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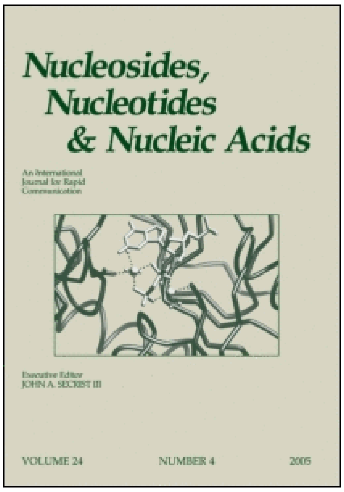

## Nucleosides, Nucleotides and Nucleic Acids

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# Synthesis of Chirally Pure Ornithine Based PNA Analogues

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NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 219-231 (1998)

# SYNTHESIS OF CHIRALLY PURE ORNITHINE BASED PNA ANALOGUES

**Alexander C. van der Laan[a], Irene van Amsterdam[a], Godefridus I. Tesser[b], Jacques H. van Boom[a*] and Esther Kuyl-Yeheskiely[a#]**

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**ABSTRACT:** A stereoselective synthesis of a chiral PNA analogue containing an ornithine based backbone is described. In this approach each elongation cycle consists of two individual coupling steps: *i.e.* extension of the free δ-amino function in the growing chain by a TOPPipU mediated coupling with Fmoc-Orn(Boc)-OH, and subsequent acylation of a free α-amine with thymin-1-ylacetic acid. Thyminyl decamers were prepared following this strategy and hybridization experiments indicated that they formed stable complexes with cRNA.

## INTRODUCTION

Peptide Nucleic Acids (PNA) in which the natural nucleobases are attached *via* a carbonyl methylene linker to a *N*-(2-aminoethyl)glycine backbone hybridize strongly and sequence specifically with complementary DNA (or RNA).[1] In addition, this type of DNA mimic is not degraded by nucleases and proteases.[2] These interesting features make PNA attractive for potential therapeutics and diagnostic tools. The favourite properties of PNA stimulated other groups to develop PNA analogs. For instance, Lenzi *et al.* prepared a PNA mimic containing aminobutyric acid and glycine as backbone.[3] It was demonstrated that this chiral peptidic DNA exhibited self recognition similar to that of DNA-DNA duplexes.

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† Dedicated to the late professor Tsujiaki Hata

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Recently, we[4] and independently Nielsen[5] and Kessler[6] reported a chiral PNA analogue, the backbone of which contains the amino acid ornithine (ONA, FIG. 1). The ornithine residue has the requisite six covalent bonds in the monomeric backbone as in PNA. The ONA monomers are linked *via* amide bonds between the carboxyl and the δ-amino function of the ornithine residues. Furthermore, the nucleobases are attached *via* a *N*-acyl (*i.e.* carbonyl methylene) linkage to the α-amino function of ornithine. Nielsen[5] and Kessler[6] assembled ONA decamers using monomer **3** (SCHEME 1). However, our preliminary studies as well as the results reported by Nielsen[5] indicated that the synthesis of ONA oligomers using monomer **3** was accompanied by a significant amount of epimerisation. The latter is due to the fact that activated $N^{\alpha}$-acyl amino acids are prone to racemisation. Furthermore, biophysical studies[5,6] showed that these ONA decamers did not hybridize to DNA and only poorly to RNA. We reasoned that the poor stability of the complexes of the ONA decamers with complementary nucleic acid fragments may be attributed to the fact that the ONA fragment was not chirally pure.

