Chemical Science

ROYAL SOCIETY OF CHEMISTRY

EDGE ARTICLE

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Cite this: *Chem. Sci.*, 2023, **14**, 3923

All publication charges for this article have been paid for by the Royal Society of Chemistry

Received 14th December 2022
Accepted 14th March 2023

DOI: 10.1039/d2sc06893a

rsc.li/chemical-science

Synthesis of functionalized 2,3-diaminopropionates and their potential for directed monobactam biosynthesis†

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The *N*-sulfonated monobactams harbor considerable potential to combat emerging bacterial infections that are problematic to treat due to their metallo-β-lactamase mediated resistance against conventional β-lactam antibiotics. Herein, we report a divergent synthesis of C3-substituted 2,3-diaminopropionates featuring an array of small functional groups and examine their potential as alternative precursors during monobactam biosynthesis in a mutant strain (*ΔsulG*) of *Pseudomonas acidophila* that is deficient in the supply of this native precursor. *In vitro* assays revealed high diastereoselectivity, as well as a substrate tolerance by the terminal adenylation domain of the non-ribosomal peptide synthetase (NRPS) SulM toward the majority of synthetic analogs. Chemical complementation of this mutant yielded a fluorinated, bioactive monobactam through fermentation as confirmed by a combination of spectrometric data and microbiological assays. This study demonstrates site-specific functionalization of a clinically important natural product and sets in place a platform for further strain improvements and engineered NRPS-biosynthesis of non-native congeners.

Introduction

Non-proteinogenic α,β-diamino acids are valuable building blocks for both natural product biosynthesis and the development of synthetic drugs.[1–3] L-2,3-Diaminopropionate (L-Dap, **1**) as well as its C3-functionalized derivatives are the structural backbone of the azetidinone warhead of monobactams, a structurally distinct class of β-lactam antibiotics. The growing emergence of Ambler class B metallo-β-lactamases (MBLs), for which no FDA approved inhibitor is yet available, has brought renewed attention to monobactams because of their intrinsic stability to MBLs and their promise to overcome otherwise resistant bacterial infections where traditional β-lactam antibiotics have failed.[4,5]

The isolation of the first monobactam sulfazecin (**2**) from *Pseudomonas acidophila*[6] and subsequent discovery of several structurally related congeners from natural sources inspired and ultimately resulted in the development of aztreonam[7] (**3**), currently the only FDA approved monobactam for clinical use.

Our group recently elucidated the biosynthesis of sulfazecin combining *in vivo* gene inactivation and *in vitro* enzyme studies.[8,9] The final module of the NRPS SulM incorporates L-Dap, which itself is derived from the primary metabolite L-phosphoserine through the successive action of two enzymes, SulG and SulH, onto the upstream D-Glu-D-Ala dipeptide precursor (Scheme 1A). Following a distinct enzymatic mechanism, this linear tripeptide is *N*-sulfonated *in trans* by the sulfotransferase SulN, a step that is essential for the unusual thioesterase-mediated cyclization to yield the sulfonated β-lactam core. Hydroxylation and *O*-methylation catalyzed by SulO and SulP, respectively, furnish the final structure of sulfazecin **2**.[8,9]

Owing to the lack of a traceable chromophore and the formally charged monobactam core that prevents facile extraction with organic solvents from the aqueous fermentation media, multi-step isolation processes are necessary to obtain these natural products from their native producers.[10,11] Therefore, synthetic procedures have historically been utilized to access derivatives of this potent pharmacophore for clinical development.[12]

Unlike naturally-occurring **2**, a prominent feature of synthetic monobactams is a substitution at C4 of the

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† Electronic supplementary information (ESI) available: Supplemental figures and schemes, materials and methods, experimental details, characterization data and NMR spectra of new compounds. Check CIF file for single crystal X-ray structure of **11**. CCDC 2207290. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d2sc06893a

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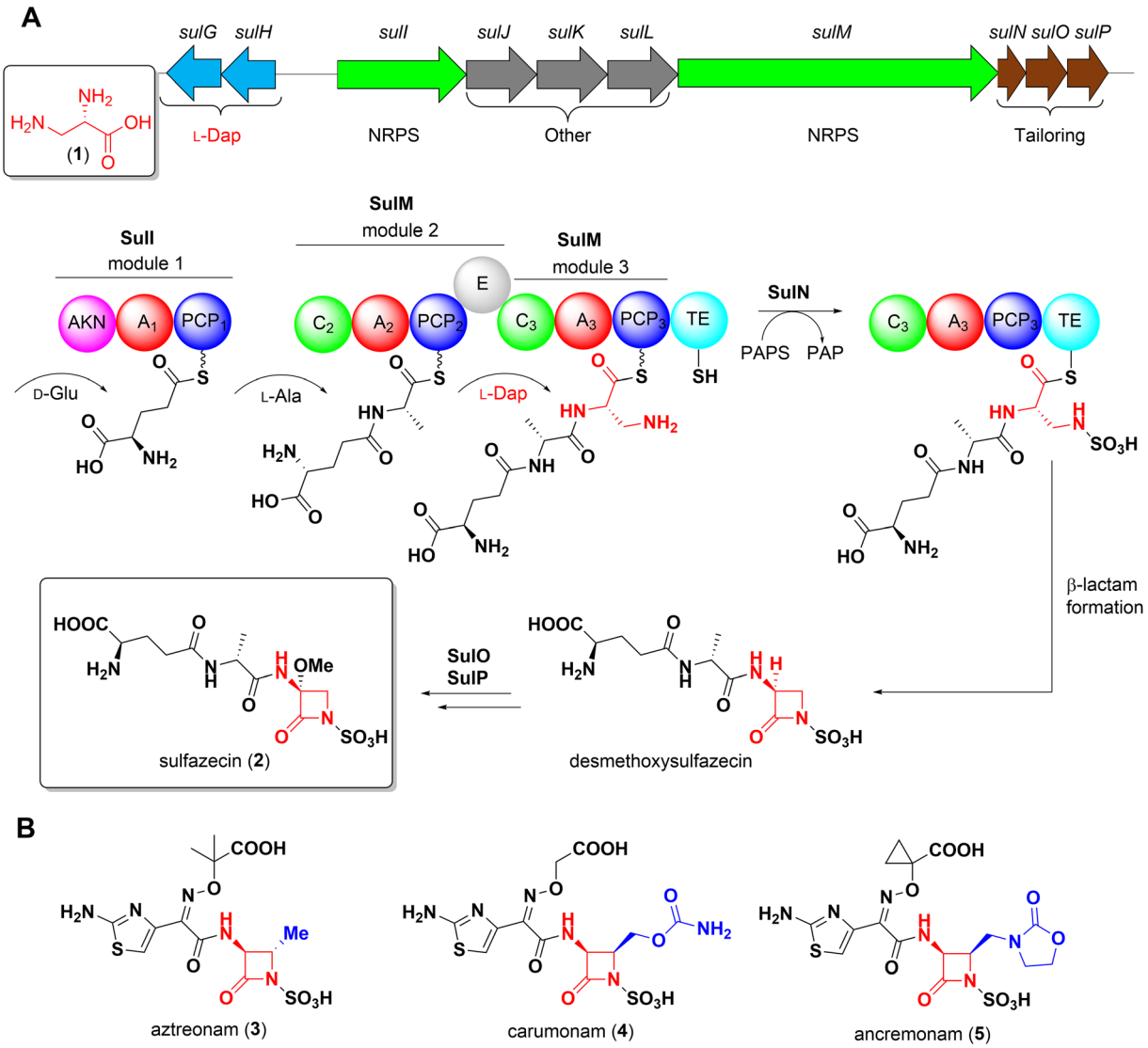


