Enantioselective Total Syntheses of (+)-Kasugamycin and (+)-Kasuganobiosamine Highlighting a Sulfamate-Tethered *Aza*-Wacker Cyclization Strategy

Gour Hari Mandal,[a] Steven P. Kelley,[b] and Shyam Sathyamoorthi[a,*]

[a]Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66047, United States

[b] Department of Chemistry, University of Missouri—Columbia, Columbia, Missouri 65211, United States

ABSTRACT: Here, we present the first enantioselective total syntheses of the natural products (+)-kasugamycin, a potent antifungal antibiotic, and (+)-kasuganobiosamine, a compound that results from kasugamycin degradation. Salient features of these syntheses include a second-generation enantioselective preparation of a kasugamine derivative (much improved in efficiency relative to our first chiral-pool effort) and our laboratory's sulfamate-tethered *aza*-Wacker cyclization.

In 1965, Umezawa and co-workers isolated an aminoglycoside antibiotic from the bacteria *Streptomyces kasugiensis*; because this bacterial strain was found in a soil sample near the Kasuga shrine in Nara, Japan, they named the natural product kasugamycin (**Figure 1**).[1-4] Remarkably, unlike many other aminoglycosides, which are potent antibacterial antibiotics, kasugamycin only exhibited weak antibacterial activity.[5-8] However, it was found to be a potent antifungal agent and continues to be agriculturally used to control rice blast disease.[9, 10] Kasugamycin is quite sensitive to heat and light exposure, and, in the environment, rapidly degrades into a related compound named kasuganobiosamine.[11] As a target for total synthesis, kasugamycin has lain dormant for more than 50 years.[12-14] Our laboratory has a deep interest in the development of new reactions to simplify the syntheses of structurally complex, anti-infective molecules.[15-17] We envision such syntheses will serve as foundations for future function-oriented[18] medicinal chemistry efforts. Thus, we chose kasugamycin as a worthy target both for its challenging, heteroatom adorned framework and for its potent antifungal activity. Here, we report the first enantioselective total syntheses of (+)-kasugamycin and (+)-kasuganobiosamine.

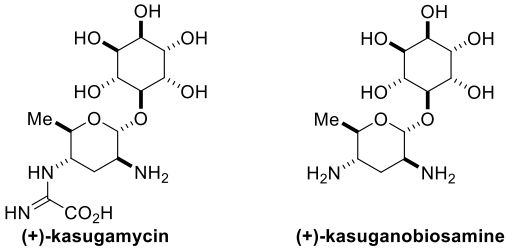
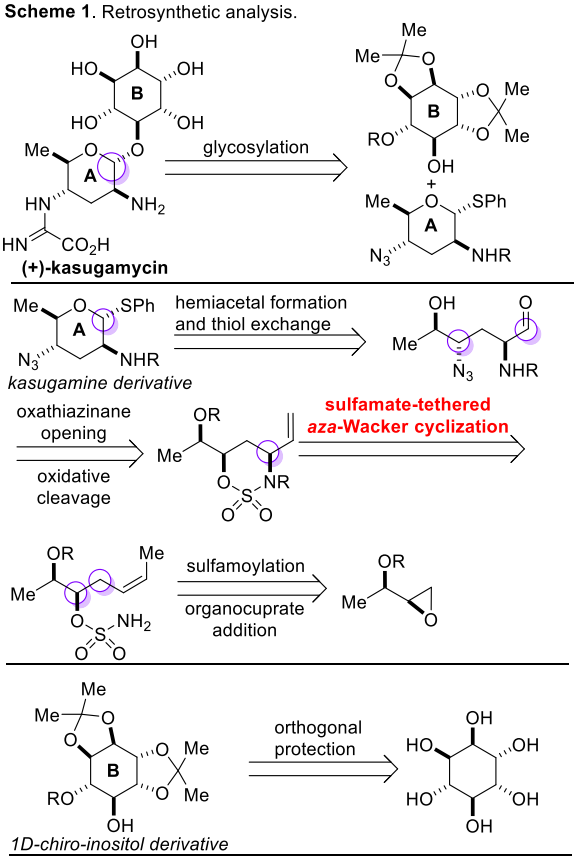


Figure 1. Kasugamycin is an aminoglycoside natural product with potent antifungal activity and modest antibacterial activity. In soil, kasuganobiosamine is one of kasugamycin's major degradation products.