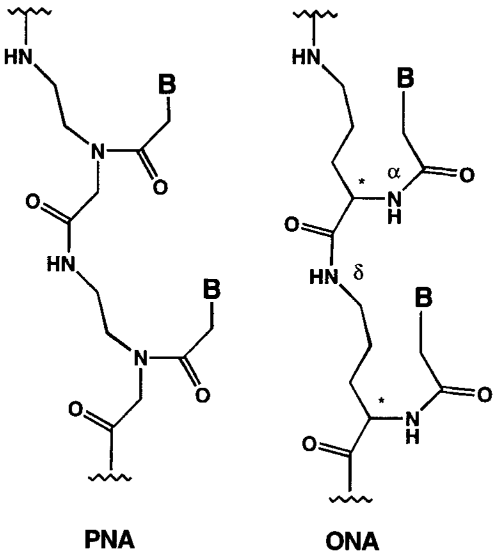
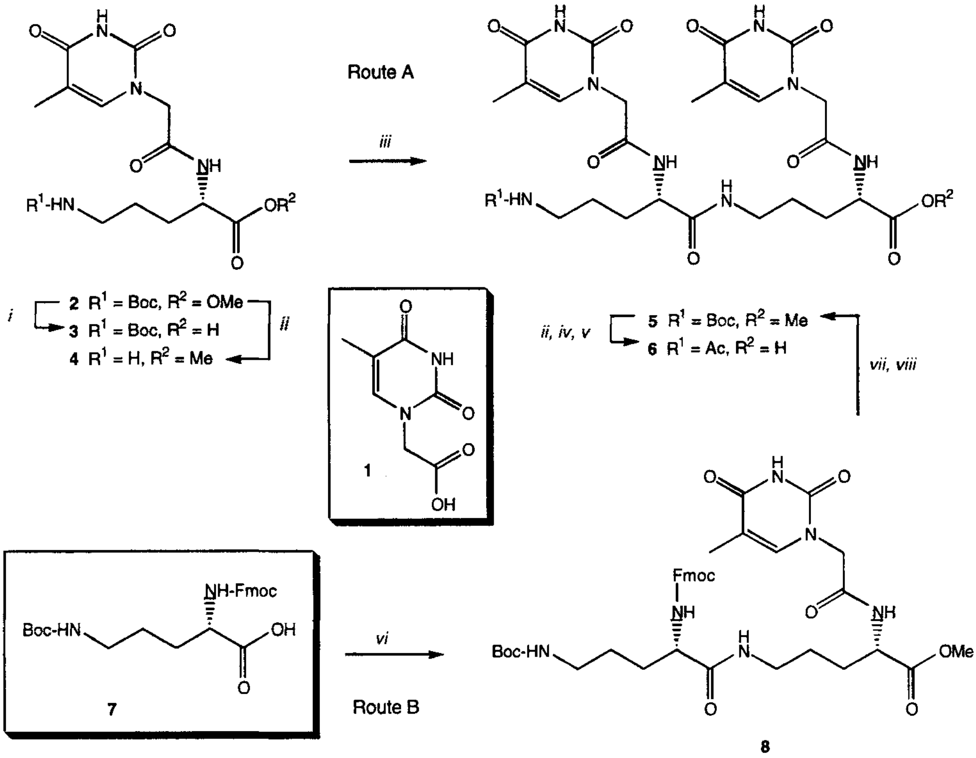


**FIG. 1.** Structures of PNA and ONA

In order to substantiate this assumption, we here report the synthesis and hybridization properties of chirally pure ONA thyminyl decamers containing either L-ornithine, *i.e.* $[\text{L-T}_{\text{ONA}}]_{10}$-Lys-$NH_2$ (**19**), D-ornithine, *i.e.* $[\text{D-T}_{\text{ONA}}]_{10}$-Lys-$NH_2$ or alternating L- and D-ornithine, *i.e.* $[\text{D-T}_{\text{ONA}}\text{-L-T}_{\text{ONA}}]_5$-Lys-$NH_2$.

## RESULTS AND DISCUSSION

It is well known[7,8] in peptide chemistry that the method of activation is one of the dominating factors for racemisation of amino acids. Therefore, we initially attempted to prepare chirally pure dimer **6** from the monomers **3** and **4** using different activation methods (SCHEME 1). To



**Reagents and Conditions:** (*i*) NaOH, 5 min, 87%; (*ii*) 25% TFA in $CH_2Cl_2$, 1.5 h, 99%; (*iii*) See Table 1; (*iv*) $Ac_2O$ (2.0 eq.) in pyridine, 2 h; (*v*) NaOH, 5 min (60% based on **5**); (*vi*) **4** (1.0 eq.), HBTU (1.0 eq.), DiPEA (1.0 eq.) in DMF, 1 h, 99%; (*vii*) 20% piperidine in DMF, 10 min; (*viii*) **1** (1.0 eq.), HBTU (1.0 eq.), DiPEA (1.0 eq.) in DMF, 1.5 h (75% based on **8**).

**SCHEME 1.** Synthesis of Ac-L-$T_{ONA}$-L-$T_{ONA}$-OH dimer **6**

this end, commercially available H-Orn(Boc)-OMe was acylated with thymin-1-ylacetic acid[9] (**1**) under the agency of 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) to give ornithine derivative **2**. Saponification or acidolysis of **2** yielded monomers **3** and **4**, respectively. Condensation of $N^{\delta}$-*t*-butyloxycarbonyl (Boc) derivative **3** with the free amino function in building block **4**, to give dimer **5**, was carried out using different coupling reagents (see TABLE 1). Acidolysis of the Boc group in **5** followed by acetylation of the resulting primary amine and saponification of the methyl ester afforded dimer **6**. The chiral purity of **6** was analysed with reversed phase HPLC. The results, summarized in TABLE 1, show that HBTU, BOP, $CF_3$-$NO_2$-PyBOP[10] or DCC/HOBt mediated peptide bond formation

