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Intercalating nucleic acids (INAs) containing insertions of 6*H*-indolo[2,3-*b*]quinoxaline

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Abstract—6*H*-Indolo[2,3-*b*]quinoxaline was studied as a covalently bound heteroaromatic intercalator. Six monomers were synthesized and incorporated into DNA oligonucleotides. Through a study of linker length dependence it was concluded that the linker between the oligo and the intercalator must consist of at least five C atoms in order to stabilize a DNA duplex. An intercalator with a 2′-deoxy-D-riboside linker to the oligo could also stabilize a DNA/RNA duplex, while (*S*)-4-(6-methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butane-1,2-diol was able to stabilize both DNA/DNA, DNA/RNA and a DNA/LNA duplex. Mismatch studies revealed a huge sensitivity to the C–C mismatch at the 5′-site of the intercalator.

1. Introduction

The term *intercalation* was first used by Lerman,[1] who had conducted a number of physical studies on interactions of DNA with planar aromatic compounds, to describe the non-covalent binding of a planar polyaromatic molecule between the nucleobases of DNA. When intercalation occurs, the torsion angles in the sugar-phosphodiester backbone of DNA are changed in order to accommodate the intercalating aromatic moiety. This causes an unwinding of the DNA helix with a maximum helix increase of 3.4 Å, which can be measured by viscosity and sedimentation of the DNA solution.[1] The degree of unwinding varies depending on the intercalator and the DNA sequence.[2–4] Normally the helix increase is less than the maximal 3.4 Å, because of other effects such as bending of the duplex around the site of intercalation. X-ray studies on intercalation were first provided by Wang et al.[5] Classical intercalators such as proflavine, acridine, ethidium bromide, daunomycin[6] and actinomycin[7,8] are known for their anti-tumour activity by inhibition of topoisomerases,[9,10] but the group of intercalators has expanded heavily over the years and now includes mono-, bis- and trisintercalators. For recent reviews, see Martinez and Chacón-Garcia,[11] Braña et al.[12] and Graves and Velea.[13]

We have used the term *intercalating nucleic acids* to define an oligonucleotide with an intercalating pseudonucleotide inserted with a covalent bond. Important factors to be considered while using intercalating nucleic acids for hybridization are the structure of the backbone, and the length of the linker and intercalator.[14] We use a vicinal dihydroxy system to incorporate the intercalator as a bulge in the DNA backbone, because it creates a distance between the intercalator and the neighbouring nucleobases of ca. 3.4 Å. Furthermore it introduces additional flexibility into the backbone. Previously we have focused on pyrene as the intercalating moiety and have achieved significant discrimination between DNA and RNA,[15] as the DNA duplex was stabilized while the RNA duplex was destabilized. The fluorescence properties of the pyrene intercalator confirmed its intercalation rather than groove binding in duplexes.[16] These findings led to commercialization of intercalating nucleic acids containing bulge insertions of (*R*)-1-*O*-(1-pyrenylmethyl)glycerol (INA™) in order to exploit the technology in diagnostics, e.g., DNA methylation screening.[17]

For this study we were looking for a heteroaromatic intercalator in order to achieve higher affinity towards ssRNA. Ren et al.[18] had done some studies on the binding affinity of small molecules to an RNA:DNA hybrid using a poly rA:poly dT assay. Among the 84 tested compounds, of which five compounds showed a potential, they found the best one to be Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**1**),[19] a naturally occurring alkaloid, known for its promising anti-tumour activities.[20]

6*H*-Indolo[2,3-*b*]quinoxaline (**2**) can be seen as an aza analogue of Ellipticine. It is a well-examined and

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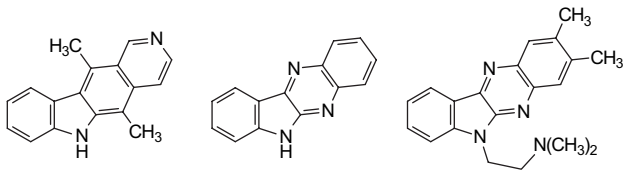


Figure 1. Structures of Ellipticine (**1**), 6*H*-Indolo[2,3-*b*]quinoxaline (**2**) and B-220 (**3**).

well-described compound, primarily by Bergman and co-workers.[21–23] It has nitrogen atoms in the third ring instead of the 5,11-methyl groups in Ellipticine. Several analogues of 6*H*-indolo[2,3-*b*]quinoxaline have been synthesized, e.g., B-220 (2,3-dimethyl-6-(2-dimethylaminoethyl)-6*H*-indolo[2,3-*b*]quinoxaline, **3**), which have shown high antiviral activity[24,25] (Fig. 1).

Ellipticine (**1**) as well as B-220 (**3**) have been used as non-covalent intercalators.[21,22,26–29] Furthermore, Arimondo et al.[30] observed a stronger stabilization of a poly(dAdT)·poly(dAdT) duplex for compound **2** than for its corresponding pyridopyrazino[2,3-*b*]indole analogues. It was therefore of interest to us to test 6*H*-indolo[2,3-*b*]quinoxaline as an intercalating nucleic acid. We also chose indolo[2,3-*b*]-quinoxaline as the intercalator for this study because of its direct synthesis.

In this paper we present the synthesis of six intercalating nucleic acids with indolo[2,3-*b*]quinoxaline as the intercalator (Fig. 2) and their evaluation by thermal stability measurements and fluorescence spectroscopy.

For monomers **4**–**7** the covalently bound linker was attached to N-6, which is the same position as the dimethylaminoethyl side chain that is attached in B-220 (**3**). Monomer **8** has a 2′-deoxy-D-ribose as the connector to the backbone of the DNA strand. It was synthesized in order to evaluate the flexibility of the acyclic linkers versus the natural furanose sugar linkage. The site of linker connection to the intercalator was changed in monomer **9** in order to investigate whether this could lead to better base stacking as the intercalator would be positioned differently in the duplex.

In this study we have investigated the dependence of linker length on the stabilities of DNA/DNA, DNA/RNA and DNA/LNA duplexes by varying the linker.

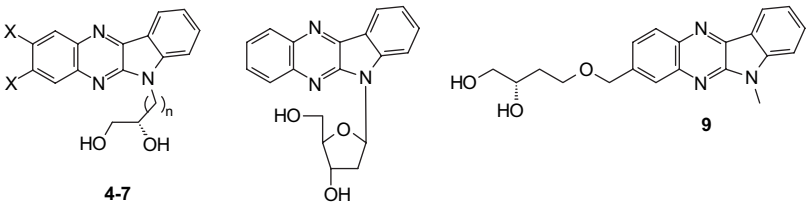
2. Results and discussion

2.1. Chemistry

The commercially available ((*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-yl)-methanol was mesylated in CH_2Cl_2/Et_3N according to the procedure described by Kim et al.[31] to give methanesulfonic acid (*R*)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl ester (**10**).[32] Enantiomerically pure methanesulfonic acid 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethyl ester (**11**)[33] was obtained from (*S*)-malic acid in four steps. (*S*)-Malic acid was converted to dimethyl (*S*)-malate according to Mori and Ikunaka.[34] The diester was reduced with $LiAlH_4$ to (*S*)-1,2,4-butanetriol and protected with an isopropylidene group as described by Hayashi et al.[35] Mesylation of 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethanol with methanesulfonyl chloride was carried out in either pyridine according to Augustyns et al.[33] or CH_2Cl_2/Et_3N according to Kim et al.[31] Using pyridine as the solvent gave **11** in 51% yield, while the latter gave **11** in 99% yield. Enantiomerically pure methanesulfonic acid 3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-propyl ester (**12**) was obtained from L-glutamic acid in four steps. L-Glutamic acid was converted to (*S*)-5-oxotetrahydrofuran-2-carboxylic acid as described by Herdeis.[36] Reduction with $LiAlH_4$ according to Brunner and Lautenschalger[37] yielded (*S*)-1,2,5-pentanetriol. This was protected with an isopropylidene group and mesylated as described above to give **12**.[38]

6*H*-Indolo[2,3-*b*]quinoxaline (**2**) and its 2,3-dichloro derivative (**13**) were easily prepared by condensation of isatin with 1,2-phenylene diamine in glacial acetic acid according to Schunck's method.[39,40] For the alkylation, **2** and **13** were deprotonated (at N-6) with NaH giving red anions. Following the procedure of Cassel et al.[41] they were then reacted with the mesylated alcohols **10** and **11** in refluxing DMF for 48 h in the presence of the phase-transfer catalyst tetra-*n*-butylammonium bromide (TBAB) giving **14b** and **c** in yields of 35–50%. A similar yield of **14d** was achieved without addition of TBAB,[42] whereas this procedure afforded **14a** in 76% yield using a long reaction time of three days.

The alkylation is supposed to take place on N-6 and this molecule will be similar to B-220 (**3**). However, there is a risk of forming an isomer due to alkylation on N-5. Bergman and co-workers[22,43] reported a 4:1 ratio of the 6-substituted versus 5-substituted derivative, by using Knotz's method.[44]



	n	X
4	1	H
5	1	Cl
6	2	H
7	3	H

Figure 2.

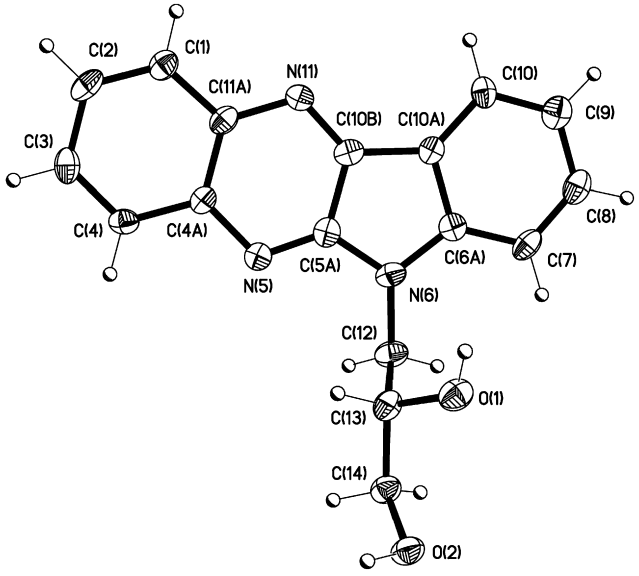
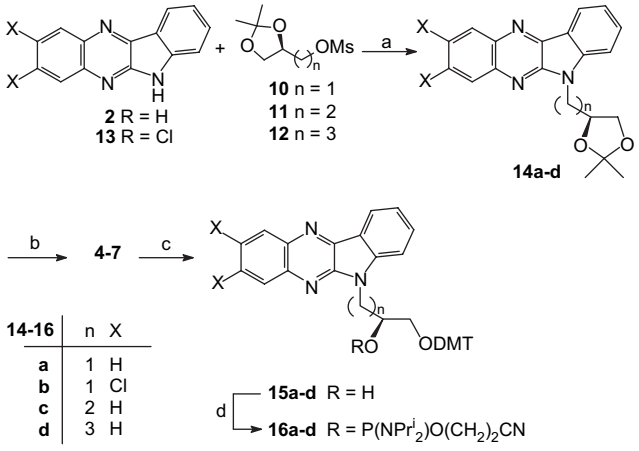


Figure 3. One independent molecule in the asymmetric unit of the X-ray structure of **4**. Displacement ellipsoids are shown at the 30% probability level for non-H atoms.

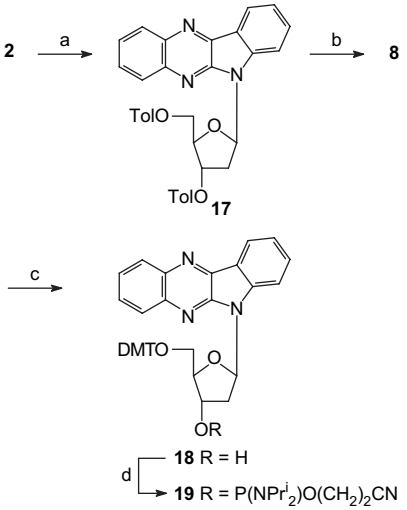
However, we did not isolate the N-5 alkylated isomer. The N-6 alkylation was proven by obtaining a synchrotron X-ray structure of the corresponding alcohol **4**, a step further in the synthetic route (see Figs. 2 and 3 and Scheme 1).



