



Geminal dialkyl derivatives of N-substituted pantothenamides: Synthesis and antibacterial activity

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ABSTRACT

As a key precursor of coenzyme A (CoA) biosynthesis, pantothenic acid has proven to be a useful backbone to elaborate probes of this biosynthetic pathway, study CoA-utilizing systems, and design molecules with antimicrobial activity. The increasing prevalence of bacterial strains resistant to one or more antibiotics has prompted a renewed interest for molecules with a novel mode of antibacterial action such as N-substituted pantothenamides. Although numerous derivatives have been reported, most are varied at the terminal N-substituent, and fewer at the β -alanine moiety. Modifications at the pantoil portion are limited to the addition of an ω -methyl group. We report a synthetic route to N-substituted pantothenamides with various alkyl substituents replacing the geminal dimethyl groups. Our methodology is also applicable to the synthesis of pantothenic acid, pantetheine and CoA derivatives. Here a small library of new N-substituted pantothenamides was synthesized. Most of these compounds display antibacterial activity against sensitive and resistant *Staphylococcus aureus*. Interestingly, replacement of the *ProR* methyl with an allyl group yielded a new N-substituted pantothenamide which is amongst the most potent reported so far.

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1. Introduction

The discovery and clinical use of antibacterial agents in the early 1900's has dramatically improved our quality of life and longevity, yet infectious diseases remain a leading cause of morbidity and mortality worldwide. The problem is exacerbated by the rapid spread of bacterial resistance to clinically used antibacterial agents.^{1–3} Since antibiotics have a finite lifetime before resistance occurs, maintaining a regular pipeline of novel antibiotics is increasingly recognized as highly important. Molecules with a novel mode of action are of special interest due to the limited possibility of cross-resistance. Although some steps in coenzyme A (CoA) biosynthesis have been validated as targets for the development of antibacterial agents, none of the currently approved antibacterials affect this pathway. The discovery of pantothenic acid (Fig. 1) as a key precursor in the biosynthesis of CoA at the turn of the century⁴ led to significant efforts towards generating analogues with antibacterial, antimalarial and antifungal activity.⁵ Modifications reported at the β -alanine fragment of pantothenic acid mainly involved replacement of the carboxylic acid end with other functional groups.^{6–12} Early on, limited variations in the pantoil fragment of pantothenic acid were also exploited.^{7,13–16}

N-Substituted pantothenamides are an important family of pantothenic acid derivatives (Fig. 1). Their antibacterial activity was

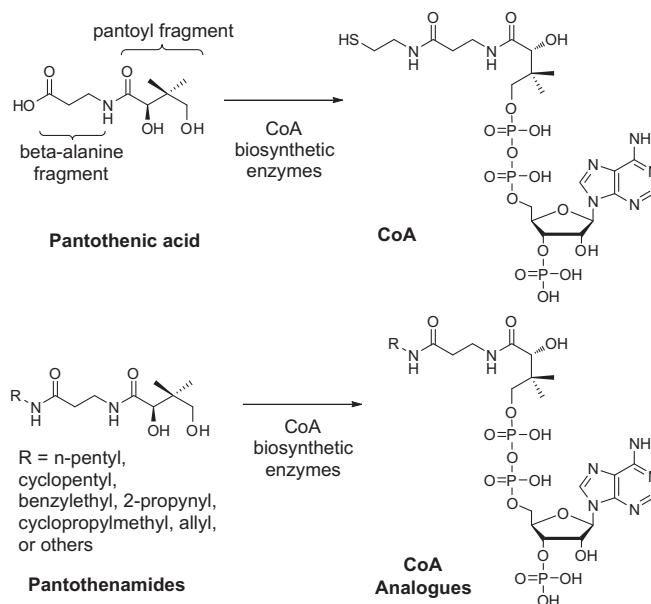


Figure 1. Structure of pantothenic acid and N-substituted pantothenamides.

first demonstrated by Skinner and coworkers¹⁷ against *Escherichia coli* and subsequently against *Staphylococcus aureus*.¹⁸ This class of

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compounds among which *N*-pentylpantothenamide (N5-pan, **9k**) is regarded as the benchmark, was originally assumed to exert its activity by inhibition of CoA biosynthesis. A study performed by Strauss and Begley however revealed that N5-pan (**9k**) is converted in vitro to ethyldethia-CoA by the *E. coli* CoA biosynthetic enzymes.¹⁹ The authors postulated that the antibacterial activity of N5-pan (**9k**) may result from the inhibition of CoA-utilizing enzymes. This hypothesis was later corroborated by Jackowski and coworkers who demonstrated that pantothenamides were converted in vivo to CoA derivatives which irreversibly inhibited the acyl carrier protein (ACP) of the fatty acid biosynthetic machinery.²⁰ Studies with *S. aureus* indicate a similar mode of action.²¹ These studies and a recent one²² also suggest that the mechanism of action of pantothenamides may be more complex than described above.

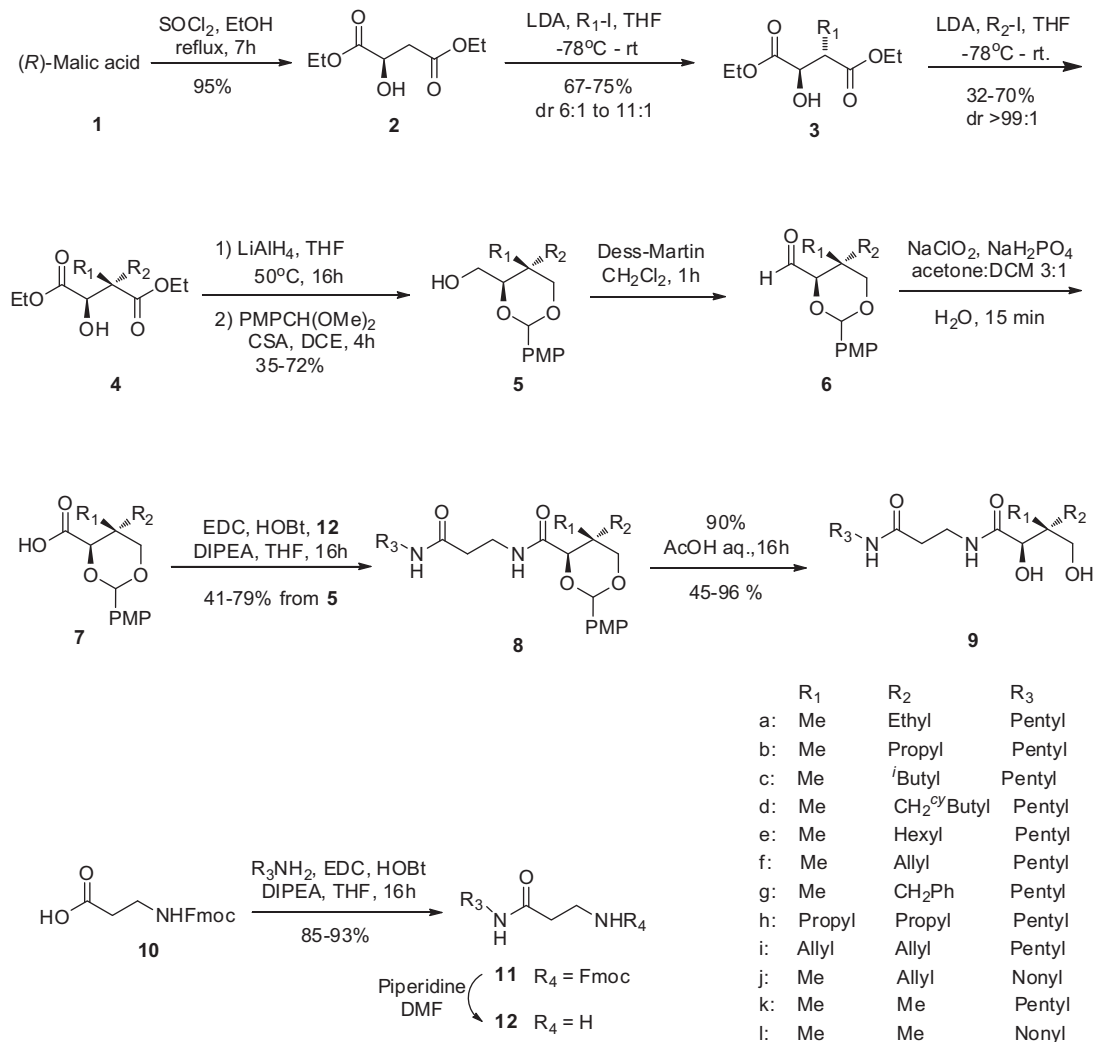
With the rapid spread of drug-resistant pathogens, there has been a renewed interest for *N*-substituted pantothenamides.^{5,23–26} Although numerous pantothenamides have been reported, the pantooyl fragment is typically conserved amongst them all, except for a few variants with an added ω -methyl group.¹³ To the best of our knowledge, there is no stereoselective methodology reported to replace the geminal dimethyl groups of pantothenic acid, pantothenamide, or pantetheine with other substituents. Pantothenic acid derivatives with one of the geminal dimethyl groups lacking or replaced with ethyl,¹⁵ phenyl⁷ or cyclopentyl¹⁶ have

been synthesized as diastereomeric mixtures, and a didemethyl CoA analogue has also been reported,²⁷ but variations at this position have otherwise not been explored. We believe that this position may provide an interesting diversification point, especially if the configuration at the quaternary carbon can be controlled. We report here a stereoselective synthetic route to *N*-substituted pantothenamide analogues with various geminal dialkyl substituents replacing the geminal dimethyl groups. This route is easily adapted to the synthesis of pantothenic acid, pantetheine, or CoA derivatives. We also report on the biological activity of the newly generated *N*-substituted pantothenamides.

2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds was achieved via a sequential double alkylation of (*R*)-diethyl malate (**2**). Compound **2** was generated from commercial (*R*)-malic acid (**1**) in 95% yield via thionyl chloride mediated esterification in refluxing absolute ethanol. Using a method reported by Seebach et al.²⁸ the lithium dianion generated by the addition of 2 equivalents of LDA to **2** was alkylated with methyl iodide, propyl iodide or allyl bromide, affording 6:1, 9:1 and 11:1 diastereomeric mixtures, respectively (**3**, Scheme 1). Initial attempts to carry out the second alkylation on



Scheme 1. Synthetic route to new *N*-substituted pantothenamides.

the lithium dianion of **3** ($R_1 = \text{Me}$) with alkyl bromides (instead of iodides) was only successful with benzyl and allyl bromides. The reaction with other bromides (propyl-, cyclobutylmethyl-, and 2-methylpropyl-) proceeded to <5% with 90% recovered starting material. Even higher concentrations and longer reaction times did not help improve the yields. Alkylation of **3** ($R_1 = \text{Me}$) with allyl bromide produced a single adduct **4f**, whereas reaction with benzyl bromide resulted in a 16:1 diastereomeric ratio of **4g** favoring the *S* configuration at the quaternary center. The *S* configuration (based on diethyl malate) is favored at the quaternary center due to the expected chelation of the lithium alkoxide generated in the reaction to the carbonyl group, thereby promoting addition from the opposite face relative to the alkoxide.^{29,30} A switch to the more reactive alkyl iodides yielded exclusively single adducts of **4a–4e** (>99% diastereoselectivity based on ¹H NMR of the crude reaction mixture). Similarly, a second alkylation of the lithium dianion of **3** ($R_1 = \text{propyl}$ or allyl) with propyl iodide and allyl iodide respectively afforded the corresponding **4h** and **4i** with both geminal dimethyl groups replaced.

