

# Development of a method for the parallel synthesis and purification of *N*-substituted pantothenamides, known inhibitors of coenzyme A biosynthesis and utilization†

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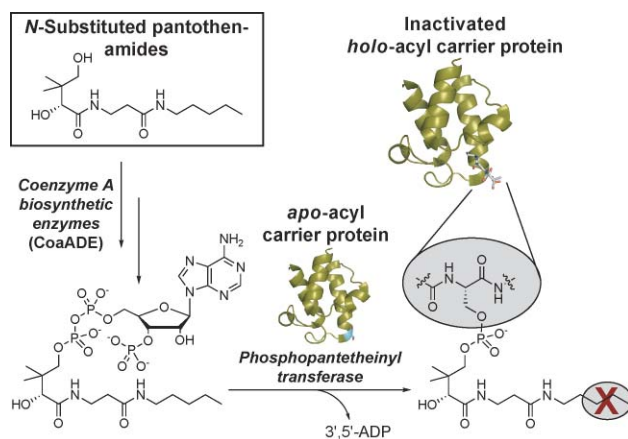
*N*-Substituted pantothenamides are a class of pantothenic acid analogues which have been shown to act as inhibitors of coenzyme A biosynthesis and utilization, especially by blocking fatty acid metabolism through formation of inactive acyl carrier proteins. To fully explore the chemical diversity and inhibitory potential of these analogues we have developed a simple method for the parallel synthesis and purification of any number of pantothenamides from a single precursor, and subsequently evaluated a small library of these compounds as inhibitors of bacterial growth to demonstrate the potential and utility of the method.

## Introduction

Coenzyme A (CoA) is a ubiquitous and essential cofactor that is mainly involved in carrying and activating acyl groups, especially those involved in fatty acid metabolism. Due to its central metabolic role, and the differences in how some of the processes that rely on CoA operate in humans and bacteria, both CoA biosynthesis and utilization have long been regarded as promising targets for antimicrobial drug development.<sup>1</sup> The search for inhibitors of these processes have mainly focused on compounds that are analogues of pantothenic acid (vitamin B<sub>5</sub>), the natural precursor of CoA. Such compounds could have a multi-faceted effect: they could potentially interfere with the cellular uptake of exogenous pantothenic acid, or inhibit one or more of the five CoA biosynthetic enzymes to reduce the levels of available intracellular CoA, or be transformed by the biosynthetic enzymes into a CoA analogue that could subsequently inhibit CoA-utilizing enzymes.

The *N*-substituted pantothenamides are a class of such pantothenic acid analogue inhibitors that have received special attention in several recent studies. Although these compounds have been known as antibacterials for some time,<sup>2</sup> it has only recently been shown that they mainly exert their inhibitory effects by being transformed *in vivo* by the CoA biosynthetic enzymes CoaADE into the corresponding CoA analogues.<sup>3</sup> Since these CoA analogues do not have the essential sulfhydryl group that confers on CoA its ability to act as an acyl carrier, they inhibit fatty acid metabolism by transferring an inactive prosthetic group to the acyl carrier proteins (ACPs) which are central to these processes (Scheme 1).<sup>4,5</sup>

Pantothenamides have also been considered promising lead compounds for the identification of pantothenate kinase (PanK,



**Scheme 1** *N*-Substituted pantothenamides (such as *N*-pentyl pantothenamide shown above) act as antimetabolites by forming coenzyme A analogues which do not contain the essential terminal thiol group of natural CoA. These analogues subsequently transfer inactive prosthetic groups to the acyl carrier proteins that are involved in fatty acid metabolism, thereby blocking these processes.

or CoaA) inhibitors.<sup>6,7</sup> As the first and committed step of CoA biosynthesis, inhibition of PanK activity presents an obvious strategy whereby CoA biosynthesis can be inhibited as an alternative approach for antimicrobial development. However, no pantothenamide characterized to date has been shown to negatively affect a PanK enzyme's ability to perform its ATP-dependent phosphorylation reaction. Instead, these compounds all seem to act as alternative (or competitive) substrates of these enzymes which transform them into 4'-phosphopantothenamides, thereby reducing their effective concentration in the cell.

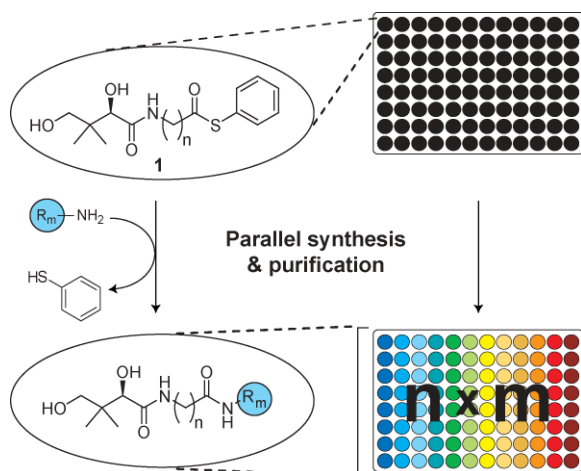
One reason why no pantothenamide that can inhibit PanK-mediated phosphorylation has been identified as yet may be due to the low-throughput manner in which they are synthesized. Each compound is usually prepared individually by activation and subsequent amidation of pantothenic acid's carboxyl group, followed

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† Electronic supplementary information (ESI) available: Detailed synthetic protocols and characterization data of thioester precursors and all *N*-substituted pantothenamides. See DOI: 10.1039/b811086g

by purification by column chromatography.<sup>3,4,6</sup> Although such a synthetic protocol is relatively simple to execute, the preparation of a large number of different *N*-substituted pantothenamides—a requirement for the effective high-throughput screening of these compounds as potential PanK inhibitors—would be a time-consuming process.

