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Azidopeptide Nucleic Acid. An Alternative Strategy for Solid-Phase Peptide Nucleic Acid (PNA) Synthesis

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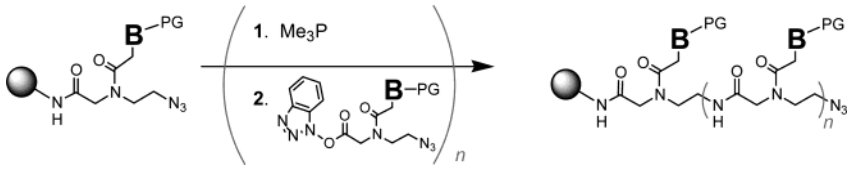
ABSTRACT



A practical and efficient method for PNA synthesis using an azide group to mask the N-terminus is reported. The deprotection was carried out in 5 min, while couplings were complete within 60 min. The near neutral conditions of the phosphine deprotection combined with the base-free coupling using hydroxybenzotriazole-activated monomers make this approach very mild.

The hybridization properties of peptide nucleic acids[1] (PNAs) have attracted widespread interest to this class of compounds.[2,3] Their biological stability has made PNAs attractive as therapeutic and biomolecular tools. Our interest in PNA chemistry stems from their use to encode compounds such as small-molecule combinatorial libraries and format such solution-based libraries into a spatially addressable arrays upon binding to an oligonucleotide microarray (see Figure 1).[4,5] This encoding strategy has also been implemented for the preparation of protein microarray.[6]

The chemical stability of PNAs and their superior hybridization properties relative to natural oligonucleotides make PNAs ideally suited as the encoding oligonucleotides. In PNAs, the phosphoribose backbone of natural oligonucleotide has been replaced by *N*-(2-aminoethyl)glycine whereby the purine/pirimidine base pair is attached to the glycine nitrogen via a methylene carbonyl linker. Their oligomerization is thus carried out using standard peptide chemistry such as Fmoc or Boc chemistry with suitable protecting groups for the purine or pyrimidine heterocycles.[3] Alternative protecting groups for the terminal amino group include Mmt[7] and DTS.[8]

A cornerstone of PNA-encoded libraries is the orthogonality of the PNA synthesis with respect to the reaction used to introduce molecular diversity. Herein, we report the use of an azide to mask the N-terminus of PNA oligomer and demonstrate that this azide can be reduced and the PNA

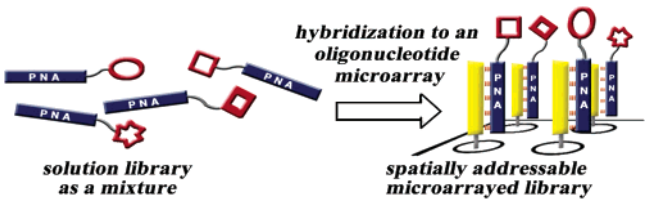


Figure 1. Libraries encoded with PNA tags (blue bars) present as a mixture in solution can be converted into spatially addressable arrays by hybridization to an oligonucleotide microarray.

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(4) Winssinger, N.; Harris, J. L.; Backes, B. J.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 3152.
(5) Winssinger, N.; Ficarro, S.; Schultz, P. G.; Harris, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11139.
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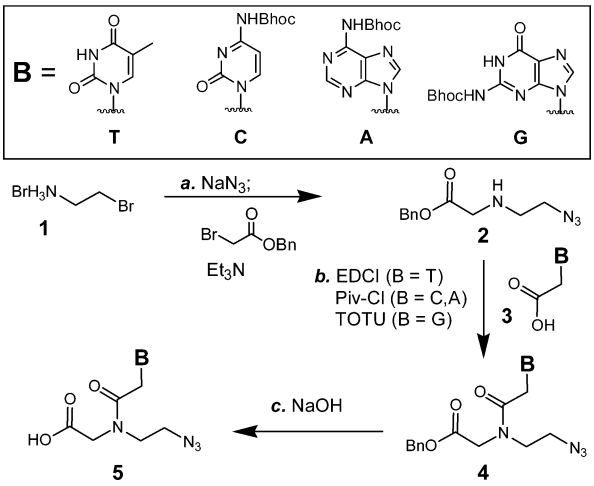
further oligomerized in rapid cycles and under mild conditions.[9]

The preparation of the four required monomers is shown in Scheme 1. The benzyl *N*-(2-azidoethyl)glycinate **2** is

Scheme 1. Synthesis of AzidoPNA Monomers **5**[10 a]



