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Authors: Sacha J Pidot, Ph.D; Mark A Rizzacasa, Ph. D

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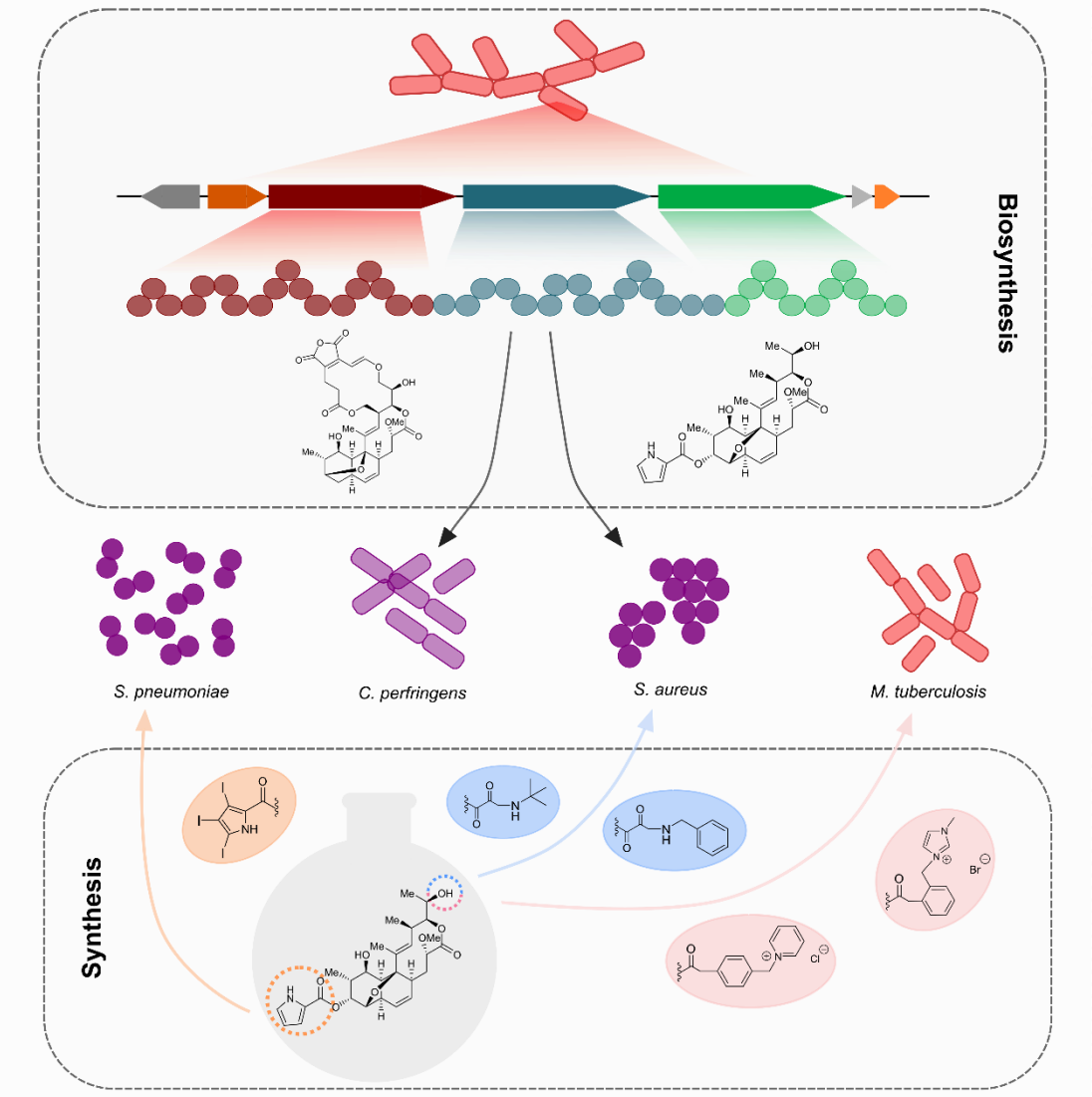
To be cited as: 10.1002/chem.201904053

Link to VoR: https://doi.org/10.1002/chem.201904053

The nargenicin family of oxa-bridged macrolide antibiotics

Sacha J. Pidot*[a] and Mark A. Rizzacasa[b]
Corresponding Author*



[a] Dr S. Pidot
Department of Microbiology and Immunology at the Doherty Institute
University of Melbourne, VIC, Australia, 3000
E-mail: sacha.pidot@unimelb.edu.au
[b] Prof M. A. Rizzacasa
School of Chemistry, The Bio21 Molecular Science and Biotechnology Institute, University of Melbourne,
VIC, Australia, 3000

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Abstract: The nargenicin family of antibiotic macrolides comprise a group of bacterial natural products with a rare ether bridged *cis*-decalin moiety and a narrow spectrum of activity. The majority of family members were identified almost four decades ago and were placed on the shelf due to the numbers of broad-spectrum compounds available at the time. However, in light of rising rates of antimicrobial resistance, there has been a renewed interest in the use of narrow-spectrum antimicrobials. Here, we review the history of this family of compounds, including synthetic approaches, and highlight the recently uncovered genetic basis for nargenicin production. Given the renewed pharmaceutical interest in these compounds, we also investigate structure-activity relationships among these molecules, with a view to the future development of members of this unusual antibiotic family.

1. Introduction

Natural products from the microbial world have provided modern society with some of the most useful medicinal drugs, especially in the area of antimicrobials.[1] In the five or more decades of successful antibiotic discovery from microbes (the “Golden Era”), broad-spectrum bioactive compounds were favored, as they could be approved for a much larger range of indications.[2] However, in recent years, there has been a much deeper appreciation of collateral damage caused to the host microbiota due to the use of broad-spectrum antimicrobials and their role in providing a fertile ground for infections with other pathogens.[3] These factors have led to renewed interest in narrow-spectrum antimicrobials (i.e. those that are genus or species specific), which were once discarded during the antibiotic discovery process.[4] One such group of narrow-spectrum antimicrobials is the nargenicin-like macrolides, which were first reported in 1980.[5] We present here a review of the discovery, activities and biosynthesis of these structurally interesting antibiotics, with a view to stimulating further research activity on these compounds.

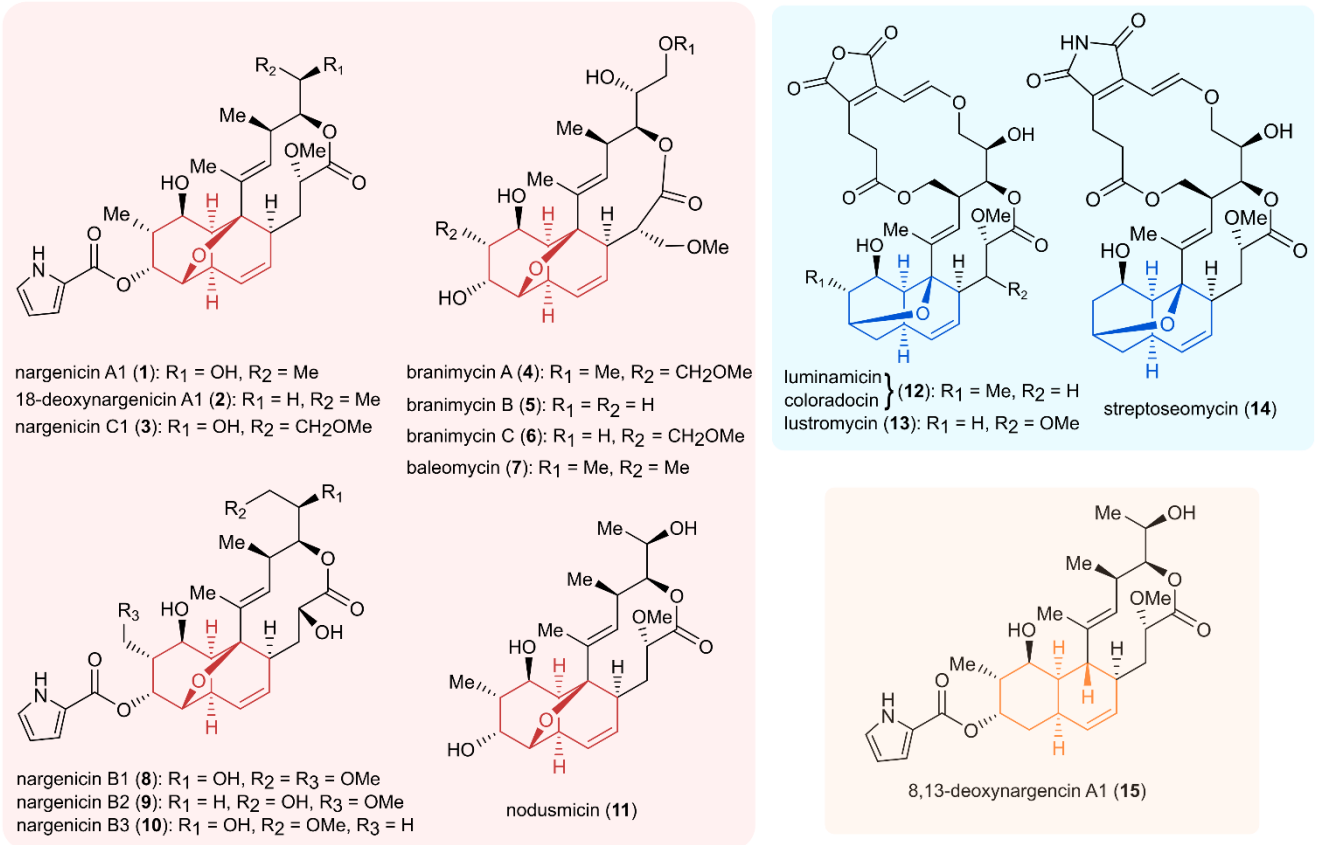

Figure 1. Nargenicin family antibiotics. Compounds are grouped based on the position of the ether bridge across the decalin scaffold. The 8,13 ether bridged decalin is coloured in red, while the 9,13 ether bridged decalin is coloured in blue. The non-ether bridged decalin of **15** is coloured in orange. Note the absolute configuration of lustromycin has not yet been determined but is most likely to be similar to that of luminamicin/coloradocin.

