

## Vinyl fluoride as an isoelectronic replacement for an enolate anion: Inhibition of type II dehydroquinases

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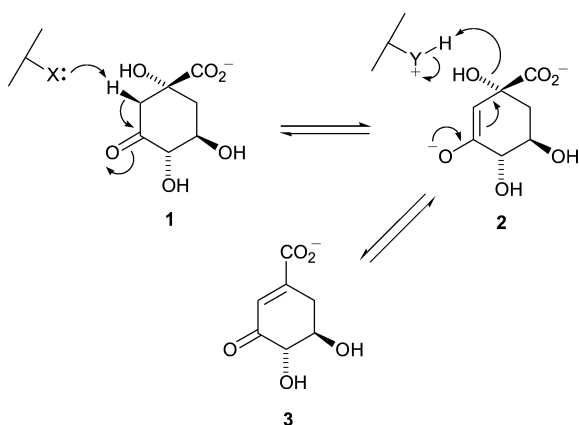
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A vinyl fluoride analogue of the intermediate in the reaction catalysed by type II dehydroquinase enzymes has been synthesized over seven steps from (–)-quinic acid and shown to be a potent enzyme inhibitor.

The shikimate<sup>1,2</sup> and quinate<sup>3</sup> pathways (the enzymatic routes from acyclic carbohydrates to essential aromatics utilized by micro-organisms, fungi and the higher plants) continue to elicit major research efforts especially since the recent discovery<sup>4</sup> of the shikimate pathway in apicomplexan parasites including the most virulent of the malarial parasites (*Plasmodium falciparum*).

Dehydroquinase [3-dehydroquinase dehydratase; E.C. 4.2.1.10] which catalyses the reversible conversion of 3-dehydroquinase (DHQ) **1** to 3-dehydroshikimate (DHS) **3** is uniquely common to both pathways. It occurs in two distinct forms each with differing chemical and biochemical properties. Type I enzymes are heat labile protein dimers<sup>5</sup> that catalyse a *syn*-elimination<sup>6</sup> of water through Schiff's base intermediates<sup>7</sup> involving a conserved lysine. Type II dehydroquinases are more thermally robust dodecameric species<sup>8</sup> that effect an *anti*-dehydration *via* an enzyme stabilized enolate **2**<sup>9</sup> (Scheme 1).



Scheme 1

The mechanistic differences of the two enzyme types led us to design inhibitors that were selective for the type II proteins which are found in a number of clinically important bacterial strains including *Mycobacterium tuberculosis*. Based upon the idea that compounds which mimicked the enolate intermediate **2** were likely to show such selectivity we prepared derivatives which, when compared to DHQ, possessed either increased  $sp^2$  character at C(2) and C(3) (alkene **4**) or had superior hydrogen bonding potential at C(3) (oxime **5**) (Fig. 1).

We were thus able to demonstrate<sup>10</sup> that **4** and **5** were potent and highly selective (up to 1000 fold) competitive reversible inhibitors of type II dehydroquinases. Recent crystallographic studies<sup>11</sup> on the complex formed between alkene **4** and the type II dehydroquinase from *Streptomyces coelicolor* (PDB accession code : 1GU1) has allowed its mode of action to be fully determined.

