Natural-Product-Directed Catalytic Stereoselective Synthesis of Functionalized Fused Borane Cluster–Oxazoles for the Discovery of Bactericidal Agents

Rajesh Varkhedkar,∥ Fan Yang,∥ Rakesh Dontha,∥ Jianglin Zhang, Jiyong Liu, Bernhard Spingler, Stijn van der Veen,* and Simon Duttwyler*

ABSTRACT: The identification of an alternative chemical space in order to address the global challenge posed by emerging antimicrobial resistance is very much needed for the discovery of novel antimicrobial lead compounds. Boron clusters are currently being explored in drug discovery due to their unique steric and electronic properties. However, the challenges associated with the synthesis and derivatization techniques of these compounds have limited their utility in the rapid construction of a library of molecules for screening against various biological targets as an alternative molecular platform. Herein, we report a transition-metal-catalyzed regioselective direct B–H alkylation–annulation of the closo-dodecaborate anion with natural products such as menthol and camphor as the directing groups. This method allowed the rapid construction of a library of 1,2,3-trisubstituted clusters, which were evaluated in terms of their antibacterial activity against WHO priority pathogens. Several of the synthesized dodecaborate derivatives displayed medium- to high-level bactericidal activity against Gram-positive and Gram-negative bacteria.

INTRODUCTION

The discovery of novel bioactive molecules is essential to overcome the impending challenges posed by emerging infectious diseases caused by multidrug-resistant pathogens worldwide. The availability of antibiotics without prescription and their prophylactic use have spurred resistance, and bacteria of concern are, among others, Staphylococcus aureus, Escherichia coli, Salmonella spp. and Neisseria gonorrhoeae. The Center of Disease Control antibiotic resistance threat report 2019 disclosed more than 2.8 million cases of antibiotic-resistant infections with more than 35000 fatalities in the US every year. Research toward the discovery of antimicrobial agents is not attractive to pharmaceutical companies due to low profits and the limited lifespan associated with antibiotics, resulting in drying up of the corresponding pipeline and the risk of returning to the preantibiotic era. In addition, studies of resistance mechanisms suggest high chances of mutations, leading to the ineffectiveness of well-established compounds. Strategies to address this challenge involve the chemical modification of natural products as well as existing drugs. Historically, screening of secondary metabolites obtained from microorganisms has been a primary source of bioactive molecules that prevent the growth of pathogens. Studies on the biosynthesis of metabolites, probes of unexplored strains of microorganisms, and the availability of genome mining tools to activate silent gene clusters have yielded numerous antibacterial compounds. Currently marketed drugs involve aminoglycosides, β-lactams, glycopeptides, polymyxins, and the corresponding semisynthetic derivatives. Additionally, molecules bearing oxazolidinone, pyrimidine, quinolone, and sulfa functionalities have provided antibacterial candidates.

The chemical space of natural products primarily comprises chiral compounds, whereas synthetic libraries often consist of flat aromatic molecules. Icosahedral boron-rich clusters exhibit a spherelike distribution of electron density and can be compared to classical arenes. The closo-dodecaborate dianion \([\text{B}_{12}\text{H}_{12}]^{2−}\) is a highly symmetrical molecule, and the installation of three different substituents leads to \(R_{\text{cap}}-1\) and \(S_{\text{cap}}-1\) stereoisomers (Figure 1a). The chirality due to such cage substitution has the potential for applications in designing molecules for medicinal...
investigated only to a limited degree. In an early review, On the other hand, their antimicrobial properties have been capture therapy (BNCT) and on the inhibition of enzymes. That set them apart from organic building blocks. Studies where polyhedral boron moieties seem to play a crucial role in antibacterial activity are the metallacarboranes: e.g., the bis(dicarbollide) K121. Recently, the groups of Šicha and Viñas have proved related compounds to that end. In 2020, Spokoyny reported on the synthesis and properties of the borane–saccharide hybrid [B9(OC6H4-1-thio-α-D-galactose)]12−, which exhibits strong binding affinity to the B subunit of Shiga toxin. Our own group has found that fused 2D/3D heterocycles based on the [B12H12]2− framework possess antimicrobial properties. We therefore wondered whether closo-dodecaborates comprising a fused N,O-heterocycle and an additional group at a boron vertex would show similar effects. Amides were anticipated to serve as starting materials for the target compounds, which can be viewed as 3D analogues of benzoxazoles with an organic handle R. This strategy requires double B–H activation, including B–C bond formation and B–O annulation. The synthesis of functionalized polyhedral boranes and carboranes by B–H activation has emerged as a powerful tool, but derivatization of anionic {CB11}H12 and {B12} clusters has only been accomplished in recent years. For dodecaborates, ureido and amide functionalities can serve as directing groups to achieve B–C and concomitant B–O bond formation.

