Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2557–2563

BIOORGANIC & MEDICINAL CHEMISTRY

Aptamers That Bind to the Antibiotic Moenomycin A

Heike Schürer,[a] Katherina Stembera,[b] Dietmar Knoll,[b] Günter Mayer,[c] Michael Blind,[c] Hans-Heinrich Förster,[a] Michael Famulok,[c,*] Peter Welzel[b,*] and Ulrich Hahn[a,*]

[a]*Universität Leipzig, Institut für Biochemie, Talstr. 33, D-04103 Leipzig, Germany*
[b]*Universität Leipzig, Institut für Organische Chemie, Johannisallee 29, D-04103 Leipzig, Germany*
[c]*Rheinische Friedrich-Wilhelms-Universität Bonn, Kekulé-Institut für Organische Chemie und Biochemie, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany*

Received 18 October 2000; revised 1 December 2000; accepted 3 December 2000

Abstract—Nuclease-resistant moenomycin-binding aptamers with dissociation constants in the range of 300 to 400 nM have been selected. Competition experiments have demonstrated that these aptamers recognize a disaccharide analogue of moenomycin. The results offer the opportunity of setting up a selective and sensitive assay for identifying moenomycin biosynthetic precursors. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

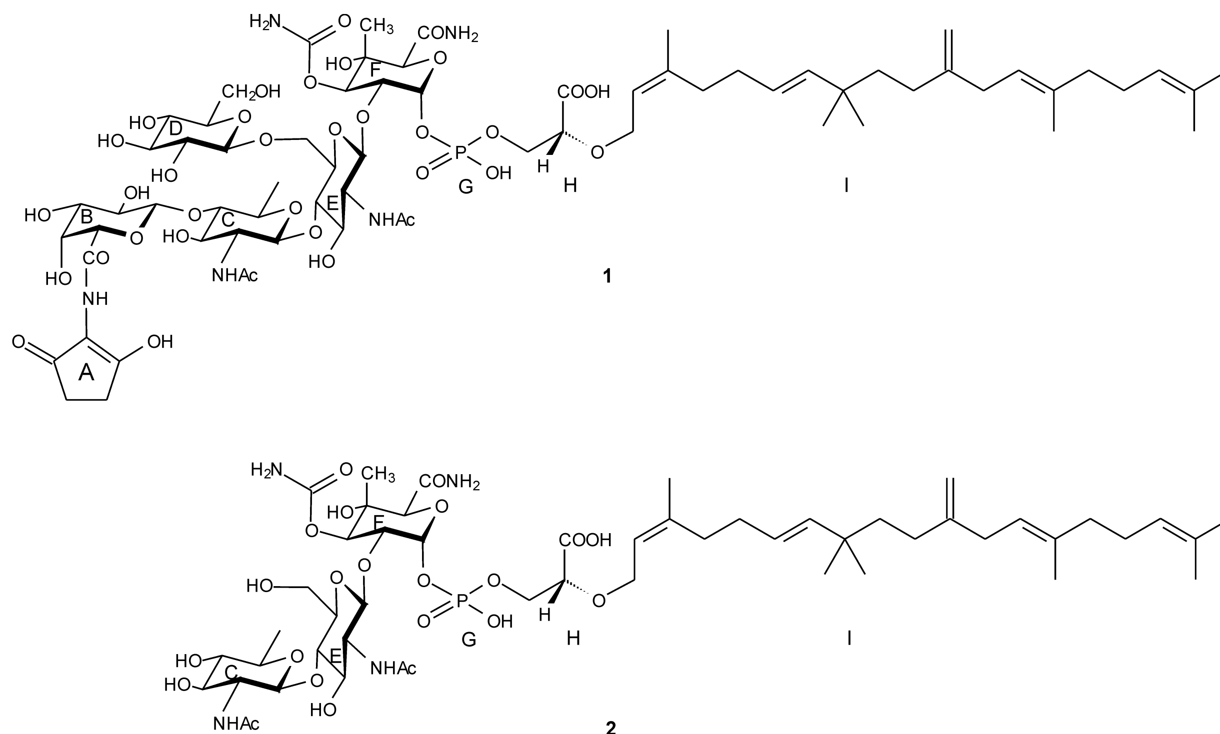
Antibiotic resistance in bacterial pathogens has become a major problem for human health and requires the development of drugs with novel modes of action.[1] One promising research area is to interfere with the biosynthesis of the peptidoglycan of the bacterial cell wall. The last two steps in peptidoglycan biosynthesis are a transglycosylation and a transpeptidation reaction, respectively. The β-lactam-antibiotics, which inhibit the transpeptidation reaction, play a major role as therapeutics (with all the resistance problems). On the other hand, the transglycosylation reaction is a new target. A number of glycopeptide antibiotics[2] and the moenomycins (see moenomycin A, **1**) have been shown to inhibit the transglycosylation. Their modes of action are, however, most probably different. For antibiotics of the moenomycin group it has been demonstrated that they interact reversibly with the transglycosylase. It is assumed that they act at the binding site of the growing polysaccharide strand and, thus, inhibit chain elongation.[3]