In an attempt to reverse the configuration at the quaternary center of compounds **4a–4g**, the order of alkylation was reversed, with the larger electrophile added first, followed by the smaller one. Unfortunately, this methodology did not proceed with good diastereoselectivity and produced inseparable mixtures of compounds.

Diastereomers **4a–4i** were next extended to the target *N*-substituted pantothenamides **9a–9l** as follows. Global reduction to the corresponding triols using LiAlH_4 , followed by selective protection of the 1,3-diol using a method reported by Shiina and coworkers,³⁰ afford the *p*-anisaldehyde acetals **5a–5l**. Dess–Martin oxidation of **5a–5l**³¹ followed by Pannick oxidation as reported by Burkart³² furnished acids **7a–7l** which were used without further purification.

In parallel, Fmoc protected β -alanine (**10**) prepared according to a literature protocol³³ was coupled to the appropriate alkyl amines in the presence of EDC to furnish **11** ($R_3 = \text{pentyl}$ or nonyl) in excellent yields. Following deprotection with piperidine in DMF, the resulting compounds **12** ($R_3 = \text{pentyl}$ or nonyl) were coupled to acids **7a–7l** using EDC or HBTU. Products **8a–8l** were obtained in 41–79% yields over the four step sequence from **5a–5l**. Not surprisingly, the yields for the formation of allyl derivatives **8f**, **8i** and **8j**

were lower than for the other derivatives (<50%). This is presumably the result of side reactions during the Pannick oxidation. Acetic acid-mediated deprotection of the *p*-anisaldehyde acetals **8a–8l** in the presence of water furnished the desired *N*-substituted pantothenamides **9a–9l**.

Compound **9m** was prepared in 33% yield over three steps from **9f** as described in Scheme 2. After TBS protection of **9f** to **13**, the product was subjected to cyclopropanation according to a protocol reported by Takemoto and co-workers.³⁴ TBAF mediated deprotection afforded the desired compound **9m**.

2.2. Biology

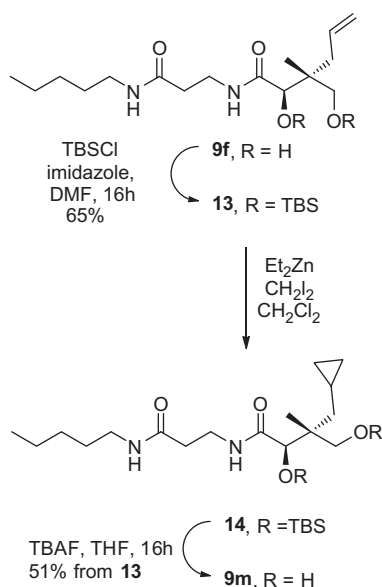
Of the antibiotic resistant microbes, *S. aureus* is one of the most notorious. In recent years this multi-drug resistant pathogen (often termed MRSA) has emerged as the major cause of nosocomial infection.³⁵ The antibacterial activity of target compounds **9a–9m** was evaluated against the bacterial *S. aureus* strains ATCC 29213 (oxacillin susceptible) and ATCC 43300 (oxacillin resistant, abbreviated MRSA), according to a standard procedure.³⁶ The MIC values obtained are listed in Table 1. A preliminary screen of these pantothenamides against other strains (*Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 13883, *E. coli* ATCC 25922 and 11775, and *Bacillus subtilis* ATCC 6051 and 6633) using the agar diffusion method suggests no antimicrobial activity (inhibition zone <1.1 cm for a 1 cm disc impregnated with the desired compound).

Varying the chain length of R_2 provides important structure–activity relationships (SARs) about the antibacterial activity of *N*-substituted pantothenamides. In our hands, the well characterized N5-pan (**9k**) shows a MIC activity of 7 μM , in agreement with previous reports.¹⁸ Replacement of the *ProR* methyl group (R_2) with small alkyl chains, such as ethyl (**9a**), appears to have little effect on the MIC. As the alkyl group gets longer however, the antibacterial activity decreases sharply, with MIC values ranging from 13 μM for $R_2 = n\text{Pr}$ (**9b**), to below detection for $R_2 = \text{hexyl}$ (**9e**). R_2 substituents with more steric bulk are also detrimental. This trend is observed with the methylcyclopropyl (**9m**), isobutyl (**9c**), methylcyclobutyl (**9d**), and benzyl (**9g**) derivatives, which show activities of 24, 101, 374 and >703 μM , respectively (MICs for **9c** and **9d** are within error). Interestingly the allyl substituted *N*-pentyl-pantothenamide **9f** makes exception to these rules. Compound **9f** is the most potent N5-pan derivative reported here (MIC of 3.2 μM), and twice as potent as the parent compound N5-pan (**9k**).

Compounds **9h** and **9i** were designed to investigate the effect of different substituents at R_1 . The diallyl substituted derivative (**9i**) had an MIC of 376 μM , which is much weaker than that of the singly substituted compound (**9f**, MIC of 3.2 μM). Confirmation of this trend was obtained with the singly (**9b**) and doubly (**9h**) substituted propyl derivatives, with respective MICs of 13 μM and >744 μM . These results are consistent with a poor tolerance of the system for variations at R_1 , and hence no further attempts were made to prepare other derivatives at R_1 .

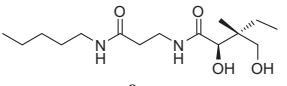
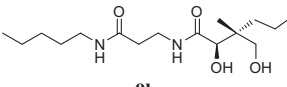
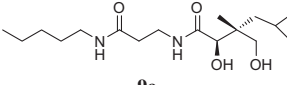
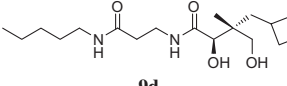
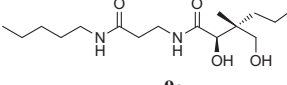
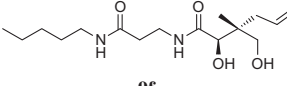
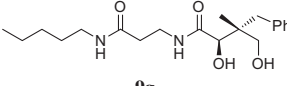
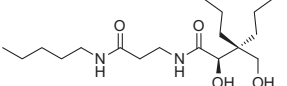
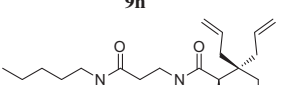
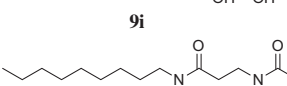
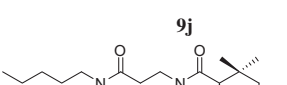
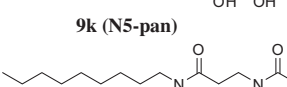
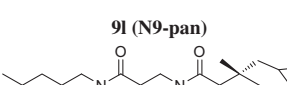
A previous report¹⁸ has shown that when the R_3 pentyl group is replaced with either a heptyl or a nonyl group, the antibacterial activity is increased. Our data for the known N9-pan (**9l**) corroborate the previous report by others.¹⁸ With this trend in mind, an R_2 -allyl substituted pantothenamide was synthesized with a nonyl (**9j**) group at R_3 . The MIC of compound **9j** (1 μM), was significantly improved over that of the parent compound **9f** (3.2 μM), consistent with the trend reported.¹⁸ In this case however, the methyl to allyl modification (**9l** vs **9j**) did not lead to an improved activity (MICs are within error).

As mentioned above, the generally accepted mechanism of action for *N*-substituted pantothenamides involves conversion to the corresponding CoA analog by the bacterial CoA biosynthetic enzymes pantothenate kinase (PanK or *coaA*), phosphopantetheine



Scheme 2. Synthesis of the methyl cyclopropyl pantothenamide analogue **9m**.

Table 1
Antibacterial activity of N-substituted pantothenamides **9a–9m**

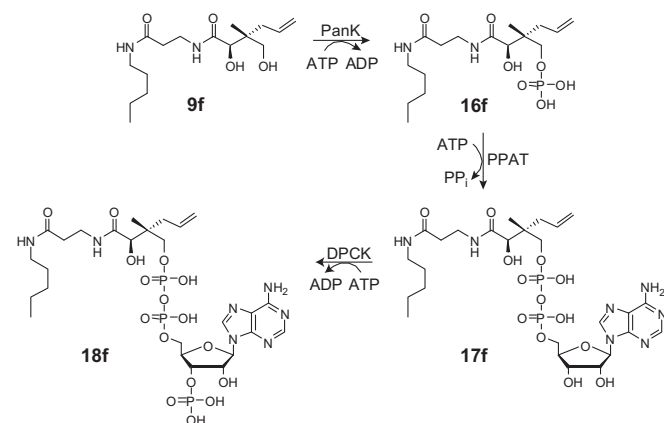
	MIC (μM) ^a	
	<i>S. aureus</i> ^b	MRSA ^c
	7 ± 6	7 ± 3
	13 ± 7	13 ± 2
	101 ± 45	51 ± 15
	374 ± 187	374 ± 153
	>715	>715
	3.2 ± 0.8	3.2 ± 0.9
	>703	>703
	>744	>744
	376 ± 217	376 ± 109
	1 ± 0.9	1 ± 0.7
	7 ± 2	7 ± 2
	0.4 ± 0.2	0.4 ± 0.2
	24 ± 14	24 ± 14

^a Minimum inhibitory concentrations (MICs) were determined using the following concentrations: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 $\mu\text{g/mL}$. The lowest concentration where complete cell death was observed was determined to be the MIC and then converted to μM using the molecular weight of each compound.

^b *Staphylococcus aureus* strains ATCC 29213 (oxacillin susceptible).

^c ATCC 43300 (oxacillin resistant or MRSA).

