The Synthesis of a Thymine Containing *E*-Olefinic Peptide Nucleic Acid (OPA) Monomer

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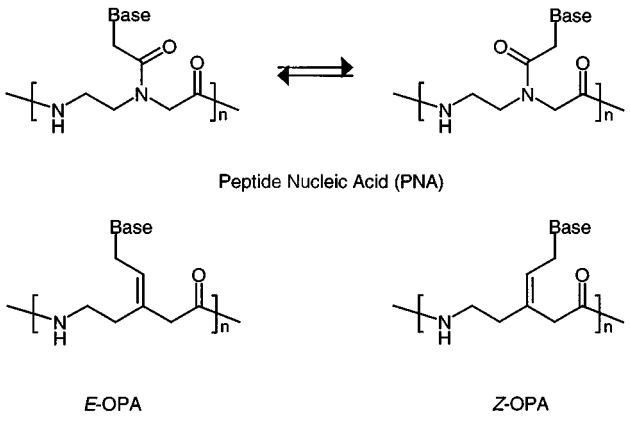
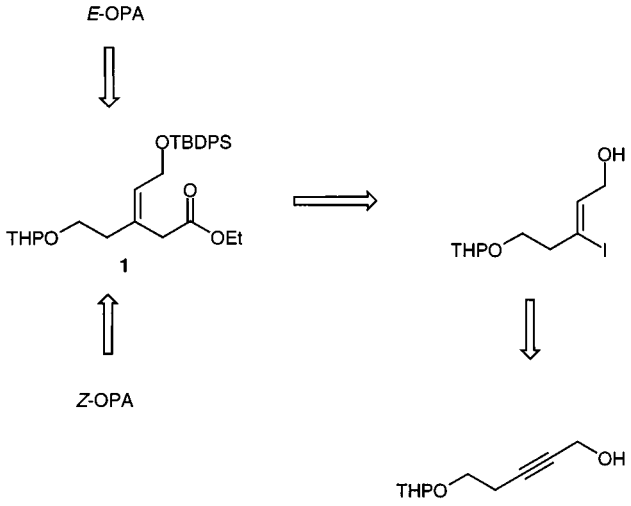
Abstract: A monomeric unit of a conformationally constrained PNA analog, *E*-olefinic peptide nucleic acid (*E*-OPA) containing the base thymine was synthesized in 14 steps. The key step involved a palladium (0) catalyzed cross-coupling reaction utilizing a Reformatsky reagent as nucleophile. A protecting group regime that is compatible with solid-phase PNA and DNA chemistries was chosen.

Key words: OPA, PNA, Reformatsky, DNA-analog, antisense

Peptide nucleic acid (PNA), reported in 1991 by Nielsen,[1] is a nucleic acid analog with remarkable pairing properties. Much work has since been invested in understanding and improving this potentially useful analog.[2] Oligomers of PNA are able to form stable duplexes with both DNA and RNA in both antiparallel and parallel orientations. Structural characterizations[3] show that when PNA is complexed with a natural nucleic acid the internal amide bond of the PNA units are uniformly aligned so that the carbonyl oxygen of the tertiary amide bond points to the carboxy terminus in an antiparallel Watson-Crick duplex. Olefinic peptide nucleic acid (OPA) monomers were designed as PNA analogs with an internal olefin of defined configuration. We reasoned that the replacement of the conformationally labile amide bond with a conformationally rigid olefin bond would allow an assessment of the role of this preorganized structural element in the duplex binding orientation observed in PNA (Scheme 1). From structural models, we anticipate that the *Z*-OPA isomer will bind in a parallel fashion, while the *E*-OPA isomer will bind in an anti-parallel orientation. The synthesis of a *Z*-OPA monomer has already been reported,[4] herein we report the synthesis of the thymine containing *E*-OPA monomer.



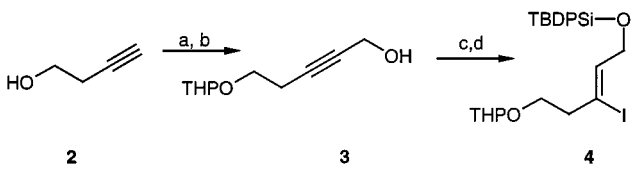
Scheme 1



Scheme 2. Retrosynthetic Design of *E*-OPA Monomers.

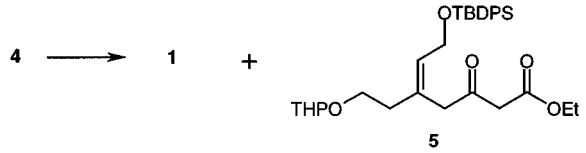
The syntheses of OPA monomers were designed to be divergent, with both *E* and *Z*-isomers utilizing a common intermediate **1** (Scheme 2). The *E*-isomer is then obtained using functional group interconversion to reverse the amino acid polarity and introduce the correct nucleobase(s). The basic OPA skeleton **1** is assembled from the vinyl iodide *via* a palladium(0) catalyzed cross-coupling reaction utilizing an ethyl α-bromoacetate derived Reformatsky reagent as the nucleophile.[5] The geometry of the olefin is defined *via* a stereospecific conversion of the propargyl alcohol to the *Z*-iodo olefin.[6] In anticipation of assembly of this monomer into oligomers utilizing solid-phase synthesis, a strategy incorporating the monomethoxytrityl amino protecting group and acyl base protection was chosen.[7] This system was selected over the standard PNA (and peptide) chemistries due to the need to avoid strongly acidic conditions which would scramble the double-bond position and geometry. Furthermore, this strategy is compatible with DNA synthesis chemistry, allowing us to construct DNA/OPA chimeras.

