DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF HETEROCYCLIC AMINOGLYCOSIDES

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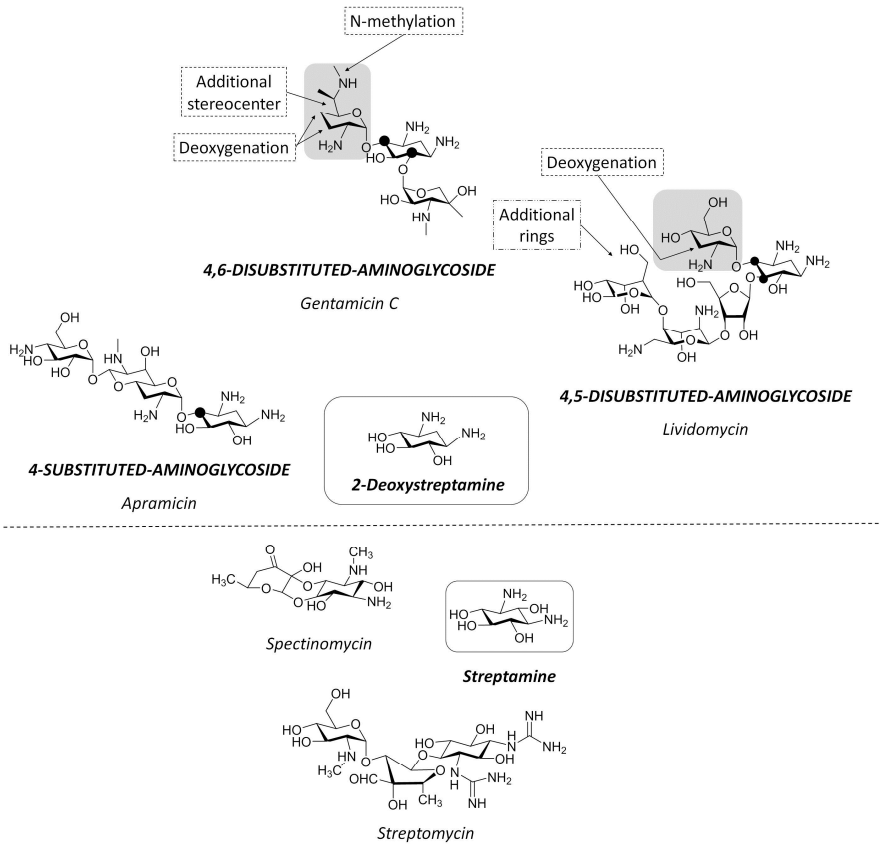
Abstract. *Aminoglycoside compounds represent a family of highly charged naturally occurring pseudo-oligosaccharides, which have been used for a long time as antibiotics that bind ribosomal RNA, but their use has been hindered by their inherent toxicity and the resistance that has emerged to these compounds. To circumvent this drawback during the last years several synthetic strategies for aminoglycoside preparation have been developed. The present review surveys the recent synthetic efforts that are focused on the preparation of heterocyclic aminoglycosides.*

Contents

1. Introduction

Aminoglycosides are highly potent, broad-spectrum antibiotics and several of them have been top selling pharmaceuticals for the treatment of a large number of infections. Their history begins in 1944 with streptomycin, that was isolated from a strain of *Streptomyces griseus*,[1] being this drug the first cure for tuberculosis. However, only few years later resistant microorganisms having the ability to withstand the effects of the antibiotic appeared. For this reason, a search for new antibacterial drugs began, leading to the discovery of a new generation of aminoglycosides, all of them containing the 2-deoxy-streptamine in its structure.[2] Until the early seventies aminoglycosides were isolated from natural sources. However, the emergence of resistant strains became a key priority in the development of new aminoglycosides.[3]

Chemically these antibiotics are composed mainly by a large variety of aminosugars. These carbohydrate moieties are 1,2-*cis* glycosidically linked to highly conserved aminocyclitol rings (1,3-diamine-cyclohexanes with three or four hydroxyl groups) traditionally termed 2-deoxy-streptamine (2-DOS) or streptamine that provide the central scaffold of any known aminoglycoside (Scheme 1). Classically, these antibiotics are divided in function of the aminocyclitol substitution pattern, affording 3 different subclasses: 4 mono-substituted (e.g. apramycin) and 4,5- (e.g. lividomycin) or 4,6- di-substituted (e.g. gentamicin C) (Scheme 1).

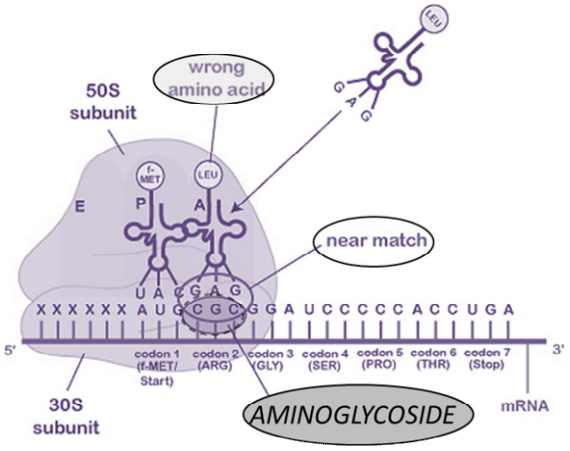


Scheme 1

In reference to the carbohydrate types, for di-substituted aminoglycosides, position 4 is occupied by a 6-amino-, 2-amino- or 2-6-diaminosugar (ring I) in both subclasses. On the other hand, the aminosugar attached to position 5 or 6 (ring III) is dependent on the subclass to which the antibiotic belongs, being most frequently a 3-amino-pyranoside for 4,6- and a ribose for 4,5- ones. Besides, some additional rings or rare carbohydrates can be identified to attach to ring III (ring IV) which in turn may be bound to other carbohydrate units (see lividomycin, Scheme 1). Finally, all rings are often modified by de-oxygenation, N- or C-methylation and even by the presence of additional stereocenters (Scheme 1). As an exception to these general rules, streptomycin and spectinomycin are shown in Scheme 1 as examples of non-deoxy-streptamine aminoglycosides in which the aminocyclitol ring in both cases is the streptamine.

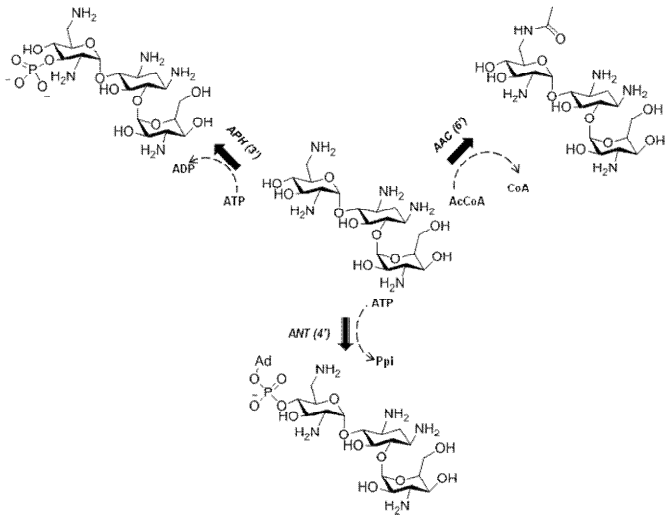
Since the discovery of aminoglycosides by Selman Waksman more than 50 years ago, most attention has focused on their binding to prokaryotic rRNA. Aminoglycosides bind to the decoding region aminoacyl-tRNA site (A-site) inducing codon misreading and inhibiting translocation, eventually resulting in cell death (Scheme 2).[4] However, the flexible and polycationic nature of these oligosaccharides allows them to bind to a variety of unrelated RNAs (eukaryotic rRNA[5] and catalytic RNAs[6] such as group I introns,[7] a hammerhead

ribozyme,[8] ribonuclease P RNA[9] and diverse HIV-RNAs: TAR, RRE1 and DIS.[10] These findings have suggested alternative pathways that can be used as novel targets for these drugs.[11]



Scheme 2

Several years after the introduction of these antibiotics in clinic, the first resistant bacteria appeared and multidrug-resistant organisms are now here to stay, having become in a serious problem over recent years. In the case of aminoglycoside antibiotics, there are three possible mechanisms of resistance: mutation of the ribosomal target, reduced permeability for the antibiotics and enzymatic modification of the drugs, thus leading to inactivation and constitutes the clinically most relevant mechanism.[12] The enzymes involved in this resistance mechanism can be classified as *N*-acetyltransferases (AACs), *O*-adenyltransferases (ANTs), and *O*-phosphotransferases (APHs) (Scheme 3).[13]



Scheme 3

Besides, due to its toxicity, the use of aminoglycosides have been clinically limited to severe infections and the more toxic antibiotics have been restricted to topical or oral administration.[14] The less toxic aminoglycosides are used for parenteral treatment of severe sepsis caused by Gram-negative bacteria.

Indeed, the search for new aminoglycoside derivatives with high affinity to several RNA targets, antibiotics with high ability to circumvent multiple resistance mechanisms, inhibitors against resistance enzymes and analogues with reduced toxicity constitutes an active field of research.[15]

In this context, the heterocyclic aminoglycoside mimetics has emerged as new promising aminoglycosides. In this review, the design, synthesis as well as their biological activities will be discussed.

