Synthesis of a Thymidine Dimer Containing a Tetrazole-2,5-diyl Internucleosidic Linkage and Its Insertion into Oligodeoxynucleotides

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Thymidine dimers in which the natural phosphodiester linkage has been replaced by a 2,5-disubstituted tetrazole ring are synthesized and incorporated into oligodeoxynucleotides (ODNs). The synthesis is accomplished by two strategies based on an alkylation of 5′-*O*-trityl-on and 5′-*O*-trityl-off 3′-deoxy-3′-(1*H*-tetrazol-5-yl)thymidines with 5′-iodo-5′-deoxythymidine in the presence of Et_3N, and the formation of only 2-substituted tetrazol-5-yl linkages is observed in 89 and 46% yields, respectively. The nucleoside dimer formed is reacted with 4,4′-dimethoxytrityl chloride (DMTCl), followed by treatment with 2-cyanoethyl tetraisopropylphosphordiamidite in the presence of *N*,*N*-diisopropylammonium tetrazolide, to afford the 5′-*O*-DMT-protected dinucleoside phosphoramidite that is used for incorporation into ODNs on an automated DNA synthesizer. The modified ODNs with one and up to five tetrazole internucleosidic linkages are obtained in good yields. The thermal stability of DNA/DNA and DNA/RNA duplexes is studied by UV experiments and reported also.

1. Introduction. – Antisense strategy as a part of controlling gene expression is a fast-growing research area. Naturally occurring oligonucleotides are unsuitable for that purpose because of the low stability towards nucleolytic degradation and poor cell penetration due to their polyanionic structure. These problems could be overcome by the chemical modification of oligonucleotides. As the main direction, the synthesis of nucleotide dimers with modified or substituted phosphodiester linkages has rapidly increased in recent years. The most promising results have been shown, when amide linkage (**I**, *Fig. 1*) as a nonionic moiety has been used. This linkage leads to oligonucleotides having increased stability towards 3′-exonucleases and thermal stability similar to unmodified ones [1]. Afterwards, to improve that result, some nucleoside dimers with various linkages containing cyclic and chain diamines [2], olefinic [3] moieties, which can mimic the overall geometry of an internucleosidic amide bond have been synthesized. To evaluate polarity and basicity, *Von Matt et al.* [4] have synthesized oligonucleotides with imidazolyl and 1,2,3-triazolyl heterocyclic moieties. Lower hybridization affinity with complementary RNA compare to wild-type was observed. *Lazrek et al.* [5] have reported the synthesis of nucleoside dimers containing 1,2,3-triazole linkage using 1,3-dipolar cycloaddition of protected 3′-azido-3′-deoxythymidine (AZT) to N(3), C(5), or C(5′) acetylene nucleosides. However, among all the heterocyclic linkages, compound **II** was the only example with substitution at the next nearest positions in the azole ring, but it was not used for

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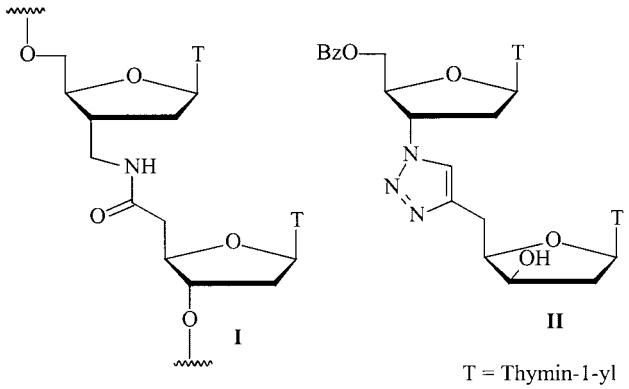


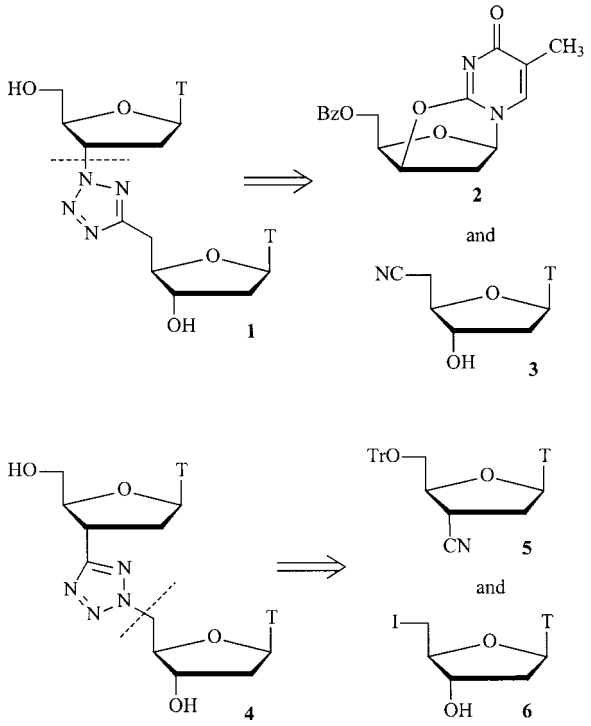
Fig. 1. *Amide* (**I**) *and 1,2,3-triazolyl* (**II**) *internucleosidic linkages*

oligonucleotide synthesis. Here, we present the synthesis of thymidine dimer having a tetrazole-2,5-diyl linkage. We believe that using a five-membered ring with the maximum number of heteroatoms can improve the solubility of the neutral nucleoside dimers due to H-bonding in H_2O solution.

