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Hybrid Aminoglycoside Antibiotics *via* Tsuji Palladium-Catalyzed Allylic Deoxygenation

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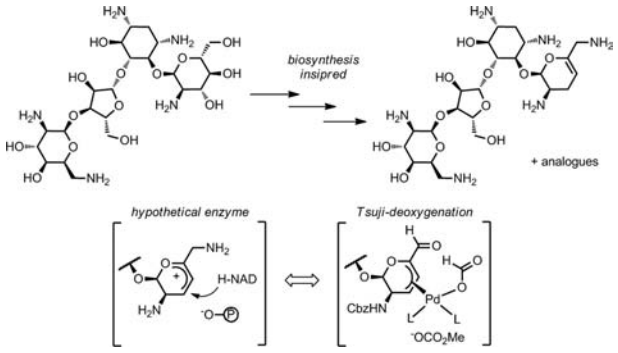
ABSTRACT



Biosynthetically inspired manipulation of the antibiotic paromomycin led, in six high-yielding steps, to a ring A harboring an α,β-unsaturated 6′-aldehyde and an allylic 3′-methylcarbonate group. Tsuji deoxygenation in the presence of 5 mol % $Pd_2(dba)_3$ and Bu_3P granted access to a novel series of 3′,4′-dideoxy-4′,5′-dehydro ring A hybrids. The neomycin−sisomicin hybrid exhibited superior *in vitro* antibacterial activity to the parent compound neomycin.

Aminoglycoside antibiotics have occupied an important position in the arsenal of antibacterial therapeutics for over half a century.[1] They are powerful broad-spectrum antibiotics which target an rRNA helix at the mRNA-tRNA decoding center of the bacterial 30S ribosomal subunit,[2] effecting their bactericidal action by inducing translation inaccuracy and inhibition.[2] As classical representatives, the gentamicin complex, amikacin, neomycin, and tobramycin have been used in clinical practice as intravenous, topical, or nebulizer drugs.[1] However, their widespread and first-line use has been compromised by the emergence of drug-resistant strains of bacteria, which express aminoglycoside modifying enzymes, classified as acetyltransferases (AACs), phosphotransferases (APHs), and adenyltransferases (ANTs).[1,3] Extensive efforts have been made over the years to overcome the growing resistance problem through chemical removal of susceptible functionalities in the 4,6-substituted 2-deoxystreptamine aminoglycosides, starting with the readily available kanamycins.[1,4] These strategies have been successful, exemplified by the marketed drugs amikacin and arbekacin.[5] In contrast, the

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4,5-disubstituted deoxystreptamine class remains relatively less explored.[5]

Paromomycin, a member of the 4,5-disubstituted 2-deoxystreptamine aminoglycosides, has been in clinical use against amoebic dysentery and leishmaniasis in underdeveloped regions of the world (Figure 1).[1,6] Its 6′-amino congener, neomycin, is widely used as a topical agent worldwide (e.g., Neosporin).[1] However, the structural features of paromomycin and neomycin make them highly susceptible to inactivating enzymes such as APH(3′) and ANT(4′) that target ring A hydroxyl groups (Figure 1).[1−4]

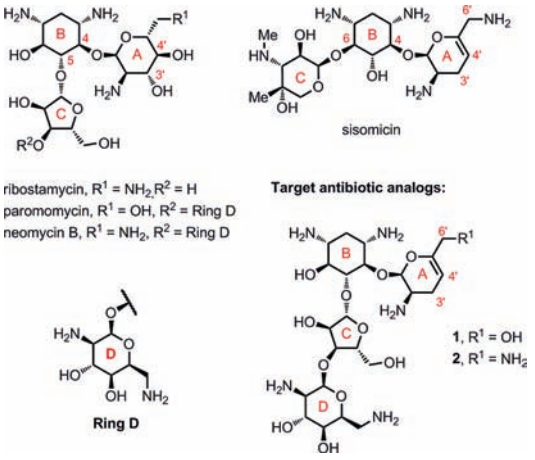


Figure 1. Aminoglycosides of the paromomycin and sisomicin families, and the designed target hybrid analogs **1** and **2**.[7−9]

