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# Site-Selective Palladium-catalyzed Oxidation of Unprotected Aminoglycosides and Sugar Phosphates

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**Abstract:** The site-selective modification of complex biomolecules by transition metal-catalysis is highly warranted, but often thwarted by the presence of Lewis basic functional groups. This study demonstrates that protonation of amines and phosphates in carbohydrates circumvents catalyst inhibition in palladium-catalyzed site-selective oxidation. Both aminoglycosides and sugar phosphates, compound classes that up till now largely escaped direct modification, are oxidized with good efficiency. Site-selective oxidation of kanamycin and amikacin was used to prepare a set of 3'-modified aminoglycoside derivatives of which two showed promising activity against antibiotic-resistant *E. coli* strains.

## Introduction

Transition metal-catalysis is an important tool in chemical biology to functionalize biomolecules. In particular palladium is used in a variety of reactions. Sonogashira, Suzuki-Miyaura and Heck-type cross-coupling reactions have been used to prepare protein conjugates[1–6] for the post-synthetic functionalization of (lanthi)peptides,[7–9] and as read-out in chemoproteomic experiments.[10–12]

Mono-, di- and oligosaccharides have been functionalized with palladium-catalyzed oxidation reactions (Figure 1 A).[13–18] In particular, Waymouth's monocationic palladium neocuproine complex **1** (Figure 1B) in combination with terminal oxidants like benzoquinone or oxygen has proven to be a valuable tool,[19–21] as it selectively oxidizes the hydroxy group at the C3 position.[17,22,23] The resulting keto group opens routes for further modification of this important class of biomolecules.[24–29]

A frequently encountered problem in palladium-catalyzed functionalization of biomolecules is catalyst inhibition. Most biomolecules have Lewis basic functional groups that can coordinate to the palladium complex. As a result, Lewis basic sites within the substrate sequester the palladium catalyst from the solution, which lowers the concentration of active catalyst (Figure 1C). This behavior is not problematic *per se* if the resulting complex retains activity or readily dissociates. In these cases, binding to Lewis basic sites can even be exploited to increase the selectivity via a proximity effect.[1] However, coordination to Lewis bases becomes problematic when the resulting complexes are non-productive and do not dissociate to form the active catalytic species.[9,30] This problem is particularly pronounced using cationic palladium, as in **1** and **2** (Figure 1B), due to its stronger Lewis acidic character.

The problems associated with catalyst inhibition can occasionally be overcome by using the palladium catalyst in excess, often in combination with excess ligand.[2,4,7,31] Under these conditions, the catalyst concentration in solution will be sufficient to perform the reaction, even when most of the catalyst forms non-productive complexes with the Lewis basic groups in the substrate (Figure 1D). High catalyst loadings are standard for palladium-mediated cross-coupling reactions on proteins,[7] and have also been used to promote other palladium-catalyzed reactions that suffer from catalyst inhibition. We showed that site-selective oxidation of unprotected glucopeptides containing a lysine residue is feasible when using an excess of monocationic palladium catalyst **1**.[31] However, the use of high catalyst loadings has its limitations. Adding excessive amounts of catalyst can promote side reactions and severely complicates both the analysis of the reaction and isolation of the product.[7,31] This problem is aggravated by the fact that palladium is toxic to cells, so should be removed to low ppm levels in the product.

An alternative for excessive amounts of the palladium catalyst is to use elevated temperatures.[32] A final strategy is to protect the Lewis basic groups in the substrate (Figure 1D). This approach is commonly used in organic synthesis and is effective, as is illustrated by our recent studies on the site-selective oxidation of aminoglycosides with monocationic palladium catalyst **1**.[26] These aminoglycoside antibiotics contain several amino groups, which had to be protected as the corresponding benzyl or *tert*-butyl carbamate to prevent catalyst

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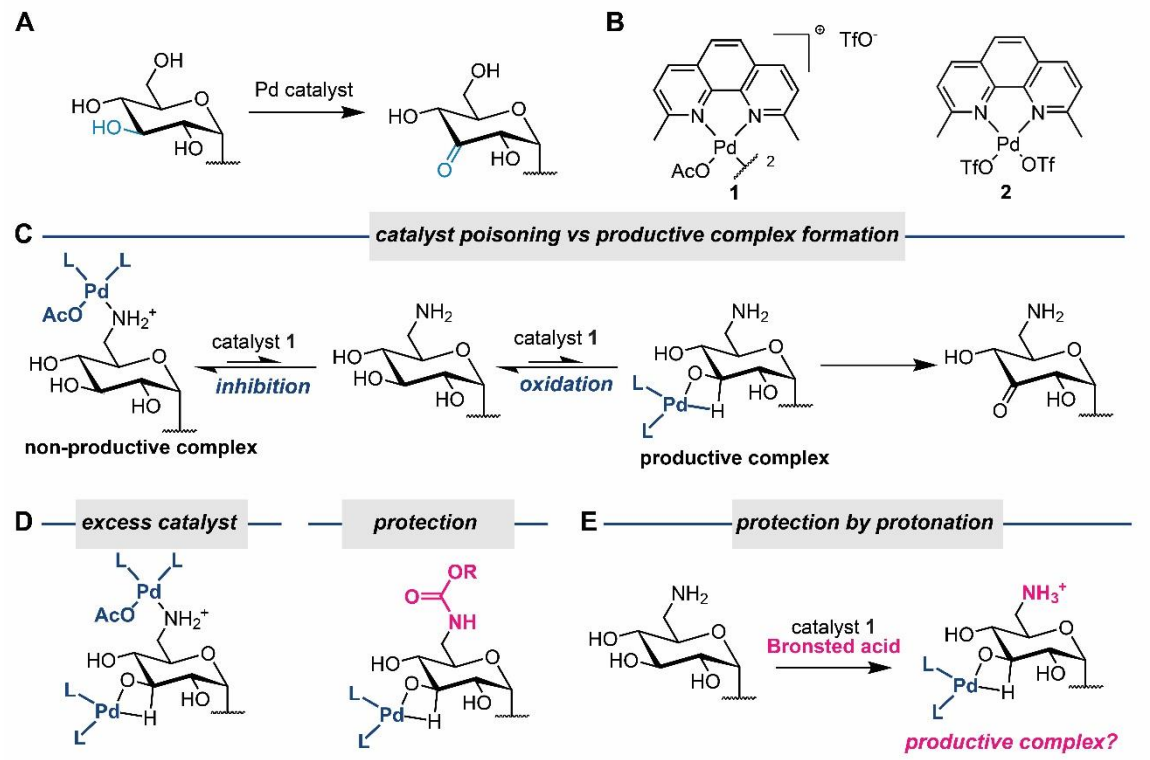


**Figure 1.** (**A**) Oxidation of glucopyranosides at the C3 position. (**B**) The structure of palladium catalyst **1** and **2**. (**C**) inhibition of the palladium catalyst by an amino group in the substrate. (**D**) Schematic representation of how an excess of catalyst or the use of protecting groups facilitates the oxidation reaction. (**E**) Representation of the proposed temporary protection by protonation.

inhibition and to allow oxidation. Protecting group strategies have their limitations, however, because full protection of all Lewis basic groups in a complex biomolecule is very difficult to achieve. A congruent problem occurs in the final deprotection step. As all protecting groups have to be removed, subtle reaction conditions are mostly ineffective and long reaction times are necessary, provoking the formation of side products. Introduction and removal of protecting groups is further complicated by the large difference in polarity of the starting material and the product because, as a result, partly protected intermediates tend to precipitate from the reaction solution. This is also observed for aminoglycosides and leads to incomplete protection and deprotection of aminoglycoside derivatives concomitant with severe purification problems.[26] A final limitation of protecting groups is that only functional groups, such as reactive groups and fluorophores, that are compatible with the deprotection conditions, can be installed.