We therefore set out to develop a simple method for the parallel synthesis of a large number of pantothenamides for use in such inhibitor screens. We decided to base our method on the known reactivity of activated thioesters towards amines, since we have successfully exploited similar aminolysis chemistry to prepare different CoA analogues from so-called pre-CoA thioesters (Scheme 2).<sup>8</sup> This strategy would involve the preparation of *S*-phenyl thiopantothenate **1b** ( $n = 2$ ) as a single activated pantothenic acid precursor; this compound can subsequently be treated with a range of structurally diverse amines to allow the simultaneous preparation of a wide array of *N*-substituted pantothenamide products. To increase the structural diversity of the resulting pool of pantothenamides even further we also looked into the utilization of *S*-phenyl thio- $\alpha$ -pantothenate **1a** ( $n = 1$ ) and *S*-phenyl thiohomopantothenate **1c** ( $n = 3$ ) as similarly activated precursors. These thioesters either have one methylene group removed from or added to the  $\beta$ -alanine moiety of natural pantothenate derivatives. Finally, to ensure the practical utility of such a method in the preparation of pure compounds for screening purposes we also set out to devise a purification protocol for the removal of all excess reagents and/or unreacted starting materials as well as any potential side products that may result from the aminolysis reaction. Here we describe the details of such a method, and demonstrate that it can be employed to prepare a structurally diverse library of *N*-substituted pantothenamides. Subsequent biological evaluation of these compounds identified two previously unknown pantothenamides as inhibitors of bacterial growth, demonstrating that this method may enable us to identify the first inhibitors of PanK activity.



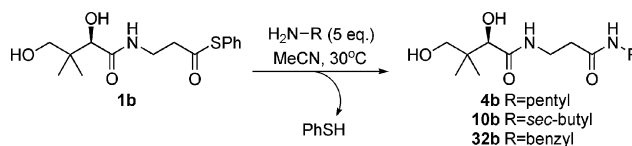
**Scheme 2** The parallel synthesis and purification of a library of *N*-substituted pantothenamides is possible by using *S*-phenyl thioesters **1** (with  $n$  number of methylene groups between the two amide groups) as precursors in aminolysis reactions with  $m$  number of amines. The resulting library would have an  $n \times m$  number of members.

## Results and discussion

### Reaction conditions for aminolysis

In the process of developing a system that will allow for the simultaneous preparation of various types of pantothenamides we set out to determine optimized conditions for the type of aminolysis reaction shown in Scheme 2. While we previously used similar aminolysis reactions to produce several CoA analogues from an *S*-phenyl thioester, the reaction design in that case was hampered by several unique limitations imposed by the chemical nature of the precursor and the resulting products.<sup>8</sup> For example, to ensure the solubility of the CoA derivatives the aminolysis reaction had to be performed in aqueous solutions, which required the use of large (up to ten equivalents) excesses of amine, as well as long (more than six hours) reaction times. Moreover, since various amines are insoluble in water this protocol precluded their use or required the addition of organic solvents to the reaction mixtures to increase their solubility. Performing the aminolysis in an aqueous solution also increased the rate of hydrolysis of the thioester precursor, and in some cases it became a significant competing side reaction. Since pantothenic acid thioesters as well as pantothenamides are soluble in organic solvents we decided to investigate whether performing the aminolysis reaction in polar solvents would address these issues. We first attempted the reaction using methanol as solvent, but excluded it since transesterification competed with the aminolysis reaction and produced methyl pantothenate as side product (data not shown). We subsequently selected acetonitrile as an alternative solvent since it has a low boiling point, allows for good solubility of reagents and was ideal for purification.

The rate of aminolysis of *S*-phenyl thiopantothenate **1b** in acetonitrile was subsequently tested in the presence of three different amines: pentylamine as an example of a primary amine, *sec*-butylamine as a typical secondary amine and benzylamine as an example of an amine with a bulky side group (Scheme 3). Tertiary amines, such as *tert*-butyl amine, have previously been shown not to be reactive and were not tested. Each reaction was performed with five equivalents of amine at 30 °C and was monitored for a decrease in the concentration of *S*-phenyl thiopantothenate **1b**. This was done by removing an aliquot of the reaction mixture at regular intervals and analyzing it by HPLC. The results are shown in Fig. 1. The data obtained for each aminolysis reaction were subsequently fit by regression analysis to the rate equation for a second-order reaction. The rate constants ( $k_{\text{obs}}$ ) and the half-lives ( $t_{1/2}$ ) of the various reactions were subsequently determined from the reaction curves obtained in this manner. These results are summarized in Table 1.

