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Synthesis of Stereoarray Isotope Labeled (SAIL) Lysine via the "Head-to-Tail" Conversion of SAIL Glutamic Acid

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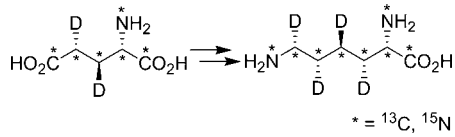
ABSTRACT



A stereoarray isotope labeled (SAIL) lysine, (2*S*,3*R*,4*R*,5*S*,6*R*)-[3,4,5,6-$^{2}H_4$;1,2,3,4,5,6-$^{13}C_6$;2,6-$^{15}N_2$]lysine, was synthesized by the "head-to-tail" conversion of SAIL-Glu, (2*S*,3*S*,4*R*)-[3,4-$^{2}H_2$;1,2,3,4,5-$^{13}C_5$;2-^{15}N]glutamic acid, with high stereospecificities for all five chiral centers. With the SAIL-Lys in hand, the unambiguous simultaneous stereospecific assignments were able to be established for each of the prochiral protons within the four methylene groups of the Lys side chains in proteins.

NMR spectroscopy has been widely used for determining protein structures in solution, despite the serious limitation of the molecular sizes for which the standard NMR methodology can be successfully applied.

In the past decade, however, concordant developments in sample preparation and spectroscopic methods have alleviated various bottlenecks, such as excessive signal overlapping and line broadening, and thus facilitated the structural studies of larger proteins that could not be analyzed by conventional methods.[1] Unfortunately, most of these new advances have extended the molecular size limit by sacrificing the structural precision, since they use the methyl and backbone amide signals as the exclusive source of the structural information, which can only be obtained by eliminating all of the other signals by extensive deuteration.[2]

We have recently developed a completely different approach, the stereoarray isotope labeling (SAIL) method, in which all *redundant* NMR signals are systematically eliminated by extensive site- and stereospecific isotope labeling.[3] One of the

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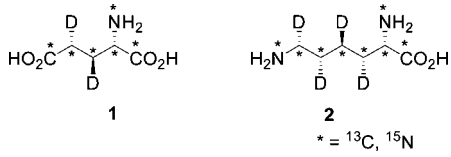
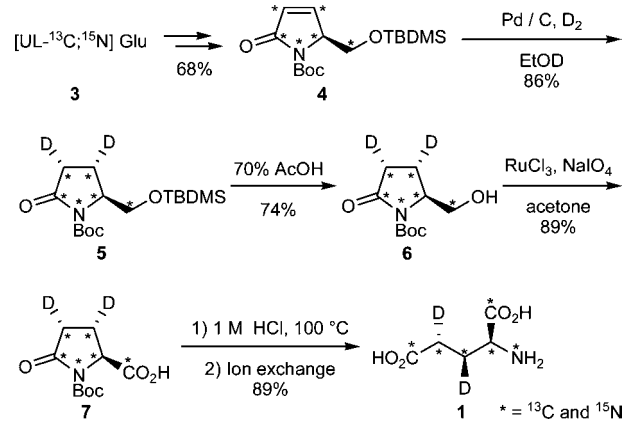
most crucial elements in the SAIL method is the synthesis of highly specific, isotope-labeled amino acids.[4] It is not trivial to synthesize, for example, a SAIL amino acid with a long aliphatic side chain, such as Lys, Arg, or Pro, in which each one of the prochiral methylene protons has to be deuterated at the high stereospecificity required for NMR applications. This is due to the fact that the chiral center of these amino acids cannot effectively control the stereocenter of the deuteration for the remote methylenes. We circumvented this problem by using a novel "head-to-tail" conversion method for SAIL-Glu **1**, (2*S*,3*S*,4*R*)-[3,4-$^{2}H_2$;1,2,3,4,5-$^{13}C_5$;2-^{15}N]glutamic acid, to synthesize SAIL-Lys **2**, (2*S*,3*R*,4*R*,5*S*,6*R*)-[3,4,5,6-$^{2}H_4$;1,2,3,4,5,6-$^{13}C_6$;2,6-$^{15}N_2$]lysine, which was otherwise very difficult to synthesize. Although a few synthetic methods exist for stereoselective lysine deuteration, no attempt to synthesize a lysine where each one of the four prochiral methylene protons is simultaneously and stereospecifically deuterated, has been reported thus far.[5]