In order to expand the use of palladium-catalyzed oxidation to the site selective functionalization of aminoglycosides and sugar phosphates, a method is required that avoids the use of protecting groups and circumvents the use of excess palladium. We hypothesized that protonation of the Lewis basic groups with a Brønsted acid during the oxidation reaction would meet this requirement, provided that conditions could be established that would not lead to decomposition of the catalyst by protonation of its ligand (Figure 1E). Temporary protonation of amine groups has been exploited to enhance the chemoselectivity of reactions,[33] and has been reported to allow ruthenium-catalyzed ring-closing metathesis of a substrate that contained a secondary amine, although in that catalyst the metal-ligand bond is considerably more stable.[34,35] We here report that the addition of defined amounts of a strong acid prevents inhibition of the palladium catalyst by amino groups and phosphate monoesters in the oxidation of hydroxy groups in carbohydrates. This allows the site-selective oxidation of aminoglycosides and sugar phosphates without the use of protecting groups. As an example, we show that *in situ* protonation greatly simplifies the site selective modification of the antibiotics kanamycin A and amikacin. Several derivatives of these antibiotics were prepared in two to three steps and in good yields. Minimal inhibitory growth assays revealed that two of the prepared derivatives were active in bacterial strains that express antibiotic resistance enzymes.

## Results and Discussion

The compatibility of the palladium catalyzed oxidation with the presence of protonated amines was first studied by adding ammonium salts to the oxidation reaction of methyl α-D-glucopyranoside **3** (Figure 2A). Weakly coordinating anions were selected, since chloride and azide are known to inhibit catalyst **1**.[36] To our delight, the palladium-catalyzed oxidation reaction proceeded in the presence of ammonium tetrafluoroborate and hexafluorophosphate, which confirmed that ammonium groups and weakly coordinating ligands do not inhibit the catalyst.

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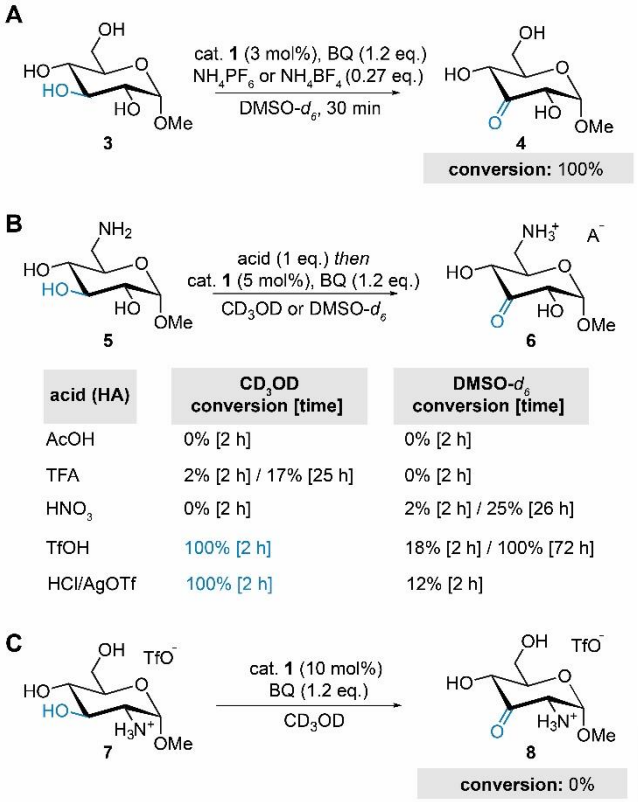


| acid (HA) | $CD_3OD$ conversion [time] | DMSO-$d_6$ conversion [time] |
|---|---|---|
| AcOH | 0% [2 h] | 0% [2 h] |
| TFA | 2% [2 h] / 17% [25 h] | 0% [2 h] |
| $HNO_3$ | 0% [2 h] | 2% [2 h] / 25% [26 h] |
| TfOH | 100% [2 h] | 18% [2 h] / 100% [72 h] |
| HCl/AgOTf | 100% [2 h] | 12% [2 h] |

**Figure 2. (A)** Oxidation of methyl α-D-glucopyranoside (**3**) in the presence of ammonium salts. (B) Oxidation of aminoglycoside **5** in the presence of 1 eq of various acids. (C) Oxidation of aminoglycoside **7** in the presence of TfOH. BQ = benzoquinone. Neocuproine = 2,9-dimethyl-1,10-phenanthroline

These initial experiments indicated that protonation of amino groups could be a viable strategy for the oxidation of aminoglycosides. Protonation, however, changes the electronic nature of the substrate considerably. A proximal ammonium group may prevent coordination of the cationic palladium catalyst due to electrostatic repulsion. Electrostatic interactions have shown to play an important role in C-H functionalization reactions by a decatungstate catalyst.[37,38] In addition, the ammonium substituent may hamper oxidation of a neighboring hydroxy group by inductive electron withdrawal.[23,39] Therefore, we selected the aminoglycosides **5** and **7** as substrates for proof-of-concept experiments (Figure 2B and 2C). In these monosaccharides, the distance between the oxidation site and the ammonium group is considerably different.

We considered the selection of a suitable acid and solvent vital for the protonation strategy. The acid needs to be sufficiently strong to ensure full protonation, and the conjugate base should not coordinate to the palladium catalyst. As both acid strength and the ability of anions to coordinate to palladium are solvent-dependent[40] we performed the oxidation of **5** in methanol and in DMSO in the presence of one equivalent of acetic acid (AcOH), trifluoroacetic acid (TFA), nitric acid or trifluoromethanesulfonic acid (TfOH). Both DMSO and methanol are commonly employed in palladium-catalyzed oxidation of saccharides, as these solvents are compatible with the reaction and dissolve the very polar substrates well. The pKa of the selected acids varies in these solvents and ranges from 12.3 (AcOH in DMSO) to –14.7 (TfOH in water).

No conversion was observed when acetic acid was used, neither in DMSO nor in methanol. For DMSO-$d_6$, this was the expected outcome, as the equilibrium between acetic acid (p$K_a$ of AcOH = 12.3 in DMSO) and amine **5** (p$K_a$ of $NH_4^+$ = 10.5 in DMSO) is on the side of the free base. For a protic medium like methanol or methanol-$d_4$, we expected that acetic acid would protonate amine **5** (p$K_a$ of $NH_4^+$ = 9.2 in water), even though acetic acid (p$K_a$ of AcOH = 4.8 in water) is a relatively weak acid.[41] Even a small amount of free amine, however, would inhibit the palladium catalyst and in addition, acetate coordinates to palladium as well, forming a less active catalyst.

The oxidation reactions in the presence of trifluoroacetic acid and nitric acid gave mixed results. In the presence of trifluoroacetic acid (p$K_a$ of TFA = –0.3 in water), minor amounts of the desired ketone **6** were detected by $^1$H-NMR after 2 h in methanol-$d_4$. The conversions increased to 17% when the reaction time was extended to 1 d. No conversion was observed in DMSO (entry 2).[42] *Vice versa*, addition of nitric acid in DMSO-$d_6$ resulted in approximately 25% conversion after 1 d (entry 3), but no conversion in methanol-$d_4$.

