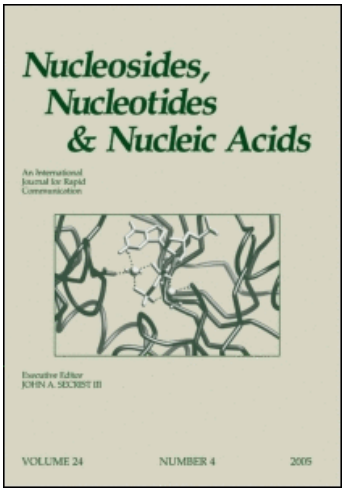

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Merita Murtola[a]; Dimitri Ossipov[a]; Jessica Sandbrink[a]; Roger Strömberg[a]
[a] Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Huddinge, Sweden

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RNA CLEAVAGE BY 2,9-DIAMINO-1,10-PHENANTHROLINE PNA CONJUGATES

Merita Murtola, Dimitri Ossipov, Jessica Sandbrink, and Roger Strömberg
□ *Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Huddinge, Sweden*

□ *We report on the synthesis of 2,9-diamino-1,10-phenanthroline PNA conjugates as well as on their action in cleavage of a target RNA. Synthesis of the PNA conjugates are performed on solid support and the phenanthroline derivative is conjugated either to the amino-end or to a centrally positioned diaminopropionic acid in the PNA via a urea linker. Cleavage of the target RNA is achieved and compared to cleavage with the corresponding 2,9-dimethyl-1,10-phenanthroline and glycine conjugates.*

Keywords PNA conjugate; solid phase synthesis; artificial nuclease; 2,9-diamino-1,10-phenanthroline

Rationally designed oligonucleotide based artificial nucleases (OBANs) may be used as artificial restriction enzymes in vitro or as novel therapeutic agents for a wide variety of diseases.[1] In the present study, 2,9-diamino-1,10-phenanthroline PNA conjugates have been synthesized and the evaluation for their use as artificial ribonucleases in the presence of Zn^{2+} ions has been initiated.

The 5-amino-2,9-dimethyl-1,10-phenanthroline Zn^{2+} complex has been shown to work as an RNA cleaving group in several artificial ribonuclease studies.[2] On the other hand, the Cu^{2+} complex of the 2,9-diamino-1,10-phenanthroline has been reported to hydrolyze 2′,3′-cAMP substantially faster than the Cu^{2+} complex of the 2,9-dimethyl-1,10-phenanthroline.[3] We therefore decided to explore the 2,9-diamino-1,10-phenanthroline as the cleaving group of PNA-based OBANs.

PNA was chosen as backbone because of its many desirable qualities.[4,5] PNA oligomers can easily be synthesized by solid phase peptide synthesis protocols[5] and readily can be modified with additional amino acids

