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Solid-phase Synthesis of PNA Monomer by Ugi Four-component Condensation Reaction

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Abstract: Peptide nucleic acids (PNA) oligomers were synthesized in most cases by peptide synthesis from N-protected monomers. In this work a new method of obtaining PNA monomer by Ugi four-component condensation reaction was tested by solid-phase synthesis. The Fmoc protected PNA monomer was build up with thymin-1-yl acetic acid, 3-methylbutyl aldehyde, Fmoc protected aminoethyl isocyanide and Gly-Wang resin.

Keywords: Peptide nucleic acids (PNA), Ugi four-component condensation reaction (U-4CR), Solid-phase synthesis, Isocyanide.

Peptide nucleic acids (PNA) are oligonucleotide analogues in which the sugar-phosphate backbone has been replaced by a polyamide chain to which the nucleobases are linked. This synthetic pseudopeptide DNA/RNA mimic, invented more than 10 years ago[1], have powerful resistance to nucleases and protease in the cellular environment while retaining the base pairing properties and high affinity for RNA and single and double-stranded DNA targets[2]. Accordingly, they could be powerful tools in molecular biology and biotechnology and potential antisense drugs[3]. However their low solubility in water and their tendency towards self-aggregation[4] limitated the use of PNAs in diagnostic and research applications. Many derivatives and analogs of PNA were designed and prepared towards improving their physico-chemical and biological properties. A systematic investigation of structure-function relationships using combinatorial chemical strategies is still an attractive research area.

PNA-oligomers are synthesized in most cases by homogeneous or solid-phase peptide synthesis from N-protected monomers[5]. The synthesis of PNA monomers has been described with different strategies using different protecting groups for the amino (Boc, Fmoc, or Mmt) and for the nucleobases. The commonly used methods for preparation of PNA monomers need several steps to generate N-(2-aminoethyl)glycine unit and followed by N-acylation of the glycine derivative by a carboxymethylated nucleobase[6]. Several quite different research reports demonstrated the usefulness of Ugi four-component condensation reaction (U-4CR) in the synthesis of the PNA monomers[7]. The multi-component condensation reactions, especially U-4CR reaction,

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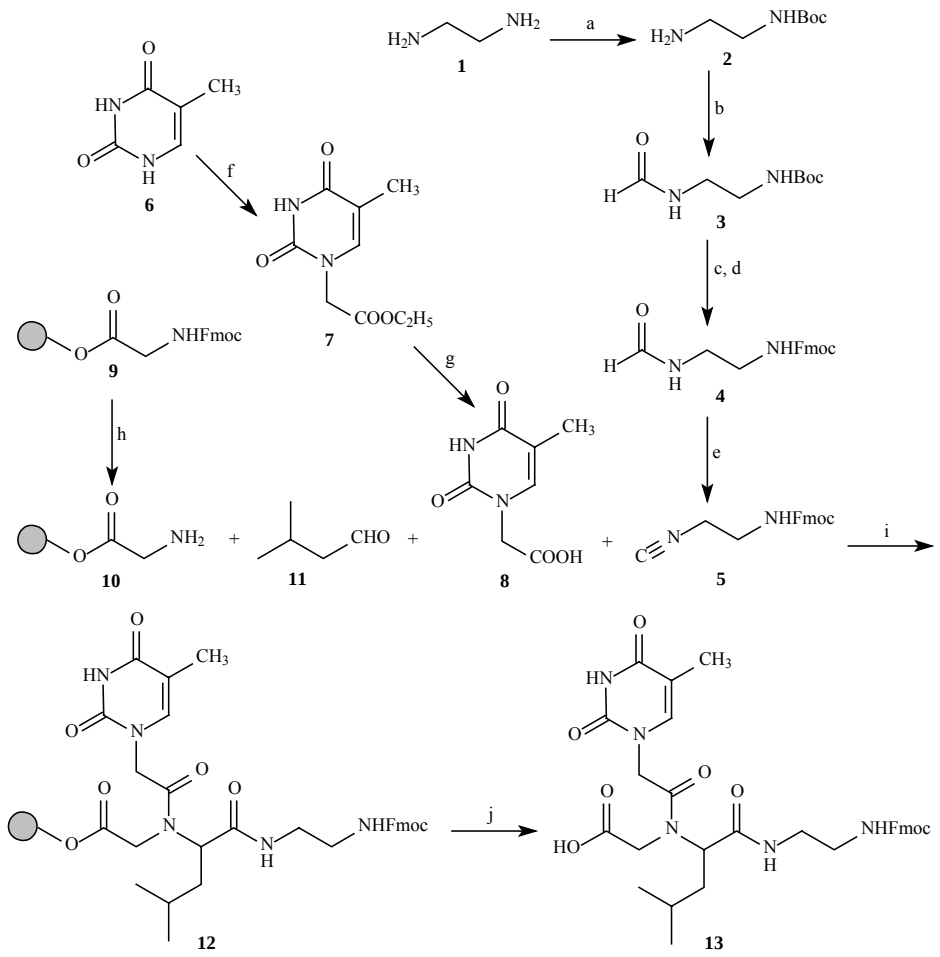
was used as a powerful tool in combinatorial chemistry[8]. Our group described a solution-phase synthetic way to obtain PNA monomer and PNA chain prolongation by progressional U-4CR reactions[9], which differed from peptide chemistry in that PNA monomers which do not need to be prepared beforehand, they would be built up along with the chain extended. In our previous work, Boc protecting strategy was chosen to synthesize the PNA in solution phase[9]. The low solubility of dimer brought some problems in the next prolonged reaction. Consequently the 9-fluorenylmethoxy-carbonyl (Fmoc) was adopted for comparison. The main advantage of using Fmoc instead of Boc implies the possibility of using an acid-cleavable resin. First of all, for the purpose of examining the protecting protocol, the thymin-1-yl acetic acid, 3-methylbutylamine, isovaleric aldehyde and Fmoc protected aminoethyl isocyanide were

