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Functionalized polystyrene supports for solid-phase synthesis of glycyl-, alanyl- and isoleucyl-RNA conjugates as hydrolysis-resistant mimics of peptidyl-tRNAs

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ABSTRACT

RNA-peptide conjugates that mimic amino acid-charged tRNAs and peptidyl-tRNAs are of high importance for structural and functional investigations of ribosomal complexes. Here, we present the synthesis of glycyl-, alanyl-, and isoleucyladenosine modified solid supports that are eligible for the synthesis of stable 3′-aminoacyl- and 3′-peptidyl-tRNA termini with an amide instead of the natural ester linkage. The present work significantly expands the range of accessible peptidyl-tRNA mimics for ribosomal studies.

1. Introduction

X-ray and cryo-electron microscopy studies of the ribosome and its subunits have revolutionized the field of ribosome research.[1–5] They bring new insights into functional and mechanistic details of the ribosome and facilitate the design of genetic and biochemical experiments due to the knowledge of exact residue positions. For many structural approaches, ribosome assembly with peptidyl- or aminoacyl-tRNA derivatives is required, however, these efforts are often impeded because of the easily hydrolizable ester group that connects the peptidyl and the tRNA moieties.[6] A stable linkage can overcome the limitations arising from hydrolysis, but also from ribose 2′-*O*/3′-*O* transesterification or transpeptidation. In analogy to the naturally occurring antibiotic puromycin, 3′-amide-linked aminoacyl- or peptidyl-tRNA mimics fulfill the requirement of higher stability during ribosome/tRNA assembly procedures (Fig. 1).[6–8] For the investigation and functional characterization of different states of ribosomal translation, such as pre- and post-peptidyl transfer states,[9–14] tRNA hybrid states,[15,16] as well as translation initiation,[17] elongation,[18,19] and termination,[20,21] nonhydrolyzable tRNA mimics are of high relevance. Furthermore, investigations of phenomena such as peptide-mediated macrolide antibiotic resistance and ribosome stalling are also dependent on this type of tRNA derivatives.[22]

For a long time, the synthesis of 3′-aminoacyl- and 3′-peptidyl-RNA conjugates represented a serious bottleneck for such studies. Although a profound fundament for their chemical synthesis was presented by Strazewski and coworkers in 2003,[23–25] significant improvements of the approach were required in terms of efficiency which have been introduced only recently.[26] The synthetic strategy for RNA-peptide conjugates relies on 3′-aminoacylamino-3′-deoxyadenosine modified solid supports. Thereby, the solid support material is attached to the 2′-hydroxyl group of the adenosine moiety (representing A76 of the tRNA 3′-terminus); the corresponding amino acid (representing the C-terminus of the peptide chain) is linked to the ribose 3′-amino-3′-deoxy modification via an amide bond.

Here, we present the synthesis of glycyl-, alanyl-, and isoleucyladenosine modified solid supports providing the above mentioned constitutional properties and following the lines of our previously published synthetic route.[26] These supports were applied in peptide and oligonucleotide synthesis and yielded the desired hydrolysis-resistant peptidyl-tRNA mimics after deprotection and purification in good yields and high purity. The present work therefore expands the range of accessible peptidyl-tRNA mimics which have been limited so far to methionine, phenylalanine, and valine at the carboxy terminus of the peptide chain.[26]

2. Results and discussion

The starting point for our undertaking was the 3′-azido-3′-deoxyadenosine derivative **1**, which was readily available from

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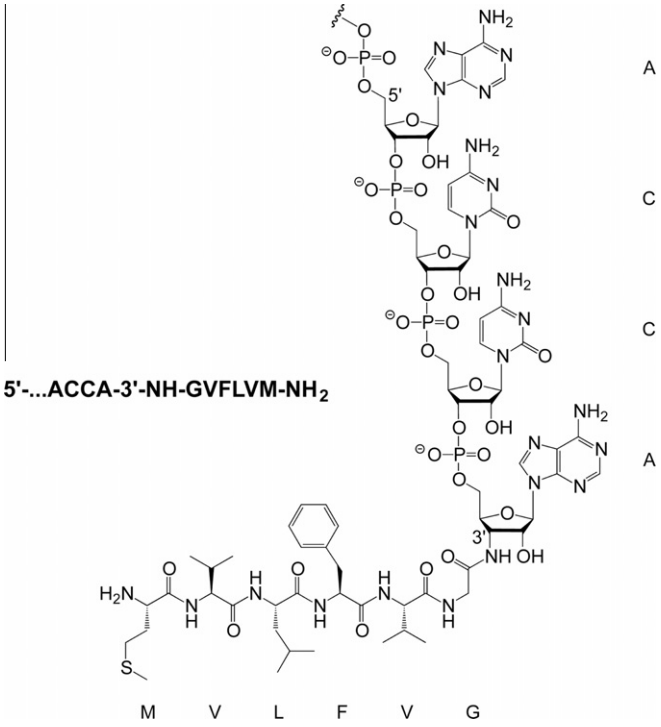
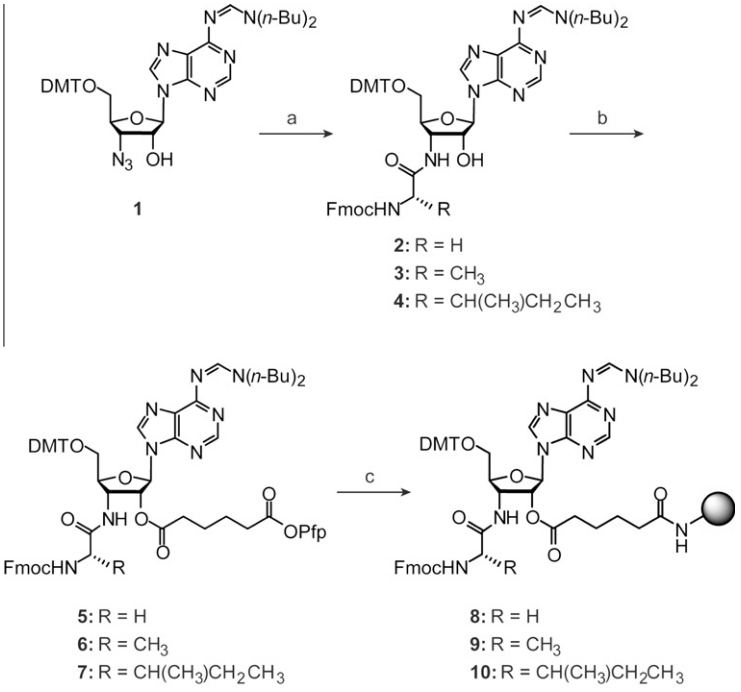


Figure 1. Constitution and sequence annotation of an exemplary target RNA-peptide conjugate that mimics 3'-peptidyl-tRNA and comprises a hydrolysis-resistant 3'-ribose amide linkage.

