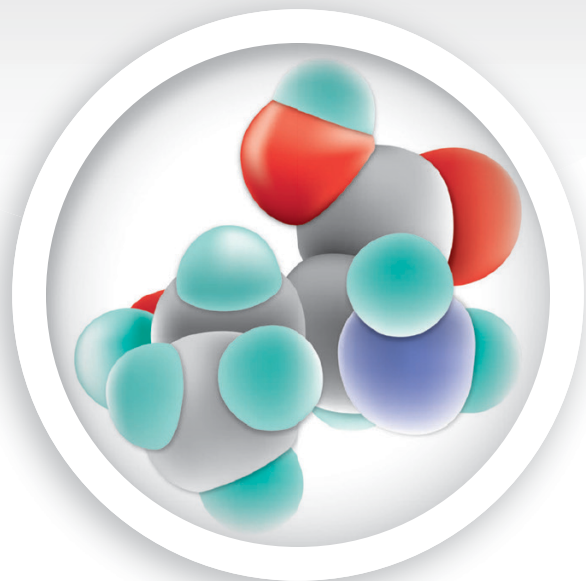


Production of U-[²H], Thr-γ2[¹³CH₃] Labeled Proteins for Methyl-TROSY NMR

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Isotope labeling has revolutionized the utility of biomolecular NMR spectroscopy, allowing the exploration of molecular interactions with high sensitivity and resolution.^{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 ¹³CH₃ in an otherwise highly deuterated background^{3,4,5,6,7} and exploiting a methyl-TROSY effect⁸ that generates high-quality spectra.

Applications to date have focused to a large extent on Ile, Leu and Val methyl probes,⁹ as the precursors for these residues are commercially available and very easy to use. More recently, however, studies utilizing Met^{10,11} and Ala¹² 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.^{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 strategy¹⁵ that starts with ¹³C-formaldehyde, natural-abundance pyruvate and D₂O, along with five enzymes that are necessary for the conversion to U-[²H], Thr-γ2[¹³CH₃]. 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-[²H], Thr-γ2[¹³CH₃]-labeled samples is straightforward. As described previously,¹⁵ the addition of Thr to growth media does lead to labeling at both Thr γ2 and Ile δ1 positions, as is expected since Thr is a precursor of Ile. Because cross peaks for Ile residues fall in an isolated region of the ¹³C, ¹H 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-[²H], Thr-γ2[¹³CH₃], 50 mg/L labeled α-ketobutyrate (¹³CH₃CD₂COONa)

(continued)

and 100 mg/L d₅-glycine as an optimal combination for production of highly deuterated proteins labeled with ¹³CH₃ 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-[²H], Thr-γ2[¹³CH₃] to probe the catalytic mechanism of a class of barrel-shaped proteases that includes both the proteasome¹⁶ and HslV¹⁷ 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 exception of the methyl groups, Figure 1 illustrates comparative spectra recorded on proteasome samples (670 kDa) that are highly deuterated and prepared labeled through the addition of either U-[¹³C, ¹H]-Thr (a) or U-[²H], Thr-γ2[¹³CH₃] (b). The difference is apparent, and the signal from the methyl group of the important catalytic residue, Thr1, is only visible in the spectrum of the protein prepared with the ²H-Thr precursor.¹⁶

The availability of U-[²H], Thr-γ2[¹³CH₃] (CDLM-9307) completes the list of compounds that are required for the production of highly deuterated proteins with ¹³CH₃ 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

