Poly(ethylene glycol) Transport Forms of Vancomycin: A Long-Lived Continuous Release Delivery System

Richard B. Greenwald,* Hong Zhao, Jing Xia, and Anthony Martinez

Enzon Pharmaceuticals Inc., 20 Kingsbridge Road, Piscataway, New Jersey 00854

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The facile reaction of vancomycin with various PEG linkers, at the V_3 position, has been selectively accomplished by using an excess of base in DMF. Using rPEG as a blocking group for V_3 provides crystalline derivatives that can be further PEGylated to give pure V_3-X_1 latentiated species (transport forms). V_3 tetrameric species were also prepared in order to increase the loading of drug on PEG. All PEG−vancomycin transport forms show significant antibacterial activity that is on the same order of native vancomycin. Significant increases in the AUC were observed for all PEG−vancomycin conjugates thus making them potential single dose therapies.

Vancomycin (**1**) is a glycopeptide antibiotic that is the drug of choice for the treatment of Gram-positive infections caused by methicillin resistant *Staphylococcus aureus*.[1] It is also used in the treatment of bacterial infections in patients allergic to β-lactam antibiotics.[2] For the safe and effective use of this drug, quantitation of its levels in patient's blood is often required to maintain therapeutic levels,[3] and dosing is usually done by infusion every 6 h for prolonged periods of time (about 10 weeks) depending on the severity of the infection being treated. Several groups have attempted to enhance the performance of **1** by continuous infusion,[4−6] but it was concluded that no significant enhancement of therapeutic efficacy was realized. However, it was observed[6] that over a 10 day interval, the cost of treatment per patient could be reduced by 30% using the continuous infusion method.

To date, we are unaware of attempts to prepare long-lived amine-based prodrugs of vancomycin (Vanco) that could be utilized for limiting the number of infusions of the drug required by the patient. Vancomycin prodrugs would therefore serve to reduce associated nursing costs and avoid the use of inserting an infusion pump, which often can increase opportunistic infections at the implant site. By definition, prodrugs are formed by modification of a chemical functionality on a drug that is necessary for activity.[7] In the case of Vanco it is not clear if either of the V_3 or X_1 amino groups (Figure 1) is necessary for activity since the degree of activity observed can be altered in either direction by acylation of one of these moieties[8] and in part depends on the structure of the acylating agent as well.[9] Modification of both positions does, however, appear to lower activity.[9] The term latentiation was popularized in 1975 by Sincula and Yalkowsky[7] in order to incorporate the idea of drug release from a conjugated species regardless of the position of blockage. Irrespective of the type of labile connection used, in the case of high molecular weight PEG (>20 000) the conjugate could transport **1** throughout the circulatory system for extended periods of time before release. Thus, the objective of the present study was to prepare PEG latentiated conjugates of **1**, either as prodrugs or transport forms, with the hope of increasing the mean residence time of the drug in the circulatory system, thereby limiting the number of treatment infusions a patient would require. We were encouraged in this endeavor by the recent report[10] that a prodrug approach to increase the circulating half-life ($t_{1/2}$) of the antibiotic gentamycin was successfully carried out by conjugation of substituted Fmoc derivatives with the available amino sugars on the molecule and resulted in a continuous release of the native drug while maintaining equivalent activity. The toxicity issue of the sulfonated hydroxymethyl fluorene byproduct formed during gentamycin release was not addressed, but in the case of a PEG latentiated drug approach to delivery, only the drug and nontoxic PEG species[11] would be formed in vivo. Our aim then was to devise unambiguous syntheses of labile PEG conjugates of **1,** establish in vivo efficacy, and, if warranted, determine the rates of release (PK).

Chemistry

Previously conjugation of V_3 and X_1 to nonpolymeric species was accomplished by reaction of **1** with chloroformates,[12] acid anhydrides,[12,13] active esters,[9,14,15] and also carboxylic acids in the presence of condensing agents such as DCC[14,15] or PyBOP.[16] In all cases, mixtures of both mono- and disubstituted acylated products were obtained, but modulation of reaction conditions were reported to favor reaction of one position to a greater extent than the other. Isolation of pure species could then be accomplished by preparative HPLC. Thus, Adamczyk and co-workers[14] using DCC to condense carboxylic acids with **1** in DMSO were able to isolate X_1 monoacylated Vanco by preparative HPLC from an enriched mixture of products. Unfortunately, the direct condensation of PEG diacid[17] with **1** using the coupling reagents DCC, EDC, and DIPC in the presence of DMAP produced only low yields of mixtures that probably consisted of both mono- and diacylated Vanco. Substitution of triethylamine (TEA) for DMAP resulted in no reaction taking place.

* To whom correspondence should be addressed. Phone: (732) 980−4924. Fax: (732) 885−2950. E-mail: richard.greenwald@enzon.com.

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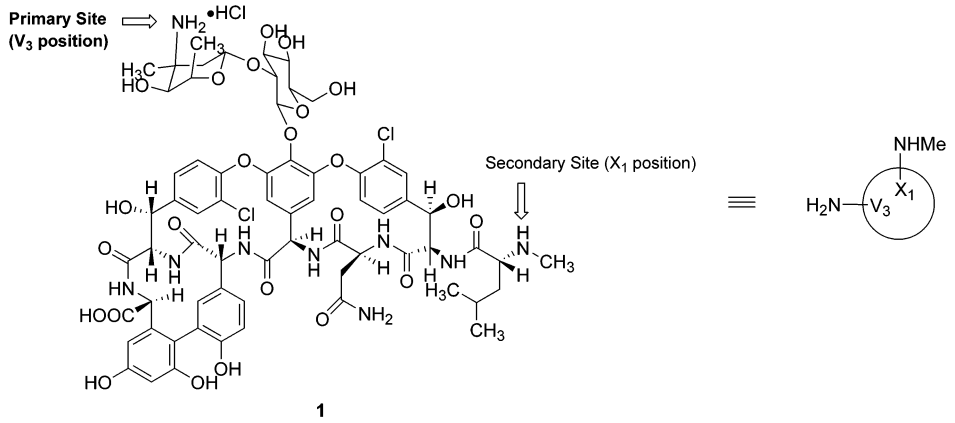
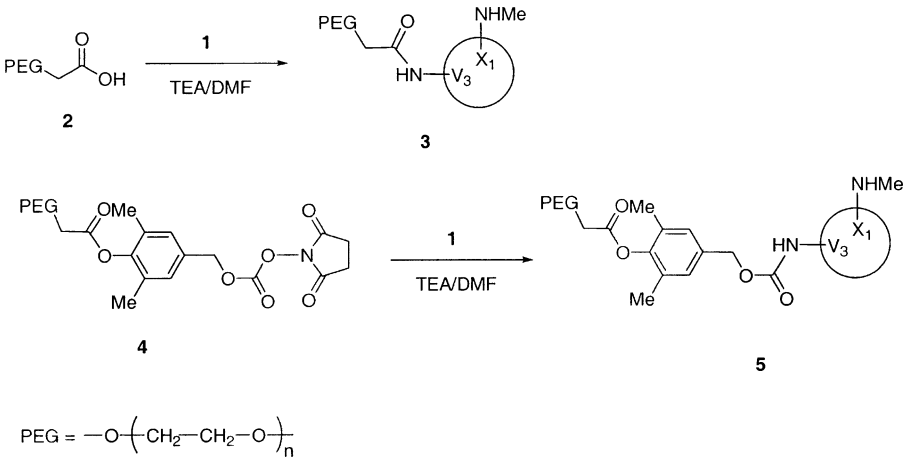


Figure 1.