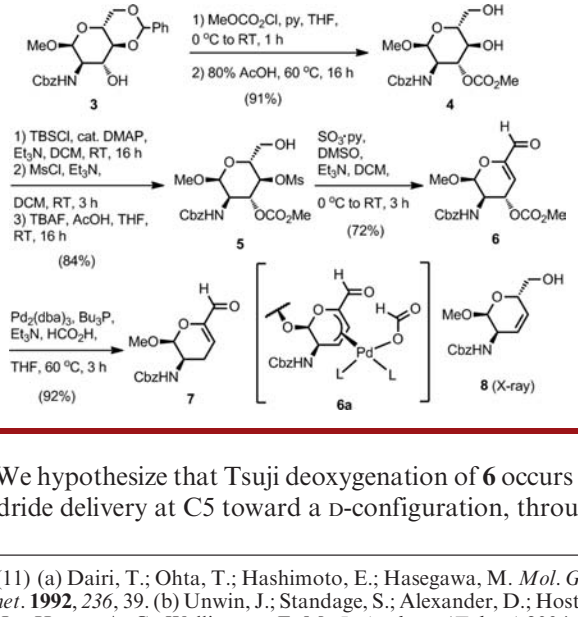
Unlike the structurally diverse 4,6-disubstituted 2-deoxystreptamine subclass,[1,7] fewer modified analogs of paromomycin and neomycin have been isolated[1,7] or chemically synthesized,[10] and instead most of their congeners feature a primordial 2-amino-2-deoxy-α-D-glucopyranosyl moiety as ring A.[8]

Sisomicin is a naturally derived congener of gentamicin that features a 6′-amino-3′,4′-dideoxy-4′,5′-dehydro ring A (Figure 1).[1,9] Intrigued by the biosynthetic transformation of aminoglycoside precursors to sisomicin and gentamicin,[8] we considered the synthesis of paromomycin and neomycin analogs in which the enzyme-susceptible ring A was exchanged for the same subunit found in sisomicin. Little detail is known of the 3′ and 4′ deoxygenation steps between gentamicin precursors and the final fermentation products.[8] However, recent reports of the surprising biosynthetic involvement of an APH(3′) kinase in these gene clusters[11] lead us to hypothesize that the 3′-hydroxyl was being activated as a leaving group to be removed profiting from the stabilization from the neighboring electron-rich olefin, which could, in turn, arise from a 6′-aldehyde-equivalent pyridoxal-catalyzed 4′,5′-dehydration.[7] Therefore, we set out to find ways in which abiotic versions of such activated intermediates could be harnessed and controlled for reduction.[7]

Herein we report on our attaining these objectives relying on the venerable Tsuji Pd-catalyzed deoxygenation reaction,[12,13] as applied to the aminoglycoside series for the first time.[12−14]

To explore the feasibility of introducing the combined features of 4′,5′-unsaturation and 3′-deoxygenation we devised a practical protocol on a ring A model, starting with known sugar **3** (Scheme 1).[7] We installed a C3- methylcarbonate, followed by liberation of the 4,6-diol to **4**. Conversion to the 4-mesylate **5**, followed by oxidation and β-elimination, led to the aldehyde **6**. During the course of a substrate scope study for Tsuji deoxygenation (Table S1),[7] we discovered that, under unoptimized conditions, treatment of aldehyde **6** with $Pd_2(dba)_3$ (20 mol %) in the presence of Bu_3P (40 mol %), Et_3N, and formic acid in degassed THF at 60 °C[12] resulted in the reliable conversion to the 3-deoxy-4,5-unsaturated aldehyde (**7**) in 92% yield.

Scheme 1. Pd-Catalyzed Allylic Deoxygenation: Model Studies



We hypothesize that Tsuji deoxygenation of **6** occurs by hydride delivery at C5 toward a D-configuration, through

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(7) See Supporting Information for further details, complete listing of aminoglycosides, and a proposed biosynthetic pathway.

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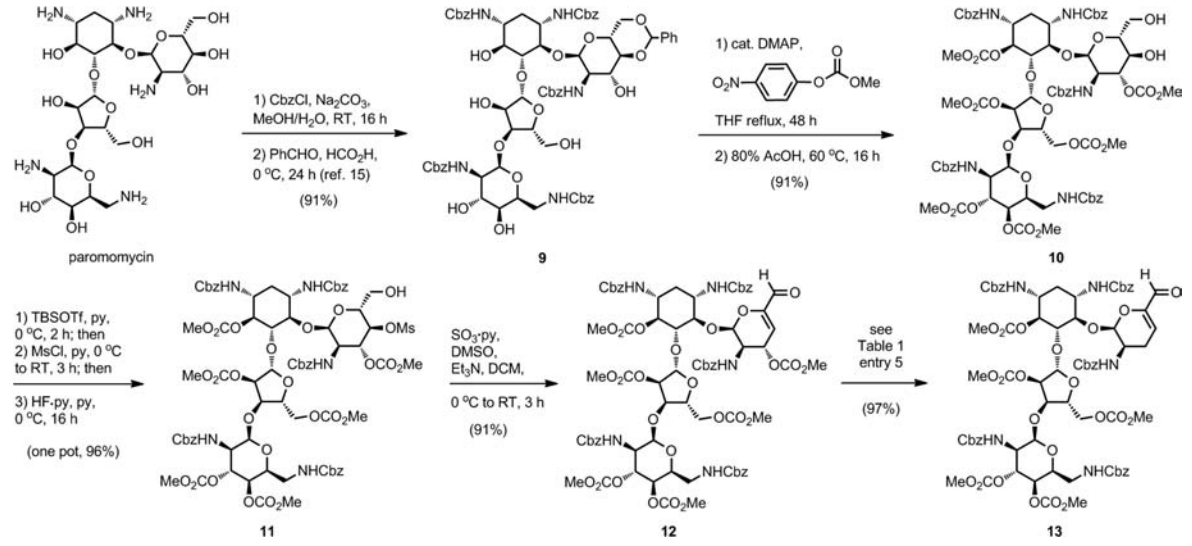
(14) For an application of a similar reaction to carbohydrates, see: Greenspoon, N.; Keinan, E. *J. Org. Chem*. **1988**, *53*, 3723.