Scheme 1 Biosynthesis of sulfazecin **2**. (A) Biosynthetic gene cluster (top) and biosynthesis of sulfazecin **2**. The L-Dap **1** derived subunit is shown in red. (B) Representative synthetic monobactams. Additional functionalization is highlighted in blue. AKN: predicted adenylylsulfate kinase of unknown function, A: adenylation domain, PCP: peptidyl-carrier-protein, C: condensation domain, E: epimerase domain, TE: thioesterase domain.

azetidinone moiety, as exemplified by carumonam **4** and ancremonam **5** (Scheme 1B).[13,14] This structural modification has been shown to improve both stability against serine β-lactamases and antibiotic activity.[15,16] However, unlike the majority of naturally occurring monobactams, the C3-methoxy substituent is absent in the synthetic compounds. This functionalization is similarly observed in the cephamycin class of β-lactam natural products and semi-synthetic drugs such as cefoxitin where it has been demonstrated to improve the stability of these cephalosporin derivatives to serine β-lactamases.[17]

In a previous key experiment disruption of *sulG* abolished sulfazecin production, but could be fully restored by exogenous supplementation of L-Dap **1** to the fermentation medium.[8] Such strains that lack supply of native building blocks have the potential to expand structural product diversity by means of mutasynthesis.[18] This approach of mutasynthesis has been developed for the production of manifold valuable compounds such as the antiparasitic ivermectin,[19] and enabled structural modification associated with enhanced bioactivity of established drugs including vancomycin, neomycin, and rapamycin.[20–22] Contemporary approaches in the field of NRPS-based mutasynthesis, however, are usually limited to substituted aromatic non-proteinogenic building blocks such as hydroxyphenylglycines, β-hydroxytyrosine or salicylates, where synthetic analogs are readily available.[20,23–26]

In this study we aimed to extend this concept through supplementation of non-native derivatives of L-Dap to *P. acidophila ΔsulG*. Here, these synthetic analogs both functionalize an unreactive sp^3-carbon and introduce an additional stereocenter into the molecule. Incorporation of any analog would allow the biosynthetic production of non-native, potentially bioactive monobactams or relay compounds for semi-synthesis

that are substituted at the azetidinone C4 position, just like their purely synthetic counterparts. These molecules would further bear the clinically relevant C3-methoxy moiety, which is not present in synthetic monobactams and provide a biosynthetic route to chemical space that is challenging to cleanly access by synthetic means.[27] Moreover, access to these densely functionalized diamino acid building blocks affords great utility in traditional synthetic and medicinal chemistry for the construction of bioactive compounds.[2]

Results and discussion

During NRPS-mediated biosynthesis the adenylation (A) domain selects its amino acid substrate in a highly chemo- and stereoselective manner before activating it for downstream processing.[28–30] Since introduction of any substituent at the β-carbon of L-Dap introduces diastereomers, we reasoned that A_3, the adenylation domain in SulM that activates L-Dap during sulfazecin biosynthesis, would be biased towards one or the other. We initially prepared the known compounds (2*S*,3*R*)-**6** and (2*S*,3*S*)-methyl-Dap **7**, the structurally simplest derivatives bearing an additional methyl group, by adapting established methodology (Scheme S1†). To probe the chemo- and stereoselectivity of A_3 toward the methyl-analogs **6** and **7**, we performed two complementary colorimetric adenylation domain activity assays *in vitro*: The first one detects inorganic pyrophosphate (PP_i) that is released during the ATP-driven amino acid activation,[31,32] whereas the second assay relies on direct detection of the aminoacyl-adenylate through capture with hydroxylamine and complexation of the resulting hydroxamate species with Fe(III) (Fig. 1A).[33,34] Both assays consistently revealed that A_3 exhibits a strong preference for the (2*S*,3*R*)-diastereomer **6** with only marginal activation of the (2*S*,3*S*)-diastereomer **7** (Fig. 1B). Interestingly, while L-serine is not activated, as shown previously,[8] both D-Dap and the L-2,4-diaminobutyric acid (L-Dab) **8** control, a non-native substrate that would preclude downstream β-lactam formation, showed lower, but clear activation.

Encouraged by these initial results we continued to construct an extended library of functionalized L-Dap derivatives bearing the preferred *threo* stereochemistry. It is worth noting that the absolute configuration at C4 of the azetidinone core in the anticipated final monobactam products would correlate with that in penicillins, cephalosporins, carbapenems and clavulanic acid where this stereocenter is essential to potent antibiotic activity.[35] Notably, the *erythro* (4*S*) stereochemistry is present in aztreonam **3**, which simplified its commercial production from readily available L-threonine. Newer synthetic monobactams, however, and the 4*R*-diasteomer of aztreonam itself have been demonstrated to have greater β-lactamase stability and antibiotic activity than the 4*S*-diastereomer.[15] The selection of the sulfazecin biosynthetic machinery for the more potent diastereomer therefore constitutes an important intrinsic advantage.