A major challenge for the transformation S → 6 was the choice of a suitable directing group. Our aim was to use a motif that provides the possibility to explore cage chirality as well as antimicrobial properties. The cage/R cage stereinduction required a substituent with saturated stereogenic centers close to the transition metal and boron vertices in the relevant transition state(s). However, aliphatic amides have not been explored in dodecaborate B–H activation. We decided to focus on directing groups involving (−)-menthol and (−)-camphamic acid on the basis of their rigid alicyclic structure, commercial availability, and reported bioactivities. We herein present a transition-metal-catalyzed, fully regioselective alkylation–annulation reaction for the construction of fused diboraazoloxoles of the closo-dodecaborate cluster by using the aforementioned directing groups and alkene coupling partners. The method enabled the synthesis of a library of diversity-oriented boron clusters 13 and 14 under mild conditions in good yields and moderate to high stereoselectivity. Antibacterial properties were observed for several molecules of the series 13 and 14, thus suggesting that multiply functionalized fused closo-dodecaborates represent a feasible alternative chemical space to traditional frameworks of organic antibiotics. Notably, one of the compounds, 14k, was found to be active with a minimum inhibitory concentration (MIC) of up to 4 μM against Gram-positive S. aureus and Enterococcus faecalis and an MIC of up to 2 μM against Gram-negative N. gonorrhoeae.

Figure 1. (a) Cage chirality of 1,2,3-trisubstituted closo-dodecaborates. (b) Boron-containing bioactive compounds. (c) Design of functionalized dodecaborates in this study. Color code: gray spheres, B; blue spheres, B–H.

The incorporation of boron as a part of bioactive compounds has recently gained much interest. Several boron-containing compounds are in clinical use, such as bortezomib (2), a proteasome inhibitor, and vaborbactam (3), an antibiotic (Figure 1b). Boron clusters are relatively nontoxic pharmacophores with steric and electronic properties that set them apart from organic building blocks. Studies on their medicinal applications have focused on boron neutron capture therapy (BNCT) and on the inhibition of enzymes. On the other hand, their antimicrobial properties have been investigated only to a limited degree. In an early review article on the potential applications of boron clusters, Pléšek postulated that derivatives resembling known antibiotics may be promising drug analogues that cannot easily be degraded by pathogens. Examples where polyhedral boron moieties seem to play a crucial role in antibacterial activity are the metallacarboranes: e.g., the bis(dicarbollide) K121 (4; Figure 1b). Recently, the groups of Šicha and Viñas have proved related compounds to that end. In 2020, Spokoyny reported on the synthesis and properties of the borane–
Scheme 1. Acylation of \([\text{B}_{12}\text{H}_{11}\text{N}]^+\) Providing Camphanyl Amide 10 and Menthol Amide 11

6. Initially, we investigated the B–H activation of 10 with styrene (12a) in the presence of Rh or Ir catalysts. A reaction with \([\text{Cp}^*\text{RhCl}_2]\) or \([\text{Cp}^*\text{IrCl}_2]\) (10 mol %) at 25 or 60 °C for 24 h indicated only a trace of the desired product by ESI-MS and mostly unchanged starting material (Table 1).

