A 'Game Changing' New Antibiotic: The Discovery of Teixobactin

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- Structure elucidation of unknown peptides
- Prediction of properties: cLogD, PSA etc

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Introduction to Teixobactin

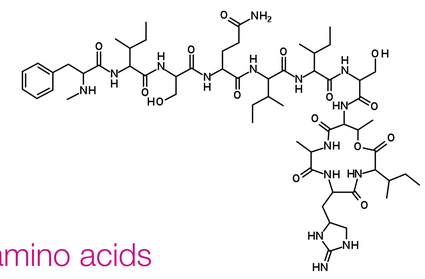
Nature, **2015**, *517*, 455-9.

The discovery of teixobactin, a novel antibacterial from a previously uncultured organism, published this year in Nature, has been recognised as a 'game changer' by infectious disease experts around the world. The golden era of antibiotic drug discovery has long since been over with a dearth of novel antibacterial classes being discovered in the last few decades. Most antibiotics introduced to the clinic were discovered by screening cultivable soil microorganisms, however over mining of this limited resource by the 1960s brought an end to the initial era of drug discovery. However, 99% of all species in external environments are uncultured (do not grow under laboratory conditions) and a novel method for isolating and growing uncultured bacteria has been developed to produce colonies of bacteria. These can then be grown in vitro, thereby giving access to novel natural products. Extracts from 10 000 isolates were screened for activity and a potent inhibitor of gram positive bacteria was identified. The compound, named teixobactin, was found to inhibit cell wall biosynthesis by interacting with multiple cell wall lipid precursors rather than interfering with the activity of one of the enzymes. Serial passage of S. aureus in the presence of sub-MIC levels of teixobactin failed to produce resistant mutants, which is an unusual and very promising resistance profile.

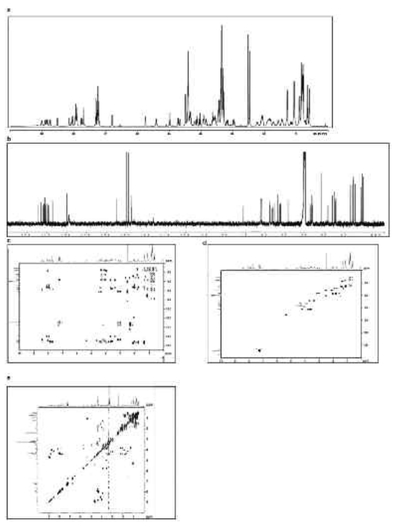
Teixobactin - A New Preclinical Antibiotic: Overview

Chemistry	Novel depsipeptide (MW 1242); potentially 1st new major antibiotic class in >25 years.
MOA	Uniquely inhibits cell wall synthesis at two readily-accessible, non-protein targets
Spectrum	Potent inhibitor of MRSA, VRE and pathogens resistant or non-susceptible to linezolid and daptomycin
Microbiology	Bactericidal
Efficacy	Excellent efficacy in murine infection models (lung, thigh, septicemia)
Safety	No adverse effects in in vitro safety profiling
Markets	IV treatment of ABSSSI, hospitalized CABP, HABP/VABP caused by S. aureus, enterococcal endocarditis, bone /joint infections

Planar Structure Determined by Extensive 2D NMR Experiments by Novobiotic Pharmaceuticals



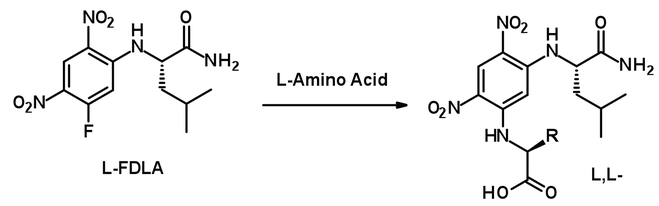
11 amino acids
13 membered macrolactone
Unusual enduracididine amino acid



Determination of Stereochemistry

The Advanced Marfey's analysis technique

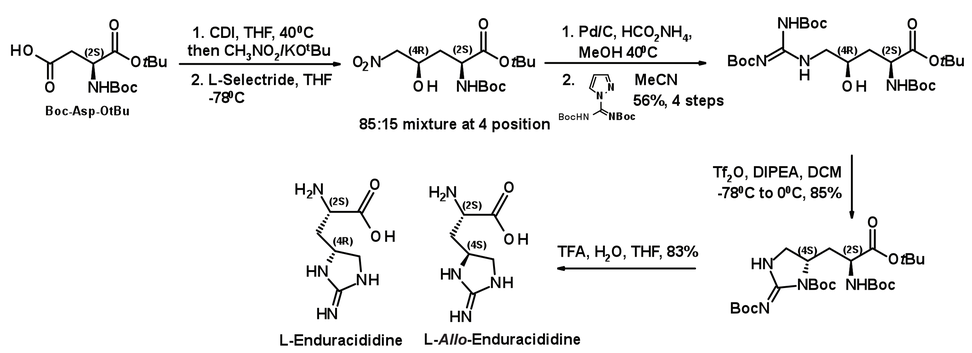
'Advanced' Marfey's analysis attaches a UV-active derivatization agent of known configuration (e.g L-FDLA: 1-fluoro-2,4-dinitrophenol-5-L-leucinamide) to the unknown hydrolysis products of a peptide. It allows the stereoisomers of the constituent amino acids to be chromatographically resolved using simple reverse phase chromatographic methodology with the added advantages that it has both a strong chromophore and is readily ionisable by electrospray ionisation. For all but one of the amino acids (Enduracididine) reference markers were available to allow determination of the configuration based on chromatographic retention time.



