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# Synthesis of Thymidine Dimer Derivatives Containing an Amide Linkage and their Incorporation into Oligodeoxyribonucleotides

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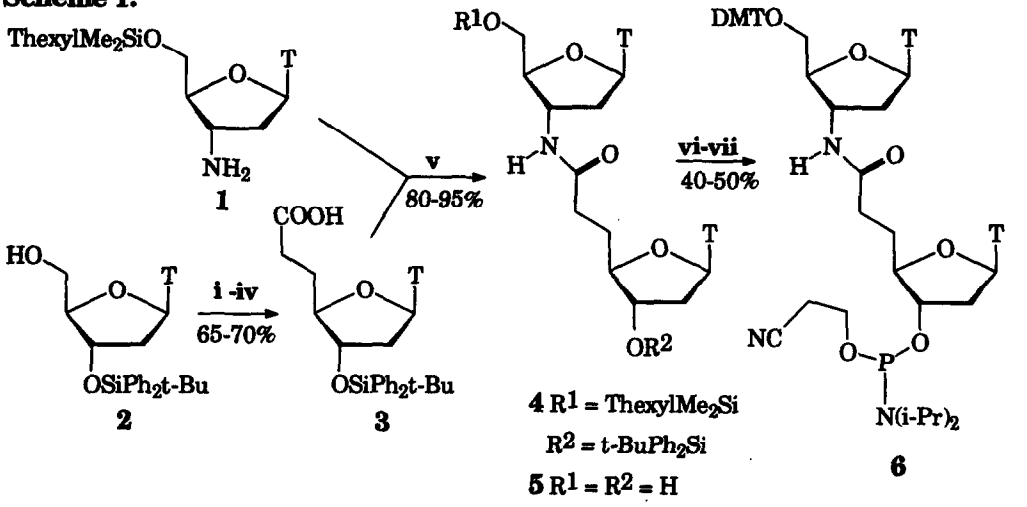
***Abstract:*** *The syntheses of thymidine dimers in which the natural phosphodiester linkage has been replaced by an amide group (3'-NR-CO-$CH_2$-5'; R=H, Me, n-Pr) are described. These new dimers were incorporated into oligonucleotides. Measurement of melting temperatures ($T_m$) of DNA/RNA duplexes and the nuclease resistance are presented. Preliminary results of molecular mechanics calculations are included.*

The syntheses of oligonucleotides with modified internucleoside phosphate linkages have recently emerged as an important goal in the antisense approach in order to regulate gene expression which offers a new and highly selective chemotherapeutic strategy to treat pathogenic diseases.[1,2] Unmodified oligodeoxyribonucleotides suffer from their instability against exo- and endonucleases. To overcome these limitations, recent studies in our laboratory have focused on the design and preparation/synthesis of new dimers with neutral amide linkages between the C(3') carbon of the upper sugar and the C(5') carbon of the lower sugar.[3] In this paper, we describe the syntheses of thymidine dimers in which the natural phosphodiester linkage has been replaced by the amides 3'-NR-CO-$CH_2$-5' (R=H, Me, n-Pr) and their incorporation into oligodeoxyribonucleotides.

