

Molybdopterin Biosynthesis: Trapping an Unusual Purine Ribose Adduct in the MoaA-Catalyzed Reaction

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S Supporting Information

ABSTRACT: MoaA/MoaC catalyze a remarkable rearrangement reaction in which guanosine-5'-triphosphate (GTP) is converted to cyclic pyranopterin monophosphate (cPMP). In this reaction, the C8 of GTP is inserted between the C2' and the C3' carbons of the GTP ribose. Previous experiments with GTP isotopomers demonstrated that the ribose C3' hydrogen atom is abstracted by the adenosyl radical. This led to a novel mechanistic proposal involving an intermediate with a bond between the C8 of guanine and C3' of the ribose. This paper describes the use of 2',3'-dideoxyGTP to trap this intermediate.

MoaA/MoaC catalyzes the first step in molybdopterin biosynthesis, converting GTP, **1**, to cyclic pyranopterin monophosphate (cPMP, **2**).¹ MoaA/MoaC catalyzes a remarkable rearrangement reaction in which the C8 of GTP, **1**, is inserted into the ribose C2'–C3' bond (Figure 1).²

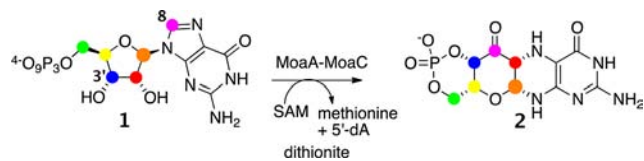


Figure 1. Early reactions in molybdopterin biosynthesis: MoaA/MoaC-catalyzed transformation of GTP (**1**) to cyclicpyranopterin monophosphate (**2**). Color shows the atom transfer pattern derived from previous labeling studies.^{2,4,8}

Previous experiments with GTP isotopomers demonstrated that the ribose C3' hydrogen atom is abstracted by the adenosyl radical. This led to a mechanistic proposal involving an intermediate with a bond between the C8 of guanine and C3' of the ribose (Figure 2).³ In this proposal, the 5'-dA radical **3**, generated by reductive cleavage of S-Adenosyl methionine (AdoMet), abstracts the 3' hydrogen atom from GTP to give **4**, which then undergoes cyclization to give **6**. Reduction of this radical by the purine liganded iron sulfur cluster to **7** followed by hydrolysis to **8** and a benzilic-like rearrangement to **9** completes the insertion of the purine carbon into the ribose. Ring opening of **9** followed by dehydration to **10** and a conjugate addition gives **12**. Cyclization to **13** followed by a final tautomerization completes the formation of the reaction product **2**.³ While ribose and deoxyribose radicals have been extensively studied in the context of enzymes such as ribonucleotide reductase and DNA damage by radiation or

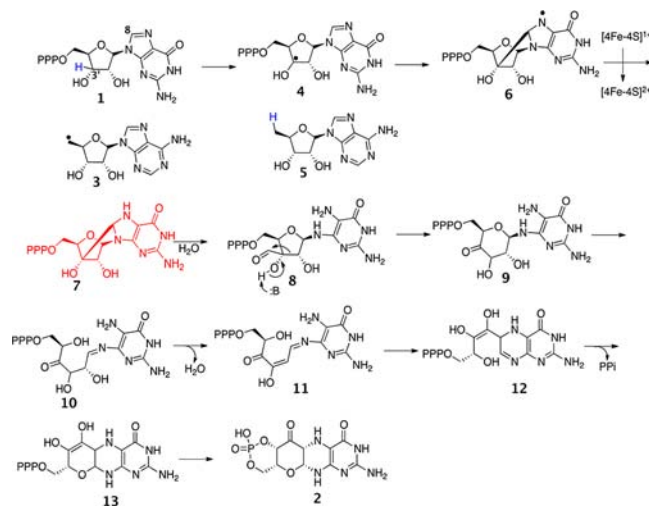


Figure 2. Mechanistic proposal for the MoaA/MoaC-catalyzed reaction.

radical-generating antibiotics, addition of a C3' centered radical to C8 of a purine has never been reported.^{5–7} The successful trapping of an analog of **8** would provide a critical test of the proposed mechanism. In this communication, we describe the use of 2',3'-dideoxy-GTP **14** as a substrate analog to achieve this trapping.

