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Structure-based design, synthesis and A-site rRNA co-crystal complexes of novel amphiphilic aminoglycoside antibiotics with new binding modes: A synergistic hydrophobic effect against resistant bacteria

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ABSTRACT

Incorporation of an hydrophobic (phenethylamino)ethyl ether at C2″ of N1-(HABA)-3′,4′-dideoxyparomomycin led to a novel analog with an excellent antibacterial profile against a host of resistant bacteria.

Introduction

The inevitable development of acquired resistance to antibiotic substances through a variety of mechanisms has been a challenging obstacle in long term human therapy.[1] Over time, bacteria are able to become resistant to the action of antibiotics, mainly through mutation, enzymatic attack at critical sites and reduced permeability. In spite of their potent activity as bactericidal compounds, the widespread use of aminoglycosides as chemotherapeutic agents has been curtailed by the development of resistance.[2] Thus, the well known naturally occurring aminoglycosides in the 4,5 and 4,6-disubstituted 2-deoxystreptamine series, such as paromomycin (**1**) (Fig. 1) or neomycin (**2**) and kanamycin (**9**) respectively are not first choice therapies, in spite of their excellent antibacterial activities in vitro against a variety of Gram-positive and Gram-negative microorganisms. Two of the more successful aminoglycosides, namely the gentamicin C complex (**8**) and tobramycin (**10**) on the other hand, are used as intravenous and inhaler formulations, respectively.[3] A key structural feature in these naturally occurring antibiotics is the absence of a C3′ and/or C4′ hydroxyl groups which are sites for enzymatic deactivation by *O*-phosphotransferases and *O*-nucleotidyltransferases.[4] With this knowledge, much effort was devoted to the chemical modification of aminoglycosides such as kanamycin (**9**) with the objective of expanding its antibacterial activity to include a larger panel of resistant bacteria. These efforts culminated with the synthesis of 3′,4′-dideoxykanamycin and related aminoglycosides with promising antibacterial profiles.[5] In this context, it should be mentioned that Nature has also been a guiding force with the discovery of butirosin (**6**), a 4,5-disubsituted 2-deoxystreptamine pseudo-trisaccharide containing the N1-(2*S*)-2-hydroxy-4-aminobutyric amide (HABA) moiety, although its further development was not pursued.[6]

A major breakthrough came with the development of amikacin (**11**), a derivative of kanamycin in which the N1 amino group of deoxystreptamine was acylated with a HABA side-chain.[7] Indeed, amikacin was found to exhibit activity against certain non-resistant strains of *Pseudomonas aeruginosa*. A logical extension toward more effective clinical candidates was the development of arbekacin (**12**),[8] in which the beneficial effects of the 3′,4′-dideoxy ring I and the N1-HABA moiety were combined. No new aminoglycoside-type antibiotic has been introduced in the market during the past two decades. The mode of action of aminoglycosides as inhibitors of protein biosynthesis at the ribosomal level has been extensively studied. Seminal contributions have recently provided insights into the structure of the ribosome, and binding sites of aminoglycoside antibiotics at the A site of the 30S subunit.[9] These studies have instigated a renewed interest in the chemistry of aminoglycosides. A large number of chemically modified analogs has been reported

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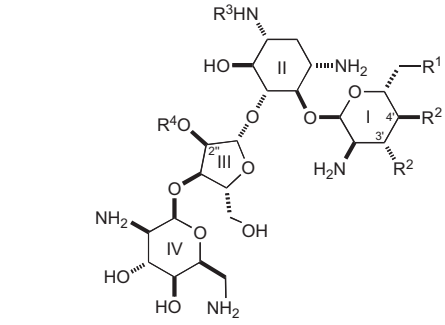


R^1 = OH, R^2 = OH, R^3 = H, R^4 = H, paromomycin (**1**)

R^1 = NH_2, R^2 = OH, R^3 = H, R^4 = H, neomycin B (**2**)

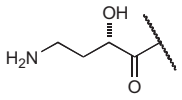
R^1 = OH, R^2 = H, R^3 = H, R^4 = H (**3**)

R^1 = OH, R^2 = OH, R^3= HABA, R^4 = H (**4**)