- Ruschak, A.M.; Kay, L.E. **2009**. Methyl groups as probes of supra-molecular structure, dynamics and function. *J Biomol NMR*, *46*, 75-87.
- Kainosho, M.; Torizawa, T.; Iwashita, Y.; Terauchi, T.; Mei Ono, A.; Guntert, P. **2006**. Optimal isotope labelling for NMR protein structure determinations. *Nature*, *440*, 52-7.
- Goto, N.K.; Gardner, K.H.; Mueller, G.A.; Willis, R.C.; Kay, L.E. **1999**. A robust and cost-effective method for the production of Val, Leu, Ile (δ1) methyl-protonated ¹⁵N-, ¹³C-, ²H-labeled proteins. *J Biomol NMR*, *13*, 369-374.
- Tugarinov, V.; Kay, L.E. **2004**. An isotope labeling strategy for methyl TROSY spectroscopy. *J Biomol NMR*, *28*, 165-72.
- Ayala, I.; Sounier, R.; Use, N.; Gans, P.; Boisbouvier, J. **2009**. An efficient protocol for the complete incorporation of methyl-protonated alanine in perdeuterated protein. *J Biomol NMR*, *43*, 111-9.
- Gans, P.; Hamelin, O.; Sounier, R.; Ayala, I.; Dura, M. A.; Amero, C.; Noirclerc-Savoye, M.; Franzetti, B.; Plevin, M.J.; Boisbouvier, J. **2010**. Stereospecific isotopic labeling of methyl groups for NMR spectroscopic studies of high molecular weight proteins. *Angew Chem Int Ed*, *49*, 1958-62.
- Fischer, M.; Kloiber, K.; Hausler, J.; Ledolter, K.; Konrat, R.; Schmid, W. **2007**. Synthesis of a ¹³C-methyl-group-labeled methionine precursor as a useful tool for simplifying protein structural analysis by NMR spectroscopy. *Chembiochem*, *8*, 610-2.
- Tugarinov, V.; Hwang, P.; Ollerenshaw, J.; Kay, L.E. **2003**. Cross-correlated relaxation enhanced ¹H-¹³C NMR spectroscopy of methyl groups in very high molecular weight proteins and protein complexes. *J Am Chem Soc*, *125*, 10420-10428.
- Rosenzweig, R.; Kay, L.E. **2014**. Bringing Dynamic Molecular Machines into Focus by Methyl-TROSY NMR. *Annu Rev Biochem*, *83*.
- Gelis, I.; Bonvin, A.M.; Keramisanou, D.; Koukaki, M.; Gouridis, G.; Karamanou, S.; Economou, A.; Kalodimos, C.G. **2007**. Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR. *Cell*, *131*, 756-69.
- Religa, T. L.; Sprangers, R.; Kay, L.E. **2010**. Dynamic regulation of archaeal proteasome gate opening as studied by TROSY NMR. *Science*, *328*, 98-102.
- Isaacson, R.L.; Simpson, P.J.; Liu, M.; Cota, E.; Zhang, X.; Freemont, P.; Matthews, S. **2007**. A new labeling method for methyl transverse relaxation-optimized spectroscopy NMR spectra of alanine residues. *J Am Chem Soc*, *129*, 15428-9.
- Sinha, K.; Jen-Jacobson, L.; Rule, G.S. **2011**. Specific labeling of threonine methyl groups for NMR studies of protein-nucleic acid complexes. *Biochemistry*, *50*, 10189-91.
- Miller, S.; Janin, J.; Lesk, A.M.; Chothia, C. **1987**. Interior and surface of monomeric proteins. *J Mol Biol*, *196*, 641-56.
- Velyvis, A.; Ruschak, A.M.; Kay, L.E. **2012**. An economical method for production of (2)H, (13)CH₃-threonine for solution NMR studies of large protein complexes: application to the 670 kDa proteasome. *PLoS One*, *7*, e43725.
- Velyvis, A.; Kay, L.E. **2013**. Measurement of Active Site Ionization Equilibria in the 670 kDa Proteasome Core Particle Using Methyl-TROSY NMR. *J Am Chem Soc*, *135*, 9259-62.
- Shi, L.; Kay, L.E. **2014**. Tracing an allosteric pathway regulating the activity of the HslV protease. *Proc Natl Acad Sci, USA*, *111*, 2140-5.

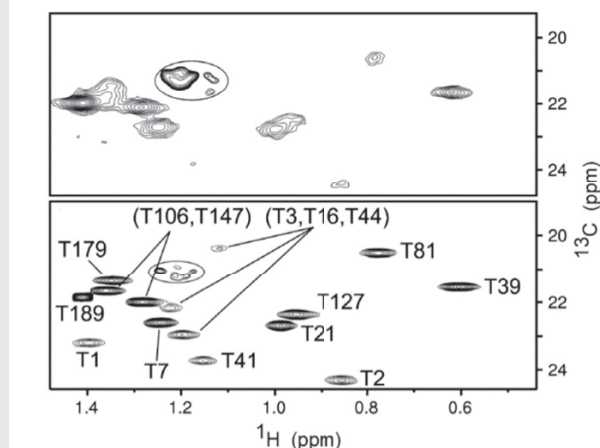


Figure 1. Comparative ¹H-¹³C HMQC spectra recorded on proteasome samples prepared with U-[¹³C, ¹H]-Thr (top) or U-[²H], Thr-γ2[¹³CH₃] (bottom). Assignment ambiguities are indicated by parentheses, and peaks derived from degradation products are indicated in ovals. Note the correlation from T1 that has been used as a probe to understand parts of the catalytic mechanism of the proteasome. See references 14 and 15 for further details.

Related Products

Catalog No.	Description
CDLM-9307	Threonine (4- ¹³ C, 97%; 2,3-D ₂ , 98%)
CDLM-7318*	α-Ketobutyrate (methyl- ¹³ C, 99%; 3,3-D ₂ , 98%)
DLM-280*	Glycine (D ₅ , 98%)

*Used together with CDLM-9307 to label both ¹³CH₃ at Thr and Ile (δ1).

