Intercalating Nucleic Acids Containing Insertions of Naphthalimide

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In a study of linker-length dependence, we evaluated naphthalimide (=1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione) and 4-bromonaphthalimide as intercalating nucleic acids. We used a vicinal dihydroxy system when incorporating the six different naphthalimide monomers into DNA, and found the minimum linker-length to be five C-atoms. With this length of the linker, naphthalimide was discriminating between DNA and RNA – stabilizing DNA, while destabilizing RNA. Furthermore, naphthalimide showed universal base character by hybridizing to the four natural bases with a range as narrow as 1.4°. When compared to pyrene, naphthalimide with the same linker-length gave significantly higher thermal meltings when hybridized to DNA.

1. Introduction. – Naphthalimide (=1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione) belongs to a group of promising DNA-targeting anticancer agents [1]. Bisnaphthalimides *Elinafide* (**1**) [2] and *Bisnafide* (**2**) [3][4] consisting of two naphthalimide residues connected by an aminoalkyl linker have been reported to inhibit topoisomerase II [5] by acting as bisintercalators in the major groove of DNA [6][7]. These symmetrical compounds are currently undergoing clinical trials [8–10].

The most active mononaphthalimides *Amonafide* (**3**) and *Mitonafide* (**4**) [1][11] have also been reported to inhibit topoisomerase II [12][13]. They were selected for clinical trials. However, the development of *Mitonafide* was stopped in phase II due to lack in efficacy in the solid tumors that were tested [14], while *Amonafide* completed clinical phase I and II trials [1]. *Amonafide* (**3**) and *Mitonafide* (**4**) differ only in the substituent at C(5), having an NH_2 and a NO_2 group, respectively.

The potency as antitumor agents made it interesting for us to test naphthalimide as an intercalating nucleic acid, which we define as an oligonucleotide with a covalently bound intercalator. Besides the type of aromatic compound used as intercalator, important factors to consider, when using intercalating nucleic acids for hybridization, are the structure of the backbone and the length of the linker. *Ikeda et al.* [15] used PNA [16] when incorporating naphthalimide as an intercalator into DNA. We chose a vicinal dihydroxy system for bulge incorporations, which we have previously described for pyr-

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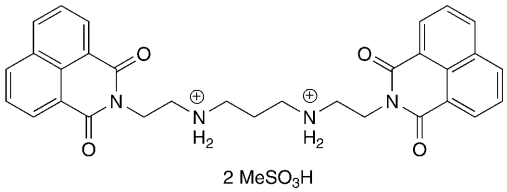

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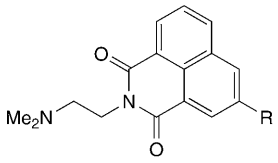
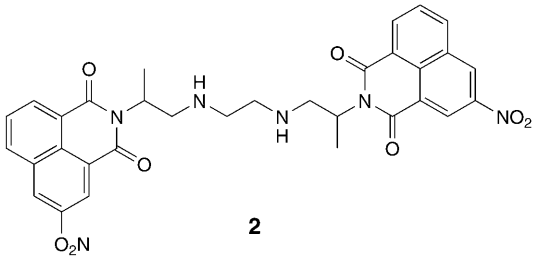


3 R = NH_2
4 R = NO_2



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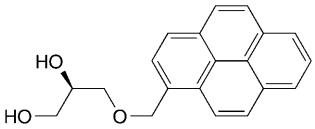
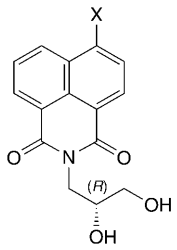


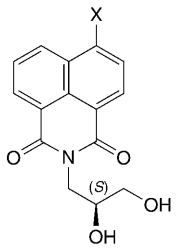
5 *INA*™

ene as the intercalator [17]. Intercalating nucleic acids containing bulge insertions of (*R*)-1-*O*-[(pyren-1-yl)methyl]glycerol (*INA*™; **5**) showed discrimination between DNA and RNA [17], as significantly by increased affinities for ssDNA were achieved, while INA/RNA duplexes were destabilized. The fluorescence properties of the pyrene intercalator confirmed its intercalation rather than groove-binding in duplexes [18]. These findings led to commercialization of *INA*™ [19].

In this paper, we present the synthesis of six intercalating nucleic acids with naphthalimide or 6-bromonaphthalimide derivatives **6**–**11** as the intercalator and their evaluation by thermal stability measurements on DNA/DNA and DNA/RNA duplexes.



6 X = Br
8 X = H



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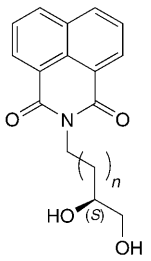
9 X = H



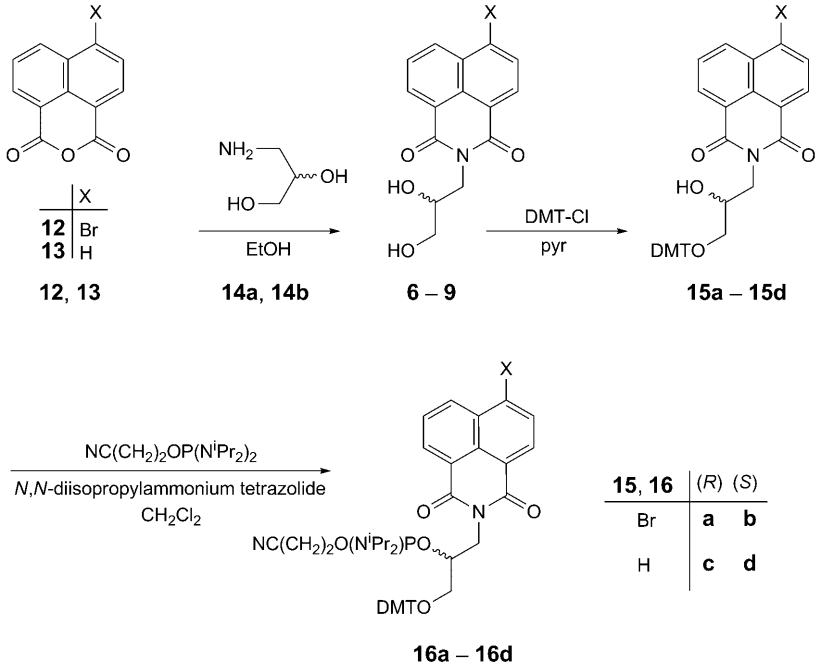
10 *n* = 1
11 *n* = 2

For monomers **6**–**9**, we have varied the configuration of the stereogenic center in the linker and the substitution at C(6) of the naphthalimide ring, in order to evaluate the dependence on configuration for the linker. *Filichev et al.* [20] found (*R*)-1-*O*-[(pyren-1-yl)methyl]glycerol to give higher stabilization than its corresponding (*S*)-isomer. Monomers **10** and **11** were synthesized only as (*S*)-isomers, because that would

give the same spatial configuration as the (*R*)-isomer of 1-*O*-[(pyren-1-yl)methyl]glycerol.

