ORIGINAL ARTICLE

Exploring the Positional Attachment of Glycopeptide/ *β*-lactam Heterodimers

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Abstract Further investigations towards novel glycopeptide/*β*-lactam heterodimers are reported. Employing a multivalent approach to drug discovery, vancomycin and cephalosporin synthons, **4**, **2**, **5** and **10**, **18**, **25** respectively, were chemically linked to yield heterodimer antibiotics. These novel compounds were designed to inhibit Gram-positive bacterial cell wall biosynthesis by simultaneously targeting the principal cellular targets of both glycopeptides and *β*-lactams. The positional attachment of both the vancomycin and the cephalosporin central cores has been explored and the SAR is reported. This novel class of bifunctional antibiotics **28**~**36** all displayed remarkable potency against a wide range of Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA). A subset of compounds, **29**, **31** and **35** demonstrated excellent bactericidal activity against MRSA (ATCC 33591) and **31** and **35** also exhibited superb *in vivo* efficacy in a mouse model of MRSA infection. As a result of this work compound **35** was selected as a clinical candidate, TD-1792.

Keywords multivalent, multivalency, bifunctional, *β*-lactam, glycopeptide, antibiotic, MRSA, TD-1792

Introduction

The medical community is increasingly concerned by the incidence of multi-drug resistant bacterial infection. The emergence of deadly and virulent Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococus pneumoniae* (PRSP) have prompted an intensified search for novel therapies [1]. Applying a multivalent approach to drug discovery we recently reported the first examples of glycopeptide/*β*-lactam heterodimers which exhibited remarkable potency against Gram-positive pathogens [2]. Due to the physical proximity of the principal cellular targets of glycopeptides (lipid intermediate II) and *β*-lactams (transpeptidase domain of penicillin binding protein, PBP), and their sequential role in the cell-wall biosynthetic pathway, we reasoned that a single molecule that inhibited both targets in a bifunctional manner may provide enhanced potency and bactericidal activity. Multivalency is a phenomenon whereby multiple, simultaneous, energetically coupled ligand: receptor interactions enhance the overall affinity and selectivity of binding [3]. Multivalency is common in

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Table 1

Compound	V	C	m/z (calcd)[a]	m/z (obsd)[b]	MIC (µg/ml)							Time to Cidality[c]	
					MSSA	MRSA	VISA	EFSVS	EFSVA	EFMVA	PRSP	0.25 µg/ml	1 µg/ml
1	—	—	—	—	≤0.05	≤0.05	≤0.05	≤0.05	3.13	>50	≤0.05	—	—
28	V_V	C_{PY}	2141.5	2141.5	≤0.05	≤0.05	≤0.05	≤0.05	3.13	>100	≤0.05	—	—
29	V_V	C_{OX}	1078.3	1078.8	≤0.05	≤0.05	≤0.05	≤0.05	0.78	>100	≤0.05	4 h	1 h
30	V_V	C_{AM}	1052.7	1052.4	≤0.05	≤0.05	≤0.05	ND	25	>100	0.2	—	—
31	V_R	C_{PY}	2170.5	2171.8	≤0.05	≤0.05	≤0.05	≤0.05	3.13	>100	≤0.05	24 h	4 h
32	V_R	C_{OX}	2184.5	2184.6	≤0.05	≤0.05	≤0.05	≤0.05	0.78	>100	≤0.05	static	4 h
33	V_R	C_{AM}	1067.2	1066.8	≤0.05	≤0.05	0.2	12.5	50	>100	0.2	static	24 h
34	V_C	C_{PY}	985.7	985.7	≤0.05	≤0.05	≤0.05	ND	1.56	>100	≤0.05	—	—
35	V_C	C_{OX}	953.2	953.3	≤0.05	≤0.05	≤0.05	≤0.05	0.78	>100	≤0.05	4 h	1 h
36	V_C	C_{AM}	966.6	966.5	≤0.05	≤0.05	≤0.05	0.2	ND	>100	≤0.05	—	—
10	—	C_{PY}	539.0	538.2	3.13	25	12.5	ND	6.25	>100	1.56	—	—
18	—	C_{OX}	553.0	553.1	1.56	25	25	1.56	3.13	>100	3.13	—	—
25	—	C_{AM}	581.0	580.3	1.56	25	25	12.5	25	>100	12.5	—	—
4	V_V	—	1493.3	1493.7	0.78	1.56	6.25	1.56	>50	>50	ND	—	—
5	V_R	—	1522.4	1522.6	0.78	1.56	ND	ND	>50	>50	ND	—	—
VCM **2**	V_C	—	—	—	0.78	0.78	6.25	0.78	>50	>50	0.78	static	static

MSSA, methicillin-susceptible *S. aureus* strain ATCC 13709; MRSA, methicillin-resistant *S. aureus* strain ATCC 33591; VISA, vancomycin-intermediate *S. aureus* strain HIP-5836; EFSVS, vancomycin-susceptible *E. faecalis* strain ATCC 29212; EFSVA, vancomycin-resistant *E. faecalis* strain MGH-01 (VanA phenotype); EFMVA, vancomycin-resistant *E. faecium* strain KPB-01 (VanA phenotype); PRSP, penicillin-resistant *Streptococcus pneumoniae* strain SU-10; VCM, vancomycin. [a] Calculated and [b] observed mass-to-charge ratios for singly charged parent ions [M⁺] for **28/31/32/10/18/25**, [M+H⁺] for **4/5**, and mass-to-charge ratios for doubly charged daughter ions ([M+H⁺]⁺) for **29/34**, ([M−pyridine(79.1)+H⁺]⁺) for **30/33/35/36**. [c] Time to achieve a 3-log CFU/ml reduction against *S. aureus* ATCC 35991 (MRSA). ND, not determined.

natural systems; for example, in controlling cell-cell interactions and in the adhesion of a virus particle to an erythrocyte [4]. Recent literature suggests that multivalency may also find applicability in the design of small molecule ligands [5]. Homodimers and oligomers of vancomycin [6], paclitaxel [7] and opioids [8] have all been reported to enhance binding relative to their constitutive monomeric components [9]. In addition to enhanced efficacy it has been proposed that multivalent molecules may also offer physicochemical advantages and allow coupling of pharmacophores with distinct mechanisms of action [10].

We previously reported the discovery of compound **1** which was designed to link a vancomycin synthon (attachment point through the vancosamine amino group) with a specific cephalosporin, β-lactam motif (attachment through the C3 pyridinium substituent) using a simple di-amide linear alkyl linkage. This compound exhibited excellent potency against a range of Gram-positive pathogens (Table 1) and displayed rapid bactericidal activity against *S. aureus* ATCC 33591 (MRSA), achieving a 3-log CFU/ml reduction after only 4 hours at a

concentration of 0.5 µg/ml.

In this paper, we describe further efforts to explore and optimize our multivalent approach to antibacterial drug discovery and report structure activity relationships with regard to the attachment points to both the glycopeptide and β-lactam components.

