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Fluorinated aminoglycosides

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Dedicated to Prof. Stephen Hanessian in honour his scientific contributions to aminoglycoside chemistry

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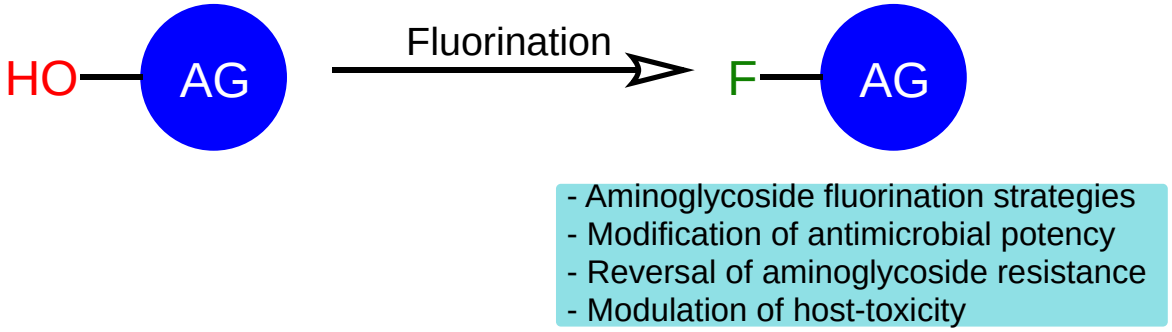
Abstract

Aminoglycosides (AGs) are natural or semisynthetic antibiotics that are inherently toxic, and their use is limited by the widespread presence of AG-resistant bacteria. However, AGs are still valued members of the antibiotic arsenal, particularly against Gram-negative bacteria. The efficiency and clinical value of this class of antibiotics have motivated the exploration of strategies to overcome or evade both the antimicrobial resistance and toxicity. The incorporation of a fluorine atom into AGs has attracted attention as a potential strategy to solve these problematics. This review provides an overview of the research on fluorinates aminoglycosides, their synthesis and, when available, their antimicrobial activity, relative toxicity toward mice or human cells and physicochemical properties.



Keywords: Fluorinated aminoglycosides, deoxyfluorination, toxicity modulation, evasion of antimicrobial resistance.

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1. Introduction

Aminoglycosides (AGs) are a class of clinically important, potent and broad-spectrum antibiotics.[1] They bind to the decoding A-site of the 16S ribosomal RNA[2-4] and interfere with protein synthesis,[3-5] which gives these types of molecules broad bactericidal effects. Examples of clinically used AGs include the natural products kanamycin A (**1**), tobramycin (**2**), and the gentamycin complex (gentamycin C2 (**6**) shown) as well as semisynthetic AGs amikacin (**4**) and dibekacin (**3**).[6] Other important AGs include paromomycin (**7**), used for the treatment of leishmaniosis and dysenteric amoebiasis,[7-8] and neomycin B (**8**), used in topical ointments[9] (Figure 1).

The extensive use of AGs as antibiotics has led to the emergence of resistant strains that express aminoglycoside-modifying enzymes (AMEs).[1, 6, 10-12] AMEs catalyse modifications of the functional groups of AGs, rendering clinically important AGs inactive. AMEs include enzymes such as *N*-acetyltransferases (AACs), *O*-phosphotransferases (APHs) and *O*-adenyltransferases (ANTs). It is possible to avoid the action of AMEs by the suitable synthetic modification of the AG. Semisynthetic dibekacin (**3**),[13] which lacks the 3' and 4' OH moieties, is not deactivated by APH (3') and ANT (4') enzymes that render kanamycin (**1**) inactive. A similar result can be obtained by deoxyfluorination. Semisynthetic AG 3',4'-dideoxy-3'-fluorokanamycin A (**144**),[14] is not deactivated by APH (3') or ANT (4') enzymes. The introduction of a fluorine atom in the structure of an AG has another advantage: it can modulate the electron density of neighbouring nitrogen atoms, reducing their basicity (*vide infra*).

In addition to resistance, another limitation on the use of AGs as drugs is their high nephrotoxicity [15] and ototoxicity.[16-19] The correlation between the acute toxicity and basicity of AGs has been established,[20] and the

introduction of an electronegative fluorine atom allows the modulation of the pK_a of a neighbouring nitrogen.[21-22]

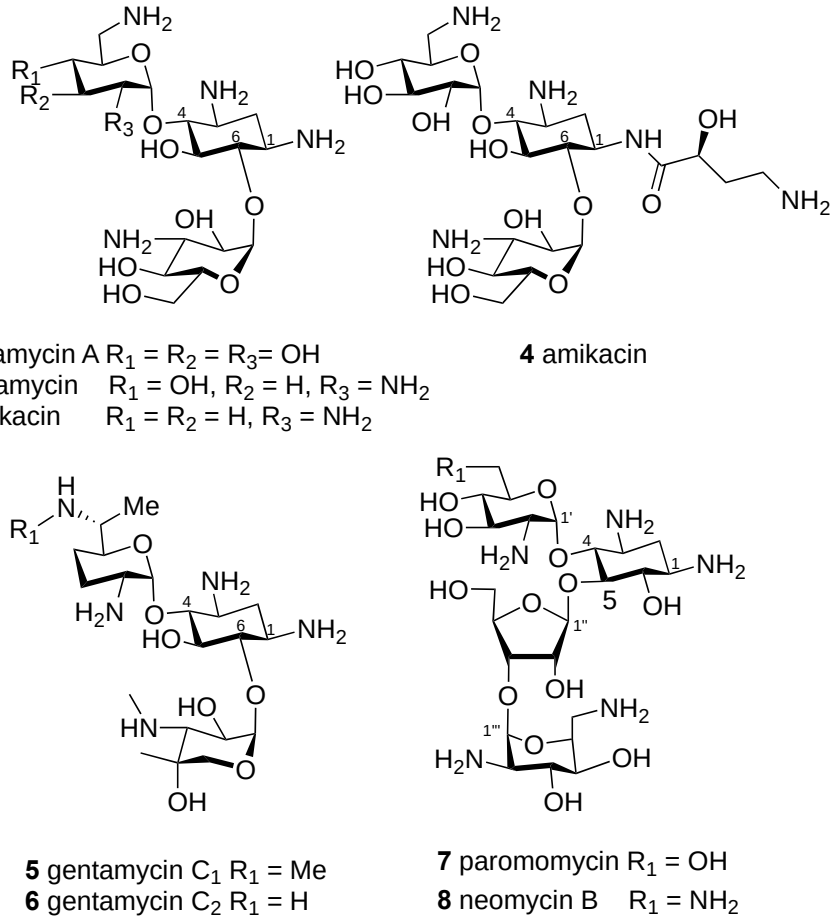


Figure 1. Clinically important AGs.

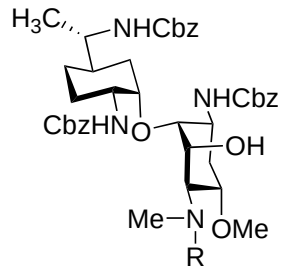
Fluorinated AGs are of interest to provide antibiotics with improved antibacterial activity,[23] better resistance to inactivation by bacterial enzymes,[24-25] and lower toxicity.[26-28] To date, the primary synthetic tool for preparing fluorinated derivatives of AGs has been deoxyfluorination using fluorinating agents, particularly DAST, or by nucleophilic displacement using a fluoride salt (organic or inorganic) [29]. The principal obstacle to fluorination of aminoglycosides is obtaining an appropriately protected molecule in which only the target group is free to transform; this process may require several well-planned protection-deprotection sequences.[29] Sometimes, the outcome of deoxyfluorination is difficult to predict *a priori*,[30] and several steps are required to achieve the desired substitution pattern, obtaining a single fluorinated AG derivative can be considered a scientific feat in and of itself.

The first reports related to fluorinated AGs appeared in the literature in the 1970s.[31-32] Since then, the topic has been of particular interest, and several studies were published between 1980 and 1998. Recently, this field has attracted new interest.[33-36] Our aim is to briefly review the synthetic procedures used to obtain fluorinated AGs described in the literature. When available, we also comment on the biological data and physicochemical parameters of the fluorinated AG.

2. Fluorinated Monosubstituted Aminocyclitol AGs

2.1. Fluorinated derivatives of sporaricin A (9) [37-38]

The structures of sporaricins A and B (**9** and **10**) are shown in Figure 2.



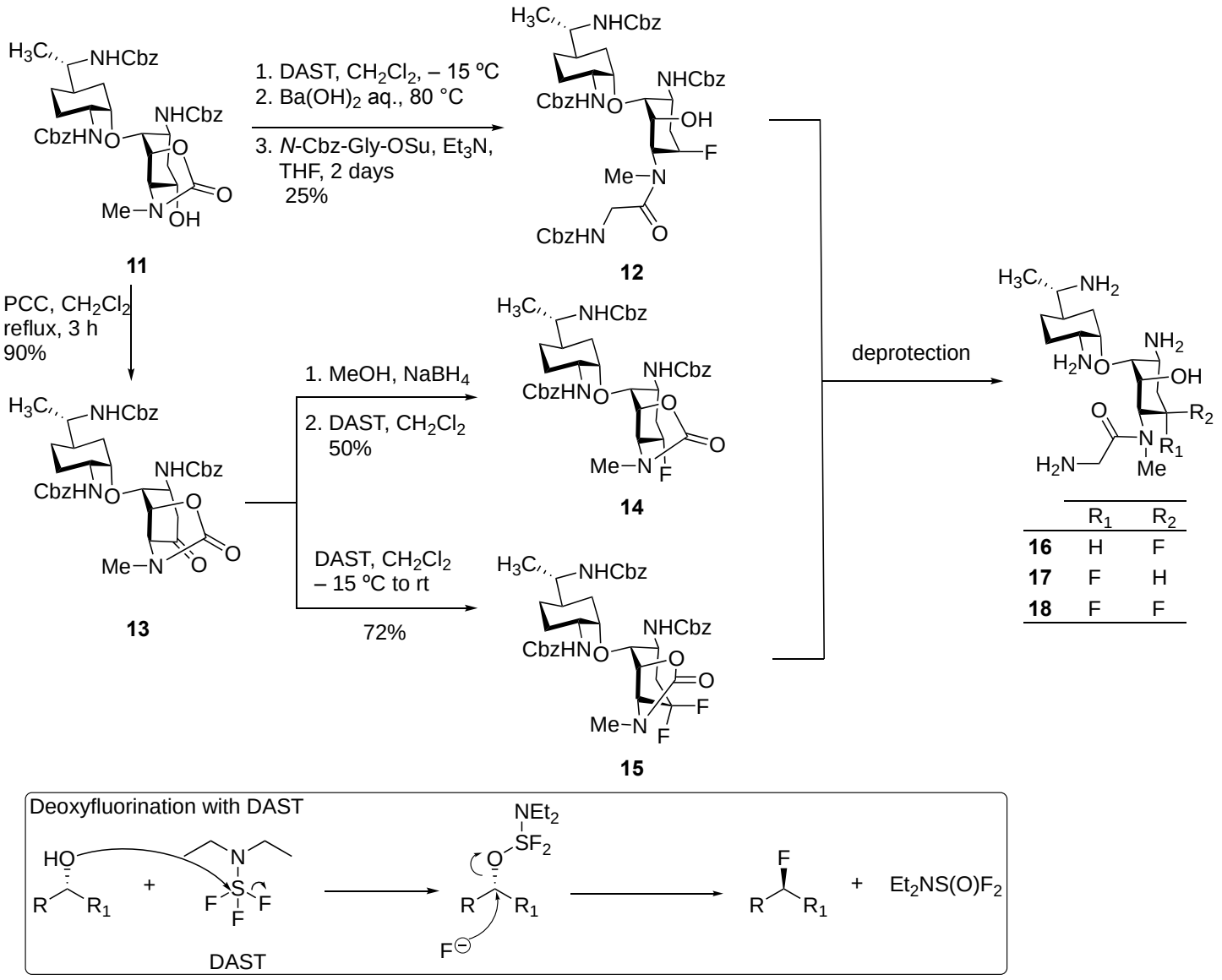
9 sporaricin A R = $COCH_2NH_2$
10 sporaricin B R = H

Figure 2. Sporaricin A and sporaricin B.

The treatment of a suitable protected sporaricin B analogue (**11**) with diethylaminosulfur trifluoride (DAST) at low temperature resulted in deoxyfluorination with inversion of the configuration (Scheme 1), the use of DAST for deoxyfluorination has been reviewed.[39-41] Selective hydrolysis of the cyclic carbamate with $Ba(OH)_2$ followed by *N*-acylation with Cbz glycine *N*-hydroxysuccinimide gave **12** in an overall yield of 25%. Removal of the *N*-Cbz protecting group by hydrogenolysis provided 3-demethoxy-3-*epi*-3-fluorosporaricin A (**16**). Treatment of **11** with pyridinium chlorochromate (PCC) gave the 3-oxo derivative (**13**) in excellent yield. The Pfitzner-Moffatt oxidation can also be used, although the yields are lower.

The reduction of the carbonyl exclusively afforded the axial alcohol, and subsequent deoxyfluorination using DAST yielded compound (**14**). Global deprotection afforded 3-fluoro-3-demethoxy sporaricin A (**17**). The reaction of **13** with DAST afforded the C-3 difluorinated analogue (**15**), which after deprotection, afforded **18**.

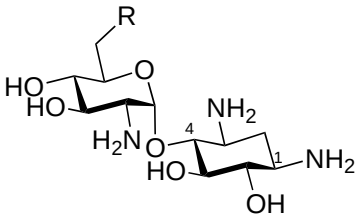
Compounds **16**, **17** and **18** were tested together with sporaricin A (**9**) against a panel of clinically important bacteria. The order of their antibacterial activity was **17** > sporaricin A (**9**) > **16** >> **18**. The fluorinated analogues (**16**, **17** and **18**) were less toxic than the parent compound, sporaricin A (**9**), with difluorinated analogue (**18**) being approximately two-fold less toxic than sporaricin A (**9**) in acute toxicity studies on mice.[37]



Scheme 1. Synthesis of 3-demethoxy-3-*epi*-3-fluorosporaricin A (**16**), 3-demethoxy-3-fluorosporaricin A (**17**) and 3-demethoxy-3,3-difluorosporaricin A (**18**).

2.2. Fluorinated analogues of neamine (19) and paromamine (20)

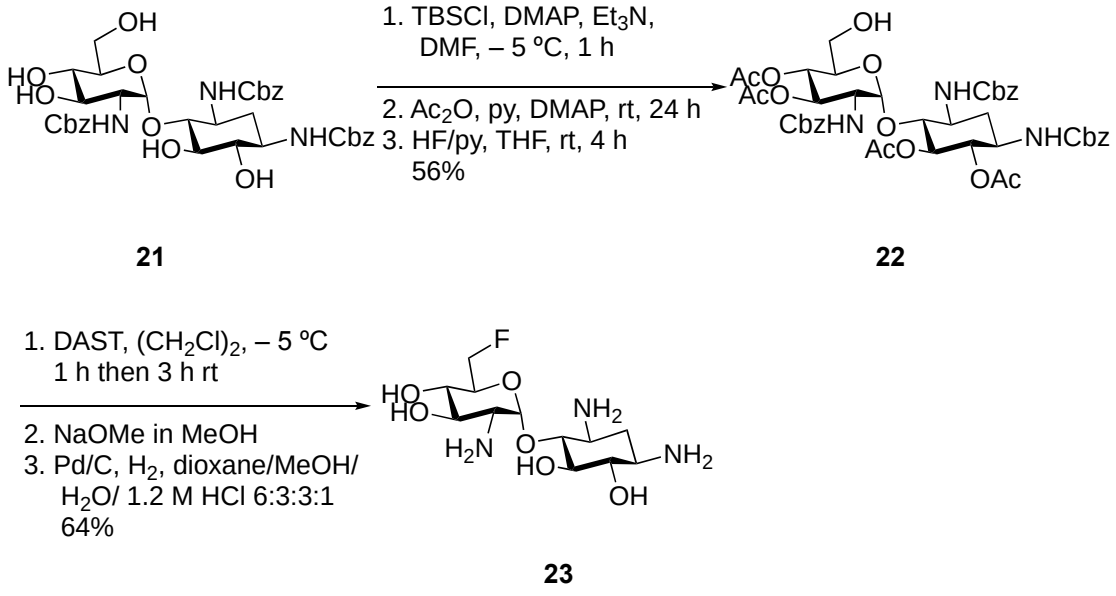
The chemical structure of neamine (**19**) and paromamine (**20**) are displayed in Figure 3.



19 neamine R = NH_2
20 paromamine R = OH

Figure 3. Neamine and paromamine.

2.2.1. Synthesis of 6'-deoxy-6'-fluoroparomamine (23).[39] The synthesis of the title compound (**23**) was performed with the aim of generating a high affinity ligand to perform ligand-based competitive binding NMR studies.[42] A suitably protected paromamine analogue (**21**) was reacted with *tert*-butyldimethylsilyl chloride (TBSCl) and then subjected to per-*O*-acetylation, affording **22** after cleavage of the silyl protecting group with HF and pyridine (Scheme 2). The reaction of **22** with DAST, followed by cleavage of the protecting groups yielded **23** in 64% yield.



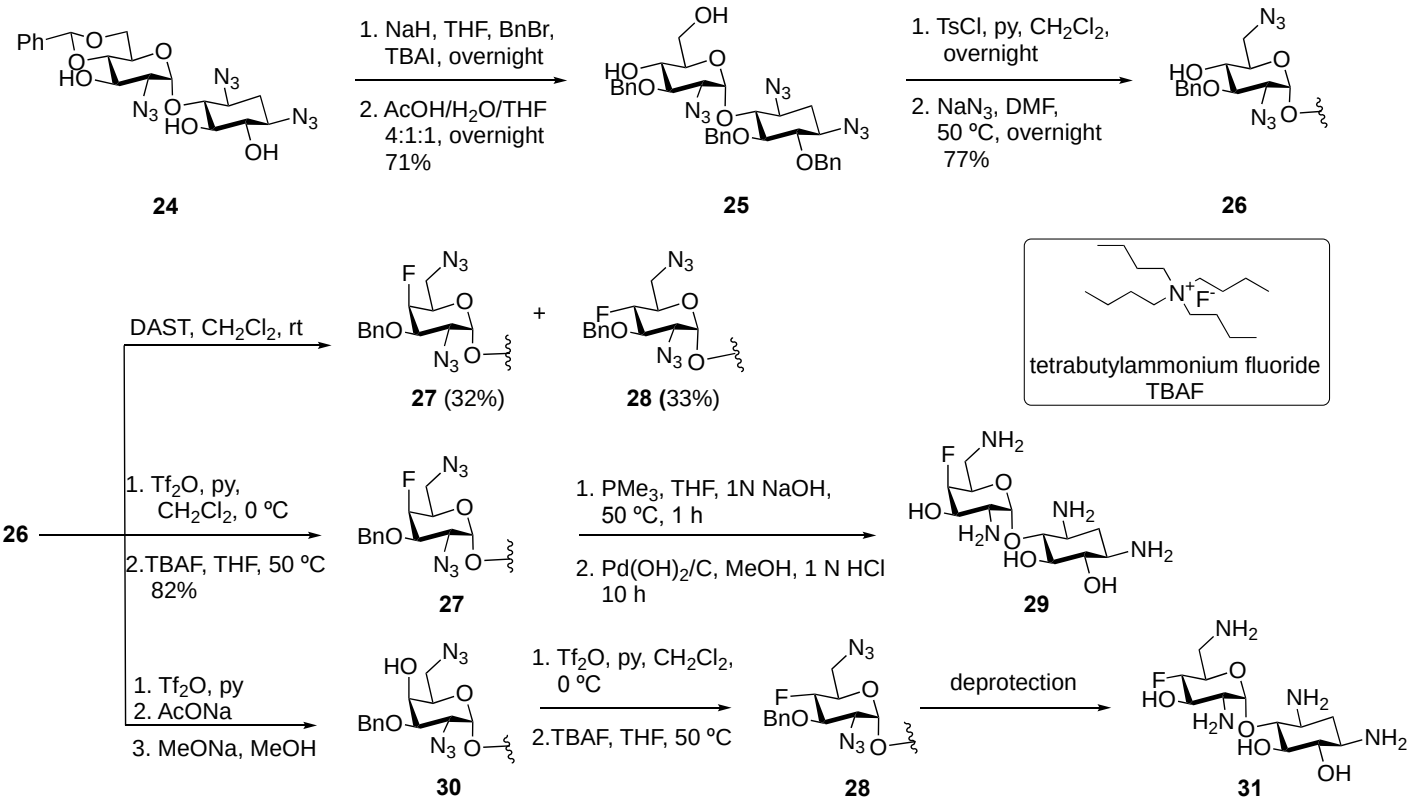
Scheme 2. Synthesis of 6'-deoxy-6'-fluoroparomamine (**23**).

Compound (**23**) proved to be a good reporter for monitoring the binding of A-site rRNA ligands. Taking advantage of the chemical-shift variations in their ^{19}F NMR spectra, the authors were able to determine the dissociation constants of paromomycin (**7**), neomycin (**8**), and neamine (**19**), and the obtained values were in good agreement with previous determinations.[42]

2.2.2. Synthesis of 4'deoxy-4'-fluoro analogues of neamine (19).[43] Two syntheses were reported by Hanessian's group as part of a systematic study to obtain fluorinated analogues of neomycin B (**8**).[25] In their first approach (Scheme 3), paromamine derivative (**24**) was used as the starting material, and their second approach (Scheme 4) used neamine derivative (**32**). The deoxyfluorination reaction was first studied using 6-azido-3-*O*-benzyl-6-deoxy-α-D-glucosamine pyranoside, a model compound that mimics ring I of neamine (**19**) (not shown). The reaction with DAST and the S_N2 displacement of 4-sulfonate esters with fluoride anion were modelled. Treatment with DAST gave a complex mixture of products, making the approach from the 4-sulfonate esters a more successful model. Both approaches were used in the synthesis of fluorinated neamine analogues.[43]

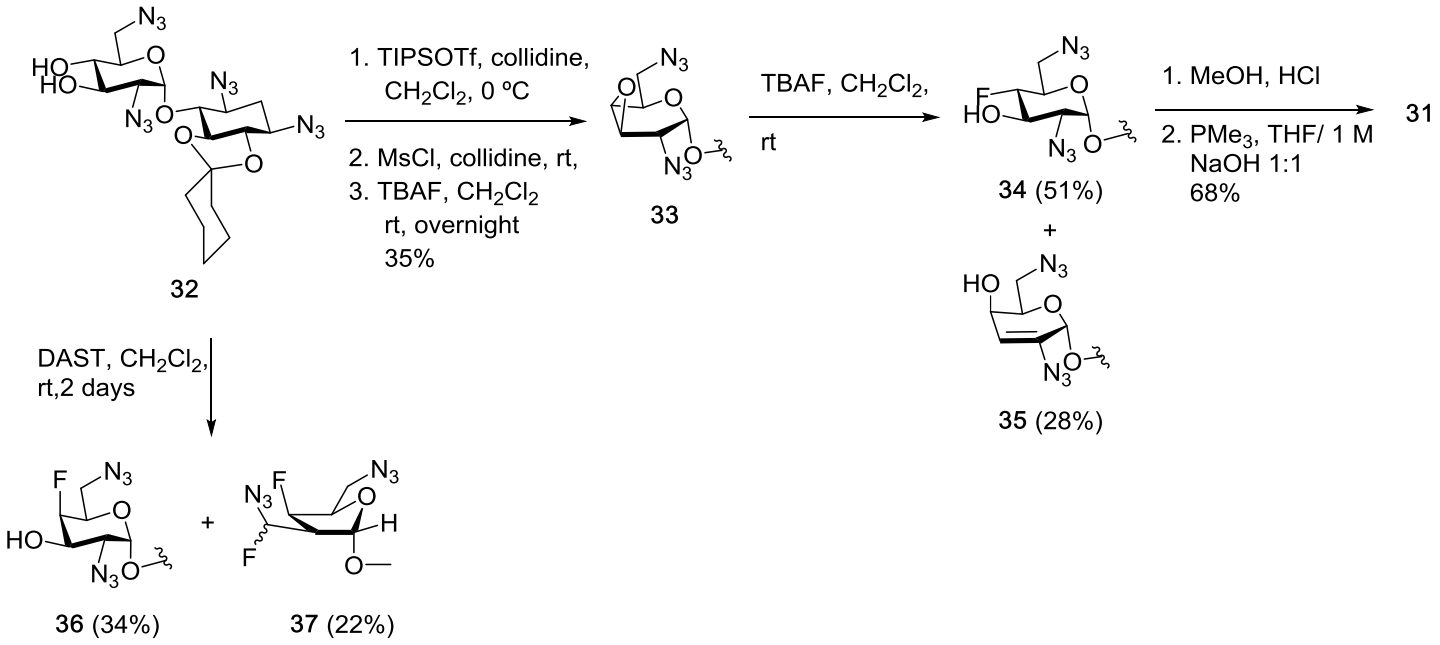
The per-*O*-benzylation of **24** using sodium hydride, benzyl bromide and tetrabutylammonium iodide (TBAI) as the catalyst, followed by cleavage of the benzylidene protecting group under acidic conditions, gave compound (**25**) (Scheme 3). The regioselective tosylation of the primary 6'-OH followed by azide displacement afforded **26**. Unlike the monosaccharide model, the reaction of compound **26** with DAST in dichloromethane afforded a separable mixture of **27** and **28** in 32% and 33% yields, respectively. Derivative (**28**), with retention of the configuration at C-4', was generated via neighbouring group participation by the C-3' benzyl ether through a 3',4'-oxiranium ring intermediate. Although the yields were reasonable, scale-up proved

challenging, and the use of 4-sulfonate esters was explored. Formation of the triflate and displacement with fluoride using tetrabutylammonium fluoride (TBAF) provided **27**. A Staudinger reduction of the azide groups followed by hydrogenolysis of the benzyl ethers gave **29**. The treatment of **26** with Tf_2O in pyridine/CH_2Cl_2 followed by S_N2 displacement with acetate and Zemplén deacetylation afforded a 4'-*epi* derivative (**30**). Compound **30** was treated with Tf_2O and TBAF to give a 4'-deoxy-4'-fluorinated derivative (**28**). Compound **31** was obtained after removal of the protecting groups under standard conditions.[43]



Scheme 3. First approach to the synthesis of 4'-deoxy-4'-epi-4'-fluoroneamine (**29**) and 4'-deoxy-4'-fluoroneamine (**31**).

