

## Novel synthesis of $\alpha$ -PNA monomers by U-4CR

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Received January 31, 2007

Accepted March 21, 2007

Published online May 14, 2007; © Springer-Verlag 2007

**Summary.** A novel synthesis of  $\alpha$ -PNA monomers was carried out by U-4CR, followed by photochemical cleavage of the 2-nitrobenzyl group and selective hydrolysis in the presence of 10% HCl in THF. Three of four functional components in the U-4CR were specially protected: cyclohexenyl isocyanide, Boc for protecting the amino group of glycine, and 2-nitrobenzyl group as a photocage (photoremovable protecting group) for ammonia. The amino group of aldehyde-containing adenine is too weak to interfere with the U-4CR, so that it is not necessary to be protected.

**Keywords:** PNA – Multi-component reaction – Peptide

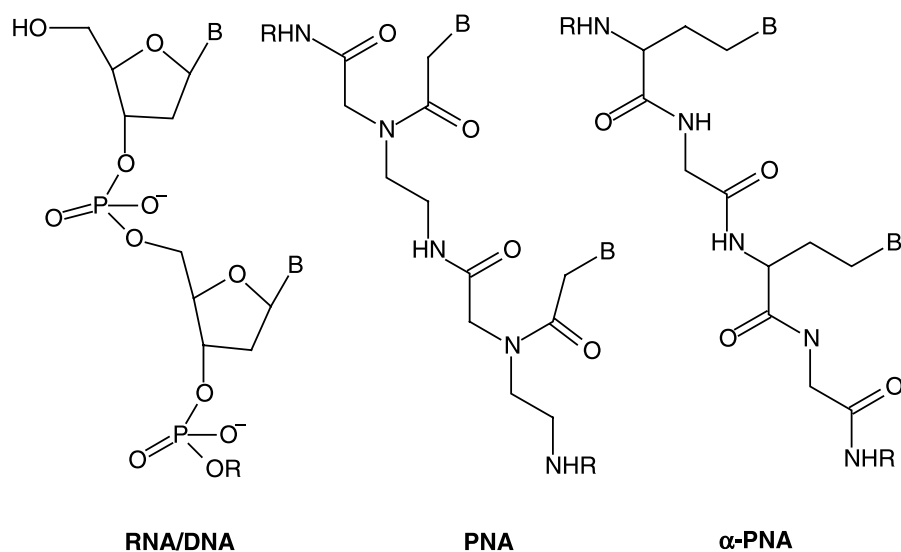
### Introduction

Modification of oligonucleotides for use in the recognition of specific DNA and RNA sequences has attracted a lot of interest and grown explosively in the last two decades (Kool, 1997; Uhlmann and Peyman, 1990). The modified oligonucleotides are of great interest in medicinal chemistry for the antisense and antigene therapy, and in molecular diagnostic strategies for identifying disease-related genes and pathogens (Braasch and Corey, 2002; De Mesmaeker, et al., 1995; Micklefield, 2001; Stein and Kreig, 1998). PNA (peptide nucleic acid), one type of modified oligonucleotides, was first created by Nielsen et al. and has sugar-phosphate backbone of DNA and RNA be completely substituted by a pseudo-peptide scaffold which is composed of *N*-(2-aminoethyl)glycine units, as shown in Fig. 1 (Nielsen et al., 1991). The purine or pyrimidine nucleobases of A/T/C/G are attached to the scaffold via carbonylmethyl spacers. The PNAs bind to complementary DNA/RNA with high sequence specificity and affinity to form duplexes via Watson-Crick base pairs and triplexes through Watson-Crick and Hoogsteen hydrogen bonding (Eriksson and Nielsen, 1996; Egholm

