# Dipyrido[3,2-*a*:2′,3′-*c*]phenazine-Tethered Oligo-DNA: Synthesis and Thermal Stability of Their DNA·DNA and DNA·RNA Duplexes and DNA·DNA·DNA Triplexes

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

Dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) derivatives were conjugated to 9-mer and 18-mer DNA (ODN) at a site without nucleobase, either at the 5′- or 3′-end or at a internucleotide position, *via* linkers of 7, 12, or 18 atoms lengths. These dppz-linked ODNs were synthesized using novel backbone glycerol phosphoramidites: Glycerol, serving as artificial nucleoside without nucleobase, was modified to amines **10**, **23**, and **24**, which were suitable for the subsequent key reaction with dppz-carboxylic acid **3** (*Schemes 2* and *3*). The products of these reactions (see **5**–**7**) were then transformed to the standard phosphoramidite derivatives (see **27**, **29**, and **30**) or used for loading on a CPG support (see **28**, **31**, and **32**). The dppz-modified ODNs were subsequently assembled in the usual manner using automated solid-phase DNA synthesis. The 9-mer ODN-dppz conjugates **35**–**43** were tested for their ability to form stable duplexes with target DNA or RNA strands (D11 (**60**) or R11 (**61**)), while the 18-mer ODN-dppz conjugates **48**–**56** were tested for their ability to form stable triplexes with a DNA target duplex D24·D24 (**62**) (see *Tables 1* and *2*). The presence of the conjugated dppz derivative increases the stability of DNA·DNA and DNA·RNA duplexes, typically by a $\Delta T_m$ of 7.3–10.9° and 4.5–7.4°, respectively, when the dppz is tethered at the 5′- or 3′-terminal (*Table 2*). The dppz derivatives also stabilize triplexes when attached to the 5′- or 3′-end, with a $\Delta T_m$ varying from 3.8–11.1° (*Table 3*). The insertion of a dppz building block at the center of a 9-mer results in a considerably poorer stability of the corresponding DNA·DNA duplexes ($\Delta T_m$ = 0.5 to 4.2°) and DNA·RNA duplexes ($\Delta T_m$ = – 1.5 to 0.9°), while the replacement of one interior nucleotide by a dppz building unit in the corresponding 8-mer ODN does not reveal the formation of any duplex at all. Different types of modifications in the middle of the 18-mer ODN, in general, do not lead to any triplex formation, except when the dppz derivative is tethered to the ODN through a 12-atom-long linker (*Entry 9* in *Table 3*).

**1. Introduction.** – Oligonucleotides (ODNs) can recognize both single-stranded (ss) DNA or RNA or double-stranded (ds) DNA in a sequence-selective manner. The high specificity of ODN-ssRNA or ODN-dsDNA recognition has led to the development of the antisense strategy [1][2] to arrest the translation of messenger RNA or antigene strategy [3–5] aimed at modulating gene expression. ODN Analogues carrying various functional groups (aromatic systems, polyamines, or metal complexes *etc.*) have been shown [6–8] to be able to assist in stabilizing the DNA triplex or DNA·DNA (DNA·RNA) duplex, which need to be efficient and specific if such materials are to be developed into an important class of diagnostics or therapeutics. On the other hand, the incorporation of DNA ligands activating the degradation of complementary DNA or RNA strands under appropriate conditions could potentially serve as powerful therapeutics to both arrest undesirable translation or transcription processes [9][10] as well as to destroy the target.

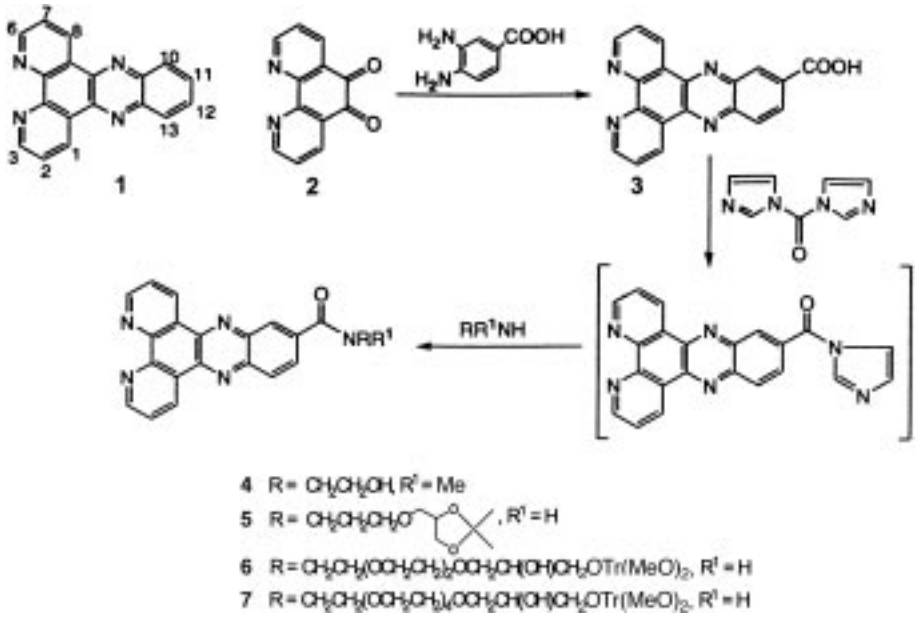
In this paper, we describe the synthesis of dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) [11] derivatives covalently linked to ODNs through linkers of different lengths. The reasons for our synthesis of dppz-tethered ODNs are as follows: *1*) $Ru^{II}$ Complexes with the dppz ligand **1** (like $[Ru(phen)_2(dppz)]^{2+}$, phen = 1,10-phenanthroline) exhibit long-lived emissions when intercalated into a DNA duplex but not when free in solution, which could be an important property for designing diagnostics [12]. *2*) It is known that $[Ru(phen)_2(dppz)]^{2+}$ intercalatively binds to double-stranded DNA at least 3 orders of magnitude more effectively than the tris-phenanthroline (phen) counterpart [13]. One of the reasons for stronger binding of $[Ru(phen)_2(dppz)]^{2+}$ than $[Ru(phen)_3]^{2+}$ is most probably due to the fact that the dppz moiety has a higher ability for intercalation into the DNA duplexes in comparison with phen. Although both the phen and the dppz ligands have the same chelating N-atoms for interaction with different metal ions, the advantage of relatively larger specificity and tighter binding by the dppz ligand lies in the fact that it has a relatively larger planar extended aromatic system compared to phen. *3*) As a result, various tetrahedral [14] and octahedral [15–17] complexes of the phen ligand, which oxidatively or in a photoinduced manner cleave the oligonucleotide chain under suitable conditions, would act more specifically and actively if the dppz ligand is used instead.

An important aspect of this work is to develop new specific $Ru^{II}$-based agents, which are both specific as well as tight binders. Hence, the present work deals with the preparation of a new type of dppz-tethered molecules which would enable us to synthesize octahedral complexes such as $[Ru(phen)_2(dppz)]^{2+}$ in which the linker is connected through the dppz moiety instead of the phen ligand. Additionally, we also wish to use the chemistry reported herein to synthesize new types of dppz-tethered derivatives such as $[Ru(dppz)_3]^{2+}$, which is also connected to the ODN chain through the dppz ligand.

We report herein our new synthetic procedure to tether a dppz chromophore to the ODN chain using the phosphoramidite chemistry, which allows its multiple introduction at any position of choice. In this study, the dppz moiety was covalently connected to the defined sequences at the 3′- or at the 5′-end or in the middle position of the phosphodiester backbone of an ODN through a linker chain of various lengths (7, 12, and 18 atoms). This permitted us to comprehensively examine the properties of these dppz-tethered ODNs with respect to their ability to stabilize DNA · DNA and DNA · RNA duplexes or triple helices. A non-nucleosidic building block based on a glycerol residue [18][19] was used for the covalent connection to the linker arms to provide maximum flexibility and to prevent stereochemical alterations of the nucleoside conformation (or hybridization properties). Two types of interior attachment of dppz to the probe strand were employed to explore the stability of duplexes and triplexes depending upon the possible configurational orientation of the dppz chromophore at the site without nucleobase: *i*) the addition of the dppz-building unit to the probe strand such that it is fully complementary to the target strand (see below, *Table 1*), while the dppz-glycerol unit is bulged out, or *ii*) the replacement of one of the interior nucleotide units by the dppz-glycerol unit in the same probe strand such that one of the nucleotide units of the target strand has no complementary nucleotide to base-pair with (*Table 1*).

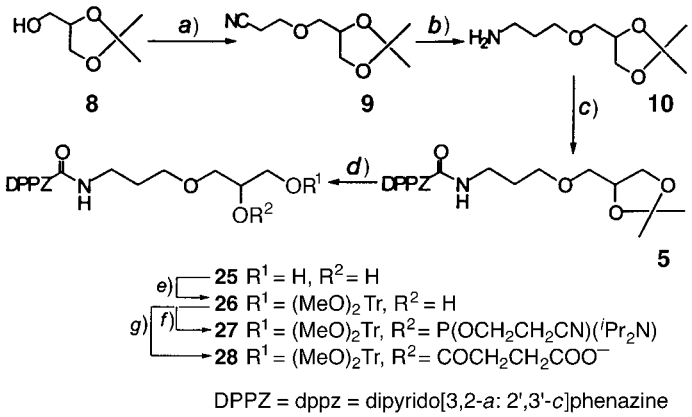
**2. Results and Discussion.** – 2.1. *Synthesis of Dppz-Tethered Phosphoramidite Blocks* **27**, **29**, *and* **30** *and of the Appropriate 3′-Succinamido-Anchored CPG Supports.* In our initial attempts to prepare ODN-dppz conjugates, we needed to find an appropriate linker. Our attempts to functionalize the parent dppz by allylic bromination of 11-methyl-dppz with *N*-bromosuccinimide (NBS) or nucleophilic substitution in *N*-alkylated-dppz failed under a variety of conditions. The alternative strategy involved the use of dipyridophenazine-11-carboxylic acid **3** (obtained from **2**) [20], which, in the activated form, formed an amide linkage upon reaction with an appropriate amine (*Scheme 1*). The task was arduous due to poor solubility of **3** in organic solvents. We, however, found that heating a suspension of **3** and 1,1′-carbonylbis[1*H*-imidazole] in pyridine at 80° for 15–20 min led to the quantitative formation of 1-acyl-1*H*-imidazole, which, upon immediate treatment with an appropriate amine for 30 min at 80°, followed by cooling to room temperature for 30 min, gave the corresponding amides **4**, **5**, **6**, or **7** in 75, 38, 72, or 44% yield, respectively. It is noteworthy that while the secondary amine gave two isomeric dynamically interconverting tertiary amides **4** at room temperature because of restricted rotation about the amide bond, the primary amines **10** (see below, *Scheme 2*) and **23** and **24** (*Scheme 3*) gave a single secondary amide **5**, **6**, or **7**, as expected.