Table 1. Optimization of the Alkylation–Annulation Reaction

| Entry | [Rh] (mol %) | [Cu] (equiv) | [Ir] (mol %) | [Ag] (equiv) | T (°C) | Solvent | Yield (%) |
|-------|-------------|-------------|-------------|-------------|--------|---------|-----------|
| 1     | [Rh] (10)   | (2.5)       |             |             | 60     | MeCN    | 85        |
| 2     | [Ir] (10)   | (2.5)       |             |             | 60     | MeCN    | 50        |
| 3     | [Rh] (10)   | (2.5)       |             |             | 60     | MeCN    | 50        |
| 4     | [Ir] (10)   | (2.5)       |             |             | 60     | MeCN    | 65        |
| 5     | [Ir] (10)   | (2.5)       |             |             | 40     | MeCN    | 65        |
| 6     | [Ir] (10)   | (2.5)       |             |             | 40     | MeCN    | 75        |
| 7     | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 72        |
| 8     | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 72        |
| 9     | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 10    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 11    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 12    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 13    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 14    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 15    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |
| 16    | [Ir] (10)   | (2.5)       |             |             | 50     | MeCN    | 82        |

Reactions were conducted on a 20 mg scale in 1 mL of the solvent in a glass vial sealed with a screw cap. Definitions: [Rh] = [RhCl\(_2\)]\(_2\); [Ir] = [IrCl\(_2\)].

Therefore, we tried addition of Cu(OAc)\(_2\) and AgOAc. The reaction of 10 in the presence of [Cp*RhCl\(_2\)] or [Cp*IrCl\(_2\)] and Cu(OAc)\(_2\)·H\(_2\)O (2 equiv) at 60 °C suggested more than 50% conversion along with a mixture of other compounds by MS, whereas using AgOAc as an additive was not found to be helpful. Thus, we lowered the temperature as well as catalyst loading. The reaction of 10 in the presence of [Cp*RhCl\(_2\)] or [Cp*IrCl\(_2\)] and Cu(OAc)\(_2\)·H\(_2\)O (2 equiv) at 40 °C significantly improved the yield of the desired product to up to 65–75%. Further lowering of the catalyst loading and temperature to 2.5 mol % at 25 °C furnished the desired product in 85% yield upon isolation by column chromatography. The reaction in other solvents such as acetone, THF, and DCE gave yields of 56% or less. EtOH or MeOH afforded primarily unchanged starting materials.

From these screening experiments, entry 11 of Table 1 was used as the basis for transformations on a larger scale and an exploration of the substrate scope. Under these conditions, we performed the reaction of 10 with 12a on a 200 mg scale to give the corresponding product 13a in 85% yield after purification by column chromatography. \(^1\)H NMR spectroscopy and mass spectrometry suggested reductive coupling of 10 with 12a, leading to a B-(CH\(_2\))\(_2\)-Ph moiety. \(^{11}\)B and \(^{11}\)B\(_{12}\)H\(_{11}\)N features are similar to those of typical organic amides; in particular, the coordination geometry around C1 is trigonal planar with a sum of angles of 360.1(2)° around this atom. The oxygen atoms O1 and O2 adopt a transoid geometry with respect to the C1=C2 axis, as indicated by the torsion angle of -178.4(2)° for O1−C1−C2−O2. Overall, the structure is similar to that of the closely related dodecaborate amide \([\text{B}_{12}\text{H}_{11}\text{N}(\text{CO})](\text{thiophen-2-yl})\). Upon attaching the desired directing groups to the closo-dodecaborate cage, we evaluated transition-metal-catalyzed coupling to explore the feasibility of formation of compounds.
NMR spectra showed desymmetrization of the cage as well as characteristic, distinct resonances at 6.6, −4.5, and −10.2 ppm corresponding to B–O, B–N, and B–C vertices, respectively (Figure 3). This peak pattern was in full agreement with that observed for related dodecaborates in earlier studies.61,64

![Figure 3. 11B NMR spectrum of [Et₃NH][13a] (128 MHz, acetone-d₆, 23 °C).](image)

Single crystals of 13a were obtained from a H₂O/EtOH solution by slow evaporation of most of the EtOH over 21 days at room temperature. An X-ray diffraction analysis revealed the composition [13a][Et₃NH]₂·5H₂O with four anions in the asymmetric unit (Figure 4). The cage showed the anticipated ellipsoids; cations, H₂O solvent molecules, and hydrogen atoms of the four anions in the asymmetric unit is shown; 25% displacement ellipsoids. A proposed mechanism is displayed and discussed in pages S9 and S10 in the Supporting Information.

