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Hydrophobic Vancomycin Derivatives with Improved ADME Properties: Discovery of Telavancin (TD-6424)

MICHAEL R. LEADBETTER*, STACY M. ADAMS, BETTINA BAZZINI, PAUL R. FATHEREE, DANE E. KARR, KEVIN M. KRAUSE, BERNICE M. T. LAM, MARTIN S. LINSELL, MATTHEW B. NODWELL†, JOHN L. PACE††, KELLY QUAST, JENG-PYNG SHAW, ELIZABETH SORIANO, SEAN G. TRAPP, JENNY D. VILLENA, TERRY X. WU, BURTON G. CHRISTENSEN and J. KEVIN JUDICE†††

Theravance, Inc.,
901 Gateway Blvd., South San Francisco, CA 94080, USA

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Novel derivatives of *N*-decylaminoethylvancomycin (**2**), containing appended hydrophilic groups were synthesized and their antibacterial activity and ADME properties were evaluated. The compounds were prepared by reacting amines with the *C*-terminus (**C**-) of **2** using PyBOP® mediated amide formation, or with the resorcinol-like (**R**-) position of **2** using a Mannich aminomethylation reaction. These analogs retained the antibacterial activity of **2** against methicillin-resistant staphylococci and vancomycin-resistant enterococci. Compounds with a negatively charged auxiliary group also exhibited improved ADME properties relative to **2**. In particular, **R**-phosphonomethylaminomethyl derivative **21** displayed good *in vitro* antibacterial activity, high urinary recovery and low distribution to liver and kidney tissues. Based on these results, **21** was advanced into development as TD-6424, and is currently in human clinical trials. The generic name telavancin has recently been approved for compound **21**.

The continuing emergence of multidrug-resistant Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE) presents an increasing challenge to the heath-care community and has intensified the search for new, more effective treatment options[1]. Of particular concern are recent reports of *S. aureus* with intermediate (VISA)[2] or high-level (VRSA)[3] resistance to vancomycin (**1**) and other glycopeptides. While some recently introduced agents such as linezolid[4] and quinupristin/dalfopristin[5] are effective against resistant organisms, they are not without limitations, including further resistance and side effects[6~8]. Accordingly, there is still a need to discover new agents to treat serious Gram-positive infections.

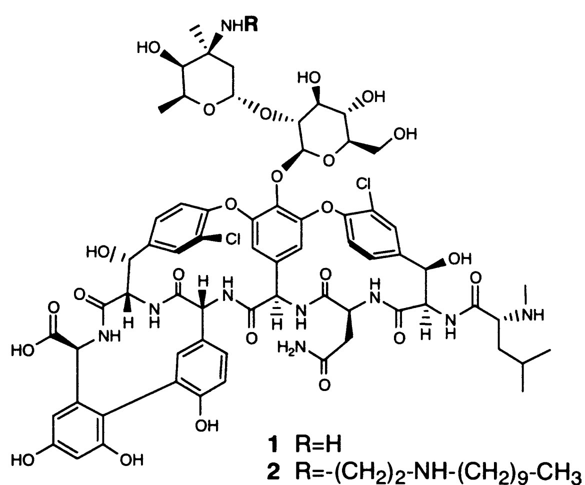
Over the past two decades, a number of groups have explored modifications to vancomycin and other glycopeptides. Through these efforts it has been established that adding hydrophobic substituents can restore antibacterial activity against VRE while retaining potency against MRSA[9~12]. As part of our effort to discover new antibacterial agents, we prepared *N*-decylaminoethylvancomycin (**2**) and found it to be active *in vitro* against staphylococci including MRSA and VISA, with MIC's equal to or better than vancomycin (Table 1). In addition, **2** was active against enterococci regardless of their susceptibility to the glycopeptides vancomycin or teicoplanin.

While the appended hydrophobic group can improve the *in vitro* antibacterial activity of vancomycin, it also can impart unfavorable absorption, distribution, metabolism

† Present address: Angiotech Pharmaceuticals, 1618 Station Street, Vancouver, B.C., Canada V6A 1B6.
†† Present address: 906 Calvert Street, St. Michaels, MD 21663.
††† Present address: Department of Medicinal Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080.
* Corresponding author: mleadbetter@theravance.com

and excretion (ADME) properties such as long elimination half life and high tissue accumulation[9,10]. Indeed, **2** proved to be poorly excreted, and distributed to liver and kidney tissues in rats (see below, Table 3). We speculated that by adding a hydrophilic group to reduce the overall lipophilicity of **2**, we could restore the favorable distribution properties of vancomycin, while retaining the activity against resistant organisms conferred by the hydrophobic group. Specifically, we hoped to reduce liver and kidney accumulation and increase urinary excretion while maintaining potent *in vitro* antibacterial activity and bactericidal properties. In this paper we report the synthesis, *in vitro* activity, and the distribution and excretion profiles of doubly-modified vancomycin derivatives bearing both hydrophobic and hydrophilic groups.

Fig. 1. Structure of vancomycin (**1**) and *N*-decylaminoethylvancomycin (**2**).



Chemistry

Derivatives of **2** were prepared that included a hydrophilic group attached through either the carboxyl terminus (**C**-) or the resorcinol-like 4′ position (**R**-) of amino acid 7 (Figure 2). The hydrophilic substituents included positively and negatively charged groups as well as neutral hydroxylated alkyl groups. All compounds were purified by reversed phase HPLC (RP-HPLC) and characterized by ion-spray mass spectrometry. Mass spectrometry data is provided in Tables 2 and 4.

