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Preparation of L-serine and L-cystine stereospecifically labeled with deuterium at the β-position

Makoto Oba,* Akira Iwasaki, Hiroshi Hitokawa, Toshinori Ikegame, Hiroyuki Banba, Kozo Ura, Tadashi Takamura and Kozaburo Nishiyama*

Department of Materials Chemistry, Tokai University, 317 Nishino, Numazu, Shizuoka 410-0395, Japan

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Abstract—The synthesis of L-serine and L-cystine stereospecifically labeled with deuterium at the β-position is described. The carboxyl group of D-serine was transformed into chirally deuterium-labeled alcohol via asymmetric reduction of 1-deuterio aldehyde, while the original hydroxymethyl group was converted into a carboxyl functionality to afford (2*S*,3*R*)-[3-^{2}H]serine. Functional group interconversions of the hydroxyl group in the obtained deuterium-labeled L-serine gave (2*R*,2′*R*,3*S*,3′*S*)-[3,3′-$^2H_2$]cystine.

1. Introduction

The stereospecific replacement of diastereotopic hydrogens with deuterium in amino acids is the most essential and the most effective method for determining the three-dimensional solution structure of peptides and proteins by NMR spectroscopy[1] and for elucidating the stereochemical course of enzymatic reactions.[2] We have recently been engaged in the stereoselective synthesis of such amino acids, in particular for NMR protein structure determination.[3–7]

For the synthesis of serine and cystine stereospecifically deuterated or tritiated at the β-position, most of the reported methods involve enzymatic and/or resolution processes in order to obtain enantiomerically pure samples.[8–18] To our knowledge, there are few examples of purely chemical methods for introducing an isotope label in a stereospecific manner.[19,20]

On the other hand, Kalvin and Woodard reported the first chemical synthesis of homoserines stereoselectively labeled with deuterium at the γ-position using asymmetric reduction of the corresponding 1-deuterio aldehyde with *R*- and *S*-Alpine-Boranes®.[21,22] We planned to use variations of this chemistry to introduce a chiral deuterium label at the β-position of both L-serine and L-cystine.

2. Results and discussion

As shown in Scheme 1, the synthesis begins with the preparation of [1-^{2}H]serinal derivative **3**. The protected D-serine **1** was condensed with 3,5-dimethylpyrazole and the pyrazolide **2** obtained was reduced with $LiAlD_4$ to give the required 1-deuterio aldehyde **3**. It is well known that the N-protected α-amino aldehydes are prone to undergo racemization.[23] Therefore aldehyde **3** obtained was immediately used in the next step without any purification. Asymmetric reduction of 1-deuterio aldehyde **3** was carried out using *S*-Alpine-Borane® (Aldrich) to yield chirally deuterium-labeled alcohol **4** in 91% yield. The absolute configuration of the newly created chiral center was determined by ^{1}H NMR spectroscopy after being converted to the final deuterium-labeled amino acids. Thus, acetylation and debenzylation of the deuterated alcohol **4** and subsequent RuO_4-oxidation of the deprotected hydroxymethyl group of compound **6** afforded protected L-serine derivative **7** in quantitative yield. Finally, compound **7** was treated with refluxing 6 M HCl followed by DOWEX® 50WX8-400 ion-exchange resin to give (2*S*,3*R*)-[3-^{2}H]serine **8** in 73% yield. The enantiomeric purity based on the α-position of the amino acid **8** was determined to be 94% ee by HPLC analysis using a chiral stationary column (SUMICHIRAL OA-6100).