Hydroxymethyl-Dap **9** was initially targeted to focus construction of our library, believing the hydroxyl moiety would be a useful handle for further functionalization (Scheme 2A). Evaluating the literature, we identified butyrolactone **11** as a key

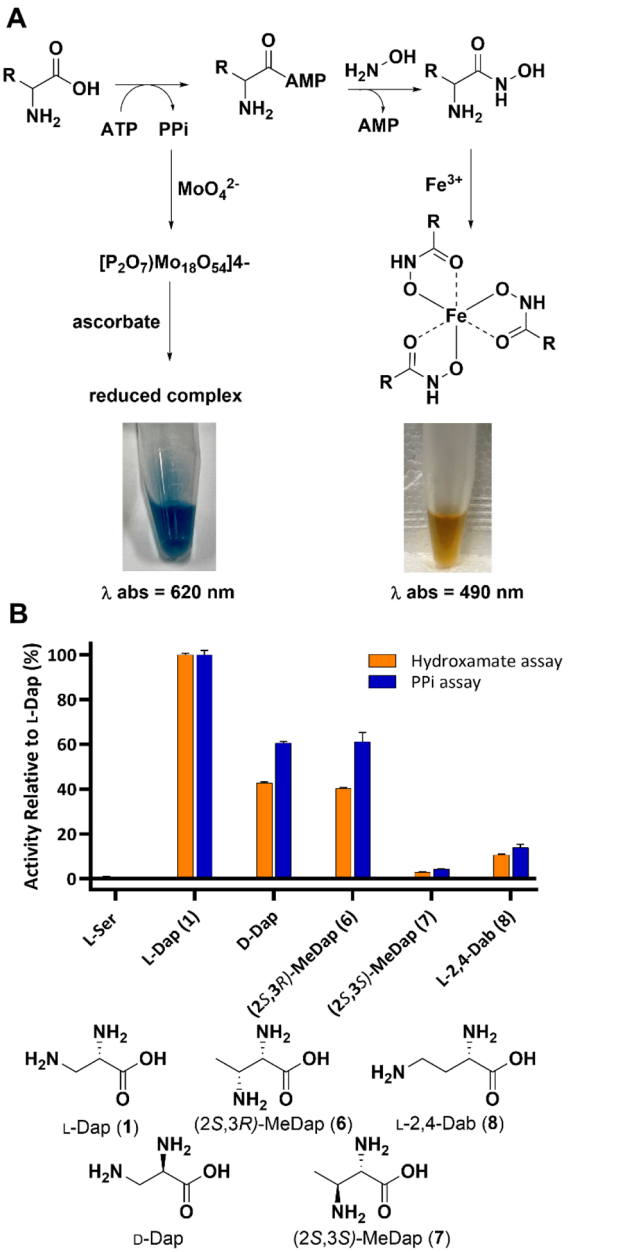
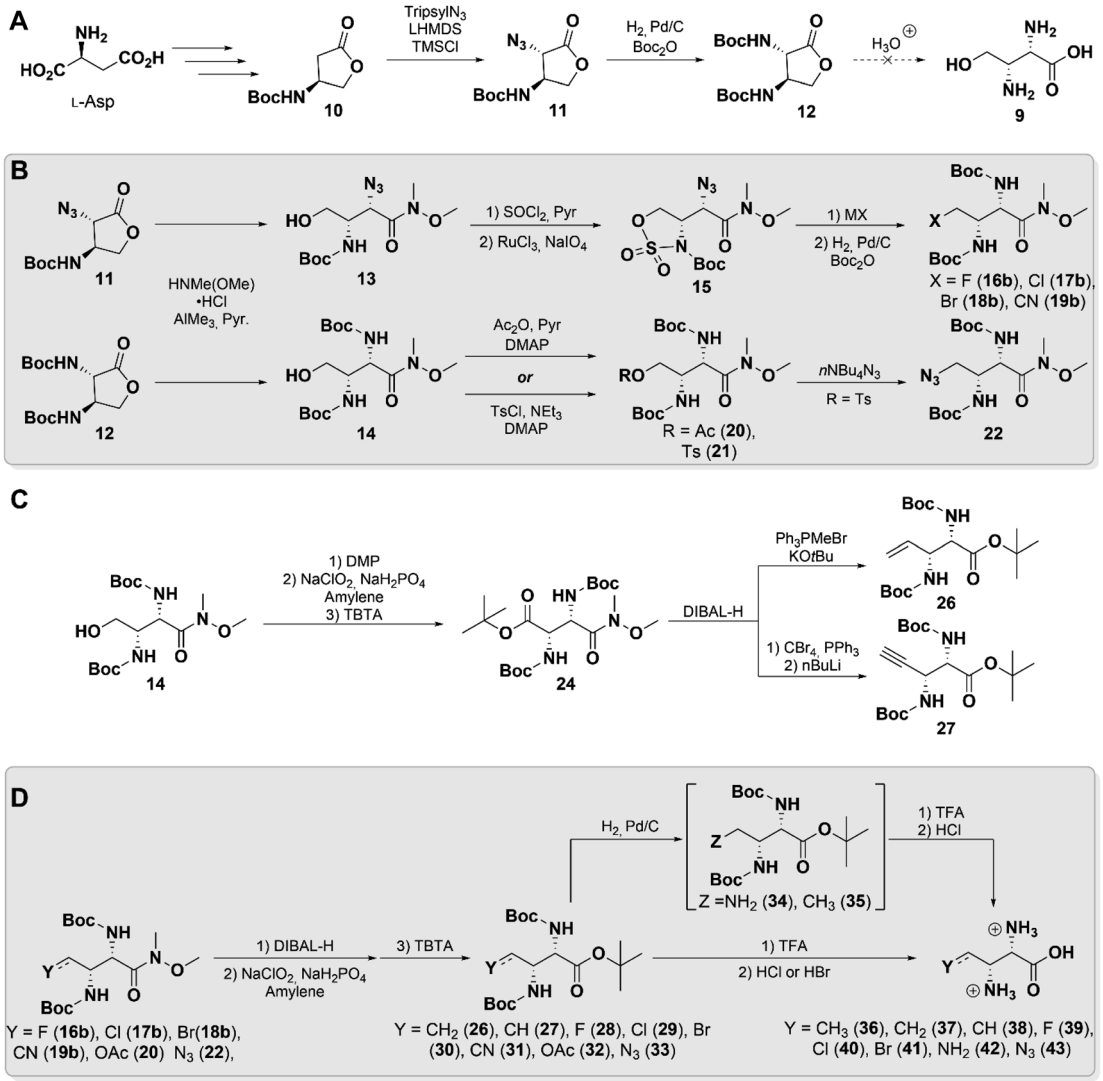


Fig. 1 (A) Principle of A-domain *in vitro* assays. (B) Substrate preference of A_3 towards Dap isomers based on *in vitro* assays.

synthetic intermediate that could be prepared by the procedure of Hanessian *et al.* from L-aspartate.[36] Unexpectedly, upon repeating the reported procedure with minor modifications we isolated a product that we believed to be **11**, but that did not agree with the reported spectral characterization. Single crystal X-ray crystallographic analysis, however, confirmed the identity of this product with the anticipated *anti* stereochemistry (Fig. S1 and Table S1†).

