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Lugdunomycin, an Angucycline-Derived Molecule with Unprecedented Chemical Architecture

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Abstract: *The angucyclines form the largest family of polycyclic aromatic polyketides, and have been studied extensively. Herein, we report the discovery of lugdunomycin, an angucycline-derived polyketide, produced by Streptomyces species QL37. Lugdunomycin has unique structural characteristics, including a heptacyclic ring system, a spiroatom, two all-carbon stereocenters, and a benzaza-[4,3,3]propellane motif. Considering the structural novelty, we propose that lugdunomycin represents a novel subclass of aromatic polyketides. Metabolomics, combined with MS-based molecular networking analysis of Streptomyces sp. QL37, elucidated 24 other rearranged and non-rearranged angucyclines, 11 of which were previously undescribed. A biosynthetic route for the lugdunomycin and limamycins is also proposed. This work demonstrates that revisiting well-known compound families and their producer strains still is a promising approach for drug discovery.*

Actinobacteria are Gram-positive, and often filamentous, bacteria that are a major source of bioactive natural products,[1,2] and most of these are produced by actinomycetes of the genus *Streptomyces*.[2,3] Despite the increasing difficulty to isolate new molecules, the biosynthetic potential of actinomycetes is far from exhausted.[4,5] Many such molecules are most likely specified by biosynthetic gene clusters (BGCs) that are poorly expressed in the laboratory, generally referred to as cryptic gene clusters.[6,7] However, novel molecules have also been identified via the "one strain—many compounds" (OSMAC) strategy,[8] which further supports the notion that actinobacteria harbor significant unexplored chemical diversity. Angucyclines and angucyclinones,[9] which bear an unsymmetrically assembled benz[a]anthracene frame, represent the largest family of polycyclic aromatic polyketides from actinomycetes, and exhibit a broad range of biological, predominantly anticancer and antibacterial, activities.[9,10] The minimal polyketide synthase (PKS) forms the initial angucycline or angucyclinone framework that is further modified by a wide array of post-PKS tailoring enzymes.[9]

Herein, we report the discovery of a novel angucycline-derived compound, lugdunomycin (**1**, Figure 1) with a striking benzaza[4,3,3]propellane-6-spiro-2′-2*H*-naphtho[1,8-*bc*]furan backbone, found in *Streptomyces* sp. QL37. OSMAC, combined with MS/MS-based molecular networking, characterized 24 other rearranged and non-rearranged angucyclines **2**–**25** (Figure 1), 11 of which were new structures featuring unique ring rearrangement, oxidation, reduction, and amidation patterns. The new structural features, and in particular the Baeyer–Villiger oxidative cleavage and expansion of the C-ring of angucycline, further enrich the existing diversity of angucycline- and/or angucyclinone-type natural products.

In our search for novel chemical diversity, seven strains showing distinctive pigmentation were prioritized from our actinomycete strain collection[11] because distinctive pigmentation is a beacon for chemical diversity.[12] The actinomycetes were grown in six different culture media, and their metabolomes were compared by thin-layer chromatography (TLC). Of these, *Streptomyces* sp QL37 yielded a rich metabolic profile, and this strain was therefore subjected to up-scale (7.5 liter) on MM agar plates. Repeated chromatography of the crude extract (2.3 g) resulted in compounds **1** (0.5 mg), **2** (27 mg), **6**/**7** (27 mg), **8** (3.4 mg), and **11** (1 mg).

