

Synthesis of (2*S*,3*R*)- and (2*S*,3*S*)-[3-²H₁]-proline via highly stereoselective hydrolysis of a silyl enol ether

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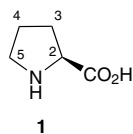
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Abstract—A straightforward synthesis of (2*S*)-[3,3-²H₂]-proline **1c** and (2*S*,3*R*)- and (2*S*,3*S*)-[3-²H₁]-proline, **1b** and **1a**, respectively, has been devised. The key step of the route to the latter compounds involves highly stereoselective hydrolysis of the silyl enol ethers **3** and **3a**, respectively, with protonation (deuteration) from the *re*-face of the silyl enol ether.
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The use of stereospecifically labelled amino acids in metabolic studies¹ and, in combination with multidimensional NMR spectroscopy, in obtaining detailed protein solution structures^{2–4} makes the ready availability of such compounds of considerable importance. The amino acid proline **1** and its post-translationally modified derivatives are constituents of antibiotics and are important for conformational constraint in proteins. Several syntheses of samples of this amino acid, stereospecifically labelled at C-3 have been completed^{1,5,6} including a chemicoenzymatic synthesis by ourselves.⁶ We now wish to report a straightforward chemical synthesis of samples of proline labelled with deuterium in the 3-*pro-R* or in the 3-*pro-S* position



During work on the synthesis of analogues of kainic acid,⁷ we prepared the silyl enol ether **3** from the 4-ketoproline derivative **2** with a view of adding a side chain at C-3 specifically from the *re*-face. We were unsuccessful in this endeavour but have now found that

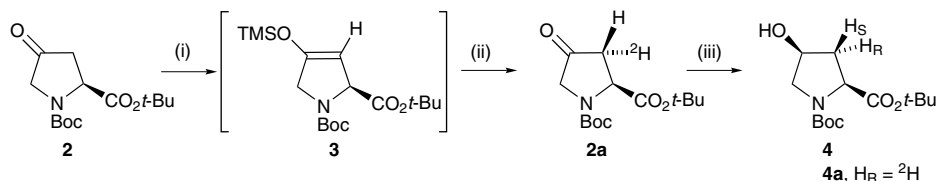
hydrolysis of a solution of the silyl enol ether **3** in THF containing excess trimethylsilyl chloride by adding excess ²H₂O gives remarkable stereoselectivity in deuteration at C-3 as shown in Scheme 1 below. Although the ¹H NMR spectrum of the unpurified product **2a** was complicated by the well-known⁸ conformational isomerism shown by *N*-acylprolines, only one of the two signals at 2.50 and 2.89 ppm due to the diastereotopic protons at C-3 in the unlabelled parent **2** was present. Since the signal at 2.89 ppm in the unlabelled compound showed a 4.5% enhancement on irradiation of the two doublets due to H-2 at ca. 4.6 ppm it could be assigned to H-3*R* and so the signal remaining at 2.5 ppm in the deuteriated compound **2a** was due to the proton, H-3*S*. Deuteration had therefore occurred from the *re*-face of the silyl enol ether **3**.

Attempts to purify compound **2a** by chromatography using silica gel ‘washed out’ the label and so the unpurified compounds **2** and **2a** were reduced directly to alcohols **4**[†] and **4a**[†] using sodium borohydride in methanol and diethyl ether at 0 °C, as shown in Scheme 1. This allowed the stereoselectivity to be confirmed using a purer and more stable compound. The ¹H NMR spectrum of the purified unlabelled compound **4** in C²HCl₃ was complicated by conformational isomerism but a simpler spectrum was obtained for a solution in C²H₃CN at 60 °C. NOE experiments in C²HCl₃, summarised in Figure 1, allowed assignment of the peaks due to hydrogens, H-3*R* and H-3*S*, since irradiation at 4.2 ppm (H-2)

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[†] This compound had the required analytical and spectroscopic properties.



Scheme 1. Reagents and conditions: (i) (a) LDA/THF/1 h, -78°C , (b) $\text{Me}_3\text{SiCl}/-78^{\circ}\text{C}$ then 1 h, rt; (ii) $^2\text{H}_2\text{O}$ rt (98% from **2**); (iii) $\text{NaBH}_4/\text{MeOH}/\text{Et}_2\text{O}/0^{\circ}\text{C}$, 5 min then 30 min, rt (98% **4** directly from **2**, 88% **4a** from **2a** as shown).

