Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 40 (2005) 798–804
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

An unexpected amide bond cleavage: poly (ethylene glycol) transport forms of vancomycin. 2

Richard B. Greenwald [1], Hong Zhao *, Ping Peng, Clifford B. Longley, Qing-Hong Dai, Jing Xia, Anthony Martinez

Enzon Pharmaceuticals, Inc., 20 Kingsbridge Road, Piscataway, NJ 08854, USA

Received 12 July 2004; received in revised form 14 January 2005; accepted 27 January 2005

Available online 22 April 2005

Abstract

PEG-amide vancomycin derivatives (V_3 position) have been synthesized and found to behave as prodrugs in vivo, demonstrating anti-microbial activity in mice when challenged with *Staphylococcus aureus*. The corresponding PEG-carbamate derivatives do not manifest this in vivo activity, although both classes of compounds have similar in vitro rat plasma stability. Thus, it appears that extra vascular cleavage of the amide bond can occur if the condition of extended circulation of the conjugate is met, resulting in the release of vancomycin.

Keywords: Vancomycin; Poly (ethylene glycol); Anti-microbial activity

1. Introduction

Our recent work [1] on poly (ethylene glycol) (PEG) prodrugs of vancomycin (Vanco, **1**, Fig. 1) established that Vanco is released in a predictable fashion using a 1,6-benzyl elimination (BE) pathway. The advantage of such a delivery system lies in its possible convenience of administration and cost effectiveness. Of several candidates synthesized, **2** (Fig. 2) was found to provide equivalent activity to Vanco, but pharmacokinetic (PK) studies demonstrated a much greater area under the plasma concentration–time curve (AUC), thus indicating that this conjugate might be dosed with less frequency than native Vanco. In vitro and in vivo studies of **2** were carried out and the results compared to **1**. In order to clearly demonstrate that the cleavable promoiety, an ester trigger, was responsible for the observed properties, it was decided to synthesize a "permanent" bonded PEG-Vanco conjugate by attachment at the V_3 position for comparison. Such a high molecular weight (hmw) PEG-Vanco derivative would not be expected to show efficacy in vivo. Amide bonds are amongst the most stable attachments found in organic compounds, and are probably the most common type of bonds employed in living organisms in the form of proteins and peptides. Therefore, it was thought that formation of a PEG-amide bond would serve as a satisfactory comparator for a stable Vanco derivative in this study. What was observed for this class of compounds in vivo was unexpected anti-microbial activity. However, other stable moieties such as PEG-carbamate derivatives of **1** demonstrated no in vivo anti-microbial activity. In this study we present our novel findings including the synthesis, in vivo activities, and possible applications of these unusual amides.

2. Chemistry

For purposes of obtaining loadings of at least 10% Vanco by weight per conjugate, only tetrameric species were employed. The syntheses of amide and carbamate PEG-Vanco conjugates were carried out using the commercially available 40,000 Da molecular weight (mw), 4 arm-PEG pentaerythritol derivative, **3** (NOF Corp.), and the linear branched PEG, **14**, [2]. As described previously [1] all conjugations occurred at the V_3 position of **1**. The preparation of amide **7** is shown in Scheme 1. Condensation of **3** with bromo-*tert*-

* Corresponding author. Tel.: +1 732 980 4902; fax: +1 732 885 2950.
E-mail address: hong.zhao@enzon.com (H. Zhao).
[1] Dr. Richard Greenwald passed away in September 2004. We would like to dedicate this paper to him.