The isolated colorless **1** was called lugdunomycin after *Lugdunum batavorum*, the Latin name for the city of Leiden. UHPLC-ToF-MS analysis of **1** identified an $[M+H]^+$ peak at *m*/*z* 474.1553, establishing its molecular composition as $C_{27}H_{23}NO_7$. The deduced chemical formula was corroborated by the attached proton test (APT) that exhibited 27 carbons. The three aromatic rings A, B, and D, were readily assigned based on the proton-splitting pattern in the ^{1}H NMR spectrum and COSY correlations (Supporting Information,

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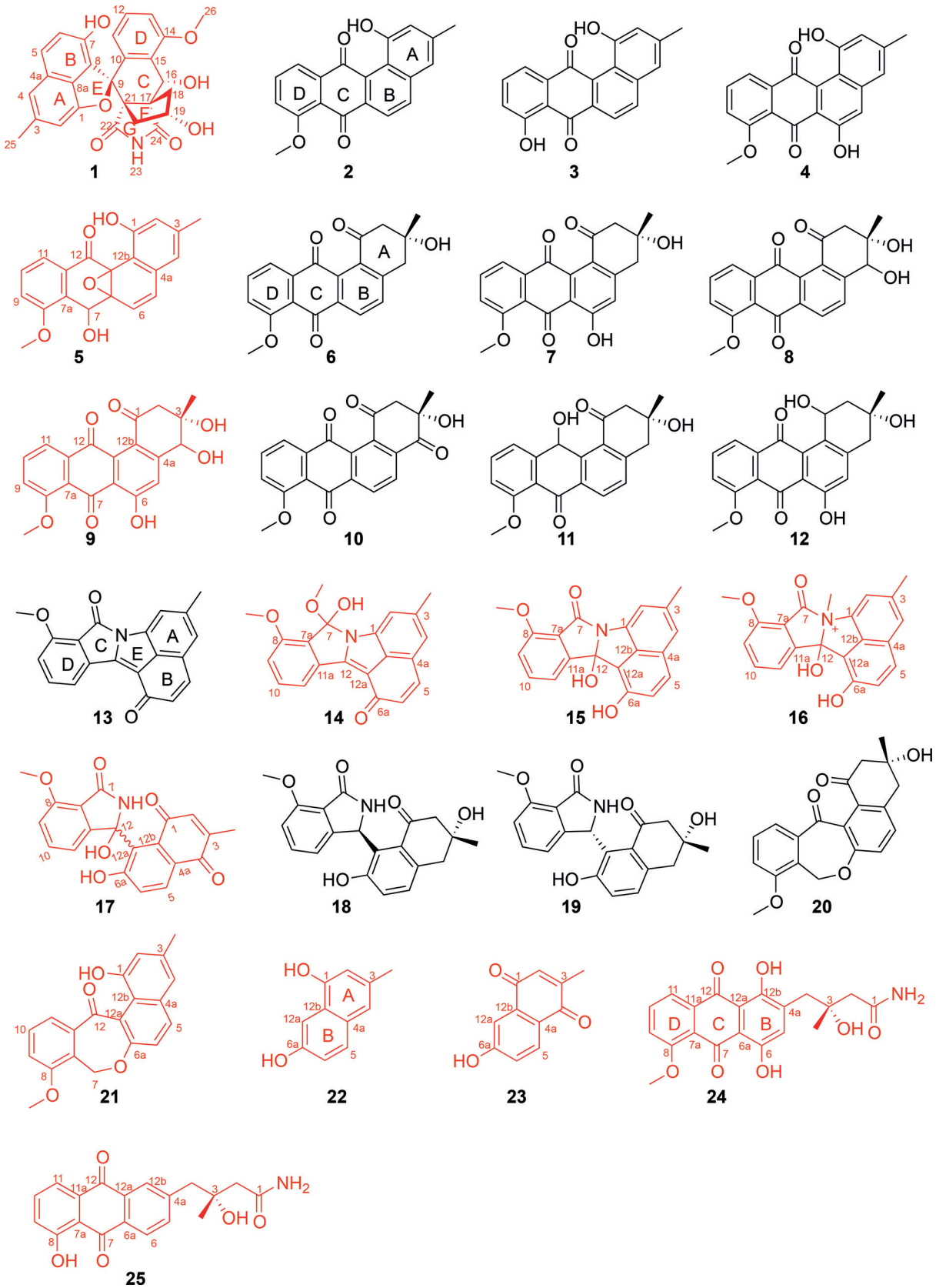

Figure 1. Angucyclines isolated from *Streptomyces sp.* QL37. Lugdunomycin (**1**) is a novel angucycline derivative. All the previously undescribed compounds are shown in red.

