PAPER

Total chemical synthesis of lassomycin and lassomycin-amide†

S. Lear,[a] T. Munshi,[b] A. S. Hudson,[a] C. Hatton,[a] J. Clardy,[c] J. A. Mosely,[a] T. J. Bull,[b] C. S. Sit*[c] and S. L. Cobb*[a]

Herein we report a practical synthetic route to the lasso peptide lassomycin (**1**) and C-terminal variant lassomycin-amide (**2**). The biological evaluation of peptides **1** and **2** against *Mycobacterium tuberculosis* revealed that neither had any activity against this bacterium. This lack of biological activity has led us to propose that naturally occurring lassomycin may actually exhibit a standard lasso peptide threaded conformation rather than the previously reported unthreaded structure.

Introduction

The pressing issue of antimicrobial resistance continues to drive the search for novel antibiotic scaffolds.[1] Emerging threats such as extensively drug-resistant tuberculosis require the use of innovative approaches in the search for potential leads.[2,3] Lassomycin (**1**), a natural product belonging to the 'lasso' peptide class, was recently isolated from an Actinomycete (*Lentzea kentuckyensis* sp.) in a screen of a library of previously uncultured soil bacteria.[4] The lasso peptide was found to possess potent and specific bactericidal activity against mycobacteria, including drug-resistant forms of *Mycobacterium tuberculosis*. The specificity of activity arises due to the fact that lassomycin targets the ClpC1 ATPase, an essential enzyme in mycobacteria. ClpC1 ATPase is a key component of protein degradation together with the ClpP1P2 proteolytic complex.[5]

The previously reported solution-state NMR structure of lassomycin is shown in Fig. 1. The peptide does not adopt the characteristic knot conformation reported for other homologous lasso peptides: here the C-terminus packs tightly against the N-terminal ring instead of passing through the macrolactam.[4] In light of this discovery, and the potential of lasso peptides as novel drug scaffolds in general,[6–8] we set out to chemically synthesize the natural product.

Herein, we report the first chemical synthesis of the recently identified lasso-peptide lassomycin (**1**) and a C-term-
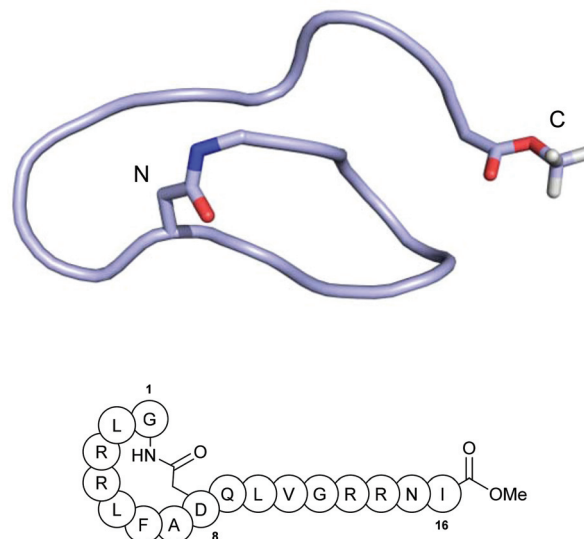
[a]*Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK. E-mail: s.l.cobb@durham.ac.uk*
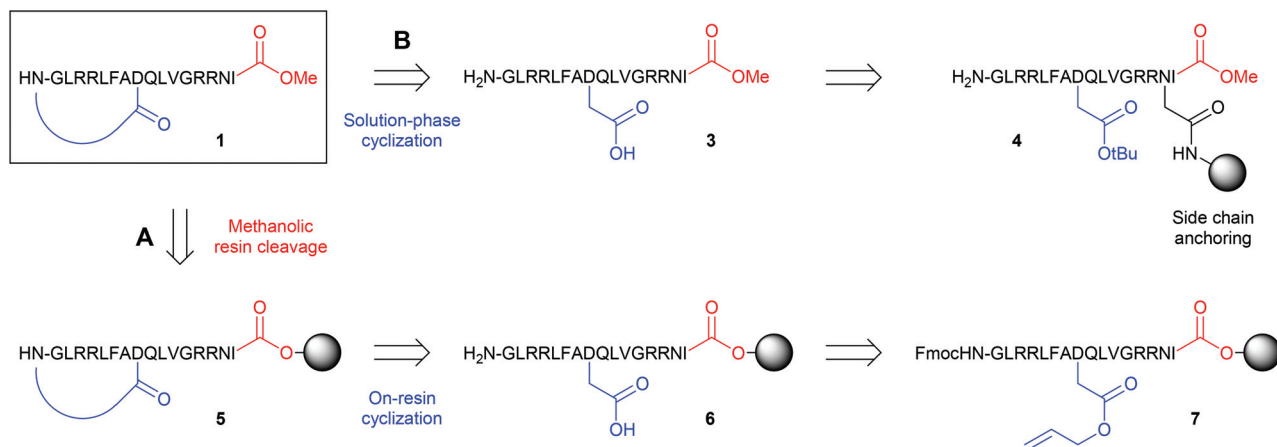
[b]*St. George's University of London, London, SW17 0RE, UK*

[c]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave, Boston, MA 02115, USA. E-mail: Clarissa_Sit@hms.harvard.edu*

†Electronic supplementary information (ESI) available: NMR, HPLC and MS analysis for all compounds and peptides prepared. See DOI: 10.1039/c6ob00631k

Fig. 1 Amino acid sequence and proposed solution-state NMR structure of lassomycin (**1**).[4,9] The N- and C-terminus are labelled.

inal amide derivative, lassomycin-amide (**2**). Synthetic peptides **1** and **2** were also evaluated for activity against *M. tuberculosis*.

