ACS OMEGA

Open Access



http://pubs.acs.org/journal/acsodf

Article

# Design and Synthesis of D-Ala-D-Ala Silica Gel for a Binding Mode-Based Physicochemical Screening Method

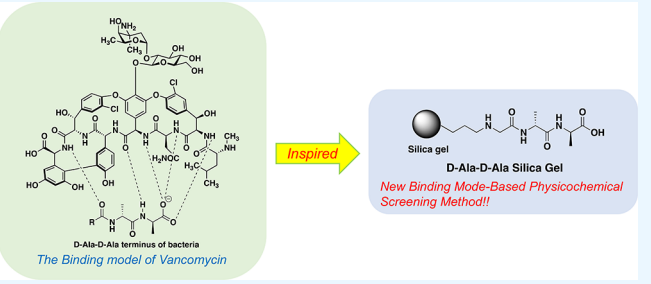
Aoi Kimishima, Yuki Suzuki, Shohei Kosaka, Masako Honsho, Sota Honma, Kazunari Sakai, Hayama Tsutsumi, Yuki Inahashi, Yoshihiro Watanabe, Masato Iwatsuki,* Yukihiro Asami,* and Takuji Nakashima*

Cite This: *ACS Omega* 2023, 8, 39035−39040

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

**ABSTRACT:** Vancomycin is a potent and broad-spectrum antibiotic that binds to the D-Ala-D-Ala moiety of the growing bacterial cell wall and kills bacteria. This fascinating binding model prompted us to design and synthesize D-Ala-D-Ala silica gels for the establishment of a new physicochemical (PC) screening method. In this report, we confirmed that vancomycin binds to D-Ala-D-Ala silica gel and can be eluted with MeOH containing 50 mM TFA. Finally, D-Ala-D-Ala silica gel enables to purify vancomycin from the culture broth of a vancomycin-producing strain, *Amycolatopsis orientalis*.




## INTRODUCTION

Many valuable and useful natural products have been isolated from microorganisms since the discovery of penicillin, and we have put numerous efforts into discovering secondary metabolites produced by microorganisms to discover new drug candidates.[1] In general, bioassay-guided fractionation (BGF) has been well-known as an approach to purify bioactive compounds.[2] However, the discovery of new compounds has gradually decreased due to the repurification of known compounds.[3] In order to discover new compounds efficiently, we have focused on a physicochemical (PC) screening method, which is based on the physicochemical properties using spectral analyses such as UV and MS, as well as color reactions.[3] As a result of PC screening, we have identified novel natural products.[3] The most notable example from these efforts is staurosporine,[4] which has been identified as a pan protein kinase inhibitor.[5] From this finding, staurosporine was derivatized to midostaurin,[6] which has been approved by the United States Food and Drug Administration (FDA).[7]

The relationship between the development of new antibiotics and the emergence of drug-resistant bacteria is a cat-and-mouse game. In contrast, vancomycin, isolated in 1957, is one of the oldest antibiotics in clinical use, being used for more than 60 years as a drug of the last resort.[8] The long-term clinical use of vancomycin would be due to its unique molecular target, contributing to its efficacy as a broad-spectrum antibiotic. Vancomycin binds to the D-Ala-D-Ala moiety of the growing bacterial cell wall (Figure 1).[9] After binding, it prevents the cell wall from forming cross-linking via transpeptidation. We envisioned that ligand binding affinity with the D-Ala-D-Ala moiety would be utilized for column chromatography to establish a binding mode-based PC screening that enables the discovery of new broad-spectrum antibiotics such as vancomycin.

