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Base-Pairing Systems Related to TNA: α-Threofuranosyl Oligonucleotides Containing Phosphoramidate Linkages[1]

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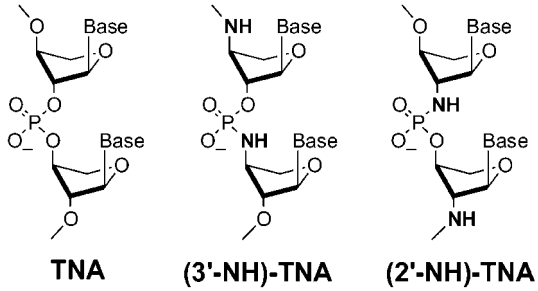
ABSTRACT



(3′NH)- and (2′NH)-TNA, two isomeric phosphoramidate analogues of TNA (α-threofuranosyl-(3′→2′) oligonucleotides), are shown to be efficient Watson–Crick base-pairing systems and to undergo intersystem cross-pairing with TNA, RNA, and DNA.

In systematic studies toward a chemical etiology of nucleic acid structure, we discovered that α-threofuranosyl-(3′→2′) oligonucleotides (TNA) represent an efficient Watson−Crick base-pairing system, able to undergo informational intersystem cross-pairing with both RNA and DNA.[2] This finding deserves our special interest since, among the potentially natural nucleic acid alternatives[3] investigated so far, TNA is structurally the simplest. More specifically, in view of its four-carbon-only carbohydrate building block, TNA may be considered to represent a simpler type of oligomer structure than RNA.[2,4] Since, in an etiological context, structural complexity of a molecule is to be assessed by criteria that define a structure's generational complexity, we previously argued that any experimental analysis of this aspect of the TNA structure should not be confined to TNA itself but include the backbones of a whole family of hypothetical oligomer structures that could derive from (C2 + C2 → C4) assembly processes involving nitrogenous starting materials and intermediates.[2] From such a point of view, the TNA backbone might represent just the oxygenous phosphodiester-type representative in a library of related nitrogenous oligomer systems, some of which might have the ability to communicate with RNA by intersystem cross-pairing. It is from this perspective that we synthesized two new members of the family, containing in place of the phosphodiester groups the isomeric phosphoramidate linkages depicted in the formulas given in the abstract. Both systems were found to indeed show base-pairing properties that are quite similar to those of TNA.

Modes of formation, synthesis, and properties of phosphoramidate analogues of oligonucleotides in the DNA and RNA series have been widely studied with regard to their potential as antisense reagents[5−7] and have also been the subject of experiments[8,9] and considerations[8,10] in an etiological context.

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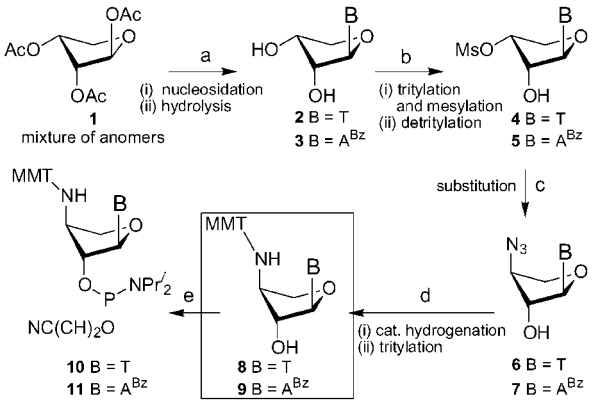
The nucleoside derivatives required for our study, namely, the α-L-3′-deoxy-3′-*N*-(4″-methoxytrityl)amino threofuranosyl nucleosides **8** and **9** and the isomeric α-L-2′-deoxy-2′-*N*-(4″-methoxy trityl)amino threofuranosyl nucleosides **16** and **22** were prepared according to Schemes 1, 2, and 3.[11] The synthesis of the nucleosides **8** and **9** starts with 1,2,3-tri-*O*-acetyl erythrose **1**[12] (Scheme 1) and proceeds by

