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An expeditious liquid-phase synthesis of cyclic peptide nucleic acids

Jeroen C. Verheijen, Gijsbert M. Grotenbreg, Ludo Hart de Ruyter, Pieter A. M. van der Klein, Gijsbert A. van der Marel and Jacques H. van Boom *

Leiden Institute of Chemistry, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands

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Abstract

Suitably protected linear precursors of cyclic PNAs can be readily obtained by BOP/DiPEA coupling of the corresponding sub-monomers. Conversion of the linear PNA dimers into the pentafluorophenyl esters allows cyclization by intramolecular attack of the deprotected primary amino function under diluted conditions. After removal of the secondary amino protecting group(s), installation of the required nucleobase-acetyl function(s) affords cyclic PNAs. In addition, the latter compounds can be prepared following a direct coupling strategy.

Keywords: cyclization; peptide nucleic acid; coupling reactions; macrocycles.

Nearly two decades ago it was disclosed that both 3′-5′-cyclic diribonucleotides c[ApUp] and c[UpUp] are inhibitors of the DNA dependent RNA polymerase in *Escherichia coli*.[1] Later on, it was revealed that c[GpGp] is an endogeneous regulator of the biological synthesis of cellulose in the Gram-negative bacterium *Acetobacter xylinum*.[2] X-Ray analysis showed that c[GpGp],[3] as well as the closely related cyclic dinucleotide c[dApdAp],[4] adopts a conformation in which the 12-membered sugar-phosphate backbone provides a rigid framework to hold the two nucleobases 6.8 Å apart in parallel planes.

The proposed intercalator potential[3] of this class of cyclic compounds was an incentive in probing the biological relevance of analogs in which the rigid cyclic framework in **I** (Fig. 1) is replaced by an isosteric cyclic bis(aminoethylglycinamide) as in the peptide nucleic acid (PNA)[5] analog **II**. It was expected that this type of analog would be readily accessible by cyclization of linear PNA dimers. However, earlier studies[6,7] have noted that condensation of PNA monomers in solution is a surprisingly low yielding process.

Recently, Di Giorgio et al. reported that liquid-phase synthesis of linear PNAs could be readily effected via an indirect strategy using orthogonally *N*-protected aminoethylglycine monomeric synthons.[8] In the first instance, we explored the feasibility of converting an orthogonally (Z/Fmoc) protected linear PNA

* Corresponding author. Fax: +31 71 527 4307; e-mail: j.boom@chem.leidenuniv.nl (J. H. van Boom)