The 400 MHz ^{1}H NMR spectrum of the deuterium-labeled L-serine **8** is shown in Figure 1 (upper). As can be seen from the spectrum, the signal for the 3*R* proton has disappeared,

* Corresponding authors. Tel.: +81 55 968 1111; fax: +81 55 968 1155; e-mail: makoto@wing.ncc.u-tokai.ac.jp

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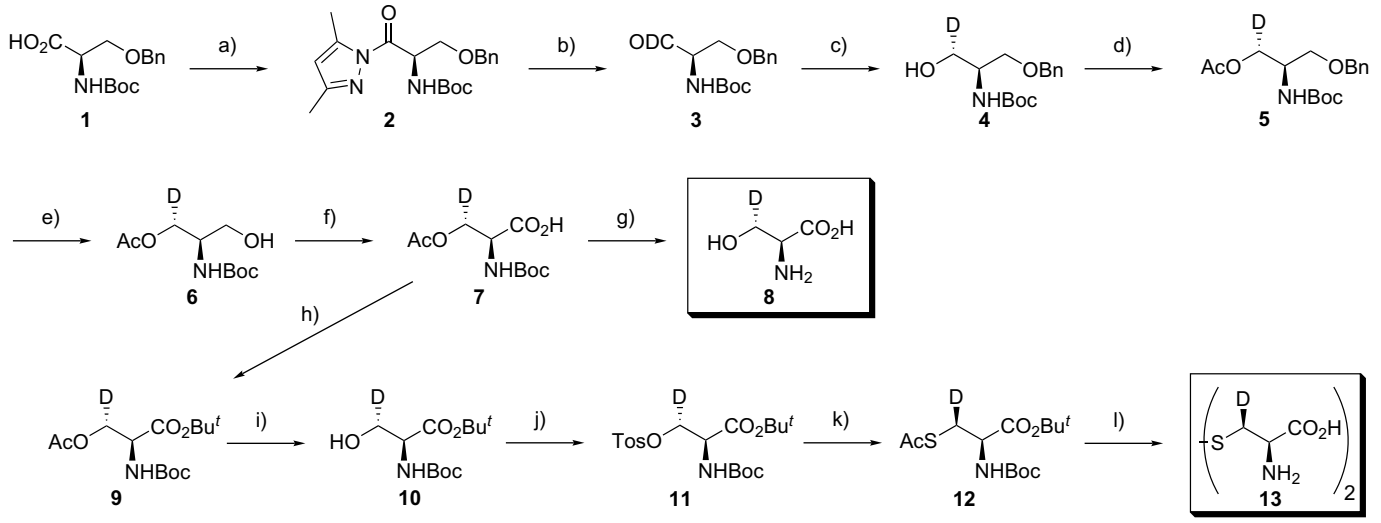

Scheme 1. Preparation of (2*S*,3*R*)-[3-^{2}H]serine **8** and (2*R*,2′*R*,3*S*,3′*S*)-[3,3′-$^2H_2$]cystine **13**. Reagents and conditions: (a) 3,5-dimethylpyrazole, DCC, chloroform, quant.; (b) $LiAlD_4$, THF, −40 °C, quant.; (c) (*S*)-Alpine-Borane®, THF, 91%; (d) Ac_2O, DMAP, pyridine, quant.; (e) H_2, 10% Pd/C, MeOH, quant.; (f) $RuCl_3$, $NaIO_4$, acetone, quant.; (g) 6 M HCl, reflux; then, DOWEX® 50WX8-400, 73%; (h) $Me_2NCH(OCH_2CH_2CHMe_2)_2$, *tert*-BuOH, benzene, reflux, 76%; (i) 1 M LiOH, THF, 90%; (j) TosCl, pyridine, 69%; (k) KSAc, DMF, 95%; (l) 6 M HCl, reflux; then, DOWEX® 50WX8-400, O_2, 63%.

