Bioorg Med Chem Lett. Author manuscript; available in PMC 2017 January 11.

Published in final edited form as:
Bioorg Med Chem Lett. 2014 August 01; 24(15): 3274–3277. doi:10.1016/j.bmcl.2014.06.013.

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], and **Karine Auclair**[*,a]

[a]Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montréal, Québec, Canada, H3A 0B8

[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 0200, The Australian National University, Canberra, Australian Capital Territory, Australia

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.

Keywords

pantothenamides; antibacterial; antiplasmodial; baker's yeast; coenzyme A

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 characterising novel therapeutic targets for antimicrobial drugs. One such target which has emerged as a promising point-of-attack is

[*]To whom correspondence should be addressed: karine.auclair@mcgill.ca; +1-514-398-2822.
[‡]These authors contributed equally to the work

Supplemental data
Supporting information available, including experimental procedures, characterization of all compounds, as well as Figure S1, NMR spectra and selected HPLC traces. This material is available via the Internet.

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 (Figure 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 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, e.g. 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 unfavourable 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 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 next. 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 favoured 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 ^{1}H 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] Thus, **7a** was reduced to the corresponding triol using $LiAlH_4$ and selectively protected as the six-membered ring anisaldehyde acetal **8**. A Dess-Martin oxidation afforded the aldehyde **9**, which was further oxidized to the acid **10** via Pinnick oxidation. Due to its instability, the crude acid (**10**) was used directly in the amide coupling to deprotected amine **13**. Finally, the acetal protecting group of **11** was cleaved using 90% aqueous acetic acid to produce the desired pantothenamide **2**.

The 2*S*,3*R*-*syn* pantothenamide **2** was tested for antibacterial activity against *S. aureus* (sensitive and MRSA) but no appreciable effect could be detected at the highest concentration tested (500 μM; data not shown). This is in sharp contrast to the 2*R*,3*R*-*anti* pantothenamide **1**, which previously showed potent antibacterial activity (MIC of 3.2 μM for both strains).[9] Interestingly, this trend was reversed when **1** and **2** were evaluated for their antiplasmodial activity. As shown in Figure 2, although both diastereomers inhibited *in vitro* parasite growth, the *syn* derivative **2** (IC_{50} of 2.4 ± 0.2 μM, n = 6) appeared to be considerably more potent ($p \ll 0.001$) when compared to its *anti*-isomer **1** (31 ± 4 μM, n = 3). Moreover, increasing the extracellular pantothenate concentration (from 1 to 50 μM) resulted in a higher IC_{50} for **2** (13 ± 1 μM, n = 3, $p \ll 0.001$), consistent with **2** inhibiting parasite growth by targeting CoA biosynthesis or utilization (Figure 2). Compounds **1** and **2** were also tested in human cells and did not show significant toxicity (see Figure S1).

A successful method was established for the synthesis of 2*S*,3*R*-*syn* 2-methyl-alkyl pantothenamide derivatives. A key step of this process involved the use of Baker's yeast for the stereoselective reduction of α-ketosuccinates. Stereochemical modification of the pantoyl fragment significantly influenced the biological activity and antimicrobial selectivity of these compounds. This suggests that such alterations may be applied to study other CoA-utilizing systems. For example, *N*-substituted pantothenamides have found use as chemical biology tools and as mechanistic probes of CoA-dependent enzymes. Specific applications include both *in vitro* and in-cell labelling of carrier proteins,[24–26] as well as prodrug activation by the CoA biosynthetic pathway.[27] Thus, we expect the methodology developed herein to find utility in various areas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Center in Green Chemistry and Catalysis, as well as by research grants from the Canadian Institute of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC) to K.A. A.H. thanks CIHR and the CIHR-McGill Drug Development Training Program (DDTP) for financial support. We are grateful to the Canberra branch of the Australian Red Cross Blood Service for the provision of erythrocytes and to Dr. Giel van Dooren (ANU) for assistance with the HFF assays.

