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# The 'fully protected backbone' approach as a versatile tool for a new solid-phase PNA synthesis strategy

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**Abstract**—The 'fully protected backbone' (FPB) strategy has been efficiently adapted to the solid-phase synthesis of homothymine, homocytosine and 'mixed' pyrimidine PNAs. This versatile and simple method avoids the preparation of PNA monomers and relies on easy available starting materials, highly efficient backbone elongations and effective nucleobase units condensations.


Peptide (or polyamide) nucleic acids are acyclic and neutral DNA or RNA surrogates, which recognize their complementary sequence with a remarkably high affinity and specificity.[1] Moreover, owing to their high chemical stability and resistance towards nucleases and proteases, they are very attractive as antigene/antisense agents, molecular biological tools and for genetic diagnosis.[2]

PNA oligomer synthesis is more often performed following standard solid-phase procedures, starting from suitable orthogonally protected PNA monomer building blocks. The elongation process has been largely studied and a variety of coupling reagents and various sets of protecting groups have been tested, leading to improvements in synthesis efficiency.[3–7] Nevertheless, this strategy requires the time-consuming and sometimes laborious synthesis of PNA monomers bearing natural or non-standard nucleobases. Moreover, reactivity and coupling rate of PNA monomers depend on the nature of the nucleobases and on the PNA sequence. To ensure coupling efficiency, a double coupling step using a large excess of PNA monomer is required. Side reactions specific to PNAs, such as intramolecular *N*-acyl migrations, may also arise.[3,7] Furthermore, in the case of chiral PNAs, partial epimerization occurs during coupling.[8]

Other convergent strategies, using submonomeric units as building blocks rather than preformed PNA monomers, have been applied to the solid-phase synthesis of PNA. One of them requires N-Fmoc N-(Boc- aminoethyl) aminoacid (Boc[Fmoc]OH) precursors and it has been successfully applied to the synthesis of chiral PNAs[9] as well as to the synthesis of labelled PNAs.[10] This strategy consists first in linking one Boc[Fmoc]OH monomer onto the growing PNA chain on the resin, then in cleaving the Fmoc group and finally, in condensing one carboxymethylnucleobase unit onto the secondary amine thus generated.

We have previously reported the liquid-phase synthesis of linear or cyclic functionalized PNAs following the 'fully protected poly (aminoethylglycinamide) backbone' (FPB) approach (Fig. 1).[11,12]

The principle consists in building a FPB bearing as many different and orthogonal protecting groups as they are different kinds of nucleobases to introduce. After selective and sequential deprotection, the simultaneous condensation of the required number of identical nucleobase acetyl moieties onto the backbone can be performed in one step. This FPB strategy avoids the synthesis of the troublesome PNA monomers, and relies on easy available starting materials (protected aminoethyglycine monomers), highly effective backbone elongations and effective nucleobase units condensations.

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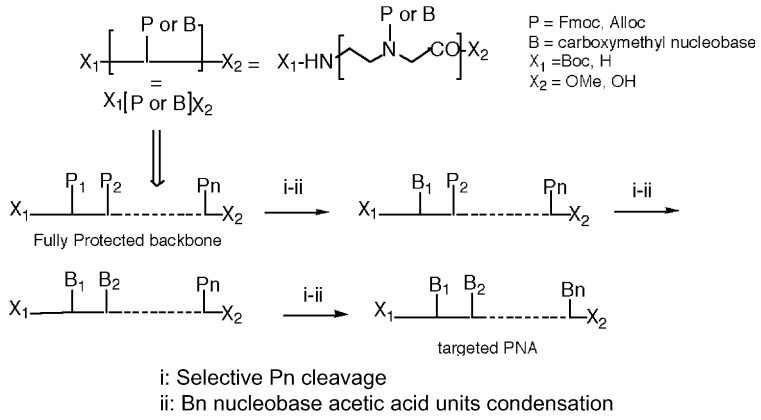