**TABLE 1.** Racemisation experiments

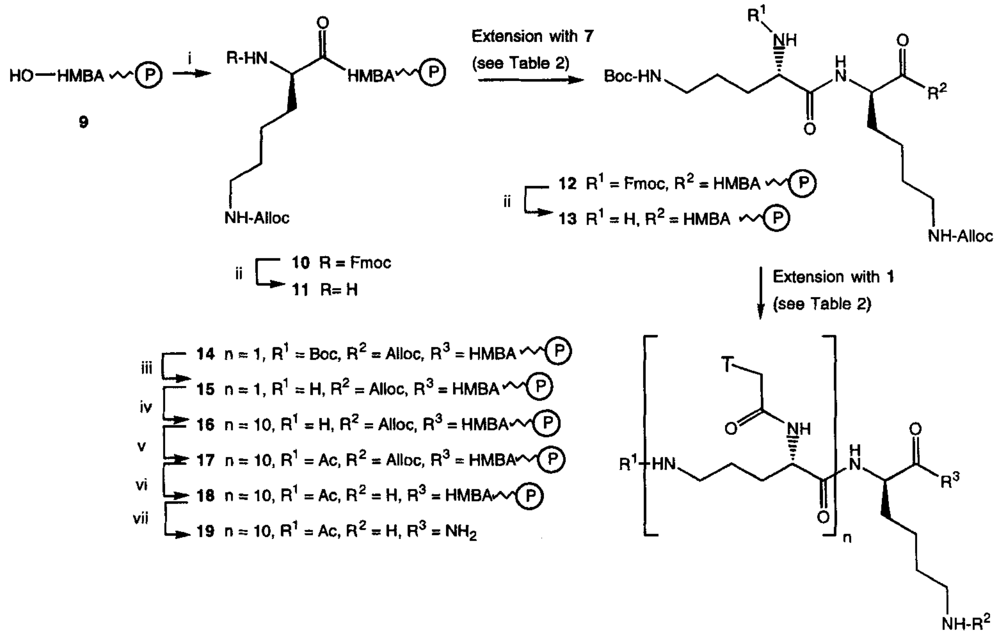
| Entry | Coupling reagents[a] | Racemisation[b] | Yield[c] |
|---|---|---|---|
| 1 | HBTU (1 eq.), DiPEA (1 eq.) | 17% | 75 % |
| 2 | BOP (1 eq.), DiPEA (1 eq.) | 10% | 78 % |
| 3 | $CF_3NO_2$PyBOP (1 eq.), DiPEA (1 eq.) | 25% | 80 % |
| 4 | DCC (1 eq.), HOBt (2 eq.) | 11% | 76 % |

[a]Coupling was performed using **3** (100 mg, 0.2 mmole) and **4** (70 mg, 0.2 mmole) in $CH_3CN$ (3 mL), 1 h. [b]Estimated by RP-HPLC. [c]After work up and precipitation from diethyl ether.

was accompanied by an extensive amount of epimerisation. On the other hand, it is also well established that protection of the α–amino function with an urethane-type group will suppress azalactone formation of the activated carboxylic acid[7] and consequently preserve the chiral purity during coupling. It occurred to us that racemisation during the assembly of ONA oligomers could be avoided following an alternative synthetic strategy using Fmoc-L-Orn(Boc)-OH (**7**) and thymin-1-ylacetic acid (**1**) as building blocks. In this strategy each elongation cycle consists of two individual coupling steps (SCHEME 1, route B). The first step entails condensation of the free δ–amino function in the growing chain with $N^{\alpha}$-fluoren-9-ylmethoxycarbonyl (Fmoc) derivative **7**. In the second step, the free α–amine, obtained after removal of the Fmoc group, is acylated with acid derivative **1**. In order to substantiate the viability of this approach, we first turned our attention to the synthesis of the ONA dimer **6**. To this end, amino component **4** was condensed with $N^{\alpha}$-Fmoc protected ornithine **7** under the agency of 2-(benzotriazol-1-yloxy)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N,N*-diisopropylethylamine (DiPEA) to afford the Fmoc derivative **8** in a near quantitative yield. Removal of the Fmoc group in **8** with 20% piperidine in *N,N*-dimethylformamide (DMF), and subsequent acylation of the resulting primary amino function with thymin-1-ylacetic acid (**1**) in the presence of HBTU/DiPEA, gave the fully protected dimer **5** in 75% yield. Cleavage of the Boc group in **5** followed by acetylation of the free amino function and saponification gave crude LL-dimer **6**. The chiral purity of LL-**6**, obtained after gel filtration (HW-40), was corroborated by RP-HPLC. It was found that the LL-**6** had a different retention time as the independently synthesized DL-diastereomer **6'**: no epimerisation was found.

