Carbohydrate Research 346 (2011) 560–568

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis and antibacterial activity of amphiphilic lysine-ligated neomycin B conjugates

Smritilekha Bera [a], George G. Zhanel [b], Frank Schweizer [a,b,*]

[a] Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
[b] Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 3P4

ARTICLE INFO

Article history:
Received 1 November 2010
Received in revised form 13 January 2011
Accepted 14 January 2011
Available online 21 January 2011

Keywords:
Antimicrobial peptides
Glycopeptides
Aminoglycosides
Antibacterials

ABSTRACT

Amphiphilic lysine-ligated neomycin B building blocks were prepared by reductive amination of a protected C5″-modified neomycin B-based aldehyde and side chain-unprotected lysine or lysine-containing peptides. It was demonstrated that a suitably protected lysine-ligated neomycin B conjugate (NeoK) serves as a building block for peptide synthesis, enabling incorporation of aminoglycoside binding sites into peptides. Antibacterial testing of three amphiphilic lysine-ligated neomycin B conjugates against a representative panel of Gram-positive and Gram-negative strains demonstrates that C5″-modified neomycin-lysine conjugate retains antibacterial activity. However, in most cases the lysine-ligated neomycin B analogs display reduced potency against Gram-positive strains when compared to unmodified neomycin B or unligated peptide. An exception is MRSA where an eightfold enhancement was observed. When compared to unmodified neomycin B, the prepared lysine-neomycin conjugates exhibited a 4–8-fold enhanced Gram-negative activity against *Pseudomonas aeruginosa* and up to 12-fold enhanced activity was observed when compared to unligated reference peptides.

1. Introduction

The rise in antibiotic resistance among pathogenic bacteria and the declining rate of novel antibacterials reaching the market are a major concern in medicine.[1–5] As a result, there is a pressing need for novel classes of antibacterial agents with new or combined mechanisms of action and reduced likelihood to lead to the development of resistance. Cationic antimicrobial peptides and their mimetics are currently investigated as a new source of potential antibacterial agents in preclinical and clinical settings.[6,7] Cationic antimicrobial peptides that contain 10–50 amino acids are amphiphilic, and are rich in lysine or arginine and hydrophobic amino acids.[8–10] However, shorter amphiphilic peptide sequences as short as di- or tri- or hexapeptides with antibacterial activity are known.[11–13] We were especially encouraged by a previous report that has shown that both dipeptides H-D-Lys-Trp-OBn (kW-OBn) and H-L-Lys-Trp-OBn (KW-OBn) display potent antibacterial Gram-positive activity against *Staphylococcus aureus* (*S. aureus*), methicillin resistant *S. aureus* (MRSA), and methicillin resistant *Staphylococcus epidermidis* (MRSE) strains.[11] Interestingly, it was observed that the nature of the *C*-terminus contributes substantially to the antimicrobial activity of these two peptides.[22] For instance, dipeptide kW-OBn exhibits strong *S. aureus* activity while this activity is abolished in peptide kW-NHBzl (see Table 1). The reasons for this peculiar behavior are currently not understood.

Numerous studies with linear, cyclic, and diastereomeric cationic antimicrobial peptides have strongly supported the hypothesis that their physicochemical properties rather than any precise sequence are responsible for their activities.[8–10] It is generally believed that the amphiphilic topology is essential for insertion into and disruption of the cytoplasmic membrane. However, other mode of actions including intracellular targets have also been suggested.[19] In particular, the ability to rapidly kill bacteria and the relative difficulty with which bacteria develop resistance in vitro make cationic antimicrobial peptides attractive targets for drug development.[9] Previously, we and others have shown that aminoglycoside antibiotics-derived amphiphiles (AADAs) form a novel class of potent antibacterial agents.[14–17,31] The physicochemical similarities between AADAs and cationic antimicrobial peptides suggest a membranolytic mode of action. This mechanism is supported by (a) the observed concentration-dependent hemolytic activity of AADAs; (b) the presence of one or more hydrophobe(s) to induce antibacterial activity and (c) the precedence of a plethora of cationic antibacterial amphiphiles with membranolytic mode of actions. In this paper we report on the synthesis of amphiphilic lysine-ligated neomycin B analogs in which the C5″-position of neomycin B is ligated to hydrophobic lysine analogs or lysine-containing hydrophobic peptide sequence

* Corresponding author. Tel.: +1 204 474 7012; fax: +1 204 474 7608.
E-mail address: schweize@cc.umanitoba.ca (F. Schweizer).

0008-6215/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2011.01.015

Table 1
Antibacterial activity (MIC) in μg/mL of various Lys-Trp dipeptides, neomycin B–lysine conjugate **5** and amphiphilic neomycin B–peptide conjugates **7** and **17** and control compounds against various Gram-positive and Gram-negative bacterial strains.

Organisms	Genta-micin	Neo-mycin B	kW-NBn	kW-OBn	Fmoc-kW-OBn	**5** (Fmoc-NeoK-OBn)	**7** (Fmoc-NeoKW-OBn)	**17** (H-WNeoKW-OH)
S. aureus[a]	1	1	512	16	2	16	8	4
MRSA[b]	2	256	512	512	2	128	32	64
S. epidermidis[c]	0.25	0.25	256	2	2	4	4	4
MRSE[d]	32	0.25	512	32	4	4	4	8
S. pneumoniae[e]	32	8	>512	32	64	128	64	32
E. coli[f]	4	2	512	64	128	32	16	32
E. coli (Gent-R)[g]	128	4	512	16	128	32	16	16
P. aeruginosa[h]	4	512	>512	512	128	>256	128	64
P. aeruginosa (Gent-R)[i]	128	512	512	>512	128	>256	64	>128

Representative minimal inhibitory concentrations (MIC) in μg/mL for various bacterial strains: [a] ATCC 29213; [b] Methicillin-resistant *S. aureus* ATCC 33592; [c] *S. epidermidis* ATCC 14990; [d] Methicillin-resistant *S. epidermidis* (CAN-ICU) 61589; [e] ATCC 49619; [f] ATCC 25922; [g] CAN-ICU 61714; [h] ATCC 27853; [i] CAN-ICU 62308.

