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Internal functionalization of PNA oligonucleotides *via* a substituted dipeptide unit

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Abstract: The solid phase synthesis of PNA oligomers with the internal dipeptide GlyPhe and the functionalization with heterocyclic ligands is described. *p*-Nitro-phenylalanine is introduced during solid phase synthesis and subsequently reduced to *p*-amino-phenylalanine. Reaction with activated acids provides the ligand conjugates in high yield and purity. This universal strategy opens up a large number of possible internal substitutions in PNA chemistry.

Introduction

Peptide nucleic acids (PNAs) are DNA analogs where the negatively charged sugar-phosphate backbone is substituted by a neutral pseudopeptide skeleton composed of *N*-(2-aminoethyl)-glycine units[1, 2] (Fig. 1). PNA possesses a remarkable chemical stability and is neither degraded by nucleases nor proteases. In addition, PNA shows high specificity in binding DNA and RNA. These properties allow PNA to act as an antisense agent.

In recent literature, metal derivatives of PNA have received increased attention. One notable example is the development of artificial nucleases, where PNA serves as the recognition domain and a metal complex as the cleavage domain.[3, 4] A metal complex can be introduced either at the *N*-terminal end of the oligomer[3-9] or at an internal site within the PNA sequence: Maiorana *et al.*[10] substituted the C2 atom of the PNA backbone and retained all nucleobases in the sequence, while Balasubramanian,[11] Achim[12, 13] and Williams[14-16] reported on PNA oligomers where one nucleobase is exchanged with a nitrogen ligand capable of metal coordination. From the synthetic point of view, a major drawback of the published internal PNA modifications is that these monomers often have to be prepared in solution by a multistep procedure.

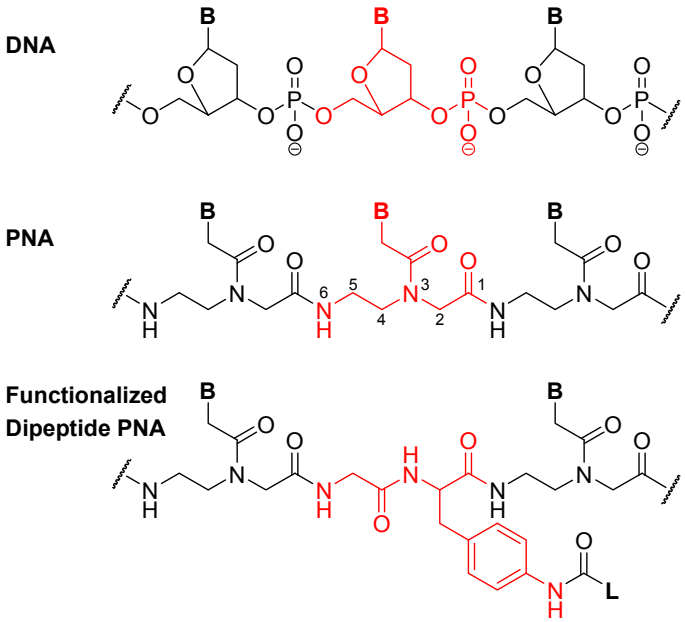

Fig. 1: Comparison of DNA, PNA and Functionalized Dipeptide PNA (**B** = a, c, g, t; **L** = Ligand)

In this communication, we present a novel approach to functionalized PNA oligomers for metal coordination. The working hypothesis is that six atoms are added to the oligomer backbone using either one PNA monomer or one dipeptide.[17] In addition, both PNA and peptide chemistry are synthetically based on amide bond formation. Therefore, one PNA monomer can be substituted with one dipeptide (Fig. 1). We chose GlyPhe, since the side chain phenyl group of Phe can be functionalized and substituted with a nitrogen ligand. If this substitution is performed on the resin in the course of SPPS, a universal strategy is obtained that opens an avenue for a vast variety of substituents and saves a time-consuming monomer synthesis in solution.

