VIP RNA–Peptide Conjugates

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Non-Hydrolyzable RNA–Peptide Conjugates: A Powerful Advance in the Synthesis of Mimics for 3′-Peptidyl tRNA Termini**

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Macrolide antibiotics, such as erythromycin, clarithromycin, roxithromycin, and telithromycin, bind close to the entrance of the ribosomal exit tunnel, adjacent to the peptidyl transferase center (PTC), and exert their action by preventing elongation of the nascent polypeptide chain (Figure 1).[1] It has been shown that translation of short peptides can render the ribosome resistant to macrolide antibiotics and that the amino acid sequence and size of the so-called resistance peptides are critical for their activity (Figure 2).[1d] Moreover, a significant correlation has been observed between different peptide consensus sequences and structurally distinct macrolide antibiotics to which resistance is conferred, suggesting a direct interaction exists between the peptide, the drug, and the ribosome. Since the peptide alone does not confer resistance, a likely scenario is that translation of these peptides is necessary to expel the macrolide antibiotic from the ribosome.

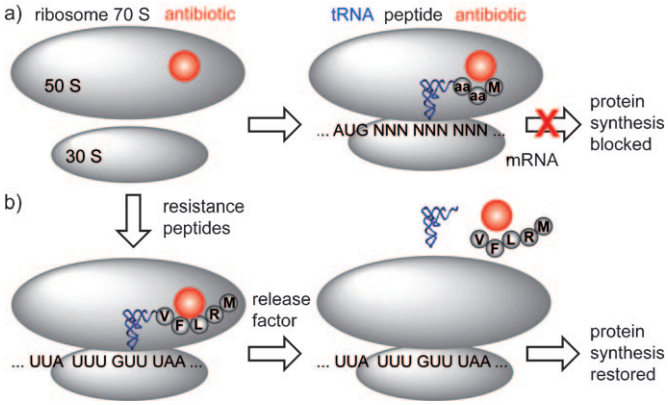

Figure 1. Resistance to macrolide antibiotics. a) Ribosomal translation is blocked by the binding of the antibiotic in the ribosomal exit tunnel, close to the PTC; b) Translation of short specific peptides confers resistance by a yet unknown mechanism. aa: amino acid, N: nucleotide.

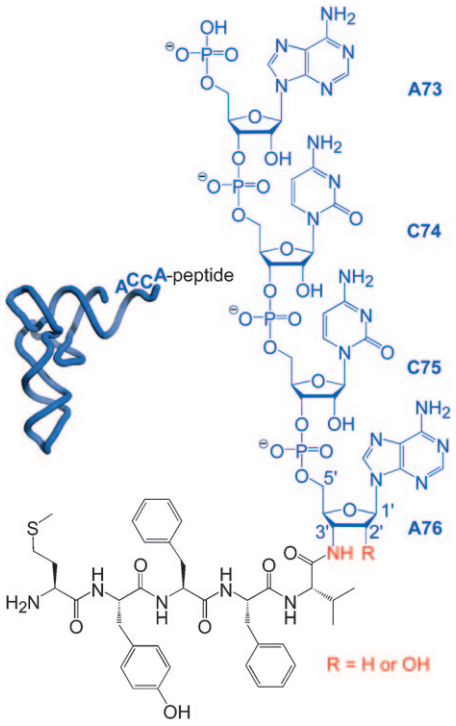

Figure 2. Sequence and chemical structure of a typical RNA–peptide conjugate investigated here; for example, 5′-p-rACC(rA$^{3'\text{-NH}}$)-VFFYM or 5′-p-rACC(dA$^{3'\text{-NH}}$)-VFFYM.

To date, there is little experimental data available addressing this hypothesis.[2,3] In particular, structural characterization of a ribosome containing a macrolide antibiotic in complex with a tRNA bearing an antibiotic resistance peptide is lacking. To reveal the structural details of these interactions would represent a major contribution not only in terms of understanding the resistance mechanism, but also for the design of novel therapeutic strategies to combat increasing bacterial antibiotic resistance.