The synthesis of the thymine *E*-OPA amino acid **10** was begun from commercially available 3-butynol **2** (Scheme 3). The alcohol was protected as the THP acetal followed



Scheme 3. (a) DHP, *p*-TsOH CH_2Cl_2, 90%. (b) *n*BuLi, THF -78°C, 10 min then $(CH_2O)_n$, 0° C -> r.t., 80%. (c) Red-Al THF, 0° C, 2 h then NIS, THF, -78° C, 95%. (d) TBDPS-Cl, imidazole, THF, 97%.

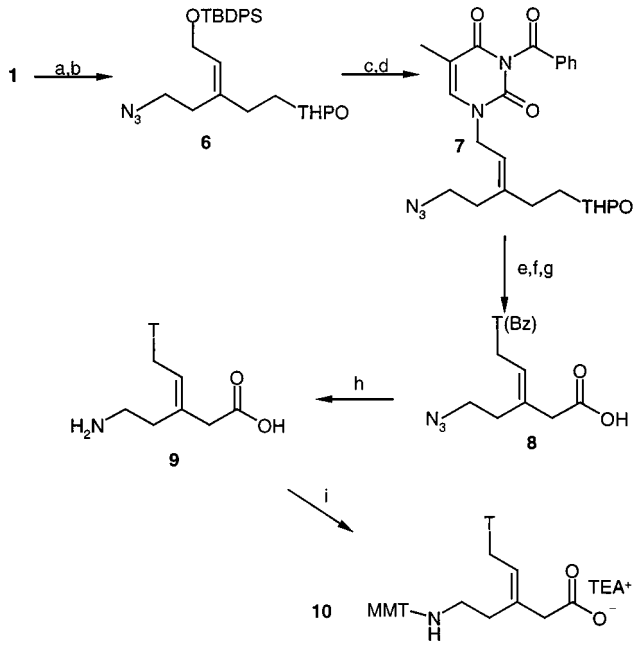
by hydroxymethylation with paraformaldehyde to afford the protected propargylic alcohol **3**. The formation of the *Z*-iodo olefin was accomplished in high yield by stereospecific reduction of the propargyl system with Red-Al followed by addition of N-iodosuccinimide. Attempts to use the significantly cheaper iodine as the I^+ source were only successful on small scale reactions. Scale up however, resulted in dramatic drops in yield (<30% total) due to scrambling of THP groups, probably due to traces of HI in solution. The allylic alcohol was then protected to afford the *tert*.-butyl-diphenyl-silyl ether **4** in high yield.



Scheme 4. Pd Catalyzed Cross-Coupling Reformatsky Reaction. Ethylbromoacetate, Zn, $CH_2(OMe)_2$, 50° C then added to **4**, $Pd(PPh_3)_4$ (20 mol%), DMPU, 70° C.

Entry	Scale (g; mmol)	Time (h)	Yield **1** (%)	Yield **5** (%)
A	1; 1.8	2	87	11
B	2.5; 4.5	2	81	10
C	16; 29	1.5	77	10
D	16; 29	1.0	76	6.6

The construction of the common intermediate **1** using the palladium(0) mediated Reformatsky reaction initially turned out to be problematic and unreliable. Eventually, several crucial factors in this reaction were elucidated, resulting in consistent and high yields of **1** on a range of reaction scales (Scheme 4).[8] We found that the activation of the zinc prior to formation of the Reformatsky reagent and the quality of the palladium(0) tetrakis-triphenylphosphine were absolutely crucial.[9] The ratio of DMPU to dimethoxymethane was also important (roughly 4:1 is optimal). And finally, the relationship between reaction time and reaction scale was important as well. As the reaction progresses, product ester **1** becomes a substrate for the Reformatsky reagent yielding the "bis-coupled" β-keto-ester **5**. As the scale of the reaction increases, the ratio of **1** to **5** decreases (Scheme 4 entries A *vs*. B *vs*. C). And with large scale reactions (Scheme 4 entries C *vs*. D), decreasing the reaction time increases the ratio of **1** to **5** while the absolute amount of **1** formed remains constant.



Scheme 5. (a) LAH, THF, 0° C, 87%. (b) $Zn(N_3)_2$-2 Pyr, DIAD, PPh_3, Toluene, 0° C, 18 h 77%. (c) TBAF, THF, 97%. (d) N^3-Benzoyl-thymine, DEAD, PPh_3, THF, 0° C, 95%. (e) p-TsOH (0.2 eq.), EtOH, 95%. (f) Dess-Martin periodinane, CH_2Cl_2. (g) $NaCl_2O$, NaH_2PO_4, 2-methyl-2-butene, tBuOH, 80%. (h) PPh_3, pyridine then NH_4OH (conc.) 2 h 75%. (i) MMT-Cl, triethylamine, DMSO, 83%.

