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Rapid solid-phase syntheses of a peptidic-aminoglycoside library

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ABSTRACT

A library of mono- and di-amino acid peptidic-aminoglycosides (PAs), with kanamycin and neomycin as the model aminoglycosides, was systematically and rapidly synthesized via solid phase peptide synthesis. Aminoglycosides were first converted into *N*-Boc protected carboxylic acids and fifteen L-amino acids were then used in the diversification of the full library. The approach outlined describes a rapid synthetic procedure where >200 PA compounds can be synthesized in a few months with 85–95% purity. UV thermal denaturation assessed the binding stabilization by PAs to model human and bacterial A-site rRNA sequences. Significant differences were found in thermal melting profiles among PAs that were attributed to specific amino acid sequences. Neomycin PAs lead to a much larger variation in the stabilization of A-site rRNA sequences ($\Delta T_m = 2.6–17.1\ ^\circ C$) as compared to kanamycin PAs ($\Delta Tm = 0.4–4.3\ ^\circ C$). Kanamycin PAs had little activity against Gram-negative and Gram-positive bacteria as compared with neomycin PAs that had significant antibacterial activity with MIC ranging from 2 to 16 μM.

1. Introduction

Aminoglycosides have been at the forefront of antimicrobial therapy for the past seven decades. In addition to their use as antibiotics for the treatment of Gram-positive and Gram-negative bacterial infections, aminoglycosides are useful as scaffolds for the recognition of nucleic acids [1–3]. Aminoglycosides bind to the A-site of the 16S bacterial ribosomal RNA (rRNA) and perturb protein synthesis causing mistranslation [4]. Prevalent use of aminoglycosides provides the selective pressure for the development of resistance via aminoglycoside modifying enzymes (AMEs) and more recently, target mutations such as rRNA methylation [5]. Various chemical modifications have been reported to address the issues of aminoglycoside resistance and target recognition of the bacterial 16S rRNA. The conjugation of peptides and aminoglycoside has been reported to show evasion of action of AMEs by circumventing AME modification of the aminoglycoside core unit in addition to building unique scaffolds for RNA and DNA recognition. We report herein solid phase chemical synthesis to gain *rapid access* to structurally well-defined aminoglycoside-dipeptide conjugate library, with kanamycin and neomycin serving as model aminoglycosides. Coincident binding of peptide and aminoglycoside to rRNA may further the diversity of RNA stabilization that can be achieved using different amino acid combinations. In general, the centrally located scaffold present in most of the clinically important aminoglycoside antibiotics is the 2-deoxystreptamine (DOS) moiety [6] (Fig. 1).

Based on the substitution pattern of the 2-DOS ring, aminoglycosides are broadly divided into two different subclasses, the 4,5- and 4,6-disubstituted DOS. Neomycin and paromomycin belong to 4,5-disubstituted subclass and kanamycin, tobramycin, amikacin, belong to 4,6- disubstituted subclass (Fig. 1).

To circumvent issues of aminoglycoside resistance, chemical modification of aminoglycosides has been used as a viable strategy, with a new drug plazomicin [7] recently receiving FDA approval. Major challenges associated with chemical syntheses of aminoglycoside-based novel antibiotics mirror the inherent difficulties related to synthesis and purification of pure and structurally well-defined carbohydrates. Challenges include: (i) the presence of multiple hydroxy and amino groups with comparable reactivity (ii) regio- and stereoselective glycosidic bonds (iii) multi-step building block synthesis (iv) the need for manipulation of numerous protecting groups and (v) tedious column purification for multiple

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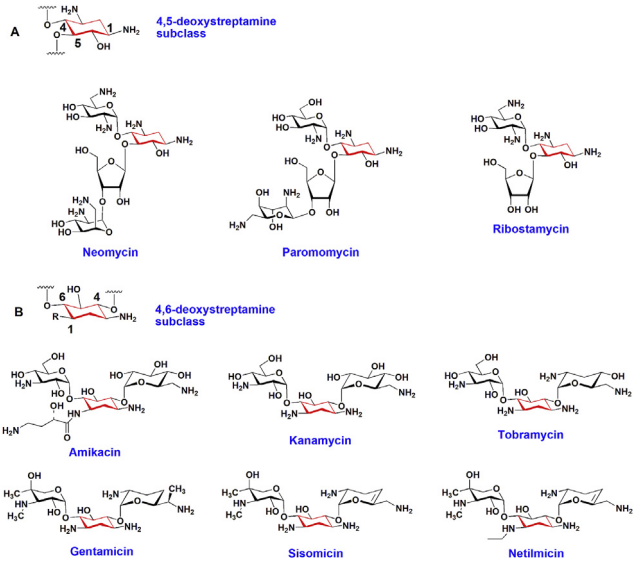

Fig. 1. A. 4,5-deoxystreptamine subclass (neomycin, paromomycin, ribostamycin); B. 4,6-deoxystreptamine subclass (amikacin, kanamycin, tobramycin, gentamicin, sisomicin, netilmicin).

intermediates. Therefore, due to the structural diversity of natural aminoglycosides, synthesis of modified aminoglycoside libraries remains an arduous challenge. A common strategy employed over the past decade has been to maintain the core aminoglycoside scaffold and incorporate additional binding/recognition moieties to synthesize structural analogs of natural aminoglycoside antibiotics.

