Artificial ribonucleases
6.* Ribonuclease activity of tetrapeptides based on amino acids involved in the catalytic site of RNase T1

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Tetrapeptides based on amino acids involved in the catalytic site of RNase T1 were synthesized. These peptides interact with a 96-mer fragment of HIV-1 RNA, which results in phosphodiester bonds splitting. The efficacy of RNA cleavage depends on the mutual arrangement of oppositely charged amino acids (Glu and Arg or Lys) in a peptide. The introduction of an additional cationic fragment (based on bis-quaternary salts of 1,4-diazabicyclooctane) into an RNase mimetic leads to a considerable increase in the efficiency of RNA depolymerization.

Key words: artificial ribonucleases, RNase active site, tetrapeptides, models of catalytic sites.

The construction of reagents that split nucleic acids is necessary for further progress in molecular biological studies, development of biotechnologies, and design of new kinds of therapeutic agents. For RNA-cleavage agents (artificial ribonucleases) to be efficient, they must have a minimum size and must not substantially change the physicochemical properties of RNA targets.

A promising approach to the construction of artificial ribonucleases, which we have extensively developed in recent years, consists in constructing structure-functional models of catalytic sites of the natural enzymes.

Earlier,[1,2] we have described the synthesis and properties of a series of artificial RNases based on short peptides containing histidine or histamine and such positively charged amino acids as Lys or Arg. It was demonstrated[3] that the resulting peptides are structural and functional analogs of the catalytic site of RNase A.

The imidazole fragments of histidine/histamine play the key role in the acid-base mechanism of RNA cleavage, which is postulated for the overwhelming majority of natural enzymes that cleave P—O bonds. The positively charged groups of the side chains of other amino acids interact with the oxygen atoms of the phosphate group, thus increasing the electrophilicity of the phosphorus atom and stabilizing the five-coordinate negatively charged intermediate.[4] The statistics on the involvement of certain amino acids in catalytic sites of various enzymes[5] is presented in Table 1. Based on these data, it can be concluded that the catalytic sites of artificial RNases functioning according to the acid-base mechanism can involve not only histidine but other amino acids as well.

Therefore, we synthesized short peptides (without histidine/histamine), which are of interest as RNase mimetics, and studied their activities.

Results and Discussion

We chose the catalytic site of well-studied G-specific RNase T1 as a natural prototype (Scheme 1).[6,7] This RNase differs from ribonuclease A in that the carboxylate anion of glutamic acid serves as a base in its catalytic site. In addition to the residue Glu 58, the residues Tyr 38, Arg 77, His 92, and Phe 100 are involved in catalysis. It should be noted that the histidine residue does not play the key role in catalysis.

In the present study, we synthesized a series of short peptides containing the following amino acids in different combinations: Arg, Glu, Ser, Thr, Lys, and Phe. The amino acid compositions of the catalytic sites of RNase mimetics were chosen such as, at neutral pH, each amino acid could have one particular function with high probability. It is known that the hydroxy group of Tyr can act as an acid-base catalyst. We used serine and threonine to replace tyrosine. Threonine is structurally similar to serine but differs substantially in the functions in catalytic sites (see Table 1). We chose phenylalanine, which is involved in the catalytic site of RNase T1, as the C-terminal amino acid for the tetrapeptides synthesized. In addition, phenylalanine possesses sufficient hydrophobicity, which facilitates isolation of intermediate di- and tripeptides.

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Table 1. Involvement of amino acid residues in the manifestation of the catalytic activity of enzymes[5]

Function of the amino acid residue	Percentage of the amino acid* (%)						
	His	Asp	Arg	Lys	Tyr	Ser	Thr
Acid/base	72	77	21	38	62	62	39
Stabilization of an intermediate/ activation of a substrate	24	16	79	53	35	20	55
Other functions	4	7	—	9	3	28	6

* The percentage of all amino acids of this type involved in the catalytic sites of enzymes.

In the first step, we synthesized six tetrapeptides containing, along with Phe, functionally important amino acid residues in different combinations.

Arg-Thr-Glu-Phe-OC_8H_{17}, RTEF (**1**)

Lys-Ser-Glu-Phe-OC_8H_{17}, KSEF (**2**)

Lys-Thr-Glu-Phe-OC_8H_{17}, KTEF (**3**)

Thr-Lys-Glu-Phe-OC_8H_{17}, TKEF (**4**)

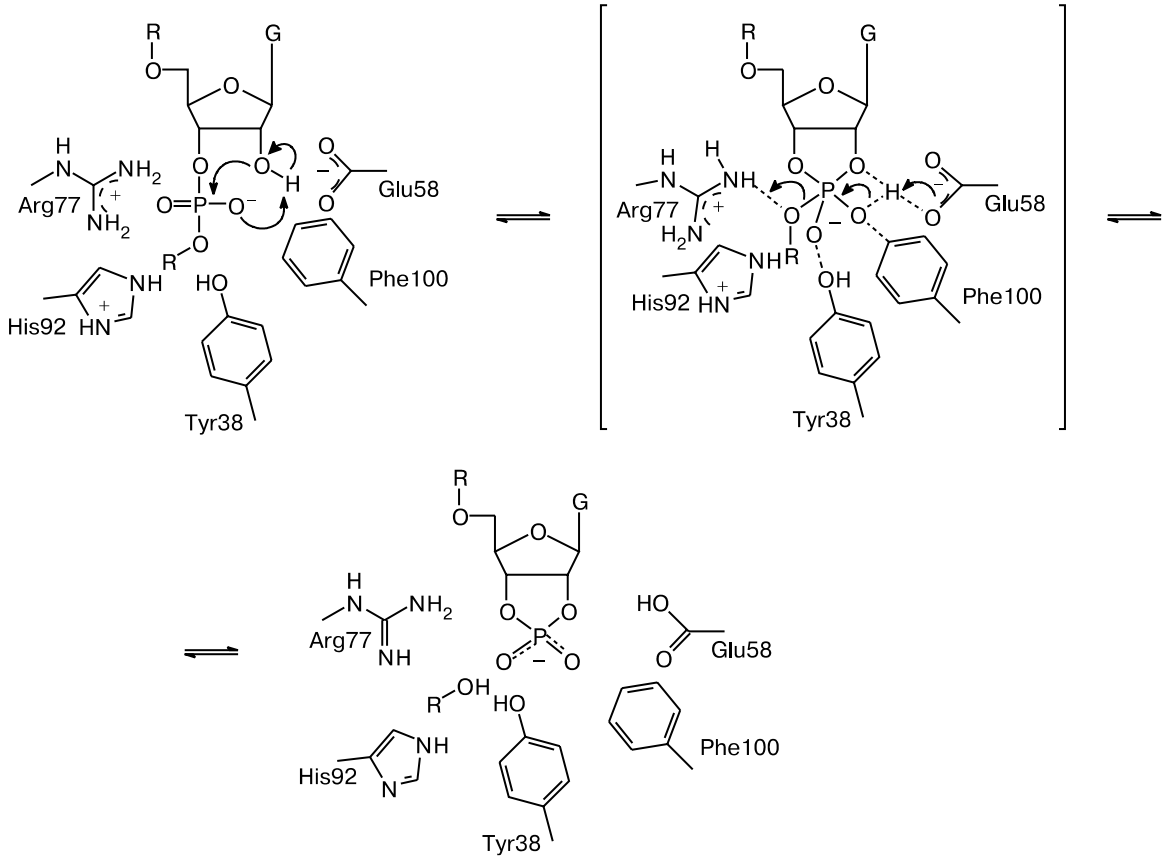
Glu-Thr-Lys-Phe-OC_8H_{17}, ETKF (**5**)