Scheme 2. Application of the Tsuji Palladium-Catalyzed Allylic Deoxygenation to Paromomycin



an *endo*-face π-allyl complex (**6a**), followed by rapid olefin isomerization *in situ*.[7] In support of this mechanism, identical Tsuji deoxygenation of analog substrates of the C6-alcohol oxidation state provided only 3,4-unsaturated products with a C5-D-configuration, exemplified by **8**, determined by X-ray crystallography.[7] However, unlike aldehyde **6**, the supplemental substrate series we explored underwent reduction sluggishly in low to moderate yields, albeit at prohibitively high catalyst loadings.[7] The properties of allylic α,β-unsaturated aldehydes such as in **6** appear streamlined for Tsuji deoxygenation, yet are not represented in the literature.[12]

We were now poised to apply our Tsuji deoxygenation protocol of allylic carbonates to highly functionalized aminoglycoside derivatives, seeking dideoxygenated analogs which could evade the APH(3′) and ANT(4′) modifying enzymes. Experience in our group and others has shown that paromomycin is a functionally malleable aminoglycoside for medicinal chemistry.[1,10,15] Paromomycin sulfate was protected with *N*-Cbz groups, followed by formation of the 4′,6′-*O*-benzylidene derivative **9** as a functional handle for ring A (Scheme 2).[15] The remaining alcohol functionalities in **9** were capped with methylcarbonates using a transesterificaiton protocol, simultaneously providing the requisite 3′-leaving group. Removal of the benzylidene acetal afforded ring-A diol **10**, which was converted to 4′-mesylate **11** and submitted to Parikh–Doering oxidation/β-elimination to give intermediate **12**, in excellent overall yield. The heavily functionalized aminoglycoside aldehyde **12** showed excellent concordance with the reactivity of the ring-A model (Scheme 1). Following optimization of the deoxygenation protocol, using 5 mol % $Pd_2(dba)_3$ and 20 mol % Bu_3P provided a nearly quantitative yield of the desired 3′-deoxy-4′,5′-unsaturated aldehyde **13** (Table 1). Changing the ligand stoichiometry to a 1:4 ratio was indispensable for this improvement (Table 1, entry 5).

Table 1. Optimization of Catalyst and Ligand Loading[a]

entry	$Pd_2(dba)_3$ (mol %)	Bu_3P (mol %)	time (h)	yield **13** (%)[b]
1	20	40	3	82
2	10	20	9	73
4	5	10	24	46
5	**5**	**20**	**3**	**97**
6	2.5	10	5	80

[a] Conditions: 0.1 M in THF, 60 °C, 10 equiv of HCO_2H, 11 equiv of Et_3N. [b] Isolated yields.

Hence, 3′,4′-dideoxygenated intermediate **13** was produced in enabling gram scale and ~60% overall yield from paromomycin sulfate. We then directed our efforts to produce the first generation of novel 3′,4′-dideoxy-4′,5′-unsaturated hybrid tetrasaccharide aminoglycosides with a sisomicin ring-A motif (Scheme 3). The 6′-position was functionalized by reduction and introduction of a 6′-azide, followed by facile cleavage of the remaining methylcarbonate groups to **14** (Scheme 3). The end-game global deprotection required Birch reduction to afford aminoglycoside analogs **1** and **2**, for it is well established that hydrogenation would lead to reduction of the olefin predominantly toward inactive C5′-L-diastereomers.[4]

Our first generation of novel sisomicin-hybrid aminoglycosides were tested against a panel of isolates representing susceptible wild-types and strains known to contain inactivating enzymes (Table 2). Preliminary data indicate

