Aminoglycoside Mimetics

Design and Synthesis of Paromomycin-Related Heterocycle-Substituted Aminoglycoside Mimetics Based on a Mass Spectrometry RNA-Binding Assay**

Yili Ding, Steven A. Hofstadler, Eric E. Swayze, Lisa Risen, and Richard H. Griffey*

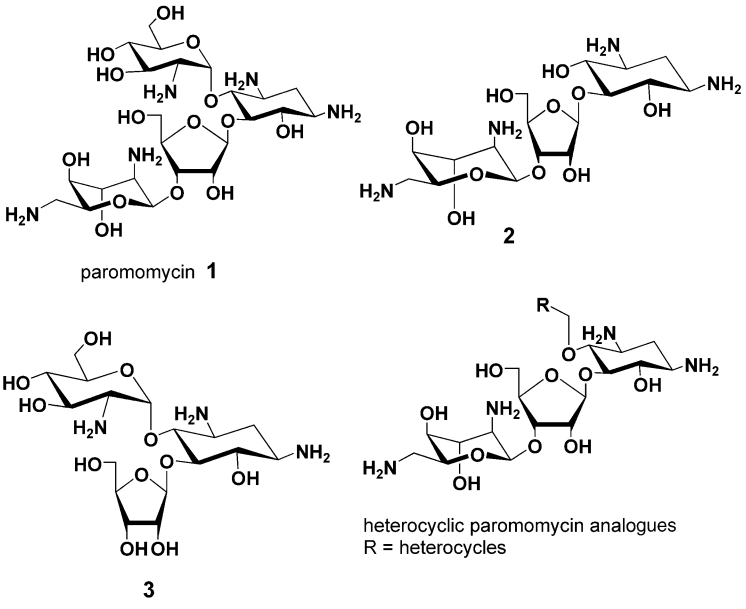
The family of aminoglycosides, which includes neomycin, paromomycin (**1**), lividomycin, kanamycin, and gentamicin, is a very potent group of bactericidal compounds that bind to the RNA of the small ribosomal subunit.[1] This bactericidal action is mediated by binding of the compound to the bacterial RNA in a way that leads to misreading of the genetic code.[2] The decoding region of the 16S ribosomal RNA contains a smaller subdomain (16S) that folds and retains the key structural features of the full-length RNA.[3] This "A-site" subdomain binds aminoglycosides at the same location as the intact rRNA.

ESIMS was used to determine solution-phase dissociation constants of RNA–ligand complexes based on gas-phase measurements of the ratio of free and bound RNA target. ESIMS was also used to determine the location of ligand binding on the RNA and to determine the specificity for 16S and 18S rRNA.[4,5] Paromomycin exhibited an excellent binding affinity and specificity for 16S rRNA in this assay.[6] Therefore, we used MS to carry out structure–activity relationship (SAR) studies to determine how the structure of the aminoglycosides affects their binding activities. Based on these results, paromomycin mimetics were designed and synthesized as new bactericidal compounds.

It has been estimated that over a half of all therapeutic agents consist of heterocyclic compounds. The heterocyclic ring system in many cases comprises the very core of the active moiety or pharmacophore. Therefore, heterocycle-substituted aminoglycoside mimetics may be ideal therapeutic agents.

To determine which rings of paromomycin are important in its 16S rRNA-binding activity, paromomycin (**1**), 5-(β-D-neobisamine)-2-deoxystreptamine (**2**), and 6′-hydroxyribostamycin (**3**) were used as standards[7] to compare their binding affinities in an MS-based RNA-binding assay. In the ESIMS RNA-binding assay, compound **2** exhibited a better binding activity than **3**, which indicated that the D ring of paromomycin may be more important than its A ring for the 16S rRNA-binding activity and specificity.[8] Based on this observation, paromomycin derivatives in which the A ring was replaced with a range of heterocycles were chosen as synthetic targets (Scheme 1).

