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Communication

Vancomycin–Teixobactin Conjugates

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ABSTRACT: Vancomycin continues to be a widely used antibiotic of last resort in treating drug-resistant pathogens despite the emergence of vancomycin-resistant strains such as vancomycin-resistant *Enterococci* (VRE). This communication reports that conjugation of vancomycin to a second antibiotic that targets a different region of lipid II enhances and rescues its antibiotic activity. Conjugation of vancomycin to a minimal teixobactin pharmacophore in which residues 1–6 are replaced with an aromatic amide results in substantial enhancement in activity over the individual components or mixtures thereof. Three conjugates with minimum inhibitory concentrations (MICs) of 0.5 μg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA) and 0.063–0.125 μg/mL against methicillin-susceptible *Staphylococcus aureus* (MSSA) were identified. Each of these conjugates is also active against VRE, even though the individual components are inactive, with the most active conjugate (Cbp-Lys_{10}-$teixo_{7-11}$-vanco) having an MIC of 2–4 μg/mL. These findings demonstrate that conjugation of vancomycin to a minimal teixobactin pharmacophore is an effective strategy for enhancing the activity of vancomycin against important Gram-positive pathogens.

Vancomycin has been an important antibiotic of last resort since its clinical introduction in 1958 (Figure 1). It is highly effective against Gram-positive pathogens such as methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) and typically has minimum inhibitory concentrations (MICs) of ≤2 μg/mL.[1] Nevertheless, resistance is rising, with vancomycin-resistant pathogens such as vancomycin-resistant *Enterococci* (VRE) now widespread and vancomycin-resistant strains of *S. aureus* (VRSA) increasingly prevalent.[2−5] Vancomycin binds to the D-Ala-D-Ala group in lipid II, inhibiting peptidoglycan cell-wall biosynthesis and ultimately leading to cell death.[6,7] Bacteria have evolved resistance to vancomycin by mutating the D-Ala-D-Ala group in lipid II to D-Ala-D-Lac, which disrupts a key hydrogen bond with vancomycin and lipid II, resulting in a 1000-fold decrease in its binding affinity.[8,9]

Teixobactin was first reported in 2015 as a promising antibiotic against Gram-positive pathogens (Figure 1).[10−12] Teixobactin is an 11-residue cyclodepsipeptide, consisting of a macrolactone ring comprising residues 8–11 and an amphiphilic tail comprising residues 1–7 (Figure 1). Teixobactin binds to the pyrophosphate group of lipid II and related cell-wall precursors, inhibits cell-wall biosynthesis, and ultimately kills bacteria by aggregating on the cell surface and lysing the bacteria. The ring of teixobactin binds the pyrophosphate group, while the amphiphilic tail of teixobactin interacts with the lipid bilayer membrane and drives aggregation by forming antiparallel β-sheets with additional teixobactin molecules.[11,12] Gram-positive bacteria cannot develop resistance to teixobactin because teixobactin targets the immutable pyrophosphate group of lipid II and related cell-wall precursors. Teixobactin is exceptionally potent, with MICs

