ACS OMEGA

Open Access

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

Article

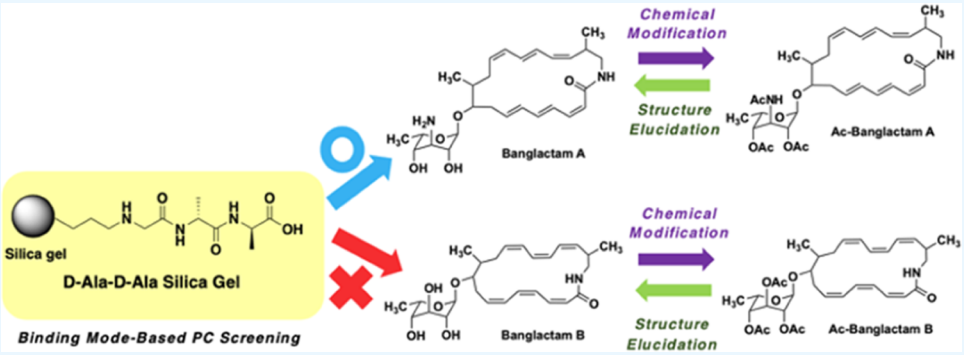
Binding Mode-Based Physicochemical Screening Method Using D-Ala-D-Ala Silica Gel and Chemical Modification Approach to Facilitate Discovery of New Macrolactams, Banglactams A and B, from *Nonomuraea bangladeshensis* K18−0086

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

Cite This: *ACS Omega* 2024, 9, 17415−17422

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

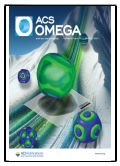


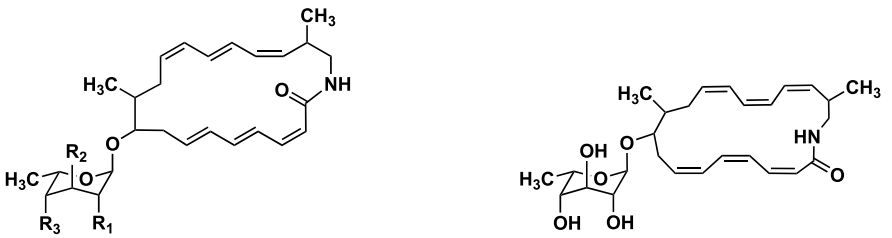
ABSTRACT: Utilizing a binding mode-based physicochemical screening method using D-Ala-D-Ala silica gel, two new macrolactams, named banglactams A (**1**) and B (**2**), were discovered from the culture broth of *Nonomuraea bangladeshensis* K18−0086. In the course of our investigation, we found that D-Ala-D-Ala silica gel precisely differentiated the chemical structures of banglactams and separated them. However, we were not able to obtain enough of **1** to elucidate the structure due to its instability and insolubility. To overcome this challenge, we chemically modified **1** to improve solubility, enabling us to obtain a sufficient material supply for the indirect determination of the structure. Antibacterial activity evaluation of banglactams revealed that **1** binding to D-Ala-D-Ala silica gel exhibited antibacterial activity against *Staphylococcus aureus*; however, this was not the case with **2**. This research indicates the utility of our original binding mode-based PC screening method, and the combination strategy of PC and chemical modifications led us to discover novel antibacterial compounds.

INTRODUCTION

Thousands of natural products have been discovered from microbial sources so far. Natural products have facilitated the development of novel drugs including agrochemicals such as pyripyropene and avermectin.[1−3] As a general approach for screening of drug candidates, bioassay-guided fractionation has been employed for decades[4] and several bioactive compounds have been identified based on their potent biological activities. However, the discovery of new natural products has progressively decreased due to the repurification of known bioactive compounds.[5] In contrast to this situation, the cutting edge of whole-genome sequencing technology has shown that diverse and unexplored biosynthetic gene clusters are encoded in many microorganisms.[6,7] They are now being recognized as a potential microbial source of new agrochemicals and drugs. To address this issue, we have worked on physicochemical (PC) screening[8] to discover new natural compounds guided by physicochemical properties instead of their biological activities. This strategy is opposite to bioassay-guided fractionation and is a superior method for efficient discovery of new compounds. Our research group has discovered several new compounds, such as hatsusamides,[9] pochoniolides,[10] and shikinefragalides,[11] based on this concept. After their initial discovery, many of them were found to show useful biological activities, and staurosporine[12] was demonstrated to be a pan protein kinase inhibitor,[13] leading to the innovation of an antineoplastic molecular target drug, midostaurin.[14] These successes encouraged us to create a new PC screening method.

Received: January 5, 2024
Revised: March 9, 2024
Accepted: March 19, 2024
Published: April 5, 2024



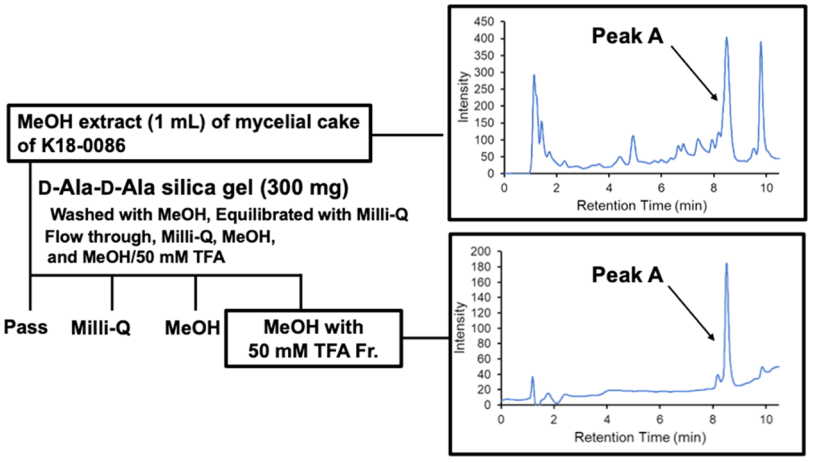


	R_1	R_2	R_3
Banglactam A (1)	−OH	$-NH_2$	−OH
Ac-banglactam A (3)	−OAc	−NHAc	−OAc

Banglactam B (2)

Figure 1. Structure of banglactams.

