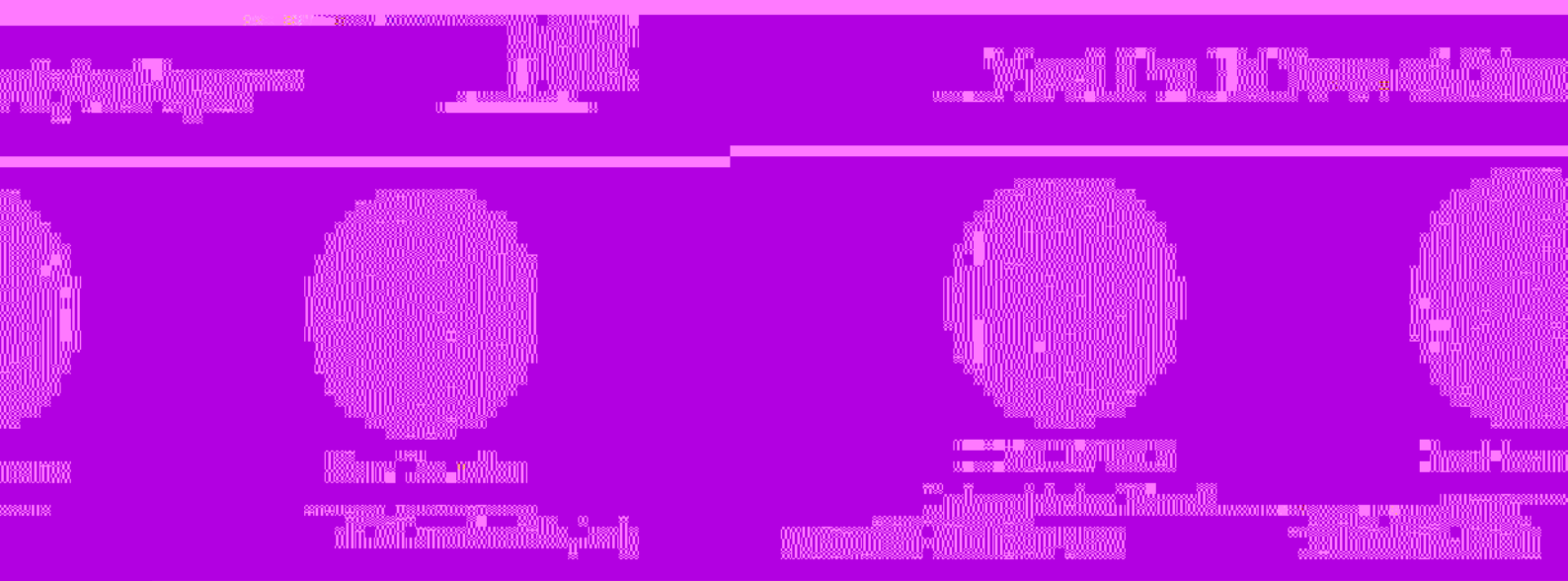


# Excellence in Chemistry Research



# Molecular Insights into Bifunctional Ambulatory DH<sub>3</sub> Substrate Specificity and Catalytic Mechanism

Zeq, Li, Y. Li, Y. Li, T. Shi\*

**Abstract:** The bifunctional ambulatory DH<sub>3</sub> (PKS) is a key enzyme in the biosynthesis of polyketide natural products. It is responsible for the formation of the polyketide chain and the subsequent cyclization to form the macrocyclic polyketide. The catalytic mechanism of the bifunctional ambulatory DH<sub>3</sub> is still unclear. In this study, we have investigated the catalytic mechanism of the bifunctional ambulatory DH<sub>3</sub> (PKS) using a combination of experimental and computational methods. We have shown that the bifunctional ambulatory DH<sub>3</sub> (PKS) is a bifunctional enzyme that can catalyze both the formation of the polyketide chain and the subsequent cyclization to form the macrocyclic polyketide. The catalytic mechanism of the bifunctional ambulatory DH<sub>3</sub> (PKS) is still unclear. In this study, we have investigated the catalytic mechanism of the bifunctional ambulatory DH<sub>3</sub> (PKS) using a combination of experimental and computational methods. We have shown that the bifunctional ambulatory DH<sub>3</sub> (PKS) is a bifunctional enzyme that can catalyze both the formation of the polyketide chain and the subsequent cyclization to form the macrocyclic polyketide.

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## Introduction

The bifunctional ambulatory DH<sub>3</sub> (PKS) is a key enzyme in the biosynthesis of polyketide natural products. It is responsible for the formation of the polyketide chain and the subsequent cyclization to form the macrocyclic polyketide. The catalytic mechanism of the bifunctional ambulatory DH<sub>3</sub> (PKS) is still unclear. In this study, we have investigated the catalytic mechanism of the bifunctional ambulatory DH<sub>3</sub> (PKS) using a combination of experimental and computational methods. We have shown that the bifunctional ambulatory DH<sub>3</sub> (PKS) is a bifunctional enzyme that can catalyze both the formation of the polyketide chain and the subsequent cyclization to form the macrocyclic polyketide.

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 Selectivities of Organic Reactions – In Celebration of Prof. Kendall N. Houk's  
 80th birthday.