2. Strategies for the preparation of heterocyclic aminoglycosides

Aminoglycoside antibiotics are constituted by a series of glicosidically linked aminocyclitols and aminosugars and can be considered from a chemical point of view as complex molecules. For this reason, synthetic modification of such natural products in the laboratory is very challenging. Maybe that is the reason why since the discovery of these compounds their chemical modification has received a great deal of attention.[16] However, and mainly due to the emergence of resistance phenomena, the search for novel antibiotics, including synthetic aminoglycosides, has become a priority line in health research, which has led to the development of new strategies in modification of these oligosaccharides.

Heterocyclic modification of aminoglycosides has been extensively employed as strategy over the last few decades for the obtainment of new potential antibiotics to be used in the treatment of a large number of bacterial infections and/or new compounds with possible inhibitory activity against aminoglycoside modifying enzymes.

Overall, there are two types of approaches reported for the obtainment of heterocyclic aminoglycosides: (a) substitution of an aminosugar and/or aminocyclitol by heterocyclic motifs and (b) conjugation of an aminoglycoside with a heterocycle through a linker. A general overview of both strategies for kanamycin and neomycin (two of the more frequent natural aminoglycosides employed in the synthesis of new heterocyclic derivatives) are showed in Scheme 4.

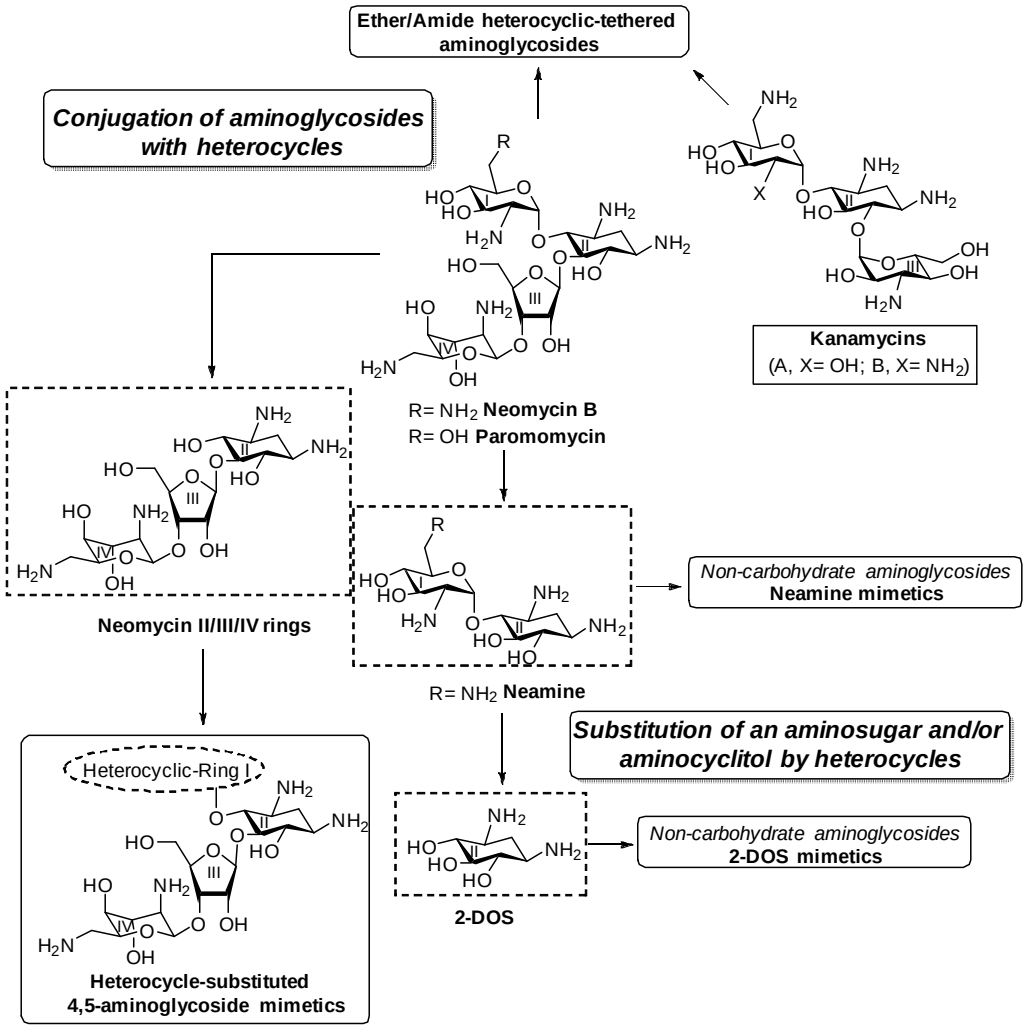
3. Substitution of an aminosugar and/or aminocyclitol by heterocycles

The total-synthesis of aminoglycoside mimetics was designed by replacement of the 2-DOS, the glycosidic substituents, or both by heterocyclic scaffolds.

The aim of replacement of 2-DOS by other amino group-carrying moieties has been the simplification or optimization of the key pharmacophore, obtaining alternative scaffolds that mimic the unique spatial arrangement of the functional groups in 2-DOS that are required for the recognition of the RNA target.[17]

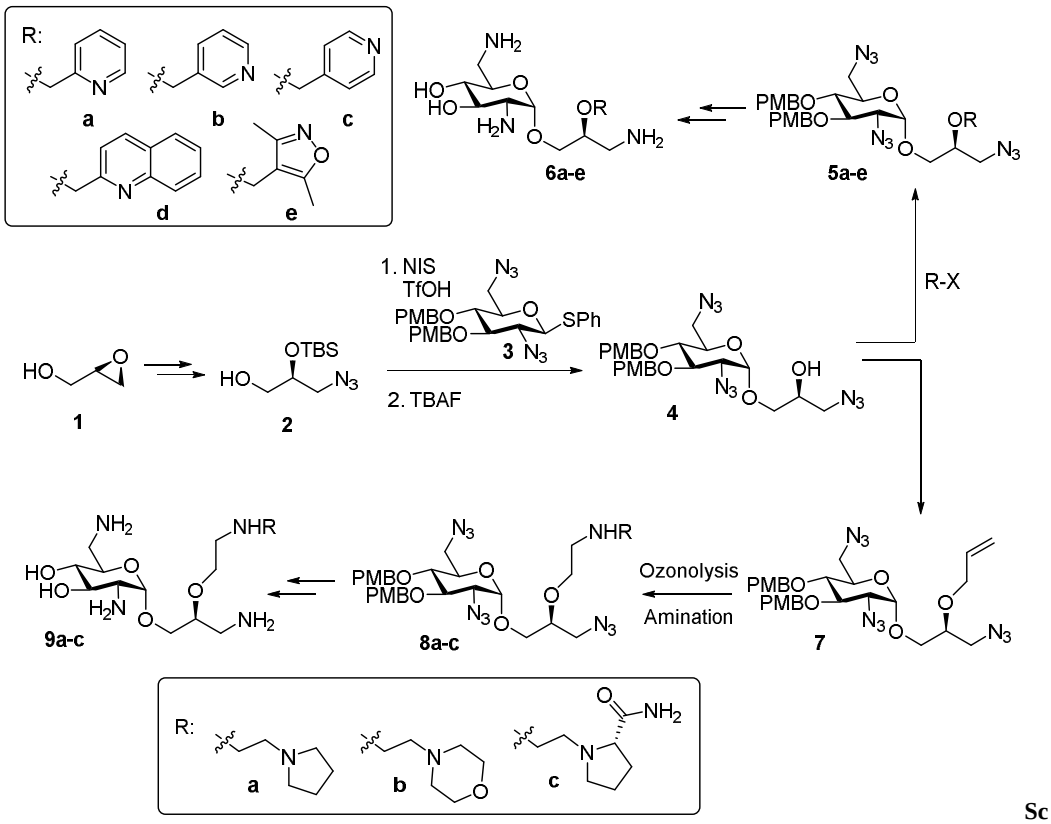
In a first approximation, Hermann and co-workers[18] have linked the 2',6'-diaminoglucosamine moiety, conserved among many potent natural aminoglycosides to two heterocyclic-types (aromatics or not) of scaffolds through the (*S*)-3-amino-1,2-propanediol (Scheme 5). In both cases, the synthesis of the desired mimetic was initiated from (*R*)-(+)-glycidol **1** which was transformed in alcohol **2** in four steps (86% overall yield).

Coupling of this with donor **3** under glycosylation conditions, followed by cleavage of the silyl ether, furnished the key glycoside **4** as the pure α-anomer (66% yield).



Scheme 4

Subsequently, in the case of aromatic heterocyclic scaffolds a variety of heterocyclic benzylic halides were coupled to alcohol **4** under basic conditions, which produced the desired ethers **5a-e** in good yields which finally were deprotected, yielding aminoglycosides mimetics **6a-e** in excellent overall yields. In a variant approach, non aromatic mimetics were obtained from allyl ether **7**. Ozonation and subsequent amination afforded the desired protected mimetics **8a-c**. These compounds were finally deprotected, which resulted in aminoglycoside mimetics **9a-c** (Scheme 5).



