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A Convenient Automated Solid-Phase Synthesis of PNA-(5')-DNA-(3')-PNA Chimera

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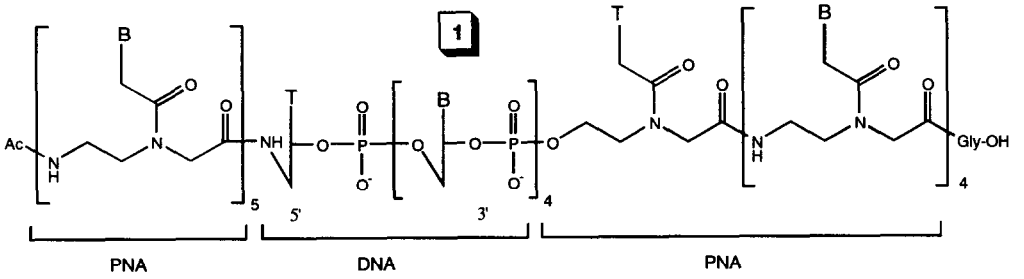
Abstract: An automated online solid-phase synthesis of Ac-cac ct T*GG TC t*ac ct-Gly-OH[1] using standard DNA and appropriately protected PNA building blocks (**2-5**) is described. This chimera forms stable duplexes with complementary DNA and RNA. © 1997 Elsevier Science Ltd.

Peptide Nucleic Acids (PNA) in which the natural nucleobases are anchored *via* a carbonyl methylene linkage to a *N*-(2-aminoethyl)glycine backbone hybridize strongly and sequence specifically to complementary DNA or RNA.[2] In addition, this type of DNA mimic is not degraded by nucleases and proteases.[3] Despite these interesting features, the potency of PNA to serve as an antisense drug is limited by its poor water solubility[4] and the fact that PNA•RNA duplexes are not recognized by the enzyme RNase H which cleaves RNA in DNA•RNA duplexes. It has been proposed that chimera of PNA and DNA would circumvent these shortcomings.[5] Moreover, attachment of PNA to both ends of DNA may lead to PNA-(5')-DNA-(3')-PNA chimera in which the DNA is fully resistant towards exonucleolytic degradation.

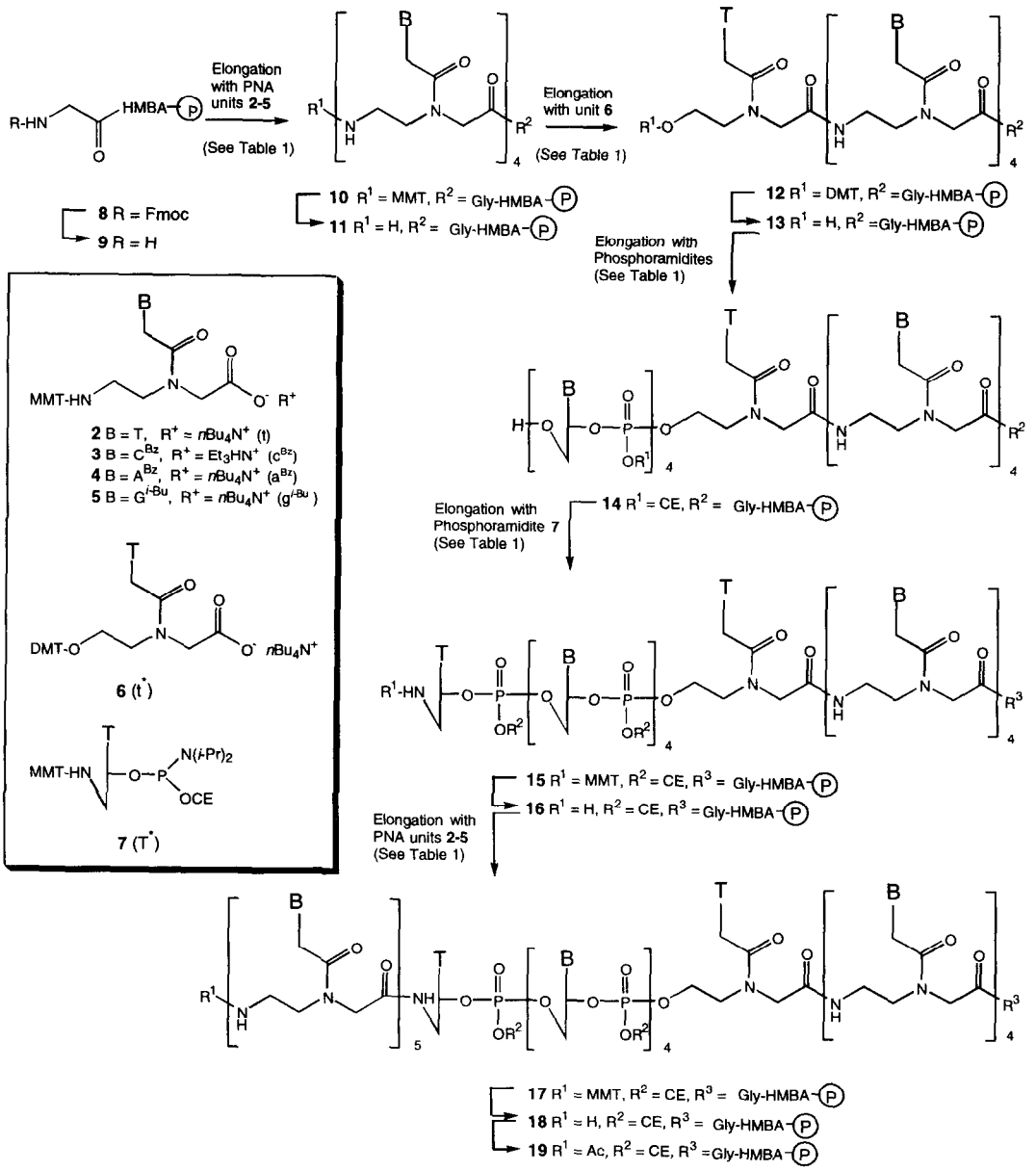
As part of a program[5a] dealing with the design and synthesis of potentially useful antisense probes, we here report a fully automated online solid-phase synthesis of a chimeric PNA-(5')-DNA-(3')-PNA[6] having the sequence[1] Ac-cac ct T*GG TC t*ac ct-Gly-OH (*i.e.* compound **1**).