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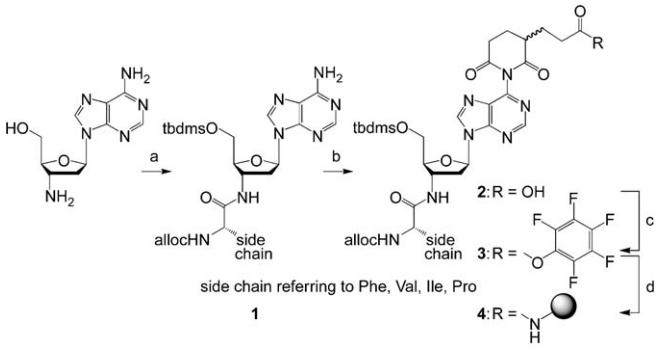
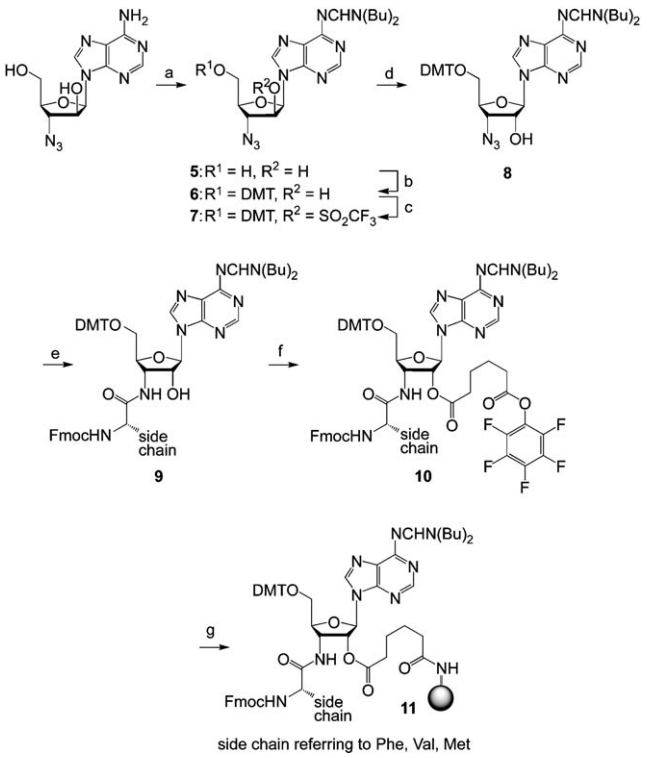
One way to gain insight into this particular mechanism of macrolide resistance would be to utilize short RNA–peptide conjugates (Figure 2) that mimic the ACCA76 tRNA terminus. The ACCA sequence directs selective binding of the conjugate to the PTC of the ribosome by formation of specific Watson–Crick base pairs between C74/C75 and ribosomal 23S rRNA nucleotides G2251/G2252.[4a–c] On the other hand, such conjugates should provide a stable analogue of the natural ester linkage that is formed by the A76 ribose 3′-hydroxyl group and the C-terminal carboxyl group of the resistance peptide to avoid hydrolysis or 2′-*O*/3′-*O* transesterification.[4d] This property is most reasonably fulfilled by an amide linkage, in analogy to the naturally occurring antibiotic puromycin.[5] Moreover, it would be favorable if both 2′-OH and 2′-deoxy A76 analogues were accessible in order to investigate the impact of this hydroxyl group (whose role in catalysis of peptide bond formation is currently disputed) on ribosome binding affinity.[6] Once such conjugates are in hand, a multitude of experiments to study the release of macrolide antibiotics would be possible, ranging from ribosome chemical probing and conjugate/macrolide competition experiments to soaking of ribosomal crystals with conjugates/antibiotics for structure determination by X-ray crystallography, enabling direct visualization of the molecular details of these interactions. Obviously, the current bottleneck of such an approach is the chemical synthesis of RNA–peptide conjugates that possess the aforementioned properties. Here, we introduce a synthetic strategy that enables reliable access to aminoacylated tRNA mimics with a variety of different peptide sequences.

Our approach started from commercially available 3′-amino-2′,3′-dideoxyadenosine to efficiently produce the functionalized solid support **4** ($dA^{3'\text{-}NH}$) in four steps (Scheme 1). We favored the tbdms (for the 5′-OH group) and alloc (for the amino acid NH_2 group) protecting groups over the analogous DMT and Fmoc protection because of the higher stability for the former observed during attachment of the linker moiety, which led to higher overall yields.



