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Artificial ribonucleases: synthesis and RNA cleaving properties of cationic conjugates bearing imidazole residues

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Abstract

Small mimics of the ribonuclease active centre were synthesized by conjugating imidazole residues to a dicationic compound. These compounds were shown to cleave tRNA under physiological conditions. The compounds provide new probes for the investigation of RNA structure in solution and potential catalytic RNA cleaving groups for antisense oligonucleotide derivatives.

Keywords: RNAse A mimics; bication; RNA cleavage; catalysis.

Introduction

Compounds capable of cleaving phosphodiester bonds in RNA under physiological conditions are needed for the development of approaches for investigation of RNA structure and for manipulating RNA nucleases. RNA cleaving compounds can serve as probes for studying RNA structure in solution. With such probes, information concerning the state of all phosphodiester bonds and the ribose in the entire RNA molecule can be obtained which is important for elucidation of the RNA secondary and tertiary structure.

The design of structures which cleave RNA with high efficacy and in a catalytic manner under physiological conditions can open new possibilities in the synthesis of antisense oligonucleotide conjugates for research and therapeutic applications [1].

Recently, the synthesis and RNA hydrolysing properties of a few RNAse A active centre mimics were reported [2-4]. RNAse A active centre was imitated by oligopeptides [2] or small peptide-like molecules [3]. Intercalating groups were used to increase affinity of the

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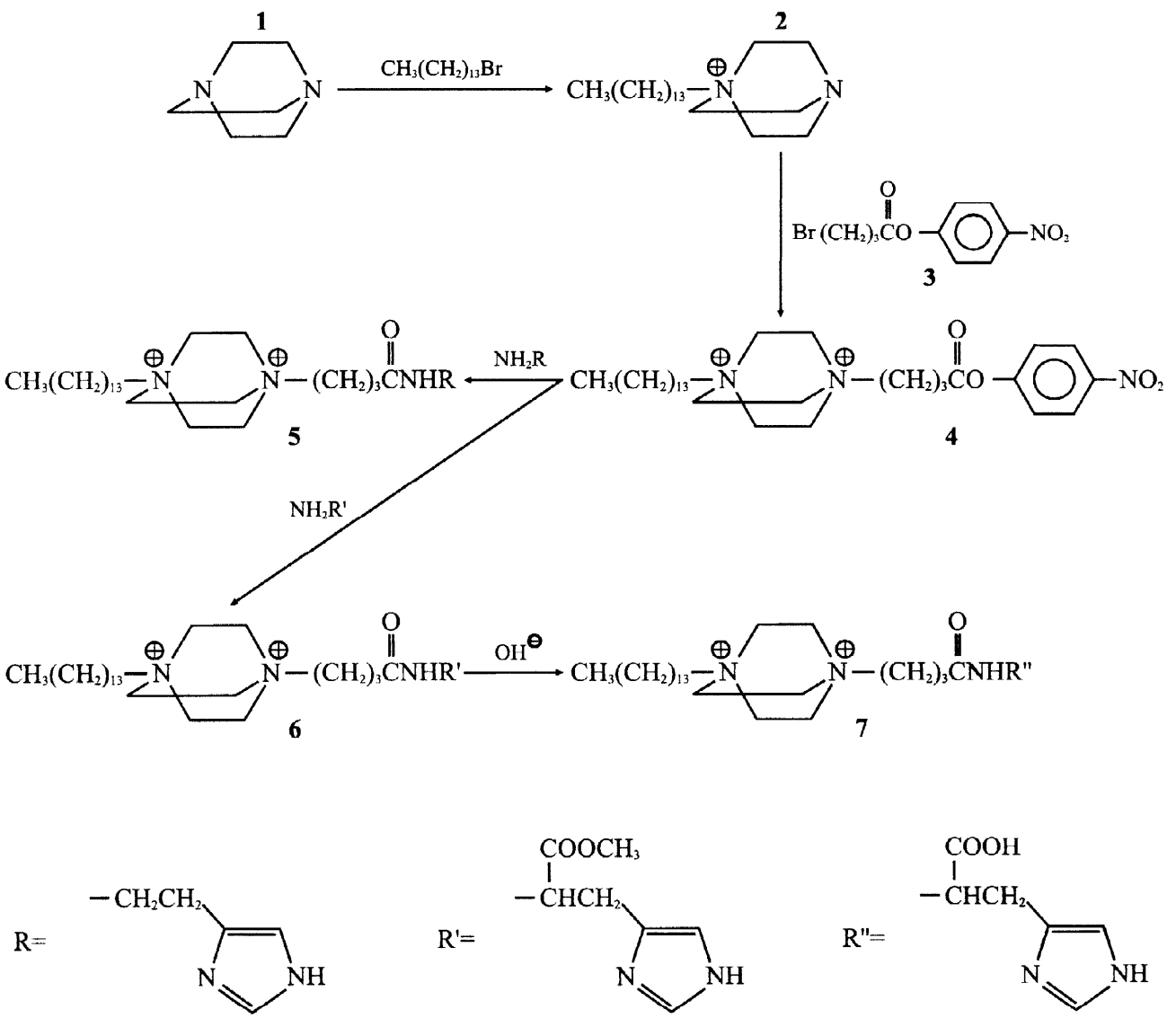
constructions to RNA. A construction bearing two imidazole residues anchored to quarternary phenazinium salt was shown to be the most effective in RNA hydrolysis [4]. The disadvantage of the mentioned compounds was the presence of intercalators as the RNA binding moiety: the intercalators tend to bind within double stranded RNA regions which are rigid and relatively more resistant to cleavage.

