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Synthesis and use of sulfonamide-, sulfoxide-, or sulfone-containing aminoglycoside–CoA bisubstrates as mechanistic probes for aminoglycoside *N*-6′-acetyltransferase

Feng Gao [†,‡], Xuxu Yan [‡], Omar Zahr, Aaron Larsen, Kenward Vong, Karine Auclair [*]

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montréal, Québec, Canada H3A 2K6

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ABSTRACT

Aminoglycoside–coenzyme A conjugates are challenging synthetic targets because of the wealth of functional groups and high polarity of the starting materials. We previously reported a one-pot synthesis of amide-linked aminoglycoside–CoA bisubstrates. These molecules are nanomolar inhibitors of aminoglycoside *N*-6′-acetyltransferase Ii (AAC(6′)-Ii), an important enzyme involved in bacterial resistance to aminoglycoside antibiotics. We report here the synthesis and biological activity of five new aminoglycoside–CoA bisubstrates containing sulfonamide, sulfoxide, or sulfone groups. Interestingly, the sulfonamide-linked bisubstrate, which was expected to best mimic the tetrahedral intermediate, does not show improved inhibition when compared with amide-linked bisubstrates. On the other hand, most of the sulfone- and sulfoxide-containing bisubstrates prepared are nanomolar inhibitors of AAC(6′)-Ii.

Aminoglycosides are broad spectrum antimicrobials.[1] Unfortunately, widespread resistance to aminoglycosides threatens the use of this important class of antibiotics, alone or in synergistic combination with β-lactams. Resistance to aminoglycosides occurs mostly via drug modifications by enzymes such as aminoglycoside *N*-6′-acetyltransferases (AAC(6′)s).[2–7] Wright and co-workers have shown that catalysis by AAC(6′)-Ii proceeds via an ordered bi–bi mechanism in which acetyl coenzyme A (AcCoA) binds before the aminoglycoside.[9] Attack of the aminoglycoside 6′-NH_2 at the thioester of AcCoA is believed to generate a tetrahedral intermediate, which subsequently collapses to yield a 6′-*N*-acetylaminoglycoside and CoA (Fig. 1).[8–10] Extensive mutagenic studies[9,10] and examination of the crystal structures of AAC(6′)-Ii[17,18] have not allowed the identification of residues that may stabilize the tetrahedral intermediate.

We recently reported the use of amide-linked aminoglycoside–CoA bisubstrate inhibitors as mechanistic and structural probes of (AAC(6′)s. An effective regio- and chemo-selective protocol for the direct *N*-6′-derivatization of unprotected aminoglycosides was used to synthesize these inhibitors in one-pot (Fig. 1, **1a** and **1b**).[11,12] These molecules exhibited nanomolar inhibition towards AAC(6′)-Ii and allowed crystallization of AAC(6′)-Ii in complex with an aminoglycoside derivative for the first time.[11]

To improve inhibition and to investigate whether AAC(6′)-Ii stabilizes the tetrahedral intermediate, we envisaged to prepare a second generation of bisubstrates containing either a sulfonamide, sulfoxide or sulfone, expected to better mimic the tetrahedral intermediate (Fig. 1, **2a, 2b, 3a**, **3b, 4a**, and **4b**). We hypothesized that if stabilization of this intermediate is important, a better mimetic would lead to increased affinity for the enzyme. Oxidized

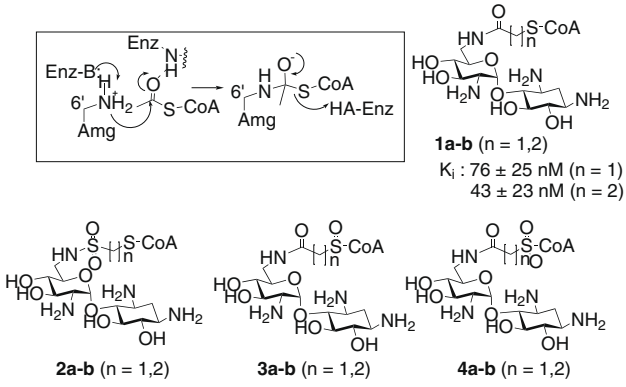

Figure 1. Proposed chemical steps catalyzed by AAC(6′)-Ii (top). Previously reported amide-linked aminoglycoside–CoA bisubstrate inhibitors (**1a** and **1b**) and bisubstrate inhibitors synthesized and tested here (**2a**, **2b**, **3a**, **3b**, **4a**, and **4b**).