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Table S1 and Figure S1). Benzene rings A and B were fused into a naphthalene system by sharing a double bond between C-4a and C-8a, based on the HMBC correlations H-4/C-8a, H-5/C-8a, H-4/C-5, H-5/C-4, and H-6/C-4a. Ring C was identified by HMBC correlations from H-16 to C-10, C-14, and C-15 of ring D. Key HMBC correlations, such as H-19/C-17, H-19/C-21, H-20/C-17, and H-20/C-21, unequivocally showed that the cyclopentanol ring F is joined to ring C by sharing the bond between C-17 and C-21. Furthermore, H-18 and H-20 showed $^{3}J_{CH}$ HMBC correlations with two carbonyl groups at δ_C 182.4, and 182.5, respectively, which indicated the presence of another ring fused to two all-carbon stereocenters,[13] C-17 and C-21, apart from rings C and F; these two carbonyls are joined by a nitrogen atom in line with both the molecular formula and the chemical shifts, consistent with succinimide ring G. Consequently, rings D/C/F/G constituted a benzaza[4,3,3]propellane motif. Ring systems A/B and D/C/F/G are linked at spiroatom C-9, as established by key HMBC correlations H-20/C-9, H-11/C-9, and H-6/C-9. An additional five-membered furan ring (ring E) is formed to make a 2*H*-naphtho[1,8-*bc*]furan module [7-methyl-2*H*-naphtho[1,8-*bc*]furan-3-ol] to join the whole structure and fit in the molecular formula. Taken together, the benzaza-[4,3,3]propellane skeleton is adorned with a spirocyclic 2*H*-naphtho[1,8-*bc*]furan moiety and two all-carbon quaternary centers embedded within five contiguous stereogenic carbons. The benzaza[4,3,3]propellane-6-spiro-2′-2*H*-naphtho[1,8-*bc*]furan architecture is unprecedented.

Single-crystal X-ray diffraction confirmed the structure of lugdunomycin (**1**) (Supporting Information, Table S2). Crystallization of **1** exhibited a centrosymmetric space group *P*42/*n*, explicitly supporting a racemic mixture. The configurations of five chiral centers in **1** were determined as 9*R**, 16*S**, 17*R**, 19*S**, and 21*S** (Figure 2 and Supporting Information, Figure S2). The racemic nature of the obtained lugdunomycin was also suggested by the measured optical rotation of zero.

To obtain insight into the diversity of angucycline-derived molecules produced by *Streptomyces* sp. QL37, we performed global natural products social (GNPS) molecular networking using MS/MS profiles[14,15] and compared the output to the GNPS database.[16] To ensure optimal chemical diversity, *Streptomyces* sp. QL37 was fermented in 77 different culture media (Supporting Information, Table S3), as described.[11] MS/MS-based molecular networking of QL37 grown on R5 + 0.8% peptone + 1% mannitol and MM + 0.5% mannitol + 1% glycerol presented the relatedness among metabolites (Supporting Information, Figure S3), including the structurally related angucyclines (Figure 1) and other families of compounds (Supporting Information, Figure S5). This observation not only dereplicated the non-rearranged angucyclines **2**, **6**–**8**, and **11** isolated from MM medium (Supporting Information, Figure S4), but also revealed many nitrogen-containing angucyclines (Figure 3B). Especially, the subnetwork for lugdunomycin identified a putative aldehyde analogue of **1** with *m*/*z* 502 (Figure 3A).