Scheme 1. (a) NaH, TBAB, DMF, 35–76%; (b) AcOH/H_2O (4:1), 67–93%; (c) DMT–Cl, pyr or CH_2Cl_2/Et_3N, 24–83%; (d) NC(CH$_2$)$_2$OP(N^iPr$_2$)$_2$, *N*,*N*-diisopropylammonium tetrazolide, CH_2Cl_2, 61–84%.

Treatment of **14a–d** with 80% aq acetic acid gave the diols **4–7**. These were DMT-protected using DMT–chloride in pyridine or in CH_2Cl_2/Et_3N and then converted to the phosphoramidites **16a–d** using 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane in CH_2Cl_2.

We also synthesized an analogue having a 2′-deoxy-D-riboside as the backbone linker (Scheme 2). 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-pentofuranosyl chloride was prepared according to Rolland et al.[45] and coupled with 6*H*-indolo-[2,3-*b*]quinoxaline (**2**) using NaH in DMF according to the procedure described above.[41] α- and β-Isomers were separated by dissolving the mixture in MeCN by which the β-isomer precipitated. Extraction with $CHCl_3$ isolated the α-isomer. Assignment of α,β-configuration was done by 2D NMR and NOE. Deprotection of the toluoyl groups were carried out using sodium methoxide in methanol as described by Abdel-Megied et al.[46] yielding β-6-(2′-deoxy-ribose)-indolo[2,3-*b*]quinoxaline (**8**). DMT-protection of the diol and conversion to the phosphoramidite (**19**) were carried out as described above.



Scheme 2. (a) 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-pentofuranosyl chloride, NaH, TBAB, DMF, 32%; (b) CH_3ONa, MeOH, 73%; (c) DMT–Cl, CH_2Cl_2/Et_3N, 58%; (d) NC(CH$_2$)$_2$OP(N^iPr$_2$)$_2$, *N*,*N*-diisopropylammonium tetrazolide, CH_2Cl_2, 21%.

Having made **4–7** with three different linker lengths and a sugar moiety (**8**), we also found it interesting to change the connection site of the intercalator to the linker. This was done in order to position the intercalator in a different way inside the duplex and thereby to investigate, whether better base stacking could be obtained. At the same time the linker length was further increased.

6*H*-Indolo[2,3-*b*]quinoxaline-3-carboxylic acid (**20**) was synthesized by condensation of isatin with 3,4-diaminobenzoic acid in glacial acetic acid according to Schunck's method.[39,40] As the diamino compound is not symmetric, there was a possibility of regioisomers. However, we did not isolate the regioisomeric 6*H*-indolo[2,3-*b*]quinoxaline-2-carboxylic acid. The regioselectivity has previously been reported by Varma and Khan.[47] However, we found it more convincing to prove the structure **20** by an X-ray structure of the corresponding alcohol **23**, a few steps further in the synthetic route (see Fig. 4 and Scheme 3).

In order to avoid alkylation on N-6, when alkylating the new site (at the 3 position), the N-6 position was blocked with a methyl group using CH_3I in DMSO in the presence of powdered potassium hydroxide. This yielded a 3:2 mixture of 6-methylindolo[2,3-*b*]quinoxaline-3-carboxylic acid methyl ester (**21**) and the carboxylic acid (**22**). A small sample of the crude product was separated into pure compounds. The rest was reduced to the corresponding alcohol (6-methyl-indolo[2,3-*b*]quinoxalin-3-yl)methanol (**23**) using $LiAlH_4$ in THF. Badger and Nelson[48] reported a 40% yield of the 6-methylated compound and 12% of the 5-methylated by

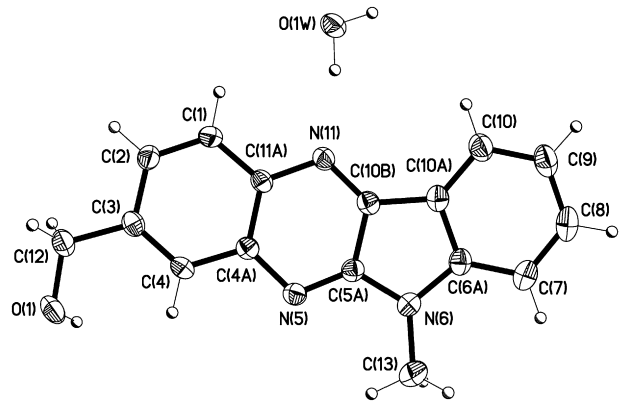
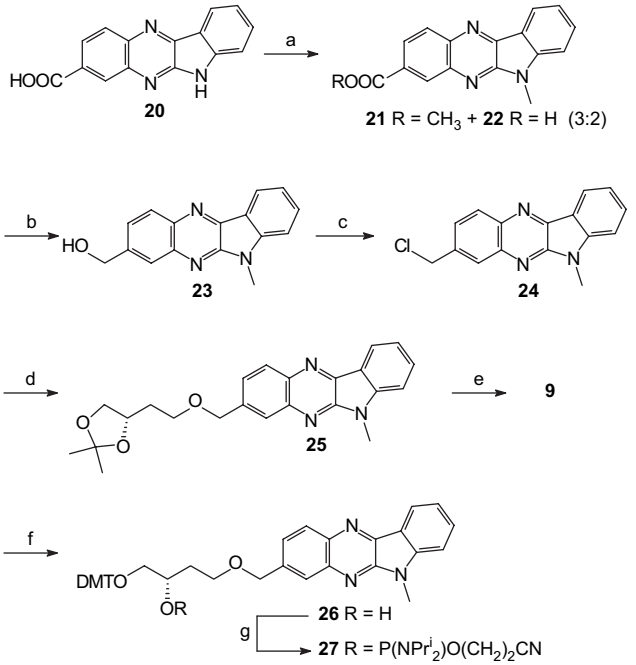


Figure 4. X-ray structure of **23**·H_2O showing displacement ellipsoids at the 50% probability level for non-H atoms.



Scheme 3. (a) CH_3I, KOH, DMSO; (b) $LiAlH_4$, THF, 44% (over two steps); (c) $SOCl_2$, CH_2Cl_2, 89%; (d) (*S*)-2,2-dimethyl-1,3-dioxolane-4-ethanol, KOH, toluene, 67%; (e) AcOH/H_2O (4:1); (f) DMT–Cl, CH_2Cl_2/Et_3N, 55%; (g) NC(CH$_2$)$_2$OP(*N*^{i}Pr$_2$)$_2$, *N*,*N*-diisopropylammonium tetrazolide, CH_2Cl_2, 47%.

using 1 equiv of KOH and CH_3I in ethanol. We used an excess of both KOH and CH_3I and DMSO as solvent, which according to Zegar et al.[22] should give a 95:5 ratio, but we isolated only the 6-methylated compound, which was also proven by the X-ray structure of **23**·H_2O (Fig. 4).

In order to have a more reactive leaving group for the subsequent alkylation reaction, the alcohol **23** was converted to 3-chloromethyl-6-methylindolo[2,3-*b*]quinoxaline (**24**) using thionyl chloride in CH_2Cl_2 according to the procedure described by Bair et al.[49] Following the procedure of Tirosh et al.,[50] **24** was reacted with 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethanol in toluene in the presence of powdered potassium hydroxide to give **25**. Subsequent treatment of **25** with 80% acetic acid as described above, followed by DMT-protection and phosphitylation gave **27** (Scheme 3).

The DMT-protected phosphoramidites of the intercalating nucleic acid monomers **4**–**9** were incorporated into two different DNA oligonucleotides using the same coupling times (2 min) in the oligo synthesis as was used for the amidites of natural nucleosides. The monomers **4**–**9** were inserted as a bulge in a 12-mer highly conserved HIV-1 strand,[51] which was modified around the site of intercalation (C-G switch) according to Christensen et al.[14,15] They were also inserted as a bulge or as an end-positioned intercalating pseudonucleotide in an 11-mer oligonucleotide earlier described by Filichev et al.[52] All modified oligonucleotides were confirmed by MALDI-TOF analysis with a variation of m/z ±3.

2.2. Thermal melting studies

The project was designed to study the linker length dependence of the intercalating nucleic acids. As it is seen from Table 1, intercalators with short linkers (**4**–**6**) were destabilizing the DNA duplex, whereas stabilization was obtained when a longer linker (**7**) or a sugar moiety (**8**) is used. Introduction of chloro substituents in the 2 and 3 positions of the 6*H*-indolo[2,3-*b*]quinoxaline (**5**) gave a marginal improvement compared to **4**. Highest stabilization was obtained for **9**, which had the longest linker, and the linker attached to C-3 instead of N-6. It is believed that the shorter linker is unable to position the intercalator optimally for base stacking in the duplex without disturbance of the backbone.

In comparison with previous studies[14] of pyrene and anthracene intercalators inserted in the same sequence and with the same linker length, 6*H*-indolo[2,3-*b*]quinoxaline (**9**) gives a higher ΔT_m (+4.5 °C vs +2.5 °C for pyrene and −1.3 °C for anthracene).

Shifting the intercalator to the complementary strand (Table 1, bottom) was expected to give higher T_m as better stacking is normally obtained when the intercalator is neighbouring two guanines in the same strand rather than two cytosines. This was also observed with the linkers **4**–**7**. However, the opposite effect is the case, when the acyclic linker is replaced with the 2-deoxyribose (**8**) or the elongated

Table 1. Melting temperatures of duplexes with **4**, **5**, **6**, **7**, **8** and **9** inserted as a bulge

	X=	—	**4**	**5**	**6**	**7**	**8**	**9**
	Sequences	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)
ODN1	5′-AGCTTG–GTTGAG-3′							
ODN2	3′-TCGAAC*X*CAACTC-5′	49.6	−9.0	−8.3	−1.0	+1.7	+4.0	+4.5
ODN3	5′-AGCTTG*X*GTTGAG-3′							
ODN4	3′-TCGAAC–CAACTC-5′	49.6	−6.0	−5.7	−1.9	+4.3	+2.2	−0.9

ΔT_m is the difference in T_m between duplexes with an intercalator (*X*) inserted and the corresponding unmodified duplex.

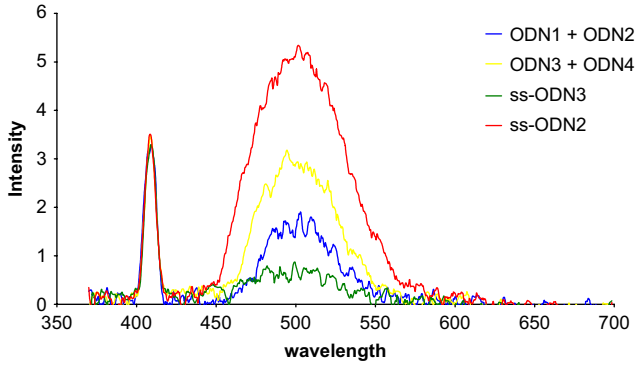


Figure 5. Fluorescence spectra of **9** inserted in **ODN2** and **ODN3**.

linker/intercalator system **9**. For **9**, a destabilization of the duplex was even observed contrary to what is seen, when the linker is connected to N-6 and when pyrene is used as the intercalator.[14] For the same linker length, shifting the pyrene intercalator to the complementary strand resulted in further stabilization of the duplex (ΔT_m=3.6 °C).[14] With the linker attached to C-3 of indolo[2,3-*b*]quinoxaline, the fourth ring of the intercalator can reach deeply into the duplex, which is not the case with pyrene because of its shape. It is thus anticipated that the intercalator **9** is placed nearer to the nucleobases of the complementary strand than those of its 'own' strand and in this way it is capable of stacking to guanines in the opposite strand in a better way.