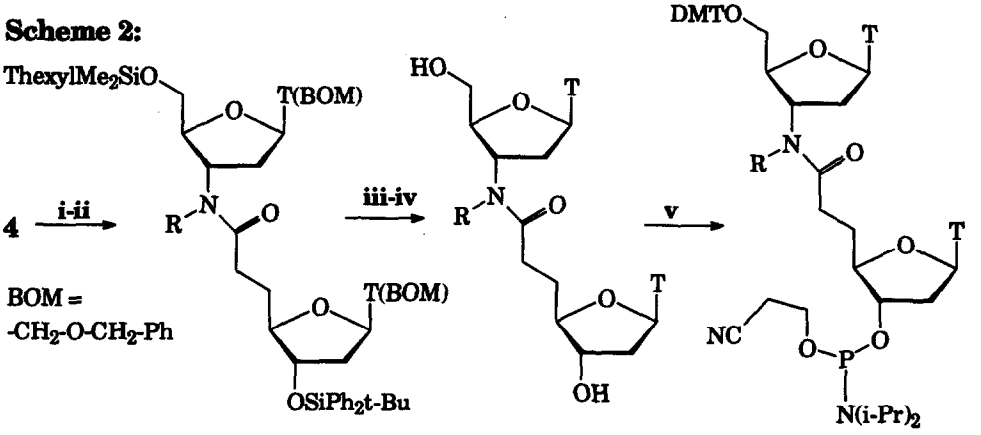
The amine **1**[4] and the alcohol **2**[5] were both prepared from thymidine, as previously reported (**Scheme 1**). To extend the carbon chain at C(5'), the alcohol **2** was oxidized to the corresponding aldehyde in DMSO by treatment with dicyclohexylcarbodiimide (DCC) and pyridinium trifluoroacetate. The *Wittig* reagent $Ph_3P{=}CHCO_2Me$ was added to a solution of the crude aldehyde to give the α,β-unsaturated ester in 85% yield. Hydrogenation of the C=C double bond, followed by saponification of the methyl ester afforded the acid **3** in 88% yield.[6] The acid **3** was treated with O-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-tetrafluoroborate (TBTU) and N-hydroxybenzotriazole to provide the corresponding activated ester[7] which was reacted *in situ* with amine **1** leading to dimer **4** in high yield. Both silyl protective groups of dimer **4** were removed with TBAF to give **5**. The 4,4'-dimethoxytrityl and the phosphoramidite groups were then introduced at the 5'- and 3'-ends, respectively, by standard methods,[8] to furnish the phosphoramidite **6**. NOE experiments indicated that dimer **5** is present in solution exclusively as the more stable *trans* rotamer. To increase the population of the *cis* rotamer and the solubility of the dimer **5**, we concentrated on the preparation of N-alkylated amide derivatives.

**Scheme 1:**




i) 4 eq. DCC, 1 eq. pyridinium trifluoroacetate, DMSO, RT, 3-5h. ii) 2 eq. $Ph_3P$=CH-$CO_2Me$, $CH_2Cl_2$, RT, 3h. iii) $H_2$, 1 atm., Pd/C 10%, MeOH, RT, 14h. iv) 2.1 eq. KOH 2M in $H_2O$, MeOH/$H_2O$ (7/3), RT, 2h. v) 1.1 eq. $Et_3N$, 1 eq. TBTU, 0.5 eq. N-hydroxybenzotriazole, $CH_3CN$, RT, 2h, then 1 eq. 1, 1.1 eq. $Et_3N$; RT, 24h. vi) 2.2 eq. TBAF, THF, RT, 12h. vii) 1.4 eq. DMTCl, pyr., RT, 18h. vii) 1.5 eq. $(i\text{-}Pr_2N)_2POCH_2CH_2CN$, 0.75 eq. $(i\text{-}Pr)_2NH_2^+$ tetrazole$^-$, $CH_2Cl_2$, RT, 12 h.

**Scheme 2:**




i) 1.8 eq. BOM-Cl, 1.9 eq. DBU, $CH_3CN$, 0°C, 2-4h. ii) 4 eq. NaH (80%), 5 eq. MeI for **7**; 5 eq. allyl iodide for **8**, THF, 55-60°C, 3h. iii) 5 eq. TBAF, THF, 0°C, 2h. iv) $H_2$, 1 atm., Pd/C 10%, MeOH, RT, 3 days. v) See vi-vii in **Scheme 1**.

For that purpose, the N(3)-H of the thymidine in dimer **4** had to be protected in order to avoid the formation of N(3)-alkylated products. Dimer **4** was treated with benzyl chloromethyl ether (BOM-Cl)[9]

to afford the fully protected dimer, which was treated with NaH and MeI to give the N-methyl dimer **7** in 75% overall yield (**Scheme 2**). N-Propylation of dimer **4** was accomplished using allyl iodide followed by hydrogenation of the C=C double bond in the consecutive deprotection step (**4** → **10**). Silyl and BOM protective groups of **7** and **8** were removed to give the fully deprotected dimers **9** and **10** in 65 and 74% yield, respectively. The unprotected dimers **9** and **10** were converted to the 3'-phosphoramidite **11** and **12** as described for **6**. The stability of dimer **5** under the basic conditions used during the solid phase oligonucleotide synthesis was experimentally verified (aq. $NH_3$ (25%), 55°C, 12h). Oligonucleotides **A-D** containing each of these dimers were synthesized[10] and the melting temperatures of the duplex strands with their RNA complements were determined[12] as summarized in the **Table**.