Up-scale refermentation (20 L) of *Streptomyces* sp. QL37 in R5 medium followed by extensive systematic isolation, via OSMAC[8] identified compounds **2**–**45**, including unrearranged benz[a]anthracene angucyclines (**2**–**12**), limamycins[17]

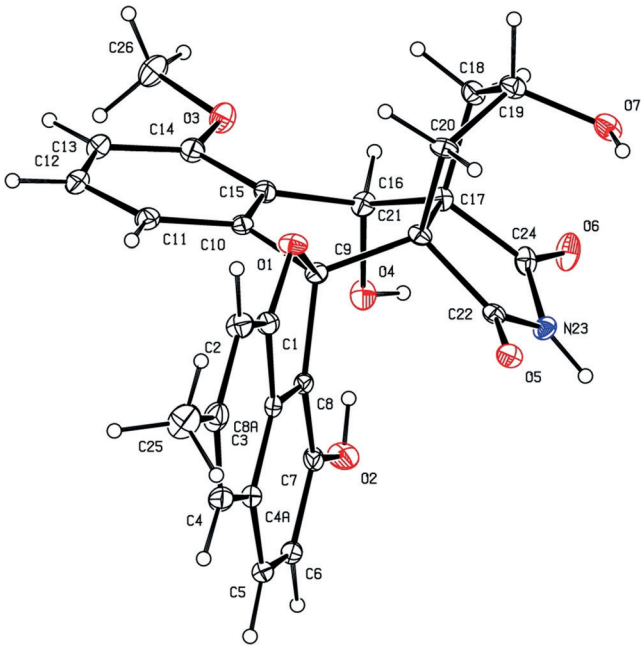

Figure 2. ORTEP drawing of the crystal structure of lugdunomycin. The absolute configurations of the five chiral centers of lugdunomycin are 9*R**, 16*S**, 17*R**, 19*S**, and 21*S**.

(**13**–**19**), emycins[17–19] (**20**, **21**), naphthalenones (**22**, **23**), anthraquinones (**24**, **25**, Figure 1), nucleosides (**26**–**33**), cyclodipeptides (**34**–**41**), 4-quinolones (**42**, **43**), vitamin K (**44**), and quinazolinone (**45**, Supporting Information, Figure S5). Of these, compounds **5**, **9**, **14**–**17**, and **21**–**25** were new structures. The NMR data assignments of new angucyclines are summarized in Tables S4–S6 in the Supporting Information, and the 2D NMR correlations (HMBC and COSY) are displayed in Figure S1 in the Supporting Information. The 11 new angucyclines featured, among others, unique ring cleavage (ring A or C) and rearrangement (**13**–**25**), hydration of double bond $\Delta^{12,12a}$ (**15**, **16**), nucleophilic addition of methanol to the ketone at C-7 (**14**), epoxidation of double bond $\Delta^{6a,12b}$ (**5**), *N*-quaternary methylation (**16**), amidation (**13**–**19**, **24**, and **25**), and reduction of the ketone at C-7 (**20**, **21**). Though **14** is likely an artifact due to the usage of methanol during chromatographic isolation, all these new structures add further chemical diversity to this important family of polyketides.

In terms of bioactivity, an agar diffusion assay whereby pure compound was spotted on filter on a lawn of the target bacterium, showed that lugdunomycin had antimicrobial activity against the Gram-positive *Bacillus subtilis* 168, but not against the Gram-negative *Escherichia coli* K12 (not shown). More extensive experiments are needed to assess the bioactivity and mode of action of lugdunomycin.

Based on the structures of the identified molecules (Figure 1), a biosynthetic route of rearranged limamycins and/or non-rearranged angucyclines is proposed (Figure 4). Versatile post-PKS oxidations are critical for the rearrangement of the precursors **2** and/or **6**. The cleavage at the C-1/12b bond in the A-ring of **2** generates the tricyclic anthraquinone scaffold in **24** and **25**. Another pivotal Baeyer–Villiger

