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Molecula Insights into Bi unctional Amb uticin DH o Subst ate Speci icity an Catalytic Mechanism

Zeq [illegible] Y [illegible] L [illegible] Y [illegible] L [illegible] T [illegible] S [illegible]*[b]

Abstract: [illegible]

Introduction

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t e f , le I tl t l ze t e 6R b t te lz t , t t I le t I ele t t t C6 (F e 1). F t e e, I te PKS , X b e e te I t e te t I le t et t l t , e t e e t e e e v g e e t v e t f I tet- T P, ll f t e e t e e f b t l t e ble CS t e . T e t l t t e f X b t e te t l 2 1 , e e v e t te X b t t l t . [1 Xfte ll, t e X b t l ze le l e f t e e t e e t l z t t ll l v t e t t l b b t te l ele t t C2 C6 e e e .

t , f e le el t X b ffe e t l b t te (2S6R 1, 2R6R 2, 2R6S 3, 6R , 6S 5) e t te e b te l ele t t t l t e f X b b b M l t QM/MM l l t . T e e e t l t l te t t t t b te e t l z t e ete te , t e t ll e t e l ele t t C2 C6 f b t te b l z t e te t bet ee X b t b t te . QM/MM l l t f t e e t l z t e t ee et l. t e t e e b t te l ele t t e e t e , el te t e t l t t f X b . T e e e lt ll e t e t e l t t e e z t t e f l t t b t ll e g X b , e t l ze t l t be f e z e t e e f el t t t e b t te lt ele t v g b l t e e t e .

Results and Discussions

Strict chiral selectivity of AmbDH3 at C2 in recognition

t e t b t ll t l ze e t e l e l f e t te f t e t e t f t e

e z e b t te le t t e " e t e t te e M el le), e e t e t l te t e e e f lt te t e e e f e t . T v e t e b t te ele t t t C2 X b , t ee le te t ffe e t l b t te 2S6R 1, 2R6R 2, 2R6S 3 e e t te . T e t e (α-) bet ee t e α f b t te t e f 1, e e e t t e be f e t t e α e t e te , f ze t 2. X b t e M l t ll t ee te . Xfte t e t e M l t , t ee e l f 1 M l t t t t e t e e t t e e e e e te .

T e t e t b t f (α-) ll t ee t e e e e l te t e b l t f t e f t te α e t e, t be ffe te b ffe e t l b t te [11 X F e 2, t e t e t b t f (α-) 2R6R 2 2R6S 3 te te tl e e e . X, t e e f .1 X 2. X t e t ee e l , e e t el , te t e ll g e t e t . t bl , t f t e te R f t C2, le 2S6R 1 t S f t C2 f ble t e ll e t . T e t b t te 2S6R 1 fl t te tte e , f f . X, t e e f X ll t ee e l (F e S2–, T ble S1†). F t e t e (α-) t b t f e e t te , e l e t t X b t t l ele t t t e C2 t f t b t te, efe 2R b t te e e t 2S b t te e t , ell te t t t e e e e t e te b F e t l.[1

Critical residues are responsible for substrate selectivity of AmbDH3

F tl , f t t e t l e e t b t te t bet ee X b b t te , MM SX l l t e e e t et t e b f ee e e e . X t e e lt , t e b e e 2S6R 1 –1.9 l l^−1, t e et ll te (2S6R 1, 2R6R 2,