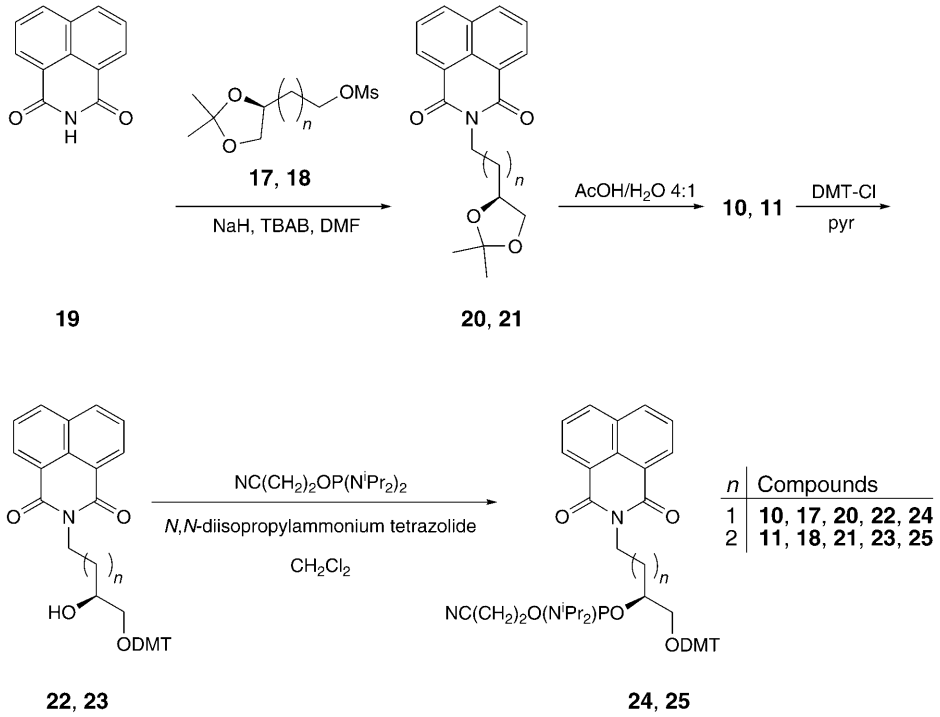
2. Results and Discussion. – 2.1. *Chemistry.* Both 4-bromonaphthalene-1,8-dicarboxylic anhydride (**12**) and naphthalene-1,8-dicarbocylic anhydride (**13**) were reacted with the (*R*)- and (*S*)-form of 3-aminopropane-1,2-diol (**14a** and **14b**, resp.) to give the monomers **6**–**9** in 60–89% yield (*Scheme 1*). The diols were prepared for oligonucleotide synthesis using standard protecting groups. The primary alcohol was protected by using 4,4′-dimethoxytrityl chloride in dry pyridine to give 5′-*O*-DMT-protected compounds **15a**–**15d** in 67–75% yield. The secondary alcohol was phosphitylated with (2-cyanoethoxy)bis(diisopropylamino)phosphane in dry CH_2Cl_2 to yield **16a**–**16d** in 22–98%.

Scheme 1



For monomers **10** and **11** another synthetic route was used (*Scheme 2*). Enantiomerically pure (*S*)-1,2-*O*-isopropylidene-4-*O*-(methylsulfonyl)butane-1,2,4-triol (**17**) [21] was obtained from (*S*)-malic acid in four steps. (*S*)-Malic acid (=hydroxybutanedioic acid) was converted to dimethyl (*S*)-malate according to *Mori* and *Ikunaka* [22]. The diester was reduced with $LiAlH_4$ to (*S*)-butane-1,2,4-triol and protected with an isopropylidene group as described by *Hayashi et al.* [23]. Mesylation of (*S*)-1,2-*O*-isopropylidenebutane-1,2,4-triol was carried out either in pyridine according to *Augustyns et al.* [21] or in CH_2Cl_2/Et_3N according to *Kim et al.* [24]. Using pyridine as the solvent, it gave **17** in a yield of 51%, while the latter solvent gave **17** in 99% yield.

Scheme 2



Enantiomerically pure (*S*)-1,2-*O*-isopropylidene-5-*O*-(methylsulfonyl)pentane-1,2,5-triol (**18**) [25] was obtained from L-glutamic acid in four steps. L-Glutamic acid was converted to (*S*)-2,3,4,5-tetrahydro-5-oxofuran-2-carboxylic acid as described by *Herdeis* [26]. Reduction with $LiAlH_4$ according to *Brunner* and *Lautenschlager* [27] yielded (*S*)-pentane-1,2,5-triol, which was protected with an isopropylidene group and mesylated as described above to give **18**.

For *N*-alkylation, naphthalimide (**19**) was deprotonated with NaH in DMF, and, in the presence of the phase-transfer catalyst Bu_4NBr (TBAB), reacted with either **17** or **18** to give 2-{2-[(*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]ethyl}-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (**20**) and 2-{3-[(*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]propyl}-1*H*-benz[*de*]-isoquinoline-1,3(2*H*)-dione (**21**) in 53–54% yield. Treatment of **20** and **21** with 80% aqueous AcOH gave the diols **10** and **11**, respectively. These were DMT-protected using DMT-Cl in pyridine or in CH_2Cl_2/Et_3N, and phosphitylated as described above to yielding **24** and **25**, respectively.

Prior to oligonucleotide synthesis, we studied the stability of the 6-Br substituent on naphthalimide under the alkaline conditions normally used for the deprotection of the protecting groups on the nucleobases (NH_3, 55°, overnight). No NH_2 substitution was observed (monitored by TLC and NMR) under these conditions.

The DMT-protected phosphoramidites of the intercalating nucleic acid monomers **6**–**11** were incorporated into DNA oligonucleotides using the same coupling times (2 min) in the oligonucleotide synthesis as was used for the amidites of natural nucleo-

sides. The monomers were inserted as a bulge in a dodecamer highly conserved HIV-1 long-term repeat region [28], which we have previously used for pyrene-intercalating nucleic acids [17][18]. All modified oligonucleotides were confirmed by MALDI-TOF analysis (*Table 1*).

Table 1. *Masses of ODNs*

Monomer	5′-CTCAAG**X**CAAGCT-3′		5′-CTCAA**X**G**X**CAAGCT-3′		5′-CTCA**X**AG**X**CAAGCT-3′		5′-CTCA**X**AGCA**X**AGCT-3′	
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
6	4025	4026	4436	4438	4436	4440	4436	4440
7	4025	4027	4436	4436	4436	4438	4436	4438
8	3946	3946	4278	4279	4278	4280	4278	4282
9	3946	3945	4278	4279	4278	4281	4278	4282
10	3960	3960	4306	4309	–	–	–	–
11	3974	3973	4334	4334	4334	4334	4334	4333

2.2. *Thermal Melting Studies.* The project was designed to study the linker-length dependence for intercalating nucleic acids with naphthalimide as the intercalating moiety. It is clearly seen from *Table 2* (*Entry 1*) that all intercalators with short linkers, *i.e.*, **6**–**10**, are destabilizing the DNA/DNA duplex. Only **11**, having a linker of five C-atoms, was able to stabilize the duplex. Studies of the configuration in the linker showed that the (*R*)-isomers, *i.e.*, **6** and **8**, were better than their corresponding (*S*)-isomers **7** and **9**. Introduction of a Br substituent at C(6) of naphthalimide did not have an effect on the (*R*)-isomer, while ΔT_m for the (*S*)-isomer was increased by 1.4° (**7** compared to **9**).

Table 2. *Melting Temperatures of DNA/DNA Duplexes with* **X**=**6**–**11** *inserted as a Bulge.* The target sequence was 5′-AGC TTG CTT GAG-3′, and the reference melting temperature for the wild-type duplex without insertions was 47.4°. Data of **X**=**5** [17] are given for comparison.

	T_m [°]						
X=	**5** [17]	**6**	**7**	**8**	**9**	**10**	**11**
3′-TCG AAC **X**GA ACT C-5′	50.4	45.4	42.4	45.4	41.0	46.1	53.0
3′-TCG AAC **X**G**X** AAC TC-5′	51.4	44.1	34.1	40.4	32.5	44.9	55.5
3′-TCG AAC **X**GA **X**AC TC-5′	55.6	46.7	40.0	44.1	31.9	–	62.7
3′-TCG A**X**A CGA **X**AC TC-5′	60.8	50.9	42.9	49.6	32.4	–	62.4

From *Table 3*, it is seen that all naphthalimides – independent of the length of the linker – destabilized the DNA/RNA duplex. Surprisingly, **10** was the most destabilizing intercalator for the DNA/RNA duplex, when only one insertion was made. It was expected that an intercalator with a linker of four C-atoms would be better than one with a three C-atom linker. (*R*)-Isomers, *i.e.*, **6** and **8**, were in general slightly favored over (*S*)-isomers **7** and **9**, and, in general, the 6-Br-substituted naphthalimides had higher T_m values than the unsubstituted ones. Intercalating nucleic acids containing insertions of **11** were discriminating between DNA and RNA, as the DNA/DNA duplexes were stabilized while DNA/RNA duplexes were destabilized.

