Control of Folding and Binding of Oligonucleotides by Use of a Nonnucleotide Linker

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Abstract: A linker containing a terephthalamide group, $-P(O)(O^-)O(CH_2)_6NHC(O)C_6H_4C(O)NH(CH_2)_6OP(O)(O^-)-$, is shown to be an effective structural element for organizing oligonucleotide chains in solution. Capping a pair of complementary oligonucleotides with this linker leads to marked enhancement in stability of the Watson–Crick duplex structure. Joining a pair of thymidylate oligomers with this linker gives a compound exhibiting unusually high affinity for oligo(dA) strands; even a tetramer unit can be recognized in dilute solution. Presumably Hoogsteen as well as Watson–Crick hydrogen bonding stabilizes a bimolecular "triplex". Both types of linkage (i.e., joining two complementary strands and joining two pyrimidine strands) are embodied in the novel compound, d(TTTTTT-X-TTTTTT-X-AAAAAA), and function jointly in stabilizing a doubly folded monomolecular triple stranded structure (T_m 58 °C, 1 M NaCl; -X- represents the terephthalamide linker group).

Introduction

Oligonucleotides containing palindromic segments linked by short runs of noncomplementary nucleotides[1] or by a hexaethylene glycol bridge[2] exhibit high T_m values. The enhanced stability of these intramolecular complexes, relative to duplex structures with equivalent base pairs, may be attributed to the high effective local concentration of the complementary intramolecular segments.

A related phenomenon is the striking stability of complexes formed between single stranded purine oligonucleotide tracts and circular oligonucleotides containing two pyrimidine oligonucleotide segments capable of forming a triplex structure with the purine tract.[3] Here, too, enhanced stability depends on the proximity of two oligonucleotide strands imposed by linker fragments.

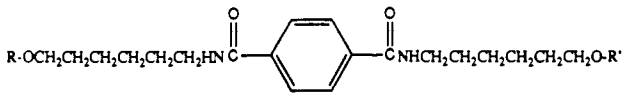
Nonnucleotide linkers can also be used to alter hybridization characteristics of oligonucleotides. Recently Schepartz et al.[4] showed that two oligonucleotide segments linked by a string of abasic phosphodiester units bind cooperatively to appropriately positioned, noncontiguous single stranded segments of a natural RNA, and Horne and Dervan[5] reported that a 1,2-dideoxyribose linker connecting two polypyrimidine oligomers at the 3′ ends serves as a switch that enables the probe to bind to alternate strands of a DNA target.

In exploring structural features that could prove useful as elements in organizing oligonucleotide frameworks, we have prepared and examined a number of oligonucleotide derivatives in which the linker $-P(O)(O^-)O(CH_2)_6NHCOC_6H_4CONH(CH_2)_6OP(O)(O^-)-$ serves as a bridge between the 3′ terminal oxygen atom of one oligomer and the 5′ terminal oxygen atom of another. This linker, designated as X in the formulas, can in principle serve as a replacement either for the single stranded oligonucleotide loop characteristic of a hairpin structure or for an oligonucleotide segment connecting two pyrimidine oligonucleotide tracts of a triple helix. Experiments designed to test both possibilities are described here.

Several features make this linker an attractive candidate for study. Molecular models indicate that it can readily span the distance between the terminal 5′ and 3′ oxygen atoms of complementary oligomers in a duplex, with the aromatic ring stacking above the terminal base pair. The terephthalamide unit provides a rigid core that reduces the overall degrees of freedom, yet the hexamethylene units permit some adjustment to conform to the preferred geometry of the adjoining oligonucleotides. In addition, this linker would be less susceptible than oligonucleotide bridges to attack by nucleases in a biological system.

Formulas for the modified oligonucleotides selected for study are indicated in Table I (compounds **1–6**). These compounds were designed to test the effect of the linker in systems potentially capable of folding into a hairpin duplex structure (compounds **1** and **6**), a bimolecuiar hairpin triplex structure (compounds **2** and **3**; in combination with appropriate complementary targets), or a monomolecular triplex structure (compound **5**).

Chart I



7	R = t-BuSi $(Me)_2$-	R' = -t-BuSi $(Me)_2$-
8	R = H-	R' = -t-BuSi $(Me)_2$-
9	R = DMT-	R' = -t-BuSi $(Me)_2$-
10	R = DMT-	R' = -H
11	R = DMT-	R' = -P (H) (O) O^-
12	R = DMT-	R' = -P (OCH_2CH_2CN) N $(iPr)_2$

Results

The monoprotected diol, **10**, was prepared from 6-aminohexanol and terephthaloyl chloride via intermediates **7–9** (Chart I). This path utilizing several protection–deprotection steps proved more satisfactory than more direct routes, where difficulties with insoluble intermediates or polymers were encountered. The alcohol was then converted to hydrogen phosphonate **11** and to phosphoramidite **12** by standard phosphitilation procedures. With a dimethoxytrityl group at one end and a reactive phosphorus function at the other, these reagents could be readily utilized in conventional protocols for synthesizing oligonucleotides on solid supports. Both **11** and **12** served satisfactorily as synthons for incorporating the terephthalamide linker into oligonucleotides.

