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

Ping Xu,* Ting Zhang, Wenhao Wang, Xiaomin Zou, Xin Zhang, Yiqiu Fu

Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100083, P. R. China
Fax +861062015584; E-mail: pingxu@bjmu.edu.cn
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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Peptide nucleic acids (PNA) are oligonucleotide analogues in which the sugar-phosphate backbone has been replaced by a polyamide chain linked to nucleobases (Figure 1).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.

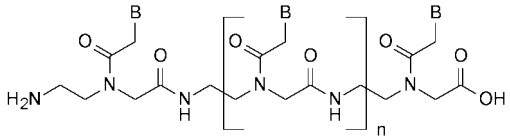

Figure 1 Structure of PNA; B = nucleobase

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 progressional 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.

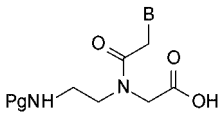

Figure 2 Structure of N-protected PNA monomer; Pg = protecting group

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 aminoethyl 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.

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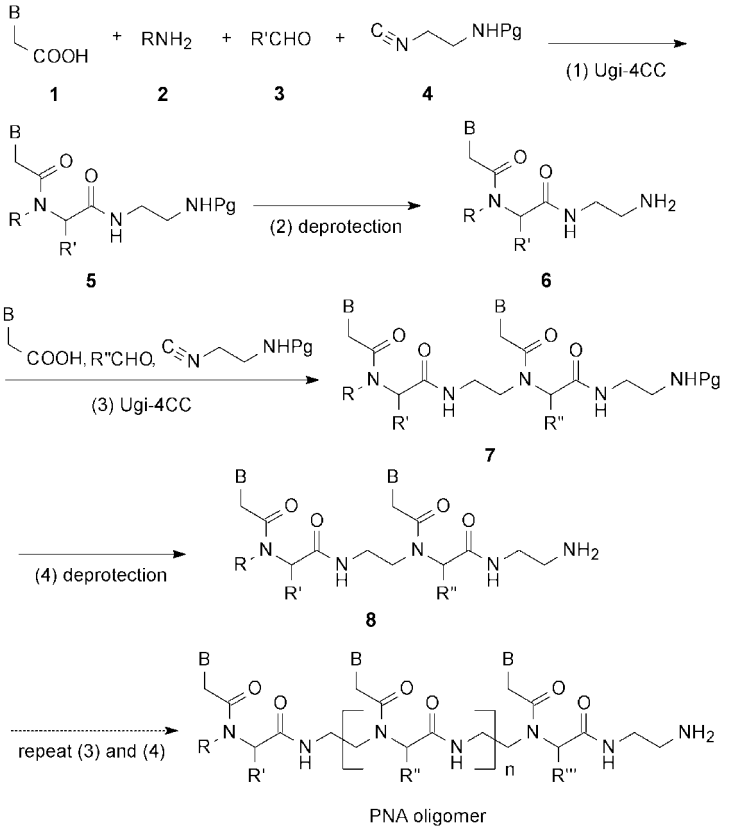
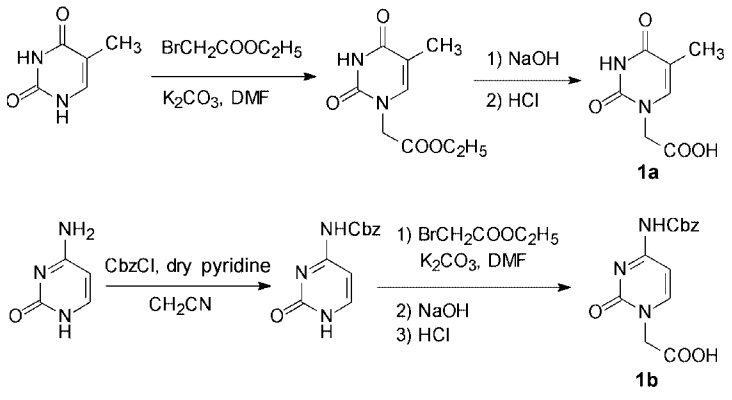