2. Results and Discussion. – The synthesis of a dinucleotide having a tetrazole ring instead of phosphodiester internucleosidic linkage was achieved in two different ways by alkylation of nucleoside N*H*-tetrazoles (*Scheme 1*). The corresponding 5-substituted tetrazoles are obtained by the classical 1,3-dipolar cycloaddition of an azide ion to a nitrile. It is known that the alkylation of N*H*-tetrazoles in the presence of base gives mixtures of 1*H*- and 2*H*-isomers in a ratio, generally, depending on the electron effect of the substituents linked to the endocyclic C-atom [6]. Recently, unusually high regioselectivity has been observed for the reaction of alkylammonium salts of 5-substituted tetrazoles with 2,3′-anhydro-5′-*O*-benzoylthymidine (**2**) to give the corresponding 3′-(2*H*-tetrazol-2-yl)nucleosides [6][7]. The formation of the N(2) isomer was explained by steric factors due to a bulky three-dimensional structure of the electrophile **2**. In an analogous synthesis of **1**, 5′-cyano-5′-deoxythymidine (**3**) is needed to obtain the corresponding azole to be reacted with the anhydro compound **2**. However, the compound **3** has been described as very sensitive to base-promoted depyrimidination [8][9]. Therefore, difficulties could be expected in reacting **3** with azides in order to obtain the N*H*-tetrazole, and, consequently, compound-**1**-type tetrazole internucleoside linker was not considered to be the first choice to be synthesised.

An alternative dinucleoside **4** can be synthesized starting from 3′-cyano-3′-deoxy-5′-*O*-tritylthymidine (**5**) as a precursor for the required tetrazole derivative and thymidine **6** having a good leaving group at C(5′). Compound **5** was obtained by modification of the *Stork* radical cyclization–cyanation reaction from 3′-deoxy-3′-iodo-5′-*O*-trityl-thymidine in a good yield [10–12]. *Koguro et al.* [13] have described a novel synthesis of 5-substituted tetrazoles from nitriles with nonpolar aromatic solvents instead of the usually used DMF, and high yields have been obtained, especially, in case of sterically blocked dipolarophiles. Treatment of the nitrile **5** with dimethylammonium azide in

Scheme 1. *Retrosynthetic Analysis for Thymidine Dimers Containing the Tetrazole-2,5-diyl Linkage*

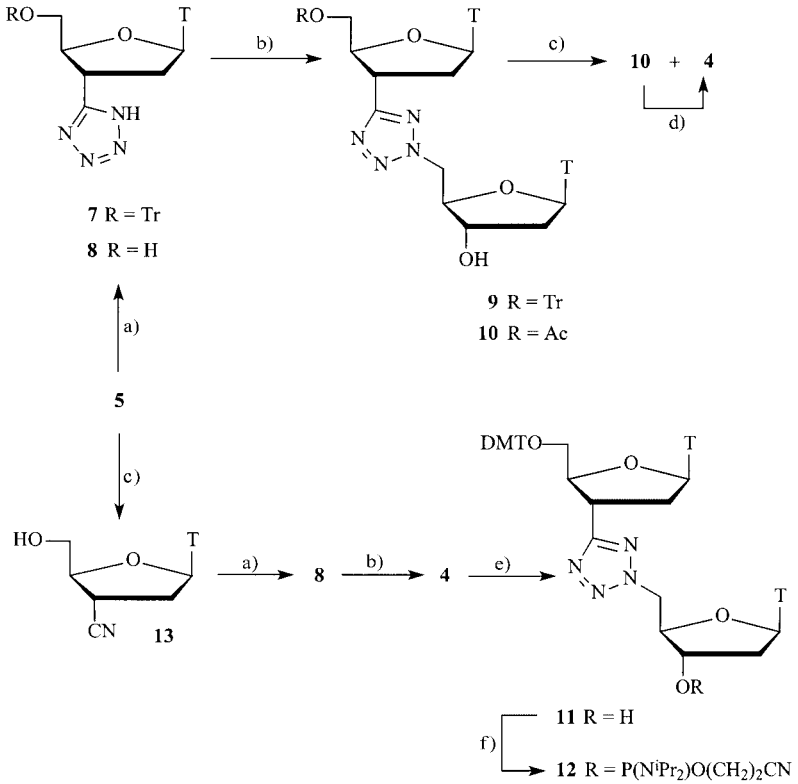


DMF (100°, 10 h) led to 3′-deoxy-5′-*O*-trityl-3′-(1*H*-tetrazol-5-yl)thymidine (**7**) in 85% yield (*Scheme 2*). A much lower conversion of the starting material **5** was observed when toluene was used as a solvent. Due to the presence of the electron-donating substituent of the obtained N*H*-tetrazole **7**, the formation of the corresponding 1*H*- and 2*H*-isomers can be expected for the alkylation reaction with primary halogenalkyl derivatives [6]. However, treatment of compound **7** with a slight excess of 5′-deoxy-5′-iodothymidine (**6**) in the presence of Et_3N in DMF at 110° for 18 h afforded only the dinucleoside **9** in 89% yield.