Scheme 5

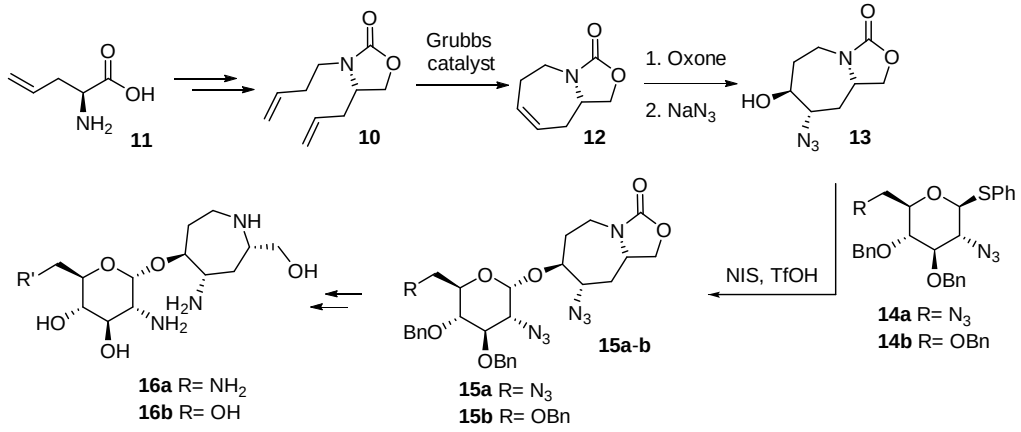
The biological activities of the novel aminoglycoside mimetics as inhibitors of bacterial (BIVT) and eukaryotic (EIVT) protein synthesis are showed in Table 1.

Table 1. Biological activities of aminoglycoside mimetics **6a-e** and **9a-c**.

Compound	**Neomycin B**	**6a**	**6b**	**6c**	**6d**	**6e**	**9a**	**9b**	**9c**
$B_{IVT}IC_{50}$ (μM)	0.032	>1000	>1000	490	19	>1000	520	690	130
$E_{IVT}IC_{50}$ (μM)	>250	>250	>250	>250	36	>250	>250	>250	1.0

Compound **6d** bound with micro-molar affinity (IC_{50}=19 μM) to the bacterial decoding-site RNA, and inhibited both bacterial and eukaryotic translation, although with a similar potency in both systems. Interestingly, the proline derivative **9c** was particularly potent against eukaryotic translation (IC_{50}=1.0 μM), but more than 100-fold less active in the bacterial system (IC_{50}=130 μM), which suggests that this compound selectively target a eukaryotic ribosomal component that is absent in bacteria.

The same authors[19] have described a series of mimetics in which 2-DOS has been replaced by an azepane, a saturated heterocycle containing one nitrogen atom in a seven-membered ring (Scheme 6).



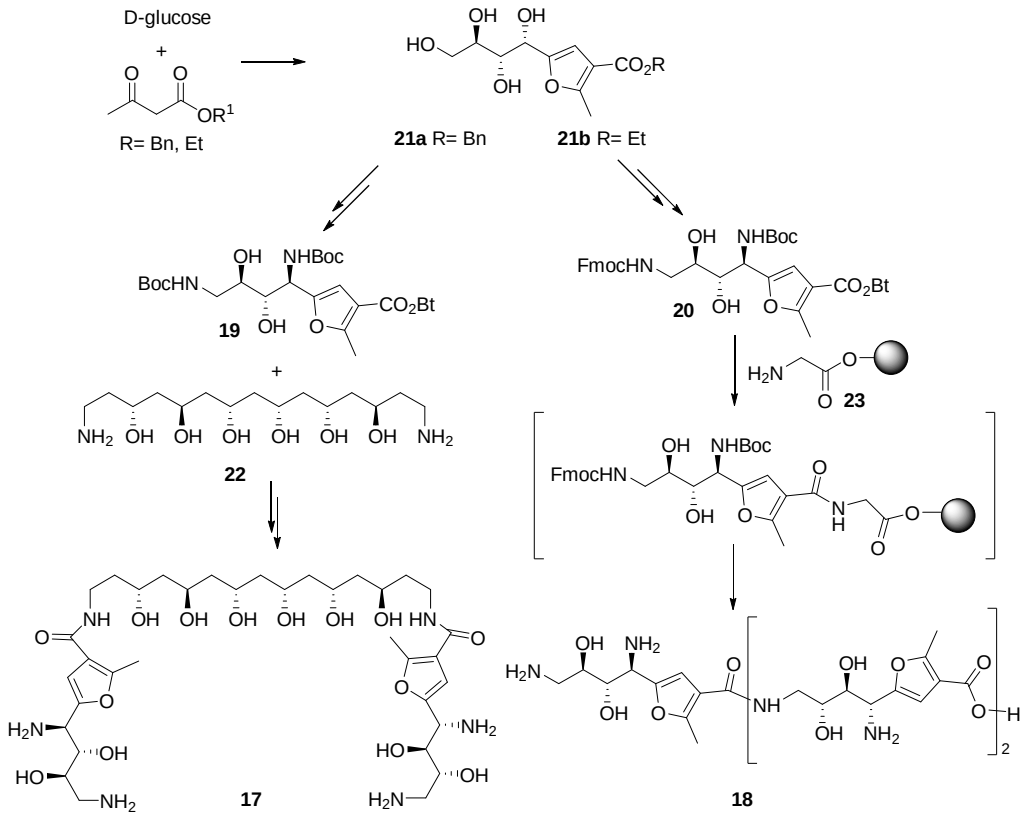
Scheme 6

The key step for the synthesis of the heterocyclic building block (4,5-disubstituted azepanes) relied on a ring-closing metathesis reaction of compound **10**, obtained in five steps from L-allyl-glycine **11**, which gave access to the seven-membered ring **12** containing a double bond. Subsequent epoxidation of **12** followed by azide opening produced the acceptor **13**. Finally, glycosylation of **13** with donors **14a-b** provided the α-*pseudo*-disaccharides **15a-b** which were deprotected in three steps, obtaining the desiderate azepane-glycosides **16a-b**. These compounds were tested against *E.coli* and *S. aureus* strains and neither of them inhibit bacterial growth (MIC>64 µg/mL).

In recent years, other strategy is the construction of dimeric aminoglycosides design by connecting two antibiotics or fragments of them with linkers differing in length and composition.[15d, 20] To test whether the 2-DOS can be replaced by other binding motifs in these dimeric compounds, Gerber-Lemaire and coworkers[21] have designed conjugates **17** and **18** between furyl residues and 1,3-hydroxyamine fragments (Scheme 7).

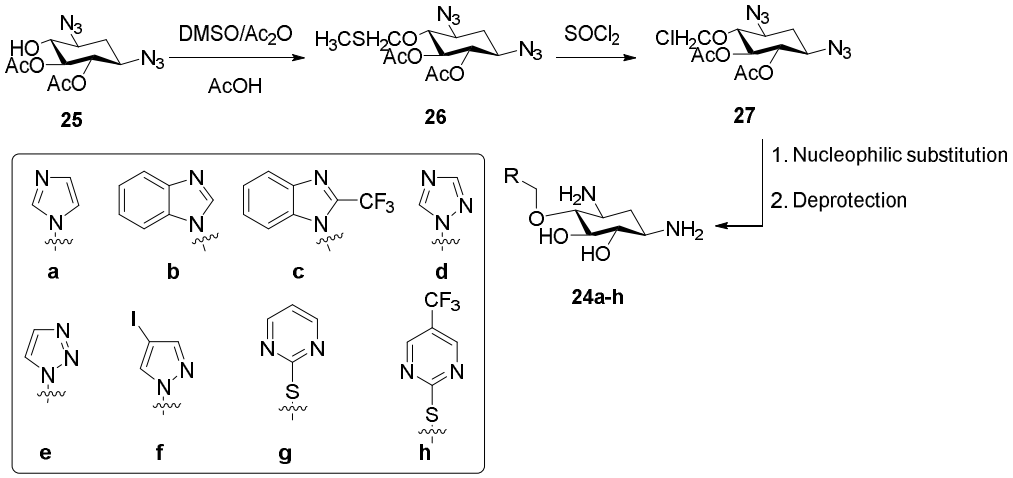
Building blocks **19** and **20** were obtained in several steps from compounds **21a-b**.[22] Furyl carbopeptoid **17** was prepared from **19** by coupling with the (+)-aminopolyol **22** upon treatment with DIPEA and subsequent acidic deprotection (overall yield 62%). On the other hand, orthogonally N-protected **20** was oligomerized using solid-phase peptide synthesis methodology, anchoring in a first step Fmoc-glycine **23** to HMBA-AM resin. Iterative couplings with **20** in the presence of DIPEA afforded the polymer-bound compound, which was removed from the solid support by treatment with MeOH-Et_3N. Final Boc deprotection with acid gave furylcarbopeptoid **18** in 87% overall yield. The antibiotic properties of both compounds **17** and **18** were tested. Although furyl moieties were expected to provide stacking interactions with rRNA, however none of the two compounds displayed high affinity toward rRNA fragments employed.

The strategies described above are limited by the absence of the 2-DOS moiety, a key structural feature of the aminoglycoside antibiotics with a crucial role for their biological activity.[2] In fact, its elimination results in a loss of antibiotic activity. In order to solve this drawback, approaches that keep up this ring have been development, leading to the synthesis of novel aminoglycoside mimetics.



