OPEN ACCESS

University of BRISTOL

Walker, P., Williams, C., Weir, A., Wang, L., Crosby, J., Race, P., Simpson, T., Willis, C., & Crump, M. (2019). Control of beta-Branching in Kalimantacin Biosynthesis: Application of 13C NMR to Polyketide Programming. *Angewandte Chemie*, *131*(36), 12576-12580. https://doi.org/10.1002/ange.201905482

Peer reviewed version

License (if available):
Other

Link to published version (if available):
10.1002/ange.201905482

Link to publication record in Explore Bristol Research
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Wiley at https://doi.org/10.1002/anie.201905482 . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research
General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/

Angewandte Chemie

GDCh

Eine Zeitschrift der Gesellschaft Deutscher Chemiker

www.angewandte.de

Akzeptierter Artikel

Titel: Control of beta-Branching in Kalimantacin Biosynthesis: Application of Direct Observe 13C NMR to Polyketide Programming.

Autoren: Paul D Walker, Christopher Williams, Angus NM Weir, Luoyi Wang, John Crosby, Paul R Race, Thomas J Simpson, Christine L Willis, and Matthew Philip Crump

Dieser Beitrag wurde nach Begutachtung und Überarbeitung sofort als "akzeptierter Artikel" (Accepted Article; AA) publiziert und kann unter Angabe der unten stehenden Digitalobjekt-Identifizierungsnummer (DOI) zitiert werden. Die deutsche Übersetzung wird gemeinsam mit der endgültigen englischen Fassung erscheinen. Die endgültige englische Fassung (Version of Record) wird ehestmöglich nach dem Redigieren und einem Korrekturgang als Early-View-Beitrag erscheinen und kann sich naturgemäß von der AA-Fassung unterscheiden. Leser sollten daher die endgültige Fassung, sobald sie veröffentlicht ist, verwenden. Für die AA-Fassung trägt der Autor die alleinige Verantwortung.

Zitierweise: *Angew. Chem. Int. Ed.* 10.1002/anie.201905482
Angew. Chem. 10.1002/ange.201905482

Link zur VoR: http://dx.doi.org/10.1002/anie.201905482
http://dx.doi.org/10.1002/ange.201905482

WILEY-VCH

10.1002/ange.201905482

WILEY-VCH

COMMUNICATION

Control of β-Branching in Kalimantacin Biosynthesis: Application of Direct Observe ^{13}C NMR to Polyketide Programming

Paul D Walker[a], Christopher Williams[a], Angus NM Weir[a], Luoyi Wang[a], John Crosby[a], Paul R Race[b], Thomas J Simpson[a], Christine L Willis*[a], Matthew P Crump*[a]

Abstract: *The presence of β-branches in the structure of polyketides that possess potent biological activity underpins their widespread importance. Kalimantacin is a polyketide antibiotic with selective activity against staphylococci and its biosynthesis involves the unprecedented incorporation of three different and sequential β-branching modifications. Here we use purified single and multi-domain enzyme components of the kalimantacin biosynthetic machinery to address in vitro how the pattern of β-branching in kalimantacin is controlled. Robust discrimination of enzyme products required the development of a generalisable assay, taking advantage of direct observe ^{13}C NMR of a single carbon-13 label incorporated into key biosynthetic mimics combined with favourable dynamic properties of an acyl carrier protein. We report a previously unassigned modular enoyl-CoA hydratase (mECH) domain and the assembly of enzyme constructs and cascades that are able to generate each specific β–branch.*

Kalimantacin **1** (Figure 1) is a polyketide antibiotic isolated from *Alcaligenes* sp. YL-02632S and *Pseudomonas fluorescens* with high selectivity for staphylococcal species and no resistant strains reported.[1] Its full structure and stereochemistry was determined by a combination of degradation and stereoselective synthesis of the resulting fragments.[2] This study also confirmed that kalimantacin and batumin, isolated from *Pseudomonas batumici* are the same compound.[3] It is produced by a hybrid type I *trans*-acyltransferase (AT) polyketide synthase (PKS) non-ribosomal peptide synthase (NRPS).[1, 4] In these systems, core assembly of a polyketide proceeds with the iterative extension of acyl units via a ketosynthase (KS) catalysed decarboxylative condensation of typically malonyl units supplied by the *trans*-acting AT as well as incorporation of amino acids. The growing intermediate is tethered as a thioester to an acyl carrier protein (ACP). Variable in-*cis* reductive domains combine with modifications from *trans*-acting domains and further downstream functionalisations to introduce structural diversity into the product. Rational manipulation of these pathways to deliver biologically optimised products remains an important goal.