Finally, trifluoromethanesulfonic acid, the strongest acid of the series (p$K_a$ of TfOH = –14.7 in water, –14.3 in DMSO),[43] was used to protonate **5**. Triflate is considered a non-coordinating anion. Full conversion of **5** to the corresponding ketone **6** was observed in methanol-$d_4$ after 2 h. The oxidation also proceeded in DMSO-$d_6$, but the reaction was considerably slower and only reached completion after 3 d. This difference underlines the importance of selecting the appropriate solvent-acid combination. Identical results were obtained when the chloride salt of **5** underwent salt metathesis with silver (I) triflate (AgOTf), to obtain the triflate salt *in situ*, prior to oxidation (entry 5).

With these conditions, oxidation of 2-amino-2-deoxyglucoside **7** with TfOH in methanol-$d_4$, was attempted (Figure 2C) but no conversion was observed. Acetyl- and carbamate-protected glucosamine derivatives are versatile substrates for this oxidation reaction,[13] so the absence of conversion confirmed that proximal ammonium groups drastically lower the reactivity of a substrate in the palladium-catalyzed oxidation reaction.

To demonstrate that the protonation strategy is more general, we applied it to phosphorylated substrates (Figure 3). Phosphate salts also inhibit the catalyst, as is evident from the failure of the oxidation of methyl α-D-glucopyranoside (**3**) in the presence of tetrabutylammonium dihydrogen phosphate (TBA $H_2PO_4$). Addition of one equivalent of TfOH, however, effectively prevented catalyst inhibition and resulted in 84% conversion of the substrate to the corresponding 3-keto product **4** (Figure 3A). Also methyl glucose-6-phosphate **9** was successfully oxidized, upon protonation of the phosphate group with triflic acid (Figure 3B). The oxidation proceeded both in methanol and in DMSO, but the reaction in methanol was considerably faster, as was also the case for aminosugars. Although the oxidation of glycerol 1-phosphate **10** in DMSO remained incomplete, this procedure gives readily access to dihydroxyacetone phosphate. Finally, we attempted the oxidation glucose 1-phosphate **11** and glucose 6-phosphate **12**. Glucose 1-phosphate **11** turned out sensitive to

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acid, rapidly hydrolyzed in DMSO and formed the *O*-methyl glycoside in methanol. Also the hemiacetal function in glucose-6-phosphate **12** reacted with methanol in the presence of TfOH. However, **12** could be oxidized in DMSO and good conversion to an anomeric mixture of the desired 3-keto-α-glucose-6-phosphate products was observed by $^{1}$H-NMR after overnight reaction. Prolonging the reaction time further led to side product formation.

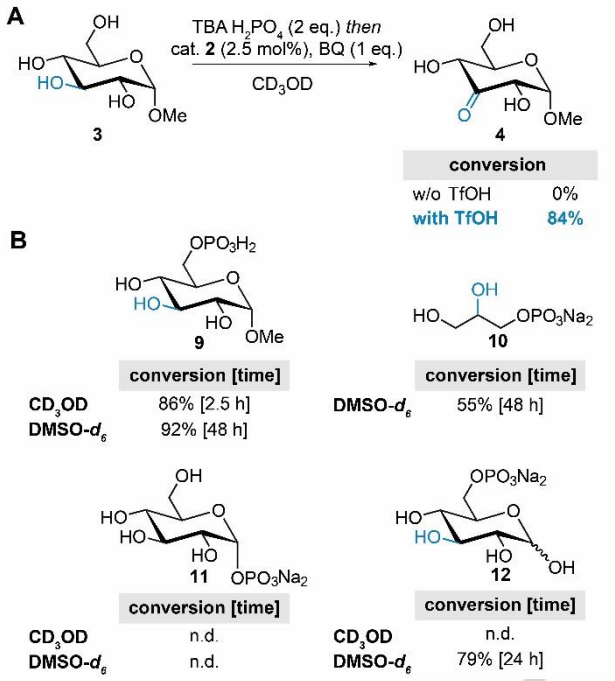


**Figure 3.** (A) Oxidation of glucopyranoside **3** in the presence of tetrabutylammonium dihydrogen phosphate. (B) Oxidation of phosphorylated substrates **9-12** in the presence of TfOH**.** Conditions used: (neocuproine)Pd(OTf)$_2$ **2** (2.5 mol%), *tert*-butyl benzoquinone (1 eq), TfOH (2 eq). n.d.: not determined because the substrate reacted with the solvent.

Having established that protonation can be used to allow oxidation of aminoglycosides by temporarily protecting Lewis basic sites, our next objective was to apply this strategy to kanamycin A **13** and its semi-synthetic analogue amikacin **14** (Figure 4A). These well-known and broadly applied aminoglycosides show broad-spectrum activity against both Gram positive and Gram negative bacteria, including *Mycobacterium tuberculosis*.[44] Kanamycin A **13** and amikacin **14** interfere with the protein translation process in bacteria by binding to the 16S rRNA of the ribosomes.[45,46] Bacterial resistance towards **13** and **14**, and antibiotics in general, is a major issue.[47],[48] The clinically most relevant bacterial resistance mechanism against **13** and **14** is structural modification of the antibiotic by aminoglycoside-modifying enzymes (AMEs).[49,50] Acetylation of one of the amino groups by *N*-acetyltransferases (AACs), and phosphorylation or adenylylation of one of the hydroxyl groups by *O*-phosphotransferases (APHs) or *O*-nucleotidyltransferases (ANTs) results in a complete loss of antibacterial activity.

Inactivation of aminoglycosides can be prevented by chemically modifying the sites that are targeted by the AMEs.[51–75] Introduction of a 4-amino-2-hydroxybutanoyl (AHB) residue on the 2-deoxystreptamine ring (ring II), as in amikacin **14**, prevents modification by most of the clinically relevant *N*-acetyltransferases.[76] Similarly, APH(3')-induced bacterial resistance has been overcome by making structural alterations at the C3' position (indicated in Figure 4A with the arrow) that circumvent phosphorylation of the 3'-OH of kanamycin A. 3'-ketokanamycin A **15**,[69] 3'-deoxykanamycin,[58,77,78] 3'-epikanamycin **17**,[26] and 3'-deoxy-3'-fluorokanamycin,[79–81] show strongly increased activity against pathogens expressing APH(3'). Modifications at the 3'-postion of kanamycin A that introduce more bulk, such as 3'-deoxy-3'-chlorokanamycin[79] and 3'-*O*-methylkanamycin,[82] have been reported as well, but these modifications are not well-tolerated and lower the antibiotic activity, compared to the parent compound kanamycin A.