The moenomycins represent a unique lead structure for novel antibiotics. The natural products themselves are not used in medicine due to their unfavorable pharmacokinetics.[4] From degradative and synthetic work it is known that trisaccharide analogues of moenomycin such as **2** represent the minimal structural requirement to elicit antibiotic activity.[5] In principle, this information could be used to design combinatorial libraries of moenomycin analogues and screen them for compounds with high antibiotic activity on one hand and favourable pharmacokinetics on the other. However, the presently available synthetic procedures are too complicated to follow this strategy (Scheme 1).[5]

A conceptionally new approach would be to isolate biosynthetic precursors with less complicated structures and use them as scaffolds for the generation of semisynthetic libraries. Although the biogenetic origin of some of the structural units has been elucidated it still has to be established how the complete structure is assembled from the precursors.[6] Based on precedent one may assume that the biosynthesis starts from membrane-anchored precursors attached to the lipid part via the phosphoglycerate unit.[7] This would mean that the UV chromophore (unit A in **1**) is introduced at a late stage of the biosynthesis. All known isolation procedures for the moenomycins are based on the chromophore moiety (UV monitoring at ≈250 nm)[8] and this may be the reason that until now no biosynthetic precursors have been identified. An assay which would allow to identify in a fermentation broth of *Streptomyces ghanaensis* compounds containing the lipid part, the phosphoglycerate moiety and a truncated sugar chain and, thus, (most of) the structural features that are responsible for the biological activity would be a real break-through for the development of new transglycosylase inhibitors. A possible strategy to identify

*Corresponding authors. (i) Fax: +49-228-735388; m.famulok@uni-bonn.de (Famulok); (ii) fax: +49-341-9736599; welzel@organik.chemie.uni-leipzig.de (Welzel); (iii) fax: +409-341-9736-988; uhahn@uni-leipzig.de (Hahn)

0968-0896/01/$ - see front matter © 2001 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(01)00030-X



Scheme 1.

and isolate such moenomycin-precursors might be the generation of antibodies or other receptor compounds that are specific for particular moenomycin epitopes and use them as scavengers or for the enrichment of these fragments from microbial extracts or fermentation broths. However, neither antibodies nor other specific receptors for moenomycin derivatives are currently available.

Here we have applied in vitro selection[9,10] of a combinatorial library of nuclease-resistant 2′-aminopyrimidine-modified RNA sequences to isolate aptameric receptor molecules that specifically bind to moenomycin.[11] Mapping experiments with the disaccharide-containing moenomycin fragment **5** revealed that isolated aptamers recognize individual epitopes of moenomycin. Thus, the aptamers described herein may serve as the basis for the development of selective and sensitive assays for the identification of biosynthetic precursors for the antibiotic (Scheme 2).

