Stereospecific Preparation of the N-Terminal Amino Acid Moiety of Nikkomycins K_X and K_Z via a Multiple Enzyme Synthesis

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Opportunistic fungal infections can be debilitating and in some cases fatal to the immunocompromised host. Infection by *Candida albicans*, a common pathogen, leads to systemic candidiasis, a significant concern for individuals suffering from acquired immune deficiency syndrome (AIDS), those receiving cancer therapy, and transplantation patients.[1,2] The target organ is often the kidney, with death resulting from renal failure. The nikkomycins are nontoxic nucleoside antifungals that selectively inhibit the fungal cell wall enzyme chitin synthase.[3−10] A nikkomycin-susceptible chitin synthase is present in *C. albicans* in the *in vivo* pathogenic state.[11−14] A variety of nikkomycins have been prepared and shown to strongly inhibit chitin synthase ($K_i = 0.16$ μM).[12] The general structure of the B, I, J, K, Q, X, and Z series nikkomoycins consists of a *N*-terminal amino acid and a nucleoside (Figure 1). The *N*-terminal amino acid moiety contains three contiguous stereogenic centers and has been the focus of numerous syntheses.[15−18] Barrett and Lebold reported the total synthesis of nikkomycin B using a chiral borane intermediate for the creation of the *N*-terminal portion.[15] König has reported a strategy for the synthesis of several nikkomycin amino acids involving a 1,3-dipolar nitrile oxide cycloaddition reaction producing diastereomers that were then resolved.[16] Using this method, König completed the sole syntheses of nikkomycins K_X and K_Z (Figure 1, X = N, Y = H, Z = H).[16,17] *Here we report a synthesis of the enantiomerically pure N-terminal amino acid of nikkomycins K_X and K_Z from achiral commodity chemicals using two consecutive enzyme-catalyzed steps.*

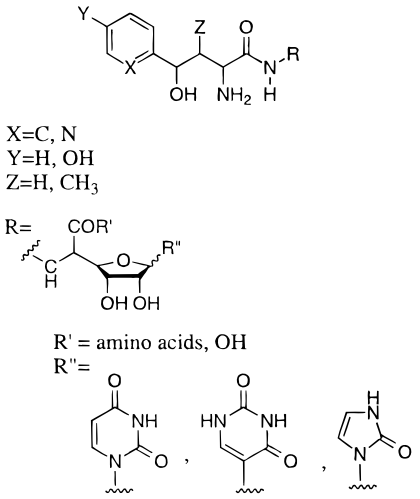

Figure 1. General structure of nikkomycins B, I, J, K, Q, X, and Z.

This study stems from continuing investigations in our laboratory of the synthetic utility of KDPG aldolase (EC 4.1.2.14).[19,20] KDPG aldolase utilizes pyruvate as the nucleophilic component in aldol addition to provide 4-substituted 4-hydroxy-2-ketobutyrate products. This fragment is both densely and differentially functionalized, providing opportunities for further synthetic transformations without the need for protecting groups. KDPG aldolase catalyzes kinetically controlled *si* face addition of pyruvate to electrophilic aldehydes to produce the *S* configuration at the new stereogenic center. The enzyme from recombinant *E. coli* (plasmid pTC 190) has been purified *via* differential dye-ligand chromatography to a specific activity of 410 U/mg.[21,22] This enzyme shows broad substrate specificity, accepting electrophiles with polar residues at positions 2, 3, or 4, or those with substituents capable of acting as hydrogen bond acceptors.[20]

Our strategy for the synthesis of the nikkomycin amino acids utilizes a pyruvate aldolase-catalyzed aldol reaction followed by a phenylalanine dehydrogenase-catalyzed reductive amination (Scheme 1). The synthesis proceeds in high yield and produces no byproducts, allowing isolation of pure material with little or no purification. We demonstrate the strategy for the synthesis of the *N*-terminal amino acid portion of nikkomycins K_X and K_Z.

