Synthesis of (+)-ribostamycin by catalytic, enantioselective hydroammoniation of benzene

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Aminoglycosides (AGs) represent a large group of pseudoglycoside natural products in which several different sugar moieties are harnessed to an aminocyclitol core. AGs constitute a major class of antibiotics that target the prokaryotic ribosome of many problematic pathogens. Hundreds of AGs have been isolated to date, with 1,3-diaminocyclohexanetriol, known as 2-deoxystreptamine (2-DOS), being the most abundant aglycon core. However, due to their diverse and complex architectures, all AG-based drugs are either natural substances or analogues prepared by late-stage modifications. Synthetic approaches to AGs are rare and lengthy; most studies involve semisynthetic reunion of modified fragments. Here we report a bottom-up chemical synthesis of the 2-DOS-based AG antibiotic ribostamycin, which proceeds in ten linear operations from benzene. A key enabling transformation involves a copper-catalysed, enantioselective, deaminative hydroamination, which sets the stage for the rapid and selective introduction of the remaining 2-DOS heteroatom functionality. This work demonstrates how the combination of a tailored, deaminative logic and strategic use of subsequent olefin functionalizations can provide practical and concise access to the AG class of compounds.

Since their initial discovery in 1943¹, aminoglycosides (AGs) have become an important class of carbohydrate-derived antibiotics obtained predominantly from actinomycetes, and several have been registered on the World Health Organization’s List of Essential Medicines². The most extensively investigated and clinically employed AGs contain a central 1,3-diaminocyclohexanetriol scaffold (Fig. 1a), known as 2-deoxystreptamine (2-DOS, 1), which is adorned with a variety of sugars, as exemplified by ribostamycin (2), neomycin B (3) and sisomicin (4). Their broad-spectrum antibiotic activity derives from the suppression of protein synthesis by binding to prokaryotic rRNA⁷–⁸, and they are particularly useful against several important Gram-negative bacteria and Mycobacterium tuberculosis. Despite their impressive anti-infective properties, AGs often suffer from the evolution of resistant bacterial strains³⁰–³¹, and exhibit undesired side effects, such as acute otic- and nephrotoxicity in patients¹⁰, restricting the prescription of AGs primarily to last-resort treatment of life-threatening infections. On the other hand, synthetically modified analogues have shown improved safety and efficacy within this class of antibiotic. However, due to their stereochemical complexity and numerous functional groups, such modifications are limited only to simple functionalizations of primary alcohols and amines on the periphery of natural products¹². Nevertheless, late-stage derivatization has provided several clinically important drugs; plazomicin (5) being the latest in this series¹³, gaining Food and Drug Administration approval in 2018.

Bottom-up synthetic approaches could potentially provide more efficient access to AG analogues and enable further investigations into this biologically important class of compounds. However, the inherent structural complexity has placed substantial practical limitations on new synthetic routes. Only a few total syntheses have been reported to date¹⁴–¹⁷, requiring numerous steps and unselective glycosylations which prevented their translation into medicinal chemistry studies. On the other hand, asymmetric syntheses of differentiated 2-DOS derivatives from different precursors are more established (Fig. 1b)¹⁸–¹⁹, ranging from ring construction strategies (6 → 7)¹⁹, elaboration of carbohydrate chiral pool precursors (8 → 9)²⁰, to functional group manipulation of carbocycles (10 → 11)²¹. However, due to the structural issues associated with the 2-DOS framework, these approaches rely on the extensive use of different modifications of pre-existing functionality to change each group one by one. Such an elaborate collection of functional group interconversions ultimately results in lengthy syntheses, regardless of the nature of the starting material, which have not been further elaborated into glycosylated AGs. Guided by this analysis and previous challenges, we realized that a global olefin functionalization approach could reduce functional group interconversions because it could directly install two desired functional groups per olefin in a stereoselective manner (Fig. 1c). According to this plan, the most logical disconnection of the 2-DOS AGs of type 12 can be traced back to reduced aniline intermediate 13, such that hydroamination of benzene (14) could serve as an ideal template for introduction of five contiguous heteroatom substituents²². Although hydroamination of olefins has been extensively developed within the last two decades²², the corresponding deaminative transformation involving amines is virtually non-existent. Only one report documents the ultraviolet-mediated addition of amines to benzene (Fig. 1c, inset)²³, delivering the 1,2-hydroaminated product 15 as a racemate in low yield as an inseparable mixture alongside the corresponding constitutional isomer 16 and rearomatized aniline 17. We imagined that the development of an efficient deaminative hydroamination would be an empowering method and also a formidable challenge in view of the reshaping of the reported example. The work reported herein features the development of a catalytic, enantioselective, deaminative hydroamination of benzene, which enables the synthesis of (+)-ribostamycin (2) in ten synthetic operations.