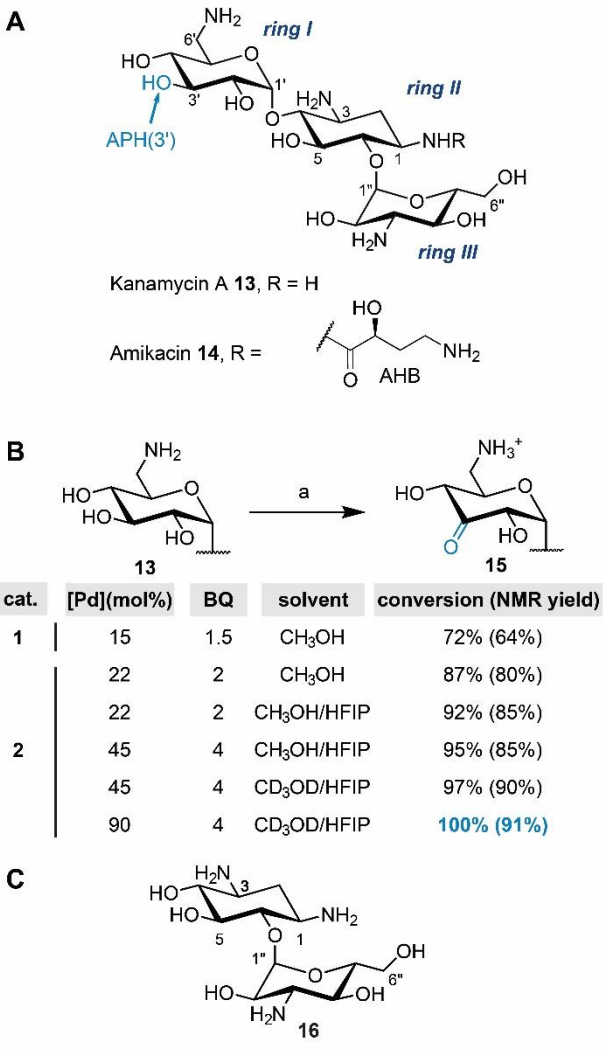


**Figure 4.** (A) Structures of kanamycin A **13** and amikacin **14**. (B) Optimization of the oxidation of kanamycin A **13**. (C) Structure of the disaccharide side product **16**.

Modification of aminoglycosides has therefore shown to be a successful approach to tackle bacterial resistance; however, preparation of modified aminoglycosides is a formidable

challenge. 3'-Ketokanamycin **15**, for example, was prepared in not less than 11 steps from kanamycin A **1**. Ten out of these eleven steps were protection and deprotection steps and just one step was the oxidation of the 3'-hydroxy group to the 3'-ketone.[69] Long synthesis routes limit the access to the amount of material that is required for thorough biological and clinical studies.

Ring I of kanamycin A **13** and amikacin **14** is similar to substrate **5** (Figure 4A and Figure 2B). We reasoned that the protonation strategy applied to **13** and **14**, would allow the introduction of small structural alterations at the 3'-position in kanamycin A and amikacin in just a few steps. Therefore, we adapted the established conditions from Figure 2B. Kanamycin A **13**, which is commercially available as the monosulfate salt, had to be converted into the tetratriflate salt, prior to oxidation. We added a slight excess of TfOH (2.2–2.5 eq) to protonate the amino groups and barium ditriflate ($BaOTf_2$) (1.2 eq) to exchange the sulfate counterion for triflate. We opted for this combination of protonation and salt metathesis, as it allows preparation of the tetratriflate salt *in situ* in methanol. Barium sulfate is extremely insoluble,[83] and after its addition only kanamycin triflate remains in solution.

The resulting suspension was used as such in the oxidation reaction and 1,4-benzoquinone (BQ) and $[(neocuproine)PdOAc]_2(OTf)_2$ **1** were added. $^1$H-NMR showed that the hydroxy group at C3' of ring I oxidized selectively and that a mixture of 3'-ketokanamycin A **15** and the corresponding hydrate had formed in 64% yield (Figure 4B). In the absence of TfOH or $Ba(OTf)_2$, the oxidation of kanamycin did not proceed, which demonstrated the necessity of both reagents to prepare the tetratriflate salt and also showed that sulfate, under the conditions used, inhibits the palladium catalyst. We envisioned that separation of remaining kanamycin A **13** from the resulting C3' derivative would be challenging; hence the conditions were further optimized to achieve complete conversion. During this process, we noted large variations in the conversion, depending on the batch of catalyst **1** used. Since $(neocuproine)Pd(OTf)_2$ **2** may be prepared *in situ* from **1** by the slight excess of TfOH used for the protonation of the amines, we also tested biscationic catalyst **2** on kanamycin A **13** tetratriflate. We were pleased to observe that $(neocuproine)Pd(OTf)_2$ **2** gave consistently high conversions, and therefore continued the optimization with this catalyst.

Oxidation of kanamycin tetratriflate **13** with two equivalents of benzoquinone and 22 mol% **2** resulted in 87% conversion. A small improvement in conversion was observed when hexafluoroisopropanol (HFIP) was used as a cosolvent ($CH_3OH$/HFIP 4/1) (entry 3). HFIP is a very polar solvent and a strong hydrogen bond donor,[84] which might assist solvation of the triflate counterions and shift the equilibrium between TfOH and kanamycin further to the tetratriflate salt. Further increasing the amount of HFIP to a ratio of 1/1 ($CH_3OH$/HFIP) resulted in a drop of conversion, due to the poor solubility of kanamycin in HFIP. Doubling the amount of benzoquinone (4 eq) and catalyst (45 mol%) gave an increase in conversion to 95% (entry 4). Interestingly, the conversion further increased when the oxidation was performed in deuterated methanol instead of regular methanol, which suggests that oxidation of methanol is a competing side reaction (entry 5). Palladium catalysts are known to oxidize methanol at room temperature with a very low rate.[19] Oxidation of the substrate normally outcompetes this side reaction.[85] In the current case, however, inductive electron withdrawal and electrostatic repulsion by the ammonium groups likely deactivate the substrate towards oxidation. Deuterated methanol competes to a lesser extent, since the C-D bond is more resistant against hydride abstraction than the C-H bond. Finally, full conversion was obtained using a near stoichiometric amount (90 mol%) of palladium catalyst (entry 6).

Pleased with this result, we attempted to purify ketone **15**. Mobashery and co-workers isolated 3'-ketokanamycin A **15** as the monotrifluoroacetate salt in 11 steps. In our hands, **15** degraded slowly when it was neutralized for workup and purification. The most prominent degradation product was disaccharide **16** in which ring I has been removed, presumably in an E1cB reaction.[86] Ketone **15** could be purified to an acceptable degree using size-exclusion chromatography, but the limited stability of **15** makes it unsuitable as an antibiotic candidate.

We reasoned that these stability problems could largely be circumvented by further modification of the ketone group, directly after the oxidation reaction (Figure 5). This indeed proved to be the case. Reduction of the ketone with $NaBH_4$ gave a mixture of 3'-epikanamycin A **17** and kanamycin A **13** in good yield. Residual salts, hydroquinone, catalyst and small amounts of degradation products were effectively removed by ion exchange chromatography using a weakly cationic exchange resin ($NH_4^+$ form) and aqueous $NH_3$ as the eluent.[87] ICP-MS analysis showed that the palladium and barium content was below 25 ppm after this step. However, the remaining kanamycin A **13** in the mixture, which originated from an imperfect stereoselectivity of the reduction of the ketone, could not be separated from the desired epimer **17** with this method. To prepare stereochemically pure 3'-epikanamycin A **17**, we hydrogenated 3'-ketokanamycin A **15** with $PtO_2$. We were pleased to observe, that this hydrogenation turned out to be considerably more selective than reduction with $NaBH_4$, and epimer **17** was obtained diastereomerically pure in 58% yield over two steps (Figure 5). This is a dramatic improvement compared to our earlier reported four-step synthesis which gave an overall yield of 10%.

