Synthesis of PNA Monomers and Dimers by Ugi Four-Component Reaction

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Abstract: Peptide nucleic acids (PNA) have received great attention as tools in molecular biology and biotechnology, diagnostic agents, and potential antisense drugs. Many derivatives and analogues of PNA were designed and prepared to improve their physico-chemical and biological properties. In most cases, PNA oligomers were synthesized by peptide synthesis from N-protected monomers. In this work, a new method to obtain PNA monomers and PNA chain prolongation by Ugi four-component condensation reaction was tested by synthesizing PNA monomers and dimers. This method differs from classical peptide chemistry in that no prior preparation of PNA monomers is needed as they are built up along with the extension of chain. The synthesis of the key component isocyanide is also presented.

Key words: peptide nucleic acids (PNA), condensation, isocyanide, Ugi four-component reaction

Peptide nucleic acids (PNA) are oligonucleotide analogues in which the sugar-phosphate backbone has been replaced by a polyamide chain linked to nucleobases (Figure 1). Invented more than 10 years ago,1 they have received great attention due to their several favorable properties, including resistance to nuclease and protease digestion, stability in serum and cell extracts, and their high affinity for RNA and single- and double-stranded DNA targets.2 For these reasons, they have become very popular, as tools in molecular biology and biotechnology, as diagnostic agents, and as potential antisense drugs. However, there are some limitations for the use of PNAs in diagnostic and research applications, for example, their low solubility in water and their tendency towards self-aggregation.3 Many derivatives and analogues of PNA were designed and prepared to improve their physico-chemical and biological properties.4 A systematic investigation of structure-function relationships using combinatorial chemical strategies is still an attractive research area.

Figure 1 Structure of PNA; B = nucleobase

PNA-oligomers are synthesized in most cases by homogeneous or solid-phase peptide synthesis from N-protected monomers (Figure 2).5 The synthesis of PNA monomers has been described with different strategies using different protecting groups for the amino function (Boc, Fmoc, or Mmt) and for the nucleobases. The commonly used methods for the preparation of PNA monomers require several steps to generate the N-(2-aminoethyl)glycine unit 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 (Ugi-4CC) reaction in the synthesis of PNA monomers.7 Herein we describe a new method to obtain PNA monomer and PNA chain prolongation by progression of Ugi-4CC reactions, which differs from classical peptide chemistry in that prior preparation of PNA monomers is not needed as they are built up along with the extended chain.

Figure 2 Structure of N-protected PNA monomer; Pg = protecting group

The multi-component condensation reactions, especially Ugi-4CC reaction, is continuously used as a powerful tool in combinatorial chemistry.8 In this work, PNA oligomer to be synthesized was designed by a repeated protocol of two steps (an Ugi reaction and a deprotection) in one turn (Scheme 1). In the first cycle, a nucleobase-acetic acid 1, an amine 2, an oxo compound (aldehyde or ketone, 3), and an N-protected aminomethyl isocyanide 4 were used as starting materials in the Ugi-4CC reaction to generate the amino-protected PNA monomer 5 in a one-pot process, followed by deprotection of the amino group. Then the deprotected product 6 reacts as the amine component with the other three components in the second cycle of Ugi reaction to yield the amino-protected PNA dimer 7. By repeating the Ugi reaction and amino-deprotection, the PNA chain can be extended. This strategy can be applied in the synthesis of a number of structural derivatives and analogues of PNA by employment of different reactants, 1, 2, 3 and 4. More expediently, the four components may be selected as four types of building blocks to construct derivatized PNA libraries by solid- or solution-phase synthesis following this strategy.
For the purpose of examining the feasibility of the synthetic route, thymin-1-ylacetic acid (1a), \([N^2-(benzyloxy-carbonyl)cytosine-1-yl]acetic\) acid (1b), 3-methylbutylamine (2a), formaldehyde (3a), 3-methylbutanal (3b), and \((N\text{-}\text{tert}-\text{butyloxycarbonyl})\text{aminoethyl isocyanide}\) (4a) were selected first to generate PNA monomers and dimers in solution phase following the above strategy. Among the four components of the Ugi reaction, 2 and 3 are commercially available, and 1a and 1b can be prepared conveniently (Scheme 2). Isocyanide 4a has to be prepared just before use because of its instability. The synthetic route to 4a is shown in Scheme 3. The mono-amidation of ethylenediamine was not easy to do because the two amino groups were often amidated at the same time. But the mono-protected \((N\text{-}\text{Boc})\text{-ethylenediamine}\) 9 was obtained conveniently with di-\text{tert}-\text{butyl} dicarbonate in moderate yield (70\%) by following the procedure described. The formylation of 9 was carried out by using HCO₂Et, HCO₂C(=O)Me, DCC/HCO₂H, respectively; and all gave the anticipated formylated product 10. But DCC/HCO₂H gave the best results. The reaction was over in 4 hours at room temperature with high yield (92\%), and no byproduct was detected. The reactants should be added in the following order: first the amine 9, then formic acid, and finally a solution of DCC in anhydrous CH₂Cl₂.

