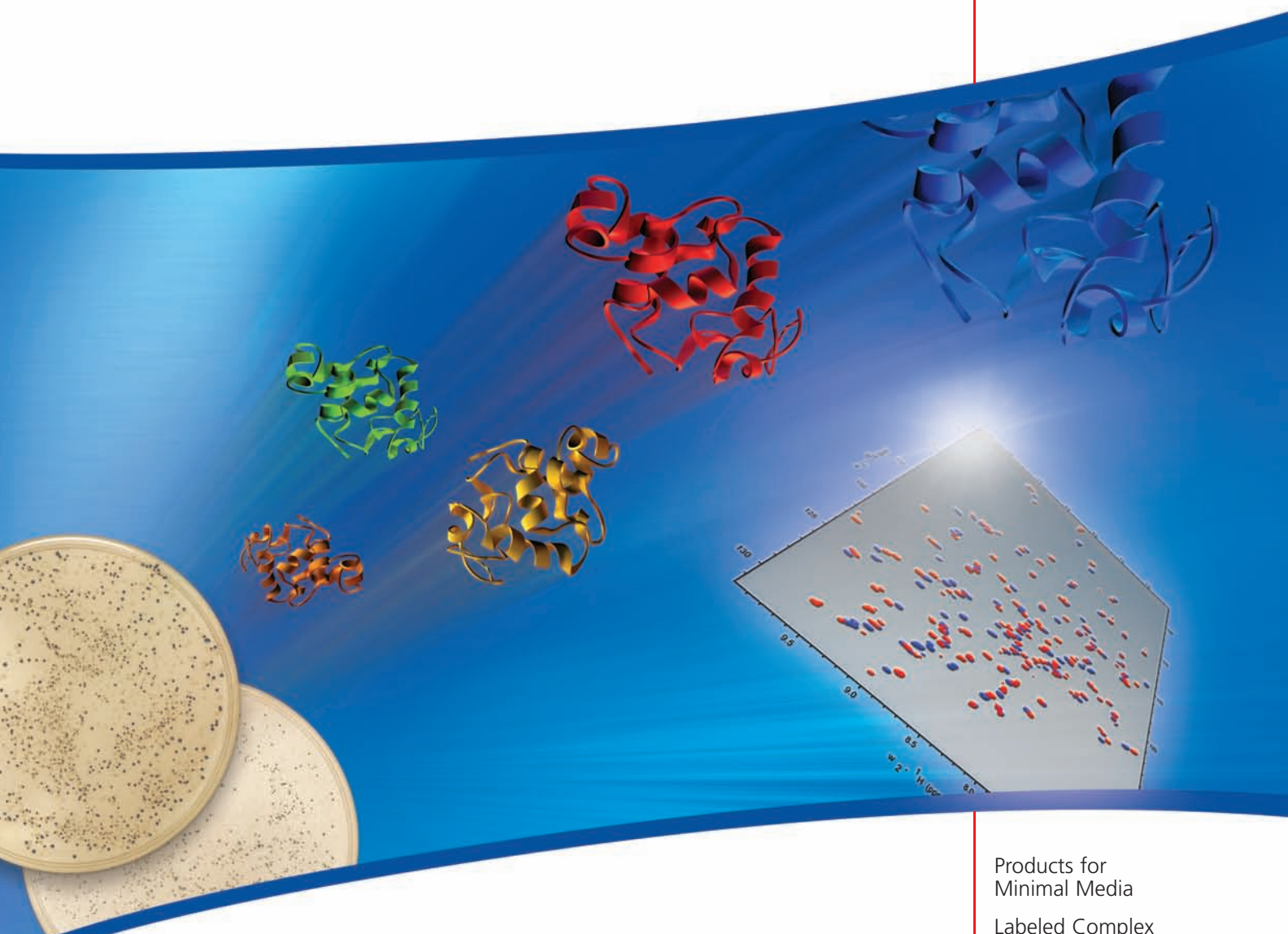


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## Isotopic Labeling in Solid-state NMR of Proteins



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### Introduction

Isotopic labeling has played a crucial role in the development of solid-state NMR spectroscopy as a method for determining the structures of proteins that reside in membranes, aggregates, or other types of assemblies. With the realization that one-third of the protein sequences in a genome correspond to

intrinsic membrane proteins, there has been a surge of interest in the further development and routine application of solid-state NMR to membrane proteins. Oriented sample methods are particularly well-suited for membrane proteins because of the highly asymmetric properties of phospholipid bilayers.<sup>1</sup>

The availability of a wide variety of labeled amino acids, carbon and nitrogen sources, as well as bacterial growth media are essential for the preparation of samples of isotopically labeled proteins.

There are three main approaches to isotopic labeling of proteins: specific, selective, and uniform labeling, and all are used in solid-state NMR studies of proteins. Most of the effort has been focused on structure determination; however, these same samples can be used to characterize the dynamics of backbone and side chain sites. An important advantage of NMR spectroscopy over other methods of studying proteins is its ability to describe both the structure and motions of individual sites.

### Specific vs. Selective Isotopic Labeling

Specific isotopic labeling refers to the placement of a single label (e.g., <sup>2</sup>H, <sup>13</sup>C, or <sup>15</sup>N) in a specified location in a polypeptide. Nearly always, specific labeling is accomplished by incorporating a labeled amino acid through chemical synthesis, which restricts this approach to relatively small polypeptides, although recent advances in synthetic methods have made it possible to specifically label an 81-residue membrane protein for NMR studies.<sup>2</sup> In favorable cases, specific labeling can be done biosynthetically if the expressed protein sequence contains only a single copy of an amino acid, and, in some cases, it can be arranged by site-directed mutagenesis. Generally, specific labeling is employed in situations where the spectroscopic experiments provide very limited or no opportunities for resolution among sites. In the initial stages of development of solid-state NMR of proteins, isotopic labeling had to carry most of the burden of the experiments. For example, a single <sup>15</sup>N label at a specific site provides the sensitivity needed to observe signals, distinguishes the signals from background, assigns the resonance to a specific site in the protein, and provides the spin-interactions that are the sources of the frequencies and other spectroscopic parameters that are measured.

The initial application of solid-state NMR to an aligned protein was performed by biosynthetically labeling the indole nitrogen of the sole tryptophan sidechain in the protein with <sup>15</sup>N.<sup>3</sup> This enabled the <sup>15</sup>N NMR spectrum of an unoriented powder sample to be used to verify that the site did not undergo motional averaging on the NMR timescales and, by comparison, to demonstrate the dramatic spectral simplifications that enabled the direct measurement of frequencies and splittings that provide orientation constraints as input for structure determination. The most comprehensive study performed with specific labeling through chemical synthesis is the structure determination of the ion channel peptide gramicidin in phospholipids bilayers.<sup>4</sup>

Nevertheless, while specific labeling was crucial in the initial stages of development, and remains useful as a tool, it is inefficient for

comprehensive studies because of the requirement for the separate preparation of many samples. As the instrumentation and experimental methods have become more powerful, it has become feasible to utilize samples where the proteins are labeled in multiple sites.