Owing to the complex nature of the target compounds, it is impractical to prepare them individually for biological activity screening.[9] However, by using MS to study the SAR, we could first screen the 4-heterocycle-substituted 2-deoxystreptamine derivatives, determine which motif has better binding properties, and then design and prepare the target



Scheme 1. Structures of paromomycin (**1**), 5-(β-D-neobisamine)-2-deoxystreptamine (**2**), 6′-hydroxyribostamycin (**3**), and the 4-heterocycle-substituted substituted paromomycin mimetics.

[*] Dr. Y. Ding
Ribapharm
3300 Hyland Avenue, Costa Mesa, CA 92626 (USA)
Fax: (+1) 714-641-7222
E-mail: yding@icnpharm.com
Dr. S. A. Hofstadler, Dr. E. E. Swayze, L. Risen, Dr. R. H. Griffey
Ibis Therapeutics, Isis Pharmaceuticals, Inc.
2292 Faraday Avenue, Carlsbad, CA 92008-7208 (USA)

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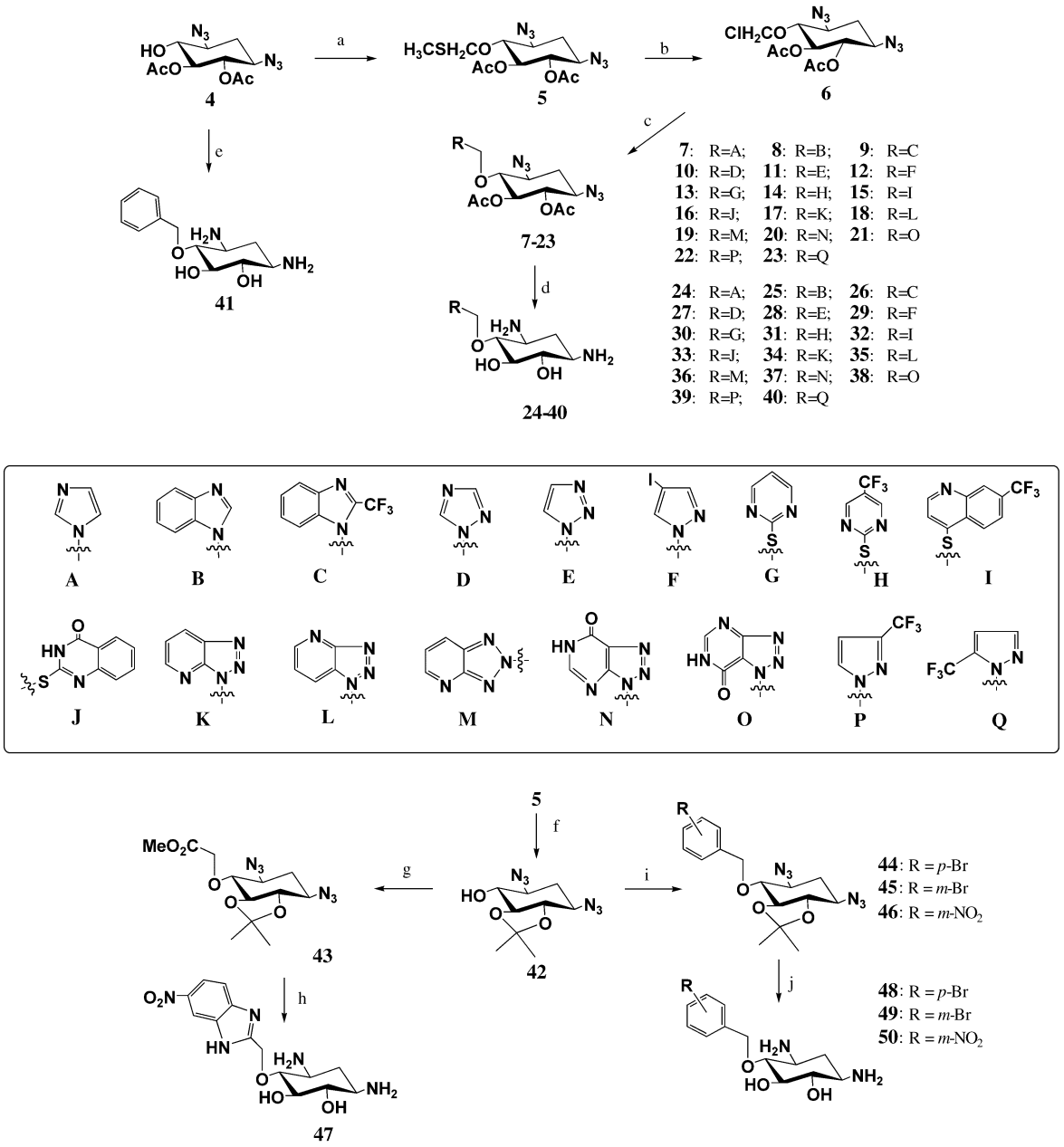
compounds. A concise synthetic strategy for these derivatives is summarized in Scheme 2. The hydroxy group of 5,6-di-*O*-acetylated diazido-2-deoxystreptamine (**4**), which was obtained from the selective enzymatic deacetylation of tri-*O*-acetyl diazido-2-deoxystreptamine,[10] was protected as an MTM ether through a Pummerer rearrangement[11] to give compound **5**. Treatment of **5** with excess SO_2Cl_2 in CH_2Cl_2 gave **6**, which was used as the core starting material for the parallel synthesis.