Multivalent Design

Consideration of the central vancomycin (V) core **2** used to construct heterodimer **1** revealed a number of potential attachment points, Fig. 1. In addition to modification of the vancosamine amino group (V_V) [2], vancomycin **2** can be selectively derivatized through the carboxyl terminus (V_C) or the 4′, resorcinol-like (V_R) position on the aromatic side chain of amino acid 7. Employing an ethyl primary amino motif attached to the vancosamine amino group as previously described [2], allows linkage *via* an amide bond to vancomycin whilst preserving the basicity at the V_V position. This has been shown to be important for the antibacterial activity of vancomycin and chloroeremomycin derivatives [11]. Amide bond formation at the *C*-terminus
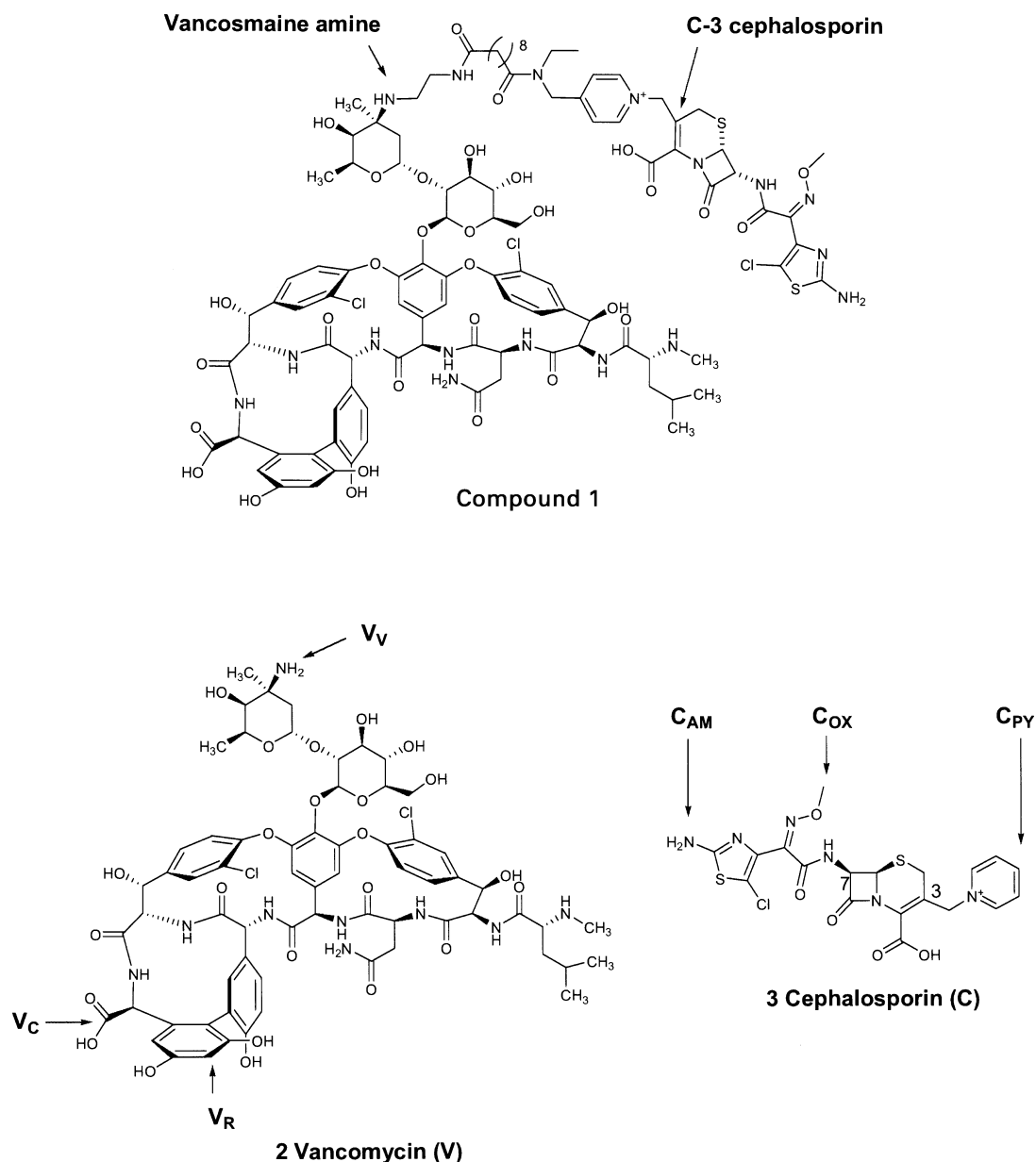
Fig. 1 Linkage points.

of vancomycin V_C is typically well tolerated [12] and Mannich aminomethylation and susbsequent amide bond formation at the V_R-position additionally provides compounds with *in vitro* activity comparable to the parent vancomycin [11, 13].

The cephalosporin (C) core **3** was previously derivatized at the C3 pyridinium susbtituent (C_{PY}) by a methyl amino moiety, Fig. 1 [2]. Introduction of a similar amino moiety at both the oxime (C_{OX}) and aminothiazole (C_{AM}) of the chloro-substituted aminothiazolyl(syn-methoxyimino)acetyl moiety at the C7 position of the cephalosporin, provides two additional attachment points.

The combination of vancomycin attachment points (V_V, V_C, V_R) with cephalosporin attachment points (C_{PY}, C_{OX}, C_{AM}) was explored by the preparation of amide-linked glycopeptide/β-lactam heterodimers **28~36**. A representation of the nine possible heterodimer combinations is presented using three specific vancomycin analogues, **4, 2, 5** (V_V, V_C, V_R respectively; Scheme 1) and three specific cephalosporin derivatives, **10, 18, 25** (C_{PY}, C_{OX}, C_{AM} respectively; Schemes 2~4). The V_V and V_R linked heterodimers (**28, 29, 30** and **31, 32, 33** respectively) use adipic acid as the connecting di-acid whilst the V_C linked heterodimers (**34, 35, 36**) are connected by direct amide
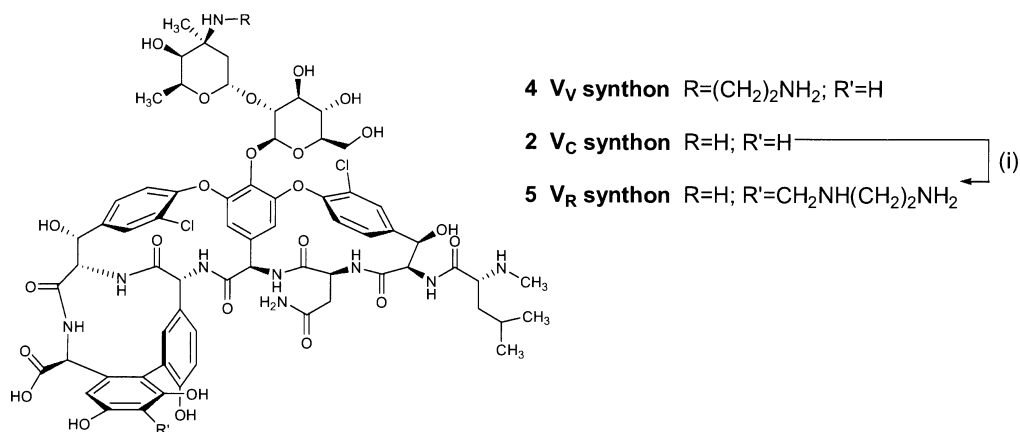
Scheme 1