Glu-Ser-Lys-Phe-OC_8H_{17}, ESKF (**6**)

C-Terminal amino acids in the resulting peptides were present as octyl (or decyl) ester. Earlier, it has been demonstrated[8] that the introduction of hydrophobic alkyl residues substantially increases the activities of artificial RNases. However, this is not associated with micellar catalysis, because efficient catalysis of RNA cleavage was observed at concentrations much lower than those required for the micelle formation.[9]

To elucidate the role of particular amino acid residues, we synthesized two series of RNase mimetics. Each peptide contained two amino acid residues bearing oppositely charged catalytically active groups (the carboxy and amino or guanidinium groups), the distance between them

Scheme 1



being varied by introducing a catalytically inert amino acid (X) as a linker. Each series contained five peptide-like compounds:

Series R: Glu-X-Arg-Gly-$OC_{10}H_{21}$ (peptides **7—11**),

Series K: Glu-X-Lys-Gly-$OC_{10}H_{21}$ (peptides **12—16**),

where X are residues of Gly, β-Ala, γ-aminobutyric acid (GABA), 6-aminohexanoic acid (AHA), or *p*-aminomethylbenzoic acid (ABA), respectively.

Earlier,[10] we have demonstrated that the efficiency of imidazole-containing artificial ribonucleases can be increased by incorporating 1,4-diazabicyclo[2.2.2]octane (DABCO)-derived bis-quaternary salts. This can be attributed to an increase in the affinity of the cationic moiety of artificial RNase for the negatively charged RNA molecule.

In the present study, we also attempted to increase the activities of cationic RNase mimetics by combining catalytically active peptides and an additional dicationic fragment in one structure. For this purpose, we synthesized compounds **17—25**. Here X stands for Gly, β-Ala, γ-aminobutyric acid (GABA), 6-aminohexanoic acid (AHA), or *p*-aminomethylbenzoic acid (ABA) residues.

The solution synthesis of tetrapeptides was carried out using the method of activated esters and the Boc strategy. The C-terminal amino acid (Phe or Gly) was alkylated with 1-bromooctane or 1-bromodecane. Due to the presence of this hydrophobic protection, the isolation and purification of the reaction product in each step becomes much easier.

Removal of the *N*-nitro and *O*-benzyl protecting groups from the functional groups of the side chains of amino acids in tetrapeptides and conjugates was carried out by catalytic hydrogenolysis. For diazabicyclo[2.2.2]octane conjugates with peptides **13** and **16**, the yields after hydrogenolysis were low due, apparently, to sorption of the resulting compounds on activated carbon. The main characteristics of the compounds are given in Tables 2 and 3. The purities of the intermediates and the completeness of the reactions were monitored by TLC. The structures of the target compounds were confirmed by ^{1}H NMR spectroscopy. In some cases, 2D (COSY) spectroscopy was used to make the correct assignment of the signals in the ^{1}H NMR spectra (data not shown).

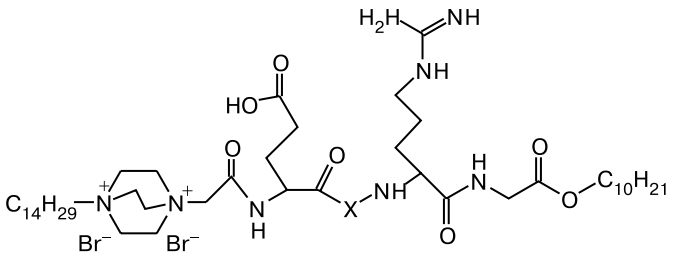
For all peptides, correct mass spectra were obtained before and after deprotection of the functional groups. The tetrapeptides are apparently good complex-forming agents because their mass spectra contained not only molecular ion peaks but also intense signals of tetrapeptide complexes with metal ions. In the mass spectra of tetrapeptide conjugates with diazabicyclo[2.2.2]octane derivatives, the molecular ion peaks of both the target compounds and the starting tetrapeptides are absent. In this case, profound destruction of the conjugates is observed.

The ribonuclease activity of compounds **1—6** was studied with the use of the [5′-^{32}P]-labeled 96-mer fragment of HIV-1 RNA as the substrate. The quantitative data on the efficacy of RNA cleavage with these peptides (Table 4) show that all molecules under examination cleave RNA. After 18 h, the degree of depolymerization varies from 35% for peptide **3** to 64% for peptide **1**. The data on total depolymerization of RNA estimated from the equation

$$(1 - RNA_{intact}/RNA_{sum}) \cdot 100$$

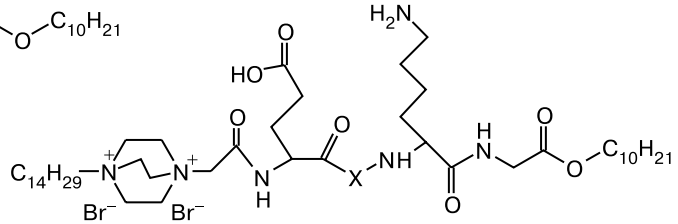
where RNA_{intact} is the amount of intact RNA after incubation and RNA_{sum} is the initial amount of RNA, are presented. Detailed data on specificity of RNA cleavage will be published elsewhere.

