SUPPORTING INFORMATION

Total Chemical Synthesis of Lassomycin and Lassomycin-Amide

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SI Figure 1 – Summary of fragment ions observed via MALDI LIFT-TOF/TOF sequencing of (a) linear (uncyclized) lassomycin, (b) lassomycin and (c) lassomycinamide. Ions resulting from amide (CO-NH) bond cleavage are shown, with the charge retained on either the N-terminal (b ion) or C-terminal (y ion) fragment (charged fragment is observed). Asterisked ions are observed only as M-17 (loss of ammonia).
**SI Figure 2** – HPLC traces for lassomycin (top left), lassomycin-amide (top right) and lassomycin(1-9) (bottom), 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).
**SI Figure 3** – MALDI-TOF mass spectra for lassomycin (top left), lassomycin-amide (top right) and lassomycin(1-9) (bottom).
**SI Figure 4** $^1$H NMR spectrum of Fmoc-Asp(OtBu)-Ile-OMe (10) recorded at 700 MHz (CDCl$_3$).

**SI Figure 5** $^{13}$C NMR spectrum of Fmoc-Asp(OtBu)-Ile-OMe (10) recorded at 176 MHz (CDCl$_3$).
SI Figure 6 – $^1$H NMR spectrum of Fmoc-Asp(OH)-Ile-OMe (12) recorded at 700 MHz (CDCl$_3$).
SI Figure 7 – Mobiliogram for lassomycin (LC ES IM MS, full scan). The collisional cross section for the lassomycin and trace hydrolysis product (13) ions in 4+ charge state are virtually identical (left-hand ellipse), whereas the 3+ charge state ions for the two peptides have different drift times and can be separated (right-hand ellipse). The peak at 50 Bins belongs to the acid hydrolysis product, and at 58 Bins to lassomycin (methyl ester). Only a single peak is 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).
**SI Figure 8** – MALDI LIFT-TOF/TOF mass spectrum showing fragmentation of linear (uncyclized) lassomycin (ions labelled).
SI Figure 9 – MALDI LIFT-TOF/TOF mass spectrum showing fragmentation of lassomycin (ions labelled).
SI Figure 10 – Overlay 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).