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Graphical Abstract

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Design, Synthesis and biological activity of novel demethylvancomycin dimers against vancomycin-resistant *enterococcus faecalis*

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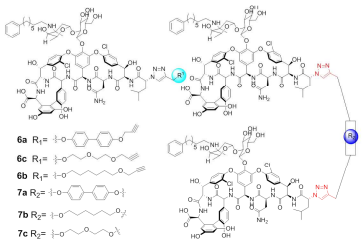

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ABSTRACT

The emergence of resistance to vancomycin and other glycopeptide antibiotics is a serious concern in clinical practice and has prompted intensive efforts to develop analogues that may overcome the resistance. One of major strategies to enhancing anti-vancomycin-resistant *enterococci* (VRE) activity emerged in recent years was connecting two vancomycin molecules by covalent linkers. Herein, we reported the design and synthesis of three different covalently linked demethylvancomycin dimers **7a-c** by applying click chemistry. Interestingly, these dimers restored their activities against VRE. Furthermore, the interactions of molecules with peptidoglycan were also investigated via computer modeling.

Introduction

Vancomycin has long functioned as an antibiotic of last resort for treating life-threatening infections caused by Gram-positive pathogens, many of which are resistant to most other antibiotics. Vancomycin targets the bacterial cell wall and inhibit peptidoglycan (PG) biosynthesis by forming complexes with PG precursors. [1, 2] The cup-shaped undersurface of the vancomycin forms five hydrogen bonds to the D-Ala-D-Ala dipeptide terminus of peptidoglycan. [3] However, its effectiveness is threatened by the emergence of resistant pathogens that are spreading rapidly and becoming a major public health concern. Thus, extensive efforts have been made to develop analogues that may overcome the resistance.

Vancomycin-resistant *enterococcus* (VRE) mutates its terminal peptides from D-Ala-D-Ala to D-Ala-D-Lac. This simple replacement of an amide NH group for an ester oxygen (NH→O) has substantially lowered its affinity to vancomycin due to the elimination of one of the hydrogen bonds in the vancomycin-PG precursor's interaction complex. [4, 5] The loss of one hydrogen bond interaction has indeed reduced 1000-fold binding affinity of vancomycin for the mutated terminal peptides (D-Ala-D-Lac), [6] which parallelly decreased the antibiotic effectiveness. [7]

In order to regain activity against resistant bacterial, several approaches have emerged in recent years. Among them, chemical modifications on vancomycin core tended to be the promising strategy to achieve the goal. The fruitful results have been obtained through addition of hydrophobic group to the amino-disaccharide moieties of vancomycin. Initially, Nagarajan *et al.* [8] synthesized lipophilic N-alkyl vancomycin derivatives which were actively against vancomycin resistant bacteria. After that, the similar principle has been applied to prepare many other vancomycin, chloroeremomycin, and teicoplanin derivatives. [9] Telavancin, Oritavancin, and Dalbavancin were demonstrated as successful examples of this principle (**Figure 1**). A second approach has sought to improve the affinity of vancomycin for D-Ala-D-Lac by covalent linking of two vancomycin molecules (vancomycin dimer). A series of studies have shown that vancomycin can self-associate via hydrogen bonding and hydrophobic interactions to form specific dimers. [10, 11] This non-covalent dimerization of glycopeptide can be beneficial for both peptide ligand binding and bacterial cell surface binding, which enhances its *in vitro* antibacterial activity.[12] However, vancomycin only self-associates weakly in solution, and this non-covalent dimerization of vancomycin alone is insufficient to act against VRE. This prompted us to examine the effectiveness of covalent dimerization. [13, 14] In vancomycin structure, at least three functional sites are accessible for covalent modification, including a primary amine group located on the vancomycin disaccharide structure (V), a C-terminal carboxylic acid (C), and a secondary amino group at the N-terminus (N).

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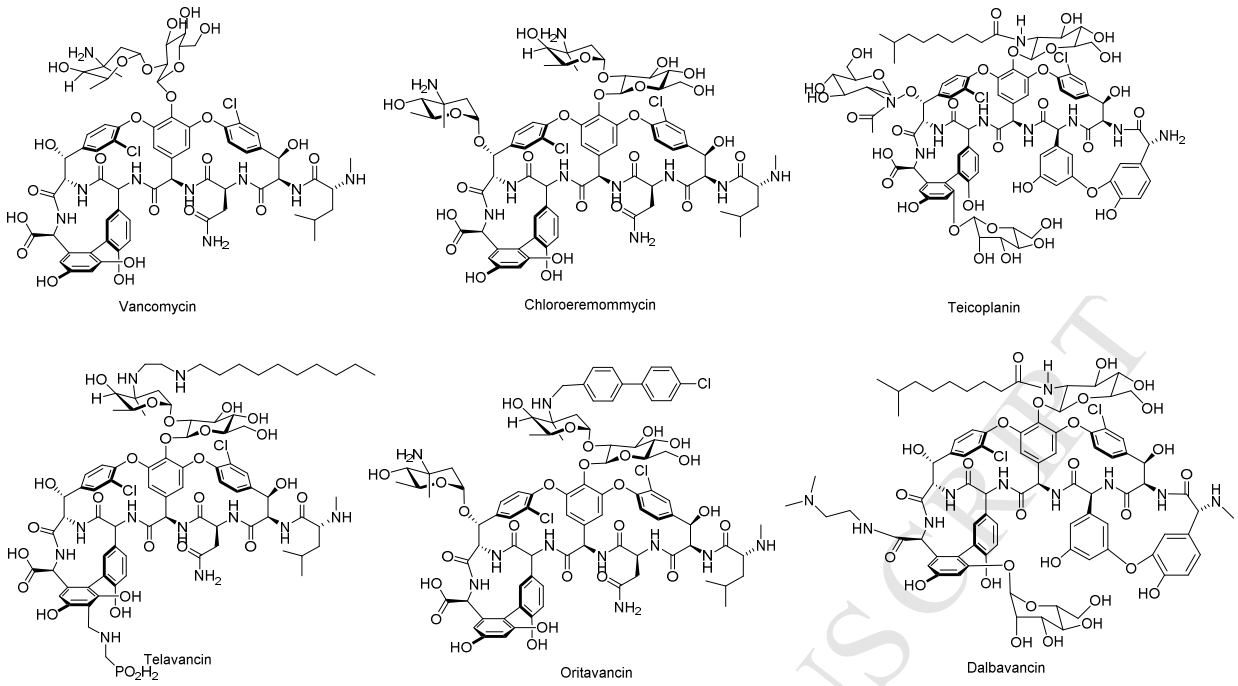