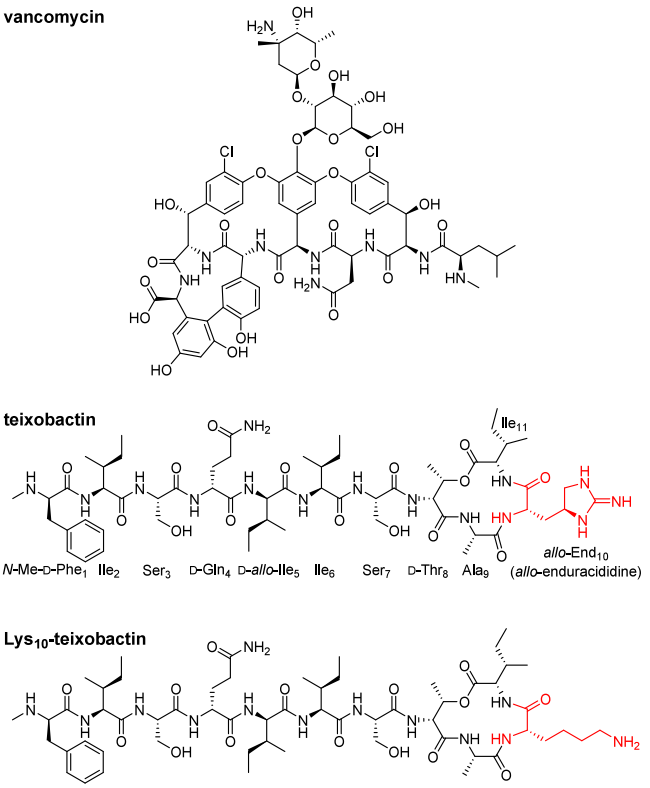

Figure 1. Structures of vancomycin, teixobactin, and Lys_{10}-teixobactin.

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of 0.005−0.5 μg/mL against many Gram-positive bacteria, including MRSA, VRE, and *Streptococcus pneumoniae*.

Modification of vancomycin can restore activity against resistant pathogens and also impart new activity.[13−20] The Boger laboratory has had great success in synthesizing modified vancomycin analogues that are active against vancomycin-susceptible and vancomycin-resistant strains of bacteria.[21−30] A notable modification is the incorporation of an amidine residue on the peptide backbone of vancomycin to restore the key hydrogen-bond interaction with lipid II in vancomycin-resistant strains.[21−24] The Boger laboratory has also demonstrated that coupling guanidinium or tetraalkylammonium moieties to the C-terminus of vancomycin can increase its membrane permeability.[24,25,27−29] Wender, Celgelski, and co-workers have also developed promising conjugates of vancomycin by appending arginine and other guanidinium containing groups to the C-terminus of vancomycin.[31−33] A number of these conjugates have shown increased activity against biofilms and persister cells formed by Gram-positive pathogens.[31−33]

In the current study, we set out to determine whether we could enhance the activity of vancomycin and overcome resistance by conjugating it to teixobactin or a minimal fragment of teixobactin that targets the pyrophosphate group of lipid II and related cell wall precursors. We envisioned the conjugates binding to both the D-Ala-D-Ala and the pyrophosphate groups of lipid II. This concept is illustrated in Figure 2. We have previously found that analogues of

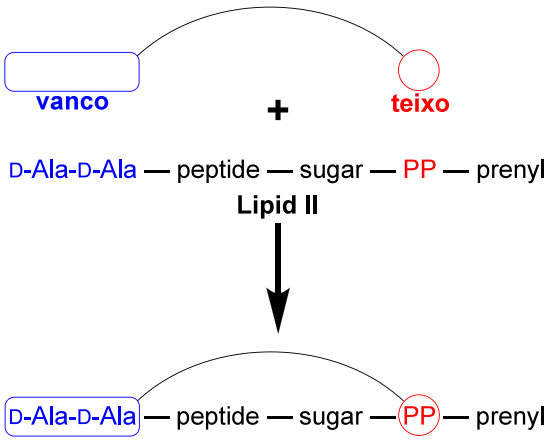

Figure 2. Envisioned binding of vancomycin−teixobactin conjugates to the D-Ala-D-Ala and pyrophosphate regions of lipid II. Similar, albeit attenuated, binding to D-Ala-D-Lac is also envisioned. This cartoon represents a simplification of the envisioned mode of binding. Although a single lipid II molecule is shown, the vancomycin−teixobactin conjugate could also bind to the D-Ala-D-Ala and pyrophosphate groups on two different lipid II molecules.

teixobactin in which the native *allo*-enduracididine residue at position 10 is replaced with lysine exhibit good antibiotic activity, albeit less than that of teixobactin (Figure 1).[34] We thus hypothesized that conjugating vancomycin to Lys_{10}-teixobactin would result in enhanced activity over that of the individual components. We further envisioned that binding of the pyrophosphate group by the teixobactin component might help restore the diminished affinity of vancomycin for D-Ala-D-Lac in VRE.