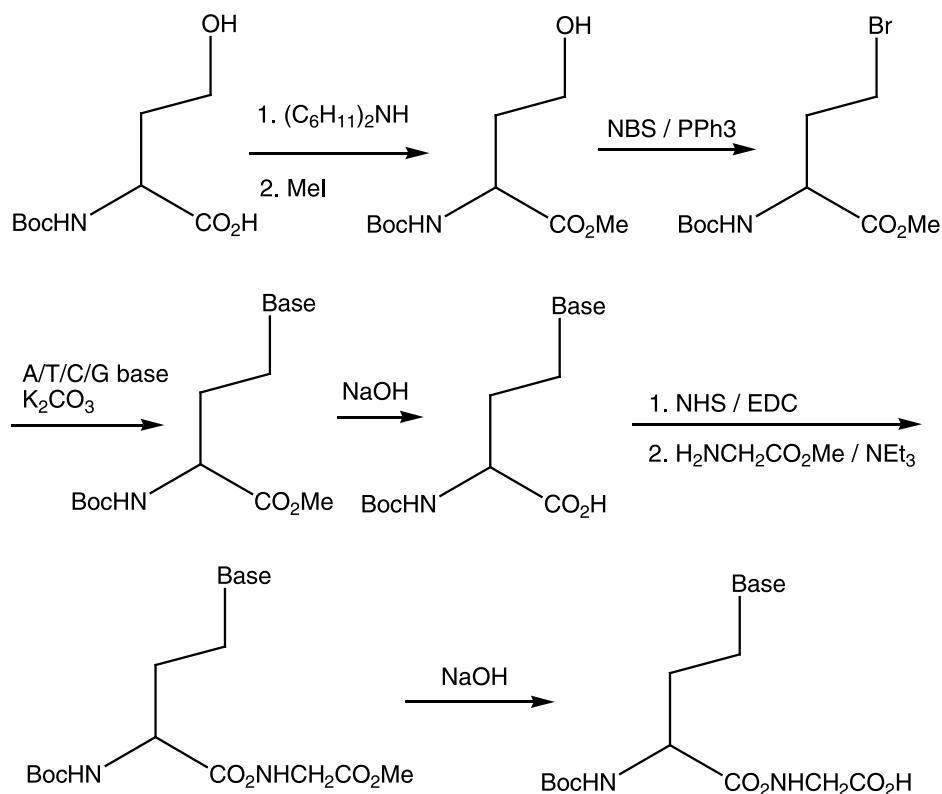
et al., 1993; Wittung et al., 1994). The PNAs show good stability to proteases and nucleases, and complexes of PNA:RNA and PNA:DNA demonstrate higher thermal stability than analogous DNA and RNA complexes (Demidov, 1994). In addition, PNAs demonstrated both good antisense and antigene activity according to in vitro studies (Bonham et al., 1995; Good, 1997; Nielsen 1994).

Attempts to understand the structure-activity relationship of PNA and improving its solubility, cell permeability, and binding orientation with RNA and DNA have resulted in several structural modifications of PNA (Ganesh and Nielsen, 2000; Kumar, 2002). These comprise ligand conjugation, introduction of chirality in the achiral PNA backbone and modifications in conformationally preorganizing the PNA strand to entropically drive the complex formation. As shown in Fig. 1,  $\alpha$ -PNA, a PNA analogue, is a true peptide mimic of DNA and involve  $\alpha$ -amino acids with side chains comprising the DNA bases attached via an ethylene linkage and interspaced with two glycines (Howarth and Wakelin, 1997; Lenzi et al., 1995).

Howarth and Wakelin (1997) prepared monomers of  $\alpha$ -PNA, as shown in Scheme 1, by coupling glycine methyl ester with nucleobase-containing amino acids which were prepared from *N*-Boc-L-homoserine, via alkylation of nucleobase with the key intermediate (*S*)-2-(*N*-Boc-amino)-4-bromobutyric acid methyl ester followed by hydrolysis. Lenzi et al. (1995) used the similar method to synthesize monomers of  $\alpha$ -PNA, too. Since adenine pairs with uracil found in RNA, in this article we show novel synthesis of adenine- and uracil-containing  $\alpha$ -PNA monomers by the U-4CR.