The first step of our synthetic scheme involves a KDPG aldolase-catalyzed aldol addition of pyruvate to 2-pyridinecarboxaldehyde; the pyridine carboxaldehydes are among the best unnatural electrophiles for KDPG aldolase-catalyzed reactions. The aldolase reaction proceeds to completion during 48 h on a 36 mmol scale using 1100

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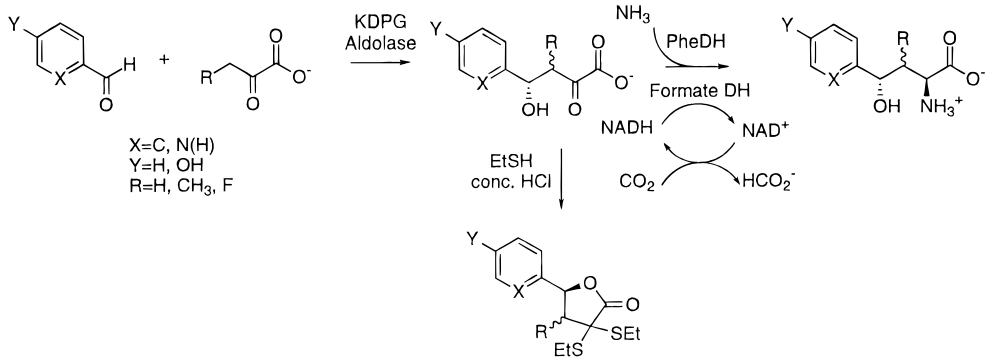
Scheme 1. Strategy for the Synthesis of the *N*-Terminal Amino Acid of Nikkomycins and Conversion of the Hydroxy Acid to the Dithioketal



U KDPG aldolase. Attempted purification of the α-keto acid by cation-exchange, anion-exchange, silica gel, or size-exclusion chromatography failed; in all cases, chromatography resulted in product decomposition. Lactonization of the adduct was unsuccessful with acid, DCC, DAEC, or Mitsunobu conditions. Esterification by Fischer conditions in methanol or ethanol, conversion of the carboxylate to a silver salt followed by treatment with iodomethane, binding the carboxylate to an anion exchanger and treating with iodomethane, and treatment of the acid with diazomethane all failed to produce the desired product.[23,24] Alternatively, precipitation with ethanol yielded pure adduct in 88% isolated yield with only minor buffer salt impurity.

The α-keto acid was converted to the dithioacetal lactone. Chiral GLC chromatography (Chrompack Chirasil-L-Val column) of the dithiolactone showed a single peak. Elution of racemic material, prepared by the method of Cornforth,[25,26] showed base-line separation of enantiomers, providing an enantiomeric excess for the enzyme-catalyzed product of >99.7%.

The α-keto acid was converted to the required α-amino acid using phenylalanine dehydrogenase from *Bacillus sphaericus* SCRC-R79a overexpressed in *E. coli*.[27−30] This enzyme accepts a variety of unnatural substrates. Yeast formate dehydrogenase was utilized to recycle NADH as previously described.[31] The enzymes were enclosed in a dialysis membrane, and the appearance of product was followed with ninhydrin. The possibility of racemization of the α-keto acid at basic pH dictated that reductive amination be conducted at pH 6.5. Because this value is significantly below optimum pH for enzyme activity, we attempted reactions at both 25 and 50 °C to increase enzymatic activity. While the room-temperature reaction proceeded to completion cleanly in 2 days, the high-temperature reaction yielded a mixture of products. The spectral data and rotation of the product produced at room temperature are consistent with those of authentic material.[16,17]

In conclusion, we have prepared the *N*-terminal amino acid moiety of nikkomycin K_X and K_Z in a two-step enzymatic reaction with 75.9% overall yield. Our synthesis represents a departure from previous KDPG aldolase-catalyzed preparations in that it generates non-carbohydrate products. The net conversion is of pyruvate, 2-pyridinecarboxaldehyde, and ammonium bicarbonate to nikkomycin amino acid plus carbon dioxide, with concomitant establishment of two stereocenters in enantiomerically and diastereomerically pure form. KDPG aldolase accepts 2-, 3-, and 4-pyridinecarboxaldehydes as well as substituted variants. Using 2-pyridinecarboxaldehyde as the electrophile, fluoropyruvate, oxobutyrate, and hydroxypyruvate are accepted as substituted nucleophiles. At least some of these unnatural nucleophiles are deprotonated stereospecifically, affording products stereoisomerically pure at C-3; we are currently studying these effects more fully. Likewise, we are continuing to explore this synthetic strategy for the preparation of related amino acid analogues and will report our results in due course.

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Supporting Information Available: Experimental details and NMR spectra for the preparation of (*S*)-4-hydroxy-2-keto-4-(2′-pyridyl)butyrate, 2,2-bis(ethylthio)-4-(2′-pyridyl)-4(*S*)-butyro-γ-lactone, (*S*,*S*)-4-hydroxy-2-amino-4-(2′-pyridyl)-butyrate (6 pages).

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