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Design and synthesis of pyrophosphate-targeting vancomycin derivatives for combating vancomycin-resistant *Enterococci*

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Abstract: Vancomycin, as the last resort for intractable Gram-positive bacterial infections, is losing the efficacy with the emergence of vancomycin-resistant bacteria especially vancomycin-resistant *Enterococci* (VRE). To combat this threat, we rationally designed and synthesized 39 novel vancomycin derivatives, *via* respective or combined modifications with metal-chelating, lipophilic, and galactose-attached strategies, for extensive structure-activity relationship (SAR) analysis. In a proposed mechanism, the conjugation of dipicolylamine (DPA) on 7th amino acid resorcinol position or C-terminus endued the vancomycin backbone with the binding activity to pyrophosphate moiety in lipid II while keeping the intrinsic binding affinity to the dipeptide terminus of the bacterial cell wall peptidoglycan precursor. The *in vitro* antibacterial activities were evaluated and the optimal compounds indicated 16-1024 fold higher activity against VRE compared with vancomycin. It was also found compound **11b** showed the synergistic effect combining two peripheral modification and mechanism especially towards VRE.

Introduction

In 2008, the Infectious Disease Society of America has stressed on a faction of antibiotic resistant bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) – acronymically dubbed "ESKAPE pathogens" – capable of "escaping" the action of antibiotics and mutually representing new paradigms in pathogenesis, transmission and resistance[1]. In 2017, the World Health Organization released for the first time a drug-resistant bacteria list which ranked 12 bacteria or bacterial families, posing the greatest threat to human health[2]. Several surveys indicated that there are approximate 700000 annual deaths worldwide due to the antibiotic resistance, and it is predicted that the number would rise to 10 million by 2050 if no proactive solutions are available. We are facing a desperate and emergent situation in combating drug-resistant bacteria[3].

For the refractory Gram-positive bacterial infections with multidrug resistance, the glycopeptide antibiotics especially vancomycin is regarded as the last resort. However, with the widespread abuse of vancomycin and other antibiotics, the bacteria gradually evolved the drug resistance against their mode of action. *Enterococcus* species are natural inhabitants in the environment and essential components of intestinal microbiota of healthy humans and animals. The *enterococci* are also facultatively anaerobic and opportunistic pathogens associated with severe infections in human such as urinary tract infections, sepsis, and endocarditis. *E. faecalis* and *E. faecium* are responsible for the majority of human enterococcal infections, and are a leading cause of hospital-acquired and multidrug-resistant infections[4]. In the late 1970s *E. faecalis* accounted for 90-95% of clinical enterococcal isolates from the hospital-associated enterococcal infections in the United States. While since early 1990s, *E. faecium* is the major cause of enterococcal infections, which is much more frequently resistant to vancomycin and ampicillin than *E. faecalis*. Until now *E. faecium* infections have been reported in various places allover the world[5]. Moreover, the percentage of vancomycin-resistant strains in all *E. faecium* isolates rose from 0% in the mid of 1980s to more than 80% in 2007. On the contrary, only ~5 % of *E. faecalis* isolates are vancomycin resistant[6]. In 1986, the emergence of enterococci with high-level resistance to glycopeptide antibiotics was astonishing, because vancomycin had been used for decades without the emergence of resistance. And now, it is apparently much more common in clinic[7].

Vancomycin binds tightly to the D-Ala-D-Ala moiety of the peptidoglycan precursor on bacterial cell wall, forming five hydrogen bonds. It inhibits the next-step transglycosylation and transpeptidation to cease the biosynthesis of mature cell wall, causing the cell lysis and death. In vancomycin-resistant enterococci cell wall, the D-Ala-D-Ala motif of peptidoglycan was mutated to D-Ala-D-Lac (*vanA*, *vanB*, and *vanD*) or D-Ala-D-Ser (*vanC*, *vanE*, *vanG* and *vanL*), that leads to about 1000-fold or 6-fold decrease in the vancomycin binding affinity respectively[8]. Therefore, development of novel and effective antibiotics against VRE especially the resistant *E. faecium* is of vital and impending emergency. Three semi-synthetic lipo-vancomycin analogues (Telavancin, Dalbavancin, and Oritavancin), as the new generation of glycopeptide antibiotics, were approved in 2009 and 2014 for treatment of MRSA infection[9]. The lipophilic groups of these drugs facilitate their insertion into the bacterial

cell membrane to disrupt the integrity of cell wall. On the other hand, the pyrophosphate component of Lipid II on bacterial surface is also an important and novel target for vancomycin analogue design. In 2008, Breukink group[10] reported a series of vancomycin derivatives conjugated with nisin, a peptide antibiotic that binds to the pyrophosphate motif, with 40-fold increase in antibacterial activity against VRE. In 2016, Haldar group[11] also reported a vancomycin derivative carrying a pyrophosphate-targeting group to combat vancomycin-resistant bacteria (VRB). According to their data, the attached dipicolylamine fragment on vancomycin C-terminus coordinated with zinc ion and specifically bound to the pyrophosphate of lipid II without disrupting vancomycin skeleton (Figure 1).

Historically, metal- or metalloid-based drugs, such as Paul Erlich's organoarsenic compound for the treatment of syphilis[12], represent a strategy for drug design and discovery. The pharmacological activities of these metal coordination compounds depend on the nature of metal ion, its ligands and the structure of the complexes. In 2006, a series of copper complexes of heterocyclic sulfonamides was reported with antibacterial activity[13]. The metal complexes showed enhanced activities than free ligands[12,13].

Herein, we designed and synthesized a series of novel vancomycin derivatives carrying different Cu^{2+}-dipicolylamine (DPA) coordination complexes and corresponding copper-free compounds. The *in vitro* antibacterial activities against VRE and pyrophosphate binding properties of these compounds were evaluated. We also investigated the combined modification strategies on vancomycin backbone and drug-combination treatment to explore the possible synergism function.

Results and Discussion

Structural design

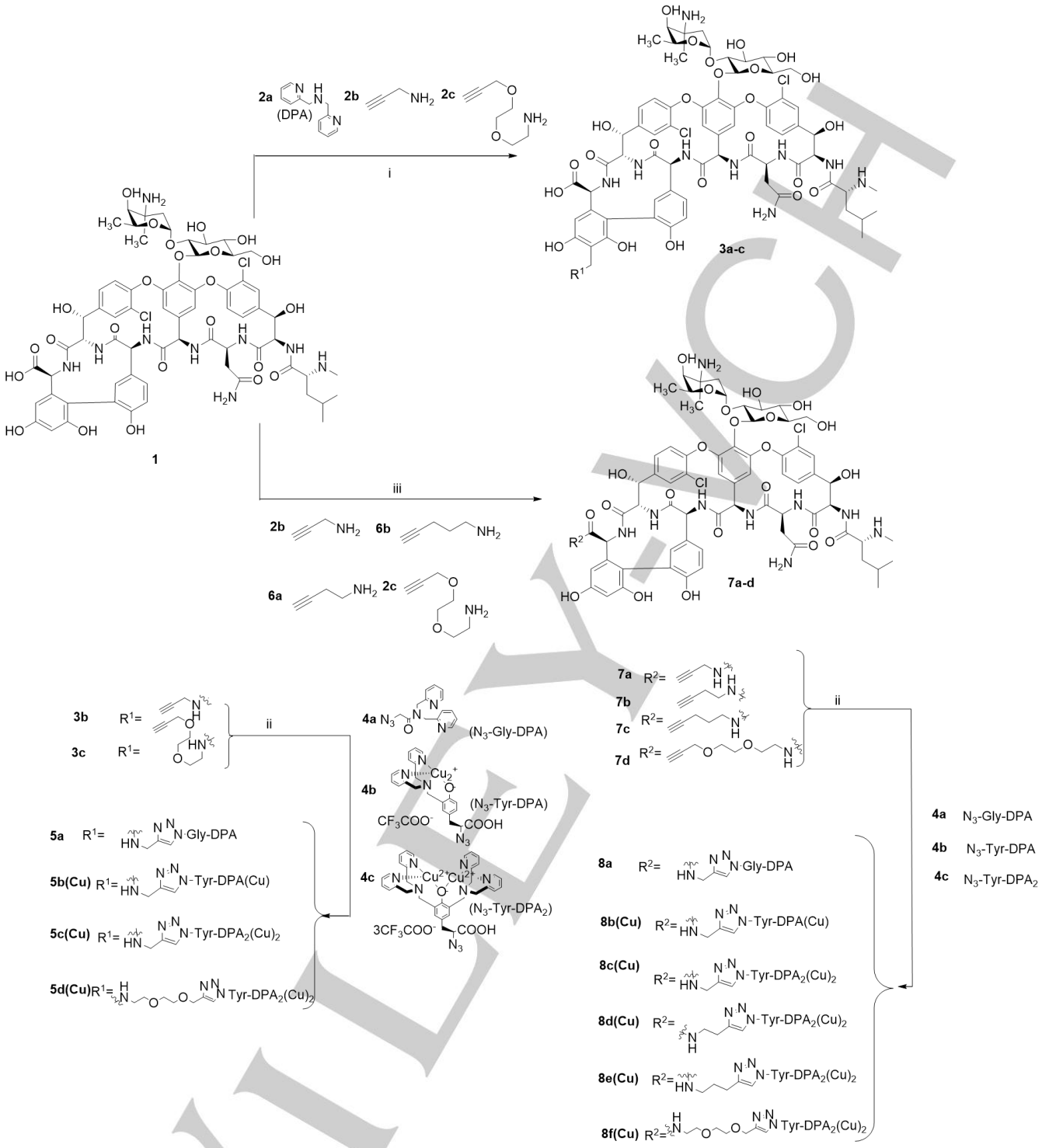
Lipid II is the precursor of bacterial cell wall peptidoglycan and plays a crucial role in the cell wall biosynthesis and bacterial viability. Lipid II consists of disaccharide pentapeptide subunit, pyrophosphate moiety, and bactoprenol (C_{55}). It has been reported that dipicolyl-Zn^{2+} complexes binds to pyrophosphates in high affinity[14]. Haldar group[11] assembled this complex structure onto vancomycin and the resulted derivatives demonstrated binding affinities towards both D-Ala-D-Ala and pyrophosphate motif with enhanced antibacterial activity. In the current work, we sought to introduce the dipicolylamine (DPA) group onto vancomycin and investigate the optimal modification strategies on conjugation site, linker structure, DPA number, metal chelation, and combined modification with lipophilic and/or sugar attachment. Hence, we designed a series of DPA-vancomycin conjugates using single DPA, double DPA on a tyrosine template, spacers in different length, conjugation on C-terminus or 7th amino acid resorcinol of vancomycin, metal chelations, and combined assembly of lipophilic or galactose (Schemes 1-3).

Chemical synthesis

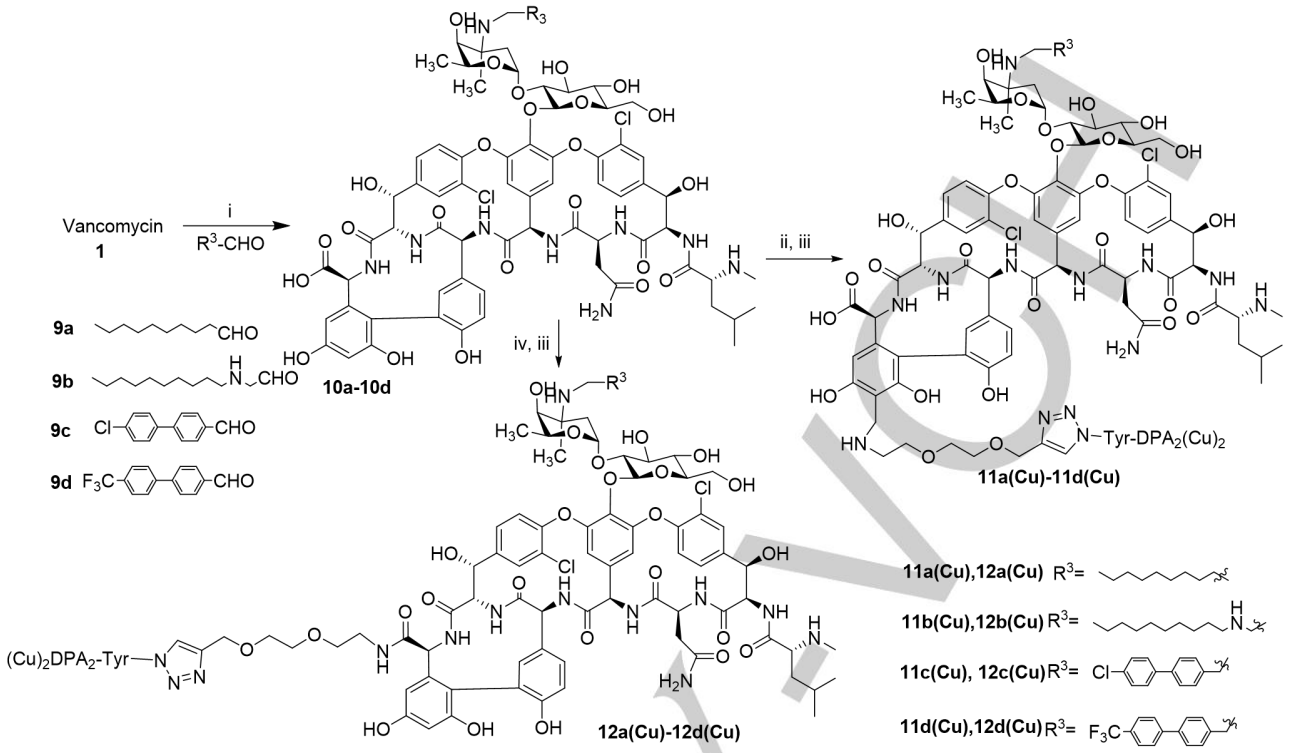
Firstly, we sought to compare various DPA structures on their enhanced antibacterial activities. We modify the 7th-amino acid resorcinol (or C-terminus) of vancomycin via a two-step synthesis by successive Mannich reaction (or amidation) and click chemistry CuAAC[15] with good yields of >70% (**5a-c**, **8a-c**, Scheme 1). During the reaction we found that copper (II) could be easily chelated with the DPA. All DPA_2-vancomycin derivatives contains two copper ions as characterized by HRMS because we used excessive copper reagents in CuAAC reaction. We used EDTA-Na to remove the copper ions and obtained the non-metal final compounds (**5b-c** and **8b-c**). Antibacterial assays against vancomycin-resistant *E.faecium* (*van*A) indicated that Cu^{2+} chelation complex with double DPA on a tyrosine template (Tyr-DPA_2, as shown in compound **4c**) is the optimal modification. Anti-VRE activities of compounds **5c(Cu)** and **8c(Cu)** were >8-fold and >64-fold better than vancomycin. Next, we hypothesized that linker length is important to place DPA moiety towards the appropriate position for pyrophosphate binding. Thus, we synthesized compounds **5d** and **8d-f** bearing Tyr-DPA_2 with linkers in different length. Antibacterial assays (Table 1, Table S1) indicated that compounds **8f** and **5d(Cu)** carrying PEG_2 linker were more effective with wide-spectrum antibacterial activities against VISA and VRE. Furthermore, we investigated the combined modifications of Tyr-DPA_2 and lipophilic fragments selected from previous work[16] (**11a-d** and **12a-d**, Scheme 2), which were proved to be able to insert into the bacterial cell membrane to disrupt the integrity[17]. As shown in Scheme 3, we also synthesized compounds **14a-b** bearing the triple modification of Tyr-DPA_2, biphenyl, and the galactose[16]. The synthesis of key intermediates is summarized in Scheme 4. Amidation of Boc-Gly with dipicolylamine gave compound **16**. Introduction of one or two DPA units onto the tyrosine phenol ring afforded compounds **18** and **19** by Mannich reaction[18]. Then the free primary amine groups on compounds **16**,**18** and **19** were converted to azido groups using efficient and stable imidazole-1-sulfonyl azide hydrochloride [19] to give the building blocks **4a-c**. The chemical structures and purities were unambiguously confirmed by spectroscopic analyses, including ^{1}H NMR, HRMS, and HPLC. The full structures of the final compounds are also listed in Table S1.