Figure 1. Structures of Vancomycin, Chloroeremomycin, Teicoplanin, Telavancin, Oritavancin and Dalbavancin

Griffin *et al.* [15] reported the covalent dimerization of vancomycin by preparing a series of dimeric vancomycin carboxamide derivatives. Interestingly, all compounds demonstrated improved *in vitro* potency against strains of *enterococci* which was highly resistant to vancomycin and other glycopeptides. Sharpless *et al.* [16] also synthesized several [1, 2, 3]-triazole vancomycin dimer derivatives by applying click chemistry at C-terminal. Some derivatives showed good or better activity against VRE compared with vancomycin. Herein, we reported the study of covalent dimerizing of glycopeptide antibiotics demethylvancomycin **1** which shows similar activity and mode of action to vancomycin against Gram-positive bacteria. We aimed to investigate N-N linked dimeric demethylvancomycin derivatives by covalently linked at N-terminal. Considering the linker rigidity and length, compounds **7a-c** were designed to confirm our hypothesis. As the first time, we reported the synthesis of 1, 4-disubstituted [1, 2, 3]-triazole N-linked dimers **7a-c** via an expedient click chemistry (**Figure 2**).

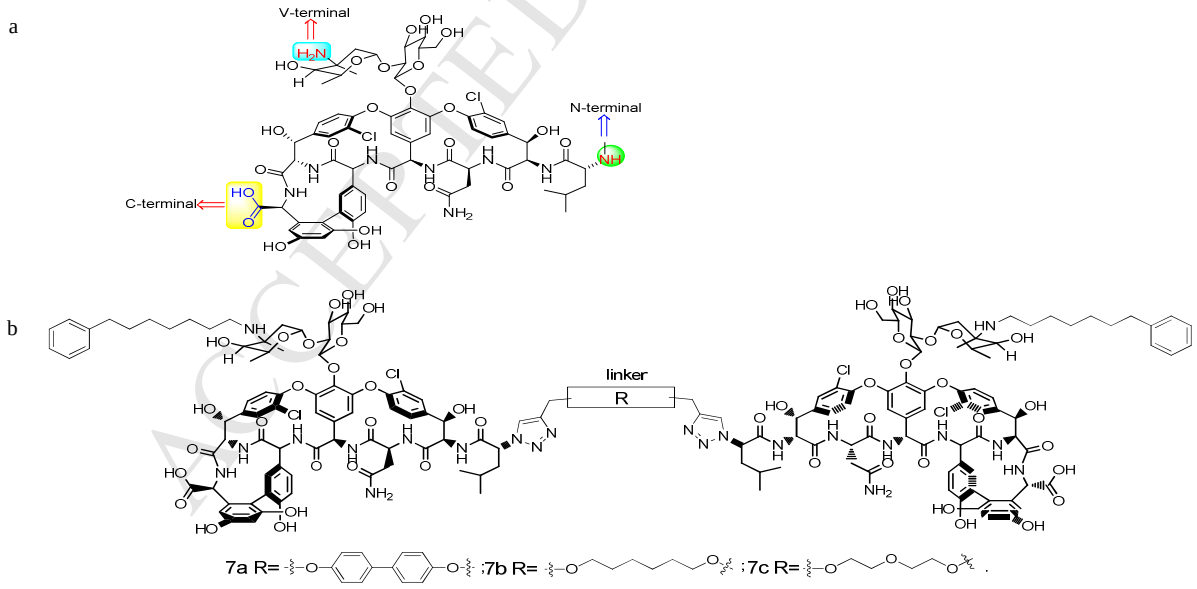
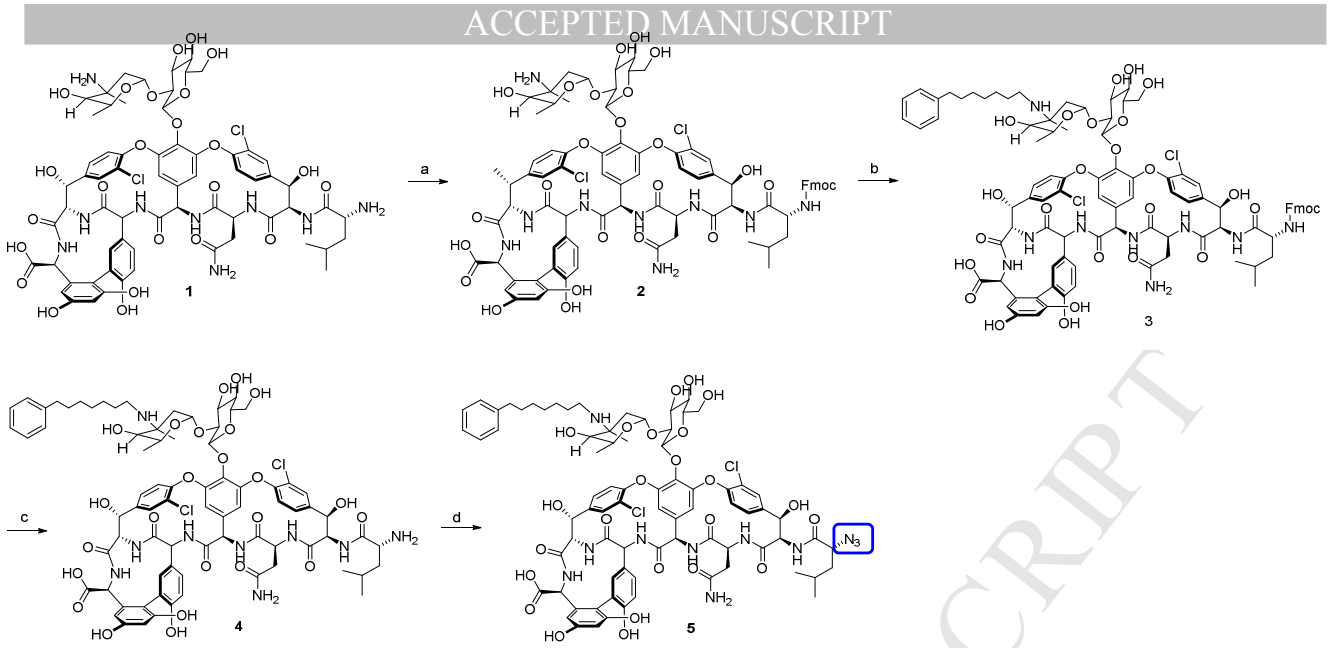


Figure 2. a) Three functional sites for covalent modifications; b) General dimer structures.