Retrosynthetic analysis of target compound **1** indicates that the introduction of the respective amide and phosphodiester bonds to the 5' and 3' end of the DNA can be realized using the building units 5'-amino-5'-deoxythymidine phosphoramidite **7** (T*)[7] and *N*-(2-hydroxyethyl)-*N*-(thymin-1-ylacetyl)glycinate **6** (t*).[5e] It is also evident that standard acyl (benzoyl and isobutyryl) groups are suitable for protection of the exocyclic amino functions of the nucleobases in both DNA and the PNA building units **2-5** (see Scheme 1),[5a,8] the *N*-monomethoxytrityl (MMT) group of which is also compatible with DNA synthesis.

The assembly of **1** commences (see Scheme 1) with the synthesis of immobilized t*ac ct-Gly PNA **12**. To this

Scheme 1 Synthesis of PNA-(5')-DNA-(3')-PNA chimera **1**



end, Fmoc protected glycine was anchored *via* an ester linkage to highly crosslinked polystyrene (PS) beads, functionalized with hydroxymethylbenzoic acid (HMBA) under the influence of DCC/DMAP,[1] to give support **8** (loading capacity: 26-28 μmole/g). Prior to the solid-phase synthesis of **1**, the Fmoc group in **8** was released with piperidine to give immobilized glycine **9**. Sequential elongation of **9** with PNA monomers **2-5**,[9] according to protocol A in Table 1, resulted in PNA fragment **10**. Acidolysis of the MMT group in PS-bound **10** and condensation of intermediate **11** with the modified PNA unit **6** (t*) led to fully protected and immobilized PNA

pentamer **12**. Extension of anchored **13**, resulting after detritylation of **12**, with cyanoethyl (CE) DNA phosphoramidites according to protocol B (see Table 1) provided, after four additional elongation cycles and final detritylation, immobilized DNA-PNA chimera **14**. At this stage, the free 5'-OH function in **14** was phosphitylated with the 3'-*O*-phosphoramidite of 5'-*N*-(4-methoxytrityl)amino-5'-deoxythymidine **7** (T*) to give, after oxidation of the intermediate phosphitetriester, decameric fragment **15**. The final stage in the online solid-phase assembly of fully protected PNA-(5')-DNA-(3')-PNA chimera **17** entails elongation of immobilized **15** with PNA monomers **2-5**. Thus, detritylation of decamer **15** and subsequent sequential extension of intermediate **16** with PNA units **2-5** by executing protocol A resulted, after five elongation cycles, in fully protected and anchored **17**. In this respect, it is of interest to note that preactivation of the incoming PNA monomers with HBTU had a beneficial effect on the condensation efficiency: *i.e.* the efficacy of each coupling step was greater than 96% as monitored colorimetrically by the amount of liberated MMT cation. Transformation of immobilized **17** into chimera **1** was effected by the following three-step procedure. Acidolysis of the MMT group in **17** (step 1 in Table 1) was followed, in order to suppress side reactions (*i.e.* cyclization or nucleobase migration)[10] under basic conditions, by acetylation of the resulting free terminal amino function in **18** (step 3 in Table 1). PS-bound **19** was then released from the solid support with aqueous ammonia (30%, v/v) for 1 h at 20°C. Finally, filtration of the solution and removal of the *N*-acyl groups by ammonolysis for 16 h at 55°C[11] gave crude **1** (see Fig. 1). Purification of the crude oligomer by reversed phase HPLC[1] yielded pentadecamer Ac-cac ct T*GG TC t*ac ct-Gly-OH (**1**) and a small quantity of the