Selective labeling refers to the biosynthetic incorporation of a single type of labeled amino acid with all of the others unlabeled. For example, if a protein has eleven leucines, then only those eleven residues will be labeled, and the labels provide sensitivity and improve resolution. As with specific labeling, the low natural background, so detrimental to the sensitivity of <sup>13</sup>C and <sup>15</sup>N, became an advantage because signals from only the labeled sites are observed in the spectra. Selective labeling is widely used to assist resonance assignments in both solution NMR and solid-state NMR experiments; however, it has special significance in solid-state NMR of aligned samples where the mapping of protein structure onto the patterns of resonances in the spectra from selectively labeled samples serves as a method to simultaneously assign resonances and determine structure.<sup>5</sup>

Combinations of specific and selective labeling are essential for the solid-state NMR methods used to measure specific distances between strategically placed pairs of nuclei in unoriented samples of proteins, including rotational resonance<sup>6</sup> and REDOR.<sup>7</sup>

### Uniform Labeling

Uniform labeling refers to biosynthetic labeling of all carbon, nitrogen, or hydrogen sites with stable isotopes. Uniform labeling of proteins with <sup>15</sup>N is particularly convenient because of the strategic locations of nitrogens in the backbone and the absence of homonuclear couplings due to the intervening carbons.<sup>8</sup> It is also possible to replace all of the carbons with <sup>13</sup>C, although in this case the spectroscopy has to deal with the network of couplings among bonded carbons. Similarly, all of the hydrogens can be replaced with deuterons in order to attenuate the dipolar couplings among the hydrogens that often present complications and difficulties in the experiments.

Until recently, the vast majority of solid-state NMR studies have been performed with specific or selective isotopic labeling; however, this was accomplished at a considerable cost in flexibility. For example, when many resonances are resolved in a spectrum from a uniformly labeled protein, assignments can be readily made by comparisons with spectra from selectively labeled samples. But this is not possible if adequate resolution is present only in spectra of selectively labeled samples. Uniform labeling has the effect of shifting the experimental burdens from the isotopic labeling to the spectroscopy, which is fundamentally more powerful and flexible and now capable of yielding completely resolved spectra of membrane proteins in phospholipids bilayers.<sup>9</sup>

Uniform labeling with <sup>13</sup>C and/or <sup>15</sup>N is readily accomplished by growing bacteria in media containing <sup>13</sup>C labeled glucose and/or <sup>15</sup>NH<sub>4</sub>Cl and opened up many avenues for NMR studies of proteins. Concurrent advances in molecular biology meant that a wide variety of prokaryotic and eukaryotic proteins could be expressed in high yields in *E. coli* grown on appropriately labeled media. This started with systematic methods for making resonance assignments in solution NMR spectra of proteins<sup>10</sup> and enabled the acquisition of the extensive chemical shift data that contributes to the constraints available for structure determination. It now includes magic angle sample spinning solid-state NMR of insoluble aggregates<sup>11, 12</sup> and polycrystalline proteins.<sup>13</sup> In some cases, there are advantages to random fractional labeling with <sup>13</sup>C.

## Combining Uniform and Selective Labeling

A common use of selectively labeled samples in both solution NMR and solid-state NMR studies of proteins is to compare spectra from uniformly and selectively labeled samples. This enables the resonances from one type of amino acid to be identified by inspection. Although limited in scope, this information is valuable because it is a reliable way of resolving ambiguities in alternative assignment schemes, and while isotopic scrambling occurs in some cases, it is generally readily recognized and can be taken into account.

The comparison of two-dimensional solid-state NMR spectra of uniformly and selectively  $^{15}\text{N}$  labeled samples is the basis for an approach to simultaneous resonance assignment and three-dimensional structure determination of membrane proteins in lipid bilayers. This method utilizes proteins aligned in the field of the NMR magnet and relies on Pisa Wheels (polarity index slant angle) to first, obtain shotgun-style resonance assignments and structure assembly of isolated polypeptide segments of the protein, and second, to assemble the segments in their correct order and obtain the full three-dimensional structure.<sup>5</sup> Pisa Wheels are circular patterns of resonances in two-dimensional PISEMA (polarization inversion spin exchange at the magic angle) spectra that mirror the helical wheels of membrane protein helices in oriented bilayers.<sup>14, 15</sup> This approach short-circuits the laborious process of obtaining complete sequential assignments, greatly accelerating the process of structure determination.

## Backbone and Side-chain Dynamics

Deuteration can be used in two distinct ways.  $^2\text{H}$  can be a very effective specific or selective label, as more commonly employed with  $^{13}\text{C}$  or  $^{15}\text{N}$ , and the quadrupolar interaction provided by this spin one nucleus can be used in solid-state NMR experiments to describe both aliphatic and aromatic side-chain dynamics.<sup>16</sup> The second way for deuteration to be used in solid-state NMR experiments is similar to the approach used in solution NMR of larger proteins because of the beneficial effects of dilution of the larger number of nearby hydrogens in a protein.

## Future Prospects

Based on the success of isotopic labeling in solution NMR<sup>17</sup> and in solid-state NMR as summarized briefly in this article, one can look forward to the implementation of even more elegant isotopic labeling schemes that will complement the development of instrumentation and experimental methods for NMR studies of proteins.

## Products for Minimal Media

Name	Isotopic Purity	Cat. No.
Ammonium- $^{15}\text{N}$ chloride	98 atom % $^{15}\text{N}$	<b>299251-250MG</b> <b>299251-1G</b> <b>299251-20G</b>
Ammonium- $^{15}\text{N}_4$ deuterioxide solution, ~ 3 N in $\text{D}_2\text{O}$	99 atom % $^{15}\text{N}$ 98 atom % D	<b>594091</b>
Ammonium- $^{15}\text{N}$ hydroxide solution, ~ 3 N in $\text{H}_2\text{O}$	98 atom % $^{15}\text{N}$	<b>488011-5G</b> <b>488011-10G</b>
Ammonium- $^{15}\text{N}_2$ sulfate	98 atom % $^{15}\text{N}$	<b>299286-250MG</b> <b>299286-1G</b> <b>299286-10G</b> <b>299286-20G</b>
Deuterium oxide	99.9 atom % D	<b>151882-10G</b> <b>151882-25G</b> <b>151882-100G</b> <b>151882-125G</b> <b>151882-250G</b> <b>151882-500G</b> <b>151882-1KG</b> <b>151882-1.107KG</b>
Deuterium oxide	99.8 atom % D	<b>617385-1KG</b> <b>617385-1.107KG</b>
Deuterium oxide	99 atom % D	<b>435767-25G</b> <b>435767-100G</b> <b>435767-1KG</b>
Deuterium oxide	70 atom % D	<b>613428</b>