![Figure 4. X-ray crystal structure of [13a][Et₃NH]₂·5H₂O (only one of the four anions in the asymmetric unit is shown; 25% displacement ellipsoids; cations, H₂O solvent molecules, and hydrogen atoms except for N–H are omitted for clarity).](image)

1,2,3-trisubstitution caused by B–C coupling and heterocycle generation upon B–O bond formation. All of the anions exhibited an R_cage configuration and similar structural features. Therefore, only one of them is described in detail in the following. The B1–B3 distance is 1.730(6) Å, slightly contracted in comparison to other B–B distances, indicative of electron delocalization within the diboraaxazole ring. Although this effect is not very strong, it is consistent with reports on similar compounds and all other distances within the ring. The coordination around C1 is trigonal planar with an internal angle of O1–C1–N1 of 118.2(4)° and a sum of angles of 360.0(4)°. The C11–C12 distance of 1.522(7) Å confirmed reductive coupling with 12a, resulting in a CH₂–CH₂ single bond.

Using the established protocol, we evaluated the generality of the reaction of 10 and 11 with various substituted styrenes as well as other olefins to generate a library of compounds. In general, the coupling–cyclization consistently provided access to products 13 and 14 in moderate to high yields under ambient conditions (Table 2). For all of the compounds, two sets of signals were observed in the ²H and ¹³C{¹H} NMR spectra (but not in the ¹¹B NMR spectra due to the naturally broadened signals), consistent with the formation of diastereomers featuring an unchanged absolute configuration of the directing group and R_cage/S_cage configuration at the cage. For 13a and 14a, 1D and 2D NMR experiments were performed to assign all ²H and ¹³C resonances. On the basis of this analysis, diagnostic signals were used to determine the diastereomeric ratios dr (see the Supporting Information for details). Although in each of the series 13 and 14 one diastereoisomer consistently dominated, at present we are unable to state whether this corresponds to the R_cage or the S_cage configuration.

Substituted styrenes with electron-withdrawing and electron-donating functionalities furnished products 13a–k and 14a–n with very high regioselectivity and control over the degree of substitution as well as moderate to good diastereoselectivity. Minor undesired compounds were dialkylated species and trace amounts of unchanged starting material. Purification by chromatography afforded isolated yields of 63–92%. Typically, diastereomeric ratios were in the range of 60:40 to 80:20. Notably, higher values of up to 91:9 were observed for styrenes with 4'-Bu and 4-OC(O)Me substitution (13f and 14f).

Coupling of 11 with the nonaromatic alkenes CH₂–CH–R (R = CO₂Me, CO₂EtBu, (CH₃)₅CH₃) proceeded in high yields of 85–92% (14l–n). However, in these cases the dr was 1:1, suggesting that the nature of the alkene coupling partner plays a decisive role in the diastereodiscriminating step.

Our previous studies suggested that the alkylation—annulation cascade occurs via B–C coupling followed by B–O bond formation as the essential steps.62,63 Both of these events require B–H activation, and several intermediates with B–H–Rh agostic-like and B–Rh direct interactions are likely to be involved. A proposed mechanism is displayed and discussed in pages S9 and S10 in the Supporting Information. Stereoinduction occurs in the second B–H activation step (affording the intermediates R_cage/V and S_cage/V in Scheme S2) and is governed by the absolute stereochemistry of the natural product moiety. Subsequently, B–O bond formation—cyclization generates R_cage·13/R_cage·14 and S_cage·13/S_cage·14 diastereomers. We intend to investigate the mechanistic manifold and the question as to which stereochemical outcome is preferred by the chiral directing groups with the assistance of calculations in a separate study.

To probe and compare the bioactivity of the trisubstituted boron clusters with that of disubstituted boron clusters, we carried out further transformations of 10 and 11. Treatment with 1.1 equiv of the iodine(III) reagent (diacetoxyiodo)benzene in MeOH gave the cyclized products 15 and 16 cleanly in 90% yield after silica gel chromatography (Scheme 2). The reaction proceeded under mild conditions in MeOH in air within 10 min, and no side reactions such as cage overoxidation and formation of B_cage=iodonium species were observed.