This hypothesis is supported by fluorescence spectroscopy (Fig. 5). Weak fluorescence was observed for the ss-DNA containing insertions of **9** between two guanines. When **9** was inserted between two cytosines in the ss-DNA, the fluorescence was stronger. Upon hybridization to the complementary strand, the monomer fluorescence of **9** inserted between the two cytosines was quenched. To the contrary, enhancement of fluorescence was observed when the ss-DNA containing insertions of **9** between two guanines was hybridized to the complementary strand. This implies that the intercalator in the first case makes base stacking with the neighbouring guanines in the opposite strand, which also leads to stabilization of the duplex.

The intercalators were also placed opposite to each other in the two strands of the duplex in a zipping manner (Table 2). We observed a significant difference between **4** and **5** as the 2,3-dichloro substituted **5** was destabilizing the duplex by −4.4 °C, while **4** gave a destabilization of −11.5 °C. Compound **7** was marginally stabilizing the duplex. The most important result was obtained with the 2′-deoxyribose linkage **8**, which gave a significant stabilization. This result is remarkable, because previous studies[53] on two pyrenes directly opposing each other in the same duplex sequence resulted in destabilization. This has also been the case in other studies with INA[54] with two pyrenes opposing each other in a slightly different DNA sequence. The stabilization of **8** is most likely due to zipping of the two indolo[2,3-*b*]quinoxaline intercalating moieties. Also **9** was stabilizing the duplex when two intercalating moieties were inserted opposite to each other. In this case zipping may be facilitated by the longer linker.

For further investigation on indolo[2,3-*b*]quinoxaline as an intercalator, monomers **4**–**9** were incorporated into a 11-mer oligonucleotide[52] either in a region of A·T base pairs in the middle of the strand or as an end positioned pseudonucleotide. Thermal stability measurements were made against DNA, RNA and LNA[55] strands (Table 3).

Also for this sequence the dependence on linker length was obvious. When incorporated in the middle of the sequence, **4**, **5** and **6** destabilized the DNA/DNA duplex. A linker with n=3 (**7**) was necessary in order to achieve stabilization. The RNA/DNA duplex stabilization was only observed for the deoxyribose linked intercalators **8** and **9** with the longest linker attached to C-3.

Incorporation of 5′-end pseudonucleotide gave stabilization to all the duplexes and for all the investigated linkers. It is seen that the chloro substituents do not lead to further stabilization of the duplex when compared with **4**. However, it is noteworthy that **8**, which gives the highest T_m when incorporated into the middle of the DNA duplex, gives a lower T_m than **7** and **9** when incorporated at the 5′-end of a duplex. This might be due to the greater flexibility of the acyclic linker.

For the affinity studies towards LNA, we did not use a fully modified duplex but incorporated three thymines with LNA (T^L) into a DNA duplex. LNA locks the sugar ring in a north-type conformation, resulting in an A-type duplex, which is typically seen for RNA/RNA duplexes. If the duplex is not fully modified with LNA monomers, there will only be A-type duplex around the insertions. In the rest of the duplex—away from LNA monomers—there will be the B-type structure like in DNA/DNA duplexes. However, in this case, with three LNA insertions evenly distributed, it is reasonable to anticipate an A-type structure of the duplex as revealed by NMR structure determination of a similar LNA/DNA duplex.[56,57] Except for **9** all intercalating nucleic acid monomers destabilized the DNA/LNA duplex when they were inserted as a bulge in the middle of the sequence. It is interesting to observe the discrimination of **7** and **8** among DNA/LNA, DNA/RNA and DNA/DNA duplexes. Compound **7** stabilized the DNA/DNA duplex, but destabilized the DNA/LNA and DNA/RNA duplexes, even though the latter was only marginally destabilized (ΔT_m=−0.7 °C).

Table 2. Melting temperatures of duplexes with zipping insertions of **4**, **5**, **7**, **8** and **9**

	X=	—	**4**	**5**	**7**	**8**	**9**
	Sequences	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)
ODN3	5′-AGCTTG*X*GTTGAG-3′						
ODN2	3′-TCGAAC*X*CAACTC-5′	49.6	−11.5	−4.4	+0.3	+9.7	+6.6

ΔT_m is the difference in T_m between duplexes with an intercalator (*X*) inserted and the corresponding unmodified duplex.

Table 3. Comparison of DNA/DNA, DNA/RNA and DNA/LNA duplexes with **4**, **5**, **7**, **8** and **9** inserted as a bulge or at the end of the duplex

X=	—	**4**	**5**	**6**	**7**	**8**	**9**
Sequences	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)
5′-TGTGAT–ATGCT-3′ 3′-ACACTA*X*TACGA-5′	42.4	−9.9	−6.8	−1.9	2.4	4.6	3.4
5′-TGTGATATGCT-3′ 3′-ACACTATACGA*X*-5′	42.4	2.4	2.4	—	3.8	3.4	4.6
5′-UGUGAU–AUGCU-3′ 3′-ACACTA*X*TACGA-5′	39.5	−13.4	−11.8	—	−0.7	1.8	2.3
5′-UGUGAUAUGCU-3′ 3′-ACACTATACGA*X*-5′	39.5	0.7	0.4	—	1.7	1.5	1.6
5′-TGT^{L}GAT^L–AT^{L}GCT-3′ 3′-ACA CTA *X*TA CGA-5′	56.7	−15.6	−16.9	−9.9	−4.4	−4.6	1.6
5′-TGT^{L}GAT^L–AT^{L}GCT-3′ 3′-ACA CTA TAC GA*X*-5′	56.7	2.1	2.9	—	3.5	3.1	4.6

T^L denotes locked nucleotide of thymine. ΔT_m is the difference in T_m between duplexes with an intercalator (*X*) inserted and the corresponding unmodified duplex.

Table 4. Melting temperatures of mismatched sequences with **4**, **7**, **8** and **9** inserted as a bulge

Sequence: 5′-AGC TTZ *Y*TT GAG-3′
3′-TCG AAC *X* CAACTC-5′

	X=		—		**4**		**7**		**8**		**9**	
	Z	*Y*	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)
Wild type	G	G	49.6		40.6		51.3		53.6		54.1	
Mut. 1	G	C	21.7	−27.9	17.9	−22.7	20.4	−30.9	22.3	−31.3	37.1	−17.0
Mut. 2	G	A	33.6	−16.0	28.3	−12.3	31.6	−19.7	34.7	−18.9	38.5	−15.6
Mut. 3	G	T	32.4	−17.2	28.4	−12.2	30.5	−20.8	33.9	−19.7	40.2	−13.9
Mut. 4	C	G	28.2	−21.4	30.3	−10.3	35.3	−16.0	37.3	−16.3	37.2	−16.9
Mut. 5	A	G	31.1	−18.5	30.5	−10.1	36.4	−14.9	38.4	−15.2	38.4	−15.7
Mut. 6	T	G	32.2	−17.4	30.5	−10.1	37.2	−14.1	39.7	−13.9	38.1	−16.0

ΔT_m is the difference in T_m between the matched sequence and the mismatched.

Compound **8** stabilized both DNA/DNA and DNA/RNA duplexes, but destabilized the DNA/LNA duplex. Compound **9**, which was stabilizing the investigated DNA/DNA, DNA/RNA and DNA/LNA duplexes, is currently being examined for stabilization of Three Way Junctions (TWJ).

Finally mismatch studies were carried out (Table 4). The specificity for hybridization was measured by the difference in the melting temperature between the fully complementary duplex and the duplex where one mismatch has been introduced. In general, when a mismatch was introduced at the 3′-site of the intercalator, this proved to be less sensitive than the unmodified oligo, while a greater sensitivity was observed when the mismatch was introduced at the 5′-site of the intercalator. However, for the C–C mismatch at the 5′-site of the intercalator, monomers **7** and **8** caused a drop in melting temperatures up to ca. 30 °C.

3. Conclusion

From the study of linker length dependence we conclude that the linker must, as a minimum, have a length corresponding to **7**, when used as a covalently bound intercalator. Shorter linkers destabilize the duplexes as they are not able to position the intercalator optimally for base stacking without disturbing the backbone. Introduction of chloro substituents in the 2,3 positions of 6*H*-indolo[2,3-*b*]quinoxaline did not increase stabilization significantly and therefore 2,3-dichloro-6*H*-indolo[2,3-*b*]quinoxaline was not coupled to any of the longer linkers.

In comparison studies of a DNA/DNA, DNA/RNA and a DNA/LNA duplex the linker length dependence was also obvious. When inserted in an A·T region in the middle of a 11-mer sequence, only **9** could stabilize DNA/LNA, while **8** and **9** stabilized DNA/RNA. Stabilization of a DNA/DNA duplex could be achieved using **7**, **8** or **9**.

In mismatch studies we found the intercalator to be less sensitive to mismatch at the 3′-site than observed for the unmodified duplex and more sensitive to the 5′-site of the intercalator. With insertions of the 6*H*-indolo[2,3-*b*]quinoxaline containing monomers **7** and **8** the maximum drop in melting temperature was ca. 30 °C for a C–C mismatch.

4. Experimental

4.1. General

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 121.5 MHz for ^{31}P with TMS as an internal standard for ^{1}H NMR, deuterated solvents $CDCl_3$ (δ 77.00), CD_2Cl_2 (δ 53.80), CD_3OD (δ 49.00), DMSO (δ 39.44) for ^{13}C NMR,

and 85% H_3PO_4 as an external standard for ^{31}P NMR. MALDI mass spectra were recorded on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Ionspec, Irvine, CA). For accurate ion mass determinations, the $[M+H]^+$ or $[M+Na]^+$ ion was peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. IR spectra were recorded on a Perkin–Elmer 1720 Infrared Fourier Transform Spectrometer. Melting points were determined on a Büchi melting point apparatus. Silica gel (0.040–0.063 mm) used for column chromatography and analytical silica gel TLC plates 60 F_{254} were purchased from Merck. UV-light or a stain of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O/Ce_2(SO_4)_3$ (50:1) in 5% sulfuric acid was used for visualization. Solvents used for column chromatography were distilled prior to use, while reagents were used as purchased. Petroleum ether (PE): bp 60–80 °C. NMR assignment follows standard nucleoside style, that is the carbon next to the intercalator is assigned C-1′.

4.2. General procedure for alkylation of 2 and 13, method A[41]

NaH (60% in oil, 1.5 equiv) was added portionwise to a solution of 6*H*-indolo[2,3-*b*]quinoxaline[39,40] (**2**, 0.139 g, 0.63 mmol) or 2,3-dichloro-6*H*-indolo-[2,3-*b*]quinoxaline[58] (**13**, 0.250 g, 0.87 mmol) in dry DMF (40 mL). After stirring at rt for 30 min, TBAB (0.2 equiv) was added and the reaction mixture was stirred for additional 30 min. The mesylated alcohol **10** or **11** (2 equiv) was added dropwise. The reaction mixture was stirred at 140 °C for 48 h. After cooling, DMF was evaporated off under reduced pressure. The residue was treated with water and extracted three times with $CHCl_3$. The combined organic phases were dried ($MgSO_4$) and the solvent evaporated off. The product was purified by silica gel column chromatography using EtOAc/PE (25:75 v/v) as an eluent.

