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Synthesis of Peptoid Nucleic Acid with Thymine as Nucleic Base

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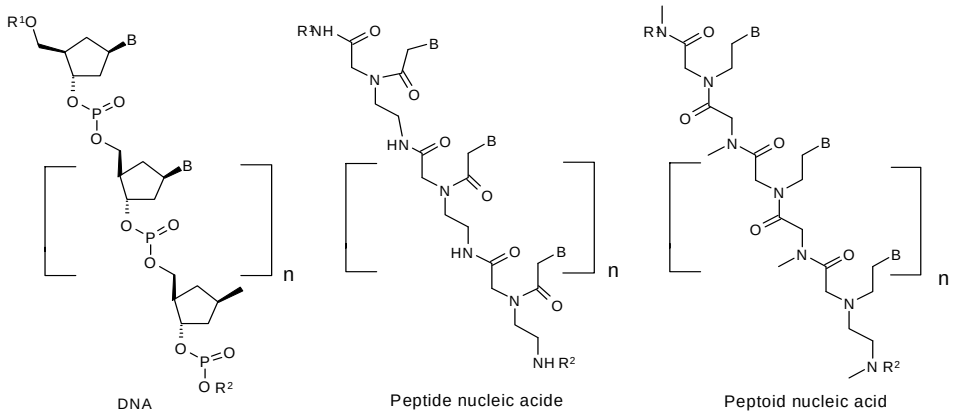
Abstract: The synthesis of peptoid nucleic acid bearing thymine as nucleobase has been achieved. This modified oligonucleotide showed good hybridization with DNA.

Keywords: Peptoid, peptide nucleic acid, solid phase synthesis, thymine.

The sequence specific bonding of oligonucleotide to RNA or double stranded DNA has attracted wide attention as the antisense and antigene strategies for treatment of diseases at the level of gene expression in medicinal chemistry[1]. Peptide nucleic acids designed as a chemira of nucleobases and polyamidic backbone bind with high affinity and sequence specifity to both complementary RNA and DNA and a number of template functions are inhibited on forming PNA/RNA and PNA/DNA complex[2-4]. In the past few years , different structures of PNAs have appearred to offer both antigene and antisense strategies for regulating gene expression.

Recently, we investigated a structurally novel type of peptide nucleic acid where the base amino acid moieties are derived from N-methyl glycine (peptoid) and in which the nucleobase containing amino acids are interspaced with one peptoid. In addition, the bases attach the backbone with ethyl linker, so they are separated from and positioned along the backbone by the same numbers of bonds found in DNA. **(Figure 1)**

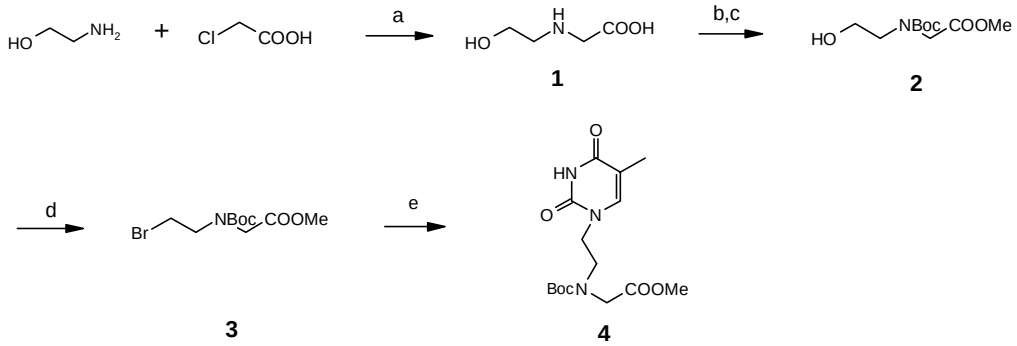
Figure 1



In these years, peptoids have received wide attention. Their specific structure featured with different chiral centres and without NH-bonds lead to special solubility, cell penetration as well as protease resistant[5]. All these unusual physical and biological properties will be important for therapeutic application[6]. Our preliminary model suggested that peptoid nucleic acid had abundant conformations and some of those be easy to bind with DNA. The result indicated that the stability of duplexes formed by this type of oligodeoxy-nucleotide analogs was found to be higher to that of DNA/DNA hybrids.