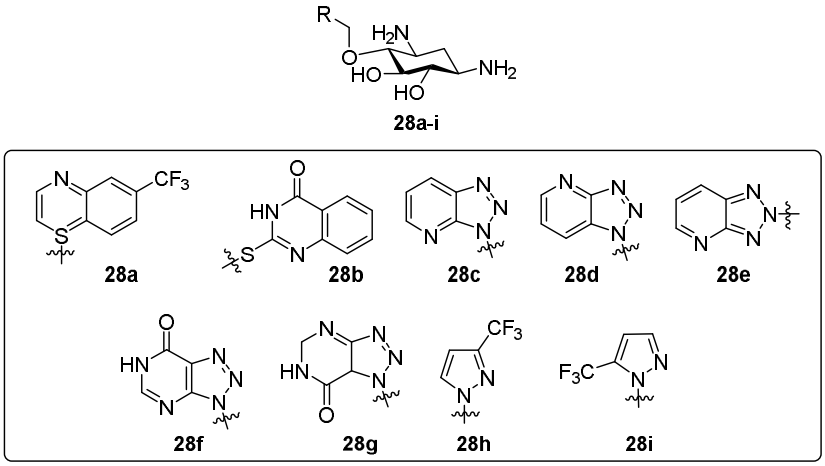
Scheme 7

The *pseudo*-disaccharide fragment I/II (neamine) is present in most aminoglycosides and has been shown to be important for specific complex formation with the prokaryotic 16S RNA.[23] Therefore, mimetics of neamine would be an ideal starting point for the synthesis of new potential antibiotics. To test this hypothesis, Ding and co-workers have synthesized several 4-heterocyclic 2-deoxy-streptamine derivatives (Scheme 8). In a first approximation, ring I of neamine was replaced by azoles and benzimidazoles (compounds **24a**-**h**).[24] These compounds were prepared from diacetylated 2-deoxy-streptamine derivative **25**,[25] on which was introduced a methylthiomethyl ether using the Pummerer rearrangement.[26] Treatment of **25** with excess DMSO/Ac_2O/AcOH at room temperature gave the 4-methylthiomethyl-protected 2-deoxy-streptamine derivative **26** in 78% yield. Subsequent treatment of **26** with $SOCl_2$ afforded the 4-chloromethyl derivative **27**. Finally, compound **27** was coupled with several nucleophilic reagents under different conditions to afford the corresponding 4-heterocyclic 2-deoxy-streptamine derivatives **24a**-**h** in satisfactory yields after total deprotection (Scheme 8). These compounds were screened against the 16S A-site by high-resolution mass spectrometry[5b] and even though they do not reach the binding affinities of neamine fragment, the synthetic strategy described in this paper provided a very efficient method to synthesize heterocyclic carbohydrate derivatives for the biological screen.



Scheme 8

In fact, the strategy described above was used by the same authors[27] for the synthesis of a large library of 4-heterocyclic 2-deoxy-streptamine derivatives **28a-i** (Scheme 9). These compounds were also screened against the 16S A-site showing the [(7-trifluoromethyl)-4-quinolinyl]sulfanyl-2-deoxy-streptamine **28a** a binding affinity (K_d=35 µm) in the order of neamine (K_d=24 µm).

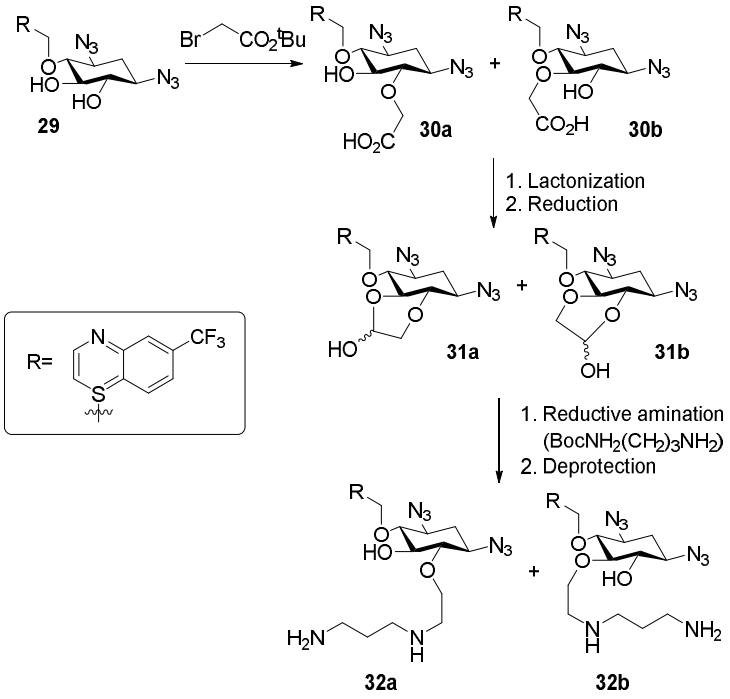


Scheme 9

In accordance with these results, the synthesis of more complex mimetics was achieved, using compound **28a** as pharmacophore.

In a first approximation, Migawa and coworkers[28] have prepared aminoglycoside analogues lacking one sugar ring by introduction of alkylamino chains at C-4 or C-5 in **28a** (Scheme 10). Alkylation of **29** (an intermediate in the synthesis of **28a**) with *tert*-butyl-α-bromoacetate afforded a mixture of carboxylic acids

30a-b exclusively, probably due to hydrolysis facilitated by the neighboring hydroxyl group. A subsequent intramolecular lactonization, followed by DIBAL reduction gave intermediates **31a-b**. Finally, reductive amination, azido reduction, and Boc removal gave the final compounds **32a** and **32b**. From a biological perspective, the addition of the alkyl amino chain improved the binding activity (**12a** and **12b**, K_d=1.5 and 2 µm respectively) over the unsubstituted derivative **28a** by a factor of 10 (K_d=35 µm).

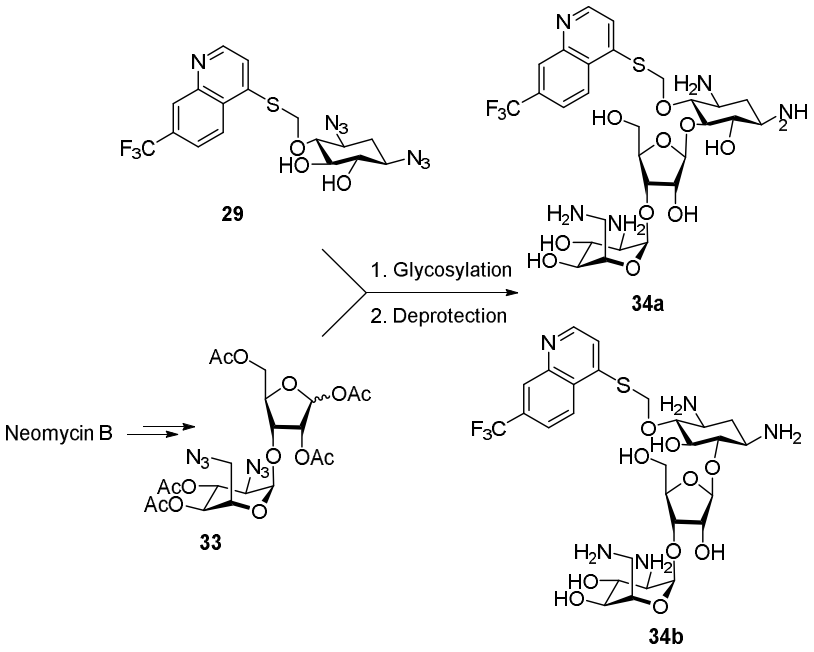


Scheme 10

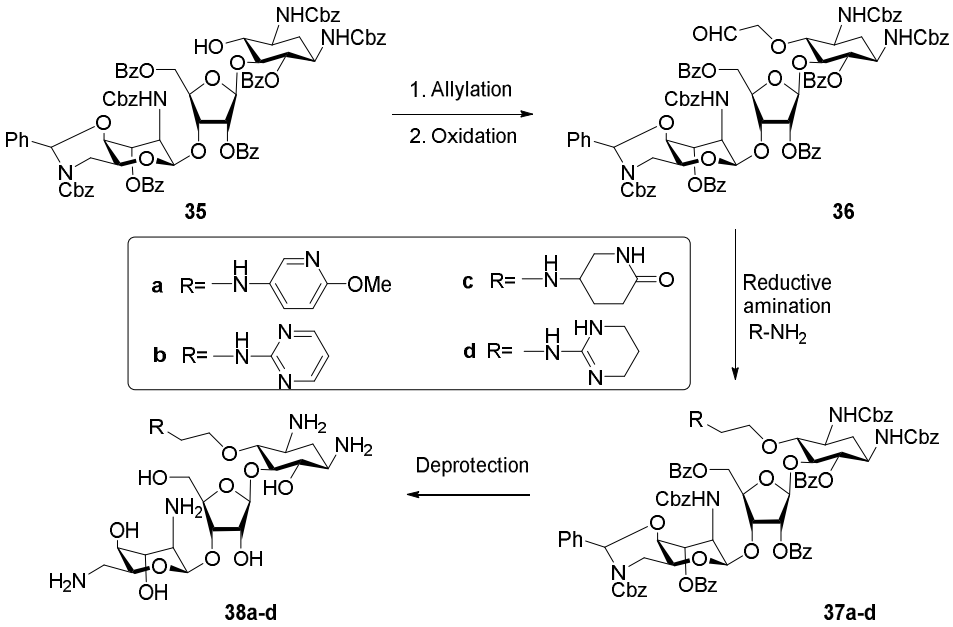
According to a similar approach, the paromomycin mimetic in which the ring of glucosamine I is replaced with [(7-trifluoromethyl)-4-quinolinyl]sulfanyl heterocycle was analyzed (Scheme 11).[27] Glycosylation of acceptor **29** with donor **33**, obtained from the acidic hydrolysis of neomycin B in three steps, and final deprotection afforded the mixture of regio-isomers **34a-b**. The 4,5-disubstituted compound **34a** has a minimum inhibitory concentration (MIC) of 3 µm against a gram-negative *E. coli* strain (ATCC 25922), a similar value to natural antibiotic paromomycin.

In agreement with these results, the use of heterocyclic appendages as spatial mimetics of ring I have demonstrated a great utility as strategy for the development of novel aminoglycosides.

