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# Identification of a novel non-carbohydrate molecule that binds to the ribosomal A-site RNA

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**Abstract**—We report the identification of a novel compound that binds to the *Escherichia coli* 16S ribosomal A-site. Binding by the compound was observed using nuclear magnetic resonance and mass spectrometry techniques. We show that the compound binds in the same position in the A-site RNA as occupied by the aminoglycoside class of antibiotics.


The aminoglycoside antibiotics such as ribostamycin, neomycin B, paromomycin, tobramycin, gentamycin, and kanamycin A are the most commonly used broad-spectrum anti-infective agents because of their high efficacy and low cost.[1] In addition to being bactericidal, aminoglycosides have predictable pharmacokinetics and often act synergistically with other antibiotics. However, these agents have the potential for harmful side effects that include deafness (ototoxicity) and kidney toxicity (nephrotoxicity).[2–4] Finally, aminoglycoside antibiotics are also prone to the development of bacterial resistance due to the action of bacterial aminoglycoside modifying enzymes.[5]

Aminoglycoside antibiotics act by binding to the aminoacyl-acceptor site (A-site) in helix 44 of the 16S ribosomal (Fig. 1) RNA and interfering with the fidelity of mRNA translation. Protein miscoding and truncation results, with this ultimately leading to bacterial cell death.[6] Chemical footprinting studies of *E. coli* rRNA has shown that neomycin, paromomycin, gentamicin, and kanamycin protect bases A1408 and G1494 in the A-site.[7] The interaction between aminoglycosides and the A-site RNA has been extensively studied by both nuclear magnetic resonance (NMR) and X-ray

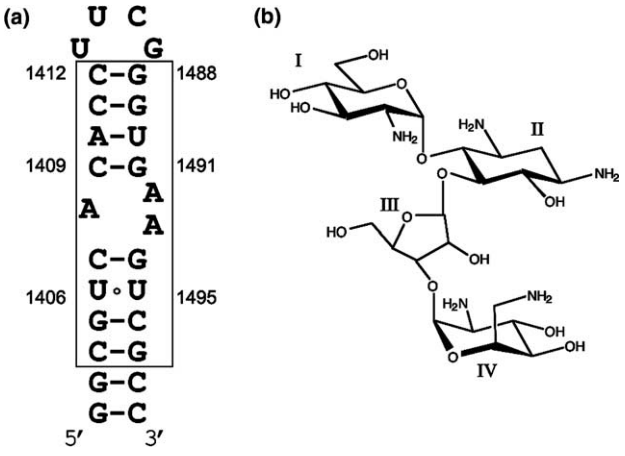


**Figure 1.** (a) Sequence and secondary structure of the *E. coli* ribosomal A-site RNA model used in this study. Nucleotides not boxed correspond to residues not present in the A-site but added for this study. The numbering scheme corresponds to that of the *E. coli* rRNA sequence. (b) Structure of the aminoglycoside paromomycin. The rings of paromomycin are numbered with Roman numerals.

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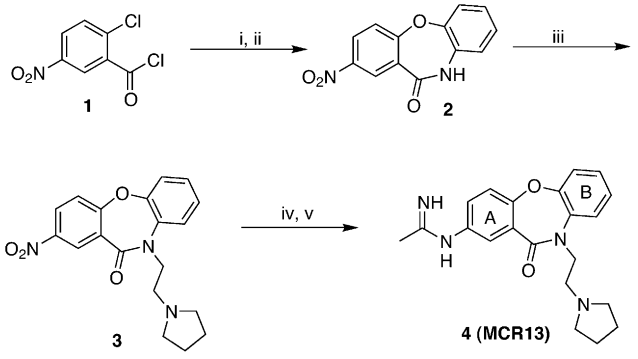
crystallographic methods using short RNA models containing the A-site nucleotides[8–13] and by a crystal structures of the intact 30S ribosome bound to paromomycin.[14] Additionally, NMR methods have been used as a screening method to discover new compounds that bind to the A-site RNA.[15] Both crystallography and NMR spectroscopy have been used to probe the interaction between RNA and a variety of non-aminoglycoside A-site binding molecules.[16,17]