**Figure 1.** Principle of the liquid-phase FPB strategy.

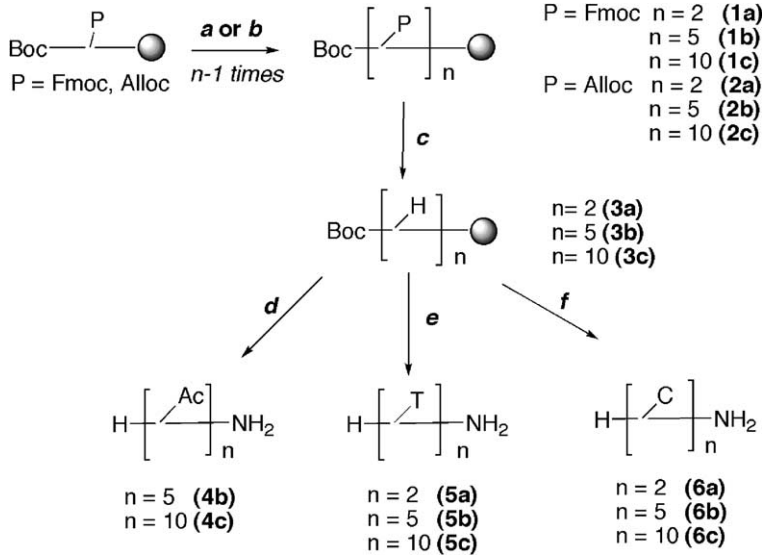
Considering the high versatility of the FPB strategy, we envisaged to adapt it to the solid-phase synthesis of PNAs, as it could be an attractive alternative to classical methods. In this letter, we report the first application of the FPB strategy to the solid-phase synthesis of homopyrimidine PNAs containing thymine (T) or cytosine (C) nucleobases, and of a mixed (heteropyrimidine) hexa-PNA (e.g., CTCTCT).

To evaluate the potential of this strategy in solid-phase, we first focused on the synthesis of di-, penta- and deca-PNAs ([T]n and [C]n with $n = 2$, 5 or 10), starting from two differently Nα-protected aminoethylglycine monomers Boc[Fmoc]OH and Boc[Alloc]OH. The synthetic routes to homo-T PNAs **5a**–**c** and homo-C PNAs **6a**–**c** are described in Scheme 1.

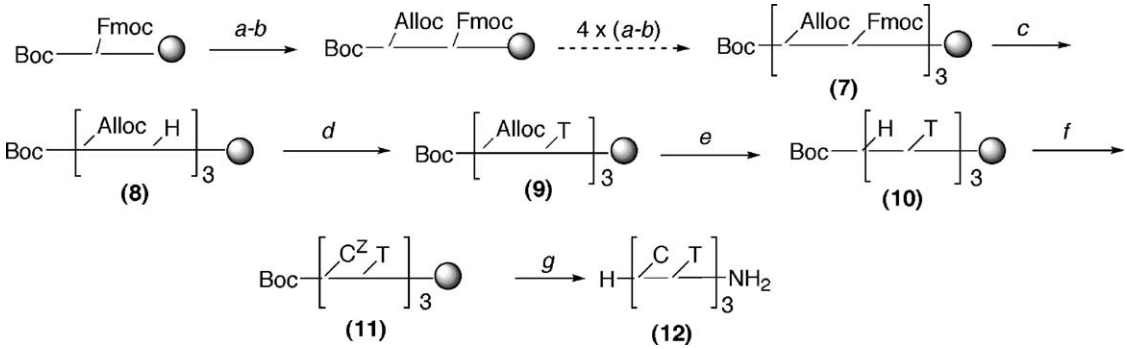
The first step consists in building the fully protected [P]n backbone on a MBHA-LL resin (P = Fmoc: **1a**–**c**; P = Alloc: **2a**–**c**). Thus, the MBHA-LL resin was first half-loaded with an appropriate amount of Boc[P]OH (Boc[Fmoc]OH or Boc[Alloc]OH), via a HBTU preactivation, in the presence DIEA as base and NMP as solvent (3 min of preactivation, then 30 min for coupling). Then, capping of the unreacted amino groups was performed by acetylation with acetic anhydride in NMP.