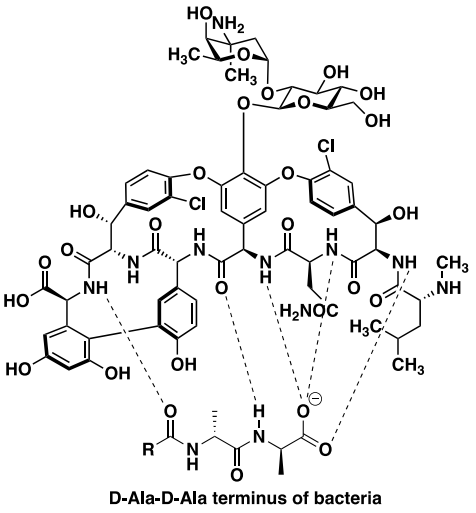
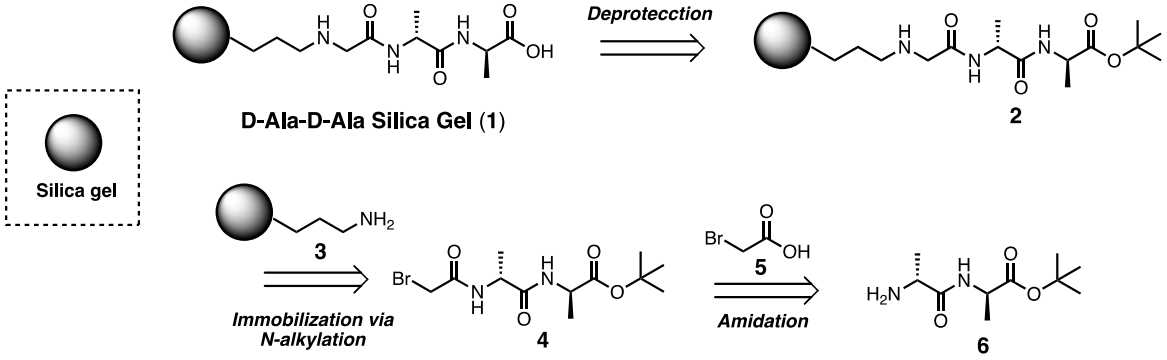


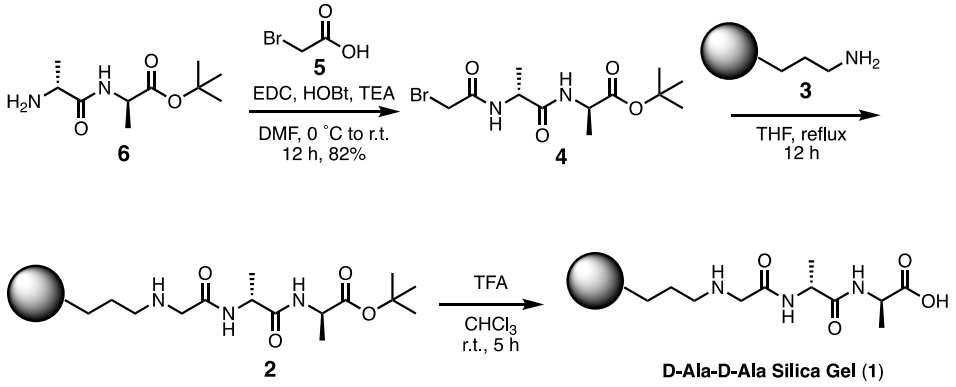

**Figure 1.** Binding model of vancomycin to the D-Ala-D-Ala terminus of bacteria.

**Received:** May 31, 2023
**Accepted:** September 20, 2023
**Published:** October 9, 2023

Scheme 1. Design of D-Ala-D-Ala Silica Gel (1)




Scheme 2. Synthesis of D-Ala-D-Ala Silica Gel (1)




In this study, we designed and synthesized D-Ala-D-Ala silica gel enabling purification of vancomycin from the culture broth of a vancomycin-producing strain, *A. orientalis* NBRC 12806, and showed the utility of our binding mode-based PC screening for the discovery of new broad-spectrum antibiotics.

## RESULTS AND DISCUSSION

**Design and Synthesis of D-Ala-D-Ala Silica Gel.** We chose NH-silica gel for D-Ala-D-Ala silica gel because immobilization of a D-Ala-D-Ala derivative would proceed under relatively mild conditions. D-Ala-D-Ala silica gel (**1**) was designed as shown in Scheme 1. D-Ala-D-Ala silica gel (**1**) would be synthesized by deprotection of the carboxylic acid group of **2**. Immobilization of D-Ala-D-Ala derivative **4** and NH-silica gel **3** progressed via *N*-alkylation. D-Ala-D-Ala derivative **4**[10] would be obtained by amidation with bromo acetic acid (**5**) from known compound **6**.[11] Our synthesis commenced with amidation of **6** with **5** using the peptide coupling condition (Scheme 2). Immobilization of **4** to amino silica gel **3** was accomplished under reflux conditions. Final deprotection of the carboxylic acid group was carried out using TFA to afford desired product **1**.