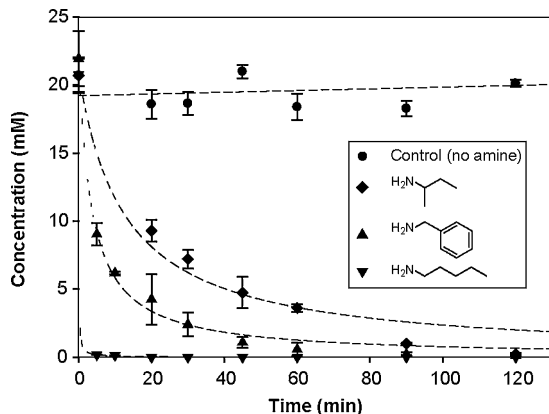


**Scheme 3** Reaction conditions used to evaluate the rate of aminolysis of *S*-phenyl thiopantothenate **1b** with different amines.

The kinetic analysis shows that the aminolysis of **1b** proceeds the fastest in the presence of a sterically unhindered primary

**Table 1** Kinetic data for the aminolysis of *S*-phenyl thiopantothenate **1b** in the presence of various amines

Amine	$k_{\text{obs}}/\text{mM}^{-1} \text{min}^{-1}$	$t_{1/2}/\text{min}$
<i>sec</i> -butylamine	$0.00380 \pm 0.0003$	12.6
benzylamine	$0.0125 \pm 0.0009$	3.65
pentylamine	$1.14 \pm 1.07$	0.0421



**Fig. 1** Evaluation of the rate of aminolysis of *S*-phenyl thiopantothenate **1b** at 30 °C in acetonitrile. The data points show the decrease in the concentration of **1b** over time in the presence of five equivalents of *sec*-butylamine (◆), benzylamine (▼) and pentylamine (▲) respectively. A control reaction (●) contained no amine. The curves (dashed lines) show the data fitted to the rate equation for a second-order reaction.

amine such as pentylamine, and that the reaction is essentially complete within five minutes (reaction half-life of ~2.5 seconds). The aminolysis with benzylamine, a primary but bulky amine is nearly a 100-fold slower, while the reaction with a secondary amine like *sec*-butylamine is nearly three-fold slower still. While the analysis indicates that the rate of an aminolysis reaction is clearly dependant on the structure of the amine, it also shows that all the reactions were more than 90% complete after two hours. Since a parallel synthesis of pantothenamides would entail the use of a large variety of structurally diverse amines we therefore decided to perform the aminolysis reactions for three hours at 30 °C to ensure the complete conversion of the thioester precursor to pantothenamide.

### Purification of pantothenamide products

With optimized aminolysis conditions in hand we set out to develop a simple purification protocol that would also be amenable for use in a parallel synthetic method. Such a protocol would mainly have to focus on the removal of the excess amine used in the aminolysis—and the thiophenol produced as a by-product of it—as the most important contaminants of the reaction. However, it may also be possible that with certain sterically hindered amines the aminolysis may not be complete after three hours, which additionally would leave some unreacted thioester in the reaction mixture. We therefore devised a purification protocol that would address the removal of all these components in step-wise fashion as shown in Scheme 4.

Two purification methods were developed. In the first and more general method (Method A), we explored the use of cation exchange chromatography with acidic resins to remove