Together these high-resolution structures provide a wealth of information about the interaction between aminoglycosides and their A-site RNA target. At the A-site the RNA backbone is distorted by the bulged nucleotides A1492 and A1493 in the paromomycin-A-site complexes. This has the effect of widening the major groove and providing a distinct binding pocket for aminoglycosides. The major interactions between the aminoglycosides and the binding pocket of the RNA include numerous electrostatic, hydrogen bonding and hydrophobic interactions. The crystal structures of paromomycin bound to the ribosomal A-site reveals several close charge–charge interactions between the amino groups of the aminoglycoside and the phosphates of the RNA backbone.[9,14] Notable interactions include those between ring II with the phosphates of A1493 and G1494 and between ring IV and the phosphate of G1405. These interactions seemed particularly noteworthy to us. With this in mind, we felt non-carbohydrate scaffolds with two basic nitrogen-containing appendages would be suitable candidates for binding to the A-site. Having examined an in-house library, we decided upon MCR13 (Scheme 1; **4**) as a potential candidate. In addition to possessing two charged sites, the two aromatic rings of the tricyclic core offered the possibility of hydrophobic or pi-stacking interactions with the RNA base pairs.

Given the inherent problems with aminoglycoside antibiotics, an unmet need exists for the development of a new class of antibiotics, which bind to the bacterial 16S ribosomal RNA but do not possess toxic side effects and that are not susceptible to bacterial resistance by enzymatic modification. Here we report the discovery of a compound (Scheme 1) with a chemical structure distinct from the aminoglycoside family that binds to the ribosomal A-site.




**Scheme 1.** Reagents and conditions: (i) 2-Aminophenol, $NaHCO_3$, ether, water; 0 °C to rt, 77%; (ii) NaOH, $H_2O$, 90 °C, 6 h, 96%; (iii) 2-Chloroethylpyrrolidine hydrochloride, aq NaOH, acetone, 60 °C; (iv) $SnCl_2$, ethanol, reflux, 1 h, 52%; (v) Thioacetimidic acid naphthalene-2-yl methyl ester hydrobromide, ethanol, rt.

The synthesis of **4** (MCR13) is outlined in Scheme 1. The acid chloride **1** was reacted with 2-aminophenol under basic conditions followed by base-mediated cyclization to the tricyclic benzoxazepinone **2**. The amide nitrogen was alkylated with 1-(2-chloroethyl)pyrrolidine to give **3**. Nitro group reduction with tin(II) chloride dihydrate in refluxing ethanol and conversion of the corresponding aniline to the acetamidine group[18] provided the dibasic compound **4**.

We have used NMR spectroscopy and mass spectrometry techniques to show that MCR13 binds to a model of the *E. coli* 16S ribosomal A-site. The A-site RNA for NMR studies was produced by in vitro transcription from a DNA template.[19] This sequence (Fig. 1) is identical to the molecule used by Fourmy et al.[8] for the NMR structure determination of the paromomycin–RNA complex. The one-dimensional $^1$H NMR spectrum of a 0.5 mM RNA sample in 10 mM sodium phosphate (pH 6.4) acquired at 25 °C is shown in Figure 2. This plot shows the downfield imino proton region and is very similar to spectra reported previously[8] for the same sequence in the same buffer conditions. To the RNA we titrated in a solution of MCR13 in identical buffer (Fig. 2) to a final concentration that is a twofold molar excess of MCR13 over RNA. We observed line broadening for a subset of the imino resonances for nucleotides primarily located near the aminoglycoside binding site (G1405, G1497, G1491, U1406, and U1495).

