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Liquid Phase Synthesis of a Peptidic Nucleic Acid Dimer

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Abstract: The first liquid phase synthesis of a peptidic nucleic acid (PNA) dimer containing guanine and adenine has been achieved in good yields. A new strategy was elaborated in order to circumvent difficult coupling of the protected PNA.

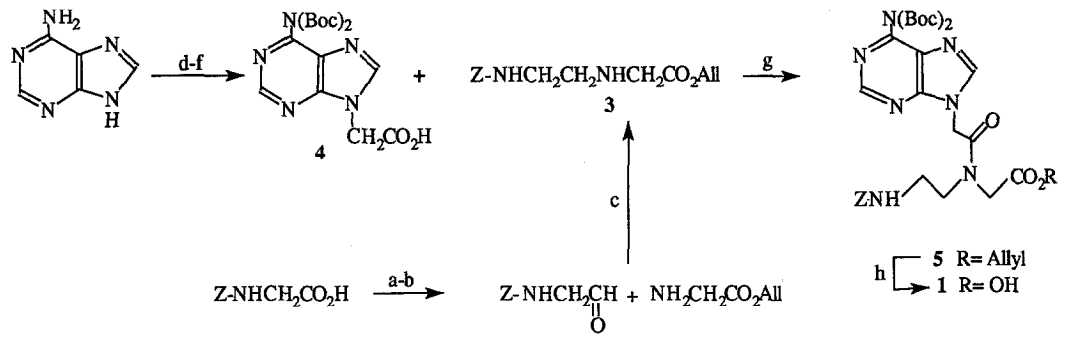
PolyPNAs are analogs of oligonucleotides which bear a N-(2-aminoethyl)-glycine backbone with the four standard nucleic acid bases as side chains[1]. PolyPNAs (about 15 subunits) attract much interest as antigene or antisens drugs: they are able to specifically recognize DNA or RNA fragments[2] and can form duplexes or triplexes via Watson-Crick or Hoogsteen interactions between complementary bases[3]. Moreover, PNAs, when compared with oligonucleotides, possess two major advantages:(i) their resistance to cellular proteases degradation, due to their non standard backbone (ii) their lipophilicity, because of the lack of the negative charges, which permits the cellular penetration[4].

The syntheses of polyPNAs, first described by Nielsen *et al*[5], follow standard solid phase peptide protocols. However, for short polyPNAs, (useful for studing *in vitro* interactions of DNA with peptides, PNA fragments, steroids...), a liquid phase synthesis is desirable as it would be both easier and more economical.

We herein describe the first liquid phase synthesis of a PNA adenine-guanine dimer.

We first prepared the two monomers of adenine **1** and guanine **2**, in order to realize their coupling by means of standard reagents. The synthesis of **1** is described in Scheme 1: the key intermediate **3** was obtained by reductive amination of Z-glycine aldehyde (which was prepared by lithium aluminium hydride reduction of the corresponding Z-glycine N,O-dimethyl hydroxylamide) with glycine allyl ester. The N,N-diprotected adenine acetic acid unit **4** was prepared in three steps: (i) alkylation of adenine at the N-9 position with methyl bromoacetate, (ii) protection of the exocyclic amino function with $(Boc)_2O$ in presence of a stoichiometric amount of DMAP (iii) alkaline hydrolysis (43% yield from adenine). The amide bond formation between the backbone **3** and the base acetic acid **4** was carried out by means of triphenyl phosphine and N-bromosuccinimide. This new method[6] affords several advantages compared to other classical coupling reagents: inexpensive starting materials, simple experimental conditions, rapid condensation (less than 15 min). It gave high yields of the protected monomer **5** (85%). Alkaline hydrolysis of alkyl esters of PNA monomers (R = Me,

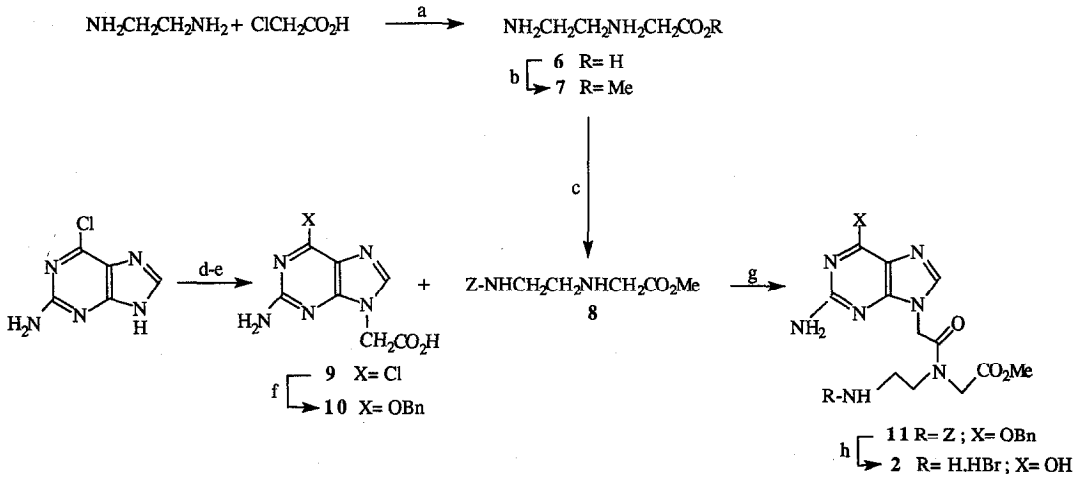
Et) generally proceeds in moderate yield[5]. In our case, smooth and clean cleavage of the allyl ester by treatment with catalytic amounts of tetrakis(triphenyl phosphine) palladium quantitatively yielded **1**.



