

Synthesis and SAR of 3,5-diamino-piperidine derivatives: Novel antibacterial translation inhibitors as aminoglycoside mimetics

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Abstract—Aminoglycoside antibiotics target an internal RNA loop within the bacterial ribosomal decoding site. Here, we describe the synthesis and SAR of novel 3,5-diamino-piperidine derivatives as aminoglycoside mimetics, and show they act as inhibitors of bacterial translation and growth.

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Bacterial resistance to antibiotics is on the rise and represents a global medical threat. In hospitals in the United States, approximately two million patients per year are infected.¹ The majority of these nosocomial pathogens are resistant to at least one antibiotic and result in about 90,000 deaths per year; a number that has increased 7-fold over the last decade. The recent and rapid spread of community acquired methicillin-resistant *Staphylococcus aureus* further highlights the threat of resistance development and illustrates the need for new antibiotics that work by novel mechanisms.² Given the broad genetic and physiological diversity of bacterial pathogens and the need for empiric therapies that cover a broad panel of organisms, it is not surprising that discovery of new antibiotics has advanced slowly. Central to antibiotic discovery is identifying broadly validated targets. One such proven target is the bacterial ribosome, which is the target for a significant number of clinically important antibiotics that bind at the ribosomal RNA (rRNA).³ Here, we expand on the description of

a novel series of antibacterial compounds that target rRNA and block bacterial translation and growth.⁴

Three-dimensional structures of different aminoglycosides bound to the decoding site, or A-site, within the 16S rRNA have been determined by X-ray crystallography.⁵ Importantly, these studies have shown that 2-deoxystreptamine (2-DOS), a conserved core scaffold among aminoglycosides, binds in a similar manner regardless of the 4,5- or 4,6-disubstitutions found in the neomycin or gentamicin families, respectively (Fig. 1).⁶ The *cis*-1,3-configured amino groups of 2-DOS are predominantly involved in base recognition

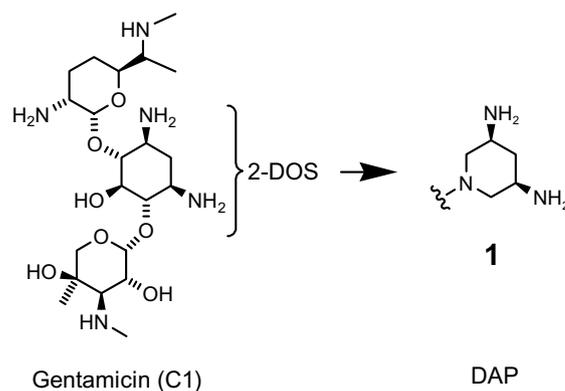


Figure 1. The aminoglycoside gentamicin and the DAP scaffold that mimics 2-DOS of the aminoglycoside.

Keywords: Aminoglycosides; Antibiotics; Translation inhibitors; 2-Deoxy-streptamine; Ribosome; Decoding site; 3,5-Diamino-piperidine.

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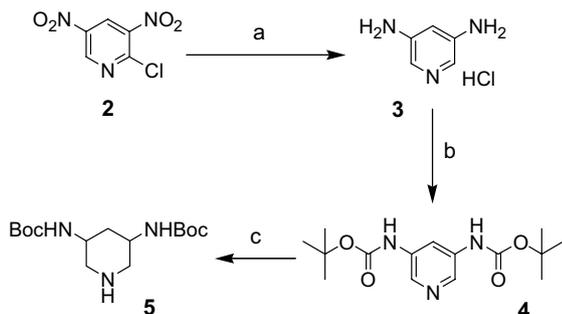
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by forming conserved hydrogen bonds with A1493, G1494, and U1495 of the 16S rRNA. These interactions anchor the aminoglycoside scaffold within the A-site internal loop and displace residues A1492 and A1493 from the RNA interior. These two adenine residues act as a molecular switch that is involved in securing the fidelity of translation by interacting with the first two bases of the mRNA–tRNA codon–anticodon hybrid. Thus, aminoglycoside binding impairs the ribosome's ability to discriminate against near-cognate tRNA–mRNA pairings, which ultimately causes cell death.

While 2-DOS is recognized as a key pharmacophore for binding rRNA, its synthesis and modification is challenging due to the five contiguous stereogenic centers.⁷ Therefore, we had developed synthetic mimetics of 2-DOS, one of which is *cis*-3,5-diamino-piperidine (DAP) (**1**) (Fig. 1).⁴ The DAP ring retains the characteristic *cis*-1,3-diamine configuration of 2-DOS that is important for RNA recognition. In contrast to aminoglycosides, the reduced chemical complexity renders the DAP series and derivatives amenable to rapid elaboration by parallel synthesis. Here, we focus on the synthesis and structure–activity relationships of DAP and other synthetic mimetics of 2-DOS.