[a] Dr S Pidot
Department of Microbiology and Immunology at the Doherty Institute
University of Melbourne, VIC, Australia, 3000
E-mail: sacha.pidot@unimelb.edu.au
[b] Prof M. A. Rizzacasa
School of Chemistry, The Bio21 Molecular Science and
Biotechnology Institute, University of Melbourne,
VIC, Australia, 3000

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2. The nargenicin antibiotics

The nargenicin family of macrolides represent a group of structurally similar antibiotics with a characteristic oxa-bridged decalin system (Figure 1). Currently, 15 nargenicin-like macrolides are known and each appears to have a slightly different antimicrobial spectrum. The best studied of these compounds is nargenicin A1 (**1**), originally known as CP 47,444, which was identified in the late 1970's at Pfizer from a culture of *Nocardia argentinensis*.[5] Initial work showed the compound to have a very narrow spectrum of activity, with nanomolar minimum inhibitory concentrations (MICs) against *Staphylococcus aureus*, including methicillin resistant strains, but with limited activity against other Gram positive organisms (including closely related Streptococci and Enterococci) or Gram negative bacteria[6], and this activity spectrum provided the impetus to study the molecule in more detail. Throughout the early 1980's much work was done to investigate the biosynthetic origins of **1** and to determine its absolute configuration.[9–11] During this same period of time, other nargenicin antibiotics were identified including 18-deoxynargenicin A1 (**2**)[12], nargenicin C1 (**3**)[13], and nargenicin B1 (**8**), B2 (**9**) and B3 (**10**)[14]; which differ only in their methylation and oxygenation patterns. Other related molecules including nodusmicin (**11**)[15,16], lustromycin (**13**)[17], streptoseomycin (**14**)[18], luminamicin[19] and coloradocin (**12**)[20] have also been identified; the latter two of which were subsequently found to be identical.[21] Interestingly, almost 30 years after the initial discovery of **1**, the branimycins (**4-7**) were identified and these are the only members of the group with a 9-membered macrolactone ring.[22–24] To date, nargenicin family antibiotics have been isolated from a range of bacterial species including *Pseudonocardia, Saccharothrix*, *Actinoplanes*, *Nocardioides, Saccharopolyspora* and *Streptomyces* species, all of which are members of the Actinobacteria, a well-known powerhouse for natural product biosynthesis.[15,18,19,23,25]

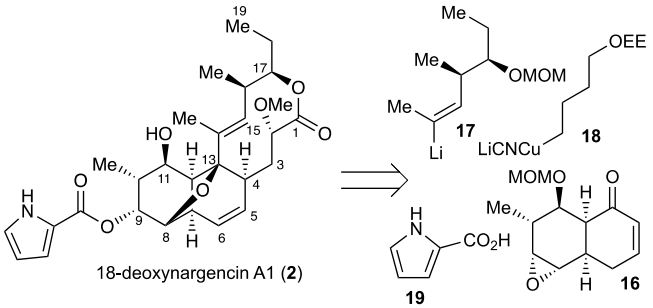
Several synthetic studies of these molecules have been attempted, which have resulted in the total synthesis of (+)-18-deoxynargencin A1[26] and branimycin A.[22,27] Many synthetic approaches have focused on efficient routes to the oxa-bridged octalin nucleus with the eventual goal of total synthesis of nargenicin A1 and these pathways are described in more detail below. An important development in the study of the nargenicins was the identification of the alpha subunit of DNA polymerase (also known as DnaE) as its molecular target in *Staphylococcus aureus.*[28] This work, by scientists at Merck, led to a patent detailing the activity of nargenicin derivatives against mycobacteria, in particular *Mycobacterium tuberculosis*.[29] In the same year, a patent on the use of branimycin derivatives as antimicrobials was also filed.[24] Taken together, these filings show that there is renewed pharmaceutical interest in the development of these narrow spectrum antibiotics for the treatment of infectious diseases.

2. Synthesis of the nargenicins

2.1. Total Synthesis of 18-Deoxynargenicin A1 (2)

Their narrow spectrum of activity combined with interesting structural features have made the nargenicin macrolides attractive targets for synthetic studies. A key challenge for total synthesis has been an efficient route to the *cis*-decalin system and the formation of the critical ether bridge across the decalin system. The first total synthesis of a member of this family of natural products, 18-deoxynargenicin A1 (**2**), was reported by the Kallmerten group and the retrosynthesis is summarized in Scheme 1.[26] The key disconnections included addition of the anion **17** to the ketone of the *cis*-decalin fragment **16** to forge the C13-14 bond with concomitant formation of the oxygen bridge *via* transannular cyclization,[30] conjugate addition of the cuprate **18** to introduce the C3-4 bond and a late stage macrolactonisation and esterification with acid **19** to install the C9 pyrrole ester.

Scheme 1. Retrosynthetic analysis of 18-deoxynargenicin A1 (Kallmerten *et al.*)



The *cis*-decalin fragment was synthesized by the route shown in Scheme 2[30] and begins with a Diels-Alder cycloaddition between the diene **21** and *p*-benzoquinone **20** to form the decalin system **22** as a single diastereoisomer. Reduction and acetal protection gave the alcohol **23**, which on mesylation and S_N' substitution, followed by acid deprotection afforded diol **24**. Selective oxidation of the allylic alcohol and protection gave ketone **25** and epoxidation of the electron rich alkene from the least hindered face afforded epoxide **16** as a single diastereoisomer in good yield. The formation of the key oxo-bridge was then tested in a model study. Addition of methyl magnesium bromide to ketone **16** generated the tertiary alkoxide, which on warming to 0°C underwent transannular cyclization epoxide ring-opening to give the desired oxygen bridged *cis*-decalin **26** in good yield.

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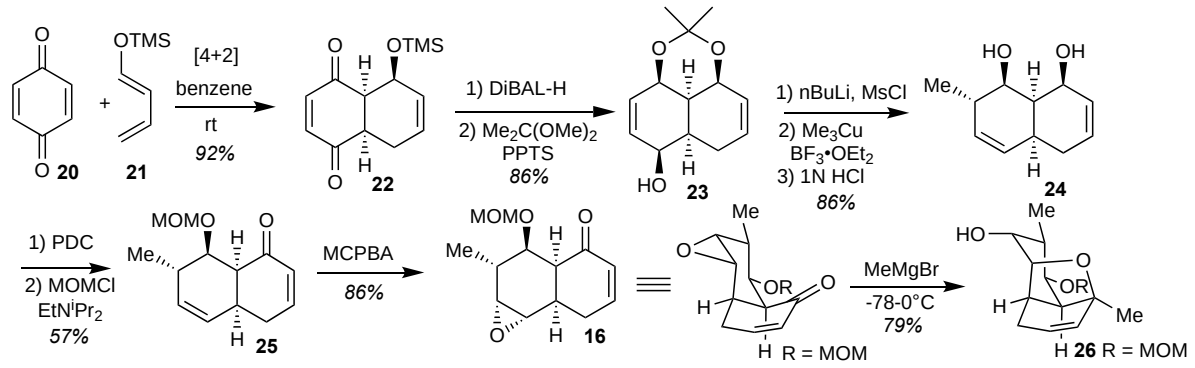
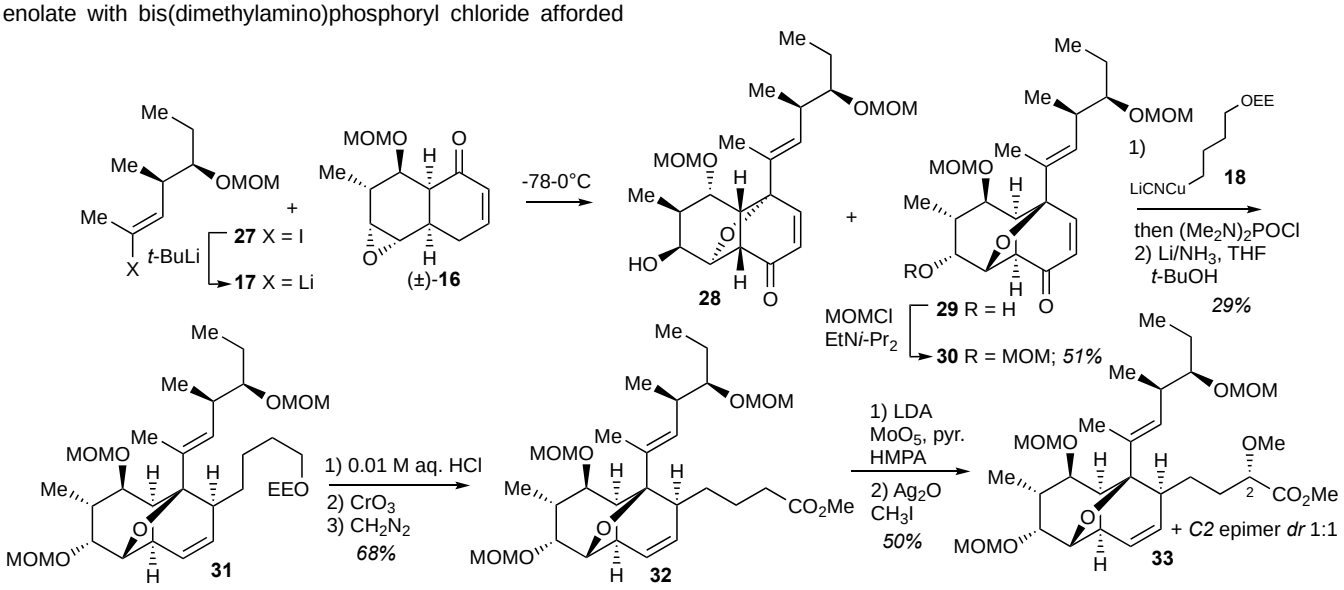


Scheme 2. Synthesis of the *cis*-decalin core **16** (Kallmerten *et al.*)

The synthesis of the macrolactone precursor (Scheme 3) began with formation of the lithium anion **17** derived from enantiopure iodide **27** via metal-halogen exchange with *t*-BuLi. Addition to the racemic ketone **16** followed by warming provided the diastereoisomeric alcohols **28** and **29** and the desired isomer **29** was protected to afford MOM ether **30**. Conjugate addition of the cuprate **18** and quenching of the enolate with bis(dimethylamino)phosphoryl chloride afforded the phosphoramide which was reduced to the alkene **31** with lithium in ammonia. Removal of the ethoxyethyl ether, oxidation and esterification gave the ester **32**. Enolate oxidation and methylation then gave the ester **33** along with the corresponding C2 epimer as a 1:1 mixture.



