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The Synthesis of the Vitamers of Biotin

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An efficient synthetic pathway for the preparation of the vitamers of biotin which provides easy access to optically pure KAPA as well as diastereomeric mixtures of DAPA, DTB, and analogs thereof has been developed.

INTRODUCTION

Biotin is a water-soluble vitamin that functions as a coenzyme in certain carboxylation and transcarboxylation reactions (*1*). Perhaps the most important of these, the carboxylation of acetyl-CoA to form malonyl-CoA, is catalyzed by acetyl-CoA carboxylase and is an initial step in fatty acid and polyketide biosynthesis. Since the proper function of at least one of the reactions in which biotin participates is a prerequisite for the growth and development of almost all organisms, biotin is an essential nutrient. Many organisms, including the majority of plants and microbes, synthesize their own biotin; others, including animals and certain microbes such as yeast and *Lactobacillus*, must obtain the vitamin from external sources. The later intermediates in the biosynthetic path, the so-called vitamers 7-keto-8-amino pelargonic acid (KAPA), 7,8-diamino pelargonic acid (DAPA), and desthiobiotin (DTB) (Scheme 1), have been identified.

Earlier reports (*2*) described the synthesis of racemic KAPA and DAPA without reporting any spectral data. Moreover, while a very large number of publications (*3*) have described the partial or total synthesis of biotin, none of these synthetic routes have been based on the natural biosynthetic path.

The final stage in the biosynthesis, at which DTB is converted into biotin, involves insertion of a S atom between two nonactivated positions (methyl and methylene groups) to form the tetrahydrothiophene ring and is of particular interest. This reaction is exceedingly difficult from a chemical standpoint. The mechanism of the enzymatic reaction is unknown. Although the enzymatic reaction has been observed *in vitro* (*4*), the sulfur donor has not been established (*5*). There is evidence that the reaction catalyzed by biotin synthase can be the rate-limiting step in the biotin biosynthetic pathway.

We were interested in developing convenient syntheses of the natural vitamers

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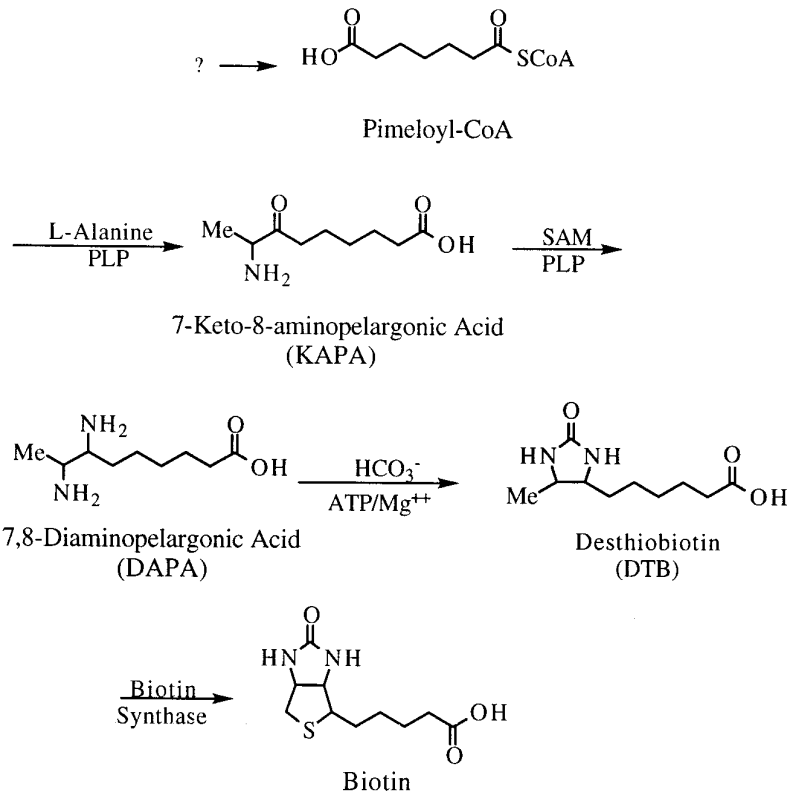


SCHEME 1. Biosynthetic pathway of biotin.

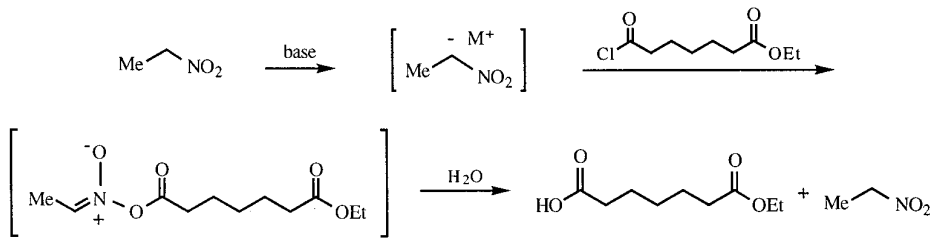
and in extending the methodology developed, for the synthesis of analogs that may be used to study the mechanism of this last biosynthetic step. We describe herein a new total synthesis of the three vitamers.