[a] Reagents and conditions: (a) **1** (1.0 equiv), NaN_3 (1.1 equiv), DMF, 60 °C, 3 h; benzyl 2-bromoacetate (0.8 equiv), Et_3N (2.0 equiv), 0 °C, 2 h, 64%. (b) **3-T** (1.0 equiv), **2** (1.0 equiv), EDCl (1.2 equiv), 4-DMAP (0.1 equiv), DMF, 23 °C, 2 h, 94%; **3-C** (1.0 equiv), NMM (2.2 equiv), Piv-Cl (1.2 equiv), **2** (1.0 equiv), 23 °C, 90 min, 50%; **3-A** (1.0 equiv), NMM (2.2 equiv), Piv-Cl (1.2 equiv), **2** (1.0 equiv), 23 °C, 90 min, 80%; **3-G** (1.0 equiv), TOTU (1.1 equiv), Et_3N (1.7 equiv), 2 (1.0 equiv), 23 °C, 2h, 49%. (c) NaOH (4.0 equiv), dioxane/H2O, 23 °C, 15 min, 77% for **4-T**, 95% for **4-C**, 76% for **4-A**, 80% for **4-G**.

prepared from 2-aminoethylbromide hydrobromide in one pot by a displacement of the bromide with NaN_3 in DMF followed by the addition of benzyl 2-bromoacetate and Et_3N to obtain the desired amine in 64% yield. As a choice of protecting group for the exocyclic nitrogen of the nucleobases, we found *N*-benzhydryloxycarbonyl (Bhoc)[11] to be ideal in terms of deactivating the nucleobase toward electrophiles and ease of deprotection.[12] Nevertheless, more resistant groups such as Cbz should also prove to be compatible with this strategy. The suitably protected nucleobases **3** were prepared according to minor modifications of known procedures[13] and coupled to amine **2** (Scheme 1). While acid **3-T** was coupled to amine **2** in excellent yield under standard carbodiimide coupling conditions (EDCI, 4-DMAP), the other acids gave poor results under these conditions. Acid **3-C** and **3-A** could be effectively coupled to amine **2** via formation of the pivaloyl mixed anhydride (50 and 80% yields, respectively), whereas TOTU[15] coupling was found to be most effective for **3-G**. The hydrolyses of the benzyl esters **4** were carried out with NaOH in H_2O/dioxane and proceeded smoothly to afford the four PNA monomers **5** as white solids in good to excellent yields.

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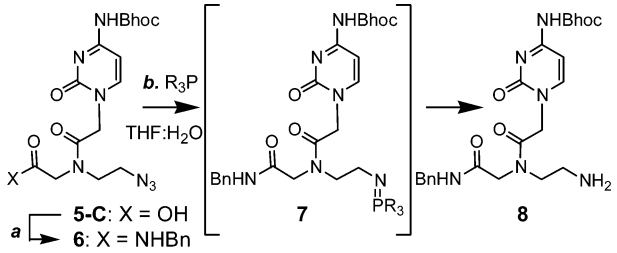
(8) Planas, M.; Bardaji, E.; Jensen, K. J.; Barany, G. *J. Org. Chem.* **1999**, *64*, 7281.

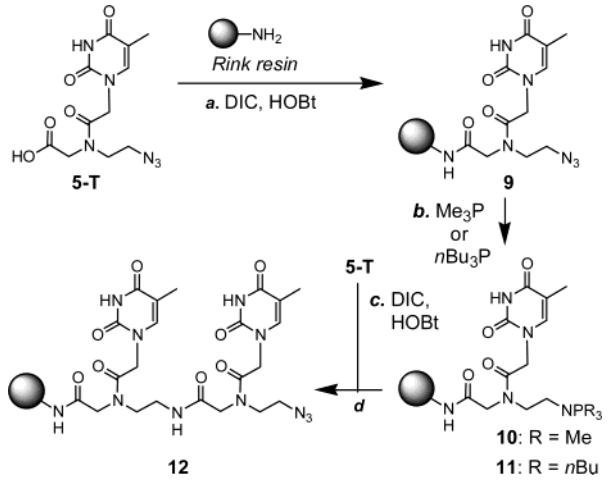
Scheme 2. Reduction of AzidoPNA[10 a]



[a] Reagents and conditions: (a) **5-C** (1.0 equiv), $BnNH_2$ (1.0 equiv), EDCl (1.0 equiv), DMF 23 °C, 30 min, 96%. (b) R_3P, THF/H_2O (9:1).