**Fig. 1.** Structures of DNA/RNA, PNA and α-PNA



**Scheme 1**

## Materials and methods

### Chemicals

All the reagents were obtained from commercial suppliers and used as received. Cyclohexenyl isocyanide and nucleobase-containing aldehydes were prepared according to the literature methods (Keating and Armstrong, 1996; Martinez and Lee, 1965).

### General procedure to prepare 2

A nucleobase-containing aldehyde (1 mmol), a 2-nitrobenzylamine (1 mmol), and 5 ml of methanol were mixed in a 10-ml quartz flask until the mixture became a clear solution. Cyclohexenyl isocyanide (1 mmol) and *N*-Boc-glycine (1 mmol) were added into the solution and the mixture was stirred at room temperature for 2 days. Then methanol was evaporated from the mixture by a rotary evaporator. The residue was dissolved by

acetone and the mixture was irradiated inside a Rayonet photochemical reactor with four 300 nm lamps at room temperature. After 6–12 h, acetone was evaporated by a rotary evaporator and the residue was purified by column chromatography to get the dipeptides **2a** or **2b**.

**2a**: Light yellow oil; yield 77%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  = 1.33 (9H, s,  $\text{CH}_3$ ), 1.46–1.63 (4H, m,  $\text{CH}_2$ ), 1.97–2.04 (4H, m,  $\text{CH}_2$ ), 3.45–3.47 (2H, m,  $\text{CH}_2$ ), 3.63–3.69 (1H, m, CH), 3.93–4.05 (1H, m, CH), 4.57–4.59 (1H, m, CH), 5.43 (1H, d,  $J$  = 7.9 Hz, CH), 5.93 (1H, m, CH), 7.52 (1H, d,  $J$  = 7.9 Hz, CH) ppm;  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  = 22.03, 22.46, 23.90, 27.37, 28.56, 43.73, 49.25, 52.12, 78.64, 100.97, 112.52, 133.52, 146.14, 151.31, 156.25, 164.10, 167.42, 169.94 ppm; IR (thin film): 1686, 1648 ( $\text{C}=\text{O}$ )  $\text{cm}^{-1}$ ; HRMS (EI,  $\text{M}^+$ )  $m/z$  calc. for  $\text{C}_{20}\text{H}_{29}\text{N}_5\text{O}_6$  435.2117, found 435.2120.

**2b**: White solid, mp 126–129 °C; yield: 41%;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  = 1.32 (9H, s,  $\text{CH}_3$ ), 1.43–1.56 (4H, m,  $\text{CH}_2$ ), 2.01–2.03 (4H, m,  $\text{CH}_2$ ), 3.48–3.83 (2H, m,  $\text{CH}_2$ ), 4.38–4.43 (1H, m, CH), 4.79–4.84 (1H, m, CH), 4.96 (1H, m, CH), 5.88 (1H, m, CH), 7.84 (1H, m, ArH), 8.34 (1H, s, ArH);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  = 21.84, 22.44, 24.00, 27.00, 28.13, 45.05, 46.18, 54.61, 80.97, 115.20, 119.56, 132.45, 142.61, 150.38, 152.95, 155.72, 156.40, 166.25, 170.23; IR (thin film): 1682, 1651 ( $\text{C}=\text{O}$ )  $\text{cm}^{-1}$ ; HRMS (EI,  $\text{M}^+$ )  $m/z$  calc. for  $\text{C}_{21}\text{H}_{30}\text{N}_8\text{O}_4$  458.2390, found 458.2382.