[a] Dr Paul Walker, Dr Christopher Williams, Angus Weir, Dr Luoyi Wang, Dr John Crosby, Prof Thomas Simpson, Prof Christine Willis, Prof Matthew Crump
School of Chemistry, University of Bristol, Cantock's Close, BS8 1TS, Bristol, UK.
E-mail: Matt.Crump@bristol.ac.uk; Chris.Willis@bristol.ac.uk;
[b] Dr Paul Race, School of Biochemistry, University of Bristol, University Walk, BS8 1TD Bristol, UK.

β-Branching is a common modification that arises through the interaction of a *trans*-acting 3-hydroxy-3-methylglutaryl CoA-synthase (HCS) containing cassette with a β-ketothioester bound to a modular acceptor ACP (ACP$_A$).[5] Kalimantacin incorporates four β-branches: an α,β unsaturated β-branch (*endo*-β-methyl) at C-3, a β,γ unsaturated β-branch (*exo*-β-methylene) at C-7 and two saturated β-methyls at C-5 and C-15. Although all three examples have been previously observed (Supplementary Figure 1 and references therein), the presence of all three types in a single molecule is unique as are the sequential introduction of branches at C-3, C-5 and C-7. The mechanisms by which this is controlled are not understood.

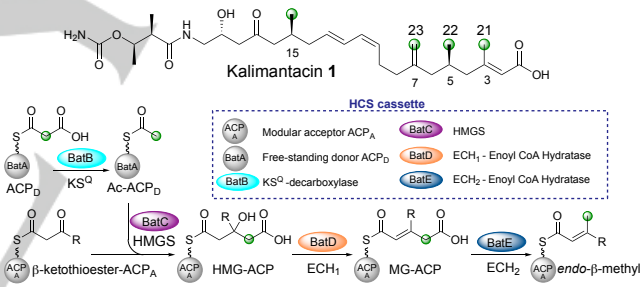


Figure 1. Incorporation of an *endo*-β-methyl branch by an HCS cassette. Kalimantacin **1** with β-branch points highlighted in green.

The most common HCS cassette that controls β-branching contains a free-standing, donor ACP (termed ACP$_D$, BatA in the kalimantacin pathway), a decarboxylating KS (KS^Q, BatB) and a 3-hydroxy-3-methylglutaryl synthase (HMGS, BatC). Previously we have shown that structural features of ACP$_A$, including a tryptophan 'flag' are essential for molecular recognition by HMGS and therefore the timing on this β-branch incorporation.[6] Two enoyl CoA hydratase (ECH) domains (BatD and BatE) that belong to the crotonase superfamily (CS) of enzymes complete the set of five enzymes (Figure 1).[5] The mechanism proceeds KS^Q catalysed generation of acetyl-ACP (Ac-ACP$_D$) and condensation with a β-ketothioester-ACP$_A$ and HMGS to give HMG-ACP$_A$. Dehydration by ECH$_1$ generates 3-methylglutaconyl (MG)-ACP$_A$ and decarboxylation by ECH$_2$ furnishes an unsaturated β-branched polyketide branch following reprotonation by ECH$_2$. Figure 1 specifically shows the formation of an *endo*-β-methyl branch, however, additional tailoring domains may also be present and influence the nature of the final β-branch introduced by the cassette.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ange.201905482

WILEY-VCH

COMMUNICATION

Almost all pathways that incorporate alternative single (eg pederin, onnamide) or multiple (phormidolide) *exo*-β-methylene branches are commonly associated with modular ECH domains with a KS-ECH-ECH-ACP$_{(1\text{-}3)}$ architecture (Supplementary Figure 1). These ECH_2 domains act in-*cis* rather than in-*trans* and are inserted specifically at the point of *exo*-β-methylene incorporation.[4b] Bat3, which incorporates the three sequential β-branches including the *exo*-β-methylene at C7, has been reported to contain four elongating modules (9-12) and an off-loading module (13) but no modular ECH_2-like domain,[1] implying that β-branching is controlled by a single *trans*-acting HCS cassette (BatA-E) and the free-standing ER (BatK)[1] (Figure 2A). However, BLAST identified a previously unassigned domain in the large inter-domain linker region (328 aa) between ACP4 and the downstream KS in module 11 with 53.9% identity to BatE. This matches the point of *exo*-β-methylene incorporation and suggests that this modular ECH_2 may control this step (Figure 2B).[7]