(i) Ethylenediamine, CH$_2$O, CH$_3$CN, H$_2$O.
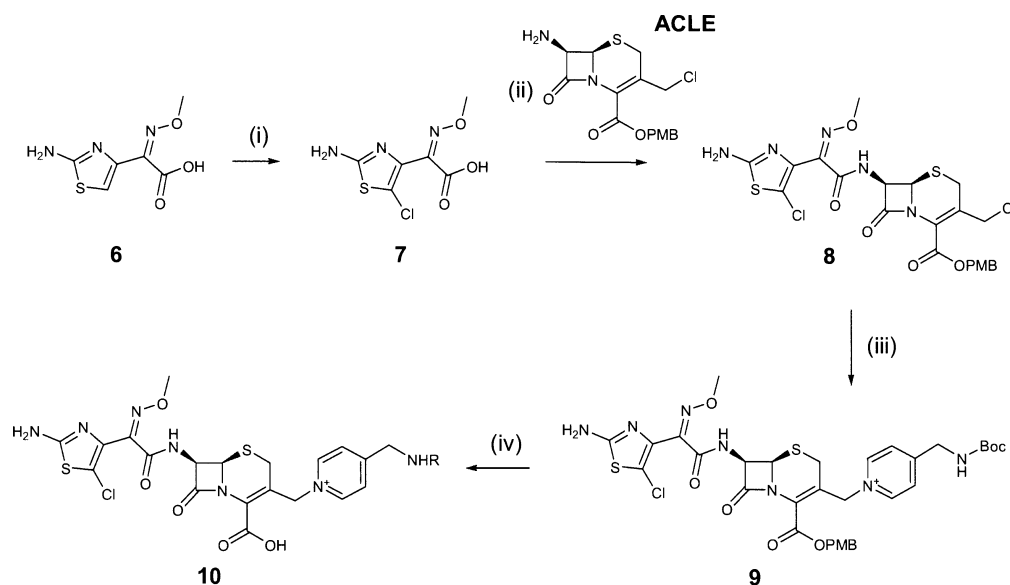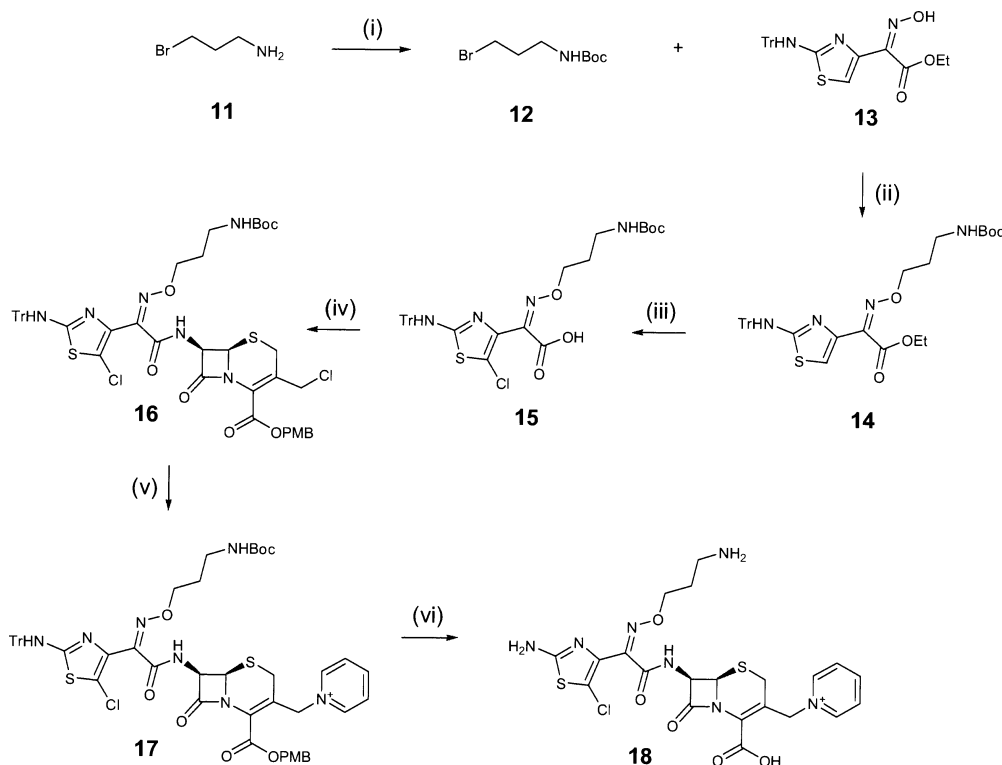

Scheme 2

(i) NCS, DMF, (ii) EDCI, 2,4,6-collidine, DMF (iii) *N*-Boc-4-(aminomethyl)-pyridine, NaI, Me$_2$CO (iv) TFA, CH$_2$Cl$_2$, anisole.

bond formation at the *C*-terminus of vancomycin **2**.

Chemistry

The synthon **4** for V$_V$ attachment of vancomycin was prepared as previously described [2]. The V$_C$ synthon **2** merely required vancomycin itself. The V$_R$ synthon **5** was prepared by Mannich aminomethylation of vancomycin **2** with ethylenediamine and formaldehyde (Scheme 1) [13, 14]. By limiting the amount of formaldehyde used we were able to suppress side reactions [15] as well as over-alkylation.

The C$_{PY}$ derivatized cephalosporin monomer core **10** (R=H) was prepared from 7-amino-3-chloromethyl-3-

cephem-4-carboxylic acid *p*-methoxybenzyl ester hydrochloride salt (ACLE) in a route similar to that previously reported for the methyl aminoethyl substituted, pyridinium compound (R=Et) used to prepare **1** (Scheme 2) [2]. The ethyl substituent was shown to have minimal effect on potency. Chlorination of aminothiazolyl(syn-methoxyimino)acetic acid **6** was effected with *N*-chlorosuccinimide (NCS) in DMF and the resulting acid **7** was coupled to ACLE using *N*-[3-dimethylaminopropyl]-*N*'-ethylcarbodiimide hydrochloride (EDCI) in the presence of collidine. The chloro leaving group at C-3 of compound **8** was displaced with *N*-Boc-4-(aminomethyl)-pyridine. Boc and *para*-methoxybenzyl (PMB) protecting group

Scheme 3

(i) Boc$_2$O, Et$_3$N, THF (ii) Cs$_2$CO$_3$, Bu$_4$NI, DMF (iii) (a) KOH, EtOH, 80°C (b) NCS, CHCl$_3$ (iv) ACLE, POCl$_3$, DIPEA, THF, −20°C (v) Pyridine, NaI, Me$_2$CO (vi) TFA, CH$_2$Cl$_2$, anisole.

deprotection of the pyridinium cephalosporin **9** was effected *in situ* with TFA in CH$_2$Cl$_2$ to afford the desired *β*-lactam C$_{PY}$ core **10**.