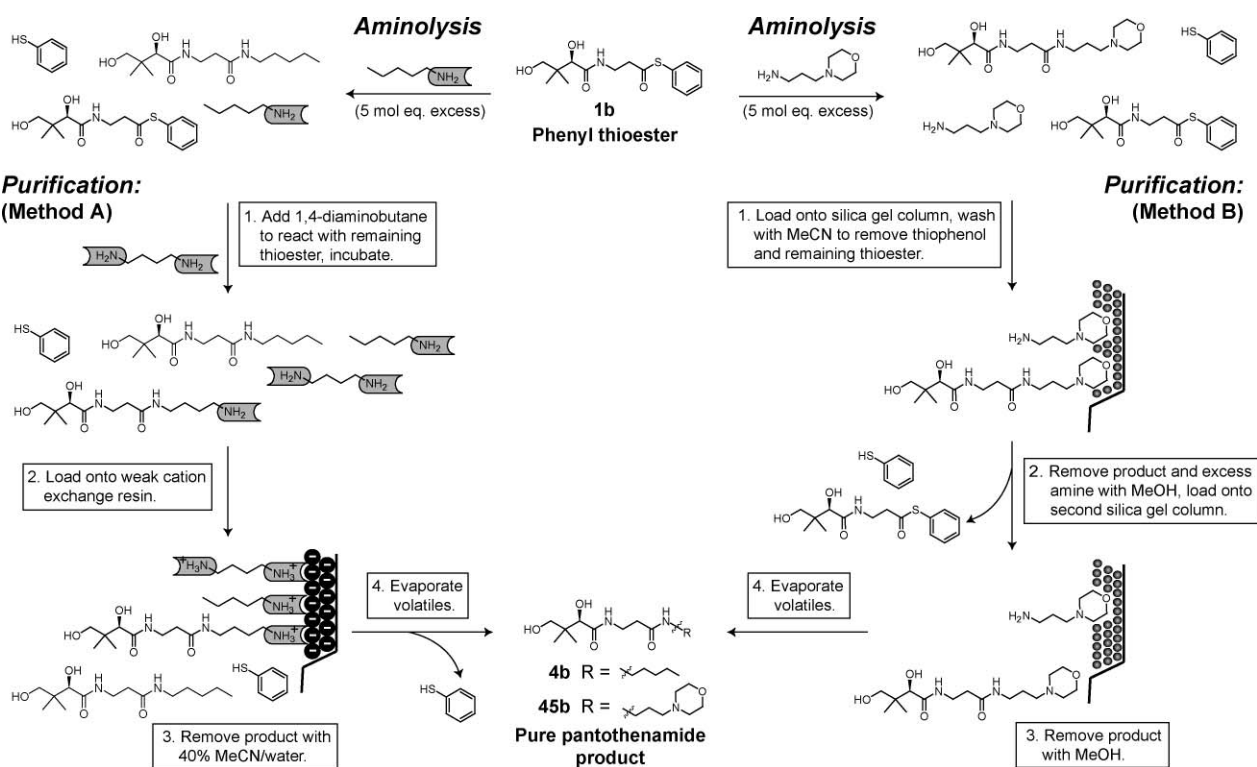
the excess amine. To enable the simultaneous removal of any potentially unreacted thioester 1,4-diaminobutane was added to the aminolysis reaction after three hours. This would result in the formation of *N*-(4-amino-butyl) pantothenamide, a compound which has a primary amine that would also be retained by a cation exchange resin. We first used strongly acidic resins in this method as such resins should be most effective in the removal of the excess amine. However, the low pH of these resins (pH < 2) was found to promote degradation of the pantothenamides to pantolactone and a  $\beta$ -alanylamine which contaminated the final product and made its purification difficult. We therefore chose to use a weakly acidic cation exchange resin which has a higher pH (pH ~ 4) and that did not cause any detectable degradation by lactonization, while still removing all the excess amine. Finally, the thiophenol produced during aminolysis as well as the reaction solvent were removed by evaporation to give the pure pantothenamides as products.

One significant limitation to this first purification method is that it cannot be applied to aminolysis reactions with amine reactants that contain an additional amine functionality (such as a tertiary amine) in its substructure as pantothenamides formed in such cases would also be retained by cation exchange resins. To address this problem we developed a silica chromatography-based purification method for such pantothenamides (Scheme 4, Method B). In this protocol, reaction mixtures were purified using two sequential silica chromatography steps: the first removes the unreacted thioester and thiophenol from the mixture, while the second separates the excess amine from the final product. Pure pantothenamides are obtained after evaporation of the solvent.

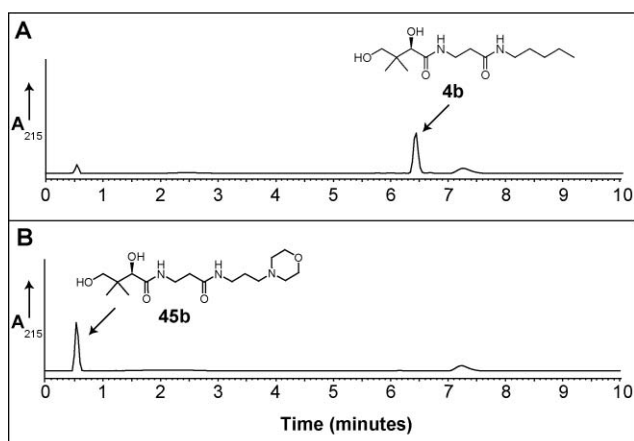
To demonstrate that these two purification methods can be successfully applied as described above, aminolysis reactions of **1b** with pentylamine and 4-(2-aminoethyl)morpholine (the examples shown in Scheme 4) were purified by following methods A and B respectively. The resulting *N*-pentyl pantothenamide **4b** and *N*-(3-morpholin-4-yl-propyl) pantothenamide **45b** purified in this manner were characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Their purity was also assessed by LC-MS analysis (Fig. 2) which indicated that the only identifiable impurity in both cases was a small amount of diphenyl phosphoric acid (the broad peak at 7.2 minutes). This contaminant is a by-product of the diphenylphosphoryl azide (DPPA)-mediated synthesis of the *S*-phenyl thioesters **1a** and **1b** which was found to be present in some batches of these compounds even after flash column chromatography. However, we found that it can be excluded through careful aqueous work-up of the coupling reaction mixtures prior to purification. Subsequent aminolysis reactions performed with thioester precursors purified in this manner and similarly analyzed by LC-MS did not show any contaminants of the aminolysis products.