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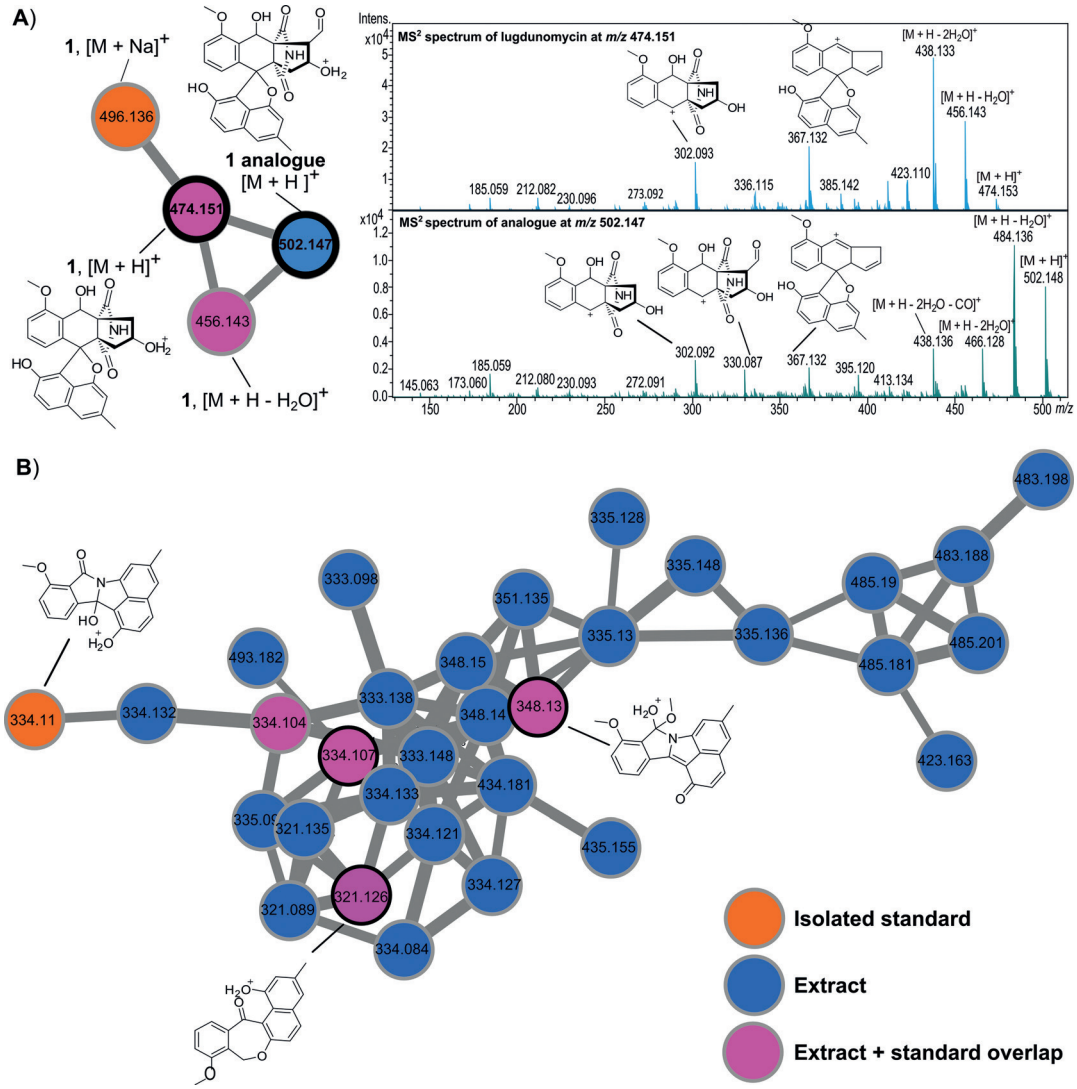

Figure 3. MS^2-based molecular networking of *Streptomyces* sp. QL37. A) Subnetwork for lugdunomycin reveals an analogue at *m*/*z* 502. Comparison of MS^2 spectra of **1** at *m*/*z* 474.151 and its analogue at *m*/*z* 502.147 suggests an additional aldehyde group in the latter; see the fragmentation pattern above the ion peak. B) Rearranged angucyclines. The edge thickness between connecting nodes corresponds to the similarity of the MS/MS spectra. The full GNPS network is presented in Figure S3 in the Supporting Information, and the subnetwork for non-rearranged angucyclines in Figure S4 in the Supporting Information.

