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Enzyme-Catalyzed Enantioselective Hydrolysis of Dihydrouracils as a Route to Enantiomerically Pure β-Amino Acids

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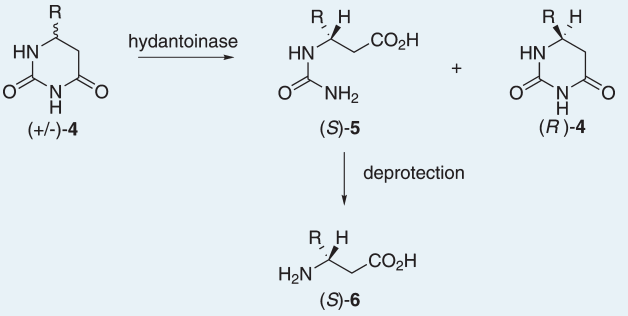
ABSTRACT: The hydantoinase from *Vigna angularis* has been shown to catalysis the hydrolysis of a range of racemic 6-substituted dihydrouracils to yield the corresponding *N*-carbamoyl-(*S*)-β-amino acids and unreacted (*R*)-dihydrouracils. High enantioselectivity (*E* >100) was achieved in cases that the C-6 substituent was an aryl group. Subsequent treatment of the *N*-carbamoyl derivatives with nitrous acid yielded the free β-amino acid.

KEYWORDS: hydantoinase, β-amino acids, enantioselective, biocatalysis, dihydrouracil, *Vigna angularis*

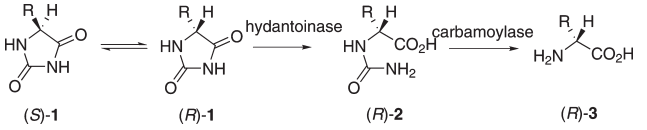


Enantiomerically pure β-amino acids are valuable building blocks for novel therapeutic agents that possess a wide range of biological activity.[1−3] Although a number of biocatalytic routes have been developed for their preparation, no single method has emerged as being universally applicable.[4] Recent approaches have been based upon the use of transaminases,[5] lipases[6,7] and aminopeptidases.[8,9] The isomerization of α- to β-amino acids using aminomutases offers a potentially attractive route, although this approach is currently hampered by the need to separate equilibrium mixtures of α-/β-amino acids.[10,11]

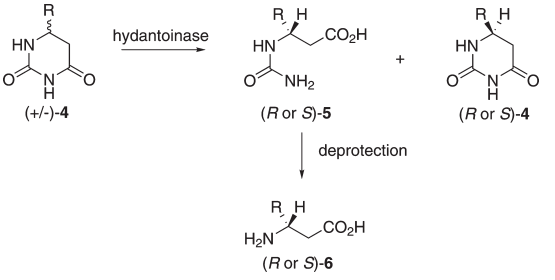
The use of hydantoinases for the enantioselective hydrolysis of racemic 5-substituted hydantoins **1** to their corresponding *N*-carbamoyl derivatives **2** is well established (Scheme 1) and has been developed to the stage that commercial processes now operate at scale for the production of specific D-(*R*)-amino acids **3** using this technology.[12] A key aspect of these processes is the in situ racemization of the unreacted enantiomer (*S*)-**1** together with carbamoylase catalyzed hydrolysis of (*R*)-**2**, leading to a dynamic kinetic resolution (DKR) reaction.

In contrast, the possibility of carrying out enantioselective hydrolysis of 6-substituted dihydrouracils **4** to their corresponding *N*-carbamoyl derivatives **5** as a route to β-amino acids **6** has received very little attention (Scheme 2). May et al.[13] described the use of a hydantoinase from *Arthrobacter aurescens* for the hydrolysis of dihydrouracil (**4**: R═H) and subsequently it was reported[14] that this hydantoinase could be applied to the resolution of 6-phenyldihydrouracil (**4**: R═Ph), although poor enantioselectivity and low reaction rates relative to 5-phenylhydantoin (**1**: R═Ph) were observed. In a separate study, a Japanese group[15] reported e.e.'s of up to 51% for the hydrolysis of 6-phenyldihydrouracil using a *Bacillus* sp.; higher selectivities (up to 93% e.e.) were obtained with substrates containing 6-alkyl, rather than 6-aryl, substituents. To assess the viability of this approach to β-amino acids, we decided to examine the hydrolysis

Scheme 1. Hydantoinase/Carbamoylase Catalyzed DKR of Racemic 5-Substituted Hydantoins 1 To Yield D-(*R*)-Amino Acids (*R*)-3



Scheme 2. Hydantoinase Catalyzed Hydrolysis of Racemic 6-Substituted Dihydrouracils



of a range of 6-substituted dihydrouracils (±)-**4** using the commercially available hydantoinase from *Vigna angularis*.[16]

The required 6-substituted dihydrouracil substrates **4a**−**i** were prepared by one of two alternative routes (Scheme 3). In method A,[17,18] urea was heated with the appropriate cinnamic acid derivative **7** at 190 °C for 2−4 h, followed by