Compound **2** was synthesized by reductive alkylation of vancomycin with *N*-fluorenylmethoxycarbonyl-*N*-decyl aminoacetaldehyde as shown in Scheme 1[13,14]. Imine formation was conducted under basic conditions and was followed by reduction with sodium cyanoborohydride and trifluoroacetic acid (TFA) to give **3**. Removal of the fluorenylmethoxycarbonyl (Fmoc) protecting group using piperidine afforded **2**. In the mass spectrum of **2** ($m/z=1633$) we observed the fragment $m/z=1305$ which corresponds to loss of vancosamine plus decylaminoethyl. We did not observe the fragment $m/z=1488$ corresponding to loss of unsubstituted vancosamine. This confirmed that the reductive alkylation to make **3** had taken place on the vancosamine nitrogen rather than the *N*-methyl-D-leucine[15].

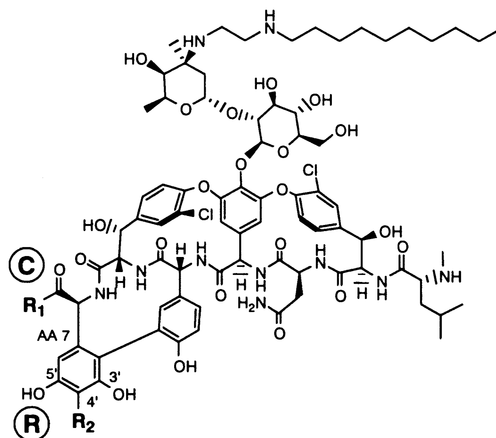
To prepare the *C*-terminal amides, we reacted intermediate **3** with the appropriate amine using benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP®), 1-hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIEA)[16] (Scheme 1).

Table 1. *In vitro* antibacterial activity of **2** against Gram-positive pathogens compared to vancomycin, teicoplanin and linezolid.

Organism	MIC (μg/mL)[a]			
	2	Vancomycin (1)	Teicoplanin	Linezolid
Staphylococcus aureus ATCC 29213	0.5	1	1	4
S. aureus ATCC 33591[b]	0.7	1	2	2
S. aureus HIP-5836[c]	1	8	8	2
Enterococcus faecalis ATCC 29212	1	2	0.5	4
E. faecalis ATCC 51575[d]	1	>256	0.5	4
E. faecalis ATCC 51575[d,e]	8	>256	32	NT[f]
E. faecalis MGH-01[g]	3	>256	>256	2
E. faecium ATCC 49624	0.5	0.3	1	4
E. faecium CDC-01[g]	8	>256	>256	4

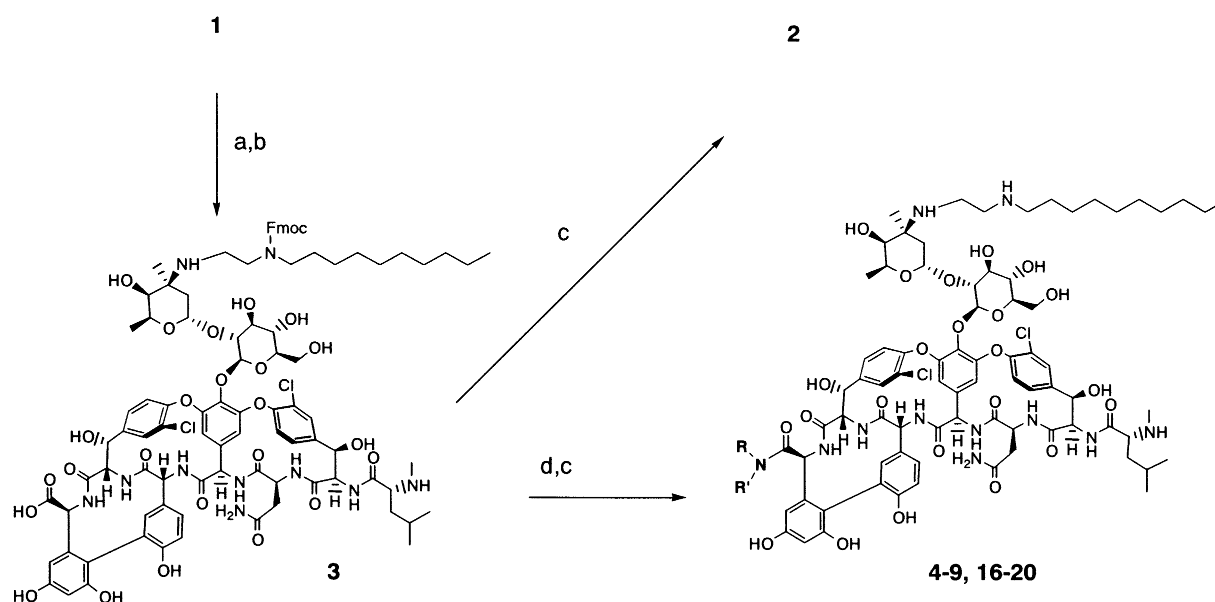
[a]Minimum inhibitory concentration as determined by broth microdilution assays *in vitro*. [b]Methicillin-resistant. [c]Vancomycin-intermediate-resistant. [d]Vancomycin-resistant (VanB). [e]Incubated with vancomycin at 10 μg/mL to induce VanB resistance. [f]Not tested. [g]Vancomycin-resistant (VanA).

