Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary information: Exploring the Catalytic Mechanism of Dihydropteroate Synthase: Elucidating the Differences between Substrate and Inhibitor.

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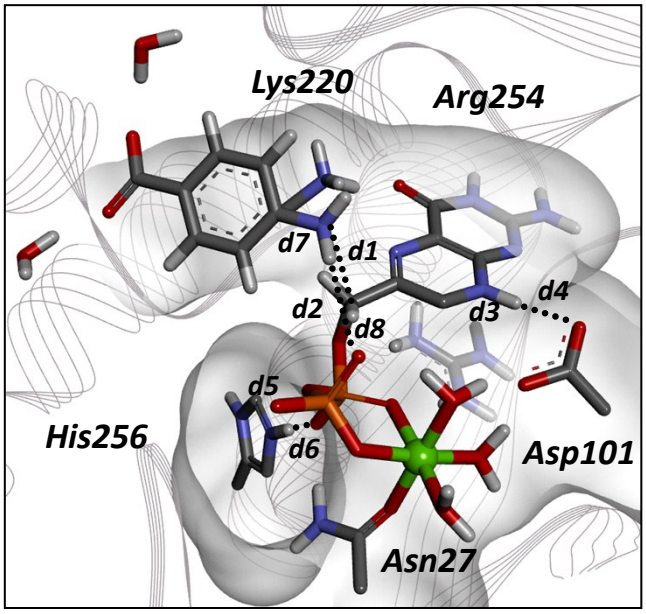

Figure S1 The key distances associated with the DHPS-PABA reaction mechanism Lys220(+1) where PPi acts as the general base.

Table S1 Key distances (Å) obtained from the Lys220(+1) based QM/MM model.

	pABA(N:)-DHP(C)	*PPi(O:)-pABA(NH)*	*pABA(N:)-Asp101(OH)*	*pABA(NH)-Asp101(O:)*	*His256(N:)-PPi(OH)*	*His256(NH)-PPi(O:)*	*pABA(N:)-PPi(OH)*	*pABA(NH)-PPi(O:)*
*p*ABA	d1	d2	d3	d4	d5	d6	d7	d8
REACT	3.06	1.45	1.03	2.06	1.08	1.46	1.01	2.81
TS1-C	2.81	2.17	1.05	1.82	1.55	1.04	1.02	2.32
TS1-N	2.92	1.98	1.80	1.02	1.50	1.06	1.02	2.26
INT1-C	2.80	2.77	1.09	1.61	1.58	1.03	1.02	2.18
INT1-N	2.84	2.83	1.86	1.00	1.57	1.03	1.02	2.17
TS2-C	2.05	2.78	1.05	1.88	1.56	1.03	1.04	1.93
TS2-N	1.91	2.86	1.85	1.01	1.54	1.04	1.04	1.90
PROD	1.47	2.99	1.03	2.16	1.62	1.03	3.40	0.98
STZ	d1	d2	d3	d4	d5	d6	d7	d8
REACT	3.01	1.44	1.04	2.00	1.07	1.47	1.04	1.86
TS1-C	-	-	-	-	-	-	-	-
TS1-N	2.94	1.94	1.85	1.01	1.39	1.11	1.04	1.83
INT1-C	-	-	-	-	-	-	-	-
INT1-N	2.82	2.82	1.91	1.00	1.55	1.04	1.04	1.81
TS2-C	-	-	-	-	-	-	-	-
TS2-N	2.16	2.78	1.06	1.81	1.52	1.05	2.48	0.98
PROD	1.48	3.00	1.04	2.04	1.61	1.03	3.68	0.97

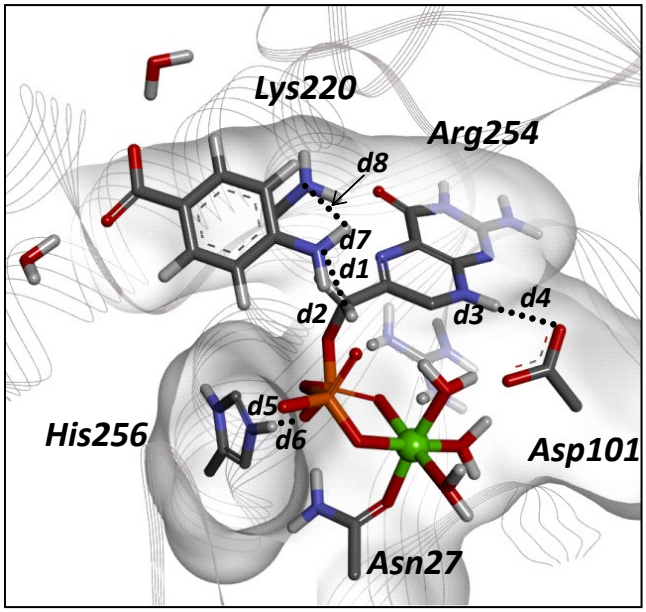

Figure S2 The key distances associated with the DHPS-PABA reaction mechanism Lys220(0) where Lys220 acts as the general base.