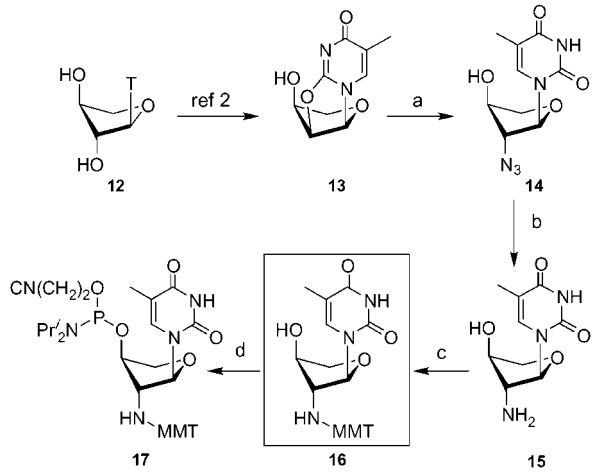
Scheme 1[a]



[a] Numbers in parentheses denote molar equivalents referring to starting compound or ratios. Numbers before these parentheses denote concentrations in M. (a) (i) 0.3 M (1.1 molar equiv) A^{Bz} or T, 0.6 (2.5) BSA, CH_3CN, 60˚C, 1 h, then 0.8 (3.0) $SnCl_4$ 60 ˚C, 20 min; (ii) for B = A^{Bz}, 1 M NaOH in THF/MeOH/H_2O 5:4:1, 4 ˚C, 10 min, 68%; for B = T, 2 M NH_3 (MeOH) in THF, rt 14 h, 65%; (b) (i) 0.3−0.9 (2.0) DMT-Cl, Py, rt, 14 h, then 0.2−0.6 (1.3) Ms-Cl, 4 °C → rt, 16 h; (ii) 0.4 (11.7) TFA, CH_2Cl_2, MeOH (3:96:1), rt, 30 min., 49% for A^{Bz}, 74% for T; (c) 2.1 (7.0) NaN_3, DMF/H_2O (4:1), 100 ˚C, 4 h, 82% for A^{Bz}, 78% for T; (d) (i) H_2, 10% Pd/C, MeOH, rt, 1 h; (ii) 0.3 (1.5) MMT-Cl, Py, rt, 2 h, 92% for A^{Bz}, 96% for T; (e) 0.3 (1.2) chloro(2-cyanoethoxy)(diisopropylethylamino)phosphine, 0.7 (2.5) NEt(*i*-Pr)$_2$, CH_2Cl_2, rt ,1 to 3h, 87% for A^{Bz}, 90% for T.

nucleosidation under Vorbrüggen−Hilbert−Johnson conditions[13] followed by selective base-catalyzed hydrolysis of the *O*-acetyl groups to **2** and **3**. Monotritylation with 4,4′-dimethoxytrityl chloride (DMT-Cl) in pyridine in both series occurs selectively at the 2′-hydroxyl (for B = thymine, 84% 2′-*O*-DMT, 3% 3′-*O*-DMT, 6% 2′,3′-bis-*O*-DMT; for B = N^6-benzoyladenine, 43% 2′-*O*-DMT, 33% of a mixture of 2′- and 3′-*O*-DMT and 6% of 2′,3′-bis-*O*-DMT; percentages refer to isolated material). Mesylation of the free 3′-hydroxyls followed by acid-catalyzed detritylation affords the 3′-mesyloxy nucleosides **4** and **5**, and substitution of the mesyloxy group by azide ions leads from the erythro into the threo series through inversion of configuration. The latter is evidenced by an X-ray analysis of the azido alcohol **6** in the thymine series.[14] Catalytic hydrogenation of the azide group, followed by protection of the amino group by mono-4-methoxytrityl chloride (MMT),[15] affords the target nucleoside **8** and **9**.

Scheme 2[a]



[a] (a) 2.1 M (8.0 molar equiv) NaN_3, 0.3 (1.2) BzOH, HMPA, 150 °C, 2 H, 82%; (b) 10% Pd−C, H_2, MeOH, rt, 2 h, 97%; (c) 1.4 (1.2) MMT-Cl, Py, rt, 1.5 h, 91%; (d) 0.3 (1.5) chloro(2-cyanoethoxy)(diisopropylethylamino)phosphine, 0.5 (4.0) NEt(*i*-Pr)$_2$, CH_2Cl_2, rt, 92%.

The synthesis of the isomeric nucleosides **16** (Scheme 2) and **22** (Scheme 3) starts from the thymine-derived anhydronucleoside **13**, the preparation of which from α-L-threofuranosyl thymine **12** was reported earlier.[2] Proton-catalyzed ring opening in **13** by reaction with sodium azide[16] at elevated temperature, catalytic reduction of the azide group of **14**, and protection of the amino group of **15** to give the thymine nucleoside **16** are all reactions that proceeded in high yield.

