Biosynthesis of the Carbamoylated D-Gulosamine Moiety of Streptothricins: Involvement of a Guanidino-N-glycosyltransferase and an N-Acetyl-D-gulosamine Deacetylase

7 AUTHORS, INCLUDING:

Zhengyan Guo
Chinese Academy of Sciences
13 PUBLICATIONS   **62** CITATIONS

SEE PROFILE

Yihua Chen
Chinese Academy of Sciences
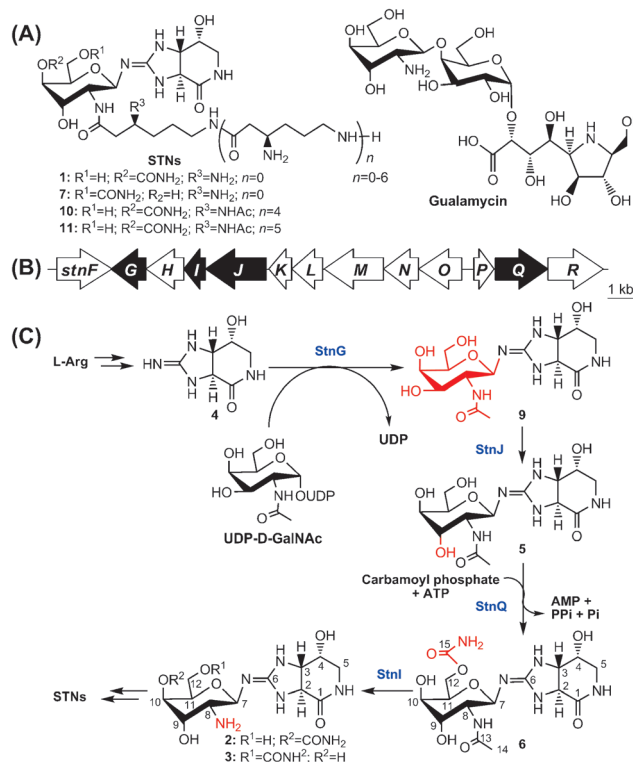**23** PUBLICATIONS   **353** CITATIONS

SEE PROFILE

Biosynthesis

Biosynthesis of the Carbamoylated D-Gulosamine Moiety of Streptothricins: Involvement of a Guanidino-*N*-glycosyltransferase and an *N*-Acetyl-D-gulosamine Deacetylase**

Zhengyan Guo, Jine Li, Hua Qin, Min Wang, Xun Lv, Xuebing Li, and Yihua Chen*

Abstract: *Streptothricins (STNs) are atypical aminoglycosides containing a rare carbamoylated D-gulosamine (D-GulN) moiety, and the antimicrobial activity of STNs has been exploited for crop protection. Herein, the biosynthetic pathway of the carbamoylated D-GulN moiety was delineated. An N-acetyl-D-galactosamine is first attached to the streptolidine lactam by the glycosyltransferse StnG and then epimerized to N-acetyl-D-gulosamine by the putative epimerase StnJ. After carbamoylation by the carbamoyltransferase StnQ, N-acetyl-D-GulN is deacetylated by StnI to furnish the carbamoylated D-GulN moiety. In vitro studies characterized two novel enzymes: StnG is an unprecedented GT-A fold N-glycosyl-transferase that glycosylates the imine nitrogen atom of guanidine, and StnI is the first reported N-acetyl-D-GulN deacetylase.*

Streptothricins (STNs) are atypical aminoglycosides featuring a streptolidine lactam base, a rare carbamoylated D-gulosamine (D-GulN) sugar, and various side chains attached to the D-GulN amino group (Scheme 1 A and see Figure S1 in the Supporting Information). The structural diversity of STNs arises from the number of β-lysine moieties (ranging from 1 to 7) attached to D-GulN and by the position of the carbamoylation.[1] STNs possess antibacterial and antifungal bioactivities and are used to prevent bacterial and fungal diseases in crops.[2] The discovery of STN F (**1**) more than 70 years ago[1a] initiated a sustained effort to elucidate the biosynthetic logic of STNs,[3] which revealed that the streptolidine lactam and

[*] Dr. Z. Guo,[+] Dr. J. Li,[+] Dr. H. Qin,[+] Dr. M. Wang, Prof. Y. Chen
State Key Laboratory of Microbial Resources
Institute of Microbiology, Chinese Academy of Sciences
Beijing 100101 (China)
E-mail: chenyihua@im.ac.cn

Dr. X. Lv, Prof. X. Li
CAS Key Laboratory of Pathogenic Microbiology & Immunology
Institute of Microbiology
Chinese Academy of Sciences
Beijing 100101 (China)

[+] These authors contributed equally to this work.