Scheme 1 Solid-phase synthesis of PNA monomer



a) $(Boc)_2O$, THF; b) HCO_2H, DCC, CH_2Cl_2; c) 2 mol/L HCl/EtOAc; d) 5% aq. $NaHCO_3$, FmocOSu, CH_3CN; e) $POCl_3$, DIPEA, CH_2Cl_2; f) $BrCH_2COOC_2H_5$, K_2CO_3, DMF; g) NaOH, then HCl; h) 20% pyridine/DMF; i) U-4CR, DMF; j) 50% TFA/CH_2Cl_2.

used as starting materials in a solution-phase U-4CR one-pot process to generate amino-protected PNA monomer, as a model reaction[10]. Considering the difficulty of purification during the sequential prolongation, an alternative solid-phase method was studied. Herein our primary results on PNA monomer solid-phase synthesis were reported.

The PNA monomer **13** was synthesized by a solid-phase U-4CR (**Scheme 1**), from Fmoc protected aminoethyl isocyanide **5**, thymin-1-yl acetic acid **8**, Gly-Wang resin **10** and isovaleric aldehyde **11**. In the four components of the reaction, isocyanide **5** has to be prepared just before use due to the instability. The preparation of **5** was described in detail in our previous work[10]. **8** can be prepared from thymine **6** by reference method[5]. **10** was yielded from Fmoc-Gly-Wang resin **9**. In order to ameliorate the poor solubility of typical PNA molecules, modified structures, *i.e.* a conjugated peptide-PNA, were considered. So an Fmoc-amino acid resin was chosen as the solid component in the Ugi reaction. This will provide a terminal for peptide attachment in the final PNA molecule. In this work, Fmoc-Gly-Wang resin **9** was purchased and preswelled in CH_2Cl_2. The Fmoc group was deprotected first by 20% pyridine/DMF to yield Gly-Wang resin **10**, and the exposed amino group was examined by Kaiser test. In the solid Ugi reaction, DMF in stead of isopropanol was used as solvent, because the resin shrank in isopropanol. The end of reaction was determined by Kaiser test, to see if there were free amino groups existent. The support linked PNA monomer **12** was cleaved from resin with TFA, and the amino-protected PNA monomer **13** was characterized by LC-MS, ^{1}H NMR, and elemental analysis.

In conclusion, a solid-phase PNA monomer synthesis strategy was designed based on Ugi four-component condensation reaction. Most of the reactants were commercially available or prepared easily. Comparison of the result of the model reaction in our previous work[9] showed that the Fmoc protection strategy was better than Boc protection in solution phase. The Fmoc protected resins can attach different amine components.

Experimental

Melting points were taken with an X-4 apparatus and the thermometer was uncorrected. ^{1}H NMR, MS and element data were taken with JNM-AL300 FT NMR SYSTEM, QSTAR ESI-TOF, FLASH EA 1112 instruments, respectively.