Scheme 2  Synthetic route to thymine and cytosine acetic acids

Scheme 1  Synthetic scheme of PNA monomer and chain extension; B = nucleobase, Pg = protecting group
The preparation of isocyanides from their formamide precursors has been reviewed. The POCl₃/Et₃N method was used in this work to transform 10 to (N-Boc)-aminoethyl isocyanide 4a. During the reaction, hydrogen chloride was produced, which may deprotect the acid labile Boc group. In fact, the deprotected compound was indeed detected in our experiment. Also, the isocyanide 4a was found to be unstable. Hence the procedure was improved to finish the reaction in shorter time (treatment with POCl₃ for 30 min instead of 1 h, then with K₂CO₃ for 30 min instead of 1 h) to reduce the unwanted deprotection. Additionally, removal of the gaseous hydrogen chloride evolved during the reaction under vacuum was also helpful. Then, the reaction mixture was worked up immediately to decrease the decomposition of isocyanide product. Isocyanide 4a was purified chromatographically and its structure was determined by ¹H NMR spectrum and the characteristic IR absorbance of isocyanide group at 2120 cm⁻¹.

Thymin-1-ylacetic acid (1a), [N⁴-(benzyloxycarbonyl)cytosine-1-yl]acetic acid (1b), 3-methylbutylamine (2a), formaldehyde (3a), 3-methylbutanal (3b), and (N-Boc)-aminoethyl isocyanide (4a) were used to build up three PNA monomers 5a–c (Table 1) by Ugi reaction (cf. Scheme 1). Equimolar amounts of the four components reacted with each other in the reaction. The structures of 5a–c were confirmed by their FAB-MS, ¹H NMR and IR spectra, and elemental analyses (see experimental section). The quantities and qualities of Ugi products were affected by reaction temperature and time. At room temperature, the reaction was slow and sometimes had to be prolonged for two days (48 h). When the reaction temperature was increased to 80 °C (refluxing in isopropanol), the reaction speeded up greatly. But when the reactions were carried out at higher temperature, the number and amount of byproducts increased gradually (monitored by TLC). Considering the purity of the products, carrying out the reaction at room temperature was better. At this temperature most reactions can be completed within 24–48 hours.

The protecting group on the main chain amino of PNA monomers 5 need to be cleaved before the extension of the chain. The typical 50% CF₃CO₂H–CH₂Cl₂ solution was used first for N-Boc deprotection of 5b. The reaction was completed easily within 1 hour to obtain the trifluoroacetate product 6a (Scheme 4). When 6a was reacted with thymine acetic acid, 3-methylbutanal and (N-Boc)-aminoethyl isocyanide 4a in a second Ugi cycle to generate the corresponding PNA dimer, the product was not the anticipated compound 7b (Mw = 832.14), but a new compound 7a (Mw = 762.00, Scheme 4). The structure of 7a was confirmed by its mass spectrum (M⁺ – Boc = 662). Its formation can be explained as follows: in the Ugi reaction, the trifluoroacetate anion, which was carried in the reaction mixture by compound 6a instead of thymine acetate anion, reacts with the amine, aldehyde, and isocyanide to yield the Ugi product 7a. This hypothesis was proved by the fact that the thymine acetic acid was recovered as expected. Anion resin was used to try to remove the trifluoroacetate anion, but to no avail. Finally HCl–DMF was

### Table 1  PNA Monomers Prepared

<table>
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<tr>
<th>PNA monomers</th>
<th>Components</th>
<th>1</th>
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used to deprotect the Boc group, and the hydrochloride product 6b (Scheme 5) is transformed to the corresponding PNA dimer successfully. Moreover, the PNA monomer 5c, which has a Cbz-protected cytosine on the side-chain, was also transformed to main chain deprotected product 6c by HCl–DMF (Scheme 5). Meanwhile the side chain protecting group Cbz was unaffected. This made it possible that the amino groups on the main chain and side-chain can be protected with Boc and Cbz, respectively, and deprotected orthogonally.

