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Stereochemical modification of geminal dialkyl substituents on pantothenamides alters antimicrobial activity

Annabelle Hoegl [a,†], Hamed Darabi [a,†], Elisa Tran [a], Emelia Awuah [a], Eleanor S. C. Kerdo [b], Eric Habib [a], Kevin J. Saliba [b,c], Karine Auclair [a,*]

[a] Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montréal, Québec H3A 0B8, Canada
[b] Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Canberra, Australian Capital Territory 0200, Australia
[c] Medical School, College of Medicine, Biology and Environment, The Australian National University, Canberra, Australian Capital Territory 0200, Australia

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ABSTRACT

Pantothenamides are *N*-substituted pantothenate derivatives which are known to exert antimicrobial activity through interference with coenzyme A (CoA) biosynthesis or downstream CoA-utilizing proteins. A previous report has shown that replacement of the *ProR* methyl group of the benchmark *N*-pentylpantothenamide with an allyl group (*R-anti* configuration) yielded one of the most potent antibacterial pantothenamides reported so far (MIC of 3.2 μM for both sensitive and resistant *Staphylococcus aureus*). We describe herein a synthetic route for accessing the corresponding *R-syn* diastereomer using a key diastereoselective reduction with Baker's yeast, and report on the scope of this reaction for modified systems. Interestingly, whilst the *R-anti* diastereomer is the only one to show antibacterial activity, the *R-syn* isomer proved to be significantly more potent against the malaria parasite (IC_{50} of 2.4 ± 0.2 μM). Our research underlines the striking influence that stereochemistry has on the biological activity of pantothenamides, and may find utility in the study of various CoA-utilizing systems.

Infectious diseases remain a major contributing factor to worldwide mortality. Moreover, the development of antimicrobial resistance is raising significant concerns about the increasingly limited efficacy of currently available treatments.[1] There have been considerable efforts towards discovering and characterizing novel therapeutic targets for antimicrobial drugs. One such target which has emerged as a promising point-of-attack is coenzyme A (CoA) biosynthesis and its associated cellular processes.[2] This ubiquitous cofactor is required for a diverse set of biological functions and is essential to all organisms. Most rely on the exogenous uptake of its natural precursor pantothenate (vitamin B_5), and extend it into CoA through a 5-step biotransformation.[3,4] The inherent differences in the CoA biosynthetic machinery between humans and microbial pathogens suggest that this pathway can be exploited for therapeutic applications.[1] In fact, a variety of pantothenate analogues have been evaluated for antibacterial, antiplasmodial and antifungal properties.[2]

An important class of such compounds are the *N*-substituted pantothenamides, including the benchmark molecule in the field, *N*-pentylpantothenamide (Fig. 1).[5] This compound was shown to have potent activity against *Escherichia coli* (in low pantothenate media)[5] and *Staphylococcus aureus*.[6] It was later found that pantothenamides were, in fact, being extended by the CoA biosynthetic enzymes into CoA analogues which affected downstream targets such as the acyl carrier protein necessary for fatty acid synthesis.[7,8] It has been suggested that pantothenamides may also act by inhibiting the CoA biosynthetic pathway.[9,10] While much of the research has focused on antibacterials, targeting the CoA pathway is also a promising strategy for antiplasmodials. For example, the provitamin pantothenol, as well as a range of other pantothenate analogues, have been shown to repress the proliferation of the malarial parasite *Plasmodium falciparum*.[11–15]

A synthetic route for accessing geminal dialkyl-substituted pantothenamide derivatives was recently reported by some of us.[16] In this study, several compounds were synthesized and evaluated for antibacterial properties which revealed that larger substituents were not well tolerated at the gem-dimethyl position. This led to the identification of a methyl-allyl derivative (**1**) with potent antibacterial activity against both sensitive and resistant *S. aureus*.[16] It was envisaged that these structure–activity relationships (SARs) could be extended through modification of the stereochemistry of the alkyl-substituted pantoyl fragment. In designing a target, we opted to focus on the 2-methyl-allyl derivative because of its clear superiority in antibacterial activity assays,[16] and to

* Corresponding author. Tel.: +1 514 398 2822; fax: +1 514 398 3797.
E-mail address: karine.auclair@mcgill.ca (K. Auclair).
† These authors contributed equally to the work.

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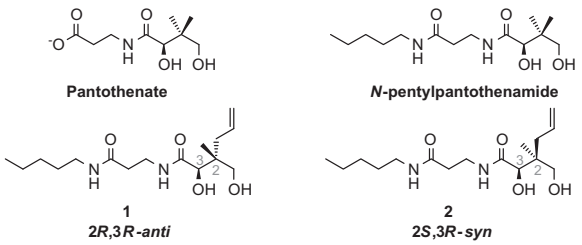
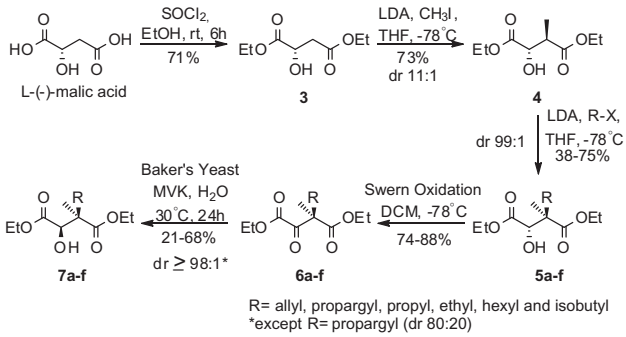


Figure 1. Structure of pantothenate and *N*-pentylpantothenamide analogues.

maintain the *R*-configuration at C-3 based on previous studies suggesting that this is the preferred stereochemistry.[2] We report here on a methodology for accessing the 2*S*,3*R*-*syn* allyl-substituted isomer (**2**), and on the contrasting profiles of diastereomers **1** and **2** with regards to antibacterial and antiplasmodial activities.