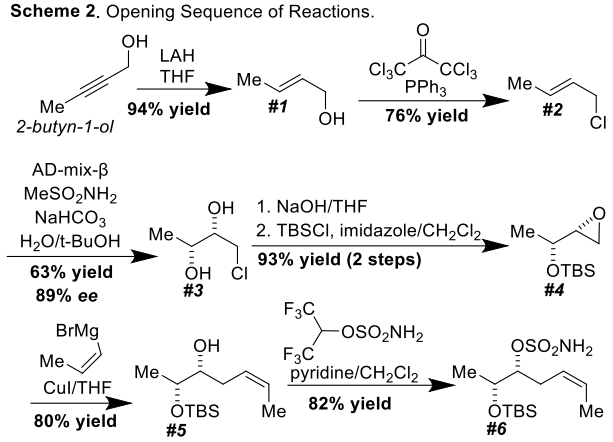
(+)-Kasugamycin is an aminoglycoside, composed of the *aza*-monosaccharide kasugamine (ring A) attached to 1D-*chiro*-inositol (ring B).[4] We planned to synthesize protected versions of each of these components and conjoin them with a late-stage glycosylation (**Scheme 1**). Following glycosylation, attachment of the amidinoformic acid moiety and global deprotection would deliver (+)-kasugamycin. Kasugamine would be systematically synthesized from a chiral epoxide synthon in a sequence highlighting our laboratory's sulfamate-tethered *aza*-Wacker cyclization.[19-22] 1D-*chiro*-inositol is commercially available but would have to be protected prior to glycosylation.

Scheme 1. Retrosynthetic analysis.



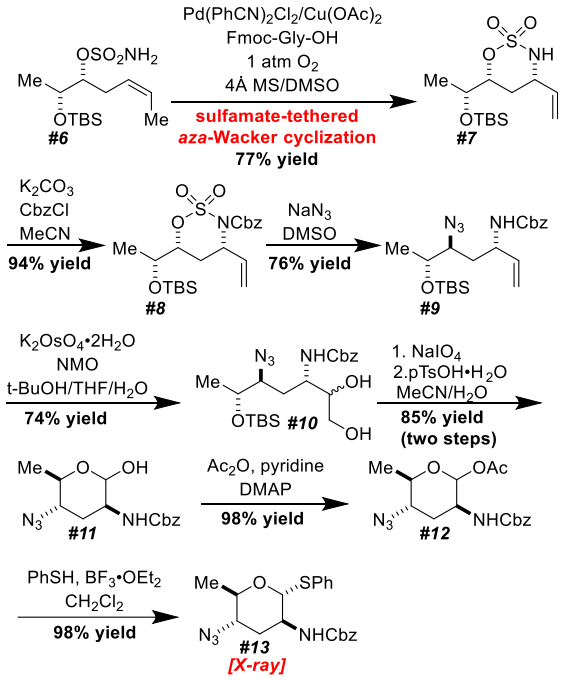
https://doi.org/10.26434/chemrxiv-2024-5hxvv **ORCID:** https://orcid.org/0000-0003-4705-7349

Scheme 2. Opening Sequence of Reactions.



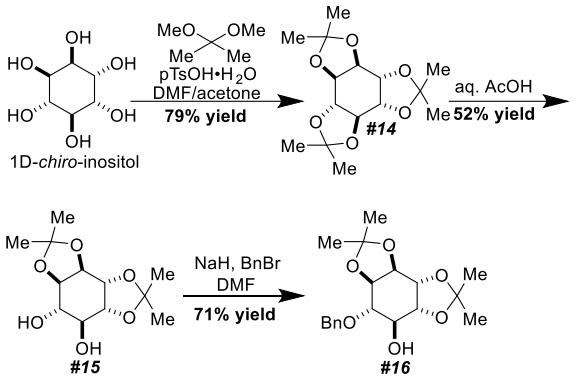
Our synthesis of (+)-kasugamycin commenced with commercially available 2-butyn-1-ol, which was reduced into (E)-2-buten-1-ol (**1**) using lithium aluminum hydride (**Scheme 2**).[23] The corresponding chloride was prepared using triphenylphosphine and hexachloroacetone (modified Appel conditions).[24] Sharpless dihydroxylation gave chloro-diol **3** in a 63% yield and 89% *ee*.[25,26] Treatment of **3** with NaOH gave an epoxy-alcohol which was converted into the corresponding TBS ether prior to purification.[27] This epoxide was regioselectively opened using *cis*-1-propenylmagnesium bromide and CuI. Resulting alcohol **5** was sulfamoylated using conditions developed by Sguazzin, Johnson, and Magolan.[28]

Scheme 3. Completion of the A ring (kasugamine).