In vitro antibacterial activity and SAR analysis

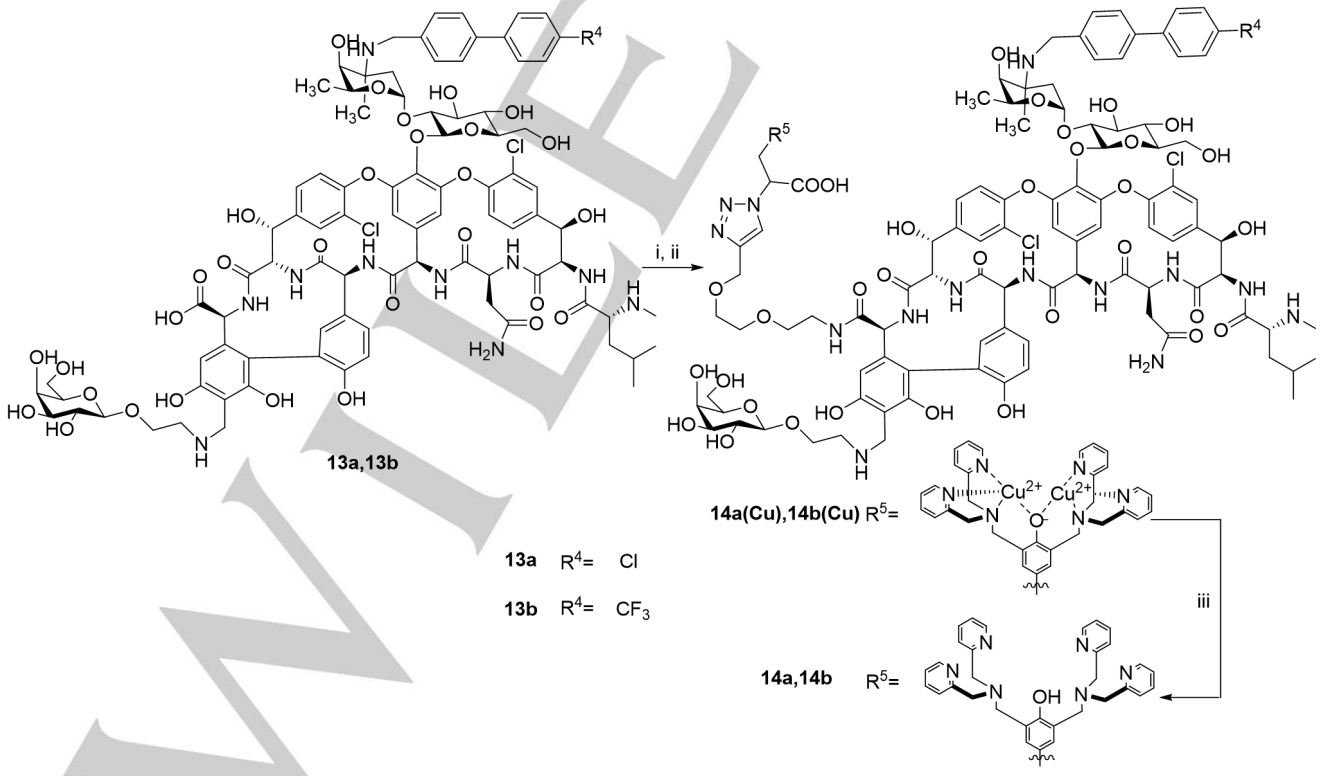
With the vancomycin derivatives in hands, we sought to study the structure-activity relationship (SAR) by *in vitro* antibacterial assays. Firstly, we chose a MSSA (Methicillin-susceptible *S. aureus*) strain (Newman), a VISA strain (Mu50)[20] and two VRE (*van*A) strains[21] (Efm-HS-0649, Efm-HS-06188) to perform the assay and measure the MIC values of our compounds (Table 1 and S2). The DPA intermediates (**18**, **19**, **4b**, **4c**) were also evaulated and did not show antibacterial activity (Table S3). Next, we selected some representative compounds to explore their antibacterial activity against two other vancomycin-susceptible strains (Table S4). Then, some representative compounds were screened on their antibacterial activities against other three VRE strains (*van*B, *van*M) (Table 2).



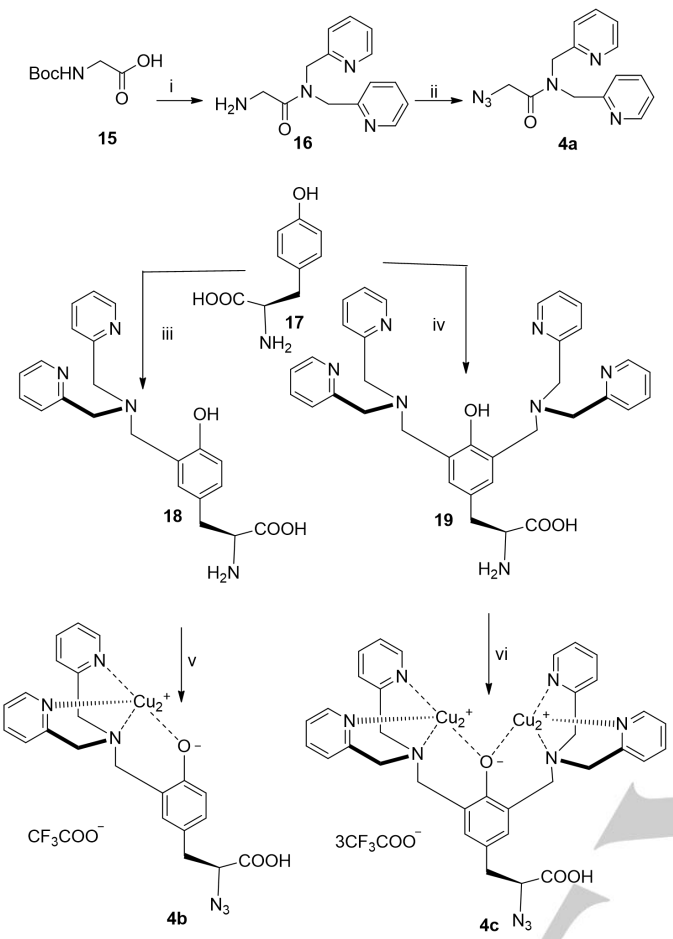
Scheme 1. Synthesis of new vancomycin derivatives **3a,5a(Cu)-5d(Cu),8a-8f(Cu)**. Reagents and conditions: i, HCHO, DIPEA, CH_3CN:H2O = 1:1; ii, $NaHCO_3$, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, tBuOH; H_2O = 1:1; iii, HATU, DIPEA, DMF:DMSO = 2:1.



Scheme 2. Synthesis of new vancomycin derivatives **12a(Cu)-12d(Cu),13a(Cu)-13d(Cu)**. Reagents and conditions: i, (a) DIPEA, DMF, 55 °C, 4h; (b) $NaCNBH_3$, TFA, MeOH, room temperature, 1h; ii, **2c**, HCHO, DIPEA, $CH_3CN:H_2O$ = 1:1; iii, **4c**, $NaHCO_3$, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, $^tBuOH:H_2O$ = 1:1; iv, **2c**, HATU, DIPEA, DMF:DMSO = 2:1.



Scheme 3. Synthesis of new vancomycin derivatives **14a-b**. Reagents and conditions: **I, 2c**, HATU, DIPEA, DMF:DMSO = 2:1; ii, **4c**, $NaHCO_3$, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, tBuOH; H_2O = 1:1; iii, EDTA-2Na, H_2O.



Scheme 4. Synthesis of **4a-4c**. Reagents and conditions: i, 1, 2,2-dipicolylamine, EDCI, HOBt, DMF; 2, 50% TFA in DCM ii, imidazole-1-sulfonyl azide·HCl, K_2CO_3, $CuSO_4$ $5H_2O$ (1% eq.), CH_3OH iii, 2,2-dipicolylamine, $(CH_2O)n$, CH_3OH, reflux for 2 days; iv, 2,2-dipicolylamine, $(CH_2O)n$, Cat. 1M HCl, CH_3CH_2OH/H_2O, reflux for 36h; v, imidazole-1-sulfonyl azide·HCl, K_2CO_3, $CuSO_4$ $5H_2O$ (1.5 eq.), CH_3OH/H_2O; vi, imidazole-1-sulfonyl azide·HCl, K_2CO_3, $CuSO_4$ $5H_2O$ (2.5 eq.), CH_3OH.

We summarized the SAR based on the assay results:

1. Vancomycin derivatives conjugated with DPA showed different antibacterial activity towards different Gram-positive bacteria. Generally, these compounds showed stronger antibacterial activity against VRE than VISA, MSSA and other vancomycin-susceptible strains. However, their antibacterial activities against *S. aureus* and *bacillus and streptococci* strains were not improved. These data demonstrated that DPA attachment is a useful strategy to enhance anti-VRE activity selectively.
2. The DPA_2-Tyr-van compounds (**5c** and **8c**) indicated better activities than DPA-Tyr-van compounds (**5b** and **8b**). The DPA-Gly-van compounds (**5a** and **8a**) did not show increased activity compared with vancomycin. It suggested the Tyr is a good template as DPA carrier since the hydroxyl of Tyr could be involved in metal chelation and enhance the pyrophosphate binding.
3. Copper (II) complexes **5d(Cu), 8b(Cu), 8c(Cu), 8e(Cu), 12b(Cu), 12c(Cu)**, and **12d(Cu)** showed better antibacterial activities in comparison of their respective ligands (**5d, 8b, 8c, 8e, 12b, 12c**, and **12d**). This is possibly due to their better binding affinity with pyrophosphate.
4. The PEG_2 structure is the optimal linker for DPA-vancomycin conjugation and compound **8f** (DPA_2-Tyr-PEG_2-vancomycin) showed the best activity against VISA and VRE (**8f** *vs* **8b, 8c, 8d**).
5. For combined modification of lipophilic and DPA groups, compound **11b** bearing DPA_2-Tyr-PEG_2 moiety on 7th-amino acid resorcinol and decylaminoethyl on vancosamine indicated the best anti-VRE (*van*A) activity (MIC 1 μg/mL), >128-fold higher than vancomycin. Impressively, its copper-complex **11b(Cu)** showed >1024 fold higher activity than vancomycin against *van*B(R) strain (MIC $\leqslant$0.0625 μg/mL). These data suggested the possible synergistic effect by combination of the lipophilic and DPA assembly. However, we did not observe the similar synergism in other compounds (**11a**, **11c-d**, **12a-d**) (Table 1 and 2), implicating the importance of proper lipophilic group and DPA conjugation position for synergism.
6. The triple modification compounds containing galactose motif remained similar anti-VRE activities compared with the corresponding non-galactose compounds (**14a-b** *vs* **12c-d**). This is in consistence with our previous results and the advantage of galactose was reported to optimize *in vivo* clearance rate and avoid accumulation.