4.2.1. 2,3-Dichloro-6-((*S*)-2,2-dimethyl-1,3-dioxolan-4-ylmethyl)-6*H*-indolo[2,3-*b*]quinoxaline (14b). Yield: 0.122 g (35%) as a yellow solid, R_f 0.94 (EtOAc/PE 2:3), mp 131 °C. ^1H NMR ($CDCl_3$): δ 1.32, 1.38 (2×s, 6H, 2×CH_3), 3.97 (dd, 1H, *J*=5.5 Hz, 8.6 Hz, H-3′), 4.15 (dd, 1H, *J*=5.5 Hz, 8.6 Hz, H-3′), 4.52 (dd, 1H, *J*=5.5 Hz, 14.6 Hz, H-1′), 4.61 (dd, 1H, *J*=5.5 Hz, 14.6 Hz, H-1′), 4.69 (quintet, 1H, *J*=5.5 Hz, H-2′), 7.40 (t, 1H, *J*=7.4 Hz, H_{arom}), 7.61–7.74 (m, 2H, H_{arom}), 8.19 (s, 1H, H_{arom}), 8.36 (s, 1H, H_{arom}), 8.39 (d, 1H, *J*=7.9 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.17, 26.70 (2×CH_3), 44.49 (C-1′), 67.24 (C-3′), 74.36 (C-2′), 109.10 ($C(CH_3)_2$), 110.70, 119.15, 121.64, 122.81, 128.29, 131.62, 132.87, 138.10, 139.18, 145.23 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{20}H_{18}Cl_2N_3O_2^+$ (MH^+): 402.0770, found 402.0783.

4.2.2. 6-[2-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl]-6*H*-indolo[2,3-*b*]quinoxaline (14c). Yield: 0.110 g (50%) as a yellow oil, R_f 0.76 (EtOAc/PE 1:4). ^1H NMR ($CDCl_3$): δ 1.32 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.13–2.25 (m, 2H, H-2′), 3.57 (dd, 1H, *J*=7.1 Hz, 8.0 Hz, H-4′), 4.01 (dd, 1H, *J*=6.0 Hz, 8.0 Hz, H-4′), 4.11–4.19 (m, 1H, H-3′), 4.52–4.70 (m, 2H, H-1′), 7.37 (m, 1H, H_{arom}), 7.56 (d, 1H, *J*=8.2 Hz, H_{arom}), 7.64–7.77 (m, 3H, H_{arom}), 8.11 (dd, 1H, *J*=1.2 Hz, 8.2 Hz, H_{arom}), 8.29 (dd, 1H, *J*=1.2 Hz, 8.2 Hz, H_{arom}), 8.46 (d, 1H, *J*=7.2 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.57, 27.05 (2×CH_3), 32.60 (C-2′), 38.36 (C-1′), 69.18 (C-4′), 73.47 (C-3′), 109.10 ($C(CH_3)_2$), 109.54, 119.42, 120.91, 122.66, 125.97, 127.75, 128.71, 129.32, 130.94, 139.29, 140.08, 140.52, 144.45, 145.47 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{21}H_{22}N_3O_2^+$ (MH^+): 348.1706, found 348.1705.

4.3. General procedure for alkylation of 2 and 13, method B[42]

NaH (60% in oil, 1.2 equiv) was added in two portions to a solution of 6*H*-indolo-[2,3-*b*]quinoxaline[39,40] (**2**, 0.230 g, 1 mmol) in dry DMF (20 mL). The reaction mixture was heated to 80 °C for 30 min, before the mesylated alcohol **10** (0.405 g, 2 mmol, 2 equiv) or **12** (0.250 g, 1 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 80 °C overnight (**14d**) or for three days (**14a**). The mixture was partitioned between CH_2Cl_2 and brine. The organic phase was dried ($MgSO_4$), and the solvent evaporated by co-evaporation with dry xylene. The product was purified by silica gel column chromatography using EtOAc/PE (25:75 v/v) as an eluent.

4.3.1. 6-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-ylmethyl)-6*H*-indolo[2,3-*b*]quinoxaline (14a). Yield: 0.268 g (76%) as a yellow solid, R_f 0.44 (EtOAc/PE 1:1), mp 135 °C. ^1H NMR ($CDCl_3$): δ 1.33 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 4.00 (dd, 1H, *J*=5.5 Hz, 8.6 Hz, H-3′), 4.13 (dd, 1H, *J*=6.0 Hz, 8.6 Hz, H-3′), 4.54 (dd, 1H, *J*=5.5 Hz, 14.4 Hz, H-1′), 4.65 (dd, 1H, *J*=5.5 Hz, 14.4 Hz, H-1′), 4.70 (quintet, 1H, *J*=5.5 Hz, H-2′), 7.38 (m, 1H, H_{arom}), 7.62–7.78 (m, 4H, H_{arom}), 8.11 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.31 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.46 (dd, 1H, *J*=0.7 Hz, 7.8 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.20, 26.68 (2×CH_3), 44.31 (C-1′), 67.32 (C-3′), 74.49 (C-2′), 109.76 ($C(CH_3)_2$), 110.41, 119.47, 121.14, 122.53, 126.13, 127.75, 128.50, 129.25, 130.90, 139.40, 139.97, 140.43, 144.89, 145.80 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{20}H_{19}N_3O_2Na^+$ (MNa^+): 356.1369, found 356.1377.

4.3.2. 6-[3-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)propyl]-6*H*-indolo[2,3-*b*]quinoxaline (14d). Yield: 0.132 g (35%) as a yellow oil. ^1H NMR ($CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.63–1.73 (m, 2H, H-3′), 2.02–2.10 (m, 2H, H-2′), 3.47 (t, 1H, *J*=8.0 Hz, H-5′), 3.99 (dd, 1H, *J*=5.9 Hz, 8.0 Hz, H-5′), 4.14–4.19 (quintet, 1H, *J*=5.9 Hz, H-4′), 4.52 (dd, 2H, *J*=6.7 Hz, 13.4 Hz, H-1′), 7.38 (t, 1H, *J*=′7.6 Hz, H_{arom}), 7.47 (d, 1H, *J*=8.3 Hz, H_{arom}), 7.64–7.77 (m, 3H, H_{arom}), 8.12 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.29 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.47 (d, 1H, *J*=7.6 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 24.83 (C-2′), 25.64, 26.90 (2×CH_3), 30.66 (C-3′), 41.12 (C-1′), 69.23 (C-5′), 75.50 (C-4′), 108.84 ($C(CH_3)_2$), 109.41, 119.46, 120.82, 122.72, 125.91, 127.75, 128.68, 129.29, 130.90, 139.25, 139.93, 140.54, 144.24, 145.58 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{22}H_{23}N_3O_2Na^+$ (MNa^+): 384.1682, found 384.1691.

4.4. General procedure for isopropylidene deprotection

Compounds **14a–d** were stirred in acetic acid/H_2O (4:1, 50 mL) at rt overnight. The solvent was evaporated under reduced pressure.

4.4.1. (*S*)-3-(Indolo[2,3-*b*]quinoxalin-6-yl)-propane-1,2-diol[59] (4). Yield: 0.194 g (93%) as yellow crystals (obtained from 0.237 g, 0.71 mmol of **14a**), R_f 0.32 (4% MeOH/CH_2Cl_2), mp 215 °C. IR (KBr): 3338 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.52 (d, 2H, J=5.3 Hz, H-3′), 4.14 (m, 1H, H-2′), 4.45 (dd, 1H, J=7.8 Hz, 14.4 Hz, H-1′), 4.56 (dd, 1H, J=4.4 Hz, 14.4 Hz, H-1′), 4.89 (br s, 1H, OH), 5.03 (br s, 1H, OH), 7.41 (t, 1H, J=7.7 Hz, H_{arom}), 7.71–7.86 (m, 4H, H_{arom}), 8.12 (dd, 1H, J=8.3 Hz, 1.1 Hz, H_{arom}), 8.28 (dd, 1H, J=8.3 Hz, 1.1 Hz, H_{arom}), 8.38 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 44.93 (C-1′), 63.96 (C-3′), 69.60 (C-2′), 111.14, 118.54, 120.77, 121.91, 125.94, 127.50, 128.88, 129.04, 131.11, 138.58, 139.63, 139.89, 145.09, 145.44 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{17}H_{15}N_3O_2Na^+$ (MNa^+): 316.1056, found 316.1054.

4.4.2. (*S*)-3-(2,3-Dichloro-indolo[2,3-*b*]quinoxalin-6-yl)-propane-1,2-diol (5). Yield: 0.080 g (92%) as a yellow solid (obtained from 0.097 g, 0.24 mmol of **14b**), R_f 0.50 (5% MeOH/$CHCl_3$), mp 212 °C. ^1H NMR (DMSO-d_6): δ 3.50 (t, 2H, J=5.4 Hz, H-3′), 4.07–4.12 (m, 1H, H-2′), 4.41 (dd, 1H, J=7.9 Hz, 14.2 Hz, H-1′), 4.51 (dd, 1H, J=4.2 Hz, 14.2 Hz, H-1′), 4.83 (t, 1H, J=5.5 Hz, OH), 4.97 (d, 1H, J=5.3 Hz, OH), 7.42 (m, 1H, H_{arom}), 7.80 (m, 3H, H_{arom}), 8.33–8.36 (m, 2H, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 44.49 (C-1′), 63.91 (C-3′), 69.44 (C-2′), 111.43, 118.01, 121.28, 122.28, 128.10, 129.58, 131.92, 137.13, 138.85, 145.19 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{17}H_{14}Cl_2N_3O_2^+$ (MH^+): 362.0457, found 362.0472.

4.4.3. (*S*)-4-(Indolo[2,3-*b*]quinoxalin-6-yl)-butane-1,2-diol (6). Yield: 0.065 g (67%) as a yellow oil (obtained from 0.110 g, 0.32 mmol of **14c**), R_f 0.20 (EtOAc/PE 1:4). ^1H NMR (DMSO-d_6): δ 1.74–1.87 (m, 1H, H-2′), 2.07–2.18 (m, 1H, H-2′), 3.26–3.57 (m, 3H, H-3′, H-4′), 4.50–4.67 (m, 2H, H-1′), 4.83 (br s, 2H, 2×OH), 7.42 (m, 1H, H_{arom}), 7.71–7.86 (m, 4H, H_{arom}), 8.14 (dd, 1H, J=8.4 Hz, 1.1 Hz, H_{arom}), 8.27 (dd, 1H, J=8.4 Hz, 1.1 Hz, H_{arom}), 8.39 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 32.22 (C-2′), 38.26 (C-1′), 65.81 (C-4′), 69.04 (C-3′), 110.43, 118.54, 120.88, 122.18, 125.99, 127.51, 128.95, 129.08, 131.37, 138.59, 139.56, 139.93, 144.35, 144.90 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{18}H_{18}N_3O_2^+$ (MH^+): 308.1393, found 308.1395.

4.4.4. (*S*)-5-(Indolo[2,3-*b*]quinoxalin-6-yl)-pentane-1,2-diol (7). Yield: 0.103 g (88%) as yellow crystals (obtained from 0.132 g, 0.37 mmol of **14d**). ^1H NMR (CD_3OD): δ 1.30–1.39 (m, 1H, H-3′), 1.44–1.50 (m, 1H, H-3′), 1.79–1.94 (m, 2H, H-2′), 3.20–3.30 (m, 2H, H-5′), 3.52 (m, 1H, H-4′), 4.25 (m, 2H, H-1′), 7.11–8.14 (m, 8H, H_{arom}). ^{13}C NMR (CD_3OD): δ 25.78 (C-2′), 31.61 (C-3′), 42.32 (C-1′), 67.23 (C-5′), 72.80 (C-4′), 111.03, 119.77, 122.04, 123.43, 127.18, 128.47, 129.44, 129.94, 132.53, 139.48, 140.67, 141.44, 145.71, 146.50 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{19}H_{19}N_3O_2Na^+$ (MNa^+): 344.1369, found 344.1363.