Scheme 1. Separation Scheme of Peak A and the HPLC Analysis



Vancomycin is a broad-spectrum antibiotic, used very commonly in the hospital to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections and empirically in severe infections of unknown cause, that binds to the D-Ala-D-Ala moiety of the bacterial cell wall.[15] This exceptional binding model inspired us to design and synthesize D-Ala-D-Ala silica gels as a new PC screening method.[16] We established the method which enabled us to purify vancomycin from the culture broth of a vancomycin-producing strain, *Amycolatopsis orientalis*.[16] In this paper, we describe our original PC screening, which led us to discover two new macrolactams, named banglactams A (**1**) and B (**2**) (Figure 1), from the culture broth of *Nonomuraea bangladeshensis* K18−0086. Throughout our investigation, we came across challenges in the isolation and characterization of **1** due to its instability and extremely low solubility. A chemical modification approach enabled us to isolate acetyl-banglactam A (**3**), facilitating the determination of the structure of **1**.

RESULTS AND DISCUSSION

Screening of Our In-House Microbial Secondary Metabolites Library Using a D-Ala-D-Ala Silica Gel and Isolation of 1. We screened our microbial library using D-Ala-D-Ala silica gel and found a hit culture broth of *N. bangladeshensis* K18−0086 (Figure S2). According to the procedure reported by us,[16] we detected a compound (Peak A) as a major component, which bound to D-Ala-D-Ala silica gel and was eluted with a solution of MeOH containing 50 mM TFA (Scheme 1). Based on liquid chromatography−mass spectrometry (LC−MS) analysis of peak A, the molecular formula of the target compound was proposed as *m*/*z* 473.3005, and we attempted to isolate the compound to characterize the structure from the 12-day-old culture broth (6 L) of the strain guided by LC-ESI-DAD-MS. The MeOH extract of the mycelial cake was separated by an HP20 column and purified by high-performance liquid chromatography (HPLC) (Scheme S1 and Figure S3−1) to afford the target compound, banglactam A (**1**, 0.9 mg), as a yellow powder. The molecular formula of **1** was determined as $C_{27}H_{40}N_2O_5$ by high-resolution-ESI-MS (HR-ESI-MS) analysis (*m*/*z* 473.3005, calculated for $C_{27}H_{41}N_2O_5$ $[M + H]^+$ 473.3010), requiring nine degrees of unsaturation (Figure S4−1−1). During our investigations, the compound was found to be unstable, especially under light, and demonstrated extremely low solubility. Attributing to these physicochemical properties, we could not obtain a sufficient amount of the compound to fully elucidate the structure. However, it seemed that **1** was still contained in the mycelial cake; therefore, we decided to modify the compound to improve the solubility.

Derivatization of the Target Compound for the Structure Elucidation. Based on HR-ESI-MS and infrared (IR) analyses, the presence of hydroxyl and amine groups in the compound was anticipated. As a first trial, the introduction of the *tert*-butoxycarbonyl group, which is a well-known protecting group for amines, was investigated. As the reaction time passed, the substrate gradually decomposed, and the corresponding product was not obtained. Due to the unexpectedly low reactivity of the nucleophilic functional

https://doi.org/10.1021/acsomega.4c00145
ACS Omega 2024, 9, 17415−17422

Scheme 2. Chemical Modification of the Mycelial Cake

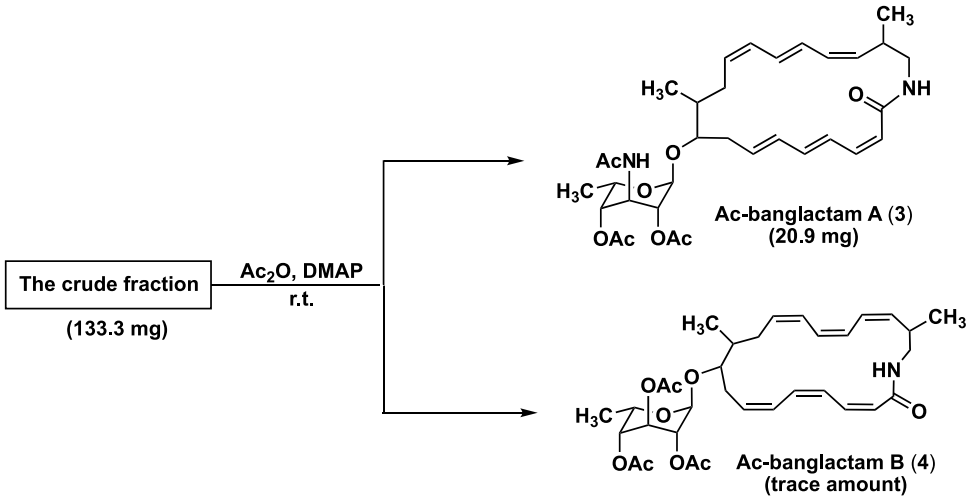


Table 1. ^{1}H and ^{13}C NMR Data of 2′*O*, 3′*N*, and 4′*O*-Triacetylbanglactam A (3) Measured in $CDCl_3$