Thermal dissociation data for the folded conformers and complexes formed by the bridged oligonucleotides are summarized in Table I, along with comparative data for unmodified oligonucleotides. The modified oligomers contain a bridge that links palindromic segments (compounds **1** and **6**), or a bridge that links oligothymidylate segments in a 3′ → 5′ orientation (compounds **2** and **3**), or two bridges, with one linking palindromic segments and the other linking oligothymidylate segments (compound **5**).

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Table I. Thermal Dissociation of Oligomer Complexes[a]

exp.	oligonucleotides	T_m,[b] °C 0.1 M NaCl	T_m,[b] °C 1.0 M NaCl
I	d(TTTTTT) + d(TTTTTT) + d(AAAAAA)		<2
II	d(TTTTTTAAAAAA)	18 (12[c])	32 (27[c])
III	d(TTTTTT-X-AAAAAA) (**1**)	42 (42[d])	50 (50[d])
IV	d(TTTTTT-X-TTTTTT) (**2**) + d(AAAAAA)	20	32
V	d(TTTTTT-X-TTTTTT) (**2**) + d(AATAAA)	<5	15
VI	d(TTTTTT-X-TTTTTT) (**2**) + d(AAAA)		17
VII	d(TTTT-X-TTTT) (**3**) + d(AAAAAA)	<5	12
VIII	d(TTTT-X-TTTT) (**3**) + d(AATAAA)		<2
IX	d(AAAAAA-X-AAAAAA) (**4**) + d(TTTTTT)		<5
X	d(TTTTTT-X-TTTTTT-X-AAAAAA) (**5**)	42 (42[d])	58 (58[d])
XI	d(GATCCGATTGTG) + d(CACAATCG)	30	35 (25[d])
XII	d(GATCCGATTGTG-X-CACAATCG) (**6**)	72 (72[d])	78

[a] The new compounds containing the linker are indicated by numbers **1**–**6**. -X- represents the terephthalamide linker group, which joins the 3'O atom of one segment to the 5'O atom of another segment. All oligomers are terminated by hydroxyl groups. [b] Tris·HCl buffer, 10 mM, pH 7.0. The total nucleotide concentration was ~1.0 A_{260} units/mL at 25 °C. [c,d] The values in parentheses are for total nucleotide base concentration of *c* 0.25 A_{260} units/mL or *d* 0.2 A_{260} units/mL; these solutions were prepared by diluting the standard solutions with *c* 3 volumes or *d* 4 volumes of the buffer–salt solution to give μmolar concentrations of oligomers of 2.3, 1.8, 1.2, and 1.0 μM for d(TTTTTTAAAAAA), **1**, **5**, and each compound in XI, respectively.

Bridge Palindromic Segments. These compounds (**1** and **6**) proved distinctive in two respects. (a) They form hypochromic structures in dilute solution that are significantly more stable than the double stranded complexes formed from model oligonucleotides lacking the bridge. One model consists of paired complementary oligonucleotides corresponding to the segments linked by the bridge, i.e., d(TTTTTT) + d(AAAAAA) and d(GATCCGATTGTG) + d(CACAATCG). The T_m values for the bridged compounds were found to be >42 °C higher than for these model compounds, both in the case of the dT/dA oligomers (compare I and III, Table I) and the mixed base oligomers (compare XI and XII). The other model is represented by the dodecamer, d(TTTTTTAAAAAA), which contains the same linked nucleotides as **1** but lacks the bridge. This compound, lacking appropriate nucleotides to form a stable hairpin structure, should form a bimolecular duplex in solution. It was selected to model the base stacking and hydrogen bonding interactions for an extended linear dimer of **1**. The enhancement in T_m for the bridged oligomer relative to this unmodified dodecamer ranged from 18 °C to 30 °C, depending on the conditions (compare II and III). (b) In contrast to the case for the complexes derived from two independent strands, the dissociation temperature for the compounds with the linked strands is independent of oligonucleotide concentrations over a 5-fold range (compare T_m values at different concentrations for II and III and XI and XII). This point is demonstrated nicely by the complete melting curves shown in Figure 1. The curves for **1** at the high and low oligomer concentrations are superimposable. In contrast, the corresponding curves for d(TTTTTTAAAAAA) differ significantly; the stability of the complex decreases with decreasing oligomer concentration, as expected for a bimolecular complex.