Scheme 1. Reagents and conditions: a) DIEA (2.0 equiv), FmocCl (1.1 equiv), Dixane:H_2O (v:v = 1:1), rt, 2.5h; b) 7-phenylheptanal (5.0 equiv), $NaCNBH_3$ (3.0 equiv), DIEA (2.0 equiv), DMF, rt, 2h; c) piperidine (15 equiv) in DMF, rt, 15min; d) trifluoromethanesulfonyl azide (3.0 equiv), DIEA (2.0 equiv), $CuSO_4 \cdot 5H_2O$ (0.1 equiv), DMF, 0 ^{0}C, 8h.

Results and Discussion

Synthesis

For the syntheses of dimers **7a-c**, demethylvancomycin was chosen as the starting material. Following previous reported procedures [17, 18] with slight modifications, intermediate **4** was prepared as follows in gram scale. The N-terminal free amino group of demethylvancomycin was protected with N-fluorenylmethoxycarbonyl (Fmoc-Cl) to obtain intermediate **2** by reductive alkylation with 7-phenylheptanal in the presence of diisopropylethylamine (DIEA) and $NaBH_3CN$. After removing the Fmoc protecting group with piperidine in DMF at room temperature, the crude compound **4** was obtained in 23% overall yield. Then, click reaction [19, 20] was used to construct the dimeric demethylvancomycin derivatives. Initially, the conversion of amino group to azide group was problematic for this complex molecule. Several diazo transfer reagents were explored, such as benzotriazol-1-yl-sulfonyl azide, [21] imidazole-1-sulfonyl azide [22] and trifyl azide (TfN_3) (**Figure 3**). [23] The best result was achieved by using trifyl azide condition in terms of mild reaction and high yield. Worth to mention, the transformation of the amino group at V-terminal to azide was unsuccessful under these conditions. Thus, **4** was subjected to the freshly prepared trifyl azide (TfN_3) in DMF in the presence of a catalytic amount of $Cu_2SO_4 \cdot H_2O$, the reaction was completed in 8h. [24] After poured the reaction mixture into dichloromethane, the resulting precipitate was centrifuged and then washed with a small amount of dichloromethane to afford crude compound **5** in 80%-90% yield (**Scheme 1**). This crude **5** was used directly for the next step click reaction without further purification. As shown in **Scheme 2**., monomers **6a-c** were synthesized by mixing the appropriate compound **5** with corresponding alkyne in DMF in the presence of CuI (0.1 equiv).

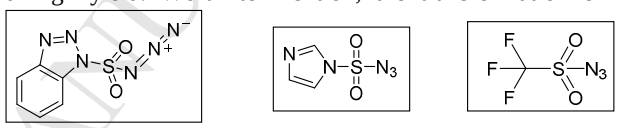
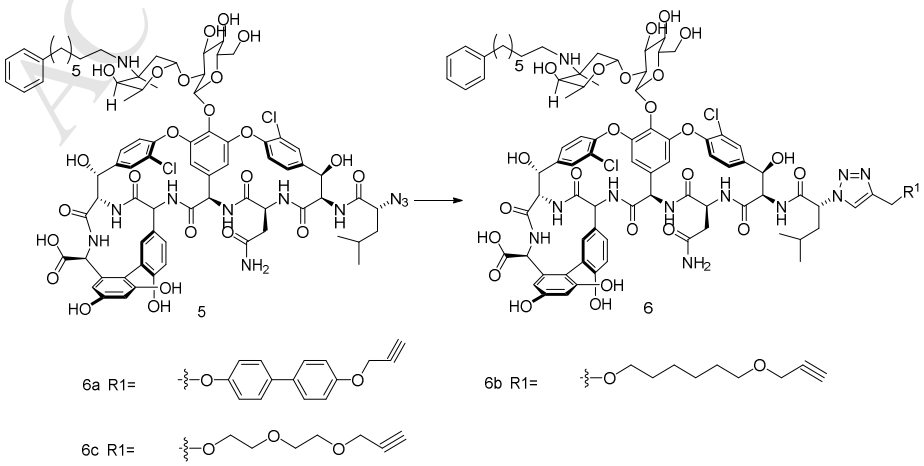
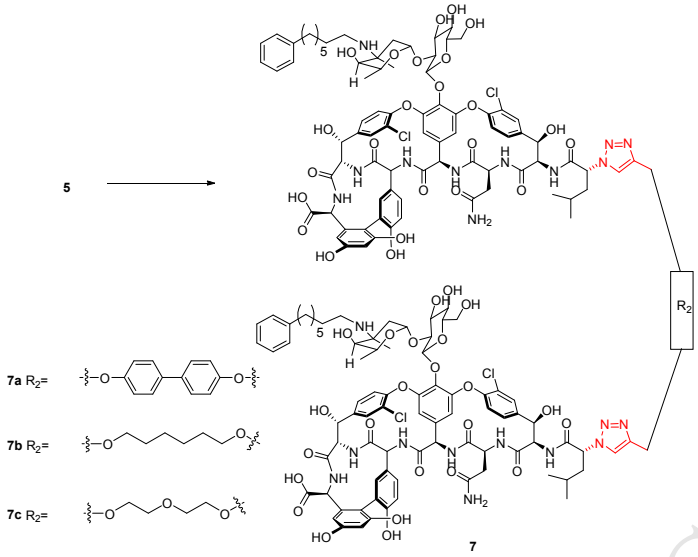


Figure 3. Structures of diazo transfer reagents: a) benzotriazol-1-yl-sulfonyl azide; b) imidazole-1-sulfonyl azide; c) trifyl azide



Scheme 2. Reagents and conditions for the preparation of **6a-6c**: **5** (1.0 equiv), alkyne (3.0 equiv), CuI (0.1 equiv), N_2, 8h



Scheme 3. Reagents and conditions for the preparation of dimers **7a-7c**: **5** (2.0 equiv), alkyne (1.0 equiv), CuI (0.1 equiv), N_2, 3-5d

After stirred at room temperature for 8h, the reaction mixture was poured into EtOAc. The resulting precipitate was isolated by centrifugation and then washed with EtOAc to remove the excess alkyne. The crude product was further purified by HPLC to obtain pure **6a-c**. Surprisingly, **6a-c** could not be converted to **7a-c** after numerous experiments, only trace of desired product was detected. Thus, we turned to use **5** (2 equiv) directly for this click reaction. As outlined in **Scheme 3**, a solution of compound **5** with corresponding alkyne in DMF was stirred for 3-5 days at room temperature. The reaction progress was monitored by HPLC on an ^{18}C column. As illustrated in **Figure 4** for the synthesis of **7a**, the starting material **5** was initially converted to monomer **6a** which subsequently cyclized with N_3-group of another compound **5** to form dimer **7a**. Finally, all pure final compounds **7a-c** were obtained by using RP-HPLC eluted with 43% of acetonitrile in water (with 0.1% trifluoroacetic acid).