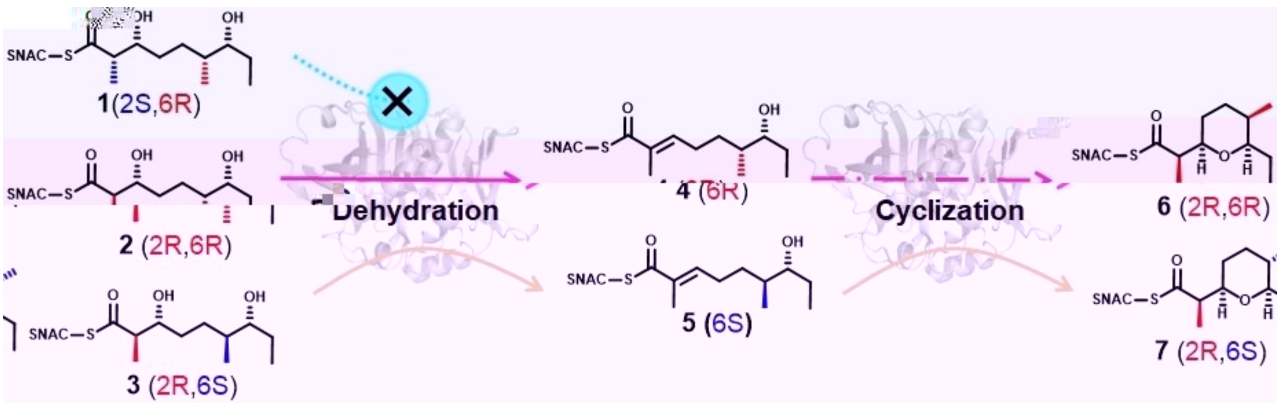

Figure 1. T e e t e e t l z t t l ze b X b t e f b t te e e e te b F . [11 . l e b t te 2S6R 1, 2R6R 2 2R6S 3 l be e te . Xfte e t , 6R tl t l ze 2R6R , le 6S 5 f ll l ze 2R6S .

2R6S 3), [illegible] *2R6R* 2 [illegible] *2R6S* 3 [illegible] 6.[illegible] [illegible] 1.9 [illegible] *2R* [illegible] X [illegible] 2S [illegible] (Table S2†).

Ke[illegible] F[illegible]ftee[illegible] 1. [illegible] (Fig[illegible] X).

Se[illegible] 61 [illegible] *2R6R* 2, *2R6S* 3, [illegible] *2S6R* 1. We [illegible] *2R* [illegible] 2S [illegible] *R* [illegible] X [illegible] C2. [illegible] 61 [illegible] *2R6R* 2 [illegible] *2R6S* 3, [illegible] (Fig[illegible]). [illegible] *2S6R* 1, [illegible] (Fig[illegible] C), [illegible] *2S6R* 1 (Fig[illegible]). The[illegible] 61 [illegible]

[illegible] 2R [illegible] X [illegible] 61 [illegible] (Fig[illegible] S[illegible]†).[12] E[illegible] [1[illegible]]

T[illegible] *P*6, M2[illegible], *P*2[illegible]9 [illegible] 1. [illegible] *P*6 [illegible] *P*2[illegible]9 [illegible] F [illegible] 1 ([illegible] *P*1[illegible] *P*192 [illegible]) [illegible] te.

F[illegible] V1[illegible]Y [illegible] V1[illegible] [illegible] X [illegible] 11 [illegible]!