#### General procedure to prepare **3**

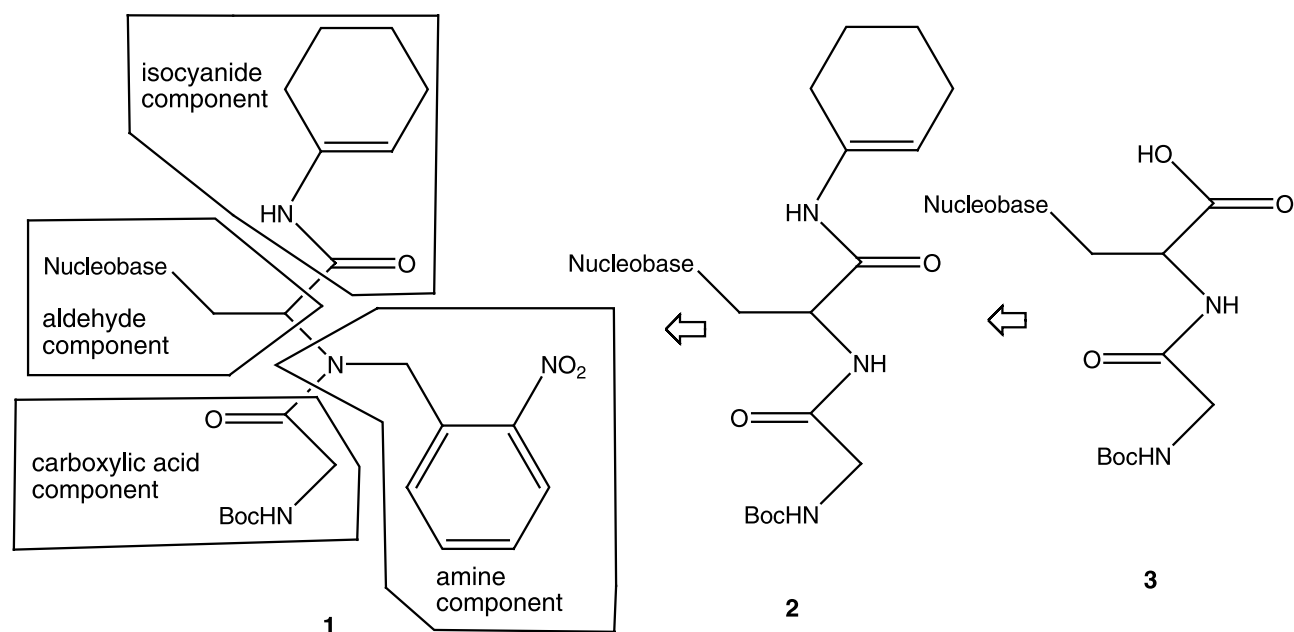
Dipeptide **2a** or **2b** was dissolved in a solution of 1 ml concentrated HCl and 9 ml THF at 0 °C. The solution was stirred for 5 h at 0 °C. Solid  $\text{Na}_2\text{CO}_3$  was added for neutralization and the solution was filtered. The filtrate was evaporated to dryness by a rotary evaporator and the residue was purified by column chromatography to get the *N*-protected  $\alpha$ -PNA monomers **3a** or **3b**.

**3a**: Light brown oil; yield 77%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  = 1.36 (9H, s,  $\text{CH}_3$ ), 3.48–3.53 (2H, m,  $\text{CH}_2$ ), 3.62–3.66 (1H, m, CH), 3.94–3.97 (1H, m, CH), 4.52–4.54 (1H, m, CH), 5.43 (1H, d,  $J$  = 7.9 Hz, CH), 7.47 (1H, d,  $J$  = 7.9 Hz, CH) ppm;  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  = 28.56, 43.86, 48.14, 51.59, 78.67, 100.93, 146.28, 151.32, 156.29, 164.13, 167.89, 170.04 ppm; IR (thin film): 1682, 1650 ( $\text{C}=\text{O}$ )  $\text{cm}^{-1}$ .