Introduction of a propyl amino group to the oxime of the aminothiazole of **3** resulted in the C$_{OX}$ synthon **18** (Scheme 3). Bromopropylamine **11** was first protected as its *N*-Boc derivative **12** which was subsequently used to alkylate trityl-protected ethyl aminothiazolyl(syn-hydroxyimino)acetate **13** in the presence of cesium carbonate and tetrabutylammonium iodide. The resultant ester **14** was hydrolyzed to the acid and chlorinated with NCS in chloroform. The acid **15** was converted to the acid chloride with phosphorous oxychloride and coupled *in situ* with ACLE to afford the cephalosporin **16**. Pyridine and sodium iodide in acetone effected displacement of the C-3 chloro leaving group of **16** and subsequent global deprotection of the resulting pyridinium compound **17** with TFA in CH$_2$Cl$_2$ yielded the C$_{OX}$ synthon **18**.

The C$_{AM}$ synthon **25** incorporated a *β*-alanine motif at the amine of the aminothiazole (Scheme 4). *N*-Boc-*β*-alanine was coupled to ethyl aminothiazolyl(syn-methoxyimino)acetate **19** with EDCI and the subsequent ester **20** hydrolyzed to the acid **21**. Following a similar

process to that described above, the acid **21** was chlorinated in DMF and coupled with ACLE using EDCI to give **23**. The C-3 chloro group was displaced with pyridine and deprotection of the Boc and PMB protecting groups with TFA afforded the C$_{AM}$ synthon **25**.

The V and C synthons **4**, **5** and **10**, **18**, **25** respectively, were covalently linked through the formation of an amide bond at each terminus of a linking adipic acid moiety. The di-1-hydroxyazabenzotriazole(HOAt)-ester **27** of adipic acid **26** was prepared and treated sequentially with the relevant V and C synthon, in the presence of collidine (Scheme 5; representative example **33**). The products were purified by reverse phase-HPLC to afford the glycopeptide/*β*-lactam heterodimers **28**~**33**. The V$_C$ synthon **2** was directly coupled with the individual C synthons **10**, **18**, **25** using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and HOAt in DMSO and DMF to afford heterodimers **34**~**36** (Scheme 6; representative example **35**).
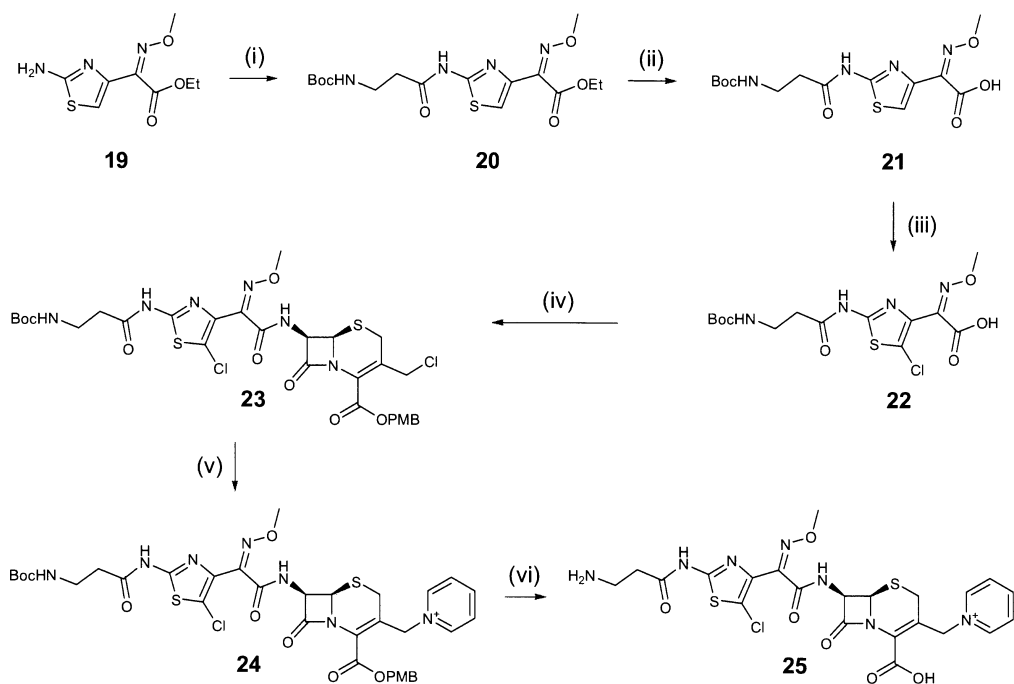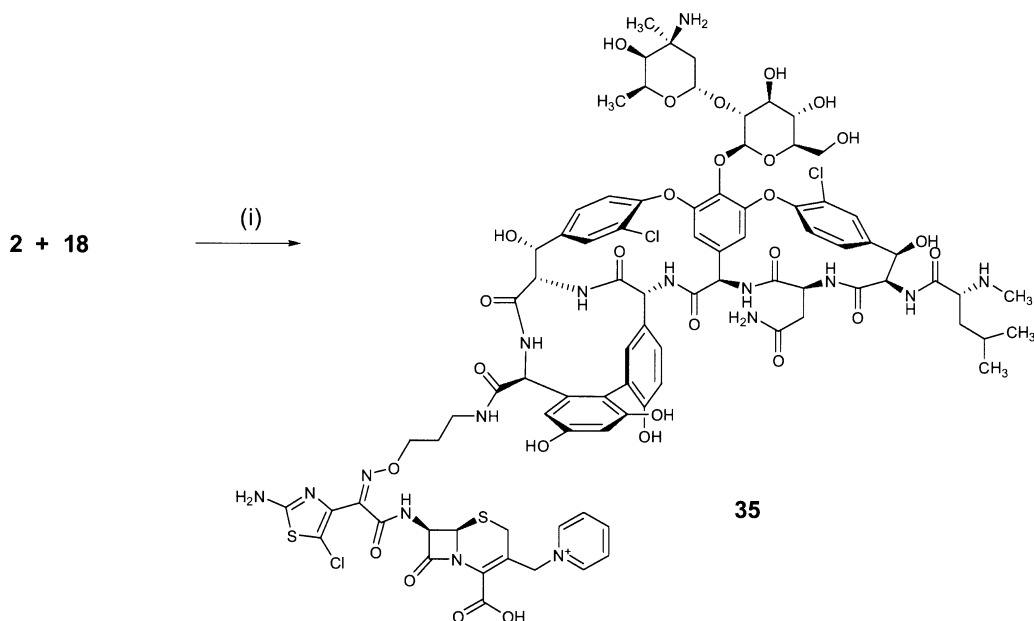
Scheme 4