Scheme 1. Synthesis of the 3′-amino-2′,3′-dideoxyadenosine-functionalized support **4** ($dA^{3'\text{-}NH}$) for the automated solid-phase synthesis of RNA–peptide conjugates. Reagents and conditions: a) 1. 1 equiv alloc-Ile-OPfp (alternatively, Phe, Val, or Pro) in DMF/pyridine (1:1), room temperature, 1 h; 2. 1.6 equiv tbdms-Cl, RT, 1 h, 54%; b) 7.5 equiv 3-(2,6-dioxotetrahydro-2*H*-pyran-3-yl)propanoic acid,[7] pyridine, 90°C, 6 h, 99%; c) 5 equiv HOPfp, 5 equiv HOBt·H_2O, 5 equiv EDC, CH_2Cl_2, RT, 16 h, 37%; d) 1.25 equiv (w/w) amino-functionalized support (GE Healthcare, Custom Primer Support 200 Amino), 2 equiv pyridine, DMF, RT, 24 h, loading: 130 μmol g^{-1}. alloc = allyloxycarbonyl, Pfp = pentafluorophenyl, tbdms = *tert*-butyldimethylsilyl, HOBt = 1-hydroxybenzotriazole, EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride.

In parallel, we investigated a pathway to obtain a functionalized support retaining the ribose 2′-OH group ($rA^{3'\text{-}NH}$): This was realized by using 9-(3′-azido-3′-deoxy-β-D-arabinofuranosyl)adenine (Scheme 2), which was synthesized in two steps from commercially available 9-(arabinofuranosyl)adenine based on a published procedure.[8] Then, amidine protection of the adenine NH_2 group (**5**), tritylation of the arabinose 5′-OH (**6**), and inversion of the configuration at C2′ (**7** and **8**) were achieved in straightforward manner; the latter transformation made use of triflate chemistry in analogy to a protocol that we originally developed for the synthesis of 2′-methylseleno-modified RNA.[9] Subsequent Staudinger–Vilarrasa[10] coupling furnished the amino acid



Scheme 2. Synthesis of the 3′-amino-3′-deoxyadenosine-functionalized support **11** ($rA^{3'\text{-}NH}$) for the automated solid-phase synthesis of RNA–peptide conjugates. Reagents and conditions: a) 4 equiv *N*,*N*-di-*n*-butylformamide dimethyl acetal, DMF, 80°C, 2.5 h, 89%; b) 2 equiv DMT-Cl, 0.3 equiv Bu_4NNO_3, pyridine, RT, 1.5 h, 79%; c) 1.5 equiv trifluoromethanesulfonyl chloride, 1.5 equiv DMAP, 2.5 equiv $(iPr)_2NEt$, CH_2Cl_2, 0°C, then 30°C, 15 min; d) 5.5 equiv $CF_3COO^-K^+$, 1.5 equiv $(iPr)_2NEt$, 2 equiv [18]crown-6, toluene, 80°C, 2.5 h (68% over (c) and (d)); e) 1.3 equiv Fmoc-Val-OBt (alternatively, Phe or Met), 2.2 equiv $P(CH_3)_3$, THF, 0° to RT, 16 h, 89%; f) 5 equiv $PfpOOC(CH_2)_4COOPfp$, 1 equiv DMAP, DMF/pyridine (1:1), RT, 1 h, 71%; g) 3 equiv (w/w) amino-functionalized support (GE Healthcare, Custom Primer Support 200 Amino), 2 equiv pyridine, DMF, RT, 22 h, loading: 46 μmol g^{-1}. DMT = 4,4′-dimethoxytrityl, DMAP = 4-(dimethylamino)pyridine, Fmoc = *N*-(9-fluorenylmethyloxycarbonyl.

linked key intermediate **9**, which was further transformed into the pentafluorophenyl adipinic acid ester **10** and finally to the desired functionalized solid support **11**.

After selective removal of the alloc group of support **4** ($dA^{3'\text{-}NH}$), or alternatively, of the Fmoc group of support **11** ($rA^{3'\text{-}NH}$), peptide synthesis on both types of support was performed with standard Fmoc-protected amino acids or their respective pentafluorophenyl ester building blocks in automated manner using a multiple peptide synthesizer based on a customized pipetting robot with a shaking device.