### Parallel synthesis

The aminolysis reaction and the purification protocol were subsequently combined to show that these methods can be used to produce a large number of structurally and functionally diverse *N*-substituted pantothenamides in parallel. The general procedure, carried out in 96-well 2 mL deep-well plates, is outlined in Scheme 5. The aminolysis reactions were performed first by incubating the thioester **1a**, **1b** or **1c** in the presence of five equivalents amine for a period of three hours at 30 °C. For purifications by method A (Scheme 4) the reaction mixtures



**Scheme 4** Protocols used for the purification of *N*-substituted pantothenamides prepared by parallel aminolysis reactions of activated thioesters. **Method A:** Aminolysis reactions with amines that do not contain other amino functionalities are purified using cation exchange chromatography with the weakly acidic resin Amberlite IRC-86 to remove excess amine and unreacted thioester (after treatment of the latter with 1,4-diaminobutane), as exemplified by the purification of pantothenamide **4b**. **Method B:** If the amine used in the aminolysis reactions contains other amino functional groups (especially tertiary amines) the resulting pantothenamide has to be purified by means of a two-step silica purification, as in the case of the morpholine-containing **45b**.

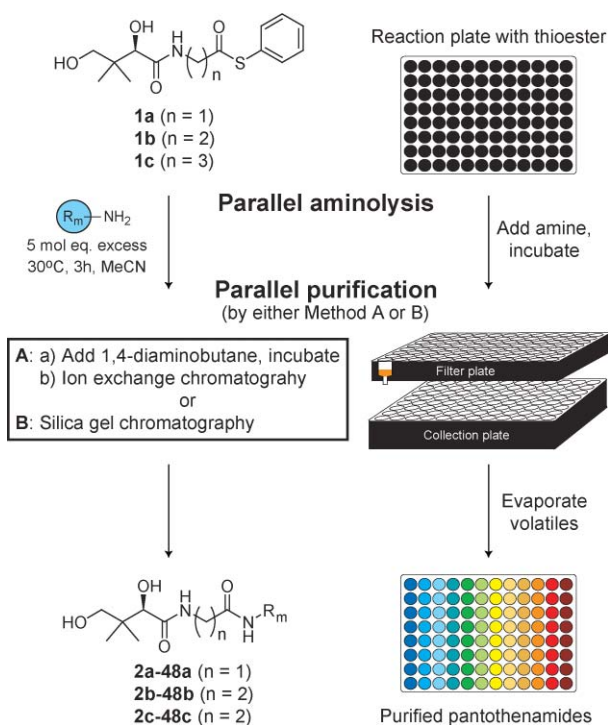


**Fig. 2** HPLC analysis of two pantothenamides formed by aminolysis of **1b** and purified by the two different methods shown in Scheme 4. *N*-pentyl pantothenamide **4b** (panel A) was purified using cation exchange resin-based method A while *N*-(3-morpholin-4-yl)propyl pantothenamide **45b** (panel B) was purified *via* silica chromatography-based method B. The only observable impurity in both cases is a small amount of diphenyl phosphoric acid, represented by the broad peak at 7.2 minutes (see text for details).

were subsequently treated with 1,4-diaminobutane to remove any unreacted thioester and then transferred to a 96-well filter plate preloaded with weakly acidic cation exchange resin. The plates were allowed to elute under gravity to ensure enough

contact time with the resin to completely remove the excess amine, as well as any *N*-(4-amino-butyl) pantothenamide formed from potentially unreacted thioester. The resin was thoroughly washed with aqueous acetonitrile and the solvent as well as the remaining thiophenol were removed by evaporation. Reaction mixtures containing pantothenamides with tertiary amine functionalities were purified by method B (Scheme 4) by transferring them to filter plates loaded with silica gel equilibrated with acetonitrile. The microcolumns were subsequently eluted with the help of a vacuum manifold. Due to the limited volume of commercially available 96-well filter plates two separate silica chromatography steps were required to remove all impurities (see Experimental section for details).

Table 2 shows the yields obtained for the aminolysis of thioesters **1a–c** with 48 structurally diverse amines to give *N*-substituted  $\alpha$ -pantothenamides **2a–49a**, *N*-substituted pantothenamides **2b–49b** and *N*-substituted homopantothenamides **2c–49c** respectively after purification. The pantothenamides **2–42** were purified by method A using cation exchange chromatography, while pantothenamides **43–49** were purified on silica gel by method B. The results show that in total 142 different pantothenamides were prepared and purified in parallel by the procedure outlined in Scheme 5, and that for the types of amines chosen the purified yields are generally in excess of 75% and in many cases above 90%. Interestingly there seems to be little correlation between the yields obtained for the three pantothenamides formed from the same amine. For example, while no product was recovered from the



**Scheme 5** Protocol for the parallel synthesis of *N*-substituted pantothenamides in 96-well format. Aminolysis reactions are performed in deep-well plates, while purifications are carried out in 96-well filter plates which act as microcolumns for chromatography. The final products are obtained after evaporation of the volatiles from the column eluates.

aminolysis reactions of *S*-phenyl pantothenate thioesters **1b** and **1c** with *tert*-butylamine (used as a supposed negative control reaction) the same reaction with *S*-phenyl  $\alpha$ -thiopantothenate **1a** gave the corresponding pantothenamide **8a** in 49% yield. However, in general the  $\alpha$ -pantothenamides and homopantothenamides were obtained in lower yields than the *N*-substituted pantothenamides formed from **1b**. This suggests that the structure of the thioester also affects the yield of the reaction, and that this phenomenon should be taken into account when such a parallel synthetic preparation of pantothenamides is performed.