Our group reported various procedures to further functionalize unprotected keto-saccharides[13,25,27–29] and we reasoned that these reactions could be used to introduce structural alterations at the 3'-position that span beyond epimerization. Protecting-group free oxidation of kanamycin A **13** followed by indium-mediated allylation of keto-glycoside **15** indeed gave the resulting 3'-allylkanamycin **18**, as a single diastereomer, in 60% yield over two steps. NOESY NMR showed that the allylation reaction exclusively afforded *allo*-configured product **18**, which is in line with earlier results obtained on glucosides. Using the earlier reported sequential oxidation-oxime formation procedure, we also prepared oximes **19** and **20** in good yields. These potential antibiotic candidates also served as precursors for the synthesis of both epimers of 3'-aminokanamycin. We previously demonstrated that reduction of C3-oxime-ethers under Birch conditions lead to the equatorial amine, whereas the axial amine can be prepared by hydrogenation of the oxime over Adams' catalyst. Indeed, reduction of methyloxime **20** with sodium yielded

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3'-aminokanamycin **21**, while hydrogenation of oxime **19** with $PtO_2$ afforded 3'-epi-aminokanamycin **22**.

To demonstrate that this oxidation method can be applied to other aminoglycosides as well, we applied the procedure to amikacin **14**. Commercial amikacin disulfate was converted into the corresponding tetratriflate salt with 2 eq of $Ba(OTf)_2$ and subsequently oxidized with catalyst **2**. NMR analysis showed full

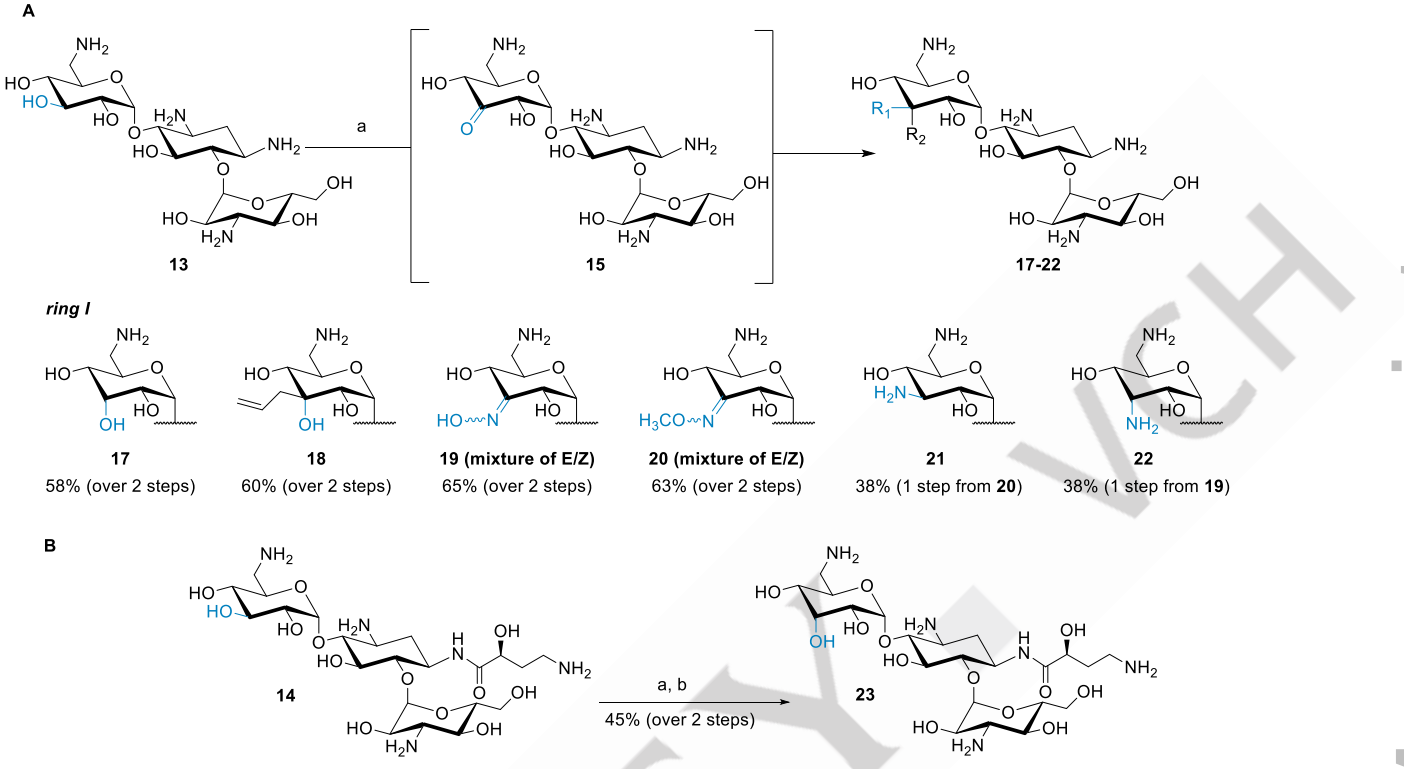


**Figure 5.** (A) Functionalization of 3'-position of kanamycin A **13**. (B) Epimerization of the 3'-position of amikacin **14**. a is Pd-catalyzed oxidation, b is Pt-catalyzed hydrogenation.

**Table 1.** MIC results of the aminoglycoside derivatives[a]

| | E. coli **ATCC 25922** | E. coli **DH5α** | DH5α **APH(3')-Ia** | DH5α **APH(3')-IIa** | DH5α **APH(3')-IIb** | DH5α **APH(3')-IIIa** | DH5α **APH(3')-VI** | DH5α **APH(3')-VI** |
|---|---|---|---|---|---|---|---|---|
| kanamycin **13** | 4 | 1-2 | 256 | 64-128 | 128-256 | >512 | 2 | 32 |
| **17** | 16-32 | 8 | 32 | 16 | 32 | 32 | 8 | 16 |
| **18** | >512 | 256-512 | >512 | >512 | >512 | >512 | 256 | >512 |
| **19** | 16 | 4 | 4 | 4 | 4 | 4-8 | 4 | 4 |
| **20** | >512 | 256-512 | 256 | 256 | 256 | 256 | 256 | 256 |
| **21** | 128-256 | 64 | 64 | 64 | 64 | 128 | 64 | 64 |
| **22** | 64 | 16 | 32 | 16-32 | 16 | 32-64 | 16 | 16 |
| amikacin **14** | 1 | 0.5-1 | 0.5-1 | ≤0.25 | 0.25-0.5 | 2-4 | 0.25-0.5 | 1-2 |
| **23** | 4-8 | 2 | 1-2 | 1 | 1 | 2 | 1 | 1-2 |

[a] values in μg/ml

conversion. The impurity level was higher compared to the oxidation of kanamycin A, but C3'-ketoamikacin was the major product. Hydrogenation with $PtO_2$ yielded diastereomerically pure 3'-epiamikacin **23** in 45% yield over two steps, confirming that the reaction can be extended to other aminoglycosides.