In the end, we investigated the combined treatment with DPA-vancomycin (**8f**) and lipo-vancomycin (**10a-d**) for anti-VRE assay. In an ideal situation, two mechanism of these compounds would work simultaneously and exhibit better efficacy than individual treatment. Unfortunately, the results were contrary to our anticipation. No significant enhancement in antibacterial activity was observed when combining two set compounds (Table S5). On the other hand, it implicated the restrictive region-chemistry of vancomycin derivatives by optimal combined modification might be important for synergism.

Mechanism validation by indicator displacement assays (IDAs)

We employed the IDA approach[22] to determine the interaction between the metal-DPA complex and pyrophosphate. The concept of this assay is shown in Figure 2. Pyrocatechol violet (PV)[23], a cation dye reagent, binds to metal-DPA chelates to give a blue solution[24] when the binding is strong. Then, pyrophosphoric acid (PPi)[25] is added, and its competitive binding with metal-DPA will release PV which is yellow in unbound form. Therefore, we can evaluate the interaction of metal-DPA and PPi based on the color alternation.

Herein, we chose compound **8f** as the model for IDA assay. Compound **8f** was mixed together with PV and different metal ions respectively. As shown in Figure 3 and Figure S1, the bottles containing Zn(II), Cu(II) and Fe(II) are blue while the control bottle without metal ion is yellow. After adding 10 equiv. PPi, the colors of bottles containing Zn(II) and Cu(II) were turned

to yellow that implicated Zn(II)-DPA and Cu(II)-DPA bound to PPi and released free PV quickly. We monitored the color alternation by adding 1, 2, 3, 5, 10 equiv. PPi in Figure S1. Zn(II)-DPA bottles turned to yellow color by less equiv. of PPi than Cu(II)-DPA, suggesting Zn(II)-DPA binds to PPi more tightly. We also conducted the assay without PV dye. Compound **19** (DPA_2-Tyr) was treated with 2 equiv. Zn(II), Cu(II), Fe(III), and Fe(II) (Figure 3c-d and S2). DPA_2-Tyr indicated better chelating effect with Cu(II), Fe(III), and Fe(II) than Zn(II). We also observed the color alternation after adding PPi.

These data validated the pyrophosphate-targeting effect of Cu(II)-DPA-Tyr-vancomycin derivatives for enhanced antibacterial activity as similar as the reported mechanism of Zn(II)-Dipi-van[11]. Based on this conclusion, we proposed the MOA of these compounds as shown in Figures 1. Cu(II)-DPA binds to pyrophosphate moiety of lipid II and the inherent mechanism of vancomycin skeleton remains to target D-Ala-D-Ala or D-Ala-D-Lac motif.

Cytotoxicity assays

To test the possible toxicity of pyrophosphate-targeting strategy, we examined the cytotoxicity of representative DPA compounds and their copper chelates (**5d**, **5d(Cu)**, **8f**, **8f(Cu)**) on two mammalian cell lines (CHO and HEK293T). As shown in Figure 4, DPA compounds did not show significant toxicity at the concentrations <50 μM, and their copper chelates indicated slightly higher toxicity especially at the concentration >50 μM. It seems the toxicity is not from pyrophosphate targeting but from copper ion. For therapeutic application, it is not necessary to use the copper chelates since the non-metal DPA-vancomycin could achieve similar anti-VRE activity. Meanwhile, compared the enhanced antibacterial activity (> 64 fold) and the increased toxicity (~30% at 100 μM), the DPA modification still demonstrated a good selectivity against the bacteria VRE than mammalian cells.

Table 1. *In vitro* activities of vancomycin derivatives against resistant *Enterococci** (*van*A)

Compound	MIC (μg/ml)		Compound	MIC (μg/ml)	
	Efm-HS-0649	Efm-HS-06188		Efm-HS-0649	Efm-HS-06188
3a	128	>128	**11b(Cu)**	2	2
5a	>128	128	**11b**	1	1
5b(Cu)	>128	128	**11c(Cu)**	2	2
5b	>128	128	**11c**	2	2
5c(Cu)	16	8	**11d(Cu)**	4	2
5c	8	4	**11d**	2	1
5d(Cu)	4	2	**12a(Cu)**	4	4
5d	8	8	**12a**	4	4
8a	128	64	**12b(Cu)**	2	1
8b(Cu)	64	32	**12b**	2	2
8b	128	64	**12c(Cu)**	2	2
8c(Cu)	2	2	**12c**	4	4
8c	8	8	**12d(Cu)**	1	1
8d(Cu)	4	4	**12d**	4	4
8d	4	4	**14a(Cu)**	4	4
8e(Cu)	2	2	**14a**	2	4
8e	4	4	**14b(Cu)**	2	4
8f(Cu)	4	4	**14b**	2	2
8f	4	4	**Dipi-van**	4	4
11a(Cu)	4	4	**Vancomycin**	>128	>128
11a	2	2	**Telavancin**	8	8

*Efm-HS-0649 and Efm-HS-06188: Glycopeptide-resistant *E.faecium*, *vanA* phenotype, isolated in China[21].

Table 2. *In vitro* activities of vancomycin derivatives against resistant *Enterococci* (*van*B, *van*M)

Compound	MIC (μg/ml)		
	Efm-HS-0847	Efm-HS-08257	Efm-HS-vb01
5c(Cu)	8	8	16
5c	8	8	8
8c(Cu)	2	2	2
8c	8	8	8
8d(Cu)	4	4	4
8d	8	4	4
8f(Cu)	4	4	4
8f	8	4	4
8e(Cu)	2	2	2
8e	2	4	4
11b(Cu)	1	1	≤0.0625
11b	1	1	1
11d(Cu)	4	4	2
11d	2	2	2
12b(Cu)	1	1	2
12b	2	2	2
12c(Cu)	1	1	1
12c	4	8	4
10b	16	8	16
10d	2	2	≤0.0625
Dipi-van	4	0.25	0.125
Vancomycin	>128	>128	>128
Telavancin	8	4	≤0.0625

Efm-HS-0847 and Efm-HS-08257: Glycopeptide-resistant *E.faecium*, *vanM* phenotype, isolated in China[21]; Efm-HS-vb01: Glycopeptide-resistant *E.faecium*, *vanB* phenotype, isolated in China.

Conclusions

In summary, a simple and rational strategy was reported to combat the acquired resistance of Gram-positive bacteria towards glycopeptide antibiotics. 39 novel vancomycin derivatives with pyrophosphate-targeting DPA chelating molecules were designed and synthesized. Then we evaluated these derivatives for their *in vitro* antibacterial activity especially towards the intractable VRE strains. Among them, 32 compounds exhibited >8-128 fold higher activity than vancomycin against VREs (*van*A, *van*B, *van*M phenotype, *E. faecium*). These compounds selectively enhanced antibaceterial activity against VREs but not for VISA, MSSA, and other two vancomycin-susceptible strains. This results generated an interesting question on mechanism of the selectivity. The SAR studies indicated that DPA_2-Tyr-PEG_2 is the optimal modification for vancomycin using pyrophosphate-targeting strategy. Combined modifications of lipophilic decylaminoethyl group attached on vancosamine and DPA_2-Tyr-PEG_2 fragment appending at resorcinol position demonstrated a synergistic effect, with enhanced activities >128 to 1024-fold than vancomycin, 2-8 fold than Telavancin, 4 fold than Dipi-van against VRE. For the mechanism of action, we validated the interaction between pyrophosphate and our DPA-Tyr-vancomycin compounds by IDA approach. These data supported our hypothetic MOA of these compounds through lipid II binding by forming the metal coordination complex with the bacterial living environmental metal ions. The Cu(II)-DPA-Tyr-vancomycin compounds indicated increased the antibacterial activity probably through this proposed mechanism. Noteworthy, the various cell wall structures of different bacteria could be a reason why these compounds have selective activity against *enterococci* but not other Gram-positive strains (*S. aureus, B. subtilis, S. pyogenes*) and the detailed mechanism will be remained in the future to elucidate. Considering the efficacy and safety of these compounds comprehensively, the copper free vancomycin derivatives bearing DPA moiety are more suitable for further research and development.

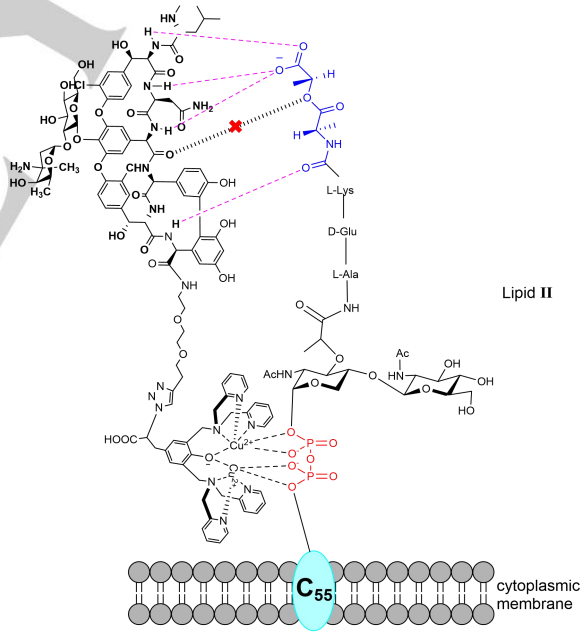


Figure 1. Proposed mode of action of **8f(Cu²⁺)** against VRE. Vancomycin backbone binds with 1000-fold lower affinity to the D-Ala-D-Lac termini (blue) of the VRE cell wall peptidoglycan's precursor lipid II through a network of four hydrogen bond (purple dashed line). The Tyr-DPA_2-Cu^{2+} complex can bind with the pyrophosphate (red) of lipid II, compensating the reduced binding affinity with the mutated dipeptide. The van-$DPA_2(Cu^{2+})$-lipid II complex sterically barricade the next-step transglycosylation and transpeptidation for cell wall biosynthesis, which renders the bacteria cell susceptible to lysis under osmotic pressure fluctuation.

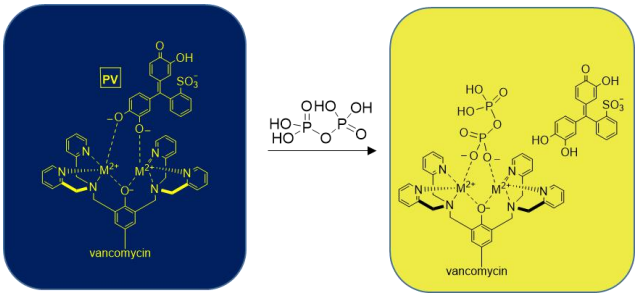


Figure 2. Concept of the indicator displacement assay (IDA).

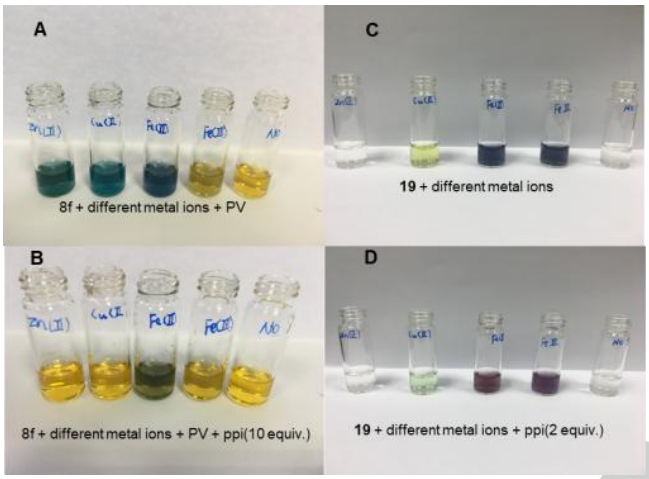


Figure 3. IDA assays for DPA-vancomycin derivatives. Panel A, **8f** + different metal ions + PV; Panel B, **8f** + different metal ions + PV + ppi (10 equiv.); Panel C, **19** + different metal ions; Panel D, **19** + different metal ions + ppi (2 equiv.). The metal ions added in the bottles from left to right: Zn^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, no-metal control.

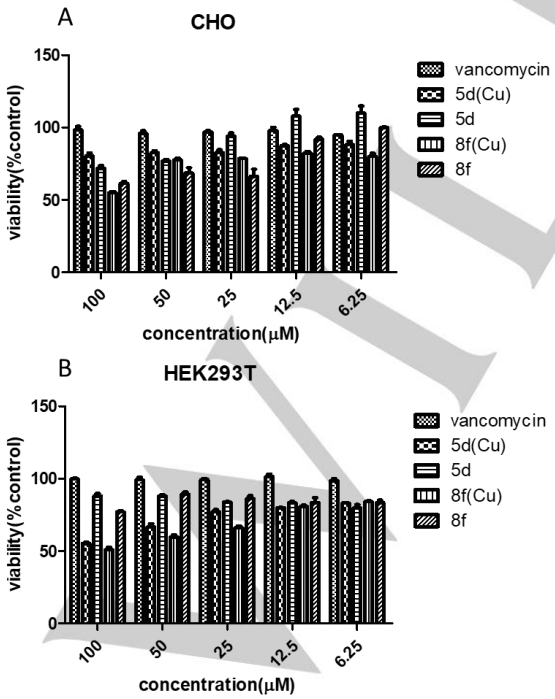


Figure 4. Cytotoxicity assays of selected compounds. Panel A: cell viability test on CHO cells; Panel B: cell viability test on HEK293T cells.