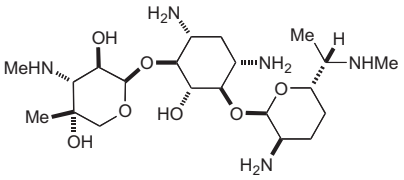
R^1 = NH_2, R^2 = OH, R^3 = HABA, R^4 = H (**5**)

R^1 = NH_2, R^2 = OH, R^3 = HABA, Ring IV= H, butirosin (**6**)

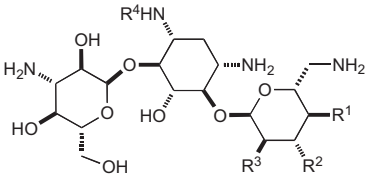
R^1 = OH, R^2 = OH, R^3 = H, R^4 = $(CH_2)_2N(CH_2)_2Ph$ (**7**)



(*S*)-2-hydroxy-4-aminobutyric acid or amide (HABA)



gentamicin complex (C_1 shown) (**8**)



R^1, R^2 = OH, R^3 = OH, R^4 =H, kanamycin A (**9**)

R^1 = OH, R^2 = H, R^3 = NH_2, R^4 = H, tobramycin (**10**)

R^1, R^2 = OH, R^3 = OH, R^4 = HABA, amikacin (**11**)

R^1, R^2 = H, R^3 = NH_2 R^4 = HABA, arbekacin (**12**)

Figure 1. Representative members of the aminoglycoside family.

with promising results, although few new insights were gained with regard to improved antibacterial profiles or diminished enzymatic resistance.

In a previous letter, we revealed a new paradigm in the design and synthesis of functionally diverse analogs of paromomycin.[10] It was found that placing hydrophobic end-groups on aminoethyl alkyl ethers at the C2″ hydroxyl group in the β-ribofuranosyl (ring III) unit of paromomycin, as in **7**, maintained the antibacterial activity in a panel of bacterial strains. Furthermore, X-ray crystal structures of complexes of some analogs with an A-site RNA model fragment revealed a new mode of binding for rings III and IV. While the precise reasons for in vitro antibacterial activity of such a modified analog of paromomycin cannot be delineated at this time, it is clear that the incorporation of a C2″ ether chain with a hydrophobic or a heteroaromatic end-group has a potentially beneficial effect. Based on these promising results,[10,11] and interesting X-ray structural data, we focused our attention on exploiting this new paradigm with other analogs in conjunction with the presence of an N1-HABA moiety. Such HABA derivatives of paromomycin **4** and neomycin **5** were reported in the literature, along with the 3′,4′-dideoxygenated version of paromomycin several years ago.[12,13] We decided to combine these features into new analogs of paromomycin. Guided by preliminary antibacterial testing,[14] we also incorporated the preferred 2″-(phenethylamino)ethyl ether in selected derivatives. These studies have led to a potent new analog with unprecedented in vitro activities against a panel of resistant bacteria in the paromomycin series. Our design paradigm was also substantiated by X-ray co-crystal structures of these modified analogs with the A-site rRNA fragment.

Chemistry

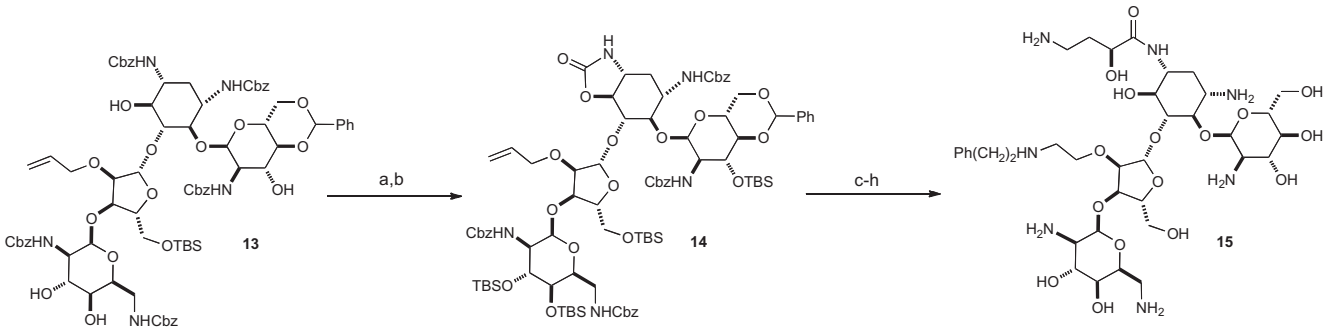
We first addressed the synthesis of an analog of N1-HABA paromomycin harboring a hydrophobic ether group at C2″ (Scheme 1). The mono TBS ether was subjected to a chemoselective *O*-allylation as previously described to give the key intermediate **13**,[10,14] and the product was converted to the cyclic carbamate **14** after silylation to the penta-*O*-TBS ether. Cleavage of the carbamate, introduction of the HABA group, followed by ozonolysis of the allylic double bond, reductive amination and global deprotection led to the analog **15**.