The tetrapeptide containing N-terminal arginine exhibited the highest activity. It can be seen that the replacement of N-terminal Lys by Glu leads to an increase in the efficacy of cleavage (peptides **2** and **6**; **3** and **5**),



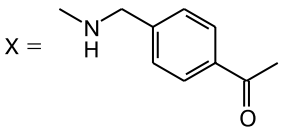
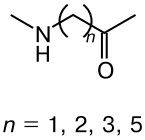
17—21

R series



22—25

K series

X =  , 

n = 1, 2, 3, 5

Table 2. Physicochemical characteristics of tetrapeptides **1—16**

Peptide	Yield* (%)	^{1}H NMR (CD_3OD), δ, *J*/Hz	MALDI-TOF MS, *m/z*		
			Found, *m/z*	Molecular formula	Calculated, M
RTEF-C_8H_{17} (**1**)	45.4	$(CD_3)_2CO$: 0.87 (t, 3 H, Me, *J* = 6.5); 1.29 (m, 15 H, Me^{Thr}, $OCH_2(C\underline{H}_2)_6Me$); 1.89 (m, 6 H, $C\underline{H}_2CH_2^{Glu}$, $CH(C\underline{H}_2)_2CH_2^{Arg}$); 2.22 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.39 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 3.08 (m, 2 H, $C\underline{H}_2^{Phe}$); 3.35 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 4.09 (m, 3 H, $OC\underline{H}_2(CH_2)_6Me$, $CHC\underline{H}(OH)Me^{Thr}$); 4.54 (m, 3 H, $C\underline{H}CH_2^{Phe}$, $C\underline{H}(CH_2)_3^{Arg}$, $C\underline{H}CH(OH)Me^{Thr}$); 7.24 (m, 5 H, CH_{apom})	664.53 $[M + H]^+$	$C_{32}H_{53}N_7O_8$	663.81
KSEF-C_8H_{17} (**2**)	35.4	$(CD_3)_2CO$: 0.87 (t, 3 H, Me, *J* = 6.5); 1.28 (m, 12 H, $OCH_2(C\underline{H}_2)_6Me$); 1.52 (m, 6 H, $CH(C\underline{H}_2)_3CH_2^{Lys}$); 1.83 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.38 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.15 (m, 4 H, $C\underline{H}_2^{Phe}$, $CH(CH_2)_3C\underline{H}_2^{Lys}$); 3.80 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 4.03 (m, 2 H, $OC\underline{H}_2(CH_2)_6Me$); 4.15 (m, 2 H, $CHC\underline{H}_2^{Ser}$); 4.56 (m, 2 H, $C\underline{H}CH_2^{Phe}$, $C\underline{H}(CH_2)_4^{Lys}$); 4.67 (m, 1 H, $C\underline{H}CH_2^{Ser}$); 7.32 (m, 5 H, CH_{apom})	622.77.80 $[M + H]^+$ 667.01 [M + 2Na]	$C_{31}H_{51}N_5O_8$	621.77
KTEF-C_8H_{17} (**3**)	34.1	$(CD_3)_2CO$: 0.87 (t, 3 H, Me, *J* = 6.5); 1.29 (m, 15 H, Me^{Thr}, $OCH_2(C\underline{H}_2)_6Me$); 1.58 (m, 6 H, $CH(C\underline{H}_2)_3CH_2^{Lys}$); 1.86 (m, 2 H, $C\underline{H}_2CH_2^{Glu}$); 2.45 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.09 (m, 4 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$, $C\underline{H}_2^{Phe}$); 3.24 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 4.12 (m, 3 H, $OC\underline{H}_2(CH_2)_6Me$, $CHC\underline{H}(OH)Me^{Thr}$); 4.58 (m, 3 H, $C\underline{H}CH_2^{Phe}$, $C\underline{H}(CH_2)_4^{Lys}$, $C\underline{H}CH(OH)Me^{Thr}$); 7.34 (m, 5 H, $C\underline{H}_{apom}$)	636.05 $[M + H]^+$	$C_{32}H_{53}N_5O_8$	635.79
TKEF-C_8H_{17} (**4**)	17.8	$(CD_3)_2CO$: 0.87 (t, 3 H, Me, *J* = 6.5); 1.28 (m, 15 H, Me^{Thr}, $OCH_2(C\underline{H}_2)_6Me$); 1.57 (m, 6 H, $CH(C\underline{H}_2)_3CH_2^{Lys}$); 1.84 (m, 2 H, $C\underline{H}_2CH_2^{Glu}$); 2.29 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.95 (m, 4 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$, $C\underline{H}_2^{Phe}$); 3.60 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 4.03 (m, 3 H, $OC\underline{H}_2(CH_2)_6Me$, $CHC\underline{H}(OH)Me^{Thr}$); 4.57 (m, 3 H, $C\underline{H}CH_2^{Phe}$, $C\underline{H}(CH_2)_4^{Lys}$, $C\underline{H}CH(OH)Me^{Thr}$); 7.34 (m, 5 H, CH_{apom})	636.42 $[M + H]^+$ 659.26 [M + Na]	$C_{32}H_{53}N_5O_8$	635.79
ETKF-C_8H_{17} (**5**)	8.9	$(CD_3)_2CO$: 0.86 (t, 3 H, Me, *J* = 6.5); 1.30 (m, 15 H, Me^{Thr}, $OCH_2(C\underline{H}_2)_6Me$); 1.54 (m, 6 H, $CH(C\underline{H}_2)_3CH_2^{Lys}$); 1.89 (m, 2 H, $C\underline{H}_2CH_2^{Glu}$); 2.47 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.09 (m, 4 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$, $C\underline{H}_2^{Phe}$); 3.54 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 4.04 (m, 3 H, $OC\underline{H}_2(CH_2)_6Me$, $CHC\underline{H}(OH)Me^{Thr}$); 4.49 (m, 3 H, $C\underline{H}CH_2^{Phe}$, $C\underline{H}(CH_2)_4^{Lys}$, $C\underline{H}CH(OH)Me^{Thr}$); 7.39 (m, 5 H, CH_{apom})	636.15 $[M + H]^+$ 642.56 $[M + Li^+]$	$C_{32}H_{53}N_5O_8$	635.79