Table S2 Key distances (Å) obtained from the Lys220(0) based QM/MM model.

	pABA(N:)-DHP(C)	*PPi(O:)-pABA(NH)*	*PABA(N:)-Asp101(OH)*	*PABA(NH)-Asp101(O:)*	*His256(N:)-PPi(OH)*	*His256(NH)-PPi(O:)*	*PABA(N:)-Lys220(NH)*	*PABA(NH)-Lys220(N:)*
pABA	d1	d2	d3	d4	d5	d6	d7	d8
REACT	3.06	1.46	1.03	2.20	1.08	1.45	1.01	3.86
TS1-C	2.85	2.12	1.04	1.99	1.57	1.03	1.01	3.98
TS1-N	2.96	1.94	1.49	1.11	1.55	1.04	1.01	3.88
INT1-C	2.85	2.81	1.06	1.76	1.57	1.03	1.01	3.95
INT1-N	2.90	2.89	1.72	1.03	1.57	1.03	1.01	3.85
TS2-C	2.20	2.72	1.04	1.96	1.66	1.01	1.02	2.84
TS2-N	2.02	2.84	1.70	1.05	1.67	1.01	1.03	2.59
PROD	1.46	3.08	1.03	2.22	1.67	1.01	2.21	1.03

STZ	d1	d2	d3	d4	d5	d6	d7	d8
REACT	3.06	1.45	1.03	2.17	1.07	1.49	1.01	3.56
TS1-C	2.88	2.07	1.05	1.88	1.53	1.04	1.01	3.91
TS1-N	2.93	1.89	1.63	1.05	1.27	1.06	1.01	3.80
INT1-C	2.82	2.83	1.07	1.68	1.57	1.03	1.01	3.90
INT1-N	2.82	2.86	1.76	1.02	1.57	1.03	1.01	3.87
TS2-C	1.96	2.79	1.04	1.93	1.64	1.02	1.02	2.83
TS2-N	1.82	2.89	1.72	1.04	1.63	1.02	1.02	2.83
PROD	1.47	3.03	1.04	2.03	1.66	1.01	2.52	1.03

Table S3 Mulliken charge distribution for Lys220(+1) stationary points for *p*ABA and STZ.

***p*ABA Carbocation INT**	**Asn 27**	**Thr 67**	**Asp 101**	**Lys 220**	**Arg 254**	**His 256**	**Mg^{2+} & H_2O**	**DHP Frag.**	**PPi Frag.**	***p*ABA Frag.**
REACT	0.20	-0.01	-0.68	0.82	0.93	0.88	1.06	0.33	-2.68	-0.86
TS1-C	0.21	-0.02	-0.66	0.86	0.92	0.15	1.06	0.66	-2.31	-0.86
TS1-N	0.22	-0.01	0.02	0.84	0.92	0.16	1.09	-0.10	-2.26	-0.87
INT1-C	0.21	-0.02	-0.64	0.87	0.91	0.14	1.05	0.77	-2.41	-0.87
INT1-N	0.21	-0.02	0.03	0.85	0.91	0.14	1.07	0.09	-2.41	-0.88
TS2-C	0.20	0.00	-0.67	0.84	0.92	0.14	1.05	0.61	-2.43	-0.66
TS2-N	0.21	0.00	0.02	0.82	0.92	0.15	1.09	-0.15	-2.44	-0.61
PROD	0.22	0.05	-0.68	0.80	0.90	0.14	1.10	0.32	-1.79	-1.07

STZ Carbocation INT	**Asn 27**	**Thr 67**	**Asp 101**	**Lys 220**	**Arg 254**	**His 256**	**Mg^{2+} & H_2O**	**DHP Frag.**	**PPi Frag.**	**STZ Frag.**
REACT	0.20	0.01	-0.66	0.81	0.94	0.89	1.22	0.33	-2.64	-0.10
TS1-C	0.22	0.01	0.03	0.83	0.92	0.20	1.24	-0.13	-2.23	-0.10
TS1-N	-	-	-	-	-	-	-	-	-	-
INT1-C	0.22	0.01	0.05	0.85	0.92	0.15	1.23	0.10	-2.40	-0.12
INT1-N	-	-	-	-	-	-	-	-	-	-
TS2-C	0.23	0.03	0.04	0.83	0.93	0.17	1.27	-0.09	-2.24	-0.18
TS2-N	-	-	-	-	-	-	-	-	-	-
PROD	0.22	0.06	-0.66	0.81	0.91	0.15	1.24	0.40	-2.08	-0.05

Table S4 Mulliken charge distribution for Lys220(0) stationary points for *p*ABA and STZ.