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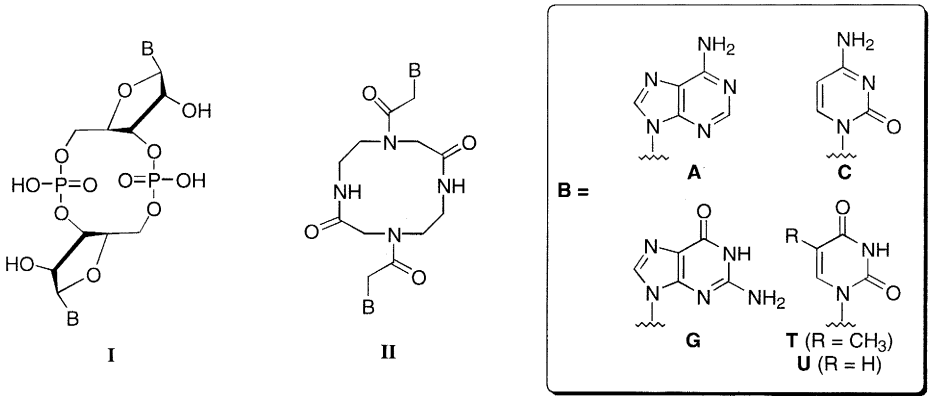
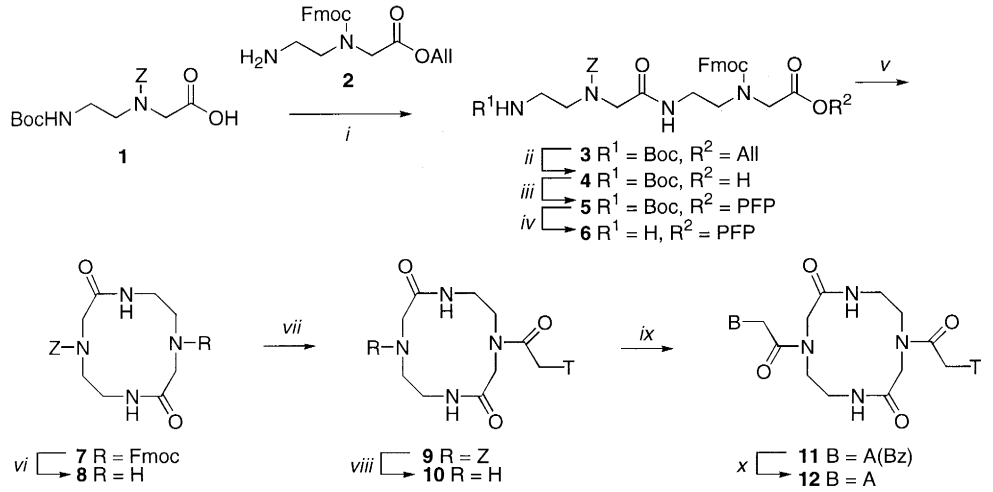


Fig. 1.

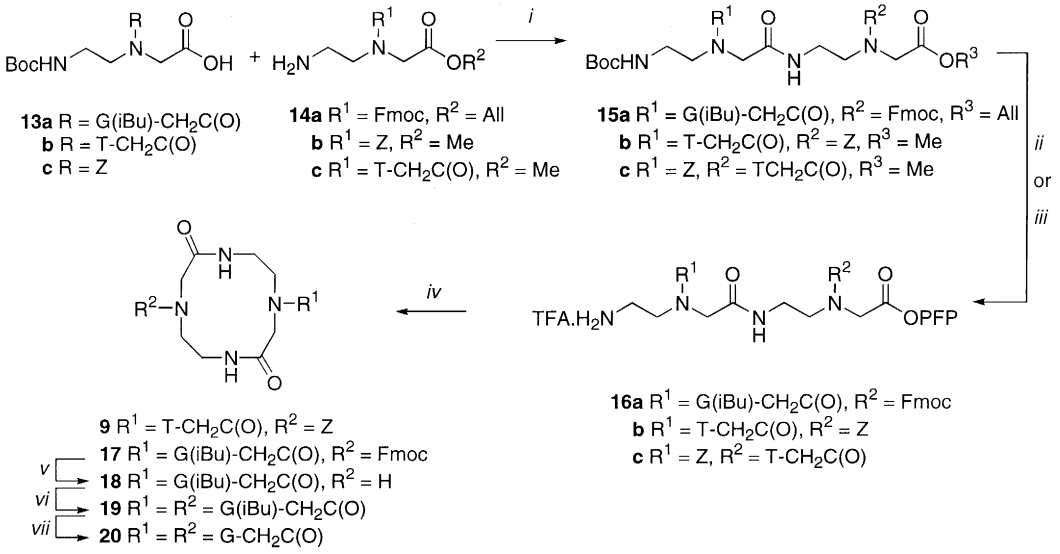
dimer into a cyclic PNA dimer. Accordingly, condensation (see Scheme 1) of *Z*-protected PNA backbone[9] **1** with Fmoc-protected PNA building block **2** under the agency of Castro's reagent gave fully protected dimer **3**. Linear dimer **3** could now be transformed into the cyclic intermediate **7** by the following four step procedure. Removal of the allyl group in **3** via palladium-catalyzed hydrostannylation was followed by EDC coupling with pentafluorophenol to give **5**. Deblocking of the *N*-Boc protective group in **5** with 50% TFA in CH_2Cl_2 afforded primary amine **6** as its TFA salt. Following a well-established procedure for the synthesis of cyclic peptides,[10] slow addition of a solution of **6** to a diluted solution of Hünig's base in acetonitrile gave orthogonally protected cyclic dimeric PNA precursor **7** in an overall yield of 57% (based on **3**). Conversion of **7** into cyclic PNA dimer **12** could be accomplished by executing the following sequence of reactions. The Fmoc protective group in **7** was removed with a 20% piperidine solution (→**8**) and the first nucleobase was installed by coupling thymin-1-ylacetic acid[11] to the free secondary amine in **8** to give **9**. Hydrogenolysis of the benzyloxycarbonyl group and condensation of the resulting **10** with *N*-benzoyl-adenin-9-ylacetic acid[12] furnished, after ammonolysis, target compound **12** in a yield of 41% over the last five steps.