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Results and Discussion

Stereochemical selectivity of AmbDH $\beta$  at C2 in recognition

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Critical residues are responsible of substrate selectivity of AmbDH $\beta$

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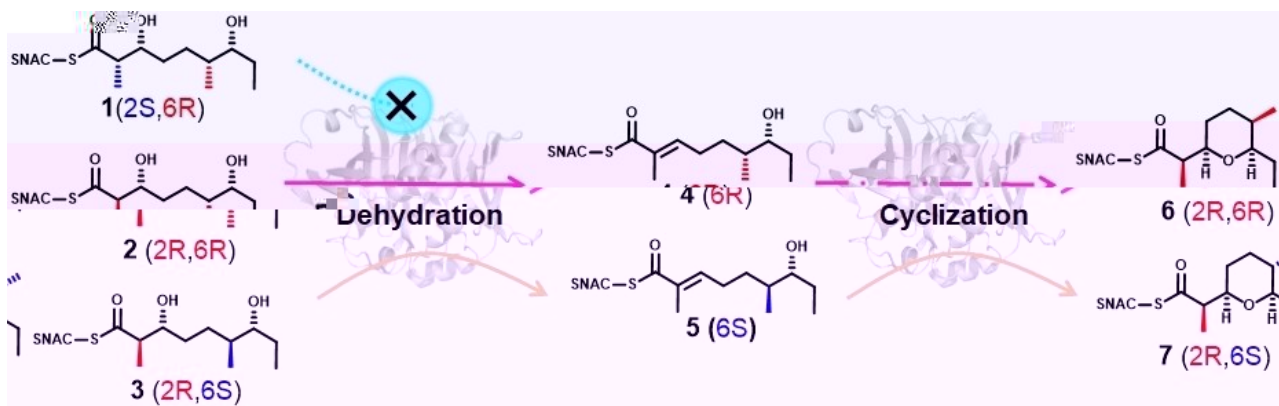
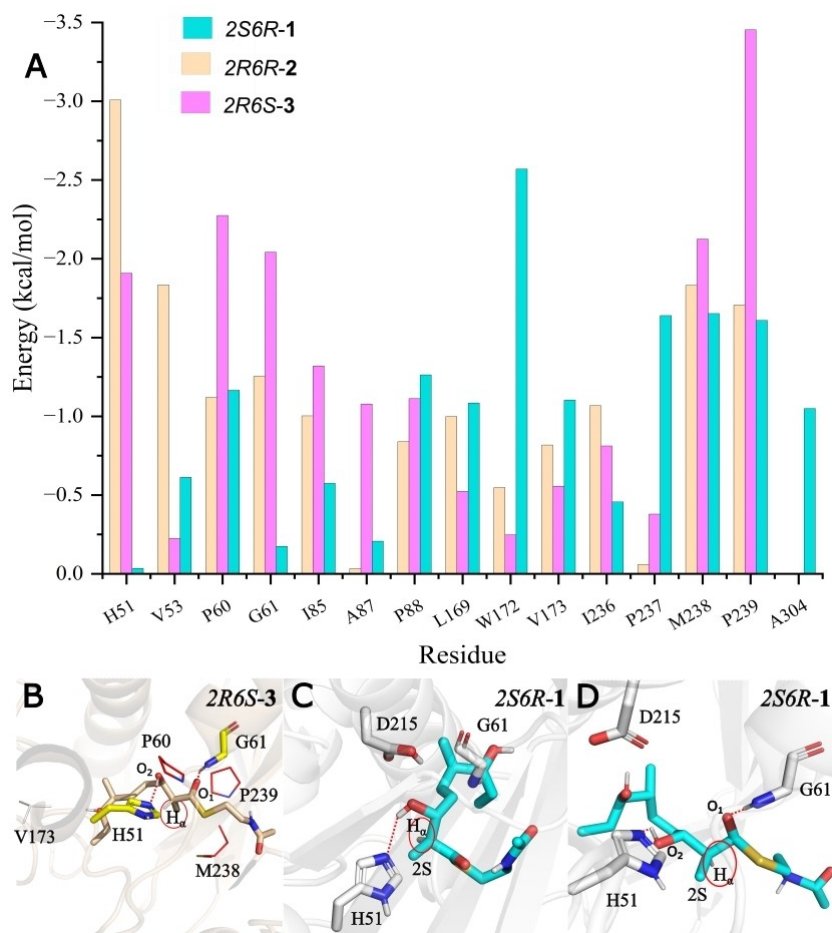


Figure 1. The stereoselectivity of AmbDH $\beta$  at C2 in recognition of the substrate 2S6R 1, 2R6R 2, 2R6S 3, 6R 4, 6S 5, 2R6R 6, 2R6S 7. The reaction scheme shows the conversion of substrate 1 to product 4 via dehydration and cyclization.