Bridged Thymidylates. Interaction of d(TTTTTT) with d(AAAAAA) is very weak (experiment I; T_m <2 °C). In sharp contrast, the linked oligomer d(TTTTTTT-X-TTTTTT) forms a stable complex (T_m 32 °C) with d(AAAAAA) under the same conditions (IV). That proper base pairing is important in this system is shown by the experiment with d(AATAAA) and d(TTTTTT-X-TTTTTT) (V); a single mismatch resulting from substitution of dT for dA in the target leads to a decrease in T_m of 17 °C.

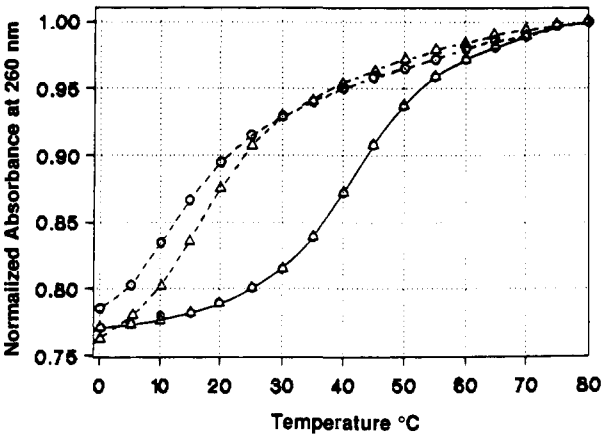

Figure 1. Melting curves in 0.1 M aqueous NaCl at pH 7.0, 10 mM Tris·HCl buffer. The dashed lines are for d(TTTTTTAAAAAA) at oligomer concentrations of 1.0 A_{260} units/mL (Δ) and 0.25 A_{260} units/mL (○). The solid line is for d(TTTTTT-X-AAAAAA), both at 1.0 A_{260} units/mL (Δ) and at 0.2 A_{260} units/mL (○).

Experiments VI–IX further delineate the properties of the linked oligonucleotide probes. Experiment VI shows that targets as small as four nucleotide units, d(AAAA), can be recognized in dilute solution, and experiments VII and VIII demonstrate that selective binding can be achieved with a probe strand as small as a pair of tetramer units. Finally, the lack of significant interaction between d(TTTTTT) and d(AAAAAA-X-AAAAAA) (experiment IX) shows that no stabilization of the complex is found when the linked overhanging oligonucleotide segment is a purine derivative. This result is consistent with the concept that the unusual stability exhibited by complexes involving the linked thymidine probes depends on formation of short triple stranded structures involving Hoogsteen as well as Watson–Crick hydrogen bonding.

Doubly-Bridged Oligonucleotides. The key properties of the doubly substituted 18-mer, compound **5**, are brought out in Figure 2 and Table I. The melting curves show a single inflection, indicative of cooperative melting. There was no evidence from any of the curves for an intermediate dissociation step. For aqueous solutions at high ionic strength (1 M NaCl), the T_m value for the helix-coil transition is 8 °C greater for compound **5** than for compound **1**, which models the Watson–Crick duplex that can form in **5**. This result shows that the extra run of six thymidylates in **5** as well as the run of six involved in Watson–Crick base pairing contributes to stabilization of the complex.

The normalized hyperchromicity observed for the "melting" of compound **5** is the same as found for compound **1** (Figure 2). Since the absorbance of single stranded oligothymidylate segments does not change on heating, this result indicates that the additional six thymidylates in **5** are also organized in a stacked conformation

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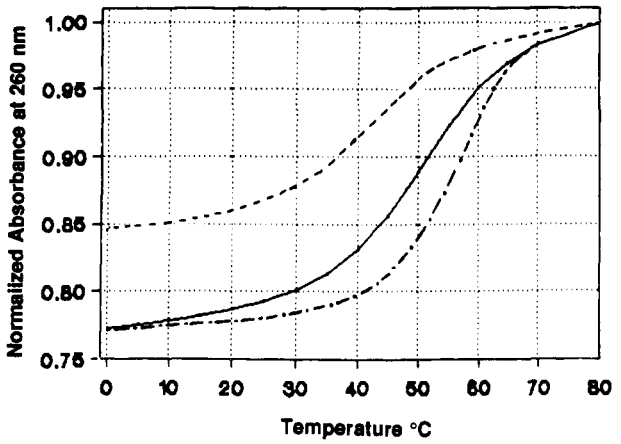


Figure 2. Melting curves for d(TTTTTT-X-AAAAAA) (—) and d(TTTTTT-X-TTTTTT-X-AAAAAA) (- - -) in 1 M NaCl at pH 7.0, 10 mM Tris·HCl buffer. The curve (- -) was calculated for d(TTTTTT-X-TTTTTT-X-AAAAAA) assuming unequal hairpin conformation at low temperature. The oligomer concentrations are ~1.0 A_{260} units/mL.