Fujii, K. et al, Tet. Lett. **1998**, *39*, 2579

Synthesis of Enduracididine

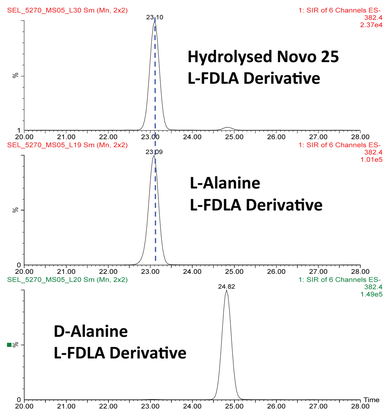
To enable Advanced Marfey's Analysis to be carried out, a synthesis of all 4 diastereomers of enduracididine was required



Synthesis also carried out with D-Boc-Asp-OtBu to afford D-enduracididine and D-*allo*-enduracididine

Representative Example of an Advanced Marfey's Analysis

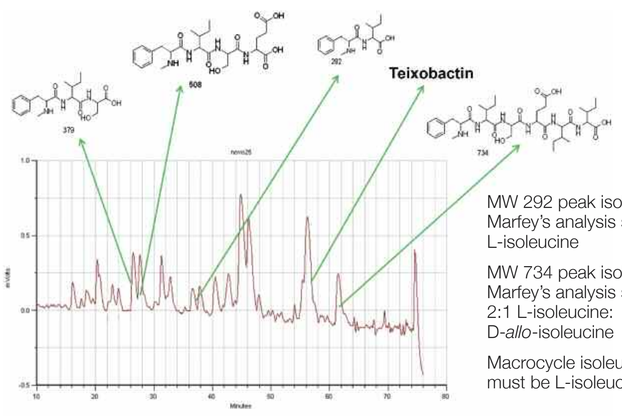
ESP-SIR Traces of m/z 382.4 for Alanine-L-FDLA Derivatives



Residue	Proposed Number in Struture	Observed Configuration
Alanine	1	L-Alanine
Enduracididine	1	L-*Allo*-enduracididine
Glutamine (As Glutamic Acid)	1	D-Glutamine
Isoleucine	4	3 x L-Isoleucine 1 x –D-*Allo*-Isoleucine
N-methyl phenylalanine	1	N-methyl-D-phenylalanine
Serine	2	2 x L-Serine
Threonine	1	D-Threonine

Positioning of the D-*Allo*-Isoleucine

3 L-isoleucines, 1 D-*allo*-isoleucine. Where is the D-*allo*-isoleucine?
Partial hydrolysis of Teixobactin with 1N HCl



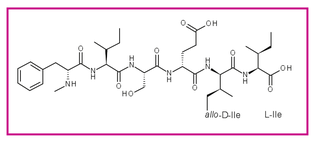
MW 292 peak isolated. Marfey's analysis showed L-isoleucine

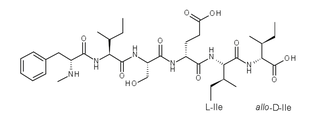
MW 734 peak isolated. Marfey's analysis showed 2:1 L-isoleucine: D-*allo*-isoleucine

Macrocycle isoleucine must be L-isoleucine

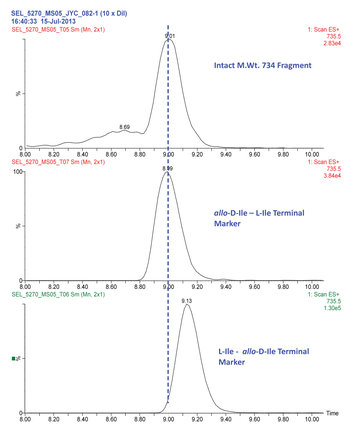
Identification of the '734' Fragment

Both the possible '734' fragment peptides were synthesised on the solid phase
Exact match with the '734' fragment identified

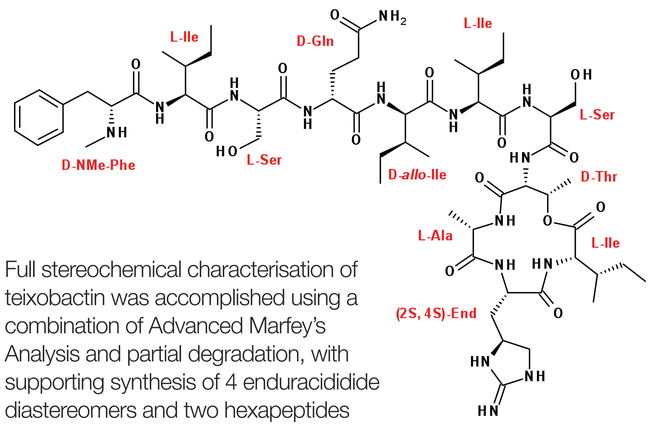




ESP+ RIC's of m/z 735.5 for Intact M.Wt. 734 Fragment



Full Structural Determination of Teixobactin Accomplished



Full stereochemical characterisation of teixobactin was accomplished using a combination of Advanced Marfey's Analysis and partial degradation, with supporting synthesis of 4 enduracididide diastereomers and two hexapeptides