9-(β-D-arabinofuranosyl)adenine in six steps according to our previously introduced synthesis.[26] Subsequent coupling[27] with alanine, glycine or isoleucine furnished the amino acid linked building blocks **2**–**4**, which were further transformed into the pentafluorophenyl active esters **5**–**7** using adipic acid bis(pentafluorophenyl)ester as linker molecule.[8] Coupling of compounds



Scheme 1. Synthesis of the modified solid supports **8**, **9**, and **10**. Reaction conditions: (a) 1.3 equiv Fmoc-Ala-OBt, Fmoc-Gly-OBt or Fmoc-Ile-OBt, 2.2 equiv Me_3P in tetrahydrofuran, 0 °C to rt, 16 h, 74–81%; (b) 5 equiv adipic acid bis(pentafluorophenyl)ester, 1 equiv DMAP in *N*,*N*-dimethylformamide/pyridine (1/1, v/v), rt, 1 h, 66–79%; (c) 3 equiv (w/w) amino-functionalized polystyrene support (*GE Healthcare*, Custom Primer Support™ 200 Amino), 2 equiv pyridine in *N*,*N*-dimethylformamide, rt, 22 h, loading: 45–63 μmol/g. Me_3P = trimethylphosphine, DMAP = 4-(*N*,*N*-dimethylamino)pyridine, HOBt = *N*-hydroxybenzotriazole, Fmoc = *N*-(9-fluorenyl)methoxycarbonyl.

5–**7** to amino-modified polystyrene (*GE Healthcare*, Custom Primer Support™ 200 Amino) finally yielded the desired 3′-aminoacylamino-3′-deoxyadenosine-functionalized solid supports **8**–**10** (Scheme 1).

These supports were then utilized for the elongation of the peptide chain by automated solid-phase peptide synthesis based on *N*-(9-fluorenyl)methoxycarbonyl (Fmoc) protected amino acids. Peptide synthesis was carried out using a multiple peptide synthesizer (*Intavis*) equipped with a shaking device under standard conditions. Alternatively, for the synthesis of tRNA mimics with a sole glycine, alanine, or isoleucine unit at the 3′-terminus, the solid supports **8**–**10** were directly supplied to RNA synthesis.

Oligoribonucleotide assembly based on 2′-*O*-[(triisopropylsilyl)oxy]methyl (2′-*O*-TOM)[28,29] protected nucleoside phosphoramidites was conducted on a DNA/RNA synthesizer in automated manner following standard synthesis protocols. Furthermore, we point out that the introduction of 5′-phosphate groups at the 5′-end of RNA-peptide conjugates was accomplished as final cycle of the automated solid-phase synthesis by using a modified phosphoramidite building block prepared according to the literature.[30]

Deprotection under basic conditions using methylamine in ethanol/water cleaved the cyanoethyl, acyl, amidine, and Fmoc protecting groups and resulted in the release of the conjugates from the solid support. Subsequent treatment with 1.0 M tetrabutylammonium fluoride trihydrate (TBAF·$3H_2O$) in tetrahydrofuran at 37 °C cleaved the 2′-*O*-TOM groups overnight. For short conjugates comprising only four nucleotides, 10% water was added to the fluoride deprotection solution (0.9 M TBAF·$3H_2O$ in tetrahydrofuran/water (9/1, v/v)) and the deprotection was carried out at room temperature. This procedure resulted in high quality crude products which were analyzed by anion-exchange HPLC. The major peak of the HPLC profiles typically represented the desired conjugate which was purified on a semi-preparative column to achieve purities of more than 95%. Finally, the constitution of the products was analyzed by LC-ESI mass spectrometry and confirmed the expected molecular weights (Fig. 2).

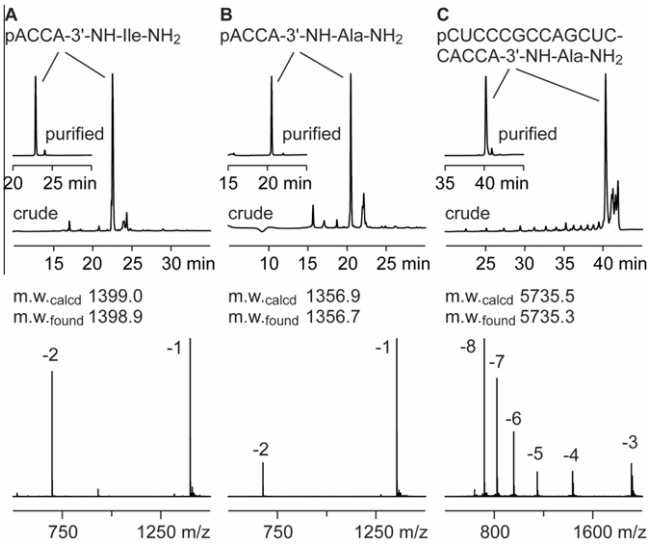


Figure 2. Synthesis of RNA-peptide conjugates based on the modified solid supports **9** and **10**. Anion-exchange HPLC profiles of crude and purified (insets) conjugates **S7**, **S4**, and **S6** (**A**–**C**, upper panels) and LC-ESI mass spectra of purified products (**A**–**C**, lower panels). Anion-exchange chromatography conditions: *Dionex* DNAPac®PA-100 (4 × 250 mm) column; temperature: 60 °C; flow rate: 1 mL/min; eluant A: 25 mM Tris–HCl (pH 8.0), 6 M urea; eluant B: 25 mM Tris–HCl (pH 8.0), 6 M urea, 500 mM $NaClO_4$; gradient: 0–35% B in A within 30 min; UV detection at 260 nm.

Following this approach, we prepared the RNA-peptide conjugates listed in Table 1. All three supports (**8**–**10**) were first tested in the construction of short conjugates (**S1**, **S4**, **S5**, and **S7**) containing the highly conserved ACCA tRNA 3′-terminus. The larger conjugate **S3** represents a peptidyl-RNA fragment whose peptide portion relates to a macrolide antibiotic resistance peptide[31,32] and whose RNA portion allows for the ligation to full-length tRNA according to a recently introduced concept for the 3′-peptidyl-tRNA semisynthesis.[7,8] Likewise, conjugate **S6** represents a peptidyl-RNA fragment appropriate for the construction of hydrolysis-resistant tmRNA derivatives.[33,34] Conjugate **S2** consists of the same RNA and peptide sequences as encountered in **S3**, however, the thioether moiety of methionine became oxidized during oligonucleotide synthesis because of repeated oxidation after each coupling step which is required for the P(III) to P(V) transformation. In the present case, about 50% of the conjugate became oxidized and **S2** and **S3** were separated during anion-exchange chromatographic purification. Alternatively, reduction of the sulfoxide moitey using *N*-methylmercapto-acetamide is conceivable in analogy to reports in the literature.[35] To handle oxidation of the methionine moiety, one may also consider replacement of the aqueous iodine solution used here by *tert*-butyl hydroperoxide solution in THF or acetonitrile during oligonucleotide solid-phase synthesis.[36] This alternative oxidizing reagent has been used for example, for synthesis of spin-labeled, nitroxide containing oligonucleotides,[37] but also for RNA containing other oxidation-sensitive moieties, such as 2-thiouridines[38] or 2′-methylseleno groups.[39] Preliminary experiments using *tert*-butyl hydroperoxide solutions for the synthesis of the RNA-peptide conjugates presented here,[40] however, were unsuccessful in our hands.