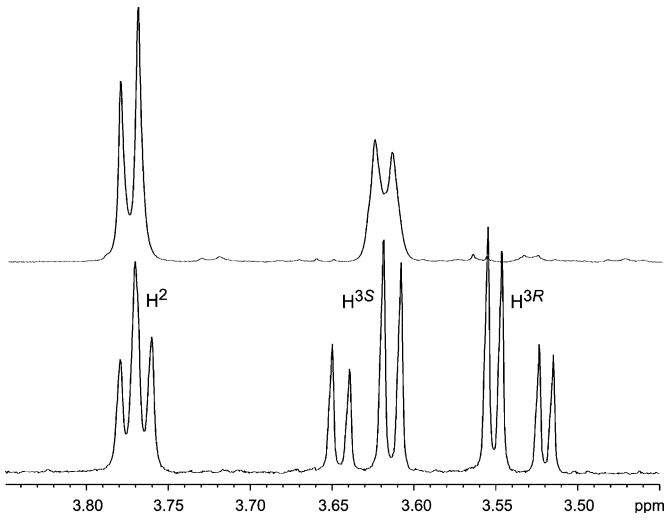

Figure 1. ^{1}H NMR spectra (at 400 MHz) of (2*S*,3*R*)-[3-^{2}H]serine **8** (upper) and unlabeled serine (lower) in 10% DCl in D_2O.

indicating that the stereochemistry at the β-position is (*R*)-configured. This stereochemical outcome is consistent with Midland's results, which show that the reduction of 1-deuterio aldehydes with (*S*)-Alpine-Borane® gives (*R*)-alcohols.[24] The diastereoselectivity of the reduction can also be estimated to be ca. 95% de by the integration of the ^{1}H NMR spectrum.

In order to obtain the deuterated L-cystine stereospecifically labeled at the β-position, we employed an S_N2-type displacement of the hydroxyl group of the corresponding deuterium-labeled L-serine derivative with an appropriate thiolate anion. After protection of the carboxyl functionality as its *tert*-butyl ester, the deuterated L-serine derivative **9** was transformed into the corresponding tosylate **11** in 62% yield (two steps) via deacetylation–tosylation processes. The tosylate **11** obtained was then treated with potassium thioacetate to give deuterium-labeled L-cystine derivative **12** in 95% yield. Deprotection of compound **12** was carried out in refluxing 6 M HCl and the resulting L-cystine hydrochloride was submitted to ion-exchange column chromatography on DOWEX® 50WX8-400. A 1 M NH_4OH eluent containing L-cystine was aerated to give highly crystalline (2*R*,2′*R*,3*S*,3′*S*)-[3,3′-$^2H_2$]cystine **13** in 63% yield.

Figure 2 (upper) shows the 400 MHz ^{1}H NMR spectrum of the deuterium-labeled L-cystine **13**. The absence of the signal assigned to the 3*S* proton verifies that the stereochemistry at the β-position is (*S*)-configuration. The diastereoselectivity of the deuterium substitution was determined to be ca. 95% de by the integration of the ^{1}H NMR spectrum.

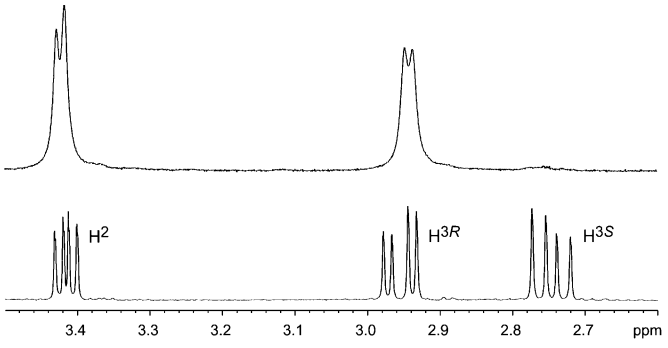

Figure 2. ^{1}H NMR spectra (at 400 MHz) of (2*R*,2′*R*,3*S*,3′*S*)-[3,3′-$^2H_2$]cystine **13** (upper) and unlabeled cystine (lower) in 2.5% NaOD in D_2O.