0223-5234/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2005.01.011

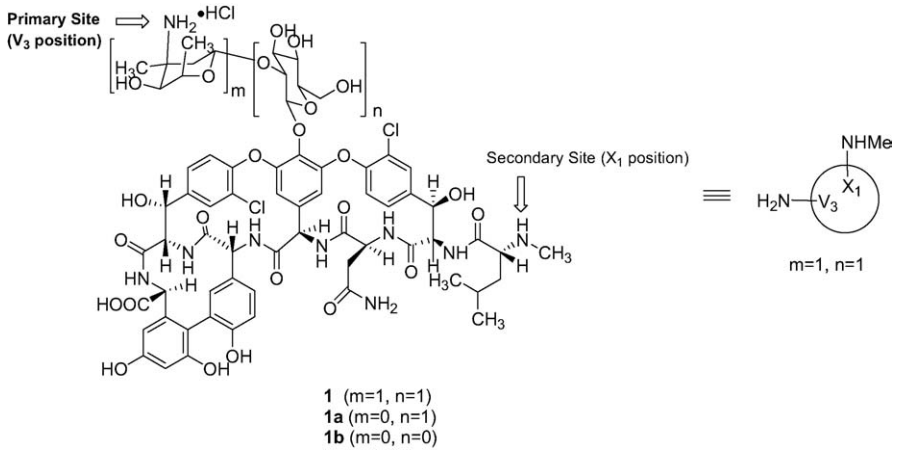


Fig. 1.

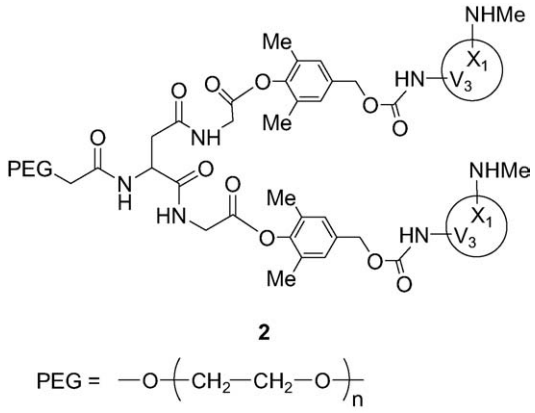


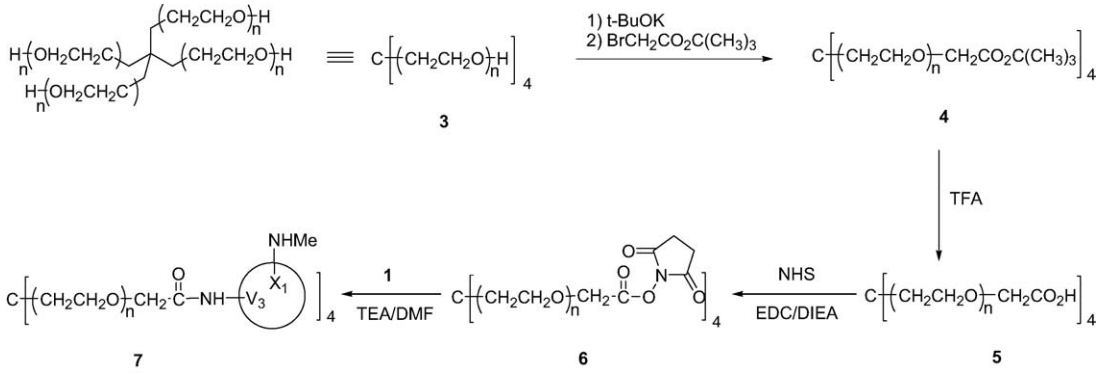
Fig. 2.

butyl acetate gave rise to the tetra ester **4**, which was cleaved by 33% TFA to yield the tetra acid, **5**. This was condensed with *N*-hydroxy succinimide (NHS) in the presence of EDC and DIEA to yield the NHS ester, **6**. Condensation of **6** with **1** was carried out in DMF in the presence of TEA [1], and produced the desired amide **7** in a yield of 71%. The preparation of **12**, an amide derivative of **1** with a glycine spacer (connected to PEG with a carbamate bond), is shown in Scheme 2. First the activated NHS carbonate, **8** was prepared by reaction of the 4 arm diol (**3**) with disuccinimidyl carbonate (DSC) to give the activated NHS derivative, **8**. This was next reacted with *tert*-butylglycine to yield the ester intermediate **9**, which was converted to the free acid **10** using 33% TFA. Activation of **10**–**11** was accomplished using NHS and EDC. Condensation of **11** with **1** in DMF/DIEA again yielded the desired amide derivative, **12**.