The corresponding adenine-containing nucleoside in this series (Scheme 3) is obtained by the (T→A)-transnucleosidation[17] **18** → **19**. The reaction is performed under modified Vorbrüggen nucleosidation conditions[13] and proceeds in a satisfactory yield of 74%, provided that the 2′-amino group is protected with the trifluoroacetyl group.[17] Base-catalyzed

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(14) The X-ray analysis was carried by Raj K. Chadha, TSRI. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Center as deposition No. CCDC 172143. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (fax +44 (1233) 336 0333; e-mail deposit@ccdc.cam.ac.uk).
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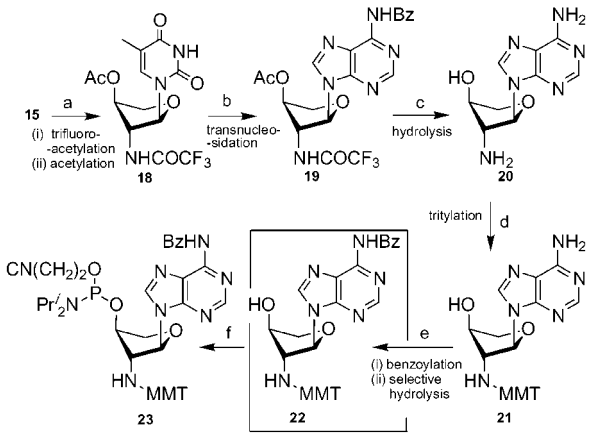
Scheme 3[a]



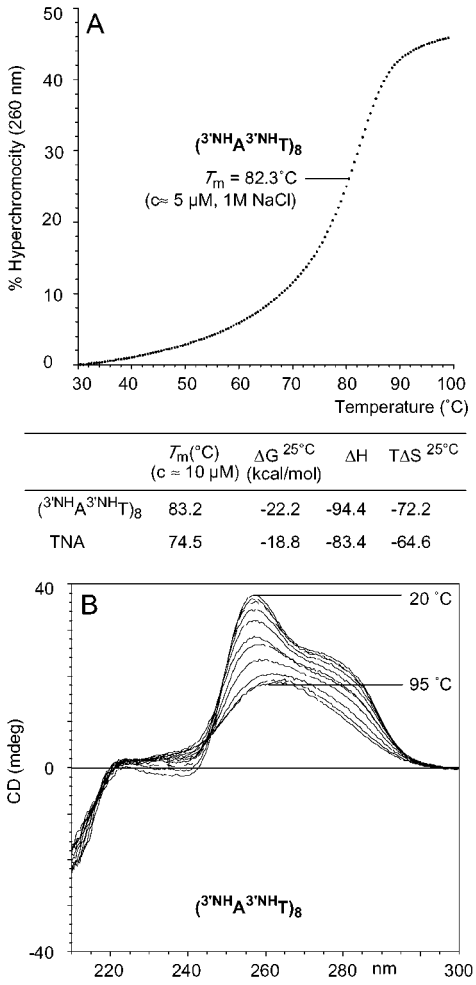
[a] (a) (i) 2.4 M (5.0 molar equiv) TFAA, DMF, rt, 14 h; (ii) 3.3 (3.2) Ac_2O, Py, rt, 3 h, 94%.; (b) 0.1 (3.0) A^{Bz}, 0.4 (9.0) BSA, 0.1 (3.0) $SnCl_4$, CH_3CN, 80 °C, 12 h, 74%; (c) 2 M NH_3 in CH_3OH, rt, 3 days, quant.; (d) 0.1 (2.5) MMT-Cl, Py, rt, 1.5 h, 73%; (e) (i) 0.5 (2.5) BzCl, Py, rt, 2 h; (ii) 1 M NaOH, THF/MeOH/H_2O 5:4:1, 20 min, 0 ˚C, 90%; (f) 0.2 (1.5) chloro(2-cyanoethoxy)(diisopropylethylamino)phosphine, 0.5 (4.0) NEt(*i*-Pr)$_2$, CH_2Cl_2, rt, 88%.

removal of all three protecting groups after the transnucleosidation step and stepwise reprotection of the two amino groups of **20** gave the desired adenine nucleoside **22** in excellent yields.