Results and discussion

Two key structural features that need to be incorporated during a chemical synthesis of lassomycin (**1**) are the isopeptide bond formed between the N-terminus and the carboxylate side chain of Asp-8, and the methylated C-terminus (Fig. 1). We envisaged two possible synthetic approaches to access lassomycin (**1**) and these are outlined in Scheme 1. Route A involved the preparation of a linear peptide and the formation of the lactam ring on resin using an allyl ester orthogonal pro-

4534 | *Org. Biomol. Chem.*, 2016, **14**, 4534–4541

This journal is © The Royal Society of Chemistry 2016

Scheme 1 Proposed synthesis routes to lassomycin (**1**): (A) on resin cyclization and (B) combined solution/on-resin approach (amino acid side chain protecting groups are omitted for clarity).

tecting group strategy for the side chain of Asp-8. The C-terminal methyl ester would be installed using 2-chlorotrityl chloride resin and the approach reported by Turner *et al.* (Scheme 1, Route A).[10] The alternative approach (Scheme 1, Route B), involved assembling the linear peptide on resin *via* side chain anchoring. In this approach a di-peptide fragment would be loaded initially onto the resin allowing the C-terminal methyl ester functionality to be installed at this point. Lactam ring formation would be carried out in solution.

Initially, Route A (Scheme 1) was investigated and the linear peptide **7** was successfully assembled *via* standard Fmoc solid phase peptide synthesis (SPPS). Next, we attempted the on-resin deprotection of the Asp-8 side chain. In our hands all attempts to remove the allyl ester and generate peptide **6** were unsuccessful, despite the use of stoichiometric amounts of tetrakis(triphenylphosphine)palladium(0) catalyst and triphenylsilane, and trialling a number of reaction solvents. The lack of success in allyl ester removal was assumed to arise from a difficulty in coordinating the allyl moiety to the catalyst, presumably due to steric hindrance around the metal centre. This effect will depend upon peptide sequence length and folding of the peptide, and as such is likely to be highly sequence dependent. In light of these difficulties alternative orthogonal protecting groups were considered (*i.e.* highly acid sensitive,[11] photolabile[12] or silicon-based[13] protection), however the requirement for more specialized deprotection conditions, or the lack of available commercial building blocks, led us to investigate Route B (Scheme 1).

The synthesis of the required orthogonally protected di-peptide (**10**) for Route B was carried out in solution. Removal of the *t*-butyl side chain from **10** under standard acidic conditions followed by attachment to Rink amide resin gave the methyl ester protected C-terminal fragment of lassomycin (Scheme 2). The peptide chain was then extended on resin using Fmoc SPPS to give a complete linear version of lassomycin (**4**). Cleavage from the resin afforded the linear peptide (**3**) which was ready for lactam ring formation.

While peptide macrocyclizations are often best performed under high dilution to minimize unwanted oligomer for-
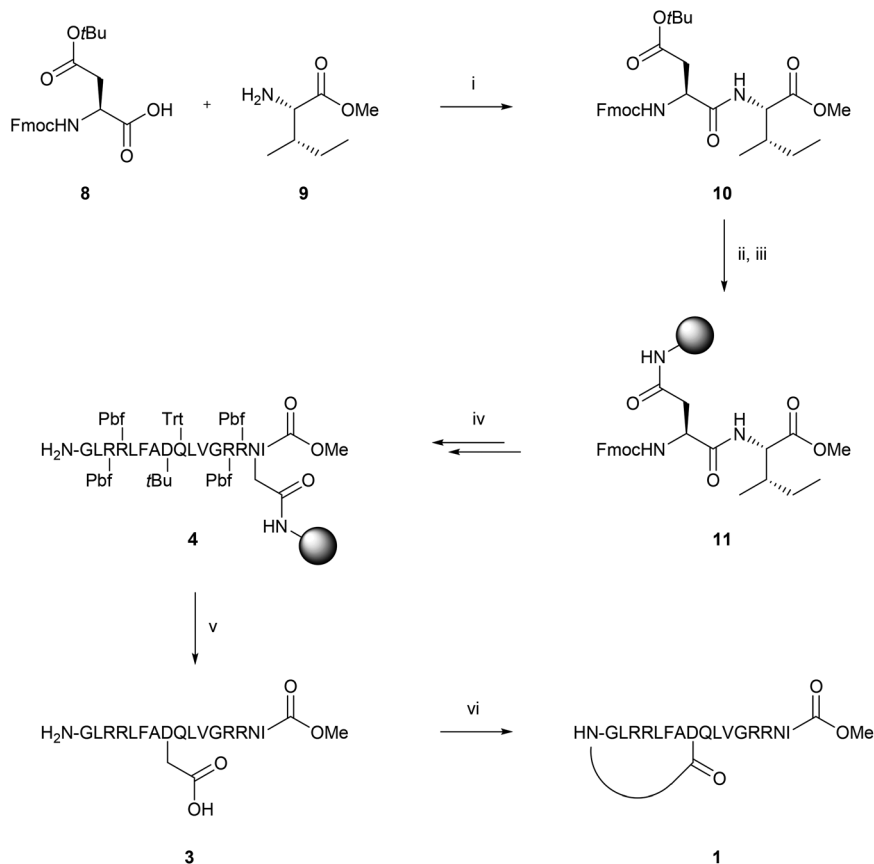
mation,[14] the 'pseudo-high dilution' conditions described by Malesevic *et al.* were instead utilized as a more convenient alternative in order to minimize solvent usage.[15] 11.3 mL each of solutions of peptide **3** (0.01 M) and HATU (0.03 M) in DMF were added to a reaction flask containing DIPEA and HOAt over a period of ~20 h at a rate of 0.01 mL min^{-1} (syringe pump controlled). Slow addition was used in order to keep the effective reaction concentration low, while requiring only a relatively small final solvent volume (34 mL). Removal of the solvent under vacuum and HPLC purification of the crude reaction product yielded lassomycin (**1**) in high purity, as confirmed by analytical HPLC and MALDI-TOF MS (Fig. 2).