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Results and discussion

With the foregoing analysis in mind, the bottom-up synthesis of (+)-ribostamycin (2) was initiated with the methodological challenge of a dearomatic hydroamination of benzene. This approach was inspired by previous dearomatic processes involving photo-activatable arenophiles\(^\text{23}\) because these have successfully served in the formation of densely functionalized, heteroatom-rich motifs. However, these examples could not be adapted to the 2-DOS core, as it is distinct from other structures due to the presence of a methylene group within the aminocyclitol framework. We hypothesized that the combination of visible-light-promoted para-cycloaddition with arenophile N-methyl-1,2,4-triazoline-3,5-dione (MTAD, 18) and copper-promoted ring opening could provide the requisite amine and methylene, formally resulting in dearomatic 1,2-hydroamination (Fig. 2a). Mechanistically, we envisioned this catalytic process could proceed through two distinct, yet convergent pathways, involving hydrocupration\(^\text{26}\) or allylic substitution\(^\text{27}\) (Fig. 2b). In a hydrocupration scenario, a ligated copper(I) hydride species (L, CuH) undergoes \(\pi\)-complexation to MTAD–benzene cycloadduct 19 followed by hydrometallation \(\text{anti}\) to the arenophile moiety (19 \(\rightarrow\) I \(\rightarrow\) II). The resulting organocupper intermediate II is poised for \(\beta\)-elimination of bridgehead arenophile motif (urazole) to deliver diene III. An additional equivalent of silane mandates concomitant regeneration of the copper hydride species and release of product 13. On the other side, copper \(\pi\)-complex I could also undergo oxidative addition, forming organocupper species IV, which upon reductive elimination delivers diene III. Finally, because of the symmetrical nature of MTAD–benzene adduct 19, a suitable chiral ligand bound to the copper centre could enable stereodifferentiation, forming the desired product through an enantioselective fashion in either mechanistic manifold (that is, I \(\rightarrow\) II or I \(\rightarrow\) IV).

Armed with this plan, we began our investigations by conducting a series of orienting studies for the desired dearomatic hydroamination (Fig. 3a and Supplementary Tables 1–3). The optimized reaction conditions involved formation of the cycloadduct between the benzene (14 \(\rightarrow\) 13) and copper salt (CuOAc or CuTC), ligand and potassium tert-butoxide with subsequent introduction of diethoxymethylsilane. Several privileged chiral ligands were viable for this asymmetric dearomatic strategy, ranging from an NHC-type ligand (20, 74% yield and 89:11 e.r.) to P,P-bidentate 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (21, 74% yield and 79:21 e.r.) and taniaphos (22, 69%, 96:4 e.r.). To gain preliminary mechanistic insight, benzene–d\(_x\) (14–d\(_x\)) was employed as the substrate to effectively track the fate of the introduced hydride (Fig. 3b). This experiment revealed the exclusive formation of 13–Me–d\(_x\), providing definitive proof of high site- and stereoselectivity, and supporting the mechanisms proposed above (Fig. 2b).

