

# Cyclobutyl-carbonyl substituted PNA: synthesis and study of a novel PNA derivative

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**Abstract**—A new, optically active, cyclobutyl-carbonyl substituted PNA monomer has been synthesized stereoselectively from a chiral amino acid prepared from (+)- $\alpha$ -pinene. A conformational search shows a lack of conformational bias for the monomer and incorporation of the monomer into a standard oligomer is tolerated without changing the binding affinity towards sequence complementary RNA, DNA or PNA targets.

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## 1. Introduction

Peptide nucleic acids (PNA), which were invented in 1990, are nucleic acid mimics in which the sugar-phosphate backbone is replaced with an uncharged, achiral pseudo peptide backbone. PNAs hybridize to complementary DNA and RNA with a high affinity and sequence specificity. These properties along with stability to proteases and nucleases make PNA a promising molecule for development as gene-targeted drugs (antigene and antisense) and for DNA diagnostic applications.<sup>1</sup>

The aminoethylglycine backbone of PNA is inherently flexible, and consequently a significant entropic loss is associated with duplex formation. Restricting rotation about the C2–C3 bond could decrease this entropic loss and increase the hybridization kinetics. Bulky substituents or ring structures have been introduced to conformationally rigidify the backbone and to preorganize the PNA strand for attaining a hybridization competent conformation. In this way, differently substituted five- and six-membered rings have been used to provide a conformational lock with varied success in terms of DNA/RNA recognition.<sup>2</sup> Furthermore, functional substituents in the PNA backbone have

produced PNA oligomers with increased bioavailability,<sup>3</sup> improved sequence discrimination,<sup>4</sup> and may also be of interest in combinatorial (aptamer) approaches using PNA.<sup>5</sup> So far only side chains from natural amino acids<sup>6</sup> and carbohydrates have been introduced.

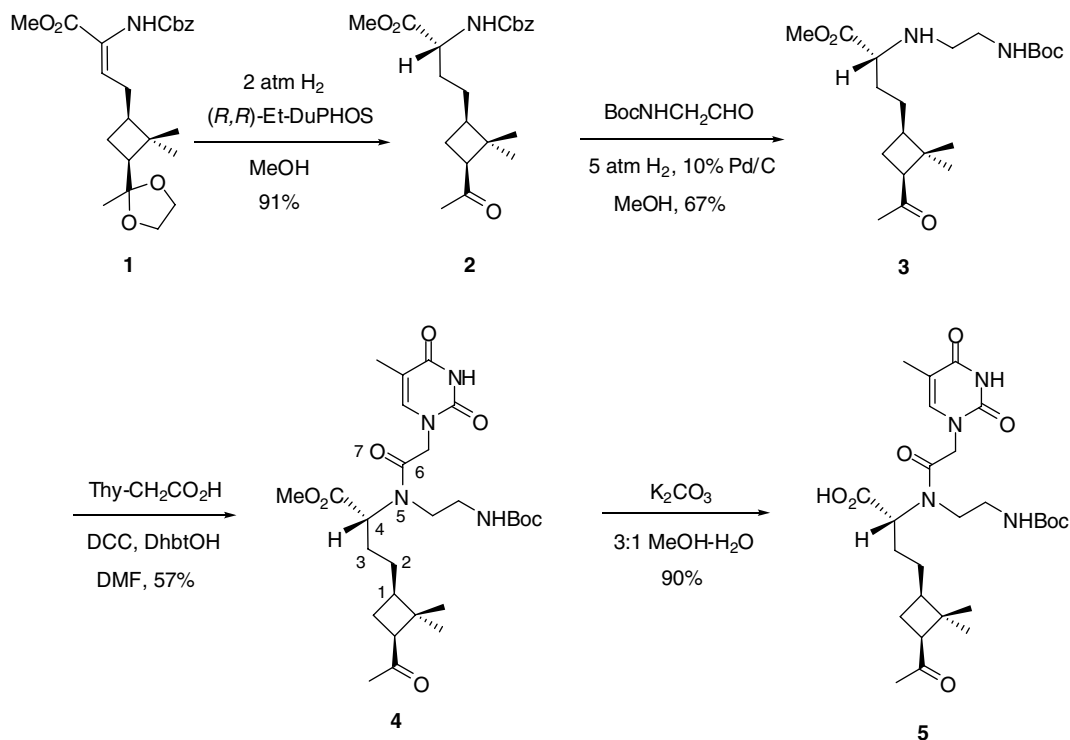
In this paper, we present the stereoselective synthesis of the first cyclobutane-carbonyl-containing PNA monomer, in enantiomerically pure form. Theoretical calculations were carried out to study its conformational rigidity. This monomer was incorporated into a decamer and DNA/RNA hybridization studies using UV- $T_m$  are reported.

