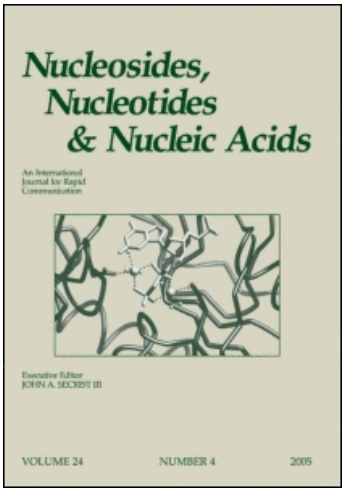

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Molecular Design of Artificial Ribonucleases Using Electrostatic Interaction

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ABSTRACT

A number of small organic ribonucleases have been synthesized with rigid polycationic structures containing an aromatic framework with two residues of bis-quaternary salts of 1,4-diazabicyclo[2.2.2]octane (DABCO) bearing various substituents. The compounds carrying positively charged groups connected via rigid linker are expected to bend the sugar-phosphate backbone and can stimulate the intramolecular phosphoester transfer reaction.

Key Words: Artificial ribonuclease; RNA cleavage; 1,4-diazabicyclo[2.2.2]octane.

INTRODUCTION

Many nature and artificial ribonucleases and ribozymes cleave RNA by an intramolecular phosphoester transfer reaction. The mechanism of this reaction involves the nucleophilic attack of the 2'-oxygen on the adjacent phosphorus center (Fig. 1). A number of artificial ribonucleases, which can accelerate the cleavage of RNA by this phosphoester transfer pathway, have been created over the last decade. As a rule artificial ribonucleases are proposed to combine both general base catalysis and general acid catalysis to promote RNA transesterification in the first step of its catalytic mechanism.

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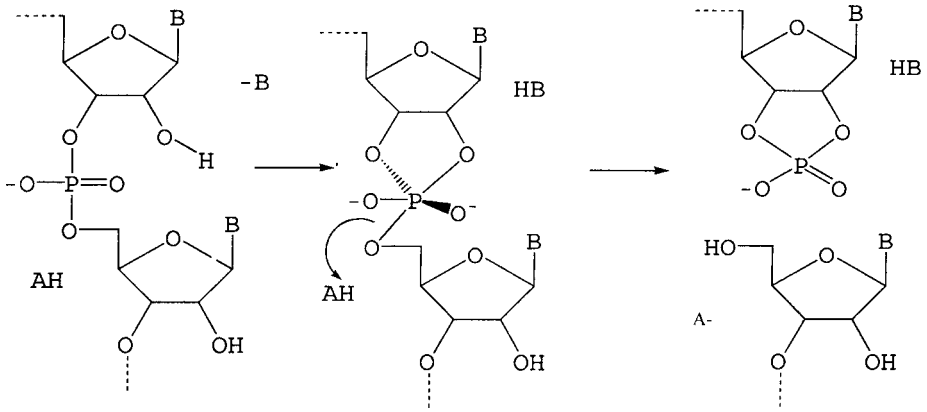


Figure 1. RNA cleavage mechanism by intramolecular phosphoester transfer.

On the other hand it is known that RNA can be spontaneously cleaved in the absence of RNA-cleaving compounds. As has been shown,[1] there is a relationship between some structural parameters of RNA linkages and the rate constants defining the spontaneous cleavage of RNA by intramolecular transesterificaion (Fig. 2).

The relative in-line character of the phosphodiester linkage in RNA needed for successful cleavage is established by the combination of two structure parameters (Fig. 2A). The torsion angles centered at C3'-O3'(ε) and O3'-P(φ) bonds determine

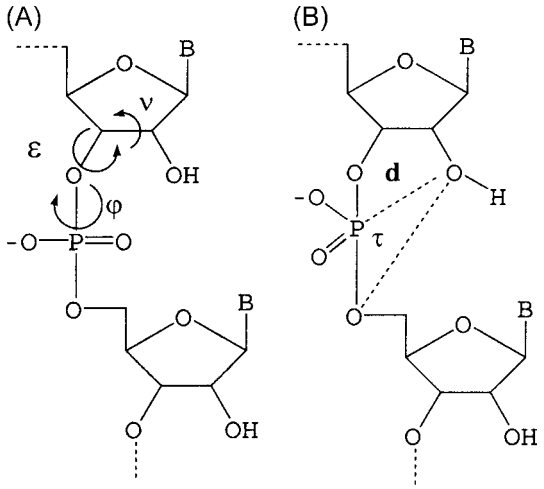
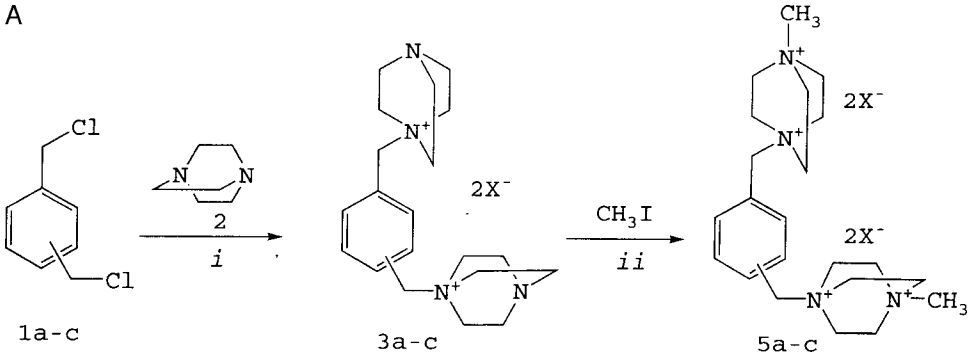


