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REPLACEMENT OF THE PHOSPHODIESTER LINKAGE IN OLIGONUCLEOTIDES BY HETEROCYCLES: SYNTHESIS OF THYMIDINE DINUCLEOTIDE ANALOGS WITH TRIAZOLE-MODIFIED BACKBONES

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Abstract: Novel backbone-modified dinucleotide analogs of types **V** and **VI** have been synthesized, where the natural phosphodiester linkage of a T-T dinucleotide has been replaced by a triazole heterocycle. The key step of the synthesis is the regioselective, thermal cycloaddition of a 2-oxoalkylidene triphenylphosphorane with an azide derivative to generate the triazole ring. © 1997 Elsevier Science Ltd.

A recent report from our laboratory has described the replacement of the phosphodiester linkage in oligonucleotides with *trans* (**II**, Figure 1) and *cis* C=C double bonds (**III**).[1] These structures can be considered as isosteric all-carbon analogues of the amide backbone modification of type **I**[2] with the amide moiety in the normal *trans* (→ **II**) or fixed in a *cis* (→ **III**) conformation. As molecular mechanics calculations on amide-**I** containing DNA/RNA heteroduplexes had not revealed any low-energy conformations with the amide group adopting a *cis* conformation,[2] we originally expected the replacement of phosphodiester linkages in oligodeoxyribonucleotides by a *cis* double bond of type **III** to result in significantly lower RNA-binding affinity than for the corresponding *trans* isomer **II**. Surprisingly, however, the incorporation of dimeric building blocks **II** and **III** into oligodeoxyribonucleotides gave rise to a rather comparable, moderate decrease in DNA/RNA duplex stability compared to the corresponding wild-type duplexes for both types of modifications (average ΔT_m/mod = -0.8 °C for **II** and -1.2 °C for **III**).[1] These results have prompted us to conduct further examinations of olefinic backbone replacements or geometrically equivalent modifications.

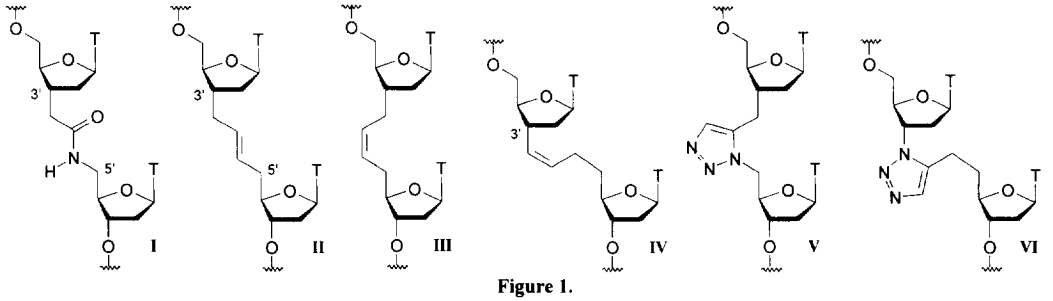
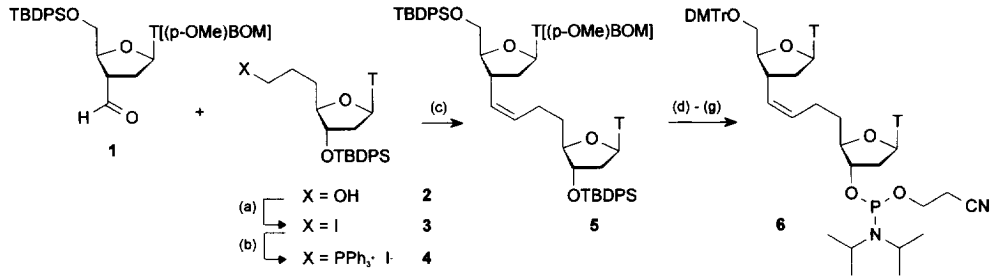


Figure 1.