Scheme 3. Synthesis of the macrolactone precursor **33** (Kallmerten *et al.*)

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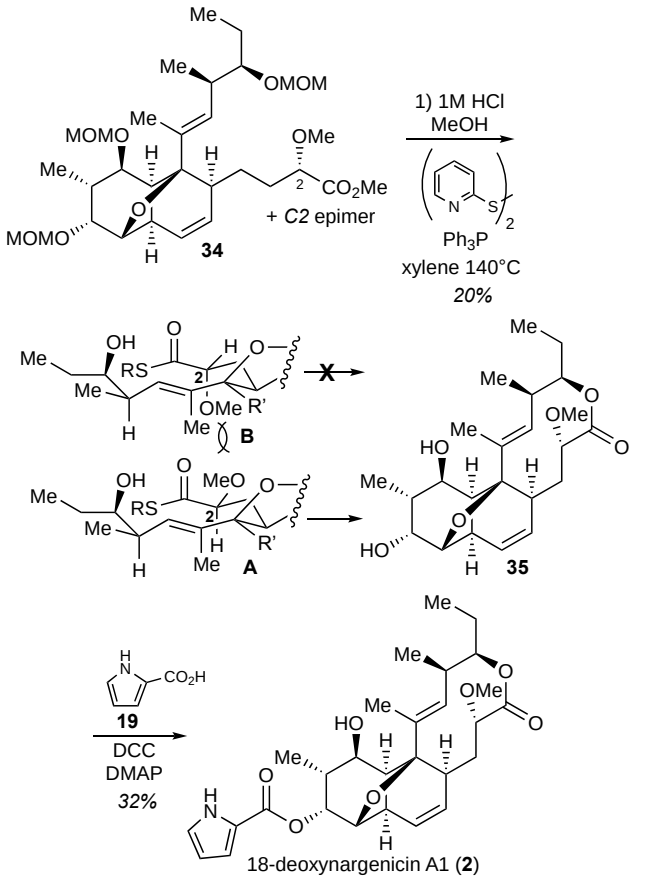
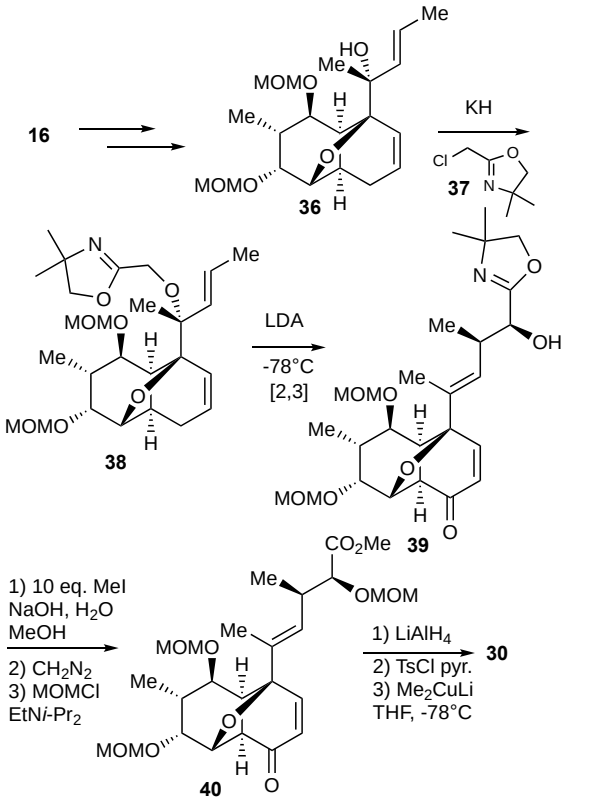


Scheme 4. Completion of the synthesis of the total synthesis of 18-deoxynargenicin A1 (**2**) (Kallmerten *et. al.*)

The final stages of the total synthesis of 18-deoxynargenicin A1 (**2**) are shown in Scheme 4. Hydrolysis of the MOM ethers and methyl ester followed by macrolactonisation under Corey-Nicolaou-Gerlach conditions[31] afforded the macrolactone **35** as the only macrolide product. It was proposed that the desired isomer **34** cyclizes via intermediate **A** whilst the undesired C2 epimer **B** failed to cyclize due to the steric interaction between the C2 methoxy group and the allylic C14 methyl group as shown. Esterification of the least hindered alcohol with acid **19** then gave 18-deoxynargenicin A1 (**2**).

A second generation synthesis of the intermediate **30** was reported by Kallmerten[32], which involved a highly stereoselective formation of the C14-C19 segment (Scheme 5). Decalin **16** was converted into the allylic alcohol **36** in a 7 step sequence and this was *O*-alkylated with the chloromethyloxazoline **37** to give **38**. Base induced [2,3] Wittig rearrangement produced the alcohol **39** as a single diastereoisomer. The stereochemical outcome of the [2,3]-Wittig rearrangement can be rationalised by the mechanism shown in Figure 2.

Hydrolysis of the quaternised oxazoline, esterification and alcohol protection gave ester **40**. Reduction followed by tosylation and cuprate displacement then afforded the nargenicin A1 intermediate **30.**



Scheme 5. Second generation synthesis of intermediate **30** (Kallmerten *et al.*)

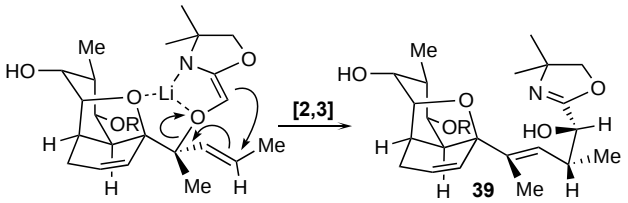


Figure 2. Rationale for the stereochemical outcome of the [2,3]-Wittig rearrangement to give compound 39 (Kallmerten *et al.*)

2.2. Total Synthesis of Branimycin (4)

The second oxo-bridged macrolide to succumb to total synthesis is branimycin reported by Mulzer et al in 2010.[22] The retrosynthetic analysis is shown in Scheme 6. The key disconnection is similar to that for nargenicin A1 (**2**). Thus, addition of the anion **41** to the ketone **42** followed by concomitant transannular cyclization would form the oxo-bridge. Conjugate addition into the enone would then form the C2-C3 bond also in a manner similar to that utilised in the total synthesis of **2**.

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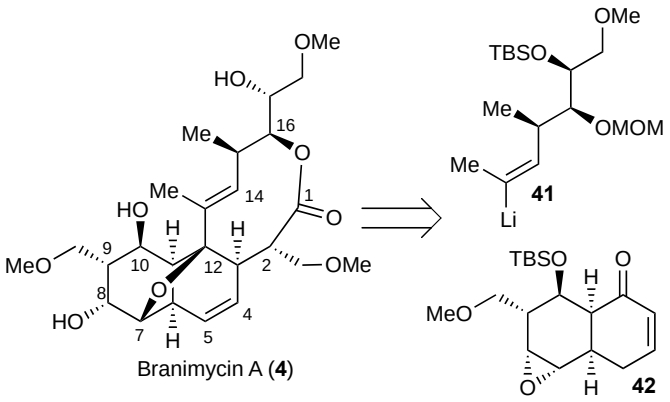
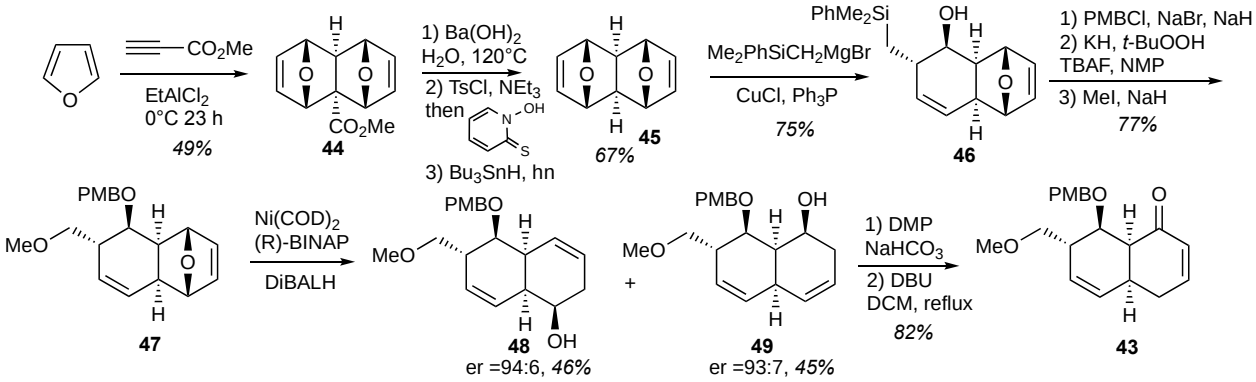