2R6S3), le t t 2R6R 2 2R6S3 -6. l l l-  
 -1.9 l l l-1, e. e t v el, t t t 2R b t te  
 t e t e. e t X b e e e f v - b l e t 2S  
 b t te (T ble S2t).  
 Ke e e e t e e t e. e e e t f e b e e  
 e e e t e. F f t e e t e t e e  
 g g t e, e f t e t b t e e t  
 1. l l l-1 ll te. The e e t t l  
 t t t l e t e e g t t l t e (Fg  
 l e BX).  
 Se l, t t e e f f t e e e, 1 61 e e  
 t e b e e f t e e t t e e e t t  
 te 2R6R 2, 2R6S3, 2S6R 1. We e f ll t  
 g e t e f f e e e e b 2R b t te 2S  
 b t te. t e t t t e e t e e g t b e  
 e v e f e t e t t R f g t g e l v t  
 f X b b t C2. e t t t e l g e t t 61  
 1 l l - b e b t 1 2 f f  
 b t te te 2R6R 2, 2R6S3,  
 t e e b e e t te (F g e B). t e t e  
 te 2S6R 1, e t e t t f (V) e e,  
 t e e t e e e e l t e (F g e BC),  
 l e e t e t t f (V) b l e, t e  
 g e b l l t e t l g f g 1  
 - t e t e t e t e t t g  
 2S6R 1 (F g e B). The e e e l z e t t t e  
 g e b e b 1 61 e t e e g t

f l l b t te, l g e l t l z e t t t l  
 e l v t f X b b 2R b t te f t e t t l  
 e e t e. W t e, 1 t e b e e f t e  
 t l t e. 61 e e e l t v e l e v e  
 t e. The e e b e b 1  
 b e e t e b t te l e t e F 1  
 l (e e t e l l r l,  
 e e t e l) (F g e S t).<sup>12</sup> E e e t v e f e t t t e l  
 f t t e b e t e b t te l f  
 v e t e e t t.<sup>18</sup>  
 T h l, P6, M2B, P2B9 e e e t b t e  
 e t t 1. l l l-1 ll t e e t e. The t t e  
 t e t f t e b e t e l e t  
 e t e b t te e t t. W t e,  
 P6 P2B9 e e g l e e e t e l  
 t t t l e b t te b g F 1 (e e  
 g P17 P192 e e t e l). T t t e e t e e  
 e e e t t t t b l z g t e b t te t e  
 te.  
 F t l, e l e t t t e e t t t t e  
 t t f V17BY V17B e t e t e l z e t v t f  
 X b b, l e t e 11 - t t !



**Figure 3.** (X) Energy of the transition state (TS) for the reaction of the iron center with the 2S ligand in the active site of the 2S6R-1 (cyan), 2R6R-2 (orange), and 2R6S-3 (magenta) variants. The energy values are in kcal/mol. (Y) 3D molecular models of the active site for the 2R6S-3 (B), 2S6R-1 (C), and 2S6R-1 (D) variants. The residues are shown as sticks and the 2S ligand is shown as a ball-and-stick model. The iron center is shown as a red sphere. The oxygen atoms are shown as red spheres. The hydrogen atoms are shown as white spheres. The nitrogen atoms are shown as blue spheres. The sulfur atom is shown as a yellow sphere. The carbon atoms are shown as grey spheres. The labels H51, V173, P60, G61, P239, M238, D215, and 2S indicate the residues and the ligand, respectively.

The energy of the transition state (TS) for the reaction of the iron center with the 2S ligand in the active site of the 2S6R-1 (cyan), 2R6R-2 (orange), and 2R6S-3 (magenta) variants. The energy values are in kcal/mol. (Y) 3D molecular models of the active site for the 2R6S-3 (B), 2S6R-1 (C), and 2S6R-1 (D) variants. The residues are shown as sticks and the 2S ligand is shown as a ball-and-stick model. The iron center is shown as a red sphere. The oxygen atoms are shown as red spheres. The hydrogen atoms are shown as white spheres. The nitrogen atoms are shown as blue spheres. The sulfur atom is shown as a yellow sphere. The carbon atoms are shown as grey spheres. The labels H51, V173, P60, G61, P239, M238, D215, and 2S indicate the residues and the ligand, respectively.

### The catalytic mechanism

In the active site, the iron center is coordinated to the 2S ligand and the oxygen atoms of the substrate. The iron center is coordinated to the oxygen atoms of the substrate and the sulfur atom of the 2S ligand. The iron center is coordinated to the oxygen atoms of the substrate and the sulfur atom of the 2S ligand. The iron center is coordinated to the oxygen atoms of the substrate and the sulfur atom of the 2S ligand.

In the active site, the iron center is coordinated to the 2S ligand and the oxygen atoms of the substrate. The iron center is coordinated to the oxygen atoms of the substrate and the sulfur atom of the 2S ligand. The iron center is coordinated to the oxygen atoms of the substrate and the sulfur atom of the 2S ligand. The iron center is coordinated to the oxygen atoms of the substrate and the sulfur atom of the 2S ligand.