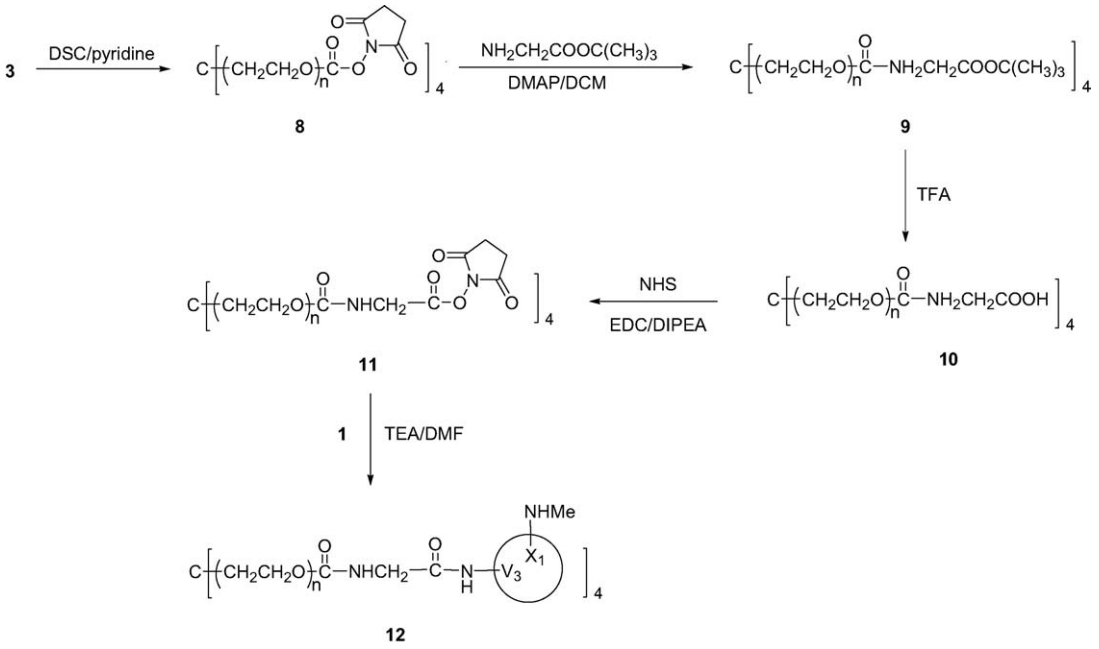
The activated 4 arm PEG carbonate **8** was also utilized for conjugation directly with **1** in DMF solution to give the carbamate, **13**, as shown in Scheme 3. A linear branched PEG-carbamate was prepared by first coupling 2-(2-aminoethoxy) ethanol with PEG aspartic acid (**14**) to give the alcohol, **15**, which was activated to the DSC **16** (Scheme 4). Reaction of **16** with **1,** at the V_3 position, was accomplished in DMF in the presence of TEA to give the desired tetra-substituted Vanco carbamate **17** (Table 1).

3. Biological results and discussion

The initial design of this study was to demonstrate that permanently linked vancomycin conjugates would be inactive, and for this reason the amide linked conjugates, **7** and **12** were prepared. It was, therefore, quite surprising to find that although there was no observable in vitro cleavage of these conjugates, significant anti-microbial activity was observed for both amide derivatives following *Staphylococcus aureus* challenge in mice (Table 2). The amide derivative

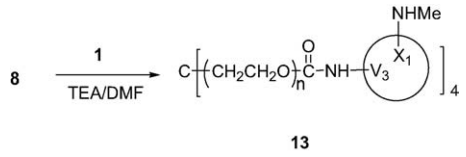


Scheme 1.

