Sulfamate Tethered *Aza*-Wacker Strategy for a Kasugamine Synthon

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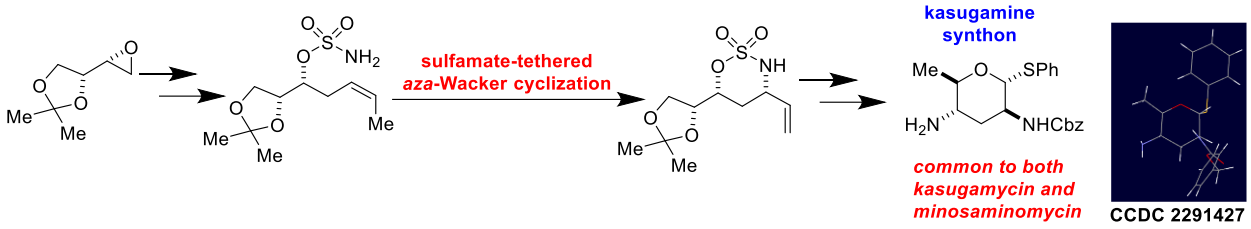
Supporting Information Placeholder



ABSTRACT: We present our preparation of a kasugamine synthon, which proceeds in 14 steps from a literature epoxide. We expect that this kasugamine derivative can be used for the total syntheses of kasugamycin, minosaminomycin, and analogue antibiotics. A key step in the synthesis is our laboratory's sulfamate-tethered *aza*-Wacker cyclization.

Kasugamycin and minosaminomycin are structurally related aminoglycoside antibiotics (**Figure 1**). Kasugamycin was first isolated in 1965 from a bacterial strain (*Streptomyces kasugiensis*) found in a soil sample near the Kasuga shrine in Nara, Japan.[1-3] Shortly after isolation, empirical testing demonstrated that kasugamycin was active against a variety of bacteria and fungi.[4, 5] While its antibacterial activity is much less than that of other members of the aminoglycoside family, its ability to control rice blast disease caused by the fungus *Pyricularia oryzae* was so remarkable that it continues to be used commercially as an agricultural fungicide (Kasumin).[6, 7] Minosaminomycin is a structurally related antibiotic to kasugamycin and has demonstrable activity against a variety of pathogenic bacteria, including mycobacteria.[8, 9]

Our interest in kasugamycin and minosaminomycin stems from both fundamental and applied considerations. These antibiotics have been synthetically unexamined for close to fifty years.[8, 10-16] Our laboratory has a deep interest in the development of new reactions which would enable the syntheses of complex antibiotics. Specifically, we envisioned that our laboratory's sulfamate-tethered *aza*-Wacker cyclization could serve as a key step for the assembly of the kasugamine portion of these antibiotics.[17-24] Our interest in designing practical synthetic routes to antibiotics with new mechanisms of action[25-31] relative to approved agents is motivated by the global rise in resistance to antibacterial and antifungal agents currently in use.[32-34] It is our hope that *de novo* syntheses will allow for the rapid preparation of analogues[35-37] and will serve as a start of a drug-discovery effort to replenish worrisome antibacterial and antifungal pipelines. In this account, we describe our preparation of a kasugamine synthon, a common building block for both kasugamycin and minosaminomycin.

There are two general strategies for the syntheses of unusual monosaccharides.[38] One is the classic "heterocycle→heterocycle" approach; in such syntheses, chemists begin with a more readily available monosaccharide and then systematically manipulate it to the end target. A second, less explored approach is to commence with a linear precursor, build functional group complexity, and then effect a late-stage cyclization ("linear→cyclic"). The former approach generally has fewer total step counts; on balance, due to existing complexity, monosaccharides can be very challenging starting materials, whose modifications generally require lengthy protection-deprotection sequences. For this reason, we prefer the latter approach, because of the greater flexibility it affords and chose kasugamine derivative **A** as our first target (**Scheme 1**).