Fig. 2. Structure of carboxy- (**C**-) and resorcinol- (**R**-) substituted *N*-decylaminoethylvancomycin analogs.



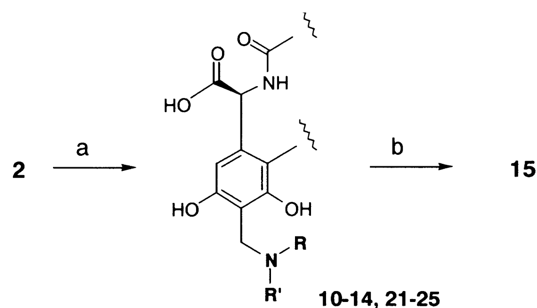
Cmpd	R1	R2
2	OH	H
4	HN—NMe2	H
5	N(—OH)(—OH)	H
6	HO, OH, N-H, OH, OH (sugar)	H
7	N-Me, OH, OH, OH, OH, OH	H
8	N-H, CO_2H	H
9	N-H, CO_2H, HO_2C	H
10	OH	H_2C-N-Me, OH, OH, OH, OH, OH
11	OH	H_2C-N-H, CO_2H
12	OH	H_2C-N-H, CO_2H, HO_2C
13	OH	H_2C-N, OH, OH
14	OH	H_2C-N-H, NMe_2
15	HO, OH, N-H, OH, OH (sugar)	H_2C-N-Me, OH, OH, OH, OH, OH
16	N-H, PO_3H_2	H
17	NH, HO_2C, O, N-H, OH, HO, OH, OH (sugar)	H
18	NH, HO_2C, O, N-Me, OH, OH, OH, OH, OH	H
19	NH, HO_2C, HO_2C, N-H, O, O, N-Me, OH, OH, OH, OH, OH	H
20	N-H, HO_2C, OH, OH, OH, OH	H
21	OH	H_2C-N-H, PO_3H_2
22	OH	H_2C-N, PO_3H_2, PO_3H_2
23	OH	H_2C-N, HO_2C, OH, OH, OH, OH, OH
24	OH	H_2C-NH, HO_2C, O, N-Me, OH, OH, OH, OH, OH
25	OH	H_2C-HN, HO_2C, OH, OH, OH, OH

Scheme 1. Synthesis of **2** and **C**-terminal amides.



Reagents: (a) *N*-Fluorenylmethoxycarbonyl-*N*-decylaminoacetaldehyde, $i\text{Pr}_2\text{NEt}$, DMF; (b) NaCNBH_3, MeOH, TFA; (c) piperidine, DMF; (d) RR'NH, PyBOP®, HOBt, DMF.

Scheme 2. Synthesis of **R**-substituted compounds and **15**.



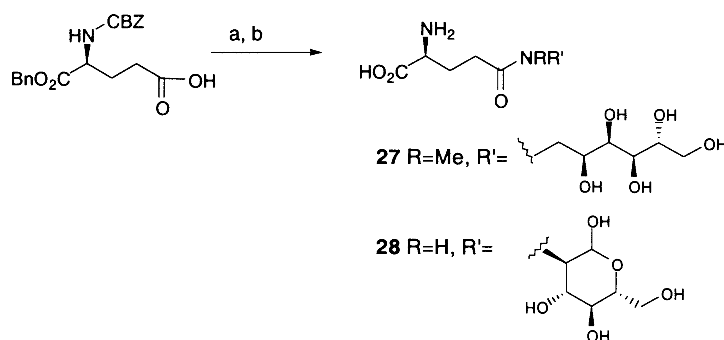
Reagents: (a) Formalin, RR'NH, $i\text{-Pr}_2\text{NEt}$, CH_3CN, H_2O; (b) D-glucosamine, PyBOP®, HOBt, DMF.

Subsequent deprotection with piperidine yielded compounds **4**~**9** and **16**~**20**.

The **R**-substituted compounds were prepared through Mannich aminomethylation of **2**, in a manner similar to that described by Pavlov *et al.* for eremomycin[12]. Compound **2**, formalin and the desired amine were reacted under basic conditions to afford compounds **10**~**14** and **21**~**25** as depicted in Scheme 2. By limiting the amount of formaldehyde used, we were able to suppress side reactions[17] as well as over-alkylation. To synthesize **15**, with substituents at both **C**- and **R**- positions, compound **10** was reacted with D-glucosamine using PyBOP® and HOBt.

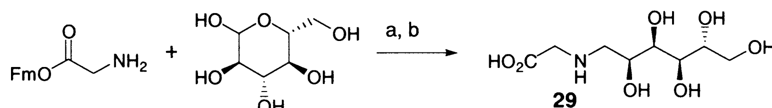
In order to confirm the point of attachment of the aminomethyl appendage of the **R**-substituted compounds, a series of NMR experiments was performed on **21**. In the proton spectrum of **21**, the resonance corresponding to the proton attached to the 4′ position of amino acid 7 was not observed. In addition, cross-peaks were observed in the

Scheme 3. Synthesis of substituted glutamic acid intermediates.



Reagents: (a) RR'NH, PyBOP®, HOBt, $i\text{Pr}_2\text{NEt}$, DMF. (b) H_2 Pd/C, MeOH.

Scheme 4. Synthesis of *N*-carboxymethyl-D-glucamine.



Reagents: (a) $NaCNBH_3$, pH 7.0 phosphate buffer, DMF. (b) NaOH, MeOH, DMF.

HMBC spectrum between the $-CH_2-$NHR protons of the phosphonic acid appendage and the 3′, 4′ and 5′ carbons, which confirmed the 4′ position as the point of attachment[12].