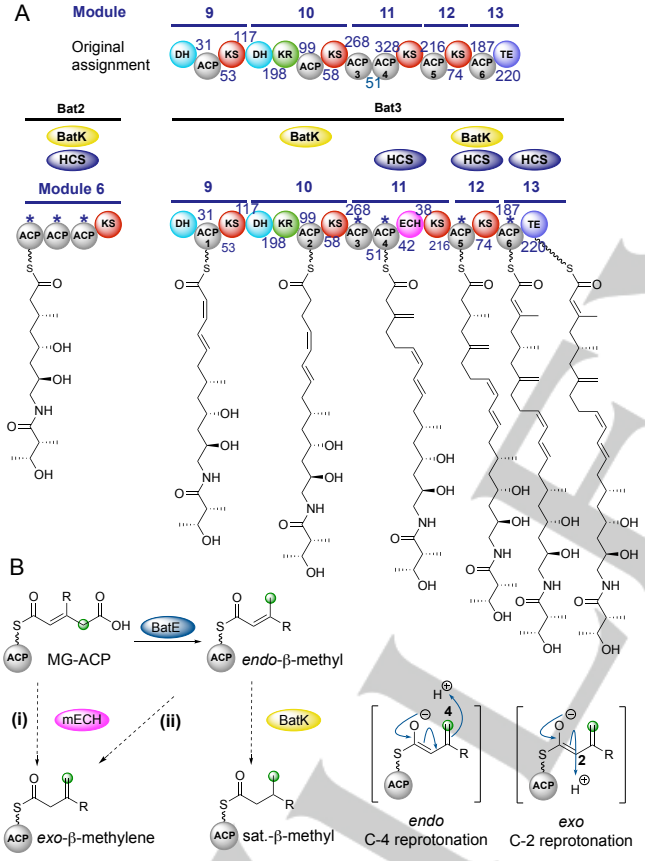
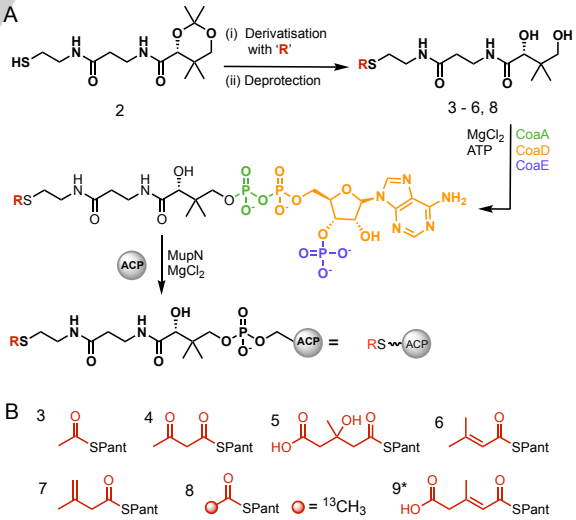


Figure 2. A) Original assigned domain order of ORF Bat3[1] and below the upated domain order to include the previously unassigned ECH domain (shown in pink). The linker lengths between catalytic domains are shown in blue. B) Proposed mechanism for the formation of β-branches in the kalimantacin biosynthetic pathway. The *exo*-β-methylene may be made via one of two routes. **(i)** Decarboxylation and C-2 reprotonation of MG-ACP by mECH. **(ii)** mECH-catalysed isomerism of an *endo*-β-methyl.

An *exo*-β-methylene may, however, arise from either (i) the modular ECH_2 intercepting the dehydrated product via decarboxylation and alternative reprotonation at the C-2 position before the *trans*-acting ECH_2 can access it or (ii) the modular ECH_2 catalysed isomerisation of an *endo*-β-methyl branch or (Figure 2B). Only the kalimantacin and phormidolide pathways are now known to have two ECH_2 domains, and therefore either route is feasible. CS family members are notoriously difficult to functionally classify based on amino acid sequence or 3D architecture[8] and homology modelling did not reveal the likelihood of mECH catalysing either of these reactions.

Previous characterisation of β–branching pathways used protein MS, in particular the phosphopantetheine (Ppant) ejection assay (MS-eject), as the primary method of determining product formation.[5, 7] However, MS cannot resolve double bond isomers so UV absorption, GC/MS of hydrolysed intermediates and ^{2}H-labelling have been applied.[9] To allow for a more detailed characterisation of β-branching pathways in general, we designed an *in vitro* NMR assay to monitor the chemical shift of an ACP-bound ^{13}C-label selectively incorporated into a biosynthetic mimic that would discriminate either regioisomer and take advantage of the favourable transverse relaxation properties of a mobile Ppant side-arm.