A critical view on the synthetic approach presented reveals that there are some limitations concerning the variability of the peptide sequence. In short, amino acid side chains that can be protected by silyl- or allyl-protecting groups during conjugate synthesis are compatible, and RNA-peptide conjugates containing serine, threonine, tyrosine, as well as glutamic or aspartic acid are accessible.[25,26] Furthermore, preliminary experiments on the incorporation of cysteine with disulfide protection were promising. More difficulties were encountered for arginine containing conjugates for which we are currently exploring alternatives based on native chemical ligation.[41]

Table 1
Selection of synthesized RNA-peptide conjugates

No	Sequence[a]	Amount [nmol]	M.W.$_{calcd}$ [amu]	M.W.$_{found}$ [amu]
S1	5′-p-GCACCA-3′-NH-Gly-NH_2	185	1993.2	1993.0
S2	5′-p-UCCCGUCAUCACCC-ACCA-3′-NH-GVFLVM^{ox}-NH_2	32	6312.2	6312.5
S3	5′-p-UCCCGUCAUCACCC-ACCA-3′-NH-GVFLVM-NH_2	35	6296.2	6296.5
S4	5′-p-ACCA-3′-NH-Ala-NH_2	118	1356.9	1356.7
S5	5′-p-GCACCA-3′-NH-Ala-NH_2	123	2007.3	2007.1
S6	5′-p-CUCCCGCCAGCUCC-ACCA-3′-NH-Ala-NH_2	186	5735.5	5735.3
S7	5′-p-ACCA-3′-NH-Ile-NH_2	112	1399.0	1398.9

[a] To indicate the correct connectivity via an amide bond, peptide sequences of the conjugates are written unconventionally (from C- to N-terminus). Amino acid one-letter code is used for RNA-peptide conjugates; amino acid three-letter code for single amino acid-RNA conjugates.

3. Conclusion

The functionalized solid supports (**8**–**10**) whose efficient synthesis is presented in this work increase amino acid side chain flexibility of hydrolysis-resistant 3′-peptidyl-tRNA mimics and therefore expand the scope of applications of these derivatives which are required to explore mechanistic details of ribosomal translation.[22] Our approach is characterized by easy handling, high efficiency and high reliability. The given examples of synthetic conjugates represent tRNA fragments that are also suited for enzymatic ligation to the corresponding full-length tRNA derivatives.[7] They are considered to further consolidate and disseminate our recently introduced semisynthetic strategy for chemically modified tRNA species that relies on site-specific cleavage of natural tRNAs by DNA enzymes followed by enzymatic ligation using synthetic fragments as prepared in this study.[7,8]

4. Experimental

4.1. Solid support synthesis

4.1.1. General remarks

^{1}H and ^{13}C NMR spectra were recorded on a *Bruker* UltraShield™ Plus 600 MHz instrument. The chemical shifts (δ) are reported relative to tetramethylsilane (TMS) and referenced to the residual proton signal of the deuterated solvent $CDCl_3$: 7.26 ppm for ^{1}H NMR spectra or 77.1 ppm for ^{13}C NMR spectra. ^{1}H and ^{13}C assignments are based on COSY and HSQC experiments. MS experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrumentation (*Thermo Fisher Scientific*) with an electrospray ion source. Samples were analyzed in the positive- or negative-ion mode. Reaction control was performed via analytical thin-layer chromatography (TLC, *Macherey–Nagel*) with fluorescent indicator. Column chromatography was carried out on Silica gel 60 (70–230 mesh). Chemical reagents and solvents were purchased from commercial suppliers (*Sigma–Aldrich*, *Acros*, *IRIS Biotech GmbH*) and used without further purification. Custom Primer Support™ 200 Amino was purchased from *GE Healthcare*. Organic solvents for reactions were dried overnight over freshly activated molecular sieves (4 Å). The reactions were carried out under argon atmosphere.

4.1.2. 6-*N*-[(Di-*n*-butylamino)methylene]-3′-{[*N*-(9-fluorenyl) methoxycarbonyl-L-glycyl]amino}-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-D-adenosine (2)

N-(9-Fluorenyl)methoxycarbonyl-L-glycine (132 mg, 0.443 mmol) was co-evaporated three times with dry tetrahydrofuran and then dissolved in 6.5 mL dry tetrahydrofuran. After cooling the solution to 0 °C, 1-hydroxybenzotriazole hydrate (78 mg, 0.509 mmol) was added. The solution was stirred for 10 min and *N*,*N*-diisopropylcarbodiimide (90 μL, 0.581 mmol) was added. After 10 more minutes, compound **1** (250 mg, 0.341 mmol), which was dissolved in 9.8 mL dry tetrahydrofuran, was slowly dropped into the solution. Then, 15 min later trimethylphosphine in tetrahydrofuran (715 μL, 0.715 mmol, 1.0 M in tetrahydrofuran) was added. The solution was allowed to warm to room temperature and stirred for 16 h. The solvent and the volatile compounds were evaporated under reduced pressure and the residue was dissolved in dichloromethane. The solution was extracted with water and with half saturated sodium bicarbonate solution. The organic layers were dried over Na_2SO_4 and evaporated. Compound **2** was purified by column chromatography on SiO_2 with dichloromethane/methanol, 99.5:0.5–97:3. Yield: 249 mg of **2** as white foam (74%). TLC (8% methanol in dichloromethane): R_f = 0.42. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.97 (q, J = 7.3 Hz, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.39 (td, J_1 = 7.7 Hz, J_2 = 15.6 Hz, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.67 (tt, J_1 = 7.3 Hz, J_2 = 14.7 Hz, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 3.40–3.52 (m, 4H, $N(CH_2CH_2CH_2CH_3)$, H(a)-C(5′), H(b)-C(5′)); 3.67–3.76 (m, 8H, $N(CH_2CH_2CH_2CH_3)$), 2× O–CH_3(DMT)); 3.79–3.94 (m, 2H, H_2C(α, Gly)); 4.22 (t, J = 6.8 Hz, 1H, H–C(9, Fmoc)); 4.41–4.43 (m, 3H, H–C(4′), O–CH_2(Fmoc)); 4.60 (m, 1H, H–C(3′)); 4.88 (s, 1H, H–C(2′)); 5.58 (s, br, 1H, HN(Gly)); 5.96 (s, 1H, H–C(1′)); 6.31 (s, br, 1H, HO–C(2′)); 6.77 (d, J = 7.7 Hz, 4H, H–C(ar)); 6.92 (m, 1H, HN–C(3′)); 7.15–7.31 (m, 13H, H–C(ar)); 7.35–7.40 (m, 2H, H–C(ar)); 7.59 (d, J = 6.6 Hz, 2H, H–C(ar)); 7.75 (d, J = 7.6 Hz, 2H, H–C(ar)); 8.14 (s, 1H, H–C(8)); 8.47 (s, 1H, H–C(2)); 9.07 (s, 1H, *HC*=N–C(6)) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.75, 13.99 ($N(CH_2CH_2CH_2CH_3)_2$); 19.85, 20.29 ($N(CH_2CH_2CH_2CH_3)_2$); 29.35, 31.05 ($N(CH_2CH_2CH_2CH_3)_2$); 44.58 (C(α, Gly)); 45.36 ($N(CH_2CH_2CH_2CH_3)$); 47.16 (C(9, Fmoc)); 52.06 ($N(CH_2CH_2CH_2CH_3)$); 52.78 (C(3′)); 55.25 (2× O–CH_3(DMT)); 63.65 (C(5′)); 67.36 (O–CH_2(Fmoc)); 74.59 (C(2′)); 80.07; 84.38 (C(4′)); 86.67; 91.36 (C(1′)); 113.22, 120.06, 125.10, 126.39, 126.90, 127.16, 127.81, 127.89, 128.21, 130.13 (C(ar)); 135.69; 135.73; 139.54 (C(8)); 141.36; 143.80; 144.54; 150.66; 152.29 (C(2)); 156.65; 158.56; 158.93; 160.50; 169.53 ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{57}H_{62}N_8O_8$, 988.15. Found 987.42.