With butyrolactone **11** in hand, we proceeded to **12** through a one-pot hydrogenation and Boc-protection. A single-step acid deprotection and hydrolysis of **12** was attempted to obtain **9**, but appreciable re-lactonization was observed upon isolation. Lactonization is a well characterized phenomenon in related



Scheme 2 Synthesis of functionalized 2,3-diaminopropionates. (A) Diastereoselective synthesis of diamino-butyrolactone **12**. (B) C4 functionalization by nucleophilic substitution. (C) Preparation of alkenyl/alkynyl analogs by head-to-tail inversion. (D) Amide cleavage and global deprotection to afford final 2,3-diaminopropionate analogues.

systems such as homoserine,[37] which we believe was made even more thermodynamically favorable by the *threo* stereochemistry in **9**. Therefore, we elected to pursue other derivatives that would not be subject to competing re-lactonization.

Literature precedence for similar systems showed that a Weinreb amide effectively suppresses this interfering reaction and permits isolation of the γ-hydroxy compound.[38] Accordingly, both lactones **11** and **12** were subjected to an $AlMe_3$-mediated amidation,[39] which cleanly generated **13** and **14** without the need for further purification (Scheme 2B). This factor proved to be particularly important, as both compounds were found to nonetheless undergo re-lactonization on silica gel as well as upon prolonged standing at room temperature. Extended reaction telescoping and careful selection of reaction conditions enabled desired downstream transformations to be carried out while minimizing re-lactonization of intermediates (see ESI† Synthetic Procedures).

From **13** and **14**, our syntheses diverged slightly to access various derivatives. Weinreb amide **13** was efficiently converted to its cyclic sulfamidate **15** in 65% yield over 3-steps from **11**, simultaneously "capping" and activating the δ-hydroxy group. Compound **15** proved to be an excellent common intermediate for the generation of derivatives through facile nucleophilic ring opening by halides (**16a–18a**, see ESI†) and cyanide (**19a**, see ESI†), followed by hydrolysis of the *N*-sulfate (Scheme 2B).[40] The α-azide in these products was then reduced and Boc-protected to yield **16b–19b** in moderate yields, likely due to steric congestion. To access additional derivatives, Boc-protected diamino lactone **12** was employed as a precursor instead. Acetylation of its Weinreb amide **14** yielded acetoxymethyl **20**, which we hoped could serve as a "masked" hydroxyl group. Sulfonylation of **14** generated tosylate **21**, which was displaced to provide azidomethyl **22**, which was not accessible in our hands from **13** (Scheme 2B).

Next, we attempted to oxidize **14** to the aldehyde (**23**, see ESI†) and further convert it to the olefin using Wittig chemistry. However, the latter reaction only yielded a small amount of the anticipated product, presumably due to competing reaction of the phosphonium ylide at the Weinreb amide. We therefore took advantage of its molecular symmetry to perform a head-to-tail inversion by oxidizing and protecting **14** as the di-amino succinate **24**. Its Weinreb amide was selectively reduced to the aldehyde **25** (see ESI†) and subjected to Wittig olefination or Corey–Fuchs reaction to yield vinyl **26** and alkynyl **27**, respectively (Scheme 2C).

For the remaining Weinreb amides basic hydrolysis proved very difficult, requiring extended reaction times and often leading to racemization, as well as destruction of labile functionalities at C4. Instead, the amide was readily reduced to the aldehyde and subsequently re-oxidized, followed by protection of the free carboxyl to simplify final purification. This protocol allowed preparation of the globally-protected Dap derivatives **26–33** in ample yields without detectable racemization. At this stage azidomethyl **33** was also converted to the tri-amine **34**, and vinyl **26** was reduced to ethyl **35**. This series of compounds, encompassing diverse chemical functionalities of small size, was then subjected to a final global deprotection and anion exchange to afford amino acids **36–43** (Scheme 2D). All of these compounds exhibited spectral signatures consistent with the *threo* stereochemistry of 2*S*,3*R*-methyl-Dap (**6**), by displaying H2–H3 vicinal coupling constants of *ca.* 3–4 Hz.[41] Unfortunately, in the case of cyanomethyl **31** and acetoxymethyl **32**, this final reaction led to decomposition, excluding them from further experiments. Bromide **41** was obtained with trace lactone, likely due to favorable 5-*exo-tet* cyclization.

With our extended substrate library in hand we employed the operationally simple hydroxamate assay to once again probe the promiscuity of SulM A_3 *in vitro*, as this domain represents the entry point of these substrate analogs into the monobactam biosynthetic machinery. *In vitro* assays revealed appreciable activation for the fluoromethyl-Dap **39**, although less than observed for the smaller (2*S*,3*R*)-methyl-Dap **6**. Except for the rigid alkyne **38**, all synthetic L-Dap analogs exhibited low but measurable activity (Fig. 2A).