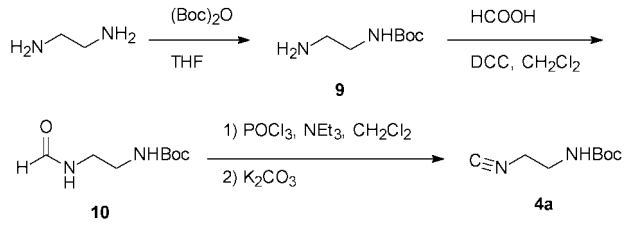
Scheme 1 Synthetic scheme of PNA monomer and chain extension; B = nucleobase, Pg = protecting group

For the purpose of examining the feasibility of the synthetic route, thymin-1-ylacetic acid (**1a**), [N^4-(benzyloxycarbonyl)cytosine-1-yl]acetic acid (**1b**), 3-methylbutylamine (**2a**), formaldehyde (**3a**), 3-methylbutanal (**3b**), and (*N*-*tert*-butyloxycarbonyl)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).[5]

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*-Boc)-ethylenediamine **9** was obtained conveniently with di-*tert*-butyl dicarbonate in moderate yield (70%) by following the procedure described.[9] The formylation of **9** was carried out by using HCO_2Et, $HCO_2C(=O)Me$, DCC/HCO_2H, respectively; and all gave the anticipated formylated product **10**. But DCC/HCO_2H 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 CH_2Cl_2.



Scheme 2 Synthetic route to thymine and cytosine acetic acids



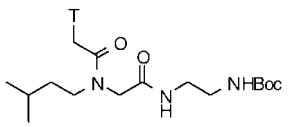
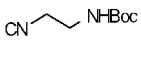
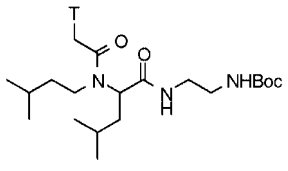
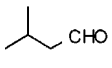
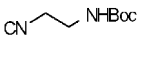
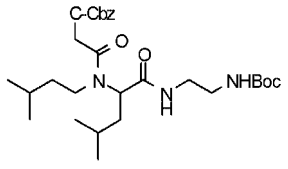
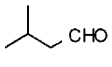
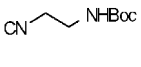
Scheme 3 Synthetic route to *N*-Boc-aminoethyl isocyanide

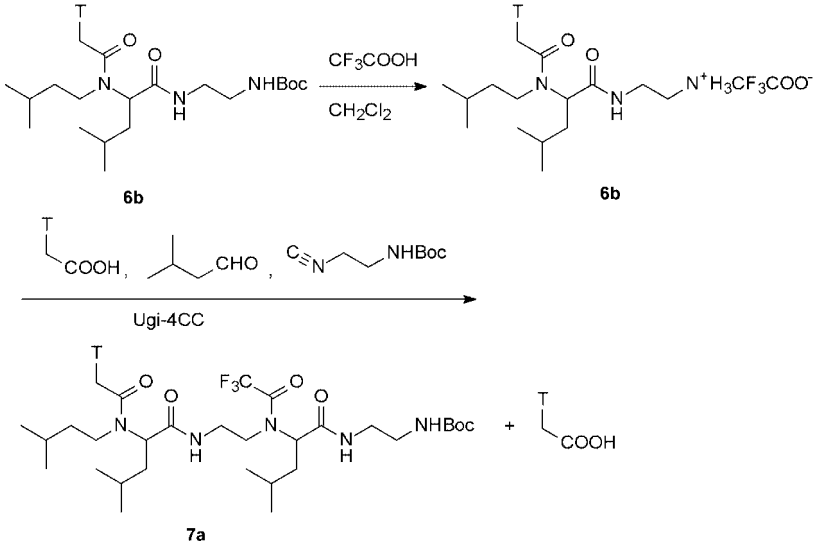
The preparation of isocyanides from their formamide precursors has been reviewed.[10] The $POCl_3/Et_3N$ method was used in this work to transform **10** to (*N*-Boc)-aminoethyl isocyanide **4a**. During the reaction, hydrogen chloride was produced, which may deprotect the acid labile Boc group. In fact 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 $POCl_3$ for 30 min instead of 1 h, then with K_2CO_3 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 ^{1}H NMR spectrum and the characteristic IR absorbance of isocyanide group at 2120 cm^{-1}.