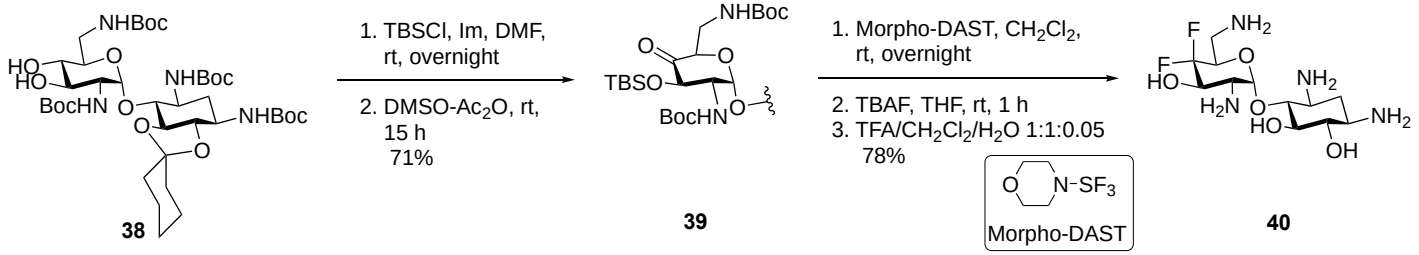
The second approach started from perazido neamine derivative **32** (Scheme 4). Treatment of **32** with triisopropylsilyl trifluoromethanesulfonate (TIPSOTf) followed by reaction with mesyl chloride (MsCl) and cleavage of the silyl protecting group with concomitant formation of the 3'-4'-oxirane moiety afforded epoxide (**33**) in 35% yield. Treatment of **33** with TBAF gave 4'-deoxy-4'-fluoroglucosamine derivative (**34**) and vinyl azide **35**. The vinyl azide is formed by *syn* elimination promoted by the basicity of the fluoride ion by a method that was previously reported in the literature.[44] The deprotection of compound **34** gave **31** in 68% yield. The reaction of **32** with DAST in dichloromethane afforded two compounds: a 4'-deoxy-4'-*epi*-4'-fluoro neamine derivative (**36**) and compound **37**, which was formed by ring contraction.



Scheme 4. Second approach to the synthesis of 4'-deoxy-4'-fluoroneamine (**31**).

Both compounds (**29** and **31**) were crystallized as complexes with the rRNA A-site,[45-46] showing that these compounds effectively interact with the macromolecule.

2.2.3. Synthesis of 4'-deoxy-4',4'-difluoroneamine (40).[47] The treatment of suitable protected neamine derivative (**38**) with *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole (Im) in DMF followed by oxidation of the 4'-OH group with DMSO-Ac_2O gave **39** (Scheme 5). Deoxyfluorination was achieved by treating **39** with Morpho-DAST, a more thermally stable version of DAST,[40, 48] and 4'-deoxy-4',4'-difluoroneamine (**40**) was obtained after removal of the protecting groups under standard deprotection conditions.

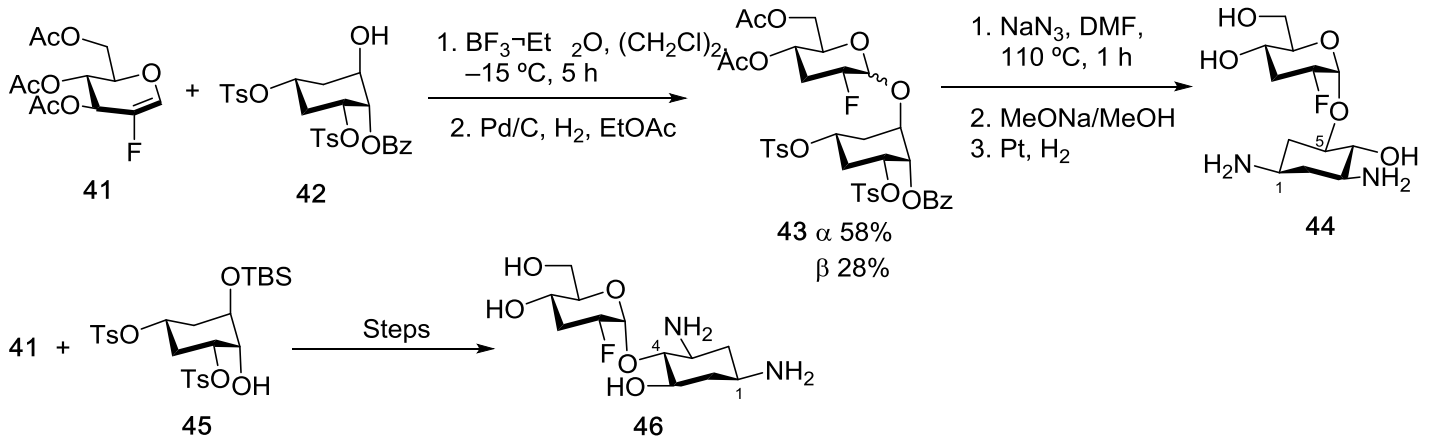


Scheme 5. Synthesis of 4'-deoxy-4',4'-difluoroneamine (**40**).

The kinetics of the reaction of Gram-negative APH(3') types I and II with neamine (**19**) and its 4'-deoxy-4',4'-difluoro analogue (**40**) were evaluated, and the results showed that difluorination severely impairs the binding of the difluorinated analogue to the enzymes. The impairment was in the same range as that observed with the D198A mutant of the APH(3')-Ia with 4'-deoxy-4',4'-difluorokanamycin A (**223**) (refer to section 3.3.18),[47] and these results were attributed to the reduction of the nucleophilicity of the 4'-OH due to the presence of the electronegative atoms.

2.3. Synthesis of α-linked 2',3'-dideoxy-2'-fluoro-pseudo-disaccharides[31]

Compounds **44** and **46** were obtained by glycosylation of the previously fluorinated derivative 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoroglycal **41**, which is accessible from tri-*O*-acetylglycal,[49-50] with cyclitol derivatives **42** and **45**, respectively (Scheme 6). The glycosylation reaction between **41** and **42** afforded a mixture of the α and β anomers. The α anomer (**43**) was obtained in 58% yield, and the β anomer was obtained in 28% yield. Compound **44** was obtained after displacement of the tosyl groups with sodium azide, hydrolysis of the ester protecting groups and hydrogenation with Pt. A similar procedure was used to obtain **46**. Both aminocyclitol derivatives were synthesized as possible precursors of more complex AGs by mutasynthesis.[31]



Scheme 6. Synthesis of fluorinated pseudo saccharides **44** and **46**.

2.4. Fluorination of apramycin (47)[34]

Apramycin (**47**, Figure 4) is a structurally unique monosubstituted aminocyclitol AG, which currently is only approved for veterinary use.[51]

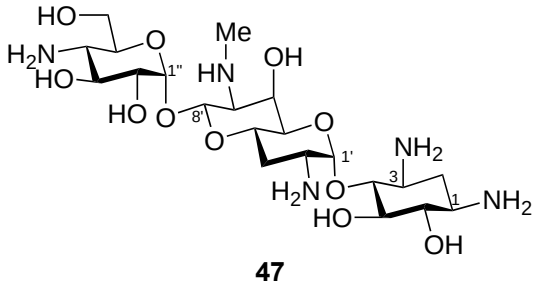
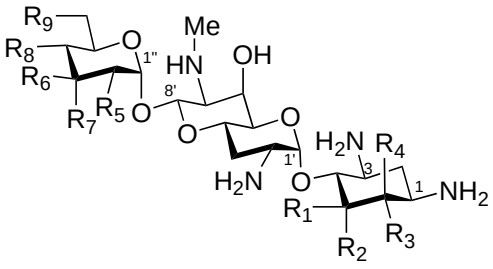


Figure 4. Apramycin (**47**).

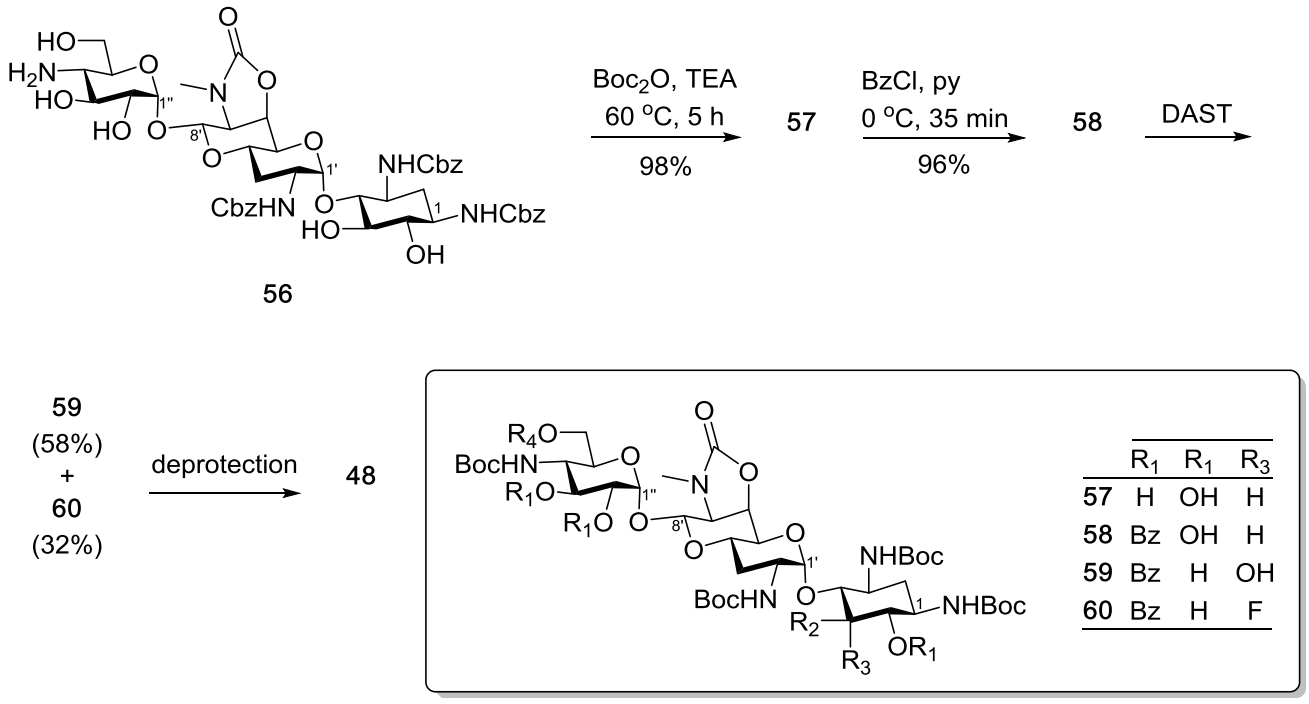
The synthesis of several fluorinated apramycin analogues (**48 – 55**) has been reported in the patent literature (Figure 5).[34] Two of the reported syntheses are discussed next (*vide infra*).



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
48	H	F	OH	H	OH	OH	H	NH_2	OH
49	H	F	OH	H	H	H	OH	NH_2	OH
50	H	F	OH	H	OH	H	H	NH_2	OH
51	H	F	H	NH_2	OH	OH	H	NH_2	OH
52	H	F	OH	H	OH	OH	H	$NH(CNH)NH_2$	OH
53	H	F	OH	H	OH	H	H	$NH(CNH)NH_2$	OH
54	F	H	H	H	OH	OH	H	NH_2	OH
55	OH	H	OH	H	OH	OH	H	NH_2	F

Figure 5. 5-Deoxy-5-*epi*-5-fluoroapramycin (**48**), 5,2''-dideoxy-5,3''-di-*epi*-5-fluoroapramycin (**49**), 5,3''-dideoxy-5-*epi*-5-fluoroapramycin (**50**), 6-amino-5,6-dideoxy-5,6-di-*epi*-5-fluoroapramycin (**51**), 4''-deamino-5-deoxy-5-*epi*-5-fluoro-4''-guanidinoapramycin (**52**), 4''-deamino-5,3''-dideoxy-5-*epi*-5-fluoro-4''-guanidinoapramycin (**53**), 5,6-dideoxy-5-fluoroapramycin (**54**) and 6''-deoxy-6''-fluoroapramycin (**55**).

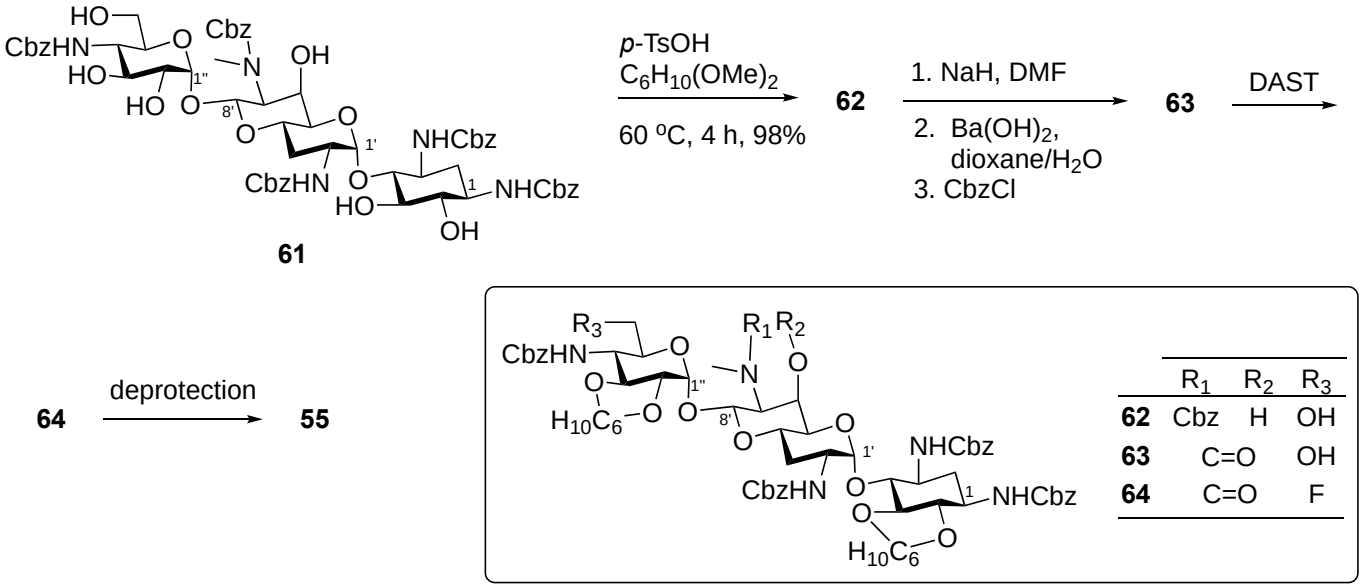
The synthesis of 5-deoxy-5-*epi*-5-fluoroapramycin (**48**)[34] started from suitably protected apramycin derivative **56**.[52] The reaction of **56** with Boc_2O and triethylamine (TEA) afforded **57**, which was further reacted with benzoyl chloride (BzCl) to give **58** (Scheme 7). Treatment of **58** with DAST gave two products, the 5-*epi* derivative (**59**) and the desired 5-deoxy-5-fluoro-5-*epi* derivative (**60**), in 58% and 32% yields, respectively. Compound (**60**) was deprotected under standard conditions, affording **48** (Figure 5).



Scheme 7. Synthesis of 5-deoxy-5-*epi*-5-fluoroapramycin (**48**).

The synthesis of 6''-deoxy-6''-fluoroapramycin (**55**)[34] started from the *N*-Cbz apramycin derivative (**61**). Treatment of **61** with 1,1-dimethylcyclohexane and *p*-toluenesulfonic acid (*p*-TsOH) gave **62** in excellent yield (Scheme 8). Treatment of **62** under the conditions described by Vasella *et al*.[52] afforded **63**, which was reacted

with DAST to afford the 6''-deoxy-6''-fluoro derivative (**64**). Compound **64** was further deprotected under standard conditions to afford **55** (Figure 5).



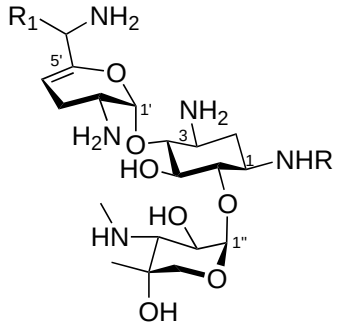
Scheme 8. Synthesis of 6''-deoxy-6''-fluoroapramycin (**55**).

Compounds (**48** - **55**) were tested against Gram-positive and Gram-negative bacteria and showed activity against *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* strains, which are resistant or show low sensitivity to amikacin (**4**), arbekacin (**93**, Figure 9) and gentamycin.

3. Fluorinated 4,6-Disubstituted 2-Deoxystreptamine AGs

3.1. Fluorinated 4,6-disubstituted 2-deoxystreptamine AGs

The structure of 4,6-disubstituted 2-deoxystreptamine AGs sisomicin (**65**), netilmicina (**66**), and verdamycin (**67**) are depicted in Figure 6.



65 sisomicin R = H, R_1 = H
66 netilmicin R = CH_2CH_3, R_1 = H
67 verdamycin, R = H, R_1 = Me

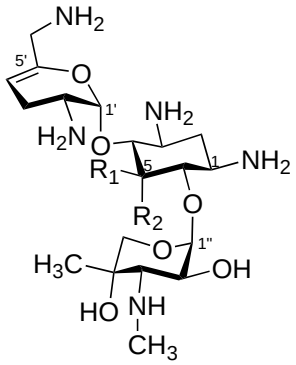
Figure 6. Sisomicin (**65**), netilmicin (**66**) and verdamicin (**67**).

3.1.1. Synthesis of fluorinated sisomicin analogues by mutasynthesis or mutational biosynthesis.[32] The first attempts toward fluorinated 4,6-disubstituted 2-deoxystreptamine aminoglycosides involved mutasynthesis or mutational biosynthesis. The process consists of selecting a microorganism, called an idiotroph, that is unable to synthesize a natural product without a mutasynthon. This precursor has to be supplemented to the microorganism for the synthesis to be successful. The most accessible mutasynthon used for generating structurally diverse AGs has been the aminocyclitol ring, particularly the 2-deoxystreptamine moiety (**69**) (Figure 7).[32]

68 streptamine R = OH
69 2-deoxystreptamine R = H
70 R = F R_1 = H
71 R = H R_1 = F

Figure 7. Streptamine derivatives.

Feeding fluorinated 2-deoxystreptamine analogues **70** or **71** to *Micronospora inyuyensis* strain 1550F afforded sisomicin derivatives **72** and **73** (Figure 8). The structures of the fluorinated sisomicin derivatives were confirmed by semisynthesis. The mutasynthetic approach was abandoned due to poor incorporation of the fluorinated mutasynthons (**70** and **71**) by the idiotroph *M. inyoensis*.[32]

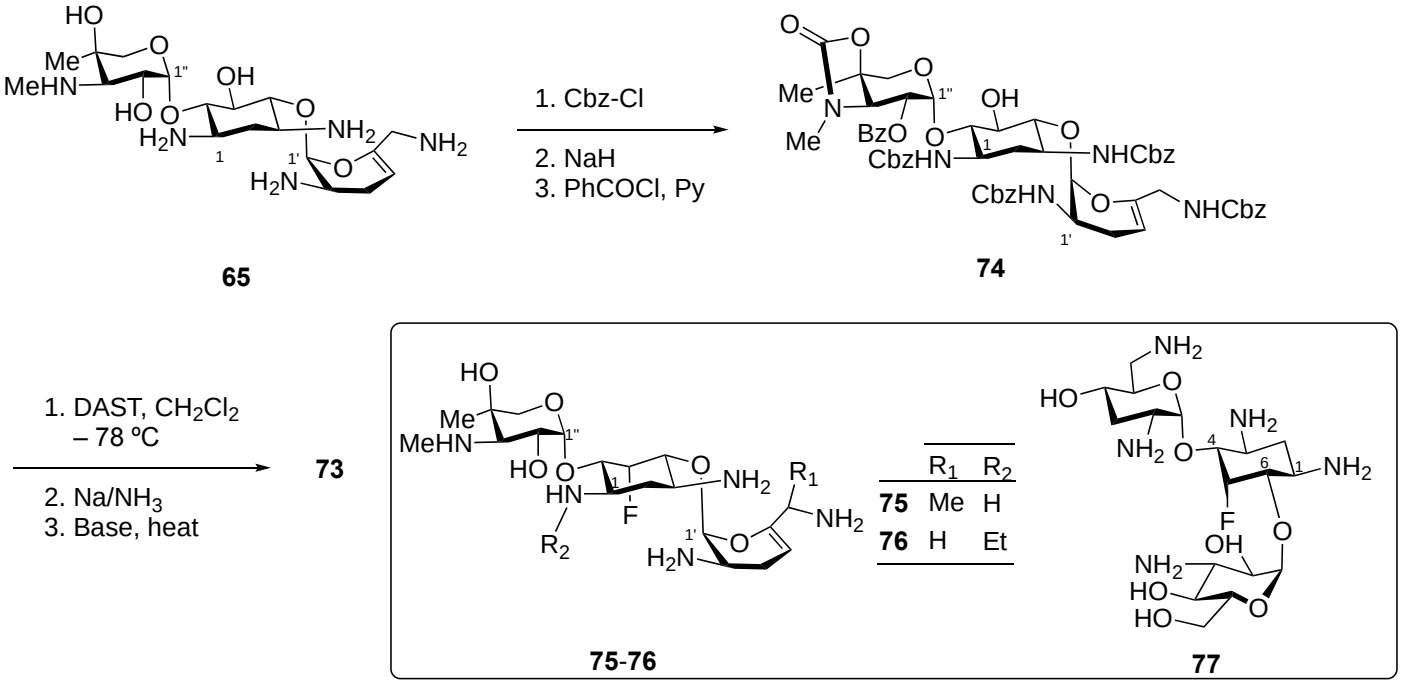


72 R_1 = F, R_2 = H
73 R_1 = H, R_2 = F

Figure 8. 5-deoxy-5-fluorosisomicin (**72**) and 5-deoxy-5-*epi*-5-fluorosisomicin (**73**).

3.1.2. Synthesis of 5-deoxy-5-fluoro and 5-deoxy-5-*epi*-5-fluoro derivatives of sisomicin (65), netilmicin (66) and verdamicin (67).[53] The synthesis of 5-deoxy-5-fluoro-5-*epi* sisomicin (**73**) is shown in Scheme 9. The fully *N*-Cbz-protected AG derivative was obtained by first reacting sisomicin (**65**) with benzyloxycarbonyl chloride (Cbz-Cl), and subsequent simultaneous pentacyclic carbamate formation promoted by sodium hydride and selective esterification with benzoyl chloride afforded intermediate **74**. Treatment of **74** with methanesulfonyl chloride followed by a reaction with a fluoride salt (Li, Na and K) was unsuccessful. However, the reaction of **74** with DAST in dichloromethane at – 78 °C led to complete clean inversion, generating exclusively the 5-deoxy-5-fluoro-5-*epi* derivative. Then, cleavage of the protecting groups afforded 5-deoxy-5-fluoro-5-*epi* sisomicin (**73**). The patent[53] also describes the synthesis of 5-deoxy-5-fluorosisomicin (**72**) (Figure 5), 5-deoxy-

5-fluoro-5-*epi* verdamicin (**75**), 5-deoxy-5-fluoro-5-*epi* netilmicin (**76**) and 5-deoxy-5-fluoro-*epi* tobramycin (**77**) using a similar protocol.



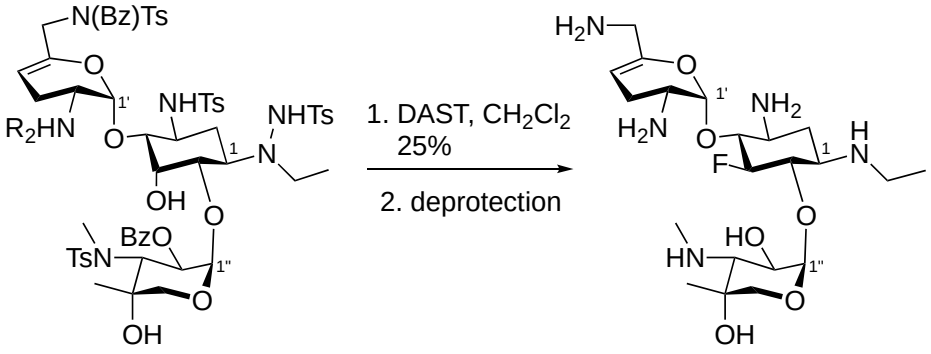
Scheme 9. 5-Deoxy-5-*epi*-5-fluorosisomicin (**73**), 5-deoxy-5-*epi*-5-fluoroverdamicin (**75**), 5-deoxy-5-epi-5-fluoronetilmicin (**76**) and 5-deoxy-5-epi-5-fluorotobramacyn (**77**).