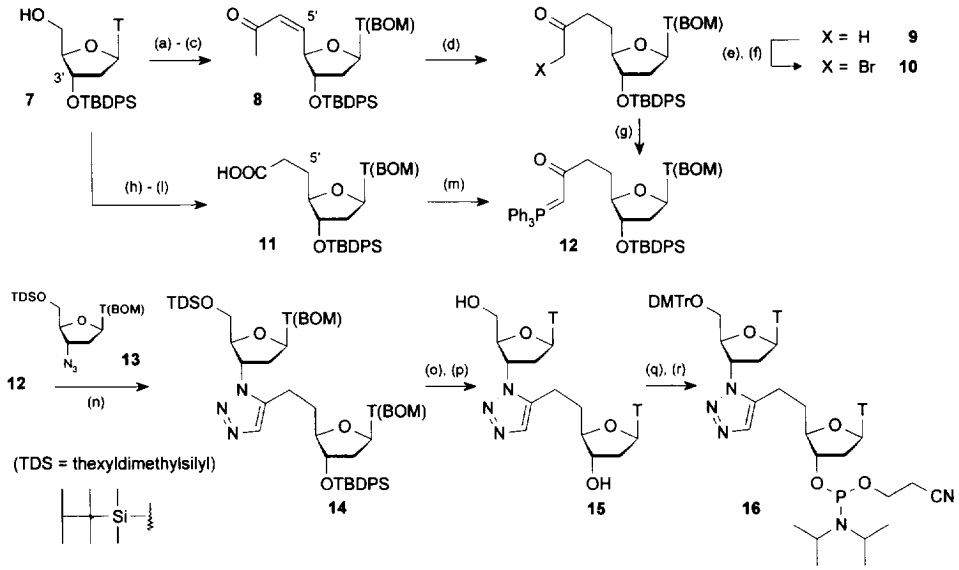
In order to evaluate the geometric constraints imposed by an internucleoside *cis* C=C double bond or its geometric equivalents, we adopted a dual approach. First, the position of the *cis* C=C double bond was moved such as to connect an olefinic carbon atom directly with C(3') of the upper sugar (see **IV**).[3] Second, in order to evaluate the influence of polarity and/or basicity of the modified backbone structure on RNA binding affinity, dimeric building blocks of types **V** and **VI** were synthesized, wherein the *cis* C=C double bond was replaced by a planar 1,2,3-triazole moiety.[4] Molecular mechanics calculations indicated that the overall geometries found in **IV** or **VI** should be well tolerated in the DNA strand of an A-type DNA/RNA heteroduplex.[5] In this communication, we report on the synthesis of backbone-modified dinucleotide analogs **IV**, **V** and **VI**. The following communication in this issue will present the synthesis of T-T dimers wherein the tri-



