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Site-Selective Displacement of Tobramycin Hydroxyls for Preparation of Antimicrobial Cationic Amphiphiles

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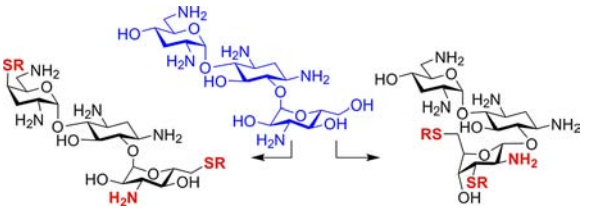
ABSTRACT



A short site-selective strategy for the activation and derivatization of alcohols of the clinically important aminoglycoside tobramycin is reported. The choice of amine protecting group affected the site-selective conversion of secondary alcohols of tobramycin into leaving groups. Temperature-dependent, chemoselective sequential nucleophilic displacements resulted in hetero- and homodithioether tobramycin-based cationic amphiphiles that demonstrated marked antimicrobial activity and impressive membrane selectivity.

Site-selective derivatization of biologically active natural products is one of the most frequently used strategies for the development of novel drugs.[1−3] This strategy is especially appealing for creation of analogues of commercially available aminoglycoside (AG) antibiotics, a large family of pseudo-oligosaccharides that contain multiple amines, and hydroxyls with similar chemical properties. AG antibiotics bind to the 16S rRNA, a component of the 30S small subunit of the bacterial ribosome and interfere with codon recognition during the translation process.[4] Unfortunately, clinical use of AGs is limited due to their toxic side effects and bacterial resistance.[4−6] As a result, the development of novel AGs with improved clinical properties has been pursued for several decades, yet semisynthetic modifications of natural AGs have led to the discovery of very few clinically relevant antibiotics.[7−9]

Previous studies demonstrated that in addition to their antimicrobial activity, AGs and their synthetic analogues have additional biological activities, further emphasizing the need for the development of methods for site-selective modifications in search of improved biological performance. Additional reported biological activities of AGs include the inhibition of several RNA-catalyzed processes, enhancement of eukaryotic ribosome read-through of premature stop-codon mutations, and the disruption of bacterial membranes.[10−16]

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We previously reported that attachment of a linear alkyl chain to the 6″ position of the AG tobramycin (**1**) results in cationic amphiphiles with potent and broad spectrum antimicrobial activity (Figure 1).[15,17] The semisynthetic route for the preparation of the cationic amphiphiles derived from **1** relied on the chemoselective conversion of the 6″-primary alcohol of this AG to a leaving group followed by a nucleophilic displacement with aliphatic chain primary thiols resulting in 6″-thioetherification of **1**.

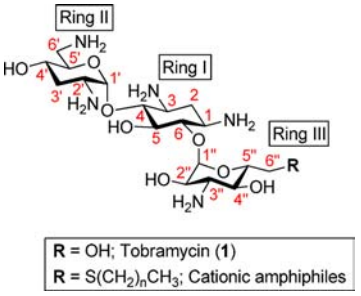

Figure 1. Structure of the clinically used AG antibiotic tobramycin and its 6″-thioether-based antimicrobial cationic amphiphiles.

In order to gain access to additional families of tobramycin-based cationic amphiphiles, we focused on the development of a site-selective method for conversion of one of the four secondary alcohols of **1** to a leaving group to facilitate the preparation of tobramycin derivatives through S_N2 nucleophilic displacement reactions. According to a previously reported procedure when penta-*N*H-Boc tobramycin derivative **2** (Scheme 1) was treated with 7 equiv of 2,4,6-triisopropylbenzenesulfonyl chloride (TIBS-Cl) in pyridine at ambient temperature, the corresponding 6″-*O*-TIBS derivative **3** (Scheme 1A) was formed in 67% isolated yield.[18] To convert one of the secondary alcohols of compound **2** to the *O*-TIBS group, we performed the reaction using an excess of TIBS-Cl (30 equiv) at ambient temperature. Under these conditions, in addition to compound **3** (31% isolated yield), the 4′, 6″-di-*O*-TIBS derivative **4** was isolated in 34% yield and the 2″, 6″-di-*O*-TIBS derivative **5** was isolated in 8% yield (Scheme 1A). The site-selectivity of the reaction was found to be dependent on the amine protecting groups. When the penta-azido-tobramycin derivative **6**[19] was treated with 15 equiv of TIBS-Cl for 18 h, 56% of the isolated products was the 6″-*O*-TIBS derivative **7**, 9% was the 4′, 6″-di-*O*-TIBS derivative **8**, and 27% was the 2″, 6″-di-*O*-TIBS derivative **9** (Scheme 1B).