**Table** : Hybridization data.[12]

| | $T_m$ (°C) | $\Delta T_m$(°C)/mod | | |
|---|---|---|---|---|
| oligomer sequence (5'→3') | wild type | **a**=**a**$_1$ | **a**=**a**$_2$ | **a**=**a**$_3$ |
| **A** C T C G T A C C **TaT** T C C G G T C C | 63.3 | -2.2 | -3.7 | -2.5 |
| **B** C T C G T A C **TaT TaT** C C G G T C C | 61.8 | -2.8 | -2.9 | -3.1 |
| **C** G C G **TaT TaT TaT TaT TaT** G C C | 50.2 | -3.9 | -4.3 | -5.4 |
| **D** T T T **TaT** C T C T C T C T C T | 43.0 | -2.7 | -2.4 | -3.9 |

(**a**$_1$ = 3'-NH-CO-$CH_2$-5', **a**$_2$ = 3'-N(Me)-CO-$CH_2$-5', **a**$_3$ = 3'-N(n-Pr)-CO-$CH_2$-5')

The introduction of the secondary amide **a**$_1$ decreases the melting temperature ($T_m$) of -2.9°C (average value over four sequences per modification). An additional substituent on the nitrogen atom of the amide (N-Me **a**$_2$, N-Pr **a**$_3$) lowers the $T_m$ value even further (**Table**). This results probably from steric interactions with the adjacent five-membered ring. The additional substituent is also increasing the population of the *cis* rotamer of amides **a**$_2$ and **a**$_3$ (*cis:trans* ≈ 1:9), as determined by $^1$H-NMR with **9** and **10** in $D_2O$.

The enzymatic stability in10% fetal calf serum[13] (exclusively 3'-exonucleases) at 37°C of the oligonucleotide TCCAGGTGTT**TaT**C (**TaT**=**Ta**$_1$**T** or **Ta**$_3$**T**) was increased by a factor of 2 to 3 compared to the unmodified oligomer.[14]

Molecular mechanics investigations using the AMBER force field[15] were carried out on octamers of the hybrid duplex CTT**Ta**$_1$TTC with complementary wild-type RNA in the standard A-form helix. Conformational analysis in the octamer duplex predicts the lowest-energy structure for the **Ta**$_1$**T** linkage with the amide bond in *trans* (**Figure**). The respective lowest-energy geometry for the octamer duplex with the amide group in *cis* was found higher in energy by 1.4 kcal/mol. Molecular dynamics computations (at 300 K) on longer oligomers with alternating ~T **Ta**$_1$**T** **Ta**$_1$**T** T~ sequences suggest that structures with the amide in *cis*, although higher in energy, behave more conservative, oscillating moderately around the minimum-energy geometry, whereas structures with the *trans* amide become

rather distorted over the first 100 picoseconds, with some base pairs definitely breaking apart. A detailed account on these investigations will be published elsewhere.[16]

In conclusion, the replacement of the phosphodiester moiety by the amide bond 3'-NR-CO-$CH_2$-5' leads to a destabilization of the duplex formed with an RNA target. The introduction of an amide group in the other positions of the backbone is under investigation in our laboratories and will be reported soon.

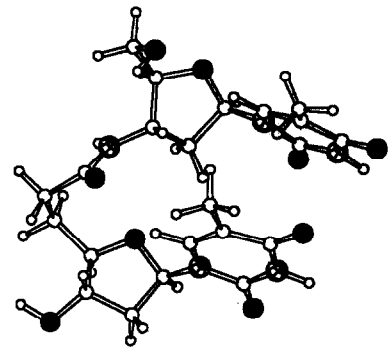


**Figure:** $Ta_1T$ cut out of the octamer duplex with the amide bond in *trans*.

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