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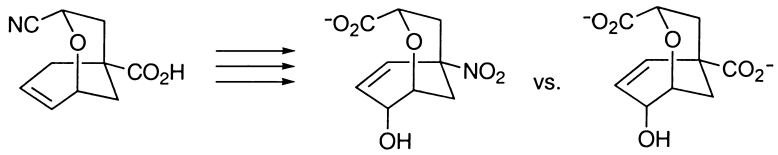

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Charge Optimization Increases the Potency and Selectivity of a Chorismate Mutase Inhibitor

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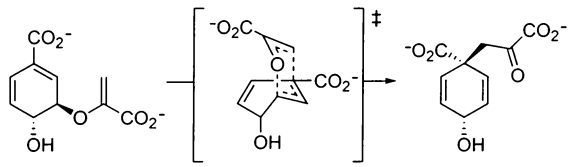
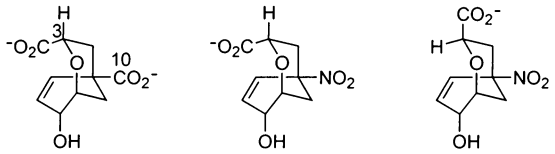
The chorismate mutase-catalyzed conversion of chorismate (**1**) to prephenate (**3**) is at the branchpoint in the shikimate pathway in bacteria, fungi, and higher plants that leads to tyrosine and phenylalanine.[1] Because mammals do not biosynthesize aromatic amino acids, this system represents an attractive target for the development of novel antibiotics, fungicides, and herbicides. Moreover, given the significant structural diversity seen for chorismate mutases from different organisms,[2−4] high selectivity should be attainable.



1 **2** **3**

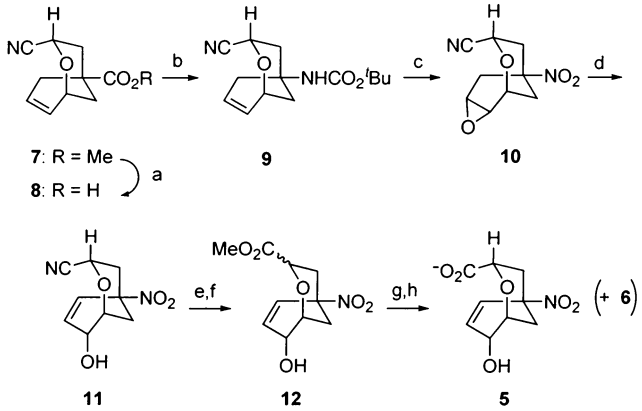


4 **5** **6**

A variety of inhibitors for chorismate mutases are already known.[5−7] The most potent of these is the endo oxabicyclic dicarboxylic acid **4**,[5] which was designed to mimic the presumed transition state (**2**) for the chorismate rearrangement. It exhibits dissociation constants in the range 0.1−3 μM for a broad spectrum of mutases.[5,8] Kangas and Tidor recently used charge optimization methods to examine the electrostatic interactions between this compound and the active site of a chorismate mutase from *Bacillus subtilis* (BsCM) computationally.[9] While most of the groups that make hydrogen bonds with the enzyme exhibit good electrostatic complementarity, the C10 carboxylate pays a substantial desolvation penalty upon binding which is apparently not compensated by strong interactions with the protein. Their calculations predict that by replacing this anionic carboxylate with an isosteric but neutral nitro group the electrostatic contribution to the binding free energy could be improved by 2−3 kcal/mol. To evaluate this prediction experimentally, we have prepared compound **5** and its exo epimer **6** and report their inhibition profiles with various chorismate mutases.

The synthetic route leading to racemic **5** is outlined in Scheme 1. Ester **7** was prepared as described by Bartlett[5] and hydrolyzed under mild conditions to afford **8**. Curtius rearrangement of **8** with diphenyl phosphoryl azide (DPPA)[10] in the presence of tBuOH gave carbamate **9**. This material was treated with trifluoroacetic acid, followed by excess dimethyldioxirane (DMDO),[11] to yield **10**.

Scheme 1 [a]



[a] Conditions: (a) NaOH, 92%; (b) DPPA, tBuOH, 73%; (c) TFA; DMDO, 87% over two steps; (d) Ph_3P, TMSBr, DBU, 66%; (e) H_2O_2, NaOH, 80%; (f) CH_2N_2, quant.; (g) separation; (h) KOH, 95%.

Epoxidation occurs exclusively on the exo face of the olefin as confirmed by X-ray crystallography (see Supporting Information). As precedented in the synthesis of **4**,[5] this epoxide furnishes the desired allylic alcohol **11** via the bromohydrin silyl ether. Saponification of nitrile **11** in 85% KOH at room temperature is accompanied by epimerization, giving the endo/exo acids (**5**/**6**) as a 2:3 mixture. Performing the reaction with 30% hydrogen peroxide in 3 N NaOH[12] minimizes the unwanted epimerization and yields a 4:1 mixture of endo/exo isomers in 80% yield. This mixture was esterified with diazomethane, and the resulting methyl esters (**12**) were separated by silica gel chromatography. Mild alkaline hydrolysis of the separated endo and exo esters cleanly afforded **5** and **6** without epimerization. Stereochemistry was assigned on the basis of the NMR coupling constants of the hydrogen at the 3-position in analogy to the corresponding oxabicyclic dicarboxylic acids:[5] endo **5**, J = 2.8, 7.5 Hz; exo **6**, J = 3.0, 12.0 Hz. This sequence provides compound **5** in eight steps from ester **7** in 29% overall yield.