Scheme 6. Retrosynthetic analysis of branimycin A (**4**) (Mulzer *et al.*)

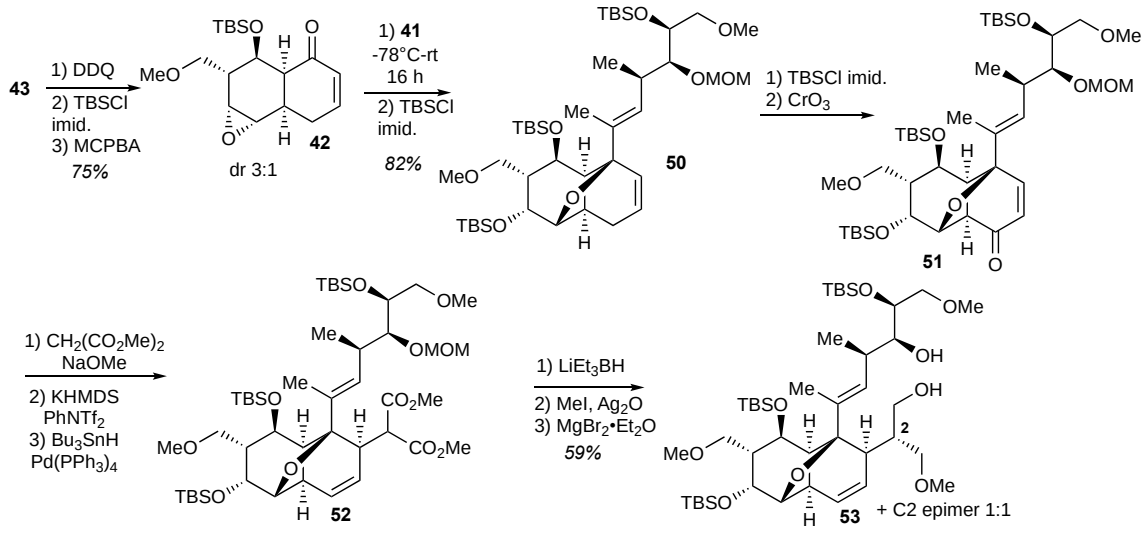
The synthesis of the precursor enone **43** to epoxide **42** is shown in Scheme 7.[33] A Lewis acid mediated [4+2]-cycloaddition between furan and methyl propiolate gave the bridged decalin **44,** which on hydrolysis and radical decarboxylation afforded **45**. Desymmetrization via epoxide ring-opening by cuprate mediated S_N' substitution afforded silane **46** in good yield. Protection, silane oxidation and methylation then gave ether **47**. Asymmetric reductive epoxide opening[34] mediated by (*R*)-BINAP gave the desired alcohol **49** in 46% yield and 93:7 er along with undesired regioisomer **48** also in good er. Oxidation and conjugation of the alkene then afforded enone **43**.

Protective group exchange and selective epoxidation of **43** gave epoxide **42** and addition of anion **41** with concomitant transannular epoxide ring-opening followed by protection of the resultant alcohol to give ether **50** (Scheme 8).



Scheme 7. Synthesis of the *cis*-decalin core **44** (Mulzer *et al.*)

Protection and allylic oxidation gave enone **51** and conjugate addition of the anion derived from dimethylmalonate followed by formation of the vinyl triflate and palladium catalysed reduction gave the diester **52**. Attempts to desymmetrize the diester by both chemical and enzymatic hydrolysis failed so this was reduced to the diol monomethylated and the MOM ether was removed to give diol **53** plus the C2 epimer in a 1:1 ratio.



Scheme 8. Synthesis of the macrolactone precursor **49** (Mulzer *et al.*)

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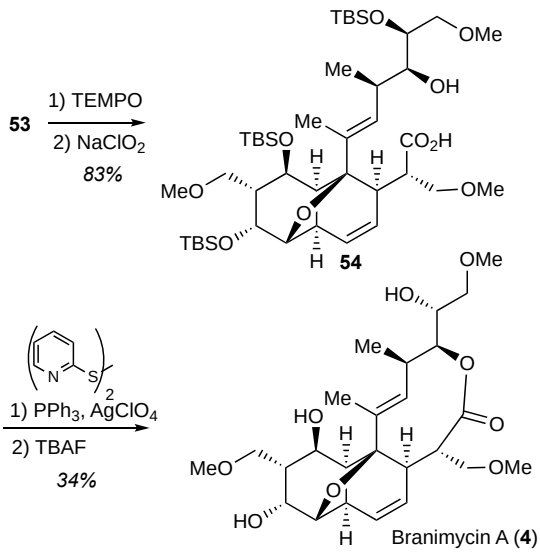
The final steps to branimycin are shown in Scheme 9 and this begins with the 2 step oxidation of alcohol **53** to afford seco acid **54**. Attempted Yamaguchi macrolactonisation only resulted in elimination of the methoxy group so Corey-Nicolaou-Gerlach conditions[31] were again utilized to form the macrolactone. TBAF induced deprotection then gave branimycin (**4**) in 34% yield for the two steps and this served to confirm the absolute configuration of this natural product.



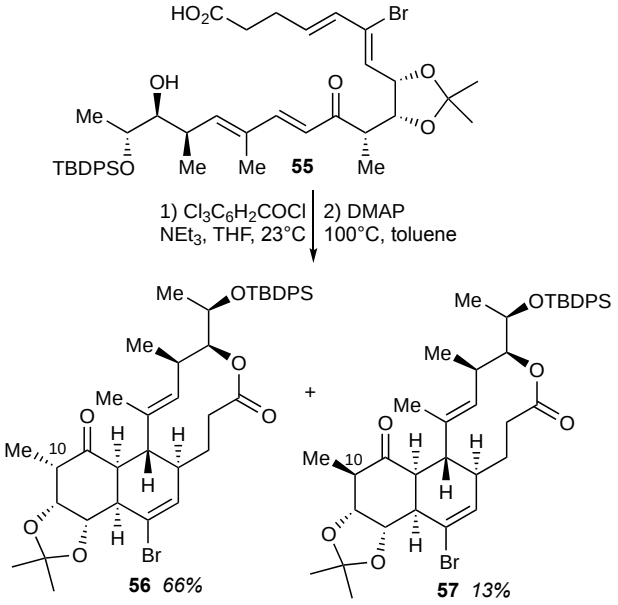
Scheme 9. Completion of the total synthesis of the branimycin A (**4**) (Mulzer *et al.*).

2.3. Synthetic approaches to the *cis*-Decalin

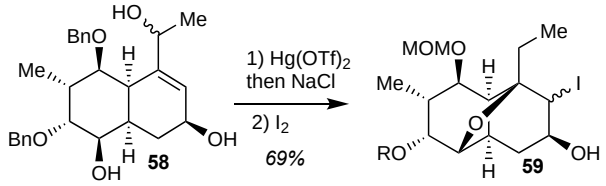
Several approaches to the *cis*-decalin core of the nargenicin natural products have also been reported. Roush[35,36] described a intramolecular Diels-Alder (IMDA) approach to assemble the *cis*-decalin system, which mirrors the postulated biosynthesis. Macrolactonisation of the acid **55** gave the 18-membered macrolide which underwent concomitant IMDA cyclisation to give the macrolide **56** and a small amount of the C10 epimer **57**, the formation of which was attributed to the weakly basic conditions (Scheme 10). The stereochemical outcome of the IMDA was explained by a preference for the endo transition state along with minimized allylic strain. Epimerisation at C10 could be reduced by conducting the reaction at 80°C to give **56** in 32% yield and less **57** but a substantial amount of the macrolide was obtained. However, this could be converted into **52** in high yield by heating to 100°C in toluene.

Gössinger and co-workers have reported an approach to the decalin fragment of coloradocin[37] as well as a synthesis of the oxo-bridged decalin of nodusmicin.[38][2] In this approach, the key step was an intramolecular oxy-mercuration reaction to form the oxo bridge. Treatment of triol **58** with mercuric triflate followed by iodination gave the oxo-bridged decalin **59** in good yield (Scheme 11).

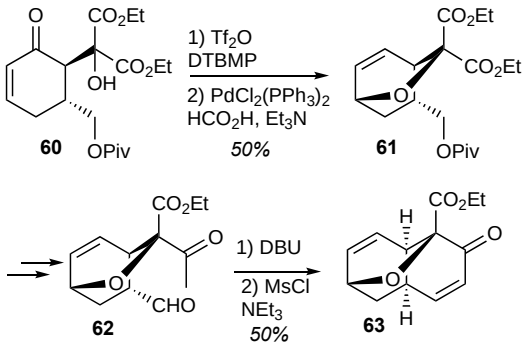
Omura and coworkers employed an intramolecular oxy-Michael reaction to form the alternative 9,13-oxo bridge *cis*-decalin system in luminamicin and lustromycin. Treatment of the ketone with triflic anhydride in the presence of 2,6-di-*t*-butyl-4-methylpyridine (DTBMP) induced an intramolecular conjugate addition of the tertiary alcohol **60** into the enone and formation of the enol triflate which was subsequently reduced under palladium catalysis to give **61** (Scheme 12). This intermediate was converted in the aldehyde **62** and exposure to DBU caused epimerisation of the aldehyde and intramolecular aldol condensation. Elimination via the mesylate then gave oxo-bridged *cis*-decalin **63**.



Scheme 10. Intramolecular Diels-Alder approach to the *cis*-decalin (Roush *et al.*).