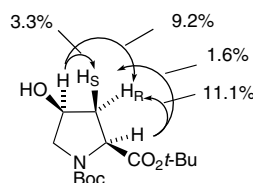
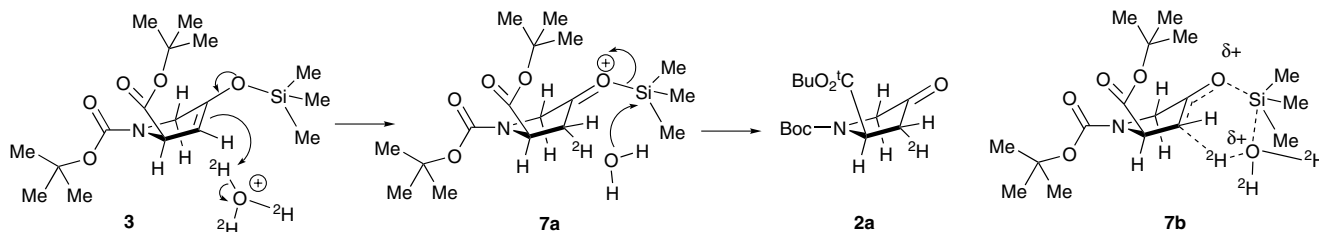


Figure 1. NOE experiments on compound **4**.

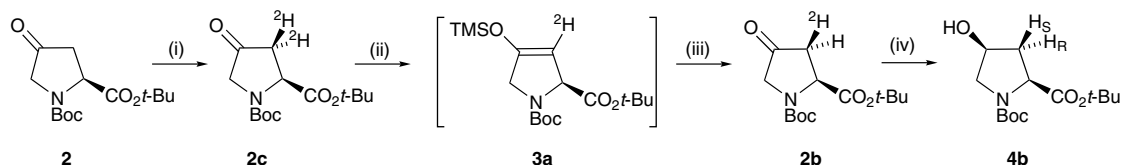
gave a considerably larger enhancement to the signal for H-3 at 2.3 ppm than that at 2.0 ppm. This defined the former signal as being due to H-3R. Irradiation at 4.3 ppm (H-4) gave a large enhancement to the signal for H-3R, thus confirming the stereochemistry at C-4. The ^1H NMR spectrum of the labelled alcohol **4a** exhibited a broad one-proton singlet for H-3S at 2.03 ppm and a residual multiplet (ca. 20%) for H-3R at 2.3 ppm. The specificity of the hydrolysis was thus confirmed. No deuterium was present in H-3S and the small amount of protium at H-3R presumably represented an isotope effect.

The high stereoselectivity found in this reaction is somewhat surprising but it is tempting to suggest that the mechanism shown in **Scheme 2** below could be partly synchronous giving the chair-like transition state **7b** shown. Interaction of the bulky trimethylsilyl and *tert*-butoxycarbonyl groups in **7b** would result in protonation occurring from the *re*-face as shown.

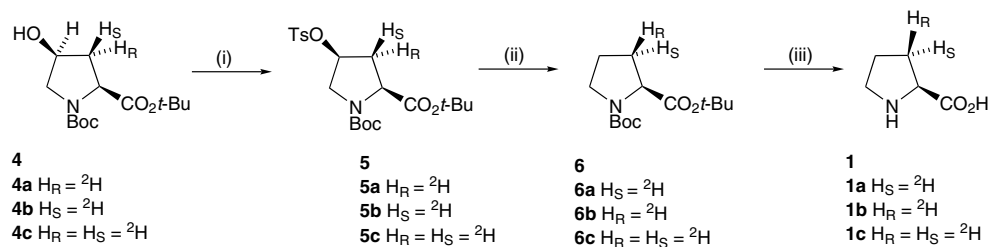
Reasoning that the isotopomer **2b** might be obtained by protonation of the deuterated silyl enol ether **3a**, we set about the preparation of the $[3,3-^2\text{H}_2]$ -ketone **2c**. We therefore treated the ketone **2** with LDA in THF and quenched the solution with $^2\text{H}_2\text{O}$. Although the ^1H NMR spectrum of the product showed that any label introduced at C-3 had been ‘washed out’ on purification using silica gel chromatography, considerable deuteration had occurred at C-5. Since the label at C-5 had remained after chromatography, we decided to ‘wash in’ deuterium at C-3 under conditions similar to those that had ‘washed’ it out. The ketone **2** was therefore stirred in $^2\text{H}_2\text{O}$ containing silica gel which had previously been washed with $^2\text{H}_2\text{O}$ as shown in **Scheme 3**. After 10 days at room temperature, almost complete exchange was obtained and the product was converted to a solution of the silyl enol ether **3a** using LDA followed by reaction with excess trimethylsilyl chloride. Addition of H_2O to the solution gave the ketone **2b**. The ^1H NMR spectrum of this compound exhibited H-3R at 2.89 ppm and a small signal for H-3S at 2.5 ppm. Because of the acidity of the ketone it was not purified but converted directly to the alcohol **4b** by reduction with NaBH_4 as before. The ^1H NMR spectrum of the purified alcohol **4b** showed a one-proton multiplet at 2.28 ppm for H-3R and ca. 30% residual protium at 2.0 ppm for H-3S. The residual protium presumably reflected incomplete deuteration in the initial dideuterated compound.



