4 s, 3H), 4.14–4.20 (m, 1H), 5.12–5.29 (m, 1H). HRMS *m/z* 303.2043 (M^+, calcd for $C_{15}H_{25}D_2NO_5$: 303.2015).

To a solution of *t*-butyl 5-methoxy[3,4-D_2]prolinate **5** (457 mg, 1.50 mmol) in CH_2Cl_2 (15 ml) was added Et_3SiD (352 mg, 3.00 mmol) and trifluoroborane etherate (468 mg, 3.30 mmol) in two portions at intervals of 0.5 h at −78°C under argon atmosphere and the resulting solution was stirred for 2 h. Then the reaction mixture was quenched with saturated aqueous Na_2CO_3 (3 ml) and the organic layer was dried over $MgSO_4$ and evaporated. Deprotection of the resultant crude [3,4,5-D_3]prolinate was carried out in 1 M HCl (25 ml) at 110°C for 3 h, followed by a treatment with Dowex 50W-X8 to give [3,4,5-D_3]proline (**6**, 166 mg, 94%) as a colorless solid, mp 217–220°C (dec.). ^{1}H NMR (D_2O) δ 1.97 (dd, *J*=8 and 7 Hz, 1H), 2.05 (dd, *J*=8 and 7 Hz, 1H), 2.34 (m, 0.08H), 3.32 (d, *J*=7 Hz, 0.9H), 3.41 (d, *J*=7 Hz, 0.1H), 4.13 (d, *J*=7 Hz, 1H). HRMS *m/z* 118.0813 (M^+, calcd for $C_5H_6D_3NO_2$: 118.0822).

3.6. (2S,5S)-[5-D]Proline **10**

Using the procedure described for the synthesis of the [3,4-D_2]prolinate **5**, *t*-butyl (2*S*,5*RS*)-*N*-*t*-butoxycarbonyl-5-methoxyprolinate **9b** was obtained as an oil from the pyroglutamate **7b** in 74% yield. ^{1}H NMR ($CDCl_3$) δ 1.43, 1.44, 1.446, 1.454, 1.47 and 1.48 (6 s, 18H), 1.71–2.46 (m, 4H), 3.33, 3.385, 3.393 and 3.42 (4 s, 3H), 4.11–4.24 (m, 1H), 5.11–5.29 (m, 1H). HRMS *m/z* 301.1936 (M^+, calcd for $C_{15}H_{27}NO_5$: 301.1936).

To a solution of 5-methoxyprolinate **9b** (300 mg, 1.00 mmol) in CH_2Cl_2 (10 ml) was added Et_3SiD (234 mg, 2.00 mmol) and trifluoroborane etherate (312 mg, 2.20 mmol) in two portions at intervals of 0.5 h at −78°C under argon atmosphere and the resulting solution was stirred for 2 h. Then the reaction mixture was quenched with saturated aqueous Na_2CO_3 and the organic layer was dried over $MgSO_4$ and evaporated. Deprotection of the resultant crude [5-D]prolinate was carried out in 1 M HCl (15 ml) at 110°C for 3 h followed by treatment with Dowex 50W-X8 to give (2*S*,5*S*)-[5-D]proline (**10**, 91.0 mg, 78%) as a colorless solid, mp 212–216°C (dec.). ^{1}H NMR ($CDCl_3$) δ 2.01 (m, 2H), 2.08 (m, 1H), 2.35 (m, 1H), 3.33 (dd, *J*=7 and 7 Hz, 0.9H), 3.41 (dd, *J*=7 and 7 Hz, 0.1H), 4.13 (dd, *J*=9 and 6 Hz). HRMS *m/z* 116.0692 (M^+, calcd for $C_5H_8DNO_2$: 116.0696).

*3.7. (2*S*,3*S*,4*R*,5*R*)-[3,4,5-D$_3$]Proline **12***

t-Butyl (2*S*,3*S*,4*R*,5*RS*)-*N*-*t*-butoxycarbonyl-5-methoxy[3,4,5-D_3]prolinate **11** was obtained as an oil in 55% yield following the procedure described for the preparation of the [3,4-D_2]prolinate **5** except that $LiEt_3BD$ was used instead of $LiEt_3BH$. ^{1}H NMR ($CDCl_3$) δ 1.44, 1.45, 1.46, 1.47, 1.486 and 1.491 (6 s, 18H), 1.85–2.11 (m, 2H), 3.35, 3.40, 3.41 and 3.44 (4 s, 3H), 4.14–4.21 (m, 1H). HRMS *m/z* 304.2116 (M^+, calcd for $C_{15}H_{24}D_3NO_5$: 304.2078).

To a solution of 5-methoxy[3,4,5-D_3]prolinate **11** (318 mg, 1.04 mmol) in CH_2Cl_2 (10 ml) was added Et_3SiH (242 mg, 2.08 mmol) and trifluoroborane etherate (324 mg, 2.28 mmol) in two portions at intervals of 0.5 h at −78°C under argon atmosphere and the resulting solution was stirred for 2 h. Then the reaction mixture was quenched with saturated aqueous Na_2CO_3 (2 ml) and the organic layer was dried over $MgSO_4$ and evaporated. Deprotection of the resultant crude [3,4,5-D_3]prolinate was carried out in 1 M HCl (17 ml) at 110°C for 3 h followed by a treatment with Dowex 50W-X8 to give [3,4,5-D_3]proline (**12**, 104 mg, 85%) as a colorless solid, mp 207–213°C (dec.). ^{1}H NMR (D_2O) δ 1.97 (dd, *J*=8 and 7 Hz, 1H), 2.05 (dd, *J*=8 and 7 Hz, 1H), 2.34 (m, 0.08H), 3.32 (d, *J*=7 Hz, 0.13H), 3.41 (d, *J*=7 Hz, 0.87H), 4.13 (d, *J*=7 Hz, 1H). HRMS *m/z* 118.0786 (M^+, calcd for $C_5H_6D_3NO_2$: 118.0822).

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