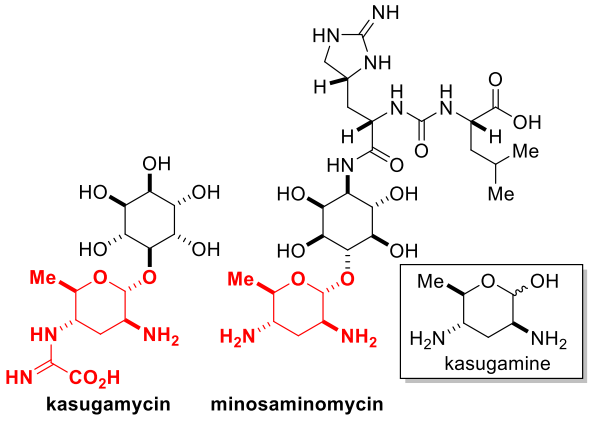


Figure 1. Kasugamycin and minosaminomycin are potent aminoglycoside antibiotics and are structurally related.

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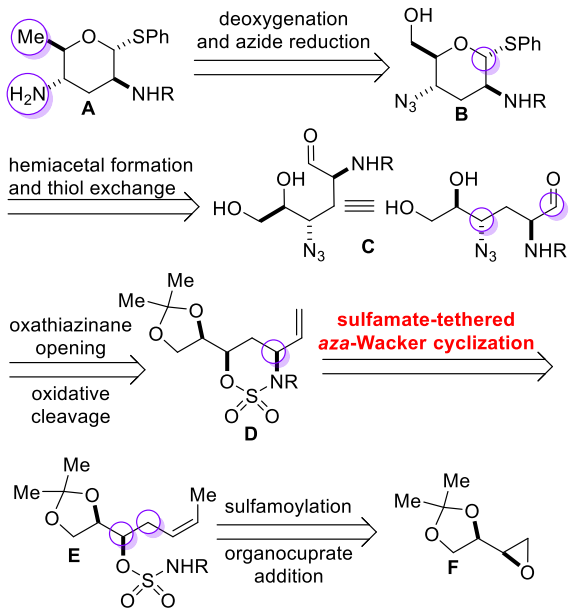
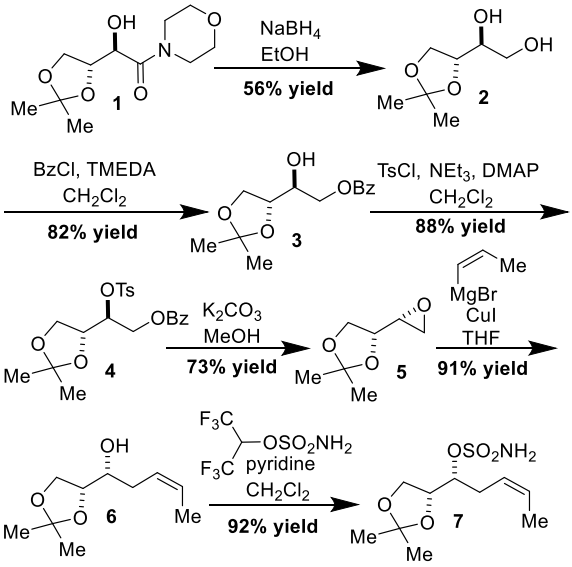


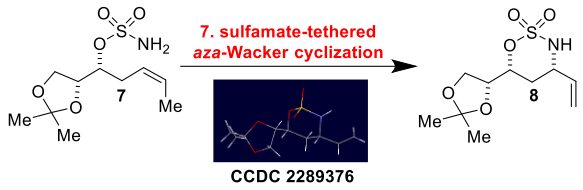
Scheme 1. Retrosynthetic analysis.