*Scheme 1*




For the design of an achiral linker unit, we chose a simple $C_3$ *sn*-glycerol backbone because it is cheap, provides maximum flexibility of the backbone to make a bulge, and possesses good $H_2O$ solubility; this $C_3$ linker also allows multiple introductions of the chromophore into synthetic ODNs at any position of choice through linkers of various lengths. Two synthetic schemes were used for the construction of linker arms of

*Scheme 2*



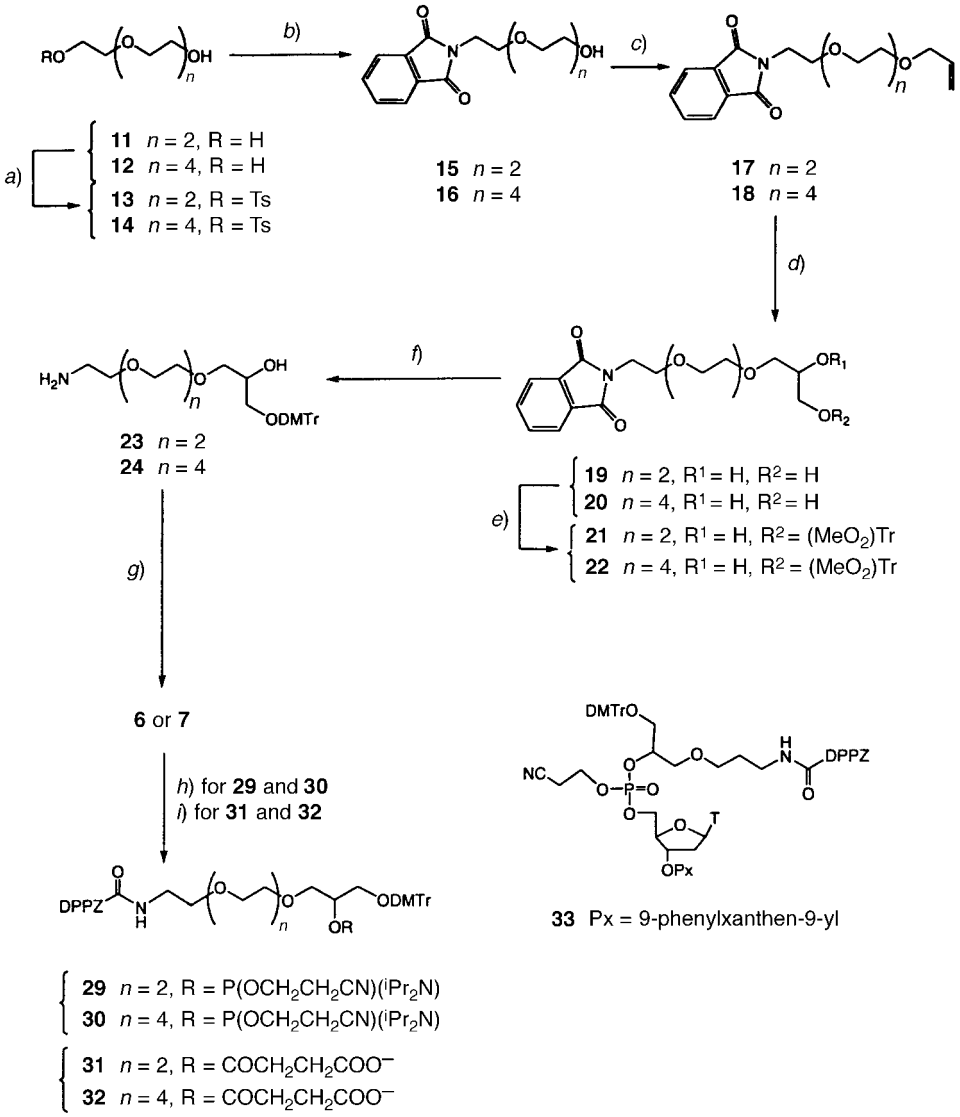

*a*) Acrylonitrile, NaH, 1 h, 20°. *b*) $NaBH_4$, $CoCl_2 \cdot 6H_2O$, MeOH, 1 h, 20°. *c*) **3**, 1,1′-carbonylbis[1*H*-imidazole], pyridine, 0.5 h, 80°, 0.5 h, 20°. *d*) 1M HCl/THF, 1.5 h, 20°. *e*) $(MeO)_2TrCl$; pyridine, 1.5 h, 20°. *f*) $PCl(OCH_2CH_2CN)(^iPr_2N)/^iPr_2NEt$, THF, 1.5 h, 20°. *g*) Succinic anhydride 4-(dimethylamino)pyridine (DMAP), $CH_2Cl_2$, 4 h, 20°.

different lengths to the 3-hydroxy group of the glycerol backbone: The shortest propane-1,3-diyl linker arm [18] was prepared from readily available solketal (= 2,2-dimethyl-1,3-dioxolane-4-methanol; **8**), which was quantitatively transformed to the 3-(alkyloxy)propanenitrile, **9** (*Scheme 2*). The latter was then reduced with $NaBH_4$ in the presence of cobalt(II) chloride to afford 3-(alkyloxy)propanamine **10** (55%).

To construct longer arms (see **6** and **7**), flexible, hydrophilic and bifunctional [21] tri- and penta(ethylene glycol) were used to tether to dppz. Because of the low coupling yield of solketal (**8**) to *n*(ethylene glycol) (in form of mesylated *n*(ethylene glycol) monoazide [19], data not shown), we built the glycerol part of the linker unit through the addition of the allyl radical [22]. This procedure involved the preparation of monotosylates **13** and **14** from tri- and penta(ethylene glycol) **11** and **12**, respectively (yield 52 and 51%, resp.; *Scheme 3*). The tosyloxy groups of **13** and **14** were then converted to the corresponding phthalimido groups of **15** (57%) and **16** (77%), which served both as the precursor and protector of the amino function. The reaction of **15** and **16** with allyl bromide in the presence of NaH in THF/DMF 15:1 afforded the corresponding olefins **17** (47%) and **18** (46%), which were then oxidized with permanganate to the respective diols **19** (52%) and **20** (38%). The permanganate treatment was carefully monitored because a prolonged reaction period led also to the removal of the phthalimido group. The primary-alcohol moiety of **19** and **20** was protected with a 4,4′-dimethoxytrityl group yielding **21** (85%) and **22** (87%), respectively. After aminolysis of the latter with methylamine, the target amines **23** and **24** were obtained in 86 and 84% yield, respectively.

The amines **10** (*Scheme 2*) and **23** and **24** (*Scheme 3*) were linked to the dppz moiety through the amide bond under the condition described in *Scheme 1* **10** → **5** (38%), **23** → **6** (72%), **24** → **7** (44%)). The dppz derivative **5** was then treated with 1M HCl/THF 1:1 to remove the isopropylidene group, and the crude product **25** (92%) was

*Scheme 3*




DMTr = $(MeO_2)Tr$; DPPZ = dppz = dipyrido[3,2-*a*: 2',3'-*c*]phenazine

*a*) TsCl pyridine/$CH_2Cl_2$, 1 h, 0°, 1.5 h, 20°. *b*) Phthalimide, DBU, DMF, 18 h, 80°. *c*) Allyl bromide, NaH, THF/DMF, 1 h, 0°, 18 h, 20°. *d*) $KMnO_4$, acetone/$H_2O$, 10 min, 20°. *e*) $(MeO)_2TrCl$/pyridine, 1.5 h, 20°. *f*) 40% $CH_2NH_2/H_2O$/MeOH, 5 h, 55°. *g*) **3**, 1,1′-carbonylbis[1*H*-imidazole], pyridine, 0.5 h, 80°, 0.5 h, 20°. *h*) $PCl(OCH_2CH_2CN)(^iPr_2N)$, $^iPr_2EtN$, THF, 1.5 h, 20°. *i*) Succinic anhydride, DMAP, $CH_2Cl_2$, 4 h, 20°.

tritylated to give **26** (91%) (*Scheme 2*). Finally, compounds **26** and **6** and **7** (*Scheme 3*) were converted in the usual manner [23] to the corresponding phosphoramidite blocks **27**, **29**, and **30** (94, 88, and 43% yield, resp.) for incorporation of these dppz-tethered units into oligodeoxynucleotides at the 5′-end or in the middle of the chain. Compounds **26**, **6**, and **7** were also treated with succinic anhydride and 4-(dimethylamino)pyridine (DMAP) in $CH_2Cl_2$ to give the corresponding succinate building blocks **28** (81%), **31**, (81%) and **32** (80%), respectively, which were then immobilized onto a 3-amino-propyl-CPG support [24], and were used for incorporation of the dppz-tethered building blocks at the 3′-termini of the oligonucleotides.

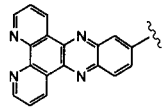
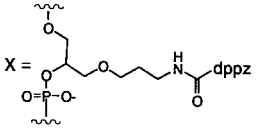
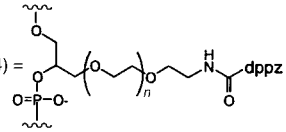
2.2. *Oligonucleotide Synthesis.* Prior to the solid-phase synthesis, we studied the stability of the amide linkage in the dppz-tethered dimeric block **33** (*Scheme 3*) under the basic conditions normally employed for the deprotection of the cyanoethyl group from the phosphotriester (ammonia, 17 h 55°). No significant degradation (< 5%) of dppz-tethered thymidine could be observed (as monitored by TLC and NMR) under these conditions.