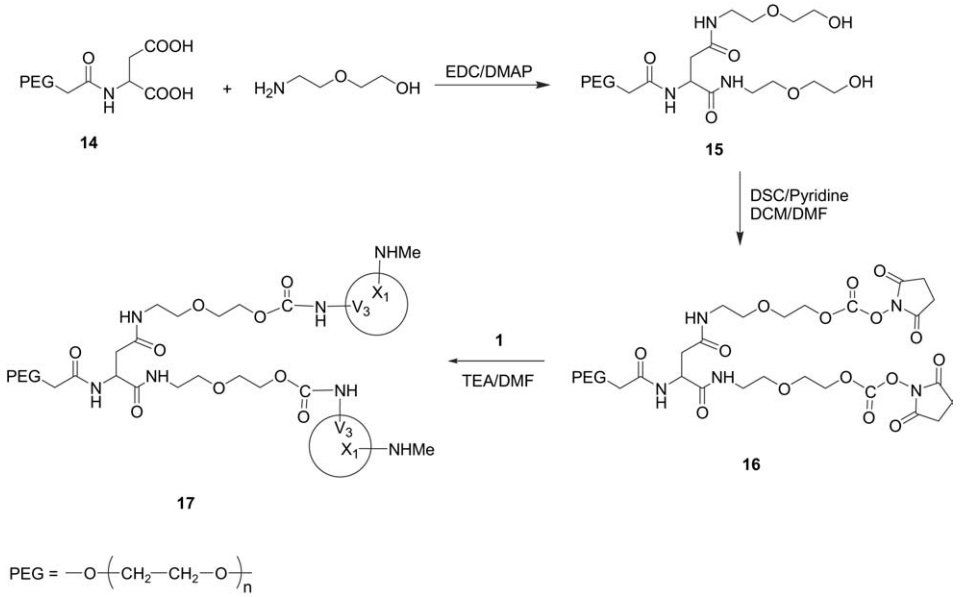


Scheme 2.

7 resulted in 80% survival at a concentration of 100 mg/kg, while at a dose of 250 mg/kg the survival rate was 100%. The carbamate-amide conjugate **12** at a dose of 100 mg/kg, was less efficacious than **7** with an observed 50% survival rate, but this result, nonetheless, indicated significant anti-microbial activity compared to untreated mice. Substituting a carbamate bond (**13**, an alternate stable bond) for the amide link of **7**, produced a conjugate that did not demonstrate any in vivo efficacy, although this compound had similar in vitro properties as the amide conjugate (Table 1). It has been pointed out that if a means of prolonging the circulating half-life of a latentiated species can be accomplished, then linking moieties of greater stability, which have previously not been considered useful, can be employed in the construction of prodrug species [3]. One-way of accomplishing this objective is to prevent rapid renal excretion of the prodrug. This can be accomplished by attachment of large ballasts such as PEG, a highly water-soluble polymer, to a particular drug. It has been demonstrated that certain PEG-drug conjugates such as camp-



Scheme 3.



Scheme 4.

Table 1
Properties of PEG-vancomycin conjugates

Compound	Mw	Percent of Vanco [a]	Solubility [b] (mg/ml)	Stability in saline (24 h) % [c]	$t_{1/2}$ (rp) (h)	$t_{1/2}$ (hp) (h)
7	45,957	12.4	134	3.3	72	182
12	46,132	10.3	102.4	2.4	110	81
13	45,901	12.9	96	4.0	78	58
17	46,568	9.2	137	3.1	161	117

[a] Percentage of vancomycin by weight.
[b] Solubility was measured in saline at 25 °C.
[c] Percent of hydrolysis after 24 h at 25 °C.