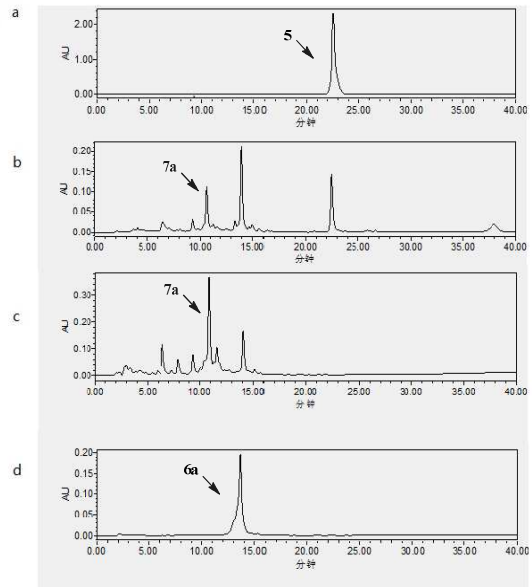


Figure 4. a) The retention time of compound **5**; b) HPLC monitor of reaction mixture of **7a** after stirring for 1.5 days; c) HPLC monitor of reaction mixture of **7a** after 5 days; d) HPLC monitor of crude compound **6a** after work-up the reaction mixture

Biological assays

The antibacterial activities of analogues **6a-c** and **7a-c** were obtained with a broth microdilution assay against methicillin-resistant *Staphylococcus aureus* (MRSA) vancomycin-resistant *Enterococcus faecalis* (VRE) and *Pneumococcus*, as shown in **table 1**. Monomers **6a-c** demonstrated slightly decreased activities against MRSA and with no activity against VRE, expects **6c** had an MIC value of 25 µg/mL for VRE2. Though **7a-c** exhibited less potent activity (higher MIC values) than demethylvancomycin for both clinical isolates MRSA and *Pneumococcus*, all of them were more potent against VRE. This was consists with previous study that the activity of N-N dimers was less potent than that of demethylvancomycin against MRSA. [25] Both **7b** and **7c** were two times more potent than **7a** against VRE. Possibly, more bulky and rigid substituent on the linker did not favour the activities against VREs.

Table 1. MIC Values of monomers **6a-6c** and dimers **7a-7c** (µg/mL)

Compd.	MRSA1	MRSA2	VRE1	VRE2	*Pneumococcus*
6a	50	50	>50	>50	50
6b	>50	>50	>50	>50	>50
6c	25	12.5	>50	25	25
7a	12.5	12.5	50	25	25
7b	12.5	12.5	25	12.5	25
7c	6.25	12.5	25	12.5	25
Devan	1.56	1.56	>50	>50	3.13

Molecular modelling

Molecular modelling was used to investigate the feasibility of simultaneous binding of the two parts (A and B). The 3D structure of peptidoglycan was constructed with the Sybyl 6.9 suite. [26, 27, 28] The docking result of dimer **7c** with peptidoglycan indicates that **7c** forms ten interactions with the peptidoglycan (**Figure 5**). The peptide backbone in Part A (dimer **7c**) forms five hydrogen bonds with the residues in the C-terminal and one hydrogen bond with $(gly)_5$. The peptide backbone of Part B forms three hydrogen bonds with the residues in the C-terminal and one hydrogen bond with $(gly)_5$. It was possible that the restoration of activity against VRE of vancomycin dimer may be due to the increase of interactions between the ligand and the dimer.

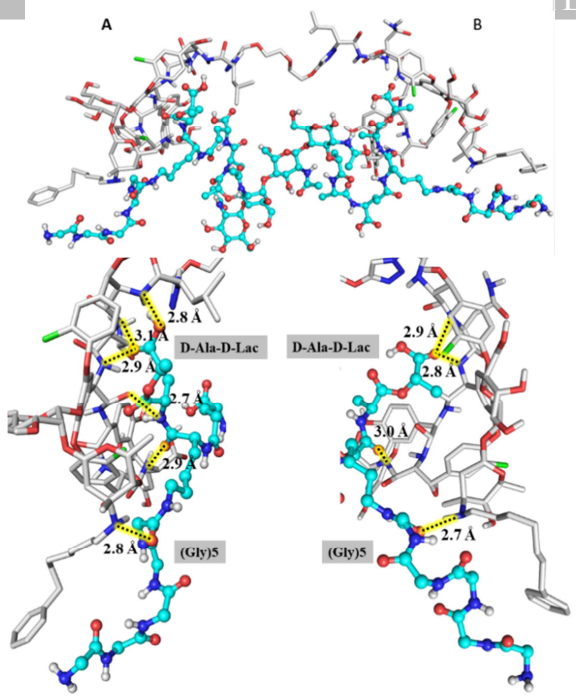


Figure 5. Molecular docking studies of **7c** with the peptidoglycan of VRE. The docking complex is shown in a stick format peptidoglycan is colored in cyan and dimer **7c** is colored in white. The **7c**-peptidoglycan complex shows ten possible interactions comprising six hydrogen bonds in part A and four hydrogen bonds in part B.

Conclusion

Since the dimerization of vancomycin could potentially enhance biological activity, dimers **7a-c** were designed and synthesized to search for potent antibacterial agents through multivalent interactions. Compounds **7a-c** was synthesized by click chemistry and their antibacterial activities were evaluated for clinical isolates of MRSA, VRE and *Pneumococcus*. All three different covalently linked dimers **7a-c** were more potent than their corresponding monomers **6a-c**, except **6c** had a similar activity against MRSA. Compared with Devan, **7a-c** showed more potent activities against VRE, though slightly weaker activities against MRSA and *Pneumococcus* were observed. More potent activities of **7b-c** than **7a** against VRE may contribute from their flexible linkers which could allow **7b-c** to adopt into more favourite conformations. This information provides us the insight which can guide future structural modifications to design more potent novel dimeric derivatives.