oxidation at the C-6a/C-7 bond of the quinone ring C is proposed to initiate the structural rearrangements of **2** and/or **6** to give compounds **13**–**23**. The lactone intermediate **6a** or **2a** is susceptible to hydration resulting in lactone ring opening, followed by the amidation at C-12 ketone to introduce a nitrogen atom in **6c** or **2c**, which is cyclized to form limamycins[17] **13**–**19** (Figure 4A). Alternatively, **2a** could go through an evolutionary pathway involved in emycin biosynthesis,[18] whereby a reduction at the ketone group of C-7 is essential to generate compounds **20** and **21**.

The co-identification of compounds **2**–**25** in *Streptomyces* sp. QL37 and elucidation of the biosynthetic pathway for the nitrogen-containing limamycins shed light on the biosynthetic logic of lugdunomycin (**1**, Figure 4B). We hypothesize that the benzaza[4,3,3]propellane skeleton of lugdunomycin is eventually constructed through a Diels–Alder [4+2] cycloaddition, and the diene and dienophile reagents for the Diels–Alder reaction are provided by the limamycin and emycin biosynthetic pathway, respectively. The putative limamycin **15a** is likely to go through a cascade of oxidative C–C bond cleavage in the D-ring, followed by decarboxylation and aldol condensation, to give an isomer of maleimycin[20] (compound **15g**) that serves as the dienophile for Diels–Alder reaction. In support of this, the identity of compound **22** was spectroscopically confirmed, and MS/MS-based molecular networking analysis of QL37 confirmed the presence of a mass consistent with that of the maleimycin isomer (**15g**) (Supporting Information, Figure S6). The hydroxy-*o*-quinodimethane intermediate **21b** is a candidate diene reagent for the Diels–Alder reaction, which is probably derived from the dehydration of **21a**. The cycloaddition of **21b** and **15g** mimics the reported Diels–Alder trapping of photochemically generated hydroxy-*o*-quinodimethanes by maleimides,[21] and this intermolecular Diels–Alder reaction