In accord with this plan, our retrosynthesis is depicted in **Scheme 1**. After a late stage hemiacetalization of heteroatom-rich linear precursor **C**, deoxygenation and azide reduction would furnish target **A**. **C** would be built systematically from epoxide **F**.[39, 40] We envisioned our laboratory's sulfamate-tethered *aza*-Wacker cyclization[17, 21] would serve as a key step during the transformation of epoxide **F** into aldehyde **C**. The successful execution of this strategy would increase the prominence of both classical and tethered *aza*-Wacker chemistry for the stereoselective assembly of complex targets.[19, 41]



Scheme 2. Opening sequence of reactions.

Our forward synthesis (**Scheme 2**) commenced with morpholine amide **1**, itself prepared in two steps from commercial D-erythrono-1,4-lactone in a procedure developed and disclosed by our laboratory.[17] Morpholine amide **1** was converted into diol **2** by heating with $NaBH_4$ in EtOH. Treatment of diol **2** with benzoyl chloride in ice-cold CH_2Cl_2 allowed for selective esterification of the primary alcohol. The secondary alcohol of benzoate **3** was tosylated using a standard mixture of NEt_3, TsCl, and catalytic DMAP. Treatment of **4** with mixture of K_2CO_3 in MeOH allowed for the hydrolysis of the benzoate ester; the resulting alkoxide anion displaced the tosylate in an intramolecular S_N2 reaction to form epoxide **5**. CuI mediated the regioselective opening of epoxide **5** by *cis*-1-propenyl-magnesium bromide. For this transformation, we have found that it is critical to freshly prepare *cis*-1-propenylmagnesium bromide; for reasons unknown, using commercial solutions of 1-propenylmagnesium bromide did not allow for clean reactivity. Sulfamoylation of alcohol **6** proceeded smoothly with Johnson and Magolan's protocol.[42]