Scheme 1 outlines the synthesis of the Boc-protected DAP (**5**). In this specific synthesis, the two NO₂ groups of 2-chloro-3,5-dinitropyridine (**2**) were hydrogenated at room temperature in the presence of 10% Pd/C to generate 3,5-diaminopyridine **3**. The amino groups were then protected as a di-Boc derivative **4** which was subsequently hydrogenated at 2200 psi using 5% Rh/C in the presence of acetic acid at 110 °C to yield the piperidine derivative **5**.^{8–10} Since aminoglycosides are relatively large molecules that engage in at least 15 contacts with rRNA,⁵ a single DAP scaffold would not allow high-affinity binding to rRNA and, hence, additional functionality was added. Guided by modeling studies and the availability of a straightforward synthetic route, DAP was linked to a triazine core which resulted in molecules (termed DAPT) that had a high affinity for A-site containing RNA oligonucleotides.⁴ The DAP scaffold and analogs (R¹ and R², termed 'headpiece') thereof were linked at the 2- and 6-positions of triazine (**6**) (Fig. 2). The so-called 'tailpiece' substituents (R³) were



Scheme 1. Reagents and conditions: (a) H₂, 10% Pd/C, rt; (b) Boc₂O, NaHCO₃, H₂O/MeOH/THF; (c) H₂, 2200 psi, 5% Rh/C, CH₃COOH, 110 °C.^{8–10}

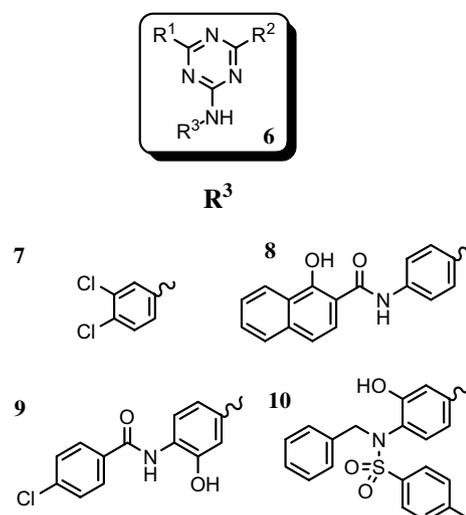


Figure 2. DAPT (**6**) and tailpiece (R³) structures.

attached at the triazine 4-position via an N-linkage. Representatives of R³ used in this study are shown in Figure 2.^{4,11}

Table 1 highlights the SAR of headpieces where R¹ and R² are identical resulting in a symmetric configuration. The symmetrical arrangement of two DAPs in compounds **11** and **12** resulted in potent translation inhibitors. Importantly, in the symmetric configuration, the acyclic headpiece (**13**) and a dimethylated DAP (**14**) retained activity (Table 1). Both of these headpieces allow the amino groups to be located in identical spatial orientation found both in DAP and 2-DOS and, hence, may account for similar hydrogen bonding with target rRNA. Although two amino groups in the five-membered ring headpiece of compound **15** have a slightly different spatial orientation than in DAP, **15** was nevertheless active. In contrast, when one of the amino groups in the headpiece is removed, or the spatial orientation is significantly altered, activity is abolished (compounds **16–18**). These results support our hypothesis that DAP can indeed substitute for the 2-DOS pharmacophore. Moreover, these findings show that the signature *cis*-3,5-diamino fragment of DAP, and by analogy the *cis*-1,3-diamino fragment of 2-DOS, can tolerate a variety of structure modifications as exemplified in compounds **11–15**.

Table 2 summarizes headpiece SAR with asymmetric configurations of R¹ and DAP (R²). For most headpiece configurations there are as many as three R³ substitutions on the triazine core (**6**), which allowed detailed insight into headpiece (R¹) SAR. Interestingly, a mono-DAP compound (**19**) (R¹ = H) has similar activity as di-DAP compounds (**12**, **20**, and **21**). Consistent with the results shown in Table 1, active headpieces in symmetric configuration (**13**, **14** and **15**) also resulted in active inhibitors when they were asymmetrically combined with DAP (**22–24**, **25**, and **29–31**) (Table 2). Conversely, a headpiece with symmetric configuration that resulted in poor activity (Table 1; **16**) was found to reduce activity when asymmetrically combined with DAP (**38–40**).

Table 1. Symmetric headpiece substitutions and protein synthesis inhibitory activity

Compound ^a	R ¹ and R ²	R ³	IC ₅₀ ^b
11		7	10
12		8	7
13		8	7
14		7	5
15		8	8
16		8	100
17		7	>1000
18		7	800
Kan			0.4
Tet			2.8

^a Kan, kanamycin; Tet, tetracycline.