In this paper we describe the design, synthesis and investigation of RNA hydrolytic activity of artificial ribonucleases, composed of a dicationic fragment and an imidazole residue.

Results and discussion

The synthesis of chemical ribonucleases is presented in Scheme 1.



Scheme 1. Synthesis of artificial ribonucleases.

The bis-quaternary salts of 1,4-diazabicyclo[2.2.2]octane **1** having a high affinity to phosphate anions [5] were used as the RNA binding part of the RNAses mimics. Compound **2** was synthesized by alkylation of diamine **1**. As a result of the reaction mono quaternary salt of **1** is formed with high yield and suitable for the further utilization without additional purification steps. The aliphatic part of **2** was not necessary for the successful synthesis and was mainly used to increase the solubility of the mono quaternary salt **2** in organic solvents. At the same time the hydrophobic part of the chemical ribonuclease could allow the molecule to penetrate through the cell membrane in further experiments with cultured cell lines *in vivo*. 4-Nitrophenyl ester **4** was obtained with high yield by refluxing of **2** with 4-nitrophenyl-γ-bromobutyrate **3** in acetone. It should be noted, that **3** and its homologues containing both strong alkylating and weak acylating groups allow the high yield synthesis of activated derivatives of **1** in one step and without laborious purification. The structure of **4** was proved by ^{1}H and ^{13}C NMR spectroscopy and the data of elemental analysis. **4** was stable under room temperature for a long time and was used as the basic precursor to conjugates with different potential cleaving active groups. In particular, we have synthesised the conjugates **5**, **6** and **7** with histamine, methyl ester of histidine and histidine respectively. The structures of the conjugates were confirmed by ^{1}H, ^{13}C NMR spectroscopy and high resolution mass spectrometry. In attempting to determine the melting points of compounds **4** - **7** they smoothly decomposed without melting.

Hydrolytic activity of artificial nucleases **5**, **6** and **7** was studied using [^{32}P]-tRNA^{Phe} as a substrate. The RNA was incubated with the compounds in 50 mM imidazole buffer at neutral pH. The positions of RNA cleavage by the nucleases **5**, **6** and **7** and the data on the cleavage efficiency at different sites are shown in Table 1.

Table 1.
Major cleavage sites and efficiencies of reactions of chemical nucleases **5**, **6**, and 7 under different conditions.[a]

Hydrolysis conditions[b]			Cleavage efficiencies[c] of the tRNA^{Phe} on the major cleavage sites[d]							
Chemical nuclease	ON1[e] (M)	$MgCl_2$ (mM)	C_{75}	C_{72}	G_{65}	C_{63}	$C_{61}/_{60}$	C_{13}	U_8	Extent of RNA cleavage (%)[f]
5	-	-	+++	(+)	++	+++	+	+	+[6]	60
5	$5\cdot10^{-5}$	-	0	0	+	+++	++++	+	+	90
6	-	-	+++	(+)	++	+++	+	+	+	60
6	$5\cdot10^{-5}$	-	0	0	+	+++	++++	+	+	100
7	-	-	+++	++	++	+++	+	+	+	95
7	-	5	+++	+	+	(+)	(+)	0	0	15
7	$5\cdot10^{-5}$	-	+	0	+	+++	++++	+	+	100
7	$5\cdot10^{-5}$	5	+++	+	0	0	0	0	(+)	20

[a] - Data of three independent experiments are shown. The rates determination and the analysis of hydrolysis products are described in Experimental;

[b] - [^{32}P]- tRNA^{Phe} was incubated with the conjugates at 37^{0}C for 18 h in 50 mM imidazole buffer at pH 7.0;

[c] - relative rates of the tRNA cleavage were determined as the ratio of the radioactivity of the corresponding cleavage fragment to the total tRNA radioactivity: 0 - no cleavage, (+) - very weak (less then 1%); + - weak (up to 5%); ++ - moderate (up to 8%); +++ - strong (up to 20%); ++++ - very strong (up to 50%);

[d] - the phosphodiester bonds after the indicated residues were hydrolysed;

[e] - the tRNA cleavage was performed in the presence of ON1 (see text) complementary to nucleotides 63-76 of the tRNA;

[f] - the extent of tRNA hydrolysis was determined as the ratio of the hydrolysed tRNA to the total amount of ^{32}P-radioactivity deposited on the gel.

All three RNAses **5**, **6** and **7** demonstrate similar specificity and high RNA hydrolytic activity: 60%, 90% and 100% of tRNA was hydrolysed during 18 h incubation at 37°C with the compounds **5**, **6** and **7** respectively. When the imidazole buffer was substituted by HEPES with the same pH and ionic strength, the cleavage yield decreased to 20-45%. The reaction of compound **5** with one imidazole group was inhibited in the HEPES buffer to a greater extent than that of **7**, with imidazole and carboxylic groups. This fact indicates that the imidazole from the buffer solution may assist the catalytic cleavage, similar to the role of protonated and deprotonated imidazoles in RNA hydrolyses by RNAse A [6,7].