## 2. Results and discussion

### 2.1. Monomer synthesis

The synthesis of the *N*-Boc protected monomer is depicted in **Scheme 1**. Dehydro amino acid **1**, easily prepared from commercial (+)- $\alpha$ -pinene,<sup>7</sup> was hydrogenated under 2 atm of pressure in the presence of (*R,R*)-Et-DuPHOS<sup>8</sup> as a catalyst and using MeOH as a solvent.<sup>9</sup> Thus, the new (*R*)-amino acid **3** was obtained as a single stereoisomer, in a 91% yield and >99.9 de. Compound **3** was prepared in a 67% yield through reductive amination<sup>10</sup> by means of in situ reaction between *N*-Boc-2-aminoacetaldehyde<sup>11</sup> and the amine resulting from the hydrogenolysis of benzyl

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Scheme 1.

carbamate in **2**. The coupling of amine **3** with commercially available 2-(thymine-1-yl)acetic acid, in the presence of DCC and DhbtOH, in DMF afforded compound **4**, which was submitted to saponification with  $K_2CO_3$  in 3:1 MeOH–H<sub>2</sub>O to provide monomer **5**, suitably protected for incorporation into oligomers by solid phase synthesis.

## 2.2. Conformational study

A conformational study<sup>12–14</sup> was undertaken on **4** to examine if any conformational bias is present in this molecule. 1982 structures with energies within a range of 3 kcal mol<sup>-1</sup> with respect to the absolute minimum were analyzed. They can be classified into four groups: **4(a)**, **4(b)**, **4(c)** and **4(d)**. The corresponding relative energies are shown in Table 1.

Table 1. Relative energies for conformers of **4**

Conformer <sup>a</sup>	<i>E</i> <sup>b</sup>
<b>4(a)</b>	0.0
<b>4(b)</b>	0.8
<b>4(c)</b>	0.9
<b>4(d)</b>	1.0

<sup>a</sup> See Figure 1.

<sup>b</sup> In kcal mol<sup>-1</sup>.

Figure 1 shows these structures. It is to be noted that no intra-molecular hydrogen bond is detected in any of them. The four structures present an anti arrangement around the C<sub>2</sub>–C<sub>3</sub> bond with a C<sub>1</sub>–C<sub>2</sub>–C<sub>3</sub>–C<sub>3</sub> dihedral angle in the 168–183° range (see Scheme 1 for atom numeration).

The conformation around the C<sub>3</sub>–C<sub>4</sub> bond is gauche in all cases, with C<sub>2</sub>–C<sub>3</sub>–C<sub>4</sub>–N<sub>5</sub> dihedral angles in the range 50–70° for all structures except **4(c)**, where this dihedral angle is 140°. The conformation around C<sub>4</sub>–N<sub>5</sub> is gauche for the three lowest energy structures (C<sub>3</sub>–C<sub>4</sub>–N<sub>5</sub>–C<sub>6</sub> dihedral angle around 50–60°) and anti for **4(d)**. The N<sub>5</sub>–C<sub>6</sub> amide bond presents a trans conformation for **4(a)** and **4(c)** and a cis arrangement for **4(b)** and **4(d)**.

For each structure, molecular dynamics at 300 K in chloroform have been performed and the evolution of all dihedral angles has been monitored. Dihedral angles corresponding to torsions around C<sub>2</sub>–C<sub>3</sub>, C<sub>3</sub>–C<sub>4</sub>, C<sub>4</sub>–N<sub>5</sub> and N<sub>5</sub>–C<sub>6</sub> vary within a small range. The dihedral angle corresponding to the torsion around C<sub>1</sub>–C<sub>2</sub> oscillates between two different values (around –70° and –170°). Finally, the remaining dihedral angles show almost free rotation.