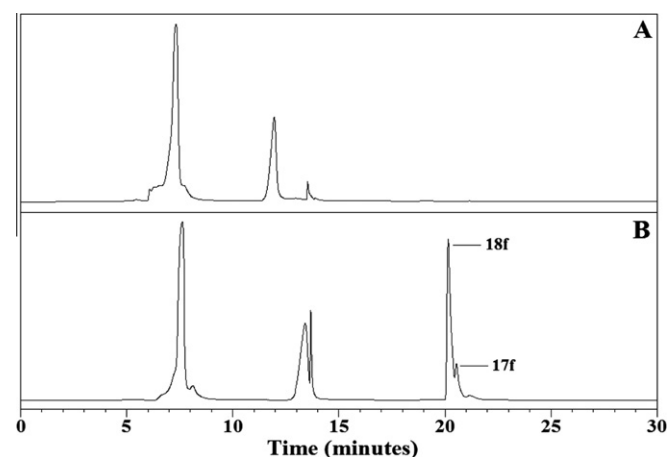
adenylyltransferase (PPAT or coaD), and dephosphocoenzyme A kinase (DPCK or coaE). The resulting CoA antimetabolites are believed to inhibit acyl carrier protein function, and thereby disrupt

**Figure 2.** Proposed cellular transformation of **9f** into **16f**, **17f**, and **18f** by the CoA biosynthetic enzymes PanK, PPAT, and DPCK.

fatty acid biosynthesis.^{19,20} To probe whether the N-substituted pantothenamides reported here proceed via a similar mechanism, compound **9f** was treated with PanK, the rate-limiting enzyme of the CoA biosynthetic pathway. The K_m and k_{cat} for this transformation were $534 \pm 159 \mu\text{M}$ and $0.87 \pm 0.04 \text{ s}^{-1}$, respectively. Although the K_m is many folds higher than that of the natural substrate pantothenic acid ($17 \pm 2 \mu\text{M}$), the k_{cat} is unaffected ($1.07 \pm 0.04 \text{ s}^{-1}$).¹⁹ Overall, the conversion of compound **9f** by the CoA biosynthetic enzymes PanK, PPAT, and DPCK is expected to yield compound **18f** (Fig. 2). An in vitro assay was performed with compounds **9f** and all three enzymes. LC–MS analysis of the reaction mixture (Fig. 3) confirms the capacity of this enzyme system to produce **18f**.

3. Conclusions

In summary, we have developed a stereoselective synthetic approach to geminal dialkyl analogues of N-substituted pantothenamides. This route should easily adapt to the preparation of various derivatives of the biologically important pantothenic acid, pantetheine, and CoA. Thus our synthetic methodology provides access to new probes for mechanistic and structural studies of the numerous pathways generating or utilizing these

**Figure 3.** LC–MS analysis of the transformation of compound **9f** by the CoA biosynthetic enzymes PanK, PPAT and DPCK. The enzymatic reaction mixtures were allowed to react either with water as a control (A) or **9f** (B). The control with natural substrate is not shown. The mass observed for **17f** and **18f** were 723.98 and 803.86, respectively.

cofactors. The antibacterial studies reported here for a small library of new pantothenamides reveal some interesting trends. Because of the numerous enzymes and pathways involved in the mode of action of pantothenamides,^{18–22} it is not possible at this time to explain these SARs in terms of the targets structures. Although replacement of the *ProS* methyl group (R_1 substituent) with larger alkyl groups was detrimental, substitution of the *ProR* methyl group (R_2 substituent) was successful at improving the antibacterial activity of N5-pan (**9k**) against *S. aureus* and MRSA. Interestingly, the size of the substituent negatively correlated with activity, except for the allyl group which was favored over methyl. Thus besides providing new SARs for the design of pantothenamides with improved antibacterial activity, this study has generated three compounds that are equivalent (**9a**) or superior (**9f** and **9j**) to N5-pan (**9k**), the model pantothenamide. Investigations into the mode of action for compound **9f** strongly suggest that, as with other N-substituted pantothenamides,^{19,20} **9f** is first converted to the corresponding CoA analogue before exerting its antibacterial effect. This is supported by kinetic studies with purified PanK, and by evidence of full conversion to the corresponding CoA derivative in the presence of PanK, PPAT, and DPCK. Prior to this study, very few modifications at the pantoyl moiety of pantothenamides had been reported. We hope that this study will encourage a more systematic exploration of this part of the molecule.

4. Experimental section

4.1. Antibacterial activity (broth dilution method)

Antimicrobial activity of compounds **9a–9m** were tested against *S. aureus* ATCC 29213 (oxacillin susceptible) and 43300 (oxacillin resistant), which were grown on Trypticase soy media (BD, Mississauga, ON). Antibacterial activity was determined using the microdilution broth method in 96-well microtiter plates according to the National Committee for Clinical Laboratory Standards (NCCLS).³⁶ For solubility purposes, compounds **9a–9m** were dissolved in 10% (v/v) acetone in water and then serially diluted to concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 $\mu\text{g/mL}$. A bacterial suspension of 10^6 cfu/mL was then used to inoculate each well, which was incubated for 18 h at 37 °C. The MIC was taken as the lowest concentration demonstrating no visible growth in the broth. All data are the average of the results from at least four separate experiments.

4.2. Antibacterial activity (agar diffusion method)

The antimicrobial activity of compounds **9a–9m** was measured against *S. aureus* ATCC 29213, *A. baumannii* ATCC 19606, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922 and 11775, and *B. subtilis* ATCC 6051 and 6633. Antibacterial activity was determined using the agar diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS).³⁶ Mueller-Hinton agar plates (Fluka) were inoculated with bacteria by streaking with a cotton swab and allowing to dry for 5 min before applying drug impregnated discs. Solutions of compounds **9a–9m** (150 $\mu\text{g/mL}$) were prepared and then aliquoted (40 μL) onto sterile discs (1 cm diameter, Fluka). Upon placement of drug impregnated discs, plates were incubated at 37 °C (*S. aureus*, *A. baumannii*, *K. pneumoniae*, and *E. coli*) or 30 °C (*B. subtilis*) for 24 h. Antibacterial activity is expressed as the diameter of the inhibition zone (mm) produced by the compounds. Ampicillin (Sigma) was used as a positive control (2.0 cm for *E. coli* 11775; 1.6 cm for *E. coli* 25922; 3.3 cm for *B. subtilis* 6051; 3.2 cm for *B. subtilis* 6633; 1.6 cm for *S. aureus* 29213; 1.1 cm for *K. pneumoniae* 13883; and 1.1 cm for *A. baumannii* 19606).

4.3. PanK enzyme activity

E. coli pantothenate kinase (PanK or *coaA* enzyme) was expressed and purified as previously described.³⁸ Enzyme activity was measured as described in the literature.¹⁹ This assay couples the production of ADP to the consumption of NADH through the activity of pyruvate kinase and lactic dehydrogenase. NADH consumption was monitored at 340 nm. Reactions were performed at 25 °C in an Agilent 8453 UV–vis spectrophotometer coupled to an Agilent 89090A Peltier temperature controller. Kinetic parameters were determined by fitting the rate data into the Michaelis–Menten equation using Prism 4.0 (GraphPad). Each reaction mixture (500 μL) contained ATP (1.5 mM), NADH (0.3 mM), phosphor(enol)pyruvate (0.5 mM), MgCl_2 (10 mM), KCl (20 mM), pyruvate kinase (5 units), lactic dehydrogenase (5 units), and PanK (5 μg , 278 nM) in Tris-HCl buffer (50 mM, pH 7.6). The reaction was initiated with the addition of the desired substrate (10–160 μM).

4.4. Transformation of N-substituted pantothenamides by a mixture of PanK, PPAT and DPCK

The *E. coli* enzymes pantothenate kinase (PanK or *coaA* enzyme), phosphopantetheine adenyltransferase (PPAT or *coaD* enzyme), and dephosphocoenzyme A kinase (DPCK or *coaE* enzyme) were expressed and purified as previously described.³⁸ Each reaction mixture (500 μL) contained ATP (5.0 mM), MgCl_2 (10 mM), PanK (5 μg , 278 nM), PPAT (5 μg , 500 nM), DPCK (5 μg , 454 nM) in Tris-Cl buffer (50 mM, pH 7.6). The reaction was initiated with the addition of pantetheine (3.0 mM) for comparison, water as a negative control, or compound **9f** (3.0 mM). Reactions were incubated for 5 hours at room temperature and then stopped by heating the mixture to 95 °C for 5 min. The precipitated protein was removed by centrifugation (13,000 rpm for 5 min) and the supernatant was analyzed by LC/MS. Reversed-phase analytical HPLC was performed with an analytical 4.60 \times 250 mm, SYNERGI 4 μ Hydro-RP 80A (Phenomenex, Torrance, CA) column coupled to an Agilent 6120 Quadrupole LC/MS system for ESI-MS analysis. The HPLC conditions had the sample elute at a flow rate of 0.5 mL min^{-1} using a combination of mobile phase A (H_2O) and mobile phase B (acetonitrile). Elution conditions are as follows: isocratic 1% phase B from 0 to 3 min; followed by linear gradients of phase B: 1–10% from 3 to 5 min; 10–15% from 5 to 10 min; 15–30% from 10 to 13 min; and finally isocratic phase B at 30% from 13 to 26 min. The detector was set to 260 nm.

4.5. Synthesis of compounds

4.5.1. (Iodomethyl) cyclobutane³⁷

To a flask containing sodium iodide (5.33 g, 35.56 mmol) was added (bromomethyl)cyclobutane (2.65 g, 17.78 mmol) and acetone (10 mL). The mixture was refluxed at 60 °C for 18 h. The solid was filtered off and the filtrate was concentrated. Diethyl ether was added to the concentrate to trigger the precipitation of sodium iodide and sodium bromide which were filtered off. This was repeated three times until no solid was seen in the oil. The filtrate was then concentrated to afford a light yellowish oil that was stored over a copper wire (2.14 g, 65% yield). ^1H NMR (CDCl_3 , 300 MHz) δ 3.23 (d, J = 7.8, 2H), 2.72–2.56 (m, 1H), 2.12–2.02 (m, 1H), 1.89–1.56 (m, 4H). ^{13}C NMR (CDCl_3 , 75 MHz) δ 38.4, 29.1, 15.8, 14.5.

4.5.2. General procedure for alkylations²⁸

To freshly prepared LDA at -78°C [2.2 equiv, prepared by adding *n*BuLi (2.2 equiv) to diisopropylamine (2.4 equiv) in THF (0.05–0.1 M total reaction concentration) at 0 °C and stirred for 15 min]

was added dropwise (*R*)-diethyl malate (1 equiv) dissolved in THF. The reaction was allowed to warm up to -40°C over 1 h, and re-cooled to -78°C before addition of the alkyl halide (2.7 equiv). The mixture was then stirred for 16 h while warming up to room temperature. The reaction was cooled down to -78°C and quenched with saturated ammonium chloride. The organic layer was separated and the aqueous layer was extracted with ethyl acetate ($3 \times 30\text{ mL}$). The combined organic layers were washed with brine, dried over sodium sulphate and evaporated to give an oil that was purified by flash chromatography on silica gel (1:1.5 diethyl ether/hexanes, v/v).

4.5.3. (2*S*,3*R*)-Diethyl-2-ethyl-3-hydroxy-2-methylsuccinate (4a)²⁸

Prepared in 40% yield from **3a** according to the general procedure. R_f 0.40 (Et₂O/hexanes, 1:1). ¹H NMR (CDCl₃, 500 MHz) δ 4.28–4.18 (m, 5H), 3.36 (d, $J = 7.9$, 1H), 1.88–1.81 (m, 1H), 1.63–1.56 (m, 1H), 1.30 (t, $J = 7.2$, 3H), 1.28 (t, $J = 7.1$, 3H), 1.15 (s, 3H), 0.88 (t, $J = 7.5$, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 174.8, 172.9, 75.6, 61.7, 60.9, 50.4, 28.2, 16.8, 14.2, 14.2, 8.7. HRMS for C₁₁H₂₀O₅ [M+Na]⁺ calcd 255.1203, found 255.1205.