Table 3. *Melting Temperatures of DNA/RNA Duplexes with* **X**=**6**–**11** *Inserted as a Bulge.* The target sequence was 5′-AGC UUG CUU GAG-3′, and the reference melting temperature for the wild-type duplex without insertions was 40.5°. Data of **X**=**5** [17] are given for comparison (reference for **X**=**5** was 42.2° [17]).

	T_m [°]						
X=	**5** [17]	**6**	**7**	**8**	**9**	**10**	**11**
3′-TCG AAC **X**GA ACT C-5′	37.8	35.4	34.9	33.0	31.8	31.7	38.0
3′-TCG AAC **X**G**X** AAC TC-5′	34.2	28.5	27.8	28.2	27.9	30.7	38.4
3′-TCG AAC **X**GA **X**AC TC-5′	32.6	30.7	34.4	29.2	26.7	–	35.8
3′-TCG A**X**A CGA **X**AC TC-5′	35.0	34.8	34.3	35.0	28.8	–	36.2

Gallego and *Reid* [7] have shown that the bisintercalator **1** is stacking mainly with G and A bases. So, to further investigate the discrimination and stabilization phenomena, intercalating nucleic acids with two naphthalimides were prepared (*Table 2*, *Entries 2–4*). The monomers were inserted with varying distances as next-nearest neighbors or with two or four nucleobases between them. Placed between two adenines four nucleobases apart, the naphthalimide (*R*)-isomers **6** and **8** with the shorter linker were actually able to stabilize the DNA/DNA duplex. In all other cases with short linkers of (*R*)-isomers, destabilization was observed when compared with the wild-type duplex. Also insertion of the (*S*)-isomers did not lead to any stabilization. In fact, double insertions of **9** led a further destabilization of *ca.* 10° when compared with a single insertion. It was interesting to observe **11** giving stabilizations up to 15.3° (7.7° per modification), when two insertions were made.

Compared to previous studies [17] of intercalating nucleic acids with pyrene as the intercalator (data are given for comparison in *Tables 2* and *3*), **11** gives higher thermal melting values than pyrene **5**.

For pyrene **5**, better stacking was achieved when two insertions were made between two adenines four nucleobases apart, but **11** was equally good, whether incorporated between the adenines four bases apart or incorporated two nucleobases apart with one insertion between two adenines and the other between a CG base pair (*Table 2*, *Entries 3–4*).

2.3. *Universal Base Studies.* We found it interesting to test naphthalimide as a universal base. Results are presented in *Table 4*. Position 7 in the 13-mer duplex was varied with all four natural bases in both strands to give a reference data set. Then *INA*™ (**5**) and ODNs with naphthalimide intercalators **6**–**11** inserted at position 7 were measured against oligonucleotides with all four natural bases.

From *Table 4*, it is obvious that the Br-substituted naphthalimides **6** and **7** were good universal bases having a narrow range of melting temperatures against all natural bases, whereas those with the same linker-length but without the Br substituents, *i.e.*, **8** and **9**, showed a broad range of melting temperatures. However, **10** and **11** having the longer linkers were the best ones. For **11**, the range was as narrow as 1.4°, and, furthermore, the melting temperatures were higher than for the bases with the shorter linkers, and they were only by 5° lower than for the wild-type duplex. For *INA*™ (**5**), the range was 2.6°.

2.4. *Mismatch Studies.* Finally, a series of mismatch studies was carried out (*Table 5*). The specificity for hybridization was measured by the difference in the melting temper-

Table 4. *Universal Base Measurements.* Melting temperatures of duplexes where a natural nucleotide was replaced by either another nucleoside or by monomers **5**–**11**.

5′-AGCTTG**Y**CTTGAG-3′
3′-TCGAAC**X**GAACTC-5′

X= / **Y**	A	C	G	T	**5**	**6**	**7**	**8**	**9**	**10**	**11**
A	39.4	37.4	45.8	50.5	43.1	38.9	40.4	36.8	37.9	40.8	42.1
C	35.5	23.6	54.3	35.1	44.1	36.7	38.1	33.8	34.3	37.9	42.6
G	45.9	52.4	46.7	43.1	41.5	36.9	38.7	32.0	34.1	38.3	41.2
T	48.9	32.5	36.1	35.8	43.1	39.6	41.7	36.8	39.2	39.4	42.0

Table 5. *Melting Temperatures* [°] *of Mismatched sequences with* **6**–**11** *Inserted as a Bulge*[a])

Sequence: 5′-AGC TT**Z** **Y**TT GAG-3′
3′-TCG AAG **X** GAA CTC-5′

	X=	–		**6**		**7**		**8**		**9**		**10**		**11**	
	Z Y	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m
Wild type	G C	47.4		45.4		42.1		45.4		41.0		46.1		53.0	
Mut. 1	C C	23.6	−23.8	34.1	−11.3	32.5	−9.6	30.2	−15.2	32.2	−8.8	32.7	−13.4	32.7	−20.3
Mut. 2	A C	30.7	−16.7	32.2	−13.2	31.5	−10.6	29.8	−15.6	31.2	−9.8	32.9	−13.2	35.3	−17.7
Mut. 3	T C	28.9	−18.5	32.0	−13.4	31.3	−10.8	30.0	−15.4	31.2	−9.8	32.5	−13.6	36.7	−16.3
Mut. 4	G G	39.8	−7.6	36.3	−9.1	32.5	−9.6	33.3	−12.1	32.5	−8.5	33.5	−12.6	39.7	−13.3
Mut. 5	G A	39.0	−8.4	34.7	−10.7	32.8	−9.3	32.2	−13.2	33.2	−7.8	34.8	−11.3	38.6	−14.4
Mut. 6	G T	35.9	−11.5	37.5	−7.9	36.4	−5.7	34.0	−11.4	33.0	−8.0	36.5	−9.6	43.1	−9.9

[a]) ΔT_m is the difference in T_m between the matched sequence and the mismatched one.

ature between the fully complementary duplex and the duplex where one mismatch has been introduced in position either **Y** or **Z**.

It is interesting to observe **9** showing almost equal specificity for all mismatches. Unfortunately, the specificity was rather low, being in the range of 7.8–9.8°, lower than the matched sequence. Compound **11** was the most sensitive monomer to mismatches especially at the 3′-site of the intercalator, where it was in the range of the unmodified sequence. Therefore, we decided to expand the studies, performing mismatch measurements with double insertions of **11** as next-nearest neighbors, and with two and four base pairs in-between, respectively (*Table 6*). Again, one mismatch was introduced in position either **Y** or **Z**.

Results for probes **II** and **III** with the intercalators separated by two and four base pairs, respectively, gave almost identical results. These probes showed in many cases even higher specificity. The C−C mismatch causes a drop in melting temperature as high as *ca.* 29°.

2.5. *Molecular Modelling*. To gain further insight into the linker-length dependence of intercalating nucleic acids, molecular modelling studies were performed with MacroModel version 5.1.016 from *Schrödinger Inc.* [29]. All calculations were conducted with OPLS-AA force field [30] and the GB/SA water model [31]. From the *Figure*, *b*, it is

Table 6. *Melting-Temperature* [°] *Data of Mismatched Sequences with Bulge Insertions of* **11** *as Next-Nearest Neighbors, and with Two and Four Base Pairs in-between, Respectively*[a])

Target: 5′-AGC TT**Z** **Y**TT GAG-3′
Probes:
I 3′-TCG AAC **X** G**X**AA CTC-5′
II 3′-TCG AAC **X** GA**X**A CTC-5′
III 3′-TCG A**X**AC GA**X**A CTC-5′

	X =	**I**		**II**		**III**	
	Z **Y**	T_m	ΔT_m	T_m	ΔT_m	T_m	ΔT_m
Wild type	G C	55.5		62.7		62.4	
Mut. 1	C C	38.8	– 16.7	33.1	– 29.6	33.4	– 29.0
Mut. 2	A C	38.9	– 16.6	41.3	– 21.4	40.4	– 22.0
Mut. 3	T C	38.6	– 16.9	38.8	– 23.9	37.9	– 24.5
Mut. 4	G G	38.8	– 16.7	52.0	– 10.7	52.2	– 10.2
Mut. 5	G A	37.5	– 18.0	50.7	– 12.0	50.7	– 11.7
Mut. 6	G T	44.9	– 10.6	46.1	– 16.6	45.6	– 16.8

[a]) ΔT_m is the difference in T_m between the matched sequence and the mismatched one.

clearly seen that **8** having the shortest linker is unable to position the intercalator optimally for base stacking with nucleobases. Furthermore, the backbone of the strand containing the intercalator is disturbed by the intercalator pulling it towards the axe of the duplex. Comparing the linker-length of three and four C-atoms (*Fig.*, *b* and *c*), it is seen that the four C-atom linker positions the intercalator deeper into the duplex. The backbone conformation looks similar to that of DNA without intercalator (*Fig.*, *a*). Comparing the linker-length of four and five C-atoms (*Fig.*, *c* and *d*) reveals that the intercalator stays in the same position between the nucleobases, but the backbone is pushed a little backwards. Thus, the linker of four C-atoms is sufficient to position the intercalator between the nucleobases and achieve stacking. By increasing the length of the linker to five C-atoms, a better stacking is not achieved. However, more favorable thermal meltings were obtained with the five C-atom linker, and this may reflect that the backbone becomes more relaxed with a longer linker.