Fully protected backbones **1a**–**c** and **2a**–**c** were then prepared by successive elongations with Boc[P]-OH units. Typically, after TFA-mediated removal of the terminal N-Boc protecting group, the coupling of the next Boc-[P]OH submonomer was performed using 2 equiv of HBTU-preactivated monomer. Each coupling step, performed with Boc[Fmoc]OH or Boc[Alloc]OH, was successful, as indicated by negative standard Kaiser tests. Consequently, no capping was performed between two coupling steps.

Next, the deprotection of all secondary amine functions of the [P]n backbones to obtain **3a**–**c** was performed by




**Scheme 1.** Reagents and conditions: (a) (i) TFA/TIS (10%), (ii) Boc[Fmoc]OH/HBTU/DIEA/NMP; (b) (i) TFA/TIS (10%), (ii) Boc[Alloc]OH/HBTU/DIEA/NMP; (c) piperidine (20%)/DMF when P = Fmoc, or $Pd(PPh_3)_4$/DEA/NMP when P = Alloc; (d) (i) $Ac_2O$/NMP, (ii) TFMSA/TFA/TIS (10%); (e) (i) $TCH_2COOH$/HBTU/DIEA/NMP, (ii) TFMSA/TFA/10% TIS; (f) (i) $C^ZCH_2COOH$/HBTU/DIEA/NMP, (ii) TFMSA/TFA/TIS (10%).




**Scheme 2.** Reagents and conditions: (a) (i) TFA/TIS (10%); (b) Boc[Alloc]OH or Boc[Fmoc]OH/HBTU/DIEA/NMP; (c) piperidine (20%)/DMF; (d) $TCH_2COOH$/HBTU/DIEA/NMP; (e) $Pd(PPh_3)_4$/DEA/NMP; (f) $C^ZCH_2COOH$/HBTU/DIEA/NMP; (g) TFMSA/TFA/TIS (10%).

means of piperidine in DMF for **1a**–**c** (P = Fmoc), or $Pd(PPh_3)_4$/DEA in NMP for **2a**–**c** (P = Alloc).

In order to assess both the efficiency of the backbone elongation and the Fmoc or Alloc deprotection processes, HPLC and MS analyses[13,14] were performed on crude polyacetylated materials **4b**–**c** obtained by acetylation ($Ac_2O$/NMP) of a small portion of the supported deprotected penta- and decamer backbones **3b**–**c**, followed by a TFA/TFMSA-mediated cleavage from the resin. These analyses demonstrated high degrees of purity of **4b**–**c**, indicating (i) no detectable deletions during the elongation process, and (ii) quantitative deprotection of backbones **1b**–**c** and **2b**–**c**.

Then, the simultaneous introduction of n thymine or $N^4$-benzyloxycarbonyl-(or Z)-cytosine acetic acid units onto the backbone was carried out using 4*n* equivalents of these base acetic acid units and HBTU as coupling reagent (3 min of preactivation). Condensations were monitored by a standard chloranil test, which turned negative after one or two condensation cycles, even in