These promising results prompted us to investigate the synthesis of thyminyl ONA decamer [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$ (*i.e.* **19**) using a fully automated peptide synthesizer. The assembly of ONA decamer **19** (SCHEME 2) is initiated by immobilization of Fmoc-Lys(Alloc)-OH to controlled pore glass (CPG) **9**, functionalized with the base-labile hydroxymethylbenzoic acid (HMBA) linker, under the agency of DCC and 4-dimethylaminopyridine (DMAP). The resin bound lysine (Lys) derivative **11**, resulting after removal of the Fmoc group in **10**, was extended with Fmoc-Orn(Boc)-OH (**7**), using 2-(2-oxo-1(2H)-pyridyl)-1,1,3,3-pentamethyleneuronium tetrafluoroborate (TOPPipU)[11] as condensing agent, to provide fully protected intermediate **12**. Cleavage of the $N^{\alpha}$-Fmoc group from CPG-bound **12** and condensation of intermediate **13** with thymin-1-ylacetic acid (**1**) led to fragment **14**. Acidolysis of the $N^{\delta}$-Boc group in **14** gave intermediate **15**. Sequential two-step elongation of **15** with building blocks **7** and **1** according to the protocol in TABLE 2 afforded, after nine elongation cycles, immobilized decameric fragment **16**. The primary amino function in **16** was acetylated with acetic anhydride to give decamer **17**. In the final stage of the synthesis, fully protected compound **17** was deblocked and released from the solid support by the following two-step procedure. Removal[12] of the ε-Alloc group from **17** with $Pd(PPh_3)_4$ in presence of HOAc and $Bu_3SnH$ afforded immobilized decamer **18**. Finally, ammonolysis ($NH_3$/EtOH) of intermediate **18** gave decamer **19**. Analysis of crude ONA fragment **19** by ion-exchange chromatography (Mono Q, FIG. 2) indicated that each coupling step, mediated by TOPPipU/DiPEA, proceeded with an average efficiency greater than 95%. Purification of the crude oligomer **19** by ion-exchange chromatography (Q-Sepharose) and gel-filtration (G-25) yielded homogeneous [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$ (**19**), as evidenced by MonoQ and mass spectrometry. The corresponding [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$ and [D-$T_{ONA}$-L-$T_{ONA}$]$_5$-Lys-$NH_2$ decamers were also prepared following the synthetic strategy as outlined in SCHEME 2.

At this stage, we examined whether [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$, [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$ and [D-$T_{ONA}$-L-$T_{ONA}$]$_5$-Lys-$NH_2$ would hybridize to complementary oligonucleotides. It was found that none of the ONA decamers hybridized with complementary deoxyribo decamer $dA_{10}$. On the other hand, [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$ and [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$ formed stable complexes with RNA decamer $rA_{10}$ (FIG. 3). In addition, it was shown that [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$ hybridizes more strongly with $rA_{10}$ (Tm = 20.1 °C) than the corresponding [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$ (Tm = 17.1 °C). Interestingly, the [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$•$rA_{10}$ complex proved to be more stable than



**Reagents and Conditions:** (*i*) Fmoc-Lys(Alloc)-OH (10 eq.), DCC (10 eq.), DMAP (1.0 eq.) in DMF, 1 h; (*ii*) 20% Piperidine in NMP, 3 min; (*iii*) 50% TFA in $CH_2Cl_2$; (*iv*) Extension with **7** and **1** (see Table 2); (*v*) $Ac_2O$ (0.5 M), DiPEA (0.125 M), HOBt (0.015 M) in NMP; (*vi*) $Pd(PPh_3)_4$ (cat.), HOAc (10 eq.), $Bu_3SnH$ (6 eq.), 1.5 h; (*vii*) $NH_3$ in EtOH, 16 h.

**SCHEME 2.** Racemisation free synthesis of $[L\text{-}T_{ONA}]_{10}$-Lys-$NH_2$ **19**

**TABLE 2.** Chemical steps involved in each elongation cycle of ONA

| Step | Manipulation | Solvents and reagents[a] | Time (min) |
|---|---|---|---|
| 1 | Coupling | Fmoc-Orn(Boc)-OH (**7**)[b], TOPPipU[c] (125 µL), DiPEA[d] (125 µL) | 30 |
| 2 | Capping | $Ac_2O$ (0.5 M), DiPEA (0.125 M), HOBt (0.015 M) in NMP | 1 |
| 3 | Deprotection | 20% Piperidine in NMP | 3 |
| 4 | Coupling | **1**[e], TOPPipU[c] (250 µL), DiPEA[d] (250 µL) | 60 |
| 5 | Capping | see Step 2 | 1 |
| 6 | Deprotection | 50% TFA in $CH_2Cl_2$ | 3 |

[a] Reactions were performed on 100 mg (5µmole) of resin. [b] 0.25 M **7** in NMP (100 µL). [c] 0.2 M TOPPipU in NMP. [d] 0.4 M DiPEA in NMP. [e] 0.25 M **1** in NMP (200 µL).