To obtain a more detailed understanding how the additional substituents affect the reactivity of A_3, we applied the commonly used continuous 6-methyl-7-thioguanosine (MesG)/hydroxylamine coupled assay[42,43] to record kinetic profiles of A_3 for its native substrate **1** and the two analogues **6** and **39**, which show the highest relative activity in end-point assays. While the size of the substituent did not have a large effect on k_{cat}, it did notably increase the K_M by approx. factor 10 for **6** (0.09 mM for **1** *vs.* 1.08 mM for **6**) and 200 for **39** (0.09 mM for **1** *vs.* 20.7 mM for **39**), in line with the observed relative activities (Fig. S2.†) Although the measured k_{cat}/K_M of 78.4 mM^{-1} min^{-1} is low for the native substrate **1**, this value might not necessarily reflect the actual catalytic efficiency of A_3 under *in vivo* conditions, as the enzyme might favour a more acidic environment that is not compatible the MesG-hydroxylamine continuous assay.[44] Furthermore, it has been shown that the turnover number can be much higher in the presence of a native acceptor molecule (here the *holo*-PCP_3) instead of hydroxylamine.[42,45]

Finally, we set out to examine whether any synthetic analog could substitute for L-Dap throughout the entire sulfazecin biosynthetic pathway *in vivo*, resulting in the production of non-native monobactams. We therefore selected the most promising candidates (2*S*,3*R*)-methyl-Dap **6** and fluoromethyl-Dap **39**, as well as chloromethyl-Dap **40** and vinyl-Dap **37** because of their potential as chemical handles for semi-synthetic modification, and performed a chemical complementation of the *P. acidophila* Δ*sulG* mutant that is deficient in L-Dap **1** biosynthesis and, therefore, sulfazecin production by itself.[8]

Initial bioassays on super-sensitive *E. coli* ESS[46] plates showed antibacterial activity for the cell-free supernatant (CFS) of the Δ*sulG* culture supplemented with **6**, whereas the inhibition zone was smaller compared to that of the positive control culture with added L-Dap **1** (Fig. S3†). Both samples also showed β-lactamase-induction activity (**1** > **6**) by nitrocefin assay,[47] further corroborating the microbiological growth inhibition data for β-lactam production (Fig. S3†). No positive signals from either bioassay were obtained for the CFS of the Δ*sulG* cultures supplemented with any other synthetic analog. We suspected that either lower production or an altered antibacterial spectrum might be the reason other potential monobactam analogs eluded detection. Therefore, semi-purified fractions of each CFS were analyzed by Ultra Performance Liquid Chromatography-High Resolution Mass Spectrometry (UPLC-HRMS) as a more sensitive detection method. Indeed, a signal corresponding to the anticipated monobactam product was obtained not just for the cultures supplemented with **1** and **6**, but also for the culture supplemented with fluoromethyl **39**, the only other analog that showed distinct activation by A_3 besides **6** (Fig. 2B). Moreover, a slightly shorter retention time (**39** < **6** < **1**) with increasing product hydrophobicity met expectations for the chromatographic parameters used.

Analysis of the tandem MS/MS (MS^e) spectra (Fig. S4–S7†) confirmed the presence of imine fragments consistent with the highly diagnostic, formal [2 + 2] cycloreversion, a fragmentation pattern common to other β-lactams[48] including aztreonam (Fig. S8†). These fragments not only bear the charged sulfamate species for detection in ESI(−) but also contain the corresponding substitution at the C4 position. The observation of these characteristic fragments leaves little doubt as to the assigned structures.

Reassured by these spectrometric data, we repeated our previous chemical complementation and bioassays using 7× concentrated CFS of Δ*sulG* cultures supplemented with either **1**, **6** or **39**. In fact, nitrocefin-positive and antibacterial activities were observed for all concentrated samples except that for the non-supplemented Δ*sulG* mutant negative control (Fig. 2B). The methylated sulfazecin analog (termed MM42842) obtained by fermentation in the presence of (2*S*,3*R*)-methyl-Dap **6**, wherein the methyl- and methoxy-substituents are *trans*, has been previously isolated from *P. cocovenenans*.[27] Our supplementation studies suggest that *P. cocovenenans* might directly employ **6**, rather than L-Dap **1**, as the native precursor during the biosynthesis of MM42842. In contrast to sulfazecin **2** its antibacterial activity has been described as mainly directed against Gram-positive bacteria, in line with our observed activity