* Corresponding author. Tel.: +1 514 398 2822; fax: +1 514 398 3797.
E-mail address: karine.auclair@mcgill.ca (K. Auclair).
† Currently at Albany Molecular Research Inc., 26 Corporate Circle, P.O. Box 15098, Albany, NY 12212, USA.
‡ These authors contributed equally to this work.

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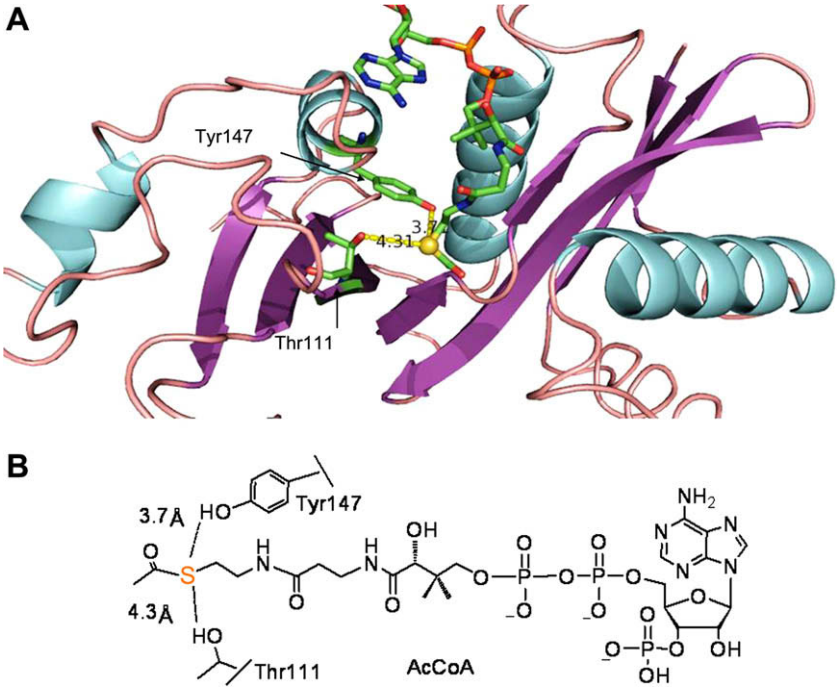


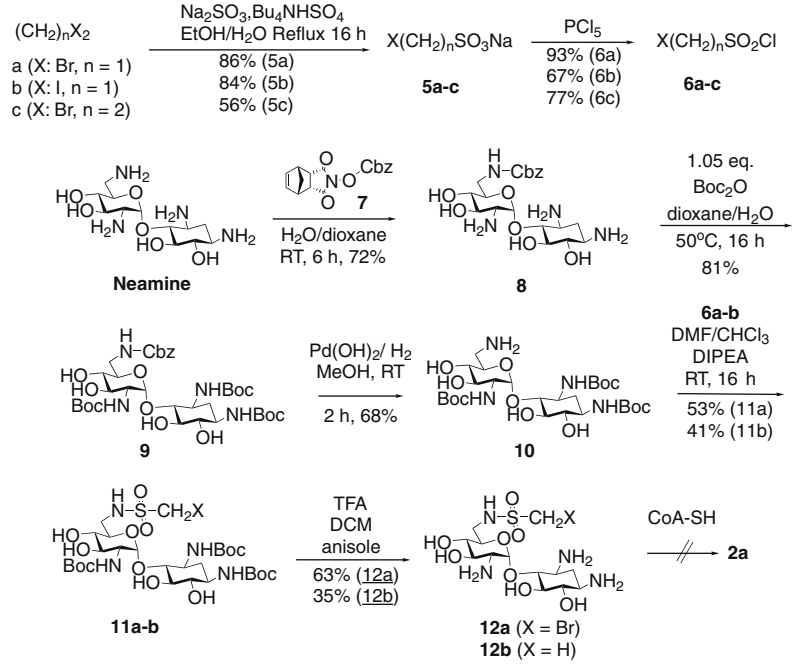
Figure 2. (A) Crystal structure of AAC(6′)-Ii in complex with AcCoA (1B87.pdb, figure produced using PyMol).[17] The protein is represented with cartoon. Tyr147, Thr111, and AcCoA are represented with line and colored by C (green), O (red), N (blue), P (purple), and the sulfur atom of AcCoA is represented as a yellow sphere. (B) Chemical structure of AcCoA and the distance between the sulfur and two amino acid residues.