Peptoid nucleic acid monomer bearing thymine was prepared from N-(2-hydroxyethyl) glycine **1** by the route shown in[7]. **(Scheme 1)** Using Boc for the N-protection and the methyl for the C-protection, we obtained the methyl ester **2**. According to the method developed by Tins *et al*, **2** was convented into bromo derivative **3** by transformation of primary hydroxy groups into corresponding bromide with NBS and PPh_3.[8]. Compound 3 was reacted with 3.5 equiv excess of the thymine in anhydrous DMSO in the presence of anhydrous potassium carbonate for 22 h. After an aqueous work up and purification by column chromatography on silica gel, **4** was obtained in 45-50% yield[9,10].

Scheme 1



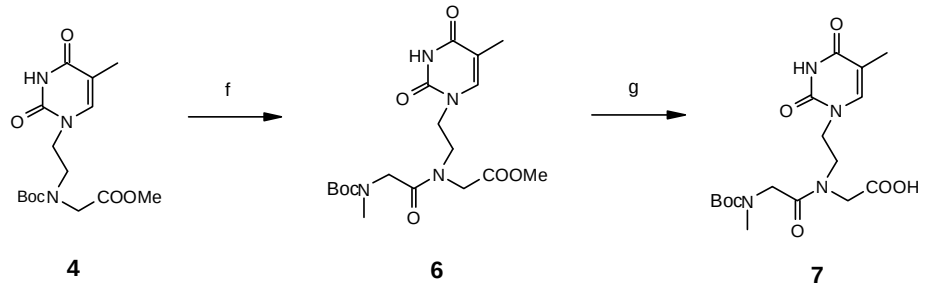
Conditions: a, in water, room temp., overnight (43%); b, Boc_2O, Bu^tOH, aq. NaOH, room temp., overnight (90%); c, CH_2N_2, 0^oC (quant); d, NBS-PPh_3 in CH_2Cl_2, room temp., over-night (40%); e, Thymine-K_2CO_3 (3.5equiv) in DMSO, 80^oC, 22hs (45-50%).

In order to reduce the solid-phase procedures, we used solution phase techniques to prepare the dipeptoid as a PNA block. Removal of the Boc group with trifluoroacetic acid, followed by coulping of the TFA salt with Boc-N-methyl glycine **5** in the presence of Bop reagent, product **6** was obtained in 90% yield. The final product **7** was obtained by the saponification of **6**[10]. **(Scheme 2)**

The assembly of the homo thymine oligomer essentially followed standard solid phase peptide synthesis (SPPS) protocols. The benahydrylamine resin (0.1mM scale) was used as the polymeric support to anchor the growing peptoid chain. Bop was used as activating reagent with the presence of a slight excess of N,N-diisopropyl-ethylamine (DIEA) as base. Each cycle used 1.5-equivalents of monomer in DMF/CH_2Cl_2, and repeating the coupling two times. After ten cycles, the peptoid was cleaved from the

resin by HF. Following the standard cleavage procedure, the crude peptoid was washed with diethyl ether, dissolved in aq. HOAc and lyophilized. After purification by

Scheme 2

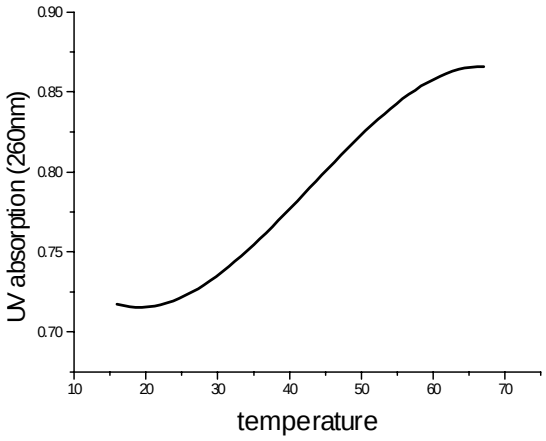


4 **6** **7**

Conditions: f, (i) TFA in CH_2Cl_2 (1:1), room temp., 30 min; (ii) N-Boc-N-methyl glycine, DIEA, BOP, in DMF, overnight (90%); g, aq. NaOH-THF (quant).standard cleavage procedure.