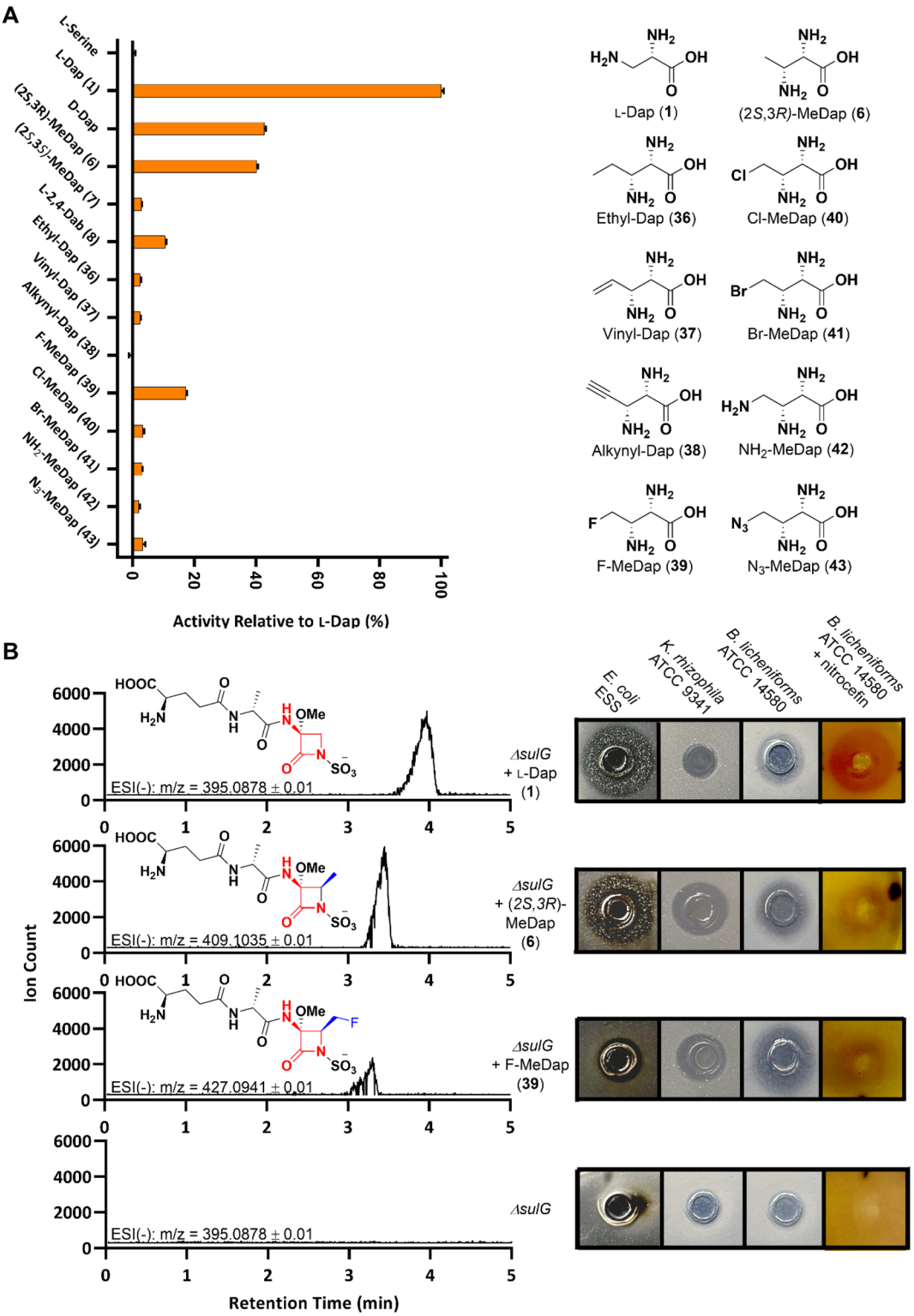


Fig. 2 (A) Hydroxamate-based *in vitro* assays with A_3 against the synthetic L-Dap library. (B) UPLC-HRMS analysis and bioassays of concentrated *P. acidophila ΔsulG* cell-free supernatant (semi-purified for UPLC-MS) supplemented with selected L-Dap derivatives.

against *Bacillus licheniformis* ATTC 14580 and *Kocuria rhizophila* ATCC 9341.[27]

A novel fluorinated monobactam, fluoromethyl-sulfazecin, was identified when the *ΔsulG* mutant was fermented in the presence of **39**. In line with the methylated analog it showed pronounced antibacterial activities against the two Gram-positive strains tested, but weak activity against *E. coli* ESS (Fig. 2B). While these data suggest that C4-substitutions alter

the bioactivity profiles compared to sulfazecin **2**, extended studies will be required to assess their antibacterial spectrum in greater detail. Nevertheless, these initial bioassay results and spectrometric data support the generation of bioactive monobactam derivatives. This outcome furthermore shows that despite the unfavourable kinetic performance of A_3 *in vitro*, under native conditions the biosynthetic machinery can successfully utilize **6** and **39** as alternative building blocks.

Currently, our ability to isolate these new products in sufficient quantities for downstream applications is met by numerous challenges. The low titre of sulfazecin **1** in wild type *P. acidophila* combined with the decreased affinity of the native biosynthetic machinery towards our unnatural substrates and an extensive purification protocol[10,49] necessitate fermentations on a larger scale. While our synthetic route (*cf.* Scheme 2) was intended to generate substantial product diversity from a common precursor, it was not designed to yield large quantities of the corresponding functionalized 2,3-diaminopropionates.

It has been long recognized that introduction of fluorine into drugs can have beneficial effects on pharmacokinetic properties due to improved metabolic stability, enhanced membrane permeation and increased target binding affinity.[50,51] The incorporation of even a single fluorine into natural products either by chemical synthesis[51] or mutasynthesis[26] has been demonstrated to significantly improve their potency. Due to its extreme scarcity among natural products, there is high interest in manipulating biosynthetic pathways to enable the site-selective incorporation of fluorine to yield compounds with potentially improved bioactivity that remain largely untapped.[52] Recent successes have utilized fluorinated extender units in engineered polyketide synthases (PKS) to generate previously unknown macrolide products bearing aliphatic C–F bonds.[53,54] In NRPS biosynthesis, fluorinated amino acids have been successfully incorporated into peptidyl natural products, however these examples consist almost exclusively of halogen-substituted aromatic α-amino acids.[25,55,56] Owing to the much simpler installation of C-sp^2–F bonds, these biosynthetic precursors are either commercially available or prepared in few synthetic steps. For instance, in order to isolate a fluorinated analog of balhimycin, Süssmuth *et al.* applied a three-step synthetic sequence to access racemic fluorinated *beta*-hydroxy tyrosine, which was required in multigram quantities to isolate the mutasynthetic product.[24] Here, although unable to obtain such quantities, we have installed fluorine at an aliphatic position to prepare fluoromethyl-Dap **39** as a single stereoisomer. Exploiting the inherent substrate flexibility of the entire native late-stage sulfazecin biosynthetic machinery, we were able to selectively produce a bioactive fluorinated monobactam using conventional fermentation methods. To the best of our knowledge, we report the first example for the generation of non-native monobactams through precursor-directed fermentation.

Conclusions

The monobactam class of antibiotics is unique among the β-lactams not only for their unusual structure and biosynthetic rationale but also due to their inherent stability against the clinical threat of Ambler class B MBLs. Here, a divergent synthesis was developed to prepare densely functionalized 2,3-diaminopropionates as biosynthetic replacements for the essential L-Dap **1** motif in sulfazecin **2**. Combining *in vitro* assays, and *in vivo* chemical complementation coupled with spectrometric and microbiological data, we demonstrate that these substituted analogs illustrate the potential to be incorporated into bioactive monobactam products.

Adenylation domain activity end-point assays of our L-Dap library roughly correlate with spectrometric data of monobactam products obtained from fermentation experiments with selected substrates. These observations imply that A_3 is flux-limiting in the efficient production of non-native sulfazecin analogs due to its decreasing activation of larger substrates (*cf.* Fig. 2A). Enzyme engineering is a powerful approach to overcome this hurdle by altering the active site to accommodate modified substrates, although rational optimization of A-domains to-date has been met with limited success.[57–59] Here the terminal NRPS module of sulfazecin biosynthesis presents unique advantages we intend to exploit – the pronounced discrimination of A_3 against cellularly ubiquitous canonical α-amino acids[8] and strict selection of α,β-diamino acids is a fortunate circumstance for reprogrammed biosynthesis. Future work will aim to determine the structural basis for this α,β-diamino selectivity and to streamline A_3 towards the effective activation of dedicated non-native L-Dap analogs.

Nevertheless, adenylation is only the first step in a short sequence of terminal biosynthetic transformations that include condensation of the non-native precursor with the upstream dipeptide, followed by *in trans N*-sulfonation and TE-catalyzed β-lactam formation with concurrent release from the NRPS. A two-step methoxylation, which is difficult to install stereoselectively using conventional synthetic methods, then furnishes the structure of the sulfazecin-like product.[9] Studies to probe and optimize the substrate tolerance of participating enzymes are currently ongoing, with the aim to improve the scope and efficiency of our biosynthetic platform *in vivo*. Additionally, establishment of a more scalable synthetic or biosynthetic route to selected precursor amino acids will enable large-scale fermentation and isolation of monobactams for further characterization. Although it was recognized from the outset that mutasynthesis of monobactams presents a difficult target due to their naturally low production titre, the present work establishes the feasibility and lays the foundation to manipulate the biosynthetic machinery to produce customized monobactams by fermentation.