Scheme 1

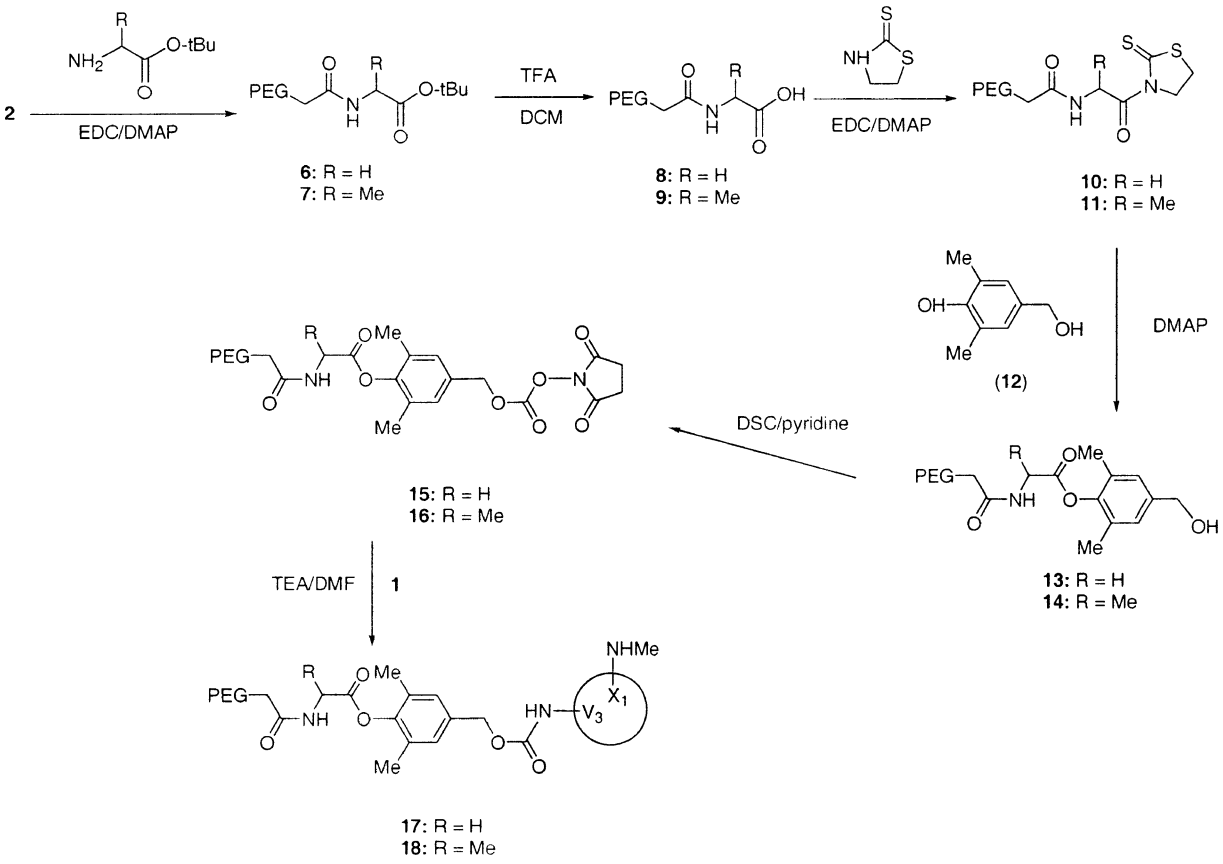


We have previously utilized 1,6-benzyl elimination (1,6-BE) as a means of releasing amino-containing bioactive substances from their PEG conjugates.[18] Application of this technology to Vanco would be flawed if more than one major conjugated product was obtained, for it is extremely difficult to separate mixtures of PEG conjugates of different molecular weights. Thus, it became of paramount importance to unearth reaction conditions that would clearly differentiate the V_3 and X_1 amino groups toward acylation not only for purification factors, but because the use of a bifunctional PEG linker could result in oligomerization.

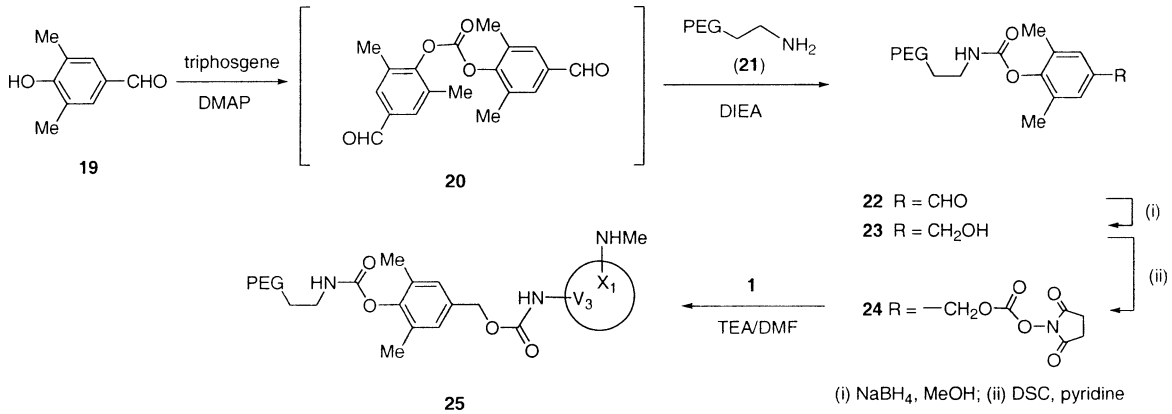
A systematic study of reaction conditions based on reported procedures[9,14,15] was undertaken to optimize reaction conditions for monofunctionalization of **1** using the succinimidyl carbonate disubstituted PEG 40 000 linker **4** (ester trigger, Scheme 1), **15** and **16** (amino acid spaced ester trigger, Scheme 2) and **24** (carbamate trigger, Scheme 3). These acylation reagents were prepared as shown in Schemes 1−4. Noteworthy in the synthesis of PEG linker **24** (Scheme 3) was the use of the novel carbonate reagent **20**.[19] This reagent was designed to provide higher yields of the PEGylated intermediate **22** than that provided by the reaction between **12** and PEG-NCO (also see ref 19 for R = H).

The coupling of PEG linker **4** to Vanco was investigated in depth, and it was first determined that the use of DMF as solvent was superior to other solvents previously employed with Vanco such as DMSO (or even mixtures of DMF−DMSO) for producing higher yields of conjugates. Next, it was found that the presence, and stoichiometry, of base was critical for maximization of yields. When DIEA or DMAP were employed in the reaction, low yields of disubstituted product resulted. Some monosubstitution was observed when pyridine was used as the base, but the most promising results were obtained when TEA was employed. The results gleaned from extensive investigation of using TEA in this reaction resulted in the determination that *at least 20 equiv of base* per Vanco molecule were required to optimize PEG conjugation. The use of less than 20 equiv resulted in incomplete reaction. It was also determined that NHS esters of PEG also reacted with Vanco in the presence of 20 equiv of TEA to yield the corresponding permanently bonded amide conjugate **3** in high yield (Scheme 1), but surprisingly the amine specific PEG linker, PEG-thiazolidine-2-thione (T-PEG),[18] was almost completely unreactive toward **1**. Using the favorable conditions established above for **1** with linkers **4**, **15**, **16**, and **24**, the resulting structures were determined by NMR spectroscopy to be **5**, **17**, **18**, and **25**, respectively. Thus selective monoacylation of the V_3 position by the various PEG 40 000 activated linkers had been achieved. In the ^{13}C NMR of the PEG derivatives (pyridine-d_5), the chemical shift (δ) of V_3 [C−NH_2] changed from 53.9 ppm to about 52 ppm. Meanwhile δ for X_1 [C−$NHCH_3$] remained intact at 62.5 ppm. The purity of these products was >95% as determined by HPLC and confirmed indirectly by GPC molecular weight determination: no higher molecular weight species were found to be present, which would be the case if the X_1 position had also reacted. With the