**Validation of D-Ala-D-Ala Silica Gel Methodology.** With D-Ala-D-Ala silica gel (**1**) in hand, we verified whether vancomycin would elute after binding to the D-Ala-D-Ala moiety. We prepared a 0.1 mg/mL solution of vancomycin and loaded the solution on a column filled with D-Ala-D-Ala silica gel and screened eluents. We attempted to elute vancomycin with water, MeOH, and 50 mM TFA in MeOH. After collecting fractions for the various eluents, we tested the antibacterial activity of each fraction against methicillin-resistant *Staphylococcus aureus* (MRSA) by a paper disk method (Table 1). The 50 mM TFA in MeOH fractions exhibited antibacterial activity, while the fractions from $H_2O$ and MeOH eluents did not. This result indicates that vancomycin binds to **1** and can be eluted with MeOH containing 50 mM TFA and prompted us to validate the utility of **1** using the culture broth of a vancomycin-producing strain, *A. orientalis* NBRC 12806. According to the literature of the production of vancomycin from *A. orientalis*,[12] we tried to make a vancomycin containing broth with minor modification. Unfortunately, no production of vancomycin was observed, and we screened culture conditions using a "one strain many compounds" (OSMAC) approach.[13] As a result of optimizations, we found the two media (glycerol-molasses medium (A) and dextrin-soybean meal medium (B)) for vancomycin production and chose the media that did not produce vancomycin (C), as a negative control. With three broths in hand, we conducted purification of vancomycin using **1** and collected each fraction to evaluate anti-MRSA activity by a paper disk method. To our delight, the fraction of 50 mM TFA in MeOH from (A) and (B) broths showed a zone of inhibition bigger than that of cultured broths, indicating that vancomycin is successfully purified by **1** and concentrated at the fractions (Table 2). Furthermore, we carried out LC-MS analysis of each fraction (Figures 2 and 3), with the desired

Table 1. Anti-MRSA Activity of Each Fraction[a]

| fraction | flow through | $H_2O$ | MeOH | 50 mM TFA in MeOH | VCM 3 μg |
|---|---|---|---|---|---|
| inhibition zone diameter (mm) | – | – | – | 7.6 | 10.6 |

[a]VCM: Vancomycin hydrochloride. –: No inhibition.



https://doi.org/10.1021/acsomega.3c03837
ACS Omega 2023, 8, 39035−39040

**Table 2. Anti-MRSA Activity of Each Fraction in Production Media A, B, and C[a]**

| production medium | amount (μL/disk) | inhibition zone diameter (mm) | | | | |
|---|---|---|---|---|---|---|
| | | cultured broth | flow through | $H_2O$ | MeOH | 50 mM TFA in MeOH |
| A | 10 | 8.0 | – | – | – | 9.8 |
| B | 10 | 7.0 | – | – | – | 8.0 |
| C | 10 | – | – | – | – | – |
| VCM (0.3 mg/mL aq.) | 10 | 11.5 | | | | |

[a]VCM: Vancomycin hydrochloride. –: No inhibition.

vancomycin peak being detected at 4.9 min ($m/z$ = 1448.44 $[M + H]^+$) (Figure 2). Predictably, the vancomycin peak was observed in the fraction of 50 mM TFA in MeOH from production medium A but not the other fractions (Figure 3b–e). These results show that D-Ala-D-Ala silica gel is an innovative new binding mode-based PC screening method for the discovery of new broad-spectrum antibiotics.

## CONCLUSIONS

The binding model of vancomycin to the bacterial D-Ala-D-Ala moiety inspired us to design and synthesize D-Ala-D-Ala silica gels for the establishment of a new PC screening method. We confirmed that vancomycin binds to **1** and can be eluted with 50 mM TFA in MeOH. Furthermore, D-Ala-D-Ala silica gel enable to purify vancomycin from the culture broth of a vancomycin-producing strain, *A. orientalis* NBRC 12806, showing the utility of this approach as a binding mode-based PC screening method. We are currently screening new bioactive compounds by using this system. The results will be reported in due course.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** High- and low-resolution mass spectra were measured using an AB Sciex Triple TOF™ 5600+ LC-MS/MS Systems (AB Sciex, Framingham, MA, USA) and carbon content of immobilized silica gel was measured with elemental analysis by the combustion method using Vario EL III (Elementar, Langenselbold, Germany).