## 2.3. Oligomer synthesis and binding

To evaluate DNA and RNA recognition of the cyclobutyl-carbonyl PNA analogue, two decamers were synthesized:

**PNA1: Ac-G-T-A-G-A-T-C-A-C-T-LysNH<sub>2</sub>**

**PNA2: Ac-G-T-A-G-A-(5)-C-A-C-T-LysNH<sub>2</sub>**

The binding to complementary RNA, DNA and PNA oligomers was analyzed by the determination of thermal stability (*T<sub>m</sub>*) of the corresponding duplexes (Table 2). The results show that incorporation of the new monomer does not significantly alter binding to complementary DNA, PNA or RNA, indicating that the bulky cyclobu-

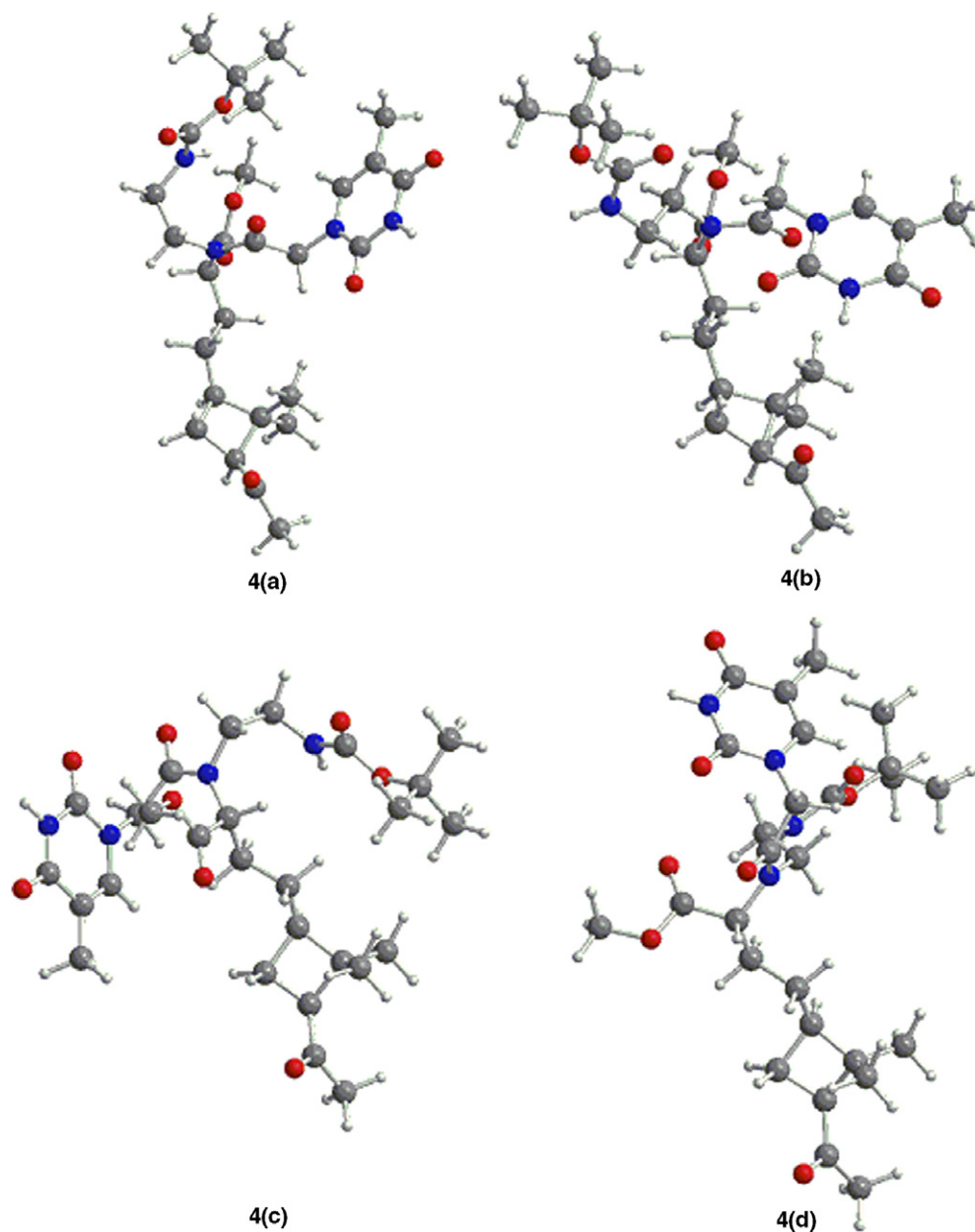


Figure 1. Structures obtained in the conformational search of 4.

Table 2. Thermal stabilities ( $T_m$  values)<sup>a</sup>

Entry	Sequence	DNA <sup>b</sup>	RNA <sup>c</sup>	PNA <sup>d</sup>
1	PNA1	54 <sup>e</sup>	57	72
2	PNA2	54 <sup>e</sup>	57 <sup>f</sup>	70 <sup>f</sup>

<sup>a</sup>  $T_m$  = melting temperature (measured in medium salt buffer: 100 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH 7.0). Heating rate: 1 K/min. UV absorbance measured at 260 nm.