We have previously found that analogues of teixobactin in which residues 1−5 are replaced with a lipophilic group exhibit partial antibiotic activity.[34] We hypothesized that the conjugation of vancomycin to Lys_{10}-teixobactin analogues in which the tail is replaced with lipophilic groups might be sufficient to achieve enhanced activity. Here, we describe our efforts to test these ideas and report that conjugation of vancomycin to the minimal pharmacophore of teixobactin results in antibiotics with excellent antibiotic activity against MSSA and MRSA and good activity against VRE.

We prepared vancomycin−teixobactin conjugates by EDC coupling of vancomycin to Lys_{10}-teixobactin and truncated teixobactin analogues (Figures S1 and S2).[31] Coupling of Lys_{10}-teixobactin occurs exclusively at the amino group of the lysine side chain, without reaction at the N-terminal amino group of the *N*-methyl-D-phenylalanine residue. We then assessed the antibiotic activity of the conjugates through MIC assays against a panel of Gram-positive bacteria. We used the Gram-negative bacteria *E. coli* as a negative control for the MIC assays. We performed the MIC assays in the presence of 0.002% polysorbate 80, which helps prevent teixobactin derivatives from being adsorbed by the plasticware used for the assays[35,10,36,35,36]

The conjugate of vancomycin and Lys_{10}-teixobactin, Lys_{10}-teixo-vanco, is active, exhibiting MICs of 4 μg/mL against both MRSA and VRE (Table 1). These values are noteworthy because acylation of Lys_{10}-teixobactin decreases its antibiotic activity: $Lys(Ac)_{10}$-teixobactin has a MIC value of 4 μg/mL against both MRSA and VRE, while Lys_{10}-teixobactin has a MIC value of 2 μg/mL. The MIC value of Lys_{10}-teixo-vanco against VRE is encouraging, because vancomycin is not active against VRE and the molar concentration of Lys_{10}-teixo-vanco at 4 μg/mL (1.3 μM) is less than the molar concentration of $Lys(Ac)_{10}$-teixobactin at 4 μg/mL (2.9 μM). Furthermore, Lys_{10}-teixo-vanco is more active than a mixture of equal weights of Lys_{10}-teixobactin and vancomycin, which exhibits MICs of 8 μg/mL against MRSA and VRE. This control experiment demonstrates that synergy is not achieved by mixing the two antibiotics and that mixing the two components without covalent conjugation may be detrimental to antibiotic activity.

The modest enhancement of vancomycin activity by conjugation to full-length Lys_{10}-teixobactin prompted us to explore truncated analogues of Lys_{10}-teixobactin in which the hydrophobic region of the tail (residues 1−6) was replaced with a lipophilic group. We focused on aromatic and chloroaromatic lipophilic groups, because aromatic and chloroaromatic groups have successfully been used to enhance the activity of vancomycin.[23,24,28,29,37,38] We thus prepared vancomycin−teixobactin conjugates to benzoyl-Lys_{10}-teixobactin$_{7-11}$ and *p*-chlorobenzoyl-Lys_{10}-teixobactin$_{7-11}$ and the corresponding biphenyl analogues Bph-Lys_{10}-teixobactin$_{7-11}$ and Cbp-Lys_{10}-teixobactin$_{7-11}$ (Figure 3). These truncated Lys_{10}-teixobactin analogues show little or no activity against Gram-positive bacteria (Table 2).

Conjugation of vancomycin to benzoyl-Lys_{10}-teixobactin$_{7-11}$ results in a modest yet promising increase in activity over vancomycin against several Gram-positive bacteria. Notably, the MIC of benzoyl-Lys_{10}-teixo$_{7-11}$-vanco against MSSA is 0.5 μg/mL, while that of vancomycin is 1 μg/mL, and the MIC against *B. subtilis* is <0.031 μg/mL, while that of vancomycin is 0.125−0.25 μg/mL. For MRSA and *S. epidermidis*, the activity of the conjugate is equal to that of vancomycin. The conjugate shows modest activity against VRE (32 μg/mL), while vancomycin is inactive.