4.1.3. 6-*N*-[(Di-*n*-butylamino)methylene]-3′-{[*N*-(9-fluorenyl) methoxycarbonyl-L-alanyl]amino}-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-D-adenosine (3)

N-(9-Fluorenyl)methoxycarbonyl-L-alanine (138 mg, 0.443 mmol) was co-evaporated three times with dry tetrahydrofuran and then dissolved in 6.5 mL dry tetrahydrofuran. After cooling the solution to 0 °C, 1-hydroxybenzotriazole hydrate (78 mg, 0.509 mmol) was added. The solution was stirred for 10 min and *N*,*N*-diisopropylcarbodiimide (90 μL, 0.581 mmol) was added. After 10 more minutes, compound **1** (250 mg, 0.341 mmol), which was dissolved in 9.8 mL dry tetrahydrofuran, was slowly dropped into the solution. Then, 15 min later trimethylphosphine in tetrahydrofuran (715 μL, 0.715 mmol, 1.0 M in tetrahydrofuran) was added. The solution was allowed to warm to room temperature and stirred for 16 h. The solvent and the volatile compounds were evaporated under reduced pressure and the residue was dissolved in dichloromethane. The solution was extracted with water and with half saturated sodium bicarbonate solution. The organic layers were dried over Na_2SO_4 and evaporated. Compound **3** was purified by column chromatography on SiO_2 with dichloromethane/methanol, 99:1–97:3. Yield: 275 mg of **3** as white foam (81%). TLC (8% methanol in dichloromethane): R_f = 0.48. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.97 (q, J = 7.0 Hz, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.36–1.44 (m, 7H, $N(CH_2CH_2CH_2CH_3)_2$, H_3C(β, Ala)); 1.68 (tt, J_1 = 7.7 Hz, J_2 = 15.4 Hz, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 3.40–3.44 (m, 3H, $N(CH_2CH_2CH_2CH_3)$, H(a)-C(5′)); 3.49 (dd, J_1 = 2.6 Hz, J_2 = 10.6 Hz, 1H, H(b)-C(5′)); 3.69–3.78 (m, 8H, $N(CH_2CH_2CH_2CH_3)$), 2× O–CH_3(DMT)); 4.21–4.24 (m, 2H, H–C(α, Ala), H–C(9, Fmoc)); 4.38–4.43 (m, 3H, H–C(4′), O–CH_2(Fmoc)); 4.60 (m, 1H, H–C(3′)); 4.86 (t, J = 4.9 Hz, 1H, H–C(2′)); 5.35 (d, J = 6.4 Hz, 1H, HN(Ala)); 5.97 (s, 2H, H–C(1′), HO–C(2′)); 6.77 (dd, J_1 = 2.2 Hz, J_2 = 8.7 Hz, 4H, H–C(ar)); 6.82 (d, J = 4.8 Hz, 1H, HN–C(3′)); 7.18–7.41 (m, 13H, H–C(ar)); 7.58 (t, J = 8.2 Hz, 2H, H–C(ar)); 7.76 (t, J = 6.6 Hz, 2H, H–C(ar)); 8.15 (s, 1H, H–C(8)); 8.50 (s, 1H, H–C(2)); 9.07 (s, 1H, *HC*=N–C(6)) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.76, 13.99 ($N(CH_2CH_2CH_2CH_3)_2$); 18.67 (C(β, Ala)); 19.85, 20.29 ($N(CH_2CH_2CH_2CH_3)_2$); 29.35, 31.06 ($N(CH_2CH_2CH_2CH_3)_2$); 45.33 ($N(CH_2CH_2CH_2CH_3)$); 47.15 (C(9, Fmoc)); 50.69 (C(α, Ala)); 52.02 ($N(CH_2CH_2CH_2CH_3)$); 52.51 (C(3′)); 55.25 (2× O–CH_3(DMT)); 63.36 (C(5′)); 67.26 (O–CH_2(Fmoc)); 74.64 (C(2′)); 80.07; 83.60 (C(4′)); 86.60; 91.33 (C(1′)); 113.22, 120.04, 120.07, 125.06, 125.11, 126.43, 126.89, 127.14, 127.16, 127.81, 127.91, 128.22, 130.13 (C(ar)); 135.67; 135.70; 139.54 (C(8)); 141.33; 141.36; 143.70; 143.83; 144.51; 150.63; 152.39 (C(2)); 156.16; 158.56; 158.87; 160.48; 173.01 ppm. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{58}H_{64}N_8O_8$, 1002.18. Found 1001.67.

4.1.4. 6-*N*-[(Di-*n*-butylamino)methylene]-3′-{[*N*-(9-fluorenyl) methoxycarbonyl-L-isoleucyl]amino}-3′-deoxy-5′-*O*-(4,4′-di methoxytrityl)-β-D-adenosine (4)