3. Conclusion

We have demonstrated an asymmetric synthesis of L-serine and L-cystine stereospecifically labeled with deuterium at the β-position without any bioorganic transformations. The key step in this synthesis to introduce a chiral label involves asymmetric reduction of the 1-deuterio aldehyde with *S*-Alpine-Borane®. Although we have not employed *R*-Alpine-Borane® as the reducing agent, we can see no reason why such reactions should not proceed likewise to give the corresponding deuterated amino acids that have the opposite stereochemistry at the β-position.

4. Experimental

4.1. General

Melting points were determined in open capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively. All chemical shifts are reported as δ values (parts per million) relative to residual chloroform (δ_H 7.26), HDO (δ_H 4.80), the central peak of deuteriochloroform (δ_C 77.0), or sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate (δ_C 0.00). High-resolution mass spectra (HRMS) were determined using perfluorokerosene as an internal standard. Optical rotations were measured on a HORIBA SEPA-200 polarimeter. HPLC analysis (monitored at 254 nm) was performed using a chiral stationary column (SUMICHIRAL OA-6100) with a 2 mM $CuSO_4$ solution as an eluent. Solvents and reagents were of commercial grade and were purified if necessary.

4.1.1. (1*R*)-[1-[(3,5-Dimethylpyrazol-1-yl)carbonyl]-2-benzyloxyethyl]carbamic acid *tert*-butyl ester 2. To a solution of (2*R*)-*N*-*tert*-butoxycarbonyl-*O*-benzylserine **1** (5.91 g, 20.0 mmol) and 3,5-dimethylpyrazole (2.11 g, 21.9 mmol) in chloroform (40 mL) was added 1,3-dicyclohexylcarbodiimide (DCC, 4.53 g, 22.0 mmol) and the mixture was stirred at room temperature overnight. After removal of the precipitated 1,3-dicyclohexylurea, the concentrated filtrate was chromatographed on silica gel. Elution with a mixture of hexane and ethyl acetate (93:7) afforded quantitative yield of the title compound **2** (7.59 g) as colorless needles (hexane), mp 69–70 °C. $[\alpha]_D^{26} = +18.2$ (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.16 (s, 3H), 2.53 (s, 3H), 3.83 (dd, $J = 10$ and 3 Hz, 1H), 4.05 (dd, $J = 10$ and 3 Hz, 1H), 4.45 (d, $J = 12$ Hz, 1H), 4.52 (d, $J = 12$ Hz, 1H), 5.63 (m, 2H), 5.93 (s, 1H), 7.18–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 13.73, 14.20, 28.31, 54.21, 70.42, 72.90, 79.84, 111.22, 127.45, 127.61, 128.29, 137.72, 144.32, 152.49, 155.51, 170.52. HRMS (EI, 30 eV) *m*/*z* 374.2059 [$(M+H)^+$, calcd for $C_{20}H_{28}N_3O_4$ 374.2080].

4.1.2. (2*R*)-*N*-*tert*-Butoxycarbonyl-*O*-benzyl[1-^{2}H]serinal 3. To a solution of 1 M $LiAlD_4$ in THF (12.0 mL, 12.0 mmol) was added a solution of compound **2** (2.24 g, 6.00 mmol) in THF (60 mL) at −40 °C under an argon atmosphere and the mixture was stirred for 1 h. The reaction was quenched with 10% aqueous citric acid and allowed to warm up to room temperature. After removal of the insoluble materials, the filtrate was diluted with ether, washed with aqueous $KHSO_4$, dried over $MgSO_4$, and concentrated to afford a quantitative yield of the title compound **3** (1.68 g) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.71 (dd, $J = 10$ and 4 Hz, 1H), 3.99 (dd, $J = 10$ and 3 Hz, 1H), 4.32 (m, 1H), 4.49 (d, $J = 12$ Hz, 1H), 4.52 (d, $J = 12$ Hz, 1H), 5.42 (d, $J = 7$ Hz, 1H), 7.26–7.37 (m, 5H).

