Reconstruction of Vancomycin by Chemical Glycosylation of the Pseudoaglycon

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Vancomycin (Figure 1) is a glycopeptide antibiotic that kills cells by binding to the D-Ala-D-Ala peptide substrate involved in cross-linking the sugar polymers that comprise the bacterial cell wall.[1] The carbohydrate portion of vancomycin is not directly involved in binding to D-Ala-D-Ala. However, it has been shown by scientists at Lilly that N-alkylation of the terminal vancosamine sugar with a hydrophobic group increases activity against vancomycin resistant strains dramatically.[2,3] Despite the importance of this carbohydrate in biological activity, no efforts to replace the vancosamine with a different sugar have been reported.[4] In fact, as far as we know, the chemical glycosylation of vancomycin at any position has never been achieved.[5,6] Vancomycin is a complex molecule which has a diverse array of functionality and is sensitive to acid, base, and oxidation.[7] Furthermore, it is soluble only in water and other polar solvents, which are not compatible with chemical glycosylation reactions. We now report a strategy for glycosylating the pseudoaglycon of vancomycin. This chemistry should permit the synthesis of large numbers of vancomycin derivatives in which the terminal carbohydrate moiety is varied.

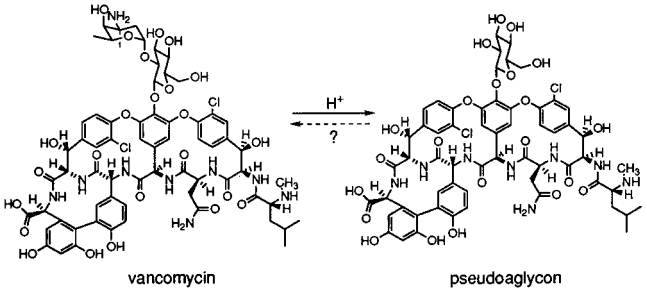
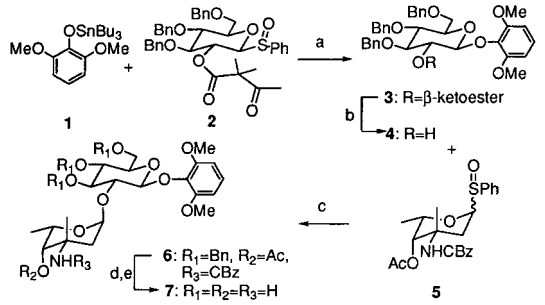


Figure 1.

We initiated our studies to develop chemistry to glycosylate vancomycin by synthesizing the vancomycin disaccharide. This structure was first synthesized by Danishefsky in 1992 and more recently by Nicolaou.[8] The disaccharide contains a β-linkage to a hindered phenol as well as an α-linkage to a 2-deoxy sugar. We used 2,6-dimethoxyphenol as a model for the hindered phenol in vancomycin.[8] Our synthesis utilized the sulfoxide glycosylation reaction[9] and proved to be both stereospecific and efficient (Scheme 1). The equatorial linkage to the 2,6-dimethoxy phenol was formed in 92% isolated yield by reacting the tin salt of the phenol[10] with a suitably protected sulfoxide **2**.[11] Stereochemical control was achieved with neighboring group participation by a β-ketoester protecting group at C2. This bulky neighboring group prevents ortho ester formation, and yet it can be readily deprotected using mild conditions (i.e., hydrazine).[12] Following the selective removal of the β-ketoester group, the resulting compound **4** was glycosylated on the free C2 hydroxyl with vancosamine derivative **5.**[13] The axial isomer was produced exclusively in 71% yield, and the disaccharide was then deprotected in two steps in an overall yield of 67%.[14] The synthesis of the vancomycin disaccharide confirmed the utility of the sulfoxide method for introducing the axial glycosidic linkage to the terminal vancosamine using protecting groups that can be removed under mild conditions compatible with the integrity of the complicated aglycone. Extending this chemistry to the synthesis of vancomycin from the pseudoaglycon, however, proved to be considerably more challenging.

Scheme 1



[a] (a) 1 equiv **1**, 2 equiv **2**, 2 equiv Tf_2O, 4 equiv DTBMP, EtOAc, −78 °C, 5 min, 92%. (b) 2.5% H_2NNH_2, MeOH/THF = 2:1, 3 h, 85%. (c) 2 equiv **5**, 1 equiv Tf_2O, 4 equiv DTBMP, Et_2O, −78 °C, 0.5 h, 71%. (d) 2% H_2NNH_2, MeOH/THF = 2:1, 8 h, 92%. (e) H_2, Pearlman's catalyst, MeOH, 1 h, 73%.