sulfides were selected for their ease of preparation, the higher polarizability of the S=O bond compared to a carbonyl, and the tetrahedral geometry at the sulfur atom. Sulfonamides have previously been used to mimic the tetrahedral intermediates involved in enzymatic catalysis by proteases[13] arginase,[14] dihydroorotase,[15] and isoleucyl tRNA synthetase (K_i = 0.04 nM).[16]

The crystal structures of AAC(6′)-Ii in complex with AcCoA (Fig. 2),[17] CoA[18] or bisubstrate inhibitors[11] all reveal the presence of two hydroxyl groups, Tyr147-OH (3.71 Å away) and Thr111-OH (4.31 Å away), near the CoA sulfur atom. We reasoned that oxidization of the sulfur atom of bisubstrates **1a and 1b** into sulfoxides **3a and 3b** or sulfones **4a and 4b** may also increase the affinity for the enzyme by allowing two extra H-bonds between the oxygen of S=O and these two hydrogen donors.

We report here the synthesis of bisubstrates **2b**, **3a, 3b**, **4a, and 4b** and their effect on the activity of AAC(6′)-Ii.

Bromomethanesulfonyl chloride (**6a**) was used as the main building block for the assembly of **1a and 1b**. Compound **6a** was assembled by reacting sodium bromomethylsulfonate (**5a**) with PCl_5 as previously reported (Scheme 1).[19] Compound **5a** was

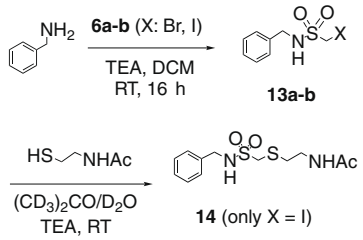


Scheme 1. First synthetic attempt to prepare bisubstrate analog **2a.**

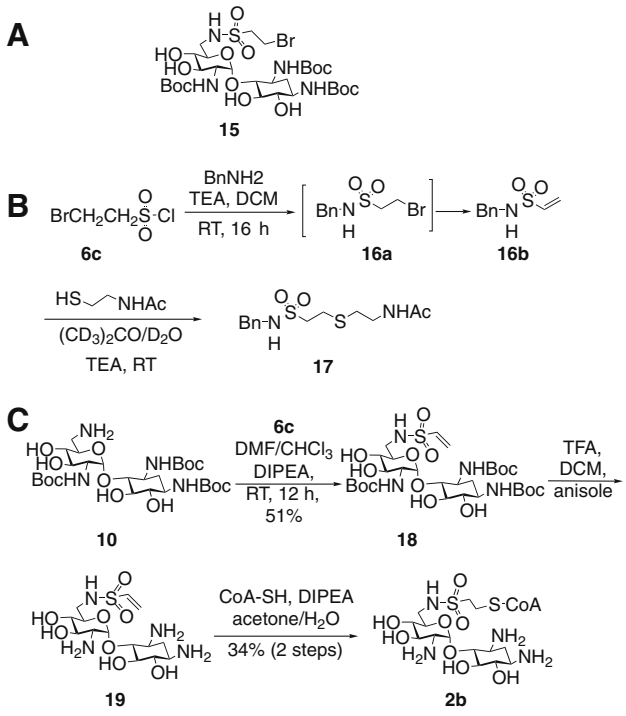
prepared using a reported procedure with some modifications.[20] Thus, sodium sulfite and dibromomethane were refluxed in a mixture of ethanol and water in the presence of a catalytic amount of tetrabutylammonium hydrogen sulfate (2 mol %), to afford crystalline product **5a**. 1,3,2′-tri-*N*-(*tert*-Butoxycarbonyl)neamine (**10**) was prepared using a known procedure.[21] *N*-Benzyloxycarbonyloxy-5-norbornene-*endo*-2,3-dicarboximide (**7**) was used to regioselectively protect the 6′-NH_2 of neamine and generate **8** in good yield. Treatment of **8** with di-*tert*-butyl dicarbonate (Boc anhydride) protected all the remaining amino groups of neamine to yield **9**, which was debenzylated to afford **10**. Compound **10** reacted with **6a** to afford **11a**, which was deprotected to give **12a**, *N*-6′-bromomethylsulfonyl neamine (Scheme 1).