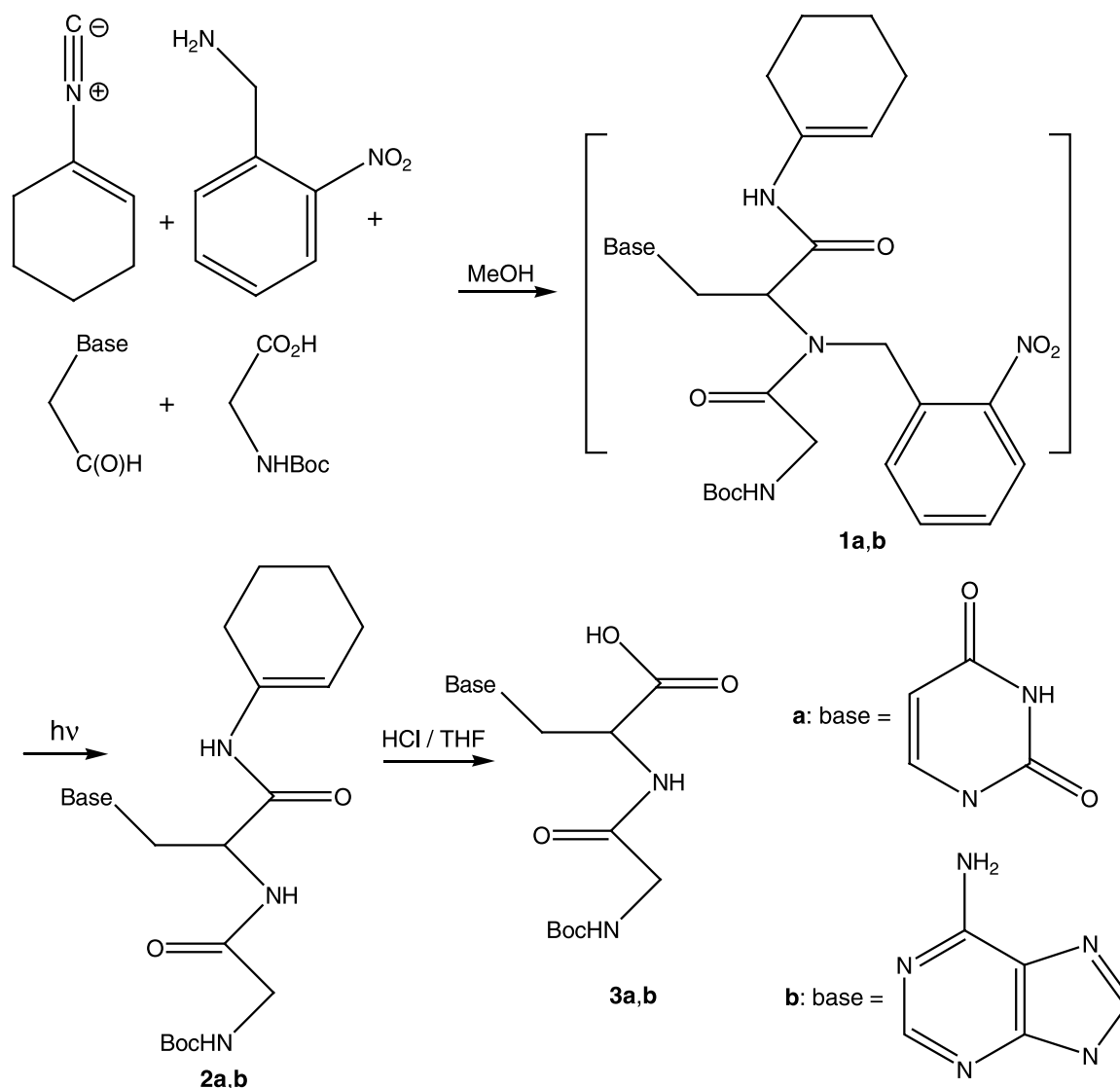
**3b**: Light brown solid, mp 195–198 °C; yield 83%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  = 1.33 (9H, s,  $\text{CH}_3$ ), 3.59–3.61 (2H, m,  $\text{CH}_2$ ), 4.46 (2H, m,  $\text{CH}_2$ ), 4.63 (1H, m, CH), 7.94 (1H, s, ArH), 8.17 (1H, s, ArH) ppm;  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  = 28.47, 44.10, 44.73, 53.08, 78.83, 118.80, 141.82, 149.98, 152.93, 156.31, 156.42, 170.18, 171.14 ppm; IR (thin film): 1684, 1653 ( $\text{C}=\text{O}$ )  $\text{cm}^{-1}$ ; HRMS (EI,  $\text{M}^+$ -H)  $m/z$  calc. for  $\text{C}_{15}\text{H}_{20}\text{N}_7\text{O}_5$  378.1525, found 378.1541.

