[2,3-$^{13}C_2$]-4-Hydroxy-L-threonine

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An eight-step synthesis of [2,3-$^{13}C_2$]-4-hydroxy-L-threonine {[2,3-$^{13}C_2$]-(2*S*,3*S*)-2-amino-3,4-dihydroxybutanoic acid} is described, starting from [1,2-$^{13}C_2$]acetylene, in an overall yield of 13%. Since a key intermediate of the synthetic sequence, 4-(benzyloxy)-(*Z*)-but-2-en-1-ol, is available commercially, the method furnishes a convenient four-step synthesis of nonenriched 4-hydroxy-L-threonine, in an overall yield of 27%.

Introduction

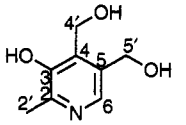
^{13}C NMR spectroscopy has opened up a powerful new dimension in biosynthetic studies. The availability as substrates of samples that are fully ^{13}C-enriched at contiguous carbon atoms, so-called "bond-labeled" samples, has made it possible to observe the transfer of intact multicarbon units from precursor into biosynthetic product. Application of ^{14}C-labeled samples provided a method for the detection of the transfer of individual carbon atoms but not of multicarbon units. Even though the sensitivity of detection of ^{13}C by NMR is approximately 3 orders of magnitude lower than that of ^{14}C by liquid scintillation counting, application of ^{13}C bond-labeled samples in investigations of precursor–product relationships is currently the method of choice in biosynthetic studies. Preconditions for success are that signal assignments within the ^{13}C NMR spectrum of the product are reliable and that the level of ^{13}C enrichment within the product is adequate for detection by high field NMR.

A serious operational difficulty with the method stems from the reality that very few ^{13}C bond-labeled compounds are commercially available. This is compounded by the fact that such samples are expensive and therefore accessible only to investigators commanding substantial research funds.

In this paper, we describe the synthesis of [2,3-$^{13}C_2$]-4-hydroxy-L-threonine (**9**), a bond-labeled substrate that was required in our investigation of the biosynthesis of vitamin B_6 in *Escherichia coli*. The rationale for the choice of the substrate and its labeling pattern will be briefly outlined, followed by a discussion of its synthesis and by brief reference to its successful application in our biosynthetic study.

[2,3-$^{13}C_2$]-4-Hydroxy-L-threonine (**9**) was synthesised in 13% overall yield in eight steps, starting from 10 L (at 1 atm, *ca.* 440 mmol) of [1,2-$^{13}C_2$]acetylene (**1**) (Cambridge Isotope Laboratories (CIL), Andover, MA, $365/L).

The Choice of [2,3-$^{13}C_2$]-4-hydroxy-L-threonine as Substrate. On the basis of indirect evidence we had postulated that the C_3N unit, N-1,C-6,5,5′, of vitamin B_6 is derived from a C_3N unit originating from 4-hydroxy-L-threonine by decarboxylation.[1] This hypothesis was based on the observed incorporation of label from [1-^{14}C]- and [1,2-^{14}C]glycolaldehyde into C-5 and C-5,5′, respectively,[2,3] and of an intact N–C unit derived from [2-^{13}C,^{15}N]-glycine into N-1,C-6 of pyridoxine,[4] giving rise to the suggestion that the two precursors may undergo aldol condensation to yield 4-hydroxy-L-threonine, in analogy with the formation of serine from glycine and formaldehyde,[5] catalyzed by serine hydroxymethylase (EC 2.1.2.1).



The notion that 4-hydroxy-L-threonine may be implicated in pyridoxine biosynthesis was developed further on the basis of genetic studies which led to the proposal of an alternative origin of the amino acid, from erythrose 4-phosphate, via erythronic acid 4-phosphate and 2-oxo-erythronic acid 4-phosphate.[6] This suggestion was entirely consistent with the observed incorporation of an intact C_3-unit, derived from glucose, into the C_3-unit, C-6,5,5′, of pyridoxine.[7] Furthermore, the presence of unlabeled 4-hydroxy-L-threonine in the medium was found to block the incorporation of label from [$^{13}C_6$]-glucose into the C_3-unit, C-6,5,5′, of pyridoxine but not into the rest of the molecule.[8] Additional evidence that 4-hydroxy-L-threonine serves as a vitamin B_6 precursor came from nutritional studies with *E. coli* mutants that are blocked in the biosynthesis of pyridoxine.[9]

To supply definitive proof of the involvement of 4-hydroxy-L-threonine as a precursor of pyridoxine, evidence for the incorporation of a labeled sample of the amino acid into the predicted carbon atoms of pyridoxine was required. More particularly, to prove that the intact carbon chain, C-2,3,4, of the amino acid is incorporated without preliminary retroaldolization to glycine plus glycolaldehyde, it was necessary to show that the intact C-2–C-3 bond of the substrate entered the product. To do so, the mode of incorporation into pyridoxine of a sample of [2,3-$^{13}C_2$]-4-hydroxy-L-threonine had to be investigated.