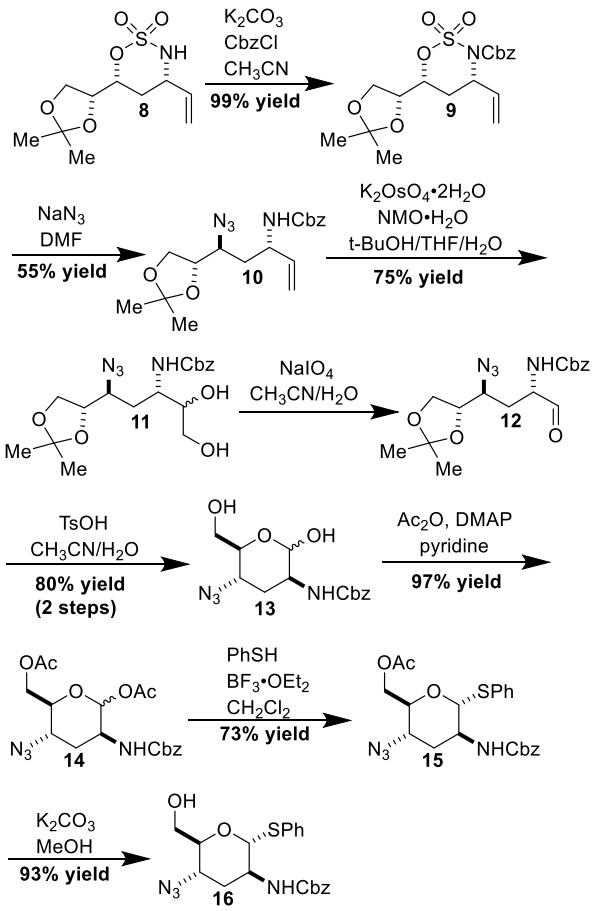


Entry	Catalyst	Additional Parameters	Solvent	Yield
1	$Pd(OAc)_2$ (10%)	$Cu(OAc)_2$ (1 equiv.) O_2 (1 atm.), 60 °C, 16 h	CH_3CN	36%
2	$Pd_2(dba)_3$ (15%)	$Cu(OAc)_2$ (1 equiv.) O_2 (1 atm.), 60 °C, 16 h	CH_3CN	50%
3	$PdCl_2(nbd)$ (15%)	$Cu(OAc)_2$ (1 equiv.) O_2 (1 atm.), 60 °C, 16 h	CH_3CN	30%
4	$Pd(CH_3CN)_2Cl_2$ (15%)	$Cu(OAc)_2$ (1 equiv.) O_2 (1 atm.), 60 °C, 16 h	CH_3CN	41%
5	$Pd(CH_3CN)_2Cl_2$ (15%)	$Cu(OAc)_2$ (1 equiv.) O_2 (1 atm.), 60 °C, 16 h	DMSO	41%
6	$Pd(CH_3CN)_2Cl_2$ (15%)	$Cu(OAc)_2$ (1 equiv.) O_2 (1 atm.) 4Å molecular sieves Fmoc-Gly-OH (1 equiv.) 60 °C, 16 h	DMSO	63%

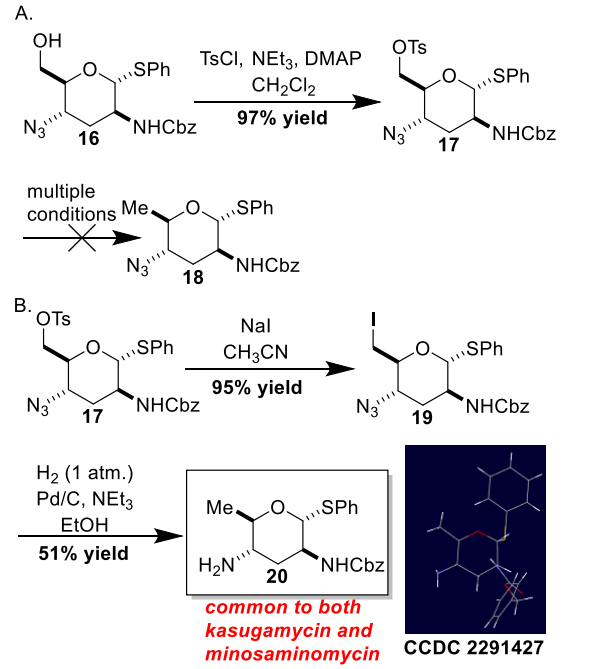
Scheme 3. Optimization of the key sulfamate-tethered *aza*-Wacker cyclization.

As a first step to evaluating the sulfamate-tethered *aza*-Wacker cyclization with **7**, we tested our standard protocol of $Pd(OAc)_2/Cu(OAc)_2$ in hot CH_3CN and a ~1 atm O_2 atmosphere. We were pleased that this delivered desired oxathiazinane **8** in a 36% yield, an excellent starting point for further optimization (**Scheme 3**, **Entry 1**). Switching to $Pd_2(dba)_3$ at a higher loading increased the yield to 50% (**Scheme 3**, **Entry 2**). Using $PdCl_2(nbd)$ and $Pd(CH_3CN)_2Cl_2$ in place of $Pd_2(dba)_3$ did not help the reaction (**Scheme 3**, **Entries 3–5**). We have previously observed a dramatic improvement in sulfamate-tethered *aza*-Wacker cyclizations when mono-protected amino acid ligands and 4Å molecular sieves are used. We hypothesize, in accord with the Yu laboratory's observations,[43-45] that mono-protected amino acids (MPAA) ligands help promote the Pd (II) – Pd (0) catalytic cycles operative during *aza*-Wacker transformations. Indeed, in line with our predictions, the yield of oxathiazinane **8** increased to 63% upon the addition of MPAA ligand Fmoc-Gly-OH and 4Å molecular sieves (**Scheme 3**, **Entry 6**). Despite the presence of molecular sieves, it was critical to use anhydrous DMSO for the best reaction yield. X-ray structural analysis of **8** (**CCDC: 2289376**) allowed us to unambiguously confirm its identity and absolute stereochemistry.

