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A New and Improved Method for Deglycosidation of Glycopeptide Antibiotics Exemplified with Vancomycin, Ristocetin, and Ramoplanin

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Abstract—A general method for the deglycosidation of glycopeptide antibiotics has been developed. Treatment of vancomycin, ristocetin, and ramoplanin with anhydrous HF results in efficient cleavage of the sugars to provide the corresponding aglycons in high yield.

In the course of several studies addressing the synthesis of glycopeptide antibiotics that act by inhibiting bacterial cell wall biosynthesis, we had the occasion to access their peptide aglycons. To date in our efforts, this has provided correlation samples for comparison with synthetic material confirming the intended structure[1–4] or has provided access to intermediates on which we could examine structural features[5,6] or properties.[7] Beyond these immediate interests of ours, the aglycons have served as antibiotic templates from which new semisynthetic derivatives or hybrids may be prepared in efforts to improve the antimicrobial properties (i.e., potency, efficacy), expand the antimicrobial spectrum including addressing resistant bacterial strains, reduce toxicity, or otherwise enhance their properties (i.e., half-life).[8] In addition, the aglycons have found use as chiral stationary phases for chromatographic resolutions and separations.[9] Thus, there is considerable interest in developing effective deglycosidation conditions for converting the readily available glycopeptides into their corresponding aglycons. Despite this interest, no single procedure appears to work across a range of the glycopeptide antibiotics and we have found that many of the reported and specialized procedures provide disappointingly low conversions or require subsequent tedious purifications. Herein, we report the development of a series of closely related deglycosidation procedures based on the use of HF which represent considerable improvements over existing protocols.

Not only has anhydrous HF been effectively used in solid-phase peptide synthesis to remove protecting groups and provide resin release of the product, but it is known to effectively cleave the glycosyl linkages of neutral and acidic sugars (0 °C, 1 h), *O*-linked aminosugars (23 °C, 3 h), and to effect *O*-glycoprotein deglycosidation.[10] We have used it in prior studies to effect both such conversions on sensitive substrates[3,11,12] and now report its use for deglycosidation of the prototypical glycopeptide antibiotics vancomycin, ristocetin, and ramoplanin.

Vancomycin

Since the disclosure of vancomycin (**1**) in 1956,[13] it has emerged as the leading member of the clinically important glycopeptide antibiotics.[8] Vancomycin is the drug of last resort for treatment of resistant bacterial infections or for use in patients allergic to β-lactam antibiotics. The emergence of bacterial strains resistant to vancomycin highlights the need to develop new antibiotics with improved activity. The vancomycin aglycon (**2**) has been shown to possess antimicrobial activity

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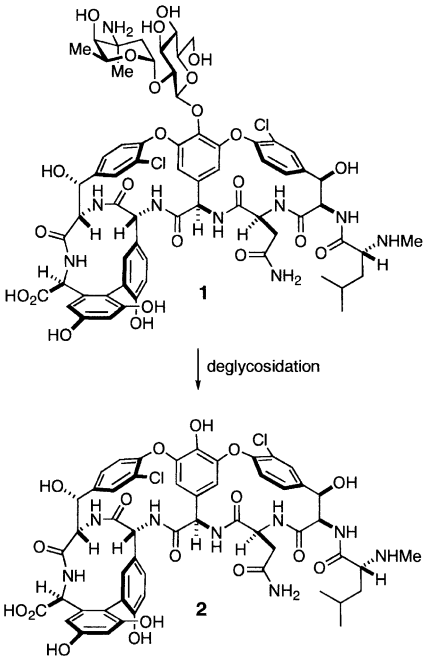
essentially equal to natural vancomycin (**1**)[14] and semi-synthetic derivatives of **2** containing alternative sugars or hydrophobic substituents are emerging as a promising strategy for the discovery and development of improved antibiotics in the fight against bacterial resistance.[15,16]