MALDI LIFT-TOF/TOF[16] MS/MS was used to confirm the correct sequence for the linear tail portion of lassomycin (ESI†). However, fragmentation was not observed for the cyclic region, but this matched the data reported by Gavrish *et al.* from MS/MS sequencing of the isolated natural lassomycin.[4] A complete set of fragment ions were observed for the uncyclized peptide.

Ion mobility (IM) mass spectrometry was also carried out on the synthetic peptide. A number of peaks with distinct collisional cross sections were resolved belonging to either lassomycin or a trace amount of the C-terminal acid hydrolysis product (**13**) (ESI Fig. 7†). Only a single peak was observed for each species suggesting that only one conformation is present, as opposed to distinct populations of threaded and unthreaded peptide (or that the collisional cross section for threaded/unthreaded ions is identical).

It was also possible to access lassomycin-amide (**2**) a C-terminal amide version of the original lassomycin (**1**) peptide. Synthesis of the required linear peptide (**14**) was carried out on Rink amide resin using Fmoc SPPS, and cleavage from the resin afforded peptide **15** (Scheme 3). Formation of the lactam ring in solution and HPLC purification to yield lassomycin-amide (**2**) were carried out as previously described for the synthesis of **1**. Purity and structure were confirmed by analytical HPLC (ESI Fig. 2†), MALDI-TOF MS (ESI Fig. 2†) and tandem mass spectrometry (ESI Fig. 1†).

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 4534–4541 | **4535**

Scheme 2 Synthesis of lassomycin. The dipeptide Fmoc-Asp(OH)-Ile-OMe (**12**) was prepared in solution and anchored to Rink amide resin *via* the Asp side chain. The required sequence was then elongated *via* SPPS, before global deprotection and cleavage from the resin. Cyclization of the linear peptide in solution yielded the cyclic target. Reagents and conditions: (i) PyBOP® (1.0 equiv.) and NMM (3.0 equiv.), DCM, rt, 18 h; SiO$_2$, 98/2% hexane/EtOAc → 50/50% hexane/EtOAc, 63%; (ii) 40% TFA, DCM, rt, 30 min, 100%; (iii) Rink amide resin (0.79 mmol g^{-1}), Fmoc-Asp(OH)-Ile-OMe (**12**) (3.1 equiv.), PyBOP® (3.1 equiv.) and DIPEA (6.2 equiv.), DMF, RT, 2 × 1 h; (iv) Fmoc SPPS (see Experimental section); (v) TFA (95%, water), TIPS, rt, 4 h; (vi) HATU (3.0 equiv.) and DIPEA (6.0 equiv.), DMF, rt, 20 h (pseudo-high dilution, see text), 14%.
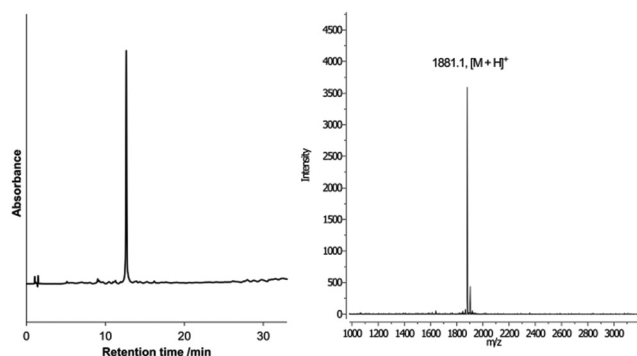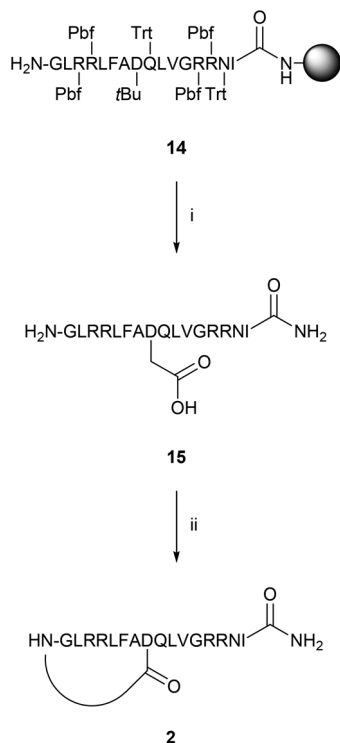

Fig. 2 HPLC trace (left) and MALDI-TOF mass spectrum (right) for synthetic lassomycin (**1**). HPLC absorbance recorded at 220 nm, gradient 0–100% B over 30 min (A = 5 : 95 : 0.05 MeCN/H$_2$O/TFA; B = 95 : 5 : 0.03 MeCN/H$_2$O/TFA).