Compounds **5** and **6** were tested as inhibitors of native BsCM and compared with **4** measured under the same conditions. The assays were performed in 50 mM potassium phosphate buffer at pH 7.5 and 30 °C as previously described.[5,13] The results are summarized in Table 1. Racemic **5** inhibits BsCM competitively with a K_i value of 300 nM; it thus binds to the enzyme >3 times more tightly than the original oxabicyclic dicarboxylic acid **4** and nearly 1000 times more tightly than the exo isomer **6**. Replacement of the C10 bridgehead carboxylate in **4** with a nitro group clearly improves the potency of inhibition as predicted by Kangas and Tidor.[9] In fact, compound **5** is the most potent inhibitor of BsCM known. Nevertheless, the magnitude of the improvement ($\Delta\Delta G \approx$ −0.7 kcal/mol) is modest compared with the expected gain of 2−3 kcal/mol in binding free energy.

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Table 1. Inhibition of Chorismate Mutases[a]

	K_i, μM		
inhibitor	BsCM	BsCM$^{1-116}$	EcCM
4	1.0	51	2.3
5	0.32	2.9	7.6
6	230	n.d.[b]	n.d.

[a] The enzymes were overproduced in *E. coli* as previously described.[14,18] In the absence of inhibitor, they had kinetic parameters in good agreement with literature values: BsCM, $k_{cat} = 41 \pm 2$ s^{-1}, $K_m = 74 \pm 8$ μM;[8,13] BsCM$^{1-116}$: $k_{cat} = 28 \pm 7$ s^{-1}, $K_m = 9.6 \pm 3$ mM;[14] EcCM: $k_{cat} = 64 \pm 3$ s^{-1}, $K_m = 390 \pm 51$ μM.[19,20] Inhibition assays were performed in 50 mM potassium phosphate buffer (pH 7.5 and 30 °C) as previously described.[5,13] Errors on the inhibition constants were less than 15%. [b] n.d., not determined.

The discrepancy between the experimental and theoretical binding free energies may reflect the fact that residues not seen explicitly in the X-ray structure were ignored in the calculations.[9] In the complex between BsCM and **4**, the C10 carboxylate of the inhibitor sits at the mouth of the binding pocket where it does not contact the protein directly.[2] However, the C terminus of the enzyme, which borders the binding pocket and is largely disordered, was only partially resolved crystallographically.[2] Biochemical characterization of several truncated proteins shows that the tail segment, while not essential for catalytic activity, does contribute substantially to ligand affinity.[14] It may also interact with inhibitors **4** and **5**, potentially invalidating the assumption that reducing the charge at the C10 position would not sacrifice significant ligand−protein interactions.[15] To assess how the tail segment influences inhibitor binding, we investigated a shortened BsCM variant, BsCM$^{1-116}$, from which the last eleven C-terminal residues were removed.[14] As found previously,[14] the k_{cat} and K_m values for this protein are 28 s^{-1} and 9.6 mM, respectively, which can be compared with the values 41 s^{-1} and 74 μM for native BsCM. The 130-fold increase in K_m for chorismate is paralleled by a somewhat smaller 50-fold increase in K_i for the conformationally constrained dicarboxylic acid **4** (Table 1). Deletion of the tail segment also increases the K_i value for the nitro derivative **5**, but only by a factor of 10. Apparently, the dianionic substrate and inhibitor gain more from their interactions with the C-terminal tail of wild-type BsCM than does compound **5**, a factor underestimated by the calculations. In the absence of the C terminus, **5** is a >10-fold better inhibitor than is **4**, corresponding to a gain of 1.7 kcal/mol in binding free energy, in reasonable agreement with the calculations.[9,15] Its relative potency with BsCM$^{1-116}$ is also reflected in the normalized ratio $K_i/K_m = 0.00030$, which represents the highest selectivity observed for any chorismate mutase inhibitor to date.

The structurally characterized chorismate mutase domain of the *Escherichia coli* P-protein (EcCM),[3] a member of the AroQ class of chorismate mutase, has a completely different protein fold and ligand-binding pocket than BsCM, an AroH mutase.[13,16] Notably, inhibitor **4** is solvent inaccessible when bound to EcCM, and its C10 carboxylate makes multiple contacts with active-site residues.[3] With this enzyme, replacement of the negatively charged carboxylate with the neutral nitro group leads to a >3-fold decrease in affinity (Table 1, $\Delta\Delta G = +0.7$ kcal/mol). Here, the reduced desolvation penalty for the nitro group does not fully compensate for the loss of stabilizing protein−ligand interactions. As a result, compound **5** achieves 10-fold greater selectivity than does **4** for AroH over AroQ mutases (Table 1). Its selectivity is thus complementary to that of the AroQ-specific inhibitor, (*S*)-(−)-dinitrobiphenic acid.[7]

In summary, charge optimization methods have afforded the most potent and selective inhibitor of BsCM known. Generalization of this approach to improving electrostatic interactions between binding partners may prove broadly useful for the design of therapeutic agents.[17]

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Supporting Information Available: Experimental details for the synthesis and characterization of **5** and **6** (PDF). X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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