Density functional theory calculations were performed to investigate the mechanism and the origin of the enantioselectivity of the
dearomative hydroamination (Fig. 4). The density functional theory calculations indicated that the hydrocupration pathway is more favourable than the allylic substitution pathway (Supplementary Fig. 4), and the density functional theory calculations indicated that the hydrocupration pathway is more favourable than the allylic substitution pathway (Supplementary Fig. 4), and the density functional theory calculations indicated that the hydrocupration pathway is more favourable than the allylic substitution pathway (Supplementary Fig. 4), and the density functional theory calculations indicated that the hydrocupration pathway is more favourable than the allylic substitution pathway (Supplementary Fig. 4).
The culmination of this work represents development of dearomatic hydroamination (Figs. 2 and 3) and a concise bottom-up approach to the 2-DOS AG (+)-ribostamycin from benzene (Fig. 5). Additional salient features include the strategic application of an arenophile motif (urazole), which served as a nitrogen source for the syn-1,3-diamine moiety, and as a controlling element during the selective introduction of oxygen functionality. Moreover, selective glycosylation introduced both carbohydrates with pertinent functionality amenable to global reduction, delivering the final product in ten operations from benzene. Most of the synthetic manipulations were conducted on a gram scale and this route already provided several hundred milligrams of the natural product. The described synthetic platform constitutes a practical and rapid preparation of a variety of 2-DOS AGs and their analogues, enabling explorations toward the development of more effective and safe antibiotics. Similarly, the dearomatic hydroamination should also provide access to tailored aminocyclitols that would be challenging to prepare using conventional chemistry.

Methods

In the procedure for enantioselective dearomatic hydroamination, MTAD (18, 2.83 g, 25 mmol, 1.0 equiv.) was dissolved in anhydrous dichloromethane (167 ml) and degassed benzene (14, 11.2 ml, 125 mmol, 5.0 equiv.) was added. The solution was cooled to −78 °C and the flask was irradiated with white light-emitting diodes at −78 °C until the pink colour disappeared. In a separate flask, a catalyst solution was prepared from CuOAc (0.138 g, 1.13 mmol, 4.5 mol%), taniaphos (22, 0.860 g, 1.25 mmol, 5.0 mol%) and KOBu (3.37 g, 30 mmol, 1.2 equiv.), dissolved in dry, degassed toluene (50 ml) and stirred for 30 min. Note: the solids should be ground to a fine powder to ensure that the cannulation process is not clogged at the low temperature. The light-emitting diodes for the reaction were turned off, and the cooled (−78 °C) catalyst solution was then
Further information on research design is available in the Reporting summary.

Large scale

-Deamortive hydroamination (see Fig. 3a)

Large scale
cannulated (14 gauge cannula) into the reaction along the wall of the flask. After catalyst addition was complete, diethoxymethylsilane (10 ml, 62.6 mmol, 2.5 equiv.) was added dropwise, and reaction mixture was allowed to slowly warm to −50 °C and stirred at this temperature for 12 h. Then the reaction was warmed to −20 °C and quenched with water (150 ml). After warming to room temperature with vigorous stirring, the reaction mixture was poured into a separatory funnel and the organic phase was drained and discarded. Then, the aqueous layer was extracted with CH2Cl2 (6 × 200 ml), dried over anhydrous MgSO4, filtered and evaporation, Pd(OH)2 (20 wt%), H2 (130 p.s.i.), AcOH/H2O, 23 °C, 6 d, 91% (isolated as an tetra-acetate salt). The Fürst–Plattner analysis reveals that the nucleophilic opening of epoxide 30 occurs with desired chemoselectivity only if nitrogen atoms are tethered. In the open form, an attack at the same position would proceed through the unfavoured twist boat-like transition state. Bn, benzyl; b.r.s.m., based on recovered starting material; Nu, nucleophile.

Fig. 5 | Synthesis of (+)-ribostamycin (2) from benzene (14). Reagents and conditions: (2) Boc2O (1.5 equiv.), DMAP (0.15 equiv.), CH2Cl2, 0 to 23 °C, 12 h, 66%; (7) Zn (10 equiv.), AcOH/MeCN/H2O, 0 to 80 °C, 4 h, 90%; (8) Pd(OH)2 (20 wt%), H2 (130 psi), EtOAc/MeOH/H2O, 23 °C, 48 h; then filtration and solvent evaporation, Pd(OH)2 (1.0 equiv.), BnBr (2.0 equiv.), DMF, 0 to 23 °C, 24 h, 54%; (4) NBS (2.0 equiv.), I2 (0.2 equiv.), MeCN/H2O, 0 to 23 °C, 16 h; then K2CO3, MeOH, 65 °C, 65%, 96:4 e.r. (3) Na2CO3 (3.0 equiv.), MeOH, 65 °C, 24 h; then removal of solvents, NaH (2.0 equiv.), AgClO4 (2.0 equiv.), BuLi, PhMe, 80 °C, 24 h; then neutralization with AcOH, CuCl2 (2.0 equiv.), 23 °C, 12 h, 60%; (10) Pd(OH)2 (20 wt%), H2 (130 psi), AcOH/H2O, 23 °C, 48 h; then filtration and solvent evaporation, Pd(OH)2 (20 wt%), H2 (130 p.s.i.), EtOAc/MeOH/H2O, 23 °C, 48 h; 91% (isolated as an tetra-acetate salt). The Fürst–Plattner analysis reveals that the nucleophilic opening of epoxide 30 occurs with desired chemoselectivity only if nitrogen atoms are tethered. In the open form, an attack at the same position would proceed through the unfavoured twist boat-like transition state. Bn, benzyl; b.r.s.m., based on recovered starting material; Nu, nucleophile.

