SUPPLEMENTARY INFORMATION

Enzyme-Catalyzed Kinetic Resolution of Chiral Precursors to Antiviral Prodrugs

Dao Feng Xiang, Andrew N. Bigley, Emily Desormeaux,

Tamari Narindoshvili, and Frank M. Raushel*

Department of Chemistry, Texas A&M University,

College Station, Texas 77843, United States

Table S1. Initial screening results with PTE variants.

Variant[a]	Selective hydrolysis	Variant	Selective hydrolysis
BHR-1	none	BHR-43	none
BHR-2	none	BHR-44	none
BHR-3	none	BHR-46	poor
BHR-4	none	BHR-47	none
BHR-5	none	BHR-48	none
BHR-7	none	BHR-49	none
BHR-9	none	BHR-50	none
BHR-11	none	BHR-53	poor
BHR-13	none	BHR-57	none
BHR-14	none	BHR-61	none
BHR-15	none	BHR-62	none
BHR-16	poor	BHR-63	none
BHR-18	poor	BHR-64	yes (S_P)
BHR-19	none	BHR-65	none
BHR-20	none	BHR-66	poor
BHR-21	none	BHR-68	none
BHR-23	none	BHR-69	none
BHR-24	none	BHR-70	yes (S_P)
BHR-25	none	BHR-71	poor
BHR-27	none	BHR-73	none
BHR-28	poor	BHR-74	none
BHR-29	none	BHR-75	none
BHR-31	none	BHR-76	yes (S_P)
BHR-33	poor	G60A-PTE	Yes (R_P)
BHR-34	poor	GGY-PTE	none
BHR-36	none	YT-PTE	none
BHR-38	none	Wild-type	poor
BHR-40	none	In1W-PTE	yes (S_P)
BHR-41	none		

[a]Variants are from references[1-5]. Screening was done as total hydrolysis of 60 µM of compound **1** in 1 mL with 50 mM Ches (pH 9.0) and 100 µM $CoCl_2$ at 30 °C.