This selective line broadening provides two pieces of information: that binding is occurring, and that the

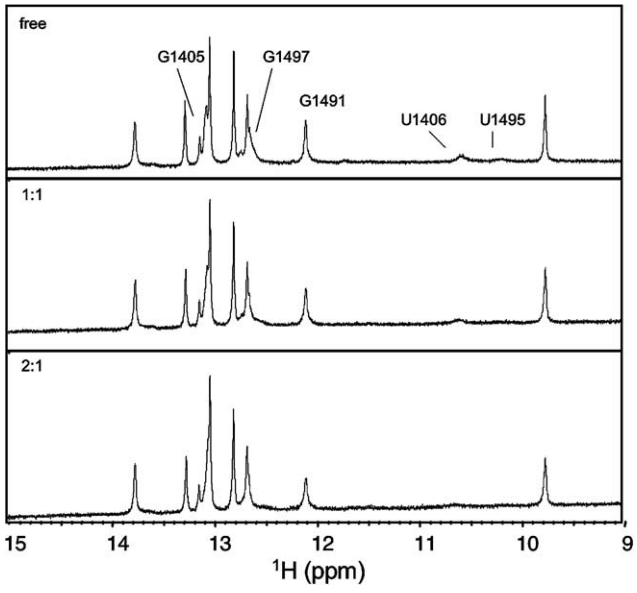



**Figure 2.** Binding of MCR13 to the A-site RNA determined by NMR spectroscopy. Shown are the NMR spectra of the A-site RNA in the absence and presence of a 1:1 and 2:1 molar ratio of MCR13 to RNA. The identity of the imino peaks that show line broadening upon binding are labeled in the unbound spectrum.

binding site is near those residues whose NMR spectrum changes. This binding-induced line broadening is indicative of either chemical exchange, such as hydrogen exchange with water, or conformational exchange taking place. The residues that experience line broadening are the same as those identified previously that show a change in chemical shift upon paromomycin binding to the A-site.[10,20] Similar NMR line-broadening effects were observed for A-site binding compounds identified by Fesik and co-workers[15] and Mayer and James.[16]

The binding of MCR13 to the A-site RNA was also tested by electrospray ionization (ESI) Fourier transform mass spectrometry (FTMS) using an ApexIII FTMS (Bruker Daltonics) equipped with a thermally assisted Apollo ESI source and an active shielded 7T superconducting magnet. The non-covalent complexes formed by different aminoglycosides with the A-site RNA have been previously investigated using this technique, which allowed for the observation of these interactions intact in the gas phase.[21,22] The mass spectra provided by a 10 μM sample of the A-site RNA (Dharmacon Inc.) in 50 mM ammonium acetate/10% isopropyl alcohol in the absence and presence of a twofold molar excess of MCR13 are shown in Figure 3a and b. A non-covalent complex formed between the RNA and MCR13 can be observed with a 1:1 stoichiometry, although with low abundance. The presence of a large percentage of unbound RNA indicates that the ligand binds weakly to the A-site RNA. On the contrary, a control experiment carried out by mixing A-site RNA with an equimolar amount of the aminoglycoside paromomycin (Fig. 3c) provided an intense signal for the 1:1 complex. A much lower percentage of unbound RNA indicates that the binding affinity is much stronger for paromomycin than for MCR13. From the work of Griffey et al. studying weak binding ligands to the A-site RNA by ESI-FTMS[23] and from estimates of the practical limit of observing non-covalent complexes by ESI-FTMS methods we estimate that MCR13 binds to the A-site RNA in the low millimolar range, with a $K_d$ of less than about 15 mM.