Both isomers (**72** and **73**) exhibited good antibacterial potency against aminoglycoside-susceptible and aminoglycoside-resistant bacteria, with **72** being the more potent of the two. In general, both fluorinated derivatives (**72** and **73**) were at least as potent as sisomicin (**65**) and slightly less potent than 5-*epi*-sisomicin, but they showed broader activities;[53-55] toxicity studies with 5-deoxy-5-*epi*-5-fluorosisomicin (**73**) revealed that this derivative was less toxic toward mice than was sisomicin (**65**) (LD_{50} of 160 mg/kg vs 30 mg/kg via i.v.).[55]

3.1.3. Synthesis of the 5-deoxy-5-fluoro- and 5-deoxy-5,5-difluoro-derivatives of netilmicin (66).[56] Compounds (**79** and **82**) where synthesized in an effort to clarify the relationship between the deoxyfluorination and toxicity of the fluorinated AGs. Treatment of a suitably protected 5-*epi* netilmicin derivative (**78**) with DAST in dichloromethane followed by cleavage of the protecting groups under standard conditions afforded 5-deoxy-5-fluoronetilmicin (**79**) (Scheme 10).

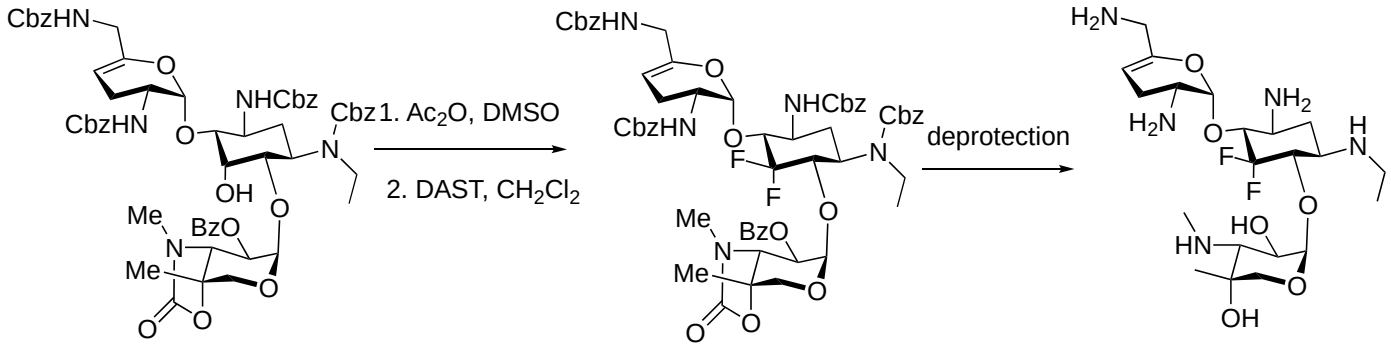
The reaction of **80** with Ac_2O in DMSO followed by treatment with DAST afforded difluorinated analogue **81**. 5-Deoxy-5,5-difluoronetilmicin (**82**) was obtained from **81** after cleavage of the protecting groups under standard conditions (Scheme 11).

Fluorinated netilmicin derivatives (**79** and **82)** have antibacterial activities comparable to the parent compound. The mono fluorinated derivative (**79**) was slightly more potent than 5-deoxy-5,5-difluoronetilmicin (**82**).[56] The effect of 5-deoxy-5-fluorination and 5-deoxy-5,5-difluorination on AGs´ toxicity is discussed in Section 3.3.13.



78 **79**

Scheme 10. Synthesis of 5-deoxy-5-fluoronetilmicin (**79**).



80 **81** **82**

Scheme 11. Synthesis of 5-deoxy-5,5-difluoronetilmicin (**82**).

3.1.4. Synthesis of 6'-deamino-6'-fluorosisomicin (87).[33] The treatment of sisomicin (**65**) with triflyl azide and $CuSO_4$ in methanol followed by the reaction with Fmoc-OSu afforded **83** (Scheme 12). The treatment of **83** under optimized selenium dioxide allylic oxidation conditions using selenium dioxide and dihydropyran (DHP) afforded a 6'-aldehyde intermediate (**84**) that was subsequently reduced to afford 1,3,2´6´-tetradeamino-6'-hydroxy-1,3,2'-triazido-3''-*N*-Fmoc sisomicin (**85**) using sodium borohydride in THF/MeOH as previously reported.[57] Deoxyfluorination of **85** was accomplished with PhenoFluor®, a deoxyfluorinating agent that shows remarkable selectivity for substituting allylic alcohols with fluorine in the presence of unprotected secondary and tertiary alcohols,[58] and the reaction afforded **86** in good yield. Final deprotection under Staudinger conditions afforded **87**.

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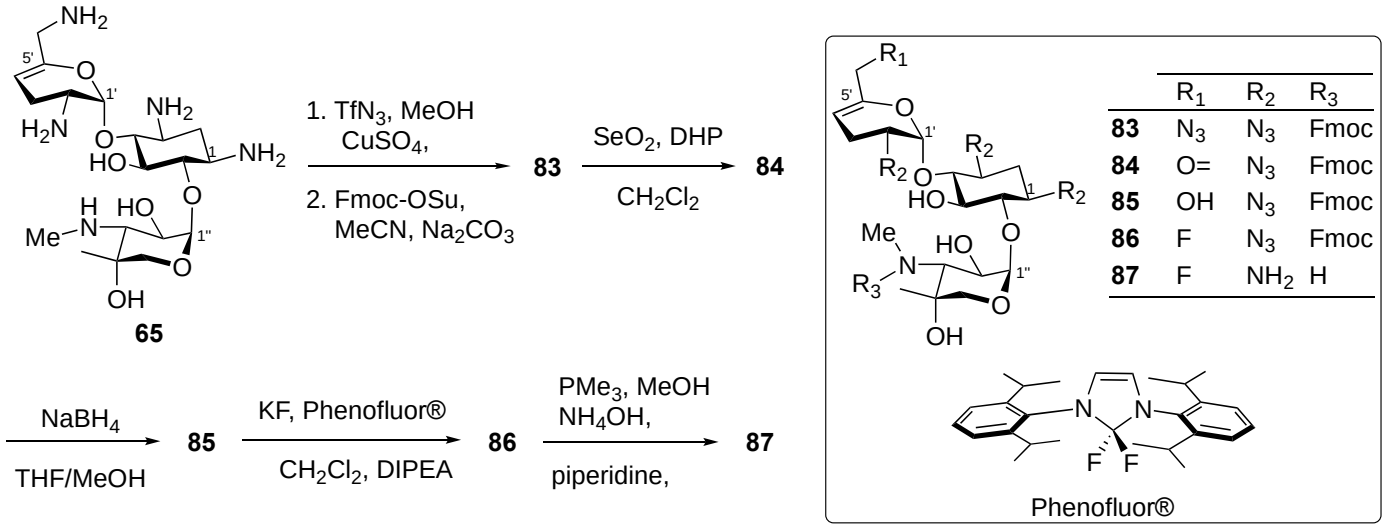
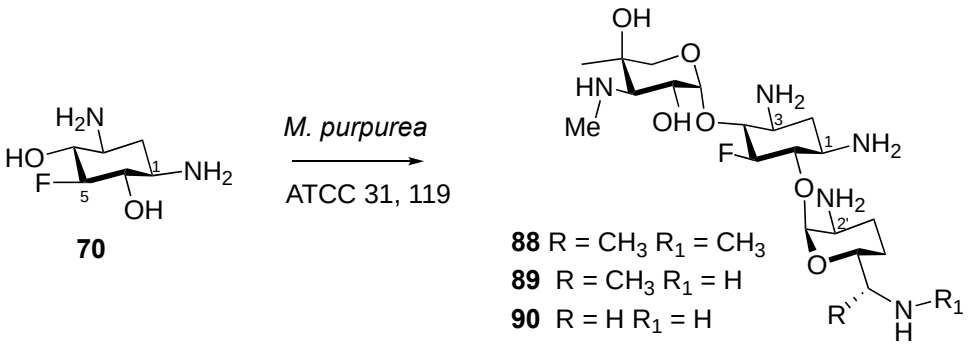


Scheme 12. Synthesis of 6'-deamino-6'-fluorosisomicin (**87**).

6´-Deamino-6´-fluorosisomicin (**87**) shows selective binding to the eukaryotic ribosome over the prokaryotic ribosome, supporting the presumption that substitution with fluoride at the C-6' position is detrimental to the antibacterial activity.[33] Compound (**87**) does not show antibacterial activity against *Escherichia coli* or *Staphylococcus aureus* strains but shows antiparasitic activity against *Tripanosoma brucei brucei*, *T. brucei rhodesiense*, *T. cruzi*, *Leishmania major*, and *Plasmodium falciparum*. Outstandingly, sisomicin (**65**) possesses both antibacterial and antiparasitic activity, being approximately three-fold more potent against parasitic cells than **87**, except for against *P. falciparum*, which showed some susceptibility only to the fluorinated derivative. These differences between the activities of sisomicin (**65**) and 6'-deamino-6'-fluorosisomicin (**87**) are attributed to their interactions within the ribosomal A-site of bacteria and protozoa. A single nucleobase change from an adenine in position 1408 in the bacterial ribosome to guanine in the protozoal ribosome A-sites seems to be responsible for the selectivity shown by **87**. Fluorine can only function as a H-bond acceptor and is unable to form a stable interaction with adenine, while ring I of the AG can interact with guanine through the C-6' fluorine, and the oxygen at C-5' that forms part of the pyranoside ring and the OH at C-1' can form a characteristic stable pseudo base-pair. Additionally, their cytotoxic activities toward HeLa cells were determined, and both sisomicin (**65**) and 6´-Deamino-6´-fluorosisomicin (**87**) are toxic in the hundreds of micromolar range, with **87** being at least 20-fold more toxic *in vitro* toward protozoan cells than toward human cells.[33]

3.2. Fluorination of gentamicin[59]

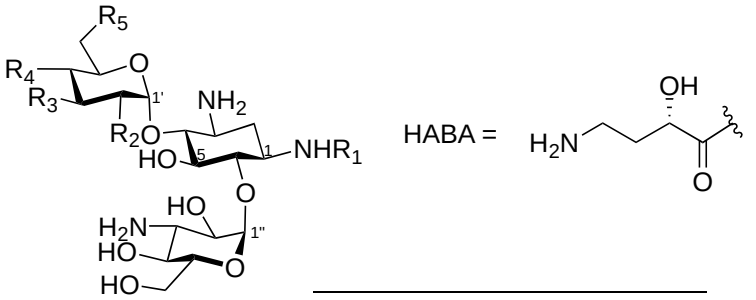
Daum *et al.* claimed[59] that the addition of 2,5-dideoxy-5-fluorostreptamine (**70**) into a fermentation culture of *Micromonospora purpurea* strain ATCC 31,119 yielded 5-deoxy-5-fluorogentamicins C_1 (**88**), C_2 (**89**) and C_{1a} (**90**) according to the reported procedure, and the compounds were most likely obtained as their 5-deoxy-5-fluoro-5-*epi* fluoro derivatives (Scheme 13). The compounds were chemically modified by the introduction of an *N*-(*S)*-4-amino-α-hydroxybutyryl (HABA) moiety in positions 1-N, 3-N and 2´-N; unfortunately, neither the yields nor the characterization data nor the results of antibacterial tests were reported for these compounds.



Scheme 13. Fluorinated derivatives of gentamicin.

3.3. Synthesis of fluorinated derivatives of the kanamycin family

The kanamycin family is an important class of 4,6-disubstituted 2-deoxystreptamine AGs that includes a series of important antibiotics, such as kanamycin A (**1**), kanamycin B (**91**), kanamycin C (**92**), tobramycin (**2**), dibekacin (**3**), amikacin (**4**) and arbekacin (**93**) (Figure 9).



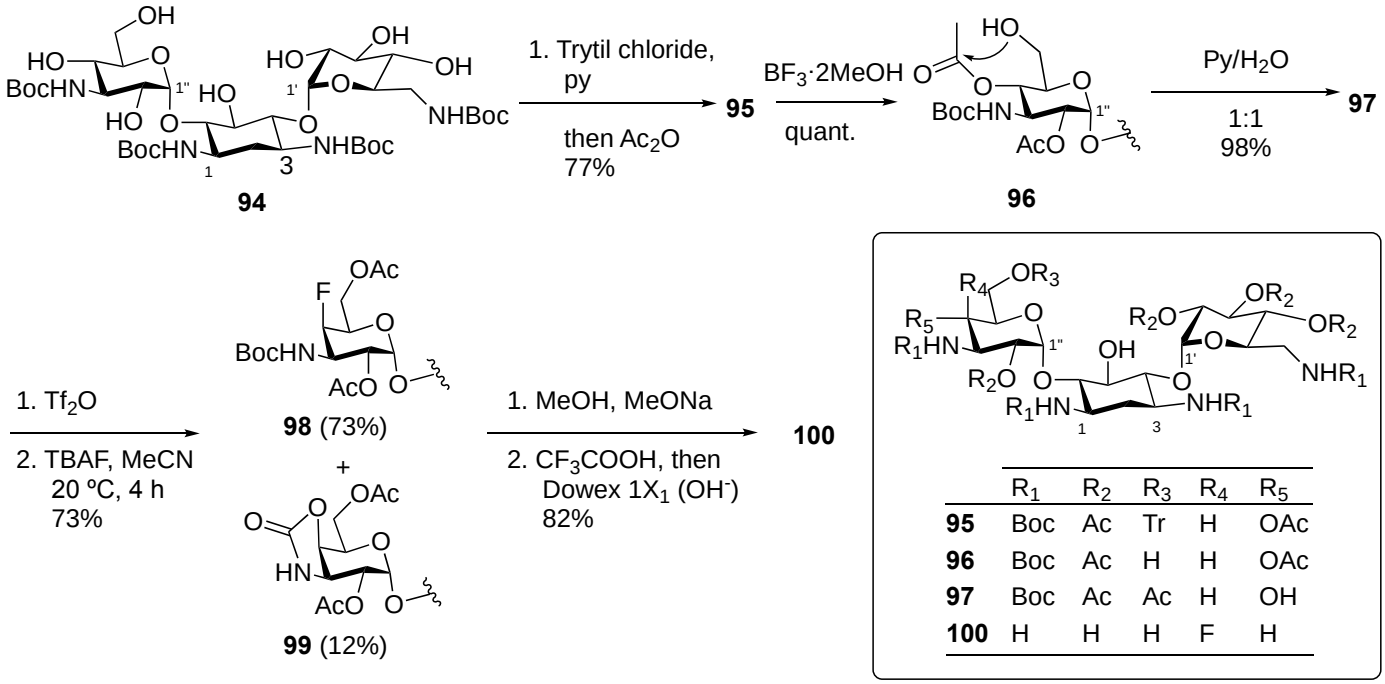
		R_1	R_2	R_3	R_4	R_5
1	kanamycin A	H	OH	OH	OH	NH_2
91	kanamycin B	H	NH_2	OH	OH	NH_2
92	kanamycin C	H	NH_2	OH	OH	OH
2	tobramycin	H	NH_2	H	OH	NH_2
3	dibekacin	H	NH_2	H	H	NH_2
4	amikacin	HABA	OH	OH	OH	NH_2
93	arbekacin	HABA	NH_2	H	H	NH_2

Figure 9. The kanamycin family of AGs.

3.3.1. Synthesis of 4''-deoxy-4''-*epi*-4''-fluorokanamycin A (100).[60] The regioselective protection of the primary OH of **94** as a trityl ether followed by per-*O*-acetylation afforded compound **95** in 77% yield (Scheme 14). Cleavage of the trityl protecting group with BF_3·2MeOH at room temperature afforded compound (**96**) in quantitative yield,[61] and this compound was treated with a 1:1 mixture of pyridine in water to promote the acetyl migration from position 4" to position 6", providing **97**. Alternatively, the slow addition of 0.1 N NaOH in ethanol also promotes the migration of the acetyl group, but in a lower yield. The reaction of **97** with trifluoromethanesulfonic anhydride followed by treatment with tetrabutylammonium fluoride (TBAF) afforded 4''-deoxy-4''-fluoro-4''-*epi* derivative (**98**) in 73% yield. Oxazolidinone (**99**) was formed as a by-product (12% yield) in this reaction. Both reactions proceeded with inversion of the configuration at C-4''. The formation of **99** can be explained by nucleophilic attack of the *N*-Boc carbonyl on the C-4'' triflate promoted by fluoride.[62]

The potential use of the *p*-bromobenzenesulfonyl (brosyl) ester as the leaving group at the C-4'' oxygen of **97** was also examined (not shown). Unfortunately, the reaction of the 4''-brosyl derivative with TBAF resulted in a complex mixture of products that was not analysed. Reactions with other nucleophiles, however, resulted either in S_N2 substitution products or elimination products, generating the C3''-C4''eno derivatives. Final cleavage of the protecting groups afforded 4''-deoxy-4''-*epi*-4''-fluorokanamycin A (**100**).

3.3.2. Synthesis of 5-deoxy-4''-*epi*-5-fluoro- (103), 6''-deoxy-6''-fluoro- (106) and 5,6''-dideoxy-5-*epi*-5,6''-difluorokanamycin A (107).[63] The reaction of **97** with DAST did not afford the expected 5,4''-dideoxy-5,4''-di*epi*-5,4''-difluorokanamycin A derivative but instead afforded 5-deoxy-5,4''-di*epi*-5-fluorokanamycin derivative **101** and the 5-deoxy-5-*epi*-5-fluoro derivative (**102**) (Scheme 15). The reaction intermediate (4''–O–SF_2–NEt_2) undergoes neighbouring group attack from the carbonyl group of the Boc moiety at C-3'' rather than attack by an external fluoride. The **101/102** ratio obtained depended on the reaction time with the formation of **102** being favoured at longer reaction times, which suggested that **101** was formed during work-up. Compounds **101** and **102** were deprotected using standard procedures to afford AGs (**103** and **104**).



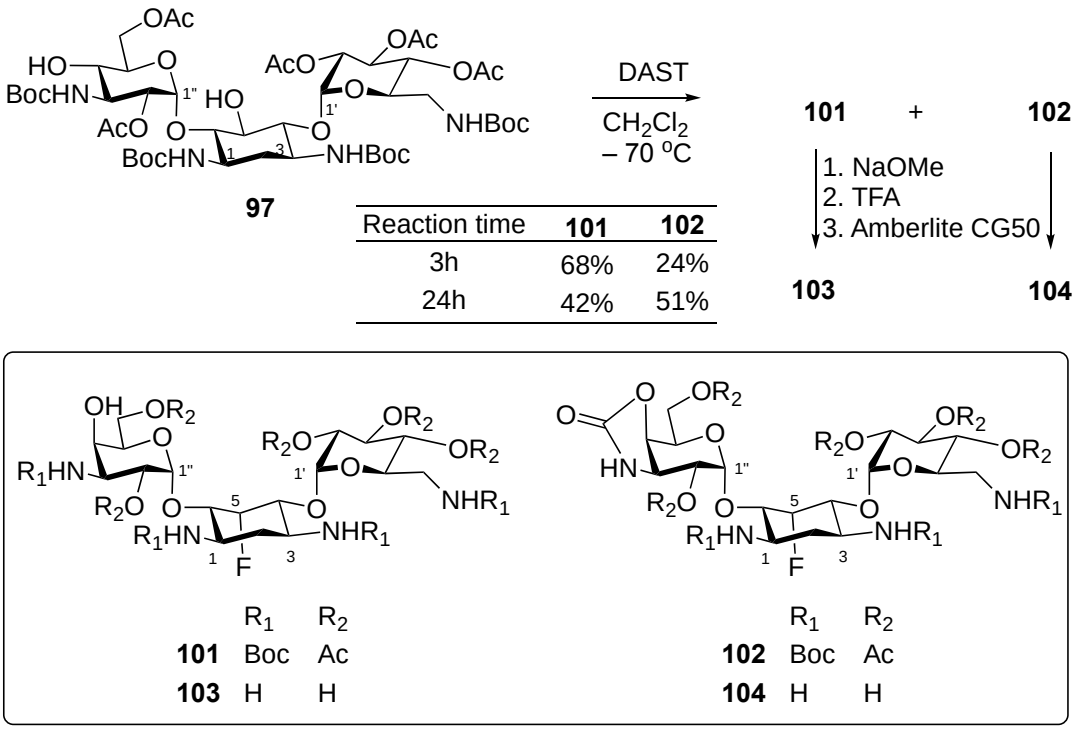
	R_1	R_2	R_3	R_4	R_5
95	Boc	Ac	Tr	H	OAc
96	Boc	Ac	H	H	OAc
97	Boc	Ac	Ac	H	OH
100	H	H	H	F	H

Scheme 14. Synthesis of 4''-deoxy-4''-epi-4''-fluorokanamycin A (**100**).

Two more fluorinated derivatives were obtained from **96** as the starting material (Scheme 16). Triflate formation followed by displacement using TBAF afforded **105** in 76% yield. Zemplén de-*O*-acetylation followed by treatment with trifluoroacetic acid gave 6''-deoxy-6''-fluorokanamycin A (**106**). The reaction of **105** with DAST followed by deprotection afforded 5,6''-dideoxy-5-*epi*-5,6''-difluorokanamycin derivative **107**.

Compounds **100**, **103**, **104**, **106**, and **107** were tested against several bacterial strains, and with the exception of compound **104**, all showed potencies comparable to kanamycin A (**1**). The pK_b values of the 3''-amino groups of **100** and kanamycin A (**1**) were calculated from the pH dependency of the chemical shift of C-2'' in the ^{13}C NMR spectrum. The obtained values were pK_b 7.4 and pK_b 8.3 for **100** and kanamycin A (**1**), respectively. The introduction of a fluorine atom in this series is compatible with their antibacterial activity and has a profound impact on the basicity of the amino groups near the introduced halogen.[63]

An alternative synthesis of **106** by the direct fluorination of tetra *N*-benzyloxycarbonyl kanamycin A and 1-*N* acyl derivatives directly with DAST was reported in the literature, but only some NMR data of this compounds are discussed.[64]

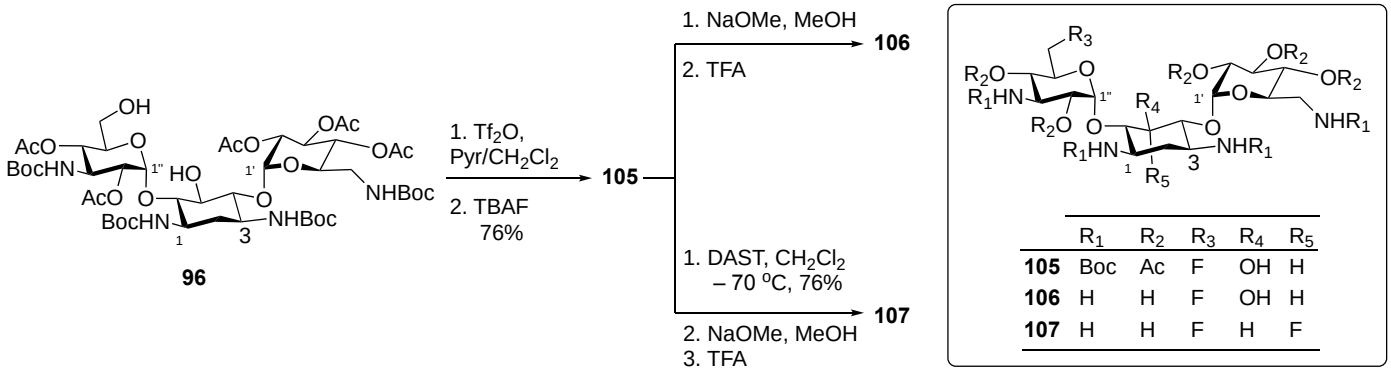


Reaction time	101	102
3h	68%	24%
24h	42%	51%

	R_1	R_2
101	Boc	Ac
103	H	H

	R_1	R_2
102	Boc	Ac
104	H	H

Scheme 15. Synthesis of 5-deoxy-4''-*epi*-5-fluorokanamycin A (**103**).