The formation of the 2,5-disubstituted 2*H*-tetrazole **9** was confirmed by the characteristic signals in ^{1}H- and ^{13}C-NMR. There are a CH_2 group attached to the N-atom (δ(H) 4.90 and δ(C) 54.5 ppm) and an endocyclic C-atom (δ(C) 165.8 ppm). In comparison, 1,5-disubstituted tetrazoles have the characteristic signals at 4.00–4.50 ppm in ^{1}H-NMR and 40–45 ppm ^{13}C-NMR for CH_2N and 153–156 ppm in ^{13}C-NMR for the C-atom of the tetrazole ring [14][15]. The regioselectivity observed in formation of the compound **9** could be explained by steric factors. Probably, a bulky structure of the starting products led to steric hindrance by which the new bond-formation between the N(1)-atom of the tetrazole ring and the C(5′) in compound **6** could not be realized.

To remove the 5′-*O*-trityl protecting group, compound **9** was treated with 80% aq. AcOH at 100°, and, only after 18 h were there no traces of the starting material

Scheme 2. *Synthesis of Phosphoramidite* **12**



a) $Me_2NH\cdots HN_3$/DMF. b) Compound **6** /Et_3N/DMF. c) 80% aq. AcOH. d) 25% NH_4OH. e) DMTCl/Py. f) $NC(CH_2)_2OP(N^iPr_2)_2$/*N*,*N*-diisopropylammonium tetrazolide/CH_2Cl_2.

according to TLC. However, 5′-*O*-acetylated and deprotected dinucleosides **10** and **4** were separated by silica-gel column chromatography, the acetate **10** probably being formed during workup. Treatment of the compound **10** with 25% aq. NH_3 for 1.5 h afforded the dinucleoside **4** in quantitative yield.

The target compound **4** was also synthesized by another strategy (*Scheme 2*). Compound **5** was heated in 80% aq. AcOH at 100° for 1 h, and the so-formed 3′-cyano-3′-deoxythymidine (**13**) was further treated with dimethylammonium azide for 24 h, affording N*H*-tetrazole **8** in 74% yield. Alkylation of **8** under the same conditions as for formation of **9** led to the dinucleoside **4** in 46% yield. As expected, the thymidine dimer **4** was easily soluble in H_2O.

The dinucleoside phosphoroamidite **12** to be used for preparation of the modified oligodeoxynucleotides was obtained in 31% overall yield by reacting **4** with 4,4′-dimethoxytrityl (DMTCl) chloride, followed by treatment with 2-cyanoethyl tetraisopropylphosphordiamidite in the presence of *N*,*N*-diisopropylammonium tetrazolide to afford **12**.

The structures of the thymine dimers **4**, **9**, and **10** were confirmed by high-resolution mass spectra, ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, and ^{1}H,^{13}C-HETCOR experiments. The structure of **12** was confirmed by ^{1}H- and ^{31}P-NMR.

The phosphoramidite **12** was incorporated into different oligonucleotide sequences on an automated solid-phase DNA synthesizer by means of an increased coupling time (12 min), and a high coupling efficiency of 98–100% for the modified amidite **12** was observed.

The thermal stability of the duplexes formed by the modified oligodeoxynucleotides and their DNA or RNA complements were determined by melting experiments (*Table* and *Fig. 2*). Incorporation of each tetrazole-type linkage resulted in lowering of the melting temperature, T_m of *ca.* 5.8° and 7.0° per modification towards DNA and RNA complementary strands, respectively. The azole backbone leads to the stepwise decrease in hyperchromicity of the formed secondary structures compared to the wild-type one and the melting curve becomes more flat (*Fig. 2*).

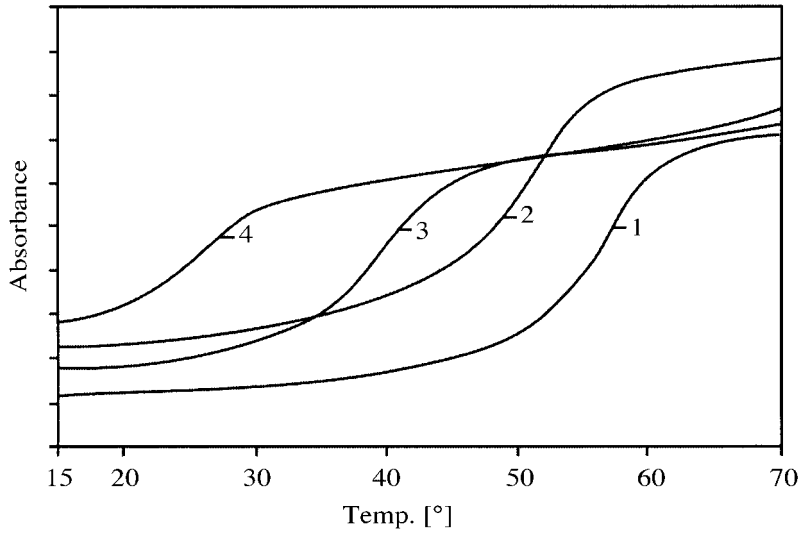


Fig. 2. *Melting curves of the DNA/DNA experiment. 1:* Unmodified duplex, *2*, *3*, *and 4:* one, three, and five inserted **TxT** (compound **4**), respectively.

