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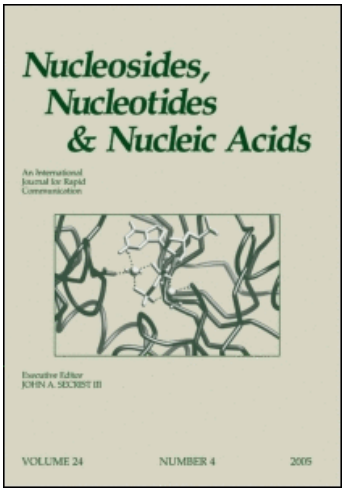

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Toward the Development of Metal-Free Synthetic Nucleases: Cleavage of a Model Substrates by 1,4-Diazabicyclo[2.2.2]Octane Derivatives

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ABSTRACT

Artificial ribonucleases of A_nBCL series were synthesized by solid-phase method. They consist of a hydrophobic alkyl radical A (n = 3–12 carbon atoms), an ''RNA-binding domain'' B (bisquaternary salt of 1,4-diazabicyclo[2.2.2]octane), a ''catalytic domain'' C (histidine residue) and a ''linker'' L that joins the domains B and C. The effect of the alkyl radical on the catalytic properties of the chemical catalyst was studied using three activated phosphate ester substrates: *p*-nitrophenyl phosphate, bis-*p*-nitrophenyl phosphate, and thymidine-3′-*p*-nitrophenyl phosphate.

Key Words: Metal-free artificial nucleases; *p*-nitrophenyl phosphate derivatives; 1,4-diazabicyclo[2.2.2]octane derivatives.

INTRODUCTION

There is much current interest in the design and development of small compounds that can mimic important enzymatic reactions under physiological conditions. One of the goals of these studies is the preparation of artificial ribonucleases, which cut a specific RNA at the target site and thus are applicable to molecular biology, therapy, and others.

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Recently we have developed a series of artificial ribonucleases (ABLC series) containing four functional domains—catalytic domain C, linker group L, RNA-binding di- or polycation B and hydrophobic radical A.[1] The optimal structure parameters and the role of B, C, L domains were determined in series of experiments.[2] According to these results, still faster RNA hydrolysis should be possible if all these domains are presented in artificial ribonucleases. However the role and the optimal structure of hydrophobic radical A have remained not studied. In this study, we have synthesized a series of compounds (Fig. 1) with different alkyl residues to optimize the structure of this domain.