Compound **6** was coupled with different nucleophilic reagents to afford the corresponding 4-heterocycle-substituted 2-deoxystreptamine derivatives **7**–**23** in satisfactory



Scheme 2. Synthesis of 2-deoxystreptamine derivatives with heterocyclic substituents at C4: a) DMSO (30 equiv), Ac_2O (15 equiv), AcOH (5 equiv), 24 h, 78%; b) SO_2Cl_2 (6 equiv), CH_2Cl_2, room temperature, 30 min, 100%; c) **A**H–**Q**H (1.5 equiv), NaH (1.7 equiv), CH_3CN/DMF, room temperature, 2 h; then **6** (1 equiv), 76–97%; d) 1) NaOMe (0.1 equiv), MeOH, room temperature, 4 h; 2) Me_3P/THF (8 equiv), H_2O/THF (1:1), 10 h, 56–75%; e) 1) benzyl 2,2,2-trichloroacetimidate (4 equiv), TfOH (2 equiv), CH_2Cl_2, room temperature, 5 h; 2) NaOM/MeOH (0.1 M), 4 h; 3) Me_3P/THF (8 equiv), H_2O, room temperature, 10 h, 62%; f) 1) NaOMe/MeOH (0.5 M), 10 h; 2) 2,2-dimethoxypropane, TsOH, room temperature, 10 h; 3) K_2CO_3 (5 equiv), MeI (5 equiv), CH_3COCH_3/H_2O (5:1), 75 °C, 15 h, 23%; g) $BrCH_2CO_2Me$ (2.0 equiv), NaH (2.2 equiv), CH_3CN, room temperature, 4 h, 75%; h) 1) CH_3CO_2H/H_2O (80%), 75 °C, 3 h; 2) NaOH/MeOH/H_2O, room temperature, 5 h; 3) 4-nitro-1,2-phenylenediamine (4 equiv), HCl/H_2O (4 N), 95 °C, 4 h; 4) Me_3P/THF (8 equiv), H_2O/THF (1:1), 10 h, 39%; i) NaH (1.3 equiv), RBnBr (1.2 equiv), room temperature, 4 h, 75–85%; j) 1) CH_3CO_2H/H_2O (80%), 75 °C, 3 h; 2) Me_3P/THF (8 equiv), H_2O/THF, room temperature, 10 h, 55–65%. DMF = *N*,*N*-dimethylformamide, Tf = trifluoromethanesulfonyl, Ts = *p*-toluenesulfonic acid.

