Biochemistry

Communication

Reductive cleavage of sulfoxide and sulfone by two radical S-adenosyl-L-methionine enzymes

Dhanaraju Mandalapu, Xinjian Ji, and Qi Zhang

Biochemistry, **Just Accepted Manuscript** • DOI: 10.1021/acs.biochem.8b00844 • Publication Date (Web): 06 Nov 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Reductive cleavage of sulfoxide and sulfone by two radical S-adenosyl-L-methionine enzymes

Dhanaraju Mandalapu, Xinjian Ji, and Qi Zhang*

Department of Chemistry, Fudan University, Shanghai, 200433, China.

Supporting Information Placeholder

ABSTRACT: Sulfoxides and sulfones are commonly found in nature as a result of thioether oxidation, whereas only a very few enzymes have been found to metabolize these compounds. Utilizing the strong reduction potential of the [4Fe-4S] cluster of radical S-adenosyl-L-methionine (SAM) enzymes, we herein report the first enzyme-catalyzed reductive cleavage of sulfoxide and sulfone. We show two radical SAM enzymes, tryptophan lyase NosL and the class C radical SAM methyltransferase NosN, are able to act on a sulfoxide SAHO and a sulfone $SAHO_2$, both of which are structurally similar to SAM. NosL cleaves all the three bonds (i.e. S-C(5′), S-C(γ), and S-O) connecting the sulfur center of SAHO, with a preference for S-C(5′) bond cleavage. Similar S-C cleavage activity was also found for $SHAO_2$, but no S-O cleavage was observed. In contrast to NosL, NosN almost exclusively cleaves the S-C(5′) bonds of SAHO and $SAHO_2$ with much higher efficiencies. Our study provides valuable insights into the [4Fe-4S] cluster-mediated reduction reactions and highlights the remarkable catalytic promiscuity of radical SAM enzymes.

Sulfoxides are an important class of compounds that are commonly used in organic synthesis. These compounds are also found in nature. For example, dimethylsulfoxide (DMSO) is naturally produced by oxidation of dimethylsulfide (DMS), and this process plays an important role in the biogeochemical cycle of sulfur;[1] L-methionine (L-Met) sulfoxide (L-MetSO), produced from L-Met oxidation, is involved in many biological processes such as aging,[2] oxidative stress protection and bacterial infection.[3, 4] Reduction of DMSO to DMS is catalyzed by DMSO reductases, a diverse class of enzymes containing a molybdenum center.[5] This reaction starts with DMSO binding to the Mo(IV) center, and the S-O bond is reductively cleaved with the O atom transferred to the Mo center (Figure 1A). Reduction of L-MetSO to L-Met by L-MetSO reductases proceeds via a very different mechanism, in which the O atom is transferred from L-MetSO to a catalytic Cys residue to produce a sulfenic acid intermediate, which is subsequently reduced by external electron donors such as thioredoxin (Figure 1B).[6, 7] Besides S-O bond cleavage, the sulfoxide S-C bonds can also be enzymatically cleaved (Figure 1C), as exemplified by Egt2 and EgtE involved in ergothioneine biosynthesis.[8, 9]

A prominent family of S-C lyases is the radical S-adenosyl-L-methionine (SAM) enzyme superfamily, which consists of more than 200,000 members found in all three domains of life.[10-12] These enzymes utilize a [4Fe-4S] cluster to reductively cleave the S-C(5′) bond of SAM, generating a highly reactive 5′-deoxyadenosyl (dAdo) radical. This radical then abstracts a hydrogen atom from the substrate to yield 5′-deoxyadenosine (dAdoH) and a radical intermediate, and the latter leads to highly diverse reactions.[11-13]