Amines that were not commercially available were prepared as outlined in Schemes 3 and 4. *N*-Benzyloxycarbonyl-*O*-benzyl-L-glutamic acid was treated with the appropriate aminosugar and PyBOP® and HOBt to form the amide. The protecting groups were removed by catalytic hydrogenation to provide the amino acids **27** and **28**. The fluorenylmethyl ester of glycine was reductively alkylated with D-glucose using sodium cyanoborohydride in water and DMF at pH 7[18]. Subsequent deprotection yielded the amino acid **29**.

Results and Discussion

We examined a set of compounds (**4**~**15**) with negatively charged, positively charged and neutral hydrophilic groups appended to **2** in order to assess the effect on antibacterial activity and distribution. Minimum inhibitory concentrations (MIC) for these compounds against a panel of staphylococci and enterococci including MRSA and VRE are presented in Table 2, along with data for vancomycin and **2**. Overall, the substituents had a modest effect on *in vitro* potency. Several compounds displayed lower MIC values than **2** against staphylococci, but no doubly substituted compound showed a significant increase in potency against VanA VRE. The most potent compound was **8**, which bears a negatively charged β-alanine at the **C** position. The MIC values for **8** were 3- to greater than 10-fold lower than **2** against staphylococci, and was equipotent with **2** against VanA VRE. Compounds with a basic nitrogen in the appended group (**4**, **14**) were less potent than **2** against both staphylococci and VanA VRE.

We selected compounds from this series to determine the effects that various substituents had on distribution and excretion. Specifically, we chose to explore compounds of varying net charge in a single-dose ADME study in rats using a dosage of 10 mg/kg. The tissue distribution data determined 24 hours after dosing is shown in Table 3. The

Table 2. Analytical and antibacterial properties of hydrophilic **C** and **R** derivitives **4~15**, vancomycin and teicoplanin.

Compound	Ion Spray MS		MIC (μg/mL)[c]				
	m/z Calc'd[a]	*m/z* Obs'd[b]	*Staphylococcus aureus* ATCC 13709	*S. aureus* ATCC 33591[d]	*Enterococcus faecalis* ATCC 51575[e]	*E. faecium* KPB-01[f]	*E. faecalis* MGH-01[f]
Vancomycin (1)	--	--	0.7	1	>50	>50	>50
Teicoplanin	--	--	0.8	2	0.5	>50	>50
2	1633.6	1632.7	0.5	0.7	1	2.5	2.8
4	1717.8	1718.2	1.2	1.5	1	3.1	3.9
5	1720.7	1720.5	0.8	1.2	4.7	2.3	3.1
6	1794.8	1794.9	0.5	0.8	0.8	2.6	3.5
7	1810.8	1810.8	0.1	0.2	1.6	3.1	3.1
8	1704.7	1705.2	<0.05	0.2	0.1	3.1	2.3
9	1748.7	1747.4	0.3	0.5	1.1	6.7	5.3
10	1840.8	1840.4	1.5	1.2	2	4.2	6.8
11	1734.7	1735.8	0.1	0.3	0.8	6.3	4.7
12	1778.7	1779.0	0.2	0.5	1	13	9.4
13	1750.8	1750.5	0.1	3.3	0.8	4.7	3.1
14	1747.8	1747.8	3.1	3.1	3.1	6.3	6.3
15	2002.0	2001.6	0.2	0.1	0.3	1.6	3.1

[a]Calculated mass-to-charge ratios for singly charged parent ions ($M+H^+$). [b]Observed mass-to-charge ratios for singly charged parent ions ($M+H^+$). [c]Minimum inhibitory concentrations in micrograms per milliliter as determined by microdilution broth assays *in vitro*. [d]Methicillin-resistant. [e]Vancomycin-resistant (VanB). [f]Vancomycin-resistant (VanA).

Table 3. Single-dose distribution study in the rat (i.v., 10 mg/kg).

Compound	Substitution[a]	Net Charge[b]	Tissue Distribution (%)[c]		
			urine	liver	kidney
vancomycin	--	+1	65	<1	<1
9	**C**	0	67	11	6
2	--	+1	16	16	6
6	**C**	+2	23	30	9
10	**R**	+2	23	10	3
4	**C**	+3	<1	28	6
15	**C+R**	+3	5	23	16

[a]Indicates where hydrophilic substituent is attached. **C** indicates carboxyl and **R** indicates resorcinol. [b]Estimated net charge at pH7.5. [c]Values are percentage of 10mg/kg dose, 24 hours after dosing.

data suggested that the net charge of these vancomycin derivatives had a substantial impact on distribution. Notably, the compound predicted to have zero net charge at physiological pH had the highest urinary recovery (**9**), while compounds with the greatest net positive charge (**4** and **15**) showed very low urinary clearance and high concentrations in liver or kidney tissues.

While none of these compounds proved suitable for advancement, we were encouraged that compound **9** was excreted in the urine much more efficiently than was **2**. In this assay, the urinary recovery of compound **9** was equivalent to that of vancomycin (**1**). To follow up on the

Table 4. Analytical and antibacterial properties of negatively charged **C** and **R** derivatives **16~25**, vancomycin and tiecoplanin.