Hanessian and coworkers[29] have expanded the level of diversity at C4 of 2-DOS by incorporating different heterocyclic motifs. Allylation and subsequent oxidation of **35** (obtained from paromomycin)[30] gave the corresponding aldehyde intermediate **36**. Subsequent reductive amination using a series of readily available amines afforded compounds **37a-d** which were finally deprotected to afford the final compounds **38a-d**. These compounds were tested against strains of *E. coli* and *S. aureus* (Scheme 12).



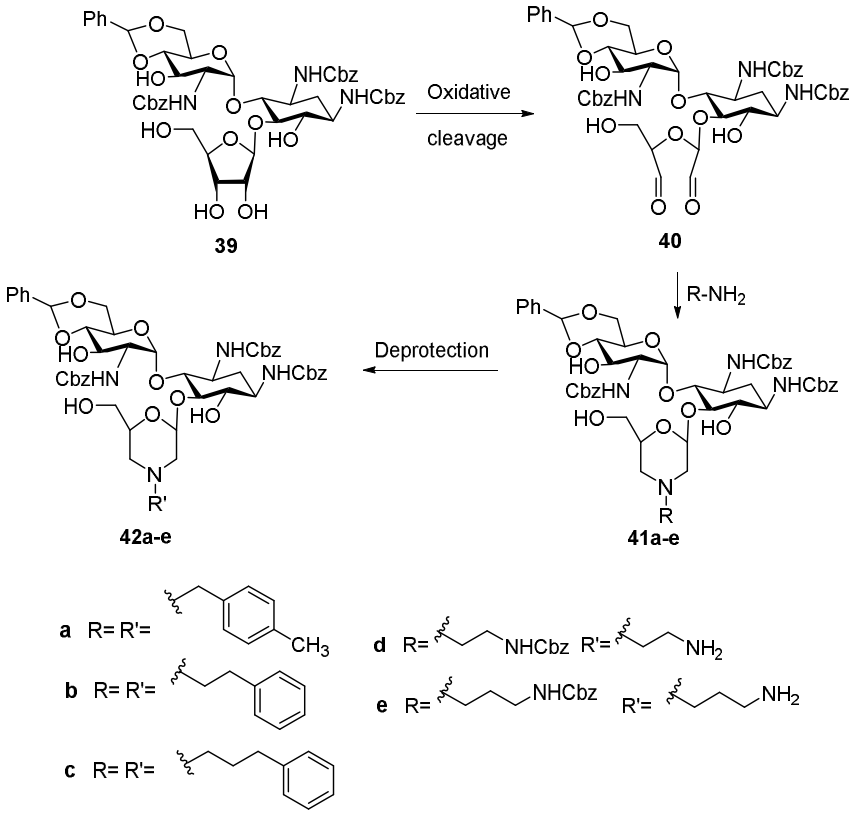
Scheme 11



Scheme 12

Only moderately weak activity was found in the case of compound **38c** (MICs=25-50 and 6-12 μg/mL for *E.coli* and *S. aureus* respectively). All other compounds **38a-b** and **38d** were inactive with MICs>100 μg/mL for both strains.

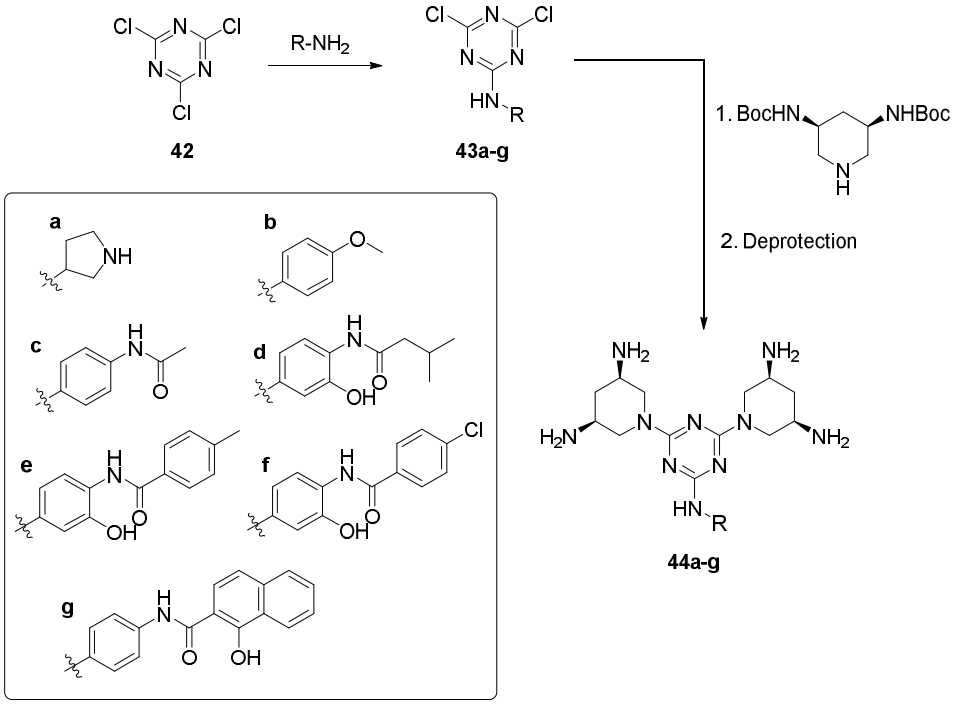
The description of several structures of aminoglycosides bound to the target ribosomal A-site RNA by X-ray and NMR methods[4b, 4d-f, 31] have provided an extremely detailed knowledge about the interactions that stabilize the drug/RNA complexes and have open the door to a whole new approximation to drug design, the structural-based approach. In this context and taking advantage of the structural information available for ribostamycin (pdb:2et5)[4g] a series of new heterocyclic mimetics has been described.[32] To this end, diverse morpholines have been considered as conformationally constrained mimics of the ribosyl ring of the natural antibiotic (Scheme 13).



Scheme 13

The oxidative cleavage of the diol in compound **39**[33] gave dialdehyde **40**, which was directly subjected to a reductive coupling with a variety of amines, obtaining the desired morpholines **41a-e** which were finally deprotected to afford **42a-e**. Unfortunately, none of these compounds exhibited antibiotic activity (MICs>100 μg/mL for strains of *P. aeruginosa*, *S. aureus*, *E. coli* and *E. faecalis*).

Finally, another strategy used to prepare ligands with higher affinity for rRNA comes from the similarity of 2-DOS with cis-3,5-diamino-piperidine (DAPT).[34] This structural moiety retains the signature *cis*-1,3-diamino fragment while disposing of additional stereocenters and have demonstrated to be a particularly suitable building block for RNA-targeted small-molecule libraries. The use of a triazine core provided access to a series of symmetrically substituted molecules that contained two DAPT scaffolds in a desirable stereochemical orientation. To this end, 1,3,5-trichloro-triazine **42** was attached to different rings providing compounds **43a-g**, which upon coupling with the piperidinyl moiety and acidic deprotection provided the final compounds **44a-g** (Scheme 14). From a biological perspective two of the obtained compounds (**44f** and **44g**) showed antibiotic activity (MICs=1-2 and 2-16 µg/mL for *E. coli* and *S. aureus* respectively).



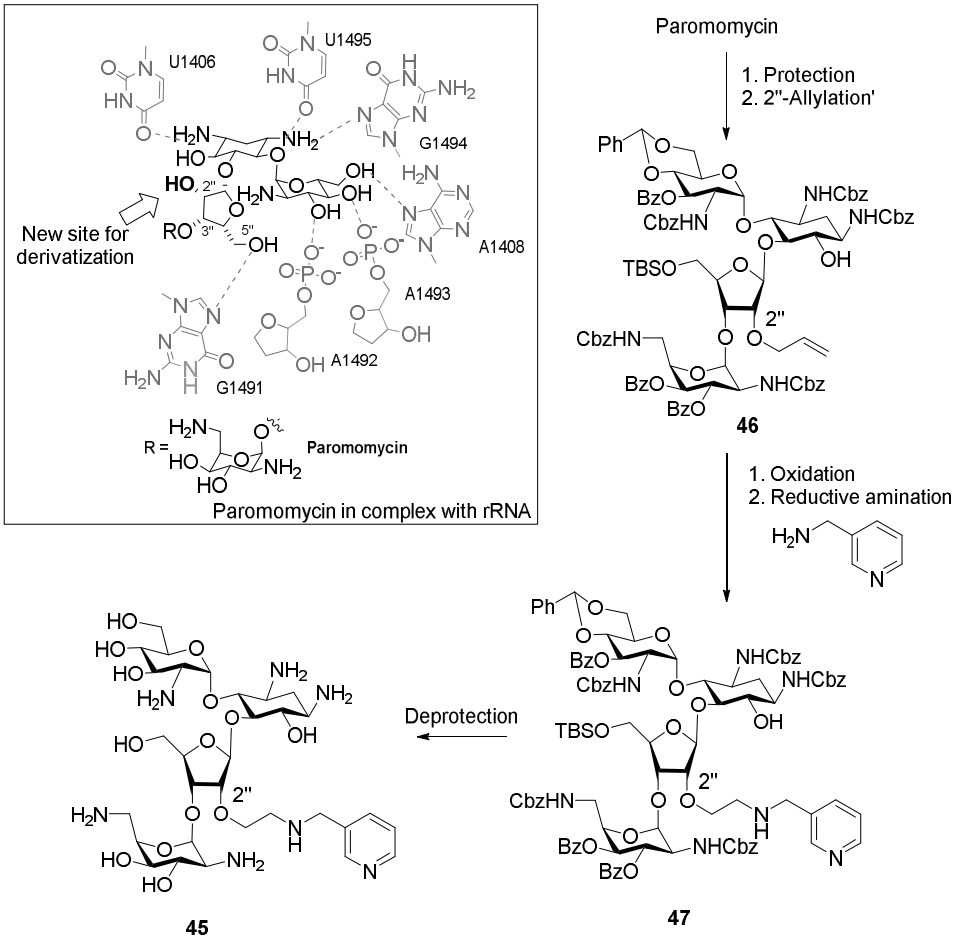
Scheme 14

4. Conjugation of aminoglycosides with heterocycles

Crystallographic structures of rRNA in complex with 4,5- and 4,6-disubstituted aminoglycosides have allowed the design of new families of semi-synthetic antibiotics. Taking advantage of the structural information available for paromomycin (pdb: 1J7T)[4e] in complex with rRNA and exploiting the reactivity pattern of this antibiotic toward new sites for derivatization to install a functionality capable of making new productive contacts, a functionalized aminoglycoside in C-2'' **45** has been described (Scheme 15).[35]