Table. *Oligodeoxynucleotides Synthesized, Melting Experiments, and Calculated and Experimental Masses of ODNs from MALDI-TOF-MS*[a])

Sequence (5′→3′)	T_m [°] (DNA/DNA)	ΔT_m [°]	T_m [°] (DNA/RNA)	ΔT_m [°]	*m/z* [Da] calc.	found
GCGTTTTTTTTTTGCG	56.8		50.6		4872.8	4872.8
GCG**TxT**TTTTTTTTGCG	51.0	– 5.8	43.4	– 7.2	4844.8	4844.6
GCG**TxTTxT**TTTTTTGCG	45.4	– 5.7	37.0	– 6.8	4788.9	4788.5
GCG**TxT**TTT**TxT**TTT**TxT**GCG	39.8	– 5.7	28.2	– 7.5	4816.9	4816.3
GCG**TxTTxTTxTTxTTxT**GCG	25.8	– 6.2	18.6	– 6.4	4733.0	4732.2

[a]) **TxT** = Modified thymidine dimer, ΔT_m = decrease in T_m per modification.

3. Conclusions. – The synthesis of phosphoramidite building blocks of the novel H_2O-soluble tetrazole-2,5-diyl-linked nucleoside dimer has been accomplished. The oligodeoxynucleotides obtained containing one and up to five modified linkages exhibit lowered ability to hybridize to a complementary DNA and RNA sequence compared to the unmodified one.

Experimental Part

General. All solvents were distilled before use. The reagents used were purchased from *Aldrich*, *Sigma*, or *Fluka*. The reagents for *Gene Assembler* were purchased from *Cruachem* (UK). Oligodeoxynucleotides (ODNs) were synthesized on an *Assembler Gene Special DNA Synthesizer* (*Pharmacia Biotech*). Purification of 5′-*O*-DMT-on and 5′-*O*-DMT-off ODNs were accomplished with HPLC (*Hamilton PRP-1*) on a *Waters Delta Prep 4000 Preparative Chromatography System*. The complementary oligonucleotide (rCGC$(A)_{10}$CGC) was purchased from *DNA Technology ApS*, Aarhus, Denmark. TLC: TLC plates *60 F_{254}* purchased from *Merck* (elution system: 10% MeOH/CH_2Cl_2), visualized in an UV light (254 nm). The silica gel (0.063 – 0.200) used for column chromatography (CC) was purchased from Merck. NMR Spectra: *Varian AC-300* FT NMR spectrometer (^{1}H: 300 MHz, ^{13}C: 75.5 MHz); internal standards used in ^{1}H-NMR spectra were TMS (0.00 ppm) for $CDCl_3$, CD_3OD and (D_6)DMSO; in ^{13}C-NMR were $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm), (D_6)DMSO (39.5 ppm). Accurate ion-mass determinations were performed with the *4.7 Tesla Ultima Fourier* transform (FT) mass spectrometer (*IonSpec*, Irvine, CA); the $[M+H]^+$ and $[M+Na]^+$ ions were peakmatched with ions derived from the 2,5-dihydroxybenzoic acid matrix; the modified ODNs were confirmed by MALDI-TOF analysis on a *Voyager Elite Biospectrometry Research Station* from *PerSeptive Biosystems*. Microanalysis were performed by *Atlantic Microlab*, *Inc.*, USA.