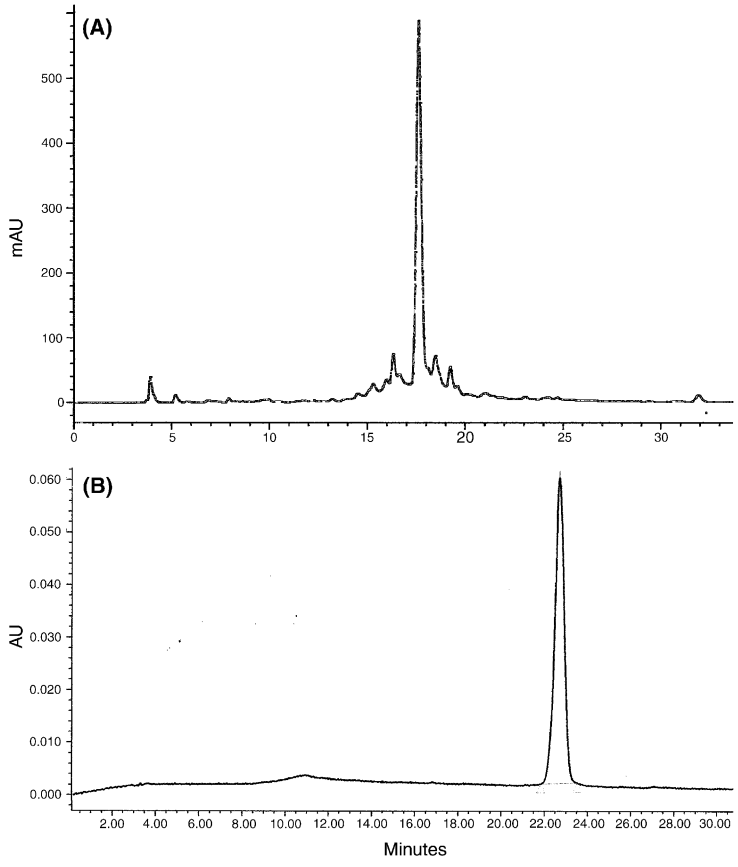



**Figure 2.** HPLC chromatograms of (A) crude and (B) purified compound **12**. Gradient from 100% (0.1% TFA/$H_2O$)/0% (0.1% TFA/$CH_3CN$) to 80% (0.1% TFA/$H_2O$)/20% (0.1% TFA/$CH_3CN$).

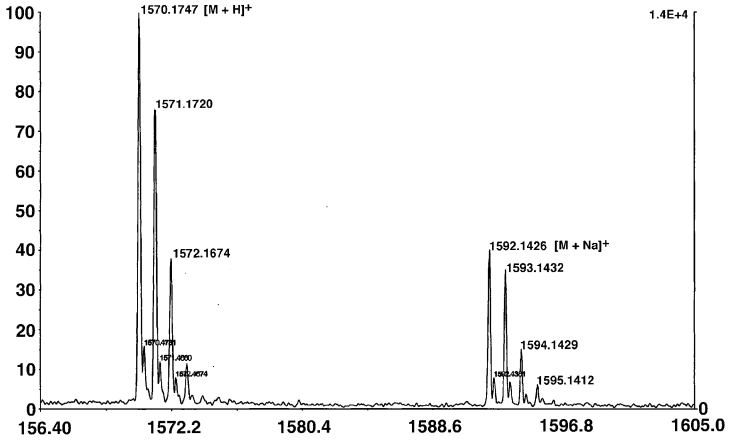



**Figure 3.** MALDI-TOF MS spectrum of purified compound **12**.

the case of the deca-PNAs. Nevertheless, a capping step was performed by acetylation with acetic anhydride in NMP (10 min).

Subsequently, the release of PNAs from the support, the cleavage of the terminal N-Boc protecting group and, when appropriate, of the N–Z exocyclic protecting groups of cytosine were simultaneously performed with TFMSA/TFA/TIS (1/3/0.1), yielding **5a–c** and **6a–c** as crude products after $Et_2O$ precipitation (as an example, 120 mg of crude deca-PNA T10 were obtained starting from 140 mg of resin, 95% yield).

Analysis by C18 reverse-phase HPLC of the crude products indicated that they consisted predominantly of PNA **5** or **6**, the degree of purity being dependent on the number of PNA monomer units, as expected (from about 70% to 95% yields). The structures were confirmed by ESI or MALDI-TOF mass spectrometry on semi-preparative HPLC purified samples.[14] The MS spectra showed the absence of partially acetylated backbones, which would result from an incomplete condensation of nucleobase units.