In the synthesis of PNA dimer by a second cycle Ugi reaction (Scheme 5), the hydrochloride 6 needed to be treated with an alkaline agent to liberate the amino group before the other three components were added. Pyridine was found to be good for this purpose. Its basicity was strong enough to neutralize the chloride ion, but insufficient to cause the self-aggregation of PNA. Considering the solubility, the reaction was carried out in DMF solution. The aldehyde is a potential diverse component in Ugi reaction and substituted aldehydes will be more meaningful than formaldehyde. So in the second Ugi cycle 3-methylbutanal instead of formaldehyde was used to test the method.

In conclusion, a novel PNA synthesis strategy was designed based on Ugi four-component condensation reaction by a repeated protocol. Three PNA monomers and two dimers were prepared in solution phase to test the method. Most of the reactants are commercially available or prepared easily. The synthesis of the key component in the Ugi reaction, (N-Boc)-aminoethyl isocyanide, is reported in detail. This strategy can be applied in the synthesis of PNA analogues, and the four components may be
selected as four types of building blocks to construct de-
rivatized PNA libraries. But this solution-phase method is
not satisfactory to build up large scale libraries and long
chain PNA oligomers because of the difficulty of purifica-
tion and the low yields. The solid-phase procedure is be-
ing studied to improve the method.

Unless otherwise mentioned, all the reagents were obtained com-
mercially and used without further purification. Petroleum ether
used had a boiling point range of 60–90 °C. Melting points were
taken with an X-4 apparatus and are uncorrected. IR (KBr), 1H
NMR, MS and elemental analyses data were taken with PE-983,
VXR-300S, VG-ZAB-HS, Carloerba-1106 instruments, rea-
sonably with H2O (37.5 mL) and aq HCl (4 mol/L, 1.6 mL) at 0 °C,
though the mixture was stirred for 30 min, and filtered to give ethyl
thymin-1-ylacetate. This solid was washed with H2O (3
and the mixture was stirred for 30 min, and filtered to give ethyl
(31%); white crystals; mp 272–274 °C (Lit. 5 mp 266–274 °C).

Thymin-1-ylactic Acid (1a)
To a stirred solution of thymine (5.00 g, 0.04 mol) and K2CO3 (5.48
A solution of (Boc)2O (2.18 g, 10 mmol) in anhyd THF (10 mL) was
added dropwise to a solution of ethyl xanthate (2.1 mL, 30 mmol)
in anhyd THF (10 mL) cooled in an ice-bath, and the mixture was
stirred for 30 min. After stirring for a further 24 h at r.t., the solven-
t was removed by evaporation under reduced pressure. The residue
was partitioned between CH2Cl2 (20 mL) and H2O (5 mL) and the aqueous
layer was washed with CH2Cl2 (2 × 5 mL). The CH2Cl2 sol-
lutions were combined and washed with 20% aq NaCl (2 × 5 mL),
dried (MgSO4), and evaporated to yield 2.4 g of a colorless oil. This
crude material was purified by column chromatography on silica gel
(CH2Cl2/ EtOH–aq NH3, 15:15:1) to give 9; yield: 1.2 g (75%); col-
orsless oil.

IR (film): 3308, 3066, 2923, 1702, 1636, 1547, 1415, 1332, 1237,
1034, 696 cm–1.

1H NMR (CDCl3); δ = 5.24 (s, 1 H, CONH, exchangeable with
D2O), 3.18 (t, 2 H, J = 5.85 Hz, NCH2), 2.82 (t, 2 H, J = 5.85 Hz,
NCH2); 2.34 (d, 2 H, J = 13.8 Hz, NH2, exchangeable with D2O).

MS (FAB): m/z = 161 (M + 1).

(N-t-Butyloxy carbonyl)-N-formyl ethylenediamine (10)
Anhyd formic acid (0.075 mL, 2 mmol) was added to a solution of 9
(320 mg, 2 mmol) in anhyd CH2Cl2 (5 mL) cooled in an ice-bath,
followed by addition of the solution of DCC (412 mg, 2 mmol) in
anhyd CH2Cl2 (5 mL). The mixture was stirred for 1–3 h at r.t. and
the solid dicyclohexylurea was filtered. The filtrate was washed
with sat. aq NaHCO3 (2 mL), dried (MgSO4), and evaporated; yield:
340 mg (90%); white solid; mp 61–63 °C.