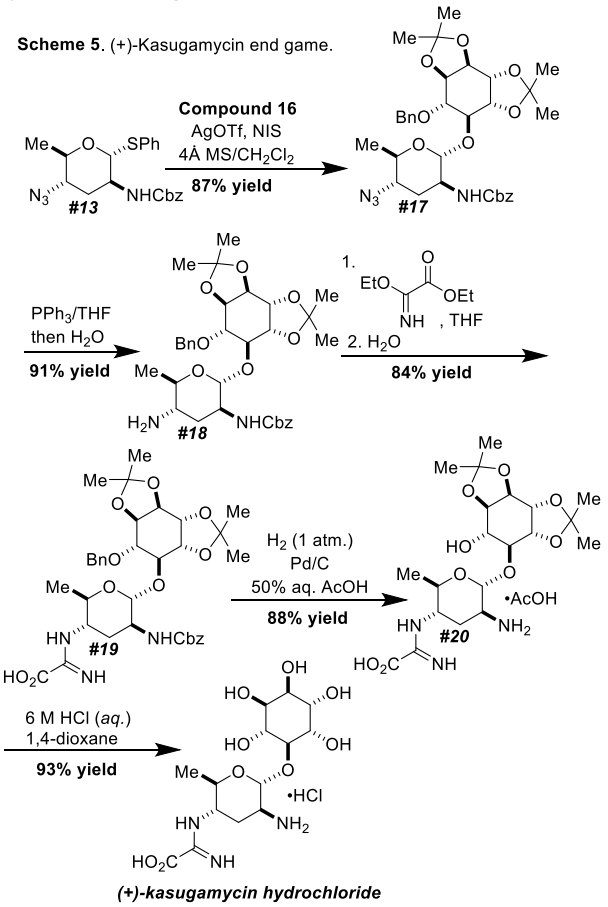
Sulfamate **6** was subjected to our laboratory's *aza*-Wacker cyclization reaction,[29] giving oxathiazinane **7** in an excellent yield and as a single diastereomer (within limits of ^{1}H NMR detection) (**Scheme 3**). **7** was activated by appending a Cbz group and ring-opened using NaN_3.[30] **9** was unstable in acidic NMR solvents but was readily di-hydroxylated using K_2OsO_4•$2H_2O$ and NMO. The diol was cleaved using $NaIO_4$. In one-pot, the TBS group was removed using pTsOH and cyclization of the resulting aldose gave kasugamine derivative **11** as a mixture of anomers. The anomeric OH group was converted into the corresponding acetate using Ac_2O/pyridine/DMAP. The acetate was exchanged with thiophenol using BF_3•OEt_2. Enantiopure thioglycoside **13** crystallized along with its racemate, consistent with the observed % *ee* for **3**, and both were confirmed by X-ray diffraction analysis (**CCDC 2335847** and **CCDC 2353400**). We note that this is enantioselective synthesis of a kasugamine derivative is much improved (10 steps) relative to our first chiral-pool attempt (~17 steps from a literature compound).[29]

Scheme 4. B ring derivative synthesis.



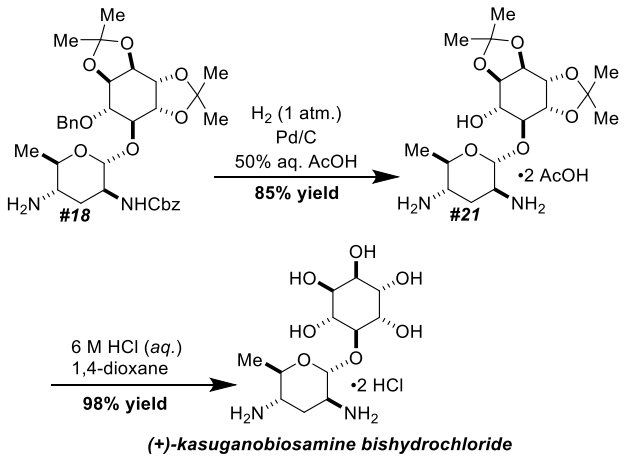
Commercially available 1D-*chiro*-inositol was converted into its *tris*-acetonide derivative using 2,2-dimethoxypropane and catalytic pTsOH (**Scheme 4**).[31] One of these acetonides was selectively removed using aqueous acetic acid to give C_2-symmetric diol **15**.[31] **15** was mono-benzylated using NaH and BnBr in DMF.