(1) Walsh, C. T.; Fisher, S. L.; Park, I.-S.; Prahalad, M.; Wu, Z. *Chem. Biol.* **1996**, *3*, 21.

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(3) Malabarba, A.; Nicas, T. I.; Thompson, R. C. *Med. Res. Rev.* **1997**, *17*, 69.

(4) However, vancosamine has been converted to epi-vancosamine.[3]

(5) The vancomycin aglycon has been enzymatically glycosylated: Solenberg, P. J.; Matsushima, P.; Stack, D. R.; Wilkie, S. C.; Thompson, R. C.; Baltz, R. H. *Chem. Biol.* **1997**, *4*, 195.

(6) A great deal of synthetic effort has been focused on the vancomycin aglycon: (a) Evans, D. A.; Barrow, J. C.; Watson, P. S.; Ratz, A. M.; Dinsmore, C. J.; Evrard, D. A.; Devries, K. M.; Ellman, J. A.; Rychnovsky, S. D.; Lacour, J. *J. Am. Chem. Soc.* **1997**, *119*, 3419. (b) Nicolaou, K. C.; Ramanjulu, J. M.; Natarajan, S.; Brase, S.; Li, H.; Boddy, C.; Rubsam, F. *Chem. Commun.* **1997**, 1899. (c) Boger, D. L.; Beresis, R. T.; Loiseleur, O.; Wu, J. H.; Castle, S. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 721. (d) For a review of work up to 1995, see: Rama Rao, R. V.; Gurjar, M. K.; Reddy, K. L.; Rao, A. S. *Chem. Rev.* **1995**, *95*, 2135.

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(9) (a) Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881. (b) Kim, S.-H.; Augeri, D.; Yang, D.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 1766. (c) Raghavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 1580. For recent studies on the mechanism of the sulfoxide glycosylation reaction see: (d) Crich, D.; Sun, S. *J. Am. Chem. Soc*. **1997**, *119*, 11217.; (e) Gildersleeve, J.; Pascal, R. A.; Kahne, D. *J. Am. Chem. Soc*. **1998**, *120*, 5961.

(10) Ogawa, T.; Matsui, M. *Carbohydr. Res*. **1976**, *51*, C13.

(11) (a) 3,4,6-Tri-O-Bn glucose[11b] is converted to **2** in six steps (66% overall). Acylation (Ac_2O, pyridine, CH_2Cl_2, 6h, rt), thiophenol displacement ($BF_3{\cdot}Et_2O$, PhSH, CH_2Cl_2, 2h, rt) and acetate hydrolysis (NaOH/MeOH, 3h, rt) gives 3,4,6-tri-O-Bn glucose phenyl sulfide. Transesterification[11c] with ethyl-2-methyl acetoacetate (DMAP, toluene, reflux, 48 h), alkylation (MeI, potassium-*tert*-butoxide, THF, 0°, 0.5 h), and oxidation (mCPBA, CH_2Cl_2, −60° to −5°, 1 h) gives **2**. (b) Betaneli, V. I.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285. (c) Taber, D. F.; Amedio, J. C.; Patel, Y. K. *J. Org. Chem.* **1985**, *50*, 3618.

(12) Less-hindered esters often lead to ortho ester side products: (a) Kunz, H.; Harreus, A. *Liebigs Ann. Chem.* **1982**, 41. (b) Sato, S.; Nunomura, S.; Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *33*, 4097. (c) Seeberger, P. H.; Eckhardt, M.; Gutteridge, C. E.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064.

(13) **5** was obtained from vancomycin in 5 steps (48% overall). Protection of vancomycin amines (Cbz-succinimide, $NaHCO_3$, H_2O, dioxane, rt, 4 h) followed by hydrolysis (1.5 M methanolic HCl, rt, 0.5 h), acylation (Ac_2O, pyridine, CH_2Cl_2, rt, 2 h), thiophenol displacement (PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0.5 h, rt), and oxidation (mCPBA, CH_2Cl_2 −78° to −20°, 1 h) gives **5**.