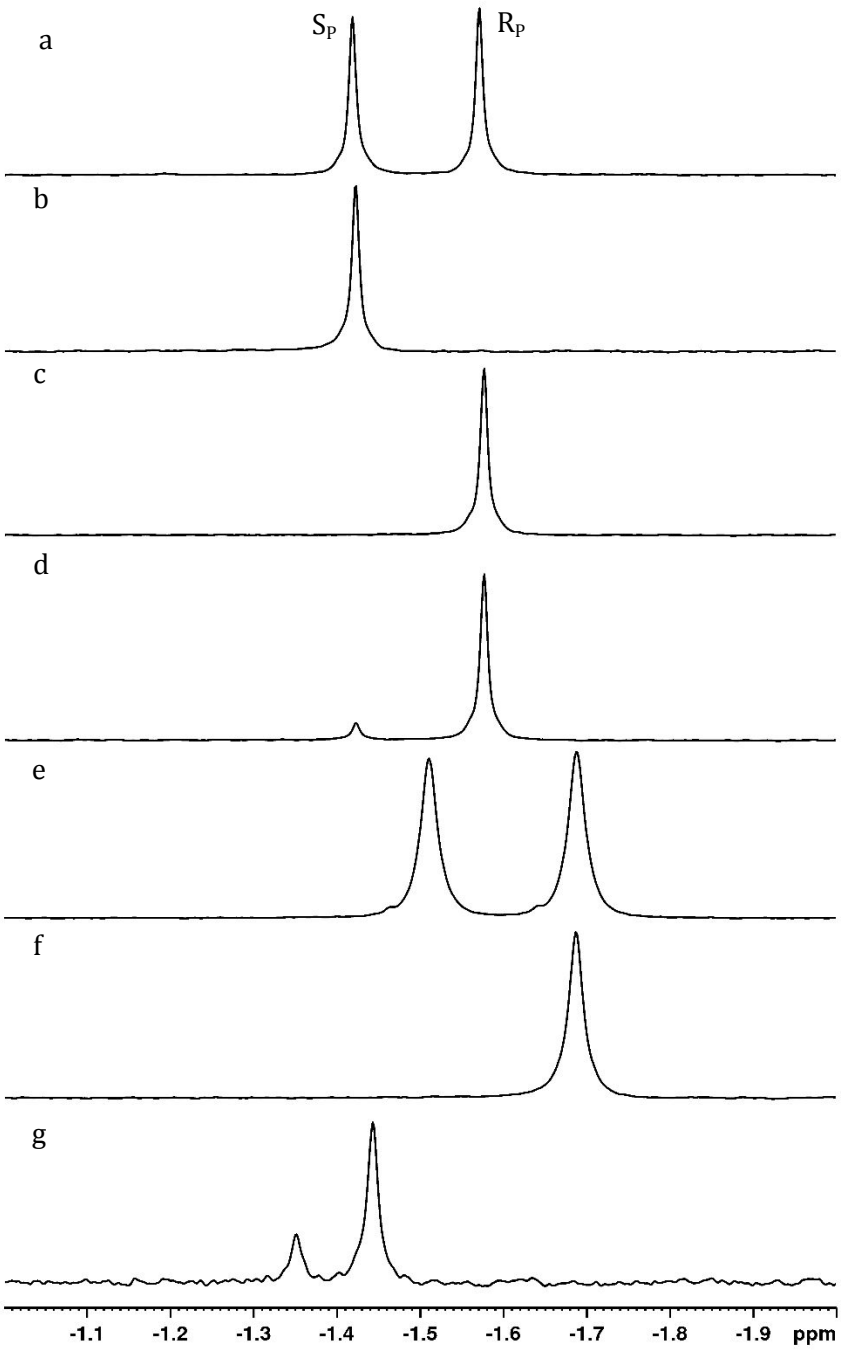

Figure S1. Chemical shifts of isomers of compound **1** in different solvents. (a) Racemate in DMSO. Resonance at -1.42 ppm is from the S_P-isomer, and the resonance at -1.58 is from the R_P-isomer. (b) S_P-isomer isolated by selective hydrolysis with G60A-PTE in DMSO. (c) R_P-isomer isolated by selective hydrolysis using In1W-PTE in DMSO. (d) Sample from **c** spiked with sample from **b**. (e) Racemate in methanol. Resonance at -1.51 ppm is due to S_P-isomer, and resonance at -1.69 ppm is due to the R_P-isomer. (f) R_P-isomer isolated by selective hydrolysis with In1W-PTE in methanol. (g) Hydrolysis of compound **1** by In1W-PTE at 40% completion in water.

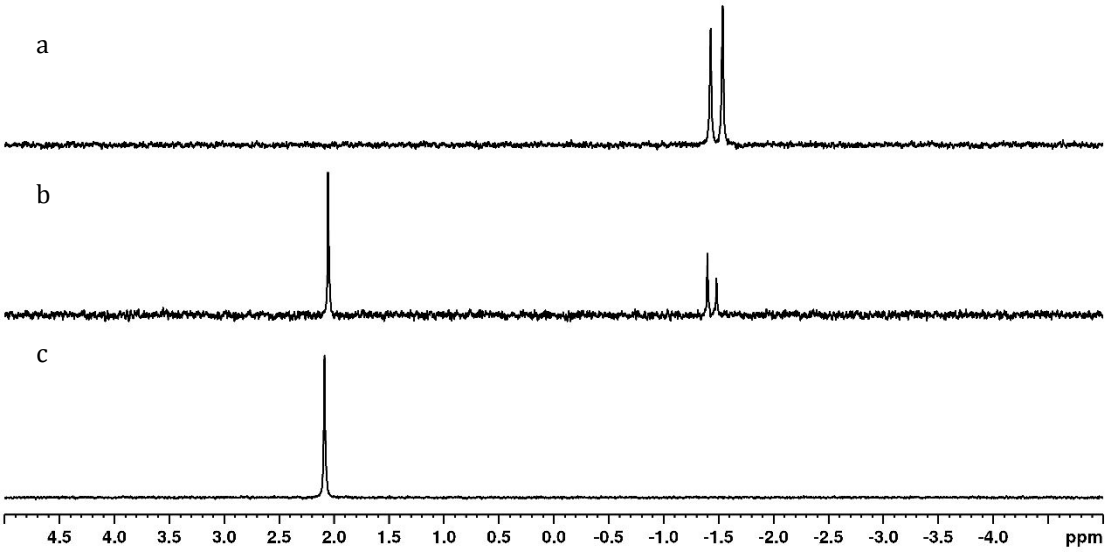

Figure S2. ^{31}P NMR spectra of compound **1** and hydrolysis product in water. (a) Two diastereomers of compound **1**. S_P-1 is at -1.43 ppm while R_P-is at -1.54 ppm. (b) Hydrolysis of compound **1** by wild-type PTE at 60% completion. The single phosphorus containing hydrolysis product is seen at 2.05 ppm. (c) Complete hydrolysis of compound **1** by wild-type PTE.