(Fig. 1). The single primary hydroxymethyl group at the ribose moiety (C5″-position) in neomycin B was chosen as a point of modification due to its expected high reactivity in chemical modifications.[21] Previous studies have indicated that modified C5″-analogs of neomycin B retain high binding affinity to RNA and antimicrobial activities.[18,30–32] Most of the previous work on C5″-modified neomycin B analogs has focused on neomycin B–lipid analogs and neomycin B–fluoroquinolone hybrid antibiotics[34] while very little data exist on amphiphilic neomycin B–amino acid or amphiphilic neomycin B–peptide conjugates that resemble short antibacterial peptides. We were particularly interested to prepare C5″-modified neomycin B conjugates linked to the ε-amino function of lysine thereby producing novel polycationic lysine mimetics, which may find utility in solid phase/solution phase antibacterial peptide synthesis. Moreover, suitably-protected lysine-ligated neomycin B building blocks may find application for incorporation of RNA-binding sites into peptides and in addition may serve as polyfunctional lysine surrogates in antimicrobial peptides.

2. Results and discussion

The chemical manipulation of aminoglycoside antibiotics provides great opportunities for medicinal chemistry due to the unique biological properties of this class of compounds.[27–31,33] However, the polyfunctional nature of aminoglycoside antibiotic scaffold frequently requires multi-synthetic protection–deprotection steps to perform selective chemical modifications. This is often perceived as a serious limitation to drug development making it difficult and expensive to access aminoglycoside analogs. We describe herein, the preparation of lysine-ligated neomycin B analogs by taking advantage of the presence of a single primary hydroxy group at the C5″-position in neomycin B. We were interested to develop a synthetic method that would permit regioselective ligation of the C5″-position of neomycin B to the side chain amino function of lysine. We envisaged that this could be achieved by regioselective oxidation of the primary alcohol into an aldehyde followed by reductive amination with a partially protected lysine building block (Fig. 1). For that purpose, the synthesis of the lysine-ligated neomycin B building block NeoK is outlined in Scheme 1. At first, commercially available neomycin B sulfate **1** was converted into Boc-protected neomycin B according to the literature procedure.[20] The primary hydroxyl group of **2** was selectively oxidized with trichlorocyanuric acid (TCCA) in the presence of catalytic amounts of TEMPO at 0 °C–rt for 3 h, according to Vasella's procedure[21h] to afford aldehyde **3**, which was directly used for the reductive amination reaction. In a model study, aldehyde **3** was ligated to Fmoc-L-Lys-OBn to form intermediate imine in the presence of molecular sieves and catalytic amount of acetic acid in minimum volume of methanol for 4 h at rt. Subsequently, the reaction mixture was diluted with methanol followed by addition of sodium cyanoborohydride and 3% methanol–acetic acid maintaining pH 5–6 and stirring at room temperature for 12 h. The reaction was monitored by TLC using methanol–CH_2Cl_2 (1:10) as solvent and developed in ethanolic ninhydrin solution. The reaction mixture was neutralized with 2% NaOH and then extracted with EtOAc to afford a crude reaction mixture containing both neomycin–lysine conjugate **4** (53%) and alcohol **2** (47%).[20] Separation was achieved by flash column chromatography using methanol–CH_2Cl_2 (1:10) as the eluent. The product was confirmed by mass as well as NMR spectroscopic data. In the ^{1}H NMR spectrum shows the appearance of aromatic protons related to the Fmoc- and benzyl-group between $\delta = 7.80$–6.41 ppm indicating the presence of lysine unit while signals at $\delta = 5.76$–1.40 indicate the neomycin B moiety. Global deprotection of all Boc-groups afforded the desired conjugate **5** as TFA salt (Scheme 1).

Once we had demonstrated that the synthetic strategy is applicable to lysine we then developed an interest to study the reductive amination of neomycin B to the ultrashort lysine-containing antimicrobial dipeptide KW-OBn.[11] For this purpose aldehyde **3** was conjugated to side chain unprotected dipeptide Fmoc-KW-OBn using the same protocol as previously applied to afford

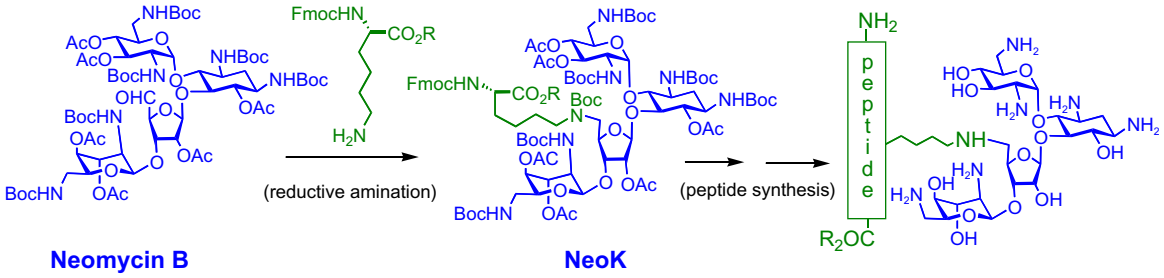

Figure 1. Synthesis of lysine-ligated neomycin B building block (NeoK) and use in peptide synthesis.