4.4.5. 6-(2′-Deoxy-3′,5′-di-*O*-(*p*-toluoyl)-β-D-ribofuranosyl)-indolo[2,3-*b*]quinoxaline (17). NaH (60% in oil, 0.274 g, 6.8 mmol, 1.5 equiv) was added portionwise to a solution of 6*H*-indolo[2,3-*b*]quinoxaline (**2**, 1.0 g, 4.6 mmol, 1 equiv) in dry DMF (40 mL) under N_2. After stirring at rt for 30 min, TBAB (0.30 g, 0.9 mmol, 0.2 equiv) was added and the reaction mixture was stirred for another 30 min. 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-pentofuranosyl chloride (1.64 g, 4.2 mmol, 0.9 equiv) was added dropwise. The reaction mixture was stirred at rt for 48 h. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/PE (40:60 v/v) as an eluent. α- and β-Isomers were separated by dissolving the mixture in MeCN by which the β-isomer precipitated. Extraction with $CHCl_3$ isolated the α-isomer.

Yield: 0.77 g (32%) as a yellow solid. ^1H NMR ($CDCl_3$): δ 2.40, 2.46 (2×s, 6H, 2×CH_3), 2.65 (ddd, 1H, J=14.3 Hz, 6.6 Hz, 2.9 Hz, H-2′), 3.76 (dt, J=14.3 Hz, 6.6 Hz, H-2″), 4.65 (q, 1H, J=3.7 Hz, H-4′), 4.74 (dd, 1H, J=11.9 Hz, 4.3 Hz, H-5′), 4.94 (dd, 1H, J=11.9 Hz, 3.7 Hz, H-5′), 6.08 (dt, 1H, J=7.4 Hz, 6.6 Hz, H-3′), 7.10 (t, 1H, J=6.6 Hz, H-1′), 7.17 (d, 2H, J=8.0 Hz, H_{arom}), 7.31 (d, 2H, J=8.0 Hz, H_{arom}), 7.35–7.42 (m, 2H, H_{arom}), 7.68–7.80 (m, 3H, H_{arom}), 7.94 (d, 2H, J=8.0 Hz, H_{arom}), 8.05 (d, 2H, J=8.0 Hz, H_{arom}), 8.16 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.29 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.46 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 21.68, 21.74 (2×CH_3), 34.73 (C-2′), 63.95 (C-5′), 74.64 (C-3′), 81.42 (C-4′), 83.43 (C-1′), 111.61, 120.33, 121.83, 122.72, 126.66, 126.73, 126.92, 128.05, 129.10, 129.12, 129.26, 129.69, 129.84, 130.94, 139.61, 140.02, 142.85, 143.87, 144.31, 145.18 (C_{arom}), 166.15, 166.29 (2×C═O). HRMS (MALDI): m/z calcd for $C_{35}H_{29}N_3O_5Na^+$ (MNa^+): 594.1999, found 594.2005.

4.4.6. 6-(2-Deoxy-β-D-ribofuranosyl)-indolo[2,3-*b*]quinoxaline (8). Sodium (0.056 g, 1 mmol) was dissolved in methanol (20 mL), and **17** (0.383 g, 0.67 mmol) was added. The reaction mixture was stirred at rt for three days. The solution was neutralized with NH_4Cl (s), and stirred for another 30 min. The mixture was filtered and product was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_2 as an eluent.

Yield: 0.165 g (73%) as a yellow solid, R_f 0.35 (8% MeOH/CH_2Cl_2), mp 226 °C. IR (KBr): 3368 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.23 (ddd, 1H, J=13.0 Hz, 6.3 Hz, 2.5 Hz, H-2′), 3.04–3.14 (m, 1H, H-2″), 3.72 (dd, 1H, J=11.6 Hz, 4.5 Hz, H-5′), 3.82 (dd, 1H, J=11.6 Hz, 3.9 Hz, H-5″), 3.99 (q, 1H, J=3.9 Hz, H-4′), 4.61–4.64 (m, 1H, H-3′), 5.23 (br s, 1H, OH), 5.42 (br s, 1H, OH), 7.01 (dd, 1H, J=8.7 Hz, 6.3 Hz, H-1′), 7.46 (t, 1H, J=7.7 Hz, H_{arom}), 7.72–7.88 (m, 3H, H_{arom}), 8.09 (dd, 2H, J=8.3 Hz, 1.2 Hz, H_{arom}), 8.28 (dd, 1H, J=8.3 Hz, 1.2 Hz, H_{arom}), 8.40 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 36.91 (C-2′), 61.76 (C-5′), 70.81 (C-3′), 82.92 (C-4′), 87.10 (C-1′), 112.72, 119.28, 121.64, 122.10, 126.63, 127.44, 128.98, 129.20, 131.32, 138.78, 139.32, 139.79, 142.71, 144.61 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{19}H_{17}N_3O_3Na^+$ (MNa^+): 358.1162, found 358.1151.

4.5. General procedure for DMT-protection of diols 4–8

The diols were dissolved in either dry pyridine (20 mL) (**5** and **6**) or CH_2Cl_2 (20 mL) and Et_3N (1 mL) (**4**, **7** and **8**), and DMT–chloride (1.1–1.5 equiv) were added. The

reaction mixture was stirred at rt for 36 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc/cyclohexane/Et_3N (33:65:2 v/v/v) as an eluent.

4.5.1. (*S*)-(4,4′-Dimethoxytriphenylmethyloxy)-3-indolo-[2,3-*b*]quinoxalin-6-yl-propan-2-ol (15a). Yield: 0.147 g (44%) as a yellow oil (obtained from 0.164 g, 0.56 mmol of **4**). ^{1}H NMR ($CDCl_3$): δ 2.81 (br s, 1H, OH), 3.16 (dd, 1H, J=6.7 Hz, 9.5 Hz, H-3′), 3.37 (dd, 1H, J=5.1 Hz, 9.5 Hz, H-3′), 3.73, 3.78 (2×s, 6H, 2×OCH_3), 4.39 (m, 1H, H-2′), 4.57 (dd, 1H, J=6.0 Hz, 14.6 Hz, H-1′), 4.64 (dd, 1H, J=3.7 Hz, 14.6 Hz, H-1′), 6.72 (d, 1H, J=8.7 Hz, H_{arom}), 6.81 (d, 4H, J=9.1 Hz, H_{arom}), 7.14–7.40 (m, 9H, H_{arom}), 7.48–7.72 (m, 4H, H_{arom}), 8.01 (dd, 1H, J=8.2 Hz, 1.3 Hz, H_{arom}), 8.25 (dd, 1H, J=8.2 Hz, 1.3 Hz, H_{arom}), 8.41 (d, 1H, J=7.6 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 46.19 (C-1′), 55.14 (2×OCH_3), 64.91 (C-3′), 70.32 (C-2′), 86.42 (C_{DMT}), 110.05, 113.03, 113.12, 119.38, 121.12, 122.50, 126.12, 126.77, 127.02, 127.43, 127.78, 127.99, 129.10, 129.24, 129.93, 130.96, 135.68, 135.81, 139.20, 139.62, 140.15, 144.71, 144.82, 145.94, 158.42, 158.59 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{38}H_{33}N_3O_4Na^+$ (MNa^+): 618.2363, found 618.2379.

4.5.2. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-3-(2,3-dichloro-indolo[2,3-*b*]quinoxalin-6-yl)-propan-2-ol (15b). Yield: 0.200 g (62%) as yellow foam (obtained from 0.175 g, 0.48 mmol of **5**). ^{1}H NMR ($CDCl_3$): δ 3.01 (br s, 1H, OH), 3.24 (dd, 1H, J=6.1 Hz, 9.7 Hz, H-3′), 3.37 (dd, 1H, J=5.1 Hz, 9.7 Hz, H-3′), 3.76, 3.79 (2×s, 6H, 2×OCH_3), 4.38–4.41 (m, 1H, H-2′), 4.53–4.56 (m, 2H, H-1′), 6.74 (d, 4H, J=8.7 Hz, H_{arom}), 6.82 (d, 1H, J=8.7 Hz, H_{arom}), 7.16–7.69 (m, 11H, H_{arom}), 8.06 (s, 1H, H_{arom}), 8.28 (s, 1H, H_{arom}), 8.59 (d, 1H, J=4.1 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 45.87 (C-1′), 55.04, 55.15 (2×OCH_3), 65.12 (C-3′), 70.01 (C-2′), 86.47 (C_{DMT}), 110.31, 113.06, 113.11, 118.86, 121.51, 122.75, 123.69, 126.84, 127.01, 127.81, 127.99, 129.11, 129.54, 129.93, 131.65, 132.86, 135.64, 135.93, 137.68, 138.56, 140.85, 144.60, 145.10, 149.76, 158.47 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{38}H_{32}Cl_2N_3O_4^+$ (MH^+): 664.1764, found 664.1791.

4.5.3. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-4-indolo[2,3-*b*]quinoxalin-6-yl-butan-2-ol (15c). Yield: 0.206 g (83%) as a yellow oil (obtained from 0.125 g, 0.41 mmol of **6**). ^{1}H NMR ($CDCl_3$): δ 1.71–2.22 (m, 2H, H-2′), 3.04–3.19 (m, 3H, H-3′, H-4′), 3.55 (br s, 1H, OH), 3.73, 3.79 (2×s, 6H, 2×OCH_3), 4.46 (m, 1H, H-1′), 4.80 (m, 1H, H-1′), 6.71–6.86 (m, 4H, H_{arom}), 7.11–7.53 (m, 11H, H_{arom}), 7.66–7.78 (m, 3H, H_{arom}), 8.09 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.31 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.49 (d, 1H, J=7.3 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 32.92 (C-2′), 37.97 (C-1′), 55.13, 55.22 (2×OCH_3), 67.29 (C-3′, C-4′), 85.98 (C_{DMT}), 109.45, 113.00, 119.51, 121.12, 122.79, 126.11, 126.66, 127.03, 127.50, 127.68, 127.74, 127.81, 128.09, 128.97, 129.11, 129.35, 129.92, 131.18, 135.99, 139.30, 139.45, 139.87, 144.37, 144.76, 158.33 (C_{arom}).

4.5.4. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-5-indolo[2,3-*b*]quinoxalin-6-yl-pentan-2-ol (15d). Yield: 0.068 g (34%) as a yellow oil (obtained from 0.103 g, 0.32 mmol of **7**), R_f 0.13 (EtOAc/cyclohexane 1:2). ^{1}H NMR ($CDCl_3$): δ 1.51–1.59 (m, 2H, H-3′), 1.97–2.13 (m, 2H, H-2′), 3.01 (dd, 1H, J=6.8 Hz, 9.3 Hz, H-5′), 3.12 (dd, 1H, J=3.7 Hz, 9.3 Hz, H-5′), 3.74 (s, 6H, 2×OCH_3), 3.91–3.93 (m, 1H, H-4′), 4.48–4.57 (m, 2H, H-1′), 6.74 (d, 4H, J=8.8 Hz, H_{arom}), 7.17 (dd, 2H, J=2.5 Hz, 6.5 Hz, H_{arom}), 7.23 (d, 6H, J=8.8 Hz, H_{arom}), 7.35 (d, 2H, J=7.4 Hz, H_{arom}), 7.45 (d, 1H, J=8.1 Hz, H_{arom}), 7.63–7.73 (m, 3H, H_{arom}), 8.07 (dd, 1H, J=8.1 Hz, 1.6 Hz, H_{arom}), 8.29 (dd, 1H, J=8.1 Hz, 1.6 Hz, H_{arom}), 8.47 (d, 1H, J=7.4 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 24.95 (C-2′), 30.30 (C-3′), 41.28 (C-1′), 55.12 (2×OCH_3), 67.44 (C-5′), 70.89 (C-4′), 85.90 (C_{DMT}), 109.48, 113.00, 119.41, 120.81, 122.68, 125.88, 126.69, 127.65, 127.71, 128.00, 128.71, 129.23, 129.91, 130.96, 135.86, 135.91, 139.16, 140.03, 140.33, 144.30, 144.72, 145.60, 158.35 (C_{arom}).