After peptide assembly, treatment with 1.0 M of tetrabutylammonium fluoride (TBAF) and 0.5 M CH_3COOH in THF selectively liberated the 5′-OH group of the 3′-amino-2′,3′-dideoxy adenosine moiety, or alternatively, standard DMT removal selectively liberated the 5′-OH group of the 3′-amino-3′-deoxy adenosine moiety of the respective support.

For both types of support, oligonucleotide assembly using 2′-*O*-[(triisopropylsilyl)oxy]methyl (2′-*O*-TOM) protected ribonucleoside phosphoramidites then proceeded in an automated manner on a DNA/RNA synthesizer following standard procedures. The solid supports were treated with methylamine in ethanol/water (1:1) at room temperature for eight hours resulting in cleavage of the cyanoethyl and acyl protecting groups from the RNA–peptide conjugates together with release from the solid support. Subsequent treatment with 1.0 M TBAF in THF/water (9:1) at room temperature for 16 h finally cleaved the 2′-*O*-TOM protecting groups and resulted in high-quality crude products; mass spectrometric analysis of these crude products revealed that the main peak of the respective HPLC profiles represented the desired RNA–peptide conjugate (Figure 3). The conjugates were purified by HPLC, predominantly using anion-exchange columns, and purities of $>95\,\%$ were obtained.

According to the strategy presented here, we were able to synthesize a series of non-hydrolyzable RNA–peptide conjugates that mimic the 3′-aminoacylated tRNA termini (Table 1). With respect to peptide side-chain functionality, our strategy proved to be tunable. So far, we have successfully incorporated tyrosine (*O*-allyl protection), serine (*O*-tbdms protection), and methionine. Thereby, the thioether moiety of methionine became partly oxidized during oligonucleotide assembly since the peptide chain was exposed to oxidative conditions required for phosphite to phosphate triester transformation after each nucleotide-coupling step (Figure 3b). However, the sulfoxide can be reduced efficiently upon treatment of the conjugate with *N*-methylmercaptoacetamide.[11] Concerning incorporation of *O*-tbdms-protected serine into RNA–peptide conjugates of the $dA^{3'\text{-}NH}$ series, the tbdms protection of the 5′-hydroxyl group from support **4·Ile** was first cleaved, and then functionalized with dimethoxytrityl group prior to conjugate synthesis (see the Supporting Information).

Furthermore by employing ribosome chemical probing experiments, we have demonstrated that the peptidyl-tRNA mimics bind to their expected binding sites in the PTC of the ribosome. The location of interacting ACCA-peptides can be identified through analysis of diagnostic nucleobase protections in the 23S rRNA. A-site- and P-site-bound tRNA