The ODN analogues **34**–**62** (*Table 1*) used in this study were synthesized by the phosphoramidite methodology [26] with a commercially available DNA/RNA synthesizer (see *Exper. Part*). After deprotection, each dppz-ODN conjugate showed a very broad peak by HPLC analysis, which did not allow an estimation of the ODN purity. The purity of the HPLC-purified oligonucleotides were however, found to be satisfactory on 20% polyacrylamide gel containing 7M urea.

2.3. *Thermal Denaturation Study of the DNA · DNA and DNA · RNA Duplexes.* The duplexes (dppz-ODN) · DNA (*Entries 2–13* in *Table 2*) and (dppz-ODN) · RNA (*Entries 15–26* in *Table 2*) were generated by hybridization of the dppz-tethered 9-mers **35**–**43** and dppz-tethered 8-mers **44**–**46** with the target 11-mer ODN D11 (**60**) and oligo-RNA R11 (**61**) in a 1 : 1 ratio (1 μM of each strand in 20 mM $PO_4^{3-}$ and 0.1M NaCl buffer at pH 7.3) (see *Table 1* for abbreviations). All the melting curves of these duplexes exhibited a monophasic dissociation. The melting temperatures obtained (*Table 2*) allow to draw the following conclusions: Tethering the dppz derivative at the 5′- or 3′-terminus increases the $T_m$ for both DNA · DNA and DNA · RNA duplexes. A 12-atom linker gives a maximal stability ($\Delta T_m = 10.9°$ for the DNA · DNA duplex (*Entry 3*, *Table 2*), and $\Delta T_m = 5.4$ for the DNA · RNA duplex (*Entry 16*) for 5′-tethered dppz duplexes compared to 7- and 18-atom linkers (*Entries 2*, *4*, *15*, and *17*). Conversely, 7-atom linkers (*Entries 5* and *18*) and 18-atoms linkers (*Entries 7* and *20*) show the maximal stability for the 3′-dppz tether compared to the 12-atoms linker (*Entries 6* and *19*) for both DNA · DNA and DNA · RNA duplexes. It is noteworthy that the stability of the DNA · RNA duplex, **40** · **61**; 3′Z9 · R11 $\Delta T_m = 7.4°$ (*Entry 20*), is comparable to the corresponding DNA · DNA duplexes in general.

The dppz incorporation at the center of the unmodified 9-mer **34** results in considerable reduction of stabilization of the DNA · DNA and DNA · RNA duplexes ($\Delta T_m = 0.5–4.2°$, *Entries 8–10*; $\Delta T_m = -1.5$ to 0.9°, *Entries 21–23*; see *Table 2*), probably because of the formation of the bulge and steric interference by the linker arm within the duplex grooves. According to the $T_m$ data, such interference is minor in the case of a 12-atoms linker (as in mY9 · D11 (**42** · **60**), *Entry 9*, and mY9 · R11 (**42** · **61**), *Entry 22*). A comparison of the two types of middle-modified DNA · DNA duplexes (*i.e.*, *Entries 8–10* with a bulge, and *Entries 11–13* without bulge) shows that the

Table 1. *Synthetic DNA-dppz Conjugates and Their Target Oligo-DNA and Oligo-RNA*

dppz =  X =  Y ($n = 2$) or Z ($n = 4$) = 

| | Oligonucleotide | Linker | | Abreviation | |
|---|---|---|---|---|---|
| | natural | 5′-d(TCCAAACAT)-3′ | – | N9 | (**34**) |
| 9-Mer | 5′-modified | 5′-d(**M**TCCAAACAT)-3′ | M = X | 5′X9 | (**35**) |
| | | | M = Y | 5′Y9 | (**36**) |
| | | | M = Z | 5′Z9 | (**37**) |
| | 3′-modified | 5′-d(TCCAAACAT**M**)-3′ | M = X | 3′X9 | (**38**) |
| | | | M = Y | 3′Y9 | (**39**) |
| | | | M = Z | 3′Z9 | (**40**) |
| 9-Mer | middle-modified | 5′-d(TCCA**M**AACAT)-3′ | M = X | mX9 | (**41**) |
| | | | M = Y | mY9 | (**42**) |
| | | | M = Z | mZ9 | (**43**) |
| 8-Mer | middle-modified | 5′-d(TCCA**M**ACAT)-3′ | M = X | mX8 | (**44**) |
| | | | M = Y | mY8 | (**45**) |
| | | | M = Z | mZ8 | (**46**) |
| 18-Mer | natural | 5′-d(TTCT$_6$CT$_6$CT)-3′ | – | N18 | (**47**) |
| 18-Mer | 5′-modified | 5′-d(**M**TTCT$_6$CT$_6$CT)-3′ | M = X | 5′X18 | (**48**) |
| | | | M = Y | 5′Y18 | (**49**) |
| | | | M = Z | 5′Z18 | (**50**) |
| | 3′-modified | 5′-d(TTCT$_6$CT$_6$CT**M**)-3′ | M = X | 3′X18 | (**51**) |
| | | | M = Y | 3′Y18 | (**52**) |
| | | | M = Z | 3′Z18 | (**53**) |
| 18-Mer | middle-modified | 5′-d(TTCT$_6$**M**CT$_6$CT)-3′ | M = X | mX18 | (**54**) |
| | | | M = Y | mY18 | (**55**) |
| | | | M = Z | mZ18 | (**56**) |
| 17-Mer | middle-modified | 5′-d(MTTCT$_6$**M**T$_6$CT)-3′ | M = X | mX17 | (**57**) |
| | | | M = Y | mY17 | (**58**) |
| | | | M = Z | mZ17 | (**59**) |
| 11-Mer | single-strand targets | 5′-d(CATGTTTGGAC)-3′ | – | D11 | (**60**) |
| | | 5′-r(CAUGUUUGGAC)-3′ | – | R11 | (**61**) |
| 24-Mer | duplex target | 5′-d(GCCAAGA$_6$GA$_6$GACGC)-3′<br>3′-d(CGGTTCT$_6$CT$_6$CTGCG)-5′ | – | D24 · D24 | (**62**) |

absence of the bulge in the middle of the duplex indeed destabilizes the duplexes enormously. This is also true for the DNA · RNA duplexes (compare *Entries 21–23* with *Entries 24–26*). This is presumably due to the fact [27] that when a site without nucleobase forms no bulge (as in *Entries 11–13* and *24–26*) and the chromophore yields a mismatch pair, the duplex becomes energetically very unfavorable because the opposite nucleobase is most probably flipped out of the helix. On the other hand, when the duplex is fully matched (as in *Entries 8–10* or *21–23*) and the site without nucleobase bulges out, the chromophore can be possibly stacked with neighboring base pairs, which is energetically more favorable. In general, DNA · DNA duplexes were more stable and revealed a much more stabilizing effect with dppz-tethering than the corresponding DNA · RNA duplexes.

Table 2. *Thermal Stability* ($T_m$) *of DNA·DNA and DNA·RNA Duplexes Obtained from a 9-Mer* (or 8-Mer) *and a 11-Mer* (1:1 mixture)

| *Entry* | DNA·DNA Duplexes | | | *Entry* | DNA·RNA Duplexes | | |
|---|---|---|---|---|---|---|---|
| | Duplex type[a]) | $T_m$ [°] | $\Delta T_m$ [°] | | Duplex type[a]) | $T_m$ [°] | $\Delta T_m$ [°] |
| *1* | N9·D11 (**34·60**) | 25.9 | – | *14* | N9·R11 (**34·61**) | 20.6 | – |
| *2* | 5′X9·D11 (**35·60**) | 33.5 | 7.6 | *15* | 5′X9·R11 (**35·61**) | 25.2 | 4.6 |
| *3* | 5′Y9·D11 (**36·60**) | 36.8 | 10.9 | *16* | 5′Y9·R11 (**36·61**) | 26.1 | 5.4 |
| *4* | 5′Z9·D11 (**37·60**) | 33.2 | 7.3 | *17* | 5′Z9·R11 (**37·61**) | 25.5 | 4.9 |
| *5* | 3′X9·D11 (**38·60**) | 34.3 | 8.4 | *18* | 3′X9·R11 (**38·61**) | 27.1 | 6.5 |
| *6* | 3′Y9·D11 (**39·60**) | 33.2 | 7.3 | *19* | 3′Y9·R11 (**39·61**) | 25.2 | 4.5 |
| *7* | 3′Z9·D11 (**40·60**) | 35.6 | 9.7 | *20* | 3′Z9·R11 (**40·61**) | 28.0 | 7.4 |
| *8* | mX9·D11 (**41·60**) | 29.0 | 3.1 | *21* | mX9·R11 (**41·61**) | 19.2 | −1.5 |
| *9* | mY9·D11 (**42·60**) | 30.1 | 4.2 | *22* | mY9·R11 (**42·61**) | 21.5 | 0.9 |
| *10* | mZ9·D11 (**43·60**) | 26.4 | 0.5 | *23* | mZ9·R11 (**43·61**) | 21.1 | 0.4 |
| *11* | mX8·D11 (**44·60**) | 14.7 | −11.2 | *24* | mX8·R11 (**44·61**) | n.f.[b]) | n.f.[b]) |
| *12* | mY8·D11 (**45·60**) | 14.6 | −11.3 | *25* | mY8·R11 (**45·61**) | n.f.[b]) | n.f.[b]) |
| *13* | mZ8·D11 (**46·60**) | n.f.[b]) | n.f.[b]) | *26* | mZ8·R11 (**46·61**) | n.f.[b]) | n.f.[b]) |

[a]) See *Table 1* for abbreviations. [b]) n.f. = duplex not formed.