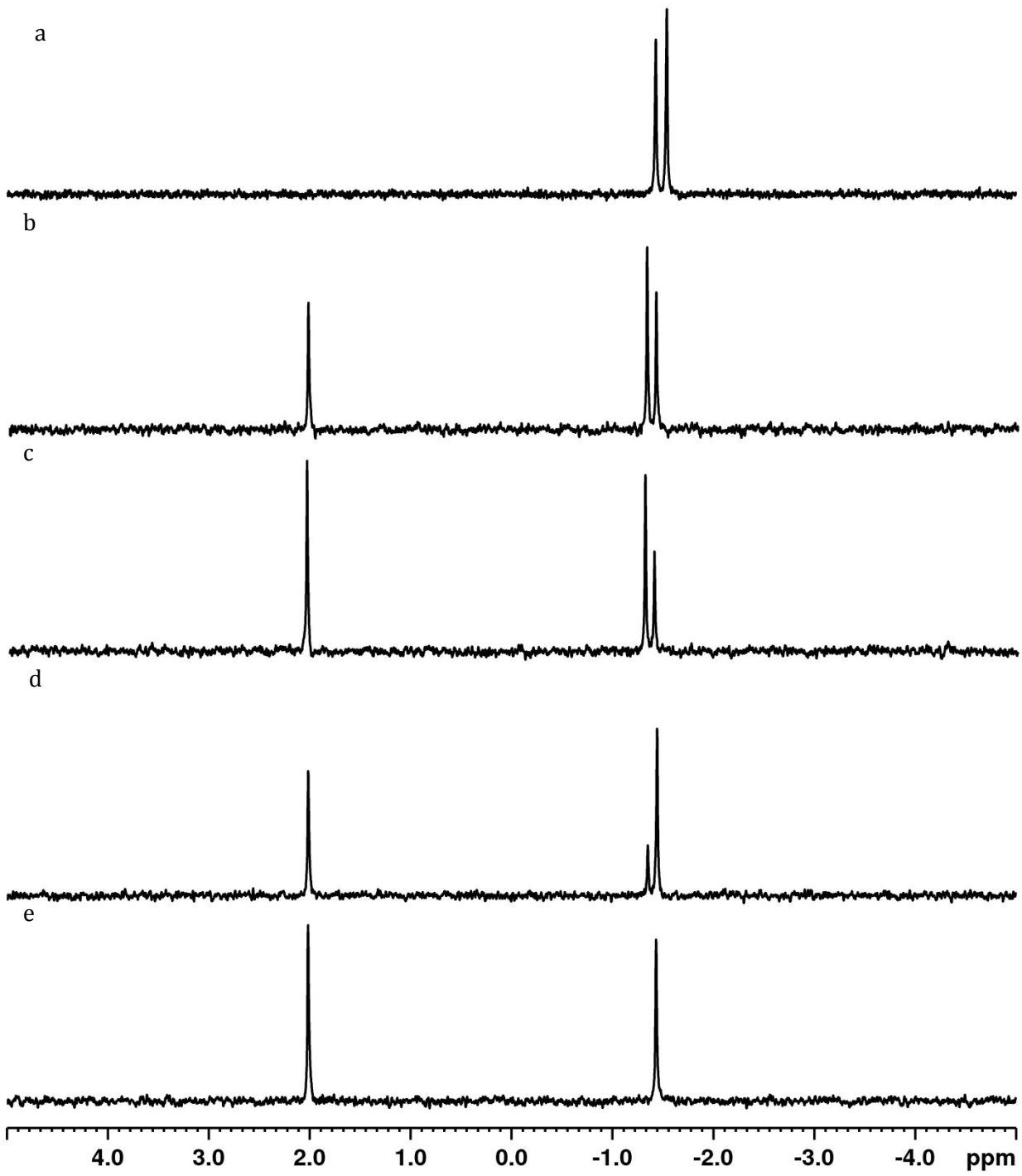

Figure S3. ^{31}P NMR spectra of the differential hydrolysis of compound **1** by variants of PTE. (a) ^{31}P NMR spectrum of 2.0 mM compound **1**. S_P-**1** is at -1.43 ppm while R_P-**1** is at -1.54 ppm. (b) Hydrolysis of 2.0 mM compound **1** by 3 μM PTE-G60A. Reaction is ~28% complete. The single phosphorus containing product is at 2.02 ppm. (c) Reaction from **b** at ~40% completion. (d) Hydrolysis of 2.0 mM compound **1** by 0.1 μM PTE-In1W. Reaction is ~38% complete. (e) Reaction from **d** at 50% completion.