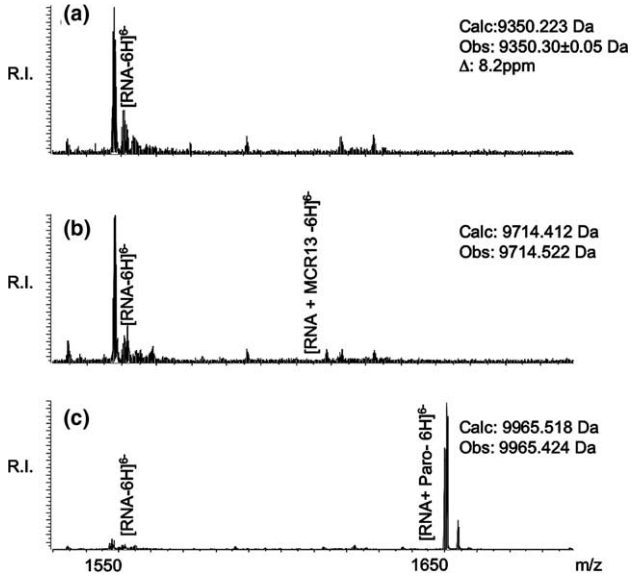



**Figure 3.** Binding of MCR13 to the A-site RNA observed by electrospray ionization Fourier transform mass spectrometry (ESI-FTMS). Shown are the mass spectra of the A-site RNA in the absence (a) and presence of a twofold molar excess of MCR13 (b). A control experiment including the aminoglycoside paromomycin is shown in (c).

A competitive binding experiment between compound MCR13 and paromomycin was then performed to see if the two molecules share a common binding site in the A-site RNA. Equimolar amounts of MCR13, paromomycin, and A-site RNA were combined and analyzed using ESI-FTMS (data not shown). The resulting mass spectrum showed only the presence of paromomycin bound to the RNA. This confirms that paromomycin binds with a higher affinity to the ribosomal A-site than MCR13. Additionally, the absence of an observable MCR13-A-site RNA complex or ternary MCR13-paromomycin-A-site RNA complex suggests that MCR13 and paromomycin share a common binding site on the A-site RNA.

To gain structural insight as to how MCR13 interacts with RNA we used Autodock 3.05[24] to model the binding of MCR13 to the A-site RNA. For the docking experiments we used both the structure of the RNA bound to paromomycin as determined by NMR[8] (PDB code: 1PBR) and X-ray crystallographic methods[9] (PDB code: 1J7T). Docking simulations were carried out on a dual 2 GHz PowerPC Macintosh G5. The initial structure of the MCR13 ligand was modeled with ab initio (6-31G*) geometry optimization using the software Gaussian 98.[25] Docking of MCR13 to both RNA structures produced similar results, the lowest energy docked structures always had MCR13 docked to the A-site in a similar position as occupied by paromomycin in the RNA–aminoglycoside complex. The lowest binding energy at 273.15 K using the NMR-derived RNA structure was $-17.51\ \text{kcal mol}^{-1}$. When the crystal structure was used as the docking target a binding energy of $-15.44\ \text{kcal mol}^{-1}$ resulted. Figure 4 shows the lowest energy docked structure obtained for the MCR13-RNA complex.

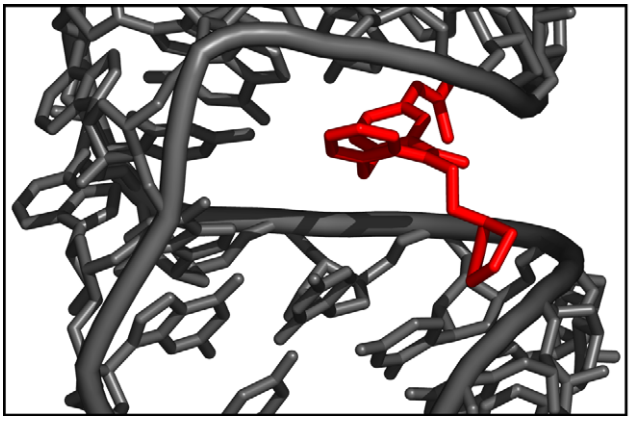


**Figure 4.** The lowest energy structure of MCR13 docked to the *E. coli* ribosomal A-site RNA (PDB code: 1PBR). MCR13 is shown in red while the RNA is in gray.