Scheme 1. STN structures, genes, and biosynthetic pathway. A) Structures of STNs and the D-GulNAc-containing compound gualamycin. B) Partial *stn* gene cluster, with genes studied in this work denoted as black arrows. C) A proposed biosynthetic pathway for the D-GulN moiety of STNs. AMP = adenosine monophosphate, ATP = adenosine triphosphate, PPi = pyrophosphate, Pi = inorganic phosphate.

the poly-β-lysine chain originate from L-arginine and L-lysine, respectively.[4] Recent genetic and enzymatic studies demonstrated that the poly-β-lysine chain is formed by atypical nonribosomal peptide synthetases and then attached to streptothrisamine (**2**) or 10-*de*-O-carbamoyl-12-O-carbamoyl-streptothrisamine (**3**; Figure 1C).[4a] However, little is known about the biosynthesis of the carbamoylated D-GulN moiety except that D-glucosamine (D-GlcN) is a precursor, based on an isotope-labeled feeding experiment.[5] In addition to STNs, the D-GulN moiety also occurs in other natural products such as gualamycin (Scheme 1 A).[6] Herein, we elucidate the biosynthetic mechanism of the carbamoylated D-GulN moiety and report StnG to be a GT-A fold *N*-glycosyltransferase that catalyzes an unprecedented glycosylation of a guanidinoimine and characterize StnI as the first *N*-acetyl-D-gulosamine (D-GulNAc) deacetylase.

We previously reported the cloning of a silent STN biosynthetic gene cluster from *Streptomyces* sp. TP-A0356 and its expression in *Streptomyces coelicolor* (Scheme 1 B).[7] Bioinformatics analyses revealed that the *stnG* and *stnQ* genes were the two best candidates for biosynthesis of the carbamoylated D-GulN moiety. StnG is a putative type-A glycosyltranferase (GT) that displays marginal homology with characterized GTs in the GT-2 family (e.g. 16.1% identity to SpsL),[8] and the DXD motif for binding divalent metal ions in GT-A family enzymes is well conserved in StnG (see Figure S2 in the Supporting Information).[9] The Δ*stnG* mutant CIM1006 was constructed by replacing *stnG* with a kanamycin resistance cassette (see Figure S3A in the Supporting Information), which abolished STN production but resulted in the accumulation of a new metabolite (**4**) (Figure 1). Notably, the Δ*stnG* mutant CIM1006 and the three
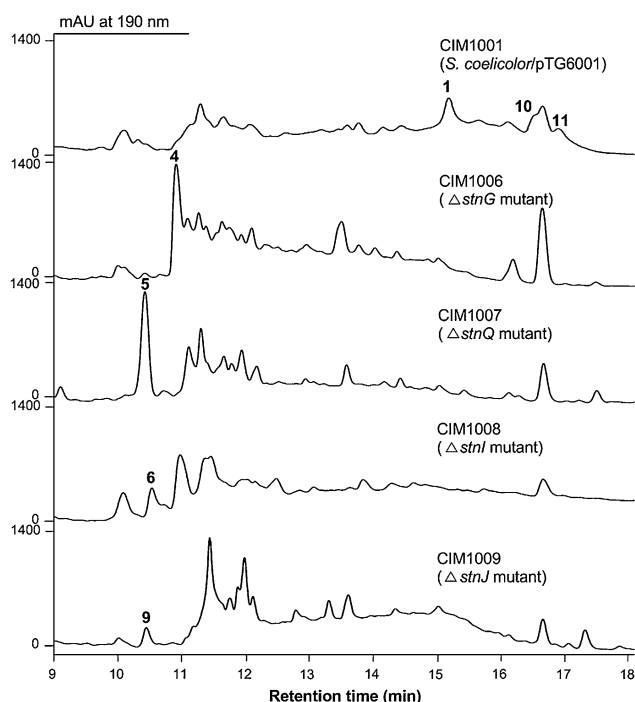