Scheme 2

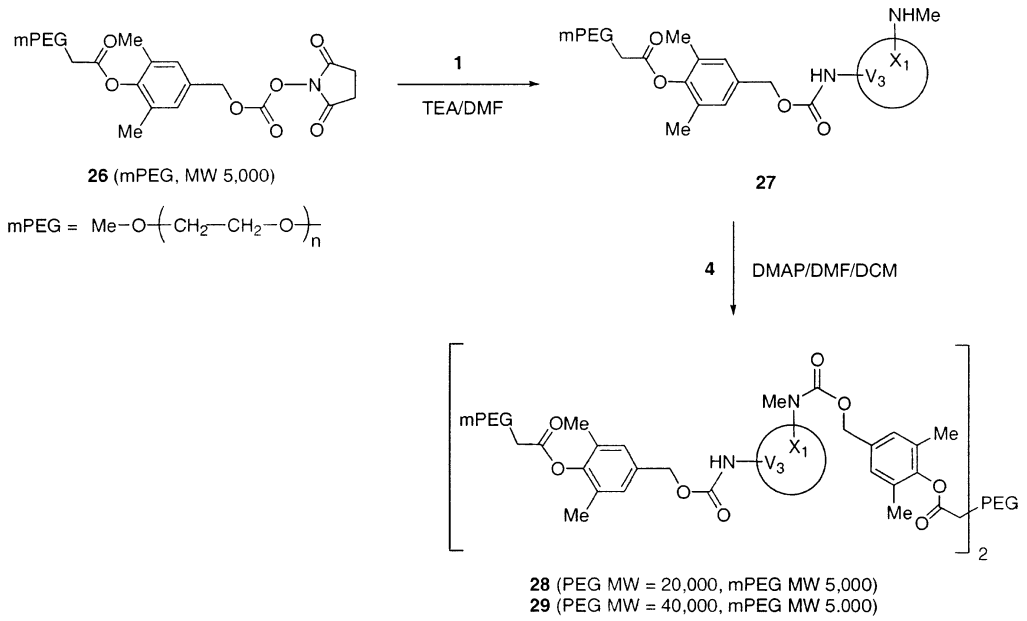


Scheme 3



knowledge that V_3 can be selectively acylated, we turned our attention to the quandary of X_1 position discrimination. It was apparent from some of our and others unsuccessful approaches,[9,15] that disubstituted product was formed during coupling and therefore both the V_3 and X_1 positions are reactive. Since the V_3 amino group can be selectively acylated, it followed that this is the more reactive of both positions under certain conditions, and in theory could be protected while conditions for the selected modification of the X_1 position were determined. Following the removal of the V_3 protecting group, monoacylated X_1 product theoretically could be isolated. It was initially found that the PEG 40 000 conjugate **5** has a much greater organic solubility than **1**, thus making this approach quite feasible. We chose to use an rPEG[19,20] as the protecting group since conditions for PEG conjugation were worked out and because the facility of the synthesis permitted modification of the trigger in order to differentiate release. Consequently, the methyl-capped rPEG linker **26** (molecular weight 5000),[20] reacted with **1** to produce the V_3 acylated derivative **27** as a crystalline solid that was, as in the case of the higher MW analogue, substantially more soluble in organic solvents than the parent. The utility of using low MW PEG in this capacity is underscored by the fact that attempts to prepare V_3 conjugates with small molecule blocking groups were not productive: the resulting conjugated compounds exhibited very poor solubility in both aqueous and organic solvents and often gelled. Compound **27** in turn was dissolved in DMF/DCM (1:1) as solvent and employing the acylation catalyst, DMAP, further reaction with the disubstituted PEG linker **4** (molecular weights 20 000 and 40 000) gave the desired symmetrically disubstituted acylated products, **28** and **29**, in excellent yield (94%) as shown in Scheme 4. It should be noted that both **28** and **29**

Scheme 4



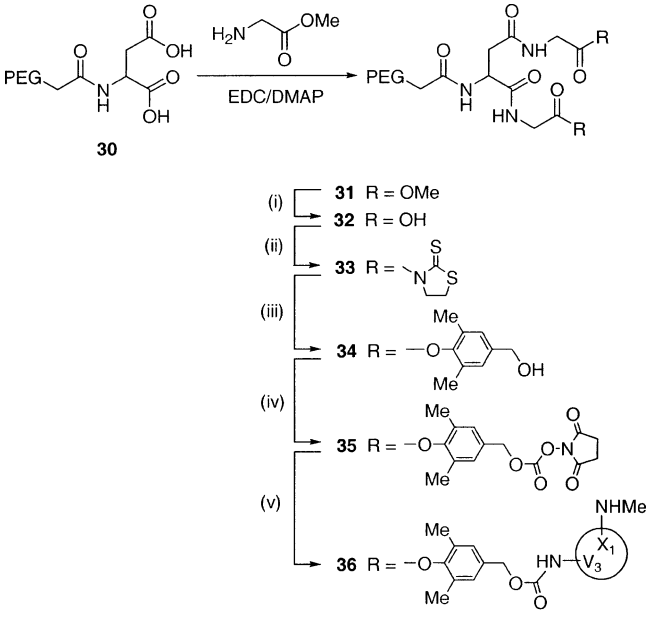
26 (mPEG, MW 5,000)

mPEG = $Me-O-(CH_2-CH_2-O)_n$

27

28 (PEG MW = 20,000, mPEG MW 5,000)
29 (PEG MW = 40,000, mPEG MW 5.000)

Scheme 5



30

(i) LiOH; (ii) EDC, DMAP, 2-mercaptothiazoline; (iii) **12,** DMAP; (iV) DSC, pyridine; (v) **1**, TEA, DMF

are necessarily doubly latentiated transport systems. Since the objective of modification of either the V_3 and X_1 positions is to adjust the PK of latentiated Vanco, then a double substitution should be a satisfactory solution for that objective if it could be shown that Vanco is regenerated from **28** and **29** under physiological conditions. By using this concept, a difficult separation of mPEG 5000 from an X_1 PEG 40 000 conjugate can be avoided. This view will be discussed in greater detail in the Discussion section.

To decrease the volume of formulated drug at a given concentration, resulting in decreased solution viscosity, as previously discussed in the case of ara-C,[21] it was necessary to increase the loading of **1** onto the polymer. Thus, tetrameric V_3-PEG transport forms were prepared using the known PEG scaffolding based on aspartic acid as shown in Scheme 5.[22] For instance conjugation of the activated tetraacid derivative **33** (Scheme 5) with **12** followed by activation of the benzyl alcohol moiety with NHS produced the tetrasubstituted 1,6-BE linker, **35**. Reaction of 1 equiv of **35** with 4 equiv of **1**, as shown in Scheme 5, gave the desired V_3 substituted tetramer **36** with a loading of four vancomycins per PEG as determined by UV analysis.[17,18] In this fashion, the % active of Vanco was increased to about 10% (theoretical, 12%).

Similarly, a tetrameric Vanco V_3 PEG carbamate derivative, **43**, was prepared as shown in Schemes 6.

Experimental Section

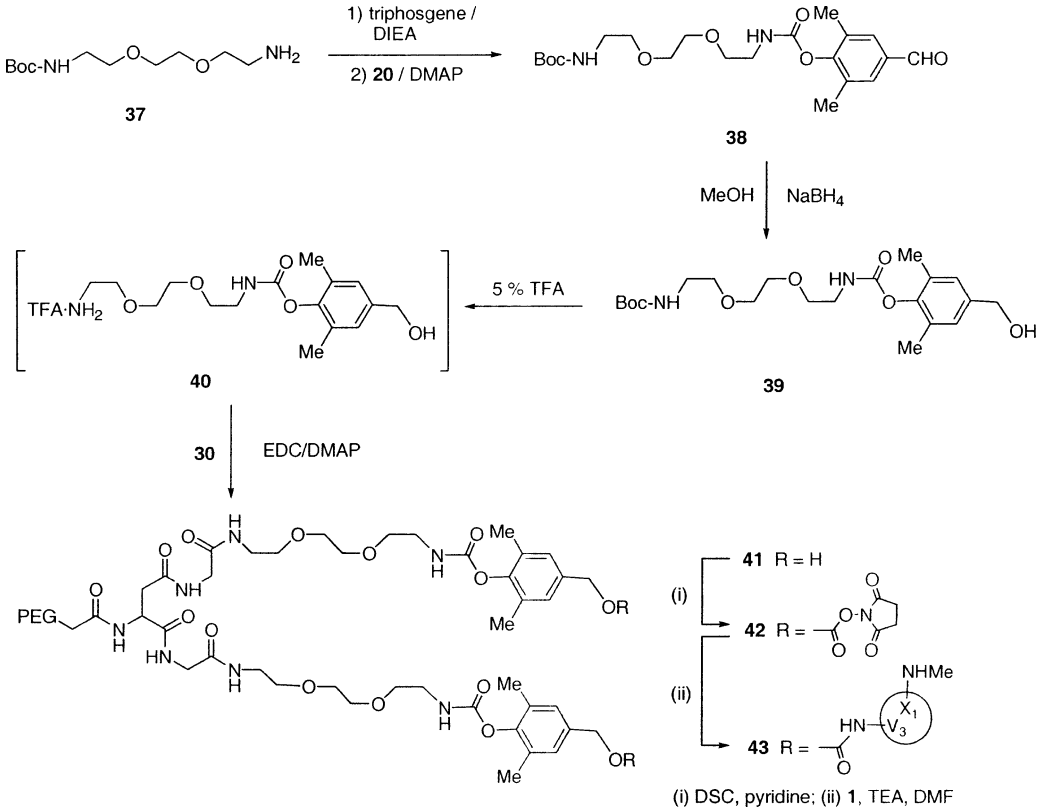
General Procedures. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation from toluene prior to use. Poly(ethylene glycol) (PEG) linkers: compounds **4**, **26**, and **30** were made according to the published procedures.[17−20] ^{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).