4.5.5. 6-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-ribofuranosyl]-indolo[2,3-*b*]quinoxaline (18). Yield: 0.148 g (58%) as a yellow oil (obtained from 0.134 g, 0.40 mmol of **8**), R_f 0.35 (4% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 2.36–2.46 (m, 2H, H-2′), 3.43–3.56 (m, 2H, H-5′), 3.72 (s, 6H, 2×OCH_3), 4.16 (m, 1H, H-4′), 5.02 (m, 1H, H-3′), 6.71 (m, 4H, H_{arom}), 7.00 (t, 1H, J=7.1 Hz, H-1′), 7.15–7.43 (m, 11H, H_{arom}), 7.66–7.71 (m, 2H, H_{arom}), 7.80 (d, 1H, J=7.6 Hz, H_{arom}), 7.92 (dd, 1H, J=7.6 Hz, 2.2 Hz, H_{arom}), 8.26 (dd, 1H, J=7.6 Hz, 2.2 Hz, H_{arom}), 8.44 (dd, 1H, J=7.6 Hz, 2.2 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 37.21 (C-2′), 55.15 (2×OCH_3), 63.64 (C-5′), 72.69 (C-3′), 83.09 (C-4′), 84.98 (C-1′), 86.46 (C_{DMT}), 111.98, 113.05, 120.11, 121.61, 122.57, 126.44, 126.80, 127.77, 127.98, 128.18, 128.72, 129.13, 130.04, 130.09, 130.97, 135.72, 135.79, 139.38, 140.02, 140.33, 143.12, 144.65, 145.19, 158.42, 158.45 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{40}H_{35}N_3O_5Na^+$ (MNa^+): 660.2469, found 660.2450.

4.6. General procedure for synthesis of phosphoramidites

The DMT-protected compound (**15a–d**, **18**) was mixed with diisopropylammoniumtetrazolide (1.7 equiv) and dissolved in dry CH_2Cl_2 (10 mL). 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane (2.5 equiv) was added, and the reaction mixture was stirred at rt overnight. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/cyclohexane/Et_3N (49:49:2 v/v/v) as an eluent.

4.6.1. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-3-indolo[2,3-*b*]quinoxalin-6-yl-propan-2-ol (16a). Yield: 0.078 g (61%) as a yellow oil (obtained from 0.095 g, 0.16 mmol of **15a**), R_f 0.58 (EtOAc/cyclohexane 1:1). ^{31}P NMR ($CDCl_3$): δ 149.96, 150.27. HRMS (MALDI): m/z calcd for $C_{47}H_{50}N_5O_5PNa^+$ (MNa^+): 818.3442, found 818.3401.

4.6.2. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-3-(2,3-dichloro-indolo[2,3-*b*]quinoxalin-6-yl)-propan-2-ol (16b). Yield: 0.146 g (66%) as a yellow oil (obtained from 0.170 g, 0.26 mmol of **15b**), R_f 0.64 (EtOAc/cyclohexane 1:1). ^{1}H NMR ($CDCl_3$): δ 0.74–1.28 (m, 12H, 4×CH_3), 2.06, 2.32 (2×t, 2H, J=6.6 Hz, CH_2CN),

3.22–3.60 (m, 6H, H-3′, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.77 (s, 6H, 2×OCH$_3$), 4.07–4.16 (m, 1H, H-2′), 4.62–4.67 (m, 2H, H-1′), 6.76–6.84 (m, 4H, H$_{arom}$), 7.16–7.69 (m, 12H, H$_{arom}$), 8.12–8.41 (m, 3H, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 20.09 (CH$_2$CN), 24.28, 24.40, 24.46, 24.54 (4×CH(CH$_3$)$_2$), 42.73, 42.92 (2×CH(CH$_3$)$_2$), 44.42 (C-1′), 55.17 (2×OCH$_3$), 57.75 (OCH$_2$CH$_2$CN), 64.87 (C-3′), 70.63 (C-2′), 86.28 (C$_{DMT}$), 110.57, 113.02 (C$_{arom}$), 119.08 (CN), 121.29, 122.76, 126.76, 127.73, 128.11, 128.27, 129.10, 129.70, 129.98, 131.50, 132.60, 135.79, 135.98, 137.83, 139.27, 144.71, 145.10, 145.93, 158.43 (C$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 150.20, 150.34. HRMS (ESI): *m/z* calcd for C$_{47}$H$_{48}$N$_5$O$_5$Cl$_2$PNa$^+$ (MNa$^+$): 886.2662, found 886.2684.

4.6.3. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-4-indolo[2,3-*b*]quinoxalin-6-yl-butan-2-ol (16c). Yield: 0.206 g (84%) as a yellow oil (obtained from 0.185 g, 0.30 mmol of **15c**). ^{1}H NMR (CDCl$_3$): δ 1.04–1.26 (m, 12H, 4×CH$_3$), 2.23–2.38 (m, 2H, H-2′), 2.40, 2.59 (2×t, 2H, J=6.5 Hz, CH$_2$CN), 3.10, 3.24 (2×dd, 1H, J=6.2 Hz, 9.2 Hz, H-4′), 3.36 (m, 1H, H-4′), 3.55–3.71 (m, 4H, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.77, 3.79 (2×s, 6H, 2×OCH$_3$), 4.51–4.61 (m, 2H, H-1′), 4.17–4.22 (m, 1H, H-3′), 6.76–6.84 (m, 4H, H$_{arom}$), 7.16–7.76 (m, 14H, H$_{arom}$), 8.04 (m, 1H, H$_{arom}$), 8.29 (dd, 1H, J=8.1 Hz, 1.3 Hz, H$_{arom}$), 8.47 (d, 1H, J=7.6 Hz, H$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 149.47, 149.70.

4.6.4. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-5-indolo[2,3-*b*]quinoxalin-6-yl-pentan-2-ol (16d). Yield: 0.066 g (73%) as a yellow oil (obtained from 0.068 g, 0.11 mmol of **15d**), R_f 0.58 (EtOAc/cyclohexane 1:1). ^{1}H NMR (CDCl$_3$): δ 1.69–2.00 (m, 2H, H-3′), 2.34, 2.64 (2×t, 2H, J=6.5 Hz, CH$_2$CN), 2.90 (dd, 1H, J=6.2 Hz, 9.2 Hz, H-5′), 3.01–3.15 (m, 1H, H-5′), 3.41–3.51, 3.56–3.70 (2×m, 4H, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.73 (s, 6H, 2×OCH$_3$), 3.97–4.06 (m, 1H, H-4′), 4.47–4.53 (m, 2H, H-1′), 6.71 (m, 4H, H$_{arom}$), 7.13–7.75 (m, 14H, H$_{arom}$), 8.07 (m, 1H, H$_{arom}$), 8.29 (dd, 1H, J=8.1 Hz, 1.2 Hz, H$_{arom}$), 8.48 (d, 1H, J=7.6 Hz, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 20.29 (CH$_2$CN), 23.71 (C-2′), 24.36, 24.46, 24.55, 24.66 (4×CH$_3$), 30.85 (C-3′), 41.35 (C-1′), 42.87, 43.04 (2×CH(CH$_3$)$_2$), 55.12 (2×OCH$_3$), 58.09 (OCH$_2$CH$_2$CN), 65.66 (C-5′), 72.70 (C-4′), 85.76 (C$_{DMT}$), 109.52, 112.91, 117.61, 119.44, 120.75, 122.66, 125.85, 126.55, 126.62, 127.62, 127.77, 127.81, 128.04, 128.11, 128.65, 129.31, 129.95, 130.91, 136.00, 139.21, 140.01, 144.34, 144.46, 144.86, 158.26, 158.31 (C$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 149.08, 149.60. HRMS (MALDI): *m/z* calcd for C$_{49}$H$_{54}$N$_5$O$_5$PNa$^+$ (MNa$^+$): 846.3755, found 846.3778.

4.6.5. Phosphoramidite of 6-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-ribofuranosyl]-indolo[2,3-*b*]quinoxaline (19). Yield: 0.033 g (21%) as a yellow oil (obtained from 0.119 g, 0.19 mmol of **18**). ^{1}H NMR (CDCl$_3$): δ 1.19–1.28 (m, 12H, 4×CH$_3$), 2.47, 2.63 (2×t, 2H, J=6.5 Hz, CH$_2$CN), 3.41–3.92 (m, 8H, H-2′, H-5′, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.73 (2×s, 6H, 2×OCH$_3$), 4.30 (m, 1H, H-4′), 5.04–5.18 (m, 1H, H-3′), 6.66–6.72 (m, 4H, H$_{arom}$), 7.01–7.43 (m, 12H, H-1′, H$_{arom}$), 7.70 (m, 2H, H$_{arom}$), 7.89–7.96 (m, 2H, H$_{arom}$), 8.27 (dd, 1H, J=7.6 Hz, 2.2 Hz, H$_{arom}$), 8.43–8.46 (m, 1H, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 20.33 (CH$_2$CN), 24.52, 24.54, 24.60, 24.67 (4×CH(CH$_3$)$_2$), 36.39 (C-2′), 43.21, 43.32 (2×CH(CH$_3$)$_2$), 55.15 (2×OCH$_3$), 58.53 (OCH$_2$CH$_2$CN), 62.98 (C-5′), 73.32 (C-3′), 83.29 (C-4′), 84.73 (C-1′), 86.30 (C$_{DMT}$), 112.29, 112.97 (C$_{arom}$), 117.45 (CN), 120.14, 121.58, 122.47, 126.37, 126.76, 127.68, 127.93, 128.31, 128.68, 129.21, 130.17, 130.97, 135.70, 135.74, 135.78, 135.81, 139.45, 140.04, 143.11, 144.64, 145.22, 158.39 (C$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 149.63, 150.02. HRMS (MALDI): *m/z* calcd for C$_{49}$H$_{52}$N$_5$O$_6$PNa$^+$ (MNa$^+$): 860.3547, found 860.3578.

4.6.6. 6-Methylindolo[2,3-*b*]quinoxaline-3-carboxylic acid methyl ester (21). 6*H*-Indolo[2,3-*b*]quinoxaline-3-carboxylic acid[58] (**20**, 2.50 g, 9.5 mmol) was suspended in dry DMSO (25 mL) and powdered KOH (1.85 g, 33 mmol, 3.5 equiv) was added. The reaction mixture turned red, as it was stirred for 15 min CH$_3$I (5.11 g, 36 mmol, 3.8 equiv) was added through a syringe. The reaction mixture was stirred at rt for 48 h. N_2 was bubbled through the reaction mixture in order to get rid of excess of CH$_3$I. H_2O (50 mL) was added, and the compound precipitated. The yellow powder was filtered, washed with water and dried in vacuo. A small sample was separated for analysis by silica gel column chromatography using EtOAc/PE 1:1 as an eluent. The rest was used for the next step without further purification. The ratio between the ester and the carboxylic acid was 3:2.

Yellow solid, R_f 0.53 (EtOAc/PE 1:1), mp 180 °C. ^{1}H NMR (CDCl$_3$): δ 3.87, 4.00 (2×s, 6H, 2×CH$_3$), 7.35 (d, 2H, J=7.3 Hz, H$_{arom}$), 7.66 (t, 1H, J=7.3 Hz, H$_{arom}$), 8.21 (m, 2H, H$_{arom}$), 8.37 (d, 1H, J=7.2 Hz, H$_{arom}$), 8.73 (s, 1H, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 27.39 (CH$_3$), 52.34 (OCH$_3$), 109.20, 118.88, 121.10, 122.91, 125.31, 129.30, 129.61, 130.30, 131.65, 139.45, 141.12, 141.38, 145.32, 145.88 (C$_{arom}$), 166.71 (COOR). HRMS (MALDI): *m/z* calcd for C$_{17}$H$_{14}$N$_3$O$_2^+$ (MH$^+$): 292.1081, found 292.1071.