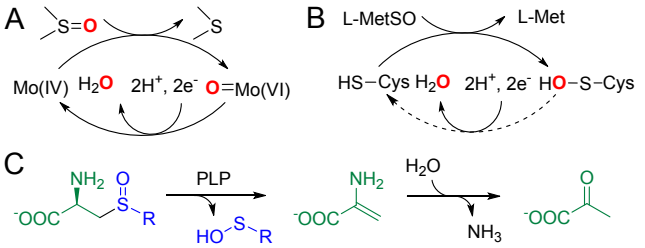


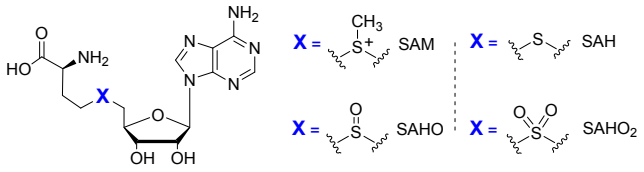
Figure 1. The reactions catalyzed by sulfoxide-metabolizing enzymes. (A) DMSO reductase utilizes a molybdenum cofactor. (B) L-Met sulfoxide reductase utilizes one or more active Cys residues to mediate L-MetSO reduction. The dotted arrow indicates different reaction routes for different L-MetSO reductases. (C) The sulfoxide S-C lyases Egt2/EgtE are PLP-dependent enzymes that cleave the S-C bond via an ionic pathway.

The S-C lyase activity of radical SAM enzymes has been tested with various SAM analogues. Magnusson and Frey showed that lysine 2,3-aminomutase (LAM) is able to cleave S-3′,4′-anhydroadenosyl-L-methionine (anSAM), and the resulting allylic radical can be well-characterized by electron paramagnetic resonance spectroscopy.[14] We showed that NosL and a class C radical SAM methyltransferase NosN are able to cleave two SAM analogues containing different nucleoside functionalities (i.e. S-guanosyl-L-methionine and S-cytidinylmethionine), and the resulting dAdo-like radicals can be captured to produce various nucleotide-linked products.[15-17] More recently, we showed that NosN is able to cleave allyl-SAM, a SAM analogue in which the methyl group is replaced by an allyl group.[18] Lin, Hoffman and coworkers showed that several SAM analogues in which the 3-amino-3-carboxylpropyl group of SAM was modified, can be cleaved by Dph2,[19-21] an unconventional radical SAM enzyme involved in diphthamide biosynthesis.[22] However, to date the tested SAM analogues are all confined to sulfonium salts, and it remains unclear whether the substrate scope of radical SAM enzymes can be expanded beyond sulfoniums.

Although sulfoxides are neutral molecules distinct from the positively charged sulfonium ions, the sulfur center of sulfoxide is isoelectronic and isosteric to that of sulfonium. We hence reasoned that radical SAM enzymes may also be able to catalyze the reductive cleavage of sulfoxides. To test this hypothesis, we synthesized SAHO, a sulfoxide derived from S-adenosyl-L-homocysteine (SAH) (Scheme 1). L-Trp lyase NosL, a radical SAM enzyme that exhibits high tolerance toward various

substrate analogues,[23-31] was used as a model enzyme in our assay.

Scheme 1. SAM and SAM structural analogues used in this study.



NosL reaction was performed by incubating the reconstituted NosL with SAHO, L-Trp and sodium dithionite. Liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) analysis of the reaction mixture clearly showed production of dAdoH, which is absent in the control assay with the supernatant of boiled enzyme (Figure 2). We also observed the production of a compound that exhibited a protonated molecular ion at m/z = 300.0760 (1.6 ppm error for a molecular formula of $C_{10}H_{13}N_5O_4S$). This compound matches well with **1**, the sulfenic acid of 5′-thioadenosine (tAdoH) (Figure 3B and Figure S1). Notably, we also observed the production of SAH in the reaction, which is clearly absent in the control assay (Figure 2). The yields of dAdoH, **1**, and SAH are roughly 10: 3: 1 according to MS intensities (Figure 2 and Table S1). We also observed the production of homocysteine sulfenic acid and α-aminobutyric acid in the reaction mixture (Figure S2), further supporting the S-C(5′) and S-C(γ) cleavage of SAHO.