The efficient 'FPB'-supported synthesis of homo-PNAs **5** and **6** led us to examine the synthesis of a 'mixed' heteropyrimidine hexa-PNA CTCTCT **12** (Scheme 2).

Thus, the corresponding supported 'mixed' protected backbone Boc[Alloc-Fmoc]$_3$ **7** was prepared by six successive elongations using alternatively Boc[Fmoc]OH, then Boc[Alloc]OH units. The efficiency of each elongation step was attested by a negative Kaiser test.

The selective cleavage of the three Fmoc groups was performed using 20% piperidine in DMF to get supported Boc[Alloc-H]$_3$ backbone **8**. Three thymine acetic acid units (12 equiv) were then condensed onto **8** using HBTU preactivation, to get Boc[Alloc-T]$_3$ **9**. Two coupling cycles were necessary to get a negative chloranil test. A capping step ($Ac_2O$ in NMP) was performed on **9** prior to the cleavage of the three Alloc protecting groups by means of $Pd(PPh_3)_4$, leading Boc[H–T]$_3$ **10**. (Z)-protected cytosine acetic acid units (12 equiv) were then condensed onto **10**, to lead Boc[$C^Z$-T]$_3$ **11**. A last capping step followed by treatment with TFMSA/TFA gave the crude hexa-PNA CTCTCT **12** after precipitation with $Et_2O$, in 92% yield.

Analysis by C18 reverse-phase HPLC of this precipitate indicated that it consisted predominantly of a single derivative (about 75% of purity; Fig. 2). The structure was confirmed by MALDI-TOF MS analysis, on a semi-preparative HPLC purified sample (Fig. 3).[14] As previously, no trace of partially acetylated backbones was detected, confirming very effective nucleobases condensations.

In summary, the FPB strategy was successfully applied to the solid-phase synthesis of di-, penta- and deca-homopyrimidine PNAs as well as of a mixed-pyrimidine hexamer. Fully protected backbones were built on the resin by very effective elongation steps, starting from easy available protected monomers. Backbone deprotections were selective and quantitative. Nucleobases condensation steps were performed efficiently, even when ten nucleobase units were condensed.

Generalization of this FPB strategy to the supported synthesis of PNAs containing all four natural nucleobases and non-natural ones is now under progress.

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## Supplementary data

HPLC analyses and mass spectral data (MALDI-TOF) for compound **4b** and PNAs **5b–c**, **6b–c**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.04.018.