We then proceeded with the synthesis of a ring A-modified analog (Scheme 2). The known intermediate **16**,[13] was *O*-silylated, then subjected to a regioselective *O*-allylation to give **17**. At this juncture, it was necessary to protect the remaining hydroxyl groups as benzoate esters. Oxidative cleavage of the allylic double bond, followed by a reductive amination with phenethylamine, then global deprotection, gave 2″-*O*-(phenethylamino)ethyl 3′,4′-dideoxy paromomycin (**18**).

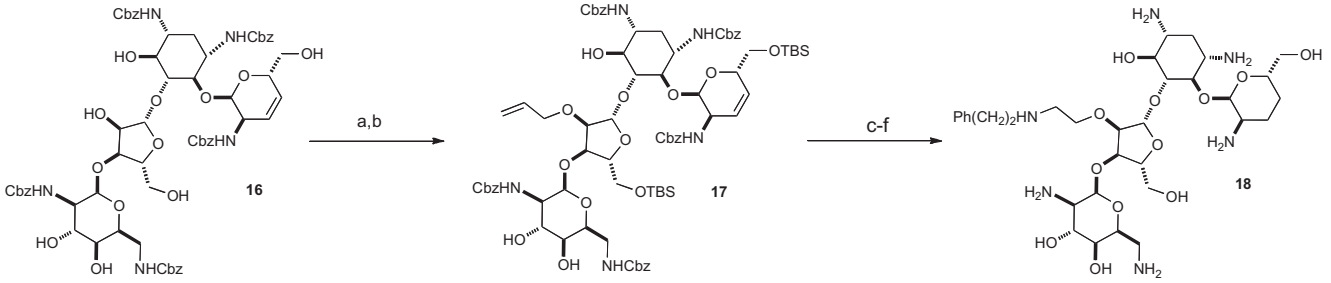
We concluded this series with the synthesis of an analog that contained the N1-HABA moiety and the 2″-*O*-(phenethylamino)ethyl ether groups within the framework of 3′,4′-dideoxy paromomycin (Scheme 3). The known intermediate **16**,[13] was treated with Wilkinson's catalyst to selectively reduce the double bond in the presence of the *N*-Cbz groups. Regioselective *O*-allylation at C2″ followed by *O*-silylation and treatment with NaH, led to the cyclic carbamate which was cleaved to give **19**. Introduction of the HABA side chain followed by ozonolysis, reductive amination, desilylation and finally hydrogenolysis afforded the analog **20**.[15]