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 multiwavelength UV detector, using a gradient of 10−90% of acetonitrile in 0.05% trifluoroacetic acid (TFA) at a flow rate of 1 mL/min.

Kinetic Studies in Rat Plasma. PEG−vancomycin (either 20 mg of 40 kDa dimer conjugate, 10 mg of 20 kDa dimer conjugate or 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 (8 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 was again added 400 μL of AcCN and MeOH (1:1, v/v), and the vial was 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.

Experimental Procedures for the Syntheses of Vancomycin−PEG Derivatives. Compound 5. To a solution of

Scheme 6



compound **1** (0.535 g, 0.369 mmol) and triethylamine (TEA, 1.026 mL, 7.36 mmol) in anhydrous dimethylformamide (DMF, 150 mL) was added PEG linker **4** (7.5 g, 0.184 mmol), and the resulting mixture was stirred at room temperature for 12 h. The solution was filtered through Celite and treated with ethyl ether (300 mL). Filtration gave crude product which was recrystallized from a mixture of DMF/TEA/2-propanol (IPA) (45 mL/1 mL/180 mL) twice to give **5** (7.61 g, 0.175 mmol, 95%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 174.44, 172.32, 170.65, 169.15, 168.74, 167.02, 159.02, 157.39, 155.37, 142.98, 142.09, 133.22, 130.37, 128.45, 127.32, 119.79, 118.28, 107.79, 102.76, 98.54, 90.25, 88.38, 78.93, 77.34, 76.41, 64.41, 62.58, 61.65, 60.71, 58.87, 56.97, 54.39, 53.35, 35.64, 25.29, 24.23, 23.48, 22.32, 18.46, 16.35, 12.08.

Compound 6. To a solution of 40 kDa PEG diacid (**2**, 22.0 g, 0.548 mmol), glycine *tert*-butyl ester hydrochloride (1.10 g, 6.58 mmol), and 4-(dimethylamino)pyridine (DMAP, 1.60 g, 13.16 mmol) in anhydrous methylene chloride (DCM, 200 mL) cooled to 0 °C in an ice bath was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 1.26 g, 6.58 mmol). This 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 with addition of ethyl ether, filtered, and crystallized from 2-propanol (IPA, 440 mL) to yield **6** (20.8 g, 0.515 mmol, 94%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 169.92, 168.38, 70.36−69.78 (PEG), 40.85, 27.68.

Compound 7. To a solution of **2** (25.0 g, 0.623 mmol), alanine *tert*-butyl ester hydrochloride (1.36 g, 7.48 mmol), and DMAP (1.82 g, 14.95 mmol) in anhydrous DCM (300 mL) cooled to 0 °C in an ice bath was added EDC (1.44 g, 7.48 mmol). This 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 (500 mL) to yield **7** (20.8 g, 0.515 mmol, 94%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.19, 168.78, 71.05−69.32 (PEG), 47.62, 27.64, 18.10.

Compound 8. A solution of **6** (20.0 g, 0.496 mmol) in DCM (200 mL) and trifluoroacetic acid (TFA, 100 mL) was stirred at room temperature for 2 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 **8** (18.2 g, 0.451 mmol, 91%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.07, 170.00, 72.91−67.29 (PEG), 39.99.

Compound 9. A solution of **7** (23.0 g, 0.570 mmol) in DCM (250 mL) and TFA (150 mL) was stirred at room temperature for 2 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 **9** (22.6 g, 0.559 mmol, 98%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.67, 168.98, 71.02−69.83 (PEG), 46.89, 17.93.

Compound 10. To a solution of **8** (17.0 g, 0.423 mmol), 2-mercaptothiazoline (2-MT, 0.20 g, 1.69 mmol), and DMAP (0.21 g, 1.69 mmol) in anhydrous DCM (150 mL) cooled to 0 °C was added EDC (0.32 g, 1.69 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 (340 mL) to yield **10** (16.5 g, 0.410 mmol, 97%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 200.96, 170.22, 170.01, 72.13−69.72 (PEG), 55.35, 45.58, 28.67.

Compound 11. To a solution of **9** (22.5 g, 0.558 mmol), 2-MT (0.266 g, 2.24 mmol), and DMAP (0.27 g, 2.24 mmol) in anhydrous DCM (250 mL) cooled to 0 °C was added EDC (0.43 g, 2.24 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 with the addition of ethyl ether, filtered, and crystallized from IPA (450 mL) to yield **11** (21.3 g, 0.525 mmol, 94%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 200.41, 174.12, 168.92, 72.93−69.34 (PEG), 56.00, 48.03, 28.45, 17.85.

Compound 13. A solution of **10** (16.0 g, 0.40 mmol), 3,5-dimethyl-4-hydroxybenzyl alcohol (0.78 g, 5.14 mmol), and DMAP (0.63 g, 5.14 mmol) in anhydrous DCM (150 mL) was refluxed for 18 h, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated with addition of ethyl ether, filtered, and crystallized from IPA to yield **13** (14.6 g, 0.36 mmol, 90%). ^{13}C NMR (67.8 MHz,

$CDCl_3$) δ 170.53, 167.47, 146.49, 138.80, 129.52, 126.61, 72.14−69.54 (PEG), 63.68, 39.96, 15.89.

Compound 14. A solution of **11** (12.4 g, 0.30 mmol), **12** (0.78 g, 5.14 mmol), and DMAP (0.63 g, 5.14 mmol) in anhydrous DCM (150 mL) was refluxed for 18 h, followed by partial removal of the solvent under reduced pressure. The PEG derivative was precipitated with addition of ethyl ether, filtered, and crystallized from IPA to yield **14** (11.8 g, 0.29 mmol, 95%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.00, 169.38, 146.36, 138.71, 129.45, 126.62, 72.93−69.06 (PEG), 63.80, 47.30, 17.73, 16.04.

Compound 15. To a solution of **13** (3.8 g, 0.094 mmol) and disuccinimidyl carbonate (DSC, 0.19 g, 0.75 mmol) in anhydrous DCM (40 mL) and DMF (4 mL) cooled to 0 °C was added pyridine (0.059 g, 0.75 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 linker was precipitated by the addition of ethyl ether, filtered, and crystallized from DCM/ethyl ether to yield **15** (3.3 g, 0.082 mmol, 87%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.52, 168.15, 167.24, 151.06, 148.02, 130.55, 130.42, 128.47, 71.83−69.64 (PEG), 40.12, 25.18, 16.05.

Compound 16. To a solution of **14** (9.0 g, 0.222 mmol) and DSC (1.14 g, 4.44 mmol) in anhydrous DCM (90 mL) and DMF (9 mL) cooled to 0 °C was added pyridine (0.351 g, 4.44 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 linker was precipitated by addition of ethyl ether, filtered, and crystallized from DCM/ethyl ether to yield **16** (6.5 g, 0.158 mmol, 71%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.05, 169.64, 168.20, 151.18, 148.07, 130.67, 130.51, 128.65, 71.98−69.93 (PEG), 47.48, 25.30, 17.82, 16.21.

Compound 17. Prepared in 88% yield by reacting **1** with **15** as described for **5**. ^{13}C NMR (67.8 MHz, C_5D_5N) δ 174.68, 171.17, 168.62, 167.14, 159.00, 157.50, 155.50, 142.00, 133.50, 130.51, 128.38, 127.24, 78.93, 65.22, 63.76, 62.49, 54.39, 52.20, 44.61, 43.29, 35.52, 25.23, 24.20, 23.46, 22.30, 18.47, 16.37.

Compound 18. Prepared in 83% yield by reacting **1** with **16** as described for **5**. ^{13}C NMR (67.8 MHz, C_5D_5N) δ 174.80, 171.46, 170.77, 144.20, 130.84, 128.75, 127.59, 119.00, 79.10, 78.38, 64.95, 62.92, 61.99, 54.69, 52.31, 51.38, 48.89, 35.85, 25.57, 24.52, 23.78, 22.65, 18.75, 16.79, 15.27.