Results and Discussion

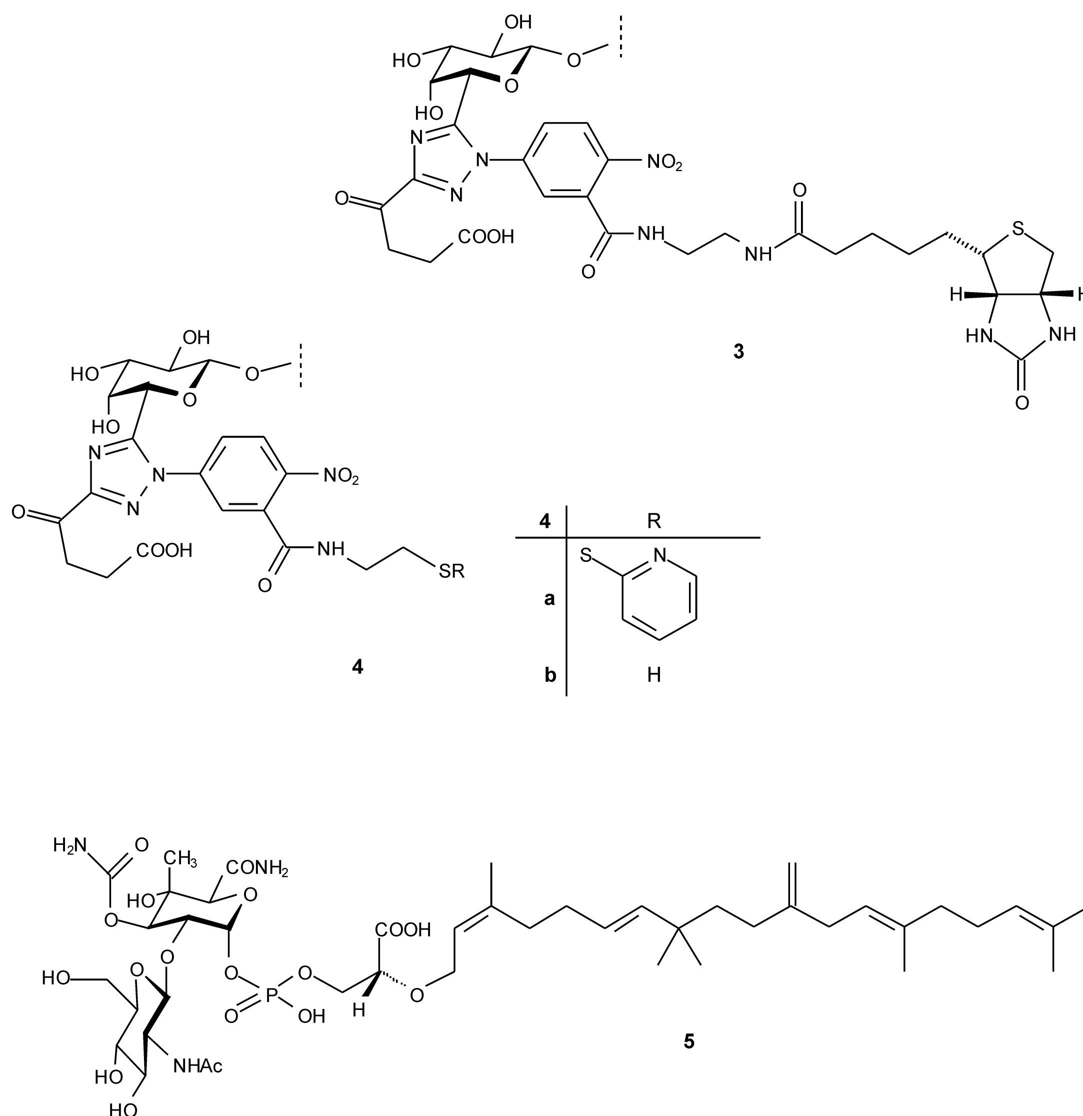
Immobilizing moenomycin on thiol-sepharose

Moenomycin A (**1**) was immobilized at thiol sepharose 4B according to the manufacturer's protocol with the following modifications: First, moenomycin A (**1**) was converted into disulfide **4a** as described previously.[12] The disulfide bridge of activated sepharose 4B was cleaved with 2-mercaptoethanol. **4a** was then covalently coupled to thiol sepharose 4B via disulfide bonds. The amount of immobilized moenomycin was determined by UV-spectroscopic quantification of the released 2-thiopyridone (343 nm) revealing a coupling efficiency of 50%. The derivatized sepharose was stable for about 2 weeks when stored at 4 °C in the binding buffer as determined by cleaving the disulfide bond with 2-mercaptoethanol and quantification of the released moenomycin thiol derivative **4b**[12] by HPLC.