We chose (2*S*,3*S*,4*R*)-[3,4-$^{2}H_2$;1,2,3,4,5-$^{13}C_5$;2-^{15}N]glutamic acid, SAIL-Glu, as our starting material, since SAIL-Glu can be prepared by the catalytic deuteration of the dehydroglutamic acid derivative prepared from L-[UL-^{13}C;^{15}N]glutamic acid. In general, one might adapt the single carbon elongation reaction to the terminal carboxyl group of glutamic acid, in order to synthesize stereoselectively deuterated lysines from the glutamic acid precursors. Obviously, it is not easy to synthesize lysines with stereoselectively deuterated methylenes at the C5 (δ) and/or C6 (ε) positions, under the stereocontrol of the remote chiral center at C2 (α). Therefore, we used a completely different scheme to deuterate the prochiral methylenes at the C5 and C6 carbons of lysine, in which the glutamic acid unit is converted into the lysine building block in the "head-to-tail" manner. With this new synthetic scheme, the C2 (α), C3 (β), C4 (γ), and C5 (δ) of Glu are converted into the C6 (ε), C5 (δ), C4 (γ), and C3 (β) of Lys, respectively, and the α-amino group of Glu is converted into the ε-amino group of Lys.

(2*S*,3*S*,4*R*)-[3,4-$^{2}H_2$;1,2,3,4,5-$^{13}C_5$;2-^{15}N]Glutamic acid, SAIL-Glu **1**, was synthesized from commercially available L-[UL-^{13}C,^{15}N]glutamic acid (Scheme 1). L-[UL-^{13}C,^{15}N]Glutamic acid **3** was converted to **4**,[6] and then the olefin **4** was subjected to catalytic deuteration at the C3 and C4 positions to afford the (2*S*,3*S*,4*R*)-[3,4,5-$^{2}H_3$;1,2,3,4,5-$^{13}C_5$;2-^{15}N]pyrrolidone derivative **5**. After removal of the TBDMS protecting group by treatment with acetic acid, the intermediate **6** was oxidized with RuO_4 ($RuCl_3$ + $NaIO_4$) into **7**, followed by acid hydrolysis in 1 M HCl at 110 °C to afford SAIL-Glu **1** in a 35% total yield.[7]

Scheme 1. Preparation of (2*S*,3*S*,4*R*)-[3,4-$^{2}H_2$;1,2,3,4,5-$^{13}C_5$;2-^{15}N]Glutamic Acid



One of the most versatile approaches to synthesize amino acids labeled with deuterium at the β-position is the asymmetric catalytic hydrogenation (or deuteration) of the corresponding α,β-dehydroamino acid (enamide) precursors. In the presence of some phosphine rhodium(I) or ruthenium(II) chiral complexes, as well as palladium- or platinum-containing chiral complexes, the asymmetric reduction proceeds in a highly stereoselective manner, with the *syn* addition of H_2 (D_2) to the C−C double bonds almost invariably occurring.[8]

Keeping this process in mind, we chose the enamide intermediate for the SAIL-Lys synthesis, which can be derived from SAIL-Glu **1**, as shown in Scheme 2. The conversion of **1** into the (2*R*,3*S*,4*R*)-[2,3,4-$^{2}H_3$;1,2,3,4-$^{13}C_4$;4-^{15}N]4-aminobutyric acid (GABA) **8** was achieved almost quantitatively by the stereospecific decarboxylation of the α carboxyl group, by enzymatic decarboxylation with glutamic acid decarboxylase in D_2O.[9]

After protecting the 4-amino group, the carboxyl group of **9** was converted, after derivatization into the carboxyl chloride with thionyl chloride, into the deuterated aldehyde **10**, by reductive deuteration with tributyltin deuteride in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium. The aldehyde intermediate **10** was converted exclusively into

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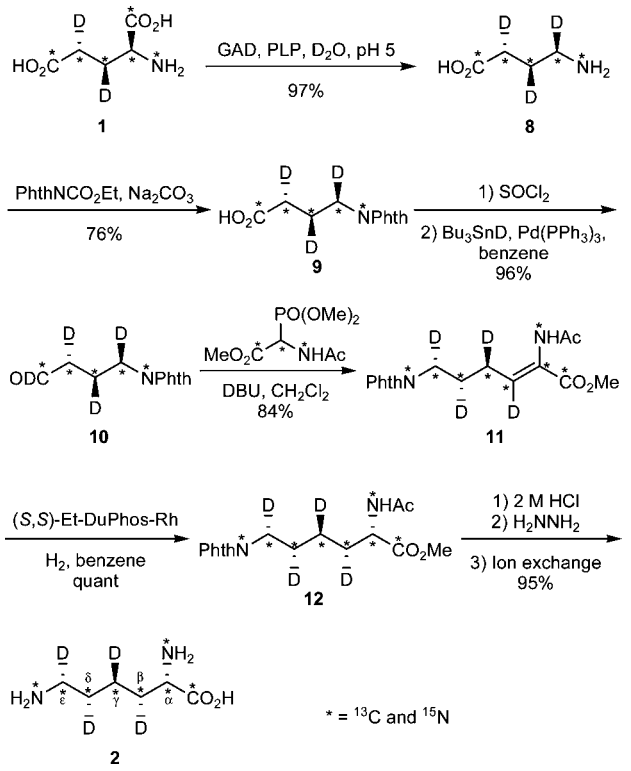
Scheme 2. Preparation of (2*S*,3*R*,4*R*,5*S*,6*R*)-[3,4,5,6-$^{2}H_4$;1,2,3,4,5,6-$^{13}C_6$;2,6-$^{15}N_2$]Lysine