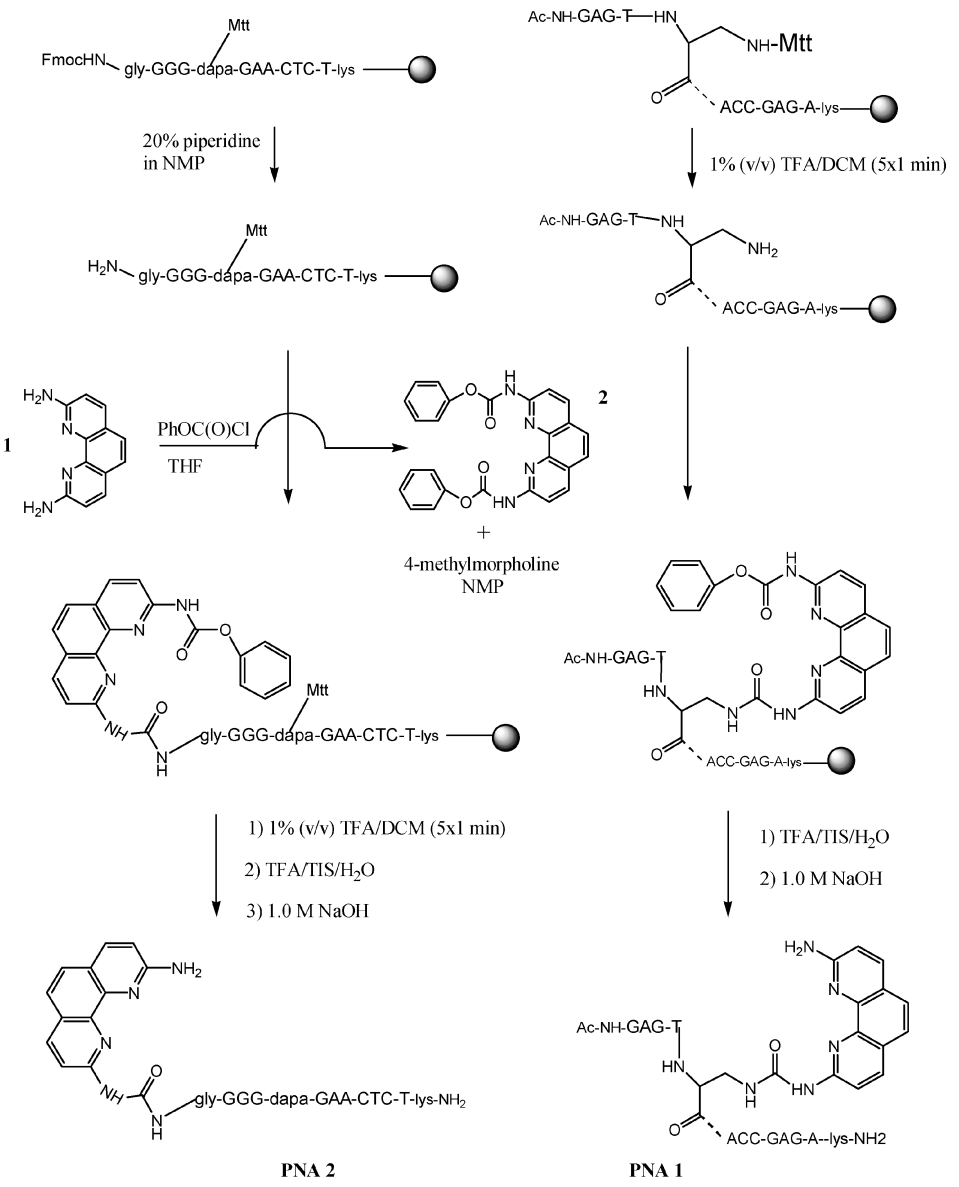
Address correspondence to Roger Strömberg, Bioorganic Chemistry, Department of Biosciences and Nutrition, Karolinska Institutet, NOVUM, S-14157 Huddinge, Sweden. E-mail: roger.stromberg@biosci.ki.se

or other functional groups. PNAs are capable of binding target RNA in a sequence-specific fashion.[4,5] PNAs are also chemically stable and highly resistant to degradation in biological fluids.[4,5] In the present study we modified PNA with an internally placed diaminopropionic acid (dapa) and with a glycine placed at the N-terminal, both serving as amino linkers for post conjugation of the phenanthroline derivatives.

2,9-Diamino-1,10-phenanthroline (**1**)[6] was converted into the bis-phenylcarbamate (**2**) in THF, by reaction with phenyl chloroformate (Scheme 1). PNA was synthesized with Fmoc chemistry using a standard protocol (for the Applied Biosystems 433A peptide synthesizer). The dapa unit was methyltrityl (Mtt) protected on the side chain. Synthesis of the different PNA 2,9-diamino-1,10-phenanthroline conjugates is outlined in Scheme 1. Synthesis of **PNA 1** from the assembled and capped PNA-dapa hybrid was carried out manually but on solid support as follows. The Mtt was first removed from the dapa unit by treatment with 1% TFA in dichloromethane (repeated 5 times). Subsequent conjugation with the diaminophenanthroline derivative was carried out with 5 eq. of bis-phenylcarbamate reagent (**2**) and 20 eq. 4-methylmorpholine in N-methylpyrrolidone (NMP) overnight at room temperature. After cleavage of the PNA-conjugate from support with trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5), the crude PNA was freeze dried, extracted with cold diethylether and then treated for 10 minutes with 1.0 M NaOH to cleave off the remaining phenyl carbamate. Synthesis of **PNA2** from the solid support bound Fmoc-glycine-PNA also was carried out manually. After removal of the Fmoc protecting group with 20% piperidine in NMP the resin bound PNA-glycine was reacted with **2** under the same condition as for **PNA1**. The Mtt protection on dapa was removed (with 1% TFA as above) prior to cleavage from support in order to conveniently wash off the protecting group. **PNA2** was then obtained after cleavage from support and hydroxide treatment in the same fashion as for **PNA1**. In order to compare the activity of the 2,9-diaminophenanthroline conjugate with a 2,9-dimethylphenanthroline derivative we synthesized **PNA3** using the same procedure as for **PNA2** except that the 5-phenoxycarbamoyl-2,9-dimethylphenanthroline reagent was used and the final NaOH treatment was omitted. **PNA4** was obtained by removing Fmoc and Mtt protection (using the same reagents as above) and then cleaving the PNA-glycine from support using the TFA/TIS/water reagent.

