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Preliminary Communication

Solid support synthesis of a PNA-DNA hybrid

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Abstract. The solid support synthesis of a homothymine PNA-DNA octamer (*i.e.* hybrid **13**) could be realized using tetrabutylammonium *N*-(2-(4-methoxytrityl)-aminoethyl)-*N*-((thymin-1-yl)acetyl)glycinate as PNA building block and the respective 2-cyanoethyl-*N,N*-diisopropylphosphoramidite derivatives of both 5'-*O*-(4,4'-dimethoxytrityl)thymidine and 5'-*N*-(4-methoxytrityl)amino-5'-deoxythymidine.

Antisense oligonucleotides (AO) have gained increasing attention as tools to inhibit gene expression[2]. In order to improve nuclease resistance and cell membrane permeability, much effort has been directed towards the design and synthesis of AO[2a,3] bearing modified internucleosidic phosphodiester or neutral and achiral

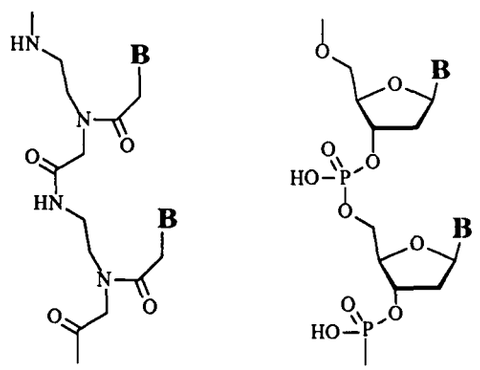
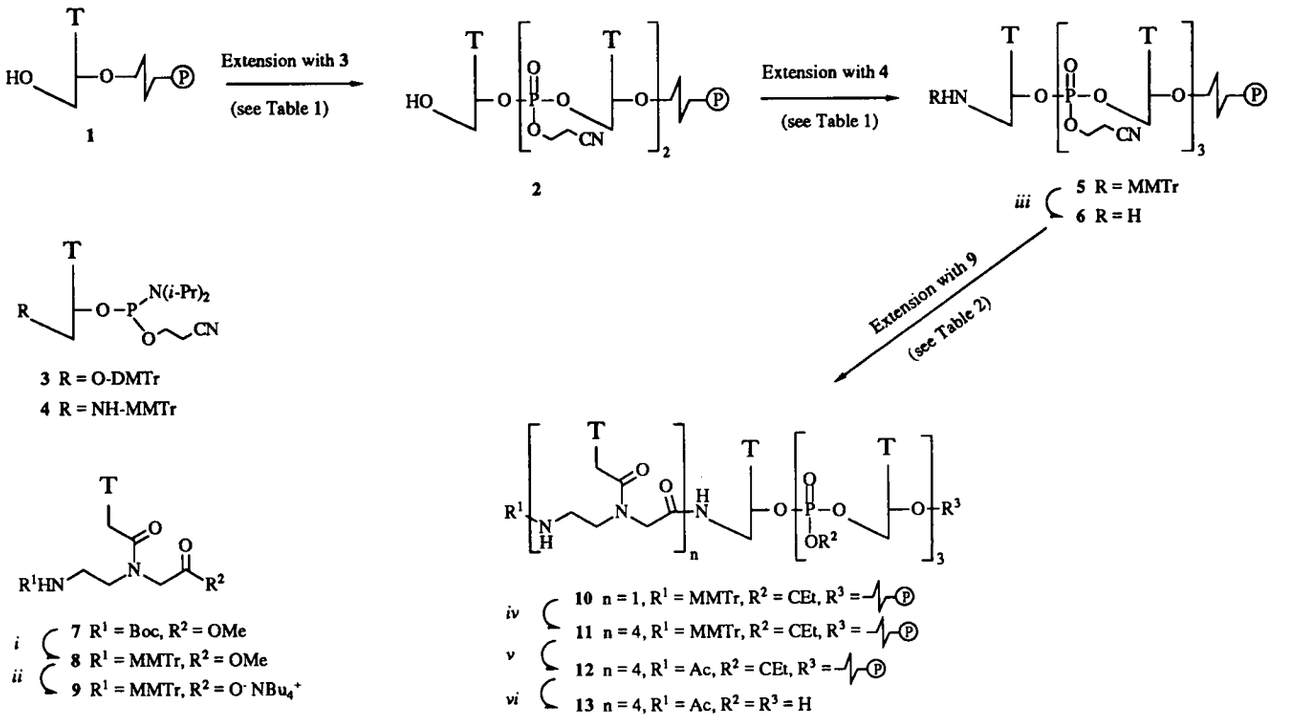