Table 2 (*continued*)

Peptide	Yield* (%)	^{1}H NMR (CD_3OD), δ, *J*/Hz	MALDI-TOF MS, *m/z*		
			Found, *m/z*	Molecular formula	Calculated, M
ESKF-C_8H_{17} (**6**)	36.6	$(CD_3)_2CO$: 0.87 (t, 3 H, Me, *J* = 6.5); 1.28 (m, 12 H, $OCH_2(C\underline{H}_2)_6Me$); 1.55 (m, 6 H, $CH(C\underline{H}_2)_3CH_2^{Lys}$); 1.78 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.48 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.08 (m, 4 H, $C\underline{H}_2^{Phe}$, $CH(CH_2)_3C\underline{H}_2^{Lys}$); 3.75 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 4.02 (m, 2 H, $OC\underline{H}_2(CH_2)_6Me$); 4.15 (m, 2 H, $CHC\underline{H}_2^{Ser}$); 4.56 (m, 2 H, $C\underline{H}CH_2^{Phe}$, $C\underline{H}(CH_2)_4^{Lys}$); 4.65 (m, 1 H, $C\underline{H}CH_2^{Ser}$); 7.33 (m, 5 H, CH_{apom})	622.74 $[M + H]^+$ 665.80 [M + 2Na]	$C_{31}H_{51}N_5O_8$	621.77
EGRG-$C_{10}H_{21}$ (**7**)	32.8	0.86 (t, 3 H, Me, *J* = 6.5); 1.27 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.61 (m, 2 H, $CHC\underline{H}_2CH_2^{Glu}$); 1.71 (m, 4 H, $CH(C\underline{H}_2)_2CH_2^{Arg}$); 2.43 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 2.54 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.32 (m, 2 H, $C\underline{H}_2^{Gly}$); 3.99 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2C(O)OAlk^{Gly}$); 4.08 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.58 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$)	557.80 $[M + H]^+$ 603.44 [M + 2Na]	$C_{25}H_{47}N_7O_7$	557.35
E(βA)RG-$C_{10}H_{21}$ (**8**)	26.1	0.86 (t, 3 H, Me, *J* = 6.5); 1.27 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.60 (t, 2 H, $CHC\underline{H}_2CH_2^{Glu}$); 1.72 (m, 4 H, $CH(C\underline{H}_2)_2CH_2^{Arg}$); 2.39 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 2.49 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.30 (m, 2 H, $CH_2C\underline{H}_2^{\beta Ala}$); 3.50 (m, 2 H, $C\underline{H}_2CH_2^{\beta Ala}$); 3.95 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2^{Gly}$); 4.07 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.54 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$)	571.82 $[M + H]^+$ 617.86 [M + 2Na]	$C_{26}H_{49}N_7O_7$	571.37
E(GABA)RG-$C_{10}H_{21}$ (**9**)	30.7	0.92 (t, 3 H, Me, *J* = 6.5); 1.33 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.73 (m, 4 H, $CH_2C\underline{H}_2CH_2^{GABA}$, $CHC\underline{H}_2(CH_2)_2^{Arg}$); 1.88 (m, 4 H, $CHCH_2C\underline{H}_2CH_2^{Arg}$, $CHC\underline{H}_2CH_2^{Glu}$); 2.34 (m, 6 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$, $CHCH_2C\underline{H}_2^{Glu}$, $CH_2CH_2C\underline{H}_2^{GABA}$); 3.26 (m, 2 H, $C\underline{H}_2CH_2CH_2^{GABA}$); 3.33 (m, 1 H, $C\underline{H}(CH_2)_2^{Glu}$); 3.97 (m, 2 H, $C\underline{H}_2^{Gly}$); 4.13 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.38 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$)	585.49 $[M + H]^+$ 631.67 [M + 2Na]	$C_{27}H_{51}N_7O_7$	585.38
E(AHA)RG-$C_{10}H_{21}$ (**10**)	36.5	0.86 (t, 3 H, Me, *J* = 6.5); 1.27 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.60 (m, 10 H, $CH_2(C\underline{H}_2)_3CH_2^{AHA}$, $CH(C\underline{H}_2)_2CH_2^{Arg}$); 1.96 (m, 2 H, $CHC\underline{H}_2CH_2^{Glu}$); 2.10 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.26 (m, 2 H, $(CH_2)_4C\underline{H}_2^{AHA}$); 2.40 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 3.22 (m, 2 H, $C\underline{H}_2(CH_2)_3CH_2^{AHA}$); 3.35 (m, 1 H, $C\underline{H}(CH_2)_2^{Glu}$); 3.94 (m, 2 H, $C\underline{H}_2^{Gly}$); 4.07 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.56 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$)	614.22 $[M + H]^+$ 659.91 [M + 2Na]	$C_{29}H_{55}N_7O_7$	613.42
E(ABA)RG-$C_{10}H_{21}$ (**11**)	7.5	0.89 (t, 3 H, Me, *J* = 6.5); 1.32 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.67 (m, 4 H, $CHC\underline{H}_2CH_2^{Glu}$, $CHC\underline{H}_2(CH_2)_2^{Arg}$); 1.79 (m, 2H, $CHCH_2C\underline{H}_2CH_2^{Arg}$); 2.12 (m, 2H, $CHCH_2C\underline{H}_2^{Glu}$); 2.42 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 3.28 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 3.98 (d, 2 H, $C\underline{H}_2^{Gly}$, *J* = 6.0); 4.15 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.48 (m, 2 H, $C\underline{H}_2^{ABA}$); 4.78 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$); 7.44, 7.90 (both m, 2 H each, CH_{apom}^{ABA})	633.90 $[M + H]^+$ 680.12 [M + 2Na]	$C_{31}H_{51}N_7O_7$	633.38

(*to be continued*)

Table 2 (*continued*)