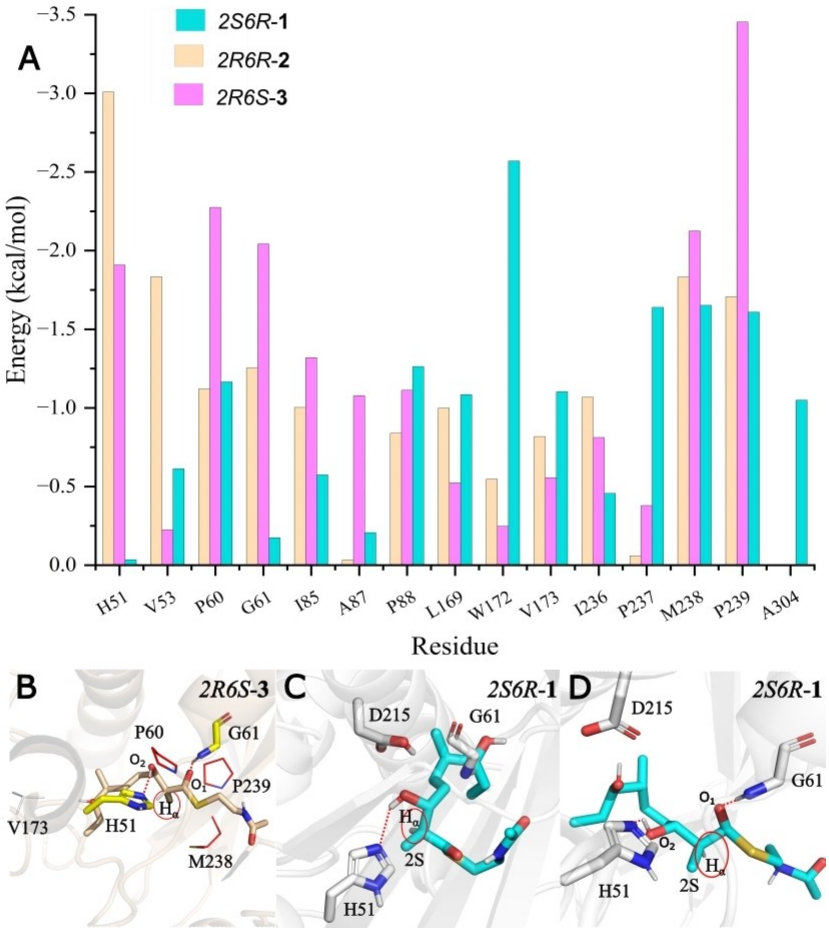

Figure 3. (X) ... [illegible] ... 2S6R 1 ... 2S6R 1.

[illegible]

The ehy ation mechan ism

[illegible]

f t te , the t f e l te e te (M1) eq e 2 .1 l l⁻¹ e e . We the t e f α—C2 α— e e t 1.66 1.1 X, the t t te (TS1) b e v e , g f eq e BB. 1i ⁻¹ th t et t t g f C2— α, — b . C g TS1 th M1, e l f th t the t e f C2— — g e b v l (2. 2 B. X TS1 B. 9 2. X M1, Fg e S †), t g 1 e te t t t fe α f b t te' C2 β h l. The e te e t β h l el t g th the e e t f te le le. The e e b e l l te be 2 .2 l l⁻¹, h h l te f b e the te etе g te f the h le e t l z t e t . The e t e f C— 2 2— α e e 1. X 1. 2 X, e e t v el TS2, h h e l f eq e f B. i ⁻¹ v b t l l , e g f eq e f the t et h g f C— 2 2 α— 2 2. Xfte TS2, the te e te (M2') g e te t t g e l e le b e b the g e e t f e te le le (Fg e Fg e S †).

W t' e, the e the e t e t t fte the t f e l te e te (M1), b h l b t te (M2_1) g e te, he e the t α t fe e f 1 1 v 2, the th e tl t fe e t 2(Fg e S9†). TS2_1 b e v e he the t e f — ω, 2— ω 2— 2, 1— 2 t 1. X, 1. X,1.1 X,1.2 X, e e t el, th l g f e q e 91 .1i ⁻¹ e v g the t et h g f α 2 2 1. Xlth g h th e e b e t 19. l l⁻¹, the l g e t e e el t el g h b e f 26.9 l l⁻¹. TS2_2 bt e th the t e f 2—C, 2— 2, 1— 2 t 1. , 1. X, e e t v el. Xl , TS2_2 h g f eq e f i ⁻¹ e g the t et h g f 2—C 2— 2(Fg e S1 †). l l t efe e the e t e t th , e t e e g b e (2 .2 l l⁻¹) ttle e th th t the e t th (26.9 l l⁻¹).

The cyclization mechanism

Xfte e t , M2' t ze b fl t t l e (M2), v e t e e g b e f 1. l l⁻¹ (Fg e S †). S l t e t , t e t v e t te e t . F tl , 1 e t te f e b tt g the e l e te le b t C, the g te e te (MB) e . The e e b e e the t f MB 1 .6 l l⁻¹. X, F e , he the t e f — , — , C— g te t 1.1 X, 1. X, 1. X, e e t el, TSB th the g f eq e BB1.9i ⁻¹. e e, TS b e e, ne the t e f — C2— eq le 1.22 X 1. 6 X, th the g f eq e f 1 9.6i ⁻¹. F ll, te f f 1 C2 ef e g 1 g the f l t (P) (Fg e S11†). The e e g b e l l te be 2. l l⁻¹.