PNA oligomers with an unfunctionalized dipeptide unit

PNA oligomers with an internal dipeptide were synthesized on TentaGel R Fmoc-Lys(Boc)-RAM resin using Fmoc-/Bhoc-protected PNA monomers and Fmoc-protected amino acids[8] (Fig. 2).The *C*-terminal lysine residue was introduced in order to enhance solubility. One synthetic cycle was composed of deprotection of the *N*-terminus (20% piperidine in DMF), and subsequent coupling of the respective PNA monomer or amino acid (20 min.), using HATU / DIPEA in DMF. After each coupling step, a Kaiser test was performed. If unreacted amine groups were present, they were acetylated with 5% acetic anhydride and 6% DIPEA in DMF. After the last coupling step, the *N*-terminus was deprotected and acetylated, followed by cleavage of the final oligomer from the resin (95% TFA, 2.5% TIS, 2.5% H_2O) and precipitation with cold ether. Oligomers **1 - 3** were purified by RP-HPLC,[18] lyophilized and characterized by MALDI-TOF[19] (Table 1, Fig. 3).

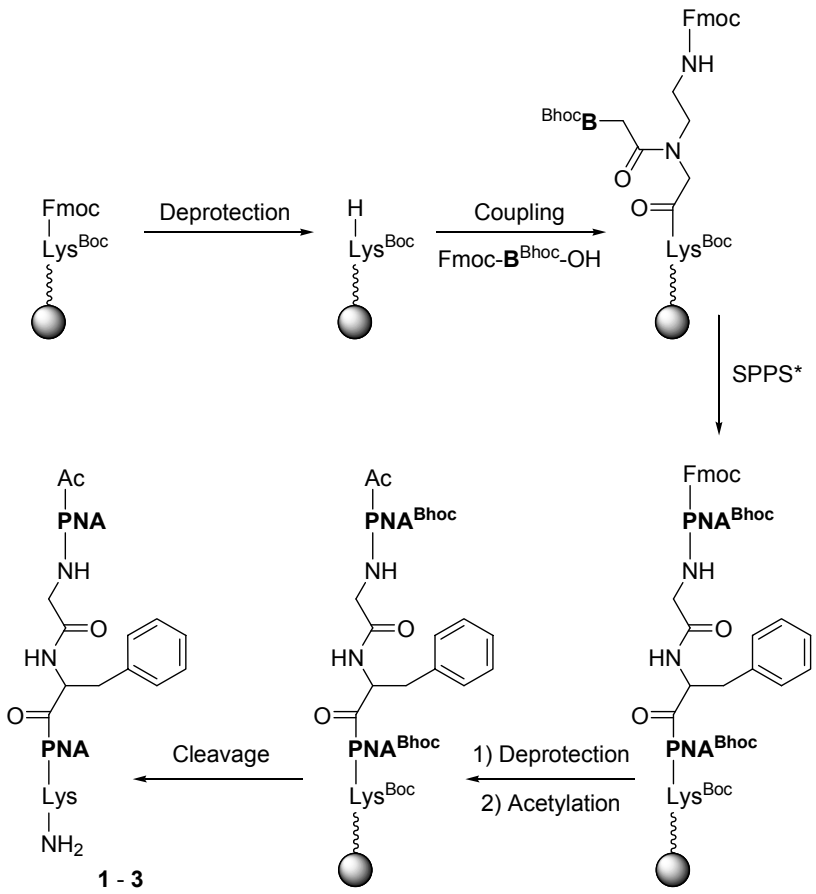

Fig. 2: Synthesis of Unsubstituted Dipeptide PNAs **1 - 3**

*monomer: Fmoc-**B**^{Bhoc}-OH, Fmoc-Gly-OH or Fmoc-Phe-OH; **B** = a, c, g, t

Table 1: Sequences and Analytical Data of Unsubstituted Dipeptide PNAs

	Sequence	M_{calc}	M_{found}
1	Ac-tacc-GlyPhe-tgtt-Lys-NH_2	2525.0	2526.7
2	Ac-ttacc-GlyPhe-tgtta-Lys-NH_2	3066.3	3067.6
3	Ac-attacc-GlyPhe-tgttat-Lys-NH_2	3608.5	3608.5