All PNA conjugates were purified by RP-HPLC,[7] freeze dried three times and characterized with Micromass LCT ESI-TOF mass spectrometry. **PNA1**: M_{obs} 3545.0, M_{calc} 3544.5, **PNA2**: M_{obs} 3542.0, M_{calc} 3541.5, **PNA3**: M_{obs} 3555.0, M_{calc} 3554.5 and **PNA4**: M_{obs} 3306.0, M_{calc} 3305.2 (Figure 1).

Kinetic cleavage studies have been initiated using equimolar amounts of artificial nuclease and RNA target. Reactions were carried out at 37°C with a 4 μM conc. of both PNA and substrate RNA (in 10 mM HEPES buffer (pH



SCHEME 1 Synthesis of PNA 2,9-diamino-1, 10-phenanthroline conjugates. Mtt = 4-methyltrityl, dapa = diaminopropionic acid, NMP = N-methylpyrrolidone, TIS = triisopropylsilane.

7.4) containing 0.1 M NaCl and 100 μM $Zn(NO_3)_2$). The target RNA was chosen to form a A_4 bulge opposite to the diaminopropionic acid (dapa) unit and with a closing G-T wobble base pair on one side. The initial results from the cleavage studies give the following half-lifes, **PNA2** 60 hours, **PNA3** 140 hours, and **PNA4** 43 hours (cleavage with **PNA1** as yet not completed). With **PNA2** cleavage appears to be more site selective than with

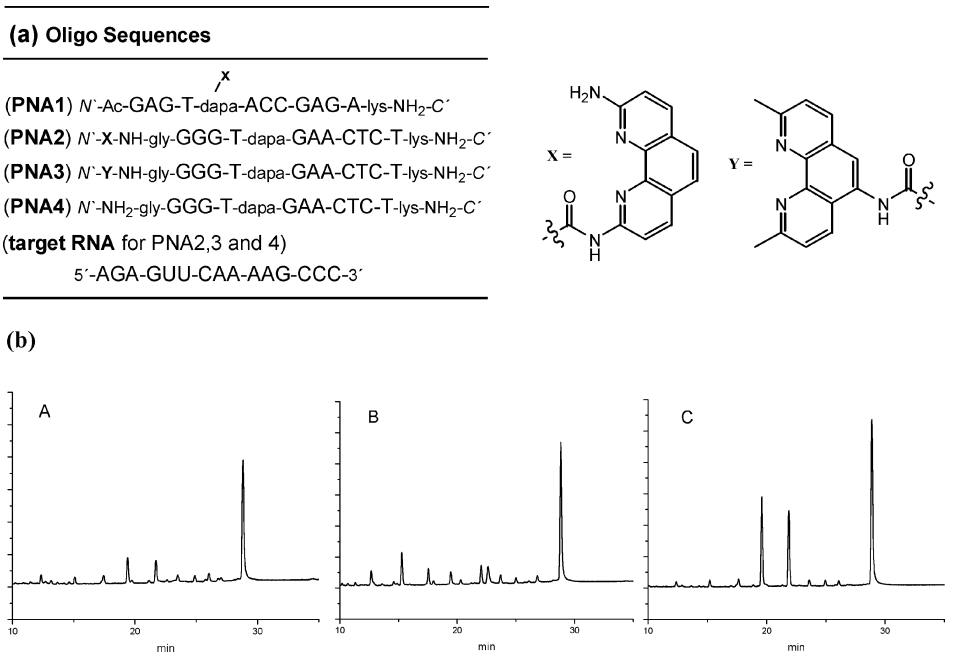


FIGURE 1 a) Schematic presentation of the PNA conjugates and the artificial ribonuclease substrate RNA; b) ion exchange-HPLC chromatograms from the analysis of reaction mixtures of PNA and RNA substrate. RNA substrate retention time is 29 minutes and the other peaks are fragments formed in the reaction. A) PNA2, after 53 hours B) PNA3, after 143 hours C) PNA4, after 32 hours.