the *Z*-isomer of the enamide precursor **11**, after condensation with the phosphoryl glycine derivative prepared from [1,2-$^{13}C_2$;2-^{15}N]Gly. After an extensive search for the proper conditions, the asymmetric hydrogenation with (+)-1,2-bis[(2*S*,5*S*)-2,5-diethylphospholano]benzene(cyclooctadiene)rhodium(I) trifluoromethanesulfonate ((*S*,*S*)-Et-DuPHOS-Rh) in benzene was found to be optimal. After the deprotection of **12** by refluxing in 2 M HCl, followed by the H_2NNH_2 treatment, (2*S*,3*R*,4*R*,5*S*,6*R*)-[3,4,5,6-$^{2}H_4$;1,2,3,4,5,6-$^{13}C_6$;2,6-$^{15}N_2$]lysine, SAIL-Lys **2**, was obtained in a total yield of 56% from SAIL-Glu **1**, after purification by ion-exchange column chromatography (DOWEX 50WX8). The enantiopurity based on the α-position of **2** was found to be 99% ee by an HPLC analysis.

SAIL-Lys **2** is extremely useful for NMR studies of proteins.[10] The 18.2 kDa protein, *E. coli* peptidyl prolyl cis−trans isomerase b (EPPIb), labeled with either [UL-^{13}C]Lys or SAIL-Lys, was prepared by the *E. coli* cell-free protein expression system, as described previously.[11] A mixture of amino acids (70 mg), containing 2 mg of either SAIL-Lys or [UL-^{13}C]Lys, and 19 other unlabeled amino acids (1 mM each), was used for cell-free expression. A comparison of their 2D-HCCH-TOCSY spectra[12] clearly shows that the connectivities for all 11 Lys residues in EPPIb are fully traceable along the SAIL-Lys labeled side chains, while they are not obvious for the protein labeled with [UL-^{13}C]Lys, as illustrated for Lys-62, highlighted in red circles in Figure 1. It is worth noting that the stereospecific assignments for all of the prochiral methylene protons are automatically implemented in the case of SAIL-Lys.

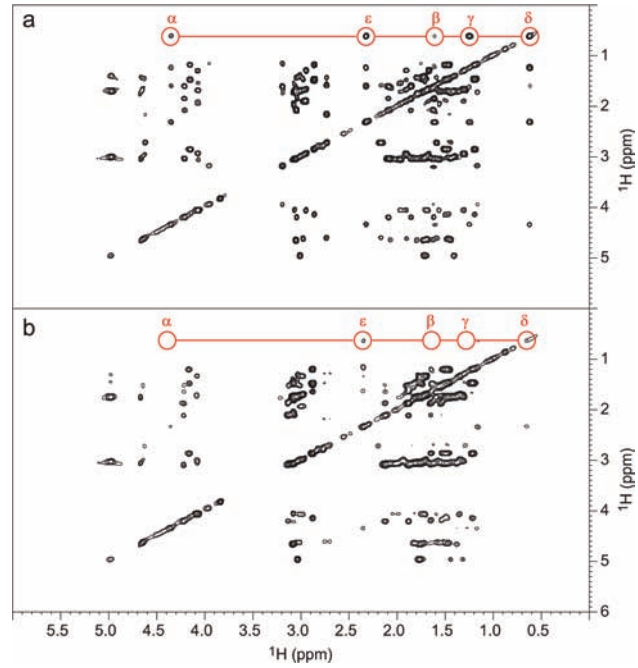


Figure 1. 2D HCCH-TOCSY NMR spectra (600 MHz for ^{1}H at 37 °C) of EPPIb labeled with SAIL-Lys (a) and with [UL-^{13}C]Lys (b). The sample concentrations were both 0.7 mM (280 μL, Shigemi microtube). Deuterium decoupling was applied during the chemical shift encoding on carbon atoms. The connectivities of Lys-62, starting from the Hδ to Hε and the Hδ to Hγ, Hβ, and Hα, are shown by red horizontal lines, although most of the cross peaks are missing in part b.

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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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