(i) Boc-β-alanine, EDCI, DMAP, DMF, CH$_2$Cl$_2$ (ii) LiOH, dioxane, H$_2$O, 50°C (iii) NCS, DMF (iv) ACLE, EDCI, HOAt, 2,4,6-collidine, DMF (v) Pyridine, NaI, Me$_2$CO (vi) TFA, CH$_2$Cl$_2$.
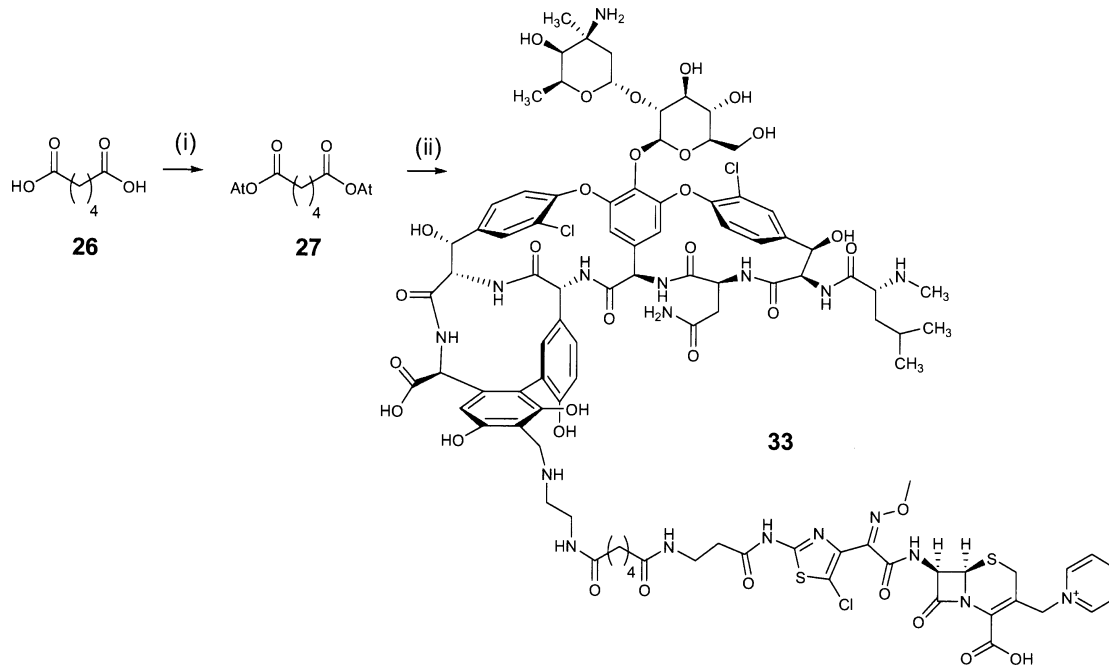
Scheme 5

(i) HOAt, EDCI, DMF (ii) (a) **25**, 2,4,6-collidine, DMF then TFA (b) **5**, 2,4,6-collidine, DMF.

2 + 18 $\xrightarrow{\text{(i)}}$

35

Scheme 6

(i) PyBOP, HOAt, DIPEA, DMSO, DMF.

Results and Discussion

The glycopeptide/β-lactam heterodimers were screened against a panel of bacterial strains, representing both the major Gram-positive human pathogens (staphylococci, enterococci and streptococci) and important drug resistance phenotypes. MICs for compound **1**, heterodimers **28~36**, C synthons **10**, **18**, **25** and V synthons **4**, **5**, **2** are presented in Table 1.

Intriguingly, it was observed that all the combinations of the V (**4**, **5**, **2**) and C synthons (**10**, **18**, **25**) resulted in heterodimers (**28~36**) with excellent potency against a range of the Gram-positive organisms tested (notably MSSA and MRSA with MIC values typically 0.05 μg/ml). The individual monomeric V synthons (**4**, **5**, **2**) and C synthons (**10**, **18**, **25**) were all considerably less potent (>30 fold) than the heterodimers (**28~36**) against all the *Staphylococcus* pathogens tested. However, against EFSVA the heterodimers (**28~36**) (0.78~25 μg/ml) were comparable in potency to the C synthons (**10**, **18**, **25**) (3.13~25 μg/ml) and against EFMVA all the compounds tested were essentially inactive (>50 μg/ml). With regard to specific heterodimer SAR, linkage *via* V_V, V_R or V_C appears to have little effect on the potency profile. For example, attaching the C_{OX} lactam **18** to the three V synthons (**4**, **5**, **2**) results in **29**, **32** and **35**, respectively, which share almost identical MIC profiles. This trend is

similarly observed for the series of C_{PY} and C_{AM} linked heterodimers (**28**, **31**, **34** and **30**, **33**, **36** respectively). Consideration of the C attachment points reveals that the C_{AM} compounds linked to V_V and V_R (**30** and **33**, respectively) show reduced potency against PRSP (0.2 μg/ml) compared to the C_{PY} and C_{OX} linked analogues (**28**, **31** and **29**, **32** respectively) (all 0.05 μg/ml). In addition, activity against the EFSVA strain demonstrates a general trend that the C_{OX} linked compounds (**29**, **32**, **35**; 0.78 μg/ml) are superior to the C_{PY} linked compounds (**28**, **31**, **34**; 1.56~3.13 μg/ml) which are in turn superior to the C_{AM} linked compounds (**30**, **33**, **36**; >25 μg/ml). The minor SAR observed between all of the heterodimer compounds (**28~36**) indicate that the relative spatial orientation (determined either by positional attachment V_V, V_C, V_R, C_{PY}, C_{OX}, C_{AM}, or linker length; V_C compounds have a shorter linker length than both V_V and V_R) of both the glycopeptide and the β-lactam component is not critical to determining the enhanced potency of the heterodimers relative to the monomeric components. This observation makes it difficult to envision simultaneous binding at both cellular targets of the heterodimers. An alternative proposal is that the heterodimers localize two active components which results in synergistic inhibition of cell wall synthesis [2].

The bactericidal activity of a set of compounds against *S. aureus* ATCC 33591 (MRSA) was determined and all were shown to be superior to vancomycin **2**, which was static at

doses of 0.25 and 1.0 μg/ml. For the C_{OX} linked set (**29**, **32**, **35**) it appears that the V_V and V_C attachment points demonstrate superior cidality with both **29** and **35** achieving a 3-log CFU/ml reduction after only 4 hours at 0.25 μg/ml or 1 hour at 1.0 μg/ml, respectively. The V_R attached compound **32** is static at 0.25 μg/ml and takes 4 hours to achieve cidality at 1.0 μg/ml. Within the V_R attached set the C_{PY} linked compound **31** shows similar cidality to the C_{OX} heterodimer **32**, both being superior to the C_{AM} derivative **33** (cidal at 1.0 μg/ml after 24 hours).

The excellent *in vitro* activity profiles described for the heterodimers **31** and **35** were tested *in vivo* using the murine neutropenic thigh model of MRSA. Upon IV administration, **31** and **35** exhibited ED_{50} values of 0.11 and 0.19 mg/kg, respectively. These are both 40 fold more efficacious than vancomycin **2** which displays an ED_{50} value of 8.1 mg/kg in this assay.

In summary, we have extended our initial multivalent approach towards glycopeptide/β-lactam heterodimers to explore the various attachment points with which to connect vancomycin and cephalosporin cores. To our surprise, all combinations of heterodimers **28**~**36** exhibited superb potency against a range of important Gram-positive bacteria. In keeping with previous observations, a subset of these potent compounds also demonstrated rapid bactericidal activity against MRSA [2]. Various SAR trends have been highlighted and two of the most attractive compounds **31** and **35** have been shown to exhibit *in vivo* efficacy >40 fold that of vancomycin against an important MRSA pathogen. As a result of this work compound **35** was selected as a clinical candidate, TD-1792. Additional SAR, further properties of these compounds and data to support the multivalent mechanism of action will be reported in future articles.

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. NMR spectra were recorded at 300 MHz. Chemical shifts (δ) are reported in ppm downfield of TMS. Abbreviations for reagents: dichloroethane (DCE), diisopropylcarbodiimide (DIC), *N,N*-diisopropylethylamine (DIPEA), *N,N*-dimethylaminopyridine (DMAP), *N*-[3-dimethylaminopropyl]-*N'*-ethylcarbodiimide hydrochloride (EDCI), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP).