***p*ABA Carbocation INT**	**Asn 27**	**Thr 67**	**Asp 101**	**Lys 220**	**Arg 254**	**His 256**	**Mg^{2+} & H2O**	**DHP Frag.**	**PPi Frag.**	**pABA Frag.**
REACT	0.19	-0.02	-0.70	-0.02	0.92	0.88	1.00	0.29	-2.67	-0.88
TS1-C	0.21	-0.02	-0.69	-0.01	0.91	0.13	0.99	0.63	-2.28	-0.88
TS1-N	0.22	-0.02	-0.06	-0.02	0.91	0.14	1.02	-0.11	-2.21	-0.88
INT1-C	0.20	-0.02	-0.68	-0.01	0.90	0.13	0.98	0.76	-2.38	-0.88
INT1-N	0.20	-0.02	-0.01	-0.01	0.91	0.13	1.00	0.06	-2.37	-0.88
TS2-C	0.19	0.00	-0.69	-0.01	0.89	0.12	1.02	0.67	-2.46	-0.73
TS2-N	0.20	0.00	-0.02	-0.01	0.89	0.12	1.04	-0.09	-2.46	-0.68
PROD	0.19	-0.02	-0.71	0.79	0.89	0.12	1.03	0.33	-2.50	-1.11

STZ Carbocation INT	**Asn 27**	**Thr 67**	**Asp 101**	**Lys 220**	**Arg 254**	**His 256**	**Mg^{2+} & H2O**	**DHP Frag.**	**PPi Frag.**	**STZ Frag.**
REACT	0.18	0.01	-0.68	-0.01	0.93	0.90	1.17	0.27	-2.67	-0.10
TS1-C	0.22	-0.01	-0.67	0.00	0.92	0.15	1.17	0.60	-2.25	-0.12
TS1-N	0.22	-0.01	-0.02	0.00	0.92	0.16	1.21	-0.17	-2.20	-0.11
INT1-C	0.21	-0.02	-0.66	0.00	0.92	0.13	1.16	0.77	-2.37	-0.14
INT1-N	0.21	-0.02	0.01	0.00	0.92	0.13	1.19	0.06	-2.37	-0.13
TS2-C	0.21	0.03	-0.49	0.01	0.91	0.13	1.20	0.37	-2.47	0.11
TS2-N	0.20	0.02	-0.25	0.01	0.91	0.13	1.17	0.18	-2.47	0.11
PROD	0.20	-0.02	-0.69	0.78	0.91	0.12	1.17	0.40	-2.50	-0.37

Table S5. QM/MM energies (ΔE) for the stationary points reported in this study (kcal/mol).

***p*ABA**	**Neutral INT**		**Carbocation INT**	
	Lys220(0)	**Lys220(+1)**	**Lys220(0)**	**Lys220(+1)**
REACT	0.0	0.0	0.0	0.0
TS1	28.8	31.2	31.9	23.0
INT	18.7	18.7	25.4	18.6
TS2	34.7	27.2	34.2	19.8
PROD	3.8	-24.9	3.8	-24.9

STZ	**Neutral INT**		**Carbocation INT**	
	Lys220(0)	**Lys220(+1)**	**Lys220(0)**	**Lys220(+1)**
REACT	0.0	0.0	-	0.0
TS1	24.3	29.0	-	23.5
INT	11.8	14.4	-	17.0
TS2	29.2	35.6	-	29.6
PROD	-4.9	-13.1	-	-13.1

Table S6. QM/MM energies (ΔE) obtained for models without and with Asn120 and Asp184 included directly in the QM region. The additional polarization provided by these residues have a small effect on the observed barriers for 3 stationary point assessed. For reasons of computational efficiency they were therefore neglected.

	Lys220(+1)	**Lys220(+1) + (Asn120 & Asp114)**
REACT	0.0	0.0
TS1-N	28.8	32.0
INT	18.7	21.9