Figure 1. Structures of PNA and DNA

"Phospho" analogues. Recently, *Nielsen et al.*[4] introduced the so-called peptide nucleic acids (PNA) as a possible alternative for backbone modified AO. This novel class of DNA analogues consists (see Fig. 1) of the natural nucleobases which are anchored *via* a carbonyl methylene linkage to N-(2-aminoethyl)glycine instead of deoxyribosyl phosphate units. Interestingly, the presence in PNA of neutral and rigid peptide bonds not only results in exceptionally strong Watson-Crick basepairing in PNA•DNA duplexes[5-9], but also renders PNA inert to the action of nucleases. Despite these interesting features, the efficacy of PNA as antisense reagents is hampered by poor water solubility[4,5]. In addition it is to be expected that RNA in a PNA•RNA duplex will not be cleaved by the enzyme RNase H. The latter process is a valuable asset in a RNase H mediated antisense approach. Moreover, the high potency of PNA to form rather stable PNA•DNA (RNA) duplexes may result in non specific hybridisation[10]. We reasoned that natural or modified AO, covalently linked at both ends to curtailed PNA (*i.e.* PNA comprising maximally four monomers), would give access to a new type of potentially useful and versatile PNA-DNA-PNA antisense probes.

As a part of a program dealing with the design and preparation of PNA-DNA-PNA hybrids, we here report a solid support synthesis of a homothymine PNA-DNA octamer bearing an acetylated terminal amino group (*i.e.* compound **13**).

The synthetic route to the target molecule **13** commences with the elongation of thymidine unit **1** immobilized *via* a 3'-O-succinyl linkage to controlled pore glass. Thus, extension of **1** with the 3'-phosphoramidite of 5'-*O*-(4,4'-dimethoxytrityl)thymidine **3** following the protocol summarized in Table 1 yielded, after final detritylation (step 1 in Table 1), the partially protected trimer **2**. At this stage, immobilized **2** was condensed (step 2 in Table 1) with the 3'-phosphoramidite of 5'-*N*-(4-methoxytrityl)amino-5'-deoxythymidine **4**[11] to give, after oxidation of the phosphitetriester intermediate and acidolysis of the MMTr-group from **5** (steps 3 and 1, respectively, in Table 1) the partially protected tetramer **6**. The final stage of the on-line



Reagents and conditions: (*i*) 6% HCl in HOAc, 3 h;, MMTr-Cl, Et_3N, in CH_2Cl_2, 2 h; (*ii*) Bu_4NOH in dioxane, 5 min at 10°C; (*iii*) step 1 in Table 1 (*iv*) Extention with 9 (see Table 2); (*v*) 2% TCA in DCE, 3.5 min, 0.25 M N-methylimidazole in Ac_2O/*sym*-collidine/CH_3CN, 2/3/15 (v/v/v), 1.2 min; (*vi*) 25% NH_4OH, 2 h at 40°C.

Scheme 1

Table 1. Chemical steps involved in each elongation cycle of the DNA

Step	Manipulation	Solvents and reagents[a]	Time (min)
1	Detritylation	2% TCA in DCE	2.5
2	Coupling	Phosphoramidite **3** or **4**[b], 1*H*-tetrazole[c] in CH_3CN	3.0
3	Oxidation	0.02 M I_2 in CH_3CN/ *sym.*-collidine/H_2O, 32/15/3 (v/v/v)	1.0
4	Capping	0.25 M *N*-methylimidazole in Ac_2O/*sym.*-collidine/CH_3CN, 2/3/15, (v/v/v)	1.2

[a] Reactions were performed on 200 mg (10 μmole) of resin (loading capacity 52 μmole/g). [b]0.1 M amidite in CH_3CN (0.4 ml). [c]0.5 M 1*H*-tetrazole in CH_3CN (0.6 ml).

Table 2. Chemical steps involved in each elongation cycle of the PNA

Step	Manipulation	Solvents and reagents[a]	Time (min)
1	Detritylation	See step 1 in Table 1	3.5
2	Coupling	HBTU[b], PNA-monomer **9**[c], DiPEA[d] in CH_3CN	15.0
3	Capping	See step 4 in Table 1	1.2

[a] Reactions were performed on 200 mg (10 μmole) of resin (loading capacity 52 μmole/g). [b]0.2 M HBTU in CH_3CN (0.4 ml). [c]0.2 M PNA in CH_3CN (0.4 ml). [d]0.2 M DiPEA in CH_3CN (0.4 ml).

solid phase assembly of the fully protected PNA-DNA adduct **11** entails elongation of tetramer **6** with the thymine PNA monomer **9**[12]. The latter compound was readily accessible by acidolysis (6% HCl/HOAc) of the *t*-butyloxycarboxyl (Boc) group from the known derivative **7**[5-8] followed by tritylation (MMTr-Cl/CH_2Cl_2/Et_3N) and subsequent saponification (*n*-Bu_4NOH/dioxane, 10°C) of **8**.Sequential elongation of **6** with the thymine PNA unit **9**, according to the protocol in Table 2, proceeded smoothly