**Fermentation.** One loop of mycelia from a slant of *A. orientalis* NBRC 12806 was inoculated into a test tube containing 10 mL of a seed culture medium (0.1% glucose, 2.4% starch, 3.0% peptone, 0.3% meat extract, 0.5% yeast extract, and 0.4% $CaCO_3$, pH 7.0). The test tube was incubated on a rotary shaker (300 rpm) at 27 °C for 3 days to give a seed culture. One-hundred microliters of the seed culture was inoculated into culture test tubes containing 10 mL of each production medium [glycerol-molasses medium (2.0% glycerol, 0.1% $CaCO_3$, 1.0% molasses, 0.5% casein, 0.1% hipolypepton) and dextrin-soybean meal medium (2.0% dextrin, 0.2% glucose, 1.5% soybean meal, 0.3% yeast extract, 0.3% $CaCO_3$)]. Fermentation was carried out on a shaker (300 rpm) at 27 °C for 6 days.

**Synthesis of 4.**[10] To a solution of hydrochloride salts **6** (16.1 g, 50.0 mmol) and **5** (7.0 g, 50.0 mmol) in DMF (400 mL) were added triethylamine (TEA) (7.0 mL, 50.0 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) (10.5 g, 55.0 mmol), and 1-hydroxybenzotriazole monohydrate (HOBt) (8.4 g, 55.0 mmol) at 0 °C. The reaction mixture was allowed to warm to rt. After stirring for 12 h, the organic solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ and washed with 5% $NaHCO_3$ aq, 1 N HCl aq, and water. The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure to afford **4** (13.83 g, 82% yield).