Scheme 11. Intramolecular oxy-mercuration approach to the nodusmicin *cis*-decalin (Gössinger *et al.*).



Scheme 12. Intramolecular conjugate approach to the lustromycin *cis*-decalin (Omura *et al.*).

A intramolecular conjugate addition was also applied in the synthesis of the bridged ether *cis*-decalin found in

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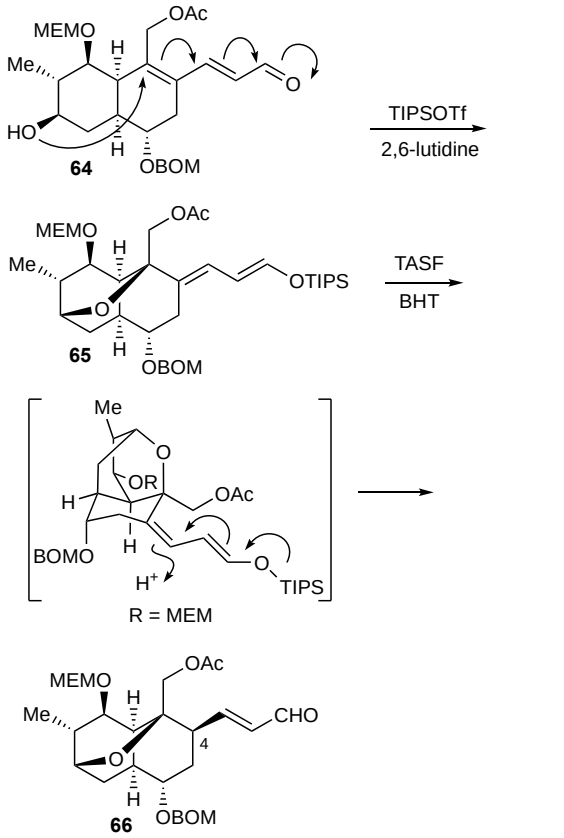
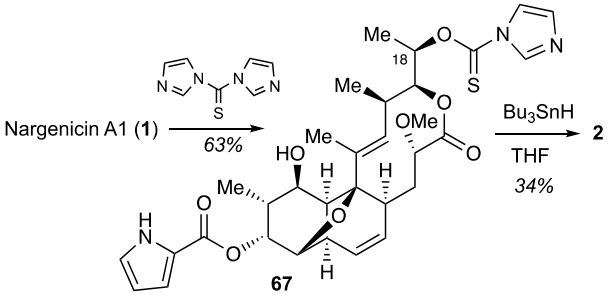
luminamicin.[39] Treatment of the dienal **64** with TIPSOTf induced an intramolecular 1,6-oxa-Michael reaction to forge the oxo-bridge and give enol ether **65** (Scheme 13). Treatment of **65** with TASF induced desilylation and stereoselective protonation as shown to give the aldehyde **66** with a stereoselectivity of 9:1 as judged by ^{1}H NMR analysis.



Scheme 13. Intramolecular 1,6-oxa-Michael reaction to form the luminamicin oxa-bridged *cis*-decalin (Omura *et al.*).

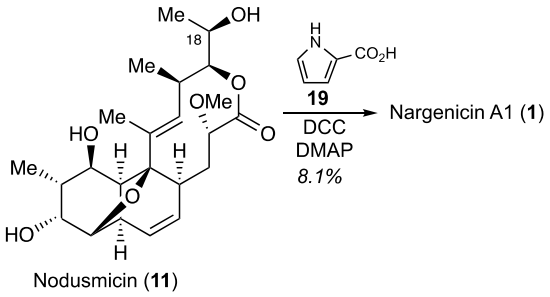
2.4. Semisynthesis

The semisynthesis of 18-deoxynargenicin A1 from nargenicin A1 was reported by Magerlein and Reid.[7] This involved formation of the 18-*O*-thioimidazole ester **67** in 63% yield by treatment of nargenicin A1 with 1,1'-thiocarbonyldiimidazole (Scheme 14). Reduction of with tributyltin hydride gave 18-deoxynargenicin A1 (**2**) in 34% yield.



Scheme 14. Synthesis of 18-deoxynargenicin A1 (**2**) from nargenicin A1 (**1**) (Magerlein and Reid).

The semisynthesis of nargenicin A1 (**1**) from nodusmicin has also been reported.[40] Esterification of nodusmicin (**11**) with 2-pyrrole-2-carboxylic acid **19** mediated DCC and DMAP gave nargenicin A1 (**1**) in low yield (8.1%) along with 18-*O*-pyrrole-2'-carbonylnodusmicin in 10% yield and 9,18-*O*-dipyrrole-2'-carbonylnodusmicin in 4% yield.



Scheme 15. Synthesis of nargenicin A1 (**1**) from nodusmicin (**11**) (Magerlein and Mizsak)

2.5. Future synthetic prospects

The total synthesis of complex nargenicin type natural products is a challenging endeavour as exemplified by there being only two total syntheses of members of this family to date. The key challenge of installing the oxa-bridged *cis*-decalin system remains the greatest hurdle to efficient synthesis. In this regard, future approaches that involve late stage oxidation (see next section) to install this biologically important functionality could provide an alternative to the multi-step sequences required. Further synthetic investigations could also lead to novel simplified analogues (diverted chemical synthesis)[41], which are not available directly from modification of the natural compounds.

3. Biosynthesis of the nargenicin macrolides

3.1. The Nar and Stm PKSs

Early ^{13}C labelling studies showed that **1** was formed from acetate and propionate building blocks akin to other known macrolides of the time.[10] Further studies suggested that the nargenicins were formed by polyketide synthases (PKSs) and this has been confirmed by recent PKS knock-out experiments in producers of both nargenicin A1 and streptoseomycin.[18,42] Genome sequencing of isolates producing **1** and **14** identified the genetic loci responsible for the production of these compounds (*nar* for nargenicin and *stm* for streptoseomycin) and allows for a direct comparison of the PKSs encoded within these biosynthetic gene clusters. Both the *nar* and *stm* loci contain three genes of almost identical total length (43 kb for *nar* versus 43.2 kb for *stm*) that together encode nine module PKSs. While direct amino acid identity between the proteins is approximately 66%, module and domain composition is almost identical, with the only exception being the acyl-transferase (AT) domain in module 4 (Figure 3). Here, the Nar PKS AT domain selects methylmalonate while the Stm PKS selects malonate. These

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differing architectures are reflected in a methyl group at C10 in **1** and a des-methyl at the same position in **14** (Figure 3). As the domain and module organisation leading to the production of the core macrolactone of these molecules has now been established, the likely PKS domain arrangements for nargenicin family members without corresponding genomic information can also be predicted (Figure 3). Given the similarity of the core lactone, the luminamicin/coloradocin-producing PKS should have a domain architecture identical to that of the nargenicin PKS. While the biosynthesis of nargenicin family members with 10-membered macrolactone cores (nargenicin, streptoseomycin, luminamicin) follows PKS biosynthetic logic, at present it is unclear how the 9-membered macrolactone rings of the branimycins are formed and further work is required in this area to enhance our understanding of branimycin biosynthesis.

The almost identical arrangement of domains and modules among the Nar and Stm PKSs, combined with the isolation of all nargenicin-family antibiotics from Actinobacteria, strongly suggests a common evolutionary origin for these megasynthases, with the acquisition/loss of accessory genes within the corresponding gene clusters. This is further

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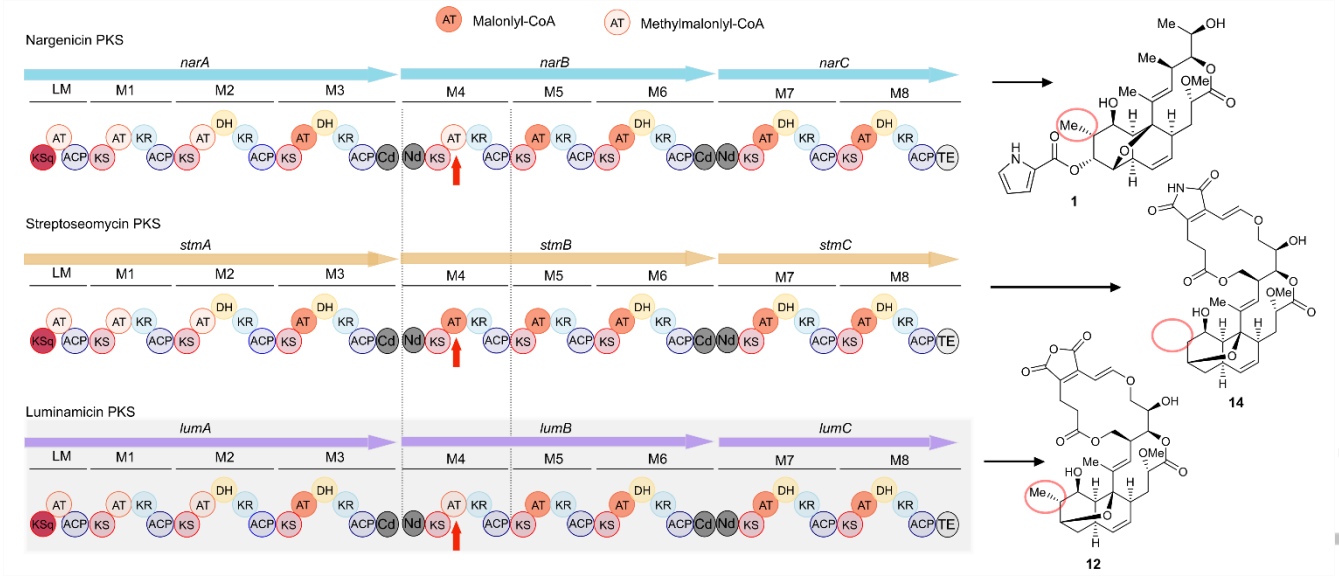

Figure 3. Structures of known and predicted nargenicin family PKSs. Each PKS is arranged with the encoding genes (coloured thick arrows), the labelled modules and domains that they encode and the resulting product. Red arrows highlight changes in the specificity of the AT domain in module 4 and the resulting change in the nargenicin core is indicated by a red circle. Note: the lustromycin PKS is predicted to be identical to that of streptoseomycin. Domain abbreviations: KSq, ketosynthase load; AT, acyltransferase; DH, dehydratase; KR, ketoreductase; ACP, acyl carrier protein; Cd, C-terminal docking; Nd, N-terminal docking.

supported by the identification of orthologous *nar* and *stm* gene clusters in the genome sequences of other *Nocardia* and *Streptomyces* strains.[42] The future genome sequencing of other nargenicin antibiotic producers, especially those that produce branimycin, lustromycin and luminamicin will provide further and much needed information on the evolution of this biosynthetic locus.