N-(9-Fluorenyl)methoxycarbonyl-L-isoleucine (179 mg, 0.506 mmol) was three times co-evaporated with dry tetrahydrofuran and then dissolved in 8.2 mL dry tetrahydrofuran. After cooling the solution to 0 °C, 1-hydroxybenzotriazole hydrate (103 mg, 0.673 mmol) was added. The solution was stirred for 10 min and *N*,*N*-diisopropylcarbodiimide (104 μL, 0.673 mmol) was added. After 10 more minutes compound **1** (286 mg, 0.389 mmol), which was dissolved in 11.1 mL dry tetrahydrofuran, was slowly dropped into the solution. Then, 15 min later trimethylphosphine in tetrahydrofuran (848 μL, 0.848 mmol, 1.0 M in tetrahydrofuran) was added. The solution was allowed to warm to room temperature and stirred for 16 h. The solvent and the volatile compounds were evaporated under reduced pressure and the residue was dissolved in dichloromethane. The solution was extracted with water and with half saturated sodium bicarbonate solution. The organic layers were dried over Na_2SO_4 and evaporated. Compound **4** was purified by column chromatography on SiO_2 with dichloromethane/methanol, 99.5:0.5–97:3. Yield: 304 mg of **4** as white foam (75%). TLC (8% methanol in dichloromethane): R_f = 0.50. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.78 (d, J = 6.7 Hz, 3H, $H_3C(\gamma'$, Ile)); 0.87 (t, J = 7.2 Hz, 3H, $H_3C(\delta$, Ile)); 0.97 (q, J = 6.5 Hz, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.07–1.12 (m, 1H, H(a)-C(γ, Ile)); 1.36–1.44 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.49 (m, 1H, H(b)-C(γ, Ile)); 1.67–1.72 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.75–1.76 (m, 1H, H–C(β, Ile)); 3.33–3.36 (m, 1H, H(a)-C(5′)); 3.41–3.46 (m, 3H, $N(CH_2CH_2CH_2CH_3)$, H(b)-C(5′)); 3.69 (dt, 1H, J_1 = 7.1 Hz, J_2 = 13.7 Hz, $N(H(a)CH(b)CH_2CH_2CH_3)$); 3.75–3.80 (m, 7H, $N(H(a)CH(b)CH_2CH_2CH_3)$, 2× $O–CH_3$(DMT)); 4.04 (t, J = 8.0 Hz, 1H, H–C(α, Ile)); 4.18 (t, J = 6.7 Hz, 1H, H–C(9, Fmoc)); 4.33–4.34 (m, 3H, H–C(4′), $O–CH_2$(Fmoc)); 4.69–4.72 (m, 1H, H–C(3′)); 4.77 (m, 1H, H–C(2′)); 5.47 (d, J = 8.9 Hz, 1H, HN(Ile)); 6.05 (s, 1H, H–C(1′)); 6.14 (s, 1H, HO–C(2′)); 6.78 (d, J = 7.8 Hz, 4H, H–C(ar)); 6.84 (d, J = 5.6 Hz, 1H, HN–C(3′)); 7.18–7.33 (m, 10H, H–C(ar)); 7.38 (t, J = 7.1 Hz, 3H, H–C(ar)); 7.55 (dd, J_1 = 7.7 Hz, J_2 = 16.8 Hz, 2H, H–C(ar)); 7.73 (dd, J_1 = 7.6 Hz, J_2 = 15.6 Hz, 2H, H–C(ar)); 8.18 (s, 1H, H–C(8)); 8.54 (s, 1H, H–C(2)); 9.07 (s, 1H, *HC*=N–C(6)) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.34 (C(δ, Ile)); 13.74, 13.98 ($N(CH_2CH_2CH_2CH_3)_2$); 15.37 (C(γ′, Ile)); 19.83, 20.27 ($N(CH_2CH_2CH_2CH_3)_2$); 25.07 (C(γ, Ile)); 29.34, 31.05 ($N(CH_2CH_2CH_2CH_3)_2$); 37.51 (C(β, Ile)); 45.31 ($N(CH_2CH_2CH_2CH_3)$); 47.17 (C(9, Fmoc)); 51.93 (C(3′)); 52.00 ($N(CH_2CH_2CH_2CH_3)$); 55.22 (2× $O–CH_3$(DMT)); 59.66 (C(α, Ile)); 63.11 (C(5′)); 67.16 ($O–CH_2$(Fmoc)); 74.64 (C(2′)); 80.06; 83.12 (C(4′)); 86.53; 91.42 (C(1′)); 113.20, 119.99, 120.05, 125.07, 126.43, 126.87, 127.10, 127.16, 127.77, 127.79, 127.89, 128.26, 130.16 (C(ar)); 135.64; 139.51 (C(8)); 141.27; 141.34; 143.66; 143.82; 144.45; 150.58; 152.47 (C(2)); 156.47; 158.55; 158.89; 160.45; 171.92 ppm. ESI-MS (*m*/*z*): $[M+H]^+$ calcd for $C_{61}H_{70}N_8O_8$, 1044.26. Found 1043.55.

4.1.5. 6-*N*-[(Di-*n*-butylamino)methylene]-3′-{[*N*-(9-fluorenyl) methoxycarbonyl-L-glycyl]amino}-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[1,6-dioxo-6-(pentafluorophenyloxy) hexyl]-β-D-adenosine (5)

Compound **2** (221 mg, 0.224 mmol) was dissolved in 2.9 mL dry *N*,*N*-dimethylformamide and 2.9 mL dry pyridine. After addition of 4-(*N*,*N*-dimethylamino)pyridine (27 mg, 0.221 mmol) and adipic acid bis(pentafluorophenyl)ester (524 mg, 1.10 mmol) the solution was stirred for 2 h at room temperature. The reaction mixture was evaporated under reduced pressure and afterwards the residue was co-evaporated three times with toluene and once with dichloromethane. Compound **5** was purified by column chromatography on SiO_2 with dichloromethane/acetone, 95:5–80:20. Yield: 192 mg of **5** as white foam (67%). TLC (15% acetone in dichloromethane): R_f = 0.31. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.97 (dt, J_1 = 7.5 Hz, J_2 = 15.7 Hz, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.35–1.41 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.65–1.68 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.73 (m, 4H, $OOCCH_2CH_2CH_2CH_2COO$); 2.42–2.44 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$); 2.65–2.67 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$); 3.41 (t, J = 7.3 Hz, 2H, $N(CH_2CH_2CH_2CH_3)$); 3.47–3.49 (m, 2H, H(a)-C(5′), H(b)-C(5′)); 3.68–3.73 (m, 2H, $N(CH_2CH_2CH_2CH_3)$); 3.78 (s, 6H, 2× $O–CH_3$(DMT)); 3.81 (t, J = 6.7 Hz, 2H, H_2C(α, Gly)); 4.14 (dt, J_1 = 3.8 Hz, J_2 = 7.7 Hz, 1H, H–C(4′)); 4.23 (t, J = 6.7 Hz, 1H, H–C(9, Fmoc)); 4.47 (d, J = 6.8 Hz, 2H, $O–CH_2$(Fmoc)); 5.23 (q, J = 7.2 Hz, 1H, H–C(3′)); 5.41 (s, br, 1H, HN(Gly)); 5.84 (d, J = 2.7 Hz, 1H, H–C(2′)); 6.16 (d, J = 2.8 Hz, 1H, H–C(1′)); 6.40 (d, J = 6.5 Hz, 1H, HN–C(3′)); 6.80 (dd, J_1 = 2.7 Hz, J_2 = 8.9 Hz, 4H, H–C(ar)); 7.19 (t, J = 7.2 Hz, 1H, H–C(ar)); 7.25–7.32 (m, 8H, H–C(ar)); 7.39–7.42 (m, 4H, H–C(ar)); 7.58 (d, J = 6.8 Hz, 2H, H–C(ar)); 7.77 (d, J = 6.1 Hz, 2H, H–C(ar)); 8.07 (s, 1H, H–C(8)); 8.52 (s, 1H, H–C(2)); 9.00 (s, 1H, *HC*=N–C(6)) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.76, 13.96 ($N(CH_2CH_2CH_2CH_3)_2$); 19.86, 20.25 ($N(CH_2CH_2CH_2CH_3)_2$); 23.77, 23.95 ($OOCCH_2CH_2CH_2CH_2COO$); 29.33, 31.08 ($N(CH_2CH_2CH_2CH_3)_2$); 32.86 ($OOCCH_2CH_2CH_2CH_2COO$); 33.26 ($OOCCH_2CH_2CH_2CH_2COO$); 44.88 (C(α, Gly)); 45.31 ($N(CH_2CH_2CH_2CH_3)$); 47.15 (C(9, Fmoc)); 50.75 (C(3′)); 51.97 ($N(CH_2CH_2CH_2CH_3)$); 55.28 (2× $O–CH_3$(DMT)); 63.22 (C(5′)); 67.32 ($O–CH_2$(Fmoc)); 75.29 (C(2′)); 82.26 (C(4′)); 86.87; 87.57 (C(1′)); 100.07; 113.24, 120.14, 124.93, 126.13, 126.96, 127.19, 127.93, 128.25, 129.22, 130.21 (C(ar)); 135.58; 135.66; 137.01; 138.79; 139.91 (C(8)); 140.38; 141.39; 142.00; 143.62; 143.65; 144.47; 151.17; 153.00 (C(2)); 156.88; 158.43; 158.60; 160.17; 169.01; 169.42; 171.50 ppm. ESI-MS (*m*/*z*): $[M+H]^+$ calcd for $C_{69}H_{69}F_5N_8O_{11}$, 1282.33. Found 1281.39.