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Table 1. MIC Values of Vancomycin and Teixobactin Derivatives and Conjugates[a]

	Bacillus subtilis ATCC 6051	*Staphylococcus epidermidis* ATCC 14990	*Staphylococcus aureus* (MSSA) ATCC 29213	*Staphylococcus aureus* (MRSA) ATCC 700698	*Enterococcus faecalis* (VRE) ATCC 51299
Individual Antibiotics					
vancomycin	0.125−0.25	2	1	2	>32
vanco-NHBu	1	2	1	4	>32
Lys_{10}-teixobactin	≤0.031	0.5	1	2	2
$Lys(Ac)_{10}$-teixobactin	1	4	4	4	4
Conjugate					
Lys_{10}-teixo-vanco	≤0.031	1	4	4	4
Mixture[b]					
Lys_{10}-teixobactin + vanco	0.5	8	4	8	8

[a]MIC values are reported in μg/mL. [b]MIC values for mixtures reflect the total concentration of antibiotic. An MIC of 4 μg/mL, for example, corresponds to 2 μg/mL of each component.

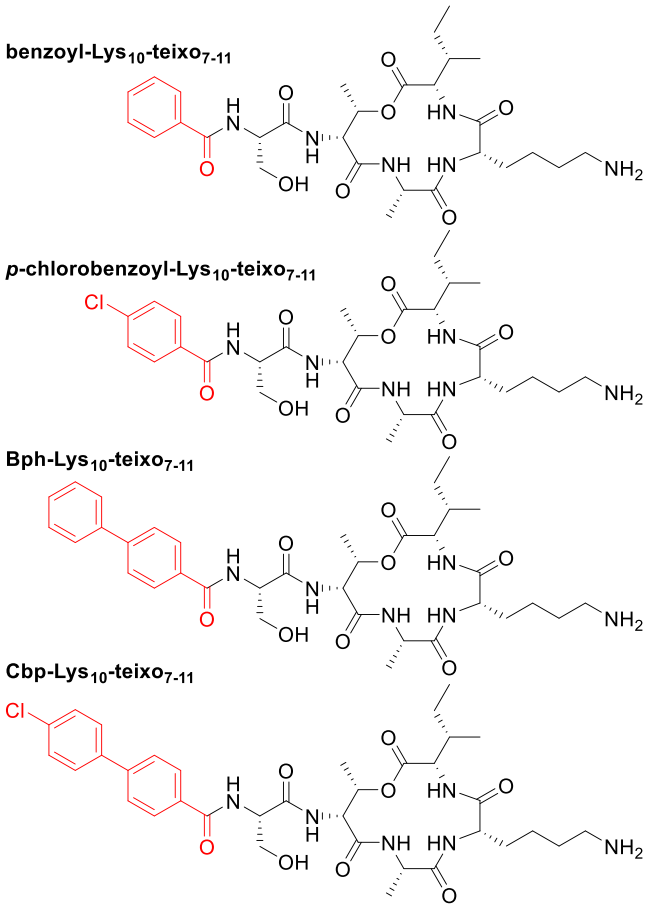

Figure 3. Chemical structures of the truncated Lys_{10}-teixobactin analogues.

The conjugates of vancomycin with *p*-chlorobenzoyl-Lys_{10}-teixobactin$_{7-11}$, Bph-Lys_{10}-teixobactin$_{7-11}$, and Cbp-Lys_{10}-teixobactin$_{7-11}$ exhibit substantially enhanced activity against all of the Gram-positive bacteria tested, including VRE (Table 2). *p*-Chlorobenzoyl-Lys_{10}-teixo$_{7-11}$-vanco is substantially more active than benzoyl-Lys_{10}-teixo$_{7-11}$-vanco, with activities of 0.063 μg/mL against MSSA, 0.5 μg/mL against MRSA, and 8−16 μg/mL against VRE. The biphenyl analogue Bph-Lys_{10}-teixo$_{7-11}$-vanco is also more active than benzoyl-Lys_{10}-teixo$_{7-11}$-vanco, with activities of 0.125 μg/mL against MSSA, 0.5 μg/mL against MRSA, and 4−8 μg/mL against VRE. The *p*-chlorobiphenyl analogue Cbp-Lys_{10}-teixo$_{7-11}$-vanco exhibits greater activity against VRE than Bph-Lys_{10}-teixo$_{7-11}$-vanco (2−4 μg/mL vs 4−8 μg/mL) and is equally active against MSSA and MRSA (0.125 μg/mL and 0.5 μg/mL). Collectively, these results demonstrate that conjugation of two components that target different regions of lipid II results in enhanced antibiotic activity.