4.1.3. (2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-*O*^{1}-benzyl[3-^{2}H]serinol 4. To a solution of compound **3** (1.68 g, 6.00 mmol) in THF (60 mL) was added a solution of 0.5 M *S*-Alpine-Borane® in THF (12.0 mL, 6.00 mmol) at room temperature under an argon atmosphere. After being stirred overnight, the reaction mixture was refluxed for 1 h and cooled to room temperature. To the mixture was added acetaldehyde (0.6 mL) and, after being stirred for 15 min, the solvent was removed under reduced pressure. The residue was dissolved in dry ether and to the solution was added diethanolamine (2.3 mL) at 0 °C under an argon atmosphere. After being stirred for 30 min, the precipitated solids were removed by filtration and the filtrate was washed with water, dried over $MgSO_4$, and concentrated. The crude product was purified by flash column chromatography on silica gel. Elution with a mixture of hexane and ethyl acetate (50:50) gave the title compound **4** (1.54 g, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.22 (br s, 1H), 3.56 (dd, $J = 9$ and 5 Hz, 1H), 3.62 (dd, $J = 10$ and 3 Hz, 1H), 3.73 (s, 1H), 3.78 (br s, 1H), 4.50 (s, 2H), 5.25 (br d, $J = 7$ Hz, 1H), 7.26–7.35 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 28.37, 51.56, 62.84 (t, $J = 21$ Hz), 70.12, 73.35, 79.61, 127.67, 127.82, 128.47, 137.80, 156.11. HRMS (EI, 30 eV) *m*/*z* 283.1726 [$(M+H)^+$, calcd for $C_{15}H_{23}DNO_4$ 283.1768].

4.1.4. (2*R*,3*R*)-*N*-*tert*-Butoxycarbonyl-*O*^{3}-acetyl-*O*^{1}-benzyl[3-^{2}H]serinol 5. To a solution of compound **4** (1.06 g, 3.75 mmol) in pyridine (5 mL) was added acetic anhydride (606 mg, 5.94 mmol) and 4-(dimethylamino)pyridine (DMAP, 48.0 mg, 0.393 mmol) and the mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with ether. The organic layer was washed with aqueous $KHSO_4$, dried over $MgSO_4$, and concentrated to give a quantitative yield of pure acetate **5** (1.24 g) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 2.02 (s, 3H), 3.49 (dd, $J = 9$ and 5 Hz, 1H), 3.56 (dd, $J = 9$ and 4 Hz, 1H), 4.01 (br s, 1H), 4.18 (d, $J = 6$ Hz, 1H), 4.49 (d, $J = 14$ Hz, 1H), 4.53 (d, $J = 14$ Hz, 1H), 4.91 (br d, $J = 8$ Hz, 1H), 7.26–7.37 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 20.78, 28.31, 48.97, 63.24 (t, $J = 22$ Hz), 68.75, 73.26, 79.64, 127.67, 127.80, 128.43, 137.79, 155.36, 170.86. HRMS (EI, 30 eV) *m*/*z* 325.1866 [$(M+H)^+$, calcd for $C_{17}H_{25}DNO_5$ 325.1874].

4.1.5. (2*R*,3*R*)-*N*-*tert*-Butoxycarbonyl-*O*^{3}-acetyl[3-^{2}H]serinol 6. A mixture of compound **5** (5.29 g, 16.3 mmol) and 10% palladium on carbon (1.50 g) in methanol (100 mL) was hydrogenated at room temperature for 1 h. After removal of the catalyst by filtration through a Celite pad, the filtrate was concentrated to give a quantitative yield of the title compound **6** (3.81 g) as a colorless oil. $[\alpha]_D^{26} = +3.2$ (*c* 1.0, $CHCl_3$) {lit.[25] $[\alpha]_D^{30} = +3.5$ (*c* 0.56, $CHCl_3$)}. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.09 (s, 3H),

3.61 (dd, $J = 11$ and 5 Hz, 1H), 3.68 (dd, $J = 11$ and 4 Hz, 1H), 3.87 (br s, 1H), 4.18 (d, $J = 5$ Hz, 1H), 4.59 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 20.80, 28.30, 51.00, 61.84, 62.73 (t, $J = 23$ Hz), 79.93, 155.79, 171.46. HRMS (EI, 70 eV) m/z 235.1378 [$(M+H)^+$, calcd for $C_{10}H_{19}DNO_5$ 235.1374].

