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Stereoselective Synthesis of Triply Isotope-Labeled Ser, Cys, and Ala: Amino Acids for Stereoarray Isotope Labeling Technology

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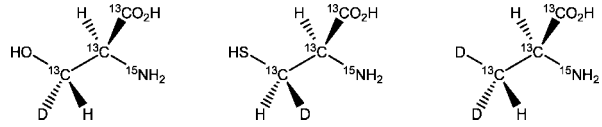
ABSTRACT



Efficient access to highly enantioselective isotope-labeled serine, cysteine, and alanine for stereoarray isotope labeling (SAIL) is described.

The sequencing of the human genome has been completed. In the postgenomic era, attention is now being focused on understanding the functions of human genes. One of the main areas of interest is high-throughput protein structure determination by nuclear magnetic resonance[1] (NMR) or X-ray crystallography. Unlike X-ray crystallography, NMR has a great advantage, in that it can be applied to proteins in solution and the natural environment. However, it is currently difficult to determine the NMR structures of proteins larger than 30 kDa because their spectra are complicated by many complex overlapping signals and significant line broadening, even when higher-field NMR spectrometers equipped with cryoprobes are used.

To overcome this problem, we have developed a new method for protein NMR spectroscopy, called stereoarray isotope labeling (SAIL) technology.[2] SAIL technology enables researchers to carry out an NMR structural analysis rapidly and with high accuracy, such as of the detailed structure of an active site, even for a 50 kDa protein. In addition, isotope labeled amino acids are useful for the estimation of the stereochemical courses of enzyme-mediated processes performed with the participation of amino acids.[3] In this paper, we describe the chemical syntheses of SAIL serine (**1**), SAIL cysteine (**2**), and SAIL alanine (**3**), which have been designed to have optimal isotope labeling for the NMR structure determination of proteins.

As shown in Figure 1, SAIL serine (**1**) and SAIL cysteine (**2**) should be stereoselectively labeled with deuterium at the β-position, and all of the carbon and nitrogen atoms should be labeled with ^{13}C and ^{15}N, respectively. This labeling pattern would increase the intensity of the ^{1}H NMR signals by decreasing the dipole−dipole relaxation and would enable

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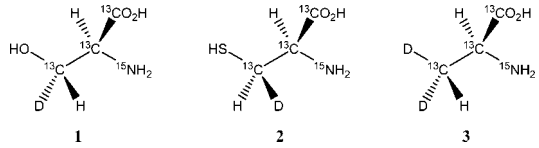


Figure 1. Structures of SAIL-Ser (1), -Cys (2), and -Ala (3).

the stereospecific assignment of a diastereotopic β-proton, based on the $^{13}C-^{13}C$ and $^{1}H-^{13}C$ correlations.

There are many other examples of methods used for the synthesis of isotope-labeled serine, cysteine, and alanine. The synthesis of a racemic mixture of serine labeled with deuterium at the β-position, via the reduction of the dehydroserine derivative, was reported by M. Kainosho[4] and D. J. Aberhart[5] more than 20 years ago. For the enantioselective synthesis of deuterium-labeled serine, Gani and co-workers reported using Baeyer−Villiger oxidation as a key reaction;[6] however, the enantiopurity of the deuterated serine is insufficient for the SAIL method. Therefore, we planned to use catalytic asymmetric hydrogenation in this experiment, for the chiral introduction of a deuterium atom at the β-position of serine, using the more easily accessible [1,2-$^{13}C_2$;^{15}N;2,2-$^{2}H_2$]glycine and ethyl [^{13}C,^{2}H]formate as our starting materials for the synthesis of SAIL serine.

Before starting the synthesis of SAIL serine, we sought to optimize the hydrogenation of the dehydroserine derivatives using various nonlabeled dehydroserine derivatives, in the presence of (+)-1,2-bis((2*S*,5*S*)-2,5-diethylphospholano)-benzene(cyclooctadiene)rhodium(I) tetrafluoroborate ((*S*,*S*)-Et-DuPHOS-Rh)[7] as the catalyst. The optical yield of the α-position during the hydrogenation was determined from serine. When the acyl group was used to protect the enol group, the deoxygenation occurred at the β-position, and the optical yields at the α-position were not sufficient. To inhibit the deoxygenation, silyl enol ether was applied to the substrate. The *t*-butyldiphenylsilyl (TBDPS) and thexyldimethylsilyl (TDS) derivatives of the protective group did not exhibit deoxygenation; however, TBDMS gave rise to a complex mixture, due to its instability. The optical purities based on the positions of the TBDPS and TDS derivatives were determined to be 99% ee and 92% ee, respectively. Therefore, to check the enantiopurity of the β-position by deuteration, we examined the catalytic deuteration of the TBDPS derivatives using deuterium gas instead of hydrogen gas, followed by deprotection, to give α,β-dideuterium-labeled serine **1b**. The 300 MHz ^{1}H NMR spectrum of serine **1b** is shown in Figure 2. As shown in the spectrum, the signals for the α-proton and the 3*S* proton have disappeared, due to the cis addition to the *re* face, indicating that the stereochemistry at the β-position is an *R* configuration.