Compound 22. To a solution of **19** (0.73 g, 4.89 mmol) and triphosgene (0.21 g, 0.70 mmol) in 100 mL of dry DCM cooled to 15 °C was added diisopropylamine (DIEA, 1.02 mL) dropwise over a period of 5 min. The reaction mixture was then allowed to warm to room temperature and stirred for 1 h to give compound **20**,[19] which was used without further purification. The reagents, 40 kDa PEG diamine hydrochloride (7.0 g, 0.175 mmol) and DIEA (0.061 mL) were added to the above solution, and the resulting mixture was stirred for 18 h at room temperature 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 **22** (5.6 g, 0.138 mmol, 79%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 190.69, 162.10, 130.31, 128.21, 124.05, 123.57, 72.11−69.49 (PEG), 39.92, 15.72.

Compound 23. To a solution of **22** (5.5 g, 0.14 mmol) in methanol (70 mL) cooled to 15 °C was added sodium borohydride (0.018 g, 0.45 mmol). The reaction mixture was allowed to warm to room temperature over a period of 2 h, followed by adjusting the pH to 6.5 with 1 N HCl. The solvent was removed under reduced pressure and the residue taken up in water. The pH was lowered to 2.0 with 0.5 N HCl and the product extracted from the water with DCM. The organic layer was dried over anhydrous sodium sulfate and filtered, followed by partial removal of the solvent under reduced pressure. The product was precipitated by addition of ethyl ether, filtered, and washed with ethyl ether to yield **23** (4.7 g, 0.12 mmol, 85%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 153.52, 130.12, 124.12, 121.33, 73.67−69.51 (PEG), 65.47, 39.91, 15.86.

Compound 24. To a solution of **23** (4.0 g, 0.99 mmol) and DSC (0.41 g, 1.60 mmol) in anhydrous DCM (40 mL) and DMF (4 mL) cooled to 0 °C was added pyridine (0.125 g, 1.60 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 linker was precipitated by addition of ethyl ether, filtered, and crystallized from DCM/ethyl ether to yield **24** (3.45 g, 0.85 mmol, 86%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 169.44, 151.34, 128.14, 126.65, 123.56, 72.38−68.90 (PEG), 41.52, 25.08, 15.79.

Compound 25. Prepared in 86% yield by reacting **1** with **24** as described for **5**. ^{13}C NMR (67.8 MHz, C_5D_5N) δ 174.44, 172.41, 170.65, 168.74, 166.18, 158.76, 157.45, 154.24, 152.74, 152.10, 148.09, 137.54, 128.77, 127.30, 90.29, 88.42, 78.84, 77.37, 76.43, 63.50, 62.66, 61.65, 60.74, 58.87, 56.99, 55.16, 54.20, 53.35, 52.81, 51.39, 35.64, 26.12, 25.26, 23.51, 22.30, 18.47, 17.43, 14.00.

Compound 27. Prepared in 91% yield by reacting **1** with **26** as described for **5**.

Compound 28. A solution of **4** (mw 20664, 4.44 g, 0.215 mmol), **27** (mw 5332, 2.73 g, 0.410 mmol), and DMAP (0.251 g, 2.05 mmol) in a mixture of anhydrous DCM:DMF (50 mL:50 mL) was stirred at room temperature for 12 h. The PEG derivative was precipitated by the addition of ethyl ether (300 mL) and filtered, and the residue was recrystallized from a mixture of ethanol:DMF:TEA (150 mL:150 mL:3.0 mL) twice to give **28** (6.82 g, 0.202 mmol, 94%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 174.68, 171.94, 170.51, 168.73, 168.62, 162.35, 155.42, 141.02, 133.16, 132.06, 130.69, 130.37, 130.08, 128.45, 127.29, 78.08, 72.5−69.0 (PEG), 65.24, 63.77, 58.88, 54.48, 35.89, 31.02, 26.21, 25.28, 24.18, 23.46, 22.33, 18.29, 16.35.

Compound 29. A solution of **4** (mw 40,664, 3.84 g, 0.0945 mmol), **27** (mw 5332, 1.20 g, 0.180 mmol), and DMAP (0.251 g, 2.05 mmol) in a mixture of anhydrous DCM:DMF (60 mL:60 mL) was stirred at room temperature for 12 h. The PEG derivative was precipitated by the addition of ethyl ether (300 mL), filtered, and the residue was crystallized from a mixture of ethanol:DMF:TEA (150 mL:150 mL:1.2 mL) to give **29** (6.82 g, 0.202 mmol, 94%).

Compound 31. To a solution of **30** (30.0 g, 0.744 mmol), glycine methyl ester hydrochloride (0.75 g, 5.95 mmol), and DMAP (1.8 g, 14.75 mmol) in DCM (300 mL) cooled to 0 °C was added EDC (1.71 g, 8.92 mmol), and the mixture was allowed to warm to room temperature and stirred overnight, followed by partial removal of the solvent in vacuo. The product was precipitated with ethyl ether, collected by filtration, and crystallized from IPA (600 mL) to give **31** (29.2 g, 97%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 170.37, 170.18, 169.82, 169.57, 169.31, 70.61−69.93 (PEG), 51.78, 48.87, 40.84, 40.80, 36.77.

Compound 32. A solution of **31** (29 g, 0.71 mmol) and lithium hydroxide hydrate (0.24 g, 5.71 mmol) in water (200 mL) was stirred overnight at room temperature and the pH adjusted to 2.5 with 0.1 N HCl. This solution was extracted with DCM and the organic layer dried (anhydrous sodium sulfate) and filtered, followed by partial removal of the solvent under reduced pressure. The product was precipitated using ethyl ether, collected by filtration, and crystallized from IPA (640 mL) to give **32** (27.4 g, 94%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 170.44, 170.32, 170.11, 169.86, 72.16−69.78 (PEG), 61.26, 49.22, 41.09, 37.30.

Compound 33. To a solution of **32** (5.0 g, 0.123 mmol), 2-MT (0.18 g, 1.51 mmol), and DMAP (0.36 g, 2.96 mmol) in anhydrous DCM (50 mL) cooled to 0 °C was added EDC (0.28 g, 1.48 mmol) and stirred overnight, followed by partial removal of the solvent under reduced pressure. The product was precipitated with ethyl ether, collected by filtration, and crystallized from IPA (100 mL) to yield **33** (4.6 g, 92%). ^{13}C NMR (67.8 MHz, C_5D_5N) δ 200.74, 200.71, 170.29, 170.17, 169.85, 169.64, 72.09−68.39 (PEG), 55.39, 48.92, 46.32, 36.67, 28.72.

Compound 34. A solution of **33** (4.5 g, 0.11 mmol), 3,5-dimethyl-4-hydroxybenzyl alcohol (0.35 g, 2.87 mmol), and DMAP (0.53 g, 2.87 mmol) in anhydrous DCM (50 mL) was refluxed for 18 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 (90 mL) to yield **34** (4.1 g, 91%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.69, 170.38, 169.96, 167.36, 167.06, 148.39, 138.72, 129.46, 126.56, 72.15−69.17 (PEG), 63.77, 49.01, 40.76, 36.83, 16.02.

Compound 35. To a solution of **34** (4.0 g, 0.097 mmol) and DSC (0.80 g, 3.11 mmol) in anhydrous DCM (40 mL) and DMF (4 mL) cooled to 0 °C was added pyridine (0.25 g, 3.11 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 linker was precipitated by the addition of ethyl ether, filtered, and crystallized from DCM/ethyl ether to yield **35** (2.8 g, 70%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.73, 170.43, 169.97, 168.17, 167.18, 166.86, 151.03, 147.96, 130.55, 130.38, 128.42, 71.78−69.78 (PEG), 49.05, 40.78, 36.92, 25.15, 15.98.

Compound 36. To a solution of **1** (0.353 g, 0.238 mmol) and TEA (0.662 mL, 4.75 mmol) in anhydrous DMF (50 mL) was added **35** (2.2 g, 0.0528 mmol), and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through Celite and the product precipitated with ethyl ether (300 mL). Filtration gave crude product which was recrystallized twice from a mixture of DMF/ethanol (1:1) to give **36** (2.0 g, 90%). HPLC purity, 98% (method described in the General Procedures).