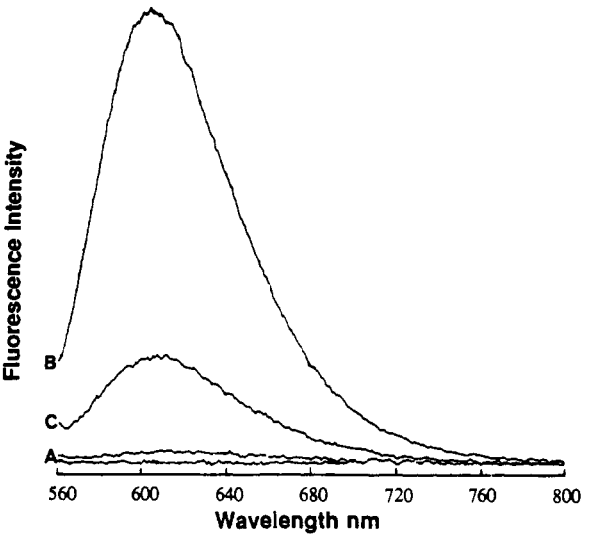


Figure 3. Fluorescent spectra for (A) ethidium bromide, (B) ethidium bromide plus d(TTTTTT-X-AAAAAA) (14 μM); and (C) ethidium bromide plus d(TTTTTT-X-TTTTTT-X-AAAAAA) (10 μM) in 0.1 M aqueous NaCl, 10 mM Tris·HCl (pH 7.0). The ethidium bromide concentration was 8 μM in all cases. The bottom line is the base line (no ethidium, no nucleotides).

in the complex. For comparison, a normalized absorbance curve was calculated for compound **5** on the assumption that the six thymidine bases and six adenine bases are stacked as in compound **1** and that the other six thymidine units are in a random coil arrangement in solution. This curve, shown by the dashed line in Figure 2, differs markedly from the observed curve.

Another significant finding is that, as in the case of compounds **1** and **6**, the T_m value for **5** is not sensitive to changes in concentration of the oligomer (experiment X, Table I). This result supports the view that the reversible transition manifested by the melting curves is unimolecular.

Ethidium bromide serves as a convenient probe for double stranded[6,7] and triple stranded[8] polynucleotides. It binds to both structures, but the affinity is greater for the double helix than the triple helix.[8] In both cases intercalative binding is indicated by an increase in fluorescence of the ethidium. To gain further information about the conformation of compound **5** we investigated the interaction of ethidium with the monobridged dodecamer, **1**, and the doubly-bridged octadecamer, **5**, under comparable conditions. The fluorescence spectra for these systems are shown in Figure 3. In both cases an increase in the intensity of fluorescence of ethidium was observed; however, the extent of the effect differed markedly. The enhancement was about five times greater for compound **1** than for compound **5**.

Discussion

The role that a nonnucleotide bridging element can play in organizing oligonucleotide tracts in dilute solution was studied with oligomers containing the linker $-P(O)(O^-)O(CH_2)_6NHC(O)C_6H_4C(O)NH(CH_2)_6O(P)(O)(O^-)-$. Systems representing three different potential folding patterns were examined.

In the simplest system the tether joins complementary oligonucleotides (compound **1** or **6**) which could form hydrogen-bonded, base-stacked structures reversibly in solution either by dimerization to an extended helix or by folding intramolecularly to a hairpin conformation. These structures are represented in Chart II for compound **1**.

The insensitivity of the T_m values to changes in oligomer concentration for **1** and **6** indicate that these bridged oligonucleotides predominantly fold to the hairpin conformation. By contrast, the thermal dissociation data for d(TTTTTTAAAAAA) indicate that this contiguous dodecamer dimerizes to an extended duplex, as expected. The linker in **1** and **6** serves as a cap to hold the nucleotide segments together for base pairing. It is noteworthy that the cap leads to an enhancement in T_m for the dT/dA oligomers of >42 °C relative to a hexamer duplex lacking the linker and to an enhancement of >18 °C relative to the bimolecular complex formed from d(TTTTTTAAAAAA). The bridged system is related to one described by Durand et al.[2] in which a hexaethylene glycol unit connecting two nonamer strands led to

Chart II

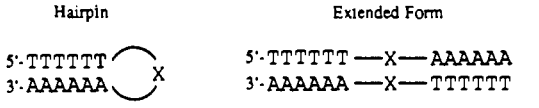
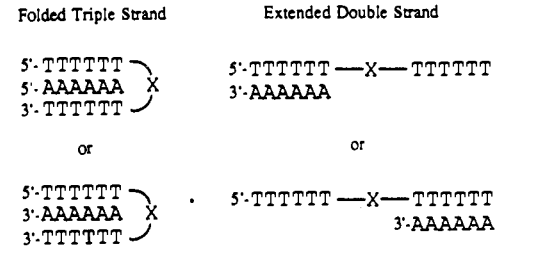