4.5.4. (2*S*,3*R*)-Diethyl-3-hydroxy-2-methyl-2-propylsuccinate (4b)^{1,2}

Prepared in 48% from **3a** according to the general alkylation procedure. R_f 0.39 (Et₂O/hexanes, 1:1, v/v). ¹H NMR (CDCl₃, 500 MHz) δ 4.81 (q, $J = 7.1$, 2H), 4.29–4.21 (m, 3H), 3.37 (d, $J = 5.9$, 1H), 1.75 (ddd, $J = 4.4$, 12.5, 13.4, 1H), 1.52 (ddd, $J = 4.6$, 12.5, 13.4, 1H), 1.40–1.32 (m, 1H), 1.31 (t, $J = 7.2$, 3H), 1.28 (t, $J = 7.1$, 3H), 1.24–1.17 (m, 1H), 1.15 (s, 3H), 0.91 (t, $J = 7.3$, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 174.9, 172.9, 75.8, 61.7, 60.9, 50.1, 37.7, 17.6, 17.4, 14.6, 14.2, 14.1. HRMS for C₁₂H₂₂O₅ [M+Na]⁺ calcd 269.1359, found 269.1361.

4.5.5. (2*S*,3*R*)-Diethyl-3-hydroxy-2-isobutyl-2-methylsuccinate (4c)

Prepared in 52% from **3a** according to the general alkylation procedure. R_f 0.34 (Et₂O/hexanes, 1:1, v/v). ¹H NMR (CDCl₃, 500 MHz) δ 4.30–4.15 (m, 5H), 3.40 (d, $J = 8.2$, 1H), 1.83 (dd, $J = 7.1$, 13.9, 1H), 1.73–1.65 (m, 1H), 1.50 (dd, $J = 5.2$, 13.9, 1H), 1.30 (t, $J = 7.1$, 3H), 1.29 (t, $J = 7.1$, 3H), 1.16 (s, 3H), 0.93 (d, $J = 6.6$, 3H), 0.85 (t, $J = 6.6$, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 175.1, 172.6, 76.7, 61.7, 60.9, 49.9, 44.0, 24.8, 24.6, 23.29, 17.04, 14.2, 14.1. HRMS for C₁₃H₂₄O₅ [M+Na]⁺ calcd 283.1516, found 283.1519.

4.5.6. (2*S*,3*R*)-Diethyl-2-(cyclobutylmethyl)-3-hydroxy-2-methylsuccinate (4d)

Prepared in 58% from **3a** according to the general alkylation procedure. R_f 0.32 (Et₂O/hexanes, 1:1, v/v). ¹H NMR (CDCl₃, 500 MHz) δ 4.27–4.14 (m, 4H), 3.42 (d, $J = 8.3$, 1H), 2.40–2.30 (m, 1H), 2.07–2.10 (m, 1H), 1.99–1.93 (m, 2H), 1.88–1.59 (m, 4H), 1.30 (t, $J = 7.2$, 3H), 1.29 (t, $J = 7.2$, 3H), 1.11 (s, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 174.9, 172.8, 76.1, 61.7, 60.9, 49.7, 42.5, 32.5, 30.3, 29.4, 19.0, 17.2, 14.2, 14.1. HRMS for C₁₄H₂₄O₅ [M+Na]⁺ calcd 295.1516, found 295.1518.

4.5.7. (2*S*,3*R*)-Diethyl-2-hexyl-3-hydroxy-2-methylsuccinate (4e)

Prepared in 60% from **3a** according to the general alkylation procedure. R_f 0.40 (Et₂O/hexanes, 1:1, v/v). ¹H NMR (CDCl₃, 500 MHz) δ 4.29–4.15 (m, 5H), 3.37 (d, $J = 8.1$, 1H), 1.80–1.74 (m, 1H), 1.52 (dd, $J = 4.0$, 13.2, 1H), 1.31–1.26 (m, 14H), 1.15 (s, 3H), 0.88 (t, $J = 7.0$, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 174.9, 172.9, 75.8, 61.7, 60.9, 50.0, 35.5, 31.6, 29.7, 24.2, 22.6, 17.3, 14.2, 14.2, 14.0. HRMS for C₁₅H₂₈O₅ [M+Na]⁺ calcd 311.1829, found 311.1834.

4.5.8. (2*S*,3*R*)-Diethyl-2-allyl-3-hydroxy-2-methylsuccinate (4f)

Prepared in 70% yield from **3a** according to the general alkylation procedure. R_f 0.33 (Et₂O/hexanes, 1:1, v/v). ¹H NMR (CDCl₃, 500 MHz) δ 5.80–5.71 (m, 1H), 5.13–5.08 (m, 2H), 4.28–4.22 (m, 3H), 4.18 (q, $J = 7.2$, 2H), 3.36 (d, $J = 6.9$, 1H), 2.54 (dd, $J = 7.0$, 13.7, 1H), 2.34 (dd, $J = 7.8$, 13.7, 1H), 1.31 (t, $J = 7.2$, 3H), 1.28 (t, $J = 7.2$, 3H), 1.18 (s, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 174.3, 172.8, 133.0, 118.9, 75.3, 61.8, 61.0, 49.9, 39.5, 17.9, 14.1, 14.1. HRMS for C₁₂H₂₀O₅ [M+Na]⁺ calcd 267.1203, found 267.1205.

4.5.9. (2*S*,3*R*)-Diethyl-2-benzyl-3-hydroxy-2-methylsuccinate (4g)^{1,2}

Prepared from **3a** according to the general alkylation procedure in 60% yield with a 16:1 ratio of diastereoisomers. R_f 0.33 (Et₂O/hexanes, 1:1, v/v). ¹H NMR (CDCl₃, 500 MHz) major isomer δ 7.29–7.19 (m, 5H), 4.28–4.21 (m, 2H), 4.17–4.31 (m, 3H), 3.70 (d, $J = 8.3$, 1H), 3.22 (d, $J = 13.3$, 1H), 3.00 (d, $J = 13.3$, 1H), 1.32 (t, $J = 7.1$, 3H), 1.23 (t, $J = 7.1$, 3H), 1.12 (s, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 174.6, 172.8, 136.3, 130.5, 130.5, 128.1, 128.1, 126.8, 75.3, 61.7, 61.1, 50.7, 41.2, 17.7, 14.2, 14.0. HRMS for C₁₆H₂₂O₅ [M+Na]⁺ calcd 317.1359, found 317.1364.

4.5.10. (*R*)-Diethyl 3-hydroxy-2,2-dipropylsuccinate (4h) and (*R*)-diethyl 2,2-diallyl-3-hydroxysuccinate (4i)

They were difficult to purify from the reaction mixture because their R_f was very close to that of the starting materials (**3h** and **3i**, respectively). A mixture containing some starting materials **3h** and **3i** was carried over to the next reaction (reduction and selective protection) from where pure **5h** and **5i** were obtained.

4.5.11. General procedure for reduction and protection (4–5)

LiAlH₄ (3 mol equivalent) was suspended in THF (0.07 M total concentration) at 0°C under an atmosphere of nitrogen. A solution of the appropriate diester (**4**) in THF was added dropwise over 3 min and the ice bath was removed. The reaction was stirred at 50°C for 16 h, cooled to room temperature and the excess reagent was quenched with solid sodium sulphate decahydrate (Na₂SO₄·10 H₂O, added until evolution of hydrogen stops). H₂SO₄ (18 N) was added until pH 2 and the reaction was neutralized with solid sodium bicarbonate (NaHCO₃). The solids were filtered off through Celite®, through Na₂SO₄, and washed with excess THF. The filtrate was concentrated to give an oil which was re-dissolved in dichloroethane (0.02 M). Anisaldehyde dimethyl acetal (1.5 mol equivalent) and camphor sulfonic acid (CSA, 0.1 mol equivalent) were added and the mixture was stirred for 4 h at room temperature. Triethyl amine (excess, 1–2 mL) was added, the mixture was concentrated and the residue was purified by flash chromatography on silica gel [EtOAc/hexanes 1:4 (v/v) → 3:7 (v/v)].

4.5.12. ((4*R*,5*R*)-5-Ethyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (5a)

Prepared in 58% yield from **4a** according to the general procedure. R_f 0.20 (EtOAc/hexanes, 3:7). ¹H NMR (CDCl₃, 500 MHz) δ 7.44 (d, $J = 8.5$, 2H), 6.91 (d, $J = 8.5$, 2H), 5.44 (s, 1H), 3.81 (s, 3H), 3.80 (d, $J = 11.0$, 1H), 3.76–3.62 (m, 4H), 1.37–1.23 (m, 2H), 1.13 (s, 3H), 0.87 (t, $J = 7.8$, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 160.1, 130.9, 127.5, 127.5, 113.7, 113.7, 101.8, 85.1, 76.8, 61.4, 55.3, 34.0, 28.2, 16.8, 7.3. HRMS for C₁₅H₂₂O₄ [M+H]⁺ calcd 267.1591, found 267.1594.

4.5.13. ((4*R*,5*R*)-2-(4-Methoxyphenyl)-5-methyl-5-propyl-1,3-dioxan-4-yl)methanol (5b)

Prepared in 72% yield from **4b** according to the general procedure. R_f 0.23 (EtOAc/hexanes, 3:7, v/v). ¹H NMR (CDCl₃, 500 MHz) δ 7.44 (d, $J = 8.5$, 2H), 6.91 (d, $J = 8.5$, 2H), 5.45 (s, 1H), 3.81 (s, 3H), 3.80 (d, $J = 11.0$, 1H), 3.75–3.63 (m, 4H), 1.35–1.16 (m, 4H),

1.14 (s, 3H), 0.91 (t, $J = 6.9$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 130.9, 127.5, 127.5, 113.7, 113.7, 101.8, 85.2, 77.1, 61.4, 55.3, 38.3, 34.2, 17.4, 16.1, 15.0. HRMS for $\text{C}_{16}\text{H}_{24}\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 281.1747, found 281.1745.

4.5.14. ((4*R*,5*R*)-5-Isobutyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (5c)

Prepared in 84% from **4c** according to the general procedure. R_f 0.24 (EtOAc/hexanes, 3:7, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.44 (d, $J = 8.5$, 2H), 6.91 (d, $J = 8.5$, 2H), 5.45 (s, 1H), 3.90 (d, $J = 11.1$, 1H), 3.81 (s, 3H), 3.73–3.71 (m, 3H), 3.64 (dd, $J = 9.8$, 11.8, 1H), 1.73 (sep, $J = 6.6$, 1H), 1.20 (dd, $J = 5.9$, 14.4, 1H), 1.18 (s, 3H), 1.14 (dd, $J = 5.6$, 14.4, 1H), 0.96 (d, $J = 6.6$, 3H), 0.93 (d, $J = 6.6$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 130.9, 127.5, 127.5, 113.7, 113.7, 101.8, 85.4, 77.3, 61.4, 55.3, 44.8, 34.9, 25.51, 25.46, 23.3, 17.8. HRMS for $\text{C}_{17}\text{H}_{26}\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 295.1904, found 295.1905.