position	2′*O*, 3′*N*, 4′*O*-triacetylbanglactam A (3) δ_C[a], type	δ_H (mult., *J* in Hz)[b]	HMBC	COSY
1	167.3, C	–		
2	121.3, CH	5.61 (d, 11.4)	1, 3, 4	3
3	139.1, CH	6.32 (dd, 11.5, 11.4)	1, 2, 4, 5	2, 4
4	127.3, CH	7.05 (dd, 14.7, 11.5)	2, 3, 5, 6	3, 5
5	138.4, CH	6.34 (m)	3, 4, 6, 7	4, 6
6	134.3, CH	6.23 (dd, 15.2, 10.8)	4, 5, 7, 8	5, 7
7	131.9, CH	5.80 (m)	5, 6, 8, 9	6, 8
8	36.3, CH_2	2.55 (m)	6, 7, 9, 10	7, 9
9	85.1, CH	3.40 (m)	7, 8, 10, 11, 20, 1′	8, 10
10	37.6, CH	1.47 (m)	8, 9 11, 12, 20	9, 11, 20
11	36.0, CH_2	1.69 (m)	9, 10, 12, 13, 20	10, 12
		2.07 (m)		10, 12
12	132.0, CH	5.68 (ddd, 14.0, 12.2, 3.9)	10, 11, 13, 14	11, 13
13	132.4, CH	6.01 (dd, 12.2, 11.2)	11, 12, 14, 15	12, 14
14	133.1, CH	6.10 (m)	12, 13, 15, 16	13, 15
15	126.9, CH	6.40 (dd, 14.8, 11.2)	13, 14, 16, 17	14, 16
16	130.5, CH	6.06 (dd, 11.2, 10.8)	14, 15, 17, 18	15, 17
17	134.5, CH	5.03 (dd, 10.8, 10.7)	15, 16, 18, 19, 21	16, 18
18	31.5, CH	3.35 (m)	16, 17, 19, 21	17, 19, 21
19	46.2, CH_2	2.49 (m)	1, 17, 18, 21	18, NH-1
		3.69 (ddd, 13.6, 7.1, 3.9)		18, NH-1
20	16.9, CH_3	1.01 (d, 6.6)	9, 10, 11	10
21	18.6, CH_3	0.97 (d, 6.6)	17, 18, 19	18
NH-1	–	5.65 (m)	1, 2, 18, 19	19
1′	99.4, CH	4.90 (br. s)	9, 2′, 3′, 5′	
2′	69.4, CH	4.78 (br. d, 3.7)	1′, 3′, 4′	
3′	44.9, CH	4.65 (ddd, 8.6, 3.7, 3.7)	1′, 2′, 4′, 5′	
NH-3′	–	5.76 (br. d, 8.6)	2‴	
4′	70.1, CH	5.11 (br. d, 3.7)	2′, 3′, 5′, 6′	
5′	65.4, CH	4.23 (br. q, 6.5)	1′, 3′, 4′, 6′	6′
6′	16.4, CH_3	1.13 (d, 6.5)	4′, 5′	5′
1″	21.2, CH_3	2.16 (s)[c]	2″	3″, NH-2″
2″	170.6, C	–		3″, NH-2″
1‴	23.2, CH_3	1.96 (s)	2‴	
2‴	169.5, C	–		3‴
1‴′	20.8, CH_3	2.17 (s)[c]	2‴′	2‴, 4‴
2‴′	170.7, C	–		

[a]Measured at 100 MHz. [b]Measured at 400 MHz. [c]Overlapped.

groups, we chose a more reactive reagent, acetic anhydride, which successfully progressed to finally furnish acetylated product **3** (Scheme 2). To our delight, this chemical modification enabled us to obtain 20.8 mg of **3** by simple preparative TLC (Figure S3−2). Interestingly, this transformation also provided a new analogue of **3**, Ac-banglactam B (**4**) as a minor product (<1 mg). Acetylation dramatically improved the solubility of **1** as we expected, and we advanced to the elucidation of the structure of **3**. The molecular formula of compounds **3** and **4** was determined as $C_{33}H_{46}N_2O_8$ by HR-ESI-MS analysis (*m*/*z* 599.3302, calculated for $C_{33}H_{47}N_2O_8$ $[M + H]^+$ 599.3327) and $C_{33}H_{45}NO_9$ by HR-ESI-MS analysis

https://doi.org/10.1021/acsomega.4c00145
ACS Omega 2024, 9, 17415−17422

(m/z 600.3178, calculated for $C_{33}H_{46}NO_9$ $[M + H]^+$ 600.3167), requiring 12 degrees of unsaturation (Figure S4−3−1 and S4−4−1), which indicated compounds **3** and **4** should be triacetylated from compounds **1** and **2**. The ^1H NMR spectrum of compound **3** in $CDCl_3$ (Table 1 and Figure S4−3−4) revealed one amide NH proton signal (δ_H 7.65), 12 olefinic proton signals (δ_H 7.05, 6.40, 6.34, 6.32, 6.23, 6.10, 6.06, 6.01, 5.80, 5.68, 5.61, and 5.03), three doublet methyl signals (δ_H 1.13, 1.01, and 0.97), and three singlet methyl signals (δ_H 2.17, 2.16, and 1.96). The ^{13}C NMR and HSQC spectra of compound **3** (Figure S4−3−5 and S4−3−7) indicated the presence of four amide or ester carbonyl carbons (δ_C 170.7, 170.6, 169.5, and 167.3), 12 olefinic carbons (δ_C 139.1, 138.4, 134.5, 134.3, 133.1, 132.4, 132.0, 131.9, 130.5, 127.3, 126.9, and 121.3), one anomeric sp^3 methine carbon (δ_C 99.4), four oxygen-bearing sp^3 methine carbons (δ_C 85.1, 70.1, 69.4, and 65.4), one nitrogen-bearing sp^3 methine carbon (δ_C 44.9), one nitrogen-bearing sp^3 methylene carbon (δ_C 46.2), two sp^3 methylene carbons (δ_C 36.3 and 36.0), two sp^3 methine carbons (δ_C 37.6 and 31.5), and six methyl carbons (δ_C 23.2, 21.2, 20.8, 18.6, 16.9, and 16.4).

Analysis of the $^1H-^1H$ COSY and HMBC spectra (Figures 2, S4−3−6, and S4−3−8) revealed the presence of a 20-membered macrolactam ring and a sugar moiety as shown in Figure 1.

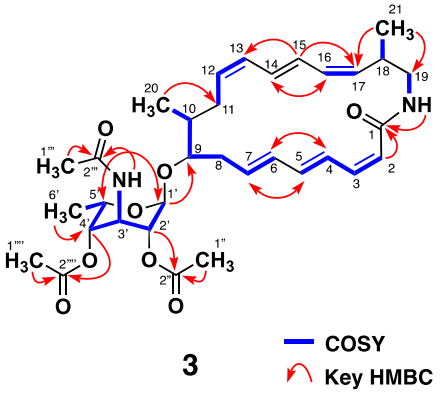


Figure 2. Structure elucidation of 2′*O*, 3′*N*, and 4′*O*-triacetylbanglactam A (**3**).