The identity of the alcohols that were transformed into the corresponding *O*-TIBS leaving groups in each isolated product was confirmed by 1D-TOCSY NMR experiments. The mixtures containing the mono-6″-*O*-TIBS and di-*O*-TIBS products were readily separable by flash chromatography, and the reactions were reproducible at milligram to gram scales. As the 2″,6″-di-*O*-TIBS compound **5** was obtained only as the minor product (8% isolated yield) when **2** was used as the starting material, sufficient amounts of this compound were obtained in two synthetic steps from compound **9** in 80% yield for the two steps (Scheme 1B).

To explain the observed product distribution, our attention was drawn to a previous study that reported that NMR chemical shifts of protons *ipso* to azides of per-azide-protected AGs may be correlated to the relative electron density of the corresponding azide groups.[19] We observed a similar correlation for the protons *ipso* to the 2″ and 4′ secondary alcohols of compounds **2** and **6** (Table 1).

Table 1. Chemical Shifts of the *Ipso*-Protons of the Secondary Alcohols of Tobramycin Derivatives **2** and **6**[a]

compd	H-4′	H-2″	H-5	H-4″
2	3.43	3.69	3.61	3.37
6	3.63	3.46	3.40	3.37

[a] 400 or 500 MHz ^{1}H NMR (CD_3OD), chemical shifts in parts per million (ppm).

Of the 2″ and 4′ secondary alcohols, the one with the more upfield *ipso*-proton resonance preferentially reacted with TIBS-Cl (Table 1). The H-4′ of the Boc-protected **2** resonated at 3.43 ppm, whereas H-2″ resonated at 3.69 ppm. An opposite order of chemical shifts was observed in the case of the azide-protected **6**; H-4′ of **6** resonated at 3.63 ppm, whereas its H-2″ resonated at 3.46 ppm. These results suggest that the electron density, and therefore the nucleophilicity, of the 4′-alcohol was higher than that of the 2″-alcohol in the case of the *N*H-Boc protected compound **2**, whereas the opposite effect was observed in the case of the azide- protected **6**. Although the chemical shifts of the ipso-protons of the 2″ and 4′-alcohols of compounds **2** and **6** were correlated with their relative nucleophilicities, there was no correlation for alcohols at the 5 and 4″ positions of these compounds (Table 1). The fact that no 5-*O*-TIBS product was observed was attributed to the known high level of steric hindrance of the C-5 alcohol of tobramycin **1** rather than to an electronic effect. This alcohol is located between the C-4 and the C-6 positions that are substituted by sugar rings II and III, respectively (Figure 1). The utility of the steric hindrance of C-5 of **1** was previously demonstrated: when penta *N*H-Cbz-protected tobramycin was