4.5.15. ((4*R*,5*R*)-5-(Cyclobutylmethyl)-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (5d)

Prepared in 35% from **4d** according to the general procedure. R_f 0.38 (EtOAc/hexanes, 1:1.5, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.5$, 2H), 6.90 (d, $J = 8.5$, 2H), 5.43 (s, 1H), 3.81 (s, 3H), 3.76 (d, $J = 11.6$, 1H), 3.72–3.61 (m, 4H), 2.43–2.37 (m, 1H), 2.07–2.03 (m, 2H), 1.91–1.83 (m, 1H), 1.78–1.72 (m, 1H), 1.69–1.59 (m, 2H), 1.40–1.25 (m, 2H), 1.11 (s, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 130.9, 127.5, 127.5, 113.7, 113.7, 101.8, 85.1, 61.5, 55.3, 43.3, 35.1, 31.6, 30.8, 30.5, 30.5, 18.9, 17.7. HRMS for $\text{C}_{18}\text{H}_{26}\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 329.1723, found 329.1727.

4.5.16. (4*R*,5*R*)-5-Hexyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (5e)

Prepared in 57% from **4e** according to the general procedure. R_f 0.30 (EtOAc/hexanes, 3:7, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.44 (d, $J = 8.8$, 2H), 6.91 (d, $J = 8.8$, 2H), 5.45 (s, 1H), 3.81 (s, 3H), 3.79 (d, $J = 12.8$, 1H), 3.75 (ddd, $J = 2.5$, 6.6, 9.0, 1H), 3.71–3.63 (m, 3H), 1.31–1.18 (m, 10H), 1.14 (s, 3H), 0.89 (t, $J = 7.0$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 130.9, 127.5, 127.5, 113.7, 113.7, 101.8, 85.2, 77.1, 61.4, 55.3, 36.0, 34.1, 31.7, 30.2, 22.7, 22.6, 17.4, 14.1. HRMS for $\text{C}_{19}\text{H}_{30}\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 323.2217, found 323.2223.

4.5.17. (4*R*,5*R*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (5f)

Prepared in 82% yield from **4f** with a minor modification to the general procedure; the excess LiAlH_4 from the reduction reaction was quenched with NaOH (5 mL of 1 M) at 0 °C and stirred for 30 min at room temperature. R_f 0.18 (EtOAc/hexanes, 3:7, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (d, $J = 8.5$, 2H), 6.91 (d, $J = 8.5$, 2H), 5.81–5.68 (m, 1H), 5.44 (s, 1H), 5.13–5.07 (m, 2H), 3.82–3.69 (m, 4H), 3.81 (s, 3H), 3.67 (dd, $J = 9.0$, 11.6, 1H), 2.06–1.96 (m, 2H), 1.16 (s, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 132.2, 130.7, 127.5, 127.5, 118.8, 113.7, 113.7, 101.8, 84.6, 77.0, 61.3, 55.3, 40.3, 34.3, 17.5. HRMS for $\text{C}_{16}\text{H}_{22}\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 279.1591, found 279.1589.

4.5.18. ((4*R*,5*R*)-5-Benzyl-2-(4-methoxyphenyl)-5-methyl-1,3-dioxan-4-yl)methanol (5g)

Prepared in 62% from **4g** according to the general procedure. ^1H NMR (CDCl_3 , 500 MHz) δ 7.41 (d, $J = 8.6$, 2H), 7.32–7.29 (m, 2H), 7.26–7.24 (m, 1H), 7.12 (d, $J = 6.8$, 2H), 6.89 (d, $J = 8.6$, 2H), 5.40 (s, 1H), 3.90–3.82 (m, 3H), 3.80 (s, 3H), 3.78–3.72 (m, 2H), 3.59 (d, $J = 11.2$, 1H), 2.59 (s, 2H), 1.17 (s, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 135.9, 130.7, 130.2, 130.2, 128.2, 128.2, 127.5, 127.5, 126.7, 113.7, 113.7, 101.7, 85.6, 77.0, 61.5, 55.3, 42.1, 34.9, 17.6. HRMS for $\text{C}_{20}\text{H}_{24}\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 329.1747, found 329.1749.

4.5.19. ((4*R*)-2-(4-Methoxyphenyl)-5,5-dipropyl-1,3-dioxan-4-yl)methanol (5h)

Prepared in 62% from **4h** according to the general procedure (90% purity). R_f 0.31 (EtOAc/hexanes, 3:7). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (d, $J = 8.8$, 2H), 6.91 (d, $J = 8.8$, 2H), 5.45 (s, 1H), 3.98 (d, $J = 11.4$, 1H), 3.86 (dd, $J = 3.2$, 8.3, 1H), 3.82–3.80 (m, 1H), 3.81 (s, 3H), 3.72 (d, $J = 12.8$, 1H), 3.62 (d, $J = 11.4$, 1H), 1.94 (ddd, $J = 4.5$, 13.0, 13.5, 1H), 1.47–1.12 (m, 8H), 0.94 (t, $J = 7.2$, 3H), 0.91 (t, $J = 6.9$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 130.9, 127.6, 127.6, 113.7, 113.7, 101.9, 85.7, 73.6, 61.4, 55.3, 36.6, 36.3, 33.6, 17.2, 16.8, 15.2, 15.1. HRMS for $\text{C}_{18}\text{H}_{28}\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 309.2060, found 309.2062.

4.5.20. ((4*R*)-5,5-Diallyl-2-(4-methoxyphenyl)-1,3-dioxan-4-yl)methanol (5i)

Prepared in 46% from **4i** with a minor modification to the general procedure; the excess LiAlH_4 from the reduction reaction was quenched with NaOH (5 mL of 1 M) at 0 °C and stirred for 30 min at room temperature. R_f 0.26 (EtOAc/hexanes, 3:7, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.44 (d, $J = 8.8$, 2H), 6.91 (d, $J = 8.8$, 2H), 5.96–5.80 (m, 2H), 5.46 (s, 1H), 5.19–5.10 (m, 4H), 3.96 (d, $J = 11.5$, 1H), 3.93 (dd, $J = 2.8$, 8.7, 1H), 3.81 (s, 3H), 3.81 (dd, $J = 2.8$, 5.2, 1H), 3.78 (d, $J = 2.8$, 1H), 3.74 (dd, $J = 8.7$, 11.7, 1H), 3.64 (dd, $J = 1$, 11.5, 1H), 2.83 (dd, $J = 7.5$, 13.8, 1H), 2.10–2.00 (m, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.2, 133.8, 132.6, 130.7, 127.6, 127.6, 118.9, 118.8, 113.7, 113.7, 102.0, 84.6, 72.9, 61.0, 55.3, 37.7, 37.3, 35.2. HRMS for $\text{C}_{18}\text{H}_{24}\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 327.1567, found 327.1576.

4.5.21. General procedure for amide coupling (5–8)

Dess–Martin periodinane (2 mol equiv) was suspended in CH_2Cl_2 and the alcohol in wet CH_2Cl_2 (1 mol equiv) was added. The mixture was stirred at room temperature for 1 h (monitored by TLC), diluted with diethyl ether and washed with a solution of $\text{Na}_2\text{S}_2\text{O}_3$ in sat. NaHCO_3 (1.5 g $\text{Na}_2\text{S}_2\text{O}_3$ in 5 mL of aqueous saturated NaHCO_3). The organic layer was dried over anhydrous sodium sulfate and concentrated to give the product which was used in the next reaction without purification. To a suspension of the crude aldehyde in a mixture of acetone and CH_2Cl_2 (3:1, v/v) was added a freshly prepared solution of NaH_2PO_4 (10 mol equiv) and NaClO_2 (5 mol equiv) in water (1:3 v/v ratio of water to the total volume of acetone/ CH_2Cl_2). The mixture was stirred at rt for 15 min. After evaporation of the solvent in vacuo, the residue was dissolved in a saturated solution of sodium sulfite (1 mL per mol equivalent of NaClO_2). The aqueous solution was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated to give the acid which was also used without purification. The appropriate Fmoc protected amine (**11**, 1.5 mol equiv) was dissolved in DMF (10 mL per 200 mg) and piperidine (1.5 mL per 200 mg sample) was added. The solvent and excess piperidine were evaporated off under vacuum. To this residue was added EDC (3 mol equiv), HOBT (3.6 mol equiv) and the flask was purged with nitrogen. A solution of the above crude acid in THF was added followed by DIPEA (10 equiv) and the mixture was stirred overnight at room temperature (16 h). The reaction mixture was poured into a separatory funnel containing an aqueous solution of saturated ammonium chloride, before extraction with ethyl acetate. The combined organic layers were dried over sodium sulfate and evaporated to give a solid. Purification by flash chromatography on silica gel (EtOAc/hexanes, 1:1 \rightarrow 5:1) afforded the desired product.

4.5.22. (4*R*,5*R*)-5-Ethyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8a)

Prepared in 58% yield from **5a** according to the general procedure. R_f 0.31 (EtOAc/hexanes 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.6$, 2H), 7.00 (t, $J = 6.2$, 1H), 6.92 (d, $J = 8.6$, 2H), 5.78 (br s, 1H), 5.43 (s, 1H), 4.17 (s, 1H), 3.85 (d, $J = 11.4$, 1H),

3.82 (s, 3H), 3.77 (d, $J = 11.4$, 1H), 3.61–3.46 (m, 2H), 3.22–3.18 (m, 2H), 2.41 (t, $J = 6.2$, 2H), 1.79–1.71 (m, 1H), 1.60–1.53 (m, 1H), 1.49–1.43 (m, 2H), 1.33–1.25 (m, 4H), 1.09 (s, 3H), 0.91 (t, $J = 7.6$, 3H), 0.87 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.6, 160.2, 130.2, 127.5, 127.5, 113.8, 113.8, 101.1, 82.7, 76.2, 55.3, 39.6, 36.3, 35.5, 35.0, 29.2, 29.0, 28.1, 22.3, 17.2, 14.0, 7.4. HRMS for $\text{C}_{23}\text{H}_{36}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 421.2697, found 421.2712.

4.5.23. (4*R*,5*R*)-5-Propyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8b)

Prepared in 67% from **5b** according to the general procedure. R_f 0.41 (EtOAc/hexanes, 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.6$, 2H), 7.01 (t, $J = 6.1$, 1H), 6.92 (d, $J = 8.6$, 2H), 5.78 (br s, 1H), 5.43 (s, 1H), 4.17 (s, 1H), 3.85 (d, $J = 11.4$, 1H), 3.82 (s, 3H), 3.78 (d, $J = 11.4$, 1H), 3.75 (ddd, $J = 6.4$, 12.5, 13.9, 1H), 3.39 (ddd, $J = 6.2$, 12.5, 13.9, 1H), 3.22–3.18 (m, 2H), 2.41 (t, $J = 6.2$, 2H), 1.66 (ddd, $J = 5.0$, 12.2, 14.0, 1H), 1.52–1.43 (m, 3H), 1.39–1.23 (m, 6H), 1.10 (s, 3H), 0.93 (t, $J = 7.2$, 3H), 0.88 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.6, 160.2, 130.2, 127.5, 127.5, 113.7, 113.7, 101.2, 82.8, 76.6, 55.3, 39.6, 38.0, 36.3, 35.6, 35.0, 29.2, 29.0, 22.3, 17.7, 16.2, 14.9, 14.0. HRMS for $\text{C}_{24}\text{H}_{38}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 435.2854, found 435.2860.