3.2. Post-PKS reactions

Modification of the core nargenicin macrolide occurs following release of the linear polyketide chain from the PKS assembly line. In the case of all nargenicin antibiotics, these modifications include cyclisation, hydroxylation and methylation. A further alteration specific to the nargenicins (**1-3, 8-10, 15**) is the esterification of a pyrrole-2-carboxylic acid group at the 9-hydroxyl group (Figure 1). Four proteins (NgnN2-5), with homology to those involved in pyrrole ring formation and attachment in clorobiocin and coumermycin A1 biosynthesis, have been shown to form the pyrrole group from L-proline and attach it to the hydroxyl at C9.[43] Nodusmicin (**11**) lacks the nargenicin A1 pyrrole group, but is identical in all other aspects, and this has been confirmed by its synthetic conversion to **1**.[40] Given that both **1** and **11** have been isolated from the same producing strain[12,16], it is highly likely that nodusmicin, while having some antibiotic activity in its own right, is actually a C9 hydroxylated intermediate in the nargenicin A1 pathway.

Hydroxylation at C2 and C18 occurs in almost all nargenicin-family molecules (with the exception of the nargenicin B series and branimycins). The *nar* biosynthetic gene cluster encodes two putative P450 hydroxylases (NarG and NarM) and a putative O-methyltransferase (NarK), which are most likely to hydroxylate the carbons at position 2 and 18, and then methylate the hydroxyl at C2.[42] Three orthologous proteins are also encoded in the *stm* gene cluster and are the prime candidates to catalyze the equivalent reactions in the biosynthesis of **14**.[18] Although the absolute configuration of **13** has not been confirmed, the presence of an additional O-methyl group at C3 suggests an extra P450 hydroxylase and O-methyltransferase are likely encoded within the genome of the producing *Streptomyces* strain.[17] Interestingly, the nargenicin B compounds have various modifications to C19 and C20, including addition of a methoxyethane or hydroxyl group and lack of methylation at the C2-hydroxyl (Figure 1). Given that these compounds have been identified from nargenicin A1 producing strains and that they are produced at lower levels than nargenicin A1, this suggests that they may be aberrantly methylated or oxygenated forms of **1**.

The trio of, luminamicin (**12**), lustromycin (**13**) and streptoseomycin (**14**) all have an additional macrolactone ring, with a unique maleimide moiety present in **14** and a maleic anhydride moiety in **12** and **13** (Figure 1). The pathway to the dialkylmaleic anhydride moiety in tautomycin biosynthesis has been established[44] and this provides some clues to potential biosynthetic pathways in these macrolides. While a plausible biosynthetic pathway to the maleimide moiety in **14** has been proposed from genome sequence data[18], experimental confirmation of this pathway is still lacking, Likewise the identity of the extra 4-carbon unit (proposed to be α-ketoglutarate) required to complete the second macrolactone ring has also not been experimentally confirmed. Future work in these areas is necessary to gain a complete picture of the biosynthetic pathway for these molecules.

3.3. Ether bridge formation

A key structural feature of the nargenicin family of antibiotics is the oxa-bridged decalin system. While an ether-bridge is present in each molecule, the nargenicins, nodusmicin and branimycin contain C8-C13 linked ether bridges, while other members of the family have a C9-C13 ether-bridge (Figure 1). The formation of the ether bridge in **1** is due to the activity of an iron and α-ketoglutarate (Fe-2OG) dependent dioxygenase family enzyme,

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NarN.[42] A NarN orthologue is present in the streptoseomycin gene cluster (StmO3) and is highly likely to catalyze the same reaction, however, NarN and StmO3 share only 53% amino acid sequence identity.[42] Despite this relative lack of identity, NarN and StmO3 are more closely related to each other than either protein is to other ether-bridge forming Fe-2OG dependent dioxygenases. This includes enzymes such as LolO, which forms a C2-C7 oxa-bridge in the fungal secondary metabolite, loline.[45,46] Indeed, limited amino acid identity appears to be a hallmark of dioxygenase family proteins due to the wide range of chemical reactions they catalyze.[47–49] This makes the identification of amino acid residues that participate in these reactions difficult, but suggests that there are likely to be residues specific to NarN and StmO3 that allow for regiospecific ether bridge formation. Alignments of these two enzymes do not provide sufficient information to discern key residues and homology models of these two proteins have also failed to yield any significant information on their structural differences (S. Pidot, data not shown). Ideally, obtaining X-ray crystal structures of these enzymes, preferably with the substrate bound (8,13-deoxynargenicin in the case of NarN), combined with mutational analysis, would provide sufficient new data to unravel the catalytic mechanisms of these intriguing enzymes.

Previous *in vitro* studies showed that purified NarN was active and maintained its ability to convert 8,13-deoxynargenicin A1 (**15**) to **1**[42], suggesting that the enzyme may be a useful tool for installing ether-bridges in enzyme-assisted synthetic studies. A previous attempt by Roush et al to synthesize **1** resulted in the complete carbon skeleton, but was unable to install the oxa-bridge across the decalin motif.[36] In light of the ability of purified NarN to catalyze the formation of the 8,13 ether bridge of **1**, revisiting the synthetic method of Roush et al[36] with a biosynthetic approach may finally yield *in vitro* nargenicin A1. Furthermore, such studies will shed light on the mechanism of action of these ether bridge forming enzymes and their place within the dioxygenase family.

3.4. Biosynthetic pathway and timing

The timing of nargenicin biosynthesis reactions is believed to be akin to that of other macrolactones, with linear polyketide synthesis followed by release, cyclisation and further modification. Folding of the polyketide chain was investigated early on and was proposed to occur by an intramolecular Diels-Alder (IMDA) reaction.[10,11] This was based on the incorporation of ^{13}C and ^{2}H labelled precursors by the nargenicin PKS.[11,50–52] Indeed, an IMDA has been employed in several attempts at chemical synthesis of the nargenicins.[30,35,36] However, recent evidence from the biosynthesis of **14** has shown that the nascent PKS product is folded via an unprecedented enzyme-catalyzed [6+4] cycloaddition.[53] This reaction is catalyzed by StmD (and its functional equivalent NarD from the nargenicin pathway (NgnD in Zhang et al[53])), a small protein containing a SnoaL-like cyclase domain. The StmD pericyclase was found to catalyze both [6+4] and [4+2] cycloadditions, with the [6+4] product transformed to the [4+2] adduct through a Cope rearrangement, which is then converted to **14** by other enzymes.[53] NarD was also found to substitute for StmD in the biosynthesis of **14**, with a similar level of efficiency, which may be expected given that they have >85% amino acid identity and highly similar crystal structures.[53] This shows that the initial biosynthetic steps in the formation of the nargenicin macrolides (chain extension, release and folding) proceed via the same general pathway, regardless of whether the eventual compounds are nargenicin- or streptoseomycin-like.

However, the order of the final steps in the biosynthesis of **1** appear to be the subject of debate. Early studies showed that the oxidations occurred at a late stage and suggested that the oxidation at C18 was the final step in the biosynthetic pathway.[9,16] However, the isolation of both **11** and **1** from the same producing isolate, shows that all oxygenations are completed prior to the addition of the pyrrole-2-carboyl group.[15,16] Furthermore, the isolation of **15** from a NarN knock-out mutant suggests that ether bridge formation is actually the final biosynthetic step.[42]

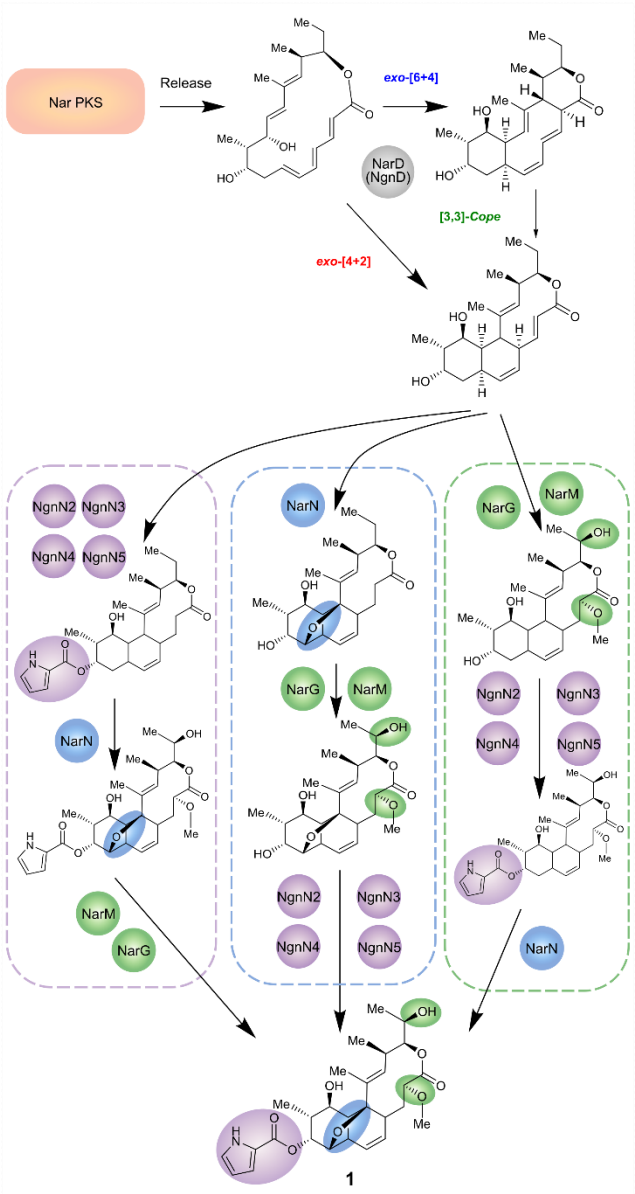

Figure 4. Timing of the final steps in the biosynthesis of **1**. The purple, blue and green dashed boxed indicate three potential pathways to **1**, with the modifications performed by various enzymes or groups of enzymes highlighted the same colour as those enzymes.