Antibacterial activity

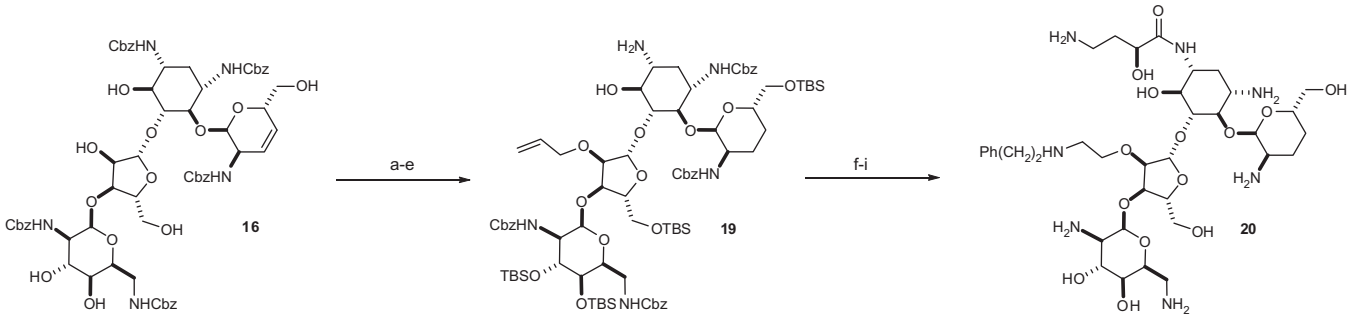
The main objective of this study was to assess the importance, if any, of combining the N1-HABA paromomycin with a hydrophobic appendage at C2″, known to modify the binding mode of paromomycin to the A-site of the rRNA subunit.[10,14] To this end, we selected from compounds with progressive substitution patterns as shown in Table 1. These were first tested against wild type strains of *Escherichia coli* (ATCC#25922) and *Staphylococcus aureus* (ATCC#13709) with paromomycin (**1**) and neomycin (**2**) as controls. We were not surprised that the incorporation of an N1-HABA moiety in paromomycin and neomycin did not significantly alter the intrinsic activities as reflected by MIC values (Table 1, entries



Scheme 1. Reagents and conditions: (a) TBSOTf, 2,4,6-collidine, CH_2Cl_2, 35%; (b) NaH, DMF, 47% (38% SM recovered); (c) LiOH aq, 63% (36% SM recovered); (d) *N*-Cbz-Haba-OSu, Et_3N, THF, 70%; (e) (i) O_3, (ii) PPh_3, 67%; (f) RNH_2, $NaBH_3CN$, MeOH, AcOH; (g) HF, pyridine, 61% (three steps); (h) AcOH:H_2O (4:1), (ii) $Pd(OH)_2/C$, H_2, 91%.



Scheme 2. Reagents and conditions: (a) TBSCl, imidazole, CH_2Cl_2, 68%; (b) allyl iodide, KHMDS, THF, 70%; (c) BzCl, DMAP, pyridine, 82%; (d) O_3, (ii) PPh_3, 62%; (e) (i) phenethylamine, $NaBH_3CN$, AcOH, MeOH, (ii) NaOMe, MeOH, 70%; (f) (i) AcOH/H_2O (4:1), (ii) $Pd(OH)_2/C$, H_2, 92%.



Scheme 3. Reagents and conditions: (a) Wilkinson's cat., EtOH/THF, 80%; (b) TBSCl, imidazole, CH_2Cl_2, 70%; (c) allyl iodide, KHMDS, THF, 68%; (d) TBSOTf, 2,4,6-collidine, CH_2Cl_2, 77%; (e) NaH, DMF, 49% (26% SM recovered); (f) LiOH aq, 88%; (g) *N*-Cbz-Haba-OSu, Et_3N, THF, 75%; (h) (i) O_3, (ii) Me_2S; (i) phenethylamine, $NaBH_3CN$, AcOH; (j) HF, pyridine, 30% (three steps); (k) $Pd(OH)_2/C$, H_2, AcOH/H_2O (4:1), quant.

Table 1
Activities of paromomycin and neomycin analogs

Entry	Compounds	MIC *E. coli* (μg/mL)	MIC *S. aureus* (μg/mL)
1	**1**	3–6	1–2
2	**2**	3–6	1–2
3	**3**	20–40	5–10
4	**4**	5–10	1.3–2.5
5	**5**	2.5–5	2.5–5.0
6	**7**	3–6	0.3–0.6
7	**15**	0.6–1.3	0.6–1.3
8	**18**	40	1–3
9	**20**	2.5–5.0	0.6–1.3

1–5). In a previous SAR study,[14] we had determined that, with few exceptions, a vast array of modifications at the C2″ position were well tolerated against *S. aureus* (ATCC#13079). Smaller, aliphatic and polar functionalities seem to be fare better against *E. coli* (ATCC#25922). One of the outstanding modifications in this series proved to be the known 2″-*O*-(phenethylamino)ethyl paromomycin (**7**) (Table 1 entry 6). It was interesting to observe that the activity of N1-HABA 2″-*O*-(phenethylamino)ethyl paromomycin (**15**) was significantly improved against *E. coli* and *S. aureus* (Table 1, entry 7). Although, a weakening of inhibitory activity was observed with 3′,4′-dideoxy paromomycin (Table 1, entry 3), it was expected that introduction of the C2″-*O*-(phenethylamino)ethyl group as in **18** would restore this loss (Table 1, entry 8). However, only modest improvement was observed against *S. aureus*. In contrast, the combination of an N1-HABA and 2″-*O*-(phenethylamino)ethyl group in the 3′,4′-dideoxygenated framework of paromomycin as in **20**, produced a potent antibiotic (Table 1, entry 9).