<sup>b</sup> Complementary DNA sequence: 5'-d(AGTGATCTAC)-3'.

<sup>c</sup> Complementary RNA sequence: 5'-d(AGUGAUCUAC)-3'.

<sup>d</sup> Complementary PNA sequence: 5'-d(AGTGATCTAC)-3'.

<sup>e</sup>  $T_m$  measured from 10 to 95 °C.

<sup>f</sup>  $T_m$  measured from 5 to 95 °C.

tyl-carbonyl substituent does not affect the conformation of the PNA backbone required for hybridization.

These results provide a route for novel backbone substituted PNA oligomers that could be of importance for optimizing cellular targeting and delivery and in vivo bioavailability<sup>3,15</sup> and for future aptamer approaches using PNA.<sup>4</sup>

### 3. Experimental

#### 3.1. Computational details

The Monte Carlo conformational search<sup>12</sup> has been done using the MMFFs force field<sup>13</sup> implemented in the Macro-model program.<sup>14</sup> The molecular dynamics simulations have been done using a time step of 1.5 fs. The equilibration time has been 250 ps and the simulation time 1 ns.

The solvation by chloroform has been taken into account through the Generalized Born/Surface Area (GB/SA) method.<sup>16</sup>

### 3.2. Methyl (1'*R*,2*R*,3'*S*)-2-benzoyloxycarbonylamino-4-(3'-acetyl-2',2'-dimethylcyclobutyl)butanoate, 2

A mixture of substrate **1** (600 mg, 1.6 mmol), prepared according to Ref. 7, and [(COD)Rh(*R,R*)-Et-DuPHOS]-OTf, (*R,R*)-Et-DuPHOS, (38.4 mg) in EtOH was stirred under a hydrogen atmosphere at 2 atm pressure for 4 days. The solvent was removed and the residue was chromatographed (1:1 CH<sub>2</sub>Cl<sub>2</sub>-EtOAc as eluent) to afford saturated **2** (548 mg, 91% yield) in >99.9 de as an oil.  $[\alpha]_D^{20} = +20.2$  (*c* 1.4, MeOH). IR (film) 3333 (br), 1699 (br) cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$  0.80 (s, 3H), 1.28 (s, 3H), 1.31–2.21 (complex absorption, 7H), 1.97 (s, 3H), 2.87 (dd, *J* = 7.7 Hz, *J'* = 9.8 Hz, 1H), 3.67 (s, 3H), 4.19 (m, 1H), 5.03–5.09 (complex absorption, 2H), 6.71 (d, *J* = 7.7 Hz, 1H), 7.27–7.36 (complex absorption, 5H). <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>)  $\delta$  17.14, 23.75, 26.88, 30.09, 30.69, 30.56, 42.26, 43.51, 52.19, 54.20, 55.03, 66.69, 128.56, 128.60, 129.16, 138.11, 156.99, 173.50, 206.48. Anal. Calcd for C<sub>21</sub>H<sub>29</sub>NO<sub>5</sub>: C, 67.18; H, 7.79; N, 3.73. Found; C, 66.78; H, 7.78; N, 3.65.

### 3.3. Methyl (1'*R*,2*R*,3'*S*)-2-[2''-*tert*-butoxycarbonylamino-1''-[(thymine-1-yl)acetyl]aminoethyl]-4-(3'-acetyl-2',2'-dimethylcyclobutyl)butanoate, 4 through compound 3

A mixture containing compound **2** (366 mg, 0.89 mmol), *N*-Boc-2-aminoacetaldehyde (214 mg, 1.3 mmol) and 10% Pd/C (30 mg) in 16 mL MeOH was stirred under a hydrogen atmosphere at 5 atm pressure for 6 h. The mixture was then filtered through *Celite* and washed exhaustively with MeOH. The solvent was removed under vacuo to afford crude **3** (230 mg, 67% yield), which was used immediately in the next step without additional purification.