1. *3′-Deoxy-3′-(1*H*-tetrazol-5-yl)-5′*-O-*tritylthymidine* (**7**). Dimethylammonium azide was synthesized from equimolar amounts of $Me_2NH_2^+Cl^-$ and NaN_3 in DMF at 70° for 4 h and subsequent removal of NaCl by filtration at r.t. and crystallization of the product at – 70° [16]. A soln. of *3′-cyano-3′-deoxy-5′*-O-*tritylthymidine* (**5**; 1.3 g, 2.64 mmol) and dimethylammonium azide (650 mg, 7.92 mmol) in DMF (50 ml) was heated at 100° for 10 h. The mixture was cooled, and the solvent was removed *in vacuo*. H_2O (50 ml) was added to the residue, and the soln. was acidified by 10% HCl to pH 1 – 2. The white precipitate formed was filtered off, washed with H_2O (2 × 30 ml), and dried to give **7** (1.2 g, 85%). M.p. 96 – 98° (H_2O). R_f 0.19. ^{1}H-NMR ((D_6)DMSO): 1.89 (*s*, Me); 2.30 – 2.80 (*m*, CH_2(2′)); 3.20 (*m*, CH_2(5′)); 3.92 (*q*, H–C(3′)); 4.16 (*m*, H–C(4′)); 6.07 (*t*, *J* = 6.0, H–C(1′)); 7.00 – 7.40 (*m*, 16 H, Tr, H–C(6)); 7.50 (*s*, N*H*-tetrazole); 11.20 (*s*, NH). ^{13}C-NMR ((D_6)DMSO): 11.9 (Me), 34.2 (C(2′)); 36.8 (C(3′)); 63.4 (C(5′)); 82.3 (C(4′)); 84.4 (C(1′)); 87.6, 127.1, 127.9, 128.2, 143.4 (Tr); 109.5 (C(5)); 136.2 (C(6)); 150.4 (C(2)); 156.7 (C(tetrazole)); 163.8 (C(4)). Anal. calc. for $C_{30}H_{28}N_6O_4 \cdot 1.5\ H_2O$ (563.6): C 63.93, H 5.55, N 14.91; found: C 64.03, H 5.40, N 14.72.

2. *3′-Deoxy-3′-[2-(5′-deoxythymidin-5′-yl)-2*H*-tetrazol-5-yl]-5′*-O-*tritylthymidine* (**9**). Compound **7** (720 mg, 1.34 mmol), *5′-deoxy-5′-iodothymidine* [17] (**6**; 570 mg, 1.62 mmol) and Et_3N (220 µl, 1.34 mmol) in DMF (40 ml) were heated at 110° for 18 h. The mixture was cooled, and the solvent was removed *in vacuo*. The residue was co-evaporated with toluene (2 × 30 ml) and purified on a silica-gel column with CH_2Cl_2/MeOH (0 → 10%) to afford **9** (905 mg, 89%). R_f 0.56. ^{1}H-NMR ($CDCl_3$): 1.49 (*s*, Me); 1.83 (*s*, Me); 2.10 (*m*, H_β–C(2′)); 2.40 (*m*, H_α–C(2′)); 2.65 (*m*, H_β–C(2″)); 2.80 (*m*, H_α–C(2″)); 3.41 (*d*, *J* = 8.8, H_a–C(5′)); 3.56 (*d*, *J* = 8.8, H_b–C(2″)); 4.10 (*q*, *J* = 7.5, H–C(3′)); 4.42 (*m*, H–C(4′), H–C(4″), OH); 4.63 (*m*, H–C(3″)); 4.90 (*m*, CH_2(5″)); 6.10 (*t*, *J* = 6.6, H–C(1″)); 6.26 (*t*, *J* = 5.0, H–C(1′)); 7.00 (*s*, H–C(6)); 7.20 – 7.50 (*m*, 18 H, Tr); 7.70 (*s*, H–C(6)); 10.00 (*m*, 2 NH). ^{13}C-NMR ($CDCl_3$): 11.9 (Me); 12.4 (Me); 35.6 (C(3′)); 38.2 (C(2′)); 39.1 (C(2″)); 54.5 (C(5″)); 63.0 (C(5′)); 71.7 (C(3″)); 83.5 (C(4′)); C(4″)); 85.2 (C(1″)); 86.5 (C(Ph_3)); 87.3 (C(1′)); 110.9, 111.1 (2 C(5)); 127.3, 127.9, 128.5, 143.2 (Tr); 135.6, 136.4 (2 C(6)); 150.4, 150.8 (2 C(2)); 164.2, 164.3 (2 C(4)); 165.8 (C(tetrazole)). HR-MS: 783.263 ($[M+Na]^+$, $C_{40}H_{40}N_8NaO_8^+$; calc. 783.256).

3. *3′-Deoxy-3′-(1*H*-tetrazol-5-yl)thymidine* (**8**). A soln. of **5** (1.74 g, 3.53 mmol) in 80% aq. AcOH (50 ml) was heated at 110° for 1 h. After cooling, the solvent was removed *in vacuo*. The residue was dissolved in 100 ml of H_2O/CH_2Cl_2 (50:50), and the H_2O layer was extracted with CH_2Cl_2 (3 × 50 ml). The H_2O phase was evaporated under reduced pressure and co-evaporated with MeCN (2 × 30 ml). The residue was dried overnight *in vacuo* to afford *3′-cyano-3′-deoxythymidine* (**13**; 762 mg, 86%). R_f 0.44. ^{1}H-NMR (CD_3OD): 1.85 (*s*, Me); 2.56 (*m*, H_β–C(2′); 2.72 (*m*, H_α–C(2′)); 3.53 (*q*, *J* = 9.0, H–C(3′)); 3.78 (*dd*, *J* = 3.0, 12.3, H_a–C(5′)); 3.95 (*dd*, *J* = 2.9, 12.5, H_b–C(5′)); 4.24 (*dt*, *J* = 8.5, 2.9, H–C(4′)); 4.80 (*m*, NH, OH); 6.16 (*dd*, *J* = 4.0, 3.4,

H–C(1′)); 7.75 (*s*, H–C(6)). ^{13}C-NMR (CD_3OD): 12.4 (Me); 28.7 (C(3′)); 37.2 (C(2′)); 61.3 (C(5′)); 84.7 (C(4′)); 86.8 (C(1′)); 111.4 (C(5)); 120.0 (CN); 138.2 (C(6)); 152.1 (C(2)); 166.4 (C(4)).