The HCS cassette (BatA, C-E), modular ACP4-6, modular ECH (mECH) and reductase (BatK) were recombinantly over-expressed in *E. coli* and purified (Supplementary Figures 2 and 3). Both BatD and BatE were trimeric by size exclusion chromatography (SEC).[8] Pantetheine-bound biosynthetic intermediates were prepared from **2**: Ac-pantetheine **3,** acetoacetyl (Acac)-pantetheine **4**, HMG-pantetheine **5**, 3-methylbut-2-enoyl-pantetheine **6** and [2-^{13}C]-Ac-pantetheine **8** (Scheme 1 and Supplementary Material and Methods, Supplementary Schemes 1-3) and used ass substrates for enzymatic reactions or converted chemoenzymatically to the corresponding CoAs for transfer onto apo-ACP.[10]



Scheme 1. A) Preparation of functionalised-ACP in a one-pot, *in situ* chemoenzymatic pantetheine upgrade and ACP-loading reaction. Biosynthetic pathway to derivatised CoA with the new functional groups added to pantetheine derivatives by CoaA, CoaD and CoaE shown in green, orange and blue respectively. The phosphopantetheine arm (black) is transferred to apo-ACP by a PPTase (here MupN) to give functionalised ACP. B) Acyl-pantetheines. * included for reference only.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ange.201905482

WILEY-VCH

COMMUNICATION

^{13}C-NMR confirmed the ^{13}C-label could be readily observed on derivatised ACPs. Apo-BatA was loaded with [^{13}C]-Ac-pantetheine **8** yielding a single ^{13}C NMR signal at 32.75 ppm (Figure 3A). Similarly, a ^{13}C-label could be observed on a modular ACP, [^{13}C]-Ac-ACP4. (Supplementary Figure 4).

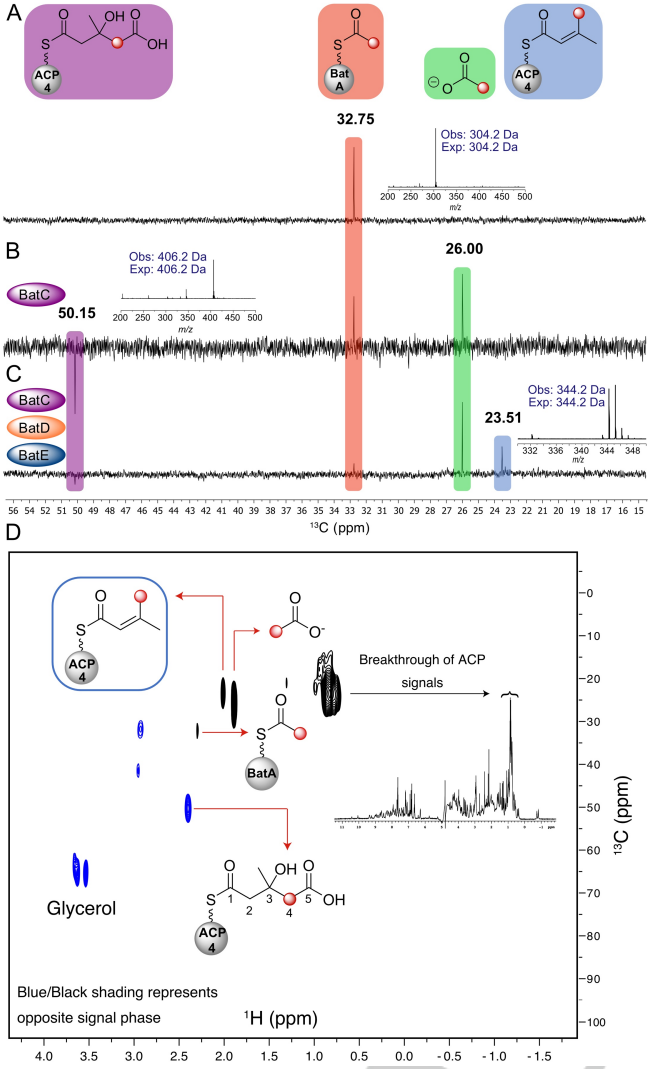


Figure 3. ^{13}C DEPT spectra and MS-eject assays for ACP4 *endo*-β-methyl branch generation. A) Acetyl ^{13}C-methyl signal of [^{13}C]-Ac-BatA (orange) with the observed ^{13}C atom highlighted with a red circle. Inset: MS-eject for [^{13}C]-Ac-BatA. B) Acac-ACP4 + [^{13}C]-Ac-BatA + BatC showing [^{13}C]-methylene of BatA-HMG (purple) and ^{13}C-methyl of hydrolysed acetate (green). C) ^{13}C DEPT and D) ^{1}H-^{13}C HSQC of Acac-ACP4 + [^{13}C]-Ac-BatA + BatC + BatD + BatE. MS-eject derived signals *endo*-β-methyl-ACP4 (344.2 Da) and Acac-ACP4 (345.2 Da).