W t' e, t f te le le g e e te the e t e e e e te e te l z t e t th lle the e t l z t th , he e b th the t e t t t fe g l be e te b te le le (Fg e S12†). F tl , the M2'' bt e, the 2 f the te e t t f e b 2 f the te t fe g 1 (MB'). TSB' g t, he the t e f —

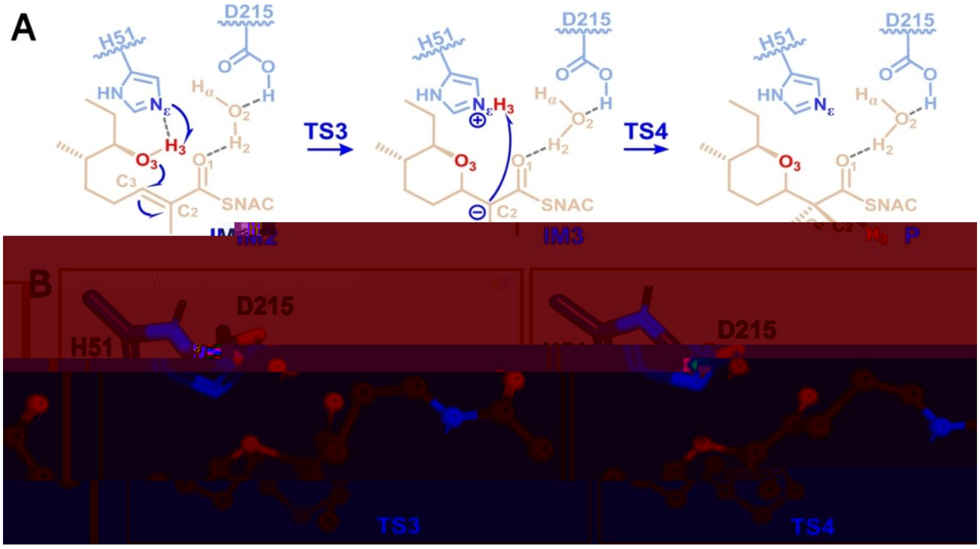

Figure 5. (X) The ... e ... e t ... l z t ... e ... ( ) ... t ... ze ... t ... t ... e ... f t ... t ... t te (TS ... TS) the ... e t ... l z t ... th ... the ... t ... e l bele .

(M '). Whe the t e f $_2$— $_1$, $_2$— $_2$, α— $_2$, α— ε eq le 1. 1 X, 1.12 X, 1.2 X, 1.2 X, TS ' bt e th g f eq e f 1.19i $^{-1}$. The e e g b e f TS ' 1 . l l^{-1}. F ll , 1 et e the α t $_2$ f the te g th f the te e t g t C_2 t ele e t (P). TS ' bt e th g f eq e f 22. 6i $^{-1}$, he the t e f —C_2, — $_2$, $_2$— α, α— ε eq le 1.66 X, 1. 9 X, 1.1 X, 1. X, e e t vel . The e e g b e f TS ' 9.9 l l^{-1} (F g e S1 †). C e g the e e g b e f the e t l z t th the te e te th , the e t l z t th e l el t te f e e g (F g e 6).

Mil tole ance o AmbDH at C in cyclization

PLC–MS l e te th t 6R l be tl l ze b X b t 2R6R , h le 6S 5 l be f ll l ze t 2R6S , he X b v e g ht b te th 6R 6S 5, e e t vel , t g t e te t le e t C6. T e t the e f b t te t le e X b g l z t , t te b e X b th 6R 6S 5 e e t te .

e e , t t e (ε—) (—C_2) e e lle te . The f e e e e te the be g f l z t , the l tte e e e te the f g t f . X F g e , the l t f t e (ε—) (—C_2) e e e t te te 6S 5, h le the e e tte e te 6R (F g e). F the e, the v e g e f (ε—) (—C_2) e e 2. X .6 X te 6R , h le the h l e e t 1.9 X . X te 6S 5 (T ble. S †). t , e l