4.1.6. 6-*N*-[(Di-*n*-butylamino)methylene]-3′-{[*N*-(9-fluorenyl) methoxycarbonyl-L-alanyl]amino}-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[1,6-dioxo-6-(pentafluorophenyloxy) hexyl]-β-D-adenosine (6)

Compound **3** (227 mg, 0.227 mmol) was dissolved in 2.9 mL dry *N*,*N*-dimethylformamide and 2.9 mL dry pyridine. After addition of 4-(*N*,*N*-dimethylamino)pyridine (28 mg, 0.229 mmol) and adipic acid bis(pentafluorophenyl)ester (533 mg, 1.11 mmol) the solution was stirred for 2 h at room temperature. The reaction mixture was evaporated under reduced pressure and afterwards the residue was co-evaporated three times with toluene and once with dichloromethane. Compound **6** was purified by column chromatography on SiO_2 with dichloromethane/acetone, 95:5–80:20. Yield: 195 mg of **6** as white foam (66%). TLC (15% acetone in dichloromethane): R_f = 0.40. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.94–0.99 (m, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.28–1.29 (m, 3H, H_3C(β, Ala)); 1.36–1.40 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.65–1.68 (m, 8H, $N(CH_2CH_2CH_2CH_3)_2$, $OOCCH_2CH_2CH_2CH_2COO$); 2.42 (t, J = 4.5 Hz, 2H, $OOCCH_2CH_2CH_2CH_2COO$); 2.60 (t, J = 4.6 Hz, 2H, $OOCCH_2CH_2CH_2CH_2COO$); 3.41 (t, J = 6.9 Hz, 2H, $N(CH_2CH_2CH_2CH_3)$); 3.46 (m, 2H, H(a)-C(5′), H(b)-C(5′)); 3.65–3.72 (m, 2H, $N(CH_2CH_2CH_2CH_3)$); 3.78 (s, 6H, 2× $O–CH_3$(DMT)); 4.15–4.22 (m, 3H, H–C(α, Ala), H–C(4′), H–C(9, Fmoc)); 4.37 (m, 1H, $O–H(a)CH(b)$(Fmoc)); 4.45–4.48 (m, 1H, O–H(a)*CH(b)* (Fmoc)); 5.28–5.25 (m, 1H, H–C(3′)); 5.27 (s, br, 1H, HN(Ala)); 5.84–5.85 (m, 1H, H–C(2′)); 6.16 (m, 1H, H–C(1′)); 6.52 (s, br, 1H, HN–C(3′)); 6.80 (d, J = 8.4 Hz, 4H, H–C(ar)); 7.19 (t, J = 6.8 Hz, 1H, H–C(ar)); 7.25–7.32 (m, 8H, H–C(ar)); 7.39–7.42 (m, 4H, H–C(ar)); 7.57 (d, J = 6.8 Hz, 2H, H–C(ar)); 7.76 (d, J = 6.8 Hz, 2H, H–C(ar)); 8.08 (s, 1H, H–C(8)); 8.52 (s, 1H, H–C(2)); 8.99 (s, 1H, *HC*=N–C(6)) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.75, 13.93 ($N(CH_2CH_2CH_2CH_3)_2$); 17.71 (C(β, Ala)); 19.86, 20.24 ($N(CH_2CH_2CH_2CH_3)_2$); 23.74, 23.97 ($OOCCH_2CH_2CH_2CH_2COO$); 29.33, 31.08 ($N(CH_2CH_2CH_2CH_3)_2$); 32.84 ($OOCCH_2CH_2CH_2CH_2COO$); 33.28 ($OOCCH_2CH_2CH_2CH_2COO$); 45.33 ($N(CH_2CH_2CH_2CH_3)$); 47.15 (C(9, Fmoc)); 50.47 (C(α, Ala)); 50.66 (C(3′)); 51.98 ($N(CH_2CH_2CH_2CH_3)$);

55.26 (2× O–CH_3(DMT)); 63.18 (C(5′)); 67.26 (O–CH_2(Fmoc)); 75.25 (C(2′)); 80.08; 82.43 (C(4′)); 86.81; 87.58 (C(1′)); 109.45; 113.26, 120.12, 124.95, 125.01, 126.05, 126.94, 127.18, 127.92, 128.30, 129.23, 130.18 (C(ar)); 135.58; 135.68; 137.12; 137.91; 138.75; 139.96; 140.34; 141.37; 142.02; 143.64; 143.71; 144.42; 151.15 (C(2)); 152.97; 156.28; 158.46; 158.63; 160.13; 169.44; 171.48; 172.11 ppm. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{70}H_{71}F_5N_8O_{11}$, 1296.35. Found 1295.30.

4.1.7. 6-*N*-[(Di-*n*-butylamino)methylene]-3′-{[*N*-(9-fluorenyl) methoxycarbonyl-L-isoleucyl]amino}-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[1,6-dioxo-6-(pentafluorophenyloxy) hexyl]-β-D-adenosine (7)