Moving forward, a Cbz group was appended to the oxathiazinane nitrogen for a subsequent ring-opening with NaN_3 (**Scheme 4**).[46, 47] The alkene of **10** was cleaved by dihydroxylation followed by treatment with $NaIO_4$. Aldehyde **12** was unstable to purification and was immediately treated with TsOH to form hemiacetal **13** as a mixture of anomers. This anomeric mixture was bis-acetylated with Ac_2O/pyridine, and the anomeric acetate was exchanged with thiophenol using $BF_3{\bullet}OEt_2$.



Scheme 4. Towards the endgame.



Scheme 5. (A) Displacement of the tosylate by hydride does not give expected product under multiple conditions. (B) Upon treatment with Pd/C and H_2 gas, deiodination and azide reduction occur in one step to furnish the desired target.

For **15**, we have assigned the configuration at the anomeric carbon based on the small coupling constant of the anomeric proton (~1.5 Hz) and by analogy to the crystal structure of a later intermediate (*vide infra*). The acetate of **15** was removed using K_2CO_3 in MeOH.

Initially, it was our plan to tosylate the alcohol of **16** and to displace it with an appropriate hydride source (**Scheme 5A**). Accordingly, tosylate **17** was prepared by stirring **16** with NEt_3 and TsCl. However, despite multiple trials, including with $NaBH_4$, $LiAlH_4$, and $NaBH_3CN$, we were unable to cleanly obtain **18**, likely because of the presence of an azide functionality. We thus sought to develop a protocol which would allow for the clean formation of the methyl group and the primary amine of kasugamine in a single step. Knowing that there was precedent for the Pd-catalyzed hydrogenolysis of azides and alkyl iodides,[48] we prepared iodide **19** using a Finkelstein reaction (**Scheme 5B**). Indeed, stirring **19** with NEt_3 and Pd-C under an atmosphere of H_2 gas cleanly delivered kasugamine derivative **20**. Crystal structure analysis of **20** (**CCDC 2291427**) allowed us to unambiguously confirm its identity and absolute stereochemistry.

In summary, we have completed a concise synthesis of a kasugamine synthon, which we envision can be used in preparations of kasugamycin, minosaminomycin, and related analogues. A highlight of the synthesis is our sulfamate-tethered *aza*-Wacker cyclization. The march towards total syntheses of these antibiotics continues in our laboratory.

ASSOCIATED CONTENT

Data Availability Statement: The data underlying this study are available in the published article and its Supporting Information.
Supporting information: Contains additional experimental details including NMR Spectra and X-ray Crystallographic Information.

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REFERENCES

1. Umezawa, H.; Hamada, M.; Suhara, Y.; Hashimoto, T.; Ikekawa, T., Kasugamycin, a new antibiotic. *Antimicrob. Agents Chemother.* **1965,** *5*, 753-757.
2. Umezawa, H.; Okami, Y.; Hashimoto, T.; Suhara, Y.; Hamada, M.; Takeuchi, T., A New Antibiotic, Kasugamycin. *J. Antibiot., Ser. A* **1965,** *18*, 101-103.
3. Suhara, Y.; Maeda, K.; Umezawa, H.; Ohno, M., Kasugamycin. In *Deoxy Sugars*, American Chemical Society **1968**; Vol. 74, pp 15-40.
4. Hamada, M.; Hashimoto, T.; Takahashi, T.; Yokoyama, S.; Miyake, M.; Takeuchi, T.; Okami, Y.; Umezawa, H., Antimicrobial Activity of Kasugamycin. *J. Antibiot. Ser. A* **1965,** *18*, 104-106.
5. Takeuchi, T.; Ishizuka, M.; Takayama, H.; Kureha, K.; Hamada, M.; Umezawa, H., Pharmacology Of Kasugamycin And The Effect On Pseudomonas Infection. *J. Antibiot. (Tokyo)* **1965,** *18*, 107-110.
6. Ishiyama, T.; Hara, I.; Matsuoka, M.; Satō, K.; Shimada, S.; Izawa, R.; Hashimoto, T.; Hamada, M.; Okami, Y.; Takeuchi, T.;