Synthetically, the pantothenamide structure has been obtained through a sequence of amide couplings on a modified pantoyl fragment.[16] In the synthesis of geminal dialkyl-substituted pantothenamide derivatives, the stereochemistry at the quaternary carbon is determined by the initial configuration of the alcohol in the starting material, and can be controlled via two successive alkylations *anti* to the alcohol.[16] This synthetic methodology, however, only provides access to *2R,3R-anti* analogues diastereoselectively. *Reversing the configuration at the quaternary center by reversing the order of the two alkylation reactions, for example adding the larger alkyl group before methylation, proceeds with poor selectivity and produces inseparable mixtures of 2R,3R-anti and 2R,3S-syn analogues.*[16,17] The difficulty in obtaining the *syn* product by reversing the alkylation sequence warrants an alternate synthetic route. In order to access the novel 2*S*,3*R* diastereomer, we envisaged inverting the stereochemistry of the fragment through oxidation, followed by diastereoselective reduction. We expected this last step to pose the greatest challenge, due to the unfavorable energetic barrier associated with forming the *syn* product. Thus, L-(−)-malic acid (3*S*-alcohol) is used here to access the *syn* isomer (**2**) through inversion of stereochemistry, while D-(−)-malic acid (3*R*-alcohol) was previously used directly to synthesize the *anti* isomer (**1**).[16]

In order to generate the alkyl-substituted pantoyl fragment with the desired stereochemistry, L-(−)-malic acid was first esterified under mild acidic conditions (Scheme 1). The Frater–Seebach method of alkylating chiral β-hydroxy esters was used to install the methyl and various alkyl groups onto **3** with excellent diastereoselectivity.[17] The addition of two equivalents of strong base generates a di-anion which forms a six-membered ring chelate.[17] The stereoconfiguration of the secondary alcohol directs the electrophilic addition from the less hindered face, thereby yielding the *anti* product with a consistently high diastereomeric ratio (dr; as measured by NMR of the crude sample). Swern oxidation was used



Scheme 1. Synthesis of compounds **7a**–**f**.

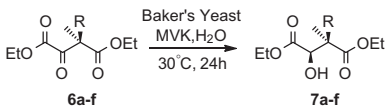
to afford the prochiral ketone **6a** in good yield, which was subsequently reduced to the *R*-alcohol **7a**.

Several commercially available chemical reducing agents were tested to evaluate their ability to yield the *syn* alcohol product from **6a**. As shown in Table S1, typical agents such as DIBAL-H, $NaBH_4$ and $Zn_2(BH_4)_2$, as well as chiral reducing agents such as (*R*)-CBS and (*S*)-CBS showed poor diastereoselectivity and yielded predominantly the *anti*-product. The potential of biocatalysts was thus explored. To this end, we envisaged utilizing a whole cell mixture of common Baker's yeast (*Saccharomyces cerevisiae*) to reduce **6a** to the *syn* product **7a** diastereoselectively. There is precedence for Baker's yeast to reduce α- and β-ketoesters to enantiopure alcohols.[18] Baker's yeast expresses several reducing enzymes which can be selectively inhibited or favored by varying the reaction conditions.[19] Pre-treatment of the yeast with cross-linking agents such as methyl-vinyl ketone (MVK), was found to favor *syn*-selectivity and prevent over-reduction to the diol.[20] Heat-denaturation (50 °C, 30 min) also achieved the same goal and even worked synergistically with MVK.[21,22]

Indeed, reduction of **6a** with Baker's yeast yielded the desired *syn* product (dr of >99:1) in 68% yield after purification. This high selectivity was only possible when the yeast was pre-incubated with MVK at 50 °C for 30 min before addition of the ketone. The dr ratio was determined by integrating the characteristic NMR peaks for the methyl group at the quaternary carbon, specifically the signal at 1.16 ppm from the *anti* product,[16] and the signal at 1.05 ppm from the *syn* product. The absolute stereochemistry of the product was confirmed by derivatization using enantiomeric auxiliary reagents and subsequent ^1H NMR analysis as described by Seco et al.[23] (*R*)- and (*S*)-methoxyphenylacetic acid (MPA) were thus coupled to **7a** to generate the diastereoisomeric MPA derivatives for NMR analysis. To evaluate the scope of the Baker's yeast reaction, a series of α-ketosuccinates (**6a**–**f**) was synthesized and reduced. The dr was measured by NMR on the crude product, and the yield was calculated after purification (Table 1). Interestingly, all compounds showed excellent diastereoselectivity (dr ⩾ 98:1, with one exception at 80:20), although the yield of the reactions generally decreased with increasing bulk of the alkyl substituents, consistent with larger groups not being well tolerated in the binding pocket of the reductase of interest. The low yields observed in some of the examples are largely attributed to product recovery issues resulting from inefficient extraction from the complex matrix. Overall, Baker's yeast shows excellent stereoselectivity for the reduction of these systems.

With the stereochemistry established, we were able to extend the intermediates into full pantothenamides as shown in Scheme 2. As mentioned above, the methyl-allyl derivative **7a** was selected for extension based on the reported antibacterial activity of **1**.[16]

Table 1
Baker's yeast reduction of di-alkyl substituted α-ketomalonates



Compound	R	dr[a]	Yield[b] (%)
7a	Allyl	>99:1	68
7b	Propargyl	80:20	65
7c	Ethyl	98:1	37
7d	Propyl	>99:1	31
7e	Hexyl	>99:1	21
7f	Isobutyl	98:1	26

[a] Diastereomeric ratio determined by NMR.
[b] Isolated yield.