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Name	Isotopic Purity	Cat. No.
d-Glucose-C-d <sub>7</sub>	97-99 atom % D	<b>552003-100MG</b> <b>552003-1G</b> <b>552003-10G</b>
d-Glucose-d <sub>12</sub>	97-99 atom % D	<b>616338-250MG</b>
d-Glucose-1- $^{13}\text{C}$	99 atom % $^{13}\text{C}$	<b>297046-250MG</b> <b>297046-1G</b> <b>297046-10G</b>
d-Glucose- $^{13}\text{C}_6$	99 atom % $^{13}\text{C}$	<b>389374-100MG</b> <b>389374-250MG</b> <b>389374-1G</b> <b>389374-2G</b> <b>389374-3G</b> <b>389374-10G</b>
d-Glucose- $^{13}\text{C}_6$ -C-d <sub>7</sub>	99 atom % $^{13}\text{C}$ 97-99 atom % D	<b>552151-500MG</b> <b>552151-1G</b> <b>552151-5G</b>
Glycerol-d <sub>8</sub>	98 atom % D	<b>447498-1G</b> <b>447498-5G</b>
Glycerol-1,3- $^{13}\text{C}_2$	99 atom % $^{13}\text{C}$	<b>492639-250MG</b>
Glycerol- $^{13}\text{C}_3$	99 atom % $^{13}\text{C}$	<b>489476-500MG</b>
Glycerol- $^{13}\text{C}_3$ , d <sub>8</sub>	99 atom % $^{13}\text{C}$ 98 atom % D	<b>NEW</b> <b>669024-500MG</b>
Sodium pyruvate-3- $^{13}\text{C}$	99 atom % $^{13}\text{C}$	<b>490733-250MG</b>



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### Typical Procedure for Growing *E. coli* Using ISOGRO® Powder

To prepare **100 mL** ISOGRO medium:

1. Dissolve 1.0 g of ISOGRO powder in about 90 mL of Millipore® water.
2. Make stock solutions of the following salts and use the quantities indicated in the medium preparation:

Salt	Conc. of Stock Soln.	Qty/100 mL medium
K <sub>2</sub> HPO <sub>4</sub>	100 g/L	1.8 mL
KH <sub>2</sub> PO <sub>4</sub>	50 g/L	2.8 mL
MgSO <sub>4</sub>	50 g/L	2.0 mL
CaCl <sub>2</sub> •H <sub>2</sub> O	37 g/L	30 µL

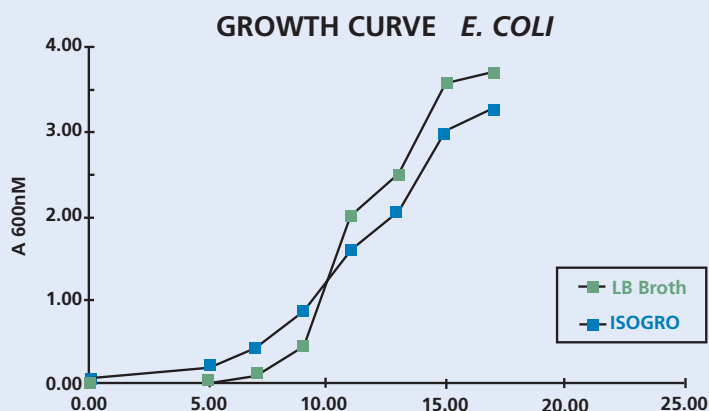
3. Adjust pH to 7.0 with NaOH and bring solution up to 100 mL with Millipore® water.
4. Pass the solution through a 0.22 µm filter and transfer the filtrate to an autoclaved shaker flask (for example: 50 mL medium in a 500 mL flask).
5. The culture is inoculated with a loop of *E. coli* which has been maintained on a nutrient agar slant.
6. Shake the culture flask in a 37 °C water bath.
7. The absorbance of the culture is measured at 600 nm with a 1:3 dilution into water.

**Note:** Researcher's specific expression applications do vary, so our preparation should serve as a guideline.

The suitability of ISOGRO as a culture medium has been demonstrated in our labs by growing *E. coli* strain W3110, ATCC 27325, in comparison with ATCC LB broth under identical conditions, with no significant differences in the two curves.

The **LB Broth**, used as a comparison medium, is made up as described in the ATCC Catalog.

**ISOTEC incorporates this comparison with LB Broth medium into our quality control testing of each batch of ISOGRO produced.**



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Name	Isotopic Purity	Cat. No.
ISOGRO®- <sup>13</sup> C Powder - Growth Medium	99 atom % <sup>13</sup> C	606863
ISOGRO-D Powder - Growth Medium	97-99 atom % D	616729
ISOGRO- <sup>15</sup> N Powder - Growth Medium	98 atom % <sup>15</sup> N	606871
ISOGRO- <sup>13</sup> C, <sup>15</sup> N Powder - Growth Medium	99 atom % <sup>13</sup> C; 98 atom % <sup>15</sup> N	606839
ISOGRO- <sup>15</sup> N, D Powder - Growth Medium	98 atom % <sup>15</sup> N; 97 atom % D	608300
ISOGRO- <sup>13</sup> C, <sup>15</sup> N, D Powder - Growth Medium	99 atom % <sup>13</sup> C; 98 atom % <sup>15</sup> N; 97-99 atom % D	608297

## $\alpha$ -Ketoacids as Biosynthetic Precursors for Protein NMR

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The state-of-the-art NMR methodology for the studies of large proteins requires full deuteration of all non-exchangeable sites in the polypeptide chain. Unfortunately, full deuteration precludes the use of NOEs for structure determination. Since methyl groups give the most intense signals in NMR spectra of proteins due to their favorable relaxation properties and are frequently located in the hydrophobic cores of protein structures, it was suggested to protonate methyl groups selectively in otherwise fully deuterated proteins.<sup>1</sup> Selective protonation of methyl groups allows to retain protons in the key elements of protein structure and to measure methyl-methyl and methyl-HN NOEs with high sensitivity and resolution<sup>2</sup> for determination of global folds of larger proteins.<sup>1,3-7</sup>

The development of strategies for selective methyl protonation of deuterated large proteins has a long history. It has been recognized that  $\alpha$ -ketoacids may serve as biosynthetic precursors for incorporation of several methyl moieties with desired isotopic labeling patterns into proteins overexpressed in minimal media. Initially, [U-<sup>13</sup>C] pyruvate was used as a primary carbon source for production of protonated Ala $\beta$ , Ile $\gamma$ , Val $\gamma$  and Leu $\delta$  methyls in highly deuterated protein molecules.<sup>1</sup> However, this approach leads to extensive generation of methyl isotopomers of other than <sup>13</sup>CH<sub>3</sub> variety (<sup>13</sup>CHD<sub>2</sub>, <sup>13</sup>CH<sub>2</sub>D and possibly <sup>13</sup>CD<sub>3</sub>) in protein samples. Due to isotope shifts, both <sup>13</sup>C and <sup>1</sup>H in each of these methyl variants resonate at different frequencies degrading resolution and sensitivity in NMR spectra. Although each of the isotopomers may be easily selected for spectroscopically, such losses in sensitivity are detrimental to NMR studies of high-molecular-mass (> 30 kDa) proteins. Later, selective protonation of isoleucine  $\delta$ 1 methyls was suggested through addition of  $\alpha$ -ketobutyrate (2-ketobutyric acid) (obtained by enzymatic reaction from threonine) to the growth medium.<sup>8</sup> It was also shown that the addition of  $\alpha$ -ketoisovalerate (2-keto-3-methylbutyric acid) leads to efficient production of methyl-protonated Val and Leu residues.<sup>9</sup> Despite that the number of methyl-containing sites is restricted to Ile $\delta$ 1, Val $\gamma$ , and Leu $\delta$  in this case, the use of  $\alpha$ -ketobutyrate and  $\alpha$ -ketoisovalerate in the growth medium does not lead to production of methyl isotopomers and is, generally, a robust and effective technique for selective protonation of methyl groups.<sup>9</sup> Sodium salts of  $\alpha$ -ketobutyric and  $\alpha$ -ketoisovaleric acids can be added to the D<sub>2</sub>O-based minimal growth medium in amounts of ~50 mg and ~80 mg/L, respectively, ~1 hour prior to induction of protein overexpression. These ketoacids can be obtained in the protonated form at position 3 and quantitatively exchanged to 3-<sup>2</sup>H at high pH in D<sub>2</sub>O (pH 12.5, 2-3 hrs. for  $\alpha$ -ketoisovalerate and pH 10.5, 12-14 hrs. for  $\alpha$ -ketobutyrate) prior to use. In addition,  $\alpha$ -ketobutyrate (the source of  $\delta$ 1-methyl protonated Ile) and  $\alpha$ -ketoisovalerate (the source of methyl protonated Val and Leu) may be used separately or together without scrambling between Ile and Val,Leu labels in the course of protein production.