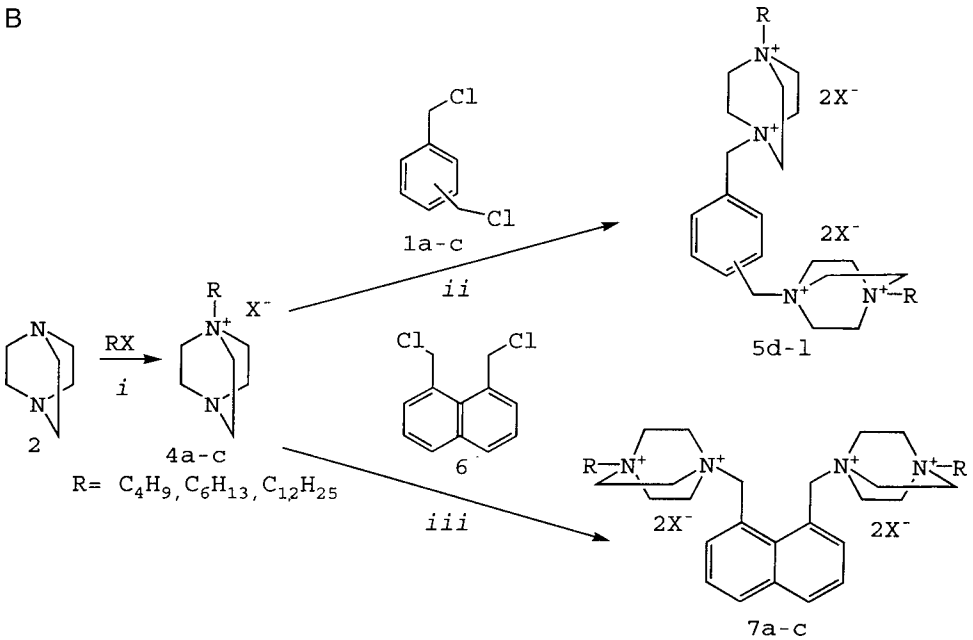
Figure 2. The internucleotide geometry of RNA. (**A**): The relative in-line conformation of each RNA linkage is defined by the torsion angles centered on three bonds identified as ε, φ, and ν. Free rotation is possible at ε and φ, whereas more restricted rotation around ν dictates the sugar pucker. (**B**): A τ angle of 180° is optimal for in-line attack. Because of steric constraints, a t angle of ≈ 45° approximates the most unfavorable orientation that can be achieved by RNA. Likewise, an attack distance of 3.0 Å is used as the closest approach that can be achieved between the 2′ oxygen and the phosphorus center in the ground state.



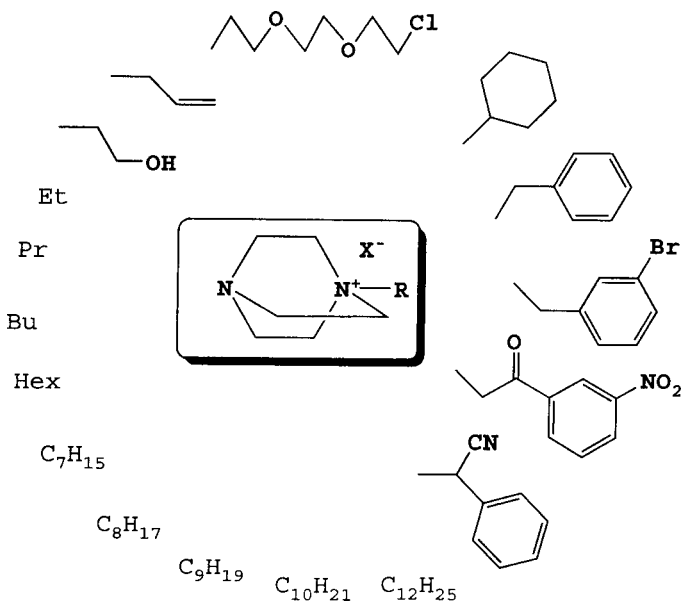
Scheme 1. Conditions: (*i*) DMF, 24°C, 30-40 h; (*ii*) H_2O-BuOH, 24°C, 15-20 h.

whether the 5'-oxygen leaving group will be positioned directly on the opposite side of the electrophilic phosphorus center relative to the nucleophilic 2'-oxygen. As a result, there may exist a continuum of structural states that range between a perfect non-in-line conformation ($\tau = 45°$) and a perfect in-line conformation ($\tau = 180°$) that defines the orientation of the 2'-oxygen, phosphorus, and 5'-oxygen centers relative to each other (Fig. 2B). The same two torsion angles that determine in-line orientation and the angle ν establish the attack distance **d** between the 2'-hydroxyl group and the phosphorus atom.