Experimental Section

Material and Instrumentation

All reagents and solvents were commercially purchased from Sinopharm, Bide Pharmatech Ltd. and Shanghai Titan Ltd. (Adamas-beta®) and used without further purification. Analytic RP-HPLC analysis was performed on a Beijing Chuang Xin Tong Heng LC-3000 (analytic model) instrument with a C-18 column (5 μm, 4.6 x 150 mm) at 40°C. The column was eluted with a gradient of 2-90% acetonitrile containing 0.1% TFA in 30 min at a flow rate of 1 mL/min. Preparative RP-HPLC preparation was performed on a Beijing Chuang Xin Tong Heng LC-3000 (preparative model) instrument with a C-18 column (10 μm, 19 x 250 mm) at room temperature. The column was eluted with a gradient of 2-70% acetonitrile containing 0.1% TFA in 30 min at a flow rate of 10 mL/min. HPLC analysis showed that the purity of all the final products were more than 95% (based on the above HPLC method). ^{1}H-NMR, ^{13}C-NMR spectra were recorded on a BRUKER Ascend™ 400 MHz or 600 MHz instrument. Chemical shifts were assigned in ppm and coupling constants were assigned in Hz. All the final products were added 20 μL D_2O to exchange the active hydrogen of the target products when the solvent was DMSO-d_6. ESI-MS spectra were measured with an Agilent 6230 LC-TOF MS spectrometer. Bacterial strains Newman and Mu50 were from laboratory stock. Efm-HS-0649, Efm-HS-06188 were obtained from Institute of antibiotics, Huashan hospital, and Fudan University.

NH_2-Gly-DPA (16)

N-Boc-L-glycine (88 mg, 0.5 mmol) was dissolved in DMF, EDCI (96 mg, 0.5 mmol) and HOBt (68 mg, 0.5 mmol) were successively added into the solution at 0 °C. Then 2,2-dipicolylamine (DPA) (100 mg, 0.5 mmol) was added and the solution was allowed to warm to room temperature and stirred for overnight. The reaction mixture was diluted with DCM. The organic layer was washed with water for 3 times and evaporated at reduced pressure. For deprotection of Boc protecting group, the residue was dissolved in 50% TFA in DCM and stirred for 1h. After completion of the reaction monitored by analytic RP-HPLC, the reaction mixture was evaporated, purified by preparative RP-HPLC and lyophilized to get the target compound **16** as slightly yellow oil (77 mg, yield 60%). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.69 – 8.52 (m, 2H), 8.40 (td, *J* = 7.9, 1.6 Hz, 1H), 8.24 (td, *J* = 7.9, 1.7 Hz, 1H), 7.84 (t, *J* = 6.8 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.74 – 7.59 (m, 2H), 5.06 (s, 2H), 4.98 (s, 2H), 4.09 (s, 2H). HRMS (ESI) calcd for $C_{14}H_{16}N_4O$ $[M+H]^+$*m/z* 257.1402, found *m/z* 257.1408.

N_3-Gly-DPA (4a)

The imidazole-1-sulfonyl azide· HCl (68 mg, 0.32 mmol) was added into the suspension of the **16** (70mg, 0.27mmol), K_2CO_3 (93 mg, 0.68 mmol), $CuSO_4 \cdot 5H_2O$ (0.7 mg, 2.7μmol) in methanol (3 ml) and stirred at room temperature. After completion of this reaction, monitored by analytic RP-HPLC, the reaction mixture was concentrated and purified by preparative RP-HPLC to get the N_3-product **4a** as dark brown oil (69mg, yield 90%). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.70 (ddd, J = 5.7, 1.7, 0.8 Hz, 1H), 8.63 (d, J = 5.5 Hz, 1H), 8.46 (td, J = 8.0, 1.6 Hz, 2H), 7.93 – 7.82 (m, 4H), 5.15 (s, 2H), 4.96 (s, 2H), 4.26 (s, 2H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 171.82 , 150.59 , 150.28 , 147.29 , 146.80 , 143.16 , 141.55 , 126.43 , 126.27 , 125.77 , 124.97 , 50.62 , 49.71 , 48.66 . HRMS (ESI) calcd for $C_{14}H_{14}N_6O$ $[M+H]^+$*m/z* 283.1307, found *m/z* 283.1309.

NH_2-Tyr-DPA (18)

A mixture of $(CH_2O)_n$ (33 mg, 1.21 mmol) and DPA (219 mg, 1.21 mmol) in methanol was heated to 65°C until the solution became homogeneous,

N-Boc-L-tyrosine (281 mg, 1.0 mmol) was added and the solution was then refluxed for 2 days. After cooling the solvent was removed, the crude compound was directly used in the next step to deprotect the Boc-group by 50% TFA in DCM. Then the mixture was concentrated at reduced pressure and purified by preparative RP-HPLC to give **18** as an oil product (274mg, 70%). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.55 (dt, *J* = 5.7, 1.3 Hz, 2H), 8.34 (td, *J* = 7.9, 1.6 Hz, 2H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.77 (td, *J* = 7.4, 1.6 Hz, 2H), 6.90 (d, *J* = 2.3 Hz, 1H), 6.80 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.45 (d, *J* = 8.3 Hz, 1H), 4.32 (s, 4H), 3.99 (t, *J* = 6.5 Hz, 1H), 3.66 (s, 2H), 3.05 – 2.82 (m, 2H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 171.98 , 154.10 , 153.01 , 146.60 , 140.52 , 133.02 , 131.11 , 126.73 , 125.87 , 125.79 , 122.82 , 115.77 , 57.18 , 55.53 , 54.46 , 34.65 . HRMS (ESI) calcd for $C_{22}H_{24}N_4O_3$ $[M+H]^+$*m/z* 393.1927, found *m/z* 393.1918.

N_3-Tyr-DPA (4b)

The imidazole-1-sulfonyl azide· HCl (128 mg, 0.61 mmol) was added into the suspension of **18** (200mg, 0.51mmol), K_2CO_3 (175 mg, 1.27 mmol), $CuSO_4 \cdot 5H_2O$ (191 mg, 0.76 mmol) in methanol (3 ml) and H_2O (1ml), and stirred at room temperature. After completion of this reaction, monitored by analytic RP-HPLC, the reaction mixture was concentrated and purified by preparative RP-HPLC to get the N_3-product **4b** as green fluffy solid which chelated with one equivalent Cu^{2+} and trifluoroacetate (120 mg, yield 40%). HRMS (ESI) calcd for $C_{22}H_{21}N_6O_3Cu^+ \cdot CF_3COO^-$[M – CF_3COO^-]$^+$*m/z* 480.0966, found *m/z* 480.0996.

NH_2-Tyr-DPA_2 (19)

1 M HCl (175 µl) and N-Boc-L-Tyrosine (200 mg, 0.71 mmol) was added into a suspension of $(CH_2O)n$ (140 mg, 1.55 mmol) and DPA (350 mg, 1.76 mmol) in ethanol (1.4 ml) and water (4.2 ml). The solution was heated to 65 ˚C to reflux for 36 hours until the completion of reaction monitored by HPLC. The reaction mixture was then cooled to room temperature and evaporated to dryness under reduced pressure. Then the residue was subjected with 50% TFA in DCM to remove the Boc-group for 1 hour and evaporated under reduced pressure. The crude compound was purified by preparative RP-HPLC to give **19** as yellow oil (364 mg, yield 85%). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.53 (dd, *J* = 5.9, 1.6 Hz, 4H), 8.32 (td, *J* = 7.9, 1.6 Hz, 4H), 7.82 (d, *J* = 8.1 Hz, 4H), 7.79 – 7.72 (m, 4H), 6.82 (s, 2H), 3.91 (dd, *J* = 8.0, 5.1 Hz, 1H), 3.58 (q, *J* = 13.1 Hz, 4H), 2.96 (dd, *J* = 14.2, 5.1 Hz, 1H), 2.84 (dd, *J* = 14.3, 8.1 Hz, 1H). ^{13}C NMR (101 MHz, Deuterium Oxide) δ 172.75, 152.61, 152.51, 146.32, 141.27, 133.12 , 127.03, 125.97, 123.28, 117.78, 114.88, 56.46, 55.05, 55.00 , 34.92. HRMS (ESI) calcd for $C_{35}H_{37}N_7O_3$ $[M+H]^+$*m/z* 604.3036, found *m/z* 604.3093.

N_3-Tyr-DPA_2 (4c)

The imidazole-1-sulfonyl azide.HCl (209 mg, 1.0 mmol) was added into the suspension of the above amine (300 mg, 0.50 mmol), K_2CO_3 (275 mg, 2.0 mmol), $CuSO_4.5H_2O$ (312 mg, 1.25 mmol) in methanol (5 ml), and stirred at room temperature. After completion of this reaction, monitored by analytic RP-HPLC, the reaction mixture was concentrated and purified by preparative RP-HPLC to get the N_3-Tyr-2DPA **4c** as slightly brown and fluffy solid which chelated with two equivalent $Cu^{2+}$and trifluoroacetate (328 mg, yield 60%). HRMS (ESI) calcd for $C_{35}H_{34}N_9O_3Cu_2^{3+} \cdot 3CF_3COO^-$ [M-CF_3COO^-]$^+$*m/z* 980.1072, found *m/z* 980.1098.

General procedures for synthesis of final vancomycin-DPA compounds. (3a, 5a(Cu)-5d(Cu), 5a-5d, 8a(Cu)-8f(Cu), 8a-8f, 11a(Cu)-11d(Cu), 11a-11d,12a(Cu)-12d(Cu), 12a-12d, 14a(Cu)-14b(Cu), 14a-14b)

Reductive amination reaction, Mannich reaction, amide condensation reaction and click chemistry (CuAAC reaction) were used in the synthesis of final compounds. Herein, we described the general procedure respectively. First, for intermediate **10a-10d**, vancomycin hydrochloride (100 mg, 67 µmol), R_3-CHO **9a-9d** (2 eq.) and DIPEA (30 µl, 172 µmol) were stirred in DMF (3 ml) for 4 hours to form Schiff base at 55°C and was monitored by analytic RP-HPLC. After completion conversion aldehyde in Schiff base, $NaCNBH_3$ (8 mg, 127 µmol) in methanol (1 ml) was added into the above reaction mixture. TFA (30 µl) was added to adjust pH to 4-5. The residue was stirred at room temperature for 2 hours to reduce the Schiff base. Then the crude product was precipitated by addition of excess of diethyl ether (50ml) and centrifuged. The supernatant was removed and the solid cake was washed with diethyl ether (30ml) once more to give the crude product. The crude was dissolved in small amount of water/acetonitrile (1:1) and was purified by preparative RP-HPLC. The fractions containing target compound was combined and lyophilized to give the product as a white fluffy solid in 70-90% yield. For final compound **3a**, intermediate **3b-3c** and corresponding alkynyl intermediates of **11a(Cu)-11d(Cu)**, Mannich reaction were performed to give corresponding product. Vancomycin hydrochloride (40 µmol) or intermediate **10a-10d** (40 µmol), R_1-NH_2 (400 µmol) and DIPEA (800 µmol) were mixed with water (1.5 ml) and CH_3CN (1.5 ml). The mixture was stirred at room temperature until the solution become homogeneous, and then the reaction mixture was kept at -10˚C for 5 minutes. Then formaldehyde (37% in water, 4.5 µl, 60 µmol) was added into the above cooled solution and monitored by analytic RP-HPLC. After 12 hours (conversion rate reached 50-80%) the residue was treated with TFA to adjust the pH to 2-3. The residue was subjected to preparative RP-HPLC purification to give corresponding intermediates as white fluffy solids in 40-80% yield. For intermediate **7a-7d** and corresponding alkynyl intermediates of **12a(Cu)-12d(Cu),14a(Cu)-14b(Cu)**, amide condensation reaction was performed to give the corresponding product. Vancomycin hydrochloride (40 µmol) or intermediate **10a-10d**,**13a-13b** (40 µmol) (**13a,13b** from previous work[14]), R_2-NH_2 (60 µmol), DIPEA (80 µmol) were mixed in 1.2 ml DMF and 600 µl DMSO, the mixture was stirred for 10 minutes at room temperature to make the solution homogeneous, then the reaction mixture was transfered to ice bath. HATU (45 µmol) was added into the cooled solution dropwise. After 30 minutes, the reaction mixture was transfered back to room temperature and monitored by analytic RP-HPLC. After 1.5 hours (when conversion rate reached 40-80%), the reaction was quenched by TFA, adjusting the pH to 3-4. Then excess diethyl ether (20 ml) was poured into the residue to precipitate the crude product and centrifuged. The crude solid product was dissolved in small amount of water/acetonitrile (1:1) and was purified by preparative RP-HPLC. The fractions containing target compound was combined and lyophilized to give the product as a white fluffy solid in 40-70% yield. For all intermediates' HRMS results were shown in the Supporting Information. For the other final compounds and corresponding copper(Cu^{2+}) chelating compounds, Copper(I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction was performed to give the corresponding final target product. Alkyn-intermediates (15 µmol) and azide-DPA intermediates **4a-4c** (16 µmol) were dissolved in H_2O (1ml) and ^{t}BuOH (1ml) to 4ml centrifuge tube. Then $NaHCO_3$ (120 µmol) and $CuSO_4.5H_2O$ (30 µmol) were added into the reaction mixture, sodium ascorbat (225 µmol) was swiftly added into the mixture to reduce the copper (II) to copper (I) and the reaction tube was kept tightly close otherwise the air would oxidize the copper(I). The reactive solution was shaked at room temperature for 2 hours and monitored with analytic RP-HPLC. After completion, the reaction was quenched with TFA (20 µl) and directly purified by preparative RP-HPLC, the target fraction was collected and lyophilized to give the product as a slight green fluffy solid in 75%-85% yield. Except compound **5a** and **8a**, the other compounds were subjected to removing the chelated copper (II). The copper-complex (10 µmol) was dissolved in EDTA-2Na (100 mM, 3 ml) and shaked for 30 minutes until all the copper was removed. The mixture was purified by preparative RP-HPLC. The fractions containing target compound was combined and lyophilized to give the product as a white fluffy solid in 80-90% yield.