These apparently contradictory results imply that there is not a single pathway or unique final step leading to nargenicin

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biosynthesis and that the enzymes performing these reactions have a certain degree of substrate tolerance (Figure 4). The use of synthetic precursors fed to each enzyme would be required to determine the extent of this tolerance and to settle the debate over the timing and introduction of these various modifications.

Table 1. MIC ranges for nargenicin family antibiotics. Note different strains of these organisms were tested for several compounds, meaning that the results are not directly comparable, but are meant to indicate structure/activity trends across this group of compounds.

	MIC [μg/ml]							
Organism	Nargenicin A1 (**1**)	Nargenicin B1 (**8**)	Branimycin (**4**, **5**, **6**)	A/B/C[a]	Streptoseomycin (**14**)	Luminamicin/ coloradocin (**12**)	Lustromycin (**13**)	Nodusmicin (**11**)
Gram positive								
S. aureus	0.25	N.D.[b]	64 (A)[c], 64 → 160 (B), 32 → 80 (C)		32	>100	>100	64
Streptococcus pneumoniae	>64	>16	>128 (B), >128 (C)		N.S.	N.D.	N.D.	N.D.
Bacillus subtilis	>32	N.D.	N.D.		64	100	>100	N.D.
Clostridium perfringens	N.D.	N.D.	>32 (B), 16 (C)		N.D.	4→32	6.25	N.D.
Gram negative								
Haemophilus influenzae	>32	>32	>64 (A), 32 → >64 (B), >64 (C)		N.D.	1 → 32	N.D.	N.D.
Escherichia coli	>32		>64 (A), >80 (B), >80 (C)		N.D.	>100	>100	N.D.

[a] Branimycin A, B or C are denoted by "(A)", "(B)" or "(C)" next to the appropriate MIC value. [b] Not determined or data not available. [c] Ranges are shown where compounds were tested against multiple strains of the same organism.

4. Structure-activity relationships

As mentioned above, each of the nargenicin antibiotics has been reported to have a slightly different antibacterial spectrum. However, a direct comparison of antibacterial activity and the magnitude of this activity (as determined by the MIC) is difficult as each compound has not been tested against the same bacterial strains. Likewise, not all reported MIC test results were performed using standardized media or testing protocols. Nevertheless, some information on structure-activity relationships (SAR) among nargenicin family members can be gleaned from the MIC values shown in Table 1.

One observation from these data is the critical role of the pyrrole-2'-cabonyl group on **1** for anti-*Staphylococcus aureus* activity, which has been confirmed through the generation of synthetic derivatives.[28] All other nargenicin family antibiotics lack this group and consequently do not have appreciable anti-*S. aureus* activity (Table 1). Interestingly, addition of a pyrrole-2'-carbonyl group to **4** greatly improved anti-*S. aureus* activity from an MIC of 64 ug/ml to 0.12 - 4 ug/ml.[24] Furthermore, it appears that the second macrolactone ring, as present on **12**, **13** and **14**, appears to confer moderate anti-anaerobe activity to these molecules, although this remains speculative as a full MIC dataset is currently unavailable.

The only compound for which extensive SAR data exists is **1**. Figure 5 outlines nargenicin derivatives with significant improvement in MIC against *S. aureus*, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*. The majority of this information is present in a 2016 Merck patent, which reports the synthesis and antimicrobial activity of >200 nargenicin derivatives.[29] These data further confirm the previously established importance of the pyrrole group and essentiality of the N-H of the pyrrole for anti-*S. aureus* activity.[28] While the pyrrole is sensitive to almost all modifications, triiodation of this group gave a molecule highly selective for *Streptococcus pneumoniae*, with almost complete loss of anti-*S. aureus* activity, suggesting that some modifications are tolerated and can cause a change in the biological selectivity of the molecule (compound 42 in the patent, Figure 5, **68**). A closer look at the modified nargenicin structures shows that the ability to bind DnaE *in vitro* is not necessarily correlated with low MICs in cell-based assays, and the strong binding of *S. aureus* DnaE is not related to

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binding to *E. coli* DnaE.[29] This suggests that there may be different binding regions for nargenicin within the two DnaE proteins. Another notable result is that compounds with potent anti-*S. aureus* activity do not often have activity against other Gram positive organisms, such as *Streptococcus pneumoniae*. Indeed, anti-*S. aureus* activity appeared to improve following addition of a range of oxopropanamide derivatives to the hydroxyl at C18, but this greatly increased *S. pneumoniae* MICs (Figure 5, **69-72**). Modifications to a keto group attached to C18, including addition of with 1-benzyl pyridinium chloride, 1-benzyl-3-methylimidazol-3-ium chloride or 1-methyl-3-[(2-methylphenyl) methyl]imidazol-1-ium bromide yielded molecules with improved activity against a range of *M. tuberculosis* strains (Figure 4, **73-75**). However, MIC values for these compounds against *S. aureus* or *S. pneumoniae* were not detailed, making it difficult to ascertain if activity against these pathogens had been lost as a result of the gain in anti-mycobacterial activity.

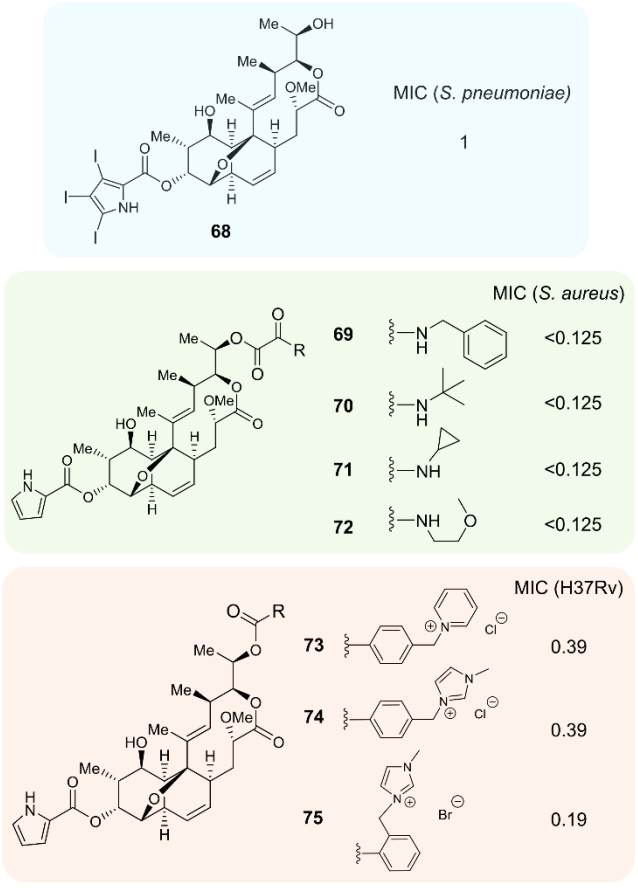

Figure 5. Structures and antimicrobial activity of derivatives of **1**. H37Rv, *M. tuberculosis* strain H37Rv.

3. Concluding remarks and future directions

The potential clinical usefulness of the nargenicins lies in their ability to attack a narrow range of pathogens, sparing the host microbiota and also potentially limiting the spread of resistance. However, early interest in the nargenicins gave way to the development of antibiotics with much broader spectra of activity. With rates of antibacterial resistance rapidly increasing through the indiscriminate use of broad-spectrum antibiotics, perhaps the time is right for a renaissance of the nargenicins. Indeed, if recent patent applications are anything to go by, it does appear as though there is renewed pharmaceutical interest in these unusual macrocycles.

However, to fully understand the biosynthesis and activity of these molecules, with a goal to improving production and clinical utility, many questions remain to be answered. Some of these include: How exactly do the nargenicins bind to DnaE and to which parts of the protein do they bind? Are there other potential binding targets? How do the biosynthetic gene clusters from other nargenicin family members differ from those already discovered? Can synthetic routes be improved to provide an efficient pathway to the nargenicins or will enzyme-assisted synthesis make inroads in nargenicin production? Finding the answers to these questions will help to improve our understanding of these narrow-spectrum antibiotics and potentially add new antimicrobial compounds to the current human armament.

Acknowledgements

This work was supported by an Australian National Health and Medical Research Council grant GNT1105522.