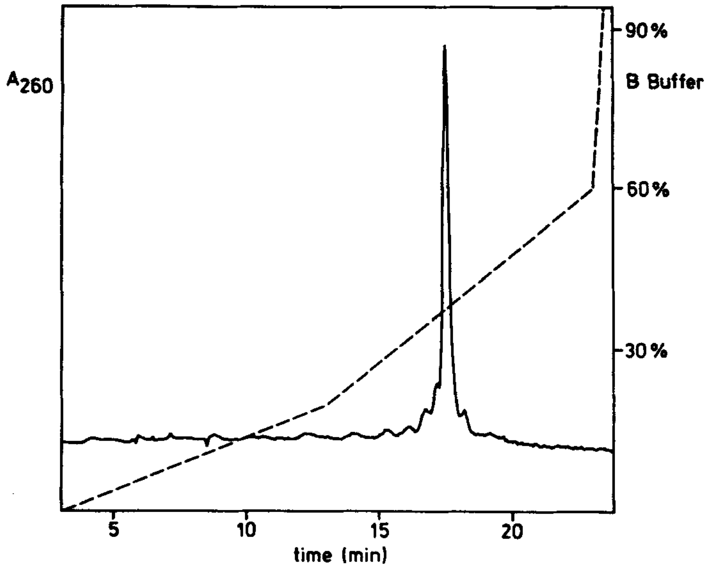


**FIG. 2.** Ion-exchange chromatograph of crude **19**.

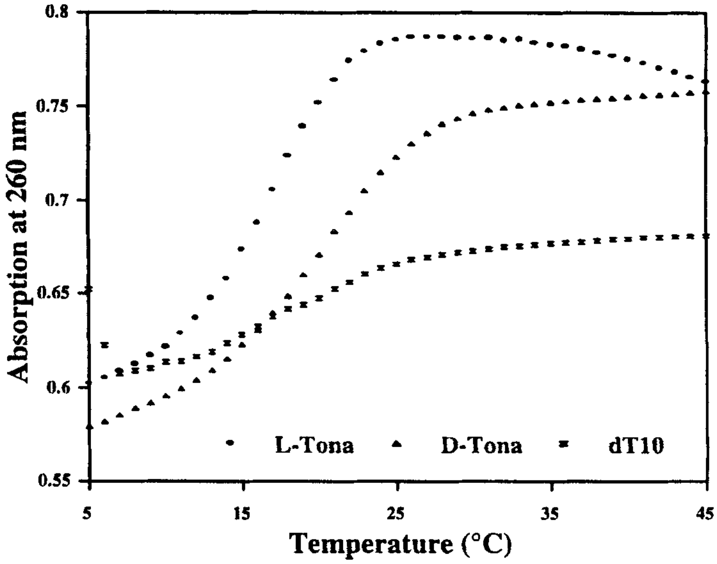


**FIG. 3.** UV melting curves for [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$, [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$ and $dT_{10}$ with $rA_{10}$.

the DNA•RNA duplexes ($dT_{10}$•$rA_{10}$: Tm = 18.5 °C). Furthermore, it is also important to note that the alternating [D-$T_{ONA}$-L-$T_{ONA}$]$_5$-Lys-$NH_2$ decamer hybridized neither with DNA nor with RNA.

## CONCLUSION

The convenient and stereoselective synthesis of thyminyl ONA decamers presented in this paper opens the way to a general solid-phase synthesis of ONA oligomers containing the common four nucleobases as well as other amino acid based oligonucleotides. It was demonstrated that both [L-$T_{ONA}$]$_{10}$-Lys-$NH_2$ and [D-$T_{ONA}$]$_{10}$-Lys-$NH_2$ formed stable complexes with complementary RNA. Furthermore, it is evident that the chirality of the ONA backbone is one of the factors determining the stability of the complexes.

## EXPERIMENTAL

DMF, dichloromethane, *N*-methylpyrrolidine (NMP) (Biosolve, peptide synthesis grade) and acetonitrile (Rathburn, HPLC grade) were stored over molecular sieves 4 Å. DiPEA and trifluoroacetic acid (TFA) were purchased from Biosolve and used as delivered. HOBt was purchased from Fluka and dried over phosphorus pentoxide for 20 h at 40°C. HBTU, DCC, BOP and TOPPipU were obtained from NOVA BIOCHEM. $CF_3NO_2$-PyBOP was prepared by published procedures[10].

NMR spectra were recordered on a JEOL JNM-FX 200 spectrometer ($^1H$ and $^{13}C$ at 200 and 50.1 MHz respectively) and a Bruker 600-DMX spectrometer ($^1H$ at 600 MHz). Chemical shifts (δ) are given in ppm relative to the signal for internal or external $Me_4Si$ for $^1H$ and to the signal for internal $CDCl_3$, DMSO-$d_6$ or external $Me_4Si$ for $^{13}C$. Mass spectra were recordered on a Finnigan MAT TSQ-70 equipped with a custom made Electron Spray Interface (ESI) and a PerSeptive Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer.

Solid Phase Syntheses were carried out on a ABI 433A peptide synthesizer. RP-HPLC analysis was performed on a AllTech Econosphere C18 column (5 micron, I.D. = 4.6 mm, length = 250 mm). Isocratic elution was performed at 40 °C by applying 0.1% TFA in $CH_3CN/H_2O$ (10/90, v/v). Ion-exchange analysis was carried out on a Pharmacia Mono Q HR 5/5 anion-exchange resin, flow rate 2.0 ml/min. Elution was effected using Buffer A: (0.01 M NaOH in $H_2O$ (pH = 12) and applying buffer B: (0.01 M NaOH + 1.2 M NaCl in $H_2O$, pH

= 12). The following gradient was used: 3 min buffer A, change to 20% B buffer in 10 min, change to 60% B buffer in 10 minutes.