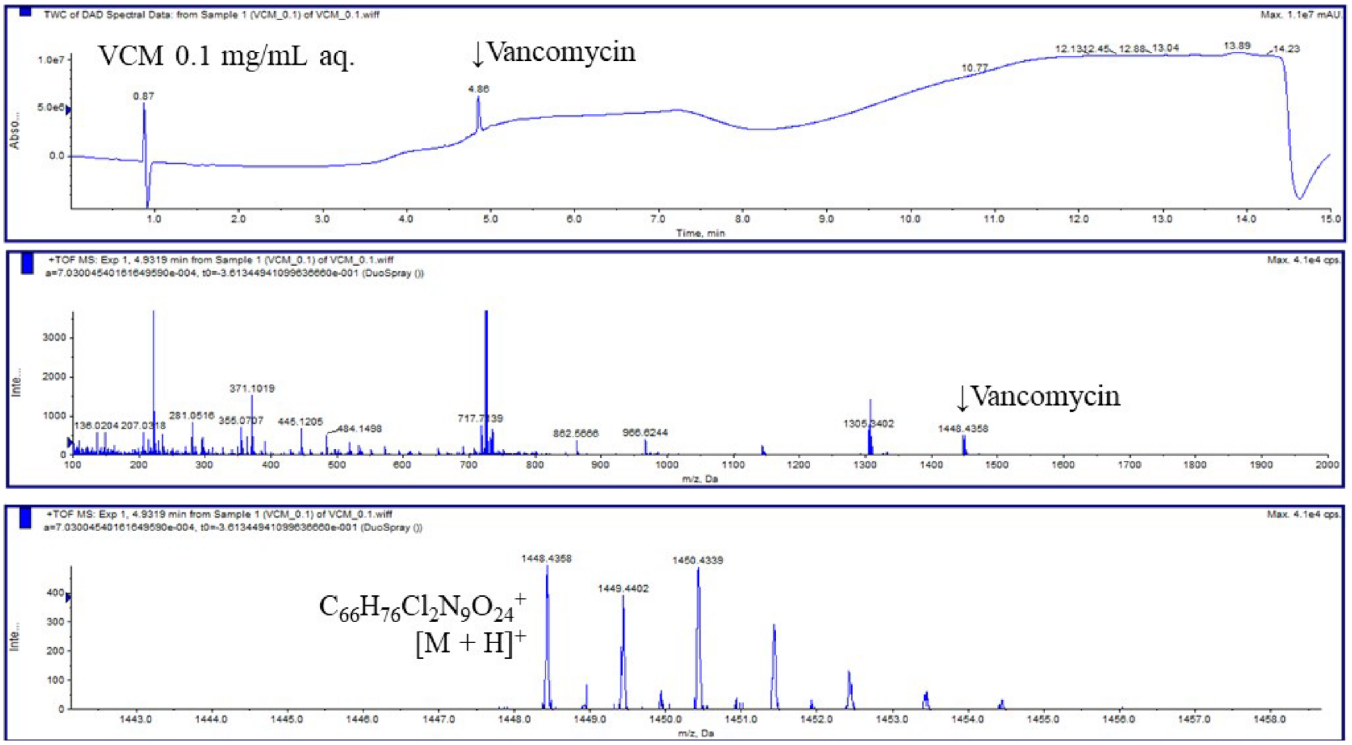



**Figure 2.** LC-MS chart of vancomycin 0.1 mg/mL aq.



https://doi.org/10.1021/acsomega.3c03837
ACS Omega 2023, 8, 39035−39040

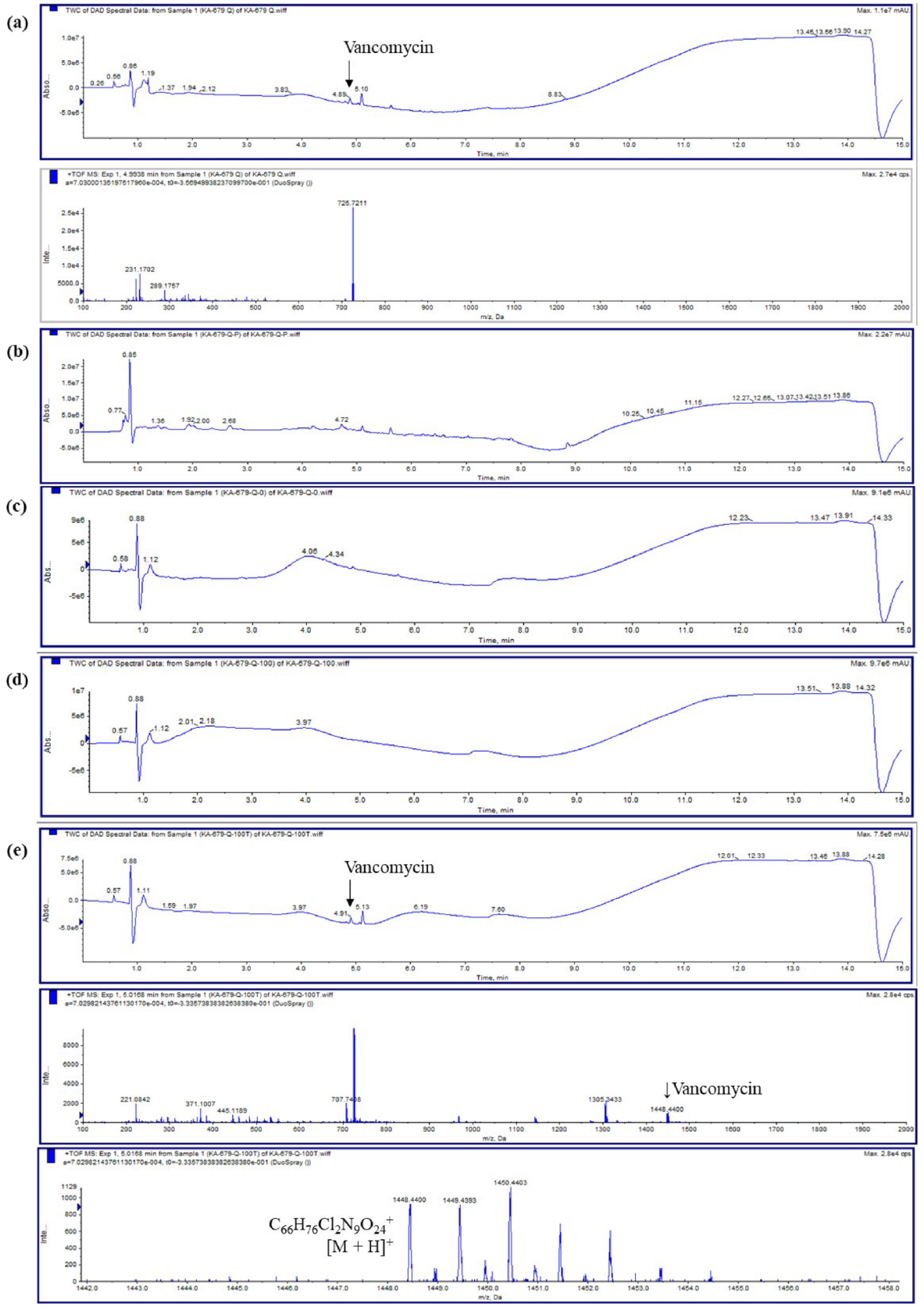



**Figure 3.** LC-MS chart of *A. orientalis* NBRC 12806 medium A cultured broth and each fraction from D-Ala-D-Ala silica gel. Vancomycin was detected at 4.9 min ($m/z$ = 1448.44 $[M + H]^+$). (a) Cultured both; (b) Flow through fraction; (c) $H_2O$ fraction; (d) MeOH fraction; (e) 50 mM TFA in MeOH fraction.



https://doi.org/10.1021/acsomega.3c03837
*ACS Omega* 2023, 8, 39035−39040

**Synthesis of 2.** To a suspension of NH-silica (Chromatorex NH MB100-75/200, Fuji Silysia Chemical Ltd.) **3** (115.0 g) in THF (500 mL) was added **4** (11.6 g, 35.0 mmol) at rt. The reaction mixture was refluxed. After stirring for 12 h, the reaction mixture was cooled to rt and filtered. The filter cake was washed with THF and MeOH and dried under reduced pressure to afford **2** (C%, increase amount rate of 3%, modification amount of 211 mmol/g), which was treated with acetic anhydride to protect unreacted amino groups. To a solution of **2** (120 g) in DMF (300 mL) was added acetic anhydride (150 mL) at rt. After the mixture was stirred for 2 h, the reaction mixture was filtered. The filter cake was washed with MeOH and dried under reduced pressure. This process was done twice.

**Synthesis of 1.