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Synthesis of [2,3-$^{13}C_2$]-(2S,3S)-2-Amino-3,4-dihydroxybutanoic Acid ([2,3-$^{13}C_2$]-4-Hydroxy-L-threonine). The synthesis of this bond-labeled sample, [2,3-$^{13}C_2$]-4-hydroxy-L-threonine, required a route that minimizes stereochemical uncertainty and employs ^{13}C-labeled starting materials that are commercially available but not excruciatingly expensive. None of the many published synthetic approaches to nonenriched 4-hydroxy-L-threonine was suitable for our purpose.

The classical synthesis, Strecker reaction on D-glyceraldehyde,[10-13] yields a mixture of 4-hydroxy-L-threonine[14] and 4-hydroxy-D-*allo*-threonine.[15] [1,2-$^{13}C_2$]-D-Glyceraldehyde is not available.

Another approach, starting from glycolaldehyde plus glycine, substrates whose appropriately labeled specimens are accessible, was unsuitable since it yields a mixture of the racemates of both diastereomers.[16]

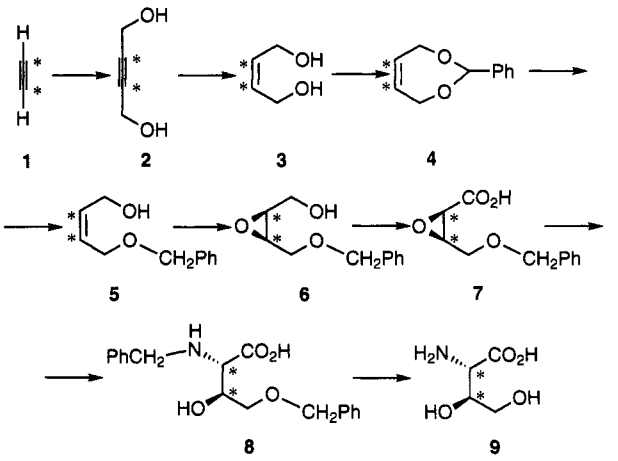
A synthesis, starting either from D-erythronic or D-threonic acid, by analogy with that for the corresponding L-isomers,[17] yields a mixture of the two diastereomers,[8] as does a synthesis starting from *N*-(benzyloxycarbonyl)-L-methionine.[18]

Appropriately labeled starting materials for the preparation of a labeled sample by either of these methods are not readily accessible. Similarly, the labeled compounds that would be required as starting materials for several stereospecific approaches are not available, *e.g.*, employing fumardialdehyde dimethyl acetal[19] derived from furan,[20] or 2,3-*O*-cyclohexylidene-[21] or 2,3-*O*-isopropylidene-D-glyceraldehyde,[22] or *N*-(benzyloxycarbonyl)-(*S*)-serinal acetonide.[23]

Another synthetic sequence had to be devised. Our sequence starts from [1,2-$^{13}C_2$]acetylene (Scheme 1).

The industrial scale synthesis of but-2-yne-1,4-diol[24] from acetylene and formaldehyde is covered by many patents. We converted [1,2-$^{13}C_2$]acetylene (**1**) into [2,3-$^{13}C_2$]but-2-yne-1,4-diol (**2**) by generating the dilithium ethynide, followed by reaction with paraformaldehyde. The subsequent steps, Lindlar hydrogenation of the yne bond, acetal formation of the (*Z*)-enediol so generated, followed by reductive cleavage and Sharpless epoxidation, exploits a series of reactions each of which is well described for nonenriched material, leading in four steps

Scheme 1[a]



[a] Key: * = 99% ^{13}C.

to (2S,3R)-3-[(benzyloxy)methyl]-2-(hydroxymethyl)-oxirane (**6**) in 90% ee and 45% overall yield from acetylene.

