Isotope labeling has revolutionized the utility of biomolecular NMR spectroscopy, allowing the exploration of molecular interactions with high sensitivity and resolution.\textsuperscript{1,2} Many different strategies are available, along with a wide array of NMR experiments that are optimized for the different labeling approaches. One scheme that has been shown to be particularly effective in studies of high-molecular-weight proteins involves labeling methyl groups as \textsuperscript{13}CH\textsubscript{3} in an otherwise highly deuterated background\textsuperscript{3,4,5,6,7} and exploiting a methyl-TROSY effect\textsuperscript{8} that generates high-quality spectra. Applications to date have focused to a large extent on Ile, Leu and Val methyl probes,\textsuperscript{9} as the precursors for these residues are commercially available and very easy to use. More recently, however, studies utilizing Met\textsuperscript{10,11} and Ala\textsuperscript{12} methyl groups have also emerged along with approaches for introducing methyls into key positions in the protein of choice.

It is of significant interest to extend the methyl-labeling methodology to include Thr residues, as Thr has a much higher propensity for surface exposure than the other methyl-containing residues.\textsuperscript{13,14} As such, Thr is often found at key molecular interfaces, including those involving in binding nucleic acids. Our laboratory has developed a biosynthetic strategy\textsuperscript{15} that starts with \textsuperscript{13}C-formaldehyde, natural-abundance pyruvate and D\textsubscript{2}O, along with five enzymes that are necessary for the conversion to U-[\textsuperscript{2}H], Thr-\textgamma 2[\textsuperscript{13}CH\textsubscript{3}]. The development of a synthetic scheme by Cambridge Isotope Laboratories, Inc. (CIL) and the commercial availability of this product is a welcome addition, since a five-enzyme synthesis is usually something that NMR spectroscopists like to avoid!

Production of U-[\textsuperscript{2}H], Thr-\textgamma 2[\textsuperscript{13}CH\textsubscript{3}] labeled samples is straightforward. As described previously,\textsuperscript{15} the addition of Thr to growth media does lead to labeling at both Thr \textgamma 2 and Ile \delta 1 positions, as is expected since Thr is a precursor of Ile. Because cross peaks for Ile residues fall in an isolated region of the \textsuperscript{13}C, \textsuperscript{1}H correlation map and are among the most well resolved of all methyl types, we prefer to include Ile labeling in all of our Thr samples (and often also Leu, Val). In this regard, we recommend using 50 mg/L labeled U-[\textsuperscript{2}H], Thr-\textgamma 2[\textsuperscript{13}CH\textsubscript{3}], 50 mg/L labeled \alpha-ketobutyrate (\textsuperscript{13}CH\textsubscript{3}CD\textsubscript{2}COCOONa)
and 100 mg/L d<sub>2</sub>-glycine as an optimal combination for production of highly deuterated proteins labeled with <sup>13</sup>CH<sub>3</sub> at Thr and Ile (δ1) methyl positions. Further details can be found in reference 15. All of these compounds can be purchased from CIL.

Thr plays a critical role in the mechanism of function of a number of important enzymes and in several eukaryotic signaling complexes where biological activity is regulated through phosphorylation. Our research using U-[<sup>2</sup>H], Thr-[<sup>2</sup>]<sup>13</sup>CH<sub>3</sub>] to probe the catalytic mechanism of a class of barrel-shaped proteases that includes both the proteasome<sup>16</sup> and HslV<sup>17</sup> has more than convinced us of the utility of this product, and one can easily imagine other systems where it would be equally useful. As an example of the importance of using precursors that are deuterated at all positions with the proteasome. See references 14 and 15 for further discussion.

The availability of U-[<sup>2</sup>H], Thr-[<sup>2</sup>]<sup>13</sup>CH<sub>3</sub>] (CDLM-9307) completes the list of compounds that are required for the production of highly deuterated proteins with <sup>13</sup>CH<sub>3</sub> labeling at any of the methyl positions. It seems likely that the strategic use of this labeling technology will allow the NMR practitioner to bring into focus the inner workings of molecular machines in ways that could not even be imagined only a few years ago.

**References**


**Related Products**

**Catalog No.** | **Description**
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CDLM-9307 | Threonine (4-<sup>13</sup>C, 97%; 2,3-D<sub>2</sub>, 98%)<sup>9</sup>
CDLM-7318* | α-Ketobutyrate (methyl-<sup>13</sup>C, 99%; 3,3-D<sub>2</sub>, 98%)<sup>9</sup>
DLM-280* | Glycine (D<sub>3</sub>, 98%)<sup>9</sup>

*Used together with CDLM-9307 to label both <sup>13</sup>CH<sub>3</sub> at Thr and Ile (δ1).

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**Figure 1.** Comparative 1<sup>H</sup>:1<sup>13</sup>C HMOC spectra recorded on proteasome samples prepared with U-[<sup>13</sup>C, <sup>2</sup>H]-Thr (top) or U-[<sup>2</sup>H], Thr-[<sup>2</sup>]<sup>13</sup>CH<sub>3</sub>] (bottom). Assignment ambiguities are indicated by parentheses, and peaks derived from degradation products are indicated in ovals. Note the correlation from T1 that has been used as a probe to understand parts of the catalytic mechanism of the proteasome. See references 14 and 15 for further details.

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**Table 1.** Crystal structure of the HslV protease. See references 14 and 15 for further discussion.

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**Table 2.** Cross-correlated relaxation enhanced 1<sup>H</sup>:1<sup>13</sup>C NMR spectroscopy of methyl groups in very high molecular weight proteins and protein complexes. J Am Chem Soc, 125, 10420-10428.

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**Table 3.** Cross-correlated relaxation enhanced 1<sup>H</sup>:1<sup>13</sup>C NMR spectroscopy of methyl groups in very high molecular weight proteins and protein complexes. J Am Chem Soc, 125, 10420-10428.

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**Table 4.** Cross-correlated relaxation enhanced 1<sup>H</sup>:1<sup>13</sup>C NMR spectroscopy of methyl groups in very high molecular weight proteins and protein complexes. J Am Chem Soc, 125, 10420-10428.