3. Conclusions. – We have tested naphthalimide as intercalating nucleic acid in a study of linker-length dependence. This dependence was obvious as shorter linkers destabilized the duplexes since they were not able to position the intercalator optimally for base stacking without disturbing the backbone, which was confirmed by molecular-modelling studies. The study revealed that naphthalimide must have a linker of at least five C-atoms, *i.e.*, **11**. Furthermore, we found this naphthalimide **11** to have a higher thermal melting than *INA*™ (**5**) when hybridized to the complementary DNA strand. Naphthalimide is discriminating between DNA and RNA like *INA*™ (**5**), as DNA is stabilized while RNA is destabilized. Naphthalimide shows universal-base character, hybridizing to all four natural bases within a range as narrow as 1.4°. Finally, **11** showed sensibility to mismatches especially if two naphthalimides were incorporated with two or four base pairs in-between.

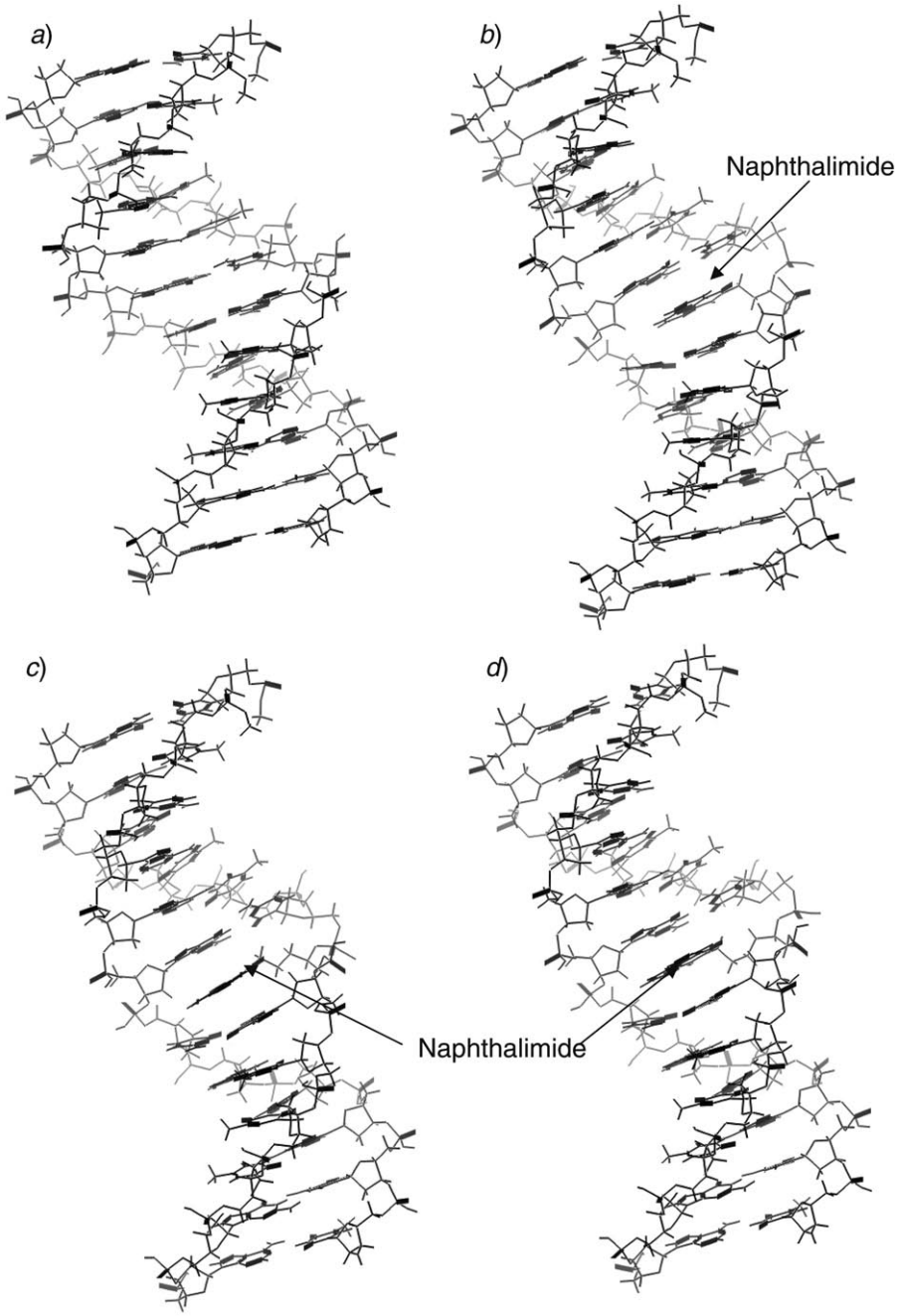


Figure. *Molecular modelling conformations of a DNA/DNA duplex* (*a*) *with insertions of one naphthalimide intercalato*r (*b*–*d*) *with a linker of three* (*b*), *four* (*c*), *and five* (*d*) *C-atoms, respectively.*

Dr. *Vyacheslav Filichev* is acknowledged for the preparation of *INA*™ used for universal-base studies.

Experimental Part

General. TLC: TLC plates *60 F_{254}* (*Merck*); visualized by UV light (254 nm) or a stain of $(NH_4)_6Mo_7O_{24}\cdot 4\,H_2O/Ce_2(SO_4)_3$ (50:1 (*v*/*v*)) in 5% H_2SO_4. Column chromatography (CC): silica-gel-packed column (silica gel *60*; 0.040–0.063 mm, *Merck*). Solvents used for CC were distilled prior to use, while reagents were used as purchased. Petroleum ether: b.p. 60–80°. M.p.: *Büchi* melting-point apparatus. NMR Spectra: *Varian Gemini 2000* spectrometer (^{1}H: 300 MHz, ^{13}C: 75 MHz, ^{31}P: 121.5 MHz); δ values in ppm relative to Me_4Si as internal standard for ^{1}H-NMR; $CDCl_3$ (δ 77.00), DMSO (δ 39.52), and CD_3CN (δ 1.32) for ^{13}C-NMR; 85% H_3PO_4 as external standard for ^{31}P-NMR, *J* in Hz. EI-MS: *Finnigan Mat SSQ 701* mass spectrometer. MALDI-MS: Accurate ion mass determinations were performed using an *Ionspec 4.7 T Ultima Fourier Transform* mass spectrometer (*IonSpec*, Irvine, CA). The M^+ or $[M+Na]^+$ ions were peak-matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. Elemental analyses were performed at H. C. Ørsted Institute, University of Copenhagen.

*General Procedure for the Synthesis of 6-Bromo-2-(2,3-dihydroxypropyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**6** and **7**) *and 2-(2,3-dihydroxypropyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**8** and **9**). To a suspension of *4-bromonaphthalene-1,8-dicarboxylic anhydride* (**12**; 1.16 g, 4 mmol) or *naphthalene-1,8-dicarboxylic anhydride* (**13**; 0.80 g, 4 mmol) in anh. EtOH (60 ml) was added *3-aminopropane-1,2-diole* (**14a** or **14b**; 0.36 g, 4 mmol), and the reaction mixture was refluxed. After 30 min, the suspension was dissolved completely, and, after 2.5 h, TLC (5% MeOH/$CHCl_3$) indicated complete consumption of the substrate. The warm soln. was filtered off, and, after cooling, precipitation occurred. The product was recrystallized from EtOH.