Without further purification, **13** was treated with dimethylammonium azide [16] (500 mg, 6 mmol) in DMF (80 ml) at 100°. Every 6 h, dimethylammonium azide (1 equiv., 250 mg) was added. After 30 h, the solvent was removed *in vacuo*, the residue was dissolved in H_2O and carefully acidified by addition of 10% HCl to pH 1–2. After 30 min, the solvent was removed *in vacuo*, and the residue was purified by CC (silica gel; CH_2Cl_2/MeOH 0 → 10%) to afford **8** (663 mg, 74%). R_f 0.05. ^{1}H-NMR (CD_3OD): 1.82 (*s*, Me); 2.50–2.70 (*m*, CH_2(2′)); 3.52 (*m*, H–C(3′)); 3.70 (*m*, H_a–C(5′)); 3.90 (*m*, H_b–C(5′)); 4.24 (*m*, H–C(4′)); 4.80 (*m*, NH, OH); 6.20 (*m*, H–C(1′)); 7.95 (*s*, H–C(6)). ^{13}C-NMR (CD_3OD): 12.4 (Me); 34.4 (C(3′)); 38.8 (C(2′)); 61.8 (C(5′)); 86.1 (C(4′)); 86.7 (C(1′)); 111.1 (C(5)); 138.5 (C(6)); 152.2 (C(2)); 158.1 (C(tetrazole)); 166.6 (C(4)). HR-MS: 317.096 ($[M+Na]^+$, $C_{11}H_{14}N_6NaO_4^+$; calc. 317.096).

4. *3′-Deoxy-3′-[2-(5′-deoxythymidin-5′-yl)-2*H*-tetrazol-5-yl]thymidine* (**4**). Compound **9** (830 mg, 1.1 mmol) was heated in 80% aq. AcOH at 80°, until the starting compound disappeared on TLC. After 18 h, the solvent was removed *in vacuo*, the residue was dissolved in 100 ml H_2O/CH_2Cl_2 (50:50), and the org. layer was extracted with H_2O (3 × 25 ml). The combined H_2O extracts were evaporated under reduced pressure, and two compounds, **10** and **4**, were separated by CC (silica gel; CH_2Cl_2/MeOH 0 → 10%).

*Data of 5′-*O*-Acetyl-3′-deoxy-3′-[2-(5′-deoxythymidin-5-yl)-2*H*-tetrazol-5-yl]thymidine* (**10**): 280 mg (46%). R_f 0.37. ^{1}H-NMR (CD_3OD): 1.89 (*s*, Me); 1.92 (*s*, Me); 2.08 (*s*, Me); 2.30 (*m*, CH_2(2′)); 2.68 (*m*, H_β–C(2″)); 2.85 (*m*, H_α–C(2″)); 3.93 (*m*, H–C(3′)); 4.35 (*m*, H–C(4′), H–C(4″)); 4.42 (*m*, CH_2(5′)); 4.52 (*m*, H–C(3″)); 4.80 (*s*, 2 NH, 2 OH); 5.00 (*d*, *J* = 5.9, CH_2(5″)); 6.17 (*t*, *J* = 7.0, H–C(1″)); 6.23 (*dd*, *J* = 4.1, 7.6, H–C(1′)); 7.39 (*s*, H–C(6)); 7.65 (*s*, H–C(6)). ^{13}C-NMR (CD_3OD): 12.5 (Me); 12.6 (Me); 20.8 (*Me*CO); 37.2 (C(3′)); 38.1 (C(2′)); 39.9 (C(2″)); 55.7 (C(5″)); 64.7 (C(5′)); 72.8 (C(3″)); 83.3 (C(4′)); 85.2 (C(4″)); 87.2 (C(1″)); 87.4 (C(1′)); 111.5, 111.8 (2 C(5)); 138.0, 138.2 (2 C(6)); 152.1, 152.2 (2 C(2)); 166.2, 166.3 (2 C(4)); 166.6 (C(tetrazole)); 172.3 (Me*C*O). HR-MS: 583.182 ($[M+Na]^+$, $C_{23}H_{28}NaO_9^+$; calc. 583.187).