References

1. Vicente M, Hodgson J, Massidda O, Tonjum T, Henriques-Normark B, Ron EZ. FEMS Microbiol Rev. 2006; 30:841. [PubMed: 17064283]
2. Spry C, Kirk K, Saliba KJ. FEMS Microbiol Rev. 2008; 32:56. [PubMed: 18173393]
3. Genschel U. Mol Biol Evol. 2004; 21:1242. [PubMed: 15014152]
4. Gerdes SY, Scholle MD, D'Souza M, Bernal A, Baev MV, Farrell M, Kurnasov OV, Daugherty MD, Mseeh F, Polanuyer BM, Campbell JW, Anantha S, Shatalin KY, Chowdhury SA, Fonstein MY, Osterman AL. J Bacteriol. 2002; 184:4555. [PubMed: 12142426]
5. Clifton G, Bryant SR, Skinner CG. Arch Biochem Biophys. 1970; 137:523. [PubMed: 4909169]
6. Choudhry AE, Mandichak TL, Broskey JP, Egolf RW, Kinsland C, Begley TP, Seefeld MA, Ku TW, Brown JR, Zalacain M, Ratnam K. Antimicrob Agents Chemother. 2003; 47:2051. [PubMed: 12760898]
7. Strauss E, Begley TP. J Biol Chem. 2002; 277:48205. [PubMed: 12372838]
8. Zhang YM, Frank MW, Virga KG, Lee RE, Rock CO, Jackowski S. J Biol Chem. 2004; 279:50969. [PubMed: 15459190]
9. Thomas J, Cronan JE. Antimicrob Agents Chemother. 2010; 54:1374. [PubMed: 20047918]
10. Virga KG, Zhang YM, Leonardi R, Ivey RA, Hevener K, Park HW, Jackowski S, Rock CO, Lee RE. Bioorg Med Chem. 2006; 14:1007. [PubMed: 16213731]
11. de Villiers M, Macuamule C, Spry C, Hyun YM, Strauss E, Saliba KJ. ACS Med Chem Lett. 2013; 4:784. [PubMed: 24900746]
12. Saliba KJ, Kirk K. Mol Biochem Parasitol. 2005; 141:129. [PubMed: 15811536]
13. Saliba KJ, Ferru I, Kirk K. Antimicrob Agents Chemother. 2005; 49:632. [PubMed: 15673744]
14. Spry C, Chai CL, Kirk K, Saliba KJ. Antimicrob Agents Chemother. 2005; 49:4649. [PubMed: 16251308]
15. Spry C, Macuamule C, Lin Z, Virga KG, Lee RE, Strauss E, Saliba KJ. PLoS One. 2013; 8:e54974. [PubMed: 23405100]
16. Akinnusi TO, Vong K, Auclair K. Bioorg Med Chem. 2011; 19:2696. [PubMed: 21440446]
17. Frater G, Mueller U, Guenther W. Tetrahedron. 1984; 40:1269.
18. Nakamura K, Yamanaka R, Matsuda T, Harada T. Tetrahedron: Asymmetry. 2003; 14:2659.
19. Shieh WR, Gopalan AS, Sih CJ. J Am Chem Soc. 1985; 107:2993.
20. Nakamura K, Kawai Y, Miyai T, Ohno A. Tetrahedron Lett. 1990; 31:3631.
21. Nakamura K, Kawai Y, Ohno A. Tetrahedron Lett. 1991; 32:2927.
22. Nakamura K, Kondo S, Kawai Y, Hida K, Kitano K, Ohno A. Tetrahedron: Asymmetry. 1996; 7:409.
23. Seco JM, Quinoa E, Riguera R. Tetrahedron: Asymmetry. 2001; 12:2915.
24. Clarke KM, Mercer AC, La Clair JJ, Burkart MD. J Am Chem Soc. 2005; 127:11234. [PubMed: 16089439]
25. Meier JL, Mercer AC, Rivera H Jr, Burkart MD. J Am Chem Soc. 2006; 128:12174. [PubMed: 16967968]
26. Worthington AS, Burkart MD. Org Biomol Chem. 2006; 4:44. [PubMed: 16357994]
27. Vong K, Tam IS, Yan X, Auclair K. ACS Chem Biol. 2012; 7:470. [PubMed: 22217014]

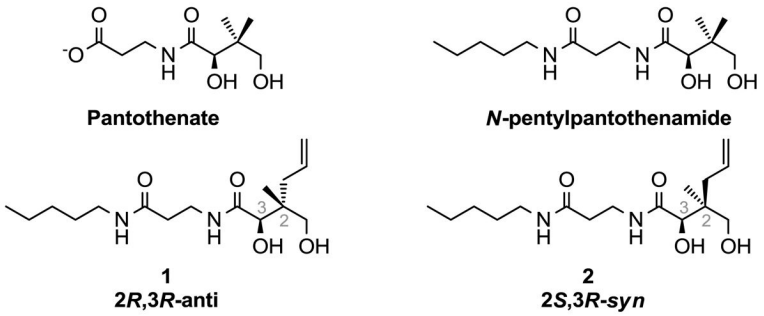


Fig. 1.
Structure of pantothenate and *N*-pentylpantothenamide analogues.