*6-Bromo-2-[(*R*)-2,3-dihydroxypropyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**6**). Yield: 1.24 g (89%). M.p. 179–180° (EtOH). $[\alpha]_D = +39.4$ ($c = 0.7$, MeOH). ^{1}H-NMR (DMSO): 3.34–3.44 (*m*, 2 H–C(3)); 3.87–3.98 (*m*, H_b–C(1), H–C(2)); 4.18 (*dd*, $J = 7.5$, 12.5, H_a–C(1)); 4.60 (*t*, $J = 5.6$, HO–C(3)); 4.77 (*d*, $J = 5.0$, HO–C(2)); 7.91 (*dd*, $J = 7.3$, 8.4, 1 arom. H); 8.10 (*d*, $J = 8.1$, 1 arom. H); 8.22 (*d*, $J = 8.1$, 1 arom. H); 8.39 (*dd*, $J = 0.8$, 8.4, 1 arom. H); 8.46 (*d*, $J = 7.3$, 1 arom. H). ^{13}C-NMR (DMSO): 43.36 (C(1)); 64.56 (C(3)); 68.47 (C(2)); 121.92, 122.70, 128.04, 128.57, 128.83, 129.50, 130.71, 131.14, 131.34, 132.23 (arom. C); 162.93, 162.99 (2 C=O). EI-MS: 350 (6, M^+), 352 $[M+2]^+$, 333 (24), 320 (93), 318 (100), 289 (40), 276 (38), 260 (25), 126 (60). Anal. calc. for $C_{15}H_{12}BrO_4N$ (350.17): C 51.45, H 3.45, N 4.00; found: C 51.45, H 3.39, N 3.98.

*6-Bromo-2-[(*S*)-2,3-dihydroxypropyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**7**). Yield: 1.23 g (88%). M.p. 179–180°. $[\alpha]_D = -40.6$ ($c = 0.7$, MeOH). Spectroscopic data identical to those of **6**.

*2-[(*R*)-2,3-Dihydroxypropyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**8**). Yield: 0.77 g (72%). R_f (5% MeOH/$CHCl_3$) 0.16. M.p. 145–149°. ^{1}H-NMR (DMSO): 3.44 (*m*, 2 H–C(3)); 3.94–4.03 (*m*, H_b–C(1), H–C(2)); 4.23 (*dd*, $J = 8.0$, 12.2, H_a–C(1)); 4.61, (br. *s*, OH), 4.80 (br. *s*, OH), 7.86 (*m*, 2 arom. H), 8.36–8.56 (*m*, 4 arom. H). ^{13}C-NMR (DMSO): 43.20 (C(1)); 64.60 (C(3)); 68.56 (C(2)); 122.18, 127.09, 127.31, 127.49, 130.53, 131.17, 131.29, 132.42, 134.03, 135.34 (arom. C); 163.63 (C=O). HR-MALDI-MS: 294.0726 ($[M+Na]^+$, $C_{19}H_{13}NaNO_4^+$; calc. 294.0736).

*2-[(*S*)-2,3-Dihydroxypropyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**9**). Yield: 0.64 g (60%). R_f (5% MeOH/$CHCl_3$) 0.20. M.p. 147–151°. ^{1}H-NMR ($CDCl_3$): 3.20, 3.37 (2 br. *s*, 2 OH); 3.67 (*m*, 2 H–C(3)); 4.11 (*m*, H–C(2)); 4.34 (*dd*, $J = 6.4$, 15.7, H_a–C(1)); 4.44 (*dd*, $J = 5.0$, 15.7, H_b–C(1)); 7.72 (*t*, $J = 8.0$, 2 arom. H); 8.16 (*d*, $J = 8.0$, 2 arom. H); 8.55 (*d*, $J = 7.4$, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 42.61 (C(1)); 63.82 (C(3)); 70.60 (C(2)); 121.93, 126.96, 127.38, 127.99, 131.39, 131.68, 133.30, 134.39, 135.25 (arom. C); 165.24 (C=O). HR-MALDI-MS: 294.0730 ($[M+Na]^+$, $C_{15}H_{13}NaNO_4^+$; calc. 294.0736). Anal. calc. for $C_{15}H_{13}NO_4$ (294.07): C 66.41, H 4.83, N 5.16; found: C 66.52, H 4.54, N 4.71.

General Procedure for DMT-Protection of Diols **6**–**9**. Diols **6**–**9** was dissolved in anh. pyridine (10–25 ml) and 4,4′-dimethoxytrityl chloride (1.5 equiv.) was added while stirring. After 24 h, the reaction was stopped by addition of MeOH (2 ml) and the solvent was removed by evaporation under

reduced pressure. The residue was dissolved in CH_2Cl_2 (50 ml), washed with sat. aq. $NaHCO_3$ (2×5 ml), dried (Na_2SO_4) and evaporated to dryness. The product was purified by CC (silica gel; cyclohexane/AcOEt/Et_3N (2:8:0.5 *v*/*v*/*v*)).

*2-{(*R*)-3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-hydroxypropyl}-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**15a**). Yield: 0.49 g (75%). Colorless solid (obtained from 0.35 g, 1.0 mmol of **6**). ^{1}H-NMR ($CDCl_3$): 2.87 (*d*, *J*=7.1, OH); 3.27 (*dd*, *J*=4.5, 9.5, H_b–C(3)); 3.34 (*dd*, *J*=4.5, 9.5, H_a–C(3)); 3.77 (*s*, 2 MeO); 4.17–4.18 (*m*, H–C(2)); 4.27 (*dd*, *J*=9.0, 13.7, H_b–C(1)); 4.59 (*dd*, *J*=9.0, 13.7, H_a–C(1)); 6.81 (*d*, *J*=8.4, 4 arom. H); 7.21–7.48 (*m*, 9 arom. H); 7.82 (*dd*, *J*=7.1, 8.5, 1 arom. H); 8.01 (*d*, *J*=8.1, 1 arom. H); 8.38 (*d*, *J*=8.1, 1 arom. H); 8.54 (*dd*, *J*=1.2, 8.5, 1 arom. H); 8.62 (*dd*, *J*=1.1, 7.4, 1 arom. H). ^{13}C-NMR ($CDCl_3$): 43.98 (C(1)); 55.15 (MeO); 65.64 (C(2)); 69.91 (C(3)); 86.09 (Ar_3C); 113.06, 122.00, 122.86, 126.72, 127.78, 128.05, 128.14, 128.99, 130.04, 130.44, 130.55, 131.07, 131.39, 132.22, 133.39, 135.89, 135.91, 144.68, 158.40 (arom. C); 164.36 (C=O).

*2-{(*S*)-3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-hydroxypropyl}-6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**15b**). Yield: 0.45 g (67%). Colorless solid (obtained from 0.35 g, 1.0 mmol of **7**). Spectroscopic data identical to those of **15a**.

*2-{(*R*)-3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-hydroxypropyl}-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione* (**15c**). Yield: 1.02 g (71%). White crystals (obtained from 0.68 g, 2.5 mmol of **8**). R_f (AcOEt/cyclohexane/Et_3N 8:2:0.05) 0.54. M.p. 83–85°. ^{1}H-NMR ($CDCl_3$): 2.94 (*d*, *J*=6.9, OH); 3.25–3.36 (*m*, 2 H–C(1)); 3.77 (*s*, 2 MeO); 4.18 (*m*, H–C(2)); 4.31 (*dd*, *J*=13.6, 3.2, H_b–C(3)); 4.59 (*dd*, *J*=13.6, 9.2, H_a–C(3)); 6.80 (*dd*, *J*=8.9, 2.7, 4 arom. H); 7.18 (*t*, *J*=7.2, 1 arom. H); 7.26 (*t*, *J*=7.2, 2 arom. H); 7.36 (*dd*, *J*=8.9, 1.5, 4 arom. H); 7.48 (*d*, *J*=7.2, 2 arom. H); 7.72 (*dd*, *J*=7.3, 8.2, 2 arom. H); 8.19 (*dd*, *J*=8.2, 0.9, 2 arom. H); 8.57 (*dd*, *J*=7.3, 0.9, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 43.94 (C(1)); 55.15 (MeO); 65.70 (C(3)); 70.10 (C(2)); 86.03 (Ar_3C); 113.05, 122.41, 126.68, 126.88, 126.99, 127.40, 127.76, 128.14, 129.08, 130.03, 131.39, 131.49, 133.33, 134.05, 135.25, 135.94, 135.97, 144.74, 158.37 (arom. C); 164.96 (C=O). HR-MALDI-MS: 596.2038 ($[M+Na]^+$, $C_{36}H_{31}NaNO_6^+$; calc. 596.2043). Anal. calc. for $C_{36}H_{31}NO_6$ (596.20): C 75.38, H 5.45, N 2.44; found: C 75.87, H 5.40, N 2.05.