LCMS analysis of the MoaA/2',3'-dideoxyGTP **14** reaction mixture demonstrated the formation of a new product eluting at 19.6 min (Figure 3A). ESI-MS (positive mode) analysis of this product demonstrated that its mass $[M+H]^+$ was 490.0 Da, 2 Da less than the $[M+H]^+$ of the substrate. We considered two possible structures for this product (**16** and **20**, Figure 3D). To test for the formation of **16**, the enzymatic product was dephosphorylated by phosphatase treatment and compared to an authentic sample of **17** (synthesis described in Supporting Information (SI)) by LCMS. Figure 3C and Figures S28 and S29 demonstrate that the retention time of **17** and its MS/MS spectrum are different from those of the enzymatic product. This excludes the possibility that MoaA catalyzes the formation of **16** from **14**.

The MS/MS spectrum of **17** showed fragmentation of the N-glycosyl bond to form guanine (152.0 Da, Figure S23). This fragmentation was not detected for the dephosphorylated enzymatic product (Figure S24) suggesting an additional bond

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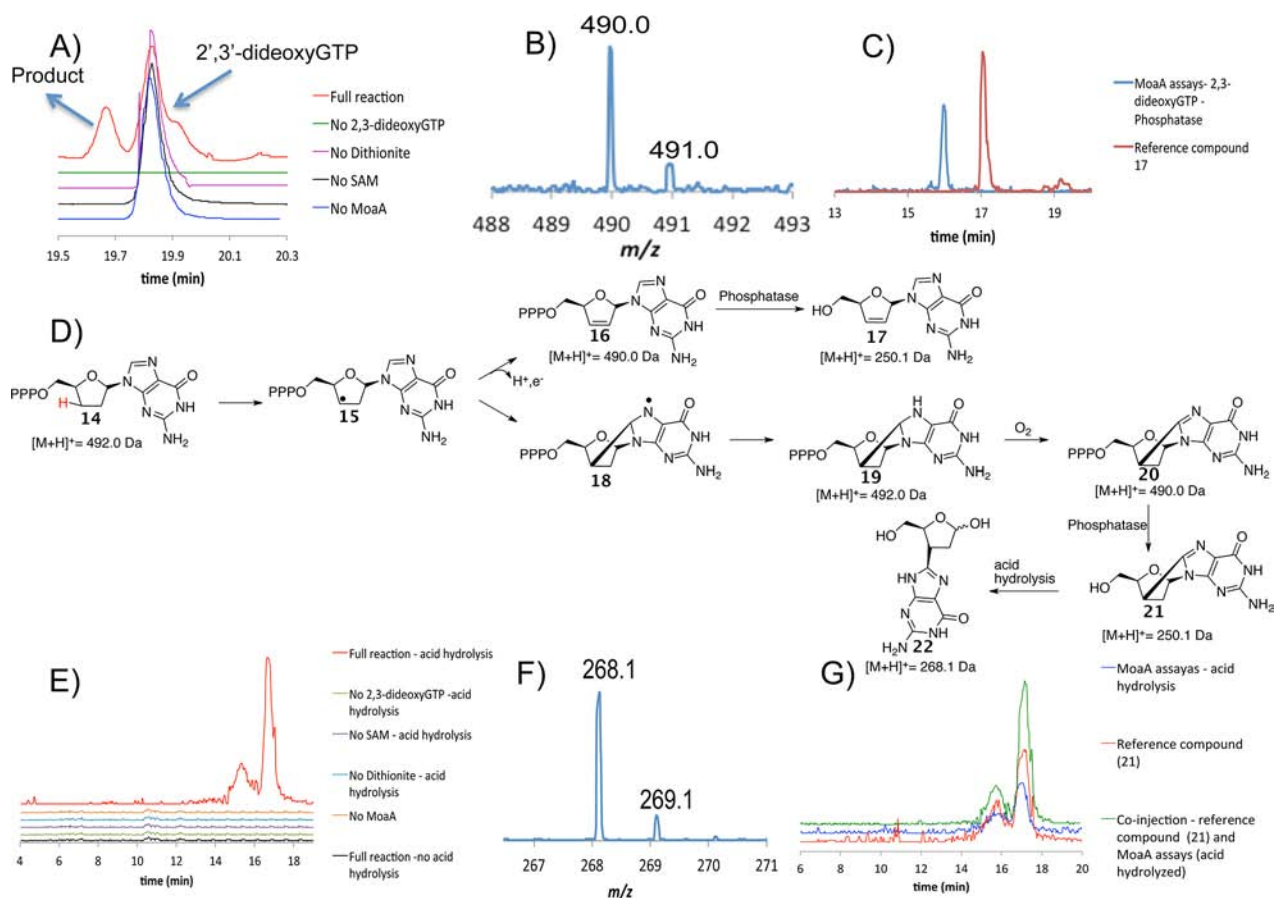