### Evaluation of *N*-substituted pantothenamides as bacterial growth inhibitors

To demonstrate the application and utility of the library of prepared pantothenamides the purified compounds were tested as inhibitors of *Escherichia coli* cell growth. Previous studies have shown that *N*-substituted pantothenamides have antimicrobial activities with minimal inhibitory concentration (MIC) values ranging between 2–200  $\mu$ M for different strains of *E. coli*.<sup>4,6</sup> In particular, the known growth inhibitor *N*-pentyl pantothenamide **4b** showed inhibition of the *E. coli* wild-type strain UB1005 with a MIC of 50  $\mu$ M.<sup>6</sup> This value can therefore be taken as the current benchmark for pantothenamide inhibition of natural bacterial strains. Consequently we decided to evaluate the inhibitory potential of the pantothenamide library relative to this value to determine if any of the compounds had a similar or improved potency.

Inhibition was tested against the K12 strain of *E. coli* as it has been sustained as a lab strain with minimal genetic manipulation<sup>9</sup>

and was available in our lab. We evaluated the potency of each pantothenamide by comparing the growth of *E. coli* K12 in 1% tryptone growth medium to its growth in the same medium with 50  $\mu$ M of pantothenamide added. The growth inhibition values for each of the prepared compounds are provided in the Electronic Supplementary Information†. However, as shown in Table 3 two pantothenamides (in addition to the known inhibitor *N*-pentyl pantothenamide **4b**) were identified that also caused complete growth inhibition (less than 5% growth relative to the controls) at this concentration. These compounds are currently being investigated to determine their specific mechanism of inhibition, *i.e.* whether like *N*-pentyl pantothenamide they are mainly inhibitors of CoA utilization, or if they perhaps inhibit one of the CoA biosynthetic enzymes such as PanK. This result demonstrates that the parallel synthetic method for the preparation of pantothenamides may successfully be applied in the identification of new members of this class of pantothenic acid analogues as potential new antimicrobials.

## Conclusion

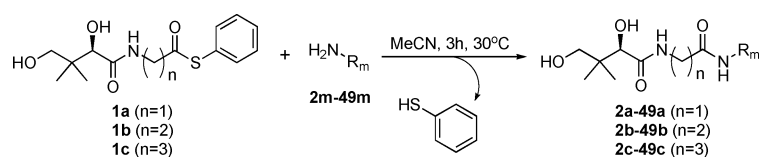
We have developed and successfully executed a general method for the parallel synthesis of a diverse array of *N*-substituted pantothenamide analogues from activated thioesters. This method is based on determined optimal aminolysis reaction times for most amines, regardless of structure. The devised protocol also addresses several purification obstacles, and has been successfully applied to the parallel purification of pantothenamides containing diverse functional groups as amide substituents. Biological evaluation of a small compound library prepared by this method allowed for the identification of two previously unknown pantothenamides as *in vivo* inhibitors of *E. coli*. This demonstrates its potential in the discovery of new pantothenic acid analogues as inhibitors of CoA biosynthesis and/or utilization.

## Experimental

### Material and methods

All chemicals, media and resins were purchased from Sigma-Aldrich (Aldrich, Sigma or Fluka), Merck Chemicals or Acros Organics and were of the highest available purity. All solvents used for reactions and purification were CHROMASOLV HPLC grade solvents from Sigma-Aldrich. Aminolysis reactions were done in 2 mL polypropylene 96-well deep-well plates from NUNC. Filtrations for purification purposes were done with AcroPrep 96-well filter plates from Pall Life Sciences. Individual polypropylene cluster tubes (Corning) were used in yield determination. Solvent evaporation from deep-well plates was done on a Labconco Centrivap concentrator. Inhibition studies were performed in Greiner Bio-one Cellstar flat-bottomed 96-well suspension culture plates. All ESI-MS and LC-MS analyses were also done at the Central Analytical Facility (CAF) at Stellenbosch University using a Waters 2690 Separations Module with a Waters 996 Photodiode Array Detector for LC separations, followed by mass analysis on a Waters Micromass Quattro mass spectrometer. All NMR analyses were performed on Varian INOVA instruments (300 MHz and 400 MHz), also at CAF.