4.6.7. 6-Methylindolo[2,3-*b*]quinoxaline-3-carboxylic acid (22). Yellow solid, R_f 0.42 (EtOAc/PE 1:1), mp 192 °C. ^{1}H NMR (DMSO-d_6): δ 3.78 (s, 3H, CH$_3$), 7.34 (t, 1H, J=7.5 Hz, H$_{arom}$), 7.57 (d, 1H, J=8.0 Hz, H$_{arom}$), 7.71 (t, 1H, J=7.5 Hz, H$_{arom}$), 7.93–8.14 (m, 2H, H$_{arom}$), 8.22 (d, 1H, J=7.5 Hz, H$_{arom}$), 8.46 (s, 1H, H$_{arom}$). ^{13}C NMR (DMSO-d_6): δ 27.30 (CH$_3$), 110.06, 117.98, 120.96, 122.26, 124.95, 129.04, 129.28, 130.29, 131.79, 138.78, 140.21, 140.64, 145.04, 145.17 (C$_{arom}$), 166.96 (COOH). HRMS (MALDI): *m/z* calcd for C$_{16}$H$_{11}$N$_3$O$_2^+$ (MH$^+$): 278.0924, found 278.0922.

4.6.8. (6-Methylindolo[2,3-*b*]quinoxalin-3-yl)methanol (23). LiAlH$_4$ (0.65 g, 17.1 mmol) was suspended in dry THF (20 mL) at 0 °C under N_2. The crude mixture of **21** and **22** was dissolved in dry THF (20 mL) and added dropwise using a syringe. The icebath was removed and the reaction was gently refluxed for 5 h. The reaction was quenched at 0 °C by carefully adding 1 mL H_2O, 1 mL of a 15% aq solution of NaOH and 3 mL of H_2O. The reaction mixture was filtered and washed with ethanol. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using 5% MeOH/CHCl$_3$ as an eluent.

Yield: 1.10 g (44% over two steps) as yellow crystals, R_f 0.34 (5% MeOH/$CHCl_3$), mp 175 °C. IR (KBr): 3371 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.89 (s, 1H, CH_3), 4.80 (d, 2H, J=5.4 Hz, CH_2), 5.53 (t, 1H, OH), 7.39 (t, 1H, J=7.7 Hz, H_{arom}), 7.66–7.80 (m, 3H, H_{arom}), 8.03 (s, 1H, H_{arom}), 8.20 (d, 1H, J=8.7 Hz, H_{arom}), 8.35 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 27.36 (CH_3), 62.77 (CH_2), 110.01, 118.44, 120.78, 121.81, 123.78, 124.91, 128.61, 130.96, 137.59, 138.80, 139.83, 143.71, 144.44, 145.16 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{16}H_{14}N_3O^+$ (MH^+): 264.1131, found 264.1128.

4.6.9. 3-Chloromethyl-6-methylindolo[2,3-*b*]quinoxaline (24). (6-Methylindolo[2,3-*b*]quinoxalin-3-yl)methanol (**23**, 0.500 g, 1.9 mmol) was suspended in a mixture of pyridine (0.23 mL) and CH_2Cl_2 (10 mL) and the suspension was cooled to 0 °C. $SOCl_2$ (0.25 mL) was added slowly with a syringe. The reaction was stirred at rt overnight. The reaction mixture was poured into water (20 mL) and CH_2Cl_2 (10 mL) was added. The two-phase system was stirred for 30 min, after which the phases were separated. The organic phase was washed with 5% $NaHCO_3$ (aq) (2×25 mL) and satd aq NaCl (2×25 mL) and dried using $MgSO_4$. The solvent was removed under reduced pressure yielding a yellow solid, which was purified by silica gel column chromatography using 1% MeOH/CH_2Cl_2 as eluent.

Yield: 0.478 g (89%) as a yellow solid, R_f 0.50 (1% MeOH/CH_2Cl_2), mp 204–205 °C. ^1H NMR (CD_2Cl_2): δ 3.84 (s, 3H, CH_3), 4.78 (s, 2H, CH_2), 7.30 (t, 1H, J=7.4 Hz, H_{arom}), 7.40 (d, 1H, J=8.2 Hz, H_{arom}), 7.59–7.64 (m, 2H, H_{arom}), 8.00 (s, 1H, H_{arom}), 8.13 (d, 1H, J=8.6 Hz, H_{arom}), 8.32 (d, 1H, J=7.9 Hz, H_{arom}). ^{13}C NMR (CD_2Cl_2): δ 27.76 (CH_3), 46.57 (CH_2), 109.83, 119.34, 121.52, 122.92, 126.71, 127.44, 129.66, 129.87, 131.80, 138.66, 138.84, 140.54, 140.79, 145.60 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{16}H_{13}ClN_3^+$ (MH^+): 282.0973, found 282.0795.

4.6.10. 3-[2-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)ethoxymethyl]-6-methylindolo[2,3-*b*]quinoxaline (25). To a solution of 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethanol (0.180 g, 1.23 mmol) in dry toluene (15 mL) was added pulverized KOH (0.736 g, 13.0 mmol) followed by 3-chloromethyl-6-methylindolo[2,3-*b*]quinoxaline (**24**, 0.205 g, 0.73 mmol). A Dean–Stark apparatus was filled with dry toluene in the side arm and used, while refluxing the reaction mixture for 48 h. H_2O (10 mL) was added and the organic phase was washed with H_2O (3×10 mL), dried using $MgSO_4$ and evaporated under reduced pressure yielding a yellow oil, which was purified by silica gel column chromatography using 2% MeOH/CH_2Cl_2 as an eluent.

Yield: 0.190 g (67%) as a yellow oil, R_f 0.46 (4% MeOH/CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 1.37, 1.41 (2×s, 6H, 2×CH_3), 1.91–1.97 (m, 2H, H-2′), 3.59–3.64 (m, 1H, H-3′), 3.69 (t, 2H, J=6.8 Hz, H-1′), 3.94 (s, 3H, NCH_3), 4.08–4.13 (m, 1H, H-4′), 4.26–4.30 (m, 1H, H-4″), 4.76 (s, 2H, CH_2), 7.34–7.43 (m, 2H, H_{arom}), 7.62–7.70 (m, 2H, H_{arom}), 7.97–8.21 (m, 1H, H_{arom}), 8.25 (d, 1H, J=8.6 Hz, H_{arom}), 8.44 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.78, 26.95 ($C(CH_3)_2$), 27.48 (CH_3), 33.92 (C-2′), 67.35 (C-1′), 69.67 (C-4′), 72.85 (CH_2), 73.80 (C-3′), 108.58 ($C(CH_3)_2$), 109.14, 119.35, 120.93, 122.54, 125.43, 125.76, 129.40, 130.90, 137.90, 138.67, 139.35, 140.39, 144.84, 145.68 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{23}H_{25}N_3O_3Na^+$ (MNa^+): 414.1788, found 414.1776.

4.6.11. (*S*)-4-(6-Methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butane-1,2-diol (9). The same procedure was used as for compounds **4**–**7**.

Quantitative yield. Yellow oil. ^1H NMR (DMSO-d_6): δ 1.53–1.62, 1.80–1.90 (2×m, 2H, H-2′), 3.23–3.32 (m, 2H, H-4′), 3.66 (t, 2H, J=6.5 Hz, H-1′), 3.82–3.95 (m, 1H, H-3′), 3.89 (s, 3H, NCH_3), 4.51 (br s, 2H, 2×OH), 4.73 (s, 2H, CH_2), 7.39 (t, 1H, J=7.3 Hz, H_{arom}), 7.62–7.83 (m, 3H, H_{arom}), 7.99–8.13 (m, 1H, H_{arom}), 8.19 (d, 1H, J=8.6 Hz, H_{arom}), 8.33 (d, 1H, J=7.6 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 27.40 (CH_3), 33.67 (C-2′), 66.04 (C-1′), 67.13 (C-4′), 68.44 (C-3′), 71.47 (CH_2), 110.09, 118.40, 120.83, 121.89, 124.94, 125.31, 128.83, 131.09, 137.77, 139.10, 139.68, 139.90, 144.58, 145.21 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{20}H_{21}N_3O_3Na^+$ (MNa^+): 374.1475, found 374.1462.

4.6.12. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-4-(6-methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butan-2-ol (26). The same procedure was used as for DMT-protection of compounds **4**–**8**.

Yield: 0.149 g (55%) as a yellow oil, ^1H NMR ($CDCl_3$): δ 1.80–1.86 (m, 2H, H-2′), 3.13–3.17, 3.68–3.73 (2×m, 4H, H-1′, H-4′), 3.76 (s, 6H, 2×OCH_3), 3.96 (s, 3H, NCH_3), 4.02–4.12 (m, 1H, H-3′), 4.74 (s, 2H, CH_2), 6.81 (d, 4H, J=8.7 Hz, H_{arom}), 7.19–7.29 (m, 3H, H_{arom}), 7.32 (d, 4H, J=8.7 Hz, H_{arom}), 7.36–7.46 (m, 4H, H_{arom}), 7.59 (dd, 1H, J=8.5 Hz, 1.8 Hz, H_{arom}), 7.70 (t, 1H, J=7.0 Hz, H_{arom}), 7.98–8.20 (m, 1H, H_{arom}), 8.25 (d, 1H, J=8.7 Hz, H_{arom}), 8.46 (d, 1H, J=7.8 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 27.50 (CH_3), 33.53 (C-2′), 55.16 (OCH_3), 67.33 (C-1′), 68.14 (C-4′), 69.43 (C-3′), 72.95 (CH_2), 85.97 (C_{DMT}), 109.16, 113.06, 120.88, 120.95, 122.58, 125.47, 125.85, 126.72, 127.77, 128.14, 129.42, 130.02, 130.93, 136.05, 138.67, 139.21, 140.38, 144.85, 158.41 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{20}H_{39}N_3O_5Na^+$ (MNa^+): 676.2782, found 676.2757.

4.6.13. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-4-(6-methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butan-2-ol (27). The same procedure was used as for phosphitylation of compounds **15a**–**d**.

Yield: 0.061 g (47%) as a yellow oil, R_f 0.52 (EtOAc/cyclohexane 1:1). ^1H NMR ($CDCl_3$): δ 1.04–1.17 (m, 12H, 4×CH_3), 2.38, 2.53 (2×t, 2H, J=6.5 Hz, CH_2CN), 1.92–1.99 (m, 1H, H-2′), 2.08–2.17 (m, 1H, H-2′), 3.02–3.07, 3.20–3.23 (2×m, 2H, H-4′), 3.49–3.72 (m, 6H, H-1′, OCH_2CH_2CN, 2×$CH(CH_3)_2$), 3.76 (s, 6H, 2×OCH_3), 3.98 (s, 3H, NCH_3), 4.19–4.22 (m, 1H, H-3′), 4.70–4.74 (m, 2H, CH_2), 6.77–6.82 (m, 4H, H_{arom}), 7.16–7.29 (m, 4H, H_{arom}), 7.34 (d, 4H, J=8.8 Hz, H_{arom}), 7.40 (t, 1H, J=7.5 Hz, H_{arom}), 7.46 (dd, 2H, J=2.9 Hz, 7.3 Hz, H_{arom}), 7.62 (m, 1H, H_{arom}), 7.71 (t, 1H, J=7.3 Hz, H_{arom}), 8.06 (d, 1H, J=8.6 Hz, H_{arom}), 8.25 (dd, 1H, J=1.0 Hz, 8.6 Hz, H_{arom}), 8.47 (d, 1H, J=7.5 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 21.03 (CH_2CN), 24.43, 24.54, 24.60, 24.73 (4×CH_3),

26.90 (CH_3), 33.84 (C-2′), 42.33, 42.81 (2×*C*H(CH_3)$_2$), 55.15 (2×OCH_3), 58.23 (O*C*H_2CH_2CN), 64.61 (C-1′), 66.31 (C-4′), 67.08 (C-3′), 72.72 (CH_2), 85.86 (C_{DMT}), 109.19, 112.97 (C_{arom}), 119.33 (CN), 120.95, 122.57, 125.56, 125.77, 126.59, 127.67, 128.24, 129.34, 130.16, 130.92, 136.19, 138.03, 139.27, 140.44, 144.06, 144.99, 158.41 (C_{arom}). ^{31}P NMR ($CDCl_3$): δ 148.57, 149.06. HRMS (MALDI): *m*/*z* calcd for $C_{50}H_{56}N_5O_6PNa^+$ (MNa^+): 876.3860, found 876.3865.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-609476 (**4**) and CCDC-609477 (**23**·H_2O). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 36033 or e-mail: deposit@ccdc.cam.ac.uk).

