An Efficient and General Route to the Synthesis of Novel Aminoglycosides for RNA Binding

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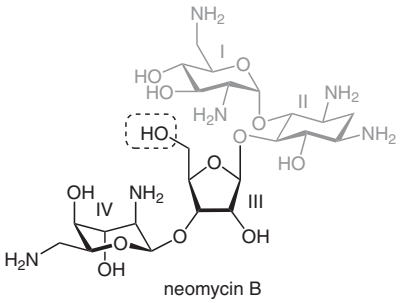
Dedicated to Professor Alberto Brandi on the occasion of his 60th birthday

Abstract: An alternative and straightforward method to prepare aminoglycoside dimers and heterodimeric conjugates is reported. The novel type of modification may provide a promising way for the development of new ligands effectively targeting to RNA.

Key words: aminoglycoside, azide, oligosaccharide synthesis, antibiotics, drug design

Protein synthesis is one of the fundamental processes in all living cells, and therefore, it is not surprising that the RNA and protein machinery of the prokaryotic ribosomes are the target of about half of the antibiotics characterized thus far.[2] Among the different classes of clinically important antibiotics that interfere with protein synthesis via this target (e.g., aminoglycosides, macrolides, and oxazolidinones), aminoglycosides (Figure 1) represent gold-standard drugs for the treatment of serious Gram-negative pathogens.



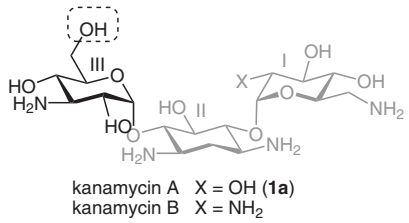


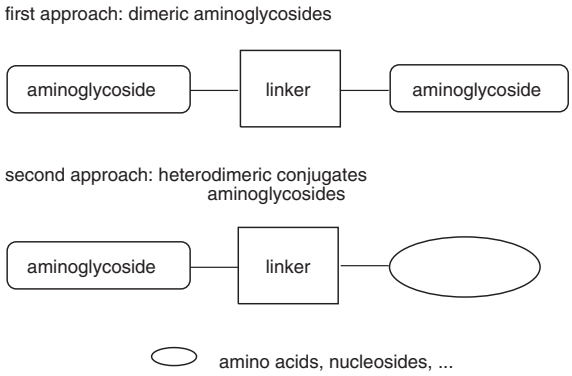
Figure 1 Natural aminoglycoside antibiotics

However, the prolonged clinical and veterinary use of aminoglycosides has resulted in the rapid spread of antibiotic resistance to this family of antibacterial agents in pathogenic bacteria.[3] The relative toxicity to mammals is another critical problem of these drugs that largely limits their intensive clinical use.[4]

Systematic studies on direct chemical modification of existing aminoglycoside drugs, with the aim of circumventing the resistance mechanisms, without either diminishing their activity or increasing their toxicity, has opened up a new era in the history of aminoglycosides.[5]

Importantly, the results of these efforts suggest that the introduction of new types of interactions between the new aminoglycosides and RNA are a crucial component in the development of new types of antibiotics.[6]

In this context, two possible approaches are the dimerization of the pharmacophore using the same or different aminoglycosides[7] and the addition of amino acids,[8] simple hydrophilic[9] or hydrophobic moieties,[10] or nucleosides and oligonucleosides[11] to make heterodimeric conjugates (Scheme 1).



Scheme 1 General approaches for the introduction of complementary interactions between aminoglycosides and RNA

According to different experimental findings, the pseudodisaccharide fragment I/II (grey fragment, Figure 1), with slightly different patterns of OH/NH_2 distribution in unit I, present in most aminoglycosides, is essential for specific complex formation with the prokaryotic 16S RNA.[12] Despite their secondary importance the amines in sugar units at the 5- or 6-positions in the 2-DOS ring provide additional contacts with the lower and upper stems, respectively, of the RNA receptor as shown by detailed NMR and crystallographic studies carried out in recent years.[13] Finally, other studies have indicated that the 6-OH in unit