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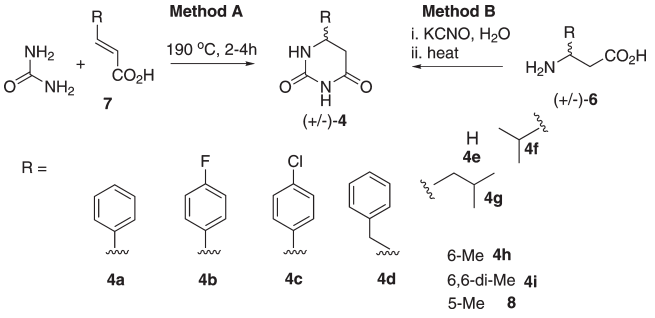
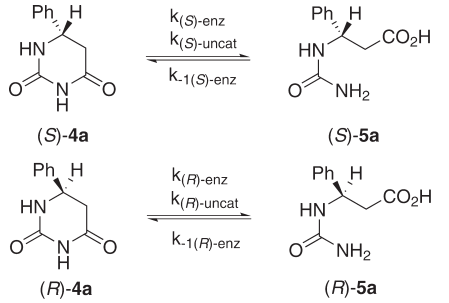
Scheme 3. Synthesis of Racemic 6-Substituted Dihydrouracils



Scheme 4. Kinetic Scheme for Hydrolysis of (±)-4a with *V. angularis* Hydantoinase



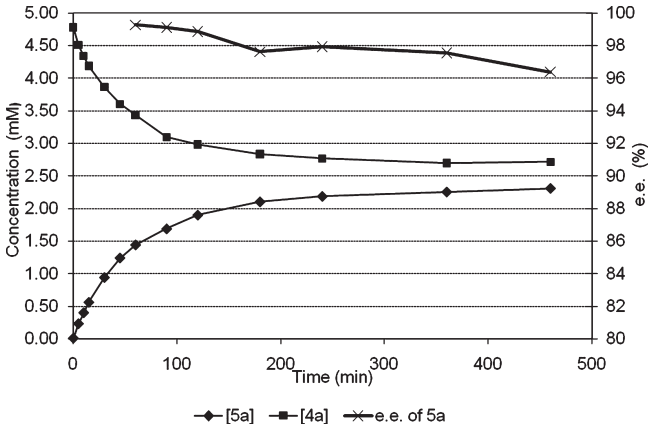


Figure 1. Progress of hydantoinase catalyzed hydrolysis of (±)-(**4a**) to (*S*)-**5a** showing composition of reaction mixture and e.e.

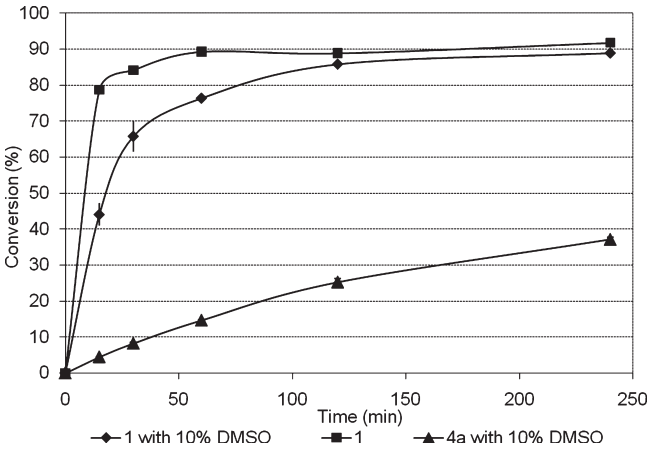


Figure 2. Relative rates of hydrolysis of **1** and **4a** using *V. angularis* hydantoinase in the presence and absence of DMSO.

recrystallization of the product, to yield **4a**−**i** in moderate yields of up to 46%. Method B[19] involved treatment of the corresponding racemic β-amino acid **6** with potassium cyanate to generate the *N*-carbamoyl derivative, followed by heating in concentrated HCl to effect cyclization to give **4a**−**i** in good overall yields. Method B, which is known to proceed without racemization, was also used to prepare enantiomerically pure (*S*)-**4a** and (*S*)-**5a**, by using enantiomerically pure (*S*)-**6a** as the starting material.

Initially, the hydantoinase-catalyzed hydrolysis of racemic 6-phenyldihydrouracil (**4a**: R═Ph) was studied as a model system. Reactions were carried out at a substrate concentration of 5 mM in TRIS buffer and monitored by reversed-phase chiral HPLC, which allowed simultaneous determination of both the extent of conversion in the reaction and also the e.e. of unreacted dihydrouracil **4a** and the *N*-carbamoyl derivative **5a**. The following observations were noted:

(i) The *V. angularis* hydantoinase was found to be highly selective (e.e. up to 98%) for the *S*-enantiomer of **4a** with an *E* value >100. The absolute configurations of both the product (*S*)-**5a** and unreacted substrate (*R*)-**4a** were assigned by comparison with authentic samples. (*S*)-**5a** is the expected enantiomer based upon the known selectivity of the *V. angularis* hydantoinase for (*R*)-hydantoins **1** (Scheme 1).