2.4. *Thermal Denaturation Studies of Triplexes.* Triplexes (*Entries 2–13* in *Table 3*) were generated by hybridization of the dppz-tethered 18-mers **48**–**56** and dppz-tethered 17-mers **57**–**59** with the target 24-mer duplex D24·D24 (**62**) [28] (18-mer (or 17-mer)/D24/D24 1:1:1; 1 μM of each strand in 20 mM $PO_4^{3-}$ and 0.1M NaCl buffer at pH 7.3). Three similar transitions were observed for all melting profiles. The transition occurring in the temperature range 12–35° was attributed to the thermal dissociation of the third strand, the transition observed in the temperature range 42–50° corresponded to the dissociation of the mismatched duplex between the 18-mer and the GA-rich 24-mer oligonucleotide, while the transition observed in the temperature range 60–63° corresponded to the dissociation of the 24-mer duplex, D24·D24 (**62**). All $T_m$s are shown in *Table 3*. These results show that all of the triple helixes formed with the oligonucleotides dppz-tethered at the 5′- or 3′-terminal are more stable than those formed with the unmodified ODN N18 (**47**), yet there are significant variations depending on the point of attachment to the ODN (3′- or 5′-) and the length of the

Table 3. *Thermal Stability* ($T_m$) *of DNA·DNA·DNA Triplexes Obtained from a 18-Mer* (or 17-Mer) *and Two 24-Mers* (1:1:1 mixture)

| *Entry* | DNA·DNA·DNA Triplexes | | | *Entry* | DNA·DNA·DNA Triplexes | | |
|---|---|---|---|---|---|---|---|
| | Triplex type[a]) | $T_m$ [°] | $\Delta T_m$ [°] | | Triplex type[a]) | $T_m$ [°] | $\Delta T_m$ [°] |
| *1* | N18·D24·24 (**47·62**) | 13.5 | – | *8* | mX18·D24·D24 (**54·62**) | n.d.[b]) | n.d.[b]) |
| *2* | 5′X18·D24·D24 (**48·62**) | 24.6 | 11.1 | *9* | mY18·D24·D24 (**55·62**) | 20.6 | 7.1 |
| *3* | 5′Y18·D24·D24 (**49·62**) | 20.9 | 7.4 | *10* | mZ18·D24·D24 (**56·62**) | n.d.[b]) | n.d.[b]) |
| *4* | 5′Z18·D24·D24 (**50·62**) | 17.3 | 3.8 | *11* | mX17·D24·D24 (**57·62**) | 15.8 | 2.3 |
| *5* | 3′X18·D24·D24 (**51·62**) | 22.6 | 9.1 | *12* | mY17·D24·D24 (**58·62**) | n.d.[b]) | n.d.[b]) |
| *6* | 3′Y18·D24·D24 (**52·62**) | 20.3 | 6.8 | *13* | mZ17·D24·D24 (**59·62**) | n.d.[b]) | n.d.[b]) |
| *7* | 3′Z18·D24·D24 (**53·62**) | 20.5 | 7.0 | | | | |

[a]) See *Table 1* for abbreviations. [b]) n.d. = not determined.

linker arm used. The most essential stabilization was seen with ODN 5′X18 (**48**; dppz attached to the 5′-end through the 7-atom linker arm). The triplex formed by it shows a $T_m$ value of 24.6°, which is an increase of 11.1° compared with the unsubstituted third strand **47**. In the case of a 5′-conjugation, the increase of the linker length up to 18 atoms leads to a sharp decrease of stabilization ($\Delta T_m$s are changed from 11.1° to 3.8°). The same trend is also observed for a 3′-attachment, albeit more gradually ($\Delta T_m$s are changed from 9.1° to 6.8°), suggesting that, in practical terms, there is no difference between 12-atom and 18-atom linker lengths as far as the triplex stability is concerned. The above data show that the increase of the linker length interferes with the triplex structure more strongly, especially in the case of 5′-modification. The increase of entropy with long linker arms could also play the destabilizing role for the triplex formation. Comparison of the 3′- and 5′-modifications for 7-atoms and 12-atoms linkers show that the 5′-dppz conjugation seems to be more desirable for stabilizing the triple helix. On the contrary, the longest linker arm (18-atoms) is preferable for a 3′-attachment of the dppz group in terms of triplex stability.

Triplexes derived from the third strand containing the dppz incorporation at the central position (mM18 · D24 · D24 and mM17 · D24 · D24 (M = X, Y, Z) gave relatively low thermal stabilities compared to a modification at the 5′- or 3′-terminus, except for two cases, namely for mY18 · D24 · D24 (**55** · **62**; *Entry 9* in *Table 3*) and mX17 · D24 · D24 (**57** · **62**; *Entry 11*). This observation shows that the optimal linker length of 12-atoms on the dppz chromophore can successfully penetrate into the interior of a triplex containing the unmodified 18-mer N18 (**47**) and thereby provide an excellent stability for the resulting triplex mY18 · D24 · D24 (**55** · **62**; ($\Delta T_m = 7.1°$). It is noteworthy that any other shorter or longer linkers with a dppz modification in the middle could not stabilize the triplex to the extent of an 18-atom linker. For triplex studies, we also chose a second type of central modification with no site without nucleobase being bulged out (as in *Entries 11–13* in *Table 3*), in which the chromophore, therefore, yields a mismatch pair. The aim was to explore if such a modification has any influence on the triplex stability in comparison with the duplex stabilization. It turned out that this type of modification does not stabilize any triplex significantly.

**3. Conclusion.** The synthetic protocol of a dppz attachment to the various ODNs was devised at the 3′- or 5′-ends as well as in the middle of an ODN chain to study the charge-transport phenomenon by a $Ru^{II}$-dppz-tethered ODN through the DNA stacks. The dppz ligand was attached to a non-nucleosidic glycerol-based building block through various linkers of different lengths. The preparation of these ODN conjugates enabled us also, in the first step, to examine how a dppz group tethered through linkers of various sizes actually stabilizes the DNA · DNA and DNA · RNA duplexes as well as DNA · DNA · DNA triplexes. The results reported here show that an ODN with a covalently 5′- or 3′-attached dppz forms more stable DNA · DNA and DNA · RNA duplexes as well as DNA · DNA · DNA triplexes as compared with non-modified counterparts. Among the set of 5′- and 3′-derivatized ODNs, the length of the linker arm and the point of attachment play a considerable role in determining the stability of the duplexes and triplexes formed. The best DNA · DNA duplex stabilization is found for 5′Y9 · D11 (**36** · **60**; *Entry 3* in *Table 2*, $\Delta T_m = 10.9°$), while the most stabilized DNA · RNA duplex is 3′Z9 · R11 (**40** · **61**; *Entry 20* in *Table 2*, $\Delta T_m = 7.4°$). The triplex

5′X18 · D24 · D24 (**48** · **62**) is the most stabilized one ($\Delta T_m = 11.1°$) among the triple helix hybrids (see *Table 3*). Insertion of a glycerol residue carrying the dppz ligand in the middle of the ODN leads to remarkably reduced DNA · DNA and DNA · RNA duplex stabilities (compare this with 3′- or 5′-modifications in *Table 2*), most probably because of a distortion by the bulged nucleotide without nucleobase. In this particular case, the stabilizing factor of the dppz chromophore competes with the destabilization arising from the bulged moiety without a nucleobase; it is likely that a more appropriate linker arm could help to enhance the stabilizing interactions of the dppz group within the duplex, which is evident from our study of the 12-atom linker arm for triplexes such as mY18 · D24 · D24 (**55** · **62**; $\Delta T_m = 7.1°$, *Entry 9* in *Table 3*). Remarkably, other dppz-tethered linkers incorporated in the middle of the oligonucleotide do not stabilize the triplex formation at all (see *Table 3*).

Work is now in progress to explore the $[Ru(phen)_2(dppz)]^{2+}$-tethered ODN and other [(metal ion)(dppz)]-tethered-directed cleavage reaction of target DNA and RNA.

The authors thank the *Swedish Natural Science Research Council* (*NFR*) and the *Swedish Engineering Research Council* (*TFR*) for generous funding.

## Experimental Part

*General*. Dry pyridine was obtained by distillation over 4-toluenesulfonyl chloride. MeCN and $CH_2Cl_2$ were distilled from $P_2O_5$ under Ar. DMF and THF were distilled over $CaH_2$. Column chromotography (CC): silica gel *Merck G60*. Semi-prep. HPLC: *Spherisorb 5ODS2* (reversed phase); *Gilson* equipment with pump model *303*, manometric module model *802C*, and dynamic mixer *811B* connected to a *Dynamax* computer program for gradient control. TLC: pre-coated silica gel $F_{254}$ plates with fluorescent indicator; eluents $CH_2Cl_2$/EtOH 95:5 (*A*), 90:10 (*B*), and 80:20 (*C*). Thermal denaturation experiments: PC-interfaced *Perkin-Elmer*-UV/VIS spectrophotometer *Lambda 40* with a *PTP-6 Peltier* temperature controller. NMR Spectra: *JNM-GX-270* spectrometer, at 270 ($^1$H) or 36 MHz ($^{31}$P); $\delta$ in ppm rel. to $SiMe_4$ as an internal standard ($^1$H) or 85% phosphoric acid as external standard ($^{31}$P); *J* in Hz.

*Dipyrido[3,2-*a*:2′,3′-*c*]phenazine-11-carboxylic acid* (**3**). A mixture of 1,10-phenanthroline-5,6-dione (**2**; 10.5 g, 50 mmol) in EtOH (370 ml) and 3,4-diaminobenzoic acid (8.37 g, 55 mmol) in EtOH (180 ml) was boiled to reflux for 10 min. After 1–2 min of heating, a grey precipitate was formed. After cooling, the precipitate was filtered and washed with EtOH: 15.35 g (94%) of **3**. $^1$H-NMR ($CF_3COOD$): 9.86 (*dt*, *J*(3,2) = *J*(6,7) = 8.2, *J*(3,1) = *J*(6,8) = 1.4, H–C(3), H–C(6)); 9.05 (*dd*, *J*(1,2) = *J*(8,7) = 5.2, *J*(1,3) = *J*(8,6) = 1.4, H–C(1), H–C(8)); 8.95 (*d*, *J*(10,12) = 1.6, H–C(10)); 8.35 (*dd*, *J*(12,13) = 8.9, *J*(12,10) = 1.6, H–C(12)); 8.27 (*d*, *J*(13,12) = 8.9, H–C(13)); 8.09 (*dt*, *J*(2,3) = *J*(7,6) = 8.2, *J*(2,1) = *J*(7,8) = 5.2, H–C(2), H–C(7)).