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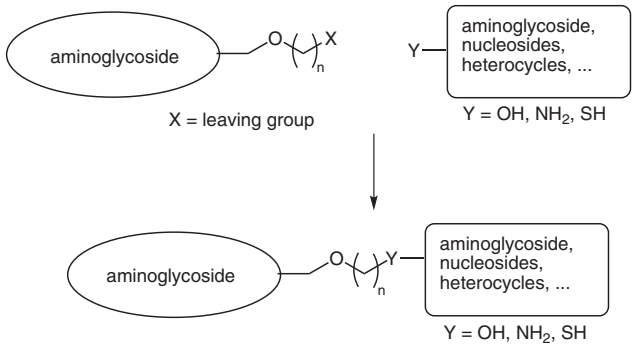
III in kanamycin and the 5-OH in unit III of neomycin are not essentials for RNA binding (selected hydroxy groups, Figure 1).[14]

Bearing in mind these results, these primary positions are optimal as tethering points between the aminoglycoside and the linker.

Here, we have developed a general selective synthetic strategy that allows us to readily synthesize homo- or heterodimers and heterodimeric conjugates derivates of aminoglycosides (Scheme 2), by coupling the functionalized aminoglycoside unit with different substrates pending nucleophiles like OH, NH_2, SH through a substitution reaction.



Scheme 2 Proposed general strategy

This approach is illustrated in this letter for the synthesis of the kanamycin–kanamycin homodimer **2b** (Figure 2).

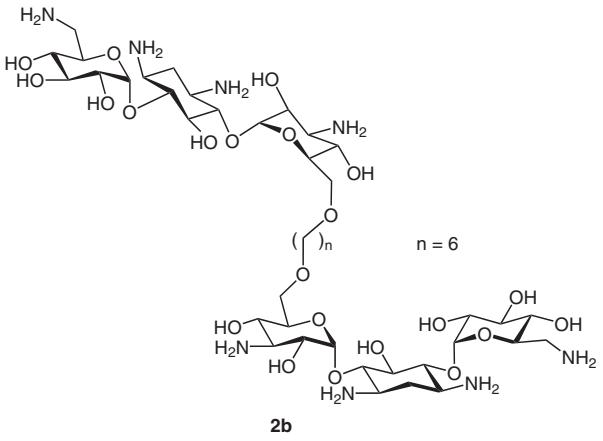
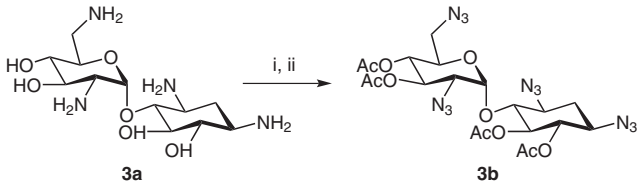


Figure 2 Structure of the kanamycin–kanamycin homodimer **2b**

As aminoglycoside precursor in this paper, compound **1d** was prepared from kanamycin A (**1a**) following the four-step procedure in Scheme 4.

Kanamycin A (**1a**) is first protected as its azido derivative **1b**. Azides have several advantages as amino protecting groups over carbamates, used in general for the synthesis of dimeric aminoglycosides.[7] These include less steric hindrance, greater solubility, and no rotamer formation or hydrogen or carbon nuclei to complicate NMR spectra. For these reasons, the preparation of azide-aminoglycosides has been a challenge for which several methods have been developed. Most of these methods employ, however, undesirable conditions or show limited success. For example, Wong and co-workers have described a methodology for the conversion of kanamycin A (**1a**) into tetraazidokanamycin (**1b**) by means of a diazotransfer reaction with freshly prepared triflyl azide (TfN_3).[15] However, in this paper for the preparation of triflyl azide and its application in the diazo transfer to amines triflic anhydride and sodium azide are used in a biphasic system of CH_2Cl_2–H_2O, and highly hazardous diazidomethane can be formed. More recently, Ernst and co-workers have developed a safe and convenient method for the copper(II)-catalyzed diazo transfer from triflyl azide to primary amines.[16] In this report toluene was identified as a solvent for the preparation of TfN_3 to avoid the formation of hazardous byproducts.