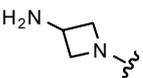
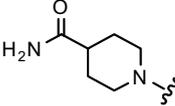
^b Coupled in vitro transcription–translation assay with *Escherichia coli* extracts (μM).⁴

In general, the replacement of an amino group with a hydroxy group on five- (32–34) or six-membered ring headpiece (38–40) slightly reduced activity compared to the di-amino substitutions (29–31 and 12, 20–21, respectively). Similarly, other mono-hydroxy substitutions exhibit moderate to weak activity (35–37, 41–43, and 44–46). An azetidin-3-ylamine substitution was tolerated (52–53), while a pyrrolidine-3,4-diamine substitutions (50–51) resulted in weak translation inhibitors. Di-hydroxy substitutions (47–49) were found to yield particularly weak inhibitors and appeared to destabilize the binding of the asymmetric DAP scaffold to its target. An acetamide substitution on the piperidine abolished activity (54–55). In summary, these results show that a symmetric substitution of two DAP moieties on the triazine core represents an optimal configuration for inhibitors of bacteria translation and growth.^{4,10} However, this work also shows that there is room to alter the headpiece configuration while retaining antibacterial

Table 2. Asymmetric (R² is DAP) headpiece substitutions

Compound ^a	R ¹	R ³	IC ₅₀	Ec ^b	Sa ^c
19	H	8	16	4	nd
12		8	10	1	2
20		9	7	2	16
21		10	3	4	8
22		8	10	4	8
23		9	18	16	32
24		10	1	16	8
25		8	8	2	8
26		8	8	2	4
27		9	11	8	16
28		10	1	16	8
29		8	10	4	2
30		9	12	8	32
31		10	13	32	2
32		8	19	4	8
33		9	13	16	32
34		10	15	16	8
35		8	10	8	16
36		9	19	16	32
37		10	21	16	8
38		8	17	2	8
39		9	40	8	64
40		10	17	16	16
41		8	310	16	16
42		9	40	64	32
43		10	44	16	8
44		8	21	4	16
45		9	31	32	32
46		10	27	32	16
47		8	72	16	32
48		9	77	32	64
49		10	43	32	32
50		9	58	16	16
51		10	36	32	16

Table 2 (continued)

Compound ^a	R ¹	R ³	IC ₅₀	Ec ^b	Sa ^c
52		9	14	8	nd
53		10	11	16	160
54		9	>1000	nd	nd
55		10	>1000	nd	nd
Cm			1.5	4	8

nd, not determined.

^a Cm, chloramphenicol.

^b *E. coli* ATCC 25922 minimum inhibitory concentration (μg/mL).⁴

^c *S. aureus* ATCC 25923 (MIC).

activity, which may be used to optimize other pharmaceutical properties such as bioavailability.

Summary and perspective. This paper focused on headpiece optimization on our novel DAPT translation inhibitors. The active DAP pharmacophore was derived from structural information of the molecular recognition between the conserved 2-DOS scaffold of aminoglycosides and the A-site RNA.^{5,6} This work expands on our efforts to rationally design 2-DOS mimetics, including the aminoazepane and novel acyclic deoxystreptamine scaffolds.¹¹ After the initial design of DAPT, the series then evolved through an iterative process of parallel synthesis and testing that led to promising lead compounds. Medicinal chemistry elaboration can now be used to generate a potential clinical candidate that may improve on the pharmaceutical properties of aminoglycosides, including persisting resistance, poor bioavailability, and toxicity.

In a future communication we will describe SAR around protein binding and the effect of serum on antibacterial potency. In the context of this study, a particularly intriguing observation was found with compound **22** in that the *Escherichia coli* MIC improved in the presence of serum protein. This synergistic activity with serum was titratable and reached a peak reduction in MIC of 16-fold. Separately, particular core and tailpiece substitutions were found to reduce the serum MIC shift. A striking feature of the DAPT series is their potent antimicrobial activity against *Pseudomonas aeruginosa*, a pathogen for which there is a unmet clinical need to develop new antibiotics.² In one study, 53 clinical isolates of *P. aeruginosa* were tested against a DAPT compound (compound **1a** in Ref. 4) and commercial antibiotics. The MIC₉₀ of the DAPT compound was 8 μg/mL, while the seven reference antibiotics that included amikacin, imipenem, ciprofloxacin, ceftazidime, and tazobactam/piperacillin all had higher MIC₉₀ values. Based on these and other findings we are particularly interested in developing the DAPT compounds as novel anti-*P. aeruginosa* agents.