Peptides **1** and **2**, as well as truncated analogue lassomycin (1–9) (**16**) representing the peptide without the tail were tested against *Mycobacterium tuberculosis* H37Rv in MIC assays utilizing both solid-agar based and liquid-media based methods.

None of the peptides showed inhibitory activity when tested, up to concentrations of 100 μg mL^{-1}. These results were surprising in particular for peptide **1**, given the previously reported biological activity profile.[4] Given the unlikely nature of spontaneous threading during the synthesis of **1** (and **2**) it is logical to assume that the peptides reported herein are unthreaded. This, in combination with the biological data, leads us to speculate that the un-threaded structure previously reported may not be correct.

The structural discrepancy from the original report[4] could possibly be due to instability of lassomycin in its threaded conformation. Several examples have been documented of lasso peptides unthreading upon heat treatment. Caulosegnin I unthreads completely after 4 hours at 95 °C, while caulosegnin III unthreads and decomposes at elevated temperatures.[17] Similarly, astexin-1 unthreads when heated to 50 °C for 4 hours[18] and astexin-2 unthreads and decomposes after heating at 95 °C for 2 hours.[19] In contrast, caulosegnin II and astexin-3 show minimal to no unthreading when heated at 95 °C for prolonged periods of time.[17,19]

4536 | *Org. Biomol. Chem.*, 2016, **14**, 4534–4541

This journal is © The Royal Society of Chemistry 2016

Scheme 3 Synthesis of lassomycin-amide (**2**). The peptide sequence was synthesized *via* SPPS on Rink amide resin (0.79 mmol g^{-1}), before global deprotection and cleavage from the resin. Cyclization of the linear peptide in solution yielded the cyclic target. Reagents and conditions: (i) TFA (95%, water), TIPS, rt, 4 h; (ii) HATU (3.0 equiv.) and DIPEA (6.0 equiv.), DMF, rt, 20 h (pseudo-high dilution, see text), 8%.

In order to further elucidate the effect of temperature on the conformational state of lassomycin, structural characterization of synthetic peptide **1** was carried out using two-dimen-

sional NMR. A comparison of ^{1}H–^{13}C HSQC spectra for **1** at two different temperatures is shown in Fig. 3. The NMR data gathered is also compared in Fig. 3 with the naturally isolated labelled [^{13}C,^{15}N]lassomycin.[4] No noticeable changes in chemical shift values are observed between the spectrum of **1** at 25 °C and at 40 °C. Furthermore, clear differences in the ^{1}H–^{13}C HSQC spectrum can be seen between the synthetic peptide (**1**) and the naturally isolated lassomycin at 40 °C (Fig. 3). These results strongly suggest that the conformation of **1** does not change appreciably upon heating, and they highlight that there are significant differences between the conformation of synthetically prepared lassomycin (**1**) and that of naturally isolated lassomycin.[4]

In light of this evidence, we propose that synthetic lassomycin (**1**) either exists in the unconstrained unthreaded form, or as a threaded lasso, but that there is no interconversion between these structures at temperatures up to 40 °C. Given the clear differences observed in the NMR between the synthetic and naturally isolated lassomycin, it seems likely that one peptide is threaded while the other is not. This idea of two distinct conformations being adopted by the synthetic and natural peptides is supported further by the biological data obtained where clear differences in activity are seen.

We propose that (i) neither peptide has undergone unthreading (or threading) upon heating during NMR data collection, or (ii) one peptide has undergone threading from an initial unthreaded state whilst the other has undergone unthreading. For case (ii) the peptide undergoing unthreading cannot be the naturally isolated lassomycin, as this would imply that the synthetic peptide (**1**) has undergone threading and that it is threaded at the point of NMR data collection, thus paradoxically implying (given the NMR study of temperature dependence already discussed) that a threaded lassomycin cannot unthread. Neither scenario therefore supports the
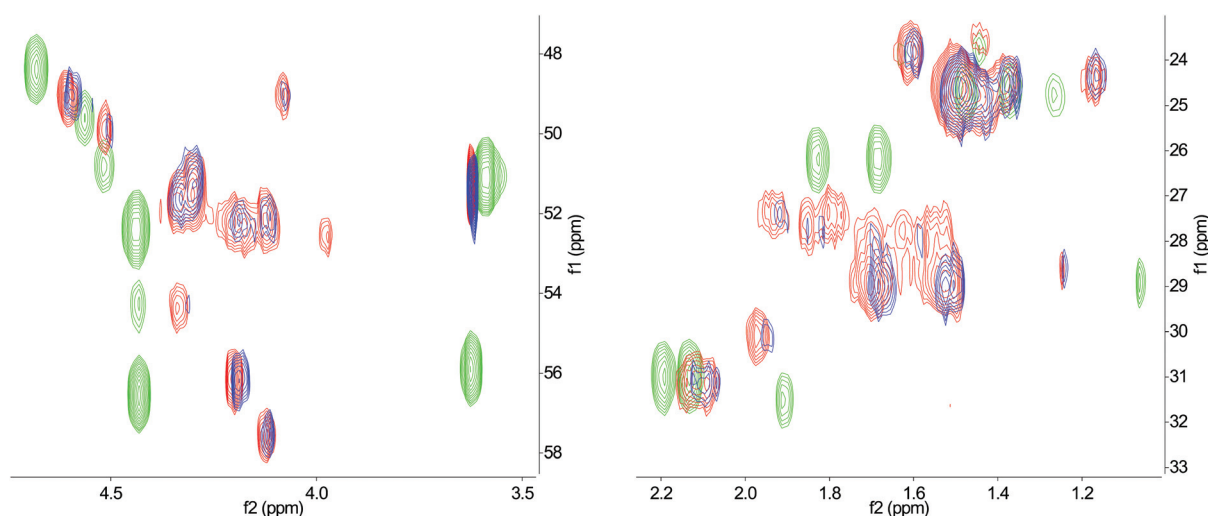