Experiment Section

Chemistry

General: Reagents were purchased from Sigma-Aldrich and TCI Chemical companies. All solvents were purified and dried in accordance with standard procedures, unless otherwise indicated. Reactions were monitored by TLC (Yantai China GF_{254} silica gel plates 5×10cm) and HPLC (Waters e2695). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-400 (400MHz) and Bruker DRX-600. Chemical shifts were recorded in ppm and splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). A Shimadzu LCMS-2012EV was used for low-resolution mass spectra (ESI) and IonSpec 4.7 Tesla FTMS (MALDI) or Bruker Daltonics APEXIII7.0 TESLA FMS (ESI) for high-resolution mass spectra.

Synthesis of 5:

Compound **4** (200 mg, 0.12 mmol) was dissolved in DMF (2 mL), then DIEA (30.2 μL, 0.24 mmol) and $CuSO_4 \cdot 5H_2O$ (2.5 mg, 0.01 mmol) were added under N_2 at 0 ^{0}C. To this mixture, freshly prepared TfN_3 (43.6 mg, 0.36 mmol) in DCM solution was added dropwise. The reaction mixture was stirred at 0 ^{0}C for 8h before the addition of DCM (20 mL). The resulting precipitate was filtered, washed with DCM, and dried in vacuo to give compound **5** as a white solid which was used directly for the next step without further purification. ESI-MS m/z: 1633.5 $[M+H]^+$.

General procedure for the preparation of 6a-c: compound **5** (0.12 mmol) and corresponding alkyne (0.36 mmol) were dissolved in DMF (4 mL), then CuI (0.01 mmol) was added under N_2. The reaction mixture was stirred at room temperature for 8 h before the addition of EtOAc (20 mL). The resulting precipitate was filtered, washed with EtOAc, and dried in vacuo to give crude compounds **6a-c**. The residue was further purified by HPLC to provide the desired pure fractions (gradient eluent: CH_3CN/H_2O, 20-55% in 0.1% TFA). The combined fractions were concentrated to a small volume (15 mL), which was lyophilized to give corresponding pure compounds **6a-c** as an off- white solid.

Compound 5: 89.8%; ^{1}H NMR (600 MHz, DMSO) δ: 9.44 (s, 1H), 9.18 (s, 1H), 9.07 (s, 1H), 8.97 (s, 1H), 8.66 (s, 1H), 8.52 (s, 1H), 8.53 (s, 1H), 8.41 (s, 1H), 7.80 (s, 1H), 7.53-7.48 (m, 2H), 7.42-7.40 (m, 2H), 7.30-7.27 (m, 1H), 7.14-7.10 (m, 2H), 7.02 (s, 1H), 6.76-6.73 (m, 1H), 6.70-6.69 (m, 2H), 6.36 (s, 1H), 6.21 (s, 1H), 5.95 (s, 1H), 5.75-5.71 (m, 1H), 5.64-5.56 (s, 1H), 5.29-5.23 (m, 2H), 5.14 (s, 3H), 5.07 (s, 1H), 4.89 (s, 1H), 4.67-4.60 (m, 1H), 4.38-4.40 (m, 2H), 4.20-4.07 (m, 2H), 3.94-3.88 (m, 1H), 3.55-3.22 (m, 6H), 2.85 (s, 2H), 2.69 (s, 1H), 2.64-2.58 (s, 3H), 2.56 (s, 1H), 2.20-2.16 (m, 1H), 2.14 (s, 1H), 2.02 (m, 1H), 1.98-1.90 (m, 3H), 1.42-1.34 (m, 4H), 1.08-1.06 (m, 2H), 1.00-0.98 (m, 2H), 0.88-0.87 (m, 3H), 0.83-0.82 (m, 3H); ESI-MS m/z: 1462.2 $[M+H]^+$.

Monomer 6a: 34.6%; ^{1}H NMR (600 MHz, DMSO) δ: 9.45 (s, 1H), 9.17 (s, 1H), 9.09 (s, 1H), 8.69 (s, 1H), 8.54 (s, 1H), 8.40 (s, 1H), 8.23 (s, 1H), 7.85 (s, 2H), 7.57 (s, 2H), 7.56 (s, 2H), 7.49-7.46 (m, 2H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.28-7.26 (m, 3H), 7.21-7.20 (m, 1H), 7.19-7.17 (m, 2H), 7.13 (s, 1H), 7.11 (s, 1H), 7.06-7.04 (m, 2H), 6.79 (d, *J* = 8.4Hz, 1H), 6.73 (d, *J* = 8.4Hz, 1H), 6.70-6.69 (m, 2H), 6.41 (s, 1H), 6.26 (s, 1H), 5.96 (s, 1H), 5.79 (s, 1H), 5.76-5.74 (m, 2H), 5.66 (s, 1H), 5.61 (s, 1H), 5.36-5.33 (m, 2H), 5.30 (m, 1H), 5.19 (s, 2H), 5.12 (s, 3H), 4.84-4.82 (m, 2H), 4.76 (s, 1H), 4.63 (d, *J* = 6.6Hz,1H), 4.46-4.44 (m, 2H), 4.29 (s, 1H), 4.21-4.19 (m, 1H), 4.10 (s, 1H), 3.59-3.56 (m, 6H), 3.27-3.25 (m, 2H), 2.77 (s, 1H), 2.70 (s, 1H), 2.58-2.55 (m, 4H), 2.17 (s, 1H), 2.08-1.98 (m, 4H), 1.91-1.90 (m, 1H), 1.81-1.79 (m, 1H), 1.76 (s, 2H), 1.57-1.55 (m, 4H), 1.36 (s, 2H), 1.30-1.26 (m, 6H), 1.20-1.16 (m, 2H), 1.10-1.09 (m, 3H), 0.90-0.89 (m, 3H), 0.87-0.85 (m, 2H), 0.83-0.82 (m, 3H); ^{13}C NMR (150 MHz, DMSO) δ:174.3, 173.0, 171.9, 170.9, 169.6, 169.0, 168.3, 157.7, 157.6, 157.0, 156.8, 155.5, 152.6, 151.7, 148.7, 142.9, 142.6, 142.2, 142.2, 140.0, 135.8, 135.1, 132.5, 132.3, 130.1, 129.9, 129.1, 128.7, 127.5, 127.0, 126.4, 126.1, 125.6, 124.2, 123.8, 123.2,

122.0, 118.5, 117.8, 116.6, 115.1, 108.1, 106.1, 105.2, 102.3, 101.3, 96.9, 78.7, 77.4, 77.3, 76.8, 76.4, 72.0, 70.6, 69.1, 68.4, 63.6, 62.2, 61.6, 59.1, 57.1, 56.4, 54.1, 53.6, 51.5, 45.5, 35.5, 33.7, 31.3, 30.7, 30.7, 29.0, 28.8, 28.8, 28.7, 28.5, 28.4, 28.1, 26.5, 25.5, 25.0, 24.7, 24.5, 22.5, 21.8, 19.0, 17.3. ESI-MS m/z: 1898.65 $[M+H]^+$; HRMS m/z: calcd for $C_{96}H_{103}Cl_2N_{11}O_{26}$ $[M+H]^+$: 1898.6543, found 1898.6554 $[M+H]^+$.