The macrolactam ring was elucidated by COSY cross-peaks of H-2 (δ_H 5.61)/H-3 (δ_H 6.32)/H-4 (δ_H 7.05)/H-5 (δ_H 6.34), H-6 (δ_H 6.23)/H-7 (δ_H 5.80)/H_2-8 (δ_H 2.55)/H-9 (δ_H 3.40)/H-10 (δ_H 1.47)/ H_3-20 (δ_H 1.01), H_2-11 (δ_H 2.07 and 1.69)/H-12 (δ_H 5.68)/H-13 (δ_H 6.01), H-16 (δ_H 6.06)/H-17 (δ_H 5.03), H-18 (δ_H 3.38)/H_3-21 (δ_H 0.97), and H_2-19 (δ_H 3.69 and 2.49)/NH-1 (δ_H 5.65), as well as cross-peaks in HMBC from NH-1 to C-1 (δ_C 167.3), from H-2 to C-1, from H-4 to C-6 (δ_C 134.3), from H-5 to C-7 (δ_C 131.9), from H-6 to C-4 (δ_C 127.3), from H-7 to C-5 (δ_C 138.4), from H_3-20 to C-11 (δ_C 36.0), from H-14 (δ_H 6.10) to C-16 (δ_C 130.5), from H-15 (δ_H 6.40) to C-13 (δ_C 132.4) and C-17 (δ_C 134.5), from H-16 to C-14 (δ_C 133.1), and from H_3-21 to C-17 and C-19 (δ_C 46.2). The geometry of the six double bonds in the macrolactam ring was determined to be 2*Z*, 4*E*, 6*E*, 12*Z*, 14*E*, and 16*Z* by $^1H-^1H$ coupling constants ($J_{H\text{-}2/H\text{-}3}$ = 11.4 Hz, $J_{H\text{-}4/H\text{-}5}$ = 14.7 Hz, $J_{H\text{-}6/H\text{-}7}$ = 15.2 Hz, $J_{H\text{-}12/H\text{-}13}$ = 12.2 Hz, $J_{H\text{-}14/H\text{-}15}$ = 14.8 Hz, and $J_{H\text{-}16/H\text{-}17}$ = 10.8 Hz).

The sugar moiety was established to be a 2′*O*, 3′*N*, 4′*O*-triacetylated 3′-amino-6-deoxypyranose by COSY cross-peaks of H-1′ (δ_H 4.90)/H-2′ (δ_H 4.78)/H-3′ (δ_H 4.65)/H-4′ (δ_H 5.11)/H-5′ (δ_H 4.23)/H_3-6′ (δ_H 1.13) and H-3′/NH-3′ (δ_H 5.76) combined with HMBC cross-peaks from H-1′ to C-5′ (δ_C 65.4), from H-2′ to C-2″ (δ_C 170.6), from H_3-1″ (δ_H 2.16) to C-2″, from NH-3′ to C-2‴ (δ_C 169.5), from H_3-1‴ (δ_H 1.96) to C-2‴, from H-4′ to C-2⁗′ (δ_C 170.7), from H_3-1⁗′ (δ_H 2.17) to C-2⁗′, and from H_3-6′ to C-4′ (δ_C 70.1). The downfield-shifted ^{13}C chemical shift (δ_C 99.6) of C-1′ suggested that this sugar moiety has a *β*-glycosidic bond. The relative configuration of this sugar moiety was established to be 1′*R**, 2′*R**, 3′*S**, 4′*S**, and 5′*S** by $^1H-^1H$ coupling constants between 1′/2′ (0 Hz), 2′/3′ (3.7 Hz), 3′/4′ (3.7 Hz), and 4′/5′ (0 Hz) (Table 1).

Finally, cross-peaks in HMBC from H-1′ to C-9 (δ_C 85.1) led us to elucidate the planar structure of compound **3** as a 20-membered macrolactam having a 2′*O*, 3′*N*, 4′*O*-triacetylated 3′-amino-6-deoxypyranose moiety at the C-9 position, and we designated compound **3** as 2′*O*, 3′*N*, 4′*O*-triacetylbanglactam A (Ac-banglactam A).

Isolation and the Structure Elucidation of 1. Based on this information, we were able to indirectly determine the structure of banglactam A (**1**) through the structure elucidation of **3**, and the discovery of **4** by the chemical modification made us realize the existence of banglactam B (**2**), which made us to isolate **2**.

Comparing the structural difference between **3** and **4** based on each mass value, we envisioned the proposed structure of **2**, where the 3′ position is substituted with a OH group compared to **1**. We also assumed that the OH group in **2** might improve its solubility and aimed to isolate **2** and directly determine the structure. In fact, the MeOH extract of the mycelial cake was separated by an HP20 column (Scheme S1) and purified by HPLC (Figure S3−1) to afford compound **2** (4.5 mg). As our assumption, **2** is soluble in DMSO, which enabled us to elucidate the structure. Banglactam B (**2**) was isolated as a yellow powder. The molecular formula of compound **2** was determined as $C_{27}H_{39}NO_6$ by HR-ESI-MS analysis (m/z 474.2861, calculated for $C_{27}H_{40}NO_6$ $[M+H]^+$ 474.2850), requiring nine degrees of unsaturation (Figure S4−2−1).