We have obtained a co-crystal structure of **18** (Fig. 2) with an A-site RNA model fragment, that shows a similar binding mode as the one previously reported for **7** and **20**.[10,11] The C2″ ether analog **18** is characterized by the absence of the hydroxyl groups at

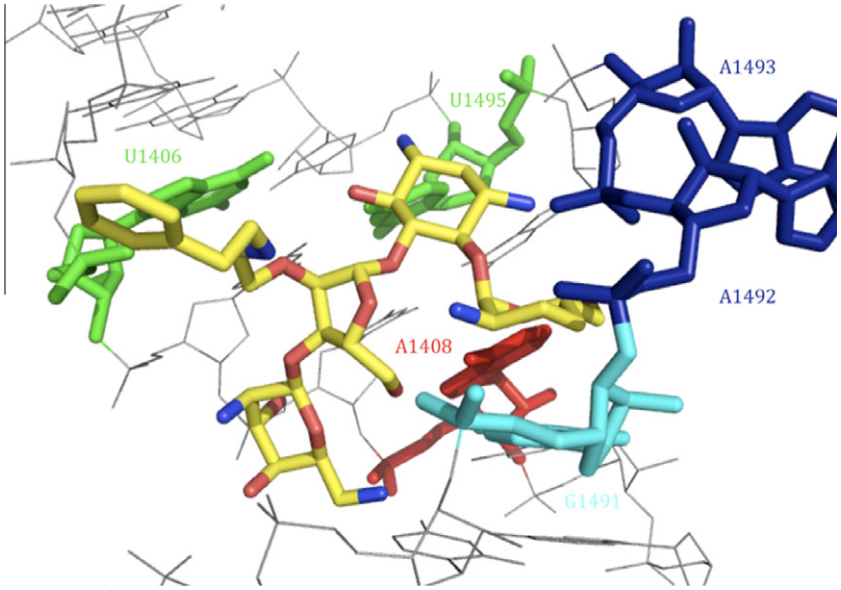


Figure 2. X-ray complex of compound (**18**) with A-site RNA. The U1406°U1495 base pair, the universally conserved A1408 residue, the G1491 residue and two bulged out adenines, A1492 and A1493, are colored in green, red, cyan, and blue, respectively.

positions 3′ and 4′ on ring I, as well as the inclusion of a hydrophobic ether appendage at C2″. The loss of direct contacts to O2P of A1492 and A1493 was partially compensated by a network of water molecules bridging N2′ of ring I and the anionic phosphate oxygens. A direct contact between N1 of ring II and O4 of U1495 observed in paromomycin complex was missing, but a new direct contact between O6 of ring II and O4 of U1406 was observed.[15]

In the co-crystal with amikacin, the HABA group forms two specific H-bonds with two stacked alternating G═C pairs beyond the conserved U°U pair, thereby anchoring firmly the aminoglycoside on the 3′ strand of the A site.[16] Although the HABA group does not improve the affinity, its incorporation together with the dideoxy modifications on ring I may compensate for the lack of contacts between the hydroxyl groups at position 3′ and 4′ of ring I which occur also on the 3′ strand of the model A-site. Thus, the energetically disfavored dideoxy modifications on ring I, appears compensated by the contacts of the HABA group two base pairs away.