RESULTS AND DISCUSSION

Synthesis of KAPA. Our initial approach involved acylation of the anion of 2-nitroethane with a suitable derivative of pimelic acid. However, hydrolytic workup of the reaction gave only the monoester of pimelic acid (Scheme 2).

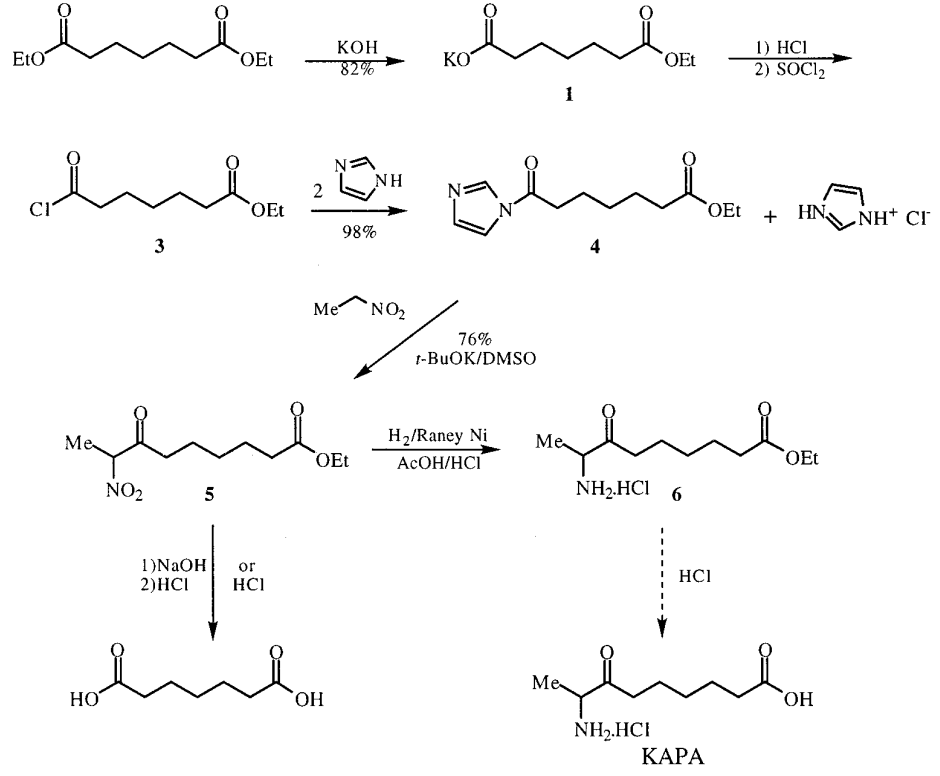
This problem was overcome by initial conversion of the acyl chloride **3** to the corresponding acyl imidazole **4** that led to the desired 7-keto-8-nitro-pelargonate **5.** Reduction of the latter with Raney Ni gave the KAPA ethyl ester **6** in poor yield (Scheme 3).

This approach suffered from several deficiencies: (a) The products obtained were racemic, (b) the yield in the reduction step was very low, and (c) pimelic acid, as

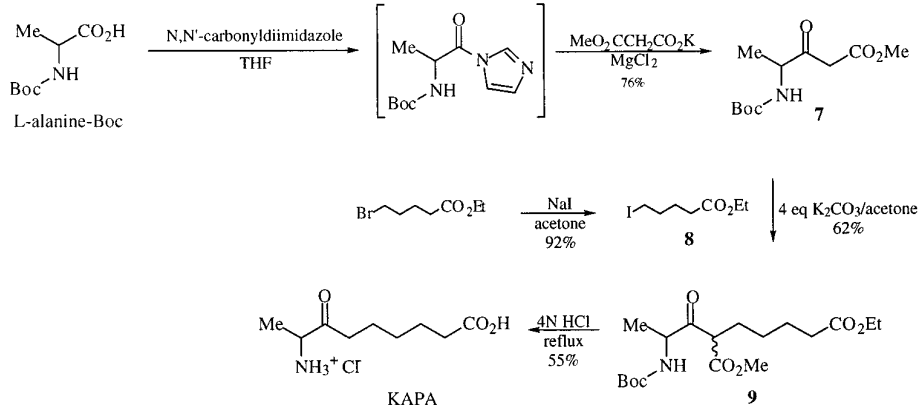


SCHEME 2

well as other 7-carbon chain unit derivatives, is expensive. To overcome these problems a new approach was taken whereby the N-containing moiety was derived from L-alanine. This approach provided the desired chirality and enabled condensation with readily available derivatives having shorter carbon chains. The most suitable L-alanine derivative in this approach was Mansour's β-keto ester **7** (*6*)