Monomer 6b: 32.5%; ^{1}H NMR (600 MHz, DMSO) δ: 9.47 (s, 1H), 9.20 (s, 1H), 9.13 (s, 1H), 8.66 (s, 1H), 8.52 (s, 1H), 8.17 (s, 1H), 7.81 (s, 2H), 7.44-7.42 (m, 2H), 7.39-7.37 (m, 2H), 7.30-7.28 (m, 3H), 7.26 (s, 2H), 7.24 (s, 2H), 7.22-7.21 (m, 1H), 7.15 (s, 4H), 7.14 (s, 4H), 6.76-6.73 (m, 1H), 6.70-6.67 (m, 3H), 6.38 (s, 1H), 6.22 (s, 1H), 5.98-5.95 (m, 1H), 5.78 (s, 1H), 5.71-5.67 (m, 3H), 5.55 (s, 1H), 5.37-5.36 (m, 1H), 5.32-5.29 (m, 2H), 5.25 (s, 1H), 5.14-5.13 (m, 2H), 5.08-5.07 (m, 2H), 4.71 (s, 1H), 4.59-4.58 (m, 1H), 4.46 (s, 2H), 4.40 (d, *J* = 7.8Hz, 2H), 4.18-4.07 (m, 2H), 4.06-4.05 (m, 1H), 3.67-3.64 (m, 2H), 3.55-3.22 (m, 6H), 3.07-3.03 (m, 1H), 2.74 (s, 1H), 2.64 (s, 1H), 2.15-2.10 (m, 1H), 2.00-1.94 (m, 4H), 1.73 (s, 4H), 1.54-1.50 (m, 6H), 1.46-1.42 (m, 6H), 1.32 (s, 3H), 1.15-1.13 (m, 4H), 1.07-1.05 (m, 3H), 0.85-0.84 (m, 3H), 0.82-0.80 (m, 2H), 0.89-0.78 (m, 3H); ^{13}C NMR (150 MHz, DMSO) δ: 173.0, 171.9, 170.9, 169.6, 169.0, 168.3, 157.7, 157.6, 157.0, 156.8, 155.5, 152.6, 151.7, 150.4, 148.7, 144.5, 142.7, 142.9, 141.6, 136.5, 135.4, 132.9, 130.1, 129.1, 128.7, 127.8, 127.5, 126.9, 126.6, 126.1, 124.6, 123.4, 122.0, 118.5, 116.7, 106.1, 102.8, 96.9, 80.8, 78.3, 77.6, 77.4, 72.0, 71.2, 70.6, 70.1, 69.9, 69.4, 68.9, 63.9, 63.6, 62.2, 61.6, 61.5, 59.6, 59.1, 57.9, 57.1, 55.4, 51.5, 46.0, 35.5, 31.7, 31.3, 30.2, 29.5, 29.4,29.4, 29.3, 29.2, 29.1,29.0, 28.8, 27.0, 26.5, 25.9, 23.0, 22.5, 21.8, 19.4, 19.0, 17.3. ESI-MS m/z: 1830.4 $[M+H]^+$; HRMS m/z: calcd for $C_{90}H_{107}Cl_2N_{11}O_{26}$ $[M+H]^+$: 1830.6853, found 1830.6773 $[M+H]^+$.

Monomer 6c: 21.7%; ^{1}H NMR (600 MHz, DMSO) δ: 9.45 (s, 1H), 9.18 (s, 1H), 9.11 (s, 1H), 8.67 (s, 1H), 8.52 (s, 1H), 8.20 (s, 1H), 7.85-7.82 (m, 2H), 7.46-7.42 (m, 2H), 7.41-7.39 (m, 2H), 7.29 (s, 2H), 7.28 (s, 1H), 7.27 (s, 1H), 7.25 (s, 1H), 7.23 (s, 1H), 7.16 (s, 4H), 7.14 (s, 4H), 7.04 (s, 2H), 6.76-6.74 (m, 2H), 6.70-6.68 (m, 2H), 6.40-6.38 (m, 1H), 6.23-6.22 (m, 1H), 5.96-5.94 (m, 1H), 5.76 (s, 1H), 5.73-5.70 (m, 2H), 5.66-5.65 (m, 1H), 5.56 (s, 1H), 5.36-5.30 (m, 3H), 5.26 (s, 1H), 5.15 (s, 1H), 5.13 (s, 1H), 5.09-5.07 (m, 2H), 4.71 (s, 1H), 4.64-4.54 (m, 1H), 4.51 (s, 2H), 4.42-4.39 (m, 2H), 4.14-4.03 (m, 3H), 3.70-3.64 (m, 1H), 3.54-3.49 (m, 10H), 3.40-3.22 (m, 6H), 2.65 (s, 1H), 2.55-2.54 (m, 3H), 2.13 (s, 1H), 2.00-1.94 (m, 2H), 1.86-1.77 (m, 1H), 1.73 (s, 1H), 1.55-1.51 (m, 4H), 1.33 (s, 2H), 1.16-1.12 (m, 3H), 1.07-1.06 (m, 3H), 0.85-0.87 (m, 3H), 0.80-0.83 (m, 2H), 0.79-0.81 (m, 3H); ^{13}C NMR (150 MHz, DMSO) δ: 173.0, 171.9, 170.9,169.6, 169.0, 168.3, 157.7,157.6, 157.0, 156.8, 155.5, 152.6, 151.7, 150.4,148.7, 144.5, 142.9, 142.7, 141.6, 140.9, 136.5, 135.4, 132.9, 132.3, 130.1, 129.1, 128.7, 127.8, 127.5, 126.9, 126.6, 126.1, 124.6, 123.8, 123.4, 122.0, 118.5, 116.7, 106.1, 102.8, 96.9, 80.8, 78.3, 77.6, 77.4, 72.0, 70.6, 70.1, 69.9, 69.4, 68.9, 68.7, 63.9, 63.6, 62.2, 61.6, 61.5, 59.6, 59.1, 57.9, 57.1, 56.4, 55.4, 54.1, 51.5, 46.0, 35.5, 31.7, 31.3, 29.5, 29.4, 29.3, 29.0, 28.8, 27.0, 26.5, 25.5, 24.7, 23.0, 22.5, 21.8, 19.4, 19.0, 17.3. ESI-MS m/z: 1818.6 $[M+H]^+$; HRMS m/z: calcd for $C_{88}H_{103}Cl_2N_{11}O_{27}$ $[M+H]^+$: 1818.6489, found 1818.6475 $[M+H]^+$.

