Pergamon

Tetrahedron Letters, Vol. 38, No. 24, pp. 4211-4214, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00871-X

Synthesis of the Monomeric Building Blocks of *Z*-Olefinic PNA (*Z*-OPA) Containing the Bases Adenine and Thymine

Michel Cantin, Rolf Schütz and Christian J. Leumann*

Department of Chemistry and Biochemistry, University of Berne, Freiestrasse 3, CH-3012 Berne, Switzerland

Abstract: The synthesis of the Boc-protected *Z*-olefinic peptide nucleic acid analog (*Z*-OPA) monomers containing the base thymine **11** and Cbz-adenine **19** is described. Key step in the synthesis of the underlying carbon framework is a Pd(0)-catalyzed coupling of a vinyl iodide, prepared in three steps from 3-butynol, with the *Reformatsky* reagent derived from ethyl α-bromoacetate.

The peptide nucleic acid analog PNA, first described in 1991 by *Nielsen* et al.[1] has attracted much attention due to its unparalleled hybridization properties with the natural nucleic acids. Structurally, PNA consists of an achiral amide-based backbone unit composed of N-(2-aminoethyl) derivatized glycine, to which the nucleobases are attached via a carboxymethyl linker (Fig. 1). PNA was shown to form strong Watson-Crick base-paired duplexes with DNA and RNA in both parallel and antiparallel strand orientations.[2] The chemical and biological properties of PNA and its structural variants have been reviewed.[3-4] Recently, detailed structural information on PNA-DNA, PNA-RNA and PNA-PNA complexes by X-ray crystallography and NMR spectroscopy became available.[5-7] From inspection of these structures it appears that all the amide bonds, connecting the base-linker units to the backbone are uniformly oriented in the *Z*-rotameric form in the complexes, whereas both the *E*- and *Z*- form exist in equilibrium on the level of the monomeric building blocks or free PNA single strands.[5,8]

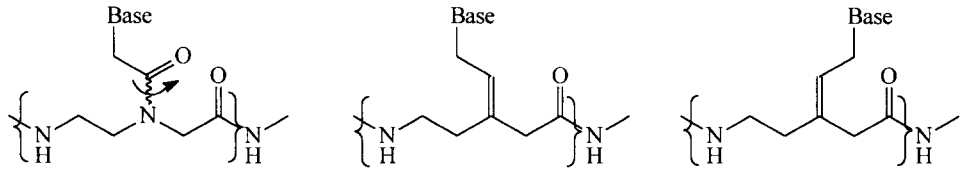
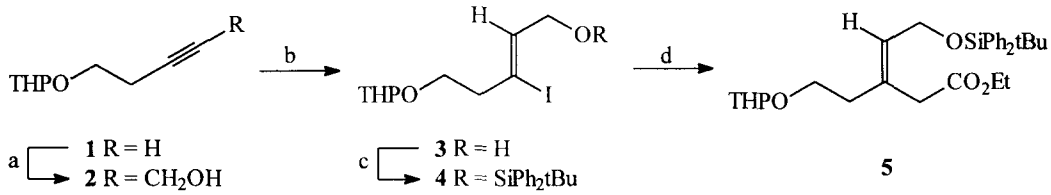


Figure 1: The structures of PNA and of the *E*- and *Z*-olefinic PNA analogs OPA.

In order to investigate the effect of structural preorganization of the monomeric PNA unit in either the E- or Z- form on pairing efficiency and orientation (parallel vs. antiparallel Watson-Crick duplex formation with DNA), we designed the analogs *Z*- and *E*-OPA in which the central amide bond of PNA is replaced by a configurationally defined C-C double bond. The fact that the deleted carbonyl group is not involved in any intramolecular H-bond in PNA complexes further supports the design of OPA as a PNA analog with retained