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% CH_3CN in H_2O 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% CH_3CN in H_2O containing 0.1% TFA over 90 minutes.

V_R Synthon 5
Ethylenediamine (7.0 ml, 100 mmol) and 1.0 N NaOH (50 ml) were added to a stirred solution of vancomycin hydrochloride (20 g, 13 mmol) in H_2O (100 ml) at 0°C. Formaldehyde [37% in H_2O] (1.3 ml, 17 mmol) was added and the reaction mixture stirred in the dark at 4°C, overnight (78% purity by HPLC). The reaction mixture was acidified with TFA at 4°C, the solid precipitate filtered and the residue purified by RP-HPLC to afford R synthon **5**·2TFA salt. IS-MS, calcd. *m/z* for [M+H$^+$]: 1522.4; obsd. 1522.6.

C_{PY} Synthon 10
N-Chlorosuccinimide (35 g, 0.26 mol) was added to a stirred solution of 2-amino-α-(methoxyimino)-4-thiazoleacetic acid **6** (50 g, 0.25 mol) in DMF (500 ml) at room temperature and the reaction mixture stirred overnight. The light brown solution of resultant **7** was used without further purification. ACLE (101.5, 0.25 mol), 2,4,6-collidine (33.5 ml, 0.25 mmol) and EDCI (53 g, 0.275 mol) were added and the reaction mixture stirred for 2 hours at room temperature. The reaction mixture was precipitated into H_2O (3 liters) and the solids filtered, washed with H_2O (2×1 liter), saturated aqueous sodium bicarbonate (500 ml), H_2O (4×500 ml) and dried. The dry solid was taken up in CH_2Cl_2 (500 ml), slowly stirred and the precipitate which formed filtered and washed with additional CH_2Cl_2 until the washings were colorless. The precipitate was collected and dried to afford **8** (74 g, 51%). ^{1}H-NMR (DMSO-d_6) δ 9.60 (d, 1H), 7.35 (m, 3H), 6.91 (d, 2H), 5.82 (m, 1H), 5.17 (m, 3H), 4.56 (m, 2H) 3.84 (s, 3H), 3.76 (s, 3H), 3.62 (m, 2H). IS-MS, calcd. *m/z* for [M+H$^+$]: 586.0; obsd. 586.2.

Me$_2$CO (250 ml) was added to a mixture of **8** (50 g, 85 mmol) and sodium iodide (13 g, 85 mmol) in the dark, under an atmosphere of nitrogen at room temperature. The reaction mixture was stirred for 30 minutes and then a solution of 4-(*N*-tert-butoxycarbonyl)aminomethyl pyridine (27 g, 130 mmol) in Me$_2$CO (30 ml) was added. The reaction mixture was stirred for 2 hours and then 0.1 N HCl (1.4 liters) was added to produce a gummy precipitate. The solvent was decanted and the gummy residue treated with H$_2$O (800 ml) to give a solid. The H$_2$O was decanted and the solid dissolved in a mixture of EtOAc/EtOH (4:1, 1 liter) and washed with brine (500 ml), dried (MgSO$_4$), filtered and concentrated *in vacuo* to afford **9** (70 g, 78% purity by HPLC). IS-MS, calcd. *m/z* for [M$^+$]: 758.2; obsd. 758.3.

Anisole (35 ml) was added to a stirred solution of crude **9** (70 g) in CH$_2$Cl$_2$ (550 ml). TFA (150 ml) was added to the reaction mixture at room temperature which was stirred for 2 hours and then concentrated *in vacuo*. The residue was diluted with diethyl ether (1 liter) and the precipitate filtered, washed with diethyl ether, stirred in H$_2$O (200 ml), filtered and dried. The residue was purified by RP-HPLC to afford **10**·2TFA salt (30 g, 46% from **8**). ^{1}H-NMR (DMSO-d_6) δ 9.53 (d, 1H), 9.29 (d, 2H), 8.21 (d, 2H), 7.46 (s, br, 2H), 5.87 (dd, 1H), 5.60 (d, 1H), 5.33 (d, 1H), 5.13 (d, 1H), 4.49 (s, 2H), 3.84 (s, 3H), 3.52 (d, 1H), 3.22 (d, 1H). IS-MS, calcd. *m/z* for [M$^+$] : 539.0; obsd. 538.2.

C$_{OX}$ Synthon 18

A solution of *tert*-butoxycarbonyl anhydride (112.6 g, 516 mmol) in THF (200 ml) was added to a stirred solution of 3-bromopropylamine hydrobromide **11** (100 g, 457 mmol) and triethylamine (190 ml) in THF (1.6 liters) at 0°C. The reaction mixture was warmed to room temperature, stirred overnight and then filtered. The filtrate was concentrated *in vacuo* and the residue diluted with hexane (1.5 liters) and stored at −20°C for 3 days. The solvent was decanted from the formed solid which was dried to afford **12** (101 g, 93%) as a white crystalline solid. ^{1}H-NMR (DMSO-d_6) δ 6.97 (t, 1H), 3.48 (t, 2H), 3.02 (t, 2H), 1.91~1.95 (m, 2H), 1.37 (s, 9H).

Cesium carbonate (230.8 g, 709 mmol) and tetrabutylammonium iodide (18.7 g, 51 mmol) were added to a stirred solution of **13** (100 g, 219 mmol) in DMF (700 ml) at room temperature. A solution of **12** (50.6 g, 213 mmol) in DMF (100 ml) was added dropwise to the reaction mixture over 30 minutes. After 2 hours, the reaction mixture was filtered and the solids washed with DMF (200 ml). The filtrate was diluted with EtOAc (2 liters) and washed with 1.0 N HCl (700 ml), saturated aqueous sodium bicarbonate (700 ml) and brine (500 ml). The organic layer

was dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to give a residue which was dissolved in boiling EtOH (250 ml). Upon cooling a solid formed which was filtered, washed with cooled EtOH (50 ml) and dried to afford **14** (117 g, 90%) as an off-white powder. ^{1}H-NMR (DMSO-d_6) δ 8.80 (s, 1H), 6.95 (m, 16H), 6.79 (t, 1H) 3.95~4.04 (m, 4H), 2.94~2.99 (m, 2H), 1.60~1.70 (t, 2H), 1.31 (s, 9H), 1.01~1.10 (t, 3H). IS-MS, calcd. *m/z* for [M+H$^+$]: 615.8; obsd. 615.4.

A solution of potassium hydroxide (23.1 g, 411 mmol) in EtOH (150 ml) was added dropwise to a solution of **14** (84.2 g, 137 mmol) in EtOH (400 ml) at 80°C. After 30 minutes, the reaction mixture (with solid precipitate) was cooled in an ice bath and diluted with EtOAc and H$_2$O. 1.0 N Phosphoric acid was added and the aqueous layer removed. H$_2$O was added to the organic layer to dissolve solid residues and the organic layer separated and further washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to afford the acid intermediate (80 g, 100%) as a tan solid which was used without further purification. *N*-Chlorosuccinimide (2.28 g, 17 mmol) was added to a stirred solution of the acid intermediate (10 g, 17 mmol) in CHCl$_3$ (70 ml) at room temperature. The reaction mixture was stirred overnight and then concentrated *in vacuo* and the residue dissolved in a minimal amount of DMF and H$_2$O added. The precipitate formed was filtered and dried to afford **15** as a tan solid (9.5 g, 90%). ^{1}H-NMR (DMSO-d_6) δ 8.97 (s, 1H), 7.18~7.41 (m, 15H), 6.83 (t, 1H) 3.97~4.05 (t, 2H), 2.94~2.99 (m, 2H), 1.63~1.74 (t, 2H), 1.37 (s, 9H). IS-MS, calcd. *m/z* for [M+H$^+$]: 622.2; obsd. 621.3.