Fig. 3 Overlay of expanded regions of ^{1}H–^{13}C HSQC (heteronuclear single-quantum correlation) NMR spectra of naturally isolated [^{13}C, ^{15}N]lassomycin at 40 °C (green), and synthetic lassomycin (**1**) at 25 °C (blue) and 40 °C (red). Clear differences in chemical shift values at 40 °C are observed between **1** and the natural isolate. In contrast, shift values for the synthetic peptide (**1**) do not change noticeably over the temperature range surveyed. (Full spectra given in the ESI.†)

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 4534–4541 | **4537**

hypothesis that the naturally isolated lassomycin has undergone unthreading upon heating to 40 °C. Furthermore, given the unlikely nature of scenario (ii), taken together with the previously discussed likelihood that synthetic lassomycin is in fact unthreaded, we propose that the previously published naturally isolated lassomycin actually exists as a threaded lasso that does not unthread upon heating to 40 °C.

Conclusions

We have developed a practical synthetic route to an unthreaded version of the lasso peptide lassomycin (**1**). Lassomycin-amide (**2**), a C-terminal amide derivative of **1** was also prepared. The biological evaluation of peptides **1** and **2** against *Mycobacterium tuberculosis* revealed that neither had any activity. A lack of biological activity and our NMR analysis of synthetic lassomycin (**1**) suggest that naturally occurring lassomycin exhibits a standard lasso peptide threaded conformation rather than the reported unthreaded structure. Further conformational studies to gain additional insight in this area are ongoing.

Experimental

All reagents were purchased from Sigma-Aldrich unless otherwise specified. Peptide synthesis grade DMF was purchased from AGTC Bioproducts (Hessle, UK) and amino acid derivatives were purchased from CEM, Novabiochem (Merck) or AGTC. PyBOP® was purchased from Apollo Scientific (Stockport, UK). All resins were purchased from Novabiochem. Peptide molecular weight calculations and mass assignments were carried out using Pep-Calc.com.[20]

Peptide synthesis

Automated SPPS was carried out at 0.10 mmol scale on a CEM Liberty1 single-channel microwave peptide synthesizer equipped with a Discover microwave unit. All reactions were carried out at room temperature in DMF using 2 × 1 h couplings for all residues except Arg, which was triple coupled (3 × 1 h). Fmoc-protected amino acids were used (5 equiv.), with PyBOP® (5 equiv.) as the activator in the presence of DIPEA (10 equiv., 2 M solution in NMP). Amino acid side chain functionality was protected as follows: Fmoc-His(Trt)-OH, Fmoc-Ser (O*t*Bu)-OH and Fmoc-Thr(O*t*Bu)-OH. The Fmoc group was removed by two successive treatments with 20% (v/v) piperidine solution in DMF (5 + 10 min). An additional 10 min deprotection was carried out for the penultimate residue (Leu-2). Bubbling with nitrogen gas was used to ensure efficient agitation of the reaction mixture during each step. Preswelling of dry resin was carried out in DMF for a minimum of 1 h.

Cleavage from solid support

Peptide-resin was shrunk in diethyl ether and treated with 2.85 mL TFA, 0.15 mL deionized water and 0.15 mL TIPS for 4 h at room temperature. The resin was then removed by filtration and the filtrate concentrated *in vacuo* before precipitation using ether and decanting of the liquid (followed by subsequent ether washes). The resulting solid peptide was dissolved in deionized water containing 0.1% TFA and lyophilized.

Solution-phase cyclization

The cyclization was carried out using the pseudo-high dilution conditions described by Malesevic *et al.*[15] Fully deprotected peptide (113 μmol) and HATU (339 μmol) were dissolved separately in 11.3 mL each of DMF which had been dried over molecular sieves. Both solutions were added simultaneously using two syringe pumps at a rate of 0.01 mL min⁻¹ each to a stirred solution of HATU (11.3 μmol) and DIPEA (678 μmol) in dry DMF (11.3 mL) over a period of ~20 h. After the addition was complete, the reaction was stirred for a further 30 min and the solvent was removed under vaccum. The resulting oil was kept at −20 °C until needed for purification.

High-performance liquid chromatography

The crude cyclization reaction product was dissolved in 2.5 mL deionized water/acetonitrile, filtered, centrifuged and injected in 0.5 mL amounts (per run) onto a Speck and Burke Analytical C18 Column (5.0 μm, 10.0 × 250 mm) attached to a PerkinElmer Series 200 LC Pump and 785A UV/Vis Detector. A linear gradient of 10–36% B (solvent A = 5 : 95 : 0.1 MeCN/H₂O/TFA; B = 95 : 5 : 0.1 MeCN/H₂O/TFA) over 40 min, followed by 36–100% B over 20 min, with a flow rate of 2 mL min⁻¹ was used for separations. Absorbance data were collected at 220 nm. Selected fractions were lyophilized and a mass assigned using MALDI-TOF MS or ESI-MS, and peak fractions of interest were pooled and lyophilized.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were collected using a Varian VNMRS 700 MHz. Multiplicities: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet, t = triplet. Chemical shifts are reported in δ units and are referenced to residual solvent peaks: CHCl₃ (¹H 7.26 ppm, ¹³C 77.0 ppm) and DMSO (¹H 2.50 ppm, ¹³C 39.5 ppm).