Building upon the efficiency and robustness of selective methyl protonation in deuterated protein structures through the use of  $\alpha$ -ketoacids, the Abbott group developed synthetic methods for production of  $\alpha$ -ketobutyric and  $\alpha$ -ketoisovaleric acids with <sup>13</sup>C enrichment exclusively at methyl positions.<sup>10</sup> The main purpose of this approach was to eliminate <sup>13</sup>C-<sup>13</sup>C scalar couplings that would allow recording very sensitive and highly resolved <sup>1</sup>H-<sup>13</sup>C methyl correlation maps for high throughput screening of ligands to larger proteins. This group demonstrated that the screening methods based on <sup>1</sup>H-<sup>13</sup>C spectroscopy of selectively [<sup>13</sup>C,<sup>1</sup>H]-labeled methyl groups of Ile,Leu and Val can be at least 3-5 times more sensitive than those based on <sup>1</sup>H-<sup>15</sup>N TROSY spectroscopy.<sup>10</sup> An alternative and more cost-effective synthetic strategy for production of these ketoacids was proposed later by Gross et al. and used for the studies of protein-ligand interactions.<sup>11</sup> Recently, Konrat and co-workers have reported a versatile synthetic procedure that allows to incorporate <sup>13</sup>CH<sub>3</sub>/<sup>12</sup>CD<sub>3</sub> (one methyl group - <sup>13</sup>CH<sub>3</sub>, the other - <sup>12</sup>CD<sub>3</sub>) isotope

labels into isopropyl moieties of Val and Leu residues with high efficiency.<sup>12</sup> Indeed, the primary advantage of using  $\alpha$ -ketobutyric and  $\alpha$ -ketoisovaleric precursors is in the possibility of incorporation of practically any desired labeling pattern into the side-chains of Ile,Leu,Val residues as well as into methyl moieties themselves. For example, using appropriate precursors the side chains of these residues may be uniformly <sup>13</sup>C-labeled or, alternatively, the <sup>13</sup>C labels can be restricted to methyl groups. The isopropyl moieties of (<sup>13</sup>CH<sub>2</sub>/<sup>13</sup>CH<sub>3</sub>) or (<sup>13</sup>CH<sub>3</sub>/<sup>12</sup>CD<sub>3</sub>) type can be produced in Val/Leu side-chains. Likewise, methyl groups of <sup>13</sup>CH<sub>3</sub>, <sup>13</sup>CHD<sub>2</sub>, and <sup>13</sup>CH<sub>2</sub>D variety can be incorporated into Ile $\delta$ 1, Val $\gamma$ , and Leu $\delta$  sites. Such isotope labeling possibilities in methyl-bearing side-chains open new perspectives for NMR of large protein molecules.

Recently, Tugarinov & Kay showed that NMR assignments can be obtained for Leu and Val groups of very large proteins using 'linearization' of the corresponding spin-systems through the use of  $\alpha$ -ketoisovalerate non-stereospecifically labeled with <sup>13</sup>C in only one of its two methyl positions.<sup>13</sup> The same laboratory showed that factors of 2-to-3 in sensitivity can be achieved through the use of HMQC as opposed to HSQC experiments on protonated Ile  $\delta$ 1 methyls (obtained using  $\alpha$ -ketobutyrate <sup>13</sup>C-enriched only in the methyl position) in a highly deuterated protein environment.<sup>14</sup> The Methyl-TROSY methodology opened new perspectives for NMR studies of methyl groups attached to very large proteins and macromolecular complexes. However, as with <sup>1</sup>H-<sup>15</sup>N TROSY, Methyl-TROSY requires extensive deuteration of the protein and a modification of standard methyl labeling strategies. The labeling strategy that maximizes the dipolar TROSY effect in Leu and Val side chains involves the <sup>13</sup>CH<sub>3</sub>/<sup>12</sup>CD<sub>3</sub> labeling of isopropyl groups.<sup>15</sup> Using the samples of a 723-residue monomeric protein Malate Synthase G (MSG, 82 kDa), selectively protonated at Ile $\delta$ 1 (<sup>13</sup>CH<sub>3</sub>), and Val $\gamma$ /Leu $\delta$  sites (<sup>13</sup>CH<sub>3</sub>/<sup>12</sup>CD<sub>3</sub>), the global fold of this protein could be derived very recently exclusively from solution NMR data.<sup>16-18</sup> Finally, the utility of <sup>13</sup>CHD<sub>2</sub> and <sup>13</sup>CH<sub>2</sub>D methyl isotopomers incorporated into the side chains of Ile/Val/Leu amino acids has been established recently for <sup>2</sup>H and <sup>13</sup>C relaxation studies in high-molecular-mass proteins in the 100 kDa range.<sup>19</sup>

Applications of the labeling methodology and the methyl-TROSY approach have been published in studies of a number of supra-molecular systems by the Kay group. Applications include the allosteric enzyme aspartate transcarbamoylase (306 kDa),<sup>20</sup> the ClpP protease (300 kDa)<sup>21</sup> and the 20S core particle proteasome (670 kDa).<sup>22</sup> A survey of the approaches used and some of the applications have recently been published as well.<sup>23</sup>