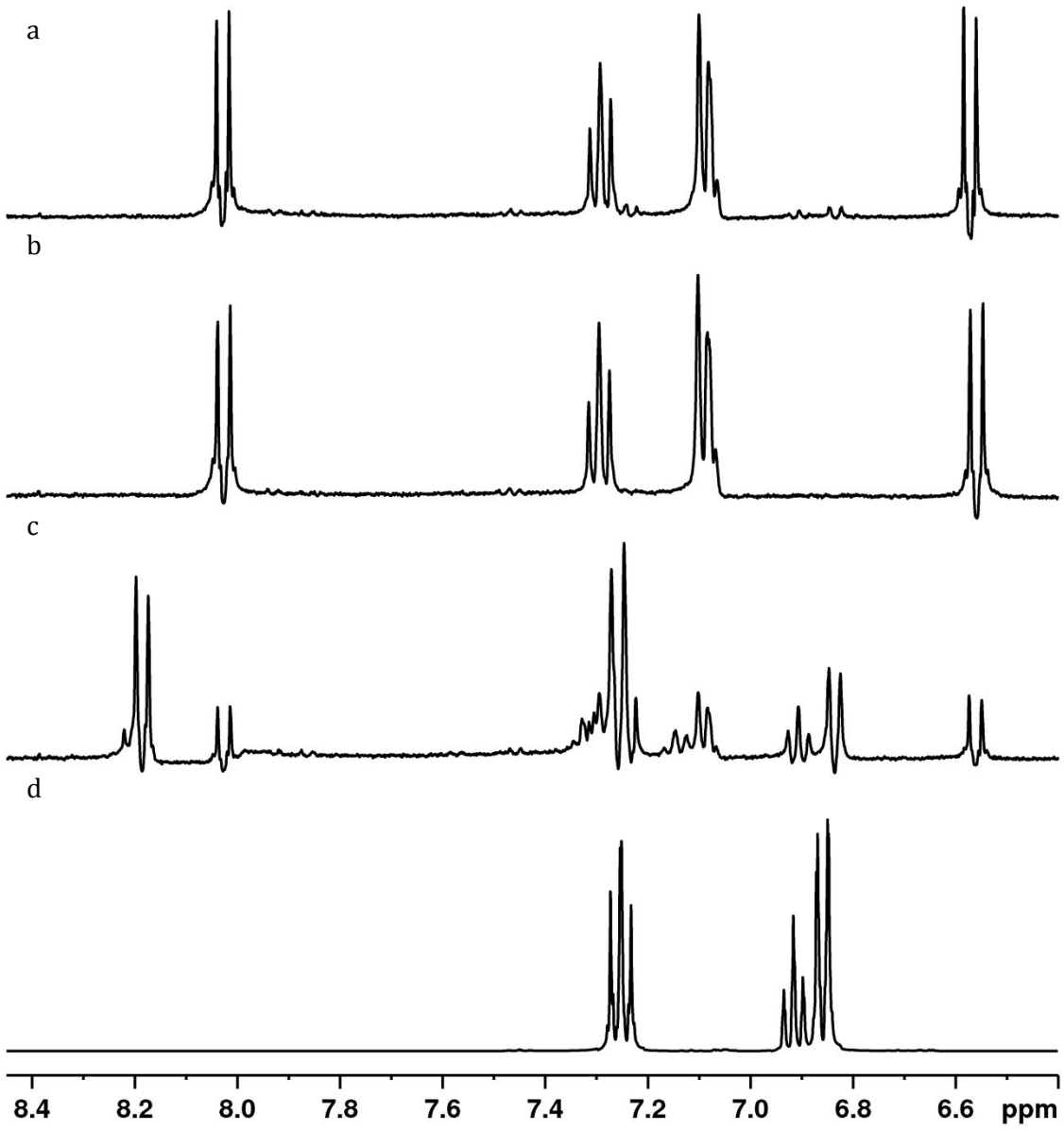

Figure S4. ^{1}H NMR spectra of the hydrolysis products of compound **1**. (a) Hydrolysis products formed by hydrolysis of the isolated R_P-isomer by Sb-PTE. (b) Hydrolysis products formed by hydrolysis of the isolated R_P-isomer by WT-PTE. The doublets at 8.03 ppm and 6.56 ppm are due to free *p*-nitrophenol, while the triplet at 7.30 ppm and doublet at 7.09 ppm are due to phenol on the phosphodiester product. (c) Hydrolysis product of the isolated S_P-isomer by *Sb*-PTE. The doublets at 8.19 ppm and 7.26 ppm are due to *p*-nitrophenol on the diester product, while the triplet at 6.91 ppm and the double at 6.83 ppm are due to free phenol. (d) Spectra of pure phenol in buffered solution.

REFERENCES

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