Oxidation of the hydroxymethyl function to a carboxylic acid, without simultaneous oxidation of the benzyl to a benzoyl group, proved to be a challenging problem. Neither pyridinium dichromate in dimethylformamide nor Jones oxidation gave the desired product in acceptable yield. Oxidation with ruthenium tetraoxide predictably gave a mixture of the benzyl- and the benzoylepoxy acids. The problem was overcome by oxidation with hypochlorite in the presence of catalytic amounts of oxoammonium salt generated from (2,2,6,6-tetramethylpiperidinyl)oxy[25] (TEMPO, free radical). The product, (2R,3R)-3-[(benzyloxy)methyl]oxirane-2-carboxylic acid (**7**) was obtained in excellent yield (88%) and high purity.

The next step, stereospecific opening of the epoxide ring of the glycidic acid (**7**), was the only reaction in the synthetic sequence that, in addition to the desired product, gave an unwanted byproduct. Predictably,[26] attack of the epoxide ring with benzylamine yielded a mixture of two regioisomers, the desired product, [2,3-$^{13}C_2$]-(2S,3S)-2-(benzylamino)-4-(benzyloxy)-3-hydroxybutanoic acid (**8**), resulting from α-attack and oxirane ring opening, together with the regioisomer, [2,3-$^{13}C_2$]-(2R,3S)-3-(benzylamino)-4-(benzyloxy)-2-hydroxybutanoic acid, product of β-attack and ring opening. Even though not regiospecific, the reaction led to a 3.5:1 excess of the desired product, most of which was then separated by recrystallization from dimethylformamide. The mixture of the two regioisomers that remained in the mother liquor could not be readily separated.

The *N*,*O*-dibenzylamino acid (**8**) was deprotected by suspension hydrogenation in water. Recrystallization yielded enantiomerically pure [2,3-$^{13}C_2$]-4-hydroxy-L-threonine (**9**) in 85% yield.

Since no chromatographic purification is involved, the preparative sequence is readily carried out on a multigram scale. Furthermore, since unlabeled 4-(benzyloxy)-(*Z*)-but-2-en-1-ol is commercially available (Aldrich, Fluka), the synthetic route to the unenriched product is shortened to four steps. The procedure, which we have

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(14) This stereoisomer, 4-hydroxy-L-threonine, has been variously described as D-*threo*-α-amino-β,γ-dihydroxy-*n*-butyric acid,[11,12] L-*threo*-α-amino-β,γ-dihydroxy-*n*-butyric acid,[16] 2-amino-2-deoxy-D-threonic acid,[13,17] L-γ-hydroxythreonine,[21,22] *threo*-β-(hydroxymethyl)-(*S*)-serine,[23] and also, erroneously, as (2*S*,3*R*)-hydroxythreonine[18] and as (2*S*,3*R*)-3-hydroxythreonine.[18]
(15) This stereoisomer, 4-hydroxy-D-*allo*threonine, has been variously described as D-*erythro*-α-amino-β,γ-dihydroxy-n-butyric acid,[11,12,16] 2-amino-2-desoxy-D-erythronic acid,[13] incompletely as *allo*-hydroxythreonine,[18] and erroneously as (2*S*,3*S*)-3-hydroxythreonine.[18]
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carried out with 25 g of 4-(benzyloxy)-(*Z*)-but-2-en-1-ol, thus provides an interesting alternative for the synthesis of unlabeled 4-hydroxy-L-threonine on a large scale.

Incubation of *E. coli* mutant WG2 with the bond-labeled substrate gave a sample of pyridoxine whose ^{13}C NMR spectrum showed doublets in the signals due to C-5 and C-6, but none in the signals of any other carbon atom.[27] The C-2,3 bond of the substrate had been incorporated intact into the predicted site of the biosynthetic product. Thus, 4-hydroxy-L-threonine serves the precursor of the C_3N unit, N-1,C-6,5,5′, of vitamin B_6.