Selection of moenomycin aptamers

Moenomycin aptamers were obtained by an in vitro selection/amplification procedure from a library of approximately 7×10^{14} different modified RNA sequences with a randomized region of 40 nucleotides using a standard in vitro selection protocol.[13] In round six 32% of the input RNA remained bound to the antibiotic on the column. After the sixth round we performed three different selection strategies in parallel using different elution methods as well as different partition methods. In method (a), elution of moenomycin-specific aptamers was achieved as in rounds one to five by reductive cleavage of the moenomycin/RNA complexes from the column with dithiothreitol, whereas in protocol (b) aptamers were affinity-eluted with $5\times200\,\mu l$ 1 mM moenomycin A. In method (c), the biotinylated moenomycin derivative **3**[14] was preincubated with streptavidin and selections continued by filter-binding. Furthermore, for the enrichment of aptamers with higher affinity from the sixth round all selection strategies were performed using more stringent conditions by an increase of washing volumes from 10 to 120 column volumes and a decrease of the concentration of immobilized material from 900 to 300 µM for methods a and b (see Experimental).

After 12 rounds of in vitro selection the DNA templates from the enriched RNA pools were cloned into pUC19 and sequences of about 35 clones from each selection strategy were determined. Representative sets of



Scheme 2.

sequences and the corresponding elution methods used for their selection are shown in Table 1.

Consensus sequences were identified which are mainly characterized by two motifs (*motif a*: GGAGG$(N)_n$G-GAGG and *motif b*: GUGGUGUGGUG). A comparison of sequences from round 6 and those from the 12th cycle revealed that an enrichment of the two motifs was already manifested in some of the round six isolates. All the RNAs from the 12th round are dominated by these two motifs. Interestingly, aptamers partitioned by filter retention are nearly exclusively characterized by *motif a* whereas aptamers derived from methods (a) and (b) are dominated by *motif b*. Most of the aptamer sequences contain more than 50% G residues. To determine whether the primer binding sites are involved in moenomycin recognition, we constructed a truncated RNA omitting any terminal primer binding sites. No difference in the binding behaviour to immobilized moenomycin was detectable, indicating that the primer binding sites are not required for moenomycin complexation.

Different methods for the determination of binding affinities

Dissociation constants were measured for two aptamers (A6 and C2) by isocratic elution from a moenomycin-sepharose column.[15] For these aptamers containing either *motif a* (C2) or *b* (A6) dissociation constants in the range of 300 to 400 nM were determined.[16]

pH-Dependence and stability

Binding of 2′-NH_2-modified RNA aptamers to moenomycin is pH-dependent, most probably because of the presence of the 2′-NH_2 group ($pK_a = 6.2$).[17] It should be noted, however, that the selection was performed at pH 7.4 whereas maximal binding efficiencies for individual RNAs were achieved at pH 7.2.

For the potential application of aptamers as recognition modules for moenomycin epitope-containing ligands in crude cell lysates these nucleic acid ligands have to be

Table 1. Sequences of moenomycin-binding aptamers[a]

Classes	Elution method	Sequences	No. of clones
***motif a*: GGAGG(N)$_n$GGAGG**			
A3	a	TGATGTCTCGAAATGAAGGAGGAGGAGGGGAACCGGATGG	1
A8	a	AGATGTCTGAAGAACTCGGAGGAGGAGGCGGTCTGGCCTG	1
A9	a	TGGATTTGATCGGAGGAGGAGGCGCCGAGCGCTGTCGTCC	1
A10	a	ACCGCGCGTGGGGGAGGAGGCGAGTGGCCTAAGTGTCCC	2
B3	b	TGGTCTGACATTGCGGAGGGGGAGGCGCAGACTCAGGCAG	1
B5	b	CGATCGAAGTAAGGAGGGGGAGGCGCCGAGCTAGCTCCT	1
B6	b	GCATGTGGAGGGGGAGGCGTGCTCGAGTATGAACTCCCGCG	1
C1	c	CATGGAGCTCTCTACGGCCCTGGAGGAGGAGGAGTGCCGT	2
C2	c	TCGACCTCGCGCGAGGAGGGTGGAGGGTCGTAGAGCGCGTA	10
C4	c	CTCGCGCGTGGAGGAGGGGGCAGATGGNCCCAGGCATCCG	4
C5	c	TGTAGACGTAGGGAGGAGGAGGCGCCAAGCTCACACCGAT	1
C8	c	TGTGCGACCGCACCGGTCGCGAACGGAGGAGGAGGCGCCA	1
C11	c	CCTGGAGGAGGAGGAGTGACTCAGGCTAGTACCGCGCCTT	3
C16	c	CCATTGCAGCGCGGGCGTAGGAGGAGGAGGCGCGTAGA	1
***motif b*: GTGGTGTGGTG**			
A1	a	GATGTCTCAGCTCGGCGGCGTGGTGA*GGTGTGGTGTTG*CG	15
A4	a	TGAACG*GTGGTGTGGTGTGGTG*CGGCGCTGCGGAGTAATG	1
A6	a	TCATCGGAGGAGC*GGTGTGGTG*AGGTGACGCGGAAAAGGT	6
A7	a	TCGTGACCGGAGGA*GTGGTGTGGTGCGGTTG*CCCTGCGTG	1
A12	a	CATCCACCGCGGTGGA*GTGGTGTGGTG*CGGTGCTGCGCGT	1
B1	b	TCGTAC*GGTGGTGT*GGAGT*GGTGTGGTG*ATGCGACGGTCC	6
B4	b	TCATCGGAGGTGC*GGTGTGGTG*AGGTGACGCGGAAAAGGT	2
B7	b	TGAACG*GTGGTGTGGTGTGGTG*CGGCGCTGCGGAGTAATG	3
B8	b	TCGTGACCGGAGGA*GTGGTGTGGTG*CGGTTGCCCTGCGTG	3