$f_{-t}$  te, t<sub>g</sub> e - t<sub>g</sub> f e | te e<sub>v</sub> te (M1) eq e<sub>v</sub>  
 2-1 | l<sup>-1</sup> e e<sub>v</sub> . W<sub>g</sub> te t e f α-C<sub>2</sub>  
 α<sub>v</sub> e e<sub>v</sub> te t<sub>g</sub> 1.66 1.17 X, t<sub>g</sub> e t<sub>g</sub> t te (TS1)  
 b e<sub>v</sub> e<sub>v</sub> , t<sub>g</sub> e<sub>v</sub> - f eq e<sub>v</sub> . 1.9 i<sup>-1</sup>  
 t<sub>g</sub> t<sub>g</sub> et t<sub>g</sub> t<sub>g</sub> f C<sub>2</sub> α<sub>v</sub> .  
 C<sub>2</sub> - g TS1 t<sub>g</sub> M1 e<sub>v</sub> | f t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> f  
 C<sub>2</sub> - g<sub>v</sub> 2.7 X M1, F<sub>v</sub> e S<sub>v</sub> t), t<sub>g</sub> z=1  
 e<sub>v</sub> te t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> fe<sub>v</sub> f<sub>v</sub> | b t te<sub>v</sub> C<sub>2</sub> β  
 l. The e<sub>v</sub> te<sub>v</sub> e<sub>v</sub> t<sub>g</sub> f te<sub>v</sub> le<sub>v</sub> le<sub>v</sub> . The  
 e e<sub>v</sub> b e<sub>v</sub> g | l<sup>-1</sup> te<sub>v</sub> be 2.2 | l<sup>-1</sup> , t<sub>g</sub> t<sub>g</sub> te<sub>v</sub>  
 e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> te<sub>v</sub> te<sub>v</sub> e<sub>v</sub> te<sub>v</sub> t<sub>g</sub> t<sub>g</sub> te<sub>v</sub> le<sub>v</sub>  
 e<sub>v</sub> t<sub>g</sub> | z t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> . The e<sub>v</sub> t e<sub>v</sub> f  
 C<sub>2</sub> - 2 e<sub>v</sub> 1. X 1.22 X, e<sub>v</sub> e t e<sub>v</sub> l TS2,  
 e<sub>v</sub> α<sub>v</sub> | f eq e<sub>v</sub> f<sub>v</sub> . 1.9 i<sup>-1</sup>  
 v b<sub>v</sub> t<sub>g</sub> | l<sup>-1</sup> , t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> f C<sub>2</sub> - 2 2  
 α<sub>v</sub> 2.2 X f te<sub>v</sub> TS2, t<sub>g</sub> t<sub>g</sub> te<sub>v</sub> e<sub>v</sub> te<sub>v</sub> (M2)  
 g e e<sub>v</sub> te<sub>v</sub> t<sub>g</sub> e<sub>v</sub> | e<sub>v</sub> e<sub>v</sub> ble b<sub>v</sub> .  
 g e b t<sub>g</sub> e e<sub>v</sub> t<sub>g</sub> f e<sub>v</sub> te<sub>v</sub> le<sub>v</sub> le<sub>v</sub> (F<sub>v</sub> e  
 F<sub>v</sub> e S<sub>v</sub> t).  
 W<sub>g</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> . t<sub>g</sub>  
 fte<sub>v</sub> t<sub>g</sub> t<sub>g</sub> f e<sub>v</sub> | te e<sub>v</sub> te (M1), b  
 t<sub>g</sub> | b t te (M2\_1) e e<sub>v</sub> te<sub>v</sub> , t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> .  
 t<sub>g</sub> fe<sub>v</sub> f<sub>v</sub> z=1 t<sub>g</sub> 2.2 t<sub>g</sub> t<sub>g</sub> e t<sub>g</sub> t<sub>g</sub>  
 fe<sub>v</sub> e<sub>v</sub> 2(F<sub>v</sub> e S<sub>v</sub> t). TS2\_1 b e<sub>v</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t e<sub>v</sub>  
 f<sub>v</sub> ω 2<sup>-ω</sup> 2<sup>-2</sup> , 1<sup>-2</sup> t 1. X,  
 1.2 X, 1.17 X, 1.27 X, e<sub>v</sub> e t e<sub>v</sub> l , t<sub>g</sub> t<sub>g</sub> l - f e<sub>v</sub>  
 q e<sub>v</sub> 91 . 1.9 i<sup>-1</sup> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> f<sub>v</sub> α 2  
 t<sub>g</sub> 1. X t<sub>g</sub> t<sub>g</sub> e e<sub>v</sub> b e<sub>v</sub> | t 19.7 | l<sup>-1</sup> ,  
 t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> | e<sub>v</sub> t e<sub>v</sub> l t<sub>g</sub> b e<sub>v</sub> .  
 f 26.9 | l<sup>-1</sup> . TS2\_2 b t e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t e<sub>v</sub> f  
 2-C<sub>2</sub> , 2<sup>-2</sup> , 1<sup>-2</sup> t 1.9 , 1. , 1.2 X, e<sub>v</sub> e t e<sub>v</sub> l .  
 Xl<sup>2</sup> , TS2\_2 t<sub>g</sub> - f eq e<sub>v</sub> f<sub>v</sub> . 1.9 i<sup>-1</sup>  
 t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> f<sub>v</sub> 2-C<sub>2</sub> 2<sup>-2</sup> (F<sub>v</sub>

e S<sub>v</sub> t). t<sub>g</sub> , t<sub>g</sub> | l<sup>-1</sup> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub>  
 e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> , e t e<sub>v</sub> e<sub>v</sub> b e<sub>v</sub> (2.2 | l<sup>-1</sup>)  
 little e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub>  
 (26.9 | l<sup>-1</sup>).