Peptide	Yield* (%)	^{1}H NMR (CD_3OD), δ, *J*/Hz	MALDI-TOF MS, *m/z*		
			Found, *m/z*	Molecular formula	Calculated, M
EGKG-$C_{10}H_{21}$ (**12**)	44.2	0.85 (t, 3 H, Me, *J* = 6.5); 1.26 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.60 (m, 4 H, $CHC\underline{H}_2CH_2{}^{Glu}$, $CHCH_2C\underline{H}_2CH_2CH_2{}^{Lys}$); 1.83 (m, 4 H, $CHC\underline{H}_2CH_2C\underline{H}_2CH_2{}^{Lys}$); 2.50 (t, 2 H, $CHCH_2C\underline{H}_2{}^{Glu}$, *J* = 7.5); 3.05 (t, 2 H, $CH(CH_2)_3C\underline{H}_2{}^{Lys}$, *J* = 7.0); 3.76 (t, 2 H, $C\underline{H}_2{}^{Gly}$, *J* = 6.5); 3.91 (m, 3 H, $C\underline{H}CH_2CH_2{}^{Glu}$, $C\underline{H}_2C(O)OAlk^{Gly}$); 4.06 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 7.0); 4.45 (m, 1 H, $C\underline{H}(CH_2)_4{}^{Lys}$)	529.93 $[M + H]^+$ 575.07 [M + 2Na]	$C_{25}H_{47}N_5O_7$	529.35
E(βA)KG-$C_{10}H_{21}$ (**13**)	61.5	0.89 (t, 3 H, Me, *J* = 6.5); 1.32 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.71 (m, 8 H, $CHC\underline{H}_2CH_2{}^{Glu}$, $CH(C\underline{H}_2)_3CH_2{}^{Lys}$); 2.52 (m, 4 H, $C\underline{H}_2C\underline{H}_2{}^{\beta Ala}$); 3.00 (t, 2 H, $CHCH_2C\underline{H}_2{}^{Glu}$, *J* = 7.5); 3.56 (t, 2 H, $CH(CH_2)_3C\underline{H}_2{}^{Lys}$, *J* = 6.0); 4.01 (m, 3 H, $C\underline{H}CH_2CH_2{}^{Glu}$, $C\underline{H}_2{}^{Gly}$); 4.15 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.40 (t, 1 H, $C\underline{H}(CH_2)_4{}^{Lys}$, *J* = 7.0)	544.12 $[M + H]^+$ 589.13 [M+2Na]	$C_{26}H_{49}N_5O_7$	543.36
E(GABA)KG-$C_{10}H_{21}$ (**14**)	25.5	0.87 (t, 3 H, Me, *J* = 6.5); 1.28 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.62 (m, 8 H, $CHC\underline{H}_2CH_2{}^{Glu}$, $CH(C\underline{H}_2)_3CH_2{}^{Lys}$); 1.84 (m, 4 H, $CHCH_2C\underline{H}_2{}^{Glu}$, $CH_2C\underline{H}_2CH_2{}^{GABA}$); 2.38 (m, 2 H, $CH_2CH_2C\underline{H}_2{}^{GABA}$); 3.18 (m, 4 H, $C\underline{H}_2CH_2CH_2{}^{GABA}$, $CH(CH_2)_3C\underline{H}_2{}^{Lys}$); 3.76 (m, 1 H, $C\underline{H}(CH_2)_2{}^{Glu}$); 3.90 (m, 2 H, $C\underline{H}_2{}^{Gly}$); 4.12 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.40 (m, 1 H, $C\underline{H}(CH_2)_4{}^{Lys}$)	557.96 $[M + H]^+$ 580.13 [M + Na]	$C_{27}H_{51}N_5O_7$	557.38
E(AHA)KG-$C_{10}H_{21}$ (**15**)	30.2	0.93 (t, 3 H, Me, *J* = 6.5); 1.33 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.64—1.80 (m, 12 H, $CH_2(C\underline{H}_2)_3CH_2{}^{AHA}$, $CH(C\underline{H}_2)_3CH_2{}^{Lys}$); 2.15 (t, 2 H, $CHC\underline{H}_2CH_2{}^{Glu}$, *J* = 6.5); 2.34 (t, 2 H, $(CH_2)_4C\underline{H}_2{}^{AHA}$, *J* = 7.6); 2.50 (t, 2 H, $CHCH_2C\underline{H}_2{}^{Glu}$, *J* = 7.0); 2.98 (t, 2 H, $CH(CH_2)_3C\underline{H}_2{}^{Lys}$, *J* = 7.1); 3.28 (t, 2 H, $C\underline{H}_2(CH_2)_3CH_2{}^{AHA}$, *J* = 7.0); 3.50 (m, 1 H, $C\underline{H}(CH_2)_2{}^{Glu}$); 3.96 (d, 2 H, $C\underline{H}_2{}^{Gly}$, *J* = 8.0); 4.15 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.40 (t, 1 H, $C\underline{H}(CH_2)_4{}^{Lys}$, *J* = 7.0)	586.08 $[M + H]^+$ 608.00 [M + Na]	$C_{29}H_{55}N_5O_7$	585.41
E(ABA)KG-$C_{10}H_{21}$ (**16**)	25.5	0.86 (t, 3 H, Me, *J* = 6.5); 1.27 (m, 16 H, $CH_2(C\underline{H}_2)_8Me$); 1.56 (m, 8 H, $CHC\underline{H}_2CH_2{}^{Glu}$, $CH(C\underline{H}_2)_3CH_2{}^{Lys}$); 2.60 (m, 2 H, $CHCH_2C\underline{H}_2{}^{Glu}$); 3.16 (m, 2 H, $CH(CH_2)_3C\underline{H}_2{}^{Lys}$); 3.96 (m, 3 H, $C\underline{H}CH_2CH_2{}^{Glu}$, $C\underline{H}_2{}^{Gly}$); 4.06 (t, 2 H, $OC\underline{H}_2(CH_2)_8Me$, *J* = 6.5); 4.45 (m, 2 H, $C\underline{H}_2{}^{ABA}$); 4.68 (m, 1 H, $C\underline{H}(CH_2)_4{}^{Lys}$); 7.40, 7.80 (both m, 2 H each, $CH_{apom}{}^{ABA}$)	605.77 $[M + H]^+$ 651.76 [M + 2Na]	$C_{31}H_{51}N_5O_7$	605.38

* The yields are given based on the salts of $GlyOC_{10}H_{21}$ and $PheOC_8H_{17}$ derivatives with trifluoroacetic acid.