Early attempts to cleave the sugars of vancomycin were carried out with refluxing 4 N HCl (Table 1).[17] These conditions resulted in the formation of many degradation products and produced the aglycon in low yield. A deglycosidation procedure utilizing TFA was disclosed in 1988 and has remained the standard method for aglycon synthesis despite the fact that HPLC purification is necessary to obtain pure **2**.[18,5] In the course of our studies, we found that anhydrous HF (AHF) is an exceptional reagent for the removal of glycopeptide sugars. When vancomycin is treated with AHF at 25 °C for 2 h (Table 1), the glycosyl linkage is cleaved, resulting in clean conversion to the aglycon and free sugars. The sugars are readily removed by simple trituration to provide the pure aglycon **2** (91%). Lower reaction temperatures were nearly as effective but the reaction was not always complete (0 °C, 2 h). Other HF reagents including HF/pyridine resulted in incomplete deglycosidation, longer reaction times, or the appearance of reaction byproducts if driven to completion. Nonetheless, competitive conversions could be obtained (Table 1), but required purification of **2** by HPLC. Thus, a new deglycosidation protocol for vancomycin that provides **2** in higher conversions (91%), permits access to the pure aglycon in quantity, and does not require arduous purification was developed employing AHF (Fig. 1).

Table 1.



Entry	Reagent	Conditions	Yield of **2** (%)
1	4 N HCl (aq)	2 h, reflux	Decomp[17]
2	TFA	7.5 h, 50 °C	55[a,18]
3	HF/Pyr	24 h, 25 °C	60[a]
4	$Et_3N(HF)_3$	24 h, 25 °C	No reaction
5	AHF	2 h, 0 °C	87[b]
6	AHF	2 h, 25 °C	91[b]

[a]Requires purification by semi-preparative HPLC.
[b]Purified by trituration of crude mixture with 4:1 EtOAc–MeOH.

Representative experimental procedures

Anhydrous HF. A Kel-F reaction vessel was charged with vancomycin (**1**, 50 mg, 34 μmol) and anisole (0.5 mL) and attached to an HF distillation apparatus. Anhydrous HF (10 mL) was distilled into the vessel and the mixture was stirred at 25 °C for 2 h. After this time, the HF and anisole were removed under vacuum to give a light pink solid. The crude product was washed with 10 mL of 4:1 EtOAc–MeOH followed by decanting the solvent and this was repeated three times. The product was dried under vacuum to give 35 mg (91%) of pure vancomycin aglycon (**2**) as a white solid.

HF/pyridine. A polypropylene reaction vial was charged with vancomycin (**1**, 50 mg, 34 μmol) and HF/pyridine (1.5 mL) and the mixture was stirred at 25 °C for 24 h. The mixture was diluted with 5 mL of MeOH and concentrated under a stream of nitrogen. The residue was washed with 10 mL of 4:1 EtOAc–MeOH followed by decanting the solvent and this was repeated three times. The crude product was further purified by HPLC (Waters semi-preparative LC 25 mm column, CH_3CN–0.07% TFA/H_2O 17:83, 10 mL/min, $R_t = 31$ min) to provide 23 mg (60%) of pure vancomycin aglycon (**2**) as a white solid.

Ristocetin A

Ristocetin A (**3**) is a member of the glycopeptide family of antibiotics related to vancomycin and it was originally isolated from *Nocardia lurida*.[19] Although it was clinically employed to treat bacterial infections in the late 1950s, undesirable platelet aggregation led to its discontinuation. However, the aglycon of ristocetin A (**4**) has been found to be slightly more active than its parent and, more interestingly, is free of the undesirable side effects.[20] In the past, the ristocetin aglycon has been prepared in two steps. Acid hydrolysis of ristocetin A produces the intermediate ristocetin ψ-aglycon (**5**), and a second triethylsilane reductive cleavage provides the