4.7. ODN and INA syntheses, 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 6*H*-indolo[2,3-*b*]quinoxaline amidits **16a–d**, **19** and **27** were dissolved in dry MeCN (if necessary a 1:1 mixture of dry MeCN and dry CH_2Cl_2), as a 0.075 M solution and inserted into the growing oligonucleotide chain using the same conditions as for normal nucleotide couplings (2 min coupling). The ODNs were synthesized with DMT and purified on 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 0.1 M NH_4HCO_3 and 50 mL of MeCN (pH=9.0)] and buffer B [250 mL of 0.1 M 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 ~35 min). The ODNs were DMT-deprotected in 80% aq acetic acid (100 μL) for 20 min, diluted with 1 M sodium acetate (150 μL), and precipitated from abs ethanol (600 μL). All modified ODNs were confirmed by MALDI-TOF analysis on a Voyager Elite Bio Spectrometry Research Station from Perceptive Biosystems.

Melting temperature measurements were performed on a Perkin–Elmer Lambda 20 UV/VIS spectrometer fitted with a PTP-6 temperature programmer. Melting temperature (T_m) measurements were determined in a 1 mM EDTA, 10 mM $Na_2HPO_4·2H_2O$, 140 mM NaCl buffer at pH=7.0 for 1.5 μM of each strand. The melting temperature was determined as the maximum of the first derivative plots of the melting curve and are with an uncertainty of ±1.0 °C as determined by repetitive experiments.

4.8. Fluorescence measurements

Fluorescence measurements were performed on a Perkin–Elmer LS-55 luminescence spectrometer fitted with a Julabo F25 temperature controller by an excitation at 360 nm and detection at 370–700 nm. All measurements were conducted at 10 °C in a 140 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA buffer at pH=7.0 with a concentration of 1.5 μM of each strand.

4.9. Single-crystal X-ray diffraction

Crystals of **4** and **23**·H_2O were obtained from solutions in MeOH/CH_2Cl_2, layered with hexane. X-ray diffraction data for **4** were collected at Beamline I911-3 at the MAX-II storage ring, MAX-lab, University of Lund, Sweden (λ=0.750 Å). Diffraction data for **23**·H_2O were collected locally with a Bruker Nonius X8APEX-II instrument. Molecule **4** crystallizes in the non-centrosymmetric space group $P2_1$ with two molecules in the asymmetric unit. The ring systems adopt a centrosymmetric arrangement (space group $P2_1/n$), but the centrosymmetry is broken by the chiral side-chains. It was not possible to determine the absolute structure: the *S* enantiomer was assigned on the basis of the chemistry and Friedel opposites were merged as equivalent data in the final cycles of refinement.

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References and notes

1. Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18–30.
2. Pigram, W. J.; Fuller, W.; Davies, M. E. *J. Mol. Biol.* **1973**, *80*, 361–365.
3. Wang, J. C. *J. Mol. Biol.* **1974**, *89*, 783–801.
4. Miller, K. J. *Biopolymers* **1979**, *18*, 2683–2719.
5. Wang, A. H. J.; Ughetto, G.; Quingley, G. J.; Rich, A. *Biochemistry* **1987**, *26*, 1152–1163.
6. Calendi, E.; Di Marco, A.; Reggiani, M.; Scarpinato, B.; Valentini, L. *Biochim. Biophys. Acta* **1965**, *103*, 25–49.
7. Gellert, M.; Smith, C. E.; Neville, D.; Felsenfeld, G. *J. Mol. Biol.* **1965**, *11*, 445–457.
8. Müller, W.; Crothers, D. M. *J. Mol. Biol.* **1968**, *35*, 251–290.
9. Glisson, B. S.; Ross, W. E. *Pharmacol. Ther.* **1987**, *32*, 89–106.
10. Ross, W. E.; Bradley, M. O. *Biochim. Biophys. Acta* **1981**, *654*, 129–134.
11. Martinez, R.; Chacón-Garcia, L. *Curr. Med. Chem.* **2005**, *12*, 127–151.
12. Braña, M. F.; Cacho, M.; Gradillas, A.; de Pascual-Teresa, B.; Ramos, A. *Curr. Pharm. Des.* **2001**, *7*, 1745–1780.
13. Graves, D. E.; Velea, L. M. *Curr. Org. Chem.* **2000**, *4*, 915–929.
14. Christensen, U. B.; Wamberg, M.; El-Essawy, F. A. G.; Ismail, A. E.-H.; Nielsen, C. B.; Filichev, V. V.; Jessen, C. H.; Petersen, M.; Pedersen, E. B. *Nucleosides Nucleotides Nucleic Acids* **2004**, *23*, 207–225.
15. Christensen, U. B.; Pedersen, E. B. *Nucleic Acids Res.* **2002**, *30*, 4918–4925.
16. Christensen, U. B.; Pedersen, E. B. *Helv. Chim. Acta* **2003**, *86*, 2090–2097.

17. Louët, S. *Nat. Biotechnol.* **23 June 2003**, http://dx.doi.org/10.1038/bioent748. INA™ is commercially available from Human Genetic Signatures Pty Ltd., Sydney, Australia.
18. Ren, J.; Qu, X.; Dattagupta, N.; Chaires, J. B. *J. Am. Chem. Soc.* **2001**, *123*, 6742–6743.
19. Goodwin, S.; Smith, A. F.; Horning, E. C. *J. Am. Chem. Soc.* **1959**, *81*, 1903–1908.
20. Kansal, V. K.; Potier, P. *Tetrahedron* **1986**, *42*, 2389–2408.
21. Patel, N.; Bergman, J.; Gräslund, A. *Eur. J. Chem.* **1991**, *197*, 597–604.
22. Zegar, I.; Gräslund, A.; Bergman, J.; Eriksson, M.; Nordén, B. *Chem. Biol. Interact.* **1989**, *72*, 277–293.
23. Bergman, J.; Damberg, C.; Valberg, H. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 31–36.
24. Harmenberg, J.; Wahren, B.; Bergman, J.; Åkerfeldt, S.; Lundblad, L. *Antimicrob. Agents Chemother.* **1988**, *32*, 1720–1724.
25. Harmenberg, J.; Åkesson-Johansson, A.; Gräslund, A.; Malmfors, T.; Bergman, J.; Wahren, B.; Åkerfeldt, S.; Lundblad, L.; Cox, S. *Antiviral Res.* **1991**, *15*, 193–204.
26. Kohn, K. W.; Waring, M. J.; Glaubiger, D.; Friedman, C. A. *Cancer Res.* **1975**, *35*, 71–76.
27. Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450–465.
28. Paoletti, C.; Lesca, C.; Cros, S.; Malvy, C.; Auclair, C. *Biochem. Pharmacol.* **1979**, *28*, 345–350.
29. Filipski, J.; Kohn, K. W. *Biochim. Biophys. Acta* **1982**, *698*, 280–286.
30. Arimondo, P. B.; Baldeyrou, B.; Laine, W.; Bal, C.; Alphonse, F.-A.; Routier, S.; Coudert, G.; Mérour, J.-Y.; Colson, P.; Houssier, C.; Bailly, C. *Chem. Biol. Interact.* **2001**, *138*, 59–75.
31. Kim, H. S.; Barak, D.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2001**, *44*, 3092–3108.
32. Kawakami, Y.; Asai, T.; Umeyama, K.; Yamashita, Y. *J. Org. Chem.* **1982**, *47*, 3581–3585.
33. Augustyns, K.; Aerschot, A. V.; Schepdael, A. V.; Urbanke, C.; Herdewijn, P. *Nucleic Acid Res.* **1991**, *19*, 2587–2593.
34. Mori, K.; Ikunaka, M. *Tetrahedron* **1984**, *40*, 3471–3479.
35. Hayashi, H.; Nakanishi, K.; Brandon, C.; Marmur, J. *J. Am. Chem. Soc.* **1973**, *95*, 8749–8757.
36. Herdeis, C. *Synthesis* **1986**, 232–233.
37. Brunner, H.; Lautenschalger, H.-J. *Synthesis* **1989**, 706–709.
38. Dostert, P.; Langlois, M.; Guerret, P.; Ancher, J.-F.; Bucher, B.; Mocquet, G. *Eur. J. Med. Chem.* **1980**, *15*, 199–206.
39. Schunck, E.; Marchlewski, L. *Chem. Ber.* **1895**, *28*, 2525–2531.
40. Buraczewski, J.; Marchlewski, L. *Chem. Ber.* **1901**, *34*, 4008–4015.
41. Cassel, S.; Debaig, C.; Benvegnu, T.; Chaimbault, P.; Lafosse, M.; Plusquellec, D.; Rollin, P. *Eur. J. Org. Chem.* **2001**, 875–896.
42. Börjesson, L.; Welch, C. J. *Tetrahedron* **1992**, *48*, 6325–6334.
43. Bergman, J. *Tetrahedron Lett.* **1989**, *30*, 1837–1840.
44. Knotz, F. *Sci. Pharm.* **1970**, *38*, 20–25.
45. Rolland, V.; Kotera, M.; Lhomme, J. *Synth. Commun.* **1997**, *27*, 3505–3511.
46. Abdel-Megied, A. E.-S.; Hansen, P.; Pedersen, E. B.; Nielsen, C. M. *Acta Chem. Scand.* **1991**, *45*, 1060–1063.
47. Varma, R. S.; Khan, I. A. *J. Indian Chem. Soc.* **1978**, *55*, 1043–1045.
48. Badger, G. M.; Nelson, J. P. *J. Chem. Soc.* **1962**, 3926–3931.
49. Bair, K. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1990**, *33*, 2385–2393.
50. Tirosh, O.; Kohen, R.; Katzhendler, J.; Gorodetsky, R.; Barenholz, Y. *J. Chem. Soc., Perkin Trans. 2* **1997**, 383–389.
51. Mujeeb, A.; Kerwin, S. M.; Kenyon, G. L.; James, T. L. *Biochemistry* **1993**, *32*, 13419–13431.
52. Filichev, V. V.; Christensen, U. B.; Pedersen, E. B.; Babu, B. R.; Wengel, J. *ChemBioChem* **2004**, *5*, 1673–1679.
53. Nielsen, C. B.; Petersen, M.; Pedersen, E. B.; Hansen, P. E.; Christensen, U. B. *Bioconjugate Chem.* **2004**, *15*, 260–269.
54. Filichev, V. V.; Hilmy, K. H. M.; Christensen, U. B.; Pedersen, E. B. *Tetrahedron Lett.* **2004**, *45*, 4907–4910.
55. Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607–3630.
56. Petersen, M.; Nielsen, C. B.; Nielsen, K. E.; Jensen, G. A.; Bondensgaard, K.; Singh, S. K.; Rajwanshi, V. K.; Koshkin, A. A.; Dahl, B. M.; Wengel, J.; Jacobsen, J. P. *J. Mol. Recognit.* **2000**, *13*, 44–53.
57. Jensen, G. A.; Singh, S. K.; Kumar, R.; Wengel, J.; Jacobsen, J. P. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1224–1232.
58. Bergman, J. O. E.; Åkerfeldt, S. G. Patent WO 8704436, 1987.
59. Alphonse, F.-A.; Routier, S.; Coudert, G.; Mérour, J.-Y. *Heterocycles* **2001**, *55*, 925–940.