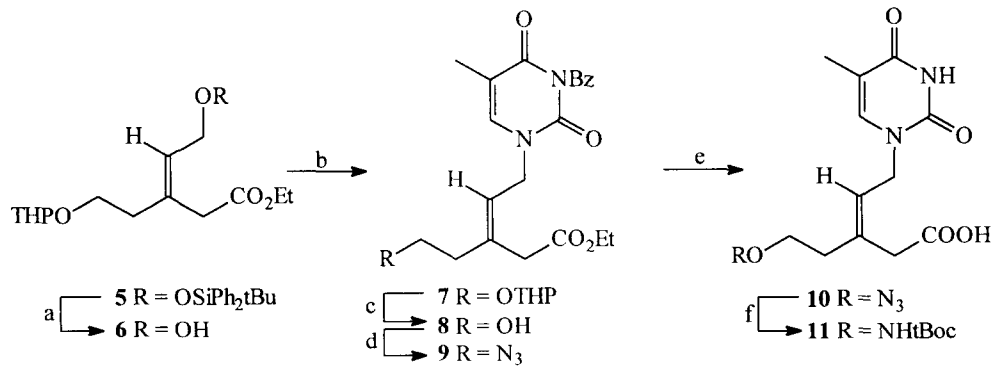
binding potential. Here we report preliminary results on the synthesis of the *Z*-OPA building blocks for oligopeptide synthesis carrying the bases adenine and thymine.

Our synthesis of the backbone unit of OPA (Scheme 1) starts from the THP-protected 3-butynol **1**, which could easily be hydroxymethylated with paraformaldehyde.[9] A stereospecific conversion of the resulting propargylic alcohol **2** into the *Z*-vinyl iodide **3** was developed based on methods reported by *Marshall* et al.[9] Silyl protection of the primary alcohol group then furnished **4**, the key intermediate for the final assembly of **5** via a palladium(0) catalyzed coupling [10] with the *Reformatsky* reagent derived from ethyl α-bromoacetate.[11] This transformation proceeded smoothly with retention of configuration of the double bond (as demostrated by ^{1}H NMR NOE experiments) and without detectable amounts of the tautomeric, conjugated olefin.



Scheme 1: *Reagents and Conditions*: a) *n*-BuLi, THF, -78 °C, 10 min, then $(CH_2O)_n$, r.t, 1.5 h, 83%; b) Red-Al, THF, addition 0 °C → r.t., 2 h; then NIS, -78 °C, 10 min, 90%; or I_2, -78 °C, 15 min, 76%; c) *t*-$BuPh_2SiCl$, imidazole, THF, r.t., 16h, 97%; d) Reformatsky reagent: $BrCH_2CO_2Et$, Zn, $CH_2(OCH_3)_2$, reflux, 30 min.; coupling: $Pd(PPh_3)_4$ (8 mol%), DMPU, 65 °C, 4 h, 66%.

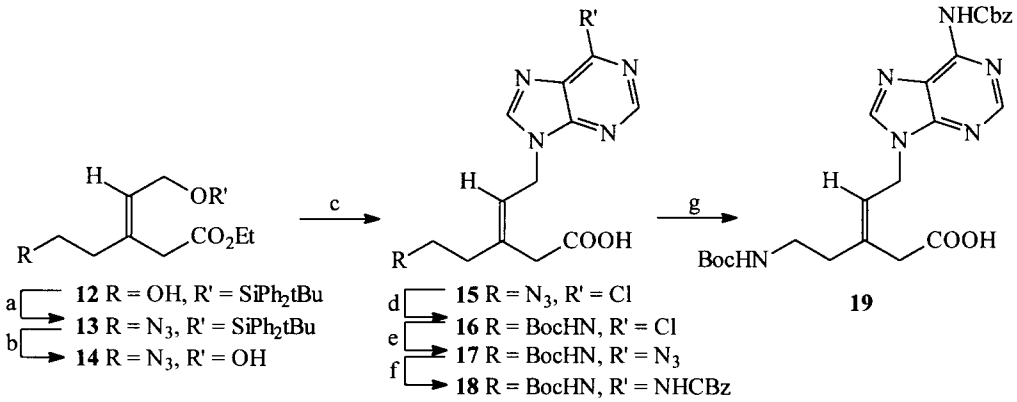
From intermediate **5**, two complementary synthetic routes were developed for the remaining functional group transformations leading to the Boc protected thymine-, and the Boc- and N^6Cbz protected adenine *Z*-OPA monomers (**11** and **19** respectively, Scheme 2 and 3). They deviate from each other by the chronology of the introduction of the nucleobase and the terminal amino group.