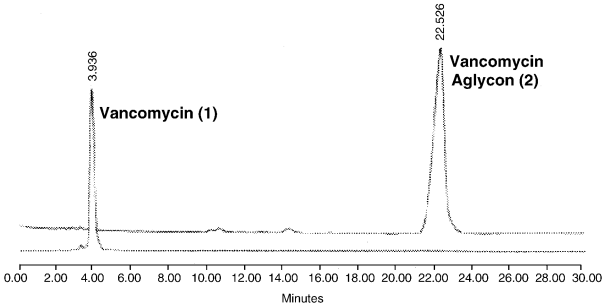
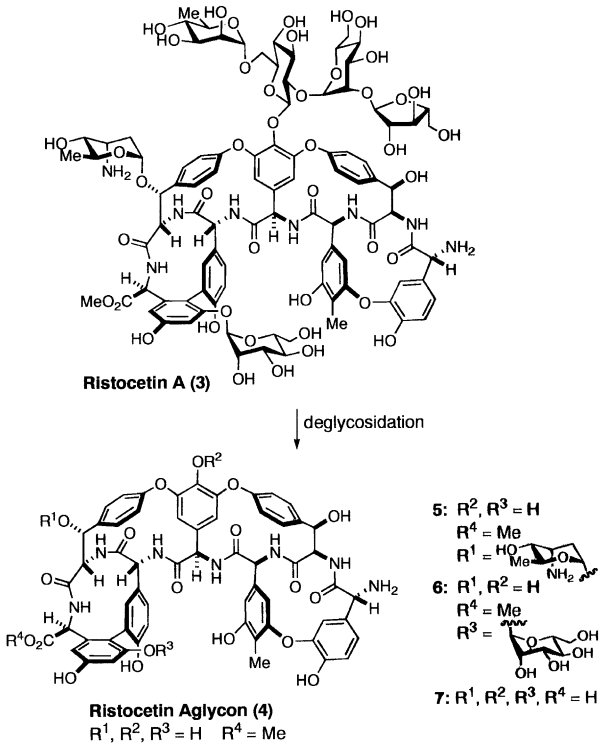


Figure 1. HPLC traces of vancomycin (**1**) and vancomycin aglycon (**2**) obtained from AHF (CH_3CN–0.07% TFA/H_2O 17:83).

ristocetin aglycon (**4**), in an overall yield of 8%.[21,22] It was ristocetin A and the difficulties in securing its aglycon that first forced us to examine new methods of deglycosidation. Thus, we examined a wide range of alternatives (Table 2) ultimately establishing that AHF or HF/pyridine provide very effective one-step procedures (92–85%) which also avoid the tedious chromatographic purifications.

Treatment of ristocetin A with 80% aqueous sulfuric acid in DMSO (1:50) afforded ristocetin ψ-aglycon (**5**) in 60% yield (entry 2). Ristocetin aglycon (**4**) was found only in very small amounts and prolonged reaction times did not noticeably change the product distribution. The use of aqueous HCl, HBr, and TFA all generated complex reaction mixtures at 80 °C (entries 3 and 5–9), and no reaction was observed at room temperature (entry 4). Anhydrous HF (AHF) containing anisole (5% v/v) as the cation scavenger provided remarkable results. Treatment of ristocetin A with AHF/anisole at −18 °C for 80 min. provided ristocetin aglycon (**4**) in 92% yield (entry 17). Moreover, the pure aglycon could be isolated by simple trituration of the reaction mixture with EtOAc. A comparable result was obtained when the reaction was run at −30 °C for 2.5 h (entry 16). Shorter reaction times result in partial deglycosidation and higher reaction temperatures (0–25 °C) provide an as yet unidentified product (entries 14 and 15). Other HF reagents including HF/Py also provided good conversions affording **4** in yields as high as 85% (entry 13), but the isolation of ristocetin aglycon in its pure form required chromatography. Treatment of ristocetin A with aqueous HF also provided ristocetin aglycon but a substantial amount of ristocetin aglycon acid (**7**) was also generated (entry 10).

Table 2.



Entry	Reagent	Conditions	Product distribution[a]
1	Et_3SiH, TFA	48 h, 25 °C	**4** (8%)[22]
2	80% H_2SO_4, DMSO	7–48 h, 85 °C	**5** (60%), **4** (<5%)[21]
3	5 N HCl	1–24 h, 80 °C	Trace of **4**[b]
4	5 N HCl	24 h, 25 °C	No reaction
5	1 N HCl	1–24 h, 80 °C	Trace of **4**[b]
6	1.2 N HCl, HOAc	1 h, 85 °C	**7** present[b]
7	TFA	2.5 h, 80 °C	**5** present[b]
8	TFA	19 h, 80 °C	**4** present[b]
9	5 N HBr	1 h, 80 °C	Trace of **4**[b]
10	48% HF (aq)	24 h, 45 °C	**4** (40%), **7** (35%)
11	HF/Py	30 min, 25 °C	**4** (60%), **6** (30%)
12	HF/Py	1 h, 25 °C	**4** (75%), **6** (15%)
13	HF/Py	2 h, 25 °C	**4** (85%)
14	AHF	1 h, 25 °C	**4** not formed[c]
15	AHF	1 h, 0 °C	**4** (55%)[c]
16	AHF	2.5 h, −30 °C	**4** (90%)
17	AHF	80 min, −18 °C	**4** (92%)[d]

[a]Unless noted otherwise, yields were determined by LC–MS of reaction mixture.
[b]Complex mixture.
[c]An unidentified side product was formed.
[d]Isolated yield.