**Antibacterial Activity.** The antimicrobial activity of all synthesized compounds was evaluated against commonly encountered "problem germs", Gram-positive and Gram-negative antimicrobial-resistant bacteria that are defined in the WHO priority list (for the complete table of all tested
The minimum inhibitory concentrations (MICs) of our compounds and the antibiotics ceftriaxone, azithromycin, and ciprofloxacin were determined against international reference strains _N. gonorrhoeae_ ATCC 49226, _S. aureus_ ATCC 25923, _E. faecalis_ ATCC 29212, _Acinetobacter baumannii_ ATCC 19606, _Klebsiella pneumonia_ ATCC 700603, _Pseudomonas aeruginosa_ ATCC 27853, _E. coli_ ATCC 25922, _Enterobacter cloacae_ ATCC 700323, _Stenotrophomonas maltophilia_ ATCC 17666, _Listeria monocytogenes_ EGD, and _Shigella sonnei_ SD10053 using the agar dilution method (see Table S1 in the Supporting Information). All compounds of the series 13 showed strong antimicrobial activity against the Gram-negative species _N. gonorrhoeae_, with compounds 13h, I displaying the best activity at an MIC of 4 μM (Table 3). Most of the series 13 compounds furthermore displayed activity against the Gram-positive species _S. aureus_ and _E. faecalis_, with the best activities being observed for compounds 13f, h, I, which displayed MICs of 8−16 μM against _S. aureus_ and 16−32 μM against _E. faecalis_. None of the series 13 compounds displayed activity against any of the other tested bacterial species (see Table S1 in the Supporting Information). Similarly, all of the series 14 compounds showed strong activity against _N. gonorrhoeae_, with the best activity being observed for compound 14k at an MIC of 2 μM (Table 3). Most of the series 14 compounds also displayed strong activity against _S. aureus_, _E. faecalis_, and _L. monocytogenes_, with the most consistent activity against all

**Table 2. Synthesis of Fused closo-Dodecaborate−Oxazoles**

| Scheme 2. Synthesis of Cyclized Compounds 15 and 16 |
|-----------------------------------------------------|
| ![Scheme 2](https://pubs.acs.org/doi/10.1021/acscentsci.1c01132) |

*Reactions were performed on a 100 mg scale in MeCN (5 mL) in a 20 mL glass vial with a screw cap. The yields noted are isolated yields after purification by chromatography. dr values were determined by NMR. See the Supporting Information for details.*

700323, _Stenotrophomonas maltophilia_ ATCC 17666, _Listeria monocytogenes_ EGD, and _Shigella sonnei_ SD10053 using the agar dilution method (see Table S1 in the Supporting Information). All compounds of the series 13 showed strong antimicrobial activity against the Gram-negative species _N. gonorrhoeae_, with compounds 13h, I displaying the best activity at an MIC of 4 μM (Table 3). Most of the series 13 compounds furthermore displayed activity against the Gram-positive species _S. aureus_ and _E. faecalis_, with the best activities being observed for compounds 13f, h, I, which displayed MICs of 8−16 μM against _S. aureus_ and 16−32 μM against _E. faecalis_. None of the series 13 compounds displayed activity against any of the other tested bacterial species (see Table S1 in the Supporting Information). Similarly, all of the series 14 compounds showed strong activity against _N. gonorrhoeae_, with the best activity being observed for compound 14k at an MIC of 2 μM (Table 3). Most of the series 14 compounds also displayed strong activity against _S. aureus_, _E. faecalis_, and _L. monocytogenes_, with the most consistent activity against all...
three species being observed for compound 14i at an MIC of 4 μM. Antimicrobial activity was also observed against the Gram-negative species *S. maltophilia*, although to a lesser degree, with compound 14h being most active with an MIC of 16 μM. No activity for the series 14 compounds was observed against the other tested bacterial species (see Table S1 in the Supporting Information). As a general trend, compounds containing the −tBu group, halides, or polar functionalities such as −OMe and −NO2 within the aryl moiety feature higher efficiency. Importantly, the antimicrobial activity of the series 13 compounds was dependent on the additional arylethyl group of these trisubstituted compounds, since the disubstituted control compound 15 did not display any antimicrobial activity. In contrast, for the series 14 compounds the additional arylethyl group did not appear to be essential for activity against *N. gonorrhoeae*, *S. aureus*, and *E. faecalis*, albeit the activity of the disubstituted control compound 16 was lower than that observed for the trisubstituted compounds that contained the additional handle. Therefore, it appears that addition of the menthylo moiety, but not the camphanic acid moiety, was beneficial for antimicrobial activity, which might explain the overall better activity observed for the series 14 compounds. In the case of noncyclized amides 10 and 11, no significant activity was detected. Similarly, the MIC values of the building blocks [Et3NH][B12H11-NH3]−, (−)-menthol, and (−)-camphanic acid were all >256 μM. This comparison...
highlights the effect of the combination of the cluster−oxazole fusion with the additional B−C derivatization of the adjacent boron vertex position.

