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Mechanistic Studies on Thiaminase I

1. The Stereochemical Course of the Reaction[1]

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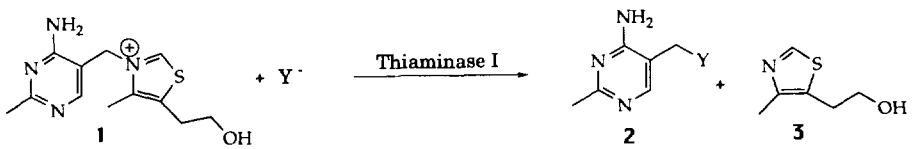
Thiaminase I catalyzes the displacement of the thiazole moiety of thiamin by a wide variety of nucleophiles. In this paper, we demonstrate that this reaction proceeds with overall retention of stereochemistry.

INTRODUCTION

Thiaminase I catalyzes the displacement of the thiazole moiety of thiamin by a wide variety of nucleophiles (Eq. [1]) (*1*).



EQUATION [1]

A mechanism for this substitution reaction is outlined in Scheme 1. Addition of an active-site nucleophile to the C6 position of the pyrimidine gives **4.** Loss of the thiazole followed by addition of the nucleophile Y gives **6.** Departure of the active site nucleophile completes the reaction. This mechanistic proposal is supported by the observation of ping-pong kinetics for the reaction (*2*) and by the mechanism-based inactivation of the enzyme with 6-chloro-2-methyl-4-aminopyrimidine (*3*). Nucleophillic attack at the planar methylene carbon of **5** may occur from the thiazole binding site, from a different site opposite to the thiazole binding site or without stereocontrol to give overall retention, inversion, or racemization of stereochemistry respectively. In this paper, we describe the stereochemical course of this reaction.

[1] This paper is dedicated to Professor Jeremy Knowles on the occasion of his 60th birthday.

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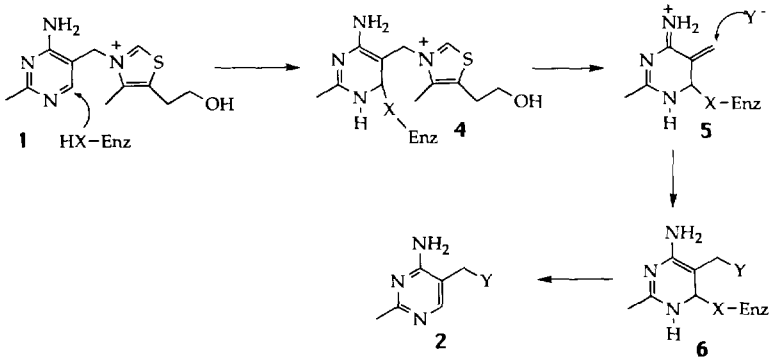
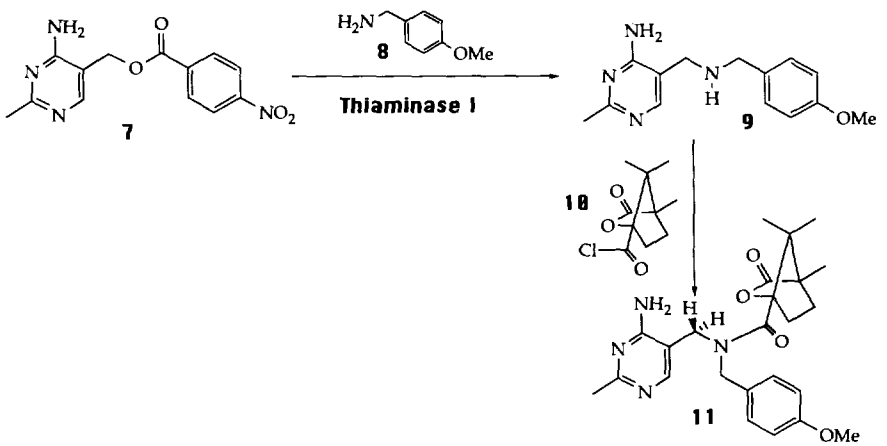


SCHEME 1

RESULTS AND DISCUSSION

Thiaminase I will catalyze the displacement of a variety of leaving groups from the pyrimidine (*4*). We have therefore been able to use the synthetically accessible *p*-nitrobenzoyl ester **7,** rather than thiamin, as our stereochemical probe (Scheme 2). This, when treated with the enzyme (*5*) in the presence of *p*-methoxybenzyl amine **8,** gives the substitution product **9** in 60% yield. This was converted to the camphanic amide **11** by treatment with (1S)-(-)-camphanic chloride. The diastereotopic C7 methylene protons of this compound can be readily differentiated by NMR analysis (Fig. 1).

The synthesis of the chiral monodeuterated ester (R)-**7** is shown in Scheme 3. Pyrimidine nitrile (*6*) **12** was reduced with $LiAlD_4$ to give deuterio-aldehyde **13.**



SCHEME 2

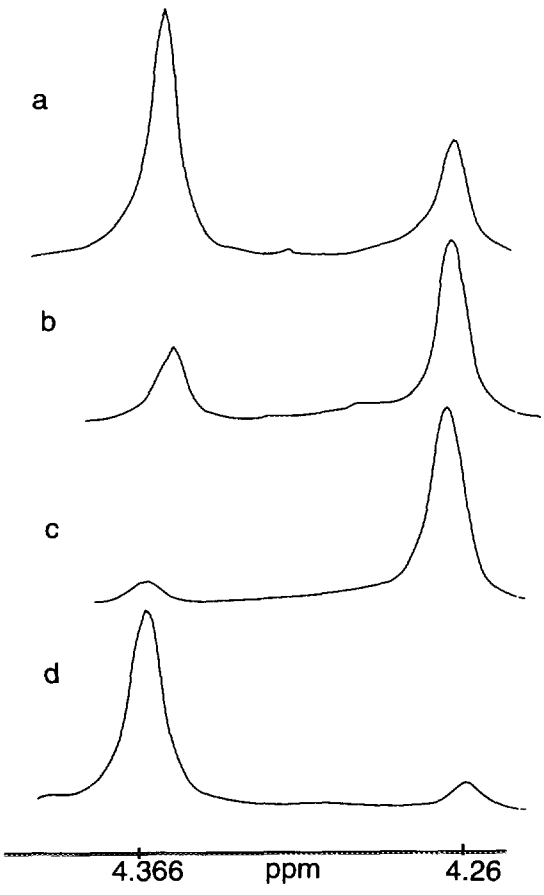
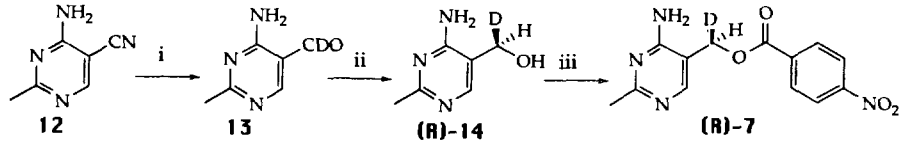


FIG. 1. Partial NMR spectra of the synthetic reference compounds and the enzymatic products: (a) reference compounds **18,** (b) reference compound **19,** (c) camphanic amide of the enzymatic product derived from (S)-monodeuterio-**7,** (d) camphanic amide of the enzymatic product derived from (R)-monodeuterio-**7.**



SCHEME 3. (i) $LiAlD_4$, THF_4; (ii) (S)-Alpine Borane; (iii) *p*-nitrobenzoyl chloride, TEA, THF.