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## $\alpha$ -Ketoacids

Name	Isotopic Purity	Structure	Cat. No.
2-Ketobutyric acid-4- <sup>13</sup> C sodium salt hydrate	99 atom % <sup>13</sup> C		571342-250MG
2-Ketobutyric acid-4- <sup>13</sup> C,4-d <sub>1</sub> sodium salt hydrate	99 atom % <sup>13</sup> C 97 atom % D		637831-1G
2-Ketobutyric acid-4- <sup>13</sup> C,4,4-d <sub>2</sub> sodium salt hydrate	99 atom % <sup>13</sup> C 98 atom % D		634727-500MG
2-Ketobutyric acid-4- <sup>13</sup> C,3,3-d <sub>2</sub> sodium salt hydrate	99 atom % <sup>13</sup> C 98 atom % D		589276-100MG
2-Ketobutyric acid-4- <sup>13</sup> C,3,3,4,4-d <sub>5</sub> sodium salt hydrate	99 atom % <sup>13</sup> C 50-70 atom % D ( <sup>13</sup> CD <sub>3</sub> ) 97 atom % D (CD <sub>2</sub> )		607533-100MG
2-Ketobutyric acid- <sup>13</sup> C <sub>4</sub> ,3,3-d <sub>2</sub> sodium salt hydrate	99 atom % <sup>13</sup> C 98 atom % D		607541-100MG
2-Keto-3-(methyl- <sup>13</sup> C)-butyric acid-4- <sup>13</sup> C sodium salt	99 atom % <sup>13</sup> C		571334-100MG
2-Keto-3-(methyl- <sup>13</sup> C,d <sub>3</sub> )-butyric acid-4- <sup>13</sup> C,d <sub>2</sub> sodium salt	98 atom % <sup>13</sup> C 98 atom % D		634379-250MG
2-Keto-3-(methyl- <sup>13</sup> C)-butyric acid-4- <sup>13</sup> C,3-d <sub>1</sub> sodium salt	99 atom % <sup>13</sup> C 98 atom % D		589063-100MG
2-Keto-3-(methyl-d <sub>3</sub> )-butyric acid-4- <sup>13</sup> C sodium salt	99 atom % <sup>13</sup> C 98 atom % D		594903-100MG
2-Keto-3-(methyl-d <sub>3</sub> )-butyric acid-4- <sup>13</sup> C, 3-d <sub>1</sub> sodium salt	99 atom % <sup>13</sup> C 97 atom % D		<b>NEW</b> 691887
2-Keto-3-(methyl-d <sub>3</sub> )-butyric acid-1,2,3,4- <sup>13</sup> C <sub>4</sub> sodium salt	99 atom % <sup>13</sup> C 98 atom % D		596418-100MG
2-Keto-3-(methyl-d <sub>3</sub> )-butyric acid-1,2,3,4- <sup>13</sup> C <sub>4</sub> , 3-d <sub>1</sub> sodium salt	99 atom % <sup>13</sup> C 98 atom % D		637858-250MG
2-Keto-3-methylbutyric acid- <sup>13</sup> C <sub>5</sub> sodium salt	99 atom % <sup>13</sup> C		663980
2-Keto-3-methylbutyric acid- <sup>13</sup> C <sub>5</sub> ,3-d <sub>1</sub> sodium salt	99 atom % <sup>13</sup> C 98 atom % D		607568-250MG

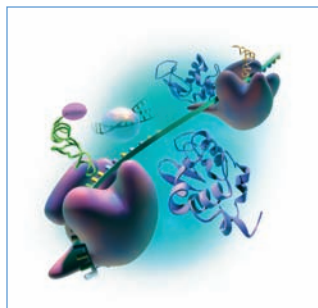
## Fully Labeled Amino Acids

### Stable Isotopes in Cell-Free Protein Synthesis

In structural genomics, the study of protein structure and function by biomolecular NMR relies on the high-throughput production of stable isotope-enriched protein. Routinely, researchers utilize microbial cells grown in defined or complex media containing (D,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) D-glucose, ammonium salts, or amino acids to uniformly label proteins.

However, *in vivo* protein expression systems have limitations:

- Many expressed proteins are insoluble and aggregate in inclusion bodies.
- Intracellular host proteases may degrade target proteins.
- Some proteins cannot be produced in living cells because of their toxicity.
- Selective amino acid labeling in cell-based systems may result in isotope dilution and diffusion.
- It may be difficult to grow organisms in deuterium-labeled media. Prokaryotes cannot glycosylate proteins.



The problems associated with living cells may be resolved by utilizing cell-free protein synthesis systems. Additional benefits of the cell-free system are its suitability for automation and high-throughput protein applications and the simplification of target protein purification.

*In vitro* protein synthesis systems (translation and/or transcription system) consist of cell extracts from *Escherichia coli*, rabbit reticulocytes, or wheat germ cells that utilize specific DNA or mRNA templates and amino acids for the production of proteins.

Cell-free synthesis systems have evolved from small batch reactions to larger scale continuous flow systems, which are capable of yielding milligram quantities of proteins.<sup>1-3</sup> Furthermore, this methodology has been adapted to produce  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labeled proteins in quantities sufficient for biomolecular NMR applications.<sup>4</sup> The utility of the cell-free system has expanded to include the development of strategies for the stereo-specific and selective labeling of proteins for NMR study.<sup>5,6</sup>

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#### Fully Labeled Amino Acids- $^{15}\text{N}$

Name	Isotopic Purity	Cat. No.
L-Alanine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>332127-100MG</b> <b>332127-500MG</b>
L-Arginine- $^{15}\text{N}_4$	96 atom % $^{15}\text{N}$	<b>653128</b>
L-Arginine- $^{15}\text{N}_4$ hydrochloride	98 atom % $^{15}\text{N}$	<b>600113-100MG</b>
L-Asparagine- $^{15}\text{N}_2$ monohydrate	98 atom % $^{15}\text{N}$	<b>485918-250MG</b>
L-Aspartic acid- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>332135-100MG</b>
L-Cysteine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>609129-100MG</b>
L-Glutamic acid- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>332143-100MG</b> <b>332143-500MG</b>
L-Glutamine- $^{15}\text{N}_2$	98 atom % $^{15}\text{N}$	<b>490032-250MG</b>
Glycine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>299294-250MG</b> <b>299294-1G</b>
L-Histidine- $^{15}\text{N}_3$	95 atom % $^{15}\text{N}$	<b>574368-100MG</b>
L-Isoleucine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>609013-500MG</b>

Name	Isotopic Purity	Cat. No.
L-Leucine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>340960-100MG</b> <b>340960-500MG</b>
L-Lysine- $^{15}\text{N}_2$ hydrochloride	98 atom % $^{15}\text{N}$	<b>609021-100MG</b>
L-Methionine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>609242-1G</b>
L-Phenylalanine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>490105-500MG</b>
L-Proline- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>608998-500MG</b>
L-Serine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>609005-500MG</b>
L-Threonine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>609099-500MG</b>
L-Tryptophan- $^{15}\text{N}_2$	95 atom % $^{15}\text{N}$	<b>574600-250MG</b>
L-Tyrosine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>332151-250MG-A</b> <b>332151-500MG-A</b>
L-Valine- $^{15}\text{N}$	98 atom % $^{15}\text{N}$	<b>490172-500MG</b>



Fully Labeled Amino Acids-<sup>13</sup>C, <sup>15</sup>N

Name	Isotopic Purity	Cat. No.
L-Alanine- <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N	98 atom % <sup>15</sup> N 98 atom % <sup>13</sup> C	<b>489883-100MG</b>
L-Arginine- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>4</sub> hydrochloride	98 atom % <sup>15</sup> N 98 atom % <sup>13</sup> C	<b>608033-100MG</b> <b>608033-250MG</b> <b>608033-500MG</b>
L-Asparagine- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N <sub>2</sub> monohydrate	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608157-100MG</b> <b>608157-500MG</b>
L-Aspartic acid- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607835-250MG</b>
L-Cysteine- <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>658057</b>
L-Glutamic acid- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607851-250MG</b> <b>607851-1G</b>
L-Glutamine- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N <sub>2</sub>	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607983-100MG</b> <b>607983-500MG</b>
Glycine- <sup>13</sup> C <sub>2</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>489522-500MG</b>
L-Histidine- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>3</sub>	97 atom % <sup>13</sup> C 95 atom % <sup>15</sup> N	<b>608009-250MG</b>
L-Isoleucine- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608092-100MG</b>
L-Leucine- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608068-100MG</b> <b>608068-250MG</b>