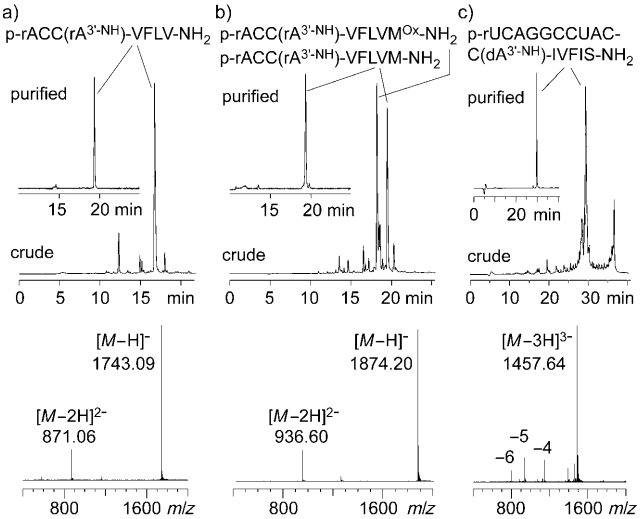


Figure 3. Representative HPLC profiles of crude and purified (insets) RNA–peptide conjugates (top) and mass spectra of purified conjugates (bottom); a) Conjugate synthesized on support **Val·11**; b) Same as (a) with additional methionine; the oxidized product can be reduced using *N*-methylmercaptoacetamide;[11] c) Conjugate synthesized on support **Ile·4.** Anion-exchange HPLC: Dionex DNAPac (4×250 mm), 80 °C, 1 mL min^{-1}, 0–35 % E2 in E1 in 30 min; E1: 25 mM Tris·HCl, pH 8.0, 10 mM $LiClO_4$; E2: same as E1 but 0.5 M $LiClO_4$ (for a, b); 60 °C, 1 mL min^{-1}, 0–35 % E2 in E1 in 30 min; E1: 25 mM Tris·HCl, 6 M urea, pH 8.0; E2: same as E1 + 0.5 M $NaClO_4$ (for c).

Table 1: Selection of synthesized nucleic acid-peptide conjugates.

Sequence[a,b]	Mass calcd	Mass found[c]	Yield[d] [nmol]
5′-HO-dACC($dA^{3'NH}$)-PPW	1571.3	1570.3	251
5′-p-rACC($dA^{3'NH}$)-FA	1488.0	1487.8	150
5′-p-rACC($dA^{3'NH}$)-VFLV	1728.4	1728.3	85
5′-p-rACC($dA^{3'NH}$)-VFFYM (K-peptide)	1957.6	1957.1	47
5′-p-rACC($dA^{3'NH}$)-IVFIS (ermC)	1829.5	1829.1	58
5′-p-rUCAGGCCUACC($dA^{3'NH}$)-IVFIS	4377.0	4377.0	80
5′-p-rACC($rA^{3'NH}$)-M	1416.0	1416.7	64
5′-p-rACC($rA^{3'NH}$)-FA	1456.1	1455.8	188
5′-p-rACC($rA^{3'NH}$)-VFLV	1744.4	1744.1	73
5′-p-rACC($rA^{3'NH}$)-VFLVM (ermC)	1875.3	1875.6	45
5′-p-dACC($rA^{3'NH}$)-VFFYM (K-peptide)	1925.6	1925.3	22
5′-p-rACC($rA^{3'NH}$)-VLLLM (erm)	1855.6	1854.8	10
5′-p-rACC($rA^{3'NH}$)-VLIVM (erm)	1841.6	1839.8	12

[a] For the chemical structure see Figure 1. [b] Term in parenthesis refers to macrolide antibiotic resistance; erm = erythromycin resistance gene. [c] Determined by ESI mass spectrometry. [d] Yield after purification.

protect characteristic nucleobases from chemical modifications: In particular, modification with 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (CMCT) of U2585 and Ψ2555 is sensitive for tRNA binding to the P-site and A-site, respectively.[12] The CMCT modifications cause a stop when the RNA is reverse transcribed, and this results in a specific band on a polyacrylamide gel when the reverse transcription products are separated by electrophoresis. Figure 4 depicts an example of a typical polyacrylamide gel of primer extension analysis of *E. coli* ribosomes exposed to dipeptide–RNA conjugates, 5′-p-rACC($rA^{3'\text{-}NH}$)-

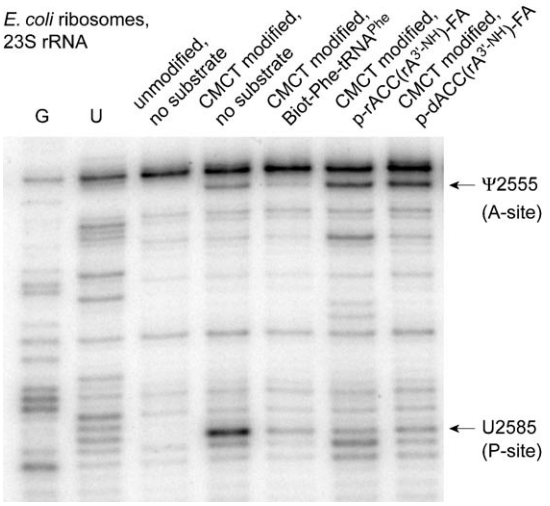


Figure 4. Chemical probing (by CMCT) and primer extension analysis of *E. coli* 23S rRNA indicate that the RNA–peptide conjugates bind to the ribosomal P-site (footprint at U2585) rather than to the A-site (Ψ2555) of the peptidyl transferase center (PTC). For details see the main text. G, U denote dideoxy sequencing lanes.