Chart III

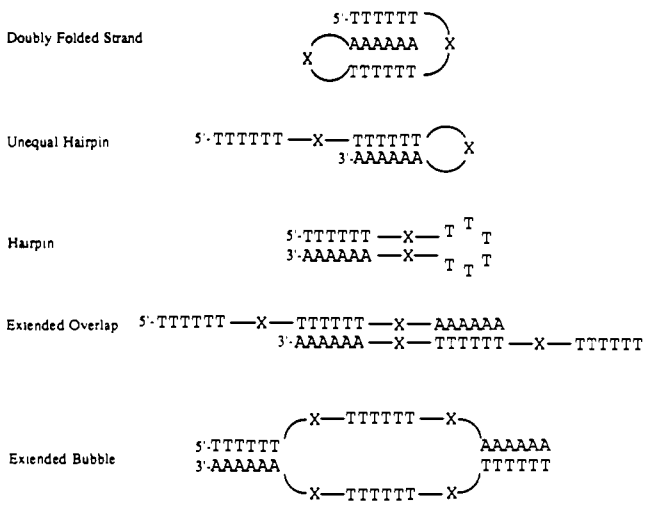


an enhancement in T_m of 35 °C relative to a duplex formed from nonlinked nonamer strands. Such bridges, which resemble single stranded oligonucleotide loops[1] in facilitating formation of hairpin structures but differ markedly from oligonucleotide loops in structure and biochemical chemical properties, offer new opportunities in the design and utilization of oligonucleotide derivatives.

In the second system two oligo(dT) strands are linked to form probes that bind selectively to oligo(dA) targets. A striking feature is the stability of the complexes that are formed. T_m values for complexes derived from the linked probes are much higher than for Watson–Crick type duplexes containing the same base pairs. Even a tetramer (dAAAA) can be recognized in dilute aqueous solution by probe **2**, and selective binding can be observed with a probe containing only four thymidine units in each arm (**3**). The types of structures that might form in these systems are depicted in Chart III.

One would expect that hydrogen bonding and base stacking interactions in the extended double stranded arrangement would be about the same as in d(TTTTTT)/d(AAAAAA); the overhanging linker and thymidylate groups should have little effect on the stability of the duplex structures. Since, in fact, the complexes formed by the linked thymidylates (**2** and **3**) are unusually stable, we conclude that the extended double stranded conformation is unimportant in this system. By contrast, the folded triple stranded form accommodates the data since the oligo(dA) strands can be held to one of the oligo(dT) segments by Watson–Crick bonding and to the other segment by Hoogsteen

Chart IV



bonding. The unusual stability stems from the fact that the two oligo(dT) segments are held in close proximity by the bridge and both bind to the oligo(dA) target. In agreement with this conclusion, no unusual stabilization was found in a system in which the overhanging strand was a purine oligomer (experiment IX, Table I). The oligo(dA) strand in the folded complex (Chart III) may, in principle, be oriented with either the 5′ or the 3′ terminus pointing toward the bridge, depending on which strand of the dT segment is held by Watson–Crick base pairing. No data on the relative stability of these two isomers is currently available.

This bridged oligonucleotide derivative resembles the circular oligonucleotides in facilitating binding of purine oligonucleotidies by aligning oligopyrimidine tracts. For comparison, Prakash and Kool[3b] recently reported a T_m of ~32° (100 mM NaCl, 10 mM $MgCl_2$) for a complex formed from d(AAAA) and a 24-mer cyclic oligonucleotide containing two d(−TTTTTTTT−) segments in a stem region. Also, since initiation of this research, another interesting example of cooperative hybridization involving tethered oligonucleotides has been described. Giovannangeli et al.[9] found that a probe consisting of two pyrimidine oligonucleotide tracts connected by a hexaethylene glycol bridge would form a triple stranded complex with a purine oligonucleotide. In contrast to the cases involving the terephthalamide type linker, however, enhancement in T_m above that for the Watson–Crick duplex was not observed.

The third system investigated [d(TTTTTT-X-TTTTTT-X-AAAAAA), compound **5**] is particularly interesting since it combines within one molecule linkers connecting both palindromic sequences and pyrimidine oligonucleotides. A priori, hybridization to one or more of the structures represented in Chart IV would be possible. These include a doubly folded monomolecular triple stranded conformer, two singly folded conformers, and two extended partial duplex structures.