Thymin-1-ylacetic acid (**1a**), [N^4-(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, ^{1}H 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% CF_3CO_2H–CH_2Cl_2 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

PNA monomers	Components			
	1	**2**	**3**	**4**
5a			HCHO	
5b				
5c				

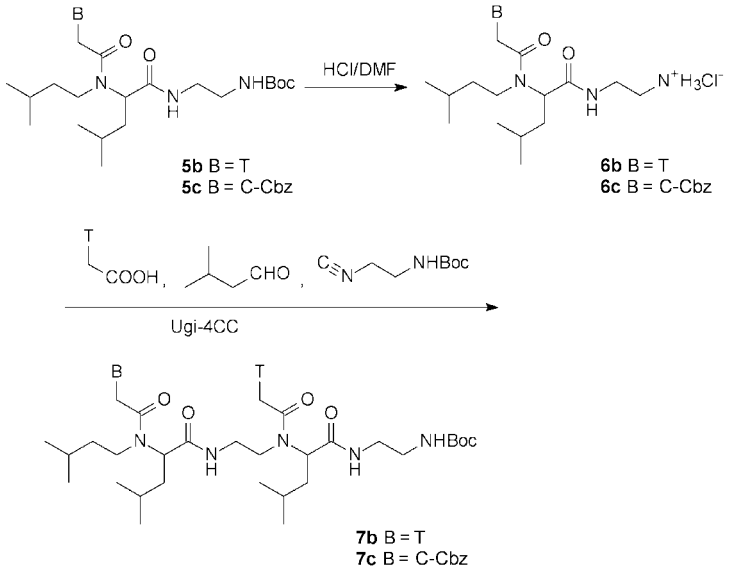


Scheme 4 Formation of compound **7a**; T = thymine

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



Scheme 5 Synthesis of PNA dimer

selected as four types of building blocks to construct derivatized 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 purification and the low yields. The solid-phase procedure is being studied to improve the method.

Unless otherwise mentioned, all the reagents were obtained commercially 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), ^{1}H NMR, MS and elemental analyses data were taken with PE-983, VXR-300S, VG-ZAB-HS, Carloerba-1106 instruments, respectively.

Thymin-1-ylacetic Acid (1a)

To a stirred solution of thymine (5.00 g, 0.04 mol) and K_2CO_3 (5.48 g, 0.04 mol) in anhyd DMF (120 mL) was added dropwise ethyl bromoacetate (4.35 mL, 0.04 mol) at r.t. under N_2, and the mixture was stirred for 20 h. After filtration, the filtrate was evaporated to dryness under reduced pressure. The residual solid was mixed thoroughly with H_2O (37.5 mL) and aq HCl (4 mol/L, 1.6 mL) at 0 °C, and the mixture was stirred for 30 min, and filtered to give ethyl thymin-1-ylacetate. This solid was washed with H_2O (3 × 20 mL), then mixed with H_2O (40 mL) and aq NaOH (1 mol/L, 20 mL), and boiled for 10 min. After cooling to 0 °C, aq HCl (4 mol/L, 13.5 mL) was added and the mixture was stirred for 30 min. After filtration, the filter cake was washed with H_2O (3 × 20 mL), and dried; yield: 4.53 g (65%); white powder; mp 260–262 °C (Lit.[5] mp not reported).

^{1}H NMR (DMSO-d_6): δ = 13.09 (s, 1 H, CO_2H), 11.33 (s, 1 H, CONH), 7.49 (s, 1 H, C=CH), 4.37 (s, 2 H, CH_2), 1.75 (s, 3 H, CH_3).

[*N*^{4}-(Benzyloxycarbonyl)cytosine-1-yl]acetic Acid (1b)

Benzyl chloroformate (52 mL, 0.36 mol) was added dropwise slowly to a suspension of cytosine (20.0 g, 0.18 mol) in anhyd pyridine (1000 mL) at 0 °C under N_2. The mixture was stirred for 20 h and evaporated under reduced pressure to remove the solvent. The solid residue was mixed thoroughly with H_2O (200 mL) and the pH was adjusted to 1 with concd HCl. The white solid was filtered off, washed with H_2O, and dried. The crude product was recrystallized from EtOH (500 mL) to give *N*^{4}-(benzyloxycarbonyl)cytosine.