a) *HCl.HNCH3(OCH3), PyBop, N-methylmorpholine (NMM), DMF (87%)* b) *LiAlH4, THF, 0°C (95%)* c) *MeOH/AcOH (99/1), NaBH3CN (45%)* d) *Adenine, DMF, NaH then BrCH2CO2CH3 (90%)* e) *(Boc)2O (3 eq.), DMAP (3 eq.), DMF (57%)* f) *Dioxane/H2O, LiOH 1N (83%)* g) *i: 4, P(C6H5)3, CH2Cl2, 0°C then NBS ii: 3.HCl, NMM (85%)* h) *Pd(P(C6H5)3)4, morpholine, THF (100%).*

SCHEME 1

The synthesis of **2** is described in Scheme 2. Condensation of chloroacetic acid with ethylene diamine taken as solvent gave compound **6** which was then esterified.

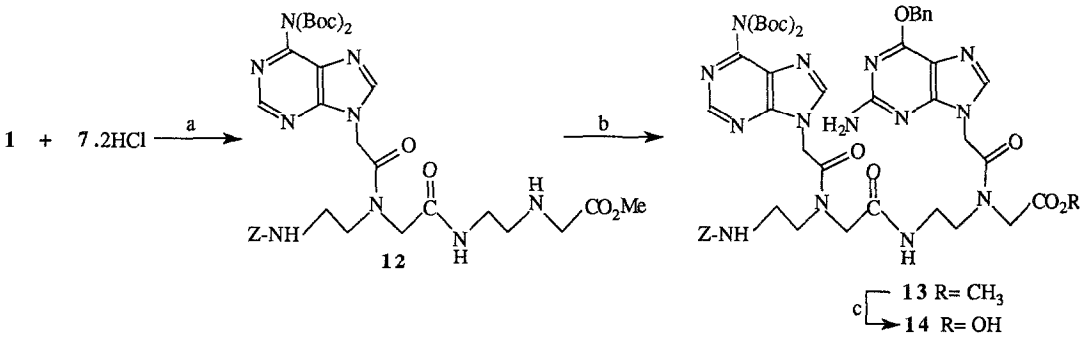


a) *(76%)* b) *MeOH/HCl$_g$, Δ (97%)* c) *Z-Cl, CH2Cl2, DMAP, 10 mn at -15°C then 2HCl.7, NMM , 2h at -15°C (48%)* d) *K2CO3, DMF, BrCH2CO2tBu, Δ (72%)* e) *TFA, (100%)* f) *C6H5CH2OH, NaH, DMF, then 9 (50%)* g) *Brop, NMM, CH2Cl2 (72%)* h) *HBr/AcOH (100%).*

SCHEME 2

Acylation, at low temperature, of the primary amine **7** with benzyl chloroformate and DMAP led to the key intermediate **8** in 37% overall yield (steps a-c). The O-protected guanine acetic acid **10** was prepared in 38% yield from 2-amino-6-chloropurine by alkylation, at the N-9 position, with tert-butyl bromo acetate, followed by TFA-mediated hydrolysis and, finally, by substitution of chlorine by sodium benzylate. Coupling between **8** and **10** by means of the Brop reagent afforded the protected PNA monomer **11** in 72% yield. Attempts to remove the Z group by hydrogenolysis failed, but the deprotection could be carried out in quantitative yield with HBr/AcOH[7].

Attempts to synthesize the PNA dimer through condensation of the two monomers **1** and **2** using various coupling reagents (Bop, PyBop, DCC/HOBt) at different temperatures[8], gave very poor yields. This led us to undertake the new strategy described in Scheme 3.



a) *i: 1, CH_2Cl_2, DCC, HOSu, 18h ii: 7.2HCl, NMM, -15°C (70%)* b) *10, Brop, NMM, DMF (80%) c) THF, LiOH 1N, 0°C (70%).*

SCHEME 3

Compound **12** was prepared by condensing, at low temperature, PNA **1** with the diamine methyl ester **7**, after a DCC/HOSu preactivation (70% yield). The amide bond formation between compounds **7** and **12** was performed with the Brop reagent, and the fully protected di PNA **13** was obtained in 80% yield after purification. Finally, saponification of the methyl ester group by LiOH gave **14** (70%).

Further elongation following the same procedure can be planned, and our new strategy should be applicable to the liquid phase syntheses of short polyPNA.

Acknowledgements:

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