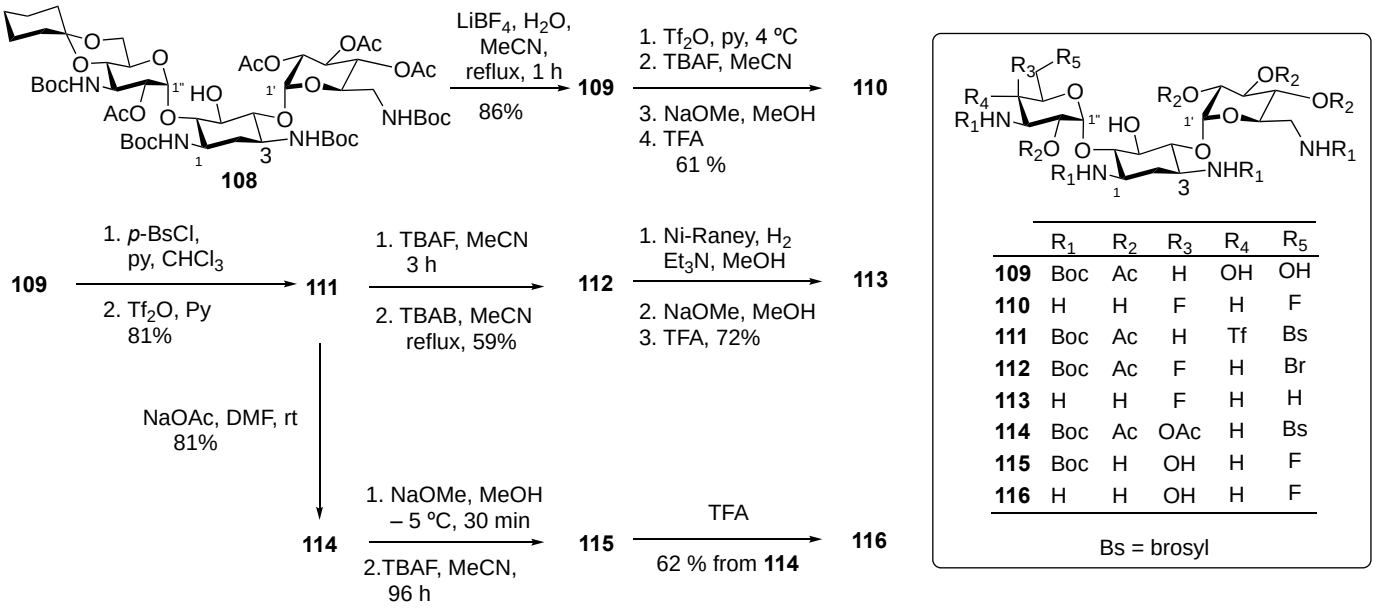


	R_1	R_2	R_3	R_4	R_5
105	Boc	Ac	F	OH	H
106	H	H	F	OH	H
107	H	H	F	H	F

Scheme 16. Synthesis of 6''-deoxy-6''-fluorokanamycin A (**106**) and 5,6''-dideoxy-5-epi-5,6''-difluorokanamycin A (**107**).

3.3.3. Synthesis of 4'',6''-dideoxy-4''-*epi*-4'',6''-difluoro- (110), 4'',6''-dideoxy-4''-*epi*-4''-fluoro- (113) and 6''-deoxy-4''-*epi*-6''-fluorokanamycin A (116)[65] The 4'',6''-*O*-cyclohexylidene moiety of **108** was cleaved with lithium tetrafluoroborate in wet MeCN,[66] affording **109** (Scheme 17). The reaction of **109** with triflic anhydride followed by treatment with TBAF in acetonitrile afforded a 4'',6''-dideoxy-4'',6''-difluoro intermediate that after protecting group cleavage and purification by ion exchange chromatography afforded **110**. Selective *O*-brosylation of **109** at position 6'' followed by *O*-triflate formation afforded **111**, a common intermediate in the

synthesis of compounds **113** and **116**. The reaction of **111** with TBAF in acetonitrile followed by treatment with tetrabutylammonium bromide (TBAB) afforded derivative **112**. The 6''-bromo moiety was reduced with Raney nickel, and the protecting groups were removed under standard conditions, affording **113**. The reaction of **111** with sodium acetate in dimethylformamide proceeded with inversion at C-4'', giving **114**. Treatment of **114** with sodium methoxide to remove the acetates and with TBAF to introduce a fluorine atom at C-6'' gave **115**, which was then deprotected using TFA to afford **116**.



	R_1	R_2	R_3	R_4	R_5
109	Boc	Ac	H	OH	OH
110	H	H	F	H	F
111	Boc	Ac	H	Tf	Bs
112	Boc	Ac	F	H	Br
113	H	H	F	H	H
114	Boc	Ac	OAc	H	Bs
115	Boc	H	OH	H	F
116	H	H	OH	H	F

Bs = brosyl

Scheme 17. Synthesis of 4'',6''-dideoxy-4''-*epi*-4'',6''-difluorokanamycin A (**110**), 4'',6''-dideoxy-4''-*epi*-4''-fluorokanamycin A (**113**) and 6''-deoxy-4''-*epi*-6''-fluorokanamycin A (**116**).

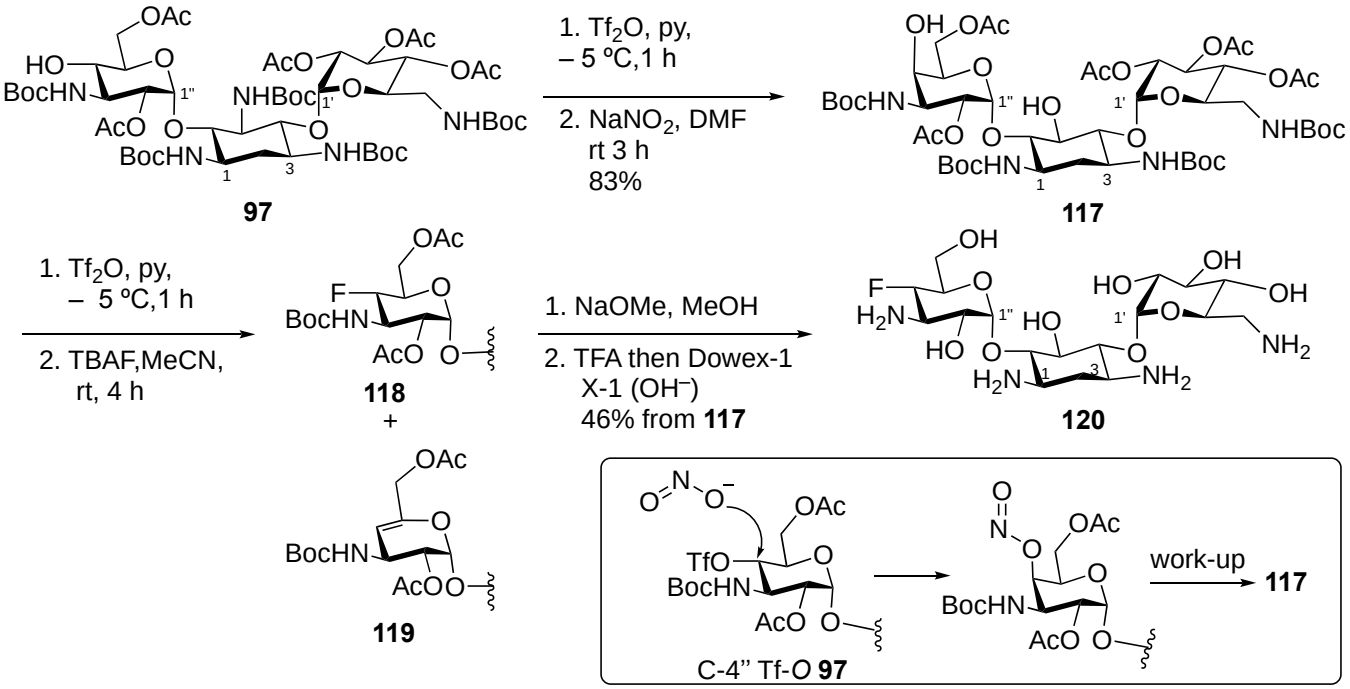
Compounds **100** (Scheme 14), **103** (Scheme 15), **110**, **113**, and **116** were tested against *S. aureus*, *Streptococcus faecalis*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis,* and in general, their potencies were higher or similar to that of kanamycin A (**1**). It is known that kanamycin A (**1**) does not possess antibacterial activity against *P. aeruginosa*, but compounds with a fluorine atom at position C-6'' presented a slight potency against this bacterial strain.[65]

3.3.4. Synthesis of 4''-deoxy-4''-fluorokanamycin A (120).[67] The reaction of **97** with triflic anhydride in pyridine followed by treatment with $NaNO_2$ in DMF[68-69] afforded the 4''-*epi* derivative (**117**) in good yield (Scheme 18). The attack of the ambident nitrite nucleophile produces the nitrite ester, which is then hydrolysed during routine work-up. The reaction of **117** with triflic anhydride followed by treatment with TBAF gave the desired compound (**118**) and derivative (**119**) as a by-product. The inseparable mixture was deprotected using standard conditions and afforded **120** after purification.

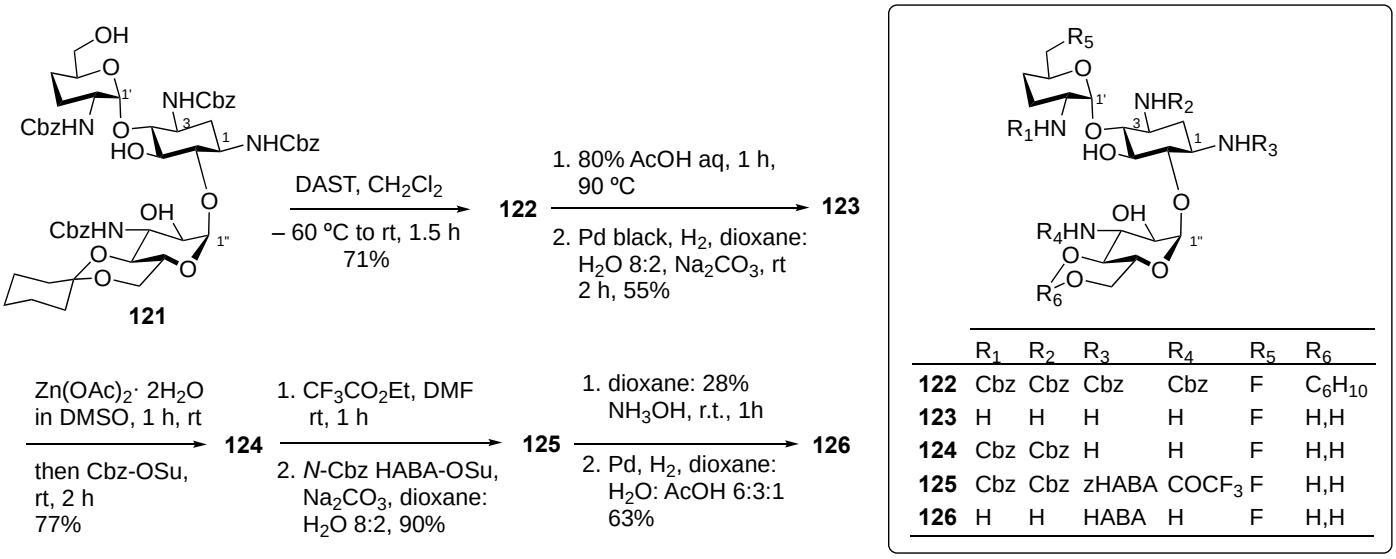
Fluorinated derivative (**120**) was less potent than its 4''-deoxy-4''-fluoro-4''-*epi* analogue (**100**) against different bacterial strains.[67]

3.3.5. Synthesis of 3',4',6'-trideoxy-6'-fluoro- and 1-*N*-HABA-3',4',6'-trideoxy-6'-fluorokanamycin C.[70] The reaction of unprotected triol **121** with DAST was fairly selective, giving the 6''-deoxyfluorinated analogue (**122**) in 71% yield (Scheme 19). Compound **122** was then deprotected under standard conditions affording **123**. Treatment of **123** with acetic acid-free zinc acetate in DMSO followed by Cbz-OSu gave **124.**[71] Compound **124** was reacted with ethyl trifluoroacetate in DMF, resulting in the selective introduction of a trifluoroacetamide moiety at 3''-N. First, a weak trifluoroacetyl ester is formed at O-2'', and subsequent migration of the

trifluoroacetyl group to the neighbouring 3''-N resulted in the more stable trifluoroacetamide. The HABA side chain was then installed using Cbz-protected HABA-OSu in *p*-dioxane, giving **125**, which afforded the 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] (HABA) derivative (**126**) after a standard deprotection.



Scheme 18. Synthesis of 4''-deoxy-4''-fluorokanamycin A (**120**).



	R_1	R_2	R_3	R_4	R_5	R_6
122	Cbz	Cbz	Cbz	Cbz	F	C_6H_{10}
123	H	H	H	H	F	H,H
124	Cbz	Cbz	H	H	F	H,H
125	Cbz	Cbz	zHABA	$COCF_3$	F	H,H
126	H	H	HABA	H	F	H,H

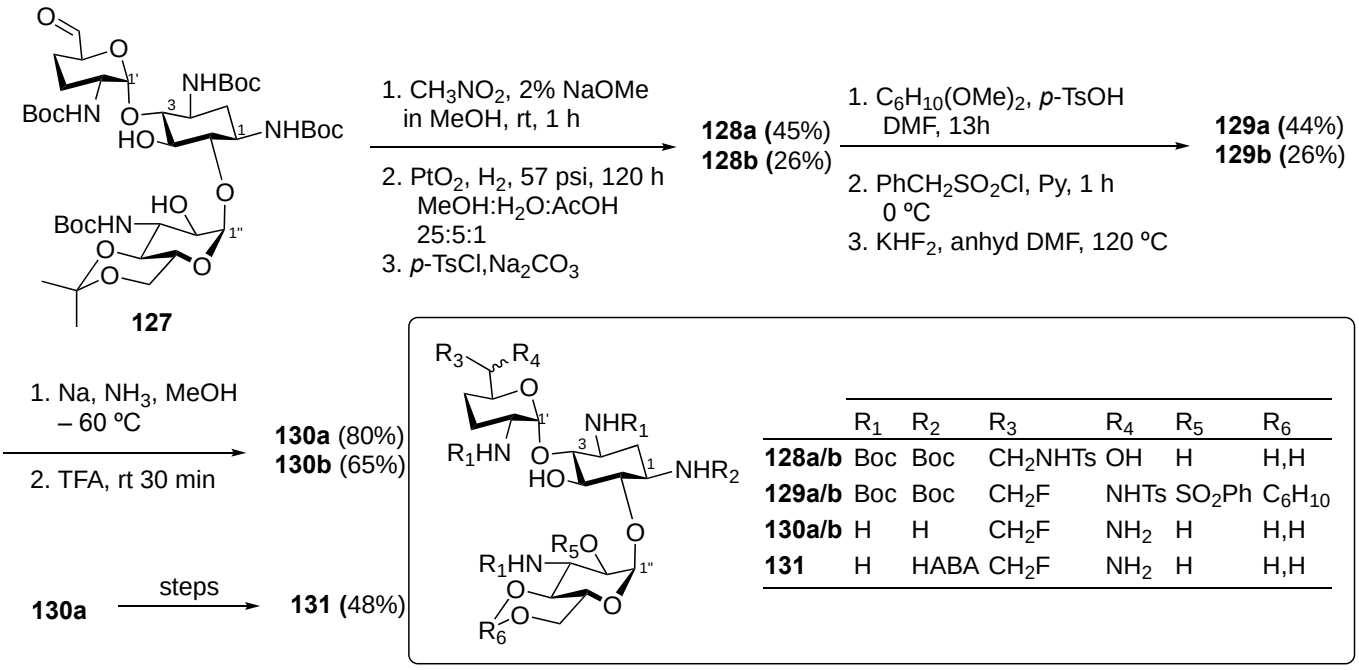
Scheme 19. Synthesis of 3',4',6'-trideoxy-6'-fluorokanamycin C (**123**) and 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-3',4',6'-trideoxy-6'-fluorokanamycin C (**126**).

Both compounds (**123** and **126**) possess very weak antibacterial potency.[61]

3.3.6. Synthesis of 6'-*C*-(fluoromethyl)kanamycin C (130 a/b) and 6'-*C*-(fluoromethyl)arbekacin (131).[70] The 6'-oxo kanamycin C derivative (**127**) was first alkylated with nitromethane and the nitro moiety was reduced

with Adam's catalyst in a 25:5:1 mixture of MeOH/water/acetic acid. Final *N*-tosylation afforded a separable diastereomeric mixture of **128a** and **128b** (Scheme 20). The isomers were separated and characterized, but the absolute stereochemistry at C-6' was not determined, and their syntheses were carried out using two parallel pathways. The reaction with 1,1-dimethoxycyclohexane and *p*-TsOH followed by phenylmethanesulfonyl chloride in pyridine and treatment with KHF_2 in DMF gave **129a** and **129b**. Final cleavage of the protecting groups of **129a** and **129b** afforded epimeric 6'-*C*-(fluoromethyl)kanamycin C **130a** and **130b**. The more active of the two isomers (**130a**) was 1-*N* acylated with a HABA under standard conditions, affording 6'-*C*-(fluoromethyl) arbekacin (**131**).

The antibacterial potencies of compounds **130a**, **130b** and **131** were generally lower than those of dibekacin (**3**) and arbekacin (**93**). Compound **131** was at least two-fold more active than **130a** and **130b**. It is theorized that the lack of activity of **123** and **126** is due to the absence of a hydrogen donor at C-6'; such a group is present in kanamycin C (**92**) and is vital for its interaction with the AG receptor. This explanation also satisfies the results obtained with compounds **130a**, **130b** and **131**, which are active but to a lesser extent than their C-6' hydroxylated counterparts. Other AGs, such as gentamicin C_1 and C_2 (**5** and **6**, Figure 1) that possess good antibacterial potency also have a methyl group at the C-6' position, indicating the fluoromethyl group would not have an adverse steric effect. Instead, the electron-withdrawing character of the fluorine atom lowers the basicity of the C-6'' amine group, decreasing its ability to form hydrogen bonds, and limiting the capacity of the molecule to bind its receptor.[70]



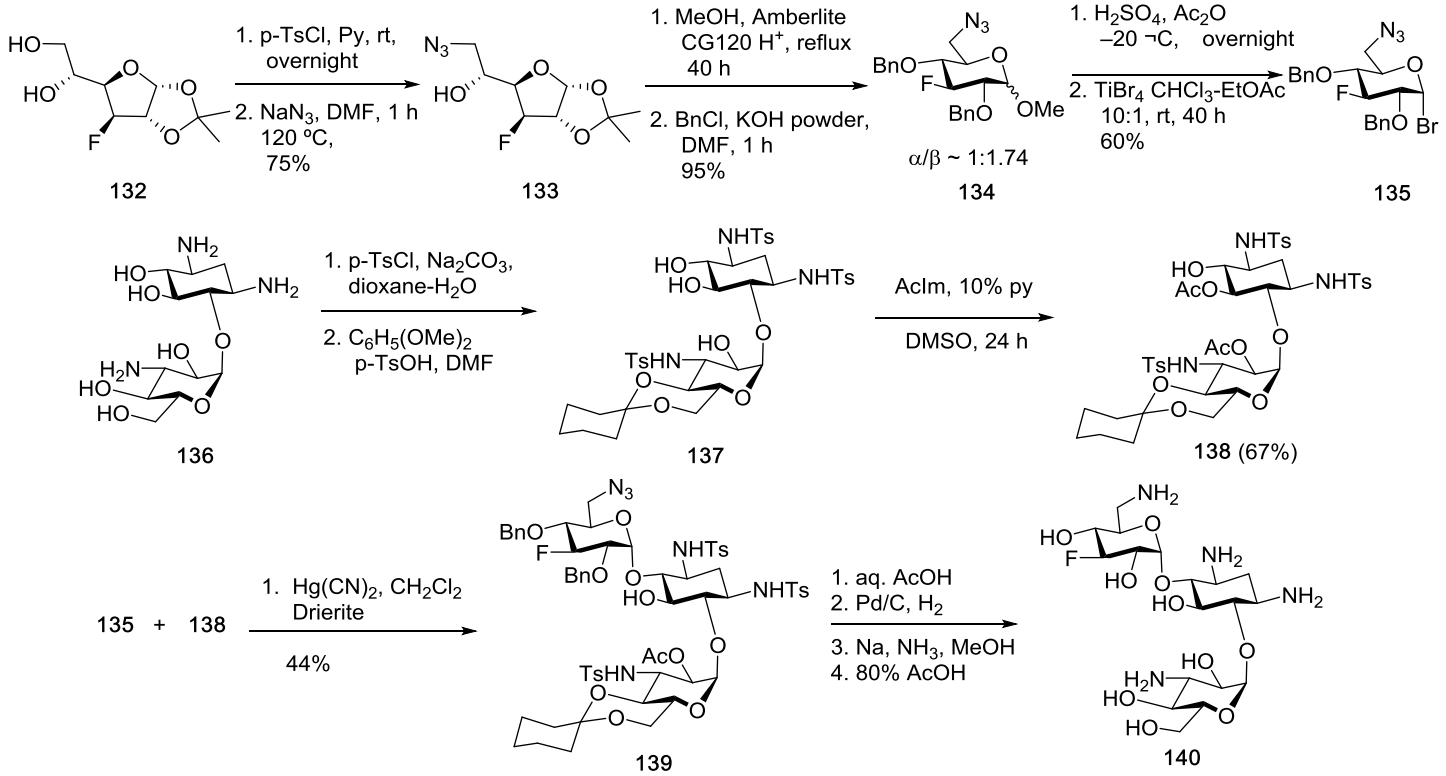
	R_1	R_2	R_3	R_4	R_5	R_6
128a/b	Boc	Boc	CH_2NHTs	OH	H	H,H
129a/b	Boc	Boc	CH_2F	NHTs	SO_2Ph	C_6H_{10}
130a/b	H	H	CH_2F	NH_2	H	H,H
131	H	HABA	CH_2F	NH_2	H	H,H

Scheme 20. Synthesis of 6'-*C*-(fluoromethyl)kanamycin C (**130a/b**) and 6'-*C*-(fluoromethyl)arbekacin (**131**).

3.3.7. Synthesis of 3'-deoxy-3'-fluorokanamycin A (140)[14, 24] and 3',4'-dideoxy-3'-fluorokanamycin A (144).[14]

3'-Deoxy-3'-fluorokanamycin A derivative (**140**) was synthesized by the condensation of 6-azido-2,4-di-*O*-benzyl-3,6-dideoxy-3-fluoro-α-D-glucopyranosyl bromide (**135**) and a protected derivative (**138**) of 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (**136**), in this approach the fluorine atom was introduced in an early stage of the synthesis (Scheme 21). The glycosyl donor (**135**) was synthesized from 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-α-D-glucofuranose (**132**). Selective tosylation followed by S_N2

displacement with sodium azide afforded **133**. Methanolysis of the 1,2-*O*-acetonide followed by treatment with benzyl bromide in anhydrous DMF yielded **134** in excellent yield as a mixture of α and β anomers. Treatment with acetic anhydride and catalytic sulfuric acid followed by exposure to $TiBr_4$ afforded bromide **135**. Glycosyl acceptor **138** was obtained from 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (**136**). Sequential treatment with *p*-TsCl and sodium carbonate followed by 1,1-dimethylcyclohexane ($C_6H_{10}(OCH_3)_2$) and *p*-TsOH acid gave **137**. The regioselective acetylation with 1-acetylimidazole (AcIm) in DMSO afforded **138** in 67% yield. The condensation of **135** and **138** in dichloromethane using mercury (II) cyanide as a promotor gave **139** in a 44% yield. The α anomer was the major product, and only traces of the β anomer were observed. Compound (**140**) was obtained after deprotection and purification.[14, 24]



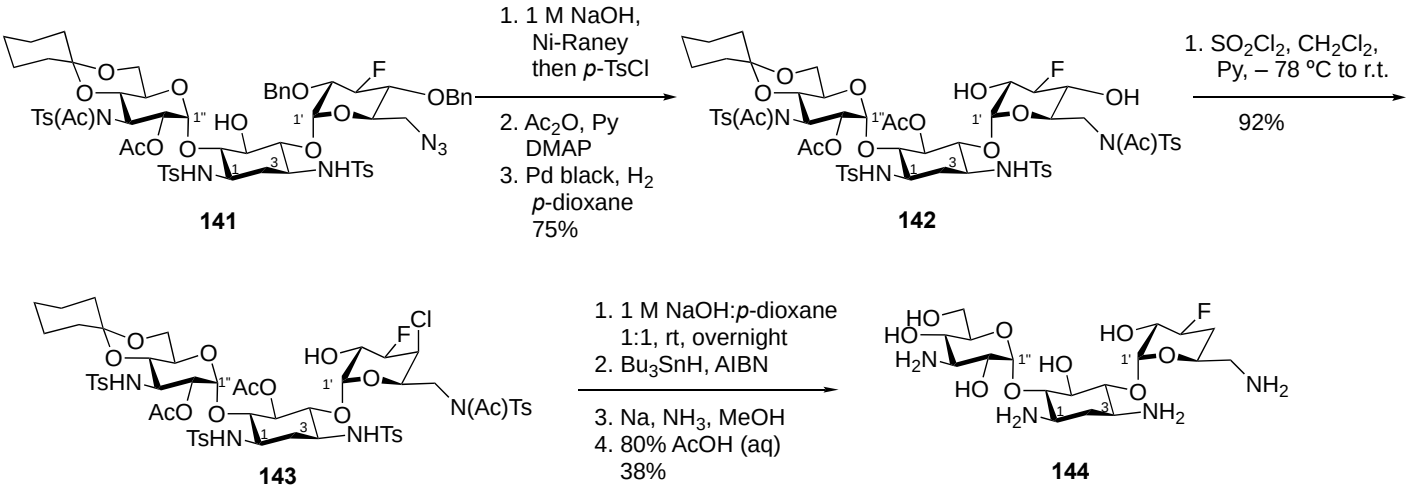
Scheme 21. Synthesis of 3'-deoxy-3'-fluorokanamycin A (**140**).