SCHEME 3

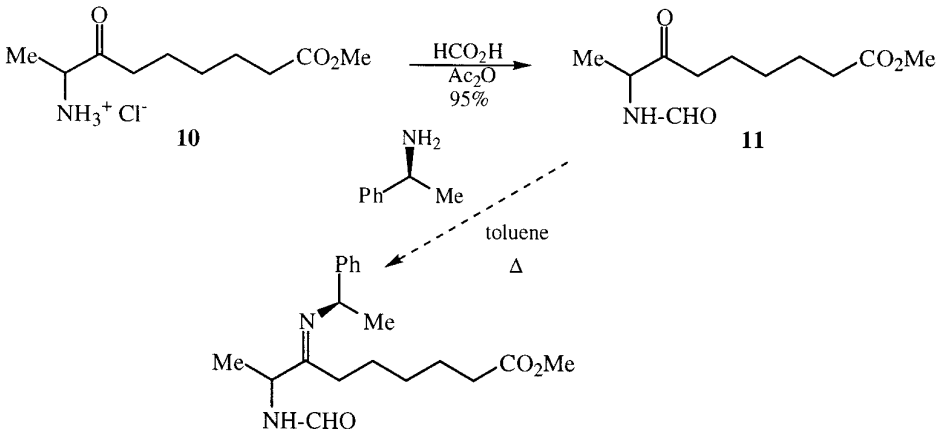


SCHEME 4

protected by an *N*-Boc group (Scheme 4). Our earlier experiments, when using the corresponding *N*-phthalimido derivatives, led to various rearrangement products (*7*). Alkylation of **7** with ethyl 5-iodovalerate **8** in the presence of 4 equivalents of K_2CO_3 in acetone (conditions found to be optimal, other bases and solvents resulting in much lower yields) gave **9.** Complete deprotection by acid hydrolysis gave optically pure KAPA, found to be identical to the natural vitamer (Scheme 4).

Synthesis of DAPA. The reductive amination of KAPA to DAPA in the biosynthetic pathway involves PLP and SAM (Scheme 1). Our initial approaches were to carry out this reaction by nonenzymatic methods taking into consideration the stereospecificity of the reductive step. Ketones have been converted to chiral amines by reduction of their corresponding chiral imines, such as those obtained by condensation with optically pure α-methylbenzyl amine, where the imine formation takes place under basic conditions (*8*). In our case, since KAPA is an α-amino ketone unstable in basic media, an initial N-protection step was required. N-protection reactions are commonly conducted in basic media; however, in this case the protective step required nonbasic conditions in order to prevent the formation of α-amino ketone intermolecular condensation products. Such an N-protection was carried out by N-formylation in the presence of formic acid/acetic anhydride (*9*) (Scheme 5). Unfortunately the subsequent ketimine formation under a variety of conditions failed.

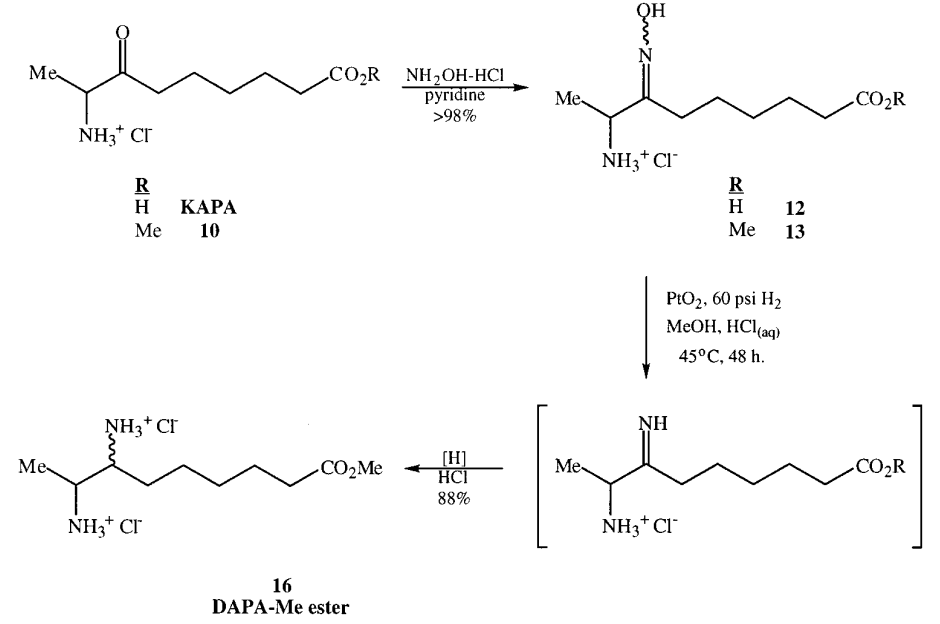
The next approach involved reduction of the readily formed oxime derivative of KAPA. Oximation of KAPA or its methyl ester **10** gave a mixture of *syn*- and *anti*-oximes (**12** or **13,** respectively) consisting mainly of the *syn* isomers. This suggested that the chiral center at the C-8 position induced stereoselectivity and it was thus hoped that a similar induction would be obtained in the course of the following reduction step. However, upon reduction an approximately equimolar mixture of diastereomers was obtained. The loss of stereospecific induction may be attributed to initial reduction of the oxime to an intermediate imine, combined with a weakening of the H bond between the imine and the neighboring ammonium ion under