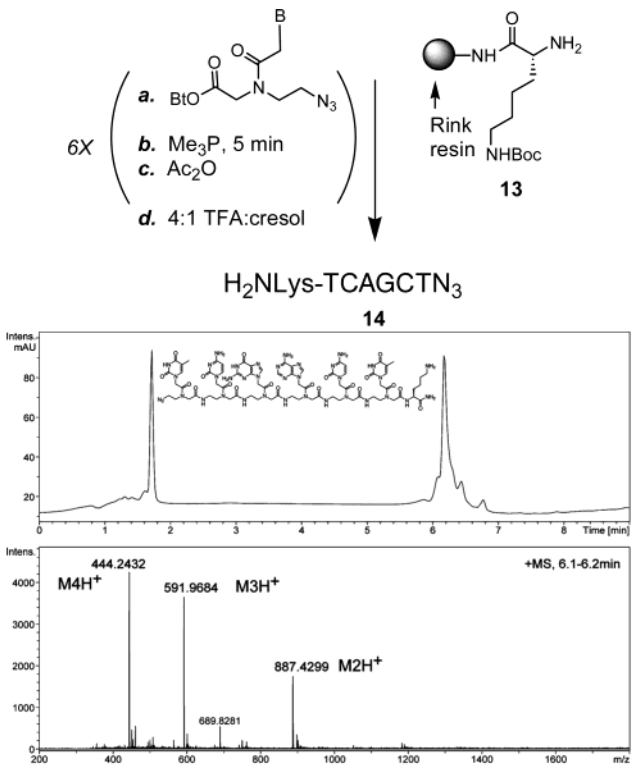
We then turned our attention to the azide reduction/PNA coupling cycle. The Staudinger[14] reduction was deemed most attractive due to its mildness; however, long reaction times were considered to be impractical for oligomerization purposes. To study the kinetics of aryl and alkylphosphine addition to the azidoPNA, we converted monomer **5-C** to the corresponding benzyl amide **6** using an EDCI-mediated coupling (Scheme 2). While the addition of triphenylphosphine to azidoPNA **6** did not go to completion at room temperature after 5 h, the addition of trimethyl and tributyl phosphine was complete within 5 min. Nevertheless, the hydrolysis of the iminophosphorane **7** to the corresponding amine **8** was relatively slow in both cases. Inspired by the direct elaboration of these azaylide intermediates into amides with carboxylic acid[15] or activated esters such as succinyl ester[16] or pentofluorophenyl ester,[17] we asked ourselves if iminophosphorane **7** would react cleanly with *N*-hydroxybenzotriazole ester generated in situ. To this end, we loaded monomer **5-T** onto Rink resin (Scheme 3) to obtain polymer-bound azido monomer **9**, which was treated with a 1 M THF

Scheme 3. Coupling with Iminophosphorane[10 a]



[a] Reagents and conditions: (a) **5-T** (3.0 equiv), HOBt (3.0 equiv), DIC (3.0 equiv), DMF 23 °C, 3 h. (b) R_3P (1.0 M), THF, 23 °C, 5 min. (c) **5-T** (3.0 equiv), HOBt (2.5 equiv), DIC (2.5 equiv), DMF, 23 °C, 60 min. (d) DMF, 23 °C, 60 min.

Scheme 4. Synthesis and LC-MS Analysis of 6-mer PNA **14**[10] [a]



[a] Reagents and conditions: (a) **5** (3.0 equiv), HOBt (3.0 equiv), DIC (2.5 equiv), DMF 23 °C, 60 min (60 min of preactivation). (b) Me_3P (0.9 M) in THF/H_2O (9:1), 23 °C, 5 min. (c) Ac_2O (4.0 equiv), pyridine (4.0 equiv), DMF, 23 °C, 5 min. (d) TFA/*m*-cresol (4:1), 23 °C, 60 min.

solution of trimethyl phosphine and tributyl phosphine for 5 min. The azaylide **10** and **11** thus obtained were treated with *N*-hydroxybenzotriazole activated ester generated in situ using DIC and HOBT for 60 min prior to addition. We were pleased to observe that compound **10** was completely consumed after 1 h to yield the desired dimer **12**. It is interesting to note that the tributyl azaylide **11** did not react under these reaction conditions.

With these results in hand, we proceeded to the solid-phase synthesis of a 6-mer PNA containing all four nucleotides. As shown in Scheme 4, double coupling cycles of 60 min with 3 equiv of monomer followed by a 5 min capping (acetic anhydride/pyridine) and 5 min trimethylphosphine deprotection were used. Aliquots of resin were removed and cleaved at each step and analyzed by LC-MS. The K-TCA-GCT-N_3 was obtained as a single major compound, eluting 6.1 min (Scheme 4, the solvent front being at 1.7 min) with an estimated average yield in excess of 92% per coupling/reduction cycle.[18]

In conclusion, we have developed an alternative strategy for the synthesis of PNA oligomers. The mildness and efficiency of the conditions reported herein complement the standard Fmoc and Boc chemistry and should facilitate cosynthesis of PNA with sensitive molecules.

Acknowledgment. We thank Human Frontiers for their support of this work (HFSP 0080/2003).

Supporting Information Available: Experimental procedures and analytical data for the preparation of PNA monomers **5-T**, **5-C**, **5-A**, and **5-G**, general procedures for PNA synthesis (deprotection and coupling), and LC-MS data of all intermediates leading to 6-mer **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Following abbreviations are used: DIC = diisopropyl carbodiimide; EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; HOBt = 1-hydroxybenzotriazole; NMM = *N*-methylmorpholine; Piv = pivaloyl; TOTU = *O*-[(ethoxycarbonyl)cyanomethylenamino]-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFA = trifluoroacetic acid.

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(18) Yield estimated on the basis of UV chromatogram integration.