Compounds of series 13 and series 14 both showed particularly strong activity against the N. gonorrhoeae reference strain. N. gonorrhoeae has developed resistance against all of the previously and currently used antimicrobials, and due to the continued emergence of multidrug-resistant strains, infections with N. gonorrhoeae have become increasingly difficult or even impossible to treat successfully.69,70 Resistance against the previously recommended antimicrobials ciprofloxacin and azithromycin is widespread, and susceptibility to the currently last available first-line therapy ceftriaxone is rapidly waning.71−75 Therefore, it is of utmost importance to develop novel antimicrobials for this multidrug-resistant bacterial pathogen. Importantly, compounds of the series 13 and 14 also showed strong activity against two recent multidrug-resistant clinical isolates, with the susceptibility being almost identical with the susceptibility displayed by the reference strain (Table 4), indicating that the molecular target of these compounds is distinct from that of previously or currently used antimicrobials.

Generally, the activity of antimicrobials can be divided into bacteriostatic compounds, which inhibit growth but do not kill, and bactericidal compounds that are able to directly kill the bacteria. This distinction is clinically relevant, since bacteriostatic compounds are dependent on an active host immune response to clear the infection, which might be problematic in immunocompromised individuals or not rapid enough for infections of the central nervous system or the heart.76,77 Therefore, we selected compound 14k, which features a phenyl-nitro moiety that showed the lowest MIC values, and tested its mode of activity against N. gonorrhoeae and S. aureus in time-kill analyses. As controls, we included the currently recommended first-line bactericidal antibiotics ceftriaxone69,70 and vancomycin76 for comparison. Compound 14k displayed rapid bactericidal activity against both N. gonorrhoeae and S. aureus (Figure 5). Exposure of N. gonorrhoeae to compound 14k at 4 × MIC resulted in >100000-fold reduction in CFU counts within the first hour, whereas ceftriaxone required 8 h to achieve similar inactivation. Of note, the MIC of ceftriaxone against N. gonorrhoeae is 250-fold lower in comparison with compound 14k, while the time-kill analyses were performed on the basis of relative × MIC values. In the case of S. aureus, exposure to compound 14k at 4 × MIC caused a >100000-fold inactivation within the first 2 h, whereas vancomycin could not effectively achieve inactivation, even after 8 h of exposure. The time-kill assays, corroborated by the results of bacterial live/dead staining (Figure 5e), thus accentuate the strong potential of 14k as a lead for novel antimicrobial compounds to treat bacterial infections caused by N. gonorrhoeae and S. aureus. For a compound to become a useful new antibiotic, it must exhibit not only high activity against pathogens but also low toxicity to the host. We are currently evaluating the fused borane−oxazoles in terms on their effects on eukaryotic cells and mice.

CONCLUSION

An efficient synthetic protocol has been developed for the stereoselective synthesis of highly functionalized fused dodecaborate−oxazoles. The protocol is mild and allows for the rapid construction of a library of compounds in moderate to high yields and stereoselectivities. This method provides access to a previously unexplored chemical space involving the natural products menthol and camphamic acid hybridized with the [B12H12]2− cage. The evaluation of antimicrobial activity against various pathogens has resulted in the identification of several active compounds. Particularly, product 14k exhibits potential for further drug development, as evidenced by its MIC values, time-kill assays showing bactericidal activity, and live/dead staining. These results lay the foundation for the further exploration of dodecaborate cage chirality as well as the study and improvement of antimicrobial properties of fused 2D/3D organic/inorganic heterocycle hybrid molecules.