[a]Selected RNAs were obtained by a SELEX procedure targeting moenomycin as described in the text. A selection of aptamer sequences is shown, along with the number of individual clones and the elution method used. All sequences of the 40 N randomized region are presented in a 5′ to 3′ orientation, nucleotides that are conserved are highlighted (*motif a*: GGAGG(N)$_n$GGAGG, *motif b*: *GTGGTGTGGTG*).

Compound 5

Moenomycin A

buffer

Figure 1. Results of the in vitro competition experiments. In each diagram, the column designated as (w) shows the radioactivity in the last wash fraction. e1-e5 represent the fractions from the competition elutions. (c) is the amount of RNA that remained bound to the column after the competitive elutions. Top panel: aptamer bound to moenomycin-sepharose eluted with **5**; bottom-left panel: aptamer bound to moenomycin-sepharose eluted with moenomycin; bottom-right panel: control elutions with buffer.

resistant against degradation by nucleases. Therefore, the stability of the 2′-NH_2-modified enriched RNA pool was tested in 10% fetal calf serum in comparison to the unmodified pool. For the latter a half life of a few seconds was determined, whilst the NH_2-modified RNA pool showed slight degradation only after 48 h (data not shown).

In vitro competition of moenomycin binding by a moenomycin fragment

In order to determine the recognition-epitope and, hence, to find out whether the isolated aptamers are suitable for the isolation of biogenetic precursors of moenomycin, competition experiments with the di-

saccharide analogue **5** were performed by loading moenomycin-sepharose with ^{32}P-labeled aptamers A6 and C2, respectively, followed by competitive elution with **5** in binding buffer. Eluted aptamers were detected by Čerenkov counting. As shown in Figure 1, elution of moenomycin by **5** was almost as efficient as elution with moenomycin A itself. This means that the aptamers do indeed recognize the disaccharide portion of moenomycin A as desired.

Moenomycin aptamers restore bacterial growth in the presence of moenomycin

As recognition of moenomycin by the selected aptamers occurs mainly via the epitope that is responsible for the antibiotic activity we reasoned that bacterial growth of a moenomycin-sensitive strain in a moenomycin-containing culture medium might be restored in the presence of the aptamers. This hypothesis was tested with *Staphylococcus aureus* cultures using a micro dilution method on micro titer plates as described previously.[18] We could clearly detect that the effect of moenomycin on bacterial growth was diminished in the presence of aptamers. The activity of moenomycin was reduced to about 50–60% under the conditions employed (Figure 2). Controls with unselected RNA did not show any effect in this assay.

