Microwave Assisted Synthesis of Peptide Nucleic Acid (PNA) Oligomers

Using Biotage® Initiator+ Alstra™

Introduction

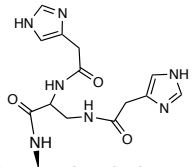
Peptide nucleic acids (PNAs) are DNA mimics where the sugar phosphate backbone of DNA is replaced by a peptide backbone consisting of *N*-(2-aminoethyl)glycine units. The purine nucleobases (Adenine A and Guanine G) and pyrimidine nucleobases (Cytosine C and Thymine T) are attached to the backbone through methylene carbonyl linkages.[1]

PNA oligomers have interesting properties such as their increased thermal and chemical stability and resistance to enzymatic degradation which drives their use in various molecular biology, molecular diagnostic, microarray, biosensor and antisense applications.[2]

Here we synthesized two PNA oligomers (Figure 1) on a small scale using the Biotage® Initiator+ Alstra™ microwave peptide synthesizer.

N-terminus *C*-terminus

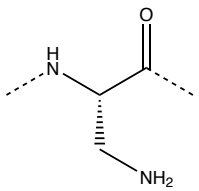
PNA **1** AcN-*GGGT-Dap-GAACTCTLys*-C(O)NH_2



PNA **2** AcN-*GGGT-Dap-GAACTCTLys*-C(O)NH_2

Figure 1. PNA oligomers synthesized.

PNA **1** incorporates a diaminoproprionic acid residue (Dap) (Figure 2) suitable for the post synthetic conjugation of different oligoethers[3] as well as catalytic groups in PNA based artificial nucleases[4] and here we present a bisimidazole derivative PNA **2**. The incorporation of linkers into PNA oligomers is used to increase solubility, introduce functionality and for the conjugation of dyes such as Cy5.



Dap

Figure 2. Diaminoproprionic acid (Dap) linker.

Experimental

Materials

All materials were obtained from commercial suppliers; Link technologies (peptide nucleic acid monomers, Fmoc-PNA-A(Bhoc)-OH, Fmoc-PNA-G(Bhoc)-OH, Fmoc-PNA-C(Bhoc)-OH and Fmoc-PNA-T-OH), Iris Biotech GmbH (Fmoc-$^{\alpha}$*N*-Lys($^{\varepsilon}$*N*-Boc)OH, N^{α}-Fmoc- N^{β}-(*p*-methyltrityl)-L-2, 3-diaminopropionic acid (Fmoc-L-Dap(Mtt)-OH),

N^{α}-Fmoc- N^{β}-Fmoc-L-2,3-diaminopropionic acid (Fmoc-L-Dap(Fmoc)-OH), trifluoroacetic acid (TFA), triisopropylsilane (TIS), and dichloromethane (DCM), *N*-methylpyrrolidone (NMP), piperidine)), Merck-Millipore (ethyl cyano(hydroxyimino) acetate (Oxyma) and *N,N'*-diisopropylcarbodiimide (DIC)), Life Technologies Europe (acetonitrile, lutidine, acetic anhydride) and Biotage (Rink Amide ChemMatrix® resin). The benzhydryloxycarbonyl (Bhoc) group was used for protecting the exocyclic amino groups of the nucleobases.

Mass spectrometry was performed on a Micromass LCT electrospray ionization time-of-flight (ESI-TOF) mass spectrometer in acetonitrile–water 1:1 (v/v), 0.1% formic acid solutions. The molecular weights of the peptide nucleic acid conjugates were reconstructed from the m/z values using the mass deconvolution program of the instrument (Mass Lynx software package).

PNA Synthesis and Analysis

The PNAs were prepared on a Biotage® Initiator+ Alstra™ microwave peptide synthesizer. PNA sequences were synthesized on Rink Amide ChemMatrix® resin (loading 0.47 mmol/g) on a 10 µmol scale in a 5 mL reactor vial. Fmoc deprotection was performed at room temperature (RT) in two stages by treating the resin with piperidine-NMP (1:4) for 3 min followed by piperidine-NMP (1:4) for 10 minutes. The resin was then washed with NMP (x5). PNA couplings were performed using 4 eq. of PNA monomer, 4 eq. oxyma and 4 eq. DIC in NMP. A coupling time of 6 min at 75 °C (microwave) was employed and then the resin was washed with NMP (x2). This was followed by an optional capping step using NMP-lutidine-acetic anhydride (89:6:5) for 1 min and then washing with NMP (x4). After the synthesis was completed, the resin was washed with NMP (x5), DCM (x5) and thoroughly dried. The PNAs were cleaved from the solid support by treatment with TFA-H_2O-TIS (95:2.5:2.5) for 1.5 h at room temperature. The PNA products evaporated to dryness and then purified with an Ascentis Express Supelco Peptide ES-C18 column (2.7 µm, 150 × 4.6 mm) at 60 °C using a flow rate of 1 mL/min. The following solvent system was used: solvent A, water containing 0.1% TFA; solvent B, CH_3CN: water containing 0.1% TFA (1:1; v/v). PNA **1** and **2** were purified using a linear gradient of 40% B for 30 min. Collected products were lyophilized. Water was added and the products were freeze dried again, which was then repeated once more.

