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Facile synthesis of 1,1-[$^2H_2$]-2-methylaminoethane-1-sulfonic acid as a substrate for taurine α ketoglutarate dioxygenase (TauD)

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ABSTRACT

Taurine α-ketoglutarate dioxygenase (TauD) is an archetypal α-ketoglutarate-dependent non-heme iron oxygenase. Here, we report a practical five-step synthetic route to 1,1-dideutero-2-methylaminoethane-sulfonic acid hydrochloride, a useful alternative substrate for the characterization of deuterium kinetic isotope effects on TauD.

TauD catalyzes the hydroxylation of taurine (aminoethanesulfonic acid) and other alkylsulfonates, alpha to the sulfonate, with the concomitant consumption of one equivalent each of α ketoglutarate (αKG) and molecular oxygen to yield the products CO_2, succinate, sulfite and aminoacetaldehyde (Fig. 1).[1] The enzyme is only expressed in microorganisms under conditions of sulfate starvation, and the product sulfite is subsequently used as a sulfur source.[2] The ease of overexpression and purification of the *E. coli* protein has led to its position as a dominant model system for the study of the family of αKG-dependent non-heme iron oxygenases, which have been implicated in processes as diverse as antibiotic biosynthesis,[3] oxygen sensing,[4] DNA repair[5–7] and the biodegradation of anthropogenic compounds, particularly herbicides.[8,9] Despite this broad array of chemical transformations, mounting evidence indicates that this class of enzymes utilizes a common mechanism (Fig. 2) to generate the reactive oxygen intermediate necessary to carry out these reactions.[10–13]

One common feature of this reaction mechanism is the use of a high-valent Fe(IV)-oxo species (species III in Fig. 2) to abstract a hydrogen atom from the prime substrate—taurine in the case of TauD. Deuterium kinetic isotope effects (KIEs) have proven to be an invaluable tool for probing the mechanism of enzymatic hydrogen atom transfer.[14] The ability to obtain reliable deuterium KIE measurements is contingent on the production of specifically deuterium-labeled substrate molecules with high isotopic purity. Previously, Bollinger and co-workers have produced 1,1-[$^2H_2$]-taurine from chlorosulfanylacetyl chloride in five steps with ~15% overall yield and 99% isotopic purity.[15] The synthesis of the N-methylated derivative indicated here was undertaken to allow the possible installation of a remote label (^3H, ^{14}C or ^{13}C) on a taurine analog for product analysis studies. *N*-methyltaurine was chosen as the simplest analog to fulfill this possibility. Prior to undertaking the synthesis, it was confirmed that the kinetic parameters of TauD with commercially available protio *N*-methyltaurine did not differ

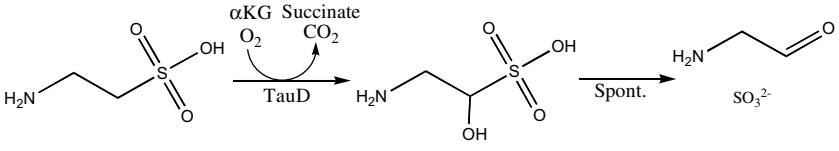

Figure 1. Reaction catalyzed by TauD.

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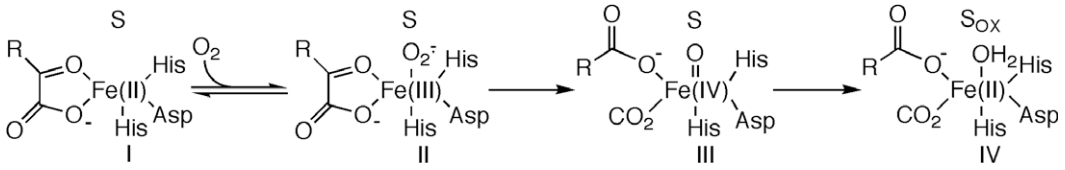


Figure 2. Abridged mechanism of the α-KG-dependent oxygenases showing the oxidation of a generic C-H bearing substrate (S) to S_{OX}. R=$CH_2CH_2CO_2^-$, His and Asp denote protein-derived histidine and aspartate ligands to the active-site iron.

Table 1
Kinetic parameters obtained with wild-type TauD and the indicated substrates