*3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propan-1-amine* (**10**) was synthesized as previously described [18].

*2,2′-[Ethane-1,2-diylbis(oxy)]bis[ethanol] Mono(4-methylbenzenesulfonate) (= Triethylene Glycol Monotosylate*; **13**). Triethylene glycol (**11**; 2.823 g, 18.8 mmol) was co-evaporated with anh. pyridine and dissolved in pyridine (75 ml). The soln. was cooled to −20° and treated dropwise within 1 h with TsCl (3.942 g, 20.68 mmol) in dry $CH_2Cl_2$ (25 ml) under vigorous stirring at −20°. After an additional hour stirring at 0° then 1.5 h at r.t., the reaction was quenched by addition of 1 ml of MeOH and the mixture stirred for 15 min. The crude material was obtained after extraction with aq. sat. $NaHCO_3$ soln./$CH_2Cl_2$ and drying of the org. phase by filtration through $MgSO_4$. CC (silica gel, 0–4% EtOH/$CH_2Cl_2$) gave 2.951 g (52%) of **13**. TLC (*B*): $R_f$ 0.50. $^1$H-NMR ($CDCl_3$): 7.73 (*d*, *J* = 8.0, 2 arom. H); 7.28 (*d*, *J* = 8.0, 2 arom. H); 4.10 (*t*, *J* = 4.8, TsOC$H_2$); 3.64 (*t*, *J* = 4.8, TsOCH$_2$C$H_2$, OC$H_2$CH$_2$OH); 3.55 (*s*, OC$H_2$C$H_2$O); 3.51 (*t*, *J* = 5.9, C$H_2$OH); 2.38 (*s*, Me).

*3,6,9,12-Tetraoxatetradecane-1,14-diol Mono(4-methylbenzenesulfonate) (= Pentaethylene Glycol Monotosylate*; **14**) was obtained from pentaethylene glycol (**12**) as described for **13**: 3.605 g (51%) of **14**. TLC (*B*): $R_f$ 0.62. $^1$H-NMR ($CDCl_3$): 7.80 (*d*, *J* = 8.3, 2 arom. H); 7.34 (*d*, *J* = 8.3, 2 arom. H); 4.16 (*t*, *J* = 4.8, TsOC$H_2$); 3.71–3.59 (*m*, 18 H); 2.6 (*t*, *J* = 5.0, OH); 2.45 (*s*, Me).

*2-{2-[2-(2-Hydroxyethoxy)ethoxy]ethyl}-1*H-*isoindole-1,3(2*H*)-dione* (**15**). To a stirred soln. of **13** (2.78 g, 9.14 mmol) in anh. DMF (40 ml), phthalimide (1.41 g, 9.59 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-en (DBu; 1.43 ml, 9.59 mmol) were added. The soln. was heated at 80° for 18 h and then evaporated. The crude yellow oil was purified by FC (silica gel, 0–5% EtOH/$CH_2Cl_2$): **13** (1.45 g, 57%). Oil. TLC: $R_f$ 0.77 (*B*), 0.48 (*A*). $^1$H-NMR ($CDCl_3$): 7.89–7.82 (*m*, 2 arom. H); 7.75–7.68 (*m*, 2 arom. H); 3.92 (*t*, *J* = 5.7, $NCH_2$); 3.76 (*t*, *J* = 5.7, $NCH_2CH_2$); 3.70–3.59 (*m*, 6 H); 3.54 (*t*, *J* = 5.9, $CH_2OH$).

*2-(14-Hydroxy-3,6,9,12-tetraoxatetradec-1-yl)-1*H-*isoindole-1,3(2*H*)-dione* (**16**). As described for **15**, from **14**: 2.5 g (77%) of **16**. TLC: $R_f$ 0.73 (*B*), 0.38 (*A*). $^1$H-NMR ($CDCl_3$): 7.88–7.81 (*m*, 2 arom. H); 7.75–7.68 (*m*, 2 arom. H); 3.90 (*t*, *J* = 5.9, $NCH_2$); 3.74 (*t*, *J* = 5.9, $NCH_2CH_2$); 3.74–3.58 (*m*, 16 H); 2.63 (*t*, *J* = 4.6, OH).

*2-{2-[2-[2-(Prop-2-enyloxy)ethoxy]ethoxy}ethyl]-1*H-*isoindole-1,3(2*H*)-dione* (**17**). Compound **15** (1.45 g, 5.24 mmol), co-evaporated from THF, was redissolved in dry THF (17 ml)/dry DMF (1.15 ml). The soln. was cooled to 0° under $N_2$, allyl bromide (0.67 ml, 7.87 mmol) and then an equimolar amount of solid NaH added, and the opaque yellow suspension stirred at 0° for 1 h and then at r.t. for 18 h. MeOH (6 ml) was added and the mixture evaporated. The crude material was submitted to CC (silica gel, 50–100% AcOEt/cyclohexane): 779 mg (47%) of **17**. TLC: $R_f$ 0.28 (*A*), 0.75 (*B*). $^1$H-NMR ($CDCl_3$): 7.88–7.81 (*m*, 2 arom. H); 7.74–7.68 (*m*, 2 arom. H); 5.96–5.82 (*m*, $CH{=}CH_2$); 5.29–5.14 (*m*, $CH{=}CH_2$); 3.99 (*d*, *J* = 5.7, $OCH_2CH{=}CH_2$); 3.90 (*t*, *J* = 5.8, $NCH_2$); 3.74 (*t*, *J* = 5.8, $NCH_2CH_2$); 3.68–3.59 (*m*, 6 H); 3.55–3.51 (*m*, $CH_2OCH_2CH{=}CH_2$).

*2-(3,6,9,12,15-Pentaoxaoctadec-17-en-1-yl)-1*H-*isoindole-1,3(2*H*)-dione* (**18**). As described for **17**, from **16**: 1.40 g (46%) of **18**. TLC: $R_f$ 0.53 (*A*), 0.77 (*B*). $^1$H-NMR ($CDCl_3$): 7.88–7.81 (*m*, 2 arom. H); 7.75–7.68 (*m*, 2 arom. H); 5.99–5.84 (*m*, $CH{=}CH_2$); 5.30–5.15 (*ddq*, *J* = 26.2, 13.85, 1.5, $CH{=}CH_2$); 4.02 (*dt*, *J* = 5.7, 1.5, $OCH_2CH{=}CH_2$); 3.90 (*t*, *J* = 5.6, $NCH_2$); 3.74 (*t*, *J* = 5.6, $NCH_2CH_2$); 3.67–3.57 (*m*, 16 H).

*2-{2-[2-[2-(2,3-Dihydroxypropoxy)ethoxy]ethoxy}ethyl]-1*H-*isoindole-1,3(2*H*)-dione* (**19**). A soln. of **17** (688 mg, 2.16 mmol) in acetone (3 ml), was cooled to 0°. An aq. soln. (6 ml of $H_2O$) of potassium permanganate (398 mg, 2.52 mmol) was added dropwise under stirring within 10 min. The mixture was stirred for a further 10 min at r.t. The resulting precipitate was filtered off through *Celite*, and the filtrate was evaporated and co-evaporated with abs. EtOH twice. The product was isolated by CC (silica gel, 0–10% EtOH/$CH_2Cl_2$): 396 mg (52%) of **19**. TLC: $R_f$ 0.49 (*B*), 0.31 (*A*). $^1$H-NMR ($CDCl_3$): 7.88–7.82 (*m*, 2 arom. H); 7.75–7.69 (*m*, 2 arom. H); 3.91 (*t*, *J* = 5.7, $NCH_2$); 3.88–3.80 (*m*, $HOCH_2CH(OH)$); 3.75 (*t*, *J* = 5.7, $NCH_2CH_2$); 3.67–3.49 (*m*, 12 H).

*2-(17,18-Dihydroxy-3,6,9,12,15-pentaoxaoctadec-1-yl)-1*H-*isoindole-1,3(2*H*)-dione* (**20**). As described for **19**, from **18**: 523 mg (38%) of **20**. TLC: $R_f$ 0.51 (*B*), 0.14 (*A*). $^1$H-NMR ($CDCl_3$): 7.88–7.82 (*m*, 2 arom. H); 7.75–7.69 (*m*, 2 arom. H); 3.90 (*t*, *J* = 5.8, $NCH_2$); 3.90–3.81 (*m*, $HOCH_2CH(OH)$); 3.74 (*t*, *J* = 5.8, $NCH_2CH_2$); 3.71–3.53 (*m*, 20 H).

*2-{2-{2-{2-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}ethoxy}ethoxy}ethyl}-1*H-*indole-1,3(2*H*)-dione* (**21**). Compound **19** (590 mg, 1.67 mmol) was dried by co-evaporation from anh. pyridine and then dissolved in dry pyridine (15 ml), and after cooling to 0° $(MeO)_2$ Tr–Cl (679 mg, 2.01 mmol) was added. The mixture was stirred for 1.5 h at r.t., then poured into aq. sat. $NaHCO_3$ soln., and extracted with $CH_2Cl_2$. The org. phase was evaporated and the residue co-evaporated with toluene/$CH_2Cl_2$ and subjected to CC (silica gel, 50–100% AcOEt/cyclohexane): 930 mg (85%) of **21**. TLC (*A*): $R_f$ 0.46. $^1$H-NMR ($CDCl_3$): 7.85–7.80 (*m*, 2 arom. H (pht)); 7.72–7.66 (*m*, 2 arom. H(pht)); 7.44–7.40 (*m*, 2 arom. H $(MeO)_2$); 7.34–7.09 (*m*, 7 arom. H ($(MeO)_2$/Tr)); 6.84–6.79 (*m*, 4 arom. H ($(MeO)_2$/Tr)); 3.99–3.91 (*m*, $(MeO)_2TrOCH_2CH(OH)$); 3.88 (*t*, *J* = 5.6, $NCH_2$); 3.78 (*s*, 2 MeO); 3.72 (*t*, *J* = 5.6, $NCH_2CH_2$); 3.64–3.45 (*m*, 10 H); 3.20–3.10 (*m*, $(MeO)_2TrOCH_2$).