Dipicolylaminomethyl vancomycin (3a)

Yield 41% (41 mg, 24.7 μmol), R.T. = 10.754 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.57 (s, 1H), 8.54 (d, *J* = 4.9 Hz, 2H), 7.88 – 7.80 (m, 2H), 7.76 (s, 1H), 7.59 (s, 1H), 7.50 (d, *J* = 9.5 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.37 (dd, *J* = 7.6, 5.0 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.10 (s, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 6.45 (s, 1H), 5.72 (d, *J* = 7.8 Hz, 1H), 5.66 (s, 1H), 5.26 – 5.19 (m, 2H), 5.13 (d, *J* = 3.1 Hz, 1H), 5.09 (d, *J* = 7.2 Hz, 2H), 4.83 (s, 1H), 4.65 (q, *J* = 6.5 Hz, 1H), 4.45 (t, *J* = 6.2 Hz, 2H), 4.41 (s, 1H), 4.39 (d, *J* = 5.5 Hz, 2H), 4.31 (d, *J* = 13.4 Hz, 1H), 4.09 (d, *J* = 10.4 Hz, 2H), 3.99 (t, *J* = 6.8 Hz, 1H), 3.65 (d, *J* = 10.9 Hz, 1H), 3.28 – 3.21 (m, 2H), 3.14 (s, 1H), 2.58 (s, 3H), 2.15 (dd, *J* = 16.5, 9.2 Hz, 1H), 1.91 – 1.84 (m, 1H), 1.70 (d, *J* = 13.1 Hz, 1H), 1.68 – 1.61 (m, 2H), 1.53 (dd, *J* = 10.7, 5.4 Hz, 1H), 1.27 (s, 3H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 6.1 Hz, 3H), 0.86 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{79}H_{88}Cl_2N_{12}O_{24}$ $[M+2H]^{2+}$*m/z* 830.2784, found *m/z* 830.2766.

Dipicolylaminoformylmethyl [1,2,3]-triazolyl methylaminomethyl vancomycin (5a)

Yield 85% (23 mg, 12.7 μmol), R.T. = 10.813 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.63 (s, 1H), 8.60 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.51 – 8.48 (m, 1H), 8.19 (s, 1H), 7.83 – 7.75 (m, 3H), 7.60 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.35 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.33 – 7.27 (m, 3H), 7.18 (d, *J* = 8.4 Hz, 1H), 7.10 (s, 1H), 5.74 (s, 2H), 5.71 (d, *J* = 7.4 Hz, 1H), 5.66 (s, 1H), 5.26 – 5.20 (m, 2H), 5.14 – 5.07 (m, 3H), 4.84 (s, 2H), 4.80 (s, 1H), 4.65 (q, *J* = 6.4 Hz, 1H), 4.59 (d, *J* = 3.3 Hz, 2H), 4.45 (d, *J* = 5.5 Hz, 1H), 4.41 (d, *J* = 5.7 Hz, 1H), 4.24 (s, 2H), 4.15 – 4.07 (m, 3H), 4.01 – 3.96 (m, 1H), 3.24 (d, *J* = 5.4 Hz, 2H), 3.15 (s, 1H), 2.57 (s, 3H), 2.09 (s, 1H), 1.88 (d, *J* = 11.6 Hz, 1H), 1.71 (d, *J* = 13.1 Hz, 1H), 1.64 (dq, *J* = 11.8, 6.4, 5.2 Hz, 2H), 1.54 – 1.47 (m, 1H), 1.28 (s, 3H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.0 Hz, 3H), 0.84 (d, *J* = 6.1 Hz, 4H). HRMS (ESI) calcd for $C_{84}H_{94}Cl_2N_{16}O_{25}$ $[M+2H]^{2+}$*m/z* 899.3055, found *m/z* 899.3035.

3'- dipicolylaminomethyltyrosine [1,2,3]-triazolyl methylaminomethyl vancomycin(Cu) (5b-Cu)

Yield 85% (27 mg, 12.7 μmol), R.T. = 11.428 min (analytical HPLC). HRMS (ESI) calcd for $C_{92}H_{101}Cl_2N_{16}O_{27}Cu^+$ $[M+2H]^{3+}$*m/z* 665.5284, found *m/z* 665.5283.

3'- dipicolylaminomethyltyrosine [1,2,3]-triazolyl methylaminomethyl vancomycin (5b)

Yield 87% (16 mg, 8.7 μmol), R.T. = 11.012 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.68 (s, 1H), 8.54 (d, *J* = 4.9 Hz, 2H), 8.27 (s, 1H), 7.83 – 7.79 (m, 3H), 7.64 (s, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 2H), 7.36 (dd, *J* = 7.6, 5.0 Hz, 2H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.13 (s, 1H), 7.08 (s, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 0H), 6.79 (d, *J* = 8.6 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 5.73 (d, *J* = 7.1 Hz, 1H), 5.71 – 5.65 (m, 2H), 5.24 (t, *J* = 6.4 Hz, 2H), 5.13 (s, 2H), 5.10 (s, 1H), 4.80 (s, 1H), 4.66 (d, *J* = 6.8 Hz, 1H), 4.46 (s, 1H), 4.43 (d, *J* = 5.6 Hz, 1H), 4.30 – 4.22 (m, 4H), 4.16 – 4.09 (m, 4H), 4.04 – 3.95 (m, 4H), 3.85 (s, 1H), 3.25 (d, *J* = 5.5 Hz, 2H), 3.17 (s, 1H), 2.57 (s, 3H), 2.17 – 2.07 (m, 1H), 1.89 (d, *J* = 11.9 Hz, 1H), 1.72 (d, *J* = 12.8 Hz, 1H), 1.64 (q, *J* = 7.7, 6.3 Hz, 2H), 1.51 (s, 1H), 1.29 (s, 3H), 1.05 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 5.9 Hz, 3H), 0.85 (d, *J* = 6.0 Hz, 3H). HRMS (ESI) calcd for $C_{92}H_{102}Cl_2N_{16}O_{27}$ $[M+3H]^{3+}$*m/z* 645.2237, found *m/z* 645.2292.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methylaminomethyl vancomycin(Cu) (5c-Cu)

Yield 83% (32 mg, 12.5 μmol), R.T. = 14.022 min (analytical HPLC). HRMS (ESI) calcd for $C_{105}H_{114}Cl_2N_{19}O_{27}Cu_2^{3+}$ M^{3+}*m/z* 756.2028, found *m/z* 756.2022.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methylaminomethyl vancomycin (5c)

Yield 80% (17 mg, 8.0 μmol), R.T. = 11.844 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.69 (s, 1H), 8.52 (d, *J* = 5.0 Hz, 4H), 8.28 (s, 1H), 7.87 – 7.79 (m, 5H), 7.62 (s, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.40 (dd, *J* = 7.6, 5.2 Hz, 4H), 7.37 (d, *J* = 7.9 Hz, 4H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 7.01 (s, 2H), 6.86 (d, *J* = 8.7 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.55 (s, 1H), 5.72 (dd, *J* = 10.3, 6.0 Hz, 2H), 5.68 (s, 1H), 5.25 (d, *J* = 8.0 Hz, 1H), 5.24 (s, 1H), 5.14 (s, 2H), 5.11 (s, 1H), 4.80 (s, 1H), 4.66 (q, *J* = 6.8 Hz, 1H), 4.49 – 4.43 (m, 2H), 4.19 – 4.06 (m, 12H), 4.03 – 3.99 (m, 2H), 3.95 (s, 5H), 3.56 – 3.49 (m, 2H), 3.47 – 3.41 (m, 2H), 3.30 (d, *J* = 11.9 Hz, 1H), 3.28 – 3.24 (m, 2H), 3.18 (s, 1H), 2.58 (s, 3H), 2.13 (s, 1H), 1.90 (d, *J* = 11.8 Hz, 1H), 1.73 (d, *J* = 13.2 Hz, 1H), 1.68 – 1.60 (m, 2H), 1.55 – 1.48 (m, 1H), 1.30 (s, 3H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 5.9 Hz, 3H), 0.85 (d, *J* = 6.0 Hz, 3H). HRMS (ESI) calcd for $C_{105}H_{115}Cl_2N_{19}O_{27}$ $[M+2H]^{2+}$*m/z* 1072.8870, found *m/z* 1072.8908.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxylethyoxyl ethylaminomethyl vancomycin(Cu) (5d-Cu)

Yield 78% (31 mg, 11.7 μmol), R.T. = 13.233 min (analytical HPLC). HRMS (ESI) calcd for $C_{109}H_{122}Cl_2N_{19}O_{29}Cu_2^{3+}$ $[M]^{3+}$*m/z* 785.5542, found *m/z* 785.5524.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxylethyoxyl ethylaminomethyl vancomycin (5d)

Yield 86% (19 mg, 8.6 μmol), R.T. = 11.898 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.63 (s, 1H), 8.50 (d, *J* = 4.9 Hz, 4H), 8.10 (s, 1H), 7.79 – 7.75 (m, 4H), 7.60 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.36 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.33 (d, *J* = 7.9 Hz, 4H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.10 (s, 1H), 6.98 (s, 2H), 6.84 (d, *J* = 8.8 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.53 (s, 1H), 5.71 (d, *J* = 7.4 Hz, 1H), 5.66 (s, 1H), 5.62 (dd, *J* = 10.7, 5.2 Hz, 1H), 5.26 – 5.20 (m, 2H), 5.12 (d, *J* = 4.2 Hz, 2H), 5.09 (s, 1H), 4.80 (s, 1H), 4.65 (q, *J* = 6.8 Hz, 1H), 4.44 (s, 1H), 4.41 (d, *J* = 5.7 Hz, 1H), 4.34 – 4.24 (m, 2H), 4.10 (s, 10H), 4.02 – 3.91 (m, 5H), 3.30 (dd, *J* = 14.3, 11.0 Hz, 1H), 3.27 – 3.22 (m, 3H), 3.16 (s, 1H), 3.05 – 3.00 (m, 2H), 2.57 (s, 3H), 2.10 (s, 1H), 1.88 (d, *J* = 12.0 Hz, 1H), 1.71 (d, *J* = 13.1 Hz, 1H), 1.68 – 1.59 (m, 2H), 1.55 – 1.46 (m, 0H), 1.28 (s, 3H), 1.20 (s, 1H), 1.03 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.0 Hz, 3H), 0.83 (d, *J* = 6.0 Hz, 3H). HRMS (ESI) calcd for $C_{109}H_{123}Cl_2N_{19}O_{29}$ $[M+3H3^{2+}$*m/z* 744.9448, found *m/z* 744.9426.

Dipicolylaminoformylmethyl [1,2,3]-triazolyl methylaminovancomycin amide (8a)

Yield 80% (21 mg, 12.0 μmol), R.T. = 11.291 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.63 (d, *J* = 5.0 Hz, 1H), 8.57 (d, *J* = 5.1 Hz, 1H), 8.46 (s, 1H), 7.94 (t, *J* = 7.7 Hz, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.82 (s, 1H), 7.79 (s, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.44 (dd, *J* = 14.9, 7.9 Hz, 5H), 7.39 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 7.21 – 7.13 (m, 2H), 6.75 (d, *J* = 8.4 Hz, 1H), 6.69 (d, *J* = 8.5 Hz, 1H), 6.34 (d, *J* = 2.4 Hz, 1H), 6.18 (d, *J* = 2.3 Hz, 1H), 5.72 (d, *J* = 7.3 Hz, 1H), 5.63 (d, *J* = 3.7 Hz, 2H), 5.56 (s, 1H), 5.28 – 5.19 (m, 3H), 5.18 – 5.13 (m, 2H), 4.89 (s, 3H), 4.66 (s, 3H), 4.45 – 4.36 (m, 4H), 4.20 (s, 2H), 3.94 (s, 1H), 3.44 – 3.38 (m, 1H), 3.24 (d, *J* = 4.9 Hz, 2H), 3.16 (s, 1H), 2.61 (s, 3H), 2.12 (s, 1H), 1.88 (d, *J* = 12.0 Hz, 1H), 1.71 (d, *J* = 13.0 Hz, 1H), 1.68 – 1.57 (m, 2H), 1.57 – 1.50 (m, 1H), 1.27 (s, 3H), 1.04 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.1 Hz, 3H), 0.83 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{83}H_{92}Cl_2N_{16}O_{24}$ $[M+2H]^{2+}$*m/z* 884.3002, found *m/z* 884.2985.

3'-Dipicolylaminomethyltyrosine [1,2,3]-triazolyl methylaminovancomycin amide(Cu) (8b-Cu)

Yield 82% (25 mg, 12.3 μmol), R.T. = 11.912 min (analytical HPLC). HRMS (ESI) calcd for $C_{91}H_{99}Cl_2N_{16}O_{26}Cu^+$ $[M+2H]^{3+}$*m/z* 655.5247, found *m/z* 655.5232.