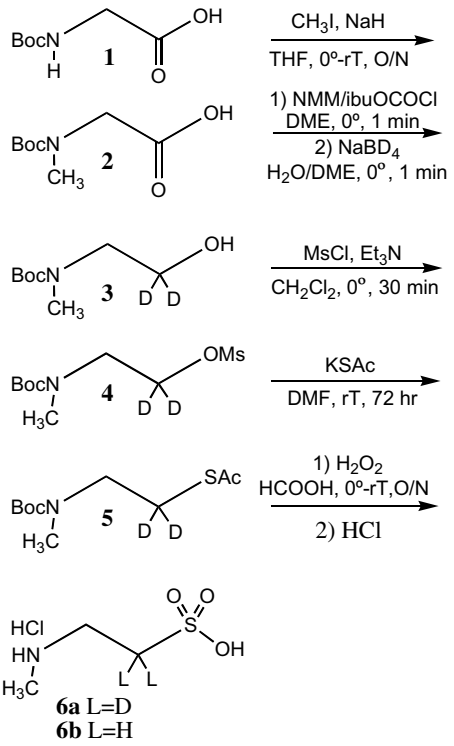
Substrate[a]	k_{cat} (s^{-1})	K_m(S) μM	k_{cat}/K_m(S)[d] $\times 10^{-5}$ $M^{-1}s^{-1}$	$K_m(O_2)$ μM	$k_{cat}/K_m(O_2)$[d] $\times 10^{-5}$ M^{-1} s^{-1}
Taurine	4.9 ± 0.2	19.4 ± 0.7	2.5 ± 0.1	41 ± 6	1.2 ± 0.2
^{H}Methyl taurine[b]	4.7 ± 0.3	54 ± 10	0.9 ± 0.2	46 ± 4	1.0 ± 0.1
^{H}Methyl taurine[c]	5.0 ± 0.2	48 ± 4	1.0 ± 0.1	41 ± 5	1.2 ± 0.2
^{D}Methyl taurine[c]	0.60 ± 0.01	5.1 ± 0.6	1.2 ± 0.1	5.6 ± 0.5	1.1 ± 0.1

[a] The superscript H indicates 1,1-[$^1H_2$] methyl taurine, and D indicates 1,1-[$^2H_2$] methyl taurine, compounds **6b** and **6a**, respectively.
[b] Commercially available.
[c] Synthetically prepared by the methods presented herein.
[d] Determined in the limit of infinite concentration of the alternate substrate.

significantly from the kinetic parameters with protio taurine (Table 1). A straightforward synthesis requiring little purification and no strictly anhydrous conditions was sought to facilitate the ease of synthesis in a largely biochemical lab. It was decided that it would be advantageous to incorporate deuterium through a reduction step with a highly isotopically enriched reducing agent, increasing the isotopic purity of the intermediate and avoiding possible dilution of isotopic enrichment by incorporation of protium from atmospheric water into an exchangeable position. The overall synthesis is shown in Scheme 1.

The designed synthesis begins with Boc-protected glycine (**1**), which is N-methylated with methyl iodide.[16] This product (**2**) is converted to a mixed carbonic–carboxylic anhydride and reduced by the action of sodium borodeuteride,[17] which is commonly available at isotopic purity of >99.9%.[18] The deuterio alcohol (**3**) is converted to the mesylate (**4**),[19] and the mesylate is displaced by thioacetate.[20] The thioacetate ester (**5**) is oxidized by performic acid[21] and converted to the hydrochloride salt, which is triturated in absolute ethanol to yield the final product (**6a**), 1,1-[$^2H_2$]-methylaminoethane-1-sulfonic acid hydrochloride, with no detectable ^1H contamination as determined by ^1H NMR or mass spectral analysis, in 23% overall yield. An analogous synthesis differing only in the use of sodium borohydride was undertaken to produce the fully protio compound (**6b**) for comparative studies with similar results. The full experimental details are given in the Supplementary data.

In order to evaluate the utility of the deuterio substrate analog, the kinetic parameters for TauD were determined using taurine, commercial protio *N*-methyltaurine, synthetic protio *N*-methyltaurine, and synthetic 1,1-dideuterio *N*-methyltaurine. The results are indicated in Table 1. The indistinguishable behavior of the enzyme with commercial and synthetic protio *N*-methyltaurine precludes the possibility of inhibitory synthetic by-products, indicating the purity and suitability of the synthetic compound for use as a substrate for TauD. The moderate KIE on k_{cat} (k_{cat}(H)/k_{cat}(D)) of 8.3 is consistent with the previous result of a significant (~35) KIE in pre-steady state experiments as well as kinetic complexity in the form of partial rate-limitation by product release,[15,22] which would lead to the diminution of the expressed isotope effect with respect to the 'intrinsic' value. The mechanism in Fig. 2 depicts an irreversible step, O–O bond scission, prior to the isotopically sensitive step of hydrogen atom abstraction. This mechanism predicts an isotope effect of unity on k_{cat}/K_m parameters, which include all steps from substrate—either prime substrate or oxygen—binding through the



Scheme 1. Synthetic route to 1,1-[$^2H_2$]-2-methylaminoethane-1-sulfonic acid.

first irreversible step,[23] as neither k_{cat}/K_m parameter includes the isotopically sensitive step. Also, as k_{cat}/K_m(S) is determined at saturating concentrations of dioxygen, dioxygen binding effectively makes substrate binding irreversible, again prediciting an isotope effect on k_{cat}/K_m(S) of unity. The determined values of $^D(k_{cat}/K_m(S))$ and $^D(k_{cat}/K_m(O_2))$ of 0.8 ± 0.1 and 1.1 ± 0.2 are within 2σ of unity, further corroborating the concensus mechanism.