Protection of the natural antibiotic paromomycin and allylation at C-2'' gave the corresponding intermediate **46**. Oxidation of this and subsequent reductive amination using 3-aminomethyl-pyridine

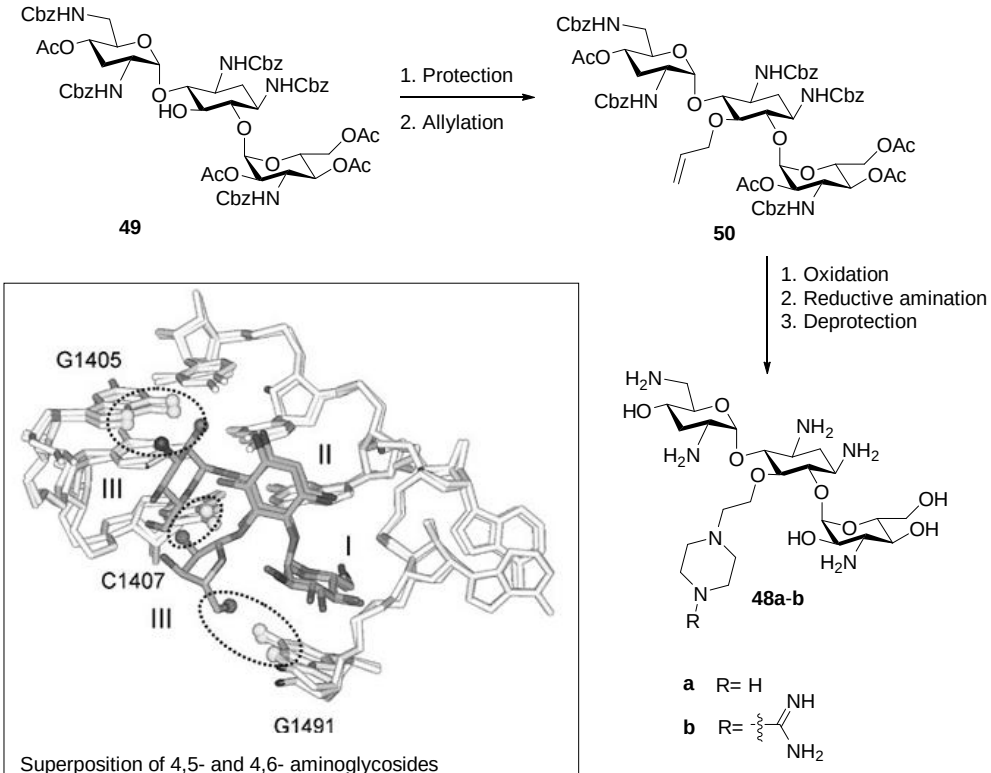
afforded compound **47** which was finally deprotected to afford the final compound **45**. Biologically, the introduction of the pyridine ring led to a diminished activity against *E. coli* (MICs=5 µg/mL and 25 µg/mL for paromomycin and **45** respectively).



Scheme 15

Making use of structural information another strategy used to prepare ligands with heterocyclic moieties in their structures comes from the structural differences between 4,5- and 4,6-disubstituted aminoglycosides. In this context, superposition of kanamycin-A (4,6-subfamily) and ribostamycin (4,5-subfamily) I/II fragments in the complex with rRNA shows that the disaccharides I/II, present in both antibiotics, occupy nearly identical positions within the RNA binding pocket, giving rise to identical interactions with the target. However, sugar units at the 5- (ribostamycin) or 6- (kanamycin A) positions exhibit very different orientations in the binding pocket, providing additional contacts with the lower and upper stems of the RNA receptor, respectively (Scheme 16). Bearing in mind these observations, 4,5,6-

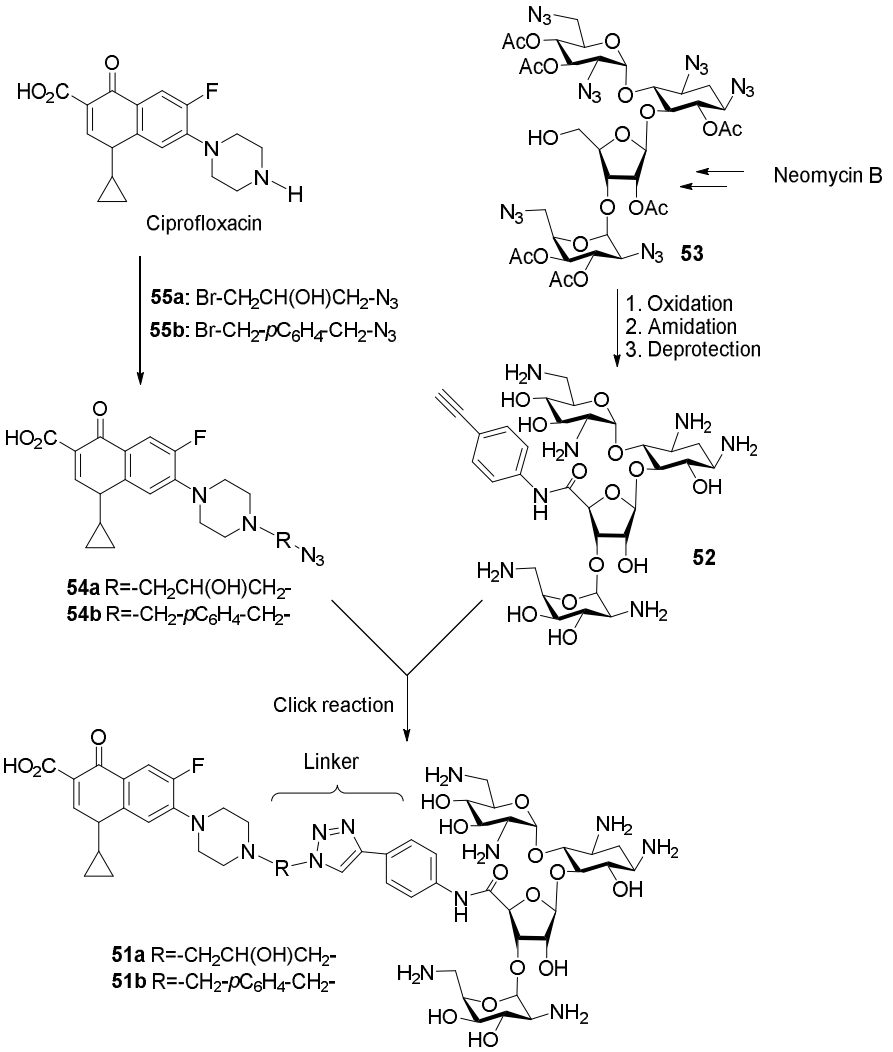
trisubstituted tobramycin derivatives have been prepared introducing ether-linked 1-ethyl or 1-ethyl-4-guanidine piperazines at C-5 in tobramycin **48a-b**.[32b] Allylation of intermediate **49** gave intermediate **50** which after oxidation, subsequent reductive amination with both 1-ethyl-piperazines and final deprotection afforded derivatives **48a-b** (Scheme 16). These compounds were as active as tobramycin against *E. coli* (MIC=0.6-1.2 μg/mL) exhibiting MIC values of 1-2 μg/mL (**48a**) and 5-10 μg/mL (**48b**) against the same strain.



Scheme 16

Another strategy that has been pursued in recent years employs a combination of two different drugs in one molecule.[15d, 36] With this strategy, each drug moiety is designed to bind independently to two different biological targets and synchronously accumulate at both target sites. In this context, two hybrid compounds **51a-b** containing a covalently linked fluoroquinolone (ciprofloxacin) and the natural aminoglycoside neomycin B have been described (Scheme 17).[37] Ciprofloxacin is a heterocyclic broad-spectrum antibiotic that functions by inhibiting DNA gyrase and TopoIV isomerase enzymes, necessary to separate bacterial DNA, thereby inhibiting cell division. The two pharmacophores (ciprofloxacin and neomycin B) were coupled via "click reactions". For the preparation of the alkyne derivative **52** the commercial antibiotic neomycin B was converted to the protecting derivative **53**. The available 5″-alcohol of this compound was

oxidized to a 5''-acid, which was coupled with an alkynyl- amine to afford the corresponding intermediate **52** after total deprotection (Scheme 17). The derivatives **54a-b** were prepared by direct coupling of the commercial ciprofloxacin with the corresponding bromoazides **55a-b** (Scheme 17).