Analytical liquid chromatography mass spectrometry (LCMS)

Analytical LCMS was carried out using an Acquity UPLC system (Waters Ltd, UK) equipped with a photodiode array detector. Samples were injected onto an Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm) and a gradient of 5–95% B (solvent A = H₂O, 0.1% formic acid; B = MeCN) was run over 3.8 min with a flow rate of 0.6 mL min⁻¹. The flow of solvent from the UPLC system was introduced into the electrospray ion source of an Acquity TQD or QToF Premier mass spectrometer, and positive ions were measured.

4538 | *Org. Biomol. Chem.*, 2016, **14**, 4534–4541

This journal is © The Royal Society of Chemistry 2016

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF mass data was collected using an Autoflex II ToF/ToF mass spectrometer (Bruker Daltonik GmBH) equipped with a 337 nm nitrogen laser. Peptides were dissolved in 1 : 1 deionized water/MeCN for MS analysis. Sample solution (1 mg mL^{-1}) was mixed with matrix solution (α-cyano-4-hydroxycinnamic acid, ~50 mg mL^{-1}) in a ratio of 1 : 9, and 1 μL of the resulting solution spotted onto a metal target and placed into the MALDI ion source. MS data was processed using FlexAnalysis 2.0 (Bruker Daltonik GmBH). MALDI MS/MS was performed using LIFT technology, which enables detection of product ions that result from elevating the laser power.

Ion-mobility (IM) mass spectrometry

Ion-mobility mass spectrometry was carried out using a Synapt G2S mass spectrometer equipped with an Acquity UPLC (Waters Ltd, UK). 1 μL of sample was injected onto a BEH C18 column (1.7 μm, 2.1 × 100 mm) and a gradient of 5–95% B (solvent A = H$_2$O, 0.1% formic acid; B = MeCN, 0.1% formic acid) was run over 6.0 min with a flow rate of 0.4 mL min^{-1}. A photodiode array detector provided absorbance data from 200 nm to 500 nm. The solvent flow was introduced into the electrospray ion source, and full scan MS carried out for positive ions. IM separation was performed with nitrogen as the gas. MS and photodiode array absorbance data was processed using MassLynx 4.1 (Waters Ltd, UK). IM results are represented as a plot of m/z against drift time in Bins (mobiliogram).

MIC testing of compounds against mycobacteria

The MICs of the compounds were determined using standard liquid-broth and solid-agar based methods. Briefly, serial dilutions of each compound were made between 100 mg L^{-1} and 0.78 mg L^{-1} with MB7H9/OADC (BD Biosciences) media in 96-well U-bottom plates. *Mycobacterium tuberculosis* H37Rv was grown to log phase (OD$_{600}$ = 1.0) and 10^4 cells were added to all wells. Solid-agar based method was carried out as previously described[21] using a 48-well plate format. Compounds (see above) were tested in triplicate by adding serial dilutions into separate wells overlaid with MB7H11/OADC (BD Biosciences) agar based media. *Mycobacterium tuberculosis* culture (5 μL containing 10^4 cells) was then inoculated into the centre of each well on top of the solidified media. Plates were sealed and incubated at 37 °C in 5% CO$_2$ for 10 days. Each plate also contained standardised serial dilution controls of first line TB drugs isoniazid (INH [Sigma, UK]; concentration range 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 μg mL^{-1}) and rifampicin (RIF [Sigma, UK]; concentration range 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 μg mL^{-1}), in addition to negative (media only) controls. The MICs were determined as the first dilution to show complete growth inhibition as determined by a total lack of colony formation within the test period. It was not possible to obtain an authentic sample of naturally isolated lassomycin to compare in this assay because the *Lentzea kentuckyensis* sub-

species from which lassomycin was originally prepared is the property of NovoBiotic Pharmaceuticals and was unavailable.