The successful transformation of linear dimer **6** into cyclic PNA precursor **7** implied that replacement of one of the urethane protective groups in **6** by a nucleobase-acetyl moiety would be an attractive alternative. To this end, we first prepared dimer **15a** by BOP-mediated condensation of guanine monomer **13a** with Fmoc-protected sub-monomer **14a** (Scheme 2). Transformation of **15a** into the corresponding pentafluorophenyl ester, followed by acidolysis of the Boc group, gave **16a**. Cyclization of the linear PNA derivative **16a**, as described for the conversion of **6** into **7**, proceeded smoothly to furnish **17** in a yield of 62%. Removal of the Fmoc protective group followed by coupling of **18** with *N-iso*-butyryl-guanin-9-yl-acetic acid[13] allowed direct isolation (34% yield) of crystalline **19** from the crude reaction mixture. Deprotection of the exocylic amines in **19** was effected with methanolic ammonia to give the homogeneous cyclic bis-guaninyl PNA dimer **20**. In a similar fashion, thymine monomer **13b** was condensed with building block **14b** to give linear dimer **15b**. Saponification of the latter dimer and subsequent formation of the pentafluorophenyl ester was followed by deprotection of the primary amine to give **16b**. Also in this case, cyclization proceeded smoothly to give **9**. At this stage, we were interested in determining whether the presence of a nucleobase in monomer **14** instead of **13** would influence the outcome of the cyclization. It was established that condensation of carboxylic acid **13c** with *N*-aminoethyl-*N*-thymin-1-yl-glycine **14c**[14] gave, after further processing of **15c** as mentioned earlier, the activated linear dimer **16c**. Subjection of this compound to DiPEA under diluted conditions gave cyclic dimer **9** as the only detectable product. In this respect, it should be noted that the cyclization products



Scheme 1. *Reagents and conditions*: (i) BOP, DiPEA, DMF, 77%; (ii) $Cl_2Pd(PPh_3)_2$, AcOH, Bu_3SnH, CH_2Cl_2, 83%; (iii) pentafluorophenol, EDC, DMF, 98%; (iv) 50% TFA, CH_2Cl_2, quant.; (v) DiPEA, dilution in ACN, 70%; (vi) 20% piperidine, DMF, 85%; (vii) T-CH_2COOH, BOP, DiPEA, DMF, 64%; (viii) H_2, Pd/C, *i*PrOH:DMF (1:1), quant.; (ix) A(Bz)-CH_2COOH, BOP, DiPEA, DMF, 83%; (x) NH_3/MeOH, 90%