***N*^{4}-(Benzyloxycarbonyl)cytosine**

Yield: 14.7 g (33%); white crystals; mp 270 °C (dec) (Lit.[5] mp >250 °C).

To a stirred suspension of the above prepared *N*^{4}-(benzyloxycarbonyl)cytosine (20.3 g, 83 mmol), and K_2CO_3 (11.4 g, 83 mmol) in anhyd DMF (230 mL) was added dropwise ethyl bromoacetate (8.9 mL, 83 mmol) at r.t. under N_2, and the mixture was stirred for 24 h. After filtration, the filtrate was evaporated under reduced pressure to dryness. The residual solid was mixed thoroughly with H_2O (80 mL) and aq HCl (4 mol/L, 3.1 mL) at 0 °C, and the mixture was stirred for 15 min, and filtered to furnish ethyl *N*^{4}-(benzyloxycarbonyl)cytosine-1-ylacetate. This solid was washed with H_2O (2 × 20 mL), then mixed with H_2O (120 mL) and aq NaOH (1 M, 620 mL), and stirred for 30 min at r.t., cooled to 0 °C, and filtered. Aq HCl (4 mol/L, 35 mL) was added, the precipitated white solid was filtered and recrystallized from MeOH (1000 mL) to give **1b**; yield: 7.76 g (31%); white crystals; mp 272–274 °C (Lit.[5] mp 266–274 °C).

1b

^{1}H NMR (DMSO-d_6): δ = 10.80 (s, 1 H, CONH, exchangeable with D_2O), 8.02 (d, 1 H, *J* = 7.0 Hz, =CH), 7.36 (m, 5 H, C_6H_5), 7.01 (d, 1 H, *J* = 7.0 Hz, =CH), 5.20 (s, 2 H, PhC*H*$_2$), 4.51 (s, 2 H, CH_2).

MS (FAB): *m*/*z* = 304 (M + 1).

(*N*-*tert*-Butyloxycarbonyl)ethylenediamine (9)

A solution of $(Boc)_2O$ (2.18 g, 10 mmol) in anhyd THF (10 mL) was added dropwise to a solution of ethylenediamine (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 solvent was removed by evaporation under reduced pressure. The residue was partitioned between CH_2Cl_2 (20 mL) and H_2O (5 mL) and the aqueous layer was washed with CH_2Cl_2 (2 × 5 mL). The CH_2Cl_2 solutions were combined and washed with 20% aq NaCl (2 × 5 mL), dried ($MgSO_4$), and evaporated to yield 2.4 g of a colorless oil. This crude materiel was purified by column chromatography on silica gel (CH_2Cl_2–EtOH–aq NH_3, 15:15:1) to give **9**; yield: 1.2 g (75%); colorless oil.

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

^{1}H NMR ($CDCl_3$): δ = 5.24 (s, 1 H, CONH, exchangeable with D_2O), 3.18 (t, 2 H, *J* = 5.85 Hz, NCH_2), 2.82 (t, 2 H, *J* = 5.85 Hz, NCH_2), 2.34 (d, 2 H, *J* = 13.8 Hz, NH_2, exchangeable with D_2O), 1.38 [s, 9 H, $C(CH_3)_3$].

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

(*N*-*tert*-Butyloxycarbonyl)-*N*′-formylethylenediamine (10)

Anhyd formic acid (0.075 mL, 2 mmol) was added to a solution of **9** (320 mg, 2 mmol) in anhyd CH_2Cl_2 (5 mL) cooled in an ice-bath, followed by addition of the solution of DCC (412 mg, 2 mmol) in anhyd CH_2Cl_2 (5 mL). The mixture was stirred for 3–4 h at r.t., and the solid dicyclohexylurea was filtered. The filtrate was washed with sat. aq $NaHCO_3$ (2 mL), dried ($MgSO_4$), and evaporated; yield: 340 mg (90%); white solid; mp 61–63 °C.