Table 1. Steps involved in chain extension of PNA and DNA on ABI 394 DNA Synthesizer

Step	Function	Solvents and Reagents [a]	Protocol A (PNA synthesis) Time (sec)	Protocol B (DNA synthesis) Time (sec)
1	Detritylation	3% CCl_3COOH in CH_2Cl_2	60	24
2	Coupling	* **PNA synthesis:** PNA units **2-6** + HBTU[b], DiPEA[c]	960	
		* **DNA synthesis:** Phosphoramidite[d], 1*H*-Tetrazole[e]		25
3	Capping	Ac_2O/Lutidine/THF + N-methyl imidazole/THF	25	15
4	Oxidation	Iodine/Pyridine/Water	not required	23

[a]Reactions were performed on a 2 μmole scale. [b]0.1 M PNA **2-6** + 0.1 M HBTU in DMF/CH_3CN (1/1, v/v) (8-9 fold excess per μmole support). [c]0.2 M DiPEA in DMF/CH_3CN (1/1, v/v) (20 fold excess per μmole support). [d]0.1 M Phosphoramidite in CH_3CN. [e]0.5 M 1*H*-Tetrazole in CH_3CN.

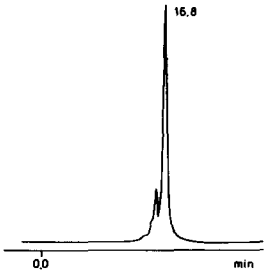


Figure 1. Crude HPLC chromatogram[13] of Ac-cac ct T*GG TC t*a cct-Gly-OH (**1**) after desalting.

Table 2. Tm values[12] of the duplexes consisting of DNA (**1'**) •DNA, DNA (**1'**)•RNA, chimera **1**•DNA and chimera **1**•RNA.

Sequence (5' → 3')	DNA[a] Tm (ΔTm)	RNA[b] Tm (ΔTm)
CAC CTT GGT CTA CCT (**1'**)	55.1 °C	59.6 °C
Ac-cac ct T*GG TC t*ac ct-Gly-OH (**1**)	40.8°C (-14.3)	52.6 °C (-7.0)

[a] Complementary DNA sequence: 5' d(AGG TAG ACC AAG GTG).
[b] Complementary RNA sequence: 5' (AGG UAG ACC AAG GUG).

glycine amide derivative of **1**. The homogeneity and identity of chimera **1** was firmly established by RP-HPLC as well as mass spectrometry.[14] In addition, it can be seen in Table 2 that the Tm values[12] of duplexes of PNA-(5')-DNA-(3')-PNA **1** with its complementary DNA and RNA are lower than those of the corresponding DNA•DNA and DNA•RNA duplexes.

A full report on the biochemical (*i.e.* RNase H activity) and biophysical properties (*i.e.* Tm values and conformational analysis) of other PNA-(5')-DNA-(3')-PNA chimeras will be published in due course.

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REFERENCES AND NOTES

1. Abbreviations: Capital and small letters stand for DNA and PNA, respectively. DCC = *N,N'*-dicyclohexylcarbodiimide. DiPEA = *N,N*-diisopropyl-*N*-ethylamine. DMAP = 4-(*N,N*-dimethylamino)pyridine. DMF = *N,N*-dimethylformamide. DMT = 4,4'-dimethoxytrityl. HBTU = 2-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. HPLC = High Performance Liquid Chromatography. THF = tetrahydrofuran.
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11. It is important to note that cleavage from solid support and concomitant removal of protecting groups in **19** with aqueous ammonia (30%, v/v) at 55°C was accompanied by the formation of a significant amount of impurities.
12. Thermal melting studies with complementary DNA and RNA were performed at 260 nm using a Perkin Elmer Lambda 12 UV/VIS Spectrometer equipped with a PTP-6 Peltier Temperature Programmer. Melting temperature (Tm) were measured in a 10 mM phosphate buffer, pH = 7.0, containing 100 mM NaCl and 0.1 mM EDTA. The solutions were heated from 25 to 80°C at a rate 0.5°C per minute and the A_{260} was recordered against temperature.
13. Reverse-Phase HPLC analysis was carried out on an ABI Aquapore RP-300, 7 µm column. Gradient elution was performed at 20°C by building up a gradient starting with buffer A (0.1 M TEAA in H_2O) and applying buffer B (CH_3CN) with a flow rate of 1.0 ml/min. The used gradient was: 0% B to 50% B in 32 min.
14. PNA-(5')-DNA-(3')-PNA **1** (*m/z*): 4280 as expected for $C_{148}H_{186}N_{69}O_{66}P_5Na_6$ (MALDI-TOF MS recordered in negative mode).

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