Thermal melting studies were performed at 260 nm using a Perkin Elmer Lambda 12 UV/VIS Spectrometer equiped with a PTP-6 Peltier Temperature Programmer.

**5-*N*-[5'-*N*-(*t*-Butyloxycarbonyl)-2'-*N*-(thymin-1-ylacetyl)-L-ornithinyl]-2-*N*-(thymin-1-ylacetyl)-L-ornithine methyl ester (5).** Method A: To a suspension of acid **3**[6] (100 mg, 0.2 mmol), amine **4** (70 mg, 0.2 mmol) and either HBTU (77 mg, 0.2 mmol), BOP (88 mg, 0.2 mmole) or $CF_3$-$NO_2$-PyBOP (127 mg, 0.2 mmol) in anhydrous $CH_3CN$ (3 mL) was added DiPEA (70 µL, 0.4 mmol). After stirring for 60 min the mixture was diluted with EtOAc (100 mL), washed with $H_2O$ (30 mL), $NaHCO_3$ (M, 2x 30 mL), $H_2O$ (30 mL), $KHSO_4$ (M, 2x 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated to dryness. The residue was dissolved in $CH_2Cl_2$ and added dropwise to diethyl ether (100 mL). The resulting precipitate was collected, washed with diethyl ether and dried *in vacuo*. HBTU: Yield 103 mg (75%) as a white solid. BOP: Yield 108 mg (78%). $CF_3NO_2$PyBOP: Yield 111 mg (80%). Method B: To a suspension of **3** (100 mg, 0.2 mmol), **4** (70 mg, 0.2 mmol) and HOBt (54 mg, 0.4 mmol) in dry $CH_3CN$ (3 mL) was added DCC (41 mg, 0.2 mmol). After stirring for 1.5 h compound **5** was isolated as described in method A. Yield 105 mg (76%). ESI-MS (*m/z*): 693 $[M+H]^+$. $^{13}C\{^1H\}$ NMR ($CDCl_3$): 8.2 ($CH_3$, thymine), 11.2 ($CH_3$, thymine), 24.8, 25.7 ($\gamma$-$CH_2$), 27.8 ($CH_3$ Boc), 28.2 ($\beta$-$CH_2$), 38.3, 38.5 ($\delta$-$CH_2$), 49.8, 50.0 ($CH_2$), 52.1 ($\alpha$-CH), 53.4 ($OCH_3$), 79.4 (C Boc), 110.3 (C-5), 141.9, 142.0 (C-6), 151.5 (C-2), 146.7 (C=O Boc), 165.0 165.1 (C-4), 167.6, 167.7 (C=O amide), 172.2 (C=O ester).

**5-*N*-[5'-*N*-Acetyl-2-*N*-(thymin-1-ylacetyl)-L-ornithinyl]-2-*N*-(thymin-1-ylacetyl)-L-ornithine (6).** To a mixture of dimer **5** (103 mg, 0.15 mmol) in $CH_2Cl_2$ (2.5 mL) was added TFA (2.5 mL). After 1.5 h stirring the reaction mixture was diluted with $CH_2Cl_2$ (50 mL) and extracted with $H_2O$ (3x 30 mL). The combined waterlayers were concentrated to dryness and coevaporated 3 times with toluene. The residue was dissolved in dry DMF (5 mL), $Ac_2O$ (90 µL, 1.0 mmol) and $Et_3N$ (138 µL, 1.0 mmol) were added and the resulting solution was stirred for 2 h. The reaction mixture was quenched with MeOH (1 mL) and concentrated *in vacuo*. To the residue was added $CH_2Cl_2$ (30 mL), the resulting precipitate was collected by filtration, dissolved in $H_2O$/MeOH (2.5 mL, 4/1, v/v) and NaOH (M, 1 mL) was added. After stirring for 5 min the pH was readjusted to pH 7 with HOAc and the resulting mixture was concentrated to dryness. The residue was purified by gelfiltration (HW 40, eluent: 1% AcOH in $H_2O$) and lyophilized. Yield 55 mg (60%) as a white fluffy solid. ESI-MS (*m/z*) 621 $[M+H]^+$. RP-HPLC: $t_R$ 32.7 min. $^1$H NMR ($D_2O$): $\delta$ 1.53-1.68 (m, 4H, 2x $\gamma$-$CH_2$), 1.69-1.84 (m, 4H, 2x $\beta$-$CH_2$), 1.88 (bs, 6H, 2x $CH_3$ thymine), 1.98 (s, 3H, $CH_3$ acetyl), 3.17-3.25 (m, 4H, 2x $\delta$-$CH_2$), 4.22-4.26 (m, 1H, $\alpha$-CH), 4.39-4.44 (M, 1H, $\alpha$-CH), 4.52-4.55 (m, 4H, 2x $CH_2$), 7.43 (s, 2H, 2x H-6). $^{13}C\{^1H\}$ NMR ($D_2O$): $\delta$ 13.6 ($CH_3$ thymine), 23.8 ($CH_3$ acetyl), 26.9, 27.0 ($\gamma$-$CH_2$), 30.2, 30.4 ($\beta$-$CH_2$), 40.9, 41.1 ($\delta$-$CH_2$), 52.7, 52.9 ($CH_2$), 54.8, 56.4 ($\alpha$-CH), 113.1 (C-5), 145.4 (C-6), 169.2 (C=O acetyl), 171.5, 171.6 (C=O amide), 175.8 (C=O acid).