Figure 1. HPLC metabolite profiles of the *stn* gene inactivation mutants. CIM1001, *S. coelicolor* M145 harboring the *stn* cluster, which produces STN F (**1**) together with *N*-acetyl-STN B (**10**) and *N*-acetyl-STN A (**11**; Figure 1).

stn mutants described below were all complemented successfully by expressing the replaced gene in trans, therefore excluding the possibility of a polar effect (see Figure S4 in the Supporting Information). The chemical formula of the water-soluble compound **4** was determined to be $C_6H_{10}N_4O_2$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS; *m/z* 171.0872 $[M+H]^+$, calcd 171.0877). Analysis of its 1D and 2D NMR data revealed that **4** has the same planar structure as the streptolidine lactam (see Table S3 and Figure S5 in the Supporting Information),[10] and its (2*S*,3*S*,4*R*) configuration was confirmed by electronic circular dichroism (ECD) analysis (see Figure S6 in the Supporting Information). In summary, although the results demonstrated

that StnG catalyzes the glycosylation of **4**, the identity of the sugar substrate was required to probe the activity of StnG in vitro.

StnQ is a putative carbamoyltransferase sharing 53% identity with TobZ, a well-characterized carbamoyltransferase involved in tobramycin biosynthesis.[11] The Δ*stnQ* mutant CIM1007 was also constructed by allelic replacement (see Figure S3B in the Supporting Information) and like CIM1006 it lost its ability to produce STNs. However, CIM1007 accumulated a new metabolite (**5**; Figure 1) with the chemical formula $C_{14}H_{23}N_5O_7$ (HR-ESI-MS *m/z* 374.1666 $[M+H]^+$, calcd 374.1676). NMR analysis of **5** revealed that its streptolidine lactam group is conjugated with a β-D-GulNAc through an N-glycosidic bond. The small coupling constants of J_{H8H9} (2.9 Hz), J_{H9H10} (< 3.5 Hz), and J_{H10H11} (< 6.0 Hz) indicate both H-9 and H-10 are in equatorial configurations.[1f,g] The acetyl group was assigned by its NMR signals (δ_H: 2.00 ppm, δ_C: 22.3 ppm (C-12 methyl) and δ_C: 174.2 ppm (C-13)) and its attachment to 8-NH$_2$ was shown by HMBC correlation from H-8 (δ_H: 4.23 ppm) to C-13 (see Table S4 and Figure S7 in the Supporting Information). The absence of a carbamoyl group in **5** implies that StnQ is a carbamoyltransferase decorating the D-GulNAc moiety, while the surprising presence of the *N*-acetyl group suggests that deacetylation is required prior to poly-β-lysine installation at the 8-NH$_2$ group en route to fully elaborated STNs.

Deacetylation may be catalyzed by the *stnI* gene, which encodes a protein that displays moderate homology with LmbE-like deacetylases (e.g. 23.0% identity to TT1542).[12] StnI possesses the HXDD motif for metal-ion binding and catalysis that is conserved in LmbE-like deacetylases (see Figure S8 in the Supporting Information).[13] Allelic replacement generated the Δ*stnI* mutant CIM1008 (see Figure S3C in the Supporting Information), which accumulated **6** as the main metabolite (Figure 1). HR-ESI-MS analysis of **6** showed an $[M+H]^+$ ion at *m/z* 417.1727, consistent with the chemical formula $C_{15}H_{24}N_6O_8$ (calcd 417.1734; see Figure S9 in the Supporting Information), and the mass difference between **5** and **6** indicates that **6** is a carbamoylated derivative of **5**. The NMR spectra of **6** confirmed the presence of a carbamoyl group (δ_C: 159.0 ppm (C-15)) connected to the 12-OH group through an ester linkage by the HMBC correlation signal from H-12 (δ_H: 4.22 ppm) to C-15 (see Table S5 and Figure S9 in the Supporting Information). These data suggest that StnI functions as a deacetylase to remove the *N*-acetyl group of **6** to form **3**. The equatorial configurations of H-9 and H-10 in the D-GulNAc moiety were determined by the small coupling constants of J_{H8H9} (2.8 Hz), J_{H9H10} (< 3.5 Hz), and J_{H10H11} (< 3.0 Hz). Interestingly, the isolation of 12-carbamoylated **6** as a main product from CIM1008 indicates that the carbamoyltransferase StnQ prefers the 12-OH position. We previously showed that the Δ*stnO* mutant CIM1004 accumulated both **2** (10-*O*-carbamoylated) and **3** (12-*O*-carbamoylated) in a ratio of about 2:5, which implies that StnQ may also carbamoylate at the 10-OH position. Moreover, the existence of both 10- and 12-carbamoylated STNs produced by STN producers (see Figure S1 in the Supporting Information)[1d,2] further supports a certain degree of promiscuous StnQ regiospecificity.