FA and 5′-p-dACC(rA$^{3'\text{-NH}}$)-FA (see the Supporting Information). From the protection pattern of U2585, it is clear that both substrates protect the P-site uridine to a comparable extent as the full-length peptidyl-tRNA analogue *N*-biotin-Phe-tRNA^{Phe} (Biot-Phe-tRNA^{Phe}). In contrast, the protection pattern of Ψ2555 is minimal, indicating that these conjugates preferentially bind to ribosomal P-site rather than A-site.

The present strategy offers reliable synthetic access to non-hydrolyzable RNA–peptide conjugates that mimic 3′-peptidyl tRNA termini. The strength of our approach combines the high sequence flexibility of the conjugates with ease of use, since assembly of both RNA and peptide rely on standard automated DMT/phosphoramidite and Fmoc solid-phase synthesis. So far, most low-molecular-weight mimics for peptidyl-tRNA termini reported in the literature have relied on puromycin (Pmn) as the crucial linker unit,[13] while only few have utilized 3′-amino- or very recently, 3′-triazolyladenosine derivatives.[14,15] For instance, CCPmn, ACCPmn, and derivatives thereof were synthesized to represent the CCA-amino acid terminus of amino acid charged tRNA (Figure 3).[13a,b] Since these compounds possess a stable 3′-amide linkage they were successfully applied in soaking experiments with ribosome crystals, and subsequent X-ray structure determination of the co-crystals provided insight into the interactions between tRNA termini and the PTC, and the process of peptide bond formation.[13c] Moreover, several transition-state analogues were synthesized based on the Pmn building block,[13c–f] and binding of the respective derivatives to the ribosomal PTC was successfully characterized at high resolution.[13c,g-j]

Using puromycin as a linker for RNA–peptide conjugates to study macrolide antibiotic resistance has a major drawback, namely that it restricts the C-terminal position of the peptide sequence to a methylated tyrosine side chain for every peptide being assembled. Tyrosine is very rarely found at the C-terminal position of the resistance peptides, whereas hydrophobic amino acids such as valine, leucine, and isoleucine are more common. Therefore, the ability to vary the amino acid present at each position was a major driving force to develop the RNA–peptide conjugates. In addition, we wanted to be able to conveniently prepare both A76 and 2′-deoxy A76 analogues of each of the respective RNA–peptide conjugates.

Another advantage of the RNA–peptide conjugates synthesized here is that they provide a free adenosine C6-NH_2 group (as in tRNA) instead of the N^6,N^6-dimethylated counterpart (as in puromycin analogues), which results in high solubility in aqueous buffer solutions. To this end, a 5′-phosphate can be easily attached through solid-phase synthesis to the ACCA moiety to confer additional solubility of the conjugates when larger hydrophobic peptides are required.

With the present flexible strategy for the convenient chemical synthesis of RNA-peptide conjugates as non-hydrolyzable peptidyl-tRNA mimics we have laid the basis for our ongoing biochemical and structural studies on macrolide antibiotic release. Furthermore, the technology presented here can be used to generate full-length non-hydrolyzable peptidyl-tRNA constructs by enzymatic ligation using T4 DNA or T4 RNA ligase.[16] Such substrates should be useful for the generation and characterization of stalled ribosomes,[17] as well as for general investigations into the interactions between the peptide chain of 3′-charged tRNA with the ribosomal exit tunnel.

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[1] a) A. S. Mankin, *Curr. Opin. Microbiol.* **2008**, *11*, 414–421; b) T. Tenson, A. S. Mankin, *Mol. Microbiol.* **2006**, *59*, 1664–1677; c) V. Vimberg, L. Xiong, M. Bailey, T. Tenson, A. S. Mankin, *Mol. Microbiol.* **2004**, *54*, 376–385; d) T. Tenson, A. S. Mankin, *Peptides* **2001**, *22*, 1661–1668; e) S. Tripathi, P. S. Kloss, A. S. Mankin, *J. Biol. Chem.* **1998**, *273*, 20073–20077; f) T. Tenson, L. Xiong, P. Kloss, A. S. Mankin, *J. Biol. Chem.* **1997**, *272*, 17425–17430; g) M. Dam, S. Douthwaite, T. Tenson, A. S. Mankin, *J. Mol. Biol.* **1996**, *259*, 1–6; h) T. Tenson, A. DeBlasio, A. S. Mankin, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 5641–5646.