The compounds **5** - **7** have demonstrated similar cleavage specificity (Fig. 1). The cleavage sites within the tRNA were phosphodiester bonds after C_{75}, C_{72}, G_{65}, C_{63}. Hydrolysis of phosphodiester bonds after U_8, C_{13}, and C_{28} occured with slower rates. Weak cleavage was observed after C_{25}, U_{52}, C_{56}. It should be mentioned that all these cleaved phosphodiester bonds are situated in the single-stranded regions of the tRNA except for C_{28} and C_{63} in the anticodon and TΨC steam of tRNA respectively.

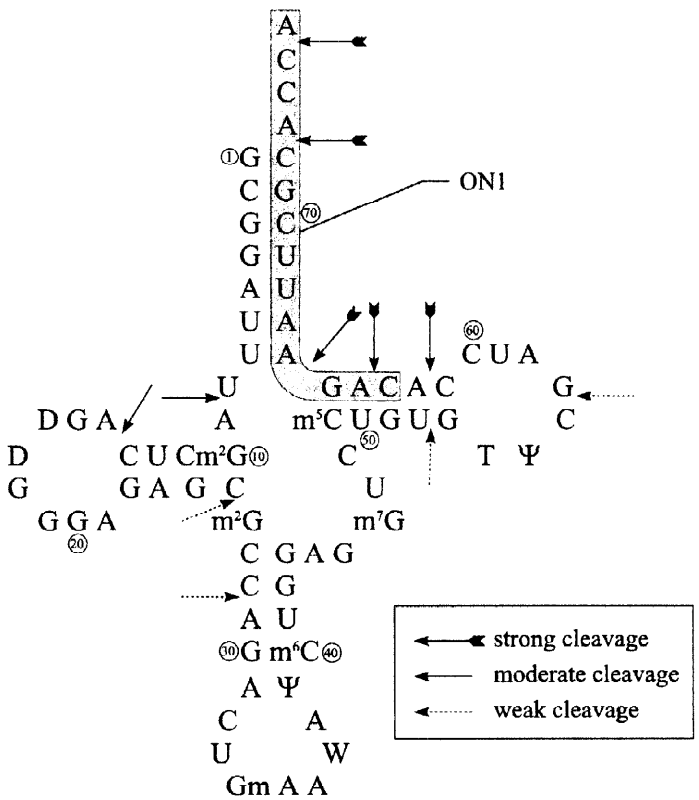


Figure 1. The Cloverleaf Structure of Yeast $tRNA^{Phe}$ and Sites of tRNA Cleavage by RNAse mimics **5**-**7**.

It is known that magnesium ions stabilize the tRNA structure [8,9]. In the presence of 5 mM of Mg^{2+} a considerable (5-fold) decrease of the total rate of RNA cleavage was observed. C_{75} and C_{72} within the open ACCA sequence at 3'-end became the major sites of RNA cleavage in the presence of magnesium, whereas the cleavage in CpA motifs in TΨC loop and steam (C_{61} and C_{63}) and D-loop was completely inhibited. Therefore the stabilization of the RNA structure by magnesium lead to masking of hydrolysis sites by secondary and tertiary interactions and to changing of the rate and positions of the RNA cleavage.

The unfolding of the acceptor stem and the T-stem of tRNA secondary structure by oligonucleotide (ON1) complementary to the 3'-end of tRNA (bases 63-76) increased the rate of tRNA hydrolysis by constructions **5** - **7** and affected the cleavage pattern. All tested compounds in the presence of ON1 cleaved tRNA quantitatively under standard incubation condition. The formation of tRNA-ON1 complex inhibited the hydrolysis of phosphodiester bonds after C_{75}, C_{72} and G_{65} and made phosphodiester bonds after C_{63} and C_{61} the major targets for the RNAses **5** - **7**.

The results obtained suggest that the synthesized RNAse mimics are sensitive to RNA structure. Compounds **5** - **7** cleave phosphodiester bonds preferentially in CpA motifs located in single stranded regions of tRNA. Less susceptible targets are phosphodiester bonds after U_8 and G_{65} in UpA and GpA sequences in the junction of RNA stems. It should be noted that **5** - **7** bind to tRNA in the same manner with imidazole residues being localized in the vicinity of internucleotide phosphates.

We have no evidence for the favourable binding of compounds **5** - **7** to CpA motifs rather than to any other sequences. Thereby, the structural and sequence preference of RNA cleavage by **5** - **7** at CpA sequences in the single-stranded regions obviously is connected with abnormal lability and/or favourable orientation of phosphodiester bonds in these Py-Pu motifs. Indirectly this is confirmed by spontaneous tRNA cleavage at UpA (U_8) site caused by different non-specific chemicals and a number of proteins [10].