tothecin derivatives with an mw of 40,000 Da circulate in the mouse for at least 15 h [4]. In the case of Vanco, in vitro half-lives in rat plasma (Table 1) are on the order of 72–161 h (extrapolated). The 4-arm branched carbamate **13**, and the terminally branched tetramer, **17** showed similar elimination half-lives ($t_{1/2}$) in the rat of 21.7 ± 9.0 and 18.3 ± 5.1 h with comparable peak plasma concentrations (C_{max}) of 8.2 ± 3.4 and 9.6 ± 2.4 mg/ml and analogous areas under the plasma concentration time curve (AUC) of 234 ± 1 and 246 ± 7 h mg/ml. The PK profile is summarized in Table 3. The 4-arm branched amide and carbamate-glycine conjugates **7** and **12** had more rapid half-lives, lower C_{max}, and smaller AUC compared to **13** and **17**. The 4-arm carbamate-glycine conjugate **12** had a 37% faster elimination half-life (15.8 ± 3.1 h), similar peak plasma concentration (8.7 ± 1.1 mg/ml) and 17% smaller AUC compared to the linear carbamate conjugate **13**. The 4-arm branched amide conjugate **7** had a 39% faster elimination half-life, 34% lower C_{max}, and 58% smaller AUC compared to the linear branched amide tetramer conjugate **17**. Clearly this suggests that in vivo hydrolysis may not readily occur in the blood; and any released **1** implies possible extra vascular cleavage, perhaps involving hepatic intervention. It is known that amides are hydrolyzed in the liver, and carbamates based on electron withdrawing moieties such as pyrimidines are also known to be hepatically cleaved as well. This is illustrated by the anti-cancer prodrug capecitabine [5]. But in general aliphatic carbamates are less susceptible to degradation in that organ. It appears as if enzymatic cleavage of these normally stable amide bonds takes place, at least in part, due to attachment to Vanco, since an analogous mw PEG-amide conjugate of doxorubicin, similarly conjugated through the amino group of its associated sugar, was shown not to manifest anti-tumor activity [6]. Rat plasma samples obtained during a PK study of compound **7** were analyzed by HPLC and MS and indicated that not only is **1** formed by cleavage of the PEG-amide bond, but even greater amounts of **1a**, desvancosamine vancomycin, resulting from the removal of the PEGylated vancosamine from the glucose portion of the antibiotic, are also present. In addition, aglucovancomycin, **1b** (both sugars removed) was also present in substantial amounts. It thus appears that not only is the PEG-amide bond cleaved in vivo, but due to the long circulating half-life of the PEG-amide conjugates, alternate metabolic pathways become available that lead to removal of the PEGylated vancosamine and the entire PEGylated disaccharide moiety from Vanco. It also seems that these metabolites have substantial biological activity since the *S. aureous* challenge test produces results that are equivalent to Vanco. A detailed study of the preparation, properties, and activity of **1b** will be discussed in subsequent papers.

Table 2
$LD_{90–100}$ anti-microbial efficacy of PEG-vancomycin tetramer conjugates in ICR mice

Compounds	Dose administered (mg/kg)	Percent survival
Saline	–	0*
Vancomycin	100	100*
7	100	80
7	150	80
7	250	100
12	100	50
13	100	10[#]
17	100	0[#]

ICR Swiss mice were administered 100, 150, or 250 mg/kg vancomycin equivalents of PEG-vancomycin conjugates in two evenly split doses 1 and 2.5 h following IP challenge with a $LD_{90–100}$ of *S. aureus* (Smith). Mice were observed for survival for 7 days. *Replicated four times; [#]replicated two times.

Table 3
Pharmacokinetic parameters of PEG-vancomycin conjugates

Compound	C_{max} (mg/ml)	$t_{1/2}$ (h)	AUC (h mg/ml)
7	6.38 ± 0.54	11.2 ± 1.1	103 ± 4
12	8.66 ± 1.11	15.8 ± 3.1	194 ± 16
13	8.21 ± 3.36	21.7 ± 9.0	234 ± 1
17	9.64 ± 2.40	18.3 ± 5.1	246 ± 7

Rats were IV administered 50 mg/kg PEG-vancomycin conjugates. Each value represents the mean ± standard deviation (n = 3).

4. Conclusions

It has been demonstrated that amide substitution at the V_3 position of **1** produces derivatives stable to buffers over a wide pH range. These PEG conjugates can be considered prodrugs, however, since these compounds are hydrolyzed in vivo, in the rat, to yield vancomycin in sufficient quantity to demonstrate good efficacy in the *S. aureus* challenge test. These observed results also were shown to be dose dependent in the case of the 4-armed branched amide tetramer **7**. This finding is in agreement with earlier speculative beliefs [5] that by increasing the PK for conjugates containing stable bonds, a corresponding increase in the pharmcodynamics (PD) will result. In the current case, half-lives of up to 161 h

were observed, and thus indicate that once a week dosing is feasible. Thus, a novel application of the amide moiety as an in vivo labile linkage has been developed for the transport and delivery of vancomycin. However, no predictive simplifications can be forthcoming as to which PEG-amide linked drugs will be active. It has already been pointed out [7] that each BE or ester PEG-drug conjugate, in a given series, must be evaluated on an individual basis. This generalization appears to be true for PEG-Vanco derivatives as well.

5. Experimental protocols

5.1. General remarks

All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation from toluene prior to use. Polyethylene glycol (PEG) linker: compounds **14** and **16** were made according to the published procedures.[1] ^{13}C NMR spectra were obtained at 75.46 MHz using a Varian Mercury® 300 NMR spectrometer and deuterated chloroform and pyridine as the solvents unless otherwise specified. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS).