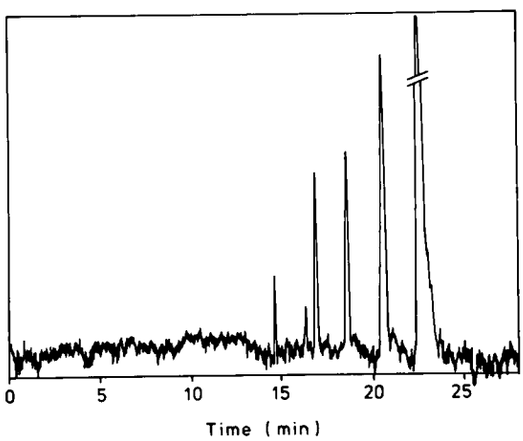


Figure 2. CGE analysis of crude octamer **13**

resulting, after four elongation cycles, in the immobilized octamer **11**. The amount of released MMTr cation, as gauged spectrophotometrically, indicated that the efficiency of the HBTU[1] mediated introduction of the amide bonds (step 2 in Table 2) was in each case greater than 85%. Acidolysis of the MMTr-group in **11** was followed, in orderto minimize the tendency of PNA to migration and (or) cyclization[13], by acetylation (see step 4 in Table 1) of the resulting primary amino function. Finally, removal of the 2-cyanoethyl groups from the phosphotriester functions and release from the solid support was effected with aq. ammonia (40°C for 2 h), to give crude **13** (see Fig. 2). Purification of the crude oligomer by reversed phase liquid chromatography yielded the PNA-DNA hybrid **13**, the homogeneity and identity of which was firmly established[14] by FPLC[1], CGE[1], ^{31}P-NMR spectroscopy as well as mass spectrometry.

In conclusion the successful on-line solid phase assembly of PNA-DNA adduct **13** presented in this paper may open the way to the future synthesis of PNA-DNA-PNA hybrids.

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References and notes.

1. **Abbreviations:** Ac, acetyl; CGE, capillary gel electrophoresis; DCE, 1,2-dichloroethane; DiPEA, *N,N*-diisopropyl-ethylamine; FPLC, fast protein liquid chromatography; HBTU, 2-(benzotriazol-1-yl-oxy)-1,1,3,3-tetramethyluronium hexafluorophosphate; TCA, trichloroacetic acid.
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12. Satisfactory mass spectrometric data were obtained for compound **9**. ^{1}H and ^{13}C NMR data (δ-values in ppm) of **9**. ^{1}H NMR 400 MHz ($CDCl_3$): 4.95 and 4.60 (s, 2H, $NCOCH_2$), 3.55 and 3.51 (t, 2H, J = 6.0 Hz, NCH_2CH_2NCO), 2.34 and 2.31 (t, 2H, J = 6.0 Hz, NCH_2CH_2NCO), 1.88 and 1.86 (s, 3H, CH_3 thymine). ^{13}C NMR ($CDCl_3$): 167.3 ($NCOCH_2$), 47.5 (NCH_2CH_2NCO), 41.5 (NCH_2CH_2NCO), 11.7 (CH_3 thymine).
13. *L. Christensen, R. Fitzpatrick, B. Gildea and J. Coull*, poster abstract presented at the 13th American Peptide Symposium (1993).
14. Octamer **13**: ^{31}P NMR (D_2O): -1.99, -2.10, -2.16 ppm. Mass spectra of **13**, recorded in both positive and negative-ion modes, give m/z values of 2327.8 and 2325.7 as expected for $C_{86}H_{110}N_{25}O_{42}P_3Na_3$ and $C_{86}H_{108}N_{25}O_{42}P_3Na_3$ respectively. FPLC analysis was carried out on a Pharmacia PepRPC HR 5/5 column (reversed phase). Gradient elution was performed at 20°C by building up a gradient starting with buffer A (0.1 M TEAA in CH_3CN/H_2O 5/95 (v/v)) and applying buffer B (0.1M TEAA in CH_3CN/H_2O 80/20 (v/v)) with a flow rate of 1.0 ml/min. The used gradient was as follows: 2 min at 0%, 10 min from 0 to 10%, 20 min from 10 to 20%. The retention time of octamer **13** is 17 min. Capillary gel electrophoresis (CGE) analysis was performed on a Applied Biosystem 270A capillary electrophoresis instrument at 15 kV using 50 μm ID capillaries, length 50 cm. Trisphosphate (75 mM in MeOH, pH 7.6) was employed as running buffer. Detection was performed at 260 nm. The retention time of **13** is 22 min.