IR (KBr): 3324, 2927, 1668, 1530, 1271, 1247, 1168 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 8.13 (s, 1 H, HCO), 6.67 (s, 1 H, CONH), 5.14 (s, 1 H, CONH), 3.34 (m, 2 H, NCH_2), 3.23 (m, 2 H, NCH_2), 1.38 [s, 9 H, $C(CH_3)_3$].

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

Anal. Calcd for $C_8H_{16}N_2O_3$ (188.26): C, 51.04; H, 8.58; N, 14.88. Found: C, 51.12; H, 8.50; N, 14.80.

(*N*-*tert*-Butyloxycarbonyl)aminoethyl Isocyanide (4a)

To a solution of **10** (1.64 g, 8.7 mmol) in anhyd CH_2Cl_2 (13 mL) was added Et_3N (3.6 mL, 26.2 mmol) and to the mixture cooled in an ice-bath was added dropwise $POCl_3$ (0.82 mL, 8.7 mmol) and stirred for 30 min. A solution of K_2CO_3 (1.21g, 8.7 mmol) in H_2O (5.7 mL) was added to the mixture and stirred for another 30 min. The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The organic layer and the CH_2Cl_2 solutions were combined and washed with H_2O (2 × 5 mL), dried ($MgSO_4$), and evaporated under reduced pressure to yield a dark-brown cream. This crude materiel was purified 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, 2120, 1630, 1510, 1250 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 4.98 (s, 1 H, CONH), 3.54 (s, 2 H, NCH_2), 3.39 (s, 2 H, NCH_2), 1.46 [s, 9 H, $C(CH_3)_3$].

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

Anal. Calcd for $C_8H_{14}N_2O_2$ (170.24): C, 56.44; H, 8.30; N, 16.46. Found: C, 56.41; H, 8.32; N, 16.43.

PNA Monomer 5a; Typical Procedure

Paraformaldehyde (117 mg, 3.9 mmol), 3-methylbutylamine (0.49 mL, 4.2 mmol), **4a** (600 mg, 3.5 mmol) and **1a** (718 mg, 4.1 mmol) were mixed with isopropanol (4.2 mL) at r.t. and stirred for 48 h. The suspension was filtered, washed thoroughly with cold EtOH to give 1.18 g of a white solid. This crude product was dissolved in a

small amount of DMF, and purified by column chromatography on silica gel (eluent: CH_2Cl_2–EtOH, 30:1); yield: 1.06 g (67%); white solid; mp 170 °C.

^{1}H NMR (DMSO-d_6): δ = 11.23 (s, 1 H, NH, exchangeable with D_2O), 8.05 (s, 1 H, CONH, exchangeable with D_2O), 7.82 (s, 1 H, CONH), 7.41 (s, 1 H, =CH), 4.22 (s, 2 H, CH_2), 3.73 (t, 2 H, *J* = 6.4 Hz, NCH_2), 3.08 (t, 2 H, *J* = 6.4 Hz, NCH_2), 1.74 (s, 3 H, $=CCH_3$), 1.55 (m, 1 H, CH), 1.36 (s, 2 H, CH_2), 1.32–1.23 (m, 10 H, 2 CH_3, 2 CH_2), 0.84 [s, 9 H, $C(CH_3)_3$].

MS (FAB): *m*/*z* = 492 (M + K).

Anal. Calcd for $C_{21}H_{35}N_5O_6$ (453.61): C, 55.60; H, 7.79; N, 15.44. Found: C, 55.70; H, 7.64; N, 15.56.

5b

Following the above typical procedure, 3-methylbutanal (5.0 mL, 47 mmol), 3-methylbutylamine (6.0 mL, 52 mmol), **4a** (7.3 g, 43 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): 3346, 2958, 1680, 1395, 1367, 1250 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 11.30 (s, 1 H, CONH, exchangeable with D_2O), 8.22 (s, 1 H, CONH), 7.83 (s, 1 H, CONH), 7.39 (s, 1 H, =CH), 4.59 (s, 2 H, CH_2), 3.10–2.90 (m, 4 H, CH_2CH_2), 1.77 (s, 3 H, $=CCH_3$), 1.50–1.30 (m, 5 H, 3 CH, NCH_2), 1.37 [s, 9 H, $C(CH_3)_3$], 0.94–0.82 (m, 16 H, 4 CH_3, 2 CH_2).