5.1.1. HPLC methods

The reaction mixtures and the purity of intermediates and final products were monitored by a Beckman Coulter System Gold® HPLC instrument. It employs a ZOBAX® 300SB C8 reversed phase column (150 × 4.6 mm) or a Phenomenex Jupiter® 300A C18 reversed phase column (150 × 4.6 mm) with a multi-wavelength UV detector, using a gradient of 10–90% of acetonitrile in 0.05% trifluoroacetic acid (TFA) at a flow rate of 1 ml/min.

5.1.2. Kinetic studies in rat plasma

PEG-Vancomycin (40 kDa tetramer conjugate) was dissolved in 1000 μl of a mixture of AcCN and MeOH (1:1, v/v). 100 μl of solution was transferred to each vial (eight vials total, StepVial System II). Solvent was removed under reduced pressure and 100 μl of rat plasma was added to each vial. The vial was sealed and vortexed for 1 min after which time the vial was incubated at 37 °C for 0, 0.5, 1, 2, 4, 6 and 20 h, respectively. To each vial 400 μl of AcCN and MeOH (1:1, v/v) was again added, and the vial vortexed for 1 min. The mixture was filtered through a 0.45 μm filter membrane and 50 μl of the filtrate was injected directly into the HPLC system.

5.2. Syntheses

5.2.1. Compound *4*

Compound **3** (30.0 g, 0.75 mmol) was first dried by azeotropic distillation of toluene (300 ml) for 2 h with the removal of 100 ml of the solvent. The remaining solution was cooled to 20 °C, followed by the addition of the 1 M potassium *tert*-butoxide solution (6.2 ml, 6.2 mmol). The resulting mixture was stirred for 2 h at 40–45 °C, followed by the addition of the *tert*-butyl bromoacetate (2.34 g, 12.0 mmol), and refluxed over night. The solvent was partially removed under reduced pressure and the PEG derivative precipitated by the addition of ethyl ether, filtered, and crystallized from *N*,*N*-dimethylformamide (DMF) and 2-propanol (IPA) to yield **4** (27 g, 0.667 mmol, 89%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 169.06, 81.00, 72.15–69.78 (PEG), 27.82.

5.2.2. Compound *5*

A solution of **4** (27 g, 0.67 mmol) in methylene chloride (DCM, 300 ml) and TFA (150 ml) was stirred at room temperature for 7 h followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered, and washed with ethyl ether to yield **5** (26 g, 0.646 mmol, 97%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.84, 70.68–68.16 (PEG), 45.19.

5.2.3. Compound *6*

To a solution of **5** (13 g, 0.32 mmol), *N*-hydroxysuccinimide (NHS, 0.3 g, 2.58 mmol), and diisopropylamine (DIEA, 1.35 ml, 7.75 mmol) in a mixture of anhydrous DCM (104 ml) and anhydrous DMF (26 ml) cooled to 0 °C, was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.74 g, 3.85 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 12 h, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered, and crystallized from DMF/IPA (48/192 ml) to give **6** (12.3 g, 0.303 mmol, 95%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 168.3, 165.47, 70.94–69.66 (PEG), 66.16, 25.29.

5.2.4. Compound *7*

To a solution of **1** (0.914 g, 0.616 mmol) and triethylamine (TEA, 3.4 ml, 24.6 mmol) in anhydrous DMF (100 ml) was added 4 Å molecular sieves (9 g), followed by PEG linker **6** (5 g, 0.123 mmol), and the resulting mixture was stirred at room temperature for 12 h. The solution was filtered through Celite and treated with ethyl alcohol (100 ml). The PEG derivative was precipitated after cooling to 0 °C, filtered and crystallized from DMF/ethyl alcohol (100/100 ml) to give **7** (4.0 g, 0.087 mmol, 71%).

5.2.5. Compound *8*

To a solution of **3** (23 g, 0.575 mmol) in anhydrous DCM (230 ml) and anhydrous DMF (23 ml), cooled to 0 °C, was added pyridine (0.7 ml, 8.9 mmol), and DSC (3.13 g, 12.2 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 12 h, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered, and crystallized from DMF/IPA (92/345 ml) to yield **8**

(21.9 g, 0.54 mmol, 94%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 168.12, 151.09, 70.70–67.95 (PEG), 45.19, 25.15.