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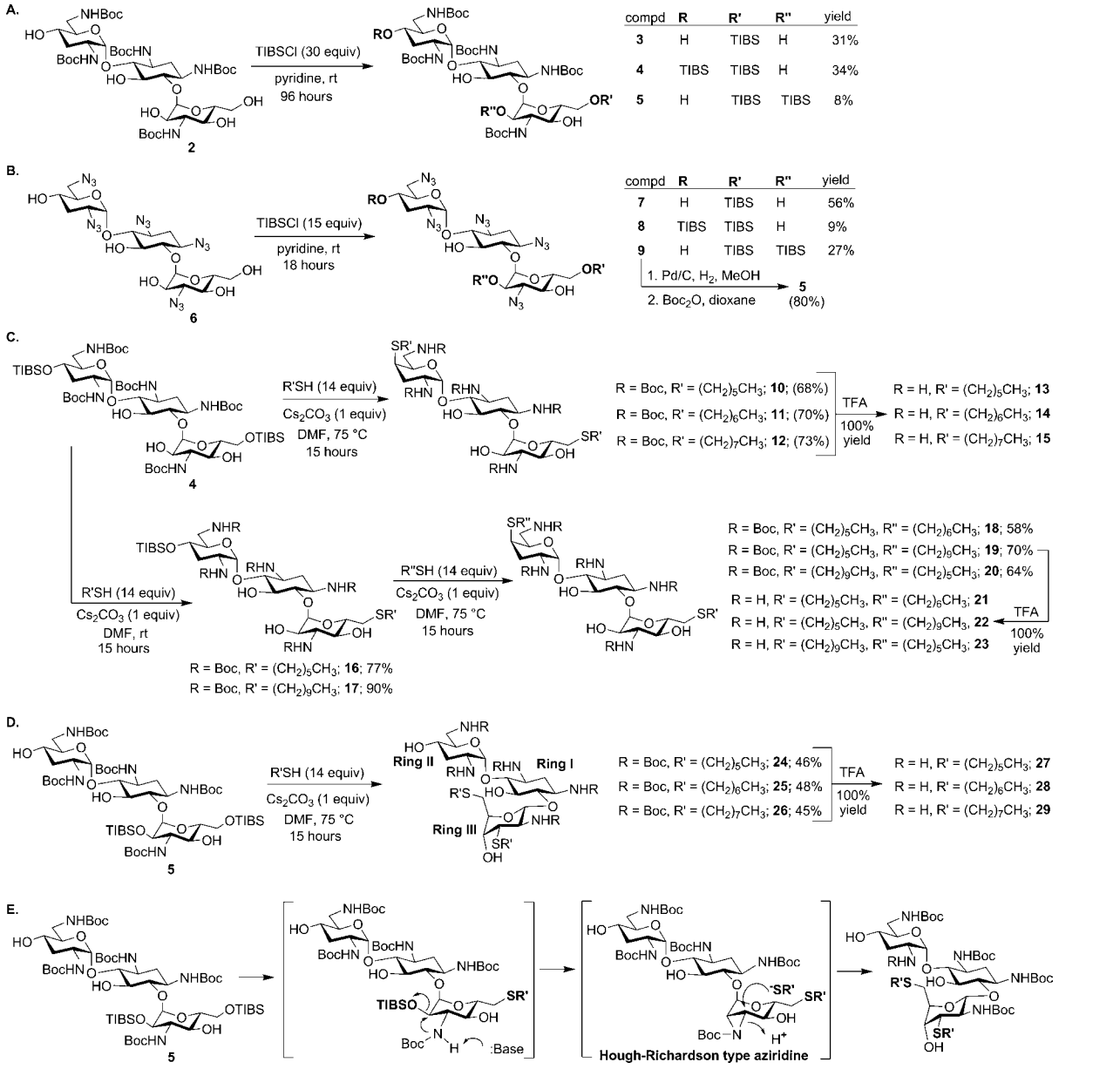
Scheme 1. Site-Selective Modification of Alcohols of Tobramycin Derivatives **2** and **6** and the synthesis of Homo- and Heterodithioether Tobramycin-Derived Antimicrobial Cationic Amphiphiles



reacted with an excess of acetic anhydride, only the C-5 alcohol was not acetylated.[20]

Finally, the C-4′ alcohol of ring II of **1** is less hindered than the ring III C-4″ alcohol as the former neighbors the C-3′ deoxy position (Figure 1). This fact may explain why 4′-*O*-TIBS and no 4″-*O*-TIBS products were isolated from the reactions with TIBS-Cl. The ^{1}H chemical shifts depend upon many factors; however, the observed differences in *ipso*-proton chemical shifts offer an explanation for the observed differences in product ratios between the 2″ and 4′-alcohols of compounds **2** and **6**.

Compounds **4** and **5** were used for the preparation of a set of homo- and heterodithioether−tobramycin-based cationic amphiphiles. Nucleophilic displacement of the two *O*-TIBS groups of **4** by *n*-alkyl-thiols at 75 °C in DMF resulted in a set of 4′,6″-homodithioether derivatives of tobramycin (**10**, **11**, and **12**, Scheme 1C) in 68−73% isolated yields. Acidic removal of the Boc groups gave the 4′,6″-dithioether tobramycin-derived cationic amphiphiles **13**−**15** as TFA salts in quantitative yields.

Selective thioetherification of the 6″-*O*-TIBS group of compound **4** was readily achieved at ambient temperature, and the 6″-thioether compounds **16** and **17** were generated (Scheme 1C). When **16** and **17** were reacted with *n*-alkyl-thiols, in DMF at 75 °C, the heterosubstituted 4′,6″-dithioether tobramycin derivatives **18**, **19**, and **20** were obtained; these compounds were treated with TFA to yield compounds **21**, **22**, and **23** in quantitative yields.