The ^1H NMR spectrum in DMSO-d_6 (Table 2 and Figure S4−2−2) revealed one amide NH proton signal (δ_H 7.94), 12 olefinic proton signals (δ_H 7.18, 6.34, 6.31, 6.28, 6.23, 6.03, 6.00, 5.95, 5.74, 5.66, 5.61, and 5.02), and three doublet methyl signals (δ_H 1.10, 0.90 and 0.86). The ^{13}C NMR and HSQC spectra (Figure S4−2−3 and S4−2−5) indicated the presence of one amide carbonyl carbon (δ_C 166.4), 12 olefinic carbons (δ_C 138.3, 137.5, 135.5, 134.3, 132.5, 132.4, 132.0, 131.8, 129.8, 127.9, 127.2, and 122.2), one anomeric sp^3 methine carbon (δ_C 103.4), five oxygen-bearing sp^3 methine carbons (δ_C 83.6, 72.5, 70.7, 67.1, and 65.5), one nitrogen-bearing sp^3 methylene carbon (δ_C 45.8), two sp^3 methylene carbons (δ_C 35.8 and 34.9), two sp^3 methine carbons (δ_C 37.7 and 30.4), and three methyl carbons (δ_C 18.4, 16.7, and 16.4).

Analysis of the $^1H-^1H$ COSY and HMBC spectra (Figures 3, S4−2−4, and S4−2−6) revealed the presence of a 20-membered macrolactam ring and a sugar moiety as shown in Figure 1.

The macrolactam ring was deduced by COSY cross-peaks of H-2 (δ_H 5.61)/H-3 (δ_H 6.23)/H-4 (δ_H 7.18)/H-5 (δ_H 6.31)/H-6 (δ_H 6.34)/H-7 (δ_H 5.74)/H_2-8 (δ_H 2.57 and 2.49)/H-9 (δ_H 3.31)/H-10 (δ_H 1.19)/H_2-11 (δ_H 2.16 and 1.53)/H-12 (δ_H 5.66)/H-13 (δ_H 6.03)/H-14 (δ_H 6.28)/H-15 (δ_H 6.00)

https://doi.org/10.1021/acsomega.4c00145
ACS Omega 2024, 9, 17415−17422

Table 2. ^{1}H and ^{13}C NMR Data of Banglactam B (2) Measured in DMSO-d_6

	banglactam B (2)			COSY
position	δ_C[a], type	δ_H (mult., J in Hz)[b]	HMBC	TOCSY
1	166.4, C	–		
2	122.2, CH	5.61 (d, 11.2)	1, 3, 4	3
3	138.3, CH	6.23 (dd, 11.2, 11.5)	1, 2, 4, 5	2, 4
4	127.9, CH	7.18 (dd, 11.5, 11.5)	2, 3, 5, 6	3, 5
5	137.5, CH	6.31 (dd, 11.5, 11.1)	3, 4, 6, 7	4, 6
6	134.3, CH	6.34 (dd, 11.2, 11.1)	4, 5, 7, 8	5, 7
7	132.0, CH	5.74 (ddd, 14.8, 11.2, 4.0)	5, 6, 8, 9	6, 8
8	35.8, CH_2	2.49 (m)	6, 7, 9, 10	7, 9
		2.57 (ddd, 14.8, 14.0, 1.8)		7, 9
9	83.6, CH	3.31 (m)	7, 8, 10, 11, 20, 1′	8, 10
10	37.7, CH	1.19 (m)	8, 9 11, 12, 20	9, 11, 20
11	34.9, CH_2	1.53 (ddd, 12.6, 12.6, 2.9)	9, 10, 12, 13, 20	10, 12
		2.16 (dd, 12.6, 11.4)		10, 12
12	131.8, CH	5.66 (ddd, 13.0, 11.4, 2.9)	10, 11, 13, 14	11, 13
13	132.4, CH[c]	6.03 (d, 13.0, 10.3)[c]	11, 12, 14, 15	12, 14
14	127.2, CH	6.28 (dd, 12.6, 10.3)	12, 13, 15, 16	13, 15
15	132.5, CH[c]	6.00 (d, 12.6, 10.5)[c]	13, 14, 16, 17	14, 16
16	129.8, CH	5.95 (dd, 10.8, 10.5)	14, 15, 17, 18	15, 17
17	135.5, CH	5.02 (dd, 10.8, 10.7)	15, 16, 18, 19, 21	16, 18
18	30.4, CH	3.38 (m)	16, 17, 19, 21	17, 19, 21
19	45.8, CH_2	2.37 (ddd, 12.4, 11.0, 5.2)	1, 17, 18, 21	18, NH-1
		3.30 (m)		18, NH-1
20	16.4, CH_3	0.90 (d, 6.0)	9, 10, 11	10
21	18.4, CH_3	0.86 (d, 6.6)	17, 18, 19	18
NH-1	–	7.94 (br. dd, 5.2, 5.2)	1, 2, 18, 19	19
1′	103.4, CH	4.74 (br. s)	9, 2′, 3′, 5′	
2′	70.7, CH	3.53 (br. s)	1′, 3′, 4′	
3′	65.5, CH	3.54 (br. d, 2.4)	1′, 2′, 4′, 5′	
4′	72.5, CH	3.45 (br. s)	2′, 3′, 5′, 6′	
5′	67.1, CH	3.85 (br. q, 6.5)	1′, 3′, 4′, 6′	6′
6′	16.7, CH_3	1.10 (d, 6.5)	4′, 5′	5′

[a]Measured at 150 MHz. [b]Measured at 600 MHz. [c]Exchangeable.

/H-16 (δ_H 5.95)/H-17 (δ_H 5.02)/H-18 (δ_H 3.38)/H_2-19 (δ_H 3.30 and 2.37)/NH-1 (δ_H 7.94), H-10/H_3-20 (δ_H 0.90), and H-18/H_3-21 (δ_H 0.86), as well as cross-peaks in HMBC from H-2 to C-1 (δ_C 166.4) and from NH-1 to C-1. The geometry of six double bonds in the macrolactam ring was determined to be all Z by small $^{1}H-^{1}H$ coupling constants ($J_{H\text{-}2/H\text{-}3}$ = 11.2 Hz, $J_{H\text{-}4/H\text{-}5}$ = 11.5 Hz, $J_{H\text{-}6/H\text{-}7}$ = 11.2 Hz, $J_{H\text{-}12/H\text{-}13}$ = 13.0 Hz, $J_{H\text{-}14/H\text{-}15}$ = 12.6 Hz, and $J_{H\text{-}16/H\text{-}17}$ = 10.8 Hz).