Name	Isotopic Purity	Cat. No.
L-Lysine- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>2</sub> hydrochloride	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608041-100MG</b>
L-Methionine- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608106-100MG</b> <b>608106-250MG</b>
L-Phenylalanine- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608017-250MG</b> <b>608017-500MG</b> <b>608017-1G</b>
L-Proline- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608114-100MG</b> <b>608114-500MG</b>
L-Serine- <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N	98 atom % <sup>15</sup> N 98 atom % <sup>13</sup> C	<b>608130-100MG</b> <b>608130-250MG</b>
L-Threonine- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607770-100MG</b> <b>607770-250MG</b> <b>607770-500MG</b>
L-Tryptophan- <sup>13</sup> C <sub>11</sub> , <sup>15</sup> N <sub>2</sub>	95 atom % <sup>15</sup> N 97 atom % <sup>13</sup> C	<b>574597-100MG</b>
L-Tyrosine- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607991-250MG</b>
L-Valine- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>600148-250MG</b> <b>600148-500MG</b> <b>600148-1G</b>

## Other Labeled Amino Acids of Interest

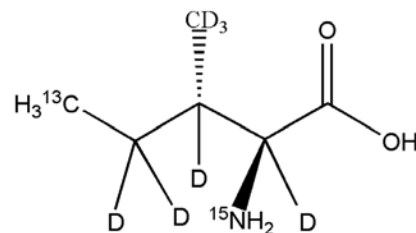
Name	Isotopic Purity	Cat. No.
L-Alanine-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>489867-500MG</b>
L-Alanine-2- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>485853-250MG</b>
Algal amino acid mixture- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>426199-1G</b>
Algal amino acid mixture- <sup>13</sup> C, <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>487910-500MG</b>
Algal amino acid mixture- <sup>13</sup> C, <sup>15</sup> N, d	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N 97 atom % D	<b>607649-500MG</b>
Algal amino acid mixture- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>608947</b>
Algal amino acid mixture- <sup>15</sup> N, d	98 atom % <sup>15</sup> N 97 atom % D	<b>596906-1G</b>
L-Asparagine- <sup>15</sup> N <sub>2</sub> , d <sub>8</sub>	98 atom % <sup>15</sup> N 98 atom % D	<b>636673-100MG</b>
L-Asparagine- <sup>15</sup> N <sub>2</sub> , d <sub>8</sub> monohydrate-d <sub>2</sub>	98 atom % D 98 atom % <sup>15</sup> N	<b>570745</b>
L-Aspartic acid-1- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>586285</b>
L-Aspartic acid-2- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607703</b>

Name	Isotopic Purity	Cat. No.
L-Aspartic acid- <sup>15</sup> N, 2,3,3-d <sub>3</sub>	98 atom % <sup>15</sup> N 98 atom % D	<b>572519-250MG</b>
L-Glutamic acid- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N, d <sub>9</sub>	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N 98 atom % D	<b>644560</b>
L-Glutamic acid- <sup>15</sup> N, d <sub>9</sub>	98 atom % <sup>15</sup> N 98 atom % D	<b>643874</b>
L-Glutamine- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N <sub>2</sub> , d <sub>10</sub>	98 atom % <sup>15</sup> N 97 atom % D 98 atom % <sup>13</sup> C	<b>635081-100MG</b>
L-Glutamine- <sup>15</sup> N <sub>2</sub> , d <sub>10</sub>	98 atom % <sup>15</sup> N 98 atom % D	<b>570737</b>
L-Leucine-1- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>490067-1G</b>
L-Leucine-2- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>607657</b>
L-Leucine-3- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>608173</b>
L-Serine-2- <sup>13</sup> C, <sup>15</sup> N	98 atom % <sup>15</sup> N 99 atom % <sup>13</sup> C	<b>485985</b>

## Don't See What You Want?

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The incorporation of selective and stereo-specific stable isotope labeled amino acids into proteins for biomolecular NMR applications is accomplished by using *in vivo* or *in vitro* techniques. Consult with ISOTEC's team of experts for the design of your labeled amino acids of interest for the production of uniformly labeled proteins. ISOTEC has the most experienced team of stable isotope custom synthesis chemists in the industry, led by an impressive group of Ph.D.s who are experts in their respective fields. ISOTEC routinely engages in the multiple step syntheses of a wide range of site and stereospecific labeling patterns for the production of isotopically labeled amino acids.



L-Isoleucine-<sup>13</sup>C, d<sub>7</sub>, <sup>15</sup>N (5-<sup>13</sup>C-3-methyl-d<sub>3</sub>, 2,3,4,4-d<sub>4</sub>)

# Isotopically Labeled Protected Amino Acids for Peptide Synthesis

## Uniformly Labeled Doubly Protected Amino Acids

Name	Isotopic Purity	Cat. No.
Boc-Glu(OBzl)-OH- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>588407</b>
Boc-Thr(Bzl)-OH- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, <b>NEW</b> 98 atom % <sup>15</sup> N	<b>672866</b>
Fmoc-Arg(Pbf)-OH- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>4</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>653659</b>
Fmoc-Asn(Trt)-OH- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N <sub>2</sub>	99 atom % <sup>13</sup> C, <b>NEW</b> 98 atom % <sup>15</sup> N	<b>668753</b>
Fmoc-Asn(Trt)-OH- <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N <b>NEW</b>	<b>668745</b>
Fmoc-Asp(OtBu)-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>594075</b>
Fmoc-Asp(OtBu)-OH- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C, <b>NEW</b> 98 atom % <sup>15</sup> N	<b>683639</b>
Fmoc-Cys(Trt)-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N <b>NEW</b>	<b>676608</b>
Fmoc-Gln(Trt)-OH- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N <sub>2</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>663956</b>
Fmoc-Glu(OtBu)-OH- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>666009</b>

## Single Protected Amino Acids

Name	Isotopic Purity	Cat. No.
Boc-Ala-OH-3,3,3-d <sub>3</sub>	99 atom % D	<b>486787</b>
Boc-Ala-OH- <sup>12</sup> C <sub>3</sub>	99.9 atom % <sup>12</sup> C	<b>492884</b>
Boc-Ala-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>486760-1G</b>
Boc-Ala-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605077</b>
Boc-Ala-OH-2- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>603449</b>
Boc-Ala-OH-3- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>492892</b>
Boc-Ala-OH- <sup>13</sup> C <sub>3</sub>	99 atom % <sup>13</sup> C	<b>586749</b>
Boc-D-Ala-OH-3- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605050</b>
Boc-Ala-OH- <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>485837</b>
Boc-Ala-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>489913-250MG</b>
Boc-Asn-OH-α- <sup>15</sup> N <sub>1</sub> (amine- <sup>15</sup> N)	98 atom % <sup>15</sup> N	<b>579785</b>
Boc-Asp-OH-3- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>586188</b>
Boc-Asp-OH-4- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>586404</b>
Boc-Asp-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>588792</b>
Boc-Gln-OH- <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N	<b>587702</b>
Boc-Glu-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>587680</b>
Boc-Glu-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>587699</b>
Boc-Gly-OH-2,2-d <sub>2</sub>	98 atom % D	<b>587710-1G</b>
Boc-Gly-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>486698-1G</b>
Boc-Gly-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>485780</b>
Boc-Gly-OH-2- <sup>13</sup> C, <sup>15</sup> N	98 atom % <sup>15</sup> N, 99 atom % <sup>13</sup> C	<b>489557-100MG</b>
Boc-Gly-OH-1- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>587729</b>
Boc-Gly-OH- <sup>13</sup> C <sub>2</sub>	99 atom % <sup>13</sup> C	<b>604992</b>
Boc-Gly-OH- <sup>13</sup> C <sub>2</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>587737</b>
Boc-Gly-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>486701-1G</b>
Boc-Leu-OH-5,5,5-d <sub>3</sub> monohydrate	99 atom % D	<b>615900</b>
Boc-Leu-OH- <sup>15</sup> N monohydrate	98 atom % <sup>15</sup> N	<b>492930</b>
Boc-Lys(Z)-OH-α- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>609161</b>
Boc-Met-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>589845</b>