This modeling demonstrates that MCR13 adopts a bent shape about the seven-member central ring and has the correct geometry to fit into the major groove pocket formed by the A-site nucleotides. MCR13 is approximately the same size as two of the carbohydrate rings of the aminoglycoside compounds. Amongst the lowest energy docked structures there is a variation in the position of MCR13 in the A-site. However, the ligand tends to bind at the same sites as occupied by rings 2 and 3 or rings 3 and 4 of paromomycin in the experimentally determined complexes. In the lowest energy docked structure we observe electrostatic interactions between the U1406 phosphate and the pyrrolidine group on MCR13 and between the C1407 and G1489 phosphate groups and the imino functionality on MCR13. Additionally, the B-aromatic ring of MCR13 (Scheme 1) stacks against the aromatic base of U1490. These interactions are consistent with MCR13 occupying the same general site as rings 2 and 3 or 3 and 4 of paromomycin. These rings on paromomycin interact with G1405, U1406, C1407, G1491, G1494, U1495, in the crystal structure of the complex.

In summary, we report the discovery of a novel non-carbohydrate molecule that binds to the *E. coli* ribosomal A-site. Binding was demonstrated using NMR spectroscopy and ESI-FTMS methods and we showed that MCR13 binds at the same site as the aminoglycoside family of antibiotics. Future work will focus on improving the affinity of MCR13 to the A-site RNA through a structure–activity relationship (SAR) study.

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## References and notes

1. Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother.* **2000**, *44*, 3249.
2. Schacht, J. *Ann. N.Y. Acad. Sci.* **1999**, *884*, 125.
3. Humes, H. D. *Ann. N.Y. Acad. Sci.* **1999**, *884*, 15.
4. Ningeot-Leclercq, M.; Tulkens, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 1003.
5. Vakulenko, S. B.; Mobashery, S. *Clin. Microbiol. Rev.* **2003**, *16*, 430.
6. Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1965.
7. Moazed, D.; Noller, H. F. *Nature* **1987**, *327*, 389.
8. Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367.
9. Vicens, Q.; Westhof, E. *Structure* **2001**, *9*, 647.
10. Fourmy, D.; Recht, M. I.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 347.
11. Vicens, Q.; Westhof, E. *J. Mol. Biol.* **2003**, *326*, 1175.
12. Lynch, S. R.; Gonzalez, R. L., Jr.; Puglisi, J. D. *Structure* **2003**, *11*, 43.
13. Vicens, Q.; Westhof, E. *Chem. Biol.* **2002**, *9*, 747.
14. Carter, A. P.; Clemons, W. M.; Brodersen, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Nature* **2000**, *407*, 340.
15. Yu, L.; Oost, T. K.; Schkeryantz, J. M.; Yang, J.; Janowick, D.; Fesik, S. W. *J. Am. Chem. Soc.* **2003**, *125*, 4444.
16. Mayer, M.; James, T. L. *J. Am. Chem. Soc.* **2004**, *126*, 4453.
17. Russell, R. J. M.; Murray, J. B.; Lentzen, G.; Haddad, J.; Mobashery, S. *J. Am. Chem. Soc.* **2003**, *125*, 3410.
18. Shearer, B. G.; Oplinger, J. A.; Lee, S. *Tetrahedron Lett.* **1997**, *38*, 179.
19. Milligan, J. F.; Groebe, D. R.; Witherell, G. W.; Uhlenbeck, O. C. *Nucleic Acids Res.* **1987**, *15*, 8783.
20. Recht, M. I.; Fourmy, D.; Blanchard, S. C.; Dahlquist, K. D.; Puglisi, J. D. *J. Mol. Biol.* **1996**, *262*, 421.
21. Hofstadler, S. A.; Sannes-Lowery, K. A.; Crooke, S. T.; Ecker, D. J.; Sasmor, H.; Manalili, S.; Griffey, R. H. *Anal. Chem.* **1999**, *71*, 3436.
22. Sannes-Lowery, K. A.; Griffey, R. H.; Hofstadler, S. A. *Anal. Biochem.* **2000**, *280*, 264.
23. Griffey, R. H.; Sannes-Lowery, K. A.; Drader, J. J.; Mohan, V.; Swayze, E. E.; Hofstadler, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 9933.
24. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
25. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A., Gaussian, Pittsburgh, PA, 2003.