Compounds

Fmoc-Asp(O*t*Bu)-Ile-OMe (10). Fmoc-Asp-(O*t*Bu)-OH (1.14 g, 1.0 equiv., 2.77 mmol), H-Ile-OMe (0.40 g, 1.0 equiv., 2.77 mmol) and PyBOP® (1.44 g, 1.0 equiv., 2.77 mmol) were suspended in DCM (30 mL) and stirred at room temperature. *N*-Methylmorpholine (0.91 mL, 3.0 equiv., 8.31 mmol) was added and the reaction was stirred at room temperature for 18 h. The crude reaction mixture was evaporated under reduced pressure and purified *via* column chromatography (SiO$_2$; 98/2% hexane/EtOAc → 50/50% hexane/EtOAc) to yield Fmoc-Asp(O*t*Bu)-Ile-OMe (**10**) as a white powder (0.93 g, 63%), δ_H (700 MHz, CDCl$_3$) 0.88–0.93 (3H, m, Ile-δCH$_3$), 0.88–0.93 (3H, m, Ile-γCH$_3$), 1.15–1.23 (1H, m, Ile-γCH$_2$), 1.38–1.44 (1H, m, Ile-γCH$_2$), 1.46 (9H, s, O*t*Bu), 1.89–1.95 (1H, m, Ile-βCH), 2.64 (1H, dd, J 17.3 and 7.3 Hz, Asp-βCH$_2$), 2.93 (1H, dd, J 17.3 and 4.1 Hz, Asp-βCH$_2$), 3.71 (3H, s, CO$_2$CH$_3$), 4.23 (1H, t, J 7.1 Hz, FmocCH), 4.41 (2H, d, J 7.1 Hz, FmocCH$_2$), 4.53 (1H, dd, J 8.6 and 4.8 Hz, Ile-αCH), 4.58–4.63 (1H, m, Asp-αCH), 6.08 (1H, d, J 8.2 Hz, AspNH), 7.16 (1H, d, J 8.6 Hz, IleNH), 7.31 (2H, t, J 7.5 Hz, FmocArH), 7.40 (2H, t, J 7.5 Hz, FmocArH), 7.59 (2H, d, J 7.5 Hz, FmocArH), 7.76 (2H, d, J 7.5 Hz, FmocArH); δ_C (176 MHz, CDCl$_3$) 11.6, 15.6, 25.1, 28.1, 37.6, 47.2, 50.9, 52.1, 56.9, 67.4, 82.0, 120.1, 125.1, 127.2, 127.8, 141.4, 143.7, 143.9, 156.0, 170.6, 171.6, 171.9; HRMS m/z (ESI) 539.2756, consistent with empirical formula C$_{30}$H$_{39}$N$_2$O$_7$ with an accuracy of 0.2 ppm (accepted as [M + H]$^+$).

Fmoc-Asp(OH)-Ile-OMe (12). Fmoc-Asp(O*t*Bu)-Ile-OMe (**10**, 0.33 g, 0.61 mmol) was treated with 20 mL of TFA solution (40% in DCM) for 30 min. Complete removal of the *t*-butyl group was confirmed using TLC. The reaction mixture was evaporated under reduced pressure to give Fmoc-Asp(OH)-Ile-OMe (**12**) as a white powder (quantitative yield, 0.29 g) which was used without further purification. δ_H (700 MHz, CDCl$_3$) 0.86–0.91 (3H, m, Ile-δCH$_3$), 0.86–0.91 (3H, m, Ile-γCH$_3$), 1.11–1.20 (1H, m, Ile-γCH$_2$), 1.35–1.44 (1H, m, Ile-γCH$_2$), 1.85–1.96 (1H, m, Ile-βCH), 2.78 (1H, dd, J 17.4 and 6.4 Hz, Asp-βCH$_2$), 3.03 (1H, d, J 17.4 Hz, Asp-βCH$_2$), 3.72 (3H, s, CO$_2$CH$_3$), 4.22 (1H, t, J 6.6 Hz, FmocCH), 4.43 (2H, d, J 6.6 Hz, FmocCH$_2$), 4.53 (1H, dd, J 8.4 and 4.7 Hz, Ile-αCH), 4.60–4.69 (1H, m, Asp-αCH), 5.97 (1H, d, J 8.0 Hz, AspNH), 7.09 (1H, d, J 8.4 Hz, IleNH), 7.31 (2H, t, J 7.5 Hz, FmocArH), 7.40 (2H, t, J 7.5 Hz, FmocArH), 7.57 (2H, d, J 7.5 Hz, FmocArH), 7.76 (2H, d, J 7.5 Hz, FmocArH); HRMS m/z (ESI) 483.2115, consistent with empirical formula C$_{26}$H$_{31}$N$_2$O$_7$ with an accuracy of 3.3 ppm (accepted as [M + H]$^+$).

Lassomycin (1). The dipeptide Fmoc-Asp(OH)-Ile-OMe (**12**) was coupled manually to Rink amide AM resin (0.79 mmol per g substitution, 0.10 mmol scale) using two 1 h couplings at rt. The sequence GLRRLFADQLVGRR was then built onto the resin-supported dipeptide *via* automated SPPS at room temperature (as described). Resin cleavage, cyclization and purification by high-performance liquid chromatography were

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 4534–4541 | 4539

carried out as detailed above to afford lassomycin (26 mg, 14%), MALDI-TOF mass: found m/z 1881.1 $[M + H]^+$, calcd 1881.2 $[M + H]^+$; HRMS m/z (ESI) 940.55785, consistent with empirical formula $C_{83}H_{144}N_{30}O_{20}$ with an accuracy of 0.3 ppm (accepted as $[M + 2H]^{2+}$).

Lassomycin-amide (2). Linear peptide GLRRLFADQLVGRRNI-amide was synthesized *via* automated SPPS at room temperature (as described) on Rink amide AM resin (0.79 mmol per g substitution, 0.10 mmol scale). Resin cleavage, cyclization and purification by high-performance liquid chromatography were carried out as detailed above to afford lassomycin-amide (4 mg, 8%), MALDI-TOF mass: found m/z 1866.2 $[M + H]^+$, calcd 1866.2 $[M + H]^+$; HRMS m/z (ESI) 933.06000, consistent with empirical formula $C_{82}H_{143}N_{31}O_{19}$ with an accuracy of 1.8 ppm (accepted as $[M + 2H]^{2+}$).