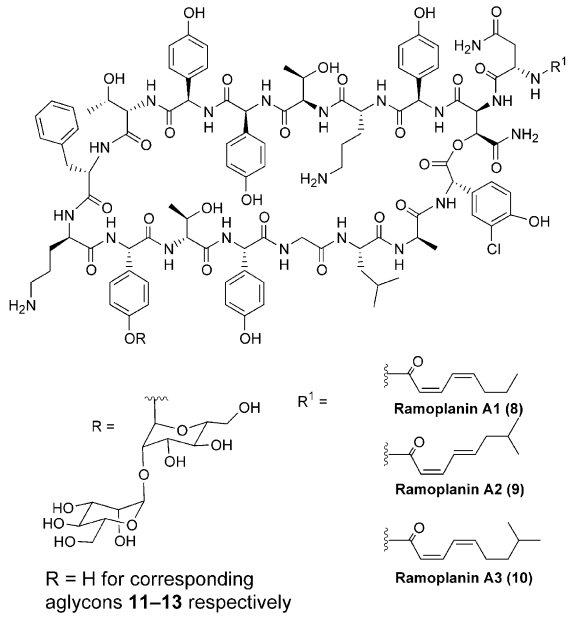
Representative experimental procedure

Following evacuation and cooling to −18 °C, a 50 mL Kel-F reaction vessel was charged with ristocetin A (**3**, 52 mg, 25 μmol) and anisole (0.5 mL) and attached to an HF distillation apparatus. Anhydrous HF (10 mL) was distilled into the reaction vessel and the mixture was stirred at −18 °C for 80 min. After this time, the HF and anisole were removed under vacuum to give a tan solid. The crude product was triturated with 5 mL of EtOAc three times and the product was dried under vacuum to give 27 mg (92%) of ristocetin aglycon (**4**) as a white solid.

Ramoplanin

Ramoplanin, a novel lipoglycodepsipeptide with potent antibacterial activity, was isolated from the fermentation broth of *Actinoplanes* sp. ATCC 33076 as a mixture of three closely related compounds **8**–**10** (Table 3).[23] The structure was disclosed in 1989, and it was established that the three compounds differ only in the acyl group attached to the Asn^1 N-terminus.[24] The ramoplanin complex was shown to be 2–10 times more active than vancomycin against Gram-positive bacteria, and the ramoplanin A2 aglycon (**12**) was reported to be equally active or more potent than the natural product in antimicrobial assays.[25,26] Shortly after its disclosure, ramoplanin was shown to disrupt bacterial cell wall biosynthesis where it inhibits the action of intracellular UDP-glcNAc transferase (MurG) and the conversion of lipid intermediate I to lipid intermediate II.[26] This inhibition was proposed to arise by ramoplanin complexation of lipid intermediate I preventing its utilization as a substrate.[27] More recently, Walker has shown that ramoplanin also inhibits the subsequent and more accessible transglycosylase-catalyzed extracellular polymerization of lipid intermediate II and forms self-associating 1:1 complexes with close analogues of the substrate, lipid intermediate II, suggesting this may

Table 3.



Entry	Reagent	Conditions	Product distribution
1	5% HCl/DMF	7 h, 70 °C	**12** (20%)[26]
2	AHF	1.5 h, 0 °C	**11** (3%), **12** (46%), **13** (3%)[a]
3[b]	AHF	1.5 h, 0 °C	**12** (92%)[c]

[a]Requires purification by semi-preparative HPLC.
[b]Reaction run with pure ramoplanin A2 (**9**).
[c]Purified by trituration with EtOAc.