Keywords: nargenicin • natural products • polyketides • ether bridge • biological activity

[1] G. M. Cragg, D. J. Newman, Biochim. et Biophys. Acta 2013, 1830, 3670–95.
[2] R. Tommasi, D. G. Brown, G. K. Walkup, J. I. Manchester, A. A. Miller, Nat. Rev. Drug. Discov. 2015, 14, 529–542.
[3] R. J. Melander, D. V. Zurawski, C. Melander, Medchemcomm 2017, 9, 12–21.
[4] G. D. Wright, Nat. Prod. Rep. 2017, 34, 694–701.
[5] W. D. Celmer, G. N. Chmurny, C. E. Moppett, R. S. Ware, P. C. Watts, E. B. Whipple, J. Am. Chem. Soc. 1980, 102, 4203–4209.
[6] W. D. Celmer, W. P. Cullen, C. E. Moppett, M. T. Jefferson, L. H. Huang, R. Shibakawa, J. Tone, Antibiotics Produced by New Species of Nocardia, 1979, US4148883A.
[7] B. J. Magerlein, R. J. Reid, J. Antibiot. 1982, 35, 254–255.
[8] J. Kallmerten, in Studies in Natural Products Chemist (Ed.: Atta-ur-Rahman), Elselvier, Amsterdam, 1995.
[9] D. E. Cane, C. C. Yang, J. Antibiot. 1985, 38, 423–6.
[10] D. E. Cane, C. Yang, J. Am. Chem. Soc. 1984, 106, 784–787.
[11] D. E. Cane, W. R. Ott, J. Am. Chem. Soc. 1988, 110, 4841–4842.
[12] H. A. Whaley, J. H. Coates, in Abstracts of Papers Intersci. Conf. Antimicr. Agents & Chemoth., Chicago, IL, 1981.
[13] W. D. Celmer, W. P. Cullen, R. Shibakawa, J. Tone, Nargenicin C1, 1984, US4436747A.
[14] J. Tone, R. Shibakawa, H. Maeda, Y. Yamauchi, K. Niki, M. Saito, K. Tsukuda, E. B. Whipple, P. C. Watts, C. E. Moppett, et al., in 20th Interscience Conference of Antimicrobial Agents and Chemotherapy, 1980.
[15] H. A. Whaley, C. G. Chidester, S. A. Mizsak, R. J. Wnuk, Tetrahedron Lett. 1980, 21, 3659–3662.
[16] W. C. Snyder, K. L. Rinehart, J. Am. Chem. Soc. 1984, 106, 787–789.
[17] H. Tomoda, R. Iwata, Y. Takahashi, Y. Iwai, R. Oiwa, S. Omura, J. Antibiot. 1986, 39, 1205–10.
[18] B. Zhang, K. B. Wang, W. Wang, S. F. Bi, Y. N. Mei, X. Z. Deng, R. H. Jiao, R. X. Tan, H. M. Ge, Org. Lett. 2018, 20, 2967–2971.
[19] S. Omura, R. Iwata, Y. Iwai, S. Taga, Y. Tanaka, H. Tomoda, J. Antibiot. 1985, 38, 1322–6.
[20] M. Jackson, J. P. Karwowski, R. J. Theriault, P. B. Fernandes, R. C. Semon, W. L. Kohl, J. Antibiot. 1987, 40, 1375–82.

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[21] R. R. Rasmussen, M. H. Scherr, D. N. Whittern, A. M. Buko, J. B. McAlpine, J. Antibiot. 1987, 40, 1383–93.
[22] S. Marchart, A. Gromov, J. Mulzer, Angew. Chem. Int. Ed. 2010, 49, 2050–3.
[23] A. F. Brana, A. Sarmiento-Vizcaino, I. Perez-Victoria, L. Otero, J. Fernandez, J. J. Palacios, J. Martin, M. de la Cruz, C. Diaz, F. Vicente, et al., J. Nat. Prod. 2017, 80, 569–573.
[24] P. J. Dudfield, J. Rowther, C. A. J. Delachaume, R. H. M. Lepine, A. P. M. Thys, J. G. P. Doyon, M. P. Toumi, F. G. Hansske, Branimycin Derivatives and Their Use for the Treatment of Bacterial Infectious Diseases, 2015, WO 2015/028094 Al.
[25] A. Cikos, N. Triballeau, P. A. Hubbard, D. Ziher, P. F. Stouten, J. G. Doyon, T. Deschrijver, J. Wouters, R. H. Lepine, L. Saniere, Org. Lett. 2016, 18, 780–3.
[26] D. J. Plata, J. Kallmerten, J. Am. Chem. Soc. 1988, 110, 4041–4042.
[27] V. S. Enev, W. Felzmann, A. Gromov, S. Marchart, J. Mulzer, Chem. Eur. J. 2012, 18, 9651–68.
[28] R. E. Painter, G. C. Adam, M. Arocho, E. DiNunzio, R. G. Donald, K. Dorso, O. Genilloud, C. Gill, M. Goetz, N. N. Hairston, et al., Chem. Biol. 2015, 22, 1362–73.
[29] K. Young, D. B. Olsen, S. B. Singh, J. Su, R. R. Wilkening, J. M. Apgar, D. Meng, D. Parker, M. Mandal, L. Yang, et al., Nargenicin Compounds and Uses Thereof as Antibacterial Agents, 2016, WO2016061772.
[30] J. Kallmerten, Tetrahedron Lett. 1984, 25, 2843–2846.
[31] E. J. Corey, K. C. Nicolaou, J. Am. Chem. Soc. 1974, 96, 5614–5616.
[32] L. T. Rossano, D. J. Plata, J. Kallmerten, J. Org. Chem. 1988, 53, 5189–5191.
[33] A. Gromov, V. Enev, J. Mulzer, Org. Lett. 2009, 11, 2884–2886.
[34] M. Lautens, T. Rovis, Tetrahedron 1998, 54, 1107–1116.
[35] J. W. Coe, W. R. Roush, J. Org. Chem. 1989, 54, 915–930.
[36] W. R. Roush, K. Koyama, M. L. Curtin, K. J. Moriarty, J. Am. Chem. Soc. 1996, 118, 7502–7512.
[37] E. Goessinger, A. Schwartz, A. Sereinig, Tetrahedron 2000, 56, 2007–2014.
[38] E. Goessinger, A. Schwartz, A. Sereinig, Tetrahedron 2001, 57, 3045–3061.
[39] H. Ando, A. Kimishima, M. Ohara, T. Hirose, T. Matsumaru, H. Takada, K. Morodome, T. Miyamoto, A. Sugawara, S. Omura, et al., J. Antibiot. 2018, 71, 268–272.
[40] B. J. Magerlein, S. A. Mizsak, J. Antibiot. 1982, 35, 111–112.
[41] R. M. Wilson, S. J. Danishefsky, J. Org. Chem. 2006, 71, 8329–8351.
[42] S. J. Pidot, M. Herisse, L. Sharkey, L. Atkin, J. L. Porter, T. Seemann, B. P. Howden, M. A. Rizzacasa, T. P. Stinear, Angew. Chem. Int. Ed. 2019, 58, 3996–4001.
[43] S. Maharjan, N. Aryal, S. Bhattarai, D. Koju, J. Lamichhane, J. K. Sohng, Appl. Microbiol. Biotechnol. 2012, 93, 687–96.
[44] W. Li, J. Ju, S. R. Rajski, H. Osada, B. Shen, J. Biol. Chem. 2008, 283, 28607–17.
[45] J. Pan, M. Bhardwaj, B. Zhang, W. Chang, C. L. Schardl, C. Krebs, R. B. Grossman, J. M. Bollinger Jr., Biochem. 2018, 57, 2074–2083.
[46] J. Pan, M. Bhardwaj, J. R. Faulkner, P. Nagabhyru, N. D. Charlton, R. M. Higashi, A. F. Miller, C. A. Young, R. B. Grossman, C. L. Schardl, Phytochemistry 2014, 98, 60–8.
[47] R. P. Hausinger, in Metallobiology (Eds.: C. Schofield, R. Hausinger), Royal Society Of Chemistry, Cambridge, 2015, pp. 1–58.
[48] L.-F. Wu, S. Meng, G.-L. Tang, Biochim. et Biophys. Acta 2016, 1864, 453–470.
[49] I. J. Clifton, McDonough, M. A., D. Ehrismann, N. J. Kershaw, N. Granatino, C. J. Schofield, J. Inorg. Biochem. 2006, 100, 644–669.
[50] D. E. Cane, P. C. Prabhakaran, W. Tan, W. R. Ott, Tetrahedron Lett. 1991, 32, 5457-5460.
[51] D. E. Cane, W. Tan, W. R. Ott, J. Am. Chem. Soc. 1993, 115, 527–535.
[52] D. E. Cane, G. Luo, J. Am. Chem. Soc. 1995, 117, 6633–6634.
[53] B. Zhang, K. B. Wang, W. Wang, X. Wang, F. Liu, J. Zhu, J. Shi, L. Y. Li, H. Han, K. Xu, et al., Nature 2019, 568, 122.

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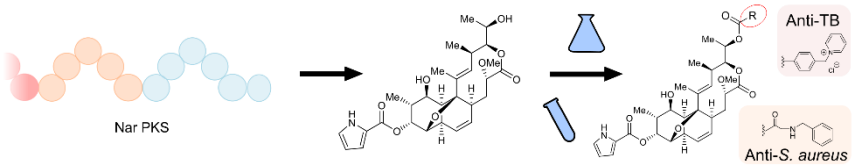

Sacha J Pidot, Mark A Rizzacasa*

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The nargenicin family of oxa-bridged macrolide antibiotics

Narrow spectrum, broad possibilities. Over the past 40 years, several narrow-spectrum antibiotics belonging to the nargenicin family have been identified and the biosynthetic pathway for the prototypical member of this family, nargenicin A1, has been uncovered. Here, we review synthetic approaches to the nargenicins, in particular the unusual oxa-bridged decalin core, and investigate biosynthetic and structure-activity aspects of these rare antibiotics.

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