Treatment of **141** with Raney nickel reduced the 6'-azide, which was then protected as a tosylate. Peracetylation followed hydrogenolysis of the benzyl groups afforded **142** (Scheme 22). The treatment of **142** with sulfuryl chloride chemoselectively halogenation C-4' and afforded **143** in 92% yield. The reduction of **143** under Birch conditions resulted in loss of both the chlorine and fluorine atoms and the formation of a 3',4´-eno derivative (not shown). Tosyl deprotection was achieved by first reducing the 4'-Cl moiety with tributyltin hydride and AIBN followed by reaction under Birch conditions to remove the tosyl groups. Compound **144** was obtained after cleavage of the remaining protecting groups under standard reaction conditions.[14]

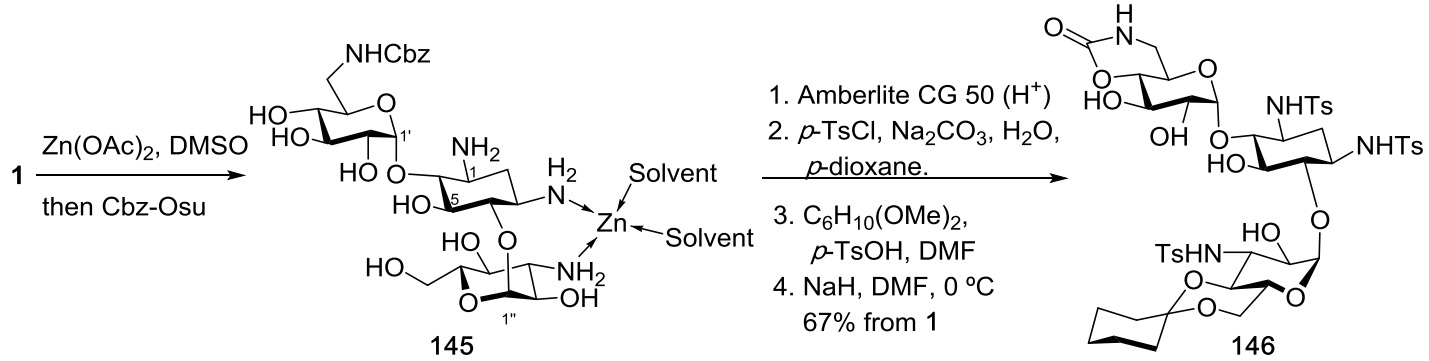
As expected, compound (**144**) was active against resistant bacteria producing both APH(3') and ANT(4'') AMEs.[14]

3.3.8. Synthesis of 3'-deoxy-3'-fluorokanamycin A (151) via a nucleophilic oxirane opening.[72] The selectivity of the *N*-acylation of AGs can be controlled by temporarily protecting vicinal amino groups as transition metal chelates and subsequently acylating of the unprotected groups.[73-76] Using this approach, the treatment of

kanamycin A (**1**) with zinc acetate in DMSO followed by the addition of Cbz-OSu allowed the selective introduction of a Cbz protecting group at 6'-N to afford **145** (Scheme 23). Subsequent treatment with Amberlite CG-50(H^+) ion exchange resin to remove the chelate, tosylation of the remaining amino moieties, 4'',6''-cyclohexylidene formation, followed by treatment with NaH in DMF to protect 4'-O as a cyclic carbamate afforded **146** in 67% overall yield.[77]



Scheme 22. 3',4'-Dideoxy-3'-fluorokanamycin A (**144**).

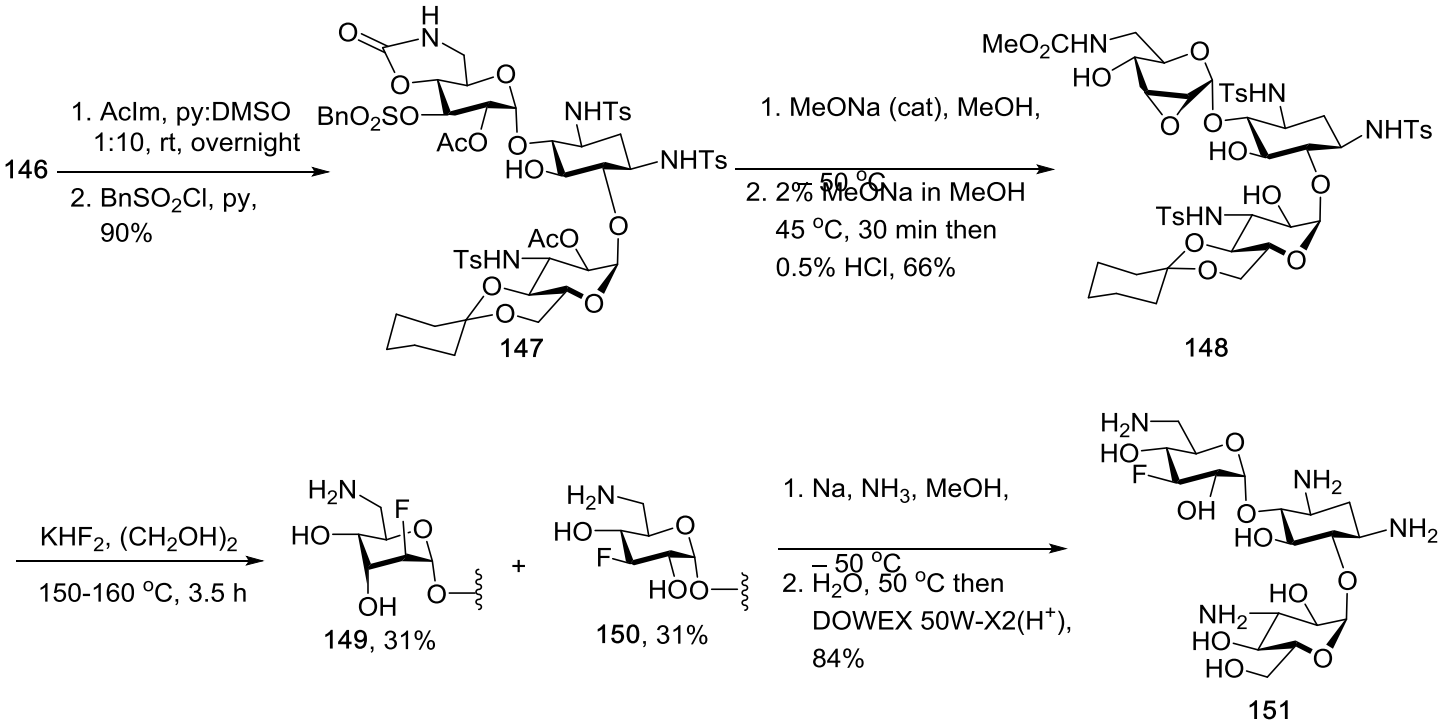


Scheme 23. Regiocontrol via chelation.

The selective *O*-acetylation of **146** with 1-acetylimidazole (AcIm) in a 1:10 mixture of pyridine-DMSO followed by treatment with benzylsulfonyl chloride in pyridine gave compound **147** (Scheme 24). The acetates were cleaved with a catalytic amount of sodium methoxide in methanol, and the 2',3'-oxirane was formed upon treatment of the deacetylated product with a hot solution of 2% sodium methoxide in methanol, affording **148**. Model experiments and molecular mechanics calculations were necessary to establish suitable conditions for maximizing the formation of the 3'-deoxy-3'fluoroglucose derivative (**150**) over the 2-deoxy-2-fluoroaltrose derivative (**149**). Based on these optimization studies, a reaction with potassium hydrogen difluoride in ethylene glycol at 150 °C for 3.5 h was used to prepare **150**. Global deprotection afforded **151**.[72, 78]

3'-Deoxy-3'-fluorokanamycin A (**151**) was tested against bacteria of the genera *Staphylococcus*, *Micrococcus*, *Bacillus*, *Escherichia*, *Mycobacterium*, *Klebsiella*, *Shigella*, *Proteus*, *Serratia*, *Providencia* and

Pseudomonas. In general, the fluorinated derivatives exhibit greater antibacterial potency than tobramycin (**2**) against sensitive and resistant bacteria containing AMEs.[24]



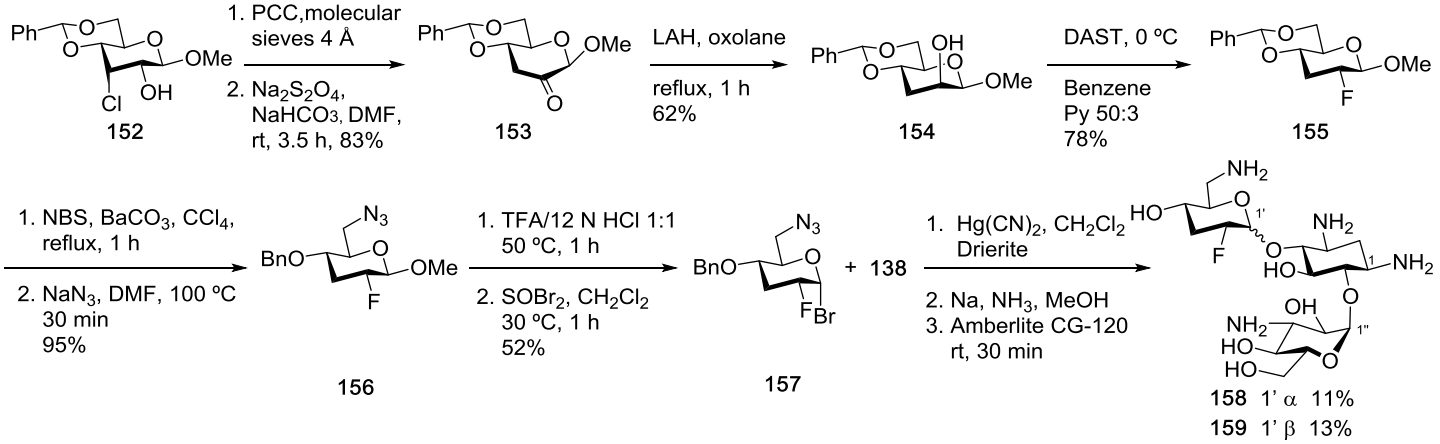
Scheme 24. Synthesis of 3'-deoxy-3'-fluorokanamycin A (**151**).

3.3.9. Synthesis of 2',3'-dideoxy-2'-fluorokanamycin A (158).[79-80] The glycosyl donor (**157**) was synthesized from methyl 4,6-*O*-benzilidene-3-chloro-3-deoxy-α-D-allopyranoside (**152**) (Scheme 25).[81] Compound **152** was treated with PCC and Drierite in dichloromethane, affording the corresponding α-chloroketo derivative, which was then reduced with sodium dithionite to obtain the 2-oxo-*ribo*-hexapiranoside derivative (**153**). The reduction of **153** with lithium aluminium hydride afforded a 2:1 ratio of C-2 epimers. The desired compound (**154**) was obtained in 62% yield. The use of other reductants gave equimolar mixtures of axial and equatorial C-2 alcohols. The DAST-mediated deoxyfluorination proceeded with inversion of the configuration at C-2, affording **155**. The 4,6-*O*-benzylidene ring of **155** was regioselectively opened[82-83] using *N*-bromosuccinimide (NBS), affording the 6-bromo 4-*O*-benzyl derivative, which was treated with sodium azide in DMF to generate the 6-azide derivative (**156**). Hydrolysis of the methyl glycoside moiety and bromination using thionyl bromide afforded glycosyl donor **157**. The condensation of **157** with **138** using mercury (II) cyanide afforded a mixture of anomers, the α-glycoside in 41% yield and the β-glycoside in 34% yield. The deprotection and reduction of the 6'-azido group afforded **158** as its C-1' β epimer **159**.

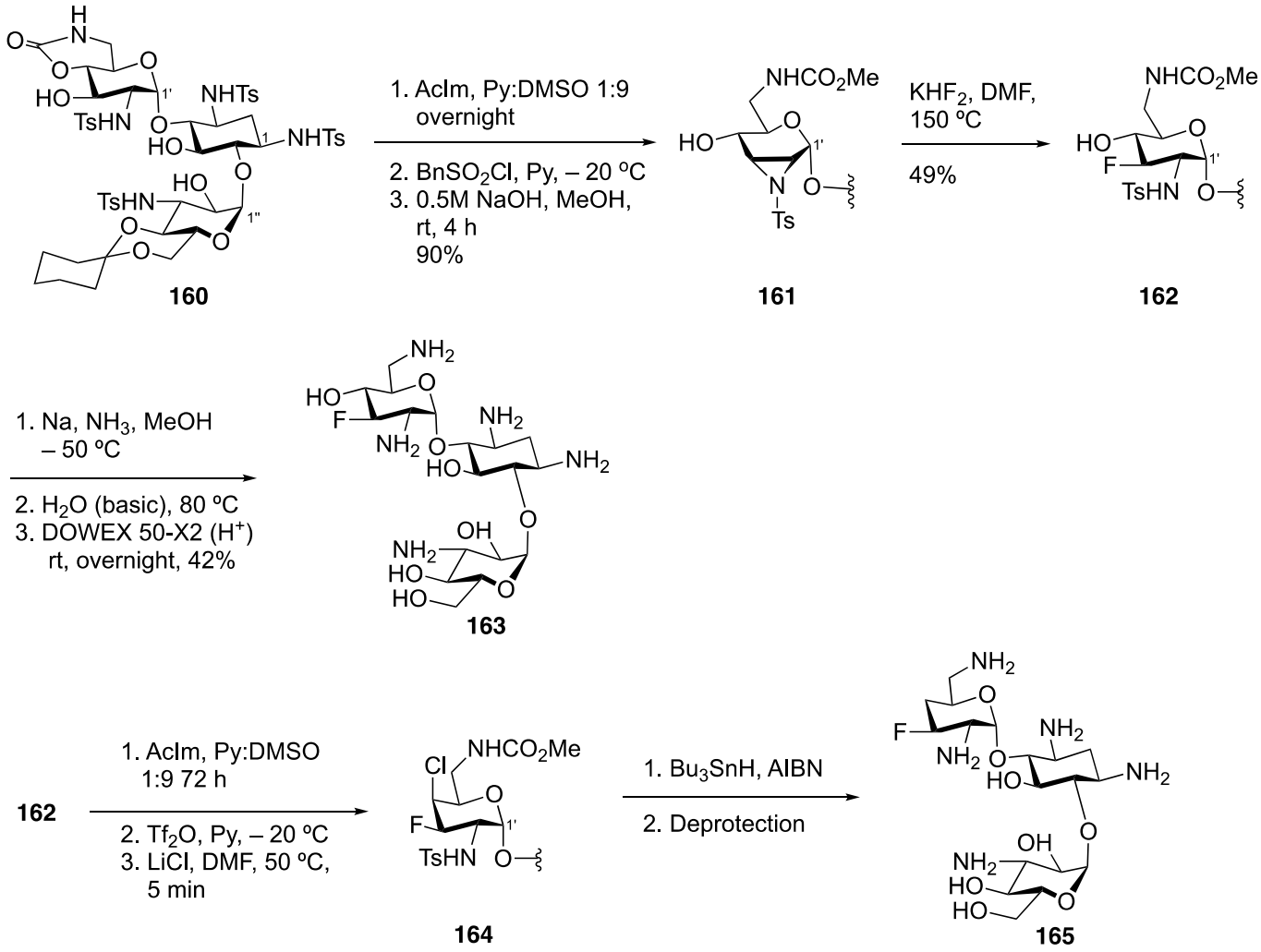
Both epimers of 2',3'-Dideoxy-2'-fluorokanamycin A (**158** and **159**) were tested for their antibacterial activities; **159** was practically inactive, and **158** was only slightly less potent than tobramycin (**2**).[79-80]

3.3.10. Synthesis of 3'-deoxy-3´-fluoro (163) and 3',4'-dideoxy-3'-fluoro (165) analogues of kanamycin B (91).[24, 84-85] Treatment of **160** with 1-acetylimidazole (AcIm) in a 1:10 mixture of pyridine-DMSO gave selective 2''-*O*-acetylation (Scheme 26). Treatment with benzenesulfonyl chloride allowed the introduction of a 3'-*O*-benzenesulfonyl moiety, which was then reacted with 0.5 M aqueous NaOH to give the 2',3'-*N*-tosylepimino derivative (**161**). The reaction of **161** with KHF_2 in DMF at 150 °C for 2 h afforded the required common intermediate (**162**). The free C-4' OH of **161** seemed to stabilize the ^5H_O conformation (half-chair with C-5 up and the oxygen down) through hydrogen bonding, favouring fluoride attack at C-3' (Figure 10, **166**). The final

product (**163**) was obtained after deprotection under standard conditions. The selective 2''-*O*-acetylation of **162** using AcIm in a 1:10 mixture of pyridine-DMSO, followed by the introduction of a 4'-triflate moiety with triflic anhydride in pyridine and subsequent treatment with lithium chloride gave **164**. The reduction of 4'-Cl with tributyltin hydride and AIBN followed by deprotection under standard conditions afforded **165**.



Scheme 25. Synthesis of 2',3'-dideoxy-2'-fluorokanamycin A (**158**).



Scheme 26. Synthesis of 3'-deoxy-3'-fluorokanamycin B (**163**) and 3',4'-dideoxy-3'-fluorokanamycin B (**165**).

Both compounds (**163** and **165**) showed antibacterial activity, and the potencies of **163** were similar to those of tobramycin (**2**),[24] while **165** was active against bacteria producing 3' and 4' AMEs.[84]

The stereochemical outcome of the attack of 2,3-(*N*-tosylepimino)-α-D-allopyranosides (**166**) by a fluoride nucleophile depends on the initial conformation (Figure 10). The attack can occur at position C-2, affording an altroside derivative (**167**), or at position C-3, affording a glucoside derivative (**168**). Model studies[84] with 2,3-(*N*-tosylepimino)-α-D-allopyranosides (**166**) revealed that while the attack to form **168** is irreversible, the altroside (**167**) is in equilibrium with the 2,3-(*N*-tosylepimino)-α-D-allopyranoside (**166**) despite the high C-F bond energy. When the derivative is rigid, for example, when an acetal is formed at C-4 and C-6 OH, compounds **167** and **168** can be isolated, but at longer reaction times, the only fluorinated product detected is **168**. When derivative (**166**) is flexible, only **168** is isolated regardless of the reaction times. It is postulated that the attack at position C-3 occurs when **166** adopts a $^{5}H_{O}$ conformation, which is stabilized by the acidic KHF_2 and the solvent, instead of the $^{O}H_{5}$ (half-chair with the oxygen up and C-5 down) conformation that favours the attack at C-2 following the Fürst-Plattner rule; this creates a torsionally stable transition state despite the stereoelectronic effects of the lone pair on the pyranoside oxygen.[86] If a nucleophile is present at C-6 (*e.g.*, OH), it could open the *N*-tosylepimino ring at C-3 to form the 3,6-anhydroglucose derivative (**169**).[84]

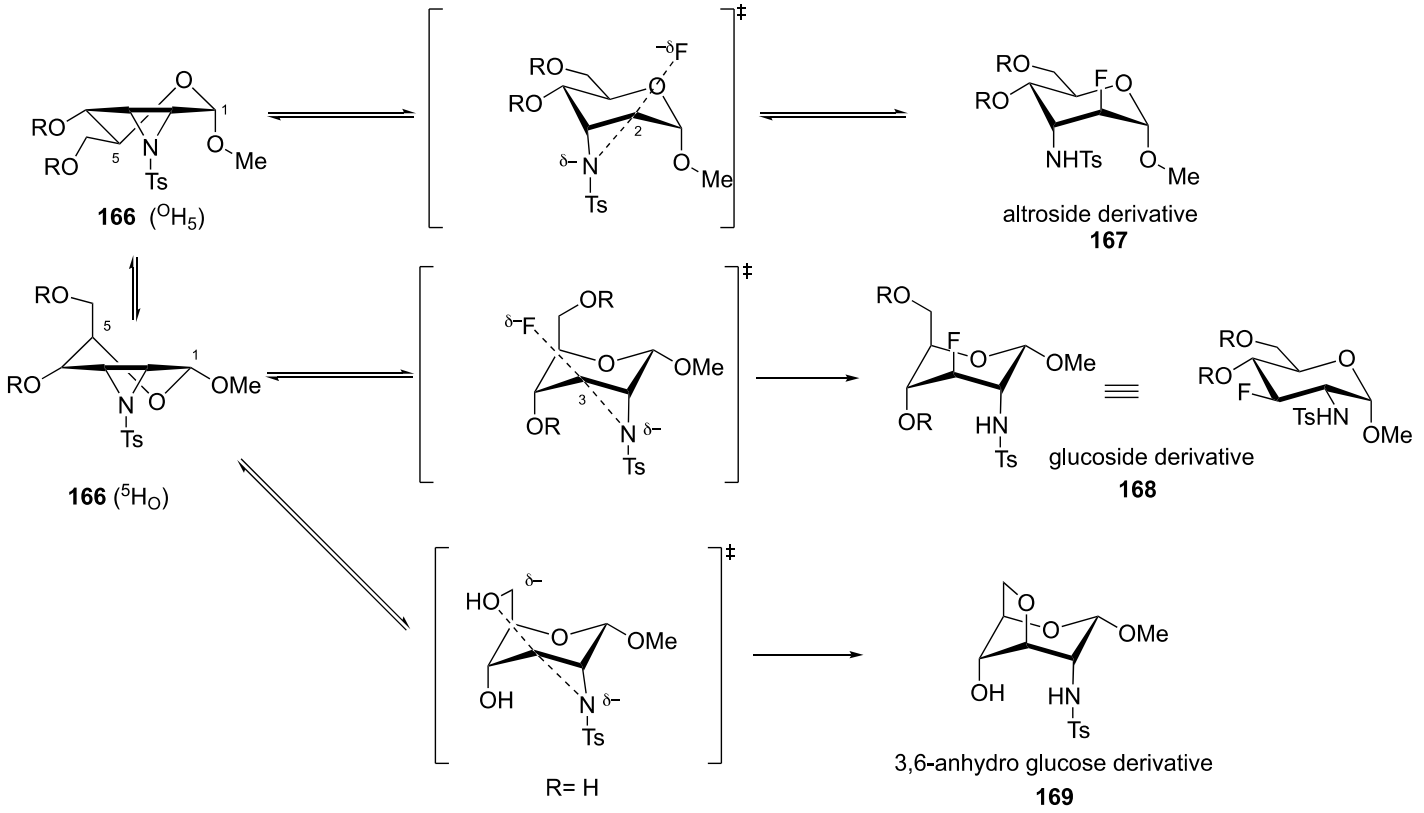
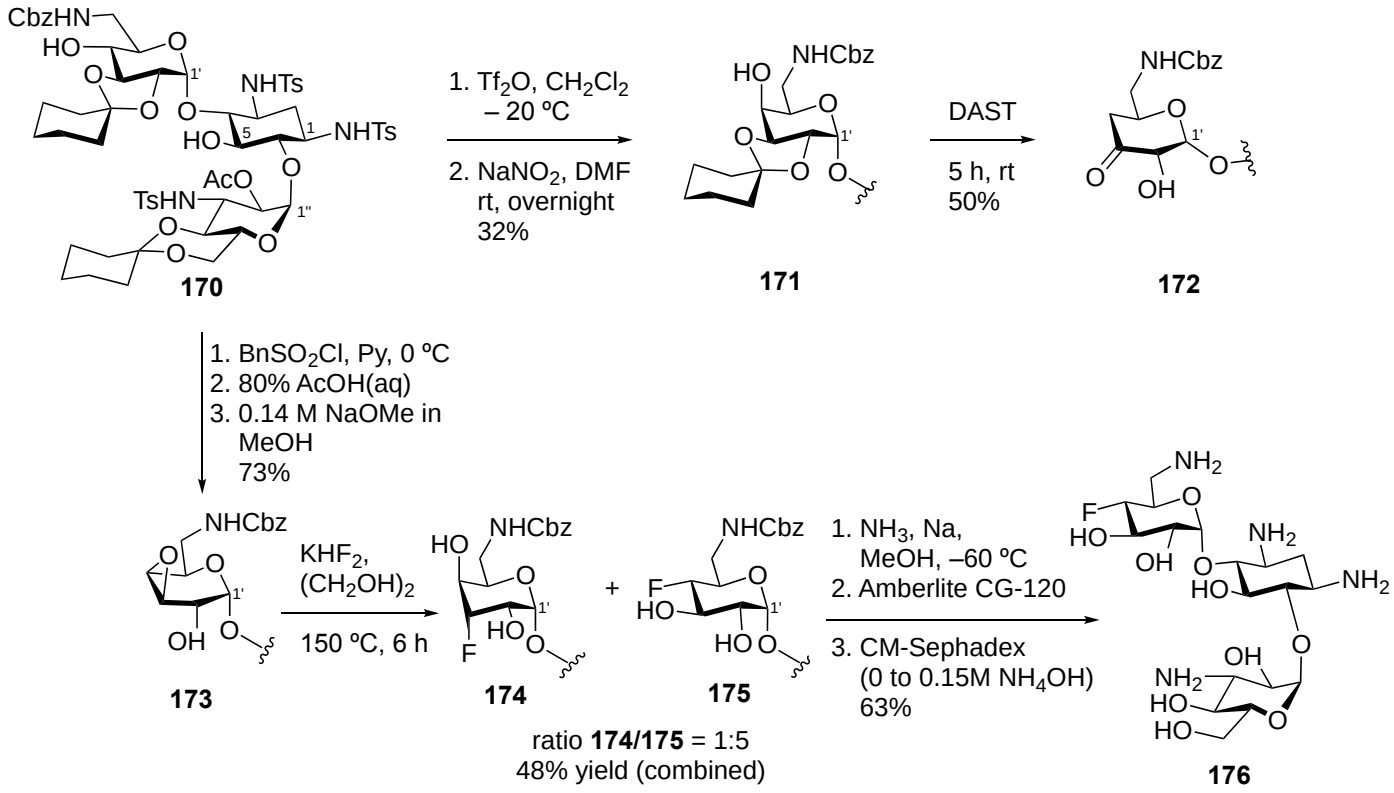


Figure 10. Stereochemical outcome of the attack of 2,3-(*N*-tosylepimino)-α-D-allopyranosides by a fluoride nucleophile.