represent the more relevant biological site of action.[28] These two steps immediately precede the transpeptidase-catalyzed cross-linking reaction and the site of action of vancomycin. Thus, mechanism-based cross-resistance between ramoplanin and vancomycin is not observed and ramoplanin represents an excellent candidate for more expansive clinical use beyond its introduction for topical infections.[29] Ramoplanin is presently in Phase III clinical trials for the oral treatment of intestinal vancomycin-resistant *Enterococcus faecium* (VREF) and Phase II trials for nasal MRSA.[25]

Past protocols for the deglycosidation of ramoplanin have relied on the treatment of ramoplanin complex with either: (1) trimethylsilyl iodide or trimethylsilyl chloride in the presence of sodium iodide followed by hydrolysis or (2) a strong mineral acid (HCl) in the presence of a lower alcohol (e.g., BuOH) under anhydrous conditions (entry 1).[26] Typically, pure ramoplanin A2 aglycon is obtained in 20–30% yield using optimized conditions for these methods. In addition to low conversions, the isolation of pure ramoplanin aglycon requires a tedious HPLC purification that results in some loss of material and contributes to the low conversions. We have found that the use of anhydrous HF cleanly cleaves the dimannose sugar moiety of ramoplanin, without affecting the sensitive ester linkage or acyl side chains.

Treatment of the ramoplanin complex with anhydrous HF (entry 2) cleanly provided the A1–A3 aglycons (**11**–**13**) contaminated only with mannose-derived reaction byproducts. Reverse-phase HPLC purification of the mixture which serves to separate the A1–A3 aglycons [20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M)] provides pure A1 (3%), A2 (46%), A3 (3%), albeit with a recovery loss due to the physical properties of the natural product aglycons.[3] Enlisting pure ramoplanin A2 (**9**), the HF deglycosidation followed by a simple EtOAc trituration to remove the deglycosidation byproducts provides the pure A2 aglycon (**12**) in 92% yield avoiding the material loss that accompanies HPLC purification (entry 3). Thus, the use of anhydrous HF for deglycosidation of ramoplanin A1–A3 is exceptionally effective providing the corresponding aglycons in conversions as high as 92% and offers a superb improvement over existing methods.

Representative Experimental Procedures

Ramoplanin A1, A2, and A3 Aglycons (**11**–**13**) from Ramoplanin Complex (**8**–**10**). Anhydrous HF (4–5 mL) was condensed in a Kel-F reaction vessel charged with the ramoplanin complex (8–10, 97 mg, 36 μmol) and anisole (0.5 mL) at −78 °C. The reaction mixture was warmed to 0 °C and stirred for 1.5 h. The HF was removed at 0 °C under a stream of N_2 over 90 min. EtOH was added (1 mL), and the solvent was removed in vacuo. The residue was dissolved in 0.1 N aqueous HCl and lyophilized. HPLC purification [Waters semi-preparative LC 25 mm column, 8 mL/min, 30 min gradient of 20–50% CH_3CN–$HCOONH_4$ (aq, 0.05 M, t_R = 17.4 min (A1); 19.2 min (A2); 21.3 min (A3))] provided pure ramoplanin A2 aglycon, and mixtures of ramoplanin A1/A2, and A2/A3 aglycons. A second HPLC purification (same conditions as above) of the recovered aglycon mixtures provided pure ramoplanin A1, A2, and A3 aglycons, as mixtures with $HCOONH_4$. The mixtures were lyophilized until they reached a constant weight and then lyophilized with 0.1 N HCl (1 mL) to give **11** (2.5 mg, 3%), **12** (40 mg, 46%), and **13** (2.3 mg, 3%) as white solids.

Ramoplanin A2 Aglycon (**12**) from Ramoplanin A2 (**9**). Anhydrous HF (2–3 mL) was condensed in a Kel-F reaction vessel charged with pure ramoplanin A2 (**9**, 4.6 mg, 1.8 μmol) and anisole (0.5 μL) at −78 °C. The reaction mixture was warmed to 0 °C and stirred for 1.5 h. The HF was removed at 0 °C under a stream of N_2 over 90 min. EtOH was added (0.5 mL), and the solvent was removed in vacuo. The residue was dissolved in 0.1 N aqueous HCl and lyophilized. EtOAc was added to the light brown solid, and the slurry was sonicated and then centrifuged. The solid was collected to give pure ramoplanin A2 aglycon (3.8 mg, 92%) as a white solid.

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