*5.2.6. Compound **9***

To a solution of **8** (6.86 g, 0.169 mmol) and *tert*-butyl glycinate (0.227 g, 1.36 mmol) in anhydrous DCM (69 ml) cooled at 0 °C was added DMAP (0.165 g, 1.3 6 mmol), and the reaction mixture allowed to warm to room temperature and stirred for 12 h. The solution was filtered, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered and crystallized from IPA to yield **9** (6.11 g, 0.151 mmol, 89%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 168.71, 156.04, 81.34, 70.61–69.2 (PEG), 63.86, 45.32, 43.08, 27.90.

*5.2.7. Compound **10***

A solution of **9** (6 g, 0.148 mmol) in DCM (60 ml) and TFA (30 ml) was stirred at room temperature for 4 h, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered and washed with ethyl ether to yield **10** (5 g, 0.124 mmol, 84%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.54, 156.04, 70.46–68.20 (PEG), 45.30, 42.20.

*5.2.8. Compound **11***

To a solution of **10** (2.5 g, 0.0619 mmol), NHS (0.057 g, 0.495 mmol), and DIEA (0.26 ml, 0.743 mmol) in anhydrous DCM (20 ml) and anhydrous DMF (5 ml) cooled at 0 °C was added EDC (0.143 g, 1.49 mmol). The reaction mixture was warmed to room temperature and stirred for 12 h, followed by partial removal of the solvents under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered and crystallized from DMF/IPA (10/75 ml) to yield **11** (2.1 g, 0.052 mmol, 84%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 168.47, 165.93, 156.06, 70.3–69.88 (PEG), 64.15, 45.33, 40.31, 25.37.

*5.2.9. Compound **12***

To a solution of **1** (0.466 g, 0.313 mmol), TEA (1.75 ml, 12.6 mmol), and 4 Å molecular sieves (5 g) in anhydrous DMF (100 ml) was added PEG linker **11** (2.54 g, 0.063 mmol), and the resulting mixture stirred at room temperature for 12 h. The solution was filtered through Celite and the ethyl alcohol (100 ml) was added to the filtrate. The PEG derivative was precipitated after cooling to 0 °C, filtered and crystallized from DMF/ethyl alcohol (50/50 ml) to give **12** (1.4 g, 0.030 mmol, 49%).

*5.2.10. Compound **13***

To a solution of **1** (0.55 g, 0.37 mmol), TEA (2.06 ml, 14.8 mmol), and 4 Å molecular sieves (5.5 g) in anhydrous DMF (100 ml) was added PEG linker **8** (3.0 g, 0.074 mmol), and the resulting mixture stirred at room temperature for 5 h. The solution was filtered through Celite and ethyl alcohol (50 ml) was added to the filtrate. The PEG derivative was precipitated after cooling to 0 °C, filtered, and crystallized from DMF/ethyl alcohol (100/100 ml) twice to give **13** (2.5 g, 0.054 mmol, 74%).

*5.1.11. Compound **15***

To a solution of **14**[1] (5 g, 0.124 mmol), 2-(2-aminoethoxy) ethanol (0.25 ml, 2.48 mmol), and DMAP (0.303 g, 2.48 mmol) in anhydrous DCM (100 ml) cooled to 0 °C was added EDC (0.476 g, 2.48 mmol) and the reaction mixture warmed to room temperature and stirred for 12 h. The reaction solution was washed with 0.1 N HCl solution, the organic layer dried (anhydrous sodium sulfate) and filtered, followed by removal of the solvent under reduced pressure. The PEG derivative was crystallized from IPA to yield **15** (3.8 g, 0.093 mmol, 75%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.91, 170.54, 155.87, 71.05–69.11 (PEG), 68.84, 64.14, 61.31, 51.44, 39.29, 37.99.

*5.2.12. Compound **16***

To a solution of **15** (4.9 g, 0.121 mmol) in anhydrous DCM (500 ml) and anhydrous DMF (5 ml) cooled to 0 °C was added pyridine (0.16 ml, 1.93 mmol) and DSC (0.494 g, 1.93 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 12 h, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated by the addition of ethyl ether, filtered, and crystallized from IPA (320 ml) to give **16**. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.03, 170.57, 169.12, 155.98, 151.62, 70.96–70.00 (PEG), 64.35, 51.85, 39.47, 38.01, 25.69.