	Ion Spray MS		MIC (μg/mL)				
AMI	m/z Calc'd[a]	m/z Obs'd[b]	*Staphylococcus aureus* ATCC 13709	*S. aureus* ATCC 33591[d]	*Enterococcus faecalis* ATCC 51575[e]	*E. faecium* KPB-01[f]	*E. faecalis* MGH-01[f]
Vancomycin	--	--	0.7	1	>50	>50	>50
Teicoplannin	--	--	0.8	2	0.5	>50	>50
2	1633.6	1632.7	0.5	0.7	1	2.5	2.8
16	1726.6	1726.6	0.3	0.4	1.6	6.3	6.3
17	1923.9	1823.8	0.5	0.4	1.2	6.3	4.7
18	1939.9	1939.7	1.0	0.6	1.6	13	9.4
19	2055.0	2054.7	1.2	0.8	1.6	13	13
20	1810.8	1810.8	0.1	0.2	0.9	6.3	4.7
21	1756.7	1756.6	0.6	0.8	1.6	6.3	9.4
22	1850.7	1850.6	2.3	3.9	4.7	13	9.4
23	1884.8	1884.7	1.2	0.8	1.6	6.3	6.3
24	1970.0	1970.8	3.1	2.3	4.7	13	13
25	1840.8	1840.8	0.4	0.8	1.2	9.3	6.3

[a]Calculated mass-to-charge ratios for singly charged parent ions ($M+H^+$). [b]Observed mass-to-charge ratios for singly charged parent ions ($M+H^+$).
[c]Minimum inhibitory concentrations in micrograms per milliliter as determined by microdilution broth assays *in vitro*. [d]Methicillin-resistant.
[e]Vancomycin-resistant (VanB). [f]Vancomycin-resistant (VanA).

suggestion that decreasing net positive charge was critical to improving clearance and distribution properties, we prepared another set of analogs containing negatively charged groups at the **C**- or **R**- position (compounds **16~25**).

As can be seen from Table 4, this set of compounds was also very active against staphylococci *in vitro*. The greatest increase in potency was seen with **20** which was 3- to 5-fold more active against MSSA and MRSA than was **2**. Reduced potency was seen with the bisphosphonic acid derivative **22**, and with glucamine glutamate **24**, which displays 3~6 fold higher MIC values against staphylococci. Against VanA VRE, none of the substituted compounds was more active than **2** and most were at least slightly less potent.

We examined a subset of these compounds in an intravenous single-dose ADME study in rats at 50 mg/kg to determine whether the distribution and elimination properties had been improved relative to **2** by the addition of the negatively charged group. We chose **21**, containing a phosphonic acid at the **R**-position[19], **18** and **24** which contained the same *N*-methyl-D-glucamine glutamate substituent at the **C**-position and the **R**-position, respectively.

The tissue distribution data measured at 24 hours after dosing are displayed in Table 5. In all cases there was a substantial increase in urinary clearance and decrease in liver and kidney distribution compared with **2**. Again, the data supported the observation that the addition of a negatively charged auxiliary hydrophilic group could increase the urinary excretion and decrease the liver and kidney distribution of a hydrophobic vancomycin derivative. Based on the results of this study, as well as its antibacterial activity, **21** was selected for further investigation.

To determine the effects of prolonged exposure to **21** on distribution and elimination, we undertook a multiple dose ADME study in the rat. Compound **21** was dosed for 7 days at 25 mg/kg/day. Efficient clearance was seen, with 58% of the last dose being recovered in the urine 24 hours after administration. Tissue accumulation was low, with 1% of the total dose detected in the liver and 0.2% of the total dose detected in the kidneys 24 hours after the last dose.

Further studies have shown that **21** has excellent

Table 5. Single-dose distribution study in the rat (i.v., 50 mg/kg).

Compound	Net Charge[a]	Tissue distribution (%)[b]		
		urine	liver	kidney
2	+1	12	16	13
21	0	40	5	2
18	+1	58	6	3
24	+1	72	4	1

[a] Estimated net charge at pH7.5. [b]Values are percentage of 50 mg/kg dose, 24 hours after dosing.

antibacterial activity against a range of clinical isolates including MSSA, MRSA and VISA and is rapidly bactericidal against these same organisms[20]. In addition, we have reported that this favorable *in vitro* profile is expressed *in vivo*, where **21** was superior to nafcillin against MSSA and superior to vancomycin against MRSA in animal models[21].

Based on its excellent antibacterial activity, both *in vitro* and *in vivo*, and favorable ADME properties, **21** was advanced into development as TD-6424, and is currently in human clinical trials. The generic name telavancin has recently been approved for compound **21**.

Experimental

General Methods

Reagents and solvents were used as received from commercial suppliers and all reactions were carried out at room temperature and without rigorous exclusion of ambient atmosphere unless otherwise noted. Ion-spray mass spectra (IS-MS) were obtained on a PE Sciex API 150EX mass spectrometer operating in positive ion mode. Nuclear magnetic resonance (NMR) specta were recorded on a Varian XL-600 NMR spectrometer in DMSO-d_6 at 70°C. Abbreviations for reagents: Cbz (benzyloxycarbonyl), Fmoc (fluorenylmethoxycarbonyl), DIEA (*N*,*N*-diisopropylethylamine).

HPLC Methods

Analytical: Reactions were monitored by analytical reversed-phase HPLC (RP-HPLC) with an HP1100 instrument using a 2.1 mm×50 mm, 3.5 μm C14 Zorbax Plus Bonus-RP column with UV detection at 214 nm. For the analytical separations, a 0.5 minutes isocratic period was followed by a 4.5 minutes gradient of 2~90% acetonitrile in water with 0.1% TFA.