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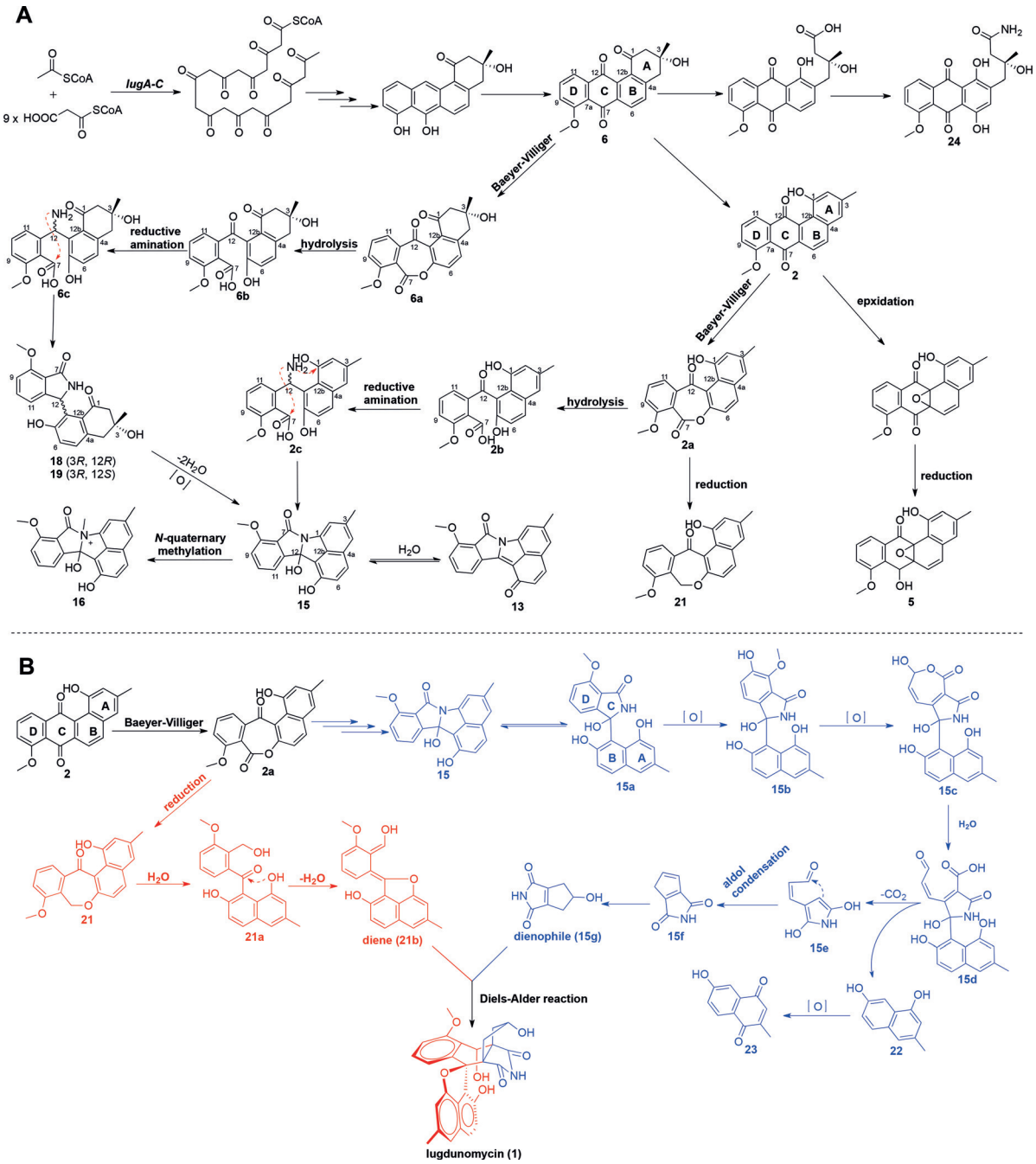

Figure 4. Biosynthetic route to limamycins and proposed pathway towards lugdunomycin. A) Biosynthesis of limamycins. Baeyer–Villiger oxidative cleavage at the quinone ring of compounds **2** and/or **6** is pivotal for the biosynthesis of lugdunomycin and rearranged limamycins **13**–**19**. B) Proposed pathway for lugdunomycin biosynthesis. As possible final step for the assembly of lugdunomycin a Diels–Alder reaction is proposed. As dienophile for the Diels–Alder reaction an isomer of maleimycin (Supporting Information, Figure S6) is a likely candidate. We propose its formation from the oxidative ring contraction in the D-ring of limamycins. The limamycin and emycin biosynthetic pathways are drawn in blue and red, respectively.

has been explored to construct complex pseudo-natural polyketides from simple intermediates.[22]

Genome sequencing of *Streptomyces* sp. QL37 allowed the identification of a type II PKS gene cluster (*lug*, Supporting Information, Table S7 and Figure S7). Genetic inactivation of the minimal PKS genes *lugA*–*C* completely abolished the production of angucyclines, limamycins, and lugdunomycin. We are investigating the precise function of the various genes of the *lug* gene cluster.

Future investigation into the genetic and synthetic knowledge underlying lugdunomycin biosynthesis, will not only guide the up-scale production of lugdunomycin and its potential variants through synthetic biology approaches, but will also offer new opportunities to expand the existing

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structural diversity of known polyketides. Thus, our work will likely form the basis of new explorations into the exciting chemical space of the angucyclines and other polyketides.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: angucycline · Baeyer–Villiger oxidation · molecular networking · natural product · polyketide