As D-GulNAc deacetylase activity has not previously been observed, we sought to characterize StnI in vitro. We purified the N-His$_6$-tagged StnI (see Figure S10 in the Supporting Information) and tested its deacetylase activity by incubating the enzyme with **5** or **6**. HPLC analyses revealed no conversion of **5**, but a new product was detected when using **6** as a substrate (Figure 2A). The chemical
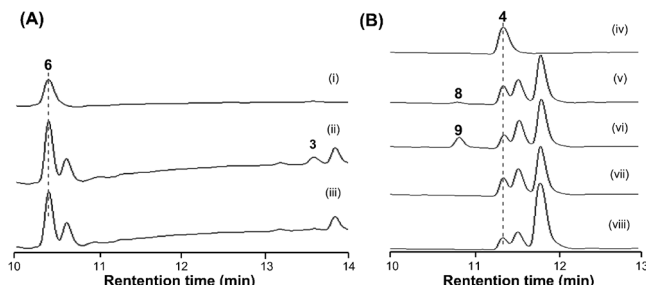

Figure 2. HPLC analysis of representative assays of StnI (A) and StnG (B). i) **6** standard; ii) **6** + StnI; iii) **6** + denatured StnI; iv) **4** standard; v) **4** + UDP-GlcNAc + StnG; vi) **4** + UDP-GalNAc + StnG; vii) **4** + UDP-GulNAc + StnG; viii) **4** + UDP-GalNAc + denatured StnG. Detection at 190 nm.

formula of the new product was $C_{13}H_{22}N_6O_7$, the same as **3**, as determined by HR-ESI-MS (m/z 375.1607 $[M+H]^+$, calcd 375.1623; see Figure S11 in the Supporting Information). These results suggest that StnQ first carbamoylates the D-GulNAc moiety of **5** to form **6**, which is then deacetylated by StnI to generate **3**. Significantly, as all the previously characterized LmbE family deacetylases catalyze the deacetylation of *N*-acetyl-D-glucosamine (D-GlcNAc),[13] StnI expands the substrate spectrum of this enzyme family.

Given that the D-GulNAc moiety exists in both **5** and **6**, we speculated that D-GlcNAc, the acetylated derivative of the identified precursor D-GlcN, is a precursor of STN biosynthesis. To validate this hypothesis, *N*-[1,2-^{13}C$_2$]-D-GlcNAc was fed to the Δ*stnI* mutant CIM1008. As anticipated, *N*-[1,2-^{13}C$_2$]-D-GlcNAc was incorporated into **6** efficiently when analyzed by MS (see Figure S12 in the Supporting Information), thereby proving the intermediacy of D-GlcNAc in the biosynthesis of the D-GulNAc moiety.

As sugars are typically activated to NDP sugars by a nucleotidyltransferase prior to GT-catalyzed installation on an aglycone,[14] we proposed that StnG recuits an NDP sugar from primary metabolism because: 1) no nucleotidyltransferase-encoding gene exists in the *stn* cluster; and 2) the *stn* cluster can be expressed in different heterologous hosts including *S. coelicolor* and *Streptomyces albus*,[7] which suggests that the substrate of StnG is an NDP sugar common in *Streptomyces*. The identification of D-GlcNAc as a precursor of STNs implicates UDP-D-GlcNAc as a potential substrate, which is one component of the ubiquitous peptidylglycoside comprising the cell wall of the Gram-positive bacteria *Streptomyces*.