3.3.11. Synthesis of 4'-deoxy-4'-fluorokanamycin A (176).[87] The C-5 configuration of **170**[88] was inverted at C-4' by the S_N2 displacement of the previously formed triflate with sodium nitrite in DMF, affording **171** in a low yield (Scheme 27). The treatment of **171** with DAST resulted in the formation of an unexpected product (**172**). The formation of this compound was postulated to occur via a 1,2-hydride shift in which the hydrogen at C-3' displaced the *anti* $-O-SF_2-NEt_2$ leaving group at C-4'.[87] However, a 3',4'-elimination reaction promoted by the

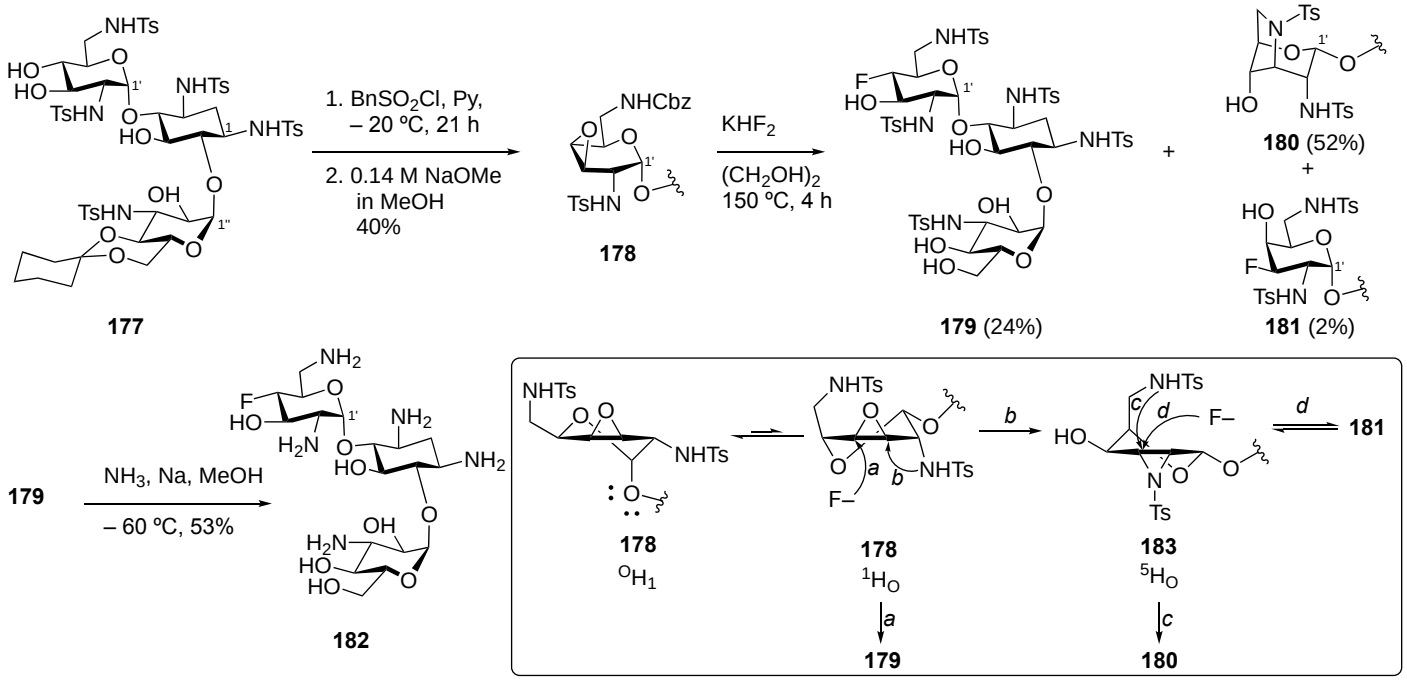
basic fluoride ion with concomitant cleavage of the 2',3'-*O*-cyclohexylidene during work-up cannot be excluded. Due to this discouraging result, a different approach was adopted. Compound **170** was treated sequentially with phenylmethanesulfonyl chloride, 80% aqueous acetic acid and 0.1 M sodium methoxide solution in methanol to afford epoxide (**173**). The treatment of **173** with KHF_2 in ethylene glycol afforded fluorinated derivatives **174** and **175** as a 1:5 mixture of inseparable compounds. This reaction also afforded non-fluorinated by-products as a result of nucleophilic attacks by ethylene glycol and water (not shown). A mixture **174** and **175** was deprotected under Birch conditions, and then the compounds were separated using ionic exchange chromatography, allowing the isolation of **176**.



Scheme 27. Synthesis of 4'-deoxy-4'-fluorokanamycin A (**176**).

3.3.12. Synthesis of 4'-deoxy-4'-fluorokanamycin B (182).[87] Compound **177** was first reacted with phenylmethanesulfonyl chloride in pyridine and then treated with sodium methoxide to afford **178** (Scheme 28). The reaction of **178** with KHF_2 in ethylene glycol resulted in a complex mixture of products, of which only three were identified. The major product was bicyclic compound **180** (52% yield), followed by the desired 4'-deoxy-4'-fluoro derivative (**179**), which was obtained in 24%. Traces of 3'-deoxy-3'-fluoro-4'-*epi* kanamycin B derivative (**181**) were also isolated. Several attempts to improve the yield of **179** by running the reaction in various protic and polar aprotic high-boiling solvents resulted only in the formation of compound **180**. Compound **178** preferentially adopts an $^{O}H_1$ conformation that exists in rapid equilibrium with a higher energy $^{1}H_O$ conformation. Following the Fürst-Plattner rule, attack of the $^{O}H_1$ conformer is not favoured because of the stereoelectronic effects of the lone pair orbital on the oxygen of the glycosidic bond hindering the approach of the nucleophile from the bottom face. Fluoride attack at C-4' occurs on the highest energy $^{1}H_O$ energy conformer of **178**, affording desired isomer (**179**). Attack at C-4' by fluorine is highly solvent-dependent, and only ethylene glycol was suitable for this transformation. Neighbouring group participation of

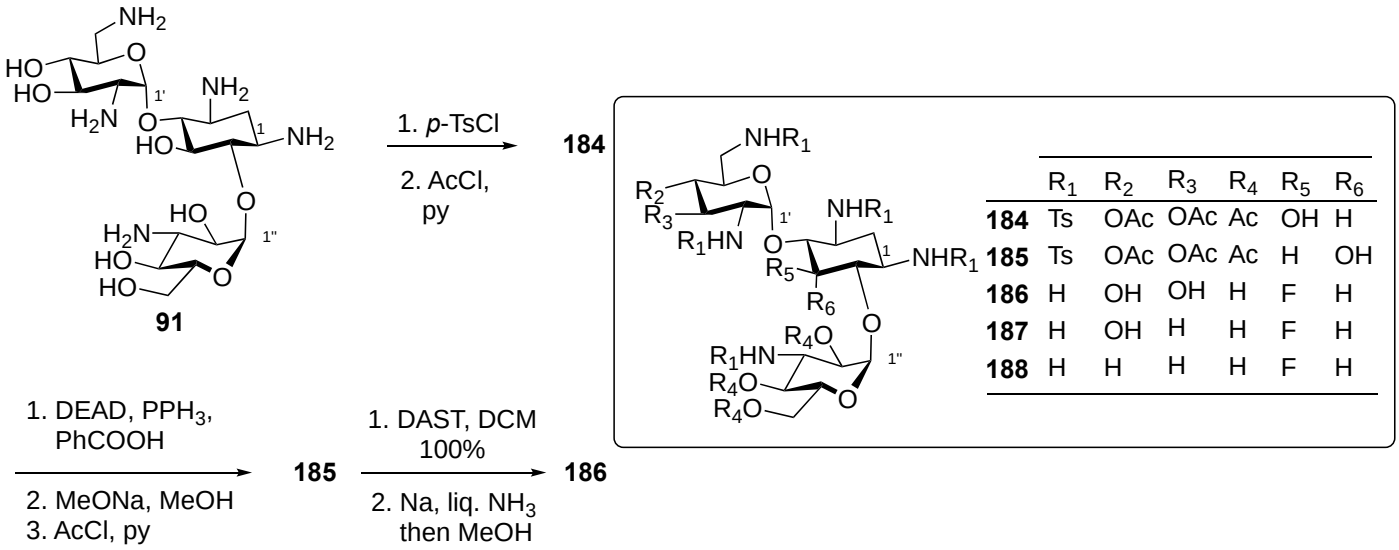
the C-2 tosylamino moiety affords 2',3'-*N*-tosylepimine compound **183**, which can be attacked intramolecularly at C-3' by the C-6' tosylamino group, affording bicyclic intermediate **180**, or intermolecularly by a fluoride anion, affording compound **181**. The attack at C-3' is reversible and favours the formation of bicyclic intermediate **180** based on the Curtin-Hammett principle.[89] Fluorinated kanamycin B analogue **182** was obtained after cleavage of the *N*-tosyl protecting groups under Birch conditions and purification by ion exchange chromatography.[87]



Scheme 28. Synthesis of 4'-deoxy-4'-fluorokanamycin B (**182**).

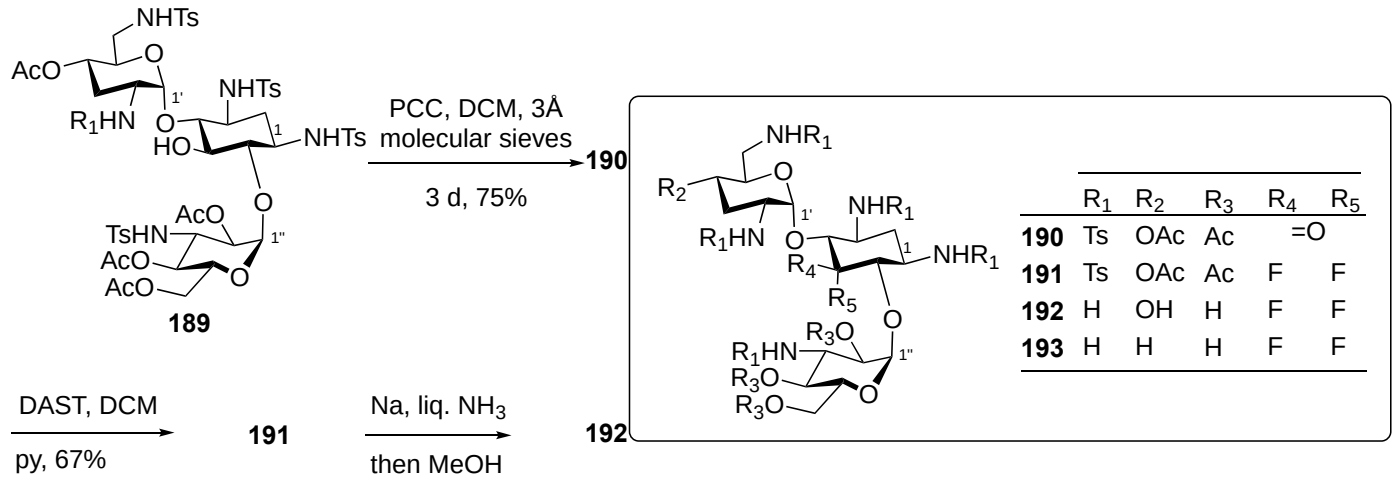
Compounds **176** and **182** were slightly less potent than kanamycins A (**1**) and B, but they showed broader activity. Bacteria expressing AAD(4') enzyme were susceptible to **176** and **182**, but bacteria expressing APH(3´) were resistant to the fluorinated AGs, indicating that the presence of an equatorial fluorine at C-4' does not influence enzymes modifying the C-3' position.[87]

3.3.13. Synthesis of 5-deoxy-5-fluoro derivatives of kanamycin B (91), tobramycin (2), and dibekacin (3).[26] The syntheses of **186**-**188** have been described elsewhere.[53] The synthesis of 5-deoxy-5-fluorokanamycin B (**186**) is described herein as an example (Scheme 29). Treatment of kanamycin B (**91**) with *p*-TsCl followed by AcCl in pyridine afforded **184**. The inversion of the C-5 stereocenter of **184** was performed under Mitsunobu conditions using DEAD, triphenylphosphine and benzoic acid, and subsequent treatment with sodium methoxide and reacetylation with AcCl in pyridine afforded intermediate (**185**). Treatment of **185** with DAST inverted the configuration, and compound (**186**) was obtained after deprotection. A similar approach was used for the syntheses of **187** and **188**.



Scheme 29. Synthesis of 5-deoxy-5-fluorokanamycin B (**186**), 5-deoxy-5-fluorotobramycin (**187**), and 5-deoxy-5-fluorodibekacin (**188**).

The synthesis of 5-deoxy-5,5-difluorotobramycin (**192**) is described as a second example (Scheme 30). The treatment of suitably protected tobramycin derivative (**189**) with pyridinium chlorochromate (PCC) in dichloromethane afforded the 5-oxo derivative (**190**). The treatment of **190** with DAST gave a difluorinated analogue (**191**) that afforded **192** after deprotection. A similar approach afforded 5-deoxy-5,5-difluoro-dibekacin (**193**).

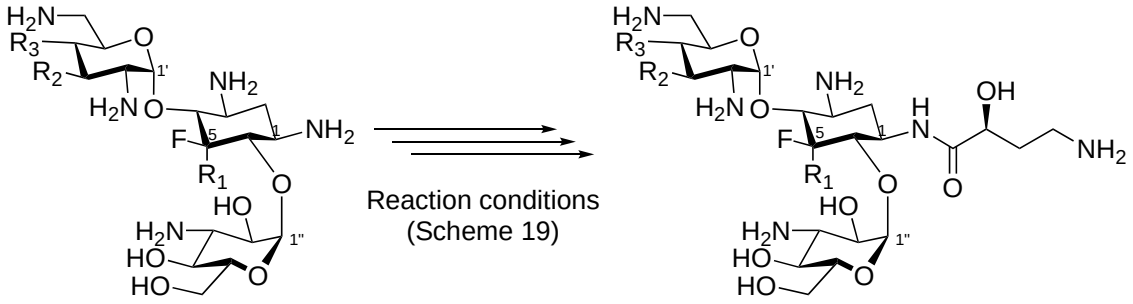


Scheme 30. Synthesis of 5-deoxy-5,5-difluorotobramycin (**192**) and 5-deoxy-5,5-difluorodibekacin (**193**).

The results of the antibacterial test suggested that the potencies of fluorinated analogues **186**-**188**, **192** and **193** were comparable to those of their parent un-fluorinated counterparts, but depending on their deoxygenation pattern, they exhibit different behaviours against resistant bacteria. Compound (**186**) was inactive against bacteria expressing the enzyme APH(3') I and II, while compounds (**187**, **188**, **192** and **193**) evaded resistance from bacteria expressing the enzymes APH(3'), ANT(2''), AAC(3), and AAC (2'). Their ability to evade AAC was attributed to the decreased basicity of the C-3 and C-2' amine groups, but the source of their ability to evade the enzyme ANT(2'') is not clear. The authors proposed that the presence of the fluorine

atom at C-5 could alter the glycosidic bond angles, impairing the fit of the AG in ANT(2'') but not in the ribosome A-site.[26] The fluorine atom can also alter the affinity of the AG towards the ANT(2'') enzyme due to the reduction of the nucleophilicity of the 2''-hydroxy group, as demonstrated by 4'-deoxy-4',4'-difluoroneamine (**40**) and 4'-deoxy-4',4'-difluorokanamycin A (**223**) (refer to Section 3.3.18).[47] The acute toxicity tests showed that the fluorinated derivatives are less toxic than their parent oxygenated AGs in mice. In general, 5-deoxy-5-fluoro derivatives **187** and **188** presented LD_{50} values approximately two-fold higher than those of their difluorinated counterparts (**192** and **193**). These results strongly suggest that the basicity of the C-1 and C-3 amines contributes to the toxicity of the AGs.

3.3.14. Synthesis of 1-*N*-HABA-5-deoxy-5-fluoro analogues of kanamycin B (91), tobramycin (2), arbekacin (93).[27] The synthesis of 1-*N*-HABA- derivatives **194–198** started from previously described fluorinated derivatives **186–188**, **192** and **193**[26] and was performed in a manner similar to what was described previously (Scheme 19). The synthesis of **195** has been described elsewhere.[53]



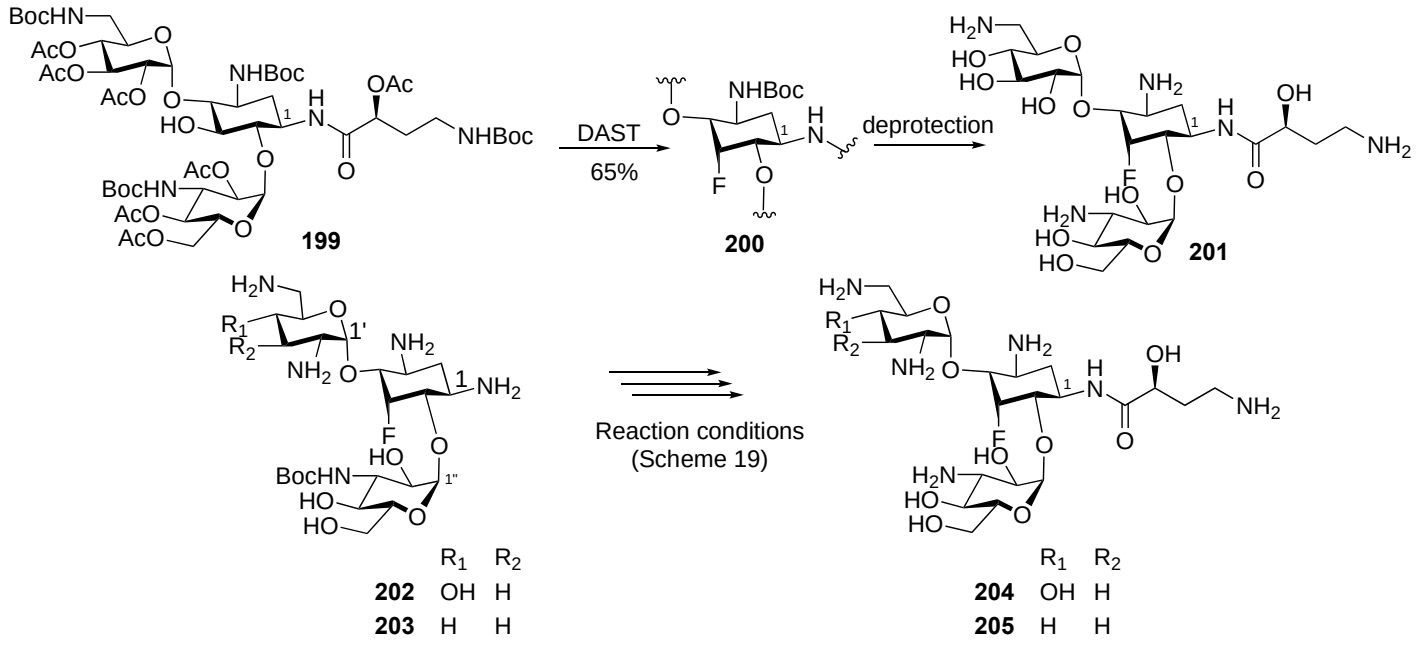
	R_1	R_2	R_3
186	H	OH	OH
187	H	H	OH
188	H	H	H
192	F	H	OH
193	F	H	H

	R_1	R_2	R_3
194	H	OH	OH
195	H	H	OH
196	H	H	H
197	F	H	OH
198	F	H	H

Scheme 31. Synthesis of 1-HABA-5-deoxy-5-fluorokanamycin B (**194**), 1-HABA-5-deoxy-5-fluorotobramycin (**195**), 1-HABA-5-deoxy-5-fluorodibekacin (**196**), 1-HABA-5-deoxy-5,5-difluorotobramycin (**197**) and 1-HABA-5-deoxy-5,5-difluorodibekacin (**198**).

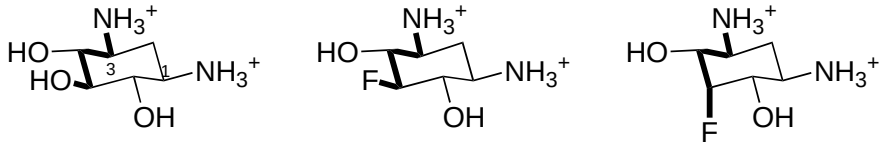
The toxicities of the AGs were in the order 5-OH>5-F>5-F,F, and based on these results, the 1-*N*-HABA group had no appreciable effect on the toxicity of this series of compounds, again suggesting that the toxicity is a consequence of the basicity of the amine groups on the deoxystreptamine core.[27]

3.3.15. 5-Deoxy-5-*epi*- derivatives of amikacin (4), tobramycin (2), dibekacin (3), 1-*N*-HABA tobramycin, and arbekacin (93).[90] The same strategy that was used for the synthesis of compound **201** was used for the synthesis of 5-deoxy-5-fluoro-5-*epi* derivatives **202** and **203** (Scheme 32). The treatment of suitably protected intermediate **199** with DAST afforded **200** with inversion of the configuration at C-5 in 65% yield. Compound (**201**) was obtained following standard deprotection procedures. The installation of the 1-*N*-HABA side chain in compounds **204** and **205** started from 5-deoxy-5-fluoro-5-*epi* fluorinated derivatives (**202** and **203**) and followed the strategy described previously (Scheme 19).



Scheme 32. 5-Deoxy-5-*epi*-5-fluoroamikacin (**201**), 5-deoxy-5-*epi*-5-fluorotobramycin (**202**), 5-deoxy-5-*epi*-5-fluorodibekacin (**203**), 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]-5-deoxy-5-*epi*-5-fluorotobramycin (**204**), 5-deoxy-5-*epi*-5-fluoroarbekacin (**205**).

All the 5-deoxy-5-*epi*-5-fluoro analogues retained the antibacterial activity of the 5-deoxy-5-fluoro-analogues and were as potent as AGs arbekacin (**93**), amikacin (**4**) and 1-*N*-HABA tobramycin (not shown). AGs **201–205** were all active against bacteria expressing the AAC(2') enzyme, and 1-*N*-HABA AGs **204–205** showed the strongest antibacterial potency against clinically isolated methicillin-resistant *S. aureus*. Surprisingly, the toxicities of 5-deoxy-5-fluoro-5-*epi* AGs (**201–205**) were the same as those of their parent compounds; this is unlike the results of 5-deoxy-5-*epi*-5-fluorosisomicin (**73**), which is less toxic than sisomicin (**65**).[55]



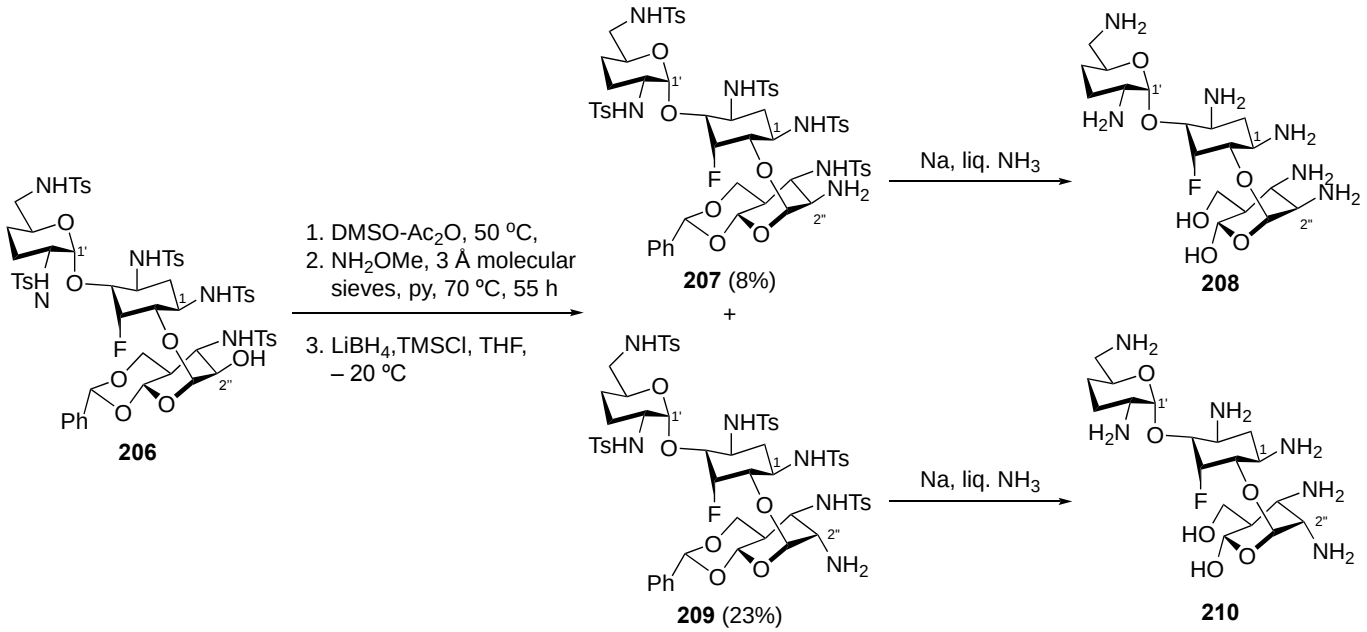
	69	**70**	**71**
pK_a	8.36	8.01	8.27
DpK_a	0	0.35	0.09

Figure 11. Influence of fluorination on pK_a.