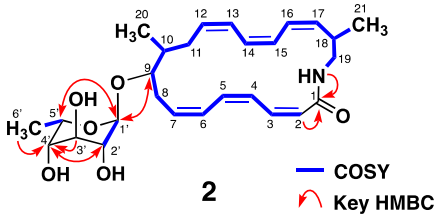


Figure 3. Structure elucidation of banglactam B (2).

The sugar moiety was established to be a 6-deoxypyranose by COSY cross-peaks of H-1′ (δ_H 4.74)/H-2′ (δ_H 3.53) and H-5′ (δ_H 3.85)/H_3-6′ (δ_H 1.10) combined with HMBC cross-peaks from H-1′ to C-5′ (δ_C 67.1), from H-2′ to C-4′ (δ_C 72.5), from H-3′ (δ_H 3.54) to C-4′, from H-4′ (δ_H 3.45) to C-2′ (δ_C 70.7), from H-5′ (δ_H 3.85) to C-1′ (δ_C 103.4), and from H_3-6′ to C-4′. The downfield-shifted ^{13}C chemical shift (δ_C 103.4) of C-1′ suggested that this sugar moiety has a β-glycosidic bond. The relative configuration of this sugar moiety was established to be 1′*S**, 2′*R**, 3′*S**, 4′*S**, and 5′*S** by $^{1}H-^{1}H$ coupling constants between 1′/2′ (0 Hz), 2′/3′ (0 Hz), 3′/4′ (2.4 Hz), and 4′/5′ (0 Hz) (Table 2).

Finally, cross-peaks in HMBC from H-1′ to C-9 (δ_C 83.6) and H-9 to C-1′ led us to elucidate the planar structure of compound **2** as a 20-membered macrolactam having a 6-deoxypyranose moiety at the C-9 position, and we designated compound **2** as banglactam B.

Antibacterial Activity Evaluation of Banglactams. The discovery of banglactams prompted us to evaluate their antibacterial activity against *S. aureus*, which has the D-Ala-D-Ala moiety in the growing cell wall. In this investigation, we sought the relevance of the binding ability of each compound and their antibacterial activity. Compounds **1**–**3** were tested for antibacterial activity against *S. aureus* on a paper disk method (Figure S5). Compound **1** displayed antibacterial activity with inhibition zones of 8.0 mm/30 μg and 7.5 mm/10 μg, respectively, while compounds **2** and **3** showed no antibacterial activity even at 30 μg/disk. This result is consistent with their binding ability to D-Ala-D-Ala silica gel. Considering the structure difference between **1** and **2**, either or both of the 3′-NH_2 moiety and the olefin geometries in the 20-membered macrolactam ring would influence the binding ability to D-Ala-D-Ala silica gel resulting in the exhibition of the antibacterial activity of **1** but not that of **2**. It was also suggested that D-Ala-D-Ala silica gel would be able to precisely recognize the chemical structures of binding compounds, which also suggests the reliability of our previous binding experiments using antibiotics such as daptomycin.[16]

In conclusion, we were fascinated by PC screening as a powerful method for the discovery of novel natural compounds and have established a new binding mode-based PC screening method using D-Ala-D-Ala silica gel. In this report, we displayed the utility of our method based on the discovery of new natural products, banglactams A (**1**) and B (**2**). Throughout their structure analysis, we encountered the issue attributed to the instability and insolubility of **1** and conducted the chemical modification to change the physicochemical property. Acetylation of the mycelial cake containing banglactams dramatically improved the solubility of **1** and made us realize the existence of **2** as a natural product, enabling the determination of the chemical structure of **1**. We believe that the combination strategy of the binding mode-based PC screening method using D-Ala-D-Ala silica gel and chemical

https://doi.org/10.1021/acsomega.4c00145
ACS Omega 2024, 9, 17415−17422

modifications pioneers a new approach in the natural product screening research area. We are currently synthesizing new banglactam A derivatives to improve the stability and solubility, and the result will be reported in due course.

EXPERIMENTAL SECTION

General Experimental Procedures. Reagents and solvents were commercially available and used without further purification. For thin-layer chromatography (TLC) analysis, precoated silica gel plates with a fluorescent indicator (Merck 60 F254, Merck KgaA, Darmstadt, Germany) were used. The Diaion HP20 resin (Mitsubishi Chemical Co., Ltd., Tokyo, Japan) was used for open column chromatography. An HPLC column, Pegasil ODS SP100 (5 μm, 20 i.d. × 250 mm), was purchased for isolation from Senshu Science Co., Ltd. (Tokyo, Japan). Liquid chromatography−ultraviolet (LC-UV) data were taken using system (Agilent Technologies, Santa Clara, CA) with an HPLC column, Symmetry C_{18} 3.5 μm (2.1 i.d. × 150 mm, Waters Co., Ltd., Milford, MA). LC-ESI-MS data were obtained using an AB Sciex Triple TOF 5600$^+$ LC-MS/MS Systems (AB Sciex, Framingham, MA) with a reversed-phase HPLC column, Capcell Core C_{18} (2.7 μm, 3.0 i.d. × 100 mm, Osaka Soda Co., Ltd., Osaka, Japan). NMR spectra were measured in DMSO-d_6 or $CDCl_3$ using an XL-400 spectrometer (Agilent Technologies) or a JNM-ECA-500 spectrometer (JEOL Ltd., Tokyo, Japan) or an AVANCE III HD-600 (Bruker, Billerica, MA) with ^{1}H NMR at 400 or 500 or 600 MHz and ^{13}C NMR at 100 or 125 or 150 MHz, respectively. The chemical shifts are expressed in ppm and are referred to as DMSO-d_6 (2.48 ppm) or $CDCl_3$ (7.26 ppm) in the ^{1}H NMR spectra and DMSO-d_6 (39.5 ppm) or $CDCl_3$ (77.0 ppm) in the ^{13}C NMR spectra, respectively. Infrared (IR) spectra were recorded using a JASCO FT-/IR-4600 spectrometer. UV spectra were measured with a U-2800 spectrophotometer (Hitachi Ltd., Tokyo, Japan). Optical rotation was measured with a JASCO P-2200 polarimeter (JASCO Corporation, Tokyo, Japan).