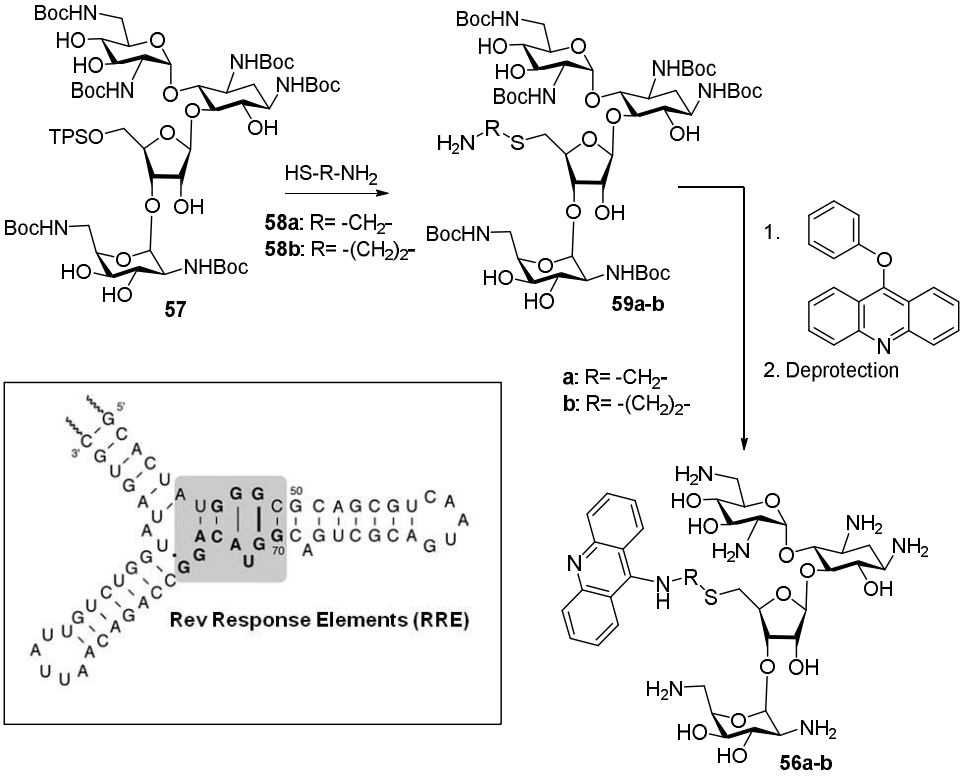
Scheme 17

Finally, the key coupling reaction between **52** and **54a-b** was performed under microwave irradiation in the presence of organic base and the Cu(I) catalyst to ensure the production of a single (anti) stereoisomer at the triazole moiety. The hybrids **51a-b** inhibited bacterial protein synthesis with potencies similar to or better than that of neomycin B and were up to 32-fold more potent inhibitors than ciprofloxacin for the fluoroquinolone targets, DNA gyrase, and TopoIV isomerase (Table 2).

Table 2. Biological activities (IC_{50}, μM) of compounds **51a-b**.

	R	R^1	DNA gyrase	Topo IV isomerase	Protein synthesis
Ciprofloxacin	-	-	1.3	10.8	Inactive
Neomycin B	-	-	Inactive	Inactive	10.5
51a	$-CH_2CH(OH)CH_2-$	$-C_6H_4-NHCO-$	0.073	0.58	2.2
51b	$-CH_2-pC_6H_4-CH_2-$	$-C_6H_4-NHCO-$	0.085	0.55	16.7

The strategies described above have as objective the preparation of new synthetic aminoglycosides with improved antibiotic activity. However, the possibility of these compounds to bind to a variety of unrelated RNAs has opened alternative pathways that can be used as novel targets for these drugs. In this context, several approaches have been explored in an attempt to increase the affinity and selectivity of aminoglycoside-based ligands to the RRE. The NMR structure of the arginine-rich RNA-binding domain of Rev complexed to a short RRE construct shows purine-purine pairing and a bulged-out pyrimidine residue.[38] According with these results, non canonical motifs can constitute favored intercalation sites and appending an intercalator to aminoglycosides can generate RNA binders with high RRE binding affinity.[39] Indeed, neomycin-acridine conjugates **56a-b** have been prepared (Scheme 18).[40]



Scheme 18

For their synthesis compound **57** was obtained using the natural antibiotic neomycin B as starting material.

Displacement of the leaving group with tiols **58a-b** afforded compounds **59a-b** which were coupled with the acridine moiety and finally were deprotected. Compound **56a** has an apparent K_i of 3nM, which is approximately the same affinity as that of the Rev peptide. However, compound **56a** displays mediocre RRE selectivity as it binds competing nucleic acids including tRNA and DNA. Modulating the selectivity of aminoglycoside-intercalator conjugates can be accomplished by varying the length of the linker. Thus compound **56b**, possessing a longer linker displays lower RRE selectivity (Table 3).

Table 3. Biological activities of compounds **56a-b**.

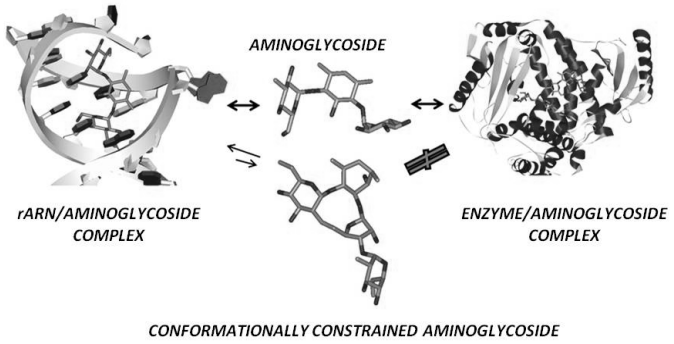
	Neomycin B	**56a**	**56b**
IC_{50} (μM) without competitors	7	0.040	0.040
IC_{50} (μM) with CT DNA	8	0.15	0.45
IC_{50} (μM) with tRNA	20	1.2	1.6
RRE specificity ratio	2	17	26

Traditionally the research interest has been focused in the synthesis of new ligands with improved affinity for RNAs as a purely academic exercise. However, the widespread and first-line use of these antibiotics in clinic 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). In this context, extensive efforts have been made over the years to overcome the growing resistance problem. Classically, chemical removal/modification of susceptible functionalities has been applied as a successful strategy for protection against specific aminoglycoside-modifying enzymes.[41]

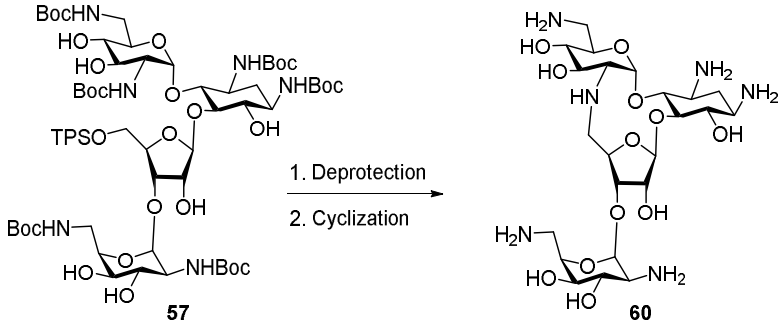
More recently, new structural-based approaches to overcome bacterial resistance have been developed. For example, based on the conformational differences exhibited by aminoglycoside ligands within the binding pockets of the ribosome and of those enzymes involved in bacterial resistance, it has been observed that some degree of conformational distortion at the rings I/II fragment is required for enzymatic activity.[42] For this reason it was possible to design conformationally locked oligosaccharides that still retain antibiotic activity, but that are not susceptible to enzymatic inactivation (Scheme 19).

Neomycin B derivative with a methylene bridge between the 2′-NH_2 of ring I and 5″-OH of ring III **60** has been prepared following a simple procedure (Scheme 20). Deprotection of the amino groups of compound **57** gave a crude sulfonate that smoothly cyclized in a highly regioselective way to afford the desired cyclic compound **60**.[15a] Compound **60** exhibits an improved activity against bacteria expressing ANT(4′) from *S. aureus* and AAC(2') from .*E.coli* (MICs= 20 and 10 μg/mL) against which neomycin B is inactive.[15a, 15b, 43]

Another approximation for overcome the resistance problem is the design of enzymatic inhibitors. These are broad-specificity agents that would target the enzymes that modify aminoglycosides and their development is a potentially much more difficult route, given that there are now over 70 known enzymes in contained in these three classes.