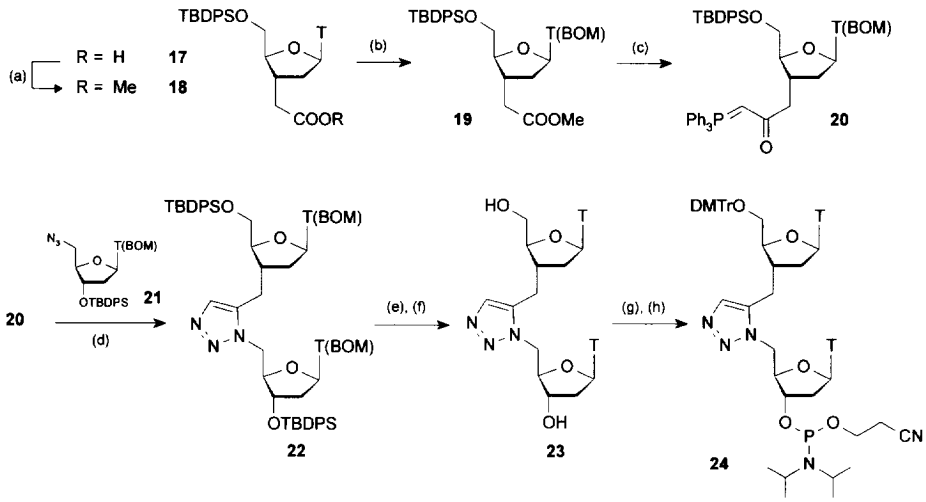
Scheme 1. (a) 4 x 0.35 equiv PPh_3, 4 x 0.35 equiv I_2, pyridine, rt, 2 h, 52%. (b) 2.0 equiv PPh_3, CH_3CN, 65 °C, 16 h, 99%. (c) 1.0 equiv **4**, 2.0 equiv $(TMS)_2NNa$, THF, -78 °C, 5 min.; + 1.0 equiv **1**, -78 °C → rt, 16 h, 81%. (d) 3.5 equiv DDQ, CH_2Cl_2 / H_2O (50 : 1), rt, 4 h, quant. (e) 2.4 equiv Bu_4NF, THF, rt, 3 h, quant. (f) 1.1 equiv DMTr-Cl, pyridine, rt, 16 h, 63%. (g) 3.0 equiv $((i\text{-}Pr)_2N)_2POCH_2CH_2CN$, 5.0 equiv diisopropylammonium tetrazolide, CH_2Cl_2, rt, 16 h, 93%.

azole units of **V** and **VI** have been replaced by imidazole rings. In addition, melting temperatures of duplexes formed by oligodeoxyribonucleotides, containing these novel dimeric building blocks, and complementary RNA will be reported.

As outlined in Scheme 1, the synthesis of *cis* olefin **6** was based on the coupling of aldehyde **1** with the ylide derived from triphenylphosphonium iodide **4**. Phosphonium salt **4** was obtained from primary alcohol **2**[6] through conversion to the corresponding iodide **3**[7] and subsequent treatment with PPh_3; deprotonation of **4** with 2 equiv of sodium hexamethyldisilazide provided the required ylide. While the in situ formed anion of the thymine unit of **4** served as an adequate base protecting group in the Wittig reaction, the thymine moiety of the



Scheme 2. (a) 1.5 equiv BOM-Cl, 1.5 equiv DBU, CH_3CN, 0 °C → rt, 4 h, 65%. (b) 6.0 equiv DCC, 1.0 equiv pyridinium trifluoroacetate, DMSO, rt, 4 h; work-up: 4.0 equiv oxalic acid. (c) 1.3 equiv $Ph_3P{=}CHCOCH_3$, THF, rt, 16 h, 76% (two steps). (d) 0.1 equiv Pt/C (5%), H_2, MeOH, rt, 2 h, 95%. (e) 2.75 equiv Li-(2,2,6,6-tetramethylpiperidide), 3.0 equiv Me_3SiCl, THF, -78 °C, 15 min.; work-up: $NaHCO_3$ (aq). (f) 1.05 equiv NBS, THF, 0 °C, 15 min., 41% (two steps). (g) 1.05 equiv PPh_3, benzene, rt, 16 h; $NaHCO_3$ (aq), 43%. (h) 6.0 equiv DCC, 1.0 equiv pyridinium trifluoroacetate, DMSO, rt, 2 h, 80%; work-up: 4.0 equiv oxalic acid. (i) 1.2 equiv $Ph_3P{=}CHCOOCH_3$, CH_2Cl_2, 0 °C, 1.5 h, 90%. (j) 10% Pd-C (10%), H_2, MeOH, rt, 1.5 h, 94%. (k) 1.5 equiv BOM-Cl, 1.5 equiv DBU, CH_3CN, 0°C → rt, 24 h, quant. (l) 30 equiv LiOH x H_2O, THF/H_2O (4:1), rt, 2 h, 92%. (m) 1. 1.05 equiv pivaloyl chloride, 1.2 equiv NEt_3, THF, -78 °C → 0 °C, 1.5 h, then -78 °C. 2. 2.2 equiv $Ph_3P{=}CH_2$, THF, -78 °C → rt, 5 h, 53%. (n) 1.0 equiv **13**, 1.2 equiv **12**, toluene, 80 °C, 48 h, 45%. (o) 2.4 equiv Bu_4NF, THF, 0 °C, 1 h, quant. (p) 1.0 equiv Pd-C (10%), H_2 (1 bar), MeOH, rt, 24 h, 83%. (q) 1.3 equiv DMTr-Cl, pyridine, rt, 24 h, 86%. (r) 3.0 equiv $((i\text{-}Pr)_2N)_2POCH_2CH_2CN$, 5.0 equiv diisopropylammonium tetrazolide, CH_2Cl_2, rt, 16 h, 80%.