*2-[18-(4,4′-Dimethoxytrityl)-17-hydroxy-3,6,9,12,15-pentaoxaoctadec-1-yl]-1*H-*isoindole-1,3(2*H*)-dione* (**22**). As described for **21** from **20**: 771 mg (87%) of **22**. TLC (A): $R_f$ 0.35. $^1$H-NMR ($CDCl_3$): 7.87–7.80 (*m*, 2 arom. H (pht)); 7.73–7.67 (*m*, 2 arom. H (pht)); 7.44–7.40 (*m*, 2 arom. H ($(MeO)_2$Tr)); 7.34–7.17 (*m*, 7 arom. H ($(MeO)_2$Tr)); 6.84–6.79 (*m*, 4 arom. H ($(MeO)_2$Tr)); 4.00–3.92 (*m*, $(MeO)_2TrOCH_2CH(OH)$); 3.89 (*t*, *J* = 5.5, $NCH_2$); 3.78 (*s*, 2 MeO); 3.73 (*t*, *J* = 5.5, $NCH_2CH_2$); 3.65–3.48 (*m*, 18 H); 3.22–3.11 (*m*, $(MeO)_2TrOCH_2$); 2.82 (*d*, *J* = 4.2, OH).

*2-{2-[2-(2-Aminoethoxy)ethoxy]ethoxy}-1-{[(4,4′-dimethoxytrityl)oxy]methyl}ethanol* (**23**). Compound **21** (961 mg, 1.47 mmol) was taken up in 40% aq. $MeNH_2$ soln. (10 ml), and MeOH (3 ml) was added to solubilize the starting material. The mixture was heated at 55° for 5 h under stirring and then evaporated and co-evaporated with abs. EtOH. The crude material was purified by CC (silica gel, 0–50% EtOH/$CH_2Cl_2$): **23** (662 mg, 86%). Pale yellow oil. TLC (*C*): $R_f$ 0.19. $^1$H-NMR ($CDCl_3$): 7.43–7.40 (*m*, 2 arom. H ($(MeO)_2$Tr)); 7.32–7.16 (*m*, 7 arom. H ($(MeO)_2$)); 6.84–6.80 (*m*, 4 arom. H ($(MeO)_2$Tr)); 4.08–4.01 (*m*, $(MeO)_2TrOCH_2CH(OH)$); 3.77 (*s*, 2 MeO); 3.72–3.50 (overlapping *m*, 12 H); 3.19–3.06 (*m*, $(MeO)_2TrOCH_2$, $CH_2CH_2NH_2$).

*17-Amino-1-{[(4,4′-dimethoxytrityl)oxy]methyl}-3,6,9,12,15-pentaoxaheptadecan-1-ol* (**24**). As described for **23** from **22**: 533 mg (84%) of **24**. TLC: $R_f$ 0.23 (*C*), 0.09 (*A*). $^1$H-NMR ($CDCl_3$): 7.44–7.40 (*m*, 2 arom. H ($(MeO)_2$Tr)); 7.32–7.16 (*m*, 7 arom. H ($(MeO)_2$Tr)); 6.83–6.79 (*m*, 4 arom. H ($(MeO)_2$Tr)); 4.12–4.05 (*m*, ($(MeO)_2$Tr)OCH$_2$C*H*(OH)); 3.89 (*t*, *J* = 5.2, C$H_2$NH$_2$); 3.79 (*s*, 2 MeO); 3.77–3.58 (overlapping *m*, 18 H); 3.26–3.01 (*m*, ($(MeO)_2$Tr)OC$H_2$, C$H_2$CH$_2$NH$_2$).

*General Method for the Preparation of dppz-Containing Amides.* Equimolar amounts of **3** and the appropriate amine were co-evaporated with dry pyridine separately. To a suspension of **3** in anh. pyridine (1 ml of pyridine for 0.06 mmol of **3**) and 1,1′-carbonylbis[1*H*-imidazole] (1.5 equiv.) was added to the suspension. The mixture was stirred under $N_2$ at 85° (oil bath) until the solid particles were solubilized (*ca.* 15 min). Subsequently, a pyridine soln. of the appropriate amine (1 ml of pyridine for 0.13 mmol of amine) was added dropwise. After a further half-hour stirring under heating and a half-hour at r.t. the pyridine was evaporated. The residue was extracted with aq. sat. $NaHCO_3$ soln./$CH_2Cl_2$ dried ($MgSO_4$), and evaporated and the residue co-evaporated with toluene and $CH_2Cl_2$ twice. The product was isolated by CC (silica gel, 0–6% EtOH/$CH_2Cl_2$): pale foam.

N-*(2-Hydroxyethyl)*-N-*methyldipyrido[3,2*-a:*2′,3′*-c*]phenazine-11-carboxamide* (**4**). From **3** (163 mg, 0.5 mmol) and 2-(methylamino)ethanol (38 mg, 0.5 mmol): 144 mg (75%) of **4**, isolated by CC (silica gel, 0–25% EtOH/$CH_2Cl_2$). $^1$H-NMR (500 MHz, $CDCl_3$, 0°): Conformer A (33.3% of the mixture at 0°): 9.25 (*ddd*, *J*(3,2) = *J*(6,7) = 8.1, *J*(3,1) = *J*(6,8) = 1.7, H–C(3), H–C(6)); 9.13 (*ddd*, *J*(1,2) = *J*(8,7) = 4.3, *J*(1,3) = *J*(8,6) = 1.7, H–C(1), H–C(8)); 8.19 (*d*, *J*(10,12) = 1.8, H–C(10)); 8.18 (*d*, *J*(13,12) = 8.7, H–C(13)); 7.92 (*dd*, *J*(12,13) = 8.7, *J*(12,10) = 1.8, H–C(12)); 7.66 (*ddd*, *J*(2,3) = *J*(7,6) = 8.1, *J*(2,1) = *J*(7,8) = 4.3, H–C(2), H–C(7)); 4.02 (br. *t*, $CH_2$,C$H_2$OH); 3.87 (br. *t*, C$H_2$CH$_2$OH); 3.19 (*s*, Me); conformer B (66.6% of the mixture at 0°): 8.93 (*dd*, *J*(1,2) = *J*(8,7) = 4.3, *J*(1,3) = *J*(8,6) = 1.7, H–C(1) or H–C(8)); 8.78 (*dd*, *J*(3,2) = *J*(6,7) = 8.0, *J*(3,1) = *J*(6,8) = 1.7, H–C(3) or H–C(6)); 8.59–8.57 (*m*, 2 H, H–C(1) or H–C(8), H–C(3) or H–C(6)); 7.76 (*d*, *J*(10,12) = 1.7, H–C(10)); 7.72 (*dd*, *J*(12,13) = 8.6, *J*(12,10) = 1.7, H–C(12)); 7.59 (*d*, *J*(13,12) = 8.6, H–C(13)); 7.57 (*dd*, *J*(2,3) = *J*(7,6) = 8.0, *J*(2,1) = *J*(7,8) = 4.3, H–C(2) or H–C(7)); 7.42 (*dd*, *J*(2,3) = *J*(7,6) = 8.0, *J*(2,1) = *J*(7,8) = 4.3, H–C(2) or H–C(7)); 3.96 (br. *t*, $CH_2$C$H_2$OH); 3.86 (br. *t*, C$H_2$CH$_2$OH); 3.29 (*s*, Me). $^1$H-NMR ((D$_6$)DMSO, 90°): 9.57 (*d*, *J*(3,2) = *J*(6,7) = 7.3, H–C(3), H–C(6)); 9.29 (br. *s*, H–C(1), H–C(8)); 8.50 (*d*, *J*(13,12) = 8.9, H–C(13)); 8.45 (*s*, H–C(10)); 8.12 (*dd*, *J*(12,13) = 8.9, *J*(12,10) = 1.6, H–C(12)); 8.00 (*dd*, *J*(2,3) = *J*(7,6) = 7.3, *J*(2,1) = *J*(7,8) = 4.6, H–C(2), H–C(7)); 3.81 (br. *t*, $CH_2$C$H_2$OH); 3.69 (br. *t*, C$H_2$CH$_2$OH); 3.26 (*s*, Me).

N-*{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}dipyrido[3,2*-a:*2′3′*-c*]phenazine-11-carboxamide* (**5**). From **3** (979 mg, 3 mmol) and **7** (567 mg, 3 mmol): 567 mg (38%) of **5**. $^1$H-NMR ($CDCl_3$): 9.54–9.50 (*ddd*, *J* = 8.2, 4.7, 2.0, H–C(3), H–C(6)(dppz)); 9.28–9.24 (*dd*, *J* = 4.7, 2.0, H–C(1), H–C(8)(dppz)); 8.61 (*s*, H–C(10)(dppz)); 8.32 (*s*, H–C(12), H–C(13)(dppz)); 7.79–7.75 (*ddd*, *J* = 8.2, 4.7, 2.0, H–C(2), H–C(7)(dppz)); 7.49 (*t*, *J* = 5.0, NHC(O)); 4.42 (*quint.* *J* = 5.0, CH); 4.13 (*dd*, *J* = 8.3, 6.56, 1 H, $CH_2$); 3.86–3.69 (*m*, 5 H, $CH_2$, OC$H_2$CH$_2$C$H_2$NH); 3.66–3.55 (*m*, C$H_2$O(CH$_2$)$_3$); 2.04 (*quint.*, *J* = 5.8, OCH$_2$C$H_2$CH$_2$NH); 1.41, 1.29 (2*s*, Me$_2$C).