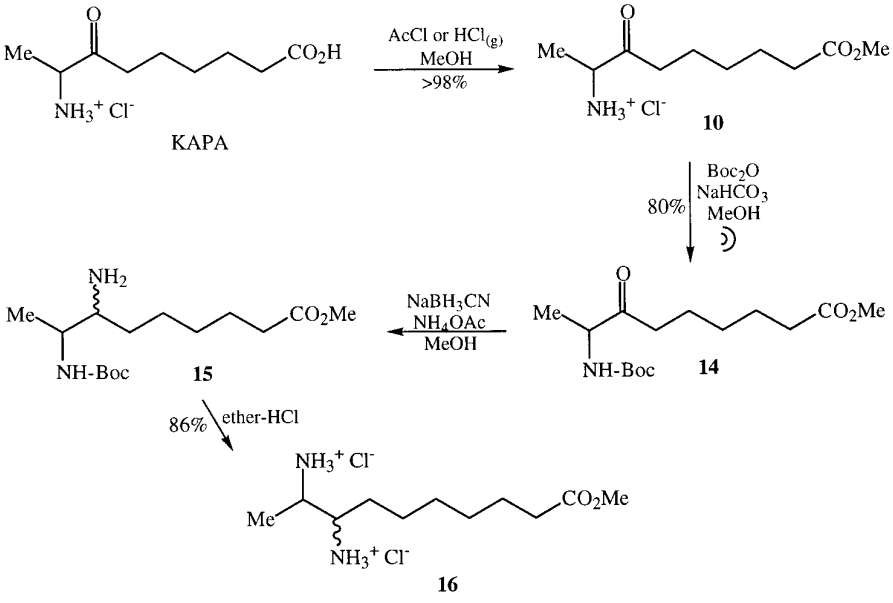
SCHEME 5

the acidic conditions of the reaction. Furthermore, under the methanolic–acidic conditions of the reaction, the corresponding DAPA methyl ester **16** was isolated (Scheme 6).

An alternative reductive amination was carried out in the presence of $NaBH_3CN$



SCHEME 6



SCHEME 7

that gave **16** also as an approximately equimolar mixture of diastereomers (Scheme 7). This approach required double protection, both of the acid as its Me ester **10,** conveniently carried out in the presence of anhydrous HCl/MeOH (*10*) and of the N as the *N*-Boc, **14.** The latter was conducted by sonication under mild basic conditions in order to avoid standard harsh basic media that would result in decomposition and/or dimerization of the free α-amino ketone.

Synthesis of DTB. The formation of the imidazolidone ring of DTB was carried out by reaction of DAPA with either triphosgene or carbonyl diimidazole (CDI). In the former case the reaction proceeded in aqueous media in the presence of base using the DAPA-free carboxylic acid or its ester **16.** In the course of the workup, the ester readily hydrolyzed. Alternatively the reaction was performed in organic media from **16** and either triphosgene or CDI, from which the yields obtained using triphosgene were quantitative (Scheme 8).

Summary. An efficient synthetic pathway for the synthesis of the vitamers of biotin, which provides easy access to optically pure KAPA as well as diastereomeric mixtures of DAPA, DTB, and analogs thereof, has been developed. As indicated, the reduction of the oximes **12** and **13** did not proceed stereospecifically. No further attempts were made at this point to separate the diastereomers since our ultimate goal is to develop a stereospecific synthesis for the vitamers. Further investigations are in progress dealing with stereospecific reductive amination of KAPA to DAPA. If this goal is attained, it should be possible to proceed to DTB maintaining complete stereospecificity using the reactions described in this paper.