3'-Dipicolylaminomethyltyrosine [1,2,3]-triazolyl methylaminovancomycin amide (8b)

Yield 85% (16 mg, 8.5 μmol), R.T. = 11.388 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.69 (s, 1H), 8.57 (d, *J* = 4.8 Hz, 2H), 8.02 (s, 1H), 7.89 – 7.81 (m, 3H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.25 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 10.9 Hz, 1H), 6.81 – 6.75 (m, 2H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.35 (d, *J* = 2.3 Hz, 1H), 5.76 (d, *J* = 7.6 Hz, 1H), 5.61 (s, 1H), 5.51 (dd, *J* = 10.5, 5.1 Hz, 1H), 5.26 (dt, *J* = 11.3, 5.8 Hz, 3H), 5.19 (s, 2H), 4.92 (s, 1H), 4.68 (d, *J* = 6.8 Hz, 1H), 4.53 – 4.47 (m, 2H), 4.43 (d, *J* = 14.0 Hz, 1H), 4.40 – 4.30 (m, 4H), 4.30 – 4.17 (m, 5H), 4.10 (d, *J* = 13.1 Hz, 1H), 3.97 (s, 1H), 3.33 (d, *J* = 12.0 Hz, 1H), 3.27 (d, *J* = 5.3 Hz, 2H), 3.19 (s, 1H), 2.63 (s, 3H), 2.14 (s, 1H), 1.91 (d, *J* = 11.9 Hz, 1H), 1.74 (d, *J* = 13.1 Hz, 1H), 1.72 – 1.60 (m, 2H), 1.60 – 1.53 (m, 1H), 1.31 (s, 3H), 1.07 (d, *J* = 6.3 Hz, 3H), 0.92 (d, *J* = 6.1 Hz, 3H), 0.87 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{91}H_{100}Cl_2N_{16}O_{26}$ $[M+2H]^{2+}$*m/z* 952.3264, found *m/z* 952.3239.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methylamino- vancomycin amide(Cu) (8c-Cu)

Yield 82% (30 mg, 11.7 μmol), R.T. = 14.202 min (analytical HPLC). HRMS (ESI) calcd for $C_{104}H_{112}Cl_2N_{19}O_{26}Cu_2^{3+}$ $[M+CF_3COO^-]^{2+}$*m/z* 1175.7915, found *m/z* 1175.7874.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methylaminovancomycin amide (8c)

Yield 87% (18 mg, 8.7 μmol), R.T. = 12.068 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.61 (s, 1H), 8.50 (dd, *J* = 4.9, 1.7 Hz, 6H), 8.04 (s, 1H), 7.84 (s, 1H), 7.78 (td, *J* = 7.7, 1.8 Hz, 5H), 7.53 (d, *J* = 8.6 Hz, 2H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.40 – 7.29 (m, 12H), 7.23 (s, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 6.36 (d, *J* = 2.4 Hz, 1H), 5.74 (s, 1H), 5.57 (s, 1H), 5.51 (dd, *J* = 10.5, 5.2 Hz, 1H), 5.27 – 5.18 (m, 3H), 5.16 (s, 2H), 4.89 (s, 1H), 4.65 (d, *J* = 6.9 Hz, 1H), 4.49 (d, *J* = 5.4 Hz, 1H), 4.46 (s, 1H), 4.34 (s, 2H), 4.25 (s, 1H), 4.14 – 4.00 (m, 10H), 3.96 (d, *J* = 13.4 Hz, 3H), 3.85 (d, *J* = 14.0 Hz, 2H), 3.33 – 3.27 (m, 1H), 3.26 – 3.22 (m, 2H), 3.16 (s, 1H), 2.60 (s, H), 2.12 (s, 1H), 1.88 (d, *J* = 12.5 Hz, 1H), 1.71 (d, *J* = 12.9 Hz, 1H), 1.68 – 1.57 (m, 2H), 1.54 (d, *J* = 10.5 Hz, 1H), 1.28 (s, 3H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.1 Hz, 3H), 0.84 (d, *J* = 6.1 Hz, 3H). HRMS (ESI) calcd for $C_{104}H_{113}Cl_2N_{19}O_{26}$ $[M+2H]^{2+}$*m/z* 1057.8819, found *m/z* 1057.8801.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl ethylaminovancomycin amide(Cu) (8d-Cu)

Yield 77% (31 mg, 11.5 μmol), R.T. = 14.181 min (analytical HPLC). HRMS (ESI) calcd for $C_{105}H_{114}Cl_2N_{19}O_{26}Cu_2^{3+}$ $[M+CF_3COO^-]^{2+}$*m/z* 1182.8001, found *m/z* 1182.7960.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl ethylaminovancomycin amide (8d)

Yield 87% (19 mg, 8.7 μmol), R.T. = 12.116 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.52 (dd, *J* = 4.9, 1.5 Hz, 4H), 8.48 (s, 1H), 7.96 (s, 1H), 7.81 (t, *J* = 7.9 Hz, 5H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.50 (s, 0H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.39 (dd, *J* = 7.6, 5.0 Hz, 4H), 7.36 (d, *J* = 7.8 Hz, 4H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.20 (s, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.97 (s, 2H), 6.75 (d, *J* = 8.1 Hz, 1H), 6.68 (d, *J* = 8.5 Hz, 1H), 6.37 (d, *J* = 2.3 Hz, 1H), 6.23 (d, *J* = 2.4 Hz, 1H), 5.73 (d, *J* = 5.7 Hz, 1H), 5.62 – 5.53 (m, 2H), 5.31 – 5.19 (m, 3H), 5.19 – 5.10 (m, 2H), 4.89 (s, 1H), 4.65 (q, *J* = 6.7 Hz, 1H), 4.44 (s, 1H), 4.37 (d, *J* = 5.5 Hz, 1H), 4.16 (s, 10H), 4.00 (s, 4H), 3.96 – 3.90 (m, 1H), 3.33 – 3.19 (m, 6H), 3.16 (s, 1H), 2.90 (s, 1H), 2.75 – 2.63 (m, 2H), 2.60 (s, 3H), 2.11 (d, *J* = 14.7 Hz, 1H), 1.88 (d, *J* = 11.7 Hz, 1H), 1.71 (d, *J* = 13.1 Hz, 1H), 1.68 – 1.57 (m, 2H), 1.56 – 1.49 (m, 1H), 1.28 (s, 3H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.88 (d, *J* = 6.2 Hz, 3H), 0.84 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{105}H_{115}Cl_2N_{19}O_{26}$ $[M+2H]^{2+}$*m/z* 1064.8898, found *m/z* 1064.8858.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl propylamino- vancomycin amide(Cu) (8e-Cu)

Yield 79% (31 mg, 11.8 μmol), R.T. = 14.151 min (analytical HPLC). HRMS (ESI) calcd for $C_{106}H_{116}Cl_2N_{19}O_{26}Cu_2^{3+}$ $[M]^{3+}$*m/z* 755.5430, found *m/z* 755.5403.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl propylamino- vancomycin amide (8e)

Yield 84% (18 mg, 8.4 μmol), R.T. = 12.230 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.52 (d, *J* = 5.0 Hz, 4H), 8.48 (s, 1H), 7.97 (s, 1H), 7.83 (t, *J* = 7.6 Hz, 6H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.50 (s, 1H), 7.46 – 7.38 (m, 7H), 7.36 (d, *J* = 8.0 Hz, 5H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.22 (s, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.96 (s, 2H), 6.75 (d, *J* = 8.2 Hz, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.37 (s, 1H), 6.25 (s, 1H), 5.72 (d, *J* = 6.2 Hz, 1H), 5.58 – 5.49 (m, 2H), 5.27 (s, 1H), 5.24 (d, *J* = 7.7 Hz, 1H), 5.21 (d, *J* = 3.4 Hz, 1H), 5.18 – 5.12 (m, 2H), 4.90 (s, 1H), 4.65 (q, *J* = 6.8 Hz, 1H), 4.44 (s, 1H), 4.37 (d, *J* = 5.3 Hz, 1H), 4.23 (s, 2H), 4.18 – 4.12 (m, 10H), 4.02 – 3.90 (m, 6H), 3.45 – 3.37 (m, 3H), 3.28 – 3.22 (m, 4H), 3.17 (s, 1H), 3.13 – 3.07 (m, 1H), 3.06 – 3.03 (m, 1H), 2.90 (s, 1H), 2.61 (s, 3H), 2.46 (m, 2H), 2.12 (d, *J* = 12.7 Hz, 1H), 1.88 (d, *J* = 11.4 Hz, 1H), 1.75 – 1.50 (m, 7H), 1.28 (s, 3H), 1.04 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.2 Hz, 3H), 0.83 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{106}H_{117}Cl_2N_{19}O_{26}$ $[M+2H]^{2+}$*m/z* 1071.8975, found *m/z* 1071.8951.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylaminovancomycin amide(Cu) (8f-Cu)

Yield 86% (34 mg, 12.9 μmol), R.T. = 14.196 min (analytical HPLC). HRMS (ESI) calcd for $C_{108}H_{120}Cl_2N_{19}O_{28}Cu_2^{3+}$ $[M]^{3+}$*m/z* 775.5506, found *m/z* 775.5463.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylaminovancomycin amide (8f)

Yield 81% (18 mg, 8.1 μmol), R.T. = 12.187 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.52 (d, *J* = 5.0 Hz, 4H), 8.44 (s, 1H), 8.10 (s, 1H), 7.85 – 7.78 (m, 5H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.39 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.36 (d, *J* = 7.9 Hz, 4H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.99 (s, 2H), 6.74 (d, *J* = 8.5 Hz, 1H), 6.68 (d, *J* = 8.4 Hz, 1H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.22 (d, *J* = 2.4 Hz, 1H), 5.72 (d, *J* = 7.0 Hz, 1H), 5.63 (dd, *J* = 11.0, 5.0 Hz, 1H), 5.57 (s, 1H), 5.26 – 5.19 (m, 3H), 5.17 – 5.11 (m, 2H), 4.89 (s, 1H), 4.65 (d, *J* = 7.1 Hz, 1H), 4.42 (s, 1H), 4.37 (d, *J* = 5.4 Hz, 1H), 4.34 – 4.24 (m, 2H), 4.13 (s, 8H), 4.00 – 3.93 (m, 6H), 3.45 – 3.35 (m, 11H), 3.28 – 3.18 (m, 3H), 3.16 (s, 1H), 2.60 (s, 3H), 2.11 (s, 1H), 1.88 (d, *J* = 11.7 Hz, 1H), 1.71 (d, *J* = 13.0 Hz, 1H), 1.68 – 1.57 (m, 2H), 1.54 (d, *J* = 9.0 Hz, 1H), 1.28 (s, 3H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.88 (d, *J* = 6.1 Hz, 3H), 0.84 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{108}H_{121}Cl_2N_{19}O_{28}$ $[M+2H]^{2+}$*m/z* 1101.9081, found *m/z* 1101.9051.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-decylvancomycin(Cu) (11a-Cu)

Yield 77% (32 mg, 11.5 μmol), R.T. = 17.455 min (analytical HPLC). HRMS (ESI) calcd for $C_{119}H_{142}Cl_2N_{19}O_{29}Cu_2^{3+}$ [M]$^{3+}$*m/z* 832.2724, found *m/z* 832.2747.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-decylvancomycin (11a)

Yield 87% (20 mg, 8.7 μmol), R.T. = 16.629 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.64 (s, 1H), 8.51 (dd, *J* = 4.9, 1.5 Hz, 4H), 8.10 (s, 1H), 7.78 (td, *J* = 7.7, 1.9 Hz, 5H), 7.60 (s, 1H), 7.48 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.36 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.34 (d, *J* = 7.9 Hz, 4H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.10 (s, 1H), 6.98 (s, 2H), 6.84 (d, *J* = 8.7 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.53 (s, 1H), 5.72 (d, *J* = 7.7 Hz, 1H), 5.69 (s, 1H), 5.62 (dd, *J* = 10.7, 5.3 Hz, 1H), 5.30 (d, *J* = 7.6 Hz, 1H), 5.27 (d, *J* = 3.6 Hz, 1H), 5.11 (s, 2H), 5.08 (s, 1H), 4.79 (s, 1H), 4.59 (d, *J* = 6.8 Hz, 1H), 4.44 (s, 1H), 4.41 (d, *J* = 5.6 Hz, 1H), 4.34 – 4.24 (m, 2H), 4.15 – 4.04 (m, 12H), 4.02 – 3.91 (m, 5H), 3.34 – 3.21 (m, 5H), 3.03 (s, 2H), 2.78 – 2.63 (m, 3H), 2.56 (s, 3H), 2.10 (s, 1H), 1.97 (d, *J* = 11.6 Hz, 1H), 1.78 (d, *J* = 13.0 Hz, 1H), 1.67 – 1.59 (m, 2H), 1.55 – 1.44 (m, 3H), 1.33 (s, 3H), 1.28 – 1.15 (m, 16H), 1.05 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.0 Hz, 3H), 0.86 – 0.78 (m, 6H). HRMS (ESI) calcd for $C_{119}H_{143}Cl_2N_{19}O_{29}$ [M+2H]$^{2+}$*m/z* 1186.9916, found *m/z* 1186.9894.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-decylaminoethylvancomycin(Cu) (11b-Cu)

Yield 75% (32 mg, 11.3 μmol), R.T. = 17.318 min (analytical HPLC). HRMS (ESI) calcd for $C_{121}H_{147}Cl_2N_{20}O_{29}Cu_2^{3+}$ [M]$^{3+}$*m/z* 846.6198, found *m/z* 846.6192.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-decylvancomycin (11b)