The synthetic procedure described here provides analytically pure *N*-methyltaurine isotopomers for use as substrates for TauD, allowing the determination of kinetic isotope effects. It is worth noting that no column purification or strictly anhydrous conditions were necessary, and that the installation of other isotopic labels either on the *N*-methyl substituent or in the alpha position could

be easily achieved through the use of appropriately labeled methyl iodide or sodium borohydride, respectively. The moderately increased yield and relative ease of this method, with respect to the existing methodology, make this a valuable technique for the preparation of isotopically labeled substrates for TauD.

Acknowledgment

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Supplementary data

Experimental procedures and spectral characterization (^{1}H NMR) of intermediates and end products, as well as HRMS of deuterated end-product are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.11.063.

References and notes

1. Eichhorn, E.; vanderPloeg, J. R.; Kertesz, M. A.; Leisinger, T. *J. Biol. Chem.* **1997**, *272*, 23031–23036.
2. vanderPloeg, J. R.; Weiss, M. A.; Saller, E.; Nashimoto, H.; Saito, N.; Kertesz, M. A.; Leisinger, T. *J. Bacteriol.* **1996**, *178*, 5438–5446.
3. Salowe, S. P.; Marsh, E. N.; Townsend, C. A. *Biochemistry* **1990**, *29*, 6499–6508.
4. Jaakkola, P.; Mole, D. R.; Tian, Y. M.; Wilson, M. I.; Gielbert, J.; Gaskell, S. J.; von Kriegsheim, A.; Hebestreit, H. F.; Mukherji, M.; Schofield, C. J.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J. *Science* **2001**, *292*, 468–472.
5. Falnes, P. O.; Johansen, R. F.; Seeberg, E. *Nature* **2002**, *419*, 178–182.
6. Trewick, S. C.; Henshaw, T. F.; Hausinger, R. P.; Lindahl, T.; Sedgwick, B. *Nature* **2002**, *419*, 174–178.
7. Aas, P. A.; Otterlei, M.; Falnes, P. O.; Vagbo, C. B.; Skorpen, F.; Akbari, M.; Sundheim, O.; Bjoras, M.; Slupphaug, G.; Seeberg, E.; Krokan, H. E. *Nature* **2003**, *421*, 859–863.
8. Fukumori, F.; Hausinger, R. P. *J. Biol. Chem.* **1993**, *268*, 24311–24317.
9. Hegg, E. L.; Whiting, A. K.; Saari, R. E.; McCracken, J.; Hausinger, R. P.; Que, L. *Biochemistry* **1999**, *38*, 16714–16726.
10. Costas, M.; Mehn, M. P.; Jensen, M. P.; Que, L. *Chem. Rev.* **2004**, *104*, 939–986.
11. Que, L.; Ho, R. Y. N. *Chem. Rev.* **1996**, *96*, 2607–2624.
12. Solomon, E. I.; Brunold, T. C.; Davis, M. I.; Kemsley, J. N.; Lee, S. K.; Lehnert, N.; Neese, F.; Skulan, A. J.; Yang, Y. S.; Zhou, J. *Chem. Rev.* **2000**, *100*, 235–349.
13. Hanauskeabel, H. M.; Gunzler, V. *J. Theor. Biol.* **1982**, *94*, 421–455.
14. Klinman, J. P. *Adv. Enzymol. Rel. Areas Mol. Biol.* **1978**, *46*, 415–494.
15. Price, J. C.; Barr, E. W.; Hoffart, L. M.; Krebs, C.; Bollinger, J. M. *Biochemistry* **2005**, *44*, 8138–8147.
16. Xu, Y. J.; Duan, X. M.; Li, M. L.; Jiang, L. Q.; Zhao, G. H.; Meng, Y.; Chen, L. G. *Molecules* **2005**, *10*, 259–264.
17. Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923–926.
18. The material available from Sigma is indicated as $\geqslant$98% isotopic purity in the specifications; however, individual lots, such as the one used in this study, can exceed 99.9% isotopic purity.
19. Higashiura, K.; Morino, H.; Matsuura, H.; Toyomaki, Y.; Ienaga, K. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1479–1481.
20. Uehling, D. E.; Donaldson, K. H.; Deaton, D. N.; Hyman, C. E.; Sugg, E. E.; Barrett, D. G.; Hughes, R. G.; Reitter, B.; Adkison, K. K.; Lancaster, M. E.; Lee, F.; Hart, R.; Paulik, M. A.; Sherman, B. W.; True, T.; Cowan, C. *J. Med. Chem.* **2002**, *45*, 567–583.
21. Labeeuw, O.; Phansavath, P.; Genet, J. P. *Tetrahedron Lett.* **2003**, *44*, 6383–6386.
22. Price, J. C.; Barr, E. W.; Glass, T. E.; Krebs, C.; Bollinger, J. M. *J. Am. Chem. Soc.* **2003**, *125*, 13008–13009.
23. Northrop, D. B. *Biochemistry* **1981**, *20*, 4056–4061.