With large amounts of ester **1** in hand the protected *E*-OPA amino acid was ready for assembly (Scheme 5). Azide **6** was produced by reduction of **1** with $LiAlH_4$ in good yield followed by azidation using modified Mitsunobu conditions with the more soluble zinc diazide dipyridine complex and diisopropyl azodicarboxylate (DIAD) in toluene.[10] The silyl ether was very efficiently cleaved using standard tetrabutylammonium fluoride treatment. The N^3-benzoyl protected thymine nucleobase[11] was subsequently introduced in high yield under Mitsunobu conditions to afford **7**.

The THP acetal of **7** was cleanly removed by acidic treatment to afford the free alcohol. Initial attempts to oxidize the alcohol directly to the acid with PDC were unsuccessful due to the inability to separate the polar acid **8** from the chromate salt by-products. Instead, a two-step oxidation was utilized. The alcohol was oxidized to the aldehyde using the Dess-Martin periodinane.[12] The crude aldehyde was then oxidized with sodium chlorite in *tert*-butyl alcohol with 2-methyl-2-butene as a Cl^+ scavenger to afford the acid **8** in good yield.[13] The next step was the reduction of the azide **8** to the primary amine. Hydrogenation using Lindlar conditions repeatedly failed in our hands. This result is interesting in view of the fact that the corresponding transformation in the *Z*-OPA series proceeds successfully and in high yield. Successful reduction to the amino acid was accomplished by treatment of the azide **8** with triphenylphosphine in pyridine,[14] followed by hydrolysis of the phosphinimine (and thymine benzoyl protecting group) with ammonium hydroxide to give the free amino

acid **9**,[15] which was subsequently protected using monomethoxytrityl chloride in DMSO with triethylamine to afford **10** as the triethylammonium salt.[16]

The herein described divergent synthesis allows incorporation of a trisubstituted olefin of defined geometry which is then efficiently assembled into the final OPA skeleton by a palladium(0) catalyzed Reformatsky reaction. Removal of the silyl protecting group from **6** will allow the introduction of any nucleobase and work in this direction is currently under way. Furthermore, the final protected amino acid salt **10** is suitable for use in solid-phase peptide synthesis and work towards incorporation into oligomers is currently being investigated.

Acknowledgement

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References and notes

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(8) Synthesis of **1**: In a dry atmosphere **4** (16.13g, 29.3 mmol) was dissolved in dry DMPU (390 ml), then tetrakis(triphenylphosphine) palladium(0) (6.82 g, 5.86 mmol) was added and the mixture heated to 70° C. In a separate flask, freshly activated zinc powder (10.0 g) was suspended in dry dimethoxymethane (15.0 ml) containing freshly distilled ethyl α-bromoacetate (3.0 ml). The flask was placed in a 50° C oilbath with vigorous stirring until initiation had occurred, at which point the reaction was removed from heat, and with continuous stirring, ethyl α-bromoacetate (12.0 ml) dissolved in dimethoxymethane (80 ml) was added at such a rate that a reflux was maintained. After addition was complete the mixture was refluxed for an additional 20 min. The resulting green mixture was then removed from the heat and stirring was stopped. After 20 min the green solution was carefully removed from the deposited zinc and added to the solution of **4** in one batch. After 1 h at 70° C the reaction was quenched by the addition of 100 ml of saturated aqueous NH_4Cl. After standard aqueous work-up, the resulting crude mixture was separated by flash chromatography (SiO_2, 5% ether/hexane to 25% ether/hexane) to give **1** (11.39 g, 22.3 mmol) in 76% yield and the bis-coupled product **5** (1.07 g, 1.9 mmol) in 7% yield.
(9) Activation of the zinc as described in Perrin, D. W.; Armarego, W. L. F.; Perrin, D. W. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon: Oxford, 1980; p 547 and use of freshly prepared tetrakis(triphenylphosphine)palladium(0), Coulson, D. R. in *Inorganic Syntheses*; Vol. 28; Angelici, R. J., Ed.; Wiley: New York, 1990; p 107. rather than the use of commercially available sources were necessary for satisfactory and reproducible results.
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(15) Spectral characterization of amino acid **9**: **^{1}H NMR** D_2O (δ): 1.73 (s, 3H), 2.47 (t, *J* = 7.35 Hz, 2H), 2.87 (s, 2H), 2.99 (t, *J* = 7.35 Hz, 2 H), 4.29 (d, *J* = 6.98 Hz, 2H), 5.37 (t, *J* = 6.98 Hz, 1H), 7.36 (s, 1H). **^{13}C NMR** D_2O (with DMSO reference) (δ): 12.81, 29.75, 38.73, 46.48, 47.45, 112.3, 127.08, 136.64, 144.18, 153.68, 168.39, 181.15. Anal. calcd. for $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72. Found: C, 53.54; H, 6.39; N, 15.63.
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