4.5.24. (4*R*,5*R*)-5-Isobutyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8c)

Prepared in 75% from **5c** according to the general procedure. R_f 0.17 (EtOAc/hexanes, 3:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.7$, 2H), 7.02 (t, $J = 6.1$, 1H), 6.92 (d, $J = 8.7$, 2H), 5.84 (br s, 1H), 5.44 (s, 1H), 4.13 (s, 1H), 3.95 (dd, $J = 11.4$, 12.8, 1H), 3.82 (s, 3H), 3.81 (d, $J = 12.1$, 1H), 3.61–3.47 (m, 2H), 3.24–3.17 (m, 2H), 2.42 (t, $J = 6.2$, 2H), 1.79–1.71 (m, 2H), 1.50–1.40 (m, 3H), 1.35–1.23 (m, 4H), 1.14 (s, 3H), 0.97 (d, $J = 6.3$, 3H), 0.96 (d, $J = 6.3$, 3H), 0.89 (t, $J = 6.9$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.7, 169.6, 160.2, 130.2, 127.5, 127.5, 113.8, 113.8, 101.1, 83.2, 76.8, 55.3, 44.2, 39.6, 36.4, 36.2, 35.0, 29.2, 29.0, 25.52, 25.48, 23.3, 22.3, 17.8, 14.0. HRMS for $\text{C}_{25}\text{H}_{40}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 449.3010, found 449.3016.

4.5.25. (4*R*,5*R*)-5-(Cyclobutylmethyl)-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8d)

Prepared in 61% from **5d** according to the general procedure. R_f 0.46 (EtOAc/hexanes, 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.41 (d, $J = 8.8$, 2H), 7.00 (t, $J = 6.2$, 1H), 6.92 (d, $J = 8.8$, 2H), 5.76 (br s, 1H), 5.42 (s, 1H), 4.12 (s, 1H), 3.83 (d, $J = 11.5$, 1H), 3.82 (s, 3H), 3.73 (d, $J = 11.5$, 1H), 3.61–3.54 (m, 1H), 3.52–3.46 (m, 1H), 3.22–3.18 (m, 2H), 2.48–2.39 (m, 3H), 2.08–1.99 (m, 2H), 1.90–1.82 (m, 2H), 1.77–1.66 (m, 3H), 1.60 (dd, $J = 7.1$, 14.7, 1H), 1.49–1.44 (m, 2H), 1.35–1.23 (m, 4H), 1.08 (s, 3H), 0.89 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.6, 160.2, 130.2, 127.4, 127.4, 113.7, 113.7, 101.1, 83.0, 77.3, 55.3, 42.8, 39.6, 36.6, 36.3, 35.0, 31.7, 30.65, 30.58, 29.2, 29.0, 22.3, 19.0, 17.6, 14.0. HRMS for $\text{C}_{26}\text{H}_{40}\text{N}_2\text{O}_5$ $[\text{M}+\text{Na}]^+$ calcd 483.2829, found 483.2836.

4.5.26. (4*R*,5*R*)-5-Hexyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8e)

Prepared in 71% from **5e** according to the general procedure. R_f 0.28 (EtOAc/hexanes, 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.8$, 2H), 7.01 (t, $J = 6.3$, 1H), 6.92 (d, $J = 8.8$, 2H), 5.97 (s, 1H), 5.43 (s, 1H), 4.18 (br s, 1H), 3.84 (d, $J = 11.5$, 1H), 3.82 (s, 3H), 3.78 (d, $J = 11.5$, 1H), 3.62–3.46 (m, 2H), 3.22–3.18 (m, 2H), 2.42–2.40 (m, 2H), 1.68–1.60 (m, 1H), 1.54–1.43 (m, 3H), 1.35–1.22 (m, 12H), 1.09 (s, 3H), 0.89 (t, $J = 7.0$, 3H), 0.88 (t, $J = 7.0$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.6, 160.2, 130.2, 127.5, 127.5, 113.7, 113.7, 101.1, 82.8, 76.6, 55.3, 39.6, 36.3, 35.7, 35.6, 35.0, 31.8, 30.2, 29.2, 29.0, 22.9, 22.7, 22.3, 17.7, 14.1, 14.0. HRMS for $\text{C}_{27}\text{H}_{44}\text{N}_2\text{O}_5$ $[\text{M}-\text{H}]^-$ calcd 475.3177, found 475.3187.

4.5.27. (4*R*,5*R*)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8f)

Prepared in 48% from **5f** according to the general procedure. R_f 0.31 (EtOAc/hexanes, 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.6$, 2H), 7.05 (t, $J = 5.2$, 1H), 6.92 (d, $J = 8.6$, 2H), 5.90–5.82 (m, 1H), 5.78 (s, 1H), 5.42 (s, 1H), 5.15–5.11 (m, 2H), 4.19 (s, 1H), 3.82 (s, 3H), 3.81 (d, $J = 11.2$, 1H), 3.78 (d, $J = 11.2$, 1H), 3.62–3.48 (m, 2H), 3.23–3.19 (m, 2H), 2.43–2.34 (m, 4H), 1.49–1.43 (m, 2H), 1.35–1.23 (m, 4H), 1.11 (s, 3H), 0.88 (t, $J = 6.9$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.5, 160.2, 132.8, 130.1, 127.5, 127.5, 118.8, 113.7, 113.7, 101.1, 82.1, 76.2, 55.3, 40.1, 39.6, 36.2, 35.5, 35.0, 29.2, 29.0, 22.3, 17.7, 14.0. HRMS for $\text{C}_{24}\text{H}_{36}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 433.2697, found 433.2714.

4.5.28. (4*R*,5*R*)-5-Benzyl-2-(4-methoxyphenyl)-5-methyl-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8g)

Prepared in 79% from **5g** according to the general procedure. R_f 0.19 (EtOAc/hexanes 3:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.38 (d, $J = 8.8$, 2H), 7.30–7.27 (m, 4H), 7.25–7.22 (m, 1H), 7.14 (t, $J = 6.0$, 1H), 6.90 (d, $J = 8.8$, 2H), 5.84 (br s, 1H), 5.30 (s, 1H), 4.26 (s, 1H), 3.82 (d, $J = 11.5$, 1H), 3.81 (s, 3H), 3.68 (d, $J = 11.5$, 1H), 3.65–3.53 (m, 2H), 3.25–3.21 (m, 2H), 3.10 (d, $J = 13.8$, 1H), 2.88 (d, $J = 13.8$, 1H), 2.46 (t, $J = 6.3$, 2H), 1.51–1.45 (m, 2H), 1.35–1.24 (m, 4H), 1.15 (s, 3H), 0.88 (t, $J = 7.1$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.8, 160.2, 136.5, 130.9, 130.9, 130.0, 128.0, 128.0, 127.4, 127.4, 126.4, 113.7, 113.7, 100.9, 81.3, 76.1, 55.3, 41.6, 39.6, 36.2, 36.0, 35.0, 29.2, 29.0, 22.3, 18.3, 14.0. HRMS for $\text{C}_{28}\text{H}_{38}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 483.2853, found 483.2864.

4.5.29. (4*R*)-5,5-Dipropyl-2-(4-methoxyphenyl)-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8h)

Prepared in 43% from **5h** according to the general procedure. R_f 0.41 (EtOAc/hexanes, 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.8$, 2H), 7.07 (t, $J = 6.0$, 1H), 6.92 (d, $J = 8.8$, 2H), 5.83 (s, 1H), 5.43 (s, 1H), 4.31 (s, 1H), 3.98 (d, $J = 11.6$, 1H), 3.82 (s, 3H), 3.76 (d, $J = 11.6$, 1H), 3.56–3.52 (m, 2H), 3.27–3.14 (m, 2H), 2.45–2.38 (m, 2H), 1.82 (ddd, $J = 4.4$, 12.5, 13.0, 1H), 1.64–1.20 (m, 13H), 0.93 (t, $J = 7.0$, 3H), 0.90 (t, $J = 7.0$, 3H), 0.88 (t, $J = 7.0$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.8, 160.2, 130.2, 127.5, 127.5, 113.7, 113.7, 101.2, 81.7, 73.3, 55.3, 39.6, 38.2, 36.2, 35.7, 34.9, 34.0, 29.2, 29.0, 22.3, 16.72, 16.69, 15.1, 15.0, 14.0. HRMS for $\text{C}_{26}\text{H}_{42}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 463.3166, found 463.3176.

4.5.30. (4*R*)-5,5-Diallyl-2-(4-methoxyphenyl)-*N*-(3-oxo-3-(pentylamino)propyl)-1,3-dioxane-4-carboxamide (8i)

Prepared in 45% from **5i** with modifications to the general procedure. HBTU was used to couple **7i** to **12a** in DMF instead of EDC in THF. R_f 0.43 (EtOAc/hexanes, 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.7$, 2H), 7.09 (t, $J = 5.8$, 1H), 6.92 (d, $J = 8.7$, 2H), 6.04–5.96 (m, 1H), 5.89–5.81 (m, 1H), 5.77 (br s, 1H), 5.42 (s, 1H), 5.18–5.10 (m, 4H), 4.35 (s, 1H), 3.92 (d, $J = 11.5$, 1H), 3.82 (s, 3H), 3.77 (d, $J = 11.5$, 1H), 3.56–3.52 (m, 2H), 3.22–3.18 (m, 2H), 2.69 (dd, $J = 7.7$, 13.5, 1H), 2.58 (dd, $J = 7.0$, 14.1, 1H), 2.41 (t, $J = 6.3$, 2H), 2.15 (dd, $J = 8.3$, 14.1, 1H), 2.03 (dd, $J = 7.5$, 13.5, 1H), 1.49–1.43 (m, 2H), 1.33–1.25 (m, 4H), 0.88 (t, $J = 6.9$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.7, 169.4, 160.3, 133.4, 133.4, 130.1, 127.5, 127.5, 119.1, 118.8, 113.8, 113.8, 101.3, 80.7, 72.2, 55.3, 39.6, 38.4, 37.3, 36.1, 35.7, 35.0, 29.2, 29.0, 22.3, 13.9. HRMS for $\text{C}_{26}\text{H}_{38}\text{N}_2\text{O}_5$ $[\text{M}-\text{H}]^-$ calcd 457.2708, found 457.2717.