Experimental Section[28]

[2,3-$^{13}C_2$]But-2-yne-1,4-diol (2). [1,2-$^{13}C_2$]Acetylene (>99% ^{13}C, CIL, 1 L, *ca.* <44 mmol) (**1**) was transferred from a break-seal container into an evacuated reaction flask using a liquid nitrogen bath, and the cold apparatus was flushed with dry nitrogen. Dry tetrahydrofuran (180 mL) was added, and the liquid nitrogen bath was then replaced by a dry ice/acetone bath. As soon as the solid mixture had melted, a pentane solution of *tert*-butyllithium (1.7 M, 50 mL, 85 mmol) was added slowly over 30 min, and the mixture was stirred for 1.5 h. The dryice/acetone bath was then removed and stirring continued for 4 h. Paraformaldehyde (1.5 g, 50 mmol), which had previously been dried for at least 16 h over phosphorus pentoxide, was added, and the mixture was stirred at room temperature overnight. More paraformaldehyde (0.9 g, 30 mmol) was added, and the mixture was kept at room temperature for 1 h and was then refluxed for 1 h. Water (3 mL, 167 mmol) was added to the boiling mixture, boiling was continued for 10 min, and the mixture was then allowed to cool to room temperature. A weakly acidic ion exchange resin, Amberlite CG 50 (30 g), was added, the suspension was stirred for 30 min, the resin was filtered off and washed with tetrahydrofuran (150 mL), and the solvent was evaporated. Distillation of the oily residue *in vacuo* (Kugelrohr, 140 °C, 1 mm Torr) (**DANGER:** but-2-yne-1,4-diol explodes on distillation in the presence of traces of alkali or alkaline earth hydroxides or halides[29]) gave the crystalline product **2** (2.10 g, 60%, purity >95%), which was used in the next reaction without further purification.

A small sample was recrystallized from ethyl acetate (2 mL/g) yielding [2,3-$^{13}C_2$]but-2-yne-1,4-diol (**2**), mp 57−58 °C (lit.[24] mp 58 °C, lit.[30] 56 °C). IR (KBr): 3270, 1990 cm^{-1}. ^1H NMR (200 MHz, $(CD_3)_2CO$) δ: 4.08−4.20 (m, 6H). ^{13}C NMR (50.3 MHz, $(CD_3)_2CO$) δ: 84.3 (enriched), 50.4 (t, $J_{C,C}$ = 43 Hz). MS (CI) *m/z*: 106 (100, M + NH_4^+).

[2,3-$^{13}C_2$]-(*Z*)-But-2-ene-1,4-diol (3).[31] [2,3-$^{13}C_2$]But-2-yne-1,4-diol (**2**) (2.49 g, 28.3 mmol) was dissolved in ethyl acetate (150 mL). Lindlar catalyst (250 mg, Aldrich) was added, and the mixture was hydrogenated until 1 equiv of hydrogen (634 mL) had been taken up.[31] The mixture was filtered, the catalyst was washed with ethyl acetate (50 mL), and the solvent was removed in vacuo. The product (2.55 g, 100%), a pale yellow oil, proved to be a 95:5 mixture of *cis* and *trans* isomers and was used in the next step without further purification.

A pure sample of **3** was obtained by column chromatography (SiO_2; diethyl ether). IR (film): 3356, 1415 cm^{-1}. ^1H NMR (200 MHz, $(CD_3)_2CO$) δ: 4.9−6.3 (dm, 2H, $^1J_{H,C}$ = 154 Hz), 4.02−4.16 (m, 6H). ^{13}C NMR (50.3 MHz, $(CD_3)_2CO$) δ: 131.4 (enriched), 58.3 (t, $J_{C,C}$ = 24 Hz). MS (EI) *m/z*: 91 (10, M + 1^+), 73 (35), 45 (100).

[5,6-$^{13}C_2$]-4,7-Dihydro-2-phenyl-1,3-dioxepin[32] **{[2,3-$^{13}C_2$]-1,4-*O*-benzylidene-(*Z*)-but-2-ene-1,4-diol, [5,6-$^{13}C_2$]-2-phenyl-1,3-dioxacyclohept-5-ene} (4).** Crude [2,3-$^{13}C_2$]-(*Z*)-but-2-ene-1,4-diol (**3**) (5.41 g, ca. 60 mmol) was triturated with dry benzene (125 mL). Benzaldehyde dimethyl acetal (12 mL, 80 mmol) and pyridinium *p*-toluenesulfonate (300 mg, 1.2 mmol) were added, and the mixture was heated (bath temperature *ca.* 80 °C), thereby removing methanol by azeotropic distillation. When removal of methanol was complete, the bath temperature was raised to 120 °C and benzene (*ca.* 75 mL) was distilled off. The reaction mixture was then cooled to room temperature, diluted with diethyl ether (100 mL), and extracted with water (2 × 25). After drying ($MgSO_4$) and removal of the solvent, the residue was distilled to yield 8.46 g (83%) cyclic acetal (**4**) (contaminated with *ca.* 3% benzaldehyde dimethyl acetal) which was used without further purification, bp 62 °C (0.1 mm Torr) (lit.[33] bp: 114 °C (3.5 mm Torr)). IR (film): 1451 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 7.43−7.47 (m, 2H), 7.19−7.31 (m, 3H), 5.26−6.03 (dm, 2H, $^1J_{H,C}$ = 104 Hz), 5.76 (s, 1H), 4.29 (d(AB) 2H, $^2J_{H,H}$ = 9.8 Hz), 4.16 (d(AB), 2H, $^2J_{H,H}$ = 9.8 Hz). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 138.7, 129.7, 128.1 (enriched), 127.9, 126.2, 101.8, 64.2 (t, $J_{C,C}$ = 21 Hz). MS (CI) *m/z*: 179 (100, M + 1^+).