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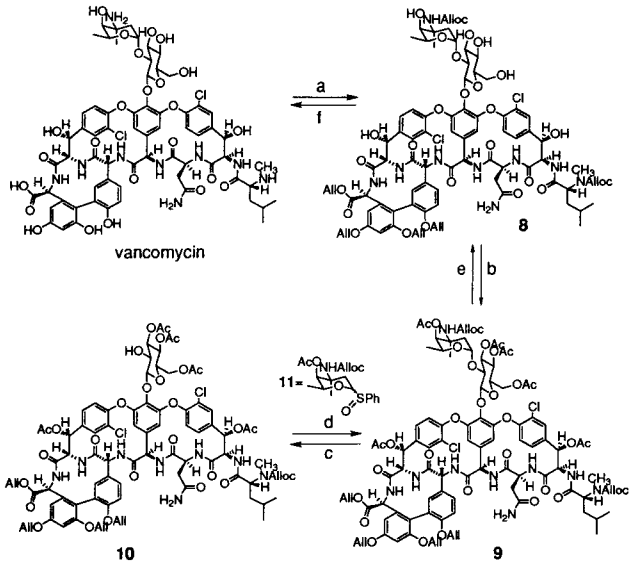
Scheme 2[a]



(a) (1) 4 equiv alloc-succinimide, 3 equiv $NaHCO_3$, H_2O/dioxane, 3 h; (2) 5 equiv allyl bromide, 2 equiv $NaHCO_3$, DMF, 2 h; (3) 10 equiv allyl bromide, 5 equiv Cs_2CO_3, DMF, 6 h, 82%, three steps. (b) 18 equiv Ac_2O, 36 equiv pyridine, 0.2 equiv DMAP, CH_2Cl_2, 5 h, 91%. (c) 30 equiv $BF_3{\cdot}Et_2O$, 10 equiv PhSH, CH_2Cl_2 rt, 0.5 h, 71%. (d) 2 equiv $BF_3{\cdot}Et_2O$, 2 equiv Tf_2O, CH_2Cl_2, then 4 equiv **11**, Et_2O, −78 °C to −20 °C, 1 h, 64% plus 23% recovered **10**. (e) 5% hydrazine, allyl alcohol/MeOH/THF = 1:1:1, 4 h, 63%. (f) 50 equiv Bu_3SnH, 1 equiv $PdCl_2(PPh_3)_3$, DMF/AcOH = 1:1, 10 min, 78%.

Glycosylation required a suitably protected vancomycin pseudoaglycon. This compound had to have protecting groups that would confer solubility in nonpolar organic solvents at −78 °C. These protecting groups had to be removable under conditions that would not damage the final product. After considerable experimentation, we established an efficient degradation route to **10** (Scheme 2) by sequentially protecting the various functional groups in vancomycin according to their reactivities, with the two amines acylated first with alloc-succinimide followed by alkylation of the carboxylic acid and then the three free phenols with allyl bromide. Allyl-derived protecting groups were selected for all of these functional groups because they can be removed cleanly in one step with $PdCl_2(PPh_3)_3$-Bu_3SnH.[15] Finally, the primary and five secondary hydroxyls were protected as acetates. The glycosidic linkage to the vancosamine was then selectively cleaved with BF_3-thiophenol to produce **10**.

Initial efforts to glycosylate the protected pseudoaglycon **10** using sulfoxide **11**[16] and triflic anhydride in the presence of base (DTBMP) yielded an unencouraging mixture of products, with the major compound having a mass consistent with loss of water from the glycosyl acceptor.[17] One of the limitations of the sulfoxide glycosylation reaction has been that it often does not proceed cleanly in systems involving amides. We believe that amide oxygens interfere with glycosylation by reacting with triflic anhydride and/or with the activated glycosyl donor. It seemed possible, therefore, that the observed loss of water could result from conversion of the primary asparagine amide to the nitrile upon treatment with triflic anhydride.[18] A model study using Cbz-Asn-OBn demonstrated that treatment with triflic anhydride and base at −78 °C cleanly produced the corresponding nitrile (80% yield).

We thought it might be possible to suppress the dehydration of primary amides in the presence of triflic anhydride by pretreating amide-containing compounds with BF_3 and leaving out the base. In fact, in the Cbz-Asn-OBn model system no nitrile was produced when BF_3 was added prior to triflic anhydride. Furthermore, a single disaccharide product (**9**) was isolated in 64% yield (along with 23% of the recovered nucleophile) when the protected pseudoaglycon was treated with 2 equiv of BF_3 prior to adding Tf_2O and donor **11**.[19] Following a two-step deprotection, the glycosylated material was found to be identical to vancomycin by ^{1}H NMR, HPLC, and mass spectral analysis.[20]

The chemistry reported above extends the utility of the sulfoxide glycosylation reaction to amide-containing systems. We have been able to achieve the first chemical glycosylation of the vancomycin pseudoaglycon, possibly the most complex system that has yet been glycosylated using a chemical approach. Our next goal is to develop chemistry to construct the glycosidic linkage to the phenol on the vancomycin aglycon, a considerable challenge in its own right. Accomplishing this objective is essential to completing the total synthesis of vancomycin.[6] In the meantime, the present work permits further exploration of the role of the carbohydrate portion of vancomycin in antibiotic activity, particularly in conferring activity against resistant microorganisms.