sephadex G-15 at 260 nm, the purity of oligomer was identified by ESI mass spectromtry. The principal mass peak at 2949.18 Da corresponds to the protonated form of the desired PNA oligomer ($C_{126}H_{175}N_{43}O_{41}$: M=2948) and the other one at 2986 Da to the potassium salt of the oligomer (*i.e.* [M+K-H]+). The mass spectraum also showed the presence of some deleted sequence. Peak at 2667 Da can be assigned to the nonamer and peak 2388 Da to octamer[11]. Then, the peptoid nucleic acid was further purified by RP-HPLC on a C-18 column using acetonitrile-water containing 0.1% TFA and its identity was confirmed by ESI-MS[11]. Interestingly, these oligomers showed an ability to form adducts with alkali metal ions especially potassium . In some case, these potassium ion adducts as the major peaks in the mass spectra[7].

Figure 2 Melting curves of the H-T_{10}- Lys-NH_2/$(dA)_{10}$



The purified peptoid nucleic acid appeared as fluffy solid after lyophilisation, was readily soluble in water. Its aqueous solution showed absorption maxima at 220 nm

(peptoid) and 272 nm (thymine). We measurred the variation of absorbance *versus* temperature of this compound at 270 nm in 0.14 M NaCl, 10^{-2}M potassium phosphate, pH 7.2 obtaining a Tm value of 42°C[12]. (**Figure 2**) The result suggested that the relative stability of a duplex of the modified peptoid nucleic acid with DNA was higher than that of the DNA/DNA (Tm=22°C).

In summary, we have accomplished the synthesis of peptoid nucleic acid by Boc strategy SPPS in good yield. The resulting oligodexoxyribonucleotide analog shows good binding affinity toward DNA strands. Extension of the chemisty to all four nucleic acid and evaluation of additional biologically relevant sequences are under current investigation.

Acknowledgment

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10. Analytical data for **4** and **7**. **4**: ^{1}H NMR (d_6-DMSO) δ7. 49 (2×s, 1H, T-CH(6)), 3. 99 and 3. 97 (2×s, 2H, CH_2COOH), 3. 71 (m, 2H, TCH_2), 3. 67 and 3. 65 (2×s, 3H, OCH_3), 3. 45 (m, 2H, TCH_2CH_2), 1. 72 and 1. 70 (2×s, 3H, T-CH_3), 1. 25 (s, 9H, Boc); mass spectrum *m/z* 342 $[M+H]^+$, 240 $[M\text{-}Boc+2H]^+$, 153 $[TCH_2CH_2]^+$, 102 $[Boc+H]^+$, 57 $[Me_3C]^+$; Anal. Calcd for $C_{15}H_{23}N_3O_6$: C, 52. 78; H, 6. 79; N, 12. 31. Found: C, 52. 89; H, 7. 05; N, 12. 14. HPLC, 97. 08%. **7**: ^{1}HNMR (d_6-DMSO) δ 12. 78 (s, 1H, COOH), 11. 32, 11. 14 and 11. 10 (3×s, 1H, T-*NH*), 7. 57 and 7. 39 (2×s,1H, T-*CH*(6)), 4. 15, 4. 14, 3. 98 (3×s,2H, CH_2COOH), 4. 04, 3. 89 and 3. 87 (3×s,2H, NCH_2CH_2), 3. 80 and 3. 73 (2×m, 2H, TCH_2CH_2), 3. 51 (m, 2H, TCH_2CH_2), 2. 73, 2. 71, 2. 65 and 2. 61 (4×s,3H, NCH_3), 1. 74 (s, 3H, T-CH_3), 1. 38, 1. 32 and 1. 30 (3×s, 9H, Boc); FAB-MS (*m/z*): 421 $[M+Na]^+$, 399 $[M+H]^+$, 299 $[M\text{-}Boc+H]^+$, 228 $[M\text{-}Boc\text{-}CH_3NCH_2CO+H]^+$, 153 $[TCH_2CH_2]^+$; Anal. Calcd. for $CH_{17}H_{26}N_4O_7$. $2H_2O$: C, 47. 00; H, 6. 96; N, 12. 90. Found: C, 47. 54; H, 6. 44; N, 12. 79. HPLC, 96. 5%.
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