[2,3-$^{13}C_2$]-4-(Benzyloxy)-(*Z*)-but-2-en-1-ol (5).[32,34] Lithium aluminum hydride (2.08 g, 56.2 mmol) was suspended in a 1:1 mixture of dry methylene chloride and diethyl ether (100 mL). The mixture was cooled in an icebath, and the benzylidene acetal (**4**) (8.46 g, 47.5 mmol) in methylene chloride (10 mL) was then added, followed by anhydrous aluminum chloride (7.77 g, 58.3 mmol) in diethyl ether (50 mL). After complete addition (30 min), the mixture was stirred for 1.25 h at room temperature and was then refluxed for another 1.25 h. Water (10 mL dropwise, followed by 150 mL) was added, and the phases were separated. The aqueous layer was extracted with methylene chloride (4 × 50 mL), and the combined organic extracts were dried ($MgSO_4$). After evaporation of the solvent the residue was distilled (Kugelrohr, 130 °C, 1 Torr) to yield 8.25 g (95.8%) (**5**) as a colorless liquid. IR (film): 3386, 1454 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 7.16−7.48 (m, 5H), 5.24−6.22 (m, 2H), 4.50 (s, 2H), 4.02−4.45 (m, 4H), 2.77 (s (br), 1H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 137.4, 132.4 (d(AB), $^1J_{C,C}$ = 70 Hz, enriched), 128.3, 127.6 (d(AB), $^1J_{C,C}$ = 70 Hz, enriched), 72.3, 65.5 (d, $^1J_{C,C}$ = 46 Hz), 58.3 (d, $^1J_{C,C}$ = 45 Hz). MS (EI) *m/z*: 105 (20), 91 (100).

[2,3-$^{13}C_2$]-(2*S*,3*R*)-3-[(Benzyloxy)methyl]-2-(hydroxymethyl)oxirane {[2,3-$^{13}C_2$]-(2*S*,3*R*)-3-[(Phenylmethoxy)methyl]oxirane-2-methanol, [2,3-$^{13}C_2$]-(2*S*,3*R*)-4-[(Benzyloxy)methyl]-2,3-epoxybutan-1-ol} (6).[35] Powdered molecular sieves (4 Å, 6 g) were suspended in dry methylene chloride (110 mL), and the mixture was cooled to −23 °C (dryice/CCl_4 bath). Titanium tetraisopropoxide (3.5 mL, 12 mmol) followed by diisopropyl L-tartrate (2.85 g, 12.1 mmol) were added, and the mixture was stirred for 5 min. Freshly distilled **5** (1.93 g, 10.7 mmol) was added, stirring was continued for 10 min, and a solution of *tert*-butyl hydroperoxide in decane (5.0−6.0 M, 10 mL, 50−60 mmol) was added dropwise. After 6 h the cooling bath was removed and the mixture was stored for 17 h at −15 to −20 °C. It was then filtered and cooled to −23 °C, an aqueous solution of L-tartaric acid (10%, 26.5 mL) was added, and the mixture was stirred vigorously for 30 min. Stirring was maintained for an additional 2 h, while the mixture was allowed to warm to room temperature. The organic phase was separated, washed with water (50 mL), and the volatile components were removed in vacuo. The residue was dissolved in diethyl ether and cooled in an ice bath. Aqueous sodium hydroxide (1 M, 35 mL) was added, and the mixture was stirred for 30 min. The phases were separated, and the organic layer was washed with water (2 × 50 mL) and dried ($MgSO_4$). After removal of the solvent the residue