The reaction of Acac-ACP4, [^{13}C]-Ac-BatA and the HMGS BatC was assayed (Figure 3B) and the ^{13}C spectrum showed the presence of two new signals. The first, at 50.15 ppm, was inverted in a DEPT experiment and was consistent with the CH_2 of an ACP-bound HMG-intermediate. The peak at 26.00 ppm was assigned to free [^{13}C]-acetate generated via hydrolysis of [^{13}C]-Ac-BatC.[11] MS-eject assay validated the formation of the [^{13}C]-HMG-ACP4 (406.2 Da).

Next BatD and BatE were incubated with [^{13}C]-HMG-ACP4 and the reaction cascade monitored by DEPT (Figure 3C). A new positive signal at 23.51 ppm emerged and by MS-eject (344.2 Da) and ^{1}H-^{13}C HSQC correlations (Figure 3D) confirmed the formation of (*Z*)- [4-^{13}C]- 3-methylbutenoyl-ACP4. Reconstitution of the same pathway using ACP5 and assay by NMR/MS (Supplementary Figure 5) and ACP6 by MS (data not shown) gave identical results.

ECH_1 and ECH_2 domains are metabolically coupled[12] and incubations with BatD alone did not yield [^{13}C]-MG-ACP4. To characterise the dehydrated MG intermediate, HMG-pantetheine **5** was incubated with BatD and BatE and monitored by ^{1}H NMR. Although reactions were significantly slower than with ACP-bound intermediates, we detected trace quantities of the MG-pantetheine **9** and 3-methylbut-2-enoyl-pantetheine **6** (Supplementary Figure 6). 2D NOESY confirmed the syn-assignment of the methylene group and alkene proton of **9**.

Initially, to determine whether mECH could complement for BatE and act as a decarboxylase, HMG-pantetheine **5** was incubated with BatD and mECH. The production of the dehydrated intermediate **9** or pantetheine **7** was measured by both NMR and MS but no unsaturated-β-methyl product was observed. Pantetheine **6** was incubated with mECH to monitor for isomerism of the α,β-unsaturated bond, but NMR detected no changes. Subsequent structural characterisation suggested incorrectly folded mECH (Supplementary Figure 7). To overcome this challenge, a di-domain of ACP4-mECH (4M) that preserved the native linker between the ACP and ECH domain was cloned, expressed and purified. The protein was dimeric by SEC (86 kDa) and ^{15}N labelling of 4M followed by acquisition of a ^{1}H-^{15}N HSQC spectrum showed improved dispersion of resonances (Supplementary Figure S7), consistent with the construct being folded. To determine whether a ^{13}C signal was still visible, [^{13}C]-Ac-pantetheine **8** was loaded onto 4M. Despite the high overall molecular mass, the ^{13}C label yielded a clear positive CH_3 peak at 32.75 ppm in the DEPT spectrum (Supplementary Figure 8).

To determine if 4M was functional and able to complement for BatE, Acac-4M was first incubated with BatC and [^{13}C]-Ac-BatA to give [^{13}C]-HMG-4M, confirming 4M to be a viable substrate for the HMGS. Freshly prepared [^{13}C]-HMG-4M was then incubated with BatD alone and a new, negatively phased peak at 118.63 ppm was observed, consistent with an *exo*-β-methylene chemical shift and matched the chemical shifts of an *exo*-β-methylene pantetheine standard **7** (Figure 4). Concurrent MS analysis yielded a MS-eject ion at 344.2 Da, consistent with a [^{13}C]-unsaturated β-branch. No *endo*-β-methyl peak was observed and analogous assays with ACP4 alone gave no MS-eject signal at 344.2 Da, ruling out spontaneous decarboxylation. A second peak at 42.10 ppm may arise from the dehydrated intermediate and was consistent with shifts recorded for free diacid equivalents.[13] This clearly demonstrated that when partnered with ACP4, mECH domain acts as a decarboxylase which produces an *exo*-β-methyl branch.

Accepted Manuscript

This article is protected by copyright. All rights reserved.