*2-{(*S*)-3-[Bis(4-methoxyphenyl)(phenyl)methoxy]-2-hydroxypropyl}-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione* (**15d**). Yield: 0.58 g (69%). White crystals (obtained from 0.40 g, 1.5 mmol of **9**). R_f (AcOEt/cyclohexane/Et_3N 2:3:0.05) 0.27. M.p. 85–88°. Spectroscopic data identical to those of **15c**. HR-MALDI-MS: 596.2014 ($[M+Na]^+$, $C_{36}H_{31}NaNO_6^+$; calc. 596.2043). Anal. calc. for $C_{36}H_{31}NO_6$ (596.20): C 75.38, H 5.45, N 2.44; found: C 74.71, H 5.36, N 1.98.

General Procedure for Synthesis of Phosphoramidites **16a**–**16d**: Compound **15a**–**15d** (1 equiv.) was dissolved in anh. CH_2Cl_2 (10 ml). (2-Cyanoethoxy)bis(diisopropylamino)phosphane (1 equiv.) was added, followed by addition of *N*,*N*-diisopropylammonium tetrazolide (1.5 equiv.). The mixture was stirred at r.t. for 12 h, diluted with CH_2Cl_2 (20 ml), washed with sat. aq. $NaHCO_3$ (2×5 ml) and brine (2×5 ml), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by CC (silica gel; cyclohexane/AcOEt/Et_3N 2:8:0.1 (*v*/*v*/*v*)) for **16a** and **16b** and cyclohexane/AcOEt/Et_3N (3:2:0.05 (*v*/*v*/*v*) for **16c** and **16d**).

Phosphoramidite **16a**. Yield: 0.23 g (90%). White foam (obtained from 0.2 g (0.3 mmol) of **15a**). ^{1}H-NMR (CD_3CN): 0.94–1.07 (*m*, 4 CH_3); 2.26, 2.46 (2*t*, *J*=6.1, CH_2CN); 3.04–3.15, 3.22–3.33 (2*m*, 2 H–C(3)); 3.35–3.66 (*m*, OCH_2CH_2CN, 2 Me_2CH); 3.69, 3.71 (2*s*, 2 MeO); 4.10–4.22 (*m*, H–C(2)); 4.34–4.50 (*m*, 2 H–C(1)); 6.65–6.79 (*m*, 4 arom. H); 7.12–7.22 (*m*, 5 arom. H); 7.25–7.29 (*dd*, *J*=2.2, 9.0, 2 arom. H); 7.39 (*m*, 2 arom. H); 7.82–7.85 (*m*, 1 arom. H); 8.04 (*dd*, *J*=3.3, 7.9, 1 arom. H); 8.25 (*t*, *J*=8.2, 1 arom. H); 8.48–8.54 (*m*, 2 arom. H). ^{13}C-NMR (CD_3CN): 20.79 (CH_2CN); 24.77, 24.80, 24.85, 24.90 (4 Me); 43.59, 43.75, 43.90 (C(1), 2 Me_2CH); 55.78 (MeO); 59.25 (OCH_2CH_2CN); 66.55 (C(3)); 71.11 (C(2)); 87.03 (Ar_3C); 113.78 (arom. C); 119.16 (CN); 122.91, 123.69, 127.57, 128.58, 128.85, 129.16, 129.40, 130.39, 130.73, 131.09, 131.76, 131.99, 132.55, 133.60, 136.62, 136.87, 145.96, 159.34, 159.42 (arom. C); 163.95, 164.10 (2 C=O). ^{31}P-NMR (CD_3CN): 149.64; 149.57.

Phosphoramidite **16b**. Yield: 0.25 g (98%). White foam (obtained from 0.2 g (0.3 mmol) of **15b**). ^{1}H- and ^{13}C-NMR: identical to those of **16a**. ^{31}P-NMR (CD_3CN): 149.61; 149.54.

Phosphoramidite **16c**. Yield: 0.60 g (59%). White foam (obtained from 0.75 g (1.3 mmol) of **15c**). R_f (AcOEt/cyclohexane/Et_3N 2:3:0.05) 0.39. ^{1}H-NMR ($CDCl_3$): 0.93, 1.03 (2*t*, *J*=6.3, 4 Me); 2.14, 2.38 (2*t*, *J*=7.0, CH_2CN); 3.14 (*dd*, *J*=5.3, 9.5, H_a–C(3)); 3.24 (*dd*, *J*=5.3, 9.5, H_b–C(3)); 3.30–3.72 (*m*,

OCH_2CH$_2$CN, 2 MeC*H*); 3.74, 3.76 (2*s*, 2 MeO); 4.11–4.23 (*m*, H–C(2)); 4.44–4.60 (*m*, 2 H–C(1)); 6.69–6.79 (*m*, 4 arom. H); 7.11–7.48 (*m*, 9 arom. H); 7.75 (*q*, *J* = 8.1, 2 arom. H); 8.21 (*t*, *J* = 8.5, 2 arom. H); 8.57 (*t*, *J* = 6.3, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 20.06 (*C*H_2CN); 24.11, 24.21, 24.33, 24.45 (4 Me); 42.99 (C(1)); 42.93, 43.17 (2 Me*C*H); 55.14 (MeO); 57.97 (O*C*H_2CH$_2$CN); 65.62 (C(3)); 70.85 (C(2)); 86.13 (Ar_3C); 112.91, 122.92, 126.56, 126.93, 127.68, 128.16, 130.09, 130.91, 131.17, 131.60, 133.73, 136.10, 136.21, 144.75, 158.25, 158.30 (arom. C); 164.13, 164.30 (2 C=O). ^{31}P-NMR ($CDCl_3$): 150.14. EI-MS: 773 (M^+).

Phosphoramidite **16d**. Yield: 0.12 g (22%). White foam (obtained from 0.40 g (0.7 mmol) of **15d**). R_f (AcOEt/cyclohecane/Et_3N 2:3:0.05) 0.39. ^{1}H- and ^{13}C-NMR: identical to those of **16c**. ^{31}P-NMR ($CDCl_3$): 150.14.

General Procedure for N-*Alkylation of* **19**. NaH (60% in mineral oil; 0.07 g, 2.9 mmol) was added portionwise to the soln. of naphthalimide (**19**; 0.40 g, 2 mmol) in dry DMF (40 ml). The mixture was stirred at r.t. for 30 min, then Bu_4NBr (TBAB) (0.13 g, 0.4 mmol) was added, and the mixture was stirred for another 30 min. *2-[(*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]ethyl methanesulfonate* (**17**; 0.88 g, 4 mmol) or *3-[(*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]propyl methanesulfonate* (**18**; 0.60 g, 2.5 mmol) was added dropwise. The mixture was stirred for 48 h at 140°. After cooling, DMF was co-evaporated with xylene under reduced pressure, and the residue was dissolved in H_2O and extracted with $CHCl_3$ (3 × 100 ml). The combined org. phases were dried ($MgSO_4$), and the solvent was removed under reduced pressure. The product was purified by CC (silica gel; AcOEt/petroleum ether 2:3 (*v*/*v*)).