Having obtained encouraging results regarding the synergistic effect of the C2″-*O*-(phenethylamino)ethyl group with an N1-HABA moiety in paromomycin, we wished to ascertain if this could be extended to bacteria having acquired resistance against a host of clinically used antibiotics such as vancomycin, oxymycin, levofloxacin, ciprofloxacin and ceftazidime (Table 2). The inclusion of a 2″-*O*-(phenethylamino)ethyl group in N1-HABA paromomycin (**15**) only modestly improved activity compared to amikacin, ceftazidime or

Table 2
Selected compounds against a panel of resistant bacteria

Organism* (ID[a])	ceft. or linz.[d] (μg/ml)	**4** (μg/mL)	**8** (μg/mL)	**11** (μg/mL)	**15** (μg/mL)	**20** (μg/mL)
E. coli (25922[b])	0.25	8	1	2	2	8
E. coli (1269687[c])	0.12	8	0.5	1	1	1
E. coli (1269640)$^{Vs,\ Cs,\ Gs}$	0.12	16	4	8	4	8
E. coli (1269620)$^{Vr,\ Cr,Gr}$	>32	8	64	2	1	4
E. coli (1269621)$^{Vr,\ Cr,Gr}$	>32	8	64	2	2	4
E. coli (1269652)$^{Vr,\ Cr,Gr}$	32	32	>128	64	8	16
E. coli (1269653)$^{Vr,\ Cr,Gr}$	16	8	>128	8	2	8
S. aureus (292213[b])	2	4	0.5	2	0.25	>0.12
S. aureus (1269615)$^{Vr,\ Cr,Gr}$	1	>128	32	16	64	0.25
S. aureus (1269616)$^{Ir,\ Cr,Gr}$	1	>128	64	16	128	0.5
S. aureus (1269617)$^{Vr,\ Cr,Gr}$	2	>128	32	8	32	0.25
S. aureus (1269618)$^{Ir,\ Cr\ ,Gr}$	1	>128	64	16	128	0.5
S. aureus (9269619)$^{Or,\ Cr\ ,Gr,Vs}$	>16	>128	128	16	>128	1
S. aureus (1269669)^{Os}	2	4	0.25	2	<0.12	0.25
S. aureus (1269670)^{Os}	2	4	0.25	1	<0.12	0.25
S. capitis (1269682)	1	0.5	0.12	0.5	<0.12	<0.12
S. epidermidis (1269663)	1	1	<0.12	0.8	<0.12	<0.12
S. epidermidis (1269675)$^{Or,\ Gr,\ Lr}$	4	64	32	8	16	<0.12
S. epidermidis (1269676)$^{Or,\ Gr,\ Lr}$	1	0.5	32	4	<0.12	<0.12
S. epidermidis (1269677)$^{Or,\ Gr}$	0.05	16	16	4	<0.12	<0.12
S. epidermidis (1269680)$^{Or,\ Gr,\ Lr}$	1	1	64	8	<0.12	<0.12
S. warneri (1269686)	1	0.25	<0.12	<0.25	<0.12	<0.12

* Resistance profile: Vr or Vs—vancomycin resistant (VRSA) or vancomycin sensitive, Gr or Gs—gentamicin resistant or sensitive, Cr or Cs—ciprofloxacin resistant or sensitive, Ir—vancomycin intermediate (VISA), Or or os-oxymycin resistant or sensitive, Lr or Ls—levofloxacin resistant or sensitive, Er or Es—ceftazidime resistant or sensitive.

[a] Study ID Number of Focus Technologies®, unless otherwise indicated.

[b] ATCC Number.

[c] *E. coli* 0157:H07.

[d] Ceftazimide in the case of *E. coli*, and linezolide in the case of *Staphylococcus*.

gentamicin against some strains. However, the combination of N1-(HABA), and 2″-*O*-(phenethylamino)ethyl groups in 3′,4′-dideoxy paromomycin (**20**), led to a remarkably improved inhibitory activity across the spectrum of resistant organisms, including VRSA and VISA strains (Table 2).

Based on our original results of the first antibacterial amphiphilic aminoglycosides represented by **7** and related analogs,[10,14] there is ample opportunity to further explore the synergistic effect of other 2″-*O*-ethers in combinations with N1-HABA derivatives of paromomycin and its chemically modified variants. In conclusion, the knowledge gained from our X-ray studies showing a new mode of binding of ring III and IV in paromomycin to rRNA fragments, has allowed us to further explore the potential of amphiphilic aminoglycosides such as **20** with much improved profiles especially against resistant bacteria. Studies involving newer modifications toward more effective and clinically tolerated aminoglycosides will be reported in due course.[17]

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.2010.09.084.

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