Scheme 5. (+)-Kasugamycin end game.



Glycosylative coupling of **13** and **16** proceeded smoothly using AgOTf and NIS (**Scheme 5**).[32] We had previously envisioned a Schmidt reaction between the trichloroacetimidate derivative of **11** and **16**; while we were able to synthesize the trichloroacetimidate of **11**, the product was very unstable and difficult to handle. The azide of **17** was converted into the corresponding amine using a Staudinger reduction. **18** was reacted with commercially available ethyl 2-ethoxy-2-iminoacetate in THF.[33] We observed that the resulting amidine ethyl carboxylate was highly unstable and was partially hydrolyzing during the attachment step, affording a mixture of products which was difficult to separate. Quenching the reaction with H_2O and stirring for a few hours gave desired amidine carboxylic acid **19** as a single product in a two-step, one-pot protocol. The Cbz and benzyl groups were removed by hydrogenolysis; here, using a 1:1 mixture of glacial acetic acid and H_2O as the solvent was far superior to earlier attempts with MeOH. The acetonides were cleaved with 6 M aqueous HCl in 1,4-dioxane. The synthetic (+)-kasugamycin hydrochloride salt was found to be identical with a commercial sample in all respects (see Supporting Information for full details).

Scheme 6. Preparation of (+)-kasuganobiosamine.



(+)-kasuganobiosamine bishydrochloride

18 was a convenient intermediate for the completion of (+)-kasuganobiosamine (**Scheme 6**). Hydrogenolysis removed the Cbz and benzyl groups. Acetonide cleavage using 6 M aqueous HCl in 1,4-dioxane delivered (+)-kasuganobiosamine as its *bis*-hydrochloride salt.

In summary, we have completed the first enantioselective total syntheses of (+)-kasugamycin and (+)-kasuganobiosamine. The synthesis of (+)-kasugamycin proceeds in a longest linear sequence of 15 steps from a known epoxide and with an approximate yield of 12%. An intermediate in this sequence was used for the preparation of (+)-kasuganobiosamine. A highlight of our syntheses is our laboratory's sulfamate tethered *aza*-Wacker reaction. This work underscores the power of tethered *aza*-Wacker chemistry for complex nitrogenous molecule assembly.

ASSOCIATED CONTENT

Supporting Information.
Additional experimental details including reaction procedures, X-ray crystallographic data, and NMR spectra.

AUTHOR INFORMATION

Corresponding Author

*ssathyam@ku.edu

ACKNOWLEDGMENT

This work was supported by National Institutes of Health grant R35GM142499 and a CMADP pilot project grant (P30GM145499) awarded to Shyam Sathyamoorthi. Justin Douglas and Sarah Neuenswander (KU NMR Lab) are acknowledged for help with structural elucidation. Lawrence Seib and Anita Saraf (KU Mass Spectrometry Facility) are acknowledged for help acquiring HRMS data.

REFERENCES

1. Umezawa, H.; Okami, Y.; Hashimoto, T.; Suhara, Y.; Hamada, M.; Takeuchi, T., A New Antibiotic, Kasugamycin. *J. Antibiot., Ser. A* **1965,** *18*, 101-103.
2. Umezawa, H.; Hamada, M.; Suhara, Y.; Hashimoto, T.; Ikekawa, T., Kasugamycin, a new antibiotic. *Antimicrob. Agents Chemother.* **1965,** *5*, 753-757.
3. Suhara, Y.; Maeda, K.; Umezawa, H.; Ohno, M., Kasugamycin. In *Deoxy Sugars*, American Chemical Society **1968**; Vol. 74, pp 15-40.
4. Suhara, Y.; Maeda, K.; Umezawa, H.; Ohno, M., Chemical studies on kasugamycin. V. The structure of kasugamycin. *Tetrahedron Lett.* **1966,** *7*, 1239-1244.
5. 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.
6. 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.*
7. 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 Letters* **1984,** *177*, 119-124.
8. 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.
9. 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.
10. 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.
11. Nakajima, M.; Shibata, H.; Kitahara, K.; Takahashi, S.; Hasegawa, A., Synthesis of kasuganobiosamine. *Tetrahedron Lett.* **1968,** *9*, 2271-2274.
12. Suhara, Y.; Sasaki, F.; Maeda, K.; Umezawa, H.; Ohno, M., The total synthesis of kasugamycin. *J. Am. Chem. Soc.* **1968,** *90*, 6559-6560.
13. Suhara, Y.; Sasaki, F.; Koyama, G.; Maeda, K.; Umezawa, H.; Ohno, M., Total synthesis of kasugamycin. *J. Am. Chem. Soc.* **1972,** *94*, 6501-6507.
14. Hanessian, S.; Masse, R., Synthetic approaches to kasugamine. *Carbohydr. Res.* **1974,** *35*, 175-185.
15. Sathyamoorthi, S., Fun With Unusual Functional Groups: Sulfamates, Phosphoramidates, and Di-tert-butyl Silanols. *Eur. J. Org. Chem.* **2024,** *27*, e202301283.