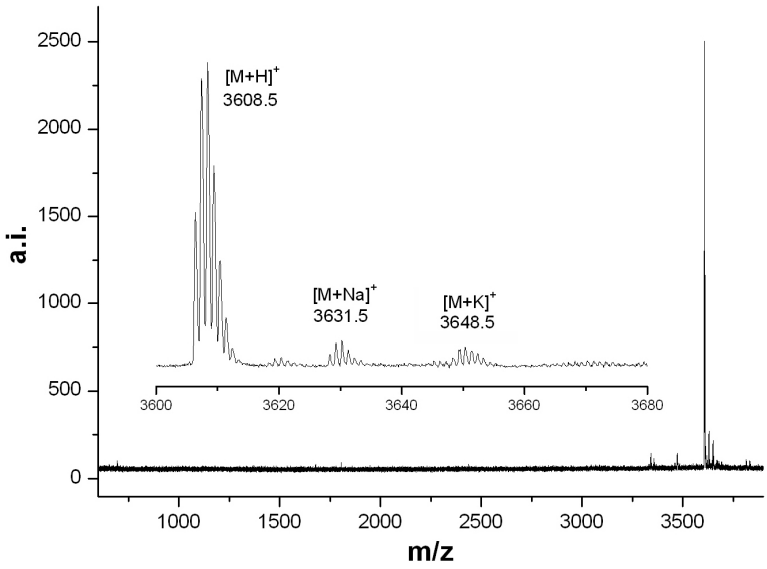

Fig. 3: MALDI-TOF Spectrum of PNA **3**

PNA oligomers with a functionalized dipeptide unit

The Fmoc-Phe(*p*-NO_2)-OH monomer **4** was used as a key precursor for the internally functionalized PNA oligomers. It was prepared by nitration of phenylalanine with HNO_3 / H_2SO_4, followed by Fmoc protection of the amine group (Fig. 4).

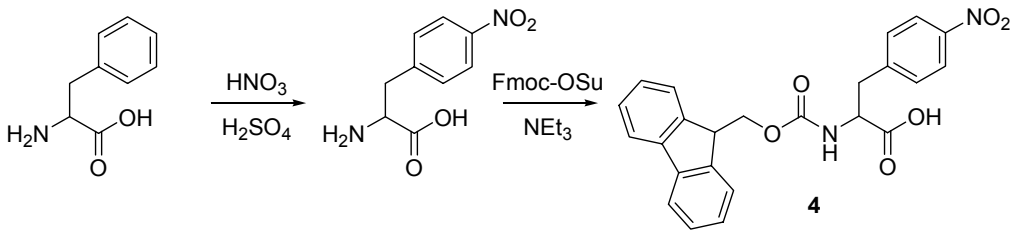

Fig. 4: Synthesis Scheme for Fmoc-Phe(*p*-NO_2)-OH **4**

As ligands, the terpyridine **5** and the bis-picolylamine **6** were chosen. Ligand **5** was synthesized by nucleophilic substitution of the corresponding chloride with ε-caprolactone using 1 eq. of H_2O and an excess of KOH in dry DMSO[20] (Fig. 5). Ligand **6** was prepared starting from commercially available unsubstituted bpa and methyl 4-(bromomethyl)benzoate[21, 22] (Fig. 6).