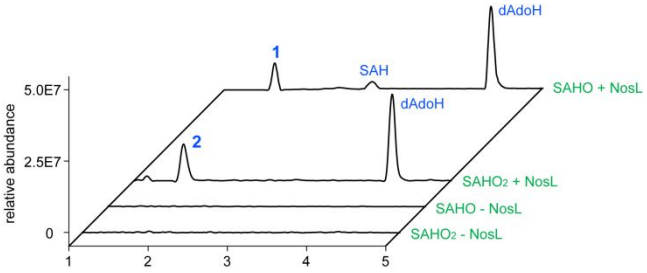


Figure 2. LC-HR-MS analysis of the NosL reaction with SAHO and $SAHO_2$. The multiple selected ion monitoring (SIM) mode includes $[M + H]^+$ = 252.1 (corresponding to dAdoH), 300.1 (corresponding to tAdoH sulfenic acid, **1**), 316.1 (corresponding to tAdoH sulfinic acid, **2**), and 385.1 (corresponding to SAH). Reactions were performed by incubation of 40 μM NosL, 5 mM sodium dithionite, 500 μM L-Trp with 1 mM SAHO or $SAHO_2$. See Figure S1 and S4 for the HR-MS/MS spectra of **1** and **2**. Conversion of L-Trp to 3-methyl-2-indolic acid (the natural activity of NosL) was not observed in the two reactions.

For most radical SAM enzymes, SAM binds to the [4Fe-4S] cluster in a conformation in which the unique Fe is close to the sulfur atom of SAM, allowing for one electron transfer via the sulfur atom to cleave the S-C(5′) bond.[32] Broderick, Hoffman, and coworkers have recently shown that dAdo radical forms an organometallic intermediate Ω with the [4Fe-4S] cluster, which is central in radical SAM chemistry.[33, 34] In contrast to most radical SAM enzymes, the unique Fe of the Dph2 [4Fe-4S] cluster is close to the C(γ) atom of SAM, thereby facilitating a Fe-based nucleophilic substitution onto the SAM C(γ) to heterolytically cleave the S-C(γ) bond.[35] We proposed that cleavages of the two S-C bonds of SAHO observed in this study may proceed similarly to those of SAM discussed above (Figure 3A and 3B).

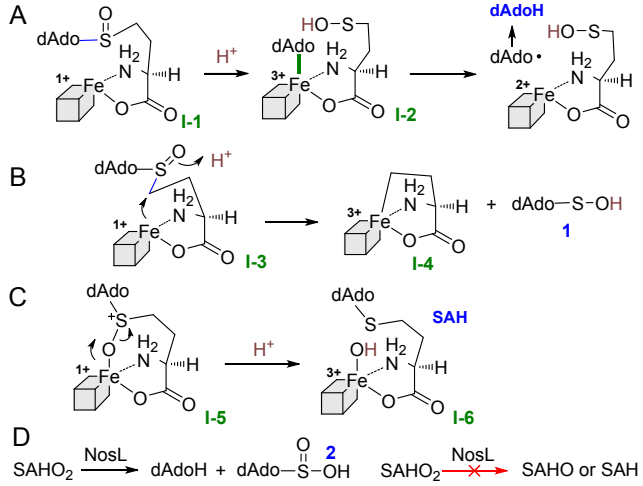


Figure 3. Working hypothesis for the reductive cleavage of SAHO by NosL. (A) NosL-catalyzed S-C(5′) bond cleavage likely starts from a conformation that the unique Fe is close to the sulfur atom (**I-1**). The Fe-C organometallic bond of Ω is highlighted in green. (B) NosL-catalyzed S-C(γ) bond cleavage likely starts from a conformation that the unique Fe is close to the C(γ) of SAHO (**I-3**) and proceeds via a nucleophilic attack reaction. (C) NosL-catalyzed SAHO reduction may proceed with oxygen transfer to the unique Fe in a way similar to DMSO reductase. (D) NosL cleaves the S-C(5′) and S-C(γ) bonds of $SAHO_2$, but not the S-O bond. A DFT model of the hexacoordinate complex **I-5** is shown in Figure S3.

Reduction of sulfoxide to thioether is a brand new reaction for the radical SAM superfamily enzymes. We proposed that this reaction may start with binding of the sulfoxide oxygen to the unique Fe to form **I-5**, a hexacoordinate complex on the unique Fe, and the reaction may possibly proceed via an S-O transfer mechanism analogous to that of DMSO reductase (Figure 3C). DFT optimization of a model of **I-5** revealed the lengths of the two Fe-O coordination bonds are equal (Figure S3), indicating the interaction between the sulfoxide oxygen and the unique Fe could be strong.