Name	Isotopic Purity	Cat. No.
Fmoc-Glu(OtBu)-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>609153-100MG</b>
Fmoc-His(Trt)-OH- <sup>15</sup> N <sub>3</sub>	98 atom % <sup>15</sup> N <b>NEW</b>	<b>676969</b>
Fmoc-Lys(Boc)-OH- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N, 98 atom % <sup>13</sup> C	<b>653632</b>
Fmoc-Lys(Boc)-OH- <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N	<b>577960</b>
Fmoc-Ser(tBu)-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>609145-100MG</b>
Fmoc-Ser(tBu)-OH- <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>658928</b>
Fmoc-Thr(tBu)-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>658162</b>
Fmoc-Thr(tBu)-OH- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, <b>NEW</b> 98 atom % <sup>15</sup> N	<b>694274</b>
Fmoc-Trp(Boc)-OH- <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N <b>NEW</b>	<b>676977</b>
Fmoc-Tyr(tBu)-OH- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>658898</b>
Fmoc-Tyr(tBu)-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>658901</b>

Name	Isotopic Purity	Cat. No.
Boc-Met-OH- <sup>13</sup> C <sub>1</sub> (methyl- <sup>13</sup> C)	99 atom % <sup>13</sup> C	<b>589853</b>
Boc-Phe-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605204</b>
Boc-Phe-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>486833</b>
Boc-Tyr-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>591092</b>
Boc-Val-OH-d <sub>8</sub>	98 atom % D	<b>616222</b>
Boc-Val-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>604976</b>
Boc-Val-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>486019</b>
Fmoc-Ala-OH-3,3,3-d <sub>3</sub>	99 atom % D	<b>485888-1G</b>
Fmoc-Ala-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>486752-1G</b>
Fmoc-Ala-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605158</b>
Fmoc-Ala-OH-3- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>489956-500MG</b>
Fmoc-Ala-OH- <sup>13</sup> C <sub>3</sub>	99 atom % <sup>13</sup> C	<b>605131-1G</b>
Fmoc-Ala-OH, <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N monohydrate	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N <b>NEW</b>	<b>667064</b>
Fmoc-Ala-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>489905-1G</b>
Fmoc-Asn-OH-α- <sup>15</sup> N <sub>1</sub> (amine- <sup>15</sup> N)	98 atom % <sup>15</sup> N	<b>609137</b>
Fmoc-Asn-OH- <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N	<b>579890</b>
Fmoc-Asp-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>588628</b>
Fmoc-Asp-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>594695</b>
Fmoc-Asp-OH-4- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605263</b>
Fmoc-Asp-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>492906</b>
Fmoc-Glu-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>490008</b>
Fmoc-Gly-OH-2,2-d <sub>2</sub>	98 atom % D	<b>485772-1G</b>
Fmoc-Gly-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605182-1G</b>
Fmoc-Gly-OH-1- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>492698-250MG</b>
Fmoc-Gly-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>489549-1G</b>
Fmoc-Gly-OH-2- <sup>13</sup> C, <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>603457-100MG</b>
Fmoc-Gly-OH- <sup>13</sup> C <sub>2</sub>	99 atom % <sup>13</sup> C	<b>587745</b>
Fmoc-Gly-OH- <sup>13</sup> C <sub>2</sub> , <sup>15</sup> N	99 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>489530-500MG</b>
Fmoc-Gly-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>485756-1G</b>

Single Protected Amino Acids *continued*

Name	Isotopic Purity	Cat. No.
Fmoc-Ile-OH- <sup>13</sup> C <sub>6</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>597228-250MG</b>
Fmoc-Ile-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>578622-250MG</b>
Fmoc-Leu-OH-5,5,5-d <sub>3</sub>	99 atom % D	<b>615943</b>
Fmoc-Leu-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>485934-1G</b>
Fmoc-Leu-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>485950-1G</b>
Fmoc-Met-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>605115</b>
Fmoc-Met-OH- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>653640</b>
Fmoc-Met-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>609196-500MG</b>
Fmoc-Phe-OH- <i>phenyl</i> -d <sub>5</sub> -2,3,3-d <sub>3</sub>	98 atom % D	<b>615994</b>
Fmoc-Phe-OH-2- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>492965</b>

Name	Isotopic Purity	Cat. No.
Fmoc-Phe-OH- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>651443-100MG</b> <b>651443-500MG</b>
Fmoc-Phe-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>609072</b>
Fmoc-Pro-OH- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>651451</b>
Fmoc-Pro-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>589519</b>
Fmoc-Tyr-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>653624</b>
Fmoc-Val-OH-d <sub>8</sub>	98 atom % D	<b>616087-1G</b>
Fmoc-Val-OH-1- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>485993</b>
Fmoc-Val-OH- <sup>13</sup> C <sub>5</sub> , <sup>15</sup> N	98 atom % <sup>13</sup> C 98 atom % <sup>15</sup> N	<b>642886</b>
Fmoc-Val-OH- <sup>15</sup> N	98 atom % <sup>15</sup> N	<b>486000-1G</b>

## Stable Isotopes in Nucleic Acid Research

Stable isotope labeled nucleic acids are playing an increasingly important role in biomolecular NMR. With the availability of isotopically labeled nucleic acids and improvements in NMR methodologies it is possible to enhance experimental sensitivity and spectral resolution.<sup>1</sup> The use of [U-<sup>13</sup>C, U-<sup>15</sup>N] DNA and RNA can facilitate the measurement of local and long-range distance restraints, thus improving the accuracy of solution structure determination.<sup>2,3</sup> The *in vitro* synthesis of [U-<sup>13</sup>C, U-<sup>15</sup>N] labeled

DNA and RNA yields nucleic acid quantities sufficient for use in NMR studies. For this application, ISOTECH® offers a full complement of high-purity [U-<sup>13</sup>C, U-<sup>15</sup>N] nucleotide monophosphates, ribonucleotides, and deoxyribonucleotides (minimal 98 atom %). For customer convenience, labeled [U-<sup>13</sup>C, U-<sup>15</sup>N] NTPs are suspended in 5 mM Tris-HCl (pH 7.0) and packaged in 1 mg, 10 mg, 25 mg, and 50 mg allotments.

## References

- Dayie K.T. J. *Biomol. NMR* (2005) **32**: 129-139.
- Jaroniec C.P., Boisbouvier J., Tworowska I., Nikonowicz E.P., and Bax A.J. *Biomol. NMR* (2005) **31**: 231-241.
- Casiano-Negroni A., Sun X., and Al-Hashimi H.M. *Biochemistry*. (2007) **46**: 6525-6535.