ACLE (37.5 g, 93 mmol) was added to a stirred solution of **15** (66.2 g, 107 mmol) in THF (850 ml) and the reaction mixture cooled to −45°C. 2,4,6-Collidine (30.6 ml, 232 mmol) was added to the reaction mixture followed by phosphorous oxychloride (12.1 ml, 130 mmol). The reaction mixture was stirred at −45°C for 10 minutes and then carefully quenched with H$_2$O (650 ml) and diluted with EtOAc (650 ml). The layers were separated and the aqueous layer further extracted with EtOAc (650 ml). The combined organic layers were washed with H$_2$O (650 ml) and brine (650 ml), dried (MgSO$_4$), filtered and concentrated *in vacuo* to afford **16** (101.2 g, >100%) as a brown-red solid. ^{1}H-NMR (DMSO-d_6) δ 9.41~9.44 (d, 1H), 8.97 (s, 1H), 7.18~7.41 (m, 17H), 6.90~6.96 (d, 2H), 6.78 (t, 1H), 5.49~5.54 (m, 1H), 5.11~5.25 (m, 3H), 4.40~4.59 (q, 2H), 3.97~4.05 (t, 2H), 3.75 (s, 3H), 3.40~3.74 (q, 2H), 2.94~2.99 (m, 2H), 1.63~1.74 (t, 2H), 1.37 (s, 9H). IS-MS, calcd. *m/z* for [M+H$^+$]: 973.0; obsd. 972.0.

Sodium iodide (7.7 g, 51.4 mmol) was added to a stirred solution of **16** (49.9 g, 51.4 mmol) in Me_2CO (220 ml) in the dark, under an atmosphere of nitrogen at room temperature. The reaction mixture was stirred for 80 minutes and then pyridine (5.82 ml, 71.9 mmol) was added. The reaction mixture was stirred for 150 minutes and then added to diethyl ether (1 liter). The resultant precipitate was filtered, washed with diethyl ether (3×100 ml) and dried *in vacuo*. The solid was further washed with diethyl ether (3×100 ml) and dried to afford **17** (46.7 g, 90%) as a tan solid. ^{1}H-NMR (DMSO-d_6) δ 9.41~9.44 (d, 1H), 8.98~9.02 (d, 2H), 8.96 (s, 1H), 8.65 (t, 1H), 8.16~8.21 (t, 2H), 7.18~7.41 (m, 17H), 6.90~6.96 (d, 2H), 6.78 (t, 1H), 5.60 (s, 2H), 5.50~5.55 (m, 1H), 5.21 (s, 2H), 5.10~5.12 (d, 1H), 3.97~4.05 (t, 2H), 3.75 (s, 3H), 3.40~3.74 (q, 2H), 3.30~3.50 (q, 2H), 2.94~2.99 (m, 2H), 1.63~1.74 (t, 2H), 1.37 (s, 9H). IS-MS, calcd. *m/z* for [M+]: 1015.6; obsd. 1014.2.

Anisole (6.2 ml) was added to a stirred solution of **17** (14.4 g, 14 mmol) in CH_2Cl_2/TFA (1 : 1, 120 ml) at room temperature. The reaction mixture stirred for 3 hours, concentrated *in vacuo* and the residue dissolved in EtOAc and extracted with H_2O. The aqueous layer was lyophilized and purified by RP-HPLC to afford **18**·2TFA salt (3.3 g, 30%) as a white powder. ^{1}H-NMR (DMSO-d_6) δ 9.59~9.62 (d, 1H), 9.00~9.08 (d, 2H), 8.65 (t, 1H), 8.17~8.22 (t, 2H), 7.77 (s, 3H), 7.39 (s, 2H), 5.83~5.92 (m, 1H), 5.41~5.63 (q, 2H), 5.17 (d, 1H), 4.02~4.15 (t, 2H), 3.29~3.57 (q, 2H), 2.79~2.92 (m, 2H), 1.80~1.97 (t, 2H). IS-MS, calcd. *m/z* for [M+]: 553.0; obsd. 553.1.

C_{AM} Synthon 25

EDCI (61.4 g, 0.320 mol) and DMAP (33.1 g, 0.271 mol) were added to a stirred solution of aminothiazole **19** (56.5 g, 0.246 mol) and Boc-β-alanine (55.9 g, 0.296 mol) in DMF/CH_2Cl_2 (4 : 1, 700 ml) at room temperature, under an atmosphere of nitrogen. After 18 hours the reaction mixture was diluted with EtOAc (1.7 liters) and washed with 1.0 M HCl (2×500 ml), saturated aqueous sodium bicarbonate (500 ml) and brine (500 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford **20** (99 g, 100%) as a foam which was used without further purification. ^{1}H-NMR (CDCl$_3$) δ 10.02 (bs, 1H), 7.21 (s, 1H) 5.18 (bs, 1H), 4.41 (q, 2H), 4.03 (s, 3H) 3.44~3.60 (m, 2H), 2.64~2.79 (m, 2H), 1.43 (s, 9H), 1.37 (t, 3H). IS-MS, calcd. *m/z* for [M+H+]: 401.4; obsd. 401.1.

2.0 M Lithium hydroxide (246.4 ml, 0.493 mol) was added to a stirred solution of **20** (99 g, 0.246 mol) in dioxane/H_2O (2 : 1 ratio, 750 ml). The reaction mixture was heated to 50°C and stirred for 6 hours. The reaction mixture was cooled, diluted with H_2O (200 ml) and washed

with EtOAc (500 and 250 ml). The aqueous layer was concentrated *in vacuo* and the crude residue partitioned between CHCl$_3$ (800 ml) and 1.0 M phosphoric acid (600 ml). The acidic aqueous layer was further extracted with CHCl$_3$ (800 ml) and the organic extracts combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford **21** (86.2 g, 94%) as a yellow foam which was used without further purification. ^{1}H-NMR (CDCl$_3$) δ 12.38 (bs, 1H), 7.26 (s, 1H) 5.16 (bs, 1H), 3.94 (s, 3H) 3.46~3.59 (m, 2H), 2.73~2.81 (m, 2H), 1.42 (s, 9H). IS-MS, calcd. *m/z* for [M+H+]: 373.4; obsd. 372.9.