Yield 86% (21 mg, 8.6 μmol), R.T. = 16.478 min (analytical HPLC). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.64 (s, 1H), 8.54 (dd, *J* = 5.0, 1.4 Hz, 4H), 8.13 (s, 1H), 7.86 – 7.75 (m, 5H), 7.61 (s, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.42 – 7.34 (m, 8H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 7.01 (s, 2H), 6.87 (d, *J* = 8.7 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.56 (s, 1H), 5.74 (d, *J* = 6.8 Hz, 1H), 5.70 (s, 1H), 5.64 (dd, *J* = 10.7, 5.2 Hz, 1H), 5.34 – 5.25 (m, 2H), 5.18 – 5.07 (m, 3H), 4.83 (s, 1H), 4.68 (d, *J* = 6.9 Hz, 1H), 4.48 (s, 1H), 4.44 (d, *J* = 5.6 Hz, 1H), 4.39 – 4.27 (m, 2H), 4.21 – 4.06 (m, 12H), 4.05 – 3.94 (m, 5H), 3.38 – 3.26 (m, 5H), 3.24 – 3.13 (m, 3H), 3.12 – 3.02 (m, 3H), 2.94 (t, *J* = 7.7 Hz, 2H), 2.73 (d, *J* = 14.6 Hz, 1H), 2.12 (s, 1H), 1.95 (d, *J* = 11.5 Hz, 1H), 1.86 (d, *J* = 13.0 Hz, 1H), 1.72 – 1.61 (m, 2H), 1.61 – 1.48 (m, 3H), 1.37 (s, 3H), 1.33 – 1.19 (m, 16H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.89 – 0.80 (m, 6H). HRMS (ESI) calcd for $C_{121}H_{148}Cl_2N_{20}O_{29}$ [M+2H]$^{2+}$*m/z* 1208.5127, found *m/z* 1208.5128.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl ethyoxyl ethylaminomethyl *N*-4'-chlorobiphenylmethyl vancomycin-(Cu) (11c-Cu)

Yield 75% (32 mg, 11.2 μmol), R.T. = 16.797 min (analytical HPLC). HRMS (ESI) calcd for $C_{122}H_{131}Cl_2N_{19}O_{29}Cu_2^{3+}$ [M]$^{3+}$*m/z* 852.2339, found *m/z* 852.2343.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-4'-chlorobiphenylmethyl vancomycin (11c)

Yield 86% (21 mg, 8.6 μmol), R.T. = 15.846 min (analytical HPLC).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.67 (s, 1H), 8.53 (d, *J* = 5.0 Hz, 4H), 8.13 (s, 1H), 7.80 (t, *J* = 8.0 Hz, 5H), 7.71 (dd, *J* = 11.1, 8.2 Hz, 4H), 7.63 (s, 1H), 7.54 (dd, *J* = 16.0, 8.2 Hz, 4H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.39 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.36 (d, *J* = 7.9 Hz, 4H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 1H), 7.12 (s, 1H), 7.00 (s, 2H), 6.86 (d, *J* = 8.7 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 5.76 – 5.70 (m, 2H), 5.64 (dd, *J* = 10.7, 5.2 Hz, 1H), 5.35 (d, *J* = 7.6 Hz, 1H), 5.30 (d, *J* = 3.8 Hz, 1H), 5.14 (s, 2H), 5.11 (s, 1H), 4.82 (s, 1H), 4.67 (d, *J* = 6.9 Hz, 1H), 4.47 (s, 1H), 4.42 (d, *J* = 5.5 Hz, 1H), 4.33 (d, *J* = 12.1 Hz, 1H), 4.28 (d, *J* = 12.2 Hz, 1H), 4.18 – 4.07 (m, 11H), 4.05 – 3.93 (m, 7H), 3.36 – 3.24 (m, 3H), 3.05 (s, 2H), 2.58 (s, 3H), 2.11 (d, *J* = 11.2 Hz, 2H), 1.83 (d, *J* = 13.2 Hz, 1H), 1.69 – 1.60 (m, 2H), 1.50 (s, 3H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.85 (d, *J* = 6.0 Hz, 3H). HRMS (ESI) calcd for $C_{122}H_{132}Cl_3N_{19}O_{29}$ [M+3H]$^{3+}$*m/z* 811.6246, found *m/z* 811.6235.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-4'-trifluromethylbiphenylmethyl vancomycin(Cu) (11d-Cu)

Yield 79% (34 mg, 11.8 μmol), R.T. = 17.387 min (analytical HPLC). HRMS (ESI) calcd for $C_{123}H_{131}Cl_2F_3N_{19}O_{29}Cu_2^{3+}$ [M]$^{3+}$*m/z* 863.5760, found *m/z* 863.5746.

3',5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolylmethoxyl-ethyoxyl ethylaminomethyl *N*-4'-trifluromethylbiphenylmethyl vancomycin (11d)

Yield 81% (20 mg, 8.1 μmol), R.T. = 16.482 min (analytical HPLC). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.67 (s, 1H), 8.54 (dd, *J* = 5.0, 1.5 Hz, 4H), 8.13 (s, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.87 – 7.76 (m, 10H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.49 (d, *J* = 8.7 Hz, 1H), 7.44 – 7.34 (m, 8H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.14 (s, 1H), 7.01 (s, 2H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.56 (s, 1H), 5.74 (d, *J* = 5.4 Hz, 2H), 5.64 (dd, *J* = 10.7, 5.2 Hz, 1H), 5.37 (d, *J* = 7.6 Hz, 1H), 5.32 (d, *J* = 4.1 Hz, 1H), 5.16 (d, *J* = 3.9 Hz, 2H), 5.13 (s, 1H), 4.84 (s, 1H), 4.69 (d, *J* = 6.8 Hz, 1H), 4.48 (s, 1H), 4.44 (d, *J* = 5.6 Hz, 1H), 4.35 (d, *J* = 12.2 Hz, 1H), 4.30 (d, *J* = 12.2 Hz, 1H), 4.15 (s, 11H), 4.04 – 3.92 (m, 4H), 3.38 – 3.16 (m, 5H), 3.06 (d, *J* = 5.7 Hz, 2H), 2.60 (s, 3H), 2.13 (d, *J* = 12.1 Hz, 2H), 1.86 (d, *J* = 13.3 Hz, 1H), 1.67 (q, *J* = 7.5, 5.5 Hz, 2H), 1.53 (d, *J* = 7.1 Hz, 3H), 1.13 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.1 Hz, 3H), 0.87 (d, *J* = 6.1 Hz, 3H). HRMS (ESI) calcd for $C_{123}H_{132}Cl_2F_3N_{19}O_{29}$ [M+2H]$^{2+}$*m/z* 1233.9462, found *m/z* 1233.9457.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-decylvancomycin amide (Cu) (12a-Cu)

Yield 80% (33 mg, 12.0 μmol), R.T. = 18.301 min (analytical HPLC). HRMS (ESI) calcd for $C_{118}H_{140}Cl_2N_{19}O_{28}Cu_2^{3+}$ [M]$^{3+}$ *m/z* 822.2695, found *m/z* 822.2682.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxyl ethylamino *N*-decylvancomycin amide (12a)

Yield 83% (19 mg, 8.3 μmol), R.T. = 17.063 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.54 (d, *J* = 5.0 Hz, 4H), 8.47 (s, 1H), 8.12 (s, 1H), 7.85 – 7.80 (m, 5H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.37 (d, *J* = 7.9 Hz, 4H), 7.29 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 2H), 7.00 (s, 2H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.70 (d, *J* = 8.5 Hz, 1H), 6.37 (d, *J* = 2.3 Hz, 1H), 6.23 (d, *J* = 2.3 Hz, 1H), 5.79 – 5.70 (m, 1H), 5.65 (dd, *J* = 11.0, 5.0 Hz, 1H), 5.60 (s, 1H), 5.32 (d, *J* = 7.7 Hz, 1H), 5.27 (s, 1H), 5.24 (s, 1H), 5.18 (s, 1H), 5.15 (s, 1H), 4.91 (s, 1H), 4.61 (d, *J* = 6.9 Hz, 1H), 4.44 (s, 1H), 4.38 (d, *J* = 5.4 Hz, 1H), 4.33 (d, *J* = 12.2 Hz, 1H), 4.29 (d, *J* = 12.1 Hz, 1H), 4.15 (s, 10H), 4.07 – 3.91 (m, 6H), 3.47 – 3.36 (m, 10H), 3.37 – 3.15 (m, 7H), 2.75 (s, 1H), 2.67 (s, 4H), 2.62 (s, 3H), 2.13 (s, 1H), 1.98 (d, *J* = 12.2 Hz, 1H), 1.79 (d, *J* = 13.0 Hz, 1H), 1.70 – 1.64 (m, 1H), 1.64 – 1.58 (m, 1H), 1.58 – 1.46 (m, 3H), 1.34 (s, 3H), 1.23 (d, *J* = 10.1 Hz, 16H), 1.07 (d, *J* = 6.3 Hz, 3H), 0.90 (d, *J* = 6.2 Hz, 3H), 0.88 – 0.78 (m, 6H). HRMS (ESI) calcd for $C_{118}H_{141}Cl_2N_{19}O_{28}$ [M+3H]$^{3+}$*m/z* 781.6602, found *m/z* 781.6575.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-decylaminoethylvancomycin amide (Cu) (12b-Cu)

Yield 79% (33 mg, 11.8 μmol), R.T. = 18.071 min (analytical HPLC). HRMS (ESI) calcd for $C_{120}H_{145}Cl_2N_{20}O_{28}Cu_2^{3+}$ $[M]^{3+}$ *m/z* 836.6169, found *m/z* 836.6160.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-decylaminoethylvancomycin amide (12b)

Yield 85% (20 mg, 8.5 μmol), R.T. = 16.792 min (analytical HPLC).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (s, 1H), 8.54 (dd, *J* = 5.0, 1.6 Hz, 4H), 8.46 (s, 1H), 8.12 (s, 1H), 7.82 (td, *J* = 7.7, 1.9 Hz, 6H), 7.56 (d, *J* = 8.8 Hz, 1H), 7.53 (s, 1H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.43 – 7.34 (m, 9H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.01 (s, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 1H), 6.39 (d, *J* = 2.4 Hz, 1H), 6.25 (d, *J* = 2.3 Hz, 1H), 5.75 (s, 1H), 5.65 (dd, *J* = 10.9, 5.0 Hz, 1H), 5.61 (s, 1H), 5.34 – 5.27 (m, 2H), 5.24 (s, 1H), 5.19 (s, 1H), 5.17 (s, 1H), 4.93 (s, 1H), 4.67 (d, *J* = 6.9 Hz, 1H), 4.46 (s, 1H), 4.40 (d, *J* = 5.5 Hz, 1H), 4.35 (d, *J* = 12.2 Hz, 1H), 4.30 (d, *J* = 12.2 Hz, 1H), 4.22 (s, 1H), 4.19 – 4.09 (m, 9H), 4.06 – 3.93 (m, 5H), 3.88 (s, 1H), 3.49 – 3.38 (m, 1H), 3.38 – 3.13 (m, 10H), 3.08 (s, 1H), 2.94 (t, *J* = 7.6 Hz, 2H), 2.63 (s, 3H), 2.14 (d, *J* = 15.0 Hz, 1H), 1.94 (d, *J* = 8.2 Hz, 1H), 1.86 (d, *J* = 12.9 Hz, 1H), 1.73 – 1.60 (m, 1H), 1.60 – 1.48 (m, 3H), 1.38 (s, 3H), 1.33 – 1.17 (m, 16H), 1.12 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.88 – 0.77 (m, 6H). HRMS (ESI) calcd for $C_{120}H_{146}Cl_2N_{20}O_{28}$ $[M+3H]^{3+}$*m/z* 796.0075, found *m/z* 796.0062.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-4'-chlorobiphenylmethylvancomycin amide(Cu) (12c-Cu)

Yield 84% (36 mg, 12.6 μmol), R.T. = 17.838 min (analytical HPLC). HRMS (ESI) calcd for $C_{121}H_{129}Cl_3N_{19}O_{28}Cu_2^{3+}$ $[M]^{3+}$ *m/z* 842.2299, found *m/z* 842.2300.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-4'-chlorobiphenylmethylvancomycin amide (12c)

Yield 84% (20 mg, 8.4 mol), R.T. = 16.333 min (analytical HPLC).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.51 (dd, *J* = 4.9, 1.6 Hz, 4H), 8.46 (s, 1H), 8.09 (s, 1H), 7.83 (s, 1H), 7.77 (td, *J* = 7.7, 1.8 Hz, 4H), 7.73 – 7.67 (m, 4H), 7.57 – 7.48 (m, 6H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.36 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.33 (d, *J* = 7.9 Hz, 4H), 7.29 (d, *J* = 8.2 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.18 (s, 1H), 6.97 (s, 2H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.68 (d, *J* = 8.6 Hz, 1H), 6.35 (d, *J* = 2.3 Hz, 1H), 6.22 (d, *J* = 2.3 Hz, 1H), 5.73 (d, *J* = 7.2 Hz, 1H), 5.65 – 5.58 (m, 2H), 5.34 (d, *J* = 7.5 Hz, 1H), 5.27 (s, 1H), 5.22 (s, 1H), 5.16 (s, 1H), 5.14 (s, 1H), 4.89 (s, 1H), 4.65 (d, *J* = 6.6 Hz, 1H), 4.43 (s, 1H), 4.37 (d, *J* = 5.3 Hz, 1H), 4.31 (d, *J* = 12.2 Hz, 1H), 4.26 (d, *J* = 12.2 Hz, 1H), 4.20 (s, 1H), 4.10 (s, 8H), 4.05 – 3.90 (m, 6H), 3.43 – 3.34 (m, 10H), 3.34 – 3.15 (m, 5H), 2.60 (s, 3H), 2.10 (s, 2H), 1.82 (d, *J* = 13.2 Hz, 1H), 1.69 – 1.57 (m, 2H), 1.56 – 1.51 (m, 1H), 1.49 (s, 3H), 1.10 (d, *J* = 6.2 Hz, 3H), 0.89 (d, *J* = 6.1 Hz, 3H), 0.84 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{121}H_{130}Cl_3N_{19}O_{28}$ $[M+3H]^{3+}$*m/z* 801.6211, found *m/z* 801.6192.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-4'-trifluromethylbiphenylmethylvancomycin amide(Cu) (12d-Cu)