**The cyclization mechanism**

X fte<sub>v</sub> e<sub>v</sub> t<sub>g</sub> , M2' t<sub>g</sub> t<sub>g</sub> z e<sub>v</sub> b fl. t t l<sub>v</sub> e  
 (M2), t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> e<sub>v</sub> g b e<sub>v</sub> f  
 1.8 | l<sup>-1</sup> (F<sub>v</sub> e S<sub>v</sub> t). S g | t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> , t<sub>g</sub>  
 e<sub>v</sub> t e<sub>v</sub> t<sub>g</sub> t e<sub>v</sub> t<sub>g</sub> . F t l , z=1 e t e<sub>v</sub> t<sub>g</sub> f  
 e<sub>v</sub> b e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> e<sub>v</sub> l e<sub>v</sub> t e<sub>v</sub> ble  
 b<sub>v</sub> t C<sub>2</sub> , t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t e (M<sub>2</sub>) e<sub>v</sub> . The  
 e e<sub>v</sub> b e<sub>v</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> f M<sub>2</sub> 1.6 | l<sup>-1</sup> . X  
 t<sub>g</sub> F<sub>v</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t e<sub>v</sub> f<sub>v</sub> . 1.9 i<sup>-1</sup>  
 C<sub>2</sub> - t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> 1.1 X, 1.2 X, 1.2 X, e<sub>v</sub> e t e<sub>v</sub> l ,  
 TS<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> - f eq e<sub>v</sub> 1.9 i<sup>-1</sup> .  
 e<sub>v</sub> e<sub>v</sub> , TS<sub>v</sub> b e<sub>v</sub> e<sub>v</sub> , g e<sub>v</sub> t<sub>g</sub> t e<sub>v</sub> f<sub>v</sub> .  
 C<sub>2</sub> - e<sub>v</sub> e<sub>v</sub> l e<sub>v</sub> 1.22 X 1.26 X, t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> f eq e<sub>v</sub>  
 f 1 9.67 i<sup>-1</sup> . F<sub>v</sub> l l , t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> f<sub>v</sub> α 1 C<sub>2</sub>  
 e<sub>v</sub> e<sub>v</sub> z=1 t<sub>g</sub> t<sub>g</sub> f l. t<sub>g</sub> t (P) (F<sub>v</sub> e S<sub>v</sub> t11).  
 The e e<sub>v</sub> b e<sub>v</sub> | l<sup>-1</sup> t e<sub>v</sub> be 2.2 | l<sup>-1</sup> .  
 W<sub>g</sub> t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> | e<sub>v</sub> e<sub>v</sub> t e<sub>v</sub>  
 t<sub>g</sub> e<sub>v</sub> t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> e<sub>v</sub> e<sub>v</sub> e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t e<sub>v</sub>  
 l z t<sub>g</sub> t<sub>g</sub> l e<sub>v</sub> t<sub>g</sub> e<sub>v</sub> t l z t<sub>g</sub> t<sub>g</sub> ,  
 e e b t<sub>g</sub> t<sub>g</sub> e<sub>v</sub> e t t<sub>g</sub> t t. fe<sub>v</sub> g<sub>v</sub> | be  
 e<sub>v</sub> t e<sub>v</sub> b<sub>v</sub> t e<sub>v</sub> | e<sub>v</sub> l e<sub>v</sub> (F<sub>v</sub> e S<sub>v</sub> t12). F<sub>v</sub> l , t<sub>g</sub> e M2'  
 b t e<sub>v</sub> , t<sub>g</sub> e<sub>v</sub> 2 f t<sub>g</sub> e<sub>v</sub> t e t t<sub>g</sub> f<sub>v</sub> .  
 e b 2 f t<sub>g</sub> e<sub>v</sub> t e t fe<sub>v</sub> g<sub>v</sub> 1 (M<sub>2</sub>') . TS<sub>v</sub>'  
 g<sub>v</sub> t, t<sub>g</sub> e<sub>v</sub> t e<sub>v</sub> f<sub>v</sub> .

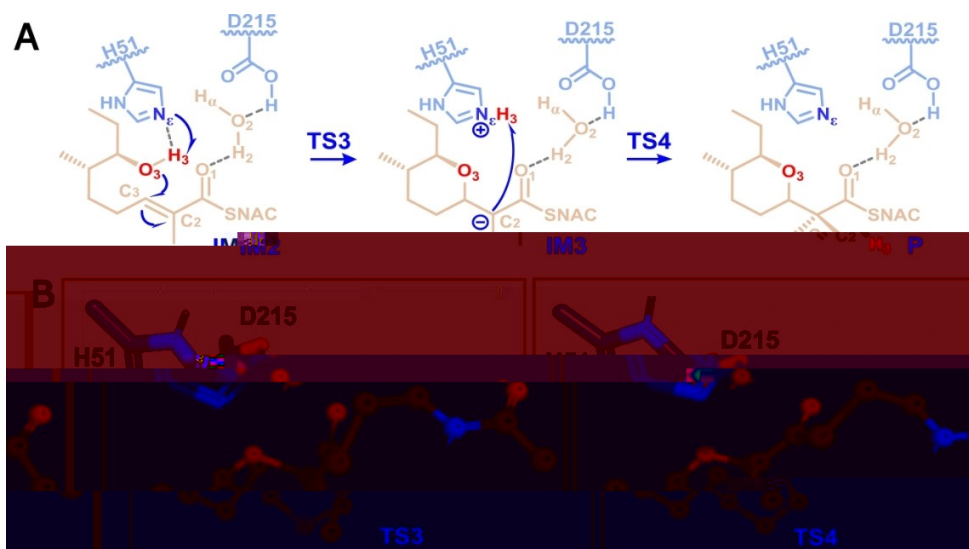


Figure 5. (X) The reaction scheme showing the conversion of reactants to products via transition states (TS) and intermediates (IM) in the presence of H51 and D215.