Figure 5. Bactericidal activity of compound 14k against Neisseria gonorrhoeae and Staphylococcus aureus. Bacterial suspensions of N. gonorrhoeae strain ATCC 49226 (NG) and S. aureus strain ATCC 29523 (SA) in GC broth supplemented with 1% Vitox were incubated with compound 14k or the control antimicrobials ceftriaxone (NG) and vancomycin (SA) at 4×, 2×, 1×, and 1/2× the minimum inhibitory concentration (MIC) or the vehicle control. Samples were taken in a time series for CFU determination or live/dead staining. (a) Survival curves of N. gonorrhoeae after exposure to compound 14k (1 × MIC: 2 μM). (b) Survival curves of N. gonorrhoeae after exposure to ceftriaxone (1 × MIC: 0.008 μM). (c) Survival curves of S. aureus after exposure to compound 14k (1 × MIC: 4 μM). (d) Survival curves of S. aureus after exposure to vancomycin (1 × MIC: 7 μM). Survival curves represent the mean and SD of three biological independent repeats. (e) Live/dead staining of N. gonorrhoeae and S. aureus after exposure to the vehicle control, compound 14k, or control antibiotics ceftriaxone and vancomycin at 1× MIC for 1 h. Viable bacteria are stained with SYTO 9 (green), whereas dead bacteria are stained with propidium iodide (red) or with both SYTO 9 and propidium iodide (yellow).
ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.1c01132.

Details of the synthesis and characterization of compounds, biological evaluation, spectra, and crystallographic data (PDF)
Crystalllographic data for 10 (CIF)
Crystalllographic data for 13a (CIF)

AUTHOR INFORMATION

Corresponding Authors
Stijn van der Veen — Department of Microbiology, and Department of Dermatology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, 310058 Hangzhou, People’s Republic of China; Email: stijnvanderveen@zju.edu.cn
Simon Duttwyler — Department of Chemistry, Zhejiang University, 310027 Hangzhou, People’s Republic of China; orcid.org/0000-0001-9851-4920; Email: duttwyler@zju.edu.cn

Authors
Rajesh Varkhedkar — Department of Chemistry, Zhejiang University, 310027 Hangzhou, People’s Republic of China; Present Address: Glenmark Life Sciences, MIDC Industrial Area, Mahape, Navi Mumbai, Maharashtra 400709, India
Fan Yang — Department of Microbiology, and Department of Dermatology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, 310058 Hangzhou, People’s Republic of China
Rakesh Dontha — Department of Chemistry, Zhejiang University, 310027 Hangzhou, People’s Republic of China; Present Address: Neuland Laboratories Ltd., 329 Veerabhadraswamy Temple Road, Bonthapally Village, 329 310027 Hangzhou, People’s Republic of China
Jianglin Zhang — Department of Microbiology, and Department of Dermatology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, 310058 Hangzhou, People’s Republic of China
Rakesh Dontha — Department of Chemistry, Zhejiang University, 310027 Hangzhou, People’s Republic of China; Present Address: Glenmark Life Sciences, MIDC Industrial Area, Mahape, Navi Mumbai, Maharashtra 400709, India
Jiyong Liu — Department of Chemistry, Zhejiang University, 310027 Hangzhou, People’s Republic of China
Bernhard Spingler — Department of Chemistry, University of Zurich, 8057 Zurich, Switzerland; orcid.org/0000-0003-3402-2016

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acscentsci.1c01132

Author Contributions
R.V., F.Y., and R.D. contributed equally to this paper.

Author Contributions
R.V., S.v.d.V., and S.D. designed the study and wrote the paper. R.V. and R.D. synthesized and characterized all new compounds. F.Y. and J.Z. evaluated the bioactivities of all compounds. J.L. and B.S. carried out the X-ray diffraction analyses. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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