Results & Discussion

The PNA sequence **1** was assembled using SPPS methods as described above with microwave heating during the coupling steps. The resin was washed and the PNA released from the solid support as described above to afford the desired PNA **1** with a crude purity of 87% and confirmed by ESI-TOF MS (Figure 3). MS (MALDI) m/z = 3290 $[M+H]^+$, calcd for $C_{130}H_{169}N_{69}O_{37}+H^+$: 3289.354.

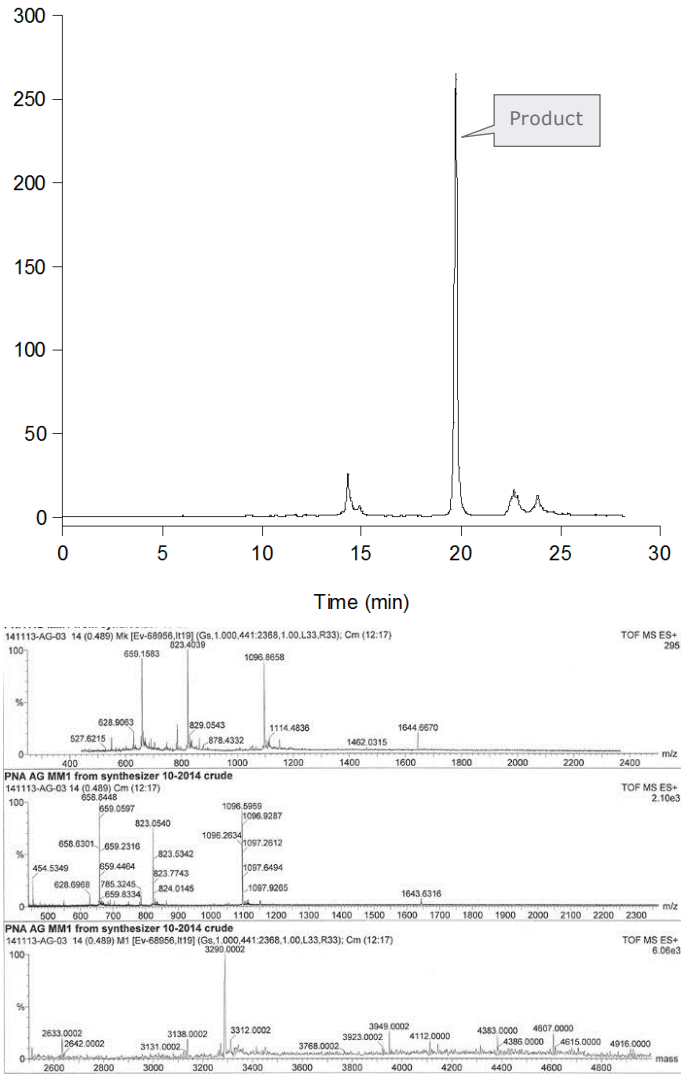
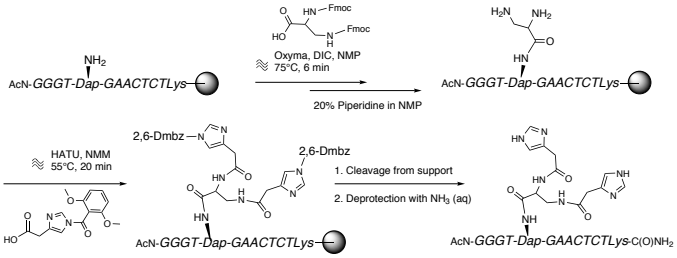


Figure 3. Analytical HPLC trace and ESI-TOF MS of crude PNA **1**.