MS (FAB): *m*/*z* = 510 (M + 1).

Anal. Calcd for $C_{25}H_{43}N_5O_6$ (509.73): C, 58.90; H, 8.52; N, 13.74. Found: C, 58.95; H, 8.50; N, 13.78.

5c

Following the above typical procedure, 3-methylbutanal (0.5 mL, 4.7 mmol), 3-methylbutylamine (0.55 mL, 4.7 mmol), **4a** (0.7 g, 4.1 mmol) and **1b** (1.5 g, 4.9 mmol) were reacted together to give **5c**; yield: 1.6 g (62%); white solid; mp 171–173 °C.

MS (FAB): *m*/*z* = 666 (M + K – 1).

Anal. Calcd for $C_{32}H_{48}N_6O_7$ (628.86): C, 61.11; H, 7.71; N, 13.37. Found: C, 61.54; H, 7.38; N, 13.41.

Deprotected PNA Monomer 6b; Typical Procedure

A solution of HCl in EtOAc (3 M, 0.33 mL) was added to a solution of **5b** (200 mg, 0.4 mmol) in DMF (3 mL) and the mixture was stirred at 80 °C for 3 h. After evaporation, the residual oil was triturated with Et_2O (10 mL) to obtain **6b** as a solid; yield: 168 mg (94%); pale yellow solid; mp 160–163 °C.

IR (KBr): 2957, 2934, 2872, 1671, 1387, 1253 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 11.31 (s, 1 H, CONH), 8.34 (s, 1 H, CONH), 7.72 (m, 3 H, NH_3^+, exchangeable with D_2O), 7.40 (s, 1 H, =CH), 4.58 (s, 2 H, CH_2), 3.41–3.22 (m, 4 H, CH_2CH_2), 1.75 (s, 3 H, CH_3), 1.58–1.46 (m, 5 H, 3 CH, NCH_2), 0.94–0.82 (m, 16 H, 4 CH_3, 2 CH_2).

MS (FAB): *m*/*z* = 410 (ammonium salt).

6c

Following the above typical procedure, **5c** (190 mg, 0.3 mmol) afforded **6c**; yield: 153 mg (90%); pale yellow solid; mp 173–175 °C.

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

PNA Dimer 7b; Typical Procedure

Pyridine (0.032 mL, 0.4 mmol) was added to a solution of **6b** (209 mg, 0.4 mmol) in DMF (4 mL), and allowed to stand overnight. 3-Methylbutanal (0.43 mL, 0.4 mmol), **4a** (68 mg, 0.4 mmol) and **1a** (147 mg, 0.8 mmol) were then added to this solution. After stirring at r.t. for 24 h, the condensed product **7b** was obtained from the reaction mixture by column chromatography on silica gel (eluent: CH_2Cl_2–EtOH, 30:1); yield: 103 mg (31%); white solid; mp 150–152 °C.

IR (KBr): 2929, 2858, 1671, 1388, 1255, 1095 cm^{-1}.

MS (FAB): *m*/*z* = 833 (M + 1).

Anal. Calcd for $C_{40}H_{65}N_9O_{10}$ (832.14): C, 57.73; H, 7.89; N, 15.15. Found: C, 57.79; H, 7.93; N, 15.18.

7c

Following the above typical procedure, **6c** (0.37 mmol), 3-methylbutanal (0.04 mL, 0.37 mmol), **4a** (63 mg, 0.37 mmol) and **1a** (130 mg, 0.74 mmol) were reacted togetrher to give **7c**; yield: 98 mg (28%); white solid; mp 157–159 °C.

MS (FAB): *m*/*z* = 952 (M + 1).

Anal. Calcd for $C_{47}H_{70}N_{10}O_{11}$ (951.27): C, 59.34; H, 7.43; N, 14.73. Found: C, 59.83; H, 7.39; N, 14.78.

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