4.5.31. (4*R*,5*R*)-5-Allyl-*N*-(3-(nonylamino)-3-oxopropyl)-2-(4-methoxyphenyl)-5-methyl-1,3-dioxane-4-carboxamide (8j)

Prepared in 41% from **5k** according to the general procedure. R_f 0.46 (EtOAc/hexanes, 3:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.8$, 2H), 7.04 (t, $J = 6.2$, 1H), 6.92 (d, $J = 8.8$, 2H), 5.90–5.82 (m,

1H), 5.77 (br s, 1H), 5.42 (s, 1H), 5.15–5.11 (m, 2H), 4.19 (s, 1H), 3.82 (s, 3H), 3.81–3.76 (m, 2H), 3.62–3.48 (m, 2H), 3.23–3.19 (m, 2H), 2.43–2.34 (m, 4H), 1.49–1.43 (m, 2H), 1.31–1.23 (m, 12H), 1.11 (s, 3H), 0.88 (t, $J = 7.1$, 3H), ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.5, 160.2, 132.8, 130.1, 127.5, 127.5, 118.8, 113.8, 113.8, 101.2, 82.1, 76.2, 55.3, 40.1, 39.7, 36.2, 35.5, 35.0, 31.8, 29.51, 29.50, 29.3, 29.2, 26.9, 22.7, 17.7, 14.1. HRMS for $\text{C}_{28}\text{H}_{44}\text{N}_2\text{O}_5$ $[\text{M}+\text{Na}]^+$ calcd 511.3142, found 511.3142.

4.5.32. (4R,5R)-5-Allyl-2-(4-methoxyphenyl)-5-methyl-N-(3-(nonylamino)-3-oxopropyl)-1,3-dioxane-4-carboxamide (8k)

Prepared in 66% from **5l** according to the general procedure. R_f 0.21 (EtOAc/hexanes 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (d, $J = 8.8$, 2H), 7.01 (t, $J = 5.8$, 1H), 6.92 (d, $J = 8.8$, 2H), 5.78 (brs, 1H), 5.46 (s, 1H), 4.07 (s, 1H), 3.82 (s, 3H), 3.71 (d, $J = 11.4$, 1H), 3.66 (d, $J = 11.4$, 1H), 3.62–3.55 (m, 2H), 3.54–3.47 (m, 2H), 3.23–3.19 (m, 2H), 2.41 (t, $J = 6.2$, 2H), 1.49–1.44 (m, 2H), 1.35–1.23 (m, 2H), 1.10 (s, 3H), 0.88 (t, $J = 7.0$, 3H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.6, 160.2, 130.1, 127.5, 127.5, 113.7, 113.7, 101.3, 83.8, 78.5, 55.3, 39.7, 36.2, 35.0, 33.1, 29.2, 29.0, 22.3, 21.8, 19.1, 14.0. HRMS for $\text{C}_{22}\text{H}_{34}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 407.2540, found 407.2547.

4.5.33. (4R)-2-(4-Methoxyphenyl)-5,5-dimethyl-N-(3-(nonylamino)-3-oxopropyl)-1,3-dioxane-4-carboxamide (8l)

Prepared in 75% from **5l** according to the general procedure. R_f 0.29 (EtOAc/hexanes 5:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.42 (d, $J = 8.8$, 2H), 7.01 (t, $J = 5.7$, 1H), 6.92 (d, $J = 8.8$, 2H), 5.85 (br s, 1H), 5.45 (s, 1H), 4.06 (s, 1H), 3.81 (s, 3H), 3.70 (d, $J = 11.4$, 1H), 3.65 (d, $J = 11.4$, 1H), 3.61–3.46 (m, 2H), 3.21–3.17 (m, 2H), 2.40 (t, $J = 6.3$, 2H), 1.48–1.43 (m, 2H), 1.31–1.20 (m, 12H), 1.10 (s, 3H), 1.09 (s, 3H), 0.87 (t, $J = 7.1$, 3H), ^{13}C NMR (CDCl_3 , 125 MHz) δ 170.6, 169.5, 160.2, 130.1, 127.5, 127.5, 113.7, 113.7, 101.3, 83.8, 78.5, 55.3, 39.6, 36.2, 35.0, 33.1, 31.7, 29.5, 29.5, 28.9, 28.9, 26.9, 22.6, 21.8, 19.1, 14.0.

4.5.34. General procedure for deprotection (8–9)

Acetic acid 90% in water was added to compounds **8a–8l** (0.03–0.05 M) at room temperature and the mixture stirred overnight (16 h). The solvent was evaporated and the residue purified by flash chromatography on silica gel (100% EtOAc \rightarrow 10% MeOH in EtOAc) to give the titled compounds. Samples for biological testing were further purified by prep TLC (10% MeOH in EtOAc) to give >95% pure compounds by reverse phase HPLC (gradient elution with $\text{H}_2\text{O}/\text{CH}_3\text{CN}$, 99:1 \rightarrow 1:99 in 30 min on analytical Phenomenex Synergi 4 μ Hydro-RP 80A C18 column)

4.5.35. (2R,3R)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-3-(pentylamino)propyl)pentanamide (9a)

Prepared in 77% yield from **8a** according to the general procedure. R_f 0.36 (EtOAc/MeOH 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (m, 1H), 5.91 (br s, 1H), 4.00 (s, 2H), 3.66–3.60 (m, 2H), 3.54–3.47 (m, 3H), 3.24–3.20 (m, 2H), 2.44–2.41 (m, 2H), 1.63–1.56 (m, 1H), 1.52–1.46 (m, 2H), 1.40–1.25 (m, 5H), 0.94 (s, 3H), 0.89 (t, $J = 6.9$, 3H), 0.87 (t, $J = 7.6$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.9, 171.4, 77.6, 68.4, 41.4, 39.7, 35.8, 35.4, 29.2, 29.0, 26.0, 22.3, 18.4, 14.0, 7.8. HRMS for $\text{C}_{15}\text{H}_{30}\text{N}_2\text{O}_4$ $[\text{M}-\text{H}]^-$ calcd 301.2133, found 301.2131. Purity, >95% ($t_R = 15.46$ min).

4.5.36. (2R,3R)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-3-(pentylamino)propyl)hexanamide (9b)

Prepared in 81% yield from **8b** according to the general procedure. R_f 0.38 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (m, 1H), 5.90 (t, $J = 4.7$, 1H), 4.00 (s, 2H), 3.66–3.59 (m, 2H), 3.56–3.48 (m, 3H), 3.25–3.18 (m, 2H), 2.44–2.41 (m, 2H), 1.53–1.46 (m, 3H), 1.35–1.23 (m, 7H), 0.96 (s, 3H), 0.92 (t, $J = 6.6$, 3H),

0.89 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.8, 171.4, 77.7, 68.8, 41.8, 39.7, 36.0, 35.9, 35.3, 29.2, 29.0, 22.3, 19.0, 16.5, 15.0, 13.9. HRMS for $\text{C}_{16}\text{H}_{32}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 339.2254, found 339.2250. Purity, >95% ($t_R = 16.60$ min).

4.5.37. (2R,3R)-2-Hydroxy-3-(hydroxymethyl)-3,5-dimethyl-N-(3-oxo-3-(pentylamino)propyl)hexanamide (9c)

Prepared in 83% yield from **8c** according to the general procedure. R_f 0.43 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.44 (m, 1H), 5.89 (t, $J = 4.6$, 1H), 3.99–3.97 (m, 2H), 3.65–3.48 (m, 5H), 3.24–3.20 (m, 2H), 2.43 (dt, $J = 1.2$, 6.5, 2H), 1.76–1.68 (m, 1H), 1.52–1.47 (m, 3H), 1.36–1.25 (m, 4H), 1.22 (dd, $J = 5.7$, 14.2, 1H), 1.01 (s, 3H), 0.94 (t, $J = 6.5$, 6H), 0.89 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.7, 171.4, 78.0, 69.0, 42.5, 42.0, 39.7, 35.8, 35.3, 29.2, 29.0, 25.5, 25.3, 23.5, 22.3, 19.4, 14.0. HRMS for $\text{C}_{17}\text{H}_{34}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 353.2411, found 353.2405. Purity >95% ($t_R = 17.66$ min).

4.5.38. (2R,3R)-4-Cyclobutyl-2-hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-3-(pentylamino)propyl)butanamide (9d)

Prepared in 60% yield from **8d** according to the general procedure. R_f 0.44 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.83 (br s, 1H), 5.80 (br s, 1H), 3.96 (s, 1H), 3.66–3.59 (m, 1H), 3.55–3.47 (m, 3H), 3.25–3.21 (m, 2H), 2.46–2.38 (m, 3H), 2.04–2.00 (m, 2H), 1.88–1.79 (m, 1H), 1.76–1.62 (m, 4H), 1.52–1.47 (m, 2H), 1.41 (dd, $J = 6.9$, 14.0, 1H), 1.35–1.25 (m, 4H), 0.95 (s, 3H), 0.90 (t, $J = 6.8$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.5, 171.3, 77.8, 68.8, 42.4, 40.6, 39.7, 35.8, 35.4, 32.0, 30.52, 30.49, 29.2, 29.0, 22.3, 19.13, 19.06, 14.0. HRMS for $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 365.2411, found 365.2412. Purity >95% ($t_R = 18.11$ min).

4.5.39. (2R,3R)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-3-(pentylamino)propyl)nonanamide (9e)

Prepared in 79% yield from **8e** according to the general procedure. R_f 0.42 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (m, 1H), 5.90 (t, $J = 4.6$, 1H), 4.00–3.98 (m, 2H), 3.66–3.59 (m, 2H), 3.56–3.48 (m, 3H), 3.25–3.18 (m, 2H), 2.47–2.38 (m, 2H), 1.55–1.46 (m, 3H), 1.35–1.26 (m, 13H), 0.96 (s, 3H), 0.89 (t, $J = 7.0$, 3H), 0.87 (t, $J = 7.0$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.8, 171.0, 77.7, 68.8, 41.7, 39.7, 35.8, 35.3, 33.8, 31.9, 30.2, 29.2, 29.0, 23.3, 22.7, 22.3, 19.0, 14.1, 13.9. HRMS for $\text{C}_{19}\text{H}_{38}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 381.2724, found 381.2720. Purity >95% ($t_R = 20.27$ min).

4.5.40. (2R,3R)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-3-(pentylamino)propyl)hex-5-enamide (9f)

Prepared in 96% yield from **8f** according to the general procedure. R_f 0.34 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.45 (m, 1H), 5.91 (t, $J = 5.3$, 1H), 5.88–5.80 (m, 1H), 5.12–5.08 (m, 2H), 4.01 (br s, 2H), 3.65–3.49 (m, 5H), 3.22 (q, $J = 7.0$, 2H), 2.43 (m, 2H), 2.29 (dd, $J = 7.5$, 13.7, 1H), 2.12 (dd, $J = 7.5$, 13.7, 1H), 1.52–1.46 (m, 2H), 1.36–1.25 (m, 4H), 0.97 (s, 3H), 0.89 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.6, 171.4, 134.0, 118.3, 77.2, 68.6, 42.0, 39.7, 38.3, 35.7, 35.3, 29.2, 29.0, 22.3, 18.8, 13.9. HRMS for $\text{C}_{16}\text{H}_{30}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 337.2098, found 337.2093. Purity, >95% ($t_R = 16.19$ min).