Umezawa, H., Studies on the Preventive Effect of Kasugamycin on Rice Blast. *J. Antibiot. Ser. A* **1965,** *18*, 115-119.
7. Yoshii, A.; Moriyama, H.; Fukuhara, T., The Novel Kasugamycin 2′-N-Acetyltransferase Gene aac(2′)-IIa, Carried by the IncP Island, Confers Kasugamycin Resistance to Rice-Pathogenic Bacteria. *Appl. Environ. Microbiol.* **2012,** *78*, 5555-5564.
8. Katsuharu, I.; Shinichi, K.; Kenji, M.; Hamao, U., Total Synthesis of Minosaminomycin. *Bull. Chem. Soc. Jpn.* **1977,** *50*, 1850-1854.
9. Hamada, M.; Kondo, S.; Yokoyama, T.; Miura, K.; Iinuma, K.; Yamamoto, H.; Maeda, K.; Takeuchi, T.; Umezawa, H., Minosaminomycin, A New Antibiotic Containing Myo-Inosamine. *J. Antibiot. (Tokyo)* **1974,** *27*, 81-83.
10. Suhara, Y.; Sasaki, F.; Maeda, K.; Umezawa, H.; Ohno, M., The total synthesis of kasugamycin. *J. Am. Chem. Soc.* **1968,** *90*, 6559-6560.
11. Suhara, Y.; Sasaki, F.; Koyama, G.; Maeda, K.; Umezawa, H.; Ohno, M., Total synthesis of kasugamycin. *J. Am. Chem. Soc.* **1972,** *94*, 6501-6507.
12. Nii, Y.; Okano, K.; Kobayashi, S.; Ohno, M., Synthesis of amidinoformic acids using benzyl cyanoformate as a synthon. *Tetrahedron Lett.* **1979,** *20*, 2517-2520.
13. Juji, Y.; Ken-ichi, S.; Hironobu, H.; Kuniaki, S., Aminosugars. XXVIII. A Facile Synthesis of Benzyl α- and β-Kasugaminides via the Corresponding Abequosides. *Bull. Chem. Soc. Jpn.* **1977,** *50*, 3305-3309.
14. Hanessian, S.; Masse, R., Synthetic approaches to kasugamine. *Carbohydr. Res.* **1974,** *35*, 175-185.
15. Yasuda, S.; Ogasawara, T.; Kawabata, S.; Iwataki, I.; Matsumoto, T., The synthesis of methyl N,N′-diacetyl-α-d-kasugaminide. *Tetrahedron* **1973,** *29*, 3141-3147.
16. Suhara, Y.; Maeda, K.; Umezawa, H.; Ohno, M., Chemical studies on kasugamycin. V. The structure of kasugamycin. *Tetrahedron Lett.* **1966,** *7*, 1239-1244.
17. Paul, D.; Mague, J. T.; Sathyamoorthi, S., Sulfamate-Tethered Aza-Wacker Cyclization Strategy for the Syntheses of 2-Amino-2-deoxyhexoses: Preparation of Orthogonally Protected d-Galactosamines. *J. Org. Chem.* **2023,** *88*, 1445-1456.
18. Nagamalla, S.; Johnson, D. K.; Sathyamoorthi, S., Sulfamate-tethered aza-Wacker approach towards analogs of Bactobolin A. *Med. Chem. Res.* **2021,** *30*, 1348-1357.
19. Thomas, A. A.; Nagamalla, S.; Sathyamoorthi, S., Salient features of the aza-Wacker cyclization reaction. *Chem. Sci.* **2020,** *11*, 8073-8088.
20. Nagamalla, S.; Mague, J. T.; Sathyamoorthi, S., Progress towards the syntheses of Bactobolin A and C4-epi-Bactobolin A using a sulfamate-tethered aza-Wacker cyclization strategy. *Tetrahedron* **2022**, 133112.
21. Shinde, A. H.; Sathyamoorthi, S., Oxidative Cyclization of Sulfamates onto Pendant Alkenes. *Org. Lett.* **2020,** *22*, 896-901.