Table 3. Physicochemical characteristics of tetrapeptide conjugates with a DABCO derivative

Conjugate	Yields* (%) in condensation (after deprotection)	^{1}H NMR (CD_3OD, δ, *J*/Hz)
$C_{14}H_{29}$-DABCO-EGRG-$C_{10}H_{21}$ (**17**)	62.5 (22.1)	0.92 (t, 6 H, 2 Me, *J* = 6.5); 1.34 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.68 (m, 2 H, $CHC\underline{H}_2CH_2^{Glu}$); 1.76 (m, 4 H, $CH(C\underline{H}_2)_2CH_2^{Arg}$); 2.60 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 2.92 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.64 (m, 2 H, $C\underline{H}_2^{Gly}$); 3.92 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2C(O)OAlk^{Gly}$); 4.00—4.40 (m, 16 H, DABCO (12H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.58 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$); 5.05 (m, 2 H, $NCH_2C(O)$-Glu)
$C_{14}H_{29}$-DABCO-E(βA)RG-$C_{10}H_{21}$ (**18**)	61.6 (27.5)	0.92 (t, 6 H, 2 Me, *J* = 6.5); 1.35 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.70 (m, 2 H, $CHC\underline{H}_2CH_2^{Glu}$); 1.88 (m, 4 H, $CH(C\underline{H}_2)_2CH_2^{Arg}$); 2.54 (m, 4 H, $CHCH_2C\underline{H}_2^{Glu}$, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 3.60 (m, 2 H, $C\underline{H}_2C\underline{H}_2^{\beta Ala}$); 3.99 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2^{Gly}$); 4.03—4.50 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.60 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$); 4.90 (m, 2 H, $NCH_2C(O)$-Glu)
$C_{14}H_{29}$-DABCO-E(GABA)RG-$C_{10}H_{21}$ (**19**)	63.3 (31.5)	0.93 (t, 6 H, 2 Me, *J* = 6.5); 1.32 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.67 (m, 4 H, $CH_2C\underline{H}_2CH_2^{GABA}$, $CHC\underline{H}_2(CH_2)_2^{Arg}$); 1.86 (m, 4 H, $CHCH_2C\underline{H}_2CH_2^{Arg}$, $CHC\underline{H}_2CH_2^{Glu}$); 2.35 (m, 6 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$, $CHCH_2C\underline{H}_2^{Glu}$, $CH_2CH_2C\underline{H}_2^{GABA}$); 3.64 (m, 3 H, $C\underline{H}_2CH_2CH_2^{GABA}$, $C\underline{H}(CH_2)_2^{Glu}$); 3.80 (m, 2 H, $C\underline{H}_2^{Gly}$); 4.03—4.50 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.60 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$); 4.90 (m, 2 H, $NCH_2C(O)$-Glu)
$C_{14}H_{29}$-DABCO-E(AHA)RG-$C_{10}H_{21}$ (**20**)	68.7 (50.4)	0.93 (t, 6 H, 2 Me, *J* = 6.5); 1.32 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.67 (m, 8 H, $CH_2(C\underline{H}_2)_3CH_2^{AHA}$, $CHC\underline{H}_2(CH_2)_2^{Arg}$); 1.88 (m, 4 H, $CHC\underline{H}_2CH_2^{Glu}$, $CHCH_2C\underline{H}_2CH_2^{Arg}$); 2.12 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.33 (m, 2 H, $(CH_2)_4C\underline{H}_2^{AHA}$); 2.52 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 3.26 (m, 2 H, $C\underline{H}_2(CH_2)_3CH_2^{AHA}$); 3.64 (m, 1 H, $C\underline{H}(CH_2)_2^{Glu}$); 3.98 (m, 2 H, $C\underline{H}_2^{Gly}$); 4.20—4.48 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.58 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$); 4.75 (m, 2 H, $NCH_2C(O)$-Glu)
$C_{14}H_{29}$-DABCO-E(ABA)RG-$C_{10}H_{21}$ (**21**)	72.9 (27.2)	0.93 (t, 6 H, 2 Me, *J* = 6.5); 1.33 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.67 (m, 2 H, $CHC\underline{H}_2CH_2^{Glu}$, $CHC\underline{H}_2(CH_2)_2^{Arg}$); 1.78 (m, 2 H, $CHC\underline{H}_2CH_2^{Glu}$); 1.88 (m, 2 H, $CHCH_2C\underline{H}_2CH_2^{Arg}$); 2.15 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.44 (m, 2 H, $CH(CH_2)_2C\underline{H}_2^{Arg}$); 3.40 (m, 1 H, $C\underline{H}CH_2CH_2^{Glu}$); 3.64 (m, 2 H, $C\underline{H}_2^{Gly}$); 4.00—4.40 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.68 (m, 1 H, $C\underline{H}(CH_2)_3^{Arg}$); 4.90 (m, 2 H, $NCH_2C(O)$-Glu); 7.46 and 7.90 (both m, 2 H each, CH_{arom}^{ABA})
$C_{14}H_{29}$-DABCO-EGKG-$C_{10}H_{21}$ (**22**)	35.8 (28.1)	0.92 (t, 6 H, 2 Me, *J* = 6.5); 1.33 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.70 (m, 4 H, $CHC\underline{H}_2CH_2^{Glu}$, $CHCH_2C\underline{H}_2CH_2CH_2^{Lys}$); 1.88 (m, 4 H, $CHC\underline{H}_2CH_2C\underline{H}_2CH_2^{Lys}$); 2.52 (t, 2 H, $CHCH_2C\underline{H}_2^{Glu}$, *J* = 7.5); 3.00 (m, 2 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$); 3.64 (m, 2 H, $C\underline{H}_2^{Gly}$); 3.98 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2C(O)OAlk^{Gly}$); 4.08—4.50 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.78 (m, 1 H, $C\underline{H}(CH_2)_4^{Lys}$); 4.90 (m, 2 H, $NCH_2C(O)$-Glu)
$C_{14}H_{29}$-DABCO-E(βA)KG-$C_{10}H_{21}$ (**23**)	45.9 (5.0)	0.93 (t, 6 H, 2 Me, *J* = 6.5); 1.32 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.70 (m, 4 H, $CHC\underline{H}_2CH_2^{Glu}$, $CHCH_2C\underline{H}_2CH_2CH_2^{Lys}$); 1.92 (m, 4 H, $CHC\underline{H}_2CH_2C\underline{H}_2CH_2^{Lys}$); 2.52 (m, 4 H, $C\underline{H}_2C\underline{H}_2^{\beta Ala}$); 3.04 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 3.64 (m, 2 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$); 4.00 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2^{Gly}$); 4.04—4.44 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.58 (m, 1 H, $C\underline{H}(CH_2)_4^{Lys}$); 4.89 (m, 2 H, $NCH_2C(O)$-Glu)
$C_{14}H_{29}$-DABCO-E(GABA)KG-$C_{10}H_{21}$ (**24**)	56.6 (23.0)	0.93 (t, 6 H, 2 Me, *J* = 6.5); 1.32 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.67 (t, 2 H, $CHCH_2C\underline{H}_2CH_2CH_2^{Lys}$, *J* = 7.0); 1.75 (t, 2 H, $CHC\underline{H}_2CH_2^{Glu}$, *J* = 7.0); 1.88 (m, 8 H, $CHC\underline{H}_2CH_2C\underline{H}_2CH_2^{Lys}$, $CHCH_2C\underline{H}_2^{Glu}$, $CH_2C\underline{H}_2CH_2^{GABA}$); 2.36 (m, 2 H, $CH_2CH_2C\underline{H}_2^{GABA}$); 2.48 (m, 2 H, $C\underline{H}_2CH_2CH_2^{GABA}$); 3.00 (m, 2 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$); 3.62 (m, 3 H, $C\underline{H}(CH_2)_2^{Glu}$, $C\underline{H}_2^{Gly}$); 4.03—4.40 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.42 (m, 1 H, $C\underline{H}(CH_2)_4^{Lys}$); 4.85 (m, 2 H, $NCH_2C(O)$-Glu)