Scheme 3. (a) 1.1 equiv DCC, 20 equiv MeOH, 0.8 equiv DMAP, CH_2Cl_2, rt, 4 h, 83%. (b) 1.8 equiv BOM-Cl, 2.0 equiv DBU, MeCN, rt, 4 h, 58%. (c) 2.1 equiv methyltriphenylphosphonium iodide, 2.2 equiv BuLi, THF, 0 °C - 50 °C, 4 h, 55%. (d) 1.0 equiv **20**, 1.6 equiv **21**, toluene, 110 °C, 36 h, 59%. (e) 2.4 equiv Bu_4NF, THF, 0 °C, 3 h, 94%. (f) 0.1 equiv Pd-C (10%), H_2 (1 bar), MeOH, rt, 48 h; then 6 equiv Na_2CO_3, quant. (g) 1.2 equiv DMTr-Cl, pyridine, rt, 16 h, 74%. (h) 3.0 equiv $((i\text{-}Pr)_2N)_2POCH_2CH_2CN$, 5.0 equiv diisopropylammonium tetrazolide, CH_2Cl_2, rt, 16 h, 94%.

aldehyde component had to be protected with a *para*-methoxybenzyloxymethyl ((*p*-OMe)-BOM) group.[8] When the coupling reaction was conducted under rigorously anhydrous conditions, *cis* dimer **5** was obtained in 81% yield.[9] NMR analysis indicated that the crude reaction mixture contained approximately 10% of the corresponding *trans* isomer, which was removed by chromatography on silica gel. Oxidative removal of the (*p*-OMe)BOM moiety of **5**[7,10] and fluoride-induced cleavage of the two *tert*-butyl-diphenylsilyl (TBDPS) groups proceeded in quantitative yield. Further elaboration using established procedures[11] furnished the dimethoxytrityl (DMTr)-protected phosphoramidite **6**.

The regioselective thermal cycloaddition of a 2-oxoalkylidene triphenylphosphorane and an azide was selected to form the triazole heterocycle in dimeric building blocks **IV** and **V**.[12] To access the triazole isomer **16** (Scheme 2) by this approach, azide **13**[13] and ylide **12** were required. Two different strategies towards **12** were evaluated. First, O(3')-TBDPS protected thymidine derivative **7** was converted to α,β-unsaturated ketone **8** by initial base protection with a benzyloxymethyl (BOM) group, Pfitzner-Moffatt oxidation to the C(5') aldehyde and subsequent Wittig reaction with acetylmethylene triphenylphosphorane. The C=C double bond in conjugation with the ketone carbonyl of **8** was then selectively hydrogenated over Pt-C without cleaving the BOM protecting group. Regioselective enolization with lithium-2,2,6,6-tetramethylpiperidide, trapping of the enolate with TMS-Cl,[14] and reaction of the resulting TMS-enol ether with NBS[15] afforded the terminal α-bromo ketone **10**. Displacement of bromide with PPh_3 and basic work-up finally gave stabilized ylide **12**.[16] The low overall efficiency of this route (6 steps, 8% yield) prompted us to examine an alternative approach to **12**. Thus, **7** was converted to carboxylic acid **11** by the sequence of (i) oxidation to the C(5') aldehyde, (ii) conversion to the α,β-unsaturated methyl ester by Wittig reaction with Ph_3P=CHCOOMe, (iii) hydrogenation of the C=C double bond, (iv) reaction with BOM-Cl, and (v) LiOH-mediated methyl ester hydrolysis. Carboxylic acid **11** was activated as its mixed anhydride with pivalic acid which was then reacted with 2.2 equiv of methylene triphenylphosphorane to afford **12** directly by an efficient in situ transylidation (6 steps from **7**, 31% yield).[17] The regiospecific cycloaddition[12] of **12** and **13** yielded triazole derivative **14**, which was further transformed to phosphoramidite **16** in high overall yield.