Preparative: Compounds were purified by preparative RP-HPLC on a Varian ProStar system using 2.5 or 5.0 cm×25 cm Rainin Dynamax columns and flow rates of 15 or 50 ml/minute, respectively. Separations were accomplished with a gradient of 5~60% acetonitrile in water containing 0.1% TFA over 90 minutes.

N'-Fluorenylmethoxycarbonyl-*N*-decylaminoethylvancomycin (**3**)

Vancomycin hydrochloride (12 g, 7.7 mmol), *N*-Fmoc-*N*-decylaminoacetaldehyde (3.2 g, 7.6 mmol) and DIEA (2.6 ml 14.9 mmol) were stirred in DMF (120 ml) for 90 minutes. Sodium cyanoborohydride (1.4 g, 22 mmol) was added, followed by methanol (120 ml) and TFA (1.8 ml, 23 mmol). Stirring was continued for one hour, and the methanol was removed under reduced pressure. The crude product was precipitated by addition to ether (600 ml), and the solid was collected on a Buchner funnel and dried under vacuum. The crude product was purified by column chromatography on C18 reverse phase silica gel, eluting with 10, 20 and 30% ACN in water containing 0.1% TFA, followed by 70% CH_3CN in water containing 0.1% TFA to elute the product. The fractions containing product were combined and lyophilized to give the TFA salt of **3** (9 g, 56%).

N-Decylaminoethylvancomycin (**2**)

Compound **3** (2.0 g, 1.0 mmol) was dissolved in DMF (10 ml) and piperidine (2.0 ml, 20 mmol) was added. After stirring for 30 minutes, the crude deprotected product was precipitated by pouring into CH_3CN (80 ml) and collected by centrifuge. The pellet was washed with ether (80 ml), collected by centrifuge, and the solid was dried under

vacuum. The crude product was purified by RP-HPLC to give the TFA salt of **2** (1.0 g, 52%).

R-(Phosphonomethyl)aminomethyl-*N*-decylaminoethylvancomycin (**21**)

(Aminomethyl)phosphonic acid (3.88 g, 35 mmol) and DIEA (6.1 ml, 35 mmol) were combined in water and stirred until homogenous. Acetonitrile (50 ml) and formaldehyde (37% solution in water, 0.42 ml, 5.6 mmol) were then added, followed by **3** (10.0 g, 5.1 mmol) and DIEA (6.1 ml, 35 mmol). After approximately 18 hours, the solution was neutralized with 20% aqueous TFA, the acetonitrile was removed under reduced pressure, and the resulting suspension was lyophilized. The recovered solid was triturated with water (100 ml), collected by filtration, dried under reduced pressure and purified by RP-HPLC to give **21** (3.05 g, 27%).

N-Decylaminoethylvancomycin, L-Aspartylamide (**9**)

To a solution of **3** (20 g, 10.4 mmol) and DIEA (5.44 ml, 31.2 mmol) in DMF (440 ml) were added sequentially HOBt (1.47 g, 10.9 mmol), PyBOP® (7.57 g, 14.6 mmol) and the TFA salt of the bis-fluorenylmethyl ester of L-aspartic acid (6.26 g, 10.4 mmol). After 1 hour, the crude product was precipitated by pouring the reaction mixture into CH_3CN (4 liters), and the solid was collected by centrifuge. The supernatant was decanted and the pellet redissolved in DMF (440 ml). Piperidine (44 ml, 44 mmol) was added, and the reaction was stirred for 1 hour. The reaction mixture was poured into ether (4 liters) to precipitate the crude deprotected product, which was collected on a Buchner funnel. The recovered solid was then triturated with CH_3CN (4 liters), collected on a Buchner funnel, and dried under vacuum. The resulting off-white solid was purified by RP-HPLC to give the TFA salt of **9** (9.8 g, 45%).

N-(γ-L-Glutamyl)-*N*-methyl-D-glucamine (**27**)

To a solution of *N*-Cbz-*O*-benzyl-L-glutamic acid (10.0 g, 27 mmol) and *N*-methyl-D-glucamine (5.3 g, 27 mmol) in DMF (60 ml) was added HOBt (4.1 g, 27 mml), PyBOP® (15.4 g, 29 mmol) and DIEA (7.0 ml, 40 mmol). The solution was stirred for 1 hour, at which time it was poured in to water (600 ml) containing K_2CO_3 (30 g). The solution was extracted with ethyl acetate, and the organics were washed with 1 N HCl in saturated NaCl, and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residual oil was dissolved in methanol (100 ml) and 10% Pd/C (1.6 g) was added under nitrogen. 4.0 N HCl in dioxane (10 ml, 40 mmol) was added, and the reaction was stirred under one atmosphere of hydrogen for 3 hours. The mixture was filtered through Celite®, which was washed with methanol. The filtrate was concentrated under reduced pressure to approximately 30 ml, and then added to CH_3CN (300 ml). The precipitate was collected on a Buchner funnel and dried *in vacuo* to give the hydrochloride salt of **27** (6.9 g, 71%) which was used without further purification.