22. Shinde, A. H.; Sathyamoorthi, S., Large Scale Oxidative Cyclization of (E)-hex-3-en-1-yl (4-methoxyphenyl)sulfamate. *Org. Synth.* **2022,** *99*, 286-304.
23. Joshi, H.; Sathyamoorthi, S., Hydroxyselenylation and Tethered Silanoxyselenylation of Allylic Silanols. *J. Org. Chem.* **2022,** *87*, 5017-5028.
24. Shinde, A. H.; Thomas, A. A.; Mague, J. T.; Sathyamoorthi, S., Highly Regio- and Diastereoselective Tethered Aza-Wacker Cyclizations of Alkenyl Phosphoramidates. *J. Org. Chem.* **2021,** *86*, 14732-14758.
25. Tanaka, N.; Yamaguchi, H.; Umezawa, H., Mechanism of Kasugamycin Action on Polypeptide Synthesis. *J. Biochem.* **1966,** *60*, 429-434.
26. Schluenzen, F.; Takemoto, C.; Wilson, D. N.; Kaminishi, T.; Harms, J. M.; Hanawa-Suetsugu, K.; Szaflarski, W.; Kawazoe, M.; Shirouzu, M.; Nierhaus, K. H.; Yokoyama, S.; Fucini, P., The antibiotic kasugamycin mimics mRNA nucleotides to destabilize tRNA binding and inhibit canonical translation initiation. *Nat. Struct. Mol. Biol.* **2006,** *13*, 871-878.
27. Tanaka, N.; Yoshida, Y.; Sashikata, K.; Yamaguchi, H.; Umezawa, H., Inhibition of polypeptide synthesis by kasugamycin, an aminoglycosidic antibiotic. *J. Antibiot. (Tokyo)* **1966,** *19*, 65-68.
28. Zhang, Y.; Aleksashin, N. A.; Klepacki, D.; Anderson, C.; Vázquez-Laslop, N.; Gross, C. A.; Mankin, A. S., The context of the ribosome binding site in mRNAs defines specificity of action of kasugamycin, an inhibitor of translation initiation. *Proc. Natl. Acad. Sci. USA* **2022,** *119*, e2118553119.
29. Poldermans, B.; Goosen, N.; Van Knippenberg, P. H., Studies on the function of two adjacent N6,N6-dimethyladenosines near the 3' end of 16 S ribosomal RNA of Escherichia coli. I. The effect of kasugamycin on initiation of protein synthesis. *J. Biol. Chem.* **1979,** *254*, 9085-9089.
30. van Buul, C. P. J. J.; Visser, W.; van Knippenberg, P. H., Increased translational fidelity caused by the antibiotic kasugamycin and ribosomal ambiguity in mutants harbouring the ksgA gene. *FEBS Lett.* **1984,** *177*, 119-124.
31. Okuyama, A.; Machiyama, N.; Kinoshita, T.; Tanaka, N., Inhibition by kasugamycin of initiation complex formation on 30S ribosomes. *Biochem. Biophys. Res. Commun.* **1971,** *43*, 196-199.
32. Ventola, C. L., The antibiotic resistance crisis: part 1: causes and threats. *P T* **2015,** *40*, 277-83.
33. Lucas, J. A.; Hawkins, N. J.; Fraaije, B. A., Chapter Two - The Evolution of Fungicide Resistance. In *Advances in Applied Microbiology*, Sariaslani, S.; Gadd, G. M., Eds. Academic Press2015; Vol. 90, pp 29-92.
34. Vitiello, A.; Ferrara, F.; Boccellino, M.; Ponzo, A.; Cimmino, C.; Comberiati, E.; Zovi, A.; Clemente, S.; Sabbatucci, M., Antifungal Drug Resistance: An Emergent Health Threat. *Biomedicines* **2023,** *11*, 1063.