Various synthetic protocols for the production of 5″-modified neomycin class of aminoglycosides have been reported for modification of antibacterial activity and nucleic acid binding [8–24]. Optimization of nucleic acid binding has been achieved by conjugating (via C5″–OH modification) B-DNA minor groove binding ligand Hoechst 33258 [25–27] and intercalators such as fluorescein [28–30], pyrene [31–34], naphthalene diimide, anthraquinone [35,36], methidium carboxylic acid [37] for selective recognition of A- and B-form DNA and binding to different nucleic acid structures [38]. Novel perylene-neomycin conjugates have been utilized for selective targeting of nucleic acid structures such as human telomeric G-quadruplex DNA through base stacking and groove recognition [39]. Various triazole linked neomycin dimers and benzimidazole-neomycin conjugates through C5″–OH group modification with varying linker length have been used for selective recognition of quadruplex [40], duplex RNA [41,42], miRNA [43], HIV TAR-RNA [44–47]. Moreover, various neomycin B dimers tethered via triazole, urea and thiourea linkages were found to be poor substrates for AMEs and selectively bind bacterial A site rRNA over human A site rRNA [48,49]. Nucleic acid binding chains such as PNA and DNA can be included in these target binding approaches [50–52]. These reports suggest that improvements in rRNA recognition can be attained by chemical modifications of naturally-occurring aminoglycosides.

Most of the synthetic approaches described above are performed using solution-phase synthesis. Compared to solid-phase synthesis, solution-phase synthesis is a slow process as it involves multiple purification steps during the course of synthesis. Thus, a methodology for rapid production of compound libraries followed by antibacterial screening and ribosomal binding analysis is highly desirable. We recently published a comprehensive approach for studying rRNA binding affinity and antibacterial activity of peptidic-neomycin library to identify novel rRNA binding antimicrobials [53]. Several PAs were quickly identified that bind with high affinity and greater selectivity to the *E. coli* A-site rRNA than the parent aminoglycoside as compared to the mammalian A-site. These promising results led us to expand the approach to include PAs using kanamycin, which belongs to 4,6-deoxystreptamine subclass. Herein, we successfully report the solid-phase assisted rapid synthesis of a 210 member mono- and diamino-acid peptidic-kanamycin library by using fifteen L-amino acids. A comparison of NMR spectra of neomycin and kanamycin conjugates with their precursor acid and PA derivative is included to identify the effect of these modifications on the electronics of the parent ring structure. We also report the antibacterial activity of select kanamycin and neomycin PAs and their effects upon binding human and *E. coli* A-site rRNA as evaluated by UV thermal denaturation.

2. Results and discussion

2.1. Synthetic strategy

Retrosynthetic analysis of covalently linked peptidic-kanamycin library is given in Fig. 2. The scheme is designed as a combination of both solid and solution phase synthesis.

In solution phase, kanamycin acid monomer **6**, as the precursor for solid phase synthesis, could be synthesized from kanamycin amine **5**, which in turn can be formed from commercially available kanamycin A, **1** in 4 steps. Simultaneously, different mono- and di-amino acid conjugates could rapidly be synthesized by modifying the standard 9-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthetic strategy [54]. Amino acid conjugates can then be coupled with kanamycin acid monomer **6**, followed by global deprotection to achieve a large PA library. The solid phase synthesis was carried out using Fmoc-PAL-PEG-PS resin by modifying Fmoc-based solid phase peptide synthetic strategy as described in the results and discussion section. This modified solid

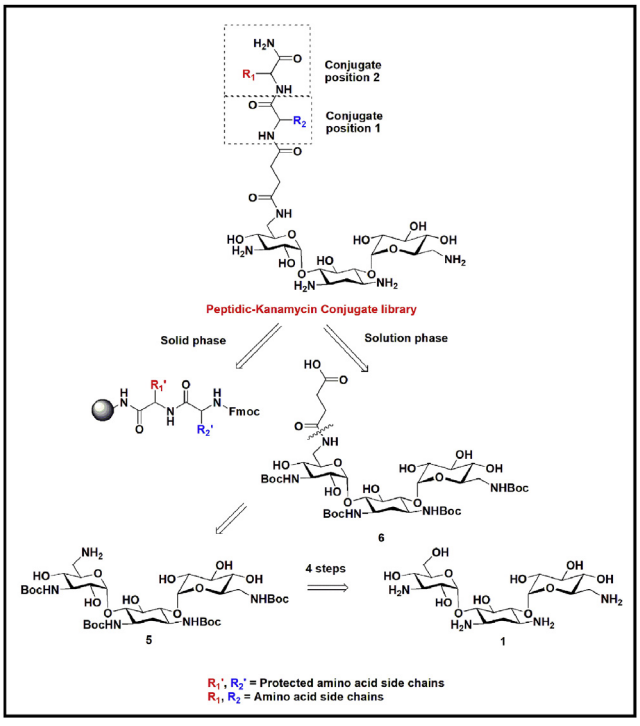

Fig. 2. Retrosynthesis of peptidic-kanamycin conjugate library.