4.5.41. (2R,3R)-3-Benzyl-2,4-dihydroxy-3-methyl-N-(3-oxo-3-(pentylamino)propyl)butanamide (9g)

Prepared in 52% yield from **8g** according to the general procedure. R_f 0.52 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.51 (m, 1H), 7.28–7.18 (m, 5H), 5.92 (t, $J = 4.9$, 1H), 4.28 (br s, 1H), 4.00 (s, 1H), 3.82 (br s, 1H), 3.67–3.61 (m, 1H), 3.56–3.48 (m, 2H), 3.51 (s, 1H), 3.20 (q, $J = 6.7$, 2H), 2.84 (d, $J = 13.2$, 1H), 2.75 (d, $J = 13.2$, 1H), 2.46–2.42 (m, 2H), 1.49–1.43 (m, 2H), 1.33–

1.22 (m, 4H), 0.87 (t, $J = 6.9$, 3H), 0.86 (s, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.9, 171.4, 137.4, 130.9, 130.9, 127.9, 127.9, 126.2, 77.3, 67.8, 42.8, 39.7, 39.0, 35.7, 35.4, 29.1, 29.0, 22.3, 18.9, 13.9. HRMS for $\text{C}_{20}\text{H}_{32}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 387.2254, found 387.2253. Purity >95% ($t_{\text{R}} = 16.19$ min).

4.5.42. (R)-2-Hydroxy-3-(hydroxymethyl)-N-(3-oxo-3-(pentylamino)propyl)-3-propylhexanamide (9h)

Prepared in 45% yield from **8h** according to the general procedure. R_f 0.35 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (m, 1H), 5.78 (br s, 1H), 4.08 (s, 1H), 3.68–3.47 (m, 4H), 3.25–3.20 (m, 2H), 2.47–2.38 (m, 2H), 1.52–1.24 (m, 16H), 0.92–0.88 (m, 9H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 174.0, 171.4, 76.7, 67.4, 44.1, 39.7, 35.7, 35.4, 34.6, 33.6, 29.2, 29.0, 22.3, 16.6, 16.3, 15.0, 15.0, 14.0. HRMS for $\text{C}_{18}\text{H}_{36}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 367.2567, found 367.2575. Purity >95% ($t_{\text{R}} = 19.05$ min).

4.5.43. (R)-3-Allyl-2-hydroxy-3-(hydroxymethyl)-N-(3-oxo-3-(pentylamino)propyl)hex-5-enamide (9i)

Prepared in 52% yield from **8i** according to the general procedure. R_f 0.16 (EtOAc/hexanes, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.25 (br s, 1H), 5.98–5.82 (m, 3H), 5.17–5.09 (m, 4H), 4.15 (s, 1H), 3.65–3.59 (m, 4H), 3.25–3.21 (m, 2H), 2.44 (t, $J = 6.7$, 2H), 2.34 (dd, $J = 7.6$, 14.1, 1H), 2.24–2.16 (m, 4H), 1.52–1.46 (m, 2H), 1.36–1.25 (m, 5H), 0.89 (t, $J = 7.2$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.6, 171.2, 134.6, 133.9, 118.5, 118.4, 75.7, 67.1, 45.1, 39.7, 37.7, 35.7, 35.6, 35.4, 29.2, 29.0, 22.3, 13.9. HRMS for $\text{C}_{18}\text{H}_{32}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 363.2254, found 363.2255. Purity >95% ($t_{\text{R}} = 18.03$ min).

4.5.44. (2R,3R)-2-Hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-(nonylamino)-3-oxopropyl)hex-5-enamide (9j)

Prepared in 48% yield from **8j** according to the general procedure. R_f 0.45 (EtOAc/MeOH, 9:1). ^1H NMR (CDCl_3 , 500 MHz) δ 7.43 (br s, 1H), 5.92 (br s, 1H), 5.89–5.81 (m, 1H), 5.13–5.09 (m, 2H), 4.02 (s, 1H), 3.66–3.52 (m, 4H), 3.25–3.21 (m, 2H), 2.47–2.44 (m, 2H), 2.30 (dd, $J = 7.7$, 14.0, 1H), 2.12 (dd, $J = 7.3$, 14.0, 1H), 1.52–1.46 (m, 2H), 1.29–1.26 (m, 14H), 0.98 (s, 3H), 0.88 (t, $J = 7.1$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.4, 171.3, 134.0, 118.4, 77.2, 68.6, 42.1, 39.7, 38.4, 35.7, 35.4, 31.8, 29.50, 29.48, 29.3, 29.2, 26.9, 22.6, 18.9, 14.1. HRMS for $\text{C}_{20}\text{H}_{38}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 393.2724, found 393.2724. Purity, >95% ($t_{\text{R}} = 21.41$ min).

4.5.45. (2R,3R)-4-Cyclopropyl-2-hydroxy-3-(hydroxymethyl)-3-methyl-N-(3-oxo-3-(pentylamino)propyl)butanamide (9m)

To a solution of compound **13** (84 mg, 0.155 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added drop wise a solution of Et_2Zn in hexanes (1.55 mL, 1.55 mmol, 1 M solution in hexanes) under nitrogen. The mixture was stirred for 15 min followed by the addition of CH_2I_2 (0.25 mL, 3.09 mmol). The reaction mixture was brought to room temperature (the formation of a white precipitate was observed) and stirred for 24 h. Saturated aqueous NH_4Cl (20 mL) was added, the mixture extracted with ethyl acetate (3 \times 15 mL) and the combined organic layer was dried (Na_2SO_4) and evaporated to afford a light yellow oil that was used directly in the next reaction. To the oil dissolved in THF (5 mL) at room temperature was added a solution of TBAF in THF (0.62 mL, 0.62 mmol, 1 M in THF). The reaction mixture was stirred overnight, diluted with saturated aqueous NaHCO_3 (5 mL) and the mixture was extracted with ethyl acetate (3 \times 10 mL). The combined organic layer was dried over Na_2SO_4 and evaporated to yield an oil that was purified by flash chromatography on silica gel (gradient elution, 9:1 EtOAc/hexanes v/v \rightarrow 19:1 EtOAc/methanol, v/v) to give the desired product (25.8 mg, 51%) over the 2 steps. R_f 0.36 (EtOAc/MeOH, 9:1, v/v). ^1H NMR (CDCl_3 , 500 MHz) δ 7.45 (br s, 1H), 5.99 (br s, 1H), 4.07 (s, 1H), 3.68 (d, $J = 11.3$, 1H), 3.65–3.60 (m, 1H), 3.61 (d, $J = 11.3$, 1H), 3.56–3.50 (m, 1H), 3.26–3.21 (m, 2H), 2.47–2.44 (m, 2H),

1.53–1.47 (m, 2H), 1.44 (dd, $J = 7.1$, 14.1, 1H), 1.35–1.26 (m, 5H), 1.07 (s, 3H), 0.90 (t, $J = 7.2$, 3H), 0.71–0.64 (m, 1H), 0.48–0.44 (m, 2H), 0.09–0.07 (m, 2H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.6, 171.3, 77.7, 68.9, 42.9, 39.7, 38.5, 35.8, 35.4, 29.2, 29.0, 22.3, 19.3, 14.0, 5.7, 4.9, 4.7. HRMS for $\text{C}_{17}\text{H}_{32}\text{N}_2\text{O}_4$ $[\text{M}+\text{Na}]^+$ calcd 351.2254, found 351.2259. Purity >95% ($t_{\text{R}} = 18.03$ min).

4.5.46. (9H-Fluoren-9-yl)methyl-3-oxo-3-(pentylamino)propyl-carbamate (11a)

Fmoc protected β -alanine (2.00 g, 6.42 mmol), EDC (1.60 g, 8.36 mmol) and HOBt (1.59 g, 10.40 mmol) were weighed into a flask which was purged with nitrogen. THF (30 mL), pentylamine (0.88 mL, 7.62 mmol) and diisopropylamine (6.04 mL, 34.68 mmol) were added sequentially and the mixture was stirred overnight under nitrogen at room temperature. The reaction mixture was poured into a separatory funnel containing saturated aqueous ammonium chloride (30 mL), the organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 \times 30 mL). The combined organic layer was washed with brine, dried over sodium sulfate and concentrated to yield a yellow solid. The solid was suspended in diethyl ether/hexanes (50 mL, 1.5/10 v/v), filtered and the residue was washed with the same solvent system (3 \times 25 mL) to afford a white solid that is homogeneous by TLC and NMR (2.04 g, 83% yield). ^1H NMR (CDCl_3 , 500 MHz) δ 7.76 (d, $J = 7.5$, 2H), 7.59 (d, $J = 7.4$, 2H), 7.40 (d, $J = 7.5$, 2H), 7.31 (d, $J = 7.5$, 2H), 5.53 (br s, 1H), 5.49 (br s, 1H), 4.37 (d, $J = 6.9$, 2H), 4.20 (t, $J = 6.9$, 1H), 3.50 (br s, 2H), 3.27–3.23 (m, 2H), 2.41 (t, $J = 5.2$, 2H), 1.53–1.47 (m, 2H), 1.35–1.23 (m, 4H), 0.90 (t, $J = 6.69$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.2, 156.6, 143.9, 143.9, 141.3, 141.3, 127.7, 127.7, 127.0, 127.0, 125.1, 125.1, 119.9, 119.9, 66.7, 47.2, 39.6, 37.1, 36.0, 29.3, 29.0, 22.3, 14.0. HRMS for $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_3$ $[\text{M}+\text{Na}]^+$ calcd 403.1992, found 403.1994.

4.5.47. (9H-Fluoren-9-yl)methyl 3-(nonylamino)-3-oxopropylcarbamate (11j)

Compound **11j** was synthesized in 84% yield from **10** and nonylamine in a manner similar to **11a**. ^1H NMR (CDCl_3 , 500 MHz) δ 7.76 (d, $J = 7.5$, 2H), 7.59 (d, $J = 7.5$, 2H), 7.40 (d, $J = 7.4$, 2H), 7.32 (d, $J = 7.4$, 2H), 5.49 (br s, 2H), 4.37 (d, $J = 7.2$, 2H), 4.20 (t, $J = 6.8$, 1H), 3.51–3.48 (m, 2H), 3.27–3.23 (m, 2H), 2.41 (t, $J = 5.7$, 2H), 1.50–1.47 (m, 2H), 1.29–1.26 (m, 12H), 0.87 (t, $J = 7.1$, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.1, 156.6, 143.9, 143.9, 141.3, 141.3, 127.7, 127.7, 127.0, 127.0, 125.1, 125.1, 119.9, 119.9, 66.7, 47.2, 39.6, 37.1, 36.0, 31.8, 29.6, 29.5, 29.3, 29.2, 26.9, 22.7, 14.1. HRMS for $\text{C}_{27}\text{H}_{36}\text{N}_2\text{O}_3$ $[\text{M}+\text{Na}]^+$ calcd 459.2618, found 459.2624.

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Supplementary data

Supplementary data (NMR spectra and HPLC traces for key intermediates and final targets) associated with this article can be found, in the online version, at [doi:10.1016/j.bmc.2011.02.053](https://doi.org/10.1016/j.bmc.2011.02.053).

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