N-*{2-[2-[2-{3‴′-[(4,4′-Dimethoxytrityl)oxy]-2‴′-hydroxypropoxy}ethoxy]ethoxy]ethyl}dipyrido[3,2*-a:*2′,3′*-c*]phenazine-11-carboxamide* (**6**). From **3** (222 mg, 0.68 mmol) and **23** (358 mg, 0.68 mmol): 409 mg (72%) of **6**. $^1$H-NMR ($CDCl_3$): 9.59 (2 *d*, *J*(3,2) = *J*(6,7) = 8.2, H–C(3), H–C(6)(dppz)); 9.31–9.24 (*m*, H–C(1), H–C(8)(dppz)); 8.86 (*d*, *J*(10,12) = 1.7, H–C(10)(dppz)); 8.43–8.40 (*dd*, *J*(12,13) = 8.9, *J*(12,10) = 1.7, H–C(12)(dppz)); 8.33 (*d*, *J*(13,12) = 8.9, H–C(13)(dppz)); 8.28–8.23 (br. *t*, NHC(O)); 7.84, 7.75 (2*m*, H–C(2), H–C(7)(dppz)); 7.30–7.26 (*m*, 2 arom. H ($(MeO)_2$Tr)); 7.15–7.06 (*m*, 7 arom. H ($(MeO)_2$Tr)); 6.64–6.58 (*m*, 4 arom. H ($(MeO)_2$Tr)); 4.18–4.08 (*m*, ($(MeO)_2$Tr)OCH$_2$C*H*(OH)); 3.84–3.56 (overlapping *m*, 14 H); 3.66 (*s*, 2 MeO); 3.19–3.06 (*m*, ($(MeO)_2$Tr)OC$H_2$).

N-*[18′-(4,4′-Dimethoxytrityl)-17′-hydroxy-3′,6′,9′,12′,15′-pentaoxaoctadec-1′-yl]dipyrido[3,2*-a:*2′,3′*-c*]phenazine-11-carboxamide* (**7**). From **3** (257 mg, 0.79 mmol) and **24** (482 mg, 0.79 mmol): 324 mg (44%) of **7**. $^1$H-NMR ($CDCl_3$): 9.61 (*td*, *J*(3,2) = *J*(6,7) = 8.2, *J*(3,1) = *J*(6,8) = 1.8, H–C(3), H–C(6)(dppz)); 9.28 (*dt*, *J*(1,2) = *J*(8,7) = 4.5, *J*(1,3) = *J*(8,6) = 1.8, H–C(1), H–C(8)(dppz)); 8.80 (*d*, *J*(10,12) = 1.7, H–C(10)(dppz)); 8.42–8.34 (*m*, H–C(12), H–C(13)(dppz)); 7.91 (br. *t*, NHC(O)); 7.79 (*dt*, *J*(2,3) = *J*(7,6) = 8.2, *J*(2,1) = *J*(7,8) = 4.5, H–C(2), H–C(7)(dppz)); 7.38–7.35 (*m*, 2 arom. H ($(MeO)_2$Tr)); 7.30–7.10 (*m*, 7 arom. H ($(MeO)_2$Tr)); 6.76–6.71 (*m*, 4 arom. H ($(MeO)_2$Tr)); 4.02–3.94 (*m*, ($(MeO)_2$TrOCH$_2$C*H*(OH)); 3.78–3.48 (overlapping *m*, 22 H); 3.73 (*s*, 2 MeO); 3.19–3.07 (*m*, ($(MeO)_2$Tr)OC$H_2$).

N-*[3-(2,3-Dihydroxypropoxy)propyl]dipyrido[3,2*-a:*2′,3′*-c*]phenazine-11-carboxamide* (**25**). To a soln. of **5** (560 mg, 1.13 mmol) in THF (4.7 ml), 1M HCl (4.7 ml) was added, and the soln. was stirred for 1.5 h. Abs. EtOH (4.7 ml) was added, the soln. evaporated, the residue redissolved in abs. EtOH, and the soln. evaporated: 474 mg

(92%) of crude **25** which was used further without purification. $^1$H-NMR ($D_2O$): 8.44 (*dd*, *J* = 7.9, 4.5, 2 H (dppz)); 7.91 (2*d*, *J* = 7.9, 2 H (dppz)); 7.30 (*m*, 2 H, dppz); 7.11 (*d*, *J*(12,13) = 8.3, H–C(12)(dppz)); 6.76 (*s*, H–C(10)(dppz)); 6.71 (*d*, *J*(13,12) = 8.3, H–C(13)(dppz)); 3.88–3.80 (*m*, CH); 3.62–3.42 (*m*, 6 H); 3.22 (*t*, *J* = 6.7, C$H_2$NH); 1.75 (*quint*. *J* = 6.7, OCH$_2$C$H_2$CH$_2$NH).

N-*{3-{3′′-[(4,4′-dimethoxytrityl)oxy]-2′′-hydroxypropoxy}propyl}dipyrido[3,2-*a:*2′,3′-*c*]phenazine-11-carboxamide* (**26**). After co-evaporation from anh. pyridine, **25** (468 mg, 1.02 mmol) was suspended in dry pyridine (9.5 ml), and after cooling to 0°, $(MeO)_2$Tr–Cl (414 mg, 1.22 mmol) was added, and the mixture was stirred for 1.5 h at r.t. Then, the reaction was quenched with MeOH, the mixture poured into aq. sat. $NaHCO_3$ soln. and extracted with $CH_2Cl_2$, the org. phase evaporated, and the residue co-evaporated with toluene/$CH_2Cl_2$ and subjected to CC (silica gel, 0–8% EtOH/$CH_2Cl_2$): 707 mg (91%) of **26**. $^1$H-NMR ($CDCl_3$): 9.52 (2 *d*, *J*(3,2) = *J*(6,7) = 8.2, H–C(3), H–C(6)(dppz)); 9.23 (2*d*, *J*(2,1) = *J*(7,8) = 4.2, H–C(1), H–C(8)(dppz)); 8.76 (*d*, *J*(10,12) = 1.6, H–C(10)(dppz)); 8.39 (*dd*, *J*(12,13) = 8.8, *J*(12,10) = 1.6, H–C(12)(dppz)); 8.39 (*dd*, *J*(13,12) = 8.8, H–C(13)(dppz)); 7.80–7.71 (*m*, H–C(2), H–C(7)(dppz)); 7.58 (2*d*, *J* = 4.6, NHC(O)); 7.31–7.28 (*m*, 2 arom. H ((MeO)$_2$Tr)); 7.18–7.08 (*m*, 7 arom. H ((MeO)$_2$Tr)); 6.67–6.63 (*m*, 4 arom. H ((MeO)$_2$Tr)); 4.21–4.13 (*m*, CH); 3.80–3.59 (overlapping *m*, 6 H); 3.68 (*s*, 2 MeO); 3.25 (*d*, *J* = 4.6, $(MeO)_2$TrOC$H_2$); 1.99 (*quint.*, *J* = 5.2, OCH$_2$C$H_2$CH$_2$NH)).

*General Method for the Phosphitylation of dppz-Containing Amides*. The dppz-containing amide (1 equiv.) was co-evaporated with dry THF twice and dissolved in dry THF (1 ml of THF for 0.07 mmol of amide) and kept under $N_2$. Then, dry $^i$Pr$_2$EtN (5 equiv.) was added, followed by 2-cyanoethyl diisopropylphosphoramidochloridite (1.5 equiv.) under vigorous stirring, which was continued for further 1.5 h. The reaction was quenched by addition of dry MeOH and the mixture stirred for 15 min. After workup with aq. sat. $NaHCO_3$ soln./$CH_2Cl_2$ drying ($MgSO_4$) of the org. phase, evaporation, and co-evaporation with toluene/$CH_2Cl_2$ (twice) the crude material was submitted to CC (silica gel, $CH_2Cl_2$/$Et_3$N 98 : 2).

N-*{3-{3′′-[(4,4′-Dimethoxytrityl)oxy]-2′′-hydroxypropoxy}propyl}dipyrido[3,2-*a:*2′,3′-*c*]phenazine-11-carboxamide 2′′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**27**). From **26** (573 mg, 0.75 mmol): 680 mg (94%) of **27**. TLC (*B*): $R_f$ 0.46. $^{31}$P-NMR ($CDCl_3$): +147.92.

N-*{2-{2-{2-[3′′′′-(4,4′-Dimethoxytrityl)-2′′′′-hydroxypropoxy]ethoxy}ethoxy}ethyl}dipyrido[3,2-*a:*2′,3′-*c*]phenazine-11-carboxamide 2′′′′-(2-cyanoethyl Diisopropylphosphoramidite)* (**29**). From **6** (215 mg, 0.26 mmol): 235 mg (88%) of **29**. TLC (*B*): $R_f$ 0.45. $^{31}$P-NMR ($CDCl_3$): +148.43.

N-*[18′-(4,4′-Dimethoxytrityl)-17′-hydroxy-3′,6′,9′,12′,15′-pentaoxaoctadec-1′-yl]dipyrido[3,2-*a:*2′,3′-*c*]phenazine-11-carboxamide 17′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**30**). From **7** (230 mg, 0.25 mmol): 120 mg (43%) of **30**. TLC (*B*): $R_f$ 0.45. $^{31}$P-NMR ($CDCl_3$): +149.11.