Taxonomic Studies of Strain K18−0086. Strain K18−0086 was isolated from a cinnamon root collected in Ome, Tokyo, Japan. The strain grew well in International *Streptomyces* Project (ISP) medium 2, forming orange colonies with white aerial mycelia (Figure S2). The 16S rRNA gene sequence analysis was performed according to our method.[17] The strain was identified as *N. bangladeshensis* based on a high similarity value of 99.9% to *N. bangladeshensis* 5−10−10^T (AB274966).

Fermentation and Isolation. We cultured and maintained strain K18−0086 on agar medium (1.0% starch, 0.3% N-Zamine, 0.1% yeast extract, 0.1% meat extract, 0.3% $CaCO_3$, 1.2% agar, and distilled water at pH 7.0). As seed culture, we inoculated a loop of strain K18−0086 into 100 mL of seed medium (2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% of 35% Ehrlich bonito extract, 0.5% yeast extract, and 0.4% $CaCO_3$) (adjusted to pH 7.0 before sterilization) in a 500 mL Erlenmeyer flask. The flask was shaken at 210 rpm for 3 days at 27 °C. We inoculated the seed culture (1 mL) into 100 mL of production medium (2.0% soluble starch, 1.0% defatted wheat germ, 0.5% glycerol, 0.3% dry yeast, 0.3% of 35% Ehrlich bonito extract, and 0.3% CaCO3) in a 500 mL Erlenmeyer flask (total 60 flasks, 6 L), which was incubated on a rotary shaker at 210 rpm for 12 days at 27 °C.

Compounds **1** and **2** were isolated from a 12-day-old cultured material of *N. bangladeshensis* K18−0086 by guided with UV and MS profiles using LC-DAD-ESI-MS analysis (Scheme S1). They were purified under light-shielded conditions due to their light sensitivity. The fermentation broth (6 L) was centrifuged to separate into the mycelial cake and supernatant. To the obtained mycelial cake was added MeOH (10 l), and the mixture was filtrated *in vacuo*. To the obtained MeOH extract solution was added H_2O (15 l), and the mixture was applied to a Diaion HP20 open column (1 L resin, Mitsubishi Chemical Co., Tokyo, Japan) equilibrated with water. The column was eluted stepwise with 80% MeOH aq (3 L) and 100% MeOH (3 L). A part (2 L) of 80% MeOH aq. fraction stocked at 4 °C was concentrated *in vacuo* to remove MeOH and extracted with EtOAc (2 L) several times. The obtained EtOAc layer was concentrated *in vacuo* to remove EtOAc and was added a small amount of MeOH. A part (5 mL) of MeOH aq. solution (20 mL) was subjected to reversed-phase HPLC using a Pegasil ODS SP100 column (20 i.d. × 250 mm, Senshu Scientific Co., Tokyo, Japan) with an isocratic solvent system of CH_3CN-H_2O-TFA (45:55:0.1) at a flow rate of 7.0 mL min^{-1} detected by UV 254 nm. The two fractions with retention times of 13 and 26 min were collected (Figure S3−1), evaporated *in vacuo*, and freeze-dried to afford banglactams A (**1**, 0.9 mg) and B (**2**, 4.5 mg), respectively.

*Banglactam A (**1**).* Yellow powder; ^{1}H, see Figure S4−1−4; HR-ESI-MS m/z 473.3005 $[M + H]^+$ (calcd for $C_{27}H_{41}N_2O_5$, 474.2850).

*Banglactam B (**2**).* Yellow powder; ^{1}H and ^{13}C NMR data, see Table 2; HR-ESI-MS m/z 474.2861 $[M + H]^+$ (calcd for $C_{27}H_{40}NO_6$, 474.2850).

Preparation and Isolation of 2′*O*, 3′*N*, 4′*O*-Triacetylbanglactam A (3). To the ODS column eluate of 100% MeOH fraction (1.5 L, Scheme S1) was added H_2O, concentrated *in vacuo*, and then freeze-dried under the light-shielded condition. The obtained powder was dissolved in pyridine (2.5 mL) and were added 4-dimethylaminopyridine (2 mg) and acetic anhydride at 0 °C. After being stirred for 16 h at room temperature, the mixture was added to EtOAc (25 mL) and 1 M HCl (25 mL). The EtOAc extract was purified by preparative TLC developed with EtOAc, and the fraction (R_f = 0.5) was extracted with MeOH/$CHCl_3$ (1:1) and concentrated *in vacuo* to yield 2′*O*,3′*N*,4′*O*-triacetylbanglactam A (**3**) with 20.8 mg as a yellow powder.

*2′O, 3′N, 4′O-Triacetylbanglactam A (**3**).* $[\alpha]_D^{21}$ −89.6 (c = 0.05, $CHCl_3$); IR (ATR, diamond prism) ν_{max} 2936, 1660, 1371, 1200, 1129, 1026, 799, 722, 529, 493, 451, 430, 418, 407 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HR-ESI-MS m/z 599.3302 $[M + H]^+$ (calcd for $C_{33}H_{47}N_2O_8$, 599.3327).

Antimicrobial Assay in Paper Disk Method. Test strain of *S. aureus* (MSSA FDA-209P), stored at − 80 °C in microbanks (IWAKI&Co., Ltd.), was inoculated into 5 mL of Mueller-Hinton Broth (BD&Co.) and incubated at 37 °C for 20 h with shaking. The test plates were prepared by mixing 1% of the seed culture into 30 mL of Mueller-Hinton Agar (BD&Co.) with 0.05% BSA in a 10−14 cm plate.