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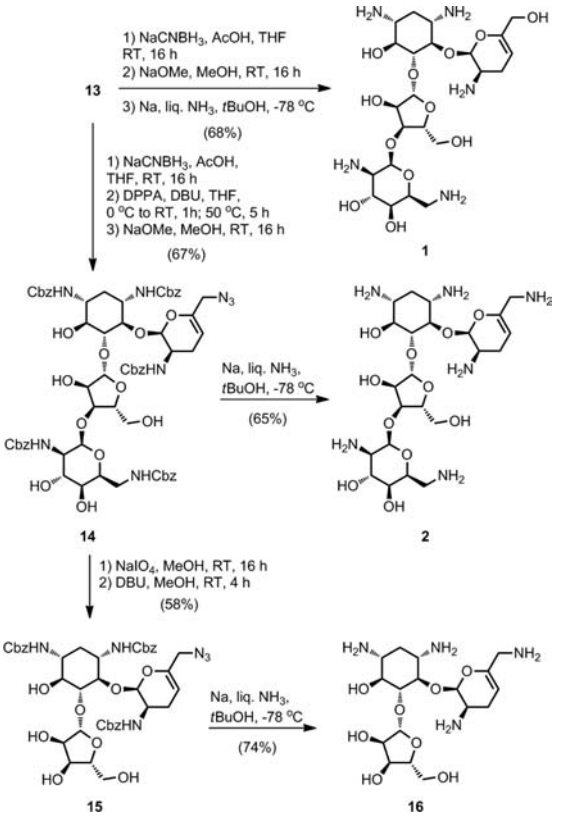
Table 2. Antibacterial Assessment: Sisomicin Ring A Hybrids, Parent Antibiotics, and Controls; Minimum Inhibitory Concentrations (μg/mL)[a]

entry	strain name	strain code/enzyme	Amk	Gent	Par	NeoB	Rib	Btr	**1**	**2**	**16**
1	*S. aureus*	ATCC 29213	2	1	2	1	16	8	8	**1**	16
2	*E. coli*	ATCC 25922	2	0.5	4	2	8	2	>64	**2**	64
3	*K. pnuemoniae*	ATCC 10031	0.5	0.25	2	0.5	2	1	>64	**0.5**	16
4	*P. aeruginosa*	ATCC 27853, APH(3′)	1	0.5	>64	32	>64	>32	>16	≤**1**	16
5	*S. aureus*	ANT(4′)	64	0.5	>64	>64	>64	>32	16	**1**	16

[a] Abbreviations: Amk, amikacin; Gent, gentamicin complex; Par, paromomycin; NeoB, neomycin B; Rib, ribostamycin; Btr, butirosin.[7]

Scheme 3. Neomycin and Paromomycin Ring A Hybrid Analogs



in vitro growth inhibition for neomycin analog **2**, with MIC values ranging from 0.5 to 2 μg/mL in *E. coli, K. pneumoniae, P. aeruginosa,* and *S. aureus*. The activity of analog **2** was not affected by two resistance enzymes prevalent in *P. aeruginosa* and *S. aureus* (from ≥32- and >64-fold improvement over neomycin and amikacin, respectively). In contrast, analog **1** displayed from 4- to greater than 32-fold less potency in four out of the five strains compared to the parent antibiotic paromomycin.[10b,15]

To extend our knowledge of the role played by ring D in these hybrid molecules, we profited from the isolated diols in **14**, to perform oxidative cleavage and β-elimination to give **15** (Scheme 3).[15a] Using this protocol, we accessed pseudotrisaccharide hybrid **16** related to the ribostamycin, previously obtained from degradation of sisomicin followed by ribosylation, but whose antimicrobial activity has not been reported.[16] The NMR spectrum was identical to that of the literature compound,[16] providing further support to the structural assignment of our novel aminoglycoside analogs. In general, we observed that ring D truncated hybrids, such as **16** were 16- to 32-fold less active compared to the tetrasaccharide congener **2**, and ribostamycin was greater than 2- to 16-fold less active than neomycin B. We did not detect growth inhibition using the 6′-OH analog of **16**.[7]

To conclude, we have demonstrated that a biosynthesis-inspired paradigm relying on transition metal control of the venerable Tsuji Pd-catalyzed deoxygenation in the aminoglycoside series can provide a novel analog series with promising antibacterial activities. Herein, we have determined that the combination of 6′-amino substitution and 3′,4′-dideoxygenation are best tolerated in the neomycin series, while providing evasion of APH(3′) and ANT(4′) enzymes shown in Table 2. Our novel deoxygenated 4,5-disubstituted 2-deoxystreptamine analogs profit from neomycin-like features such as the L-idose ring D, which can explore an extended binding pocket and provide additional beneficial RNA contacts.[2,10] Meanwhile lacking the third accessory ring C of gentamicins and kanamycins may hinder recognition by widespread subtypes of inactivating enzymes.[3,4] Further details on second generation analogs of sisomicin–neomycin hybrids **2** and **16** will be reported in due course.

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Supporting Information Available. Experimental procedures, supplemental substrate scope table, ORTEP of **8**, and full spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.