Scheme 19



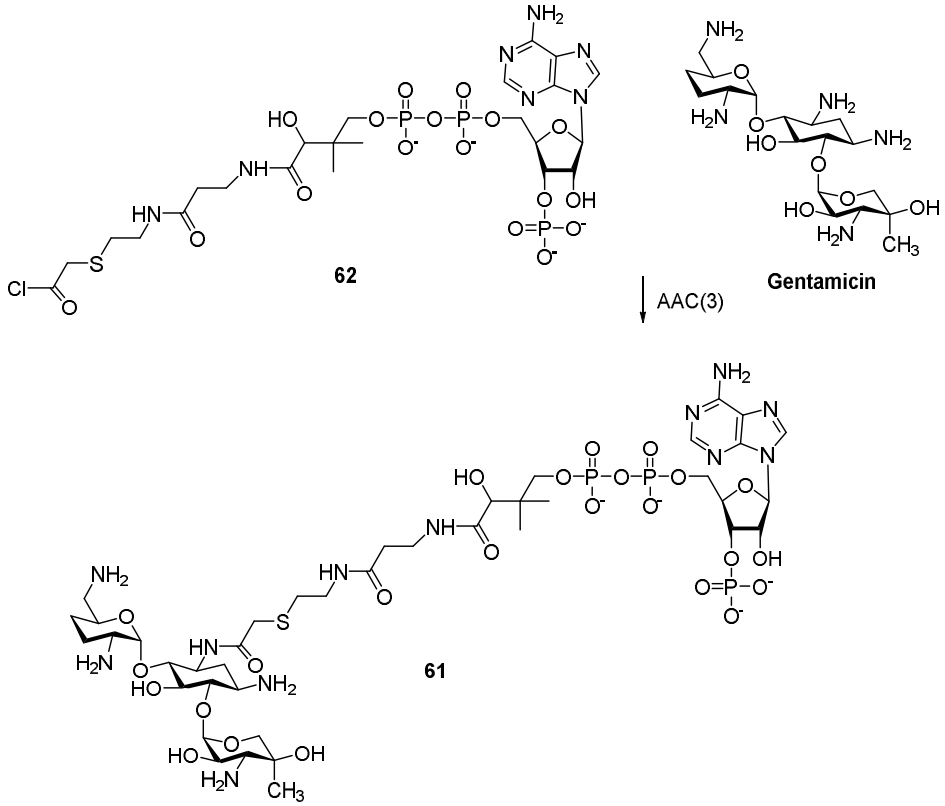
Scheme 20

Nevertheless, now that structural information is available for all three families, it might be possible to design inhibitors that are effective against a range of enzymes as opposed to just the one. If successful, such a strategy would allow some or all of the drugs that were once effective, including kanamycin, gentamicin and streptomycin, to play again a role in antimicrobial chemotherapy. In this context, it is also possible the development of antisense oligonucleotides or oligonucleotide analogs that interfere with gene expression.

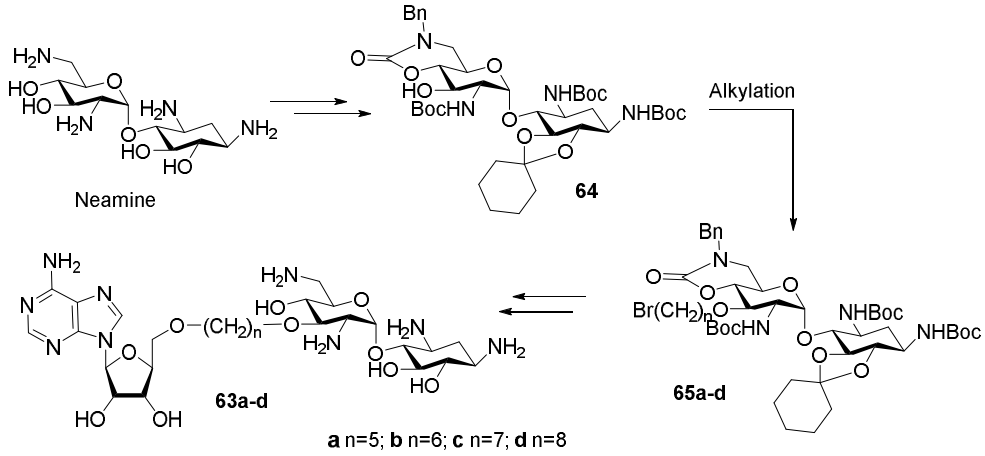
Inhibitor design can be targeted at either the aminoglycoside or cofactor binding sites, or both. In this latter case such an approach has been reported for diverse enzymes. Compounds consisting of both substrates covalently linked, known as bisubstrates, are potential tools to inhibit enzymatic reactions that involve the initial formation of a ternary complex through ordered or random binding of the substrates.

As a first approximation an aminoglycoside-CoA bisubstrate **61** was synthesized by gentamicin acetyltransferase catalyzed coupling of acetyl-CoA chloride **62** and gentamicin (Scheme 21). Compound **61** inhibit the activity of AAC(3) *in vitro*, but not *in vivo*, probably due to the inability of the compound to penetrate the cell wall.[44]

Further research led to the synthesis of other bisubstrates of smaller size by using truncated aminoglycosides or CoA. Scheme 22 shows the structure of the family of molecules formed by linking adenosine to the 3'-hydroxyl of neamine (**63a-d**). For their synthesis, compound **64** was obtained using the neomycin B fragment neamine as starting material.



Scheme 21



Scheme 22

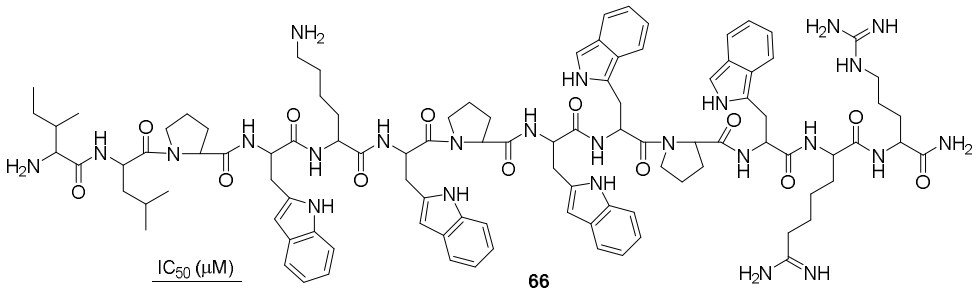
The aminoglycoside and the adenosine are connected by a methylene bridge of varying length (between 5 and 8 carbon atoms) which were introduced by alkylation of C-3' hydroxyl of compound **64** to afford intermediates **65a-d**. Finally, adenosine introduction in these molecules and subsequent total deprotection gave the desired molecules **63a-d**.

These compounds are inhibitors of both APH(3')-Ia and APH(3')-IIa, with the best inhibition obtained with bridge lengths of 6 and 7 carbon atoms (Table 4).[45]

Table 4. Inhibition constants of **63a-d** against APH(3')-Ia and APH(3')-IIa.

	n		**K*i*(μM)**	
		Variable substrate	APH(3')-Ia	APH(3')-IIa
63a	5	ATP	558±65	10±4
		Kanamycin A	32±5	18±11
63b	6	ATP	10±8	3±2
		Kanamycin A	3±2	5±3
63c	7	ATP	22±14	6±5
		Kanamycin A	9±1	17±4
63d	8	ATP	35±26	9±5
		Kanamycin A	224±61	14±2

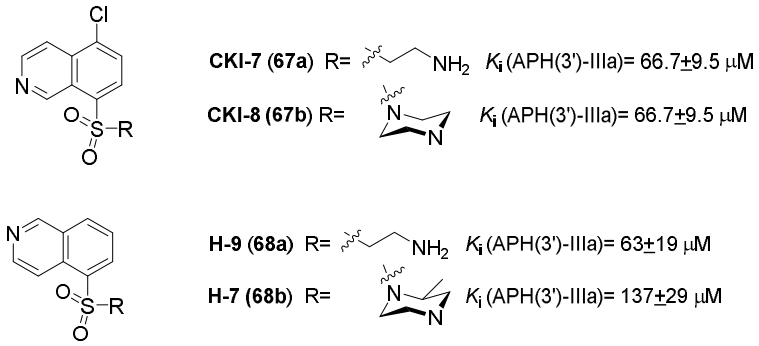
Fewer efforts have been directed towards non-carbohydrate inhibitors. Cationic antimicrobial peptides were tested as inhibitors of APH(3')-IIIa, AAC(6')-Ii, and AAC(6')-APH(2''). The results showed that the bovine peptide indolicidin **66** and analogs thereof have an inhibitory effect against both aminoglycoside phosphotransferases and aminoglycoside acetyltransferases, albeit by different mechanisms (Scheme 23).[48] These peptides represented the first example of broad-spectrum inhibitors for aminoglycoside resistance enzymes. However, although this research showed enormous potential for therapeutic purposes none of the peptides showed inhibitory effect *in vivo.*



Scheme 23

Finally, in the case of aminoglycoside-phosphotrasferases one of most promising approaches to their inhibition takes advantage of the remarkable degree of structural similarity between these enzymes and the eukaryotic protein kinases. Numerous inhibitors have been designed to target the nucleotide binding sites of protein kinases, and it is possible that this knowledge could be transferred to aminoglycoside phosphotransferases. In this context, known inhibitors of eukaryotic protein kinases were tested to determine if they had also activity against aminoglycoside phosphotransferases.[49]

Examples of protein kinase inhibitors with demonstrated activity against APHs include isoquinoline sulphonamides, such as the casein kinase inhibitors CKI-7 **67a** and CKI-8 **67b**, the cAMP kinase inhibitor H-7 **68a** and the casein kinase inhibitor H-9 **68b** (Scheme 24).[50]



Scheme 24

5. Conclusions

Ever since the discovery of aminoglycoside antibiotics, their challenging synthesis has attracted much attention. With the advancements in studies of resistance mechanisms and structural information from the binding of aminoglycosides with the target, the A-site decoding region of 16S rRNA and with other RNAs, new strategies have been developed that aim to revive antibacterial activity against aminoglycoside-resistant bacteria or as novel drugs for other RNA targets. Compounds containing carbohydrate and heterocyclic motifs have been designed as a new family of aminoglycosides. For their synthesis two alternative strategies have been described: (a) substitution of an aminosugar and/or aminocyclitol by heterocyclic motifs and (b) conjugation of an aminoglycoside with a heterocycle through a linker. Both strategies have yielded a large variety of compounds that have been probed against different biological targets.

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