**Table 2** Aminolysis of phenyl thioesters **1a**, **1b** and **1c** with a variety of amines (**2m–49m**) to form the corresponding *N*-substituted  $\alpha$ -pantothenamides (**2a** to **49a**), *N*-substituted pantothenamides (**2b** to **49b**) and *N*-substituted homopantothenamides (**2c** to **49c**). Yields were determined by weight determination of each purified compound, followed by their characterization by <sup>1</sup>H NMR



Entry	R-group	% Yields			Entry	R-group	% Yields		
		<b>a</b> ( $n=1$ )	<b>b</b> ( $n=2$ )	<b>c</b> ( $n=3$ )			<b>a</b> ( $n=1$ )	<b>b</b> ( $n=2$ )	<b>c</b> ( $n=3$ )
2		84	98	78	26		86	94	82
3		85	96	75	27		87	94	94
4		85	95	76	28		96	98	89
5		90	94	74	29		91	87	88
6		86	91	74	30		81	98	68
7		86	94	75	31		84	88	74
8		49	NR	NR	32		80	94	75
9		82	97	65	33		88	90	75
10		88	95	70	34		91	96	71
11		90	94	78	35		78	97	82
12		83	95	75	36		91	85	74
13		88	91	78	37		~90 <sup>a</sup>	~90 <sup>a</sup>	~61 <sup>a</sup>
14		94	92	75	38		78	92	81
15		65	99	73	39		81	95	80
16		80	94	77	40		48	87	71
17		77	78	72	41		80	93	75
18		83	91	73	42		74	84	43
19		86	94	78	43		87	79	83
20		86	94	82	44		89	87	76

**Table 2** (Contd.)

$\text{HO-CH}_2\text{-CH(OH)-CH}_2\text{-C(=O)-NH-(CH}_2\text{)}_n\text{-C(=O)-S-Ph} + \text{H}_2\text{N-R}_m \xrightarrow[\text{HS-Ph}]{\text{MeCN, 3h, 30}^\circ\text{C}}$

**1a** ( $n=1$ )  
**1b** ( $n=2$ )  
**1c** ( $n=3$ )

**2a-49a** ( $n=1$ )  
**2b-49b** ( $n=2$ )  
**2c-49c** ( $n=3$ )

Entry	R-group	% Yields			Entry	R-group	% Yields		
		a ( $n=1$ )	b ( $n=2$ )	c ( $n=3$ )			a ( $n=1$ )	b ( $n=2$ )	c ( $n=3$ )
21		88	90	84	45		85	75	65
22		85	92	87	46		80	70	72
23		81	93	74	47		75	78	66
24		79	98	78	48		81	77	67
25		86	97	78	49		55	97	78

<sup>a</sup> Yield not accurate due to impurities present in amine starting material (based on <sup>1</sup>H NMR analysis).

**Table 3** *N*-substituted pantothenamides that showed inhibition of *E. coli* K12 growth in 1% tryptone at a concentration of 50 μM

Compound	Name	Bacterial Growth (%) <sup>a</sup>
<b>4b</b>	<i>N</i> -pentyl pantothenamide	-2 ± 2
<b>14a</b>	<i>N</i> -cyclopentyl α-pantothenamide	-2 ± 5
<b>16a</b>	<i>N</i> -cyclopropylmethyl α-pantothenamide	-3 ± 1

<sup>a</sup> Inhibition was evaluated by determining cell densities (OD<sub>600</sub>) of cultures grown in the presence of inhibitor relative to control samples with no inhibitor added. The reported values are the averages of four separately tested samples, with the associated standard deviation.

### Aminolysis reaction conditions

**Sample analysis.** The rates of aminolysis were determined by adding 20 mM *S*-phenyl thioester pantothenate **1b** to a solution of 100 mM amine (either pentylamine, benzylamine or *sec*-butylamine) in acetonitrile in a total volume of 1 mL. A negative control reaction contained no amine. Periodically 50 μL aliquots were removed from the solution and immediately loaded onto 30 mg (dry weight) of Amberlite IRC-86 ion exchange resin prewashed with water. The resin was washed with 250 μL 40% aqueous acetonitrile, and the eluent was concentrated until all the solvent was removed. The residue was redissolved in 500 μL 50% acetonitrile and 15 μL of each sample was analyzed by HPLC on a Hewlett-Packard series 1100 HPLC system with dual wavelength detection at 215 and 254 nm by using a Gemini 5 μ C<sub>18</sub> (110A), 2.0 × 30 mm column equilibrated with 100% solution A (0.01% TFA in MilliQ water) and 0% solution B (acetonitrile). The column was eluted with a linear gradient increasing to 100% B over 10 minutes, followed by an isocratic elution with 100% solution B for a further 5 minutes. A flow rate of 0.35 mL min<sup>-1</sup> was maintained. The

concentration of the *S*-phenyl thioester pantothenate **1b** remaining in each sample was determined by integration of the corresponding peak area, followed by correlation of this area to a standard curve of known concentrations of **1b** prepared as described below. All samples were analyzed in triplicate.

**Thioester standard curve.** A standard curve of known *S*-phenyl thioester pantothenate concentrations was obtained by HPLC analysis of thioester samples with concentrations ranging between 0 and 25 mM. From each of these samples 50 μL aliquots were removed which were treated and analyzed exactly as described above. Samples were injected in triplicate, and the standard curve obtained by integration of the resulting peak areas.

**Data fitting and analysis.** The resulting data were analyzed by plotting the determined concentrations of **1b** against time, followed by nonlinear regression analysis of the data using a second-order rate eqn (1)

$$f(x) = \frac{[A]_0}{1 + [A]_0 k_{\text{obs}} x} \quad (1)$$

The reaction rate constants ( $k_{\text{obs}}$ ) were obtained directly in this manner (as one of the equation parameters) while the half-life of each reaction was calculated from the rate constants. Data analysis and plotting were performed using SigmaPlot 9.0 (Systat software).