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(14) Spectral data for **7**: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.03 (t, J = 8.2 Hz, 1 H), 6.68 (d, J = 9.2 Hz, 2 H), 5.39 (d, J = 4.0 Hz, V_{H-1}, 1 H), 5.16 (J = 7.6 Hz, G_{H-1}, 1 H), 4.55−4.52 (m, V_{H-5}, 1 H), 3.81 (s, OMe, 6 H), 3.68−3.60 (m, G_{H-2}, $G_{H-6'}$, G_{H-6}, 3 H), 3.51 (t, J = 9.1 Hz, G_{H-3}, 1 H), 3.42 (t, J = 9.5 Hz, G_{H-4}, 1 H), 3.20 (s, V_{H-4}, 1 H), 3.14−3.11 (m, G_{H-5}, 1 H), 2.04−1.93 (m, V_{H-2}, $V_{H-2'}$, 2 H), 1.65 (s, CH_3, 3 H), 1.07 (d, J = 6.7 Hz, CH_3, 3 H); ^{13}C NMR (CD_3OD, 500 MHz) δ 154.90, 134.00, 125.66, 107.30, 101.95, 98.59, 79.50, 79.27, 78.17, 73.29, 71.65, 64.97, 62.62, 56.91, 55.60, 35.14, 23.68, 17.08. HRMS (FAB): calcd for $C_{21}H_{33}NNaO_{10}$ [M + Na$^+$] 482.1991, found 482.2002.

(15) Dangles, O.; Guibe, F.; Balavoine, G.; Lavielle, S.; Marquet, A. *J. Org. Chem.* **1987**, *52*, 4984.

(16) **11** was prepared in the same way as **5**, substituting alloc-succinimide for Cbz-succinimide.

(17) The IR specrum of the major product shows a weak stretch at 2248 cm^{-1}.

(18) Dehydration of amides upon treatment with triflic anhydride and pyridine has been reported: Charette, A. B.; Chua, P. *Synlett* **1998**, 163.

(19) To produce **9**, **10** (22.4 mg, 0.0127 mmol) was azeotroped 3× with toluene, dissolved in 1 mL of CH_2Cl_2 and cooled to −78 °C. BF_3Et_2O (2.3 μL, 0.0191 mmol) was added followed by triflic anhydride (4.3 μL, 0.0255 mmol). Sulfoxide **11** (20 mg, 0.0509 mmol) in 0.5 mL of CH_2Cl_2 was then added dropwise over 1 min, and the reaction was allowed to warm to −25 °C over 1.5 h. After the reaction was quenched with a 1:1 solution of MeOH/DIEA (100 μL), the solvent was removed under vacuum, and the residue was chromatographed over silica gel (10 mm × 5 cm column; 20 mL of 100% $CHCl_3$; 20 mL of 2.5% MeOH/$CHCl_3$; 20 mL of 5% MeOH/$CHCl_3$). Impure product (25 mg) was obtained and further purified by reverse-phase HPLC (PHENOMENEX LUNA C18 column, 21 × 25 mm, 5 μM particle size) using a linear gradient (80% CH_3CN/0.1% CH_3COOH/19.9% H_2O to 99.9% CH_3CN/0.1% CH_3COOH over 30 min). **9** (16.5 mg, 64%) was obtained along with 5 mg of recovered **10** (23%). R_f = 0.3 (5% MeOH/$CHCl_3$). HRMS (FAB): calcd for $C_{98}H_{111}N_9NaO_{34}Cl_2$ [M + Na$^+$] 2050.6508, found 2050.6458.

(20) Synthetic vancomycin coeluted with authentic vancomycin on reverse-phase analytic HPLC using a PHENOMENEX PRODIGY 5 μm ODS(3) 100 Å column (250 × 4.6 mm), eluting with 0.1% TFA in water for 2 min followed by a linear gradient of 0.1% TFA in water to 20% CH_3CN/0.1% TFA in water over 28 min. Vancomycin elutes at 27.9 min. Mass: [M + H]$^+$, 1449.3, [M − V + H] $^+$, 1305.2, [M − V − G + H] $^+$, 1144.3.