To evaluate and compare their antimicrobial activities, the minimum inhibitory concentration (MIC) was determined for the synthesized aminoglycoside derivatives. The compounds were tested on several *E. coli* strains, including engineered strains carrying different APH(3') genes (Table 1). The MIC assays revealed that, against wild type *E. coli*, none of the derivatives showed increased activity compared to parent kanamycin A **13** and amikacin **14**. However, 3'-epikanamycin **17** and especially the oxime-derived kanamycin **19** showed overall the lowest MIC values of the kanamycin derivatives against APH(3')-expressing strains. Thus, **19** displayed a more than 64-fold increase in activity against the strain expressing APH(3')-IIIa compared to kanamycin **13**. Allyl- **18** and methyloxime-derived kanamycin **20** showed high MIC values, indicating that their relatively large substituents at the 3'-position hinder binding to the ribosome. Another notable observation is that the activity of 3'-epi-aminokanamycin **22** is comparable to that of 3'-epikanamycin **17** and that 3'-aminokanamycin **21** is overall less active than **17** and **22**. An equatorial amino-group apparently is not well-tolerated and negatively affects the antibacterial activity. 3'-Epiamikacin **23** retained high activity against resistant strains, though slightly lower than its parent amikacin (**14**). The introduction of the AHB residue on the 2-deoxystreptamine ring makes amikacin already refractory to APH(3') enzymes, but we were pleased to see that epimerization at C'3 had a limited impact on the antibacterial activity.

## Conclusion

Palladium-catalyzed oxidation has shown to be an excellent method for the site-selective modification of carbohydrates, and this method has now been extended to aminoglycosides and sugar phosphates. The *in situ* masking of Lewis basic sites by protonation with triflic acid, allows these oxidation reactions to proceed successfully without the use of protecting groups. It avoids the cumbersome introduction and removal of multiple protecting groups in complex carbohydrates and in addition expands the scope of the functional groups that can be introduced via the generated carbonyl function. These introduced functional groups don't need to be orthogonal with deprotection conditions. This provides considerable incentive to apply the here developed strategy in chemical biology.

Proof of concept was provided for the well-known broad-spectrum antibiotics kanamycin A and amikacin that were oxidized site-selectively at the 3'-position upon protonation and salt metathesis of their commercial sulfate salts. The ketone function was used successfully to prepare a range of kanamycin A derivatives and 3'-epiamikacin in excellent yield in merely two steps. MIC assays revealed that the 3'-epimer and 3'-oxime derivatives of kanamycin A are active against *E. coli* strains resistant to the parent antibiotic. This provides considerable incentive to apply the here developed strategy for the synthesis of novel aminoglycoside antibiotics.

## Supporting Information

The authors have cited additional references within the Supporting Information.[88–93]

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**Keywords:** aminoglycosides • palladium • oxidation • antibiotics • site-selective

[1] J. Willwacher, R. Raj, S. Mohammed, B. G. Davis, *J. Am. Chem. Soc.* **2016**, *138*, 8678–8681.
[2] J. M. Chalker, C. S. C. Wood, B. G. Davis, *J. Am. Chem. Soc.* **2009**, *131*, 16346–16347.
[3] Q. Zhao, G. Guo, W. Zhu, L. Zhu, Y. Da, Y. Han, H. Xu, S. Wu, Y. Cheng, Y. Zhou, et al., *Chem. Eur. J.* **2020**, *26*, 15938–15943.
[4] M. E. Ourailidou, J. Y. Van Der Meer, B. J. Baas, M. Jeronimus-Stratingh, A. L. Gottumukkala, G. J. Poelarends, A. J. Minnaard, F. J. Dekker, *ChemBioChem* **2014**, *15*, 209–212.
[5] S. Hauke, M. Best, T. T. Schmidt, M. Baalmann, A. Krause, R. Wombacher, *Bioconjug. Chem.* **2014**, *25*, 1632–1637.
[6] K. Kodama, S. Fukuzawa, H. Nakayama, T. Kigawa, K. Sakamoto, T. Yabuki, N. Matsuda, M. Shirouzu, K. Takio, K. Tachibana, S. Yokoyama, *ChemBioChem* **2006**, *7*, 134–139.
[7] A. D. de Bruijn, G. Roelfes, *Chem. Eur. J.* **2018**, *24*, 12728–12733.
[8] N. A. Nguyen, V. Agarwal, *Biochemistry* **2023**, *62*, 1838–1843.
[9] T. K. Lee, B. Manandhar, K. J. Kassees, J. M. Ahn, *J. Org. Chem.* **2020**, *85*, 1376–1384.
[10] J. Cao, L. M. Boatner, H. S. Desai, N. R. Burton, E. Armenta, N. J. Chan, J. O. Castellón, K. M. Backus, *Anal. Chem.* **2021**, *93*, 2610–2618.
[11] M. E. Ourailidou, P. Dockerty, M. Witte, G. J. Poelarends, F. J. Dekker, *Org. Biomol. Chem.* **2015**, *13*, 3648–3653.
[12] N. Eleftheriadis, S. A. Thee, M. R. H. Zwinderman, N. G. J. Leus, F. J. Dekker, *Angew. Chem. Int. Ed.* **2016**, *55*, 12300–12305.
[13] M. Jäger, M. Hartmann, J. G. de Vries, A. J. Minnaard, *Angew. Chem. Int. Ed.* **2013**, *52*, 7809–7812.
[14] K. Chung, R. M. Waymouth, *ACS Catal.* **2016**, *6*, 4653–4659.
[15] W. C. Ho, K. Chung, A. J. Ingram, R. M. Waymouth, *J. Am. Chem. Soc.* **2018**, *140*, 748–757.
[16] N. N. H. M. Eisink, J. Lohse, M. D. Witte, A. J. Minnaard, *Org. Biomol. Chem.* **2016**, *14*, 4859–4864.
[17] N. N. H. M. Eisink, M. D. Witte, A. J. Minnaard, *ACS Catal.* **2017**, *7*, 1438–1445.
[18] S. Ramsay-Burrough, D. P. Marron, K. C. Armstrong, T. J. Del Castillo, R. N. Zare, R. M. Waymouth, *J. Am. Chem. Soc.* **2023**, *145*, 2282–2293.
[19] D. M. Pearson, R. M. Waymouth, *Organometallics* **2009**, *28*, 3896–3900.
[20] R. M. Painter, D. M. Pearson, R. M. Waymouth, *Angew. Chem. Int. Ed.* **2010**, *49*, 9456–9459.
[21] K. Chung, S. M. Banik, A. G. De Crisci, D. M. Pearson, T. R. Blake,

J. V. Olsson, A. J. Ingram, R. N. Zare, R. M. Waymouth, *J. Am. Chem. Soc.* **2013**, *135*, 7593–7602.

[22] I. C. (Steven) Wan, T. A. Hamlin, N. N. H. M. Eisink, N. Marinus, C. de Boer, C. A. Vis, J. D. C. Codée, M. D. Witte, A. J. Minnaard, F. M. Bickelhaupt, *Eur. J. Org. Chem.* **2021**, 632–636.

[23] N. Marinus, N. N. H. M. Eisink, N. R. M. Reintjens, R. S. Dijkstra, R. W. A. Havenith, A. J. Minnaard, M. D. Witte, *Chem. Eur. J.* **2023**, *29*, e202300318.

[24] J. Zhang, N. N. H. M. Eisink, M. D. Witte, A. J. Minnaard, *J. Org. Chem.* **2019**, *84*, 516–525.