References

1. Schatz, A., Bugle, E. & Waksman, S. A. Streptomycin, a substance exhibiting antibiotic activity against Gram-positive and Gram-negative bacteria. Proc. Soc. Exp. Biol. Med. 55, 66–69 (1944).

2. Kirst, H. A. & Marinelli, F. in Antimicrobials: New and Old Molecules in the Fight Against Multi-resistant Bacteria (eds Marinelli, F. & Genilouid, O.) 193–209 (Springer, 2014).

3. Becker, B. & Cooper, M. A. Aminoglycoside antibiotics in the 21st century. ACS Chem. Biol. 8, 105–115 (2013).

4. Shomura, T. et al. Studies on antibiotic SF-733, a new antibiotic. I. Taxonomy, isolation and characterization. J. Antibiot. 23, 155–161 (1970).

5. Waksman, S. A. & Lechevalier, H. A. Neomycin, a new antibiotic active against streptomycin-resistant bacteria, including tuberculous organisms. Science 109, 305–307 (1949).

6. Weinstein, M. J. et al. Antibiotic 6640, a new Micromonospora-produced aminoglycoside antibiotic. J. Antibiot. 23, 551–554 (1970).
7. Magnet, S. & Blanchard, J. S. Molecular Insights into aminoglycoside action and resistance. *Chem. Rev.* 105, 477–498 (2005).
8. Borovinskaya, M. A. et al. Structural basis for aminoglycoside inhibition of bacterial ribosomes. *Nat. Rev. Mol. Cell Biol.* 18, 727–737 (2017).
9. Garneau-Tsodikova, S. & Labby, J. K. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. *Med. Chem. Comm.* 7, 11–27 (2016).
10. Ramirez, M. S. & Tolmasy, M. E. Aminoglycoside modifying enzymes. *Drug Resist. Updat.* 13, 151–171 (2010).
11. Wargo, K. A. & Edwards, J. D. Aminoglycoside-induced nephrotoxicity. *J. Pharm. Pract.* 27, 573–577 (2014).
12. Chandrika, N. T. & Garneau-Tsodikova, S. Comprehensive review of chemical strategies for the preparation of new aminoglycosides and their biological activities. *Chem. Soc. Rev.* 47, 1189–1249 (2018).
13. Abdul-Mutakabbir, J. C., Kebriaei, R., Jorgensen, S. C. J. & Rybak, M. J. Teaching an old class new tricks: a novel semi-synthetic aminoglycoside, plazomicin. *Infect. Dis. Ther.* 8, 155–170 (2019).
14. Umezawa, S. Recent advances in the synthesis of aminoglycoside antibiotics. *Pure Appl. Chem.* 50, 1453–1476 (1978).
15. Usai, T. & Umezawa, S. Total synthesis of neomycin B. *Carbohydr. Res.* 174, 133–143 (1988).
16. Fukami, H., Kitahara, K. & Nakajima, M. Total synthesis of ribostamycin. *Tetrahedron Lett.* 17, 545–548 (1976).
17. Yoshikawa, M., Ikeda, Y., Takenaka, K., Torihara, M. & Kitagawa, I. Synthesis of ribostamycin. An application of a chemical conversion method from carbohydrate to aminocyclitol. *Chem. Lett.* 13, 2097–2100 (1984).
18. Busscher, G. F., Rutjes, F. P. J. T. & van Delft, F. L. Synthesis of a 2-deoxystreptamine: central scaffold of aminoglycoside antibiotics. *Chem. Rev.* 105, 775–792 (2005).
19. Busscher, G. F., Rutjes, F. P. J. T. & van Delft, F. L. Synthesis of a protected enantiomerically pure 2-deoxysterptamine derivative from 2-allylglycine. *Tetrahedron* 45, 3629–3632 (2004).
20. Kitagawa, I., Yoshikawa, M., Ikeda, Y. & Kayakiri, H. Reductive one-step elimination of an acetoxyl residue at β-position of a nitro group: syntheses of (-)-shikimic acid from d-mannose and 2-deoxysterptamine pentacetate from N-acetyl-d-glucosamine. *Heterocycles* 17, 209–214 (1982).