To verify the proposed activity of StnG toward UDP-D-GlcNAc, we purified N-His$_6$-tagged StnG (see Figure S10 in the Supporting Information) and incubated the enzyme with **4**

and UDP-D-GlcNAc as substrates. To our delight, although the reaction was slow, a new product was detected by HPLC analysis of an overnight assay. HR-ESI-MS analysis revealed its chemical formula is $C_{14}H_{23}N_5O_7$ (m/z 374.1675 $[M+H]^+$, calcd 374.1676), consistent with the expected product **8** (Figure 2B and see S13B in the Supporting Information). We then examined StnG activity towards UDP-D-GulNAc (synthesized as described in the Supporting Information) and UDP-*N*-acetyl-D-galactosamine (UDP-D-GalNAc). Although no reaction occurred when using the former substrate, which indicates that the STN D-GulNAc moiety is formed after glycosyl transfer, the effective conversion of **4** into a new product **9** was observed with the latter substrate (Figure 2B). Compound **9** shares the same chemical formula $C_{14}H_{23}N_5O_7$ as **5** and **8** (HR-ESI-MS m/z 374.1664 $[M+H]^+$, calcd 374.1676; see Figure S13D in the Supporting Information), but could be clearly differentiated by careful HPLC analysis (see Figure S16D in the Supporting Information). The reaction conditions of StnG were then optimized using **4** and UDP-D-GalNAc as substrates (see Figure S14 in the Supporting Information). Steady-state kinetic analyses under the optimized conditions (pH 8.0, 30°C with 0.05 mm Mn^{2+}) revealed Michaelis–Menten behavior for all substrates, and the resulting steady-state kinetic constants clearly demonstrate a strong preference of UDP-D-GalNAc over UDP-D-GlcNAc as the sugar donor for StnG (Table 1 and see

Table 1: StnG kinetic parameters for different substrates.

Toward UDP-GlcNAc and UDP-GalNAc as Donors[a]			
Donor	K_m [mm]	k_{cat} [min^{-1}]	k_{cat}/K_m [s^{-1} m^{-1}]
UDP-GlcNAc	1.15 ± 0.12	3.2 ± 0.31	46.4 ± 4.8
UDP-GalNAc	0.27 ± 0.03	72.2 ± 6.9	$4.46 \times 10^3 \pm 412$
Toward 4 as an Acceptor[b]			
Acceptor	K_m [mm]	k_{cat} [min^{-1}]	k_{cat}/K_m [s^{-1} m^{-1}]
4	0.42 ± 0.04	86.7 ± 8.5	$3.44 \times 10^3 \pm 347$

[a] With saturating **4** (6 mm) as the acceptor. [b] With saturating UDP-GalNAc (5 mm) as the donor.

Figure S15 in the Supporting Information). UDP-D-GalNAc is also abundant in *Streptomyces*, and normally originates from UDP-D-GlcNAc through UDP-D-GlcNAc 4-epimerase (e.g. SCO3137 and SCO2988 in *S. coelicolor* M145). Consequently, we propose that StnG attaches D-GalNAc to the guanidine of **4** to form **9**, which is then converted into **5** by an epimerase.

Most GTs involved in secondary metabolite biosynthesis belong to the GT-B family,[15] which possess one domain for NDP-sugar binding and another for aglycone recognition. In contrast, the GT-A family enzymes are single-domain proteins,[15] and to our knowledge ValG is the only characterized GT-A fold GT involved in secondary metabolite biosynthesis. While ValG is an O-GT that adds D-glucose to validoxylamine to form validamycin,[16] StnG represents the first reported GT-A fold N-GT involved in secondary metabolite biosynthesis, and furthermore catalyzes the unprecedented attachment of a sugar to the imine nitrogen atom of

guanidine. Considering that the guanidine moiety is widespread in biological molecules such as proteins (e.g. Arg) and nucleic acids (e.g. guanine), we speculate that this unusual N-glycosylation may not be limited to natural products. Intriguingly, glycosylation of Arg residues in proteins has recently been shown to be required for bacterial colonization of enteropathic *E. coli* in a mouse model,[17] but the exact position and mechanism of glycosylation remains unknown. Our finding therefore provides a potential GT-catalyzed route to this post-translational modification.