We next synthesized SAH sulfone ($SAHO_2$), which contains a tetrahedral and achiral sulfur, in contrast to the pyramidal sulfur of SAHO (Scheme 1). Assays were then performed by incubation of NosL, L-Trp, and dithionite with $SAHO_2$. HR-LCMS analysis of the reaction mixture showed that dAdoH was produced with a yield similar to that from SAHO, demonstrating that both sulfoxide and sulfone can be cleaved by NosL with similar efficiencies (Figure 2 and Table S1). We also found a compound exhibiting protonated molecular ion at m/z = 316.0702 (4.4 ppm error for a molecular formula of $C_{10}H_{13}N_5O_5S$). This compound is consistent with **2**, the sulfinic acid of tAdoH (Figure 3D and Figure S4). Notably, neither SAH nor SAHO was found in the $SAHO_2$ reaction (Figure 3D), suggesting that unlike SAHO, NosL does not catalyze the S-O cleavage of $SAHO_2$.

To expand our sulfoxide/sulfone biochemistry to other radical SAM enzymes, we tested the SAHO reaction with NosN, a class C radical SAM methyltransferase.[36, 37] LC-HRMS analysis of the reaction mixture containing the reconstituted NosN, SAHO and dithionite showed that dAdoH was produced, which is about 8-fold higher than that produced by NosL in a similar assay condition (Figure 4). Although **1** and SAH were also found in the reaction mixture, the yields of these two compounds are less than

1% compared to dAdoH (Table S1). NosN also efficiently cleaved $SAHO_2$, converting it predominantly to dAdoH (Figure 4).

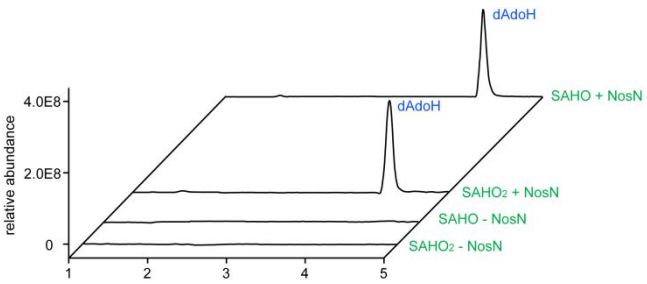


Figure 4. LC-HR-MS analysis of the NosN reaction with SAHO and $SAHO_2$. The multiple selected ion monitoring (SIM) mode was set exactly the same to that of Figure 3 ($[M + H]^+$ = 252.1 (corresponding to dAdoH), 300.1 (corresponding to **1**), 316.1(corresponding to **2**), and 385.1 (corresponding to SAH). Reactions were performed by incubation of 40 μM NosN, 5 mM sodium dithionite, and 1 mM SAHO or $SAHO_2$. Only trace amounts of **1** and SAH (from SAHO), and **2** (from $SAHO_2$) were produced in the corresponding reactions.

In summary, we have demonstrated herein, to the best of our knowledge, the first enzyme-catalyzed reductive cleavage of sulfoxide and sulfone. We showed that NosL cleaves all the three bonds connecting the sulfur center of SAHO to produce three different sets of products, whereas NosN almost exclusively cleaves the S-C(5′) bond of SAHO and $SAHO_2$. We would like to point out that, compared to the canonical activity of SAM cleavage, the reactions for SAHO and $SAHO_2$ are much less efficient (Table S1). Nevertheless, the novel activities reported here expands the catalytic repertoire of Fe-S proteins and opens the way to explore these enzymes for novel bioengineering applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxx.xxx/acs.biochem.xxx

AUTHOR INFORMATION

Corresponding Author

*E-mail: qizhang@sioc.ac.cn.

ORCID

Qi Zhang: 0000-0002-8135-2221

Funding Sources

This work is supported in part by grants from the National Key Research and Development Program (2016 Y F A0501302), and from National Natural Science Foundation of China (31500028 and 31670060).