## References and notes


1. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500.
2. (a) Nielsen, P. E. *Methods Mol. Biol.* **2005**, *288*, 343–358; (b) Nielsen, P. E. *Curr. Opin. Mol. Ther.* **2000**, *3*, 282–287; (c) Jacob, A.; Brandt, O.; Stephan, A.; Hoheisel, J. D. *Methods Mol. Biol.* **2004**, *283*, 283–293; (d) Pellestor, F.; Paulasova, P. *Int. J. Mol. Med.* **2004**, *13*, 521–525; (e) Nielsen, P. E. *Mol. Biotechnol.* **2004**, *26*, 233–248; (f) Kaihatsu, K.; Janowski, B. A.; Corey, D. R. *Chem. Biol.* **2004**, *11*, 749–758.
3. (a) Kofoed, T.; Hansen, H. F.; Orum, H.; Koch, T. *J. Pept. Sci.* **2001**, *7*, 402–412; (b) Sugiyama, T.; Kittaka, A.; Takemoto, Y.; Takayama, H.; Kuroda, R. *Nucleic Acids Res. Suppl.* **2002**, *1*, 145–146.
4. Dwaine, A. B.; Corey, D. R. *Methods* **2001**, *23*, 97–107.
5. Mayfield, L. D.; Corey, D. R. *Anal. Biochem.* **1999**, *268*, 401–404.
6. Christensen, L.; Fitzpatrick, R.; Gildea, B.; Petersen, K. H.; Hansen, H. F.; Koch, T.; Egholm, M.; Buchardt, O.; Nielsen, P. E.; Coull, J.; Berg, R. H. *J. Pept. Sci.* **1995**, *3*, 175–183.
7. (a) Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzio, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179–6194; (b) Will, D. W.; Breipohl, G.; Langner, D.; Knolle, J.; Uhlmann, E. *Tetrahedron* **1995**, *51*, 12069–12082.
8. Corradini, R.; Sforza, S.; Dossena, A.; Palla, G.; Rocchi, R.; Filira, F.; Nastri, F.; Marchelli, R. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2690–2696.
9. Sforza, S.; Tedeschi, T.; Corradini, R.; Ciavardelli, D.; Dossena, A.; Marchelli, R. *Eur. J. Org. Chem.* **2003**, 1056–1063.
10. (a) Seitz, O.; Kohler, O. *Chem. Eur. J.* **2001**, *7*, 3911–3925; (b) Seitz, O. *Tetrahedron Lett.* **1999**, *40*, 4161–4164.
11. (a) Di Giorgio, C.; Pairot, S.; Schwergold, C.; Patino, N.; Condom, R.; Farese-Di Giorgio, A.; Guedj, R. *Tetrahedron* **1999**, *55*, 1937–1958; (b) Schwergold, C.; Depecker, G.; Di Giorgio, C.; Patino, N.; Jossinet, F.; Ehresmann, B.; Terreux, R.; Cabrol-Bass, D.; Condom, R. *Tetrahedron* **2002**, *58*, 5675–5687; (c) Caldarelli, S.; Depecker, G.; Patino, N.; Di Giorgio, A.; Barouillet, T.; Doglio, A.; Condom, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4435–4438.
12. Verheijen, J. C.; Grotenbreg, G. M.; Hart de Ruyter, L.; Van der Klein, P. A. M.; Van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **2000**, *41*, 3991–3995.
13. HPLC analyses were performed with either a HP1100 (UV detector set at 260 nm) or a Waters 996 Photodiode Array Detector (PDA, UV detector from 195 to 290 nm), using a RP-18 (5 mm) Licrospher 100 ($250 \times 4$ mm) column as support. Elution solvents: 0.1% TFA $H_2O$ and 0.1% TFA $CH_3CN$.
14. $[T]_2$: $C_{22}H_{31}N_9O_8$ MS (ESI+) *m/z* 550.4 $[M+H]^+$. $[C]_2$: $C_{20}H_{29}N_{11}O_6$ MS (ESI+) *m/z* 519.8 $[M+H]^+$; For $[Ac]_{10}$, PNA $[T]_5$, $[C]_5$, $[TC]_3$, $[T]_{10}$ and $[C]_{10}$: MALDI-TOF MS. Calcd exact mass for $[Ac]_{10}$ ($C_{60}H_{103}N_{21}O_{20}$) 1437.77, found 1460.39 (M+Na)+; calcd exact mass for $[T]_5$ ($C_{55}H_{73}N_{21}O_{20}$) 1347.53, found 1348.41 (M+H)+ and 1370.37 (M+Na)+; calcd exact mass for $[C]_5$ ($C_{50}H_{68}N_{26}O_{15}$) 1272.54, found 1273.39 (M+H)+ and 1295.37 (M+Na)+; calcd exact mass for $[T]_{10}$ ($C_{110}H_{143}N_{41}O_{40}$) 2678.04, found 2679.10 (M+H)+ and 2701.03 (M+Na)+; calcd exact mass for $[C]_{10}$ ($C_{100}H_{133}N_{51}O_{30}$) 2528.05, found 2529.08 (M+H)+ and 2550.99 (M+Na)+; calcd exact mass for $[CT]_3$ ($C_{63}H_{84}N_{28}O_{21}$) 1568.64, found 1570.10 (M+H)+ and 1592.14 (M+Na)+.