Data of **4**: 110 mg, 20%. M.p. 234° (MeOH). R_f 0.21. ^{1}H-NMR (CD_3OD): 1.90 (*s*, 2 Me); 2.30 (*m*, CH_2(2′)); 2.64 (*m*, H_β–C(2″)); 2.86 (*m*, H_α–C(2″)); 3.83 (*dd*, *J* = 3.1, 12.3, H_a–C(5′)); 3.90 (*m*, H–C(3′), H_b–C(5′)); 4.29 (*dt*, *J* = 8.5, 2.7, H–C(4′)); 4.37 (*dd*, *J* = 5.9, 9.4, H–C(4″)); 4.53 (*m*, H–C(3″)); 4.80 (*s*, 2 NH, 2 OH); 5.00 (*d*, *J* = 5.9, CH_2(5″)); 6.21 (*t*, *J* = 7.0, H–C(1″)); 6.28 (*dd*, *J* = 3.7, 7.0, H–C(1′)); 7.42 (*s*, H–C(6)); 8.08 (*s*, H–C(6)). ^{13}C-NMR (CD_3OD): 12.5 (2 Me); 35.6 (C(3′)); 38.9 (C(2′)); 39.6 (C(2″)); 55.7 (C(5″)); 61.9 (C(5′)); 72.8 (C(3″)); 82.3 (C(4′)); 86.6 (C(4″), C(1″)); 87.4 (C(1′)); 111.2, 111.8 (2 C(5)); 138.2, 138.4 (2 C(6)); 152.1, 152.3 (2 C(2)); 166.2, 166.3 (2 C(4)); 167.0 (C(tetrazole)). HR-MS: 541.181 ($[M+Na]^+$, $C_{21}H_{26}N_8NaO_8^+$; calc. 541.177).

From **10**. Compound **10** (77 mg, 0.14 mmol) was dissolved in 25% aq. NH_3 (5 ml). After stirring at r.t. for 1.5 h, the solvent was removed *in vacuo*, the residue was purified by CC (silica gel; CH_2Cl_2/MeOH 0 → 10%) to give **4** (71 mg, 100%).

From **8**. Compound **8** (663 mg, 2.3 mmol), **6** (952 mg, 2.7 mmol), and Et_3N (312 µl, 2.3 mmol) in DMF (40 ml) were heated at 110° for 18 h. After cooling, the solvent was removed *in vacuo*. The residue was co-evaporated with toluene (2 × 30 ml) and purified by CC (silica gel; CH_2Cl_2/MeOH 0 → 10%) to afford **4** (533 mg, 46%).

5. *3′-Deoxy-3′-[2-(5′-deoxythymidin-5′-yl)-2*H*-tetrazol-5-yl]-5′-*O*-(4,4′-dimethoxytrityl)thymidine* (**11**). Thymidine dimer **4** (70 mg, 0.14 mmol) was dissolved in anh. pyridine (3 ml) and DMTCl (70 mg, 0.21 mmol) was added under N_2. After 18 h, more DMTCl (70 mg, 0.21 mmol) was added to complete the reaction. After 4 h, anal. TLC showed no more starting material, and the reaction was quenched with MeOH (1 ml). The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (10 ml) and extracted with sat. aq. $NaHCO_3$ (3 × 5 ml). The org. layer was dried (Na_2SO_4), and evaporated *in vacuo*, and the product was purified by CC (silica gel; CH_2Cl_2/MeOH 0 → 10%) to afford **11** as a foam (78 mg, 70%), which was used in the next step without further purification. R_f 0.36. ^{1}H-NMR ($CDCl_3$): 1.50 (*s*, Me); 1.95 (*s*, Me); 2.30 (*m*, CH_2(2′)); 2.65 (*m*, H_β–C(2″)); 2.89 (*m*, H_α–C(2″)); 3.13 (br. *s*, OH); 3.39 (*d*, *J* = 8.6, H_a–C(5′)); 3.60 (*d*, *J* = 8.6, H_b–C(5′)); 3.70 (*m*, H–C(3′)); 3.80 (*s*, 2 MeO), 4.36 (*m*, H–C(4′)); 4.46 (*m*, H–C(4″)); 4.52 (*m*, H–C(3″)); 4.90 (*m*, CH_2(5″)); 6.10 (*t*, *J* = 6.7, H–C(1″)); 6.28 (*t*, *J* = 5.5, H–C(1′)); 6.79 (*d*, *J* = 8.8, 2 H, DMT); 6.81 (*d*, *J* = 9.1, 2 H, DMT); 7.03 (*s*, H–C(6)); 7.20–7.45 (*m*, 10 H, DMT); 7.74 (*s*, H–C(6)); 9.80 (*s*, 2 NH). ^{13}C-NMR ($CDCl_3$): 11.9 (Me); 12.4 (Me); 35.5 (C(3′)); 38.3 (C(2′)); 39.2 (C(2″)); 54.4 (C(5″)); 55.2 (MeO); 62.7 (C(5′)); 71.5 (C(3″)); 83.4 (C(4′)); 83.5 (C(4″)); 85.1 (C(Ar_3)); 86.4 (C(1″)); 86.7 (C(1′)); 110.9, 111.0 (2 C (5)); 113.1, 127.0, 127.9, 128.0, 130.0, 138.7 (Ar); 135.3, 135.4 (2 C(6)); 144.3 (Ar); 150.2, 150.5 (2 C(2)); 158.5 (Ar); 163.9, 164.0 (2 C(4)); 165.7 (C(tetrazole)). HR-MS: 843.288 ($[M+Na]^+$, $C_{42}H_{44}N_8NaO_{10}^+$; calc. 843.284).