The conversion of the target nucleosides **8**, **9**, **16**, and **22** to the corresponding phosphoroamidites **10**, **11**, **17**, and **23**—the activated derivatives for solid support oligomer synthesis—was carried out under standard conditions.[18]

The assembly on a DNA synthesizer[19] of oligonucleotide (3′NH)- and (2′NH)-TNA strands containing exclusively phosphoramidate linkages was accomplished using a modified version of the phosphoroamidite method described by Gryaznov.[7] Nucleoside derivatives **8**, **9**, **16**, and **22** were attached to CPG solid support via a succinate linker.[20] 4,5-Dicyanoimidzaole (pK_a 5.2)[21] was used as coupling activator instead of the more common 5-ethylthio-1-*H*-tetrazole (pK_a 3.8)[22] in order to cope with the relative lability of the MMT protecting group toward protons. In performing the automated synthesis, unreacted amino groups were capped with 0.5 M isobutyric anhydride in 2,6-lutidine/THF (1:1), and the detachment of the oligos from the solid support with concomitant deprotection of the phosphate and nucleobase protecting groups was achieved by treatment with 1.0 M methylamine in H_2O/EtOH (1:1) at rt during 2 h. Oligos were purified by HPLC (SAX 1000-8 (Macherey-Nagel)) up to a minimal purity of 95% and shown to have the expected molecular mass by MALDI-TOF MS.

An exploratory base-pairing study was carried out in the (3′NH)-TNA series on the self-complementary sequence ($^{3'NH}A^{3'NH}T)_8$, a sequence that was chosen because TNA strands with alternating purine and pyrimidine bases had been observed to form the most stable duplexes among isomeric sequences.[2] Remarkably, ($^{3'NH}A^{3'NH}T)_8$ was found to form a duplex that turned out to be both thermally and thermodynamically more stable than the corresponding TNA duplex (Figure 1).



	T_m(°C) (c ≈ 10 μM)	ΔG 25°C (kcal/mol)	ΔH	TΔS 25°C
($^{3'NH}A^{3'NH}T)_8$	83.2	-22.2	-94.4	-72.2
TNA	74.5	-18.8	-83.4	-64.6

Figure 1. (A) UV-spectroscopic melting curve (heating) and (B) temperature-dependent CD curves of the duplex formed by the self-complementary (3′NH)-TNA sequence ($^{3'NH}A^{3'NH}T)_8$ (temperature ranges are 20−95 °C). T_m values and thermodynamic data were determined in 1.0 M NaCl, 10 mM NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0. All T_m curves were reversible (no hysteresis). T_m values are taken from the maxima of the first-derivative curve (software Kaleidagraph). Thermodynamic data are derived from plots of T_m^{-1} with versus ln (*c*) (experimental error estimated in ΔH values ±5%). T_m values and thermodynamic data for the TNA duplex are taken from ref 2.

The main part of our study focused on the pairing properties of the non-selfcomplementary but antiparallel-complementary sequences 3′-($A_4T_3ATAT_2AT_2A$)-2′ and 3′-

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$(TA_2TA_2TATA_3T_4)$-2′ prepared before in the TNA as well as in the RNA and DNA series.[2] Table 1 summarizes the T_m values and the thermodynamic data of duplexes formed by intrasystem as well as intersystem cross-pairing of these two sequences in all possible combinations involving the backbones of TNA, RNA, DNA, (3′NH)-TNA, and (2′NH)-TNA. The data in the shaded rectangles refer to intrasystem pairing and reveal the (3′NH)-TNA duplex to be the thermally (but not thermodynamically) most stable duplex among all, while the (2′-NH)-isomer is the thermally (as well as thermodynamically) weakest. Intersystem cross-pairings of both (3′NH)- and (2′NH)-TNA are stronger with RNA than with DNA; in fact, the combinations (3′NH)-TNA/RNA and (2′NH)-TNA/RNA turn out to be comparable in strength to that of the natural DNA/RNA combination. Rather remarkably, (2′NH)-TNA forms a distinctly stronger cross-pairing duplex with TNA than its (3′-NH)-isomer. A rationale for these subtle behavioral differences is not in sight. It is, however, of interest to juxtapose these differences with the pairing properties of Gryaznov's amidate−DNA- and −RNA-type oligomers: there, the (3′NH)-DNA[6] and the (3′NH)-RNA[7] were found to pair strongly with the natural parent systems, whereas the (5′NH)-isomer of DNA was reported not to pair with DNA and RNA at all.[10]

The similarity of CD curves in Figure 2 of the sequence-identical hexadecamer duplexes formed by the (2′NH)-TNA sequence $A_4T_3ATAT_2AT_2A$ with its complementary sequence $TA_2TA_2TATA_3T_4$ from the RNA, DNA, TNA, and (3′-NH)- and (2′NH)-TNA series further characterize the structural kinship between the TNA family and the natural systems (see also Figure 1).