IR (KBr): 3324, 2927, 1668, 1530, 1271, 1247, 1168 cm–1.

1H NMR (CDCl3); δ = 5.14 (s, 1 H, CONH), 3.34 (m, 2 H, NCH2),
3.23 (m, 2 H, NCH2), 1.38 [s, 9 H, C(CH3)3].

MS (FAB): m/z = 189 (M + 1).

Found: C, 51.12; H, 8.50; N, 14.80.

(N-t-Butyloxy carbonyl)-N-methyleneisocyanide (4a)
A solution of 10 (1.64 g, 8.7 mmol) in anhyd CH2Cl2 (13 mL) was
added Et3N (3.6 mL, 26.2 mmol) and to the mixture cooled in a
ice-bath was added dropwise POCI5 (0.82 mL, 8.7 mmol) and
stirred for 30 min. A solution of K2CO3 (1.21g, 8.7 mmol) in H2O
(5.7 mL) was added to the mixture and stirred for another 30 min.
The aqueous layer was extracted with CH2Cl2 (2 × 5 mL). The or-
ganic layer and the CH2Cl2 solutions were combined and washed
with H2O (2 × 5 mL), dried (MgSO4), and evaporated under reduced
pressure to yield a dark-brown cream. This crude material was pu-
fified by column chromatography on silica gel (petroleum ether–
EtOAc, 3:1); yield: 1.0 g (68%); pale yellow solid; mp 46–47 °C.

IR (KBr): 2960, 2160, 1630, 1510, 1250 cm–1.

1H NMR (CDCl3); δ = 4.98 (s, 1 H, CONH), 3.54 (s, 2 H, NCH2),
3.39 (s, 2 H, NCH2), 1.46 [s, 9 H, C(CH3)3].

MS (FAB): m/z = 171 (M + 1).

Found: C, 56.41; H, 8.32; N, 16.43.
small amount of DMF. and purified by column chromatography on silica gel (eluent: CH₂Cl₂–EtOH, 30:1); yield: 1.06 g (67%); white solid; mp 170 °C.

1H NMR (DMSO-d₆): δ = 11.23 (s, 1 H, NH, exchangeable with D₂O), 8.05 (s, 1 H, CONH, exchangeable with D₂O), 7.82 (s, 1 H, CONH), 7.41 (s, 1 H, =CH), 4.22 (s, 2 H, CH₂), 3.73 (t, 2 H, J = 6.4 Hz, NCH₂), 3.08 (t, 2 H, J = 6.4 Hz, NCH₂), 1.74 (s, 3 H, =CHCH₃), 1.55 (m, 1 H, CH), 1.36 (s, 2 H, CH₂), 1.32–1.23 (m, 10 H, 2 CH₃, 2 CH₂), 0.84 [s, 9 H, C(CH₃)₃].

MS (FAB): m/z = 492 (M + K̅).

Anal. Calcd for C₂₅H₄₃N₅O₆ (509.73): C, 58.90; H, 8.52; N, 13.74. Found: C, 55.70; H, 7.64; N, 15.56.

Following the above typical procedure, 3-methylbutanal (5.0 mL, 47 mmol), 3-methylbutylamine (0.55 mL, 4.7 mmol), and 1a (8.6 g, 49 mmol) were reacted to give 5b; yield: 1.6 g (62%); white solid; mp 171–173 °C.

MS (FAB): m/z = 529 (ammonium salt).

Anal. Calcd for C₂₅H₄₃N₅O₆ (509.73): C, 58.90; H, 8.52; N, 13.74. Found: C, 55.70; H, 7.64; N, 15.56.

Following the above typical procedure, 3-methylbutanal (5.0 mL, 47 mmol), 3-methylbutylamine (0.55 mL, 4.7 mmol), and 1a (8.6 g, 49 mmol) were reacted to give 5b; yield: 17.0 g (80%); white solid; mp 161–163 °C.

IR (KBr): 2957, 2934, 2872, 1671, 1387, 1253 cm⁻¹.

Anal. Calcd for C₂₅H₄₃N₅O₆ (509.73): C, 58.90; H, 8.52; N, 13.74. Found: C, 55.70; H, 7.64; N, 15.56.

References