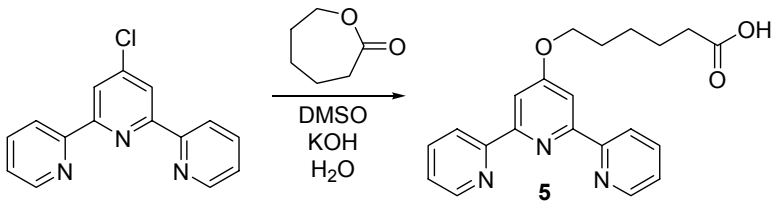

Fig. 5: Synthesis of tpy **5**

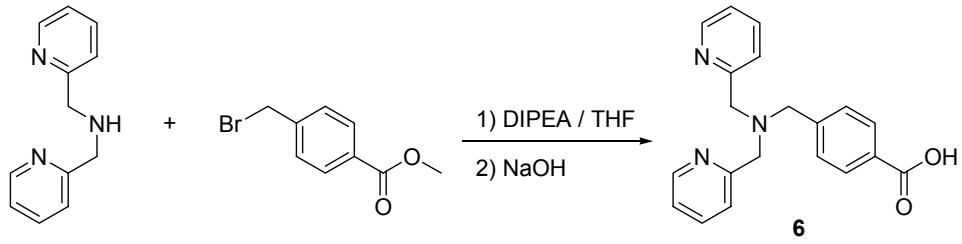

Fig. 6: Synthesis of bpa **6**

The functionalized PNA oligomers were assembled using the procedure described above; the nitro group was inert under these conditions (Fig. 7). After the coupling of Gly, the reduction of the nitro group to an amine function was performed on the resin using 0.9M $SnCl_2$ in DMF.[23-25] Then, the ligands **5** or **6** were coupled to this amino function *via* their carboxylic group in standard conditions for amide bond formation (HATU / DIPEA). Finally, oligomer synthesis, isolation and purification were continued as described for **1** - **3**. The analytical data for oligomers $\mathbf{1_L}$ - $\mathbf{3_L}$ (**L** = tpy, bpa) are listed in Table 2, and a MALDI-TOF spectrum of $\mathbf{3_{tpy}}$ is exemplified in Fig. 8.

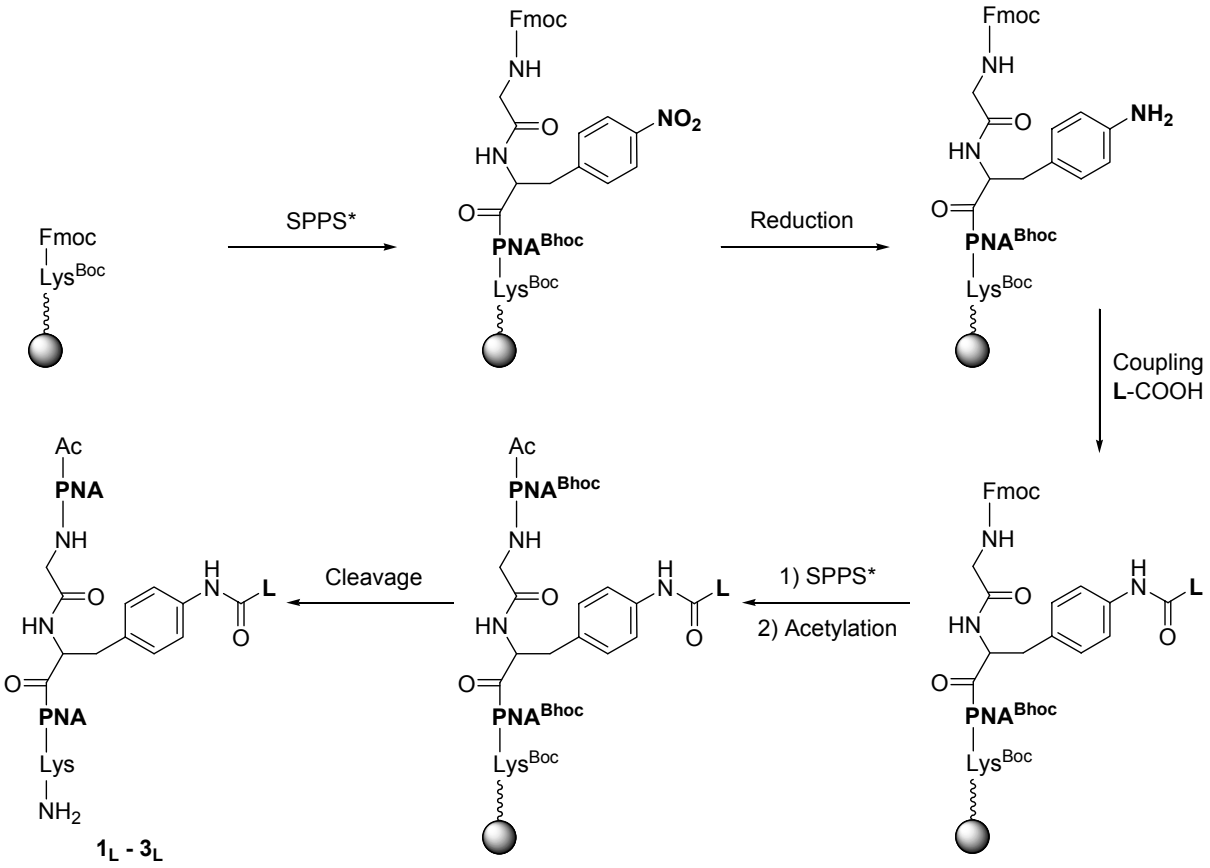

Fig. 7: Synthesis of Substituted Dipeptide PNAs: Internal Coupling Strategy of a Ligand **L**