Compound **4** (209 mg, 0.200 mmol) was dissolved in 2.9 mL dry *N*,*N*-dimethylformamide and 2.9 mL dry pyridine. After addition of 4-(*N*,*N*-dimethylamino)pyridine (25 mg, 0.205 mmol) and adipic acid bis(pentafluorophenyl)ester (470 mg, 0.983 mmol) the solution was stirred for 2 h at room temperature. The reaction mixture was evaporated under reduced pressure and afterwards the residue was co-evaporated three times with toluene and once with dichloromethane. Compound **7** was purified by column chromatography on SiO_2 with dichloromethane/acetone, 95:5–85:15. Yield: 213 mg of **7** as white foam (79%). TLC (15% acetone in dichloromethane): R_f = 0.58. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.73 (d, J = 6.7 Hz, 3H, $H_3C(\gamma'$, Ile)); 0.86 (t, J = 7.2 Hz, 3H, $H_3C(\delta$, Ile)); 0.97 (q, J = 7.3 Hz, 6H, $N(CH_2CH_2CH_2CH_3)_2$); 1.04–1.07 (m, 1H, H(a)-C(γ, Ile)); 1.37–1.43 (m, 4H, $N(CH_2CH_2CH_2CH_3)_2$); 1.47–1.49 (m, 1H, H(b)-C(γ, Ile)); 1.63–1.71 (m, 8H, $N(CH_2CH_2CH_2CH_3)_2$, $OOCCH_2CH_2CH_2CH_2COO$); 1.79–1.80 (m, 1H, H–C(β, Ile)); 2.44–2.46 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$); 2.57–2.67 (m, 2H, $OOCCH_2CH_2CH_2CH_2COO$); 3.40–3.43 (m, 3H, $N(CH_2CH_2CH_2CH_3)$, H(a)-C(5′)); 3.47 (dd, J_1 = 5.1 Hz, J_2 = 10.6 Hz, 1H, H(b)-C(5′)); 3.70–3.76 (m, 2H, $N(CH_2CH_2CH_2CH_3)$); 3.78 (s, 6H, 2× O–CH_3 (DMT)); 3.83 (t, J = 6.9 Hz, 2H, H–C(α, Ile)); 4.19–4.23 (m, H–C(9, Fmoc), H–C(4′)); 4.33 (t, J = 8.4 Hz, 1H, O–*H(a)*CH(b)(Fmoc)); 4.44 (dd, J_1 = 7.0 Hz, J_2 = 10.5 Hz, 1H, O–H(a)*CH(b)*(Fmoc)); 5.26–5.27 (m, 2H, H–C(3′), HN(Ile)); 5.83 (dd, J_1 = 2.7 Hz, J_2 = 6.0 Hz, 1H, H–C(2′)); 6.17 (d, J = 2.8 Hz, 1H, H–C(1′)); 6.27 (d, J = 7.5 Hz, 1H, HN–C(3′)); 6.6.77–6.79 (m, 4H, H–C(ar)); 7.19 (t, J = 7.3 Hz, 1H, H–C(ar)); 7.25 (t, J = 7.6 Hz, 2H, H–C(ar)); 7.28–7.31 (m, 6H, H–C(ar)); 7.37–7.42 (m, 4H, H–C(ar)); 7.57 (t, J = 6.5 Hz, 2H, H–C(ar)); 7.76 (dd, J_1 = 3.3 Hz, J_2 = 7.5 Hz, 2H, H–C(ar)); 8.05 (s, 1H, H–C(8)); 8.52 (s, 1H, H–C(2)); 8.99 (s, 1H, *HC*=N–C(6)) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ 10.98 (C(δ, Ile)); 13.70, 13.93 $(N(CH_2CH_2CH_2CH_3)_2)$; 15.39 (C(γ′, Ile)); 19.80, 20.23 $(N(CH_2CH_2CH_2CH_3)_2)$; 23.67, 23.91 $(OOCCH_2CH_2CH_2CH_2COO)$; 24.75 (C(γ, Ile)); 29.28, 31.04 $(N(CH_2CH_2CH_2CH_3)_2)$; 32.77 $(OOCCH_2CH_2CH_2CH_2COO)$; 33.25 $(OOCCH_2CH_2CH_2CH_2COO)$; 36.38 (C(β, Ile)); 45.22 $(N(CH_2CH_2CH_2CH_3))$; 47.11 (C(9, Fmoc)); 50.75 (C(3′)); 51.87 $(N(CH_2CH_2CH_2CH_3))$; 55.18 (2× O–CH_3(DMT)); 59.70 (C(α, Ile)); 63.21 (C(5′)); 67.10 (O-CH_2(Fmoc)); 75.15 (C(2′)); 82.34 (C(4′)); 86.62; 87.62 (C(1′)); 100.01; 113.16, 120.02, 124.99, 126.23, 126.86, 127.11, 127.80, 127.86, 128.21, 130.10 (C(ar)); 135.44; 135.57; 137.01; 138.70; 139.83; 141.29; 141.94; 143.65; 144.33; 151.19; 153.04 (C(2)); 156.36; 158.31; 158.52; 160.18; 169.36; 171.20; 171.36 ppm. ESI-MS (*m/z*): $[M+H]^+$ calcd for $C_{72}H_{77}F_5N_8O_{11}$, 1338.43. Found 1337.29.

4.1.8. DMTO-rA-3′-NH-Gly-NHFmoc solid support (8)

To a solution of active ester **5** (177 mg, 0.138 mmol) in 3.0 mL dry *N*,*N*-dimethylformamide was added amino-functionalized support (*GE Healthcare*, Custom Primer Support™ 200 Amino, 530 mg) and pyridine (22 μL, 0.271 mmol). The suspension was agitated for 22 h at room temperature and the beads were collected on a Büchner funnel. The beads were washed with *N*,*N*-dimethylformamide, methanol, and dichloromethane and dried. Capping was performed by treatment of the beads with a mixture of 6.0 mL of solution A (acetic anhydride/2,4,6-trimethylpyridine/acetonitrile, 2/3/5) and 6.0 mL of solution B (4-(*N*,*N*-dimethylamino)pyridine/acetonitrile, 0.5 M) for 10 min at room temperature. The suspension was filtrated again and the beads were washed with acetonitrile, methanol, and dichloromethane and dried under vacuum. Loading of the support **10** was 63 μmol/g.

4.1.9. DMTO-rA-3′-NH-Ala-NHFmoc solid support (9)

To a solution of active ester **6** (175 mg, 0.135 mmol) in 3.0 mL dry *N*,*N*-dimethylformamide was added amino-functionalized support (*GE Healthcare*, Custom Primer Support™ 200 Amino, 525 mg) and pyridine (22 μL, 0.271 mmol). The suspension was agitated for 22 h at room temperature and the beads were collected on a Büchner funnel. The beads were washed with *N*,*N*-dimethylformamide, methanol, and dichloromethane and dried. Capping was performed by treatment of the beads with a mixture of 6.0 mL of solution A (acetic anhydride/2,4,6-trimethylpyridine/acetonitrile, 2/3/5) and 6.0 mL of solution B (4-(*N*,*N*-dimethylamino)pyridine/acetonitrile, 0.5 M) for 10 min at room temperature. The suspension was filtrated again and the beads were washed with acetonitrile, methanol, and dichloromethane and dried under vacuum. Loading of the support **8** was 45 μmol/g.

4.1.10. DMTO-rA-3′-NH-Ile-NHFmoc solid support (10)

To a solution of active ester **7** (223 mg, 0.167 mmol) in 3.5 mL dry *N*,*N*-dimethylformamide was added amino-functionalized support (*GE Healthcare*, Custom Primer Support™ 200 Amino, 668 mg) and pyridine (27 μL, 0.332 mmol). The suspension was agitated for 22 h at room temperature and the beads were collected on a Büchner funnel. The beads were washed with *N*,*N*-dimethylformamide, methanol, and dichloromethane and dried. Capping was performed by treatment of the beads with a mixture of 6.0 mL of solution A (acetic anhydride/2,4,6-trimethylpyridine/acetonitrile, 2/3/5) and 6.0 mL of solution B (4-(*N*,*N*-dimethylamino)pyridine/acetonitrile, 0.5 M) for 10 min at room temperature. The suspension was filtrated again and the beads were washed with acetonitrile, methanol, and dichloromethane and dried under vacuum. Loading of the support **10** was 55 μmol/g.