The relationship between the orientation of the 5-fluoro substituent and the basicity of the amino groups at C-1 and C-3 in the 5-deoxy-5-fluoro and 5-deoxy-5-fluoro-5-*epi* series was studied with a model system. Three compounds, namely, 2-deoxystreptamine (**69**), 2,5-dideoxy-5-fluoro-2-deoxystreptamine (**70**) and 2,5-dideoxy-5-*epi*-5-fluoro-2-deoxy*streptamine* (**71**), were prepared, and the pK_a values of the 1,3-amino groups were determined by ^{13}C NMR spectroscopy and by classic titration methods (Figure 11). The obtained pK_a values showed that the equatorial 5-fluoro atom of **70** significantly reduced the basicity of the amino groups (Δ = 0.35) when compared to the value obtained for 2-deoxystreptamine (**70**). The axial 5-fluoro of **71**,

however, reduced the basicity only slightly (Δ = 0.09). This difference in basicity correlates well with the difference in acute toxicity found between the 5-deoxy-5-fluoro and 5-deoxy-5-fluoro-5-*epi* series.[90] The effect of the difluorination at C-5 still needs to be assessed.

3.3.16. Synthesis of 2''-amino-5,2''-dideoxy-5-*epi*-5-fluorodibekacin (208) and 2''-amino-5,2''-dideoxy-5,2''-*diepi*-5-fluorodibekacin (210).[91] As part of a study on the effect of 5-fluorination on the ^{13}C NMR chemical shifts of C-4 and C-6, several 2''-amino-2''-deoxy and 2''-acylamino-2''-deoxy derivatives of 5-deoxy-5-fluoro-5-*epi*-dibekacin (**203**) were prepared. The syntheses of **208** and **210** are described as examples (Scheme 33). A suitably protected derivative (**206**) was treated with DMSO and acetic anhydride to give the 2''-oxo derivative, which was the reacted with methoxyamine hydrochloride and reduced with $LiBH_4$-TMSCl[92] to afford a diastereomeric mixture of **207** and **209** that was separated by flash chromatography. The deprotection of **207** and **209** afforded AGs (**208** and **210**).



Scheme 33. Synthesis of 2''-amino-5,2''-dideoxy-5-epi-5-fluorodibekacin (**208**) and 2''-amino-5,2''-dideoxy-5,2''-diepi-5-fluorodibekacin (**210**).

Intermediates (**207** and **209**) were acylated at 2''-N with HABA, β-alanine or 4-butanoic acid (not shown), and the compounds were analysed by NMR spectroscopy. Deoxyfluorination at C-5 causes an upfield shift in the ^{13}C NMR signals of C-4 and C-6 relative to the values determined for the non-fluorinated AG. The effect is more significant at C-4, indicating that despite both carbons being vicinal to the halogen atom, the electron-withdrawing effect is more pronounced at this position than at C-6. These results, together with MM2UEC refined by MOPAC93/MP3 calculations, revealed that this difference can be explained by a through-space interaction between the 5-fluoro atom and the 4-O and 6-O species (Figure 12) that shield the vicinal C-4 and C-6 atoms.[91]

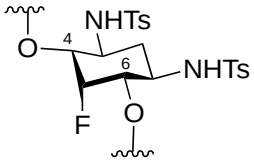
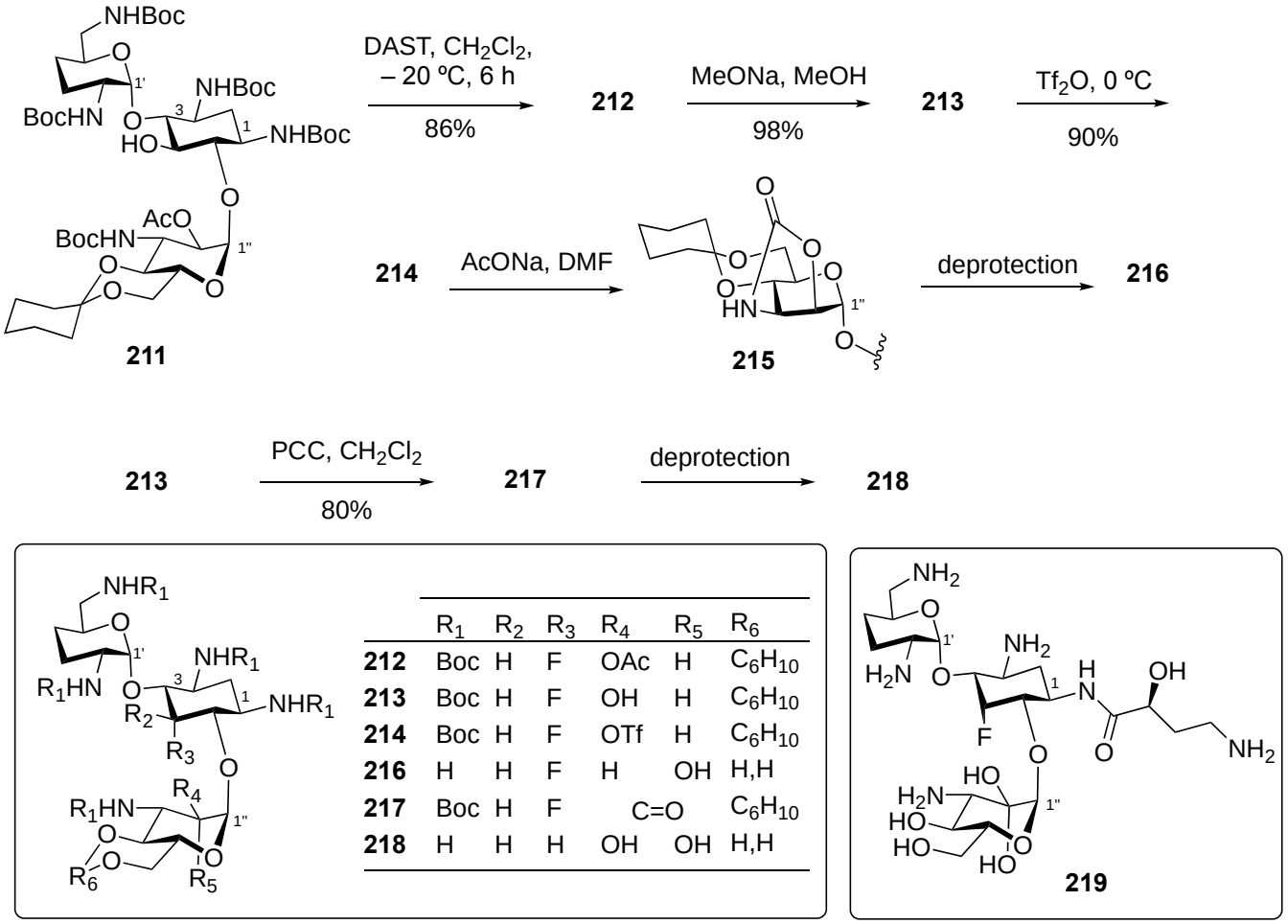


Figure 12

With the exception of **208**, which showed some potency, the **210** and the 2''-acylamino-2''-deoxy derivatives (not shown) exhibited lower potencies or a complete absence of antibacterial activity, indicating that *N*-acylation at C-2'' is detrimental to the bacteriotoxic nature of these molecules.[91]

3.3.17. Synthesis of 5-deoxy-5,2''-di*epi*-5-fluorodibekacin (216), 5-deoxy-5-*epi*-5-fluoro-2''-hydroxy dibekacin (218) and 5-deoxy-5-*epi*-5-fluoro-2''-hydroxyarbekacin (219).[93] A suitably protected dibekacin analogue (**211**) was treated with DAST in dichloromethane to afford **212** in 80% yield (Scheme 34). Deacetylation of **212** followed by triflation gave **214** in a high yield. Treatment of **214** with sodium acetate in DMF afforded cyclic carbamate (**215)** with inversion of the configuration at C-2''. Finally, deprotection under standard procedures afforded **216**. Treatment of **213** with PCC in dichloromethane gave compound **217** in 80% yield, and deprotection of this compound gave 2''-oxo derivative **218** in the form of a hydrate. Arbekacin analogue (**219**) was prepared in a similar manner starting from a suitably protected arbekacin analogue (not shown).

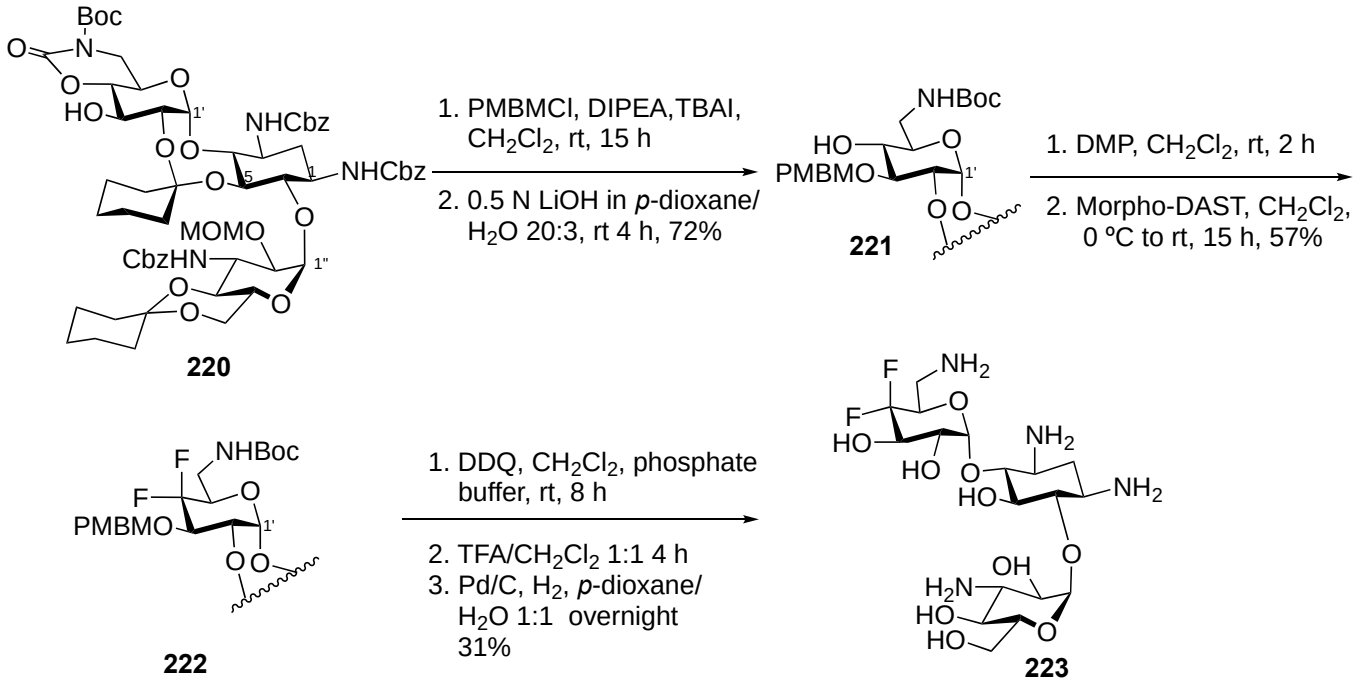


	R_1	R_2	R_3	R_4	R_5	R_6
212	Boc	H	F	OAc	H	C_6H_{10}
213	Boc	H	F	OH	H	C_6H_{10}
214	Boc	H	F	OTf	H	C_6H_{10}
216	H	H	F	H	OH	H,H
217	Boc	H	F	C=O		C_6H_{10}
218	H	H	H	OH	OH	H,H

Scheme 34. Synthesis of 5-deoxy-5,2''-diepi-5-fluorodibekacin (**216**), 5-deoxy-5-epi-5-fluoro-2''-hydroxydibekacin (**218**) and 5-deoxy-5-epi-5-fluoro-2''-hydroxyarbekacin (**219**).

The antibacterial activity potencies of **216**-**219** were considerably diminished compared with that of arbekacin (**93**); this effect was attributed to the absence of the equatorial OH-2" of the AG when in the oxo form.

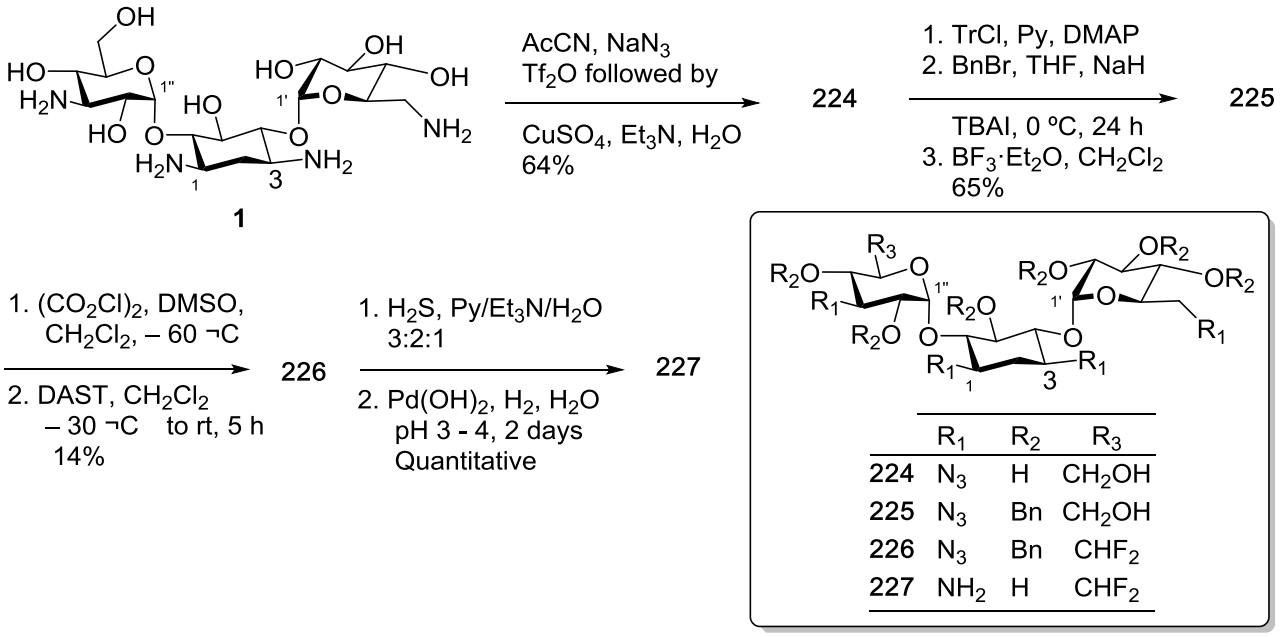
3.3.18. Synthesis of 4'-deoxy-4',4'-difluorokanamycin A (223).[47] 4'-Deoxy-4',4'-difluorokanamycin A (**223**) was synthesized to determine the importance of the nucleophilicity C-3' OH group in the phosphorylation mechanism of the enzyme APH(3'). The synthesis started from compound **220**, which was obtained in six steps from kanamycin A (**1**)[94] (Scheme 35). Treatment of **220** with *p*-methoxybenzyl chloromethyl ether (PMBMCl) followed by base-promoted hydrolysis of the 5',6'-*N*,*O*-carbonyl moiety afforded **221**. The reaction of **221** with Dess-Martin periodinane (DMP) gave the 4'-oxo derivative, which was treated with Morpho-DAST to afford **222**. Difluorinated kanamycin A derivative (**223**) was obtained after protecting group cleavage under standard conditions.



Scheme 35. Synthesis of 4'-deoxy-4',4'-difluorokanamycin A (**223**).

4'-Deoxy-4',4'-difluorokanamycin A (**223**) and 4'-deoxy-4',4'-difluoroneamine (**40**) (Scheme 5, Section 2.2.3) presented moderate antibacterial potencies. Remarkably, the MIC determined for a resistant *E. coli* strain that expresses the APH(3')IIa enzyme was the same as that determined for the wild-type bacteria when **223** and **40** were used for testing. Enzyme kinetics analysis also demonstrated that **40** and **223** were impaired substrates for the APH(3')IIa enzyme. These results show that the reduction in the nucleophilicity of C-3' OH caused by the electronegativity of the adjacent fluoride atom renders these compounds poor substrates for phosphorylation by the APH(3')IIa enzyme.[47]

3.3.19. Synthesis of 6''-deoxy-6'',6''-difluorokanamycin A (227).[35] A diazo transfer reaction afforded perazido derivative **224** from kanamycin A (**1**) in 64% yield (Scheme 36). The regioselective protection of the primary OH of **224** as a trityl ether followed by per-*O*-benzylation and removal of the trityl protecting group with $BF_3{\cdot}Et_2O$ afforded compound **225** in 65% overall yield. A Swern oxidation followed by treatment with DAST afforded difluorinated analogue **226**, which was fully deprotected to afford **227**.

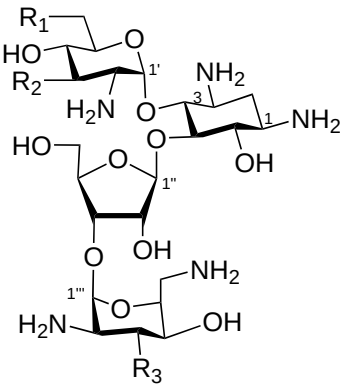


Scheme 36. Synthesis of 6''-deoxy-6'',6''-difluorokanamycin A (**227**).

Compound **227** was two-fold more active against AG-sensitive bacteria but was practically inactive against the tested AG-resistant bacteria.

4. Fluorinated 4,5-Disubstituted 2-Deoxystreptamine AGs

Fluorinations of the 4,5-disustituted-2-deoxystreptamine family of AGs are rare. Examples of this family of AGs include paromomycin (**7**), neomycin (**8**), and lividomycin (**228**) (Figure 13).

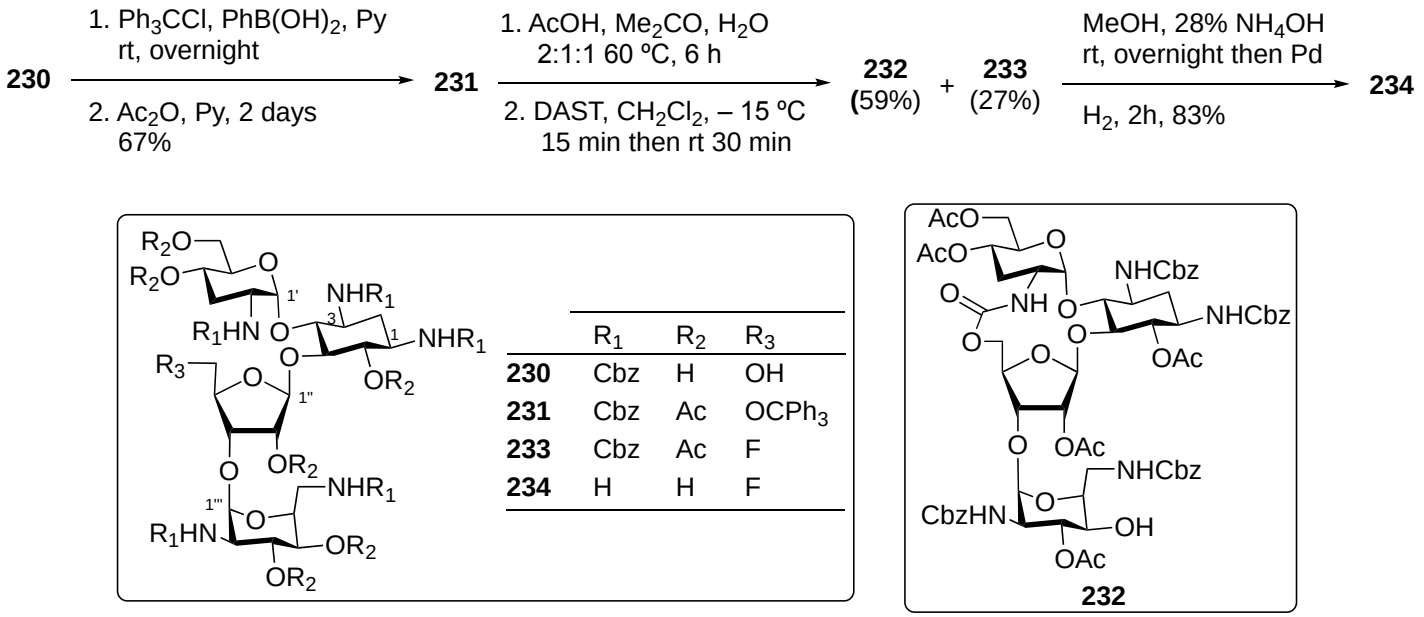


	R_1	R_2	R_3
228 lividomycin	OH	H	a-mannopyranoside
229 lividomycin B	OH	H	H
7 paromomycin	OH	OH	H
8 neomycin B	NH_2	OH	H

Figure 13. The neomycin family of AGs.

4.1. Synthesis of 5''-deoxy-5''-fluorolividomycin B (234)[95]

The selective tritylation of the C-5'' OH of **230** with phenyl boronate and trityl chloride followed by peracetylation afforded **231** (Scheme 37). The removal of the C-5'' trityl group under acidic conditions followed by reaction with DAST produced **232** (59% yield) as the major component and desired 5''-deoxy-5''-fluoro derivative **233** in 27% yield. Standard protecting group removal afforded **234**.

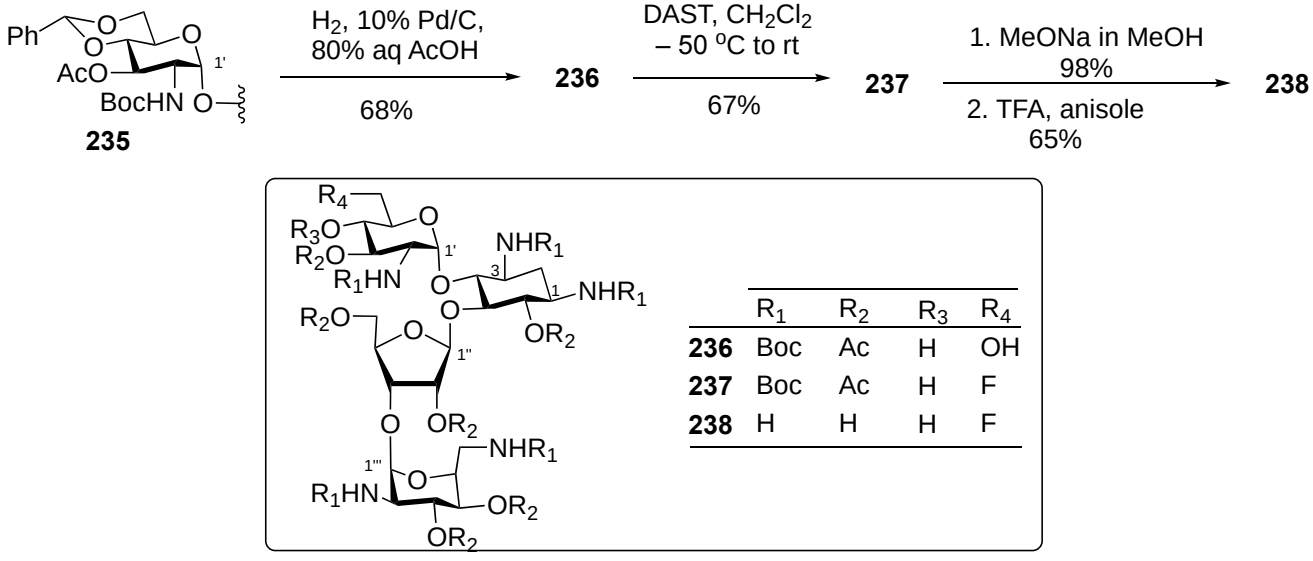


Scheme 37. Synthesis of 5''-deoxy-5''-fluorolividomycin B (**234**).