** To a solution of acetylated **2** in $CHCl_3$ (90 mL) was added TFA (270 mL) at rt. After being stirred for 5 h, the reaction mixture was filtered. The filter cake was washed with THF and MeOH. The residue was dissolved in 5% $NaHCO_3$ and filtered again. The filter cake was washed with 0.5 N HCl and water. The residue was dried under reduced pressure to afford **1** (C%, decrease amount rate 1%, modification amount 210 mmol/g).

**Vancomycin Purification using D-Ala-D-Ala Silica Gel.** D-Ala-D-Ala silica gel of 0.3 g in a syringe (13 mm i.d. × 10 mm) was swelled with MeOH and then equilibrated with Milli-Q. The 0.1 mg/mL vancomycin solution and each production broth supernatant of 1 mL were loaded on the equilibrated D-Ala-D-Ala silica gel column. Vancomycin bound to the column was sequentially eluted with Milli-Q, MeOH, and 50 mM TFA in MeOH of 4 mL each. The flow through fraction, the water, the MeOH, and the 50 mM TFA in MeOH fractions were collected and used in the antimicrobial activity assay.

**Antimicrobial Activity.** The antimicrobial activity against *S. aureus* ATCC 43300 was determined using the paper disk method (6 mm disc from Advantec Co., Ltd., Tokyo, Japan). Sterile filter discs impregnated with compound solutions were placed on agar plates, and the plates were incubated. After incubation, inhibitory zones were determined. Culture conditions were as follows: 3.8% Mueller Hinton II agar (Becton Dickinson Co., Japan), 1.0% inoculation, 37 °C, 16 h.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03837.