10.1002/ange.201905482

WILEY-VCH

COMMUNICATION

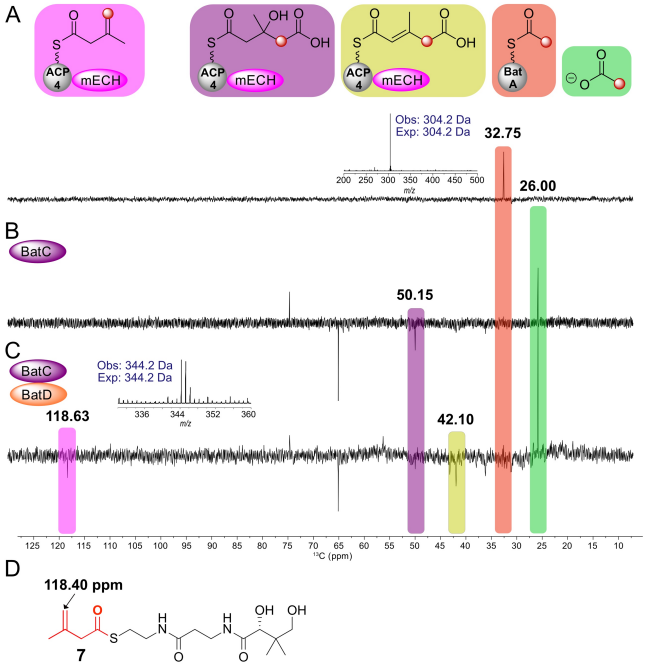


Figure 4. ^{13}C NMR (DEPT) and MS-eject of the β-branching pathway for the di-domain 4M reaction cascade. A) [^{13}C]-Ac-BatA (orange). Inset: MS-eject of Ac-BatA. B) Acac-4M + [^{13}C]-Ac-BatA + BatC assay. The [^{13}C]-CH_2 of BatA-HMG is shown in purple and the $^{13}CH_3$ signal of hydrolysed acetate in green. C) Acac-4M + [^{13}C]-Ac-BatA + BatC + BatD assay. The NMR signal of the ^{13}C *exo*-methylene group is shown in pink and the dehydrated MG in yellow. Inset: eject assay of decarboxylated product. D) *exo*-β-methylene pantetheine standard **7.**

Finally, [4-^{13}C]-HMG-4M was incubated with both BatD and BatE. The reaction contains both possible decarboxylase domains, creating the possibility of competition for the formation of either an *endo*-β-methyl or *exo*-β-methylene. ^{13}C-DEPT spectra yielded the expected signal corresponding to the *exo*-β-methylene but the *endo*-β-methyl product was also observable (Supplementary Figure 9). In this case both signals were very weak and the presence of [^{13}C]-Ac-BatA and [^{13}C]-HMG-4M, in addition to the dehydrated intermediate at 42.10 ppm, suggested that the flux through the pathway was inefficient or stalled and it was not possible to validate this result by MS due to low yields.

Two saturated β-methyl branches are also formed in kalimantacin biosynthesis, firstly, when the biosynthetic intermediate is attached to the ACP in module 6 located on Bat2, and secondly, when attached to ACP5 on Bat3 (module 12). The saturated β-methyl is hypothesised to be formed via the reduction of an HCS cassette derived *endo*-β-methyl by a *trans*-acting ER domain (BatK).[1, 14] To elucidate the selectivity of BatK, ACPs derivatised with **6** were incubated with BatK and NADH and assayed by MS (Figure 5).[15]

The MS-eject assay with ACP5 showed the production of a new ion (345 Da) after 1 h and complete consumption of the starting material (343 Da) confirming reduction as expected. Enoyl-ACP6 was not reduced under the same conditions, or after 6 h of monitoring, which was consistent with the presence of the unsaturated *endo*-β-methyl group in kalimantacin. Surprisingly ACP4 which would not normally bear a substrate for BatK, was fully reduced after 1h. No reduction was observed by incubating the ACPs with NADH in the absence of BatK.

In this study we have combined carbon-13 isotopic labelling, NMR and MS to dissect the mechanism of the different β-branching pathways. Importantly this approach takes advantage of the fundamental property of an ACP to 'display' polar intermediates at the end of a dynamic phosphopantetheine arm rather than sequester them.[16] Combined with single site ^{13}C enrichment and ^{13}C-observe cryoprobe technology, we have demonstrated facile monitoring of polyketide intermediate processing in an extended enzyme cascade. We observe no background ^{13}C signal from natural abundance protein signals when attached to either a single ACP or the dimeric 4M di-domain (86 kDa) and the ^{13}C signal remains sharp. Uniquely this ACP based approach avoids the problems associated with fast transverse relaxation rates that would normally be associated with studying systems of this size and should be a useful tool for studying other related PKS systems.

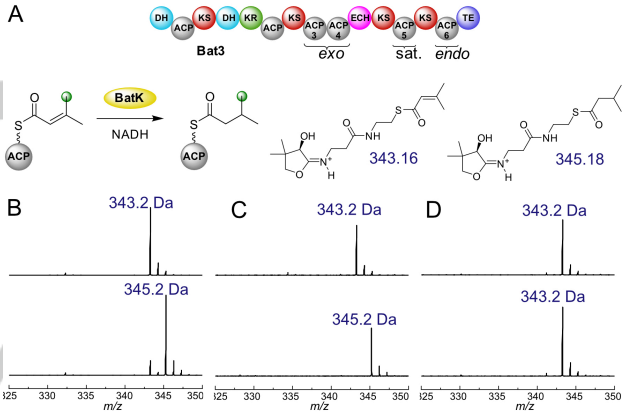


Figure 5. A) BatK catalysed reduction of enoyl-ACP and position of ACP4, 5 and 6 in the PKS and MS-eject ions are shown. (B-D) MS-eject assay of enoyl-ACP4, 5 or 6 respectively before (top row) and after (bottom) addition of BatK.