N-Decylaminoethylvancomycin, Amide with *N*-(γ-L-Glutamyl)-*N*-methyl-D-glucamine (**18**)

Intermediate **3** (1.8 g, 0.91 mmol), PyBOP® (0.52 g, 1.0 mmol) and HOBt (0.15 g, 1.0 mmol) were dissolved in DMF (12 ml), and *N*-methylmorpholine (0.10 ml, 0.91 mmol) was added. The reaction was stirred for 20 minutes, at which time an additional portion of *N*-methylmorpholine (0.10 ml, 0.91 mmol) was added. After 5 minutes, a solution of the amine hydrochloride **27** (0.66 g, 1.8 mmol) in DMF (2.0 ml) was added, followed by another portion of *N*-methylmorpholine (0.40 ml, 3.6 mmol). The reaction mixture was stirred for 20 minutes, and then poured in to ether (150 ml). The solid was collected on a Buchner funnel, washed with ether and dried under vacuum. The crude material was slurried in DMF (30 ml), and piperidine (6 ml, 6 mmol) was added. The reaction was stirred for 20 minutes, at which time it was poured into ether (150 ml). The solid was collected on a Buchner funnel, rinsed with ether and dried *in vacuo*. The crude deprotected product was purified by RP-HPLC to give **18** (0.45 g, 22%).

R-[*N*-(γ-L-Glutamyl)-*N*-methyl-D-glucamino]methyl-*N*-decylaminoethylvancomycin (**24**)

Amino acid **27** (3.15 g, 8.8 mmol), intermediate **3** (1.8 g, 0.91 mmol) and DIEA (2.9 ml, 17 mmol) were dissolved in CH_3CN (7.5 ml) and water (7.5 ml), and 37% formaldehyde (55 μl, 0.73 mmol) was then added. After stirring for 4 hours, the CH_3CN was removed under reduced pressure and the mixture was neutralized with TFA. The resulting thick slurry was dried under vacuum. The residue was dissolved in DMF (9.6 ml) and piperidine (1.2 ml, 1.2 mmol) was added. The reaction mixture was stirred for 30 minutes at which time it was added to CH_3CN (100 ml). The resulting solid was collected on a Buchner funnel, dried *in vacuo* and purified by RP-HPLC to give the TFA salt of **24** (0.26 g, 12%).

N-Carboxymethyl-D-glucamine (**29**)

A solution of the fluorenylmethyl ester of glycine (10.0 g, 27.2 mmol) and D-glucose (7.4 g, 41 mmole) in DMF

(70 ml) and pH 7.0 phosphate buffer (1.0 M, 30 ml) was stirred for 30 minutes. Sodium cyanoborohydride (1.7 g, 27.2 mmol) was added, and the reaction stirred for 1.5 hours. The mixture was concentrated to dryness under reduced pressure, and the residue redissolved in DMF (70 ml) and water (30 ml). Another charge of sodium cyanoborohydride (1.7 g, 27.2 mmol) was added and the reaction was stirred for 1 hour, at which time the mixture was concentrated to dryness under reduced pressure. The residue was purified by RP-HPLC to give the fluorenylmethyl ester of *N*-carboxymethyl-D-glucamine (5.75 g 33%).

A portion of the fluorenylmethyl ester of *N*-carboxymethyl-D-glucamine (1.60 g, 3.0 mmol) was dissolved in DMF (16 ml) and a solution of NaOH in methanol (1.0 M, 6.0 ml, 6.0 mmol) was added. After 40 minutes, the product was precipitated by adding the reaction mixture to CH_3CN (90 ml). The crude product was isolated by centrifugation and the pellet was washed with CH_3CN (90 ml). The resulting solid was dried *in vacuo* to give the sodium salt of **29** (0.80 g , 99%).

R-*N′*-Carboxymethyl-D-glucaminomethyl-*N*-decylamino-ethylvancomycin (**23**)

Amine **29** (0.52 g, 2.0 mmol) and the TFA salt of **2** (790 mg, 0.4 mmol) were dissolved in CH_3CN (3 ml) and water (4 ml). DIEA (0.28 ml, 1.6 mmol) was added, followed by formaldehyde (3.7% aqueous solution, 0.24 ml, 0.32 mmol). The reaction mixture was stirred for 13 hours, at which time HPLC showed that starting material had not been completely consumed. Another portion of formaldehyde was added (0.10 ml, 0.13 mmol), and the reaction was stirred for 3 hours. The reaction mixture was acidified to pH 2 with TFA and purified by RP-HPLC to give the TFA salt of **23** (147 mg, 16%).

Determination of *In Vitro* Antimicrobial Activity

Minimum inhibitory concentrations (MICs) were determined by broth microdilution methodology as described by the National Committee for Clinical Laboratory Standards[22].

Single- and Multi-Dose Tissue Distribution Studies

For the single dose studies, female Sprague-Dawley rats (n=3 per dose group) were dosed at 10 or 50 mg/kg in 5% dextrose or 25% hydroxypropyl-β-cyclodextrin (HP-βCD) respectively. For the multiple-dose study, female Sprague-Dawley rats (n=5 per dose group) were dosed once daily at 25 mg/kg in 5% HP-βCD for 7 days. Urine samples were cage collected over the 24-hour period following the final dose. Drug concentrations in the urine were determined by LC-MS analysis, and are expressed in the tables as a percentage of a single dose. Twenty-four hours after the last dose, the animals were sacrificed and the liver and kidneys were removed for further analysis. One kidney and part of the liver were homogenized for concentration analysis using reverse phase HPLC with UV detection. The results for liver and kidney distribution given are a percent of the total drug given over the study.