[25] N. Marinus, N. Tahiri, M. Duca, L. M. C. M. Mouthaan, S. Bianca, M. van den Noort, B. Poolman, M. D. Witte, A. J. Minnaard, *Org. Lett.* **2020**, *22*, 5622–5626.

[26] A. A. Bastian, M. Bastian, M. Jäger, M. Loznik, E. M. Warszawik, X. Yang, N. Tahiri, P. Fodran, M. D. Witte, A. Thoma, , J. Köhler, A.J. Minnaard, A. Herrmann, *Chem. Eur. J.* **2022**, *28*, e202200883.

[27] M. Ahmadian-Moghaddam, N. R. M. Reintjens, M. D. Witte, A. J. Minnaard, *Eur. J. Org. Chem.* **2023**, *26*, e202300281.

[28] N. R. M. Reintjens, M. D. Witte, A. J. Minnaard, *Org. Biomol. Chem.* **2023**, *21*, 5098–5103.

[29] J. Zhang, N. R. M. Reintjens, J. Dhineshkumar, M. D. Witte, A. J. Minnaard, *Org. Lett.* **2022**, *24*, 5339–5344.

[30] D. T. Bong, M. R. Ghadiri, *Org. Lett.* **2001**, *3*, 2509–2511.

[31] N. R. M. Reintjens, L. Yakovlieva, N. Marinus, J. Hekelaar, F. Nuti, A. M. Papini, M. D. Witte, A. J. Minnaard, M. T. C. Walvoort, *Eur. J. Org. Chem.* **2022**, e202200677.

[32] M. Kuriyama, S. Nakashima, T. Miyagi, K. Sato, K. Yamamoto, O. Onomura, *Org. Chem. Front.* **2018**, *5*, 2364.

[33] N. A. Afagh, A. K. Yudin, *Angew. Chem. Int. Ed.* **2010**, *49*, 262–310.

[34] J. Teichert, B. Feringa, *Synthesis* **2010**, 1200–1204.

[35] D. L. Wright, J. P. Schulte, M. A. Page, *Org. Lett.* **2000**, *2*, 1847–1850.

[36] "Unpublished observation from our group."

[37] J. Zeng, T. Torigoe, Y. Kuninobu, *ACS Catal.* **2022**, *12*, 3058–3062.

[38] Z. Yuan, H. Yang, N. Malik, M. Čolović, D. S. Weber, D. Wilson, F. Bénard, R. E. Martin, J. J. Warren, P. Schaffer, R. Britton, *ACS Catal.* **2019**, *9*, 8276–8284.

[39] M. Salamone, G. Carboni, M. Bietti, *J. Org. Chem.* **2016**, *81*, 9269–9278.

[40] A. D. Westland, *Can. J. Chem.* **1968**, *46*, 2097.

[41] D. H. Ripin, D. A. Evans, , "Evans pKa table," https://organicchemistrydata.org/hansreich/resources/pka/pka_data/evans_pKa_table.pdf**.**

[42] C. D. Ritchie, S. Lu, *J. Am. Chem. Soc.* **1989**, *111*, 8542–8543.

[43] A. Trummal, L. Lipping, I. Kaljurand, I. A. Koppel, I. Leito, *J. Phys. Chem. A* **2016**, *120*, 3663–3669.

[44] K. D. Green, S. Garneau-Tsodikova, *Front. Microbiol.* **2013**, *4*, 208.

[45] D. Moazed, H. F. Noller, *Nature* **1987**, *327*, 389–394.

[46] A. P. Carter, W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly, V. Ramakrishnan, *Nature* **2000**, *407*, 340–348.

[47] J. O'Neill, *Rev. Antimicrob. Resist.* **2014**, 1–16.

[48] M. Miethke, M. Pieroni, T. Weber, M. Brönstrup, P. Hammann, L. Halby, P. B. Arimondo, P. Glaser, B. Aigle, H. B. Bode, R. Moreira, Y. Li, A. Luzhetskyy, M. H. Medema, J. L. Pernodet, M. Stadler, J. R. Tormo, O. Genilloud, A. W. Truman, K. J. Weissman, E. Takano, S. Sabatini, E. Stegmann, H. Brötz-Oesterhelt, W. Wohlleben, M. Seemann, M. Empting, A. K. H. Hirsch, B. Loretz, C. M. Lehr, A. Titz, J. Herrmann, T. Jaeger, S. Alt, T. Hesterkamp, M. Winterhalter, A. Schiefer, K. Pfarr, A. Hoerauf, H. Graz, M. Graz, M. Lindvall, S. Ramurthy, A. Karlén, M. van Dongen, H. Petkovic, A. Keller, F. Peyrane, S. Donadio, L. Fraisse, L. J. V. Piddock, I. H. Gilbert, H. E. Moser, R. Müller, *Nat. Rev. Chem.* **2021**, *5*, 726–749.

[49] D. D. Boehr, I. F. Moore, G. D. Wright, in *Front. Antimicrob. Resist.*, ASM Press, Washington DC, **2005**, pp. 85–100.

[50] M.-P. Mingeot-Leclercq, Y. Glupczynski, P. M. Tulkens, *Antimicrob. Agents Chemother.* **1999**, *43*, 727–737.

[51] J. B. Aggen, E. S. Armstrong, A. A. Goldblum, P. Dozzo, M. S. Linsell, M. J. Gliedt, D. J. Hildebrandt, L. A. Feeney, A. Kubo, R. D. Matias, S. Lopez, M. Gomez, K. B. Wlasichuk, R. Diokno, G. H. Miller, H. E. Moser, *Antimicrob. Agents Chemother.* **2010**, *54*, 4636–4642.

[52] G. G. Zhanel, C. D. Lawson, S. Zelenitsky, B. Findlay, F. Schweizer, H. Adam, A. Walkty, E. Rubinstein, A. S. Gin, D. J. Hoban, J. P. Lynch, J. A. Karlowsky, *Expert Rev. Anti. Infect. Ther.* **2012**, *10*, 459–473.

[53] G. C. Sati, V. A. Sarpe, T. Furukawa, S. Mondal, M. Mantovani, S. N. Hobbie, A. Vasella, E. C. Böttger, D. Crich, *ACS Infect. Dis.* **2019**, 1718–1730.

[54] A. A. Bastian, E. M. Warszawik, P. Panduru, C. Arenz, A. Herrmann, *Chem. Eur. J.* **2013**, *19*, 9151–9154.

[55] A. R. Mandhapati, G. Yang, T. Kato, D. Shcherbakov, S. N. Hobbie, A. Vasella, E. C. Böttger, D. Crich, *J. Am. Chem. Soc.* **2017**, *139*, 14611–14619.

[56] R. B. Yan, M. Yuan, Y. Wu, X. You, X. S. Ye, *Bioorg. Med. Chem.* **2011**, *19*, 30–40.

[57] J. L. Asensio, A. Hidalgo, A. Bastida, M. Torrado, F. Corzana, J. L. Chiara, E. García-Junceda, J. Cañada, J. Jiménez-Barbero, *J. Am. Chem. Soc.* **2005**, *127*, 8278–8279.

[58] S. Umezawa, T. Tsuchiya, in *Amin. Antibiot.*, **1982**, pp. 37–110.

[59] S. Zárate, M. De la Cruz Claure, R. Benito-Arenas, J. Revuelta, A. Santana, A. Bastida, *Molecules* **2018**, *23*, 284.