*monomer: Fmoc-$\mathbf{B}^{Bhoc}$-OH, Fmoc-Gly-OH or Fmoc-Phe(*p*-NO_2)-OH **4**; **B** = a, c, g, t; **L**-COOH = **5**, **6**

Table 2: Sequences and Analytical Data of Substituted Dipeptide PNAs

	Sequence	M_{calc}	M_{found}
$\mathbf{1}_{tpy}$	Ac-tacc-GlyPhe(tpy)-tgtt-Lys-NH_2	2885.2	2886.2
$\mathbf{2}_{tpy}$	Ac-ttacc-GlyPhe(tpy)-tgtta-Lys-NH_2	3426.4	3428.6
$\mathbf{3}_{tpy}$	Ac-attacc-GlyPhe(tpy)-tgttat-Lys-NH_2	3967.6	3968.2
$\mathbf{1}_{bpa}$	Ac-tacc-GlyPhe(bpa)-tgtt-Lys-NH_2	2855.2	2855.1
$\mathbf{2}_{bpa}$	Ac-ttacc-GlyPhe(bpa)-tgtta-Lys-NH_2	3396.4	3397.8

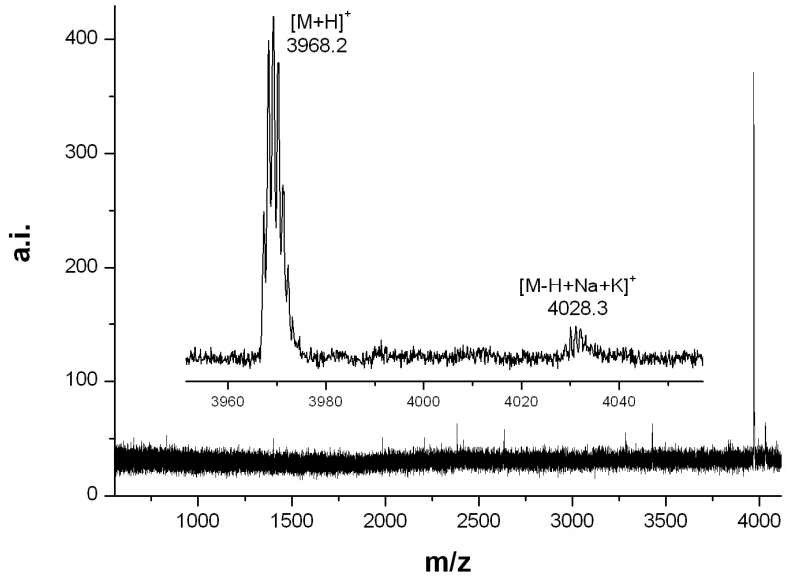

Fig. 8: MALDI-TOF Spectrum of PNA $\mathbf{3}_{\mathbf{tpy}}$

Conclusion

The solid phase synthesis of PNA oligomers is described, where an internal monomer is substituted by the dipeptide GlyPhe(*p*-NO_2). The key step is the reduction of the *p*-nitro group to a *p*-amino group on the resin. This universal strategy opens up a large number of possible substitutions by reaction of this amino group with activated acids, which is demonstrated by coupling of the chelating ligands **5** and **6**. The use of such conjugates as artificial nucleases for site specific DNA cleavage is presently under investigation. Other applications might include labelling with fluorescent markers or the synthesis of branched PNA oligomers.

Abbreviations: Ac: acetyl-; B: nucleobase (a, c, g, t); Bhoc: benzhydryloxycarbonyl; Boc: *tert*-butyloxycarbonyl; bpa: *N*-(4-carboxymethyl)benzyl-*N*,*N*-bis(2-picolyl)amine **6**; DCM: dichloromethane; DIPEA: di-isopropyl-ethylamine; DMF: dimethylformamide; Fmoc: fluorenyl-9-methoxycarbonyl; Gly: glycine; HATU: 2-(1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Lys: lysine; Phe: phenylalanine; PNA: peptide nucleic acid; SPPS: solid phase peptide synthesis; Su: succinimidyl; tpy: 4-[4'-oxa-(2,2':6',2''-terpyridinyl)]hexanoic acid **5**; TFA: trifluoro-acetic acid; TIS: tri-isopropylsilane

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