6. *3′-Deoxy-3′-(2-{5′-deoxy-3′*-O-*[2-cyanoethoxy(diisopropylamino)phosphino]thymidin-5′-yl}-2*H-*tetrazol-5-yl)-5′*-O-*(4,4′-dimethoxytrityl)thymidine* (**12**). Compound **11** (240 mg, 0.29 mmol) was dissolved under N_2 in anh. CH_2Cl_2 (5 ml). *N,N*-Diisopropylammonium tetrazolide (82 mg, 0.47 mmol) was added, followed by dropwise addition of 2-cyanoethyl tetraisopropylphosphordiamidite (0.190 ml, 0.59 mmol). After 2.5 h, anal. TLC showed no more starting material, and the reaction was quenched with H_2O (1 ml), followed by addition of CH_2Cl_2 (10 ml). The mixture was washed with sat. aq. $NaHCO_3$ (2 × 10 ml). The org. phase was dried (Na_2SO_4), and the solvents were removed under reduced pressure. The residue was purified by CC (silica gel; cyclohexane/AcOEt 50 → 100%). Combined UV-active fractions were evaporated *in vacuo* to afford **12** (132 mg, 44%) as a foam that was co-evaporated with dried MeCN (3 × 30 ml) before using in ODN synthesis. R_f 0.59. ^{1}H-NMR ($CDCl_3$): 1.20 (*m*, 2 *Me*$_2$CH); 1.50 (*s*, Me); 1.95 (*s*, Me); 2.25 (*m*, CH_2(2′)); 2.57 (*m*, H_β–C(2″)); 2.68 (*m*, CH_2CN); 2.85 (*m*, H_α–C(2″)); 3.40 (*m*, H_a–C(5′)); 3.60 (*m*, H_b–C(5′), H–C(3′), O*C*H_2*C*H_2CN, 2 Me_2C*H*); 3.85 (*s*, 2 MeO); 4.40 (*m*, H–C(4′), H–C(4″)); 4.65 (*m*, H–C(3″)); 4.90 (*m*, CH_2(5″)); 6.10 (*t*, *J* = 6.7, H–C(1″)); 6.35 (*m*, H–C(1′)); 6.79 (*d*, *J* = 8.8, 2 H, DMT); 6.81 (*d*, *J* = 9.1, 2 H, DMT); 6.95 (*s*, H–C(6)); 7.20–7.45 (*m*, 10 H, DMT); 7.75 (*s*, 1 H, H–C(6); 9.50 (*s*, 2 H, 2 × NH). ^{31}P-NMR ($CDCl_3$): 150.3 (*s*).

7. *Synthesis and Purification of* **TxT**-*Modified and Unmodified Oligodeoxynucleotides.* The oligodeoxynucleotides were synthesized on a *Pharmacia Gene Assembler*® special synthesizer in 0.2-μmol scale (5.0 μmol embedded per cycle, *Pharmacia Primer Support*™) with commercially available 2-cyanoethyl phosphoramidites and **12**. The coupling time was increased from 2 min for the commercial amidites to 12 min for the modified dimer **12**. The 5′-*O*-DMT-on ODNs were removed from the solid support and deprotected with 32% aq. NH_3 (1 ml) at 55° overnight and then purified on prep. HPLC. The solvent systems were buffer *A* (950 ml of 0.1M NH_4HCO_3 and 50 ml of MeCN (pH 9.0)) and buffer *B* (250 ml of 0.1M NH_4HCO_3 and 750 ml of MeCN (pH 9.0)), which were used in the following order: 5 min 100% *A*, 30 min linear gradient of 0 → 70% *B* in *A*, 5 min linear gradient of 70 → 100% *B* in *A*. Flow rate was 1 ml min^{-1}. The purified 5′-*O*-DMT-on ODNs eluted as one peak after *ca*. 30 min (UV control 254 nm). The fractions were concentrated *in vacuo*, followed by treatment with 10% aq. AcOH for 20 min, and further purification on HPLC under the same conditions afforded detritylated ODNs eluted at 23–28 min. The resulting solns. were evaporated *in vacuo* and co-evaporated twice with H_2O to remove volatile salts to afford ODNs, which were used in melting-temp. measurements.

8. *Melting Experiments.* Melting-temp. measurements were performed on a *Perkin-Elmer* UV/VIS spectrometer fitted with a *PTP-6 Peltier* temp.-programming element. The oligodeoxynucleotides were dissolved in a medium salt buffer (pH 7.0, 1 mM EDTA, 10 mM $Na_2HPO_4 \cdot 2\,H_2O$, 140 mM NaCl) to a concentration of 1.5 μM for each strand. Before each experiment, all samples were heated at 95° in a H_2O bath for 5 min and then cooled slowly to r.t. The absorbance at 260 nm was measured from 18° to 85° in 1-cm cells. The melting temp. was determined as the maximum of the derivative plots of the melting curve. All melting temp. are within the uncertainty ± 1.0° as determined by repetitive experiments.

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