The properties of compound **1**, d(TTTTTT-X-AAAAAA), serve as reference points for evaluating the relative importance of the conformations that compound **5** might assume. Analysis of several lines of evidence points to the doubly folded structure as the predominant conformation. (a) Perhaps the most definitive is the hyperchromicity exhibited by **5** on melting. The normalized increase in absorbance is the same as observed for compound **1**, in which all nucleoside bases are in a stacked arrangement at low temperature. We conclude that all bases in **5** are also stacked in solution at low temperature (0 °C). This condition is satisfied only by the doubly folded conformation. (b) As in the case of **1**, the temperature of dissociation of **5** remains constant over a 5-fold range of nucleotide concentration. This behavior is consistent with expectations for the doubly and singly folded conformations but not for extended duplexes formed from separate strands. (c) At high ionic strength (1 M NaCl) the stacked form of **5** is more stable than the duplex formed by **1**. This enhanced stability is readily explained in terms of the doubly folded structure, which is an intramolecular version of the folded triple strand structure pictured in Chart III. As demonstrated by the unusual stability of that complex (experiments IV–VII, Table I), Hoogsteen and Watson–Crick pairing can act cooperatively. (d) In the stacked conformation, compound **5** is less effective than compound **1** in enhancing the fluorescence of ethidium. Since triple stranded complexes bind ethidium[8] less efficiently than related double stranded complexes, this property points to the doubly folded conformation. The other forms shown in Chart IV contain double stranded segments that would be expected to mimic **1** in binding ethidium. (e) Finally, the T_m values for **5** increase more than the T_m values for **1** with increasing salt concentration. Although not definitive for distinguishing among all the conformations shown in Chart IV, this result is consistent with a doubly folded, triple stranded structure for **5** and a singly folded, double stranded structure for **1**.

In summary, these examples, illustrating three different folding patterns, demonstrate that a tailored nonnucleotide linker can be used to stabilize hybridization and to direct folding of oligonucleotide strands. Such linkers may prove useful in designing new molecular systems based on oligonucleotides.

Experimental Section

Materials and Methods. Pyridine, tetrahydrofuran (THF), and acetonitrile (MeCN; HPLC grade) were dried over calcium hydride. Reactions leading to compounds **11** and **12** were carried out with stirring under a nitrogen atmosphere. Compounds **7–10** were purified by chromatography on a silica gel column (silica/sample 40/1 w/w). Thin layer chromatography (TLC) was carried out on analytical Merck silica plates. Thermal dissociation data were obtained on a Perkin-Elmer 570 UV–vis spectrophotometer equipped with a Peltier temperature controller. Absorbance values were recorded after equilibration as the temperature was increased incrementally 5 °C at 5-min intervals. Fluorescence spectra were obtained on a Perkin-Elmer fluorescence spectrometer using an excitation wavelength of 550 nm. NMR spectra were run in $CDCl_3$ on a Varian XL400 instrument; ppm are relative to Me_4Si. FAB mass spectra were obtained on a VG-70-250SE mass spectrometer by Dr. Doris Hung. Elemental analyses were made by the Searle Laboratory.

Denaturing polyacrylamide gel electrophoresis (PAGE) was carried out with a standard Tris/boric acid/EDTA buffer and a 20% gel (acrylamide/bisacrylamide 29/1, w/w, 165 × 165 × 0.75 mm plate) run 1.5 h at 400 V.

***N,N′*-Bis[6-(*tert*-butyldimethylsiloxy)hexyl]terephthalamide (7).** A solution of 6-aminohexanol (5.85 g, 50 mmol) and imidazole (6.8 g, 100 mmol) in DMF (50 mL, dried with 4A molecular sieves) was cooled to 0 °C and treated with *tert*-butyldimethylsilyl chloride (16.0 g, 110 mmol). The mixture was allowed to warm to room temperature, stirred overnight, concentrated under vacuum, and partitioned between water and CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated to give a thick oil (11.2 g): R_f (TLC; $CHCl_3$/MeOH/Et_3N, 88/10/2, v/v/v) 0.5. Presence of a free amino group was shown by a positive ninhydrin test.

To a portion of this material (1.39 g, ~6 mmol) dissolved in benzene/ether (1/1 v/v; 40 mL) was added ice (15 g), K_2CO_3 (0.41 g, 3 mmol), and a cold solution of terephthaloyl chloride (0.30 g, 1.5 mmol) in benzene/ether (1/1, v/v, 20 mL). The next day the organic layer was dried and evaporated. Purification of the solid product (0.88 g) by chromatography (EtOAc eluant) afforded the title compound: 0.704 g (80%); mp 155–156 °C; R_f (TLC EtOAc) 0.78; FAB MS, M + 1 593, M calcd 592; ^1H NMR ($CDCl_3$) ppm, 0.03 (s, $2Me_2Si$), 0.86 (s, 2*t*-BuSi), 1.28–1.50 (m, $2(CH_2)_4$), 3.23–3.56 (m, $2CH_2N$, $2CH_2O$), 6.34 (t, 2NH), 7.75 (s, C_6H_4).