*2-{2-[(*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]ethyl}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**20**). Yield: 0.359 g (54%). Yellow solid. R_f (AcOEt/petroleum ether 2:3) 0.60. M.p. 185°. ^{1}H-NMR ($CDCl_3$): 1.33, 1.41 (2*s*, 2 Me); 1.90–1.99 (*m*, 1 H–C(2)); 2.05–2.15 (*m*, 1 H–C(2)); 3.65 (*dd*, *J* = 6.0, 7.7, H_a–C(4′)); 4.12 (*dd*, *J* = 6.0, 7.7, H_b–C(4′)); 4.19–4.42 (*m*, 2 H–C(1), H–C(3)); 7.75 (*t*, *J* = 7.7, 2 arom. H); 8.21 (*d*, *J* = 8.4, 2 arom. H); 8.59 (*d*, *J* = 6.9, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 25.61, 26.95 (2 Me); 32.11 (C(2)); 37.36 (C(1)); 69.24 (C(4)); 74.28 (C(3)); 108.91 (Me_2C); 122.59, 126.89, 131.16, 133.90 (arom. C); 164.09 (C=O). HR-MALDI-MS: 348.1217 ([*M* + Na]$^+$, $C_{19}H_{19}NaNO_4^+$; calc. 348.1206).

*2-{3-[(*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl]propyl}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**21**). Yield: 0.453 g (53%). Yellow oil (obtained from 0.492 g (2.5 mmol) of **19**). R_f (AcOEt/petroleum ether 2:3) 0.35. ^{1}H-NMR ($CDCl_3$): 1.34, 1.39 (2*s*, 2 Me); 1.65–1.90 (*m*, 2 H–C(2), 2 H–C(3)); 3.54 (*t*, *J* = 7.5, H–C(4′)); 4.11–4.25 (*m*, 2 H–C(1), 2 H–C(5′)); 7.75 (*t*, *J* = 7.4, 2 arom. H); 8.21 (*dd*, *J* = 1.0, 8.3, 2 arom. H); 8.58 (*dd*, *J* = 1.0, 7.4, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 24.45 (C(2)); 25.70, 26.89 (2 Me); 30.90 (C(3)); 40.07 (C(1)); 69.33 (C(5)); 75.71 (C(4)); 108.70 (Me_2C); 122.58, 126.87, 128.08, 131.17, 131.52, 133.87 (arom. C); 164.10 (C=O). HR-MALDI-MS: 362.1349 ([*M* + Na]$^+$, $C_{19}H_{19}NaNO_4^+$; calc. 362.1363).

*2-[(*S*)-3,4-Dihydroxybutyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (***10***)*. Compound **20** (0.13 g, 0.4 mmol) was stirred in acetic AcOH/H_2O 4:1 (50 ml) at r.t. overnight. The solvent was co-evaporated two times with toluene, and the product was purified by CC (silica gel; AcOEt/petroleum ether 3:7 (*v*/*v*)) to afford **10** (0.1 g, 87%). Yellow solid. R_f (AcOEt/petroleum ether 3:7) 0.20. M.p. 160°. ^{1}H-NMR (DMSO): 1.55–1.68, 1.78–1.86 (2*m*, 2 H–C(2)); 3.22–3.26 (*m*, 2 H–C(4)); 3.51–3.58 (*m*, H–C(3)); 4.02–4.12 (*m*, H_a–C(1)); 4.20–4.30 (*m*, H_b–C(1)); 4.58 (br. *s*, 2 OH); 7.82–7.88 (*m*, 2 arom. H); 8.41–8.48 (*m*, 4 arom. H). ^{13}C-NMR (DMSO): 31.94 (C(2)); 37.50 (C(1)); 65.92 (C(4)); 69.88 (C(3)); 122.09, 127.16, 127.29, 130.61, 131.24, 134.18 (arom. C); 163.34 (C=O). HR-MALDI-MS: 308.0888 ([*M* + Na]$^+$, $C_{16}H_{15}NaNO_4^+$; calc. 308.0893).

*2-[(*S*)-4,5-Dihydroxypentyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione* (**11**). Compound **21** (0.359 g, 1 mmol) was stirred in AcOH/H_2O 4:1 (40 ml) at r.t. overnight. The solvent was evaporated under reduced pressure: **11** (quant.). Yellow oil. ^{1}H-NMR (DMSO): 1.32–1.81 (*m*, 2 H–C(2), 2 H–C(3)); 3.21–3.33 (*m*, H–C(4), 2 H–C(5)); 4.05 (*t*, *J* = 7.3, 2 H–C(1)); 4.46 (br. *s*, 2 OH); 7.85 (*dd*, *J* = 7.4, 8.2, 2 arom. H); 8.45 (*ddd*, *J* = 1.0, 7.4, 11.4, 4 arom. H). ^{13}C-NMR (DMSO): 24.08 (C(2)); 30.95 (C(3)); 39.92 (C(1)); 65.79 (C(5)); 70.98 (C(4)); 122.02, 127.20, 130.70, 131.26, 134.25 (arom. C); 163.37 (C=O). HR-MALDI-MS: 322.1059 ([*M* + Na]$^+$, $C_{17}H_{17}NaNO_4^+$; calc. 322.1050).

*2-{(*S*)-4-[Bis(4-methoxyphenyl)(phenyl)methoxy]-3-hydroxybutyl}-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione* (**22**). Compound **10** (0.1 g, 0.34 mmol) was dissolved in dry pyridine (20 ml), and 4,4′-dimethoxytrityl chloride (DMT-Cl; 0.15 g, 0.4 mmol) was added portionwise at 0°. The mixture was stirred over-

night and co-evaporated with toluene/MeOH 1:1 (20 ml) under reduced pressure. The residue was purified by CC (silica gel; cyclohexane/AcOEt/Et_3N 49:49:2 (*v/v/v*)) to yield **22** (0.12 g, 60%). Yellow foam. R_f (cyclohexane/AcOEt/Et_3N 49:49:2) 0.60. ^{1}H-NMR ($CDCl_3$): 1.78–1.94 (*m*, 2 H–C(2)); 3.06–3.18 (*m*, 2 H–C(3)); 3.30 (br. *s*, H–C(4)); 3.75 (*s*, 2 MeO); 4.45 (*t*, *J* = 6.2, 2 H–C(1)); 6.77 (*d*, *J* = 8.8, 4 arom. H); 7.13 (*t*, *J* = 7.3, 2 arom. H); 7.23 (*t*, *J* = 7.7, 2 arom. H); 7.29 (*dd*, *J* = 7.3, 1.9, 4 arom. H); 7.41 (*d*, *J* = 8.2, 2 arom. H); 7.76 (*t*, *J* = 7.7, 2 arom. H); 8.22 (*d*, *J* = 8.2, 2 arom. H); 8.60 (*d*, *J* = 7.0, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 32.48 (C(1)); 37.18 (C(2)); 55.15 (2 MeO); 67.41 (C(4)); 68.32 (C(3)); 85.93 (Ar_3C); 113.03, 122.46, 126.63, 126.95, 127.72, 128.15, 130.00, 131.42, 134.09, 136.09, 144.84, 158.34 (arom. C); 164.57 (C=O). HR-MALDI-MS: 610.2220 ($[M+Na]^+$, $C_{37}H_{33}NaNO_6^+$; calc. 610.2200).