DCC (94.5 mg, 0.46 mmol) was added to a solution of 2-(thymine-1-yl)acetic acid (84.3 mg, 0.46 mmol) and DhbtOH (74.7 mg, 0.46 mmol) in 0.8 mL dry DMF, and the mixture was stirred under a nitrogen atmosphere at room temperature for 40 min. Then a solution of compound **3** (110 mg, 0.29 mmol) in 1.3 mL dry DMF was added and the resultant mixture was stirred overnight. The reaction mixture was filtered and the solvent was removed at a reduced pressure. The residue was poured into 10 mL water and extracted with ethyl acetate (4 × 5 mL). The organic layers were washed with saturated aqueous NaHCO<sub>3</sub> (1 × 10 mL) and this aqueous phase was extracted with ethyl acetate (2 × 5 mL). The combined organic extracts were dried over MgSO<sub>4</sub> and the solvent was removed under vacuo. Crude **4** was purified by column chromatography (4:1 EtOAc-CH<sub>2</sub>Cl<sub>2</sub>) to afford 90 mg (57% yield) of the pure compound as a colourless oil.  $[\alpha]_D^{20} = +33.0$  (*c* 1.0, MeOH). IR (film) 2591 (br), 1667 (br) cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, acetone-*d*<sub>6</sub>)  $\delta$  for the major rotamer, 0.80 (s, 3H), 1.28 (s, 3H), 1.26–2.10 (complex absorption, 7H), 1.41 (6, 9H), 1.81, (s, 3H), 1.97 (s, 3H), 2.71–2.99 (m, 1H), 3.30–3.77 (complex absorption, 4H), 4.30 (dd, *J* = 5.9 Hz, *J'* = 8.7 Hz, 1H), 4.63–4.81 (complex

absorption, 2H), 6.14 (s, 1H), 7.23 (br s, 1H), 9.91 (br s, 1H). <sup>13</sup>C NMR (62.5 MHz, acetone-*d*<sub>6</sub>)  $\delta$  for the major rotamer, 11.35, 16.30, 22.93, 26.84, 27.68, 29.20, 29.92, 39.21, 41.58, 42.68, 47.07, 48.09, 51.41, 53.37, 59.60, 78.37, 108.75, 141.78, 150.99, 155.93, 164.03, 167.40, 171.21, 206.11. HRMS (EI, 70 eV), calcd for C<sub>27</sub>H<sub>42</sub>N<sub>4</sub>O<sub>8</sub> [M]: 550.3004; experimental 550.3003. Calcd for C<sub>22</sub>H<sub>34</sub>N<sub>4</sub>O<sub>6</sub> [M-C<sub>5</sub>H<sub>8</sub>O<sub>2</sub>]: 450.2478; experimental 450.2478.

### 3.4. (1'*R*,2*R*,3'*S*)-2-[2''-*tert*-Butoxycarbonylamino-1''-[(thymine-1-yl)acetyl]aminoethyl]-4-(3'-acetyl-2',2'-dimethylcyclobutyl)butanoic acid, 5

Ester **4** (140 mg, 0.25 mmol) was added to a solution of K<sub>2</sub>CO<sub>3</sub> (174 mg, 1.3 mmol) in 3:1 MeOH-H<sub>2</sub>O (2.4 mL) and the resulting mixture was stirred at room temperature for 12 h. Methanol was evaporated and the aqueous layer was washed with ether and, subsequently, 5% HCl was added to reach a pH of 2. The acid aqueous phase was extracted with ether (5 × 5 mL). The combined organic extracts were dried over MgSO<sub>4</sub> and the solvent was removed at reduced pressure to afford acid **5** as a solid that was purified by crystallization (33 mg, 24% yield). Crystals, mp 115–121 °C (from ether-pentane).  $[\alpha]_D^{20} = +14.5$  (*c* 1.1, MeOH). IR (film) 3500–2900 (br), 2952 (br), 1672 (br) cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, acetone-*d*<sub>6</sub>)  $\delta$  for the major rotamer, 0.80 (s, 3H), 1.28 (s, 3H), 1.22–2.10 (complex absorption, 7H), 1.41 (s, 9H), 1.80 (s, 3H), 1.97 (s, 3H), 2.72–3.80 (complex absorption, 5H), 4.26–4.41 (m, 1H), 4.59–4.82 (complex absorption, 2H), 6.20 (s, 1H), 7.27 (br s, 1H), 10.02 (br s, 1H). <sup>13</sup>C NMR (62.5 MHz, methanol-*d*<sub>4</sub>)  $\delta$  for the major rotamer, 12.20, 17.35, 24.83, 28.20, 28.75, 30.23, 30.89, 40.26, 43.03, 44.48, 48.67, 49.84, 55.04, 62.26, 80.58, 110.84, 143.74, 152.91, 158.34, 166.98, 169.59, 174.22, 210.95. FAB<sup>+</sup>MS: 559.29 (M+Na<sup>+</sup>).

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