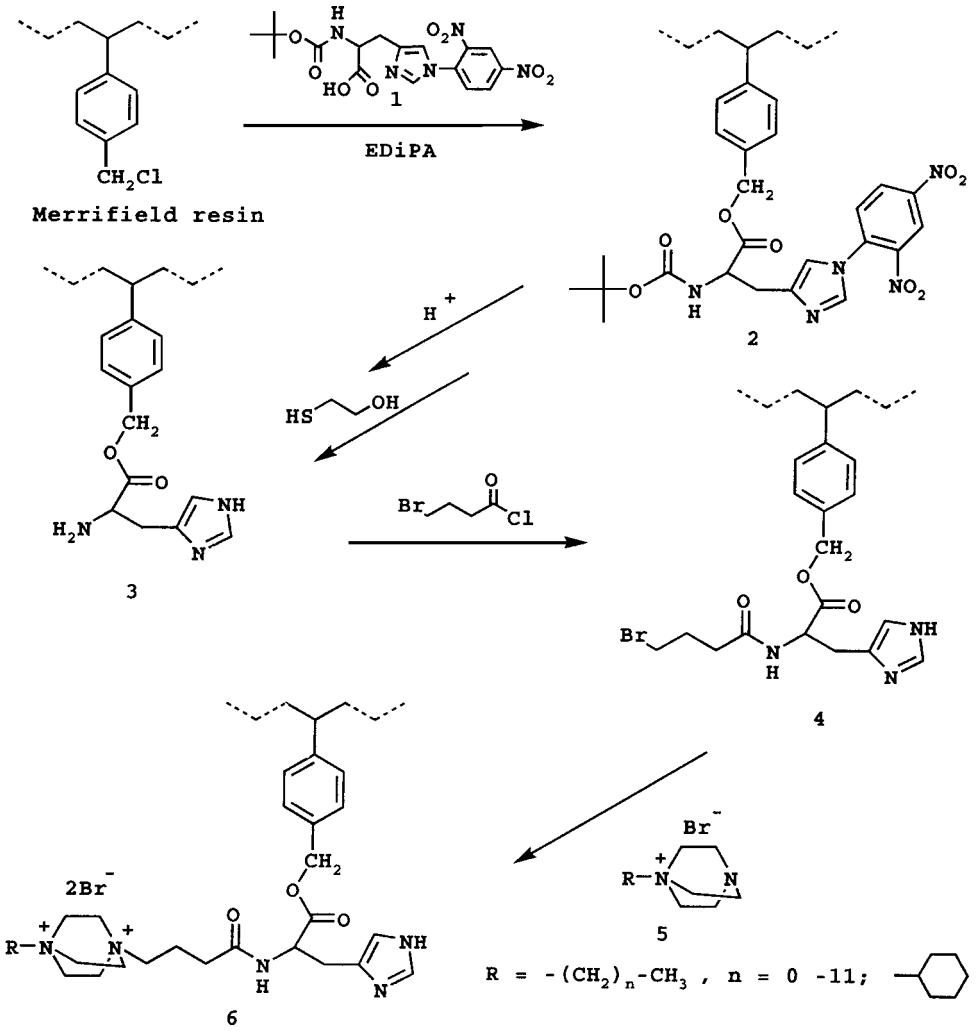

Figure 1. Solid-phase synthesis of artificial ribonucleases of ABCL series. 6a–h: a: n = 2, b: n = 3; c: n = 5; d: n = 6; e: n = 8; f: n = 9; g: n = 11; h: R—cyclohexyl.

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RESULTS AND DISCUSSION

The synthesis of chemical ribonucleases is presented in Fig. 1. Merrifield resin was used as a polymer carrier. Treatment of Merrifield resin with excess N^{α}-Boc-N^{im}-DNP-L-Histidine and EDiPA results in compound 2. Subsequent attachment of the linker group was carried out by N-acylation of amino acid's α-amino groups by 4-bromobutyryl chloride. Compounds 5 were synthesized by alkylation of 1,4-diazabicyclo[2.2.2]octane using different alkyl halides. Mono-quantery salts of 1,4-diazabicyclo[2.2.2]octane were joined to compound 4. Thus compounds differing in length of alkyl residue were obtained.

Kinetic studies were performed by using three activated phosphate ester substrates: *p*-nitrophenyl phosphate (*p*NPP), bis-*p*-nitrophenyl phosphate (BNPP), and thymidine-3′-*p*-nitrophenyl phosphate (TNPP) which is considered a reference substrate for the cleavage of phosphomono-and phosphodiesters.[3] Pseudo-first-order rate constants were determined for the three phosphate ester substrates.

Catalytic activity of RNase mimetics was determined on the surface of polymer carrier. Thus, the influence of micelle formation was excluded. The kinetic measurements were followed spectroscopically, monitoring the release of *p*-nitrophenolate ion at 400 nm. The rate constants were obtained by curve fitting of the initial data of the kinetic curve. Kinetics parameters were calculated by Origin program. Eq. 1 described experimental data in the best way.

$$A(t) = a + b^{*} \exp(t/k') \tag{1}$$

A (t)—absorption of *p*-nitrophenolate at 400 nm, $k' = 1/k_{obs}$, a, b—constants.

The rate constants of the model substrates hydrolysis as a function of the alkyl fragment length are represented in Fig. 2. The hydrolysis efficiency by artificial ribonucleases depends on substrate structures. Hydrolysis rate of p-nitrophenylphosphate weakly depends on structure of alkyl residue of catalyst. The highest value of k_{obs} in this

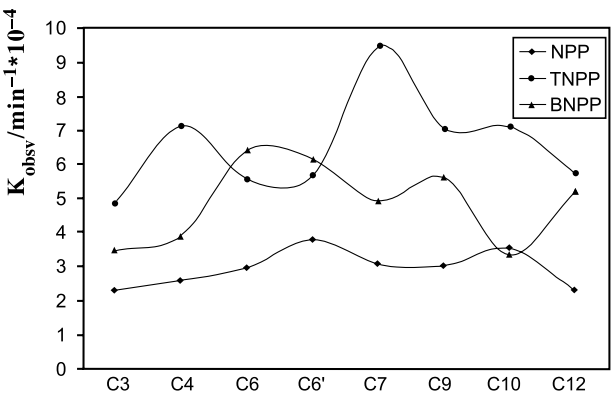

Figure 2. Dependence hydrolysis rate on the number of carbons in alkyl residue in RNasemimetics.

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experiment is observed for compounds A_6BLC and A_{10}BLC. In the case of bis-*p*-nitrophenylphosphate an increase of hydrolysis rate for all catalysts is observed. The influence of alkyl residue length becomes more marked. The most active derivatives are A_6BLC, A_9BLC and A_{12}BLC. The rate constants are changed in the range from $3.9*10^{-4}$ min^{-1} to $8.3*10^{-4}$ min^{-1}. For thymidine-3′-*p*-nitrophenyl phosphate further growth of efficiency is observed, but in this case compound A_{10}BLC possesses maximum activity. The rate constants are changed in the range from $4.8*10^{-4}$ min^{-1} to $9.5*10^{-4}$ min^{-1}.

Catalysts of A_nBLC series cleave phosphodiester bonds more efficiently than phosphomonoesters. Even in the case of simple model substrates, the influence of alkyl residue structure on efficiency of cleavage is displayed. In this study conditions excluding micelle formation are used, therefore it can be considered that the role of alkyl residues consists in promoting a suitable spatial complex structure based on hydrophobic interactions.

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