**5-*N*-[5'-*N*-(*t*-Butyloxycarbonyl)-2'-*N*-(9-fluorenylmethoxycarbonyl)-L-ornithinyl]-2-*N*-(thymin-1-ylacetyl)-L-ornithine methyl ester (8).** Amine **4** (700 mg, 2.0 mmole), Fmoc-Orn(Boc)-OH **7** (900 mg, 2.0 mmol) and HBTU (765 mg, 2.0 mmol) were dissolved in $CH_3CN$ (30 mL) and the coupling was initiated by adding DiPEA (650 µL, 4.0 mmole). After stirring for 1 h the mixture was diluted with EtOAc (200 mL), washed with $H_2O$ (50 mL), $NaHCO_3$ (M, 2x 50 mL), $H_2O$ (50 mL), $KHSO_4$ (M, 2x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, evaporated to dryness, redissolved in $CH_2Cl_2$/MeOH (10 mL, 1/1 v/v) and added dropwise to diethyl ether. The precipitate was collected by filtration and dried *in vacuo*. Yield 1.49 mg (99%). ESI-MS (*m/z*): 749 $[M+H]^+$. $^1$H NMR ($CDCl_3$): $\delta$ 1.36 (s, 9H, $CH_3$ Boc), 1.50 (m, 2H, $\gamma$-$CH_2$), 1.59 ($\gamma$-$CH_2$), 1.76 (m, 2H, $\beta$-$CH_2$ Orn-1), 1.86 (s, 3H, $CH_3$ Thymine), 1.86 (m, 2H, $\beta$-$CH_2$ Orn-2), 2.81 (m, 1H, NH), 3.12 (m, 2H, $CH_2$-T), 3.64 (m, 1H, $\alpha$-CH Orn-2), 3.66 (s, 3H, $OCH_3$), 4.15 (m, 1H, $\alpha$-CH Orn-1), 4.19 (m, 1H, CH Fmoc), 4.23 (m, 2H, $\delta$-$CH_2$ Orn-1), 4.38 (m, 2H, $CH_2$ Fmoc), 7.15 (s, 1H, H-6), 7.31 (m, 2H, arom-CH Fmoc), 7.39 (m, 2H, arom-CH Fmoc), 7.63 (m, 2H, arom-CH Fmoc), 7.75 m, 2H, arom-CH Fmoc). $^{13}C\{^1H\}$ NMR ($CDCl_3$): $\delta$ 12.0 ($CH_3$ thymine), 25.5, 26.1 ($\gamma$-$CH_2$), 28.4 ($CH_3$ Boc), 28.7, 29.6 ($\beta$-$CH_2$), 38.7 ($\delta$-$CH_2$ Orn-2), 47.3 (CH Fmoc), 50.5 ($\delta$-$CH_2$ Orn-1), 52.4, ($OCH_3$), 54.3, 54.8 ($\alpha$-CH), 67.2 ($CH_2$ Fmoc), 79.5 (C Boc), 110.8 (C-5), 120.1, 125.2, 127.3, 127.9 (arom-CH Fmoc), 141.4 (arom-C Fmoc), 142.0 (C-6), 144.0 (C=O Boc), 151.8 (C-2), 157. (C=O Fmoc), 165.3 (C-4), 167.8 (C=O amide), 172.7 (C=O ester).

**5-*N*-[5'-*N*-(*t*-Butyloxycarbonyl)-2'-*N*-(thymin-1-ylacetyl)-L-ornithinyl]-2-*N*-thymin-1-ylacetyl)-L-ornithine methyl ester (5).** To a solution of **8** (0.75 mg, 1.0 mmole) in DMF (8 mL) was added piperidine (2 mL) and the mixture was stirred for 15 min. $H_2O$ (50 mL) was added, the mixture was washed with diethyl ether (3x 20 mL) and the waterlayer was evaporated to dryness. The residue, after coevaporation with $CH_3CN$ (2x) was dissolved in $CH_3CN$ (15 mL) and thymin-1-ylacetic acid (276 mg, 1.5 mmole), HBTU (573 mg, 1.5

mmole) and DiPEA (325 µL, 2.0 mmole) were added. After stirring for 1.5 h the mixture was diluted with EtOAc (200 mL), washed with $H_2O$ (50 mL), $NaHCO_3$ (M, 2x 50 mL), $H_2O$ (50 mL), $KHSO_4$ (2x 50 mL) and brine (50 mL). The organic layer was evaporated to dryness, the residue was dissolved in $CH_2Cl_2$ and added dropwise to diethyl ether (100 mL). The precipitate was collected, washed with diethyl ether and dried *in vacuo*. Yield 510 mg (75%).