yields.[12] Deprotection and reduction of **7**–**23** gave the derivatives **24**–**40**, respectively. Direct alkylation of **4** with benzyl 2,2,2-trichloroacetimidate followed by deprotection gave the 4-benzyl-2-deoxystreptamine derivative **41**. Deacetylation of compound **5** with methanolic sodium methoxide was followed by protection of the free hydroxy groups as an isopropylidene. Subsequent cleavage of the MTM ether group afforded the product **42**. Alkylation of compound **42** with $BrCH_2CO_2Me$, *o*-BrBnBr, *p*-BrBnBr, and *o*-NO_2BnBr under basic conditions yielded 4-alkylated 2-deoxystreptamine derivatives **43**–**46**. Treatment of **43** with NaOMe/MeOH/H_2O followed by condensation with 3-nitrophenylenediamine (Philips reaction)[13] and subsequent deprotection gave the 4-benzimidazolyl-2-deoxystreptamine derivative **47**. Deprotection of **44**–**46** provided derivatives **48**–**50**, respectively.

The ESIMS RNA-binding assay was then used to evaluate the binding affinities of compounds **24**–**40** and **47**–**50** for a 27-mer RNA representing the 16S A site. The compounds were screened against the 16S A site at equal concentrations in separate experiments. [(7-Trifluoromethyl)-4-quinolinyl]sulfanyl-2-deoxystreptamine (**32**) showed better binding affinity (68 μM) than other 4-heterocycle-substituted 2-deoxystreptamine derivatives.[14]

Based on this result, the paromomycin mimetic in which the A ring is replaced with heterocycle **I** was selected as the target. Its concise synthesis is summarized in Scheme 3. Treatment of **15** with methanolic sodium methoxide gave the product **51**. The glycosylation donor **52** was obtained from the acidic hydrolysis of neomycin B in three steps,[7] which is a more simple and effective method than the total synthesis approach. The glycosylation reaction between **51** and C,D-ring donor **52** was performed in CH_2Cl_2 in the presence of TMSOTf. After deacetylation, the desired product **53** was isolated as a minor product and **54** was isolated as the major product. Reduction of the azido groups of **53** with Me_3P/NaOH/THF/H_2O gave the final target **55**. By using a similar method, **54** was converted into **56**.[15]

The ESIMS RNA-binding assay was used to evaluate the binding activity of **55**. This aminoglycoside mimetic exhibited good RNA-binding activity ($K_d < 1$ μM).[16] Its K_d value is higher than that of paromomycin (110 nM), but lower than those of apramycin (2 μM), bekanamycin (2 μM), and tobramycin (2 μM).[4–6]

Compound **55** was tested in a coupled bacterial transcription/translation assay in which the ability of a compound to inhibit either the transcription of a DNA template into mRNA or the subsequent translation of this mRNA into functional luciferase protein is evaluated. Compound **55** inhibited this coupled assay with an IC_{50} of 2 μM, and was shown to have a minimum inhibitory concentration (MIC) of 3 μM against a gram-negative *E. coli* strain (ATCC 25922).

In summary, we used MS to study SAR and were able to design heterocyclic aminoglycoside mimetics more efficiently. A concise synthesis route was used to prepare heterocyclic paromomycin mimetics from neomycin B. The use of this strategy to synthesize more heterocyclic aminoglycoside mimetics will be reported in due course.