Compound 38. To a solution of **37 (**4.0 g, 16.1 mmol) and triphosgene (1.92 g, 6.46 mmol) in anhydrous DCM (50 mL) cooled to 15 °C was added DIEA (7.6 mL, 43.6 mmol) dropwise over a period of 5 min. This mixture was allowed to warm to room temperature over a period of 1 h, followed by the addition of **20** (1.97 g, 16.13 mmol) and DMAP (2.4 g, 16.1 mmol), and then stirred at room-temperature overnight. The mixture was washed with 0.1 N HCl solution, the organic layer dried (anhydrous sodium sulfate) and filtered, and the solvent removed from the filtrate under reduced pressure to give crude **38**. ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 191.18, 155.64, 152.99, 152.82, 133.42, 132.06, 129.76, 78.99, 41.00, 40.17, 28.21, 16.12.

Compound 39. To a solution of **38** (1.0 g, 2.36 mmol) in methanol (30 mL) cooled to 15 °C was added sodium borohydride (0.1 g, 2.63 mmol), and the reaction mixture was stirred at room temperature for 1 h, followed by acidification with 0.1 N HCl solution. The solvent was removed from the filtrate in vacuo, and the residue was taken up in water (20 mL) and extracted with DCM. The organic layer was dried (anhydrous sodium sulfate) and filtered and the solvent removed under reduced pressure. The crude product was purified by silica gel chromatography to yield **39** (0.9 g, 90%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 155.65, 153.95, 146.81, 138.01, 130.33, 126.56, 78.86, 63.89, 40.69, 39.96, 28.09, 15.92.

Compound 41. To a solution of **39** (0.9 g, 2.11 mmol) in DCM (57 mL) was added trifluoroacetic acid (3 mL), and the reaction mixture was stirred for 1.5 h at room temperature followed by removal of the solvent in vacuo. The residue was dissolved in anhydrous DCM (70 mL), and to this solution was added **30** (7.4 g, 0.18 mmol) and DMAP (1.26 g, 10.3 mmol). The mixture was cooled to 0 °C followed by the addition of EDC (0.56 g, 2.93 mmol). The solution was allowed to warm to room temperature and stirred overnight followed by partial removal of the solvent under reduced pressure. The product was precipitated with ethyl ether, collected by filtration, and crystallized from DCM/ethyl ether to yield **41** (6.45 g, 85%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.34, 170.23, 169.82, 153.96, 147.06, 146.91, 138.39, 138.02, 130.61, 130.55, 126.79, 72.30−69.29(PEG), 64.35, 64.26, 49.17, 39.19, 39.05, 16.16.

Compound 42. To a solution of **41** (6.4 g, 0.154 mmol) and DSC (1.26 g, 4.92 mmol) in a mixture of anhydrous DCM (120 mL) and DMF (12 mL), cooled to 0 °C, was added pyridine (0.39 g, 4.92 mmol), and the mixture was allowed to warm to room temperature and stirred overnight. The solvent was partially removed under reduced pressure and the product precipitated with ethyl ether, filtered, and crystallized from DCM/ethyl ether to give **42** (5.8 g, 89%). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.26, 169.91, 168.25, 153.59, 151.15, 148.51, 131.31, 130.04, 128.53, 72.21−68.18(PEG), 49.40, 40.96, 39.18, 37.19, 25.29, 16.11.

Compound 43. This was prepared in 79% yield by reacting **1** with **42** as described for **36**. HPLC purity 98% (method described in the General Procedures).

Methods and Results

LD_{90-100} Examination of PEG−Vancomycin Conjugates 5, 17, 18, 25, 28, 29, 36, and 43 in ICR Swiss Mice. Studies examining the in vivo anti-infective potential of PEG−vancomycin conjugates in ICR Swiss mice were performed. In the initial study, PEG−vancomycin conjugates **5**, **17**, **18**, **25**, **28**, **29**, **36**, and **43** were administered at 100 mg/kg vancomycin equivalents in two equal split doses given 1 and 2.5 h following a LD_{90-100} inoculation of *Staphylococcus aureus* (Smith) to ICR Swiss mice. As controls, *S. aureus* (Smith)-challenged mice were administered either saline or 100 mg/kg vancomycin given in equal split doses. Ten mice were used per compound tested. Mice were observed for 1 week for survival. Significant antimicrobial activity was indicated by greater than 50% survival of the mice. (Table 4).

A second study was also performed to examine the sustained anti-infective protection of PEG−vancomycin conjugate **18**. ICR Swiss mice were administered with either 100 mg/kg vancomycin equivalents of **18**, 100 mg/kg vancomycin, or saline in two equally split doses 1.5 h apart. Ten mice per treatment group were used. Mice were challenged with an LD_{90-100} inoculum of *S. aureus* (Smith) 8 h after initial administration of test compounds. Mice were observed for survival as before.

The studies showed that PEG−vancomycin conjugates **5**, **17**, **18**, **25**, **28**, **29**, **36**, and **43** when administered at 100 mg/kg vancomycin equivalents showed significant activity against *S. aureus*-challenged mice. Likewise, the PEG−vancomycin conjugate **18** showed sustained 8 h antimicrobial protection against *S. aureus* challenge in ICR Swiss mice and significant antimicrobial activity with 80% survival of the mice. Native vancomycin treatment and saline control showed no protection with 0% survival. All the data are summarized in Table 4.

Pharmacokinetics of Vancomycin (1) and PEG−Vancomycin Conjugates 17, 18, 25, 28, 29, 36, and 43 in Rats. This study was performed to determine the circulatory pharmacokinetics of PEG−vancomycin conjugates (**17**, **18**, **25**, **28**, and **29**) in rats. PEG−vancomycin conjugates containing a 50 mg/kg vancomycin equivalent dose and 50 mg/kg native vancomycin (**1**) were injected as a single intravenous dose into the tail vein of conscious rats 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. A 250 μL bleed was done on rats sedated with 30% O_2/70% CO_2 via the retro-orbital plexus into EDTA containing 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 free vancomycin by a fluorescence polarization immunoassay using an Abbott Laboratories TD_x analyzer within 2 h of thawing. A single compartment intravenous, first-order elimination model using WinNonlin software was used to determine the plasma pharmacokinetic parameters for conjugates **1**, **28**, and

Table 1. Properties of PEG−Vancomycin Conjugates (V_3 Dimers)

compd	mw	% of Vanco×*bb*	solubility[b] (mg/mL)	stability in saline (24 h), %[c]	$t_{1/2}$ (rp) (h)	$t_{1/2}$ (hp) (h)
2	42936	6.75	128	≪1	≫24	- -
5	43332	6.60	131	<3	2	6.6
17	42669	6.56	145	<2	19	27
18	43457	6.10	122	<2	16	>24
25	43275	5.15	141	<1	>24	≫24

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

Table 2. Properties of PEG−Vancomycin Conjugates (X_1-Substituted V_3 Dimers)

compd	mw	% of Vanco	solubility[b] (mg/mL)	stability in saline (24 h)[c]	$t_{1/2}$ (rp) (h)	$t_{1/2}$ (hp) (h)
28	33764	8.80	180	<1	4.6	32
29	53764	5.39	128	<1	5.0	22

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

29 and a two-compartment intravenous, first-order elimination model for conjugates **17**, **18**, **25**, **36**, and **43**. The plasma concentration−time curves showed coefficients of determination (r^2) of greater than 0.96 for all PEG−vancomycin conjugates and 1.00 for vancomycin itself.

In this study, vancomycin showed a circulatory half-life ($t_{1/2}$) of 0.34 h with a C_{max} of 162 μg/mL and an area under the curve (AUC) of 78.8 h·μg/mL. Vancomycin had a clearance (CL) of 642 mL/h/kg and a volume of distribution at a steady state (V_{ss}) of 309 mL/kg in rats.

Overall the pharmacokinetic estimates of the PEG−vancomycin conjugates uniformly showed a longer sustained, although lower concentration, of circulating free vancomycin in rat plasma. All of the PEG−vancomycin conjugates showed a 12 to 64-fold longer $t_{1/2}$ (4.08 h to 21.9 h). All of the conjugates achieved C_{max} values that were 16% to 66% (26 μg/mL to 107 μg/mL) of those observed for unmodified vancomycin. The AUC for the PEG−vancomycin conjugates were 3.6−27.7 fold greater (282.2 h·μg/mL to 2184 h·μg/mL) than that observed for vancomycin and had concomitantly slower clearance rates (71 mL/h/kg to 293.9 mL/h/kg) that were 11% to 45% of vancomycin's. The V_{ss} of the PEG−vancomycin conjugates were 3−9-folds greater (938 mL/kg − 2723 mL/kg) than that observed for vancomycin. The PK profile is summarized in the following Table 5.