PNA3 (Figure 1b). Interestingly **PNA4** gives considerably higher selectivity than both **PNA2** and **PNA3** in cleavage of the target RNA (Figure 1b). It appears that under these conditions and linked in this way the glycine moiety actually excerts higher cleavage activity than the phenanthroline derivatives. This is unlikely to be general as it is bound to be highly dependent on positioning of the cleaving groups relative to cleaved phosphates, but further investigation are need to clarify this. The result with **PNA4** suggest that, for the purpose of developing more selective cleavers, this finding is worth a look at in more detail.

REFERENCES

1. Niittymäki, T.; Lönnberg, H. Artificial ribonucleases. *Org. Biomol. Chem.* **2006**, 4, 15–25.
2. a) Whitney, A.; Gavory, G.; Balasubramanian, S. Site-specific cleavage of human telomerase RNA using PNA-neocuproine-Zn(II) derivatives. *Chem. Commun.* **2003**, 36–37. b) Åström, H.; Williams, N.H.; Strömberg, R. Oligonucleotide based artificial nuclease (OBAN) systems. Bulge size dependence and positioning of catalytic group in cleavage of RNA-bulges. *Org. Biomol. Chem.* **2003**, 1, 1461–1465. c) Åström, H.; Strömberg, R. Synthesis of new OBAN's and further studies on positioning of the catalytic group. *Org. Biomol. Chem.* **2004**, 2, 1901–1907.
3. Wall, M.; Linkletter, B.; Williams, D.; Lebuis, A.-M.; Hynes, R.C.; Chin, J. Rapid hydrolysis of 2′,3′-cAMP with a Cu(II) complex: Effect of intramolecular hydrogen bonding on the basicity and reactivity of a metal-bound hydroxide. *J. Am. Chem. Soc.* **1999**, 121, 4710–4711.

4. Lundin, K.E.; Good, L.; Strömberg, R.; Gräslund, A.; Smith, C.I.E. Biological activity and biotechnological aspects of peptide nucleic acid. *Advances in Genetics* **2006**, 56, 1–51.
5. Nielsen, P. E. (ed.), 2004. *Peptide Nucleic Acids, protocols and applications* Horizon Bioscience, Wymondham.
6. a) Summers, L.A. Chemical constitution and activity of bipyridylium herbicides. *Tetrahedron* **1968**, 24, 5433–5437. b) Yamada, M.; Nakamura, Y.; Kuroda, S.; Shimao, I. Synthesis of 2,9-dichloro-1,10-phenanthroline from *N*, *N*′-annelated phenanthrolinediones. *Bull. Chem. Soc. Jpn.* **1990**, 63, 2710–2712. c) Ogawa, S.; Yamaguchi, T.; Gotoh, N. Preparation of a conjugated tautomer of 1,14:7,8-diethenotetrapyrido-[2,1,6-*de*: 2′, 1′, 6′-*gh*: 2″, 1″, 6″-*kl*: 2‴, 1‴, 6‴-*na*] [1,3,5,8,10,12] hexaazacyclotetradecine and it's metal derivatives.*J. Chem. Soc.Perkin I* **1974**, 976–978.
7. **PNA1** and **PNA2** were purified on a Phenomenex Jupiter Proteo (4 μm 250 × 4.6 mm) column at 60°C using a flow rate of 1ml/min and a linear gradient of 10% B for 20 min, then 10–37% B for 20 min was used. Retention times were 35 min for **PNA1** and 32 min for **PNA2**. **PNA3** and **PNA4** were purified on a Phenomenex Jupiter Proteo (4 μm 250 × 10 mm) column at 60°C using a flow rate of 4 ml/min and a linear gradient of 10% B for 20 min, then 10–37% B for 20 min was used. Retention times were 32 min for **PNA3** and 27 min for **PNA4**.