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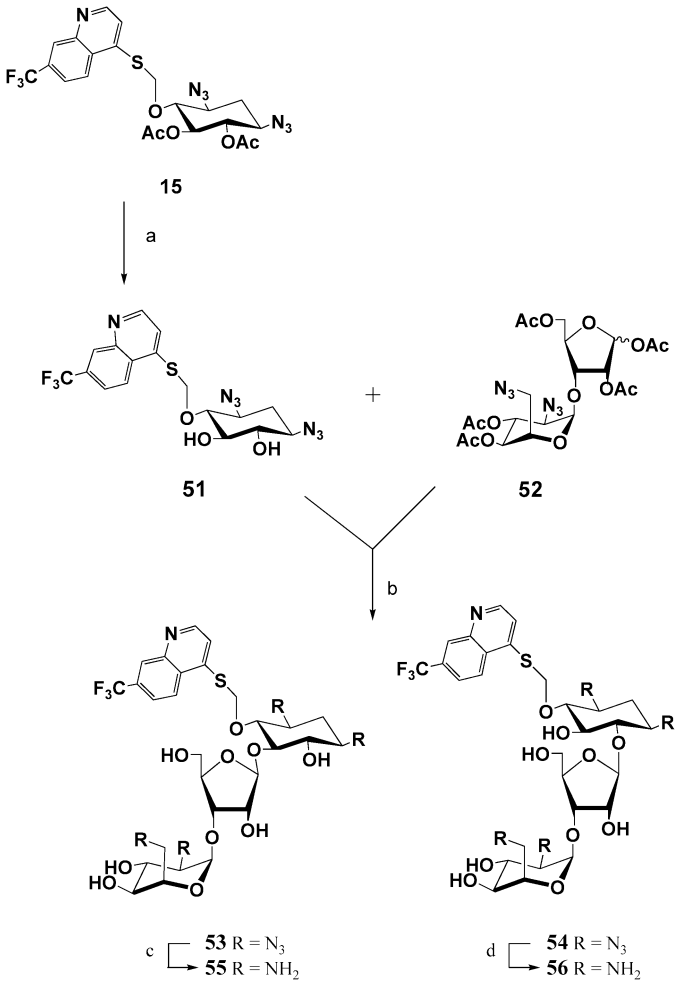

Scheme 3. Synthesis of **55** and **56**: a) NaOMe/MeOH (0.5 M), room temperature, 10 h, 85%; b) 1) **51** (1 equiv), **52** (2 equiv), molecular sieves (4 Å), TMSOTf (1 equiv), 4 h; 2) NaOMe/MeOH (0.5 M), 2 h, 10% (**53**), 31% (**54**); c) Me_3P/THF (8 equiv), THF/H_2O (2:1), 10 h, 46%; d) Me_3P/THF (8 equiv), THF/H_2O (2:1), 10 h, 52%. TMS = trimethylsilyl.

Keywords: aminoglycosides · antibiotics · heterocycles · mass spectrometry · synthesis design

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[14] The MS RNA-binding data were obtained from the assay described in reference [4], and neamine was used as the standard compound. Some of the MS RNA-binding data were reported in reference [12].
[15] Selected data: **53**: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.69$ (d, 1H, $J = 12.4$ Hz), 5.55 (d, 1H, $J = 12.4$ Hz), 5.16 (d, 1H, $J = 2.0$ Hz), 5.12 ppm (d, 1H, $J = 5.2$ Hz); **54**: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.82$ (d, 1H, $J = 12.4$ Hz), 5.49 (d, 1H, $J = 12.4$ Hz), 5.37 (d, 1H, $J = 2.0$ Hz), 5.04 ppm (d, 1H, $J = 1.6$ Hz); **55**. ^{1}H NMR (400 MHz, D_2O): $\delta = 5.67$ (d, 1H, $J = 12.4$ Hz), 5.45 (d, 1H, $J = 10.8$ Hz), 5.16 (s, 1H), 5.07 ppm (s, 1H); **56**: ^{1}H NMR (400 MHz, D_2O): $\delta = 5.51$ (d, 1H, $J = 12.0$ Hz), 5.31 (d, 1H, $J = 12.4$ Hz), 5.20 (s, 1H), 4.72 ppm (d, 1H, $J = 7.6$ Hz).
[16] The MS RNA-binding data were obtained from the assay described in reference [4], and paromomycin was used as the standard compound.