Discussion

After completion of the synthesis of the various latentiated disubstitued 40 000 mw PEG V_3 derivatives of **1** as described above, the physical properties of these derivatives were determined and are listed in Table 1. Very little hydrolysis occurs for all derivatives in saline (pH 7.0) thus enabling stable formulations to be prepared prior to use. However, the rate of decomposition ($t_{1/2}$) in rat plasma for the PEG transport forms varies significantly from 1 to >24 h: a similar trend is observed in the case of human plasma (Tables 1−3). But, as shown in Table 4, efficacy for all the PEG conjugates is virtually equipotent to that of Vanco itself.

It can be seen that 5−6 wt % of **1** is present in the PEG 40 000 conjugates, as shown in Table 2. Substitution of PEG 20 000 which provides higher loading (8.8%, compound **28**, Table 2) results in a very poor PK profile

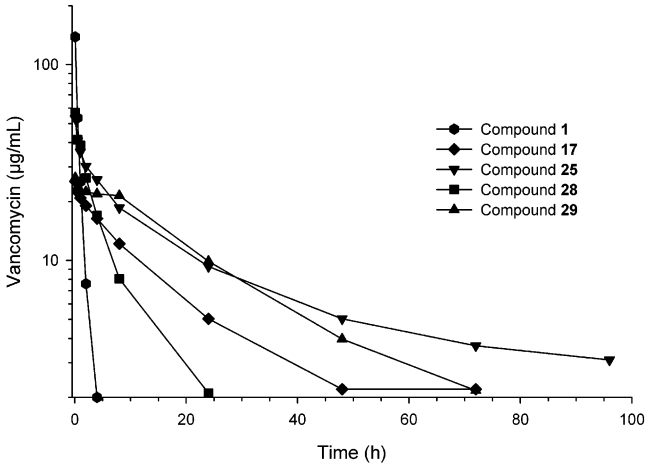


Figure 2. Vancomycin plasma concentration−time curve (dimers). Rats were administered 50 mg/kg of compound intravenously and bled 250 μL at 5 min, 1, 2, 4, 8, 24, 38, 72, and 96 h via retro-orbital plexus. Plasma was analyzed for vancomycin concentration by a fluorescent polarization immunoassay.

Table 3. Properties of PEG−Vancomycin Conjugate (V_3 Tetramer)

compd	mw	% of Vanco	solubility[b] (mg/mL)	stability in saline (24 h)[c]	$t_{1/2}$ (rp) (h)	$t_{1/2}$ (hp) (h)
36	47011	9.06	120	<2%	20	>24
43	47483	10.4	141	<1%	36	≫24

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

Table 4. Compound Description and Summary of LD_{90-100} Results

compound	post[a] challenge % survival	prechallenge % survival
saline	10	0
1	100	0
5	100	- -
17	100	- -
18	100	80[b]
25	90	- -
28	100	- -
29	80	- -
36	100	- -
43	100	- -

[a] Compound given 1h following *S. aureus* (Smith) challenge, survival after 1 week. [b] Compound given 8 h prior to *S. aureus* (Smith) challenge, survival after 1 week (single experiment).

since its CL is the highest observed in the study (Table 5, Figure 2). This may be the result of more rapid renal and endoreticular clearance which is known to occur as the MW of PEG changes.[23]

Compound **18,** with a 4-fold greater AUC than **1**, was chosen at random for a prechallange experiment. Particularly noteworthy is that **18**, with an in vitro $t_{1/2}$ of 16 h (PK $t_{1/2}$ 4.1 h) shows significant activity (80% survival) when given 8 h prior to bacterial challenge (*S. aureus*), while native **1** demonstrates 0% survival.

A comparison of the similar conjugates **17** (gly ester trigger) and **18 (**ala ester trigger**)**, which have very similar in vitro properties, shows a dissimilar in vivo plasma $t_{1/2}$. This may be due to an enzyme specific cleavage of the alanine derivative. Conjugate **25** (PEG 40 kDa, carbamate trigger,V_3 substitution) decomposes due to the base-catalyzed hydrolysis of a phenolic carbamate[18] and results in a $t_{1/2}$ of 9.1 h and gives rise

Table 5. Pharmacokinetics of Intravenous Bolus Administration of 50 mg/kg Vancomycin Equivalents of PEG−Vancomycin Conjugates in Rats

compound	C_{max} (μg/mL)	plasma $t_{1/2}$ (hr)	CL (mL/h/kg)	V_{ss} (mL/kg)	AUC (h·μg/mL)
1	162.0 ± 9.0	0.34 ± 0.04	642.0 ± 83.7	309.3 ± 17.2	78.8 ± 10.8
17	25.5 ± 2.1	10.48 ± 0.58	131.1 ± 18.2	2723.3 ± 97.3	386.4 ± 51.4
18	47.9 ± 4.3	4.09 ± 0.25	178.3 ± 13.4	2640.8 ± 23.9	282.2 ± 28.5
25	54.6 ± 2.5	9.12 ± 0.98	70.0 ± 5.4	1648.3 ± 81.5	716.9 ± 56.8
28	53.7 ± 5.4	2.23 ± 0.38	293.9 ± 26.1	938.0 ± 94.3	171.0 ± 15.2
29	24.8 ± 7.7	21.19 ± 6.90	70.9 ± 6.3	2128.6 ± 560.1	708.4 ± 61.0
36	29.3 ± 5.1	12.37 ± 2.18	97.8 ± 7.3	3598 ± 462	513 ± 40
43	107.1 ± 16.5	14.2 ± 1.40	23.0 ± 2.1	1341 ± 52.5	2184 ± 189

to the largest AUC found for dimeric PEG−vanco latentiated derivatives in this study (Table 5, Figure 2). Pharmacokinetic (PK) values for most of the conjugates were determined as described in the Methods and Results section and are shown in Table 5. Examination of the PK data indicate an impressive increase in circulating half-life and a much larger AUC for all PEG conjugates.

Conjugation (blocking) of theV_3 position with **26,** gave latentiated **27 (**α-alkoxyester trigger**),** which was further functionalized at the X_1 position with a PEG 40 000 to yield the Vanco transport form, **29** (double ester trigger, X_1-protected with V_3 substitution). This conjugate has a very similar AUC as that found for **25** and an extremely long plasma $t_{1/2}$ (21.2 h, Table 5, Figure 2). The conjugate **29** while providing an excellent PK profile (Table 5) carries only about 5% of **1** because of the increase in MW. Note that an approximate $t_{1/2}$ for X_1 PEG conjugates can be arrived at by comparing the V_3 substituted PEG 40 000 compound **5**, $t_{1/2}$ =2 h, with **29 (**V_3 protection and X_1 substitution), $t_{1/2}$ = 5 h, (Tables 1 and 2). Fortunately, since **29** had a longer $t_{1/2}$, then it follows that this will also be the rate-determining step in the breakdown of the X_1 derivative itself. Thus, there is no need to remove the V_3 blocking PEG group in order to investigate an X_1 latentiated Vanco derivative-especially in light of the fact that the lower MW PEG (5,000) can hydrolyze either at the same or more rapid rate, but never at a slower rate.[17]

Increased loading adjustments of the PEG 40 000 conjugates were therefore done by preparing tetramers whose water solubilities are still adequate for formulation (Table 3). For a decreased dosing schedule to be perfected for the various conjugates, the variables to be considered would include a combination of high loading, low C_{max} (no spiking), and long $t_{1/2}$. Generally, it is far simpler to directly synthesize the V_3 substituted compounds; therefore further conversions to the more highly loaded tetrameric forms were based on this consideration. From Table 5 it is clear that the two dimeric compounds with the greatest AUC, longest $t_{1/2}$, and lowest CL were **17** and **25**. Also from inspection of Figure 2 detectable amounts of **1** could still be observed to be greater than 2 μg/mL for both conjugates after 100 h. On the basis of these factors the tetrameric latentiated conjugate, **36** analogous to **17** was synthesized as shown in Schemes 5. The tetrameric compound **36**, utilizing a glycine ester trigger with a loading of 9% Vanco, had an AUC of 513, a reasonable $t_{1/2}$ of 12.4 h, and a relatively low C_{max} of 29.3 μg/mL. A second tetramer, **43** (analogous to **25**), with a 10% loading of **1**, as expected (**43**) had a large AUC of >2000 (Table 3, Figure 3).