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[1] D. A. Hopwood, *Streptomyces in Nature and Medicine: The Antibiotic Makers*, Oxford University Press, New York, **2007**.
[2] E. A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H. Klenk, C. Clément, Y. Ouhdouch, G. P. van Wezel, *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 1 – 43.
[3] J. Bérdy, *J. Antibiot. (Tokyo)* **2012**, *65*, 385 – 395.
[4] R. Kolter, G. P. van Wezel, *Nat. Microbiol.* **2016**, *1*, 15020.
[5] M. A. Cooper, D. Shlaes, *Nature* **2011**, *472*, 32.
[6] H. Zhu, S. K. Sandiford, G. P. van Wezel, *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 371 – 386.
[7] P. J. Rutledge, G. L. Challis, *Nat. Rev. Microbiol.* **2015**, *13*, 509 – 523.
[8] H. B. Bode, B. Bethe, R. Höfs, A. Zeeck, *ChemBioChem* **2002**, *3*, 619 – 627.
[9] M. K. Kharel, P. Pahari, M. D. Shepherd, N. Tibrewal, S. E. Nybo, K. A. Shaaban, J. Rohr, *Nat. Prod. Rep.* **2012**, *29*, 264 – 325.
[10] J. Rohr, R. Thiericke, *Nat. Prod. Rep.* **1992**, *9*, 103 – 137.
[11] H. Zhu, J. Swierstra, C. Wu, G. Girard, Y. H. Choi, W. van Wamel, S. K. Sandiford, G. P. van Wezel, *Microbiology* **2014**, *160*, 1714 – 1725.
[12] S. F. Brady, C. J. Chao, J. Handelsman, J. Clardy, *Org. Lett.* **2001**, *3*, 1981 – 1984.
[13] Y. Minko, M. Pasco, L. Lercher, M. Botoshansky, I. Marek, *Nature* **2012**, *490*, 522 – 526.
[14] J. Watrous, P. Roach, T. Alexandrov, B. S. Heath, J. Y. Yang, R. D. Kersten, M. van der Voort, K. Pogliano, H. Gross, J. M. Raaijmakers, et al., *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 1743 – 1752.
[15] D. D. Nguyen, C.-H. Wu, W. J. Moree, A. Lamsa, M. H. Medema, X. Zhao, R. G. Gavilan, M. Aparicio, L. Atencio, C. Jackson, et al., *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E2611 – 2620.
[16] M. Wang, J. J. Carver, V. V. Phelan, L. M. Sanchez, N. Garg, Y. Peng, D. D. Nguyen, J. Watrous, C. A. Kapono, T. Luzzatto-Knaan, et al., *Nat. Biotechnol.* **2016**, *34*, 828 – 837.
[17] S. Fotso, T. Mahmud, T. M. Zabriskie, D. A. Santosa, P. J. Proteau, *J. Antibiot. (Tokyo)* **2008**, *61*, 449 – 456.
[18] M. Gerlitz, G. Udvarnoki, J. Rohr, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1617 – 1621; *Angew. Chem.* **1995**, *107*, 1757 – 1761.
[19] M. Ma, M. E. Rateb, Q. Teng, D. Yang, J. D. Rudolf, X. Zhu, Y. Huang, L. X. Zhao, Y. Jiang, X. Li, et al., *J. Nat. Prod.* **2015**, *78*, 2471 – 2480.
[20] E. F. Elstner, D. M. Carnes, R. J. Suhadolnik, G. P. Kreishman, M. P. Schweizer, R. K. Robins, *Biochemistry* **1973**, *12*, 4992 – 4997.
[21] L. Dell'Amico, A. Vega-Peñaloza, S. Cuadros, P. Melchiorre, *Angew. Chem. Int. Ed.* **2016**, *55*, 3313 – 3317; *Angew. Chem.* **2016**, *128*, 3374 – 3378.
[22] T. Asai, K. Tsukada, S. Ise, N. Shirata, M. Hashimoto, I. Fujii, K. Gomi, K. Nakagawara, E. N. Kodama, Y. Oshima, *Nat. Chem.* **2015**, *7*, 737 – 743.

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