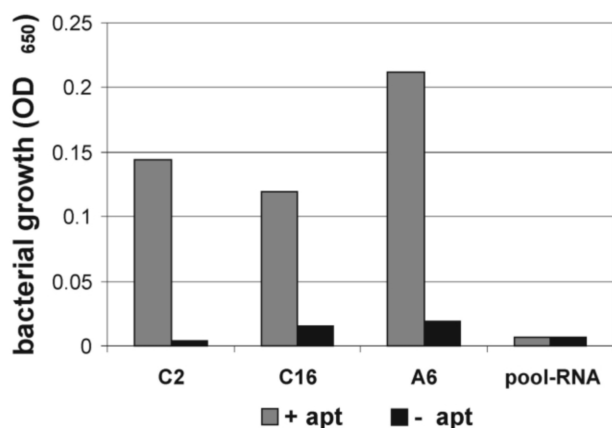


Figure 2. Effect of aptamers on the antibiotic activity of moenomycin A. The antibiotic effect of moenomycin (7.9×10^{-8} mol/l) was reduced by aptamers C2, C16, and A6 at concentrations of 3.3×10^{-6} mol/l, resulting in restoration of bacterial growth (grey columns). Unselected RNA was ineffective in this assay (black columns). Bacterial growth in the absence of moenomycin A resulted in an $OD_{650\ nm}$ of 0.7 after 6 h under the conditions employed.

Outlook

We have isolated aptamers that are capable of recognizing a disaccharide analogue of moenomycin A. This property should be useful as an analytical tool for the isolation of biogenetic precursors of moenomycin A from cultures of deletion mutants of *Streptomyces ghanaensis* that do not produce the full-length moenomycins. Work in this direction is in progress. If it will be successful very interesting perspectives for the synthesis of semisynthetic libraries of moenomycin analogues are obvious.

Experimental

Materials

4a, **4b** and **5** were prepared and purified as described previously.[5,12] [α-^{32}P]GTP was from NEN Life Science Products, 2′-NH_2-modified CTP and UTP were purchased from Amersham. The DNA pool Mic 40 was synthesized using an Expedite synthesizer, primers were purchased from Metabion.

Immobilizing moenomycin on thiol-sepharose

The disulfide bridge of activated sepharose 4B was cleaved with 2-mercaptoethanol. Then **4a** was covalently coupled to thiol sepharose 4B via disulfide bonds. Thus, cleavage of the disulfide bridge of activated thiolsepharose 4B was performed by incubating the pre-swollen matrix in the following buffer: 300 mM $NaHCO_3$, 1 mM EDTA, 5 mM 2-mercaptoethanol, pH 8.0. The suspension was gently mixed for 1 h at 4 °C followed by intense washing with 50 mM NaCl, 1 mM EDTA, adjusted to pH 3.0 with acetic acid. The coupling reaction was performed by incubating the thiol matrix with **4a** and gentle swirling for 3 h at 4 °C in immobilization buffer (500 mM NaCl, 1 mM EDTA, 10 mM sodium acetate, pH 5.0). The matrix was washed with sodium acetate, pH 6.0 and the amount of immobilized moenomycin was determined by UV-spectroscopic quantification of the released 2-thiopyridone (343 nm). The coupling efficiency was about 50%. The affinity-sepharose could be used for about 2 weeks when stored at 4 °C in the binding buffer.

Control of the quality of the material was performed by cleaving off the thiol derivative **4b** of moenomycin A with 2-mercaptoethanol and determining the released **4b** by HPLC (5 μm LiChrosper®100, RP-18e, 40:60 acetonitrile-buffer [sodium heptanesulfonate (3 g), $K_2HPO_4\times3$ H_2O (26.2 g), KH_2PO_4 (0.6 g) in water, final volume 1 L, pH 8.2], flow rate 0.5 mL min^{-1}, diode array detection at 278 nm). Thiol sepharose utilized for preselection (to remove matrix binders) was prepared as described above.

DNA-and RNA pool synthesis

Synthesized DNA includes a cassette of 40 completely randomized nucleotides flanked by constant regions that were required for enzymatic amplification. The synthetic single stranded DNA template was PCR amplified using the following primers: 5′-*TCT AAT ACG ACT CAC TAT A*GG GAG AGA C**AA GCT T**GG GTC-3′; 3′-primer: 5′-GTG A**GG ATC C**TT AAT TAA CTT CTC TTT CTC-3, PCR-amplified DNA pool: 5′-*TCT AAT ACG ACT CAC TAT A*GG GAG AGA C**AA GCT T**GG GTC-N40-AGA AGA GAA AGA GAA GTT AAT TAA **GGA TCC** TCA C-3′ (*italic*, T7-promotor; bold and underlined, restriction sites). The RNA library with a complexity of 7×10^{14} different molecules was synthesized by in vitro transcription from the PCR-amplified synthetic DNA pool. The reaction was performed by incubating approximately 1,2 nmol of the PCR-derived template DNA with T7-RNA polymerase (100 units/μl) in the presence of ATP, GTP (3 mM each), 10 μCi [α]^{32}P-GTP (800 Ci/

mmol), 2′-NH_2-CTP and 2′-NH_2-UTP (1 mM each) for 4 h at 37 °C in 80 mM HEPES-buffer (pH 8.0) containing 12 mM $MgCl_2$, 5 mM dithiothreitol and 2 mM spermidine. After in vitro transcription the RNA was purified on 8 % polyacrylamide gels under denaturing conditions, eluted from the gel by the crush and soak method and ethanol precipitated. In subsequent rounds of selections, in vitro transcriptions were carried out with 200 pmol template DNA.