Conclusion

RNA cleaving conjugates were synthesized using the same scheme of synthesis based on the peptide chemistry approaches. The first evaluation of the hydrolytic activity of the synthesised RNAse mimics showed that all the compounds are promising both as probes for studying the RNA structure and for biomedical applications due to their high specificity and high efficiency in RNA hydrolysis.

Experimental

General. Radioactivity of the samples was determined by counter Minibetta (Pharmacia, Sweden). Flash column chromatography was performed using Silpearl (100-200 mesh, Sklo Union k. p., Czechoslovakia). Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed using the Elemental Analyzer Model 1106 (Carlo Erba, Italy). Infrared (IR) spectra were recorded on a Specord M-80 spectrometer (Karl Zeiss Iena, GDR). Unless otherwise noted, NMR spectra were obtained using a Bruker AM-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C spectra respectively or a Bruker WP-200 spectrometer at 200 MHz for ^1H spectra in other cases. All coupling constants are given in Hertz, chemical shifts - in ppm. ^{13}C spectra were recorded using J-modulated spin echo. All spectra were recorded in Dimethylsulfoxide-D6 (99% D, Aldrich) with TMS as an internal standard. The NMR spectra catalogue of Sadtler Research Laboratories, Inc. and other widely known sources of NMR spectral information were used for the interpretation of obtained NMR spectra.

High Resolution Electrospray Mass Spectra were recorded using a home built Time-of Flight Mass Spectrometer with Orthogonal Extraction of ions injected from Electrospray Ion Source (ES O-TOF-MS) [11]. The ES O-TOF-MS has mass resolving power ~3000 and accuracy of ion mass determination better than 10^{-4} in case a calibration was performed in the day of

analysis. All samples for mass spectrometry were dissolved in methanol at a concentration of $5 \cdot 10^{-5}$M. The homogeneity of synthesized substances was proved by NMR spectroscopy.

1-Tetradecyl-1-azonia-4-azabicyclo[2.2.2]octane bromide (2) [62634-13-3]. To the suspension of 5.6 g (0.05 moles) 1,4-diazabicyclo[2.2.2]octane (**1**) in 30 ml of boiling ether, a minimal amount of acetone needed for the complete dissolving of **1** was added, followed by dropwise addition of 11.08 g (0.04 moles) of tetradecylbromide. The mixture was refluxed for 6 h, then cooled. The formed precipitate was filtered off, washed with ether and dried in vacuo over P_2O_5 to give 13.8 g (89%) of white small-crystal powder of **2**. M.P. 163-166^{0}C.
^{1}H-NMR (200 MHz) δ: 0.84 (t, J=6.7, 3H, $C\underline{H}_3$), 1.24 (br, 22H, $CH_3(C\underline{H}_2)_{11}$), 1.64 (m, 2H, $(CH_2)_{11}C\underline{H}_2CH_2N^+$), 3.00 (m, 6H, $N(C\underline{H}_2CH_2)_3N^+$), 3.20 (t, J=10.2, 2H, $(CH_2)_{11}CH_2C\underline{H}_2N^+$), 3.30 (m, 6H, $N(CH_2C\underline{H}_2)_3N^+$).
^{13}C-NMR δ: 13.70 (1C, $\underline{C}H_3$), 22.05 (1C, $(CH_2)_{11}\underline{C}H_2CH_2N^+$), 23.10 (1C, $CH_3\underline{C}H_2$), 28.10 (1C, $(CH_2)_{10}\underline{C}H_2(CH_2)_2N^+$), 29.00-31.50 (9C, $CH_3CH_2(\underline{C}H_2)_9(CH_2)_3N^+$), 44.69 (3C, $N(\underline{C}H_2CH_2)_3N^+$), 54.35 (3C, $N(CH_2\underline{C}H_2)_3N^+$), 61.23 (1C, $\underline{C}H_2N^+$).

4-Nitrophenyl-γ-butyrate (3) [79376-77-5]. Freshly distillated thionylbromide (10.92 g, 0.05 moles) was added dropwise to the 4.3 g (0.05 mole) of γ-butyrolactone at 0^{0}C. After incubation at room temperature for 30 min the reaction mixture was heated at 60^{0}C until the gas evolution stopped, then cooled. 6.95 g (0.05 moles) of dried 4-nitrophenol was added to the obtained non-purified γ-brombutyrylbromide. The reaction mixture was slowly heated up to 120^{0}C, then incubated at this temperature for 30 min and cooled, followed by dilution by 20 ml of chloroform. After purification by flash liquid chromatography on silicagel with chloroform as eluent and solvent removing the obtained yellow oil was dissolved in 10 ml of dried ethanol. 7.92 g (55%) of **3** was crystallized at -5^{0}C as colourless needle-like crystals. M.P. 54^{0}C (55-58^{0}C in literature [12]).