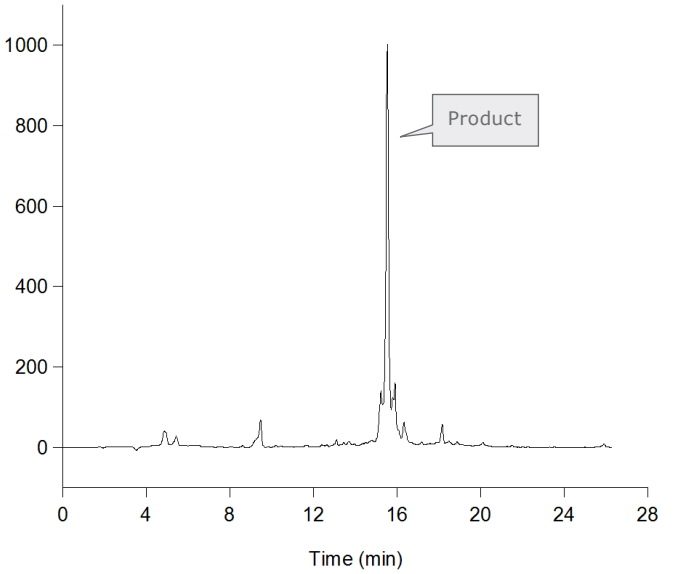
For the synthesis of the conjugate PNA **2**, the PNA sequence **1** was assembled using SPPS methods as described above with microwave heating during the coupling steps. This PNA remained attached to the resin (Scheme 1). The N^{β}-methyltrityl protection of diaminopropionic acid was removed by treatment with 1% TFA in DCM for 1 min (x5), followed by washing with DCM and NMP. N^{α}-Fmoc- N^{β}-Fmoc-L-2,3-diaminopropionic acid (5 eq.) was then coupled to the PNA using the standard coupling method described above followed by the standard removal of the two Dap Fmoc protecting groups.

Conjugation of a DMBz-imidazole moiety (2,6-dimethoxybenzoyl-imidazole-4-acetic acid prepared separately. Prof. Roger

Strömberg *et al.*) to the resin bound PNA was carried out using DMBz-imidazole (17 eq.), HATU (15 eq.), NMM (15 eq.) in 250 µl of NMP under microwave irradiation for 20 min at 55 °C. The resin was washed and the peptide released from the solid support as described above to afford the desired PNA **2** and confirmed by ESI-TOF MS (Figure 4). MS (MALDI) m/z = 3589 $[M]^+$, calcd for $C_{143}H_{183}N_{75}O_{40}$: 3590.459.



Scheme 1. Synthesis of PNA **2**, a bisimidazole conjugate.



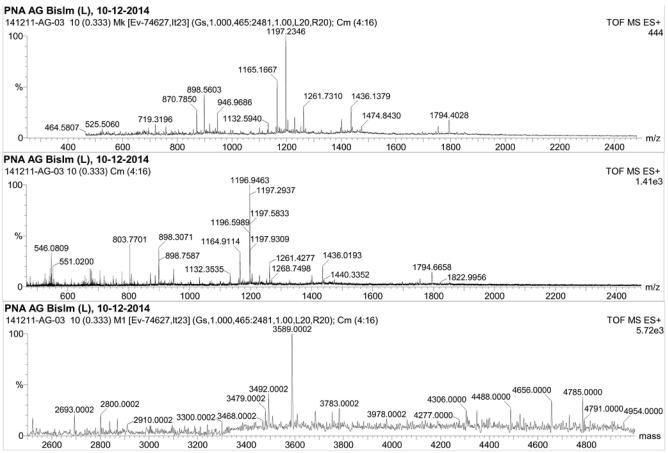


Figure 4. Analytical HPLC trace and ESI-TOF MS of crude PNA **2**.

Conclusion

Here we have demonstrated the use of microwave heating for the small scale synthesis (10 µmol) of PNA oligomers where microwave heating was applied during coupling steps only.

The Biotage® Initiator+ Alstra™ microwave peptide synthesizer is the ideal tool for the synthesis of PNAs and conjugates. With the ability to use low volumes and accurate dispensing using digital syringe pumps, makes the Initiator+ Alstra perfectly suited for small scale microwave peptide and peptidomimetic synthesis, especially when the use of expensive building blocks is required such as during PNA synthesis.

References

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2. Lundin, K. Good, L., Strömberg R., Gräslund A., and Smith E. C., *Adv. Genetics*, **2006**, *56*, 1-51.; Nielsen, P., *Chemistry and Biodiversity*, **2010**, *7*, 786-804.
3. Ghidini, A; Steunenberg, P; Murtola, M; Strömberg, R., *Molecules*, **2014**, *19*, 3135–3148.
4. Dogandzhiyski, P; Ghidini, A; Danneberg, F; Strömberg, R; Göbel, M. W., *Bioconjugate Chem.*, **2015**, *26*, 2514–2519.

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