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Synthesis of saccharocin from apramycin and evaluation of its ribosomal selectivity†

 Vikram A. Sarpe,^{*a} Michael G. Pirrone,^a Klara Haldimann,^b Sven N. Hobbie,^b
 Andrea Vasella^c and David Crich ^{*a}

We describe a straightforward synthesis of the apramycin biosynthetic precursor saccharocin from apramycin by regioselective partial azidation followed by stereoretentive oxidative deamination. Saccharocin was found to exhibit excellent selectivity for inhibition of the bacterial ribosome over the eukaryotic ribosomes indicating that its presence as a minor impurity in apramycin itself should not be problematic in the development of the latter as a clinical candidate.

The atypical 2-deoxystreptamine class aminoglycoside antibiotic (AGA) apramycin **1** (Fig. 1) enjoys broad activity against methicillin-resistant *Staphylococcus aureus* and Gram-negative bacterial infections including the carbapenem resistant enterobacteriaceae (CREs) and the ESKAPE pathogens.^{1–7} Due to its unusual structure apramycin blocks the action of the vast majority of aminoglycoside modifying enzymes (AMEs) and so circumvents most common AGA resistance mechanisms.^{1,8} This structural protection from resistance mechanisms extends to the action of the ribosomal methyl transferases acting on G1405 in the AGA binding site, which are increasingly found encoded on the same plasmid as metallocarbapenemases,^{4,9–11} and which effectively suppress the action of all 4,6-disubstituted AGAs in current clinical practice, including the recently introduced plazomicin.^{5,12–14} These attributes and the excellent selectivity of apramycin for the target bacterial over eukaryotic ribosomes, which results in reduced ototoxicity in the guinea pig model,⁸ combine to make apramycin a clinical candidate for the treatment of CREs.¹⁵

Saccharocin, 4"-desamino-4"-hydroxy apramycin **2** (Fig. 1) is a late stage biosynthetic precursor of apramycin¹⁶ with established antibacterial activity,^{17,18} that is a potential contaminant of apramycin. As such we required a straightforward synthesis of saccharocin to evaluate its ribosomal selectivity profile and potential for toxic side effects. Accordingly, we describe the facile synthesis of saccharocin from apramycin and

a comparison of their ribosomal selectivities. The synthesis is achieved by controlled regioselective triazidation of three of the four primary amino groups followed by stereospecific oxidative deamination^{19,20} of the remaining 4"-amine under conditions developed in our laboratories for use in sialic acid chemistry,^{21–24} following the seminal work of Zbiral.²⁵ The selective azidation method should also greatly facilitate the preparation of other apramycin 4"-derivatives, such as have previously only been accessible by circuitous routes.^{26,27}

Results and discussion

Extensive experimentation revealed that quenching the copper sulfate-catalyzed reaction of **1** with triflyl azide^{28,29} before complete conversion afforded the known³⁰ tetraazide **3** in 52% yield, and a crude preparation of a largely single triazide **4**. For practical purposes the mixture of **3** and **4** were acetylated with acetic anhydride in pyridine and then separated chromatographically resulting in the isolation of the peracetyl tetraazide **5** in 26% yield, and the triazido-hepta-acetyl derivative **6** in 37% yield for the two steps (Scheme 1). Alternatively, treatment of the crude preparation of **4** with trifluoroacetic anhydride and potassium acetate at 50 °C followed by release of the trifluoroacetyl esters under acidic conditions afforded the triazido trifluoroacetamide **7** in 29% yield (Scheme 1). The advantage of **7** derives from its clean rotamer-free NMR

1, X = NH₂, apramycin
2, X = OH, saccharocin

Fig. 1 Apramycin (**1**) and saccharocin (**2**).

^a Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA. E-mail: vasarpe@gmail.com, dcrich@chem.wayne.edu

^b Institut für Medizinische Mikrobiologie, Universität Zürich, Gloriastrasse 30, 8006 Zürich, Switzerland

^c Laboratorium für Organische Chemie, ETH Zürich, Vladimir-Prelog-Weg 1-5/10, 8093 Zürich, Switzerland

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Scheme 1 Regioselective triazidation of apramycin.

spectra which greatly facilitate characterization and localization of the amide group at the 4''-position. Hydrolysis of 7 with sodium methoxide in methanol provided pure 4 suitable for characterization purposes in 55% yield. Clearly, the 4''-amino group of apramycin is significantly less nucleophilic toward the electrophilic triflyl azide-copper sulfate combination than any of the other amino groups enabling direct preparations of 4, 6, 7 and potentially many other derivatives with useful antibacterial activity.^{26,27} The reduced nucleophilicity of the 4''-amino group is presumably a function of the greater number of electron-withdrawing and sterically bulky C–O bonds in its immediate vicinity than is the case for the other amino groups in the parent. This is consistent with the well-known reduction of basicity in amines by β - and γ -C–O bonds,³¹ the experimental pK_a values of the various basic amines in apramycin,^{32,33} and the general low nucleophilicity of the glucopyranose 4-hydroxy group.³⁴

Treatment of 6 with nitrosyl tetrafluoroborate and pyridine in dichloromethane at -10°C gave an *N*-nitrosoamide 8, identified by EI-mass spectrometry, that was exposed to preformed sodium trifluoroethoxide and 18-crown-6 at the same temperature, as described in the sialic acid series,^{21–24} followed rapidly by the addition of glacial acetic acid and resulting overall in the isolation of the desired desacetamido-acetoxy derivative 9 in 59% overall yield (Scheme 2). Importantly, this oxidative deamination affords a single diastereoisomer of the product and proceeds with overall retention of configuration as is apparent from the pseudo-triplet nature