1-Tetradecyl-4-γ-(carb-4-nitrophenoxy)-propyl-1,4-diazoniabicyclo[2.2.2]octane dibromide (4). 1.95 g (5 mmole) of **2** was suspended in the solution of 1.51 g (5.25 mmole) of **3** in 10 ml of acetone. The mixture was refluxed for 18 h. After cooling the precipitate was filtered off, washed with acetone and dried in vacuo over P_2O_5 to give 3.08 g (91%) of light-cream hygroscopic crystals.
Anal. Calc. for $C_{30}H_{51}N_3O_4Br_2$: C, 53.2; H, 7.5; N, 6.2. Found: C, 52.5; H, 7.7; N, 6.1%.
^{1}H-NMR δ: 0.843 (t, J=6.7, 3H, $C\underline{H}_3$), 1.232 (br, 22H, $CH_3(C\underline{H}_2)_{11}$), 1.682 (m, 2H, $(CH_2)_{11}C\underline{H}_2CH_2N^+$), 2.137 (m, 2H, $N^+CH_2C\underline{H}_2CH_2CO$), 2.812 (t, J=7.1, 2H, $C\underline{H}_2CO$), 3.324 (t, J=7.3, 6H, $CH_3(CH_2)_{13}N^+(C\underline{H}_2CH_2)_3N^+$), 3.534 (t, J=7.3, 6H, $N^+(CH_2C\underline{H}_2)_3N^+(CH_2)_3CO$), 3.551-3.698 (2m, 2H + 2H, $C\underline{H}_2N^+$), 7.506 (d, J=9.2, 2H, H-2,6 Ph), 8.322 (d, J=9.2, 2H, H-3,5 Ph).
^{13}C-NMR δ: 13.84 (1C, $\underline{C}H_3$), 21.09 (1C, $N^+CH_2\underline{C}H_2CH_2CO$), 21.26 (1C, $(CH_2)_{11}\underline{C}H_2CH_2N^+$), 21.98 (1C, $CH_3\underline{C}H_2$), 25.67 (1C, $(CH_2)_{10}\underline{C}H_2(CH_2)_2N^+$), 28.35-28.95 (9C, $CH_3CH_2(\underline{C}H_2)_9(CH_2)_3N^+$), 31.19 (1C, $N^+CH_2CH_2\underline{C}H_2CO$), 50.33-50.76 (3C + 3C, $N(\underline{C}H_2\underline{C}H_2)_3N^+$), 62.17-63.28 (1C + 1C, $\underline{C}H_2N^+$), 123.14 (2C, C-2,6 Ph), 125.19 (2C, C-3,5 Ph), 145.02 (1C, C-4 Ph), 155.13 (1C, C-1 Ph), 170.03 (1C, $\underline{C}OO$).
IR (KBr pellet; only the most characteristics absorbance bands are shown) cm^{-1}:
CH_2-groups: 2925 (s., $\nu_{asym}CH_2$), 2855 (s., $\nu_{sym}CH_2$), 1463 (s., $\delta_{asym}CH_2$);
ester-group: 1720 (s., νC=O), 1205 (s., νC-O-C), 1113 (s., νC-O-C);
nitro-group: 1528 (s., $\nu_{asym}NO_2$), 1334 (s., $\nu_{sym}NO_2$);
1,4-disubstituted aryl: 3020 (sm., ν(CH aryl)), 1750 (m., δ(CH), aryl), 1705 (m., ν(C=C aryl)), 1614 (s., ν(C=C aryl)), 1592 (s., ν(C=C aryl)), 1444 (s., ν(C=C

aryl)), 1155 (m., δ(CH aryl)), 1058 (m., δ(CH aryl)), 853 (s., δ(CH 1,4-aryl).

1-Tetradecyl-4-γ-[N-(2-(imidazole-4-yl)-ethyl)-carbamyl]-propyl-1,4-diazoniabicyclo[2.2.2] octane dibromide (5). The solution of 12 mg (0.11 mmoles) of histamine in 0.5 ml of dimethylformamide (DMF) and 11.1 mg (0.11 mmoles) triethylamine (TEA) were added to the solution of 69.3 mg (0.1 mmoles) of **4** in 1 ml of DMF. The mixture was incubated at 45°C for 12 h then evaporated up to the volume of 0.15-0.20 ml and the product was precipitated by acetone. For purification the reaction product was reprecipitated by acetone from ethanol solution, filtered off, dried in vacuo over P_2O_5 to give 30 mg (45%) of **5** as a lightly coloured highly hygroscopic amorphic substance. The sample contained trace amount of dimethylformamide.

^{1}H-NMR δ: 0.848 (t, J=6.8, 3H, $C\underline{H}_3$), 1.238 (br., 22H, $CH_3(C\underline{H}_2)_{11}$), 1.695 (m, 2H, $(CH_2)_{11}C\underline{H}_2CH_2N^+$), 1.933 (t, J=7.2, 2H, $C\underline{H}_2CO$), 2.188 (m, 2H, $N^+CH_2C\underline{H}_2CH_2CO$), 2.682 (t, J=7.0, 2H, $C\underline{H}_2Im$), 3.303 (dt, J_d=5.8, J_t=7.0, 2H, $CONHC\underline{H}_2$), 3.505-3.531 (2t, J=10.5, 2H + 2H, $C\underline{H}_2N^+$), 3.936 (s, 12H, $N^+(C\underline{H}_2C\underline{H}_2)_3N^+$), 6.978 (s, 1H, H-5 Im), 7.944 (s, 1H, H-2 Im), 8.091 (t, J=5.8, 1H, $CON\underline{H}$).