Unfortunately, bromide **12a** did not react with CoA (shown as CoA-SH in synthetic schemes). We suspected that this lack of reactivity was due to the low electrophilicity of the bromomethylene carbon. To confirm this hypothesis, we carried out two model reactions (Scheme 2) to compare the reactivity of bromomethyl sulfonamide and iodomethanesulfonamide toward sulfhydryl nucleophile. Indeed, when *N*-benzyl bromomethanesulfonamide (Scheme 2, **13a**) was treated with *N*-acetylcysteamine (a surrogate for CoA) in an aqueous solvent for 2 days at RT, no product (**14**) was detectable. The iodomethanesulfonamide **13b**, however, reacted with *N*-acetylcysteamine to yield product **14** under the same conditions after 2 days at RT. Encouraged by this, we set out to prepare the corresponding iodide. A synthetic pathway similar to that described for **12a** was used. Surprisingly, removal of the Boc group followed by chromatography on silica gel led to reduction of the product to 6′-*N*-methanesulfonylneamine (**12b**). Reduction of the iodomethanesulfonamide was also observed in the model reaction but only as a minor product.[22]

Previous studies with amide-linked bisubstrates[11] show that linkers with $n = 2$ are slightly more potent than those with $n = 1$. We thus embarked on the synthesis of bisubstrate **2b** (Fig. 1). Retrosynthetic analysis suggests compound **15** (Scheme 3A) as a reasonable intermediate. Again, a model reaction was used to evaluate the feasibility of this synthetic approach (Scheme 3B). Not surprisingly, the β-bromosulfinamide **16a** easily eliminated to give a mixture of **16a** and the vinylsulfonamide **16b**. With 2.5 equivalents of base, **16b** was the only isolated product. Vinylsulfinamides are known to be susceptible to Michael addition by sulfhydryl groups.[23–26] To optimize the reaction conditions, vinylsulfinamide **16b** was reacted with *N*-acetyl cysteamine (Scheme 3B). In the presence of triethylamine (TEA), the reaction was complete within 30 min and afforded adduct **17**. Next, 2-bromoethanesulfonyl chloride (**6c**) was reacted with protected neamine **10** to yield the vinylsulfinamide **18** after spontaneous bromide elimination (Scheme 3C). Deprotection of **18** yielded **19** as the trifluoroacetate salt. Attempts to purify this intermediate on silica gel led to decomposition of the product. Crude **19** was therefore used directly in a reaction with CoA, which yielded bisubstrate **2b** in >35% yield after reverse phase HPLC purification (only part of the sample was purified).



Scheme 2. Model reaction for thiol attack at halomethanesulfonamides.



Scheme 3. Model reaction and synthesis of bisubstrate **2b**.

Bisubstrates **3a**, **3b** and **4a**, **4b** were next prepared by direct oxidation of the known sulfides **1a** and **1b**. Selective oxidation of sulfides to sulfoxides has interested chemists for many years.[27–35] Magnesium monoperoxyphthalate (MMPP) was reported to selectively oxidize glycosyl sulfides to sulfoxides[31,36] or sulfones[37] in good yields. Thus MMPP appeared as the most suitable oxidant for our purpose. Unfortunately, the oxidation of **1b** with MMPP was very slow. Half of the starting material remained unchanged after one hour at RT in the presence of 3 equivalents of MMPP. Furthermore, only the sulfoxide product was observed under these conditions. Longer reaction times led to decomposition of the reactants and product. *m*-Chloroperbenzoic acid (*m*-CPBA),[38–40] sodium periodate,[41–44] *tert*-butylhydroperoxide (TBHP),[34,45–47] Oxone,[34,48–51] *N*-sulfonyloxaziridine,[33] and dioxirane[50] have also been used for the selective oxidation of sulfides to sulfoxides.

Table 1
Screening of oxidants for the selective sulfide oxidation of **1b**

Oxidant[a]	Equiv.	Results[b]		
		Sulfide (%)	Sulfoxide (%)	Sulfone (%)
MMPP	3	>90	<10	0
*m*CPBA	3	60	40	0
TBHP	3	90	10	0
H_2O_2	3	60	40	0
H_2O_2·Urea	3	100	0	0
H_2O_2·Na_2CO_3	3		Decomposition	
H_2O_2·Na_2BO_2	3		Decomposition	
$NaIO_4$	3		Decomposition	
$NaIO_4$	1	50	50	0
$(NH_4)_2S_2O_8$	1	50	50	0
$(NH_4)_2S_2O_8$	2	0	100	0
Oxone[c]	1	0	60	40
Oxone	2	0	0	100