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was distilled (Kugelrohr, 160 °C, 1 Torr) to yield 1.80 g of epoxy alcohol **6** (85.7%) as a colorless oil. The optical purity (89 ± 1% ee) was determined by ^{19}F NMR of the corresponding Mosher ester and by comparison of the optical rotation of the sample with the reported value.[36] $[\alpha]^{25}_D$: −23.5 (*c* 1.54, $CHCl_3$) (lit.[36] $[\alpha]^{25}_D$ −27 (*c* 1.5, $CHCl_3$)). IR (film): 3422 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 7.19−7.34 (m, 5H), 4.56 (d(AB), 1H, $^2J_{H,H}$ = 12 Hz), 4.46 (d(AB), 1H, $^2J_{H,H}$= 12 Hz), 3.54−3.68 (m, 5H), 2.69−2.82 (m, 1H), 2.05 (s (br), 1H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 131.7, 128.5, 128.0, 127.8, 73.4, 68.0 (d, $^1J_{C,C}$ = 47 Hz), 60.7 (d, $^1J_{C,C}$ = 48 Hz), 55.7 (d(AB), $^1J_{C,C}$ = 28 Hz, enriched), 54.6 (d(AB), $^1J_{C,C}$ = 28 Hz, enriched). MS (CI) *m/z*: 214 (100, M+ NH_4^+).

[2,3-$^{13}C_2$]-(2*R*,3*R*)-3-[(Benzyloxy)methyl]oxirane-2-carboxylic Acid (7). To a solution of the epoxy alcohol **6** (2.00 g, 10.5 mmol) in methylene chloride (12 mL) were added, in sequence, (tetramethylpiperidyl)oxy (TEMPO) (0.016 M in methylene chloride, 12 mL, 0.2 mmol), Aliquat 336 (0.08 M in methylene chloride, 8 mL, 0.64 mmol), and aqueous KBr (0.5 M, 4 mL), and the mixture was cooled in an icebath. An ice cold 1:1 mixture of saturated aqueous $NaHCO_3$ and NaOCl (Cl content > 5%, Aldrich) (96 mL) was then added, and the reaction mixture was stirred vigorously for 20 min. Cooling in ice was continued while aqueous NaOH (5 M) was added until the pH exceeded 12. After 10 min of stirring the phases were separated and the aqueous layer was washed with methylene chloride (2 × 10 mL) and acidified to pH 4. (**Danger**: evolution of chlorine gas). The solution was extracted with methylene chloride (4 × 50 mL), and the combined organic extracts were dried ($MgSO_4$). Solvent was removed in vacuo to yield 1.89 g (88.2%) crude epoxy acid (**7**) as a yellow oil (purity > 90%) which was used without purification in the next step. $[\alpha]^{25}_D$: −6.3 (*c* 1.95, $CHCl_3$; crude product). IR (film): 1742 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 11.0−11.4 (s (br), 1H), 7.20−7.35 (m, 5H), 4.72 (d(AB), 1H, $^2J_{H,H}$ = 12.0 Hz), 4.62 (d(AB), 1H, $^2J_{H,H}$ = 12.0 Hz), 3.85−4.16 (m, 3H), 3.12−3.24 (m, 1H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 172.9 (d, $^1J_{C,C}$ = 76 Hz), 137.1, 128.4, 127.9, 127.8, 73.3, 66.5 (d, $^1J_{C,C}$ = 51 Hz), 56.0 (d(AB), $^1J_{C,C}$ = 25 Hz, enriched), 50.9 (d(AB), $^1J_{C,C}$ = 25 Hz, enriched). MS (CI) *m/z*: 228 (90, M + NH_4^+), 90 (100). HRMS (EI): calcd for $^{12}C_9{}^{13}C_2H_{12}O_4$ (M^+) 210.0802, found 210.0793.