General procedure for the preparation of 7a-c: Compound **5** (0.18 mmol) and corresponding alkyne (0.09 mmol) were dissolved in DMF (5 mL), then CuI (0.036 mmol) and ascorbate (0.045 mmol) were added under N_2. The reaction mixture was stirred at room temperature for 3-5 d before the addition of EtOAc (25 mL). The resulting precipitate was filtered, washed with EtOAc, and dried in vacuo to give crude compounds **7a-c**. The residue was further purified by HPLC to provide the desired pure fractions (isocratic eluent: CH_3CN/H_2O, 43% in 0.1% TFA) with Waters SunFire Prep C18 (5 μm 10×250 mm) column. The combined fractions were concentrated to a small volume (15 mL) which was lyophilized to give corresponding pure compounds **7a-c** as a white solid.

Dimer 7a: 18.6%, ^{1}H NMR (600 MHz, DMSO) δ: 9.45 (s, 2H), 9.17 (s, 2H), 9.10 (s, 2H), 8.66 (s, 2H), 8.52 (s, 2H), 8.38 (s, 2H), 8.14 (s, 2H), 7.83 (s, 4H), 7.68 (d, *J* = 12 Hz, 2H), 7.56-7.54 (m, 4H), 7.50-7.48 (m, 2H), 7.46-7,44 (m, 4H), 7.41 (s, 2H), 7.31-7.28 (m, 2H), 7.29-7.24 (m, 6H), 7.25 (d, *J* = 8.6 Hz, 6H), 7.17-7.15 (m, 4H), 7.11-7.08 (m, 3H), 7.09 (s, 2H), 6.75 (d, *J* = 12 Hz, 2H), 6.71 (d, *J* = 12 Hz, 2H), 6.40 (s, 2H), 6.24 (s, 2H), 5.96 (s, 2H), 5.81 (s, 2H), 5.76-5.74 (m, 4H), 5.57 (s, 2H), 5.34-5.32 (m, 2H), 5.27 (s, 2H), 5.17 (s, 4H), 5.10 (s, 4H), 4.91 (s, 2H), 4.85 (s, 2H), 4.82 (s, 3H), 4.75 (s, 3H), 4.65-4.61 (m, 2H), 4.50-4.51 (m, 6H), 4.42-4.41 (m, 4H), 4.28(s, 4H), 4.20-4.17 (m, 4H), 4,12-4.10 (m, 2H), 3.86-3.06 (m, 12H), 2.77-2.73 (m, 4H), 2.67-2.65 (m, 4H), 2.18-2.15 (m, 6H), 1.99-1.96 (m, 6H), 1.53-1.47 (m, 6H), 1.48-1.44 (m, 6H), 1.35 (s, 6H), 1.24-1.17 (m, 6H), 1.08-1.07 (m, 6H), 0.88-0.86 (m, 6H), 0.85-0.84 (m, 4H), 0.81-0.79 (m, 6H); ^{13}C NMR (150 MHz, DMSO) δ: 174.4, 172.4, 171.8, 170.4, 169.1, 168.5, 167.7, 157.8, 157.6, 157.4, 157.2, 156.5, 155.0, 152.1, 151.2, 148.2, 142.6, 142.4, 142.2, 142.1, 140.4, 135.9, 135.6, 132.5, 132.3, 131.8, 129.9, 129.5, 128.1, 127.2, 127.0, 126.3, 126.1, 125.5, 124.1, 123.7, 123.2, 121.5, 118.2, 117.9, 116.2, 115.0, 107.5, 105.6, 104.6, 102.3, 100.8, 96.4, 78.5, 77.7, 76.9, 76.8, 72.4, 70.1, 68.5, 67.6, 63.0, 61.7, 61.1, 59.1, 58.5, 56.6, 54.8, 53.5, 51.0, 45.5, 35.0, 33.5, 31.2, 30.8, 30.7, 29.0, 28.9, 28.7, 28.5, 28.3, 28.3, 28.2, 26.4, 25.9, 25.0, 24.3, 24.2, 22.5, 21.2, 18.8, 16.8. ESI-MS m/z: 1767.5 $[M+2H]^{2+}$; HRMS m/ z: calcd for $C_{174}H_{192}Cl_4N_{22}O_{50}$ $[M+2H]^{2+}$: 1766.6043, found 1766.6071 $[M+2H]^{2+}$.

Dimer 7b: 9.8% ^{1}H NMR (600 MHz, DMSO) δ: 9.46 (s, 2H), 9.21 (s, 2H), 9.12 (s, 2H), 8.69 (s, 2H), 8.52 (s, 2H), 8.19 (s, 2H), 7.95 (s, 3H), 7.88-7.84 (m, 3H), 7.47-7.46 (m, 2H), 7.45-7.38 (m, 4H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.27-7.26 (m, 4H), 7.22 (s, 2H), 7.18 (s, 4H), 7.17 (s, 4H), 6.79-6.77 (m, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 6.70-6.66 (m, 4H), 6.40 (s, 2H), 6.26 (s, 2H), 5.98-5.97 (m, 2H), 5.75-5.73 (m, 4H), 5.67-5.65 (m, 4H), 5.59 (s, 2H), 5.36-5.34 (m, 4H), 5.33-5.31 (m, 2H), 5.29 (s, 2H), 5.18 (s, 2H)), 5.11 (s, 6H), 4.75 (s, 2H), 4.62-4.61 (m, 2H), 4.49 (s, 4H), 4.46-4.45 (m, 2H), 4.44-4.43 (m, 2H), 4.41-4.39 (m, 4H), 4.20-4.19 (m, 4H), 4.13-4.11 (m, 4H), 4.09-4.08 (m, 4H), 3.22-3.45 (m, 12H), 2.89 (s, 6H), 2.73 (s, 4H), 2.62 (s, 2H), 2.17-2.15 (m, 2H), 2.03-2.01 (m, 2H), 1.98-1.96 (m, 8H), 1.91-1.88 (m, 2H), 1.80-1.76 (m, 2H), 1.56-1.53 (m, 6H), 1.49-1.45 (m, 6H), 1.37-1.35 (m, 6H), 1.10-1.09 (m, 6H), 1.07-1.05 (m, 8H), 0.89-0.87 (m, 6H), 0.85-0.84 (m, 4H), 0.83-0.82 (m, 6H); ^{13}C NMR (150 MHz, DMSO) δ: 173.0, 171.9, 170.9, 169.6, 169.0, 168.3, 157.7, 157.6, 157.0, 156.8, 155.5, 152.6, 151.7, 150.4, 148.7, 143.3, 142.9, 142.7, 136.5, 136.1, 135.1, 133.3, 133.0, 132.3, 130.1, 129.1, 128.7, 127.8, 127.5, 126.9, 126.6, 126.1, 124.6, 124.2, 123.8, 122.0, 118.5, 115.7, 115.6, 105.2, 106.1, 105.2, 102.8, 101.3, 96.9, 79.8, 78.7, 78.3, 77.5, 77.4, 72.0, 70.6, 69.1, 62.2, 61.6, 59.6, 59.1, 57.1, 56.5, 55.9, 55.4, 54.1, 51.5, 35.5, 33.2, 31.7, 31.3, 29.5, 29.4, 29.2, 29.2, 29.1, 29.0, 28.8, 28.8, 27.0, 26.5, 25.5, 24.7, 23.0, 22.5, 21.8, 19.4, 17.3. ESI-MS m/z: 1733.0 $[M+2H]^{2+}$; HRMS m/ z: calcd for $C_{168}H_{196}Cl_4N_{22}O_{50}$ $[M+2H]^{2+}$: 1732.0907, found 1732.0916 $[M+2H]^{2+}$