21. Trost, B. M. & Malhotra, S. Asymmetric stereodivergent strategy towards aminocyclitols. *Chem. Eur. J.* 20, 8288–8292 (2014).
22. Roche, S. P. & Porco, J. A. Deearomatization strategies in the synthesis of complex natural products. *Angew. Chem. Int. Ed.* 50, 4068–4093 (2011).
23. Müller, T. E., Hultzsch, K. C., Yus, M., Foubelo, F. & Tada, M. Hydroamination: direct addition of amines to alkenes and alkyynes. *Chem. Rev.* 108, 3795–3892 (2008).
24. Bryce-Smith, D., Gilbert, A. & Manning, C. 1,2-Photoaddition of primary and secondary amines to benzene. *Angew. Chem. Int. Ed.* 13, 341–342 (1974).
25. Southgate, E. H., Pospech, J., Fu, J., Holycross, D. R. & Sarlah, D. Dearomatative dihydroxylation with arenoephiles. *Nature Chem.* 8, 922–928 (2016).
26. Liu, R. Y. & Buchwald, S. L. CuH-catalyzed olefin functionalization: from hydroamination to carbonyl addition. *Acc. Chem. Res.* 53, 1229–1243 (2020).
27. Langlois, J.-B. & Alexakis, A. In *Transition Metal Catalyzed Enantioselective Allylic Substitution in Organic Synthesis* (ed. Kazmaier, U.) 255–268 (Springer, 2012).
28. Xi, Y. & Hartwig, J. F. Mechanistic studies of copper-catalyzed asymmetric hydroboration of alkenes. *J. Am. Chem. Soc.* 139, 12758–12772 (2017).
29. Yang, Y., Shi, S. L., Niu, D., Liu, P. & Buchwald, S. L. Catalytic asymmetric hydroamination of unactivated internal olefins to aliphatic amines. *Science* 349, 62–66 (2015).
30. Lodh, R. S., Borah, A. J. & Phukan, P. Synthesis of bromohydrins using NBS in presence of iodine as catalyst. *Indian J. Chem.* 53B, 1425–1429 (2014).
31. Malik, G., Ferry, A., Guinchard, X. & Crich, D. Synthesis of β-hydroxy O-alkyl hydroxylamines from epoxides using a convenient and versatile two-step procedure. *Synthesis* 45, 65–74 (2013).
32. Shimomura, N. & Mukaiyama, T. Catalytic synthesis of β-d-ribofurcanosides from d-ribofurcanose and alcohols. *Chem. Lett.* 22, 1941–1944 (1993).
33. Gilibert, E. & Pedersen, C. M. Scalable synthesis of anomerically pure orthogonal-protected GlcN3 and GalN3 from d-glucosamine. *Org. Lett.* 18, 4424–4427 (2016).
34. Dey, R. T. & Sarkar, T. K. On the [3 + 2] annulation of cyclic allylsilanes with N-phenyltriazolinedione: an enantio- and diastereoselective synthesis of cis-1,3-diaminocycloptolols. *J. Org. Chem.* 75, 4521–4529 (2010).
35. Clique, B. et al. Synthesis of a library of stereo- and regiochemically diverse aminoglycoside derivatives. *Org. Biomol. Chem.* 3, 2776–2785 (2005).

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Author contributions

C.N.U. and D.S. conceived the idea, designed the experiments, analysed the data and prepared the manuscript with the input of all authors. P.G. assisted with optimization and screening efforts involving dearomative hydroamination. Y.Z. carried out the computational studies with P.L. providing guidance. S.L., K.S.L. and J.M.N. assisted with preparation of key intermediates.

Competing interests

The authors declare no competing interests.

Additional information

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