In vitro selection

Selections were performed using moenomycin-sepharose 4B affinity columns containing 900 μM (round 1 to 5) and 300 μM (round 6 to 12) moenomycin A, respectively, equilibrated with binding buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$). Prior to incubation with moenomycin the pool RNAs were dissolved in binding buffer, denatured at 70 °C for 10 min and left at room temperature for 30 min. For the preselection, the RNA-pool was applied to 200 μl of moenomycin-less Sepharose 4B to remove non-specific RNAs. In the first round of selection 7.4 nmol of the initial pool were incubated with 400 μl of the moenomycin-sepharose column for 1 h at room temperature with gentle mixing. In the second and third round of selection 1 nmol and 500 pmol, respectively, in all subsequent rounds 250 pmol of the RNA were incubated with 100 μl of the column material. The slurry was transferred into a chromatography column and unbound RNAs were removed by washing the column with 4 to 10 column volumes (round one to five) or 50 to 120 column volumes (round 6–12) of binding buffer. Elution of moenomycin specific aptamers was achieved with 5×200 μl 200 mM DTT in binding buffer (round 1-5). From the sixth round three different strategies were performed in parallel using different elution methods as well as different partition methods. According to method (a), elution of moenomycin-specific aptamers was performed by reductive cleavage of the moenomycin/RNA complexes from the column with dithiothreitol as described for round 1–5, whereas in protocol (b) aptamers were affinity-eluted with 5×200 μl 1 mM moenomycin A. Using method (c), the biotinylated moenomycin derivative **3**[19] was preincubated with streptavidin. From the sixth round on the moenomycin-biotin-streptavidin complex was incubated with the RNA pool and bound RNAs were separated from unbound by filter separation.

Eluted fractions were quantified by Čerenkov counting and ethanol precipitated in the presence of glycogen. The eluted RNAs were reverse transcribed in a total volume of 50 μl with Superscript reverse transcriptase (200 units/μl). The cDNA molecules were than amplified by PCR, purified by phenol-chloroform extraction and transcribed in vitro using T7 RNA polymerase to synthesize the RNA pool for the next round as described above.

Sequence analysis

The PCR-amplified DNA from the final rounds of selection was digested with *Bam*HI and *Hind*III (corresponding restriction sites were included in the primer regions) and the fragments were ligated to similarly digested pUC19. *Escherichia coli* JM 109 cells were transformed and plasmids from individual bacterial clones were sequenced following the standard dideoxy sequencing protocol using the M13 universal primer. Ninety clones with inserts were sequenced and consensus sequences were derived. 12 clones were chosen for further study.

Generation of minimal-RNA

The construction of a truncated RNA omitting any terminal primer binding sites was achieved by hybridization of synthetic DNA sequences, containing only the 40n minimal sequence of A4 aptamer and the T7 promotor. For the determination of binding properties moenomycin-labeled sepharose was loaded with minimal and full length RNA. Then, the column was washed with 20 column volumes of binding buffer. The remaining bound RNA was eluted with DTT, each fraction was then analyzed by Čerenkov counting.

In vitro competition assay with 5

The in vitro competition assay was performed by loading moenomycin-coated sepharose with ^{32}P-labeled aptamers. The moenomycin disaccharide analogue **5** was used in a three fold molar excess (1 mM) to the immobilized moenomycin (350 μM). Elution of aptamers mediated by **5** was detected by Čerenkov counting.