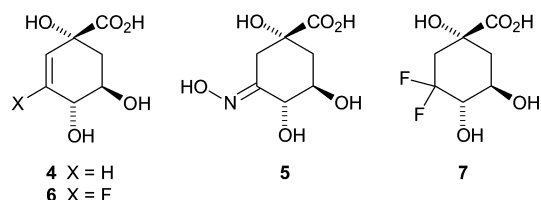
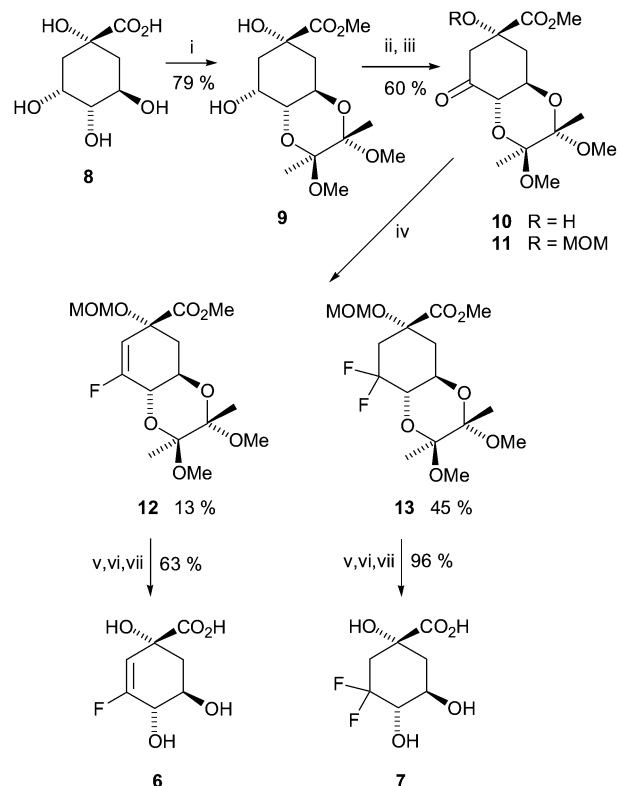


Fig. 1

Our search for even more effective inhibitors focused on derivatives in which the true geometric and electronic features of enolate **2** were more accurately mimicked, particularly the electron distribution and high electron density generated at C(3). Despite the relative rarity of the vinyl fluoride functionality we chose to replace the enolate oxyanion with its closest chemical equivalent, the isosteric and isoelectronic fluorine atom. Herein we describe the use of the vinyl fluoride moiety as an isosteric and isoelectronic replacement for an enolate anion and report the syntheses of fluorovinyl **6** and difluoro **7**<sup>12</sup> acids.

Acids **6** and **7** were prepared over seven steps from (–)-quinic acid **8** (Scheme 2). Esterification of **8** was



**Scheme 2** Reagents and conditions: i, MeC(OMe)<sub>2</sub>C(OMe)<sub>2</sub>Me, HC(OMe)<sub>3</sub>, CSA, MeOH, 65 °C, Ar, 16 h; ii, RuCl<sub>3</sub>, KIO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O, CHCl<sub>3</sub>, 20 °C, 48 h; iii, CH<sub>2</sub>(OMe)<sub>2</sub>, P<sub>2</sub>O<sub>5</sub>, CHCl<sub>3</sub>, 20 °C, 16 h; iv, DAST, DME, 80 °C, Ar, 6 h; v, TFA, H<sub>2</sub>O, 60 °C, 2 h; vi, NaOH, H<sub>2</sub>O, 20 °C, 30 min; vii, Amberlite IR-120(H), H<sub>2</sub>O, 20 °C, 10 min.

accomplished concomitantly with protection of the 4,5-*trans*-diol as the cyclic bis-ketal **9** as described previously.<sup>13</sup> Ruthenium(III) catalysed periodate oxidation to the C(3) ketone **10** followed by MOM protection of the tertiary alcohol at C(1) afforded the fully protected 3-dehydroquinone **11**.<sup>14</sup> Incorporation of fluorine at C(3) proved to be somewhat capricious. It was ultimately reproducibly achieved<sup>15</sup> upon treatment of ketone **11** in boiling anhydrous DME under argon with multiple portions of DAST to afford a mixture of two chromatographically separable products **12** and **13**. Extensive NMR analysis (<sup>1</sup>H, <sup>13</sup>C and <sup>19</sup>F in deuteriochloroform) showed the less polar compound to be the desired vinyl fluoride **12** ( $\delta_{\text{F}} -115.9$  coupled to  $\delta_{\text{C}} 159.6$ ). Determination of the structure and relative stereochemistry of the more polar, crystalline product **13** ( $\delta_{\text{F}} -113.3$  and  $-103.3$  coupled to  $\delta_{\text{C}} 119.7$ ) proved possible by X-ray crystallographic analysis<sup>16,17</sup> (Fig. 2). The product was thereby unequivocally shown to be the corresponding C(3) *gem*-difluoride.