4.1.6. (2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-*O*^{3}-acetyl[3-^{2}H]serine 7. To a suspension of sodium metaperiodate (40.0 g, 187 mmol) and $RuCl_3 \cdot nH_2O$ (1.21 g) in H_2O (100 mL) was added a solution of compound **6** (3.81 g, 16.3 mmol) in acetone (100 mL). The resulting two-phase mixture was vigorously stirred at room temperature for 1.5 h. The layers were separated. To the organic phase was added 2-propanol (50 mL) and the mixture was stirred for 1 h. After removal of the precipitated RuO_2 using a Celite pad, the filtrate was extracted with chloroform and dried over $MgSO_4$. Evaporation of the solvent gave a quantitative yield of the title compound **7** (4.04 g) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.18 (s, 3H), 4.46 (d, $J = 3$ Hz, 1H), 4.59 (dd, $J = 9$ and 3 Hz, 1H), 5.31 (d, $J = 9$ Hz, 1H).

4.1.7. (2*S*,3*R*)-[3-^{2}H]Serine 8. Deprotection of compound **7** (505 mg, 2.03 mmol) was carried out in refluxing 6 M HCl (20 mL) overnight followed by treatment with DOWEX® 50WX8-400 to give (2*S*,3*R*)-[3-^{2}H]serine (**8**, 157 mg, 73%) as a colorless solid, mp 237 °C dec (lit.[26] 228 °C dec). $[\alpha]_D^{23} = +15.4$ (c 1.0, 1 M HCl) {lit.[26] $[\alpha]_D^{25} = +14.95$ (c 9.34, 1 M HCl)}. ^{1}H NMR (10% DCl in D_2O) δ 3.56 (d, $J = 4$ Hz, 1H), 3.72 (d, $J = 4$ Hz, 1H). ^{13}C NMR (D_2O) δ 56.40, 59.95 (t, $J = 22$ Hz), 172.49.

4.1.8. (2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-*O*^{3}-acetyl[3-^{2}H]serine *tert*-butyl ester 9. To a refluxing solution of compound **7** (4.04 g, 16.3 mmol) in benzene (60 mL) was added a mixture of *N*,*N*-dimethylformamide dineopentyl acetal (7.38 g, 31.9 mmol) and *tert*-butyl alcohol (3.73 g, 50.3 mmol) and the reaction mixture was stirred for 2 h. Then the cooled reaction mixture was diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$ and brine, 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 (90:10) afforded the title compound **9** (3.77 g, 76%) as a colorless oil. $[\alpha]_D^{26} = +24.05$ (c 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.46 (s, 9H), 2.05 (s, 3H), 4.43–4.45 (m, 2H), 5.28 (br d, $J = 7$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 20.89, 28.13, 28.52, 53.56, 64.67 (t, $J = 24$ Hz), 80.29, 82.93, 155.44, 168.96, 170.76. HRMS (EI, 70 eV) m/z 305.1826 [$(M+H)^+$, calcd for $C_{14}H_{25}DNO_6$ 305.1823].

4.1.9. (2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl[3-^{2}H]serine *tert*-butyl ester 10. To a solution of compound **9** (3.76 g, 12.4 mmol) in THF (60 mL) was added a solution of LiOH (505 mg, 12.0 mmol) in H_2O (45 mL). After being stirred for an additional 2 h, the mixture was extracted with ethyl acetate. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated to afford the title compound **10** (2.93 g, 90%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.48 (s, 9H), 2.31 (br s, 1H), 3.87 (d, $J = 4$ Hz, 1H), 4.25 (m, 1H), 5.41 (m, 1H).