N-Chlorosuccinimide (6.9 g, 52 mmol) was added to a stirred solution of **21** (19.3 g, 52 mmol) in DMF (100 ml) at room temperature, under an atmosphere of nitrogen. The reaction mixture was stirred for 18 hours and the light brown solution of resultant **22** was used without purification. The reaction mixture was diluted with DMF (40 ml) and EDCI (14.9, 78 mmol), HOAt (10.6 g, 78 mmol) and 2,4,6-collidine (20.6 ml, 156 mmol) added. The reaction mixture was stirred at room temperature for 20 minutes and then cooled to 0°C and a solution of ACLE (22.1 g, 54 mmol) and 2,4,6-collidine (10.3 ml, 78 mmol) in DMF (170 ml) added. The reaction mixture was warmed to room temperature over 4 hours, and then diluted with EtOAc (1.2 liters) and washed with 1.0 M HCl (2×400 ml) and saturated aqueous sodium bicarbonate (2×400 ml). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford **23** (14.94 g, 38%) as an off-white solid which could be used crude, without further purification. A sample was purified by flash chromatography (EtOAc/hexane, 1 : 1 to 5 : 4 to 3 : 2 to 2 : 1). ^{1}H-NMR (CDCl$_3$) δ 10.65 (bs, 1H), 8.31 (bs, 1H) 7.33 (d, 2H), 6.90 (d, 2H), 6.09 (m, 1H), 5.08~5.32 (m, 4H), 4.64 (d, 1H), 4.44 (d, 1H), 4.09 (s, 3H), 3.84 (s, 3H) 3.45~3.76 (m, 4H), 2.62~2.70 (m, 2H), 1.45 (s, 9H). IS-MS, calcd. *m/z* for [M+H+]: 758.7; obsd. 757.4.

Sodium iodide (1.88 g, 12.6 mmol) was added to a stirred solution of **23** (9.7 g, 12.8 mmol) in Me_2CO (70 ml) in the dark, at room temperature for 1 hour under an atmosphere of nitrogen. After 1 hour pyridine (1.35 ml, 16.6 mmol) was added and the reaction mixture stirred for a further 2 hours. The reaction mixture was added portionwise to diethyl ether (700 ml) and the resulting precipitate filtered and dried to afford **24** (10.14 g, 99%) as a crude brown solid. IS-MS, calcd. *m/z* for [M+H+]: 801.3; obsd. 800.2.

A solution of the crude **24** in TFA/CH_2Cl_2 (2 : 3, 70 ml) was stirred at room temperature for 1 hour. The reaction mixture was concentrated *in vacuo* and the residue partioned between EtOAc (70 ml) and H_2O (70 ml). The organic layer was further extracted with H_2O (2×50 ml) and the combined aqueous extracts lyophillized. The

residue was purified by RP-HPLC to afford **25**·2TFA salt (2.1 g, 20% from **23**) as an off-white solid. ^{1}H-NMR (DMSO-d_6) δ 9.78 (d, 1H), 9.26 (bs, 2H) 8.72 (t, 1H), 8.25 (t, 2H), 7.88 (bs, 3H), 5.92~5.96 (m, 1H), 5.70 (d, 1H), 5.42 (d, 1H), 5.22 (d, 1H), 3.95 (s, 3H), 3.60 (d, 1H) 3.32 (d, 1H), 3.18 (bs, 2H), 2.87 (t, 2H). IS-MS, calcd. *m/z* for [M$^+$]: 581.0; obsd. 580.3.

Di-HOAt Ester of Adipic Acid 27

A solution of adipic acid (6.63 g, 45.4 mmol), HOAt (15.28 g, 99.8 mmol) and EDCI (19.13 g, 99.8 mmol) in DMF (80 ml) was stirred at room temperature, under an atmosphere of nitrogen, overnight. The reaction mixture was diluted with CH_2Cl_2 (500 ml) and washed with saturated aqueous sodium bicarbonate (2×200 ml) and brine (2×200 ml). The organic phase was dried (MgSO$_4$), filtered and concentrated *in vacuo* to afford **27** as a white solid was used without further purification.

Representative Example: Glycopeptide-*β*-lactam Heterodimers 31

10·2TFA salt (1.0 g, 1.3 mmol) was added to a stirred solution of crude di-HOAt adipic ester **27** (2.48 g, 6.5 mmol) in DMF (50 ml) at room temperature. The reaction mixture was cooled to 0°C and 2,4,6-collidine (0.34 ml, 2.6 mmol) added and stirring continued for 15 minutes. TFA (0.3 ml, 3.9 mmol) was added to quench the reaction which was added to EtOAc (400 ml) and the resultant precipitate centrifuged washed with EtOAc, the EtOAc decanted and the solid dried. A solution of **5** (3.86 g, 1.95 mmol) in DMF (40 ml) was added to the solid and the reaction mixture stirred and cooled to 0°C. 2,4,6-Collidine (1.03 ml, 7.8 mmol) was added and the reaction mixture stirred for 20 minutes and then quenched with TFA (0.80 ml, 10.4 mmol) and added to CH_3CN (400 ml). The resultant precipitate was filtered and purified by RP-HPLC to afford **31**·3TFA salt (740 mg, 23%) as a white powder. IS-MS, calcd. *m/z* for [M$^+$]: 2170.5; obsd. 2171.8.

Representative Example: Glycopeptide-*β*-lactam Heterodimers 35

A solution of PyBOP (1.3 g, 2.6 mmol) and HOAt (0.35 g, 2.6 mmol) in DMF (40 ml) was added to a stirred solution of vancomycin hydrochloride **2** (4.2 g, 2.8 mmol) in DMSO (40 ml) at room temperature. DIPEA (0.98 ml, 5.7 mmol) was subsequently added and the reaction mixture stirred for 30 minutes and then quenched with TFA (0.44 ml, 5.7 mmol). The reaction mixture was cooled to 0°C and a solution of **18**·2TFA salt (2.6 mmol) in DMF (20 ml) added followed by 2,4,6-collidine (1.5 ml, 11.4 mmol). The reaction mixture was stirred for 4 hours and then quenched with TFA (1.1 ml) and added to diethyl ether. The resultant precipitate was centrifuged, washed with diethyl ether, the ether decanted and the solid dried and then purified by RP-HPLC. The anion of the salt was exchanged using Amberlyte resin to afford **35**·3HCl salt (1.4 g, 26%) as a white powder. IS-MS, calcd. *m/z* for ([M−pyridine(79.1)+H$^+$]$^+$): 953.2; obsd. 953.3.

Determination of *in Vitro* Antimicrobial Activity

MIC susceptibility determinations were performed using a standard broth microdilution assay according to the recommendation of the National Committee for Clinical Laboratory Standards [16].

Determination of Bactericidal Activity

Time-kill assays were performed in broth media. Log-phase cultures of bacteria with a final density of approximately 10^6 CFU/ml were tested. Immediately after inoculation, the first samples (100 μl) were taken and constituted the time point 0 hour. Test tubes were incubated in ambient air at 35°C with constant agitation for 24 hours and were sampled at various time points. Samples were diluted in saline and colony counts were performed by applying 100 μl of samples directly on solid media. Colonies were counted after 24 hours incubation.

Determination of *in Vivo* Efficacy in Neutropenic Mouse Thigh Assay

Female mice were rendered neutropenic with cyclophosphamide and infected in the thigh with the organism. Infected animals were dosed intravenously beginning at 1 hour post-infection with vehicle or the appropriate dose of **31/35**. At 24 hours post-infection, animals were euthanized and thighs were harvested, homogenized and processed to determine bacterial titer [17].

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