We performed a time-kill assay against MRSA with Cbp-Lys_{10}-teixo$_{7-11}$-vanco, to assess whether this conjugate is bactericidal. We performed this assay by culturing MRSA in the presence of the conjugate at 1 μg/mL (2 × MIC), determined the concentration of colony-forming units (CFUs) as a function of time, and compared the conjugate to vancomycin (1 μg/mL) and no antibiotic. Over the course of 4 h, the concentration of bacteria decreased by ca. three $\log_{10}$ units in the presence of the conjugate (Figure 4). In contrast, the concentration of bacteria increased ca. 3-fold in the presence of vancomycin, and the bacteria grew rapidly in the absence of antibiotic. These results show that Cbp-Lys_{10}-teixo$_{7-11}$-vanco is bactericidal at concentrations above the MIC.

To determine the effect of the truncated Lys_{10}-teixobactin conjugates on mammalian cells, we assessed their hemolytic activity and cytotoxicity against human red blood cells and HEK-293 cells. Although none of these compounds proved highly lytic or toxic, they do exhibit increasing hemolytic activity and cytotoxicity with increasing hydrophobicity of the aromatic amide group (Figures S3−S8). Benzoyl-Lys_{10}-teixo$_{7-11}$-vanco and *p*-chlorobenzoyl-Lys_{10}-teixo$_{7-11}$-vanco exhibit no hemolytic activity at concentrations as high as 100 μg/mL and no cytotoxicity at concentrations as high as 50 μM (113 and 115 μg/mL). Bph-Lys_{10}-teixo$_{7-11}$-vanco exhibits no hemolytic activity at concentrations as high as 50 μg/mL and slight hemolytic activity (2%) at 100 μg/mL, as well as no cytotoxicity at concentrations as high as 25 μM (59 μg/mL). Cbp-Lys_{10}-teixo$_{7-11}$-vanco exhibits no hemolytic activity at concentrations as high as 25 μg/mL and a slight hemolytic activity (4%) at 100 μg/mL, as well as no cytotoxicity at concentrations as high as 6.25 μM (15 μg/mL) and a slight cytotoxicity at 12.5 μM (30 μg/mL). The far greater activity of the conjugates against bacteria than mammalian cells suggests that they may be good candidates for preclinical *in vivo* studies in which the antibiotic is administered intravenously and evaluated for efficacy.

In summary, conjugation of vancomycin to the macrocyclic pharmacophore of teixobactin significantly enhances antibiotic activity against Gram-positive bacteria compared to the components alone and mixtures of the two components.

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Table 2. MIC Values of Vancomycin and Truncated Teixobactin Derivatives and Conjugates[a]

	Bacillus subtilis ATCC 6051	*Staphylococcus epidermidis* ATCC 14990	*Staphylococcus aureus* (MSSA) ATCC 29213	*Staphylococcus aureus* (MRSA) ATCC 700698	*Enterococcus faecalis* (VRE) ATCC 51299
Individual Antibiotics					
vancomycin	0.125−0.25	2	1	2	>32
benzoyl-Lys_{10}-teixobactin$_{7-11}$	>32	>32	>32	>32	>32
p-chlorobenzoyl-Lys_{10}-teixobactin$_{7-11}$	>32	>32	>32	>32	>32
Bph-Lys_{10}-teixobactin$_{7-11}$	32	>32	>32	>32	>32
Cbp-Lys_{10}-teixobactin$_{7-11}$	16	16	16	32	>32
Conjugates					
benzoyl-Lys_{10}-teixo$_{7-11}$-vanco	≤0.031	2	0.5	2	32
p-chlorobenzoyl-Lys_{10}-teixo$_{7-11}$-vanco	≤0.031	0.063	0.063	0.5	8−16
Bph-Lys_{10}-teixo$_{7-11}$-vanco	≤0.031	≤0.031	0.125	0.5	4−8
Cbp-Lys_{10}-teixo$_{7-11}$-vanco	≤0.031	≤0.031	0.125	0.5	2−4
Mixtures[b]					
benzoyl-Lys_{10}-teixobactin$_{7-11}$ + vanco	0.5	8	4	8	>32
p-chlorobenzoyl-Lys_{10}-teixobactin$_{7-11}$ + vanco	0.5−1	8	4	8	>32
Bph-Lys_{10}-teixobactin$_{7-11}$ + vanco	1	8	4	8	>32
Cbp-Lys_{10}-teixobactin$_{7-11}$ + vanco	0.5−1	8	4	8	>32