[60] N. Thamban Chandrika, S. Garneau-Tsodikova, *Chem. Soc. Rev.* **2018**, *47*, 1189–1249.

[61] K. J. Labby, S. Garneau-Tsodikova, *Future Med. Chem.* **2013**, *5*, 1285–1309.

[62] S. Duscha, H. Boukari, D. Shcherbakov, S. Salian, S. Silva, A. Kendall, T. Kato, R. Akbergenov, D. Perez-Fernandez, B. Bernet, S. Vaddi, P. Thommes, J. Schacht, D. Crich, A. Vasella, E. C. Böttger, *MBio* **2014**, *5*, 1–10.

[63] T. Matsushita, W. Chen, R. Juskeviciene, Y. Teo, D. Shcherbakov, A. Vasella, E. C. Böttger, D. Crich, *J. Am. Chem. Soc.* **2015**, *137*, 7706–7717.

[64] N. T. Chandrika, S. Garneau-Tsodikova, *Medchemcomm* **2016**, *7*, 50–68.

[65] T. Matsushita, G. C. Sati, N. Kondasinghe, M. G. Pirrone, T. Kato, P. Waduge, H. S. Kumar, A. C. Sanchon, M. Dobosz-Bartoszek, D. Shcherbakov, M. Juhas, S. N. Hobbie, T. Schrepfer, C. S. Chow, Y. S. Polikanov, J. Schacht, A. Vasella, E. C. Böttger, D. Crich, *J. Am. Chem. Soc.* **2019**, *141*, 5051–5061.

[66] W. Zhang, Y. Chen, Q. Liang, H. Li, H. Jin, L. Zhang, X. Meng, Z. Li, *J. Org. Chem.* **2013**, *78*, 400–409.

[67] E. C. Böttger, D. Crich, *ACS Infect. Dis.* **2020**, *6*, 168–172.

[68] L. P. Kotra, J. Haddad, S. Mobashery, *Antimicrob. Agents*

*Chemother.* **2000**, *44*, 3249–3256.

[69] J. Haddad, S. Vakulenko, S. Mobashery, *J. Am. Chem. Soc.* **1999**, *121*, 11922–11923.

[70] D. Lubriks, R. Zogota, V. A. Sarpe, T. Matsushita, G. C. Sati, K. Haldimann, M. Gysin, E. C. Böttger, A. Vasella, E. Suna, S. N. Hobbie, D. Crich , *ACS Infect. Dis.* **2021**, *7*, 2413–2424.

[71] J. C. K. Quirke, G. C. Sati, A. Sonousi, M. Gysin, K. Haldimann, E. C. Böttger, A. Vasella, S. N. Hobbie, D. Crich, *ChemMedChem* **2022**, *17*, e202200120.

[72] R. Mohamad-Ramshan, C. Ande, T. Matsushita, K. Haldimann, A. Vasella, S. N. Hobbie, D. Crich, *Tetrahedron* **2023**, *135*, 133330.

[73] D. Lubriks, K. Haldimann, S. N. Hobbie, A. Vasella, E. Suna, D. Crich, *Antibiotics* **2023**, *12*, 25.

[74] D. Lubriks, K. Haldimann, F. Kiliç, M. Hartmann, E. C. Böttger, S. N. Hobbie, E. Suna, D. Crich, *Helv. Chim. Acta* **2023**, *106*, e202300138.

[75] S. Jana, P. Rajasekaran, K. Haldimann, A. Vasella, E. C. Böttger, S. N. Hobbie, D. Crich, *ACS Infect. Dis.* **2023**, *9*, 1622–1633.

[76] M. Ramirez, M. Tolmasky, *Molecules* **2017**, *22*, 2267.

[77] T. Yoneta, T. Matsuno, H. Nanahoshi, S. Fukatsu, *Chem. Pharm. Bull.* **1981**, *29*, 3469–3473.

[78] T. Jikihara, T. Tsuchiya, H. Umezawa, S. Umezawa, *Carbohydr. Res.* **1981**, *89*, 91–101.

[79] E. Umemura, T. Tsuchiya, Y. Kobayashi, K. Tanaka, *Carbohydr. Res.* **1992**, *224*, 141–163.

[80] Y. Takahashi, T. Tsuchiya, S. Umezawa, H. Umezawa, *Carbohydr. Res.* **1991**, *210*, 221–232.

[81] T. Tsuchiya, Y. Takahashi, Y. Kobayashi, S. Umezawa, H. Umezawa, *J. Antibiot. (Tokyo).* **1985**, *38*, 1287–1290.

[82] H. Umezawa, T. Tsuchiya, R. Muto, S. Umezawa, *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2842–2847.

[83] N. E. Dixon, G. A. Lawrance, P. A. Lay, A. M. Sargeson, H. Taube, in *Inorg. Synth.*, **1990**, pp. 70–76.

[84] V. Pozhydaiev, M. Power, V. Gandon, J. Moran, D. Lebœuf, *Chem. Commun.* **2020**, *56*, 11548–11564.

[85] N. Marinus, M. T. C. Walvoort, M. D. Witte, A. J. Minnaard, H. M. van Dijk, in *Carbohydr. Chem. Proven Synth. Methods, Vol. 5* (Eds.: P. Kosma, T. Wrodnigg, A. Stütz), CRC Press, **2021**, p. Chapter 16.

[86] H. M. Liu, Y. Tsuda, *Chem. Pharm. Bull.* **1996**, *44*, 80–87.

[87] H. Umezawa, S. Kondo, in *Methods Enzymol.*, **1975**, pp. 263–278.

[88] W. P. Gallagher, A. Vo, *Org. Process Res. Dev.* **2015**, *19*, 1369–1373.

[89] N. R. Conley, L. A. Labios, D. M. Pearson, C. C. L. McCrory, R. M. Waymouth, *Organometallics* **2007**, *26*, 5447–5453.

[90] P. A. Burland, H. M. I. Osborn, A. Turkson, *Bioorg. Med. Chem.* **2011**, *19*, 5679–5692.

[91] G. Sosnovsky, C. Thomas Gnewuch, *J. Pharm. Sci.* **1994**, *83*, 989–998.

[92] M. K. Patel, B. G. Davis, *Org. Lett.* **2013**, *15*, 346–349.

[93] *CLSI (2015). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically-Tenth Edition: Approved Standard M07-A10CLSI, Wayne, PA, USA.* **2015**, 2015.

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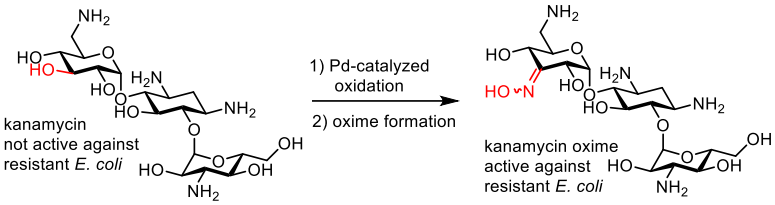


Protonation of amino groups and phosphates in aminoglycosides and sugar phosphates allows direct palladium-catalyzed site-selective oxidation without the use of protecting groups. The site-selective oxidation of kanamycin and amikacin allowed the preparation of a set of 3'-modified aminoglycoside derivatives of which two show promising activity against antibiotic-resistant *E. coli* strains.

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