Fmoc-Gly-Wang resin **9** (500 mg, 1 mmol/g) was swelled in dry CH_2Cl_2 (5 mL) over night. The solvent was filtered off, 20% pyridine/DMF (5 mL) was added and the mixture was shaken for 15 min. This deprotection procedure was repeated twice. Then the resin was washed successively with DMF (5 mL × 5), ethanol (5 mL × 3), DMF (5 mL × 3), till the Kaiser test showed blue. Isovaleric aldehyde **11** (0.48 mL, 4.5 mmol), the solution of thymin-1-ylacetic acid **8** (850 mg, 4.5 mmol) in DMF (7.5 mL) and the solution of isocyanide **5** (1.3 g, 4.5 mmol) were added. The mixture was shaken for 48 h and filtered, washed orderly with DMF (5 mL × 3), ethanol (5 mL × 3), CH_2Cl_2 (5 mL × 3). Kaiser test showed no blue solution and resin. The resin **12** linked PNA monomer was cleaved by 50% TFA/CH_2CL_2 (5 mL) at room temperature for 20 min.

The filtered solution was evaporated in vacuum and the residue was purified by sillica column chromatography to give 52 mg (34.6%) of **13** as a white crystal; mp 120°C. ^{1}H NMR (DMSO-d_6, δ ppm): 11.320 (s, 1 H, CONHCO), 8.912 (s, 1 H, NHCO), 7.889 (d, 2 H, J = 7.5 Hz, ArH), 7.721 (d, 2 H, J = 7.5 Hz, ArH), 7.669 (s, 1 H, NHCO), 7.423 (t, 2 H, J = 7.5 Hz, ArH), 7.333 (t, 2 H, J = 7.5 Hz, ArH), 7.243 (s, 1 H, ArH), 5.153 (m, 3 H, NCHCO, NCH_2CO), 4.532 (d, 2 H, J = 6.9 Hz, CH_2O), 4.327 (s, 2 H, $COCH_2Ar$), 4.306 (t, 1 H, J = 6.9 Hz, CH), 3.336 (m, 4 H, CH_2CH_2), 1.728 (s, 3 H, T-CH_3), 1.147 (m, 2 H, CH_2), 0.846 (m, 7 H, $CH(CH_3)_2$). MS (ESI-TOF): *m/z* = 620.45 (M^+). Anal. calcd for $C_{32}H_{37}N_5O_8$ (619.67): C 62.02; H 6.02; N 11.30. Found: C 62.12; H 6.08; N 11.09.

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References

1. P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt, *Science*, **1991**, *254*, 1497.
2. (a) M. Egholm, O. Buchardt, L. Christensen, *et al*., *Nature*, **1993**, *365*, 556. (b) P. Wittung, P. E. Nielsen, O. Buchardt, M. Egholm, B. NordeÂn, *Nature*, **1994**, *368*, 561. (c) P.E. Nielsen, M. Egholm, O. Buchardt, *Gene*, **1994**, *149*, 139.
3. P. E. Nielsen, *Curr. Opin. Biotechnol.*, **1999**, *10*, 71.
4. M. Egholm, O. Buchardt, P.E. Nielsen, R. H. Berg, *J. Am. Chem. Soc.*, **1992**, *114*, 9677.
5. K. L. Dueholm, M. Egholm, C. Behrends, L. Christensen, H. F. Hansen, T. Vulpius, K. Petersen, R. H. Berg, P. E. Nielsen, O. Buchardt, *J. Org. Chem.*, **1994**, *59*, 5767.
6. (a) B. Hyrup, M. Egholm, P.E. Nielsen, P. Wittung, B. Nordén, O. Buchardt, *J. Am. Chem. Soc.*, **1994**, *116*, 7964. (b) D.W. Will, G. Breipohl, D. Langner, J. Knolle, E. Uhlmann, *Tetrahedron*, **1995**, *51*(44), 12069.
7. (a) H. Gröger, M. Hatam, J. Kintscher, J. Martens, *Synth. Commun.*, **1996**, *26*(18), 3383. (b) A. Dömling, W. Richter, I. Ugi, *Nucleosides & Nucleotides*, **1997**, *16*(7-9), 1753. (c) W. Maison, I. Schlemminger, O. Westerhoff, J. Martens, *Bioorg. Med. Chem. Lett.*, **1999**, *9*(4), 581. (d) W. Maison, I. Schlemminger, O. Westerhoff, J. Martens, *Bioorg. Med. Chem.*, **2000**, *8*(6), 1343. (e) A. Dömling, *Nucleosides & Nucleotides*, **1998**, *17*(9-11), 1667. (f) A. Dömling, K-Z. Chi, M. Barrère, *Bioorg. Med. Chem. Lett.*, **1999**, *9*(19), 2871.
8. A. Dömling, I. Ugi, *Angew. Chem. Int. Ed. Engl.*, **2000**, *39*(18), 3168.
9. P. Xu, T. Zhang, W. Wang, X. Zou, X. Zhang, Y. Fu, *Synthesis*, **2003**, 1171.
10. W. Wang, T. Zhang, P. Xu, *J. Chinese Pharm. Sci.*, **2003**, *12*(2), 66.

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