To test the scope of this version of the diazo transfer reaction in aminoglycosides, protection of neamine (**3a**) was investigated (Scheme 3).



Scheme 3 *Reagents and conditions*: (i) TfN_3 (2.5 equiv for amine), $NaHCO_3$, $CuSO_4 \cdot 5H_2O$ (0.05 equiv), different solvents (see Table 1), r.t., 18 h; (ii) Ac_2O–pyridine (1:2, v/v), DMAP (0.05 equiv), r.t., 16 h.

As a first approximation, we carried out the reaction under heterogeneous conditions, but the desired compound was not obtained due to the limited solubility of aminoglycosides in toluene. When the neamine hydrochloride was subjected to the monophasic conditions (H_2O–PhMe–MeOH) previously described,[16] the aminoglycoside precipitated from the solution, which prevented any reaction with TfN_3. In order to avoid this, we chose pyridine as co-solvent because it dissolves the aminoglycoside, and TfN_3 is stable under these conditions.[17]

The reaction was tested in three different conditions (Table 1). The triflyl azide solution in toluene was added to a mixture of neamine (**3a**), sodium hydrogencarbonate and copper(II) sulfate in water, followed by a mixture of methanol and pyridine to obtain a homogeneous turquoise solution. The TLC indicated the reactions to be complete within 20 hours.

Table 1 Diazo Transfer Reaction Using Different Solvent Ratios

Entry	Solvent ratio (H_2O–PhMe–MeOH–pyridine)	Yield of **3b** (%)[a]
1	1:1.7:3:3, homogeneous	75
2	1.7:1:3:3, homogeneous	50
3	1:4:5:5, homogeneous	63

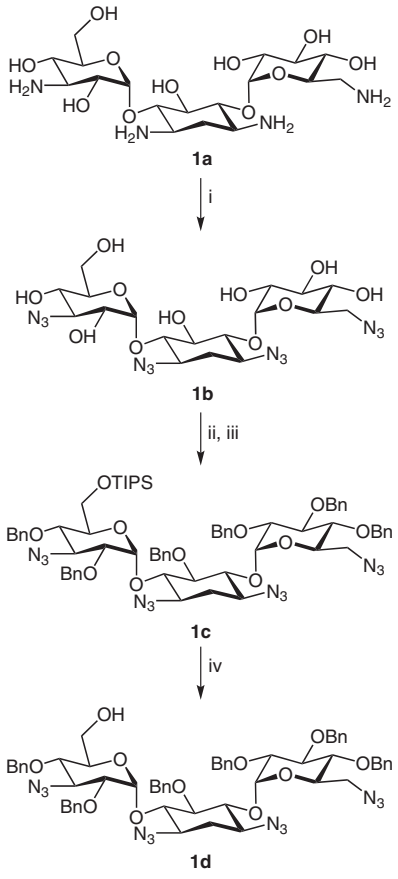
[a] Isolated yields of two steps.

In order to avoid problems during the purification process, the crude reaction mixture was subsequently treated with acetic anhydride and a catalytic amount of DMAP in pyridine yielding **3b** in moderate to good yields.

After the successful conversion of neamine (**3a**) into **3b**, the diazotransference of kanamycin A (**1a**) was undertaken. Free kanamycin A (**1a**) was treated with excess of TfN_3 in the presence of $CuSO_4{\cdot}5H_2O$ in a mixture of H_2O–PhMe–MeOH–pyridine (1:1.7:3:3). After 18 hours and aqueous workup the tetraazidokanamycin (**1b**), without isolation, was acetylated. Finally, O-deacetylation gave tetraazidekanamycin (**1b**) with an overall yield 63% from kanamycin A (**1a**).

This compound was treated with triisopropylsilyl trifluoromethanesulfonate and 2,6-lutidine providing the silyl ether intermediate, which was subsequently benzylated to yield **1c**. Finally, acidic desilylation afforded compound **1d**, the key component in this methodology (Scheme 4).

This monomer **1d** that has a specific free hydroxy group is very useful for the synthesis of dimeric aminoglycosides or heterodimeric conjugates by coupling the functionalized main unit with different linkers (Scheme 2).