Yield 77% (33 mg, 11.5 μmol), R.T. = 18.358 min (analytical HPLC). HRMS (ESI) calcd for $C_{122}H_{129}Cl_2F_3N_{19}O_{28}Cu_2^{3+}$ $[M]^{3+}$ *m/z* 853.5720, found *m/z* 853.5714.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino *N*-4'-trifluromethylbiphenylmethylvancomycin amide (12d)

Yield 83% (20 mg, 8.3 μmol), R.T. = 16.938 min (analytical HPLC).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.53 (d, *J* = 5.1 Hz, 4H), 8.46 (s, 1H), 8.11 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.85 – 7.79 (m, 7H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.51 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.24 – 7.14 (m, 2H), 6.99 (s, 2H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.69 (d, *J* = 8.5 Hz, 1H), 6.36 (d, *J* = 2.3 Hz, 1H), 6.22 (d, *J* = 2.3 Hz, 1H), 5.78 – 5.70 (m, 1H), 5.64 (dd, *J* = 11.0, 5.0 Hz, 1H), 5.60 (s, 1H), 5.34 (d, *J* = 7.6 Hz, 1H), 5.28 (d, *J* = 3.8 Hz, 1H), 5.24 (s, 1H), 5.17 (d, *J* = 3.7 Hz, 1H), 5.15 (s, 1H), 4.91 (s, 1H), 4.66 (q, *J* = 6.9, 6.5 Hz, 1H), 4.47 – 4.40 (m, 1H), 4.37 (d, *J* = 5.4 Hz, 1H), 4.32 (d, *J* = 12.1 Hz, 1H), 4.27 (d, *J* = 12.2 Hz, 1H), 4.20 (s, 1H), 4.15 (s, 8H), 4.08 – 3.90 (m, 6H), 3.53 – 3.35 (m, 10H), 3.34 – 3.16 (m, 5H), 2.61 (s, 3H), 2.11 (d, *J* = 12.5 Hz, 2H), 1.83 (d, *J* = 13.1 Hz, 1H), 1.69 – 1.58 (m, 1H), 1.57 – 1.52 (m, 1H), 1.49 (s, 3H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.88 (d, *J* = 6.2 Hz, 3H), 0.84 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) calcd for $C_{122}H_{130}Cl_2F_3N_{19}O_{28}$ $[M+3H]^{3+}$*m/z* 812.9632, found *m/z* 812.9698.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino (β-D-galactopyranosylethyl) aminomethyl-*N*-4'-chlorobiphenylmethyl vancomycin amide(Cu) (14a-Cu)

Yield 79% (36 mg, 11.8 μmol), R.T. = 17.091 min (analytical HPLC). HRMS (ESI) calcd for $C_{130}H_{146}Cl_3N_{20}O_{34}Cu_2^{3+}$ $[M]^{3+}$ *m/z* 920.5989, found *m/z* 920.6036.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl ethoxylethylamino (β-D-galactopyranosylethyl) aminomethyl-*N*-4'-chlorobiphenylmethyl vancomycin amide (14a)

Yield 82% (21 mg, 8.2 μmol), R.T. = 15.669 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.63 (s, 1H), 8.54 (dd, *J* = 4.8, 1.6 Hz, 4H), 8.13 (s, 1H), 7.83 (td, *J* = 7.8, 1.8 Hz, 6H), 7.74 – 7.67 (m, 5H), 7.63 (s, 1H), 7.56 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.41 (dd, *J* = 7.6, 5.1 Hz, 4H), 7.37 (d, *J* = 7.9 Hz, 4H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.16 (s, 1H), 7.01 (s, 2H), 6.85 (d, *J* = 8.7 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 6.50 (s, 1H), 5.74 (s, 1H), 5.71 (s, 1H), 5.65 (dd, *J* = 10.9, 5.1 Hz, 1H), 5.36 (d, *J* = 7.5 Hz, 1H), 5.30 (s, 1H), 5.27 (s, 1H), 5.14 (s, 1H), 5.11 (s, 1H), 4.82 (s, 1H), 4.66 (d, *J* = 6.7 Hz, 1H), 4.46 (s, 1H), 4.40 (d, *J* = 5.4 Hz, 1H), 4.33 (d, *J* = 12.2 Hz, 1H), 4.28 (d, *J* = 12.2 Hz, 1H), 4.21 – 4.09 (m, 15H), 4.09 – 3.91 (m, 8H), 3.50 – 3.36 (m, 12H), 3.37 – 3.18 (m, 10H), 3.09 (s, 2H), 2.58 (s, 3H), 2.11 (s, 2H), 1.83 (d, *J* = 13.2 Hz, 1H), 1.70 – 1.60 (m, 2H), 1.51 (s, 3H), 1.12 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.86 (d, *J* = 6.0 Hz, 3H). HRMS (ESI) calcd for $C_{130}H_{147}Cl_3N_{20}O_{34}$ $[M+3H]^{3+}$*m/z* 879.9896, found *m/z* 879.9851.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino (β-D-galactopyranosylethyl) aminomethyl-*N*-4'-trifluoromethylbiphenylmethyl vancomycin amide(Cu) (14b-Cu)

Yield 80% (37 mg, 12.0 μmol), R.T. = 17.626 min (analytical HPLC). HRMS (ESI) calcd for $C_{132}H_{147}Cl_2F_3N_{20}O_{34}Cu_2^{3+}$ $[M]^{3+}$ *m/z* 931.9405, found *m/z* 931.9397.

3'5'-Bis(dipicolylaminomethyl) tyrosine [1,2,3]-triazolyl methoyl-ethoxylethylamino (β-D-galactopyranosylethyl) aminomethyl-*N*-4'-trifluoromethylbiphenylmethyl vancomycin amide (14b)

Yield 84% (22 mg, 8.4 μmol), R.T. = 16.283 min (analytical HPLC). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.63 (s, 1H), 8.53 (dd, *J* = 5.0, 1.5 Hz, 4H), 8.12 (s, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.86 – 7.77 (m, 12H), 7.63 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.42 – 7.34 (m, 12H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.24 (d, *J* =

8.3 Hz, 1H), 7.16 (s, 1H), 7.00 (s, 3H), 6.85 (d, *J* = 8.7 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 6.50 (s, 1H), 5.77 – 5.73 (m, 1H), 5.71 (s, 2H), 5.64 (dd, *J* = 10.9, 5.1 Hz, 1H), 5.36 (d, *J* = 7.6 Hz, 1H), 5.30 (s, 1H), 5.27 (s, 1H), 5.14 (s, 1H), 5.11 (s, 1H), 4.82 (s, 1H), 4.67 (d, *J* = 6.8 Hz, 1H), 4.46 (s, 1H), 4.40 (d, *J* = 5.4 Hz, 1H), 4.32 (d, *J* = 12.2 Hz, 1H), 4.27 (d, *J* = 12.2 Hz, 1H), 4.19 – 4.09 (m, 15H), 4.09 – 3.93 (m, 11H), 3.84 – 3.77 (m, 1H), 3.47 – 3.37 (m, 12H), 3.36 – 3.22 (m, 10H), 3.09 (s, 2H), 3.04 (d, *J* = 5.8 Hz, 1H), 2.84 (t, *J* = 8.8 Hz, 1H), 2.64 (s, 1H), 2.58 (s, 3H), 2.15 – 2.08 (m, 2H), 1.84 (d, *J* = 13.0 Hz, 1H), 1.69 – 1.60 (m, 2H), 1.51 (s, 3H), 1.12 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 5.9 Hz, 3H), 0.87 (d, *J* = 6.1 Hz, 3H). HRMS (ESI) calcd for $C_{131}H_{147}Cl_2F_3N_{20}O_{34}$ $[M+3H]^{3+}$ *m/z* 891.3317, found *m/z* 891.3360.

***In vitro* Antibacterial Activity**

Minimum Inhibitory Concentration (MIC) determination[26]

The MIC values for all antimicrobial agents were measured by broth microdilution, using Mueller-Hinton II broth (cation-adjusted, BD 212322). Generally, compounds were dissolved in DMSO to 5.12 mg/ml as stock solutions. All samples were diluted with culture broth to 128 μg/ml as the initial concentration. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 128 μg/ml to 0.0625 μg/ml. 150 μL of each dilution was distributed in 96-well plates, as well as sterile controls, growth controls (containing culture broth plus DMSO, without compounds) and positive controls (containing culture broth plus control antibiotics such as vancomycin, Telavancin, etc). Each test and growth control well was inoculated with 5 μL of an exponential phase bacterial suspension (about 105 CFU/well). The 96-well plates were incubated at 37 ºC for 24 h. MIC values of these compounds was defined as the lowest concentration to inhibit the bacterial growth completely. All MIC values were interpreted according to recommendations of the Clinical and Laboratory Standards Institute (CLSI).

Cytotoxicity assays

Cell viability kit CCK-8 (Cell Counting Kit-8)[27] was used to evaluate the cytotoxicity of newly synthesized vancomycin derivatives. Generally, 100 μl of CHO cells (Chinese hamster ovary cells) and HEK293t cells (human embryonic kidney cells) suspension (~6000 cells/well) were distributed into 96-well plate. After overnight incubation, add 10 μl of various concentrations of 5**d**(Cu), 5d, 8f(Cu), 8f, Vancomycin were added to the plate well (final concentration:6.25μM, 12.5μM, 25 μM, 50 μM and 100 μM), and further incubated for 72h. After that, 10 μl of CCK-8 solution were added into each well of the plate, and the whole plate were incubated at 37 °C for 1.5h. Finally, optical density at 450 nm (OD450) was recorded using VERSMax microplate reader. To calculate the cell viability, growth controls (sterile PBS buffer without compounds) and blank controls (culture broth only, no cells) were also included. Each set was performed triplicated. Cell viability was defined as: $OD_C/OD_{C=0} \times 100$. For which, OD_C represents the optical density of cells treated with different concentration of compounds, $OD_{C=0}$ represents the optical density with no compounds added.

Indicator displacement assays (IDAs)[23]

Pyrocatechol violet (PV) is a catechol-type pH-sensitive dye, as the chromogenic indicator in this assay. The sensing ensemble was prepared by simply mixing compound **8f** (100 μM, 1ml), $CuSO_4.5H_2O$ (4 mM, 50 μl) and PV (20 mM, 5 μl) in a 1:2:1 molar ratio in an aqueous solution of 10 mM HEPES buffer pH 7.0. Then PPi was incrementally added into every bottle, one bottle was kept as control without metal ion. After every addition of quantitative PPi, the bottles were shacked slightly for 60 seconds then then their photos were taken with white background.

Color developing assay without chromogenic indicator

Compound **19** was dissolved in appropriate ddH_2O to prepare the 2 mM solution. Transfer 1 ml of this solution to 5 bottle respectively, 100 μl (40 mM) of metal ion stocks were successively added into above bottles except the right one (as control). Then quantitative PPi (1eq. 300 mM, 7μl) was added into these five bottles respectively and color change was noted. One additional equivalent of PPi was added again and color changes were compared.

Abbreviations

R.T., retention time; HATU, *N,N,N',N'*-tetramethyl-*O*-(1*H*-pyridintriazol-1-yl)-uronium hexafluorophosphate; MIC, minimum inhibitory concentration; DIPEA, N,N-diisopropylethylamine; MSSA, methicillinsensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*; VISA: vancomycin-intermediate resistant *S. aureus*; VRE: vancomycin-resistant *enterococci*; DMF: dimethylformamide; CuAAC: Copper(I)-Catalyzed Azide-Alkyne Cycloaddition; DPA: dipicolylamine; PPi: pyrophosphoric acid; PV: pyrocatechol violet; HEPES: 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid

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Keywords: vancomycin • pyrophosphate • antibacterial activity • VRE • Click chemistry • Mannich reaction

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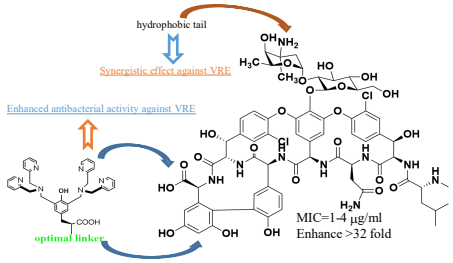

The introduction of optimal pyrophosphate-targeting moiety enhanced the antibacterial activity >32 fold than vancomycin against VRE and the appropriate collocation of hydrophobic tail provided synergistic effect (>128 fold than vancomycin) in antibacterial activity towards VRE.