Scheme 2.



Scheme 3. Reagents and conditions: (i) $^2\text{H}_2\text{O}$ -silica gel/10 days/rt (93%); (ii) (a) LDA/THF -78°C , 1 h, (b) $\text{Me}_3\text{SiCl}/-78^{\circ}\text{C}$, 5 min then 1 h, rt; (iii) H_2O rt (98% from **2c**); (iv) $\text{NaBH}_4/\text{MeOH}/\text{Et}_2\text{O}/0^{\circ}\text{C}$, 5 min then 30 min, rt (40% **4b** from **2b** as shown; 89% **4c** directly from **2c**).



Scheme 4. Reagents and conditions: (i) TsCl/pyridine/rt, 20 h (58% **5**, 45% **5a**, 71% **5b**, 50% **5c**); (ii) NaBH₄/DMSO/85 °C, 6.5 h (94% **6**, 87% **6a**, 59% **6b**, 89% **6c**); (iii) 6 N HCl/rt, 2 h (98% **1**, 30% **1a**, 79% **1b**, 57% **1c**).

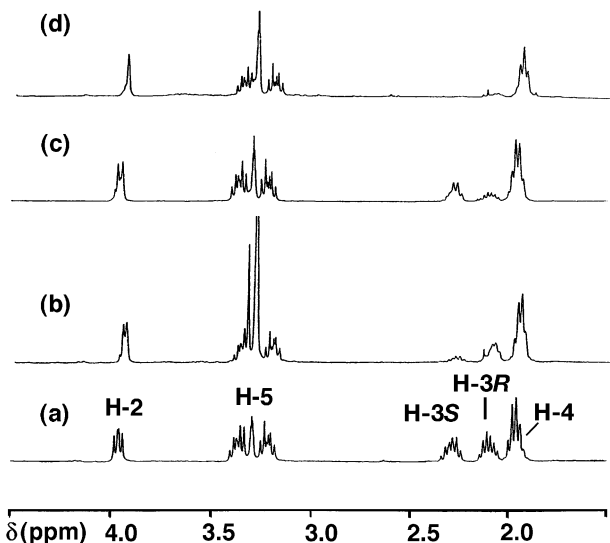


Figure 2. 360 MHz ¹H NMR spectra in C²H₃O²H of (a) (2*S*)-proline **1**; (b) (2*S*,3*S*)-[3-²H₁]-proline **1a**; (c) (2*S*,3*R*)-[3-²H₁]-proline **1b** and (d) (2*S*)-[3,3-²H₂]-proline **1c**.

Conversion of the unlabelled and labelled alcohols **4** to the corresponding samples of proline **1** proceeded as described in Scheme 4 above. The alcohols **4** were first converted to the *para*-toluenesulfonates **5**[†] using *para*-toluenesulfonyl chloride in pyridine at room temperature. These were then reduced to the protected proline derivatives **6**^{†,‡} by heating at 85 °C with NaBH₄ in DMSO for 6.5 h. Deprotection using 6 N aqueous hydrochloric acid for 2 h at room temperature gave the

samples of proline, **1**, **1a**, **1b** and **1c**. The ¹H NMR spectra of these compounds are shown in Figure 2, (2*S*,3*S*)-[3-²H₁]-proline **1a** having 22% protium at H-3*S*, and (2*S*,3*R*)-[3-²H₁]-proline having ca. 33% protium at H-3*R*. The same synthetic route was used to convert (2*S*)-[3,3-²H₂]-4-ketoproline **2c** to (2*S*)-[3,3-²H₂]-proline **1c**.

Acknowledgements

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[†]Because of the change in group priority on removal of the oxygen from C-4, the nomenclature requires that H-3*S* and H-3*R* be redefined after step (ii) in Scheme 3.