(*to be continued*)

Table 3 (*continued*)

Conjugate	Yields* (%) in condensation (after deprotection)	^{1}H NMR (CD_3OD, δ, *J*/Hz)
$C_{14}H_{29}$-DABCO-E(ABA)KG-$C_{10}H_{21}$ (**25**)	61.0 (5.9)	0.93 (t, 6 H, 2 Me, *J* = 6.5); 1.32 (m, 40 H, $CH_2(C\underline{H}_2)_8Me$, $CH_2(C\underline{H}_2)_{12}Me$); 1.52 (m, 4 H, $CHC\underline{H}_2CH_2^{Glu}$, $CH(C\underline{H}_2)_2CH_2CH_2^{Lys}$); 1.90 (m, 4 H, $CHC\underline{H}_2CH_2^{Glu}$, $CH(CH_2)_2C\underline{H}_2CH_2^{Lys}$); 2.40 (m, 2 H, $CHCH_2C\underline{H}_2^{Glu}$); 2.98 (m, 2 H, $CH(CH_2)_3C\underline{H}_2^{Lys}$); 3.60 (m, 3 H, $C\underline{H}CH_2CH_2^{Glu}$, $C\underline{H}_2^{Gly}$); 3.98—4.40 (m, 16 H, DABCO (12 H), $OC\underline{H}_2(CH_2)_8Me$, $NC\underline{H}_2(CH_2)_{12}Me$); 4.62 (m, 1 H, $C\underline{H}(CH_2)_4^{Lys}$); 4.82 (m, 2 H, $NCH_2C(O)$-Glu); 7.47 and 7.92 (both m, 2 H each, CH_{arom}^{ABA})

* The yields are given based on the corresponding tetrapeptide (containing protected functional groups without Boc protection).

Table 4. Degree of cleavage of the [5′-^{32}P]-labeled 96-mer fragment of HIV 1 RNA with tetrapeptides **1—6** after 6 and 18 h*

Peptide	Cleavage (%)	
	6 h	18 h
1	31	64
2	25	49
3	15	35
4	32	50
5	34	54
6	35	52

* Reaction conditions: 50 *mM* Tris—HCl (pH 7.0), 0.2 *M* KCl, 0.5 *mM* EDTA, *C* (with total yeast tRNA as a carrier) = 0.1 mg mL^{-1}, *C* (of tetrapeptides) = 10^{-3} mol L^{-1}, *T* = 37 °C.

Table 5. Degree of cleavage of the [5′-^{32}P]-labeled 96-mer fragment of HIV 1 RNA with tetrapeptides of the R series (K series) after 6 and 18 h*

Peptide	Cleavage (%)	
	6 h	18 h
7 (**12**)	13 (37)	24 (80)
8 (**13**)	15 (49)	31 (87)
9 (**14**)	25 (50)	51 (84)
10 (**15**)	32 (71)	56 (98)
11 (**16**)	29 (21)	62 (60)

* Reaction conditions: 50 *mM* Tris—HCl (pH 7.0), 0.2 *M* KCl, 0.5 *mM* EDTA, *C* (with total yeast tRNA as a carrier) = 0.1 mg mL^{-1}, *C* (of tetrapeptides) = 10^{-4} mol L^{-1}, *T* = 37 °C. The values for the K series are given in parentheses.

which is most pronounced at short hydrolysis times. The replacement of Thr by Ser (peptides **3** and **2**) leads to an increase in activity, whereas this replacement has no effect in the case of peptides **5** and **6** (the degree of cleavage remains unchanged).

The catalytic activity of compounds **7—16** was studied also in experiments with the [5′-^{32}P]-labeled 96-mer fragment of HIV-1 RNA (Table 5).

All constructions exhibit catalytic activity and split the RNA substrate more efficiently than RNase mimetics **1—6**. Presumably, the efficiency of catalysis by RNase mimetics **7—16** is determined by the optimum arrangement of the key amino acids (Glu and Arg/Lys), whereas hydroxy-containing amino acids are apparently of little importance in hydrolysis and serve only as linkers between catalytically active groups. In addition, as can be seen from the above data, the replacement of Phe by Gly has no effect on the degree of cleavage.

The efficacy of cleavage depends substantially on the nature of the positively charged amino acid. Compounds **12—16** (K series) (the degree of depolymerization is 60—98%) are much more active than the corresponding analogs of the R series (the degree of depolymerization is 24—62%). The influence of the length of the linker between catalytically active amino acids on the degree of cleavage is most pronounced for compounds of the R series. The total percentage of RNA cleavage increases with increasing distance (due to an increase in the number of methylene units) between Glu and the positively charged amino acid. In the K series, an analogous dependence is clearly observed at an incubation time of 6 h. After incubation for 18 h, the percentage of depolymerization of RNA is virtually the same for all compounds of the series, which is apparently associated with the fact that the reaction reaches a plateau (see Table 5).