^{13}C-NMR δ: 13.85 (1C, $\underline{C}H_3$), 17.64 (1C, $N^+CH_2\underline{C}H_2CH_2CO$), 21.25 (1C, $(CH_2)_{11}\underline{C}H_2CH_2N^+$), 21.98 (1C, $CH_3\underline{C}H_2$), 25.45 (1C, $(CH_2)_{10}\underline{C}H_2(CH_2)_2N^+$), 26.18 (1C, $\underline{C}H_2Im$), 28.30-29.00 (8C, $CH_3(CH_2)_2(\underline{C}H_2)_8(CH_2)_3N^+$), 31.06 (1C, $CH_3CH_2\underline{C}H_2$), 31.19 (1C, $N^+CH_2CH_2\underline{C}H_2CO$), 38.39 (1C, $CONH\underline{C}H_2$), 50.34 (6C, $N(\underline{C}H_2\underline{C}H_2)_3N^+$), 62.79-63.30 (1C + 1C, $\underline{C}H_2N^+$), 116.52 (1C, C-5 Im), 133.37 (1C, C-4 Im), 134.32 (1C, C-2 Im), 170.29 (1C, $\underline{C}ONH$).

ES-TOF-MS (Table 2.)

Table 2.
Mass spectrum of **5**.

Fragment	M/Z (calc.)[a]	M/Z (exp.)	I (%)
$[M\text{-}Br]^+$	570.359	570.418	3.7
$[M\text{-}Br]^+$	568.359	568.415	3.6
		384.348	5.5
		383.848	10.3
$[C_{14}H_{29}N(CH_2CH_2)_3N]^+$ (^{13}C)	310.328	310.365	3.5
$[C_{14}H_{29}N(CH_2CH_2)_3N]^+$	309.327	309.368	13.7
$[M\text{-}Br+H]^{2+}$ (^{13}C)	286.184	286.221	2.6
$[M\text{-}Br+H]^{2+}$	285.683	285.720	7.3
$[M\text{-}Br+H]^{2+}$ (^{13}C)	285.184	285.225	2.4
$[M\text{-}Br+H]^{2+}$	284.683	284.733	6.6
$[M\text{-}2Br+DMFA]^{2+}$ (^{13}C)	281.748	281.781	3.6
$[M\text{-}2Br+DMFA]^{2+}$	281.247	281.286	10.0
$[M\text{-}2Br]^{2+}$ (^{13}C)	245.221	245.263	31.0
$[M\text{-}2Br]^{2+}$	244.720	244.757	100.0
$[M\text{-}C_{14}H_{29}N(CH_2CH_2)_3N\text{-}2Br]^+$	180.114	180.149	5.0

[a] M/Z values are given for most abundant isotopes in all tables.

1-Tetradecyl-4-γ-[N-(1-carbmethoxy-2-(imidazole-4-yl)-ethyl)-carbamyl]-propyl-1,4-diazoniabicyclo[2.2.2]octane dibromide (6). 138 mg (0.2 mmole) of **4** and 37 mg (0.22 mmole) of free base of methyl ester of histidine were used to synthesized 66 mg of **6** (47%) as described for **3**. Compound **6** looks like lightly coloured amorphic highly hygroscopic substance.

^{1}H-NMR (200 MHz) δ: 0.85 (t, J=6.5, 3H, $C\underline{H}_3$), 1.24 (br., 22H, $CH_3(C\underline{H}_2)_{11}$), 1.67 (m, 2H, $(CH_2)_{11}C\underline{H}_2CH_2N^+$), 1.89 (m, 2H, $N^+CH_2C\underline{H}_2CH_2CO$), 2.24 (t, J=6.4, 2H, $C\underline{H}_2CONH$), 3.00 (d, J=7.5, 2H, CH_2Im), 3.48 (m, 4H, $C\underline{H}_2N^+$), 3.63 (s, 3H, $COOC\underline{H}_3$), 3.90 (s, 12H, $N^+(C\underline{H}_2C\underline{H}_2)_3N^+$), 4.50 (m, 1H, $C\underline{H}COO$), 7.18 (s, 1H, H-5 Im), 8.37 (s, 1H, H-2 Im), 8.48 (s, 1H, H-1 Im), 8.60 (d, J=7.9, 1H, $CON\underline{H}$).
^{13}C-NMR δ: 13.72 (1C, $\underline{C}H_3$), 17.52 (1C, $N^+CH_2\underline{C}H_2CH_2CO$), 22.07 (1C, $(CH_2)_{11}\underline{C}H_2CH_2N^+$), 23.18 (1C, $CH_3\underline{C}H_2$), 26.50 (1C, $(CH_2)_{10}\underline{C}H_2(CH_2)_2N^+$), 28.23 (1C, $\underline{C}H_2Im$), 29.00-31.50 (9C, $CH_3(CH_2)_2(\underline{C}H_2)_8(CH_2)_3N^+$), 33.25 (1C, $N^+CH_2CH_2\underline{C}H_2CO$), 51.90 (1C, $C\underline{H}_3O$), 52.72 (6C, $N(\underline{C}H_2\underline{C}H_2)_3N^+$), 56.35 (1C, $\underline{C}HCOO$), 61.81-63.25 (1C + 1C, $\underline{C}H_2N^+$), 120.15 (1C, C-5 Im), 129.77 (1C, C-4 Im), 132.71 (1C, C-2 Im), 169.21 (1C, $\underline{C}OOCH_3$), 172.15 (1C, $\underline{C}ONH$).
ES-TOF-MS (Table 3.)