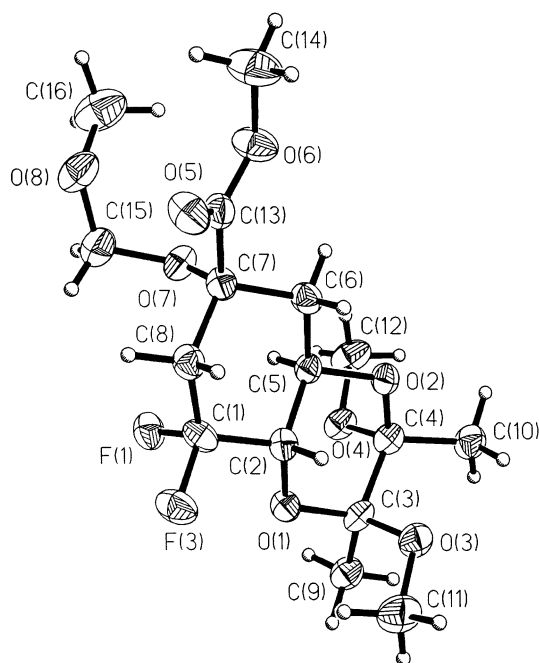


Fig. 2 Single crystal X-ray structure of **13**.

The desired product **12** was the minor of the two (**12** 13%, **13** 45%). Attempted dehydrofluorination of **13** to afford the required fluoride **12** proved to be unsuccessful under a variety of basic conditions both with and without the addition of silver salts (added to encourage fluoride precipitation). Nonetheless, three step deprotection of **12** and **13** (acid catalysed bis-ketal and MOM deprotection, saponification and ion-exchange) afforded the fluorovinyl **6** and difluoro **7** acids (63% and 96% respectively).

The fluorovinyl acid **6** was assayed against both type I and type II dehydroquinases and was shown to be highly selective for the type II proteins. Specifically **6** has a  $K_i$  of 10  $\mu\text{M}$  against the dehydroquinase from *Mycobacterium tuberculosis*, making it the most potent inhibitor reported for this enzyme. Significantly, **4** which has a hydrogen instead of a fluorine at C(3), has a  $K_i$  of 200  $\mu\text{M}$  against the same enzyme. Full biochemical

results together with details of X-ray crystallographic studies of complexes of **6** with type II enzymes will be reported elsewhere.

Fluorine has been widely utilized to alter both the chemical and biochemical properties of many compounds, chiefly as a replacement for H, OH or neutral oxygen atoms (as part of a CHF or CF<sub>2</sub> group); those pertinent to the shikimate pathway include 2-fluoro,<sup>18</sup> 6-fluoro<sup>19</sup> (both H replacements), 3-fluoro<sup>20</sup> and 3,5-difluoroshikimates<sup>21</sup> (OH groups). This is the first report of fluorine as a replacement for an oxyanion, which it resembles more closely than a hydroxy group, by being both isosteric and isoelectronic.

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- 15 For optimal results, recrystallized ketone **11** [from hexane and diethyl ether] was used with fresh samples of DAST and anhydrous DME.
- 16 Crystal data for **13**: C<sub>16</sub>H<sub>26</sub>F<sub>2</sub>O<sub>8</sub>,  $M = 384.37$ , tetragonal,  $a = b = 19.184(3)$ ,  $c = 10.165(4)$  Å,  $U = 3740.9(17)$  Å<sup>3</sup>,  $T = 180(2)$  K, space group  $P4_12_12$  (no. 92),  $Z = 8$ ,  $\mu$  (Mo-K $\alpha$ ) = 0.12 mm<sup>-1</sup>, 3845 reflections measured, 3296 unique ( $R_{\text{int}} = 0.028$ ) which were used in all calculations. The final  $wR(F^2)$  were 0.047 [ $I > 2\sigma(I)$ ] and 0.074 (all data).
- 17 Full crystallographic data including lists of bond length, bond angles, hydrogen coordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, CCDC 184932. See <http://www.rsc.org/suppdata/cc/b2/b205105m/> for crystallographic files in .cif or other electronic format.
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