Scheme 2: *Reagents and Conditions*: a) HF·Py:Py 1:9, CH_3CN, r.t., 2.5 h; b) DEAD, PPh_3, N^3-benzoyl thymine, THF, 0 °C, 1 h, 74% over 2 steps; c) *p*-TSA, EtOH, r.t., 3 h, 87%; d) DIAD, PPh_3, $Zn(N_3)_2$·2Py, toluene-THF (1:1), 0 °C-r.t., 4 h, 68% e) LiOH (1.0 M), dioxane:H_2O 1:1, 0 °C, 16 h, 74%; f) Lindlar catalyst, H_2, MeOH, r.t., 4 h, then $(Boc)_2O$, Et_3N, r.t. → 40 °C, 40 min, 73%.

The silyl ether function of intermediate **5** was cleaved using the hydrofluoric acid - pyridine complex in pyridine. Under these conditions competing lactone formation could largely be suppressed to a ratio of ca. 9:1

in favor of the alcohol **6**. Subsequent coupling of **6** with N^{3}benzoyl thymine [12] in a *Mitsunobu* type reaction [13] furnished compound **7**. The *N*-1 connection of the base to the backbone unit in **7** was verified by ^{1}H NMR NOE spectroscopy. The tetrahydropyranyl group in **7** was then removed and the resulting primary alcohol **8** converted into the azide **9**, using the procedure reported by *Viaud & Rollin.*[14] Finally, both the ester and the benzoyl group on the base were hydrolyzed, and the acid **10** converted into the thymine-*Z*-PNA monomer **11** by catalytic hydrogenation with Lindlar catalyst, followed by Boc protection of the resulting amine. Under these conditions neither saturation of the trisubstituted double bond nor lactam formation was observed.



Scheme 3: *Reagents and Conditions*: a) DIAD, PPh_3, $Zn(N_3)_2$·2Py, toluene, r.t., 3 h, 74%; b) HF·Py-Py (1:9), CH_3CN, r.t., 2.5 h, 75%; c) DEAD, PPh_3, 6-chloropurine, THF, r.t., 6 h, 67%; d) Lindlar catalyst, H_2, $(Boc)_2O$, EtOH, r.t., 48 h, 78%; e) NaN_3, DMF, 50 °C, 6 h, 60%; f) i: Lindlar catalyst, H_2, EtOH, r.t., 4 h, ii: Rapoport's reagent, CH_2Cl_2, r.t, 16h, 70%; g) LiOH (1.0 M), dioxane:H_2O 1:1, r.t., 4h, 92%.

For obtaining the Boc- and Cbz-protected adenine-*Z*-OPA monomer (Scheme 3), alcohol **12**, obtained from **6** by acid catalyzed removal of the THP group as described above, was first converted into azide **13**. Again, cleavage of the silyl ether group in **13** led to **14**, together with traces of the corresponding lactone. Although different protected adenine derivatives were tried in the subsequent *Mitsunobu* coupling, 6-chloropurine gave the best results leading to compound **15** in 67% yield. Reduction of the azide function in **15** followed by introduction of the Boc-group furnished compound **16**. Conversion of the 6-chloropurine residue into the Cbz-protected adenine moiety in **16** worked best via the azide **17**, which again after hydrogenolytic reduction and protection with *Rapoport's* reagent ($PhCH_2OCOIm^+Et$, BF_4^-)[15] yielded **18** that could be hydrolyzed to the *Z*-OPA monomer **19** containing the Cbz protected base adenine.[16]

With these monomer building blocks in hand we now aim at the solid phase synthesis of PNA oligomers doped with single *Z*-OPA units, using commercially available Boc/Cbz protected PNA monomers, as well as at the synthesis of pure *Z*-OPA oligomers by the solid phase methodology used for PNA synthesis.[17] Parallel to these experiments a different protection scheme for amino- and base protection in OPA monomers, allowing for the synthesis of copolymers with DNA[18] will be elaborated.

Furthermore, the synthesis presented here opens a facile route to the corresponding *E*-OPA monomers, starting from intermediate **12** by oxidation of the primary hydroxyl group to a carboxylic acid function and by

transforming the ester group into a primary amino group. Experiments in this direction are currently under way in our laboratory.

Acknowledgements

Financial support from the Swiss National Science foundation as well as from the Wander-Stiftung, Berne, is gratefully acknowledged.

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(*Received in Germany* 4 *April* 1997; *accepted* 30 *April* 1997)