*2-{(*S*)-5-[Bis(4-methoxyphenyl)(phenyl)methoxy]-4-hydroxypentyl}-1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-dione* (**23**). Compound **11** (0.300 g, 1.0 mmol) was dissolved in dry CH_2Cl_2 (20 ml) and dry Et_3N (1 ml), and DMT-Cl (0.341 g, 1.0 mmol) was added. The mixture was stirred under N_2 overnight and concentrated under reduced pressure. The residue was purified by CC (silica gel; AcOEt/petroleum ether/Et_3N 24:74:2 (*v/v/v*)) to yield **23** (0.273 g, 45%). Yellow oil. R_f (AcOEt/petroleum ether 1:3) 0.27. ^{1}H-NMR ($CDCl_3$): 1.50–1.61 (*m*, 2 H–C(3)); 1.71–1.94 (*m*, 2 H–C(2)); 3.03 (*dd*, *J* = 7.5, 9.1, H_a–C(5)); 3.15 (*dd*, *J* = 3.5, 9.1, H_b–C(5)); 3.77 (*s*, 2 MeO); 3.82–3.90 (*m*, H–C(4)); 4.20 (*t*, *J* = 7.4, 2 H–C(1)); 6.80 (*d*, *J* = 9.0, 4 arom. H); 7.18–7.26 (*m*, 3 arom. H); 7.30 (*d*, *J* = 9.1, 4 arom. H); 7.41 (*d*, *J* = 7.2, 2 arom. H); 7.74 (*t*, *J* = 8.4, 2 arom. H); 8.22 (*dd*, *J* = 1.0, 8.4, 2 arom. H); 8.58 (*dd*, *J* = 1.0, 7.2, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 24.32 (C(2)); 30.68 (C(3)); 40.07 (C(1)); 55.11 (2 MeO); 67.42 (C(5)); 70.71 (C(4)); 85.92 (Ar_3C); 113.03, 122.58, 126.66, 126.84, 127.72, 128.04, 129.95, 131.12, 131.49, 133.83, 135.93, 144.79, 158.35 (arom. C); 164.11 (C=O). HR-MALDI-MS: 624.2352 ($[M+Na]^+$, $C_{38}H_{35}NaNO_6^+$; calc. 624.2357).

General Procedure for Synthesis of Phophoramidites **24** and **25**. The DMT-protected compound, **22** or **23**, was mixed with *N,N*-diisopropylammonium tetrazolide (1.5 equiv) in dry CH_2Cl_2 (7 ml). (2-Cyanoethoxy)bis(diisopropylamino)phosphane (1.5 equiv. for **22**, 3 equiv for **23**) was added, and the mixture was stirred under N_2 for 24 h. The solvent was evaporated under reduced pressure, and the residue was purified by CC (silica gel; cyclohexane/AcOEt/Et_3N 49:49:2 (*v/v/v*)).

Phosphoramidite **24**. Yield: 0.076 g (81%). Yellow foam (obtained from 0.070 g (0.119 mmol) of **22**). ^{1}H-NMR ($CDCl_3$): 1.05–1.22 (*m*, 4 Me); 2.04–2.09 (*m*, 2 H–C(2)), 2.39, 2.69 (2*t*, CH_2CN); 3.05–3.32 (*m*, 2 H–C(4)); 3.53–3.72, 3.84–4.02 (2*m*, OCH_2CH_2CN, 2 Me_2CH); 3.78 (2*s*, 2 MeO); 4.13–4.18, 4.31–4.41 (2*m*, 2 H–C(1), H–C(3)); 6.78–6.83 (*m*, 4 arom. H); 7.17–7.29 (*m*, 3 arom. H); 7.35 (*d*, *J* = 8.8, 4 arom. H); 7.47 (*dd*, *J* = 1.0, 8.4, 2 arom. H); 7.74 (*t*, *J* = 7.7, 2 arom. H); 8.19 (*dd*, *J* = 1.0, 8.4, 2 arom. H); 8.58 (*d*, *J* = 6.9, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 20.01 (CH_2CN); 24.42, 24.52, 24.62, 24.72 (4 Me); 32.82 (C(2)); 37.27 (C(1)); 42.99, 43.08 (2 Me_2CH); 55.16 (2 MeO); 58.30 (OCH_2CH_2CN); 66.19 (C(4)); 72.74 (C(3)); 85.98 (Ar_3C); 112.99, 122.79, 126.62, 126.84, 127.67, 128.32, 130.15, 131.09, 131.55, 133.77, 136.80, 144.93, 158.36 (arom. C); 163.92 (C=O). ^{31}P-NMR ($CHCl_3$): 149.46; 149.78. HR-MALDI-MS: 810.3309 ($[M+Na]^+$, $C_{46}H_{50}N_3NaO_7P^+$; calc. 810.3278).

Phosphoramidite **25**. Yield: 0.141 g (39%). Colorless foam (obtained from 0.273 g (0.45 mmol) of **23**). R_f (cyclohexane/AcOEt 1:1) 0.57. ^{1}H-NMR ($CDCl_3$): 0.99–1.15 (*m*, 4 Me); 1.60–1.93 (*m*, 2 H–C(2), 2 H–C(3)); 2.36, 2.60 (2*t*, *J* = 6.8, CH_2CN); 2.94 (*dd*, *J* = 6.6, 8.9, H_a–C(5)); 3.08–3.19 (*m*, H_b–C(5)); 3.45–3.87 (*m*, OCH_2CH_2CN, 2 Me_2CH); 3.76 (*s*, 2 MeO); 4.04–4.08 (*m*, H–C(4)); 4.19–4.23 (*m*, 2 H–C(1)); 6.74–6.79 (*m*, 4 arom. H); 7.14–7.26 (*m*, 3 arom. H); 7.29 (*dd*, *J* = 2.3, 9.1, 4 arom. H); 7.41 (*d*, *J* = 7.2, 2 arom. H); 7.74 (*t*, *J* = 8.4, 2 arom. H); 8.19 (*d*, *J* = 8.4, 2 arom. H); 8.58 (*ddd*, *J* = 1.1, 3.5, 7.2, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 20.22 (CH_2CN); 23.45 (C(2)); 24.38, 24.46, 24.60, 24.69 (4 Me); 30.91 (C(3)); 40.28 (C(1)); 42.88, 43.05 (2 Me_2CH); 55.14 (2 MeO); 58.45 (OCH_2CH_2CN); 65.86 (C(5)); 72.95 (C(4)); 85.86 (Ar_3C); 112.95, 122.71, 126.58, 126.89, 127.63, 128.15, 130.08, 131.09, 131.55, 133.78, 136.18, 144.99, 158.28 (arom. C); 164.05, 164.09 (2 C=O). ^{31}P-NMR ($CDCl_3$): 149.06; 149.60.

ODN and INA Synthesis, Purification, and Measurement of Melting Temperatures. The ODN and INA syntheses were carried out on an *Expedite*™ nucleic acid synthesis system model *8909* from *Applied Biosystems*. The appropriate naphthalimide phosphoramidite (**16a**–**16d**, **24**, and **25**) was dissolved in a 1:1 mixture of dry MeCN and dry CH_2Cl_2, as a 0.1M soln., and inserted into the growing oligonucleotide chain using the same conditions as for normal nucleotide couplings (2-min coupling). The ODNs were

synthesized with Trityl-on and purified by a *Waters Prep LC 4000* HPLC with a *Waters Prep LC* controller and a *Waters 2487 Dual λ* absorbance detector on a *Waters Xterra™ MS C_{18}* column. Buffer *A*: 950 ml of 0.1M NH_4HCO_3 and 50 ml of MeCN, (pH 9.0), and buffer *B*: 250 ml of 0.1 NH_4HCO_3 and 750 ml of MeCN, (pH 9.0). Gradients: 5 min 100% *A*, linear gradient to 70% *B* in 30 min, 2 min. with 70% *B*, linear gradient to 100% *B* in 8 min and then 100% *A* in 15 min (product peak at *ca*. 35 min). The ODNs were DMT-deprotected in 100 µl of 80% AcOH. After 20 min, 100 µl of H_2O, 50 µl of a 3M AcONa, and 600 µl of abs. EtOH were added. The ODNs were kept at $-20°$ overnight, by which precipitation occurred. They were then centrifuged for 15 min upon cooling. The supernatant was decanted off, and the ODNs were dried in a vacuum centrifuge. All modified ODNs were confirmed by MALDI-TOF analysis on a *Voyager Elite Bio* spectrometry research station from *Perceptive Biosystems*.

Melting-temp. measurements were performed on a *Perkin-Elmer Lambda* 20 UV/VIS spectrometer fitted with a *PTP-6* temp. programmer. Melting temp. (T_m) measurements were determined in a 1 mM EDTA, 10 mM $Na_2HPO_4 \cdot 2\ H_2O$, 140 mM NaCl buffer at pH 7.0 for 1.5 µM of each strand. (For mismatch measurements, a concentration of 1.0 µM of each strand was used.) The melting temp. was determined as the maximum of the first derivative plots of the melting curves obtained by measuring the absorbance at 260 nm against increasing temp. (1.0°/min) and is with an uncertainty $\pm 1.0°$ as determined by repetitive experiments.

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