Data availability

ESI† contains everything needed to repeat any experiment.

Author contributions

MSL, LK, RFL and CAT conceptualized experiments, analysed data, and wrote the manuscript. MSL, LK and JY carried out chemical synthesis. LK, RFL and MSL performed *in vitro* assays.

RFL performed fermentation experiments and bioassays. TAZ and MSL developed and carried out UPLC-HRMS analyses.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This study was funded by National Institutes of Health Research Grant R01 AI121072. LK acknowledges financial support from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 492438365. TAZ was supported by T32 GM135131. We would like to thank Dr M. A. Siegler for the collection of X-ray crystallographic data. We would also like to thank Dr I.P. Mortimer and Dr J. Catazaro for their help with ESI-MS and NMR experiments, respectively.

Notes and references

1 A. Viso, R. Fernández de la Pradilla, A. García and A. Flores, *Chem. Rev.*, 2005, **105**, 3167–3196.
2 A. Viso, R. F. De La Pradilla, M. Tortosa, A. García and A. Flores, *Chem. Rev.*, 2011, **111**, 3167–3196.
3 C. Müller, S. Nolden, P. Gebhardt, E. Heinzelmann, C. Lange, O. Puk, K. Welzel, W. Wohlleben and D. Schwartz, *Antimicrob. Agents Chemother.*, 2007, **51**, 1028–1037.
4 K. Bush and P. A. Bradford, *Clin. Microbiol. Rev.*, 2020, **33**(2), DOI: **10.1128/cmr.00047-19**.
5 P. Hinchliffe, D. M. Moreno, M. A. Rossi, M. F. Mojica, V. Martinez, V. Villamil, B. Spellberg, G. L. Drusano, C. Banchio, G. Mahler, R. A. Bonomo, A. J. Vila and J. Spencer, *ACS Infect. Dis.*, 2021, **7**, 2697–2706.
6 R. B. Sykes, C. M. Cimarusti, D. P. Bonner, K. Bush, D. M. Floyd, N. H. Georgopapadakou, W. H. Koster, W. C. Liu, W. L. Parker, P. A. Principe, M. L. Rathnum, W. A. Slusarchyk, W. H. Trejo and J. S. Wells, *Nature*, 1981, **291**, 489–491.
7 R. B. Sykes, D. P. Bonner, K. Bush and N. H. Georgopapadakou, *Antimicrob. Agents Chemother.*, 1982, **21**, 85–92.
8 R. Li, R. A. Oliver and C. A. Townsend, *Cell Chem. Biol.*, 2017, **24**, 24–34.
9 R. A. Oliver, R. Li and C. A. Townsend, *Nat. Chem. Biol.*, 2018, **14**, 5–7.
10 M. Asai, K. Haibara, M. Muroi, K. Kintaka and T. Kishi, *J. Antibiot.*, 1981, **34**, 621–627.
11 W. L. Parker, W. H. Koster, C. M. Cimarusti, D. M. Floyd, W. C. Liu and M. L. Rathnum, *J. Antibiot.*, 1982, **35**, 189–195.
12 C. M. Cimarusti, D. P. Bonner, H. Breuer, H. W. Chang, A. W. Fritz, D. M. Floyd, T. P. Kissick, W. H. Koster, D. Kronenthal, F. Massa, R. H. Mueller, J. Pluscec, W. A. Slusarchyk, R. B. Sykes, M. Taylor and E. R. Weaver, *Tetrahedron*, 1983, **39**, 2577–2589.
13 A. Imada, M. Kondo, K. Okonogi, K. Yukishige and M. Kuno, *Antimicrob. Agents Chemother.*, 1985, **27**, 821–827.
14 M. Osborn, N. Stachulski, H. Sun, J. Blais, V. Venishetty, M. Raccuglia, M. Kankam, R. Colvin and F. Segal, *Antimicrob. Agents Chemother.*, 2019, **63**(7), e02592–18.
15 R. B. Sykes and D. P. Bonner, *Rev. Infect. Dis.*, 1985, **7**(Suppl 4), S579–S593.
16 F. Reck, A. Bermingham, J. Blais, V. Capka, T. Cariaga, A. Casarez, R. Colvin, C. R. Dean, A. Fekete, W. Gong, E. Growcott, H. Guo, A. K. Jones, C. Li, F. Li, X. Lin, M. Lindvall, S. Lopez, D. McKenney, L. Metzger, H. E. Moser, R. Prathapam, D. Rasper, P. Rudewicz, V. Sethuraman, X. Shen, J. Shaul, R. L. Simmons, K. Tashiro, D. Tang, M. Tjandra, N. Turner, T. Uehara, C. Vitt, S. Whitebread, A. Yifru, X. Zang and Q. Zhu, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 748–755.
17 H. R. Onishi, D. R. Daoust, S. B. Zimmerman, D. Hendlin and E. O. Stapley, *Antimicrob. Agents Chemother.*, 1974, **5**, 38–48.
18 K. L. Rinehart, *Pure Appl. Chem.*, 1977, **49**, 1361–1384.
19 C. D. Denoya, R. W. Fedechko, E. W. Hafner, H. A. I. McArthur, M. R. Morgenstern, D. D. Skinner, K. Stutzman-Engwall, R. G. Wax and W. C. Wernau, *J. Bacteriol.*, 1995, **177**, 3504–3511.
20 S. Weist, C. Kittel, D. Bischoff, B. Bister, V. Pfeifer, G. J. Nicholson, W. Wohlleben and R. D. Süssmuth, *J. Am. Chem. Soc.*, 2004, **126**, 5942–5943.
21 W. T. Shier, K. L. Rinehart and D. Gottlieb, *Proc. Natl. Acad. Sci. U. S. A.*, 1969, **63**, 198–204.
22 M. A. Gregory, H. Petkovic, R. E. Lill, S. J. Moss, B. Wilkinson, S. Gaisser, P. F. Leadlay and R. M. Sheridan, *Angew. Chem., Int. Ed.*, 2005, **44**, 4757–4760.
23 Z. Hojati, C. Milne, B. Harvey, L. Gordon, M. Borg, F. Flett, B. Wilkinson, P. J. Sidebottom, B. A. M. Rudd, M. A. Hayes, C. P. Smith and J. Micklefield, *Chem. Biol.*, 2002, **9**, 1175–1187.
24 S. Weist, B. Bister, O. Puk, D. Bischoff, S. Pelzer, G. J. Nicholson, W. Wohlleben, G. Jung and R. D. Süssmuth, *Angew. Chem., Int. Ed.*, 2002, **41**, 3383–3385.
25 Y. Shi, Z. Jiang, X. Lei, N. Zhang, Q. Cai, Q. Li, L. Wang, S. Si, Y. Xie and B. Hong, *Microb. Cell Fact.*, 2016, **15**, 77.
26 F. Xie, S. Dai, Y. Zhao, P. Huang, S. Yu, B. Ren, Q. Wang, Z. Ji, G. Alterovitz, Q. Zhang, J. Zhang, X. Chen, L. Jiang, F. Song, H. Liu, F. M. Ausubel, X. Liu, H. Dai and L. Zhang, *Cell Chem. Biol.*, 2020, **27**, 1532–1543.e6.
27 S. J. Box, A. G. Brown, M. L. Gilpin, M. N. Gwynn and S. R. Spear, *J. Antibiot.*, 1988, **41**, 7–12.
28 T. Stachelhaus, H. D. Mootz and M. A. Marahiel, *Chem. Biol.*, 1999, **6**, 493–505.
29 G. L. Challis, J. Ravel and C. A. Townsend, *Chem. Biol.*, 2000, **7**, 211–224.
30 B. R. M. Villiers and F. Hollfelder, *ChemBioChem*, 2009, **10**, 671–682.
31 H. Katano, H. Watanabe, M. Takakuwa, C. Maruyama and Y. Hamano, *Anal. Sci.*, 2013, **29**, 1095–1098.
32 C. Maruyama, H. Niikura, M. Takakuwa, H. Katano and Y. Hamano, in *Methods in Molecular Biology*, Humana Press, New York, NY, 2016, vol. 1401, pp. 77–84.