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- (a) Synthesis of 3,5-diaminopyridine hydrochloride (**3**): 2-chloro-3,5-dinitro-pyridine (27.36 g, **2**) was divided into two equal portions and added into two 2 L round-bottomed flasks. Methanol (700 mL) was then added to each flask. Nitrogen gas was bubbled through the solution while stirring and 7.15 g of 10% palladium on carbon was divided into two equal portions and added to each flask portion-wise while under nitrogen. The solutions were cooled in an ice-water bath under N₂. Hydrogen balloons then replaced the N₂ inlets. After evacuation, the solutions were exposed to H₂ gas at slightly positive pressure. This vacuum-hydrogen exchange was done at least five times. The reaction mixtures were then stirred under slight positive pressure of H₂ for 2 days. Then the solutions were filtered through Celite under reduced pressure to remove the catalyst. The filtrate was concentrated in vacuo. The crude product (24.27 g, 100% yield) was a dark green solid and was immediately used in the next step without purification. Product **3** was confirmed by LC-MS.
(b) Synthesis of di-Boc-protected 3,5-diamino-pyridine (**4**): Compound **3** (19.56 g, 0.13 mol) was dissolved in 150 mL of methanol in a 2 L reaction vessel. Nitrogen was used to purge the flask and the reaction solution was cooled to 0 °C. A solution of 58.67 g (0.27 mol) of di-*tert*-butyldicarbonate in 85 mL of methanol, was added dropwise. 18.9 mL of triethylamine (0.13 mol) was diluted with 20 mL of methanol and added dropwise. Then another 58.67 g of di-*tert*-butyldicarbonate dissolved in 85 mL of methanol was added dropwise. After that another 29.34 g of di-*tert*-butyldicarbonate, dissolved in 40 mL of

methanol was added dropwise. The reaction mixture was warmed to rt and stirred overnight. TLC analysis showed that no starting material and very little mono-Boc-protected diaminopyridine remained. The methanol was removed in vacuo. The resulting solid was dissolved in ethyl acetate and washed with brine. The aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried with MgSO₄ and passed through a layer of silica gel (135 cm × 3 cm). The silica was thoroughly washed with ethyl acetate. The ethyl acetate solution was concentrated in vacuo and the resulting solid was dissolved in a minimal amount of refluxing methylene chloride and then cooled to rt. The crystallized solid was filtered under reduced pressure and washed with a small amount of CH₂Cl₂ and hexane, and dried in a vacuum oven at 50–60 °C. The first three batches of crystals weighed a total of 26.99 g, and a fourth batch of crystals (from methanol) weighed 3.59 g, yielding a total of 30.58 g of product **4** (73.6% yield). ¹H NMR (CDCl₃): δ 8.22 (d, 2H, *J* = 2.4 Hz), 8.16 (t, 1H, *J* = 2.4 Hz), 6.66 (s, 2H, NH), 1.54 (s, 18H, Boc).

(c) Synthesis of (3*R*, 5*S*)-3,5-bis-(*tert*-butoxycarbonylamino)-piperidine (**5**): Compound **4** (10 g, 32.34 mmol) was dissolved in 350 mL of methanol. Glacial acetic acid (1.85 mL, 32.34 mmol) was added, followed by 5% Rh-C (wet, 6.66 g, 5 mol%), purged with N₂, and transferred to a high-pressure hydrogenator. The vessel was flushed twice with H₂ gas at 500 psi. The H₂ pressure was then increased to 1000 psi, and the vessel was heated to 110 °C. The H₂ pressure was subsequently increased to 2200 psi and the mixture was stirred for 28 h, followed

by cooling to rt. The catalyst was filtered through Celite under reduced pressure. The filtrate was concentrated in vacuo to remove methanol. Na₂CO₃ (3.43 g, 32.34 mmol) in 100 mL H₂O was added followed by 100 mL EtOAc. After stirring for 90 min, a thick white precipitate formed. The mixture was filtered under reduced pressure and the solid was washed with EtOAc and dried in a vacuum oven overnight at 60 °C. The desired product **5** (5.6 g) was obtained as an off-white solid whose identity was confirmed by LC–MS and ¹H NMR. MS *m/e* 316 [M+1]⁺ (exact MS: 315). ¹H NMR (DMSO-*d*₆): 6.70 (d, 2H, *J* = 7.2 Hz, 2NHCO), 3.16–3.28 (m, 2H, H_{3,5ax}), 2.76–2.84 (m, 2H, H_{2,6eq}), 2.17 (br, 1H, NH), 1.99 (t, 2H, *J* = 11.6 Hz, H_{2,6ax}), 1.84–1.90 (m, 1H, H_{4eq}), 1.37 (s, 18H, Boc), 1.10 (q, 1H, *J* = 11.6 Hz, H_{4ax}). HPLC has a single peak. Melting point: 214.6 °C (decompose). The above process produces the *cis*-isomer in high purity.

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