*General Method for the Preparation of the dppz-Functionalized Support*. The dppz-containing amide (1 equiv). and 4-(dimethylamino)pyridine (2.1 equiv.) were dissolved in dry $CH_2Cl_2$ (1 ml of solvent for 0.18 mmol of amide). Then, succinic anhydride (2.03 equiv.) was added, and the soln. was stirred at r.t. for 4 h. The mixture was extracted with 0.1M citric acid and then with aq. sat. $NaHCO_3$ soln., and the org. phase dried ($MgSO_4$) and evaporated: the crude product was used directly for coupling to CPG. $^i$Pr$_2$EtN (34 mg, 263.2 mmol) in THF (1.8 ml) was added to the succinate derivative of the dppz-containing amide (138.5 mmol), followed by isobutyl carbonochloridate (= isobutyl chloroformate; 18.9 mg, 138.5 mmol) in THF (0.5 ml). After stirring for 2 h, a soln. of $^i$Pr$_2$EtN (0.6 ml) in dry THF (1.54 ml) and 3-aminopropyl-CPG (1 g) were added to the mixture. The suspension was shaken for 2 h and then filtered and the solid thoroughly washed with THF, $CH_2Cl_2$ (3 times), and $Et_2O$ (4 times). $(MeO)_2$Tr release with acid and measurement at 498 nm were performed at this stage to determine the loading. The support was then suspended in dry pyridine (8 ml), 4-(dimethylamino)pyridine (167 mg) and $Ac_2O$ (0.77 ml) were added, and the suspension was shaken for 2 h, after which the suspension was filtered and the solid thoroughly washed with pyridine, $CH_2Cl_2$ (4 times), and $Et_2O$ (4 times) and then dried under vacuum over $P_2O_5$.

N-*{3-{3′′-[(4,4′-Dimethoxytrityl)oxy]-2′′-hydroxypropoxy}propyl}dipyrido[3,2-*a:*2′,3′-*c*]phenazine-11-carboxamide 2′′-(Hydrogen Butanedioate)* (**28**) *and the Corresponding 2′′-{4-[(3-CPG-propyl)amino]-4-oxobutanoate}*. From **26** (133 mg, 0.17 mmol): 122 mg (81%) of **28**. $^1$H-NMR ($CDCl_3$): 9.47 (br. *s*, H–C(3), H–C(6)(dppz)); 9.22 (br. *s*, H–C(1), H–C(8)(dppz)); 8.73 (br. *s*, H–C(10)(dppz)); 8.39–8.27 (*m*, H–C(12), H–C(13)(dppz)); 7.86 (br. *s*, NHC(O)); 7.71 (br. *s*, H–C(2), H–C(7)(dppz)); 7.37–7.10 (*m*, 9 arom. H ((MeO)$_2$Tr)); 6.77–6.74 (*m*, 4 arom. H ((MeO)$_2$Tr)); 5.28 (br. *s*, $(MeO)_2$TrOCH$_2$C*H*); 3.80–3.58 (overlapping *m*, 20 H); 3.19–3.26 (*m*, $(MeO)_2$TrOC$H_2$); 2.68 (br. *s*, $(CH_2)_2$COO); 1.75 (*quint.* *J* = 5.2, OCH$_2$C$H_2$CH$_2$NH).

The loading of the support (1 g) was 21.9 mmol/g of CPG.

N-*{2-{2-{2-{3''''-[(4,4'-Dimethoxytrityl)oxy]-2''''-hydroxypropoxy}ethoxy}ethoxy}ethyl}dipyrido[3,2-*a:*2',3'-*c*]phenazine-11-carboxamide 2''''-(Hydrogen Butanedioate)* (**31**) *and the Corresponding 2''''-{4-[(3-CPG-propyl)amino]-4-oxobutanoate}*. From **6** (52 mg, 0.06 mmol): 47 mg (81%) of **31**. $^1$H-NMR ($CDCl_3$): 9.47 (br. *s*, H–C(3), H–C(6)(dppz)); 9.22 (br. *s*, H–C(1), H–C(8)(dppz)); 8.73 (br. *s*, H–C(10)(dppz)); 8.39–8.27 (*m*, H–C(12), H–C(13)(dppz)); 7.86 (br. *s*, NHC(O)); 7.71 (br. *s*, H–C(2), H–C(7)(dppz)); 7.37–7.10 (*m*, 9 arom. H ((MeO)$_2$Tr)); 6.77–6.74 (*m*, 4 arom. H ((MeO)$_2$Tr)); 5.28 (br. *s*, (MeO)$_2$TrOC$H_2$,C*H*); 3.80–3.58 (overlapping *m*, 20 H); 3.19–3.17 (*m*, (MeO)$_2$TrOC$H_2$); 2.66 (br. *s*, (C$H_2$)$_2$COO).

The loading of the appropriate support (369 mg) was 22.7 mmol/g of CPG.

N-*[18'-(4,4'-Dimethoxytrityl)-17'-hydroxy-3',6',9',12',15'-pentaoxaoctadec-1'-yl]dipyrido[3,2-*a:*2',3'-*c*]phenazine-11-carboxamide 17'-(Hydrogen Butanedioate)* (**32**) *and the Corresponding 17'-{4-[(3-CPG-propyl)amino]-4-oxobutanoate}*. From **7** (100 mg, 0.107 mmol): 89 mg (80%) of **32.** $^1$H-NMR ($CDCl_3$): 9.57 (br. *s*, H–C(3), H–C(6)(dppz)); 9.26 (br. *s*, H–C(1), H–C(8)(dppz)); 8.78 (br. *s*, H–C(10)(dppz)); 8.42–8.33 (*m*, H–C(12), H–C(13)(dppz)); 7.77 (br. *s*, NHC(O), H–C(2), H–C(7)(dppz)); 7.39–7.14 (*m*, 9 arom. H ((MeO)$_2$Tr)); 6.83–6.72 (*m*, 4 arom. H (MeO)$_2$Tr)); 5.22 (br. *s*, (MeO)$_2$TrOCH$_2$C*H*); 3.80–3.51 (overlapping *m*, 28 H); 3.20–3.18 (*m*, (MeO)$_2$TrOC$H_2$); 2.67 (br. *s*, (C$H_2$)$_2$COO).

The loading of the appropriate support (369 mg) was 24.3 mmol/g of CPG.

*Synthesis, Deprotection, and Purification of Oligonucleotides.* All ODNs were synthesized on a 1.0-µmol scale with an 8-channel *Applied-Biosystems-392*-DNA/RNA synthesizer using conventional 2-cyanoethyl phosphoramidite chemistry. Modified oligonucleotides 3'M9 (M = X, Y, or Z; **38**–**40**) and 3'M18 (M = X, Y, or Z; **51**–**53**) (*Table 1*) were synthesized on the modified supports containing the dppz chromophore tethered to the glycerol residue through linkers of 7 (M = X), 12 (M = Y), or 18 (M = Z) atoms length. Other ODNs were obtained using standard CPG-dT support. The preparation of target ODNs D11 (**60**), R11 (**61**), and D24·D24 (**62**) involved the use of standard CPG-dC or -C supports. Phosphoramidite blocks **27**, **29**, and **30** were dissolved in dry MeCN with a final concentration of 0.15M and used after filtration for solid-phase synthesis with a coupling time of 10 min (25 s for standard nucleoside phosphoramidites).

After each synthesis of the protected oligomers, the solid support was transferred directly out from the cassette to a 50-ml round-bottomed flask containing 20 ml of conc. aq. $NH_3$ soln. and was shaken for 2 h at 20°. After removal of CPG by filtration and evaporation of the filtrate, the residue was redissolved in conc. aq. $NH_3$ soln. and stirred at 55° for 17 h. The crude ODNs were purified by reversed-phase HPLC (gradient elution with A (0.1M (Et$_3$NH)OAc, 5% MeCN, pH 7.0) and *B* (0.1M (Et$_3$NH)OAc, 50% MeCN, pH 7.0)), and the purities of the dppz-ODN conjugates were checked by denaturing 20% polyacrylamide gel electrophoresis. The reversed-phase HPLCs of all dppz-ODN conjugates indicated the presence of several colorless fractions, and one slightly yellow fraction. The UV/VIS spectra of each colored fraction showed the characteristic bands at 369 nm and 387 nm (dppz). The detritylated oligomers were evaporated and co-evaporated with $H_2O$ 5 times and then directly lyophilized (5 × 1 ml $H_2O$). All ODNs were subsequently submitted to $Na^+$ exchange by passing then through a column of *Dowex-50* ($Na^+$ form).

Concentrations of dppz-ODNs were determined accounting for the contribution to the absorbance at 260 nm from the dppz moiety itself. This was done by taking the ratio of the area under the UV curve for compound **25** (or products of detritylation of **6** and **7**) at 235–335 nm to that at 335–415 nm. The absorption of the dppz-labeled oligomers at 260 nm was then corrected for the dppz absorption by using the above ratio (3.78–4.06) to estimate the contribution of the dppz chromophore to the absorbance area for a given oligonucleotide at 235–335 nm. Starting from 1 µmol of thymidine residues linked to controlled-pore glass, the following amounts, measured in $A_{260}$ units (*OD*), were obtained after purification: 3'M9: (**38**–**40**), 10.9–24.1; 5'M9 (**35**–**37**), 12.1–14.8; mM9 or mM8 (**41**–**46**), 12.1–29.5; 3'M18 (**51**–**53**), 17.1–34.8; 5'M18 (**48**–**50**), 10.4–29.5; mM18 or mM17 (**54**–**59**), 14.0–57.3.

*Thermal Denaturation Experiments.* UV Melting profiles were obtained by scanning $A_{260}$ absorbance *vs.* time at a heating rate of 0.5°/min from 5° to 70° for triplexes and of 1.0°/min from 10° to 55° for duplexes. The melting temperature $T_m$ (± 0.5°) was determined as the maximum of the first derivative of melting curves. The triplex and duplex melting experiments were performed in 20 mM $Na_2HPO_4/NaH_2PO_4$ and 0.1M NaCl at pH 7.3 at a hybrid concentration of *ca.* 1 mM. The approximate extinction coefficients for natural ODNs were calculated as previously described [30][31]. In cases of the dppz-tethered ODNs, the extinction coefficients were corrected for the absorbance contribution of the dppz moiety at 260 nm by subtraction. After preparation, the solns. consisting of three components (for the formation of triplexes) were heated to 70° for 5 min and then allowed to cool down to 20° for 30 min and then kept at 0° overnight. The appropriate solns. consisting of two components (for the formation of duplexes) were heated to 70° for 5 min, and then allowed to cool down to 20°

for 30 min under shaking. During the melting experiments at temp. $< ca.$ 15°, $N_2$ was continuously passed through the sample compartment to prevent moisture condensation.

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*Received September 13, 1999*