The *stnJ* gene is the only candidate encoding an epimerase in the *stn* cluster. StnJ is a putative bifunctional protein with its N-terminus showing moderate homology with dehydrogenases (e.g. 20.7% identity with WlbA)[18] and C-terminus showing moderate homology with NAD-dependent epimerases (e.g. 20.2% identity with GalE).[19] The ΔstnJ mutant CIM1009 (see Figure S3D in the Supporting Information) accumulated a metabolite possessing the same HPLC retention time as **9** (Figure 1 and see Figure S16D in the Supporting Information). As expected, the chemical formula of the accumulated compound is $C_{14}H_{23}N_5O_7$, the same as **9**, when analyzed by HR-ESI-MS (m/z 374.1672 for $[M+H]^+$ and 396.1488 for $[M+Na]^+$, calcd 374.1676 and 396.1490, respectively; see Figure S16C in the Supporting Information), which indicates that StnJ catalyzes the epimerization of the 9-OH of **9** to form **5**.

Based on these results, we propose a biosynthetic pathway for the carbamoylated D-GulN moiety of STNs. The unprecedented guanidino-*N*-glycosyltransferase StnG attaches UDP-D-GalNAc to **4** to generate **9**, which is converted into **5** by the putative bifunctional dehydrogenase/epimerase StnJ; StnQ then decorates **5** by adding the carbamoyl group to its 12-OH group to generate **6**; finally, StnI catalyzes the deacetylation of the carbamoylated D-GulNAc moiety to form **3** (Scheme 1C). In the proposed pathway, an unexpected acetylated sugar UDP-D-GalNAc is used as the precursor and the *N*-acetyl group is detached just prior to installation of the poly-β-lysine chain, in a process reminiscent of the protecting group chemistry employed during the chemical synthesis of amino sugars. Interestingly, similar protecting group logic has been used in nature for the biosynthesis of teicoplanin.[20]

Finally, the availability of several new STN intermediates motivated experiments to test the antibiotic activity of **4**, **5**, and **6** against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. No inhibition was observed (see Table S6 in the Supporting Information), thus indicating that the poly-β-lysine chain is indispensible for the antibiotic activity of STNs.

In summary, we have elucidated the biosynthetic mechanism of the carbamoylated D-GulN moiety of STNs. Two novel enzymes, StnG and StnI, were characterized as a GT-A fold GT adding a sugar to the guanidino-imine group and an LmbE-like D-GulNAc deacetylase, respectively.

Keywords: aminoglycoside · biosynthesis · deacetylation · D-gulosamine · glycosylation

[1] a) S. A. Waksman, *J. Bacteriol.* **1943**, *46*, 299–310; b) V. Zbinovsky, W. K. Hausmann, E. R. Wetzel, D. B. Borders, E. L. Patterson, *Appl. Microbiol.* **1968**, *16*, 614–616; c) Y. Kido, T. Furuie, K. Suzuki, K. Sakamoto, Y. Yokoyama, M. Uyeda, J. Kinjyo, S. Yahara, T. Nohara, M. Shibata, *J. Antibiot.* **1987**, *40*, 1698–1706; d) M. Gan, X. Zheng, L. Gan, Y. Guan, X. Hao, Y. Liu, S. Si, Y. Zhang, L. Yu, C. Xiao, *J. Nat. Prod.* **2011**, *74*, 1142–1147.

[2] Z. Ji, M. Wang, J. Zhang, S. Wei, W. Wu, *J. Antibiot.* **2007**, *60*, 739–744.

[3] a) Y. Sawada, S. Nakashima, H. Taniyama, Y. Inamori, *Chem. Pharm. Bull.* **1977**, *25*, 1478–1481; b) T. K. Thiruvengadam, S. J. Gould, D. J. Aberhart, H. J. Lin, *J. Am. Chem. Soc.* **1983**, *105*, 5470–5476; c) M. D. Jackson, S. J. Gould, T. M. Zabriskie, *J. Org. Chem.* **2002**, *67*, 2934–2941.