***N*-[6-(*tert*-Butyldimethylsiloxy)hexyl]-*N′*-(6-hydroxyhexyl)terephthalamide (8).** A solution of compound **7** (2.96 g, 5 mmol) and Bu_4NF (3.75 mmol) in THF (100 mL) was stirred 1 h, the solvent was removed at reduced pressure, and the residue was taken up in CH_2Cl_2 and washed with water. Chromatography afforded, after elution of some starting bis-silyl derivative (7, 1.3 g), the desired monosilyl compound, **8**: 0.95 g (40%); mp 127–128 °C; R_f (TLC, EtOAc) 0.33; FAB MS, M + 1 479, calcd M 478; ^1H NMR (DMSO-d_6) ppm 0.03 (s, Me_2Si), 0.84 (s, *t*-BuSi), 1.29–1.50 (m, $2(CH_2)_4$), 2.45 (DMSO impurity), 3.23–3.57 (m, $2CH_2N$ and $2CH_2O$), 4.34 (t, 2NH), 7.88 (s, terephthalyl).

***N′*-[6-(*tert*-Butyldimethylsiloxy)hexyl]-*N*-[(6-(dimethoxytrityl)oxy)-hexyl]terephthalamide (9).** A mixture of compound **8** (0.92 g, 1.9 mmol) and 4,4′-dimethoxytrityl chloride in pyridine (25 mL) was stirred overnight with a catalytic amount of 4-(dimethylamino)pyridine, evaporated at reduced pressure, and partitioned between water and CH_2Cl_2. The solid obtained from the organic layer was taken up in EtOAc and purified

by column chromatography. Elution with EtOAc/hexane/Et_3N (20/78/2 v/v/v; to elute dimethoxytritanol), followed by EtOAc/hexane/Et_3N (50/48/2 v/v/v), afforded the DMT derivative of compound **9**: 0.9 g (65%); R_f (TLC hexane/EtOAc/Et_3N 80/19/1 v/v/v) 0.24, positive DMT+ test; ^1H NMR ($CDCl_3$) ppm 0.02 (s, Me_2Si), 0.87 (s, *t*-BuSi), 1.30–1.62 (m, $2(CH_2)_4$), 3.04 (t, CH_2OC), 3.40 (m, $2CH_2N$), 3.56 (t, CH_2OSi), 3.74 (s, 2MeO), 6.12 (m, 2NH), 6.8 (d, 4 H [J_{HH} 8.8 Hz], H3 and H5 of anisyl), 7.2–7.5 (m, rest of DMT), 7.77 (s, terephthalyl).

***N*-(6-((Dimethoxytrityl)oxy)hexyl]-*N'*-(6-hydroxyhexyl)terephthalamide (10).** A portion of compound **9** (0.61 g, 0.78 mmol) was stirred with Bu_4NF (1 mmol) in dry THF (25 mL) for 3 h, and then the mixture was concentrated under reduced pressure and chromatographed to give 0.52 g (98%) of compound **10**: mp 89–90 °C; R_f (TLC, EtOAc/Et_3N 98/2 v/v) 0.25; FAB MS, M + 1 667, M calcd 666; ^1H NMR ($CDCl_3$) ppm 1.30–1.62 (m, $2(CH_2)_4$), 3.04 (t, CH_2OC), 3.46 (m, $2CH_2N$), 3.63 (m, CH_2-hydroxyl) 3.78 (s, $2CH_3O$), 6.19 (t, HN), 6.30 (t, HN), 6.8 (d, 4 H [J_{HH} 8.8 Hz], H3 and H5 of 2 anisyls), 7.2–7.5 (m, rest of DMT), 7.77 (s, terephthalyl). Anal. Calcd for $C_{41}H_{50}O_6N_2 \cdot 0.5H_2O$: C, 72.89; H, 7.56; N, 4.15. Found: C, 72.65; H, 7.78; N, 4.11.

Hydrogen Phosphonate Derivative of Compound 10 (11). To imidazole (0.73 g, 11 mmol) in MeCN (20 mL) at ice temperature was successively added with stirring PCl_3 (0.28 mL, 3.2 mmol), Et_3N (1.6 mL, 11 mmol), and compound **10** (0.50 g, 0.75 mmol in 5 mL of MeCN). After removing the ice bath, stirring for 4 h, addition of water (5 mL), and stirring for 30 min, the mixture was evaporated under reduced pressure, coevaporated with pyridine/Et_3N, and partitioned between $CHCl_3$ and water. Purification of the recovered solid by chromatography ($CHCl_3$/MeOH/Et_3N, 87/10/3, v/v/v) gave 0.535 g (84%) of the desired triethylammonium hydrogen phosphonate salt (compound **11**): R_f (TLC, $CHCl_3$/MeOH/Et_3N 87/10/3 v/v/v). The ^{31}P NMR spectrum showed two peaks (+4.95 and −0.12 ppm) at 121 MHz for (P resonance split by H).