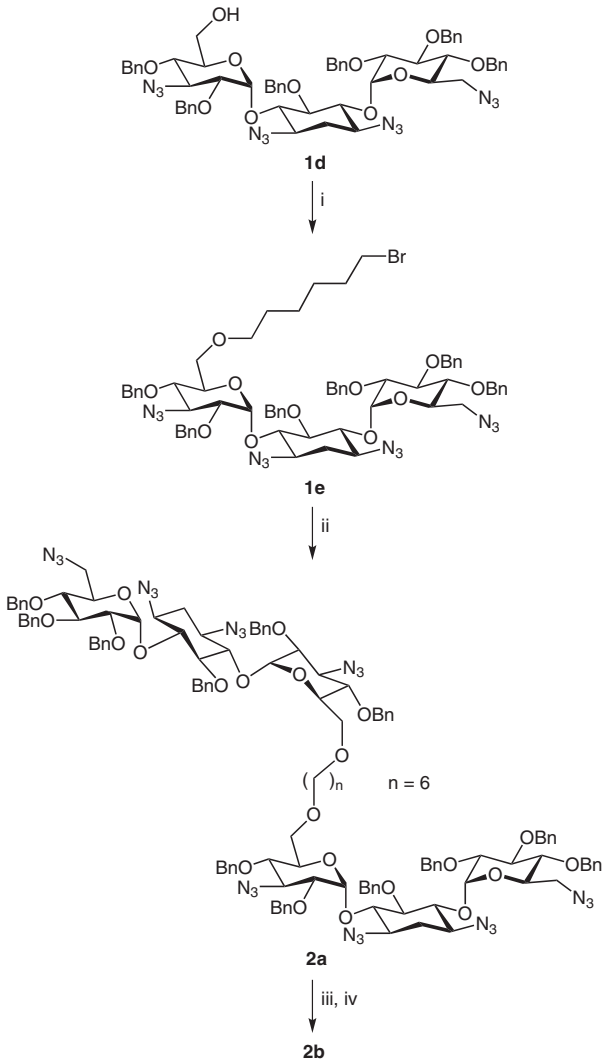


Scheme 4 *Reagents and conditions*: (i) (a) TfN_3, $CuSO_4{\cdot}5H_2O$, toluene, pyridine–MeOH–H_2O, r.t., 18 h; (b) Ac_2O, DMAP, pyridine, 70% (two steps); (c) NaOMe, MeOH, 90%; (ii) TIPSOTf, 2,6-lutidine, THF, 10 min, 65%; (iii) BnBr, NaH, TBAI, DMF, r.t., 18 h, 50%; (iv) HF·pyridine, THF, r.t., 1 h, 90%.

For example, in this letter, to test the scope of this methodology, compound **1d** was alkylated with 1,6-dibromohexane to afford compound **1e** in 75% yield.

Finally, a new monomer unit was alkylated with compound **1d**, obtaining the protected homodimer kanamycin–kanamycin (**2a**), in 50% yield.

The resulting dimer was first reduced under Staudinger conditions to convert the azides into amines. In the last step this compound was debenzylated by hydrogenolysis in the presence of trifluoroacetic acid. The reaction mixture was filtered, concentrated, and purified by silica gel chromatography using NH_4OH–*n*-BuOH–EtOH–CH_2Cl_2 (5:2:2.5:1), followed by cation-exchange chromatography to give the pure aminoglycoside dimer kanamycin–kanamycin **2b** (Scheme 5).



Scheme 5 *Reagents and conditions*: (i)1,6-dibromohexane, NaH, NaI, DMF, r.t., 16 h, 75%; (ii) **1d**, NaH, NaI, DMF, r.t., 48 h, 50%; (iii) Me_3P, NaOH, THF, 55 °C, 24 h; (iv) H_2, $Pd(OH)_2$/C, TFA, MeOH–H_2O, r.t., 72 h, 55% (two steps).

In conclusion, we have developed an efficient and general route to novel homo- or heterodimers and heterodimeric conjugates derivates of aminoglycosides. In addition, we

have reported a safe and convenient method for the synthesis of azide-aminoglycosides.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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