The presence of a functional modular ECH_2 domain at the point of *exo*-methylene β-branching in the kalimantacin brings this pathway in line with other PKSs. A comparison with related pathways (Supplementary Figure 1) shows that the di-domain ACP3-4 followed by the modular ECH_2 is reversed compared to all other characterised systems and only contains a single modular ECH. All other related modules contain two apparent sequential modular ECHs although invariably the first of these is truncated and most likely non-functional. The modular ECH_2 required the presence of the upstream ACP4 for acitivity and unlike other members of this decarboxylase family, it is dimeric rather than trimeric.[17] It is therefore likely that the ECH_2 folds via the 'self-association' mechanism whereby the active site is formed by residues within the monomeric units, supplemented by

Accepted Manuscript

This article is protected by copyright. All rights reserved.

stabilising interactions with either or both the ACP4 and linker region.

Formation of the C-7 *exo*-β-methylene branch at ACP4 therefore proceeds via an in-*cis* mECH mediated decarboxylation and C2 selective reprotonation. Our *in vitro* results show, however, that BatE - ACP4/4M protein-protein interactions are compatible and can yield an *endo*-β-methyl branch. It is therefore possible for BatE (and subsequently BatK) to access ACP4 so this must be efficiently suppressed *in vivo*. Subsequently ACP5 and ACP6 interact with BatC-E but only ACP5 is recognised by BatK.

β-Branching is therefore partially directed by ACP selectivity but must include elements of structural and substrate control. For example, increased flux of biosynthetic intermediates channelled from ACP4 to the in-*cis* mECH rather than BatE can ensure *exo*-β-methylene branch formation. However, the synthase must first permit BatD access to ACP4 and subsequently occlude BatE. Conformational dynamics of the PKS and proximity effect may ensure the dehydrated product is rapidly channelled to the mECH. Conformational rearrangements of a module from the pikromycin modular *cis*-AT PKS have been shown to be important to allow an ACP-bound intermediate to interact with different catalytic domains and similar mechanisms may operate here.[18] *trans*-AT PKSs may represent an additional level of sophistication however, requiring control of the order of both *cis* and *trans* functionalities.[6, 19] The control required for the selective interaction of modular ACPs with *trans*-acting domains is poorly understood and offers an exciting area for future research.

Acknowledgements

We thank the BBSRC and EPSRC for funding through the Bristol Centre for Synthetic Biology (BB/L01386X/1), Core Capability for Chemistry Research (2), the Bristol Chemical Synthesis Centre for Doctoral Training for PDW (EP/L015366/1) and the doctoral training grant for ANMW.

Keywords: Polyketide • antibiotic • NMR spectroscopy • β-branching • kalimantacin

[1] W. Mattheus, L.-J. Gao, P. Herdewijn, B. Landuyt, J. Verhaegen, J. Masschelein, G. Volckaert, R. Lavigne, *Chem. Biol.* **2010**, *17*, 149-159.

[2] I. R. G. Thistlethwaite, F. M. Bull, C. Cui, P. D. Walker, S. S. Gao, L. Wang, Z. Song, J. Masschelein, R. Lavigne, M. P. Crump, P. R. Race, T. J. Simpson, C. L. Willis, *Chem. Sci.* **2017**, *8*, 6196-6201.

[3] V. V. Smirnov, L. N. Churkina, V. I. Perepnikhatka, N. S. Mukvich, A. D. Garagulia, A. N. Kiprianova, A. N. Kravets, S. A. Dovzhenko, *Prikl. Biokhim. Mikrobiol.* **2000**, *36*, 55-58.

[4] a L. H. Du, C. Sanchez, B. Shen, *Metab. Eng.* **2001**, *3*, 78-95; b E. J. N. Helfrich, J. Piel, *Nat. Prod. Rep.* **2016**, *33*, 231-316.

[5] C. T. Calderone, W. E. Kowtoniuk, N. L. Kelleher, C. T. Walsh, P. C. Dorrestein, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8977-8982.