Figure 3. Analysis of the product formed when 2',3'-dideoxyGTP **14** is treated with MoaA. (A) LC analysis of the MoaA reaction mixture and controls. Red trace is the full reaction where all the components are present. Green, pink, black, and blue traces are for reaction mixtures where either 2',3'-dideoxyGTP, dithionite, SAM, or MoaA is absent respectively. The unidentified signal at 19.9 min has the same $[M+H]^+$ as 2',3'-dideoxyGTP. (B) MS of the compound eluting at 19.6 min. The $[M+H]^+$ is 490.0 Da, 2 Da less than the $[M+H]^+$ of the substrate **14**. (C) Extracted ion chromatograms for $[M+H]^+ = 250.0$ Da demonstrate that compound **17** is different from the dephosphorylated enzymatic product. (D) Mechanistic analysis to suggest possible products formed from 2',3'-dideoxyGTP **14**. (E) Extracted ion chromatograms for $[M+H]^+ = 268.1$ Da demonstrate that the $[M+H]^+ = 268.1$ Da signal is seen only in reaction mixtures with all the components present after phosphatase treatment and acid hydrolysis. The two compounds with $[M+H]^+ = 268.1$ Da are most likely a consequence of hemiacetal isomerization during the acid hydrolysis. (F) MS of the compound formed by acid hydrolysis of the dephosphorylated enzymatic product ($[M+H]^+ = 268.1$ Da). (G) Extracted ion chromatograms for $[M+H]^+ = 268.1$ Da demonstrate that the derivatized enzymatic product has the same mass and coelutes with compound **22**.

between the sugar and the base consistent with **20**. Unfortunately, NMR characterization of the enzymatic product was not possible. Only small quantities were formed because 2',3'-dideoxyGTP **14** is a poor substrate (there is no evidence for enzyme modification by MS analysis). An identification strategy, involving comparison with a reference compound, was therefore adopted. If **19** is the product structure, oxidation and dephosphorylation, followed by N-glycosyl bond cleavage, would give **22** which can be synthesized as a reference (see SI). To test this, the enzymatic product was derivatized by treating the MoaA reaction mixture with phosphatase followed by acid at 65 °C for 2 h. The extracted ion chromatograms for 268.1 Da (**22** + H⁺ + H₂O) are shown in Figure 3E and demonstrate that the acid treatment generates isomers of the product as expected from ring opening of the hemiacetal. The derivatized enzymatic product coelutes with the synthetic reference (also generates isomers on acid treatment (see SI) and is identical by LCMS analysis). Thus, the enzymatic reaction of 2',3'-dideoxyGTP **14** supports the formation of the remarkable reaction intermediate **7** in the MoaA-catalyzed reaction.

A mechanistic proposal for the formation of **22** is described in Figure 4. The 5'-dA radical **4** abstracts the 3' hydrogen atom

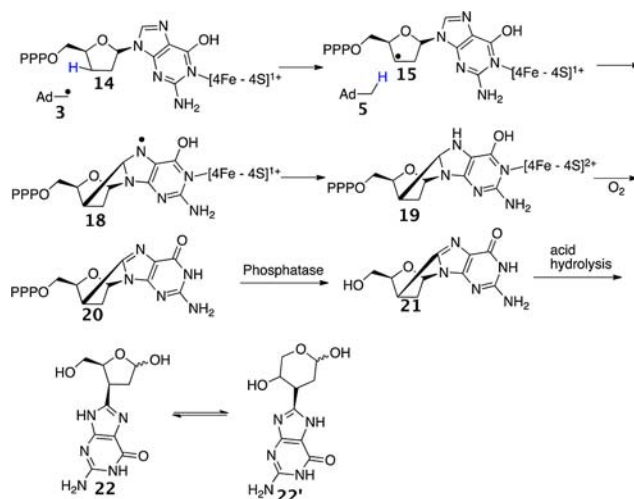


Figure 4. Mechanistic proposal for the MoaA catalyzed reaction and subsequent derivatization of 2',3'-dideoxyGTP **14**.

from 2',3'-dideoxyGTP **14** to give **15**, which then undergoes cyclization to give **18**. Reduction of this radical by the purine liganded iron sulfur cluster gives **19** which on aerobic oxidation results in the formation of **20**. Phosphatase treatment of **20** followed by acid hydrolysis gives **22** as a mixture of isomers.

While this paper was under review, an important new paper on the mechanism of MoaA/MoaC appeared in the literature.⁹ This paper suggests that compound **7** is the product of MoaA and demonstrates that **7** is a substrate for MoaC. Both papers provide support for the unprecedented bond formation between C3' of the ribose and C8 of the purine using different but complementary approaches.

■ ASSOCIATED CONTENT

📄 Supporting Information

The experimental procedures are described in the Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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