Lassomycin(1–9) (17). Linear peptide GLRRLFADQ-amide was synthesized *via* automated SPPS on Rink amide AM resin (0.79 mmol per g substitution, 0.10 mmol scale). Resin cleavage, cyclization and purification by high-performance liquid chromatography afforded lassomycin(1–9) (31 mg, 29%), MALDI-TOF mass: found m/z 1056.6 $[M + H]^+$, calcd 1057.2 $[M + H]^+$; HRMS m/z (ESI) 1056.6082, consistent with empirical formula $C_{47}H_{78}N_{17}O_{11}$ with an accuracy of 1.4 ppm (accepted as $[M + H]^+$).

Abbreviations

AM	Aminomethyl
ATP	Adenosine triphosphate
DCM	Dichloromethane
DIPEA	*N,N*-Diisopropylethylamine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
ES	Electrospray
ESI	Electrospray ionization
Fmoc	Fluorenylmethyloxycarbonyl
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium 3-oxid hexafluorophosphate
HOAt	3*H*-[1,2,3]-Triazolo[4,5-*b*]pyridin-3-ol
HPLC	High-performance liquid chromatography
IM	Ion-mobility
LC	Liquid chromatography
LIFT	LIFT
MALDI	Matrix-assisted laser desorption/ionization
MeCN	Acetonitrile
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
PyBOP®	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
rt	Room temperature
SPPS	Solid-phase peptide synthesis
*t*Bu	*tert*-Butyl
TFA	Trifluoroacetic acid
TIPS	Triisopropylsilane
TLC	Thin-layer chromatography
TOF	Time of flight
Trt	Triphenylmethyl
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
HRMS	High-resolution mass spectrometry

Acknowledgements

Notes and references

1 M. A. Fischbach and C. T. Walsh, *Science*, 2009, **325**, 1089.
2 S. E. Dorman and R. E. Chaisson, *Nat. Med.*, 2007, **13**, 295.
3 T. Kaeberlein, K. Lewis and S. S. Epstein, *Science*, 2002, **296**, 1127.
4 E. Gavrish, C. S. Sit, S. Cao, O. Kandror, A. Spoering, A. Peoples, L. Ling, A. Fetterman, D. Hughes, A. Bissell, H. Torrey, T. Akopian, A. Mueller, S. Epstein, A. Goldberg, J. Clardy and K. Lewis, *Chem. Biol.*, 2014, **21**, 509.
5 T. Akopian, O. Kandror, R. M. Raju, M. Unnikrishnan, E. J. Rubin and A. L. Goldberg, *EMBO J.*, 2012, **31**, 1529.
6 M. O. Maksimov, S. J. Pan and A. J. Link, *Nat. Prod. Rep.*, 2012, **29**, 996.
7 M. Zimmermann, J. D. Hegemann, X. Xie and M. A. Marahiel, *Chem. Biol.*, 2013, **20**, 558.
8 H. Nar, A. Schmid, C. Puder and O. Potterat, *ChemMed-Chem*, 2010, **5**, 1689.
9 *The PyMOL Molecular Graphics System, Version 1.2r1*, Schrödinger, LLC.
10 R. A. Turner, R. J. Weber and R. S. Lokey, *Org. Lett.*, 2010, **12**, 1852.
11 (*a*) S. M. Ocampo, F. Albericio, I. Fernández, M. Vilaseca and R. Eritja, *Org. Lett.*, 2005, 7, 4349; (*b*) M. Mergler, F. Dick, B. Sax, P. Weiler and T. Vorherr, *J. Pept. Sci.*, 2003, **9**, 36; (*c*) C. Yue, J. Thierry and P. Potier, *Tetrahedron Lett.*, 1993, **34**, 323.
12 (*a*) A. G. Russell, M.-E. Ragoussi, R. Ramalho, C. W. Wharton, D. Carteau, D. M. Bassani and J. S. Snaith, *J. Org. Chem.*, 2010, **75**, 4648; (*b*) R. S. Givens, J. F. W. Weber, P. G. Conrad, G. Orosz, S. L. Donahue and S. A. Thayer, *J. Am. Chem. Soc.*, 2000, **122**, 2687.
13 (*a*) B. Mothia, A. N. Appleyard, S. Wadman and A. B. Tabor, *Org. Lett.*, 2011, **13**, 4216; (*b*) C. K. Marlowe, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 437.
14 C. J. White and A. K. Yudin, *Nat. Chem.*, 2011, **3**, 509.

4540 | *Org. Biomol. Chem.*, 2016, **14**, 4534–4541

This journal is © The Royal Society of Chemistry 2016

15 M. Malesevic, U. Strijowski, D. Bächle and N. Sewald, *J. Biotechnol.*, 2004, **112**, 73.

16 D. Suckau, A. Resemann, M. Schuerenberg, P. Hufnagel, J. Franzen and A. Holle, *Anal. Bioanal. Chem.*, 2003, **376**, 952.

17 J. D. Hegemann, M. Zimmermann, X. Xie and M. A. Marahiel, *J. Am. Chem. Soc.*, 2013, **135**, 210.

18 M. Zimmermann, J. D. Hegemann, X. Xie and M. A. Marahiel, *Chem. Biol.*, 2013, **20**, 558.

19 M. O. Maksimov and A. J. Link, *J. Am. Chem. Soc.*, 2013, **135**, 12038.

20 S. Lear and S. L. Cobb, *J. Comput. Aided Mol. Des.*, 2016, **30**, 271.

21 D. Evangelopoulos and S. Bhakta, *Methods Mol. Biol.*, 2010, **642**, 193.