The introduction of the (4-tetradecyl-1,4-diazoniabicyclo[2.2.2]oct-1-yl)acetyl residue leads to a substantial increase in the degree of hydrolysis (Table 6). In most cases, virtually quantitative cleavage is achieved after 19 h. In addition, some compounds split RNA to the extent of ~80% after incubation for 6 h (peptides **21**, **22**, and **24**). The influence of the distance between the catalytically active groups in tetrapeptide (unit X) on the cleavage rate is substantially eliminated.

The exception is a *p*-aminobenzoic acid-based linker. The catalytic activity of peptide **25** appeared to be an order of magnitude lower than that of other compounds.

Table 6. Degree of cleavage of the [5′-^{32}P]-labeled 96-mer fragment of RNA HIV 1 with conjugates of DABCO derivatives with tetrapeptides of the R series (K series) after 6 and 19 h*

Peptide	Cleavage (%)	
	6 h	19 h
17 (**22**)	62 (89)	82 (98)
18 (**23**)	77 (49)	99 (93)
19 (**24**)	69 (82)	99 (98)
20	40	82
21 (**25**)	79 (6)	98 (15)

* The reaction conditions are given in the note to Table 5; *C* (conjugates with tetrapeptides) = 10^{-4} mol L^{-1}. The values for the K series are given in parentheses.

Catalytically active tetrapeptide **16**, which was used for the synthesis of conjugate **25**, also exhibited the lowest activity. Apparently, the *p*-aminobenzoic linker used in the present study hinders the optimum arrangement of the catalytically active groups with the respect to the ribose phosphate backbone of RNA.

Thus, based on the results of the present study, it can be concluded that the development of structure-function models of catalytic sites of natural enzymes is an efficient approach to the design of artificial ribonucleases. The addition of catalytically active groups to molecules possessing high affinity for RNA increases the efficacy of the substrate cleavage.

Experimental

In the present study, we used dicyclohexylcarbodiimide, *N*-hydroxysuccinimide, Boc-β-alanine, di-*tert*-butyl pyrocarbonate, *N*-ethyldiisopropylamine, N^{ω}-NO_2-L-arginine (Fluka, Germany), *N*-Boc-glycine, N^{α}-Boc-*O*-Bzl-L-serine, N^{α}-Boc-*O*-Bzl-L-threonine, N^{α}-Boc-N^{ε}-(2Cl-Cbz)-L-lysine, *N*-Boc-*O*-Bzl-L-glutamic acid, and *N*-Boc-L-phenylalanine (FisherBiotech USA). Other chemical reagents and solvents (of reagent grade and special purity grade; manufactured in Russia) were purified, if necessary, according to standard procedures.[11] 1-Tetradecyl-4-(4-nitrophenoxycarbonyl)methyl-1,4-diazoniabicyclo[2.2.2]octane dibromide was kindly provided by D. A. Konevets (Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences).

Thin-layer chromatography was performed on DC-Alufolien Kieselgel 60 F_{254} plates (Merck, Germany) using the CH_2Cl_2—methanol (9 : 1) (*A*) and ethyl acetate—methanol (3 : 1) (*B*) solvent systems.

The ^{1}H NMR spectra were recorded on a Bruker WP-200-SY spectrometer (200 MHz) with Me_4Si as the internal standard. The chemical shifts are given on the δ scale. The MALDI-TOF mass spectra were obtained on a Bruker FLEX III spectrometer (Bruker Analytical Systems).

***N*$^{\alpha}$-Boc-Amino acid octyl/decyl esters (general procedure).** N^{α}-Boc-Amino acid (20 mmol) was dissolved in ethyl acetate (15 mL). Triethylamine (22 mmol) and 1-bromooctane/1-bromodecane (20 mmol) were added. The reaction mixture was refluxed for 1 day. Triethylamine hydrobromide that precipitated was filtered off. The solution was washed successively with water, a 2% citric acid solution, water, a saturated $NaHCO_3$ solution, and water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The resulting oily compound was used in subsequent experiments without additional purification.

N-Hydrosuccinimide esters of protected amino acids were synthesized according to a known general procedure.[12]

Boc-Deprotection (general procedure). A Boc-protected amino acid or peptide (1 mmol) was dissolved in a 1 : 1 TFA—CH_2Cl_2 mixture (5 mL). The reaction mixture was kept at ~20 °C for 1.5 h, the solvent was removed *in vacuo*, and ethanol was added to, and distilled from, the residue (3×10 mL).

Peptide synthesis (general procedure). A solution of *N*-hydrosuccinimide esters of a protected amino acid (1.2 mmol) in ethyl acetate (5 mL) was added with stirring to a solution of TFA salt of an *N*-nonprotected amino acid or peptide (1 mmol) and triethylamine (2 mmol, 278 μL) in ethyl acetate (10 mL). The reaction mixture was stirred until the amino component was completely consumed (TLC control) and then *N*,*N*′-dimethylethylenediamine (1 mmol, 110 μL) was added. The reaction mixture was stirred for 30 min and washed with a 2% citric acid solution (20 mL), water (20 mL), a saturated $NaHCO_3$ solution (20 mL), and water (20 mL). The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The solution of the protected tetrapeptide in ethyl acetate (15 mL), after Boc-deprotection, was washed with a saturated $NaHCO_3$ solution (10 mL) and water (10 mL) and then dried with anhydrous Na_2SO_4. The solvent was removed *in vacu*o.

***N*,*O*-Deprotection of tetrapeptides (general procedure).** After Boc-deprotection, tetrapeptides (0.1 mmol) were dissolved in methanol (10 mL) and subjected to hydrogenolysis in the presence of 5% Pd/C (50 mg) (TLC control). After completion of the reaction, the catalyst was filtered off and the solvent was removed *in vacuo*.

Synthesis of peptide conjugates with 1,4-diazabicyclooctane derivatives (general procedure). A protected tetrapeptide (0.1 mmol), after Boc-deprotection, was dissolved in distilled DMF (1 mL). Then triethylamine (0.1 mmol, 13.9 μL) was added. A suspension of 1-tetradecyl-4-(4-nitrophenoxycarbonyl)methyl-1,4-diazoniabicyclo[2.2.2]octane dibromide (0.1 mmol, 64.9 mg) in DMF (1 mL) was added to the reaction mixture and the mixture was stirred at ~20 °C for 48 h. The product was precipitated with 10 volumes of diethyl ether. The white flake-like precipitate was separated by centrifugation, washed with diethyl ether, and dried *in vacuo*.

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