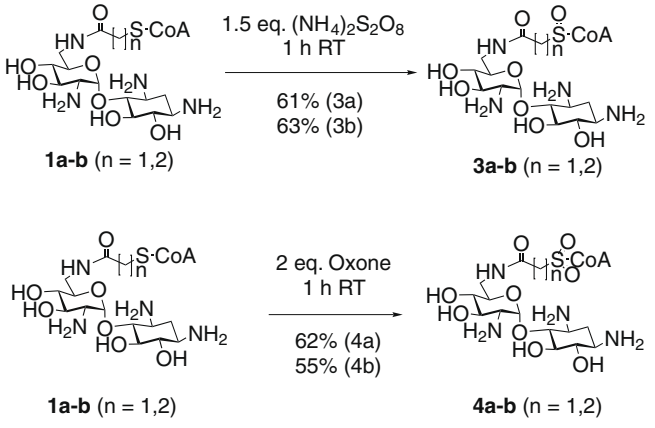
[a] Reaction time is 1 h at RT in water.
[b] Composition was normalized according to corresponding peak area from LC–MS.
[c] Potassium monopersulfate as in $2KHSO_5$·$KHSO_4$·K_2SO_4.

Except for *N*-sulfonyloxaziridines, all of these reagents, and more, were explored for the oxidation of **1b**. The results are summarized in Table 1.

Ammonium persulfate was the best oxidant for the selective oxidation of the sulfide **1a** and **1b** to the corresponding sulfoxides **3a** and **3b** (Scheme 4). The reactions were complete within 1 h when 2 equivalents of oxidant were used. To our knowledge, this is the first time that $(NH_4)_2SO_8$ is successfully used for the selective oxidation of a sulfide containing multiple functionalities, to a sulfoxide under aqueous conditions. Bisubstrates **3a** and **3b** were purified by reverse-phase HPLC. One of two possible diastereoisomers was major (>90%) and the minor isomer was discarded. No attempt was made to determine the absolute stereochemistry at the sulfur atom because of the prohibitive cost of CoA. As for the oxidation of the sulfides **1a** and **1b** to sulfones **4a** and **4b**, respectively, oxone appeared to be the most efficient oxidant (Scheme 4).

The bisubstrates **2b**, **3a**, **3b**, **4a**, and **4b** were tested for inhibition of AAC(6′)-Ii. The results are shown in Table 2. The large error reported for the K_i of **4a** can be explained by the hygroscopic nature of this compound, which decreased the accuracy of weight measurements. All compounds tested were potent competitive inhibitors with K_is ranging from low micromolar to nanomolar. Surprisingly, the bisubstrate with a sulfonamide linker (**2b**) showed a decreased inhibition compared to the corresponding amide-linked bisubstrate (**1b**). This result suggests either that the enzyme does not stabilize the tetrahedral intermediate or that **2b** is a poor mimic of the tetrahedral intermediate. The synthesis of phosphonate-linked bisubstrates is currently under way to verify these hypotheses.

In conclusion, we report here the synthesis of five new bisubstrates containing a sulfonamide linker, or an amide linker adjacent to sulfoxide or sulfone groups. Four of these bisubstrates were assembled in only two steps. We demonstrate for the first time the utility of $(NH_4)_2S_2O_8$ in the selective oxidation of highly functionalized sulfides to sulfoxides under aqueous conditions. Although sulfonamides are expected to better mimic tetrahedral intermediates than amides, sulfonamide-linked bisubstrate **2b** showed poorer inhibition of AAC(6′)-Ii than amide-linked inhibitor **1b**. This supports the hypothesis that AAC(6′)-Ii may catalyze the reaction mainly via proximity effects.[9,10]



Scheme 4. Optimized conditions for the syntheses of bisubstrates **3a**, **3b** and **4a**, **4b** via the selective oxidation of the sulfides **1a** and **1b**.

Table 2
AAC(6′)-Ii inhibition constants (K_i) for bisubstrates **2b**, **3a**, **3b**, **4a**, and **4b**

Inhibitor	**2b**	**3a**	**3b**	**4a**	**4b**
Type	Competitive	Competitive	Competitive	Competitive	Competitive
K_i (μM)	1.6 ± 0.6	0.06 ± 0.03	2.0 ± 0.7	0.27 ± 0.2	0.09 ± 0.06

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.004.

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