35. Wu, Z.-C.; Boger, D. L., Maxamycins: Durable Antibiotics Derived by Rational Redesign of Vancomycin. *Acc. Chem. Res.* **2020,** *53*, 2587-2599.
36. Wright, P. M.; Seiple, I. B.; Myers, A. G., The Evolving Role of Chemical Synthesis in Antibacterial Drug Discovery. *Angew. Chem. Int. Ed.* **2014,** *53*, 8840-8869.
37. Privalsky, T. M.; Soohoo, A. M.; Wang, J.; Walsh, C. T.; Wright, G. D.; Gordon, E. M.; Gray, N. S.; Khosla, C., Prospects for Antibacterial Discovery and Development. *J. Am. Chem. Soc.* **2021,** *143*, 21127-21142.
38. Skarbek, K.; Milewska, M. J., Biosynthetic and synthetic access to amino sugars. *Carbohydr. Res.* **2016,** *434*, 44-71.
39. White, J. D.; Badger, R. A.; Kezar, H. S.; Pallenberg, A. J.; Schiehser, G. A., Structure, Sythesis and Absolute Configuration of Leptosphaerin, a Metabolite of the Marine Ascomycete Leptosphaeria oraemaris. *Tetrahedron* **1989,** *45*, 6631-6644.
40. Vargeese, C.; Abushanab, E., Chemistry of L-ascorbic and D-isoascorbic acids. 4. An efficient synthesis of 2-deoxypentofuranoses. *J. Org. Chem.* **1990,** *55*, 4400-4403.
41. Weinstein, A. B.; Schuman, D. P.; Tan, Z. X.; Stahl, S. S., Synthesis of Vicinal Aminoalcohols by Stereoselective Aza-Wacker Cyclizations: Access to (−)-Acosamine by Redox Relay. *Angew. Chem. Int. Ed.* **2013,** *52*, 11867-11870.
42. Sguazzin, M. A.; Johnson, J. W.; Magolan, J., Hexafluoroisopropyl Sulfamate: A Useful Reagent for the Synthesis of Sulfamates and Sulfamides. *Org. Lett.* **2021,** *23*, 3373-3378.
43. Shao, Q.; Wu, K.; Zhuang, Z.; Qian, S.; Yu, J.-Q., From Pd(OAc)2 to Chiral Catalysts: The Discovery and Development of Bifunctional Mono-N-Protected Amino Acid Ligands for Diverse C–H Functionalization Reactions. *Acc. Chem. Res.* **2020,** *53*, 833-851.
44. Engle, K. M.; Wang, D.-H.; Yu, J.-Q., Ligand-Accelerated C−H Activation Reactions: Evidence for a Switch of Mechanism. *J. Am. Chem. Soc.* **2010,** *132*, 14137-14151.
45. Shi, B.-F.; Maugel, N.; Zhang, Y.-H.; Yu, J.-Q., PdII-Catalyzed Enantioselective Activation of C(sp2)-H and C(sp3)-H Bonds Using Monoprotected Amino Acids as Chiral Ligands. *Angew. Chem. Int. Ed.* **2008,** *47*, 4882-4886.
46. Espino, C. G.; Wehn, P. M.; Chow, J.; Du Bois, J., Synthesis of 1,3-Difunctionalized Amine Derivatives through Selective C−H Bond Oxidation. *J. Am. Chem. Soc.* **2001,** *123*, 6935-6936.
47. Paradine, S. M.; Griffin, J. R.; Zhao, J.; Petronico, A. L.; Miller, S. M.; Christina White, M., A manganese catalyst for highly reactive yet chemoselective intramolecular C(sp3)–H amination. *Nat. Chem.* **2015,** *7*, 987-994.
48. Weerapana, E.; Glover, K. J.; Chen, M. M.; Imperiali, B., Investigating Bacterial N-Linked Glycosylation: Synthesis and Glycosyl Acceptor Activity of the Undecaprenyl Pyrophosphate-Linked Bacillosamine. *J. Am. Chem. Soc.* **2005,** *127*, 13766-13767.