4.1.10. (2*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-*O*^{3}-(4-toluenesulfonyl)[3-^{2}H]serine *tert*-butyl ester 11. To a solution of compound **10** (2.93 g, 11.2 mmol) in pyridine (10 mL) was added 4-toluenesulfonyl chloride (3.00 g, 15.7 mmol) at 0 °C and the solution was refrigerated overnight. The mixture was quenched with water (10 mL) and extracted with ethyl acetate. The organic layer was washed successively with 1 M HCl, water, and brine, 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 (80:20) afforded the title compound **11** (3.21 g, 69%) as colorless needles (hexane), mp 90–92 °C. ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 1.43 (s, 9H), 2.44 (s, 3H), 4.35–4.38 (m, 2H), 5.26 (br d, $J = 7$ Hz, 1H), 7.34 (d, $J = 8$ Hz, 2H), 7.76 (d, $J = 8$ Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 21.87, 28.03, 28.45, 53.49, 64.93 (t, $J = 24$ Hz), 80.38, 83.54, 128.19, 130.14, 132.67, 145.29, 155.21, 167.60. HRMS (EI) m/z 417.1835 [$(M+H)^+$, calcd for $C_{19}H_{29}DNO_7S$ 417.1806].

4.1.11. (2*R*,3*S*)-*N*-*tert*-Butoxycarbonyl-*S*-acetyl[3-^{2}H]cystine *tert*-butyl ester 12. To a solution of compound **11** (979 mg, 2.35 mmol) in DMF (5 mL) was added potassium thioacetate (442 mg, 3.87 mmol) under an argon atmosphere at 0 °C and the mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated. The crude product was purified by flash column chromatography on silica gel. Elution with a mixture of hexane and ethyl acetate (80:20) gave 95% yield of the title compound **12** (712 mg, 2.22 mmol) as colorless needles (hexane), mp 47–50 °C. ^{1}H NMR ($CDCl_3$) δ 1.44 (br s, 9H), 1.46 (br s, 9H), 2.34 (s, 3H), 3.39 (br d, $J = 4$ Hz, 1H), 4.41 (br dd, $J = 7$ and 4 Hz, 1H), 5.20 (br d, $J = 7$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 28.12, 28.52, 30.75, 31.40 (t, $J = 22$ Hz), 53.79, 80.05, 82.83, 155.36, 169.66, 194.68. HRMS (EI) m/z 321.1644 [$(M+H)^+$, calcd for $C_{14}H_{25}DNO_5S$ 321.1594].

4.1.12. (2*R*,2′*R*,3*S*,3′*S*)-[3,3′-^{2}H$_2$]Cystine 13. A solution of compound **12** (609 mg, 1.90 mmol) in 6 M HCl (20 mL) was refluxed overnight. The cooled reaction mixture was washed with chloroform and concentrated. The residue was treated with an ion-exchange resin (DOWEX® 50WX8-400) and the adsorbed amino acid was eluted with 1 M ammonia solution. The fractions, which gave a positive ninhydrin reaction, were combined, aerated overnight, and concentrated to give 63% yield of the title compound **13** (143 mg, 0.595 mmol) as a colorless solid, mp >250 °C (lit.[27] 260–261 °C dec (sealed tube)). $[\alpha]_D^{23} = -220.0$ (c 0.1, 1 M HCl) (lit.[27] $[\alpha]_D^{20} = -223.4$ (c 1.0, 1 M HCl)). ^{1}H NMR (2.5% NaOD–D_2O) δ 3.01 (d, $J = 4$ Hz, 1H), 3.49 (d, $J = 4$ Hz, 1H). ^{13}C NMR (2.5% NaOD–D_2O) δ 46.05 (t, $J = 22$ Hz), 57.59, 183.60.

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