Scheme 2 Stereoretentive oxidative deamination and completion of the synthesis of saccharocin (2).

of H4'' with $J = 9.9\text{ Hz}$ in the ^1H -NMR spectrum in $\text{DMF-}d_7$ at 60°C . This is consistent with the precedent for the oxidative deamination of 4-aminoglucose derivatives and with that in the sialic acid series.^{20,25,35} Based on our extensive work on this reaction in the sialic acid series we believe this outcome is the result of *trans*-annular participation by the pyranoside ring oxygen as suggested originally for cognate reactions by Stevens and Horton,^{36–40} as also observed in the 5-thiaglucopyranosides,⁴¹ and related to the more familiar participation by the ring nitrogen in the interconversion of 3-substituted piperidines and prolinol derivatives.⁴² Mass spectrometry of the crude reaction mixture from this oxidative deamination indicates the formation of a byproduct(s) arising from formal elimination of the acetamido group, again consistent with work in the sialic acid series, that was not isolated. Finally, 9 was heated to 60°C with barium hydroxide in aqueous 1,4-dioxane, followed by hydrogenolysis over palladium hydroxide on carbon to give saccharocin 2, which was isolated in 40% yield in the form of its tetraacetate salt after chromatography over Sephadex and lyophilization (Scheme 2), and whose spectral data were consistent with those in the literature.^{17,18}

Ribosomal selectivity

Apramycin 1 and saccharocin 2 were screened for their ability to inhibit luciferase production by bacterial and eukaryotic ribosomes in a series of cell-free assays using isolated bacterial and humanized bacterial ribosomes (Table 1). The humanized bacterial ribosomes incorporate a complete decoding A site from the human mitochondrial ribosome (Mit 13), from the A1555G mutant of the human mitochondrial ribosome (A1555G), and the human cytoplasmic ribosome (Cyt 14) (Fig. 2).⁴³ The ratio of the IC_{50} values for the humanized ribosomes to those for the bacterial ribosome provides an estimation of the selectivity and so of toxicity at the level of the target decoding A site. High selectivity for the bacterial over the mitochondrial and A1555G mutant mitochondrial ribosomes is suggestive of minimal ototoxicity, it having been demonstrated that inhibition of the mitochondrial ribosome and its A1555G mutant found in a subset of the population that is hypersusceptible to drug-induced ototoxicity is a major cause of ototoxicity.⁴⁴ The absence of selectivity for the bacterial over the cytoplasmic ribosome on the other hand would be indicative of more broad and systemic toxicity.

Table 1 Antiribosomal activity and selectivity of apramycin (1) and saccharocin (2)

	IC_{50}^a ($\mu\text{g ml}^{-1}$)				Selectivity		
	wt	Mit13	A1555G	Cyt14	Mit13	A1555G	Cyt14
1	0.078	67	40	69	859	513	885
2	0.16	>500	307	437	>3125	1918	2731

^a All measurements were made in duplicate.

Fig. 2 Decoding A sites of bacterial and eukaryotic ribosomes (the AGA binding pocket is boxed). The prokaryotic numbering scheme is illustrated for the AGA binding pocket. Changes in the binding pocket from the bacterial ribosome are coloured green. The A1555G mutant conferring hypersusceptibility to aminoglycoside ototoxicity is coloured red.

Table 2 Antibacterial activity against relevant bacterial pathogens ($\mu\text{g mL}^{-1}$)^a

	Strain	1	2
<i>E. coli</i>	AG001	4	8–16
<i>K. pneumoniae</i>	AG215	1–2	4
<i>E. cloacae</i>	AG290	2–4	4–8
<i>A. baumannii</i>	AG309	4	8
<i>P. aeruginosa</i>	AG220	4	8–16
MRSA	AG038	4	32

^a All measurements were made in duplicate.

As is clear from Table 1, saccharocin is two-fold less active than apramycin for the inhibition of the bacterial ribosome suggesting a minor reduction in antibacterial activity. It is more than two-fold less active than apramycin against each of the humanized ribosomes leading to a generally increased selectivity. On this basis, and as the selectivity of apramycin is already such that only very minimal ototoxicity is seen in the guinea pig model at doses up to ten times the estimated clinical dose, saccharocin is not expected to display any significant ototoxicity.

In keeping with the two fold reduction in IC_{50} values for the bacterial ribosome as compared to apramycin, saccharocin displayed approximately two-fold reductions in antibacterial activity against MRSA and a selection of Gram-negative pathogens (Table 2) consistent with the earlier literature.^{17,18} No attempt was made to test the antibacterial activity of saccharocin in the presence of aminoglycoside modifying enzymes or of ribosomal transferases, as none of these act at or near the 4"-position of apramycin.

Conclusion

Saccharocin is readily accessed by partial regioselective azidation of apramycin followed by stereoretentive oxidative deamination of the remaining free amino group at the 4"-po-

sition, and eventual deprotection. Saccharocin shows increased selectivity for the bacterial over humanized bacterial ribosomes compared to apramycin suggestive of little or no ototoxic side effects. Saccharocin is approximately two-fold less active than apramycin against MRSA and typical ESKAPE pathogens consistent with their relative IC_{50} values for the inhibition of the bacterial ribosome. Together these factors suggest that the possible presence of minor quantities of saccharocin in apramycin does not present any cause for concern from either a toxicity or an activity standpoint. The simple chemistry developed for the differentiation of the 4"-amino group of apramycin should also be applicable to the improved synthesis of new and existing apramycin derivatives modified at that position.

Conflicts of interest

SNH, AV, and DC are cofounders of and have an equity interest in Juvabis AG, a biotech startup developing aminoglycoside antibiotics.

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