**5-*N*-[5'-*N*-Acetyl-2-*N*-(thymin-1-ylacetyl)-L-ornithinyl]-2-*N*-(thymin-1-ylacetyl)-L-ornithine (6').** Compound **6'** was prepared analogous to **6** using Fmoc-D-Orn(Boc)-OH. RP-HPLC: $t_R$ = 34.2 min.

**Solid Phase Synthesis of [L-$T_{ONA}$]$_{10}$-Lys(Alloc)-HMBA-CPG (17).** Immobilized undecapeptide **17** was assembled using the Boc/Fmoc strategy. The synthesis was carried out on 100 mg Fmoc-Lys(Alloc)-HMBA-CPG resin **10** (5 µmole). The assembly of the peptide was performed, after cleavage of the first Fmoc group with 20% piperidine in NMP, using Fmoc-Orn(Boc)-OH (**7**) and thymin-1-ylacetic acid (**1**). Condensation was performed with TOPPipU in presence of DiPEA. The protocol for one elongation/deprotection cycle consisted of (1) wash: NMP (10 mL); (2) coupling: Fmoc-Orn(Boc)-OH (**7**, 5 eq.), TOPPipU (5 eq.), DiPEA (10 eq.) in NMP (0.35 mL), 30 min; (3) wash: NMP (2.5 mL); (4) capping: $Ac_2O$ (0.5 M), DiPEA (0.125 M), HOBt (0,015 M) in NMP (2.0 mL), 1 min; (5) wash: NMP (10 mL); (6) Fmoc cleavage: 20% piperidine in NMP (1.5 mL), 3 min; (7) wash: NMP (2.5 mL); (8) coupling: thymin-1-ylacetic acid (**1**, 10 eq.), TOPPipU (10 eq.), DiPEA (20 eq.) in NMP (0.7 mL), 60 min; (9) see step 3; (10) see step 4 (11) see step 5; (12) Boc cleavage: 50% TFA in $CH_2Cl_2$ (1.5 mL), 3.5 min; (13) wash: $CH_2Cl_2$ (10 mL). After the last elongation cycle the free terminal amino function was capped with $Ac_2O$ as described in step 4 in the elongation cycle, washed with NMP (10 mL), $CH_2Cl_2$ (10 mL) and air dried.

**Deprotection and Cleavage from Resin.** The obtained anchored peptide **17** was deprotected by treatment with HOAc (10 eq.), $Bu_3SnH$ (6 eq.), $Pd(PPh_3)_4$ (cat.) in $CH_2Cl_2$ (3.5 mL) for 1 h. The immobilized oligomer **18** was collected by filtration and washed with $CH_2Cl_2$ (3x 1 mL), DMF (2x 1 mL), 0.5% DiPEA in DMF (3x 1 mL), DMF (1 mL), 0.5% sodium diethyl dithiocarbamate in DMF (3x 1 mL), DMF (2x 1 mL) and $CH_2Cl_2$ (3x 1 mL). To the resulting immobilized oligomer **18** was added saturated $NH_3$ in EtOH (3 mL) and shaken for 16 h. The support was removed by filtration, $H_2O$ (15 mL) was added and the mixture was evaporated to dryness. The crude oligomer **19** was purified on Q-Sepharose, desalted on G-25 and lyophilized to obtain pure **19**, as a white fluffy solid, according to ion-exchange chromatography (MonoQ: $t_R = 17.5$ min). MALDI-TOF MS: (*m/z*) 2992 $[M+H]^+$.

**Thermal Melting Studies.** Melting temperature (Tm) was measured with 1:1 molar mixtures of $[L\text{-}T_{ONA}]_{10}$-Lys-$NH_2$, $[D\text{-}T_{ONA}]_{10}$-Lys-$NH_2$ or $[D\text{-}T_{ONA}\text{-}L\text{-}T_{ONA}]_5$-Lys-$NH_2$ and complementary DNA or RNA, each at a concentration of 3 μM, in a 10 mM phosphate buffer, pH = 7.0, containing 100 mM NaCl and 0.1 mM EDTA. Prior to recording the melting profile, the solutions were heated to 90 °C for several minutes and then cooled to 25°C over 1.5 h.. The solutions were heated from 25 to 80°C at a rate 0.5°C per minute and the $A_{260}$ was recordered against temperature. The Tm values were determined from the maxima of the first-derivative plots of absorbance versus temperature.

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