Paper disks (6 mm, thin, Advantec Toyo Kaisha, Ltd., Tokyo, Japan) soaked with 10 μL of each sample were placed on the test plates. After incubation at 37 °C overnight, the diameter (mm) of the inhibition zone was measured. Paper disk soaked with 10 μL of 0.3 mg/mL vancomycin aqueous solution was used as a positive control.

https://doi.org/10.1021/acsomega.4c00145
ACS Omega 2024, 9, 17415−17422

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00145.

PC screening using D-Ala-D-Ala silica gel; taxonomy of K18−0086 strain; isolation of banglactams A (**1**) and B (**2**) and derivatization from **1** to **3**; spectral data of banglactams A (**1**) and B (**2**) and triacetylbanglactams A (**3**) and B (**4**); and antibacterial activity on paper disk method (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

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

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

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

Authors

Aoi Kimishima − *Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan; Graduate School of Infection Control Sciences, Kitasato University, Tokyo 108-8641, Japan*; orcid.org/0000-0002-2625-1356

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

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

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

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

Yoshihiro Watanabe − *Ōmura Satoshi Memorial Institute, Kitasato University, Tokyo 108-8641, Japan; Graduate School of Infection Control Sciences, 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.4c00145

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Distinguished Emeritus Professor Satoshi Ōmura (Kitasato University) for his helpful support and valuable guidance and suggestions. They thank Dr. Kenichiro Nagai, Reiko Seki, and Noriko Sato (School of Pharmacy, Kitasato University) for various instrumental analyses. This research was partially supported by Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research [BINDS]) from AMED under Grant Number JP21am0101096 (Phase I) and JP22ama121035 (Phase II). This study was supported by funds from the Institute for Fermentation, Osaka (IFO), Japan.

■ REFERENCES

(1) Newman, D. J.; Cragg, G. M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83* (3), 770−803.

(2) Kandasamy, R.; London, D.; Stam, L.; von Deyn, W.; Zhao, X.; Salgado, V. L.; Nesterov, A. Afidopyropen: New and potent modulator of insect transient receptor potential channels. *Insect Biochem. Mol. Biol.* **2017**, *84*, 32−39.

(3) Burg, R. W.; Miller, M. B.; Baker, E. E.; Birnbaum, J.; Currie, A. S.; Hartman, R.; Kong, Y-L.; Monaghan, L. R.; Olson, G.; Putter, I.; Tunac, B. J.; Wallick, H.; Stapley, O. E.; Oiwa, R.; Ōmura, S. Avermectins, New Family of Potent Anthelmintic Agents: Producing Organism and Fermentation. *Antimicrob. Agents Chemother.* **1979**, *15*, 361−367.

(4) Weller, M. G. A unifying review of bioassay-guided fractionation, effect-directed analysis and related techniques. *Sensors* **2012**, *12* (7), 9181−9209.

(5) Bérdy, J. Thoughts and facts about antibiotics: where we are now and where we are heading. *J. Antibiot.* **2012**, *65* (8), 385−395.

(6) Keller, N. P. Fungal secondary metabolism: regulation, function and drug discovery. *Nat. Rev. Microbiol.* **2019**, *17* (3), 167−180.

(7) Kikuchi, Y.; Kawashima, M.; Iwatsuki, M.; Kimishima, A.; Tsutsumi, H.; Asami, Y.; Inahashi, Y. Comprehensive analysis of biosynthetic gene clusters in bacteria and discovery of *Tumebacillus* as a potential producer of natural products. *J. Antibiot.* **2023**, *76* (6), 316−323.

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

(9) Miyano, R.; Matsuo, H.; Nonaka, K.; Mokudai, T.; Niwano, Y.; Shiomi, K.; Takahashi, Y.; Ōmura, S.; Nakashima, T. Pochoniolides A and B, new antioxidants from the fungal strain *Pochonia chlamydosporia* var. *spinulospora* FKI-7537. *J. Biosci. Bioeng.* **2018**, *126* (5), 661−666.

(10) Koike, R.; Watanabe, Y.; Kato, K.; Mikasa, Y.; Shimizu, K.; Nonaka, K.; Honsho, M.; Suga, T.; Hokari, R.; Ishiyama, A.; Asami, Y.; Iwatsuki, M. Shikinefragalides A-D, new tricyclic macrolides produced by *Stachybotryaceae* sp. FKI-9632. *J. Antibiot.* **2022**, *75* (4), 199−206.

(11) Izuta, S.; Kosaka, S.; Kawai, M.; Miyano, R.; Matsuo, H.; Matsumoto, A.; Nonaka, K.; Takahashi, Y.; Ōmura, S.; Nakashima, T. Dipyrimicin A and B, microbial compounds isolated from *Amycolatopsis* sp. K16−0194. *J. Antibiot.* **2018**, *71* (5), 535−537.

(12) 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.

(13) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. Staurosporine, a potent inhibitor of phospholipid/Ca++dependent protein kinase. *Biochem. Biophys. Res. Commun.* **1986**, *135* (2), 397−402.

(14) Lena, A.; Toppino, A.; Miele, K.; Roletto, J.; Paissoni, P. Process for the Preparation of Midostaurin with High Purity. WO Patent, WO2020200945A12023.
(15) Perkins, H. R. Vancomycin and related antibiotics. *Pharmacol. Ther.* **1982**, *16* (2), 181−197.
(16) Kimishima, A.; Suzuki, Y.; Kosaka, S.; Honsho, M.; Honma, S.; Sakai, K.; Tsutsumi, H.; Inahashi, Y.; Watanabe, Y.; Iwatsuki, M.; Asami, Y.; Nakashima, T. Design and synthesis of d-Ala-d-Ala silica gel for a binding mode-based physicochemical screening method. *ACS Omega* **2023**, *8* (42), 39035−39040.
(17) Inahashi, Y.; Matsumoto, A.; Danbara, H.; Ōmura, S.; Takahashi, Y. *Phytohabitans suffuscus* gen. nov., sp. nov., an actinomycete of the family *Micromonosporaceae* isolated from plant roots. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2652−2658.

https://doi.org/10.1021/acsomega.4c00145
ACS Omega 2024, 9, 17415−17422