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

We thank Prof. T. Begley (Texas A&M U) for a stimulating discussion on the catalytic promiscuity of radical SAM enzymes.

ABBREVIATIONS

anSAM, 3′,4′-anhydroadenosyl-L-methionine; DFT, density functional theory; DMS, dimethylsulfide; HR-LCMS, liquid chromatography coupled with high resolution mass spectrometry; HR-MS/MS, high resolution tandem MS; L-MetSO, L-methionine sulfoxide; SAH, S-adenosyl-L-homocysteine; SAHO, SAH sulfoxide; $SAHO_2$, SAH sulfone; tAdoH, 5′-thioadenosine.

REFERENCES

1. Kappler, U., and Schafer, H. (2014) Transformations of dimethylsulfide, *Met Ions Life Sci 14*, 279-313.

2. Petropoulos, I., and Friguet, B. (2005) Protein maintenance in aging and replicative senescence: a role for the peptide methionine sulfoxide reductases, *Biochim Biophys Acta 1703*, 261-266.

3. Zhao, C., Hartke, A., La Sorda, M., Posteraro, B., Laplace, J. M., Auffray, Y., and Sanguinetti, M. (2010) Role of methionine sulfoxide reductases A and B of Enterococcus faecalis in oxidative stress and virulence, *Infect Immun 78*, 3889-3897.

4. Romsang, A., Atichartpongkul, S., Trinachartvanit, W., Vattanaviboon, P., and Mongkolsuk, S. (2013) Gene expression and physiological role of Pseudomonas aeruginosa methionine sulfoxide reductases during oxidative stress, *J Bacteriol 195*, 3299-3308.

5. Schindelin, H., Kisker, C., Hilton, J., Rajagopalan, K. V., and Rees, D. C. (1996) Crystal structure of DMSO reductase: redox-linked changes in molybdopterin coordination, *Science 272*, 1615-1621.

6. Weissbach, H., Resnick, L., and Brot, N. (2005) Methionine sulfoxide reductases: history and cellular role in protecting against oxidative damage, *Biochim Biophys Acta 1703*, 203-212.

7. Achilli, C., Ciana, A., and Minetti, G. (2015) The discovery of methionine sulfoxide reductase enzymes: An historical account and future perspectives. *Biofactors 41*, 135-152.

8. Song, H., Hu, W., Naowarojna, N., Her, A. S., Wang, S., Desai, R., Qin, L., Chen, X., and Liu, P. (2015) Mechanistic studies of a novel C-S lyase in ergothioneine biosynthesis: the involvement of a sulfenic acid intermediate, *Sci Rep 5*, 11870.

9. Irani, S., Naowarojna, N., Tang, Y., Kathuria, K. R., Wang, S., Dhembi, A., Lee, N., Yan, W., Lyu, H., Costello, C. E., Liu, P., and Zhang, Y. J. (2018) Snapshots of C-S Cleavage in Egt2 Reveals Substrate Specificity and Reaction Mechanism, *Cell Chem Biol*.

10. Gerlt, J. A., Bouvier, J. T., Davidson, D. B., Imker, H. J., Sadkhin, B., Slater, D. R., and Whalen, K. L. (2015) Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST): A web tool for generating protein sequence similarity networks, *Biochim Biophys Acta 1854*, 1019-1037.

11. Broderick, J. B., Duffus, B. R., Duschene, K. S., and Shepard, E. M. (2014) Radical S-adenosylmethionine enzymes, *Chem Rev 114*, 4229-4317.

12. Landgraf, B. J., McCarthy, E. L., and Booker, S. J. (2016) Radical S-Adenosylmethionine Enzymes in Human Health and Disease, *Annu Rev Biochem 85*, 485-514.

13. Frey, P. A., Hegeman, A. D., and Ruzicka, F. J. (2008) The radical SAM superfamily, *Crit. Rev. Biochem. Mol. Biol. 43*, 63-88.

14. Magnusson, O. T., Reed, G. H., and Frey, P. A. (2001) Characterization of an allylic analogue of the 5'-deoxyadenosyl radical: an intermediate in the reaction of lysine 2,3-aminomutase, *Biochemistry 40*, 7773-7782.