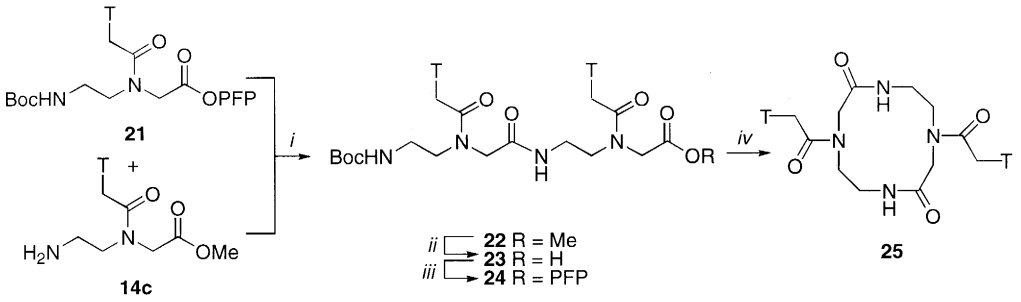
from **16b** and **16c** were identical in all aspects (i.e. ^{1}H NMR, ^{13}C NMR and mass spectrometry) with earlier prepared **9** (cf. Scheme 1).



Scheme 2. *Reagents and conditions*: (i) BOP, DiPEA, DMF, 75% (**15a**), 76% (**15b**), 66% (**15c**); (ii) (1) $Cl_2Pd(PPh_3)_2$, AcOH, Bu_3SnH, CH_2Cl_2, 76%; (2) pentafluorophenol, EDC, DMF, 70%; (3) 50% TFA in CH_2Cl_2, quant. (**16a**); (iii) (1) NaOH (1N), MeOH:H_2O (1:1, v/v); (2) HCl; (3) pentafluorophenol, EDC, DMF; (4) 50% TFA, CH_2Cl_2, 90% (**16b**), 60% (**16c**); (iv) DiPEA, dilution in ACN, 62% (**17**), 67% (**9** from **16b**), 60% (**9** from **16c**); (v) 20% piperidine, DMF, 85%; (vi) G(iBu)-CH_2COOH, BOP, DiPEA, 34%; (vii) NH_3/MeOH, 96%

The results obtained thus far clearly show that the cyclization of linear pentafluorophenyl esters is not influenced by the nature of the substituents at the secondary amine (i.e. R^1 and R^2 in **16**). We surmised that the poor yields, observed in the introduction of an amide bond between PNA monomers in a direct coupling strategy, could be improved using pentafluorophenyl active esters. In order to validate this

assumption, we prepared the linear bis-thyminyl[15] PNA dimer **22** in an excellent yield by condensing the pentafluorophenyl ester **21**[16] with primary amine **14c** (Scheme 3). Hydrolysis of the methyl ester in **22** with sodium hydroxide, followed by neutralization with Dowex-H^+, afforded dimeric carboxylic acid **23**. Subjection of **23** to the same sequence of reactions as described earlier for the cyclization of PNA precursors gave access to the bis-thyminyl cyclic PNA **25**. HPLC–MS analysis of the crude reaction mixture revealed that the only detectable side-products formed in the cyclization reaction were a hydrolyzed linear dimer and a cyclized tetramer. The latter macrocycle is formed through intermolecular coupling of two dimers, followed by cyclization of the resulting tetrameric pentafluorophenyl ester. The latter finding indicates that the methodology described here may be successfully extended to the construction of larger ring-systems.



Scheme 3. *Reagents and conditions*: (i) DiPEA, ACN, 90%; (ii) (1) NaOH (1N), MeOH:H_2O (1:1, v/v); (2) Dowex-H^+; (iii) pentafluorophenol, EDC, DMF, 51% (three steps); (iv) (1) 50% TFA in CH_2Cl_2; (2) DiPEA, dilution in ACN, 68% (isolated yield)

In conclusion, we have shown that the liquid-phase synthesis of cyclic PNA dimers may be readily effected by three different routes, each of which has its own merit in the construction of cyclic bis(aminoethylglycinamide) frameworks carrying a variety of functional groups. Adaptation of this methodology also opens the way to a liquid-phase synthesis of cyclic oligo-PNAs. The biological relevance of cyclic PNA dimers as well as extension of the reported strategy to the solid-phase synthesis of cyclic PNAs is currently under investigation.

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