33 N. Kadi and G. L. Challis, *Methods Enzymol.*, 2009, **458**, 431–457.
34 R. Hara, R. Suzuki and K. Kino, *Anal. Biochem.*, 2015, **477**, 89–91.
35 C. A. Townsend, *Curr. Opin. Chem. Biol.*, 2016, **35**, 97–108.
36 S. Hanessian, B. Vanasse, H. Yang and M. Alpegiani, *Can. J. Chem.*, 1993, **71**, 1407–1411.
37 M. D. Armstrong, *J. Am. Chem. Soc.*, 1949, **71**, 3399–3402.
38 C. Pearson, K. L. Rinehart and M. Sugano, *Tetrahedron Lett.*, 1999, **40**, 411–414.
39 B. T. Kelley and M. M. Joullié, *Org. Lett.*, 2010, **12**, 4244–4247.
40 S. Bolek and J. Ignatowska, *J. Fluorine Chem.*, 2019, **217**, 13–21.
41 H. Han, J. Yoon and K. D. Janda, *J. Org. Chem.*, 1998, **63**, 2045–2048.
42 D. J. Wilson and C. C. Aldrich, *Anal. Biochem.*, 2010, **404**, 56–63.
43 B. P. Duckworth, D. J. Wilson and C. C. Aldrich, in *Methods in Molecular Biology*, Humana Press Inc., 2016, vol. 1401, pp. 53–61.
44 M. R. Webb, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 4884–4887.
45 D. E. Ehmann, C. A. Shaw-Reid, H. C. Losey and C. T. Walsh, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 2509–2514.
46 H. Aoki, H. I. Sakai, M. Kohsaka, T. Konomi, J. Hosoda, E. Iguchi, H. Imanaka and Y. Kubochi, *J. Antibiot.*, 1976, **29**, 492–500.
47 C. H. O'Callaghan, A. Morris, S. M. Kirby and A. H. Shingler, *Antimicrob. Agents Chemother.*, 1972, **1**, 283–288.
48 L. A. Mitscher, H. D. H. Showalter, K. Shirahata and R. L. Foltz, *J. Antibiot.*, 1975, **28**, 668–675.
49 A. Imada, K. Kitano and M. Asai, *US Pat.*, US4229436A, 1980.
50 S. Purser, P. R. Moore, S. Swallow and V. Gouverneur, *Chem. Soc. Rev.*, 2008, **37**, 320–330.
51 D. O'Hagan, *J. Fluorine Chem.*, 2010, **131**, 1071–1081.
52 M. C. Walker and M. C. Y. Chang, *Chem. Soc. Rev.*, 2014, **43**, 6527–6536.
53 S. Sirirungruang, O. Ad, T. M. Privalsky, S. Ramesh, J. L. Sax, H. Dong, E. E. K. Baidoo, B. Amer, C. Khosla and M. C. Y. Chang, *Nat. Chem. Biol.*, 2022, **18**, 886–893.
54 A. Rittner, M. Joppe, J. J. Schmidt, L. M. Mayer, S. Reiners, E. Heid, D. Herzberg, D. H. Sherman and M. Grininger, *Nat. Chem.*, 2022, 1–7.
55 C. Wang, C. Lambert, M. Hauser, A. Deuschmann, C. Zeilinger, K. Rottner, T. E. B. Stradal, M. Stadler, E. J. Skellam and R. J. Cox, *Chem. - Eur. J.*, 2020, **26**, 13578–13583.
56 K. M. J. De Mattos-Shipley, C. Greco, D. M. Heard, G. Hough, N. P. Mulholland, J. L. Vincent, J. Micklefield, T. J. Simpson, C. L. Willis, R. J. Cox and A. M. Bailey, *Chem. Sci.*, 2018, **9**, 4109–4117.
57 H. Kries, R. Wachtel, A. Pabst, B. Wanner, D. Niquille and D. Hilvert, *Angew. Chem., Int. Ed.*, 2014, **53**, 10105–10108.
58 S. Meyer, J. C. Kehr, A. Mainz, D. Dehm, D. Petras, R. D. Süssmuth and E. Dittmann, *Cell Chem. Biol.*, 2016, **23**, 462–471.
59 D. L. Niquille, D. A. Hansen, T. Mori, D. Fercher, H. Kries and D. Hilvert, *Nat. Chem.*, 2017, **10**, 282–287.