[2,3-$^{13}C_2$]-(2*S*,3*S*)-2-(Benzylamino)-4-(benzyloxy)-3-hydroxybutanoic Acid (8). Epoxy acid **7** (2.08 g, 9.9 mmol) was suspended in water (2.25 mL) under an atmosphere of nitrogen, and the solution was cooled in an icebath. Benzylamine (3.5 mL, 32 mmol) was added followed by aqueous NaOH (5 M, 1.8 mL, 9 mmol). The mixture was heated to reflux for 2 h and was then cooled to room temperature. More NaOH solution (5 M, 1 mL) was added, and the mixture was diluted with water (*ca.* 10 mL). After extraction with diethyl ether (10 mL) the aqueous phase was acidified to pH 5.6 with hydrochloric acid. After being stirred in an icebath for 1.5 h the colorless precipitate was filtered off, washed with ice-cold acetone and diethyl ether, and dried.

The product (2.77 g, 87.4%) was a 3.5:1 mixture of the desired product **8**, resulting from α-attack and oxirane ring opening, and the isomer, [2,3-$^{13}C_2$]-(2*R*,3*S*)-3-(benzylamino)-4-(benzyloxy)-2-hydroxybutanoic acid, the product of β-attack and ring opening. Recrystallization of the mixture from dimethylformamide (20 mL) yielded 1.32 g (42.1%) of α-opening product **8** (purity > 98%), mp 205 °C. $[\alpha]^{25}_D$ −25.2 (*c* 1.11, DMSO). IR (KBr): 3320, 1621 cm^{-1}. ^1H NMR (200 MHz, $(CD_3)_2SO$) δ: 7.21−7.46 (m, 10H), 4.34−4.51 (m, 1H), 4.42 (s, 2H), 3.94 (dd, 1H, $^2J_{H,H}$ = 13.3 Hz, $^3J_{H,H}$ = 4.0 Hz), 3.30−3.73 (m, 6.5H), 2.77−2.92 (m, 0.5H). ^{13}C NMR (50.3 MHz, $(CD_3)_2SO$) δ: 172.0 (d, $^1J_{C,C}$ = 53 Hz), 138.4, 137.7, 129.3, 129.1, 128.7, 128.3, 128.2, 127.5, 127.4, 127.1, 72.2, 71.5 (d, $^1J_{C,C}$ = 35 Hz), 69.5 (d(AB), $^1J_{C,C}$ = 39 Hz, enriched), 61.4 (d(AB), $^1J_{C,C}$ = 39 Hz, enriched), 50.9. MS (CI) *m/z*: 318 (100, M + 1^+). HRMS (CI): calcd for $^{12}C_{16}{}^{13}C_2H_{22}NO_4$ 318.1615, found 318.1609.

[2,3-$^{13}C_2$]-4-Hydroxy-L-threonine {[2,3-$^{13}C_2$]-(2*S*,3*S*)-2-Amino-3,4-dihydroxybutanoic Acid} (9). The dibenzylamino acid (**8**) (3.00 g, 9.45 mmol) was suspended in water (50 mL). Pearlman's catalyst (20% $Pd(OH)_2$ on carbon, 2 g, Aldrich) was added, and the mixture was hydrogenated at 40 psi for 6 h. The catalyst was filtered off and washed with hot water (20 mL), and the solvent was removed in vacuo. The light yellow residue was dissolved in boiling water (6 mL) and induced to crystallize by addition of methanol (12 mL). Pure amino acid **9** (1.10 g, 85.3%) was obtained after the mixture was kept in the refrigerator overnight, mp 210−211 °C dec (lit.[13] mp 209−11 °C dec, lit.[12] mp 214−5 °C dec). $[\alpha]^{25}_D$ −13.9 (*c* 1.61 H_2O) (lit.[13] $[\alpha]^{25}_D$ −13.4 (*c* 1.60, H_2O), lit.[11] $[\alpha]^{25}_D$ −13.7). IR (KBr): 1680 cm^{-1}. H NMR (200 MHz, D_2O) δ: 4.00 (dm, 1H, $^1J_{H,C}$ = 150.3 Hz), 3.61 (dm, 1H, $^1J_{H,C}$ = 144.0 Hz), 3.56−3.62 (m, 2H). ^{13}C NMR (50.3 MHz, D_2O) δ: 172.6 (d, $^1J_{C,C}$ = 53 Hz), 69.3 (d, $^1J_{C,C}$ = 38 Hz, enriched), 63.5 (d, $^1J_{C,C}$ = 41 Hz), 56.8 (d, $^1J_{C,C}$ = 38 Hz, enriched). MS (CI) *m/z*: 138 (100, M + 1^+). HRMS (CI): calcd for $^{12}C_2{}^{13}C_2H_{10}NO_4$ 138.0677, found 138.0680.

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