*5.2.13. Compound **17***

To a solution of **1** (0.465 g, 0.313 mmol) and TEA (1.75 ml, 12.5 mmol) in anhydrous DMF (100 ml) was added 4 Å molecular sieves (5 g), followed by PEG linker **16** (2.58 g, 0.063 mmol), and the resulting mixture stirred at room temperature for 12 h. The solution was filtered through Celite and treated with ethyl alcohol (100 ml). The PEG derivative was precipitated after cooling 0 °C, filtered, and crystallized from DMF/ethyl alcohol (100/100 ml) to give **17**.

5.3. Efficacy assay

Studies examining the in vivo anti-infective potential of PEG-vancomycin conjugates **7**, **12, 13** and **17** were performed in ICR mice (MDS Breeding Center, Taiwan, ROC). The PEG-vancomycin conjugates were administered intravenously in two equal split doses given 1 and 2.5 h following intraperitoneal inoculation of a $LD_{90–100}$ challenge of *S. aureus* (Smith). As controls, *S. aureus* (Smith) challenged mice were administered either saline or 100 mg/kg vancomycin given in two equal split doses. Ten mice were used per compound tested. Mice were observed for 1 week for survival. Significant anti-microbial activity was indicated by greater than 50% survival of the mice. The dose of vancomycin required for 50% survival of *S. aureus* (Smith) challenged mice was 0.3 mg/kg when given intraperitoneally (MDS Pharma Services, reference data).

5.4. Pharmacokinetics assay

To elucidate the circulatory pharmacokinetics of these PEG-vancomycin conjugates, rats were administered 50 mg/kg vancomycin equivalent doses of the tetramer conjugates (**7**, **12**, **13** and **17**) or native vancomycin (**1**). The compounds were administered as a single intravenous dose into the tail vein of the rat at a rate of 0.5 ml/min [~2.5 min]. Blood samples were obtained 72 h prior to treatment and at 0.08, 0.5, 1, 2, 4, 8, 24, 48, 72, and 96 h after compound administration. The rats were sedated with 30% O_2/70% CO_2 and bled 250 μl via the retro-orbital plexus into EDTA coated vials. Blood was immediately processed for plasma and frozen on dry ice. Plasma samples were stored at –80 °C until analyzed. The plasma samples were analyzed for the PEG-vancomycin conjugate by HPLC followed by mass spectrometer analysis (Version 4.1). The plasma pharmacokinetics for the conjugates was determined using a single compartment intravenous, first order elimination model employing WinNonlin software. The plasma concentration–time curves showed coefficients of determination (r^2) of greater than 0.86 for 4-arm carbamate conjugates **13** and linear branched amide **17** and > 0.95 for 4-arm amide conjugate **7** and 4-arm carbamate-amide conjugate **12**.

Acknowledgements

We wish to thank the Analytic and Biology Groups for their assistance and Carol Aitken for her help in the preparation of this manuscript. We also wish to thank Dr. Ying Gao for her technical assistance.

References

[1] R.B. Greenwald, H. Zhao, J. Xia, A. Martinez, J. Med. Chem. 46 (2003) 5021–5030.

[2] Y.H. Choe, C.D. Conover, D. Wu, M. Royzen, R.D. Greenwald, J. Control. Release 79 (2002) 41–53.

[3] R.B. Greenwald, C.W. Gilbert, A. Pendri, C.D. Conover, J. Xia, J. Med. Chem. 39 (1996) 424–431.

[4] C.D. Conover, R.B. Greenwald, A. Pendri, K.L. Shum, Anticancer Drug Des. 14 (1999) 499–506.

[5] Y. Tsukamoto, Y. Kato, M. Ura, I. Holii, H. Ishit Suka, H. Kusuhara, et al., Pharm. Res. 18 (2001) 1190–1202.

[6] R.B. Greenwald, Unpublished results.

[7] R.B. Greenwald, Y. Choe, J. McGuire, C.D. Conover, Adv. Drug Deliv. Rev. 55 (2000) 217–250.