[2] M. Lovmar, K. Nilsson, V. Vimberg, T. Tenson, M. Nervall, M. Ehrenberg, *J. Biol. Chem.* **2006**, *281*, 6742–6750.

[3] L. Verdier, J. Gharbi-Benarous, G. Bertho, P. Mauvais, J.-P. Girault, *Biochemistry* **2002**, *41*, 4218–4229.

[4] a) K. H. Nierhaus in *Protein Synthesis and Ribosome Structure* (Eds.: K. H. Nierhaus, D. N. Wilson), Wiley-VCH, Weinheim, **2004**, pp. 323–336; b) D. N. Wilson, K. H. Nierhaus, *Angew. Chem.* **2003**, *115*, 3586–3610; *Angew. Chem. Int. Ed.* **2003**, *42*, 3464–3486; c) Y. Lapidot, N. De Groot, S. Rappoport, *Biochim. Biophys. Acta Nucleic Acids Protein Synth.* **1969**, *182*, 105–112.

[5] M. A. Darken, *Pharmacol. Rev.* **1964**, *16*, 223–243.

[6] a) M. Koch, Y. Huang, M. Sprinzl, *Angew. Chem.* **2008**, *120*, 7352–7355; *Angew. Chem. Int. Ed.* **2008**, *47*, 7242–7245; b) K. Lang, M. Erlacher, D. N. Wilson, R. Micura, N. Polacek, *Chem. Biol.* **2008**, *15*, 485–492; c) K. S. Huang, N. Carrasco, E. Pfund,

S. A. Strobel, *Biochemistry* **2008**, *47*, 8822 – 8827; d) S. Chládek, Sprinzl, *Angew. Chem.* **1985**, *97*, 377 – 398; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 371 – 391.

[7] S. R. Waldvogel, W. Pfleiderer, *Helv. Chim. Acta* **1998**, *81*, 46 – 58.

[8] Y. C. Jack Chen, F. Hansske, K. D. Janda, M. J. Robins, *J. Org. Chem.* **1991**, *56*, 3410 – 3413.

[9] a) C. Höbartner, R. Rieder, C. Kreutz, B. Puffer, K. Lang, A. Polonskaia, A. Serganov, R. Micura, *J. Am. Chem. Soc.* **2005**, *127*, 12035 – 12045; b) H. Moroder, C. Kreutz, K. Lang, A. Serganov, R. Micura, *J. Am. Chem. Soc.* **2006**, *128*, 9909 – 9918; c) R. Micura, C. Höbartner, R. Rieder, C. Kreutz, B. Puffer, K. Lang, H. Moroder, *Curr. Protoc. Nucl. Acid Chem.* **2007**, Wiley, C1.15.1.–C1.15.34.

[10] a) H. Chapuis, P. Strazewski, *Tetrahedron* **2006**, *62*, 12108 – 12115; b) A. Charafeddine, W. Dayoub, H. Chapuis, P. Strazewski, *Chem. Eur. J.* **2007**, *13*, 5566 – 5584; c) B. Y. Michel, K. S. Krishnakumar, P. Strazewski, *Synlett* **2008**, 2461 – 2464; d) H. Saneyoshi, B. Y. Michel, Y. Choi, P. Strazewski, V. E. Marquez, *J. Org. Chem.* **2008**, *73*, 9435 – 9438.

[11] V. Marchán, C. Rodríguez-Tanty, M. Estrada, E. Pedroso, A. Grandas, *Eur. J. Org. Chem.* **2000**, 2495 – 2500.

[12] a) K. H. Nierhaus in *Protein Synthesis and Ribosome Structure* (Eds.: K. H. Nierhaus, D. N. Wilson), Wiley-VCH, Weinheim, **2004**, pp. 207 – 217; b) D. Moazed, H. F. Noller, *Cell* **1989**, *57*, 585 – 597.