The (3′NH)-phosphoramidate−TNA sequence TA_2TA_2-$TATA_3T_4$ has been shown to be stable toward hydrolytic cleavage in slightly basic medium (1.0 M NaCl, 0.25 M $MgCl_2$, 0.1 M HEPES buffer, 37 °C, pH 8, 25 days) quite similar to TNA itself.[2] Not unexpectedly,[5] such is not the case under acidic conditions; the half-life of the hexadecamer (2′NH)-phosphoramidate−TNA sequence $A_4T_3ATAT_2AT_2A$ in 80% aqueous acetic acid (pH ≈ 0.3) at rt is about 1 h.

We consider the experimental demonstration of the base-pairing capacity of the (2′NH)- and (3′NH)-analogues of TNA as a step in a systematic experimental mapping of the base-pairing properties and the potential for self-assembly of the members of a family of threose-related informational oligomer systems that, in principle, can all be derived from a narrow set of C2- and C1-starting materials.[2]

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Table 1

3′–AAAATTTATATTATTA–2′
2′–TTTTAAATATAATAAT–3′

T_m (°C) / ΔG (25 °C) / ΔH / TΔS (Kcal/mol)	TNA	RNA	DNA	(3′–NH) TNA	(2′–NH) TNA
TNA	56 *	57 *	47 *	32	46
	-14.5	-17.3	-13.3	-8.9	-11.2
	-73.7	-93.9	-77.3	-39.8	-63.4
	-59.2	-76.6	-64.0	-30.9	-52.2
RNA	52 *	59 *	44 *	47	45
	-15.8	-20.0	-12.3	-13.3	-12.2
	-95.2	-131.9	-73.9	-76.9	-81.9
	-79.4	-111.9	-61.6	-63.6	-69.5
DNA	41 *	43 *	48 *	17	23
	-11.0	-13.7	-16.8	-7.6	-6.9
	-57.0	-98.0	-129.2	-45.7	-52.7
	-46.0	-84.3	-112.4	-38.1	-45.8
(3′–NH) TNA	29	49	27	63	26
	-8.4	-14.3	-8.3	-15.8	-7.1
	-36.6	-82.1	-43.5	-67.9	-38.4
	-28.2	-67.8	-35.2	-52.1	-31.3
(2′–NH) TNA	46	52	35	30	36
	-11.1	-17.1	-9.4	-8.1	-10.2
	-62.9	-118.9	-77.9	-52.7	-80.1
	-51.8	-101.8	-68.5	-44.6	-69.9

[a] (3′NH)-TNA = 3′-$^{3'NH}A_4{}^{3'NH}T_3{}^{3'NH}A{}^{3'NH}T{}^{3'NH}A{}^{3'NH}T_2{}^{3'NH}A{}^{3'NH}T_2{}^{3'NH}$A-2′; (2′NH)-TNA is defined in an analogous manner. The color of the acronyms relates to oligonucleotide sequence indicated by the same color. The labels 3′ and 2′ denote strand orientation in TNA sequences; for RNA and DNA sequences, the corresponding labels are 5′ and 3′. Base sequences are written in the 3′→2′ or 5′→3′ direction. T_m values in the shaded diagonal refer to intrasystem pairing. For conditions, see caption of Figure 1. T_m values refer to a concentration of ca. 5 + 5 μM. An asterisk indicates that T_m values were taken from ref 2.

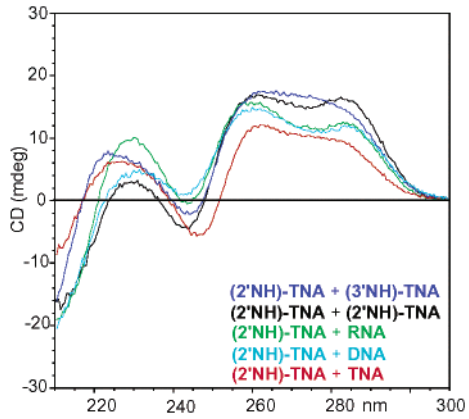

Figure 2. Comparison of CD spectra of duplexes formed by the (2′NH)-TNA sequence $A_4T_3ATAT_2AT_2A$ with its complementary sequence $TA_2TA_2TATA_3T_4$ in the DNA, RNA, TNA,and (2′NH)- and (3′NH)-TNA series ($c \approx 5 + 5$ μM, $T \approx 0$ °C). For conditions, see the caption of Figure 1.