Deoxyfluorination at C-5'' was detrimental to the antibacterial potency of lividomycin B. The antibacterial potency of compound (**234**) is comparable to the potency of 5''-deoxylividomycin B, indicating that C-5'' OH moiety is important for biological activity.[95]

4.2. Fluorination of paromomycin (7) and neomycin (8)

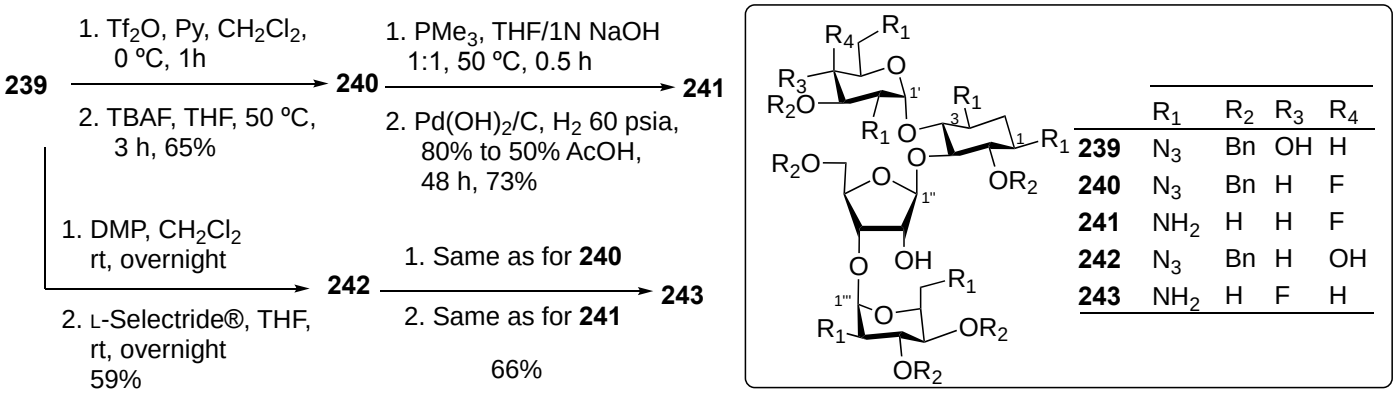
4.2.1. Synthesis of 6'-deoxy-6'-fluoroparomomycin (238).[96] Hydrogenolysis of the benzylidene protecting group of compound **235** afforded **236** (Scheme 38). A selective deoxyfluorination with DAST in dichloromethane followed by cleavage of the protecting groups using standard procedures afforded **238**.



Scheme 38. Synthesis of 6'-deoxy-6'-fluoroparomomycin (**238**).

Compared with paromomycin (**7**), 6'-deoxy-6'-fluoro analogue (**238**) showed a lower potency against both wild-type 1408A and 1408G mutant ribosomes[96] and against methicillin-resistant strains of *S. aureus*, *E. coli* and *P. aeruginosa.*[97] Compound **238** was also screened in cell-free assays for its ability to inhibit translation by bacterial wild-type and recombinant hybrid ribosomes carrying complete A site cassettes of the human mitochondrial (Mit13), mitochondrial A1555G, and cytoplasmic ribosomes (Cyt14), and it showed no significant potency.[97]

4.2.2. Synthesis of 4'-deoxy-4'-*epi*-4'-fluoro- (241), 4'-deoxy-4'-fluoro- (243), and 1-*N*-HABA-4'-deoxy-4'-*epi*-4'-fluoroneomycin B (246).[25] The synthesis started with a suitably protected neomycin B analogue (**239**, Scheme 39). Treatment of **239** with DAST gave a complex mixture of products (not shown). Compound **239** was then reacted with triflic anhydride in dichloromethane and then treated with TBAF in THF to give **240**. Cleavage of the protecting groups under standard conditions afforded **241**. To synthesize **243,** the alcohol at 4'-C was epimerized by sequential oxidation with Dess-Martin periodinane in dichloromethane followed by stereoselective reduction using L-Selectride®, affording 4-*epi* derivative **242**. The reaction of **242** with triflic anhydride followed by treatment with fluoride using TBAF in THF and deprotection gave compound (**243**).



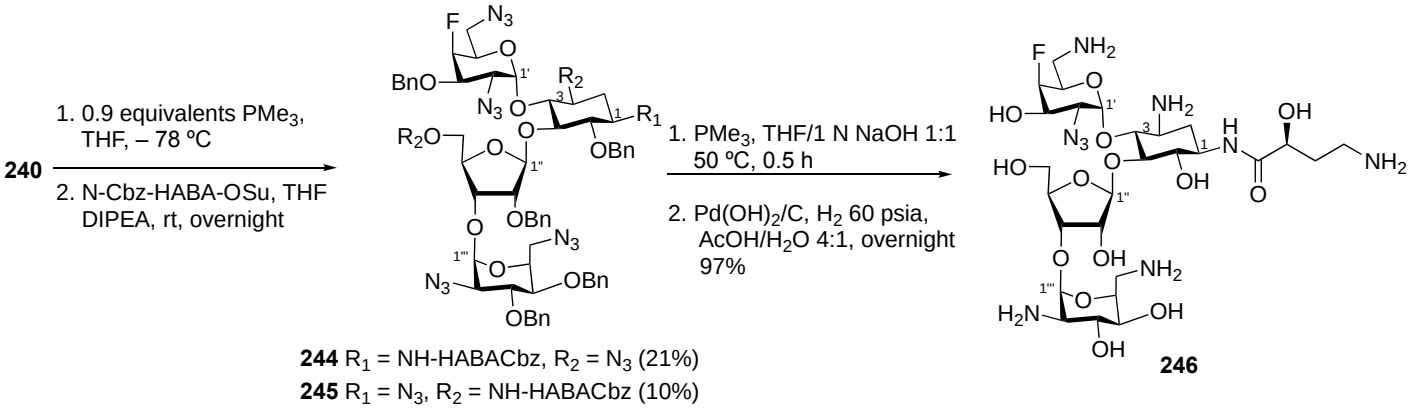
Scheme 39. Synthesis of 4'-deoxy-4'-epi-4'-fluoroneomycin B (**241**), 4'-deoxy-4'-fluoroneomycin B (**243**).

The selective reduction of the C-1 and C-3 azides of **240** under Staudinger conditions[98-99] followed by acylation with *N*-Cbz-HABA-OSu afforded compounds **244** and **245** (Scheme 40). The reaction gave a 1:0.8 ratio of **244**:**245**. The regioselectivity of the Staudinger reduction correlates with the electron density at the corresponding α-nitrogen atoms as determined by natural abundance ^{15}N NMR; the azide bearing the most electron-deficient α-nitrogen was preferentially reduced.[100] Compound **244** was deprotected under standard conditions, affording **246**.

Isomeric 4'-fluoro analogues **241** and **243** showed potencies similar to those of their parent compound, neomycin B (**8**), when tested against susceptible isolates of *S. aureus*, *K. pneumoniae* and *E. coli* strains. Both compounds were also active towards bacterial isolates expressing the ANT(4')-I and ANT(4')-II enzymes. Remarkably, 4'-deoxy-4'fluoro-4'-*epi* derivative **243** was also active towards two *P. aeruginosa* strains expressing chromosomal APH(3')-IIb enzymes. Similarly, these properties were also observed in its analogue, 1-*N*-HABA-4'-deoxy-4'-fluoro-4'-*epi*-neomycin (**246**), which is active against *P. aeruginosa* expressing the APH(3')-IIb enzyme.[25] Similar behaviour was observed in the kanamycin series in which 4'-deoxy-4',4'-difluorokanamycin A (**223**) and 4'-deoxy-4',4'-difluoroneamine (**40**) were found to be impaired substrates for the APH(3')IIa enzyme.[47] On the other hand, 4'-deoxy-4'-fluorokanamycin A (**176**) and 4'-deoxy-4'-fluorokanamycin B (**182**) are susceptible to inactivation by APH(3') enzyme.[87] These results, together with

those obtained for compounds **241**, **243** and **246**, seem to indicate that an axial fluorine atom at C-4' is required to impair binding with the APH(3')IIa enzyme.

A co-crystal of **246** with A-site rRNA showed that the compound is accommodated inside the A-site and the fluorine atom at C-4' stacks with a guanine residue inside the receptor.[25]



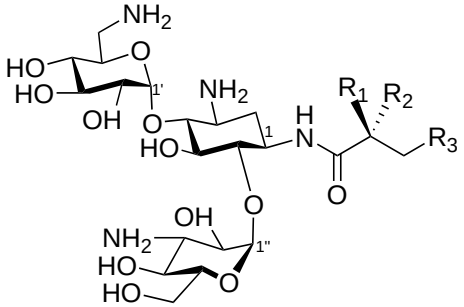
244 R_1 = NH-HABACbz, R_2 = N_3 (21%)
245 R_1 = N_3, R_2 = NH-HABACbz (10%)

Scheme 40. Synthesis of 1-*N*-HABA-4'-deoxy-4'-epi-4'-fluoroneomycin B (**246**).

5. AGs with Fluorinated ω-Amino Acid Lateral Chains at Position 1

5.1. Synthesis of amikacin analogues with a ω-amino-α-fluoroalkanoyl side chain[23]

Kanamycin A (**1**) was 1-*N*-acylated with a series of ω-amino-α-fluoro amino acids to determine their impact on the antibacterial activity (Figure 14). The fluorinated amino acids required for the side chains were synthesized by deoxyfluorination of suitably protected α-hydroxy or α-oxo esters with DAST. The 1-*N*-acylation was performed following the zinc-chelate ethyl trifluoroacetate method (see Scheme 19).[71]



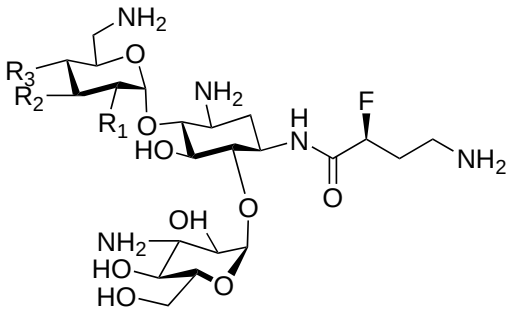
	R_1	R_2	Configuration	R_3
247	F	H	*(S)*	NH_2
248	F	H	*(S)*	CH_2NH_2
249	H	F	*(R)*	CH_2NH_2
250	F	F		CH_2NH_2
251	F	H	*(S)*	$CH_2CH_2NH_2$

Figure 14. Kanamycin ω-amino-α-fluoro analogues: 1-*N*-[(S)-3-amino-2-fluoropropanoyl]kanamycin A (**247**), 2'''-deoxy-2'''-fluoroamikacin (**248**), 2'''-deoxy-2'''-*epi*-2'''-2'''-fluoroamikacin (**249**), 2'''-deoxy-2''',2'''-difluoroamikacin (**250**) and 1-*N*-[(S)-5-amino-2-fluorovaleryl]kanamycin A (**251**).

The most promising chain in terms of biological activity, the (*S*)-4-amino-2-fluorobutyric acid moiety, was also introduced to other AGs, to get acylated derivatives of kanamycin B (**252**), tobramycin (**253**), dibekacin (**254**), and gentamycin B (not shown) (Figure 15).

Of all the ω-amino-2-fluoro amino acids coupled with kanamycin A, 2'''-deoxy-2'''-fluoroamikacin (**248**) showed the best biological activity profile (similar to that of kanamycin). The other analogues showed

dramatically lower antibacterial potencies. It was also noted that the antibacterial potency of the fluorinated analogues parallel the potencies of the corresponding 2-hydroxybutyryl analogues, leading to the conclusion that the 2-fluoro group may play the same role as the 2-hydroxy group when present in the side chain. The acute toxicity of **248** (LD_{50} 280 mg/kg, iv mice) was found to be the same as that reported for amikacin (**4**, LD_{50} 280 mg/kg).[23]

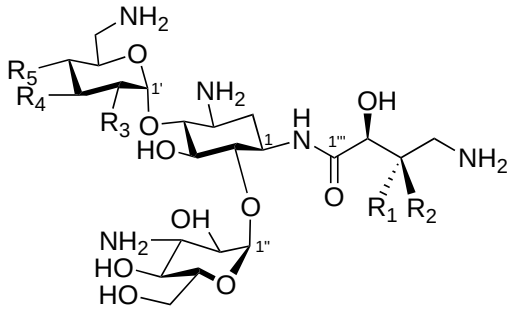


	R_1	R_2	R_3
248	OH	OH	OH
252	NH_2	OH	OH
253	NH_2	H	OH
254	NH_2	H	H

Figure 15. 1-*N*-[(*S*)-4-Amino-2-fluorobutanoyl] series: 2'''-deoxy-2'''-fluoroamikacin (**248**), 1-*N*-[(*S*)-4-amino-2-fluorobutanoyl]kanamycin B (**252**), 1-*N*-[(*S*)-4-amino-2-fluorobutanoyl]tobramycin (**253**), 1-*N*-[(*S*)-4-amino-2-fluorobutanoyl]dibekacin (**254**).

5.2. Synthesis of 1-*N*-[(*2R*,*3R*)- and 1-*N*-[(*2R*,*3R*)-4-amino-3-fluoro-2-hydroxybutanoyl] derivatives of kanamycin A[101]

The required (*2R*,*3R*)-4-amino-3-fluoro-2-hydroxybutanoic acids were synthesized from the appropriate carbohydrate precursors[101] and were coupled to the N-1 group of suitably protected kanamycin A and kanamycin B analogues to give compounds **255** - **258** (Figure 16).



	R_1	R_2	Configuration	R_3	R_4	R_5
255	F	H	*(R)*	OH	OH	OH
256	F	H	*(R)*	NH_2	H	OH
257	F	H	*(R)*	NH_2	H	H
258	H	F	*(S)*	NH_2	H	H

Figure 16. 1-*N*-[(2*R*,3*R*)-4-amino-3-fluoro-2-hydroxybutanoyl]kanamycin A (**255**), 1-*N*-[(2*R*,3*R*)-4-amino-3-fluoro-2-hydroxybutanoyl]tobramycin (**256**), 1-*N*-[(2*R*,3*R*)-4-amino-3-fluoro-2-hydroxybutanoyl]dibekacin (**257**) and 1-*N*-[(2*R*,3*S*)-4-amino-3-fluoro-2-hydroxybutanoyl]dibekacin (**258**).

The antibacterial tests showed that the (*2R*, *3R*)-analogues (**255–258**) were slightly more potent as antibacterial agents compared with amikacin (**4**), arbekacin (**93**) and 1-*N*-HABA tobramycin, while the (*2R*,*3S*) diastereomer (**258**) was less potent. Additionally, compounds **255**-**258** were slightly more potent against bacteria producing AAC(3) enzyme and remarkably also against AAC(2') and AAC(6'), which are located far from the fluorine atom.

The pK_a values of the C-4''' amino groups of amikacin (**4**) and arbekacin (**93**) were measured by ^{13}C NMR spectroscopy and were approximately two units higher than the pK_a values determined for compounds **255**

and **257** (10.2 vs 8.7). The acute toxicity values determined for **255** (LD_{50} 250 mg/kg) and **257** (LD_{50} 80 mg/kg), on the other hand, were quite similar to those of the reference compounds (**4**, LD_{50} 220 mg/kg and **93**, LD_{50} 80 mg/kg), indicating that the toxicity was not influenced by the presence of the fluorine atom. It was postulated that the toxicity may be largely influenced by the ratio of C-4''' NH_2 present as the free base or as the ammonium salt and not simply by the basicity and that a pK_a value of ~ 8 is required for a meaningful reduction in the toxicity by analogy with compounds **201**–**205** (refer to Section 3.3.15.).[101]

5.3. Synthesis of 1-*N*-[(*2'''S,4'''S*) and (*2'''S,4'''R*)-5-amino-4-fluoro-2-hydroxypentanoyl] derivatives of dibekacin[102]

The homologous (*2S*,4S) and (*2R*,4*R*)-5-amino-4-fluoro-2-hydroxypentanoic acids (prepared from malic acid) were coupled to 1-N of the aminoglycoside dibekacin (**3**) to obtain compounds **259** and **260** (Figure 17). Compounds **259** and **260** had the same potency as arbekacin (**93**). The acute toxicities in mice determined for **259** (~130 mg/kg) and **260** (~125 mg/kg) were approximately 1.7-fold weaker than that of arbekacin (**93**) (~75 mg/kg). This decrease in toxicity was attributed to the length of the chain attached to the AG. To further test this hypothesis, compounds **93**, **261**, **262** and **263** were synthesized (Figure 17), and a direct relationship between the chain length and the toxicity was found. The acute toxicities of these compounds were in the order **261**>**93**>**262**>**263**. The LD_{50} for **262** (~120 mg/kg), which possesses a 5-carbon chain, is similar to that of **259** (~130 mg/kg), indicating that the chain length and not the presence of the fluorine atom at C-4''' controls the toxicity. With respect to antibacterial activity, **263** was less potent than arbekacin (**93**), while **261** and **260** are almost equipotent to the aforementioned AG,[102] indicating that a butanoic derivative presents the best balance between high antibacterial potency and low acute toxicity.

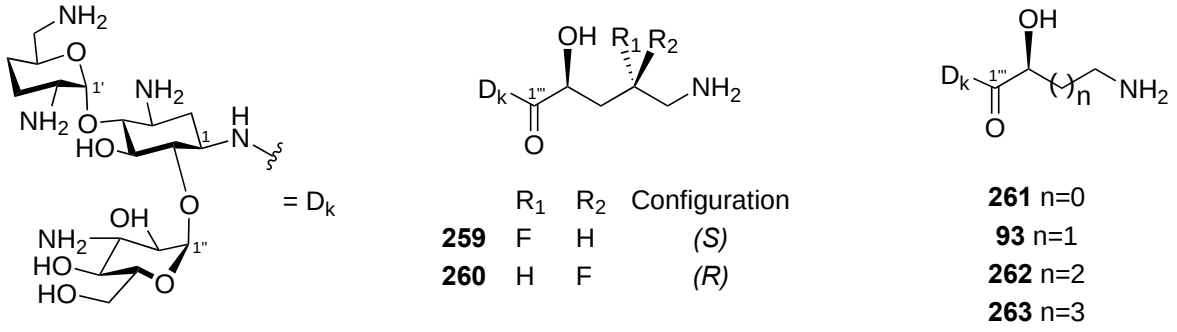


Figure 17. 1-*N*-[(2'''*S*,4'''*S*)-5-amino-4-fluoro-2-hydroxypentanoyl]dibekacin (**259**) and 1-*N*-[(2'''*S*,4'''*R*)-5-amino-4-fluoro-2-hydroxypentanoyl] (**260**) and homologous ω-amino-α-hydroxyalkanoyl analogues.

5.4. Synthesis of 3''''-fluorinated 1-*N*-(4''''-amino-2''''-hydroxybutanoyl)-3'',4'',3''',4'''-tetradeoxy neomycin B analogues[28]

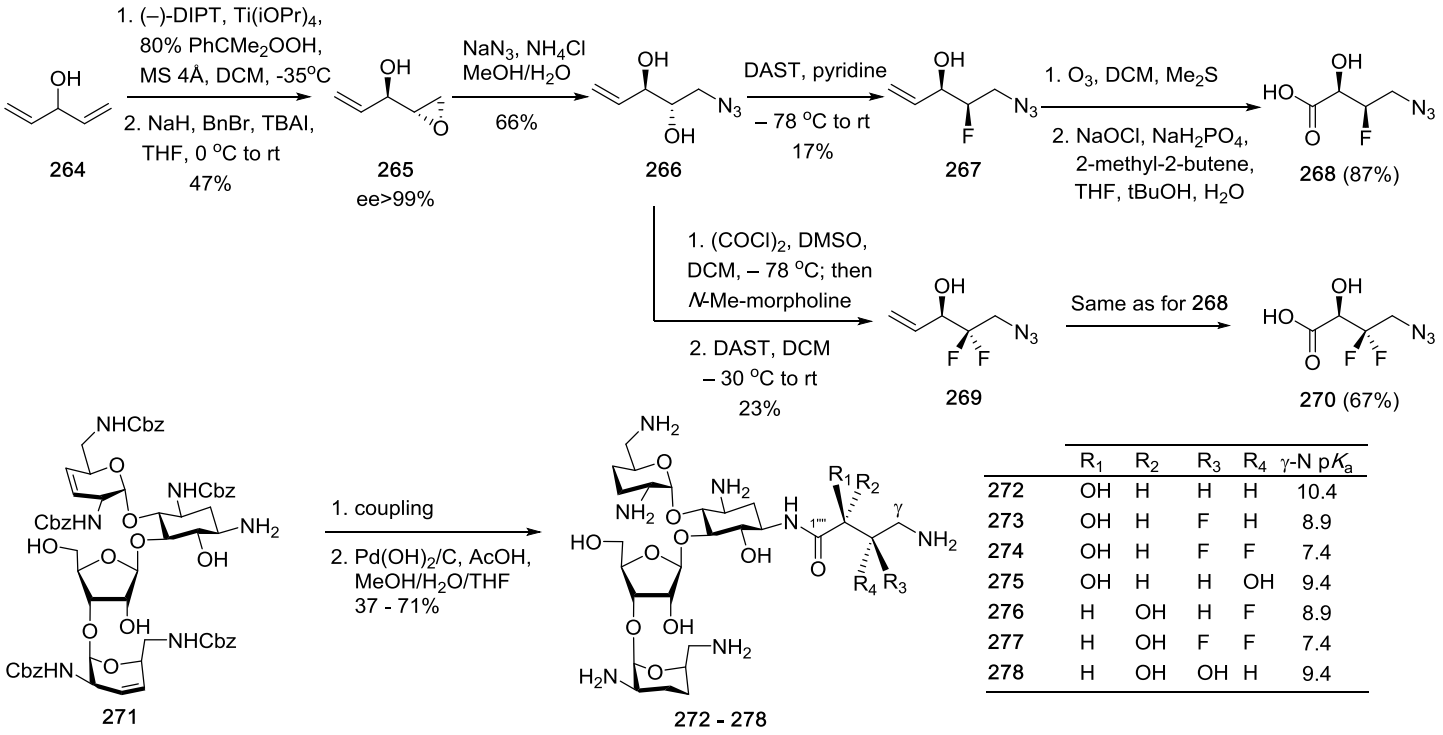
Suitably protected fluorinated acids (exemplified by **268** and **270**, Scheme 41) were synthesized and coupled to a tetradeoxy neomycin precursor (**271**)[103] to afford a series AGs that were tested for biological activity. Divinyl-carbinol **264** was treated with (–)-diisopropyl-tartrate (DIPT), $Ti(iOPr)_4$ and $PhCMe_2OOH$ and then benzylated to afford optically pure epoxide **265**. Treatment of **265** with sodium azide gave **266**. Further treatment of **266** with DAST gave a deoxyfluorinated analogue (**267**). Ozonolysis followed by a Pinnick oxidation afforded **268**. The oxidation of **266** under Swern conditions followed by deoxyfluorination with DAST gave difluorinated compound **269**, which was then converted to **270**. Compounds **268** and **270** were coupled

to **271**, and subsequent reduction of the azides and olefins by catalytic hydrogenation afforded **273** and **274**. A similar procedure was used to prepare **276** and **277**. Diols **275** and **278** were also synthesized.

Compounds **272–275**, which have the natural L-HABA configuration at C-2'''', and unnatural D-HABA analogues (**276–278**) were tested against a broad panel of susceptible and resistant strains of ESKAPE pathogens to examine the effects of γ-*N* pK_a modulation on their antibacterial potencies. The L-HABA analogues (**273–275**) were as potent as the parent compound (**272**) against wild-type strains and retained the activity of the parent compound through a panel of AG-resistant strains. The D-HABA analogues (**276–278**) were generally less potent than the analogues in the L-series but much more potent than the reference compounds.[28] A strong correlation between the γ-*N* pK_a and the antibiotic potency was observed in the D-HABA series.

Compound **273** was co-crystallized with the A-site decoding rRNA region. The data showed that the fluorinated acyl groups adopt the same conformation inside the A-site as the HABA chain as was observed for amikacin (**4**), and the (3''''*R*) fluorine atom forms hydrogen bonds to two cytosine residues inside the A-site rRNA.

Dose-response caspase-3/7 activation on the human kidney cell line HK2 triggered by compounds **272**-**278** revealed that apoptosis induction depends on the pK_a of the derivative. Compounds with higher γ-*N* pK_a values elicited apoptosis with an average EC_{50} = 26 mg/mL, which is equal to that of the parent compound (**272**) (EC_{50} = 27 mg/mL) within assay error. Difluorinated analogues **274** and **277** were less toxic, requiring a 2-fold higher concentration to elicit apoptosis (EC_{50} = 47 and 58 mg/mL, respectively).



	R_1	R_2	R_3	R_4	γ-N pK_a
272	OH	H	H	H	10.4
273	OH	H	F	H	8.9
274	OH	H	F	F	7.4
275	OH	H	H	OH	9.4
276	H	OH	H	F	8.9
277	H	OH	F	F	7.4
278	H	OH	OH	H	9.4

Scheme 41. 3'',4'',3''',4'''-Tetradeoxy neomycin B analogues.

6. Conclusions

The present review provides an overview of the methods used for the incorporation of fluorine into AGs. It can be seen that the major synthetic challenge continues to be the somewhat cumbersome protecting-group strategies employed herein. The development of milder and more selective fluorinating agents, *e.g.*, PhenoFluor®, which allows the fluorination of unprotected complex molecules, will undoubtedly simplify this process in the future. The biological importance of the fluorination of AGs parallels the importance of the introduction of fluorine into biologically active molecules and remains an active field of research given the importance of AGs as antibiotics.

Additionally, current progress in the biosynthetic pathways of aminoglycosides [104-105] could furnish tools to access fluorinated aminoglycosides by designed mutasynthesis.

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