Our synthesis began with the condensation of ethyl [1,2,3-^{13}C;^{15}N;3-^{2}H]hippurate (**4**) with ethyl [^{13}C;^{2}H]formate, followed by the silylation of the enolate **5**, to give exclusively the Z isomer of the dehydroserine derivative **6** in 64% yield. The obtained derivative **6** was examined using asymmetric catalytic hydrogenation, followed by deprotection to give SAIL serine **1** (Scheme 1). The enantiopurity based on the

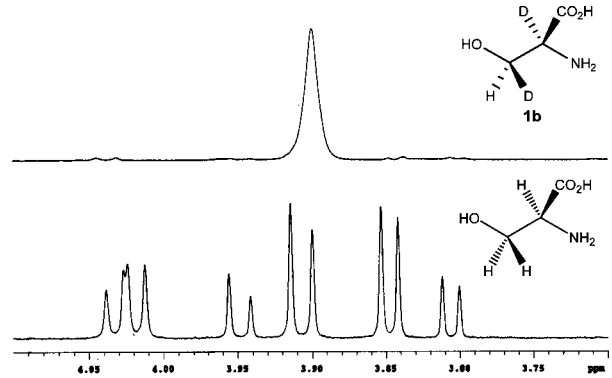
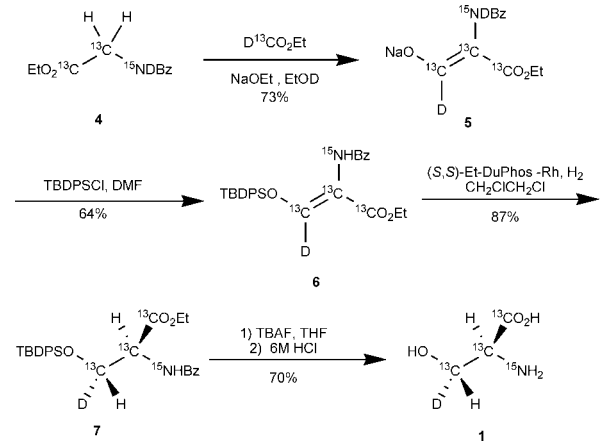


Figure 2. ^{1}H NMR Spectra of (2*S*,3*R*)-[2,3-$^{2}H_2$]serine and [^{1}H]serine.

Scheme 1



α-position was determined to be 95% ee by an HPLC analysis using a chiral stationary column (SUMICHIRAL OA-6100). We are not sure why **1** had a slightly low optical yield, but it probably resulted from racemization at the hydrolysis step,[8] since the NMR experiment revealed that the NMR signal of the β-proton of (2*S*,3*R*) or (2*R*,3*S*)-**7** was not detected; however, that of **1** was detected.[9]

The technique of stereoselective labeling with deuterium at the β-position of cysteine was developed by Axelesson et al., starting with the enzymatic reaction of fumaric acid to produce malic acid, which would form (2*S*,3*S*)-[3-^{2}H]cystine.[10] Our latest paper also dealt with the preparation of