50 pmol of ^{32}P-labeled renatured RNA aptamers were applied in binding buffer (20 mM HEPES, pH 7,4, 150 mM NaCl, 5 mM $MgCl_2$) to moenomycin-sepharose (300 μM) pre-equilibrated with binding buffer. After incubation for 1 h at room temperature with gentle mixing the column was washed with 20 column volumes of binding buffer. The remaining bound RNA was affinity eluted with 5×200 μl of 1 mM of **5**, moenomycin A, and binding buffer as control. Each fraction was then analyzed by Čerenkov counting. All experiments were performed under selection conditions at pH 7.4 in order to avoid all problems connected with any pH-dependent conformational changes of 2′-amino-modified aptamers (pK of the 2′-amino group = 6.4).[17]

Antibiotic activity of the moenomycin aptamer complexes

The microdilution assay was performed on microtiterplates using 3.3×10^{-6} mol aptamers per l (C2, C16, A6) as well as pool RNA (control) in buffered medium (isosensitest broth, Oxoid), *Staphylococcus aureus* and moenomycin A at minimal inhibitory concentrations (7.9×10^{-8} mol/l). Bacterial growth was inspected over a period of 6 h at 37 °C determining OD values at 650 nm with an ELISA reader.

Acknowledgements

Support by the Deutsche Forschungsgemeinschaft ('Innovationskolleg 'Chemisches Signal und biologische

Antwort', to P.W. and U.H.), the priority program 'RNA Biochemistry' (to M.F.), the Karl-Ziegler foundation (to M.F.), and the Fonds der Chemischen Industrie is gratefully acknowledged.

References and Notes

1. Schroeder, R.; Waldsich, C.; Wank, H. *EMBO* **2000**, *19*, 1.
2. Goldman, R. C.; Baizman, E. R.; Longley, C. B.; Branstrom, A. A. *FEMS Microbiol. Lett.* **2000**, *183*, 209 and references therein..
3. For leading references, see Vogel, S.; Buchynskyy, A.; Stembera, K.; Richter, K.; Hennig, L.; Müller, D.; Welzel, P.; Maquin, F.; Bonhomme, C.; Lampilas, M. *Bioorg. Med.-Chem.Lett.* **2000**, *10*, 1963.
4. For a discussion of this point, see Anikin, A.; Buchynskyy, A.; Kempin, U.; Stembera, K.; Welzel, P.; Lantzsch, G. *Angew. Chem. 111, 3931.; Angew. Chem. Int. Ed.* **1999**, *1999*, 38 3703.
5. For leading references, see El-Abadla, N.; Lampilas, M.; Hennig, L.; Findeisen, M.; Welzel, P.; Müller, D.; Markus, A. *van Heijenoort, J. Tetrahedron* **1999**, *55*, 699.
6. Schuricht, U.; Endler, K.; Hennig, L.; Findeisen, M.; Welzel, P. *J.Prakt.Chem.* **2000**, *342*, 761.
7. Bugg, T. D. H.; Brandish, P. E. *FEMS Microbiol. Lett.* **1994**, *119*, 255.
8. Donnerstag, A.; Marzian, S.; Müller, D.; Welzel, P.; Böttger, D.; Stärk, A.; Fehlhaber, H.-W.; Markus, A.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1995**, *51*, 1931 and references therein.
9. Reviews: Osborne, S. E.; Ellington, A. D. *Chem. Rev. 97, 349; Famulok, M. Curr. Opin. Struct. Biol.* **1997**, *1999*, 9 324.
10. Hermann, T.; Patel, D. J. *Science* **2000**, *287*, 820.
11. For the recognition of aminoglycoside antibiotics by aptamers, see ref 10.
12. Kempin, U.; Hennig, L.; Knoll, D.; Welzel, P.; Müller, D. *Markus, A., van Heijenoort, J. Tetrahedron* **1997**, *53*, 17669.
13. Famulok, M. *J. Am. Chem. Soc.* **1994**, *116*, 1698.
14. The synthesis of this compound will be reported elsewhere.
15. Arnold, F. H.; Schofield, S. A.; Blanch, H. W. *J. Chromatogr.* **1986**, *355*, 13. Geiger, A.; Burgstaller, P.; Etz, H. v.d.; Roeder, A.; Famulok, M. *Nucleic Acids Res.* **1986**, *1996*, 24 1029.
16. See related results obtained by fluorescence correlation spectroscopy: Schürer, H.; Buchynskyy, A.; Korn, K., Famulok, M., Welzel, P.; Hahn, U. submitted
17. Lin, Y.; Qin, Q.; Gill, S. C.; Jayasena, S. D. *Nucleic Acids Res.* **1994**, *22*, 5229.
18. See ref 3.
19. The synthesis of this compound will be reported elsewhere.