## Results and discussion

We used the U-4CR to synthesize monomers of  $\alpha$ -PNA. The U-4CR is a multi-component reaction (MCR) which needs four functional groups to work on: isocyanide, amine, carboxylic acid, and aldehyde. As shown in Scheme 2, retrosynthesis of  $\alpha$ -PNA monomers was planned. The *N*-cyclohexenyl amide in **1** comes from isocyanide component of the U-4CR and it can be selectively hydrolyzed into the corresponding carboxylic acid in a mild acidic condition (Keating and Armstrong, 1996) in the presence of *N*-Boc group in **2**, so cyclohexenyl isocyanide was used in the U-4CR. Ammonia as an amine component in the U-4CR was not successful, so several approaches were proposed to solve the problem (Costa et al., 2003; Kazmaier and Christina, 2003; Kunz and Pfrengle, 1988; Kunz et al., 1989; Lehnhoff et al., 1995; Linderman et al., 1999; Oertel et al., 2000; Pick et al., 2005; Siegmüller and Ugi, 1986; Urban and Ugi, 1975; Ziegler et al., 1999). In this article, we use 2-nitrobenzyl amine as ammonia equivalent in the U-4CR, followed by photochemical cleavage of 2-nitrobenzyl group (Sung et al., 2006). Glycine provides the



Scheme 2



Scheme 3

carboxylic acid component in the U-4CR, but its amino group needs to be protected. The Boc was chosen to protect the amino group of glycine because it is not deprotected at the hydrolysis condition of *N*-cyclohexenyl amide but it can be deprotected at the condition that peptide bonds remain intact. An aldehyde group with attachment of a nucleobase serves as an aldehyde component in the U-4CR.

As shown in Scheme 3, after cyclohexenyl isocyanide, 2-nitrobenzyl amine, *N*-Boc-glycine, and nucleobase-containing aldehyde were mixed in methanol at room temperature for 2 days, crude dipeptide **1** was formed in good yield. We then investigated whether the amino group of aldehyde-containing adenine interferes with the U-4CR. What we found is that reactivity of the amino group in

adenine is much smaller than that of 2-nitrobenzyl amine, so it does not interfere with the U-4CR and it is not necessary to protect the amino group of adenine. Without isolation of **1**, we tried to photochemically cleave 2-nitrobenzyl group in the solvent of methanol with 254 nm of UV light, but degradation became significant in a long reaction time. Therefore, we replaced the solvent of methanol with acetone, which usually serves as a photosensitizer, and did the photochemical cleavage with 300 nm of UV light. The product **2** was produced in a good yield.

Selective hydrolysis of *N*-cyclohexenyl amide moiety of **2** depends on concentration of HCl, reaction temperature, and reaction time. We found that hydrolysis of **2** in 10% HCl of THF solution at 0 °C for 5 h is the best condition, so that *N*-cyclohexenyl is hydrolyzed but *N*-Boc remains

intact. After the selective hydrolysis at the acidic condition,  $\alpha$ -PNA monomer **3** was produced in a good yield.

## Conclusion

Special strategies were adopted in protecting three of four functional components of the U-4CR: cyclohexenyl isocyanide, Boc for protecting the amino group of glycine, and 2-nitrobenzyl group as a photocage for ammonia. The amino group of aldehyde-containing adenine is not reactive enough to interfere with the U-4CR, so that it is not necessary to be protected. By means of this special design for the U-4CR, we carried out novel synthesis of two complementary  $\alpha$ -PNA monomers in two pots.

## Acknowledgment

Financial support from the National Science Council of Taiwan (NSC 94-2113-M-006-009) is gratefully acknowledged.

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