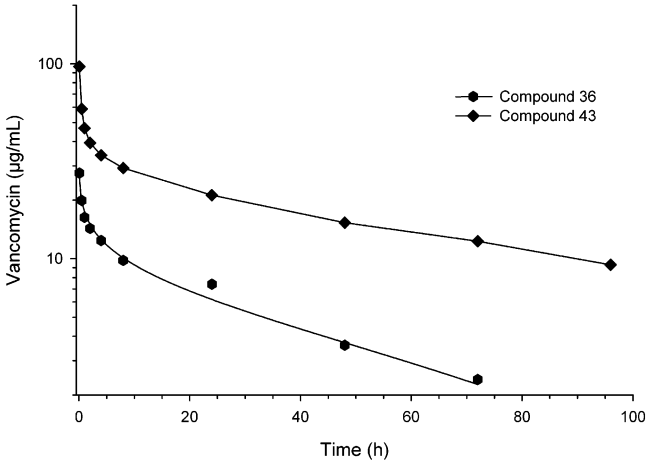


Figure 3. Vancomycin plasma concentration−time curve (tetramers). Rats were administered 50 mg/kg of compound intravenously and bled 250 μL at 5 min, 1, 2, 4, 8, 24, 48, 72, and 96 h via retro-orbital plexus. Plasma was analyzed for vancomycin concentration by fluorescent polarization immunoassay.

Conclusions

Exclusive V_3 conjugation of PEG with Vanco can be done in DMF as solvent with a large excess of TEA. Although the same selectivity could not be demonstrated for the X_1 position, it was found that low MW PEG could be used effectively as a blocking group at the V_3 position, which then allowed PEG conjugation of the X_1 position to take place. Furthermore, doubly latentiated PEG−Vanco derivatives (V_3-X_1 substitution) had a greater $t_{1/2}$ than the V_3 conjugates, thus demonstrating that hydrolysis of X_1 in the V_3-X_1 transport form is in fact the rate controlling step in the plasma kinetic study. Therefore, the PK profile of V_3-X_1 is essentially equivalent to X_1 substituted derivatives alone. The more highly loaded V_3 tetramer is preferable for further development since the synthetic routes are more facile than the preparation of the double latentiated forms. The current protocols for Vanco administration gives a rapid peak concentration (spike) that is rapidly cleared from the bloodstream and thus results in the need for mutidosing regimens. PEG−Vanco transport forms may provide a novel and useful form of Vanco delivery, because slow release of Vanco from these conjugates can be used in place of continuous infusion type therapies. This approach now offers a drug delivery system that is convenient, efficacious, less painful to the patient and at dramatically reduced costs. Predosing experiments have clearly demonstrated that while native **1** has no effect under these conditions, latentiated **1** provides short range protection from future bacterial exposure.

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References

(1) Kirby, W. M. M. Vancomycin therapy in severe staphyloccal infections. *Rev. Infect. Dis.* **1981**, *3*, 5236−5239.
(2) Ingerman, M. J.; Santoro, J. Vancomycin a new old agent. *Infect. Dis. Clin. North Am.* **1989**, *3*, 641−651.
(3) Pryka, R. D.; Rodveld, K. A.; Erdman, S. M. An updated comparison of drug dosing methods. IV. Vancomycin. *Clin. Pharmacokinet.* **1991**, *20*, 463−476.
(4) James, J. K.; Palmer, S. M.; Levine, D. P.; Rybac, M. J. Comparison of conventional dosing versus continuous-infusion vancomycin therapy for patients with suspected or documented gram-positive infections. *Antimicrob. Agents and Chemother.* **1996**, *40*, 696−700.
(5) Klepser, M. E.; Patel, K. B.; Nicolau, D. P.; Quintiliani, R.; Nightingale, C. H. Comparison of bactericidal activities of intermittent and continuous infusion dosing of vancomycin against methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis*. *Pharmacotherapy* **1998**, *18*, 1069−1074.
(6) Wysocki, M. et al. Continuous versus intermittent infusion of vancomycin in severe Staphyloccal infections: Prospective multicenter randomized study. *Antimicrob. Agents Chemother.* **2001**, *45*, 2460−2467.
(7) Sinkula, S. S.; Yalkowsky, S. H. Rationale for design of biologically reversible drug derivatives: Prodrugs. *J. Pharm. Sci.* **1975**, *64*, 181−210.
(8) Pavlov, A. Y. Preobrazhenskaya. Chemical modification of glycopeptide antibiotics [VC1]. *Russian J. Bioorg. Chem*. (Eng. Version). **1998**, *24*, 570−587.
(9) Nagarajan, R.; Schabel, A. A.; Occolowitz, J. L.; Counter, F. T.; Ott, J. L. Synthesis and antibacterial activity of N-acyl vancomycins. *J. Antibiot.* **1988**, XLI, 1430−1438.
(10) Schecter, Y.; Tsubery, H.; Fridkin, M. *J Med. Chem*. **2002**, *45*, 4264−4270.
(11) Pang, S. J. Final report on the safety assessment of poly(ethylene glycol) (PEGs). *J. Am. College Toxicol.* **1993**, *12*, 429.
(12) Kannan. R.; Harris, C. M.; Harris, T. M.; Waltho, J. P.; Skelton, N. J.; Williams, D. H. Function of the amino sugar and N-terminal amino acid of the antibiotic vancomycin in its complexation with cell wall peptides. *J. Am. Chem. Soc.* **1988**, *110*, 2946−2953.
(13) Perkins, H. R. Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. *Biochem. J.* **1969**, *111*, 195−205.
(14) Adamczyk, M.; Grote, J.; Moore, J. A.; Rege, S. D.; Yu, Z. Structure-binding relationships for the interaction between a vancomycin monoclonal antibody fab fragment and a library of vancomycin analogues and tracers. *Bioconjugate Chem*. **1999**, *10*, 176−185.
(15) Ghosh, M.; Miller, M. J. Synthesis and in vitro antibacterial activity of spermidine-based amixed catechol- and hydroxamate-containing siderophore-vancomycin conjugates. *Bioorg. Med. Chem*. **1996**, *4*, 43−48.
(16) Staroske, T.; Williams, D. H. Synthesis of covalent head-to-tail dimers of vancomycin. *Tetrahedron Lett.* **1998**, *39*, 4917−4920.
(17) Greenwald, R. B.; Gilbert, C. W.; Pendri, A.; Conover, C. D.; Xia, J.; Martinez, A. Drug delivery systems: Soluble taxol 2′-poly (ethylene glycol) ester progroup − design and in vitro effectiveness. *J. Med. Chem*. **1996**, *39*, 424−431.
(18) Greenwald, R. B.; Pendri, A.; Conover, C. D.; Zhao, H.; Choe, Y. H.; Martinez, A.; Shum, K.; Guan, S. Drug delivery systems employing 1,4-or 1,6-elimination: poly (ethylene glycol) prodrugs of amine containing compounds. *J. Med. Chem*. **1999**, *42*, 3657−3667.
(19) Greenwald, R. B.; Yang, Y.; Zhao, H.; Conover, C. D.; Lee, S.; Filpula, D. Controlled release of proteins from their poly-(ethylene glycol) conjugates: Drug delivery systems employing 1,6-elimination. *Bioconjugate Chem*. **2003**, *14*, 395−403.
(20) Lee, S.; Greenwald, R. B.; McGuire, J.; Yang, K.; Shi, C. Drug delivery systems employing 1,6-elimination: releasable poly-(ethylene glycol) conjugates of proteins. *Bioconjugate Chem*. **2001**, *12*, 163−169.
(21) Choe, Y. H.; Conover, C. D.; Wu, D.; Royzen, M.; Greenwald, R. B. Anticancer drug delivery systems: N^4-acyl poly (ethylene glycol) prodrugs of ara-C. I. Efficacy in solid tumors. *J. Controlled Release* **2000**, *79*, 41−53.
(22) Choe, Y. H.; Conover, C. D.; Wu, D.; Royzen, M.; Gervacio, Y.; Borowski, V.; Mehlig, M.; Greenwald, R. B. Anticancer drug delivery systems: Multi-loaded N^4-acyl poly (ethylene glycol) prodrugs of ara-C. II. Efficacy in ascites and solid tumors. *J. Controlled Release* **2000**, *79*, 55−70.
(23) Yamaoka, T.; Tabata, Y.; Ikada, Y. Distribution and tissue uptake of poly (ethylene glycol) with different molecular weights after intravenous administration to mice. *J. Pharm. Sci.* **1994**, *83*, 601−606.

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