[a]MIC values reported in μg/mL. [b]MIC values for mixtures reflect the total concentration of antibiotic. An MIC of 4 μg/mL, for example, corresponds to 2 μg/mL of each component.

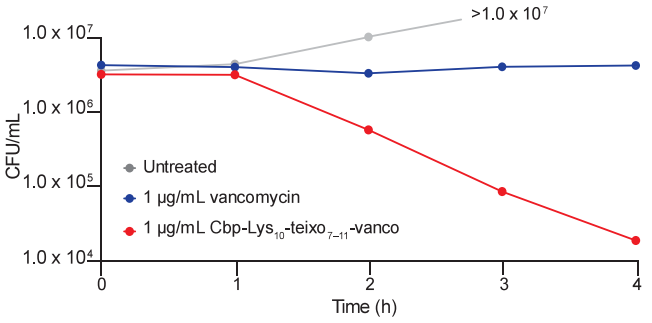

Figure 4. Time kill assay of Cbp-Lys_{10}-teixo$_{7-11}$-vanco and vancomycin at 1 μg/mL against MRSA cells. This concentration is 2 times the MIC of Cbp-Lys_{10}-teixo$_{7-11}$-vanco against MRSA.

Lys_{10}-teixobactin analogues containing the minimal pharmacophore of the macrolactone ring and an aromatic amide in place of the aggregation-prone peptide tail show little or no activity on their own. Coupling vancomycin to this minimal pharmacophore creates a conjugate with binding sites for both the D-Ala-D-Ala group and the pyrophosphate group of lipid II. Even though the D-Ala-D-Ala group is mutated to D-Ala-D-Lac in VRE, conjugation of the two inactive components imparts respectable antibiotic activity against this problematic pathogen. Notably, mixtures of vancomycin and minimal teixobactin pharmacophores do not show enhanced activity against any of the Gram-positive bacteria tested.

Although Figure 2 provides a cartoon of the concept that guided these studies, the Lys_{10} side chain is likely too short to allow the vancomycin and teixobactin components to bind the D-Ala-D-Ala and pyrophosphate groups within a single lipid II molecule. The distance between the D-Ala-D-Ala and pyrophosphate groups of lipid II is approximately 12 Å, while the distance between vancomycin and the macrolactone ring of teixobactin is about 6 Å (Figure S9).[12] We thus envision that the conjugates described herein may interact with two different lipid II molecules.

The small number of vancomycin−teixobactin conjugates described here represent a proof-of-concept, and *p*-chlorobenzoyl-Lys_{10}-teixo$_{7-11}$-vanco, Bph-Lys_{10}-teixo$_{7-11}$-vanco, and Cbp-Lys_{10}-teixo$_{7-11}$-vanco exhibit promising antibiotic activity against important Gram-positive pathogens. These three compounds are four to eight times more active against MSSA and MRSA than vancomycin. Cbp-Lys_{10}-teixo$_{7-11}$-vanco shows the most promise against VRE, with MIC values of 2−4 μg/mL. The vancomycin−teixobactin conjugates described herein represent only our initial efforts at exploring the concept of vancomycin−teixobactin conjugates. We anticipate that further optimization will result in conjugates with low toxicity and activities of ≤1 μg/mL against VRE and other important pathogens.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.4c17175.

Supplementary figures, materials and methods, and RP-HPLC analytical traces and mass spectra (PDF)

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Notes

The authors declare the following competing financial interest(s): J.S.N. and M.S.T.L.P. have filed a patent application on vancomycin-teixobactin conjugates through the Regents of the University of California.

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