16. Nagamalla, S.; Johnson, D. K.; Sathyamoorthi, S., Sulfamate-tethered *aza*-Wacker approach towards analogs of Bactobolin A. *Med. Chem. Res.* **2021,** *30*, 1348-1357.
17. 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.
18. Wender, P. A.; Verma, V. A.; Paxton, T. J.; Pillow, T. H., Function-Oriented Synthesis, Step Economy, and Drug Design. *Acc. Chem. Res.* **2008,** *41*, 40-49.
19. Thomas, A. A.; Nagamalla, S.; Sathyamoorthi, S., Salient features of the *aza*-Wacker cyclization reaction. *Chem. Sci.* **2020,** *11*, 8073-8088.
20. Shinde, A. H.; Sathyamoorthi, S., Oxidative Cyclization of Sulfamates onto Pendant Alkenes. *Org. Lett.* **2020,** *22*, 896-901.
21. 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.
22. 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.
23. Denmark, S. E.; Harmata, M. A.; White, K. S., Studies on the addition of allyl oxides to sulfonylallenes. Preparation of highly substituted allyl vinyl ethers for carbanionic Claisen rearrangements. *J. Org. Chem.* **1987,** *52*, 4031-4042.
24. Suen, L. M.; Steigerwald, M. L.; Leighton, J. L., A new and more powerfully activating diamine for practical and scalable enantioselective aldehyde crotylsilylation reactions. *Chem. Sci.* **2013,** *4*, 2413-2417.
25. Vanhessche, K. P. M.; Wang, Z.-M.; Sharpless, K. B., Asymmetric dihydroxylation of primary allylic halides and a concise synthesis of (−)-diepoxybutane. *Tetrahedron Lett.* **1994,** *35*, 3469-3472.
26. Baldauf, S.; Schauenburg, D.; Bode, J. W., A Threonine-Forming Oxazetidine Amino Acid for the Chemical Synthesis of Proteins through KAHA Ligation. *Angew. Chem. Int. Ed.* **2019,** *58*, 12599-12603.
27. Li, X.; Manjunatha, U. H.; Goodwin, M. B.; Knox, J. E.; Lipinski, C. A.; Keller, T. H.; Barry, C. E.; Dowd, C. S., Synthesis and antitubercular activity of 7-(R)- and 7-(S)-methyl-2-nitro-6-(S)-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazines, analogues of PA-824. *Bioorg. Med. Chem. Lett.* **2008,** *18*, 2256-2262.
28. 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.
29. Mandal, G. H.; Sathyamoorthi, S., Sulfamate-Tethered *Aza*-Wacker Strategy for a Kasugamine Synthon. *J. Org. Chem.* **2024,** *89*, 793-797.
30. 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.
31. Cousins, G.; Falshaw, A.; Hoberg, J. O., Monoesterification of di-O-isopropylidene and di-O-cyclohexylidene chiro-inositols. *Carbohydr. Res.* **2003,** *338*, 995-998.
32. Mendlik, M. T.; Tao, P.; Hadad, C. M.; Coleman, R. S.; Lowary, T. L., Synthesis of l-Daunosamine and l-Ristosamine Glycosides via Photoinduced Aziridination. Conversion to Thioglycosides for Use in Glycosylation Reactions. *J. Org. Chem.* **2006,** *71*, 8059-8070.
33. Gómez, E.; Avendaño, C.; McKillop, A., Ethyl carboethoxyformimidate in heterocyclic chemistry. *Tetrahedron* **1986,** *42*, 2625-2634.

TOC Graphic