[6] A. S. Haines, X. Dong, Z. Song, R. Farmer, C. Williams, J. Hothersall, E. Ploskon, P. Wattana-amorn, E. R. Stephens, E. Yamada, R. Gurney, Y. Takebayashi, J. Masschelein, R. J. Cox, R. Lavigne, C. L. Willis, T. J. Simpson, J. Crosby, P. J. Winn, C. M. Thomas, M. P. Crump, *Nat. Chem. Biol.* **2013**, *9*, 685-692.

[7] L. C. Gu, B. Wang, A. Kulkarni, T. W. Geders, R. V. Grindberg, L. Gerwick, K. Hakansson, P. Wipf, J. L. Smith, W. H. Gerwick, D. H. Sherman, *Nature* **2009**, *459*, 731-735.

[8] C. T. Lohans, D. Y. Wang, J. Wang, R. B. Hamed, C. J. Schofield, *ACS Catalysis* **2017**, *7*, 6587-6599.

[9] a C. T. Calderone, D. F. Iwig, P. C. Dorrestein, N. L. Kelleher, C. T. Walsh, *Chem. Biol.* **2007**, *14*, 835-846; b S. T. Slocum, A. N. Lowell, A. N. Tripathi, V. V. Shende, J. L. Smith, D. H. Sherman, *Meth. Enzymol.* **2018**, *604*, 207-236.

[10] A. S. Worthington, M. D. Burkart, *Org. Biomol. Chem.* **2006**, *4*, 44-46.

[11] a F. P. Maloney, L. Gerwick, W. H. Gerwick, D. H. Sherman, J. L. Smith, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 10316-10321; b H. M. Miziorko, M. D. Lane, *J. Biol. Chem.* **1977**, *252*, 1414-1420.

[12] L. C. Gu, J. Y. Jia, H. C. Liu, K. Hakansson, W. H. Gerwick, D. H. Sherman, *J. Am. Chem. Soc.* **2006**, *128*, 9014-9015.

[13] H. M. Miziorko, M. D. Lane, *J. Biol. Chem.* **1977**, *252*, 1414-1420.

[14] B. Uytterhoeven, T. Lathouwers, M. Voet, C. W. Michiels, R. Lavigne, *Front. Microbiol.* **2016**, *7*.

[15] S. B. Bumpus, N. A. Magarvey, N. L. Kelleher, C. T. Walsh, C. T. Calderone, *J. Am. Chem. Soc.* **2008**, *130*, 11614-11616.

[16] S. E. Evans, C. Williams, C. J. Arthur, E. Ploskon, P. Wattana-amorn, R. J. Cox, J. Crosby, C. L. Willis, T. J. Simpson, M. P. Crump, *J. Mol. Biol.* **2009**, *389*, 511-528.

[17] T. W. Geders, L. C. Gu, J. C. Mowers, H. C. Liu, W. H. Gerwick, K. Hakansson, D. H. Sherman, J. L. Smith, *J. Biol. Chem.* **2007**, *282*, 35954-35963.

[18] J. R. Whicher, S. Dutta, D. A. Hansen, W. A. Hale, J. A. Chemler, A. M. Dosey, A. R. H. Narayan, K. Hakansson, D. H. Sherman, J. L. Smith, G. Skiniotis, *Nature* **2014**, *510*, 560-564.

[19] S. Kosol, M. Jenner, J. R. Lewandowski, G. L. Challis, *Nat. Prod. Rep.* **2018**, *35*, 1097-1109.

Accepted Manuscript

WILEY-VCH

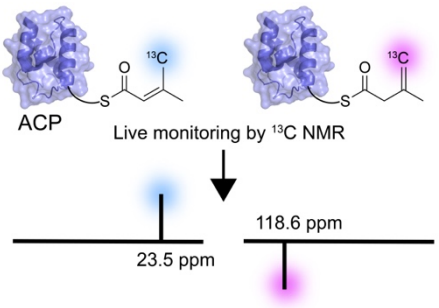
This article is protected by copyright. All rights reserved.

10.1002/ange.201905482

WILEY-VCH

COMMUNICATION

A direct ^{13}C observe NMR assay monitors model biosynthetic intermediates bound to an acyl carrier protein and is used in conjunction with multi-enzyme cascades to show how the pattern of β-branching in kalimantacin is controlled.



*Dr. Paul Walker, Dr. Christopher Williams, Angus Weir, Dr. Luoyi Wang, Dr. John Crosby, Dr. Paul Race, Prof. Thomas Simpson, Prof. Christine Willis, Prof. Matthew Crump**

Page No. – Page No.

Control of β-Branching in Kalimantacin Biosynthesis: Application of Direct Observe ^{13}C NMR to Polyketide Programming

WILEY-VCH

Accepted Manuscript

This article is protected by copyright. All rights reserved.