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References

1) NEU, H. C.: The crisis in antibiotic resistance. Science 257: 1064～1073, 1992
2) SMITH, T. L.; M. L. PEARSON, K. R. WILCOX, C. CRUZ, M. V. LANCASTER, B. ROBINSON-DUNN, F. C. TENOVER, M. J. ZERVOS, J. D. BAND, E. WHITE & W. R. JARVIS: Emergence of vancomycin resistance in *Staphylococcus aureus*. Glycopeptide-intermediate *Staphylococcus aureus* working group. N. Engl. J. Med. 340: 493～501, 1999
3) SIEVERT, D. M.; M. L. BOULTON, G. STOLTMAN, D. JOHNSON, M. G. STOBIERSKI, F. P. DOWNES, P. A. SOMSEL, J. T. RUDRIK, W. BROWN, W. HAFEEZ, T. LUNDSTROM, E. FLANAGAN, R. JOHNSON, J. MITCHELL & S. CHANG: *Staphylococcus aureus* resistant to vancomycin-United States, 2002. Morbid. Mortal. Weekly Rep. 51: 565～567, 2002
4) DIEKEMA, D. J. & R. N. JONES: Oxazolidinone antibiotics. Lancet 357: 1179, 2001
5) LAMB, H. M.; D. P. FIGGITT & D. FAUDS: Quinupristin/Dalfopristin. A review of its use in the management of serious Gram-positive infections. Drugs 58: 1061～1097, 1999
6) RUBENSTEIN, E.; P. PROKOCIMER & G.H. TALBOT: Safety and tolerability of quinupristin/dalfopristin: administration guidelines. J. Antimicrob. Chemother. 44A: 37～46, 1999
7) GONZOLES, R. D.; P. C. SCHRECKENBERGAR, M. B. GRAHAM, S. KELKAR, K. DEBENSTEN & J. P. QUINN: Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. Lancet 357: 1179, 2001
8) LIVERMORE, D. M.: Quinupristin/dalfopristin and linezolid: where, when, which and whether to use? J. Antimicrob. Chemother. 46: 347～350, 2000
9) NAGARAJAN, R.; A. A. SCHABEL, J. L. OCCOLOWITZ, F. T. COUNTER, J. L. OTT & A. M. FELTY-DUCKWORTH: Synthesis and antibacterial activity of *N*-alkylvancomycins. J. Antibiotics 42: 63～72, 1989
10) NAGARAJAN, R.: Structure-activity relationships of vancomycin-type glycopeptide antibiotics. J. Antibiotics 46: 1181～1195, 1993
11) PAVLOV, A. Y. & M. N. PREOBRAZHENSKAYA: Chemical

modification of glycopeptide antibiotics. Russ. Biorg. Chem. 24: 570~587, 1998
12) Pavlov A. Y.; E. I. Lazhko & M. N. Preobrazhenskaya: A new type of chemical modification of glycopeptide antibiotics: aminomethylated derivatives of eremymomycin and their antibacterial activity. J. Antibiotics 50: 509~513, 1997
13) Judice, J. K.; P. R. Fatheree, B. M. T. Lam, M. R. Leadbetter, M. S. Linsell, S. G. Trapp, G. Yang & Y. Zhu (Theravance, Inc): Glycopeptide derivatives and pharmaceutical compositions containing the same. U. S. 6,392,012, May 21, 2002
14) Cooper, R. D. G.; N. J. Snyder, M. J. Zweifel, M. A. Staszak, S. C. Wilkie, T. I. Nicas, D. L. Mullen, T. F. Butler, M. J. Rodriguez, B. E. Huff & R. C. Thompson: Reductive alkylation of glycopeptide antibiotics: synthesis and antibacterial activity. J. Antibiotics 49: 575~58, 1996
15) Roberts, G. D.; S. A. Carr, S. Rotterschaefer & P. W. Jeffs: Structural characterization of glycopeptide antibiotics related to vancomycin by fast atom bombardment mass spectrometry. J. Antibiotics 38: 713~720, 1985
16) Zweifel, M. J.; N. J. Snyder, R. D. G. Cooper, T. I. Nicas, D. L. Mullen, T. F. Butler & M. J. Rodriguez: Glycopeptide carboxamides active against vancomycin-resistant enterococci. J. Antibiotics 56: 289~295, 2003
17) Heck, A. J.; P. J. Bonnici, E. Breukink & M. Wills: Modification and inhibition of vancomycin group antibiotics by formaldehyde and acetaldehyde. Chemistry 7: 910~916, 2001
18) Walton, D. J.; E. R. Ison & W. A. Szarek: Synthesis of *N*-(1-deoxyhexitol-1-yl)amino acids, reference compounds for the nonenzymatic glycosylation of proteins. Carbohydrate Res. 128: 37~49, 1984
19) Leadbetter, M. R. & M. S. Linsell (Theravance, Inc.): Glycopeptide phosphonate derivatives. U. S. 6,635,618, October 21, 2003
20) Pace, J. L.; K. M. Krause, D. L. Johnston, D. V. Debabov, T. X. Wu, L. Farrington, C. E. Lane, D. L. Higgins, B. G. Christensen, J. K. Judice & K. Kaniga: *In vitro* activity of TD-6424 against *Staphylococcus aureus*. Antimicrob. Agents Chemother. 47: 3602~3604, 2003
21) Hegde, S. S.; N. Reyes, T. Wiens, R. Skinner, J. McCullough, N. Vanasse & J. K. Judice: *In vitro* pharmacodynamics of TD-6424, a novel rapidly bactericidal concentration-dependent antibiotic, in preclinical species. 43rd Interscience Conference of Antimicrob. Agents and Chemother. Chicago, Abstract F2108, 2003
22) National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved guideline, 5th ed., M7-A5. The Committee, Wayne, Penn., 2000