15. Ji, X., Li, Y., Xie, L., Lu, H., Ding, W., and Zhang, Q. (2016) Expanding Radical SAM Chemistry by Using Radical Addition Reactions and SAM Analogues, *Angew Chem Int Ed55*, 11845-11848.

16. Ji, X., and Zhang, Q. (2017) Using radical SAM chemistry to access nucleoside-containing compounds, *Synlett 28*, 143-147.

17. Ding, W., Wu, Y., Ji, X., Qianzhu, H., Chen, F., Deng, Z., Yu, Y., and Zhang, Q. (2017) Nucleoside-linked shunt products in the reaction catalyzed by the class C radical S-adenosylmethionine methyltransferase NosN, *Chem Commun 53*, 5235-5238.

18. Ji, X., Mandalapu, D., Cheng, J., Ding, W., and Zhang, Q. (2018) Expanding the Chemistry of the Class C Radical SAM Methyltransferase NosN by Using an Allyl Analogue of SAM, *Angew Chem Int Ed 57*, 6601-6604.

19. Dong, M., Horitani, M., Dzikovski, B., Pandelia, M. E., Krebs, C., Freed, J. H., Hoffman, B. M., and Lin, H. (2016) Organometallic Complex Formed by an Unconventional Radical S-Adenosylmethionine Enzyme, *J Am Chem Soc 138*, 9755-9758.

20. Dong, M., Horitani, M., Dzikovski, B., Freed, J. H., Ealick, S. E., Hoffman, B. M., and Lin, H. (2017) Substrate-Dependent Cleavage Site Selection by Unconventional Radical S-Adenosylmethionine Enzymes in Diphthamide Biosynthesis, *J Am Chem Soc 139*, 5680-5683.

21. Dong, M., Zhang, Y., and Lin, H. (2018) Noncanonical Radical SAM Enzyme Chemistry Learned from Diphthamide Biosynthesis, *Biochemistry, 57, 3454-3459*.

22. Zhang, Y., Zhu, X., Torelli, A. T., Lee, M., Dzikovski, B., Koralewski, R. M., Wang, E., Freed, J., Krebs, C., Ealick, S. E., and Lin, H. (2010) Diphthamide biosynthesis requires an organic radical generated by an iron-sulphur enzyme, *Nature 465*, 891-896.

23. Nicolet, Y., Zeppieri, L., Amara, P., and Fontecilla-Camps, J. C. (2014) Crystal structure of tryptophan lyase (NosL): evidence for radical formation at the amino group of tryptophan, *Angew Chem Int Ed 53*, 11840-11844.

24. Ji, X., Li, Y., Ding, W., and Zhang, Q. (2015) Substrate-Tuned Catalysis of the Radical S-Adenosyl-L-Methionine Enzyme NosL Involved in Nosiheptide Biosynthesis, *Angew Chem Int Ed 54*, 9021-9024.

25. Bhandari, D. M., Xu, H., Nicolet, Y., Fontecilla-Camps, J. C., and Begley, T. P. (2015) Tryptophan Lyase (NosL): Mechanistic Insights from Substrate Analogues and Mutagenesis, *Biochemistry 54*, 4767-4769.

26. Ji, X. J., Li, Y. Z., Jia, Y. L., Ding, W., and Zhang, Q. (2016) Mechanistic Insights into the Radical S-adenosyl-l-methionine Enzyme NosL From a Substrate Analogue and the Shunt Products, *Angew Chem Int Ed 55*, 3334-3337.

27. Sicoli, G., Mouesca, J. M., Zeppieri, L., Amara, P., Martin, L., Barra, A. L., Fontecilla-Camps, J. C., Gambarelli, S., and Nicolet, Y. (2016) Fine-tuning of a radical-based reaction by radical S-adenosyl-L-methionine tryptophan lyase, *Science 351*, 1320-1323.

28. Ding, W., Ji, X., Li, Y., and Zhang, Q. (2016) Catalytic promiscuity of the radical S-adenosyl-L-methionine enzyme NosL, *Front Chem 4*, 27.