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Table 2. Minimal Inhibitory Concentration (MIC) and Hemolysis Values

	MIC values (μg/mL)									
bacteria[a]	1	13	14	15	21	22	23	27	28	29
A	16	16	4	4	4	4	4	32	32	8
B	>32	32	8	4	8	>32	>32	>32	>32	>32
C	0.3	4	2	4	2	2	4	8	16	4
D	>32	8	4	8	4	4	4	32	32	8
E	16	>32	>32	>32	8	4	4	>32	32	8
F	32	32	>32	32	32	>32	>32	8	16	4
G	>32	8	4	1	4	2	2	8	16	4
H	8	4	4	4	2	2	2	4	16	8
hemolysis[b]										
128 μg/mL	0	29.9 ± 1.6	94.9 ± 3.7	88.2 ± 3.2	15.8 ± 3.2	96.5 ± 2.5	100 ± 3.2	10.9 ± 1.0	45.4 ± 0.5	97.7 ± 2.1

[a] Bacterial strains: (A) *S. aureus Oxford*, NCTC 6571; (B) *MRSA*; (C) *S. epidermidis* ATCC 12228 (biofilm negative); (D) *S. epidermidis* ATCC 35984/RP62A (biofilm positive); (E) *S. aureus Cowan*, ATCC 12598; (F) *S. pyogenes* M1T1; (G) *S. pyogenes* JRS75; (H) *S. pyogenes* T5. [b] The results are expressed as percentage of hemoglobin released relative to the positive control (sample treated with Triton X100).

According to Richardson's rules, nucleophilic displacement of 2-aryl sulfonate in an α- pyranoside sugar ring such as the 2″-*O*-TIBS group of ring III of compound **5** is disfavored due to unfavorable alignment of dipoles in the transition state of the reaction.[21] It was not that surprising, therefore, that treatment of **5** with *n*-alkyl-thiols and Cs_2CO_3 in DMF resulted in compounds **24**, **25**, and **26** which, after deprotection, gave the final compounds **27**, **28**, and **29** (Scheme 1D). 500 MHz ^{1}H NMR analysis in D_2O revealed that in these three compounds C-3″ and C-6″ were substituted by thioether groups and C-2″ was substituted by an *N*H-Boc group or by an amine in the α-D-altro-configuration. This can be rationalized by an intramolecular nucleophilic attack of the C-3″ *N*H-Boc of **5** on its C-2″ *O*-TIBS group to form a Hough–Richardson-type aziridine (Scheme 1E).[22,23] The aziridine then underwent a thiolate-mediated ring-opening in Furst–Plattner fashion at C-3″ to yield the products with α-D-altro-configuration in accordance with Richardson's rules.[24]

The *N*H-Boc group at C-3″ of compound **5** was found to be essential for this reaction to proceed via the aziridine intermediate. When the 2″,6″-di-*O*-TIBS penta-azido tobramycin derivative **9** was treated with NaN_3 and crown-5 in DMF, the 2″-*O*-TIBS of **9** was not displaced even at 100 °C and only decomposition of this compound was observed. Ring III adopted a $^1C_4$ chair conformation in compounds **27**, **28**, and **29** as was indicated by ^{1}H NMR ($J_{1''-2''}$ = 8.0 Hz indicating trans diaxial relationships between H-1″ and H-2″). In a $^1C_4$ chair conformation, the C-1″, C-2″, and C-3″ hindered substituents are equatorial, therefore minimizing disfavored 1,3-diaxial interactions that would take place if ring III adopted a $^4C_1$ chair conformation.

The antimicrobial activity was tested on eight bacterial strains (Table 2).[25] Although none of the 3″,6″-dithioether compounds **27**–**29** were effective against the tested methicillin-resistant *Staphylococcus aureus* strain (strain **B**), the 4′,6″-dithioether compounds **14**, **15**, and **21** were potent against this pathogen. On the other hand, none of the 4′,6″-dithioether derivatives (**13**–**15** and **21**–**23**) had activity against *Streptococcus pyogenes* M1T1 (strain **F**); however, the 3″,6″-dithioether compounds **27**–**29** exhibited moderate to good activity against this pathogen. Of the nine tested antimicrobial cationic amphiphiles, compound **21** caused both low percentage of hemolysis (Table 2) and demonstrated potent antimicrobial activity against seven of the eight bacterial strains tested.

In conclusion, we report a method for site selective nucleophilic displacement of secondary alcohols of tobramycin. Site-selectivity was affected by the amine protecting groups used and facilitated the generation of homo- and heterodithioether tobramycin-derived cationic amphiphiles. By altering the position and length of the aliphatic thioether chains it was possible to develop potent antimicrobial cationic amphiphiles that exhibited impressive selectivity for bacterial compared to red blood cell membranes. The reported methodology can be further extended by using other nucleophiles.

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Supporting Information Available. Synthetic procedures and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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