Table 3.
Mass spectrum of **6**.

Fragment	M/Z (calc.)[a]	M/Z (exp.)	I (%)
$[M\text{-}Br]^+$	628.364	628.340	0.6
$[M\text{-}Br]^+$	626.364	626.358	0.6
$[M\text{-}Br\text{-}CH_3+H]^+$	614.349	614.384	0.3
$[M\text{-}Br\text{-}CH_3+H]^+$	612.349	612.352	0.3
$[M\text{-}2Br\text{-}CH_3]^+$	532.422	532.423	1.4
$[2(M\text{-}2Br\text{-}CH_3)\text{-}CO_2\text{-}C_{14}H_{29}+2H]^{2+}$ (^{13}C)	413.323	413.285	7.0
$[2(M\text{-}2Br\text{-}CH_3)\text{-}CO_2\text{-}C_{14}H_{29}+2H]^{2+}$	412.822	412.785	14.4
		315.725	7.9
$[C_{14}H_{29}N(CH_2CH_2)_3N]^+$	309.327	309.331	9.4
		301.114	8.4
$[M\text{-}2Br]^{2+}$ (^{13}C)	274.223	274.222	33.5
$[M\text{-}2Br]^{2+}$	273.723	273.739	100.0
$[M\text{-}2Br\text{-}CH_3+H]^{2+}$ (^{13}C)	267.215	267.210	19.1
$[M\text{-}2Br\text{-}CH_3+H]^{2+}$	266.715	266.730	65.5

[a] M/Z values are given for most abundant isotopes in all tables.

Triethylammonium salt of 1-Tetradecyl-4-γ-[N-(1-carboxy-2-(imidazole-4-yl)-ethyl)-carbamyl]-propyl-1,4-diazoniabicyclo[2.2.2]octane dibromide (7). Compound **6** was dissolved in the mixture of ethanol-water-triethylamine 4:5:1 and incubated at 45°C for 12 h. The solution was evaporated and the residue was rubbed with acetone to give quantitatively lightly coloured amorphic highly hygroscopic compound **7**.
^{1}H-NMR δ: 0.852 (t, J=6.5, 3H, $C\underline{H}_3$), 1.182 (t, J=7.3, 9H, $C\underline{H}_3$ TEA), 1.241 (br., 22H, $CH_3(C\underline{H}_2)_{11}$), 1.673 (m, 2H, $(CH_2)_{11}C\underline{H}_2CH_2N^+$), 1.901 (m, 2H, $N^+CH_2C\underline{H}_2CH_2CO$), 2.226 (t, J=6.4, 2H, $C\underline{H}_2CONH$), 2.876 (d, J=8.0, 2H, CH_2Im), 3.051 (q, J=7.3, 6H, $CH_3C\underline{H}_2$ TEA), 3.254-3.468 (2t, J=10.0, 2H + 2H, CH_2N^+), 3.874 (s, 12H, $N^+(C\underline{H}_2C\underline{H}_2)_3N^+$), 4.442 (q, J=8.0, 1H, $C\underline{H}COO$), 6.831 (s, 1H, H-5 Im), 7.609 (s, 1H, H-2 Im), 8.074 (s, 1H, H-1 Im), 8.350 (d, J=7.9, 1H, $CON\underline{H}$).
^{13}C-NMR δ: 8.69 (3C, $\underline{C}H_3$ TEA), 13.65 (1C, $\underline{C}H_3$), 17.50 (1C, $N^+CH_2\underline{C}H_2CH_2CO$), 22.45 (1C, $(CH_2)_{11}\underline{C}H_2CH_2N^+$), 23.02 (1C, $CH_3\underline{C}H_2$), 26.06 (1C, $(CH_2)_{10}\underline{C}H_2(CH_2)_2N^+$), 28.20 (1C, $\underline{C}H_2Im$), 29.00-31.50 (9C, $CH_3(CH_2)_2(\underline{C}H_2)_8(CH_2)_3N^+$), 32.19 (1C, $N^+CH_2CH_2\underline{C}H_2CO$), 47.05 (3C, $CH_3\underline{C}H_2$ TEA), 51.68 (6C, $N(\underline{C}H_2\underline{C}H_2)_3N^+$), 57.65 (1C, CHCOO), 64.53-65.50 (1C + 1C, $\underline{C}H_2N^+$), 117.00 (1C, C-5 Im), 131.66 (1C, C-4 Im), 134.80 (1C, C-2 Im), 173.21 (1C, $\underline{C}OO$), 177.04 (1C, $\underline{C}ONH$).

ES-TOF-MS (Table 4).

Table 4.
Mass spectrum of 7.