Based on these facts and molecular modelling studies, we have supposed that compounds carrying positively charged groups conjugated by rigid linker would be



Scheme 2. Conditions: (*i*) $(CH_3)_2CO$, 24°C, 4-48 h; (*ii*) CH_3CN, 24°C, 30-40 h; (*ii*) CH_3CN, 24°C, 24 h.



Scheme 3. Structures of mono-quarternary salts of 1,4-diazabicyclo[2.2.2]octane used for preparation of the combinatorial library of artificial ribonucleases.

capable of introducing the necessary distortion into the sugar-phosphate framework and would stimulate the intramolecular phosphoester transfer reaction.

In the present work we describe a series of novel artificial ribonucleases based on aromatic frameworks with two 1,4-diazabicyclo[2.2.2]octanes substitutes. (Schemes 1–3).

RESULTS AND DISCUSSION

A number of cationic molecule structures possessing high affinity to phosphate anions have been reported.[2] In our work we have used bis-quaternary salts of 1,4-diazabicyclo[2.2.2]octane as cationic groups. The high affinity of these salts to phosphate-anions is due to steric factors.[3] On the other hand the DABCO motif is a simple method of introducing a positive charge into a designed cationic host. This motif is readily alkylated[4] and, since the two nitrogen atoms are effectively insulated from each other, double differential alkylation is readily accomplished.[5]

Hydrophobic interactions involving bases of nucleic acids are key processes in both chemical and biological recognition. A series of studies has shown that bis-quaternary salts of 1,4-diazabicyclo[2.2.2]octane containing hydrophobic residues can be used for separation of the mono- or triphosphate derivatives of nucleosides.[3,6] It was found that the UpA-specific self-cleavage reaction of some RNA structures can occur in the presence of ammonia cations and non-ionic or zwitter-ionic detergents.[7] We have supposed that in our case hydrophobic radicals can also be ensured similar selectivity of interaction between RNA-target and artificial ribonucleases too.

For this research, a combinatorial approach was used to expand the library of such cleaving constructs to analyze structure-based cleaving properties and, therefore, to obtain a '*Structure-Function*' correlation.

Two strategies were used to synthesize polycationic molecules as shown in Scheme 1 (route A) and Scheme 2 (route B). The α,α′-dichlorxylols 1a–c were treated with DABCO 2 in aprotonic solvents (DMF or acetone) to result in derivatives 3a–c. The ^{1}H NMR spectra of 3a–c showed the methylene protons as a singlet at δ 4.5 (for 3a), δ 4.71 (for 3b) or δ 4.89 (for 3c) with the DABCO protons as two broad triplets in the region δ 3.20–3.60. Experimental difficulties arised on alkylation step these compounds with alkyl halides as compounds 3a–c didn't dissolve in aprotonic solvents. Attempts to alkylate the nitrogen of the two DABCO moieties in the salts 3a–c in alcoholes were unsuccessful and resulted in formation of monoalkylated products due to low solubility. Alkylation compounds 3a–c in H_2O-n-butanol system led to products 5a–c in high yields only for short alkyl halides. The ^{1}H NMR spectra of compounds 5a–c showed that all signals shifted at lower field and singlet at δ 3.56 for methyl group was appeared. It was found that the route B was preferable. Consequently, DABCO was alkylated with a number of alkyl halides to give the corresponding alkyl-DABCO salts 4a–c which were then treated with α,α′-dichlorxylols (compounds 2a–c) to give corresponding tetracations 5d-l. Products 5d-l were formed in high yields to be independent of structure of alkyl-substitute. The ^{1}H NMR spectra were in agreement with the assigned structures and the ^{13}C NMR spectra showed the expected number of carbons. A similar sequence of reactions (route B) was carried out with 1,8-bis(bromomethyl)-naphthalene (compound 6) to prepare the desired compounds 7a–c in good yield as shown in Scheme 2. The ^{1}H and ^{13}C NMR spectra are consistent with the assigned structures. All structures were proved by the data of elementary analysis. Full chemical description of synthesized compounds will be produced in additional publication.

Combinatorial library of compounds with others hydrophobic residues was synthesized by reacting of mixture of seventeen monosubstituted 1,4-diazabicyclo[2.2.2]octane and α,α′-dichlor-p-xylol in acetonitrile. The substituetes were illustrated in Scheme 3.

The novel artificial ribonucleases have been tested (8) and the results will be published separately.

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