(M<sup>-</sup>). We then performed a series of calculations to determine the relative energies of the reactants, transition states, and products. The energy of the reactants was set to 0.0 kcal/mol. The energy of the transition states was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures. The energy of the transition states was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures. The energy of the transition states was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures.

### Mil tolerance of AmbDH3 at C<sup>4</sup>n cyclization

PLC-MS I.e. the transition state for the cyclization of the substrate. The energy of the transition state was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures. The energy of the transition states was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures. The energy of the transition states was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures.

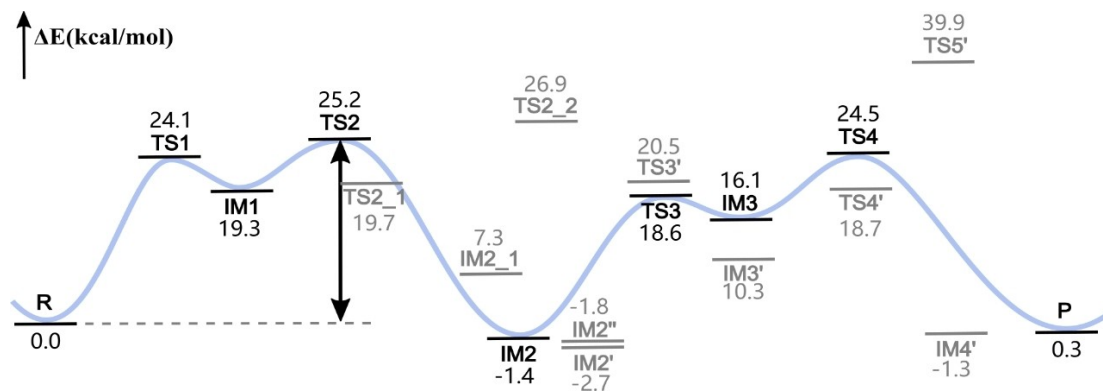


Figure 6. The free energy profile of the reaction showing the energy of the reactants, transition states, and products. The energy of the reactants was set to 0.0 kcal/mol. The energy of the transition states was determined by calculating the energy of the transition state structures. The energy of the products was determined by calculating the energy of the product structures.

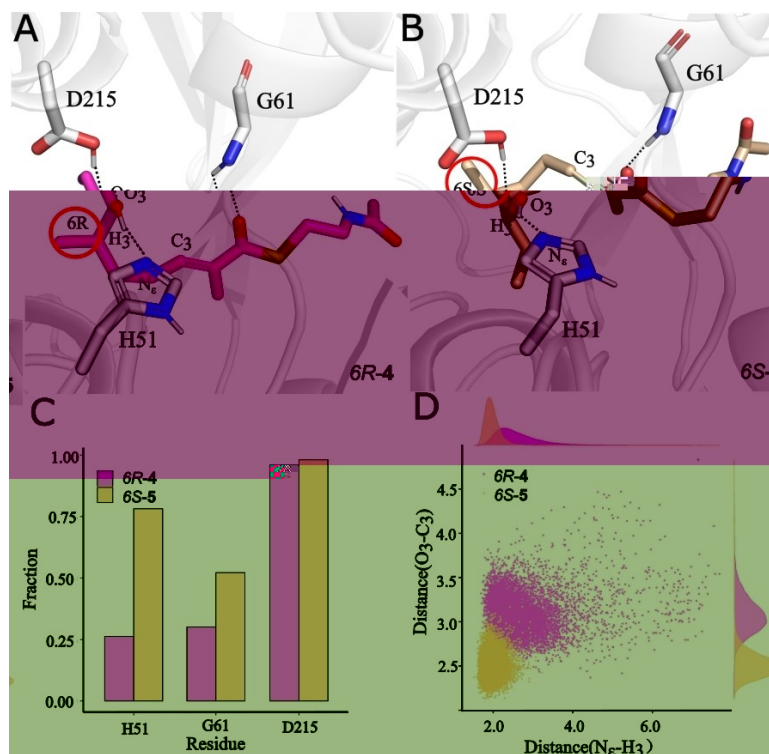


Figure 1. Structural analysis of the 6R-4 (X-ray crystallographic) and 6S-5 (molecular dynamics simulation) transition states. (A) 6R-4 transition state structure showing residues D215 and G61. (B) 6S-5 transition state structure showing residues D215 and G61. (C) Fraction of residues H51, G61, and D215 in the 6R-4 (purple) and 6S-5 (yellow) transition states. (D) Scatter plot of Distance(O3-C3) vs Distance(Nε-H3) for 6R-4 (purple) and 6S-5 (yellow) transition states.

... 6R-4 transition state structure showing residues D215 and G61. (B) 6S-5 transition state structure showing residues D215 and G61. (C) Fraction of residues H51, G61, and D215 in the 6R-4 (purple) and 6S-5 (yellow) transition states. (D) Scatter plot of Distance(O3-C3) vs Distance(Nε-H3) for 6R-4 (purple) and 6S-5 (yellow) transition states.