### Parallel synthesis and purification

**General procedure.** The parallel aminolysis of *S*-phenyl thioesters **1a**, **1b** and **1c** with amines **2m–49m** were performed using 48 different amines (in fivefold excess) in 100% acetonitrile as solvent. Solutions of the amines (100 mM final) in acetonitrile were loaded into a 2 mL 96-well deep-well plate, followed by addition of

the desired thioester (20 mM final) also dissolved in acetonitrile. The resulting solutions had a final volume of 1 mL. The deep-well plate was capped and incubated at 30 °C while shaking vigorously for 3 hours.

**Purification method A.** After 3 hours the reaction mixtures of compounds **2a–42a**, **2b–42b** and **2c–42c** were treated with 1,4-diaminobutane (100 µL; 550 mM). The resulting solutions were incubated with shaking at 30 °C for another 60 minutes before being loaded onto pre-washed Amberlite IRC-86 weak cation exchange resin (300 mg dry weight per well) contained in a 1 mL AcroPrep 96-well filter plate. The resin was eluted under gravity, and was washed twice with 300 µL 40% aqueous acetonitrile. The combined eluates were dried overnight on a Centrivap centrifugal concentrator under reduced pressure. The resin was subsequently washed with another 1200 µL 40% aqueous acetonitrile and the eluate added to the dried products. The resulting solutions were transferred to individual pre-weighed 96-well cluster tubes and dried for another 72 hours by centrifugal concentration under reduced pressure to remove all the solvent and thiophenol present in the mixture. The cluster tubes containing the dried products were subsequently weighed individually to determine the purified yield of each compound.

**Purification method B.** The reaction mixtures of compounds **43a–49a**, **43b–49b** and **43c–49c** were transferred to 1 mL AcroPrep 96-well filter plates pre-loaded with silica gel (300 mg dry weight per well) that had been equilibrated with 100% acetonitrile. The microcolumns were subsequently eluted by using a 96-well plate vacuum manifold. The silica was then washed with 500 µL acetonitrile, followed by elution of the product as well as some remaining amine from the silica with ~3.6 mL methanol. The eluates were captured in a new 96-well plate and were dried overnight by centrifugal concentration under reduced pressure. The resulting residues were resuspended in 150 µL methanol before being loaded onto a second 1 mL AcroPrep 96-well filter plate pre-loaded with silica gel (300 mg dry weight per well) that had been equilibrated with 100% methanol. The products were eluted with 2 mL methanol for **44a–c**, **45a–c**, **48a–c** as well as **49a–c**. Compounds **43a–c**, **46a–c** and **47a–c** were only fully removed from the silica after elution with 6 mL of methanol. The eluates were collected in individual pre-weighed 96-well cluster tubes, followed by removal of the solvent by centrifugal concentration over 48 hours. The yields of the purified compounds were determined by individual weighing of the cluster tubes containing the products.

**Characterization of *N*-substituted pantothenamides.** Pantothenamides **4a**, **4b**, **4c**, **45a**, **45b** and **45c** were fully characterized by <sup>1</sup>H and <sup>13</sup>C NMR to demonstrate the efficacy and utility of the two purification methods. The purity of these compounds were further verified by LC-MS analysis on a X-bridge C<sub>18</sub> (110A) 3.5 µm, 2.1 × 50 mm column. The column was equilibrated with 100% solution A (0.01% TFA in MilliQ water) and 0% solution B (acetonitrile). The products eluted with a linear gradient increasing to 100% B over 10 minutes, followed by a isocratic elution with 100% solution B for a further

5 minutes. A flow rate of 0.35 mL min<sup>-1</sup> was maintained. In all cases the compounds gave single peaks at 215 nm in these analyses. The optical purity of pantothenamides **4b** and **45b** was assessed by performing <sup>1</sup>H NMR analysis in the presence of increasing amounts of tris-[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium (Eu(tfc)<sub>3</sub>). All other *N*-substituted pantothenamides were characterized by <sup>1</sup>H NMR. The detailed characterization data are provided in the Electronic Supplementary Information (ESI)†.

#### Growth of *E. coli* K12 with *N*-substituted pantothenamides

Inhibition assays were performed by preparing a starter culture of *E. coli* K12 in 1% tryptone containing four separate colonies grown on LB agar plates. The starter culture was grown to mid-log phase and then diluted 30 000-fold in the same medium. A 10 µL aliquot of the diluted cell suspension was used to inoculate each well of a 96-well flat-bottomed plate containing 100 µL of 1% tryptone broth supplemented with a specific *N*-substituted pantothenamide in final concentration of 50 µM. The plates were incubated at 37 °C for 20 hours before the cell densities were measured by reading the absorbance in each well at 600 nm. The extent of growth in each well was determined by normalizing the OD<sub>600</sub> values relative to those of the negative control (containing 3% acetonitrile instead of pantothenamide), which were taken as 100% growth. Each compound was tested in quadruplicate.

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