[13] a) K. Okuda, A. C. Seila, S. A. Strobel, *Tetrahedron* **2004**, *60*, 12101 – 12112; b) K. Okuda, A. C. Seila, S. A. Strobel, *Biochemistry* **2005**, *44*, 6675 – 6684; c) T. M. Schmeing, A. C. Seila, J. L. Hansen, B. Freeborn, J. K. Soukup, S. A. Scaringe, S. A. Strobel, P. B. Moore, T. A. Steitz, *Nat. Struct. Biol.* **2002**, *9*, 225 – 230; d) K. S. Huang, J. S. Weinger, E. B. Butler, S. A. Strobel, *J. Am. Chem. Soc.* **2006**, *128*, 3108 – 3109; e) J. S. Weinger, D. Kitchen, S. A. Scaringe, S. A. Strobel, G. W. Muth, *Nucleic Acids Res.* **2004**, *32*, 1502 – 1511; f) A. V. Manuilov, S. S. Hixson, R. A. Zimmermann, *RNA* **2007**, *13*, 793 – 800; g) T. M. Schmeing, K. S. Huang, D. E. Kitchen, S. A. Strobel, T. A. Steitz, *Mol. Cell* **2005**, *20*, 437 – 448; h) P. Nissen, J. Hansen, N. Ban, P. B. Moore, T. A. Steitz, *Science* **2000**, *289*, 920 – 930; i) M. Zhong, S. A. Strobel, *Org. Lett.* **2006**, *8*, 55 – 58; j) M. Zhong, S. A. Strobel, *J. Org. Chem.* **2008**, *73*, 603 – 611.

[14] a) S. Terenzi, E. Biała, N. Q. Nguyen-Trung, P. Strazewski, *Angew. Chem.* **2003**, *115*, 3015 – 3018; *Angew. Chem. Int. Ed.* **2003**, *42*, 2909 – 2912; b) A. N. Lazar, A. W. Coleman, S. Terenzi, P. Strazewski, *Chem. Commun.* **2006**, 63 – 65; c) N. Q. Nguyen-Trung, S. Terenzi, G. Scherer, P. Strazewski, *Org. Lett.* **2003**, *5*, 2603 – 2606; d) N. Q. Nguyen-Trung, O. Botta, S. Terenzi, P. Strazewski, *J. Org. Chem.* **2003**, *68*, 2038 – 2041; e) B. Zhang, L. Zhang, L. Sun, Z. Cui, *Org. Lett.* **2002**, *4*, 3615 – 3618.

[15] a) M. Chemama, M. Fonvielle, M. Arthur, J. M. Valéry, M. Etheve-Quelquejeu, *Chem. Eur. J.* **2009**, *15*, 1929 – 1938; b) M. Chemama, M. Fonvielle, R. Villet, M. Arthur, J. M. Valéry, M. Etheve-Quelquejeu, *J. Am. Chem. Soc.* **2007**, *129*, 12642 – 12643.

[16] K. Lang, R. Micura, *Nat. Protoc.* **2008**, *3*, 1457 – 1466.

[17] N. Vazquez-Laslop, C. Thum, A. S. Mankin, *Mol. Cell* **2008**, *30*, 190 – 202.

Communications

RNA–Peptide Conjugates

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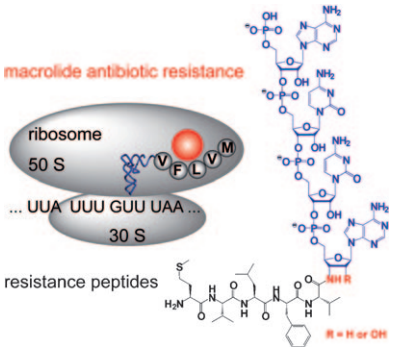
Non-Hydrolyzable RNA–Peptide Conjugates: A Powerful Advance in the Synthesis of Mimics for 3′-Peptidyl tRNA Termini



Translation of specific small peptides on the ribosome can confer resistance to macrolide antibiotics. To reveal the molecular details of this and related phenomena, stable RNA–peptide conjugates that mimic peptidyl-tRNA would be desirable, especially for ribosome structural biology. A flexible solid-phase synthesis strategy now allows efficient access to these highly requested derivatives without restriction on the RNA and peptide sequences.