[4] a) C. Maruyama, J. Toyoda, Y. Kato, M. Izumikawa, H. Takagi, K. Shin-ya, H. Katano, T. Utagawa, Y. Hamano, *Nat. Chem. Biol.* **2012**, *8*, 791–797; b) C. Y. Chang, S. Y. Lyu, Y. C. Liu, N. S. Hsu, C. C. Wu, C. F. Tang, K. H. Lin, J. Y. Ho, C. J. Wu, M. D. Tsai, T. L. Li, *Angew. Chem. Int. Ed.* **2014**, *53*, 1943–1948; *Angew. Chem.* **2014**, *126*, 1974–1979.

[5] V. A. Palaniswamy, S. J. Gould, *J. Chem. Soc.* **1988**, *8*, 2283–2286.

[6] K. Tsuchiya, S. Kobayashi, T. Kurokawa, T. Nakagawa, N. Shimada, H. Nakamura, Y. Iitaka, M. Kitagawa, K. Tatsuta, *J. Antibiot.* **1995**, *48*, 630–634.

[7] J. Li, Z. Guo, W. Huang, X. Meng, G. Ai, G. Tang, Y. Chen, *Sci. China Life Sci.* **2013**, *56*, 619–627.

[8] T. J. Pollock, W. A. van Workum, L. Thorne, M. J. Mikolajczak, M. Yamazaki, J. W. Kijne, R. W. Armentrout, *J. Bacteriol.* **1998**, *180*, 586–593.

[9] a) N. Tarbouriech, S. J. Charnock, G. J. Davies, *J. Mol. Biol.* **2001**, *314*, 655–661; b) L. L. Lairson, B. Henrissat, G. J. Davies, S. G. Withers, *Annu. Rev. Biochem.* **2008**, *77*, 521–555.

[10] M. Kinoshita, Y. Suzuki, *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2375–2378.

[11] C. Parthier, S. Gorlich, F. Jaenecke, C. Breithaupt, U. Brauer, U. Fandrich, D. Clausnitzer, U. F. Wehmeier, C. Bottcher, D. Scheel, M. T. Stubbs, *Angew. Chem. Int. Ed.* **2012**, *51*, 4046–4052; *Angew. Chem.* **2012**, *124*, 4122–4128.

[12] N. Handa, T. Terada, Y. Kamewari, H. Hamana, J. R. Tame, S. Y. Park, K. Kinoshita, M. Ota, H. Nakamura, S. Kuramitsu, M. Shirouzu, S. Yokoyama, *Protein Sci.* **2003**, *12*, 1621–1632.

[13] A. Deli, D. Koutsioulis, V. E. Fadouloglou, P. Spiliotopoulou, S. Balomenou, S. Arnaouteli, M. Tzanodaskalaki, K. Mavromatis, M. Kokkinidis, V. Bouriotis, *FEBS J.* **2010**, *277*, 2740–2753.

[14] C. J. Thibodeaux, C. E. Melancon 3rd, H.-w. Liu, *Angew. Chem. Int. Ed.* **2008**, *47*, 9814–9859; *Angew. Chem.* **2008**, *120*, 9960–10007.

[15] a) Y. Hu, S. Walker, *Chem. Biol.* **2002**, *9*, 1287–1296; b) S. Singh, G. N. Phillips, Jr., J. S. Thorson, *Nat. Prod. Rep.* **2012**, *29*, 1201–1237.

[16] H. Xu, K. Minagawa, L. Bai, Z. Deng, T. Mahmud, *J. Nat. Prod.* **2008**, *71*, 1233–1236.

[17] S. Li, L. Zhang, Q. Yao, L. Li, N. Dong, J. Rong, W. Gao, X. Ding, L. Sun, X. Chen, S. Chen, F. Shao, *Nature* **2013**, *501*, 242–246.

[18] J. B. Thoden, H. M. Holden, *Biochemistry* **2011**, *50*, 1483–1491.

[19] H. Sakuraba, T. Kawai, K. Yoneda, T. Ohshima, *Arch. Biochem. Biophys.* **2011**, *512*, 126–134.

[20] a) J. Y. Ho, Y. T. Huang, C. J. Wu, Y. S. Li, M. D. Tsai, T. L. Li, *J. Am. Chem. Soc.* **2006**, *128*, 13694–13695; b) A. W. Truman, L. Robinson, J. B. Spencer, *ChemBioChem* **2006**, *7*, 1670–1675.