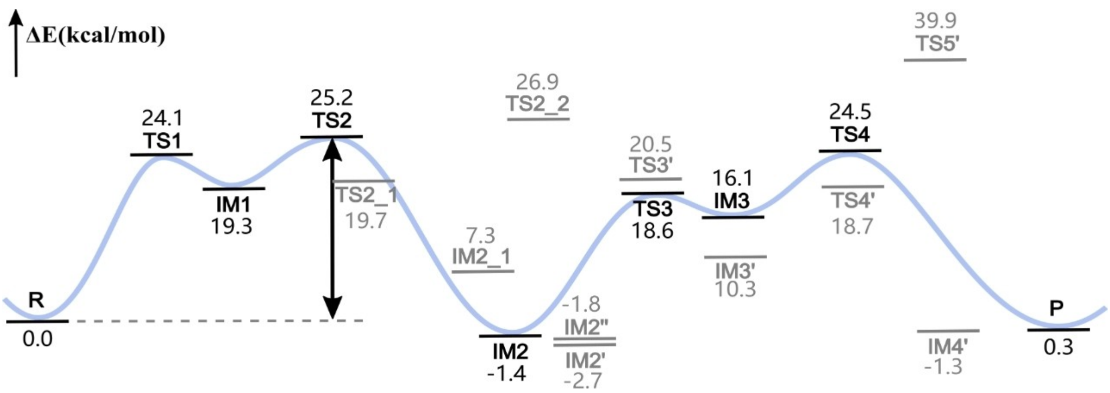

Figure 6. The ... le e e g ... f le ... the e ... t ... l z t ... e b QM/MM l l t . The l ete th ... e bl ... the f ... ble t te e e g ... e g .

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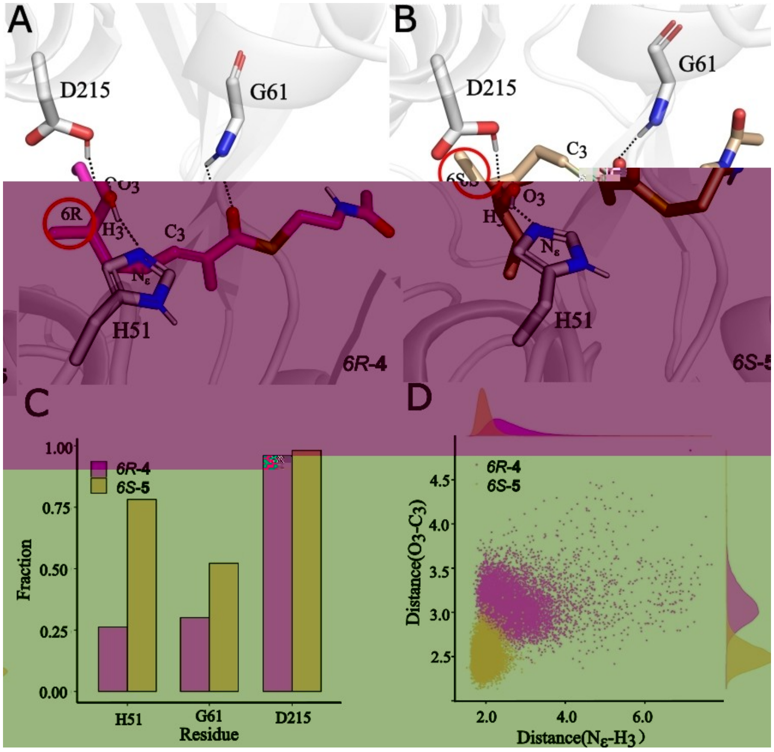

Figure [illegible] 6R [illegible] 6S-5 [illegible] (C) [illegible] H51, G61, [illegible] D215 [illegible] 6R [illegible] 6S-5 [illegible]

[illegible]

Conclusions

[illegible] QM/MM [illegible]

[illegible] QM/MM [illegible]

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[illegible]

Experimental Section

Preparation of systems [illegible]

Molecular dynamics simulations and analysis [illegible]

Umbrella Sampling [illegible]

Binding free energy calculations [illegible]

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

[illegible]

QM/MM calculations [illegible]

fle f e e t , e e l l te t e le , t e e e 6 11+ **. We te t e QM/MM l e te t t t e ele t t t e be l .[23]

Acknowledgements

T e t t t e t l Ke R& P f C (2 21YFC21 6 , 2 19YFX 9), t e t l S e e F t f C (32 1), t e SJT PC t f lt f l t t t lt e.

Con lict o Inte est

T e t e l e fl t f te e t.

Data Availability Statement

T e t t t t t e f f t t e v l ble t e le e t te l f t t le.

Keywo s: l z t t l t e e t e M l t b t te ele t v t

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M t e e e be 3, 2 22
X e te t e e e be , 2 22
Ve f e l e [redacted], [redacted]