Cyanoethyl *N*,*N*-Diisopropylphosphoramidite Derivative of Compound 10 (12). Chloro-β-cyanoethoxy-*N*,*N*-diisopropylaminophosphine (0.23 mL, 1 mmol) was slowly added to a solution of compound **10** (500 mg, 0.75 mmol), diisopropylamine (0.28 mL, 2 mmol), and 4-(dimethylamino)pyridine (10 mg) in tetrahydrofuran (10 mL). After 4 h (as indicated by TLC, the reaction was virtually complete) the mixture was poured into ethyl acetate (100 mL, prewashed with brine) and washed successively with dilute sodium bicarbonate and brine. The ethyl acetate layer was dried (sodium sulfate) and concentrated, and the product was isolated by chromatography on a silica gel column with a solvent of ethyl acetate/hexane/triethylamine 47/50/3 v/v/v: yield 390 mg (60%); R_f (Merck silica plates) 0.5 in the solvent used for the preparative separation; ^1H NMR ($CDCl_3$) ppm 1.12–1.29 (m, 6Me of 2*i*Pr), 1.35–1.64 (m, $8CH_2$), 2.64 (m, 2 CH of 2*i*Pr), 3.40 (m, $CH_2OPOCH_2CH_2CN$), 3.45 (m, $2CH_2N$), 3.40 (t, CH_2O-DMT), 3.79 (s, $2CH_3O$), 6.15–6.27 (m, 2 NH), 6.80–7.44 (m, 13 aromatic H of DMT), 7.80 (s, terephthalyl); ^{31}P NMR 145.4 ppm (85% H_3PO_4 as external reference).

Synthetic Oligonucleotides. Oligonucleotides were synthesized on long chain alkylamine controlled pore glass supports (80–100 mesh, 500 A, from Glen Research) using nucleoside cyanoethyl phosphoramidite reagents (Milligen/Biosearch) for addition of nucleotide units and hydrogen phosphonate reagent **11** (preparation of compounds **2**–**4** and **6**) or phosphoramidite reagent **12** (preparation of compounds **1** and **5**) for addition of the linker. Conventional protocols for syntheses with phosphoramidite reagents[10] and hydrogen phosphonate reagents[11] were followed, starting with 1 μmol of loaded nucleoside. A Cyclone DNA synthesizer was employed for the amidite couplings. For the hydrogen phosphonate coupling with compound **11**, the support was removed from the DNA synthesizer and coupling carried out manually by the syringe technique. The yields in the coupling steps, as indicated by the DMT+ test, averaged >97% for the cycles with phosphoramidite reagents and 90% for condensations with the hydrogen phosphonate linker reagent. The oligonucleotides, worked up in a conventional manner and isolated by reversed phase HPLC, were better than 95% pure by analysis by HPLC and polyacrylamide gel electrophoresis. That the terephthalamide linkage is stable to ammonium hydroxide under the synthesis conditions was demonstrated by treating compounds **1** and **10** with concentrated ammonium hydroxide at 55 °C for 15 h. Analysis by TLC and HPLC showed that the compounds were unchanged (<5% degradation).

Acknowledgment. This work was supported by Grant GM10265 from the National Institute of General Medicine of the National Institutes of Health. We are grateful to Dario M. Bassani and Prof. F. D. Lewis for assistance with the fluorescence experiments.

Stereoselectivity of Enzymatic and Chemical Oxygenation of Sulfur Atoms in 2-Methyl-1,3-benzodithiole

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Abstract: S-Monooxygenation of 2-methyl-1,3-benzodithiole, to yield *cis*- and *trans*-2-methyl-1,3-benzodithiole 1-oxide, has been studied as a new stereochemical probe using achiral and chiral chemical oxidants and enzymes from microbial and animal sources. Chemical oxidants showed a preference for trans *S*-oxide formation, and this preference was enhanced in the presence of a chiral matrix. The ability of pure enzymes to stereodifferentiate between geminal lone pairs on a prochiral sulfur atom, or geminal sulfur atoms on a prochiral carbon atom, has been observed. Thus, intact fungal and bacterial oxidations showed a marked selectivity (87–96%) for formation of the cis *S*-oxide isomers. In addition, highly purified hog liver and rabbit lung flavin-containing monooxygenases showed a marked preference for formation of the cis *S*-oxide and the *pro-R* sulfur atom. Other monooxygenase-catalyzed sulfoxidations of 2-methyl-1,3-benzodithiole, including cytochrome P-450 2B1 ($P450_{PB-B}$), showed a markedly lower stereoselectivity.

Introduction

Sulfoxidation is a very common biotransformation process which has been widely observed in xenobiotic metabolism.[1] The ability of monooxygenase enzymes to stereodifferentiate between prochiral lone pairs on a sulfur atom has previously been examined using fungal, bacterial, and animal enzymes.[1,2] The two sulfur atoms present in the thioacetal group provide a more rigorous challenge to the monooxygenase enzymes in stereoselecting between each geminal lone pair on a prochiral sulfur atom and between geminal sulfur atoms attached to a prochiral carbon atom.

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