Dimer 7c: 15.7%, ^{1}H NMR (600 MHz, DMSO) δ: 9.46 (s, 2H), 9.19 (s, 2H), 9.12 (s, 2H), 8.66 (s, 2H), 8.52 (s, 2H), 8.36 (s, 2H),

8.20 (s, 2H), 7.82 (s, 2H), 7.42-7.44 (m, 2H), 7.39-7.41 (m, 4H), 7.30-7.32 (m, 6H), 7.28 (s, 2H), 7.24 (d, J = 8.6 Hz, 4H), 7.22 (s, 4H), 7.19 (s, 4H), 7.16 (s, 4H), 7.14 (s, 4H), 7.07 (s, 6H), 6.76 (d, J = 12 Hz, 2H), 6.70 (m, 4H), 6.38 (s, 2H), 6.22 (s, 2H), 5.97-5.92 (m, 2H), 5.77 (s, 1H), 5.73-5.67 (m, 4H), 5.55 (s, 1H), 5.37-5.36 (m, 2H), 5.31-5.30 (m, 2H), 5.26 (s, 2H), 5.15-5.12 (m, 4H), 5.07 (s, 4H), 4.69 (s, 1H), 4.60-4.58 (m, 2H), 4.51 (s, 3H), 4.43-4.39 (m, 4H), 4.17-4.11 (m, 4H), 3.42-3.22 (m, 12H), 3.04- 3.02 (m, 4H), 2.73-2.64 (m, 2H), 2.55-2.51 (m, 4H) 2.14-2.10 (m, 2H), 1.98-1.96 (m, 8H), 1.84-1.77 (m, 4H), 1.73 (s, 4H), 1.54-1.51 (m, 10H), 1.33 (s, 6H), 1.17-1.14 (m, 6H), 1.07-1.05 (m, 6H), 0.86-0.84 (m, 6H), 0.84-0.82 (m, 4H), 0.80-0.78 (m, 6H); ^{13}C NMR (150 MHz, DMSO) δ: 173.0, 171.9, 171.0, 169.6, 169.0, 168.3, 158.2, 158.0, 157.6, 157.0, 156.8, 155.6, 152.6, 151.7, 150.4, 148.7, 144.5, 142.9, 142.7, 136.5, 136.1, 135.1, 133.3, 133.0, 132.3, 130.1, 129.1, 128.7, 127.8, 127.5, 126.9, 126.7, 126.1, 124.6, 124.6, 124.2, 123.8, 123.5, 122.0, 118.5, 116.6, 115.7, 115.6, 106.1, 105.0, 102.8, 101.3, 96.9, 79.8, 18.7, 78.3, 77.6, 77.3, 72.0, 70.6, 69.9, 68.9, 62.2, 61.6, 59.0, 57.9, 57.1, 54.1, 51.5, 35.5, 33.2, 31.7, 31.3, 29.5, 29.3, 29.1, 29.0, 28.8, 28.8, 27.0, 26.5, 25.5, 24.7, 23.0, 22.5, 21.8, 19.3, 17.3. ESI-MS m/z: 1727.4 $[M+2H]^{2+}$; HRMS m/ z: calcd for $C_{166}H_{192}Cl_4N_{22}O_{51}$ $[M+2H]^{2+}$: 1725.5947, found 1725.5961 $[M+2H]^{2+}$.

Biological assays

The minimun inhibitory concentration (MIC) values were measured to investigate the antibacterial activity of the test compounds against MRSA, VRE, and Pneumococcus. Demethylvancomycin (Devan) was used as a positive control. MIC values were investigated using an agar dilution method according to the methods of CLSI. Compound stock solutions (320 μg ml^{-1}) were prepared in DMSO/H_2O (50%). Serial twofold dilutions prepared from the stock solutions with sterile H_2O were diluted ten-fold with Mueller-Hinton (MH) agar medium to obtain a concentration range of 0.024-50 μg ml^{-1}. The test organisms were grown in MH broth medium at 35 □ for 8 h and were adjusted to a turbidity of 0.5 using the McFarland standard. The bacterial suspensions were inoculated onto the drug-supplemented MH agar plates with a multipoint inoculator and incubated at 35 □ for 16 h.

Molecular docking and modelling

The 3D structure of **7c** was constructed with the Sybyl 6.9 suite using the crystal structure of vancomycin as a template. Energy minimization was conducted by using the Powell method with Tripos force field and MMFF94 charge for 1000 steps iterations. 3D structure of the receptor (peptidoglycan) was constructed and optimized using the AMBER force field. Three pairs of glycosidic dihedral angles defined the tetrasaccharide conformation in the receptor. Docking procedure was conducted with AutoDock4.2, during the docking process, the number of generation, energy evaluation, and docking runs were set to 380000, 1500000, and 10 respectively. The atom type, generation, and the number of runs for the LGA algorithm were edited and assigned according to the requirement of the AMBER force field. The best estimated binding conformations of dimer **7c** were selected considering the low binding energy and geometrical complementarity.

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