### Conclusion

The 6R-4 transition state structure showing residues D215 and G61. (B) 6S-5 transition state structure showing residues D215 and G61. (C) Fraction of residues H51, G61, and D215 in the 6R-4 (purple) and 6S-5 (yellow) transition states. (D) Scatter plot of Distance(O3-C3) vs Distance(Nε-H3) for 6R-4 (purple) and 6S-5 (yellow) transition states.

... 6R-4 transition state structure showing residues D215 and G61. (B) 6S-5 transition state structure showing residues D215 and G61. (C) Fraction of residues H51, G61, and D215 in the 6R-4 (purple) and 6S-5 (yellow) transition states. (D) Scatter plot of Distance(O3-C3) vs Distance(Nε-H3) for 6R-4 (purple) and 6S-5 (yellow) transition states.



el t<sup>1</sup> b t<sup>2</sup> e e t<sup>3</sup> f t<sup>4</sup> e l e l e  
 l g e t<sup>5</sup> t<sup>6</sup> t<sup>7</sup> e g b e f 2-2 l l<sup>-1</sup>.  
 e l t e e e t t<sup>8</sup> t<sup>9</sup> e b e e t<sup>10</sup>  
 e t<sup>11</sup> t<sup>12</sup> e e t<sup>13</sup> e e t<sup>14</sup> b e  
 t<sup>15</sup> t<sup>16</sup> f t<sup>17</sup> e l e l e.  
 L t, t<sup>18</sup> e z e b t. t<sup>19</sup> e t<sup>20</sup> 6R 6S 5  
 e e t<sup>21</sup> t<sup>22</sup> e t<sup>23</sup> e t<sup>24</sup> e l e e f X b t  
 C6. F ll, 6S 5 t<sup>25</sup> t<sup>26</sup> b l e e b t  
 f v b l e t<sup>27</sup> t<sup>28</sup> . T t e t e g t b t<sup>29</sup> f e  
 e t<sup>30</sup> t<sup>31</sup> t<sup>32</sup> e t<sup>33</sup> t<sup>34</sup> t<sup>35</sup> 6R 6S 5 l l b e  
 l z e, l e t e t e f 6S 5 e e f f e t t<sup>36</sup>  
 t<sup>37</sup> t<sup>38</sup> f 6R t<sup>39</sup> t<sup>40</sup> e l l t<sup>41</sup> t<sup>42</sup> e e e t.  
 e v l l e l t e l b t e l t e l e t<sup>43</sup>  
 e v f t<sup>44</sup> e l q e b f t<sup>45</sup> l ( e t<sup>46</sup> t<sup>47</sup> )  
 l z t<sup>48</sup> ) e t<sup>49</sup> t e X b l l e e e  
 g t t<sup>50</sup> t<sup>51</sup> t<sup>52</sup> e t<sup>53</sup> f e t<sup>54</sup> e v e  
 g e t<sup>55</sup> t<sup>56</sup> f v l t t e e t e t<sup>57</sup> e l l  
 v e t<sup>58</sup> e t<sup>59</sup> t<sup>60</sup> t<sup>61</sup> e e z t  
 e f l t g t b t l l  
 e g g e t e P K S g e .

## Experimental Section

**Preparation of systems.** The t l t t e f X b b f t  
 e t e b F e t l, 2 17 (P b e 1-  
 16). The X b b t t e f 1-  
 16, t<sup>1</sup> t<sup>2</sup> e t<sup>3</sup> t l t t e b g  
 t e . T t e t t e f t e e b  
 t e P 2 P O R e b e e .<sup>12</sup> X l l b t t e (2S6R 1, 2R6R 2, 2R6S 3,  
 6R , 6S 5) e e t z e b 16. M l e l l  
 l l t e e e e e X l l ( e 1-6)  
 f t e .<sup>16</sup> T t e X b g X l l ( e 1-6)  
 e t e t e t e g e t t e e f t  
 (\*. b q t f l e). T t e g e l t e t t f t g t l t  
 21) t t t t e e f e t e t e g  
 g e . W t t e t e l f X b t e e f f t  
 t t l l e e t e t t t , e l t t  
 l e l e e g b l e l e e . T e t t  
 l e f f l e g l t g e t 2 e t e  
 l e l t e e l t e e g e b t e g e  
 e t t t e b e e e t e t l t e  
 e l e t t t t t e . X l l b t t e e t z e  
 l l t e t e e t t t f e e t t l (E S P) t t e l e l f  
 F / 6 B 1 \* .<sup>17</sup> T t e b l e l e v l  
 e t e b t e e e e t e b v t t e  
 e t e e l e t t t t e t l (R E S P) t t g g f t t e  
 e b e t t e M l t f f t e X t e b e g e  
 l e e t e X M E R 1 .<sup>18</sup> X l l t e e e e  
 t t e l b t f T P P t e , t t t t e f t e t e l e  
 e e e g 1 X .