(ii) Despite the high *S* enantioselectivity observed, conversions did not proceed to 50% as expected, even after prolonged reaction times. All reactions gave an equilibrium ratio of **4a**:**5a** = 54:46 after 6 h with an e.e. for **5a** of ∼97% (Figure 1).

(iii) To further probe the equilibrium issue, the reverse reaction, namely, hydantoinase-catalyzed cyclization of *N*-carbamoyl-β-phenylalanine **5a**, was investigated. At pH 7.5 with (±)-**5a**, ∼5% conversion to **4a** was observed after 6 h, confirming the equilibrium position at this pH. As expected, the cyclization was found to be highly *S*-selective with no appreciable cyclization of the *R* enantiomer ($k_{-1S\text{-enz}} > k_{-1R\text{-enz}}$). No cyclization occurred in the absence of the hydantoinase.

(iv) In contrast to hydantoins, appreciable background hydrolysis of **4a** occurred with a strong pH and buffer dependency. At pH 7 in TRIS buffer, only 0.5% hydrolysis was observed, rising to 20% at pH 9. The hydantoinase was found to exhibit maximum activity at pH = 7.5 with respect to hydrolysis of (±)-**4a**.

The diagram in Scheme 4 summarizes the overall kinetic scheme for the hydrolysis of (±)-**4a** to **5a**. In this process $k_{S\text{-enz}} \gg k_{R\text{-enz}}$ and $k_{-1S\text{-enz}} > k_{-1R\text{-enz}}$ with $k_{S\text{-uncat}} = k_{R\text{-uncat}}$. Although reversibility has also been demonstrated for hydantoinase-catalyzed hydrolysis of hydantoins,[20] this reaction has an appreciable rate only at low pH, in contrast to cyclization of **5a**.

Direct comparison with 5-phenylhydantoin (**1**: R═Ph) as substrate revealed that **4a** was hydrolyzed with ∼10% of the rate when the reaction was carried out in DMSO (Figure 2).

The substrate specificity of the hydantoinase was then examined with respect to a range of 6-substituted dihdrouracils **4a**−**j** (Table 1).

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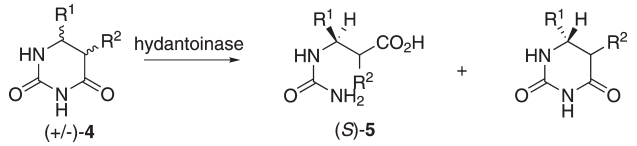
Table 1. Hydrolysis of 4i−4j with *V. angularis* Hydantoinase



(+/-)-**4** (*S*)-**5**

substrate	R^1	R^2	rel. rate	*E*
4a	C_6H_5	H	7	>200
4b	*p*-F-C_6H_4	H	15	>100
4c	*p*-Cl-C_6H_4	H	nd	>100
4d	$C_6H_5CH_2$	H	79	0
4e	H	H	100	
4f	*i*-Pr	H	46	3
4g	*i*-Bu	H	55	2
4h	Me	H	52	5
4i	$(Me)_2$	H	0	
4j	H	Me	23	

The reaction was found to be highly enantioselective ($E > 100$), provided that the substrate contained an aryl group as the C-6 substituent (**4a**−**c**). Replacement of phenyl- (**4a**) with benzyl- (**4d**) resulted in a high rate of hydrolysis but no apparent selectivity. In cases that the C-6 substituent is an alkyl group (**4f**−**h**), the reactions proceed quickly but with low selectivity ($E_R = 2-5$). Relocating the substituent at the C-5 rather than C-6 position (**4j**) led to a reduced rate of hydrolysis with no apparent selectivity.

Finally, a preparative scale reaction was carried out at 20 mM substrate concentration. After 4 h, the conversion of (±)-**4a** to the product (*S*)-**5a** reached 46% with 97% e.e. Subsequent extraction of the reaction mixture with 2-butanol gave (*S*)-**5a** in 42% yield based on (±)-**4a**. Cleavage of the *N*-carbamoyl group with nitrous acid yielded (*S*)-*β*-phenylalanine **6a** in 79% yield. An attempt to remove the *N*-carbamoyl group by treatment of (*S*)-**5a** with a commercially available carbamoylase[21] was not successful, in contrast to a previous report using the carbamoylase from *Agrobacterium tumefaciens*.[22]

In summary, we have demonstrated that racemic 6-substituted dihydrouracils can be enantioselectively hydrolyzed by the hydantoinase from *V. angularis*. In cases that the substituent is an aryl group, the selectivity is very high (E >100), thereby providing a new and practical method for the preparation of this class of enantiomerically pure *β*-amino acids.

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