Fragment	M/Z (calc.)[a]	M/Z (exp.)	I (%)
$[M\text{-}Br\text{-}TEA+H+CO]^+$ (^{13}C)	643.345	643.430	0.8
$[M\text{-}Br\text{-}TEA+H+CO]^+$	642.344	642.440	1.8
$[M\text{-}Br\text{-}TEA+H+CO]^+$ (^{13}C)	641.345	641.410	1.0
$[M\text{-}Br\text{-}TEA+H+CO]^+$	640.344	640.433	1.8
$[M\text{-}Br\text{-}TEA+H]^+$	614.349	614.391	1.2
$[M\text{-}Br\text{-}TEA+H]^+$	612.349	612.418	1.0
$[M\text{-}2Br\text{-}TEA]^+$	532.422	532.456	2.1
$[M\text{-}2Br\text{-}TEA+C_{14}H_{29}N(CH_2CH_2)_3N\text{-}2H]^{2+}$ (^{13}C)	420.368	420.379	4.6
$[M\text{-}2Br\text{-}TEA+C_{14}H_{29}N(CH_2CH_2)_3N\text{-}2H]^{2+}$	419.867	419.851	9.8
$[C_{14}H_{29}N(CH_2CH_2)_3N(CH_2)_3CO+H]^+$ (^{13}C)	381.378	381.378	2.7
$[C_{14}H_{29}N(CH_2CH_2)_3N(CH_2)_3CO+H]^+$	380.377	380.369	10.0
$[M\text{-}2Br\text{-}TEA+NH_2(CH_2)_2Im+H]^{2+}$ (^{13}C)	323.760	323.226	10.0
$[M\text{-}2Br\text{-}TEA+NH_2(CH_2)_2Im+H]^{2+}$	322.759	322.784	23.5
$[C_{14}H_{29}N(CH_2CH_2)_3N]^+$ (^{13}C)	310.328	310.357	28.0
$[C_{14}H_{29}N(CH_2CH_2)_3N]^+$	309.327	309.356	100.0
$[M\text{-}2Br\text{-}TEA+H+CO]^{2+}$ (^{13}C)	281.213	281.227	17.0
$[M\text{-}2Br\text{-}TEA+H+CO]^{2+}$	280.712	280.764	37.5
$[M\text{-}2Br\text{-}TEA+H]^{2+}$ (^{13}C)	267.215	267.246	10.3
$[M\text{-}2Br\text{-}TEA+H]^{2+}$	266.715	266.748	25.3
$[M\text{-}2Br\text{-}TEA\text{-}CO]^{2+}$	252.214	252.207	30.0
$[M\text{-}C_{14}H_{29}N(CH_2CH_2)_3N\text{-}TEA\text{-}2Br+H]^+$	224.103	224.130	11.7
$[C_{14}H_{29}N(CH_2CH_2)_3N(CH_2)_3CO+3H]^{2+}$	191.196	191.167	78.0

[a] M/Z values are given for most abundant isotopes in all tables.

Cleaving of tRNA^{Phe} by compounds 5-7 (general procedure). 3'-[^{32}P]-tRNA^{Phe} was obtained according to published protocols [13,14]. Briefly, 15 μl reaction mixture contained 50 mM HEPES-KOH pH 7.5; 10 mM $MgCl_2$; 10% DMSO; 0.1 mM ATP; 2 mM DTE; 100 μg/ml BSA; 160 pmole of tRNA; 200 μCu [^{32}P]-pCp and 20 U. T4 RNA ligase. The reaction was performed at 4^{0}C overnight. The labelled tRNA was isolated by using denatured 12% polyacrylamide gel electrophoresis, eluted from the gel by 0.5 M ammonium acetate and precipitated by ethanol. Obtained [^{32}P]-tRNA^{Phe} was $5\cdot10^5$ cpm/pmole of specific activity.

3'-[^{32}P]-tRNA^{Phe} hydrolysis by **5-7** was performed at 37^{0}C for 18 h. The reaction mixture contained 50 mM imidazole buffer pH 7.0; 200 mM KCl; 1 mM EDTA; 1 μg of RNA carrier (total tRNA from Escherichia coli); $5\cdot10^{-7}$ M 3'-[^{32}P]-tRNA^{Phe} and $5\cdot10^{-5}$ M one of chemical nucleases **5-7**. Some reaction mixtures contained 5 mM $MgCl_2$ and/or $5\cdot10^{-5}$ M ON1 (TGGTGCCGAATTCTG, complementary to the 63-76 sequence of tRNA). These experiments are indicated in the Table 1. For some experiments the buffer was 50 mM HEPES-KOH pH 7.0 instead of 50 mM imidazole; these experiments are also indicated. After incubation, the tRNA and their hydrolysis products were precipitated by ethanol and resolved on 12% polyacrylamide gel containing 8 M urea. Gel was radioautographed on the X-Ray film (Fuji). To obtain quantitative data the band of tRNA or tRNA fragments were cut out of the gel and the radioactivity of the gel slices was determined.

To localise the sites of tRNA cleavage, partial RNAse T1 digestion of tRNA under denatured condition was run on the same gel in parallel with experimental samples.

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