4.2. Solid-phase peptide and oligonucleotide synthesis, deprotection, and purification of RNA-peptide conjugates

4.2.1. Solid-phase peptide synthesis on the solid supports 8, 9, and 10

Peptides were synthesized on an *Intavis* MultiPep RS peptide synthesizer with a shaking device following slightly modified standard synthesis protocols. The 3 mL reaction vessels were equipped with a filter membrane. After each reaction step the supernatant was extracted by suction. Initial wash: the resin (~35 mg) was washed with *N*,*N*-dimethylformamide (5 × 1.5 mL, 2 min each). Fmoc deprotection: 20% (v/v) piperidine in DMF was administered (2 × 450 μL, 8 and 12 min). Then, the resin was washed with *N*,*N*-dimethylformamide (8 × 800 μL, 1 min each). Coupling: 2 coupling cycles were accomplished with a reaction time of 60 min for each step. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 0.6 M in *N*,*N*-dimethylformamide, 210 μL), 4-methylmorpholine (45% in *N*,*N*-dimethylformamide, 60 μL), 1-methyl-2-pyrrolidinone (5 μL) and Fmoc-protected amino acid (0.6 M in *N*,*N*-dimethylformamide, 220 μL) were mixed (activation time: 2 min) and administered onto the resin. After coupling, the resin was treated with dichloromethane (1 × 200 μL, 5 min). Capping: 5% (v/v) acetic anhydride, 6% (v/v) 2,6-lutidine in *N*,*N*-dimethylformamide was administered (2 × 450 μL, 5 min each). Afterwards the resin was washed with *N*,*N*-dimethylformamide (7 × 800 μL, 1 min each). Final wash: the resin was washed

with dichloromethane (3 × 600 μL, 1 min each). All peptides were synthesized Fmoc-ON.

4.2.2. Solid-phase oligonucleotide synthesis on the peptide charged supports 8, 9, and 10

All oligonucleotides were synthesized on a *Pharmacia* Gene Assembler Plus following standard synthesis protocols. Detritylation (2.0 min): dichloroacetic acid/1,2-dichloroethane (4/96, v/v). Coupling (3.0 min): phosphoramidites/acetonitrile (0.1 M × 120 μL) were activated by 5-(benzylthio)-1*H*-tetrazole/acetonitrile (0.3 M × 360 μL). Capping (3 × 0.4 min): A: acetic anhydride/2,4,6-trimethylpyridine/acetonitrile (2/3/5, v/v/v), B: 4-(dimethylamino)pyridine/acetonitrile (0.5 M), A/B = 1/1. Oxidation (1.0 min): iodine (10 mM) in acetonitrile/2,4,6-trimethylpyridine/water (10/1/5). Solutions of amidites, tetrazole solutions, and acetonitrile were dried over activated molecular sieves (4Å) overnight. All sequences were synthesized trityl-OFF.

4.2.3. Deprotection of RNA-peptide conjugates

The beads (~35 mg) were transferred into an *Eppendorf* tube and equal volumes of methylamine in ethanol (8 M, 0.65 mL) and methylamine in water (40%, 0.65 mL) were added. The mixture was kept at room temperature for 8 h. After the supernatant was filtered and evaporated to dryness, the 2′-*O*-silyl ethers were removed. RNA-peptide conjugates with a length of 4 nucleotides were treated with a solution of 0.9 M tetrabutylammonium fluoride trihydrate (TBAF·$3H_2O$) in tetrahydrofuran/water (9/1, 1.0 mL) for 16 h at room temperature. Larger RNA-peptide conjugates were treated with a solution of 1.0 M TBAF·$3H_2O$ in tetrahydrofuran (1.0 mL) for 16 h at 37 °C. The reaction was quenched by the addition of triethylammonium acetate buffer (1 M, pH 7.3, 1.0 mL). The volume of the solution was reduced to 0.5 mL and directly applied onto a purification system (ÄKTAprime™ plus, *GE Healthcare*) equipped with a size exclusion column (HiPrep™ 26/10 desalting column, *GE Healthcare*). The crude conjugate was eluted with water, evaporated to dryness, and dissolved in 1.0 mL water.

4.2.4. Analysis and purification of RNA-peptide conjugates

Analysis of crude products after deprotection was performed by anion-exchange HPLC on a *Dionex* DNAPac®PA-100 column (4x250 mm) at 60 °C. Flow rate: 1 mL/min; eluant A: 25 mM Tris–HCl (pH 8.0), 6 M urea; eluant B: 25 mM Tris-HCl (pH 8.0), 6 M urea, 500 mM $NaClO_4$; gradient: 0–35% B in A within 30 min; UV-detection at 260 nm. Crude products were purified on a semi-preparative *Dionex* DNAPac®PA-100 column (9x250 mm) at 60 °C. Flow rate: 2 mL/min; gradient: δ5–10% B in A within 20 min; UV-detection at 260 nm. Fractions containing the oligonucleotide were loaded on a C18 SepPak®Plus cartridge (*Waters*), washed with 0.15 M triethylammonium bicarbonate in water ($(Et_3NH)^+HCO_3^-$), water, and eluted with water/acetonitrile (1/1, v/v)). Combined fractions of the oligonucleotide were lyophilized to dryness and dissolved in 1.0 mL water. The RNA yield was determined as units of optical density at 260 nm by UV spectroscopy (*Implen* NanoPhotometer) at room temperature. The product quality was verified by anion-exchange chromatography as described in this paragraph.

4.2.5. LC-ESI mass spectrometry of RNA-peptide conjugates

All experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrumentation (*Thermo Fisher Scientific*) connected to an Amersham Ettan micro LC system (*GE Healthcare*). RNA-peptide conjugates were analyzed in the negative-ion mode with a potential of -4 kV applied to the spray needle. LC: sample: 200 pmol lyophilized conjugate dissolved in 30 μL water for conjugates ‹ 5 nt, 200 pmol lyophilized conjugate dissolved in 30 μL 20 mM ethylenediaminetetraacetic acid (EDTA) in water for conjugates › 5 nt; column: XTerra®MS, C18 2.5 μm, 1.0 × 50 mm at 21 °C; flow rate: 30 μL/min; eluant A: 8.6 mM triethylamine, 100 mM 1,1,1,3,3,3-hexafluoroisopropanol in water (pH 8.0); eluant B: methanol; gradient: 0–100% B in A within 20 min; UV-detection at 254 nm. Prior each injection, column equilibration was performed by eluting buffer A for 30 min at a flow rate of 30 μL/min.

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A. Supplementary data

Supplementary data (^1H and ^{13}C NMR spectra for compounds **2**–**7**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.018.

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