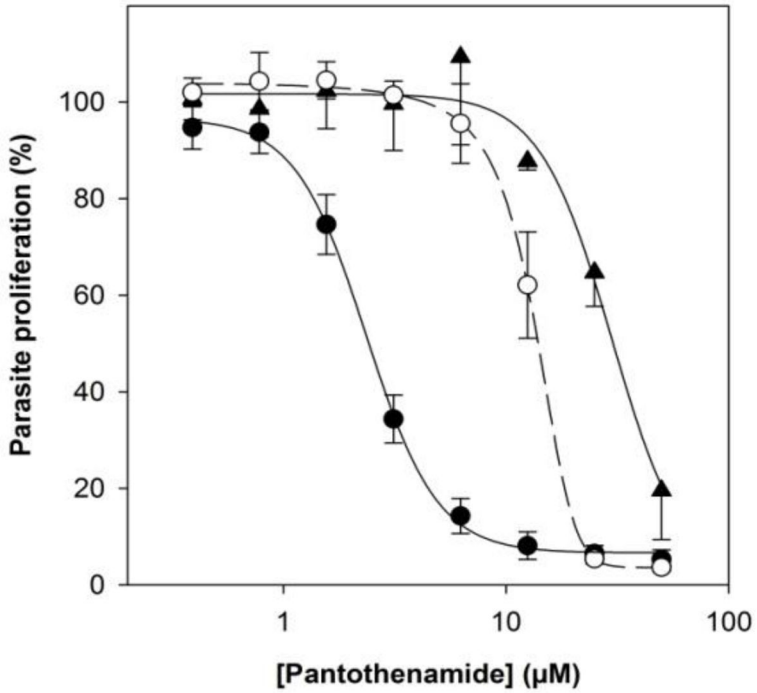
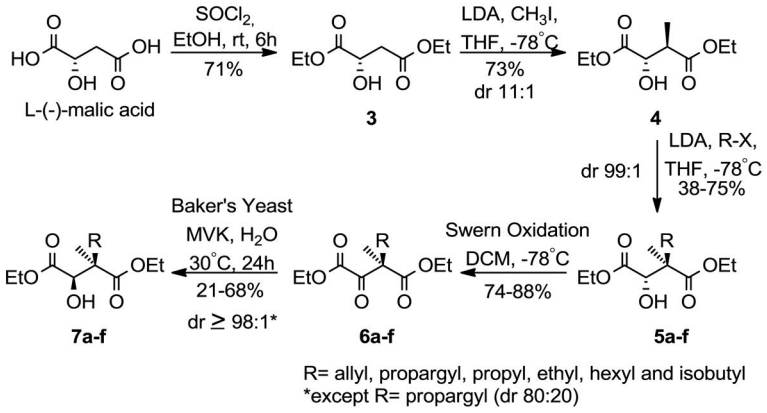


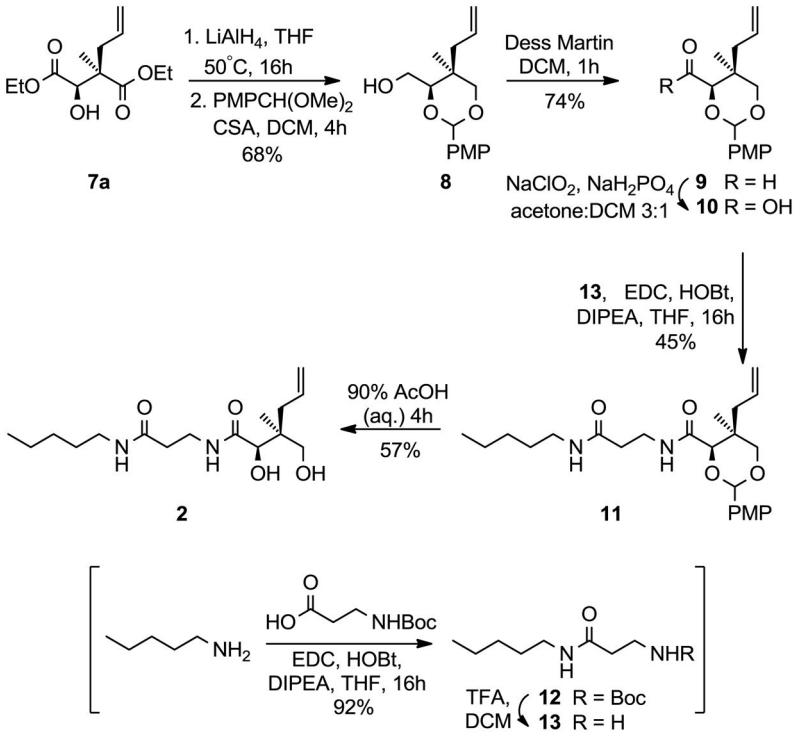
Fig. 2.

Antiplasmodial activity of the *syn*-isomer **2** in medium containing 1 μM pantothenate (black circles) or 50 μM pantothenate (white circles), in comparison with the *anti*-isomer **1** in medium containing 1 μM pantothenate (black triangles). Error bars represent SEM from 3–6 independent experiments.



Scheme 1.

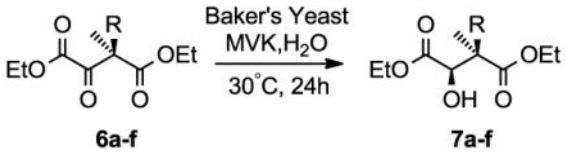
Synthesis of compounds **7a–f**.



Scheme 2.
Synthesis of compound **2**.[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.