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(8) Manning, J. M. *J. Am. Chem. Soc.* **1970**, *92*, 7449.
(9) See Supporting Information.
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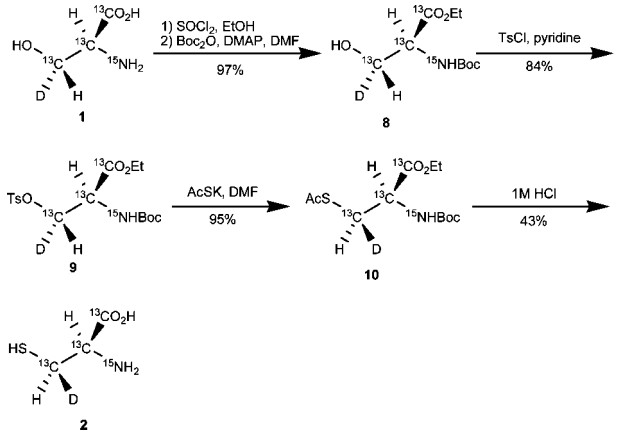
(2*S*,3*R*)-[3-^{2}H]serine and (2*R*,2′*R*,3*S*,3′*S*)-[3,3′-$^{2}H_2$]cystine starting from (2*R*)-serine.[11] Although these methods have the advantage of being sufficiently affordable for application to both SAIL serine and SAIL cysteine with minor variations, it could not be applied to the SAIL method because of difficulties in the procurement of materials and low yield. We have developed the synthesis of SAIL cysteine, using SAIL serine 2,6,7-trioxabicyclo[2,2,2]octyl ester.[6] However, this method suffers from poor reproducibility, and similar to SAIL serine starting from [1,2,3-^{13}C;^{15}N]serine, it is difficult to increase the scale of the production capacity. Therefore, we planned to use the abovementioned SAIL serine and convert the hydroxyl group of serine into a thiol group, by an S_N2-type displacement, to yield SAIL cysteine. For the synthesis of SAIL cysteine, we followed the scheme developed by Arnold et al.[12] As a result, we accomplished the synthesis of SAIL cystine ((2*R*,2′*R*,3*S*,3*S*)-[1,1′,2,2′,3,3′-$^{13}C_6$,2,2′-$^{15}N_2$;3,3′-$^{2}H_2$]cystine) with a 35% overall yield, although potentially risky chemicals, such as dimethyl azodicarboxylate, were used. To avoid potentially hazardous reaction materials, we also examined a more easily accessible means to synthesize the stereoisomer at the β-position of SAIL cysteine (Scheme 2). First, we selected the (2*S*,3*R*)-

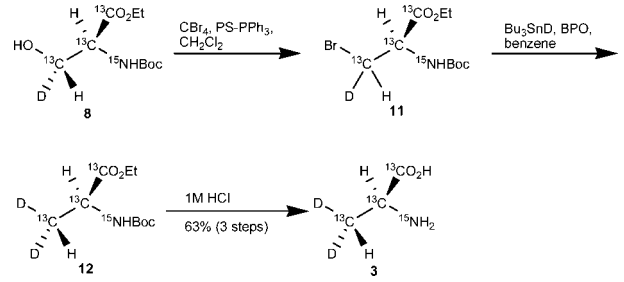
Scheme 2



N-benzoyl serine ethyl ester **7**, which was converted to tosylate and then treated with potassium thioacetate to give the (2*R*,3*S*)-cysteine derivative. Although the displacement at the β-position by the thioacetate anion proceeded with inversion, unfortunately, the yield was low, due to byproduct formation. We attempted to exchange the protection with the *t*-butoxycarbonyl group, to prevent the byproduct formation. Thus, derivative **8**, derived from SAIL serine **1**, was then subjected to tosylation followed by thioacetylation by potassium thioacetate, to yield the deuterium-labeled cysteine derivative **10** (Scheme 2). The deprotection of compound **10** was accomplished by refluxing with 1 M HCl, and the resulting SAIL cysteine hydrochloride **2** ((2*R*,3*R*)-[1,2,3-$^{13}C_3$;2-^{15}N;3′-^{2}H] cysteine) was purified by ion-exchange column chromatography on an DOWEX 50W-X8 column with a 43% yield. The enantiopurity based on the α-position of amino acid **2** was determined to be 95% ee, by HPLC analysis using a chiral stationary column (DAICEL CROWNPAK CR+).

SAIL alanine **3** should be dideuterated at the β-position, to increase the signal intensity. In particular, this labeling pattern could be more effective for larger proteins than for smaller ones. We employed deoxygenation of the hydroxyl group of the SAIL serine derivative **8** (Scheme 3). Derivative

Scheme 3



8 was treated with polymer-supported triphenylphosphine and carbon tetrabromide for conversion to the bromoalanine derivative **11**, after which derivative **11** was reduced with tributyltin deuteride, to give derivative **12**. The deprotection of compound **12** was carried out by refluxing with 1 M HCl, and the resulting alanine hydrochloride was subjected to ion-exchange column chromatography, using a DOWEX 50W-X8 column, to give SAIL alanine **3** ((2*S*)-[1,2,3-$^{13}C_3$;2-^{15}N;3,3-$^{2}H_2$]alanine) with a 63% yield from derivative **8**. The enatiopurity based on the α-position was determined to be 93% ee by an HPLC analysis using a chiral stationary column (SUMICHIRAL OA-6100).

In conclusion, we have developed the efficient syntheses of isotope-labeled serine, cysteine, and alanine. The starting materials uniformly labeled with ^{13}C and ^{15}N are now commercially available. This approach proved more amenable to scale-up, enabling the preparation of significant quantities of these amino acids. Structural determinations of proteins using the 20 SAIL amino acids are underway in our laboratory. Details of the syntheses of other SAIL amino acids will be reported in due course.

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Supporting Information Available: Experimental details and spectral data for all key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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