In order to apply the same cycloaddition strategy to the synthesis of the triazole positional isomer **24** (Scheme 3), access to phosphonium ylide **20** and azide **21**[18] was required. It was found that the BOM-protected methyl ester **19**, obtained from acid **17**[2] by initial DCC-mediated methyl ester formation (→ **18**) fol-

lowed by protection of the thymine moiety with a BOM group, reacted cleanly with 2.1 equiv of methylenetriphenylphosphorane to yield the stabilized ylide **20**, thus obviating the need to prepare a more strongly activated carboxylate derivative.[19] Thermal cycloaddition of **20** with azide **21** afforded triazole **22** regioselectively and proceeded in markedly higher yield than the corresponding reaction between **12** and azide **13** leading to triazole **14** (Scheme 2). This probably reflects the lower steric hindrance around azide **21** as compared to azide **13**. All four protecting groups of **22** could be removed in an overall yield of 94%. Dimer **23** was then converted to the DMTr-protected phosphoramidite **24**.

In summary, we have devised a straighforward synthesis of modified dinucleotide analogs **6**, **16** and **24**. The following communication in this issue will present the synthesis of T-T dimers wherein the triazole units of **V** and **VI** are replaced by imidazole rings. In addition, the results of thermal melting experiments with duplexes formed by oligodeoxyribonucleotides, incorporating triazole and imidazole modified dimeric building blocks, and complementary RNA will be reported.

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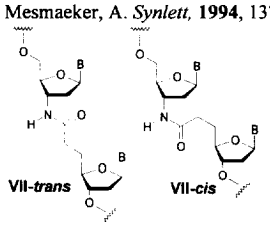
3. This particular class of backbone modifications (**IV** and **VI**) was designed so as to serve as a conformational mimic for the "amide-1" modification (**VII**) constrained to a *cis* conformation.[20] Conformational analysis of the duplex d(CTT**Ta**TTC) (**a**: "amide-1" linkage) with its complementary RNA indicated that the lowest-energy structure with the amide group *cis* (as in **VII**-*cis*) was only 1.4 kcal/mol higher than the most stable conformation with the amide bond *trans*.[20] In addition, the *cis* amide conformer yielded a stable duplex structure in molecular dynamics computations.



4. 3'-Deoxy-3'-(1,2,3-triazol-1-yl)thymidines have been synthesized as potential anti-HIV agents. See, e.g. (a) Hirota, K.; Hosono, H.; Kitade, Y.; Maki, Y.; Chu, C. K.; Schinazi, R. F.; Hideo, N.; Ono, K. *Chem. Pharm. Bull.* **1990**, *38*, 2597. (b) Häbich, D.; Barth, W. *Heterocycles*, **1989**, *29*, 2083. (c) Wigerinck, P.; Van Aerschot, A.; Claes, P.; Balzarini, J.; De Clercq, E.; Herdewijn, P. *J. Heterocyclic Chem.* **1989**, *26*, 1635. See also: (d) Lazrek, H. B.; Taoprirte, M.; Kabbaj, Y.; Oulih, T.; Barascut, J. L.; Imbach, J. L.; Almasoudi, N. A.; Pfleiderer, W. *Abstract of Papers*, XII IRT Nucleosides, Nucleotides And Their Biological Applications, La Jolla, CA, September 1996; PPI 27.
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