LC-MS chart of each fraction from culture broths (B and C), daptomycin, and teicoplanin applied to D-Ala-D-Alasilica gel (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Authors

**Masato Iwatsuki** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*; orcid.org/0000-0001-8373-4314; Email: iwatuki@lisci.kitasato-u.ac.jp

**Yukihiro Asami** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*; orcid.org/0000-0003-1821-9169; Email: yasami@lisci.kitasato-u.ac.jp

**Takuji Nakashima** − *Research Organization for Nano and Life Innovation, Waseda University, Tokyo 162-0041, Japan*; Email: takuji@aoni.waseda.jp

### Authors

**Aoi Kimishima** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*

**Yuki Suzuki** − *Graduate School of Infection Control Sciences, Kitasato University, Tokyo 108-8641, Japan*

**Shohei Kosaka** − *Research & Development Group, Fuji Silysia Chemical Ltd., Kasugai, Aichi 487-0013, Japan*

**Masako Honsho** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*

**Sota Honma** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*

**Kazunari Sakai** − *Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*

**Hayama Tsutsumi** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*

**Yuki Inahashi** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*

**Yoshihiro Watanabe** − *Graduate School of Infection Control Sciences and Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan*; orcid.org/0000-0002-5756-0530

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c03837



### Funding

This study was partially supported by the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS), the Japan Agency for Medical Research & Development (AMED) under grant number JP21am0101096.


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We are grateful to Distinguished Emeritus Professor S. Ōmura (Kitasato University, Tokyo, Japan) for his helpful support and valuable suggestions.

## ■ REFERENCES


(1) Ōmura, S. A Splendid Gift from the Earth: The Origins and Impact of the Avermectins (Nobel Lecture). *Angew. Chem., Int. Ed. Engl.* **2016**, *55* (35), 10190−10209.

(2) Weller, M. G. A Unifying Review of Bioassay-Guided Fractionation, Effect-Directed Analysis and Related Techniques. *Sensors* **2012**, *12* (7), 9181−9209.

(3) Nakashima, T.; Takahashi, Y.; Ōmura, S. Search of for new compounds from Kitasato microbial library by physicochemical screening. *Biochem. Pharmacol.* **2017**, *134*, 42−55.

(4) Ōmura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchya, H.; Takahashi, Y.; Masuma, R. A new alkaloid AM-2282 of *Streptomyces* origin. Taxonomy, fermentation, isolation and preliminary characterization. *J. Antibiot.* **1977**, *30* (4), 275−282.

(5) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. Staurosporine, a potent inhibitor of phospholipid/$Ca^{2+}$

dependent protein kinase. *Biochem. Biophys. Res. Commun.* **1986**, *135* (2), 397−402.
(6) Lena, A.; Toppino, A.; Miele, K.; Roletto, J.; Paissoni, P. Process for the preparation of midostaurin with high purity. 2023, WO2020200945A1.
(7) Manley, P. W.; Weisberg, E.; Sattler, M.; Griffin, J. D. Midostaurin, a Natural Product-Derived Kinase Inhibitor Recently Approved for the Treatment of Hematological Malignancies. *Biochemistry* **2018**, *57* (5), 477−478.
(8) Rubinstein, E.; Keynan, Y. Vancomycin Revisited - 60 Years Later. *Front. Public Health* **2014**, *2*, 217.
(9) Perkins, H. R. Vancomycin and related antibiotics. *Pharmacol. Ther.* **1982**, *16*, 181−197.
(10) Goodacre, J.; Jeffries, L.; Nayler, J. H.; Ponsford, R. J.; Stirling, I. Antibacterial halogenoacetyl derivatives of amino acids and simple peptides. *J. Med. Chem.* **1977**, *20* (11), 1445−1448.
(11) Nakashima, E.; Yamamoto, H. Biomimetic Peptide Catalytic Bond-Forming Utilizing a Mild Brønsted Acid. *Chem. - Eur. J.* **2022**, *28* (35), No. e202103989.
(12) Jung, H. M.; Kim, S. Y.; Hyun, H. H.; Lee, J. K. $Ca^{2+}$ and $Cu^{2+}$ supplementation augments vancomycin production by *Amycolatopsis orientalis*. *Biotechnol. Lett.* **2002**, *24*, 293−296.
(13) Bode, H. B.; Bethe, B.; Höfs, R.; Zeeck, A. Big Effects from Small Changes: Possible Ways to Explore Nature's Chemical Diversity. *ChemBioChem* **2002**, *3* (7), 619−627.


https://doi.org/10.1021/acsomega.3c03837
*ACS Omega* 2023, 8, 39035−39040