29. Bhandari, D. M., Fedoseyenko, D., and Begley, T. P. (2016) Tryptophan Lyase (NosL): A Cornucopia of 5'-Deoxyadenosyl Radical Mediated Transformations, *J Am Chem Soc 138*, 16184-16187.

30. Bhandari, D. M., Fedoseyenko, D., and Begley, T. P. (2018) Mechanistic Studies on Tryptophan Lyase (NosL): Identification of Cyanide as a Reaction Product, *J Am Chem Soc 140*, 542-545.

31. Wang, X. Y., Zhu, W. Y., and Liu, Y. J. (2017) Tryptophan lyase (NosL): mechanistic insights into amine dehydrogenation and carboxyl fragment migration by QM/MM calculations, *Catal Sci Technol 7*, 2846-2856.

32. Walsby, C. J., Hong, W., Broderick, W. E., Cheek, J., Ortillo, D., Broderick, J. B., and Hoffman, B. M. (2002) Electron-nuclear double resonance spectroscopic evidence that S-adenosylmethionine binds in contact with the catalytically active [4Fe-4S](+) cluster of pyruvate formate-lyase activating enzyme, *J Am Chem Soc 124*, 3143-3151.

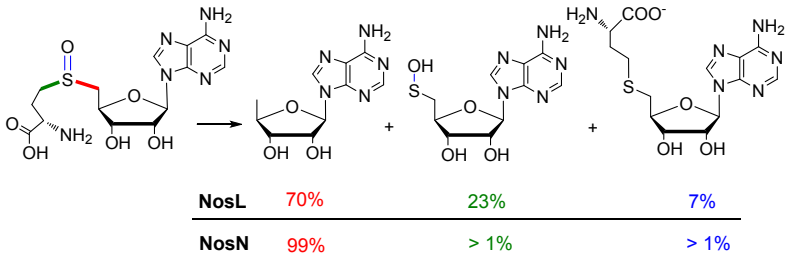
33. Horitani, M., Shisler, K., Broderick, W. E., Hutcheson, R. U., Duschene, K. S., Marts, A. R., Hoffman, B. M., and Broderick, J. B. (2016) Radical SAM catalysis via an organometallic intermediate with an Fe-[5'-C]-deoxyadenosyl bond, *Science 352*, 822-825.

34. Byer, A. S., Yang, H., McDaniel, E. C., Kathiresan, V., Impano, S., Pagnier, A., Watts, H., Denler, C., Vagstad, A. L., Piel, J., Duschene, K. S., Shepard, E. M., Shields, T. P., Scott, L. G., Lilla, E. A., Yokoyama, K., Broderick, W. E., Hoffman, B. M., and Broderick, J. B. (2018) Paradigm Shift for Radical S-Adenosyl-l-methionine Reactions: The Organometallic Intermediate Omega Is Central to Catalysis, *J Am Chem Soc 140*, 8634-8638.

35. Dong, M., Kathiresan, V., Fenwick, M. K., Torelli, A. T., Zhang, Y., Caranto, J. D., Dzikovski, B., Sharma, A., Lancaster, K. M., Freed, J. H., Ealick, S. E., Hoffman, B. M., and Lin, H. (2018) Organometallic and radical intermediates reveal mechanism of diphthamide biosynthesis, *Science 359*, 1247-1250.

36. Ding, W., Li, Y., Zhao, J., Ji, X., Mo, T., Qianzhu, H., Deng, Z., Yu, Y., Chen, F., and Zhang, Q. (2017) The Catalytic Mechanism of the Class C radical S-Adenosylmethionine Methyltransferase NosN, *Angew Chem Int Ed 56*, 3857-3861.

37. LaMattina, J. W., Wang, B., Badding, E. D., Gadsby, L. K., Grove, T. L., and Booker, S. J. (2017) NosN, a Radical S-Adenosylmethionine Methylase, Catalyzes Both C1 Transfer and Formation of the Ester Linkage of the Side-Ring System during the Biosynthesis of Nosiheptide, *J Am Chem Soc 139*, 17438-17445.



TOC Figure