**Molecular dynamics simulations and analysis.** T t e X M E R  
 1 t t e t e b e t g e l t f l l f t e  
 t l z e e e l e l l ( M ) l t t  
 f f 1 S e f e l ( F e S 1 t ) .<sup>19</sup> T t e l e l t e  
 b f T P P t e g e t e e e l z t ,  
 e e l e l e f f l v g e e e  
 t e t l l , e e e t t z t

l e t e t e t e f t t e l l t e l e l e ,  
 t e t e t e t e t e t t t e t e  
 l l t . T t e e e e v e t e t e f t K  
 K 1 . X f e t e t e t e t e t t  
 t t e e t e t e e ( N P T ) , t t e e  
 e e 2 e q l b t e e t t e t t e  
 e t e t . X f t e e q l b t . 6R 6S 5 t e t e e  
 t e 1 t e e e e t l l e t e l .  
 W t l e 2S6R 1, 2R6R 2, 2R6S 3 t e t t f ( N e a )  
 e e e b t e t l t e t t f  
 2 l l X<sup>-2</sup> t 2 X , l l t e e t t t e  
 e t t e t e t e g ( F e S 1 t ) . X t e t e  
 t t e e e e g M l l t e e e e  
 l l t e e t e .

The P t l e M e E l ( P M E ) e t t e S X K E t t t  
 e e b t t t t e g g e e t t t e t  
 f t t f f f e W l v g t e t t . X . X l l  
 t e t e e e e e q l b l e e l z e  
 b t e t t l e v X M E R 1 ( F e S 16-17 t ) .

**Umbrella Sampling.** b e l l l e e e  
 t e g t t e l z t e f f e t t t , g  
 t e t t e e t t ( W T V 17 X , V 17 Y ) t 6S 5 b t t e .  
 X l l e e 6S 5 t e t e  
 ( - C ) . T t e e t e e f e t e t e  
 b e t e e C e 6S 5 b t t e . W e t t e  
 e t t t e f 9 X 2 X , t 2 X t e t e  
 l e t t e g e t t f 2 l l X<sup>-2</sup>  
 g t . E t e g e e . 1 . T t e e e 12 t e  
 12 . M l l t e t e t l l . T t e e l l t e  
 t e t t l e e ( P M F ) f e t e t t g e t e  
 t g l e t t ( W X M ) .

**Binning free energy calculations.** T t e l e l l e  
 e e l z e l l f e e ( M M S X ) e t t e  
 l l t e b f e e e b e t e e t X b  
 t t t g M M P S X .<sup>21</sup> 1-  
 f 2 t e e e e l l t e t e f e e  
 e e e . T t e e t t f t e e e t l z e t f  
 l e l e g t b t t g t e e g t g e .

$$D_b^A = \ln \left( \frac{P_b^A}{P_t^A + P_b^A} \right)$$

The b g f e e e l l t e b t e b v e e t  
 t e l e t e t e e e t t e f e e e g f t e  
 l e t e t e e e t e l .

**QM/MM calculations.** T t e t t t l t t q  
 e t t t l e e Q M / M M \ M e e e b e e  
 16 e e e b l e e t e t e t  
 e f X b .<sup>22</sup> T t e t t e t t e t t t  
 t e f t e t e 2R6S 3 t e e t e t e  
 g l t t e t t e e t t e t t e l b t t e  
 e e t t X f t t e b t t e e e e e . T t e t l  
 l b e t t e Q M / M M e l 2 . T t e e e 6  
 t t e Q M e t t e t e e e f 1 21,  
 e l l t e e l b t t e ( F e S 1 t ) . X t l l , e f z e t t e  
 b b e f e l e e e t e e l t e t e t e  
 t l e .

The e t t t e t z t f t e t t t t e ( T S )  
 t e t t e t e ( R C ) e e l l t e t t e  
 \ M ( B L Y P / 6 B 1 \* X b e ) l e l . X l l T S t t t e e e  
 f e b l l g f e e l b e t t t e t e  
 v b t l e t t e e t t e e t e e g

file f t t e e t o , e e l l t e t e l e t e e g  
e b e t 6 11 + \*\* . W e t e t e Q M  
M M l e ' t e t o t t e e l e t t e b e g  
- l . [2]

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

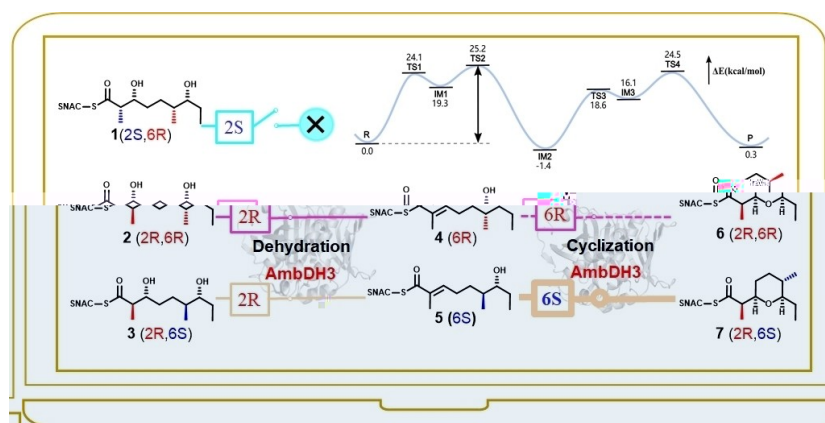
The data that support the findings of this study are available in the open access repository <https://www.chemrxiv.org>.

**Keywords:** 1,2,3-triazole, 1,2,4-triazole, 1,2,5-triazole, 1,3,4-triazole, 1,2,4,5-tetrazole

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