## Nucleotides

Name	Isotopic Purity	Cat. No.
AMP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>15</sup> N, 98 atom % <sup>13</sup> C	<b>650676</b>
AMP- <sup>15</sup> N <sub>5</sub>	98 atom % <sup>15</sup> N	<b>662658</b>
ATP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>645702-1MG</b> <b>645702-10MG</b> <b>645702-25MG</b>
Supplied as sodium salt in 100 mM soln in H <sub>2</sub> O, with 5 mM Tris buffer		
CMP- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>3</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>650692</b>
CMP- <sup>15</sup> N <sub>3</sub>	98 atom % <sup>15</sup> N	<b>662682</b>
CTP- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>3</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>645699-1MG</b> <b>645699-10MG</b> <b>645699-25MG</b>
Supplied as sodium salt in 100 mM soln in H <sub>2</sub> O, with 5 mM Tris buffer		
dAMP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>648620</b>
dATP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>646237-1MG</b> <b>646237-10MG</b> <b>646237-25MG</b>
Supplied as sodium salt in 100 mM soln in H <sub>2</sub> O, 5 mM Tris HCl buffer		
dCMP- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>3</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>648612</b>
dCTP- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>3</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>646229-1MG</b> <b>646229-10MG</b> <b>646229-25MG</b>
Supplied as sodium salt in 100 mM soln in H <sub>2</sub> O, 5 mM Tris HCl buffer		

Name	Isotopic Purity	Cat. No.
dGMP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>648604</b>
dGTP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>646210-1MG</b> <b>646210-10MG</b> <b>646210-25MG</b>
Supplied as sodium salt in 100 mM soln in H <sub>2</sub> O, with 5 mM Tris buffer		
GMP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>650684</b>
GMP- <sup>15</sup> N <sub>5</sub>	98 atom % <sup>15</sup> N	<b>662674</b>
GTP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>5</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>645680-1MG</b> <b>645680-10MG</b> <b>645680-25MG</b>
Supplied as sodium salt in 100 mM soln in H <sub>2</sub> O, with 5 mM Tris buffer		
TMP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>2</sub>	98% <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>648590</b>
TTP- <sup>13</sup> C <sub>10</sub> , <sup>15</sup> N <sub>2</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>646202-1MG</b> <b>646202-10MG</b> <b>646202-25MG</b>
UMP- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>2</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>651370</b>
UMP- <sup>15</sup> N <sub>2</sub>	98 atom % <sup>15</sup> N	<b>662666</b>
UTP- <sup>13</sup> C <sub>9</sub> , <sup>15</sup> N <sub>2</sub>	98 atom % <sup>13</sup> C, 98 atom % <sup>15</sup> N	<b>645672-1MG</b> <b>645672-10MG</b> <b>645672-25MG</b>
Supplied as sodium salt, in 100 mM soln in H <sub>2</sub> O, with 5 mM Tris buffer		

## Additional Products of Interest

Name	Isotopic Purity	Cat. No.
Acetic acid-d <sub>4</sub>	99.5 atom % D	<b>151785-5G</b> <b>151785-10G</b> <b>151785-25G</b> <b>151785-50G</b>
Ammonium-d <sub>4</sub> bromide	98 atom % D	<b>176575-5G</b>
Ammonium-d <sub>4</sub> chloride	98 atom % D	<b>175676-5G</b> <b>175676-10G</b>
Ammonium-d <sub>4</sub> deuterioxide solution, 25 wt. % in D <sub>2</sub> O	99 atom % D	<b>176702-5G</b> <b>176702-10G</b> <b>176702-50G</b> <b>176702-100G</b>
Bis-tris-d <sub>19</sub>	98 atom % D	<b>655392-500MG</b> <b>655392-1G</b>
DL-Dithiothreitol-d <sub>10</sub>	98 atom % D	<b>485535-500MG</b>
Dodecylphosphorylcholine-d <sub>38</sub>	98 atom % D	<b>485616-500MG</b>
Ethylenediaminetetraacetic-d <sub>12</sub> acid	98 atom % D	<b>489379-1G</b>
Formic acid-d <sub>2</sub> , 95 wt. % in H <sub>2</sub> O	98 atom % D	<b>426229-1G</b> <b>426229-5G</b>
Glycerol-1,1,2,3,3-d <sub>5</sub>	98 atom % D	<b>454524-1G</b> <b>454524-5G</b>
Glycine-d <sub>5</sub>	98 atom % D	<b>175838-1G</b> <b>175838-5G</b>
HEPES-d <sub>18</sub>	98 atom % D	<b>643823</b>
Imidazole-d <sub>4</sub>	95 atom % D	<b>366021</b>

Name	Isotopic Purity	Cat. No.
2-Mercaptoethanol-d <sub>6</sub>	96 atom % D	<b>615226-500MG</b>
MES-d <sub>13</sub>	98 atom % D <b>NEW</b>	<b>687022</b>
Methanol- <sup>13</sup> C	99 atom % <sup>13</sup> C	<b>277177-1G</b> <b>277177-5G</b>
Methanol- <sup>13</sup> C, d <sub>4</sub>	99.5 atom % D 99 atom % <sup>13</sup> C	<b>293865-1G</b> <b>293865-5G</b>
Octyl-β-D-glucopyranoside-d <sub>24</sub>	98 atom % D	<b>658863</b>
PIPES-d <sub>18</sub>	98 atom % D <b>NEW</b>	<b>696633</b>
Sodium acetate-d <sub>3</sub>	99 atom % D	<b>176079-5G</b> <b>176079-25G</b>
Sodium acetate- <sup>13</sup> C <sub>2</sub>	99 atom % <sup>13</sup> C	<b>282014-250MG</b> <b>282014-1G</b>
Sodium acetate- <sup>13</sup> C <sub>2</sub> , d <sub>3</sub>	99 atom % D 99 atom % <sup>13</sup> C	<b>299111-100MG</b> <b>299111-500MG</b>
Sodium dodecyl sulfate-d <sub>25</sub>	98 atom % D	<b>451851-100MG</b> <b>451851-500MG</b>
Sodium formate-d	99 atom % D	<b>373842-1G</b> <b>373842-5G</b>
Succinic acid-d <sub>6</sub>	98 atom % D	<b>488356-5G</b>
Tris-d <sub>11</sub> solution, 1 M in D <sub>2</sub> O	98 atom % D	<b>486248-10ML</b>
Tris(hydroxymethyl-d <sub>3</sub> )amino-d <sub>2</sub> -methane	98 atom % D	<b>449105-1G</b> <b>449105-5G</b>

## Need more information?

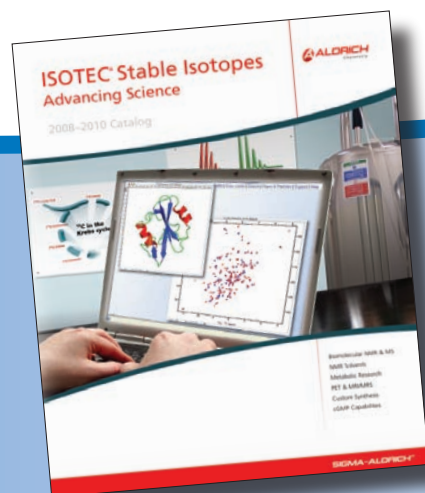
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